Supplementary Information

1 Stability of the slow manifold and derivation of the expression of $\mathcal{R}(\bar{X})$

Consider the system in standard singular perturbation form

$$\begin{aligned} \frac{dy}{dt} &= k(t) - \delta(y - C) \\ \epsilon \frac{dC}{dt} &= -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C). \end{aligned} \tag{1}$$

For a variable x involved in system (1), we denote by $\bar{x}$ the value of the variable x once we have set $\epsilon = 0$ in system (1). Let $g(C, y) := -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C)$ and let $\bar{C} = \gamma(\bar{y})$ be the smallest root of $g(\gamma(\bar{y}), \bar{y}) = 0$. Then, $\bar{C} = \gamma(\bar{y})$ defines the slow manifold. Model (1) reduced to the slow manifold leads to the reduced model

$$\frac{d\bar{y}}{dt} = k(t) - \delta(\bar{y} - \gamma(\bar{y})). \tag{2}$$

Let $\tau = t/\epsilon$ and let $e_C = C - \bar{C}$ be the error between C and its approximation $\bar{C}$, the dynamics of such an error, called the boundary layer system, is given by

$$\frac{de_C}{d\tau} = -\delta(e_C + \bar{C}) + \frac{\delta}{k_d}(p_{TOT} - e_C - \bar{C})(\bar{y} - e_C - \bar{C}), \tag{3}$$

and describe the dynamics of the error of C with respect to $\bar{C}$, in which $\bar{y}$ and thus $\bar{C}$ are considered frozen at the initial condition. Since we desire C to tend to $\bar{C}$, we study the stability of the equilibrium point $e_C = 0$ of equation (3).

Proposition 1. *The equilibrium $e_C = 0$ of the boundary layer system (3) is asymptotically stable uniformly in $\bar{y}$ and $\partial g/\partial C|_{\bar{C}(t),\bar{y}(t)}$ has real part smaller than a fixed negative number.*

Proof. One can easily verify that $\partial g/\partial C|_{\bar{C}(t),\bar{y}(t)} \leq -\delta$ and that $\frac{de_C}{d\tau} = -K(\bar{y})e_C + \frac{\delta}{k_d}e_C^2$, in which $K(\bar{y}) \geq K_0$ with K_0 independent of $\bar{y}$. Therefore, the local asymptotic stability is uniform in $\bar{y}$. □

This proposition implies that the slow manifold is stable asymptotically, that is, after a fast transient y is well approximated by $\bar{y}$ and C is well approximated by $\bar{C}$. More formally, it follows that (Theorem 3.1 [5]) if $e_C(0)$ is in the region of attraction of the equilibrium $e_C = 0$, then for a fixed $T > 0$ we have that $y(t) = \bar{y}(t) + O(\epsilon)$, for all $t \in [0, T]$ and for each fixed $t_2 > t_1 > 0$ we have that $C(t) = \bar{C}(t) + O(\epsilon)$, for all $t \in [t_1, t_2]$. As a consequence, we also have that $X(t) = \bar{X}(t) + O(\epsilon)$, for all $t \in [t_1, t_2]$. Since $\bar{X}(t) = \bar{y}(t) - \bar{C}(t)$, the differential equation that $\bar{X}$ satisfies is given by $\frac{d\bar{X}}{dt} = \frac{d\bar{y}(t)}{dt} - \frac{d\gamma(\bar{y})}{d\bar{y}}\frac{d\bar{y}(t)}{dt}$, which finally leads to

$$\frac{d\bar{X}}{dt} = (k(t) - \delta\bar{X})\left(1 - \frac{d\gamma(\bar{y})}{d\bar{y}}\right). \tag{4}$$

After a fast transient $X(t)$ will follow $\bar{X}(t)$ solution of equation (4).

Since when $\frac{d\gamma(\bar{y})}{dy} = 0$, the dynamics of equation (4) is the same as the dynamics of the isolated system, we determine a more useful expression for $\frac{d\gamma(\bar{y})}{d\bar{y}}$ as follows.

