# The Interplay Between Feedback and Buffering in Homeostasis: Supplementary Information 1

**Notation:** The equations used in the main text are boxed in the supplementary information. An unmarked symbol, such as $\Gamma$, refers to a linearization constant resulting from deviations about the nominal steady state, while a symbol with an overline, such as $\bar{\Gamma}$, refers to the constant at the nominal steady state. Commonly used constants throughout this section are:

$$\begin{aligned} B &= \frac{b_y}{b_x + \gamma_x} \quad \text{(buffering equilibrium ratio)} \\ \Gamma &= \gamma_y + B\gamma_x \quad \text{(removal constant)} \\ H &= \frac{h_y}{\Gamma} \quad \text{(non-dimensionalized feedback gain)} \\ \frac{1}{c} &= \frac{1}{b_x + \gamma_x} \quad \text{(buffering speed)} \\ R &= \frac{b_x + \gamma_x}{\gamma_y} \quad \text{(normalized buffering speed)} \\ T_{\mathrm{u}} &= \frac{1}{\gamma_y} \quad \text{(unregulated system time scale)}\,. \end{aligned} \tag{1}$$

## S1 Minimal Model Analysis

*This SI section analyzes a minimal model of a buffering-feedback system. This analysis includes deriving a minimal model for analysis (S1.1-S1.2), followed by analyzing the effect of reactions on different time scales (S1.3), the effect of disturbances (S1.4-S1.8), stability (S1.9), noise (S1.10), the comparison with technological controllers (S1.11), disturbances acting on the buffering species (S1.12), and feedback occurring from the buffering species (S1.13).*

We consider the minimal model

$$\begin{aligned} \dot{y} &= \underbrace{p_y(y)}_{\substack{\text{production} \\ \text{with feedback}}} - \underbrace{g_y(y,x) + g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_y(y)}_{\text{removal}} + \underbrace{d_y(t)}_{\text{disturbance}} \\ \dot{x} &= \underbrace{g_y(y,x) - g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_x(x)}_{\text{removal}}\,, \end{aligned} \tag{2}$$

where $y$ is the regulated species, $x$ is the buffering species, $p_y$ is the production rate of $y$, $\nu_y(y)$ and $\nu_x(x)$ are the removal rates of $y$ and $x$, the (lumped) buffering reactions are $g_y(y,x)$ representing $y \to x$ and $g_x(x,y)$ representing $x \to y$, and $d_y$ is a disturbance. Incorporation of

feedback into this class of systems is represented by the $y$ dependence of the production term $p_y$. We initially restrict our focus to feedback exclusively on the production of species $y$.

For the case presented in the main text, we assume linear removal of species $y$, such that $\nu_y(y) = \gamma_y y$, and no removal of species $x$, such that $\nu_x(x) = 0$. In assuming $\nu_x(x) = 0$, we look at the class of cellular biochemical systems where the time scale is much faster than the growth rate of the cell. This includes many metabolic processes.

## S1.1 Nominal Steady State

*The following section establishes the steady state (set point) of the minimal model. This is required later for the analysis of deviations about the steady state. The section also defines the steady-state production, the steady-state buffer equilibrium ratio, and the steady-state removal constant. These quantities prove useful for non-dimensionalization in later sections.*

The nominal steady state $(\bar{y}, \bar{x})$ of Equation (2) occurs when $\dot{y} = \dot{x} = 0$ and $d_y = 0$. For this case, the buffering reactions are in equilibrium and production matches removal when

$$\begin{aligned} g_y(\bar{y}, \bar{x}) &= g_x(\bar{x}, \bar{y}) + \nu_x(\bar{x}) \quad \text{(buffering rates at steady state)} \\ p_y(\bar{y}) &= \nu_y(\bar{y}) + \nu_x(\bar{x}) \quad \text{(production/removal rates at steady state).} \end{aligned}$$

The solution $(\bar{y}, \bar{x})$ is implicit, and if required can be determined numerically when an analytical solution cannot be found. In the following, we assume that there is one isolated nominal steady state of interest, and study the properties of the solution as it deviates from this steady state.

For later analysis, it is useful to define the steady-state buffering ratio:

$$\bar{B} = \frac{\bar{x}}{\bar{y}} \,,$$

the nominal production rate with feedback:

$$\bar{p}_y = p_y(\bar{y}) \,,$$

and the steady-state removal constant:

$$\bar{\Gamma} = \frac{\bar{p}_y}{\bar{y}} = \frac{\nu_y(\bar{y}) + \nu_x(\bar{B}\bar{y})}{\bar{y}} \,. \tag{3}$$

An important case, used in later sections, occurs where removal is linear (i.e., $\nu_y(\bar{y}) = \gamma_y \bar{y}$ and $\nu_y(\bar{x}) = \gamma_x \bar{x} = \gamma_x \bar{B}\bar{y}$), leading to

$$\bar{\Gamma} = \gamma_y + \bar{B}\gamma_x \,.$$

## S1.2 Minimal Model Linearization

*The following section determines the linearized minimal model—a useful approximation that permits an analytical and more thorough mathematical analysis.*

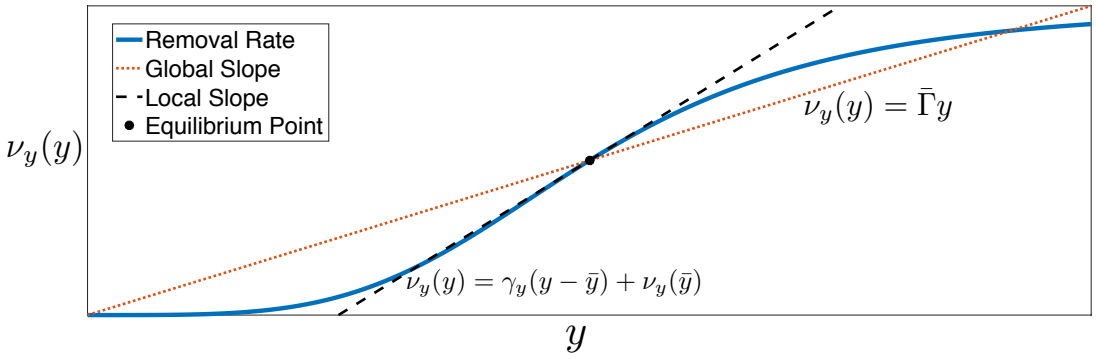



Figure S1.1: Example of a removal rate function, illustrating the significance of the linearization constant $\gamma_y$ and the steady-state constant $\bar{\Gamma}$ (assuming $\nu_x(x) = 0$). $\gamma_y$ is the slope of the removal rate function linearized about the equilibrium point. $\bar{\Gamma}$ is the slope of the line connecting the equilibrium point to the origin.

We next linearize the non-linear model. To carry this out, we define deviations from the nominal steady state as

$$\Delta y = y - \bar{y}$$
$$\Delta x = x - \bar{x}\,.$$

Linearizing our full model around the nominal steady state, we obtain

$$\begin{aligned} \Delta\dot{y} &= -h_y \Delta y - b_y \Delta y + b_x \Delta x - \gamma_y \Delta y + d_y(t) \\ \Delta\dot{x} &= b_y \Delta y - b_x \Delta x - \gamma_x \Delta x\,, \end{aligned} \tag{4}$$

where

$$\begin{aligned} h_y &= -\frac{\partial}{\partial y} p_y(y)|_{y=\bar{y}}\,, \\ b_y &= \frac{\partial}{\partial y}(g_y(y,x) - g_x(x,y))|_{(y,x)=(\bar{y},\bar{x})}\,, \\ b_x &= \frac{\partial}{\partial x}(g_x(x,y) - g_y(y,x))|_{(y,x)=(\bar{y},\bar{x})}\,, \\ \gamma_y &= \frac{d\nu_y}{dy}(y)|_{y=\bar{y}}\,, \\ \gamma_x &= \frac{d\nu_x}{dx}(x)|_{x=\bar{x}}\,. \end{aligned}$$

## S1.3 Rapid Buffering

*The following section analyzes the effect of buffering reactions that occur on a time scale significantly faster than the feedback process. The buffering reaction can be considered to rapidly obtain an equilibrium state, which it maintains while the rest of the system changes more slowly. This 'rapid-equilibrium' approximation reduces the model from two variables to one. Conditions for the rapid-equilibrium approximation to hold are then obtained using singular perturbation theory.*

We initially study the special case of rapid buffering, where we assume that the buffering reactions reach their equilibrium much faster than the production reaches equilibrium with removal. We find a simplified model for the rapid buffering case and find two assumptions for rapid buffering to hold. The first 'weak' assumption (see Equation (6)) is used when the disturbance is bounded. The second 'strong' assumption, presented in a later section (see Equation (21)), is used for unbounded impulse disturbances.

We can use the total quasi steady-state approach [2] to determine a reduced model for rapid buffering, where interconversion reactions between species are 'fast' while the production and removal reactions are 'slow'. This approach allows more accurate reduced models with more general assumptions [13] for enzyme kinetics [2], signalling network [13] and gene regulation [8]. This approach enables us to reduce our two-state model (Equation (4)) to the following one-state model:

$$(1+B)\Delta\dot{y} = -(\Gamma + h_y)\Delta y + d_y(t)\,, \tag{5}$$

where

$$\Gamma = \gamma_y + B\gamma_x \quad \text{and} \quad B = \frac{b_y}{b_x + \gamma_x}\,,$$

assuming that disturbance is bounded and that 'weak' rapid buffering occurs, where $b_x$ is sufficiently large such that

$$\frac{h_y + \Gamma}{1+B} \ll b_y + b_x + \gamma_x \quad \text{and} \quad \frac{h_y + \gamma_y}{1+B} \ll b_x + \gamma_x\,, \tag{6}$$

where Equation (6) can be related to buffering speed using

$$b_y + b_x + \gamma_x = (1+B)\frac{1}{c}, \quad b_x + \gamma_x = \frac{1}{c}, \quad \text{and} \quad c = \frac{1}{b_x + \gamma_x}\,.$$

We refer to Equation (6) as weak rapid buffering due to the extra condition for a bounded disturbance. This allows for a standard vector field definition and non-dimensionalization using a general estimate of the largest typical $\Delta y$.

We refer to $1/c = b_x + \gamma_x$ as the buffering speed. Since $b_y = B/c$, we can describe buffering parameters in terms of the buffering equilibrium ratio $B$ and buffering speed $1/c$, instead of $b_x$ and $b_y$.

