

**Imperial College
London**

**Phase Transitions in the Cell Cytoplasm:
A Theoretical Investigation**

Jean-David Wurtz

Thesis submitted for the degree of Doctor of Philosophy PhD

Department of Bioengineering
Imperial College London
September 2017

Abstract

Biological cells organise their interior into compartments called organelles in order to function. The familiar ones are the mitochondria, the Golgi apparatus and the lysosomes, which are surrounded by a lipid membrane. There are also membrane-less organelles that are currently receiving intense attention from the biology and physics communities. Membrane-less organelles are ubiquitously present, from yeast cells to mammalian cells, and play key roles in biological functions. One of these are the stress granules (SG) that form in the cytoplasm when the cell is under stress, and are indispensable to the cell's survival. Membrane-less organelles are proteinaceous liquid drops that assemble by phase separation in the cytoplasm. Phase separation under non-equilibrium conditions in the cell cytoplasm is poorly understood as a physical phenomenon, limiting our understanding of membrane-less organelles. In this thesis, we investigate the physics of non-equilibrium phase separation. Specifically, we study a ternary fluid model in which phase-separating proteins can be converted into soluble proteins, and vice versa, via ATP-driven chemical reactions. We elucidate using analytical and simulation methods how drop size, formation and coarsening are controlled by the reaction rates, and categorize comprehensively the qualitative behaviour of the system into distinct regimes. We then apply our formalism to SG formation. Guided by experimental observations, we consider minimal models of SG formation based on phase separation regulated by ATP-driven chemical reactions. We also provide specific predictions that can be tested experimentally. The model studied in this thesis is a minimal model of membrane-less organelle regulation in the cytoplasm, and can also be applied to chemically-controlled drops in emulsions in the engineering setting.

Acknowledgements

I am indebted to my supervisor Dr Chiu Fan Lee for his continuous guidance and patience. I could not have imagined having a better mentor. I am also grateful to my parents for their support throughout my studies. Finally, I would like to thank my friends and colleagues Marta Costa Braga, Jacopo Bono, Margherita Mia Ciano, Nikola Ciganovi, Alice Spellanhour, Antonio Stanziola and Sara Zannone for the countless passionate discussions that have contributed to this thesis.

DECLARATION OF ORIGINALITY

I certify that this thesis constitutes my own work. All material which is not my own work has been properly acknowledged. This thesis was carried out between October 2013 and September 2017 under the supervision of Dr. Chiu Fan Lee in the Department of Bioengineering at Imperial College London UK.

Parts of the chapters 4 and 5 of this thesis have been published in a refereed journal:

J.D. Wurtz and C.F. Lee, “Chemical-reaction-controlled phase separated drops: Formation, size selection, and coarsening,” *Physical Review Letter*, vol. 120, iss. 7, pp. 078102, 2018

Parts of the chapter 6 of this thesis have been submitted to a refereed journal and are under review:

J.D. Wurtz and C.F. Lee, ‘Stress granule formation via ATP depletion-triggered phase separation,’ awaiting referee reports in *New Journal of Physics*.

Parts of this thesis have been presented, as oral or poster presentations, in the following conferences

19th IUPAB congress and 11th EBSA congress (oral and poster presentation)
British Biophysical Society and IOP Institute of Physics
Edinburgh International Conference Centre, Edinburgh, UK
16-20 July 2017

CDT Fluid Dynamics Student Symposium (oral presentation)
Imperial College London, London, UK
12 July 2017

Crick Computational and Physical Biology afternoon workshop (poster presentation)
The Francis Crick Institute, London, UK
5 December 2016

The Physics of Soft and Biological Matter (poster presentation)
IOP Institute of Physics

Homerton College, Cambridge, UK
6-8 April 2016

From Molecules to Systems 2016 Winter School (poster presentation)
St. Catherines College, Oxford University, Oxford, UK
5-8 January 2016

Phase Transitions and Scale Invariance in Biology (oral presentation)
Complex Systems Dynamics (CoSyDy)
Imperial College London, London, UK
28 September 2015

Physics of Living Matter Symposium 10th Edition (poster presentation)
Cambridge, UK
24-25 September 2015

Physics of Emergent Behaviour II from molecules to planets (oral presentation)
IOP Institute of Physics Science Museum, London, UK
9-10 July 2015

COPYRIGHT DECLARATION

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

Contents

1	Introduction	15
1.1	Liquid drops as organelles	16
1.2	Active drop behaviour	17
1.3	Stress granules	18
1.4	Phase separation & chemical reactions	19
1.5	Thesis overview	21
2	Liquid-Liquid Phase Separation in Binary Fluids	22
2.1	Free energy of a homogeneous system	22
2.2	Free energy of a multi-phase system	25
2.3	Two-phase coexistence	26
2.3.1	Flat interface and infinitely large drops	27
2.3.2	Curved interface and finite drops	28
2.4	Maximum number of phases	28
2.5	Phase diagram	29
2.6	Instability and metastability of the homogeneous state	31
2.6.1	Spinodal decomposition	31
2.6.2	Nucleation and growth	31
2.7	Dynamics of a multi-drop system	33
2.7.1	Ostwald ripening	33
2.7.2	Coalescence	36
2.8	Summary & discussion	37
3	Arrest of Ostwald Ripening in Binary Fluids	38
3.1	Volume fractions profiles inside and outside drops	40
3.2	Drop growth and shrinkage	42
3.3	Steady-state drop radius	44
3.4	Linear stability of the steady-state	46
3.5	Summary & discussion	48

4	Active Phase-Separated Drops in a Ternary Fluid	49
4.1	Model of cytoplasmic phase separation	49
4.2	Concentration profiles inside and outside drops	53
4.3	Drop growth, shrinkage and stability	56
4.3.1	Steady-state drop radius	57
4.3.2	Linear stability of the steady-state	58
4.4	Numerical determination of the stability of a multi-drop system . .	59
4.5	Small drops	62
4.5.1	Vanishing drop number density	62
4.5.2	High drop number density	64
4.6	Large drops	65
4.7	Stability diagrams	68
4.8	Comparison with existing results	68
4.9	Summary & discussion	72
5	Monte Carlo Simulations of Phase-Separated Drops	73
5.1	General method	73
5.2	Volume fraction profiles in a single-drop system	74
5.3	Determination of the equilibrium parameters	76
5.4	Relation between physical and simulation units	77
5.5	Stability-instability boundary radius	78
5.6	Comparison between theory and simulations	78
5.7	Summary	78
6	Application of Formalism: Stress Granule Formation	81
6.1	Experimental observations	81
6.2	Minimal models	82
6.3	Dynamics of a multi-drop system	85
6.4	Model selection	85
6.4.1	Model B	85
6.4.2	Model C	86
6.4.3	Model A	88
6.5	Summary & discussion	91
6.6	Predictions	93
7	Conclusion	95
	List of Figures	97

Bibliography	99
Appendix A Arrest of Ostwald Ripening in Binary Fluids	106
A.1 Validity of the small drop approximation	106
Appendix B Active Phase-Separated Drops in a Ternary Fluid	107
B.1 Concentrations controlled by chemical reactions	107
B.2 Linear stability of the steady-state	108
B.3 Passive systems	110
B.4 Chemical reactions cause drops shrinkage and nucleus radius increase	112
B.5 Small drops and vanishing drop number density	114
B.6 Critical forward rate constant k_c	115
B.7 Small drops and high drop number density	116
B.8 Large drops	119
B.9 Jump of the concentration S at the drop interface	122
B.10 Two-dimensional systems	122
Appendix C Application of Formalism: Stress Granule Formation	124
C.1 Difference of size between a stable drop and the nucleus close to the dissolution rate	124
Appendix D Copyright and Permissions	128

Chapter 1

Introduction

To function, a biological cell has to organise its interior contents into compartments called organelles. Typically these structures are enclosed within a lipid membrane, which forms a physical barrier regulating the exchange of the constituents with the exterior. However, there exist many organelles that are *membrane-less*, yet they are dynamically regulated. These structures, composed of proteins and often RNA, include stress granules, centrosomes and P granules contained in the cytoplasm [1], as well as nucleoli and Cajal bodies found in the nucleus [2]. They are present in most organisms, from yeast cells to mammalian cells, and play key biological roles. For example, stress granules promote cell survival when the cell experiences environmental stress by protecting cytoplasmic RNA from degradation [3], and centrosomes form the mitotic spindles in dividing cells [4]. Membrane-less organelles are also associated with neurodegenerative diseases such as amyotrophic lateral sclerosis [5] and Alzheimer disease [6]. Membrane-less organelles have received a lot of attention recently by the scientific community, but the physics associated with these structure has only began to be investigated [7–11]. Membrane-less organelles resemble liquid drops, in that they are spherical, they fuse upon contact when two of them collide and they deform under shearing forces [7, 9]. Recent experiments have provided strong evidence that the intriguing mechanism behind their formation, in the absence of a membrane, is based on the physical phenomenon of phase separation [10, 12–15]. Phase separation refers to the spontaneous partitioning of a system into compartments of distinct macroscopic properties, such as the condensation of water vapour into drops when fog forms. Phase separation, under the equilibrium condition, is a well-understood phenomenon [16]. However, the cell is driven out of equilibrium by a myriad of energy-consuming processes, including adenosine triphosphate (ATP)-driven chemical reactions, that can potentially affect the phase-separating behaviour of the organelles constituents [10, 14, 17, 18]. As

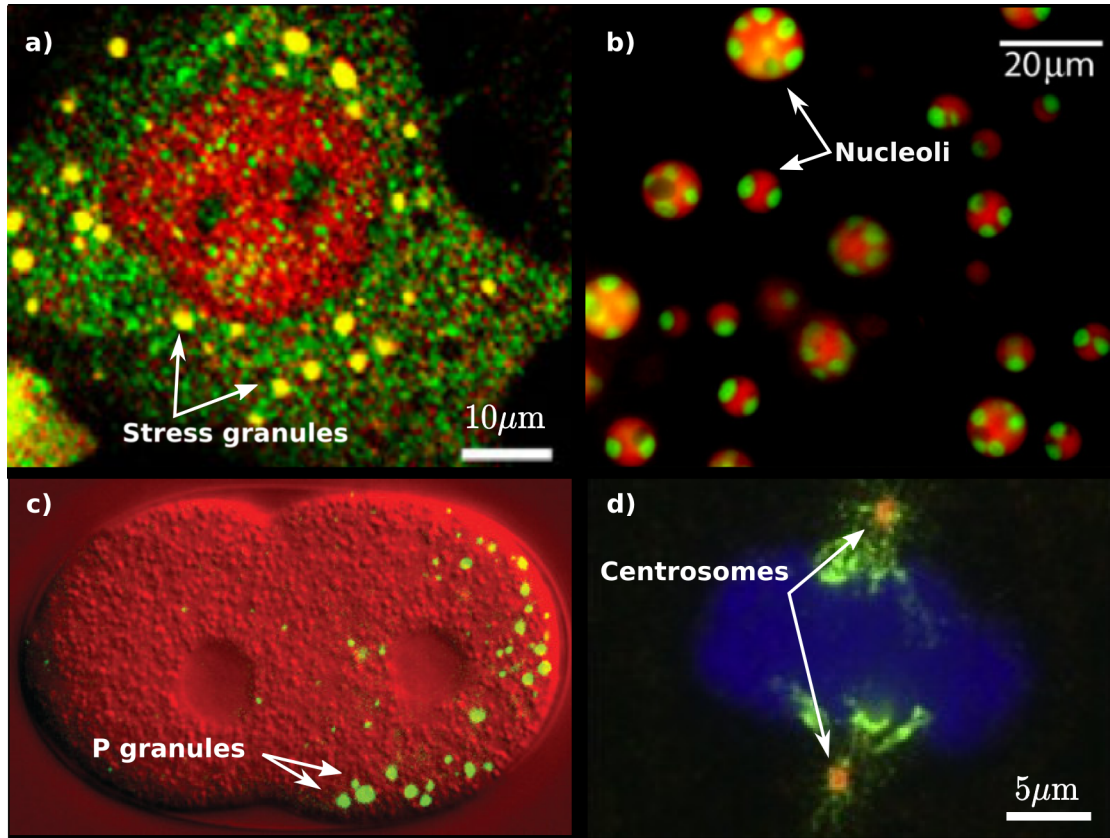


Figure 1.1: *Membrane-less organelles*. a) Stress granules form in the cell cytoplasm when a cell is subjected to environmental stress. Panel modified from Ref. [19]. b) Nucleoli are nuclear organelles involved in ribosomal biogenesis. Panel modified from Ref. [8]. c) P granules localise to the posterior side of the *C. elegans* embryo prior to asymmetric cell division. The embryo is $\sim 50\mu\text{m}$ long. Panel modified from Ref. [7]. d) Centrosomes are the organizing centres of the microtubule network. Panel modified from Ref. [4].

the physics of non-equilibrium phase separation is poorly understood, our understanding of membrane-less organelles is limited. In this thesis, we investigate the physics of non-equilibrium phase separation and apply our findings to the formation of stress granules.

1.1 Liquid drops as organelles

Membrane-less organelles are found both in the cytoplasm [1] and in the nucleus [2]. They are dense and complex aggregates, typically made of dozens of different proteins and RNA [1]. Despite the lack of a membrane, the interior contents are tightly regulated. Membrane-less organelles are liquid drops with a viscosity that is comparable to runny honey, in the case of P granules [7], or significantly larger

for the nucleoli [8] (Fig. 1.1d)). Membrane-less components are not confined in the interior, but rapidly shuttle in and out, with components having a turnover time as short as a few seconds [7, 20, 21].

The formation of membrane-less organelles is dependent on proteins that contain intrinsically disordered regions [3, 22]. This term refers to regions lacking a well defined secondary structure. In vitro, these structures are prone to phase separate into liquid drops [23] and can transition into a gel at high concentrations [24, 25]. The driving force for the protein phase separation is provided by the large number of weakly adhesive elements contained in the disordered regions [26]. The tendency to phase separate increases with the number of interacting sites, and by tuning the protein valence using phosphorylation, it is possible to control the transition from a soluble protein assembly to a phase-separated, droplet state [27, 28]. This provides a potential mechanism for the control of phase-separated liquid drops in vivo. In support of this mechanism, the dissolution of P granules and stress granules can be mediated by phosphorylation [29, 30].

1.2 Active drop behaviour

In a passive system, i.e. a system without energy input, phase separation is a well understood physical phenomenon. For instance in a freshly made vinaigrette, the vinegar forms drops of various sizes dispersed in oil. This system is unstable, and if left alone, the vinegar drops will coarsen until a unique drop remains in equilibrium with the surrounding oil. This coarsening is due to two effects: drops coalesce upon collision [31], and undergo Ostwald ripening which is the process by which small drops evaporate while the larger ones grow [32, 33]. The single-drop state corresponds to the state of minimal energy. To restore a multi-drop state, one must put energy into the system, e.g. by stirring the mixture vigorously. Cells are continually driven out of equilibrium by energy-consuming processes, and membrane-less organelles, display various non-equilibrium behaviours, as we will see in the two examples below.

P granules are membrane-less organelles that localise to the posterior side of the *C.elegans* embryo (Fig. 1.1(a)), prior to asymmetric cell division [34]. This preferential localization depends on the existence of an ATP-dependent concentration gradient of the MEX-5 protein across the cell [7]. A simple mechanism has been proposed to explain this behaviour, where the MEX-5 protein inhibits P granule formation [11]. This leads to a position-dependent phase separation where drops

are created on one side of the cell and dissolve on the opposite side. Even a weak concentration gradient, exploiting the high non-linearity of phase separation, results in the strong segregation of the drops. Therefore, energy-consuming processes drive P granules out of equilibrium to provide a biological function, the asymmetric localisation of P granules in the embryo.

Centrosomes are the organization centre of the microtubule network (Fig. 1.1b)). In the early stage of the cell cycle, two centrosomes are nucleated, each located at a centriole [35]. After a first stage where centrosomes grow in size, both drops end up having a similar size. Zwicker *et al.* [10] have proposed a model that couples protein phase separation to ATP-driven chemical reactions. Specifically, a phase-separating protein is converted to a soluble protein, and vice versa, via phosphorylation-dephosphorylation reactions. One of the virtues of this model is that Ostwald ripening can be arrested [17], allowing the coexistence of two identical stable drops in the system. Hence, as for P granules, centrosomes are driven out of equilibrium by energy-consuming processes, resulting in a biological function.

1.3 Stress granules

Stress granules (SG) are another type of membrane-less organelles that are dynamically regulated, yet we lack a model based on non-equilibrium phase separation for their regulation. SG form quickly, on the order of 10 min, when the cell is under stress (e.g., heat shock, chemical stress, osmotic shock, etc.), and also dissolve away rapidly when the stress is removed [36, 37]. The cell's reaction to external stresses by forming SG is critical for its survival [3, 38, 39]. Although specific functions of SG remain unclear, they are thought to be involved in protecting RNA by recruiting them into SG, away from harmful conditions [20, 40, 41]. In addition, SG malfunction is associated with several degenerative diseases such as amyotrophic lateral sclerosis and multisystem proteinopathy [42].

Stress conditions are often associated with cytoplasmic ATP depletion [43, 44], and it has been shown that ATP depletion alone can trigger SG formation [45]. Additionally, SG dissolution is promoted by ATP-dependent reactions [29, 46, 47]. Together, these observations suggest that SG formation and dissolution may be controlled by the ATP-level. In this thesis, guided by these experimental observations, we will we apply the concept of protein phase separation coupled to ATP-dependent chemical reactions to study SG formation, and propose minimal models.

1.4 Phase separation & chemical reactions

To understand how chemical reactions influence phase separation in the cytoplasm, we consider here chemical reactions converting proteins from a phase-separating state P , to a soluble state S , and vice versa. While states S do not interact, nearby states P form energetic bonds with each others, driving the phase separation. Therefore, the energy U associated to a given protein depends on its state, P or S , but also on the local concentration of P . For a chemical reaction to transform a P into a S , an *activation energy* ΔU needs to be supplied. The higher the P concentration, the larger the number of bonds, and therefore the higher the activation energy ΔU , as shown in the schematic in Fig. 1.2.

The activation energy ΔU can be provided by thermal energy, emanating from molecular agitation, or can be supplied by an external source, like ATP molecules. In the case of thermal energy, the rate constant k of the reaction $P \rightarrow S$ decays exponentially with the activation energy: $k \propto e^{-\Delta U/(k_B T)}$ [48], where k_B is Boltzmann's constant and T is the temperature. Since ΔU increases as the concentration of P increases, the reaction rate constant k is a decreasing function of the concentration of P (lower curve in b)).

In contrast, if another energy source, for example ATP molecules, powers the chemical reaction, the magnitude of the activation energy ΔU is irrelevant if ATP molecules carry enough energy. The reaction rate constant k is therefore independent of the concentration of P (upper line in b)). Therefore, in a phase-separated system containing drops rich in P states, these ATP-driven chemical reactions inject energy preferentially inside the drops, where the activation energy ΔU is the highest (Fig. 1.2a)).

In this thesis we use this reaction scheme to investigate the non-equilibrium physics of phase-separating proteins coupled to ATP-driven chemical reactions in the cytoplasm and apply our formalism to SG formation.

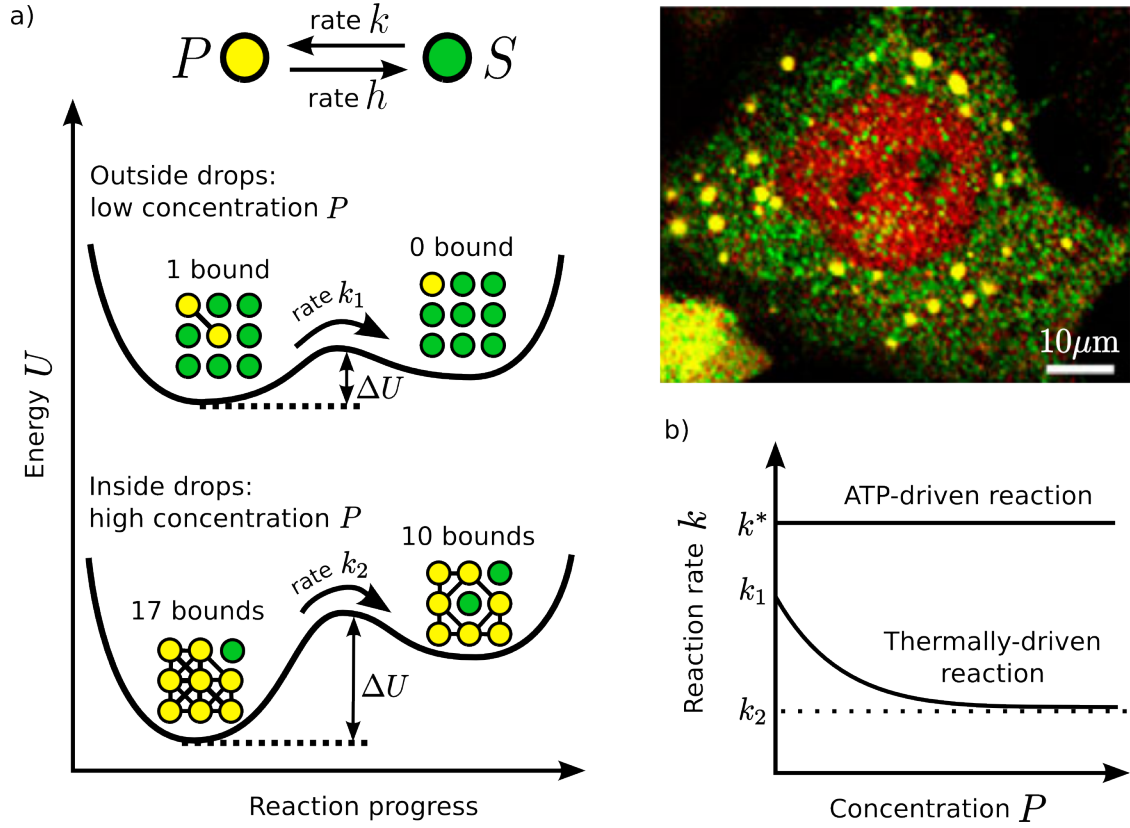


Figure 1.2: *Phase separation with chemical reactions.* a) A phase-separating mixture made of components P (yellow beads) and S (green beads) form drops rich in P . Neighbouring P form bonds (black lines) while S do not interact. For a chemical reaction to convert a P (central molecule in each group) into a S , an activation energy ΔU must be provided to break the bonds. The higher the local concentration in P , the more bonds exist, and the higher the activation energy ΔU . b) If ΔU is provided by thermal energy, the reaction rate constant k decays exponentially with ΔU : $k \propto e^{-\Delta U/T}$ [48], where T is the temperature. Therefore k decreases with the concentration of P (lower curve). In energy-driven reactions, the activation energy ΔU is supplied by another source, such as ATP molecules. The rate constant k can therefore be arbitrary, and in particular it can be concentration independent (upper line).

1.5 Thesis overview

Phase separation under non-equilibrium conditions is exploited by biological cells for the assembly of membrane-less organelles but remains poorly understood as a physical phenomenon. In this thesis we investigate the non-equilibrium physics of phase separation proteins coupled to ATP-driven chemical reactions and apply our formalism to the study of stress granule (SG) formation.

We will now give an outline of this thesis. In Chapter 2, we introduce the basic physical principles of equilibrium phase separation in a binary fluid. In Chapter 3, we present the state-of-the-art understanding of phase-separating systems coupled to ATP-driven chemical reactions. In Chapter 4 we study a ternary fluid model in which phase-separating molecules can be converted into soluble molecules, and vice versa, via ATP-driven chemical reactions. We elucidate how drop size, formation and coarsening can be controlled by the chemical reaction rates, and categorize the qualitative behaviour of the system in distinct regimes via analytical and numerical methods. Our model is a minimal representation of the cell cytoplasm. In Chapter 5 we support our theoretical predictions by simulating our ternary mixture on a 2D lattice using Monte Carlo methods. In Chapter 6 we use the formalism developed in Chapter 4 and, guided by experimental observations on SG, consider minimal models of SG formation based on the mechanism of phase separation regulated by ATP-driven chemical reactions. We identify a minimal model of SG formation triggered by ATP depletion, and provide specific predictions that can be tested experimentally. Finally in Chapter 7, we summarize our findings and discuss potential extension of our work.

Chapter 2

Liquid-Liquid Phase Separation in Binary Fluids

Phase separation refers to the spontaneous partitioning of a system into multiple phases of distinct properties such as concentration. This ubiquitous phenomenon can be observed in our daily life, in oil drop formation in salad dressings for example (Fig. 2.1), or in the separation of milk into liquid whey and solid curdle. In this chapter, we present the fundamental principles of phase separation of an incompressible liquid binary mixture into liquid phases. This is referred to as liquid-liquid phase separation. We will restrict ourselves to passive systems, i.e. isolated systems without energy input.

For a closed system at constant temperature T the thermal equilibrium state is such that the *Helmholtz free energy* F is minimal [16]:

$$F = U - k_B T \ln \Omega , \quad (2.1)$$

where $k_B \ln \Omega$ is the Boltzmann entropy, with Ω the number of possible molecular arrangements leading to that particular state, k_B is the Boltzmann constant, and U is the system potential energy, also depending on the particular state. The free energy F therefore captures a competition between the tendency of a system to increase its entropy while reducing its potential energy.

2.1 Free energy of a homogeneous system

We start by deriving the free energy F of a homogeneous (one phase) binary fluid, composed of elements P and S , on a lattice. N_P , N_S are the number of molecules P and S , respectively, and all lattice sites are occupied, so $M = N_P + N_S$ is the

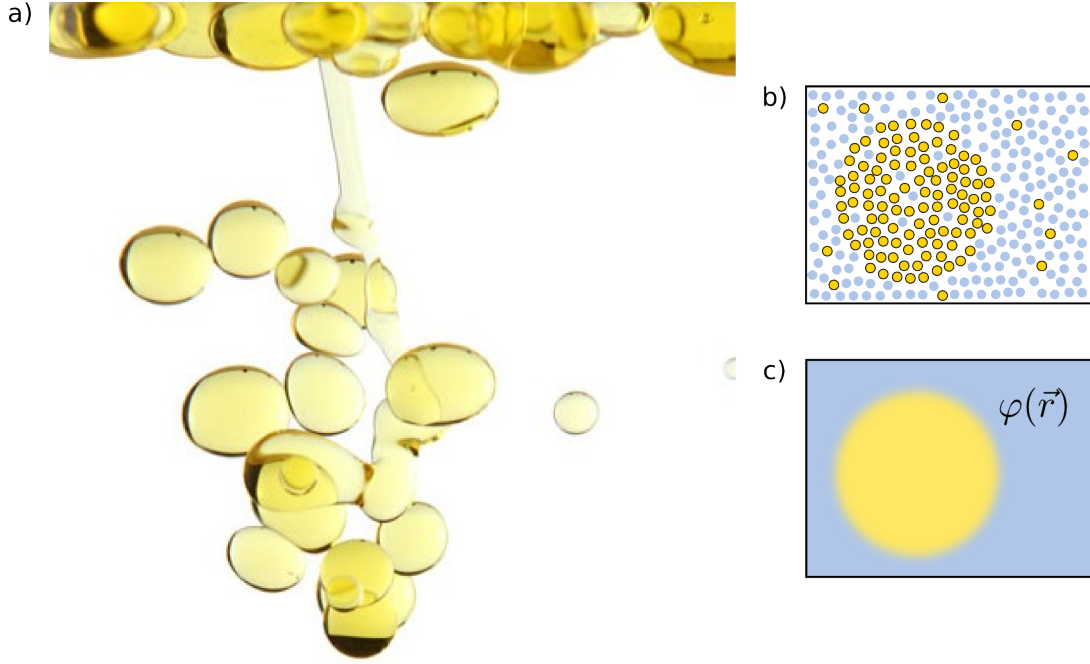


Figure 2.1: a) A mixture of oil and water phase separates into oil-rich drops surrounded by an oil-poor phase. ©Nikola Bilic, Dreamstime.com b) Schematic representation of the distribution of oil molecules (yellow discs) and water molecules (blue discs) inside and outside a drop. c) Continuous representation of the oil volume fraction $\varphi(\vec{r})$ where $\vec{r}$ is the position vector.

number of lattice sites. A molecular pair is defined by two adjacent molecules, and has one of the three possible interaction energies: e_{PP} if the pair is constituted of two P s, e_{SS} if two S s, and e_{PS} if an P and a S form the pair. We restrict ourselves to nearest neighbour interactions. The affinity between two molecules is determined by the sign of the corresponding interaction energy. For example, if e_{PP} is negative then the grouping of P is favoured energetically. These interactions can result from various physical interactions such as van der Waals interactions or entropy driven hydrophobic interactions. We calculate the total potential energy of the system using a mean-field approximation: for every lattice site that is occupied by an P molecule, each of its nearest neighbour has a probability N_P/M or N_S/M to be occupied by an P or a S molecule, respectively. Using the same argument for the sites occupied by S molecules, and neglecting the spatial correlations of the concentrations, we find the total potential energy [49]

$$U = \frac{zM}{2} (e_{PP}\varphi_{\text{tot}}^2 + e_{SS}(1 - \varphi_{\text{tot}})^2 + 2e_{PS}\varphi_{\text{tot}}(1 - \varphi_{\text{tot}})) \quad (2.2)$$

where z is the number of nearest neighbours per lattice site (for exemple $z = 6$ for a cubic lattice) and $\varphi_{\text{tot}} = N_P/M$ is the volume fraction of P and therefore

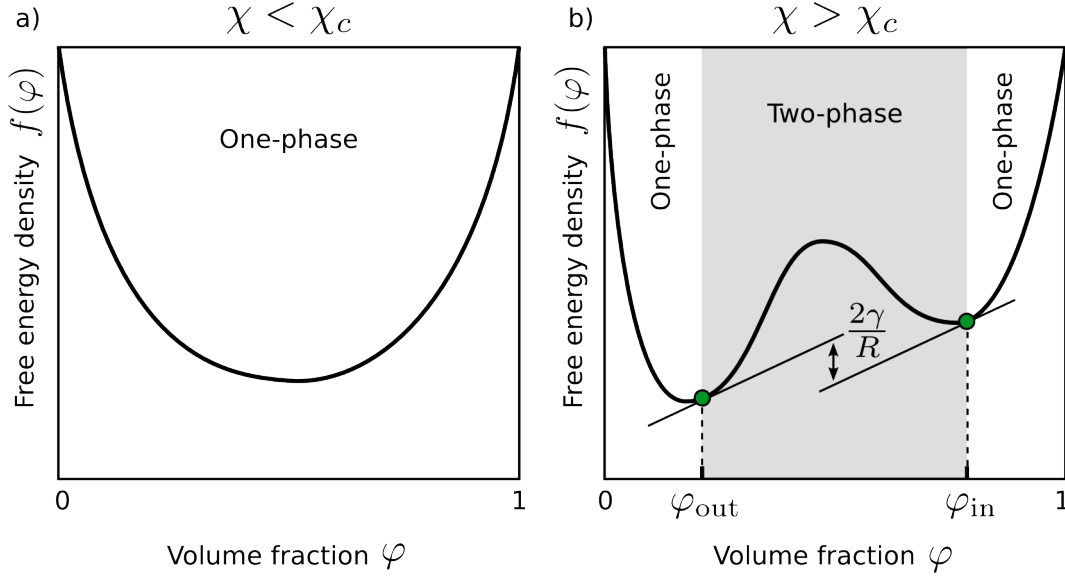


Figure 2.2: *Free energy density of a binary mixture made of components P and S . The shape of the free energy density $f(\varphi_{\text{tot}})$ function of the volume fraction φ_{tot} of P molecules depends on the magnitude of the mixture interaction parameter χ (Eq. (2.4)). For χ smaller than a critical value χ_c , $f(\varphi_{\text{tot}})$ is convex everywhere and the system has a unique phase (a)). For $\chi > \chi_c$, $f(\varphi_{\text{tot}})$ contains both convex and concave regions. This shape allows for the system to separate into two phases of volume fractions φ_{in} and φ_{out} , at the condition that $\varphi_{\text{out}} < \varphi_{\text{tot}} < \varphi_{\text{in}}$ (2.20). In the case where the dense phase forms a spherical drop, $\varphi_{\text{in/out}}$ are determined by the Maxwell tangent construction (Eqs 2.15, 2.16) and depend on the drop radius R and the interface surface tension γ (b)).*

$1 - \varphi_{\text{tot}} = N_S/M$ is the volume fraction of S . The number Ω of possible molecular configurations is the number of ways that N_P molecules can be distributed on M sites:

$$\Omega = \binom{M}{N_P} = \frac{M!}{N_P!(M - N_P)!} . \quad (2.3)$$

Note that the permutations of two molecules of the same type have not been accounted for. This amounts to consider that molecules of a same type are indistinguishable [16]. Using the Stirling's formula ($\ln x! \simeq x \ln x - x$) we find the free energy density:

$$f(\varphi_{\text{tot}}) \equiv \frac{F}{V} \simeq \frac{z}{2\nu} (e_{PP}\varphi_{\text{tot}}^2 + e_{SS}(1 - \varphi_{\text{tot}})^2 + 2e_{PS}\varphi_{\text{tot}}(1 - \varphi_{\text{tot}})) \quad (2.4)$$

$$+ \frac{k_B T}{\nu} (\varphi_{\text{tot}} \ln \varphi_{\text{tot}} + (1 - \varphi_{\text{tot}}) \ln(1 - \varphi_{\text{tot}})) , \quad (2.5)$$

where $V = \nu M$ is the volume of the system with ν the volume of a lattice site.

The mixing free energy density f_{mix} , often preferred, is defined as the free energy difference between the free energy f of the homogeneous state and the free energy of a system that is partitioned into two pure phases ($\varphi_{\text{tot}} = 0$ and $\varphi_{\text{tot}} = 1$) [16]. In the latter case the free energy density is

$$\varphi_{\text{tot}}f(1) + (1 - \varphi_{\text{tot}})f(0) , \quad (2.6)$$

and therefore

$$f_{\text{mix}} = \frac{k_B T}{\nu} [\varphi_{\text{tot}} \ln \varphi_{\text{tot}} - (1 - \varphi_{\text{tot}}) \ln(1 - \varphi_{\text{tot}}) + \chi \varphi_{\text{tot}}(1 - \varphi_{\text{tot}})] \quad (2.7)$$

where $\chi = z(2e_{PS} - e_{PP} - e_{SS})/(2k_B T)$ is the Flory-Huggins interaction parameter [50,51]. The first two terms inside the square brackets in the right hand side are the entropic contribution, minimal when both components are equally mixed ($\varphi_{\text{tot}} = 0.5$). The third term is the potential energy contribution that favour demixing of P and S if $\chi > 0$ or favour mixing if $\chi < 0$.

We show in Fig. 2.2 the free energy density f as a function of the volume fraction φ_{tot} and for different values of the interaction parameter χ . In the absence of molecular interactions ($\chi = 0$) the free energy density is convex. If χ is greater than a positive critical value χ_c , then f contains convex and concave regions, suggesting that the total free energy can be lowered by the formation of additional phases of different volume fractions.

2.2 Free energy of a multi-phase system

We now allow the system to form multiple phases within which the volume fractions are homogeneous. The total free energy can be written as

$$F = \sum_{i=1}^N V_i f(\varphi_i) + F_{\mathcal{I}} , \quad (2.8)$$

where N is the number of phases, V_i is the volume of the i -th phase and φ_i is the volume fraction of molecules P in the i -th phase, respectively. $F_{\mathcal{I}}$ is the free energy of the interface regions that separate adjacent phases, where the volume fraction is not homogeneous. If the interface is sharp, i.e. the variation of volume fraction occurs on a short distance compared to the system size, the interface region can be

approximated by a surface:

$$F_{\mathcal{I}} = \sum_{k,l}^N A_{k,l} \gamma_{k,l} , \quad (2.9)$$

with $A_{k,l}$ and $\gamma_{k,l}$ being the surface area and the free energy per surface area of the interface between the k -th and the l -th phase, respectively. $\gamma_{k,l}$ is usually referred to as the surface tension.

2.3 Two-phase coexistence

We concentrate on a two-phase system, where a phase enriched in P forms a spherical drop of radius R , surrounded by a phase depleted in P . The volume fractions of P inside and outside the drop are φ_{in} and φ_{out} , respectively. From Eqs (2.8) and (2.9) the total free energy is

$$F = \frac{4\pi R^3}{3} f(\varphi_{\text{in}}) + \left(V - \frac{4\pi R^3}{3} \right) f(\varphi_{\text{out}}) + 4\pi R^2 \gamma , \quad (2.10)$$

where V is the total volume of the system and γ is the interface surface tension. The total number of molecules P in the system must be conserved so φ_{in} , φ_{out} and R are constrained by the relation

$$\frac{4\pi R^3}{3} \varphi_{\text{in}} + \left(V - \frac{4\pi R^3}{3} \right) \varphi_{\text{out}} = V \varphi_{\text{tot}} . \quad (2.11)$$

where φ_{tot} is the global volume fraction of P . To find the set $\{\varphi_{\text{in}}, \varphi_{\text{out}}, R\}$ that minimizes the free energy F (Eq. (2.10)) while respecting the constraint in Eq. (2.11), we use the Lagrange multiplier method and we seek for the minima of the unconstrained following quantity:

$$\tilde{F} = F - \lambda \left[\frac{4\pi R^3}{3} \varphi_{\text{in}} + \left(V - \frac{4\pi R^3}{3} \right) \varphi_{\text{out}} \right] , \quad (2.12)$$

where λ is the Lagrange multiplier constant. Taking the derivatives of $\tilde{F}$ with respect to φ_{in} , φ_{out} , R to be zero leads to:

$$f'(\varphi_{\text{in}}) = f'(\varphi_{\text{out}}) \quad (2.13)$$

$$f(\varphi_{\text{in}}) - f(\varphi_{\text{out}}) - f'(\varphi_{\text{in}})(\varphi_{\text{in}} - \varphi_{\text{out}}) = -\frac{2\gamma}{R} . \quad (2.14)$$

The interpretation of these relations becomes clear if we introduce the chemical potential $\mu = \nu \partial f / \partial \varphi$ and the pressure p , related to f and μ from first thermodynamics principles: $f = \varphi \mu / \nu - p$. Eqs (2.13) and (2.14) then become

$$\mu_{\text{in}} = \mu_{\text{out}} \quad (2.15)$$

$$p_{\text{in}} - p_{\text{out}} = \frac{2\gamma}{R}, \quad (2.16)$$

with $\mu_{\text{in/out}} \equiv \nu \partial f / \partial \varphi|_{\varphi_{\text{in/out}}}$ and $p_{\text{in/out}} \equiv \mu_{\text{in/out}} \varphi_{\text{in/out}} / \nu - f(\varphi_{\text{in/out}})$ the chemical potential and pressure inside and outside the drop, respectively. The first relation implies that the chemical potentials are identical inside and outside the drop, and the second relation, known as the Laplace's law, states that the difference of pressure inside and outside the drop is proportional to the surface tension γ and the drop curvature $1/R$ [16].

