

IMPERIAL COLLEGE LONDON

DOCTORAL THESIS

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**Dissection of modularity and spatial organisation  
in natural and engineered cellular contexts:  
Systems analysis at the junction of systems and  
synthetic biology**

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for the degree of Doctor of Philosophy*

*in the*

Centre for Process Systems Engineering,  
Department of Chemical Engineering

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IMPERIAL COLLEGE LONDON

# *Abstract*

Faculty of Engineering

Department of Chemical Engineering

Doctor of Philosophy

## **Dissection of modularity and spatial organisation in natural and engineered cellular contexts: Systems analysis at the junction of systems and synthetic biology**

by Govind MENON

Research in systems and synthetic biology in the past decade has resulted in important advances in understanding as well as engineering cellular systems through the underpinning molecular networks. Furthermore, there is an increasing blurring of boundaries between natural and engineered cellular systems. Dissecting information processing through these molecular networks is a central aspect of this. This brings up a number of systems challenges to be tackled. In this thesis, we focus on the dissection of two features of information processing molecular networks, modularity and spatial organisation. Modularity, a basic and cross-cutting theme in engineering is a key underlying theme for both systems and synthetic biology. It is a basis for both building complex circuits in synthetic biology using bottom up approaches, and for understanding the behaviour of complex molecular networks in terms of their constituent building blocks. Spatial organisation is a hallmark of cellular systems ranging from bacteria to eukaryotes and an ingredient actively exploited in evolution. It is also a vital tool in manipulating and engineering the behaviour of molecular networks through multiple, recently developed tools focussing on compartmentalisation. In the first part of this thesis, we develop a systems engineering framework to understand the behaviour of a module within a network, accounting for different aspects of the module and the ambient network. In spatial organisation, we focus on multiple themes (i) the analysis and evaluation of compartmental models as appropriate descriptions of compartmentalised reacting systems, (ii) developing systems engineering frameworks for engineering spatially organised genetic circuits, (iii) a theoretical and systems framework for elucidating the effect of spatial organisation in molecular networks. Taken together, these studies provide systems tools and approaches for dissecting and engineering vital aspects of the complexity of cellular information processing systems.





# Declaration of Authorship

I, Govind MENON, declare that this thesis titled, “Dissection of modularity and spatial organisation in natural and engineered cellular contexts: Systems analysis at the junction of systems and synthetic biology” and the work presented in it are my own. I confirm that:

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Govind Menon

21 January 2019

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# Chapter 1

## Introduction

One of the fundamental aspects of cellular life is the ability of living cells to survive and proliferate by perceiving their environment and regulating their function in response to stimuli. In populations and multicellular organisms, a cell also needs to communicate and coordinate their response with other cells in different contexts. Intracellular processes - signal transmission, gene expression, metabolic processes, and other specialised functions - are all regulated in response to a variety of internal and external stimuli. Some of these stimuli consist of environmental stresses - for example heat shock or osmotic stress, while others are chemical signals, in the form of messenger molecules such as hormones and growth factors, that elicit diverse responses within the cell, affecting its growth and proliferation. Other molecules in the environment, such as nutrients, also dictate cell movement. For example, single-celled organisms such as bacteria regulate their movement in response to chemoattractant concentration gradients in the environment. All of these stimulus-response behaviours, central to biology, are increasingly viewed from the perspective of information processing biochemical systems, where these behaviours represent information processing functions that cells must perform.

Cells use complex networks of biomolecular interactions to carry out such information processing tasks - receiving environmental cues, transmitting and amplifying signals, integrating and storing this information, and making decisions that determine the response of the cell - regulating metabolism, growth, division, movement, or programmed cell death. These networks consist of a vast array of interacting components, including nucleic acids, proteins, lipids, ions, and small molecules, forming different classes of reaction pathways - signalling pathways involving receptors on the membrane and signal transduction cascades within, metabolic pathways involving enzyme mediated biosynthesis or breakdown of molecules, and gene regulatory pathways involving various mechanisms for regulating gene expression.

In addition to the diversity and number of molecular components, there are many sources of complexity in these networks, as we see below.

Feedback regulation is a central feature of cellular information processing (Wolkenhauer and Mesarović, 2005). In many instances, feedback loops occur at multiple levels in biochemical pathways, giving rise to biological function by generating bistability, oscillation, polarization, and robustness (Brandman and Meyer, 2008). Well known examples of feedback regulation include the negative regulation in the Lac operon, the negative feedback loop formed by the p53 tumour suppressor and its negative regulator Mdm2, (Lahav et al., 2004), and the positive feedback loop responsible for cell-fate switching in *Xenopus* oocytes (Ferrell and Machleder, 1998).

Another key feature of cellular networks that is critical for realising information processing functions, is the highly non-linear nature of interactions. Co-operative phenomena in biochemical interactions are a common source of non-linearity (Qian, 2012). The existence of feedback loops embedded in modification cascades, the multistep nature of some reactions, and the sequestration of species in the form of complexes, are efficient mechanisms for producing and amplifying nonlinear responses (Louis and Becskei, 2002). Cyclic modification of proteins through phosphorylation and dephosphorylation, catalyzed by kinases and phosphatases, are also able to produce switch-like responses (Zhang, Bhattacharya, and Andersen, 2013).

Spatial organisation of biochemical pathways is another fundamental ingredient of information processing biochemical networks, being a ubiquitous feature of cells across the evolutionary scale. Experimental and theoretical investigations into the regulation of cellular processes by biochemical networks, over the last decade, have increasingly highlighted how important the intracellular spatiotemporal distribution of network components is to orchestrating cellular function (Kholodenko, Hancock, and Kolch, 2010). The precise organization of cell organelles, proteins, nucleic acids, ions, and other small molecules in time and space, is now known to play a central role in various signalling processes (Kholodenko and Kolch, 2008).

Stochastic fluctuations are another central aspect of cell function (Balázsi, Oudenaarden, and Collins, 2011; Bruggeman and Teusink, 2018), with molecular noise effects recognised as a key factor in regulating gene expression (Raser and Shea, 2005; Munsky, Neuert, and Oudenaarden, 2012; Wolf, Silander, and Nimwegen, 2015), and even in signalling pathways (Lee et al., 2009; Flores et al., 2012). For example, stochastic switching in gene expression is known to shape cell fate determination by microbes, and is the focus of a

large body of literature, as reviewed in (Norman et al., 2015).

In addition to these factors, biochemical information processing networks are shaped by evolution, with changes in protein structure and function possibly leading to new interactions and capabilities. All of these factors present new systems challenges to understanding and engineering cellular information processing, that demand dedicated modelling and systems frameworks.

The motivation to understand biochemical information processing, comes from essentially two viewpoints: (i) that of systems biology, where the focus is on natural pathways: understanding aspects such as dynamic behaviour, robustness, crosstalk or evolution, and (ii) that of synthetic biology, which is aimed at engineering biological systems, and focuses on rewiring pathways, achieving programmable functionality, reconstituting minimal systems in vitro to understand them, or even engineering artificial cells. Importantly, recent approaches that combine natural and engineered pathways to engineer new capabilities represent a blurring of boundaries between the natural and the synthetic, and this gives additional impetus to unravelling the complexity of biochemical information processing.

A compelling systems theme that emerges in both the natural and the synthetic contexts is the modularity of biochemical pathways. For natural systems, this question arises directly, when trying to understand pathway behaviour in terms of core functional modules. For synthetic systems, it is vital to understand the extent of modularity for building more complex systems out of engineered modules. Theoretical investigations into the interconnection of gene regulatory circuits (Del Vecchio, Ninfa, and Sontag, 2008; Gyorgy and Del Vecchio, 2014) have revealed essential limitations to the modularity of components, while the literature describes multiple instances of cross-talk between signalling modules and the sharing of components by different modules. Thus, while it is apparent that there are certain limits to the modular nature of biochemical pathways, there is also a clear need to understand the extent of their modularity.

In light of this, we focus on two critical systems aspects of cellular information processing - modularity of biochemical pathways, and the spatial organisation of pathways. Our approach in each of these cases is guided by both the need to elucidate the functioning of natural pathways and the need to develop design principles for engineering synthetic pathways.

## 1.1 Modules and networks

Biochemical modules performing specific functions form core building blocks for the function and organisation of information processing pathways. Different kinds of characteristic information processing modules have been identified as core generators of behaviour in various natural pathways. The modules studied include different types of switches, oscillators, and adaptive modules. Modules exhibiting these characteristics have also been engineered in multiple instances using synthetic approaches, and with different classes of pathways, including gene regulatory and enzymatic circuits.

These modules may be thought of as loosely analogous to the modular components of an electronic circuit. However, there are essential differences arising from the fact that they consist of biochemical reactions, and are subject to evolution. The key aspects in this regard are: (i) how such modules function as part of a larger biochemical network in the cell, (ii) their ability to maintain characteristic behaviour when embedded within the ambient network, and (iii) how they interface with each other to facilitate new information processing functions. Although specific modules have been the subject of numerous studies in natural biology, they are typically studied in isolation, with no examination of either the effect of the ambient network.

There is a great deal of interest today, in designing and building synthetic regulatory circuits in cells to control various biological processes (Elowitz and Lim, 2010). The design of such circuits has to incorporate modularity if they are to reliably carry out their function within the cellular environment, independent of the pathway they are connected to (Levskaya et al., 2009). Moreover, a key focus of synthetic biology today is on using engineered modules to build more complex systems (Purnick and Weiss, 2009). The constraints and capabilities of network function, arising from modular interconnection, need to be clearly understood for the design of such synthetic regulatory circuits. Modules and their interactions also arise as a central aspect in modelling and simulation on the scale of the whole cell. In this case, the complexity and scale of the problem necessitates the use of a modular approach to constructing a model (Miller et al., 2012).

These considerations suggest multiple open questions, including (i) how do modules function as part of a network?, (ii) how do modules interface with each other?, (iii) how might synthetic circuits be designed to achieve modularity in engineered pathways? We are faced with substantial challenges in trying to answer these questions - a particular type of information

processing behaviour can be realised in different ways, depending on the biochemical nature of the components involved, the network structure, and the dynamical characteristics. There is no systematic framework to understand the behaviour of modules in a network, that accounts for all of these aspects.

We address this gap by developing a systems framework, rooted in dynamical systems, to elucidate the behaviour of modules as part of networks. We begin by isolating a set of canonical modules from the literature, based on their information processing characteristics. In doing this, we consider multiple realisations of each type, based on network structure, biochemical nature, and dynamical characteristics. We then examine module interaction with the environment - different possible characteristics of the ambient network are considered by examining pairwise interaction between different types of modules. We also consider different modes of interaction - sequential and oblique interaction of modules, and especially focus on the effects of sequestration of species in enzyme-substrate complexes - a recurring theme in cell signalling over the last decade. We use nonlinear ODE models to represent the modules, and use a combination of computational results (including simulation and bifurcation analysis) and supporting analytical results.

This framework allows us to draw a number of generalisable conclusions about the effect of the ambient network on module behaviour in multiple contexts, as well as elucidate capabilities and constraints for engineering robust synthetic modules.

## 1.2 Spatial organisation

Spatial organisation is a common feature of intracellular processes across the evolutionary scale- ranging from compartmentalised metabolism in bacterial microcompartments and the intricate spatio-temporal regulation of cell division processes in bacteria, to the ubiquitous compartmentalisation of metabolic pathways within membrane bound organelles and the dynamic localisation of components of signalling pathways in eukaryotic cells. With the advent of imaging techniques that give spatial resolution of intracellular processes, the spatial organisation of biochemical pathways is being increasingly appreciated.

For instance, spatial regulation plays an important role in polarity generation by extracellular cues in eukaryotic chemotaxis; for example, in budding yeast, the Ras protein remains evenly distributed around the plasma membrane, but in response to chemoattractants it is activated in a spatially

restricted manner at the leading edge (Berzat and Hall, 2010). Furthermore, studies have shown that the spatial organisation of MAP Kinase pathways is integral to pheromone response functions in budding yeast (Doncic et al., 2015). The activity dependent localization of G-proteins has emerged as a key aspect of multiple signalling processes, such as vesicular trafficking Vartak and Bastiaens, 2010. The spatial organization of lipid rafts in the plasma membrane has been shown to play an important role in regulating the clustering of transmembrane receptors for signal transduction Bethani et al. (2010), and mislocalisation of cyclin-dependent kinases has been implicated in oncogenesis. These are just a few of the many instances in eukaryotic cells, where spatial organisation is a crucial aspect of signalling.

Even, in bacteria, which lack membrane bound organelles, regulation of key signalling processes involves the dynamic localization and spatial oscillation of protein complexes (Shapiro, McAdams, and Losick, 2009). Well known examples include the regulatory system of proteins controlling bacterial chromosome deployment, placement of the cell division plane, and asymmetric cell division in *Caulobacter crescentus* (Thanbichler, 2009). Spatial concentration gradients of protein modified forms within the cell have emerged as a central feature in prokaryotes, exerting dynamic control over the the spatial organization of intracellular regulatory factors (Kiekebusch and Thanbichler, 2014).

Spatial organisation by compartmentalisation is also a common feature of metabolic pathways in bacteria and eukaryotes. While many autotrophic bacteria are known to have microcompartments such as the carboxysome, that encapsulate metabolic pathways, others such as filamentous cyanobacteria, exhibit compartmentalisation at the intercellular level. The spatial organisation of microbial communities in the context of metabolic interactions has also been highlighted. In eukaryotes, for example, plant metabolic pathways are spread over multiple compartments, meaning that the transport and exchange of multiple metabolites between compartments is a central aspect of their regulation (Sweetlove and Fernie, 2013).

The problem of understanding the role of spatial localization and compartmentalization, has recently been described as one of the most important open questions in cell biology (Howard, 2014). However, while part of the motivation to elucidate the role of spatial organisation comes from the need to understand natural pathways and possibly manipulate this organisation -

for instance to maximise biosynthetic fluxes in metabolic engineering, a significant impetus for understanding these aspects is generated by the growing interest in engineering spatially organised synthetic pathways *in vitro*. This is the focus of several recent studies, developing functional bottom up constructs for compartmentalising gene regulatory and enzymatic pathways in a cell free setting. They use a variety of approaches, including liposomes (Elani, Law, and Ces, 2015; Adamala et al., 2016), water-in-oil droplets (Torre, Keating, and Mansy, 2014), and etched compartments Karzbrun et al., 2014, to organise these pathways in space, with certain components allowed to diffuse between compartments. In addition to facilitating programmable pattern formation and modular design, such constructs also form an essential ingredient in any approach to build artificial cells.

Given the cross-cutting relevance of spatial organisation in natural pathways and the interest in building compartmentalised synthetic pathways, there are multiple open questions that need to be addressed, including: (i) What is the effect of compartmentalising certain molecular components in a pathway, and how does this depend on the biochemical nature of the components? (ii) What are the complexities associated with using compartmentalisation to manipulate more complex pathways? (iii) What framework should be used to model compartmentalised pathways so as to give reliable predictions for analysis and design? (iv) What does spatial organisation bring to the design space for engineering cell-free synthetic systems? (v) How does spatial organisation shape the function of complex information processing networks? We take a multi-pronged approach to address these questions.

Accurate understanding of the role of compartments and designing compartmentalized systems needs reliable modelling/systems frameworks. Compartmental ODE models present a tractable way of capturing the essential features - localised reaction and transport. However, it is not clear how reliably they can be used in these contexts for analysis and design. We examine a series of building blocks of signalling and metabolic pathways with compartmental organization, considering different classes of pathways, different kinetic regimes, and multiple compartmentalisation scenarios. These include covalent modification cycles, open pathways, two-tier cascades and multisite modification systems. We systematically analyse when compartmental ODE models can be used in these contexts, by comparing this model with detailed reaction-transport models, and establishing a correspondence between the two. We build on this (and on previous work (Alam-Nazki and Krishnan, 2013; Alam-Nazki and Krishnan, 2015)) to examine additional complexities

associated with these pathways, and also examine sample problems in the engineering of these pathways. Through this approach, we are able to identify regimes where compartmental ODE models can and cannot be used reliably, suggest augmentations and better procedures make them reliable in these contexts, and reveal various trade-offs that emerge in engineering the compartmentalisation of more complex signalling and metabolic pathways.

Many recent studies have focussed on building spatially distributed realisations of cell free genetic circuits, motivated by the possibility of engineering programmable pattern formation, modular circuit construction, and for creating artificial cells. While the various studies on engineering cell-free synthetic pathways have demonstrated important experimental capabilities, the design principles underpinning compartmentalization of nucleic acid based circuits remains to be addressed. There are many kinds of such circuits, and different ways of compartmentalisation and spatial organisation. Functional circuits have been built from multiple classes of nucleic acid based pathways, both within cells and in cell free contexts, and there are systematic approaches to designing some of these. However, there is no framework that addresses the interplay of circuit designs, spatial organisation, and the operation of compartments. In order to address this gap, we have taken a multi-pronged approach to elucidate the basic aspects of spatial organisation of these circuits. Our approach combines basic template based regulatory circuits, canonical motifs, and examines organising them in different spatial configurations, including well mixed compartments connected by channels, and a uniform channel, both of which reflect configurations that have been realised experimentally. In each case, the communicating species (proteins or nucleic acids) diffuse across the spatial domain while the transcription/translation processes are compartmentalised. We examine different levels of organisation, including different ways of distributing the nodes in a motif, and different ways of organising compartments in a spatial domain. This allows us to reveal a number of features associated with compartmentalising synthetic and template-based circuits, including the consequences of distributing circuits, including basic trade-offs and how they may be circumvented, hidden constraints in realizing a distributed circuit, and appealing new features of compartmentalised circuits. By focussing on the most basic considerations of compartmentalising genetic circuits, we obtain insights that are relevant in a broad range of settings.

Given that the combination of the network structure formed by the interactions and the spatial characteristics/organisation of the components lies



at the heart of how biochemical networks process information, we need a systematic approach to dissect the myriad possibilities that this entails, in such a way that we can elucidate (i) How spatial organization shapes the behaviour of a motif, possibly endowing it with new capabilities? (ii) How spatio-temporal information processing functions and new control structures emerge from patterns of activation/inhibition between components with different spatial characteristics? We develop a systems framework to understand the role of spatial organization in biochemical information processing networks, that combines two complementary lines of approach: (i) A motif centric approach that proceeds by considering a set of basic network motifs, and examining the effect of introducing spatial organisation at the level of nodes or interactions (ii) An approach centred on patterns of interactions between three different spatial classes (see below). In the first approach by identifying a set of two and three node motifs, involving patterns of activation and inhibition between nodes consisting of interconverting modified forms. Each motif is associated with certain kinds of dynamic behaviour in the ODE context. We then systematically examine the effect of introducing spatial complexity in a motif. This includes combining localised and diffusing elements at the level of both nodes and interactions. In the second approach, we focus three distinct spatial classes - local (essentially non-diffusing), global (diffusible) and localised - and examine different patterns of interaction between these classes. Our two-pronged approach yields multiple classes of insights into the role of spatial organisation in information processing networks, and the interplay of different spatial classes in giving rise to information processing functions.

## 1.3 Organisation of the thesis

In the next chapter, we address the modularity of information processing biochemical pathways. First, we describe the systems framework that we use to study the functioning of biochemical modules as part of networks - the set of modules chosen for the study, the models used, and how we model different types of interactions. Next, we present a selection of results, covering different combinations of modules and different types of interaction. The results are also summarized in tabular form. Additional cases of modular interaction, along with supporting computational and analytical results, are presented in the appendix. In the last section, we discuss the salient points that

emerge from our study, draw connections to concrete instances in natural biology, and highlight insights into the modular design of synthetic circuits.

In chapter 3, we address the question of reliable modelling frameworks for compartmentalised biochemical pathways. We first describe our approach to evaluating compartmental ODE models, and the different classes of pathways considered. We begin with the simplest case of a single covalent modification cycle, and use this to identify the basic sources of limitations in compartmental ODE models. We then extend these insights to other classes of pathways and more complex extensions, highlighting further aspects that may limit the reliable use of compartmental models. In light of this, we also examine an exemplar problem in engineering the spatial organisation of metabolic pathways. We conclude with a discussion of when compartmental models may be used reliably for design and analysis in these contexts, and suggest augmentations/better procedures for their use outside these regimes. We present supporting analytical results and other extensions in the appendix.

In chapter 4, we address the question of what spatial organisation brings to the design of synthetic cell-free genetic circuits. We begin with a discussion of the types of regulatory motifs considered, and the different types of spatial configurations examined in the study. The next section focusses on the distribution of motifs in systems of well-mixed compartments connected by channels. This is followed by a study of circuits distributed in a 1-D channel. In each case, we start with a discussion of basic effects and trade-offs, followed by analysis of more complex cases involving bistable or oscillatory behaviour, and other design factors such as resources.

In chapter 5, we address the role of spatial organisation in information processing networks. We begin with a discussion of the different classes of motifs considered, and the models used. We then discuss the two lines of approach that we use, laying out the structure of the investigation in each case. In the next section we discuss results emerging from the first approach - focusing on introducing spatial complexity into a given motif. The results of the approach centred on spatial classes is presented in the next section. We end with a brief summary of the different classes of insights that emerge from this study.

For chapters 3 and 4, supporting analytical results are presented in an appendix immediately following each chapter. The appendices at the end of the thesis contain additional discussions, supporting computational results, and other supporting information, including model equations and parameter

values used to generate the results shown in each chapter.

### **List of papers from this thesis:**

1. G. Menon and J. Krishnan, Bridging the gap between modules in isolation and as part of networks: A systems framework for elucidating interaction and regulation of signalling modules, *Journal of Chemical Physics*, 145, 2016
2. G. Menon, C. Okeke, and J. Krishnan, Modelling compartmentalization towards elucidation and engineering of spatial organization in biochemical pathways, *Scientific Reports*, 7, 2017
3. G. Menon and J. Krishnan, Design principles for compartmentalisation and spatial organisation of synthetic genetic circuits, Submitted, 2018
4. G. Menon and J. Krishnan, Paper in preparation, 2018



## Chapter 2

# Bridging the gap between modules in isolation and as part of biochemical networks

### 2.1 Introduction

The functioning of cells and their response to the external environment is governed by gene regulatory and protein networks, whose complexity has many sources. To start with, the number of entities in such networks is very large. However, what makes the elucidation of network function especially challenging, is that the networks involve highly non-linear interactions and widespread feedback. Stochastic and spatial effects can play unexpected and important roles. The network interactions have been partially shaped by evolution, making them difficult to elucidate. In addition, there are many examples of crosstalk and interactions between pathways. All these (and other) factors contribute many challenges to understanding the organization, functioning, and information processing in such networks. Systems biology has come up against these challenges in a wide variety of specific contexts, where the approach has usually been to focus on parts of a network/system that are experimentally probed, and either simplify or accept as reasonable, nominal descriptions of other interactions. Thus in many respects, at every level systems biology functions in a partly correlative fashion.

In order to understand the behaviour of cells and how they respond to the environment, it is essential to understand information processing in such networks, and further, the dynamical element to information processing is very important (Marks, Klingmüller, and Müller-Decker, 2008; Alexander et al., 2009). A useful starting point for studying information processing in these networks, is the understanding of “building blocks” in signal transduction and information processing (Hartwell et al., 1999). Of course, what

may be regarded as building blocks, to an extent, depends on the level of the investigation. In signal transduction, building blocks include (i) Covalent modification cycles (ii) Various elementary network “modules” and “motifs”, which repeatedly occur in multiple contexts. (Alon, 2003; Bhattacharyya et al., 2006).

Again, there are different ways in which one may identify such modules (Kaltenbach and Stelling, 2012). One is based on a causal pattern of connections that may be ubiquitous in networks (Alon, 2007). Another way of isolating modules is by the nature of information processing that they achieve (Tyson, Chen, and Novak, 2003). Neither category is a subset of the other: firstly, similar network patterns may give rise to different kinds of information processing, because of the nature of the non-linear interactions, and secondly, particular information processing can be realized by different network structures (Tyson and Novak, 2010). Nonetheless it is possible to use some basic modules as a vantage point for understanding different aspects of information processing through networks.

It is clear that biology is not strictly modular in the same sense as engineering. However, studying information processing in networks in relation to modules is useful for multiple reasons. First, elucidation of information processing and model building/conceptualization, in concrete contexts, relies on many simplifications: these include coarse graining and ignoring a number of auxiliary factors. So, in many cases, an implicit modularization is assumed. While this may not be strictly true, such assumptions allow for simplification and effective understanding of processes of interest, and have been used effectively in conjunction with experiments. Secondly, since modules are embedded in networks, to understand the complex, non-linear information processing in networks, it is useful to understand modules and how they function in the network. Finally, modules (especially in gene regulatory and signalling processes) have been successfully realized in synthetic biology, and the focus in this field is on moving from modules to systems (Chin, 2006; Purnick and Weiss, 2009).

We will focus on signalling modules as determined by the nature of information processing, rather than structural features. A number of examples of characteristic information processing modules have been studied, including covalent modification cycles (and extensions thereof), monostable switches, bistable switches, oscillators and adaptive modules (Tyson, Chen, and Novak, 2003). The information processing in each of these modules (apart from covalent modification cycles) is highly non-linear. Each of these modules has

been observed in multiple cellular contexts (Tyson, Chen, and Novak, 2001). Further, analogous gene regulatory modules have also been built for each distinct type of information processing (Andrianantoandro et al., 2006).

In synthetic biology, since the modules are engineered, essentially minimal networks (subject to the constraints of the biological building blocks) have been used in the design, though of course, additional factors and complexities have also been incorporated (Purcell et al., 2010). In systems biology, such information processing modules are realized in different, perhaps complex ways, and they interact with other parts of the signalling networks.

There are substantial challenges in bridging the gap between understanding the behaviour of modules in isolation, and embedded in networks that contain them. There are potentially many interactions and connections between the module and the surrounding network, of a more or less arbitrary nature. Elements of the module may participate in other pathways (Seaton and Krishnan, 2011b). These interactions may have stochastic and even spatial dimensions to them. Elements of the module may be substantially sequestered in other pathways and modules, an effect referred to as “retroactivity” (Del Vecchio, Ninfa, and Sontag, 2008). When this is combined with the highly non-linear nature of the modules, we realize that there are highly non-trivial differences between modules functioning in isolation and as parts of networks (Krishnan, Mois, and Suwanmajo, 2014).

In this chapter, building on Seaton and Krishnan, 2011a; Seaton and Krishnan, 2011b, we develop a systems framework that begins to bridge the gap between understanding the behaviour of modules in isolation and within networks. This leads us to examine different aspects of module interaction with the environment. We begin by isolating a few representative modules: covalent modification cycles, monostable switches, bistable switches, adaptive modules, and oscillators, and considering different realizations of these modules. We then examine the effects of perturbation of these modules by the environment. We examine the interaction of modules, connected in multiple ways, both sequential and oblique. Our study generates a number of insights into the behaviour of modules in networks and how they differ from the module in isolation.

Such an approach has multiple potential uses. Firstly, it allows us to better understand what a module is, and how it functions as part of a pathway. Secondly, it provides a basis for understanding modular interaction, crosstalk and other complex effects that arise from the presence of the module in a network. Thirdly, it allows for a broader appreciation of information

processing in such modules, their capabilities and constraints, as well as how new modes of information processing may arise. Fourthly, such an approach, which is synthetic in essence, is of relevance in synthetic biology as well (discussed later), especially with its current focus on moving from modules to systems. Such an approach also provides a template for beginning to design more complex circuits therein. Finally, there has recently been an interest in whole cell modelling (Karr et al., 2012; Sanghvi et al., 2013; Ma'ayan, Blitzer, and Iyengar, 2005), and no reasonable effort of that kind can be soundly carried out without an understanding of these factors. Overall, this approach contributes steps towards a genuine systems understanding of the complexity of signal transduction. Since the approach is rooted strongly in dynamical systems, it is able to discern qualitative and quantitative effects and trends that may be relevant in multiple contexts.

## 2.2 Models and Methodology

In this section, we outline the models that we use for our study, and describe how we analyse the interaction of modules. At the outset, we emphasize that our goal is to understand the effect of the network environment of a signalling module, on its behaviour. For this purpose, we will focus on signalling modules based on their information processing, and therefore, when we discuss modules, we are referring to representative circuits that exhibit certain characteristic information processing capabilities. The modules we will focus on are (i) Covalent modification cycles (ii) Monostable switches (iii) Bistable switches (iv) Adaptive modules (v) Oscillatory modules. The covalent modification cycle can be regarded as a simple building block, representative of the most basic information processing. Its role will be seen subsequently.

**Choice of models:** Having chosen a set of information processing modules, we focus on the models chosen to realize these modules. We choose generic biochemically-based circuits that have been used in the literature to represent these modules in one context or another. A few things must be noted in this regard. Firstly, we take an information processing dynamical systems approach in our study. This means that we focus on modules based on their information processing capabilities (rather than any purely structural aspects). In addition, our entire systems approach is rooted in dynamical systems, so that we focus on the dynamical systems underpinning of the



modules. Thus, we employ representative circuit descriptions that exhibit the dynamical systems characteristics typical of that module.

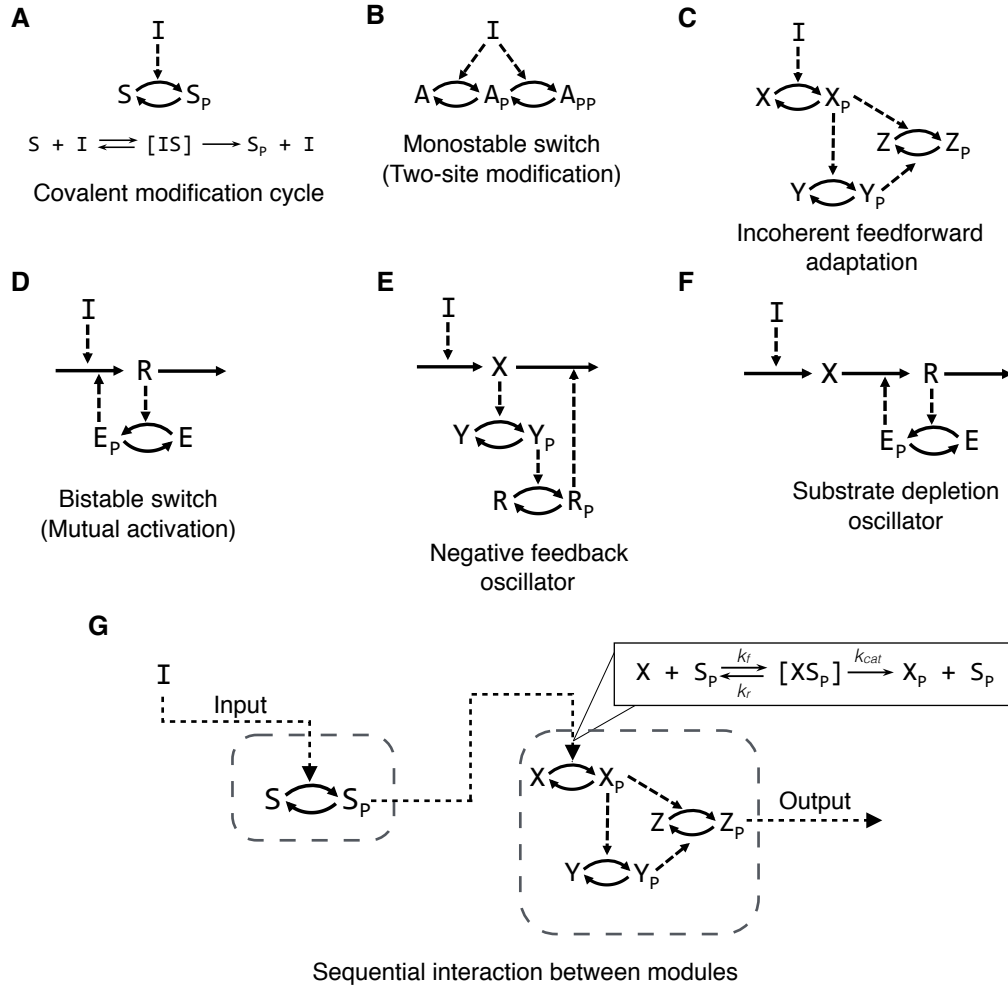


FIGURE 2.1: (A)-(F) Schematics of the modules used. (G) A schematic of sequential interaction between an upstream covalent modification cycle and a downstream adaptive incoherent feedforward module, illustrating how coupling with sharing is described.

Secondly, we choose essentially simplified models. This allows us to focus on essential aspects of the interplay between a module and its environment, without the consideration, at this stage, of any extraneous and essentially arbitrary complexity (also see Conclusions and Discussion). Thirdly, there may be multiple models that satisfy these criteria, and whose separate examination may be relevant, in the context of our study. In such cases, we employ multiple models in our study, with a careful consideration of whether any essential differences arise. As a simple example, in the case of oscillators,

we consider two different models, which have essential differences from a dynamical systems perspective, as will be seen below. Finally it is worth pointing out that when we consider modules, we employ signalling circuit models representative of the behaviour we desire. We note that similar behaviour in some cases could also be obtained from (complex) enzymatic reactions (such as multisite phosphorylation), but such models are specific to enzymatic modification and consideration of complexes (for example, see Fellu and Wiuf, 2011), and we do not consider them here.

**Module structure:** The models, for the modules under consideration, are all described by ordinary differential equations. Stochastic as well as spatial effects could indeed play important roles, but we do not examine these effects at this stage. We also note the following: (i) The basal parameter set is chosen so that the model is in the appropriate parameter regime representing the behaviour of interest. Most of the parameters are kept fixed in our study. (ii) Since our approach is rooted in dynamical systems, the focus is on key qualitative trends and characteristics, and less on absolute numbers. These are arguably the characteristics that are easiest to discern unambiguously in biological systems. (iii) While in principle any variable or parameter may be perturbed by the environment, there is usually a designated parameter (or variable) that serves as the input to the module. This is especially true of “input-driven” modules such as adaptive modules and monostable switches. In a similar way, while multiple variables may be regarded as the output of the module, there is typically a designated variable that serves as the output (again this is especially true for modules such as adaptive modules and monostable switches). (iv) Our modules involve sequences of biochemical reactions. In general, there are multiple ways to model such reactions, such as mass action kinetics, Michaelis-Menten kinetics etc. We use modules that involve primarily mass-action kinetics, and employ Michaelis-Menten kinetics only in places where the corresponding enzyme is an isolated one (i.e. not regulated by any other step). In such cases the Michaelis-Menten description can be easily and directly justified by unpacking the steps. We note however, that many of our results also apply to models that use Michaelis-Menten kinetics in other steps.

**The module and its network environment - Interaction of modules:** Having laid out the module structure, we define how we examine the interaction of modules. We examine the most basic interaction of modules from an information processing and dynamical systems approach. We start with sequential interactions. In these interactions, the output (variable) of one

module regulates the input of another module. This regulation can be either positive or negative, and we usually consider positive regulation, for specificity. In the basic case, the output of the first module is not affected by the interaction. This can happen, for instance, if intermediate enzymatic reactions are of mass action type. We then systematically expand our study of such sequential interactions, to consider the effects of the sequestration of species of the first module in the second. This builds on the previous analysis, and indicates what new features emerge by consideration of such effects. Finally, we consider the oblique interaction of modules, where the output of the first module affects some parameter in the second module that may not be its input. Naturally, it is possible to examine other kinds of interactions between modules, including direct interactions between variables of modules. We defer a detailed study of this scenario to future work.

### **2.2.1 Models**

We first present equations for the module in isolation. All the modules are biochemical modules, and can be “unpacked” in an unambiguous way. The modules are also subject to conservation conditions, which can be incorporated to reduce the number of equations. We subsequently discuss how the module is connected to other modules. Schematics of the modules and their input-output responses (in isolation) are shown in Figs. 1,2.

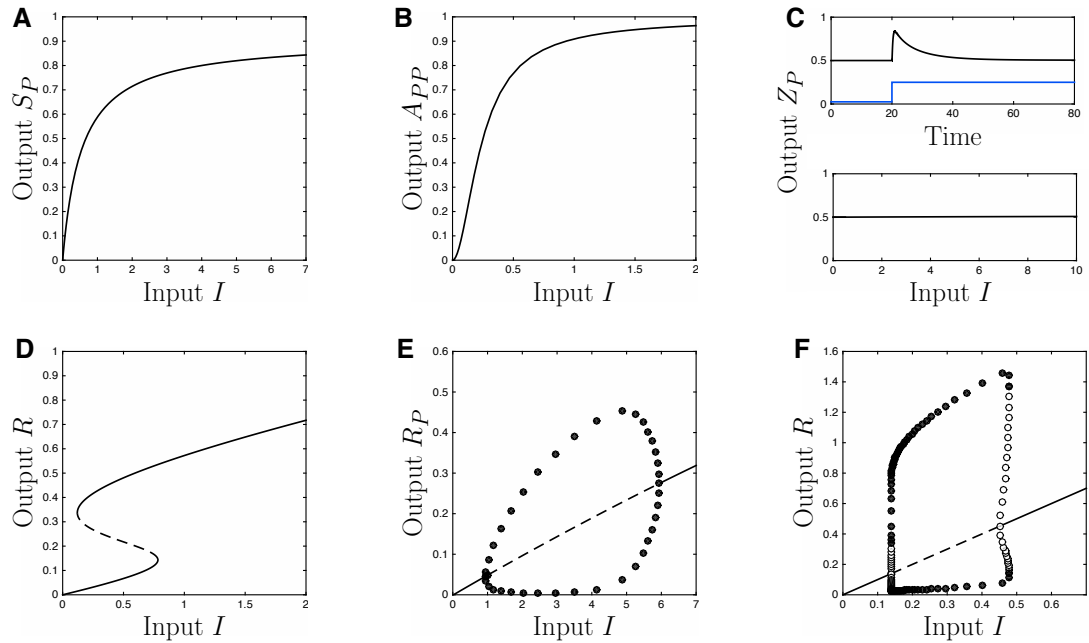


FIGURE 2.2: Steady state input-output curves for the modules in isolation. (A) Covalent modification cycle (B) Monostable switch (Two-site modification) (C) Incoherent feedforward adaptive module (Showing both bifurcation diagram and response to step input) (D) Bistable switch - Mutual activation (E) Negative feedback oscillator (F) Substrate depletion oscillator. In each bifurcation diagram, a solid line indicates stable steady states; a dashed line indicates unstable steady states; for limit cycles, the maximum and minimum values between which a variable oscillates, are marked by circles. Filled circles: stable limit cycles, Unfilled circles: unstable limit cycles.

**Covalent modification cycle:** We use a widely used description of the covalent modification cycle (CMC), describing enzyme substrate binding and modification. For the module in isolation, the total amount of enzymes (both kinase and phosphatase) is conserved, and so is the total substrate. Further, for simplicity we will assume dephosphorylation of the modified substrate to act via mass action kinetics. For our purposes, this is not an important restriction, since we use this module as a basic module, and are not concerned about the possibility of switch-like behaviour in this context (those modules

are studied separately). The model is:

$$\begin{aligned}\frac{d[S]}{dt} &= k_4[S_P] + k_2[IS] - k_1[S][I] \\ \frac{d[IS]}{dt} &= k_1[S][I] - k_2[IS] - k_3[IS] \\ \frac{d[S_P]}{dt} &= k_3[IS] - k_4[S_P] \\ [S_T] &= [S] + [S_P] + [IS], [I_T] = [I] + [IS]\end{aligned}$$

where  $[S_P]$  is the concentration of the phosphorylated substrate, and  $[IS]$  is the concentration of the kinase complex.  $[S_T]$  is the total substrate concentration. The total amount of kinase  $[I_T]$  is the input, and the concentration of the modified substrate  $S_P$  is the output.

It is worth pointing out that a special parameter regime for this module is one where the phosphorylation occurs via mass-action kinetics. Here, all the kinase is free kinase and thus the free kinase can be manipulated as an independent parameter. This has consequences for the connection of this module to other modules, as discussed later.

**Monostable switches:** We will consider modules with moderate as well as more sharp switch-like behaviour. There are multiple ways in which such switch-like behaviour can be generated (Zhang, Bhattacharya, and Andersen, 2013), which from the perspective of our analysis does not make much difference. We examined two prototypical modules. The first one ( Fig. 1) is described by:

$$\begin{aligned}\frac{d[A_P]}{dt} &= k_1[I][A] + k_4[A_{PP}] - k_3[I][A_P] - k_2[A_P] \\ \frac{d[A_{PP}]}{dt} &= k_3[I][A_P] - k_4[A_{PP}] \\ [A_T] &= [A] + [A_P] + [A_{PP}]\end{aligned}$$

This model depicts a sequential distributive two site mechanism, with all enzymatic reactions acting according to mass-action kinetics. The input is the concentration of the kinase  $I$  and the output is the concentration of the fully modified form  $A_{PP}$ .  $A_P$  is the singly modified substrate concentration and  $A_T$  is the total substrate concentration. This module describes only a moderate level of switch-like behaviour (roughly a Hill coefficient 2) and this can be increased by increasing the number of modification sites, or by adding other components.

The other module we consider is a 3-tier cascade used to model a MAP Kinase cascade in Huang and Ferrell, 1996, also studied in Ventura, Sepulchre, and Merajver, 2008. As has been extensively studied, this cascade is capable of exhibiting switch like behaviour. In our studies we have examined both these as well as other kinds of switch-like modules, and will present results for the module discussed above. We briefly discuss some aspects of the MAP Kinase model in the Appendix.

**Bistable switches:** Bistable circuits are typically realized by strong positive feedback, which may include co-operative interactions or saturation. We consider two models of bistability. In the first model, the positive feedback is realized by a species activating (for specificity) a second species that in turn serves to activate the first species (mutual activation). The model mutual activation model (similar to Tyson, Chen, and Novak, 2003) is given by

$$\begin{aligned}\frac{d[R]}{dt} &= k_0[E_P] + k_1[I] - k_2[R] \\ \frac{d[E_P]}{dt} &= k_3[R][E] - \frac{k_4[E_P]}{K_4 + [E_P]} \\ [E_T] &= [E] + [E_P]\end{aligned}$$

Here  $I$  is the input,  $R$  is the output and  $E_P$  and  $R$  form a positive feedback loop. Note that the Michaelis-Menten step in the equation for  $E_P$  can be easily unpacked.

We also examine a slightly different module discussed in Ferrell Jr and Xiong, 2001, involving positive feedback with saturated degradation. The module consists of a fixed total amount of species. It is briefly discussed in Section III of the supplemental material.

**Oscillatory circuits:** We consider two different oscillatory modules presented in Tyson, Chen, and Novak, 2003, with some minor modifications. The modifications are made to ensure that most steps occur via mass action kinetics, except some isolated reactions that are described by Michaelis-Menten kinetics, which can be unpacked easily. This in turn allows the module to be easily interfaced with other modules. While both modules produce robust sustained oscillations, their dynamical systems characteristics are different. The first module is a simple negative feedback oscillator (NFO), which

is described by the following equations:

$$\begin{aligned}\frac{d[X]}{dt} &= k_0 + k_1[I] - k_2[X] - k'_2[R_P][X] \\ \frac{d[Y_P]}{dt} &= k_3[X][Y] - \frac{k_4[Y_P]}{K_4 + [Y_P]} \\ \frac{d[R_P]}{dt} &= k_5[Y_P][R] - \frac{k_6[R_P]}{K_6 + [R_P]} \\ [Y_T] &= [Y] + [Y_P], [R_T] = [R] + [R_P]\end{aligned}$$

This module consists of a species  $X$  (whose production is promoted by the input signal  $I$ ), which activates the species  $Y_P$  in a covalent modification cycle.  $Y_P$  in turn, activates the output species  $R_P$  in a second covalent modification cycle. Both cycles are governed by mass action kinetics in the forward reaction while the backward reaction is governed by Michaelis-Menten kinetics. The activated species of the second cycle promotes the degradation of  $X$ , thus completing a negative feedback loop. For basal parameter values, the system has two supercritical Hopf bifurcations at positive values of the input.

The second module is a substrate depletion oscillator (SDO) described by the model

$$\begin{aligned}\frac{d[X]}{dt} &= k_1[I] - k'_0[X] - k_0[E_P][X] \\ \frac{d[R]}{dt} &= k'_0[X] + k_0[E_P][X] - k_2[R] \\ \frac{d[E_P]}{dt} &= k_3[R][E] - \frac{k_4[E_P]}{K_4 + [E_P]} \\ [E_T] &= [E] + [E_P]\end{aligned}$$

This module involves mutual activation between the output species  $R$  and the species  $E_P$ . While  $R$  activates  $E_P$  in a covalent modification cycle,  $E_P$  promotes the production of  $R$  from the substrate  $X$ . This input  $I$  promotes production of  $X$ . For the chosen parameter values, the system has two subcritical Hopf bifurcation points at positive values of the input.

**Adaptive circuits:** Adaptation is studied in many contexts in signal transduction. We consider two different models of adaptation, a negative feedback model and a feedforward model. Each of these has been seen in specific biological contexts. A detailed study of three node motifs in Ma et al., 2009 shows that the negative feedback (NF) and the so-called incoherent feedforward (IFF) are the two adaptive circuits that arise in these motifs.

For our purposes, we consider an incoherent feedforward module, which

relies on co-regulation of antagonistic pathways. There are different variants of this module, which are essentially similar for our purposes. The model of our incoherent feedforward module is:

$$\begin{aligned}\frac{d[X_P]}{dt} &= k_1[I][X] - k_2[X_P] \\ \frac{d[Y_P]}{dt} &= k_3[X_P][Y] - k_4[Y_P] \\ \frac{d[Z_P]}{dt} &= k_5[X_P][Z] - k_6[Y_P][Z_P] \\ [X_T] &= [X] + [X_P], [Y_T] = [Y] + [Y_P], [Z_T] = [Z] + [Z_P]\end{aligned}$$

This module consists of three nodes, X, Y, and Z, each with active ( $X_P$ ) and inactive forms (X). The input activates  $X_P$ , which in turn activates  $Y_P$  and  $Z_P$ . The active form  $Y_P$  de-activates  $Z_P$ . We use a model that employs mass action kinetics everywhere. At steady state, the output  $Z_P$  adapts to the level of the input  $I$ . The adaptation is close to exact over a wide range of input signal. There are other variants of such a model that involve Michaelis-Menten kinetics, or somewhat different structures of the module above (for instance the signal regulating X and Y directly), but these are minor differences that do not affect the essential information processing capability of the module. We also examine a negative feedback model of adaptation, where the output negatively regulates itself through a buffer species. We discuss this model in the Section III of the supplemental material.

**Coupling of Modules:** We examine coupling of modules that involves the output of one module acting as an enzyme in another. If this enzymatic reaction is not in the mass action regime, sequestration effects can result. In this case, this reaction is described in a standard way, including binding to/unbinding from substrate, and irreversible catalytic conversion (see Section II of the supplemental material).

**Parameters:** For the modules in isolation, nominal parameter values (representing comparable time scales of all modules) are chosen to reflect the representative module behaviour; furthermore, the effects of parameters on module behaviour are well understood at the outset. In studying the coupling of modules, we focus on key qualitative behaviour and transitions. Therefore, we choose parameters associated with the coupling to allow for non-trivial effects from the interaction. The effects of varying the coupling parameter follow directly from our study: for instance if this parameter is not within certain ranges, certain qualitative transitions may not be triggered by the upstream input. For coupling with sequestration, we choose nominal



values for rate constants of enzyme-substrate unbinding and catalytic conversion. We directly examine the effect of the catalytic constant to assess the effect of sequestration. Parameter values, used in the simulations shown, are given in Section V of the supplemental material, where we note any assumptions on parameter ranges (also discussed below where necessary), otherwise the behaviour is generally representative.

We perform both simulation (in MATLAB) and bifurcation analysis (using Matcont (Dhooge, Govaerts, and Kuznetsov, 2003)). A range of alternative parameter values and alternative models have been studied, underpinning this work. The trends and transitions (the focus of our work) which we demonstrate, are equally relevant in these other cases, for comparable kinds of coupling.

## 2.3 Results

We first discuss the way we present the results. We have five different classes of modules, some of which are represented by different models. All the models have been characterized in isolation, and have well defined inputs and outputs. We first consider sequential interconnection of modules, without sharing of species. We then revisit this to examine the effects of shared components in the interaction. Finally, noting that modules may interact in a non-sequential manner, we examine basic aspects of what may be regarded as oblique interactions of modules. For each type of interaction, we group the results under different subheadings that reflect the common insights relevant across different module combinations. These insights are also highlighted in the chart presented in Fig. 3. We conclude our analysis of each type of interaction by highlighting some of the insights relevant to systems and synthetic biology. Readers not interested in the detailed discussion of the various coupling scenarios may refer to tables I and II, for a summary of the possible behaviour arising from coupling different pairs of modules, with or without sharing of components. Additional analysis and discussion are presented in the supplemental material .

Each of the chosen modules can function as an upstream or a downstream module. In the case of the covalent modification cycle, we also consider a special case: one where the forward reaction acts via mass action kinetics. In this case, the covalent modification cycle can be connected downstream

without sharing of components. In the case of other modules, different possible models for these modules were examined as part of our study, and we comment on any important differences subsequently.

**Nature of the Results:** The focus of our work is to understand qualitative capabilities, constraints, and new effects associated with modular coupling and the effects of sequestration therein. This is reflected in the nature of the results we present. We present the results as a sequence of modular coupling scenarios, highlighting new effects. The parameters we vary are: the input, coupling parameter (as appropriate), and the catalytic rate constant of the coupling reaction (to study sequestration). We emphasise the following points:

- (1) For sequential coupling without sharing, the upstream module output plays the role of a parameter in the downstream module. Therefore, all our results can be easily understood in terms of the dynamical systems (bifurcation) characteristics of the modules in isolation, and they generalize to other choices of modules with the same bifurcation characteristics.

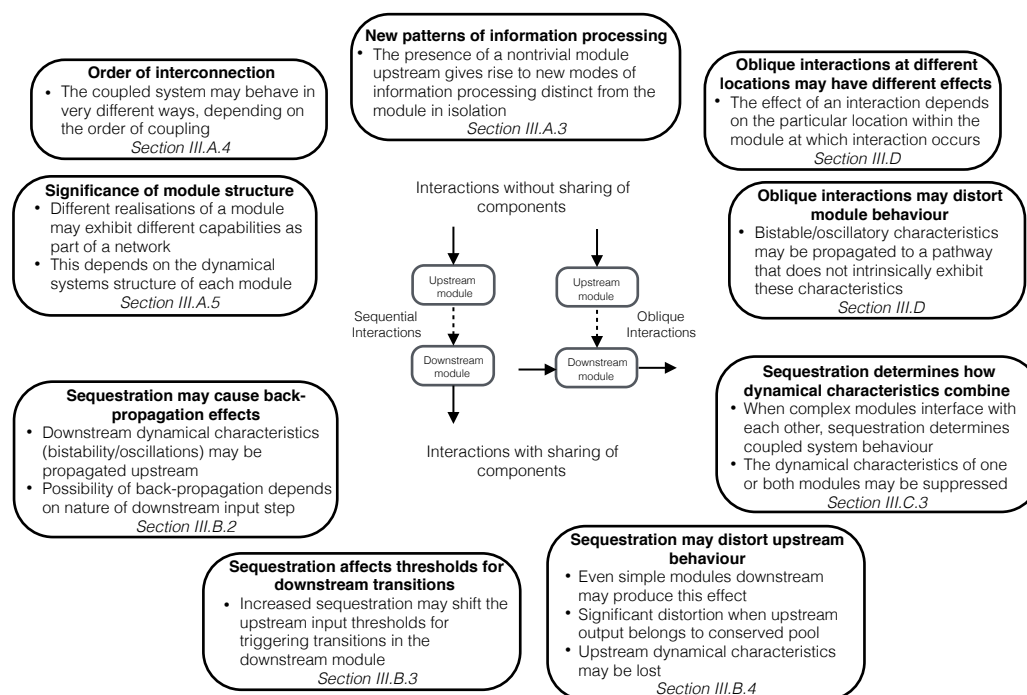


FIGURE 2.3: Summary of essential insights on modular interactions.

- (2) Our results for coupling of modules with shared components are understood by studying the effects of sharing, progressing from simpler cases

(involving CMCs) to more complex cases; additionally we have analogous scenarios for coupling without sharing as a reference. Our analysis brings out the importance of both dynamical systems characteristics and relevant biochemical aspects of the coupled modules.

- (3) For oblique coupling, we do not consider shared components, so the results here may also be understood in terms of the dynamical systems characteristics of the modules in isolation. The approach and the insights, suitably interpreted, generalize to other choices of modules.

### 2.3.1 Sequential interaction without shared components

We now investigate sequential interconnection between modules, where the output variable of one (upstream) module acts essentially as the input parameter for another (downstream) module. This may be realized by having the upstream output species act as an enzyme for the downstream module, with mass action kinetics. As there is no sequestration, the behaviour of the upstream module is left unchanged by such an interconnection.

In order to understand the input-output response of a given system of coupled information processing modules, we start by fixing a basal level of input signal to the system, and examine the effects of step and rectangular pulse changes with respect to this basal level. We note that the response to such step and pulse inputs would, in general, also depend on the chosen basal level of the input, and we account for this in our analysis.

We note that disparities in the intrinsic time scales of coupled modules may play a role, both in the case of time varying inputs, and when the module dynamics contains vital information (adaptive and oscillator modules). However, from the perspective of this chapter, we examine this aspect only where it leads to new qualitative effects.

We first consider coupling involving the CMC and the monostable switch.

#### CMC or Monostable switch upstream

Consider the coupling of a CMC to a downstream module, with the output species concentration  $[S_P]$  playing the role of the input parameter in the downstream module. Since the species  $S_P$  is not sequestered in the downstream module, the input-output response of the CMC is not affected by the coupling.

The behaviour of the coupled system is largely determined by the characteristics of the downstream module in this case. However, we note that the

non-linear response of the CMC still has a part to play. Both dynamics of the CMC, and the saturation of  $[S_P]$  with increasing upstream input, can introduce significant qualitative differences in the response of the coupled system, relative to the scenario where the downstream module is directly regulated by the signal. The upper limit on  $[S_P]$  restricts the range of signal received by the downstream module, and this may prevent certain transitions in the downstream module being triggered by the upstream input.

The effect of coupling a monostable switch upstream of a given module may be understood in an analogous way to the CMC. Again, the fact that the output of the switch is constrained by a fixed total amount, may prevent certain downstream transitions being triggered by the upstream input (for a fixed value of the coupling parameter). This insight extends to any case where the output species of an upstream module derives from a fixed pool.

### **CMC or Monostable switch downstream**

In this case, the input-output behaviour of the coupled system may be understood from the upstream module in isolation. Qualitatively, the behaviour of the coupled system is no different from that of the upstream module (See Section I of the supplemental material for a more detailed discussion). Coupled systems with the monostable switch downstream may be understood in an analogous way.

The remaining cases involve interactions between oscillatory, bistable or adaptive modules. In the following subsections, we describe particular instances of these coupled systems, which serve to highlight important aspects of sequential interconnection. In all these cases, a detailed analysis, covering all possible behaviours for a given interaction, is provided in Section I of the supplemental material.

### **New patterns of information processing can result from coupling**

Modular coupling gives rise to information processing that is distinct from the modules in isolation.

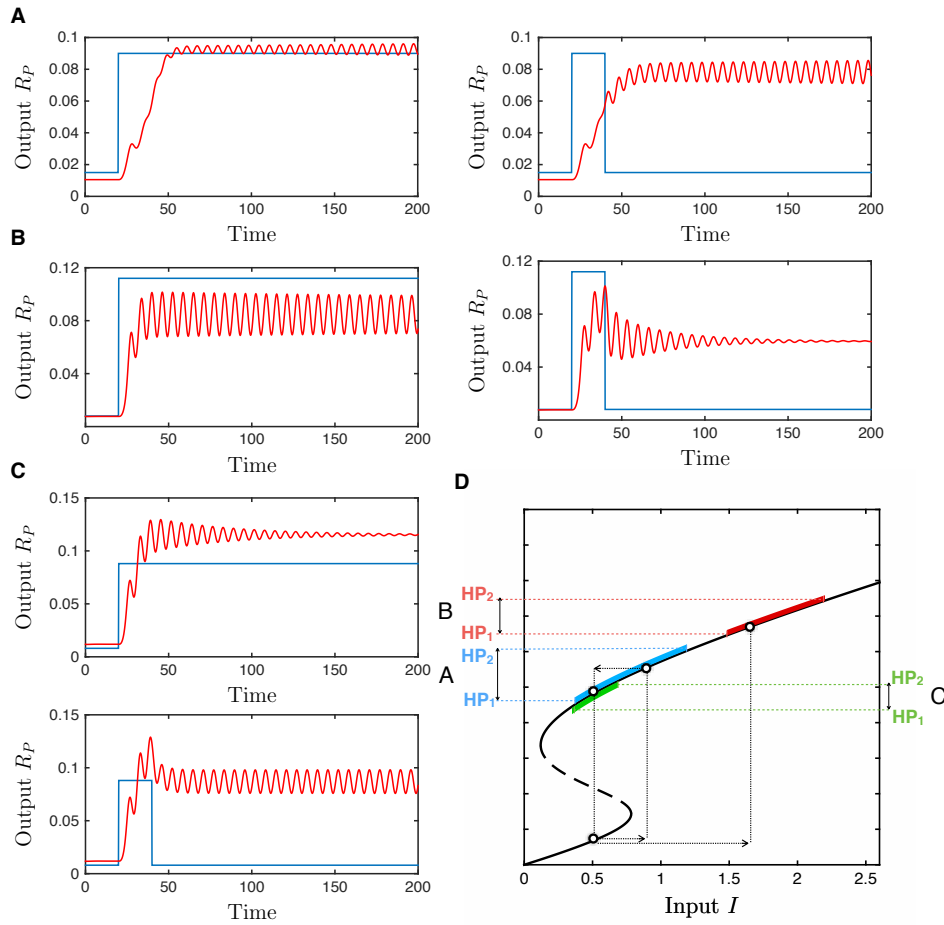


FIGURE 2.4: Sequential interaction without sharing: Bistable switch upstream - Negative feedback oscillator downstream. In each case, the input signal (scaled) is depicted in blue, and the downstream output in red. (A) Both step and pulse inputs may switch on sustained oscillations. (B) Only step inputs can switch on sustained oscillations. (C) Only pulse inputs can switch on sustained oscillations. (D) The diagram illustrates how the different scenarios in (A),(B),(C) may be realised, using the steady state input-output curve for the upstream bistable module. Clearly, the response depends on which part of the upper steady state branch corresponds to the oscillatory input range for the downstream module (A:Blue, B:Red, or C:Green; HP: Hopf points).

**Bistable switch upstream - NFO downstream:** We start by noting that switching in the bistable module may be induced by either a step or a pulse signal, provided that the basal input lies in the bistable regime. When this module is coupled to a downstream NFO, the bistable characteristics, combined

with the oscillatory behaviour downstream, gives rise to multiple possibilities, including coexisting steady states and oscillatory states (and even multiple oscillatory states) of the coupled system output, over certain ranges of upstream input. Here we describe a scenario illustrating some of these possibilities. An exhaustive analysis of all scenarios is provided in the Section I of the supplemental material.

In the present scenario, the parameters of the NFO, and the coupling parameter, are such that oscillatory behaviour occurs when the bistable module output is in a range of steady states on the upper branch. An upstream input that induces switching of branches in the bistable module, could therefore switch on stable oscillations of the downstream output. We consider a basal input lying in the bistable regime for the bistable module, with the upstream module initially at a steady state on the lower branch. The coupling gives rise to three possible types of responses to step and rectangular pulse inputs. In the first case, sustained oscillations of the downstream output may be switched on by either a step or a pulse input to the bistable module (Fig. 4(A)). Either of these inputs can produce a switching to the higher branch of steady states, triggering oscillations downstream.

The remaining possibilities (Fig. 4(B,C)) correspond to situations where: (1) a step input can switch on oscillations, but a pulse cannot (since downstream oscillations correspond to a range of steady states outside the bistable regime); (2) a pulse input can start oscillations, but a step input cannot (since downstream oscillations correspond to a range of steady states completely within the bistable regime).

**Bistable switch upstream - Adaptive module downstream:** We examine the effect that a switching in the upstream module (in response to either a step or pulse input) can have on the downstream output. If a switch does occur, then it has two possible effects. One possibility is that the output adapts, and the adaptation is close to exact. In this case, the output of the downstream module exhibits a transient of substantial amplitude, due to the switching in the upstream module. We note that the adaptive module largely, or completely filters out the effect of permanent switching in the bistable module.

It is worth noting here, that when an input pulse is able to elicit a substantial response (due to the bistable switch being triggered), a subsequent input pulse of the same characteristics may not do so, simply because there is no

further switching in the bistable module (Fig. 5(A)) (Also see supplemental material - Section I).

The other possibility is that the adaptive module is pushed away from the regime of exact adaptation. Depending on the nature of the module and the parameters, an adaptive module can exhibit either underadaptation (seen in both IFF and NF adaptive modules) or overadaptation (seen in IFF) (Krishnan, 2011).

**NFO upstream - Adaptive module downstream:** We consider a basal input that corresponds to stable oscillations for the NFO. These oscillations are propagated downstream due to the coupling, and the adaptive output no longer adapts to a steady state, but exhibits sustained oscillations. However, the adaptive module, loosely speaking, filters out any change in the mean of the oscillations. That is, even for different levels of input, which correspond to different limit cycles of the upstream NFO, the mean of oscillations of the downstream output remains almost the same (Fig. 5(B)). It is worth mentioning here, that if the dynamics of the adaptive module is fast relative to the NFO, the oscillations are very minor, since the adaptive module essentially reaches a quasi-steady state for a slow modulation of its input. On the other hand if the adaptive module dynamics is slow relative to the oscillator, again the output involves low amplitude oscillations as the adaptive module effectively samples the mean of the oscillations. This can be demonstrated more formally but we do not do that here.

All the above instances crystallize the fact that the presence of a nontrivial upstream module fundamentally affects information processing in the system.

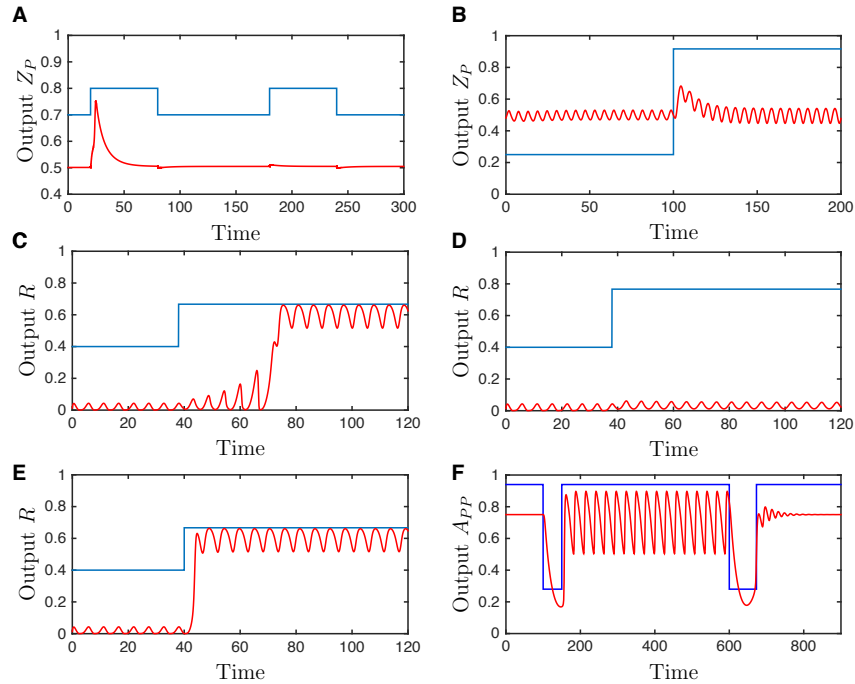


FIGURE 2.5: Sequential interaction without sharing. In each case, the input signal (scaled) is depicted in blue. (A) Bistable switch upstream - Adaptive module downstream. Significant transient response to first pulse input, but no response to subsequent identical pulse input. (B) Negative feedback oscillator upstream - Adaptive module downstream. Any change in the mean of oscillations is largely filtered out in the downstream adaptive output. (C),(D),(E) Negative feedback oscillator upstream - bistable switch downstream. (C) A step input can cause a switching of oscillatory branches (Step input at  $t=38$ ). (D) A step input of larger amplitude may not cause a switching of branches. (E) The contrast to (C) shows that timing of the step input can significantly affect the time taken to reach the asymptotic state (Step input at  $t=40$ ). (F) Substrate depletion oscillator upstream - Monostable switch (Two-site modification) downstream. Sustained oscillations may be switched on and switched off by pulse inputs.

### Order of Interconnection is Important

Reversing the order of modular interconnection significantly affects behaviour, as seen in the contrast between the results shown below and the earlier cases.

**NFO upstream - Bistable switch downstream:** In analysing this coupling, we restrict ourselves to a range of upstream input that corresponds to stable oscillations in the NFO. We note that, in this case, the asymptotic state for the coupled system output is always oscillatory. For the NFO in isolation,



the amplitude of oscillations depends on the input, and this is also seen in the coupled system. As a consequence of bistability in the downstream module, we can get oscillations about multiple steady states, for instance, when the amplitude of oscillations is small. Thus, it is possible to have multiple branches of oscillatory states for the downstream output. We note however, that, in contrast to the coupling of these modules in the reverse order, it is not possible to have coexistence of stable oscillations with a stable steady state, for the same input.

We present a scenario that illustrates some basic features of this coupling. When multiple oscillatory branches exist, variation in the upstream input (step or pulse) can induce switching between these branches. Fig. 5(C) depicts a switching of oscillatory branches, triggered by a step input. Two points need to be noted with regard to this switching.

1. Whether a step or pulse input can switch the output to a different oscillatory state, depends non-monotonically on the size of the step/pulse (compare Figs. 5(C) and 5(D)). This is because of the non-monotonic dependence of amplitude of oscillations in the NFO, on its input. This indicates how the bifurcation structure of the oscillator (in particular the variation in amplitude of oscillations) can determine the response of the coupled system.
2. The timing of the step/pulse input (relative to the phase of oscillations) can dramatically affect the time taken to reach the new oscillatory state. This is effect illustrated, for a step input, by the contrast between Figs. 5(C) and 5(E).

A detailed explanation for the behaviour described above, as well as a discussion of other possible scenarios, is provided in Section I of the supplemental material.

**Adaptive module upstream - Bistable switch/NFO downstream:** A step input can result in a switching in the bistable module, as studied in Seaton and Krishnan, 2011a. This of course depends on the adaptive module, and its time scale relative to the bistable module. If the adaptive module is very fast relative to the bistable module, the input to the bistable module is a rapid transient that is less likely to induce switching. The fact that a step input can produce a permanent effect (i.e. a switching) in the downstream output is in direct contrast to the coupling of these modules in the reverse order, where the downstream output is expected to adapt.

When an adaptive module is upstream of an NFO, a step input can produce transient oscillations of the downstream output (if the basal conditions correspond to no oscillations), or a transient disruption of oscillations (if the basal conditions correspond to oscillations).

### Significance of Module Structure

The results for coupling without sharing, pertaining to the NFO, would be equally applicable to any other oscillatory module exhibiting a similar bifurcation structure, i.e. where the oscillations arise due to a supercritical Hopf bifurcation (with the input signal as bifurcation parameter). However, oscillatory behaviour in a given module can also arise in other ways, notably, through a subcritical Hopf bifurcation. When oscillations arise in this way, we have the possibility of stable steady states coexisting with stable oscillatory states over a range of input (Strogatz, 2014), for the module in isolation. To examine the differences in input-output response, relative to the NFO, that might arise in such cases, we include a second oscillatory module in our study - a substrate depletion oscillator (SDO), that exhibits a subcritical Hopf bifurcation. For simplicity, we couple this to a simple monostable switch module downstream, to illustrate the basic differences.

**SDO upstream - Monostable switch downstream:** Suppose the basal input signal lies in a regime of co-existence of stable steady states and oscillations, and the coupled system is initially at a stable steady state.

1. Pulse inputs can switch on stable oscillations, for instance, by taking the system, transiently, into a regime where only oscillations exist. Whether a given pulse input switches on oscillations in this scenario, depends on the duration of the pulse in a non-monotonic way (see Section I of the supplemental material).
2. It is possible to both switch on and switch off oscillations through pulse inputs (even of the same magnitude) as illustrated in Fig. 5(F). In this regard, the timing (and duration) of the pulse can be very important in determining whether it can switch off existing oscillations

The fact that a pulse can switch on and switch off oscillations permanently, highlights a clear difference between this oscillator and the NFO, where a pulse input does not do so. This basic insight can be extended to the case of coupling of the SDO module with other downstream modules (See supplemental material).

We make an additional comment about bistable and oscillatory modules: in the above discussion, we essentially associate the output of the upstream module with the designated input of the downstream module. There are other parameters in the bistable and NFO modules, that could serve equally well as alternative inputs, giving the same qualitative behaviour for the coupled system. However, as we will see below, the choice of input can give rise to qualitative differences, when we consider coupling with sharing.

**Biological Relevance:** The cases discussed above suggest ways in which evolutionary processes can generate new information processing behaviour by introducing new interactions between existing modules. For instance,

- a) The simplest cases, with a CMC upstream of other modules, suggest a way for the cell to prevent downstream transitions being triggered under unfavourable circumstances, by regulating the total amount in the CMC.
- b) Having an adaptive module downstream of an oscillator allows the system to essentially filter out any variations in the mean of the upstream oscillations, while allowing the output to oscillate.
- c) The conjunction of a bistable switch with a downstream adaptive module allows part of a network to switch to a different steady state, while certain downstream pathways are buffered against this change of steady state.
- d) An upstream adaptive module may be used by the cell to insulate a downstream network, from variations in an upstream input signal so that these variations only have a transient effect. For example, an adaptive module upstream of an oscillator can be used either to prevent sustained oscillations being triggered by an input, or to create a robust oscillations within a range of input.
- e) Our analysis suggests it is possible to tune a bistable switch and an oscillator so that oscillations in the coupled system are triggered only by certain specific types of stimuli (for instance, only pulse inputs), something which could be exploited in synthetic biology.

### 2.3.2 Sequential interaction with shared components: CMC upstream or downstream

We now examine the coupling of modules with shared components, building on the previous study. In the present instance, the coupling reaction is no

longer described by mass action kinetics, rather it involves explicit enzyme-substrate binding-unbinding, and catalytic conversion. This results in sequestration effects. We first examine, in detail, the effect of coupling modules to a covalent modification cycle (CMC), as this reveals many of the essential features, and may be generically expected in signalling circuits. Coupling to CMCs with sharing involves the situation where the phosphorylation is not in the mass action regime.

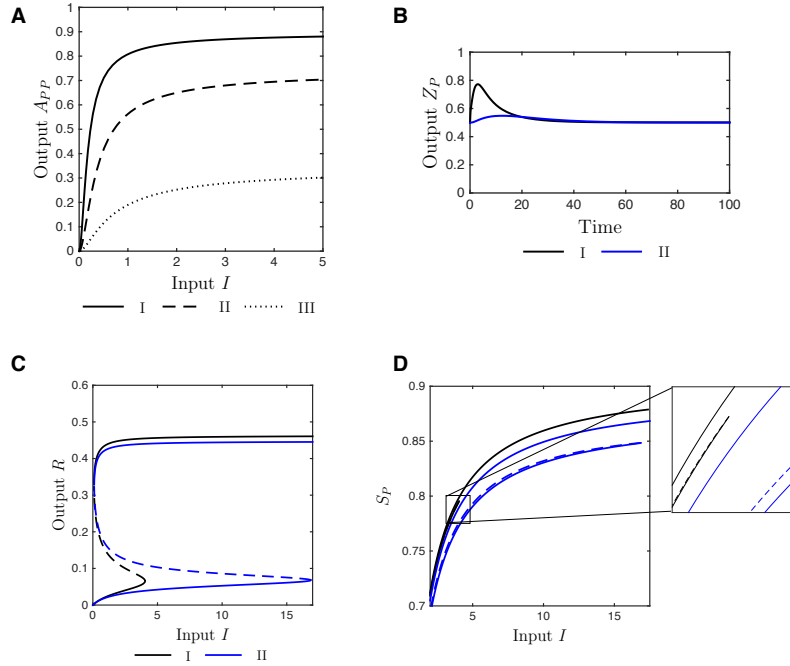


FIGURE 2.6: Sequential interaction with sharing, with the covalent modification cycle upstream. (A) Monostable switch (Two-site modification) downstream. The sharpness of the switching is lost as the catalytic constant of the coupling,  $k_{cat}$ , is lowered ( $I > II > III$ ). (B) Adaptive module downstream. The magnitude of the transient response becomes diminished as  $k_{cat}$  is lowered ( $I > II$ ). (C) Bistable switch downstream. As  $k_{cat}$  is lowered, initially, the range of bistability (with respect to upstream input) increases ( $I > II$ ). Eventually, as  $k_{cat}$  is lowered further, the bistable regime, as a whole, moves to higher values of the input (not shown). (D) The backpropagation of bistable effects also increases as  $k_{cat}$  decreases, with the steady state branches of the upstream output  $S_P$  moving further apart.

### CMC upstream: Increasing sequestration distorts input response

We study the effects of sequestration on the input-output behaviour of the coupled system by varying the catalytic constant of the coupling reaction -

$k_{cat}$ . A low  $k_{cat}$  results in greater sequestration of the upstream output, while a high  $k_{cat}$  (relative to binding/unbinding) is essentially equivalent to the sequential coupling without sharing.

**Monostable switch downstream:** For high  $k_{cat}$  of the coupling reaction, the steady state input-output relationship is qualitatively similar to the switch module in isolation. The switch-like behaviour becomes progressively less sharp as  $k_{cat}$  is lowered (Fig. 6(A)). For low  $k_{cat}$ , the input-output response becomes more gradual.

**Adaptive module downstream:** Here we examine the adaptive behaviour of the coupled system, by focussing on the response to step inputs. When  $k_{cat}$  is high, the downstream output exhibits almost perfect adaptation to step inputs. When  $k_{cat}$  is reduced, the response remains adaptive, but the amplitude of the transient response to a step input is considerably reduced. The adaptive module is in a regime of almost exact adaptation and this is reflected in both these results (Fig. 6(B)). The effects seen in both the above cases may be demonstrated analytically, but we do not do this here.

We now turn to the coupling of the CMC to a downstream bistable switch/NFO.

#### **CMC upstream: Sequestration causes back-propagation of downstream characteristics**

When the coupling involves sequestration, the dynamical characteristics of the downstream module (bistability/oscillations) may be propagated to the upstream CMC. The possibility of such a back-propagation effect depends on the *input step* of the downstream module, through which the CMC is coupled. If the coupling occurs through a zeroth order reaction, with the input acting as an enzyme on a substrate present in large excess, then the upstream CMC is not qualitatively affected, except for a degree of uptake of its output species  $S_P$  in the downstream module. Thus, coupling through a zeroth order reaction essentially insulates the upstream module from downstream behaviour. The designated input steps to both the bistable switch and the NFO, are of this type (see Section 2). If the CMC is coupled to these modules through a different step, one not involving a zeroth order reaction, then downstream characteristics (bistability or oscillations) are propagated to the CMC. In such cases, the back-propagation effect becomes stronger as the extent of sequestration is increased (i.e. as  $k_{cat}$  is reduced). This is shown in Figs. 6(D) and 7(B).

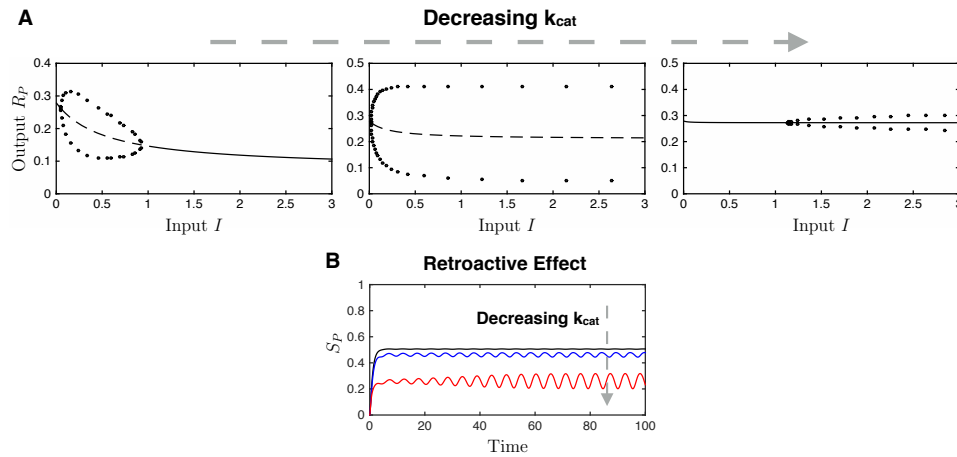


FIGURE 2.7: Sequential interaction with sharing, negative feedback oscillator downstream of a covalent modification cycle. (A) The input range for oscillatory behaviour changes significantly as the catalytic constant decreases, with the onset of oscillations increasing to higher values (right hand panel). (B) Increased sequestration leads to higher amplitude oscillations of the upstream species.

### CMC upstream: Sequestration affects thresholds for input-driven transitions

Increasing sequestration produces significant changes in the input thresholds for qualitative transitions in the coupled system. These effects are broadly similar whether the coupling occurs through a zeroth order step or not. For the CMC coupled to the bistable switch, increasing sequestration affects the locations of the limit points (LPs) in the steady state input-output curve. Lowering  $k_{cat}$  causes both LPs to move higher values of input: initially, the change in the left LP is much smaller than the right LP, but eventually, the left LP also moves away at a faster rate. Thus, as  $k_{cat}$  is lowered, the bistable regime initially becomes extended over a larger range of input (Fig. 6(C)), but eventually, the regime as a whole moves to progressively higher values of input, so that bistability is not attained at reasonable input values.

For the CMC coupled to the NFO, increasing sequestration affects the locations of the Hopf bifurcation points for the coupled system, similar to the effect on LPs for the bistable switch. Thus, initially, lowering  $k_{cat}$  extends the oscillatory regime over larger ranges of input, but eventually, the regime as a whole moves to progressively higher values of input (Fig. 7(A)), making oscillatory behaviour unattainable at reasonable values of input.

The results above illustrate how sequestration of a species, belonging to a conserved pool in the CMC, can impose additional constraints on information processing by the coupled system, relative to coupling without sharing. The effects observed for both the bistable switch and the NFO downstream, can be clearly understood with reference to the mathematical analysis presented in Section II of the supplemental material.

#### **CMC downstream: Extent of sequestration determines distortion of upstream behaviour**

Here we examine the effect of sequestration of the upstream output in a downstream CMC, by varying the total amount of species,  $[S_T]$ , in the CMC. The catalytic constant of the coupling reaction,  $k_{cat}$ , is fixed for these interactions. Similar behaviour may be observed by varying  $k_{cat}$ , while keeping the total amount fixed downstream.

**Monostable switch upstream:** As seen in Fig. 8(A), a switch like behaviour is still observed, irrespective of  $[S_T]$ ; however, the magnitude of the switch is lowered significantly for high amounts of downstream species.

**Adaptive module upstream:** The upstream output exhibits almost perfect adaptation, irrespective of  $[S_T]$  (Fig. 8(B)). As  $[S_T]$  is increased, the amplitude of the transient in response to a step input is lowered, and the steady state of the adaptive output is reduced by increased uptake downstream.

**Bistable switch upstream:** For the switch employed, bistable behaviour is retained, and the input range for bistability is unaffected, as  $[S_T]$  is increased (Fig. 8(C)). This occurs because  $R$ , the output species of the switch, comes from an open chemical reaction (which insulates against the sequestration effect). The situation is different if we consider coupling with a bistable module where the output species is part of a conserved pool (see supplemental material - Section III). Even for the present module, if the coupling occurs through a species ( $E_P$ ), associated with a conserved pool in the module, then a distortion of bistable behaviour is possible. As  $[S_T]$  is increased, the input range for bistability becomes narrower, and eventually, bistable behaviour is lost (Fig. 8(D)).

**NFO upstream:** The output of the NFO belongs to a conserved pool. As a result, increasing  $[S_T]$  in the downstream CMC significantly distorts the behaviour of the coupled system. Increasing  $[S_T]$  reduces both the input range for oscillations and the amplitude of the oscillations, and eventually destroys oscillatory behaviour.

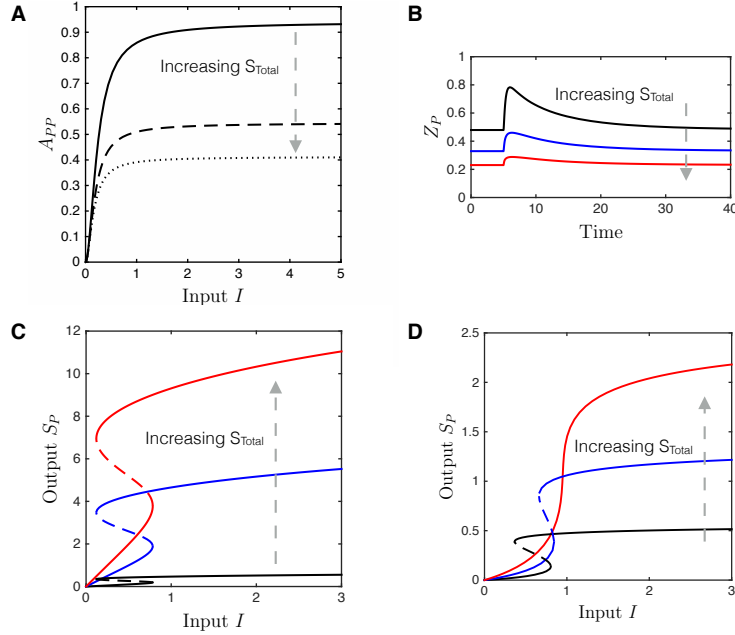


FIGURE 2.8: Sequential interaction with sharing, with the covalent modification cycle downstream. (A) Monostable switch upstream. As the total amount of downstream species increases, the sharpness of the switch is retained, but the upstream variable saturates at a lower steady state. (B) Adaptive module upstream. As the total amount of downstream species increases, the upstream output adapts to a lower steady state value, and the response amplitude is diminished. (C) Bistable switch downstream. The range of bistability is unaffected by an increase in the total amount of downstream species. See text for details. (D) If the species  $E_P$  (part of a conserved pool) of the bistable module participates in the coupling, instead of the species  $R$ , the upstream bistability is destroyed as the total amount of downstream species increases.

The above results reveal the possibility of significant alteration of qualitative behaviour, due to sequestration, when a module is coupled even to a simple CMC. In all the above scenarios, the effects may be understood to have been caused by a reduction in the total amount, in a conserved pool of species in the upstream module, due to increased sequestration. We present computational results in the Supplementary Information, which demonstrate



that similar effects are observed for the modules in isolation, when the corresponding total amount in the output step is reduced.

**Biological Relevance:** Both kinds of scenarios examined above: (1) upstream CMC (2) downstream CMC, may be expected in many signalling contexts. Having an upstream CMC allows the cell to modulate the signal received by a downstream module. Depending on the extent of sequestration, a cell may regulate the total amount in the CMC, as a means to control the input thresholds for a downstream transition, to counteract the effects of sequestration. Sequestration of the module output in a downstream CMC significantly distorts the dynamical characteristics, when the module output belongs to a conserved pool. This effect gives rise to the possibility of certain ‘frustrated’ modules existing within networks, where a module is prevented from exhibiting its intrinsic complex dynamical capabilities purely due its downstream coupling. In a given biological system, such ‘frustrated’ modules may be expected to emerge from time to time as signalling networks evolve (caused by connection to a new downstream target or by changes in the kinetics of an existing downstream interaction). A cell may mitigate such distortion in different ways, such as by having the module output produced by an open reaction step, by having the downstream interaction in a mass action regime, or by regulating the total amount in the downstream CMC.