For the case in the main text, where $\gamma_x = 0$, then Equation (5) simplifies to

$$\boxed{(1+B)\Delta\dot{y} = -(\gamma_y + h_y)\Delta y + d_y(t)}$$

and $B = b_y/b_x$. For later analysis, we primarily use

$$(1+B)\Delta\dot{y} = -\Gamma(1+H)\Delta y + d_y(t)\,,$$

where the feedback gain can be written

$$H = \frac{h_y}{\Gamma}.$$

In our analysis, we treat $H$ and $B$ as free parameters in order to study their particular effect on regulation. Note our treatment of $H$ as a free parameter despite its dependence on $B$ (via $\Gamma$). In any scenario where $B$ is understood as varying independently of $H$, it is therefore

assumed that either $h_y$ varies correspondingly, in order to keep $H$ constant, or that $\gamma_x$ and $\gamma_y$ vary accordingly, in order to keep $\Gamma$ constant. $H$ and $B$ are always independent for the case $\gamma_x = 0$, as presented in the main text.

We can also note that for linear removal, $\Gamma = \bar{y}/\bar{p}_y$, and so the feedback gain can be written in the form

$$\boxed{H = -\frac{\bar{y}}{\bar{p}_y}\frac{\partial p_y(\bar{y})}{\partial y}}\,.$$

To derive the reduced model in Equation (5), we use the total quasi steady-state approach [2]. We first transform the two-state model in Equation (4) to

$$\begin{aligned} \Delta\dot{y}_\mathrm{T} &= -(h_y + \gamma_y)\Delta y - \gamma_x \Delta x + d_y(t) \\ \Delta\dot{x} &= b_y \Delta y - (b_x + \gamma_x)\Delta x\,, \end{aligned} \tag{7}$$

where $\Delta y_\mathrm{T} = \Delta y + \Delta x$ is the total concentration. Treating $\Delta y_\mathrm{T}$ as the slow variable and $\Delta x$ as the fast variable, we assume that $\Delta x$ is at quasi-steady state ($\Delta\dot{x} \approx 0$), and so

$$\begin{aligned} \Delta\dot{y}_\mathrm{T} &= -(\gamma_y + h_y)\Delta y - \gamma_x \Delta x + d_y(t) \\ \Delta x &= B\Delta y\,. \end{aligned}$$

We note that the buffering equilibrium ratio at quasi-steady state is

$$\boxed{B = \frac{\Delta x}{\Delta y}}$$

for non-zero $\Delta y$. Substituting, we have

$$\begin{aligned} \Delta\dot{y}_\mathrm{T} &= -(\gamma_y + B\gamma_x + h_y)\Delta y + d_y(t) \\ &= -(\Gamma + h_y)\Delta y + d_y(t)\,. \end{aligned}$$

Applying $\Delta y_\mathrm{T} = \Delta y + \Delta x$ to the slow time scale, we have

$$\begin{aligned} \Delta\dot{y}_\mathrm{T} &= \Delta\dot{y} + \Delta\dot{x} \\ &= (1 + B)\Delta\dot{y} = -(\Gamma + h_y)\Delta y + d_y(t)\,, \end{aligned}$$

and thus obtain Equation (5).

To derive the time-scale separation condition in Equation (6), we need to place the two-state model into standard singular-perturbation form [9]. Eliminating $\Delta y$ from Equation (7), we have the two-state model

$$\begin{aligned} \Delta\dot{y}_\mathrm{T} &= -(h_y + \gamma_y)(\Delta y_\mathrm{T} - \Delta x) - \gamma_x \Delta x + d_y(t) \\ \Delta\dot{x} &= b_y \Delta y_\mathrm{T} - (b_y + b_x + \gamma_x)\Delta x\,. \end{aligned}$$

We next non-dimensionalize the equations with the aim of ensuring that all terms are of a similar scale, with the exception of a small parameter $\varepsilon$. We initially choose an arbitrary time scale, arbitrary maximum of $\Delta y_\mathrm{T}$, and scale $\Delta x$ in terms of $\Delta y_\mathrm{T}$. We then select a typical maximum value of $\Delta y_\mathrm{T}$ and a typical time scale.

Non-dimensionalizing the variables, we have

$$\begin{aligned}\frac{d\Delta y_{\mathrm{T}n}}{d\tau} &= -T_{\mathrm{s}}(h_y+\gamma_y)\Delta y_{\mathrm{T}n} + T_{\mathrm{s}}\frac{B}{1+B}(h_y+\gamma_y-\gamma_x)\Delta x_n + T_{\mathrm{s}}\frac{D_{\max}}{\Delta\tilde{y}_{\mathrm{T}}}d_n(\tau)\\ \frac{1}{T_{\mathrm{s}}}\frac{1}{b_y+b_x+\gamma_x}\frac{d\Delta x_n}{d\tau} &= \Delta y_{\mathrm{T}n} - \Delta x_n\,,\end{aligned}$$

such that $\tau$ is the non-dimensionalized time scale and

$$\begin{aligned}\Delta y_{\mathrm{T}} &= \Delta\tilde{y}_{\mathrm{T}}\Delta y_{\mathrm{T}n}\,, & \Delta x &= \frac{B\Delta\tilde{y}_{\mathrm{T}}}{1+B}\Delta x_n\,,\\ \tau &= \frac{t}{T_{\mathrm{s}}}\,, & d_y &= D_{\max}d_n\,,\\ \frac{B}{1+B} &= \frac{b_y}{b_y+b_x+\gamma_x}\,, & D_{\max} &= \sup(|d_y(t)|)\,,\end{aligned}$$

where the disturbance is non-dimensionalized to normalize the vector field. We next select $\tilde{y}_{\mathrm{T}}$, $T_{\mathrm{s}}$, and $T_{\mathrm{f}}$ based on a typical maximum value of $\Delta y$ and $d_y$, which we can use to transform the equations into a standard singular perturbation form. We have

$$\begin{aligned}\frac{d\Delta y_{\mathrm{T}n}}{d\tau} &= -\lambda_1\Delta y_{\mathrm{T}n} + (\lambda_2-\lambda_3)\Delta x_n + d_n(\tau)\\ \varepsilon\frac{d\Delta x_n}{d\tau} &= \Delta y_{\mathrm{T}n} - \Delta x_n\,,\end{aligned} \tag{8}$$

where

$$\begin{aligned}\Delta\tilde{y}_{\mathrm{T}} &= T_{\mathrm{s}}D_{\max}\,, & \varepsilon &= \frac{T_{\mathrm{f}}}{T_{\mathrm{s}}} \ll 1\,,\\ T_{\mathrm{s}} &= \min\left(\frac{1+B}{h_y+\Gamma}, \frac{1}{h_y+\gamma_y}\right), & T_{\mathrm{f}} &= \frac{1}{b_y+b_x+\gamma_x}\,,\\ \lambda_1 &= T_{\mathrm{s}}(h_y+\gamma_y)\,, & \lambda_2 &= T_{\mathrm{s}}(h_y+\gamma_y)\frac{B}{1+B}\,,\\ \lambda_3 &= T_{\mathrm{s}}\frac{B\gamma_x}{1+B}\,,\end{aligned} \tag{9}$$

and where $T_{\mathrm{s}}$ and $T_{\mathrm{f}}$ are the slow and fast time scales, respectively. The assumption in Equation (6) follows from writing $\varepsilon \ll 1$ in expanded form.

### S1.4 Sensitivity Functions and Steady-State Disturbances

*The following section analyzes the effect of steady-state disturbances on the minimal model.*

To understand the effect of disturbances, we first analyze steady-state disturbances. This is an important regulatory case, and also illustrates the different sensitivity functions used to characterize disturbance rejection.

To obtain the steady-state deviation from the nominal under a constant and persistent disturbance $d_y = \bar{d}_y$, we set $\Delta\dot{y} = \Delta\dot{x} = 0$ in Equation (4). This yields

$$\begin{aligned}\Delta\bar{y} &= \frac{1}{\Gamma+h_y}\bar{d}_y\\ &= \frac{1}{\Gamma}\frac{1}{1+H}\bar{d}_y\,,\end{aligned} \tag{10}$$

where $\bar{d_y}$ is a constant. It is important to note that $\Gamma$ is dependent upon $B$ only if $\gamma_x \neq 0$.

In order to evaluate the effectiveness of regulation strategies for steady-state disturbance rejection, we introduce a sensitivity function, defined for two separate cases. One for the case where *disturbances scale proportionally to $\bar{p}_y$, the steady-state production of $y$*:

$$\phi_{\text{ss}} = \frac{\bar{p}_y}{\bar{y}} \frac{|\Delta y|}{|d_y|}, \tag{11}$$

and another for the case where *disturbances are independent from other signals*:

$$\phi_{\text{ss}} = \frac{1}{\bar{y}} \frac{|\Delta y|}{|d_y|}. \tag{12}$$

In both expressions, $|\Delta y|$ is normalized by $\bar{y}$, its nominal steady state. In the former case, we also normalize the disturbance, $|d_y|$, by $\bar{p}_y$, the nominal production rate (with feedback) of $y$. This allows us to compare two systems that achieve the same nominal $\bar{y}$ using different rates of production $\bar{p}_y$. For example, the addition of a buffer to a system requires, as compensation, an increase in the rate of production of $y$ or a decrease in its effective rate of removal in order to maintain the same $\bar{y}$. In the case of an increased nominal production rate, disturbances that enter the system via $y$ production will be amplified. Note that other scenarios, such as when disturbances scale with the rate of removal of $y$, are not analyzed here.

Using Equations (3) and (10), we can simplify Equations (11) and (12) to

$$\phi_{\text{ss}} = \frac{\bar{\Gamma}}{\Gamma} \frac{1}{1+H}$$

and

$$\phi_{\text{ss}} = \frac{1}{\bar{p}_y} \frac{\bar{\Gamma}}{\Gamma} \frac{1}{1+H}.$$

For the linear removal case presented in the main text, where $\bar{\Gamma} = \Gamma$, then

$$\phi_{\text{ss}} = \frac{1}{1+H}$$

and

$$\phi_{\text{ss}} = \frac{1}{\bar{p}_y} \frac{1}{1+H}.$$

For the case of independent disturbances, increasing the amount of buffer typically increases the total removal rate as both species are degraded, and so in steady state, the total production also increases to keep the steady state at the required concentration. This increase in production makes the relative effect of a perturbation smaller. However, this form of regulation can also be achieved by any increase in production/removal, without requiring the need of a buffer.