Using these two relations, the equilibrium concentrations inside and outside the drop, φ_{in} and φ_{out} , can be determined graphically on the $f(\varphi)$ plot, via the *Maxwell construction* (Fig. 2.2 b)). Eq. (2.15) expresses that the tangents of f in φ_{in} and φ_{out} are parallel (see straight lines). Eq. (2.16) expresses that the distance separating these two tangents, in the f axis direction, is equal to $2\gamma/R$ (see double arrow). Note that, in the case where the drop is infinitely large, $2\gamma/R \rightarrow 0$ and a unique line is tangent to f in φ_{in} and φ_{out} simultaneously.

For the conditions Eqs (2.15) and (2.16) to be respected, and therefore for phase separation to be possible, $f(\varphi)$ must contain both concave and convex regions, which is true for a large enough interaction parameter χ .

2.3.1 Flat interface and infinitely large drops

In the limiting case where the drop radius R is infinite, the interface is flat and the surface tension term γ/R in Eq. (2.14) vanishes. In this scenario we denote the volume fractions inside and outside the drop by $\hat{\varphi}_{\text{in}}$ and $\hat{\varphi}_{\text{out}}$, respectively, and Eqs (2.13) and (2.14) become:

$$f'(\hat{\varphi}_{\text{in}}) = f'(\hat{\varphi}_{\text{out}}) \quad (2.17)$$

$$f(\hat{\varphi}_{\text{in}}) - f'(\hat{\varphi}_{\text{in}})\hat{\varphi}_{\text{in}} = f(\hat{\varphi}_{\text{out}}) - f'(\hat{\varphi}_{\text{out}})\hat{\varphi}_{\text{out}}. \quad (2.18)$$

The drop radius R is given by the conservation of the number of molecules (Eq. (2.11)):

$$\frac{4\pi R^3}{3} = \frac{\varphi_{\text{tot}} - \hat{\varphi}_{\text{out}}}{\hat{\varphi}_{\text{in}} - \hat{\varphi}_{\text{out}}} V, \quad (2.19)$$

and since R must be positive, we find the range of volume fractions φ_{tot} allowing phase separation:

$$\hat{\varphi}_{\text{out}} < \varphi_{\text{tot}} < \hat{\varphi}_{\text{in}} . \quad (2.20)$$

2.3.2 Curved interface and finite drops

For a drop or finite size, but still large so that the surface tension term γ/R is small compared to the other quantities in Eq. (2.14), we can expand the volume fractions $\varphi_{\text{in/out}}$ around the volume fractions for a flat interface [49]:

$$\varphi_{\text{in}} = \hat{\varphi}_{\text{in}} + \delta\hat{\varphi}_{\text{in}} \quad (2.21)$$

$$\varphi_{\text{out}} = \hat{\varphi}_{\text{out}} + \delta\hat{\varphi}_{\text{out}} , \quad (2.22)$$

with $\delta\varphi_{\text{in/out}} \ll \hat{\varphi}_{\text{in/out}}$. Expanding Eqs (2.13) and (2.14) to the first order in $\delta\varphi_{\text{in/out}}$ leads to

$$\varphi_{\text{in/out}} \simeq \hat{\varphi}_{\text{in/out}} \left(1 + \frac{l_{c,\text{in/out}}}{R} \right) \quad (2.23)$$

with

$$l_{c,\text{out}} = \frac{2\gamma}{\hat{\varphi}_{\text{out}} f''(\hat{\varphi}_{\text{out}})(\hat{\varphi}_{\text{in}} - \hat{\varphi}_{\text{out}})} \quad (2.24)$$

$$l_{c,\text{in}} = \frac{\hat{\varphi}_{\text{out}} f''(\hat{\varphi}_{\text{out}})}{\hat{\varphi}_{\text{in}} f''(\hat{\varphi}_{\text{in}})} l_{c,\text{out}} . \quad (2.25)$$

$l_{c,\text{in/out}}$ are the capillary lengths. Eq. (2.23) is known as the *Gibbs-Thomson relation* and shows that the presence of a curved interface influence the coexistence volume fractions $\varphi_{\text{in/out}}$. A consequence in a multi-drop system is that the volume fraction outside drops is larger close to small drops than close to large drops. As we shall see this has an important consequence the dynamics of a multi-drop system. Note that in the case of strong phase separation, $\hat{\varphi}_{\text{in}}$ is much larger than $\hat{\varphi}_{\text{out}}$ and $l_{c,\text{in}}$ can be neglected. Therefore the interface curvature mostly affects the volume fraction φ_{out} outside drops.

2.4 Maximum number of phases

We have shown that the formation of two phases with distinct volume fractions can lower the system free energy F (Eq. (2.8)). Here we ask whether F can be lowered

further by the formation of additional phases. Let us therefore consider a system of global volume fraction φ_{tot} of P molecules that contains three phases labelled $i = 1, 2, 3$. The P volume fraction in the i -th phase is noted φ_i . Neglecting the surface tension for simplicity, the free energy F is equal to $V_1 f(\varphi_1) + V_2 f(\varphi_2) + (V - V_1 - V_2) f(\varphi_3)$ with V_i the volume of the i -th phase and V the total volume of the system. The conservation of the total number of molecules P imposes the constraint $\varphi_1 V_1 + \varphi_2 V_2 + \varphi_3 (V - V_1 - V_2) = \varphi_{\text{tot}} V$. Using again the Lagrange multiplier method the constrained minimization of F is equivalent to the unconstrained minimization of the following quantity:

$$\tilde{F} = F - \lambda (\varphi_1 V_1 + \varphi_2 V_2 + \varphi_3 (V - V_1 - V_2)) , \quad (2.26)$$

where λ is the Lagrange multiplier constant. Equating to zero the derivatives of $\tilde{F}$ with respect to $\varphi_1, \varphi_2, \varphi_3, V_1, V_2$ leads to

$$\begin{aligned} f'(\varphi_1) &= f'(\varphi_2) = f'(\varphi_3) \\ f(\varphi_1) - f'(\varphi_1)\varphi_1 &= f(\varphi_2) - f'(\varphi_2)\varphi_2 = f(\varphi_3) - f'(\varphi_3)\varphi_3 \end{aligned} \quad (2.27)$$

These relations imply that a unique line is tangent to $f(\varphi)$ in φ_1, φ_2 and φ_3 simultaneously. This is generically impossible as it requires an infinitely fine tuning of the shape of the free energy density. The formation of three distinct phases in a binary fluid is therefore impossible. This argument can be generalised for an arbitrary number of phases and we conclude that a binary mixture can form at most two distinct phases. The maximal number of phases that can form in a multi-component fluid is related to the number of its components by the *Gibbs phase rule* [52].

2.5 Phase diagram

In a binary fluid, we have shown by minimizing the free energy (Eq. (2.1)), that at thermal equilibrium the system can either be in a one-phase (homogeneous) or in a two-phase state, depending on the volume fraction φ_{tot} of P molecules and the interaction parameter χ . In a two-phase system, the phase volume fractions $\hat{\varphi}_{\text{in}}$ and $\hat{\varphi}_{\text{out}}$ are solutions of Eqs (2.17) and (2.18). We show in the phase diagram in Fig. 2.3 the volume fractions $\hat{\varphi}_{\text{in/out}}$ for different values of the interaction parameter χ . The curves $\hat{\varphi}_{\text{in/out}}(\chi)$ constitute the phase boundary (green curve) inside which the system can phase separate.

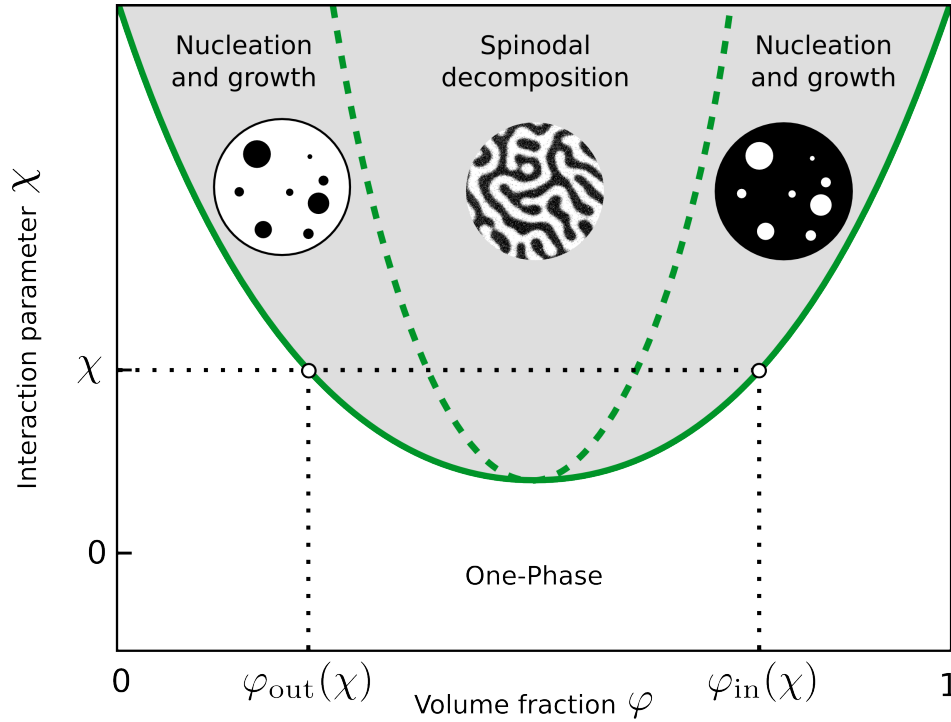


Figure 2.3: Phase diagram of a binary mixture composed of P and S molecules, as a function of the volume fraction φ_{tot} of P and the mixture interaction parameter χ . When the system is inside the phase boundary (green continuous curve) the formation of two phases can occur. For a given value of χ , the phase volume fractions $\varphi_{\text{in/out}}$ are given by the two locations of the phase boundary at the same value χ (see dashed straight lines). In the spinodal decomposition region, enclosed by the green dashed curve the homogeneous state is unstable to infinitesimal perturbations. Therefore phase separation occurs spontaneously throughout the system, leading to a characteristic interconnected phase pattern (see insert). The spherical inserts show systems shortly after the initiation of phase separation, where the black regions represent are the P -rich phase ($\varphi = \varphi_{\text{in}}$) and the white regions the P -poor phase ($\varphi = \varphi_{\text{out}}$). Outside the spinodal decomposition region but still within the phase boundary, is the nucleation and growth regime. Here the homogeneous state is meta-stable, and drop formation is suppressed by an energy barrier. Drops larger than the nucleus overcome this barrier and grow spontaneously, initiating the phase separation.

2.6 Instability and metastability of the homogeneous state

In the previous sections we have investigated the thermal equilibrium state of a binary fluid. Here we consider a homogeneous system that is brought inside the phase-separating region (Fig. 2.3), and we study the instabilities that initiate the phase separation.

2.6.1 Spinodal decomposition

Suppose that in a homogeneous system with volume fraction φ_{tot} of P molecules, local fluctuations of the volume fraction result in the formation of two small domains, labelled $i = 1, 2$, of identical volume δV and volume fractions $\varphi_1 = \varphi_{\text{tot}} + \delta\varphi$ and $\varphi_2 = \varphi_{\text{tot}} - \delta\varphi$ with $\delta\varphi$ positive and infinitesimal. Note that the average volume fraction remains unchanged, as required by the conservation of the total number of molecules P . The change δF of the system total free energy F is:

$$\delta F = \delta V f(\varphi_{\text{tot}} + \delta\varphi) + \delta V f(\varphi_{\text{tot}} - \delta\varphi) - 2\delta V f(\varphi_{\text{tot}}) \quad (2.28)$$

$$\underset{\delta\varphi \rightarrow 0}{=} \delta V (\delta\varphi)^2 f''(\varphi_{\text{tot}}) . \quad (2.29)$$

If $f(\varphi_{\text{tot}})$ is convex ($f'' < 0$) the perturbation is energetically favourable ($\delta F < 0$), and will spontaneously grow. In this mode of phase separation known as *spinodal decomposition*, the mixture is unstable and phase-separated domains will appear throughout the system leading to a characteristic interconnected pattern [53] (Fig. 2.3). The region of spinodal decomposition is bounded by the green dashed curve in the phase diagram (Fig. 2.3).

Outside the spinodal region, but still within the phase boundary, the free energy density $f(\varphi)$ is concave ($f''(\varphi) > 0$) so the fluctuation is energetically unfavourable ($\delta F > 0$), and will decay spontaneously. The homogeneous state is therefore metastable and we show in Section 2.6.2 that an energy barrier must be overcome before phase separation can take place.

2.6.2 Nucleation and growth

We now focus on the phase-separating region of the phase diagram that is outside the spinodal region (Fig. 2.3). We showed in Section 2.6.1 that the homogeneous state in this region is stable against infinitesimal fluctuations. Let us therefore consider finite perturbations of the homogeneous state: suppose the formation of a

small spherical domain of radius R and of volume fraction φ_{in} . The volume fraction outside this domain is only slightly affected and can be written as $\varphi_{\text{tot}} - \delta\varphi$ with $\delta\varphi \ll \varphi_{\text{tot}}$. The change of the free energy F resulting from the domain formation is:

$$\Delta F = \frac{4}{3}\pi R^3 f(\varphi_{\text{in}}) + \left(V - \frac{4}{3}\pi R^3\right) f(\varphi_{\text{tot}} - \delta\varphi) + \gamma 4\pi R^2 - V f(\varphi_{\text{tot}}) \quad (2.30)$$

where γ is the surface tension of the interface between the new domain and the surrounding phase, and V is the system volume. The conservation of the total number of molecules P in the system reads

$$4/3\pi R^3 \varphi_{\text{in}} + (V - 4/3\pi R^3)(\varphi_{\text{tot}} - \delta\varphi) = \varphi_{\text{tot}} V, \quad (2.31)$$

and we find

$$\delta\varphi = \varphi_{\text{tot}} - \frac{V\varphi_{\text{tot}} - 4/3\pi R^3 \varphi_{\text{in}}}{V - 4/3\pi R^3}. \quad (2.32)$$

Expanding the free energy ΔF (Eq. (2.30)) to the first order in $\delta\varphi$, we find

$$\Delta F \simeq \frac{4}{3}\pi R^3 [f(\varphi_{\text{in}}) - f(\varphi_{\text{tot}}) - f'(\varphi_{\text{tot}})(\varphi_{\text{in}} - \varphi_{\text{tot}})] + \gamma 4\pi R^2. \quad (2.33)$$

If the square bracketed term in the right hand side is negative the bulk energy ($\sim R^3$) favours the drop formation. On the other hand, the surface energy ($\sim R^2$) penalises the creation on an interface between the drop and the surrounding phase. For a small drop, the surface energy dominates and the drop growth is energetically unfavourable ($d\Delta F/dR > 0$) leading to the spontaneous drop dissolution. Above a critical radius R_n the bulk energy dominates. The drop growth is thus energetically favourable ($d\Delta F/dR < 0$), leading to further growth by diffusive transport of molecules from the surrounding phase to the drop, initiating the phase separation. The drop growth eventually stops when the depletion of the surrounding phase is such that its volume fraction equates φ_{in} , so that the drop and the surrounding phase are in equilibrium (Eqs (2.13),(2.14)). The radius R_n of the critical drop, also called nucleus, is the solution of $d\Delta F/dR|_{R=R_n} = 0$:

$$R_n = \frac{2\gamma}{f(\varphi_{\text{in}}) - f(\varphi_{\text{tot}}) - f'(\varphi_{\text{tot}})(\varphi_{\text{in}} - \varphi_{\text{tot}})}. \quad (2.34)$$

Note that the energy barrier for drop formation vanishes ($R_n = 0$) when the surface tension γ is zero.

We have shown that in this regime called *nucleation and growth* regime, phase separation can occur at the condition that an energy barrier is overcome by the formation of a nucleus of the dense phase. The drop nucleation, i.e. the formation of a nucleus, can be achieved by random fluctuations of the volume fraction emerging from thermal energy (homogeneous nucleation), or can be assisted by third parties such as impurities that assist the local increase of the volume fraction (heterogeneous nucleation). Typically multiple nuclei appear in the system, and after an initial growth stage, the system is composed of multiple drops of various sizes in equilibrium with the surrounding phase. In Section 2.7 we study the interactions between drops and show that a multi-drop system is always unstable.

2.7 Dynamics of a multi-drop system

Suppose now a phase-separated, multi-drop system. A single-drop configuration is energetically favourable compared to a multi-drop configuration, as it minimizes the total surface energy while keeping the drop volume constant (Eq. (2.10)). Therefore only a single drop can exist at thermal equilibrium. In this section we present the mechanisms that lead to the coarsening of a multi-drop system.

2.7.1 Ostwald ripening

We have seen in Section 2.3.2 that the Gibbs-Thomson relation dictates that the volume fraction outside a small drop is larger than that outside a large drop (Eq. (2.23)). Intuitively we expect that this effect leads to diffusive fluxes feeding the growth of large drops at the expense of small drops (Fig. 2.4 a)). In the case of small supersaturation $\varphi_{\text{tot}} - \hat{\varphi}_{\text{out}}$, the drop density is small (Eq. (2.19)), and drops are in average far from each other. The volume fraction far from drops φ_{∞} can thus be approximated to be homogeneous, and drops interact with each other only via this common far-field. When the spatial gradients of the volume fraction are small, which is assumed to be true inside and outside drops, and far from the interfaces, the dynamics of the volume fraction profile is well approximated by an ideal gas diffusion equation [49]:

$$\frac{\partial \varphi}{\partial t} = D \nabla^2 \varphi . \quad (2.35)$$

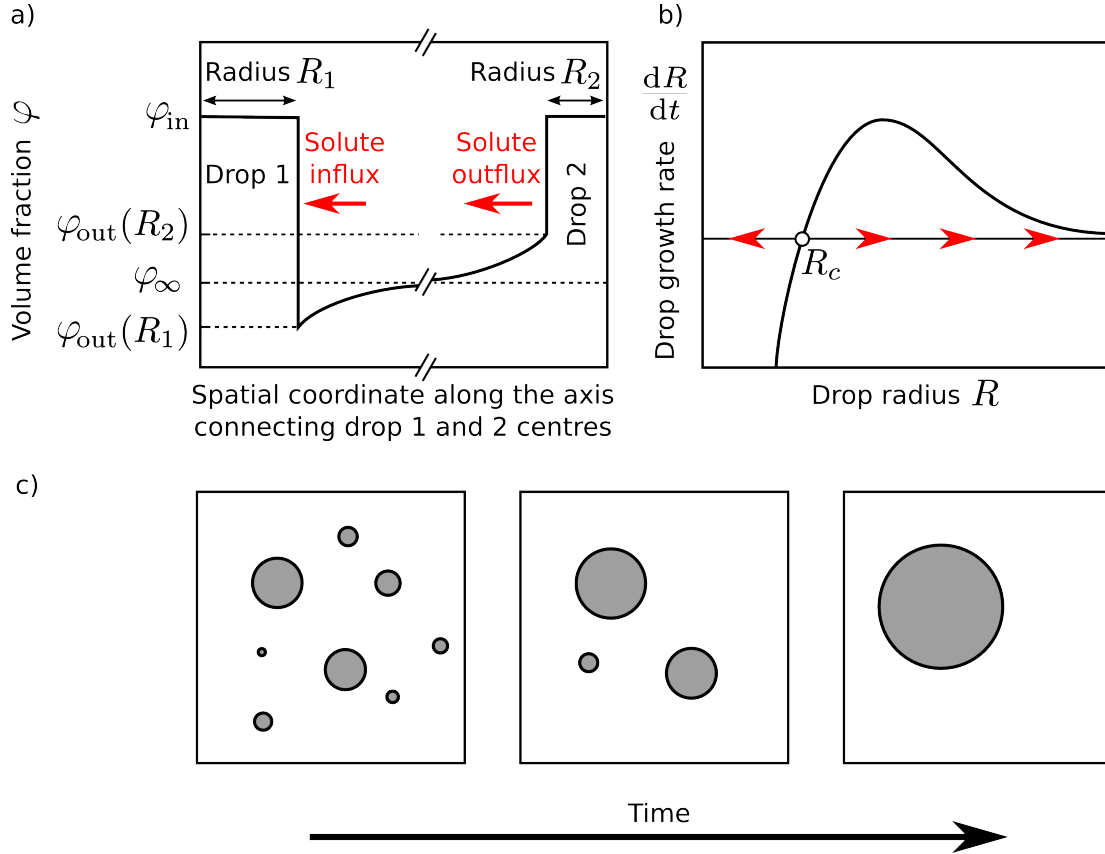


Figure 2.4: *Ostwald ripening destabilises multi-drop systems.* For small supersaturation $\varphi_{\text{tot}} - \hat{\varphi}_{\text{out}}$, the distance between drops is large so drops interact with each other only via the common far-field volume fraction φ_{∞} (a)). Outside drops, the volume fraction φ_{out} close to small drops is larger than that close to large drops (Eq. (2.23)). This causes a diffusive transport of molecules from small drops to large drops. Thus there exist a critical radius R_n above which drops grow while smaller drops dissolve (Eqs (2.40),(2.41)) (b)). As a result multi-drop systems undergo coarsening until a unique drop remains [33] (c)).

The boundary conditions must be as follow:

$$\varphi(r = R) = \hat{\varphi}_{\text{out}} \left(1 + \frac{l_c}{R} \right) \quad (2.36)$$

$$\varphi(r \rightarrow \infty) = \varphi_{\infty} , \quad (2.37)$$

with r being the distance from the drop centre. The first condition is the Gibbs-Thomson relation (Eq. (2.23)) with l_c the capillary length given by Eq. 2.24. In the case of strong phase separation ($\hat{\varphi}_{\text{in}} \gg \hat{\varphi}_{\text{out}}$) the effect of the interface curvature on the profile inside drops can be neglected, which amounts to take $\varphi_{\text{in}} = \hat{\varphi}_{\text{in}}$ (Section 2.3.2). To solve the diffusion equation Eq. (2.35), we will use a crucial assumption known as the *quasi-static approximation*: when the dynamics of the drop radii is much slower than the equilibration of the volume fraction profile we can approximate the profile by its steady-state ($\partial\varphi/\partial t = 0$). Assuming moreover spherical symmetry around drops, the resolution of the system of equations (2.35)-(2.37) leads to the following volume fraction profile.

$$\varphi(r) = -\frac{R}{r} \left(\varphi_{\infty} - \hat{\varphi}_{\text{out}} - \frac{\hat{\varphi}_{\text{out}} l_c}{R} \right) + \varphi_{\infty} . \quad (2.38)$$

The drop growth rate dR/dt is linked to the diffusion current of material $D\nabla\varphi|_{r=R}$ across the drop interface per unit surface [33]:

$$\frac{dR}{dt} = \frac{D}{\varphi_{\text{in}}} \nabla\varphi(r)|_{r=R} \quad (2.39)$$

$$= \frac{D}{\varphi_{\text{in}} R} \left(\varphi_{\infty} - \hat{\varphi}_{\text{out}} - \frac{\hat{\varphi}_{\text{out}} l_c}{R} \right) , \quad (2.40)$$

with D the molecular diffusion coefficient, and we used $\varphi_{\text{in}} \gg \varphi_{\text{out}}$. As we have intuitively expected, small drops dissolve ($dR/dt < 0$), while drops larger than a critical size grow ($dR/dt > 0$). The critical radius R_n is solution of $dR/dt|_{R=R_n} = 0$:

$$R_n = \frac{\hat{\varphi}_{\text{out}} l_c}{\varphi_{\infty} - \hat{\varphi}_{\text{out}}} . \quad (2.41)$$

Note that R_n is time dependent: the far field volume fraction φ_{∞} must be fixed by the conservation of the total number of molecules P in the system:

$$\varphi_{\text{in}} \sum_{i=1}^N \frac{4\pi}{3} R_i^3(t) + \varphi_{\infty} \left(V - \sum_{i=1}^N \frac{4\pi}{3} R_i^3(t) \right) = \varphi_{\text{tot}} V , \quad (2.42)$$

where N is the number of drops, i is the drop index, V the system total volume and φ_{tot} the global volume fraction of P . In the drop neighbourhood we have ignored the deviation of the volume fraction from the far-field φ_{∞} since drops are far from each other and the volume fraction $\varphi(r)$ converges to φ_{∞} at large r . We can expand this relation to the first order in the drop density ($\propto \sum_i R_i^3/V$):

$$\varphi_{\infty} \simeq \varphi_{\text{tot}} - \frac{\varphi_{\text{in}}}{V} \sum_{i=1}^N \frac{4\pi}{3} R_i^3(t) , \quad (2.43)$$

where we used $\varphi_{\text{in}} \gg \varphi_{\text{tot}}$. Using Eqs (2.40), (2.41) and (2.43) we find the complete set of dynamical equations that describe the dynamics of the multi-drop system [33]:

$$\frac{dR_i}{dt} = \frac{Dl_c \hat{\varphi}_{\text{out}}}{R_i} \left(\frac{1}{R_n} - \frac{1}{R_i} \right) \quad (2.44)$$

$$\frac{d\varphi_{\infty}}{dt} = -\frac{\hat{\varphi}_{\text{in}}}{V} \sum_{i=1}^N \frac{4\pi}{3} \frac{d}{dt} R_i^3(t) \quad (2.45)$$

with

$$R_n = \frac{\hat{\varphi}_{\text{out}} l_c}{\varphi_{\text{tot}} - \hat{\varphi}_{\text{out}} - \frac{\varphi_{\text{in}}}{V} \sum_{i=1}^N \frac{4\pi}{3} R_i^3(t)} . \quad (2.46)$$

Lifshitz and Slyozov showed in the limit of vanishing supersaturation $\varphi_{\text{tot}} - \hat{\varphi}_{\text{out}}$ that an infinite multi-drop system always converges to the same drop radius distribution when normalized by the mean drop radius, irrespectively of the initial distribution. The mean drop radius $\langle R \rangle$ scales with time as $\langle R \rangle \sim t^{1/3}$ [33]. In a finite system, drops will coarsen until a single drop remains at equilibrium.

2.7.2 Coalescence

Due to thermal energy, drops also undergo Brownian motion. When two drops collide the surface tension drives their coalescence into a larger spherical drop, thus reducing the total surface energy [31]. This effect is expected to be slow in the cell cytoplasm, as molecular crowding strongly suppresses the diffusion of macromolecular structures [54]. In this work we will ignore drop coalescence and concentrate on drop coarsening by Ostwald ripening.

2.8 Summary & discussion

In this chapter we have presented important results of the physics of liquid-liquid phase separation in incompressible and passive binary fluids. Passive refers to the fact that no energy is input in the system. An important result is that once phase separation is initiated and drops are formed, the system will inevitably evolve toward a single-drop state by Ostwald ripening. In Chapter 3 we show that Ostwald ripening can be arrested in the presence of active chemical reactions.

Chapter 3

Arrest of Ostwald Ripening in Binary Fluids

In this chapter, we study the effect of active chemical reactions on binary mixtures. The term “active” refers to the fact that the reactions are energy-consuming. Contrary to the passive case (without reactions) studied in Chapter 2, we show that the drop coarsening via Ostwald ripening can be arrested. This chapter is based on the work of Zwicker et al. [17]. The same first author has also developed a formalism for ternary mixtures in his PhD thesis [55]. For simplicity we concentrate on the binary case in this chapter. However we do not restrict ourselves to the case of infinitely strong segregation ($\hat{\varphi}_{\text{in}} = 1$ and $\hat{\varphi}_{\text{out}} = 0$ in Eq. (2.17), (2.18)) that is considered in the Ref. [17].

We consider the following chemical reaction scheme:



where P and S are the labels of the two molecular species. k and h are the forward and backward reaction rate constants, respectively, assumed to be independent of the local concentrations of molecules P and S . This entails that the reactions are energy consuming (Section 1.4). In the biological context, P and S can represent two states of the same protein with different binding affinities. The state conversion can be achieved, for example, by phosphorylation-dephosphorylation reactions, where energy is supplied by ATP molecules [56]. We focus on mixtures that are diluted in P , so that in the phase-separating state, drops that are rich in P are surrounded by a phase that is depleted from P and rich in S . Moreover we restrict ourselves to the strong separation regime where the volume fraction of P inside drops is much larger than outside. We will assume that the chemical reactions

rates, k and h (Eq. (3.1)), are small enough so that thermal equilibrium is locally established. With regards to the interface, this implies that the P volume fraction of is given by the Gibbs-Thomson relations (Eq. (2.23)). Namely, for a drop of radius R we have:

$$\varphi_{\text{in}}(r = R) = \hat{\varphi}_{\text{in}} \quad (3.2)$$

$$\varphi_{\text{out}}(r = R) = \hat{\varphi}_{\text{out}} \left(1 + \frac{l_c}{R} \right), \quad (3.3)$$

where $\varphi_{\text{in}}(r)$ and $\varphi_{\text{out}}(r)$ denote the volume fractions of P inside and outside the drop, respectively, and r is the distance from the drop centre. $\hat{\varphi}_{\text{out}}$ and $\hat{\varphi}_{\text{in}}$ are the phase coexistence volume fractions in an infinite, single-drop system, without chemical reactions. l_c is the capillary length given by Eq. (2.24). The validity of this local thermal equilibrium approximation at the interface will be demonstrated in Section 5.2, using simulation methods, in the context of a ternary mixture. In the work of Zwicker and al. [17], it has been assumed that $\hat{\varphi}_{\text{in}} = 1$ and $\hat{\varphi}_{\text{out}} = 0$, leading to $\varphi_{\text{out}}(R) = l_c \hat{\varphi}_{\text{out}}/R$. Note that in this case $l_c \hat{\varphi}_{\text{out}}$ is non-zero, because l_c depends on $\hat{\varphi}_{\text{out}}$ (Eq. 2.24). Here we will add generality by relaxing these constraints. However we still impose $\hat{\varphi}_{\text{in}} \gg \hat{\varphi}_{\text{out}}$ due to strong phase separation.

We now focus on the volume fraction profiles away from interfaces, i.e inside and outside drops. In the absence of chemical reactions, we have argued in Section 2.7.1, that the volume fraction profiles are well described by the ideal gas diffusion equation (Eq. (2.35)). When chemical reactions are present, we assume again local thermal equilibrium. Hence, we account for the local conversion of molecules P into S , and vice versa, by simply adding a source and a sink term to the ideal gas diffusion equation. We obtain the following reaction-diffusion equation:

$$\frac{\partial \varphi_{\text{in/out}}}{\partial t} = -k\varphi_{\text{in/out}} + h(1 - \varphi_{\text{in/out}}) + D\nabla^2 \varphi_{\text{in/out}} \quad (3.4)$$

with D the molecular diffusion coefficient. The first, and the second term in the right hand side, account for the destruction, and the creation of P , respectively. Note that in our binary fluid, $1 - P$ is the volume fraction of S .

Contrary to the case without chemical reactions, the global P volume fraction $\varphi_{\text{tot}} \equiv \int P(r)$ is not fixed, but depends on the reaction rate constants k and h . Since the rate constants are independent of the volume fractions we must have

$$\frac{d\varphi_{\text{tot}}}{dt} = -k\varphi_{\text{tot}} - h(1 - \varphi_{\text{tot}}). \quad (3.5)$$

A chemical equilibrium is reached at the steady-state ($d/dt = 0$), and the global P volume fraction is given by

$$\varphi_{\text{tot}} = \frac{h}{k + h} . \quad (3.6)$$

3.1 Volume fractions profiles inside and outside drops

The volume fraction profiles, if they equilibrate much faster than the drop radius dynamics, can be approximated to be at steady-state [33]. Using this quasi-static approximation, and assuming moreover spherical symmetry in the drop and in its neighbourhood, the generic solution to the reaction-diffusion equation (3.4) is

$$\varphi_{\text{in/out}}(r) = \frac{h}{k + h} + \frac{1}{r} (A_{\text{in/out}} e^{r/\xi} + B_{\text{in/out}} e^{-r/\xi}) , \quad (3.7)$$

with r the distance from the drop centre, and

$$\xi = \sqrt{\frac{D}{k + h}} \quad (3.8)$$

is the length scale of the profile gradient. Therefore, the chemical reactions introduce a new length scale (ξ) in the system. As we shall see, the magnitude of this length scale compared to the system other characteristic lengths is important. One relevant length is obviously the drop radii R . Using a mean-field approximation, we neglect the effect of the particular spatial distribution of drops. Therefore another relevant length is the average inter-drop distance L , given by

$$L = \left(\frac{3}{4\pi\rho} \right)^{1/3} \quad (3.9)$$

with $\rho = N/V$ the drop number density, N the number of drops and V the system volume. The volume fraction profile $\varphi_{\text{in/out}}(r)$ for each drop and its surrounding region, is given by Eq. (3.7) for $0 < r < L$. Importantly, note that r is always upper bounded by L .

Volume fraction profile inside drops

Inside drops, we impose the no flux boundary condition in the drop centre ($\nabla\varphi_{\text{in}}|_{r=0}$), and the interface boundary condition is given by the Gibbs-Thomson relation (Eq.

(3.2)). Enforcing these conditions in Eq. (3.7), the volume fraction profile inside a drop of radius R is:

$$\varphi_{\text{in}}(r) = \frac{h}{k+h} + \left(\hat{\varphi}_{\text{in}} - \frac{h}{k+h} \right) \frac{R \sinh(r/\xi)}{r \sinh(R/\xi)} . \quad (3.10)$$

Let us assume, for the time being, that the drop radius R is small compared to the gradient length scale ξ . We can then expand for the small quantities r/ξ and R/ξ :

$$\varphi_{\text{in}}(r) = \frac{h}{k+h} + \left(\hat{\varphi}_{\text{in}} - \frac{h}{k+h} \right) \left(1 + \mathcal{O}\left(\frac{R^2}{\xi^2}\right) \right) \quad (3.11)$$

$$\simeq \hat{\varphi}_{\text{in}} \quad (3.12)$$

We find that for small drops ($R \ll \xi$), the profile inside $\varphi_{\text{in}}(r)$ is homogeneous. We will determine the expression of the steady-state drop radius in Section 3.3 and show that the condition $R \ll \xi$ is indeed true in a mixture diluted in P .

Volume fraction profile outside drops

Outside drops, we will consider two distinct regimes depending on the magnitude of the inter-drop distance L relative to the gradient length scale ξ .

Large drop number density ρ , or small inter-drop distance ($L \ll \xi$). We start with the regime where the drop number density ρ is large, so that the inter-drop distance L is much smaller than the gradient length scale ξ (Eq. (3.9)). Therefore, r is also always much smaller than ξ , since $r < L$. Far from drops ($R \ll r \ll \xi$), the volume fraction $\varphi_{\text{out}}(r)$ approaches the far-field volume fraction φ_{∞} . Using this boundary condition, we can solve Eq. (3.7), and expand the solution $\varphi_{\text{out}}(r)$ in the small quantities r/ξ , R/ξ and R/L :

$$\varphi_{\text{out}}(r) \simeq \varphi_{\infty} - \frac{R}{r} (\varphi_{\infty} - \varphi_{\text{out}}(R)) , \quad (3.13)$$

with $\varphi_{\text{out}}(R)$ the interface volume fraction given by the Gibbs-Thomson relation (Eq. (3.3)). Note that this profile is the same as in the case where no chemical reactions are present (Eq. (2.38)). This can be understood as follow: since this regime is defined by $L \ll \xi$, this implies that the reaction rates k and h are small (Eq. (3.8)), to the extent that the profile is not significantly affected by the chemical reactions. However, chemical reactions cannot be neglected all together, as we shall see. The far-field volume fraction φ_{∞} has to be determined from the conservation of the total number of molecules P in the system, as done in Section 2.7.1. Plugging

the global P volume fraction φ_{tot} (Eq. (3.6)) into the expression of φ_{∞} (Eq. (2.43)) we get

$$\varphi_{\infty} = \frac{h}{k+h} - \frac{\hat{\varphi}_{\text{in}}}{V} \sum_{i=1}^N \frac{4}{3} \pi R_i^3, \quad (3.14)$$

with N the number of drops, i the drop index and V the system volume. As we expect intuitively, since drops are rich in P , the larger the total drop volume is, the smaller the far-field concentration φ_{∞} is.

Small drop number density ρ , or large inter-drop distance ($L \gg \xi$). We now turn to the regime where the drop number density ρ is small, so that the inter-drop distance L is much larger than the gradient length scale ξ (Eq. (3.9)). Far from drops ($r \gg R$), and also such that $r \gg \xi$, the volume fraction $\varphi_{\text{out}}(r)$ converges toward the far-field volume fraction φ_{∞} . We use this boundary condition to determine the profile $\varphi_{\text{out}}(r)$ from Eq. (3.7), and expanding in the small parameter L/ξ , we find

$$\varphi_{\text{out}}(r) \simeq \varphi_{\infty} - \frac{R}{r} (\varphi_{\infty} - \varphi_{\text{out}}(R)) e^{(R-r)/\xi}, \quad (3.15)$$

with

$$\varphi_{\infty} = \frac{h}{k+h}, \quad (3.16)$$

and $\varphi_{\text{out}}(R)$ is the interface volume fraction given by the Gibbs-Thomson relation (Eq. (3.3)). Note that, contrary to the large drop number density ρ regime, the far-field volume fraction φ_{∞} no longer depends on the number N of drops and the drop radii R . Instead, φ_{∞} is solely determined by the reaction rates k and h , and the far-field is at chemical equilibrium, since $\varphi_{\infty} = \varphi_{\text{tot}}$ (Eq. (3.6)).

3.2 Drop growth and shrinkage

Due to the spatial gradient of the volume fraction $\varphi_{\text{out}}(r)$ outside a drop, there is a flux $j = D \nabla P|_{r=R}$ of P molecules per unit area, at the drop interface ($r = R$). In both regimes, large and small drop number density ρ (Eqs (3.13) and (3.15)), we

find the identical flux j :

$$j = D \nabla P|_{r=R} \quad (3.17)$$

$$= \frac{D}{R} (\varphi_\infty - \varphi_{\text{out}}(R)) . \quad (3.18)$$

with $\varphi_{\text{out}}(R)$ given by the Gibbs-Thomson relation (Eq. (3.3)). Inside the drop, the volume fraction $\varphi_{\text{in}}(r)$ is homogeneous and equal to $\hat{\varphi}_{\text{in}}$ (Eq. (3.12)). Therefore, the rate of change of φ_{in} due to chemical reactions, is

$$\frac{d\varphi_{\text{in}}}{dt} = -k\hat{\varphi}_{\text{in}} + h(1 - \hat{\varphi}_{\text{in}}) \quad (3.19)$$

$$\simeq -k\hat{\varphi}_{\text{in}} \quad (3.20)$$

$$< 0 \quad (3.21)$$

and we recall that $1 - \hat{\varphi}_{\text{in}}$ is the volume fraction of S inside drops. We have supposed that the forward reaction ($P \rightarrow^k S$) dominates inside drops, and we neglected the backward reaction, since drops are rich in P and poor in S . This intuitive argument will be validated in Appendix A.1 (Eq. (A.2)). Since $d\varphi_{\text{in}}/dt$ is negative, the chemical reactions deplete the drop from P molecules. On the other hand, the drop receives a flux j of P molecules from the outside (Eq. (3.18)). When the depletion is larger than the influx, or vice versa, since the volume fraction inside the drop is fixed at the interface by the Gibbs-Thomson relation ($\varphi_{\text{in}}(r) = \hat{\varphi}_{\text{in}}$, Eq. (3.2)), the drop radius R must evolve to accommodate. Namely, the drop growth rate is [10]:

$$\frac{dR}{dt} = \frac{1}{\hat{\varphi}_{\text{in}} - \varphi_{\text{out}}(R)} \left[\frac{D}{R} \left(\varphi_\infty - \hat{\varphi}_{\text{out}} - \frac{\hat{\varphi}_{\text{out}} l_c}{R} \right) - \frac{k\hat{\varphi}_{\text{in}} R}{3} \right] . \quad (3.22)$$

This result resembles the growth rate in passive systems, i.e., without chemical reactions (Eq. (2.40)), with an additional term proportional to the forward reaction rate constant k and the drop radius R . This term accounts for the chemical conversion from P to S inside drops. We show, in Fig. 3.1, the drop growth rate dR/dt as a function of the drop radius R , in two scenarios: in the passive case ($k = 0$, left figure) and in the active case ($k > 0$, right figure). In the passive case, all drops larger than a critical radius R_n grow ($dR/dt > 0$). In the active case, chemical reactions introduce a stable fixed point radius R^* , such that smaller drops grow while larger drops shrink ($dR/dt < 0$). In Section 3.3, we will study quantitatively the drop growth rate (Eq. (3.22)), and determine the critical radii R^* and R_n in our multi-drop system.

