Supplementary Information for

Molecular switch architecture determines response properties of signaling pathways

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S1. Dose-responses for activation, derepression, and concerted mechanisms

S1-A. Ordinary differential equations. The ordinary differential equations (ODEs) that govern the dynamics of X, X^*, Y, and Y^* for the concerted mechanism described in the main text are:

$$\frac{dX}{dt} = -k_1 SX + k_2 X^*, \tag{S1.1a}$$
$$\frac{dX^*}{dt} = k_1 SX - k_2 X^*, \tag{S1.1b}$$
$$\frac{dY}{dt} = -(k_3 + k_5 X^*)Y + (k_4 + k_6 X)Y^*, \tag{S1.1c}$$
$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)Y - (k_4 + k_6 X)Y^*. \tag{S1.1d}$$

We assume that $X + X^* = X_T$ and $Y + Y^* = Y_T$, which reduce the above ODEs to

$$\frac{dX^*}{dt} = k_1 S(X_T - X^*) - k_2 X^*, \tag{S1.2a}$$
$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X^*))Y^*. \tag{S1.2b}$$

Re-arranging terms, we obtain the following system of ODEs

$$\frac{dX^*}{dt} = k_1 X_T S - (k_1 S + k_2)X^*, \tag{S1.3a}$$
$$\frac{dY^*}{dt} = k_3 Y_T + k_5 Y_T X^* - (k_3 + k_4 + k_6 X_T)Y^* - (k_5 - k_6)X^* Y^*, \tag{S1.3b}$$

which is Eq. (1) in the main text.

S1-B. Dose-responses. We term the steady-state responses obtained by varying the stimulus level as dose-responses. The number of active receptors in steady-state, $\overline{X^*}$, and the active switch molecules at steady-state, $\overline{Y^*}$, may be computed by setting the ODEs in Eq. (S1.3) to zero:

$$\overline{X^*} = \frac{k_1 S X_T}{k_1 S + k_2}, \tag{S1.4a}$$
$$\overline{Y^*} = \frac{k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2}{k_1 S + k_2}}. \tag{S1.4b}$$

Rearranging terms gives Eq. (2) of the main text:

$$\overline{X^*} = \frac{S X_T}{\frac{k_2}{k_1} + S}, \tag{S1.5a}$$
$$\overline{Y^*} = \frac{\frac{k_2 k_3}{k_1(k_3 + k_4 + k_5 X_T)} + \frac{k_3 + k_5 X_T}{k_3 + k_4 + k_5 X_T} S}{\frac{k_2(k_3 + k_4 + k_6 X_T)}{k_1(k_3 + k_4 + k_5 X_T)} + S} Y_T. \tag{S1.5b}$$

It may now be seen that $\overline{X^*}$ and $\overline{Y^*}$ have the form:

$$\overline{X^*} = \frac{X_0^* \Theta_{X^*} + X_\infty^* S}{\Theta_{X^*} + S}, \tag{S1.6a}$$
$$\overline{Y^*} = \frac{Y_0^* \Theta_{Y^*} + Y_\infty^* S}{\Theta_{Y^*} + S}, \tag{S1.6b}$$

where

$$X_0^* = 0, \tag{S1.6c}$$
$$X_\infty^* = X_T, \tag{S1.6d}$$
$$\Theta_{X^*} = \frac{k_2}{k_1}, \tag{S1.6e}$$
$$Y_0^* = \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}, \tag{S1.6f}$$
$$Y_\infty^* = \frac{(k_3 + k_5 X_T) Y_T}{k_3 + k_4 + k_5 X_T}, \tag{S1.6g}$$
$$\Theta_{Y^*} = \Theta_{X^*} \frac{k_3 + k_4 + k_6 X_T}{k_3 + k_4 + k_5 X_T}. \tag{S1.6h}$$

We first note that the dose-response of the receptor (i.e., the receptor occupancy) and that of the switch are of similar form. Below, we describe the effects of various parameters on these dose-responses.

1. The parameters k_1 and k_2 do not affect the minimum response X_0^* and the maximum response X_T. The ratio $\Theta_{X^*} = \frac{k_2}{k_1}$ determines the stimulus level at which $X^* = \frac{X_T}{2}$.

2. Increasing the lumped parameter $k_6 X_T$ decreases the minimum response of the switch Y_0^*. We term $k_6 X_T$ the (total) repression strength and it has no effect on the maximum response of the switch Y_∞^*.

3. Increasing the lumped parameter $k_5 X_T$ increases the maximum response of the switch Y_∞^*. We term $k_5 X_T$ the (total) activation strength and it has no effect on the minimum response of the switch Y_0^*.

4. If the basal activation rate of the switch k_3 is zero then $Y_0^* = 0$. A non-zero k_3 results in a non-zero Y_0^* and therefore shifts the dose-response curve upwards. It is worth noting that $k_3 = 0$ implies that $k_5 X_T \neq 0$ for the switch to generate a response at all.

5. If the basal deactivation rate of the switch k_4 is zero then $Y_\infty^* = Y_T$. Note that if we set $k_4 = 0$ then $k_6 X_T \neq 0$ has to be true to repress the switch in the absence of the stimulus. A non-zero k_4 results in $Y_\infty^* < Y_T$ and therefore shifts the dose-response curve downwards.

S1-C. A comment on parameter choice for Fig. 2. Our choice of parameters in Fig. 2 of the main text is guided by the above observations on dose-responses of X^* and Y^*. We also assume that basal rate k_3 is much smaller than the 'total activation strength' $k_5 X_T$. Likewise, the basal rate k_4 is much smaller than the 'total repression strength' $k_6 X_T$. Relaxing these assumptions would result in effects described by points 4 and 5 above. In addition to ignoring the basal rates wherever possible ($k_3 = 0$ for activation; $k_4 = 0$ for derepression; $k_3 = k_4 = 0$ for concerted), we normalize the stimulus strength by the binding affinity of the receptor and we only plot fractional responses in Fig. 2. Thus, the qualitative features of our results do not depend on the exact values of the parameters k_1, k_2, S, X_T and Y_T.

S2. Background results for transient signaling response and response time

Let $R(t)$ be a signaling response at time t. One convenient way to define the response time is through the center of mass:

$$\mathcal{T}_R = \frac{\int_0^\infty tR(t)dt}{\int_0^\infty R(t)dt}. \quad \text{[S2.1]}$$

This definition is the same as the one used for 'signaling time' in (1). One advantage of this definition is that it is often analytically tractable if $R(t)$ is available. Equivalently, $\mathcal{T}_R$ may also be computed in the Laplace (frequency) domain. To that end, let us define the Laplace transform

$$\mathcal{R}[\omega] = \int_0^\infty e^{-\omega t} R(t)dt. \quad \text{[S2.2]}$$

The integrals corresponding to the numerator and the denominator in the above definition of $\mathcal{T}_R$ may be computed as:

$$\int_0^\infty tR(t)dt = -\left.\frac{d\mathcal{R}[\omega]}{d\omega}\right|_{\omega=0}, \quad \text{[S2.3a]}$$

$$\int_0^\infty R(t)dt = \left.\mathcal{R}[\omega]\right|_{\omega=0}. \quad \text{[S2.3b]}$$

Thus, we have that

$$\mathcal{T}_R = -\frac{\left.\frac{d\mathcal{R}[\omega]}{d\omega}\right|_{\omega=0}}{\left.\mathcal{R}[\omega]\right|_{\omega=0}} = -\left.\frac{d\log\left(\mathcal{R}[\omega]\right)}{d\omega}\right|_{\omega=0}. \quad \text{[S2.4]}$$

Because $R(t) \geq 0$ to be biologically meaningful response, one limitation of the above definition of $\mathcal{T}_R$ is that the integrals do not converge if $\overline{R} = \lim_{t\to\infty} R(t) \neq 0$. So the definition needs to be modified for responses that have non-zero steady-states, which is the case for models considered in this work. As done in (2), one can extend the definition to a response with non-zero steady-state:

$$\mathcal{T}_R = \frac{\int_0^\infty t\left|\overline{R} - R(t)\right| dt}{\int_0^\infty \left|\overline{R} - R(t)\right| dt}. \quad \text{[S2.5]}$$

Here $|.|$ takes the absolute value or the modulus of its argument. Note that both definitions are equivalent when $\overline{R} = 0$. The new definition works with the absolute value of the error signal $E(t) = \overline{R} - R(t)$. The absolute value, $|.|$, may be dropped from the above definition if $E(t) \geq 0, \forall t \geq 0$, or $E(t) \leq 0, \forall t \geq 0$. In such cases, we can use the frequency domain version of the definition:

$$\mathcal{T}_R = \mathcal{T}_E = -\left.\frac{d\log\left(\mathcal{E}[\omega]\right)}{d\omega}\right|_{\omega=0}. \quad \text{[S2.6]}$$

Here $\mathcal{E}[\omega]$ is the Laplace transform of $E(t)$ that is related with $\mathcal{R}[\omega]$ as

$$\mathcal{E}[\omega] = \frac{\overline{R}}{\omega} - \mathcal{R}[\omega]. \quad \text{[S2.7]}$$

Generally speaking, if $E(t)$ changes sign then Eq. (S2.6) only expression serves as an approximation of the response time in Eq. (S2.1).