Proposition 2.

$$\frac{d\gamma(\bar{y})}{d\bar{y}} = \frac{1}{1 + \frac{(1+\bar{X}/k_d)^2}{p_{TOT}/k_d}} =: \mathcal{R}(\bar{X}) \tag{5}$$

and $\mathcal{R}(\bar{X}) < 1$.

Proof. We remove the bar from the variables to simplify notation. Suppose that $\gamma(y)$ satisfies that $g(\gamma(y), y) = 0$, where $g(C, y) = \delta\left[-C + \frac{1}{k_d}(p_{TOT} - C)(y - c)\right]$. We want to calculate $d\gamma/dy$.

$$d\gamma/dy = -\frac{\partial g/\partial y}{\partial g/\partial C} = \frac{\frac{1}{k_d}(p_{TOT} - C)}{1 + \frac{1}{k_d}(p_{TOT} - C) + \frac{1}{k_d}(y - C)}$$

so substituting

$$\frac{1}{k_d}(y - C) = \frac{C}{p_{TOT} - C}$$

this equals

$$\frac{1}{1 + \frac{k_d p_{TOT}}{(p_{TOT}-C)^2}} = \frac{1}{1 + \frac{k_d}{p_{TOT}}\left(1 + \frac{C}{p_{TOT}-C}\right)^2}$$

and now substituting $\frac{C}{p_{TOT}-C} = \frac{1}{k_d}(y - C)$ we conclude that this equals

$$\frac{1}{1 + \frac{k_d}{p_{TOT}}\left(1 + \frac{1}{k_d}(y - C)\right)^2},$$

in which $y - C = X$.

□

2 Attenuation of the retroactivity to the output by feedback

Lemma 1. *Consider the system*

$$\frac{dX}{dt} = G(t)(u(t) - KX)$$

in which $G(t) \geq G_0 > 0$ *and* $|u'(t)| \leq V$ *uniformly in* t*. Then,*

$$|X(t) - \frac{u(t)}{K}| \leq \exp(-tG_0K)|X(0) - \frac{u(0)}{K}| + \frac{V}{G_0K^2}.$$

Proof. Let $e = X - u/K$. The error dynamics is given by $\dot{e} = -G(t)Ke - \frac{\dot{u}(t)}{K}$. The solution of such a differential equation is provided by

$$e(t) = e(0)\exp(-\int_0^t KG(\tau)d\tau) + \int_0^t \exp(-\int_\tau^t KG(\sigma)d\sigma)\frac{u'(t)}{K}d\tau.$$

Since $|u'(t)| \leq V$ and $G(t) \geq G_0 > 0$ for all t, we have that

$$|X(t) - \frac{u(t)}{K}| \leq \exp(-tG_0K)|X(0) - \frac{u(0)}{K}| + (1 - \exp(-tG_0K))V/(G_0K^2).$$

Hence, we obtain the desired result. □

Then, we can give the following simple corollary to Lemma 1.

Corollary 1. *Consider the two systems*

$$\frac{dX_r}{dt} = G(u(t) - KX_r) \text{ and } \frac{dX}{dt} = \bar{G}(t)(u(t) - KX), \tag{6}$$

in which $|u'(t)| \le V$, $\bar{G}(t) > G_0$, *and* $G \ge G_0$ *for* $G_0 > 0$. *Then*

$$|X(t) - X_r(t)| \le \exp(-tG_0K)C_0 + 2\frac{V}{G_0K^2},$$

for a suitable nonnegative constant C_0.