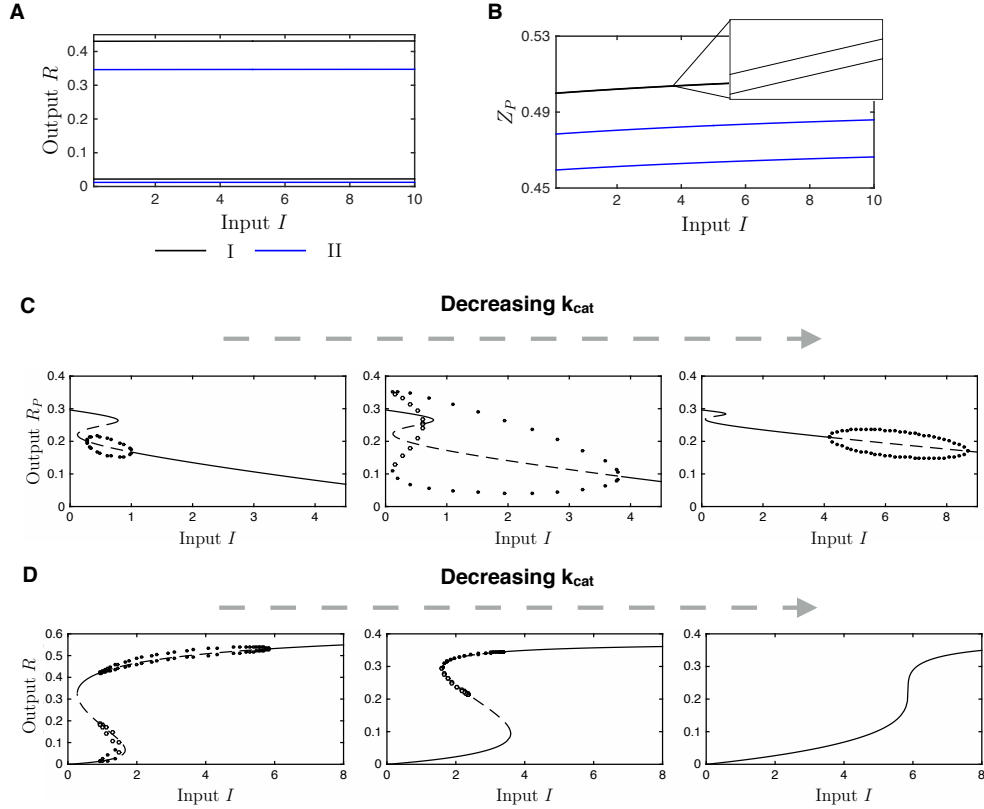


FIGURE 2.9: Sequential interaction with sharing. (A),(B) Adaptive module upstream - Bistable switch downstream. (A) The adaptive module continues to essentially filter out the effects of the upstream input, even in the presence of sequestration ( $k_{cat}$  for  $I > k_{cat}$  for II). (B) Backpropagation of bistability: The two steady state branches for the adaptive variable are almost indistinguishable at high  $k_{cat}$  (black curves). They move apart as  $k_{cat}$  decreases (blue curves). (C) Bistable switch upstream - NFO downstream (Bifurcation diagrams shown for NFO output). The input range for oscillatory behaviour varies significantly as the catalytic constant decreases, and increased sequestration causes the oscillatory branch to extend onto the middle branch of steady states. Oscillatory behaviour is eventually lost, but the system retains bistability (not shown). See text for details. (D) NFO upstream - Bistable switch downstream (Bifurcation diagrams shown for bistable switch output). We see two distinct oscillatory regions, and different branches of stable limit cycles that coexist over a range of upstream input. The oscillatory regions merge as the catalytic constant decreases, followed by the complete disappearance of oscillations, and eventually, the loss of bistability.

### 2.3.3 Sequential interaction with shared components: Coupling of strongly nonlinear modules

We now examine the interactions involving adaptive, oscillatory and bistable modules.

#### **Adaptive module upstream: Bistability/oscillations may be propagated upstream**

If the adaptive module is coupled to a downstream bistable switch or NFO, then, even if the adaptive module is in a regime of close to exact adaptation, sequestration causes downstream characteristics (bistability/oscillations) to be propagated to the adaptive output, so that it no longer exhibits exact adaptation (see Fig. 9(B) and S7(B)).

We note that the back-propagation of bistability/oscillations in this case affects only on the output variable  $[Z_P]$  of the adaptive module, while  $[X_P]$  and  $[Y_P]$  do not exhibit multiple steady states/oscillations. This is due to the feedforward structure of the module and the fact that all interactions within the module are in the mass action regime. In contrast, for a negative feedback adaptive module (see supplemental material - Section III), downstream characteristics are propagated to *all the variables within the feedback loop*, even if all interactions are mass action.

#### **Adaptive module downstream: Effect on upstream module is similar to a CMC**

We see that, in addition to the affect on the upstream module (similar to the effect of a downstream CMC) sequestration also affects the dynamic response of the adaptive module. Even with sequestration, the effect of bistability is largely filtered out by the adaptive module (i.e. different steady states are practically indistinguishable). This is due to the feedforward structure of the adaptive module and the fact that it is in a regime of close to exact adaptation. The negative feedback adaptive module we consider, exhibits a degree of under-adaptation, and therefore the effects of an upstream bistable switch are propagated to a greater extent in this case.

**Bistable switch upstream:** For high  $k_{cat}$ , the downstream output exhibits bistable characteristics, where the different steady state branches of the output are very close to one another (a consequence of adaptation). For lower  $k_{cat}$ , these branches become more separated. Reducing  $k_{cat}$  can significantly

diminish amplitude of the transient in the downstream output, for step inputs that cause switching (Fig. S7(A)).

**NFO upstream:** As  $k_{cat}$  is lowered, the input range for oscillations is reduced, and oscillatory behaviour may be destroyed, due to sequestration effects (Fig. S7(C)). This effect is analogous to the destruction of oscillations in the NFO due to increased sequestration in a downstream CMC.

### **Bistable switch and NFO: Sequestration determines combination of dynamical characteristics**

**Bistable switch upstream - NFO downstream:** The coupling of these modules (through the designated input to the NFO), without sharing of components, gives rise to multiple scenarios, including coexisting steady states and oscillations over a range of input (see section III.A.3), as well as coexisting oscillatory states (see Section II of the supplemental material). Analogous scenarios are observed when they are coupled through the alternative input to the NFO in the absence of sharing (see supplemental material). To study the effects of sequestration, we examine coupling through the alternative input. Here we report on an analogue of the case presented earlier and examine the effects of sequestration. At high  $k_{cat}$  (similar to the non-sharing case), the output exhibits bistable characteristics, with an oscillatory regime on the upper branch. As seen in Fig. 9(C), on reducing  $k_{cat}$ , the oscillatory regime is significantly distorted, and may extend to other branches. Further decrease of  $k_{cat}$  can lead to the shrinking of the region back onto the upper branch and ultimate destruction of oscillations. The system retains bistable characteristics, since the output of the bistable module comes from an open step (and is hence relatively insensitive to the effects of sequestration).

Similar effects are observed in the other scenarios (see Section II of the supplemental material). These, along with the results above, indicate that the extent of sequestration, for this coupling, can determine (1) the possibility of oscillatory behaviour (2) the possibility of coexisting steady states and oscillatory states, for a given input.

**NFO upstream - Bistable switch downstream:** Similar to the above case, where these modules were coupled in the reverse order, we observe that the extent of sequestration determines the possibility of oscillations, and the coexistence of multiple oscillatory states. This is illustrated, for a particular scenario, in Fig. 9(D). Lowering  $k_{cat}$  causes the input range for oscillations

to become narrower, and eventually the possibility of coexisting oscillatory states is lost. Further increase in sequestration completely destroys oscillatory behaviour and ultimately even bistable characteristics are lost.

Taken together, the analysis of sequestration in both cases (also see supplemental material - Section II) shows that sequestration can result in a complex combination of bistability and oscillations, only oscillations, only bistability, or neither oscillations nor bistability. When the modules are coupled through species belonging to conserved pools (closed steps), the relative total amounts in the two participating steps, together with sequestration, determines what combination of bistable and oscillatory characteristics the coupled system exhibits. For example, if the total amount in the output step of the oscillator is large relative to the total amount in the input step for the bistable switch, sequestration can cause the coupled system to exhibit only oscillatory behaviour, while showing no bistable characteristics (see Figure S3 in the supplemental material).

It is evident that the particular bifurcation structure of the modules, as well as the nature of coupling and the sharing of components all play important, and in some cases unintuitive roles. Naturally, the response of the coupled modules to particular stimuli also depend on other transient features and which basin of attraction a given trajectory ends up in.

**Biological relevance:** For the complex modules seen in systems biology, the above results tell us what may happen when the core generators of dynamical characteristics in such modules are interfaced directly with each other. The resulting distortion illustrates the need for buffering layers in the interfacing of modules. In the context of building synthetic biochemical circuits (to achieve new functionality), our analysis of the above scenarios shows that, when trying to interface different synthetic signalling modules designed in isolation, the dynamical characteristics of one or both modules may end up being suppressed in the resulting coupled system. Our study also suggests ways in which such an effect may be mitigated in a synthetic context, such as adjusting the total amount in a conserved pool, or by designing the interaction through open steps. A synthetic module designed with open steps both at the input location and output (thus mitigating the effects of sequestration), would have the capability to interface with multiple modules, both upstream and downstream, without having the intrinsic dynamical characteristics of the module distorted by the interaction.

### 2.3.4 Oblique Interaction

In sections 2.3.1 to 2.3.3, we examined how a module interacts with its environment through its designated input and output steps. Since each module also contains other biochemical steps, the interaction with the environment could also occur through one of these steps. Interactions of this kind could significantly affect the variation of module output in response to its input. To complete our study of modular interaction, we examine some aspects of such interactions between modules, that we refer to as *oblique* interactions.

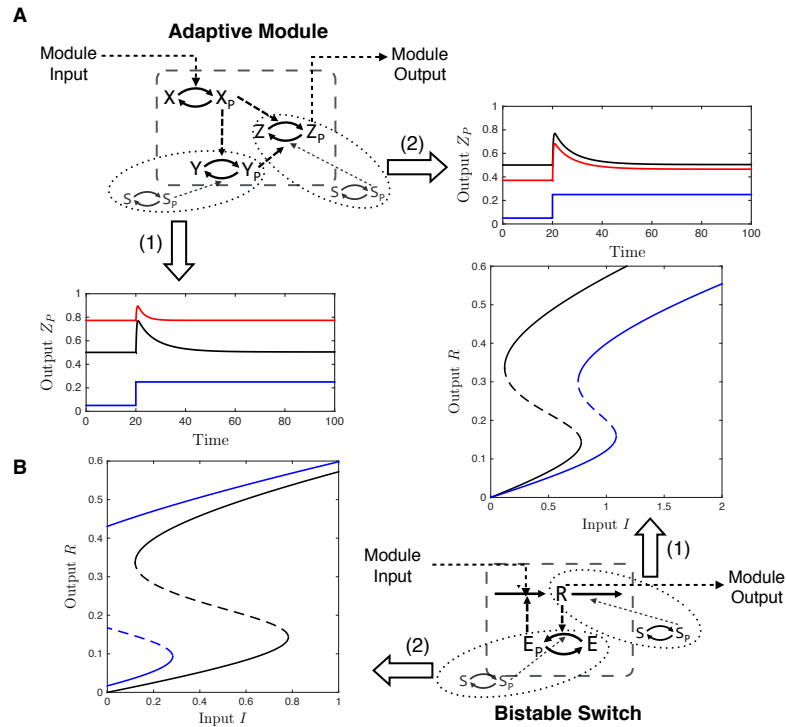


FIGURE 2.10: Oblique interactions with a CMC. (A) Adaptive Module. An oblique interaction at one location - (1) causes adaptation to a steady state (red line) different from the basal steady state for the module (black line). A similar interaction at a different location - (2) causes under-adaptation. (B) Bistable Switch. An oblique interaction at one location - (1) reduces the bistable range (blue line) relative to the module in isolation (black line). A similar interaction at a different location - (2) shifts the bistable range, possibly causing the switch to become 'one-way'.

Here, we will focus on the relationship between the input and output of a given module, in the presence of an oblique interaction with another module. Such an interaction occurs through a different location/step (i.e. involving a different reaction) in the module, from that of the designated

module input. For simplicity, we do not consider sharing of components here, and the interactions are assumed to be mass action. Our focus will be on how the input-output behaviour of a given module is affected by such an oblique interaction.

In a module, an oblique interaction can occur at different locations. As part of our investigation, we identify and highlight cases where the location of oblique interaction can cause significant qualitative differences. As in the case of sequential coupling, we continue to choose coupling parameters, to allow for non-trivial effects of such interactions.

When we study oblique interactions, we will regard one of the modules (the one whose input-output behaviour is the focus of interest) as the downstream module and examine how it is affected by an oblique interaction with the other (upstream) module (see Figure 10(A,B) for a schematic representation of oblique interaction with a CMC). For simplicity we keep the input of the upstream module fixed (a parameter) and vary the input of the downstream module to examine its input-output behaviour. Naturally, it is possible to vary both inputs simultaneously, but we defer this to a subsequent investigation.

Many basic aspects of oblique interaction are transparently seen in the case when the upstream module is a CMC. We discuss this first.

### Oblique interaction with a CMC

**Oblique interaction alters module behaviour:** An oblique interaction of a module, even with a simple CMC, is seen to produce significant differences in the way the module responds to its input (relative to its behaviour in isolation). For example, such an oblique interaction at one location on the adaptive module (at the step  $Y_P$  to  $Y$ ) results in close to exact adaptation, to a steady state different from that for the module in isolation (Figure 10(A)-(1)). The extent to which the steady state differs depends on the strength of the oblique interaction. Similar oblique interactions with the CMC, in the bistable switch (in the degradation of  $R$ ) and the NFO (in the step  $R_P$  to  $R$ ), are found to reduce the input range for bistability (Figure 10(B)-(1)) and oscillations, even in the absence of sequestration.

**Different locations for oblique interaction can produce different effects:** The effect of an oblique interaction with the CMC, on a module, depends on the location at which this interaction occurs. This aspect is highlighted by the following examples. In the case of the adaptive module, a similar interaction

with the CMC, to the one considered above, but at the step  $Z_P$  to  $Z$ , results in the module exhibiting under-adaptation to changes in its input (Figure 10(A)-(2)). The qualitative difference in the effect seen here, compared to that seen above, is due to the different roles of the two steps ( $Y_P$  to  $Y$  and  $Z_P$  to  $Z$ ), relative to the structure of the module. For a similar reason, in the bistable switch, we find that an oblique interaction with the CMC at the step  $E$  to  $E_P$ , has a different effect to the oblique interaction seen above. Here, it causes a shift of the bistable regime to lower input values, rather than reducing its extent. We note that this effect could cause a part of the bistable range to correspond to negative values of the input, meaning that switching of steady states in the bistable module will be ‘one-way’ (Figure 10(B)-(2)).

We now examine oblique interactions with the more complex modules. The effects of such interactions are of the same nature as for those involving the CMC, but with additional qualitative aspects characteristic of the upstream module involved.

### **Oblique interaction with strongly nonlinear modules**

**With an Adaptive module:** When a module is subject to an oblique interaction with an adaptive module (in a regime of close to exact adaptation), its behaviour is essentially determined by the steady state of the adaptive output (any deviations from this are transient). This steady state influence of the adaptive module may significantly alter downstream module behaviour. For example, the presence of an oblique interaction with the adaptive module can significantly distort the bistable/oscillatory regime in a bistable switch/NFO, possibly even leading to a destruction of bistability/oscillations.



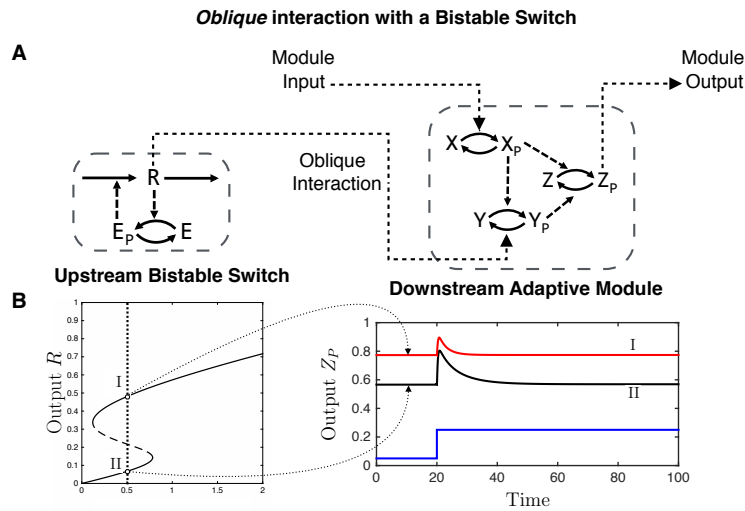


FIGURE 2.11: Propagation of bistability through an oblique interaction. (A) Schematic depicting an oblique interaction of the adaptive module with the bistable switch. (B) The downstream output may adapt to different steady state values (I or II), depending on the history of the upstream bistable switch.

**With a Bistable switch/NFO:** An oblique interaction with a bistable switch causes bistable characteristics to be propagated to the downstream module. If the switch is in the bistable regime, the response of the downstream module, to its input, would depend on the history of the bistable module that it is interacting with. For example, if the bistable switch interacts with the adaptive module at the step  $Y_p$  to  $Y$ , then the adaptive output could exhibit adaptation to either of two distinct steady states, depending on the state of the upstream switch (Figure 11(B)). It is also clear that bistable characteristics will be propagated to a downstream module, irrespective of the location at which the oblique interaction occurs. Similarly, an oblique interaction with an NFO would cause oscillations to be propagated to downstream, irrespective of the location of the interaction.

**Biological Relevance:** In examining the behaviour of a particular signalling pathway, possible oblique interactions must be taken into account, as these can potentially result in the dynamical characteristics from a different part of the network being propagated to this pathway. This can result in a module exhibiting mixed behaviour, for example, by exhibiting adaptation about multiple steady states as seen above. The perceived behaviour of a particular pathway may not be intrinsic to it, but may be the result of an oblique interaction with another module. Consequently, observed behaviour may be

misattributed to a given pathway. Oblique interactions also provide an avenue for the cell to regulate the input response of a given pathway between different regimes depending on the circumstances.

When a given synthetic module is inserted into an existing cellular network, oblique interactions with the cellular machinery may occur. In such cases, our analysis can suggest ways in which the module may be tuned to achieve desired functionality. Furthermore, our study of oblique interactions suggests an avenue for systematically increasing the complexity of a given synthetic module, by subjecting the module to a secondary input, designed to switch the module between different regimes of input-output behaviour.

## 2.4 Conclusions and Discussion

In this chapter, we developed a systems engineering framework for understanding the interaction between a signalling module and its network environment. We examine such interactions for different possibilities of the nature of the surrounding network, which leads us to focus on the interaction between a signalling module and other modules. Thus, we build a bridge between understanding a signalling/biochemical module in isolation and as part of a network.

Taking a global approach, we examined different kinds of interaction of modules, with and without sharing of components, creating a library of scenarios which was analysed. Our models were fairly simple realisations of these modules, representing the common denominator for more complex modules exhibiting similar behaviour. Thus, our study serves as a platform for examining more complex cases. We were able to transparently analyse and understand different aspects of modular interaction, so that these insights are relevant to alternative and more complex modules. We note that for interaction (sequential, oblique) with negligible sharing, similar effects could be seen, even if multiple intermediate steps of a cascade (close to mass-action kinetics) are involved in the interaction.

Our results generalise in multiple directions, for a number of reasons: (1) Those for coupling without sharing arise solely from the dynamical systems characteristics of the modules, with different possible scenarios depending on the coupling parameter. These results are also applicable for the coupling of modules through intermediate steps. (2) Coupling to the CMC with sharing represents a generic scenario of a biochemical module interfacing with its environment (3) Our study of complex modules provides a framework for

understanding synthetic constructs combining these modules, with sharing of components.

**Effects of modular coupling:** Through our integrated approach, we are able to obtain multiple insights into how a module may interact with the ambient network. The first and most basic point is that the behaviour of a module in isolation is very different from that when it is embedded in an ambient network, as the interactions with the surrounding network can substantially, and in some cases drastically, affect its behaviour. Secondly, genuinely new modes of information processing can result from the interaction of modules, and we have seen a number of cases of this. Bistability or oscillatory behaviour can be combined with other kinds of modules and their effects can be propagated to these modules. In some cases, the combination of modules can also serve to buffer against the propagation of dynamics of modules.

**Effects of sequestration:** The sharing of components can introduce a significant retroactive effect, which depends on the modules being coupled and the way they are coupled. Our study reveals another dimension to the effects of sequestration, by incorporating the complexity and characteristics of the participating modules. In cases where the coupled system without sharing exhibits the dynamical systems features of both modules, the analogous coupling with sharing may not exhibit all of these features, purely due to sequestration.

**Oblique coupling:** Our study of oblique interactions between modules provides some insights into how even indirect interactions can significantly affect module behaviour. Such oblique coupling of modules can be regarded as a form of crosstalk between pathways (Komarova et al., 2005), extended to the case of information processing modules.

**Important module characteristics:** Our results illustrate the roles played by both the dynamical systems characteristics of a module, and the biochemical details, in the context of modular interaction. The qualitative features arising from modular coupling ultimately depends on the dynamical systems characteristics of the coupled modules; for coupling without sharing, it depends entirely on these characteristics. Where similar behaviour may be realised in different ways, the dynamical systems structure of a particular module determines both how it is affected by the environment, and how it performs its

role within the network, as exemplified by our study of different oscillators. The biochemical aspects of a module rise to prominence in coupling with sequestration. A zeroth-order input step (implicitly assuming a fixed pool of substrate) insulates against back propagation to an upstream module, while an open step producing the output buffers against distortion by increased sequestration downstream.

**Consolidated insights into an exemplar module:** As a synthesis of our results in the context of one module, our studies show, for bistable modules: (i) how this bistability can be propagated upstream in a signalling pathway (ii) how even simple pathways downstream can destroy bistability due to sequestration of species (iii) how the propagation of bistability may be prevented if a zeroth order reaction/open pathway is involved (iv) how adaptation downstream can buffer/insulate against the propagation of bistability (v) how “oblique” interactions can result in bistability being propagated to other pathways/modules, significantly altering their behaviour (vi) Our consideration of multiple bistable modules sheds light into exactly what aspects of the modules themselves is responsible for their behaviour in a network. (vii) Finally this also provides insights into what factors may allow for robust functioning of a bistable module in a network.

The models we employed are fairly simple ODE based models, which encapsulate interactions giving rise to the qualitative behaviour observed. These serve as a common foundation for both systems biology and synthetic biology (where simple modules are the norm). From our discussions above, it is clear that many of the essential insights generalise to more complex realisations of these modules in systems biology. Furthermore, this analysis suggests a procedure for studying the effects of the ambient network, on a given complex realisation: this can be done by systematically accounting for both the core driving circuit and additional embellishments, and assessing the contribution of each of these, in this regard. Stochastic analogues of such coupling are another aspect to be examined. The role of spatial organization of biochemical circuits is being increasingly recognised (for example, see Alam-Nazki and Krishnan, 2012; Alam-Nazki and Krishnan, 2015). These aspects could play a particularly important role in the present context, and will be examined subsequently.

**Modules and Modularity.** Our study provides different insights into modules and modularity. As we have seen, interactions with the ambient network can drastically alter a module behaviour. How is the behaviour of

a module (particularly characteristic information processing modules) maintained in the face of this? It has been suggested in Alexander et al., 2009 and elsewhere, that evolutionarily conserved species constitute the core of a module, which is responsible for its behaviour and that other embellishments through evolution may modulate this. Our studies provide a complementary perspective. Different mechanisms can buffer a signalling module from its environment. The effect of sequestration downstream can be buffered against, by having, for instance, covalent modification cycles acting in the mass action regime. With regard to oblique and extraneous upstream interactions, having intermediate covalent modification cycles in low amounts, interacting weakly, buffers against these interactions. On the other hand this should not buffer against the designated input, which should be able to trigger the characteristic behaviour of the module. Evolution brings with it an arsenal of multiple additional possibilities to insulate and maintain a module behaviour, including open pathways, adaptive modules, redundant pathways, multiple isoforms and spatial compartmentalization. Some of these ingredients can also expand the core components of a module, or even lead to essential rewiring of the core module. Naturally, the modules seen are those which have been buffered against substantial environmental interaction. There could also be cases where such additional interaction has completely altered/destroyed the characteristics of the module. Our analysis can, in conjunction with a study of networks in different species, both bacteria and eukaryotes, provide a new window into understanding the evolution and organization of cellular modules. In this manner it may allow us to appreciate the actual realization of different modules in cellular contexts, and trace the alteration in behaviour of modules with evolutionarily conserved components.

**Systems biology.** Our analysis is relevant to systems biology in multiple ways. Firstly there are multiple contexts in which the above modules and their combinations are seen in cell signalling. Bistable modules are found in cell cycle transitions (Xiong and Ferrell, 2003; Sha et al., 2003), and these interact with other pathways or modules (Swat, Kel, and Herzl, 2004; Doncic et al., 2015). They are also implicated in irreversible decision making, for instance, at the level of caspase pathways in apoptosis and in stem cell differentiation (Eissing et al., 2004; Harris et al., 2011). Oscillators are present at different levels and in multiple cell signalling contexts ranging from p53-Mdm2 to the Cdc2/APC system to MPF oscillations (Bar-Or et al., 2000; Pomerening, Kim, and Ferrell, 2005; Tyson, 1991), with both negative feedback and

substrate-depletion implicated. Adaptation is seen in many signalling contexts, including at the sensory level (for instance in chemotaxis (Chang and Levchenko, 2013)) and also embedded in signalling circuits. The interaction of adaptive and switch like modules is implicated in contexts such as osmoregulation in yeast (Muzzey et al., 2009), while adaptive signalling interfaces with the complexity of the cell cycle, that includes bistable and oscillatory processes (Chen et al., 2000). The interaction between covalent modification cycles or monostable switches with other modules can be generically expected in signalling. Many different interactions between modules may be expected in realistic cellular networks, noting that no signalling module is isolated. Our study here provides insights into different interactions between basic biochemical modules, providing a way of appreciating the extra layers of organization involved in these processes, as well as understanding cellular decision making through such pathways and networks, and the robustness of such behaviour.

There is currently substantial interest in whole cell modelling (Karr et al., 2012; Macklin, Ruggero, and Covert, 2014). While there are many challenges involved in this enterprise, any reasonable attempt at whole cell modelling will have to seriously confront some of the issues we have considered. These include non-linearity in pathways and the presence of non-linear modules, and the interaction between the modules and the ambient network. Our study provides a platform, centred around the basic modules for this purpose.

**Synthetic biology.** Given the implicit synthetic underpinning of this work, it is clear that the results have relevance for synthetic biology as well. Bistable, oscillatory, and adaptive modules, and complex variations thereof have been engineered through synthetic approaches (Purnick and Weiss, 2009) as well as in chemical biology (Semenov et al., 2016; Lanchuk et al., 2018). The natural progression in synthetic biology is to go from basic modules, both genetic and biochemical, to more complex and sophisticated information processing systems, which in turn can be utilized in different contexts (Elowitz and Lim, 2010; Bashor et al., 2010; Purnick and Weiss, 2009). The realization of this step is a very substantial challenge that relies on both building reliable, complex circuits through the design cycle, and ensuring that it works reasonably in the cellular environment. In this regard it should be noted that even within desired circuits, the effect of retroactivity needs to be managed or countered (Mishra et al., 2014; Lyons et al., 2014), and secondly the circuits may have a number of extraneous interactions with the cellular chassis. Therefore it

is necessary to understand the interaction between a given module and the surroundings in a systematic manner. The building of compound circuits through transcription factor modules is discussed in Gyorgy and Del Vecchio, 2014. In the context of signalling and biochemical networks, our study provides a basis for the realization of complex logic in signalling circuits and also for realizing complex interactions between information processing modules (and understanding the effects of sequestration). It can also provide insights into how modules can be designed in a robust manner to buffer from interaction with the surroundings. The interaction and integration of modules (not just signalling) in cell free environments is an emerging theme in both synthetic biology (Caschera and Noireaux, 2014) and chemical biology (Semenov et al., 2015; Lanchuk et al., 2018). Finally this is also relevant in a range of related settings such as molecular communication (Nakano, Eckford, and Haraguchi, 2013), biomolecular information processing and molecular computing (Katz, 2013a; Katz, 2013b), as all of these settings rely on information processing, molecular modules and networks, and their interaction.

TABLE 2.1: Sequential interactions without sharing

| Upstream Module                          | Downstream Module                                                                                                                                                |                                                                                              |                                                                                                                                                                                                             |                                                                                                                                                                                                        |
|------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                          | Monostable Switch                                                                                                                                                | Adaptive Module                                                                              | Bistable Switch                                                                                                                                                                                             | Negative Feedback Oscillator                                                                                                                                                                           |
| <b>Covalent Modification Cycle (CMC)</b> | Switch-like change in the downstream output for a step input of sufficient magnitude.                                                                            | Close to exact adaptation of the downstream output to a step change in the upstream input.   | Switching of the downstream output may be possible, depending on the level at which the output of the covalent modification cycle saturates. A pulse input may produce a permanent response.                | A step change in the upstream input may switch on/off sustained oscillations, depending on the level at which the output of the covalent modification cycle saturates.                                 |
| <b>Monostable Switch</b>                 | <b>Covalent Modification Cycle</b>                                                                                                                               | <b>Adaptive Module</b>                                                                       | <b>Bistable Switch</b>                                                                                                                                                                                      | <b>Negative Feedback Oscillator</b>                                                                                                                                                                    |
|                                          | Switch-like change in the downstream output for a step input of sufficient magnitude.                                                                            | Close to exact adaptation of the downstream output to a step change in the upstream input.   | Switching of the downstream output may be possible, depending on the level at which the output of the upstream switch saturates. A pulse input may produce a permanent response.                            | A step change in the upstream input may switch on/off sustained oscillations, depending on the level at which the output of the upstream switch saturates.                                             |
| <b>Adaptive Module</b>                   | <b>Covalent Modification Cycle</b>                                                                                                                               | <b>Monostable Switch</b>                                                                     | <b>Bistable Switch</b>                                                                                                                                                                                      | <b>Negative Feedback Oscillator</b>                                                                                                                                                                    |
|                                          | Close to exact adaptation of the downstream output to a step change in the upstream input.                                                                       | Close to exact adaptation of the downstream output to a step change in the upstream input.   | A step or a pulse input upstream may produce a permanent switching of the downstream output, though the upstream output continues to be adaptive.                                                           | A step or a pulse input upstream produces transient oscillations/transient disruption of stable oscillations of the downstream output.                                                                 |
| <b>Bistable Switch</b>                   | <b>Covalent Modification Cycle</b>                                                                                                                               | <b>Monostable Switch</b>                                                                     | <b>Adaptive Module</b>                                                                                                                                                                                      | <b>Negative Feedback Oscillator</b>                                                                                                                                                                    |
|                                          | A step or a pulse input upstream may produce a permanent switching of the downstream output.                                                                     | A step or a pulse input upstream may produce a permanent switching of the downstream output. | The amplitude of the transient response in the downstream output, elicited by an upstream input, may vary significantly, depending on whether the input causes a switching in the upstream bistable module. | Possible scenarios include coexisting steady states and oscillatory states at a fixed input, and switching between steady states and oscillatory states induced by step/pulse variations of the input. |
| <b>Negative Feedback Oscillator</b>      | <b>Covalent Modification Cycle</b>                                                                                                                               | <b>Monostable Switch</b>                                                                     | <b>Adaptive Module</b>                                                                                                                                                                                      | <b>Bistable Switch</b>                                                                                                                                                                                 |
|                                          | If the upstream input is in the oscillatory regime, the asymptotic state of the output is oscillatory. The amplitude of oscillations depends on the input level. | Essentially similar to having the covalent modification cycle downstream of the oscillator.  | If the upstream input is in the oscillatory regime, the asymptotic state of the output is oscillatory. Any change in the mean of the oscillations upstream, is filtered out by the adaptation downstream.   | Possible scenarios include coexisting steady states and oscillatory states at a fixed input, and switching between steady states and oscillatory states induced by step/pulse variations of the input. |

Table I: Summary of results for sequential interactions without sharing, involving different combinations of modules. See text for details.



TABLE 2.2: Sequential interactions with sharing

| Upstream Module                          | Downstream Module                                                                                                                                                                                                                        |                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                      |
|------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                          | Monostable Switch                                                                                                                                                                                                                        | Adaptive Module                                                                                                                                                                      | Bistable Switch                                                                                                                                                                                                                                                                    | Negative Feedback Oscillator                                                                                                                                                                                                         |
| <b>Covalent Modification Cycle (CMC)</b> | Increased sequestration (lower catalytic constant of coupling reaction) may reduce the sharpness of the switching.                                                                                                                       | With increased sequestration (lower catalytic constant), the downstream output continues to exhibit adaptation, but the transient response, to a step input upstream, is diminished. | Increased sequestration (lower catalytic constant) distorts the range of upstream input over which the system is bistable, and may cause the bistable regime to become unattainable at finite values of the input. Bistable characteristics may be propagated upstream.            | The size and location of the oscillatory region, with respect to the upstream input, varies significantly as the extent of sequestration increases. Oscillations may be propagated upstream. See text for details.                   |
| <b>Monostable Switch</b>                 | <b>Covalent Modification Cycle</b>                                                                                                                                                                                                       | <b>Adaptive Module</b>                                                                                                                                                               | <b>Bistable Switch</b>                                                                                                                                                                                                                                                             | <b>Negative Feedback Oscillator</b>                                                                                                                                                                                                  |
|                                          | Increased sequestration of the output in the downstream CMC causes the magnitude of the switch to be lowered.                                                                                                                            | Essentially similar to having a covalent modification cycle upstream.                                                                                                                | Essentially similar to having a covalent modification cycle upstream.                                                                                                                                                                                                              | Essentially similar to having a covalent modification cycle upstream.                                                                                                                                                                |
| <b>Adaptive Module</b>                   | <b>Covalent Modification Cycle</b>                                                                                                                                                                                                       | <b>Monostable Switch</b>                                                                                                                                                             | <b>Bistable Switch</b>                                                                                                                                                                                                                                                             | <b>Negative Feedback Oscillator</b>                                                                                                                                                                                                  |
|                                          | Increased sequestration of the output in the downstream CMC causes both the steady state of the adaptive module, and the amplitude of the transient response to decrease.                                                                | Essentially similar to having a covalent modification cycle downstream.                                                                                                              | Bistable characteristics may be propagated to the adaptive module output. The back propagation effect becomes more significant as the extent of sequestration increases.                                                                                                           | If the basal level is oscillatory, oscillations may be propagated to the adaptive module output. The feedforward structure of the adaptive module prevents downstream oscillations being propagated to the other upstream variables. |
| <b>Bistable Switch</b>                   | <b>Covalent Modification Cycle</b>                                                                                                                                                                                                       | <b>Monostable Switch</b>                                                                                                                                                             | <b>Adaptive Module</b>                                                                                                                                                                                                                                                             | <b>Negative Feedback Oscillator</b>                                                                                                                                                                                                  |
|                                          | Increased sequestration of the output in the downstream CMC can distort upstream bistable characteristics, if the output variable of the bistable module belongs to a fixed pool. In such a scenario, bistability may even be destroyed. | Essentially similar to having a covalent modification cycle downstream.                                                                                                              | Even with sequestration, the adaptive module (due to its feedforward structure) can filter out the effects of bistability upstream. Increased sequestration reduces the amplitude of the downstream transient response elicited by an upstream input.                              | The extent of sequestration can determine the possibility of oscillatory behaviour and the possibility of coexisting steady states and oscillatory states, for a given input.                                                        |
| <b>Negative Feedback Oscillator</b>      | <b>Covalent Modification Cycle</b>                                                                                                                                                                                                       | <b>Monostable Switch</b>                                                                                                                                                             | <b>Adaptive Module</b>                                                                                                                                                                                                                                                             | <b>Bistable Switch</b>                                                                                                                                                                                                               |
|                                          | Increased sequestration of the output in the downstream CMC distorts oscillatory behaviour upstream, possibly even destroying oscillatory behaviour.                                                                                     | Essentially similar to having a covalent modification cycle downstream.                                                                                                              | If the upstream input lies in the oscillatory regime, the downstream output exhibits oscillations. As the extent of sequestration increases, the input range for oscillations becomes narrower, and oscillatory behaviour may be lost (similar to the effect of a downstream CMC). | Sequestration can result in a complex combination of bistability and oscillations, only oscillations, only bistability, or neither oscillations nor bistability.                                                                     |

Table II: Summary of results for sequential interactions with sharing of components, involving different combinations of modules. See text for details.



## Chapter 3

# Modelling compartmentalization

### 3.1 Introduction

Information processing, and indeed, most vital aspects of cellular life are underpinned by complex and sophisticated protein and genetic networks. These networks have many hallmarks which distinguish them from networks in non-living contexts: they are shaped by evolution and consequently not parsimonious in construction, they contain abundant nonlinearity including feedback regulation at many levels; they make use of different information processing modules; and finally are generally robust in function (Marks, Klingmüller, and Müller-Decker, 2008). In addition, due to small numbers of molecules, they are subject to stochastic fluctuations (Elowitz et al., 2002).

Dissecting these information processing networks, to connect their realization to their function in different contexts, is a major theme of systems biology. Engineering information processing towards various applications, or even towards a better understanding of the underlying biology is the goal of synthetic biology.

In spite of considerable strides in understanding the organization and functioning of these networks in a range of contexts, there are still a number of basic questions and issues which remain unanswered, and in some cases even unquestioned. One aspect that needs to be addressed is the spatial organization of pathways (Howard, 2014). It is well known that spatial organization of pathways, with components distributed between different locations: the membrane, the cytoplasm (in different locations therein), and membrane bound compartments such as organelles, vesicles, and the nucleus, is ubiquitous in both signaling (Mor and Philips, 2006; McCormick and Baillie, 2014; Gao et al., 2011; Doncic et al., 2015) and metabolic processes (Mintz-Oron et al., 2012; Sweetlove and Fernie, 2013). In fact, even bacterial cells, which lack organelles, exhibit precise spatial organization of processes (Thanbichler, 2009; Kiekebusch and Thanbichler, 2014), including

compartmentalization of metabolic pathways (Kerfeld, Heinhorst, and Cannon, 2010). While this spatial organization is generally recognized, its effect on the functioning of the pathways is generally not well explored. Very often, it is either explicitly or implicitly assumed that spatial organization has a minor role, and can be subsumed within a purely temporal (kinetic) description of the pathways. Compartmentalization is a hallmark of cellular pathways, and there have been attempts to incorporate this aspect in models of different intracellular signalling processes (Higgins et al., 2007; Eungdamrong and Iyengar, 2007; Doncic et al., 2015). In spite of this, there is still a vast chasm which needs to be bridged to effectively address this issue. In recent work, we developed a framework to understand the effect of compartmentalization on different types of canonical biochemical modification sequences and pathways (Alam-Nazki and Krishnan, 2015; Alam-Nazki and Krishnan, 2013).

A considerable impetus for understanding the effect of compartmentalization comes from trying to engineer spatial organization in synthetic biology and beyond. The use of proteins and nucleic acids as synthetic scaffolds for spatial organization of enzymatic modification pathways has been realized in multiple studies (Dueber et al., 2009; Sachdeva et al., 2014). Engineered microcompartments which localize the steps of a metabolic pathway have been suggested as a means of increasing pathway flux (Chen and Silver, 2012; Kim and Tullman-Ercek, 2013). Multi-compartment lipid vesicles (Elani et al., 2013; Elani, Law, and Ces, 2014) are an example of membrane bound compartments developed as a platform to regulate the functioning and behaviour of biochemical pathways. Here, particular steps of a metabolic cascade are localized in certain compartments, and the remaining steps in other compartments. The fact that only some entities can cross between compartments is an experimental basis for segregating reactions. Compartmentalization of genetic circuits in different cell free environments has also been investigated, including encapsulation by lipid vesicles (Nomura et al., 2003; Noireaux and Libchaber, 2004; Elani, Law, and Ces, 2015) and by compartments carved into a silicon chip (Karzbrun et al., 2014; Tayar et al., 2015). Finally, there have also been attempts to spatio-temporally control the location of entities, using optical or magnetic means (Levskaya et al., 2009; Lasker et al., 2014; Etoc et al., 2013).

In addition to the regular systems and synthetic biology motivations, compartmentalization is also of considerable interest from an evolutionary perspective (Diekmann and Pereira-Leal, 2013; Gabaldón and Pittis, 2015). It

is clear that there is a certain degree of localization/compartmentalization in bacteria, but that this is very much greater in eukaryotes. How this new feature is exploited in different cell types and to what end is still an open question. This of course depends on the type of pathway and the type of information processing. Assessing compartmentalization from this angle may provide vital clues into why pathways are organized the way they are. Finally, there are experimental studies which explore the origins of life, using vesicles to compartmentalize reactions and thereby capture the essential features of proto-cells.

Taken together, it is clear that compartmentalization is ubiquitous in natural systems, a key ingredient in evolution, and a very promising tool for engineering biochemical pathways in cells and cell free environments. Ultimately a clear understanding of the role of compartmentalization and an effective basis for engineering them, needs an appropriate modelling and systems framework. The choice of the modelling framework dictates what kinds of conclusions can be drawn, and whether they are sound and generalizable. Compartmental ODE models are a widespread framework for analyzing compartmental systems in the broadest sense. Even in studying compartmentalization, they are a natural choice as they allow for the modelling framework to focus on the key underlying and measurable features.

In this chapter, our goal is two-fold. On the one hand we investigate the effects of compartmentalization in a series of typical biochemical pathways. This includes building blocks for both signalling and metabolic pathways, and extensions thereof. On the other hand, we investigate the most appropriate modelling framework for describing these compartmental systems, and whether compartmental ODE models provide realistic depictions as well as reliable conclusions. Through a series of investigations we establish under which conditions these compartmentalized pathways can be described by compartmental models. Our study is relevant in both systems and synthetic biology. In systems biology the fact that pathways are compartmentalized is increasingly realized. On the other hand models in systems biology at all levels need simplifications, to focus on the key underlying aspects. We address when and if compartmental models can reasonably capture the effects of compartmentalization, and make sound conclusions and reliable predictions therefrom. In synthetic biology, the design of compartments and manipulation of pathways need sound and robust platforms for design, analysis and engineering. In this regard the choice of modelling framework must enable this in a transparent way, while respecting the underlying physics and

chemistry of the process.

This chapter is organized as follows. In the next section we outline the models we use and the approach we take. We then present various results of our study. We conclude with a summary and discussion of the results. Analytical results supporting the computations are presented in the Supplementary Information.

## 3.2 Models

In this section, we first describe the essential features of the compartmentalized biochemical reaction pathways that are of interest in this study. Next, we present the basic PDE framework that we use to model these systems, and describe the general form of the corresponding compartmental ODE descriptions. Following this, we discuss the criteria that we use to examine to what extent a compartmental ODE model is consistent with the system it attempts to describe.

### 3.2.1 Compartmentalized pathways with diffusive transport

The kinds of compartmentalized biochemical pathways of interest in our study share the following features:

- A spatial domain that consists of compartments separated by intermediate spaces.
- Reactions confined to one or more compartments (realised through the compartmentalization of corresponding enzymes or substrates).
- Transport of species between compartments by diffusion.
- No chemical reactions in the intermediate spaces.

The purpose of our study is to examine the capabilities of compartmental ODE models, as tools for understanding such compartmentalized systems of biochemical pathways. PDE descriptions of these systems form a reference point.

For simplicity, we restrict ourselves to a 1-D PDE framework, and assume a single diffusivity for each species in the entire domain. However, as we explain later, most of our insights continue to be relevant to systems in 2-D and 3-D.

### 3.2.2 1-D PDE description

For simplicity, we consider a system with two compartments. The framework is easily extended to systems involving more compartments. For a system with  $n$  species, the PDE description consists of a set of coupled reaction-diffusion equations, with the following general form:

$$\begin{aligned}
 &\text{In compartment 1 } (\theta \in \Omega_1), \\
 &\quad \frac{\partial X_j}{\partial t} = f_{j1}(X_1, X_2, \dots, X_n) + D_j \frac{\partial^2 X_j}{\partial \theta^2} \\
 &\quad \text{where } j = 1, 2, \dots, n \\
 &\text{Between compartments } (\theta \in \Omega_{12}), \\
 &\quad \frac{\partial X_j}{\partial t} = D_j \frac{\partial^2 X_j}{\partial \theta^2} \\
 &\quad \text{where } j = 1, 2, \dots, n \\
 &\text{In compartment 2 } (\theta \in \Omega_2), \\
 &\quad \frac{\partial X_j}{\partial t} = f_{j2}(X_1, X_2, \dots, X_n) + D_j \frac{\partial^2 X_j}{\partial \theta^2}
 \end{aligned} \tag{3.1}$$

Here,  $\theta$  represents the spatial variable and  $t$  represents time.  $X_j$  represents the concentration of species  $j$ , and  $D_j$  represents its diffusivity. The spatial domain  $\Omega$  is partitioned into 3 intervals -  $\Omega_1$  and  $\Omega_2$  representing the compartments, and  $\Omega_{12}$  representing the intermediate space between compartments. No-flux boundary conditions are used at the edges of the domain.

The function  $f_{j1}(X_1, X_2, \dots, X_n)$  represents the reaction kinetic terms for species  $j$ , associated with the reactions occurring in compartment 1, and similarly for the function  $f_{j2}(X_1, X_2, \dots, X_n)$ . If all the reactions are described by mass-action kinetics of first-order, the functions  $f_j$  will be linear. When the system involves closed steps, as is common in biochemical pathways, the PDE description implies a conservation of species. In such cases, the total amount of a conserved species needs to be specified when solving the for the steady states of the PDE.

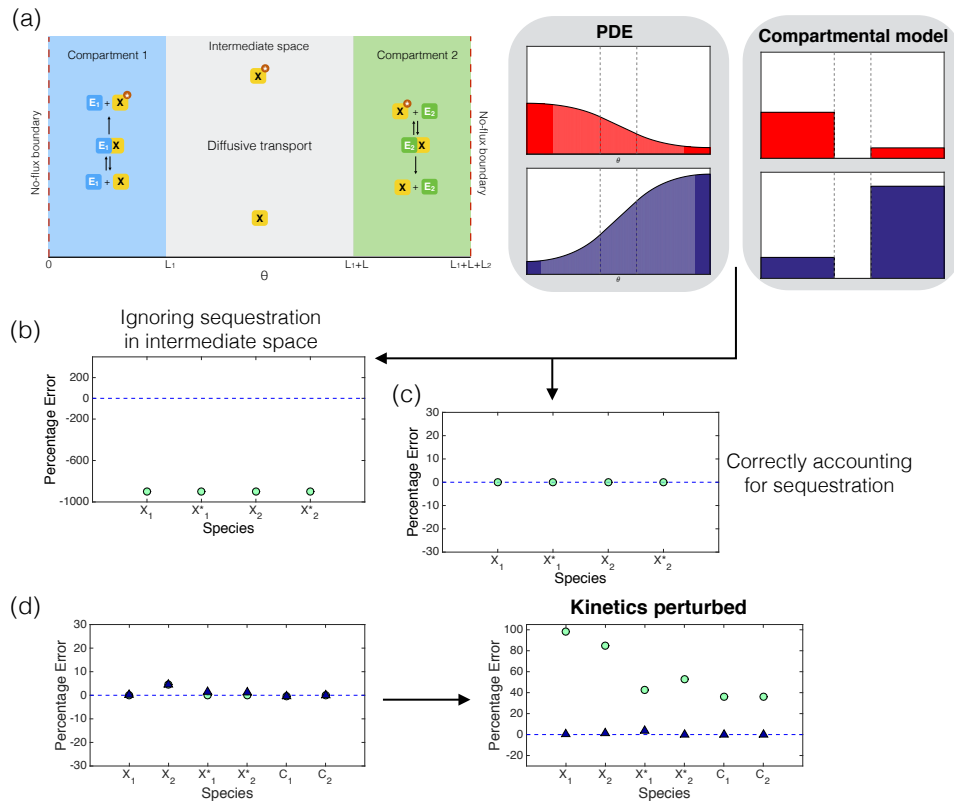


FIGURE 3.1: **(a)** Schematic for the compartmentalized pathway. The PDE description yields steady state concentration profiles of  $X$  and  $X^*$ . The compartmental ODE gives steady state compartmental concentrations of  $X$  and  $X^*$ . We examine the error between the PDE compartmental averages and the ODE steady state. **(b)** Percentage error for each species, between the steady state compartmental averages of the PDE model, and the steady state of a compartmental model using the same total amount of substrate as the PDE model. We consider a system with two compartments, each occupying 5 percent of the whole domain. **(c)** In the mass action regime, if the total amount of substrate in the compartments is exactly accounted for, transport parameters can be computed, which result in an exact match. **(d)** In the non-mass action regime, an exact fit cannot be obtained, even if the total amount of substrate in the compartments is accounted for. Errors are shown for both the basic compartmental model calibrated with the exact total amount of substrate in the compartments under basal conditions - green circles, and the compartmental model with modified conservation to account for amount of substrate in the intermediate space - blue triangles. For the basal kinetics, both cases match the PDE closely. When the kinetic parameter is changed (phosphatase concentration in compartment 2 increases), the modified model (blue triangles) continues to give a good fit, while the basic compartmental model with calibration under basal conditions (green circles) produces large errors.



The different biochemical pathways examined in this study, are described below (for a two compartment scenario). Readers not interested in the details may skip directly to the section on compartmental ODE models.

### Simple closed system: Single modification cycle

This is a closed system consisting of a single enzymatic modification cycle involving species  $X$  and  $X^*$ . Both species diffuse across the whole domain, and may have different diffusivities.  $E_1$ , the enzyme catalyzing  $X \rightarrow X^*$  is non-diffusing and uniformly distributed in compartment 1 (see Figure 1(a)).  $E_2$ , the enzyme catalyzing  $X^* \rightarrow X$  is non-diffusing and uniformly distributed in compartment 2. The enzyme substrate complexes,  $XE_1$  and  $X^*E_2$  (referred to as  $C_1$  and  $C_2$ ) are also non-diffusing. The total amount of substrate within the whole domain, consisting of free substrate ( $X$  and  $X^*$ ) and the complexes, is conserved.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= -k_1[X][E_1] + k_{-1}[C_1] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= k_2[C_1] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[C_1]}{\partial t} &= k_1[X][E_1] - k_{-1}[C_1] - k_2[C_1]\end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_4[C_2] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_3[X^*][E_2] + k_{-3}[C_2] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[C_2]}{\partial t} &= k_3[X^*][E_2] - k_{-3}[C_2] - k_4[C_2]\end{aligned}$$

These result in the conservation equations,

$$\begin{aligned} X^{Total} &= (\langle [X] \rangle + \langle [X^*] \rangle)l + \langle [C_1] \rangle_1 L_1 + \langle [C_2] \rangle_2 L_2, \\ E_1^{Total} &= (\langle [E_1] \rangle_1 + \langle [C_1] \rangle_1) L_1 \\ E_2^{Total} &= (\langle [E_2] \rangle_2 + \langle [C_2] \rangle_2) L_2 \end{aligned}$$

where  $\langle \cdot \rangle$  represents the spatial average over the whole domain, and  $\langle \cdot \rangle_i$  represents the spatial average over the compartment  $i$ .

### Simple open system

This is an open system involving a single species  $X$  that is produced in compartment 1 and degraded in both compartments 1 and 2.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\frac{\partial [X]}{\partial t} = k_0 - k_1 [X] + D_X \frac{\partial^2 [X]}{\partial \theta^2}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\frac{\partial [X]}{\partial t} = D_X \frac{\partial^2 [X]}{\partial \theta^2}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\frac{\partial [X]}{\partial t} = -k_2 [X] + D_X \frac{\partial^2 [X]}{\partial \theta^2}$$

### Two-site modification

This is a closed system involving interconversion between the species  $X$  and its modified forms,  $X^*$  and  $X^{**}$ , with the three forms belonging to a conserved pool. As in the previous models, both enzymes and complexes are non-diffusing. Enzyme  $E_1$ , uniformly distributed in compartment 1, catalyzes both the forward steps  $X \rightarrow X^*$  and  $X^* \rightarrow X^{**}$ , while  $E_2$ , uniformly distributed in compartment 2, catalyzes both the reverse steps.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= -k_1[X][E_1] + k_{-1}[C_1] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_3[X^*][E_1] + k_{-3}[C_2] + k_2[C_1] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= k_4[C_2] + D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2} \\ \frac{\partial[C_1]}{\partial t} &= k_1[X][E_1] - k_{-1}[C_1] - k_2[C_1] \\ \frac{\partial[C_2]}{\partial t} &= k_3[X^*][E_1] - k_{-3}[C_2] - k_4[C_2]\end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_8[C_4] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_7[X^*][E_2] + k_{-7}[C_4] + k_6[C_3] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= -k_5[X^{**}][E_2] + k_{-5}[C_3] + D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2} \\ \frac{\partial[C_3]}{\partial t} &= k_5[X^{**}][E_2] - k_{-5}[C_3] - k_6[C_3] \\ \frac{\partial[C_4]}{\partial t} &= k_7[X^*][E_2] - k_{-7}[C_4] - k_8[C_4]\end{aligned}$$

These result in the conservation equations,

$$\begin{aligned}X^{Total} &= (\langle[X]\rangle + \langle[X^*]\rangle + \langle[X^{**}]\rangle)l + \langle[C_1]\rangle_1 L_1 + \langle[C_2]\rangle_1 L_1 + \langle[C_3]\rangle_1 L_2 + \langle[C_4]\rangle_1 L_2, \\ E_1^{Total} &= (\langle[E_1]\rangle_1 + \langle[C_1]\rangle_1 + \langle[C_2]\rangle_1) L_1 \\ E_2^{Total} &= (\langle[E_2]\rangle_1 + \langle[C_3]\rangle_2 + \langle[C_4]\rangle_2) L_2\end{aligned}$$

### 3.2.3 Compartmental ODE models

A compartmental ODE description conceptualizes systems of the above form as two compartments with species transported between them. It represents

diffusive transport of species between compartments using a linear term, in which the difference in species concentrations between compartments is multiplied by a proportionality constant (assumed positive). The proportionality constant for the transport from compartment 1 to 2 is the associated transport parameter. The compartmental ODE model corresponding to the system described by equations (1), takes the following form:

For compartments 1 and 2,

$$\begin{aligned} V_1 \frac{dX_{j1}}{dt} &= V_1 f_{j1}(X_{11}, X_{21}, \dots, X_{n1}) - V_1 tr_{1 \rightarrow 2}^j X_{j1} + V_2 tr_{2 \rightarrow 1}^j X_{j2} \\ V_2 \frac{dX_{j2}}{dt} &= V_2 f_{j2}(X_{12}, X_{22}, \dots, X_{n2}) - V_2 tr_{2 \rightarrow 1}^j X_{j2} + V_1 tr_{1 \rightarrow 2}^j X_{j1} \end{aligned} \quad (3.3)$$

where  $V_i$  represents the volume of compartment  $i$  ( $i = 1, 2$ ) and the variables  $X_{ji}$  represent the concentration of species  $j$  in compartment  $i$ .  $tr_{1 \rightarrow 2}^j$  is the rate constant associated with the transport of species  $j$  from compartment 1 to compartment 2. The reaction kinetic terms, represented by the functions  $f_{ji}(X_{1i}, X_{2i}, \dots, X_{ni})$ , are the same as those in the PDE model. As in the case of the PDE description, for closed steps, the compartmental model implies a conservation condition, and the corresponding total amounts need to be specified when solving for the steady states of the compartmental ODE.

The corresponding compartmental ODE models, for the different pathways considered in this study, are presented in the Supplementary Information.

### 3.2.4 Evaluating compartmental ODE models

We examine compartmental ODEs to understand whether they can consistently describe systems of the form (1). We will primarily focus on steady state behaviour since this is the primary aspect of interest and is a natural first step of examination. In general, we can expect a certain mismatch in dynamic variation because of the differences in the way the transport is described.

We examine the quantitative correspondence between the ODE model and the PDE description, within the compartments, at steady state. We look for a quantitative match between steady-state species concentrations in the compartments in the compartmental ODE model, and the steady-state compartmental averages in the PDE description.

In any given instance, the effectiveness of a compartmental model is indicated by the degree to which the ODE steady-state matches the PDE spatial averages in the relevant compartments. One way of quantifying this is using a relative error, calculated as:

$$e_{X_{ji}} = \frac{|X_{ji}^{ss} - \langle X_j^{ss} \rangle_i|}{\langle X_j^{ss} \rangle_i}, \quad (3.4)$$

which gives the relative error produced by the compartmental ODE in describing the steady state concentration of species  $j$  in compartment  $i$ . Here,  $X_{ji}^{ss}$  is the steady-state concentration of species  $j$  in compartment  $i$ , as given by the ODE, and  $\langle X_j^{ss} \rangle_i$  is the steady-state spatial average of the concentration  $X_j$  in compartment  $i$ , as given by the PDE.

We write the equations in dimensionless form. We choose representative parameters which are sufficient for the purposes of our study. While quantitative results do depend on parameters, the main underlying conclusions do not, and are valid across multiple parameter sets. In this regard, we note the following points. (i) We have examined multiple regimes of enzymatic reactions, both mass action (no complex formation) and non-mass action (with enzyme-substrate complexes). (ii) We span a range of compartmental sizes, relative to the size of the domain. (iii) We examine a range of diffusion coefficients. We note that when the diffusivity becomes large, the concentration of diffusing species is essentially the same in both compartments. In such a special case, it may be possible to do without a compartmental description. (iv) We use analytical work to explain and consolidate these conclusions. Where possible, we use analytical solutions to explain the parameter dependence transparently. This is sufficient for the nature of the conclusions that we draw.

The PDE models are numerically solved in MATLAB (using `ode15s`), using a finite difference scheme, to find the steady-state concentration profiles of the different species. Spatial averages within the compartments and the intermediate space are then computed using a trapezoidal method. For the single modification cycle and the simple open pathway, analytical solutions are obtained in the limit where all the enzymatic reactions follow mass-action kinetics (see Supplementary Information).

### 3.3 Results

Compartmentalized reaction systems are routinely encountered in biology, and compartmental ODE models are used to describe such systems. In such settings, the reactions and their consequences are the main point of interest. Such models keep the focus on the compartments where reactions occur, by describing inter-compartmental transport in a simplified way (and focussing on average concentrations in the compartments). However, movement of species between compartments occurs through transport (diffusion, and other modes). This therefore brings up a number of questions. (i) When is a compartmental ODE model an accurate and reliable description of such a compartmentalized reaction scenario? (ii) How well does it work for closed reaction systems (as seen in signalling, with conservation of species) and open reaction systems (as seen in metabolism, which involves production and degradation of species). (iii) Does this change depending on the kinetic regime of reactions (mass-action regime, non mass action regime)? (iv) How do factors such as the relative sizes of the compartments and the intervening space affect this? (v) What consequence does this have for compartmentalized reactions in systems and synthetic biology, noting both the myriad additional complexities present in cellular systems, and potentially broad types of new designs in synthetic biology? Addressing such questions demands a systematic systems investigation, which we undertake below.

Our goal is to investigate compartmentalization in canonical building blocks of signalling and metabolic pathways, focussing on when and to what extent compartmental ODE models are adequate for this. We present the investigation in three stages: (i) investigation of simple open and closed pathways, (ii) investigation of more complex closed pathways, which are building blocks for signalling cascades, and (iii) an expansion of (i) to examine a basic design problem involving compartmentalization of metabolic reactions. In all these cases, we consider reactions compartmentalized in two compartments and employ PDE and compartmental ODE models as described above.

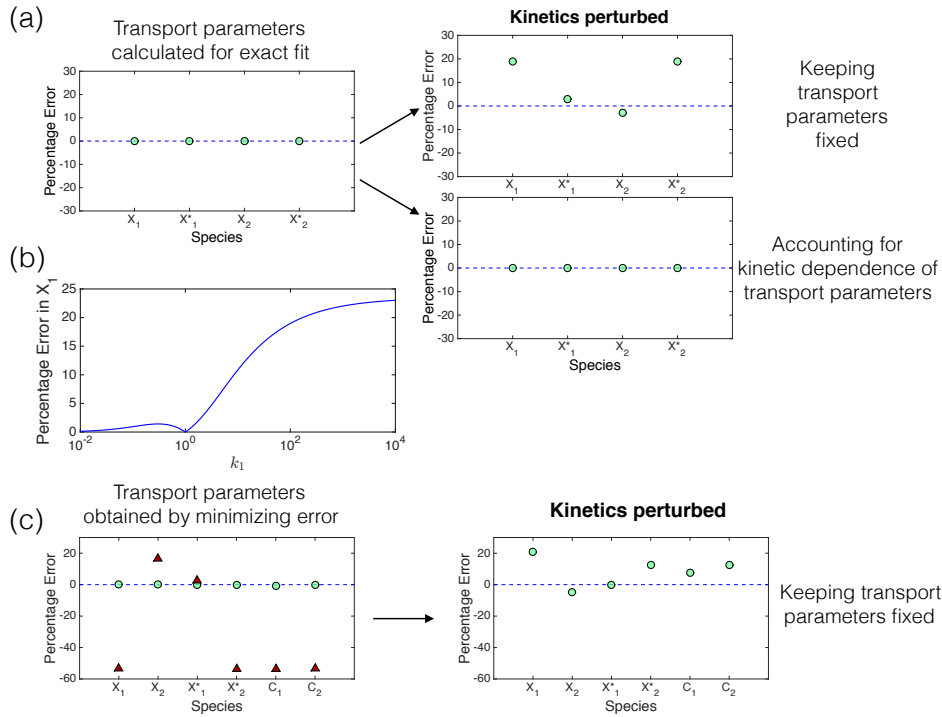


FIGURE 3.2: The transport parameters that produce an exact match depend on kinetic parameters (non-thin compartment regime). **(a)** In the mass action regime, transport parameters can be computed, which can guarantee an exact match, provided that sequestration in the intermediate space is exactly accounted for. However, this exact match is not robust to changes in kinetics, even if sequestration is correctly accounted for. On the other hand, if we account for the kinetic dependence of the transport parameters, the compartmental model continues to produce an exact fit. **(b)** The percentage error in  $X_1$  (average concentration of  $X$  in compartment 1) is zero at the basal value of kinetic parameter  $k_1$ , and increases when  $k_1$  is perturbed. **(c)** Away from the thin compartment regime,  $D/LL_1$  is a poor choice of transport parameter (triangles). In the non-mass action regime, an exact fit is impossible, but transport parameters may be computed by minimizing the error (sum-squared error). Transport parameters obtained by minimizing the error produce a good match (green circles - maximum error 0.6 percent). However, for these transport parameters, the ability to match the PDE is lost if the kinetics is perturbed.

We note that, for any biochemical pathway that we study, the kinetics and compartment sizes are common to both types of models. With regard to transport, diffusion is explicitly described in the PDE model, while the compartmental model has transport parameters. For each species, there are

two transport parameters in principle, describing transport between the compartments in both directions. As employed in the literature, these transport parameters are equal if the volumes of the compartments are equal. We will examine this transport parameter in our analysis.

## Simple open and closed systems

In this subsection, we focus on simple reactions (both closed and open) which are building blocks for signalling and metabolic pathways. We consider (i) A covalent modification cycle and (ii) A single reaction with production and degradation. In each case, reactions are compartmentalized. We wish to assess compartmental ODE models of such systems. In so doing, we span a range of compartment sizes (relative to the intervening space), from the thin compartment regime (small compartment size relative to intervening space) to the non-thin compartment regime. Spanning this entire range allows us to tease out multiple factors at play. We also examine the consequences of kinetic regimes, such as a mass-action kinetic regime and a non-mass action kinetic regime. Finally, since the goal of a model is to be able to make new predictions and provide new understanding, we examine the role of kinetic parameters in assessing the reliability of compartmental ODE models.

**Simple closed systems.** We start by examining a sample closed system: a covalent modification cycle, with phosphorylation and dephosphorylation in two compartments, with modified and unmodified species diffusing between compartments (see Fig. 1). Such scenarios are seen in multiple cellular contexts, including bacterial pathways where the two compartments represent the two poles of the bacteria (Kiekebusch and Thanbichler, 2014). We start the system with a specified amount of kinase and phosphatase in the respective compartments, and (for specificity) all the substrate in one of the compartments. For simplicity we assume mass-action kinetics of the enzymatic modification, and examine other cases subsequently. Appropriate transport parameters for the compartmental models are chosen (discussed in detail later). We wait till the system reaches a steady state. As seen in Fig. 1(b), there is a substantial mismatch between the steady state of the compartmental model and steady state average concentrations of the PDE.

A careful examination of both models indicates why this is the case. In the PDE model, both substrates are present not only in the compartments, but also in the intervening space. Substrates are subject to a conservation condition (since there is no *de novo* production or degradation of substrate



in the relevant timescales), and this is implicit in both models. However, the compartmental model does not accurately describe the conservation of substrate. This is the reason for the mismatch. The greater the separation between compartments, the greater the mismatch in general.

**Populating the compartmental model with correct total species amounts.**

Noting the source of the disparity between the models, there are different ways to reduce or remove this disparity. One way is to populate the compartmental model with a total amount of substrate which matches the total amount of substrate in the two compartments in the PDE solution. For instance, this information could be obtained from an experiment and used in the compartmental model. As shown in Fig. 1(c), using this approach (and an appropriate choice of transport parameters), we are able to obtain an exact match. Now if the modification kinetics are not mass-action, the same approach can be used. Here, for fundamental reasons, the compartmental model can never exactly match the PDE model: this is because of the presence of complexes and the associated nonlinearity in the kinetics. The presence of nonlinear kinetics necessarily implies that compartment averages of substrate and complex concentrations in the PDE cannot match that of the compartmental models. This is discussed analytically in the Supplementary Information. Indeed, changing kinetics of modification away from mass action (keeping transport parameters fixed) does lead to a degree of mismatch. This implies, that the best one can achieve with a compartmental model, is to reduce this mismatch and get as close as possible to the PDE model (for average species concentrations). The only flexibility in the compartmental model resides in the transport parameters. We can vary the transport parameters of the modified and unmodified substrate to see how close a match can be obtained. Numerically minimizing the error (see Supplementary Information) indicates that there are transport parameters which can give a very small error (discussed later). In multiple cases considered, the transport parameters for the species, happen to be in the ratio of their diffusivities. Further aspects about transport parameters are discussed later.

**Lack of kinetic robustness.** Now if we use the above approach to populate the compartmental model with the correct total amount of substrate from the PDE model (exact fit for mass action kinetics, inexact for non-mass action kinetics), we can use this compartmental model in a predictive way. We examine the response of the model to a change of kinetic parameters, which would be a natural use of such a model. How good are the predictions? We find that there is an increasing error between the compartmental

model prediction and the PDE model, as the kinase concentration is changed from basal levels (Fig. 1(d): green circles). This suggests that even with the correct total amount of substrate, compartmental models may be subject to non-trivial levels of error when used predictively. We refer to this as kinetic non-robustness of the compartmental model. It is instructive to examine the reasons for this error. To do this, we examine two specific regimes.

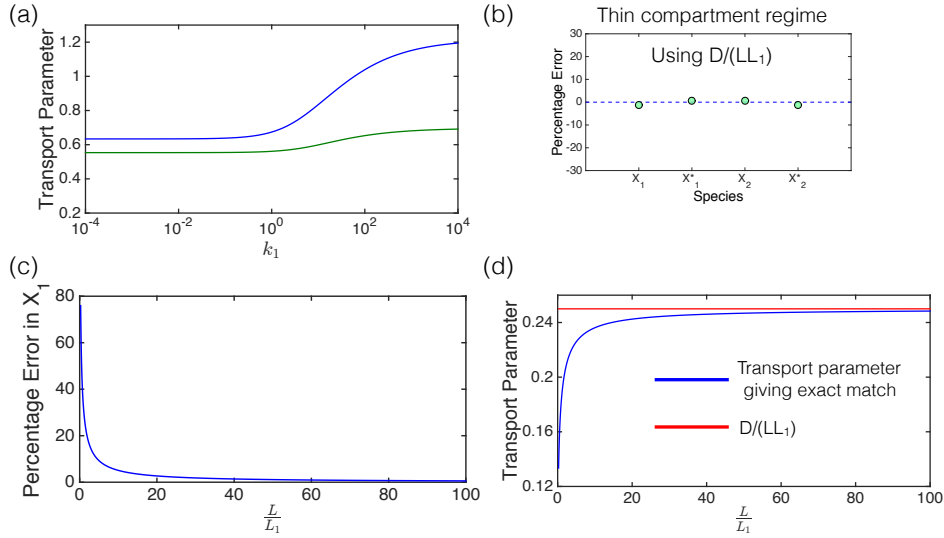


FIGURE 3.3: Kinetic dependence of the transport parameters in the mass action regime. **(a)** The transport parameters that produce an exact match between the compartmental model and the PDE, depend on kinetics (see text). For a given change in kinetics, the corresponding variation of transport parameters depends on compartment sizes relative to the intermediate space—green line:  $\frac{L}{L_1} = 1$ ; blue line:  $\frac{L}{L_1} = 0.1$ . **(b)** Using  $D/LL_1$  as the transport parameter produces a good approximation in the thin compartment regime (the maximum error here is about 1 percent). **(c)** Using  $D/LL_1$  as the transport parameter, away from the thin compartment regime (small  $L/L_1$ ), can result in a significant mismatch between the compartmental model and the PDE model at steady state. **(d)** The effect of varying  $L$  and consequently  $L/L_1$ , keeping  $D/L$  constant. Away from the thin compartment regime (small  $L/L_1$ ), the transport parameter that produces an exact match differs significantly from  $D/LL_1$ . It approaches this value as  $L/L_1$  becomes large (i.e. as the compartment sizes become smaller relative to the intermediate space).

**Thin compartment: The effect of sequestration.** If the size of the compartment is small relative to the size of the domain, then the transport parameters are determined as  $D/LL_1$  where  $D$  is the diffusivity of the species,

$L$  is the length of the intervening space and  $L_1$  the size of the compartment (see schematic in Fig. 1, and analytical discussion in Supplementary Information). With regard to sequestration, we note that the total amount of substrate in the compartments can, in general, vary with the kinetic parameters. Therefore, the assumption of a fixed total amount of substrate in the two compartments will contribute increasing errors when kinetic parameters are changed. This aspect can however, be exactly accounted for analytically: we can determine the amount of substrate species in the intervening space. The amount of species in the intervening space is equal to the average of edge concentrations of the species (right edge of first compartment and left edge of the second), multiplied by the length of the intervening space. This is determined analytically (see Supplementary Information). Now, in the thin compartment regime, the edge concentrations of species in a compartment are practically equal to the average concentration of the species in the compartment. This means that we can account for sequestration through an analytical equation, which depends only on compartmental concentrations. This very substantially improves the predictive capability of the compartmental model (Fig. 1(d)). Furthermore, since the conservation condition is explicit in terms of model variables, this modified compartmental model can easily be used for sensitivity and bifurcation analysis. Note that the factor which prevented an exact match of the compartment model with the PDE model in the non-mass action kinetic regime (errors introduced in averages through nonlinearity), becomes insignificant in the case of thin compartments, and so we expect this augmentation to be effective in both mass-action and non-mass action cases.

**Non-thin compartment: A new factor emerges.** In this case we note that the transport parameter associated with a species is not  $D/LL_1$ . As we have seen above, there is an exact match (mass-action kinetics) and close to exact match (non-mass action kinetics) for basal kinetic parameter values (having calibrated the model by accounting for sequestration in the intermediate space under basal conditions). When the compartmental model is used in a predictive way through variation of kinetic parameters there is a steadily increasing error (see Fig. 2(a,b)). Sequestration does contribute to this, as seen above. If we adopt the approach above to systematically account for sequestration, we find that, while the amount in the intervening space can be written in terms of compartmental edge concentrations of species, this cannot in general be written exactly in terms of average compartmental concentration. Therefore, there will be a level of approximation associated with

this approach to accounting for sequestration.

If we examine a special case where the intervening space is much smaller than the compartmental size, we expect the effect of this sequestration to be minimized. Interestingly even here, the model shows kinetic non-robustness, with a steadily increasing error as kinetic parameters are changed (this is true even when the kinetics is mass-action). Thus there has to be another contributing factor. This factor is in fact, the transport parameter.

**What the transport parameter represents.** Examining the PDE model in the case of mass action kinetics, and averaging over compartments, indicates that compartmental average concentrations at steady state do satisfy a compartmental-type equation (see Supplementary Information). In other words, the actual average concentrations in the PDE can emerge as the steady state of the compartmental ODE model. This establishes a correspondence between the PDE model and the compartmental ODE model. What emerges from this correspondence, however, is the fact that in such a case, the transport parameter in the corresponding ODE model demonstrates a clear dependence on both diffusivity and kinetic parameters. This means that when kinetic parameters change, so do the transport parameters. In fact, we determine transport parameters which are applicable across the spectrum of compartmental sizes and reduce to  $D/LL_1$  in the thin compartment case (see Supplementary Information). Incorporating the kinetic dependence of the transport parameter, results in a substantially reduced error (which would be zero if sequestration was exactly accounted for). Additionally, if the intervening space is small, the effect of distortion due to sequestration is also minimized, and in this case, the compartmental model with kinetically-dependent transport parameters provides a good approximation, and the error is close to zero. It is worth pointing out that the transport parameters of different species, determined as above, are in the ratio of their diffusivities, even though the transport parameter is not proportional to the diffusion coefficient. We also note that the transport parameter is in general not directly proportional to  $1/L$  either. The variation of the transport parameter with kinetics, and the error incurred in using a transport parameter  $D/LL_1$  is depicted in Fig. 3(a) and 3(c).

In the non-mass action kinetics regime, while the correspondence of the PDE with a compartmental ODE is not exact, the transport parameters that produce the closest match show a clear dependence on kinetics, echoing the mass-action case. There are two ways to account for this kinetic dependence: one is to do this computationally. This then makes the compartmental model

solution as involved as solving the PDE. The other option, which is approximate, is to use transport parameters and their dependence on kinetic parameters, from the mass action regime.

Taken together, by making a correspondence between the PDE model and the compartmental ODE model, we find that in the mass action kinetics case, the average compartmental concentrations of species in the PDE emerge exactly as the steady state of the compartmental ODE model, provided that (i) the transport parameters have a particular dependence on kinetics (and diffusivities and sizes), which can be determined, (ii) the effect of sequestration is exactly accounted for.

**Open reaction systems.** For completeness, we also consider a sample open reaction pathway, with production of a substrate ("inflow") in one compartment, and degradation "outflow" in both the first and second compartments (Fig. 4(a), (b)). In this case, there is no conservation condition, and so sequestration does not play a role. Exactly as seen above, in the thin compartment case, the transport parameter of a species is  $D/LL_1$ . In the non-thin compartment case, for mass action kinetics (of degradation) the transport parameter can be determined as a function of kinetic parameters (though it is independent of the rate of production), diffusivity, and sizes of the compartments and the intervening space. This can be done analytically, and is discussed in the Supplementary Information. Therefore in this case, the model using the modified transport parameters can be used to obtain an exact match.

**Comments on sequestration.** A number of compartmental models employed in the literature, including ones which implicitly work in the thin compartment limit (using a transport parameter  $D/LL_1$ ), do not systematically account for sequestration. In fact the effects of sequestration are strongest in the thin compartment regime. Our analysis above shows how this can be accounted for at steady state analytically, while still keeping the focus on the reaction compartments. It is possible to introduce the intervening space as another compartment, and arrive at the same result, through this expanded model (the equivalence of the two approaches is demonstrated in the Supplementary Information). We note, in passing, that in certain special cases (Mass action kinetics, equal diffusivities), the total amount of species in the compartments can be determined directly (see Supplementary Information). One aspect common to both the 1-D case and higher dimensions is the fact that focussing only on the reaction compartments (rather than introducing the intervening space as another compartment) allows for greater

conceptual simplicity, while accounting for sequestration. Note that having additional compartments requires the computation of additional inter-compartmental transport parameters in all these cases. Incidentally, it is worth pointing out that in 2-D, when the number of reaction compartments is greater than 2, introducing the intervening space as another compartment may lead to a simplification of the model structure.

**Comments on the transport parameter.** Our analysis in the mass-action kinetic regime demonstrates a steady state correspondence between the PDE model and a compartmental model with transport parameters having kinetic dependence (which can be determined). This has multiple important consequences. Errors introduced by using a compartmental ODE model with fixed transport parameters can be seen by comparing it with another ODE model of the same type, thus explicitly revealing the source of the disparity. The mass-action regime allows us to explicitly lay bare a factor at play, which is equally relevant in the non-mass action regime. The factor responsible for the disparity is the fundamental reason for the degradation in predictive capacity of such a model when kinetic parameters are changed, and is revealed explicitly through our analysis. Another qualitative effect explicitly revealed by the kinetic dependence of transport parameters in the “corresponding” ODE model, is discussed in the next section.

**Relevance to Systems and Synthetic Biology.** We note that the biochemical modules studied above are basic building blocks of complex pathways in systems biology. Sequestration in particular has been a recurring theme in the context of signalling, in the past decade (Blüthgen et al., 2006; Ventura, Sepulchre, and Merajver, 2008). The fact that the transport parameter (which may guarantee an exact match) is not constant (even with mass action kinetics) indicates a fundamental trade-off in modelling: choosing a simpler framework necessarily needs non-constant transport parameters to guarantee accuracy, away from the thin compartment regime. Further, in both systems and synthetic biology there is an interest in the effect of multiple “levers” in affecting pathways. Thus it is clear that having a description which is not robust to changes in kinetic parameters is fundamentally limiting. Finally depending on the context, either the regime of thin compartments (eg: poles of bacteria) or the regime of relatively large compartments separated by small intermediate spaces (calcium microdomains (Higgins et al., 2007), multi-compartment vesicles) may be encountered.

### 3.3.1 Extensions to more complex systems

Having studied the behaviour of compartmental ODE models of simple open and closed systems, we expand our study to a range of more complex scenarios with a view towards building a bridge towards applications in systems and synthetic biology. To that end we study (i) A two tier enzymatic modification cascade, which is an extension of a covalent modification cycle (ii) The effect of introducing additional compartments, as an example of a modular strategy of spatial organization in synthetic biology (iii) Multisite substrate modification, which is another distinct extension of covalent modification cycles (iv) A pathway design problem relevant to both metabolic engineering and synthetic biology. Each of these scenarios presents a new context/application, while also revealing new consequences of the effects studied in the previous section.

**A two tier cascade.** We now build on the above results to examine slightly more complex reaction systems. Thus far we have considered only a single tier of a modification cascade. We now briefly examine what happens when we have multiple stages of modification. To do this, we consider a two stage closed system, where the first modification tier occurs through mass action kinetics, and the second tier is completely localized in the second compartment (Fig. 4(e)). The modified substrate of the first tier, serves as an enzyme (kinase) for the second tier. This is representative of biological settings where downstream pathways are activated by the modified substrate at the first stage. It is already known that a particular modification stage can affect a stage above it, an effect referred to as retroactivity. This in turn, arises because some of the substrate species in the first tier is sequestered in a complex downstream.

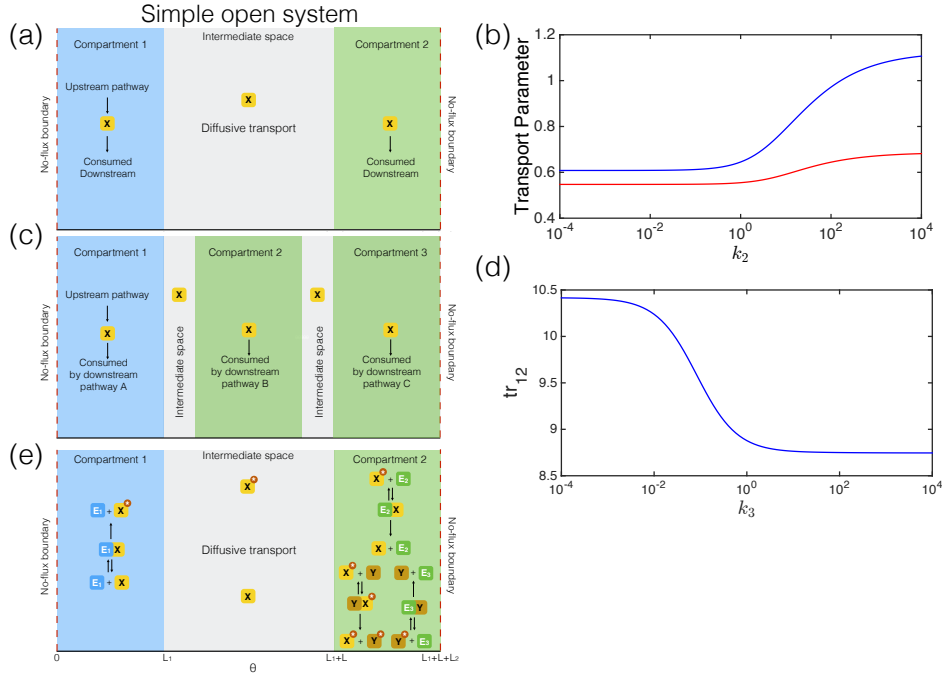


FIGURE 3.4: Simple open system, and pathways with added complexity. **(a)** Schematic for a simple open system with production in the first compartment and degradation in both first and second compartments. **(b)** For a given change in kinetics, the corresponding variation of transport parameters depends on compartment sizes relative to the intermediate space- green line:  $\frac{L}{L_1} = 1$ ; blue line:  $\frac{L}{L_1} = 0.1$ . **(c)** Open system with  $X$  produced in compartment 1 and consumed in compartments 1, 2, and 3. **(d)** Using the analytical solution to the PDE model, we see that modulating the kinetics in compartment 3, can alter the transport parameter associated with transport of  $X$  between compartments 1 and 2 ( $tr_{12}$ ). **(e)** Schematic for the compartmentalized two-step cascade.

As before we look at two regimes.

**Thin compartment.** In this case, the transport parameters are  $D/LL_1$  (the same analytical calculation holds good), and the additional sequestration of the first tier output in the second tier of the cascade is accounted for by describing the second tier modification in the compartmental model. The essential insight is that profiles of all species within a given compartment are practically flat. This means that (i) sequestration in the intermediate space can be easily accounted for (ii) the nonlinearity associated with the sequestration downstream leads to negligible error. Therefore, the compartmental model can achieve a close match to the PDE model with regard to the diffusing species, and consequently also for the output of the cascade.



**Non-thin compartment.** Here, as noted earlier, the transport parameters for the diffusing species are no longer  $D/LL_1$ . Looking at the first stage of the modification, we see that the only change introduced by the second tier modification, is to reduce the total amount of substrate species in the first step, due to an additional sequestration. Now, as discussed above, the transport parameters (i.e transport parameters in the compartmental ODE model which would allow for an exact match with the PDE model) of the diffusing species in the first stage of the modification depend on diffusivity, compartment sizes, size of intervening space, but not the total amount of species. Thus the transport parameter remains exactly the same. In other words, through this choice of transport parameters, and exactly accounting for sequestration of the species of the first tier, both in the intervening space and downstream, one can ensure that the compartmental ODE model exactly matched the PDE model in the first tier. In this case, there is however, a certain degree of error in analytically trying to account for the sequestration, both in the intermediate space (since we cannot approximate edge concentrations by averages) and in the second tier (due to the nonlinear kinetics). Both of these can be traced to the fact that concentration profiles are not uniform in a compartment.

The essential insight from the above is that the effect of adding a second tier of modification localized in one of the compartments has two consequences: (i) The transport parameters of the diffusing species (which move between compartments) in the “corresponding” compartmental ODE model, is unaffected by the second tier reaction. This is relevant in many biological contexts, where the output of one stage may be involved in triggering many pathways downstream (ii) There is an effect of sequestering of the output of the first tier downstream. As long as the downstream reaction is not in the mass action regime, the second tier reaction introduces a nonlinearity which prevents an exact match between the compartmental model and the PDE.

**A modular approach to compartmentalization.** In trying to design metabolic pathways, it is natural to attempt a modular design through spatial organization. We examine such a case here, building on the simple open system examined previously. We noted that it was possible to obtain a compartmental description of this system, even when the compartments were not thin. We now consider an augmentation of the original system, with an extra compartment downstream (where the substrate degrades). Analyzing this system exactly as before shows that it is possible to obtain a compartmental description similar to that above. Strikingly, here the transport parameter

(which guarantees an exact match with the PDE) associated with transport between the first and second compartments is actually altered by the kinetics in the third compartment (Fig. 4(d)). This illustrates that adding a compartment downstream can actually affect transport parameters upstream, even though it does not really affect the transport upstream. This further highlights how the transport parameter in general depends on both kinetics and diffusivity, and demonstrates that global changes in kinetics can effect local transport parameters.

The essential insight here is that since transport parameters (i.e. those which can guarantee exact matches with the PDE) in general depend on kinetics (of all reactions), a change in the kinetics in the third compartment can affect the transport parameters associated with the first and second compartments. This follows from our study of the previous section. This occurs generally as long as (i) the system is not in the thin compartment regime (transport parameters would be independent of kinetics) and (ii) the diffusivities are not large for the length scale of the system (otherwise spatial gradients would not be sustained). Both conditions can occur in a wide range of contexts.

**Multisite modification: Transport parameters and diffusivity.** We now examine a second type of complexity in the reaction pathway: multisite phosphorylation of substrate by a kinase-phosphatase pair. In contrast to the simple covalent modification cycle considered above, we have two possible modifications, and consequently three substrate species which cycle between compartments (Fig. 5(a)). This represents a different kind of extension of a covalent modification cycle: one where there are additional cycles of substrate modification. Analyzing this system (and obtaining a correspondence between the PDE model and compartmental ODE models) shows that we can obtain a compartmental model which exactly matches the PDE at steady state, when modifications are through mass action kinetics. The transport parameters, in such a case, in general can depend on the kinetic parameters, but interestingly, the transport parameters of different species are not in the ratio of their diffusivities. For instance, as shown in the table in Fig. 5(b), equally diffusing species have different transport parameters. This insight, which stems from the influence of kinetics on transport parameters, is valid for other reaction systems as well.