In what follows, we compute the response time for a few representative examples of linear dynamical systems that are pertinent to the models considered in the main text.

S2-A. A simple switch. Consider a protein that transitions between two states A and A^* as

$$A \underset{k_2}{\overset{k_1 S}{\rightleftarrows}} A^*. \quad \text{[S2.8]}$$

Let $A(t)$ and $A^*(t)$denote the number of molecules that are in states A and A^*, respectively, at time t. We assume that the total number of molecules is conserved, i.e., $A_T = A(t) + A^*(t)$. We quantify the signaling through the switch by $A^*(t)$, i.e., the number of molecules in the state A^*. The ordinary differential equation (ODE) governing the dynamics of A^* is:

$$\frac{dA^*}{dt} = k_1 S(A_T - A^*) - k_2 A^*. \quad \text{[S2.9]}$$

Let $\mathcal{A}^*[\omega]$ denote the Laplace transform of $A^*(t)$, then

$$\mathcal{A}[\omega] = \frac{k_1 S A_T}{k_1 S + k_2}\left(\frac{1}{\omega} - \frac{1}{\omega + k_1 S + k_2}\right) + \frac{A^*(0)}{\omega + k_1 S + k_2}, \quad \text{[S2.10]}$$

where $A^*(0) < A_T$ is the initial condition. Taking inverse Laplace transform, the solution to this ODE is

$$A^*(t) = A^*(0)e^{-(k_1 S + k_2)t} + \frac{k_1 S A_T}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right). \quad \text{[S2.11]}$$

While we can use $A^*(t)$ to compute the response time as

$$\mathcal{T}_{A^*} = \frac{\int_0^\infty t\left|\overline{A^*} - A^*(t)\right| dt}{\int_0^\infty \left|\overline{A^*} - A^*(t)\right| dt}, \quad \text{[S2.12]}$$

we instead use the Laplace transform $\mathcal{A}[\omega]$ to do so. In frequency domain, we have that

$$\mathcal{T}_{A^*} = -\left.\frac{d\log\left(\mathcal{E}_{\mathcal{A}}[\omega]\right)}{d\omega}\right|_{\omega=0}, \quad \text{[S2.13]}$$

where $\mathcal{E}_A = \frac{\overline{A}}{\omega} - \mathcal{A}[\omega]$ is the Laplace transform of the error signal $E_A(t) = \overline{A} - A(t)$. The steady-state value $\overline{A^*} = \lim_{t\to\infty} A(t)$ may be computed by taking the limit of the time domain solution or by applying the final value theorem in frequency domain

$$\overline{A^*} = \lim_{t\to\infty} A(t) = \lim_{\omega\to 0} \omega\mathcal{A}[\omega] = \frac{k_1 S A_T}{k_1 S + k_2}. \quad \text{[S2.14]}$$

With these, we have

$$\mathcal{E}_{\mathcal{A}}[\omega] = \left(\frac{k_1 S A_T}{k_1 S + k_2} - A^*(0)\right)\frac{1}{\omega + k_1 S + k_2}, \quad \text{[S2.15]}$$

which results in the following for the response time

$$\mathcal{T}_{A^*} = -\left.\frac{d\log\left(\mathcal{E}_{\mathcal{A}}[\omega]\right)}{d\omega}\right|_{\omega=0} = \frac{1}{k_1 S + k_2}. \quad \text{[S2.16]}$$

We thus deduce that if the response is determined by a single kinetic step, the response time defined above is reciprocal of the rate constant for that step. It is also worth noting that the error signal is zero if $A^*(0) = \frac{k_1 S A_T}{k_1 S + k_2} = \overline{A^*}$ and the response time has no meaning in that case.

There are other definitions of response time that are based on the time it takes for the response to start from $A^*(0)$ and reduce its deviation from its steady-state by a factor $0 < f < 1$. More specifically, we define $\mathcal{T}_f$ as the solution to the following equation

$$\frac{A^*(\mathcal{T}_f) - A^*(0)}{\overline{A^*} - A^*(0)} = f \quad \text{[S2.17a]}$$

$$\implies A^*(0)e^{-(k_1 S + k_2)\mathcal{T}_f} + \frac{k_1 S A_T}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)\mathcal{T}_f}\right) = A^*(0) + f\left(\frac{k_1 S A_T}{k_1 S + k_2} - A^*(0)\right). \quad \text{[S2.17b]}$$

For $A^*(0) \neq \frac{k_1 S A_T}{k_1 S + k_2}$, the above equation reduces to

$$1 - e^{-(k_1 S + k_2)\mathcal{T}_f} = f, \tag{S2.18}$$

which has a straightforward solution

$$\mathcal{T}_f = -\frac{\log(1-f)}{k_1 S + k_2}. \tag{S2.19}$$

Notably, the response time is set by $1/(k_1 S + k_2)$ up to a scale which depends on the specific value of f. We discuss three cases. First, setting $f = 1/2$ corresponds to the time at which half of the deviation from the steady-state has been reduced. The corresponding response time is given by

$$\mathcal{T}_{50\%} = \frac{\log 2}{k_1 S + k_2}. \tag{S2.20a}$$

Second, $f = (e-1)/e \approx 0.632$ is also frequently used for which we obtain

$$\mathcal{T}_{63.2\%} = \frac{1}{k_1 S + k_2}. \tag{S2.20b}$$

Lastly, a third definition concerns computing the time it takes for the response to travel from 10% to 90% of the difference between its initial value $A^*(0)$ and steady-state $A^* = \frac{k_1 S A_T}{k_1 S + k_2}$. In this case, we get

$$\mathcal{T}_{90\%} - \mathcal{T}_{10\%} = \frac{\log 9}{k_1 S + k_2}. \tag{S2.20c}$$

S2-B. Two-tier linear system. We next consider a two-tier linear system

$$\frac{dR_1}{dt} = \eta_0 - \eta_1 R_1, \tag{S2.21a}$$

$$\frac{dR_2}{dt} = \xi_0 + \xi_1 R_1 - \xi_2 R_2, \tag{S2.21b}$$

with initial conditions $(R_1(0), R_2(0))$. The steady-state solution to this system is given by

$$\left(\overline{R_1}, \overline{R_2}\right) = \left(\frac{\eta_0}{\eta_1}, \frac{\xi_0 + \xi_1 \frac{\eta_0}{\eta_1}}{\xi_2}\right). \tag{S2.21c}$$