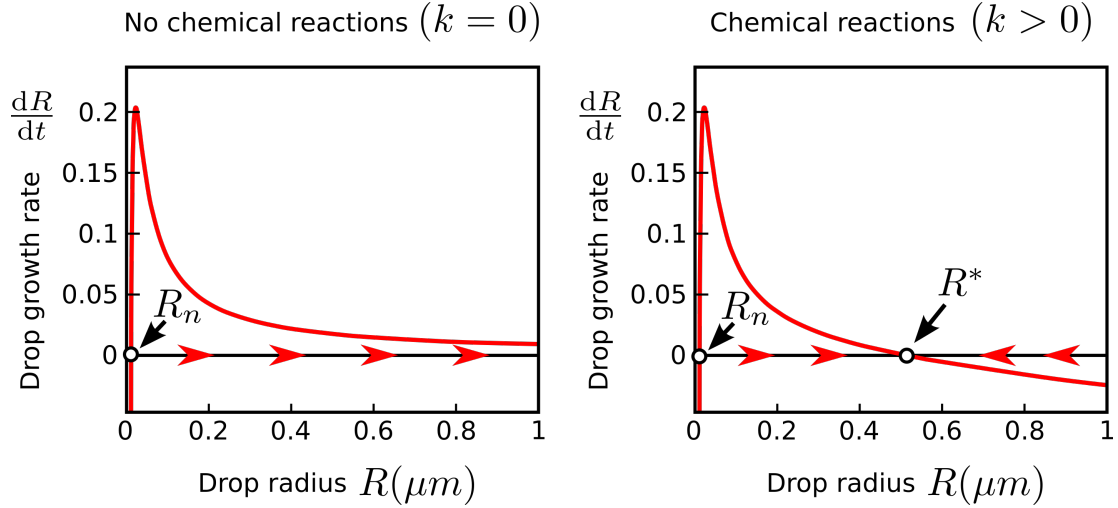


Figure 3.1: *Effect of chemical reactions on the drop growth rate.* The drop growth rate dR/dt is shown as a function of the drop radius R (Eq. (3.22)). Without chemical reactions ($k = 0$, left figure), the growth rate has an unstable fixed point radius R_n , such that smaller drops shrink ($dR/dt < 0$) and larger drops grow ($dR/dt > 0$). With chemical reactions ($k > 0$, right figure), a stable fixed point radius R^* is introduced: smaller drops grow while larger drops dissolve. Parameters: $D = 1 \mu\text{m}^2.\text{s}^{-1}$, $l_c = 1 \mu\text{m}$, $\hat{\varphi}_{\text{in}} = 10^{-1}$, $\hat{\varphi}_{\text{out}} = 10^{-4}$, $\varphi_{\infty} = 10^{-3}$, $k = 0$ (left figure), $k = 0.1 \text{ s}^{-1}$ (right figure).

3.3 Steady-state drop radius

The steady-state drop radius R^* is the solution of $dR/dt = 0$ (Eq. (3.22)):

$$\varphi_{\infty} - \hat{\varphi}_{\text{out}} - \frac{\hat{\varphi}_{\text{out}} l_c}{R^*} - \frac{k \hat{\varphi}_{\text{in}} (R^*)^2}{3D} = 0. \quad (3.23)$$

Considering drops large enough so that we can neglect the effect of the surface tension term ($\propto l_c/R$), we find the stable point radius R^* :

$$R^* = \sqrt{\frac{3D(\varphi_{\infty} - \hat{\varphi}_{\text{out}})}{k \hat{\varphi}_{\text{in}}}}. \quad (3.24)$$

If on the contrary we consider small drops, so that the term proportional to R^2 is negligible in Eq. (3.23), we find the unstable point radius R_n :

$$R_n = \frac{\hat{\varphi}_{\text{out}} l_c}{\varphi_{\infty} - \hat{\varphi}_{\text{out}}}. \quad (3.25)$$

Therefore, we found the steady-state radii R^* and R_n (Eqs (3.24) and (3.25)) as functions of the far-field volume fraction φ_{∞} , which we will examine in the two regimes of small and large drop number density ρ .

Small drop number density The maximal stable drop radius, labelled R_u , and the minimal critical radius R_n , are obtained when the far-field volume fraction φ_∞ is maximal. This occurs in the low drop number density ρ regime (compare Eqs (3.16) and (3.14)). In this regime, by inserting φ_∞ (Eq. (3.16)) in the expressions of R^* and R_n (Eqs (3.24) and (3.25)), we find:

$$R_u = \sqrt{\frac{3D \left(\frac{h}{k+h} - \hat{\varphi}_{\text{out}} \right)}{k\hat{\varphi}_{\text{in}}}} \quad (3.26)$$

$$R_n = \frac{\hat{\varphi}_{\text{out}} l_c}{\frac{h}{k+h} - \hat{\varphi}_{\text{out}}} . \quad (3.27)$$

Note that this scenario also corresponds to a single drop in an infinite system, since in that case the drop number density ρ vanishes. By comparing R_u to the gradient length scale ξ , we show in Appendix A.1 that $R_u \ll \xi$ is always true in a mixture diluted in P , validating the expression of the profile $\varphi_{\text{in}}(r)$ inside drops (Eq. (3.12)). As the rate constant k increases, the drop radius R_u decreases and R_n increases. When R_u becomes smaller than R_n , no steady-state radius exists. The critical rate constant k_c is the solution of $R_u(k_c) = R_n(k_c)$:

$$k_c = \frac{3D \left(\frac{h}{h+k_c} - \hat{\varphi}_{\text{out}} \right)^3}{\hat{\varphi}_{\text{in}} \hat{\varphi}_{\text{out}}^2 l_c^2} \quad (3.28)$$

In other words, when the forward rate constant k is larger than k_c , no drops exist and phase separation is destroyed. We will see that this is also true in the large drop number density ρ regime.

Large drop number density In the high drop number density ρ regime, the value of the far-field volume fraction φ_∞ is smaller compared to the small ρ regime. This leads to smaller steady-state radius R^* (Eq. (3.24)), and larger critical drop radius R_n (Eq. (3.25)). As a result, the critical forward rate constant above which the drops dissolve in this regime is even smaller than k_c . This demonstrates that k_c is the maximal forward reaction rate constant k above which no drops can exist, irrespective of the drop number density ρ .

We show in Fig. 3.2 the region of existence of a steady-state radius R (enclosed by a continuous red curve), as a function of the forward reaction rate constant k , and at fixed backward reaction rate constant h . Outside this region all drops dissolve (downward arrows). The analytical expression for the maximal drop radius

R_u and the critical drop radius R_n (Eqs (3.26), (3.27)) are shown in red dotted curves. The estimate of the maximal rate constant k_c (Eq. (3.28)) above which all drops dissolve is shown with a black arrow and a vertical line.

3.4 Linear stability of the steady-state

So far we have considered a multi-drop system at steady-state, with drops of identical radius R^* . Here we study the linear stability of the steady-state by perturbing the drop radii. We do so in such a way that the total drop volume remains constant:

$$R_i(t) = R^* + \delta R_i(t) \quad (3.29)$$

with

$$\delta R_i(t) = (-1)^i \epsilon(t) \quad (3.30)$$

and $0 < \epsilon(t=0) \ll R^*$, $i = 1, \dots, N$ is the drop index. Without loss of generality, N is an even number, so the drop total volume is conserved, up to the linear order in ϵ . Therefore, the far-field volume fraction φ_∞ remains constant as well (Eqs (3.16) and (3.14)). Plugging Eq. (3.29) in the drop growth rate dR/dt (Eq. (3.22)), and expanding to the first order in ϵ , we find the evolution of the drop radius perturbation $\epsilon(t)$:

$$\frac{d\epsilon}{dt} = \left(\frac{2D\hat{\varphi}_{\text{out}}l_c}{(R^*)^3} - \frac{D(\varphi_\infty - \hat{\varphi}_{\text{out}})}{(R^*)^2} - \frac{k\hat{\varphi}_{\text{in}}}{3} \right) \epsilon. \quad (3.31)$$

Using the steady-state relation Eq. (3.23) to eliminate φ_∞ , we find

$$\frac{d}{dt}\epsilon = \alpha\epsilon, \quad (3.32)$$

with

$$\alpha = \left(\frac{D\hat{\varphi}_{\text{out}}l_c}{(R^*)^3} - \frac{2k\hat{\varphi}_{\text{in}}}{3} \right). \quad (3.33)$$

If the steady-state drop radius R^* is small, since the first term ($\propto 1/(R^*)^3$) dominates, $\alpha > 0$ hence the perturbation will grow spontaneously. Specifically, some drops, the ones which radius has increased due to the perturbation, will grow, while the others will shrink. The steady-state is therefore linearly unstable to Ostwald ripening. On the contrary, if the steady-state radius is large, the first term

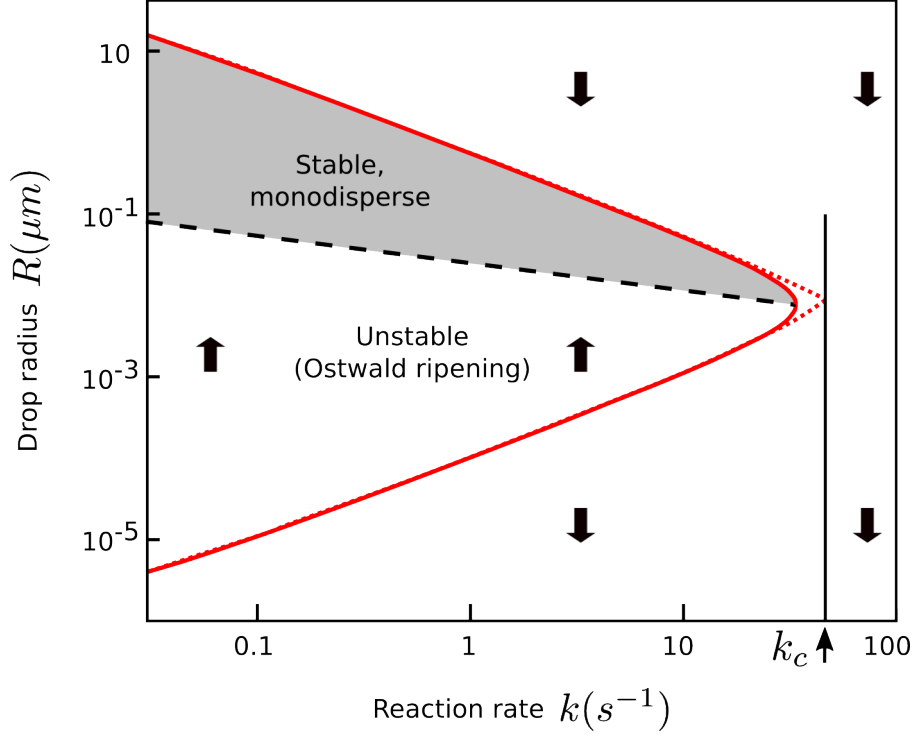


Figure 3.2: *Stability diagram of a multi-drop system at fixed backward reaction rate constant h .* The range of possible steady-state drop radius R^* given by Eq. (3.23) are enclosed by the continuous red curve. The drop radius R^* is function of the drop number density ρ . Outside this curve, no steady-state radius exist, hence drops dissolve (downward arrows). In the grey region, the steady-states are stable against Ostwald ripening. Outside the grey region, but still within the red continuous curve, the steady-states are unstable against Ostwald ripening: drops coarsen with time, leading to an increase of the average drop radius R (upward arrows). The stability-instability boundary is shown with a black dashed line (Eq. (3.34)). The analytical expressions for the maximal drop radius R_u (Eq. (3.26)) and the critical drop radius R_n (Eq. (3.27)) are shown with red dotted lines. The estimate of the critical forward rate constant k_c (Eq. (3.28)), beyond which no drops exist, is shown with a black arrow and a vertical line. Note that the minimum forward rate constant k showed in this diagram is $k = 3h$. For smaller rates, the mixture is not in the P -diluted regime, and the validity of the predictions presented in this chapter breaks down (Appendix A.1). Parameters: $D = 1 \mu\text{m}^2\text{s}^{-1}$, $l_c = 10^{-2} \mu\text{m}$, $\hat{\varphi}_{\text{in}} = 10^{-1}$, $\hat{\varphi}_{\text{out}} = 10^{-4}$, $h = 10^{-2} \text{s}^{-1}$.

($\propto 1/(R^*)^3$) is negligible and $\alpha < 0$, so the perturbation spontaneously decays. The steady-state is thus linearly stable against Ostwald ripening. The critical radius R_l at which the transition occurs is solution of $\alpha = 0$:

$$R_l = \left(\frac{3D\hat{\varphi}_{\text{out}}l_c}{2k\hat{\varphi}_{\text{in}}} \right)^{1/3}. \quad (3.34)$$

In the stability diagram in Fig. 3.2, we show the stability of the system for varying forward rate constant k and fixed backward rate constant h . Steady-state drops can exist within the continuous red curve. The region that is stable against Ostwald ripening is shown in grey. Outside this region, but within the red continuous curve, drops coarsen, leading to an increase of the average drop radius R over time (upward arrow). All drops dissolve outside the red curve (downward arrows).

3.5 Summary & discussion

In this chapter, we have used the arguments developed by Zwicker et al. [17] to study the effect of active chemical reactions in a phase-separating binary fluid. We found that the drops have an upper bounded size, and in a multi-drop system, the drop coarsening by Ostwald ripening is arrested if the drops are larger than a critical size. Additionally, there are critical reaction rates above which the chemical reactions destroy the phase separation.

In the biological context of membrane-less organelle formation, this active binary system can represent two states of the same protein, one being phase-separating, and the other being soluble. Each state can be converted into the other by ATP-dependent chemical reactions, such as phosphorylations-dephosphorylations. However, the cell cytoplasm is composed in majority of other constituents that are not involved in drop formation, e.g. other proteins and water molecules. Therefore, in a minimal model of phase separation in the cytoplasm, a third component, the solvent, should be included. In Chapter 4 we therefore extend the formalism to ternary mixtures.

Chapter 4

Active Phase-Separated Drops in a Ternary Fluid

In a passive, phase-separating system, drops always coarsen via Ostwald ripening (Chapter 2). In a binary fluid, this ripening process can be arrested and drop sizes can be controlled by active chemical reactions (Chapter 3). In the cellular context, the two components of the fluid can be interpreted as two states of the same protein, but with different interaction properties. Each state can be converted into the other, via, for example, phosphorylation and dephosphorylation reactions [56]. However, the cell cytoplasm consists mainly of other components, such as the proteins that are not involved in the drop formation, water molecules etc. Therefore, a minimal model for chemical reaction-controlled cytoplasmic phase separation arguably contains three components: two protein states plus the solvent.

Here we study the effect of the active chemical reactions on a ternary mixture. Contrary to previous works [17, 55] we allow for the existence of spatial gradients of the protein concentration inside the drops and we go beyond the small supersaturation limit (large drop-drop distance limit) discussed in Chapter 3 to elucidate the system dynamics at intermediate drop density. Additionally, we consider arbitrary equilibrium concentrations $\hat{P}_{\text{in}}$ and $\hat{P}_{\text{out}}$ inside and outside drops. We find new regimes with qualitative differences in the system behaviour.

4.1 Model of cytoplasmic phase separation

Our ternary mixture consists of two molecular states, one phase-separating (P) and one soluble (S), plus the solvent or cytosol (C). States P and S can be converted

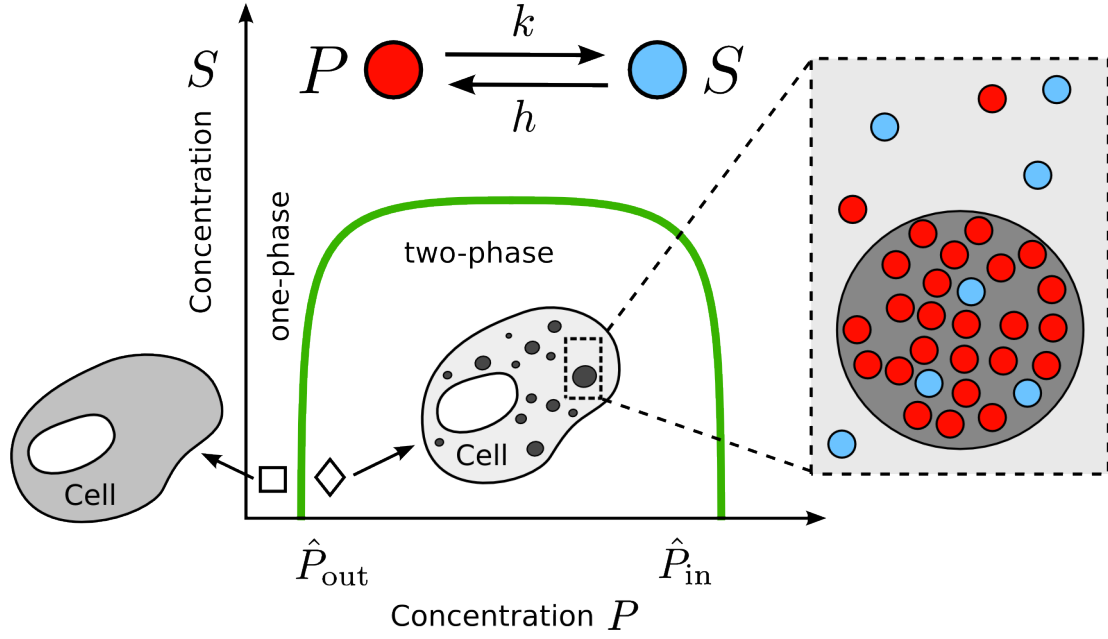


Figure 4.1: *Model of cytoplasmic phase separation.* The cell cytoplasm is modeled by a ternary fluid composed of phase-separating (P) and soluble (S) molecular states, and other cytoplasmic components (C). Chemical reactions convert P into S with the rate constant k , and S into P with the rate constant h (Eq. (4.1)). In the passive case ($k = h = 0$), the system is well mixed (‘ $\square$ ’) if the concentrations of P and S lie outside the phase boundary (green line in the phase diagram); the system phase separates otherwise (‘ $\diamond$ ’). In the latter case, we assume that S does not phase separate and remains homogeneous.

into each other by the chemical reactions



where k and h are the reaction rate constants. The non-equilibrium nature, or active nature, of these reactions lies in the fact that both reaction rates are independent of the local concentrations and thus have to be driven by free energy consumption (Section 1.4). In the context of the cell, these reactions can be, e.g., ATP-driven protein phosphorylation and dephosphorylation.

In the passive case ($k, h = 0$), as discussed in Section 2.7, a finite system will inevitably coarsen via Ostwald ripening [33] and drop coalescence [31]. Here, we assume that drop diffusion is negligible so we will focus exclusively on the Ostwald ripening. In the cell context, this is motivated by the strong suppression of macromolecular diffusion in the cell cytoplasm [54]. As we have seen in Section 2.7.1, Ostwald ripening results from two effects: 1) the Gibbs-Thomson relation dictating that for a drop of size R , the concentrations of solute inside and outside the drop

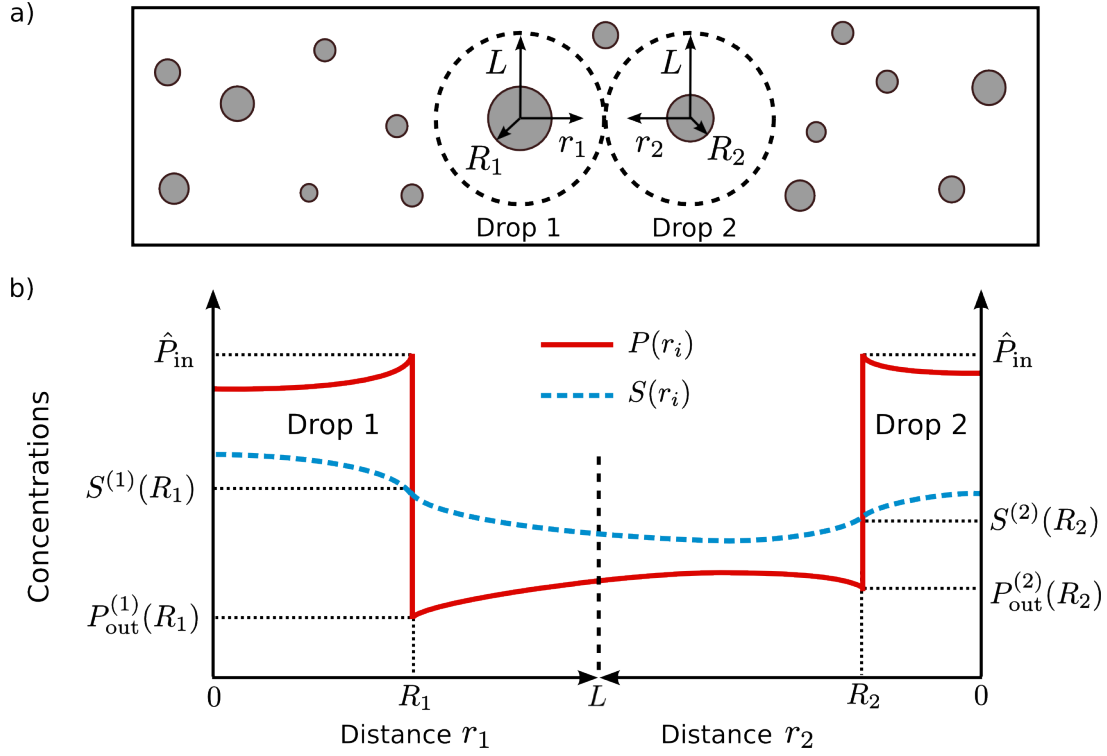


Figure 4.2: *Model of cytoplasmic phase separation.* a) A multi-drop system with drop number density ρ is studied by considering two interacting subsystems ($i = 1, 2$) of radius $L = [3/(4\pi\rho)]^{1/3}$, each having a drop of radius R_i in their center. b) Schematics of the concentration profiles of P and S in the subsystems when chemical reactions are present ($k, h > 0$, Eqs (4.29) and (4.25)). At the subsystems' boundaries ($r_i = L$) the profiles and their derivatives are matched by assumption (Eq. (4.5)).

next to the interface are $\hat{P}_{in}$ and $\hat{P}_{out}(1 + l_c/R)$ respectively, where l_c is the capillary length and $\hat{P}_{in/out}$ are the phase coexistence concentrations in a passive system (see Fig. 4.1); and 2) the concentration profile of the solute in the dilute phase is given by the steady-state solution to the diffusion equation (the quasi-static assumption, see Section 2.7.1). These two effects combined lead to a diffusive flux of solute from small drops to big drops (Fig. 2.4) [33].

In the active case, i.e., when chemical reactions are switched on ($k, h > 0$), we assume that local thermal equilibrium remains valid so that the interface boundary conditions for P are unchanged. We will show later that these conditions are verified, using simulation methods in Chapter 5. In addition, assuming local thermal equilibrium also away from interfaces, the effect of chemical reactions on the concentration profiles are accounted for by adding sink and source terms to the steady-state diffusion equation. The concentration profiles inside and outside the

drops are given by [57]:

$$D\nabla^2 P_{\text{in/out}} - kP_{\text{in/out}} + hS_{\text{in/out}} = 0 \quad (4.2)$$

$$D\nabla^2 S_{\text{in/out}} + kP_{\text{in/out}} - hS_{\text{in/out}} = 0 \quad (4.3)$$

where $P_{\text{in/out}}$ and $S_{\text{in/out}}$ denote the concentration profiles of P and S inside and outside drops with subscripts “in” and “out”, respectively. D is the diffusion coefficient, assumed to be identical for both P and S and both inside and outside drops.

For simplicity, we will first focus on two spherical subsystems of radius L , each having a spherical drop in their centre (Fig. 4.2a)). We assume that the concentrations and their gradients at the boundaries of the two subsystems match (Fig. 4.1b)). The rationale for this approximation is that in a multi-drop system, the actual boundary conditions are influenced by many neighbouring drops and we treat these fluctuating boundary conditions in a *mean-field* manner by assuming spherical symmetry around the drops. The corresponding boundary conditions are

$$P_{\text{out}}^{(1)}(L) = P_{\text{out}}^{(2)}(L) \quad (4.4)$$

$$\nabla_{\mathbf{r}_1} P_{\text{out}}^{(1)}|_{\mathbf{r}_1=L} = -\nabla_{\mathbf{r}_2} P_{\text{out}}^{(2)}|_{\mathbf{r}_2=L}, \quad (4.5)$$

and the same apply to $S_{\text{in/out}}^{(i)}$. The subscript $i = 1, 2$ denotes the drop index. Note that we use two different coordinate systems r_1 and r_2 , each having their respective drop centre as the origin (Fig. 4.1c)). Importantly, this description goes beyond the small supersaturation limit: in the small supersaturation limit, L must be much larger than R so that the concentration profiles approach a far-field concentration P_∞ far from drops. The boundary condition in this scenario is $P_{\text{out}}(r \rightarrow \infty) = P_\infty$. In our description however, the constraint on L is relaxed. For any $L > R$ the profiles are connected at the subsystem boundary ($r = L$) (Eqs (4.4) and (4.5)). This makes our model less restrictive and allows intermediate supersaturations to be considered.

We impose no flux boundary conditions in the centre of drop ($r_i = 0$), and, with the Gibbs-Thomson relations at the interface ($r_i = R_i$), we have the additional boundary conditions:

$$\nabla_{\mathbf{r}_i} P_{\text{in}}^{(i)}|_{r=0} = 0 \quad (4.6)$$

$$P_{\text{in}}^{(i)}(R_i) = \hat{P}_{\text{in}} \quad (4.7)$$

$$P_{\text{out}}^{(i)}(R_i) = \hat{P}_{\text{out}} \left(1 + \frac{l_c}{R_i} \right). \quad (4.8)$$

In a multi-drop system with drop number density $\rho = N/V$, with N the number of drops and V the system volume, the subsystem radius L represents half of the mean inter-drop distance. Hence L is related to the drop number density ρ :

$$L = \left(\frac{3}{4\pi\rho} \right)^{1/3}. \quad (4.9)$$

In other words, we will approximate multi-drop systems with drop number density ρ by our two-drop system with subsystem radius L given by Eq. (4.9). Using simulations methods in Chapter 5, we will show the validity of this approximation.

4.2 Concentration profiles inside and outside drops

We have shown in Section 3 (Eq. (3.6)) that since the reaction rate constants k, h are independent of the concentrations, the global concentrations of P and S are

$$P_{\text{tot}} = \frac{\phi h}{k + h} \quad (4.10)$$

$$S_{\text{tot}} = \frac{\phi k}{k + h}, \quad (4.11)$$

respectively, where $\phi \equiv P_{\text{tot}} + S_{\text{tot}}$ is the total solute concentration, independent of the reaction rate constants k, h . We also demonstrate this result from the reaction diffusion equations (4.2) and (4.3) in Appendix B.1.

In our two-drop system, the concentration profiles of P and S are given by (Eqs (4.2), (4.3)):

$$D\nabla^2 P_{\text{in}}^{(i)} - kP_{\text{in}}^{(i)} + hS_{\text{in}}^{(i)} = 0 \quad 0 \leq r_i < R_i \quad (4.12)$$

$$D\nabla^2 S_{\text{in}}^{(i)} + kP_{\text{in}}^{(i)} - hS_{\text{in}}^{(i)} = 0 \quad 0 \leq r_i < R_i \quad (4.13)$$

inside the drop, and

$$D\nabla^2 P_{\text{out}}^{(i)} - kP_{\text{out}}^{(i)} + hS_{\text{out}}^{(i)} = 0 \quad R_i < r_i \leq L \quad (4.14)$$

$$D\nabla^2 S_{\text{out}}^{(i)} + kP_{\text{out}}^{(i)} - hS_{\text{out}}^{(i)} = 0 \quad R_i < r_i \leq L \quad (4.15)$$

outside the drop. Adding Eqs (4.12) + (4.13), and Eqs (4.14) + (4.15), gives

$$\nabla^2 \left(P_{\text{in/out}}^{(i)}(r_i) + S_{\text{in/out}}^{(i)}(r_i) \right) = 0. \quad (4.16)$$

The generic solution, with spherical symmetry, is

$$P_{\text{in/out}}^{(i)}(r_i) + S_{\text{in/out}}^{(i)}(r_i) = \frac{a_{\text{in/out}}^{(i)}}{r_i} + b_{\text{in/out}}^{(i)} , \quad (4.17)$$

with $a_{\text{in/out}}^{(i)}$ and $b_{\text{in/out}}^{(i)}$ constants.

Inside a drop, the total concentration $P_{\text{in}}(r_i) + S_{\text{in}}(r_i)$ must not diverge in the drop centre ($r_i = 0$), therefore $a_{\text{in}}^{(i)} = 0$. As a result, the total concentration $P_{\text{in}}(r_i) + S_{\text{in}}(r_i)$ inside drops is homogeneous, and equal to a constant I_i :

$$I_i \equiv P_{\text{in}}^{(i)}(r_i) + S_{\text{in}}^{(i)}(r_i). \quad (4.18)$$

Outside the drop, the total concentration $P_{\text{out}}(r_i) + S_{\text{out}}(r_i)$ must be continuous at the boundary between the two subsystems ($r_i = L$). In the case where $R_1 = R_2$, this implies that $a_{\text{out}}^{(i)} = 0$. In our study we will focus on small differences in drop radii ($R_1 \simeq R_2$) and we will make the approximation that $a_{\text{out}}^{(i)}$ remains zero. Therefore, outside drops, the total concentration $P_{\text{out}}(r_i) + S_{\text{out}}(r_i)$ is equal to a same constant O in both subsystems:

$$O \equiv P_{\text{out}}^{(i)}(r_i) + S_{\text{out}}^{(i)}(r_i) \quad i = 1, 2. \quad (4.19)$$

We can therefore express the total concentrations inside (I_i), and outside (O) drops, in terms of the concentrations at the drop interfaces ($r_i = R_i$):

$$I_i = P_{\text{in}}^{(i)}(R_i) + S_{\text{in}}^{(i)}(R_i) \quad (4.20)$$

$$O = P_{\text{out}}^{(i)}(R_i) + S_{\text{out}}^{(i)}(R_i) . \quad (4.21)$$

Using the fact that the total concentrations are homogeneous inside and outside drops (Eqs (4.18), (4.19)), we can decouple the reaction-diffusion systems (Eqs (4.12), (4.13) and (4.14), (4.15)):

$$D\nabla^2 P_{\text{in}}^{(i)}(r_i) - (k + h)P_{\text{in}}^{(i)}(r_i) + hI_i = 0 \quad (4.22)$$

$$D\nabla^2 P_{\text{out}}^{(i)}(r_i) - (k + h)P_{\text{out}}^{(i)}(r_i) + hO = 0 , \quad (4.23)$$

and $S_{\text{in}}^{(i)}(r_i) = I_i - P_{\text{in}}^{(i)}(r_i)$, and $S_{\text{out}}^{(i)}(r_i) = O - P_{\text{out}}^{(i)}(r_i)$. Note that these two equations are coupled by their boundary conditions (Eqs (4.4)-(4.8)). The generic

solutions, in spherical symmetry, are

$$P_{\text{in}}^{(i)}(r_i) = \frac{I_i h}{k + h} + H_{\text{in}}^{(i)} \frac{R_i}{r_i} (A_{\text{in},i} e^{r_i/\xi} + B_{\text{in},i} e^{-r_i/\xi}) \quad (4.24)$$

$$P_{\text{out}}^{(i)}(r_i) = \frac{O h}{k + h} + H_{\text{out}}^{(i)} \frac{R_i}{r_i} (A_i e^{r/\xi} + B_i e^{-r_i/\xi}) , \quad (4.25)$$

with

$$\xi \equiv \sqrt{\frac{D}{k + h}} \quad (4.26)$$

the length scale of the concentration gradients, resulting from the chemical reactions. Note that in the absence of chemical reactions ($k = h = 0$) the gradient length scale is infinite, however gradients may still exist due to the term $1/r_i$ in the profiles (Eqs (4.24), (4.25)).

$$H_{\text{in}}^{(i)} \equiv P_{\text{in}}^{(i)}(R_i) - \frac{I_i h}{k + h} \quad (4.27)$$

$$H_{\text{out}}^{(i)} \equiv P_{\text{out}}^{(i)}(R_i) - \frac{O h}{k + h} . \quad (4.28)$$

The coefficients A_i , B_i , $A_{\text{in},i}$, $B_{\text{in},i}$ are independent of r_i , and are determined by the boundary conditions.

Inside drops, enforcing the boundary conditions (Eqs (4.6),(4.7)), the concentration profile (Eq. (4.24)) becomes

$$P_{\text{in}}^{(i)}(r) = \frac{I_i h}{k + h} + H_{\text{in}}^{(i)} \frac{R_i}{r} \frac{\sinh(r/\xi)}{\sinh(R_i/\xi)} . \quad (4.29)$$

Outside drops, plugging the boundary conditions (Eqs (4.4),(4.5),(4.8)) in the concentration profiles (Eq. (4.25)), we find that the coefficients A_i , B_i are solutions of the system:

$$A_i e^{R_i/\xi} + B_i e^{-R_i/\xi} = 1 \quad i = 1, 2 \quad (4.30)$$

$$H_{\text{out}}^{(1)} (A_1 e^{L/\xi} + B_1 e^{-L/\xi}) = H_{\text{out}}^{(2)} (A_2 e^{L/\xi} + B_2 e^{-L/\xi}) \quad (4.31)$$

$$H_{\text{out}}^{(1)} (A_1 e^{L/\xi} - B_1 e^{-L/\xi}) = -H_{\text{out}}^{(2)} (A_2 e^{L/\xi} - B_2 e^{-L/\xi}) . \quad (4.32)$$

In order to completely describe the P concentration profiles, we also need to determine the concentrations of S at the drop interface ($S_{\text{in/out}}^{(i)}(R_i)$), on which the total concentrations inside and outside drops (I_i , O) depend (Eqs (4.18), (4.19)). We can find $S_{\text{in/out}}^{(i)}(R_i)$ by imposing the conservation of the total number of molecules

P and S in the system:

$$I_1 R_1^3 + I_2 R_2^3 + O(2L^3 - R_1^3 - R_2^3) = 2\phi L^3. \quad (4.33)$$

Plugging the expression of I_i and O (Eqs (4.18), (4.19)) in this equation, and imposing that the S concentration is continuous across a drop interface ($S_{\text{in}}^{(i)}(R_i) = S_{\text{out}}^{(i)}(R_i)$), we find:

$$\begin{aligned} S_{\text{in}}^{(i)}(R_i) &= S_{\text{out}}^{(i)}(R_i) \\ &= \phi - P_{\text{out}}^{(i)}(R_i) - \frac{1}{2} \sum_{j=1}^2 \left(\hat{P}_{\text{in}} - P_{\text{out}}^{(j)}(R_j) \right) \left(\frac{R_j}{L} \right)^3. \end{aligned} \quad (4.34)$$

This assumption is not essential and we describe the more general case where S is discontinuous at the drop interface in Appendix B.9.

We have expressed, in our two-drop system, the concentration profiles $P_{\text{in/out}}^{(i)}$ and $S_{\text{in/out}}^{(i)}$. They depend on the total solute concentration ϕ , the concentration of P at the interface, given by the Gibbs-Thomson relations (Eq. (4.5), (4.6)), the chemical reaction rate constants k and h , the drop radii R_1 and R_2 , and the inter-drop distance L . Importantly, the chemical reactions cause spatial gradients of length scale ξ (Eq. (4.26)) inside and outside drops.

4.3 Drop growth, shrinkage and stability

An important consequence of having gradients of the concentration profiles, is the subsequent existence of fluxes of molecules across the system. At a drop interface, if the flux of P from the outside ($\propto \nabla P_{\text{out}}|_{r=R}$) is not identical to the flux from the inside ($\propto \nabla P_{\text{in}}|_{r=R}$), there will be an accumulation, or a depletion, of molecules P at the interface. Since the interface concentrations of P are fixed by the Gibbs-Thomson relations (Eqs (4.5),(4.6)), the interface must move so that it remains at the prescribed concentration. Thus, it leads to a growth or a shrinkage of the drop. By comparing the gradients inside and outside the drop, at the interface ($r = R$), one can therefore determine the drop growth rate. The volumetric growth rate of

the i -th drop in this two-drop system is [10]:

$$G^{(i)}(R_i, R_j) = \frac{4\pi}{3} \frac{dR_i^3}{dt} \quad (4.35)$$

$$\begin{aligned} &= \frac{4\pi D R_i^2}{\hat{P}_{\text{in}} - P_{\text{out}}^{(i)}(R_i)} \left(\left. \frac{dP_{\text{out}}^{(i)}}{dr_i} \right|_{R_i^+} - \left. \frac{dP_{\text{in}}^{(i)}}{dr_i} \right|_{R_i^-} \right) \\ &= \frac{4\pi D R_i}{\hat{P}_{\text{in}} - P_{\text{out}}^{(i)}(R_i)} \left[H_{\text{out}}^{(i)} \frac{R_i}{\xi} (A_i e^{R_i/\xi} - B_i e^{-R_i/\xi}) - H_{\text{in}}^{(i)} \left(\frac{R_i}{\xi} \coth \frac{R_i}{\xi} - 1 \right) \right]. \end{aligned} \quad (4.36)$$

Note that the growth of the i -th drop depends of both radii R_1 and R_2 via the coefficients A_i and B_i , which are the solutions of Eqs (4.30)-(4.32).