**Compartmental averages and the direction of transport.** Our analysis of the multisite phosphorylation pathway above reveals a new feature hitherto not encountered. Even when the kinetics is in the mass-action regime (and

even when diffusivities of species are equal), we find (see Fig. 5(c)) that non-monotonic concentration profiles are possible within a compartment. This has the important consequence that there can be a significant difference between compartmental edge and average concentrations. Furthermore, the difference between (nearest) edge concentrations of two compartments may not even have the same sign as the difference in average concentrations (Fig. 5(d)). Diffusive transport in the intervening space is driven by the difference in edge concentrations, while transport is conceptualized in the compartmental model in terms of differences in average concentrations. This has the consequence that in such situations the transport parameter (to exactly fit the PDE solution) has got to be negative. In other words, populating a compartmental model with positive transport parameters will be guaranteed to lead to a mismatch. This is because it is fundamentally incapable of capturing both average concentrations and the direction of transport simultaneously, for certain species. Building on this, we find that it is possible for (a) nearest edge concentrations to be the same, while average concentrations are different, in which case the transport parameter is zero (b) nearest edge concentrations are different while the average concentrations are the same, in which the transport parameter (which guarantees a match with the PDE) diverges. Taken together this shows that there may be fundamental limitations to compartmental ODE models exactly matching the PDE model, if the transport parameters are positive. We note in passing that having transport parameters being negative could lead to unphysical behaviour in dynamic simulations such as negative concentrations.

**Multistability in compartmentalized biochemical pathways.** Multisite phosphorylation systems have attracted a lot of attention, because of their potential to exhibit complex information processing characteristics, such as bistability (Ortega et al., 2006; Thomson and Gunawardena, 2009). When we consider multisite phosphorylation in thin compartments, in a kinetic parameter regime which allows for bistability, we readily find two different steady states (Fig. 5(e)). Note that these two steady states do not have the same total amount of substrate in the two compartments. This means that any ad hoc, or even experimentally based calibration of the compartmental model to account for sequestration, will necessarily fall short in capturing the two steady states (even in a relatively thin compartment) (Fig. 5(f)). On the other hand, in this case, using the analytically augmented sequestration condition, allows for both steady states to be accurately captured.

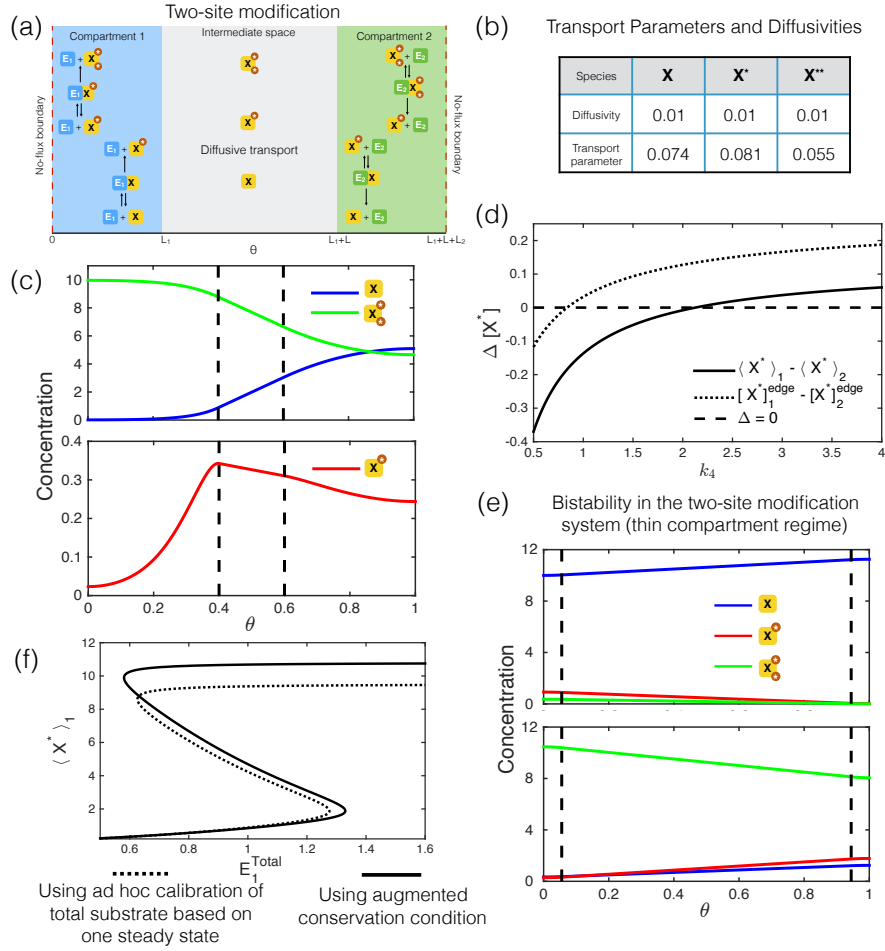


FIGURE 3.5: Multi-site modification. **(a)** Schematic for compartmentalized distributive two-site modification pathway (kinase in compartment 1 and phosphatase in compartment 2). **(b)** With multiple diffusing species in a pathway (two-site substrate modification: see text), their associated transport parameters need not be in the same ratio as their diffusivities, even when the species belong to the same conserved pool. In **(c)** and **(e)**, where steady state concentration profiles are shown, the dashed lines mark the compartment boundaries. **(c)** An example of steady state concentration profiles where  $[X^*]$  is clearly non-monotonic, and the difference in edge concentrations and the difference in average concentrations between compartments, have opposite signs. Note that the maximum of  $[X^*]$  is inside compartment 1 and not on the boundary. **(d)** Plot showing how the difference in average concentrations and the difference in edge concentrations vary with kinetics (rate constant  $k_4$ ). Notice that they have opposite signs over a certain range of  $k_4$ . **(e)** The two-site modification pathway shows bistability with compartmentalized enzymes (The system considered is in the thin compartment regime:  $\frac{L}{L_1} = 18$ ). Spatial concentration profiles of the modified forms, for the two different stable steady states are shown. **(f)** Comparing a compartmental description that systematically accounts for sequestration in the intermediate space with another which is calibrated to account for total amount of species in the compartments in an *ad hoc* manner (the calibration is based on one of the two steady states at basal conditions:  $E_1^{Total} = 0.6$ ). We find that the model with *ad hoc* calibration introduces significant errors in capturing the other steady state. In fact, under basal conditions, the model with *ad hoc* calibration does not even exhibit a second steady state.

Another aspect of bistability is worth highlighting in the context of compartmental systems. In our earlier study (single covalent modification, non-mass action kinetics, non-thin compartment) we noted that a compartmental model would not exactly match a PDE model. We demonstrated that we could obtain transport parameters using an optimization approach, to realize a “best fit”. When there are potentially multiple steady states, getting the transport parameters to best fit a given steady state in the compartmental model with that in the PDE may still allow for the possibility of other steady states (though this is not guaranteed). However with this choice of transport parameters, the second steady state of the compartmental model may not optimally match that of the PDE (or even be close). This means that if a single set of transport parameters is used with a desire to accurately obtain both steady states, then the best fit criteria must necessarily simultaneously incorporate both steady states.

The essential insights from our study of the multisite substrate modification are as follows. (i) With two species diffusing (i.e a single covalent modification cycle, mass action regime, studied earlier), the transport parameters of the “corresponding” ODE model are in the ratio of their diffusivities. The number of species in this case places a mathematical constraint which ensures this. When more than two species diffuse (same regime), this constraint is removed, and transport parameters are no longer in the ratio of their diffusivity. (ii) Non-monotonicity in concentration profiles can result in a qualitative mismatch between differences in (nearest) edge concentrations and differences in average concentrations of two compartments. This can occur even in the mass action regime. Consequently a compartmental model with positive transport parameters will result in a mismatch with the PDE. The conceptualization of transport being driven by differences in average concentrations can result in significant errors. (iii) There is considerable interest in the capacity of multi-site modification systems to generate bistability and sequestration plays a central role (even when the system is localized). Consequently, a compartmental model which does not correctly account for sequestration is liable to make incorrect inferences regarding this.

**Exemplar pathway design problems.** A particular interest in compartmentalization comes from metabolic engineering, where compartmentalization (for example manipulating or reorganizing pathways within naturally available compartments) offers a potentially important tool for manipulating metabolic systems (Zecchin et al., 2015). We examine two exemplar cases related to this. In the first case, a metabolite is produced in one compartment,

diffuses to the other and is consumed in the second compartment (an undesired pathway). A enzyme (of fixed amount) is localized in one of the compartments resulting in the conversion of the metabolite to a desired product there. The goal is to determine, which compartment the enzyme  $E$  should be targeted to, to maximize the flux of the desired conversion. Questions of this kind have been examined in multiple instances in the literature (Hinzpeter, Gerland, and Tostevin, 2017). As seen in Fig. 6(b), a choice of transport parameter  $D/LL_1$  results in a conclusion which is opposite to that suggested by the PDE model. On the other hand, incorporating the effects of the basal production/transport/consumption in the transport parameters of the compartmental model, results in the right conclusion. This shows how a plausible but incorrect choice of transport parameters can qualitatively affect the result. We note that the second reaction (desired pathway mediated by the enzyme) will affect the transport parameters as well. For the greatest accuracy, it may be necessary to incorporate the effects of this pathway in the transport parameter.

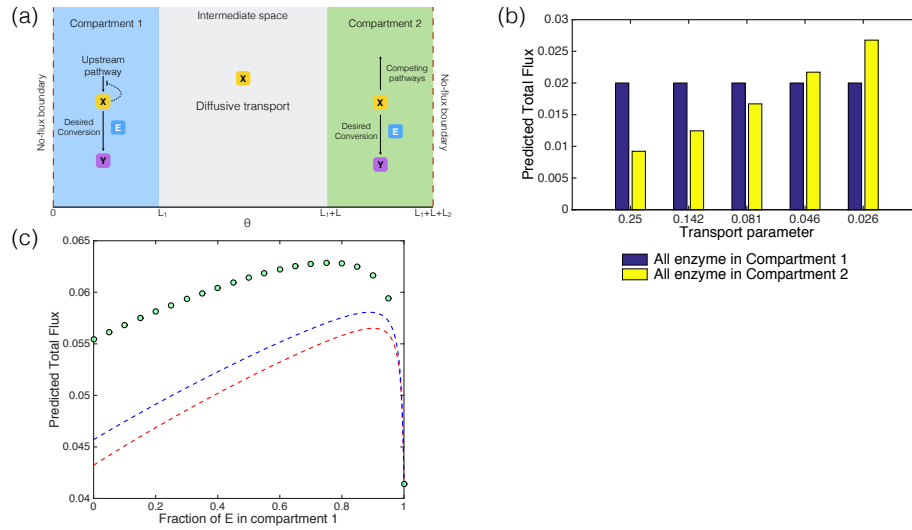


FIGURE 3.6: Sample design problem, using compartmental models to optimally target a metabolic enzyme to a compartment. **(a)** Schematic of the compartmentalized metabolic pathway, with upstream pathway in compartment 1 producing a metabolite X, and reactions consuming X in both compartments, including the desired conversion (by enzyme E) and competing pathways. In the text, we examine two scenarios, one with negative feedback inhibition (depicted) and one where this is absent. **(b)** The case of no feedback inhibition. The choice of transport parameter is crucial for the correct prediction of fluxes for the two extreme distributions (no feedback inhibition). An incorrect transport parameter might lead to a completely misleading impression as to which distribution is better. The fluxes are shown as computed using a range of transport parameters between  $D/(LL_1)$  (which is 0.25 here) and the transport parameter accounting for the basal reactions (which is 0.026). **(c)** The case with feedback inhibition. There is significant disparity between the flux of product obtained from the compartmental model and the PDE. The green dots represent the flux of the desired reaction, as given by the PDE. If a transport parameter  $D/(LL_1)$  is used in the compartmental model, the disparity is substantial (red dashed line), and further, even an improved estimate of the transport parameter (accounting for the basal reactions) still leaves a clear degree of disparity (blue dashed line).

In the second exemplar case, we consider the setup above, but with an extra ingredient: the substrate inhibits its production (Fig. 6(a)). This is observed in multiple contexts. Here we examine what the optimal distribution of enzyme between the compartments should be, to maximize total flux of

the output. Here as seen in Fig. 6(c), we find that there is a significant disparity between the flux of product obtained from the compartmental model and the PDE. Furthermore, the compartmental model predicts that if all the enzyme is in either of the compartments, the flux is roughly the same, a conclusion which is misleading. We note that if a transport parameter of  $D/LL_1$  is used in the compartmental model, the disparity is substantial, and further, even an improved estimate of the transport parameter (as done above) still leaves a clear degree of disparity. In such cases, it is necessary to incorporate the effects of the second reaction on the transport parameter to obtain reliable predictions.

The essential insight is that non-trivial pathway optimization problems usually involving a trade-off between multiple competing factors. When the compartments are not small (i.e. transport parameters are not fixed) and when the diffusivities are not large (i.e. non-trivial gradients can exist), the kinetic dependence of transport parameters (which guarantee a fit with the PDE model) can significantly affect the balance between competing factors in determining the optimal solution. In such a case the optimal solution may be very different from that in a case where the transport parameters were constant. Consequently there can be an essential mismatch between the optimal solution in the PDE model and that of a compartmental ODE model with constant transport parameters.

**Relevance to Systems and Synthetic Biology.** Even in more complex synthetic and systems biology applications, the presence of multiple levers enhances the applicability of the associated pathway, and their role needs to be accurately understood. Optimizing and manipulating pathways is a major theme in synthetic biology and metabolic pathways, and compartmentalization is seen as a vital tool. Compartmentalization of modules is seen as a basic ingredient in the realization of proto-cells. In all these cases, the choice of a modelling framework must enable accurate understanding and design. Finally, design (whether of multiple compartments or multiple pathways) is usually implemented in a modular way, and our analysis indicates the pitfalls associated with that, in the context of compartmentalization.



## 3.4 Discussion

Compartmentalization is a basic ingredient of cellular systems and a key focal point of interest, for multiple reasons. This stems from the role of compartmentalization in cellular information processing networks, compartmentalization as a key ingredient in evolution, and the interest in using compartmentalization as a key engineering “lever” to regulate and design biochemical pathways. It is clear in all these cases, that progress needs both dedicated experiments as well as modelling and systems approaches. Compartmental ODEs are a popular choice for models, as they incorporate essential features about compartments, focus on the reaction compartments (which are the focus of interest anyway) and are tractable: they represent a manageable balance between complexity and analyzability. However since the modelling framework sits right at the foundation of the investigation and serves as the basis for analysing and designing compartments, it is vital that effect of the choice of modelling framework in affecting the results is well understood.

We analyzed compartmental ODE models compared with detailed PDE models in a range of systems, with reactions in two compartments, separated by an intervening space. We spanned an entire range, with thin compartments (with substantial intervening space) at one extreme, and fat compartments (with relatively small intervening space) at the other. We primarily compared the models at steady state. Our main conclusion is that there are two factors which can significantly affect whether compartmental ODE models yield accurate results: correct determination of total compartmental concentrations (something which is independent of a model) and the transport parameters (which arise from the model description). In the thin compartment regime, due to conservation of species, there may be a considerable amount of species in the intervening space, and further this amount may depend on reaction kinetics and other parameters. We demonstrate how analytically incorporating the conservation results in an augmented compartmental model, will give much more accurate results. In the other regime, one of the key factors which affects the accuracy of the compartmental models is the choice of transport parameters. Through a careful analysis of the correspondence between compartmental ODE models and PDE models (with mass action kinetics), we find that the the steady state solution of the PDE model corresponds to that of a compartmental ODE model where the transport parameters actually involve a combination of diffusion and kinetics. Consequently

any compartmental model in this regime, which incorporates constant transport parameters will make predictions which are not robust to changes in the kinetics. For compartmental sizes which are at neither extreme, both factors can contribute significantly. We note in passing, that reductions from PDE to ODE models have also been considered in other settings, such as fronts in reaction-diffusion systems, in the context of cell polarization (Edelstein-Keshet et al., 2013; Holmes, Mata, and Edelstein-Keshet, 2015).

These same core factors play very significant roles when biochemical pathways with additional complexity are considered, whether multisite substrate modification, the effects of bistability, the effects of adding further compartments, or designing metabolic pathways to maximize yield. In general, when the kinetics of the reactions follow mass action kinetics, it is possible to obtain a compartmental model, with transport parameters which depend on kinetics to exactly match the PDE model at steady state. As seen from our studies, in certain situations even with mass action kinetics, non-monotonic concentration profiles may arise in compartments, and consequently the gradient of species between compartments may not be in the direction from higher average concentration to lower average concentration. In other words, there may be a fundamental limitation in such cases for a compartmental ODE model with positive transport parameters, to match the PDE model and this can be traced to a mismatch between how the “driving force” of transport is conceptualized in compartmental ODE models and how it actually occurs. When the kinetics is strongly nonlinear, this factor can prevent an exact match, for fundamental reasons. We note that sequestration is a dominant underlying theme in theoretical and experimental studies of signalling in the past decade. From our results, we note that for compartmental models to make accurate predictions and conclusions, both these factors must be appropriately incorporated, failing which very significant inaccuracies could result.

Our approach has involved systematically dissecting and analyzing compartmental systems proceeding from the simple to the complex, while also studying both closed and open systems, kinetic regimes, compartment sizes, as well as a range of more complex scenarios. Through this unified approach, we are able to systematically and explicitly disentangle multiple contributing factors, to better understand their interplay. As a consequence, we are (i) able to identify some very basic sources which limit the efficacy of compartmental ODE models (ii) identify how and when that can be corrected for (iii) obtain clear insights relevant to each category of model/context (iv) sharply reveal what the consequences of additional complexities may be in this regard, and

how they depend on the type of augmentation.

Our focus in this chapter was on the steady state behaviour of compartmental models. It is clear that when dynamic behaviour of models is concerned, there will in general be additional differences between the compartmental model and the PDE model (so that even if steady state behaviour is the same, the dynamic behaviour may be different) This in turn is due to the essentially different way in which the transport is described. Naturally, if there is a mismatch in steady state behaviour, there will have to be a difference in dynamic behaviour as well. We point out that if dynamic behaviour is desired, it would be preferable to describe the intermediate space as an additional compartment.

Cellular systems are complex and modelling in systems biology necessarily involves simplifications: what is the relevance of our analysis here? Compartmental models of biochemical pathways are already being used in many contexts simply because it is recognized that compartmentalization is a vital ingredient, playing a potentially important role. This being the case, a compartmental ODE representation runs the risk of severe qualitative and quantitative distortions. Sequestration and conservation have already been shown to be a key ingredient in qualitatively altering signal transduction characteristics in multiple contexts (Blüthgen et al., 2006; Thomson and Gunawardena, 2009). The fact that the transport parameter (guaranteeing an exact match) in such a representation (when the compartments are not thin) actually depends on kinetics has serious and profound implications. It simply indicates, that at this level of description (to ensure an exact match) the inter-compartmental transport parameters and reactions are not independent, and multiple entities in a reaction network can affect the transport parameters. In closed systems, there is, in fact, no regime of compartmental sizes, which is immune to both these distortions. In synthetic biology, engineering compartmentalized pathways necessarily progresses starting from simpler tractable cases. Given the centrality of compartmentalization in the design goal and the relative simplicity (in the initial stages of the setup), adopting a naive compartmental ODE model is harder to justify.

We now examine a range of contexts of current interest in compartmentalization, which goes beyond understanding their effect in cellular information processing. In cell-free synthetic biology, compartmentalized biochemical pathways, including coupled systems of lipid vesicles and spatially coupled compartments on silicon chips, have been engineered. Cell-free transcription-translation systems encapsulated in lipid vesicles (Caschera and

Noireaux, 2016) and compartmentalized biochemical modules, (Miller et al., 2013) have been investigated in the context of building artificial cells. Spatially organized chemical systems, relying on compartmentalization and diffusive transport, are a central theme in exploring the origins of life. Active droplets, with chemical reactions compartmentalized by phase separation, and maintained far from thermodynamic equilibrium by the supply of a chemical fuel, have been shown to be capable of spontaneous division, and suggested as candidates for early proto-cells (Zwicker et al., 2017).

Optimizing spatial organization of metabolism is an emerging theme in synthetic biology (Agapakis, Boyle, and Silver, 2012). Among the natural strategies used by a cell are spatial organization and compartmentalization at multiple levels - pathways compartmentalized at the intercellular level (different pathways in different cells e.g. symbiotic microbial communities), reactions compartmentalized at the level of sub-cellular compartments (peroxisomes, mitochondria in eukaryotes; proteinaceous microcompartments in prokaryotes), and enzymes localised at the level of multifunctional enzyme complexes by scaffold proteins. These strategies have been the basis for synthetic biology designs including engineering microbial co-cultures (Kim, Du, and Ismagilov, 2011) -where the focus is on realizing complex processes and achieving new functions without genetic engineering by spatially organizing communities of multiple microbes. Designing the spatial ordering of metabolic pathways in communities of competing/cooperating microbial cells, cross-feeding between co-operating strains of *E.coli* (Wintermute and Silver, 2010) and engineered cross-feeding of essential metabolites between yeast strains resulting in a synthetic cooperative system within a yeast population are different directions which are being pursued. Finally, another tool in metabolic engineering is manipulating the natural compartmentalization of pathways (DeLoache and Dueber, 2013; Avalos, Fink, and Stephanopoulos, 2013; Brat et al., 2012; Farhi et al., 2011).

By comparing and establishing a correspondence between compartmental ODE and PDE models for various classes of typical reaction systems, we (i) provide analysis which can be employed in using these models, (ii) establish limits and cases where the compartmental ODE models do and do not work, (iii) present augmentation which reduces/eliminates the mismatch between the ODE model and the PDE, (iv) suggest better procedures for the use of these compartmental models. We discuss this last point briefly. In general, in a system of diffusive transport, directly using a PDE model, along with its tools of analysis, may be preferable. If the advantage of a compartmental

model is desired, then another possibility is that it can be used (with potential augmentations) alongside a PDE model, so that the relative advantage of each approach can be employed. For some applications, the situation may be such that only a compartmental model may be feasible. In such a case our analysis may still suggest ways in which it can be used more effectively. For instance (say in a non-mass action regime) the question arises as to how to choose the transport parameter: our analysis (for non-thin compartments) indicates that the transport parameter would depend on kinetics. In such a case it may be possible to obtain “best estimates” of transport parameters at different points over a range of kinetic parameters, and then interpolate (rather than just use one basal value). In the context of pathway optimization, it may be possible to at least do some *a posteriori* analysis of the robustness of results to changes in transport parameters. Depending on whether the resulting behaviour is robust or not, the modelling approach may need to be refined or revisited. Finally, even if the focus is on compartmental behaviour, obtaining data which resolves the spatial behaviour in a compartment, may be a useful way of performing *a posteriori* checks.

Any modelling/systems framework involves a trade-off between tractability and effective representation of important features. A compartmental ODE model is a tractable framework which aims to capture the essential features of compartmentalization reliably. Our analysis indicates that while a compartmental ODE model may be useful as a nominal description in systems biology, one must be very careful about when it can and cannot be applied, and also when it can be used in a predictive manner reliably. In multiple instances, even with all the calibration, data fitting and parameter estimation this model will fall short for fundamental reasons. In some cases it can be augmented or improved, and our analysis indicates exactly how that can be done. In the context of engineering compartments, our study indicates when this type of model is and isn’t a reliable framework for undertaking any systematic design. In the ultimate analysis getting the interaction between the physics and chemistry involved in compartmentalization right, should be the key to successfully understanding and engineering compartmentalization, and this should dictate the choice of the foundational framework which is the basis of all the investigations.

## 3.5 Appendix

The following sections are organised as follows. We first outline the models for the compartmentalized pathways that we examine. Following this, we present a detailed analytical investigation of multiple aspects discussed in the main text. We note that the analytical solutions in all relevant cases have been cross-validated against numerical solutions of the PDE models.

### 3.5.1 Models

The different biochemical pathways examined here are described below (for the two compartment scenario). Schematics for the various pathways are presented in the main text and Figure S1.

#### Single modification cycle

This is a closed system consisting of a single enzymatic modification cycle involving species  $X$  and  $X^*$ . Both species diffuse across the whole domain, and may have different diffusivities.  $E_1$ , the enzyme catalyzing  $X \rightarrow X^*$  is non-diffusing and uniformly distributed in compartment 1 (see Figure 1(a)).  $E_2$ , the enzyme catalyzing  $X^* \rightarrow X$  is non-diffusing and uniformly distributed in compartment 2. The enzyme substrate complexes,  $XE_1$  and  $X^*E_2$  (referred to as  $C_1$  and  $C_2$ ) are also non-diffusing. The total amount of substrate within the whole domain, consisting of free substrate ( $X$  and  $X^*$ ) and the complexes, is conserved.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= -k_1[X][E_1] + k_{-1}[C_1] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= k_2[C_1] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[C_1]}{\partial t} &= k_1[X][E_1] - k_{-1}[C_1] - k_2[C_1]\end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_4[C_2] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_3[X^*][E_2] + k_{-3}[C_2] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[C_2]}{\partial t} &= k_3[X^*][E_2] - k_{-3}[C_2] - k_4[C_2]\end{aligned}$$

With the conservation equations,

$$\begin{aligned}X^{Total} &= (\langle[X]\rangle + \langle[X^*]\rangle)l + \langle[C_1]\rangle_1 L_1 + \langle[C_2]\rangle_2 L_2, \\ E_1^{Total} &= (\langle[E_1]\rangle_1 + \langle[C_1]\rangle_1) L_1 \\ E_2^{Total} &= (\langle[E_2]\rangle_2 + \langle[C_2]\rangle_2) L_2\end{aligned}$$

where  $\langle \cdot \rangle$  represents the spatial average over the whole domain, and  $\langle \cdot \rangle_i$  represents the spatial average over the compartment  $i$ .

The corresponding compartmental model is as follows.

**Compartmental ODE model:** In compartment 1,

$$\begin{aligned}L_1 \frac{d[X_1]}{dt} &= -k_1[X_1][E_1]L_1 + k_{-1}[C_1]L_1 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2 \\ L_1 \frac{d[X_1^*]}{dt} &= k_2[C_1]L_1 - tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1 + tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 \\ \frac{d[C_1]}{dt} &= k_1[X_1][E_1] - k_{-1}[C_1] - k_2[C_1]\end{aligned}$$

In compartment 2,

$$\begin{aligned} L_2 \frac{d[X_2]}{dt} &= k_4[C_2]L_2 - tr_{2 \rightarrow 1}^X[X_2]L_2 + tr_{1 \rightarrow 2}^X[X_1]L_1 \\ L_2 \frac{d[X_2^*]}{dt} &= -k_3[X_2^*][E_2]L_2 + k_{-3}[C_2] - tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 + tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1 \\ \frac{d[C_2]}{dt} &= k_3[X_2^*][E_2] - k_{-3}[C_2] - k_4[C_2] \end{aligned}$$

With corresponding conservation equations for the substrate and the enzymes. The form of the conservation equation for the diffusing substrate will be examined as part of our analysis.

In the compartmental ODE equations, the variables  $[X_i]$  and  $[X_i^*]$  represent the concentrations of species  $X$  and  $X^*$  in compartment  $i$ .

### Two-step cascade

This is a closed system consisting of two enzymatic modification cycles - the first involving species  $X$  and  $X^*$ , and the second involving  $Y$  and  $Y^*$  (See Fig. S1(a)). As before, the  $X$  cycle involves enzymes  $E_1$  and  $E_2$ . Both of these are non-diffusing and uniformly distributed in compartments 1 and 2 respectively.  $X$  and  $X^*$  diffuse over the whole domain, with  $X^*$  acting as the enzyme for  $Y \rightarrow Y^*$ .  $Y$  and  $Y^*$  are non-diffusing and confined to compartment 2.  $E_3$ , the enzyme catalyzing  $Y^* \rightarrow Y$  is also non-diffusing and uniformly distributed in compartment 2. All enzyme-substrate complexes are non-diffusing. The total amount of substrate over the whole domain, in each of the cycles, is conserved.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned} \frac{\partial[X]}{\partial t} &= -k_1[X][E_1] + k_{-1}[C_1] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= k_2[C_1] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[C_1]}{\partial t} &= k_1[X][E_1] - k_{-1}[C_1] - k_2[C_1] \end{aligned}$$



Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_4[C_2] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_3[X^*][E_2] + k_{-3}[C_2] - k_5[X^*][Y] + k_{-5}[C_3] + k_6[C_3] + D_X \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[Y]}{\partial t} &= -k_5[X^*][Y] + k_{-5}[C_3] + k_8[C_4] \\ \frac{\partial[Y^*]}{\partial t} &= -k_7[Y^*][E_3] + k_{-7}[C_3] + k_6[C_4] \\ \frac{\partial[C_2]}{\partial t} &= k_3[X^*][E_2] - k_{-3}[C_2] - k_4[C_2] \\ \frac{\partial[C_3]}{\partial t} &= k_5[X^*][Y] - k_{-5}[C_3] - k_6[C_3] \\ \frac{\partial[C_4]}{\partial t} &= k_7[Y^*][E_3] - k_{-7}[C_4] - k_8[C_4]\end{aligned}$$

With the conservation equations,

$$\begin{aligned}X^{Total} &= (\langle[X]\rangle + \langle[X^*]\rangle)l + \langle[C_1]\rangle_1 L_1 + \langle[C_2]\rangle_1 L_1 + \langle[C_3]\rangle_1 L_2, \\ Y^{Total} &= (\langle[Y]\rangle_2 + \langle[Y^*]\rangle_2) L_2 + \langle[C_3]\rangle_2 L_2 + \langle[C_4]\rangle_2 L_2, \\ E_1^{Total} &= (\langle[E_1]\rangle_1 + \langle[C_1]\rangle_1) L_1 \\ E_2^{Total} &= (\langle[E_2]\rangle_2 + \langle[C_2]\rangle_2) L_2 \\ E_3^{Total} &= (\langle[E_3]\rangle_2 + \langle[C_4]\rangle_2) L_2\end{aligned}$$

The corresponding compartmental model is as follows.

**Compartmental ODE model:** In compartment 1,

$$\begin{aligned}L_1 \frac{d[X_1]}{dt} &= -k_1[X_1][E_1]L_1 + k_{-1}[C_1]L_1 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2 \\ L_1 \frac{d[X_1^*]}{dt} &= k_2[C_1]L_1 - tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1 + tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 \\ \frac{d[C_1]}{dt} &= k_1[X_1][E_1] - k_{-1}[C_1] - k_2[C_1]\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2$ ),

$$L_2 \frac{d[X_2]}{dt} = k_4[C_2]L_2 - tr_{2 \rightarrow 1}^X[X_2]L_2 + tr_{1 \rightarrow 2}^X[X_1]L_1$$

$$L_2 \frac{d[X_2^*]}{dt} = -k_3[X_1^*][E_2]L_2 + k_{-3}[C_2]L_2 - k_5[X_2^*][Y]L_2 + k_{-5}[C_3]L_2 + k_6[C_3] - tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 + tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1$$

$$\frac{d[Y]}{dt} = -k_5[X_2^*][Y] + k_{-5}[C_3] + k_8[C_4]$$

$$\frac{d[Y^*]}{dt} = -k_7[Y^*][E_3] + k_{-7}[C_3] + k_6[C_4]$$

$$\frac{d[C_2]}{dt} = k_3[X_1^*][E_2] - k_{-3}[C_2] - k_4[C_2]$$

$$\frac{d[C_3]}{dt} = k_5[X_2^*][Y] - k_{-5}[C_3] - k_6[C_3]$$

$$\frac{d[C_4]}{dt} = k_7[Y^*][E_3] - k_{-7}[C_4] - k_8[C_4]$$

With corresponding conservation equations for the substrates and the enzymes.

### Two-site modification

This is a closed system involving interconversion between the species  $X$  and its modified forms,  $X^*$  and  $X^{**}$ , with the three forms belonging to a conserved pool. As in the previous models, both enzymes and complexes are non-diffusing. Enzyme  $E_1$ , uniformly distributed in compartment 1, catalyzes both the forward steps  $X \rightarrow X^*$  and  $X^* \rightarrow X^{**}$ , while  $E_2$ , uniformly distributed in compartment 2, catalyzes both the reverse steps.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned} \frac{\partial[X]}{\partial t} &= -k_1[X][E_1] + k_{-1}[C_1] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_3[X^*][E_1] + k_{-3}[C_2] + k_2[C_1] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= k_4[C_2] + D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2} \\ \frac{\partial[C_1]}{\partial t} &= k_1[X][E_1] - k_{-1}[C_1] - k_2[C_1] \\ \frac{\partial[C_2]}{\partial t} &= k_3[X^*][E_1] - k_{-3}[C_2] - k_4[C_2] \end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_8[C_4] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_7[X^*][E_2] + k_{-7}[C_4] + k_6[C_3] + D_{X^*} \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= -k_5[X^{**}][E_2] + k_{-5}[C_3] + D_{X^{**}} \frac{\partial^2[X^{**}]}{\partial \theta^2} \\ \frac{\partial[C_3]}{\partial t} &= k_5[X^{**}][E_2] - k_{-5}[C_3] - k_6[C_3] \\ \frac{\partial[C_4]}{\partial t} &= k_7[X^*][E_2] - k_{-7}[C_4] - k_8[C_4]\end{aligned}$$

With the conservation equations,

$$\begin{aligned}X^{Total} &= (\langle[X]\rangle + \langle[X^*]\rangle + \langle[X^{**}]\rangle)l + \langle[C_1]\rangle_1 L_1 + \langle[C_2]\rangle_1 L_1 + \langle[C_3]\rangle_1 L_2 + \langle[C_4]\rangle_1 L_2, \\ E_1^{Total} &= (\langle[E_1]\rangle_1 + \langle[C_1]\rangle_1 + \langle[C_2]\rangle_1) L_1 \\ E_2^{Total} &= (\langle[E_2]\rangle_1 + \langle[C_3]\rangle_2 + \langle[C_4]\rangle_2) L_2\end{aligned}$$

The corresponding compartmental model is as follows.

**Compartmental ODE model:** In compartment 1,

$$\begin{aligned}L_1 \frac{d[X]}{dt} &= -k_1[X_1][E_1]L_1 + k_{-1}[C_1]L_1 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2 \\ L_1 \frac{d[X_1^*]}{dt} &= -k_3[X_1^*][E_1]L_1 + k_{-3}[C_2]L_1 + k_2[C_1]L_1 - tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1 + tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 \\ L_1 \frac{d[X_1^{**}]}{dt} &= k_4[C_2]L_1 - tr_{1 \rightarrow 2}^{X^{**}}[X_1^{**}]L_1 + tr_{2 \rightarrow 1}^{X^{**}}[X_2^{**}]L_2 \\ \frac{d[C_1]}{dt} &= k_1[X_1][E_1] - k_{-1}[C_1] - k_2[C_1] \\ \frac{d[C_2]}{dt} &= k_3[X_1^*][E_1] - k_{-3}[C_2] - k_4[C_2]\end{aligned}$$

In compartment 2,

$$\begin{aligned}
 L_2 \frac{d[X_2]}{dt} &= k_8[C_4]L_2 - tr_{2 \rightarrow 1}^X[X_2]L_2 + tr_{1 \rightarrow 2}^X[X_1]L_1 \\
 L_2 \frac{d[X_2^*]}{dt} &= -k_7[X_2^*][E_2]L_2 + k_{-7}[C_2]L_2 + k_6[C_3]L_2 - tr_{2 \rightarrow 1}^{X^*}[X_2^*]L_2 + tr_{1 \rightarrow 2}^{X^*}[X_1^*]L_1 \\
 L_2 \frac{d[X_2^{**}]}{dt} &= -k_5[X_2^{**}][E_2]L_2 + k_{-5}[C_3]L_2 - tr_{2 \rightarrow 1}^{X^{**}}[X_2^{**}]L_2 + tr_{1 \rightarrow 2}^{X^{**}}[X_1^{**}]L_1 \\
 \frac{d[C_3]}{dt} &= k_5[X_2^{**}][E_2] - k_{-5}[C_3] - k_6[C_3] \\
 \frac{d[C_4]}{dt} &= k_7[X_2^*][E_2] - k_{-7}[C_4] - k_8[C_4]
 \end{aligned}$$

With corresponding conservation equations for the substrates and the enzymes.

### Simple open system

This is an open system involving a single species  $X$  that is produced in compartment 1 and degraded in compartment 2.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\frac{\partial[X]}{\partial t} = k_0 + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\frac{\partial[X]}{\partial t} = -k_1[X] + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

The corresponding compartmental model is as follows.

**Compartmental ODE model:** In compartment 1,

$$L_1 \frac{d[X]}{dt} = k_0 L_1 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2$$

In compartment 2,

$$L_2 \frac{d[X_2]}{dt} = -k_1[X_2]L_2 - tr_{2 \rightarrow 1}^X[X_2]L_2 + tr_{1 \rightarrow 2}^X[X_1]L_1$$

### Metabolic pathway

This system consists of part of a metabolic pathway, involving two successive enzymatic conversions of metabolites. The two enzymes, as well as the initial metabolite  $R$ , are non-diffusible, while the product of the first conversion  $X$ , is diffusible across the whole domain.  $R$  is present in compartment 1, with the first enzyme  $P$  also confined to this compartment. The concentration of  $R$  is assumed to be maintained at a fixed level in compartment 1. The second enzyme  $E$ , that converts  $X$  to another metabolite  $Y$ , is distributed between the two compartments (uniformly in each compartment), with a possible difference in its kinetics in compartments 1 and 2. There are competing reactions, consuming  $X$ , in the second compartment. The enzymatic reactions are assumed to operate at the mass-action regime.

**PDE model:** In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\frac{\partial[X]}{\partial t} = k_1[P][R] - k_{21}[E][X] + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\frac{\partial[X]}{\partial t} = -k_{22}[E][X] - k_3[X] + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

The total amount of the enzyme  $F$  is fixed,

$$E^{Total} = \langle [E] \rangle_1 L_1 + \langle [E] \rangle_2 L_2$$

The corresponding compartmental model is as follows.

**Compartmental ODE model:** In compartment 1,

$$L_1 \frac{d[X_1]}{dt} = k_1[P][R]L_1 - k_{21}[E_1][X]L_1 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2$$

In compartment 2,

$$L_2 \frac{d[X]}{dt} = -k_{22}[E_2][X]L_2 - k_3[X]L_2 - tr_{1 \rightarrow 2}^X[X_1]L_1 + tr_{2 \rightarrow 1}^X[X_2]L_2,$$

with the total amount of the enzyme  $E$ ,

$$E^{Total} = [E_1]L_1 + [E_2]L_2$$

We also examine a variant of the above pathway, where the product metabolite  $X$  negatively regulates the enzyme  $E$ . This type of negative feedback by a metabolite on the upstream enzymes in a pathway may be realised through allosteric regulation. In this case, the production term for species  $X$  in compartment 1 is changed to  $\frac{k_1[P][R]}{K+[X]}$  in the PDE and  $\frac{k_1[P][R]}{K+[X]}L_1$  in the compartmental model.

### 3.5.2 Analytical Investigations

In this section, we present a series of investigations to consolidate and further discuss points made in the main text. This includes solution of the relevant PDEs, correspondence with the relevant compartmental models (and obtaining relevant parameters therein, as well as other related discussions). In all cases, analytical solutions to the PDE are cross-validated against numerical solutions. Furthermore, parametrizing or modifying compartmental models to account for sequestration or changing transport parameters is done through a correspondence between compartmental ODE models and the PDE. This is subject to an additional level of cross-validation. Indeed, as discussed in the main text, these changes have demonstrated a marked improvement in matching the compartmental ODE model with the PDE in many different parameter regimes and scenarios. Numerical evidence in the text substantiates other analytical discussions.

#### Transport parameters in the thin compartment regime

For the kinds of compartmentalized reaction pathways examined in this study, compartmental ODE models naturally correspond to the limit of well mixed compartments connected by pure diffusive transport. This limit is usually assumed when using compartmental ODE models. This limit may be realised in different ways - for the type of scenario examined here, this limit corresponds to having compartment sizes much smaller than the intervening space, i.e. the thin compartment regime.

Consider any 1-D, two compartment system (the following discussion is equally applicable to systems with more compartments) of the kind examined here (for example, see Figure 1). The net transport rate of a species  $X_j$  out of compartment 1 may be expressed as the product of the diffusivity  $D_j$  and the concentration gradient of  $X_j$  at the edge of the compartment. Since the intervening space involves only diffusion, this transport rate, at the limit of well mixed compartments, is given by

$$D_j \frac{X_{j1} - X_{j2}}{L}, \quad (3.5)$$

where  $X_{j1}$  and  $X_{j2}$  are the concentrations of  $X_j$  in compartments 1 and 2, and  $L$  is the size of the space between compartments. Now, the net transport rate of the same species  $X$  out of compartment 1, in the corresponding compartmental ODE model is represented as

$$L_1 tr_{1 \rightarrow 2}^X X_{j1} - L_2 tr_{2 \rightarrow 1}^X X_{j2}. \quad (3.6)$$

From (1) and (2), we see that the transport parameters for  $X$  corresponding to the limit of well mixed compartments is given by

$$tr_{1 \rightarrow 2}^X = \frac{D_X}{LL_1}$$

$$tr_{2 \rightarrow 1}^X = \frac{D_X}{LL_2}.$$

In the following discussion, we use analytical solutions to the PDE (for the simple closed and open systems) to obtain expressions for the transport parameters, and show that the above values for the transport parameters are attained in the limit of thin compartments.

#### Accounting for sequestration: Modified conservation condition

Consider the simple closed system, the compartmentalized single modification cycle discussed in the text (see Figure 1(a)). In the thin compartment regime, the total amount of substrate in the compartments depends on kinetics, while the transport parameters may be approximated by  $D/LL_1$ . In order for such a compartmental ODE model to completely capture the effect of changing kinetics, it needs a way of accounting for the variation in total amount of substrate (in the compartments).

Now, although the total amount in the compartments, i.e.  $\tilde{X}^{Total}$ , may depend on kinetics, the total amount in the whole domain is still conserved

and remains invariant. That is,

$$X^{Total} = \tilde{X}^{Total} + X^{Inter}$$

is fixed independent of the kinetics, where  $X^{Inter}$  represents the total amount of  $X$  and  $X^*$  in the intermediate space. Note that the enzyme-substrate complexes, being non-diffusible, are not present in the intermediate space. Since the concentration profiles of  $X$  and  $X^*$  are linear in the intermediate space (due to pure diffusion in the intermediate space),  $X^{Inter}$  can be computed exactly using the concentrations of  $X$  and  $X^*$  at the edges of the compartments (i.e. at  $\theta = L_1$  and  $\theta = L_1 + L$ ).

$$X^{Inter} = \frac{X(L_1) + X(L_2)}{2}L + \frac{X^*(L_1) + X^*(L_2)}{2}L$$

In the thin compartment regime, in the absence of significant gradients within the compartments, these edge concentrations may be reasonably approximated by the compartmental averages. This means that, for the compartmental ODE, we can write a conservation condition for the whole domain:

$$\begin{aligned} X^{Total} = & [X_1]L_1 + [X_2]L_2 + [X_1^*]L_1 + [X_2^*]L_2 + [C_1]L_1 + [C_2]L_2 \\ & + \frac{[X_1] + [X_2]}{2}L + \frac{[X_1^*] + [X_2^*]}{2}L \end{aligned}$$

For the single modification cycle, there is only one enzyme-substrate complex in each compartment -  $C_1$  in compartment 1 and  $C_2$  in compartment 2.

The above conservation condition involves only the compartmental variables, but still accounts for the sequestration of species in the intermediate space. Note that we have explicitly used steady state conditions in obtaining this conservation condition.

### Accounting for sequestration: Using a third compartment for the intermediate space

The modified conservation condition described above approximates the total amount in the intermediate space well, for a system in the thin compartment regime. However, this approximation only describes the steady state. Away from steady state conditions, the total amount in the intermediate space may not be equal to the average of the compartmental concentrations multiplied by the size of the intermediate space. One way to overcome this limitation, is to use a third compartment to describe the intermediate space.



Consider a general pathway involving two compartments in 1-D. In the thin compartment regime, the basic (two) compartmental model is given by:

$$\begin{aligned} L_1 \frac{dX_{j1}}{dt} &= L_1 f_{j1}(X_{11}, X_{21}, \dots, X_{n1}) - L_1 tr_{1 \rightarrow 2}^j X_{j1} + L_2 tr_{2 \rightarrow 1}^j X_{j2} \\ L_2 \frac{dX_{j2}}{dt} &= L_2 f_{j2}(X_{12}, X_{22}, \dots, X_{n2}) - L_2 tr_{2 \rightarrow 1}^j X_{j2} + L_1 tr_{1 \rightarrow 2}^j X_{j1} \end{aligned} \quad (3.7)$$

where  $j = 1, 2, \dots, n$

where the variables  $X_{ji}$  represent the (average) concentration of species  $j$  in compartment  $i$ . The reaction kinetic terms are represented by the functions  $f_{ji}(X_{1i}, X_{2i}, \dots, X_{ni})$ . Each species  $j$  has a pair of transport parameters associated with it -  $tr_{1 \rightarrow 2}^j$  and  $tr_{1 \rightarrow 2}^j$ . As seen above, in the thin compartment regime, these are well approximated by  $\frac{D_j}{LL_1}$  and  $\frac{D_j}{LL_2}$  respectively, where  $D_j$  is the diffusivity of species  $j$ . Thus, the transport rate of  $X_j$  out of compartment 1 (into compartment 2) has the form (1).

Now, instead of just using a modified conservation condition for each diffusing species that is conserved, we introduce a third compartment representing the intermediate space. The compartmental model is now given by

$$\begin{aligned} L_1 \frac{dX_{j1}}{dt} &= L_1 f_{j1}(X_{11}, X_{21}, \dots, X_{n1}) - L_1 tr_{1 \rightarrow m}^j X_{j1} + L tr_{m \rightarrow 1}^j X_{jm} \\ L \frac{dX_{jm}}{dt} &= L_1 tr_{1 \rightarrow m}^j X_{j1} - L tr_{m \rightarrow 1}^j X_{jm} + L_2 tr_{2 \rightarrow m}^j X_{j2} - L tr_{m \rightarrow 2}^j X_{jm} \\ L_2 \frac{dX_{j2}}{dt} &= L_2 f_{j2}(X_{12}, X_{22}, \dots, X_{n2}) - L_2 tr_{2 \rightarrow m}^j X_{j2} + L tr_{m \rightarrow 2}^j X_{jm} \end{aligned} \quad (3.8)$$

where  $j = 1, 2, \dots, n$

Here,  $X_{jm}$  represents the (average) concentration of species  $j$  in the intermediate space denoted by  $m$ . Suppose that transport parameters are chosen

in the following way:

$$\begin{aligned} tr_{1 \rightarrow m}^j &= \frac{2D_j}{LL_1}, \\ tr_{2 \rightarrow m}^j &= \frac{2D_j}{LL_2}, \\ tr_{m \rightarrow 1}^j &= tr_{m \rightarrow 2}^j = \frac{2D_j}{L^2}. \end{aligned}$$

At steady state, this gives  $X_{jm} = \frac{X_{j1} + X_{j2}}{2}$ , and the transport rate of  $X_j$  out of compartment 1 (into compartment 2) continues to have the form (1). Now, the conservation condition for a conserved, diffusing species may be correctly approximated by incorporating the corresponding amount in the third compartment, when computing the total amount of substrate. Note, that the above model fails completely if any reactions involving net production or removal of  $X_j$  are present in the intermediate space, even if the corresponding kinetic terms are incorporated into (4). This is because the resulting system would no longer correspond to the limit of thin compartments.

Now we discuss analytical solutions to the PDE models for the single modification cycle and the simple open system discussed in the main text.

## Solutions to PDE models

### Case 1: Single modification cycle(mass-action)

#### 1-D PDE model:

We assume that both phosphorylation and dephosphorylation occur through mass action kinetics. Note that this can be formally obtained from the model in the previous section by making the catalytic constants high. The kinase and phosphatase are uniformly distributed in the regions  $0 \leq \theta < L_1$  and  $L_1 + L \leq \theta < l < L_1 + L + L_2$  respectively (see Figure 1(a)). We assume that both the phosphorylation and dephosphorylation involve mass action kinetics. We first consider the case where  $X$  and  $X^*$  are equally diffusible.

In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned} \frac{\partial [X]}{\partial t} &= -k_1[X] + D \frac{\partial^2 [X]}{\partial \theta^2} \\ \frac{\partial [X^*]}{\partial t} &= k_1[X] + D \frac{\partial^2 [X^*]}{\partial \theta^2} \end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D \frac{\partial^2[X^*]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_2[X^*] + D \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= -k_2[X^*] + D \frac{\partial^2[X^*]}{\partial \theta^2}\end{aligned}$$

With the conservation condition,

$$X^{Total} = (\langle[X]\rangle + \langle[X^*]\rangle)l,$$

where  $\langle \cdot \rangle$  represents the spatial average over the whole domain. In the above,  $k_1$  represents the product of the phosphorylation rate constant and the total kinase concentration, while  $k_2$  represents the product of the dephosphorylation rate constant and the total phosphatase concentration

Since  $X$  and  $X^*$  are equally diffusible, the PDE model implies that the quantity  $[X] + [X^*]$  must be constant throughout the domain at steady state. That is,  $[X] + [X^*] = X_T$ , where  $X_T = X^{Total}/l$ . This also means that the total amount of substrate sequestered in the middle is independent of the kinetic parameters in the compartments. Also note that the equations for  $X$  and  $X^*$  are linear. Combined with the above condition, this means that  $[X]$  and  $[X^*]$  are linearly proportional to  $X_T$  (and hence to  $X^{Total}$ ).

### 1-D PDE Solution

Implementing no-flux boundary conditions we find:

$$\begin{aligned}
 \text{For } 0 \leq \theta < L_1 : [X] &= c_1 X_T (e^{\omega_1 \theta} + e^{-\omega_1 \theta}) \\
 [X^*] &= X_T (1 - c_1 (e^{\omega_1 \theta} + e^{-\omega_1 \theta})) \\
 \text{For } L_1 \leq \theta < L_1 + L : [X] &= X_T (c_2 \theta + c_3) \\
 [X^*] &= X_T (1 - c_2 \theta - c_3) \\
 \text{For } L_1 + L \leq \theta < l : [X^*] &= c_4 X_T (e^{\omega_2 \theta} + e^{\omega_2 (2l - \theta)}) \\
 [X] &= X_T (1 - c_4 (e^{\omega_2 \theta} + e^{\omega_2 (2l - \theta)})) \\
 \text{where } l = L_1 + L + L_2; \omega_1^2 &= \frac{k_1}{D}; \omega_2^2 = \frac{k_2}{D}
 \end{aligned}$$

$$\begin{aligned}
 c_1 &= \frac{1}{(e^{\omega_1 L_1} + e^{-\omega_1 L_1}) + \omega_1 L (e^{\omega_1 L_1} - e^{-\omega_1 L_1}) - \frac{\omega_1 (e^{\omega_1 L_1} - e^{-\omega_1 L_1}) (e^{\omega_2 (L_1 + L)} + e^{\omega_2 (2l - L_1 - L)})}{\omega_2 (e^{\omega_2 (L_1 + L)} - e^{\omega_2 (2l - L_1 - L)})}} \\
 c_2 &= c_1 \omega_1 (e^{\omega_1 L_1} - e^{-\omega_1 L_1}) \\
 c_3 &= c_1 (e^{\omega_1 L_1} + e^{-\omega_1 L_1}) - c_2 L \\
 c_4 &= \frac{-c_2}{\omega_2 (e^{\omega_2 (L_1 + L)} - e^{\omega_2 (2l - L_1 - L)})}
 \end{aligned}$$

### Compartmental averages

$$\begin{aligned}
 \langle X \rangle_1 &= \frac{c_1 X_T}{\omega_1 L_1} (e^{\omega_1 L_1} - e^{-\omega_1 L_1}) \\
 \langle X^* \rangle_2 &= \frac{c_4 X_T}{\omega_2 L_2} (e^{\omega_2 (L_1 + L)} - e^{\omega_2 (2l - L_1 - L)})
 \end{aligned} \tag{3.9}$$

Since the diffusivities of  $X$  and  $X^*$  are equal, the compartmental averages must also satisfy the equations

$$\begin{aligned}
 \langle X \rangle_1 + \langle X^* \rangle_1 &= X_T \\
 \langle X \rangle_2 + \langle X^* \rangle_2 &= X_T
 \end{aligned} \tag{3.10}$$

We now consider the corresponding compartmental ODE model for the case where the volumes of the two compartments are equal, i.e.  $L_1 = L_2$ . The case where  $L_1 \neq L_2$  is essentially no different. Since the volumes are equal,

we have a single transport parameter associated with each species -  $tr^X$  for  $X$  and  $tr^{X^*}$  for  $X^*$ . The model is as follows.

In compartment 1,

$$\begin{aligned}\frac{d[X_1]}{dt} &= -k_1[X_1] - tr^X[X_1] + tr^X[X_2] \\ \frac{d[X_1^*]}{dt} &= k_1[X_1] - tr^{X^*}[X_1^*] + tr^{X^*}[X_2^*]\end{aligned}$$

In compartment 2,

$$\begin{aligned}\frac{d[X_2]}{dt} &= k_2[X_2^*] - tr^X[X_2] + tr^X[X_1] \\ \frac{d[X_2^*]}{dt} &= -k_3[X_2^*] - tr^{X^*}[X_2^*] + tr^{X^*}[X_1^*]\end{aligned}\quad (3.11)$$

### Transport parameter

The values of the transport parameters that make the steady state of the compartmental model exactly match the PDE averages may then be computed as follows:

$$\begin{aligned}tr^X &= \frac{k_1\langle X \rangle_1}{\langle X \rangle_2 - \langle X \rangle_1} \\ tr^{X^*} &= \frac{-k_1\langle X \rangle_1}{\langle X^* \rangle_2 - \langle X^* \rangle_1}\end{aligned}$$

As we show below, the two transport parameters must be in the same ratio as the diffusivities of  $X$  and  $X^*$ . Thus, for the case of equal diffusivities, we have a single transport parameter  $tr$ . Using equations (5) and (6), we have

$$tr = \frac{k_1 \frac{c_1}{\omega_1 L_1} (e^{\omega_1 L_1} - e^{-\omega_1 L_1})}{1 - (1 + \frac{k_1}{k_2}) \frac{c_1}{\omega_1 L_1} (e^{\omega_1 L_1} - e^{-\omega_1 L_1})} \quad (3.12)$$

Taking the limit  $L \rightarrow \infty$ , keeping  $\frac{D}{L}$  constant, we find that

$$\begin{aligned}\lim_{L \rightarrow \infty} \langle X \rangle_1 &= \lim_{L \rightarrow \infty} 2c_1 X_T = \frac{X_T}{\left(1 + \frac{k_1}{k_2} + \frac{k_1 L L_1}{D}\right)} \\ \implies \lim_{L \rightarrow \infty} tr &= \frac{D}{L_1 L}\end{aligned}$$

This shows how the expression  $D/LL_1$  can be reached naturally from the expression for the transport parameter.

We now examine the case where  $X$  and  $X^*$  have different diffusivities,  $D_X$  and  $D_{X^*}$ . To find the steady state solution to this system, we use a change of variables:  $[\tilde{X}] = D_X[X]$  and  $[\tilde{X}^*] = D_{X^*}[X^*]$ . At steady state, this gives us a system of the same form as the equal diffusivity case (with  $D = 1$ ), in the new variables  $[\tilde{X}]$  and  $[\tilde{X}^*]$ . Note that, in the new system, the rate constants multiplying  $[\tilde{X}]$  and  $[\tilde{X}^*]$  are  $\frac{k_1}{D_X}$  and  $\frac{k_2}{D_{X^*}}$  respectively. Now, we can use the solution previously obtained in the equal diffusivity case, with these rate constants, and setting  $D = 1$ , to compute the profiles of the new variables  $[\tilde{X}]$  and  $[\tilde{X}^*]$ . The actual profiles are then given by  $[X] = \frac{[\tilde{X}]}{D_X}$  and  $[X^*] = \frac{[\tilde{X}^*]}{D_{X^*}}$ .

The compartmental model for the system is given by (7). The values of the transport parameters that produce an exact match with the PDE averages at steady state are computed as follows:

$$tr^X = \frac{k_1 \langle X \rangle_1}{\langle X \rangle_2 - \langle X \rangle_1} = \frac{k_1 \frac{\langle \tilde{X} \rangle_1}{D_X}}{\frac{1}{D_X} (\langle \tilde{X} \rangle_2 - \langle \tilde{X} \rangle_1)} = \frac{k_1 \langle \tilde{X} \rangle_1}{\langle \tilde{X} \rangle_2 - \langle \tilde{X} \rangle_1} \quad (3.13)$$

$$tr^{X^*} = \frac{-k_1 \langle X \rangle_1}{\langle X^* \rangle_2 - \langle X^* \rangle_1} = \frac{-k_1 \frac{\langle \tilde{X} \rangle_1}{D_X}}{\frac{1}{D_{X^*}} (\langle \tilde{X}^* \rangle_2 - \langle \tilde{X}^* \rangle_1)} = \frac{k_1 \langle \tilde{X} \rangle_1}{\langle \tilde{X} \rangle_2 - \langle \tilde{X} \rangle_1} \left( \frac{D_{X^*}}{D_X} \right), \quad (3.14)$$

where we have also used the fact that  $\langle \tilde{X} \rangle$  and  $\langle \tilde{X}^* \rangle$  satisfy equations of the form (6). From (9) and (10), we see that the transport parameters are in the same ratio as the diffusivities.

### Case 2: Simple open system

This is an open system involving a single species  $X$  that is produced in compartment 1 (rate constant  $k_0$ ) and degraded/consumed in compartment 2 (rate constant  $k_1$ ).

#### 1-D PDE Model

In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\frac{\partial [X]}{\partial t} = k_0 + D_X \frac{\partial^2 [X]}{\partial \theta^2}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\frac{\partial[X]}{\partial t} = -k_1[X] + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

### 1-D PDE Solution

The solution is as follows:

$$\text{For } 0 \leq \theta < L_1 : X = k_0 \left( \frac{-\theta^2}{2D} + c_2 \right)$$

$$\text{For } L_1 \leq \theta < L_1 + L : X = k_0(c_3\theta + c_4)$$

$$\text{For } L_1 + L \leq \theta < l : X = k_0 c_5 (e^{\omega_1 \theta} + e^{\omega_1 (2l - \theta)})$$

$$\text{where } l = L_1 + L + L_2; \omega_1^2 = \frac{k_1}{D};$$

$$c_2 = \left( \frac{L_1^2}{2D} + \frac{k_0 L_1 L}{D} - \frac{k_0 L_1 (e^{\omega_1 (L_1 + L)} + e^{\omega_1 (2l - L_1 - L)})}{\omega_1 D (e^{\omega_1 (L_1 + L)} - e^{\omega_1 (2l - L_1 - L)})} \right)$$

$$c_3 = \frac{-L_1}{D}$$

$$c_4 = \frac{L_1^2}{D} + \frac{L_1 L}{D} - \frac{L_1 (e^{\omega_1 (L_1 + L)} + e^{\omega_1 (2l - L_1 - L)})}{\omega_1 D (e^{\omega_1 (L_1 + L)} - e^{\omega_1 (2l - L_1 - L)})}$$

$$c_5 = \frac{-L_1}{\omega_1 D (e^{\omega_1 (L_1 + L)} - e^{\omega_1 (2l - L_1 - L)})}$$

### Compartmental averages

$$\langle X \rangle_1 = k_0 \left( \frac{-L_1^2}{6D} + c_2 \right)$$

$$\langle X \rangle_2 = k_0 \left( \frac{-c_5 (e^{\omega_1 (L_1 + L)} - e^{\omega_1 (2l - L_1 - L)})}{\omega_1 L_2} \right) = \frac{k_0 L_1}{k_1 L_2} \quad (3.15)$$

We now consider the corresponding compartmental model for the case where the volumes of the two compartments are equal, i.e.  $L_1 = L_2$ . The case where  $L_1 \neq L_2$  is essentially no different. Since the volumes are equal, we have a single transport parameter associated the species  $X$ . The model is given by:

**Compartmental ODE model:** In compartment 1,

$$\frac{d[X]}{dt} = k_0 - tr^X[X_1] + tr^X[X_2]$$

In compartment 2,

$$\frac{d[X_2]}{dt} = -k_1[X_2] - tr^X[X_2] + tr^X[X_1]$$

### Transport parameter

The values of the transport parameters that make the steady state of the compartmental model exactly match the PDE averages may then be computed as follows:

$$tr^X = \frac{k_0}{\langle X \rangle_1 - \langle X \rangle_2}, \quad (3.16)$$

where  $\langle X \rangle_1$  and  $\langle X \rangle_2$  are given by (11). Substituting for  $\langle X \rangle_1$  and  $\langle X \rangle_2$  and taking the limit  $L \rightarrow \infty$ , keeping  $\frac{D}{L}$  constant, we find that

$$\begin{aligned} \lim_{L \rightarrow \infty} \langle X \rangle_1 &= \lim_{L \rightarrow \infty} k_0 c_2 = k_0 L_1 \left( \frac{1}{k_1 L_2} + \frac{L}{D} \right) \\ \implies \lim_{L \rightarrow \infty} tr^X &= \frac{D}{L_1 L} \end{aligned}$$

### Case 3: Simple open system (with degradation in both compartments)

This is an open system involving a single species  $X$  that is produced in compartment 1 (rate constant  $k_0$ ) and degraded/consumed in both compartment 1 (rate constant  $k_1$ ) and compartment 2 (rate constant  $k_2$ ).

### 1-D PDE Model

In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\frac{\partial [X]}{\partial t} = k_0 - k_1[X] + D_X \frac{\partial^2 [X]}{\partial \theta^2}$$



Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\frac{\partial[X]}{\partial t} = -k_2[X] + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

### 1-D PDE Solution

The solution is as follows:

$$\text{For } 0 \leq \theta < L_1 : X = k_0 \left( c_1 (e^{\omega_1 \theta} + e^{-\omega_1 \theta}) + \frac{1}{k_1} \right)$$

$$\text{For } L_1 \leq \theta < L_1 + L : X = k_0 (c_3 \theta + c_4)$$

$$\text{For } L_1 + L \leq \theta < l : X = k_0 c_5 (e^{\omega_2 \theta} + e^{\omega_2 (2l - \theta)})$$

$$\text{where } l = L_1 + L + L_2; \omega_1^2 = \frac{k_1}{D}; \omega_2^2 = \frac{k_2}{D};$$

$$\begin{aligned} c_3 &= \frac{1}{k_1 \left( \frac{(e^{\omega_2(L_1+L)} + e^{\omega_2(2l-L_1-L)})}{\omega_2(e^{\omega_2(L_1+L)} - e^{\omega_2(2l-L_1-L)})} - \frac{(e^{\omega_1 L_1} + e^{-\omega_1 L_1})}{\omega_1(e^{\omega_1 L_1} - e^{-\omega_1 L_1})} - L \right)} \\ c_1 &= \frac{c_3}{\omega_1(e^{\omega_1 L_1} - e^{-\omega_1 L_1})} \\ c_4 &= c_1(e^{\omega_1 L_1} + e^{-\omega_1 L_1}) + \frac{1}{k_1} - c_3 L_1 \\ c_5 &= \frac{c_3}{\omega_2(e^{\omega_2(L_1+L)} - e^{\omega_2(2l-L_1-L)})} \end{aligned}$$

### Compartmental averages

$$\begin{aligned} \langle X \rangle_1 &= k_0 \left( \frac{c_1}{\omega_1 L_1} (e^{\omega_1 L_1} - e^{-\omega_1 L_1}) + \frac{1}{k_1} \right) \\ \langle X \rangle_2 &= k_0 \left( \frac{c_5}{\omega_2 L_2} (e^{\omega_2(L_1+L)} - e^{\omega_2(2l-L_1-L)}) \right) \end{aligned}$$

We now consider the corresponding compartmental model for the case where the volumes of the two compartments are equal, i.e.  $L_1 = L_2$ . The case where  $L_1 \neq L_2$  is essentially no different. Since the volumes are equal, we have a single transport parameter associated the species  $X$ . The model is given by:

**Compartmental ODE model:** In compartment 1,

$$\frac{d[X]}{dt} = k_0 - k_1[X_1] - tr^X[X_1] + tr^X[X_2]$$

In compartment 2,

$$\frac{d[X_2]}{dt} = -k_2[X_2] - tr^X[X_2] + tr^X[X_1]$$

### Transport parameter

The values of the transport parameters that make the steady state of the compartmental model exactly match the PDE averages may then be computed as follows:

$$tr^X = \frac{k_0 - k_1 \langle X \rangle_1}{\langle X \rangle_1 - \langle X \rangle_2},$$

### Case 4: Compartmentalized two-site modification (Mass-action)

We consider the system involving interconversion between the species  $X$  and its modified forms,  $X^*$  and  $X^{**}$ , with the three forms belonging to a conserved pool. The phosphorylation reactions are assumed to be in the mass action regime, with kinases restricted to compartment 1 and phosphatases in compartment 2. All species are assumed to be equally diffusible.

### 1-D PDE Model

In compartment 1 ( $0 \leq \theta \leq L_1$ ),

$$\begin{aligned} \frac{\partial [X]}{\partial t} &= -k_1[X] + D_X \frac{\partial^2 [X]}{\partial \theta^2} \\ \frac{\partial [X^*]}{\partial t} &= k_1[X] - k_2[X^*] + D_X \frac{\partial^2 [X^*]}{\partial \theta^2} \\ \frac{\partial [X^{**}]}{\partial t} &= k_2[X^*] + D_X \frac{\partial^2 [X^{**}]}{\partial \theta^2} \end{aligned}$$

Between the compartments ( $L_1 \leq \theta \leq L_1 + L$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= D_X \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= D_X \frac{\partial^2[X^{**}]}{\partial \theta^2}\end{aligned}$$

In compartment 2 ( $L_1 + L \leq \theta \leq L_1 + L + L_2 = l$ ),

$$\begin{aligned}\frac{\partial[X]}{\partial t} &= k_4[X^*] + D_X \frac{\partial^2[X]}{\partial \theta^2} \\ \frac{\partial[X^*]}{\partial t} &= k_3[X^{**}] - k_4[X^*] + D_X \frac{\partial^2[X^*]}{\partial \theta^2} \\ \frac{\partial[X^{**}]}{\partial t} &= -k_3[X^{**}] + D_X \frac{\partial^2[X^{**}]}{\partial \theta^2}\end{aligned}$$

With the concentration profiles satisfying a conservation condition of the form,

$$\frac{X^{Total}}{l} = [X] + [X^*] + [X^{**}] \text{ for } 0 \leq \theta \leq l$$

### 1-D PDE Solution

For  $0 \leq \theta < L_1$  :  $[X] = X_T c_1 \cosh(\omega_1 \theta)$

$$[X^*] = X_T \left( c_2 \cosh(\omega_2 \theta) - \frac{k_1 c_1}{k_1 - k_2} \cosh(\omega_1 \theta) \right)$$

$$[X^{**}] = X_T - [X] - [X^*]$$

For  $L_1 \leq \theta < L_1 + L$  :  $[X] = X_T(c_3 \theta + c_4)$

$$[X^*] = X_T(c_5 \theta + c_6)$$

$$[X^{**}] = X_T - [X] - [X^*]$$

For  $L_1 + L \leq \theta < l$  :  $[X] = X_T - [X^*] - [X^{**}]$

$$[X^*] = X_T \left( c_8 \cosh(\omega_4(l - \theta)) - \frac{k_3 c_7}{k_3 - k_4} \cosh(\omega_3(l - \theta)) \right)$$

$$[X^{**}] = X_T c_7 \cosh(\omega_3(l - \theta))$$

where  $l = L_1 + L + L_2$ ;

$$\omega_1^2 = \frac{k_1}{D_X}; \omega_2^2 = \frac{k_2}{D_X}; \omega_3^2 = \frac{k_3}{D_X}; \omega_4^2 = \frac{k_4}{D_X}$$

The constants  $c_i$  ( $i = 1, \dots, 8$ ) that determine the solution, are obtained by using the boundary conditions - no-flux conditions at the outer boundaries of the domain, and continuity of the concentration profiles and their first derivatives, at the compartment boundaries. This yields eight linear equations in the variables  $c_i$ , which are then solved by symbolic computation using Maple. The expressions thus obtained for the steady state concentration profiles of  $[X]$ ,  $[X^*]$ , and  $[X^{**}]$  are then validated by comparison with the steady state profiles obtained by numerical solution of the PDE system.

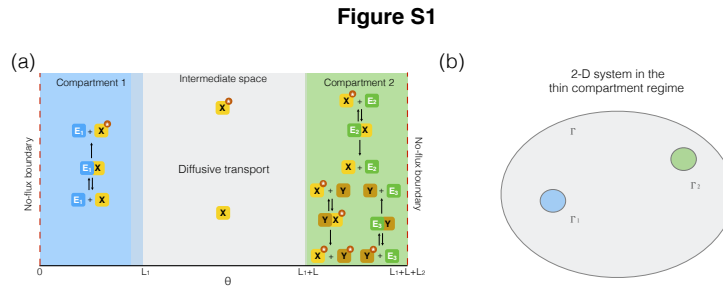


FIGURE 3.7: Figure S1.(a) Schematic of compartmentalised two-tier cascade. (b) Schematic of a compartmentalised system in two dimensions.

### Thin compartment regime in a 2-D system

We have seen that a compartmental model, with fixed transport parameters of the form  $D/LL_i$ , is a good approximation for a 1-D system in the thin compartment regime, at steady state. Here we show how a similar ODE model may be obtained for the same kind of system in a general 2-D case, with smooth boundaries for the compartments and the spatial domain. Consider the 2-D system shown in Fig. S1(b). Since the kinetics play no role in representing transport in the thin compartment regime, we can focus on a single species  $X$  to get the general picture. At steady state, let  $X(\vec{r})$  denote the concentration of species  $X$  outside the compartments. Within the compartments, and on the compartment boundaries ( $\Gamma_1$  and  $\Gamma_2$ ), the concentrations are given by the compartmental averages -  $X_1$  and  $X_2$ .

Since the intermediate space involves only diffusion of  $X$ , at steady state we have

$$\nabla^2 X(\vec{r}) = 0$$

*with the boundary conditions*

$$\nabla X(\vec{r}) = 0 \text{ on the outer boundary } \Gamma \text{ (no-flux)}$$

$$X(\vec{r}) = X_1 \text{ on the boundary } \Gamma_1$$

$$X(\vec{r}) = X_2 \text{ on the boundary } \Gamma_2$$

The solution to this boundary value problem may be expressed as

$$X(\vec{r}) = X_1 f_1(\vec{r}) + X_2 f_2(\vec{r})$$

where the functions  $f_1(\vec{r})$  and  $f_2(\vec{r})$  are solutions to the above boundary problem for the basal cases  $X_1 = 1, X_2 = 0$  and  $X_1 = 0, X_2 = 1$  respectively. As long as the geometry of the system remains unchanged, these functions only need to be solved for once (perhaps numerically), and can be used in the following way to obtain a compartmental description in the thin compartment regime. Note that the gradient of  $X(\vec{r})$  is given by

$$\nabla X(\vec{r}) = X_1 \nabla f_1(\vec{r}) + X_2 \nabla f_2(\vec{r}) \quad (3.17)$$

At steady state, the net transport of  $X$  out of compartment 1 is given by averaging the flux over the boundary  $\Gamma_1$ :

$$\text{Net Transport} = -D_X \int_{\Gamma_1} \nabla X(\vec{r}) \cdot \hat{n} ds,$$

where  $\hat{n}$  is the unit outward normal to  $\Gamma_1$ . This can be rewritten as:

$$\text{Net Transport} = -D_X \left( \int_{\Gamma_1} \nabla f_1(\vec{r}) \cdot \hat{n} ds \right) X_1 - D_X \left( \int_{\Gamma_1} \nabla f_2(\vec{r}) \cdot \hat{n} ds \right) X_2, \quad (3.18)$$

In the compartmental model for this 2-D system, the net transport of  $X$  out of compartment 1 is represented by:

$$\text{Net Transport} = A_1 tr_{1 \rightarrow 2}^X X_1 - A_2 tr_{2 \rightarrow 1}^X X_2 \quad (3.19)$$

where  $X_i$  are the compartmental (average) concentrations and  $A_i$  are the volumes (areas) of the compartments. Comparing (14) and (15), we see that the two transport parameters associated with the species  $X$  in the compartmental model are given by:

$$\begin{aligned} tr_{1 \rightarrow 2}^X &= -\frac{D_X}{A_1} \left( \int_{\Gamma_1} \nabla f_1(\vec{r}) \cdot \hat{n} ds \right), \\ tr_{2 \rightarrow 1}^X &= \frac{D_X}{A_2} \left( \int_{\Gamma_1} \nabla f_2(\vec{r}) \cdot \hat{n} ds \right) \end{aligned}$$

### Finding a compartmental ODE description to match the PDE

#### 1-D PDE at steady state

Consider a general pathway with first-order mass-action kinetics and possible zeroth order production, distributed between two equally sized compartments ( $L_1 = L_2$ ), in a 1-D domain (see Figure 1 for the geometry). At steady state, the PDE model in vector form gives:

$$\begin{aligned} A_1 u + b_1 + D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in compartment 1} \\ D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in the intermediate space} \\ A_2 u + b_2 + D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in compartment 2} \end{aligned} \quad (3.20)$$

Here,  $u$  is the vector of species concentrations at a point in the domain, and  $A_1$  and  $A_2$  are square matrices containing the rate constants corresponding to the mass-action reactions in compartments 1 and 2 respectively. The vectors  $b_1$  and  $b_2$  represent possible zeroth order production terms, and  $D$  is a diagonal matrix containing the diffusivities of the different species. For example, for the single modification cycle discussed in the main text, with kinetics at the mass-action regime,

$$\begin{aligned} A_1 &= \begin{bmatrix} -k_1 & 0 \\ k_1 & 0 \end{bmatrix}; \quad A_2 = \begin{bmatrix} 0 & k_2 \\ 0 & -k_2 \end{bmatrix} \\ b_1 &= b_2 = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \\ D &= \begin{bmatrix} D_X & 0 \\ 0 & D_{X^*} \end{bmatrix} \end{aligned}$$

No-flux boundaries are present at the ends of the domain. Averaging over compartments 1 and 2 at steady state, we have

$$A_1 \langle u \rangle_1 + b_1 + \frac{1}{L_1} D \left( \frac{\partial u}{\partial \theta} \right)_{L_1} = 0 \quad (3.21)$$

$$A_2 \langle u \rangle_2 + b_2 - \frac{1}{L_1} D \left( \frac{\partial u}{\partial \theta} \right)_{L_1+L} = 0 \quad (3.22)$$

Here,  $\langle u \rangle_1$  and  $\langle u \rangle_2$  are vectors of compartmental averages for compartments 1 and 2. Since no reactions occur in the intermediate space, we also have

$$\left( \frac{\partial u}{\partial \theta} \right)_{L_1} = \left( \frac{\partial u}{\partial \theta} \right)_{L_1+L} \quad (3.23)$$

This also means that

$$A_1 \langle u \rangle_1 + b_1 = -A_2 \langle u \rangle_2 - b_2 \quad (3.24)$$

### Compartmental ODE

For the system described by (16), we consider a compartmental ODE model where each species has a single transport parameter associated with it. The compartmental model has the form:

$$\begin{aligned} \frac{d\bar{u}_1}{dt} &= A_1 \bar{u}_1 + b_1 + \Delta \sigma \\ \frac{d\bar{u}_2}{dt} &= A_2 \bar{u}_2 + b_2 - \Delta \sigma \end{aligned} \quad (3.25)$$

Here,  $\bar{u}_1$  and  $\bar{u}_2$  denote vectors of species concentrations corresponding to compartments 1 and 2 respectively.  $\sigma$  is a vector containing the transport parameters, and  $\Delta$  is a diagonal matrix having the components of the vector  $\bar{u}_2 - \bar{u}_1$  as its diagonal.

Since we want the compartmental model to match the PDE compartmental averages at steady state, the steady state equations for the compartmental model must satisfy the equations:

$$\begin{aligned} A_1 \langle u \rangle_1 + b_1 + \Delta_u \sigma &= 0 \\ A_2 \langle u \rangle_2 + b_2 - \Delta_u \sigma &= 0 \end{aligned}$$

where  $\Delta_u$  is a diagonal matrix having the vector  $\langle u \rangle_2 - \langle u \rangle_1$  as its diagonal. Thus, the matrix  $\Delta_u$  is non-singular, assuming that the system corresponds to

a case where all diffusing species exhibit gradients between compartments. This fact, along with (20), allows us to compute the unique vector of transport parameters-  $\sigma_{ss}$ , that produces an exact match between the compartmental model and the PDE averages at steady state.

$$\sigma_{ss} = -\Delta_u^{-1}(A_1 \langle u \rangle_1 + b_1) \quad (3.26)$$

Note that there are exceptional cases where the compartmental averages may be equal for certain species (i.e. certain components of the vector  $\langle u \rangle_2 - \langle u \rangle_1$  may be zero), even when there is a non-zero concentration gradient for this species between compartments. In such cases, the transport parameter associated with this species diverges (See main text).

Of course, in addition to using these transport parameters, the ability of the compartmental model to match the PDE at steady state also relies on correctly describing the conservation conditions for any conserved species, as seen previously.

### Compartmental ODEs: Nonlinear kinetics

We now examine the case similar to (16), but where the kinetics can have arbitrary form, i.e. possibly including nonlinear terms such as second-order mass action steps or Michaelis-Menten kinetics. At steady state we have:

$$\begin{aligned} f_1(u) + D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in compartment 1} \\ D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in the intermediate space} \\ f_2(u) + D \frac{\partial^2 u}{\partial \theta^2} &= 0 \text{ in compartment 2} \end{aligned} \quad (3.27)$$

where  $f_1(u)$  and  $f_2(u)$  are nonlinear, vector valued functions of  $u$  representing the sum of the kinetic terms in compartments 1 and 2. Averaging over the compartments, we find

$$\begin{aligned} \langle f_1(u) \rangle_1 + \frac{1}{L_1} D \left( \frac{\partial u}{\partial \theta} \right)_{L_1} &= 0 \\ \langle f_2(u) \rangle_2 - \frac{1}{L_1} D \left( \frac{\partial u}{\partial \theta} \right)_{L_1+L} &= 0 \end{aligned}$$



As before, we have

$$\langle f_1(u) \rangle_1 = -\langle f_2(u) \rangle_2$$

Note however, that these are spatial averages of  $f_1(u)$  and  $f_2(u)$ , and not functions of the average of  $u$ , i.e.  $f_1(\langle u \rangle_1)$  and  $f_2(\langle u \rangle_2)$ . In general, these are not equal, and therefore,

$$f_1(\langle u \rangle_1) \neq -f_2(\langle u \rangle_2) \quad (3.28)$$

Now consider the compartmental model for the system (23), given by

$$\begin{aligned} \frac{d\bar{u}_1}{dt} &= f_1(\bar{u}_1) + \Delta\sigma \\ \frac{d\bar{u}_2}{dt} &= f_2(\bar{u}_2) - \Delta\sigma \end{aligned} \quad (3.29)$$

At steady state, the vectors of compartmental concentrations,  $\bar{u}_1$  and  $\bar{u}_2$  must satisfy

$$f_1(\bar{u}_1) = -f_2(\bar{u}_2) \quad (3.30)$$

From (24) and (26), we can conclude that the compartmental model cannot exactly match the PDE averages at steady state, when nonlinear kinetic terms are involved. The best that can be done in this case is to choose the vector of transport parameters such that the mismatch is minimized in some sense. In the present study, we choose to minimize the sum-squared error  $\|(\bar{u}_1 - \langle u \rangle_1)\|^2 + \|(\bar{u}_2 - \langle u \rangle_2)\|^2$ . For the compartmental model (10), this gives us a nonlinear optimization problem with a quadratic objective function and nonlinear equality constraints:

$$\begin{aligned} \min_{\sigma} \quad & \|(\bar{u}_1 - \langle u \rangle_1)\|^2 + \|(\bar{u}_2 - \langle u \rangle_2)\|^2 \\ \text{s.t.} \quad & f_1(\bar{u}_1) + \Delta\sigma = 0 \\ & f_2(\bar{u}_2) - \Delta\sigma = 0 \end{aligned} \quad (3.31)$$

Any conservation conditions would form additional equality constraints in (27).

### Transport Parameters and Diffusivities

We now examine the possibility that, for the first-order mass-action system in (16), the transport parameters for the different species, that produce an exact steady state match, are in the same ratio as the diffusivities of the corresponding species. For this to be true, at steady state we need the vectors of compartmental averages,  $\langle u \rangle_1$  and  $\langle u \rangle_2$  to satisfy an equation of the form:

$$A_1 \langle u \rangle_1 + b_1 + \lambda D (\langle u \rangle_2 - \langle u \rangle_1) = 0 \quad (3.32)$$

for some positive  $\lambda$ . Thus, from equations (17) and (28), we need

$$\frac{1}{L_1} \left( \frac{\partial \bar{u}}{\partial \theta} \right)_{L_1} = \lambda (\langle \bar{u} \rangle_2 - \langle \bar{u} \rangle_1) \quad (3.33)$$

**Single modification cycle (X to X\*).** Since the kinetics in each compartment conserves the total amount of species, the species concentrations satisfy

$$D_X X + D_{X^*} X^* = X_T \quad (3.34)$$

at all points in the domain.  $X_T$  is constant within the domain but its value depends on both kinetic and spatial parameters (including compartment size, compartment separation, and diffusivities). Thus, the compartmental averages satisfy,

$$D_X \langle X \rangle_1 + D_{X^*} \langle X^* \rangle_1 = D_X \langle X \rangle_2 + D_{X^*} \langle X^* \rangle_2 = X_T$$

Rewriting this,

$$D_X (\langle X \rangle_1 - \langle X \rangle_2) + D_{X^*} (\langle X^* \rangle_1 - \langle X^* \rangle_2) = 0 \quad (3.35)$$

Now, the steady state mass balance for compartment 1 implies

$$D_X \left( \frac{\partial X}{\partial \theta} \right)_{L_1} + D_{X^*}^* \left( \frac{\partial X^*}{\partial \theta} \right)_{L_1} = 0 \quad (3.36)$$

i.e. the net flux out of the compartment is zero.

Equations (31) and (32) imply that the differences in compartmental averages are related to the concentration gradients in the intervening space, as follows

$$\begin{bmatrix} \left(\frac{\partial X}{\partial \theta}\right)_{L_1} \\ \left(\frac{\partial X^*}{\partial \theta}\right)_{L_1} \end{bmatrix} = \gamma \begin{bmatrix} \langle X \rangle_1 - \langle X \rangle_2 \\ \langle X^* \rangle_1 - \langle X^* \rangle_2 \end{bmatrix} \quad (3.37)$$

Thus, the compartmental fluxes and compartmental averages satisfy an equation of the form (29). This means that a compartmental ODE model that exactly matches the PDE compartmental averages at steady state, must have transport parameters in the same ratio as the diffusivities. For a given set of kinetic and spatial parameters, these transport parameters would be uniquely determined.

**Two-site modification cycle (X to X\* to X\*\*).** Since the kinetics in each compartment only results in an interconversion between species, adding the equations for X, X\*, and X\*\* at steady state results in:

$$D_X X + D_{X^*} X^* + D_{X^{**}} X^{**} = X_T \quad (3.38)$$

at all points in the domain, where  $X_T$  is constant across the domain. As in the previous case, the compartmental averages for the different species satisfy

$$D_X (\langle X \rangle_1 - \langle X \rangle_2) + D_{X^*} (\langle X^* \rangle_1 - \langle X^* \rangle_2) + D_{X^{**}} (\langle X^{**} \rangle_1 - \langle X^{**} \rangle_2) = 0 \quad (3.39)$$

Considering the steady state mass balance for compartment 1, we have

$$D_X \left(\frac{\partial X}{\partial \theta}\right)_{L_1} + D_{X^*}^* \left(\frac{\partial X^*}{\partial \theta}\right)_{L_1} + D_{X^{**}}^{**} \left(\frac{\partial X^{**}}{\partial \theta}\right)_{L_1} = 0 \quad (3.40)$$

However, unlike the case of the single modification cycle, equations (35) and (36) do not suffice to give us a relationship of the form

$$\begin{bmatrix} \left(\frac{\partial X}{\partial \theta}\right)_{L_1} \\ \left(\frac{\partial X^*}{\partial \theta}\right)_{L_1} \\ \left(\frac{\partial X^{**}}{\partial \theta}\right)_{L_1} \end{bmatrix} = \gamma \begin{bmatrix} \langle X \rangle_1 - \langle X \rangle_2 \\ \langle X^* \rangle_1 - \langle X^* \rangle_2 \\ \langle X^{**} \rangle_1 - \langle X^{**} \rangle_2 \end{bmatrix} \quad (3.41)$$

This means that a compartmental ODE, which matches the PDE compartmental averages at steady state, need not have transport parameters in the same ratio as the diffusivities. However, the transport parameter associated with each species would still be uniquely determined. The transport parameters for the exact match, obtained numerically (solving the PDE and computing compartmental averages) for a sample case, are shown in Fig. 5(b).