### S1.5 Oscillatory Disturbance Rejection for Rapid Buffering

*The following section analyzes the effects of oscillatory disturbances on the minimal model, which generalizes the analysis in the previous section. Fourier (and the related Laplace) transforms are used*

*for analysis. Fourier transforms allow a disturbance to be decomposed into its constituent oscillatory signals (frequencies), and for the effect of each constituent frequency on the system to be quantified.*

In this section, we determine the sensitivity of the class of rapid buffering systems to oscillating disturbances of different frequencies. Oscillating disturbances with zero frequency are the special case of a constant disturbance analyzed in S1.4. For dynamic disturbances, we define the sensitivity function to be

$$\phi_\omega = \frac{\|\Delta y\|_{\text{pow}}}{\bar{y}} \Big/ \frac{\|d_y\|_{\text{pow}}}{\bar{p}_y}\,, \tag{13}$$

where the square-root of the power of a signal is defined as

$$\|\Delta y\|_{\text{pow}} = \sqrt{\lim_{T\to\infty} \frac{1}{T}\int_0^T \Delta y^2 dt}\,. \tag{14}$$

The power and corresponding sensitivity function are useful for analyzing persistent disturbances, i.e., those that do not fade away over time.

To determine the sensitivity to oscillating disturbances, we apply a frequency domain approach with Laplace/Fourier transforms. From Equation (5), the model for rapid buffering is

$$(1+B)\Delta\dot{y} = -\Gamma(1+H)\Delta y + d_y(t)\,,$$

which has a frequency domain representation of

$$(1+B)sY(s) = -\Gamma(1+H)Y(s) + D_y(s)\,,$$

where $Y(s) = \mathcal{L}\{\Delta y(t)\}$ is the output and $D_y(s) = \mathcal{L}\{d_y(t)\}$ is the disturbance in the frequency domain, $\mathcal{L}(\cdot)$ is the Laplace transform, and $s$ is the complex frequency. The transfer function from the disturbance $D_y(s)$ to the output $Y(s)$ is

$$G(s) = \frac{Y(s)}{D_y(s)} = \frac{1}{(1+B)s + \Gamma(1+H)}\,. \tag{15}$$

Setting $s = j\omega$ (where $j$ is the imaginary unit number), we have

$$G(j\omega) = \frac{1}{(1+B)j\omega + \Gamma(1+H)}\,, \tag{16}$$

where $G(j\omega)$ denotes the frequency response from $D_y(j\omega)$ to $Y(j\omega)$. The magnitude of this frequency response is given by

$$|G(j\omega)|^2 = \frac{1}{(1+B)^2\omega^2 + \Gamma^2(1+H)^2}\,. \tag{17}$$

Using the properties of the transfer function (Parseval's Theorem for the equivalence of the power of a signal in the time or the frequency domain [5]) and Equation (3), we obtain

$$\begin{aligned}
\phi_\omega &= \frac{\bar{p}_y}{\bar{y}}|G(j\omega)| \\
&= \frac{\bar{\Gamma}}{\sqrt{(1+B)^2\omega^2 + \Gamma^2(1+H)^2}} \\
&= \frac{\bar{\Gamma}}{\Gamma}\frac{1}{\sqrt{(1+B)^2\frac{1}{\Gamma^2}\omega^2 + (1+H)^2}}\,.
\end{aligned}$$

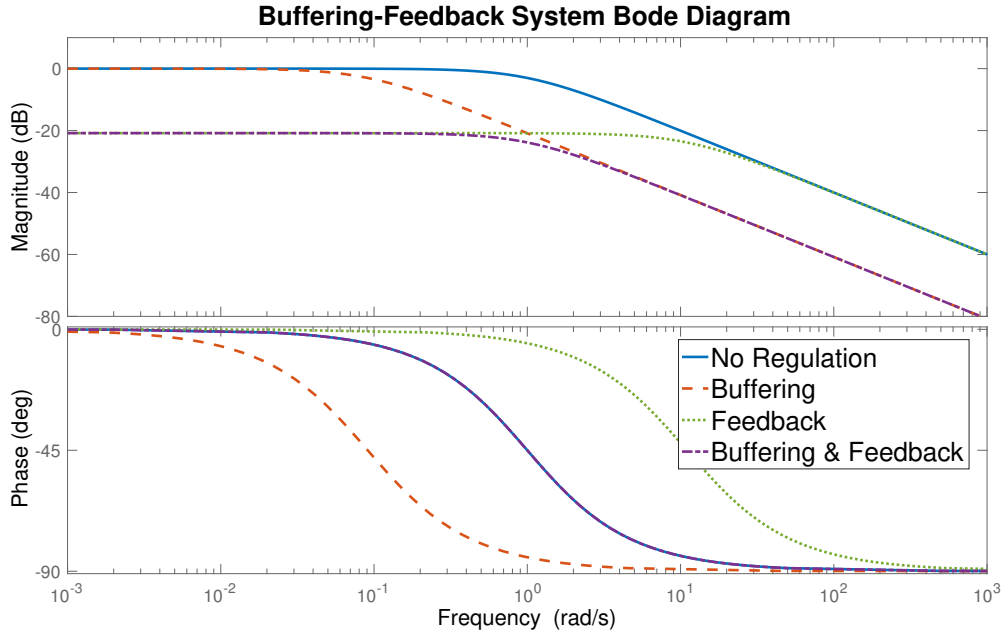



Figure S1.2: A Bode plot of the transfer function given by Equation (16) with $\gamma_y = \Gamma = 1$. Feedback gain $H = 0$ or 10 and buffering equilibrium ratio $B = 0$ or 10.

Normalizing the disturbance frequency $\omega$ by the unregulated system time scale $T_\mathrm{u} = 1/\gamma_y$, we have

$$\phi_\omega = \frac{\bar{\Gamma}}{\Gamma} \frac{1}{\sqrt{(1+B)^2 \frac{\gamma_y^2}{\Gamma^2} \omega_n^2 + (1+H)^2}},$$

where $\omega_n = \omega T_\mathrm{u}$. If we assume that $\gamma_x = 0$ and that the removal is linear, then $\Gamma = \gamma_y + B\gamma_x = \gamma_y$ and $\bar{\Gamma} = \Gamma = \gamma_y$. Thus, for the case presented in the main text we have

$$\boxed{\phi_\omega = \frac{1}{\sqrt{(1+B)^2 \omega_n^2 + (1+H)^2}}}. \tag{18}$$

## S1.6 Impulse Disturbance Rejection for Rapid Buffering

*The following section analyzes the effect of impulse disturbances on the minimal model. The analysis is completed using Fourier transforms, allowing the overall effect to be calculated by summing (via integration) the effect from all the constituent signals of the frequency-decomposed impulse disturbance.*

We next determine the sensitivity of the model in Equation (2) to an impulse with rapid buffering. The sensitivity function $\phi_\mathrm{imp}$ used for analysis is

$$\phi_\mathrm{imp} = \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_\mathrm{u}}} \Big/ \frac{|\hat{d}_y|}{\bar{p}_y T_\mathrm{u}} \tag{19}$$

where $T_\mathrm{u} = 1/\gamma_y$ is the time scale of the unregulated system, $|\hat{d}_y|$ is a measure of the impulse

disturbance, and the $L_2$ norm is defined as

$$\|\Delta y\|_{L_2} = \sqrt{\int_0^\infty \Delta y^2 dt}\,, \tag{20}$$

which is a metric of the accumulated deviation of $\Delta y$ over time. The impulse disturbance is $d_y(t) = \hat{d}_y \delta(t)$ where $\delta(t)$ is a unit impulse and $|\hat{d}_y|$ is a constant. The unit impulse is (informally) defined to be $\delta(t) = \infty$ when $t = 0$ and $\delta(t) = 0$ when $t \neq 0$, such that $\int_{-\infty}^{\infty} \delta(t) dt = 1$ [14]. The non-dimensionalization of $\|\Delta y\|_{L_2}$ by $\sqrt{\bar{y}^2 T_\mathrm{u}} = \bar{y}\sqrt{T_\mathrm{u}}$ is based on $\bar{y}^2$ corresponding to the integrand $\Delta y^2$ in Equation (20) and $T_\mathrm{u}$ corresponding to the integration over time. The non-dimensionalization of $d_y(t)$ is by $\bar{p}_y T_\mathrm{u}$, where $\bar{p}_y$ corresponds to the integrand and $T_\mathrm{u}$ is due to the integration over time.

For linear removal with $\gamma_x = 0$, as presented in the main text, then $\Gamma = \gamma_y + B\gamma_x = \gamma_y$ and so $\bar{\Gamma} = \gamma_y$. This gives $\bar{p}_y T_\mathrm{u} = \bar{p}_y/\gamma_y = \bar{y}$, and the sensitivity function reduces to

$$\phi_\mathrm{imp} = \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_u}} \Big/ \frac{|\hat{d}_y|}{\bar{y}}\,.$$

For an impulse $d_y = \hat{d}_y \delta(t)$, we cannot use the 'weak rapid buffering' assumption given by Equation (6), as the impulse does not have a finite maximum. Instead, we provide the alternate 'strong rapid buffering' assumption

$$h_y + \Gamma \ll b_x + \gamma_x = \frac{1}{c} \quad \text{and} \quad h_y + \gamma_y \ll b_y + b_x + \gamma_x = \frac{1+B}{c}\,, \tag{21}$$

which we derive in S1.7.

The 2-norm in the frequency domain is determined by integrating Equation (17), such that [5]

$$\begin{aligned} \|G(s)\|_2^2 &= \frac{1}{2\pi}\int_{-\infty}^{\infty} |G(j\omega)|^2 d\omega \\ &= \frac{1}{2(1+B)\Gamma(1+H)}\,. \end{aligned}$$

For the general case ($\gamma_x \neq 0$), the sensitivity function for strong rapid buffering (Equation (21)) becomes

$$\begin{aligned} \phi_\mathrm{imp} &= \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_\mathrm{u}}} \Big/ \frac{\|D_y\|}{\bar{p}_y T_\mathrm{u}} \\ &= \frac{\bar{\Gamma}}{\sqrt{\gamma_y}} \|G(s)\|_2 \\ &= \frac{\bar{\Gamma}}{\Gamma}\sqrt{\frac{\Gamma}{\gamma_y}\frac{1}{2(1+H)(1+B)}}\,, \end{aligned} \tag{22}$$

where $\bar{\Gamma}$ is the steady-state removal rate, $T_\mathrm{u} = 1/\gamma_y$ is the unregulated open-loop time scale, and $\Gamma = \gamma_y + B\gamma_x$ is the local removal rate.