Let $\mathcal{R}_1[\omega]$ and $\mathcal{R}_2[\omega]$ respectively denote the Laplace transforms of $R_1(t)$ and $R_2(t)$. Then the solution in Laplace domain to the above system of ODEs, after some algebraic manipulation, is given by

$$\mathcal{R}_1[\omega] = \frac{\eta_0}{\eta_1}\left(\frac{1}{\omega} - \frac{1}{\omega + \eta_1}\right) + \frac{R_1(0)}{\omega + \eta_1}, \tag{S2.22a}$$

$$\mathcal{R}_2[\omega] = \frac{\xi_0}{\xi_2}\left(\frac{1}{\omega} - \frac{1}{\omega + \xi_2}\right) + \frac{\eta_0 \xi_1}{\eta_1 \xi_2}\left(\frac{1}{\omega} - \frac{\omega + \eta_1 + \xi_2}{(\omega + \eta_1)(\omega + \xi_2)}\right) + \frac{\xi_1 R_1(0)}{(\omega + \eta_1)(\omega + \xi_2)} + \frac{R_2(0)}{\omega + \xi_2}. \tag{S2.22b}$$

The transient solution may be computed by taking the inverse Laplace transform:

$$R_1(t) = \frac{\eta_0}{\eta_1}\left(1 - e^{-\eta_1 t}\right) + R_1(0) e^{-\eta_1 t}, \tag{S2.23a}$$

$$R_2(t) = \frac{\xi_0 + \xi_1 \frac{\eta_0}{\eta_1}}{\xi_2} - \left(\frac{\xi_0 + \xi_1 \frac{\eta_0}{\eta_1}}{\xi_2} - R_2(0)\right) e^{-\xi_2 t} - \frac{\xi_1 \left(\frac{\eta_0}{\eta_1} - R_1(0)\right)}{\eta_1 - \xi_2}\left(e^{-\xi_2 t} - e^{-\eta_1 t}\right). \tag{S2.23b}$$

The solution for the limiting case when $\eta_1 = \xi_2$ may also be obtained by taking the limit $\eta_1 \to \xi_2$. Another special case that is relevant to our discussion in this manuscript is when the initial conditions are specified as $R_1(0) = 0$ and $R_2(0) = \frac{\xi_0}{\xi_2}$. For this case, we have the following

$$R_1(t) = \frac{\eta_0}{\eta_1}\left(1 - e^{-\eta_1 t}\right), \tag{S2.24a}$$

$$R_2(t) = \frac{\xi_0 + \xi_1 \frac{\eta_0}{\eta_1}}{\xi_2} - \frac{\eta_0 \xi_1}{\eta_1 \xi_2}\left(\frac{\eta_1 e^{-\xi_2 t} - \xi_2 e^{-\eta_1 t}}{\eta_1 - \xi_2}\right). \tag{S2.24b}$$

Next we compute the response time. To that end, we note that the error signals $E_{R_1} = \overline{R_1} - R_1$ and $E_{R_2} = \overline{R_2} - R_2$ have the Laplace transforms

$$\mathcal{E}_{R_1}[\omega] = \frac{\frac{\eta_0}{\eta_1} - R_1(0)}{\omega + \eta_1}, \tag{S2.25a}$$

$$\mathcal{E}_{R_2}[\omega] = \frac{\frac{\xi_0 + \xi_1 \frac{\eta_0}{\eta_1}}{\xi_2} - R_2(0)}{\omega + \xi_2} + \frac{\xi_1 \left(\frac{\eta_0}{\eta_1} - R_1(0)\right)}{(\omega + \eta_1)(\omega + \xi_2)}. \tag{S2.25b}$$

Using these, the response times are given by

$$\mathcal{T}_{R_1} = -\left.\frac{d\log\left(\mathcal{E}_{R1}[\omega]\right)}{d\omega}\right|_{\omega=0} = \frac{1}{\eta_1}, \qquad \text{[S2.26a]}$$

$$\mathcal{T}_{R_2} = -\left.\frac{d\log\left(\mathcal{E}_{R2}[\omega]\right)}{d\omega}\right|_{\omega=0} = \frac{1}{\xi_2} + \frac{1}{\eta_1}\frac{\left(\frac{\eta_0}{\eta_1} - R_1(0)\right)\xi_1}{\left(\frac{\eta_0}{\eta_1} - R_1(0)\right)\xi_1 + \left(\frac{\xi_0+\xi_1\frac{\eta_0}{\eta_1}}{\xi_2} - R_2(0)\right)\eta_1}. \qquad \text{[S2.26b]}$$

We note that the response time $\mathcal{T}_{R_2}$ depends upon the initial conditions $R_1(0)$ and $R_2(0)$. Recalling the steady-state values of R_1 and R_2 from Eq. (S2.21c), we recognize $\frac{\eta_0}{\eta_1} - R_1(0)$ and $\frac{\xi_0+\xi_1\frac{\eta_0}{\eta_1}}{\xi_2} - R_2(0)$ as $\overline{R_1} - R_1(0)$ and $\overline{R_2} - R_2(0)$, respectively. Thus, if $R_1(0) = \overline{R_1}$, meaning that the upstream component is at its steady-state, then we obtain $\mathcal{T}_{R_2} = 1/\xi_2$. The dependence on $R_1(0)$ and $R_2(0)$ also drops for the special case when $R_1(0) = 0$ and $R_2(0) = \frac{\xi_0}{\xi_2}$. In this case, $\mathcal{T}_{R_2}$ simplifies to

$$\mathcal{T}_{R_2} = \frac{1}{\xi_2} + \frac{\xi_2}{\eta_1+\xi_2}\frac{1}{\eta_1}, \qquad \text{[S2.27]}$$

where the first-term is the response time if R_1 were at steady-state, and the second term is the time-averaged $\mathcal{T}_{R_1}$.

S2-C. Two-tier linear system with two inputs. Next, we consider a variant of the two-tier linear system in Eq. (S2.21). Here the dynamics of R_2 is affected by two upstream components R_{11} and R_{12} as below:

$$\frac{dR_2}{dt} = \xi_0 + \xi_{11}R_{11} + \xi_{12}R_{12} - \xi_2 R_2. \qquad \text{[S2.28]}$$

We do not specify the dynamics of R_{11} and R_{22}, but assume that their Laplace transforms, $\mathcal{R}_{11}[\omega]$ and $\mathcal{R}_{12}[\omega]$ are known. Consequently, we can compute the steady-states, $\overline{R_{11}}$ and $\overline{R_{12}}$, using the final value theorem. Furthermore the Laplace transforms of the error signals may also be computed as

$$\mathcal{E}_{R_{11}}[\omega] = \frac{\overline{R_{11}}}{\omega} - \mathcal{R}_{11}[\omega], \qquad \text{[S2.29a]}$$

$$\mathcal{E}_{R_{12}}[\omega] = \frac{\overline{R_{12}}}{\omega} - \mathcal{R}_{12}[\omega]. \qquad \text{[S2.29b]}$$

Taking Laplace transform of Eq. (S2.28) gives

$$\mathcal{R}_2[\omega] = \frac{\frac{\xi_0}{\omega} + R_2(0)}{\omega+\xi_2} + \frac{\xi_{11}}{\omega+\xi_2}\mathcal{R}_{11}[\omega] + \frac{\xi_{12}}{\omega+\xi_2}\mathcal{R}_{12}[\omega]. \qquad \text{[S2.30]}$$

The transient solution may be computed by taking the inverse Laplace transform of the above equation once $\mathcal{R}_{11}[\omega]$ and $\mathcal{R}_{22}[\omega]$ are specified. We can, however, compute a generic form for the the response time of R_2.