### 3.5.3 Additional Information

#### Adding a third compartment

We examine the case of adding a third compartment to an existing two compartment open pathway (see schematic in Fig. 4(c)). The three compartments are of the same size, and are equally spaced. The species  $X$  is produced at a constant rate (zeroth order kinetics) in compartment 1, and diffuses across the domain.  $X$  is consumed by reactions in compartments 1, 2 and 3 (represented by first order reactions). We obtain the analytical solution to this three compartment system at steady state (not shown), and use the steady state concentration profile of  $X$  to compute spatial averages within each compartment. We use these averages to calculate the two transport parameters  $tr_{12}^X$  and  $tr_{23}^X$  representing transport between compartments 1 and 2 and between 2 and 3 respectively, as shown in (22) for the general case. Each of these transport parameters depend on the diffusivity, compartment sizes, compartment separation, and all the kinetic parameters. We fix the diffusivity, compartment sizes, compartment separation, and the kinetic parameters in compartments 1 and 2 (see table below for parameter values), and examine the effect of varying the rate constant of  $X$  consumption in compartment 3. The result is shown in Fig. 4(d). Note that the analytical solution to the three compartment system was cross-validated by comparison to the numerical solution of the PDE.

#### Bistable two-site modification

As discussed in the text, we consider a compartmentalized two-site modification pathway (see Fig. 5(a)), in a kinetic regime where it exhibits two stable steady states, over a range of values of  $E_1^{Total}$  - the total amount of kinase in compartment 1. We consider this system in the thin compartment regime ( $L/L_1 = 18$ ). The concentration profiles for the two steady states are obtained by numerical solution of the PDE model, with a fixed total amount of substrate  $X^{Total} = 10$  and enzymes  $E_1^{Total} = 0.6$ ;  $E_2^{Total} = 5$ . We note

that the total amount of substrate (free substrate + complexes) in the compartments is different for the two steady states. Now, for a given steady state, there are two ways of calibrating the compartmental model with the correct total amount of substrate in the compartment. One way is to obtain this total amount from the concentration profiles of the given steady state. However, since this amount is different for the two steady states, the resulting compartmental description can only capture one of these accurately (i.e. the steady state used for the calibration). As shown in Fig. 5(f) this may not even capture the second steady state. The other way is to use the modified conservation condition discussed previously, which accounts for substrate in intermediate space as well. This is a good approximation because the system is in the thin compartment regime. By using this conservation condition, the compartmental model is able to accurately capture both steady states.



## Chapter 4

# Design principles for compartmentalization and spatial organisation of synthetic genetic circuits

### 4.1 Introduction

It is evident that cells have exploited spatial organization as a basic mode of manipulating information processing, increasingly through evolution. However, despite the impressive strides made in systems biology, the role of spatial organization in the functioning and robustness of information processing networks is generally not well understood. In contrast, synthetic biology has undertaken a number of strides in the context of designing compartmentalization, and this is emerging as a vital new tool in the current wave of synthetic biology progressing from modules to systems.

Compartmentalization through synthetic approaches has already been realized in both cellular and cell-free systems. Transcription-translation systems and circuits, involving the localization of both DNA templates and the transcription-translation machinery, have been realized in multiple cell free systems (Noireaux and Libchaber, 2004; Schwarz-Schilling et al., 2016; Lentini et al., 2017). These include systems involving communication between compartments, where certain global diffusing species serve as key regulators (growthfactor/inducer/quorum sensing molecule) (Karzbrun et al., 2014; Tayar et al., 2015; Adamala et al., 2016). Spatial organization has also been engineered in other transcription based enzymatic DNA circuits. One such class of circuits are those constructed using the PEN-toolbox (Baccouche

et al., 2014). Such circuits (including switches and oscillators) have been compartmentalised using water in oil droplets (Genot et al., 2016). A spatially distributed configuration has also been engineered through localization of DNA templates by attachment to microparticles (Gines et al., 2017). A related class of DNA-based circuit, also involving strand displacement (Weitz and Simmel, 2012), has been realised in a compartmentalised configuration (Weitz et al., 2014b). Hybrid systems involving compartmentalized cell-free gene expression and compartmentalised/non-compartmentalized cell cultures have also been constructed (Schwarz-Schilling et al., 2016; Lentini et al., 2017). Compartmentalization has also been extended to other biochemical pathways, including a rationally designed and tunable metabolic pathway (Elani, Law, and Ces, 2014; Nijemeisland et al., 2016; Hindley et al., 2018; Peters Ruud et al., 2013).

The mechanisms for compartmentalization include liposomes (Elani, Law, and Ces, 2015; Adamala et al., 2016), water in oil emulsions/droplets to encapsulate cell-free gene expression systems (Torre, Keating, and Mansy, 2014), bacterial colonies (and in some cases each of these in communication (Weitz et al., 2014a)), microemulsions (for nucleic acid based circuits), droplets in hydrogels (Bayoumi et al., 2017), coacervates (Dora. Tang et al., 2015), shape changing polymersomes (enabling reversible compartmentalisation)(Abdelmohsen et al., 2016), porous microparticles (Gines et al., 2017), compartments etched on a silicon chip connected by microchannels (Karzbrun et al., 2014), and proteinosomes (also employed in combination with coacervates and vesicles (Qiao et al., 2016; Tang et al., 2018)). It is clear that a considerable technology for compartmentalizing genetic circuits and reaction pathways is emerging.

The motivation for compartmentalization in cell-free and artificial cells arises from diverse sources. Cell free systems are appealing environments for the characterization/forward engineering of synthetic circuits (Niederholtmeyer et al., 2015; Takahashi et al., 2015). Compartmentalization can facilitate modular bottom-up approaches to building novel functional pathways/pathways that emulate regulatory functions of natural cells, and is a basic ingredient of artificial cells. Compartmental organization in cell free systems facilitates the creation of localized communicating systems, for instance communicating bacterial cultures, and especially communicating hybrid systems (bacterial cultures and cell free gene expression compartments). Spatial organization has been suggested as a basic tool for molecular programming, facilitating speed, modularity and reusability of parts (Chatterjee



et al., 2017). It affords methodologies for programmable pattern formation, distributed organization of information processing and distributed manufacturing, with potentially broad applications in biotechnology and biomedicine. Finally, it also serves as a platform for elucidating the effect of compartmentalization of natural pathways.

While the impressive advances above have created a robust experimental platform for compartmentalization, there are a number of issues to be addressed before compartmentalization can be used in an effective and broad-based way to facilitate the applications listed above. One of the important ingredients missing is a systems-based understanding of the interplay between the potential circuit designs, the spatial organization of compartments, the operational state of compartments and well as the distribution of resources. In a nutshell, even in cell-free environments and artificial cells, the underpinning design principles, and associated framework to guide the choice, rational design and even consideration of spatially distributed transcriptional and related circuits, is largely missing.

We develop a systems framework to elucidate the interplay between the transcriptional circuit, the spatial organization and the operational state of compartments (well-mixed or not). These are some of the most basic common factors involved in the realization of any circuit in cell-free environments and artificial cells. We study a series of prototypical circuits which have already been realized in synthetic biology, with or without spatial organization. We consider both genetic circuits through transcription and translation, as well as other template-based circuits employed in the literature. We study the effect of the organization of compartments in the spatial domain, including spatial topology and boundaries. We consider two broad classes of configurations (both experimentally constructed, one where all compartments are well mixed, and the other where they are not).

The simultaneous consideration of these various factors reveals design principles, constraints and tradeoffs which emerge in the process of either spatially distributing an already designed circuit or designing a priori, a spatially distributed circuit. Since any non-trivial spatially distributed circuit may involve a combination of these factors, dictated by the context, understanding the interplay of these factors is important. It could also be useful in making important basic choices of experimental settings within which to ground the design. Furthermore, new hidden capabilities and features arise from the study which could guide new designs to directly exploit them. In making the progression from modules to systems and developing modular

design, a key question is how spatial organization fits in with this and enables new or improved possibilities. This is also directly relevant to the goal of realizing scalable distributed circuits for applications. Our approach provides a platform for integrating spatial aspects of design with existing design methodologies for these circuits. Finally we point out that while we focus on transcriptional circuits, many of the underlying design principles are also applicable to hybrid circuits involving combinations of genetic circuits and cell populations.

## 4.2 Methods

### 4.2.1 Models

Our analysis focusses on the spatial organisation of information processing biochemical pathways based on a cell-free transcription-translation system. These pathways are constructed using motifs based on transcriptional activation and inhibition between gene regulatory nodes. Each node consists of a gene encoding a specific protein, with this protein either regulating gene expression at other nodes through activation or repression (acting as a transcription factor or sigma-factor at these nodes), or acting as an output signal (eg. fluorescent protein). The gene expression at a node may be either constitutive or regulated by a signal. Thus, each node may also include a mechanism for regulating the expression of its gene. The transcription-translation machinery and resources, as well as degradation machinery for the mRNA and possibly for the proteins, are also an essential component of each node. Thus, the biochemical components of this type of system may be divided into essentially four components - the DNA template, the mRNA, the transcription-translation and degradation resources, and the communicating species, i.e. the proteins.

For the basic case where the entire system is confined to a single, well-mixed compartment, a standard widely-used ODE model can be used to represent the kinetics. In a regime where the transcription-translation resources are non-limiting, the model represents the kinetics of three species at a given node - the promoter sites bound to a transcription factor, the mRNA, and the protein. If we assume that transcription regulation is carried out by a protein dimer, rather than a monomeric protein, the model also has to include the kinetics of the dimer. The equations describing the kinetics at a single node  $X$  take the following form:

$$\begin{aligned}
\frac{dP_X}{dt} &= k_X^b Y_2 (P_X^T - P_X) - k_X^u P_X \\
\frac{dM_X}{dt} &= r_X^c (P_X^T - P_X) + r_X^y P_X - r_X^d M_X \\
\frac{dX}{dt} &= k_X^t M_X - 2k_X^2 X^2 + 2k_X^{-2} X_{dim} - k_X^d X \\
\frac{dX_{dim}}{dt} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_2}^d X_{dim}
\end{aligned}$$

where  $P_X$  represents the concentration of bound promoter sites,  $M_X$  represents the concentration of mRNA encoding the protein, and  $X$  and  $X_{dim}$  represent the concentration of the protein and its dimer respectively.  $Y_2$  represents the concentration of the protein dimer acting as the transcription factor regulating expression at this node. A motif is composed of multiple nodes of this form, interacting with each other through transcription regulation. Whether an interaction constitutes activation or repression depends on the relative values of the transcription rate constants - in this case  $r_X^c$ , the rate constant for transcription from the unbound promoter, and  $r_X^y$ , the rate constant for transcription promoter bound to the transcription factor ( $r_X^c \ll r_X^y$  constitutes activation). Models of the above form can be used to describe different types of gene regulatory motifs, including cascades, positive and negative feedback (including autoregulation), feedforward motifs, with multiple nodes, and also to examine more complex extensions of these. These models also allow us to capture characteristic information processing behaviour associated with such motifs, including bistability and oscillations.

In addition to transcription-translation systems, we also examine purely transcription-based circuits, such as those constructed using the PEN toolbox (Baccouche et al., 2014), which have also been used to realise information processing characteristics such as bistability and oscillations. We use models described in (Genot et al., 2016; Gines et al., 2017) as the basic representation for these systems.

In our analysis, we use the solver ode15s in MATLAB R2014b to simulate the spatially discretized PDE. We use the Matcont package to perform bifurcation analysis of the ODE models. We use custom written code in MATLAB to perform equilibrium continuation for the PDE models.

### 4.2.2 Spatial Organisation

For a given gene regulatory motif (as described above), the obvious approach to spatial organisation, and the one that has been used in multiple experimental studies, is to localise/compartmentalise the DNA templates, at different locations in a spatial domain. Transcription-translation resources are supplied to these template locations (usually in the form of cell extract). If the templates for different nodes are separated in space, this localises the transcription process. This necessitates the transport of the protein (or the associated mRNA) within the spatial domain, from the location of its encoding template, to the location of any other template where this protein regulates transcription, in order to maintain the interactions that make up the motif. For simplicity, we will examine the case where the *communication* between spatially separated nodes is achieved by proteins (and their dimers) that diffuse across the spatial domain to reach their target nodes. We will not consider diffusion of mRNA in the spatial domain (this is consistent with a regime where the timescale of mRNA degradation is much greater than its timescale of diffusion).

For a given motif, there are multiple ways of organising its constituent nodes in a spatial domain. Each of the nodes could be localised in a different spatial location, or multiple nodes could be localised in the same location, and we could even have the same nodes (i.e. the same template) localised at multiple locations within the spatial domain. We examine multiple spatial designs of these kinds for each motif.

The removal of the communicating species/protein from the spatial domain by degradation/leaks is an essential element of the design of these circuits.

The spatial organisation of cell-free DNA template based biochemical pathways (including both transcription-translation systems and purely transcription based systems) has been achieved using various different experimental approaches. These include compartmentalisation by compartments etched on a silicon chip (Karzbrun et al., 2014), by liposomes (Adamala et al., 2016), by water in oil droplets (Schwarz-Schilling et al., 2016), and by binding DNA templates to porous microparticles (Gines et al., 2017). In order to capture the essential features of a spatially organised pathway in different frameworks, and to examine the different regimes that they represent, we focus two types of spatial configurations. The first consists of essentially well-mixed compartments connected by 1-D channels, and the second consists of localised

patches of DNA template in a uniform 1-D spatial domain. The former corresponds to the spatial configuration realised in Karzbrun et al., 2014 while the latter is reminiscent of the scenario in Gines et al., 2017.

**Well-mixed configuration** In this configuration, the DNA templates are localised in well-mixed compartments connected by 1-D channels, with a possible “leak” of the protein (and dimer) from each compartment. The model in this case a combination of the relevant transcription kinetics in the well-mixed compartments (which has a leak) and diffusion of the proteins along the channel between compartments. The leak is modelled by a first order linear term, which incorporates the basic effect of the leak (Tayar et al., 2015). We assume that there is a continuous supply of transcription-translation resources. In our simulations, to start with we ignore degradation of protein in the system, although this could be easily incorporated, and assume equal diffusivities for the protein and its dimer. For a two compartment configuration, the model equations for a protein X are as follows:

In compartment 1 ,

$$\begin{aligned}\frac{dP_X}{dt} &= k_X^b Y_2(P_X^T - P_X) - k_X^u P_X \\ \frac{dM_X}{dt} &= r_X^c(P_X^T - P_X) + r_X^y P_X - r_X^d M_X \\ \frac{\partial X}{\partial t} &= k_X^t M_X - 2k_X^2 X^2 + 2k_X^{-2} X_{dim} - k_X^d X + D_X \frac{A}{V} \left( \frac{\partial X}{\partial \theta} \right)_{\theta=0} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_2}^d X_{dim} + D_{X_{dim}} \frac{A}{V} \left( \frac{\partial X_{dim}}{\partial \theta} \right)_{\theta=0}\end{aligned}$$

Between compartments ( $\theta \in [0, L]$ ),

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_{dim} + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} + D_{X_{dim}} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

In compartment 2 ,

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_{dim} - k_X^d X - D_X \frac{A}{V} \left( \frac{\partial X}{\partial \theta} \right)_{\theta=L} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_2}^d X_{dim} - D_{X_{dim}} \frac{A}{V} \left( \frac{\partial X_{dim}}{\partial \theta} \right)_{\theta=L}\end{aligned}$$

This is similar to models used in the literature, even in conjunction with experiments. Here,  $\theta$  represents the spatial coordinate for the channel,  $L$  is

the length of the channel, and  $D_X$  is the diffusivity of the protein. When the  $X$  dimer regulates transcription in any given compartment, the kinetic equation for  $X_{dim}$  in that compartment will include terms describing binding and unbinding to the promoter sites. The systems we examine in the well-mixed configuration consist of multiple compartments coupled as shown above, with the nodes of a given motif distributed among these compartments in different ways, including cases where multiple nodes are placed in the same compartment, or where the same node occurs in multiple compartments. We will also assume that the compartments are “open” in the sense that there is a continuous supply of TX-TL resources. However, this would also imply the possibility of a “leak” of the communicating species from every compartment, in addition to possible degradation by enzymes. For simplicity, we will use a first order degradation term to represent both these modes of removal of the communicating species. This can be rigorously justified in certain regimes. One of the aspects we will examine is the effect of spatial pattern of organisation (purely in terms of compartment connectivity) on the behaviour of a spatially distributed motif.

**Uniform 1-D channel** In this configuration, the DNA template for a node is localised to a region (compartment) within a 1-D spatial domain, with the protein and dimer allowed to diffuse across the whole domain. Both transcription and translation are assumed to occur only within the localised region of the template, though spatial gradients can exist in this compartment. The model in this case involves transcriptional kinetics in the compartments, with diffusion of proteins in the 1-D channel. While we start by considering open boundaries and ignoring protein degradation in the channel, we subsequently examine this as well.

For a single node  $X$ , the equations are as follows:

In the  $X$  patch ( $\theta \in \Omega_X$ ),

$$\begin{aligned}\frac{dP_X}{dt} &= k_X^b \gamma_2(P_X^T - P_X) - k_X^u P_X \\ \frac{dM_X}{dt} &= r_X^c(P_X^T - P_X) + r_X^y P_X - r_X^d M_X \\ \frac{\partial X}{\partial t} &= k_X^t M_X - 2k_X^2 X^2 + 2k_X^{-2} X_2 - k_X^d X + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= 2k_X^2 X^2 - 2k_X^{-2} X_{dim} - k_{X_{dim}}^d X_{dim} + D_{X_{dim}} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

Outside the  $X$  patch ( $\theta \notin \Omega_X$ ),

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_2 - k_X^d X + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_{dim}}^d X_{dim} + D_{X_2} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

were,  $\theta$  represents the spatial coordinate for the channel, and  $D_X$  is the diffusivity of the protein.

We note in the above, that localisation/compartmentalisation occurs purely due to localisation of transcription/translation, with proteins free to diffuse in the channel. We note that it is possible to consider compartments bounded by membranes in a 1-D channel. Analysis of such a case leads to similar insights from the vantage point of our study. We note however, that physical compartments could, in certain cases, selectively localise proteins as well. We do not examine this case here.

**Boundary conditions and spatial domain.** The spatial domain in each case involves the placement of compartments (equispaced) in the domain. We have studied both circular domains and linear domains. In the former case periodic boundary conditions are applied. In the latter case, we have examined both closed boundaries (in the case of well-mixed compartments) which involve no-flux boundary conditions, as well as open boundaries, which are modelled by zero concentration of species at the boundaries.

We have not examined other modes of biochemical regulation employed in such synthetic circuits, such as sigma factor regulation, strand displacement reactions, post-translational regulation by miRNA, or direct interactions between the signal species from different nodes. We also note that our study, focussing on design principles, has employed deterministic descriptions which best reveals the basic interplay of factors; this could serve as a

platform for a subsequent consideration of stochastic effects.

**Parameters.** The parameters in the model involve transcriptional kinetic parameters as well as parameters associated with compartments and spatial organization. For the motifs we study, we have employed parameters drawn from the literature, wherever possible corresponding to already realized synthetic circuits. These parameters are not varied: in a few places we consider the perturbation of the kinetic parameters to make some specific points. With regard to spatial parameters, parameters such as diffusivities, compartmental sizes and channel lengths are chosen based on values employed in such synthetic distributed circuits. Furthermore, we vary these parameters to study the effect of the interplay of these spatial factors and the transcriptional kinetics. We emphasize that our study focusses on basic design principles and qualitative features. These emerge directly from the interplay between transcriptional kinetics and spatial organization: in many cases they are observed in multiple scenarios (of choice of motif and operation of compartment) further testifying to the essential robustness of the conclusions. In some cases, certain conclusions arise from the interplay of specific motifs and/or spatial organization and we identify those as such and analyze them. We consolidate our computational results with analytical work to pin down the reasons for the behaviour observed. Further details are discussed in the Supplemental information.

## 4.3 Results and discussion

### 4.3.1 Models

We represent transcription-translation circuit motifs using models that are widely employed in the literature for design and engineering of synthetic gene regulatory circuits. We examine simple two node activation/repression, two node feedback motifs, including a bistable system, as well as two oscillatory motifs (a three node ring oscillator and an activator-inhibitor oscillator). Each node in a motif consists of a gene (DNA template) whose expression is regulated by a transcription factor. Spatial organisation of a motif involves localising the DNA templates in different compartments, and having them interact through diffusing proteins. All the transcription-translation machinery and resources are assumed to be non-limiting and available at the location of transcription (and protein synthesis is assumed to be confined



at this location, given the negligible diffusion of mRNA: see Methods section). We consider two types of spatial configurations: (i) well-mixed compartments connected by channels (similar to the configurations constructed in Karzbrun et al., 2014: discussed below), and (ii) localised templates in a uniform 1-D channel (where the localisation could also potentially involve compartment boundaries - see Methods). Both configurations involve some form of removal/degradation of proteins: a leak (and possible degradation) in the former case, and open boundaries/degradation in the latter. These two configurations together capture the essential features of many spatially distributed designs realised experimentally. A detailed discussion of these configurations and the models used is presented in the methods section.

We use kinetic parameters within ranges used in the literature. We keep these parameters fixed while focussing on the interplay between spatial organisation and the nature of the circuit. The basic insights emerge directly from this interplay: in many cases they are observed across different motifs and over both spatial configurations, testifying to the essential robustness of the conclusions.

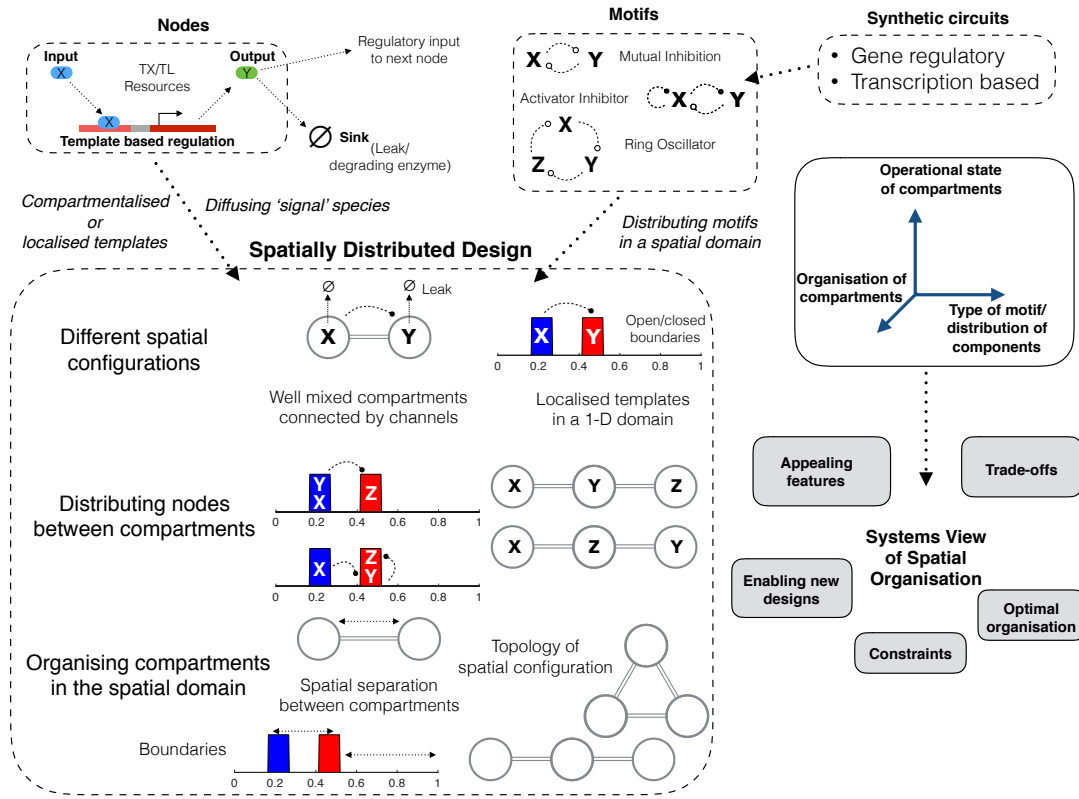


FIGURE 4.1: A schematic representation of the basic ingredients of distributed genetic circuits: a single node (transcription-translation), motifs, and spatial organisation of a motif. A spatially distributed design involves localisation of transcription in (well-mixed) compartments or regions in a 1-D domain: some associated aspects are depicted. Our study provides a systems view of spatially distributed circuit design.

We examine three ingredients: (a) the operational state of the compartments - well mixed or not (b) the nature of the circuit (motif structure) and different ways of distributing its components (c) the effects of spatial factors on circuit behaviour: these factors include compartment separation (relative to other compartments as well as boundaries), topology of spatial organisation, and modular augmentations to a configuration. Some factors (e.g. compartment separation) are examined for all circuits, whereas the effect of other factors is presented in the context of specific circuits, to reveal essentially new insights. This strikes a balance between a comprehensive approach and avoiding redundancy in presentation.

We present the salient insights which emerge from our simultaneous consideration of all these features. We identify which insights are independent

of the nature of the compartment and which ones depend on this. Additional computational and supporting analytical results, are presented in the Appendix at the end of the chapter and the Supplementary Information (also see Table S1 for a summary).

We now present the computational results, starting with the case of well-mixed compartments before proceeding to the uniform 1-D channel. In each case we have studied in detail: (i) the effect of spatially organizing multiple motifs, (ii) the organization of compartments/topology of the spatial domain (Fig. 1).

### 4.3.2 Well-mixed compartments

Certain experimental configurations - for instance (Karzbrun et al., 2014)- involve essentially well-mixed compartments that localise circuit components. The approximately well-mixed operational state of the compartments here (demonstrated experimentally) arises from having connecting channels whose dimensions are small relative to the volume of the compartments.

#### **Basic effects of compartmental organization are revealed through the study of simple motifs**

In all the cases of compartmental organization for well mixed compartments studied below, we assume that two compartments (terminal compartments) are placed at the ends of the spatial domain, in a linear configuration, unless otherwise mentioned. In these cases, a placement of terminal compartments at locations not corresponding to the ends of the domain, give rise to essentially similar results (in fact, exactly the same results for no-flux boundary conditions).

We start by examining some basic aspects associated with spatially organizing circuits in well-mixed compartments. Some of the basic insights pertain to the interplay between the motif, the effect of leak/degradation in the compartments, and spatial separation. These can already be seen clearly in the case of simple motifs. We study the most basic spatially distributed circuit, which involves the DNA template in one compartment, the protein diffusing in the channel, and a second compartment representing an output compartment (where the protein can potentially regulate protein production from a second template). Note that the sequestration of protein through reversible binding to downstream templates does not affect steady state behaviour.

**The presence of a trade-off associated with changing compartment spacing:** The spacing of compartments is one of the most basic design parameters in distributing circuits. Consider the simplest case with two compartments, and a template, expressing a protein X, localised in one of them. We see that the variation of protein concentration as a function of compartment spacing is monotonic in each of the compartments: increasing in the compartment containing the template, and decreasing in the output compartment. This is easily established analytically. While the decrease in the output compartment is expected, it is worth analyzing the reason for an increase in the input ("sender") compartment. The compartments are associated with leaks, representing effectively localized degradation/depletion of the proteins. By moving the output compartment further away, the effect of the depletion in this compartment, is reduced. It is worth emphasizing that this behaviour is essentially different from the case of closed systems (eg: signalling pathways) in a spatial domain: there, an increase in the size of the domain (for the case of a single species diffusing) leads to a reduction in concentration everywhere due to the accentuation of dilution (Alam-Nazki and Krishnan, 2015).

We now explicitly introduce an output node Y (regulated by the first node X) in the second compartment. Here we find that the steady state concentration of the output protein Y can vary non-monotonically with spatial separation (Fig. 2(A)). Interestingly, if the regulation of Y by X involves activation, this non-monotonic behaviour can be seen only in the output compartment. On the other hand, if the regulation involves repression, this non-monotonic behaviour is seen only in the input (X) compartment (Fig. 2(B)).

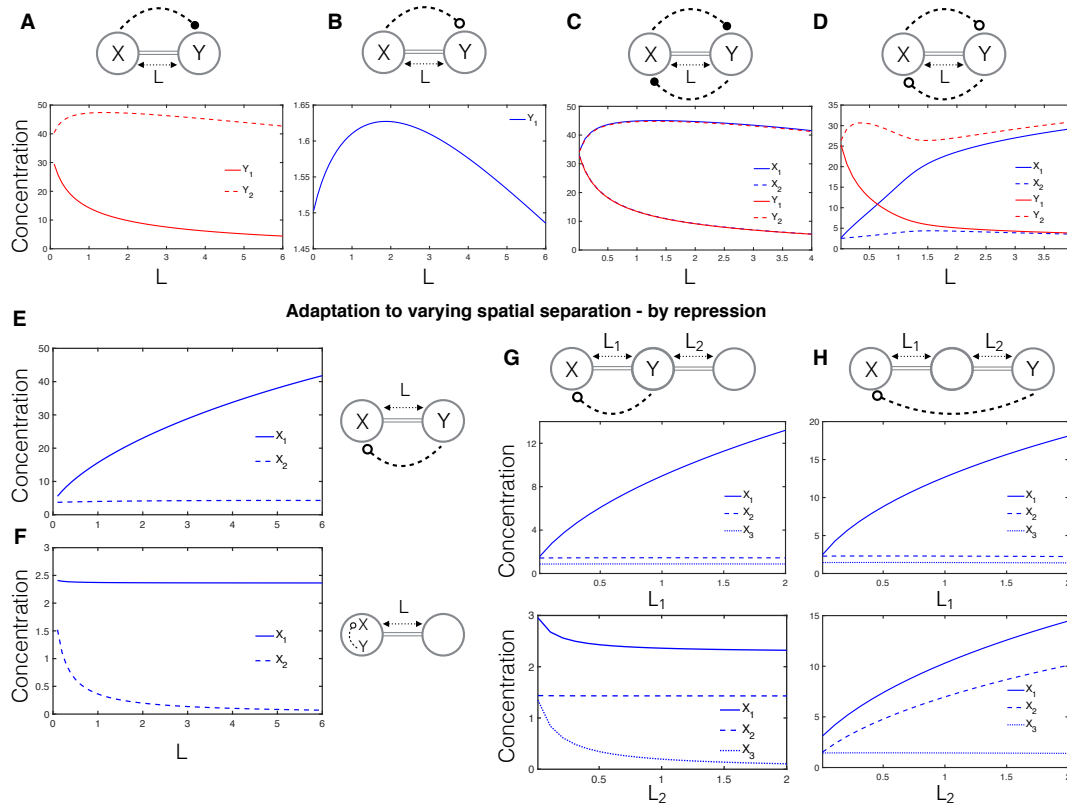


FIGURE 4.2: X produced in compartment 1 and Y in compartment 2. As channel length increases (A) Y in compartment 2 can exhibit a non-monotonic variation if Y is activated by X (B) Y in compartment 1 can exhibit a non-monotonic variation if Y is repressed by X. (C) Positive feedback - Mutual activation: Y in compartment 2 exhibits only a maximum with increasing channel length. (D) Positive feedback - Mutual inhibition: Y in compartment 2 exhibits a maximum and a minimum with increasing channel length. (E) Adaptation to spatial separation. X and Y are expressed constitutively in their respective compartments. X is repressed by Y. The steady state concentration of X in compartment 2 adapts (not exact) to changes in channel length. (F) If X and Y are co-localised, then X is found to adapt in the production compartment. (G,H) This mechanism for adaptation continues to hold good when multiple compartments are involved (illustrated in a three compartment case). In these cases, it is only the X level in the Y compartment that adapts to variation in the lengths of both channels.

The activation case clearly reveals an underlying trade-off associated with increasing compartment spacing: increasing the spacing reduces the effective leak of Y from the X compartment, but also results in reduced expression of Y due to a lower level of X in the Y compartment. This also defines an optimal distance which maximizes the output (Y) in its compartment (analytically

established). On the other hand, in the input compartment, there is no such trade-off.

In the case of repression, increasing compartmental spacing always increases the output in the output compartment, simply because both factors (reduced leak due to input compartment, weakened signal) act in the same way. In contrast, a trade-off is seen for  $Y$ , in the  $X$  compartment. Increase spacing involves reduced repression of  $Y$ , but also a lower level of  $Y$  in the  $X$  compartment. In this context, we note the subtle role of protein dimerisation. The computational results shown are based on dimeric regulation. In the absence of dimeric regulation (if the regulation occurs through the monomer), the trade-offs mentioned above can result in non-monotonic behaviour in the case of activation, but not in the case of repression. All the results presented here are established analytically in the Appendix (Section 4.5.2).

**Consequences for feedback:** We build on this to examine the consequences of the above result for feedback regulation, focussing on a two node positive feedback circuit.

In the case of mutual activation, it is possible to observe non-monotonic variation of either species in its production compartment, associated with a maximum in the species concentration (Fig. 2(C)). Each species exhibits non-monotonic behaviour only in the compartment where it is produced, simply because it is only in these compartments that there is a trade-off (reduction in effective leak versus reduced expression) associated with increasing separation (See Appendix, Section 4.5.2). In contrast, with a mutual inhibition motif, it is possible to observe non-monotonicity of concentration of each species in either of the compartments. Furthermore this non-monotonic behaviour can manifest as a trend associated with both a maximum and a minimum in concentration (Fig. 2(D)). Overall, this demonstrates the reinforcement of the basic tradeoff in the case of feedback, showing how it manifests in different ways depending on the way the feedback is realized.

The insights seen here arise from the the basic ingredients of compartmentalised production/regulation, and localised leaks from compartments. Depending on the design context, the presence of competing effects can define optimal ranges of separation for the desired operation of the compartmentalised circuit. In the context of modular design, where additional compartments may need to be added to an existing circuit, while maintaining its ability to function, similar trade-offs may constrain the design.

### Adaptation to spatial separation

We now return to the basic case of repression without feedback. We have already seen that in this case the output level in the input compartment can exhibit a non-monotonic response to changing compartment separation. However, there is another important aspect here. As seen in (Fig. 2(E)), it is possible to obtain an output which depends very weakly on spatial separation, over a broad range of spacing. This behaviour, reminiscent of (inexact) adaptation, is exhibited by X concentration in the Y (repressor) compartment. Incidentally, even if both species were produced in a common compartment with no production in the other compartment, then this adaptation occurs in the compartment where production occurs (Fig. 2(F)). In essence adaptation of the output species occurs in the compartment where its repressor is produced. We demonstrate the origins of this behaviour, in a slightly simplified model that does not involve dimerisation, in the Appendix (Section 4.5.4). Our computational results show that similar behaviour can be seen even with dimerisation. A basic ingredient for this behaviour, for both monomeric and dimeric regulation, is the saturation of the promoter of the output species and the equal diffusivity of both species.

We now examine how such adaptive behaviour can occur when these species and associated compartments are part of a bigger configuration of species and compartments. At the outset, we note that, as long as the transcription of these species is not regulated by other species, the same behaviour holds good.

Adaptation of steady state compartmental concentrations to varying separation can be achieved by this approach, even when the source template X in the above scenario is subject to regulation from a different node. Achieving adaptation in this case involves dealing with two factors - (i) ensuring the level of the regulating signal (let us call the regulating node Z) is maintained in the X compartment, even as spatial separation is varied, and (ii) having a mechanism of combinatorial regulation of X expression by Z and Y, that allows for repression by Y to have the same effect as before. The first factor can be dealt with by having Z regulated by a repressor that is produced in the X compartment (with similar assumptions). This would produce the same adaptive effect, allowing the level of Z to be maintained in the X compartment. The second factor can be dealt with by incorporating a combinatorial regulation mechanism that does not involve competitive binding. This may be realised in multiple ways, for example, with multiple binding sites on the promoter or by having one of the regulatory signals work as a sigma-factor

controlling the transcription rate.

Building on our analysis of the simple two compartment case above, we now examine whether such adaptation of steady state compartmental concentration occurs in more complex spatial configurations - involving more compartments and channels. Here we consider a three compartment linear configuration - with two connecting channels, and observe that depending on where the repressor Y is produced, the level of X adapts in that compartment to variation in the length of either of the channels. Thus, the adaptive behaviour described above can be achieved even within larger configurations of compartments, and the level of the output can adapt to variations in the lengths of multiple channels. This can be demonstrated analytically using a simplified model (See Appendix, Section 4.5.4). Furthermore, our analysis also implies that adaptation can be achieved in the face of modular augmentations to a given configuration of compartments. If a compartment is added to an existing configuration, the level of X in the Y compartment is maintained, so long as the newly added compartment does not introduce additional production or transcriptional regulation of X.

Overall, enabling adaptation to spatial separation could be a valuable design tool, especially since it can be achieved in a broad range of settings.

### Compartmental organization of bistable motifs

**The consequence of distributing a bistable motif and the effect of spatial separation of compartments:** We now turn to the case of a bistable circuit realized by mutual inhibition (Fig.3). If we contrast the completely localized circuit in a single (well-mixed) compartment, with that distributed between two compartments, we find that the switching thresholds associated with the input are significantly distorted. In the present context, the input signal is assumed to be the level of constitutive expression of the node X, regulated by an external signal (Note that our conclusions are valid for other input signals as well). In fact, it is possible to have a localized circuit realizing a one-way switch, converted to a bistable circuit exhibiting a two-way switch.

For fixed kinetic parameters, increasing the spatial separation can result in a loss of bistability. Bistability can however be restored by increasing the template concentrations - a natural tunable parameter for synthetic circuits. This is further consolidated in Fig.3 by the 2-parameter diagram for a simplified model (see Supplementary Information), which neglects dimer degradation and dimerization in the channel, though the basic effect does not depend on these simplifications.



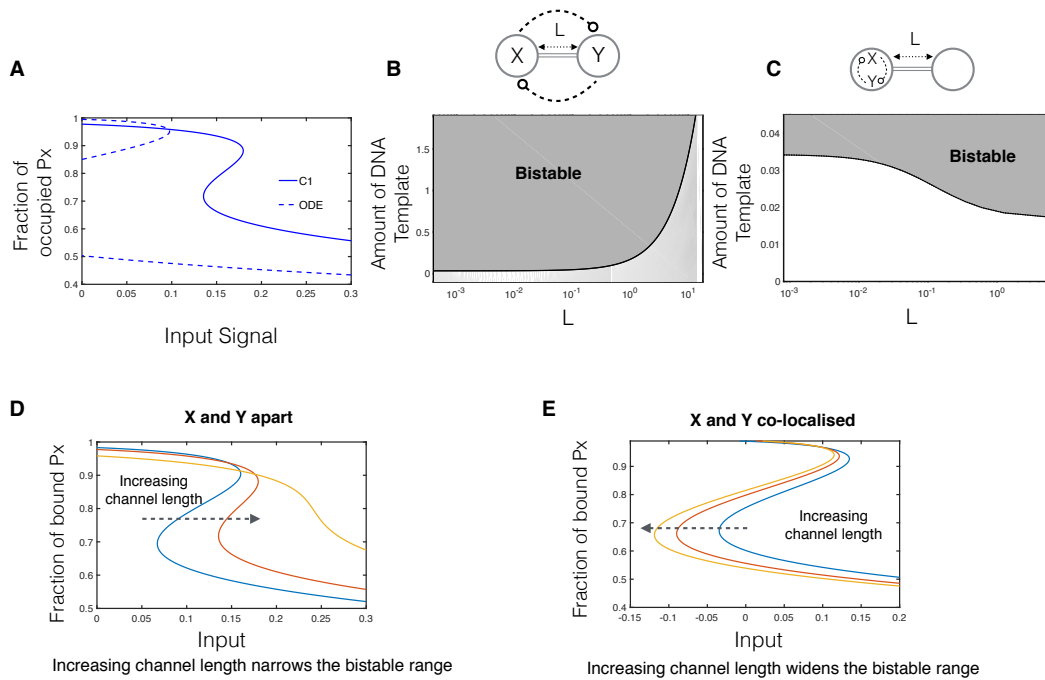


FIGURE 4.3: (A) Bistable motif - two node mutual inhibition: In going from localised to distributed organization, both the thresholds and the reversibility of switching may be affected (all kinetic parameters kept fixed). (B) Increasing channel length can destroy bistable characteristics but this can be restored by increasing the amount of DNA template (Two parameter bifurcation diagrams generated using a simplified model: see text). (C) If the two nodes of the bistable motif are co-localised, increasing channel length now makes it possible to get bistability at lower total amounts of template. (D) and (E). One parameter bifurcation diagrams depicting different effects of increasing compartment separation in the two scenarios - when X and Y are separate, increasing channel length narrows the range of bistability, while in the co-localised case, it widens the range.

**The qualitative effect of spatial separation depends on the distribution of circuit nodes across compartments:** Finally, by way of contrast we examine a different spatial organisation of the bistable motif, where the production of both species occurs in the same compartment, with a second “output” compartment. Here we find that increasing compartmental spacing has a qualitatively different effect: the bistability is reinforced, with an increased range (Fig.3E). Increasing channel length essentially reduces the effect of the

leak from the second compartment, in contrast to the former case, where increased separation weakens the interaction between nodes.

This is demonstrated both through bifurcation analysis of the PDE, as well as a 2-parameter diagram of the simplified model (Fig.3C) (also see Supplementary Information). These results indicate the possibility of using spatial parameters, as a lever to tune bistable behaviour, for example adjusting compartment separation to transition between irreversible switching behaviour and a toggle switch. Furthermore we see that, for tuning a given circuit, the nature of the perturbation introduced by varying a particular spatial parameter can depend on the chosen spatial organisation of the nodes, in addition to the network structure of the circuit. Given that it is possible control circuit function through spatial parameters, these considerations may be relevant in deciding the original spatial distribution of the circuit.

**Modular augmentation of a bistable circuit:** We can build on these insights to study the effect of a modular augmentation of a bistable circuit: the incorporation of an additional compartment (and associated connecting channel) connected to the X compartment (see Supplementary Information). In this context we note (i) this modular augmentation can completely destroy bistability due to the distortion introduced by an extra leak (ii) bistability can be restored by, for instance, introducing a degree of X production in the new compartment. However, if this production of X in the new compartment is increased beyond a certain range, bistability can again be lost due to a disruption of the balance between X and Y, which is responsible for the creation of bistability in the first place.

Finally we find that, in cases where the whole motif is localized in a particular compartment, within a network of connected compartments, the possibility of bistable behaviour may depend on the location of this compartment within the entire compartmental configuration (see Appendix, Section 4.5.3).

### Compartmental organization of oscillators

**Different ways of distributing an oscillatory circuit has qualitatively different implications:** We focus on a three compartment system connected through channels (in a linear configuration), and examine the effect of organizing a standard negative feedback repressilator circuit in this distributed set-up (Fig. 4(A)-(D)). We employ a repressilator circuit with kinetic parameters corresponding to oscillations. Now if we distribute the three nodes in

sequence in the three compartments, we find that the circuit sustains oscillations for the chosen compartment spacing, though this may be destroyed by increasing the spacing. We find that increasing the template concentrations of the species (equally) can restore oscillatory behaviour. However, even with increased template concentration, increasing compartment spacing will eventually destroy oscillations.

For the given compartment spacing, a redistribution of the motif with 2 nodes co-localised in one compartment and one in the other (both terminal compartments) was found to abolish oscillations (Fig.4(C)). A second point to make is that if the nodes are not symmetric (i.e. not identical values for kinetic parameters), the order in which nodes are placed in compartments can also have a significant effect: swapping the locations of the second and third nodes was found to destroy oscillations (Fig.4(D)). This shows that with a given compartmental configuration in a spatial domain, the manner of distribution of the motif can be critical. In effect, the differences in distribution of interaction strengths/delays between nodes, associated with different designs, can produce qualitatively different behaviour.

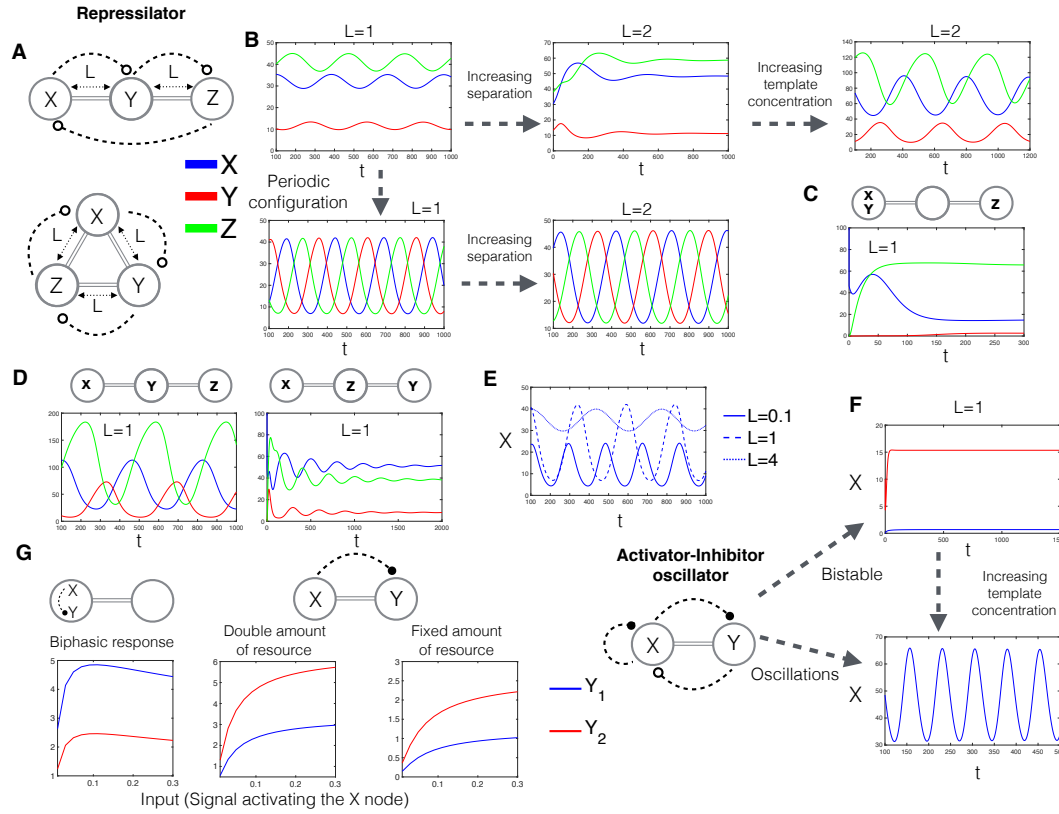


FIGURE 4.4: (A) Schematics of different spatial topologies for the distributed negative feedback oscillator. (B) Linear vs periodic configuration - both configurations exhibit sustained oscillations for the chosen compartment spacing and template concentration. The periodic configuration exhibits commensurate oscillations for all three species. Doubling channel length disrupts oscillations in the linear configuration (but not the periodic configuration), but this can be restored by increasing template concentration. (C) For a compartment spacing where the basic linear configuration produces oscillations, co-localising two of the nodes (X and Y here) does not produce sustained oscillations. (D) When the nodes are not symmetric, arranging the compartments in a different order can disrupt the oscillatory behaviour. (E) Compartment separation can be used as a lever to tune frequency of oscillations. Here we see a significant difference in the frequency of oscillations for three different channel lengths. (F) Activator-inhibitor (see schematic)-localised to distributed design: the localised system gives oscillations (not shown), but the distributed (two compartment) system is bistable. Simulations with two different initial conditions exhibit different steady states. Oscillatory behaviour may be restored by increasing template concentration. (G) For a two compartment scenario with X and Y co-localised, the resource sharing gives rise to a biphasic response to the input. Placing Y in the second compartment, and doubling the amount of enzyme prevents the biphasic response. If the initial total amount of enzyme is redistributed between the two compartments, the biphasic response is prevented, but the output level is lower than the co-localised configuration.

**The role of topology of spatial organisation:** We then examined the effect of a change of topology of compartments from linear to circular. For a fixed compartment spacing, it was found that the circular topology was able to generate oscillations in the case above, where the linear topology did not. Furthermore, multiple computational studies for a symmetric repressilator point to an increased range of oscillations with respect to compartment spacing (assumed uniform). Note that the periodic distribution introduces commensurate perturbations to all three nodes, rather than incommensurate distortions. More generally, a periodic configuration allows for the maintenance of the symmetry of a (symmetric) repressilator, in contrast to the linear configuration (see Fig. 4(B)). The above insight suggests the possibility that manipulating topology of compartment connectivity in a flexible manner may be used to control circuit function.

**The roles of leak and separation in determining oscillatory behaviour:** In our transition from a motif in one compartment to a motif distributed between three compartments connected by channels, two new factors emerge: an additional delay caused by the fact that nodes (templates) are spatially separated, and a depletion/leak of proteins in compartments other than that of their production (in effect an additional leak). We have seen that this can destroy oscillations, and we have also seen that oscillations could be restored by changing template amounts. The leak of proteins through additional compartments (based on the study of simpler cases above) could be expected to destabilize oscillations, leading to a steady state. Interestingly, we find that the additional depletion can also play positive role in sustaining oscillations. This is seen by examining *in silico*, the case where oscillations are observed in the distributed configuration and restricting the leak of each species to the compartment where it is produced: this by itself can destroy oscillations. More generally, it suggests that the spatial organisation of protein removal can be a key determinant of system behaviour. Additionally, depending on the design, the compartment spacing may be an accessible dial for tuning frequency (Fig. 4(E)).

By way of contrast, we examine a different oscillator: an activator-inhibitor system exhibiting relaxation oscillations (similar to Stricker et al., 2008). In this case (Fig. 4(E)-(F)), by distributing the oscillator across two compartments, we note (i) it is possible to get oscillations if the compartments are not too far apart and (ii) if the compartment spacing is increased, oscillations are abrogated, with the distributed system exhibiting bistability. This behaviour

is easily rationalised, noting that the circuit implements positive feedback by direct self-activation, with negative feedback realised through a different species.

The above analysis highlights the important role of spatial organization in tuning some of the critical factors that determine the behaviour of synthetic oscillatory circuits - distribution of depletion/degradation rates for the different nodes (Page and Perez-Carrasco, 2018), the strength of interaction and time delay between nodes. This insight is also relevant in the context of building more complex circuits involving oscillators - coupling oscillators to other circuits, engineering oscillators based on a larger number of nodes - or implementing the basic oscillatory circuit in more complex spatial topologies.

### **Spatial organization and competition for resources:**

Synthetic genetic circuits involve resources (for example transcription-translation machinery, or degrading enzymes) that may be shared by different nodes. With multiple nodes competing for a limited amount of resource, this can introduce additional level of interactions between the nodes, which may significantly distort the expected behaviour of the circuit. Spatial organization that separates nodes and decouples resources can mitigate these effects (Fig. 4(G)). Here we consider a simple two node (sender-receiver) motif which we use to illustrate this point. We contrast the co-localized circuit in one compartment with a distributed circuit in two compartments. For illustrative purposes we consider one resource, the RNA polymerase enzyme, though this applies to other resources as well. Regarding resources, we examine two scenarios: (i) one where the total amount of enzyme is conserved and (ii) the other where the second compartment is associated with an extra supply of enzyme. For the co-localized circuit in one compartment, resource limitation can result in biphasic response of the output to varying input signal. This biphasic response can be eliminated in both the two-compartment scenarios. If the total enzyme is conserved, this could reduce the level of the output. On the other hand if an extra resource is added in the second compartment, this reduction of output can be partially alleviated.

#### **4.3.3 Uniform channel**

We now examine circuits distributed in a uniform 1-D channel. This represents a broadly occurring scenario of spatial organisation without well mixed

compartments. The templates for production of various species are localized at particular locations (compartments). Open boundaries and spatially distributed degrading enzymes provide an outflow. Our analysis reveals both distinct aspects which emerge when considering these set-ups and results which draw parallels with the well mixed compartment scenario.

Our examination of a uniform channel does not explicitly incorporate the effects of membranes bounding compartments. However, the steady state behaviour in scenarios involving such membranes is in many respects essentially similar, and many of the insights obtained here may be expected to hold good in such cases (see Appendix, Section 4.5.6).

In the well-mixed configuration, our focus was entirely on the levels of proteins inside the compartments. In the current instance, we focus on (1) local concentration of proteins in compartments (2) spatial profiles and global features of protein concentration, such as spatial average of the protein over the whole domain. The latter may be particularly important in cases where the proteins regulate downstream processes outside the production compartments.

### **Basic effects of compartmental organization**

We focus on two basic measures of output species concentration that may be relevant: the concentration in a given compartment/location and the average concentration over the length of the channel. When we consider the most basic case of a single compartment, we find that both local and spatial average concentration of species concentration depends on the location of the compartment relative to the boundaries. Both measures of concentration are maximized when the compartment is placed in the middle of the domain (minimizing the net effect of leak to boundaries) (see Appendix, Section 4.5.7). This has implications for the case of a simple two node (sender-receiver) motif. If the sender is at a fixed location, not in the centre of the domain, the configuration that maximises the spatial average of the receiver output may involve placing the two nodes apart at a well defined distance (Fig. 5(A)). This results from a trade-off between a reduced sender signal (at the location of the receiver) and increased proximity to a boundary (increasing the effect of leak to the boundaries).

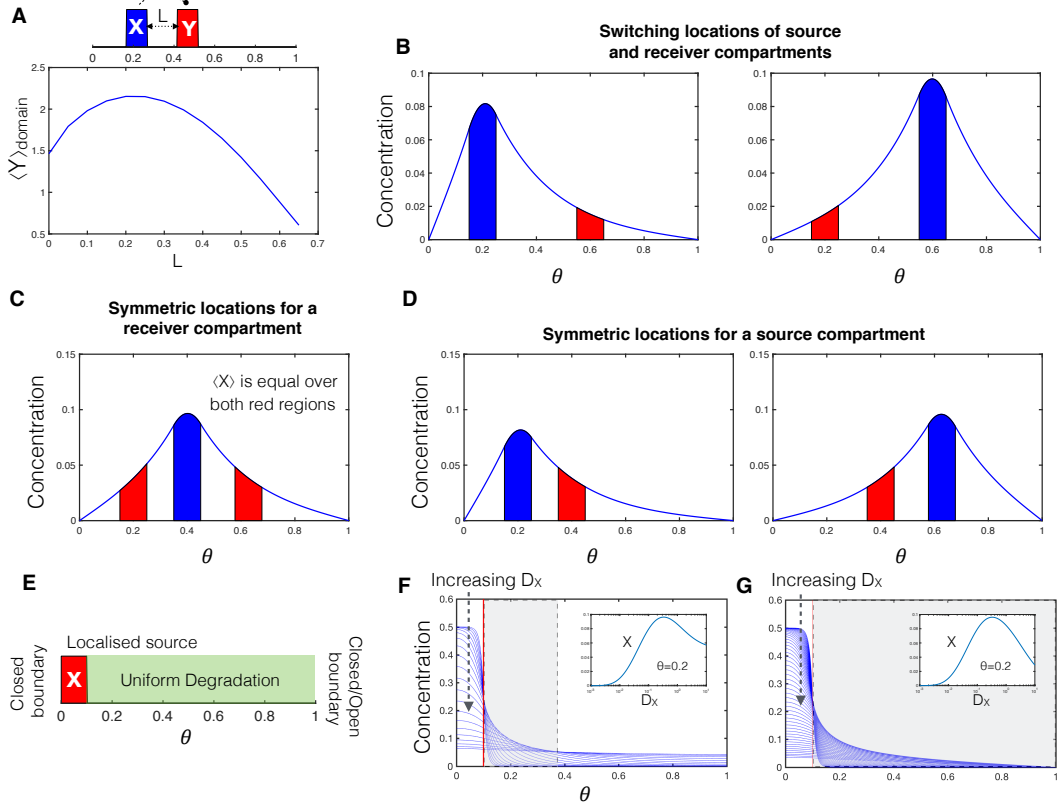


FIGURE 4.5: (A) 1-D channel with open boundaries and no degradation or dimerisation: A maximum of the spatial average of Y in the domain is obtained for an intermediate separation between X and Y (X positively regulates Y), indicative of a trade-off. (B) 1-D channel with open boundaries and uniform degradation in the domain (no dimerisation): Swapping of sender and receiver locations maintains the average of the sender signal over the receiver patch. (C,D) To obtain the same average concentration over a receiver compartment there exist (C) two "symmetric" locations of the receiver for fixed location of sender (D) two "symmetric" locations of the sender, for a given receiver location (see text). (F) Non-monotonic dependence of steady-state concentration profiles on diffusivity. In a closed domain, the region of non monotonicity only covers part of the domain outside the production patch. (G) With an open boundary, the region of non-monotonicity always extends from the edge of the production patch to the open boundary, irrespective of the size of the production patch.



### Multiple spatial configurations that preserve interaction strengths

A distinct aspect of relative compartmental organization appears in the 1-D channel case. This is associated with the multiplicity of configurations of sender and receiver locations which guarantee the same sender signal level in the receiver compartment (averaged over the receiver compartment). For a given location of sender and receiver (i) an interchange of locations leaves the average of sender signal over the receiver compartment unchanged (Fig. 5(B))(ii) if we keep the receiver location fixed, there is another location of sender (guaranteed to lie in the spatial domain if there are open boundary conditions: see Appendix, Section 4.5.5) that will give the same average sender signal level in the receiver compartment (Fig. 5(D)) (iii) keeping the sender compartment fixed, there is an alternate location of receiver to maintain the same average of sender signal it receives (Fig. 5(C)). This indicates underlying symmetries which can be exploited to achieve flexibility in design. This result, which is based on an analysis of relatively small compartments, and assuming no overlap between them, also applies when the compartment size is larger. However, depending on the width of the compartments, (in relation to the size of the domain) there could be additional constraints associated with realising some of these designs (for example compartments overlapping or not being contained within the spatial domain). For a given circuit involving multiple nodes, the above multiplicity of configurations gives the flexibility to maintain interaction strengths between some nodes, while changing others.

The above analysis was based on basic circuits which incorporate only the protein monomer. We note that many of the transcription based circuits described in the literature involve only monomeric regulation (Baccouche et al., 2014; Weitz and Simmel, 2012). Even in the context of transcription-translation systems, it has been shown that non-linear behaviour like bistability can be achieved through monomeric regulation alone (Widder, Macía, and Solé, 2009; Padirac, Fujii, and Rondelez, 2012). The insights we have obtained continue to hold good even with protein dimerisation, if the dimer is non-diffusible and does not degrade. Furthermore, if the dimer diffuses and degrades at the same rate as the monomer, the same insights hold good for the total concentration of protein (see Appendix, Section 4.5.5). Note that, if the focus is on the average of the dimer, the corresponding symmetric location may be different to that computed above.

### Effects of diffusivity of species

In the case of well-mixed compartments, the effects of diffusivity of species are easy to understand at steady state. In fact if there is no degradation of protein in the connecting channels, this can be investigated through a compartmental model. Localisation in a uniform channel brings new features when protein degradation is present. For a single compartment in a domain with either open or closed boundaries, increasing diffusivity leads to a non-monotonic variation in steady state concentration over a range of locations, arising from the fundamental interplay of diffusion and degradation. With open boundaries, the “spatial zone” where non-monotonicity occurs, was always found to be outside the compartment and extends all the way from the compartment boundary to the boundary of the domain (Fig. 5(G)). With closed boundaries, depending on compartment size, this region could even be within the compartment. Even when outside, it may not extend all the way to the boundary (Fig. 5(F)).

The presence of these regions of non-monotonic variation may have important consequences for design. It suggests that there are possible locations of the receiver node, where tuning the steady state signal level by changing diffusivity involves a fundamental trade-off.

### Spatial organization of degradation

Degradation of proteins (and DNA/mRNA for other nucleic acid based circuits), is a key tunable dial of many synthetic circuits (Gines et al., 2017; Tomazou et al., 2018). Here we focus on the effect of spatially organizing a degrading enzyme (common to all nodes of the circuit: mirroring the approach used in experiments). We consider a two-node (sender-receiver) motif with sender and receiver localised at different locations within a 1-D channel, with closed boundaries (Fig. 6(C)). At the outset, one can consider different spatial distributions of this degrading enzyme: either it is present everywhere in the domain, or localized in specific regions, for instance co-localised with one or more nodes.

**Localised degrading enzyme:** We first make some broad observations regarding the presence or absence of gradients of protein concentration. If the degrading enzyme is localized in only one of the compartments, the corresponding species (whose production occurs there) exhibits a flat steady state concentration profile steady state throughout the domain. Furthermore in

this case, the other species exhibits a gradient only in the region between the place of its production and that of its degradation (Fig. 6(C)). Now, irrespective of whether the degrading enzyme is co-localized with the sender or the receiver, the average of the sender signal over the receiver compartment remains the same. However, the receiver output is different because the enzyme also degrades the receiver output. This demonstrates, how altering the localisation of degrading enzyme can affect the output without altering the interaction between nodes. However the spatial average of the sender signal over another location or over the entire domain is different.

**Degrading enzyme distributed between sender and receiver:** We now examine the effect of distributing a fixed total amount of degrading enzyme between the two compartments. Here we find (Fig. 6(E)), that (i) the average of the sender concentration over the receiver compartment varies symmetrically with the distribution of enzyme in the two compartments, exhibiting a minimum at equal distribution and maximum at the two extreme distributions, (ii) the average sender concentration over the spatial domain varies asymmetrically with the distribution of enzyme, exhibiting a maximum when the enzyme is co-localised with the receiver, (iii) the average of the receiver output over the receiver compartment varies asymmetrically with the distribution of enzyme, also exhibiting a maximum when the enzyme is co-localised with the sender. The last feature arises from the fact that, while the production rate of the receiver output varies symmetrically (as a result of (i)), its degradation rate varies asymmetrically (maximum when all the degrading enzyme is co-localised with receiver).

**Uniformly distributed degrading enzyme:** Finally we note that the uniform distribution of the same total amount of enzyme over the whole domain, is another natural design. In this case, gradients of both sender and receiver signal exist throughout the domain. Furthermore the spatial average of the sender is the same as that in the scenario where all the enzyme was in the sender location (in which case the sender profile was spatially uniform). A similar conclusion applies to the receiver as well. The distribution of the degrading enzyme over the whole domain can lead to a higher average of the receiver output over its compartment than any co-localised design of degrading enzyme. Thus if maximisation of receiver output is desired, a completely non-localised distribution of degrading enzyme may be an appealing option.

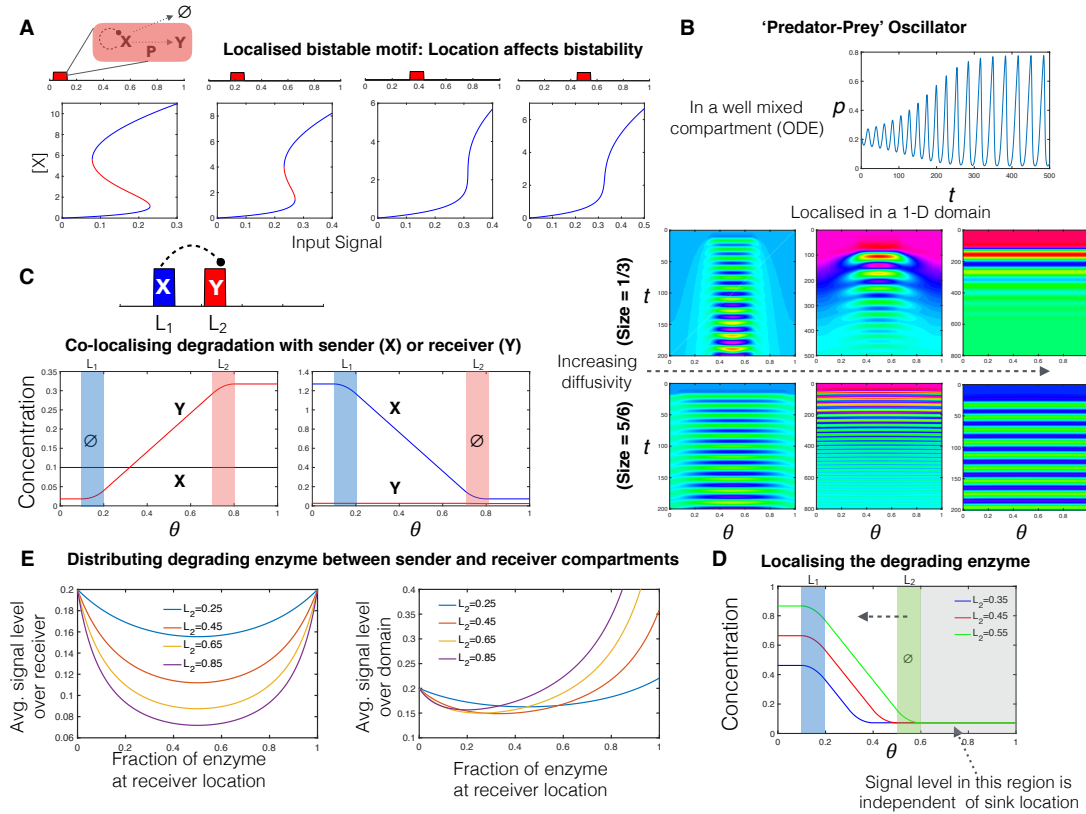


FIGURE 4.6: (A) Localising a single node bistable motif in a 1-D domain with closed boundaries and uniform degradation. Depending on the location of the compartment within the domain, the motif may or may not exhibit bistability. (B) Localising an oscillator in a 1-D domain, with closed boundaries and no degradation outside the compartment. One of the species is allowed to diffuse throughout the domain. Depending on the diffusivity, this can disrupt oscillatory behaviour. For low diffusivities, the system produces oscillations essentially localised within the compartment. For higher diffusivities, the oscillatory behaviour is de-stabilised and the system exhibits damped oscillations. In certain cases, depending on compartment size higher diffusivity can cause the system to revert to oscillatory behaviour (global oscillations). (C) Spatial organisation of a common degrading enzyme in a sender-receiver system (fixed separation). Co-localising the degrading enzyme with the sender or receiver affects (i) the possibility of forming concentration gradients (ii) the spatial average of a species in the domain. Co-localising with the sender (receiver) produces a flat profile for the sender (receiver) and a graded profile for the receiver (sender). (D) A localised sink in the domain creates a region where the concentration of the sender species is spatially uniform and independent of separation between the sender compartment and the sink location. (E) Distributing the degrading enzyme between the sender and receiver compartments. Both the domain average of the sender species and its average over the receiver compartment vary non-monotonically as the fraction of degrading enzyme in the receiver compartment is increased. The variation of the domain average is asymmetric, while the variation of the average over the receiver compartment is symmetric.

**Effect of localisation/non-localisation of degrading enzyme on global features of output:** We note that (i) a uniformly distributed degrading enzyme results in the same spatial average of the sender over the full domain as localising the degrading enzyme in the sender production compartment (ii) localisation of degrading enzyme in one compartment (sender/receiver) results in a gradient of only one of those species, and only in the region between the two compartments, depending only on compartment separation. This is in contrast with uniform degrading enzyme resulting in gradients everywhere, which depend on the size of the overall domain.

Degradation pathways for species are a vital tuner for the behaviour of synthetic circuits (Rondelez, 2012; Niederholtmeyer et al., 2015; Tomazou et al., 2018). There are several key aspects to its role in design, which have been studied, including the possibility of saturation of degrading enzymes, competition for degrading enzymes between multiple species, and maintaining commensurate degradation rates for all species. The results above indicate that the spatial organisation of degrading enzymes may be just as important, and expands the design space for synthetic circuits. Species-specific degrading enzymes have also been employed in synthetic circuits in the literature. The use of such enzymes may present additional possibilities for tuning circuit behaviour in combination with spatial organisation.

### **Adaptation to spatial separation**

We focus on the design goal of maintaining a protein concentration at a location, independent of the distance from its production location: an analogous problem was studied in the well-mixed configuration. Building on the results above, we note that this is achieved, if (i) the degrading enzyme is co-localised with the location of transcription (the steady state concentration profile is uniform), or (ii) the degrading enzyme is entirely localised at the desired output location. On the other hand, if the degrading enzyme is present in more than one location/region, or even present uniformly, this will not happen. Can the repressor circuit we have employed previously work here? We find that this can work, subject to two restrictions (i) the compartment sizes are small relative to that of the domain (ii) the degradation rate constant and the diffusivity must be the same for both species (iii) the repressor compartment cannot be too close to an open boundary, as this would violate the assumption of regulation in the saturated regime. The insights relating to

adaptation in configurations with more than two compartments, and adaptation to modular augmentations, seen for the well-mixed compartments, are also valid here.

### Localised bistable and oscillatory circuits in a uniform channel

For a synthetic circuit such as a bistable switch or an oscillator, designed to perform a specific function, the ultimate goal may be to have them regulate certain ‘downstream’ processes, or communicate with other synthetic constructs (or even microbial populations). In these contexts, when such a motif is compartmentalised in its entirety, its ‘output’ is required to diffuse out of the compartment and spread to other locations in the spatial domain, where its targets may be located. Here we demonstrate that letting an ‘output’ species diffuse out of the compartment and across the spatial domain (with possible degradation in these regions) can have important consequences, in that circuit behaviour may now depend on spatial parameters such as location relative to boundaries, and diffusivity.

**Bistable behaviour can depend on location relative to boundaries:** In order to examine the basic effects of localizing a bistable motif in a spatial domain, while letting its output diffuse across the whole domain, we examine a single node, auto-catalytic bistable motif as experimentally used by Gines et al., 2017. This system is particularly suitable for analysis in the present context, as it has only one output species, and involves no dimerisation. In addition, a similar spatially distributed design (involving localised templates, diffusing output, and uniform degradation) has been realised experimentally. With uniform degradation over the domain, we find that the possibility of the motif exhibiting bistability depends on the location of the compartment relative to the boundaries (Fig. 6(A)): in fact, the propensity for bistability is greater when the compartment is closer to the boundaries. This is because these locations correspond to the lower “effective” degradation (see Appendix, Section 4.5.9). There is an interesting contrast in the effect of compartment location, in the case of an open 1-D domain (with no degrading enzyme: the boundary acts as the “leak”). Here the closer the compartment is to a boundary, the greater the propensity for destruction of bistability.

**The effect of diffusivity on circuits:** In addition to boundary effects, the diffusivity of species, can play an important role, in affecting circuit behaviour, as seen earlier. This is also seen in the bistable circuit. Here we focus on a

distinctive aspect of the effect of diffusivity, which is seen in the case of oscillatory circuits. We examine an oscillatory motif whose components are produced in a single compartment in the domain (closed boundaries, for specificity) with the degrading enzyme also localised there. If the compartment was “sealed off”, oscillations would result. We now examine the effect of allowing one of the species to diffuse out of the compartment, into the surrounding domain. We find that this can destabilize oscillations, giving rise to a spatially uniform steady state (the steady state corresponds to that of the ODE model incidentally). This destabilization depends on the surrounding domain being sufficiently large: if the compartment occupies a sizeable fraction of the domain, this destabilization may not happen. In terms of species diffusivity, we see that a larger diffusivity generally increases the propensity for the attainment of a steady state and the destruction of oscillations. Interestingly, as seen in Fig. 6(B), in some cases, increasing the diffusivity further, can result in the reappearance of oscillations. Furthermore in these cases, oscillations may persist for high diffusivity, in which case they represent global, essentially uniform oscillations in space (see Supplementary Information).

#### 4.3.4 Applications

We now consolidate and extend our earlier results in the context of exemplar applications.

##### **Adaptation to template concentration**

An important aspect of compartmentalising genetic and other nucleic acid based circuits is robustness to variations in template amounts. The nature of the process used for spatial organisation of components may limit precise control of the amount of DNA or RNA templates encapsulated in a compartment. We now discuss how adaptation to template concentrations can be obtained at different spatial locations, and furthermore, how this can be combined with another appealing feature that we have discussed - adaptation to spatial separation.

Negative regulation has been used to design (and experimentally realise) synthetic gene regulatory circuits capable of adaptation to template concentrations (Bleris et al., 2011). This type of circuit involves repression of the output by a second protein that is expressed from the same template.



We now examine how this functions in a spatially distributed circuit involving a well-mixed configuration. We first consider a basic implementation of the Bleris et al., 2011 type circuit, where a protein X and its repressor Y are produced from the same template. In a two compartment configuration, with this template in one compartment, we see that, if both X and Y diffuse equally: (i) adaptation of X to template concentrations occurs in both compartments, (ii) while X adapts to spatial separation in the production compartment, it does not adapt in the second compartment.

Our analysis in previous sections shows that adaptation to spatial separation in the second compartment can be achieved by having a repressor produced there. Combining such a repressor (which we call Z) with the above circuit may allow for adaptation to both spatial separation and template amounts in the second compartment. Achieving this requires working within certain constraints (see Appendix, Section 4.5.4): (i) the combined regulation by the two repressors Y and Z must not involve competition for binding sites (ii) the repressor Y must be confined to the production compartment. A possible way of constructing the above features, would be to combine regulation at both transcriptional and translational levels - the former through transcription factor regulation and the latter through miRNA regulation involving an RNA induced silencing complex (as demonstrated by Bleris et al., 2011). Having Y as the miRNA regulator and Z as the transcription factor is a way of realising these features. Thus we see how adaptation to template concentration can be combined with adaptation to spatial separation, in a given compartment, for any choice of template location.

### **Communication with a target**

A basic application of a distributed circuit is one where a circuit communicates with a target, which may be at a different location. A further level of complexity arises from the possible spatial organisation of circuit components themselves. We examine these aspects in the context of a representative class of examples involving communication between a circuit and a target.

The placement of a synthetic circuit relative to its target may involve multiple considerations, including the nature of the target and the circuit, the compatibility of the circuit with the target environment, possible barriers around the target, degradation/dilution affecting the diffusing signal species. A natural criterion for determining the placement of a circuit in this context might be to to maximise the level of the signal at the target location, thereby ensuring effective communication to the target. Here we examine



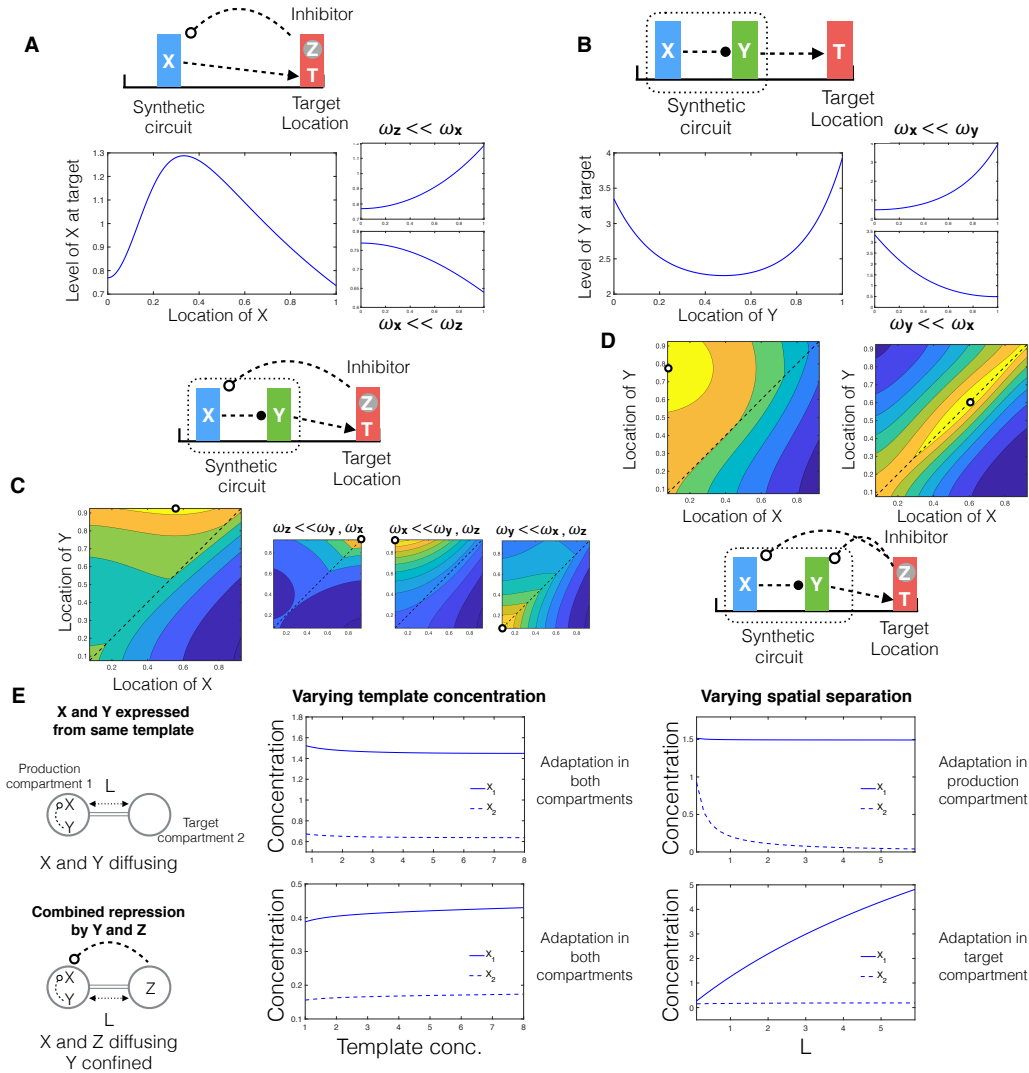


FIGURE 4.7: (A)-(D) Communication with a target in a 1-D closed spatial domain. (A) Single node circuit (output X) with diffusing inhibitor (Z) produced at target location. With comparable  $\omega_X$  and  $\omega_Z$ , the optimal location may be an intermediate one. With  $\omega_X$  much smaller than  $\omega_Z$ , optimal placement requires maximising the separation between the circuit and the target. On the other hand, co-localisation of circuit with the target performs best if  $\omega_Z$  is much smaller than  $\omega_X$ . (B) Two node circuit (output Y). With  $\omega_Y$  much smaller than  $\omega_X$ , optimal placement requires co-localising the two nodes. With  $\omega_X$  much smaller, it is better to separate the two nodes. (C) Inhibitor targeting only node X. Depending on the  $\omega$  for the two nodes and the inhibitor, the optimal placement may involve co-localisation away from the target, co-localisation at the target, or separating the two nodes. Marker indicates optimal location. (D) Inhibitor targeting both node X and Y. Even with the freedom to separate the two nodes, the optimal placement may involve co-localising the two nodes at an intermediate location. (E) Adaptation to template concentration. With X and its repressor Y produced from the same template (and both equally diffusible), steady state levels of X adapt to changing template concentration in both compartments. However, adaptation to spatial separation is achieved only in the production compartment. (F) With the combined action of two repressors Y and Z (see text for details), and Y non-diffusible, adaptation to both template concentration and spatial separation is achieved.

the problem of optimal placement with respect to this criterion, in the presence of two basic restricting factors - negative regulation of the circuit by the target through a diffusing inhibitor produced at the target, and degradation/dilution of the diffusing species. These factors are also representative of more general restricting factors associated with the target location, which may constrain the placement of a synthetic construct in different applications.

We examine this system in a 1-D channel, which is broadly representative and does not necessitate special assumptions such as well mixed compartments. We consider closed boundaries, although similar effects can be seen when boundary effects are absent. We focus on the level of the circuit output signal at the target location, which is assumed to be at one end of the channel.

**Single node circuit:** In the simplest case - that of a single node circuit - the parameter determining the optimal circuit location is the diffusivity scaled by the degradation rate (referred to as  $\omega$ ), for the circuit output ( $\omega_o$ ) and the inhibitor ( $\omega_i$ ). With  $\omega_o$  much smaller than  $\omega_i$  - for instance if the circuit output is highly diffusible relative to the inhibitor, with comparable degradation rates - optimal placement requires maximising the separation between the circuit and the target. On the other hand, co-localisation of circuit with the target performs best if  $\omega_i$  is much smaller than  $\omega_o$ . This is simply because the limiting factor is inhibitor diffusion in the former case, and circuit output diffusion in the latter. If  $\omega_o$  and  $\omega_i$  are comparable, the optimal location may be an intermediate one.

**Two node circuit:** Next we consider cases where the circuit may have more than one node, which brings up the possibility of distributing the circuit itself. We examine a two node circuit, with the first node activating the second node, with the outputs of both nodes diffusing across the domain - with associated parameters  $\omega_1$  and  $\omega_2$ . In order to highlight the most basic effect of distribution of the circuit on communication to the target, we first examine a case where the target offers no inhibition. We fix the first node at one end of the domain and focus on how the location of the second node affects the level of its output at the target location.

Here we see that, for  $\omega_1$  much smaller than  $\omega_2$ , it is optimal to co-localise the second node with the target, while for  $\omega_2$  much smaller than  $\omega_1$ , co-localisation with the first node does better. This is because the diffusivity

of species 2 is a limiting factor in the first case, while that of species 1 is the limiting factor in the second case.

Next we introduce inhibition of the first node, as before, and consider optimal placement of the two nodes in the spatial domain (Inhibition of the second node is essentially the same as the one node case). In some situations, the optimal placement of the two nodes involves co-localising them (no distribution of the circuit). This is seen when (i) for  $\omega_2$  much less than the others, co-localisation of both nodes far from the target works best, (ii) for  $\omega_i$  much less than the others, co-localisation of both nodes with the target works best. This simply emphasises the fact that if either the inhibitor or the circuit output are highly diffusible relative to the others, then there is no advantage in distributing the circuit. This further illustrates that in the presence of one dominant (highly diffusible) factor in the communication - the optimal distribution is determined by maximising/minimising the other factor (minimising inhibition or maximising communication).

On the other hand, distribution of the circuit is the best option in other situations. For instance, as seen in the figure, for  $\omega_1$  much less than the others the optimal placement involves placing the first node far from the target, while co-localising the second with the target. This scenario minimises both the inhibition effect on the circuit, and the limitations of the communicating nodes (node 2). With comparable  $\omega$  for all three species we see that it is possible that the optimal placement that involves co-localising the second node with the target, while the first node is placed at an intermediate location (Fig.7(C)).

Now, if we allow for inhibition of both nodes by the diffusing inhibitor, we find that (i) in certain situations, the optimal placement involves co-localising both nodes at an intermediate location (ii) In other situations, the optimal placement involves different locations for each of the nodes and the target.

Overall, our analysis reveals how, depending on the context, the best spatial organisation may span an entire range from completely co-localised and completely distributed.

## 4.4 Discussion

Compartmentalization and spatial organisation of gene regulatory circuits has emerged as a vital new tool in synthetic biology, and is being explored in multiple ways. This leads to a number of basic questions of both conceptual and practical interest: What is the effect of spatially distributing a circuit,

|                                                    | Effects seen in both well-mixed compartment and uniform channel configurations                                                                                                                                                                                                 | Essential differences between the configurations                                                                                                                                                                                                                       |
|----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Basic Trade-offs</b>                            | Non-monotonic behaviour arising from trade-offs between compartment separation, leak/degradation in compartments, and strength of interaction between nodes. The nature of the trade-offs and the locations where they occur can depend on the type of regulatory interaction. | In the uniform channel, when the compartment size is not small relative to the overall domain, the effect of diffusivity and compartment size on steady state profiles may be essentially different from the case of well-mixed compartments.                          |
| <b>Distributing a bistable circuit</b>             | Perturbations introduced by spatial separation of the nodes may be mitigated by changing template concentrations. Different ways of a circuit can give qualitatively different dependence on spatial parameters.                                                               | In the uniform channel, different organisations of degrading enzymes allow for a whole spectrum of dependences on spatial parameters.                                                                                                                                  |
| <b>Distributing an oscillator circuit</b>          | Perturbations introduced by spatial separation of the nodes may be mitigated by changing template concentrations. Different ways of distributing nodes can give designs that exhibit qualitatively different dependence on spatial parameters.                                 | A distinctive aspect that emerges for a completely localised oscillator circuit in the uniform channel is the formation of completely static patterns within the compartment (this persists even if species are not allowed to leave the compartment).                 |
| <b>Modular addition of new nodes/ compartments</b> |                                                                                                                                                                                                                                                                                | Adding new compartments in the well-mixed case can distort steady state behaviour of components (due to added leak). In the uniform channel, it is possible to add compartments, which do not distort steady state behaviour of existing nodes.                        |
| <b>Mitigating competition for resources</b>        | Resource competition effects can be mitigated through spatial separation, however, this introduces a trade-off that can result in reduced output levels.                                                                                                                       |                                                                                                                                                                                                                                                                        |
| <b>Spatial organisation of protein depletion</b>   | For a given spatial organisation of a circuit, the spatial organisation of leaks/ degrading enzymes determines steady state and dynamic behaviour.                                                                                                                             | The uniform channel presents greater flexibility for spatially organising degradation of species, which can further be tailored to particular species. Furthermore, the effect of basic trade-offs also depend on the distribution of degrading enzyme in the channel. |
| <b>Topology/Ordering of circuit components</b>     | Topology of compartment configuration and the ordering of compartments with the domain can affect circuit behaviour.                                                                                                                                                           |                                                                                                                                                                                                                                                                        |
| <b>Adaptation to spatial separation</b>            | Negative regulation of the output node by a signal produced in a given compartment can be used to maintain the output level in this compartment independent of channel lengths.                                                                                                | In the uniform channel: (i) other designs resulting in adaptation to spatial separation are possible (co-localising degrading enzyme with production) (ii) even with negative regulation, deviations from adaptation, can be observed.                                 |

TABLE 4.1: Summary of the main results from our study.

and how does it depend on the nature of the circuit? What are the effects of spatial parameters therein and can these be used as effective tunable dials? How are these dependent on the particular spatial configuration (including boundaries), as well as the operational state of compartments - well-mixed or not? In which respects does spatial organisation provide useful new ways of creating circuits? In a nutshell, what does spatial distribution entail, and what are the associated design principles?