4.3.1 Steady-state drop radius

Given the drop growth rate $G^{(i)}$ (Eq. (4.36)), we can study the steady-state drop radius R^* at which the two drops of the same size are in the steady-state. R^* is the solution of $G^{(i)}(R^*, R^*) = 0$:

$$H_{\text{in}} (y \coth(y) - 1) + H_{\text{out}} (A(1 - y)e^y + B(1 + y)e^{-y}) = 0 \quad (4.37)$$

with $x \equiv L/\xi$, $y \equiv R^*/\xi$, $H_{\text{in/out}} \equiv H_{\text{in/out}}^{(i)}(R_1 = R_2 = R^*)$, and

$$A = \frac{(x + 1)e^{-x}}{(x - 1)e^{x-y} + (x + 1)e^{-(x-y)}} \quad (4.38)$$

$$B = \frac{(x - 1)e^x}{(x - 1)e^{x-y} + (x + 1)e^{-(x-y)}}. \quad (4.39)$$

For a more convenient analysis of the steady-state relation Eq. (4.37), it is useful to decouple x and y . We do so using the identity $ae^c + be^{-c} = (a + b) \cosh(c) + (a - b) \sinh(c)$:

$$\frac{\sinh x - x \cosh x}{\cosh x - x \sinh x} = \frac{(\lambda + 1) (\sinh y - y \cosh y)}{\lambda (\cosh y - y \sinh y) - \cosh y (y \coth y - 1)} \quad (4.40)$$

with

$$\begin{aligned} \lambda &\equiv -H_{\text{out}}/H_{\text{in}} \\ &= \frac{\phi - P_{\text{out}}(R^*)(1 + k/h) - \frac{(R^*)^3}{L^3}(\hat{P}_{\text{in}} - P_{\text{out}}(R^*))}{\hat{P}_{\text{in}}k/h + P_{\text{out}}(R^*) - \phi + \frac{(R^*)^3}{L^3}(\hat{P}_{\text{in}} - P_{\text{out}}(R^*))}. \end{aligned} \quad (4.41)$$

The steady-state drop radius R^* is the solution of Eq. (4.40), and we recall that $P_{\text{out}}(R)$ is given by the Gibbs-Thomson relation Eq. (4.8).

4.3.2 Linear stability of the steady-state

We can now analyse the steady-state stability by calculating the growth rates $G^{(i)}$ of the drops upon perturbing their size:

$$R_1 = R^* + \epsilon \quad (4.42)$$

$$R_2 = R^* - \epsilon. \quad (4.43)$$

Performing a linear stability analysis, we take $0 < \epsilon \ll R^*$, and expand the growth rate $G^{(i)}$ (Eq. (4.36)) with respect to ϵ . For the drop $i = 1$ we have

$$G^{(1)}(R_1, R_2) = g_0(R^*) + g_1(R^*)\epsilon + \mathcal{O}(\epsilon^2) \quad (4.44)$$

with

$$g_0(R^*) = G^{(1)}(R^*, R^*) = G^{(2)}(R^*, R^*) \quad (4.45)$$

$$g_1(R^*) = \left. \frac{\partial G^{(1)}}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial G^{(1)}}{\partial R_2} \right|_{R^*, R^*}. \quad (4.46)$$

Note that in g_0 and g_1 , the subscript 0, 1 refers to the order of the $G^{(1)}$ expansion in ϵ , not the drop label i . We have perturbed the drops in such a way that the first drop is slightly larger than the second drop ($R_1 > R_2$). If $g_1 > 0$, the first drop will continue to grow. The system is therefore unstable against Ostwald ripening. If, on the contrary, $g_1 < 0$, the first drop will shrink. Therefore the perturbation is spontaneously suppressed and the system is linearly stable against Ostwald ripening. We provide the details of the calculation of g_1 in Appendix B.2, and we find:

$$g_1(R^*) = 4\pi D [f_1 H_{\text{in}} + f_2 H_{\text{out}} + R^* (f_3 \mathcal{H}_{\text{in}} + f_4 \mathcal{H}_{\text{out}})] , \quad (4.47)$$

with

$$f_1 = y^2 \operatorname{csch}^2 y - 1 \quad (4.48)$$

$$f_2 = \frac{(1-x)e^{2(x-y)} + (1+x)e^{-2(x-y)} + 4y(x-y) - 2}{(1-x)e^{2(x-y)} + (1+x)e^{-2(x-y)} - 2} \quad (4.49)$$

$$f_3 = 1 - y \coth y \quad (4.50)$$

$$f_4 = -\frac{(1+y)e^{x-y} + (y-1)e^{-(x-y)}}{e^{x-y} - e^{-(x-y)}} \quad (4.51)$$

$$\mathcal{H}_{\text{in}} = -\frac{\hat{P}_{\text{out}} l_c}{(k/h + 1)R^2} \quad (4.52)$$

$$\mathcal{H}_{\text{out}} = -\frac{\hat{P}_{\text{out}} l_c}{R^2}. \quad (4.53)$$

Solving for $g_0(R^*) = 0$, or equivalently Eq. (4.40), allows to determine the steady-state drop radius R^* and the sign of $g_1(R^*)$ indicates the stability of the system: coarsening will occur if $g_1 > 0$ while the system is stable if $g_1 < 0$.

4.4 Numerical determination of the stability of a multi-drop system

We obtain the drop steady-state radius R and its stability by solving numerically the steady-state relation Eq. (4.40) and $g_1(R)$ (Eq. (4.47)). The surface plot in Fig. 4.3 shows the steady-state radius R for a fixed drop number density ρ and varying reaction rate constants k, h . The stable region ($g_1 < 0$) is enclosed by a dashed line. In Fig. 4.4, we show, for fixed backward rate constant h , the region within which the steady-state radius R exists (enclosed by the continuous line), and the region of stability (grey). Note that ρ is not fixed in Fig. 4.4. The stability-instability boundary is shown by a dashed line. Inside the stable region, the system consists of monodisperse drops, whose sizes are controlled by the reaction rate constants k, h and the drop number density ρ . Outside the stable region but still within the continuous curve, the monodisperse system is in an unstable steady-state. Outside the continuous curve, drops always shrink.

Interestingly, there are qualitative changes in the system's behaviour as k varies with fixed h as shown in Fig. 4.4. When $k < k_l$ (blue arrow), the system is in the Lifshitz-Slyozov regime and coarsens (upward arrows), while for $k_l < k < k_u$ (green arrow), the system can be stable (grey region), with co-existing drops of radius determined by ρ . In other words, k_l is the critical rate constant beyond which Ostwald ripening is arrested. Between k_u and k_c (red arrow), the system can

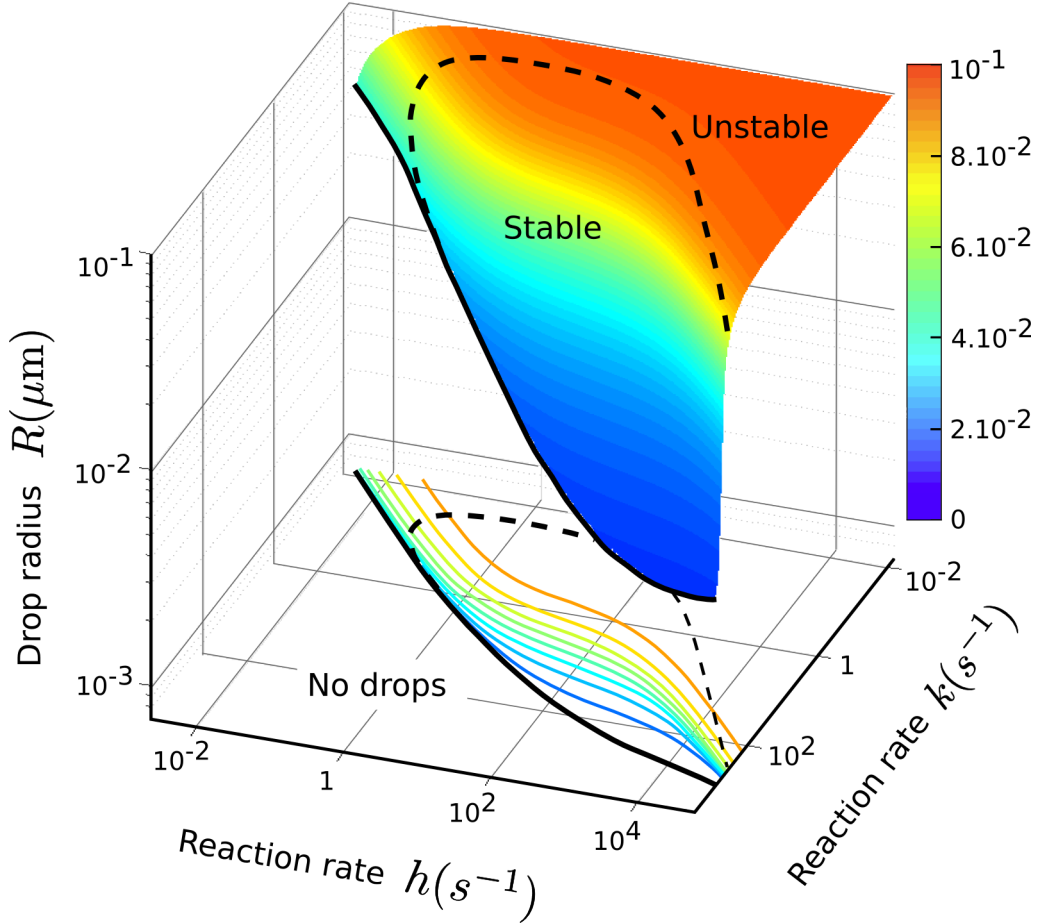


Figure 4.3: *The stability of a multi-drop system at fixed drop number density ρ . The steady-state radius R (solution of $g_0(R) = 0$, Eq. (4.40)) is controlled by the reaction rate constants k and h . The continuous line delimits the region where R exists. The steady-state is stable ($g_1(R) < 0$, Eq. (4.47)) inside the region enclosed by the dashed line and the continuous line, and unstable ($g_1(R) > 0$) outside this region. Parameters: $\rho = 1\mu\text{m}^{-3}$, $l_c = 10^{-2}\mu\text{m}$, $D = 1\mu\text{m}^2\text{s}^{-1}$, $\phi = 5 \cdot 10^{-4}/\nu$, $\hat{P}_{\text{in}} = 10^{-1}/\nu$, $\hat{P}_{\text{out}} = 10^{-4}/\nu$, where ν is the molecular volume of P and S and can be chosen arbitrarily.*

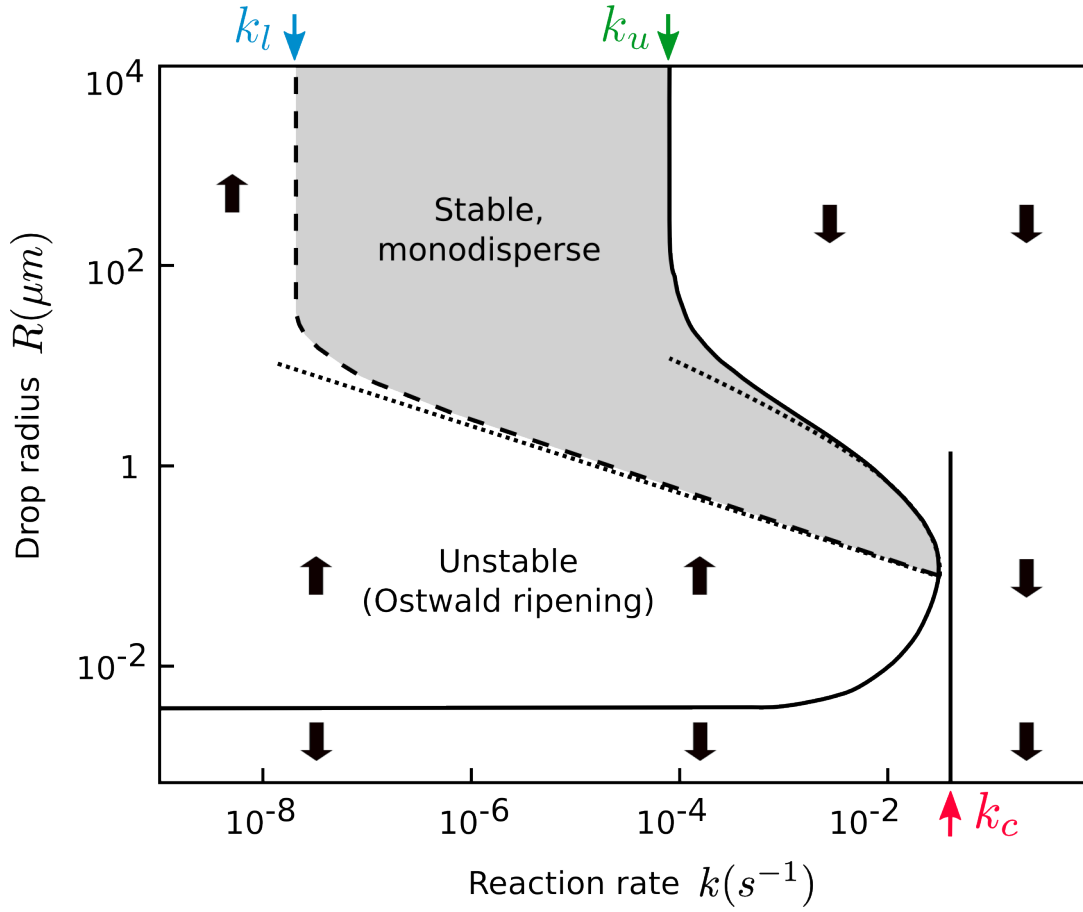


Figure 4.4: *The stability of a multi-drop system at fixed backward reaction rate constant h . A steady-state drop radius R exists in the region enclosed by the continuous line and depends on the rate constant k and the drop number density ρ . Outside this region no steady-states exist and drops dissolve (downward arrows). The lower part of this line represents the smallest possible drop, or nucleus. The steady-state is stable inside the grey region. Outside this region the steady-state is unstable to Ostwald ripening causing the average radius to increase (upward arrows). The stability-instability boundary ($g_1(R) = 0$, Eq. (4.47)) is shown with a dashed line. For rate constants k smaller than k_l (blue arrow) the system is always unstable (Lifshitz-Slyosov regime). For rate constants larger than k_l , a system of small drops is unstable and coarsen (upward arrows). For rate constants larger than k_u (green arrow) there exists an upper bound on R^* . For rate constants larger than k_c (red arrow and black straight line) no steady-states exist and drops dissolve. The values of k_l , k_u and k_c displayed by the arrows are analytical results from Sections 4.5 and 4.6. The analytical expressions for the upper bound radius (R_u in Eq. (4.55)) and the stability-instability boundary (R_l in Eq. (4.65)) are shown by the dotted lines. There is a good agreement between our analytical calculation and the numerical solutions. Parameters: $h = 10^{-2} \text{ s}^{-1}$ and the rest are as in Fig. 4.3 with ρ being variable.*

also be stable, but with an upper bound on the radius. Beyond k_c , no drops can exist in the system as all drops evaporate (downward arrows).

Using the formalism developed in Sections 4.1 to 4.3, we now will explain analytically the salient features of the stability diagram (Fig. 4.4) by focusing on distinct limits in the small supersaturation limit ($R \ll L$), and the strong phase separation regime ($\hat{P}_{\text{in}} \gg \hat{P}_{\text{out}}$).

4.5 Small drops

By small drops, we will refer to drops that are much smaller than the gradient length scale ξ ($R \ll \xi$, Eq. (4.26)). Within this regime, we will distinguish between two sub-regimes based on the magnitude of the drop number density ρ : the large ρ regime, where the inter-drop distance L (Eq. (4.9)) is much smaller than the gradient length scale ξ , and the vanishing ρ regime where $L \rightarrow \infty$.

4.5.1 Vanishing drop number density

We start with the vanishing drop number density ρ regime. Here, $L/\xi \rightarrow \infty$ and $R/\xi \ll 1$. Note that this scenario also corresponds to a single drop in an infinite system, since $L \rightarrow \infty$ in this case.

Steady-state drop radius. By expanding the steady-state condition Eq. (4.40) for $L/\xi \rightarrow \infty$ and $R/\xi \ll 1$, we find that the steady-state drop radius R_u is given by the relation (see Appendix B.5 for the calculation details):

$$R_u^2 \simeq \frac{3D}{k\hat{P}_{\text{in}}} \left[\frac{\phi h}{k+h} - \hat{P}_{\text{out}} \left(1 + \frac{l_c}{R_u} \right) \right], \quad (4.54)$$

and we remind that l_c is related to the surface tension of the drop interface (Section 2.3.2). For drops much larger than the capillary length l_c , we can neglect the surface tension:

$$R_u \simeq \sqrt{\frac{3D \left(\frac{\phi h}{k+h} - \hat{P}_{\text{out}} \right)}{k\hat{P}_{\text{in}}}}, \quad (4.55)$$

which is indicated by the upper dotted line in Fig. 4.4. Note that the drop radius R_u is independent of the drop number density ρ .

In this regime, the steady-state drop radius in ternary mixtures is identical than

that in binary mixtures diluted in P (see Eq. (3.24)). For $\phi = 1$, $\hat{P}_{\text{out}} = 0$ and $\hat{P}_{\text{in}} = 1$, we recover the results in [17].

Critical forward reaction rates. The radius R_u given by Eq. (4.55) has been obtained by assuming that R_u is much smaller than the gradient length scale ξ . Since R_u increases as k decreases, k must have a lower bound in this regime ($R \ll \xi$, $\rho \rightarrow 0$). By solving $R_u \ll \xi$ (Eqs (4.55), (4.26)) we find the condition on the forward rate constant k :

$$k \gg \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} h . \quad (4.56)$$

For rate constants smaller than that, the drop radius R_u becomes comparable or larger than the gradient length scale ξ . Therefore drops are no more adequately described by (4.55). We will treat this scenario in Section 4.6.

On the other hand, as the forward reaction rate constant k increases, the steady-state drop radius R_u decreases. When k is larger than a critical value k_c the steady-state radius relation Eq. (4.54) admits no solutions so all drops dissolve. The value of k_c is bounded as follow (see Appendix B.6 for calculation details):

$$k_c \leq \min \left\{ \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} h \quad ; \quad \frac{4D \left(\phi - \hat{P}_{\text{out}} \right)^3}{9l_c^2 \hat{P}_{\text{in}} \hat{P}_{\text{out}}^2} \right\} . \quad (4.57)$$

Note that $k > (\phi - \hat{P}_{\text{out}})/\hat{P}_{\text{out}}h$ corresponds to the situation where the conversion $P \rightarrow S$ is so strong that the system is outside the passive phase-separating region ($P_{\text{tot}} < \hat{P}_{\text{out}}$, see Fig. 4.1). The rate constant $k = (\phi - \hat{P}_{\text{out}})/\hat{P}_{\text{out}}h$ is shown with a red arrow in Fig. 4.4.

Critical backward reaction rate. When the reaction rate constants k and h are large enough, the gradient length scale ξ (Eq. (4.26)) becomes smaller than the nucleus radius R_n in passive systems ($k, h = 0$), which expression is (Appendix B.3, Eq. B.38)

$$R_n \simeq \frac{\hat{P}_{\text{out}} l_c}{\phi - \hat{P}_{\text{out}}} . \quad (4.58)$$

In an active system ($k, h > 0$), drops radius can only be larger than R_n (Appendix B.4). Therefore, since we must have $R \ll \xi$ in this regime, we also have the

requirement that $\xi \gg R_n$, or equivalently:

$$k < \frac{D(\phi - \hat{P}_{\text{out}})^2}{l_c^2 \hat{P}_{\text{out}}^2} - h. \quad (4.59)$$

Combining the two conditions Eqs (4.56) and (4.59), it results that the backward reaction rate constant h must be smaller than the critical rate

$$h_0 = \frac{D}{l_c^2} \left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} \right)^2. \quad (4.60)$$

where we have used the strong phase separation approximation $\hat{P}_{\text{in}} \gg \hat{P}_{\text{out}}$.

We have shown that if the backward rate constant h is larger than h_0 , or if the forward rate constant k is too small (Eq. (4.56)), drops leave the small drop regime defined by $R \ll \xi$. We will treat the large drop regime ($R \gg \xi$) in Section 4.6. On the other hand, if the forward rate constant k is larger than k_c , drops dissolve and phase separation is destroyed.

Multi-drop stability. We can now study the stability of a multi-drop system at steady-state, with drops of radius R_u (Eq. (4.55)). Taking again $L/\xi \rightarrow \infty$ and $R/\xi \ll 1$ and expanding the stability relation $g_1(R_u)$ (Eq. (4.47)), we find (see Appendix B.5 for the derivation details):

$$g_1 \simeq -\frac{8\pi D H_{\text{in}} R_u^2}{3\xi^2} \quad (4.61)$$

$$< 0 \quad (4.62)$$

where H_{in} is given by Eq. 4.27. Since $g_1 < 0$, we conclude that a multi-drop system in the regime of small drops and vanishing drop number density ($R \ll \xi$, $\rho \rightarrow 0$) is stable against Ostwald ripening. This result was also demonstrated in binary mixtures [17].

4.5.2 High drop number density

We now study the regime where the drop radii R and the inter-drop distance L are both small compared to the gradient length scale ξ . In other words, both R/ξ and L/ξ are small quantities. For simplicity we focus only on the region $k \gg (\phi - \hat{P}_{\text{out}})/\hat{P}_{\text{in}} h$ (Eq. (4.56)).

Steady-state drop radius. Expanding for $R/\xi \ll L/\xi \ll 1$, the steady-state condition Eq. (4.40) gives the steady-state drop radius (see Appendix B.7 for calculation details):

$$R^3 \simeq \frac{\frac{\phi h}{k+h} - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} L^3 \quad (4.63)$$

Therefore in this regime, contrary to the regime of vanishing drop number density (Section 4.5.1), the drop size scales with the system size ($\propto L$).

Multi-drop stability Using again $R/\xi \ll L/\xi \ll 1$ we expand the stability relation $g_1(R)$ (Eq. (4.47)) (see Appendix B.7 for calculation details):

$$g_1 = 4\pi D \left(\frac{\hat{P}_{\text{out}} l_c}{R} - \frac{2R^2 k \hat{P}_{\text{in}}}{3D} \right). \quad (4.64)$$

The system is unstable for small radius R ($g_1 > 0$) and stable for large R ($g_1 < 0$). The stability-instability boundary radius R_l is the solution of $g_1(R_l) = 0$:

$$R_l \simeq \left(\frac{3D l_c \hat{P}_{\text{out}}}{2k \hat{P}_{\text{in}}} \right)^{\frac{1}{3}}. \quad (4.65)$$

which is indicated by the lower dotted line in Fig. 4.4. We recover the result previously found for binary mixtures (Section 3.4, Eq. 3.34). Note that $\hat{P}_{\text{out}} = 0$ does not implies $R_l = 0$ because l_c has a dependence in $\hat{P}_{\text{out}}$ (Eq. 2.24). A multi-drop system is stable if the drop radius is larger than R_l .

4.6 Large drops

We saw in Section 4.5 that the small drop regime ($R \ll \xi$) breaks down for some reaction rate constants k and h (Eqs (4.56), (4.60)). We focus here on the large drop regime, defined by drops much larger than the gradient length scale ξ ($R \gg \xi$). Since the inter-drop distance L is always larger than R , this regime also implies that $L \gg \xi$. Again, we assume small supersaturation ($R \ll L$) and strong phase separation ($\hat{P}_{\text{in}} \gg \hat{P}_{\text{out}}$).

Steady-state drop radius We expand the steady-state condition Eq. (4.40) for $L/\xi \gg R/\xi \gg 1$ and find (see Appendix B.8 for calculation details):

$$R^3 \simeq \left(a + \frac{b}{R}\right) L^3, \quad (4.66)$$

with

$$a = \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} - \frac{k}{2h} \quad (4.67)$$

$$b = -\frac{\hat{P}_{\text{out}} l_c}{\hat{P}_{\text{in}}} + \frac{\xi k}{2h}. \quad (4.68)$$

The steady-state drop radius R scales as the system size ($\propto L$), with a finite size correction (b/R). When $k = 0$ and $h > 0$ no chemical reactions occur and we recover the passive steady-state radius (Appendix B.3, Eq. (B.37)), because $P_{\text{tot}} = \phi$ (Eq. (4.10)). In particular, the finite size correction is negative ($b = -\hat{P}_{\text{out}} l_c / \hat{P}_{\text{in}}$) and originates from the Gibbs-Thomson relation (Eq. (4.8)). Interestingly when chemical reactions are switched on ($k, h > 0$) the correction b/R becomes positive if the rate constant k is larger than a critical value, which is the solution of $b(k) = 0$:

$$k = \frac{2l_c \hat{P}_{\text{out}} h^{3/2}}{D^{1/2} \hat{P}_{\text{in}}}. \quad (4.69)$$

We have used the fact that $k < (\phi - \hat{P}_{\text{out}}) / \hat{P}_{\text{in}} h$, since otherwise the system is in the small drop regime (Eq. (4.56)), which implies that $k \ll h$. We note that the situation where b is positive is not associated to the existence of an effective negative surface tension. Indeed, we recall that thermal equilibrium is assumed to hold at the drop interfacial region, even in the presence of chemical reactions. Therefore the surface tension is always identical to the equilibrium surface tension. In other words, the local stability of the interface is always ensured, irrespective of the value of b . We note that the presence of fluxes across the interface, caused by concentration gradients (Eqs (4.24) and (4.25)), may however destabilise the spherical shape of the interface, leading to potential deformation or division of large drops [58]. This analysis is beyond the scope of this thesis, and the Monte Carlo simulations conducted in Chapter 5 did not reveal such instabilities. Nonetheless, we shall see that the transition to the “inverse Gibbs-Thomson regime” ($b > 0$) affects the system coarsening behaviour when multiple drops are present.

Multi-drop stability. We expand the stability relation $g_1(R)$ (Eq. (4.47)) for $L/\xi \gg R/\xi \gg 1$ (see Appendix B.8 for calculation details):

$$g_1 = 4\pi D \left(-\frac{k\hat{P}_{\text{in}}}{h} + \frac{2l_c\hat{P}_{\text{out}}h^{1/2}}{D^{1/2}} \right), \quad (4.70)$$

The stability in this regime is independent of the drop radius R . The system is unstable at small k ($g_1 > 0$) and stable at large k ($g_1 < 0$). The critical rate constant k_l at which the stability-instability transition occurs is the solution of $g_1(k_l) = 0$:

$$k_l = \frac{2l_c\hat{P}_{\text{out}}h^{3/2}}{D^{1/2}\hat{P}_{\text{in}}}, \quad (4.71)$$

which is indicated by the blue arrow in Fig. 4.4. When the rate constant k is larger than k_l , a multi-drop system becomes stable. Interestingly, at this rate, the system also transitions into the “inverse Gibbs-Thomson regime” (Eq. (4.69)).

Critical reaction rates. In large drop limit, $b/R \rightarrow 0$, and there is a critical rate constant k_u above which drops cease to exist ($R < 0$):

$$k_u = 2\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}h \quad (4.72)$$

which is indicated by the green arrow in Fig. 4.4. This is consistent with the previous result that drops are in the small drop regime for $k \gg k_u$ (Eq. (4.56)).

As the backward rate constant h increases, the stability-instability boundary rate constant k_l (Eq. (4.71)) increases faster than the critical rate constant k_u (Eq. (4.72)) does. When h becomes larger than a critical value h'_0 , we have $k_l > k_u$. Since no large drops can exist for $k > k_u$, large drops are always unstable when $h > h'_0$. We find h'_0 by solving $k_u(h'_0) = k_l(h'_0)$:

$$h'_0 = \frac{D}{l_c^2} \left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} \right)^2 \quad (4.73)$$

$$= h_0. \quad (4.74)$$

Interestingly, the critical rate constant h'_0 is equal to the critical backward rate constant h_0 in the small drop regime (4.60), above which no small drops ($R \ll \xi$) can exist. Therefore, for $h > h_0$, large drops are always stable, and all drops dissolve for $k > k_u$ since no small drop regime exist in this case.

4.7 Stability diagrams

In section 4.5 and 4.6 we have described analytically the steady-state radius and the stability of our active multi-drop system. Depending on the magnitude of the reaction rate constants k and h , relative to the critical forward rate constants k_l , k_u , k_c (Eqs (4.71), (4.72), (4.57)), and backward rate constant h_0 (Eqs (4.73)), the system exhibits various qualitative features. We showed in Fig. 4.4 the stability diagram for varying forward rate constant k and fixed backward rate constant h ($h < h_0$, Eq. (4.73)).

We can also summarize our results in the reaction rate constant space, as shown in the stability diagram in Fig. 4.5. We focus first on the scenario where $h < h_0$ (see black arrow). When k is smaller than the critical rate constant k_l (Eq. (4.71), blue regular dashed line), a multi-drop system is always unstable to Ostwald ripening. For $k_l < k < k_u$ (green irregular dashed line, Eq. (4.72)), large drop systems ($R \gg \xi$) are stable. For $k_u < k < k_c$ (red continuous curve, Eq. (4.57) and Eq. (B.57) in Appendix B.6), drop radii are maximally bounded by R_u (Eq. 4.55), and the multi-drop system stability depends on the drop radius. All drops dissolve for $k > k_c$. In the case where $h > h_0$, drops are always unstable, have no upper bound radii, and dissolve for $k > k_u$.

4.8 Comparison with existing results

In this chapter we have improved and generalized upon multiple assumptions adopted in the work of Zwicker et al. [17]. In particular, we have analysed the large drop regime, the regime of non-negligible supersaturation, we have allowed for the presence of solvent by considering a ternary fluid, and considered arbitrary equilibrium concentrations $\hat{P}_{\text{in}}$, $\hat{P}_{\text{out}}$, inside and outside the drops. In Figs 4.6 and 4.7 we show how these generalisations affect qualitatively the stability diagrams by exploring the effect of solvent inclusion. In Fig. 4.6, we show the stability diagram for varying solvent concentration, keeping the other parameters as in the Ref. [17]. In particular, we find that for non-zero solvent concentration the arrest of Ostwald ripening occurs above a forward reaction rate constant threshold (k_l , blue line and blue arrows, Eq. (4.71)), demonstrating that this transition belongs to the non-equilibrium regime entirely. Additionally for $k_l < k < k_u$ (green curve, Eq. (4.72)), there is a regime where a multi-drop assembly can be stable, with the drop radius R not upper bounded and given by Eq. (4.66) if $R \gg \xi$. In Fig. 4.7 we compare the stability diagrams without solvent (a)) and with solvent (b)), for varying forward reaction

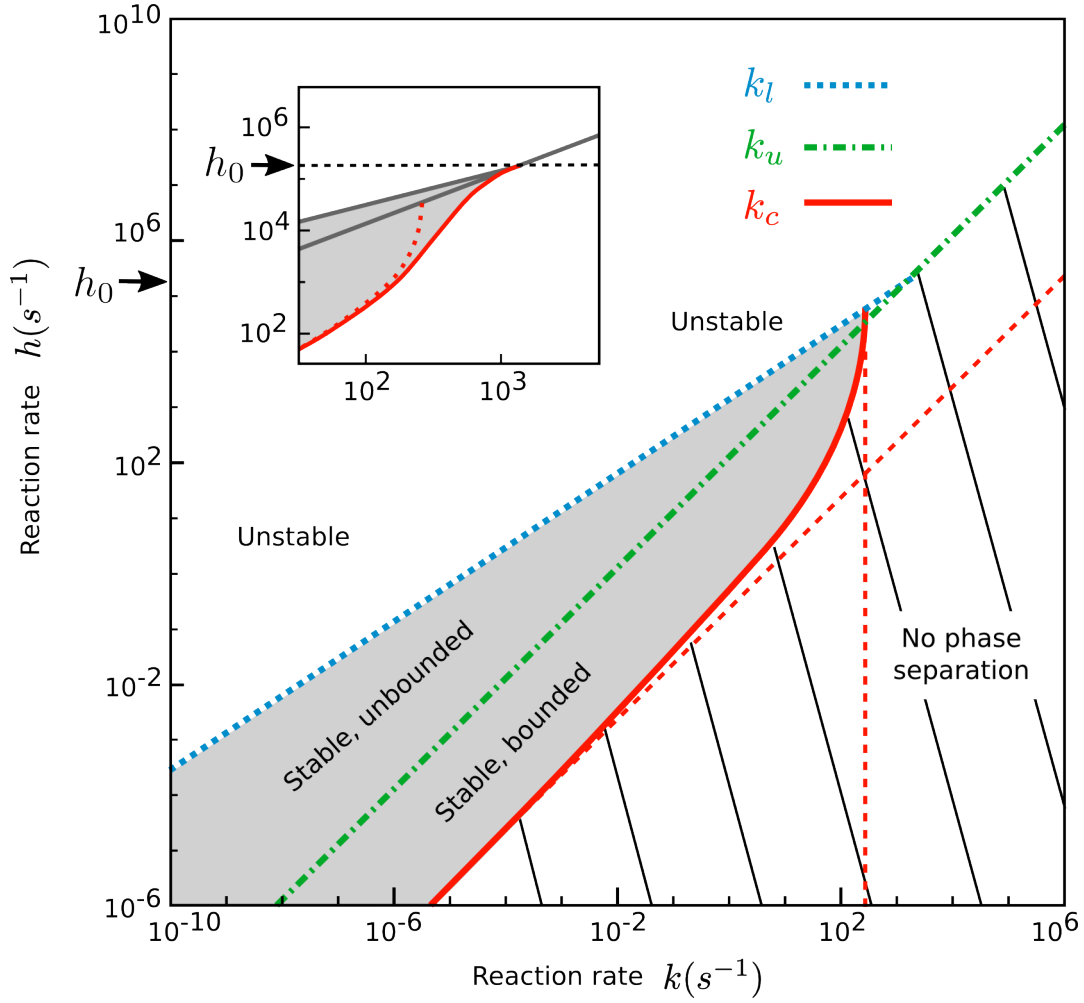


Figure 4.5: *Stability diagram of a multi-drop system in the reaction rate constant space.* In a multi-drop system, drop existence, radius and stability, depend on the chemical reaction rate constants k and h . For backward rate constants h smaller than h_0 (black arrow, Eq. (4.73)), multi-drop systems are stable against Ostwald ripening (grey region) if the forward rate constant k is between k_l (blue dotted line, Eq. (4.71)) and k_c (red continuous line, Eq. (B.57)). The upper bounds of k_c estimated in Eq. (4.57) are shown in red dashed lines. In the white region, multi-drop systems are unstable. The drop radius is upper bounded (Section 4.5.1) if $k > k_u$ (green irregular dashed line, Eq. (4.72)), or unbounded otherwise. Phase separation is destroyed and drops dissolve in the hashed area. The validity of the expression of k_c given by Eq. (B.57) breaks down when the drop radius R approaches the gradient length-scale ξ . In this region, we determined k_c by solving exactly the steady-state relation Eq. (4.40) (continuous red line in the insert figure. For comparison, k_c determined from (B.57) is showed by the dotted red line). Parameters: $l_c = 10^{-2}\mu\text{m}$, $D = 1\mu\text{m}^2\text{s}^{-1}$, $\phi = 5 \cdot 10^{-4}/\nu$, $\hat{P}_{\text{in}} = 10^{-1}/\nu$, $\hat{P}_{\text{out}} = 10^{-4}/\nu$, where ν is the molecular volume of P and S and can be chosen arbitrarily.

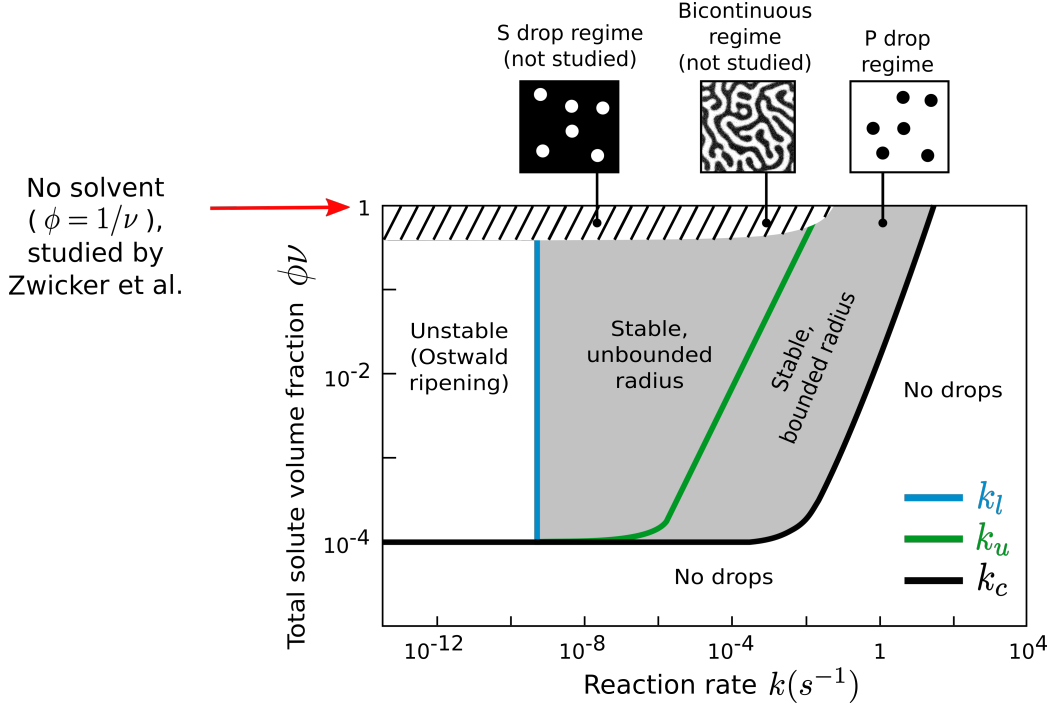


Figure 4.6: *Effect of the solvent inclusion on the stability diagram.* In the case studied by Zwicker et al. [17] where no solvent is present ($\phi = 1/\nu$, red arrow), the steady-state can only be of two kinds: for $k > k_c$ (black curve, Eq. (4.57)) no drops exist, and for $k < k_c$ stable drops can form whose radii are upper bounded (see Fig. 4.7 a), and Section 4.5). For small enough k (hashed area) the volume fraction of P is so large that the system forms bi-continuous structures, or S -rich drops surrounded by a P -rich phase. Therefore the current model (P -rich drops surrounded by an S -rich phase) breaks down. As we allow for the presence of solvent by relaxing the constraint on ϕ , we discover two novel system behaviors: for $k_l < k < k_u$ (k_l and k_u are shown by the blue and green curves, respectively, Eqs (4.71) and (4.72)), multiple drops can exist at stable steady-state, which radii are not upper bounded (see Fig 4.7 b) and Section 4.6), and for $k < k_l$ drops are always unstable against Ostwald ripening. Parameters: $l_c = 10^{-2}\mu m$, $D = 1\mu m^2 s^{-1}$, $h = 10^{-2}s^{-1}$, $\hat{P}_{in} = 1/\nu$, $\hat{P}_{out} = 10^{-4}/\nu$, $h = 10^{-2}s^{-1}$, $\Delta S = 0$, where ν is the molecular volume of P and S and can be chosen arbitrarily. $\Delta S = 0$ signifies that the S concentration is zero inside drops (Eq. (B.99)), as in Zwicker et al. [17].