To compute the response time of R_2, we first note that the steady-state response $\overline{R_2}$ is given by

$$\overline{R_2} = \frac{\xi_0 + \xi_{11}\overline{R_{11}} + \xi_{12}\overline{R_{12}}}{\xi_2}. \qquad \text{[S2.31]}$$

Therefore, the Laplace transform of the error signal may be computed as

$$\mathcal{E}_{R_2}[\omega] = \frac{\overline{R_2}}{\omega} - \mathcal{R}_2[\omega] \qquad \text{[S2.32a]}$$

$$= \frac{\frac{\xi_0+\xi_{11}\overline{R_{11}}+\xi_{12}\overline{R_{12}}}{\xi_2}}{\omega} - \frac{\frac{\xi_0}{\omega}+R_2(0)}{\omega+\xi_2} - \frac{\xi_{11}}{\omega+\xi_2}\mathcal{R}_{11}[\omega] - \frac{\xi_{12}}{\omega+\xi_2}\mathcal{R}_{12}[\omega] \qquad \text{[S2.32b]}$$

$$= \frac{\frac{\xi_0}{\xi_2} - R_2(0)}{\omega+\xi_2} + \frac{\frac{\xi_{11}}{\xi_2}\overline{R_{11}} + \xi_{11}\mathcal{E}_{R_{11}}[\omega]}{\omega+\xi_2} + \frac{\frac{\xi_{12}}{\xi_2}\overline{R_{12}} + \xi_{12}\mathcal{E}_{R_{12}}[\omega]}{\omega+\xi_2}, \qquad \text{[S2.32c]}$$

$$= \frac{\overline{R_2} - R_2(0)}{\omega+\xi_2} + \frac{\xi_{11}\mathcal{E}_{R_{11}}[\omega]}{\omega+\xi_2} + \frac{\xi_{12}\mathcal{E}_{R_{12}}[\omega]}{\omega+\xi_2}, \qquad \text{[S2.32d]}$$

where we have used Eq. (S2.29) to substitute for $\mathcal{R}_{11}[\omega]$ and $\mathcal{R}_{12}[\omega]$. The response time is then given by

$$\mathcal{T}_{R_2} = -\left.\frac{d\log\left(\mathcal{E}_{R2}[\omega]\right)}{d\omega}\right|_{\omega=0} \qquad \text{[S2.33a]}$$

$$= \left.\frac{1}{\omega+\xi_2}\right|_{\omega=0} - \left.\frac{\xi_{11}\frac{d\mathcal{E}_{R11}[\omega]}{d\omega} + \xi_{12}\frac{d\mathcal{E}_{R12}[\omega]}{d\omega}}{\overline{R_2} - R_2(0) + \xi_{11}\mathcal{E}_{R11}[\omega] + \xi_{12}\mathcal{E}_{R12}[\omega]}\right|_{\omega=0} \qquad \text{[S2.33b]}$$

$$= \frac{1}{\xi_2} - \frac{\xi_{11}\left.\frac{d\mathcal{E}_{R11}[\omega]}{d\omega}\right|_{\omega=0} + \xi_{12}\left.\frac{d\mathcal{E}_{R12}[\omega]}{d\omega}\right|_{\omega=0}}{\overline{R_2} - R_2(0) + \xi_{11}\mathcal{E}_{R11}[0] + \xi_{12}\mathcal{E}_{R12}[0]} \qquad \text{[S2.33c]}$$

$$= \frac{1}{\xi_2} + \mathcal{T}_{R_{11}}\frac{\xi_{11}\mathcal{E}_{R11}[0]}{\overline{R_2} - R_2(0) + \xi_{11}\mathcal{E}_{R11}[0] + \xi_{12}\mathcal{E}_{R12}[0]} + \mathcal{T}_{R_{12}}\frac{\xi_{12}\mathcal{E}_{R12}[0]}{\overline{R_2} - R_2(0) + \xi_{11}\mathcal{E}_{R11}[0] + \xi_{12}\mathcal{E}_{R12}[0]}. \qquad \text{[S2.33d]}$$

If $R_{11}(0) = \overline{R_{11}}$, then $\mathcal{E}_{R_{11}}[0] = 0$. Likewise, if $R_{12}(0) = \overline{R_{12}}$ implies $\mathcal{E}_{R_{12}}[0] = 0$. Thus, if R_{11} and R_{12} are at steady-state then the response time is given by $1/\xi_2$. The second and the third terms in the above expression are the time-averaging terms.

S3. Transient solution and response time for molecular switch architectures

In this section, we consider two-tier cascades of Fig. 1 in the main text. Because activation and derepression are special cases of the concerted mechanism, we concern ourselves only with the ODEs of a concerted mechanism here.

The ordinary differential equations (ODEs) that govern the dynamics are

$$\frac{dX^*}{dt} = k_1 S(X_T - X^*) - k_2 X^*, \tag{S3.1a}$$

$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6 (X_T - X^*)) Y^*, \tag{S3.1b}$$

with initial conditions

$$X^*(0) = 0, \quad Y^*(0) = \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}. \tag{S3.1c}$$

The steady-states of X^* and Y^* are computed by setting the derivatives to zero.

$$\overline{X^*} = \frac{k_1 S X_T}{k_1 S + k_2}, \quad \overline{Y^*} = \frac{k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}} Y_T. \tag{S3.2}$$

Recall that plugging $k_6 = 0$ and $k_5 = 0$, result in ODEs for the activation and derepression mechanisms, respectively. Furthermore, we term the special case $k_5 = k_6$ as perfect concerted mechanism, where the activation and repression strengths match.

Analytical solutions for nonlinear ODEs such as those in Eq. (S3.1) typically do not exist. However, a careful look at Eq. (S3.1) shows that the nonlinear term is $(k_5 - k_6) X^* Y^*$. Thus for a special case when $k_5 = k_6$ (perfect concerted mechanism), the system is linear, which has an analytical solution. The solutions for other cases can be computed numerically. We also provide an approximate solution using linearization around the steady-state solution $(\overline{X^*}, \overline{Y^*})$.

S3-A. Transient solution for a perfect concerted model. A perfect concerted model is characterized by $k_5 = k_6$. Substituting $k_5 = k_6$ in Eq. (S3.1) results in

$$\frac{dX^*}{dt} = k_1 S X_T - (k_1 S + k_2) X^*, \tag{S3.3a}$$

$$\frac{dY^*}{dt} = k_3 Y_T + k_6 Y_T X^* - (k_3 + k_4 + k_6 X_T) Y^*, \tag{S3.3b}$$

with initial condition $(X^*(0), Y^*(0)) = \left(0, \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}\right)$. We note that the form of Eq. (S3.3) is same as that of Eq. (S2.21), with parameters $\eta_0 = k_1 S X_T$, $\eta_1 = k_1 S + k_2$, $\xi_0 = k_3 Y_T$, $\xi_1 = k_6 Y_T$, and $\xi_2 = k_3 + k_4 + k_6 X_T$. Thus, we can use Eq. (S2.24) to get the transient solution

$$X^*(t) = \frac{k_1 S X_T}{k_1 S + k_2} \left(1 - e^{-(k_1 S + k_2) t}\right), \tag{S3.4a}$$

$$Y^*(t) = \frac{k_3 + k_6 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T} Y_T - \frac{k_6 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T} Y_T \frac{(k_1 S + k_2) e^{-(k_3 + k_4 + k_6 X_T) t} - (k_3 + k_4 + k_6 X_T) e^{-(k_1 S + k_2) t}}{k_1 S + k_2 - (k_3 + k_4 + k_6 X_T)}. \tag{S3.4b}$$

For the special case when $k_1 S + k_2 = k_3 + k_4 + k_6 X_T$, we have

$$Y^*(t) = \frac{k_3 + k_6 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T} Y_T - \frac{k_6 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T} Y_T e^{-(k_3 + k_4 + k_6 X_T) t} \left(1 + (k_3 + k_4 + k_6 X_T) t\right). \tag{S3.4c}$$

S3-B. Approximate transient solution using linearization. The ODE system in Eq. (S3.1) contains the nonlinear term $X^* Y^*$, which can be linearized around the steady-state solution $(\overline{X^*}, \overline{Y^*})$ as

$$X^* Y^* \approx \overline{Y^*} X^* + \overline{X^*} Y^* - \overline{X^*}\, \overline{Y^*}, \tag{S3.5a}$$

where

$$\overline{X^*} = \frac{k_1 S X_T}{k_1 S + k_2}, \tag{S3.5b}$$

$$\overline{Y^*} = \frac{k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T + (k_5 - k_6) \frac{k_1 S X_T}{k_1 S + k_2}} Y_T. \tag{S3.5c}$$

Substituting this for the nonlinear term in Eq. (S3.1), we get the following

$$\frac{dX^*}{dt} = k_1 S X_T - (k_1 S + k_2)X^*, \tag{S3.6a}$$

$$\frac{dY^*}{dt} = \left(k_3 Y_T + (k_5 - k_6)\overline{X^*}\,\overline{Y^*}\right) + \left(k_5 Y_T - (k_5 - k_6)\overline{Y^*}\right) X^* - (k_3 + k_4 + k_6 X_T + (k_5 - k_6)\overline{X^*})Y^*. \tag{S3.6b}$$