We address these questions through the creation of a dedicated systems framework which examines spatial organisation of cell-free transcription-translation and other template-based circuits. Spatial organisation is examined along three main axes: the type of operational state (well-mixed compartments or uniform channel), the type of motif and how it is distributed, and the placement of compartments in the ambient spatial domain.

We emphasise the fact that the three axes along which the systems framework is developed represent arguably the most basic features involved in the design of distributed circuits. A systems framework examining the interplay of these central features thus provides a range of basic insights, of both conceptual and engineering interest. We note that specific application contexts may dictate particular choices of compartmental construction/operational state/interconnection. A majority of currently developed synthetic circuits do not explicitly incorporate spatial organisation at the level of design. A systems framework provides a window into what distribution entails and how it may be systematically exploited.

Our analysis is based on widely used and validated models of circuits; furthermore analogous spatial models have both been validated and used in the analysis and experimental construction of distributed cell-free circuits. Our study of distributed circuits in a uniform channel does not explicitly incorporate the effects of membranes bounding compartments. However, the essential insights we present are relevant in such cases. We note that transport across membranes can itself be manipulated, but these aspects fall beyond the scope of the current study. The basic insights from our study of the uniform channel are also relevant in 2-D/3-D, though the study of specific compartment geometries need a dedicated study of their own, which can build on these insights.

**Basic effects of distributing circuits:** The diverse effects of compartmentalisation highlighted through our study are summarised in Table 1. The effect of spatial organisation on different circuits representing similar dynamical characteristics can be significantly different. This role of the underlying realisation of a circuit, is further reinforced by examining the trade-offs arising from varying spatial separation of compartments. Spatial separation and spatial organisation (placement of compartments, location of boundaries, spatial topology of interconnection) all affect circuit behaviour and could represent tunable dials. Importantly, the effects of these tunable dials can strongly depend not only on the nature of the circuit but also on how it is distributed. Spatial organisation affords multiple advantages, including alleviation of resource competition, eliminating unnecessary interactions, and new ways of shaping and tuning the response.

Another aspect of interest in synthetic circuits is retroactivity and its mitigation (Gyorgy and Del Vecchio, 2014). In this context, spatial organisation

can potentially reduce retroactivity, but at the cost of a reduced output (see Supplementary Information) (Alam-Nazki and Krishnan, 2015).

**Well-mixed compartments vs uniform channel:** We find both parallels and important differences which arise from the basic designs associated with operational state of the compartments. The essential differences arise from two main sources: (i) the uniform channel presents greater flexibility for spatially organising degradation of species, which can also be tailored to particular species (ii) when the compartment size is not small relative to the overall domain, non-trivial gradients within a compartment can exist. Distinct aspects stemming from the compartment size in a uniform channel include (i) the effect of diffusivity on steady state profiles is essentially different from the case of well-mixed compartments, and cannot be captured by a compartmental ODE description (ii) deviations from adaptation to spatial separation, even with promoter saturation, can be observed (iii) for certain circuits it is possible for pattern formation (arising from an instability) to occur within a compartment (something which is even seen when the compartment is “sealed off”). Different organisations of degrading enzymes in the uniform channel lead to entirely different location dependences in a bistable circuit: degrading enzyme co-localised with production removes the dependence on location whereas uniform degrading enzyme results in location dependence similar to well-mixed compartments.

**Adaptation to spatial separation:** We demonstrate that negative regulation by repression, involving promoter saturation, allows the steady state concentration of a particular species to adapt to spatial separation, at a chosen location/compartment, and could even be combined with adaptation to template amounts. This essential insight is valid for both the well mixed and the uniform channel (with the restriction that compartment sizes are small relative to the size of the domain). In a uniform channel with closed boundaries, another possible strategy is to localise the degrading enzyme uniformly over a region. This allows the average of the steady state output concentration over this region to adapt to changes in spatial separation between any compartments and/or closed boundaries. The above strategies can also produce adaptation in the face of modular augmentation of an existing configuration by the addition of new compartments and channels. Adaptation is achieved in such cases, as long as (i) modular augmentation does not involve further production of the species under consideration (ii) the key underlying factor

enabling adaptation (promoter saturation, single region of the degrading enzyme) is maintained.

**Spatial organisation of degradation:** In spatially distributed circuits, the spatial organisation of degrading enzymes emerges as a natural approach for regulating circuit behaviour. We find that localisation of degrading enzymes can be used to create asymmetries between nodes. This has consequences for both local and average concentration of species over the domain. It also determines whether spatial profiles are graded or uniform in parts of the domain. The latter feature is an appealing aspect which could be exploited in multiple ways, for instance it could be used to ensure that the concentration of such species are independent of the size of certain parts of the domain. The spatial organisation of degradation is particularly important in regulating bistable and oscillatory circuits, expanding on purely temporal studies.

Our simultaneous consideration of kinetic factors as well as multiple aspects of spatial organisation (which may be broadly encountered) brings to the fore (i) new capabilities of distributed circuits (ii) multiple hidden constraints involved, (iii) the presence of multiple trade-offs that arise, (iv) new circuit designs with appealing features. This is foundational to the conceptualisation, design, augmentation and optimisation of distributed synthetic circuits. Many of these insights naturally emerge in a scenario that is typical of many applications - a compartmentalised synthetic circuit communicating with a target at different location. The presence of restricting factors dictates the location of the synthetic circuit for best performance. In certain instances, distribution of the synthetic circuit is fundamentally better than a localised circuit. Analysis of basic models reveals transparent design principles underpinning the locations of circuit elements and target. We expect these insights to be relevant in multiple application contexts which might involve additional considerations and restricting factors.

Overall, a systems framework for the analysis and design of spatial organisation in genetic circuits, sets the stage for its systematic exploitation in application areas as diverse as biomedicine and distributed manufacturing, while also providing insights into the organization of gene regulation in natural systems.

## 4.5 Appendix

In the following sections, we present analytical arguments based on the mathematical models that support the main results of the study. Additional discussion and computational results are presented in the supplementary information for this chapter.

### 4.5.1 Steady state analysis of the well-mixed configuration

The models used for transcription-translation circuits in all cases involves the formation of protein dimers. The diffusivity and rate constant of leak/degradation are assumed to be equal for both monomeric and dimeric forms. Dimerisation is assumed to occur wherever the protein monomer is present (i.e. both in the compartments and in the connecting channels). For a two compartment configuration (equal sized compartments), the model equations for a protein X are as follows (similar to Karzbrun et al., 2014):

In compartment 1 ,

$$\begin{aligned}\frac{dP_X}{dt} &= k_X^b Y_2(P_X^T - P_X) - k_X^u P_X \\ \frac{dM_X}{dt} &= r_X^c(P_X^T - P_X) + r_X^y P_X - r_X^d M_X \\ \frac{\partial X}{\partial t} &= k_X^t M_X - 2k_X^2 X^2 + 2k_X^{-2} X_{dim} - k_X^d X + D_X \frac{A}{V} \left( \frac{\partial X}{\partial \theta} \right)_{\theta=0} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_2}^d X_{dim} + D_{X_{dim}} \frac{A}{V} \left( \frac{\partial X_{dim}}{\partial \theta} \right)_{\theta=0}\end{aligned}$$

Between compartments ( $\theta \in [0, L]$ ),

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_{dim} + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} + D_{X_{dim}} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

In compartment 2 ,

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_{dim} - k_X^d X - D_X \frac{A}{V} \left( \frac{\partial X}{\partial \theta} \right)_{\theta=L} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_2}^d X_{dim} - D_{X_{dim}} \frac{A}{V} \left( \frac{\partial X_{dim}}{\partial \theta} \right)_{\theta=L}\end{aligned}$$

where  $A$  is the area of the channel cross-section and  $V$  is the compartment volume. We assume  $D_X = D_{X_{dim}}$  and  $k_X^d = k_{X_{dim}}^d$  in all cases. Now, consider



the steady state concentration profiles between compartments given by the above model. These must satisfy the equations:

$$\begin{aligned} -2k_X^2 X^2 + 2k_X^{-2} X_2 + D_X \frac{\partial^2 X}{\partial \theta^2} &= 0 \\ k_X^2 X^2 - k_X^{-2} X_{dim} + D_{X_{dim}} \frac{\partial^2 X_{dim}}{\partial \theta^2} &= 0 \end{aligned}$$

For  $D_X = D_{X_{dim}}$ , multiplying the second equation by two and adding this to the first, gives us the following equation for the total concentration of protein  $X_T = X + 2X_{dim}$  ([monomer]+2[dimer]):

$$D_X \frac{\partial^2 X_T}{\partial \theta^2} = 0.$$

This implies a constant gradient for  $X_T$  in the channel. Thus, at steady state, we have constant gradients in the channels for the total concentration of protein, while the monomer and dimer concentration profiles generally do not have constant gradients by themselves. However, if the model does not involve any dimer formation (i.e. we only have the monomeric form), then the steady state concentration profiles of the monomers in the channels satisfy the equation

$$D_X \frac{\partial^2 X}{\partial \theta^2} = 0,$$

and therefore have constant gradients between compartments. This means that, when there is no dimer formation, we can replace the gradient  $\frac{\partial X}{\partial \theta}$  appearing in the transport term for  $X$  by the term  $\left(\frac{X_2 - X_1}{L}\right)$ , at steady state, in both compartments. This yields a transport term of the form  $p(X_2 - X_1)$  where  $X_1, X_2$  are the concentrations of species  $X$  in two adjacent compartments, and  $p = \frac{DA}{VL}$  is the transport coefficient. This yields a compartmental description of the system that exactly maps onto the PDE at steady state. We will use this description repeatedly in the analysis that follows.

#### 4.5.2 Analysis of non-monotonic steady state behaviour

We have examined the non-monotonic variation of steady state concentrations of the species (proteins) in response to increasing compartment separation, for basic two-node positive and negative regulation. This behaviour, and the underlying trade-offs that cause it, are discussed in the main text.

Here we give analytical arguments to support these conclusions, using simplified models of the kinetics - (i) we neglect transcription from the inactive form of the gene, (ii) when dimeric regulation is involved, we ignore leak/degradation of the protein dimers, dimerisation in the channel, and transport of the dimers. For both types of regulation, we first examine the simpler case of monomeric regulation - where dimerisation is completely absent. With no dimerisation, the steady state concentration gradients are constant between compartments, so the steady state of the compartmental model can be exactly mapped onto that of the PDE. When dimeric regulation is involved (ii) means that, at steady state, the protein gradients are constant between the compartments, and we can use a compartmental description for the transport terms. Note that, in the following analysis, all kinetic parameters take non-negative values. In all cases, the expressions for the steady state compartmental concentrations ( $X_1, Y_1$  etc.) have been checked by comparison with steady states obtained by numerical solution of the ODE model. The expressions for the derivatives (of steady state compartmental concentrations with respect to the transport coefficient  $p$ ) were calculated using MAPLE 2017. In the following analysis, we assume that the two proteins X and Y have equal diffusivity, although the results continue to hold good when this is not the case.

### Activation - Monomeric regulation

For the simple activation motif (X activating Y) with X produced in compartment 1 and the Y template in compartment 2, we first examine the case with monomeric regulation (no dimerisation). Here,  $p$  is a parameter ( $p = \frac{DA}{VL}$ ) representing the diffusive transport rate between the compartments. Note that  $p$  is positive and inversely proportional to the length  $L$ , of the channel connecting the compartments (i.e. the separation between the compartments in the spatial domain). The steady state equations for the X and Y concentrations in the two compartments take the following form:

$$\begin{aligned} k_0 - k_d X_1 - p(X_1 - X_2) &= 0 \\ -k_d X_2 - p(X_2 - X_1) &= 0 \\ -k_d Y_1 - p(Y_1 - Y_2) &= 0 \\ \frac{k_1 X_2}{K + X_2} - k_d Y_2 - p(Y_2 - Y_1) &= 0 \end{aligned}$$

Using these equations, we find

$$X_1 = \frac{k_0 (p + k_d)}{k_d (2p + k_d)}, \quad X_2 = \frac{pk_0}{k_d (2p + k_d)}$$

$$\frac{dX_1}{dp} = -\frac{k_0}{(2p + k_d)^2}, \quad \frac{dX_2}{dp} = \frac{k_0}{(2p + k_d)^2}$$

Thus, increasing channel length/compartment separation leads to an increase in  $X_1$  and a decrease in  $X_2$ .  $X_1$  eventually saturates while  $X_2$  approaches zero. On examining  $Y$ , we have

$$Y_2 = \frac{k_1 pk_0 (p + k_d)}{k_d^2 (2p + k_d)^2} \left( K + \frac{pk_0}{k_d (2p + k_d)} \right)^{-1}$$

$$\frac{dY_2}{dp} = \frac{(2Kpk_d^2 + Kk_d^3 - p^2k_0) k_0 k_1}{(2Kpk_d + Kk_d^2 + pk_0)^2 (2p + k_d)^2}$$

For non-negative values of the parameters, the polynomial in the numerator will always have a single real positive root. Thus,  $Y_2$  can vary non-monotonically with changing compartment separation. Suppose the positive root is at  $p = p^*$ . It is easy to see that the second derivative of  $Y_2$  at  $p^*$  must have the same sign as the derivative of the polynomial at this point ( $\frac{dY_2}{dp}$  has the form  $\frac{f(p)}{g(p)}$  where  $f = 0$  at  $p = p^*$ , and  $g > 0$ . Thus,  $\frac{d^2Y_2}{dp^2} = \frac{f'(p^*)}{g(p^*)}$ . Thus,  $Y_2$  has a maximum at  $p^*$ .

Similarly examining  $Y_1$ , we find that

$$\frac{dY_1}{dp} = \frac{(4Kpk_d + 2Kk_d^2 + pk_0) k_0 pk_1}{(2Kpk_d + Kk_d^2 + pk_0)^2 (2p + k_d)^2}$$

Since the polynomial in the numerator can have no positive real roots,  $Y_1$  must vary monotonically for  $p > 0$ .

### Activation - Dimeric regulation

If we ignore leak/degradation of the protein dimers, dimerisation in the channel, and transport of the dimers, the steady state equations for the  $X$

and  $Y$  concentrations in the two compartments take the following form:

$$\begin{aligned} k_0 - k_d X_1 - p(X_1 - X_2) &= 0 \\ -k_d X_2 - p(X_2 - X_1) &= 0 \\ -k_d Y_1 - p(Y_1 - Y_2) &= 0 \\ \frac{k_1 X_2^2}{K + X_2^2} - k_d Y_2 - p(Y_2 - Y_1) &= 0 \end{aligned}$$

On examining  $Y_2$ , we have

$$Y_2 = \frac{k_1 p^2 k_0^2 (p + k_d)}{k_d^3 (2p + k_d)^3} \left( K + \frac{p^2 k_0^2}{k_d^2 (2p + k_d)^2} \right)^{-1}$$

$$\frac{dY_2}{dp} = \frac{\left( 4Kp^3 k_d^2 + 12Kp^2 k_d^3 + 9Kp k_d^4 + 2Kk_d^5 - p^3 k_0^2 \right) p k_0^2 k_1}{\left( 4Kp^2 k_d^2 + 4Kp k_d^3 + Kk_d^4 + p^2 k_0^2 \right)^2 (2p + k_d)^2}$$

The polynomial in the numerator may be rearranged to give

$$f_2(p) = \left( 4Kk_d^2 - k_0^2 \right) k_0^2 k_1 p^3 + 12Kk_d^3 k_0^2 k_1 p^2 + 9Kk_d^4 k_0^2 k_1 p + 2Kk_d^5 k_0^2 k_1$$

Using Descartes' Rule of Signs or otherwise, it can be seen that this polynomial has a single positive real root if  $4Kk_d^2 - k_0^2 < 0$ . As before, we also find that the second derivative of  $Y_2$  at this root is negative. Thus  $Y_2$  can exhibit a maximum if the kinetic parameters satisfy the above condition.

Similarly examining  $Y_1$ , we find that

$$\frac{dY_1}{dp} = \frac{\left( 12Kp^2 k_d^2 + 12Kp k_d^3 + 3Kk_d^4 + p^2 k_0^2 \right) p^2 k_0^2 k_1}{\left( 4Kp^2 k_d^2 + 4Kp k_d^3 + Kk_d^4 + p^2 k_0^2 \right)^2 (2p + k_d)^2}$$

Since the polynomial in the numerator can have no positive real roots,  $Y_1$  must vary monotonically for  $p > 0$ . Note that our analysis of the dimeric activation relies on certain simplifying assumptions that do not describe the full system (for which we present computational results in the text). Thus, while this analysis demonstrates the possibility of non-monotonic behaviour shown in the results, it does not guarantee a single maximum, although this is the only behaviour observed computationally.

### Repression - Monomeric regulation

Now we examine the simple repression motif (X repressing Y) with X produced in compartment 1 and the Y template in compartment 2. For monomeric regulation, the steady state equations for the X and Y concentrations in the two compartments take the following form:

$$\begin{aligned} k_0 - k_d X_1 - p(X_1 - X_2) &= 0 \\ -k_d X_2 - p(X_2 - X_1) &= 0 \\ -k_d Y_1 - p(Y_1 - Y_2) &= 0 \\ \frac{k_1}{K + X_2} - k_d Y_2 - p(Y_2 - Y_1) &= 0 \end{aligned}$$

On examining Y, we have

$$Y_2 = \frac{k_1 (p + k_d)}{k_d (2p + k_d)} \left( K + \frac{pk_0}{k_d (2p + k_d)} \right)^{-1}$$

$$\frac{dY_2}{dp} = - \frac{(Kk_d + k_0) k_d k_1}{\left( 2Kpk_d + Kk_d^2 + pk_0 \right)^2}$$

This implies that  $Y_2$  varies monotonically with  $p$ . Similarly examining  $Y_1$ , we find that

$$\frac{dY_1}{dp} = \frac{Kk_d^2 k_1}{\left( 2Kpk_d + Kk_d^2 + pk_0 \right)^2}$$

Thus, with monomeric repression,  $Y_1$  varies monotonically with  $p$ , although the trade-off (see main text) is still present.

**Repression - Dimeric regulation**

With repression of Y by the dimeric form of X, the steady state equations for the X and Y concentrations in the two compartments take the following form:

$$\begin{aligned} k_0 - k_d X_1 - p(X_1 - X_2) &= 0 \\ -k_d X_2 - p(X_2 - X_1) &= 0 \\ -k_d Y_1 - p(Y_1 - Y_2) &= 0 \\ \frac{k_1}{K + X_2^2} - k_d Y_2 - p(Y_2 - Y_1) &= 0 \end{aligned}$$

On examining  $Y$ , we have

$$Y_2 = \frac{k_1 (p + k_d)}{k_d (2p + k_d)} \left( K + \frac{p^2 k_0^2}{k_d^2 (2p + k_d)^2} \right)^{-1}$$

$$\frac{dY_2}{dp} = - \frac{\left( 4Kp^2 k_d^2 + 4Kpk_d^3 + Kk_d^4 + 3p^2 k_0^2 + 2pk_0^2 k_d \right) k_d^2 k_1}{\left( 4Kp^2 k_d^2 + 4Kpk_d^3 + Kk_d^4 + p^2 k_0^2 \right)^2}$$

The numerator polynomial cannot have a real positive root, and therefore  $Y_2$  must vary non-monotonically with  $p$ . Similarly examining  $Y_1$ , we find that

$$\frac{dY_1}{dp} = \frac{\left( 4Kp^2 k_d^2 + 4Kpk_d^3 + Kk_d^4 - p^2 k_0^2 \right) k_d^2 k_1}{\left( 4Kp^2 k_d^2 + 4Kpk_d^3 + Kk_d^4 + p^2 k_0^2 \right)^2}$$

The numerator polynomial has a single positive real root if  $4Kk_d^2 - k_0^2 < 0$ . We also find that the second derivative of  $Y_1$  at this root is negative. Thus,  $Y_1$  can exhibit a maximum if the kinetic parameters satisfy the above condition.

In conclusion, we see that, as the separation between compartments (channel length) is varied, (i) non-monotonic variation of steady state Y concentration in compartment 2 is possible for the simple activation motif (with either monomeric or dimeric regulation), (ii) non-monotonic variation of steady state Y concentration in compartment 1 is possible for the simple repression motif (with dimeric regulation but not with monomeric regulation).

### Mutual-activation motif: effect of spatial separation

In the case of the mutual activation motif distributed between two compartments, varying compartment separation can produce non-monotonic variation of steady state concentrations only in the production compartments. The fact that such behaviour is not possible the other compartments can be demonstrated as follows, using a compartmental description as above. The steady state equations for the two species X and Y take the following form:

$$\begin{aligned} f_1(Y_1) - k_d X_1 - p(X_1 - X_2) &= 0 \\ -k_d X_2 - p(X_2 - X_1) &= 0 \\ -k_d Y_1 - p(Y_1 - Y_2) &= 0 \\ f_2(X_2) - k_d Y_2 - p(Y_2 - Y_1) &= 0 \end{aligned}$$

where  $f_1$  and  $f_2$  are Hill functions representing activation, and  $p$  is the transport parameter as before.

These can be simplified to take the form:

$$\begin{aligned} f_1(Y_1) - g(p)X_1 &= 0 \\ X_2 - h(p)X_1 &= 0 \\ Y_1 - h(p)Y_2 &= 0 \\ f_2(X_2) - g(p)Y_2 &= 0 \end{aligned}$$

where  $g(p) = \frac{k_d^2 + 2k_d p}{k_d + p}$  and  $h(p) = \frac{p}{k_d + p}$ . On differentiating the above with respect to  $p$ , we have the following:

$$\begin{aligned} Y_1' \frac{df_1}{dY_1} - g'X_1 - gX_1' &= 0 \\ X_2' - h'X_1 - hX_1' &= 0 \\ Y_1' - h'Y_2 - hY_2' &= 0 \\ X_2' \frac{df_2}{dX_2} - g'Y_2 - gY_2' &= 0 \end{aligned}$$

The derivatives  $\frac{df_1}{dY_1}$  and  $\frac{df_2}{dX_2}$  are known to be positive. Now, assuming a positive steady state, we consider the possibility that  $X_2$  exhibits non-monotonic

behaviour. Thus, for some positive value of  $p$ ,  $X_2' = 0$ . At this point, it can be seen from the last equation that  $Y_2' = -\frac{g'}{g}Y_2$ . Substituting for  $Y_2'$  in the third equation gives  $Y_1' = \frac{gh' - g'h}{g}Y_2$ . For  $p > 0$ , it can be shown that  $gh' - g'h > 0$ . This implies that  $Y_1' > 0$ . Now, setting  $X_2' = 0$  in the second equation gives  $X_1' = -\frac{h'}{h}X_1$ . Substituting for  $X_1'$  in the first equation gives  $Y_1' \frac{df_1}{dY_1} = \frac{g'h - gh'}{h}X_1$ . This implies that  $Y_1' < 0$ . Thus we have a contradiction, and this indicates that non-monotonic behaviour is impossible for  $X_2$ . A similar analysis demonstrates the same for  $Y_1$ .

### 4.5.3 Localizing a bistable motif: Well-mixed compartments

In order to examine the basic effects of localizing a bistable motif in a spatial domain, while letting its output diffuse (and degrade) across the whole domain, we examine a single node, auto-catalytic bistable motif as used by Gines et al., 2017. This system particularly is suitable for analysis in the present context, as it has only one output species, and involves no dimerisation. In addition, a similar spatially distributed design (involving localised templates, diffusing output, and uniform degradation) has been realised experimentally. A simplified ODE model for this motif (as described in Gines et al., 2017), takes the form:

$$\frac{dx}{dt} = \frac{k_1x}{K_1 + x} - \frac{k_2x}{K_2 + x} - k_d x \quad (4.1)$$

where  $x$  is the concentration of the output species. The first term describes the autocatalytic production of the signal species, the second term describes a saturating degradation pathway for the signal species (realised through a 'pseudo-template'), while the last term corresponds to a second degradation pathway realised through an exonuclease, that is assumed not to saturate. Before we study the possible spatial organisation of this type of motif, we briefly examine how the degradation rate constant  $k_d$  in the last term, affects bistable behaviour. As seen in Fig. S1(A), increasing  $k_d$  can bring about a saddle node bifurcation that takes the system out of the bistable regime. On the other hand, reducing  $k_d$  cannot take the system out of this regime. This can also be seen analytically, by setting the RHS of (4.1) to zero, which yields the following cubic equation in  $x$ :

$$-k_d x^3 + (-K_1 k_d - K_2 k_d + k_1 - k_2)x^2 + (-K_1 K_2 k_d - K_1 k_2 + K_2 k_1)x = 0$$



$x = 0$  is one of the roots. For bistability, this polynomial must have two other positive roots. If the remaining parameters are chosen so that the system is bistable for a basal value of  $k_d$ , then it can be demonstrated that there are two positive roots for  $k_d < \frac{k_1 - k_2}{K_1 + K_2}$  and no positive roots otherwise.

Clearly, this condition is violated for large enough  $k_d$ . Thus it is clear that, if we start with a set of parameter values that make the system bistable, then increasing  $k_d$  can make the system monostable. The conclusions that we draw in the following analysis rely on this observation.

### Spatial Separation of the degrading enzyme

Localizing the templates (both the autocatalytic template and the pseudo-template) in one compartment (well-mixed compartment with no leak) and the degrading enzyme in the other (see Fig. S1(B)), creates the possibility of tuning the bistable behaviour of the motif by varying the separation between the compartments. A compartmental description of this system (which is a valid approximation at steady state) is as follows:

$$\begin{aligned} \frac{k_1 x_1}{K_1 + x_1} - \frac{k_2 x_1}{K_2 + x_1} - tr(x_1 - x_2) &= 0 \\ -k_d x_2 - tr(x_2 - x_1) &= 0 \end{aligned}$$

This means that the first equation has the form

$$\frac{k_1 x_1}{K_1 + x_1} - \frac{k_2 x_1}{K_2 + x_1} - \tilde{k}_d x_1 = 0$$

where  $\tilde{k}_d = \frac{k_d tr}{k_d + tr}$ . Thus, the steady state equation for  $x_1$  has the same form as the RHS of (4.1), with an effective degradation rate constant that is lower than  $k_d$ . This implies two possibilities: (i) If the motif exhibits bistability with co-localised degradation in a single compartment configuration (i.e. the kinetic parameters are such that the basic ODE described above gives bistability), then the motif continues to exhibit bistable behaviour in the distributed configuration (assuming that resource limitations do not come into play); (ii) If the motif is monostable in the co-localised configuration, then it may exhibit bistable behaviour in the distributed configuration, where the effective degradation rate constant is lower. This also indicates the possibility of tuning the switching thresholds of the bistable motif by varying the separation of the templates from the degrading enzyme.

### Placement in a linear array of compartments

If the templates (both the autocatalytic template and the 'pseudo-template') are localised in one of a linear array of compartments, all of which have the same rate constant of leak/degradation associated with them, there is a possibility that the bistable behaviour of the motif may depend on its location within the array. (An equivalent result for the same motif in a uniform 1-D channel is illustrated in the main text (see Figure 6) with the help of bifurcation curves computed through equilibrium continuation for the PDE system.) Here we give an analytical argument that demonstrates the possibility of such behaviour. We will consider a linear configuration of three compartments. The analysis can however, be extended to any number compartments.

In the three compartment configuration, there are two possible ways of placing the motif (see Fig. S1(C)): (i) in the middle compartment; (ii) in one of the terminal compartments. Consider case (i). At steady state, a compartmental description of the transport is a valid approximation, and the  $X$  concentrations in the three compartments are related by the following equations:

$$\begin{aligned} -\delta x_1 - tr(x_1 - x_2) &= 0 \\ \frac{k_1 x_2}{K_1 + x_2} - \frac{k_2 x_2}{K_2 + x_2} - \delta x_2 - tr(x_2 - x_1) - tr(x_2 - x_3) &= 0 \\ -\delta x_3 - tr(x_3 - x_2) &= 0 \end{aligned}$$

This yields the following equation for steady state  $x_2$ , that has the same form as the RHS of (4.1):

$$\frac{k_1 x_2}{K_1 + x_2} - \frac{k_2 x_2}{K_2 + x_2} - \tilde{k}_d x_2 = 0$$

where the effective degradation rate constant  $\tilde{k}_d = \delta + \frac{2\delta tr}{\delta + tr}$ . Similarly, in case (ii), the steady state concentrations are related by:

$$\begin{aligned} \frac{k_1 x_1}{K_1 + x_1} - \frac{k_2 x_1}{K_2 + x_1} - \delta x_1 - tr(x_1 - x_2) &= 0 \\ -\delta x_2 - tr(x_2 - x_1) - tr(x_2 - x_3) &= 0 \\ -\delta x_3 - tr(x_3 - x_2) &= 0 \end{aligned}$$

This yields the following equation for steady state  $x_1$ , that again has the same form as the RHS of (4.1):

$$\frac{k_1 x_1}{K_1 + x_1} - \frac{k_2 x_1}{K_2 + x_1} - \hat{k}_d x_1 = 0$$

where the effective degradation rate constant  $\hat{k}_d = \delta + \frac{tr\delta(\delta+2tr)}{\delta^2+tr^2+3\delta tr}$ . It can be shown that  $\hat{k}_d < \tilde{k}_d$ . This implies that it is possible that (for certain parameter values) the motif may be bistable in case (ii) and not in case (i). This implies that, even with uniform degradation/leak in all compartments, the location of the motif within a linear array of compartments can determine its behaviour.

### Localizing the two node mutual inhibition motif

The basic bistable circuit examined in this study is the two node mutual inhibition motif. Here we present an analytical argument that allows us to extend the above analysis for the single node bistable motif to the mutual inhibition motif, under certain conditions. In the analysis above, we have used the ODE model for the bistable motif, and focused on the form of the RHS of (4.1). Keeping the kinetic parameters fixed, the effect of the spatial organisation is captured by an effective degradation rate constant. We follow the same approach here, and start by examining the ODE model. If dimer leak/degradation is ignored, and we use a quasi-steady state approximation for, the mRNA kinetics, promoter binding and the dimer reactions, the X and Y concentrations satisfy equations of the form:

$$\begin{aligned} \frac{dx}{dt} &= \frac{k_x}{K_x + y^2} - k_d x \\ \frac{dy}{dt} &= \frac{k_y}{K_y + x^2} - k_d y \end{aligned} \tag{4.2}$$

where we have assumed that the degradation rate/leak is equal for both species. Note that, if X and Y nodes are co-localised in one of the above configurations, at steady state, the  $k_d$  in the above equations is essentially replaced by an 'effective' degradation rate  $k_d^{eff}$ . If X and Y have the same diffusivity,  $k_d^{eff}$  is also the same for both.

Now we examine how the parameter  $k_d$  affects bistability in the system (4.2). Consider the variable  $u = K_y + x^2$ , for which we have

$$\begin{aligned}\frac{du}{dt} &= 2x \frac{dx}{dt} \\ &= \frac{2xk_x}{K_x + y^2} - 2k_d x^2 \\ &= \frac{2k_x(u - K_y)^{\frac{1}{2}}}{K_x + y^2} - 2k_d u + 2k_d K_y\end{aligned}$$

At steady state, we have

$$y = \frac{\gamma}{u} \text{ where } \gamma = \frac{k_y}{k_d}$$

Notice that for non-zero values of  $x$ ,  $\frac{du}{dt} = 0$  implies  $\frac{dx}{dt} = 0$ . Thus, the steady states of the original system correspond to the solutions of the following equation, for  $u > K_y$ :

$$\frac{k_x u^2 (u - K_y)^{\frac{1}{2}}}{K_x u^2 + \gamma^2} - k_d u + k_d K_y = 0$$

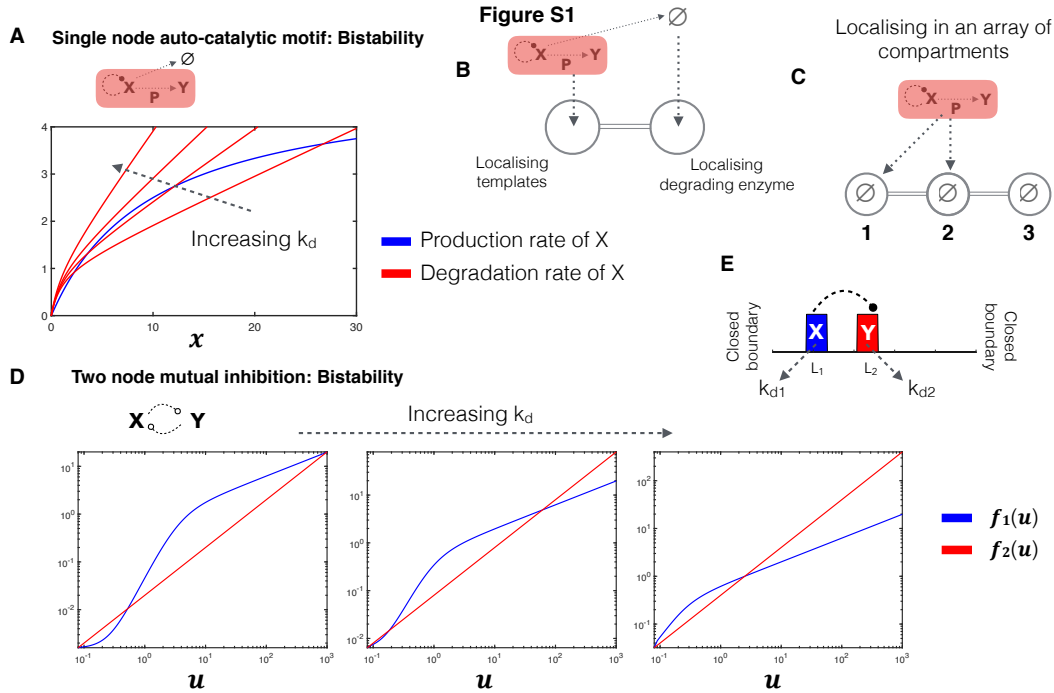
Note that  $k_d$  appears in all three of the terms in this equation (in the first term through  $\gamma$ ). We examine the effect of  $k_d$  by plotting two curves for  $u \geq K_y$ :

$$\begin{aligned}f_1(u) &= \frac{k_x u^2 (u - K_y)^{\frac{1}{2}}}{K_x u^2 + \gamma^2} + k_d K_y \\ f_2(u) &= k_d u\end{aligned}$$

The points where the curves intersect (other than  $u = K_y$ ) correspond to the steady states of the system (4.2). Analysis indicates that we always have  $f_1(u) > f_2(u)$  for  $u$  sufficiently close to  $K_y$ . We also see that, for large  $u$ ,  $f_1(u)$  is  $O(u^{\frac{1}{2}})$ , while  $f_2(u)$  is  $O(u)$ . This means that  $f_1(u) < f_2(u)$  for sufficiently large  $u$ , so that we are guaranteed to have at least one other point of intersection (i.e. steady state). As seen in Fig. S1(D), for kinetic parameters chosen in the bistable regime,  $f_1(u)$  gives a sigmoidal curve that intersects the  $f_2(u)$  curve at three points (other than  $u = K_y$ ). Now we can see what happens when the degradation rate  $k_d$  is increased, keeping the other parameters fixed. As  $k_d$  increases,  $\gamma$  becomes smaller, and this causes the  $f_1(u)$  curve to

eventually lose its sigmoidal shape, thus making it impossible to have multiple points of intersection. On the other hand, reducing  $k_d$  preserves the sigmoidal shape of  $f_1(u)$ , and bistability is maintained.

Thus, the effect of the parameter  $k_d$  is essentially similar to that seen in the single node bistable motif discussed previously. This means that the insights relating to spatial separation from the degrading enzyme and location dependent bistability in an array of compartments are also applicable to the two node mutual inhibition motif.



**Figure S1:** (A) ODE analysis of the single node autocatalytic motif (Supplemental section 4). Increasing  $k_d$  takes the system out of the bistable regime. (B) Schematic for supplemental section 4.1 (C) Schematic for supplemental section 4.2; the templates can be localised in the middle compartment or in one of the terminal compartments. (D) ODE analysis of the two-node mutual inhibition motif (Supplemental section 4.3). Increasing  $k_d$  takes the system out of the bistable regime. (E) Schematic for Supplemental section 8.

#### 4.5.4 Adaptation to spatial separation

Here we demonstrate the possibility of achieving adaptation of steady state concentration to varying spatial separation between compartments. We do this using simplified models for the kinetics and for simplicity consider the case of monomeric regulation. However, the conclusions do not rely on having monomeric regulation - the results shown in the main text were generated using the full model with dimeric regulation. These results, as well as the following analysis relies on having equal diffusivities for X and Y and equal leak/degradation rate constants for both species in a given compartment.

**Two compartment configuration: X and Y apart**

For the two compartment scenario with the X template in the first compartment and its repressor Y being produced in the second compartment, a compartmental model for the system (essentially equivalent to the PDE system at steady state) is given by:

$$\begin{aligned}\frac{dx_1}{dt} &= \frac{k_x}{K_x + y_1} - k_d x_1 - tr(x_1 - x_2) \\ \frac{dx_2}{dt} &= -k_d x_2 - tr(x_2 - x_1) \\ \frac{dy_1}{dt} &= -k_d y_1 - tr(y_1 - y_2) \\ \frac{dy_2}{dt} &= k_y - k_d y_2 - tr(y_2 - y_1)\end{aligned}$$

where  $tr = \frac{DA}{LV}$  is the transport coefficient.

At steady state, the repressor Y concentrations in the compartments are given by,

$$\begin{aligned}y_2 &= \frac{k_y}{H}, \text{ where } H = \frac{k_d^2 + 2k_d tr}{k_d + tr} \\ y_1 &= G y_2 = \frac{G}{H} k_y, \text{ where } G = \frac{tr}{k_d + tr}\end{aligned}$$

The X concentrations are:

$$\begin{aligned}x_1 &= \frac{1}{H} \frac{k_x}{K_x + y_1} \\ x_2 &= G x_1 = \frac{G}{H} \frac{k_x}{K_x + y_1} \\ &\approx \frac{G}{H} \frac{k_x}{y_1} \quad (\text{in the saturated regime}) \\ &= \frac{k_x}{k_y}\end{aligned}$$

Thus, we see that the steady state concentration of X in the second compartment (where its repressor Y is produced) is approximately independent of the transport coefficient  $tr$ , and therefore of the spatial separation (channel length). This behaviour is also observed computationally, over a range of channel lengths, in the full model (see Figure 2).

### Two compartment configuration: X and Y co-localised

For the two compartment scenario with the both X and Y templates in the first compartment and an 'empty' second compartment (potentially containing a template regulated by X or Y), a compartmental model for the system is given by:

$$\begin{aligned}\frac{dx_1}{dt} &= \frac{k_x}{K_x + y_1} - k_d x_1 - tr(x_1 - x_2) \\ \frac{dx_2}{dt} &= -k_d x_2 - tr(x_2 - x_1) \\ \frac{dy_1}{dt} &= k_y - k_d y_1 - tr(y_1 - y_2) \\ \frac{dy_2}{dt} &= -k_d y_2 - tr(y_2 - y_1)\end{aligned}$$

At steady state the repressor Y concentrations in the compartments are given by,

$$\begin{aligned}y_1 &= \frac{k_y}{H}, \text{ where } H = \frac{k_d^2 + 2k_d tr}{k_d + tr} \\ y_2 &= G y_1 = \frac{G}{H} k_y, \text{ where } G = \frac{tr}{k_d + tr}\end{aligned}$$

The X concentrations are:

$$\begin{aligned}x_1 &= \frac{1}{H} \frac{k_x}{K_x + y_1} \\ &\approx \frac{1}{H} \frac{k_x}{y_1} \quad (\text{in the saturated regime}) \\ &= \frac{k_x}{k_y} \\ x_2 &= G x_1 = \frac{G}{H} \frac{k_x}{K_x + y_1}\end{aligned}$$

Thus, we see that the steady state concentration of X in the first compartment (where both X and its repressor Y are produced) is approximately independent of the transport coefficient  $tr$ , and therefore of the spatial separation (channel length). This behaviour is also observed computationally, over a range of channel lengths, in the full model (see Figure 2).

### Effect of additional upstream regulation of X

Here we have considered a case where the expression of X is regulated only by the node Y. However, we note that the both the above result and the subsequent ones below, are also valid when X is subject to additional regulation by another input (say Z) produced in a different compartment, as long as this regulation does not involve competition for binding sites with the repressor Y, and does not interfere with the Y regulation in any other way. Under such conditions, the basal production rate of X,  $k_x$  becomes  $k_x(z)$ , i.e. a function of the input Z, while the rest of the model remains the same. The level of Z in the X compartment would, of course, also have to be maintained in order for X to adapt as desired. If we require X to adapt in a different compartment to where it is produced, this can be achieved by the same approach, having a different repressor (i.e. distinct from Y) for Z, that is produced in the X compartment. If we require X to adapt in its production compartment, then the repressor node Y is co-localised with X, and we only need Y to also repress Z, in order to maintain level of Z in the X compartment.

**Note:** Note that negative autoregulation (i.e. repression of X transcription by the X protein) does not produce the type of adaptive behaviour described above.

We now examine a three compartment configuration to demonstrate that this approach to achieving adaptation to varying spatial separation (channel lengths) is also applicable to configurations involving more compartments.

### Three compartments: Case 1

For the three compartment scenario with the X template in the first compartment, its repressor Y being produced in the second compartment, and a third compartment potentially containing a template that is regulated by X and/or Y. Since the presence/absence of a template in the third compartment does not affect the steady state concentrations of X or Y in any of the compartments, and only contributes through the leak of X and Y from this compartment, we will treat this compartment as empty for the purposes of this analysis. A compartmental model for the system (essentially equivalent to the PDE system at steady state) is given by:



$$\begin{aligned}
\frac{dx_1}{dt} &= \frac{k_x}{K_x + y_1} - k_d x_1 - t_1(x_1 - x_2) \\
\frac{dx_2}{dt} &= -k_d x_2 - t_1(x_2 - x_1) - t_2(x_2 - x_3) \\
\frac{dx_3}{dt} &= -k_d x_3 - t_2(x_3 - x_2) \\
\frac{dy_1}{dt} &= -k_d y_1 - t_1(y_1 - y_2) \\
\frac{dy_2}{dt} &= k_y - k_d y_2 - t_1(y_2 - y_1) - t_2(y_2 - y_3) \\
\frac{dy_3}{dt} &= -k_d y_3 - t_2(y_3 - y_2)
\end{aligned}$$

At steady state the repressor Y concentrations in the compartments are given by,

$$\begin{aligned}
y_1 &= G_1 y_2, \text{ where } G_1 = \frac{t_1}{k_d + t_1} \\
y_3 &= G_2 y_2, \text{ where } G_2 = \frac{t_2}{k_d + t_2} \\
y_2 &= \frac{k_y}{F}, \text{ where } F = k_d + t_1(1 - G_1) + t_2(1 - G_2)
\end{aligned}$$

The X concentrations are:

$$\begin{aligned}
x_3 &= G_2 x_2 \\
x_2 &= H x_1, \text{ where } H = \frac{t_1}{k_d + t_1 + \frac{t_2 k_d}{k_d + t_2}} \\
x_1 &= \frac{1}{k_d + t_1(1 - H)} \frac{k_x}{K_x + y_1}
\end{aligned}$$

Thus, in a regime where  $y_1$  is saturating, we have

$$\begin{aligned}
x_1 &\approx \frac{1}{k_d + t_1(1 - H)} \frac{k_x}{y_1} \\
&= \frac{1}{k_d + t_1(1 - H)} \frac{k_x}{G_1 y_2}
\end{aligned}$$

This means that  $x_2$  and  $x_3$  are given by:

$$x_2 = Hx_1 \approx \frac{H}{k_d + t_1(1 - H)} \frac{k_x F}{G_1 k_y} = \frac{k_x}{k_y}$$

$$x_3 = G_2 x_2 \approx \frac{k_x t_2}{k_y (k_d + t_2)}$$

Thus, we see that  $x_2$  adapts to changes in  $t_1$  or  $t_2$  (i.e. varying the length of either channel), while  $x_3$  adapts only to changes in  $t_1$ .

### Three compartments: Case 2

Now we examine the case where X and Y are in the terminal compartments of a three compartment configuration, with the middle compartment treated as empty (as we have done above). A compartmental model for the system (essentially equivalent to the PDE system at steady state) is given by:

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{k_x}{K_x + y_1} - k_d x_1 - t_1(x_1 - x_2) \\ \frac{dx_2}{dt} &= -k_d x_2 - t_1(x_2 - x_1) - t_2(x_2 - x_3) \\ \frac{dx_3}{dt} &= -k_d x_3 - t_2(x_3 - x_2) \\ \frac{dy_1}{dt} &= -k_d y_1 - t_1(y_1 - y_2) \\ \frac{dy_2}{dt} &= -k_d y_2 - t_1(y_2 - y_1) - t_2(y_2 - y_3) \\ \frac{dy_3}{dt} &= k_y - k_d y_3 - t_2(y_3 - y_2) \end{aligned}$$

At steady state the repressor Y concentrations in the compartments are given by,

$$y_2 = H_y y_3, \text{ where } H_y = \frac{t_2}{k_d + t_2 + \frac{t_1 k_d}{k_d + t_1}}$$

$$y_1 = G_y y_2, \text{ where } G_y = \frac{t_1}{k_d + t_1}$$

$$y_3 = \frac{k_y}{k_d + t_2(1 - H_y)}$$

The X concentrations are:

$$\begin{aligned} x_2 &= H_x x_1, \text{ where } H_x = \frac{t_1}{k_d + t_1 + \frac{t_2 k_d}{k_d + t_2}} \\ x_3 &= G_x x_2, \text{ where } G_x = \frac{t_2}{k_d + t_2} \\ x_1 &= \frac{k_x}{K_x + y_1} \left( \frac{1}{k_d + t_1(1 - H_x)} \right) \end{aligned}$$

Thus, in a regime where  $y_1$  is saturating, we have

$$\begin{aligned} x_1 &\approx \frac{1}{k_d + t_1(1 - H_x)} \frac{k_x}{y_1} \\ &= \frac{1}{k_d + t_1(1 - H_x)} \frac{k_x}{G_y H_y y_3} \end{aligned}$$

This means that  $x_3$  is given by:

$$\begin{aligned} x_3 &= G_x H_x x_1 \approx \left( \frac{k_x}{k_y} \right) \left( \frac{G_x H_x}{G_y H_y} \right) \left( \frac{k_d + t_2(1 - H_y)}{k_d + t_1(1 - H_x)} \right) \\ &= \frac{k_x}{k_y} \end{aligned}$$

Thus, the concentration of X in the third compartment (where Y is produced) is essentially independent of both the transport coefficients (and therefore also independent of the channel lengths). Also note that  $x_2 = \frac{x_3}{G_x}$ , and therefore  $x_2$  is independent of the transport coefficient  $t_1$ .

**Note:** The above results, for the two and three compartment configurations, can also be demonstrated through a more general approach involving an arbitrary network of compartments. Consider a node X, whose template is localised in multiple compartments within such a network. Using a compartmental model (as above), it can be seen that the vector of steady state compartmental concentrations of X -  $x = [x_1, x_2, \dots, x_n]^T$  (the compartments are numbered from 1 to  $n$ ) - is the solution to a system of linear equations of the form  $A_X x = b_X$ . The  $i^{th}$  component of vector  $b_X$  here is the (zeroth order) production rate of X in compartment  $i$ . The matrix  $A_X$  is symmetric (since the network of compartments has the structure of an undirected graph), and its entries are inter-compartmental transport parameters and rate constants of leak from compartments. Now suppose the second node Y (the repressor) is

located in compartment  $j$  (only in this compartment). The steady state levels of  $Y$  also satisfies a system of linear equations  $A_Y y = b_Y$ .

We note the following points: (i) if  $X$  and  $Y$  have essentially the same diffusivity (hence also the same transport parameters and rate constants of leak), then the matrices  $A_X$  and  $A_Y$  are identical - we call it  $A$  (ii) the vector  $y$  of steady state concentrations is given by  $y_i = k_y A_{i,j}^{-1}$  where  $k_y$  is the production rate of  $Y$  in compartment. (iii) If the production rate of  $X$  in the  $i^{th}$  compartment is given by  $\frac{k_i^X}{K_X + y_i}$ , then in a saturated regime (substituting for the  $y_i$  from the previous step), the vector  $b_X$  is approximately given by  $b_X = [\frac{k_1^X}{k_y A_{1,j}^{-1}}, \frac{k_2^X}{k_y A_{2,j}^{-1}}, \dots, \frac{k_n^X}{k_y A_{n,j}^{-1}}]^T$ . (iv) The level of  $X$  in compartment  $j$  (where  $Y$  is produced) is therefore given by  $x_j = \sum_i \frac{k_i^X A_{j,i}^{-1}}{k_y A_{i,j}^{-1}}$ . Since the matrix  $A$  is symmetric, so is its inverse, and therefore we have  $x_j = \frac{1}{k_y} \sum_i k_i^X$ .

The above result suggests the following interpretations: (i)  $x_j$  adapts to changing the connectivity between compartments, as long as none of the  $X$  producing compartments becomes disconnected (ii)  $x_j$  adapts to the addition of a new compartment, as long as this does not introduce additional  $X$  production (iii)  $x_j$  adapts to changes in the separation between compartments (assuming that the resulting change in transport parameters is the same for  $X$  and  $Y$ ) (iv)  $x_j$  adapts to changing the location  $j$  of repressor production

We note that the above results are valid only if the assumption of saturation in the regulation by the repressor continues to hold good.

### Adaptation to template concentrations

The circuits implemented in Bleris et al., 2011, for adaptation to template concentration, are based on incoherent feedforward regulation of the output, at either the transcriptional or the post-transcriptional level, by a second species produced from the same template. In either case, under certain assumptions, the steady state relationship between the input  $x$  (concentration of template) and the output  $y$  (protein concentration) takes the form:

$$y = \frac{Vx}{K + x}$$

Now, in the context of the present discussion (see above), it is easy to see that the same type of circuit (i.e. with  $X$  and  $Y$  produced from the same template) could also exhibit adaptation to spatial separation, as seen for the case where  $X$  and  $Y$  are co-localised. In this case, again assuming monomeric

regulation of X by Y, the steady-state compartmental description reads

$$\begin{aligned}\frac{dx_1}{dt} &= \frac{k_x S}{K_x + y_1} - k_d x_1 - tr(x_1 - x_2) \\ \frac{dx_2}{dt} &= -k_d x_2 - tr(x_2 - x_1) \\ \frac{dy_1}{dt} &= k_y S - k_d y_1 - tr(y_1 - y_2) \\ \frac{dy_2}{dt} &= -k_d y_2 - tr(y_2 - y_1)\end{aligned}$$

where  $S$  represents the template concentration. As before, the steady-state value of  $x_1$  is given by:

$$\begin{aligned}x_1 &= \frac{1}{H} \frac{k_x S}{K_x + y_1} \\ &\approx \frac{1}{H} \frac{k_x S}{\left(\frac{k_y S}{H}\right)} \quad (\text{in the saturated regime}) \\ &= \frac{k_x}{k_y}\end{aligned}$$

This essentially means that the steady state concentration of X, in the compartment where it is produced, adapts to both variation in channel lengths, and variation in the template concentration.

If we now want to achieve this type of combined adaptation of X to both spatial separation and X template concentration in a different compartment (i.e. not where the X template is located), then we find that this requires a combined regulation of X production by two repressors (say Y and Z). A possible circuit design that achieves this is as follows: one repressor (Y) is produced from the same template as X, while the other (Z) is produced in the compartment where we require adaptation of X. Here we examine such a circuit, with monomeric regulation, in a two compartment configuration, with X and Y produced (from the same template) in compartment 1 and Z produced in compartment 2. We first examine the case where Z only represses X, and does not regulate Y. If we assume equal diffusivities, and that the combined regulation of X by Y and Z does not involve competition for binding sites, we have the following model:

$$\begin{aligned}
\frac{dx_1}{dt} &= \frac{k_x S}{(K_{xy} + y_1)(K_{xz} + z_1)} - k_d x_1 - tr(x_1 - x_2) \\
\frac{dx_2}{dt} &= -k_d x_2 - tr(x_2 - x_1) \\
\frac{dy_1}{dt} &= k_y S - k_d y_1 - tr(y_1 - y_2) \\
\frac{dy_2}{dt} &= -k_d y_2 - tr(y_2 - y_1) \\
\frac{dz_1}{dt} &= -k_d z_1 - tr(z_1 - z_2) \\
\frac{dz_2}{dt} &= k_z - k_d z_2 - tr(z_2 - z_1)
\end{aligned}$$

Solving for the steady state as before, we have

$$\begin{aligned}
z_2 &= \frac{k_y}{H}, \text{ where } H = \frac{k_d^2 + 2k_d tr}{k_d + tr} \\
z_1 &= G y_2 = \frac{G}{H} k_z, \text{ where } G = \frac{tr}{k_d + tr} \\
y_1 &= \frac{k_y S}{H} \\
y_2 &= \frac{G}{H} k_y S
\end{aligned}$$

This means that, for X, we have

$$\begin{aligned}
x_1 &= \frac{1}{H} \frac{k_x S}{(K_{xy} + y_1)(K_{xz} + z_1)} \\
&\approx \frac{1}{H} \frac{k_x S}{\left(\frac{k_y S}{H}\right) \left(\frac{G k_z}{H}\right)} \quad (\text{in the saturated regime}) \\
&= \frac{k_x}{k_y k_z} \left(\frac{H}{G}\right) \\
x_2 &= G x_1 \approx \frac{k_x}{k_y k_z} H
\end{aligned}$$

This means that, although  $x_2$  is approximately independent of the template concentration  $S$ , it still depends on the transport coefficient, and therefore does not adapt to changing compartment separation. However, if  $Y$  is essentially non-diffusible, we have  $y_1 = \frac{k_y S}{k_d}$ , in which case, we have  $x_1 \approx \frac{k_x}{k_y k_z} \left(\frac{k_d}{G}\right)$  and  $x_2 \approx \frac{k_x}{k_y k_z} k_d$ . Thus, if  $Y$  does not diffuse,  $x_2$  is approximately

independent of both the template concentration and the template concentration, and we get the desired behaviour.

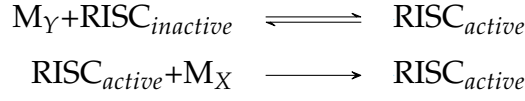
If we now examine the case where both X and Y production is repressed by Z, a similar analysis (again in the saturated regime) shows that while  $x_2$  approximately adapts to the template concentration, it continues to depend on the transport coefficient, regardless of whether Y is diffusible or not.

To summarise, for the case of monomeric regulation, we need Y and Z to meet the following conditions: (i) while Z represses X production, it must not repress Y production, (ii) the combined regulation of X by Y and Z should not involve competition for binding sites, (iii) Y must be essentially confined to the compartment where it is produced. A circuit that satisfies these constraints will allow the steady state concentration of X in the Z compartment to adapt to both variation in compartment spacing and variation in template concentration.

#### **Exemplar case: combined adaptation to template concentration and spatial separation**

We present computational results in the main text, showing how adaptation to both of the above factors can be achieved by one possible realisation of such a circuit. The particular realisation considered here involves having one of the repressor nodes (Y) act at the translational level - with the mRNA from node Y activating an RNA induced silencing complex (RISC)- and the other (Z) acting at the transcriptional level - the protein from node Z acting as a transcription factor. While the protein from node Z inhibits transcription of node X (through dimeric regulation), the RISC activated by mRNA expressed constitutively from node Y degrades the mRNA produced by transcription at node X. Having the two repressing nodes act at different steps precludes any competitive effects in their regulation of X. In addition, having the mRNA from the Y node act as the regulator allows its effect to be essentially confined to its production compartment, since the mRNA diffusion is assumed to be slow relative to its degradation.

For the exemplar case shown in the text, in addition to all the basic ingredients of our model, we assume a fixed total concentration (active and inactive forms) of the silencing complex in the compartment where the template for X (and Y) is placed. In addition to the basic transcription/translation reactions, the following reactions involving the RISC are assumed to take place in this compartment (following the simplified model used in Bleris et al., 2011):



where  $M_Y$  and  $M_X$  denote the mRNA transcript associated with nodes Y and X. The kinetics of these mRNA transcripts and the RISC (in the compartment containing the X-Y template) is modelled using mass action kinetics (similar to Bleris et al., 2011):

$$\begin{aligned}
\frac{dM_Y}{dt} &= r_Y^c P_X^T - r_X^d M_Y - k_b M_Y [\text{RISC}_{inactive}] + k_u [\text{RISC}_{active}] \\
\frac{d[\text{RISC}_{active}]}{dt} &= k_b M_Y [\text{RISC}_{inactive}] - k_u [\text{RISC}_{active}] \\
\frac{dM_X}{dt} &= r_X^c (P_X^T - P_X) + r_X^z P_X - r_X^d M_X - r_X^{\text{RISC}} [\text{RISC}_{active}] M_X
\end{aligned}$$

with the conservation condition

$$[\text{RISC}_{total}] = [\text{RISC}_{inactive}] + [\text{RISC}_{active}]$$

For the computational results shown, the  $[\text{RISC}_{total}]$  is taken to be high so that saturation of the RISC does not come into play.

#### 4.5.5 Single production compartment in a 1-D channel

##### Open boundaries and no degradation

For a single node, with constant production of signal species, localised over  $\theta \in [\theta_1, \theta_2]$  in a 1-D domain of  $L = 1$  with no degradation, the simplified model is as follows: For  $\theta \in [0, \theta_1)$ ,

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in [\theta_1, \theta_2]$ ,

$$\frac{\partial[X]}{\partial t} = k_0 + D_X \frac{\partial^2[X]}{\partial \theta^2}$$



For  $\theta \in [\theta_2, 1)$ ,

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

With homogeneous Dirichlet boundaries, the solution is given by:

For  $\theta \in [0, \theta_1)$ ,

$$X = \frac{k_0}{D} \theta (\theta_2 - \theta_1) \left(1 - \frac{\theta_1 + \theta_2}{2}\right)$$

For  $\theta \in [\theta_1, \theta_2]$ ,

$$X = \frac{k_0}{D} \theta (\theta_2 - \theta) \left(1 - \frac{\theta + \theta_2}{2}\right) + \frac{k_0}{2D} (1 - \theta) (\theta^2 - \theta_1^2)$$

For  $\theta \in [\theta_2, 1)$ ,

$$X = \frac{k_0}{2D} (1 - \theta) (\theta_2^2 - \theta_1^2)$$

### Closed boundaries and uniform degradation

For a single node, with constant production of signal species, localised over  $\theta \in [\theta_1, \theta_2]$  in a 1-D domain of  $L = 1$  with uniform degradation, the simplified model is as follows:

For  $\theta \in [0, \theta_1)$ ,

$$\frac{\partial[X]}{\partial t} = -k_d X + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in [\theta_1, \theta_2]$ ,

$$\frac{\partial[X]}{\partial t} = k_0 - k_d X + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in [\theta_2, 1)$ ,

$$\frac{\partial[X]}{\partial t} = -k_d X + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

With homogeneous Neumann boundaries, the solution is given by:

For  $\theta \in [0, \theta_1)$ ,

$$X = \frac{k_0 \cosh(\omega \theta)}{k_d \sinh(\omega)} (\sinh(\omega(1 - \theta_1)) - \sinh(\omega(1 - \theta_2)))$$

For  $\theta \in [\theta_1, \theta_2]$ ,

$$X = \frac{k_0 \cosh(\omega\theta)}{k_d \sinh(\omega)} (\sinh(\omega(1-\theta)) - \sinh(\omega(1-\theta_2))) \\ + \frac{k_0 \cosh(\omega(1-\theta))}{k_d \sinh(\omega)} (\sinh(\omega\theta) - \sinh(\omega\theta_1))$$

For  $\theta \in [\theta_2, 1)$ ,

$$X = \frac{k_0 \cosh(\omega(1-\theta))}{k_d \sinh(\omega)} (\sinh(\omega\theta_2) - \sinh(\omega\theta_1))$$

where  $\omega = \sqrt{\frac{k_d}{D}}$ .

### Open boundaries and uniform degradation

In this case, the model is the same as above, but we have homogeneous Dirichlet boundaries. The solution is given by:

For  $\theta \in [0, \theta_1)$ ,

$$X = \frac{k_0 \sinh(\omega\theta)}{k_d \sinh(\omega)} (\cosh(\omega(1-\theta_1)) - \cosh(\omega(1-\theta_2)))$$

For  $\theta \in [\theta_1, \theta_2]$ ,

$$X = \frac{k_0 \sinh(\omega\theta)}{k_d \sinh(\omega)} (\cosh(\omega(1-\theta)) - \cosh(\omega(1-\theta_2))) \\ + \frac{k_0 \sinh(\omega(1-\theta))}{k_d \sinh(\omega)} (\cosh(\omega\theta) - \cosh(\omega\theta_1))$$

For  $\theta \in [\theta_2, 1)$ ,

$$X = \frac{k_0 \sinh(\omega(1-\theta))}{k_d \sinh(\omega)} (\cosh(\omega\theta_2) - \cosh(\omega\theta_1))$$

where  $\omega = \sqrt{\frac{k_d}{D}}$ .

In all three cases, the expressions for the solutions have been validated by comparison with steady state concentration profiles obtained by numerical solution of the PDE models, for fixed values of parameters.

### Finding symmetric locations

Using the above solutions, and calculating the spatial average of the concentration profile over potential compartment locations, we see that, for a fixed

location of the sender(receiver) compartment, there can be a pair of 'symmetric' locations for the receiver(sender) compartment (i.e. a pair of locations such that placing the receiver(sender) compartment at either one would result in the same average signal level over the receiver compartment). Suppose the sender location (midpoint of the compartment) is at  $p$ , then the symmetric pair of locations  $p_1$  and  $p_2$  (where  $p_1 < p < p_2$ ) are related by:

### Open boundaries and no degradation

$$p_1(1 - p) = p(1 - p_2)$$

### Closed boundaries and uniform degradation

$$\cosh(\omega p) \cosh(\omega(1 - p_2)) = \cosh(\omega p_1) \cosh(\omega(1 - p))$$

In this case, depending on the value of  $p$ , it is possible that a symmetric location corresponding to a given location  $p_1$  may not exist (the expression  $\frac{\cosh(\omega p_1) \cosh(\omega(1 - p))}{\cosh(\omega p)}$  may be less than 1).

### Open boundaries and uniform degradation

$$\sinh(\omega p) \sinh(\omega(1 - p_2)) = \sinh(\omega p_1) \sinh(\omega(1 - p))$$

With open boundaries, a symmetric location  $p_2$  exists for any given  $p$  and  $p_1$  within the domain. Note that the above analysis involves only the locations of the midpoints of the compartments assumes that the size of the compartments is such that they may be placed at these locations without creating an overlap between compartments or crossing the boundaries of the domain. The above expressions relating the symmetric locations have been validated through numerical solution of the PDE models.

**Note:** The full system examined in our study includes a dimer form for each protein, that is also diffusible and subject to degradation. If we consider the case of constant production of X within a compartment as above, and include

dimer formation, transport and degradation, the equations are as follows:

In the X compartment ( $\theta \in \Omega_X$ ),

$$\begin{aligned}\frac{\partial X}{\partial t} &= k_0 - 2k_X^2 X^2 + 2k_X^{-2} X_2 - k_X^d X + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_{dim}}^d X_{dim} + D_{X_{dim}} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

Outside the X compartment ( $\theta \notin \Omega_X$ ),

$$\begin{aligned}\frac{\partial X}{\partial t} &= -2k_X^2 X^2 + 2k_X^{-2} X_2 - k_X^d X + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X_{dim}}{\partial t} &= k_X^2 X^2 - k_X^{-2} X_{dim} - k_{X_{dim}}^d X_{dim} + D_{X_2} \frac{\partial^2 X_{dim}}{\partial \theta^2}\end{aligned}$$

For the purpose of our study, we assume that  $D_X = D_{X_{dim}}$  and  $k_X^d = k_{X_{dim}}^d$  in all cases. As seen for the channel between compartments in section 4.5.1 above, with these assumptions, multiplying the dimer equation by two and adding this to the monomer equation (both within and outside the compartment) gives us equations for the total concentration of protein  $X_T = X + 2X_{dim}$  ([monomer]+2[dimer]). These equations have the same form as those used above. Thus, when we have dimer formation, the above results (i.e pertaining to symmetric locations) can apply to the total amount of protein.

#### 4.5.6 Effect of membranes bounding compartments

Our model of the uniform 1-D channel does not incorporate compartment boundaries, and this is reflected in the fact that all proteins can in principle diffuse along the channel. We briefly comment on the effect of membranes and compartment boundaries. By examining a model of spatial organisation in a 1-D channel, involving compartments with boundaries, we find that at steady state, the primary effect of the membrane is to create a possible difference in the concentration gradient of proteins inside and outside the compartment. While this can quantitatively perturb the behaviour of the system, many of the essential qualitative insights continue to hold good. The presence of a membrane allows for multiple other features - the localisation of a component in a compartment, and additional tunable dials for manipulating the transport of species across compartments. The former can be incorporated within the existing model in a simple way by localising those species

and regarding them as non-diffusible. The latter is beyond the scope of the current study.

### 4.5.7 Spatial organisation of degradation

In order to examine the effect of co-localising a common degrading enzyme with the sender or the receiver compartment in a uniform channel, as well as to study the possible distribution of a fixed amount of this degrading enzyme into the sender and receiver compartments, we consider the scenario shown in Fig. S1(E). We have a source compartment, with co-localised degradation (rate constant  $k_{d1}$ ) and a receiver compartment, also with co-localised degradation (rate constant  $k_{d2}$ ), placed in a uniform 1-D channel with closed boundaries. Both compartments are of equal size  $w_L$ . The model is as follows:

For  $\theta \in (0, \theta_1)$

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in (\theta_1, \theta_2)$ ,

$$\frac{\partial[X]}{\partial t} = k_0 - k_{d1}X + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in (\theta_2, \theta_3)$ ,

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in (\theta_3, \theta_4)$ ,

$$\frac{\partial[X]}{\partial t} = -k_{d2}X + D_X \frac{\partial^2[X]}{\partial \theta^2}$$

For  $\theta \in (\theta_4, L)$

$$\frac{\partial[X]}{\partial t} = D_X \frac{\partial^2[X]}{\partial \theta^2}$$

With no flux boundary conditions, the solution takes the following form:

$$\begin{aligned}
 X &= c_1 \text{ for } \theta \in (0, \theta_1) \\
 X &= \frac{k_0}{k_{d1}} + c_2 \cosh(\omega_1(\theta - \theta_1)) \text{ for } \theta \in (\theta_1, \theta_2) \\
 X &= c_3\theta + c_4 \text{ for } \theta \in (\theta_2, \theta_3) \\
 X &= c_5 \cosh(\omega_2(\theta_4 - \theta)) \text{ for } \theta \in (\theta_3, \theta_4) \\
 X &= c_6 \text{ for } \theta \in (\theta_4, L)
 \end{aligned}$$

where  $\omega_1 = \sqrt{\frac{k_{d1}}{D}}$  and  $\omega_2 = \sqrt{\frac{k_{d2}}{D}}$ . The constants  $c_i$  are given by:

$$\begin{aligned}
 c_2 &= -\left(\frac{k_0}{k_{d1}}\right) / \left(\cosh(\omega_1 w_L) + \omega_1 \sinh(\omega_1 w_L) \left(\theta_3 - \theta_2 + \frac{\cosh(\omega_2 w_L)}{\omega_2 \sinh(\omega_2 w_L)}\right)\right) \\
 c_1 &= \left(\frac{k_0}{k_{d1}}\right) + c_2 \\
 c_3 &= c_2 \omega_1 \sinh(\omega_1 w_L) \\
 c_5 &= \frac{-c_3}{\omega_2 \sinh(\omega_2 w_L)} = c_6
 \end{aligned}$$

We use the above solution to compute spatial averages of the concentration profile over both the 'sender' and 'receiver' compartments, and over the whole domain. These are given by:

$$\begin{aligned}
 \langle X \rangle_{\text{sender}} &= \frac{1}{w_L} \left( \frac{k_0 w_L}{k_{d1}} + \frac{c_2 \sinh(\omega_1 w_L)}{\omega_1} \right) \\
 \langle X \rangle_{\text{receiver}} &= \frac{1}{w_L} \left( \frac{c_5 \sinh(\omega_2 w_L)}{\omega_2} \right)
 \end{aligned}$$

If the common degrading enzyme is assumed to act via mass action kinetics, then we can use the above solution to analyse the distribution of a fixed amount of enzyme between the sender and receiver compartments. In this case, the degradation rate constants  $k_{d1}$  and  $k_{d2}$  will be proportional to the concentration of enzyme in the respective compartments.

**Note:** The average of the sender signal over the receiver compartment is the same for the cases where all the degrading enzyme is co-localised with the sender or the receiver. To see why, consider the mass balance for the sender species in the whole domain. If the degrading enzyme is co-localised with the sender, then at steady state, the concentration of the sender output

is uniform over the whole domain (which is closed), and the mass balance gives us  $k_0 w_L = k_d w_L \langle X \rangle_{\text{sender}}$ . Thus, we have  $\langle X \rangle_{\text{receiver}} = \langle X \rangle_{\text{sender}} = \frac{k_0}{k_d}$ . If the degrading enzyme is co-localised with the receiver, then at steady state, the mass balance gives us  $k_0 w_L = k_d w_L \langle X \rangle_{\text{receiver}}$ . Thus, the average of the sender output over the receiver is again  $\langle X \rangle_{\text{receiver}} = \frac{k_0}{k_d}$ . We also note that, by the same principle, the average of the sender output over the whole domain is also equal to  $\frac{k_0}{k_d}$  if the same total amount of enzyme is distributed over the whole domain (and assumed to function in the unsaturated regime)

#### 4.5.8 Location dependence in a uniform channel: Single production compartment

For a single production compartment located in a uniform channel (see Section 4.5.5), the average concentration of the output, (both average over the domain and average over the production compartment), can depend on the location of the compartment within the domain. With open boundaries, both of these measures of output are maximised when the compartment is placed at the centre of the domain, i.e. furthest from the boundaries. With closed boundaries and uniform degradation, only the average over the production compartment exhibits location dependence, while the domain average is independent of the compartment location. Here we use the analytical solutions presented in Section 4.5.5, to examine this position dependent behaviour, focussing on the average of the output over the production compartment.

##### With open boundaries and no degradation

Let the domain size be normalised to 1 and let the compartment of size  $l$  be at location  $p$  (midpoint of the compartment). At steady state, the concentration profile has a constant gradient outside the compartment, and equals zero at the two boundaries. For simplicity, we will examine the case where the compartment size is small relative to the size of the domain ( $l \ll 1$ ). In this case, the steady concentration profile is relatively flat within the compartment, and can be approximated by its average  $x_c$ . Now, the removal of the species from the compartment happens via diffusion out of the compartment through the boundaries. The steady state mass balance for the compartment thus has the form:

$$lk_0 - R_{\text{boundaries}} = 0$$

where the first term represents localised production and the second term represents the net outflow through the compartment boundaries. From the solution presented in section 4.5.5, the steady state fluxes at the compartment boundaries are:

$$D \left. \frac{dx}{d\theta} \right|_{\theta_1} = k_0 l (1 - p); \quad D \left. \frac{dx}{d\theta} \right|_{\theta_2} = -k_0 l p,$$

while the average concentration in the compartment (approximated by the concentration at the midpoint) is given by

$$x_c \approx \frac{k_0}{D} l p (1 - p)$$

where the approximation is valid for  $l \ll 1$ . Thus, the net outflow through the compartment boundaries can be expressed as:

$$\begin{aligned} R_{\text{boundaries}} &= D \left. \frac{dx}{d\theta} \right|_{\theta_1} - D \left. \frac{dx}{d\theta} \right|_{\theta_2} \\ &= \frac{D}{p} x_c + \frac{D}{(1 - p)} x_c \end{aligned}$$

In this instance, this expression could be obtained directly, exploiting the fact that the boundaries have zero concentrations.

Using this approximation for the effective outflow of the species, the steady state mass balance for the compartment thus has the form:

$$l(k_0 - k_d^{\text{eff}} x_c) = 0$$

where  $k_d^{\text{eff}} = \frac{D}{p(1-p)l}$ . The compartmental average at steady state is therefore given by  $x_c = \frac{k_0}{k_d^{\text{eff}}}$ . Now,  $k_d^{\text{eff}}$  is minimum when  $p = 0.5$  (i.e. the compartment is at the centre of the domain), and increases towards the boundaries. Thus,  $x_c$  must be maximum when the compartment is placed at the centre of the domain.

### With closed boundaries and uniform degradation

Proceeding exactly as above, we see that the steady state mass balance for the compartment has the form:

$$lk_0 - lk_d x_c - R_{\text{boundaries}} = 0$$



where the first term represents localised production, the second term represents degradation within the compartment, and the third term represents the net outflow through the compartment boundaries. From the solution presented in section 4.5.5, for  $l \ll 1$ , the compartmental average (approximated by the concentration at the midpoint) is given by:

$$x_c \approx \frac{2k_0 \sinh(\frac{\omega l}{2}) \cosh(\omega p) \cosh(\omega(1-p))}{k_d \sinh(\omega)}$$

where  $\omega = \sqrt{\frac{k_d}{D}}$ . Again, using the analytical solution and neglecting appropriate terms for  $l \ll 1$ , the total removal rate of the species through the boundaries is approximately given by:

$$\begin{aligned} R_{\text{boundaries}} &\approx \frac{k_0 D \omega \sinh(\omega l)}{k_d \sinh(\omega)} ((\cosh(\omega(1-p)) \sinh(\omega p) + \sinh(\omega(1-p)) \cosh(\omega p)) \\ &= \left[ \frac{D \omega \sinh(\omega l)}{2 \sinh(\frac{\omega l}{2})} \left( \frac{\sinh(\omega p)}{\cosh(\omega p)} + \frac{\sinh(\omega(1-p))}{\cosh(\omega(1-p))} \right) \right] x_c \end{aligned}$$

Using this approximation for the effective outflow of the species, the steady state mass balance for the compartment thus has the form:

$$l(k_0 - k_d^{\text{eff}} x_c) = 0$$

where

$$k_d^{\text{eff}} = \left[ k_d + \frac{D \omega \sinh(\omega l)}{2l \sinh(\frac{\omega l}{2})} \left( \frac{\sinh(\omega p)}{\cosh(\omega p)} + \frac{\sinh(\omega(1-p))}{\cosh(\omega(1-p))} \right) \right]$$

The compartmental average at steady state is again given by  $x_c = \frac{k_0}{k_d^{\text{eff}}}$ . However, in this case, we see that  $k_d^{\text{eff}}$  has a minimum when the compartment is placed closed to the boundaries, and is maximum when it is placed at the centre. Thus, in contrast to the previous case,  $x_c$  is maximised by placing the compartment close to the boundaries of the domain.

#### 4.5.9 Location dependence in a uniform channel: Localizing a bistable motif

Here we examine the localisation in a uniform channel, of the single node, auto-catalytic bistable motif (Gines et al., 2017) discussed in section 4.5.3. We

again assume that both the autocatalytic template and the ‘pseudo-template’ are co-localised.

### Placement in an open domain

With open boundaries (homogeneous Dirichlet BCs), the degrading enzyme is not essential to achieve a steady state since there is a removal of species through the boundaries. We therefore perform the analysis of the PDE model without the degrading enzyme, while noting that qualitatively similar insights hold good if a degrading enzyme is present along with open boundaries. Let the domain size be normalised to 1 and let the compartment of size  $l$  be at location  $p$  (midpoint of the compartment). At steady state, the concentration profile is linear outside the compartment, and equals zero at the two boundaries. For simplicity, we will examine the case where the compartment size is relatively small relative to the size of the domain ( $l \ll 1$ ). In this case, the steady concentration profile is relatively flat within the compartment, and can be approximated by its average  $x_c$ . Now, the removal of the species from the compartment happens in two ways (i) diffusion out of the compartment through the boundaries and (ii) conversion by pseudo-template within the compartment. The steady state mass balance for the compartment thus has the form:

$$l \left( \frac{k_1 x_c}{K_1 + x_c} - \frac{k_2 x_c}{K_2 + x_c} \right) - R_{\text{boundaries}} = 0$$

where the first two terms represent localised production and removal (production from the autocatalytic template and conversion through the pseudo-template, and the last term represents the net outflow through the compartment boundaries. Since the only difference with the system considered in Section 4.5.8 is inside the compartment, the gradients outside, as a function of the compartment concentration  $x_c$ , remain exactly the same.

Thus, the net outflow through the compartment boundaries can be expressed as:

$$\begin{aligned} R_{\text{boundaries}} &= D \left. \frac{dx}{d\theta} \right|_{\theta_1} - D \left. \frac{dx}{d\theta} \right|_{\theta_2} \\ &= \frac{D}{p} x_c + \frac{D}{(1-p)} x_c \end{aligned}$$

This is obtained by noting that the gradient is a constant, and the boundaries have zero concentration.

Using this approximation for the effective outflow of the species in the present scenario (i.e. the localised bistable motif), the steady state mass balance for the compartment has the form:

$$\frac{k_1 x_c}{K_1 + x_c} - \frac{k_2 x_c}{K_2 + x_c} - k_d^{eff} x_c = 0$$

where  $k_d^{eff} = \frac{D}{p(1-p)l}$ . This equation has the same form as the RHS of (4.1). Now suppose the kinetic parameters are such that (4.1) is bistable only for  $k_d < k_d^*$ . This would imply that, in the present scenario, we need  $k_d^{eff} < k_d^*$  for the motif to exhibit bistability. It can be seen that locations within the domain satisfying this condition exist only when  $4D < k_d^* l$ . If this condition is satisfied, then there exists a range of locations in the middle of the domain (and symmetric about the centre of the domain), where the system is bistable.

### Placement in an closed domain with uniform degradation

Localising the templates (both the autocatalytic template and the 'pseudo-template') in a domain with closed boundaries, with the degrading enzyme uniformly distributed over the whole domain, creates the possibility that the bistable behaviour of the motif depends on its location within the domain. This is illustrated in the main text (see Figure 6) with the help of bifurcation curves computed by equilibrium continuation for the PDE model. Here we give an analytical argument to support these computational results. We look for an argument that is similar to the ones used above: by approximating the steady state mass balance for the compartment when  $l \ll 1$ , and thereby obtaining an equation for the compartmental average  $x_c$ , that has the same form as the RHS of (4.1). Proceeding as before, from the analytical solution for a single production compartment given in section 4.5.5, we find that, for  $l \ll 1$ , this compartmental average is given by

$$x_c \approx \frac{2k_0 \sinh(\frac{\omega l}{2}) \cosh(\omega p) \cosh(\omega(1-p))}{k_d \sinh(\omega)}$$

where  $\omega = \sqrt{\frac{k_d}{D}}$ .

Here we have used the analytical solution shown in section 4.5.5, and neglected such terms as are consistent with the assumption  $l \ll 1$ . Now, the removal of the species from the compartment happens in two ways (i) diffusion out of the compartment through the boundaries and (ii) conversion by pseudo-template/degradation within the compartment. The diffusive flux

out of the compartment equals the diffusivity times the gradient of the concentration profile at the compartment boundaries. Again, using the analytical solution and neglecting appropriate terms for ( $l \ll 1$ ), the total removal rate of the species through the boundaries is approximately given by:

$$\begin{aligned} R_{\text{boundaries}} &\approx \frac{k_0 D \omega \sinh(\omega l)}{k_d \sinh(\omega)} ((\cosh(\omega(1-p)) \sinh(\omega p) + \sinh(\omega(1-p)) \cosh(\omega p)) \\ &= \left[ \frac{D \omega \sinh(\omega l)}{2 \sinh(\frac{\omega l}{2})} \left( \frac{\sinh(\omega p)}{\cosh(\omega p)} + \frac{\sinh(\omega(1-p))}{\cosh(\omega(1-p))} \right) \right] x_c \end{aligned}$$

When  $l \ll 1$ , this simplifies to

$$R_{\text{boundaries}} = \left[ D \omega \left( \frac{\sinh(\omega p)}{\cosh(\omega p)} + \frac{\sinh(\omega(1-p))}{\cosh(\omega(1-p))} \right) \right] x_c$$

The expression for  $R_{\text{boundaries}}$  as a function of  $x_c$  can also be obtained by solving two boundary value problems (diffusion-degradation), with one boundary at value  $x_c$  and the other boundary insulated. This yields exactly the same result (the solution in each segment being  $x_c \frac{\cosh(\omega \theta)}{\cosh(\omega p)}$  and  $x_c \frac{\cosh(\omega(1-\theta))}{\cosh(\omega(1-p))}$ ).

This means that the steady state equation for  $x_c$  can again be approximately expressed in the form:

$$\frac{k_1 x_c}{K_1 + x_c} - \frac{k_2 x_c}{K_2 + x_c} - k_d^{\text{eff}} x_c = 0$$

where

$$k_d^{\text{eff}} = \left[ k_d + \frac{D \omega \sinh(\omega l)}{2l \sinh(\frac{\omega l}{2})} \left( \frac{\sinh(\omega p)}{\cosh(\omega p)} + \frac{\sinh(\omega(1-p))}{\cosh(\omega(1-p))} \right) \right]$$

The term in square brackets forms an ‘effective’ rate constant of degradation within the compartment, again leaving us with a steady state equation having the same form as the RHS of (4.1). This term varies symmetrically as  $p$  ranges from 0 to 1 - it has a maximum for  $p = 0.5$  i.e. when the compartment is at the centre of the domain, and is minimum at  $p = 0$  and  $p = 1$ . This implies that it is possible that, for fixed kinetic parameters, the motif may be bistable when the compartment is placed closer to the ends of the domain, and lose bistability when the compartment is closer to the centre of the domain (due to the higher ‘effective’ degradation rate). Although the above analysis assumes  $l \ll 1$ , we note that this behaviour (position dependent bistability) is observed computationally through bifurcation analysis of the

PDE model, for a compartment of size  $l = 0.1$  (Fig. 6(A)). Here we use a constant (zeroth order) production of the species as an ‘input’ to the motif (i.e. the bifurcation parameter).

This type of position dependent behaviour, of a bistable motif in the uniform channel, is also seen (computationally) for a transcription translation motif of the type studied here (single node self activation), with uniform (and equal) degradation of both monomer and dimer forms across the domain. This means that the above insights may continue to hold good even in the presence of dimer formation and leak/degradation/transport of dimer.

#### 4.5.10 Application: Communication with a target

For our analysis of circuit placement relative to a target, we make the following basic assumptions: (i) closed boundaries for the domain, with the target adjacent to one of the boundaries (target location  $p_T$  close to 1)(ii) small compartment sizes ( $size = l$ ) relative to the size of the domain: this allows us to approximate the average concentration of a species within a compartment by the concentration at the midpoint of the compartment (iii) spatially uniform degradation rate constants for all diffusing species (iv) monomeric regulation for both the action of the inhibitor and the regulation of one node by the other within the synthetic circuit (v) constant production of the inhibitor at the target location (zeroth -order reaction). Note that there is no feedback interaction between the circuit and the target.

The circuit output is labelled  $X$ , the target  $T$ , and the inhibitor  $Z$  (see Fig. 7 in the main text). The steady state concentration profiles calculated in the Section 7 can be used to calculate both the level of the inhibitor at the circuit location, and subsequently, the circuit output at the target location. For fixed target location  $p_T$  (midpoint of target region) and variable circuit location  $p_X$ , the level of the inhibitor at the circuit location takes the form

$$Z(p_X) = f_Z(\omega_Z) \cosh(\omega_Z p_X)$$

$$\text{where } f_Z(\omega_Z) = \frac{2k_Z \cosh(\omega_Z(1 - p_T)) \sinh\left(\frac{\omega_Z l}{2}\right)}{k_Z^d \sinh(\omega_X)}$$

The resulting production rate of  $X$  takes the form  $\frac{k_X}{K_X + Z(p_X)}$ , the steady state level of  $X$  at the target. Thus, the steady state level of  $X$  at the target location

takes the form

$$X(p_T) = \frac{f_X(\omega_X) \cosh(\omega_X p_X)}{K_X + f_Z(\omega_Z) \cosh(\omega_Z p_X)}$$

$$\text{where } f_X(\omega_X) = \frac{2k_X \cosh(\omega_X(1 - p_T)) \sinh\left(\frac{\omega_X l}{2}\right)}{k_X^d \sinh(\omega_X)}$$

On examining the expression for  $\frac{dX(p_T)}{dp_X}$ , and considering its Taylor series expansion in terms of  $\omega_X$ , we see that the leading order term is negative. This indicates that, for small  $\omega_X$  (and therefore  $\omega_X$  small relative to  $\omega_Z$ ),  $X(p_Z)$  is maximised by lowering  $p_X$ , i.e. ensuring maximum separation from the target. Similarly, by examining the Taylor series expansion of  $\frac{dX(p_T)}{dp_X}$  in terms of  $\omega_Z$ , we see that the leading order term is positive. This indicates that, for  $\omega_Z$  small relative to  $\omega_X$ ,  $X(p_Z)$  is maximised by increasing  $p_X$ , i.e. by co-localising with the target. This confirms the intuitive understanding of how  $\omega_X$  and  $\omega_Z$  determine optimal circuit placement (see text).