For the case where $\gamma_x = 0$ and removal is linear, $\Gamma = \gamma_y + B\gamma_x = \gamma_y$ and $\bar{\Gamma} = \Gamma = \gamma_y$. For this case, we also note that $\bar{p}_y T_\mathrm{u} = \bar{p}_y/\gamma_y = \bar{y}$. Thus, for the case presented in the main text, we have

$$\boxed{\phi_\mathrm{imp} = \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_\mathrm{u}}} \Big/ \frac{\|D_y\|}{\bar{y}} = \frac{1}{\sqrt{2(1+H)(1+B)}}}\,.$$

## S1.7 Impulse Disturbance Rejection when Buffering is Not Rapid

*The following section carries out further analysis on the effect of impulse disturbances on the minimal model. The analysis is similar to Section S1.6, but with a higher-order model.*

We next characterize impulse disturbance rejection when buffering is no longer assumed to be rapid. For this, we determine the frequency response associated with the linearized two-state minimal model

$$\begin{aligned}\Delta\dot{y} &= -b_y\Delta y + b_x\Delta x - \gamma_y\Delta y - h_y\Delta y + d_y(t)\\ \Delta\dot{x} &= b_y\Delta y - b_x\Delta x - \gamma_x\Delta x\,.\end{aligned}$$

The frequency domain representation is

$$\begin{aligned}sY(s) &= -b_yY(s) + b_xX(s) - \gamma_yY(s) - h_yY(s) + D_y(s)\\ sX(s) &= b_yY(s) - b_xX(s) - \gamma_xX(s)\,,\end{aligned}$$

which leads to

$$Y(s) = \frac{(s+b_x+\gamma_x)}{(s+b_y+h_y+\gamma_y)(s+b_x+\gamma_x) - b_yb_x}D_y(s)\,,$$

where the transfer function from $D_y(s)$ to $Y(s)$ is

$$\begin{aligned}G(s) = \frac{Y(s)}{D_y(s)} &= \frac{(s+b_x+\gamma_x)}{(s+b_y+h_y+\gamma_y)(s+b_x+\gamma_x) - b_yb_x}\\ &= \frac{(s+b_x+\gamma_x)}{s^2 + (b_y+h_y+\gamma_y+b_x+\gamma_x)s + (h_y+\gamma_y)(b_x+\gamma_x) + b_y\gamma_x}\\ &= \frac{(1+cs)}{cs^2 + (1+B+c(\gamma_y+h_y))s + \Gamma + h_y}\\ &= \frac{(s+\frac{1}{c})}{s^2 + \frac{1}{c}(1+B+c(\gamma_y+h_y))s + \frac{1}{c}(\Gamma+h_y)}\,,\end{aligned} \tag{23}$$

where $1/c = b_x + \gamma_x$ is the buffering speed.

To calculate the impulse-response sensitivity function for the general case, we need to determine the 2-norm of $G(s)$. A generic second-order transfer function

$$\begin{aligned}G(s) &= \frac{s+e}{(s+a)(s+b)}\\ &= \frac{s+e}{s^2+(a+b)s+ab}\end{aligned} \tag{24}$$

has a 2-norm given by [5]

$$\|G(s)\|_2^2 = \frac{e^2+ab}{2ab(a+b)}\,. \tag{25}$$

Therefore, matching coefficients of Equations (23) and (24), we have

$$\|G(s)\|_2^2 = \frac{1+c(\Gamma+h_y)}{2(\Gamma+h_y)(1+B+c(h_y+\gamma_y)}$$

$$\|G(s)\|_2^2 = \frac{1 + c(\Gamma + h_y)}{2(\Gamma + h_y)(1 + B)\left(1 + c\frac{\gamma_y + h_y}{1+B}\right)}. \tag{26}$$

From Equation (19), the sensitivity function is

$$\begin{aligned} \phi_{\text{imp}} &= \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_{\text{u}}}} \Big/ \frac{\|D_y\|}{\bar{p}_y T_{\text{u}}} \\ &= \frac{\bar{\Gamma}}{\sqrt{\gamma_y}} \|G(s)\|_2^2 \\ &= \frac{\bar{\Gamma}}{\Gamma} \sqrt{\frac{\Gamma}{\gamma_y} \frac{\kappa}{2(1+H)(1+B)}}, \end{aligned} \tag{27}$$

where

$$\kappa = \frac{1 + c(\Gamma + h_y)}{1 + c\frac{\gamma_y + h_y}{1+B}}$$

and $T_{\text{u}} = 1/\gamma_y$. If $\kappa \to 1$, then the non-rapid sensitivity function approaches the rapid case in Equation (22). For $\kappa \approx 1$, we require that

$$c(\Gamma + h_y) \ll 1 \quad \text{and} \quad c\frac{\gamma_y + h_y}{1 + B} \ll 1,$$

which is an alternative form of the strong buffering assumption given by Equation (21). The strong rapid buffering condition in Equation (21) requires a larger $b_x$ than the weak rapid buffering condition given in Equation (6). We cannot use Equation (6) as impulses do not have a maximum, and so do not fit into the standard singular-perturbation form in Equation (8).

Writing Equation (27) in terms of normalized buffering speed $R$ instead of $c$, we have

$$\kappa = \frac{R + \frac{\Gamma}{\gamma_y}(1 + H)}{R + \frac{1 + \frac{\Gamma}{\gamma_y} H}{1+B}}$$

where

$$R = \frac{b_x + \gamma_x}{\gamma_y}.$$

For the non-rapid case where removal is linear and $\gamma_x = 0$, resulting in $\bar{\Gamma} = \Gamma = \gamma_y + B\gamma_x = \gamma_y$, we have

$$\begin{aligned} \phi_{\text{imp}} &= \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_{\text{u}}}} \Big/ \frac{\|D_y\|}{\bar{y}} \\ &= \sqrt{\frac{\kappa}{2(1+H)(1+B)}}, \end{aligned}$$

where

$$\kappa = \frac{R + (1 + H)}{R + \frac{1+H}{1+B}}, \quad B = \frac{b_y}{b_x}, \quad \text{and} \quad R = \frac{b_x}{\gamma_y}.$$

## S1.8 Rapid Buffering is Optimal for Rejection of Impulse Disturbances at $d_y$

*The following section carries out further analysis on the effect of impulse disturbances on the minimal model. Specifically, it uses the results of the previous section to minimize $\phi_{imp}$ and show that the optimal regulation occurs when the buffering speed is extremely rapid.*

We next show that strong rapid buffering (Equation (21)) is optimal for rejection of an impulse disturbance at $d_y$. In Equation (27), changing the buffering speed only changes $\kappa$. Following from Equation (21), the strong rapid buffering limit is

$$\frac{h_y+\gamma_y}{b_x+\gamma_x} \to 0 \quad \text{and} \quad \frac{h_y+\Gamma}{b_y+b_x+\gamma_x} \to 0\,. \tag{28}$$

We have

$$\kappa = \frac{1+c(\Gamma+h_y)}{1+c\frac{h_y+\gamma_y}{1+B}} \geq 1$$

as

$$\Gamma + h_y \geq h_y + \gamma_y \geq \frac{h_y+\gamma_y}{1+B}\,,$$

therefore, for fixed $B$ and $H$ and removal constants, if we take the limit of strong rapid buffering, then $\kappa \to 1$ and $\phi_{\text{imp}}$ in Equation (27) approaches its infimum value.

## S1.9 Stability Analysis in the Presence of Feedback Delays

*The following section analyzes the stability of the minimal models. To do so, the models of the previous sections are extended to incorporate a feedback delay. Stability conditions are then determined using the Nyquist criterion [11]—a method using Fourier/Laplace transforms of the open-loop system (i.e., with disconnected feedback) to determine the properties of the closed-loop system (i.e., with connected feedback).*

In this section, we analyze the stability of a class of systems with feedback delay and show that buffering can help stabilize an otherwise unstable feedback system. We carry out this analysis for both rapid and non-rapid buffering.

### S1.9.1 Stability Analysis in the Presence of Feedback Delays when Buffering is Rapid

We first complete stability analysis under the assumption that buffering is rapid. With rapid buffering, the model with a feedback delay is a modified version of Equation (5),

$$\begin{aligned}(1+B)\Delta\dot{y}(t) &= -\Gamma\Delta y(t) + d_y(t) + u_{\text{f}}(t-\tau)\\ u_{\text{f}}(t) &= -h_y\Delta y(t)\,,\end{aligned} \tag{29}$$

where $\tau$ is a delay. Taking the Laplace transform of Equation (29), we have

$$\begin{aligned}(1+B)sY(s) &= -\Gamma Y(s) + D_y(s) + e^{-s\tau}U_{\text{f}}(s)\\ U_{\text{f}}(s) &= -h_y Y(s)\,.\end{aligned} \tag{30}$$

The closed-loop transfer function is thus given by

$$\frac{Y(s)}{D_y(s)} = \frac{1}{(1+B)s+\Gamma+h_y e^{-s\tau}}\,. \tag{31}$$

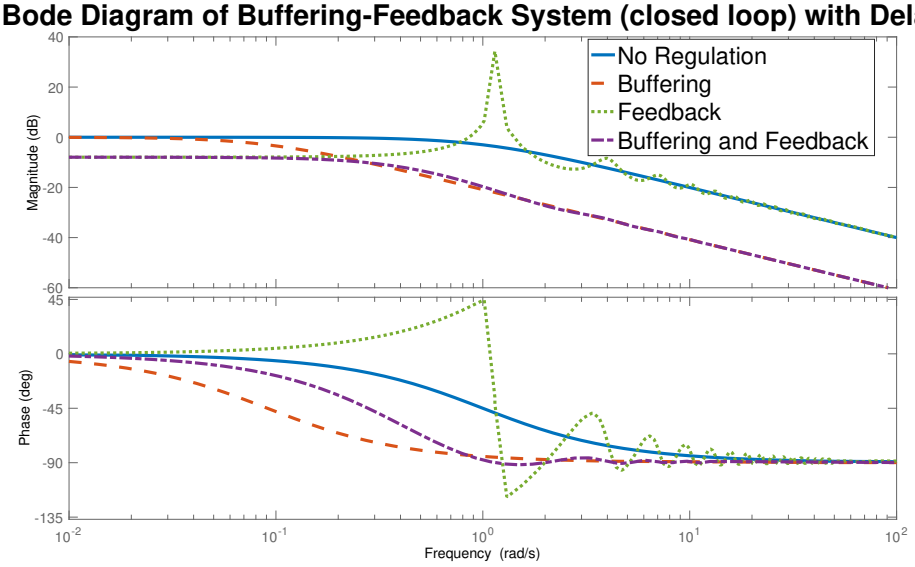



Figure S1.3: A Bode plot of the closed-loop transfer function given by Equation (31) with non-dimensionalized time. Feedback gain $H = 0$ or 1.5, buffering equilibrium ratio $B = 0$ or 10, and delay $\tau = 2$. The feedback plot shows a stable case near instability, where the magnitude response contains a 'resonance spike'. The plot also shows that buffering removes the resonance spike and highlights the requirement for buffering to reject high-frequency disturbances rather than feedback.