These ODEs are similar to those in Eq. (S2.21). The parameters are: $\eta_0 = k_1 S X_T$, $\eta_1 = k_1 S + k_2$, $\xi_0 = k_3 Y_T + (k_5 - k_6)\overline{X^*}\,\overline{Y^*}$, $\xi_1 = k_5 Y_T - (k_5 - k_6)\overline{Y^*}$, and $\xi_2 = k_3 + k_4 + k_6 X_T + (k_5 - k_6)\overline{X^*}$. With the initial conditions $(X^*(0), Y^*(0)) = \left(0, \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}\right)$, the solution same as that in Eq. (S2.24) and is given by.

$$X^*(t) = \frac{k_1 S X_T}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right), \tag{S3.7a}$$

$$Y^*(t) = \frac{k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}}{k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}} Y_T - \frac{k_1 S X_T}{k_1 S + k_2} \frac{(k_4 k_5 + k_3 k_6 + k_5 k_6 X_T) Y_T}{\left(k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}\right)^2} \times$$
$$\frac{(k_1 S + k_2) e^{-\left(k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}\right)t} - \left(k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}\right) e^{-(k_1 S + k_2)t}}{k_1 S + k_2 - \left(k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}\right)}. \tag{S3.7b}$$

The special case when the timescales match may be computed by taking the limit of the above solution.

S3-C. Response time for a perfect concerted mechanism. For this case, we can simply adapt the results of Eq. (S2.26a) and Eq. (S2.27).

$$\mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2}, \tag{S3.8a}$$

$$\mathcal{T}_{Y^*} = \frac{1}{k_3 + k_4 + k_6 X_T} + \frac{1}{k_1 S + k_2} \times \frac{k_3 + k_4 + k_6 X_T}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T}. \tag{S3.8b}$$

S3-D. Response time for the linear approximation. As with the response time for the perfect concerted mechanism, here too we adapt the results of Eq. (S2.26a) and Eq. (S2.27).

$$\mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2}, \tag{S3.9a}$$

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}} + \frac{1}{k_1 S + k_2} \times \frac{k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}}. \tag{S3.9b}$$

How good is the above approximation of response time? One check is to plug in $k_5 = k_6$ to obtain the approximation for the perfect concerted model for which we have the exact expression of the response time in Eq. (S3.8). Indeed, substituting $k_5 = k_6$ in Eq. (S3.9) yields

$$\mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2}, \tag{S3.10a}$$

$$\mathcal{T}_{Y^*} = \frac{1}{k_3 + k_4 + k_6 X_T} + \frac{1}{k_1 S + k_2} \times \frac{k_3 + k_4 + k_6 X_T}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T}, \tag{S3.10b}$$

which is exactly same as Eq. (S3.8). Thus the linear approximation is exact for the perfect concerted model. This is not surprising because the perfect concerted model is linear by construction.A second check of how good the approximation in Eq. (S3.9) is through numerical computation, which is discussed in a later section.

S3-E. Response time for ratiometric signaling. Ratiometric signaling is the special case where the signaling output does not depend upon the total number of receptors X_T. In Eq. (5) of the main text, we show that when $k_3 = 0$ and $k_4 = 0$, then the steady-state response is independent of X_T. Here we ask whether setting $k_3 = 0$ and $k_4 = 0$ also result in the response time being independent from X_T. To this end, we plug these values in the expression of $\mathcal{T}_{Y^*}$ in Eq. (S3.9):

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}} + \frac{1}{k_1 S + k_2} \times \frac{k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}}{k_1 S + k_2 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}}. \tag{S3.11}$$

Clearly, the response time depends upon X_T, thereby establishing that the ratiometric signaling is only applicable for the dose-response. We further ask how X_T affects the response time. To this end, the most convenient limit to check is when the receptor dynamics is fast, i.e., $k_1 S + k_2 \gg k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}$, which gives us

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k_2}}. \tag{S3.12}$$

Thus, if everything else is constant then increasing X_T decreases the response time. Even when the receptor dynamics is not fast, we can verify this effect by looking at the sign of the derivative of $\mathcal{T}_{Y^*}$ with respect to X^*

$$\frac{d\mathcal{T}_{Y^*}}{dX_T} = -\frac{(k_1S+k_2)^5 + 2(k_1S+k_2)^3(k_2k_6X_T + k_1Sk_5X_T)}{X_T(k_2k_6X_T + k_1Sk_5X_T)\left((k_1S+k_2)^2 + k_2k_6X_T + k_1Sk_5X_T\right)^2} < 0. \quad \text{[S3.13]}$$

Next, we discuss the numerical method to compute response time which we use to validate our approximations.

S3-F. Numerical computation of the response time. One convenience in using the center of mass definition of the response time

$$\mathcal{T}_{Y^*} = \frac{\int_0^\infty t\left|\overline{Y^*} - Y^*(t)\right| dt}{\int_0^\infty \left|\overline{Y^*} - Y^*(t)\right| dt} \quad \text{[S3.14]}$$

is that it can be computer numerically via solution of an augmented ODE system

$$\frac{dX^*}{dt} = k_1S(X_T - X^*) - k_2X^*, \quad \text{[S3.15a]}$$

$$\frac{dY^*}{dt} = (k_3 + k_5X^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X^*))Y^*, \quad \text{[S3.15b]}$$

$$\frac{dV_1}{dt} = \left|\overline{Y^*} - Y^*\right|, \quad \text{[S3.15c]}$$

$$\frac{dV_2}{dt} = 1, \quad \text{[S3.15d]}$$

$$\frac{dV_3}{dt} = V_1V_2. \quad \text{[S3.15e]}$$

Here $V_1(t)$, $V_2(t)$ and $V_3(t)$ are the augmented states to the original ODE system. The initial conditions are given by

$$(X^*(0), Y^*(0), V_1(0), V_2(0), V_3(0)) = \left(0, \frac{k_3Y_T}{k_3+k_4+k_6X_T}, \frac{\left(k_3 + k_5\frac{k_1SX_T}{k_1S+k_2}\right)Y_T}{k_3+k_4+k_5\frac{k_1SX_T}{k_1S+k_2} + k_6\frac{k_2X_T}{k_1S+k_2}} - \frac{k_3Y_T}{k_3+k_4+k_6X_T}, 0, 0\right). \quad \text{[S3.15f]}$$

Note that the state $V_1(t)$ computes the integral in the denominator upto a time horizon t, $V_2(t)$ tracks the time, and $V_3(t)$ computes the numerator up to time horizon t. If we choose t to be large enough such that the system has reached steady-state, then $\frac{V_3(t)}{V_1(t)}$ computes the response time. It is easy to see that the approximation gets better with a larger t. We can use the approximation of response time in Eq. (S3.9) to set a time for the integration.

S3-G. Validity of linear approximation. Except for the case of the perfect concerted mechanism for which the nonlinearity in the dynamics of Y^* drops out, we have to approximate the dynamics of our ODE system. We have relied upon linearization of the dynamics around the steady-state to obtain an approximate transient solution as well as analytically tractable results on response time. Strictly speaking, linear approximation of a dynamical system is only valid in a small neighborhood around the point of linearization (the steady-state solution in our case). Therefore, our approximations of the response times are technically valid for small perturbations around steady-states. Thus, it is imperative that we validate our results using computer simulations.

Our results on response time in the Fig. 3 of the main text show that the analytical results are reasonably accurate even for activation and derepression mechanisms. The quality of approximation is excellent in the regime where receptor dynamics is much faster than the switch dynamics and deteriorates as the receptor dynamics becomes slow. This could be explained by the fact that when receptor dynamics is much faster, we can assume that Y^* only see a constant $X^* = \overline{X^*}$. The dynamics of Y^* thus becomes linear in this limit, providing accurate match between our approximation and the numerical results.