Next we examine the two node circuit with no inhibition (the second node is labelled  $Y$ ). Here (assuming  $p_X$  fixed and  $p_X \leq p_Y$ ), we have the following expressions for  $X$  at the location of node  $Y$ , and for  $Y$  at the target location

$$X(p_Y) = \frac{2k_X \cosh(\omega_X(1 - p_Y)) \sinh\left(\frac{\omega_X l}{2}\right)}{k_X^d \sinh(\omega_X)} \cosh(\omega_X p_X)$$

$$Y(p_T) = \frac{2k_Y \cosh(\omega_Y(1 - p_T)) \sinh\left(\frac{\omega_Y l}{2}\right)}{k_Y^d \sinh(\omega_Y)} \cosh(\omega_Y p_Y)$$

$$\text{where } k_Y = \frac{k_{0Y} X(p_Y)}{K_Y + X(p_Y)}$$

Here again, by examining the Taylor series expansions of  $\frac{dY(p_T)}{dp_Y}$  in terms of  $\omega_Y$  and  $\omega_X$ , we see that for  $\omega_Y$  small relative to  $\omega_X$ ,  $Y(p_T)$  is maximised by minimising  $p_Y$  (i.e. co-localising the two circuit nodes), while for  $\omega_X$  small relative to  $\omega_Y$ , it is maximised by increasing  $p_Y$  (i.e. co-localising node  $Y$  with the target).

Now, in the case of the two node circuit with inhibition of the first node,  $Y$  at the target location again takes the form:

$$Y(p_T) = \frac{2k_Y \cosh(\omega_Y(1 - p_T)) \sinh\left(\frac{\omega_Y l}{2}\right)}{k_Y^d \sinh(\omega_Y)} \cosh(\omega_Y p_Y)$$

where  $k_Y = \frac{k_{0Y} X(p_Y)}{K_Y + X(p_Y)}$

Here  $X(p_Y)$  is given by:

$$X(p_Y) = \frac{f_X(\omega_X) \cosh(\omega_X(1 - p_Y)) \cosh(\omega_X p_X)}{K_X + f_Z(\omega_Z) \cosh(\omega_Z p_X)}$$

where  $f_X(\omega_X) = \frac{2k_X \sinh\left(\frac{\omega_X l}{2}\right)}{k_X^d \sinh(\omega_X)}$

where the expression for  $Z(p_X)$  is exactly as in the first case (single node circuit with inhibition).

Similar to the two previous cases, here we examine Taylor series expansions of both  $\frac{\partial Y(p_T)}{\partial p_X}$  and  $\frac{\partial Y(p_T)}{\partial p_Y}$ , in terms of  $\omega_Z$ ,  $\omega_X$ , and  $\omega_Y$ . By examining the leading order terms in each case, we see that: (i) for  $\omega_Z$  much smaller than the others,  $Y(p_T)$  is maximised by maximising  $p_X$  and  $p_Y$ , (ii) for  $\omega_X$  much smaller than the others,  $Y(p_T)$  is maximised by maximising  $p_Y$  and minimising  $p_X$ , (i) for  $\omega_Y$  much smaller than the others,  $Y(p_T)$  is maximised by minimising  $p_X$  and  $p_Y$ .

The above results are validated by comparison to computational results. Computational results also indicate that these insights continue to hold good even for larger compartment sizes. For illustrative purposes, we also show computational results for the case involving inhibition of both nodes.





## Chapter 5

# Spatial Networks

### 5.1 Introduction

Biochemical information processing is carried out by complex networks of molecular interactions. These networks may span multiple levels of cellular organisation - consisting of pathways involved in intracellular decision making/regulation, as well as those responsible for cell to cell communication at a population level, or for coordination/organisation at the tissue level. We can identify a variety of regulatory motifs within these networks, consisting of simple patterns of interaction (activation or inhibition) among a small number of nodes/components. These motifs may be of different types, depending on the patterns of interaction, including simple signal transduction, coherent/incoherent feedforward regulation, positive/negative feedback, and more complex combinations/extensions of these. Furthermore, individual motifs may involve interactions spanning different levels of cellular organization.

Different types of regulatory motifs are capable of different types of dynamic behaviour/information processing characteristics, including multi-stability, adaptation, and oscillations. See Tyson and Novak, [2010](#) for a classification of two and three node motifs, and a discussion of the possible dynamical characteristics of each class. There are several examples of basic motifs, which known to perform their expected function in living cells. An understanding of functional motifs is essential to predict, manipulate, or engineer information processing/decision making capabilities of biochemical networks.

While the behaviour/capabilities of basic motifs (and some moderately complex extensions), considered as well mixed systems of reactions, are well understood, the fact remains that spatial organization is an undeniable feature of the way these molecular networks are realised in cells, and is known to play a vital role in many processes. The molecular components forming a motif may have very different spatial characteristics. Some may be localised

at specific locations in space, for instance at the cell membrane, or confined to specific compartments such as the nucleus; others may be diffusible or be trafficked by active transport mechanisms; and still others, while not being localised in space, may be essentially non-diffusible, for instance due their size and crowding effects, or because they form complexes with other immobile species.

The spatial characteristics and organization of the components can combine with the dynamical capabilities of the network motif formed by the interactions between these components, in highly non-trivial ways, to realise important cellular functions. The resulting behaviour of a given motif may involve a direct combination of the dynamical characteristics of the well mixed motif with the spatial context, including possible modulation/distortion of these characteristics by spatial factors, or self-organised spatio-temporal behaviour, for example pattern formation and travelling wave phenomena.

Given that the combination of the network structure formed by the interactions and the spatial characteristics/organisation of the components lies at the heart of how these biochemical networks process information, we need a systematic approach to dissect the myriad possibilities that this entails, in such a way that we can elucidate (i) How spatial organization shapes the behaviour of a motif, possibly endowing it with new capabilities? (ii) How spatio-temporal information processing functions and new control structures emerge from patterns of activation/inhibition between components with different spatial characteristics? We develop a systems framework to understand the role of spatial organization in biochemical information processing networks, that combines two lines of approach: (i) A motif centric approach that proceeds by considering a set of basic network motifs (including feed-forward and feedback and regulation and combinations of the two), and examining the effect of introducing spatial organisation at the level of nodes or interactions (ii) An approach centred on patterns of interactions between three different spatial classes (see below).

## 5.2 Methodology

In order to be able to identify clear patterns in the interplay between space and network structure, we consider three limiting cases for the spatial characteristics of components, forming essentially three spatial classes (i) *Local* components: these components are present everywhere in the spatial domain and are essentially non-diffusible (ii) *Global* components: these components are

highly diffusible across the whole domain (iii) *Localized* components: these components are non-diffusible and confined to one or more subdomains. In specific cases, where a clear trend may be discerned, we will also consider the effect of varying the diffusivity of a *Global* component over a range of values.

The nodes/components in a network motif, and the interactions between them, may be realised in different ways. In a signalling pathway for example, possible realisations include a node consisting of a pair of interconverting modified forms (with other nodes possibly regulating the forward and reverse modification reactions) or a single species (with its production and/or degradation reactions regulated by other nodes). For the purpose of our study, we will consider nodes consisting of interconverting modified forms (one ‘active’ and the other ‘inactive’), with a conserved total amount at each node, and the active form at a given node regulating the interconversion (through enzymatic modification) at one or more of the other nodes. The nature of the regulation can be activation or inhibition, both of which can be realised in various different ways. For this study, we restrict ourselves to the following ways of realising activation and inhibition: for activation, the active form of the ‘regulating’ node converts the inactive form at the ‘target’ node to the active form; for inhibition, the the active form of the ‘regulating’ node converts the active form at the ‘target’ node to the inactive form. This type of enzymatic regulation, in principle, involves the formation of enzyme substrate complexes, and could result in the sequestration of the active and inactive/active forms at interacting nodes. It is well known sequestration effects can have non-trivial consequences for the behaviour of a motif. However, since our focus is on the interplay between regulatory patterns and the spatial characteristics of the interacting components, we will eschew such additional complexity by working in the limit where the enzyme kinetics is such that the amounts of these complexes is negligible.

### 5.2.1 Models: Kinetics

We use models based on mass action kinetics to represent the networks. For a given motif, we have one equation associated with each node, describing the time derivative of the concentration of the ‘active’ form. The reactions to be accounted for at each node include both interconversion reactions (inactive to active) and (active to inactive), each of which may be regulated (with or

without a Hill function) by the active forms at other nodes. In addition, there may be basal rates for the same reactions (independent of other nodes).

For node X activating node Y

$$\begin{aligned}\frac{dX}{dt} &= -k_x X - k_{sx} SX + k_{-x} X^* \\ \frac{dX^*}{dt} &= k_x X + k_{sx} SX - k_{-x} X^* \\ \frac{dY}{dt} &= -k_y Y - k_{xy}(X^*)Y + k_{-y} Y^* \\ \frac{dY^*}{dt} &= k_y Y + k_{xy}(X^*)Y - k_{-y} Y^*\end{aligned}$$

For node X inhibiting node Y

$$\begin{aligned}\frac{dX}{dt} &= -k_x X - k_{sx} SX + k_{-x} X^* \\ \frac{dX^*}{dt} &= k_x X + k_{sx} SX - k_{-x} X^* \\ \frac{dY}{dt} &= -k_y Y - k_y Y + k_{-xy}(X^*)Y^* \\ \frac{dY^*}{dt} &= k_y Y + k_y Y - k_{-xy}(X^*)Y^*\end{aligned}$$

where  $S$  represents the input signal, activating the  $X$  node. We use the same notation for the parameters throughout the study:  $k_i$  and  $k_{-i}$  represents the basal rate of conversion to and from the active form, for species  $i$ , while parameters of the form  $k_{ij}$  and  $k_{-ij}$  represent activation and inhibition of node  $j$  by node  $i$ . With a Hill function, the same interactions take the following

form:

For node X activating node Y

$$\begin{aligned}\frac{dX}{dt} &= -k_x X - k_{sx} SX + k_{-x} X^* \\ \frac{dX^*}{dt} &= k_x X + k_{sx} SX - k_{-x} X^* \\ \frac{dY}{dt} &= -k_y Y - k_{xy} Y \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) + k_{-y} Y^* \\ \frac{dY^*}{dt} &= k_y Y + k_{xy} Y \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) - k_{-y} Y^*\end{aligned}$$

For node X inhibiting node Y

$$\begin{aligned}\frac{dX}{dt} &= -k_x X - k_{sx} SX + k_{-x} X^* \\ \frac{dX^*}{dt} &= k_x X + k_{sx} SX - k_{-x} X^* \\ \frac{dY}{dt} &= -k_y Y - k_y Y + k_{-xy} \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) Y^* \\ \frac{dY^*}{dt} &= k_y Y + k_y Y - k_{-xy} \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) Y^*\end{aligned}$$

where the Hill coefficient  $n$  may be 2 or 4.

**Parameters:** Our focus in this study is to identify broad qualitative trends in the functioning of motifs with spatially organised components. To this end, we choose kinetic parameter values for the motifs such that they exhibit the characteristic dynamic behaviour associated with them in the temporal context (as ODEs). The results we present do not rely on non-generic values for these parameters. In all cases, we see that the behaviour we report is observed over a range of values of these parameters, and thus essentially emerges from the structure of the interactions themselves. In addition, in many cases, we are guided in our analysis of a motif by the bifurcation analysis of the ODE. The values of spatial parameters - diffusivity, domain size, and size of localised subdomain, are chosen appropriately for the particular spatial realisation being studied. In some cases, we choose these parameters to highlight certain capabilities or constraints, however, we ensure that the behaviour observed does not rely on a specific set of parameter values, but can be seen over a range. In many contexts, we highlight qualitative trends that emerge from varying these spatial parameters. Furthermore, in most

cases, these trends are observed across different classes of motifs and spatial scenarios, and this further testifies to the robustness of our results.

**Note:** The parameter values for all the results presented here are given in Appendix D. Although the model equations in each case are not presented, the notation adopted for labelling the parameters, together with knowing the structure of a given motif should make it possible to construct the model equations.

### 5.2.2 Models: Space

For simplicity, we confine ourselves to examining these reaction systems realised in a 1-D spatial domain. Note however, that the basic aspects of the interplay between spatial organisation and interaction patterns will carry through to higher dimensions. We combine the kinetics and spatial organization in an explicit spatial description (PDE) consisting of reaction-diffusion equations. We can have local nodes where the species are present everywhere in the domain, with their diffusivity set to a relatively low value; global nodes where the species are present everywhere in the domain, with relatively high diffusivity; localised nodes with species confined to certain subdomains, with their diffusivity set to zero. Note that, with all three classes of nodes, the diffusivity of the two interconverting forms are chosen to be equal. This simplifies the model, allowing us to describe only the active forms, (equal diffusivity of the two modified forms implies that, if the total concentration of the two species is initially uniform in space, then it will remain so) by using the total concentration to eliminate the concentration of the inactive form. Throughout the study, for all simulations, we will confine our investigation to initial conditions where the total concentration of modified forms, for any node, is uniform within the spatial domain containing that node (whole domain or localised subdomain). As a result, the equations for each node take the following form (We drop the  $*$  in the notation - for the rest of the chapter, and when referring to the plots, we will refer to the active forms as  $X$  or  $Y$  rather than  $X^*$  or  $Y^*$ ):

For localised node X activating node Y

Within the localised subdomain

$$\begin{aligned}\frac{\partial X}{\partial t} &= -k_x X - k_{sx} SX + k_{-x} X^* + D_x \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial X^*}{\partial t} &= k_x X + k_{sx} SX - k_{-x} X^* + D_x \frac{\partial^2 X^*}{\partial \theta^2}\end{aligned}$$

with  $D_x = 0$

$$\begin{aligned}\frac{\partial Y}{\partial t} &= -k_y Y - k_{xy} Y \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) + k_{-y} Y^* + D_y \frac{\partial^2 Y}{\partial \theta^2} \\ \frac{\partial Y^*}{\partial t} &= k_y Y + k_{xy} Y \left( \frac{(X^*)^n}{K_{xy} + (X^*)^n} \right) - k_{-y} Y^* + D_y \frac{\partial^2 Y^*}{\partial \theta^2}\end{aligned}$$

with  $D_y > 0$

Outside the localised subdomain

(X and  $X^*$  being absent here)

$$\begin{aligned}\frac{\partial Y}{\partial t} &= -k_y Y + k_{-y} Y^* + D_y \frac{\partial^2 Y}{\partial \theta^2} \\ \frac{\partial Y^*}{\partial t} &= k_y Y - k_{-y} Y^* + D_y \frac{\partial^2 Y^*}{\partial \theta^2}\end{aligned}$$

For global/local node Y activating a localised node X

Within the localised subdomain

$$\frac{\partial Y}{\partial t} = -k_y Y - k_{sy} SY + k_{-y} Y^* + D_y \frac{\partial^2 Y}{\partial \theta^2}$$

$$\frac{\partial Y^*}{\partial t} = k_y Y + k_{sy} SY - k_{-y} Y^* + D_y \frac{\partial^2 Y^*}{\partial \theta^2}$$

with  $D_y > 0$

$$\frac{\partial X}{\partial t} = -k_x X - k_{yx} X \left( \frac{(Y^*)^n}{K_{yx} + (Y^*)^n} \right) + k_{-x} X^* + D_x \frac{\partial^2 X}{\partial \theta^2}$$

$$\frac{\partial X^*}{\partial t} = k_x X + k_{yx} X \left( \frac{(Y^*)^n}{K_{yx} + (Y^*)^n} \right) - k_{-x} X^* + D_x \frac{\partial^2 X^*}{\partial \theta^2}$$

with  $D_x = 0$

Outside the localised subdomain

(X and  $X^*$  being absent here)

$$\frac{\partial Y}{\partial t} = -k_y Y - k_{sy} SY + k_{-y} Y^* + D_y \frac{\partial^2 Y}{\partial \theta^2}$$

$$\frac{\partial Y^*}{\partial t} = k_y Y + k_{sy} SY - k_{-y} Y^* + D_y \frac{\partial^2 Y^*}{\partial \theta^2}$$

We consider both closed and periodic boundaries for the domain, with the former possibly giving rise to position dependent behaviour in certain cases involving localised components.

Note: At any given node participating in an interaction, at least one of the interconversion reactions is regulated either by an input signal or by a different node. If only one of the reactions is regulated in this way, then we assume that its reverse reaction (a) is associated with a fixed rate constant (b) this rate constant is uniform in the spatial domain (uniform within the specified subdomain for a localised node).

### 5.2.3 Input Signal

An important aspect of the behaviour arising from a pattern of interactions between nodes, is how a designated output (i.e. the concentration of the active form at a specific node) is regulated by an input signal. In this study,



we consider input signals acting through the same type of enzymatic regulation as the interactions between nodes. Furthermore, unless mentioned otherwise, we do not examine dynamically varying input signals. In the spatial context, we can have spatially homogeneous as well as spatially graded concentration profiles for the signal species, each of which can elicit different types of response from a given motif. This aspect will be examined in detail when considering different spatial realisations of a motif. When examining spatially graded signals, we will always assume a sinusoidal signal profile of wavelength equal to domain size (unless mentioned otherwise). The input signal can in principle regulate either of the conversion reactions, at any of the nodes in a motif. In some cases, for instance with feedforward motifs, it is possible to make a sensible choice of input nodes, but this is not so in the case of feedback motifs. In such cases, it is possible that the input location can make a difference depending on the spatial class to which the targeted node belongs. This aspect is examined in detail.

#### 5.2.4 Motif centric approach

In this approach we start with a set of two and three node motifs involving a maximum of four interactions between them (maximum two interactions for the two node motifs). This allows us to keep to a tractable level of complexity, while still allowing motifs that include multiple feedback loops, and combinations of feedforward and feedback loops. The motifs in this chosen set are capable of exhibiting a wide range of behaviours - from simple signal transduction and adaptive behaviour, to combinations of multi-stability and oscillations in the purely temporal context, and self organised spatio-temporal behaviour such as pattern formation and travelling waves.

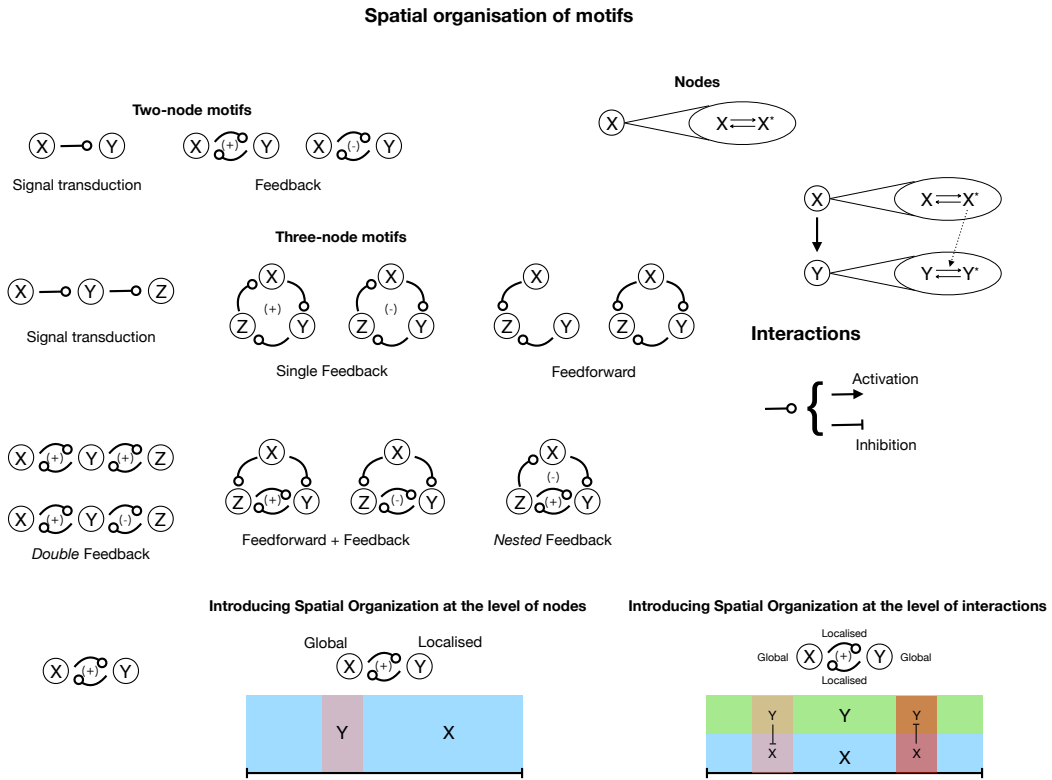


FIGURE 5.1: Schematic describing the first approach, centred on motifs. The individual nodes consist of pairs of interconverting forms, and the interactions may be activation or inhibition. We examine different classes of two and three node motifs, with up to four interactions. We introduce spatial complexity by localising either nodes or interactions, with other components either diffusing across the domain or being essentially non-diffusible.

Limiting ourselves to three nodes can be quite restrictive in the context of spatial organisation, for instance, localising two interacting nodes at different locations would prevent their direct interaction, thus breaking the motif. In order to overcome such constraints, and to allow us to examine cases with multiple localised components in this part of our study, we also introduce some spatial organisation in the interactions themselves. In the basic case, a given interaction is allowed to occur at any location where the two interacting nodes are present. In addition, we will also examine the following possibilities: (i) the interaction is facilitated by a diffusing (global) species - this allows interactions between separated localised nodes to be maintained (ii) the interaction (between global or local nodes) is itself localised, i.e. restricted to occur in one or more subdomains; such a localised interaction between nodes can be facilitated in natural pathways, for example, by having an intermediate step (essentially an additional node) in the interaction, that

involves a localised species, or if the interacting species are required to bind to a localised scaffold for the reaction to take place. (iii) the interaction (between local or global nodes) involves a combination of localised and diffusing components in the following way - if the network structure of the motif involves a node X (global or local) regulating a node Y (global or local), then such an interaction is realised by introducing a pair of implicit nodes, say a localised node Z, and a global (highly diffusible) node W, and having X activate Z, which in turn activates W, which then regulates Y.

In each case, we first characterise the behaviour of the motif in the purely temporal context (using the ODE model). As discussed above, we introduce nonlinearity to the interactions using Hill functions in such a way as to allow a motif to realise particular types of dynamical behaviour, including multistability and oscillations. Then we examine the different possible spatial realisations, assigning different nodes to different spatial classes. For any localised nodes, we consider a maximum of two different locations. In a 1-D spatial domain, having two locations of localisation is sufficient, both to divide the whole domain into distinct regions, and to allow for basic effects such as source sink separation, as well as delay effects arising from diffusion between locations.

In examining the spatial realisations of a given motif, we focus on (i) how the well mixed behaviour may be distorted in the spatial realisation (ii) what new capabilities result from introducing spatial organisation (including processing of graded input signals), and how the different spatial classes contribute to this (iii) what constraints may be imposed on motif function by different spatial realisations (including dependence on spatial parameters). We start with the two node motifs (simple signal transduction and feedback), followed by three node motifs with a up to of three interactions (feedforward and feedback), before moving on to three node motifs with up to four interactions. The last of these includes the most complex motifs that we examine, including (i) what we call as double feedback motifs, with one node common to two two-node feedback loops (see figure), (ii) what we call nested feedback motifs, with one two node feedback loop contained within a three node feedback loop, (iii) motifs with a two node feedback loop having feedforward interactions with a third node.

### 5.2.5 Space centric approach

Here we start with the three different spatial classes - local, global, and localised, and examine different patterns of interactions between them. We use our study from the motif point of view to guide our choice of interaction patterns. We start by examining pairwise interactions between the different classes - i.e. localised and local, localised and global, and local and global. In each case the interactions occur between one or more nodes in each spatial class. The structure of the nodes and the nature of the interactions is exactly as in the motif based approach described above. We examine the following patterns of interaction (see figure): (i) Simple signal transduction, (ii) feedforward regulation, (iii) simple feedback interaction, (iv) a feedback loop completely within one class, with simple signal transduction or feedforward interaction between classes, (v) a feedback loop completely within one class, with a second feedback loop between classes (vi) two feedback loops between classes, with a common node in one class. In (v) and (vi), it is possible to have both loops positive, both negative, or one positive and one negative forming an activator-inhibitor type of system. While we examine all three possibilities in (v), we only consider the activator-inhibitor type interaction in (vi).

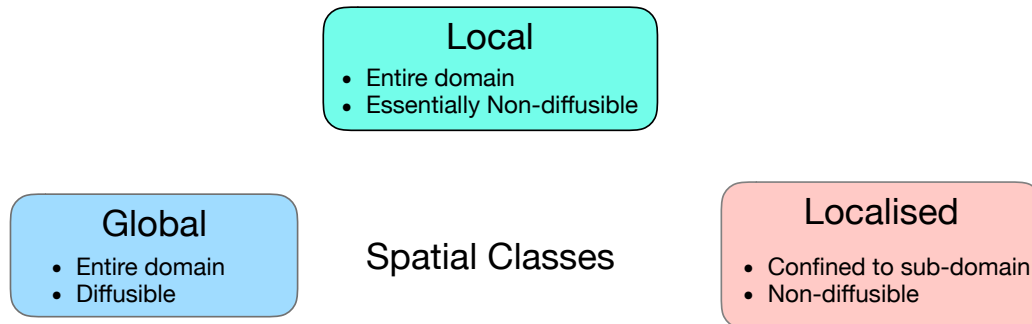


FIGURE 5.2: Schematic showing the three different “spatial classes”.

In our study of interaction patterns between the different spatial classes, for simplicity, we will restrict the number of nodes involved to the minimum number required to realise a set of interactions. A single feedback loop need involve only two nodes. However, we make an exception in the case of negative feedback loops, where we allow a maximum of three nodes in a single feedback loop, to allow for oscillatory behaviour.

We build on our study of pairwise interactions between classes to examine cases where all three classes are involved. We begin with simple signal transduction and feedforward interactions before examining feedback interactions. In order to keep the complexity tractable in this part of the study, we consider cases involving a maximum of two feedback loops. We make two exceptions to this rule, to allow for pattern forming and travelling wave (excitable medium) phenomena. In each of these cases, the characteristic behaviour requires a minimum of two feedback loops (one positive and the other negative), and we allow an additional feedback loop involving the localised class, for the purpose of the study.

In examining these patterns of interaction between the three spatial classes,

we focus on (i) the role of each spatial class in shaping the resulting behaviour, examining what capabilities each class provides, for instance in receiving different kinds of input signals, and how this depends on the nature of its interactions with the other classes (ii) how self organised spatio-temporal behaviour arising from interactions between the local and global classes combines with localised components (iii) control structures combining all three classes in a feedback loops.

Note: The spatial class of local components consists of essentially non-diffusible species. However, for all the results that we present here involving such components, we also check that our observations are valid not just for diffusivity exactly equal to zero, but also when these components have a non-zero value of diffusivity that is still negligible compared to the global components.

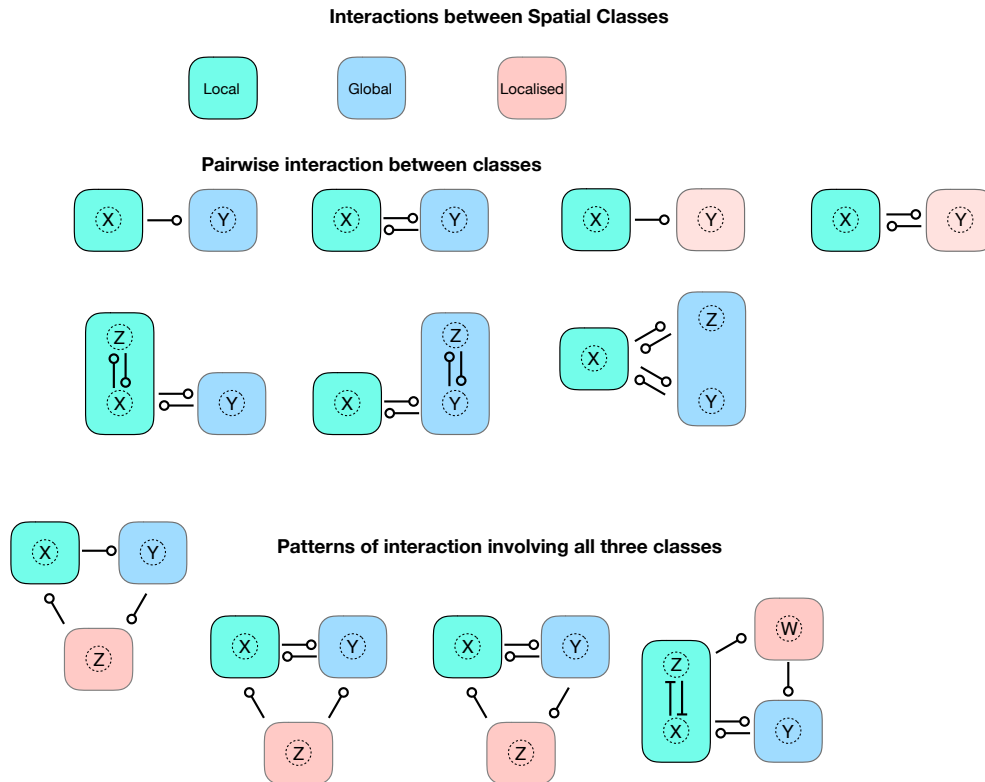


FIGURE 5.3: Schematic showing the second approach, focused on patterns of interactions between different spatial classes. We examine multiple patterns of interaction, including those involving multiple feedback loops between spatial classes.

## 5.3 Results

### 5.3.1 Motif centric approach

We begin by considering the spatial organisation of two node motifs. We examine all possible ways of incorporating localised elements (nodes or interactions), and use this as a basis to guide our study of three node motifs, where the number of possible combinations is much greater. Note that, as discussed in the methods, this part of the study includes the possibility of localised interactions between nodes. When examining such cases, we restrict localisation to a maximum of two interactions. Furthermore, when considering localised interactions, all the nodes will be assumed to be either local or global.

#### Two-node motifs - Basic effects

With simple signal transduction motifs (i.e. activation or inhibition), we observe that localisation of either the regulating node (the node that receives the signal) or the interaction between the nodes, introduces a gradient in the steady state profile of the output node. This gradient may be negligible if the diffusivity of this node is very high. Now if a localised regulating node is subject to a gradient signal, then it may be said to transmit spatial information from the signal profile to the output, in the following sense: if the phase of the signal changes, keeping its spatial average the same, then this change is reflected in the level of the output. This is due to the fact that the 'local' signal level at the location of the localised node also changes. The same result follows when we have a localised interaction, provided that the diffusivity of the regulating node is not so high as to essentially average out the spatial profile of the signal.

#### Two-node motifs - Feedback

We begin by considering the localisation of one of the nodes in a two node positive or negative feedback loop. The two basic effects seen above – of possible location dependence in a closed domain (even when the signal is uniform), and the sensitivity of the output level to changes in the phase of a graded signal - are also seen here. The only difference is that the feedback regulation causes the former to be reflected in the steady state of both nodes. Thus, the (average) steady state levels of both the localised and the diffusing

node (in response to a fixed uniform signal) depend on the location of the localised node within the domain.

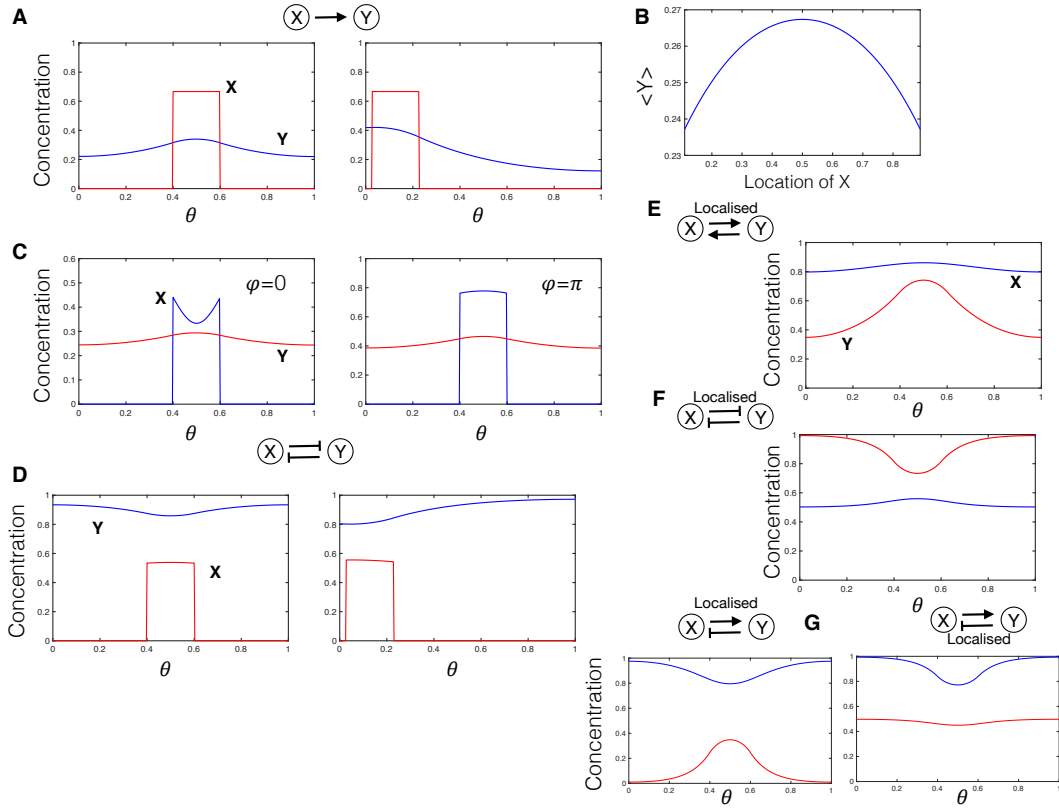


FIGURE 5.4: Simple signal transduction and feedback. (A) Localised regulation of a global node Y by a localised node X. The profile of Y depends on the location of the X node relative to the boundaries. (B) The spatial average of the node Y across the domain depends on the location of node X, with a maximum when it is at the centre of the domain. The level of X is independent of location. (C) The steady state level of both nodes depends on the phase of a signal. (D) With a feedback, (mutual inhibition in this case) the level of both nodes exhibits position dependence. (E,F,G) Localising one of the interactions. The spatial profiles of X and Y may be similar or contrasting, depending on which interaction is global (see text)

Next we examine the case of localising an interaction. Here again, the localised interaction causes gradients to form at steady state, and we observe the same effects as above. In addition, on examining the nature of the spatial profiles at the two nodes, in response to a uniform signal, we find an interesting pattern. We see that the two nodes can exhibit either similar or contrasting gradients, depending on the nature of the interaction that is localised. Let us start with the two node mutual activation motif. Here it is easy to see that, if one of the interactions is localised, then the two nodes exhibit similar gradients at steady state. Similarly, with the mutual inhibition



motif, localising one of the interactions results in contrasting gradients for the two nodes. This behaviour may be understood in the following way: the localised interaction merely introduces the spatial heterogeneity that creates a steady state gradient. It is the remaining interaction (which occurs across the whole domain) that determines whether the gradients are similar (if activation) or contrasting (if inhibition). This is clearly reflected in the case of the two node negative feedback loop, where we see that localising the inhibition results in similar gradients, while a localised activation produces contrasting gradients (see Fig. 4).

**Bistability:** A two node positive feedback motif (mutual activation or mutual inhibition) is capable of bistable behaviour. We first examine the case of a localised input signal with nothing else localised. In this case the possibility of bistable behaviour depends on the diffusivity of the two nodes (for simplicity, nodes assumed equally diffusible). Here we see such behaviour in a two node mutual activation motif, subjected to a localised input signal. As the diffusivity is increased over a range, the motif loses bistable behaviour and then regains it (see Fig. 5). While the bistable behaviour is essentially localised at low diffusivity (with the two steady state profiles almost identical away from the localised subdomain), its effect is seen globally (across the whole domain) at high diffusivity.

Next we consider the localisation of one of the nodes. Here, in addition to its dependence on diffusivity, we see that the bistable behaviour also depends on the location of the localised node within a closed spatial domain. For example, we see that (for fixed diffusivity), a system with the localised node close to a boundary may be bistable, while one with this node at the centre of the domain is not. This is reminiscent of the ‘location-dependent’ bistable behaviour seen for spatially organised genetic circuits, in the previous chapter (see Appendix C for an analysis of this behaviour).

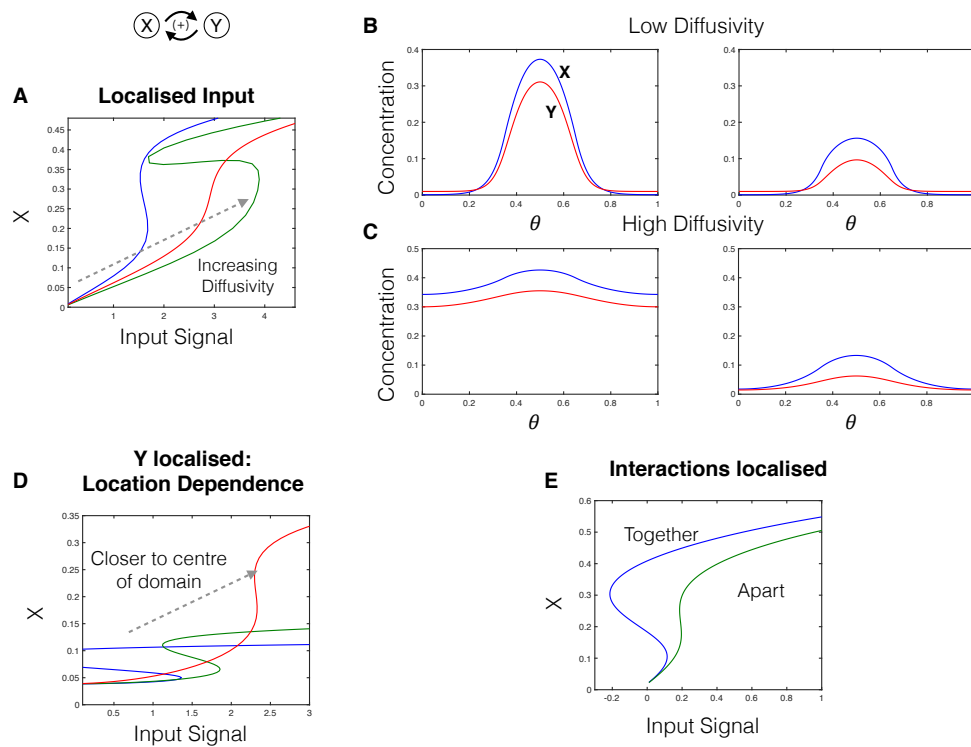


FIGURE 5.5: Two node positive feedback motif (mutual activation). (A) With localised activating signal on X. The bistable behaviour is affected by the diffusivity of the nodes - bistable at low diffusivity, no bistability at intermediate diffusivity, bistable at high diffusivity. (B) Stable steady state profiles at low diffusivity. The level of X and Y outside the localised domain is essentially the same at both steady states. (C) Stable steady state profiles at high diffusivity. The steady states are significantly different across the whole domain. (D) With the Y node localised, and X diffusing, bistable behaviour depends on the location of node Y relative to the boundaries (as seen in the variation of switching thresholds). The size of the bistable regime is negligible when node Y is at the centre of the domain. (E) With localised interactions, we see that the bistable behaviour may depend on whether they are co-localised or apart (for fixed diffusivity of the nodes).

We now come to the case of localised interactions. Here we see that the diffusivity dependence of bistable behaviour can exhibit different trends, depending on whether the two interactions are co-localised - in which case low diffusivity may produce bistability but high diffusivity may not - or apart - in which case a minimum level of diffusivity is required to maintain bistable behaviour.

**Oscillations:** A two node negative feedback motif (even with Hill function dependence) is incapable of limit cycle oscillations in the ODE case. This

can be demonstrated using Bendixson's negative criterion for systems on the plane. However, we see that by localising the interactions at different in a spatial domain, the motif becomes can exhibit stable spatio-temporal oscillations. Separating the interactions (sufficiently) in space, effectively introduces a time delay that essentially overcomes the restriction imposed by the two node negative feedback structure. In this case, the oscillatory behaviour depends on the diffusivity of the two nodes (assumed equal). As the diffusivity is varied, we see that stable oscillations are absent at low diffusivity, emerge at intermediate diffusivity, and disappear at high diffusivity (see Fig. 6).

With the above system (i.e. with the two interactions at different locations), we also see that it is possible to control the behaviour using a spatially graded signal. In fact it is possible that, for the same signal average (over the domain), an oscillatory response may be elicited only by a graded signal, while a uniform signal does not. In such cases, the possibility of oscillations may also depend on the phase of the signal.

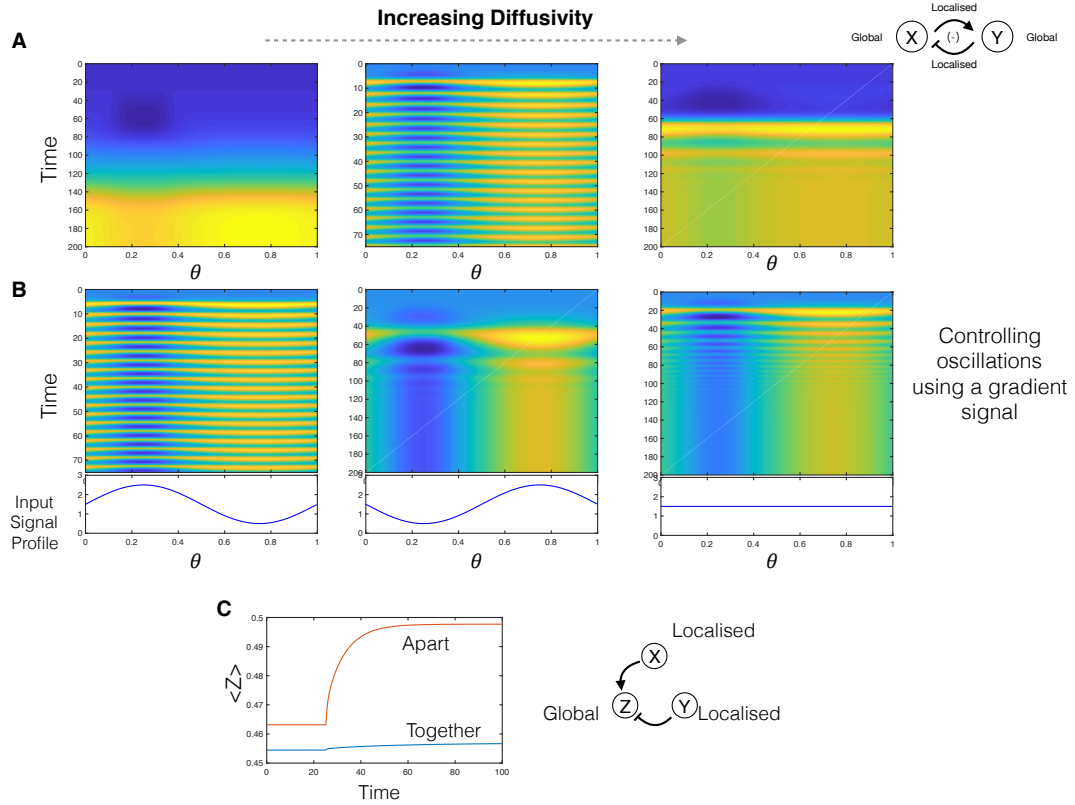


FIGURE 5.6: Two node negative feedback motif with interactions localised at different locations. (A) Behaviour depends on diffusivity of the nodes. We see damped oscillations at both low diffusivities and high diffusivities, with stable spatiotemporal oscillations at intermediate diffusivities. (B) The oscillatory behaviour may be controlled by applying a gradient signal. Here we see a case where a graded signal can produce stable oscillations, depending on its phase, while a uniform signal with the same average level does not. (C) Adaptation by an incoherent feedforward motif. Responses to identical step inputs, with the localised input nodes co-localised and apart. While we have close to exact adaptation with the inputs co-localised, this is not possible when they are apart.

### Three-node motifs with three interactions - Feedforward

Here we examine the simplest realisation of a feedforward interaction - an 'output' node regulated by two 'input' nodes, both of which are targeted by the same input signal. It is instructive to examine the case of an incoherent feedforward (with one of the nodes activating the output and the other inhibiting it), which is capable of (inexact) adaptive behaviour in the ODE. Let us first consider the case where only one of the input nodes is localised. Here we see that, if the output node has relatively low diffusivity, then the effective

'source-sink separation' created by localising one of the input nodes can produce significant gradients in the steady state output profile. As a result, the output (including its spatial average) does not exhibit adaptive behaviour, even to a uniform signal.

Now, with both input nodes localised, we see (assuming that the basal rates of interconversion at the output node are negligible relative to those controlled by the interactions) the following possibilities: (i) With the nodes co-localised, the steady state output profile is essentially uniform, and it essentially exhibits adaptation to a uniform signal. (ii) With the nodes apart, we again have a 'source-sink separation' that results in steady state gradients, and may prevent adaptive behaviour (see Fig. 6).

### **Three-node motifs with three interactions - Feedback**

A three node negative feedback motif may be capable of oscillations (with sufficient nonlinearity in the interactions - we use a Hill coefficient of 4). With one of the nodes localised, we see that (i) the possibility of stable oscillations depends on the diffusivity - as the diffusivity is increased over a range, we find essentially localised oscillations at low diffusivity, no stable oscillations at intermediate diffusivity, and essentially global oscillations at high diffusivity (see Fig. 7). Note the contrast between this trend and what we have seen previously for oscillatory behaviour in the two node negative feedback motif.

A three node positive feedback motif is capable of bistability, however, having a localised node here does not add any new capabilities or introduce new effects, apart from those already seen in the two node case discussed above.

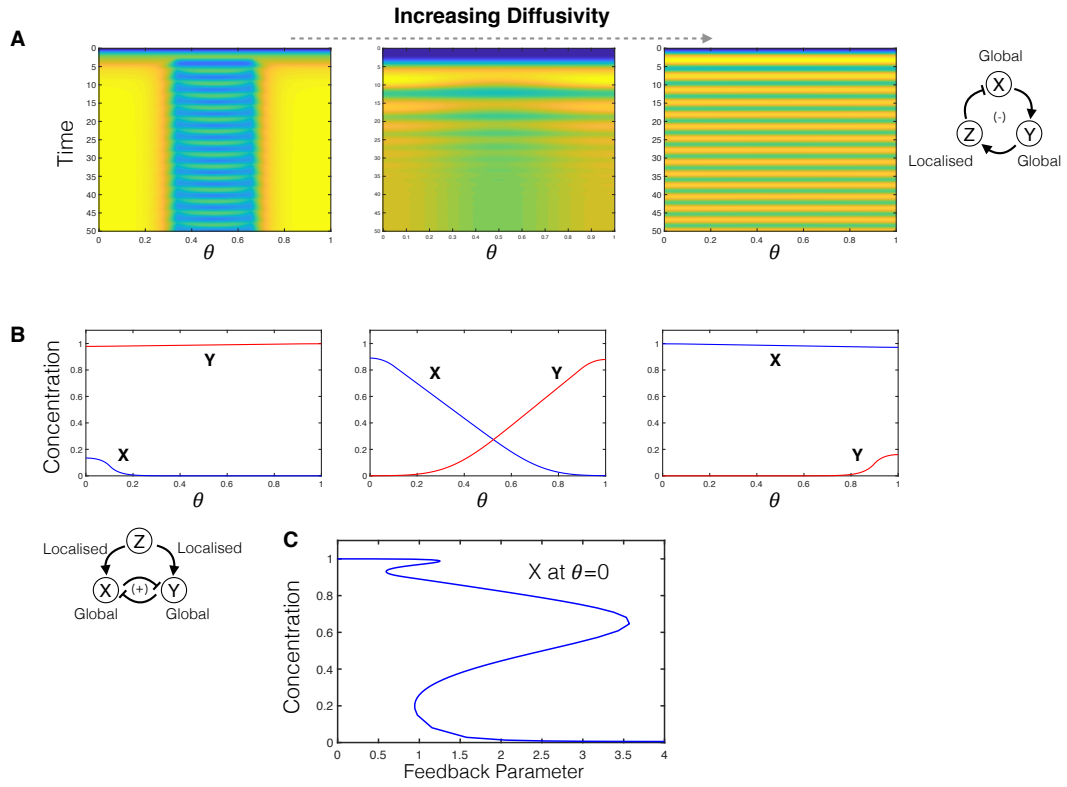


FIGURE 5.7: Three node motifs. (A) Three node negative feedback with one node localised. Diffusivity dependent behaviour. Stable oscillations at low and high diffusivities, lost at intermediate diffusivities. (B) Two node positive feedback (mutual inhibition), with localised feedforward activation. With the activation, by local node Z, localised at opposite ends of the domain, the motif exhibits three stable steady state, two of which essentially correspond to its steady states in the ODE, and a third that allows essentially localised coexistence of X and Y. (C) Bifurcation diagram with respect to a feedback parameter, showing the coexistence of all three steady states over a range of values.

### Three node motifs with four interactions

We now consider a maximum of four interactions between the three nodes. This allows us to realise essentially two classes of motifs - (i) A two node feedback loop augmented by a feedforward interaction with a third node. (ii) A combination of a two node and a three node feedback loop. The former can be understood by building our understanding of the two node feedback loops, and we examine these cases first, and discuss some of the significant cases below.

### Feedforward interaction of a two-node feedback with a third node

**Two-node mutual inhibition with a coherent feedforward:** Here the two nodes forming the mutual inhibition loop are both activated by a third, 'input' node. In this case, we use a specific model from the literature for the two node feedback - the Cdc2/Wee1 system discussed in Angeli, Ferrell, and Sontag, 2004. The parameters (also taken from this paper) are chosen so that the two node feedback loop by itself, is capable of bistability (in the ODE). Each stable steady state has one of the nodes 'high' and the other 'low'.

Now, with all three nodes having relatively low diffusivity, we observe that localisation of the two feedforward (activation) interactions, at different locations (at the two ends of a closed domain for instance), can add a new feature - by introducing a third stable steady state. Specifically, the system can have two (heterogeneous) stable, 'high-low' steady states that essentially correspond to the stable steady states of the two node feedback - i.e. where one node is 'high' and essentially uniform, and the other is 'low' and essentially localised (see figure) - as well as a third stable 'co-existence' steady state, where the two nodes exhibit significant (and contrasting) gradients (see Fig. 7). The latter has both nodes being 'high' in a certain part of the domain (around the subdomain where each one is activated), allowing a localised co-existence of the two active forms within the domain. Bifurcation analysis of the PDE model, with respect to the 'feedback' parameter, indicates the co-existence of all three steady states over a range of parameter values. Furthermore, simulations indicate that, for the chosen parameters., a uniform signal can only switch the system between two of the steady states - specifically, between one of the 'high-low' steady states and the 'co-existence' steady state. A spatially graded signal is required to switch between the two 'high-low' steady states. Simulations over a large range of input signal indicates that this restriction with respect to uniform signals is not due to a saturation effect at the 'input' node, and cannot be overcome even by making the total amount at the 'input' node arbitrarily high.

The above case clearly demonstrates the potential for localisation to introduce new capabilities when combined with a motif and diffusing components.

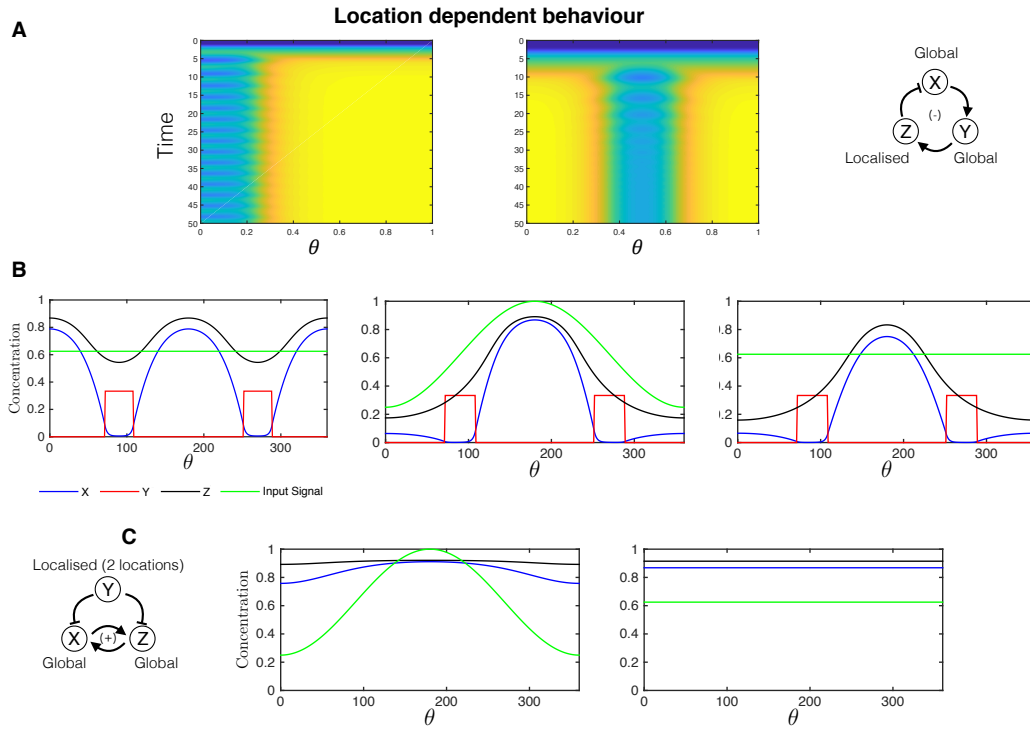


FIGURE 5.8: Three node motifs. (A) Three node negative feedback with one node localised. Location dependent behaviour. Stable oscillations when the node Z is localised near a boundary; damped oscillations when it is localised at the centre of the domain. (B) Localised inhibition of a two node mutual activation bistable motif, can trap bistable fronts. With localised inhibition at two separate locations, this enables irreversible spatial switching by a graded signal. Switching is not possible for the same signal, in the absence of localised inhibition.

**Two-node mutual activation with a coherent feedforward:** Here the two nodes forming the mutual activation loop are both inhibited by a third node, and the input signal is assumed to directly target one of the nodes in the feedback loop. Again, we choose parameter values so that the two node feedback is capable of bistability. Now, it has been shown that a bistable motif with weakly diffusible species can exhibit 'spatial switching' in response to a gradient signal (for instance, see Alam-Nazki and Krishnan, 2012). Here we see that, in a periodic domain, localising the third feedforward node at two separate locations can facilitate such 'spatial switching', even when the two mutually activating nodes have higher diffusivity and are incapable of



'spatial switching' by themselves. In this case, the 'spatial-switching' essentially results from having bistable fronts trapped by the heterogeneity created by localised inhibition by the third node. With the possibility of fronts being trapped at two separate locations, the domain is essentially divided into two independent subdomains, each of which can maintain one of two steady states (see Fig. 8).

### **Feedback interaction a two-node feedback with a third node**

With two feedback loops between three nodes, we have three possible combinations of feedback loops. Motifs combining two positive feedbacks are capable of multistable behaviour, with three distinct stable steady states. Those combining two negative feedback loops can exhibit oscillations depending on their structure and spatial organisation. However, since we have already examined the basic aspects of how spatial organisation combines with both these types of dynamical characteristics (multistability and oscillations) individually, and since the number of possible configurations is very large when we also bring in spatial organisation, here we will focus on motifs that combine a positive and a negative feedback. Such motifs bring new capabilities in the spatial context, including self-organised behaviour patterns and wave phenomena, and therefore we might also expect these motifs to exhibit non-trivial behaviour when localised elements are introduced.

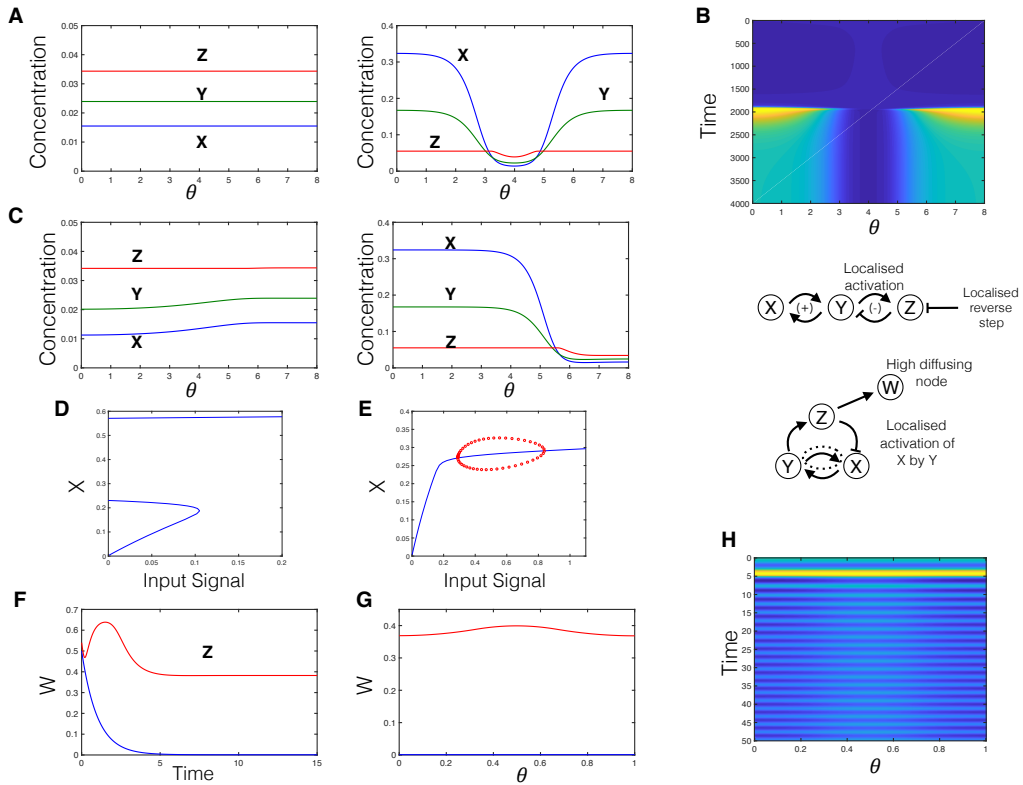


FIGURE 5.9: Three node motifs combining a positive and a negative feedback loop. (A) Localisation can destabilise a homogeneous steady state. All nodes are equally diffusible. With no localisation, we have a stable homogeneous steady state corresponding to the steady state of the ODE. By localising the forward and backward steps at the Z node, the same homogeneous steady state is still an equilibrium for this system. However, it may be unstable depending on the size of the localised subdomain, with the system reaching an inhomogeneous steady state. Note that the Z node is still uniform outside the localised subdomain. (B) Time evolution of the Y node across the domain, with the homogeneous steady state as initial condition, and an inhomogeneous final steady state. (C) With the homogeneous steady state unstable, the system is capable of exhibiting distinct stable steady states, depending on initial conditions (Shown here with the localised subdomain near one of the boundaries). (D-H) Three node negative feedback containing a positive feedback within. (D) With the positive feedback between X and Y, the system is bistable (ODE). (E) In the absence of the positive feedback, the three node negative feedback loop is capable of oscillations. (F,G,H) With the positive feedback localised, it is possible to access both bistable and oscillatory regimes by varying the same input signal (uniform signal profile). Here we show how each of these characteristics may be captured by a high diffusing output node W, that is activated by Z. (F) Bistability: Time evolution of the spatial average of W depending on initial conditions. (G) Two stable steady state profiles of W. (H) Essentially global oscillations of W when the input signal is in the oscillatory regime.

**Localisation can destabilise a homogeneous equilibrium solution:** Perhaps the most significant result we see in this section, is that localisation - to be specific, localised interactions between equally diffusible nodes - can bring about instability of a homogeneous equilibrium solution. The particular case in which we observe this behaviour is that of a motif with a two-node positive feedback loop and a two-node negative feedback loop sharing a common node. All nodes are assumed to be equally diffusible.

Let us first label the nodes for clarity in the discussion. Consider nodes Y and X, mutually activating each other, with node Y also activating node Z, which in turn inhibits Y. Now suppose that one of the interactions - specifically the activation of Z by Y is localised in a subdomain. This localisation by itself will necessarily introduce steady state gradients in node Z, and this gradient will also be reflected at the other nodes due to the other interactions. Thus, there is no possibility of a homogeneous equilibrium in this case.

Now consider the case where the reverse step at node Z (i.e. the conversion from  $Z^*$  to Z) is also localised within the same subdomain. Assuming that there is no basal rate of conversion of Z to  $Z^*$  outside this subdomain, it may be seen that this system can have homogeneous equilibrium solutions exactly corresponding to the stable equilibrium of the ODE. Indeed, without any localisation, this is also a stable homogeneous equilibrium solution for the spatial realisation. However, with localisation of the reactions at the Z node, simulations indicate that this equilibrium solution may in fact be unstable to arbitrarily small spatial perturbations. In multiple simulations with initial conditions corresponding to this solution (with a small spatial perturbation added), we see that the system evolves away from this solution and reaches a heterogeneous steady state (see Fig. 9).

We note that, whatever heterogeneous steady state the system reaches, the spatial profile of node Z outside of the localised domain must be uniform (since node Z is not subject to any reactions outside this domain). Its profile can (and does) exhibit heterogeneity only within the localised subdomain.

Further investigation indicates that:

1. The instability may depend on the size of the subdomain. As the size of the subdomain is increased, the homogeneous equilibrium eventually becomes stable.
2. When the homogeneous equilibrium is unstable, there may be multiple heterogeneous steady states that the system can reach, depending on initial conditions. This is true for both closed and periodic domains.

3. In a closed domain, for a fixed size of localised subdomain, the instability may depend on the location of the subdomain relative to the boundaries. For instance, the homogeneous equilibrium may be unstable when the subdomain is close to the centre of the domain, while it is stable when the subdomain is close to one of the boundaries.

**Combining oscillatory and bistable behaviour:** Here we consider a motif that consists of a three node negative feedback, containing a two-node positive feedback (see figure). The input signal activates the node X. For the ODE with the parameter values chosen, we see that, while the negative feedback loop by itself is capable of oscillatory behaviour over a range of input, the presence of the added positive feedback causes the motif to be bistable, with no oscillatory behaviour at any (positive) value of signal.

We now examine a spatial realisation of this motif, with the feedback interaction between X and Y localised (the three node feedback loop remains intact across the whole domain). In this case, we see that for relatively low diffusivities of the nodes (assumed equally diffusible) it may be possible to access regimes of both bistable and oscillatory behaviour, just by varying the same (uniform) input signal. However, in each of these regimes, behaviour is essentially confined to a part of the domain - bistability is confined to the domain of localisation of the positive feedback interaction, while oscillatory behaviour is confined to the rest of the domain. Both these behaviours may be communicated across the whole domain by having a highly diffusible node that is regulated by one of the motif nodes. Whether the motif is in the bistable or the oscillatory regime, such an output node would exhibit essentially uniform steady states or oscillations across the domain (see Fig. 9). This case demonstrates how a single localised component can allow a motif to exhibit a combination of characteristics that may not coexist in the well-mixed (ODE) scenario.

**Pattern formation:** Although the three-node feedback motifs in this section are capable of pattern forming behaviour, we note that (within our framework of motifs) three nodes with four interactions only provides the minimal set of ingredients necessary for this behaviour, without allowing for the possibility of any additional regulation. Therefore, not only is localisation of any of the components certain to distort pattern formation, but we are also restricted in terms of which node may be localised. Since pattern formation by such a motif essentially relies on 'local' activation and 'global' inhibition,

localising the inhibitor node will prevent such behaviour. It is possible however, for instance, to essentially localise pattern formation by localising one of the other nodes or by localising interactions. We do not discuss these cases in detail here, but defer the examination of such systems to the next section of this chapter, where we examine interactions between spatial classes. There we take a constructive approach to add additional localised regulation and feedback to a pattern forming system.

### **Combining localised and global elements within an interaction:**

As a way to systematically introduce slightly more complexity into the spatial realisations of motifs examined so far, we consider a case where an interaction between two nodes can involve a combination of a localised and a highly diffusible element. This essentially allows the localised component of the interaction to sample 'locally' and communicate this information across the whole domain through the diffusible element.

**New capabilities:** To demonstrate how such an interaction can introduce new capabilities, we examine the case of a bistable two-node mutual inhibition motif (X and Y are global and inhibit each other) subject to a feedforward regulation by a third, non diffusing node Z. Z activates both X and Y, with parameters chosen so that variation of the signal regulating Z cannot take the system out of the bistable regime. This essentially means that the signal cannot induce a switching of steady states regardless of its spatial profile. We now introduce a localised and a global element to the activation of Y by Z. The global element here is assumed to be highly diffusible (essentially flat at steady state). With such an interaction, we observe that, while varying a uniform signal (even up to saturation of the Z node) does not produce a switching of steady states, a graded signal can induce a switching. Moreover, this depends on the phase of the signal (see Fig. 10).

**Delay effects:** Now we examine the possibility that the global element in the interaction has relatively low diffusivity. In this case, we see that although the steady state behaviour of the motif is essentially similar to that above (with bistability and X and Y essentially uniform at steady state), the lower diffusivity of the global element now introduces a delay effect that changes the transient behaviour. This allows even uniform input signals to induce switching in the motif.

Thus we see how a combination of localised and global elements can (i) introduce new capabilities (ii) make the transient behaviour of a motif dependent on the spatial organisation while keeping steady state behaviour essentially unaffected.

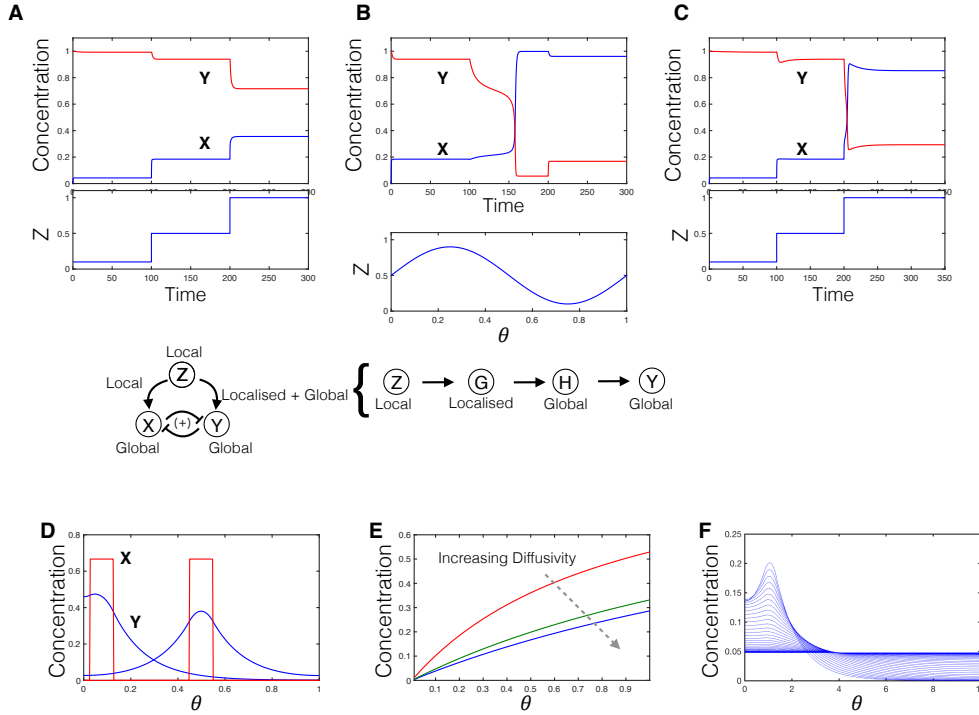


FIGURE 5.10: Spatial organisation of motifs. (A) Two node mutual inhibition with activating feedforward from node Z. Nodes X,Y and Z are global. The activation of node Y occurs through a combination of a localised node G and a high diffusing global node H. Plots show time profile of evolution of X and Y spatial averages. (A) shows that switching between steady states cannot be induced by step changes with a uniform signal profile. (B) Switching is possible with a gradient signal (this also depends on the phase of signal profile). (C) If the diffusivity of the global node H is lower, then it may be possible for a step change in a uniform signal to induce switching. (D) Basic location dependent behaviour. Localised node X activates global node Y. The level of Y within the localised subdomain depends on its locations relative to the boundaries. (E) Global node communicating between two localised nodes. When the nodes are co-localised, the signal response of the localised output node is reduced by increasing diffusivity of the global node. (F) When the nodes are apart, there exists a range of locations for the output node for which its steady state response varies non-monotonically as the diffusivity of the global node is increased. This is because the level of the global component at these locations varies non-monotonically. Here we show steady state profiles for this component over a range of diffusivity.

### 5.3.2 Space centric approach

We now examine pairwise interactions between the three spatial classes – local, global, and localised – following the approach laid out in section 2. An important point needs to be made here regarding the distinction between local and global classes, and how this dictates the way we examine their interactions with the localised class. Local nodes consist of essentially non-diffusible species. When the diffusivity of such a node is strictly equal to zero, there is a clear distinction between this class and the global class of nodes, in terms of their interaction with a localised node. The former results in a completely localised realisation of the motif, and the effects of the motif interactions are confined to the subdomain containing the localised node. With the local node being completely decoupled in space, for uniform initial conditions, the behaviour within this subdomain is identical to the ODE realisation of the motif. Outside this subdomain, the local node exhibits a steady state that is dictated by the basal interconversion rates. In the latter case, with a global node, the effect of the motif interactions is propagated across the whole domain. Moreover, even with uniform initial conditions, the presence of non-zero basal interconversion rates outside of the localised subdomain, and the spatial coupling by diffusion can make the behaviour within the subdomain, quite distinct from that of the ODE realisation of the motif. Now, when the local node instead has a negligible, but non-zero diffusivity, it is no longer decoupled in space, and so, with respect to its interaction with a localised node, is no different from a global node with low diffusivity. The examination of such cases can therefore be subsumed within the study of localised + global interactions, by considering a range of diffusivity for the global class, ranging from low to high. The case of local nodes with zero diffusivity can be understood in terms of the ODE behaviour, as mentioned above, and needs no examination in a spatial context. For interactions between local and global classes, the two are clearly distinguished by their diffusivity – zero, or a negligible but non-zero diffusivity for the local class, and a significantly higher diffusivity for the global class. Note that the relatively high diffusivity of the global class does not preclude the formation of significant gradients/localised structures involving nodes in this class, as this is determined not only by the diffusivity, but also by the reaction rates corresponding to these nodes. Thus, we are left with two types of pairwise interactions, namely local and global, and localised and global.

### 5.3.3 Localised and Global

#### Simple signal transduction

**Localised targets Global:** The regulation of a global node by a localised node ensures that the steady state profile of the global node is graded. Its gradient may or may not be negligible – this depends on the interplay between its diffusivity, and its basal reaction rates across the domain. Even for high values diffusivity, a global node with one of its conversion reactions regulated by a localised node, can exhibit significant gradients if the basal reaction rate constant associated with the reverse reaction is high enough (assuming that the reverse reaction occurs everywhere in the domain). For graded signals, the response of the localised node depends on the phase of the signal, and therefore, so does the (average) level of the global output. An interesting point to note is that, in a domain with closed boundaries, at steady state, the (average) level of the global output over the whole domain depends on the location of the localised subdomain relative to the boundaries (see Figure 4(B)). This dependence, which essentially arises as a boundary effect, becomes negligible at high diffusivities. This effect also distinguishes a global node involving a conserved total amount of interconverting forms, from one involving a single species being produced and degraded. It can be shown analytically that the latter cannot exhibit such position dependence of average output. Also note that, in the present case, for low diffusivities, the (average) output level within the subdomain of localisation also depends on the location of the localised subdomain relative to the boundaries. This can be significant in the context of feedback regulation between a localised and a global node (see below). Note that varying the level of the input signal at the location of the localised node has a pure scaling effect on the profile of the global node outside the localised subdomain. This can be demonstrated using the analytical solution for the case of a single node subject to localised activation. From the expression for the concentration profile outside the region of localisation, we see that the localised activation rate only appears in a multiplicative term that does not depend on the spatial variable.

**Global targets Localised:** For a global node with high diffusivity (relative to reaction rate constants), its steady state profile is essentially flat, and therefore no spatial information (from a graded signal) reaches the localised node. However, if it has relatively low diffusivity, a graded input signal can produce significant gradients in its steady state profile. In this case, the response



of the localised node would depend on the phase of the signal.

**Localised targets Global targets Localised:** When a global node plays the role of a communicating node between two localised nodes, the response of the motif depends on the diffusivity of the global node. Without loss of generality, we focus on the case where both interactions involve activation (Similar insights hold for cases involving inhibition). There are essentially two cases: (i) when the localised nodes are co-localised, the steady state signal response of the motif is significantly reduced in magnitude as the diffusivity is increased (see Fig. 10); in addition, the localised output level also depends on the location of the localised nodes within a closed domain (a consequence of the location dependence seen above). (ii) When the localised nodes are apart, the steady state signal response of the motif is typically reduced at low diffusivity, with the two localised nodes becoming disconnected in the limit of zero diffusivity. However, as the diffusivity is increased, we note that, depending on the location of the localised output node, the response may be non-monotonic - i.e. as the diffusivity increases, there is a certain range of locations in the domain where the steady state level of the global node over increases first, reaches a maximum at a certain value of diffusivity, and then decreases again, with the profile becoming essentially flat and unchanging at very high diffusivity (see Fig. 10).

#### **Feedforward interactions:**

**Localised input and output nodes; Global feedforward:** In this case, there is a direct interaction between the two localised nodes, and so they must be co-localised for this interaction to occur. The global nature of one of the feedforward arms means that the steady state input response of the motif to a graded signal must depend on the diffusivity of this global element. For an incoherent feedforward interaction, depending on parameters, the steady state level of the localised output can exhibit adaptation to signal levels (inexact adaptation). However, in addition to the kinetic parameters, the adaptive steady state also depends on the spatial parameters (diffusivity, domain size, location within the domain). This can be seen as a direct consequence of the effects discussed previously.

**Localised and Global input nodes; Global output:** The presence of a localised feedforward interaction makes the steady state profile of the output node graded (depending on parameters, the gradient may be negligible). For

an incoherent feedforward interaction, depending on parameters the output node may exhibit capable of adaptive behaviour with respect to a uniform input signal. This requires the output node to have relatively high diffusivity (no significant gradients at steady state). However, the output cannot adapt to spatially graded signals, since the localised arm of the feedforward only transmits the local signal level, while the global arm essentially transmits the average level.

**Localised input nodes; Global output:** Here we find possible adaptive behaviour, regardless of the diffusivity of the global node, when the two localised input nodes are co-localised. If however, the input nodes are apart, adaptation to a uniform signal relies on relatively high diffusivity of the global node, while there is no adaptation to a graded signal.

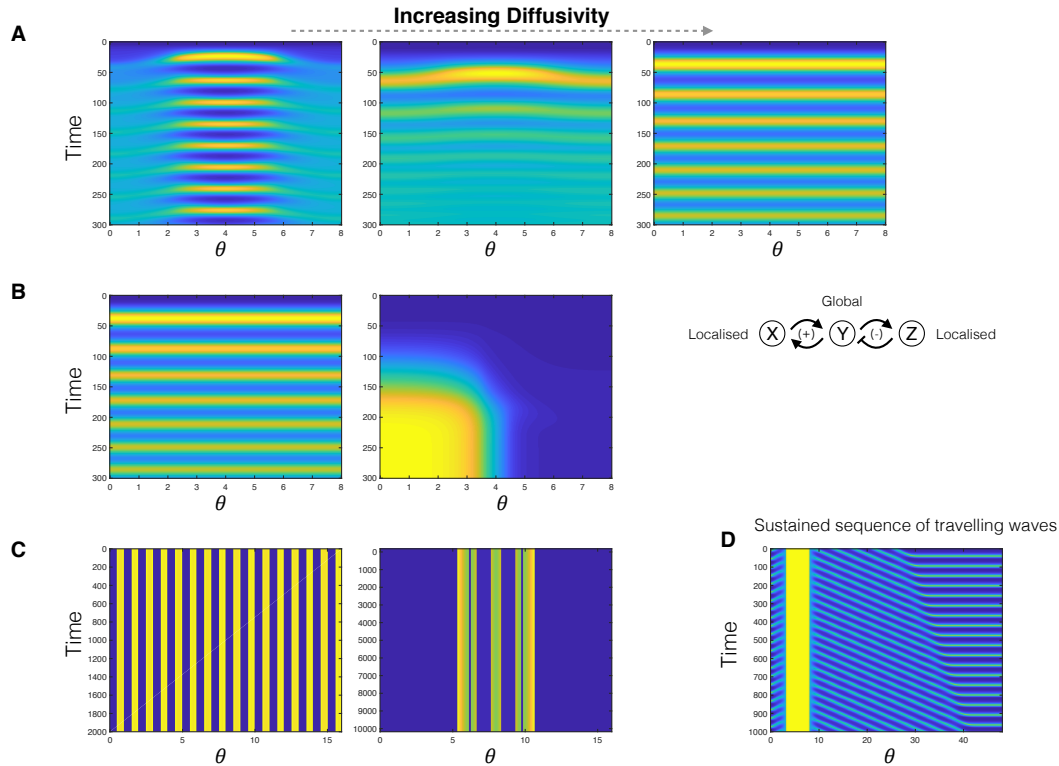


FIGURE 5.11: (A) Activator inhibitor type motif, with positive and negative feedback elements (X and Z) localised together at the centre of the domain. We again see diffusivity dependent oscillatory behaviour. (B) With the two feedback loops localised apart, stable oscillations are only possible at high diffusivity of the global node Y. (C) Localising X and Y leads to pattern formation within the localised region. However, this pattern is quite distinct from that observed when X and Y are non-diffusing, but present everywhere. (D) With all three nodes global (with equal diffusivity), activation of this motif by a localised node can produce a sustained sequence of travelling waves.

### Feedback interactions

**Single feedback loop – basic observations:** In the simplest case, of a two node feedback (positive or negative) between a localised node and a global node, we see that the steady state level of the localised node depends on both the diffusivity of the global node and the location of the subdomain (within a spatial domain with closed boundaries). Both these dependences become negligible at high diffusivities of the global node (in fact, the location dependence due to the boundaries is also negligible at very low diffusivities). This may be understood by examining the simpler case of a localised node regulating a global node, where we see that the (average) level of the global

output over the subdomain of localisation depends on both its diffusivity and the location of the localised subdomain relative to the boundaries. In a feedback loop, with the global node now also regulating the localised node, this dependence is reflected in the steady state of the localised node. In fact, such a feedback interaction with a global node is the only way the output level of a localised node can exhibit location dependence.

**Single feedback loop – bistability and oscillations:** A two node positive feedback loop (mutual activation or mutual inhibition), with suitable non-linearity in the interaction, is capable of bistable behaviour in the ODE. We see echoes of this behaviour in a two node feedback involving a global and a localised node, i.e. two distinct, stable steady state profiles for the same set of parameter values. Furthermore, the possibility of such behaviour depends on the spatial parameters – (i) the diffusivity of the global node: where the “bistable” behaviour may be seen for low diffusivity and disappears for high diffusivity and (ii) the location of the localised node within a closed domain: for a fixed diffusivity of the global node, the “bistable” behaviour may be seen when the location is close to a boundary, but disappears if the location is close to the centre of the domain. Similar “location dependent” bistable behaviour is also observed (and has been analysed) in the context of other spatially organised bistable circuits with localisation in a closed domain (see Chapter 3). A three node negative feedback loop, again with suitable non-linearity in the interactions, is capable of exhibiting oscillatory behaviour in the ODE. Now, with two of the nodes global and the other localised, it is possible to see echoes of the ODE behaviour, with sustained spatio-temporal oscillations. Similar to the case of bistability, the possibility of oscillatory behaviour depends on both (i) the diffusivity of the global nodes: with sustained oscillations possible for very low and for high diffusivity of the global nodes, and a stable steady state profile at intermediate diffusivities; and (ii) the location of the localised node within a closed domain: for a fixed diffusivity of the global node, sustained oscillations may be possible when the location is close to a boundary, but disappears if the location is close to the centre of the domain.

**Single feedback loop completely within Global or Localised - Bistability:** Consider a two node positive feedback loop completely within the global class, i.e. between two global nodes, capable of bistability. If this system is subject to regulation by a localised node that transmits an input signal, we

see that the “bistable” behaviour of the resulting system depends on the diffusivity of the global nodes. Interestingly, as the diffusivity is increased, we find that the behaviour may appear for low diffusivity, disappear for higher values, and reappear at even higher diffusivities. Localised regulation of a global positive feedback loop can also shape “bistable” behaviour, as the resulting heterogeneity may be capable of essentially trapping a bistable front. We have already seen such effects - for a mutual activation system consisting of two global nodes, we see that inhibition of one or both global nodes by a localised node, located at multiple distinct locations, can divide the domain into regions where it is possible to simultaneously sustain distinct steady states of the switch. Significantly, the localised element brings this capability to a system where the diffusivity is too high for spatial switching to be sustained by a graded signal profile alone.

**Single feedback loop completely within Global or Localised class - Oscillations:** We examine localised regulation of a three node negative feedback oscillator formed by global nodes. Here, depending on the diffusivity of the global nodes, the system may exhibit so called ‘phase waves’ emitted from the region of heterogeneity, i.e. the localised subdomain. The wave here consists of a localised spatiotemporal variation in the phase of oscillations. This phenomenon has been examined in multiple studies – see for example (Ortoleva and Ross, 1973).

### Double feedback systems

These cases involve two feedback loops sharing a common node.

**Two distinct feedback loops between Global and Localised elements:** For simplicity, we only examine the activation-inhibition type of interaction here. There are two possible realisations of this pattern.

1. A global node interacting with two distinct localised nodes. The activation-inhibition motif is capable of oscillatory behaviour in the ODE, and this may be echoed in such a spatial realisation – resulting in spatiotemporal oscillations. The localisation of the two feedback loops produces diffusivity dependent behaviour, with the possibility of oscillations depending on the diffusivity of the global element. If the two localised nodes are co-localised, we observe that oscillatory behaviour is seen both at very low (essentially localised oscillations) and very high

diffusivities (essentially global oscillations) oscillations may be abrogated over an intermediate range of diffusivities. The dependence of oscillatory behaviour on diffusivity thus exhibits a broad trend that is also observed in other contexts in this study where localisation combines with an oscillatory motif. Furthermore, we also have the possibility (in a closed spatial domain) of location dependent behaviour (also seen in multiple contexts in this study). Now, if the localised nodes are apart, the two feedback loops are separated in space, and oscillatory behaviour is no longer realisable at low diffusivities. Thus, for the same set of interactions involving the same spatial classes, the diffusivity dependence can exhibit qualitatively different trends, depending on the spatial organisation (see Fig. 11).

2. A localised node interacting with two distinct global nodes. For simplicity, we assume that the two global nodes are equally diffusible. As above, spatiotemporal oscillations may be possible, and we see similar diffusivity dependent and location dependent behaviour.

**One feedback completely within Global or Localised:** We examine multiple combinations of positive and negative feedback loops, with one of the loops completely within one spatial class. Cases involving two negative feedback loops were not found to exhibit any clear qualitative trends apart from the ones already observed for a single negative feedback loop.

With two positive feedback loops, it is possible for a motif to exhibit tristability (coexistence of three stable steady states). With two of the nodes localised and the other global, we see that the possibility of such behaviour again depends on the diffusivity of the global node. This is reminiscent of the diffusivity dependent bistable behaviour seen previously when localised nodes are involved. Although we have not observed it for the chosen parameter values, we expect that location dependent behaviour in a closed domain will also emerge here.

We have more possibilities with a combination of positive and negative feedback. Here again we find possible spatio-temporal oscillatory behaviour, possibly shaped by diffusivities of the global species, and location within a closed domain. In addition, with the positive feedback loop within the localised class and the negative feedback through the global element, it is also possible to obtain pattern formation within the localised subdomain (see Fig. 11).

### 5.3.4 Local and Global

#### Simple signal transduction

Here we focus on the response to a graded signal. We will assume that the diffusivity of the global node is high relative to its associated reaction rate constants, so that its gradient is negligible gradient at steady state. With a local node regulating a global node, the essential point is that, while the output does not reflect the spatial profile of the input signal there is still the possibility that the (average) level of the output might depend, not just on the spatial average of the input, but also on its gradient. This is essentially a consequence of the fact that “locally”, the steady state of the local node depends non-linearly on the level of the input signal. This means that the level of output can reflect changes in the gradient of a signal whose spatial average remains constant. In such a scenario, depending on the diffusivity of the local node, the average steady state level of this node may exhibit significant dependence on the phase of the signal. Since the global output node essentially produces an averaged “readout” of the local node, its level also depends on the phase of the signal. With a global node regulating a local node, however, the global element the output captures essentially no information about the spatial profile.

#### Feedforward interactions

Incoherent feedforward interactions involving a local input and output node, with a feedforward regulation through a global node, are known to be capable of adaptive behaviour in response to spatially uniform signals, while still capturing graded signals.