To analyze the class of system with delays, we use the Nyquist stability criterion and the open-loop transfer function [11]. The open-loop transfer function from $U_f(s)$ to the output $Y(s)$ is given by (including buffering but not feedback and considering $d_y(t) = 0$ in Equation (29))

$$G(s) = \frac{1}{\Gamma}\frac{e^{-s\tau}}{sT+1},$$

where $T = \frac{1+B}{\Gamma}$. For the stability of this case, the Nyquist stability criterion requires that $h_y|G(j\omega)| < 1$ at the frequency $\omega = \omega_c$ where $\angle G(j\omega_c) = \pi$.

Starting with the characterization of $\angle G(j\omega_c) = -\pi$, we have

$$\angle G(j\omega_c) = -\omega_c\tau - \tan^{-1}(\omega_c T) = -\pi,$$

where $T = \frac{1+B}{\Gamma}$. If we asymptotically approximate $\tan^{-1}(\cdot)$ using

$$\tan^{-1}(\omega_c T) \approx \begin{cases} \omega_c T, & \text{if } \omega_c T \le \frac{\pi}{2} \\ \frac{\pi}{2}, & \text{if } \omega_c T > \frac{\pi}{2} \end{cases},$$

we obtain

$$\angle G(j\omega_c) \approx \begin{cases} -\omega_c(T+\tau), & \text{if } \omega_c \le \frac{\pi}{2T} \\ -\omega_c\tau - \frac{\pi}{2}, & \text{if } \omega_c > \frac{\pi}{2T} \end{cases}.$$

This yields

$$\omega_c \approx \begin{cases} \frac{\pi}{2\tau}, & \text{if } \tau < T \\ \frac{\pi}{\tau+T}, & \text{if } \tau \ge T \end{cases}. \tag{32}$$

The Nyquist stability criterion [11] tells us that the feedback system is stable if and only if $h_y|G(j\omega_c)| < 1$. This imposes the condition

$$|G(j\omega_c)| = \frac{h_y}{\Gamma}\sqrt{\frac{1}{\omega_c^2 T^2 + 1}} < 1\,,$$

which is equivalent to

$$H < \sqrt{1 + \omega_c^2 T^2}\,,$$

resulting in the constraint

$$\phi_{\omega,\text{low}} = \frac{1}{1+H} > \frac{1}{1+\sqrt{1+w_c^2 T^2}}\,,$$

where $\phi_{\omega,\text{low}}$ represents $\phi_\omega$ for low frequencies, i.e., for values of $\omega$ where $(1+B)\omega \ll (1+H)$ in Equation (18). Thus, the feedback system is stable if and only if the feedback gain, $H$, is smaller than a specific upper bound (given in Equation (34)) that depends on the amount of delay in the feedback loop ($\omega_c$ depends on $\tau$ in Equation (32)).

From Equation (32), we can write

$$\begin{aligned}\omega_c T &= \begin{cases} \frac{\pi}{2\Gamma}\frac{1+B}{\tau}, & \text{if } \tau < T \\ \frac{\pi\tau}{(\tau+T)\Gamma}\frac{1+B}{\tau}, & \text{if } \tau \geq T \end{cases} \\ &= \frac{\alpha}{\Gamma}\frac{1+B}{\tau}\,,\end{aligned}$$

where

$$\alpha = \max\left[\frac{\pi}{2}, \frac{\pi\tau}{\tau+T}\right].$$

Therefore, we can also write

$$H < H_{\text{crit}} \quad \text{and} \quad \phi_{\omega,\text{low}} > \frac{1}{1+H_{\text{crit}}}\,, \tag{33}$$

where

$$H_{\text{crit}} = \sqrt{1 + \frac{\alpha^2}{\Gamma^2}\frac{(1+B)^2}{\tau^2}}\,. \tag{34}$$

For the case of $\gamma_x = 0$ presented in the main text, Equations (33) and (34) can be rewritten as

$$\boxed{H < H_{\text{crit}} \quad \text{and} \quad \phi_{\omega,\text{low}} > \frac{1}{1+H_{\text{crit}}}}, \tag{35}$$

where

$$\boxed{H_{\text{crit}} = \sqrt{1 + \frac{\alpha^2}{\gamma_y^2}\frac{(1+B)^2}{\tau^2}}},$$

$\alpha = \max\left[\pi/2, \pi\tau/(\tau+T)\right]$, and $T = (1+B)/\gamma_y$.

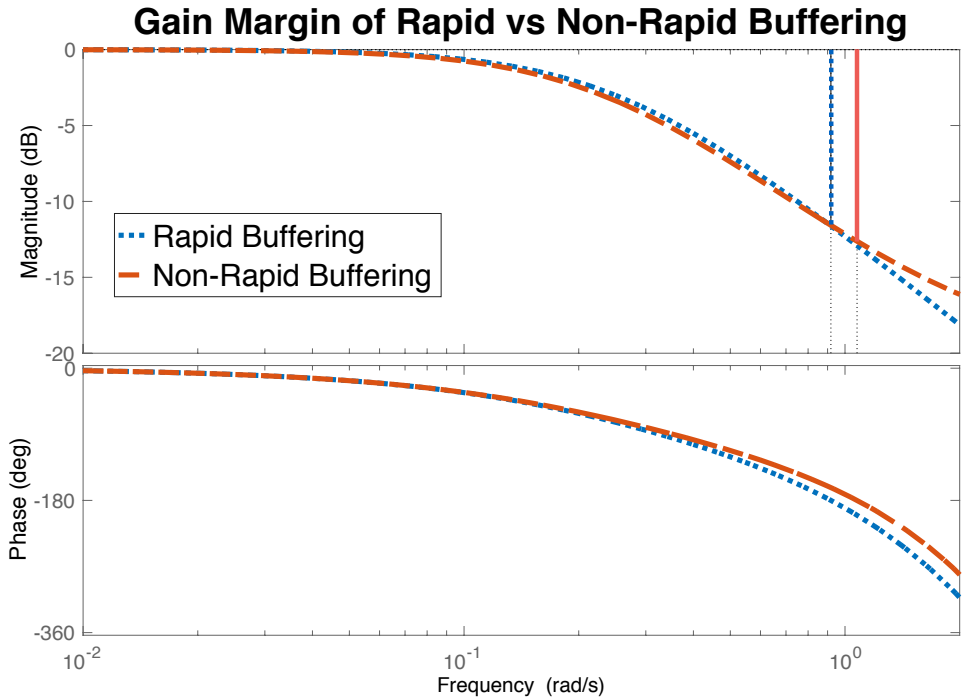



Figure S1.4: A Bode plot of the open-loop transfer function given by Equation (36). Buffering equilibrium ratio $B = 3$, delay $\tau = 2$, removal rate $\Gamma = 1$, and buffering speed $1/c = 2$. It can be observed that the gain margin is increased for non-rapid buffering.

### S1.9.2 Graphical Stability Analysis in the Presence of Feedback Delays when Buffering is Not Rapid

We next look at how stability conditions are changed for the case where buffering is not rapid. To do so, we derive the transfer function for the two-state model in Equation (4), modified to include feedback delay. We then graphically show an increase in stability gain margin as buffering is slowed from rapid buffering.

Starting from the initial two-state model in Equation (4), the two-state model with delay can be written as

$$\begin{aligned} \Delta\dot{y} &= u_f(t-\tau) - b_y\Delta y + b_x\Delta x - \gamma_y\Delta y + d_y(t) \\ \Delta\dot{x} &= b_y\Delta y - b_x\Delta x - \gamma_x\Delta x \\ u_f(t) &= -h_y\Delta y(t)\,. \end{aligned}$$

Its Laplace Transform is given by

$$\begin{aligned} sY(s) &= e^{-s\tau}U_f(s) - b_yY(s) + b_xX(s) - \gamma_yY(s) + \nu(s) \\ sX(s) &= b_yY(s) - b_xX(s) - \gamma_xX(s) \\ U_f(s) &= -h_yY(s)\,, \end{aligned}$$

and the open-loop transfer function from $U_f(s)$ to $Y(s)$ is

$$G_{ol}(s) = \frac{Y(s)}{U_f(s)} = \frac{(1+cs)e^{-s\tau}}{cs^2 + (1+B+c\gamma_y)s + \Gamma}, \tag{36}$$

where $1/c = b_x + \gamma_x$ is the buffering speed. In Figure S1.4, we can see an example of the increase in gain margin from slowing buffering from the rapid limit. The increase in gain margin occurs due to the increase in phase of the open-loop system.

## S1.10 Molecular Noise Analysis

*The following section analyzes the molecular noise in the minimal models. The system of chemical reactions is simulated for the case where the variables represent discrete numbers of molecules. We use large volume/numbers of molecules and linearization approximations to derive a linear Chemical Langevin Equation, in which variables are continuous concentrations, from the biochemical reactions. This simplified equation is then analyzed to determine the magnitude and frequency composition of the noise at the regulated species.*

In this section, we determine the molecular noise due to the discrete molecular nature of the reactions. For simplicity and to better complement preceding sections, we use Chemical Langevin Equations (CLE) with the linear noise approximation to analyze molecular noise with linear removal terms. The CLE with the linear noise approximation is a larger-volume, linearization approximation of the Chemical Master Equation [10, 3, 6]—equations representing the stochastic system for discrete molecular levels. The linear noise approximation also exactly agrees with the Chemical Master Equation up to second-order moments for chemical systems composed of zero, first, and some second order reactions [7]. We use the Gillespie algorithm [10] for simulations of discrete molecular levels presented in the main text.

If we assume linear removal of species $y$ and ignore removal of species $x$, the biochemical system has the transitions

$$\begin{aligned}
y_n &\overset{p_{y_n}(y_n)}{\rightarrow} y_n + 1 \\
y_n &\overset{\gamma_y y_n}{\rightarrow} y_n - 1 \\
(y_n, x_n) &\overset{g_{y_n}(y_n,x_n)}{\rightarrow} (y_n - 1, x_n + 1) \\
(y_n, x_n) &\overset{g_{x_n}(x_n,y_n)}{\rightarrow} (y_n + 1, x_n - 1),
\end{aligned}$$

where $y_n = \Omega y$ is the number of molecules of $y$ in a system with volume $\Omega$, $x_n = \Omega x$ is the number of molecules of $x$, $p_{y_n}(y_n) = \Omega p_y(y_n/\Omega)$, $g_{y_n}(y_n, x_n) = \Omega g_y(y_n/\Omega, x_n/\Omega)$, and $g_{x_n}(x_n, y_n) = \Omega g_x(x_n/\Omega, y_n/\Omega)$. Linear buffering rate equations are used in the simulations.