S3-H. A comment on parameter choice for Fig. 3. We note that both steady-state response and the response time may be decoupled in the case of the receptor. Specifically, the response time is $1/(k_1S+k_2)$ whereas the steady-state receptor occupancy is $k_1SX_T/(k_1S+k_2)$. Thus, it is possible to maintain same steady-state fractional occupancy of the receptor by maintaining k_1S/k_2 while varying k_2 to change the response time. We exploit this feature to mathematically control the comparison of different signaling mechanisms.

S4. Stochastic analysis of two-tier cascades

Here we consider a two-tier model for signal transduction as described in Table 1 in the main text. Let $P_{m,n}(t)$ denote the probability of finding m molecules of X^* and n molecules of Y^* at time t. Then, we can write the chemical master equation (CME) that describes the time evolution of $P_{m,n}$

$$\begin{aligned}\frac{dP_{m,n}(t)}{dt} &= k_1S(X_T-(m-1))P_{m-1,n} + k_2(m+1)P_{m+1,n} + k_3(Y_T-(n-1))P_{m,n-1} \\ &+ k_5m(Y_T-(n-1))P_{m,n-1} + k_4(n+1)P_{m,n+1} + k_6(X_T-m)(n+1)P_{m,n+1} \\ &- \left(k_1S(X_T-m) + k_2m + k_3(Y_T-n) + k_5m(Y_T-n) + k_4n + k_6(X_T-m)n\right)P_{m,n},\end{aligned} \quad \text{[S4.1]}$$

where $m = 0, 1, \ldots, X_T$ and $n = 0, \ldots, Y_T$ (3, 4). It is often difficult to analytically solve the CME. Because the dynamics of X^* is linear and it does not depend upon Y^*, it is possible to provide an analytical solution P_m. As for $P_{m,n}$, we only provide approximate and exact computations of its first two moments.

S4-A. Stochastic solution to receptor dynamics. The CME that governs the time evolution of $P_m(t)$ is:

$$\frac{dP_m(t)}{dt} = k_1 S\left(X_T - (m-1)\right) P_{m-1}(t) + k_2(m+1)P_{m+1}(t) - \left(k_1 S\left(X_T - m\right) + k_2 m\right) P_m(t). \qquad \text{[S4.2]}$$

We define a generating function

$$G(z) = \sum_{m=0}^{\infty} z^m P_m, \quad |z| \leq 1 \qquad \text{[S4.3]}$$

to solve Eq. (S4.2). Multiplying both sides by z^m and summing over m yields

$$\frac{\partial G}{\partial t} = k_1 S X_T \sum_{m=0}^{\infty} z^m P_{m-1} - k_1 S \sum_{m=0}^{\infty} z^m \left(m-1\right) P_{m-1} + k_2 \sum_{m=0}^{\infty} z^m \left(m+1\right) P_{m+1} - k_1 S X_T \sum_{m=0}^{\infty} z^m P_m + \left(k_1 S - k_2\right) \sum_{m=0}^{\infty} z^m m P_m. \qquad \text{[S4.4]}$$

The above equation becomes the following partial differential equation (PDE)

$$\frac{\partial G}{\partial t} = k_1 S X_T (z-1) G + \left(-k_1 S z^2 + k_2 + \left(k_1 S - k_2\right) z\right) \frac{\partial G}{\partial z}. \qquad \text{[S4.5]}$$

We solve this PDE using method of characteristics, assuming the initial condition $G(z, 0) = 1$ which corresponds to 0 molecules of X^*. The solution is given by

$$G(z,t) = \left(1 - \frac{k_1 S}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right) + \frac{k_1 S}{k_1 S + k_2} z \left(1 - e^{-(k_1 S + k_2)t}\right)\right)^{X_T}. \qquad \text{[S4.6]}$$

Using Binomial theorem, the above expression can be written as

$$G(z,t) = \sum_{m=0}^{X_T} \binom{X_T}{m} \left(\frac{k_1 S}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right)\right)^m \left(1 - \frac{k_1 S}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right)\right)^{X_T - m} z^m. \qquad \text{[S4.7]}$$

The probability $P_m(t)$ is given by the coefficient of z^m

$$P_m(t) = \binom{X_T}{m} \left(\frac{k_1 S}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right)\right)^m \left(1 - \frac{k_1 S}{k_1 S + k_2}\left(1 - e^{-(k_1 S + k_2)t}\right)\right)^{X_T - m}. \qquad \text{[S4.8]}$$

The stationary distribution $\overline{P_m}$ is computed by taking limit $t \to \infty$

$$\overline{P_m} = \binom{X_T}{m} \left(\frac{k_1 S}{k_1 S + k_2}\right)^m \left(1 - \frac{k_1 S}{k_1 S + k_2}\right)^{X_T - m}, \qquad \text{[S4.9]}$$

which is a Binomial distribution with parameters X_T and $\frac{k_1 S}{k_1 S + k_2}$ (5). The stationary moments of this distribution are given by

$$\langle X^* \rangle = \frac{k_1 S X_T}{k_1 S + k_2}, \qquad \text{[S4.10a]}$$

$$\left\langle X^{*2} \right\rangle - \langle X^* \rangle^2 = \frac{k_1 S k_2 X_T}{(k_1 S + k_2)^2}, \qquad \text{[S4.10b]}$$

$$CV_{X^*}^2 = \frac{\left\langle X^{*2} \right\rangle - \langle X^* \rangle^2}{\langle X^* \rangle^2} = \frac{k_2}{k_1 S X_T}. \qquad \text{[S4.10c]}$$

S4-B. Moment dynamics. We are specifically concerned with moments of the two-tier model. To this end, we take the well-established approach of using the ODEs that govern the moment dynamics (e.g., see (6, 7)). A generic moment may be written as

$$\begin{aligned}\frac{d\left\langle X^{*m_1} Y^{*m_2}\right\rangle}{dt} = {} & \left\langle k_1 S (X_T - X^*) \left((X^* + 1)^{m_1} Y^{*m_2} - X^{*m_1} Y^{*m_2}\right)\right\rangle + \left\langle k_2 X^* \left((X^* - 1)^{m_1} Y^{*m_2} - X^{*m_1} Y^{*m_2}\right)\right\rangle \\ & + \left\langle \left(k_3 + k_5 X^*\right)\left(Y_T - Y^*\right)\left(X^{*m_1}(Y^* + 1)^{m_2} - X^{*m_1} Y^{*m_2}\right)\right\rangle \\ & + \left\langle \left(k_4 + k_6 (X_T - X^*)\right) Y^* \left(X^{*m_1}(Y^* - 1)^{m_2} - X^{*m_1} Y^{*m_2}\right)\right\rangle. \end{aligned} \qquad \text{[S4.11]}$$

Here we have used $\langle . \rangle$ to denote the expected value of a random variable. Our focus in this work is to compute the first two moments in steady-state. However, due to the nonlinearity $X^* Y^*$ in these equations, the moment dynamics is not closed in that a lower-order moment depends upon a higher-order moment (6–8). It turns out that for the special case $k_5 = k_6$ (perfect concerted model), the moments may be computed exactly. We provide approximate formulas for moments using a linear approximation when $k_5 \neq k_6$.