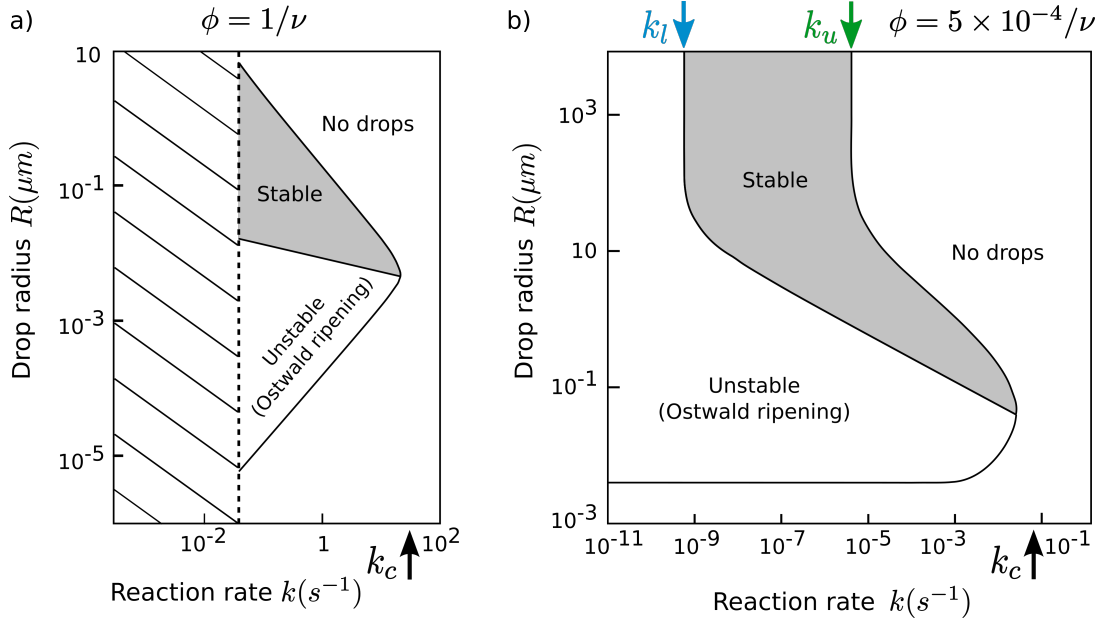


Figure 4.7: *Stability diagrams for varying forward rate constant k and drop radius R , with and without solvent.* a) Scenario without solvent ($\phi = 1/\nu$), studied by Zwicker et al. [17]. For $k > k_c$ (black arrow, Eq. (4.57)) no drops exist. For $k < k_c$ drops can form and the steady-state (grey area) is composed of stable drops with upper bounded radii R (Section 4.5). At small enough k (hashed area) the P-rich drop model breaks down (see hashed area and caption in Fig. 4.6). b) Scenario with solvent ($\phi < 1/\nu$), which study is made possible with the model developed in this chapter. Compared to the no-solvent case (a)), two novel behaviors are discovered. For $k_l < k < k_u$ (blue and green arrows, Eqs (4.71) and (4.72)), the steady-state (grey area) is composed of stable drops which radii are not upper bounded (Section 4.6), and for $k < k_l$ drops are always unstable against Ostwald ripening. Parameters: a) $\phi = 1/\nu$, b) $\phi = 5 \times 10^4/\nu$ and the rest are as in Fig. 4.6.

rate constant k and drop radius R .

4.9 Summary & discussion

In summary, we have described theoretically the drop formation, radius and stability in a ternary mixture with active chemical reactions. In particular, we have categorised the qualitative behaviour of the system into distinct regimes based on the reaction rates. We have generalized upon assumptions from previous work [17, 55], and have identified qualitatively distinct regimes. Importantly, in order to arrest Ostwald ripening, the reaction rate constants have to belong to a particular range, demonstrating that such transition belongs entirely to the non-equilibrium regime. Additionally, our formalism goes beyond the negligible supersaturation limit and allows the study of systems with intermediate drop density.

Our calculation is based on the assumption that a multi-drop system can be described by our two drop-system with a mean-field matching at the system boundaries (Eqs (4.4), (4.5)). In Chapter 5, we test this assumption using Monte Carlo simulations.

Our work is of direct importance to cytoplasmic organisation. In Chapter 6, we apply our results to the formation of stress granules, a class of cytoplasmic organelles which lack a membrane. Our work is also relevant to the control of emulsions in the engineering setting. Interesting future directions include the incorporation of drop coalescence into our coarsening picture, the study of potential shape instabilities in chemically active drops [58], and the generalization of our formalism to many-component mixtures [59, 60].

Chapter 5

Monte Carlo Simulations of Phase-Separated Drops

We described theoretically, in Chapter 4, the drop formation, radius and stability in a ternary mixture in the presence of active chemical reactions. We based our calculation on a number of assumptions. First, we assumed that even in the presence of active chemical reactions, thermal equilibrium remains valid locally. At a drop interface, this led us to describe the coexistence concentrations with the Gibbs-Thomson relations (Eqs (4.7), Eqs (4.8)). Inside and outside a drop, we assumed that the concentration profiles obey reaction diffusion equations (Eqs (4.2), (4.3)). Finally, we approximated a multi-drop system by a two-drop system, with a mean-field matching assumption at the system boundaries (Eqs (4.4), (4.5)). In this chapter we test these assumptions by performing Monte Carlo simulations of our ternary model on a 2D lattice (see Appendix B.10 for details about the theory in two dimensions).

5.1 General method

We study the dynamics of chemically active drops in a ternary fluid using Monte Carlo simulation methods [61]. We consider a ternary mixture P, S, C on a two dimensional square lattice where each site has the dimension Δd . Each particle P interact with its 8 nearest neighbours so that every $P - P$ pair contributes to the system energy by e_{PP} . The total system Hamiltonian is

$$H = N_{PP}e_{PP} \tag{5.1}$$

where N_{PP} is the total number of $P - P$ pairs in the system.

We enumerate the simulation steps carried out within a simulation time unit Δt . To simulate the system, we use the Metropolis-Hastings algorithm together with the Kawasaki exchange scheme [62]. The entire lattice is searched sequentially for sites occupied by a P or S . When such site is found, one of its 8 nearest neighbour is randomly selected. The two sites are then exchanged with probability

$$p = \begin{cases} e^{-\Delta H/(k_b T)} & \Delta H > 0 \\ 1 & \Delta H \leq 0 \end{cases} \quad (5.2)$$

where ΔH is the change in Hamiltonian caused by the exchange, T the temperature and k_b the Boltzmann constant. We then consider the chemical reactions that convert P into S and vice versa:



where k and h are the reaction rate constants. The entire lattice is again sequentially searched for sites occupied by a P or S . When a site with a P is found, the P is destroyed and replaced by a newly created S , with the probability k . If a site with a S is found, the S is destroyed and replaced with a newly created P , with the probability h .

5.2 Volume fraction profiles in a single-drop system

We first test the local thermal equilibrium assumption. We study the volume fraction profiles in a single-drop system in passive ($k = h = 0$) and active ($k, h > 0$) conditions (Fig. 5.1). We find that the coexistence volume fractions of P at the interface are roughly similar both in the passive and active case. In addition, the profiles inside and outside drops are no longer flat, as predicted theoretically (Eqs (4.29), (4.25)). This supports the assumption of local thermal equilibrium in our system.

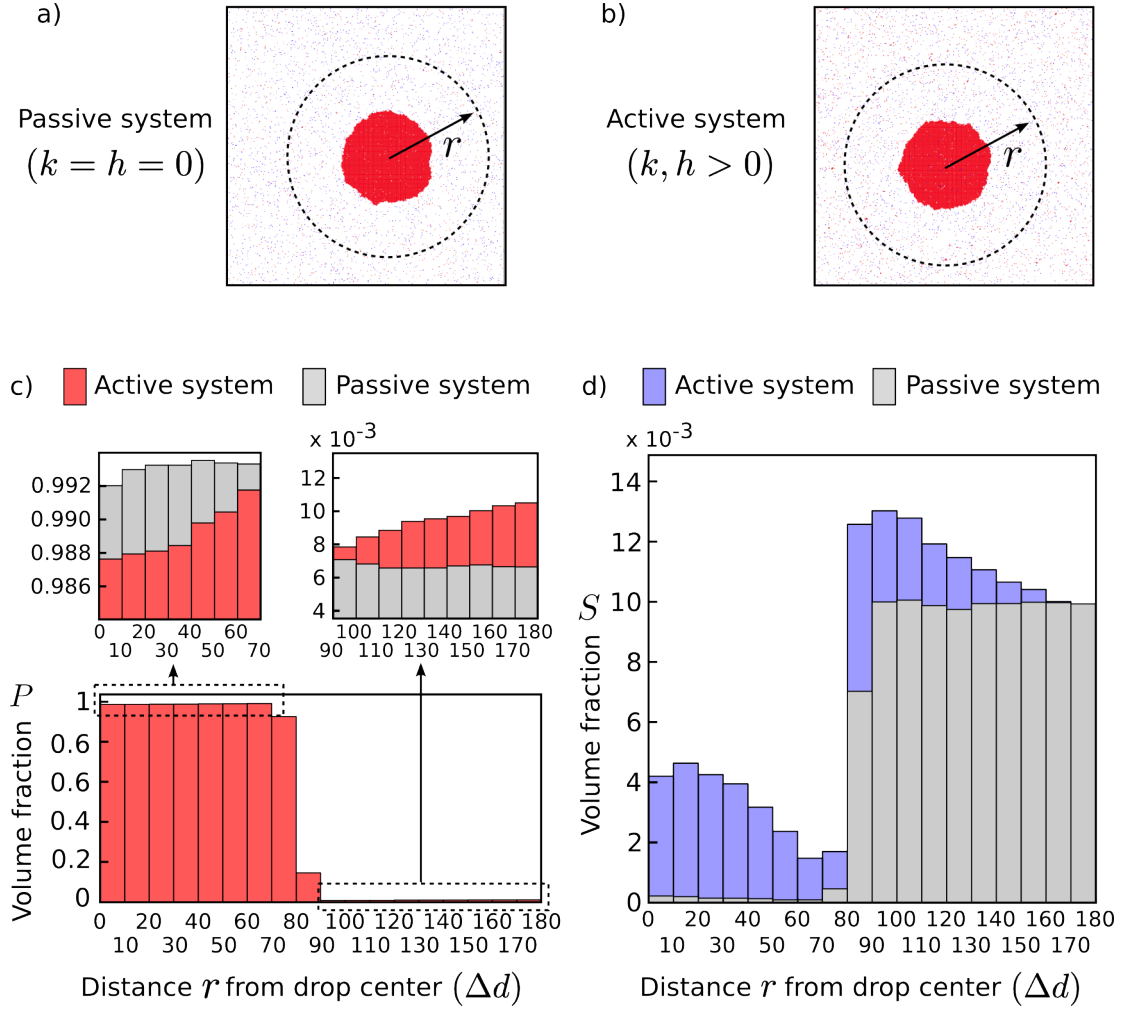


Figure 5.1: Volume fraction profiles in a single-drop, passive system ($k = h = 0$) (a)), and active system ($k, h > 0$) (b)). c) At the drop interface, the P profiles are similar both in the active and the passive case. In the active system, volume fraction gradients in P and S exist inside and outside drops (c) and d)). The profiles are radially averaged inside a disc centred on the drop centre of mass (dashed line in a) and b)), then averaged over multiple samples. Parameters: system size= $500 \times 500 (\Delta d)^2$, disc radius= $180 \Delta d$, $\phi = 0.1$. $\epsilon_{PP} = -9/7$. Passive parameters: $P_{\text{tot}} = 1/11$, $S_{\text{tot}} = 1/110$. Active parameters: $k = 2 \times 10^{-6}(\Delta t)^{-1}$, $h = 2 \times 10^{-5}(\Delta t)^{-1}$.

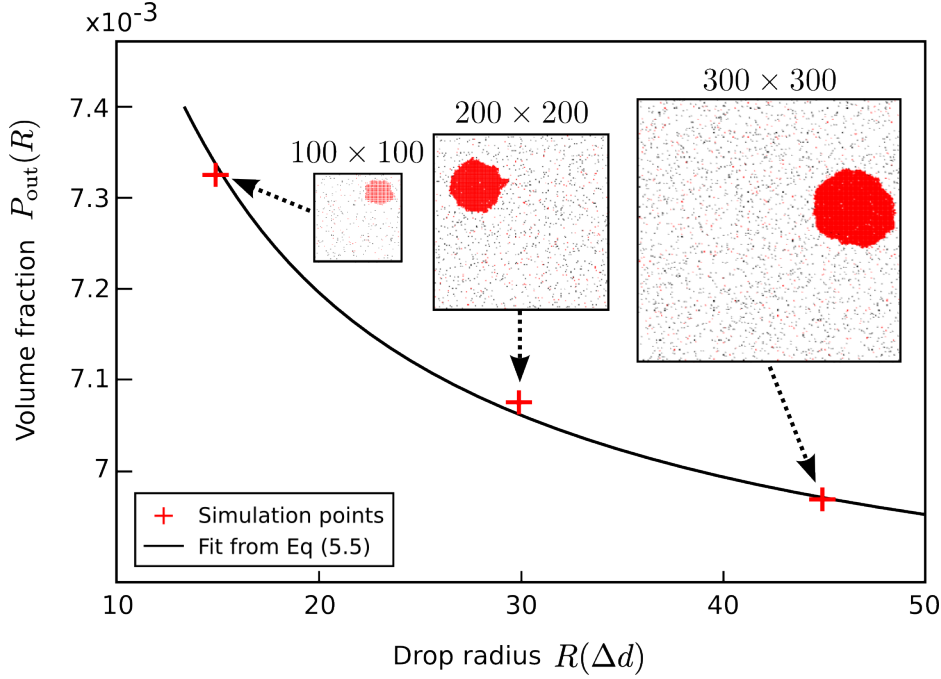


Figure 5.2: *Determination of the equilibrium parameters in the Monte Carlo simulations: capillary length l_c and saturation volume fraction $\hat{P}_{\text{out}}$.* We simulate three single-drop, passive ($k = h = 0$) systems, of different sizes (snapshots). We extract their drop radius R and the volume fraction of P outside drops $P_{\text{out}}(R)$. We fit the results to the Gibbs-Thomson relation (Eq. (5.5)) (red points). We perform a linear regression of $P_{\text{out}}(R) \times R = \hat{P}_{\text{in}} \times R + l_c$ and find $l_c = 1.2 \pm 0.1 \Delta d$ and $\hat{P}_{\text{out}} = (6.79 \pm 0.02) \cdot 10^{-3}$ (see black curve for best fit). To cancel out fluctuations in R and $P_{\text{out}}(R)$ we calculate their mean values by averaging a large number of samples. Moreover $P_{\text{out}}(R)$ is also spatially averaged in a square region in the dilute phase. Parameters: $P_{\text{tot}} = 1/13$, $S_{\text{tot}} = 3/130$, $e_{PP} = -9/7$, system sizes = 100×100 , 200×200 , 300×300 $(\Delta d)^2$.

5.3 Determination of the equilibrium parameters

In our theoretical description we used the Gibbs-Thomson relations to describe the interface:

$$P_{\text{in}}(R) = \hat{P}_{\text{in}} \quad (5.4)$$

$$P_{\text{out}}(R) = \hat{P}_{\text{out}} \left(1 + \frac{l_c}{R} \right) \quad (5.5)$$

with $P_{\text{in}}(R)$ and $P_{\text{out}}(R)$ the interface volume fraction of P , inside and outside the drop, respectively. R is the drop radius, l_c is the capillary length, and $\hat{P}_{\text{in/out}}$ are the coexistence volume fractions in a passive system (Fig. 4.1). In order to compare our simulations with our theory, we need to determine the value of the capillary length l_c and the phase coexistence volume fractions $\hat{P}_{\text{in/out}}$ associated to

our simulations.

We simulate single-drop systems of different sizes, and extract for each system the drop radius R and the P volume fraction $P_{\text{out}}(R)$ in the dilute phase. We then fit the results to the Gibbs-Thomson relation (Eq. (5.5)) (Fig. 5.2) and find:

$$l_c = 1.2 \pm 0.1 \Delta d \quad (5.6)$$

$$\hat{P}_{\text{out}} = (6.79 \pm 0.02) \times 10^{-3} \quad (5.7)$$

See Fig. 5.2 for the simulation parameters.

From the volume fractions profiles at the interface shown in Fig. 5.1, we approximate

$$\hat{P}_{\text{in}} = 1 \quad (5.8)$$

$$\Delta S = 0.1 \quad (5.9)$$

5.4 Relation between physical and simulation units

We establish the correspondence between the time and length units in the simulation (Δt , Δd) and the physical units (seconds, meters). The diffusion coefficient associated to a random walk on our lattice is given by

$$D = \frac{(\Delta d)^2}{2\Delta t} . \quad (5.10)$$

Equating D to the typical protein diffusion coefficient in the cytoplasm, $1\mu\text{m}^2.\text{s}^{-1}$, and Δd to the typical protein size, 10nm, we express the physical time and length in terms of Δt and Δd

$$1\text{s} = 2.10^4 \Delta t \quad (5.11)$$

$$1\mu\text{m} = 10^2 \Delta d . \quad (5.12)$$

Using this correspondence, the capillary length l_c (Appendix 5.3, Fig. 5.2), expressed in physical unit, is:

$$l_c = 1.2 \times 10^{-2} \mu\text{m} . \quad (5.13)$$

5.5 Stability-instability boundary radius

We seek the instability-stability boundary radius (dashed line in Fig. 4.4 and Eq. (4.65)). At time $t = 0$ we randomly distribute P and S molecules on the lattice in such a way that the system is inside the phase boundary ($P_{\text{tot}} > \hat{P}_{\text{out}}$, Fig. 4.1) and globally at chemical equilibrium ($P_{\text{tot}} = h\phi/(k+h)$, $S_{\text{tot}} = k\phi/(k+h)$, Eqs (4.10), (4.11)). In the early stage drops nucleate, grow, and undergo coarsening by fusion, when two drops collide, and by Ostwald ripening, leading to an increase of the average drop radius. Eventually coarsening is arrested and the system reaches a steady-state composed of drops with similar radii. This particular steady-state radius, that is reached by starting from small drops, is defined as the stability-instability boundary radius (Fig. 5.3(b)). The coarsening and steady-state regimes are shown in Fig. 5.3.

5.6 Comparison between theory and simulations

We now compare our simulations to our theoretical predictions for 2D systems (see Appendix B.10 for calculation details in 2D). Specifically, we focus on the stability-instability boundary radius (Section 5.5). We show the stability diagrams in Fig. 5.4, where the dashed line represents the stability-instability boundary radius R ($g_1 = 0$, Eq. (B.107)). We compare this boundary to our simulation results (red points in Fig. 5.4). In order to avoid excessively large simulation times, we studied the small drop regime ($R \ll \xi$, Section 4.5, Fig. 5.4(a)) and the large drop regime ($R \gg \xi$, Section 4.6, Fig. 5.4(b)) with two different choices of the backward rate constant h . We find a good agreement, between our theoretical predictions and Monte Carlo simulations.

5.7 Summary

We have performed Monte Carlo simulations to test the theory developed in Chapter 4. We found a good agreement with our theoretical predictions, thus supporting the validity of the mean-field assumption we employed (Section 4.1, Eqs (4.4), (4.5)). In particular, we confirmed that for a multi-drop system to be stable, the forward reaction rate constant must be larger than a critical value. Thus, the arrest of Ostwald ripening can belong entirely to the non-equilibrium regime. In Chapter 6, we apply the formalism developed in Chapter 4 to study the formation of stress granules, a class of cytoplasmic organelles that were presented in Chapter 1.

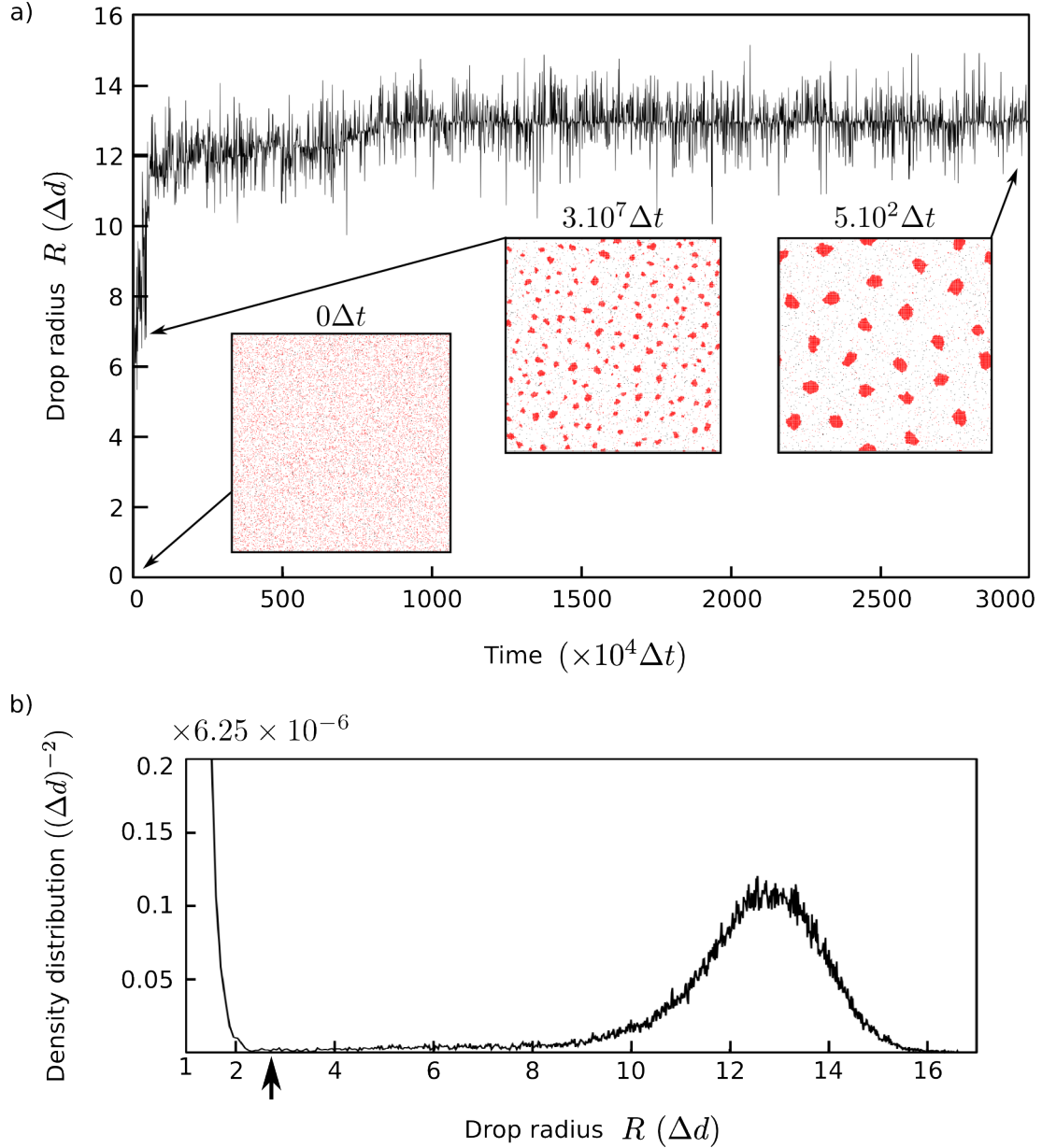


Figure 5.3: *Determination of the stability-instability boundary radius in Monte Carlo simulations.* In the initial conditions, particles P and S are randomly distributed on the lattice, ensuring global chemical equilibrium (Eqs (4.10), (4.11)). a) In the early stage drops nucleate and grow, then drops undergo coarsening via coalescence and Ostwald ripening leading to an increase of the average drop radius. Eventually coarsening is arrested and the system reaches a steady-state composed of drops with similar radii. This particular steady-state radius, that is reached when starting with small drops, is defined as the stability-instability boundary radius. Snapshots (inserts) are taken at different times and P and S and shown with red dots and blue dots, respectively. b) The steady-state radius is defined by the location of the highest peak in the drop radius distribution. The radius distribution is averaged during the second half of the simulation. We neglect the small drops that form transiently due to the stochastic fluctuations of the volume fractions by ignoring drops that contain less than 20 P molecules (arrow). Parameters: system size = $400 \times 400 (\Delta d)^2$, $\phi = 0.1$, $e_{PP} = -9/7$, $h = 10^{-4}(\Delta t)^{-1}$, $k = 10^{-5}(\Delta t)^{-1}$.

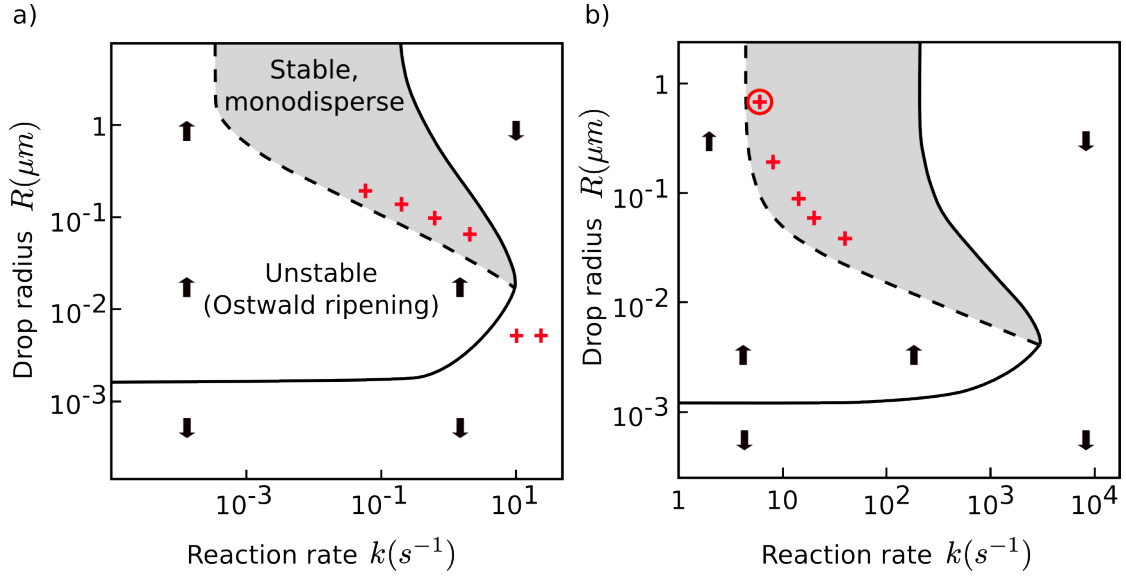


Figure 5.4: *Comparison between 2D theoretical predictions and Monte Carlo simulations.* The rate constant k is varied keeping the rate constant h fixed. A steady-state drop radius R (solution of $g_0(R) = 0$, Eq. (B.107)) exists in the region enclosed by the continuous line. Outside this region no steady-states exist and drops dissolve (downward arrows). The steady-state R is stable inside the grey region ($g_1(R) < 0$, Eq. (B.107)). Outside the grey region the steady-state is unstable to Ostwald ripening ($g_1(R) > 0$) causing the average drop radius to increase (upward arrows). The stability-instability boundary ($g_1(R) = 0$) is shown with a dashed line. Regarding the simulations, the lattice is initialized at $\Delta t = 0$ by randomly distributing P and S on the lattice in such a way that the system is inside the phase boundary ($P_{\text{tot}} > \hat{P}_{\text{out}}$, and see Fig. 4.1 in main text) and globally at chemical equilibrium (Eqs (4.10), (4.10)). In the early stage drops nucleate, grow and coarsen, leading to an increase of the mean drop radius, then coarsening is stopped and the system reaches a steady-state defined as the stability-instability boundary (Fig. 5.3). Simulation data are shown in red. The two rightmost crosses in a) represent the size of the lattice site ($\sim 10^{-2}\mu\text{m}$), i.e., there are no drops in system. The encircled cross in b) indicates that the system coarsened until a single drop remained even in the largest system simulated. There is a good agreement between theory and simulations. The duration of simulations range from $1.8 \times 10^7 \Delta t$ to $2 \times 10^8 \Delta t$. Parameters: $\phi = 0.1$, $D = 1\mu\text{m}^2\text{s}^{-1}$, $\hat{P}_{\text{in}} = 1$, $\hat{P}_{\text{out}} = 7.79 \times 10^{-3}$, $\Delta S = 0.1$, $l_c = 1.2 \times 10^{-2}\mu\text{m}$, $e_{PP} = -9/7$. Figure a): system size= $400 \times 400 (\Delta d)^2$, $h = 2 \text{ s}^{-1}$. Figure b): system size= $300 \times 300 (\Delta d)^2$ to $400 \times 400 (\Delta d)^2$, $h = 2 \times 10^3 \text{ s}^{-1}$. See Section 5.4 for the equivalence between simulation and physical units.

Chapter 6

Application of Formalism: Stress Granule Formation

We have developed in Chapter 4 a theoretical description of drops in a ternary fluid in the presence of energy-consuming chemical reactions, and verified in Chapter 5 the validity of our predictions by using Monte Carlo simulations. We now apply our formalism to investigate the formation of stress granules (SG), a class of cytoplasmic membrane-less organelles that were introduced in Chapter 1. Guiding our analysis with experimental observations, we present minimal models of SG regulation based on chemical reaction-controlled phase separation.

6.1 Experimental observations

Experimental studies have shown that SG assemble in response to multiple types of stress situations [45, 63, 64]. Several pathways for SG formation have been identified but the precise mechanisms of SG formation remain unclear. Interestingly, while various stress conditions causing SG assembly also cause a depletion of the cytoplasmic ATP concentration [43, 44, 63], ATP depletion alone has been shown to trigger SG formation [45]. Here, we will focus exclusively on how changes in ATP levels can regulate SG formation. In other words, we will make the assumption that the ATP concentration directly triggers SG formation through ATP-dependent biochemical reactions. In addition to this central premise, we will use two other pieces of biological observations to guide our modelling: 1) during normal conditions, i.e. without imposed stress, the ATP concentration is at the normal level and SG are absent, or at least are so small that they are undetectable microscopically. 2) When external stress is imposed, ATP can fall by 50% [44, 63], and SG assemble, with sizes of the order of a micrometer [38]. Hence a relatively mild change in the ATP level

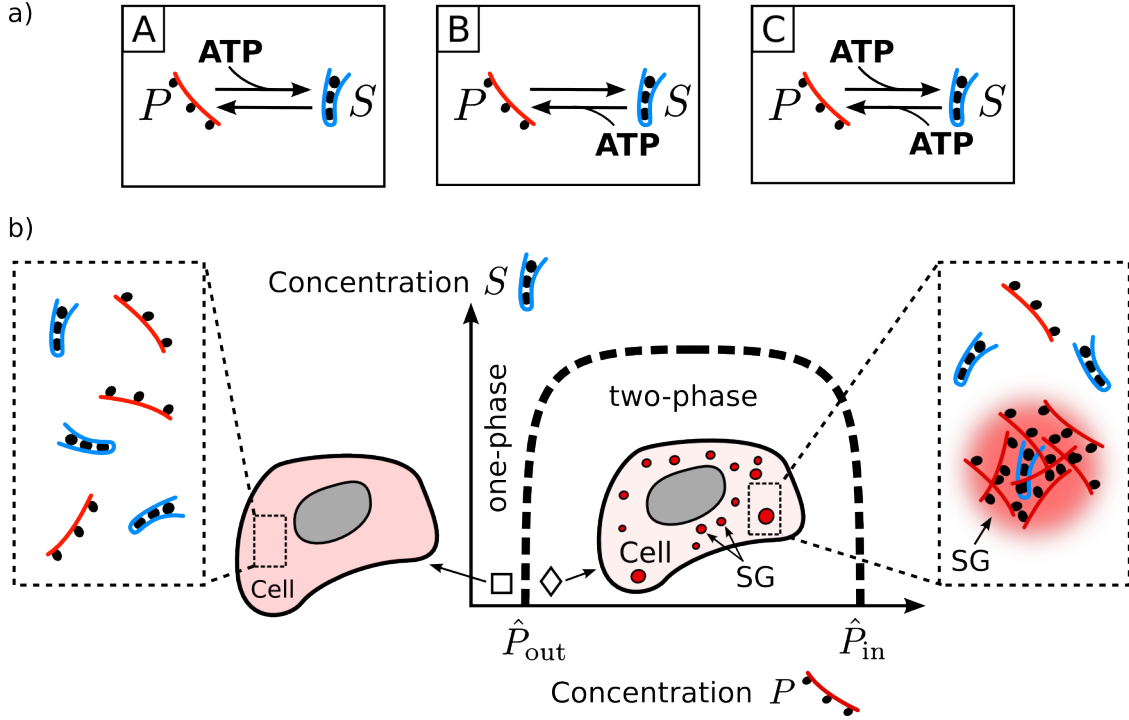


Figure 6.1: (a) *Distinct schemes of ATP-driven chemical reactions that control cytoplasmic SG formation.* The phase-separating form of the constituent molecules in SG is denoted by P , and the soluble form is denoted by S . In model A, ATP promotes conversion from P to S , while it is the reverse in model B. In model C, ATP drives both conversions. (b) *Phase diagram.* At low P concentration, molecules distribute homogeneously ($\square$ symbol). When the concentration of P increases beyond the phase boundary denoted by the dashed line, P -rich drops form and are surrounded by a P -poor phase ($\diamond$ symbol).

can lead to a very large change in SG size. We will therefore impose the following two constraints on our modelling: 1) *a decrease of the ATP level leads to the growth of SG*, and 2) *the response is switch-like*.

We now set out to construct minimal models that are compatible with these salient experimental findings on SG regulation.

6.2 Minimal models

We first describe a set of minimal models of SG regulation based on the principle of phase separation controlled by ATP-driven chemical reactions. We consider the cell cytoplasm as a ternary mixture of molecules: the phase-separating form of the SG constituent molecules (P), the soluble form of the same molecules (S), and the remaining molecules in the cytoplasm (C). By S being soluble, we mean that the concentration of S is continuous at a drop interface. Note that the constituents of

SG consist of many distinct proteins and mRNA. Therefore, P and S are meant to represent the average behaviour of the set of proteins and mRNA responsible for SG formation via phase separation [60]. The same applies to the component C , which represents the average behaviour of the cytoplasmic molecules not involved in SG formation. To control phase separation, we further assume that P and S can be inter-converted by chemical reactions that are potentially ATP-driven:



where k and h denote the forward and backward reactions. In the biological context these reactions can be protein post-transcriptional modifications. For example the phase behaviour (phase-separated or homogeneous) of intrinsically disordered proteins, a class of proteins that lack a well defined secondary structure, can be controlled via their phosphorylation/dephosphorylation [27,28]. In our minimal description, we assume that there is no cooperativity in the chemical reactions, and that the forward, backward, or both reactions are ATP-driven so that the reactions rate constants k, h do not depend on the local concentrations of the molecular components (see Section 1.4). This implies also no cooperativity in the chemical reactions. However, the rate constants can be influenced by the ATP concentration, denoted by α , in a linear manner. With these simplifications, we can categorise the distinct schemes into three models: A, B, and C (Fig. 6.1(a)). In model A, ATP promotes the conversion from the phase-separating state P to the soluble state S . In model B, ATP promotes the reverse reaction, and in model C, both reactions are driven by ATP. Specifically, we have:

$$\text{Model A : } \quad k_A(\alpha) = \alpha K_A, \quad h_A(\alpha) = H_A \quad (6.2)$$

$$\text{Model B : } \quad k_B(\alpha) = K_B, \quad h_B(\alpha) = \alpha H_B \quad (6.3)$$

$$\text{Model C : } \quad k_C(\alpha) = \alpha K_C, \quad h_C(\alpha) = \alpha H_C, \quad (6.4)$$

with K_i, H_i being constants, where $i = A, B, C$ refers to the model under consideration. Given these three minimal models, depicted in Fig. 6.1(a), we now aim to determine which one of them is the most compatible with experimental observations.

We assume that, in the presence of chemical reactions, our system remains close to equilibrium, so that the formalism developed in Chapter 4 applies. We briefly summarise the main results relevant to our analysis. At the interface, local thermal equilibrium implies that the concentration of P at each side of the interface are given by the Gibbs-Thomson relations. Denoting the concentrations of P and S by

the same symbols, we have, for a drop of radius R :

$$P_{\text{in}}(R) = \hat{P}_{\text{in}} \quad (6.5)$$

$$P_{\text{out}}(R) = \hat{P}_{\text{out}} \left(1 + \frac{l_c}{R} \right), \quad (6.6)$$

where the subscripts “in” and “out” denote the concentration profiles inside and outside the drop, and where we have assumed spherical symmetry with the centre of the drop being the origin of our coordinate system. $\hat{P}_{\text{in/out}}$ are the equilibrium coexistence concentrations given by the phase boundary (see Fig. 6.1(b)) with l_c being the capillary length (Section 2.3.2). Inside and outside drops the concentration profiles are given by (Eq. (4.24), (4.25)):

$$P_{\text{in/out}}(r) = U_0 + \frac{R}{r} \left(U_1 e^{r/\xi} + U_{-1} e^{-r/\xi} \right), \quad (6.7)$$

where U_n are independent of r but model-parameter dependent, and

$$\xi \equiv \sqrt{\frac{D}{k_i + h_i}} \quad (6.8)$$

with $i = A, B, C$, corresponds to the length scale of the concentration gradients induced by the chemical reactions, and D is the protein diffusion coefficient assumed to be equal for both molecules P and S , both inside and outside the drop. The profiles inside and outside drops thus have spatial gradients, as shown in Fig. 6.2. The overall concentrations of P and S in the whole system, denoted by P_{tot} and S_{tot} , respectively, are controlled solely by the chemical reaction rate constants (Eqs (4.10), (4.11)):

$$P_{\text{tot}} = \frac{h_i(\alpha)}{k_i(\alpha) + h_i(\alpha)} \phi \quad (6.9)$$

$$S_{\text{tot}} = \frac{k_i(\alpha)}{k_i(\alpha) + h_i(\alpha)} \phi, \quad (6.10)$$

where $\phi \equiv P_{\text{tot}} + S_{\text{tot}}$ is the overall concentration of SG constituent molecules (whether phase-separating or soluble). In particular, the total concentration of phase-separating molecules P_{tot} depends on the chemical reaction rates, the ATP concentration α as well as the model under consideration (A, B or C).

6.3 Dynamics of a multi-drop system

At thermal equilibrium ($k, h = 0$), a finite, phase-separating system in the nucleation and growth regime (which is the regime relevant to our biological context) can only be in two steady-states: either the system is well-mixed (i.e., no granules) or a single granule enriched in P co-exists with the surrounding cytoplasm that is dilute in P (Section 2.7). Furthermore, due to surface tension, there exists a critical radius R_n below which drops are no longer thermodynamically stable (Eq. (2.34)). The critical radius can be estimated as a trade off between the surface energy ($\propto R^2$) that penalises having two phases and the bulk free energy in the drop ($\propto R^3$) that promotes drop formation. As a result, in the early stage of phase separation when the mixture is homogeneous, drops larger than R_n need to be nucleated either by the stochastic fluctuations of the concentrations in the case of homogeneous nucleation, or by the help of a third party such as impurities or other proteins or RNA acting as an aggregation site in the case of heterogeneous nucleation (Section 2.6.2). Once multiple drops are nucleated, a multi-drop system is always unstable and coarsens by Ostwald ripening (Section 2.7.1) – the mechanism by which large drops grow while small drops dissolve – and/or coalescence of drops upon encountering via diffusion [31]. Since the diffusion of protein complexes in the cytoplasm is strongly suppressed [54], we will ignore drop diffusion completely here and focus on Ostwald ripening. Eventually a single drop survives in a finite system (Fig. 2.4).