We next use a linear noise approximation to determine the stationary variance. The CLE used for analysis with large volume/numbers of molecules is [10, 3]

$$\begin{aligned}
\frac{dy}{dt} &= p_y(y) - g_y(y,x) + g_x(x,y) + b_x x - \gamma_y y \\
&\quad + \Omega^{-1/2}\left(\sqrt{p_y(y)}\xi_1 - \sqrt{g_y(y,x)}\xi_2 + \sqrt{g_x(y,x)}\xi_3 - \sqrt{\gamma_y y}\xi_4\right) \\
\frac{dx}{dt} &= g_y(y,x) - g_x(x,y) + \Omega^{-1/2}\left(\sqrt{g_y(y,x)}\xi_2 - \sqrt{g_x(y,x)}\xi_3\right),
\end{aligned}$$

where $\xi_i$s are Gaussian white noise sources with zero mean and unit variance, $p_y$ includes feedback, and the equations are presented using a standard derivative notation for Langevin equations. We next use the linear noise approximation about the steady state of the deterministic system [10, 3, 6], noting that the mean of each concentration is assumed to be equal to its deterministic steady state. We set $y = \bar{y} + \Omega^{-1/2}\hat{y}$ and $x = \bar{x} + \Omega^{-1/2}\hat{x}$, which results in

$$\begin{aligned}\frac{d\hat{y}}{dt} &= -h_y\hat{y} - b_y\hat{y} + b_x\hat{x} - \gamma_y\hat{y} + \sqrt{\bar{p}_y}\xi_1 - \sqrt{\bar{g}_y}\xi_2 + \sqrt{\bar{g}_x}\xi_3 - \sqrt{\gamma_y\bar{y}}\xi_4 \\ \frac{d\hat{x}}{dt} &= b_y\hat{y} - b_x\hat{x} + \sqrt{\bar{g}_y}\xi_2 - \sqrt{\bar{g}_x}\xi_3\,,\end{aligned} \tag{37}$$

where $\bar{g}_y = g_y(\bar{y}, \bar{x})$, $\bar{g}_x = g_x(\bar{x}, \bar{y})$, and the remaining coefficients are defined in Equation (4). When buffering is linear, then $\bar{g}_y = b_y\bar{y}$ and $\bar{g}_x = b_x\bar{x}$.

We can write Equation (37) in its state-space representation [10] as

$$\begin{aligned}\frac{d\hat{z}}{dt} &= \hat{A}\hat{z} + \hat{B}\xi \\ \hat{y} &= \hat{C}\hat{z}\,,\end{aligned}$$

where $\hat{z} = (\hat{y}, \hat{x})^T$ is the vector of states, $\xi$ is the vector of Gaussian white noise sources,

$$\begin{aligned}\hat{A} &= \begin{pmatrix} -(b_y + h_y + \gamma_y) & b_x \\ b_y & -b_x \end{pmatrix}, \\ \hat{B} &= \begin{pmatrix} \sqrt{\bar{p}_y} & -\sqrt{\bar{g}_y} & \sqrt{\bar{g}_x} & -\sqrt{\gamma_y\bar{y}} \\ 0 & \sqrt{\bar{g}_y} & -\sqrt{\bar{g}_y} & 0 \end{pmatrix}, \\ \hat{C} &= \begin{pmatrix} 1 & 0 \end{pmatrix}.\end{aligned}$$

The variance of $\hat{z}$ can be determined from the covariance matrix $Q = \mathrm{cov}(\hat{z})$ by solving [10, 3, 6]

$$\hat{A}Q + Q\hat{A}^T = -BB^T\,, \tag{38}$$

and the covariance matrix $Q_n$ of $(y_n, x_n)$ can be determined by solving [12, 6]

$$\hat{A}Q_n + Q_n\hat{A}^T = -\Omega BB^T\,, \tag{39}$$

where

$$\hat{B}\hat{B}^T = \begin{pmatrix} (\bar{p}_y + \gamma_y\bar{y}) + (\bar{g}_y + \bar{g}_x) & -(\bar{g}_y + \bar{g}_x) \\ -(\bar{g}_y + \bar{g}_x) & (\bar{g}_y + \bar{g}_x) \end{pmatrix}.$$

Solving for the variance $\sigma^2_{y_n}$ of $y_n$, we have

$$\begin{aligned}\sigma^2_{y_n} &= \frac{\Omega(\bar{p}_y + \gamma_y\bar{y})(b_x + \gamma_y + h_y)}{2(\gamma_y + h_y)(b_x + b_y + \gamma_y + h_y)} + \frac{\Omega(\bar{g}_y + \bar{g}_x)}{2(b_x + b_y + \gamma_y + h_y)} \\ &= \frac{\Omega\bar{p}_y}{\gamma_y}\frac{(1+\rho)}{(1+H)(1+B+\rho)} + \frac{\Omega\bar{g}_y}{b_x(1+B+\rho)} \\ &= \langle y_n\rangle\frac{(1+\rho)}{(1+H)(1+B+\rho)} + \langle y_n\rangle\frac{\bar{g}_{y_n}}{b_y\langle y_n\rangle}\frac{B}{(1+B+\rho)}\,,\end{aligned} \tag{40}$$

where $\bar{g}_{y_n} = \Omega\bar{g}_y$, $\langle y_n\rangle = \Omega\langle y\rangle = \Omega\bar{y}$ and

$$\rho = (1+H)\frac{\gamma_y}{b_x} = \frac{1+H}{R}\,.$$

The first term in Equation (40) represents noise from production and removal reactions, while the second term represents noise due to the buffering reactions. The resulting noise intensity is

$$\phi_{\text{noise}} = \frac{\sigma_{y_n}}{\langle y_n \rangle} = \frac{1}{\sqrt{\langle y_n \rangle}} \sqrt{\frac{1+\rho}{(1+B)(1+H+\rho)} + \frac{\bar{g}_y}{b_y \bar{y}} \frac{B}{(1+B+\rho)}}\,.$$

The function $\phi_{\text{noise}}$ is also known as the noise strength or coefficient of variation.

The noise intensity for rapid buffering ($\rho \to 0$) and linear buffering ($\bar{g}_y = b_y \bar{y}$), which is presented and discussed in the main text, is

$$\boxed{\phi_{\text{noise}} = \frac{\sigma_{y_n}}{\langle y_n \rangle} = \frac{1}{\sqrt{\langle y_n \rangle}} \sqrt{\frac{1}{(1+B)(1+H)} + \frac{B}{(1+B)}}}\,.$$

It can be seen that for slower buffering (where $\rho > 0$), feedback can regulate the buffering molecular noise term. In this case, the production/removal noise can be attenuated by both increased feedback gain, $H$, and increased buffering equilibrium ratio, $B$. Also, non-linear buffering can reduce the contribution of buffering noise if $\bar{g}_y < b_y \bar{y}$, under linear noise approximation assumptions.

We next analyze the linearized equation using a frequency-domain approach, noting that the Laplace transform is used interchangeably with the Fourier transform by setting $s = j\omega$. The equivalence of a frequency-domain methodology can be seen by noting that [5]

$$\text{Var}(\hat{y}) = \hat{C} Q \hat{C}^T = \|\hat{G}\|_2^2 \quad \text{where} \quad \hat{G}(j\omega) = \hat{C}(j\omega I - \hat{A})^{-1} \hat{B}\,.$$

Using the independence of white noise sources, the frequency-domain representation of Equation (37) is

$$\hat{Y} = \sqrt{\bar{p}_y} G_y N_1 - \sqrt{\bar{g}_y} G_b N_2 + \sqrt{\bar{g}_x} G_b N_3 - \sqrt{\gamma_y \bar{y}} G_y N_4\,, \tag{41}$$

where $\hat{Y}$ is the frequency-domain representation of $\hat{y}$, $N_1$ to $N_4$ are the frequency-domain representations of $\xi_1$ to $\xi_4$, respectively, and

$$\begin{aligned} G_y &= \frac{1+cs}{cs^2 + (1+B+c(\gamma_y+h_y))s + \gamma_y + h_y} \\ G_b &= \frac{cs}{cs^2 + (1+B+c(\gamma_y+h_y))s + \gamma_y + h_y}\,, \end{aligned} \tag{42}$$

where $c = 1/b_x$.

To analyze the noise bandwidth for rapid buffering, we set $s = j\omega$ in Equation (42) transfer functions such that

$$\begin{aligned} G_y &= \frac{1+jc\omega}{c(j\omega)^2 + (1+B+c\gamma_y(1+H))j\omega + \gamma_y(1+H)} \\ G_b &= \frac{jc\omega}{c(j\omega)^2 + (1+B+c\gamma_y(1+H))j\omega + \gamma_y(1+H)}\,. \end{aligned}$$

Using $1+B \gg (1+H)/R$, these approximate to

$$\begin{aligned} G_y &\approx \frac{1+jc\omega}{\left(\frac{c}{1+B}j\omega + 1\right)\left((1+B)s + \gamma_y(1+H)\right)} \\ G_b &\approx \frac{jc\omega}{\left(\frac{c}{1+B}j\omega + 1\right)\left((1+B)j\omega + \gamma_y(1+H)\right)}\,. \end{aligned}$$

We can observe that $G_y$ acts as a low-pass filter with a bandwidth between 0 and $\gamma_y(1+H)/(1+B)$, while $G_b$ acts as a band-pass filter with a bandwidth between $\gamma_y(1+H)/(1+B)$ and $(1+B)/c$. By noting that white noise has a uniform spectral density, we can see that the buffering reaction introduces higher-frequency noise while the production and removal reactions introduce lower-frequency noise.

## S1.11 Relating Buffering to Negative Derivative Control used in Technological Feedback Systems

*The following section compares buffer-feedback systems to technological controllers. Similarities and differences are shown using Laplace transforms, as this transform is commonly used for the design and representation of technological controllers.*

In this section, we compare buffering to derivative feedback in the linear case. We first show that rapid buffering is mathematically equivalent to negative derivative feedback. This finding generalizes a previous *in silico* observation that creatine acts as derivative control for ATP [4]. We also show the equivalence of (general) buffering to either derivative filtering or lead controllers, both closely related to pure derivative controllers. Finally, we represent the class of buffering-feedback systems in block diagram form and show the similarity between measurement noise and the molecular noise produced by buffering.