#### Feedback interactions

**Single feedback loop – basic observations:** Simple positive and negative feedback loops involving a local and a global element have been studied in previous work (Alam-Nazki and Krishnan, 2012). The point of interest here is the response to a graded signal, when the signal targets the local element and the feedback occurs through the global node. It has been shown that the presence of the global element essentially “weakens” the spatial contrast (at the local node) created by the signal in the case of positive feedback regulation, while it “strengthens” the spatial contrast in the case of a negative feedback.

**Single feedback loop – bistability and oscillations:** Both of these cases, with local and global elements, have been examined by Alam-Nazki and Krishnan, 2012. With bistability, the essential insight is that the presence of the global node prevents spatial switching behaviour in response to graded signal profiles. Similarly, in the case of an oscillatory system involving a single feedback loop, the presence of the global node can restrict the triggering of oscillations by a gradient signal, causing it to depend on the signal average across the whole domain rather than whether it locally crosses the oscillatory threshold.

### Double feedback systems

Systems involving both a positive and a negative feedback loop between local and global elements provide the basic ingredients for many different types of self-organised behaviour. Here we will focus on two of the most basic types – excitable systems and stationary pattern forming systems. However, we start by examining a more basic case, where the pattern of interactions results in oscillatory behaviour independent of the spatial characteristics of the nodes.

**Oscillator:** In regimes where the system is capable of sustained oscillations, it is possible that a spatially graded signal can disrupt oscillatory behaviour, even when its magnitude at every point in the domain lies completely within the oscillatory range of the system with respect to a spatially uniform signal. We observe that this capability, i.e. to control oscillations using a gradient depends on both the input location (i.e. the node targeted by the signal) and the spatial nature of the nodes. For example, here we see how a spatially graded signal targeting the positive feedback node can produce this behaviour, with all nodes equally diffusible.

**Excitable systems:** With the positive feedback loop within the global class and the negative feedback through a local node, we have the possibility of an excitable medium, with a uniform, stable steady state that is susceptible to finite perturbations. Different kinds of spatio-temporal patterns may be triggered in these systems, depending on the nature of the perturbation provided by an input signal. These include solitary travelling waves and continuous wavetrains in one dimension, and target patterns and spiral waves in two dimensions. Such systems have been studied extensively, and form one of



the main classes of reaction-diffusion systems where self-organized spatio-temporal behaviour results from the interplay between local processes and diffusive transport. Our intention here is not to discuss or examine the behaviour of these systems in any detail, but rather to note the capabilities arising from such a pattern of interactions between the local and global classes, and to set the stage for examining how the introduction of a localised element, either through feedforward or feedback regulation, can shape such behaviour.

**Pattern forming systems:** Another important class of systems where the local and global classes combine to produce self-organised structures, is that of pattern forming systems involving a Turing instability, where an infinitesimal inhomogeneous perturbation destabilises a uniform steady state, resulting in a stationary pattern. Here the behaviour arises from a combination of “local” self-activation and long range inhibition. With respect to the patterns of interactions considered here, this implies having the positive feedback loop within the local class, and the negative feedback occurring through a global element. We note that more complex behaviour may be possible, for instance oscillating patterns resulting from a combination of the Turing instability with an oscillatory instability. Other instabilities such as the wave instability, may also result from such an interaction between local and global classes, but we do not examine these possibilities here. A relevant point to note here, in the context of the study, is the fact that the particular stationary pattern formed depends on the initial conditions.

### 5.3.5 Interactions involving all three classes

#### **Simple signal transduction:**

Here we assume that the diffusivity of the global element is high enough for gradients to be negligible at steady state. There are six different ways of ordering the three spatial classes in a signal transduction cascade:

**Localised output with local or global input node:** In either case, the node regulating the localised node is essentially uniform at steady state. As a result, there can be no location dependent behaviour, i.e. the steady state does not depend on the location of the localised subdomain relative to the boundaries. If the input targets the local node (which in turn regulates the global node), then as seen previously, the steady state level of the global node can

depend not just on the average signal level, but also on its spatial profile. This dependence will also be reflected in the localised output. If the input targets the global node, then the signal profile is of course, essentially averaged out, and the output captures no spatial information.

**Localised input with local or global output node:** (i) If the localised input node directly regulates the local node, then for uniform input signals, the level of the global output depends on the location of the localised node relative to the boundaries. This is a direct consequence of the location dependent behaviour discussed previously for localised regulation of a node with low diffusivity. (ii) If the localised input node directly regulates the global node, then there is essentially no location dependent behaviour with respect to uniform input signals. In both the above cases, the localised node does capture the phase of the signal and this will be reflected in the output level.

**Global and Local nodes communicating through a localised node:** (i) If the input targets the global node, the signal profile is averaged out, and the output level is essentially determined only by the average signal level. However, even here, the interplay between a localised node regulating a weakly diffusible local node, and the boundaries can cause the average output level to depend on the location of the localised node within a closed domain. (ii) If the input targets the local node, this node captures the spatial profile of the signal and this case is essentially the same as having the signal directly regulating the localised node. The output is uniform, but the localised node ensures that its level captures changes in the phase of the signal.

### Feedforward interactions

Here we consider the simplest pattern of feedforward interaction, with two nodes receiving a common input signal, and regulating a third 'output' node. Again, we assume that the diffusivity of the global node is high enough for steady state gradients to be negligible. There are three possibilities: (i) With a global output node, the presence of the localised input node ensures that the spatial profile of the signal (phase of signal) is reflected in the output level. Depending on parameters, with an incoherent feedforward, the output level may exhibit an adaptive response to uniform signals, but not, in general, to spatially graded ones. (ii) With a weakly diffusible local output node, the regulation by the localised input node produces a gradient in the output and again, the phase of the signal is reflected in the output level. An

incoherent feedforward in this case results in what is effectively a ‘source-sink separation’ for the local output node, and as a result, its (average) level cannot exhibit adaptation even to uniform signals. (iii) With a localised output node, the presence of the local input node ensures that the spatial profile of the signal is transmitted to the output, so that the output level depends on the phase of the signal.

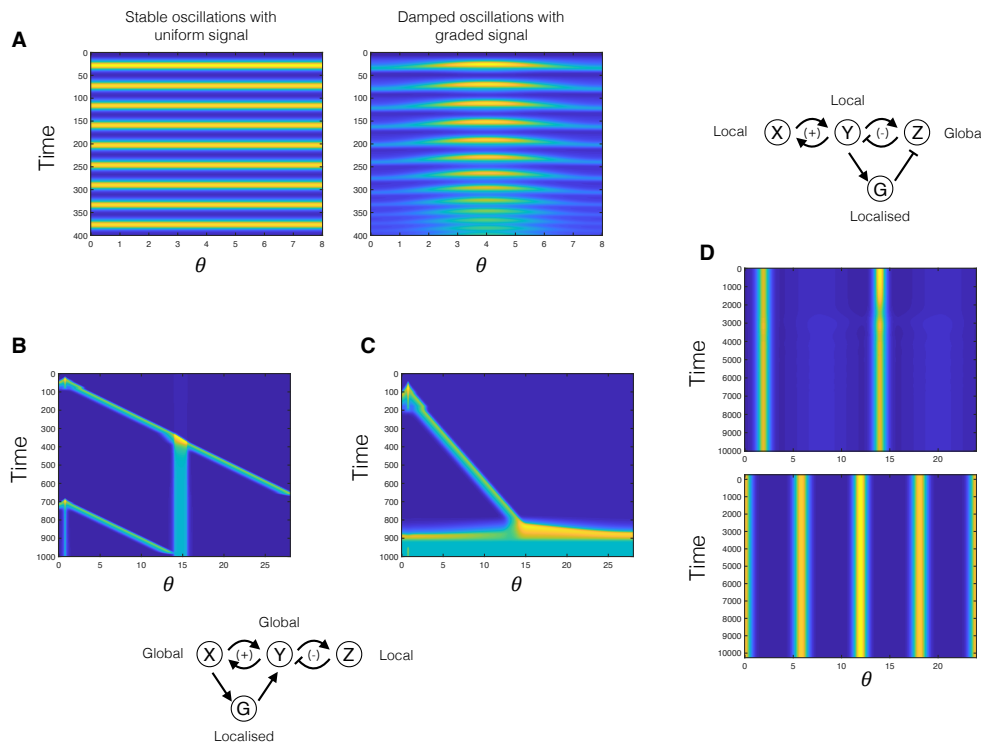


FIGURE 5.12: (A) Stable oscillations with uniform signal; damped oscillations with graded signal (B) Excitable system with localised positive feedback regulation. Here a localised switching is induced by the passage of a travelling wave through the localised domain. The localised structure thus formed annihilates subsequent travelling waves, stopping them from traversing the whole domain. (C) Excitable system with localised+global positive feedback regulation. In this case we see a global switching instead of a localised switching. This prevents any further triggering of travelling waves by localised perturbations. (D) Feedback control of pattern formation. (see text).

## Feedback interactions

### Localised forward regulation of feedback systems involving Local and Global

**nodes:** Consider a bistable system formed by a local and a global node (positive Feedback between a local and a global node, capable of bistable

behaviour for uniform input signals). If we now add localised regulation, with the input signal transmitted through a localised node, we observe two effects: (a) position dependent behaviour, where the switching thresholds with respect to the input signal depend on the location of the localised node within the domain, and (b) switching thresholds with respect to an input signal may be significantly different, depending on which node is local and which is global. Next we consider the activator-inhibitor type system examined previously, with a positive feedback between global nodes X and Y, and a negative feedback on Y through a local node Z. With kinetic parameter values chosen in the oscillatory regime, we observe that adding a localised activation to Y can produce a sustained sequence of travelling wave solutions, with waves propagating from the location of the localised node to the boundary (see Fig. 11).

**Localised regulation of excitable systems:** With the same system in the purely excitable regime, a localised activation of the global node Y (or X) can generate a single travelling wave solution when the localised node preforming the activation is subject to a step signal. Here we assume a closed domain, with the localised node adjacent to one of the boundaries. The travelling wave generated is annihilated at the opposite boundary. Now, we also see that a localised inhibition of Y (or X) can disrupt and annihilate a travelling wave solution upon reaching this location. This suggests the interesting possibility of combining these two features to form an incoherent feedforward type regulation, with the same input signal regulating both the activating and the inhibiting node, with the two nodes placed apart. In this case, it is possible that a graded signal may trigger only the activating node, thus generating a travelling wave that is not intercepted by the inhibiting node, while a uniform signal may trigger both nodes, and produce a travelling wave whose trajectory is cut short by the localised inhibition.

**Localised regulation of pattern forming systems:** In such systems, depending on the size of the localised subdomain, relative to the characteristic wavelength of the pattern, localised activation can either act as a spatial cue that determines the phase of the pattern formed (if the localised region is relatively small) or distort the pattern (if this region is relatively large). We also see that localised regulation can determine the wavelength of the pattern formed. In such cases, the effect of the localised regulation may be irreversible, meaning that the resulting pattern persists even if the input to this

node is switched off. Furthermore, we observe that by combining such localised regulation at two locations, we can irreversibly switch between patterns of different phase and wavelength. This depends on the spacing between the localised regulatory nodes and the characteristic wavelength that the (Local+Global) system is capable of.

**Single feedback loop involving all three classes:** If we ignore the nature of the interactions, there are essentially two different ways of realising a single feedback loop involving all three classes: (i) The Local node is directly regulated by the Localised node (ii) The localised node is directly regulated by the local node We first examine the response to a uniform signal. In the first case, (i) we observe that (a) unless the input signal directly regulates the local node, its response is essentially confined to the localised subdomain where it is being regulated (strictly confined only if the local node has zero diffusivity); (b) however, any perturbation of the local node at a different location, for instance by another localised node, would be propagated across the whole domain by the global element; (c) if the local node has non-zero diffusivity, then for a fixed signal level, the steady state levels of all three nodes depend on the location of the localised subdomain within a closed domain. This again, is location dependent behaviour (seen in multiple contexts so far) resulting from a localised node regulating a weakly diffusible local node. In the second case (ii), we see that (assuming that the diffusivity of the global element is large enough for any steady state gradients to be negligible), (a) the local node responds to the input signal across the whole domain, irrespective of which node the signal targets (the local node is regulated by the global node), and the system exhibits essentially no gradients at steady state; (b) perturbations of the local node (activation or inhibition) at other locations (i.e. away from the localised node) leave the steady state of the other nodes essentially unaffected.

To summarise, in the first case, although the steady state of the local node is essentially independent of the motif in certain parts of the domain, perturbing it in these locations will affect the behaviour of the motif. In contrast, for the second scenario, although the steady state of the local node throughout the domain depends on the motif, it may be perturbed in certain parts of the domain, without having any effect on the behaviour of the motif.

The above insights continue to be relevant when the feedback loop exhibits bistable or oscillatory behaviour. With bistable behaviour, if the local node is regulated by the localised node, the local element essentially exhibits

a single steady state level outside the subdomain of localisation, even when the motif is in the bistable regime. However, note that since the local node does regulate the global element, the transient behaviour of the motif may depend on the initial profile of the local node across the whole domain. In contrast, if the local node regulates the localised element, then although it exhibits significantly different steady state levels outside the localised subdomain, its initial profile in this region has essentially no effect on the transient behaviour of the motif.

**Adding localised feedback interactions to the excitable and pattern forming systems:** We now examine the possible effects of introducing additional feedback regulation to excitable and pattern forming systems through a localised node. We also consider the possibility of a feedback realised through a combination of localised and a global, highly diffusible element. This is worth examining because, although both excitable and pattern forming systems involve both local and global elements, the global elements in these cases are only highly diffusible relative to the local nodes. In fact they are capable of exhibiting significant gradients, and forming localised structures, for instance a travelling wave. As we have already seen, the combination of a localised element and a highly diffusible element in an interaction, can introduce new capabilities, essentially by sampling 'locally' and communicating this information across the whole domain. As this could have non-trivial consequences when combined with the localised structures here, we also examine this possibility.

**Excitable System:** For the excitable system described previously, we introduce a fourth, localised node  $G$  that is activated by  $X$  and in turn activates  $Y$  (We do not include cooperative effects in this interaction). This constitutes an added positive feedback between these two global nodes. In this case we observe that a travelling wave, when passing through this location, can trigger a localised switching, forming a stationary structure - a localised peak for the two global nodes. The wave continues to propagate to the boundary of the domain. Furthermore, we see that subsequent travelling waves reaching this location are annihilated by interaction with this localised structure (see Fig. 12).

Introducing a similar localised interaction between either of the global nodes and the local node  $Z$ , creates an additional, localised negative feedback. We find that it is possible for such an interaction to annihilate, and

disrupt the passage of a travelling wave.

Returning to the former case of a localised positive feedback, and introducing a global element to this interaction (where the node G activates a highly diffusible node H), we see that the localised switching induced by the passage of a travelling wave is replaced here by a 'global' switching, i.e. the nodes X and Y switch irreversibly across the whole domain. This is significant, because it prevents any further triggering of travelling waves by localised perturbations (see Fig. 12).

**Pattern forming system:** Here we introduce a localised node G that is activated by X and inhibits the inhibitory node Z. We assume that the phase of the pattern formed is set by a spatial cue in the form of a localised interaction (discussed previously). We observe that, depending on the size of the localised domain, this localised regulation can disrupt the intrinsic pattern formed by the basal system. This also depends on the phase of the pattern. Specifically, the feedback through the localised node is essentially triggered only if a crest in the pattern coincides with its location. However, the distorting effect of this node remains localised. The introduction of a global element, through which the localised node can regulate Z, allows the distorting effect of this feedback to become essentially global. This leads to a scenario where the frequency of the pattern formed depends on the location of the spatial cue that sets the phase of the pattern. If its location is such that a crest in the pattern overlaps with the localised node G, then this triggers the feedback, which now has a global effect - it essentially allows the formation of a pattern with higher frequency relative to the case where there is no overlap. Thus we see that, by a combination of localised and global elements, it is possible to control pattern formation in such a way that localised spatial cues at different locations can produce distinct patterns (see Fig. 12).

## 5.4 Conclusions

In this chapter we develop a systems approach to dissect information processing by networks with spatially distributed components. We combine localised, diffusing, and essentially non-diffusible elements (nodes or interactions) with basic regulatory motifs. We use two complementary approaches, one focused on introducing spatial organisation into the nodes and interactions of a motif, and the other focussed on the role of essentially different

|                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Basic Effects of localised components in simple motifs                     | <p><b>Spatial averages:</b> Simple signal transduction by a localised node allows spatial information from a signal to be captured by a diffusible output node. Variations in the phase of a gradient signal may be reflected in changes to the spatial average of the output.</p> <p><b>Boundary effects:</b> A localised node can introduce boundary effects, where the (average) level of a diffusible output may depend on location relative to boundaries, even with spatially uniform signals.</p> <p><b>Gradient responses:</b> With simple feedback regulation, localised interactions can dictate whether components form opposing or similar gradients. With more than two nodes, localising a particular interaction can also dictate which subset of nodes form opposing or similar gradients</p>                                                                                                                                                                                                                                                                                                                               |
| Basic Effects of localised components in Feedforward motifs                | <p><b>Adaptation by IFF:</b> Localised feedforward regulation imposes constraints on adaptation of a diffusible output. Gradients created by localised regulation can prevent adaptation to step changes in input signal. This also depends on the particular spatial organisation of the nodes - co-localised or apart.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Basic Effects of localised components in Feedback motifs with bistability  | <p><b>Localised components introduce new capabilities:</b> Possible tristable behaviour for a bistable module with localised activation of nodes; robust 'spatial switching' with localised inhibition of nodes; <b>Response to gradient signals:</b> Localised components introduce possibility of gradient dependent switching behaviour. <b>Effect of localised components depends on details of realisation:</b> Mutual activation and mutual inhibition motifs may be endowed with different capabilities when localised components are introduced. <b>Position dependent behaviour:</b> Bistable behaviour may depend on placement of localised components relative to the boundaries. For instance, bistability may be destroyed by placing a localised node at the centre of the domain while it is preserved by placing it close to a boundary. <b>Diffusivity dependent behaviour:</b> Bistability may depend on diffusivity of global components. The diffusivity dependence may be non-monotonic, i.e. with qualitatively similar behaviour at high and low diffusivity, and distance behaviour at intermediate diffusivity</p> |
| Basic Effects of localised components in Feedback motifs with oscillations | <p><b>Localised components introduce new capabilities:</b> Localising interactions allows spatiotemporal oscillatory behaviour in a two node negative feedback motif, which is incapable of such behaviour in the well mixed case; <b>Response to gradient signals:</b> Localised components introduce possibility of controlling oscillations using the gradient of a signal. <b>Position dependent behaviour:</b> Oscillatory behaviour may depend on placement of localised components relative to the boundaries. For instance, oscillations may be destroyed by placing a localised node at the centre of the domain while it is restored by placing it at a boundary. <b>Diffusivity dependent behaviour:</b> Oscillatory behaviour may depend on diffusivity of global components. In many cases, the diffusivity dependence may be non-monotonic.</p>                                                                                                                                                                                                                                                                               |
| Spatial organisation and order of interconnection                          | <p><b>Spatial organisation:</b> The same motif (same kinetic parameters) can exhibit distinct behaviour depending on the spatial organisation and the spatial characteristics of its components. Specifically, diffusion dependent and position dependent behaviour (for instance in an activator-inhibitor type oscillator, depends on the particular spatial realisation). <b>Order of interconnection:</b> The information processing capability of even simple motifs (i.e. ability to capture spatial features of the signal) can depend on the order in which the different spatial classes interact. It can also affect certain aspects of pathway modularity - localised perturbations to a local element may or may not be propagated further down the pathway, depending on the order of interaction.</p>                                                                                                                                                                                                                                                                                                                         |
| Localisation can destabilise a homogeneous equilibrium                     | <p>For a given motif with all nodes diffusible, localising certain combinations of interactions can result in homogeneous equilibrium. This equilibrium is necessarily equivalent to the steady state of the well-mixed system. However, depending on the nature of the motif, the stability of this equilibrium may be affected by the localisation. Specifically, such a combination of localised reactions destabilises a homogeneous equilibrium in an activator inhibitor type motif, even with all nodes diffusing equally.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Modularity and Multifunctional motifs                                      | <p>Certain types of motifs (nested feedback) combine positive and negative feedback loops in an essentially non-modular way - i.e. the feedback loops have multiple nodes in common. Such motifs may be capable of exhibiting both bistable and oscillatory behaviour. However, due to the non-modular nature of the feedback loops, it may be that in a given parameter regime, only one of these characteristics is exhibited by the well-mixed system. In the spatially distributed system, we see that localising one of the interactions makes it possible to switch between oscillatory and bistable characteristics by varying a single input signal.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Control structures for pattern formation                                   | <p>Localised nodes can regulate pattern formation in multiple ways. <b>Activation by a localised node</b> can (a) distort a pattern by creating gradients (b) provide a spatial cue to set the phase of a pattern (c) localised activation at multiple predefined locations can be used to reversibly switch between patterns of different phase and wavelength. <b>Feedback regulation through a localised node</b>, regulating a diffusible node acting as the 'control signal', yields a mechanism to ensure robust pattern formation, where only patterns whose phase lies in a certain range can be triggered by localised perturbations.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

TABLE 5.1: Summary of the main results from our study.

spatial classes - local, global, and localised. While we take a more constructive approach to introducing spatial complexity in the former, in the latter the focus is on patterns of activation and inhibition between the different classes.

Our study leads to a range of insights into (i) the role of localised and diffusing elements in shaping the behaviour of a motif, and the interplay of spatial factors with the dynamic characteristics of a motif (ii) how the interplay of distinct spatial elements can produce new capabilities for a motif, (iii) qualitative trends, that depend on spatial parameters, which emerge from combining localised and diffusible elements, (iv) the potential for localised interactions to destabilise a homogeneous equilibrium, (v) how trends depending on both kinetics and spatial parameters may be qualitatively different depending on spatial organisation, even with the same motif structure and dynamical characteristics (vi) the different capabilities that emerge



purely from certain patterns of interaction between spatial classes, and (vii) realising feedback control structures that combine different spatial classes. The main insights are summarised in Table 5.1.

Taken together, these insights point to a systems dissection of the role of spatial organisation in information processing networks, with implications far beyond the context of biochemical pathways. We aim to develop these insights further, and establish connections to concrete biological contexts, as part of future work.



## Chapter 6

# Conclusions

Biochemical information processing by molecular networks is a fundamental feature of cellular life. In addition to involving a vast array of molecular components, there are many sources of complexity in these networks - feedback regulation at multiple levels, nonlinearity in the interactions, intricate spatial organisation, stochastic effects, and the role of evolution in shaping their behaviour. These features are not confined to a modulatory role in shaping biochemical information processing, rather they are central to realising cellular function.

The motivation to elucidate biochemical information processing arises from what are essentially two complementary perspectives: that of natural biology, where an understanding of pathway function and evolution is a central theme, and that of synthetic and chemical biology, where the focus is on engineering pathways with desired functionality in both natural cells and synthetic/cell-free constructs.

The complexity arising from the combination of nonlinearity, feedback regulation, spatial organisation, stochastic effects and evolution, poses new systems challenges to understanding and engineering information processing by biochemical pathways. In this thesis, we have focussed on two basic systems aspects of cellular information processing: (1) biochemical information processing modules as part of networks, and (2) spatial organisation of pathways. We develop systems approaches to address each of these aspects.

### **Information processing modules as part of networks**

Elucidating how complex information processing biochemical modules function as part of a larger biochemical network is essential for understanding pathway function in multiple cellular contexts (for instance in yeast pheromone

response (Atay and Skotheim, 2017) or the control of mitotic transitions (Hutter et al., 2017)). Synthetic biology brings a myriad of possibilities for integrating synthetic biochemical modules with existing cellular machinery, or combining engineered modules to achieve new functions (Purnick and Weiss, 2009), while the latter is also an emerging theme in chemical biology (Semenov et al., 2015). A framework that enables the systematic analysis of module function as part of a network, as well as interaction between modules, is indispensable in all of these contexts.

The fact that a particular type of module may be realised in multiple ways, differing in terms of network structure of interactions, biochemical nature of the components, and dynamical characteristics, poses a significant challenge to creating such a framework. We address these challenges by developing a systems framework that brings together a set of canonical modules from the literature, including multiple realisations (different network structure, biochemical nature, and dynamical characteristics) of the same type of module, as well as different types of interaction - sequential or oblique, depending on where a module is targeted/regulated by another module.

Our study yields insights into new information processing behaviour arising from modular interactions, as well as into what makes a module - how its network structure, biochemical nature, and dynamical characteristics shape its interaction with the ambient network. In addition, by incorporating sequestration effects, our framework generalises the concept of retroactivity to complex interactions of signalling modules. Natural pathways are shaped by evolution, and the evolutionary process may bring in additional layers of regulation in realising such modules - our framework provides a way to dissect such added complexity, and appreciate its role in particular cellular contexts. Further work involving specific pathways will also have to engage with greater complexity, for instance the combination of signalling and gene regulatory components in a module, the compartmentalisation of certain components, or even intergenerational aspects of module function - all of which may be found in a single pathway (Doncic et al., 2015).

Cross-talk between signalling pathways is an important theme in multiple cellular contexts (Lozano-Durán and Zipfel, 2015; Totaro et al., 2018), with different aspects of interest, including signal integration (Wang, Bai, and Wang, 2014) and information transmission in the presence of noise (Tareen,

Wingreen, and Mukhopadhyay, 2018; Wang et al., 2018). In the context of signal integration, which involves interaction between modules separately regulated by distinct signals, our framework for oblique interactions between modules (without sequestration of components) can be directly adapted to the analysis of cross-talk phenomena. The study of oblique interactions with sequestration could also contribute useful insights in these contexts, and presents an avenue for future work. The investigation of noise effects brings added complexity. The analysis of cross-talk without noise, using our framework, could form a suitable starting point for such studies. In addition, our framework also suggests a systematic way of structuring such an investigation.

## **Spatial organisation**

Understanding the role of compartmentalization and its use in evolution is an emerging theme in natural biology. The interest in compartmentalization is also reinforced by recent efforts to engineer spatial organisation of synthetic and cell-free biochemical systems. From the point of view of spatial organisation, we examine multiple aspects, including (i) how compartmentalisation affects the basic building blocks of pathways (ii) choice of modelling frameworks for analysis and design of compartmentalised biochemical pathways (iii) the role of spatial organisation in engineering cell free synthetic circuits and (iv) information processing by spatially distributed networks.

## **Modelling compartmentalization**

Any systematic understanding of compartmentalization relies both on experimental work as well as modelling and systems frameworks. We assess the applicability of a basic modelling tool - compartmental ODE models - in elucidating and engineering compartmentalization of biochemical pathways. To do this we examine a range of basic building blocks of signalling and metabolic pathways, and investigate the effects of compartmentalising them. We examine when and to what extent compartmental models can provide an accurate description and predictive understanding, by comparing them with detailed models of reaction and transport. We then build on this to examine additional complexities in biochemical pathways as well as basic exemplar design problems in metabolic pathways.

Through a systematic analysis, combining both computational and analytical work, applied to a range of basic signalling and metabolic circuits with

compartmentalization, we draw a number of insights. We find conditions under which compartmental ODE models provide a reliable description of the process. We uncover a common underlying theme, which we refer to as “kinetic dependence of transport parameters” where we find that even if a compartmental model is calibrated (even with experimental data), it cannot be used reliably in a predictive way. Through detailed analysis, we trace the source of this problem and suggest ways in which this limitation can be addressed. We also find that basic compartmental models do not describe sequestration of species accurately, resulting in potentially substantial error. We indicate a way in which this can be overcome and demonstrate this in an exemplar case. By examining additional complexities in signalling networks, as well as exemplar design problems in synthetic biology, we are able to uncover the subtle consequences of using such compartmental models in multiple biological contexts. A key underlying factor that emerges from our study: the significance of inhomogeneous concentration profiles within a compartment, is also relevant to any investigation that uses compartmental models to examine stochastic effects such as the propagation of noise between compartments.

### **Design principles for compartmentalised cell-free genetic circuits**

There are established frameworks for the design and analysis cell-free genetic and other kinds of nucleic acid based circuits, that allow circuits with desired information processing characteristics to be constructed and tuned. Compartmentalization/spatial organisation of such circuits is an essential ingredient in several potential applications, including the construction of artificial cells, pattern forming systems, distributed manufacturing, or even for enabling modular design. While various experimental studies have constructed robust and versatile frameworks for compartmentalizing these circuits, a framework of design principles for spatial organisation of these systems has been lacking. An elucidation of basic design principles is essential for enabling the systematic construction of spatially organised circuits using these experimental tools.

We developed a systems framework to address compartmentalization, that accounts for potential circuit designs, spatial organisation of compartments, operational state of compartments, and the distribution of resources. Our study yields a range of basic insights and provides a window into what distribution entails and how it may be systematically exploited. We reveal basic constraints and trade-offs associated with distributing circuits in space,

including the effect of modular augmentations to a configuration and ways of mitigating its effect. We also identify new capabilities, such as the use of negative regulation to achieve adaptation of concentrations to spatial separation, within a target compartment. In addition to highlighting the interplay between the nature of a motif and spatial organisation, we demonstrate how spatial organisation expands the design space for circuits: for instance by exploiting the distribution of specific degradation pathways. In addition, our analysis of a prototypical application scenario reinforces many of the basic insights emerging from our study.

The insights from our study provide a means to address several basic questions that arise in constructing a distributed cell-free circuit - What types of compartmentalization framework to use? What components to co-localise or separate? Can distribution be exploited to achieve new capabilities/provide accessible dials for tuning pathway function? How to achieve modularity in the design? By elucidating the effect of different spatial topologies and modular augmentations to a compartmental configuration, our framework suggests ways of further exploiting existing experimental capabilities such as re-configurable networks of compartments (Bolognesi et al., 2018), to shape the behaviour of a circuit. The ability to control transport into and out of compartments using a biochemical signal is a key feature that can take the information processing capabilities of cell-free circuits even further - our framework can be extended to incorporate this aspect.

### **Information processing by spatially distributed networks**

Our attempts to elucidate the functioning of spatially organised biochemical pathways leads us to the broader theme of information processing by networks with spatially distributed components. While information processing by biochemical pathways has been mapped out in terms of interaction motifs in many contexts, we are still faced with the question: What does space bring to these networks. There is a need to understand how spatial organisation and the spatial characteristics of components - localised, diffusing or non-diffusing - shapes the behaviour of these motifs, and possibly enables new capabilities. Furthermore, while it is well known that the interplay of different classes of spatial components (high diffusing and weakly diffusing) can give rise to self-organised spatio-temporal behaviour, their interplay with localised components is not understood to the same extent.

We develop a comprehensive systems framework to understand the role of spatial organisation in networks, combining two complementary approaches:

bringing space to networks - by introducing spatial complexity into motifs (localised or diffusing nodes and localised interactions), and bringing network structure to space - by examining on patterns of interaction between components with distinct spatial characteristics. Our study yields a range of insights into the constraints and capabilities conferred on a motif by different spatial configurations, the interplay between the spatial organisation and the dynamical characteristics of a motif - for instance how location relative to a boundary affects bistability or oscillatory behaviour, and the role of the different spatial classes in shaping information processing. By examining more complex motifs with multiple feedback loops, we also reveal the interplay between modularity and spatial organisation - with spatial organisation an essential feature for achieving robust multi-functionality.

Information processing by distributed networks of biochemical components is an emerging theme in materials science, with recent studies focussed on integrating synthetic information processing circuits into polymer materials to create smart materials with new functionality and modular design (Beyer et al., 2018; Wagner et al., 2018). The spatial organisation and spatial characteristics of circuit components are key aspects for constructing such 'biohybrid' materials systems. By systematically bringing these ingredients together with network structure, our framework provides a way to think about the design of such systems - exploiting spatial organisation to achieve desired information processing characteristics or modular design, using localised components to control global behaviour, or even identifying new capabilities that can be engineered.

In the context of developmental pattern formation, our framework brings together the basic ingredients of both positional information systems (localised, diffusing, and non-diffusing components - see for instance Durrieu et al., 2018), and Turing systems (low-diffusing and high-diffusing components). There is evidence to suggest that certain pattern formation processes combine these essentially distinct mechanisms, with one upstream of the other, or both operating in parallel (Green and Sharpe, 2015). By combining the basic spatial ingredients with complex motif structures, our framework provides a way to think about such processes and how these mechanisms interface with each other. Through our analysis control structures involving different spatial classes our framework also encompasses other mechanisms of feedback regulation in developmental systems, such as "expansion-repression" mechanisms for scaling morphogen gradients in response to tissue growth (Ben-Zvi and Barkai, 2010).



## 6.1 Future work:

**Developing links to specific contexts in natural biology and connection to experimental data:** Our analysis of modules is relevant to systems biology in multiple contexts. Modules and their combinations are seen in multiple pathways in natural systems, and are the focus of several recent experimental studies - see for instance Doncic et al., 2015; Atay, Doncic, and Skotheim, 2016, focussing on a pheromone signalling pathway in budding yeast, which combines feedforward regulation of a protein with a bistable switch involving the same protein, and Rata et al., 2018 which examines the role of two interlinked bistable modules in controlling control mitotic transitions in mammalian cells. Our study provides a way of appreciating the extra layers of organization seen in natural contexts, the robustness of pathway function, and its ability to evolve. Spatial organisation also plays a role here - compartmentalization of a module can be a key ingredient (Doncic et al., 2015), while module function may also control (and be regulated by) cell geometry (Atay and Skotheim, 2017). Thus, the study of such natural pathways offers fertile ground for bringing together the two main strands of our work. Furthermore, crosstalk between pathways is a ubiquitous feature of signalling processes, and our study of oblique interactions provides a means to address this aspect.

The publication of our work on modules has also led to external collaborations, involving the analysis of disease models. Multiple feedback interactions between modules, reflecting the multifactorial nature of the disease, are a key feature in this context. Our analysis of interactions between complex information processing modules, and the resulting insights into how their dynamical characteristics combine, forms a starting point both for understanding how multiple feedback loops combine in such systems, and for elucidating the role of different feedback interactions.

Our study of spatial organisation gives multiple insights into how compartmentalisation affects basic building blocks of multiple classes of pathways, including metabolic pathways, where we reveal the interplay between product inhibition effects and compartmentalization. Building on these insights, and examining specific instances of compartmentalised metabolic and signalling pathways, we may be able to approach the problem of understanding how the spatial organisation of such pathways has been shaped by the evolutionary process (Diekmann and Pereira-Leal, 2013; Alam et al., 2017).

**Parametric analysis:** In our study of modules and spatial organisation, particularly in the section on spatial networks, we have restricted ourselves to examining motifs in regimes where they exhibit characteristic behaviour in the ODE. However, in the spatial realisation the motif may be capable of exhibiting a variety of complex characteristics. This means that a broader exploration of the parameter space, possibly involving parameter sampling strategies, may be required to completely map out its capabilities. A focussed parametric analysis, especially in cases involving a combination of multiple positive and negative feedback regulation, could potentially reveal other regimes with distinct information processing behaviours of interest.

**Connection to bottom-up approaches in synthetic biology and chemical biology:** Several recent studies have developed experimental frameworks for the bottom-up construction of spatially organised biochemical pathways. These include compartmentalising different kinds of pathways, different types of compartments, as well as new ways of manipulating and forming networks of these compartments. The insights from our study of the basic effects of compartmentalising pathways and modelling frameworks for compartmentalised systems are directly applicable in these contexts. These insights will find direct applications in the design of compartmentalised synthetic systems through existing experimental collaborations within the group.

The design principles that emerge from our study of compartmentalised cell-free genetic circuits can be adapted to enable a design flowsheet for spatial organisation in multiple contexts, including the construction of hybrid systems that combine cell-free circuits with microbial populations.

Bottom up engineering of chemical information processing is also an emerging theme in chemical biology, where bistable and oscillatory modules been built (Semenov et al., 2016; Semenov et al., 2014), and there is significant interest in interfacing such modules to achieve new information processing functions (Semenov et al., 2015; Lanchuk et al., 2018). By providing sharp insights into how the biochemical nature of components shapes module interactions, our framework can guide attempts to identify suitable reactions and components that may be used as building blocks for robust chemical modules in such approaches.

**Examining the effects of noise:** The role of noise, resulting from fluctuations in the concentration of molecules, has been examined in multiple contexts, including signalling pathways and gene regulatory networks (Bruggerman and Teusink, 2018), with noise effects potentially playing distinct roles in information processing at the intracellular and population levels (Suderman et al., 2017). The compartmentalisation of pathways has been suggested as an important mechanism of passive noise filtering within cells (reviewed in Stoeger, Battich, and Pelkmans, 2016). At the level of tissues/cell populations, the role of stochastic effects in developmental pattern forming processes is of significant interest (Oates, 2011): while robustness to noise is a key aspect (Maini et al., 2012; Di Patti et al., 2018), the presence of noise can potentially have qualitative effects on patterning (Perez-Carrasco et al., 2016), or even generate fluctuation-induced patterns, (Biancalani, Jafarpour, and Goldenfeld, 2017). Each of these contexts involves a confluence of networks, spatial organization, and stochastic effects. By bringing together networks and spatial organisation, and elucidating the basic aspects that emerge from their interplay, our framework provides a foundation for further investigations that bring in the added complexity of stochastic effects. The insights emerging from our study form a common starting point for multiple investigations, for instance, aimed at understanding how spatial organisation shapes noise propagation in different types of information processing motifs at the intracellular level, or examining how localised components shape pattern formation in the presence of noise, at the level of populations/tissues.



## Appendix A

# Supplemental Material for Chapter 2

The Supplemental Material is organised as follows:

- In section I, we present a detailed discussion on sequential interconnection, without sharing of components. This discussion is intended to complement the results presented in the text.
- Section II consists of two parts. The first contains a detailed description of how we model modular interaction with sharing of components, including mathematical analysis of a general case involving an upstream CMC. In the second part we examine the effects of sequestration for each of the scenarios discussed in section I.
- Section III contains brief discussions on alternative bistable, adaptive, and monostable switch modules.
- In Section IV, we present a brief mathematical analysis of the effects of oblique interactions on the IFF adaptive module.
- Section V contains a discussion on the parameter values for the models, used in the chapter. The particular values used in all the figures are also presented.
- Section VI contains additional plots and computational results.

### A.1 Sequential interaction without sharing

As we have noted in the case of coupled networks with the CMC or the monostable switch upstream of another module, the response of the downstream output to the upstream input signal may be transparently understood in the following way:

- 1) Any variation in the upstream input signal is reflected in the steady state output of the upstream module, in a manner that is completely determined by the input-output behaviour of the upstream module in isolation (hyperbolic response for CMC or sigmoidal response for Monostable switch).
- 2) The output of the upstream module functions as the input parameter for the downstream module. Thus, any change in this 'upstream' output (in response to an upstream input), causes a corresponding variation in the output of the downstream module. This variation is completely determined by the input-output response of the downstream module in isolation.

When we explore the more complex instances of modular coupling, involving interconnections between adaptive modules, bistable switches, and oscillators, this insight allows us to map out the qualitatively distinct steady state/asymptotic behaviour, that the coupled system may exhibit. This includes any variations that may be seen by varying the coupling parameter. It also provides a means to explain the behaviour that we observe, based on the input-output responses of the modules in isolation.

### CMC or Monostable switch downstream

As mentioned in the text, the input-output response of the coupled system in this scenario is qualitatively similar to that of the upstream module in isolation. For example, when the upstream module is adaptive, the adaptive output  $[Z_P]$  adapts to a step change in the upstream input. Since  $[Z_P]$  acts as the input parameter for the CMC, the downstream output  $[S_P]$  must also adapt. However, depending on the coupling parameter, the amplitude of the transient response, of the downstream output, may be quite different from that of the adaptive module in isolation, subject to the same step input.

In a similar way, a bistable switch upstream makes the coupled system bistable. Associated with each steady state value of the bistable switch output  $[R]$ , there is a steady state value for the CMC output  $[S_P]$ . Since there is no sequestration, the bistable regime for the coupled system (with respect to input), is identical to the bistable regime of the bistable switch in isolation. Again, there may be quantitative differences between the output of the coupled system versus that of the bistable module in isolation, such as in the relative differences between the steady state values on different branches.

It is also easy to see that having an oscillator upstream would make the output of the coupled system oscillate. Similar to the case of bistability, the range of upstream input for which the coupled system exhibits stable oscillations is identical to the oscillatory range of the oscillator module in isolation.

### **Adaptive module - Bistable switch**

Here, the output  $[Z_P]$  of the adaptive module acts as the input parameter to the bistable switch. We consider inputs to the coupled system that keep the adaptive module in a regime of close to exact adaptation. The coupling parameter is chosen in such a way that the steady state value of  $[Z_P]$  constitutes an input to the switch that lies in the bistable range. With the above restrictions, it is clear that the response of the coupled system to a given input depends on (1) the transient response that this input elicits in  $[Z_P]$  (the steady state value remaining nearly the same), and (2) whether this transient causes a permanent switching of steady states in the downstream module.

Since there is no sharing of components, the transient response of  $[Z_P]$  in the coupled system is identical to that for the adaptive module in isolation. For the bistable module in isolation, we know that the response to a transient, pulse like input, depends on both the magnitude and duration of such an input. In the present case, the magnitude of the transient signal to the switch depends on the upstream input (to the adaptive module). Step or pulse inputs of greater magnitude, to the adaptive module, would result in a correspondingly higher magnitude transient signal to the switch. The duration of this transient signal depends on the dynamics of the adaptive module, and therefore, the relative timescales of the two modules come into play. If the adaptive module dynamics is fast relative to the bistable module, then the duration of the transient may be too short to trigger a switching of steady state branches downstream, even if the amplitude of the transient is substantial (Seaton and Krishnan, 2011a).

### **Bistable switch - Adaptive module**

This scenario is discussed in detail in Seaton and Krishnan, 2011a. For the purpose of our study, it may be understood in the following way: First, if we focus on basal input levels in the bistable range of the switch in isolation, we know that step and pulse inputs of sufficient magnitude and duration, can cause the bistable module output  $[R]$  to switch to a different branch of stable

steady states. Input signals of insufficient magnitude can cause a permanent (step) or transient (pulse) change between steady states on the same branch.

Second, since  $[R]$  acts as the input parameter to the adaptive module, all we need, to understand the behaviour of the coupled system, is to examine what effect the above changes in  $[R]$ , can produce in the output of the adaptive module. Note that such changes may regulate the adaptive module out of a regime of essentially exact adaptation Seaton and Krishnan, 2011a, but we do not discuss this here. These effects depend on the coupling parameter, the shape of the input-output curve for the switch (i.e. the slopes of the different branches), and the parameters of the adaptive module. For simplicity, we restrict ourselves to a scenario where the adaptive module exhibits close to exact adaptation over the range of steady states of the bistable switch.

We can see that, in general, a switching of steady state branches in the bistable module will result in a 'step' input of greater amplitude to the adaptive module, than any variation of steady states on the same branch. Thus, the amplitude of the transient response of the downstream output may differ significantly, depending on whether the upstream input has triggered a switching or not. This produces an interesting effect in the case of pulse inputs. Suppose a pulse signal to the coupled system causes a switching of branches upstream, and thus elicits a significant transient response downstream. A subsequent identical pulse signal would produce a significantly smaller downstream response, simply because there is no further switching between branches.

## Adaptive module - NFO

The adaptive module upstream of an NFO can be understood in terms of a transient adaptive variation of a parameter in the NFO. Here, we consider only such inputs that keep the adaptive module in a regime of close to exact adaptation. The coupling parameter may be fixed so that either of the two following scenarios is realised:

- (1) The steady state value of  $[Z_P]$  constitutes an input to the oscillator that lies in the oscillatory regime. In this case, it is possible that an input signal that produces a significant transient response in the adaptive output  $[Z_P]$ , will result in a transient disruption of oscillations of the downstream output.



- (2) The steady state value of  $[Z_P]$  constitutes an input to the oscillator that lies outside the oscillatory regime. In this case, it is possible that an input signal that produces a significant transient response in the adaptive output  $[Z_P]$ , will result in transient oscillations of the downstream output.

Note that, in this case, the effect on the NFO is purely transient.

## NFO - Adaptive module

When an oscillator is coupled to a downstream adaptive module, we have the output of the oscillator  $R_P$  acting as the input to the adaptive module. We choose the coupling parameter so that this input corresponds to a regime of close to exact adaptation for the adaptive module in isolation.

We know that for the oscillator in isolation, its output exhibits stable limit cycle oscillations over a range of values of its input parameter. For the purpose of our analysis, we will restrict ourselves to the case where the upstream input to the coupled system always lies in this range. We note that, if the upstream input lies outside this range, the behaviour may be easily understood.

Since the NFO output  $[R_P]$  acts as the input parameter to the adaptive module, stable oscillations of  $[R_P]$ , would cause the downstream output to also exhibit stable oscillations, rather than adapting to a steady state. Two special cases merit further discussion. If there is a disparity of time scales, we have two possibilities:

- (1) The oscillator dynamics are slow relative to the adaptive module: The downstream output exhibits oscillations of negligible amplitude. This is because the adaptive module essentially reaches a quasi steady state for a slow modulation of its input.
- (2) The oscillator dynamics are fast relative to the adaptive module: the downstream output again exhibits oscillations of negligible amplitude, as the adaptive module effectively samples the mean of the oscillations. These aspects can be demonstrated analytically.

## Bistable switch - NFO

When a bistable switch is coupled to a downstream oscillator, the output of the switch acts as the input parameter for the oscillator. As we know for the oscillator in isolation, its output exhibits stable limit cycle oscillations over a range of values of this input parameter. We call this range/interval 'W'.

Now, since this input is also the output of the bistable switch, the interval  $W$  corresponds to some part of the steady-state input-output curve ' $G$ ', for the switch. The existence of three branches of steady states over a certain range of input gives rise to the following cases (see Figure S1(a-f)):

- (a)  $W$  corresponds to a part of the upper steady state branch
- (b)  $W$  corresponds to a part of the middle steady state branch
- (c)  $W$  corresponds to a part of the lower steady state branch
- (d)  $W$  corresponds partly to the upper and partly to the middle branches
- (e)  $W$  corresponds partly to the lower and partly to the middle branches
- (f)  $W$  corresponds partly to the lower and upper branches, and the entire middle branch

In all cases except (b), there also arises the question of whether the part of the curve  $G$  corresponding to  $W$  lies completely in the bistable regime, or extends into a monostable regime. In (a) and (c) it is also possible that this part of the curve lies completely in a monostable regime. This covers all possibilities for a given bistable switch and NFO.

Exactly one of these possibilities will be realised by a given coupling between the bistable switch and the NFO. The parameters of the individual modules, along with the coupling parameter, determine which of these alternatives is realised. However, we emphasise that the list of cases constructed above does not depend on the particular values of the parameters used. In fact, it relies only on the bifurcation structure of the two modules. Indeed, the same list of cases would apply to the interconnection (of the kind we consider here) of any bistable and oscillator modules, that share the same bifurcation structure.

Having mapped out the different possibilities, we note that the long term behaviour of the output of the coupled system (i.e. the output of the oscillator) is completely determined by the steady state of the upstream switch (i.e. a point lying on one of the three branches). This makes it easy to analyse the possible effects of step and pulse inputs to the coupled system. Note that, when considering step and pulse inputs in the following analysis, we particularly focus only on those of sufficient magnitude and duration to cause a permanent switching of steady states for the bistable module in isolation.

To start, we know that the middle branch of steady states for the bistable switch is unstable, and therefore, no input signal can bring the switch to one

of the steady states on this branch asymptotically. This means that no upstream input can switch on stable oscillations of the downstream output in case (b). More generally, we can say that only those parts of  $W$  that correspond to the lower or upper branches can affect the long term behaviour of the downstream output. In this sense, the cases (d) and (e) are essentially no different from (a) and (c), and need not be considered separately.

Now, if the bistable switch is at a steady state on the lower branch for the basal input, a step signal of sufficient magnitude can always cause a permanent transition to the upper branch. A pulse input of sufficient duration and magnitude may also cause such a transition, if the basal input lies in the bistable regime. If we allow for step and pulse signals that involve a *decrease* from the basal input, it is easy to see that the same reasoning applies to transitions from the upper branch to the lower branch. This means that it will be sufficient for us to consider either (a) or (c), to examine any non-trivial aspects associated with the bistability. Case (a) is discussed in detail, in the text, for a basal input lying in the bistable regime. For a basal input lying in a monostable regime, it should be clear that the results for step inputs remain qualitatively the same, while pulse inputs can no longer cause a permanent transition in the output behaviour.

Case (f) is different to the others, as it can exhibit multiple limit cycles for the same input. Here, the fact that  $W$  corresponds partly to the upper and lower branches, makes possible the coexistence of two distinct oscillatory states for the downstream output, for a given upstream input. If the basal input lies in this range, a pulse signal could permanently switch the output from one oscillatory state to the other.

We have two possible sub-cases here, whose response to pulse and step inputs are qualitatively different, though the discussion in the previous paragraph applies equally to both. The first of these is when  $W$  lies completely within the bistable regime. In this case, we have, in addition to having a range of input with two oscillatory states, another range of input where a stable steady state and stable oscillatory state coexist. If the basal input were to lie within this second input range, a pulse signal of sufficient duration and magnitude could cause permanent switching between a steady state and an oscillatory state. A step input that causes a switching in the bistable module, on the other hand, would only switch the output to a steady state (Since any step signal that causes a switching in the bistable module has to take the system out of the bistable regime.).

The second sub-case is when  $W$  also extends into the monostable regime

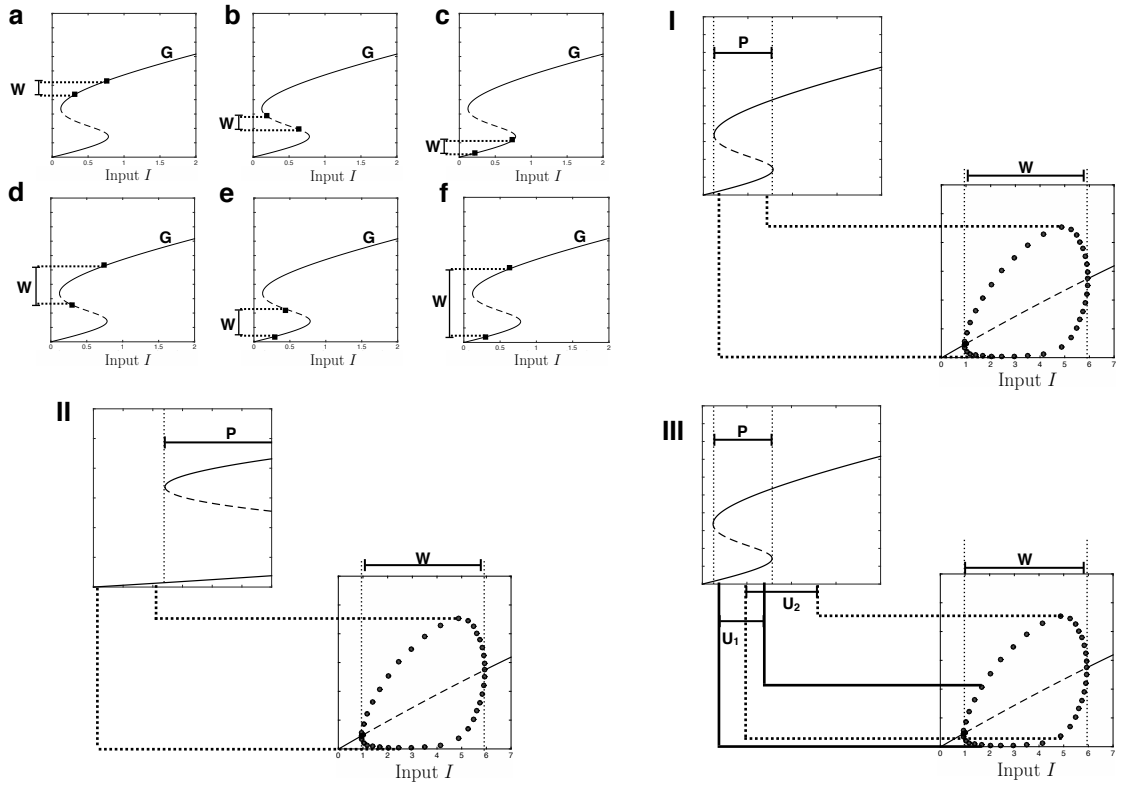


Figure S1: Sequential interactions without sharing: Bistable switch and NFO. The first part, (a) - (f), pertains to the coupling of the bistable switch to a downstream NFO. (a) - (f) are schematics representing the relationship between the input output curve of the switch and the oscillatory regime of the NFO, for the six cases discussed. I - III are schematics representing the three cases discussed for the coupling of the NFO to a downstream bistable switch. In I, the interval  $U$  (see text) corresponding to any of the oscillatory states always lies within the bistable range  $P$ . In II, the interval  $U$  corresponding to any of the oscillatory states always lies outside the bistable range  $P$ . In III, we depict the interval  $U$  corresponding to two different oscillatory states.  $U_1$ , which corresponds to the oscillatory state (limit cycle) at one input level, lies entirely within the bistable range  $P$ .  $U_2$ , which corresponds to the oscillatory state at a different input level, lies partly outside the bistable range  $P$ .

on one of the branches. In this case, a pulse signal produces the same effect as above, while a step input that causes a switching in the bistable module could also permanently switch the output to a different oscillatory state.

## **NFO - Bistable switch**

When the NFO is coupled to a downstream bistable switch, we have the output variable of the oscillator acting as the input parameter for the switch. As we know for the bistable switch in isolation, its output exhibits coexisting stable steady states over a range of values of this input parameter. We call this range/interval 'P'.

We also know that, for the oscillator in isolation, its output exhibits stable limit cycle oscillations over a range of values of its input parameter. We call this range/interval 'W'. Within this range, the amplitude of the limit cycles exhibits continuous and non-monotonic dependence on the input. For the purpose of our analysis, we will restrict ourselves to the case where the input to the coupled system always lies in the range W. We note that, if the upstream input lies outside this range, the behaviour may be easily understood.

The following results can be most transparently understood in the case of a slow modulation of the input to the bistable switch, i.e. where the oscillator dynamics is slow relative to the bistable switch.

Since the output of the oscillator module is the input to the bistable switch, one of the first conclusions we can make is that, if the input to the oscillator is constant, and lies in the range W, then the output of the bistable switch (i.e. the output of the coupled system) will exhibit oscillations. Note, however, that these oscillations could occur about either of the steady state branches of the switch, as long as the system is in a regime where they coexist. Thus, we have the possibility of coexisting stable oscillatory states for the downstream output, just as in the case of the bistable switch coupled upstream of the NFO.

The essentially new aspect of this coupling, relative to the modules in isolation, is the possibility of multiple coexisting oscillatory states. Therefore we focus on possible switching between these oscillatory states, which may be induced by the upstream input, noting that this input comes through the oscillator module.

For a fixed upstream input in the oscillatory range, the oscillator output varies over a range of values associated with a particular limit cycle. If the

oscillator dynamics is slow relative to the bistable module, this amounts to a slow modulation of the input parameter to the bistable module, over a range which we call 'U'. This range, of course, would depend on the particular limit cycle associated with the current input to the oscillator. Now, when the oscillator dynamics are slow, the relationship between the interval P and the interval U corresponding to a particular limit cycle will determine whether a given input signal can cause a switching between oscillatory states in the downstream output (These insights come from the effects of the slow modulation of a parameter, on the bistable switch). This consideration gives rise to three different possibilities (see Figure S1(I-III)):

- (I) For all limit cycles of the oscillator (irrespective of the input), the corresponding interval U lies completely within the interval P (i.e. within the bistable range).
- (II) For all limit cycles of the oscillator (irrespective of the input), the corresponding interval U lies completely outside the interval P (i.e. outside the bistable range).
- (III) There exist some limit cycles for the oscillator, for which the corresponding interval U lies partly within and partly outside the range P. The remaining limit cycles may correspond to either (I) or (II). (It is also possible that, for some limit cycles, the interval U contains the whole of the range P, but for simplicity, we do not examine this possibility here.)

Restricting ourselves to upstream input signals that lie within the interval W (i.e. the oscillatory range), we can make the following conclusions.

In case (I), the output of the coupled system can be at either of two distinct oscillatory states (corresponding to two different stable limit cycles), and no gradual variation of the upstream input (within the interval W) will cause a switching between them.

In case (II), the output of the coupled system has only one stable oscillatory state, and any variation of the upstream input (within the interval W) merely modulates this oscillatory state.

In case (III) (see main text), a change in the input (even a slow variation), can cause the amplitude of oscillations to change, in such a way that the input parameter of the bistable module is taken out of the bistable regime. For a slow modulation of this parameter, this causes a switching of branches in the bistable module (this is depicted in the main text), so that, even if the upstream input is brought back to its basal level, the downstream output is now at a different oscillatory state.

Due to the non-monotonic variation in the amplitude of the NFO limit cycles, with respect to its input (see bifurcation diagram in Figure 2), it is possible that the possibility switching between oscillatory states can depend non-monotonically on the size of a step input.

For inputs within the interval  $W$ , the asymptotic state of the coupled system is always oscillatory. Thus, the dynamic response of the system to a step input of given magnitude would depend on the *phase of oscillations* at the instant the step input is given. Therefore, the magnitude of the step determines the asymptotic state of the system, but the timing of the step affects the dynamic response.

### SDO - CMC/monostable switch

For the SDO upstream of a CMC or monostable switch, the phase of oscillations at a given instant of time comes into play in a slightly different way to the above case. We consider whether a pulse input can cause a permanent switching to oscillations from a steady state. As discussed in the main text (also see below), a pulse input (when the pulse is switched on) can push the system into an oscillatory trajectory. Now, when the pulse is switched off, the phase of these oscillations will determine whether an asymptotic oscillatory state is reached, and consequently whether a permanent transition to oscillations is realised. This may be understood as follows. At the basal input level  $I_0$ , the system has both a stable steady state ' $SS_{I_0}$ ', and a stable limit cycle ' $LC_{I_0}$ ', each with its own *basin of attraction*. Assume that the system is initially at  $SS_{I_0}$ . When the input is 'switched on' (i.e.  $I_0$  to  $I_1$ ), the system moves to regime where only stable limit cycles exist (for a pulse of sufficient magnitude). Some part of the corresponding limit cycle ' $LC_{I_1}$ ' lies within the basin of attraction of  $LC_{I_0}$ , while the remaining part of  $LC_{I_1}$  lies within the basin of attraction of  $SS_{I_0}$ . Therefore, depending on which part of  $LC_{I_1}$  the system is traversing, at the instant when the input is switched back to  $I_0$ , the asymptotic state could be either oscillatory ( $LC_{I_0}$ ) or a steady state ( $SS_{I_0}$ ). The possibility of a pulse input switching off oscillations in an SDO, arises for the same reasons.

## A.2 Sequential interaction with shared components

The coupling of modules, with shared components, amounts to the output species of the upstream module acting as the enzyme in one of the reactions

of the downstream module, in a non-mass action regime. Such an interaction allows sequestration of the upstream output in a downstream complex, the extent of which can significantly alter the behaviour of the coupled system (See main text). We describe one instance of this coupling - an upstream covalent modification cycle coupled to the bistable switch - to illustrate how this is modelled.

As discussed in the main text, we examine two distinct input locations for the bistable switch. The models used to describe the coupling of the switch to an upstream covalent modification cycle, through each of these inputs, are discussed below.

### A.2.1 Coupling through Designated Input: CMC- Bistable switch

The designated input to the bistable switch appears as a zeroth order reaction - a term of the form  $k_1[I]$  (See Models and Methods). We interpret this term as follows: the input species  $I$  acts as an enzyme, on a substrate  $M$  present in large excess ( $[M]$  is essentially constant). The input species binds to the substrate to form a complex, which then yields the reaction product  $R$ , and the enzyme. The binding of the input species to the substrate is assumed to be reversible ( $k_f$  binding/  $k_r$  unbinding), while the production of  $R$  (catalytic constant  $k_{cat}$ ) is not. The equations for an upstream covalent modification cycle coupled to the bistable switch, through these steps, are as follows:

$$\begin{aligned}\frac{d[S]}{dt} &= r_4[S_P] + r_2[IS] - r_1[S][I] \\ \frac{d[IS]}{dt} &= r_1[S][I] - r_2[IS] - r_3[IS] \\ \frac{d[S_P]}{dt} &= r_3[IS] - r_4[S_P] - k_f[M][S_P] \\ &\quad + k_r[MS_P] + k_{cat}[MS_P]\end{aligned}$$



$$\begin{aligned}
\frac{d[MS_P]}{dt} &= k_f[M][S_P] - k_r[MS_P] - k_{cat}[MS_P] \\
\frac{d[R]}{dt} &= k_0[E_P] + k_{cat}[MS_P] - k_2[R] \\
\frac{d[E_P]}{dt} &= k_3[R][E] - \frac{k_4[E_P]}{K_4 + [E_P]} \\
[I_T] &= [I] + [IS]; \quad [S_T] = [S] + [S_P] + [IS] + [MS_P] \\
[E_T] &= [E] + [E_P] \\
[M] &\text{ is a constant}
\end{aligned}$$

Here, the steady state value of  $[S_P]$  depends on  $[M]$ , but is independent of the downstream variables  $[R]$  and  $[E_P]$ . Since only  $[R]$  and  $[E_P]$  exhibit multiple steady states, while  $[M]$  is fixed,  $[S_P]$  does not exhibit bistable characteristics. (The parameters of the upstream covalent modification cycle are labelled  $r_i$  here, in order to avoid confusion with the parameters of the bistable switch.)

### A.2.2 Coupling through Alternative Input: CMC- Bistable switch

The alternative input to the bistable switch consists of the input species  $I$  modifying  $E$  to produce  $E_P$ . Again, the binding of the input species to the substrate is assumed to be reversible ( $k_f$  binding/  $k_r$  unbinding), while the production of  $E_P$  from the complex (catalytic constant  $k_{cat}$ ) is not. The equations for an upstream covalent modification cycle coupled to the bistable

switch, through the alternative input, are as follows:

$$\begin{aligned}
 \frac{d[S]}{dt} &= r_4[S_P] + r_2[IS] - r_1[S][I] \\
 \frac{d[IS]}{dt} &= r_1[S][I] - r_2[IS] - r_3[IS] \\
 \frac{d[S_P]}{dt} &= r_3[IS] - r_4[S_P] - k_f[E][S_P] + k_r[ES_P] + k_{cat}[ES_P] \\
 \frac{d[ES_P]}{dt} &= k_f[E][S_P] - k_r[ES_P] - k_{cat}[ES_P] \\
 \frac{d[R]}{dt} &= k_0[E_P] + k_1[Fixed Input] - k_2[R] \\
 \frac{d[E_P]}{dt} &= k_3[R][E] - \frac{k_4[E_P]}{K_4 + [E_P]} + k_{cat}[ES_P] \\
 [I_T] &= [I] + [IS], [S_T] = [S] + [S_P] + [IS] + [ES_P] \\
 [E_T] &= [E] + [E_P] + [ES_P]
 \end{aligned}$$

Here, the steady state value of  $[S_P]$  depends on the downstream variable  $[E_P]$ . Thus we have a steady state value of  $[S_P]$  corresponding to each steady state value of  $[E_P]$  (i.e.  $[S_P]$  exhibits bistable characteristics). Note that, when coupling through the alternative input, the value of the designated input is kept fixed (denoted by *Fixed Input*).

**Significance of open and closed steps:** When two modules are coupled with sharing, the effect of sequestration of upstream output, on the behaviour of the coupled system, depends on whether the upstream output species belongs to an open or a closed step. As we have seen for the bistable module, its output species  $R$  is not part of a conserved pool (i.e. an open step), and therefore, its sequestration in a downstream module does not affect the bistability of the coupled system (See text). However, if the conserved species  $E$  or  $E_P$  (part of a closed step), of the same module, is sequestered downstream, then increased sequestration can cause loss of bistability. A similar observation may be drawn for the two oscillators upstream of other modules. The output species of the NFO belongs to a conserved pool, while the output of the SDO arises from an open step. Thus, increased downstream sequestration of the output can destroy oscillations in a coupled system with the NFO, while it has no such effect with the SDO. Taken together, with the discussion of the previous paragraph, we can conclude that having open steps in a module has significant implications for the connection of the module both upstream and downstream.

### A.2.3 Effects of sequestration on thresholds for downstream transitions: With a CMC upstream

The following analysis aims to explain the effects discussed in Section III.B.3 of the main text, which is relevant to modules that exhibit bifurcations with the input as bifurcation parameter (bistable and oscillator modules). Consider an upstream CMC coupled to a downstream module through a zeroth order reaction as shown in Figure S2(A). The equations for the variables pertinent to the interaction are:

$$\begin{aligned}
 \frac{d[IS]}{dt} &= r_1[I]([S_T] - [IS] - [MS_P] - [S_P]) \\
 &\quad - r_2[IS] - r_3[IS] \\
 \frac{d[S_P]}{dt} &= r_3[IS] - r_4[S_P] - k_f[M][S_P] \\
 &\quad + k_r[MS_P] + k_{cat}[MS_P] \\
 \frac{d[MS_P]}{dt} &= k_f[M][S_P] - k_r[MS_P] - k_{cat}[MS_P] \\
 \frac{d[R]}{dt} &= f([R], \sim) + k_{cat}[MS_P] \\
 [I_T] &= [I] + [IS]
 \end{aligned}$$

The upstream input in this case is the total enzyme concentration,  $[I_T]$ . Here,  $\sim$  denotes the remaining downstream module variables, that play no part in the coupling. Note that the equations for the downstream module variables remain unchanged, except that the ‘input term’  $k_1[I]$ , which forms an additive bifurcation parameter for the module in isolation (see bistable and oscillatory modules described in Section II.A of the main text), is now replaced by  $k_{cat}[MS_P]$ . Since  $[MS_P]$  is independent of other downstream variables, this term continues to form an additive bifurcation parameter with respect to the downstream module. At steady state, we find

$$\begin{aligned}
 [MS_P] &= \frac{k_f[M][S_P]}{k_r + k_{cat}} = \mu[S_P] \\
 [IS] &= \frac{r_1[I]}{r_1[I] + r_2 + r_3}([S_T] - (1 + \mu)[S_P])
 \end{aligned}$$

For the downstream module in isolation, suppose a transition occurs (i.e. there is a bifurcation point) at  $k_{input}I = \delta^*$ . In the present case, the same transition must occur exactly when  $k_{cat}[MS_P] = \delta^*$ . The value of  $[S_P]$  corresponding to this transition is given by  $\delta^*/k_{cat}\mu$ . Thus, the corresponding

value of the upstream input may be calculated to give:

$$[I]^* = \left( \frac{r_2 + r_3}{r_1} \right) \frac{\gamma \delta^*}{(k_{cat} \mu S_T - (1 + \mu + \gamma) \delta^*)} \quad (\text{A.1})$$

where  $\gamma = r_4/r_3$

This allows us to calculate  $[I]^*$  (the value of  $[I]$  corresponding to a transition in the downstream module) in the limiting case where  $k_{cat} \rightarrow \infty$ . This corresponds to sequential interconnection without sharing.

$$\lim_{k_{cat} \rightarrow \infty} [I]^* = \left( \frac{r_2 + r_3}{r_1} \right) \frac{\gamma \delta^*}{(k_f M S_T - (1 + \gamma) \delta^*)} \quad (\text{A.2})$$

Clearly, from (1), as  $k_{cat}$  is reduced,  $[I]^*$  becomes undefined when  $k_{cat} \mu S_T = (1 + \mu + \gamma) \delta^*$ . Below this value of  $k_{cat}$ ,  $[I]^*$  is negative. Thus we have  $\tilde{k}_{cat}$ , the positive value of the catalytic constant, below which this transition becomes impossible for finite upstream input (finite  $[I_T]$ ).

$$\tilde{k}_{cat} = \frac{((1 + \gamma)k_r + k_f M) \delta^*}{(k_f M S_T - (1 + \gamma) \delta^*)} \quad (\text{A.3})$$

If the downstream module exhibits multiple transition points (bifurcation points), we have a value of  $\delta^*$  corresponding to each one of these, and the corresponding values of  $[I]^*$  may be calculated from (1). Note that, according to (3),  $\tilde{k}_{cat}$  will be different for each of these transitions. This means that, as  $k_{cat}$  is lowered, different transition points become unattainable at different values of  $k_{cat}$ .

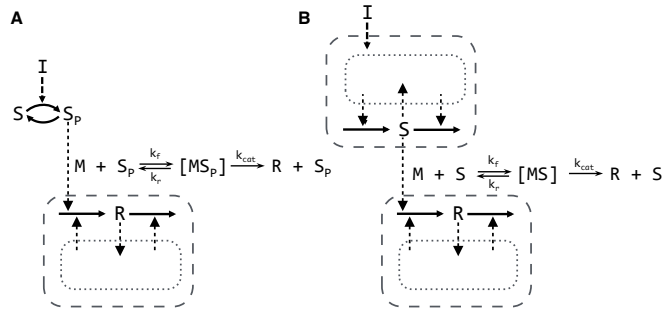


Figure S2: Schematics for coupling of modules with sharing of components.

(A) Upstream CMC coupled to the downstream module through a zeroth-order reaction. (B) Upstream output from an open step coupled to the downstream module through a zeroth-order reaction.

The above analysis applies to the sequential coupling of the CMC to any

downstream module whose input parameter appears in the same way. In the present study, this is applicable in the case of the bistable module and the two oscillators, when the coupling occurs via the designated input. The results of our analysis completely explain all the observed effects of varying the catalytic constant in these three cases, for coupling through the designated input. We note that similar effects are observed for coupling through the alternative input, although we do not analyse those cases here. In general, we also observe similar effects whenever the output species of the upstream module belongs to a conserved pool, such as when the upstream module is a monostable switch or the NFO.

#### A.2.4 Effects of sequestration on thresholds for downstream transitions: Open step coupled to zeroth-order step

Consider the output of an upstream module, coming from an open step, coupled to a downstream module through a zeroth order reaction, as shown in Figure S2(B). The equations for the variables pertinent to the interaction are:

$$\begin{aligned}\frac{d[S]}{dt} &= g([S], [I], \sim) - k_f[M][S] + k_r[MS] + k_{cat}[MS] \\ \frac{d[MS]}{dt} &= k_f[M][S] - k_r[MS] - k_{cat}[MS] \\ \frac{d[R]}{dt} &= f([R], \sim) + k_{cat}[MS]\end{aligned}$$

In  $g([S], [I], \sim)$ ,  $\sim$  denotes the remaining upstream module variables, that play no part in the coupling, while in  $f([R], \sim)$ ,  $\sim$  denotes the remaining downstream module variables. Clearly, the steady state value of  $[S]$  is unaffected by the coupling, and therefore its relationship with the upstream input does not depend on  $k_{cat}$ . As in the previous case, the term  $k_{cat}[MS]$  plays the role of an additive bifurcation parameter in the downstream module. At steady state,

$$[MS] = \frac{k_f[M][S]}{k_r + k_{cat}}$$

Thus, the additive bifurcation parameter for the downstream module is given by:

$$k_{cat}[MS] = \frac{k_f k_{cat}[M][S]}{k_r + k_{cat}}$$

For specificity, we assume that  $[S]$  at steady state is a monotonically increasing function of the input  $[I]$ . If a downstream transition (i.e. bifurcation point) occurs at  $[I]^*$  for a given  $k_{cat}$ , it may be easily understood from the above expression, that at a lower  $k_{cat}$ , the same transition would be observed at a value of  $[I]$  that is higher than  $[I]^*$ . Thus, as  $k_{cat}$  is lowered, the downstream bifurcation points move away to higher values of the upstream input. However, unlike the previous case, it is possible that these transitions become unattainable (i.e. unreachable at finite input) only when  $k_{cat}$  becomes equal to zero. (This depends on whether  $[S]$  at steady state is bounded.)

**Monostable switch upstream or downstream** :The effects of coupling the monostable switch upstream are qualitatively similar to having a covalent modification upstream, as may be expected. In all cases, the upstream variables saturate at a lower level as  $k_{cat}$  is lowered, and the downstream output behaviour is affected in much the same way as with an upstream covalent modification. The only difference is that the steady state input-output curve of monostable switch in isolation is sigmoidal, and this aspect is seen (with suitable distortions) in the coupled case.

**Bistable switch-NFO:** For simplicity, we consider two scenarios that are analogous to cases (a) and (f) in the coupling of the bistable switch and the NFO without sharing. As explained in the previous section, cases (a) and (f) cover the essential aspects of the coupling.

For coupling with sharing, again, all the three sub-cases of the non-sharing scenario (case (a)) are possible. While it is not our intention to focus on responses to step and pulse inputs here, we note that qualitatively similar responses to step and pulse inputs may be seen, depending on the catalytic constant.

First we examine coupling through the designated input to the NFO, interpreting the zeroth order step producing X in the manner discussed above. Oscillations are not propagated upstream for this coupling. The system exhibits bistable characteristics in both cases. Coupling parameters are chosen so that at high  $k_{cat}$ , in the first case (analogous to (a)), an oscillatory region

corresponds to the higher branch of steady states, and in the second (analogous to (f)), it extends from the lower to the higher branch. In the first case, as  $k_{cat}$  is lowered, the extent of the oscillatory regime increases, with respect to the input. The upper limit of the oscillatory range moves to larger values of the input signal, while the lower limit only increases slightly. Further lowering  $k_{cat}$  causes the lower limit also to increase substantially, so that the oscillatory regime as a whole moves to higher values of input signal. In the second case, similar effects are observed, with the upper limit of the oscillatory range moving to larger input values first, followed by the lower limit moving up onto the middle branch and eventually the upper branch of steady states.

Since the switch output  $R$  comes from an open step, the effects seen for the coupling through the designated input may be easily explained with reference to the analysis presented earlier.

Next we focus on coupling through the alternative input to the NFO, where the input acts as an enzyme, promoting the degradation of species  $X$ . Here again, the system exhibits bistable characteristics, and oscillations are propagated to the upstream variables.

For the scenario analogous to case (a), which is discussed in the main text, stable oscillations correspond only to a part of the upper steady state branch. As the catalytic constant is lowered, the extent of the oscillatory regime (with respect to upstream input) first increases, and then reduces. Eventually, at very low  $k_{cat}$ , the Hopf bifurcation points approach each other and oscillations disappear completely. The system continues to exhibit bistability.

For case (f), in the non-sharing scenario, we have the possibility of coexisting oscillatory states. For the analogous coupling with sharing, through the alternative input, the coupling parameters (with a basal *high* value for  $k_{cat}$ ) are such that the coupled system exhibits coexistence of two stable oscillatory states over a range of input. As seen in [Fig], for the basal catalytic constant (high), we have coexisting stable oscillatory states. As the catalytic constant of the coupling reaction is lowered, the extent of the oscillatory regime (with respect to input) is seen to increase, while the coexistence of distinct stable oscillatory states no longer occurs. We instead see coexistence of a stable steady state and oscillations, in a certain range of catalytic constant. Further lowering the catalytic constant reduces the extent of the oscillatory regime, and eventually oscillations disappear (the Hopf bifurcation points approach each other, and the oscillatory regime narrows, and finally disappears). Again, bistable behaviour is retained.

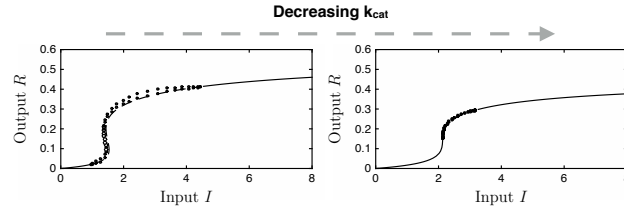


Figure S3: Sequential interaction, with sequestration, between an upstream NFO and a downstream bistable switch. In the scenario shown here, as the extent of sequestration increases, the coupled system first loses bistable characteristics, while oscillatory behaviour persists. Eventually, increasing sequestration also destroys oscillatory behaviour (not shown).

In both the above scenarios for the alternative input, if the input lies in an oscillatory regime, the upstream variables are seen to oscillate. The amplitude of upstream oscillations increases initially as the catalytic constant is lowered from its basal high value. This behaviour is analogous to the propagation of oscillations to an upstream CMC, discussed previously.

**NFO-Bistable switch:** For coupling the bistable switch to a downstream oscillator with sharing of components, we consider three scenarios analogous to the three possibilities discussed in the non-sharing case. We first examine coupling through the alternative input, where back propagation of bistable characteristics may be possible.

In the first scenario, analogous to case (I), the coupled system exhibits bistable characteristics with respect to the upstream input, and two distinct stable oscillatory states coexist for a range of upstream input. There are no oscillatory states outside this range. The extent of this oscillatory range becomes diminished as  $k_{cat}$  is lowered, and eventually, oscillations disappear. Further reducing  $k_{cat}$  causes the system to lose bistability.

The second scenario is analogous to case (II) in the non-sharing case. Here, the system exhibits a single stable oscillatory state over a certain range of input, and bistable behaviour over a different range of input. As in the previous case, the oscillatory range becomes smaller as  $k_{cat}$  is reduced, and eventually oscillations disappear. Further reducing  $k_{cat}$  also causes the system to lose bistability.

The third scenario, analogous to case (III) is discussed in the main text.



The common pattern in all three cases discussed above, in terms of the effect of  $k_{cat}$ , consists in the narrowing of the oscillatory range, followed by the destruction of oscillations, and eventually the loss of bistability, as  $k_{cat}$  is reduced. The destruction of oscillations may be seen as analogous to the case of the NFO upstream of a CMC, where increased sequestration of the NFO output (part of a conserved pool) causes a similar effect. Since the alternative input location for the switch also involves a conserved pool, the loss of bistability may also be expected to result from increased sequestration, as seen in Section III.B.4.

For coupling through the designated input of the switch, which involves a zeroth order step, we observe largely similar trends, except for the absence of back propagation of bistable characteristics here. The only other significant qualitative difference is that, as  $k_{cat}$  is lowered, bistable behaviour disappears, not via a narrowing of the bistable regime as above, but through the regime itself moving to higher values of the input, with bistability eventually becoming impossible for finite values of the input. It may be shown analytically, that this complete disappearance of oscillatory behaviour occurs at a positive value of  $k_{cat}$ .

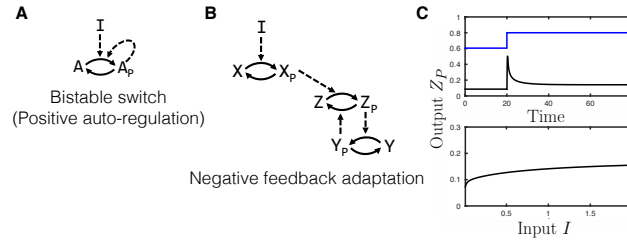


Figure S4:(A) Alternative bistable switch. (B) Negative feedback adaptive module. (C) Input-output response for the negative feedback adaptive module (Showing both bifurcation diagram and response to step input).

## A.3 Alternative realizations of modules

### A.3.1 Alternative bistable module

This module consists of two interconvertible species:  $A$  and  $A_p$  (Fig. S4(A)). The total amount of the two forms is conserved. The input  $I$  activates  $A_p$ , and the reaction is governed by simple mass action kinetics. The species  $A_p$  also promotes its own production through a linear positive feedback. The rate

of  $A_P$  degradation saturates with increasing amount of the  $A_P$  (see Ferrell Jr and Xiong, 2001). This saturation causes the system to be bistable.

$$\frac{d[A_P]}{dt} = k_1[I][A] + k_3[A_P][A] - \frac{k_2[A_P]}{K_2 + [A_P]}$$

$$[A_T] = [A] + [A_P]$$

The behaviour of this module, when connected sequentially to other modules, without sharing of components, is similar to the bistable module we consider in the main text. The main difference arises in the case of coupling with sharing, when the bistable switch is upstream. Since this bistable module consists of an interconversion between species that belong to a fixed pool, the sequestration of the output species in a downstream module may destroy bistability in the coupled system. When the switch is downstream, the effect of bistability is propagated to the upstream module, as we see in the case of the other bistable switch.

### A.3.2 Negative-feedback adaptation

This module consists of three nodes, two of them forming a negative feedback loop (Fig. S4(B)). Each node consists of an interconversion between two species. The total amount of the two species is conserved at each node. The input node (X) is activated by the input signal  $I$ . This node activates the second node (Z), which acts as the output node. The output node activates the buffer node (Y). (Y) in turn, deactivates (Z), thus forming a negative feedback loop. We consider a model that uses mass action kinetics everywhere, except for the degradation of (Y), which is described by Michaelis-Menten kinetics. The Michaelis-Menten description can be easily justified in such a case. The module exhibits a moderate level of underadaptation for the chosen parameter values (Fig. S4(C)). However, we note that close to exact adaptation may be achieved, in an appropriate range of parameter values, if Michaelis-Menten kinetics are used everywhere (see Ma et al., 2009). The model is as

follows:

$$\begin{aligned}\frac{d[X_P]}{dt} &= k_1[I][X] - k_2[X_P] \\ \frac{d[Y_P]}{dt} &= k_3[Z_P][Y] - \frac{k_4[Y_P]}{K_4 + [Y_P]} \\ \frac{d[Z_P]}{dt} &= k_5[X_P][Z] - k_6[Y_P][Z_P] \\ [X_T] &= [X] + [X_P], [Y_T] = [Y] + [Y_P], [Z_T] = [Z] + [Z_P]\end{aligned}$$

*Sequential interactions without sharing:* The behaviour of this module is broadly similar to that of the incoherent feedforward (IFF) module when coupled downstream of other modules, the only difference being moderate under-adaptation of the output. When coupled upstream of another module, this underadaptation of the NF adaptive module means that it does not insulate the downstream module completely, from variations in the upstream input.

*Sequential interactions with sharing:* The NF module behaves in much the same way as the IFF module when coupled downstream of other modules. However, when coupled to an upstream bistable switch, the NF module does not filter out the effects of bistability to the same extent as the IFF adaptive module. This is due to the under-adaptation of the NF adaptive module. When coupled upstream of a CMC, similar to the IFF module, the extent of sequestration of the adaptive output can reduce the amplitude of the transient response to a step signal. A contrast with the IFF module may be seen in the case of coupling to a downstream bistable switch or NFO. While the back-propagation effect in the IFF module is restricted to the output node, in the NF module, bistability or oscillations will be propagated to all the nodes within the negative feedback loop. The fact that all the interactions within the feedback loop are governed by mass-action kinetics, does not prevent these downstream effects being propagated to all the nodes.

### A.3.3 Alternative monostable switch - MAPK cascade:

An alternative monostable switch module that may be considered, which gives sharper switching, is the three tier modification cascade, used to model a MAPK cascade. This has been studied by Huang and Ferrell, 1996 and in greater detail by Ventura, Sepulchre, and Merajver, 2008. Though this module has multiple stages, the effects of sequential coupling, both without and with sharing, are broadly similar to what is seen here.

The supplementary information is organised as follows: We discuss (1) further results associated with the substrate depletion oscillator (2) parameters used in our computational analysis.

### A.3.4 Substrate Depletion Oscillator

#### Sequential interaction without shared components

When the SDO is coupled upstream of another module, without shared components, the subcritical Hopf bifurcation exhibited by this module gives rise to important differences, relative to analogous coupling involving the NFO. As discussed in the main text, these differences are particularly significant in the case of response to pulse inputs. In particular, that pulse inputs can switch on and switch off oscillations. Consequently, the coupling of the SDO to any downstream module may be mapped out in an analogous way to the NFO, noting this essential difference.

When the SDO is coupled downstream of a CMC or monostable switch, a step or pulse input upstream may switch the downstream output from a stable steady state to stable oscillations, or from stable oscillations to a stable steady state. Here again, the main difference with the NFO stems from the fact that a pulse input can trigger a permanent transition. This aspect is also relevant when the SDO is coupled downstream of the adaptive module, where the transient response triggered by an input, in the adaptive module, may cause a permanent transition between a steady state and oscillations downstream. The coupling the SDO downstream of the bistable switch can give rise to multiple scenarios, analogous to those detailed above for the NFO, with added complexity resulting from the subcritical nature of the Hopf bifurcation. As seen for the NFO, there may be scenarios with a single stable oscillatory state for a given input, as well as scenarios with multiple stable oscillatory states for the same input. Since we consider only step and pulse inputs to the upstream module, and the effects of these inputs to the bistable module are well understood, it is easy to see what the resulting inputs to the downstream SDO will be (for a fixed coupling parameter). This allows us to transparently map out the response of the SDO, for scenarios with a single stable oscillatory state for a given input. The more complex scenarios, involving multiple stable oscillatory states for the same input, may be examined along the same lines as for the NFO (see above), but we do not discuss this here.

### Sequential interaction with shared components

For coupling with shared components, we examine the basic scenario of coupling the SDO to a CMC. The effects seen for this coupling are consistent with those discussed in the main text for the other modules, and may be explained in an analogous way.