Surprisingly, Ostwald ripening can be suppressed when non-equilibrium chemical reactions are present ($k, h > 0$), as demonstrated in Chapter 3 and 4. We will now employ the formalism discussed to study the behaviour of the three minimal models introduced.

6.4 Model selection

6.4.1 Model B

We will start with model B. In this model, ATP drives the S to P conversion (i.e., $k_B = K_B$ and $h_B = \alpha H_B$). As such, a reduction in ATP will naturally suppress this conversion and thus lead to a decrease in P and restrain phase separation. Therefore, depleting ATP cannot promote SG formation. As a result, we can eliminate this model since it contradicts our first biological constraint (Section 6.1).

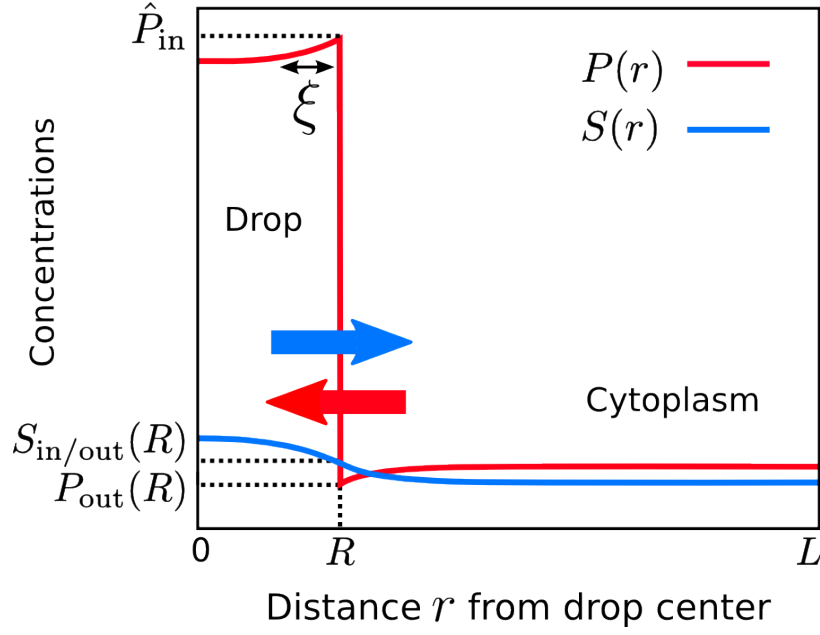


Figure 6.2: *Concentration gradients inside and outside a phase-separated drop.* The concentration profiles of P and S inside and outside drops, have concentration gradients of length scale ξ (double arrow, Eq. (6.8)). Subsequently, there is an in-flux of P (red arrow) and an out-flux of S (blue arrow) at the drop interface.

6.4.2 Model C

In model C, ATP drives both conversions (i.e., $k_C = \alpha K_C$ and $h_C = \alpha H_C$). As a result, the overall concentrations P_{tot} and S_{tot} are independent of α (Eq. (6.9)). In fact, there are two qualitatively distinct regimes depending on the relative magnitude of K_C/H_C and the parameter (Section 4.7, Eq. (4.72))

$$\eta \equiv \frac{2(\phi - \hat{P}_{\text{out}})}{\hat{P}_{\text{in}}} . \quad (6.11)$$

$K_C/H_C \gg \eta$ regime (Fig. 6.3(a)).

This regime corresponds to the situation where the gradient length scale ξ is much larger than the drops (Section 4.5), so drops can be assumed to be homogeneous in concentrations ($P_{\text{in}}(r) = \hat{P}_{\text{in}}$). However concentration gradients in the cytoplasm can be significant and by increasing α , the subsequent decrease in ξ leads to steep gradients in the cytoplasm (Fig. 6.2). Since the concentration of P is fixed at the interface (Eqs (6.5), (6.6)), the gradients result in a higher cytoplasmic concentration of P away from the drop. As the total number of P is constant in this model, the drop must shrink to compensate. Indeed, using the quantitative method developed in Chapter 4, we know that drops tend to shrink as α is increased. In Fig. 6.3(a)

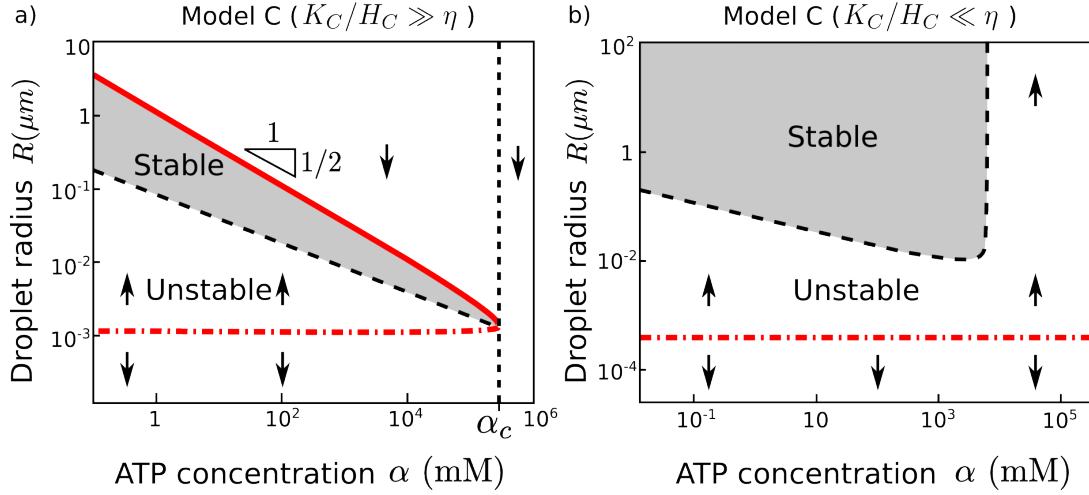


Figure 6.3: *Stability diagram of model C.* Two regimes can be distinguished depending on the magnitude of K_C/H_C with respect to the parameter η (Eq. 6.11). (a) $K_C/H_C \gg \eta$: drops can exist below a critical ATP concentration α_c (vertical dashed line). Drops of radius smaller than the nucleus radius R_n (discontinuous red curve), or larger than the maximal radius R_u (continuous red curve) are unstable and dissolve (downward arrows). Drops larger than R_n but smaller than a critical radius (black slanted dashed line) are unstable and coarsen via Ostwald ripening, leading to an increase of the average drop radius (upward arrows). Above the critical radius and below R_u drops are stable (grey region). All drops dissolve for $\alpha > \alpha_c$. (b) $K_C/H_C \ll \eta$: α controls the stability of the drops but not their formation and dissolution. Parameters: $\phi = 0.2 \mu\text{M}$, $\hat{P}_{\text{out}} = 0.04 \mu\text{M}$, $\hat{P}_{\text{in}} = 40 \mu\text{M}$, $l_c = 1 \text{ nm}$, $D = 1 \mu\text{m}^2\text{s}^{-1}$. (a): $K_C = 5 \times 10^{-3} \text{ mM}^{-1}\text{s}^{-1}$, $H_C = 5 \times 10^{-3} \text{ mM}^{-1}\text{s}^{-1}$. (b): $K_C = 5 \times 10^{-3} \text{ mM}^{-1}\text{s}^{-1}$, $H_C = 10 \text{ mM}^{-1}\text{s}^{-1}$. These parameters are meant to be generic in order to elucidate the system's behaviour.

we show the range of stable drop radii in a multi-drop system as α varies. Note that the drop number density is variable and depends on the nucleation process and potentially the coarsening kinetics.

Similar to the equilibrium case ($k, h = 0$), there exists a critical radius R_n (red irregular dashed line) below which drops dissolve (downward arrows). Systems with drops larger than R_n are unstable and coarsen via Ostwald ripening leading to an increase of the drop size (upward arrows). Above a critical radius (black slanted dashed line, Eq. (4.65)) there is a region where multi-drop systems are stable (grey area) and this region is bounded by a maximal radius R_u (red continuous line) such that drops larger than R_u shrink (downward arrows). Specifically, R_u has the following scaling form (Eq. (4.55)):

$$R_u \propto \alpha^{-1/2}. \quad (6.12)$$

Namely, a fall of α increases the size of stable SG, thus satisfying our first constraint discussed in Section 6.1. However, this ATP-controlled growth is sub-linear. For instance, to decrease the maximal SG radius R_u by two-fold, a four-fold decrease in ATP concentration is required. Therefore, depletion of ATP according to the scaling relation in Eq. (6.12) alone cannot account for the switch-like behaviour, which is our second biological constraint.

However, we cannot yet rule out this model because of another intriguing feature of this type of non-equilibrium phase-separating systems. When α is greater than a critical value α_c , even though the overall concentrations P_{tot} and S_{tot} remain constant, one can still eliminate drops completely by quenching the stable radii bellow the nucleus radius R_n . An estimate of an upper bound of R_n can be given by the smallest granule observed, which we take to be of the order 100 nm [38]. As we demonstrate in Appendix C.1, the scaling law (Eq. (6.12)) remains valid until $R_u \simeq R_n$ so we can use it to estimate the maximal SG size that would form upon varying α by a factor of two in the vicinity of α_c . As a conservative estimate, if we assume that the tip of the phase boundary where $\alpha = \alpha_c$ (Fig. 6.3) corresponds to the ATP concentration in normal conditions, and that the corresponding drop size is R_n , then a reduction of 50% of α can only lead to a maximal SG radius of around $100 \times 2^{1/2} \simeq 140$ nm according to the upper bound law R_u . This radius is too small compared to experimental observations (Section 6.1) and we thus rule out model C in this regime.

$K_C/H_C \ll \eta$ regime (Fig. 6.3(b)).

This regime corresponds to the case where gradients are significant inside drops ($\xi \ll R$, Section 4.6). Here, α also controls the drop stability but drops have unbounded radii and cannot be dissolved irrespectively of α . Since one cannot control drop assembly and dissolution based on the magnitude of α we can eliminate model C in this regime as well.

In summary, we have shown that model C does not provide the switch-like response compatible with our second biological constraint (Section 6.1). We can therefore rule out this particular model.

6.4.3 Model A

In model A only the forward reaction rate constant k is amplified by an increase of α and the backward rate constant is unchanged (i.e., $k_A = \alpha K_A$ and $h_A = H_A$). Again, there are two distinct regimes, depending on the magnitude of H_A relative

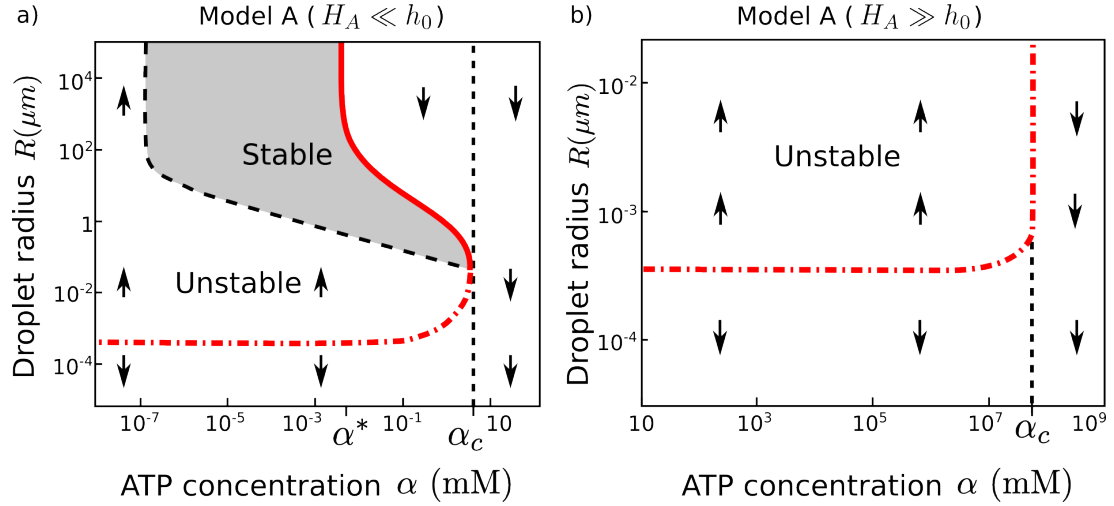


Figure 6.4: *Stability diagram of model A.* Two regimes can be distinguished depending on the magnitude of h_0 with respect to the parameter h_0 (Eq. 6.13). (a) $H_A \ll h_0$: drops can exist below a critical ATP concentration α_c (black vertical dashed line). Drops of radius smaller than the nucleus radius R_n (discontinuous red curve), or larger than the maximal radius R_u (continuous red curve) are unstable and dissolve (downward arrows). Drops larger than R_n but smaller than a critical radius (black dashed curve) are unstable and coarsen via Ostwald ripening, leading to an increase of the average drop radius (upward arrows). There exists another critical ATP concentration α^* below which drops radius are not maximally bounded. (b) $K_A/H_A \ll \eta$: drops can exist only below a critical ATP concentration α_c and are always unstable and coarsen *via* Ostwald ripening (upward arrows). Parameters: $\phi = 0.2 \mu\text{M}$, $\hat{P}_{\text{out}} = 0.04 \mu\text{M}$, $\hat{P}_{\text{in}} = 40 \mu\text{M}$, $l_c = 1 \text{ nm}$, $D = 1 \mu\text{m}^2\text{s}^{-1}$, $K_A = 5 \times 10^{-3} \text{ mM}^{-1}\text{s}^{-1}$. (a): $H_A = 5 \times 10^{-3} \text{ s}^{-1}$. (b): $H_A = 5 \times 10^7 \text{ s}^{-1}$. These parameters are meant to be generic in order to elucidate the system's behaviour.

to the parameter (Section 4.7, Eq. (4.73))

$$h_0 \equiv \frac{D}{l_c^2} \left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} \right)^2. \quad (6.13)$$

$H_A \ll h_0$ regime (Fig. 6.4(a)).

When α is larger than the value $\alpha^* \equiv \eta H_A / K_A$, drops are always much smaller than ξ (Section 4.7, Eq. (4.72)) so the mechanism that control drop size is similar to the one discussed in model C: increasing α increases cytoplasmic gradients that enrich the cytoplasm in P , leading to drop shrinkage. However a major difference in model A is that the overall P concentration (P_{tot}) is not fixed any more but decreases as α increases (Eq. (6.9)). Compared to model C we therefore expect that the drop radius decreases more drastically as α increases. This is indeed the case and the

maximal radius of a stable drop is (Eq. (4.55)):

$$R_u \propto \sqrt{\frac{P_{\text{tot}} - \hat{P}_{\text{out}}}{\alpha}} \quad (6.14)$$

$$= \sqrt{\frac{1}{\alpha} \left(\frac{H_A \phi}{\alpha K_A + H_A} - \hat{P}_{\text{out}} \right)}. \quad (6.15)$$

The drop shrinkage as α increases is more pronounced than in model C due to the additional α in the denominator of P_{tot} (Eq. (6.9)). When α is greater than a critical value α_c (vertical dashed line in Fig. 6.4(a)), then $P_{\text{tot}} \leq \hat{P}_{\text{out}}$ and all drops dissolve ($R_u = 0$), which corresponds to the situation where the system falls outside the equilibrium phase-separating region (Fig. 6.1(b), ‘ $\square$ ’ symbol). By equating R_u to zero we find the expression of α_c :

$$\alpha_c = \left(\frac{\phi}{\hat{P}_{\text{out}}} - 1 \right) \frac{H_A}{K_A}. \quad (6.16)$$

As a result, in model A drop dissolution can be achieved by depleting P to the extent that the system crosses the equilibrium phase boundary. This suggests a stronger response than in model C which we quantify in Appendix C.1. In particular we show that the ratio R_u/R_n (R_n being the nucleus radius, see Section 6.3) for $\alpha \lesssim \alpha_c$ does not have to be of order 1 as in model C but is a function of the system parameters $\hat{P}_{\text{out}}, \hat{P}_{\text{in}}, \phi, D, l_c$ and H_A . Therefore in the $H_A \ll h_0$ regime of model A, drops can be formed in a switch-like manner by a two-fold decrease of α , satisfying both our biological constraints (Section 6.1). Interestingly, for $\alpha < \alpha^*$ the size of stable drops is not bounded ($R_u \rightarrow \infty$), which provides a strong experimental prediction that we will discuss in Section 6.6.

$H_A \gg h_0$ regime (Fig. 6.4(b)).

This regime corresponds to the situation where the chemical reactions are of rate constants fast enough that the gradient length scale ξ is smaller than the nucleus radius R_n (see Eq. (4.60)). Although there exists a critical ATP concentration α_c beyond which all drops dissolve (vertical dashed line), a multi-drop system is always unstable and coarsen via Ostwald ripening (upward arrows). This is not a desirable feature for the formation of stable cytoplasmic organelles and we therefore discard model A in this regime.

In summary, we conclude that among the three minimal models introduced, model A in the $H_A \ll h_0$ regime is the best suited to describe the physics of ATP-

triggered SG formation.

6.5 Summary & discussion

Starting from experimental observations of SG formation in the cell cytoplasm, we have formulated three minimal models based on chemical reaction-controlled phase separation to account for the appearance of SG upon ATP depletion. Applying the formalism developed in Chapter 4, we compared the models based on their qualitative features to salient experimental observations.

We eliminated model B because it does not predict SG growth when ATP concentration falls. Model C was discarded because although SG grow during ATP depletion, the response is not switch-like. Finally, we found that model A, where ATP drives only the $P \rightarrow S$ conversion, can satisfy both biological constraints. However, we have ruled out the $H_A \gg h_0$ regime in model A because drops cannot be stable as they always coarsen via Ostwald ripening.

We are thus left with a unique scenario, i.e., model A with $H_A \ll h_0$ and we will now use estimates of physiological parameters to elucidate a particular scenario of our model (see Fig. 6.5 for the parameter details). In normal conditions the ATP concentration α is at its basal value (blue arrow) leading to a low concentration of molecule P . As a result, the system is outside the phase-separating region ($\square$ in the insert figure) and no drops can exist. Upon a two-fold decrease of ATP (red arrow) the concentration of P increases, thus taking the system inside the phase-separating region ($\diamond$). SG can form via the nucleation of small drops of radius ~ 10 nm. The nuclei then grow and coarsen (upward black arrow) leading to stable SG with much larger radii, between ~ 1 and ~ 10 μm (grey region), depending of the number of drops nucleated. We thus find that the two biological constraints that SG must form when the ATP concentration decreases by two-fold, and in a switch-like manner, are both satisfied (Section 6.1). Furthermore, the quantitative predictions for the stable SG radii are consistent with experimental observations. Therefore, model A reproduces salient experimental observations of SG formation and dissolution based on the ATP level.

A peculiar feature of model A is that under normal condition, ATP is continuously hydrolysed to keep SG from forming. Superficially, it may seem wasteful energetically. However, this is in fact not dissimilar to any insurance schemes that we are familiar with. For instance, we pay a car insurance premium every month so that when an accident occurs, the damage cost is covered. This perspective is particularly pertinent for SG regulation since the timing of environmental stresses

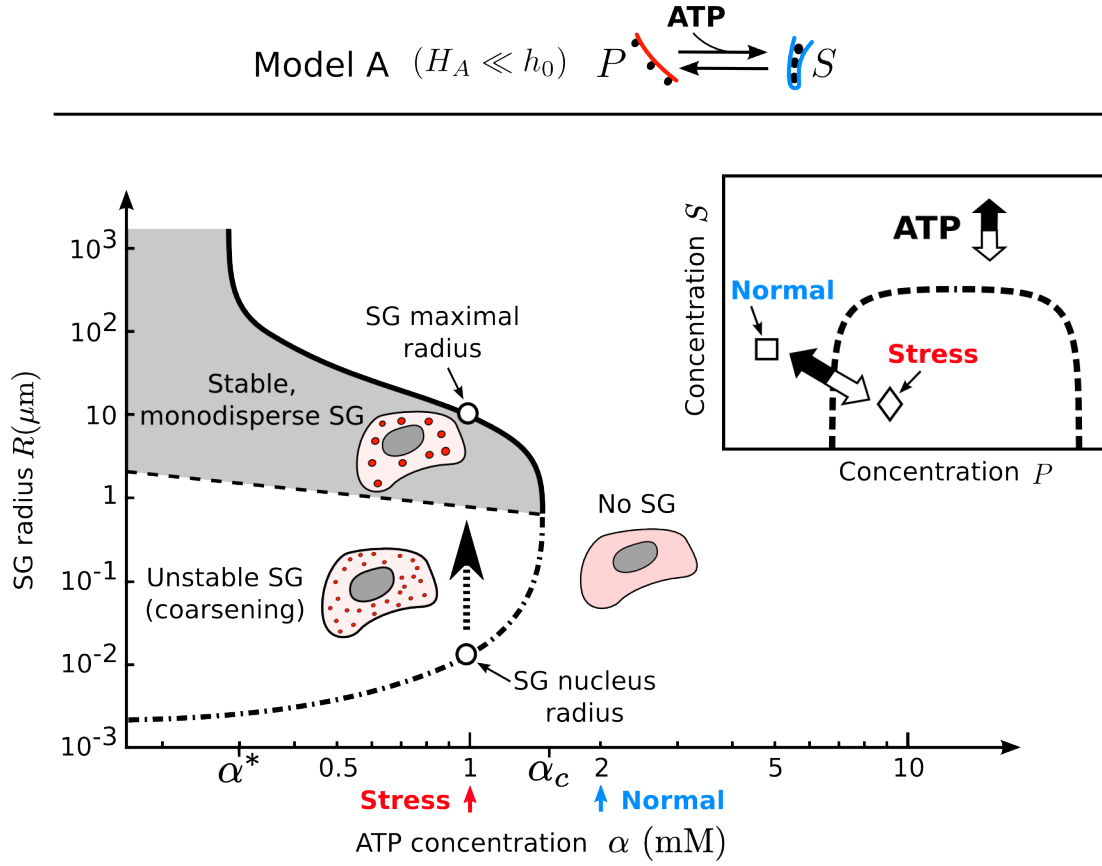


Figure 6.5: *Model of stress granule formation.* Among the three minimal models proposed, only model A in the $H_A \gg h_0$ regime can describe SG formation and dissolution upon two-fold variations of the ATP concentration α . In this model phase-separating states P are converted into soluble states S at a rate proportional to α . During normal conditions α is high (blue arrow) and there are few molecules P so that the system does not phase separate (‘□’ symbol in insert). When α falls by a two-fold during environmental stresses (red arrow) the concentration of P increases, making the system cross the phase boundary and SG assemble by phase separation (‘◇’ symbol). SG nucleate from small drops of radius of about 10 nm then grow and coarsen (black upward arrow) until they reach a stable radius between 1 and 10 μm (grey region). Parameters: $\phi = 2 \mu\text{M}$, $\hat{P}_{\text{out}} = 0.4 \mu\text{M}$, $\hat{P}_{\text{in}} = 40 \mu\text{M}$, $D = 6.5 \mu\text{m}^2\text{s}^{-1}$, $l_c = 3 \text{ nm}$, $H_A = 5 \times 10^{-3} \text{ s}^{-1}$, $K_A = 4/3 \times 10^{-2} \text{ mM}^{-1}\text{s}^{-1}$. These parameters are chosen such that $\alpha_c = 1.5 \text{ mM}$ (Eq. (6.16)) is a typical ATP concentration, D , ϕ and $\hat{P}_{\text{out}}$ are typical protein diffusion coefficient and concentrations in the cytoplasm, $\hat{P}_{\text{in}} = 100\hat{P}_{\text{out}}$ [27, 65] and l_c is according to [66]. K_A and H_A are such that when ATP is varied, the times needed for the concentrations P_{tot} and S_{tot} to reach a steady state ($\simeq 1/k$, $1/h$) are smaller than the time scale of SG formation and dissolution ($\simeq 15\text{min}$ [36, 37], Section 6.1).

can be unpredictable. Furthermore, due to physical constraints such as cell size, storage of ATP for a long period of time is difficult. It may therefore be desirable to have survival mechanisms, such as SG formation, that are spontaneous and do not require additional ATP consumption for the formation of SG.

Indeed, there is already experimental evidence suggesting that ATP can promote SG disassembly by ATP-dependent protein phosphorylation, via the activity of focal adhesion kinase (FAK) [46], Casein Kinase 2 [47] and dual specificity kinase DYRK3 [29]. On the other hand, while energy depletion often accompanies stress conditions [43, 44], SG formation [63] or even cause SG formation [45], ATP may also be necessary to SG assembly in some situations [67]. We note as well that there is substantial evidence that energy depletion-independent pathways may also exist, such as via the phosphorylation of eukaryotic initiation factor alpha (eIF2-alpha) [68]. Given all these evidence, the cell seems to have multiple mechanisms to ensure SG assembly and disassembly at stressful times [69] and our work may describe a particular pathway of SG regulation. Nevertheless, the virtue of our model is that it leads to specific predictions that can be tested experimentally, which we will now enumerate.

6.6 Predictions

Our model provides the following experimental predictions:

1. Since the $P \rightarrow S$ reaction is the one that requires the input of ATP, it is natural to relate the conversion to the ubiquitous ATP-driven phosphorylation reaction. In other words, our model suggests that the soluble state of the SG assembling constituents corresponds to the phosphorylated form of these constituents.
2. We predict the existence of a concentration gradient of the phase-separating constituent P outside the SG, with the gradient length scale of the form

$$\xi = \sqrt{\frac{D}{\alpha K_A + H_A}} , \quad (6.17)$$

3. If the inter-granule distance is much larger than the gradient length scale ξ obtained in 2), then within the SG formation regime we predict a relationship between the maximal SG radius R_u and the ATP level α (Eq. (4.55)):

$$R_u \sim \alpha^{-1/2} . \quad (6.18)$$

4. Finally, we predict that there exists a critical value of ATP concentration below which the upper bound on SG radius diverges. Specifically, the critical concentration is (Eq. (4.72)):

$$\alpha^* = 2 \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} \frac{H_A}{K_A} . \quad (6.19)$$

The first prediction may be tested by screening the purified constituents of SG in an in vitro setting. The second, third and fourth predictions can be tested using imaging techniques with well regulated ATP concentration either in vivo or in vitro.

Chapter 7

Conclusion

Membrane-less organelles are tightly regulated structures formed by liquid-liquid phase separation of the cytoplasm. Membrane-less organelles are driven out of equilibrium by multiple energy-consuming processes. The current theoretical knowledge about phase separation under non-equilibrium conditions remains poor, which limits our understanding of membrane-less organelle regulation. In this thesis we investigated a minimal model of cytoplasmic phase separation, composed of a ternary fluid in which ATP-driven chemical reactions convert phase-separating proteins into soluble proteins, and vice versa. Using a mean-field approximation to describe a multi-drop system, we elucidated analytically how drop size, formation, and coarsening can be controlled by the chemical reaction rates. Compared to previous works [17, 55] our formalism goes beyond the small supersaturation limit and include the existence of protein concentration gradients inside the drops. We found that the presence of concentration gradients inside drops has a significant impact on the stability diagrams. In particular, the phenomenon of drop coarsening by Ostwald ripening is arrested only for a particular range of chemical reaction rates, demonstrating that this transition belongs entirely to the non-equilibrium regime. Furthermore, we categorized comprehensively the qualitative behaviour of the system into distinct regimes based on the reaction rates. We tested our theoretical predictions using Monte Carlo simulation methods. We then applied our formalism to the study of SG formation and considered minimal models based on the mechanism of phase separation regulated by ATP-driven chemical reactions. Comparing our predictions to experimental observations, we identified a minimal model of SG formation triggered by ATP depletion. Our model suggests that ATP is continuously hydrolysed to deter SG formation under normal conditions. As a result of environmental stress, ATP is depleted in the cytoplasm leading to the spontaneous assembly of SG.

Our formalism can be applied to cytoplasmic organisation in general. Furthermore, interesting future directions include the study of chemically-induced shape instability of the drops [58], and the extension of our formalism to many-component mixtures [59, 60] to account for the full complexity of the cytoplasm. Our work is also relevant to the control of emulsions by chemical reactions in the engineering setting.

List of Figures

1.1	Membrane-less organelles	16
1.2	Phase separation with chemical reactions	20
2.1	Phase separation in an oil-water mixture	23
2.2	Free energy density of a binary mixture	24
2.3	Phase diagram of a binary mixture	30
2.4	Ostwald ripening destabilises multi-drop systems	34
3.1	Effect of chemical reactions on the drop growth rate	44
3.2	The stability a multi-drop system at fixed backward reaction rate .	47
4.1	Model of cytoplasmic phase separation	50
4.2	Concentration profiles in a two-drop system	51
4.3	The stability of a multi-drop system at fixed drop number density .	60
4.4	The stability of a multi-drop system at fixed backward reaction rate	61
4.5	Stability diagram of a multi-drop system in the reaction rate constant space	69
4.6	Effect of the solvent inclusion on the stability diagram	70
4.7	Stability diagrams for varying forward rate constant and drop radius, with and without solvent.	71
5.1	Volume fraction profiles in a single-drop system in the Monte Carlo simulations	75
5.2	Determination of the equilibrium parameters in the Monte Carlo sim- ulations	76
5.3	Determination of the stability-instability boundary radius in Monte Carlo simulations	79
5.4	Comparison between theory and Monte Carlo simulations	80
6.1	Distinct schemes for control of cytoplasmic SG formation and phase diagram	82

6.2	Concentration gradients inside and outside a phase-separated drop .	86
6.3	Stability diagram of model C	87
6.4	Stability diagram of model A	89
6.5	Model of stress granule formation	92
C.1	The drop radius determines the drop growth or shrinkage	125

Bibliography

- [1] P. Anderson and N. Kedersha, “RNA granules,” *The Journal of cell biology*, vol. 172, pp. 803–808, mar 2006.
- [2] Y. S. Mao, B. Zhang, and D. L. Spector, “Biogenesis and function of nuclear bodies,” *Trends in Genetics*, vol. 27, no. 8, pp. 295–306, 2011.
- [3] D. S. Protter and R. Parker, “Principles and Properties of Stress Granules,” *Trends in Cell Biology*, vol. 26, no. 9, pp. 668–679, 2016.
- [4] A. Krämer, B. Maier, and J. Bartek, “Centrosome clustering and chromosomal (in)stability: A matter of life and death,” *Molecular Oncology*, vol. 5, no. 4, pp. 324–335, 2011.
- [5] A. C. Fan, A. K. L. Leung, and U. States, *RNA Granules and Diseases A Case Study of Stress Granules in ALS and FTL D*, vol. 907. Springer, 2016.
- [6] C. A. Brunello, X. Yan, and H. J. Huttunen, “Internalized Tau sensitizes cells to stress by promoting formation and stability of stress granules,” *Scientific Reports*, vol. 6, no. 1, p. 30498, 2016.
- [7] C. Brangwynne, C. Eckmann, D. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, and A. Hyman, “Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation,” *Science*, vol. 324, no. 5935, pp. 1729–1732, 2009.
- [8] C. P. Brangwynne, T. J. Mitchison, and A. A. Hyman, “Active liquid-like behavior of nucleoli determines their size and shape in *Xenopus laevis* oocytes,” *Proceedings of the National Academy of Sciences*, vol. 108, pp. 4334–4339, mar 2011.
- [9] C. P. Brangwynne, “Phase transitions and size scaling of membrane-less organelles,” *Journal of Cell Biology*, vol. 203, no. 6, pp. 875–881, 2013.

- [10] D. Zwicker, M. Decker, S. Jaensch, A. a. Hyman, and F. Jülicher, “Centrosomes are autocatalytic droplets of pericentriolar material organized by centrioles,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, pp. E2636–E2645, jul 2014.
- [11] C. F. Lee, C. P. Brangwynne, J. Gharakhani, A. A. Hyman, and F. Jülicher, “Spatial organization of the cell cytoplasm by position-dependent phase separation,” *Physical Review Letters*, vol. 111, no. 8, p. 88101, 2013.
- [12] A. A. Hyman, C. A. Weber, and F. Jülicher, “Liquid-liquid phase separation in biology,” *Annual review of cell and developmental biology*, vol. 30, pp. 39–58, jan 2014.
- [13] C. P. Brangwynne, P. Tompa, and R. V. Pappu, “Polymer physics of intracellular phase transitions,” *Nature Physics*, vol. 11, no. 11, pp. 899–904, 2015.
- [14] S. Saha, C. A. Weber, M. Nusch, O. Adame-Arana, C. Hoege, M. Y. Hein, E. Osborne-Nishimura, J. Mahamid, M. Jahnel, L. Jawerth, A. Pozniakovski, C. R. Eckmann, F. Jülicher, and A. A. Hyman, “Polar Positioning of Phase-Separated Liquid Compartments in Cells Regulated by an mRNA Competition Mechanism,” *Cell*, vol. 166, pp. 1572–1584, sep 2016.
- [15] A. Molliex, J. Temirov, J. Lee, M. Coughlin, A. P. Kanagaraj, H. J. Kim, T. Mittag, and J. P. Taylor, “Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization,” *Cell*, vol. 163, no. 1, pp. 123–133, 2015.
- [16] T. L. Hill and J. Gillis, *An Introduction to Statistical Thermodynamics*, vol. 14. Courier Corporation, 1961.
- [17] D. Zwicker, A. A. Hyman, and F. Jülicher, “Suppression of Ostwald ripening in active emulsions,” *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, vol. 92, no. 1, pp. 1–12, 2015.
- [18] C. A. Weber, C. F. Lee, and F. Jülicher, “Droplet ripening in concentration gradients,” *New Journal of Physics*, vol. 19, p. 53021, mar 2017.
- [19] M. G. Thomas, L. J. M. Tosar, M. A. Desbats, C. C. Leishman, and G. L. Boccaccio, “Mammalian Staufen 1 is recruited to stress granules and impairs their assembly,” *Journal of Cell Science*, vol. 122, no. 4, pp. 563–573, 2009.

- [20] N. Kedersha, G. Stoecklin, M. Ayodele, P. Yacono, J. Lykke-Andersen, M. J. Fritzler, D. Scheuner, R. J. Kaufman, D. E. Golan, and P. Anderson, “Stress granules and processing bodies are dynamically linked sites of mRNP remodeling,” *The Journal of cell biology*, vol. 169, pp. 871–884, jun 2005.
- [21] S. Mollet, N. Cougot, A. Wilczynska, F. Dautry, M. Kress, E. Bertrand, and D. Weil, “Translationally Repressed mRNA Transiently Cycles through Stress Granules during Stress,” *Molecular biology of the cell*, vol. 19, pp. 4469–4479, 2008.
- [22] N. Gilks, N. Kedersha, M. Ayodele, L. Shen, G. Stoecklin, L. M. Dember, and P. Anderson, “Stress Granule Assembly Is Mediated by Prion-like Aggregation of TIA-1,” *Molecular Biology of the Cell*, vol. 15, no. December, pp. 5383–5398, 2004.
- [23] Y. Lin, D. S. Protter, M. K. Rosen, and R. Parker, “Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins,” *Molecular Cell*, vol. 60, no. 2, pp. 208–219, 2015.
- [24] T. W. Han, M. Kato, S. Xie, L. C. Wu, H. Mirzaei, J. Pei, M. Chen, Y. Xie, J. Allen, G. Xiao, and S. L. McKnight, “Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies,” *Cell*, vol. 149, pp. 768–779, may 2012.
- [25] M. Kato, T. W. Han, S. Xie, K. Shi, X. Du, L. C. Wu, H. Mirzaei, E. J. Goldsmith, J. Longgood, J. Pei, N. V. Grishin, D. E. Frantz, J. W. Schneider, S. Chen, L. Li, M. R. Sawaya, D. Eisenberg, R. Tycko, and S. L. McKnight, “Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels,” *Cell*, vol. 149, pp. 753–767, may 2012.
- [26] V. N. Uversky, “Protein intrinsic disorder-based liquidliquid phase transitions in biological systems: Complex coacervates and membrane-less organelles,” *Advances in Colloid and Interface Science*, vol. 239, pp. 97–114, 2016.
- [27] P. Li, S. Banjade, H.-C. Cheng, S. Kim, B. Chen, L. Guo, M. Llaguno, J. V. Hollingsworth, D. S. King, S. F. Banani, P. S. Russo, Q.-X. Jiang, B. T. Nixon, and M. K. Rosen, “Phase transitions in the assembly of multivalent signalling proteins,” *Nature*, vol. 483, pp. 336–40, mar 2012.
- [28] A. Bah and J. D. Forman-Kay, “Modulation of intrinsically disordered protein function by post-translational modifications,” *Journal of Biological Chemistry*, vol. 291, no. 13, pp. 6696–6705, 2016.