Proof. We can apply Lemma 1 to the two systems in equation (6), separately. This along with the triangular inequality $|X(t) - X_r(t)| \le |X(t) - u(t)/K| + |X_r(t) - u(t)/K|$ leads to $|X(t) - X_r(t)| \le \exp(-tG_0K)C_0 + 2\frac{V}{G_0K^2}$, for a suitable nonnegative constant C_0 depending on the initial conditions. □

Let us now consider the isolated system

$$\frac{dX}{dt} = k(t) - \delta X, \tag{7}$$

and the connected system (4) and assume that we can amplify with gain G the input $k(t)$ and apply an additional negative feedback $-G'X$, in which $G' = \alpha G$ for some $\alpha = O(1)$. Then, we obtain the two systems (isolated an connected) as

$$\frac{dX_r}{dt} = G(k(t) - (\alpha + \delta/G)X_r) \tag{8}$$

and

$$\frac{dX}{dt} = G(k(t) - (\alpha + \delta/G)X)(1 - d(t)) \tag{9}$$

respectively, in which $d(t) = \left|\frac{d\gamma(y)}{dy}\right|$ and $y(t)$ given by the reduced system

$$\frac{dy}{dt} = Gk(t) - (G' + \delta)(y - \gamma(y)).$$

We can apply Corollary 1 to the two systems (8) and (9) with $\bar{G}(t) = G(1-d(t))$, $K = (\alpha+\delta/G)$, and $k(t) = u(t)$, to obtain that $X(t)$ can be made close to $X_r(t)$ by increasing the gain G.

2.1 Design 1: Amplification through transcriptional activation

The differential equations modeling the insulation device are given by

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z + \boxed{k_- Z_p - k_+ Z(p_{0,TOT} - Z_p)} && (10)\\
\frac{dZ_p}{dt} &= k_+ Z(p_{0,TOT} - Z_p) - k_- Z_p && (11)\\
\frac{dm_X}{dt} &= GZ_p - \delta_1 m_X && (12)\\
\frac{dX}{dt} &= \nu m_X - \eta_1 YX + \eta_2 W - \delta_2 X + \boxed{k_{off} C - k_{on} X(p_{TOT} - C)} && (13)\\
\frac{dW}{dt} &= \eta_1 XY - \eta_2 W - \beta W && (14)\\
\frac{dY}{dt} &= -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W && (15)\\
\frac{dC}{dt} &= -k_{off} C + k_{on} X(p_{TOT} - C), && (16)
\end{aligned}$$

in which we have assumed that the expression of gene z is controlled by a promoter with activity $k(t)$. These equations will be studied numerically and analyzed mathematically in a simplified form. The variable Z_p is the concentration of protein Z bound to the promoter controlling gene x, $p_{0,TOT}$ is the total concentration of the promoter p_0 controlling gene x, m_X is the concentration of messenger RNA of X, C is the concentration of X bound to the downstream binding sites with total concentration p_{TOT}, γ is the decay rate of the protease. The value of G is the production rate of X mRNA per unit concentration of Z bound to the promoter controlling x; the promoter controlling gene y has strength αG, in which α is a constant so that the promoter controlling y has the same order of magnitude strength as the promoter controlling x. The dynamics of equations (10)–(16) without the elements in the box in equation (13) describe the dynamics of X with no downstream system, which we call X_r.

We mathematically explain why system (10)–(16) allows to have $X \approx X_r$ thus attenuating the effect of s on the X dynamics. Equations (10) and (11) simply determine the signal $Z_p(t)$ that is the input to equations (12)–(16). For the discussion regarding the attenuation of the effect of s, it is not relevant what the specific form of signal $Z_p(t)$ is. Let then $Z_p(t)$ be any bounded signal $v(t)$. Since equation (12) takes $v(t)$ as an input, we will have that $m_X = G\bar{v}(t)$, for a suitable signal $\bar{v}(t)$. Let us assume for the sake of simplifying the analysis that the protease reaction is a one step reaction, that is, $\mathrm{X} + \mathrm{Y} \xrightarrow{\beta} \mathrm{Y}$. Therefore, equation (15) simplifies to $\frac{dY}{dt} = \alpha G - \gamma Y$ and equation (13) simplifies to $\frac{dX}{dt} = \nu m_X - \beta YX - \delta_2 X + k_{off} C - k_{on} X(p_{TOT} - C)$. If we consider the protease to be at its equilibrium, we have that $Y(t) = \alpha G/\gamma$. As a consequence, the X dynamics becomes