S4–B–a. Moment computation for a perfect concerted model. For the concerted model, $k_5 = k_6$. Let us write moment dynamics for first two moments.

$$\frac{d\langle X^*\rangle}{dt} = k_1 S X_T - (k_1 S + k_2)\langle X^*\rangle, \tag{S4.12a}$$
$$\frac{d\langle Y^*\rangle}{dt} = k_3 Y_T + k_6 Y_T\langle X^*\rangle - (k_3 + k_4 + k_6 X_T)\langle Y^*\rangle, \tag{S4.12b}$$
$$\frac{d\left\langle X^{*2}\right\rangle}{dt} = k_1 S X_T + (k_1 S(2X_T - 1) + k_2)\langle X^*\rangle - 2(k_1 S + k_2)\left\langle X^{*2}\right\rangle, \tag{S4.12c}$$
$$\frac{d\langle X^*Y^*\rangle}{dt} = k_3 Y_T\langle X^*\rangle + k_1 S X_T\langle Y^*\rangle + k_6 Y_T\left\langle X^{*2}\right\rangle - (k_1 S + k_2 + k_3 + k_4 + k_6 X_T)\langle X^*Y^*\rangle, \tag{S4.12d}$$
$$\frac{d\left\langle Y^{*2}\right\rangle}{dt} = k_3 Y_T + k_6 Y_T\langle X^*\rangle + (k_3(2Y_T - 1) + k_4 + k_6 X_T)\langle Y^*\rangle + 2k_6(Y_T - 1)\langle X^*Y^*\rangle - 2(k_3 + k_4 + k_6 X_T)\left\langle Y^{*2}\right\rangle. \tag{S4.12e}$$

We can solve for steady-state moments by setting each of the derivatives equal to zero. For example, the means are given by

$$\langle X^*\rangle = \frac{k_1 S}{k_1 S + k_2} X_T, \tag{S4.13a}$$
$$\langle Y^*\rangle = \frac{k_3 + k_6\langle X^*\rangle}{k_3 + k_4 + k_6 X_T} Y_T = \frac{k_3 + k_6\frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T. \tag{S4.13b}$$

Next, we compute second order moments. $\left\langle X^{*2}\right\rangle$ is given by

$$\left\langle X^{*2}\right\rangle = \left(\frac{k_1 S}{k_1 S + k_2} X_T\right)^2 + \frac{k_1 S k_2 X_T}{(k_1 S + k_2)^2}, \tag{S4.14}$$

where the first term is $\langle X^*\rangle^2$. The cross moment $\langle X^*Y^*\rangle$ is

$$\langle X^*Y^*\rangle = \frac{k_1 S k_2 k_6 X_T Y_T}{(k_1 S + k_2)^2(k_1 S + k_2 + k_3 + k_4 + k_6 X_T)} + \left(\frac{k_1 S}{k_1 S + k_2} X_T\right)\left(\frac{k_3 + k_6\frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T\right). \tag{S4.15}$$

Here the second term is $\langle X^*\rangle\langle Y^*\rangle$. Finally, the second order moment $\left\langle Y^{*2}\right\rangle$ in terms of the other moments is

$$\left\langle Y^{*2}\right\rangle = \frac{k_3 Y_T}{2(k_3 + k_4 + k_6 X_T)} + \frac{k_6 Y_T\langle X^*\rangle}{2(k_3 + k_4 + k_6 X_T)} + \frac{(k_3(2Y_T - 1) + k_4 + k_6 X_T)\langle Y^*\rangle}{2(k_3 + k_4 + k_6 X_T)} + \frac{2k_6(Y_T - 1))\langle X^*Y^*\rangle}{2(k_3 + k_4 + k_6 X_T)} \tag{S4.16a}$$
$$= \frac{k_3 Y_T + k_4 + k_6 X_T}{k_3 + k_4 + k_6 X_T}\langle Y^*\rangle + \frac{k_6(Y_T - 1)\langle X^*Y^*\rangle}{k_3 + k_4 + k_6 X_T}. \tag{S4.16b}$$

Using the moments computed above, we can compute the centered moments. For example, the variance of X^* is

$$\left\langle X^{*2}\right\rangle - \langle X^*\rangle^2 = \frac{k_1 S k_2 X_T}{(k_1 S + k_2)^2}, \tag{S4.17}$$

the centered cross moment is

$$\langle X^*Y^*\rangle - \langle X^*\rangle\langle Y^*\rangle = \frac{k_1 S k_2 k_6 X_T Y_T}{(k_1 S + k_2)^2(k_1 S + k_2 + k_3 + k_4 + k_6 X_T)}, \tag{S4.18}$$

and the variance of Y^* is

$$\left\langle Y^{*2}\right\rangle - \langle Y^*\rangle^2 = \left(\frac{k_3 Y_T + k_4 + k_6 X_T}{k_3 + k_4 + k_6 X_T}\right)\left(\frac{k_3 + k_6\frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T\right) + \frac{k_6(Y_T - 1)}{k_3 + k_4 + k_6 X_T}\times$$
$$\left(\frac{k_1 S k_2 k_6 X_T Y_T}{(k_1 S + k_2)^2(k_1 S + k_2 + k_3 + k_4 + k_6 X_T)} + \frac{k_1 S X_T}{k_1 S + k_2}\frac{k_3 + k_6\frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k X_T} Y_T\right) - \left(\frac{k_3 + k_6\frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T\right)^2. \tag{S4.19}$$

We use the centered moments computed above to quantify noise in X^* and Y^* using coefficient of variation squared.

Coefficient of variation squared. Let $CV^2_{X^*}$ and $CV^2_{Y^*}$ respectively are the coefficient of variation squared for X^* and Y^*. Then

$$CV^2_{X^*} = \frac{\left\langle X^{*2}\right\rangle - \left\langle X^*\right\rangle^2}{\left\langle X^*\right\rangle^2} = \frac{k_2}{k_1 S X_T}, \quad \text{[S4.20]}$$

and

$$CV^2_{Y^*} = \frac{\left\langle Y^{*2}\right\rangle - \left\langle Y^*\right\rangle^2}{\left\langle Y^*\right\rangle^2} \quad \text{[S4.21]}$$

$$= \left(\frac{k_3 Y_T + k_4 + k_6 X_T}{k_3 + k_4 + k_6 X_T}\right)\left(\frac{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T\right)\frac{1}{Y_T^2}\left(\frac{k_3 + k_4 + k_6 X_T}{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T}\right)^2$$
$$+ \frac{1}{Y_T^2}\left(\frac{k_3 + k_4 + k_6 X_T}{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T}\right)^2 \frac{k_6(Y_T - 1)}{k_3 + k_4 + k_6 X_T}\left(\frac{k_1 S k_2 k_6 X_T Y_T}{(k_1 S + k_2)^2 (k_1 S + k_2 + k_3 + k_4 + k_6 X_T)}\right)$$
$$+ \frac{1}{Y_T^2}\left(\frac{k_3 + k_4 + k_6 X_T}{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T}\right)^2 \frac{k_6(Y_T - 1)}{k_3 + k_4 + k_6 X_T}\left(\frac{k_1 S X_T}{k_1 S + k_2}\frac{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 X_T} Y_T\right) - 1. \quad \text{[S4.22]}$$

On simplifying, we get

$$CV^2_{Y^*} = \frac{1}{Y_T}\frac{k_3 Y_T + k_4 + k_6 X_T}{k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T} + \frac{Y_T - 1}{Y_T}\frac{k_6\left(k_3 + k_4 + k_6 X_T\right)\left(k_1 S k_2 k_6 X_T\right)}{(k_1 S + k_2)^2\left(k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T\right)^2 \left(k_1 S + k_2 + k_3 + k_4 + k_6 X_T\right)}$$
$$+ \frac{Y_T - 1}{Y_T}\frac{k_6 k_1 S X_T}{\left(k_3 + k_6 \frac{k_1 S}{k_1 S + k_2} X_T\right)\left(k_1 S + k_2\right)}. \quad \text{[S4.23]}$$

Decomposing the coefficient of variation squared into different sources. We expect that $CV^2_{Y^*}$ has two sources of noise: activation/deactivation events for X^* and activation/deactivation events for Y^*. To tease out the contribution from activation/deactivation events for Y^*, we consider a scenario the dynamics of X^* is deterministic. In this case, the moment dynamics is given by

$$\frac{dX^*}{dt} = k_1 S X_T - (k_1 S + k_2) X^*, \quad \text{[S4.24a]}$$
$$\frac{d\left\langle Y^*\right\rangle}{dt} = k_3 Y_T + k_6 Y_T X^* - (k_3 + k_4 + k X_T)\left\langle Y^*\right\rangle, \quad \text{[S4.24b]}$$
$$\frac{d\left\langle Y^{*2}\right\rangle}{dt} = k_3 Y_T + k_6 Y_T X^* + (k_3(2Y_T - 1) + k_4 + k_6 X_T)\left\langle Y^*\right\rangle + 2k_6(Y_T - 1)X^*\left\langle Y^*\right\rangle - 2(k_3 + k_4 + k_6 X_T)\left\langle Y^{*2}\right\rangle. \quad \text{[S4.24c]}$$

The steady-state solution for the coefficient of variation squared computed from these equations is given by

$$CV^2_{Y^*}\Big|_{act./deact.} = \frac{1}{Y_T}\frac{k_4 + k_6 X_T \frac{k_2}{k_1 S + k_2}}{k_3 + k_6 X_T \frac{k_2}{k_1 S + k_2}}. \quad \text{[S4.25]}$$

We do not provide detailed calculations here. One sanity check is that this expression is consistent with coefficient of variation squared for a binomial distribution, which is expected if X^* were constant.