- [29] F. Wippich, B. Bodenmiller, M. G. Trajkovska, S. Wanka, R. Aebersold, and L. Pelkmans, “Dual specificity kinase DYRK3 couples stress granule condensation/ dissolution to mTORC1 signaling,” *Cell*, vol. 152, no. 4, pp. 791–805, 2013.
- [30] J. T. Wang, J. Smith, B.-C. Chen, H. Schmidt, D. Rasoloson, A. Paix, B. G. Lambrus, D. Calidas, E. Betzig, and G. Seydoux, “Regulation of RNA granule dynamics by phosphorylation of serine-rich, intrinsically disordered proteins in *C. elegans*,” *eLife*, vol. 3, pp. 1–23, 2014.
- [31] E. D. Siggia, “Late stages of spinodal decomposition in binary mixtures,” *Physical Review A*, vol. 20, no. 2, pp. 595–605, 1979.
- [32] W. Ostwald, “Studien uber die Bildung und Umwandlung fester Korper,” *Zeitschrift fur physikalische Chemie*, vol. 22, no. 1, pp. 289–330, 1897.
- [33] I. Lifshitz and V. Slyozov, “The kinetics of precipitation from supersaturated solid solutions,” *Journal of Physics and Chemistry of Solids*, vol. 19, no. 1, pp. 35–50, 1961.
- [34] D. Updike and S. Strome, “P granule assembly and function in *Caenorhabditis elegans* germ cells,” *Journal of andrology*, vol. 31, no. 1, pp. 53–60, 2010.
- [35] M. Bornens, “The Centrosome in Cells and Organisms,” *Science*, vol. 335, no. 6067, pp. 422–426, 2012.
- [36] J. Wheeler, “Distinct stages in stress granule assembly and disassembly,” *eLife*, pp. 1–25, 2016.
- [37] D. Ohshima, K. Arimoto-Matsuzaki, T. Tomida, M. Takekawa, and K. Ichikawa, “Spatio-temporal Dynamics and Mechanisms of Stress Granule Assembly,” *PLOS Computational Biology*, vol. 11, no. 6, pp. 1–22, 2015.
- [38] P. Anderson and N. Kedersha, “Stress granules,” *Current Biology*, vol. 19, no. 10, pp. 397–398, 2009.
- [39] S. H. Kwon, Y. Zhang, and P. Matthias, “The deacetylase HDAC6 is an essential component of stress granules and plays a critical role in the cellular response to stress,” *Genes & Development*, vol. 21, pp. 3381–3394, 2007.
- [40] A. Aulas, G. Caron, C. G. Gkogkas, N.-V. Mohamed, L. Destroismaisons, N. Sonenberg, N. Leclerc, J. A. Parker, and C. Vande Velde, “G3BP1 promotes

- stress-induced RNA granule interactions to preserve polyadenylated mRNA,” *The Journal of Cell Biology*, vol. 209, no. 1, pp. 73–84, 2015.
- [41] J. R. Buchan and R. Parker, “Eukaryotic Stress Granules: The Ins and Outs of Translation,” *Molecular Cell*, vol. 36, no. 6, pp. 932–941, 2009.
- [42] M. Ramaswami, J. P. Taylor, and R. Parker, “Altered ribostasis: RNA-protein granules in degenerative disorders,” *Cell*, vol. 154, no. 4, pp. 727–736, 2013.
- [43] M. B. Lilly, J. R. Durant, T. C. Ng, W. T. Evanochko, N. G. Kumar, G. A. Elgavish, J. D. Glickson, R. Hiramoto, V. Ghanta, and C. R. Katholi, “Loss of High-Energy Phosphate following Hyperthermia Demonstrated by in Vivo ^{31}P -Nuclear Magnetic Resonance Spectroscopy,” *Cancer Research*, vol. 44, no. 2, pp. 633–638, 1984.
- [44] J. Chang, a. a. Knowlton, F. Xu, and J. S. Wasser, “Activation of the heat shock response: relationship to energy metabolites. A (^{31}P) NMR study in rat hearts,” *American journal of physiology. Heart and circulatory physiology*, vol. 280, pp. H426–H433, 2001.
- [45] N. Kedersha, S. Chen, N. Gilks, W. Li, I. J. Miller, J. Stahl, and P. Anderson, “Evidence That Ternary Complex (eIF2-GTP-tRNAⁱ Met) Deficient Preinitiation Complexes Are Core Constituents of Mammalian Stress Granules,” *Molecular biology of the cell*, vol. 13, no. January, pp. 195–210, 2002.
- [46] N.-P. Tsai, P.-C. Ho, and L.-N. Wei, “Regulation of stress granule dynamics by Grb7 and FAK signalling pathway,” *The EMBO journal*, vol. 27, no. 5, pp. 715–26, 2008.
- [47] R. E. Reineke, L. C., Tsai, W. C., Jain, A., Kaelber, J. T., Jung, S. Y., Lloyd, “Casein Kinase 2 is linked to stress granule dynamics through phosphorylation of the stress granule nucleating protein G3BP1,” *Molecular and cellular biology*, vol. 37, no. 4, pp. e00596–16, 2017.
- [48] P. Hänggi, P. Talkner, and M. Borkovec, “Reaction-rate theory: fifty years after Kramers,” *Rev. Mod. Phys.*, vol. 62, pp. 251–341, apr 1990.
- [49] F. J. C.A. Weber, D. Zwicker and C. Lee, “Liquid droplets regulated by chemical gradients and reactions,” *Invited review in the Journal of Physics: Condensed Matter*.

- [50] P. J. Flory, “Thermodynamics of high-polymer solutions,” *The Journal of chemical physics*, vol. 1, no. May 2016, pp. 25–30, 1945.
- [51] M. Huggins, “Some Properties of Solutions of Long-chain Compounds,” *The Journal of Physical Chemistry*, 1942.
- [52] J. W. Gibbs, “On the equilibrium of heterogeneous substances,” *American Journal of Science*, vol. s3-16, no. 96, pp. 441–458, 1878.
- [53] A. J. Bray, “Theory of phase-ordering kinetics,” *Advances in Physics*, vol. 51, no. 2, pp. 481–587, 2002.
- [54] M. Weiss, M. Elsner, F. Kartberg, and T. Nilsson, “Anomalous subdiffusion is a measure for cytoplasmic crowding in living cells,” *Biophysical journal*, vol. 87, no. 5, pp. 3518–24, 2004.
- [55] D. Zwicker, *Physical Description of Centrosomes as Active Droplets*. PhD thesis, TU Dresden.
- [56] B. J. Alberts, A. Lewis, and J. Raff, *Molecular biology of the cell*. No. 574.87 M6/2008, Garland Science, 2008.
- [57] S. C. Glotzer, E. A. D. Marzio, and M. Muthukumar, “Reaction-Controlled Morphology of Phase-Separating Mixtures,” *Phys. Rev. Lett.*, vol. 74, no. 11, 1995.
- [58] D. Zwicker, R. Seyboldt, C. A. Weber, A. A. Hyman, and F. Jülicher, “Growth and division of active droplets provides a model for protocells,” *Nature Physics*, vol. 13, no. 4, pp. 408–413, 2016.
- [59] R. P. Sear and J. A. Cuesta, “Instabilities in Complex Mixtures with a Large Number of Components,” *Physical Review Letters*, vol. 91, no. 24, p. 245701, 2003.
- [60] W. M. Jacobs and D. Frenkel, “Phase Transitions in Biological Systems with Many Components,” *Biophysical Journal*, vol. 112, no. 4, pp. 683–691, 2017.
- [61] J. S. Liu, *Monte Carlo strategies in scientific computing*. Springer Science & Business Media, 2008.
- [62] K. Kawasaki, *Phase transitions and critical phenomena*, vol. 2. Academic, New York, 1972.

- [63] S. Hofmann, V. Cherkasova, P. Bankhead, B. Bukau, and G. Stoecklin, "Translation suppression promotes stress granule formation and cell survival in response to cold shock," *Molecular Biology of the Cell*, vol. 23, no. 19, pp. 3786–3800, 2012.
- [64] N. Kedersha, M. Gupta, W. Li, I. Miller, and P. Anderson, "RNA-binding Proteins TIA-1 and TIAR Link the Phosphorylation of eIF-2 to the Assembly of Mammalian Stress Granules," *The Journal of Cell Biology*, vol. 147, no. 7, pp. 1431–1441, 1999.
- [65] T. J. Nott, E. Petsalaki, P. Farber, D. Jervis, E. Fussner, A. Plochowitz, T. D. Craggs, D. P. Bazett-Jones, T. Pawson, J. D. Forman-Kay, and A. J. Baldwin, "Phase Transition of a Disordered Nuage Protein Generates Environmentally Responsive Membraneless Organelles," *Molecular Cell*, vol. 57, no. 5, pp. 936–947, 2015.
- [66] Y. Shin, J. Berry, N. Pannucci, M. P. Haataja, J. E. Toettcher, and C. P. Brangwynne, "Spatiotemporal Control of Intracellular Phase Transitions Using Light-Activated optoDroplets," *Cell*, vol. 168, no. 1, pp. 159–171, 2017.
- [67] S. Jain, J. R. Wheeler, R. W. Walters, A. Agrawal, A. Barsic, and R. Parker, "ATPase-Modulated Stress Granules Contain a Diverse Proteome and Substructure," *Cell*, vol. 164, no. 3, pp. 487–498, 2016.
- [68] E. McEwen, N. Kedersha, B. Song, D. Scheuner, N. Gilks, A. Han, J. J. Chen, P. Anderson, and R. J. Kaufman, "Heme-regulated inhibitor kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure," *Journal of Biological Chemistry*, vol. 280, no. 17, pp. 16925–16933, 2005.
- [69] Y. Dang, N. Kedersha, W. K. Low, D. Romo, M. Gorospe, R. Kaufman, P. Anderson, and J. O. Liu, "Eukaryotic initiation factor 2 α -independent pathway of stress granule induction by the natural product pateamine A," *Journal of Biological Chemistry*, vol. 281, no. 43, pp. 32870–32878, 2006.

Appendix A

Arrest of Ostwald Ripening in Binary Fluids

A.1 Validity of the small drop approximation

The calculation in Chapter 3 is based on the assumption that drops radii are much smaller than the gradient length scale ξ (Eq. (3.8)). At steady-state, the largest possible drop radius R_u is given by Eq. (3.26). By setting $R_u \ll \xi$, we find the reaction rate constants k, h for which the assumption is valid [17]:

$$\frac{3D \left(\frac{h}{k+h} - \hat{\varphi}_{\text{out}} \right)}{k\hat{\varphi}_{\text{in}}} \ll \frac{D}{k+h} . \quad (\text{A.1})$$

In a system dilute in P ($\varphi_{\text{tot}} \ll 1$), we must have $k \gg h$ (Eq. (3.6)). Therefore, taking $k+h \simeq k$, and since $\hat{\varphi}_{\text{in}} \gg \hat{\varphi}_{\text{out}}$ in the strong phase separation regime, we get

$$\frac{k}{h} \gg \frac{3}{\hat{\varphi}_{\text{in}}} \quad (\text{A.2})$$

$$\Rightarrow \frac{k}{h} \gg 3 , \quad (\text{A.3})$$

since a volume fraction is smaller or equal to 1 by definition. This condition is equivalent to $\varphi_{\text{tot}} \ll 1/4$ (Eq. (3.6)), which is true, by definition, for a mixture diluted in P .

We conclude that, in a binary mixture diluted in P , such that $\varphi_{\text{tot}} \ll 1/4$, drops are always much smaller than the gradient length scale ξ . This validates the small drop approximation in Chapter 3.

Appendix B

Active Phase-Separated Drops in a Ternary Fluid

B.1 Concentrations controlled by chemical reactions

We demonstrate the expressions of the global concentrations P_{tot} and S_{tot} in Eqs (4.10) and (4.11) from the reaction diffusion equations (4.2) and (4.3). In a single-drop system,

$$P_{\text{tot}}V = \int_{0 \leq r < R} d^3\mathbf{r} P_{\text{in}}(\mathbf{r}) + \int_{R \leq r \leq L} d^3\mathbf{r} P_{\text{out}}(\mathbf{r}) \quad (\text{B.1})$$

where $V = 4\pi L^3/3$ is the system's volume. Now, by integrating over the solution to Eqs (4.2), we obtain

$$0 = \int_{0 \leq r < R} d^3\mathbf{r} [D\nabla^2 P_{\text{in}}(\mathbf{r}) - kP_{\text{in}}(\mathbf{r}) + hS_{\text{in}}(\mathbf{r})] \quad (\text{B.2})$$

$$+ \int_{R \leq r \leq L} d^3\mathbf{r} [D\nabla^2 P_{\text{out}}(\mathbf{r}) - kP_{\text{out}}(\mathbf{r}) + hS_{\text{out}}(\mathbf{r})] . \quad (\text{B.3})$$

Since in the steady-state, the gradients of P_{in} and P_{out} at the interface have to be identical ($\nabla P_{\text{in}}|_{r=R^-} = \nabla P_{\text{out}}|_{r=R^+}$), the diffusion terms in the squared brackets above cancel each other. The same conclusion applies to S_{tot} . Therefore, we have

$$0 = -kP_{\text{tot}} + hS_{\text{tot}} , \quad (\text{B.4})$$

and therefore:

$$P_{\text{tot}} = \frac{\phi h}{k + h} \quad (\text{B.5})$$

$$S_{\text{tot}} = \frac{\phi k}{k + h} , \quad (\text{B.6})$$

with $\phi \equiv P_{\text{tot}} + S_{\text{tot}}$ the global solute concentration. As a stable multi-drop system can be viewed as a system with many copies of the one-drop system, the above conclusion also holds when multiple drops co-exist.

B.2 Linear stability of the steady-state

Given a two-drop system at steady-state, we detail the calculation of the linear stability analysis of the steady-state, leading to the stability relation g_1 Eq. (4.47), and the quantities in Eqs (4.27), (4.28), and (4.48)-(4.51).

We perturb the drop sizes about the steady-state:

$$R_1 = R^* + \epsilon \quad (\text{B.7})$$

$$R_2 = R^* - \epsilon , \quad (\text{B.8})$$

$$(\text{B.9})$$

with R_i the radius of the i -th drop, R^* the steady-state radius, and $\epsilon \ll R^*$. We focus on the growth rate of the drop 1 ($G^{(1)}$, Eq. (4.36)). Expanding for the small parameter ϵ/R^* :

$$G^{(1)}(R_1, R_2) = g_0(R^*) + \epsilon g_1(R^*) + \mathcal{O}(\epsilon^2) \quad (\text{B.10})$$

with

$$g_0(R^*) \equiv G^{(1)}(R^*, R^*) = G^{(2)}(R^*, R^*) \quad (\text{B.11})$$

$$g_1(R^*) \equiv \left. \frac{\partial G^{(1)}}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial G^{(1)}}{\partial R_2} \right|_{R^*, R^*} . \quad (\text{B.12})$$

This leads to

$$\begin{aligned} g_1 = & 4\pi D [H_{\text{in}} (y^2 \text{csch}^2 y - 1) \\ & + H_{\text{out}} ((\mathcal{A}(y-1)e^y - \mathcal{B}(1+y)e^{-y}) R + 2 + y^2 - 2y (Ae^y - Be^{-y})) \\ & + \mathcal{H}_{\text{in}} (1 - y \coth y) R + \mathcal{H}_{\text{out}} [A(y-1)e^y - B(1+y)e^{-y}] R] . \end{aligned} \quad (\text{B.13})$$

with $\mathcal{A} \equiv \left. \frac{\partial A_1}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial A_1}{\partial R_2} \right|_{R^*, R^*}$, $\mathcal{B} \equiv \left. \frac{\partial B_1}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial B_1}{\partial R_2} \right|_{R^*, R^*}$ and $\mathcal{H}_{\text{in/out}} \equiv \left. \frac{\partial H_{\text{in/out}}^{(1)}}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial H_{\text{in/out}}^{(1)}}{\partial R_2} \right|_{R^*, R^*}$. The coefficients A_i and B_i are solution of the system of equations (4.30)-(4.32), and the coefficients A and B are given by Eqs (4.38) and (4.39). Plugging the expressions of H_{in} and H_{out} (Eqs (4.27) and (4.28)) in the definitions of $\mathcal{H}_{\text{in/out}}$, we find

$$\mathcal{H}_{\text{in}} = -\frac{\hat{P}_{\text{out}} l_c h}{(k+h)R^2} \quad (\text{B.14})$$

$$\mathcal{H}_{\text{out}} = -\frac{\hat{P}_{\text{out}} l_c}{R^2}. \quad (\text{B.15})$$

We have recovered Eqs (4.52) and (4.53).

Expanding A_i for small ϵ/R^*

$$A_1(R_1, R_2) = A + \epsilon \left(\left. \frac{\partial A_1}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial A_1}{\partial R_2} \right|_{R^*, R^*} \right) + \mathcal{O}(\epsilon^2) \quad (\text{B.16})$$

$$A_2(R_1, R_2) = A + \epsilon \left(\left. \frac{\partial A_2}{\partial R_1} \right|_{R^*, R^*} - \left. \frac{\partial A_2}{\partial R_2} \right|_{R^*, R^*} \right) + \mathcal{O}(\epsilon^2) \quad (\text{B.17})$$

The two-drop system must be unchanged by the permutation of the two drops, therefore

$$\left. \frac{\partial A_2}{\partial R_1} \right|_{R^*, R^*} = \left. \frac{\partial A_1}{\partial R_2} \right|_{R^*, R^*} \quad (\text{B.18})$$

$$\left. \frac{\partial A_1}{\partial R_1} \right|_{R^*, R^*} = \left. \frac{\partial A_2}{\partial R_2} \right|_{R^*, R^*}, \quad (\text{B.19})$$

and it follows that

$$A_1(R_1, R_2) = A + \epsilon \mathcal{A} + \mathcal{O}(\epsilon^2) \quad (\text{B.20})$$

$$A_2(R_1, R_2) = A - \epsilon \mathcal{A} + \mathcal{O}(\epsilon^2). \quad (\text{B.21})$$

This can be generalized for the other quantities:

$$B_1(R_1, R_2) = B + \epsilon \mathcal{B} + \mathcal{O}(\epsilon^2) \quad (\text{B.22})$$

$$B_2(R_1, R_2) = B - \epsilon \mathcal{B} + \mathcal{O}(\epsilon^2) \quad (\text{B.23})$$

$$H_{\text{in}}^{(1)}(R_1, R_2) = H_{\text{in}} + \epsilon \mathcal{H}_{\text{in}} + \mathcal{O}(\epsilon^2) \quad (\text{B.24})$$

$$H_{\text{in}}^{(2)}(R_1, R_2) = H_{\text{in}} - \epsilon \mathcal{H}_{\text{in}} + \mathcal{O}(\epsilon^2) \quad (\text{B.25})$$

$$H_{\text{out}}^{(1)}(R_1, R_2) = H_{\text{out}} + \epsilon \mathcal{H}_{\text{out}} + \mathcal{O}(\epsilon^2) \quad (\text{B.26})$$

$$H_{\text{out}}^{(2)}(R_1, R_2) = H_{\text{out}} - \epsilon \mathcal{H}_{\text{out}} + \mathcal{O}(\epsilon^2) . \quad (\text{B.27})$$

Using these results, the system of equations (4.30)-(4.32) reduces to

$$(A + \mathcal{A}\xi) e^y - (B - \mathcal{B}\xi) e^{-y} = 0 \quad (\text{B.28})$$

$$H_{\text{out}} (\mathcal{A}e^x + \mathcal{B}e^{-x}) + \mathcal{H}_{\text{out}} (\mathcal{A}e^x + \mathcal{B}e^{-x}) = 0 \quad (\text{B.29})$$

with $x \equiv L/\xi$ and $y \equiv R^*/\xi$. We solve for $\mathcal{A}, \mathcal{B}$:

$$\mathcal{A} = \frac{-\frac{\mathcal{H}_{\text{out}}}{H_{\text{out}}} (Ae^x + Be^{-x}) e^{-y} + \frac{1}{R} (A(y-1)e^y - B(1+y)e^{-y}) e^{-x}}{e^{x-y} - e^{-(x-y)}} \quad (\text{B.30})$$

$$\mathcal{B} = \frac{\frac{\mathcal{H}_{\text{out}}}{H_{\text{out}}} (Ae^x + Be^{-x}) e^y - \frac{1}{R} (A(y-1)e^y - B(1+y)e^{-y}) e^x}{e^{x-y} - e^{-(x-y)}}. \quad (\text{B.31})$$

We rearrange in the more convenient form:

$$g_1 = 4\pi D [f_1 H_{\text{in}} + f_2 H_{\text{out}} + f_3 R^* (\mathcal{H}_{\text{in}} + f_4 \mathcal{H}_{\text{out}})] , \quad (\text{B.32})$$

with

$$\begin{aligned} f_1 &= y^2 \text{csch}^2 y - 1 \\ f_2 &= \frac{(1-x)e^{2(x-y)} + (1+x)e^{-2(x-y)} + 4y(x-y) - 2}{(1-x)e^{2(x-y)} + (1+x)e^{-2(x-y)} - 2} \\ f_3 &= 1 - y \coth y \\ f_4 &= -\frac{(1+y)e^{x-y} + (y-1)e^{-(x-y)}}{e^{x-y} - e^{-(x-y)}} , . \end{aligned} \quad (\text{B.33})$$

and we thus recover Eqs (4.47)-(4.51).

B.3 Passive systems

We use the formalism developed in Sections 4.1 to 4.3 to recover some well known results in passive systems, i.e. without chemical reactions: drop radius, nucleus

size, and Ostwald ripening in multi-drop systems.

When $k = 0$ or/and $h = 0$ no reactions occur in the steady-state and the system is in passive conditions. From Eqs (4.10)-(4.11) it can be seen that in the scenario $\{k = 0; h > 0\}$, then $P_{\text{tot}} = \phi$, $S_{\text{tot}} = 0$. In the other scenario $\{k > 0; h = 0\}$, then $P_{\text{tot}} = 0$, $S_{\text{tot}} = \phi$. Finally, when $k = h = 0$, Eqs (4.10) and (4.11) are undefined. We can nonetheless study such systems at concentrations P_{tot} , S_{tot} using our formalism by making k and h converge to zero while keeping the ratio $\Gamma \equiv k/h$ in such a way that we recover the desired $P_{\text{tot}}, S_{\text{tot}}$ from Eqs (4.10)-(4.11):

$$\Gamma = \frac{\phi - P_{\text{tot}}}{P_{\text{tot}}} . \quad (\text{B.34})$$

Using this prescription we now calculate the steady-state drop radius R^* and determine its stability. Taking k and h to zero implies that $\xi \rightarrow \infty$ (Eq. (4.26)), thus $x \equiv L/\xi \rightarrow 0$, $y \equiv R^*/\xi \rightarrow 0$ (but $y/x = R/L$) and therefore the steady-state condition Eq. (4.40) becomes

$$(R^*)^3 = \frac{\lambda}{\lambda + 1} L^3 , \quad (\text{B.35})$$

with λ given by Eq. (4.41). Plugging the expression of λ and Γ in this result we find:

$$(R^*)^3 = \frac{P_{\text{tot}} - P_{\text{out}}}{\hat{P}_{\text{in}}} L^3 \left(1 + \mathcal{O} \left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} \right) \right) . \quad (\text{B.36})$$

Since in the passive case there are no concentration gradients (this can be seen by taking $k = h = 0$ in Eqs (4.12)-(4.15)), this result can also be recovered simply by imposing the conservation of the number of molecules P in the system: $R^3 P_{\text{in}} + (L^3 - R^3) P_{\text{out}} = L^3 P_{\text{tot}}$. Plugging the Gibbs-Thomson relation (Eq. (4.8)) in this result, we find the influence of the surface tension on the drop radius:

$$(R^*)^3 = \left(\frac{P_{\text{tot}} - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} - \frac{\hat{P}_{\text{out}} l_c}{\hat{P}_{\text{in}} R^*} \right) \left(1 + \mathcal{O} \left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} \right) \right) L^3 . \quad (\text{B.37})$$

The drop radius thus scales as the system size ($\propto L$) with a negative finite size correction ($\propto 1/R$). We also find the radius R_n of the nucleus, which is the smallest drop that can exist:

$$R_n \simeq \frac{\hat{P}_{\text{out}} l_c}{P_{\text{tot}} - \hat{P}_{\text{out}}} . \quad (\text{B.38})$$

Smaller drops dissolve because the concentration of P outside the drop next to interface is larger than the total concentration P_{tot} . We now study the stability of a multi-drop system by taking $k, h = 0$ (leading to $x, y \rightarrow 0$ but $y/x = R/L$), and Γ given by (B.34). Thus from Eqs (4.47) we find:

$$f_1 = 0 \quad (\text{B.39})$$

$$f_2 = 0 \quad (\text{B.40})$$

$$f_3 = 0 \quad (\text{B.41})$$

$$f_4 = -1 + \mathcal{O}\left(\frac{R}{L}\right), \quad (\text{B.42})$$

and therefore

$$g_1 \simeq \frac{4\pi D \hat{P}_{\text{out}} l_c}{R^*} > 0. \quad (\text{B.43})$$

Since $g_1 > 0$ for all radii R^* we recover the result that a passive multi-drop system is always unstable to Ostwald ripening [33].

B.4 Chemical reactions cause drops shrinkage and nucleus radius increase

We will demonstrate qualitatively that chemical reactions cause drops to shrink, while the radius of the smallest possible drop in a steady-state system, or nucleus, increases (see Eq. (B.38) for the passive case).

Drop shrinkage. We consider a single-drop system that is initially passive ($k = h = 0$), with total concentrations P_{tot} and S_{tot} . The total solute concentration $\phi \equiv P_{\text{tot}} + S_{\text{tot}}$ is small, and the phase separation is strong so $\hat{P}_{\text{in}} \gg \hat{P}_{\text{out}}$. The drop radius is therefore given by Eq. (B.37). We now switch on the chemical reactions ($k, h > 0$), in such a way that P_{tot} and S_{tot} remain unchanged (k/h being given by Eq. (B.34)). Let us first proceed to a simple intuitive argument: outside the drop, the concentrations of both P and S are small and we neglect the chemical reactions. Inside the drop however, the P concentration is high so we expect that the reaction $P \xrightarrow{k} S$ dominates, and depletes P from the drop. This will lead to the drop shrinkage, as molecules P are converted into S and evacuated outside by diffusive flux. As the drop shrink, the supersaturation outside the drop must increase, in order to conserve the total number of molecules P in the system. The increase of

the supersaturation must result in an influx of P toward the drop (Section 2.7.1, Eq. (2.40)). At steady-state, the drop radius is such that both efflux and influx are balanced. From this intuitive argument, we expect that drops are smaller when chemical reactions are switched on.

We now show that this argument is indeed correct. At the exact time $t = 0$ at which chemical reactions are switched on, the concentration profiles are flat inside and outside the drop. We can predict qualitatively how the system reacts after a small time interval $t = dt$. Using the reaction-diffusion equations Eqs (4.12) and (4.14) with $\nabla^2 P_{\text{in/out}} = 0$ we find the variation of the concentration P inside and outside the drop:

$$\frac{dP_{\text{in}}}{dt} = -k\hat{P}_{\text{in}} + hS_{\text{tot}} \quad (\text{B.44})$$

$$\frac{dP_{\text{out}}}{dt} = -kP_{\text{out}} + hS_{\text{tot}} , \quad (\text{B.45})$$

and since $S_{\text{tot}} = P_{\text{tot}}k/h$ (Eq. (4.11)):

$$\frac{dP_{\text{in}}}{dt} = -k(\hat{P}_{\text{in}} - P_{\text{tot}}) \quad (\text{B.46})$$

$$\frac{dP_{\text{out}}}{dt} = k(P_{\text{tot}} - P_{\text{out}}) . \quad (\text{B.47})$$

$\hat{P}_{\text{in}} > P_{\text{tot}} > P_{\text{out}}$ is a condition for a phase separation to occur due to the conservation of the number of molecules P in the system. Moreover we focus only on systems where the drop density is small ($R^3/L^3 \ll 1$) so from Eq. (B.37) we must have $P_{\text{tot}} \ll \hat{P}_{\text{in}}$. As a result, the decrease in concentration inside the drop must be larger than the increase in concentration outside the drop ($|dP_{\text{in}}/dt| > |dP_{\text{out}}/dt|$). Because of the fixed interfacial boundary conditions (Eqs (4.7), (4.8)), we expect that the gradient inside the drop next to the interface is greater than that right outside the drop, i.e.,

$$\left. \frac{\partial P_{\text{in}}}{\partial r} \right|_R > \left. \frac{\partial P_{\text{out}}}{\partial r} \right|_R . \quad (\text{B.48})$$

Therefore, the concentration of P is depleted at the interface. In order for the interface concentration to remain fixed, the drop radius must shrink.

Increase of the nucleus radius. Let us now see the effect of chemical reactions on the nucleus radius. Consider a nucleus of a system in passive condition ($k = h = 0$). Its radius R_n is given by Eq. (B.38). From the Gibbs-Thomson relation (Eq. (4.8)) we know that the P concentration right outside the nucleus is identical to P_{tot} . When chemical reactions are turned on (keeping $P_{\text{tot}}, S_{\text{tot}}$ constant), the

nucleus must shrink from the argument we have just exposed. As a result, the concentration of P just outside the nucleus will exceed P_{tot} (Eq. (4.8)), breaking down the requirement that the total number of molecules P must be conserved. This must lead to the drop dissolution. To compensate for this effect, the nucleus in active systems is necessarily larger than the nucleus in passive system.

We have shown qualitatively that when chemical reactions are turned on, drops shrink while the size of the smallest possible drop that can exist, the nucleus, increase.

B.5 Small drops and vanishing drop number density

We present the calculation leading to the steady-state drop expression (Eq. (4.54)), in the regime of small drops ($R \gg \xi$) and vanishing drop number density $\rho \rightarrow 0$. Here, $x \equiv L/\xi \rightarrow \infty$ and $y \equiv R^*/\xi \ll 1$. By expanding the steady-state condition (Eq. (4.40)) and the stability relation g_1 (Eq. (4.47) for $x \gg 1$ and $y \ll 1$, we find:

$$\frac{y^2}{3} = \lambda \left(1 + \mathcal{O}\left(\frac{1}{x}\right) + \mathcal{O}(y) \right), \quad (\text{B.49})$$

and λ , from Eq. (4.41), is:

$$\lambda = \frac{\phi - P_{\text{out}}(1 + \Gamma)}{\hat{P}_{\text{in}}\Gamma} \left(1 + \mathcal{O}\left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}\Gamma}\right) + \mathcal{O}\left(\frac{\hat{P}_{\text{in}}}{\frac{\phi}{1+\Gamma} - \hat{P}_{\text{out}}} \frac{R}{L^3}\right) + \mathcal{O}\left(\frac{1}{\Gamma} \frac{R}{L^3}\right) \right) \quad (\text{B.50})$$

with $\Gamma \equiv k/h$. Plugging λ into y we find an expression of the steady-state drop radius:

$$R_u = \sqrt{\frac{3D \left(\frac{\phi}{1+\Gamma} - \hat{P}_{\text{out}} \left(1 + \frac{l_c}{R} \right) \right)}{k\hat{P}_{\text{in}}}} \times \left(1 + \mathcal{O}\left(\frac{1}{x}\right) + \mathcal{O}(y) + \mathcal{O}\left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}\Gamma}\right) + \mathcal{O}\left(\frac{\hat{P}_{\text{in}}}{\frac{\phi}{1+\Gamma} - \hat{P}_{\text{out}}} \frac{R^3}{L^3}\right) + \mathcal{O}\left(\frac{1}{\Gamma} \frac{R^3}{L^3}\right) \right), \quad (\text{B.51})$$

and we recover (Eq. (4.54)). We check self-consistently that the “ $\mathcal{O}(\cdot)$ ” quantities here and in Eq. (B.50) are indeed small. We start with the condition $\mathcal{O}(y) \ll 1$,

equivalent to

$$R_u \ll \xi \quad (\text{B.52})$$

$$k \gg \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} h . \quad (\text{B.53})$$

This requirement together with the fact that $\hat{P}_{\text{in}} > \phi > \hat{P}_{\text{out}}$ must be true in a phase-separating system, shows that $\mathcal{O}((\phi - \hat{P}_{\text{out}})/\hat{P}_{\text{in}}\Gamma)$ is small. Finally, $\mathcal{O}(1/x)$, $\mathcal{O}[\hat{P}_{\text{in}}/(\phi/(1 + \Gamma) - \hat{P}_{\text{out}})R^3/L^3]$ and $\mathcal{O}[R^3/(\Gamma L^3)]$ can be set arbitrary small by increasing L , or equivalently by decreasing the drop number density ρ .

B.6 Critical forward rate constant k_c

We estimate the upper-bounds of the critical forward rate constant k_c above which dissolve (Eqs (4.57)), in the regime of small drops and vanishing drop number density ($R \ll \xi$, $\rho \rightarrow 0$, Section 4.5.1). We can re-write the expression for the drop radius, Eq. (4.54), as:

$$aR^3 + bR^2 + cR + d = 0 , \quad (\text{B.54})$$

with

$$a = \frac{k_c \hat{P}_{\text{in}}}{3D} , \quad b = 0 , \quad c = - \left(\frac{\phi h}{k + k_c} - \hat{P}_{\text{out}} \right) , \quad d = \hat{P}_{\text{out}} l_c . \quad (\text{B.55})$$

Since $a > 0$, $b = 0$, and $d > 0$, this cubic equation in R admits no real solution if the determinant $\Delta = 18abcd - 4b^3d + b^2c^2 - 4ac^3 - 27a^2d^2$ is negative, and two real positive solutions if $\Delta > 0$. We have ignored complex or negative solutions since they are unphysical. The expression of Δ is:

$$\Delta = \frac{k \hat{P}_{\text{in}}}{D} \left[\frac{4}{3} \left(\frac{\phi h}{k + k_c} - \hat{P}_{\text{out}} \right)^3 - \frac{3k \hat{P}_{\text{in}} \hat{P}_{\text{out}}^2 l_c^2}{D} \right] . \quad (\text{B.56})$$

At small rate constant k the discriminant Δ is positive so two steady-state radii R exist. At large k the discriminant Δ becomes negative so there are no steady-state radii and therefore no drops can exist in the system. The critical rate constant k_c

at which this transition occurs is the solution of $\Delta(k_c) = 0$:

$$\frac{k_c}{\left(\frac{\phi h}{k_c + h} - \hat{P}_{\text{out}}\right)^3} = \frac{4D}{9l_c^2 \hat{P}_{\text{in}} \hat{P}_{\text{out}}^2} . \quad (\text{B.57})$$

We can find upper bounds on k_c by noticing the two following elements: first, this equation admits a solution only if $\phi h/(k_c + h) - \hat{P}_{\text{out}} > 0$; and second, k_c is a monotonic and increasing function of h , therefore k_c is upper bounded by $k_c(h \rightarrow \infty)$. Using these two arguments, the critical rate constant k_c is thus bounded as follow:

$$k_c < \min \left\{ \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} h \quad ; \quad \frac{4D \left(\phi - \hat{P}_{\text{out}}\right)^3}{9l_c^2 \hat{P}_{\text{in}} \hat{P}_{\text{out}}^2} \right\} . \quad (\text{B.58})$$

Importantly, note that the validity of the expression of k_c and its upper-bounds (Eqs (B.57) and (B.58)) break down as R approaches ξ (see insert in Fig. 4.5).

B.7 Small drops and high drop number density

We detail the calculation leading to the steady-state drop radius (Eq. (4.63)) and the stability-instability boundary radius (Eq. (4.65)), in the regime small drop ($R \ll \xi$) and high drop number density ($L \ll \xi$) regime. Moreover we focus only on forward rate constants k such that $k \gg k_u$ (Eq. (4.72)).

Steady-state

Expanding for $y \ll x \ll 1$, the steady-state condition Eq. (4.40) becomes

$$x^3 = \frac{(\lambda + 1)y^3}{\lambda - \frac{y^2}{3}} (1 + \mathcal{O}(x^2)) . \quad (\text{B.59})$$

with λ given by Eq. (4.41). Imposing $y \ll x \ll 1$ on this result leads to the following requirements:

$$\frac{y^2}{3} \ll \lambda \ll 1 \quad (\text{B.60})$$

$$\Rightarrow \quad \mathcal{O}\left(\frac{y^3}{x^3}\right) = \mathcal{O}(\lambda) \quad (\text{B.61})$$

$$\Rightarrow \quad x^3 \ll y , \quad (\text{B.62})$$

and Eq. (B.59) thus becomes:

$$x^3 = \frac{y^3}{\lambda} \left(1 + \mathcal{O}(x^2) + \mathcal{O}\left(\frac{x^3}{y}\right) \right). \quad (\text{B.63})$$

Plugging the expression of λ (Eq. (4.41)) into this result, we find the steady-state drop radius R :

$$\begin{aligned} R^3 = & \frac{\frac{\phi}{1+\Gamma} - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} L^3 \left[1 + \frac{\Gamma}{1+\Gamma} \left(\mathcal{O}(x^2) + \mathcal{O}\left(\frac{x^3}{y}\right) + \mathcal{O}\left(\frac{R^3}{L^3\Gamma}\right) + \mathcal{O}\left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}\Gamma}\right) \right) \right. \\ & \left. + \mathcal{O}\left(\frac{1}{1+\Gamma} \frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) \right]. \end{aligned} \quad (\text{B.64})$$

with $\Gamma \equiv k/h$, hence Eq. (4.63).

We now show that the terms “ $\mathcal{O}(\cdot)$ ” are indeed small. Since $\hat{P}_{\text{in}} > \phi > \hat{P}_{\text{out}}$ must be true in a phase-separating system, and taking $k \gg k_u$ (Eq. (4.72)) shows that $\mathcal{O}[(\phi - \hat{P}_{\text{out}})/\hat{P}_{\text{in}}\Gamma]$ and $\mathcal{O}[1/(1+\Gamma)\hat{P}_{\text{out}}/\hat{P}_{\text{in}}]$ are small. Using Eq. (B.64) and keeping only the dominant order, the condition $\mathcal{O}(R^3/(L^3\Gamma)) \ll 1$ becomes:

$$\Gamma + \Gamma^2 - \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} \gg 0 \quad (\text{B.65})$$

$$\Gamma \gg \frac{1}{2} \left(\sqrt{1 + 4 \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}} - 1 \right) \simeq \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} \quad (\text{B.66})$$

$$k \gg k_u, \quad (\text{B.67})$$

which is a condition we have already imposed. Finally, using again Eq. (B.64) and keeping only the dominant order, the condition $\mathcal{O}(x^3/y) \ll 1$ leads to a lower bound on the drop number density ρ :

$$\rho \gg \frac{3}{4\pi} \left(\frac{k+h}{D} \right)^{3/2} \left(\frac{\hat{P}_{\text{in}}}{\frac{\phi h}{k+h} - \hat{P}_{\text{out}}} \right)^{1/2}. \quad (\text{B.68})$$

Stability

Expanding for $y \ll x \ll 1$ and keeping in mind that $x^3 \ll y$ (Eq. (B.60)), Eqs (4.48)-(4.51) become

$$f_1 = -\frac{y^2}{3} (1 + \mathcal{O}(y^2)) \quad (\text{B.69})$$

$$f_2 = \frac{x^3}{3y} \left(1 + \mathcal{O}(x) + \mathcal{O}\left(\frac{y}{x}\right) + \mathcal{O}\left(\frac{x^3}{y}\right) \right) \quad (\text{B.70})$$

$$f_3 = -\frac{y^2}{3} (1 + \mathcal{O}(y^2)) \quad (\text{B.71})$$

$$f_4 = -1 + \mathcal{O}(x) + \mathcal{O}\left(\frac{y}{x}\right). \quad (\text{B.72})$$

and using these results together with the steady-state condition Eq. (B.63), the stability relation Eq. (4.47) becomes

$$g_1 = 4\pi D \left[-\frac{2y^2 H_{\text{in}}}{3} \left(1 + \mathcal{O}(x) + \mathcal{O}\left(\frac{y}{x}\right) + \mathcal{O}\left(\frac{x^3}{y}\right) \right) + \frac{\hat{P}_{\text{out}} l_c}{R} \left(1 + \mathcal{O}(x) + \mathcal{O}\left(\frac{y}{x}\right) \right) \right]. \quad (\text{B.73})$$

The term $\mathcal{O}(x)$ is always small by definition in this regime (high drop number density). $\mathcal{O}(y/x)$ is small because the drop density is small (equivalently, the supersaturation is small). Finally, $\mathcal{O}(x^3/y)$ is small from Eq. (B.60).

The system is unstable for small radii ($g_1 > 0$) and stable for large radii ($g_1 < 0$). The stability-instability boundary radius R_l is the solution of $g_1(R_l) = 0$:

$$R_l y^2 \simeq \frac{3\hat{P}_{\text{out}} l_c}{2H_{\text{in}}}. \quad (\text{B.74})$$

Expanding H_{in} (Eq. (4.27)) gives

$$H_{\text{in}} = \frac{\Gamma \hat{P}_{\text{in}}}{1 + \Gamma} \left(1 + \mathcal{O}\left(\frac{\phi - \hat{P}_{\text{out}}}{\Gamma \hat{P}_{\text{in}}}\right) + \mathcal{O}\left(\frac{R^3}{L^3 \Gamma}\right) \right). \quad (\text{B.75})$$

We have shown previously that, in this regime, the terms $\mathcal{O}(..)$ that appear in this relations are small. Plugging this result into Eq. (B.74):

$$R_l \simeq \left(\frac{3Dl_c \hat{P}_{\text{out}}}{2k\hat{P}_{\text{in}}} \right)^{\frac{1}{3}}, \quad (\text{B.76})$$

hence Eq. (4.65).