$$\frac{dX}{dt} = \nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X + \boxed{k_{off}C - k_{on}X(p_{TOT} - C)},$$

with C determined by equation (16). By using the same singular perturbation argument employed in the previous section, we obtain that the dynamics of X will be after a fast transient approximatively equal to

$$\frac{dX}{dt} = (\nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X)(1 - d(t)), \tag{17}$$

in which $d(t) < 1$. In the case in which $d(t) = 0$, we obtain the dynamics of the isolated system as

$$\frac{dX_r}{dt} = \nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X_r. \tag{18}$$

We can thus apply Corollary 1 to systems (18) and (17) with $u(t) = \nu\bar{v}(t)$, $K = \beta\alpha/\gamma + \delta_2/G$, and $\bar{G}(t) = G(1 - d(t))$, to conclude that $X(t)$ can be made closer to $X_r(t)$ by increasing G.

2.2 Design 2: Amplification through phosphorylation

A one step model for the phosphorylation reactions is considered to apply Corollary 1:

$$\mathrm{Z} + \mathrm{X} \xrightarrow{k_1} \mathrm{Z} + \mathrm{X}_p,$$

and

$$\mathrm{Y} + \mathrm{X}_p \xrightarrow{k_2} \mathrm{Y} + \mathrm{X}.$$

The conservation of X gives $X + X_p + C = X_{TOT}$, in which X is the inactive protein, X_p is the phosphorylated protein that binds to the downstream sites p, and C is the complex of the phosphorylated protein X_p bound to the promoter p. The X_p dynamics can be described by the first equation in the following model

$$\begin{aligned} \frac{dX_p}{dt} &= k_1 X_{TOT} Z(t)\left(1 - \frac{X_p}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) - k_2 Y X_p + \boxed{k_{off}C - k_{on}X_p(p_{TOT} - C)} && (19)\\ \frac{dC}{dt} &= -k_{off}C + k_{on}X_p(p_{TOT} - C). && (20) \end{aligned}$$

The boxed terms represent the retroactivity s to the output. For a weakly activated pathway ([2]), $X_p \ll X_{TOT}$. Also, if we assume that the concentration of total X is large compared to the concentration of the downstream binding sites, that is, $X_{TOT} \gg p_{TOT}$, equation (19) is approximatively equal to

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t) - k_2 Y X_p + k_{off} C - k_{on} X_p (p_{TOT} - C).$$

Denote $G = k_1 X_{TOT}$ and $G' = k_2 Y$. Exploiting again the difference of time scales between the X_p dynamics and the C dynamics, after a fast initial transient, the dynamics of X_p can be well approximated by

$$\frac{dX_p}{dt} = (GZ(t) - G' X_p)(1 - d(t)), \tag{21}$$

in which $0 < d(t) < 1$ is the effect of the retroactivity s to the output after a short transient. System (21) with $d(t) = 0$ determines the isolated system. We call X_r the output signal to the isolated system. We thus apply Corollary 1 to system (21) with $d(t) = 0$ and to system (21) with $u(t) = Z(t)$, $\bar{G}(t) = G(1 - d(t))$, and $K = k_2 Y/(k_1 X_{TOT})$ to conclude that $X(t)$ can be made closer to $X_r(t)$ by increasing the gain G.

3 A general formulation of attenuation of retroactivity

We briefly discuss here a formalization of the "low-retroactivity" property, described in terms of the general system model:

$$\begin{aligned} \frac{dx}{dt} &= f(x, u, s) \\ y &= Y(x, u, s) \\ r &= R(x, u, s). \end{aligned} \tag{22}$$

We view the input signal u and the retroactivity s to the output as belonging to sets $\mathcal{U}$ and $\mathcal{V}$ respectively. These sets summarize all prior information available about the signals, such as their ranges of values, or their maximal rates of change. The initial conditions at time $t = 0$ for the state variables x are supposed to lie in a subset $\mathcal{X}$ of the set of possible states. The definitions will be stated relative to a given a number $\Delta > 0$ (in practice, a small number) which specifies the tolerated level of retroactivity, and an interval $I \subseteq (0, +\infty)$ which specifies on what time interval the retroactivity should be small.