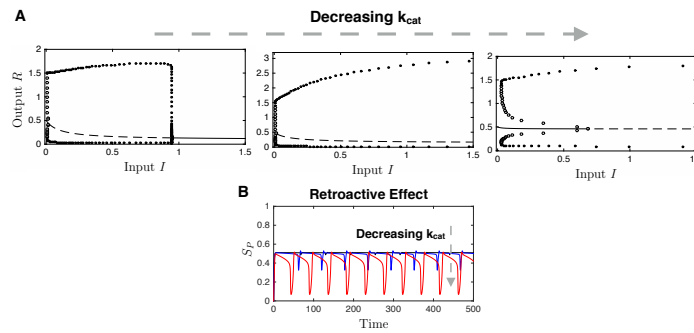


Figure S5: Sequential interaction with sharing, substrate depletion oscillator downstream of a covalent modification cycle. (A) The input range for oscillatory behaviour changes significantly as the catalytic constant decreases. (B) Increased sequestration leads to higher amplitude oscillations of the upstream species.

*CMC upstream - SDO downstream:* For the same reasons as in the case of the NFO, coupling through the designated input to the SDO does not allow back-propagation of oscillations to the CMC. Here we examine coupling through an alternative input, where back-propagation of oscillations occurs. At high  $k_{cat}$ , the coupled system undergoes a subcritical Hopf bifurcation at a positive value of the signal, with the oscillatory regime closed by a supercritical Hopf bifurcation. As the  $k_{cat}$  is reduced, we see a trend similar to the analogous case for the NFO (see Figure S5): the extent of the oscillatory regime (with respect to the upstream input) initially increases, but eventually, the oscillatory regime, as a whole, moves to higher input values so that oscillatory behaviour becomes unattainable at reasonable values of input.

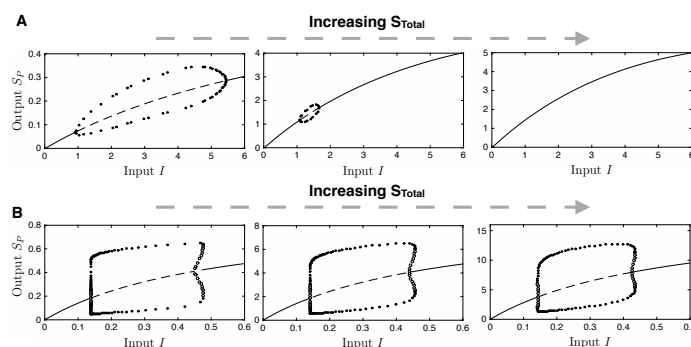


Figure S6: Sequential interaction with sharing, with the covalent modification cycle downstream. (A) Negative feedback oscillator upstream.

The oscillatory regime, with respect to the upstream input, becomes narrower and eventually disappears, as the total amount of downstream species increases (See text). (B) Substrate depletion oscillator. The range of oscillatory behaviour and the nature of the transitions remains unaffected by an increase in the total amount of downstream species.

*SDO upstream - CMC downstream:* When coupled to a downstream CMC, increased sequestration of the SDO output in the downstream module does not distort the oscillatory behaviour in the SDO (see Figure S6). The range of input for oscillatory behaviour remains unaffected by the sequestration. It is essentially due to the fact that the output of this oscillator is associated with an open chemical reaction. This effect is similar to what is observed case of the bistable switch coupled to a downstream CMC. However, as mentioned in the case of the bistable switch, if the SDO were coupled to the CMC through a different species, one belonging to a conserved pool, then oscillatory behaviour would be significantly distorted, or even destroyed, by increased sequestration downstream.

We can examine the SDO coupled to the other modules, with shared components, in an manner exactly analogous to the NFO. There are a number of parallel insights which emerge in this case, but we do not discuss this here.

## A.4 Oblique interactions: Analytical results

### Adaptive module

For the adaptive module in isolation, we have the following equation for its output  $[Z_P]$

$$\frac{d[Z_P]}{dt} = k_5[X_P]([Z_T] - [Z_P]) - k_6[Y_P][Z_P]$$

At steady state,  $[Y_P]$  is given by

$$[Y_P] = \frac{k_3[X_P]}{k_3[X_P] + k_4}$$

Thus, we can calculate  $[Z_P]$  at steady state, which is given by

$$[Z_P] = \frac{k_5[X_P]Z_T}{k_5[X_P]Z_T + k_6\left(\frac{k_3[X_P]}{k_3[X_P] + k_4}\right)} \quad (\text{A.4})$$

Since  $[X_P]$  is a monotonically increasing function of the module input, calculating  $\frac{d[Z_P]}{d[X_P]}$  allows us to understand general trends in the variation of steady state  $[Z_P]$  as the upstream input is varied.

$$\frac{d[Z_P]}{d[X_P]} = \frac{k_5k_6k_3^2[X_P]^2Z_T}{(k_3[X_P] + k_4)^2}$$

Note that the derivative is positive. For close to exact adaptation, we want this derivative to be close to zero, in which case the above derivative is approximately given by

$$\frac{d[Z_P]}{d[X_P]} = k_5k_6[X_P]^2Z_T\left(\frac{k_3}{k_4}\right)^2 \quad (\text{A.5})$$

Now suppose we have an oblique interaction with a CMC, where the CMC output  $S_P$  promotes the conversion  $Y_P$  to  $Y$ . We now have

$$[Z_P] = \frac{k_5[X_P]Z_T}{k_5[X_P]Z_T + k_6\left(\frac{k_3[X_P]}{k_3[X_P] + k_4 + k_{ob}[S_P]}\right)} \quad (\text{A.6})$$

and the derivative is approximately given by

$$\frac{d[\overline{Z_P}]}{d[\overline{X_P}]} = k_5 k_6 [\overline{X_P}]^2 Z_T \left( \frac{k_3}{k_4 + k_{ob}[S_P]} \right)^2 \quad (\text{A.7})$$

From (6) and (7), it is clear that any increase in  $[S_P]$  will result in an increase in  $[\overline{Z_P}]$  for a fixed  $[X_P]$ , while it makes  $\frac{d[\overline{Z_P}]}{d[\overline{X_P}]}$  even smaller, so that the adaptation is closer to exact, than for the module in isolation. Next we examine the scenario where the oblique interaction occurs through a different location, with  $S_P$  promoting the conversion  $Z_P$  to  $Z$ . This gives us

$$[\overline{Z_P}] = \frac{k_5 [\overline{X_P}] Z_T}{k_5 [\overline{X_P}] Z_T + k_6 \left( \frac{k_3 [\overline{X_P}]}{k_3 [\overline{X_P}] + k_4} \right) + k_{ob}[S_P]} \quad (\text{A.8})$$

and the derivative is approximately given by

$$\frac{d[\overline{Z_P}]}{d[\overline{X_P}]} = k_5 k_6 [\overline{X_P}]^2 Z_T \left( \frac{k_3}{k_4} \right)^2 + k_5 k_{ob}[S_P] \quad (\text{A.9})$$

From (8), it is clear that any increase in  $[S_P]$  will result in a decrease in  $[\overline{Z_P}]$ , for a fixed  $[X_P]$ . By comparing (9) to (5), we see that increasing  $[S_P]$  will produce an increase in the derivative, so that the response becomes under-adaptive.

## Bistable switch

When an oblique interaction with the CMC occurs through the degradation step for species  $R$  in the bistable module, (the CMC output species  $S_P$  promotes the degradation of  $R$  via a mass action step),  $[E_P]$  at steady state satisfies the equation

$$\begin{aligned} \frac{k_3}{k_4} (K_4 + [E_P]) (k_0[E_P] + k_1[I]) ([E_T] - [E_P]) \\ = (k_2 + k_{ob}[S_P]) [E_P] \end{aligned} \quad (\text{A.10})$$

The LHS is a function of  $[E_P]$ , which we call  $H([E_P], [I])$ . The input  $[I]$  is a parameter here. It is clear that the steady state values of  $[E_P]$  are given by the points at which  $H([E_P], [I])$  intersects the straight line passing through the origin, with slope  $\lambda = (k_2 + k_{ob}[S_P])$ . For the module to be in the bistable regime, there must be three such points, corresponding to positive values of  $[E_P]$ . It is easy to show that, as  $\lambda$  increases, correspondingly higher values



$[I]$  are needed to realize multiple points of intersection. Thus, for a stronger oblique interaction, the bistable regime is shifted to higher values of the input  $[I]$ .

Now, a necessary condition for the existence of three intersection points corresponding to positive values of  $[E_P]$ , is that the point of inflection for  $H([E_P], [I])$  should also correspond to a positive value of  $[E_P]$ . This condition, after rearrangement, gives the necessary condition

$$[I] < \frac{k_0}{k_1}([E_T] - K_4) \quad (\text{A.11})$$

Note that (11) is unaffected by the oblique interaction. This condition, together with the discussion in the previous paragraph implies that the presence of the oblique interaction reduces the extent of the bistable regime (with respect to input  $[I]$ ), and may even destroy bistability.

When an oblique interaction with the CMC occurs through the step converting  $E$  to  $E_P$  in the bistable module, (the CMC output species  $S_P$  converts  $E$  to  $E_P$  via a mass action step),  $[E_P]$  at steady state satisfies the equation

$$\begin{aligned} & \left( \frac{k_3}{k_2}(k_0[E_P] + k_1[I]) + k_{ob}[S_P] \right) ([E_T] - [E_P]) \\ &= \frac{k_4[E_P]}{K_4 + [E_P]} \end{aligned} \quad (\text{A.12})$$

Rearranging to express  $[I]$  as a function of  $[E_P]$ , we get the following expression for the steady state input-output curve:

$$I = \frac{k_2 k_4 [E_P]}{k_1 k_3 (K_4 + [E_P]) ([E_T] - [E_P])} - \frac{k_0}{k_1} [E_P] - \frac{k_2}{k_1 k_3} k_{ob} [S_P] \quad (\text{A.13})$$

The above expression tells us that the presence of the oblique interaction, at this location, translates the steady state input-output curve to lower values of the input  $[I]$ , without affecting the extent of the bistable range.

## A.5 Parameter values

The parameter values for the various results presented in the main text and in the supplementary material, are summarised below. In this regard, we

summarise a few important points:

1. When we couple modules, we focus on the qualitative behaviour and transitions which can arise. Thus, we focus on the capabilities and constraints associated with coupling.
2. For purposes of illustration of these results, we choose parameter values associated with the coupling, which reveal such behaviour. In some cases, the model behaviour is representative and typical, whereas, in others, certain ranges of coupling parameters are needed. In the latter case, we note any relevant points in the main text and in the information below.
3. The parameters associated with the isolated modules are usually kept fixed (see discussion in text).
4. When we consider coupling without shared components, the coupling parameter is chosen to reveal potential new behaviour which can arise. It is clear that, if the coupling parameter is very small, the coupling may have a very minor effect (no qualitative change).
5. In the case of coupling with sharing we describe the enzymatic modification of the coupling reaction explicitly, through description of enzyme-substrate binding/unbinding and catalytic conversion. The enzyme-substrate binding reaction rate is chosen so as to allow the aforementioned qualitative transitions when the catalytic constant is very high (an infinite catalytic constant essentially corresponds to the case with no sharing). The effects of sequestration are then studied by reducing the catalytic constant.
6. Again, in the case of oblique coupling, we focus and report on new qualitative behaviour which can arise, and the coupling is chosen appropriately.

**Comments on parameter ranges in computational results:** We discuss further points relevant to parameter ranges used in computational studies. At the outset, we note that all modules have roughly comparable timescales. We also note that in most of our studies, we vary the upstream input. In the case of coupling without sharing, we discuss the coupling parameter range in relation to the upstream module:

1. If the upstream module is a CMC or monostable switch, the coupling parameter is chosen in a range (when the downstream module is a

bistable switch or oscillator) so that the key transitions therein can be triggered. Otherwise there is no restriction.

2. If the upstream module is adaptive, for a downstream bistable or oscillatory module, the coupling is chosen so that the downstream module is in the bistable/oscillatory regime.
3. If the upstream module is an oscillator and the downstream module is bistable, we discuss the different possible scenarios arising from different ranges of the coupling parameter, in the supplemental information.
4. For the bistable module upstream and oscillatory module downstream, different qualitative possibilities associated with coupling are already discussed in detail, in the supplemental information.
5. The parameter choices in all other cases give rise to broadly representative behaviour.

In the case of coupling with sharing, our focus is on the effects of sequestration. In this regard:

1. We choose the coupling (enzyme-substrate binding parameter) so that when the catalytic constant is very high, the various transitions can be triggered as in the non-sharing case. Thus, the points in the previous paragraph are relevant here.
2. When we consider the covalent modification cycle, we choose binding, unbinding, and catalytic constants for the phosphorylation in comparable ranges. When the CMC is downstream, we vary the total amount of species to assess the effects of sequestration.

For oblique interactions, in the case of the adaptive module downstream of a bistable switch, the coupling parameter is chosen in a suitable range so that neither stable steady state of the bistable switch causes a saturation in the adaptive module.

The parameter values for the modules used, and the coupling parameters used for all the plots are given below. For the modules in isolation: for oscillator and bistable modules, parameters are chosen as used in (Tyson et al., 2003), with minor modifications as a result of our changing certain steps to mass action kinetics. For the other modules, a representative set of parameters is used.

## List of Parameter Values

### Covalent Modification Cycle

$$k_1 = 1$$

$$k_2 = 1$$

$$k_3 = 5$$

$$k_4 = 0.5$$

$$[S_T] = 1$$

$$[I_T] = 1$$


---

### Monostable Switch (Two-site Modification)

$$k_1 = 1$$

$$k_2 = 1$$

$$k_3 = 1$$

$$k_4 = 0.05$$

$$[A_T] = 1$$


---

### Incoherent Feedforward Adaptation

$$k_1 = 1$$

$$k_2 = 20$$

$$k_3 = 0.01$$

$$k_4 = 0.1$$

$$k_5 = 15$$

$$k_6 = 150$$

$$[X_T] = 1$$

$$[Y_T] = 1$$

$$[Z_T] = 1$$


---

**Negative Feedback Adaptation**

$$k_1 = 10$$

$$k_2 = 10$$

$$k_3 = 0.8$$

$$k_4 = 0.05$$

$$k_5 = 5$$

$$k_6 = 50$$

$$[X_T] = 1$$

$$[Y_T] = 1$$

$$[Z_T] = 1$$

---

**Sigmoidal Positive Feedback:  
Mutual Activation**

$$k_0 = 0.7$$

$$k_1 = 0.1$$

$$k_2 = 1$$

$$k_3 = 1$$

$$k_4 = 0.2$$

$$K_4 = 0.05$$

$$[E_T] = 1$$

**Linear Positive Feedback  
with Saturated Degradation**

$$k_1 = 0.25$$

$$k_2 = 0.2$$

$$k_3 = 1$$

$$K_2 = 0.01$$

$$[A_T] = 1$$

---

**Negative Feedback Oscillator**

$$k_0 = 0$$

$$k_1 = 0.4$$

$$k_2 = 0.01$$

$$k'_2 = 4$$

$$k_3 = 0.1$$

$$k_4 = 0.2$$

$$k_5 = 5$$

$$k_6 = 0.5$$

$$K_4 = 0.01$$

$$K_6 = 0.01$$

$$[Y_T] = 1$$

$$[R_T] = 1$$

**Substrate Depletion Oscillator**

$$k_0 = 0.4$$

$$k'_0 = 0.01$$

$$k_1 = 1$$

$$k_2 = 1$$

$$k_3 = 10$$

$$k_4 = 3$$

$$K_4 = 0.05$$

$$[E_T] = 1$$

**Figure 3** (Interactions without sharing)Bistable switch - NFO

## Part A

*Coupling parameter :  $k_c = 3.8122$*

*NFO parameter :  $[R_T] = 0.605$*

*Step/Pulse Input :*

*On :  $t = 20$ ; Off :  $t = 40$  ( $I = 0.5$  to  $1$ )*

## Part B

*Coupling parameter* :  $k_c = 2.8857$

*NFO parameter* :  $[R_T] = 0.6$

*Step/Pulse Input* :

*On* :  $t = 20$ ; *Off* :  $t = 40$  ( $I = 0.5$  to  $1.8$ )

## Part C

*Coupling parameter* :  $k_c = 4.2630$

*NFO parameter* :  $[R_T] = 0.6$

*Step/Pulse Input* :

*On* :  $t = 20$ ; *Off* :  $t = 40$  ( $I = 0.5$  to  $1.2$ )

**Figure 4** (Interactions without sharing)Part A: Bistable switch - Adaptive module

*Coupling parameter* :  $k_c = 10$

*Pulse Input* :

*First* :  $t = 20 - 80$ ; *Second* :  $t = 180 - 240$   
( $I = 0.7$  to  $0.8$ )

Part B: NFO - Adaptive module

*Coupling parameter* :  $k_c = 1$

*Step Input* :

*On* :  $t = 100$  ( $I = 1.5$  to  $5.5$ )

Parts C,D and E: NFO - Bistable switch

*Coupling parameter* :  $k_c = 1.9$

*Step Input* :

$I = 3$  to  $5$  in C ( $t = 38$ ) and E ( $t = 40$ )

$I = 3$  to  $5.7$  in D ( $t = 38$ )

Part E: SDO - Monostable switch

*Coupling parameter :  $k_c = 1$*

*Pulse Input :*

*First :  $t = 100 - 150$ ; Second :  $t = 600 - 673$*

*( $I = 0.47$  to  $0.14$ )*

---

**Figure 5** (Interactions with sharing)Part A: CMC - Monostable switch

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 100, 0.1, 0.01$$

Part B: CMC - Adaptive module

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 10, 0.1$$

Part C: CMC - Bistable switch

Alternative input to bistable switch: Input acts on  $E$  to produce  $E_P$

*Designated Input to Bistable switch = 0*

$$k_f = 0.1$$

$$k_r = 0.01$$

$$k_{cat} = 10, 2$$

**Figure 6** (Interactions with sharing)CMC - NFO

Alternative input to NFO: Input acts as an enzyme degrading  $X$

*Designated Input to NFO = 6*

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 100, 1, 0.07$$


---



**Figure 7** (Interactions with sharing)Part A: Monostable switch - CMC

$$[S_T] = 1, 10, 15$$

Part B: Adaptive module - CMC

$$[S_T] = 1, 10, 20$$

Part C: Bistable switch - CMCSpecies  $R$  as input to CMC

$$[S_T] = 1, 10, 20$$

Part D: Bistable switch - CMCSpecies  $E_P$  as input to CMC

$$[S_T] = 1, 2.5, 5$$

**Figure 8** (Interactions with sharing)Parts A,B: Adaptive module - Bistable switchAlternative input to bistable switch: Input acts on  $E$  to produce  $E_P$ *Designated Input to Bistable switch* = 0

$$k_f = 0.1$$

$$k_r = 0.01$$

$$k_{cat} = 100, 0.5$$

Part C: Bistable switch - NFOAlternative input to NFO: Input acts as an enzyme degrading  $X$ *Designated Input to NFO* = 6.4

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 10, 2, 0.06$$

Part D: NFO - Bistable switchAlternative input to bistable switch: Input acts on  $E$  to produce  $E_P$

*Designated Input to Bistable switch = 0*

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 10, 0.4, 0.3$$

**Figure 9** (Oblique interactions)

Part A (1): CMC - Adaptive module

(CMC input = 0.7,  $k_{ob} = 0.5$ )

Adaptive Module Equations with

Oblique interaction :

$$\frac{d[X_P]}{dt} = k_1[I]([X_T] - [X_P]) - k_2[X_P]$$

$$\begin{aligned} \frac{d[Y_P]}{dt} &= k_3[X_P]([Y_T] - [Y_P]) - k_4[Y_P] \\ &\quad - k_{ob}[S_P][Y_P] \end{aligned}$$

$$\frac{d[Z_P]}{dt} = k_5[X_P]([Z_T] - [Z_P]) - k_6[Y_P][Z_P]$$

Part A (2): CMC - Adaptive module

(CMC input = 0.7,  $k_{ob} = 1$ )

Adaptive Module Equations with

Oblique interaction :

$$\frac{d[X_P]}{dt} = k_1[I]([X_T] - [X_P]) - k_2[X_P]$$

$$\frac{d[Y_P]}{dt} = k_3[X_P]([Y_T] - [Y_P]) - k_4[Y_P]$$

$$\begin{aligned} \frac{d[Z_P]}{dt} &= k_5[X_P]([Z_T] - [Z_P]) - k_6[Y_P][Z_P] \\ &\quad - k_{ob}[S_P][Z_P] \end{aligned}$$

**Part B (1): CMC-Bistable switch**(CMC input = 0.7,  $k_{ob} = 0.4$ )

Bistable Module Equations with

Oblique interaction :

$$\begin{aligned}\frac{d[R]}{dt} &= k_0[E_P] + k_1[I] - k_2[R] - k_{ob}[S_P][R] \\ \frac{d[E_P]}{dt} &= k_3[R]([E_T] - [E_P]) - \frac{k_4[E_P]}{K_4 + [E_P]}\end{aligned}$$

**Part B (1): CMC-Bistable switch**(CMC input = 0.7,  $k_{ob} = 0.4$ )

Bistable Module Equations with

Oblique interaction :

$$\begin{aligned}\frac{d[R]}{dt} &= k_0[E_P] + k_1[I] - k_2[R] \\ \frac{d[E_P]}{dt} &= k_3[R]([E_T] - [E_P]) + k_{ob}[S_P]([E_T] - [E_P]) \\ &\quad - \frac{k_4[E_P]}{K_4 + [E_P]}\end{aligned}$$

**Figure 10** (Oblique interactions)**Part A (1): Bistable switch - Adaptive module**(Bistable switch input = 0.5,  $k_{ob} = 0.5$ )

Adaptive Module Equations with

Oblique interaction :

$$\begin{aligned}\frac{d[X_P]}{dt} &= k_1[I]([X_T] - [X_P]) - k_2[X_P] \\ \frac{d[Y_P]}{dt} &= k_3[X_P]([Y_T] - [Y_P]) - k_4[Y_P] \\ &\quad - k_{ob}[R][Y_P] \\ \frac{d[Z_P]}{dt} &= k_5[X_P]([Z_T] - [Z_P]) - k_6[Y_P][Z_P]\end{aligned}$$

**Figure S3** (Interactions with sharing)**NFO - Bistable switch**

Alternative input to bistable switch: Input acts on  $E$  to produce  $E_p$

*Designated Input to Bistable switch* = 0

$$\begin{aligned}[R_T] &= 5 \\ [E_T] &= 0.85 \\ k_f &= 1 \\ k_r &= 0.1 \\ k_{cat} &= 10, 1\end{aligned}$$


---

**Figure S5** (Interactions with sharing)

CMC - SDO

Alternative input to SDO: Input acts as an enzyme degrading  $R$

*Designated Input to SDO* = 0.5

$$\begin{aligned}k_f &= 5 \\ k_r &= 0.1 \\ k_{cat} &= 10, 1, 0.045\end{aligned}$$


---

**Figure S6** (Interactions with sharing)

Part A: NFO - CMC

$$[S_T] = 1, 15, 20$$

Part B: SDO - CMC

$$[S_T] = 1, 10, 20$$


---

**Figure S7** (Interactions with sharing)

Part A: Bistable switch - Adaptive module

$$\begin{aligned}k_f &= 10 \\ k_r &= 1 \\ k_{cat} &= 100, 5, 1\end{aligned}$$

Part B: Adaptive module - NFO

Alternative input to NFO: Input acts as an enzyme degrading X

*Designated Input to NFO = 6*

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 100, 10, 5$$

Part C: NFO - Adaptive module

$$k_f = 1$$

$$k_r = 0.1$$

$$k_{cat} = 100, 2, 0.8$$

## A.6 Additional Plots

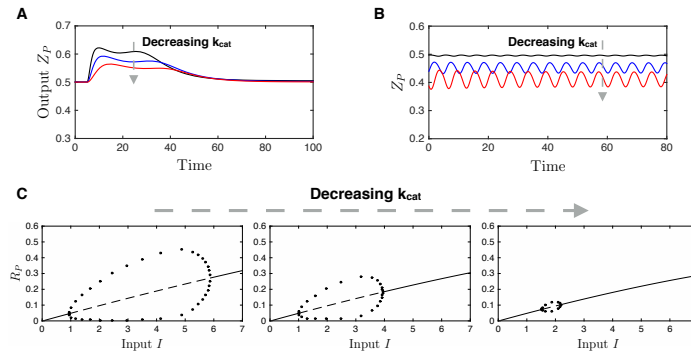


FIGURE A.1: Sequential interaction with sharing. (A) Bistable switch - Adaptive module. The amplitude of response to a step input that causes a switching of steady states, is diminished as  $k_{cat}$  decreases. (B) Adaptive module - NFO. As the  $k_{cat}$  decreases, the upstream adaptive variable oscillates with increasing amplitude. (C) NFO - Adaptive module. As  $k_{cat}$  decreases, the range of upstream input for oscillations becomes narrower, and eventually oscillations disappear (not shown).

### Effects of downstream sequestration on an upstream module

The effects of increased sequestration of module output species in a downstream covalent modification cycle are discussed in Section III.B.4. The behaviour of the upstream module is shown to be significantly distorted by the sequestration of its output species when this species belongs to a conserved pool of interconvertible forms. The designated output belongs to such a conserved pool in the case of the monostable switch, the adaptive module, and the NFO. This is also the case for the bistable switch, if the coupling to the CMC takes place through  $E_P$  instead of  $R$ . Here we show, computationally, that similar distortion of module behaviour may be observed, for each of these modules in isolation, if the total amount in the conserved pool containing the output species, is reduced.

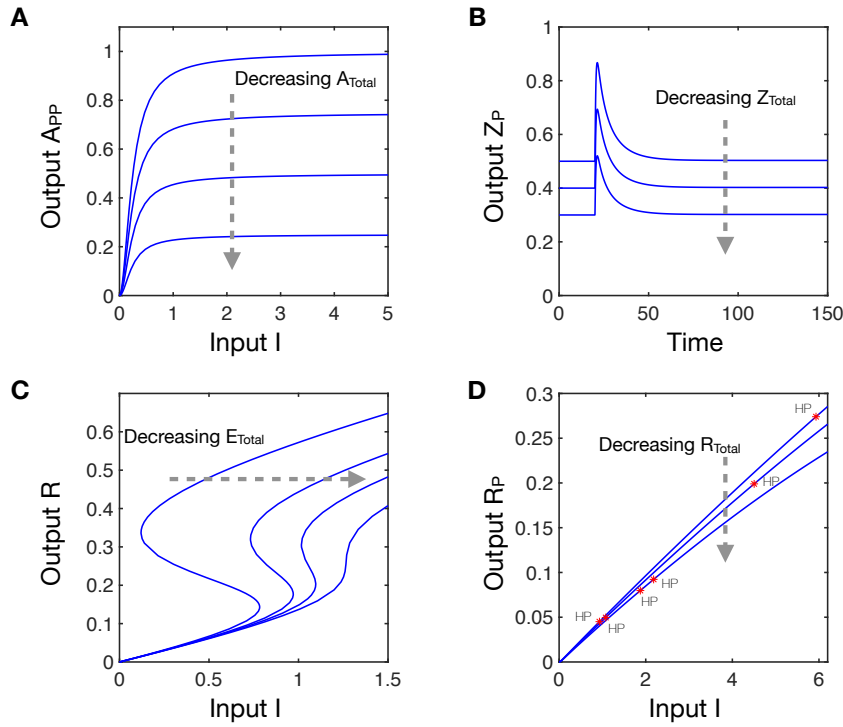


FIGURE A.2: (A) Monostable switch: The steady state input-output relationship is distorted by a reduction in  $A_T$ . The change is qualitatively similar to the effect of increased sequestration of the output in a downstream CMC. (B) Adaptive module: Both the basal steady state value and the amplitude of the transient response, to a given step signal, are reduced by a reduction in  $Z_T$ . This effect is qualitatively similar to the effect of increased sequestration of the output in a downstream CMC. (C) Bistable switch: The steady state input-output relationship is distorted by a reduction in  $E_T$ . The effect seen here is qualitatively similar to that of increased sequestration of the output in a downstream CMC (When the bistable switch is coupled to the CMC through species  $E_P$ ). (D) NFO: HP denotes a Hopf bifurcation point. The range of input signal over which stable oscillations are observed, is significantly reduced by a reduction in  $R_T$ . The observed variation is qualitatively similar to that of increased sequestration of the output in a downstream CMC.





## Appendix B

# Supplemental Material for Chapter 3

Here we present additional information related to the plots shown, including the parameter values used.

**Parameter values**

|              |                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 1 (b) | Total_amount of substrate = 2<br>$k_1 = 1$<br>$k_2 = 2$<br>$D_X = 0.05$<br>$D_{X^*} = 0.1$<br><br>$L/L1 = 18$<br>Domain size = 1                                                                                                                                                                                                                                                                            |
| Figure 1(c)  | Total_amount of substrate = 2<br>$k_1 = 1$<br>$k_2 = 2$<br>$D_X = 0.05$<br>$D_{X^*} = 0.1$<br><br>$L/L1 = 18$<br>Domain size = 1                                                                                                                                                                                                                                                                            |
| Figure 1(d)  | Total_amount of substrate = 10<br>$k_1 = 10$<br>$k_{-1} = 0.1$<br>$k_2 = 1.5$<br>$k_3 = 2$<br>$k_{-3} = 0.2$<br>$k_4 = 0.4$<br>$E_1^{Total} = 10$<br>$E_2^{Total} = 1$<br>$D_X = D_{X^*} = 0.1$<br><br>$L/L1 = 18$<br>Domain size = 1<br><b>Note:</b> The sequestration in the intermediate space is accounted for exactly, in both the basal and perturbed cases, using the numerical solution of the PDE. |

|                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 2(a), (b) | <p>Total amount of substrate = 2<br/> <math>k_1 = 1</math> (basal); <math>k_1 = 100</math> (perturbed)<br/> <math>k_2 = 2</math><br/> <math>D_X = 0.05</math><br/> <math>D_{X^*} = 0.1</math></p> <p><math>L/L_1 = 0.5</math><br/> Domain size = 1<br/> <b>Note:</b> The sequestration in the intermediate space is accounted for exactly, in both the basal and perturbed cases, using the analytical solution.</p>                                                                                                                                                                                                                                                                                                                  |
| Figure 2(c)      | <p>Total amount of substrate = 10<br/> <math>k_1 = 1</math> (basal); <math>k_1 = 100</math> (perturbed)<br/> <math>k_{-1} = 0.1</math><br/> <math>k_2 = 300</math><br/> <math>k_3 = 2</math><br/> <math>k_{-3} = 0.2</math><br/> <math>k_4 = 400</math><br/> <math>E_1^{Total} = 1</math><br/> <math>E_2^{Total} = 1</math><br/> <math>D_X = D_{X^*} = 0.1</math></p> <p><math>L/L_1 = 0.5</math><br/> Domain size = 1<br/> <math>D/(L_1 * L) = 1.25</math><br/> Transport parameter = 0.54 (basal); 0.68 (perturbed)<br/> (Computed by minimizing error)<br/> <b>Note:</b> The sequestration in the intermediate space is accounted for exactly, in both the basal and perturbed cases, using the numerical solution of the PDE.</p> |

|                                             |                                                                                                                                                                                                                           |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 3(a)                                 | <p>Total amount of substrate = 2<br/> <math>k_2 = 2</math><br/> <math>D_X = 0.1</math><br/> <math>D_{X^*} = 0.1</math></p> <p><math>L/L_1 = 1, 0.1</math><br/> Domain size = 1</p>                                        |
| Figure 3(b)                                 | <p>Total amount of substrate = 2<br/> <math>k_1 = 10</math><br/> <math>k_2 = 2</math><br/> <math>D_X = 0.1</math><br/> <math>D_{X^*} = 0.1</math></p> <p><math>L/L_1 = 18</math><br/> Domain size = 1</p>                 |
| Figure 3(c) and Figure 3(d)                 | <p>Total amount of substrate = 2<br/> <math>k_1 = 10</math><br/> <math>k_2 = 2</math><br/> <math>D_X = 0.1</math><br/> <math>D_{X^*} = 0.1</math></p> <p>Vary L, keeping D/L constant<br/> Domain size = 1</p>            |
| Figure 4(b) (Simple open pathway)           | <p><math>k_0 = 1</math><br/> <math>D_X = 0.1</math></p> <p><math>L/L_1 = 1, 0.1</math><br/> Domain size = 1</p>                                                                                                           |
| Figure 4(d) (Three compartment open system) | <p><math>k_0 = 1</math> (Compartment 1)<br/> <math>k_1 = 1</math> (Compartment 1)<br/> <math>k_2 = 0.1</math> (Compartment 2)<br/> <math>D_X = 0.1</math><br/> Equal sized compartments, <math>L_1=L_2=L_3=1/3</math></p> |
|                                             | Equally spaced, separation = 1/15                                                                                                                                                                                         |

|                                     |                                                                                                                                                        |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 5(b) (Two-site modification) | Mass action limit<br>$k_1 = 1.5$<br>$k_2 = 0.02$<br>$k_3 = 0.1$<br>$k_4 = 2$<br>$D_X = D_{X^*} = D_{X^{**}} = 0.01$<br>Domain size = 1<br>$L/L1 = 0.5$ |
| Figure 5(c) (Two-site modification) | Mass action limit<br>$k_1 = 1.5$<br>$k_2 = 2$<br>$k_3 = 0.05$<br>$k_4 = 1$<br>$D_X = D_{X^*} = D_{X^{**}} = 0.01$<br>Domain size = 1<br>$L/L1 = 0.5$   |
| Figure 5(d) (Two-site modification) | Mass action limit<br>$k_1 = 1.5$<br>$k_2 = 2$<br>$k_3 = 0.05$<br>$D_X = D_{X^*} = D_{X^{**}} = 0.01$<br>Domain size = 1<br>$L/L1 = 0.5$                |

|                      |                                                                                                                                                                                                                                                                                                                                                 |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 5 (e) and (f) | Total amount in whole domain = 10<br>$k_1 = 45$<br>$k_{-1} = 0.5$<br>$k_2 = 4$<br>$k_3 = 125$<br>$k_{-3} = 0.5$<br>$k_4 = 80$<br>$k_5 = 15$<br>$k_{-5} = 0.05$<br>$k_6 = 1$<br>$k_7 = 25$<br>$k_{-7} = 0.05$<br>$k_8 = 3.5$<br>$E_1^{Total} = 0.6$<br>$E_2^{Total} = 5$<br>$D_X = D_{X^*} = D_{X^{**}} = 0.1$<br>$L/L1 = 18$<br>Domain size = 1 |
| Figure 6(b)          | $k_1 = 0.02$<br>$k_{21} = 0.1$<br>$k_{22} = 1$<br>$k_3 = 0.5$<br>$E^{Total} = 1$<br>$F^{Total} = 1$<br>$D_X = 0.05$                                                                                                                                                                                                                             |
|                      | $L1=L2=2$<br>$L=0.1$                                                                                                                                                                                                                                                                                                                            |

|             |                                                                                                                                                                       |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 6(c) | $k_1 = 0.02$<br>$K = 0.01$<br>$k_{21} = 0.1$<br>$k_{22} = 10$<br>$k_3 = 0.001$<br>$E^{Total} = 1$<br>$F^{Total} = 2$<br>$D_X = 0.1$<br><br>$l_1=l_2=9/20$<br>$l=0.1;$ |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## Appendix C

# Supplemental Material for Chapter 4

### C.1 Two-node mutual inhibition motif: Simplified model

In order to perform a bifurcation analysis of the two node mutual inhibition motif in the well-mixed configuration, we will use a simplified model, again ignoring leak/degradation of the protein dimers, dimerisation in the channel, and transport of the dimers. Since we are on the steady states, we will use a compartmental description (as described above). With X in compartment 1 and Y in compartment 2, the model used for bifurcation analysis of steady state behaviour, is given by:

$$\begin{aligned}\frac{dX_1}{dt} &= k_{0x} + \frac{k_{1x}\alpha Pt_x}{1 + K_y Y_1^2} + \frac{k_{2x}\alpha Pt_x K_y Y_1^2}{1 + K_y Y_1^2} - k_d X_1 - tr(X_1 - X_2) \\ \frac{dX_2}{dt} &= -k_d X_2 - tr(X_2 - X_1) \\ \frac{dY_1}{dt} &= -k_d Y_1 - tr(Y_1 - Y_2) \\ \frac{dY_2}{dt} &= \frac{k_{1y}\alpha Pt_y}{1 + K_x X_2^2} + \frac{k_{2y}\alpha Pt_y K_x X_2^2}{1 + K_x X_2^2} - k_d Y_1 - tr(Y_2 - Y_1)\end{aligned}$$

As before, we have assumed equal diffusivity for the proteins X and Y - hence the same transport parameter  $p$  appears in all equations. With X and Y co-localised in compartment 1, the model is given by:

$$\begin{aligned}\frac{dX_1}{dt} &= k0_x + \frac{k1_x \alpha P t_x}{1 + K_y Y_1^2} + \frac{k2_x \alpha P t_x K_y Y_1^2}{1 + K_y Y_1^2} - k_d X_1 - tr(X_1 - X_2) \\ \frac{dX_2}{dt} &= -k_d X_2 - tr(X_2 - X_1) \\ \frac{dY_1}{dt} &= \frac{k1_y \alpha P t_y}{1 + K_x X_1^2} + \frac{k2_y \alpha P t_y K_x X_1^2}{1 + K_x X_1^2} - k_d Y_1 - tr(Y_1 - Y_2) \\ \frac{dY_2}{dt} &= -k_d Y_2 - tr(Y_2 - Y_1)\end{aligned}$$

In this model, we have three production terms for X, representing (i) expression from a constitutive promoter (this represents an 'input' to the motif (ii) expression from the Y regulated promoter in the unbound state (iii) expression from the same promoter when bound to the Y dimer (transcription factor). For repression, the rate constant of expression for the unbound promoter is much higher than that for the bound promoter ( $k1_x \gg k2_x$ ).

We use bifurcation analysis of the above system to demonstrate the basic role of template concentration and spatial separation in realising bistable behaviour. For this purpose, we fix the ratio of template concentrations of X and Y by fixing  $P t_x$  and  $P t_y$ , and keep this ratio fixed while using  $\alpha$  as a bifurcation parameter. The transport parameter ( $tr = \frac{DA}{LV}$ ) is the other bifurcation parameter in this analysis. All other parameters are kept fixed.

The bifurcation analysis of the simplified model is used to obtain the two-parameter plots (see Figure 3) illustrating the interplay between the template concentration and the separation between compartments (channel length). Similar qualitative trends are observed in simulations of the full PDE model, for both the distributed and co-localised configurations.

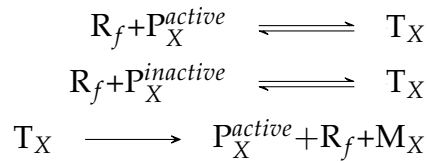
### C.1.1 Modular Augmentation

We use the above model to examine the possibility of a modular augmentation of the two compartment configuration of this motif (with X and Y apart) by connecting this system to a third compartment (see Fig. S2), assuming equal channel lengths. This new compartment (which we connect to the X compartment here) could represent an 'output' node connected to the existing bistable motif, where we have a template that is regulated in some way by the protein X. Since we focus on the steady state behaviour of the system,

we will not introduce this template explicitly. We make the following observations: (i) the behaviour of the motif in the original configuration may be perturbed by the addition of the third compartment, possibly taking it out of the bistable regime (ii) this effect can be mitigated by including a certain amount of X template in the new compartment. In the case examined here, we see that the perturbation introduced by the addition of the new compartment is effectively counteracted by adding a relatively small amount of X template to this compartment (10 percent of the X template present in the original motif).

## C.2 Effect of competition for resources - Fixed total RNAP

The effect of resource competition between different nodes in a genetic activation cascade, including competition for RNAPs and ribosomes, have been examined in detail by Qian et al., 2017. Here we demonstrate both the possible use of compartmentalisation (that decouples the resources) to mitigate these effects, and a resulting trade-off that may result in lower output levels. For simplicity, we focus on a single resource - the RNAP enzyme, for which fix the total concentration (free + bound to template). Other resources are assumed to be non-limiting. We consider a three node activation cascade where X activates Y and X itself is activated by an input I. We consider the following reactions involving the RNAP:



where  $R_f$  is the free RNAP,  $P_X^{active}$  is the promoter activator complex (i.e. bound to the input transcription factor),  $P_X^{inactive}$  is the inactive form of the promoter,  $T_X$  is the transcription complex, and  $M_X$  is the mRNA transcript for X. The rate constants, for binding and unbinding of the free RNAP to form the transcription complex, are chosen to reflect the high binding affinity of the active form relative to the inactive form. We consider the same reactions for the RNAP at the Y node. The total concentration of RNAP, which we keep

fixed, is therefore given by

$$[R]_{total} = [R_f] + [T_X] + [T_Y]$$

The basic effect, which is essentially a biphasic response of the output  $Y$  to increasing concentration of input  $I$ , results from the fact that the nodes  $X$  and  $Y$  (with transcription factor bound to the promoter) compete for the RNAP. We note that, in the context of co-localised nodes in a spatially distributed configuration (for instance the well-mixed compartments), this type of effect could potentially combine with the basic trade-offs that give rise to non-monotonic behaviour (Section 4.5.2).

As discussed in the main text, spatial separation that decouples the resources used by the two nodes is one possible way of mitigating this type of competition effect. However, a trade-off arises in this case due to potential signal attenuation between nodes, depending on the compartment separation and the leaks. This is illustrated in the main text (Figure 5) using computational results obtained for the full model.

### C.3 Localised oscillator in a uniform channel: ‘Predator-Prey’ Oscillator

A predator-prey type oscillatory circuit built using the PEN toolbox has been compartmentalised within water-in-oil compartments (Genot et al., 2016). We use a model of this oscillator to examine the basic effects of localising an oscillator in a uniform channel. A simplified model of this oscillator (Genot et al., 2016) is given by:

$$\begin{aligned}\frac{dn}{dt} &= pol.tem.n(1 - \beta.pol.tem.n) - pol.p.n - \lambda.exo\frac{n}{1+p} \\ \frac{dp}{dt} &= pol.p.n - exo\frac{p}{1+p}\end{aligned}$$

where  $n$  and  $p$  are the ‘prey’ and ‘predator’ concentrations,  $\beta$ ,  $\lambda$  are parameters, and  $tem$ ,  $pol$ , and  $exo$  are the (fixed) concentrations of template, DNA polymerase, and exonuclease respectively. The unstable equilibrium



point is given by (Genot 2016):

$$n_{ss} = \frac{1 + tem - \sqrt{\Delta}/pol}{2(pol.tem^2.\beta + \lambda)}$$

$$p_{ss} = -1 + tem + \frac{\sqrt{\Delta}}{2pol}$$

The Jacobian for this system of ODEs,  $J_{ij}$  may be calculated as follows:

$$J_{11} = pol.tem(1 - 2 * \beta.pol.tem) - pol.p - \frac{\lambda.exo}{1 + p}$$

$$J_{12} = -pol.n + \frac{\lambda.exo.n}{(1 + p)^2}$$

$$J_{21} = pol.p$$

$$J_{22} = pol.n - \frac{exo}{(1 + p)^2}$$

We first analyse the behaviour of this system (with parameters chosen in the oscillatory regime) in a spatial configuration involving two well-mixed compartments, with species allowed to diffuse between the compartments. If the timescale of diffusion is assumed to be much faster than the timescale of reactions, the dynamics can be approximately captured by a compartmental model. Through a bifurcation analysis of this compartmental description, we make the following observations: (i) If the whole system (including degradation) is localised in a single compartment and one of the species is allowed to diffuse between compartments, this can destabilise the oscillations and lead to a steady state where the diffusing species is equal in both compartments. (ii) This destabilisation of the oscillations depends on both the diffusivity and the relative sizes of the compartments. (iii) For fixed compartment sizes, starting at a low diffusivity, increasing diffusivity can de-stabilise the oscillations, leading to a stable steady state (through a Hopf bifurcation). Further increase in diffusivity can de-stabilise this steady state, restoring oscillatory behaviour.

Next, we analyse the behaviour of the analogous system in the uniform 1-D channel with closed boundaries. That is, we localise the whole system (all reactions including degradation) to a compartment in the 1-D domain, and let one of the species diffuse across the whole domain. The kinetic parameters are the same as above. We note that, for these parameter values, if the prey species is allowed to diffuse, while keeping the predator species non-diffusible, then the system exhibits a Type 1 Turing bifurcation. However,

since pattern forming behaviour is not the focus of this study, we examine the alternative case, where the predator diffuses and the prey does not. In this case, depending on the size of the compartment relative to the domain, we observe the same qualitative trends as above, for the effect of diffusivity on oscillatory behaviour. Computational results illustrating these trends are shown in Figure 6, and discussed in the main text. We also note that we observed similar behaviour with respect to changing diffusivity and compartment size, in the same spatial configuration (both uniform channel and well-mixed compartments) for other models of chemical oscillators, including (i) the transcription-translation based activator-inhibitor oscillator used in this study (assuming equal degradation of protein and dimer within the compartment), (ii) the Brusselator model (Kuznetsov, 2004), (iii) the Oregonator model (Mazzotti, Morbidelli, and Serravalle, 1995).

## C.4 Retroactive effect in a uniform 1-D channel:

To demonstrate the basic trends associated with spatial organisation and the retroactive effect of an output protein binding to a downstream promoter, we examine a two-node system in a uniform 1-D channel, with closed boundaries and uniform degradation (Fig. S3). The protein  $X$  is produced in a compartment at location  $L_1$ , and diffuses across the domain. The template for the node  $Y$  is localised in a compartment at location  $L_2$ .  $X$  binds with the promoter sites on the  $Y$  template to form a complex  $P_X$ . We consider a production rate of  $X$  that varies sinusoidally in time, and examine the dynamics of the protein  $X$  and the complex, as in Del Vecchio, Ninfa, and Sontag, 2008. The model is as follows:

In the X compartment

$$\frac{\partial X}{\partial t} = kx - k_d X + D_X \frac{\partial^2 X}{\partial \theta^2}$$

Outside the two compartments

$$\frac{\partial X}{\partial t} = -k_d X + D_X \frac{\partial^2 X}{\partial \theta^2}$$

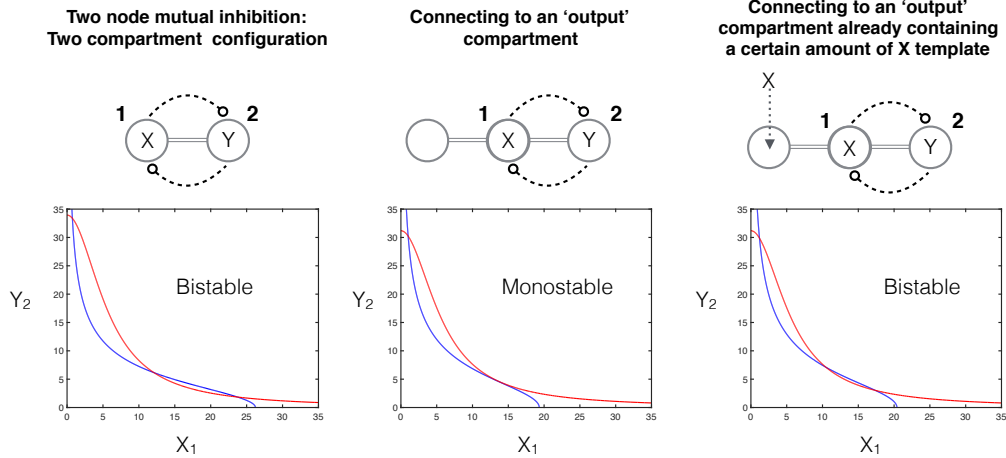
In the Y compartment

$$\begin{aligned} \frac{\partial X}{\partial t} &= -k_b(P_T - P_X)X + k_u P_X - k_d X + D_X \frac{\partial^2 X}{\partial \theta^2} \\ \frac{\partial P_X}{\partial t} &= k_b(P_T - P_X)X - k_u P_X \end{aligned}$$

where  $P_T$  is the total concentration of promoter, and  $k_x = 0.05(1 + \sin(\omega t))$ , with  $\omega = 0.005$ . On examining the dynamic variation of X concentration at its production compartment, we find that binding to the Y promoter distorts both the amplitude and phase of the oscillations, relative to the case where the Y template is absent (X in isolation). We observe that, depending on the diffusivity, this distortion may be reduced, as the Y compartment is moved away from the X compartment (see Fig. S3). For fixed kinetic parameters, this effect is more pronounced at low diffusivity, and is negligible at high diffusivity.

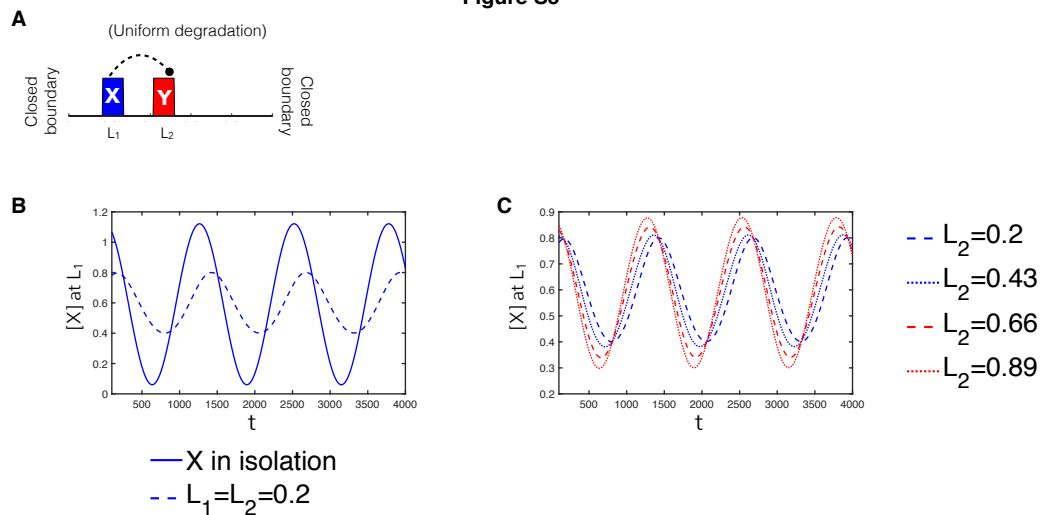
## Modular augmentation

Figure S2



**Figure S2:** Modular augmentation of a two-node distributed bistable circuit (Supplemental section 3.1). The blue and red curves represent null-clines plotted for the simplified model.

Figure S3



**Figure S3:** Effect of spatial separation on retroactivity. **(A)** Schematic of the spatial configuration: uniform 1-D channel with closed boundaries and uniform degradation. X produced (sinusoidal production rate) in a compartment at location L1. X reversibly binds to Y template placed in a second compartment at location L2. **(B)** Distortion in X oscillations at location L1, when Y template is co-localised with X. Both amplitude and phase are affected. **(C)** The distortion caused by the binding to Y template is reduced as the Y compartment is moved away from the X compartment.

## C.5 Parameters

The parameter values used in the study (including those used in the computational results presented) are presented in the table below. The values of transcription, translation, and degradation rate constants are in ranges taken from the literature, while the rate constants of the dimer reactions and the binding/unbinding reactions to the promoters, are assumed to take relatively larger values, as has been done elsewhere in the literature. In these cases, the rates are chosen so that the equilibrium constants are within ranges described in the literature. The concentrations are assumed to be scaled by a factor of 1 nM, and time by a factor of 1 min.

### C.5.1 Spatial Parameters

We assume diffusivities to be of the order of  $10^3 \text{ m}^2/\text{min}$ , as described in the literature (Karzbrun et al., 2014). The well-mixed compartments are assumed to have a radius of  $50 \text{ m}$ , with channel width ranging from 1 to  $10 \text{ m}$ . Lengths are assumed to be scaled by a factor of  $50 \text{ m}$ .

Tables of parameter values used

Well-mixed configuration: Spatial parameters

| Spatial Parameters        | For oscillators | For all other figures |
|---------------------------|-----------------|-----------------------|
| A (Channel cross-section) | 0.2h            | 0.02h                 |
| V (Compartment volume)    | $\pi h$         | $\pi h$               |

h is the 'depth' of the spatial configuration, uniform across compartments and channels.

Figure 2

A

| Node            | X    | Y     |
|-----------------|------|-------|
| pT              | 6    | 10.5  |
| k <sup>b</sup>  | -    | 100   |
| k <sup>u</sup>  | -    | 100   |
| k <sup>i</sup>  | 0.1  | 0.1   |
| k <sup>d</sup>  | 0.02 | 0.02  |
| k <sup>2</sup>  | 10   | 10    |
| k <sup>-2</sup> | 100  | 100   |
| r <sup>0</sup>  | 1.7  | 2E-04 |
| r <sup>TF</sup> | -    | 1.1   |
| r <sup>d</sup>  | 0.1  | 0.1   |
| D               | 0.4  | 0.4   |
| gconst          | -    | -     |

B

| Node            | X    | Y     |
|-----------------|------|-------|
| pT              | 6    | 10.5  |
| k <sup>b</sup>  | -    | 100   |
| k <sup>u</sup>  | -    | 100   |
| k <sup>i</sup>  | 0.1  | 0.1   |
| k <sup>d</sup>  | 0.02 | 0.02  |
| k <sup>2</sup>  | 10   | 10    |
| k <sup>-2</sup> | 100  | 100   |
| r <sup>0</sup>  | 1.7  | 1.1   |
| r <sup>TF</sup> | -    | 2E-04 |
| r <sup>d</sup>  | 0.1  | 0.1   |
| D               | 0.4  | 0.4   |
| gconst          | -    | -     |

C

| Node            | X     | Y     |
|-----------------|-------|-------|
| pT              | 6     | 8     |
| k <sup>b</sup>  | 100   | 100   |
| k <sup>u</sup>  | 100   | 100   |
| k <sup>i</sup>  | 0.1   | 0.1   |
| k <sup>d</sup>  | 0.02  | 0.02  |
| k <sup>2</sup>  | 10    | 10    |
| k <sup>-2</sup> | 100   | 100   |
| r <sup>0</sup>  | 2E-04 | 2E-04 |
| r <sup>TF</sup> | 1.7   | 1.3   |
| r <sup>d</sup>  | 0.1   | 0.1   |
| D               | 0.4   | 0.4   |
| gconst          | 0.3   | -     |

D

| Node            | X     | Y     |
|-----------------|-------|-------|
| pT              | 6     | 8     |
| k <sup>b</sup>  | 100   | 100   |
| k <sup>u</sup>  | 100   | 100   |
| k <sup>i</sup>  | 0.1   | 0.1   |
| k <sup>d</sup>  | 0.02  | 0.02  |
| k <sup>2</sup>  | 10    | 10    |
| k <sup>-2</sup> | 100   | 100   |
| r <sup>0</sup>  | 1.7   | 1.3   |
| r <sup>TF</sup> | 2E-04 | 2E-04 |
| r <sup>d</sup>  | 0.1   | 0.1   |
| D               | 0.4   | 0.4   |
| gconst          | -     | -     |

E,F,G,H

| Node            | X     | Y    |
|-----------------|-------|------|
| pT              | 6     | 10.5 |
| k <sup>b</sup>  | -     | 100  |
| k <sup>u</sup>  | -     | 100  |
| k <sup>i</sup>  | 0.1   | 0.1  |
| k <sup>d</sup>  | 0.02  | 0.02 |
| k <sup>2</sup>  | 10    | 100  |
| k <sup>-2</sup> | 100   | 100  |
| r <sup>0</sup>  | 1.7   | 1.1  |
| r <sup>TF</sup> | 2E-04 | -    |
| r <sup>d</sup>  | 0.1   | 0.1  |
| D               | 0.4   | 0.4  |
| gconst          | -     | -    |

Figure 3

| Node            | X     | Y     |
|-----------------|-------|-------|
| pT              | 6     | 8     |
| k <sup>b</sup>  | 100   | 100   |
| k <sup>u</sup>  | 100   | 100   |
| k <sup>i</sup>  | 0.1   | 0.1   |
| k <sup>d</sup>  | 0.02  | 0.02  |
| k <sup>2</sup>  | 10    | 10    |
| k <sup>-2</sup> | 100   | 100   |
| r <sup>0</sup>  | 1.7   | 1.3   |
| r <sup>TF</sup> | 2E-04 | 2E-04 |
| r <sup>d</sup>  | 0.1   | 0.1   |
| D               | 0.4   | 0.4   |
| gconst          | -     | -     |

Basal Parameter values for bistable mutual inhibition motif. Parameters for simplified model obtained from the above, using a quasi-steady state approximation for dimer formation and binding to promoter.

Figure 4

A to E

| Node     | All nodes |
|----------|-----------|
| $p^T$    | 5, 20     |
| $k^b$    | 100       |
| $k^u$    | 15        |
| $k^i$    | 0.5       |
| $k^d$    | 0.054     |
| $k^2$    | 10        |
| $k^{-2}$ | 1E+03     |
| $r^0$    | 0.4       |
| $r^{TF}$ | 0         |
| $r^d$    | 0.087     |
| <b>D</b> | 0.4       |

Basal Parameter values for repressilator motif (Niederholtmeyer et al. 2015)

| Node     | All nodes |
|----------|-----------|
| $p^T$    | 1         |
| $k^b$    | 100       |
| $k^u$    | 15        |
| $k^i$    | 6.93      |
| $k^d$    | 0.0693    |
| $k^2$    | 10        |
| $k^{-2}$ | 1E+04     |
| $r^0$    | 29.97     |
| $r^{TF}$ | 0.03      |
| $r^d$    | 0.347     |
| <b>D</b> | 0.4       |

Second set of parameter values for repressilator motif (Elowitz 2000, Biomodels Database)

F

| Node     | X     | Y     |
|----------|-------|-------|
| $p^T$    | .4    | 1     |
| $k^b$    | 1000  | 1000  |
| $k^u$    | 100   | 100   |
| $k^i$    | 1     | 1     |
| $k^d$    | 0.2   | 0.2   |
| $k^2$    | 100   | 1E+03 |
| $k^{-2}$ | 1E+03 | 1E+03 |
| $r^0$    | 0.02  | 2E-04 |
| $r^X$    | 5     | 10    |
| $r^Y$    | 2E-04 | -     |
| $r^d$    | 1     | 1     |
| <b>D</b> | 0.4   | 0.4   |

Basal Parameter values for activator-inhibitor oscillator

G

| Node                           | X     | Y    |
|--------------------------------|-------|------|
| $p^T$                          | 2     | 1    |
| $k^b$                          | 100   | 1    |
| $k^u$                          | 100   | 100  |
| $k^i$                          | 0.1   | 0.1  |
| $k^d$                          | 0.02  | 0.02 |
| $k^2$                          | 10    | 10   |
| $k^{-2}$                       | 100   | 100  |
| Transcription rate const.      | 1.7   | 1.1  |
| $r^d$                          | 0.1   | 0.1  |
| <b>D</b>                       | 0.4   | 0.4  |
| $r_{const}$                    | -     | -    |
| Total RNAP                     | 1     |      |
| RNAP binding inactive promoter | 1E-02 |      |
| RNAP binding active promoter   | 10    |      |
| RNAP unbinding                 | 1     |      |

Figure 5

A

| Node        | X                     | Y     |
|-------------|-----------------------|-------|
| $p^T$       | 6                     | 10.5  |
| $k^b$       | -                     | 100   |
| $k^u$       | -                     | 100   |
| $k^i$       | 0.1                   | 0.1   |
| $k^d$       | 0.02                  | 0.02  |
| $k^2$       | 10                    | 10    |
| $k^{-2}$    | 100                   | 100   |
| $r^0$       | 1.7                   | 2E-04 |
| $r^{TF}$    | -                     | 1.1   |
| $r^d$       | 0.1                   | 0.1   |
| <b>D</b>    | 0.1                   | 0.1   |
| $r_{const}$ | -                     | -     |
| Boundaries  | Homogeneous Dirichlet |       |

B

| Node              | X                     |
|-------------------|-----------------------|
| $k_0$             | 1                     |
| $k_d$             | 2                     |
| <b>D</b>          | 0.1                   |
| Domain Size       | 1                     |
| Compartment Size  | 0.1                   |
| Sender Location   | 0.2                   |
| Receiver Location | 0.6                   |
| Boundaries        | Homogeneous Dirichlet |

C,D

| Node              | X                     |
|-------------------|-----------------------|
| $k_0$             | 1                     |
| $k_d$             | 2                     |
| <b>D</b>          | 0.1                   |
| Domain Size       | 1                     |
| Compartment Size  | 0.1                   |
| Sender Location   | 0.4                   |
| Receiver Location | 0.2                   |
| Boundaries        | Homogeneous Dirichlet |

F

| Node             | X                                 |
|------------------|-----------------------------------|
| $k_0$            | 0.5                               |
| $k_d$            | 1                                 |
| <b>D</b>         | 1e-4 to 10                        |
| Domain Size      | 1                                 |
| Compartment Size | 0.1                               |
| Boundaries       | No-flux and Homogeneous Dirichlet |

G

| Node             | X          |
|------------------|------------|
| $k_0$            | 0.5        |
| $k_d$            | 1          |
| <b>D</b>         | 1e-4 to 10 |
| Domain Size      | 1          |
| Compartment Size | 0.1        |
| Boundaries       | No-flux    |

Figure 6

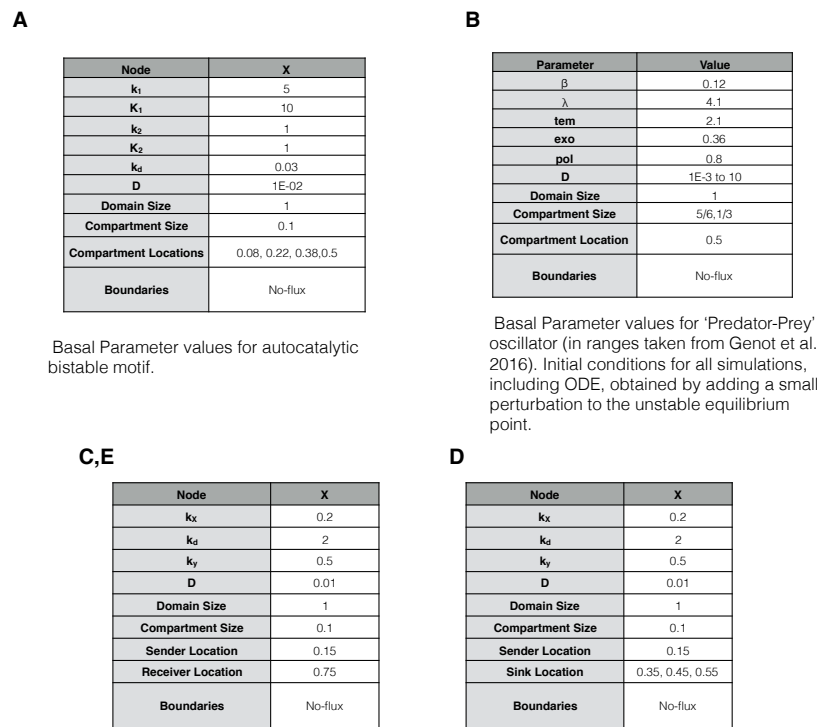


Figure 7

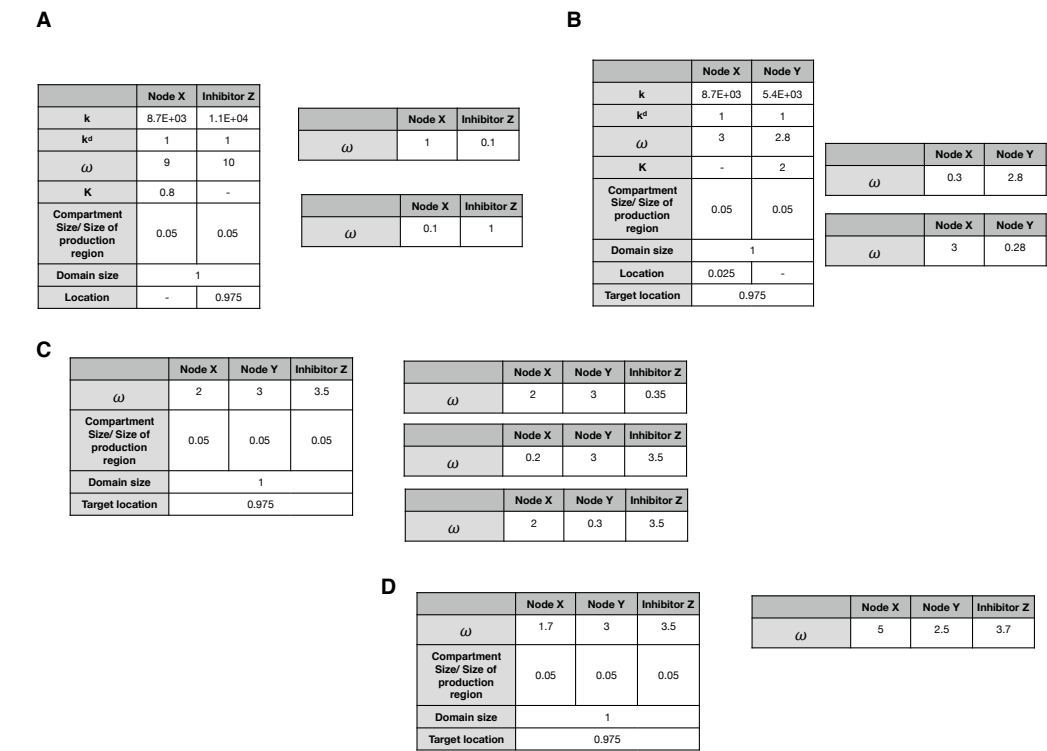


Figure 7

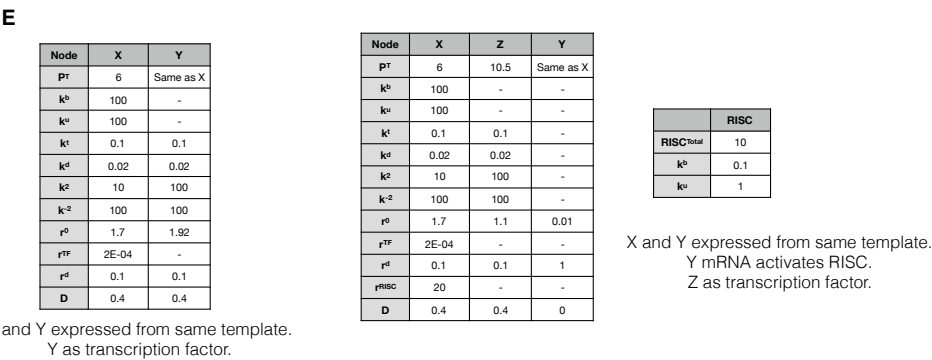


Figure S1

A

| Node           | X                   |
|----------------|---------------------|
| k <sub>1</sub> | 4                   |
| K <sub>1</sub> | 100                 |
| k <sub>2</sub> | 100                 |
| K <sub>2</sub> | 0.1                 |
| k <sub>d</sub> | 0.1, 0.15, 0.2, 0.3 |

D

| Node           | X               | Y    |
|----------------|-----------------|------|
| k              | 0.05            | 0.02 |
| K              | 0.08            | 0.08 |
| k <sub>d</sub> | 0.02, 0.08, 0.4 |      |

Figure S2

| Node            | X     | Y     |
|-----------------|-------|-------|
| P <sup>T</sup>  | .4    | 8     |
| k <sup>b</sup>  | 100   | 100   |
| k <sup>u</sup>  | 100   | 100   |
| k <sup>t</sup>  | 0.1   | 0.1   |
| k <sup>d</sup>  | 0.02  | 0.02  |
| k <sup>2</sup>  | 10    | 10    |
| k <sup>-2</sup> | 100   | 100   |
| r <sup>0</sup>  | 1.7   | 1.3   |
| r <sup>TF</sup> | 2E-04 | 2E-04 |
| r <sup>d</sup>  | 0.1   | 0.1   |
| D               | 0.4   | 0.4   |
| gconst          | -     | -     |
| L               | 0.1   |       |

Figure S3

| Node           | X    |
|----------------|------|
| k <sub>b</sub> | 10   |
| k <sub>u</sub> | 10   |
| P <sub>T</sub> | 100  |
| k <sub>d</sub> | 0.01 |
| D <sub>x</sub> | 0.01 |



| Result                                             | Justification                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Well mixed compartments</b>                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Non-monotonic effects in simple motifs</b>      | Analysis using simplified models (ignoring dimerisation in channel/leak of dimer) demonstrates this behaviour. Computational results for the full model.                                                                                                                                                                                                                                                                                                           |
| <b>Non-monotonic effects with feedback</b>         | Building on the understanding gained from the simple motifs above. Computational results for the full model.                                                                                                                                                                                                                                                                                                                                                       |
| <b>Adaptation to spatial separation</b>            | Analysis using monomeric regulation - clearly demonstrates the effect. Computational results for the full system with dimerisation.                                                                                                                                                                                                                                                                                                                                |
| <b>Bistable motif.</b>                             | Analysis (including 1 and 2 parameter bifurcation analysis) using simplified model explains the qualitative trends. Computational results (including PDE equilibrium continuation) for the full model.                                                                                                                                                                                                                                                             |
| <b>Repressilator</b>                               | Computational results for two different sets of parameters - the original repressilator model parameter values (Biomodels page) and a set of values used in a cell-free realisation of the repressilator (Niederholtmeyer et al. 2015). Analogous results obtained for both sets of parameters.                                                                                                                                                                    |
| <b>Resource sharing</b>                            | We incorporate sharing of RNAP by two nodes. Computational results for the full model. The basic effect of resource sharing shown here - biphasic response - has been demonstrated in other studies (Qian et al. 2017)                                                                                                                                                                                                                                             |
| <b>Uniform Channel</b>                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Non-monotonic effects</b>                       | Building on the understanding gained from well-mixed compartment, now including the effect of the boundary. Analysis of a single 'sender' compartment - analytical solution for a constant production of output and no dimerisation. Analytical solutions for (i) open boundaries with no degradation (ii) open boundaries with uniform degradation (iii) closed boundaries with uniform degradation.                                                              |
| <b>Symmetric locations</b>                         | Obtained using the above analytical solutions.                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Non-monotonic effect of varying diffusivity</b> | Obtained using the above analytical solutions.                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Basic effects of degradation</b>                | Obtained using analytical solutions for a model without dimerisation.                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Localised bistable motif in uniform channel</b> | Analysis of a single node autocatalytic bistable motif (Gines et al. 2017) in (i) a 1-D domain with open boundaries and no degradation (ii) a 1-D domain with closed boundaries and uniform degradation. Computational results for the full PDE model, including numerical bifurcation analysis. Similar qualitative trends are observed for a single node, self-activating TX+TL motif. This is seen using numerical bifurcation analysis for the full PDE model. |
| <b>Localised oscillator in a uniform channel</b>   | Analysis in the well-mixed configuration. Computational results for full PDE system in the 1-D channel. Similar qualitative trends across multiple oscillatory reaction systems, including (i) the activator-inhibitor TX+TL motif used in the study (ii) Ronglez' predator prey oscillator (using model) (iii) the Oregonator model (iv) the Brusselator model (v) a chemical oscillator (Strogatz 1994)                                                          |

TABLE C.1: Summary of supporting analysis and computational results.



## Appendix D

# Supplemental Material for Chapter 5

**Note:** In all cases, the total amounts at each node, are equal to one, unless mentioned otherwise. Similarly, the size of the spatial domain is assumed to be one, unless mentioned otherwise.

FIG. 4

A : SIMPLE ACTIVATION  $X \rightarrow Y$

$k_x = 1, k_{xy} = 1, k_{-x} = 0.1, k_{-y} = 0.5$ , Size of localised subdomain:  $P_w = 0.2$ ,  $X_t = 1, D_x = 1$ , Location of the centre of the localised subdomain:  $P_1 = 0.5, P_2 = 0.125$ .

B

$k_x = 1, k_{xy} = 1, k_{-x} = 0.1, k_{-y} = 0.5$ , Size of localised subdomain:  $P_w = 0.2$ ,  $X_t = 1, D_x = 1$ , Location of the centre of the localised subdomain:  $P = 0.11 - 0.89$ .

C

$k_x = 1, k_{-x} = 0.5, k_{xy} = 1, k_{-y} = 0.2, D_y = 0.1$ . Size of localised subdomain (placed at centre):  $P_w = 0.2$ , Signal:  $S = 1 + 0.75\cos(2 * \pi * \theta + \phi)$

D

## MUTUAL INHIBITION

## X LOCALIZED

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_x = k_{-yx} = k_y = k_{-xy} = 1, D_y = 0.1$ .

## E

## MUTUAL ACTIVATION

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_{yx} = 1, k_{-x} = 0.1, k_{xy} = 1, k_{-xy} = 0.1, D_x = D_y = 1e - 2$ . X activation of Y localised.

## F

## MUTUAL INHIBITION

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_x = k_{-yx} = k_y = k_{-xy} = 1, D_x = D_y = 1e - 2$ . X inhibition of Y localised.

## G

## NF

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_x = k_{-yx} = k_y = k_{-xy} = 1, D_x = D_y = 1e - 2$ . One of activation or inhibition localised in each case.

## FIG. 5

## A

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_{sx} = 0.04, k_{xy} = 1.8, X_t = Y_t = 1, k_{xy} = 0.8, k_{xy} = 0.4, D_x = D_y = 0.001|0.005|0.01$ .

## B

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_{sx} = 0.04, k_{xy} = 1.8, X_t = Y_t = 1, k_{xy} = 0.8, k_{xy} = 0.4, D_x = D_y = 0.001, S = 0.25$ .

C

Size of localised subdomain (placed at centre):  $P_w = 0.2, k_{sx} = 0.04, k_{xy} = 1.8, X_t = Y_t = 1, k_{xy} = 0.8, k_{xy} = 0.4, D_x = D_y = 0.01, S = 2.5$ .

D

MUTUAL ACTIVATION - LOCATIONS:  $\theta = 0.1, 0.2, 0.5$

Size of localised subdomain:  $p_w = 0.2, k_{xy} = 1.8, K_{xy} = 0.4, k_x = 0.01, k_{-y} = 1, k_{sy} = 4e - 2, k_{yx} = 0.8, K_{yx} = 0.2, D_x = 1e - 2, k_{-x} = 0.25$ .

E

MUTUAL ACTIVATION - INTERACTIONS LOCALISED AT LOCATIONS:  $\theta = 0.2, 0.8$

Size of localised subdomain:  $p_w = 0.2, k_{xy} = 1.8, K_{xy} = 0.4, k_x = 0.01, k_{-y} = 0.2, k_{sy} = 4e - 2, k_{yx} = 0.8, K_{yx} = 0.2, D_x = 0.25, D_y = 0.25, k_{-x} = 0.1$ .

FIG. 6

A

NEGATIVE FEEDBACK - INTERACTIONS LOCALISED AT LOCATIONS:  $\theta = 0.25, 0.75$

Size of localised subdomain:  $p_w = 0.2, k_x = k_y = 1e - 3, K_{sx} = 0.1, K_{yx} = 1e - 3, k_{-yx} = 100, k_{xy} = 0.02, k_{xy} = 10, k_{-y} = 1$ .

B

Size of localised subdomain:  $p_w = 0.2, k_{sx} = 0.5, k_{sy} = 0.6, k_{xz} = 100, k_{-x} = 10, k_{-y} = 10, k_{yz} = 100, D_z = 0.01$ .

FIG. 7

A

Size of localised subdomain (placed at centre):  $P_w = 0.2$ ,  $k_x = 0.2$ ,  $k_{sx} = 1$ ,  $k_{-zx} = 6$ ,  $k_{-zx} = 0.01$ ,  $n = 4$ ,  $p_w = 1/3$ ,  $k_{xy} = 1$ ,  $K_{xy} = 0.2$ ,  $k_{-y} = 1$ ,  $k_{yz} = 2$ ,  $K_{yz} = 0.01$ ,  $k_{-z} = 1$ ,  $D = 1e - 3|1e - 1|10$ .

B,C

MUTUAL INHIBITION

Size of localised subdomain:  $P_w = 0.2$ ,  $k_x = k_y = 1$ ,  $k_{-xy} = 10$ ,  $k_{-xy} = 1$ ,  $k_{-yx} = 200$ ,  $K_{-yx} = 30$ ,  $D_x = D_y = 1e - 2$ . Activation interactions localised at two ends of the domain.

FIG. 8

A

Size of localised subdomain (placed at centre or at one end):  $P_w = 0.2$ ,  $k_x = 0.2$ ,  $k_{sx} = 1$ ,  $k_{-zx} = 6$ ,  $k_{-zx} = 0.01$ ,  $n = 4$ ,  $p_w = 1/3$ ,  $k_{xy} = 1$ ,  $K_{xy} = 0.2$ ,  $k_{-y} = 1$ ,  $k_{yz} = 2$ ,  $K_{yz} = 0.01$ ,  $k_{-z} = 1$ ,  $D_x = D_y = 0.005$ .

B

2 NODE MUTUAL ACTIVATION WITH LOCALIZED FEEDFORWARD INHIBITION BY Y

Size of localised subdomains:  $P_w = 0.1$ , placed at  $\theta = 0.25, 0.75$   $k_x = k_z = k_y = 0.5$ ,  $n = 4$ ,  $k_{zx} = 10$ ,  $k_{xz} = 100$ ,  $K_{zx} = K_{xz} = 0.5$ ,  $k_{-yx} = 20$ ,  $k_{-zx} = 1$ .

FIG. 9

A

$k_{yx} = 2$ ,  $k_{yx} = 0.05$ ,  $k_{-x} = 1.5$ ,  $k_{xy} = 0.1$ ,  $k_{-zy} = 3$ ,  $k_x = k_y = k_z = k_{-y} = 1e - 3$ ,  $k_{yz} = 0.5$ ,  $K_{yz} = 0.1$ ,  $K_{-z} = 0.08$ ,  $D_x = D_y = D_z = 1$ ,  $p_w = 0.2$ ,  $L = 8$ ,  $location = 0.5$ .

B

$k_{yx} = 2, k_{yx} = 0.05, k_{-x} = 1.5, k_{xy} = 0.1, k_{-zy} = 3, k_x = k_y = k_z = k_{-y} = 1e - 3, k_{yz} = 0.5, K_{yz} = 0.1, K_{-z} = 0.08, D_x = D_y = D_z = 1, p_w = 1/5, L = 8, location = 0.5.$

C

$k_{yx} = 2, k_{yx} = 0.05, k_{-x} = 1.5, k_{xy} = 0.1, k_{-zy} = 3, k_x = k_y = k_z = k_{-y} = 1e - 3, k_{yz} = 0.5, K_{yz} = 0.1, K_{-z} = 0.08, D_x = D_y = D_z = 1, p_w = 1/5, L = 8,$   
(location at the edge).

D

$k_{sx} = input, k_{-x} = 0.5, k_x = k_y = k_z = k_w = 1e - 3, k_{-zx} = 6, K_{-zx} = 0.01, k_{yx} = 10, K_{yx} = 0.01, k_{xy} = 1, K_{xy} = 0.02, K_{-y} = 1, k_{yz} = 2, K_{yz} = 0.01, k_{-z} = 1, k_{zw} = 2, K_{zw} = 0.01, D = 0.001$  (for all species),  $P_w = 1/3, k_{-w} = 1.$

E

$k_{sx} = input, k_{-x} = 0.5, k_x = k_y = k_z = k_w = 1e - 3, k_{-zx} = 6, K_{-zx} = 0.01, k_{yx} = 10, K_{yx} = 0.01, k_{xy} = 1, K_{xy} = 0.02, K_{-y} = 1, k_{yz} = 2, K_{yz} = 0.01, k_{-z} = 1, k_{zw} = 2, K_{zw} = 0.01, D = 0.001$  (for all species),  $P_w = 1/3, k_{-w} = 1.$

F, G, H

Same as D or E, but with the positive regulation of X by Y localised in a subdomain of size 0.2 at the centre.

FIG. 10

A

$k_{zx} = 2, k_{-yx} = 5, K_{-yx} = 0.1, k_{zy} = 10, k_{-xy} = 4, K_{-xy} = 4, k_{zg} = 1, k_{-g} = 1, k_{gh} = 7.5, k_{-h} = 20, s = 0.1 - 0.5 - 1, P_w = 0.2, location = 0.3, D_x = D_y = 1, D_h = 100.$

B

$k_{zx} = 2, k_{yx} = 5, K_{yx} = 0.1, k_{zy} = 10, k_{xy} = 4, K_{xy} = 4, k_{zg} = 1, k_{-g} = 1,$   
 $k_{gh} = 7.5, k_{-h} = 20, \text{Graded input } S = 0.5 + 0.4 * \sin(2\pi\theta), \text{location} = 72, 72,$   
 $D_x = D_y = 1, D_h = 100.$

C

$k_{zx} = 2, k_{yx} = 5, K_{yx} = 0.1, k_{zy} = 10, k_{xy} = 4, K_{xy} = 4, k_{zg} = 1, k_{-g} = 1,$   
 $k_{gh} = 7.5, k_{-h} = 20, s = 0.1 - 0.5 - 1, \text{location} = 72, 72, D_x = D_y = 1,$   
 $D_h = 0.01.$

E

SINGLE NODE WITH LOCALIZED ACTIVATION (USING ANALYTICAL SOLUTION)

$D = 0.005, 0.1, 10, k_1 = [0.01, 1], P_w = 72, \text{located at the centre of the domain},$   
 $k_2 = 0.5, X_T = 1.$

F

$k_1 = 1, k_2 = 1, X_T = 1, D = [1e - 2 \text{ to } 10], L = 10, \text{Location: } P = 0.11,$   
 $P_w = 0.05.$

FIG. 11

A AND B

$k_{yx} = 2, K_{yx} = 0.05, k_{-x} = 1.5, k_{xy} = 0.5, k_{-zy} = 3, k_{yz} = 0.5, K_{yz} = 0.1,$   
 $k_{-z} = 0.08, P_w = 0.44, \text{Together - } [D_y = 1e - 1, 1, 1e4], \text{Apart (at opposite}$   
 $\text{ends of domain- } [D_y = 1e - 4].$

C

$L = 16, k_{xy} = 0.7, k_{-zy} = 3, k_{yx} = 0.5, K_{yx} = 0.05, k_{-x} = 1.5, k_{yz} = 0.5.$   
 $K_{yz} = 0.1, k_{-z} = 0.4, \text{Localized} = 1/3.$



D

Localised activation (size=0.1 of domain), located at 0.12 of the domain.  $D_x = D_y = 1e - 2$ ,  $D_z = 1e - 4$ ,  $L = 48$ ,  $k_{xy} = 0.3$ ,  $k_{-zy} = 3$ ,  $k_{yx} = 2$ ,  $K_{yx} = 0.05$ ,  $k_{-x} = 1.5$ ,  $k_{yz} = 0.5$ ,  $K_{yz} = 0.1$ ,  $k_{-z} = 0.08$ .

FIG. 12

A

$D_x = D_y = D_z = 0.1$ ,  $L = 8$ ,  $k_{xy} = 0.5$ ,  $k_{-zy} = 3$ ,  $k_{yx} = 2$ ,  $K_{yx} = 0.05$ ,  $k_{-x} = 1.5$ ,  $k_{yz} = 0.5$ ,  $K_{yz} = 0.1$ ,  $k_{-z} = 0.1$ ,  $s = 1.75$  or  $1.75 + 0.75 \cos(2 * \pi * \theta / 8)$ .

B

$D_x = D_y = 0.01$ ,  $D_z = 1e - 4$ ,  $D_g = 0$ ,  $k_{xy} = 1$ ,  $k_{-zy} = 5$ ,  $k_{yx} = 2$ ,  $K_{xy} = 0.01$ ,  $k_{-x} = 0.5$ ,  $k_{yz} = 0.05$ ,  $K_{yz} = 0.1$ ,  $k_{-z} = 0.008$ ,  $n = 4$  for yx,  $n = 2$  for yz. Size of localised subdomain for feedback interaction is  $\frac{1}{18}$  of domain size ( $L = 28$ )

C

$D_x = D_y = 0.01$ ,  $D_z = 1e - 4$ ,  $D_g = 0$ ,  $k_{xy} = 1$ ,  $k_{-zy} = 5$ ,  $k_{yx} = 2$ ,  $K_{xy} = 0.01$ ,  $k_{-x} = 0.5$ ,  $k_{yz} = 0.05$ ,  $K_{yz} = 0.1$ ,  $k_{-z} = 0.008$ ,  $n = 4$  for yx,  $n = 2$  for yz,  $D_h = 10$ ,  $k_{gh} = 5$ ,  $k_{-h} = 0.06$  (Localized).

D

$D_x = D_y = 1e - 2$ ,  $D_z = 1$ ,  $D_h = 10$ ,  $k_{xy} = 0.7$ ,  $k_{-zy} = 3$ ,  $k_{yx} = 0.5$ ,  $K_{yx} = 0.05$ ,  $k_{-x} = 1.5$ ,  $k_{yz} = 0.5$ ,  $K_{yz} = 0.1$ ,  $k_{-z} = 0.06$ ,  $k_{yg} = 1$ ,  $K_{yg} = 0.001 / (3^4)$ ,  $k_{gh} = 5$ ,  $k_{-h} = 2.16$ , Spatial cue locations: at 0.58 of the domain and exactlt at the centre. Size of localised subdomains, both for feedback interaction and the spatial cue for setting the pattern, is  $P_w = 0.028$  of the domain.



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