PID (Proportional-Integral-Derivative) feedback controllers provide a straightforward method for considering and combining past (integral), present (proportional), and future (derivative) state information [1]. In particular, derivative feedback works by opposing and cancelling a portion of the would-be (i.e., short-term future) rate of change of the regulated species, and in a manner that is not dependent on current states (i.e., current concentrations). In our biochemical model, rates of change represent net fluxes. When $d_y$ and therefore the inflow rate of $y$ increases, a buffer reduces the would-be rate of increase to the net flux of $y$ by conversion to the buffer species $x$. Conversely, when $d_y$ decreases, a buffer reduces the would-be rate of decrease to the net flux of $y$ by $x \to y$ conversion. The rapid equilibrium of the buffering reaction ensures that the rate of conversion between $y$ and $x$ depends only on the net flux of $y$ and not its current concentration; this is because the concentration-dependent contributions to the $y \leftrightarrow x$ conversion rates, $b_y y$ and $b_x x$, are always balanced.

To show the equivalence with derivative feedback, we use the model for rapid buffering in Equation (5) with $\gamma_x = 0$:

$$\boxed{(1+B)\Delta\dot{y} = -(\gamma_y + h_y)\Delta y + d_y}. \tag{43}$$

Treating the buffer as a second 'feedback' term, this model can be rewritten as

$$\boxed{\Delta\dot{y} = -\gamma_y \Delta y - h_y \Delta y - B\Delta\dot{y} + d_y}, \tag{44}$$

in which we observe that the buffer term, $-B\Delta\dot{y}$, is equivalent to a negative derivative feedback term with gain $B$. The model in Equation (43) is thus equivalent to a negative feedback model comprising a proportional feedback term $-h_y\Delta y$ with proportional feedback gain $h_y$, and a derivative feedback term $-B\Delta\dot{y}$ with derivative feedback gain $B$.

If we cannot assume rapid buffering or that $\gamma_x = 0$, we use the two-state model in Equation (4), which in the frequency domain can be written as

$$\begin{aligned} sY(s) &= -h_y Y(s) - b_y Y(s) + b_x X(s) - \gamma_y Y(s) + D_y(s) \\ sX(s) &= b_y Y(s) - b_x X(s) - \gamma_x X(s)\,, \end{aligned}$$

where $Y(s)$ and $X(s)$ are the Laplace transforms of $\Delta y$ and $\Delta x$, respectively. The second equation can be rearranged as

$$X(s) = \frac{b_y}{s + b_x + \gamma_x} Y(s),$$

and substituting this into the first equation gives us

$$sY(s) = -h_y Y(s) - \gamma_y Y(s) + \left( b_x \frac{b_y}{s + b_x + \gamma_x} - b_y \right) Y(s) + D_y(s).$$

Isolating the contribution of buffering into a feedback term called $U_{\mathrm{b}}(s)$, we can rewrite the model as

$$\begin{aligned} sY(s) &= -h_y Y(s) - \gamma_y Y(s) + U_{\mathrm{b}}(s) + D_y(s) \\ U_{\mathrm{b}}(s) &= -C_{\mathrm{d}}(s) Y(s) \\ C_{\mathrm{d}}(s) &= b_y - b_x \frac{b_y}{s + b_x + \gamma_x}\,, \end{aligned}$$

where $C_{\mathrm{d}}(s)$ represents the buffering subsystem. This subsystem can be rewritten as

$$\begin{aligned} C_{\mathrm{d}}(s) &= \frac{b_y}{s + b_x + \gamma_x} [s + b_x + \gamma_x - b_x] \\ &= b_y \frac{s + \gamma_x}{s + (\gamma_x + b_x)}\,, \end{aligned} \tag{45}$$

which is the standard form of a lead controller [11]. Thus for $\gamma_x \neq 0$, buffering is equivalent to a lead controller, a form of controller closely related to derivative controllers [11].

Alternatively, the buffering subsystem in Equation (45) can be written as

$$C_{\mathrm{d}}(s) = \frac{B(s + \gamma_x)}{1 + cs}\,,$$

with $B = \frac{b_y}{b_x + \gamma_x}$ and $c = \frac{1}{b_x + \gamma_x}$, as before. If $\gamma_x = 0$, then

$$C_{\mathrm{d}}(s) = \frac{Bs}{1 + cs}\,, \tag{46}$$

which is the standard form for derivative filtering, i.e., the combination of a low-pass pre-filter with a derivative control action [11].

We next determine a block diagram representation for the $\gamma_x = 0$ class of buffering-feedback systems. To complete this, we decompose the various transfer functions (which relate noise/disturbances to the output) into their various subsystems. From Equation (23), when $\gamma_x = 0$, the transfer function from disturbances to the output is

$$G_1(s) = \frac{1 + cs}{cs^2 + (1 + B + c(\gamma_y + h_y))s + (\gamma_y + h_y)}\,.$$

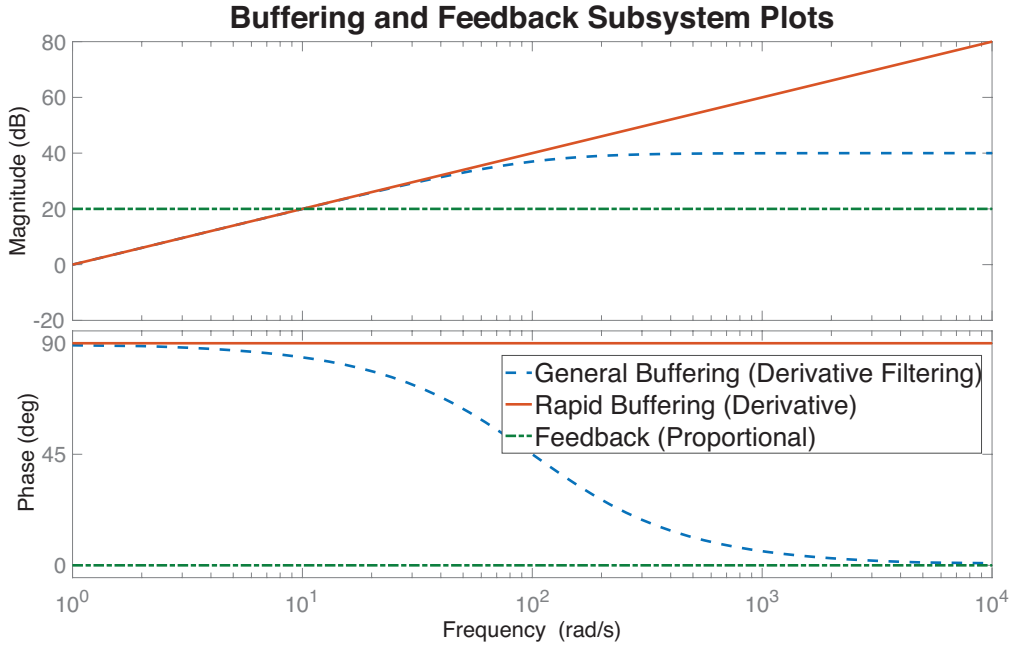



Figure S1.5: A Bode plot of the buffering and feedback subsystems using either Equation (47) for feedback and general buffering, or $C_{\mathrm{d}} = Bs$ for rapid buffering. $H = 10$, $B = 1$, and $c = 0$ or 0.01.

Defining the open-loop and regulation transfer function as

$$G_{\mathrm{ol}}(s) = \frac{1}{s + \gamma_y} \quad \text{and} \quad C(s) = C_{\mathrm{p}} + C_{\mathrm{d}}(s)\,, \tag{47}$$

respectively, where

$$C_{\mathrm{d}}(s) = \frac{Bs}{1 + cs} \quad \text{and} \quad C_{\mathrm{p}} = h_y\,, \tag{48}$$

we obtain

$$G_1(s) = \frac{G_{\mathrm{ol}}(s)}{1 + C(s)G_{\mathrm{ol}}(s)}\,, \tag{49}$$

which is the standard form for the closed-loop transfer function from a load disturbance to an output [11].

We next analyze the molecular noise generated by buffering reactions for the case where $\gamma_x = 0$ and the buffering rates are linear. The output noise from the buffering reactions in the case of linear buffering, given by setting $\bar{g}_x = \bar{g}_y = b_y\bar{y}$ in Equation (41), is

$$Y(s) = \frac{2\sqrt{b_y}cs\Omega^{-1/2}N}{cs^2 + (1 + B + c(\gamma_y + h_y))s + (\gamma_y + h_y)}\,,$$

where $N$ is the frequency-domain representation of the buffering reaction white noise sources, $Y = \Omega^{-1/2}\hat{Y}$ and $\Omega$ is the system volume. Using the notation $Y = 2G_2\Omega^{-1/2}N$, the transfer

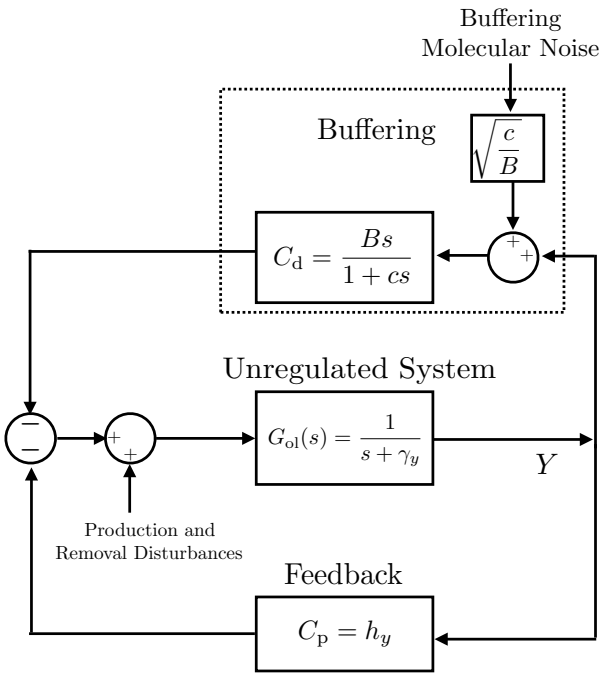



Figure S1.6: The buffering-feedback regulation system when $\gamma_x = 0$, presented in block-diagram representation. $Y$ is the frequency transform of set point deviation $\Delta y$.

function is

$$\begin{aligned} G_2(s) &= \frac{\sqrt{b_y}cs}{cs^2 + (1 + B + c(\gamma_y + h_y))s + (\gamma_y + h_y)} \\ &= \sqrt{\frac{c}{B}} \frac{Bs}{cs^2 + (1 + B + c(\gamma_y + h_y))s + (\gamma_y + h_y)}, \end{aligned} \tag{50}$$

where $\sqrt{b_y}c = \sqrt{cB} = B\sqrt{c/B}$. Using a similar decomposition to Equations (47)-(49), we have

$$G_2(s) = \sqrt{\frac{c}{B}} \frac{C_{\mathrm{d}}(s)G_{\mathrm{ol}}(s)}{1 + C(s)G_{\mathrm{ol}}(s)}, \tag{51}$$

which, excluding the multiplier $\sqrt{c/B}$, is identical to the standard closed-loop transfer function from measurement noise to an output. Therefore, molecular noise due to buffering is similar to measurement noise for PID controllers in technology. However, in the class of buffering-feedback systems we study here, the measurement noise only occurs for the derivative control channel equivalent, and not the proportional channel.