We check self-consistently the condition $\mathcal{O}(y) \ll 1$:

$$R_l \ll \xi \quad (\text{B.77})$$

$$\frac{D^2 l_c^2 \hat{P}_{\text{out}}^2}{k^2 \hat{P}_{\text{in}}^2} \ll \frac{D^3}{h^3 (1 + \Gamma)^3} \quad (\text{B.78})$$

$$k \gg \frac{l_c \hat{P}_{\text{out}} h^{3/2}}{D^{1/2} \hat{P}_{\text{in}}} \left(1 + \mathcal{O} \left(\frac{l_c \hat{P}_{\text{out}} h^{1/2}}{D^{1/2} \hat{P}_{\text{in}}} \right) \right) \simeq k_l . \quad (\text{B.79})$$

When the backward rate constant h is smaller than the critical rate constant h_0 (4.73), then we have $k_l < k_u$. Therefore the above condition is always true in the $k \gg k_u$ regime. Using the expression of h_0 (Eq. (4.73)) we also see that $\mathcal{O}(l_c \hat{P}_{\text{out}} h^{1/2} / (D^{1/2} \hat{P}_{\text{in}}))$ is always small when $h < h_0$. If on the contrary $h > h_0$ we have shown in Section 4.5.1 that only large drops ($R \gg \xi$) exist, so R_l is undefined.

B.8 Large drops

We will present, in the large drop regime ($R \gg \xi$), the details of the derivation leading the steady-state drop radius (Eq. 4.66), the stability-instability boundary rate constant k_l (Eq. (4.71)), and the maximal forward rate constant k_u above which large drops dissolve. Since the inter-drop distance L is always larger than R this regime also implies that $L \gg \xi$.

Steady-state

We expand the steady-state condition Eq. (4.40) for $x \gg y \gg 1$ and find:

$$y = \frac{1 + \lambda}{1 - \lambda} \left(1 + \mathcal{O} \left(\frac{1}{x} \right) + \mathcal{O} \left(e^{-2(x-y)} \right) + \mathcal{O} \left(e^{-2y} \right) \right). \quad (\text{B.80})$$

with λ given by Eqs (4.41). For $y \gg 1$ to be true we must also have

$$\lambda < 1, \quad \mathcal{O}(\lambda) = \mathcal{O}(1), \quad (\text{B.81})$$

and we further expand using $\lambda - 1$ as a small parameter:

$$y = \frac{2}{1 - \lambda} \left(1 + \mathcal{O} \left(\frac{1}{y} \right) + \mathcal{O} \left(e^{-2(x-y)} \right) \right). \quad (\text{B.82})$$

Using Eqs (4.41) and expanding further in the small parameters l_c/R and $1/y$ we get the steady-state radius R :

$$(R^*)^3 = \left(a + \frac{b}{R}\right) L^3, \quad (\text{B.83})$$

with

$$a = \left(\frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{in}}} - \frac{\Gamma}{2} \left(1 + \mathcal{O}\left(\frac{1}{y}\right) \right) \right) \left(1 + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) \right) \quad (\text{B.84})$$

$$b = -\frac{\hat{P}_{\text{out}} l_c}{\hat{P}_{\text{in}}} \left(1 + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) \right) + \frac{\Gamma \xi}{2} \left(1 + \mathcal{O}\left(\frac{1}{y}\right) + \mathcal{O}(e^{-2(x-y)}) \right) \quad (\text{B.85})$$

In large drop limit, $b/R \rightarrow 0$, and there is a critical rate constant k_u above which drops cease to exist ($R < 0$):

$$k_u = \frac{2(\phi - \hat{P}_{\text{out}})h}{\hat{P}_{\text{in}}} \left[1 + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) \right], \quad (\text{B.86})$$

hence Eq. (4.72). Solving $b(k) = 0$ gives the rate constant k for which the finite size correction b/R switch sign (Eq. (4.69)):

$$k = \frac{2l_c \hat{P}_{\text{out}} h^{3/2}}{D^{1/2} \hat{P}_{\text{in}}} \left[1 + \mathcal{O}\left(\frac{k}{h}\right) + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) + \mathcal{O}\left(\frac{1}{y}\right) + \mathcal{O}(e^{-2(x-y)}) \right], \quad (\text{B.87})$$

where we have used the fact that in the large drop regime k must be smaller than k_u therefore k/h is always small.

Stability

We expand Eqs (B.33) for $x \gg y \gg 1$:

$$f_1 = -1 + \mathcal{O}(ye^{-2(x-y)}) \quad (\text{B.88})$$

$$f_2 = 1 + \mathcal{O}\left(\frac{1}{x}\right) + \mathcal{O}(ye^{-2(x-y)}) \quad (\text{B.89})$$

$$f_3 = -y \left(1 + \mathcal{O}\left(\frac{1}{y}\right) \right) \quad (\text{B.90})$$

$$f_4 = -y \left(1 + \mathcal{O}\left(\frac{1}{y}\right) + \mathcal{O}(e^{-2(x-y)}) \right). \quad (\text{B.91})$$

From the definitions of $H_{\text{in/out}}$ (Eqs (4.27),(4.28)) we have

$$H_{\text{out}} - H_{\text{in}} = -\Gamma \hat{P}_{\text{in}} \left(1 + \mathcal{O}(\Gamma) + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) \right), \quad (\text{B.92})$$

Again, Γ is small in this regime since $k \ll k_u$ Eq. (B.86). Using the steady-state radius Eq. (4.66) in the definitions of $H_{\text{in/out}}$ we find

$$\mathcal{O}(H_{\text{in}}) = \mathcal{O}(H_{\text{out}}) = \mathcal{O}(\hat{P}_{\text{in}}\Gamma). \quad (\text{B.93})$$

Therefore Eq. (4.47) becomes

$$g_1 = 4\pi D \left(-\Gamma \hat{P}_{\text{in}} (1 + \delta_1) + \frac{2l_c \hat{P}_{\text{out}}}{\xi} (1 + \delta_2) \right) \quad (\text{B.94})$$

$$= 4\pi D \left(-\Gamma \hat{P}_{\text{in}} (1 + \delta_1) + \frac{2l_c \hat{P}_{\text{out}} h^{1/2}}{D^{1/2}} (1 + \delta_2) \right), \quad (\text{B.95})$$

with

$$\delta_1 = \mathcal{O}(\Gamma) + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) + \mathcal{O}\left(\frac{1}{x}\right) + \mathcal{O}(ye^{-2(x-y)}) \quad (\text{B.96})$$

$$\delta_2 = \mathcal{O}(\Gamma) + \mathcal{O}\left(\frac{1}{y}\right) + \mathcal{O}(e^{-2(x-y)}). \quad (\text{B.97})$$

Remembering that $\Gamma \equiv k/h$, we see that the system is unstable at small k ($g_1 > 0$) and stable at large k ($g_1 < 0$). We seek the critical rate constant k_l at which the stability-instability transition occurs ($g_1(k_l) = 0$):

$$k_l = \frac{2l_c \hat{P}_{\text{out}} h^{3/2}}{D^{1/2} \hat{P}_{\text{in}}} \left(1 + \mathcal{O}\left(\frac{k_l}{h}\right) + \mathcal{O}\left(\frac{\hat{P}_{\text{out}}}{\hat{P}_{\text{in}}}\right) + \mathcal{O}\left(\frac{1}{y}\right) + \mathcal{O}(ye^{-2(x-y)}) \right) \quad (\text{B.98})$$

If the backward rate constant h is smaller than the critical rate constant h_0 (Eq. (4.73), then $k_l < k_u$, and $\mathcal{O}(k_l/h)$ is always small (Eq. (4.72)). On the contrary, if $h > h_0$, $k_l > k_u$ so k_l is not defined, since large drops dissolve for $k > k_u$. In this case large drops are always unstable.

B.9 Jump of the concentration S at the drop interface

In the case where the concentration S has a discontinuity at a drop interface, we define

$$\Delta S \equiv \frac{S_{\text{in}}^{(i)}(R_i)}{S_{\text{out}}^{(i)}(R_i)}, \quad (\text{B.99})$$

and solving the system of equations Eqs (B.99), (4.33), (4.18), (4.19) leads to

$$S_{\text{in}}^{(i)}(R_i) = \Delta S \frac{\phi - P_{\text{out}}^{(i)}(R_i) - \frac{1}{2} \left[\left(\hat{P}_{\text{in}} - P_{\text{out}}^{(1)}(R_1) \right) \left(\frac{R_1}{L} \right)^d + \left(\hat{P}_{\text{in}} - P_{\text{out}}^{(2)}(R_2) \right) \left(\frac{R_2}{L} \right)^d \right]}{1 - \frac{1}{2} \left(\frac{R_j}{L} \right)^d + \frac{1}{2} \Delta S \frac{R_1^d + R_2^d}{L^d}}, \quad j \neq i \quad (\text{B.100})$$

$$S_{\text{out}}^{(i)}(R_i) = \frac{S_{\text{in}}^{(i)}(R_i)}{\Delta S}. \quad (\text{B.101})$$

Plugging these results in the definitions of $H_{\text{in/out}}$ (Eqs (4.27), (4.28)), we find:

$$H_{\text{in}} = P_{\text{in}} - \frac{\Delta S \phi + \left(\hat{P}_{\text{in}} - \Delta S P_{\text{out}}(R^*) \right) \left(1 - \left(\frac{R}{L} \right)^d \right)}{(\chi + 1) \left(1 - (1 - \Delta S) \left(\frac{R}{L} \right)^d \right)} \quad (\text{B.102})$$

$$H_{\text{out}} = P_{\text{out}} - \frac{\phi - \left(\hat{P}_{\text{in}} - \Delta S P_{\text{out}}(R^*) \right) \left(\frac{R}{L} \right)^d}{(\chi + 1) \left(1 - (1 - \Delta S) \left(\frac{R}{L} \right)^d \right)} \quad (\text{B.103})$$

$$\mathcal{H}_{\text{in}} = -\frac{\Delta S \hat{P}_{\text{out}} l_c}{(\chi + 1) R^2} \quad (\text{B.104})$$

$$\mathcal{H}_{\text{out}} = -\frac{\hat{P}_{\text{out}} l_c}{R^2}. \quad (\text{B.105})$$

The other quantities appearing in the steady state relation Eq. (4.40) and the stability relation g_1 Eq. (4.47) are unchanged.

B.10 Two-dimensional systems

We present the theoretical results of Chapter 4 in two dimensions and for an arbitrary jump ΔS (B.99) of the concentration S at the interface. The steady-state condition (Eq. (4.40)) becomes

$$H_{\text{out}} [A J_1(\iota y^*) - B Y_1(-\iota y^*)] - H_{\text{in}} \frac{J_1(\iota y^*)}{J_0(\iota y^*)} = 0, \quad (\text{B.106})$$

with J_0 and Y_0 are the 0-th order Bessel functions of the first and second kind, respectively, and ι is the imaginary unit $\sqrt{-1}$, and $y^* \equiv R^*/\xi$. The stability condition (Eq. (4.47)) becomes

$$\begin{aligned} \frac{g_1(R^*)}{4\pi D R^{*2}} = & \mathcal{H}_{\text{out}} \frac{\iota}{\xi} [-AJ_1(\iota y^*) + BY_1(-\iota y^*)] + H_{\text{out}} \frac{\iota}{\xi} [-\mathcal{A}J_1(\iota y^*) + \mathcal{B}Y_1(-\iota y^*)] \\ & + H_{\text{out}} \frac{1}{\xi^2} \left[A \left(J_0(\iota y^*) + \frac{\iota}{y^*} J_1(\iota y^*) \right) + B \left(Y_0(-\iota y^*) - \frac{\iota}{y^*} Y_1(-\iota y^*) \right) \right] \\ & + \mathcal{H}_{\text{in}} \frac{\iota}{\xi} \frac{J_1(\iota y^*)}{J_0(\iota y^*)} - H_{\text{in}} \frac{1}{\xi^2} \left[\frac{J_0(\iota y^*) + \frac{\iota}{y^*} J_1(\iota y^*)}{J_0(\iota y^*)} + \left(\frac{J_1(\iota y^*)}{J_0(\iota y^*)} \right)^2 \right] \end{aligned} \quad (\text{B.107})$$

with $x \equiv L/\xi$ and

$$A = \frac{Y_1(-\iota x)}{J_1(\iota x)Y_0(-\iota y^*) + Y_1(-\iota x)J_0(\iota y^*)} \quad (\text{B.108})$$

$$B = \frac{Y_1(\iota x)}{J_1(\iota x)Y_0(-\iota y^*) + Y_1(-\iota x)J_0(\iota y^*)} \quad (\text{B.109})$$

$$\mathcal{A} = \frac{\iota J_1(\iota x)Y_1(-\iota y^*)Y_0(-\iota x) - \iota J_1(\iota y^*)Y_1(-\iota x)Y_0(-\iota x) - \xi \frac{\mathcal{H}_{\text{out}}}{H_{\text{out}}} [J_0(\iota x)Y_1(-\iota x)Y_0(-\iota y^*) + J_1(\iota x)Y_0(-\iota x)Y_0(-\iota y^*)]}{\xi [J_0(\iota x)Y_0(-\iota y^*) - Y_0(-\iota x)J_0(\iota y^*)] [J_1(\iota x)Y_0(-\iota y^*) + Y_1(-\iota x)J_0(\iota y^*)]} \quad (\text{B.110})$$

$$\mathcal{B} = \frac{-\iota J_1(\iota x)Y_1(-\iota y^*)J_0(\iota x) + \iota J_1(\iota y^*)Y_1(-\iota x)J_0(\iota x) + \xi \frac{\mathcal{H}_{\text{out}}}{H_{\text{out}}} [J_0(\iota x)Y_1(-\iota x)J_0(\iota y^*) + J_1(\iota x)Y_0(-\iota x)J_0(\iota y^*)]}{\xi [J_0(\iota x)Y_0(-\iota y^*) - Y_0(-\iota x)J_0(\iota y^*)] [J_1(\iota x)Y_0(-\iota y^*) + Y_1(-\iota x)J_0(\iota y^*)]} \quad (\text{B.111})$$

$$H_{\text{in}} = \hat{P}_{\text{in}} - \frac{\Delta S \phi + \left(\hat{P}_{\text{in}} - \Delta S P_{\text{out}}(R^*) \right) \left(1 - \left(\frac{R^*}{L} \right)^2 \right)}{(\chi + 1) \left(1 - (1 - \Delta S) \left(\frac{R^*}{L} \right)^2 \right)} \quad (\text{B.112})$$

$$H_{\text{out}} = P_{\text{out}} - \frac{\phi - \left(\hat{P}_{\text{in}} - \Delta S P_{\text{out}}(R^*) \right) \left(\frac{R^*}{L} \right)^2}{(\chi + 1) \left(1 - (1 - \Delta S) \left(\frac{R^*}{L} \right)^2 \right)} \quad (\text{B.113})$$

$$\mathcal{H}_{\text{in}} = -\frac{\Delta S \hat{P}_{\text{out}} l_c}{(\chi + 1) R^2} \quad (\text{B.114})$$

$$\mathcal{H}_{\text{out}} = -\frac{\hat{P}_{\text{out}} l_c}{R^2} . \quad (\text{B.115})$$

Note that we are interested only in the real parts of Eqs (B.106) and (B.107).

Appendix C

Application of Formalism: Stress Granule Formation

C.1 Difference of size between a stable drop and the nucleus close to the dissolution rate

In model A for $H_A \ll h_0$ and model C for $K_C/H_C \gg \eta$ we have seen that there exists a critical ATP concentration α_c beyond which no drops can exist, and below which drops can nucleate from a minimal radius R_n and grow until they reach their stable radius that is upper bounded by R_u . Therefore one can control drop formation and dissolution via small variations of α in the vicinity of α_c (Figs 6.3(a) and 6.4(a)). From experimental observations we know that during stress α vary by two-fold and $R_u \gg R_n$, providing a constraint on our modelling (Sec. 6.1). Here we examine this constraint by quantifying the size ratio R_u/R_n in the vicinity of α_c .

We consider a single drop of radius R in an infinite system. Its nucleus radius R_n and stable radius R_u are equal to the nucleus radius and maximal drop radius in a multi-drop system (Section B.4). When drops are much smaller than the gradient length scale ξ which is true in the regimes under consideration, the net flux J of molecules P at the drop interface is composed of an in-flux from the medium and an out-flux due to the chemical conversion $P \xrightarrow{k} S$ inside the drop that depletes P (Section 3.2):

$$J = 4\pi DR \left(\Delta - \frac{\hat{P}_{\text{out}} l_c}{R} \right) - \frac{4\pi R^3}{3} k \hat{P}_{\text{in}} , \quad (\text{C.1})$$

where Δ is the supersaturation set by the chemical reaction rate constants k, h :

$$\Delta = \frac{\phi}{1 + k/h} - \hat{P}_{\text{out}} . \quad (\text{C.2})$$

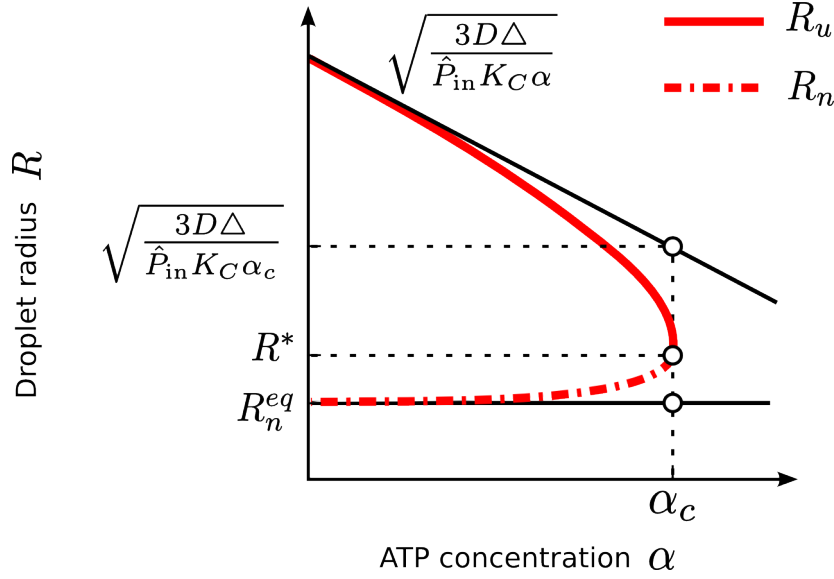


Figure C.1: Fixed points of the drop growth rate Eq. (C.1) in an infinite single-drop system for varying ATP concentration α , for model C in the $K_C/H_C \gg \eta$ regime. The stable drop radius R_u (red continuous line) and the nucleus radius R_n (red dashed line) correspond to the maximal stable radius and nucleus radius in a multi-drop system (Fig. 6.3 (a)). No drops exist for α larger than a critical value α_c . For $\alpha \ll \alpha_c$, $R_u \simeq \sqrt{3D\Delta/(\hat{P}_{\text{in}}K_C\alpha)}$ (upper black line, Eq. (C.5)) and $R_n \simeq R_n^{\text{eq}}$ (lower black line, Eq. (C.4)). When $\alpha \lesssim \alpha_c$, small variations of k lead to strong variations of R_u and R_n . In this strong response regime the ratio R_u/R_n is bounded by $\sqrt{3D\Delta/(\hat{P}_{\text{in}}K_C\alpha_c)}/R_n^{\text{eq}}$.

The drop grows when $J > 0$ and shrink otherwise. At equilibrium ($k = h = 0$ but k/h is still defined by Eqs (6.2) or (6.4)) there is a unique fixed point radius ($J = 0$):

$$R_c^{\text{eq}} = \frac{\hat{P}_{\text{out}}l_c}{\Delta}. \quad (\text{C.3})$$

R_c^{eq} is unstable ($dJ/dR|_{R_n} > 0$) and is the nucleus radius at equilibrium: smaller drops dissolve while larger drops grow.

When chemical reactions are switched on ($k, h > 0$), Eq. (C.1) admits two fixed points, shown in Fig. C.1 for varying k . For small R we can neglect the reaction term ($\propto kR^3$) and find the unstable fixed point, or nucleus radius,

$$R_n \simeq R_n^{\text{eq}}, \quad (\text{C.4})$$

and for large R we neglect the surface tension term ($\propto l_c/R$) and find the stable

fixed point.

$$R_u \simeq \sqrt{\frac{3D\Delta}{\hat{P}_{\text{in}}k}}. \quad (\text{C.5})$$

Additionally there exist a critical rate constant k_c above which no fixed points exist and $J < 0$ for all R meaning that all drops dissolve.

We will now examine these results in model A and C, seeking for the ratio R_u/R_n for $k \simeq k_c$.

Model C

In model C, $k = \alpha K_C$ and $h = \alpha H_C$ so the ratio k/h and Δ are constant. Therefore $R_u \propto \alpha^{-1/2}$ and we recover Eq. (6.12). Since this scaling is sub-linear we saw that it cannot explain SG formation and dissolution upon small variations of α . However when α approaches α_c the separation between R_n and R_u becomes small so the above approximations cease to be valid and a strong response regime exists: small variations of α lead to strong variations of R_u and R_n (Fig. C.1).

Qualitatively, it can be seen from Eq. (C.1) that since we omitted the term $\propto l_c/R$ in the determination of R_u we have overestimated R_u , and since we neglected the term $\propto kR^3$ in the determination of R_n we have underestimated R_n . Therefore the exact value of R_u is bounded from above by $\sqrt{3D\Delta/(\hat{P}_{\text{in}}k)}$ while the exact value of R_n is bounded from below by R_n^{eq} . The ratio R_u/R_n in the strong response regime is therefore also bounded (see Fig. C.1):

$$\frac{R_u}{R_n} < \frac{\sqrt{3D\Delta/(\hat{P}_{\text{in}}k)}}{R_n^{\text{eq}}} \bigg|_{k=K_C\alpha_c}. \quad (\text{C.6})$$

At $\alpha = \alpha_c$ the two fixed points R_n and R_u intersect at the radius R^* and since R_n and R_u are unstable and stable fixed points, respectively, we have

$$J(\alpha_c, R^*) = 0 \quad (\text{C.7a})$$

$$\frac{dJ}{dR} \bigg|_{\alpha_c, R^*} = 0, \quad (\text{C.7b})$$

and solving for α_c gives

$$\alpha_c = \frac{4D\Delta^3}{9l_c^2 \hat{P}_{\text{out}}^2 \hat{P}_{\text{in}} K_C}. \quad (\text{C.8})$$

We then find

$$\frac{R_u}{R_n} < \frac{3\sqrt{3}}{2}. \quad (\text{C.9})$$

In other words, the size of the stable drops and nuclei are of the same order. This shows independently of the system parameters that the strong response regime in model C cannot account for the switch-like response observed experimentally.

Model A

We now concentrate on model A. Here the supersaturation Δ is no more constant since only the backward rate is constant (i.e. $k = \alpha K_A$, $h = H_A$):

$$\Delta = \frac{\phi}{1 + \alpha \frac{K_A}{H_A}} . \quad (\text{C.10})$$

Therefore there exist a critical ATP concentration α_c above which $\Delta = 0$ and all drops dissolve ($R_u = 0$). From this equation the expression of α_c is:

$$\alpha_c = \frac{\phi - \hat{P}_{\text{out}}}{\hat{P}_{\text{out}}} \frac{H_A}{K_A} . \quad (\text{C.11})$$

We define $\alpha_{\text{stress}} \equiv 2\alpha_c/3$ and $\alpha_{\text{normal}} = 4\alpha_c/3$ the ATP concentrations during stress and normal condition, respectively, in agreement with the biological constraint that $\alpha_{\text{normal}} = 2\alpha_{\text{stress}}$ (Sec. 6.1). Moreover we assumed these concentrations to be equidistant from α_c for simplicity. During normal conditions $\alpha = \alpha_{\text{normal}} > \alpha_c$ so no drops can exist. During stress condition $\alpha = \alpha_{\text{stress}} < \alpha_c$ and using Eqs (C.4),(C.5), (C.10) and (C.11), we find the size ratio R_u/R_n during stress conditions:

$$\frac{R_u(\alpha_{\text{stress}})}{R_n(\alpha_{\text{stress}})} = \frac{3}{4} \sqrt{\frac{D}{l_c^2 H_A} \frac{(\hat{P}_{\text{out}})^2}{(\phi - \hat{P}_{\text{out}}) \hat{P}_{\text{in}}} \left(\frac{\phi/\hat{P}_{\text{out}} - 2}{\phi/\hat{P}_{\text{out}} + 1} \right)^3} \quad (\text{C.12})$$

Therefore we find that in model A and contrary to model C, the size ratio between stable drops and nuclei is function of the system parameters and can be arbitrarily large. This can potentially provide the switch-like response observed experimentally which we discuss quantitatively in Sec. 6.5 using physiologically relevant parameters.

Appendix D

Copyright and Permissions

25/09/2017

RightsLink Printable License

**The Company of Biologists Ltd LICENSE
TERMS AND CONDITIONS**

Sep 25, 2017

This is a License Agreement between Imperial College London -- Jean David Wurtz ("You") and The Company of Biologists Ltd ("The Company of Biologists Ltd") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by The Company of Biologists Ltd, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	4196090843300
License date	Sep 25, 2017
Licensed content publisher	The Company of Biologists Ltd
Licensed content title	Journal of cell science
Licensed content date	Jan 1, 1966
Type of Use	Thesis/Dissertation
Requestor type	Academic institution
Format	Print, Electronic
Portion	chapter/article
Title or numeric reference of the portion(s)	Chapter 1, Figure 1.1a)
Title of the article or chapter the portion is from	Phase Transitions in the Cell Cytoplasm: A Theoretical Investigation
Editor of portion(s)	Imperial College London
Author of portion(s)	Jean David Wurtz
Volume of serial or monograph.	N/A
Page range of the portion	
Publication date of portion	2017
Rights for	Main product
Duration of use	Life of current edition
Creation of copies for the disabled	no
With minor editing privileges	yes
For distribution to	Worldwide
In the following language(s)	Original language of publication
With incidental promotional use	no
The lifetime unit quantity of new product	Up to 499
Made available in the following markets	
The requesting person/organization is:	Jean David Wurtz
Order reference number	

<https://s100.copyright.com//CustomerAdmin/PLF.jsp?ref=cf326f52-9d7c-44a6-bd93-263571819e92>

1/5

Figure D.1: Permission for Fig. 1.1a) 1/5

25/09/2017 RightsLink Printable License

Author/Editor

Title of New Work

Publisher of New Work

Expected publication date Oct 2017

Estimated size (pages)

Billing Type Credit Card

Credit card info Visa ending in 6663

Credit card expiration 03/2021

Total (may include CCC user fee) 2.59 GBP

Terms and Conditions

TERMS AND CONDITIONS

The following terms are individual to this publisher:

The acknowledgement should state "Reproduced / adapted with permission" and give the source journal name. The acknowledgement should either provide full citation details or refer to the relevant citation in the article reference list. The full citation details should include authors, journal, year, volume, issue and page citation.

Where appearing online or in other electronic media, a link should be provided to the original article (e.g. via DOI):

Development: dev.biologists.org

Disease Models & Mechanisms: dmm.biologists.org

Journal of Cell Science: jcs.biologists.org

The Journal of Experimental Biology: jeb.biologists.org

Other Terms and Conditions:

STANDARD TERMS AND CONDITIONS

1. Description of Service; Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.

2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.

3. Scope of License; Limitations and Obligations.

3.1 All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.

3.2 General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291. Payments Due: Invoices are payable

Figure D.2: Permission for Fig. 1.1a) 2/5

25/09/2017

RightsLink Printable License

upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.

3.3 Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies printed on paper in accordance with this license and still in User's stock at the end of such period).

3.4 In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any of such third party materials; without a separate license, such third party materials may not be used.

3.5 Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc. " Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republished Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.

3.6 User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties' rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.

4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, including legal fees and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.

5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event, the total liability of the Rightsholder and CCC (including their

<https://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=cf326f52-9d7c-44a6-bd93-263571819e92>

3/5

Figure D.3: Permission for Fig. 1.1a) 3/5

25/09/2017

RightsLink Printable License

respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.

6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER; USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.

7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder's ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder's ordinary license price for the most closely analogous licensable use plus Rightsholder's and/or CCC's costs and expenses incurred in collecting such payment.

8. Miscellaneous.

8.1 User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

8.2 Use of User-related information collected through the Service is governed by CCC's privacy policy, available online here:

<http://www.copyright.com/content/cc3/en/tools/footer/privacypolicy.html>.

8.3 The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User's rights in the new material which includes the Work(s) licensed under this Service.

8.4 No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC's standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.

8.5 The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers

<https://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=cf326f52-9d7c-44a6-bd93-263571819e92>

4/5

Figure D.4: Permission for Fig. 1.1a) 4/5

25/09/2017

RightsLink Printable License

the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to info@copyright.com.

v 1.1

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Requests for Permission to Reuse Material Published in PNAS

 www.pnas.org/site/aboutpnas/rightperm.xhtml

PNAS

The author(s) retains copyright to individual PNAS articles, and the National Academy of Sciences of the United States of America (NAS) holds copyright to the collective work and retains an [exclusive License to Publish](#) these articles, except for open access articles submitted beginning September 2017. For such open access articles, NAS retains a [nonexclusive License to Publish](#), and these articles are distributed under a [CC BY-NC-ND](#) license.

For volumes 106–114 (2009–September 2017), the author(s) retains copyright to individual articles, and NAS retains an exclusive License to Publish these articles and holds copyright to the collective work. Volumes 90–105 (1993–2008) are copyright National Academy of Sciences. For volumes 1–89 (1915–1992), the author(s) retains copyright to individual articles, and NAS holds copyright to the collective work.

Authors whose work will be reused should be notified. Use of PNAS material must not imply any endorsement by PNAS or NAS. The full journal reference must be cited and, for articles published in Volumes 90–105 (1993–2008), "Copyright (copyright year) National Academy of Sciences."

The PNAS listing on the Sherpa RoMEO publisher copyright policies and self-archiving detail pages can be found [here](#).

Additional information and answers to frequently asked questions about author rights and permissions are available on our [FAQ page](#).

Requesting Permission

Anyone may, without requesting permission, use original figures or tables published in PNAS for noncommercial and educational use (i.e., in a review article, in a book that is not for sale), provided that the full journal reference is cited and, for articles published in volumes 90–105 (1993–2008), "Copyright (copyright year) National Academy of Sciences." Commercial reuse of figures and tables (i.e., in promotional materials, in a textbook for sale) requires permission from PNAS.

Text and data mining are permitted for noncommercial institutions with an active institutional site license to PNAS for internal noncommercial research purposes. Other requests should be sent to PNASpermissions@nas.edu.

PNAS authors need not obtain permission for the following cases:

1. to use their original figures or tables in their future works;
2. to make copies of their articles for their own personal use, including classroom use, or for the personal use of colleagues, provided those copies are not for sale and are not distributed in a systematic way;
3. to include their articles as part of their dissertations; or
4. to use all or part of their articles in printed compilations of their own works. The full journal reference must be cited and, for articles published in volumes 90–105 (1993–2008), "Copyright (copyright year) National Academy of Sciences."

For permission to reuse material in volumes 1–89 (1915–1992), requests should be sent to the original authors, who hold the copyright. The full journal reference must be cited.

1/2

Figure D.6: Permission for Fig. 1.1b) 1/2

For permission to reuse material in volumes 90–114 (1993–2017), requests should be sent to PNASpermissions@nas.edu and must include the following information:

1. Your full name, affiliation, and title
2. Your complete mailing address, phone number, and email
3. PNAS volume number, issue number, and issue date
4. PNAS article title
5. PNAS authors' names
6. Page numbers of items to be reprinted
7. Figure/table number or portion of text to be reprinted

Requests must also include the following information about the intended use of the material:

1. Title of work in which PNAS material will appear
2. Authors/editors of work
3. Publisher of work
4. Retail price of work
5. Number of copies of work to be produced
6. Intended audience
7. Whether work is for nonprofit or commercial use

PNAS cannot supply original artwork.

For permission to reprint material in volumes 114–present (2017–present), requests relating to articles published under the exclusive PNAS License to Publish should be sent to PNASpermissions@nas.edu. For open access articles that are distributed under the [CC BY-NC-ND license](#), permission is not required to reuse the content for noncommercial purposes on the condition that no derivative works are created by the users and that the resulting work must be made available under the same terms. Users are advised to check each article for its publication license and corresponding distribution policies.

Requests for Permission to Photocopy Material Published in PNAS

For permission to photocopy beyond that permitted by [Section 107 or 108 of the US Copyright Law](#), contact:

[Copyright Clearance Center](#)
222 Rosewood Drive
Danvers, MA 01923
Phone: 978-750-8400
Fax: 978-750-4770
Email: info@copyright.com

Authorization to photocopy items for the internal or personal use of specific clients is granted by the National Academy of Sciences provided that the proper fee is paid directly to the Copyright Clearance Center.

25/09/2017

RightsLink - Your Account

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Sep 25, 2017

This Agreement between Imperial College London -- Jean David Wurtz ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4193271240311
License date	Sep 20, 2017
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation
Licensed Content Author	Clifford P. Brangwynne, Christian R. Eckmann, David S. Courson, Agata Rybarska, Carsten Hoege, Jöbin Gharakhani, Frank Jülicher, Anthony A. Hyman
Licensed Content Date	Jun 26, 2009
Licensed Content Volume	324
Licensed Content Issue	5935
Volume number	324
Issue number	5935
Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	1
Order reference number	
Title of your thesis / dissertation	Phase Transitions in the Cell Cytoplasm: A Theoretical Investigation
Expected completion date	Oct 2017
Estimated size(pages)	150
Requestor Location	Imperial College London Flat 7, 2 Gateforth Streets London, NW88EH United Kingdom Attn: Imperial College London
Billing Type	Invoice
Billing Address	Imperial College London Flat 7, 2 Gateforth Streets London, United Kingdom NW88EH Attn: Imperial College London
Total	0.00 GBP

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

<https://s100.copyright.com/MyAccount/web/jsp/viewprintablelicensefrommyorders.jsp?ref=48aabc8-3fa2-4d8c-8fcd-aae1c426...> 1/4

Figure D.8: Permission for Fig. 1.1c) 1/4

25/09/2017

RightsLink - Your Account

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time.

In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION

If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:

Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:

If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

<https://s100.copyright.com/MyAccount/web/jsp/viewprintablelicensefrommyorders.jsp?ref=48aabc8-3fa2-4d8c-8fcd-aae1c426...> 2/4

Figure D.9: Permission for Fig. 1.1c) 2/4

25/09/2017

RightsLink - Your Account

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:

Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:

Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:

Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way.

No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted.

They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:

The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:

Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:

These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

<https://s100.copyright.com/MyAccount/web/jsp/viewprintablelicensefrommyorders.jsp?ref=48aabc8-3fa2-4d8c-8fcd-aae1c426...> 3/4

Figure D.10: Permission for Fig. 1.1c) 3/4

25/09/2017

RightsLink - Your Account

FOR CLASSROOM PHOTOCOPIES:

Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:

These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written. Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:

Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:

Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:

Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:

Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

25/09/2017

Molecular Oncology - Permissions - Wiley Online Library



Molecular Oncology ▼

Molecular Oncology - Open Access License and Copyright

All *Molecular Oncology* articles are published under the terms of the Creative Commons Attribution License (CC BY) which allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC BY license permits commercial and non-commercial re-use of an open access article, as long as the author is properly attributed.

Use of the article, in whole or part, in any medium requires attribution suitable in form and content as follows: [Title of Article/Author/Journal Title and Volume/Issue]. Published under a Creative Commons Attribution (CC BY) License. Links to the final article on Wiley's website are encouraged where applicable.

Further Details Related to Re-Use of Articles:

Copyright on any research article published by a Wiley Open Access journal is retained by the author(s).

Authors grant Wiley a license to publish the article and identify itself as the original publisher.

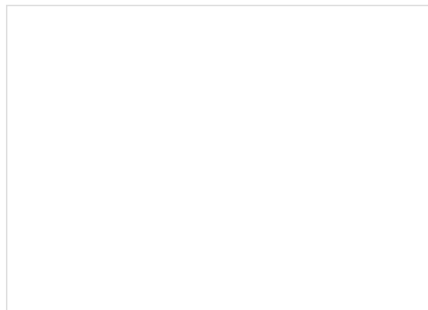
Authors also grant any third party the right to use the article freely as long as its original authors, citation details and publisher are identified.

The Creative Commons Attribution License does not affect the moral rights of authors, including without limitation the right not to have their work subjected to derogatory treatment. It also does not affect any other rights held by authors or third parties in the article, including trademark or patent rights, or the rights of privacy and publicity. Use of the article must not assert or imply, whether implicitly or explicitly, any connection with, endorsement or sponsorship of such use by the author, publisher or any other party associated with the article.

For any reuse or distribution, users must include the copyright notice and make clear to others that the article is made available under a Creative Commons Attribution license, linking to the relevant Creative Commons web page. Users may impose no restrictions on use of the article other than those imposed by the Creative Commons Attribution license.

To the fullest extent permitted by applicable law, the article is made available as is and without representation or warranties of any kind whether express, implied, statutory or otherwise and including, without limitation, warranties of title, merchantability, fitness for a particular purpose, non-infringement, absence of defects, accuracy, or the presence or absence of errors.

Advertisement



[http://febs.onlinelibrary.wiley.com/hub/journal/10.1002/\(ISSN\)1878-0261/about/permissions.html](http://febs.onlinelibrary.wiley.com/hub/journal/10.1002/(ISSN)1878-0261/about/permissions.html)

1/4

Figure D.12: Permission for Fig. 1.1d)

24/09/2017

Proof of License

Proof of License
(accurate as of September 24, 2017)

Dreamstime LLC
1616 Westgate Circle
Brentwood, TN 37027
United States

Customer name: J Wurtz
Location: London, United Kingdom
Address: Flat 7, 2 Gateforth Street
Phone: 07786006211

Dreamstime.com LLC hereby confirms that the buyer, J Wurtz, and/or the buyer's client(s) as indicated, are entitled to use the media listed below, beginning on the date listed next to each and under the license indicated, for commercial/editorial purposes listed on our site at the dates listed below. This document shall serve as proof that the specified licenses for usage of each media listed below have been properly purchased from Dreamstime.com LLC, and such usage is authorized subject and according to the rights and restrictions set forth on the Terms & Conditions page of its website (available at <http://www.dreamstime.com/terms>).

Image	File ID	Title	Author	Downloaded	Size	License
	8140294	Oil drops	Nikola Bilic Dreamstime.com	09/23/2017 04:41 PM	Extralarge	Royalty Free

Figure D.13: Permission for Fig. 2.1a)