The system (22) will be said to have Δ-*level retroactivity to the output, on the time interval* I, provided that, for any initial condition ξ in $\mathcal{X}$, any signals $u \in \mathcal{U}$ and $s \in \mathcal{V}$, and any time instant $t \in I$:

$$|y(t) - y_0(t)| \leq \Delta \qquad \text{and} \qquad |r(t) - r_0(t)| \leq \Delta,$$

where x, y, r are as in (22) with $x(0) = \xi$, and:

$$\begin{aligned} \frac{dx_0}{dt} &= f(x_0, u, 0), \qquad x_0(0) = \xi \\ y_0 &= Y(x_0, u, 0) \\ r_0 &= R(x_0, u, 0). \end{aligned}$$

In words, the difference between the output y and the output $y = y_0$ that would have been measured had the retroactivity signal s not been present ($s = 0$) is not larger than the number Δ; and also the retroactivity r to the input is not substantially different than if s was not there.

Similarly, the system (22) will be said to have Δ-*level retroactivity to the input, on the time interval* I, if for any initial condition $\xi \in \mathcal{X}$, any signal $u \in \mathcal{U}$, and any time instant $t \in I$:

$$|r_0(t)| \leq \Delta$$

where, as earlier, $r_0 = R(x_0, u, 0)$. In words, the retroactivity to the input is small, assuming that the system is not subject to retroactivity to its outputs.

Observe that when the system has both Δ-level retroactivity to the input and the output, from $|r(t) - r_0(t)| \leq \Delta$ and $|r_0(t)| \leq \Delta$ one has that $|r(t)| \leq 2\Delta$, that is, the input retroactivity is "small" even if retroactivities to its outputs are present.

These formulations are very general, and apply to arbitrary systems.

The properties are a variant of the control theory property of *almost disturbance decoupling* [4, 9], and their study and verification is naturally carried out using techniques based on *gains* and *input to state stability* [1, 6, 7]. In this paper, we described but one particular approach, which is useful whenever time-scale separation techniques can be employed. For simplicity, we presented our calculations for finite time intervals, but entirely analogous calculations based on singular perturbation theory are possible on infinite intervals, appealing to the methods described in [3] and [8].

References

[1] J.C. Doyle, B. Francis, and A. Tannenbaum. *Feedback Control Theory*. MacMillan publishing Co., 1990.

[2] R. Heinrich, B. G. Neel, and T. A. Rapoport. Mathematical models of protein kinase signal transduction. *Molecular Cell*, 9:957–970, 2002.

[3] F.C. Hoppensteadt. Singular perturbations on the infinite interval. *Trans. Amer. Math. Soc.*, 123:521–535, 1966.

[4] A. Isidori, B. Schwartz, and T. J. Tarn. Semiglobal L_2 performance bounds for disturbance attenuation in nonlinear systems. *IEEE Trans. Automat. Control*, 44(8):1535–1545, 1999.

[5] P. Kokotovic, H. K. Khalil, and J. O'Reilly. *Singular Perturbation Methods in Control*. SIAM, 1999.

[6] E. Sontag. Input to state stability: Basic concepts and results. In P. Nistri and G. Stefani, editors, *Nonlinear and Optimal Control Theory*, pages 163–220. Springer-Verlag, Berlin, 2007.

[7] E.D. Sontag. *Mathematical Control Theory*. Springer-Verlag, New York, 1998.

[8] A.R. Teel, L. Moreau, and D. Nešić. A unified framework for input-to-state stability in systems with two time scales. *IEEE Trans. Automat. Control*, 48(9):1526–1544, 2003.

[9] S. Weiland and J.C. Willems. Almost disturbance decoupling with internal stability. *IEEE Trans. Automatic Control*, 34:277–286, 1989.