Supplemental Data: Feedback Regulation of Opposing Enzymes Generates Robust, All-or-None Bistable Responses

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Here we show how we arrived at the curves shown in the rate balance plots.

Inactivation rate, one-loop system. For the one-loop system where the activator is feedback-activated but the inactivator is not, the rate of inactivation of *A* is given by:

$$Rate_{inact} = k_1 x \qquad \text{[Eq 1]}$$

where *x* represents the fraction of *A* that is active (and hence can be inactivated). Varying the rate constant k_1 simply changes the slope of the red line (Fig. 2AB).

Activation rate, one-loop or two-loop system. The rate of activation of *A* is more complicated. It depends upon the activity of the activator enzyme, which in turn depends upon the activity of *A*. Here we will assume that the response of the activator to *A* is rapid, allowing us to write the rate equation in terms of a single variable, *x*, which represents the fraction of *A* that is active.

We assume that the direct regulation of the activator by *x* is described by a Hill equation. The rate of activation of *A* will be proportional to a rate constant, the fraction of *A* that is unactivated (1-*x*), and the basal and activated activities of the activator. This yields Eq 2:

$$Rate_{act} = k_2\left(\beta_2 \frac{K_2^{n_2}}{K_2^{n_2} + x^{n_2}} + \frac{x^{n_2}}{K_2^{n_2} + x^{n_2}}\right)(1 - x) \qquad \text{[Eq 2]}$$

where k_2 is the rate constant for activation of *A* by the fully-active activator, β_2 is the basal activity the activator as a fraction of the maximal activity, K_2 is the fractional activity of *A* at which the activator is half-maximally active, and n_2 is the Hill coefficient for the activation of the activator by active *A*. Note that the Hill functions allow the activation curve to wrap around the straight-line inactivation curve; ultrasensitivity is required for bistability in this case. However, if we had used a hyperbolic (Michaelian) inactivation rate function, we could have generated bistability without making the feedback ultrasensitive. See [S1] for further discussion of this point.

Note that varying the rate constant k_2 would simply stretch the blue curve up or down (Fig. 2BC).

Inactivation rate, two-loop system. In the two-loop system, the activation rate is still given by Eq 2, but the inactivation rate is different. If the inactivation of the inactivator enzyme by active *A* is well-approximated by a Hill function, then the inactivation rate is given by:

$$Rate_{inact} = k_1\left(\beta_1 \frac{x^{n_1}}{K_1^{n_1} + x^{n_1}} + \frac{K_1^{n_1}}{K_1^{n_1} + x^{n_1}}\right)x \qquad \text{[Eq 3]}$$

where k_1 is the rate constant for inactivation of A by the maximally-active inactivator enzyme, β_1 is the basal activity of the inactivator as a fraction of the maximal activity, K_1 is the fractional activity of A at which the inactivator is half-maximally inactivated, and n_1 is the Hill coefficient for the inactivation of the inactivator by A.

Varying the rate constant k_1 stretches the red curve up or down (Fig. 2C).

Parameters. For the calculations shown in Fig. 2 we took k_1 to be a range of values, k_2=1, n_1=3, n_2=3, K_1=0.3, K_2=0.3, β_1=0, and β_2=0.05. These parameter values made the red and blue curves be approximately mirror images of each other (Fig. 2C). However, even for less symmetrical parameter choices, the robustness of the two-loop system can be very high.

Note that with the two-loop system it is possible to choose parameters that make the curves intersect at five points—the system becomes tristable—but the tristability is relatively brittle.

Alternative strategies for improving robustness. One can also improve the robustness of the one-loop system (Fig. 2B) by assuming that the inactivation rate curve is hyperbolic rather than a straight line, as would be the case if the inactvator enzyme is running close to saturation.

Supplemental Reference

S1. Ferrell, J.E., Jr., and Xiong, W. (2001). Bistability in cell signaling: how to make continuous processes discontinuous, and reversible processes irreversible. Chaos *11*, 227-236.