## S1.12 Low-Pass Filtering of Buffering Species Disturbances

*The following section carries out further analysis on the effect of a disturbance acting on the buffering species instead of the regulated species. The section shows that the buffer also acts as a further low-*

*pass filter for buffering disturbances. The analysis is similar to Sections S1.5 and S1.7, where fourier transforms are used.*

In this section, we determine the sensitivity functions for the case where the disturbance is acting on the buffering species.

For the case where the disturbance is on the buffering species and feedback is on the regulated species (Figure 1A in the main section of the paper with disturbance $d_x$ and not $d_y$), we consider the minimal model

$$\begin{aligned}\dot{y} = &\underbrace{p_y(y)}_{\substack{\text{production}\\ \text{with feedback}}} - \underbrace{g_y(y,x) + g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_y(y)}_{\text{removal}}\\ \dot{x} = &\underbrace{g_y(y,x) - g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_x(x)}_{\text{removal}} + \underbrace{d_x(t)}_{\text{disturbance}} ,\end{aligned}$$

with nominal steady state $(\bar{y}, \bar{x})$ and deviations $\Delta y = y - \bar{y}$ and $\Delta x = x - \bar{x}$. Linearization of this model yields

$$\begin{aligned}\Delta\dot{y} &= -b_y\Delta y + b_x\Delta x - \gamma_y\Delta y - h_y\Delta y\\ \Delta\dot{x} &= b_y\Delta y - b_x\Delta x - \gamma_x\Delta x + d_x ,\end{aligned} \tag{52}$$

where

$$\begin{aligned}h_y &= -\frac{\partial}{\partial y}p_y(y)|_{y=\bar{y}} ,\\ b_y &= \frac{\partial}{\partial y}(g_y(y,x) - g_x(x,y))|_{(y,x)=(\bar{y},\bar{x})} ,\\ b_x &= \frac{\partial}{\partial x}(g_x(x,y) - g_y(y,x))|_{(y,x)=(\bar{y},\bar{x})} ,\\ \gamma_y &= \frac{d\nu_y}{dy}(y)|_{y=\bar{y}} ,\\ \gamma_x &= \frac{d\nu_x}{dy}(x)|_{x=\bar{x}} .\end{aligned}$$

Taking the Laplace transform gives us

$$\begin{aligned}(s + b_y + \gamma_y + h_y)Y(s) &= b_xX(s)\\ (s + b_x + \gamma_x)X(s) &= b_yY(s) + D_x(s) ,\end{aligned}$$

which can be rearranged as

$$[(s + b_y + \gamma_y + h_y)(s + b_x + \gamma_x) - b_xb_y]Y(s) = b_xD_x(s) ,$$

or furthermore as

$$\begin{aligned}\frac{Y(s)}{D_x(s)} = G_x(s) &= \frac{b_x}{(s + b_y + \gamma_y + h_y)(s + b_x + \gamma_x) - b_xb_y}\\ &= \frac{b_x}{s + b_x + \gamma_x}\frac{s + b_x + \gamma_x}{(s + b_y + \gamma_y + h_y)(s + b_x + \gamma_x) - b_xb_y}\\ &= \frac{b_x}{s + b_x + \gamma_x}\frac{Y(s)}{D_y(s)} ,\end{aligned}$$

using $Y(s)/D_y(s)$ from Equation (23). Setting $s = j\omega$ gives us

$$G_x(j\omega) = \frac{b_x}{j\omega + b_x + \gamma_x} \frac{Y(j\omega)}{D_y(j\omega)}\,.$$

Normalizing the disturbance frequency $\omega$ by the unregulated system time scale $T_\mathrm{u} = 1/\gamma_y$, such that $\omega_n = \omega T_\mathrm{u}$, we have

$$\begin{aligned} G_x &= \frac{b_x}{j\gamma_y\omega_n + b_x + \gamma_x} G(j\omega) \\ &= \frac{\theta}{1 + j\frac{\omega_n}{R}} G\,, \end{aligned}$$

where

$$R = \frac{b_x + \gamma_x}{\gamma_y} \quad \text{and} \quad \theta = \frac{b_x}{b_x + \gamma_x}\,.$$

For an oscillating disturbance, we have the sensitivity function

$$\begin{aligned} \phi_\omega^{d_x} &= \frac{\|\Delta y\|_\mathrm{pow}}{\bar{y}} \Big/ \frac{\|d_x\|_\mathrm{pow}}{\bar{p}_y} \\ &= \frac{\bar{p}_y}{\bar{y}} |G_x| \\ &= \frac{\theta}{\sqrt{1 + \left(\frac{\omega_n}{R}\right)^2}} \frac{\bar{p}_y}{\bar{y}} |G| \\ &= \frac{\theta}{\sqrt{1 + \left(\frac{\omega_n}{R}\right)^2}} \phi_\omega\,. \end{aligned}$$

For the case presented in the main text where $\gamma_x = 0$ and so $\theta = 1$, the sensitivity function becomes

$$\boxed{\phi_\omega^{d_x} = \frac{1}{\sqrt{1 + \left(\frac{\omega_n}{R}\right)^2}} \phi_\omega}\,.$$

We next look at the impulse response. Calculating the norm of $G_x(s)$ using Equations (24) and (25), we have

$$\begin{aligned} \|G_x(s)\|_2^2 &= \frac{\theta^2}{2(1 + B + c(\gamma_y + h_y))(\Gamma + h_y)} \\ &= \frac{1}{2\Gamma(1+H)(1+B)} \frac{\theta^2}{\left(1 + \frac{c(\gamma_y + h_y)}{1+B}\right)}\,, \end{aligned} \tag{53}$$

where $\theta = \frac{b_x}{b_x + \gamma_x}$, $c = \frac{1}{b_x + \gamma_x}$, and $B = \frac{b_y}{b_x + \gamma_x}$. For an impulse, we have $\|\Delta y\|_{L_2} = \|G(s)\hat{d}_x\|_{L_2}$ where $d_x(t) = \hat{d}_x \delta(t)$ and $\delta(t)$ is a unit impulse. This leads to the sensitivity function

$$\begin{aligned} \phi_\mathrm{imp}^{d_x} &= \frac{\|\Delta y\|_{L_2}}{\bar{y}\sqrt{T_\mathrm{u}}} \Big/ \frac{\|\hat{d}_x\|}{\bar{p}_y T_\mathrm{u}} \\ &= \frac{\bar{\Gamma}}{\Gamma} \sqrt{\frac{\Gamma}{\Gamma_u} \frac{\kappa}{2(1+H)(1+B)}}\,, \end{aligned} \tag{54}$$

where

$$\kappa = \frac{R\theta^2}{R + \frac{1+\frac{\Gamma}{\gamma_y}H}{1+B}}, \quad R = \frac{b_x + \gamma_x}{\gamma_y}.$$

From Equation (54), it can be seen that reducing $R$ for a given $B$ improves the rejection of impulse disturbances at $d_x$.

### S1.13 Slow Buffering with Reservoir Feedback Acts as Integral Feedback

*The following section carries out analysis on the effect of feedback from the buffering species instead of the regulated species. The section shows that the feedback can act as a form of integral feedback for slow buffering. The analysis uses Laplace tranforms to show the similarities to proportional and integral control, in a similar approach to Section S1.11.*

We consider the revised minimal model

$$\begin{aligned}
\dot{y} = &\underbrace{p_y(x)}_{\substack{\text{production}\\ \text{with feedback}}} - \underbrace{g_y(y,x) + g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_y(y)}_{\text{removal}} + \underbrace{d_y(t)}_{\text{disturbance}} \\
\dot{x} = &\underbrace{g_y(y,x) - g_x(x,y)}_{\text{buffering}} - \underbrace{\nu_x(x)}_{\text{removal}},
\end{aligned}$$

in which the feedback has been altered from $p_y(y)$ to $p_y(x)$. Assuming equivalent steady states to Equation (2), this model can be linearized as

$$\begin{aligned}
\Delta\dot{y} &= -h_x\Delta x - b_y\Delta y + b_x\Delta x - \gamma_y\Delta y + d_y(t) \\
\Delta\dot{x} &= b_y\Delta y - b_x\Delta x - \gamma_x\Delta x,
\end{aligned}$$

where $h_x = \frac{\partial}{\partial y}p_y(x)|_{x=\bar{x}}$. Written in the frequency domain, we have

$$\begin{aligned}
sY(s) &= -h_xX(s) - b_yY(s) + b_xX(s) - \gamma_yY(s) + D_y(s) \\
sX(s) &= b_yY(s) - b_xX(s) - \gamma_xX(s),
\end{aligned}$$

which leads to

$$X(s) = \frac{b_y}{s + b_x + \gamma_x}Y(s).$$

Thus, the feedback term can be written as

$$-h_xX(s) = -\frac{h_xb_y}{s + b_x + \gamma_x}Y(s).$$

For rapid buffering, we have the feedback approximation

$$U_h = -h_xX = -h_xBY,$$

which is the similar to feedback from $y$, only differing by the gain. We observe that there is a pole at $s_p = -(b_x + \gamma_x)$, where the transfer function is infinite. For the slow buffering limit, the pole $s_p = -(b_x + \gamma_x) \to 0$, and so we have the feedback approximation

$$U_h = -h_xX = -\frac{h_xb_y}{s}Y,$$

which is a form of integral feedback.

Combining the effect of both buffering and feedback from the buffering species, we have

$$\begin{aligned} U_{h+b} &= -h_x X - b_y Y + b_x X \\ &= -b_y \frac{s + h_x + \gamma_x}{s + b_x + \gamma_x} Y \,, \end{aligned}$$

which can take the more general lead or lag controller form, rather than just being limited to a lead controller. For the slow buffering limit, where the pole $s_p = -(b_x + \gamma_x) \to 0$, we have an approximate PI (proportional plus integral) regulator

$$U_{h+b} \approx -\left( b_y + \frac{h_x b_y}{s} \right) Y \,.$$

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