Subtracting Eq. (S4.25) from Eq. (S4.23), we obtain the contribution of noise in X^* to noise in Y^*:

$$CV^2_{Y^*} - CV^2_{Y^*}\Big|_{act./deact.} = \frac{Y_T - 1}{Y_T}\frac{(k_3 + k_4 + k_6 X_T)(k_1 S k_2 k^2 X_T)}{(k_1 S + k_2 + k_3 + k_4 + k_6 X_T)(k_1 S + k_2)^2\left(k_3 + k_6 X_T \frac{k_1 S}{k_1 S + k_2}\right)^2}. \quad \text{[S4.26]}$$

We expect that the term on the right hand side should have contribution from $CV^2_{X^*}$, which is time-averaged. Recall Eq. (S3.8) that $k_1 S + k_2$ is response time of the receptor and that $k_3 + k_4 + k_6 X_T$ is response time of the switch if the receptor dynamics is fast. Thus, $\frac{k_3 + k_4 + k_6 X_T}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T}$ can be interpreted as the timescale averaging. Therefore, we write

$$CV^2_{Y^*} = \underbrace{\frac{1}{Y_T}\frac{k_4 + k_6 X_T \frac{k_2}{k_1 S + k_2}}{k_3 + k_6 X_T \frac{k_1 S}{k_1 S + k_2}}}_{\text{contribution from act./deact. of } Y^*} + \underbrace{\frac{k_3 + k_4 + k_6 X_T}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T}}_{\text{time-averaging}}\underbrace{\frac{Y_T - 1}{Y_T}\left(\frac{k_6 X_T \frac{k_1 S}{k_1 S + k_2}}{k_3 + k_6 X_T \frac{k_1 S}{k_1 S + k_2}}\right)^2}_{\text{coupling}} CV^2_{X^*}, \quad \text{[S4.27]}$$

where $CV^2_{X^*} = \frac{k_2}{k_1 S X_T}$.

S4–B–b. Approximate moment dynamics using linear approximation. As discussed earlier, the moment dynamics is not closed when $k_5 - k_6$ is non-zero. To estimate moments, we first linearize the nonlinear term around the solution of the deterministic model (9). Let (X^*_{det}, Y^*_{det}) be solution to the ODE model

$$\frac{dX^*_{det}}{dt} = k_1 S X_T - (k_1 S + k_2) X^*_{det}, \tag{S4.28a}$$

$$\frac{dY^*_{det}}{dt} = k_3 Y_T + k_5 Y_T X^*_{det} - (k_3 + k_4 + k_6 X^*_{det}) Y^*_{det} - (k_5 - k_6) X^*_{det} Y^*_{det}. \tag{S4.28b}$$

The stochastic model with linearized transition rates is shown in Table S1.

Event	Update	Transition rate
$X \to X^*$	$X^* \mapsto X^* + 1$	$k_1 S (X_T - X^*)$
$X^* \to X$	$X^* \mapsto X^* - 1$	$k_2 X^*$
$Y \to Y^*$	$Y^* \mapsto Y^* + 1$	$(k_3 Y_T + k_5 Y_T X^*) - k_3 Y^* - k_5 \left(Y^*_{det} X^* + X^*_{det} Y^* - X^*_{det} Y^*_{det}\right)$
$Y^* \to Y$	$Y^* \mapsto Y^* - 1$	$(k_4 + k_6 X_T) Y^* - k_6 \left(Y^*_{det} X^* + X^*_{det} Y^* - X^*_{det} Y^*_{det}\right)$

Table S1. Transitions and associated rates for the stochastic model.

The second order moments with the above linearized propensity model satisfy the following differential equations

$$\frac{d\left\langle X^{*2}\right\rangle}{dt} = k_1 S X_T + (2k_1 S X_T - k_1 S + k_2) X^*_{det} - 2(k_1 S + k_2)\left\langle X^{*2}\right\rangle, \tag{S4.29a}$$

$$\begin{aligned}\frac{d\left\langle X^* Y^*\right\rangle}{dt} &= (k_3 Y_T + k_5 X^*_{det} Y^*_{det} - k_6 X^*_{det} Y^*_{det}) X^*_{det} + k_1 S X_T Y^*_{det} + (k_5 Y_T - k_5 Y^*_{det} + k_6 Y^*_{det})\left\langle X^{*2}\right\rangle \\ &- (k_1 S + k_2 + k_3 + k_4 + k_5 X^*_{det} + k_6 X_T - k_6 X^*_{det})\left\langle X^* Y^*\right\rangle,\end{aligned} \tag{S4.29b}$$

$$\begin{aligned}\frac{d\left\langle Y^{*2}\right\rangle}{dt} &= k_3 Y_T + k_5 X^*_{det} Y^*_{det} + k_6 X^*_{det} Y^*_{det} + (k_5 Y_T - k_5 Y^*_{det} - k_6 Y^*_{det}) X^*_{det} \\ &+ (2k_3 Y_T - k_3 + k_4 + 2k_5 X^*_{det} Y^*_{det} - k_5 X^*_{det} + k_6 X_T - k_6 X^*_{det}) Y^*_{det} + 2(k_5 Y_T - k_5 Y^*_{det} + k_6 Y^*_{det})\left\langle X^* Y^*\right\rangle \\ &- 2(k_3 + k_4 + k_5 X^*_{det} + k_6 X_T - k_6 X^*_{det})\left\langle Y^{*2}\right\rangle.\end{aligned} \tag{S4.29c}$$

Computing these moment equations, along with the solutions to the deterministic dynamics, approximates the moments. Using a symbolic solver to solve for moments in steady-state, we get the following for the coefficient of variation of X^*.

$$CV^2_{X^*} = \frac{k_2}{k_1 S X_T}. \tag{S4.30}$$

The formula for $CV^2_{Y^*}$ can be obtained in the same manner as done for the perfect concerted model and is given by

$$\begin{aligned} CV^2_{Y^*} \approx & \underbrace{\frac{1}{Y_T}\frac{k_4 + k_6 X_T \frac{k_2}{k_1 S + k_2}}{k_3 + k_5 X_T \frac{k_1 S}{k_1 S + k_2}}}_{\text{contribution from act./deact. of } Y^*} + \\ & \underbrace{\frac{k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}}{k_1 S + k_2 + k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}}}_{\text{time-averaging}} \times \underbrace{\frac{\left(\frac{k_1 S X_T}{k_1 S + k_2}\right)^2 (k_4 k_5 + k_6 (k_3 + k_5 X_T))^2}{\left(k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}\right)^2 \left(k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}\right)^2}}_{\text{coupling}} CV^2_{X^*}. \end{aligned} \tag{S4.31}$$

Because we already have exact moment formulas when $k_5 = k_6$, we can immediately check the validity of linear approximation for that case. Plugging $k_5 = k_6$ shows that the noise approximation above differs from Eq. (S4.27) by a factor $(Y_T - 1)/Y_T$ that multiplies $CV^2_{Y^*}$. Typically $(Y_T - 1)/Y_T \approx 1$ for large Y_T, indicating that our linear approximation is reasonably good for a concerted model.

S4–B–c. Coefficient variation squared for ratiometric signaling. For ratiometric signaling, in which the steady-state response does not depend upon the total number of receptors X_T, we need $k_3 = 0$ and $k_4 = 0$. Substituting these in the expression of $CV^2_{Y^*}$ in Eq. (S4.31), we get

$$CV^2_{Y^*} \approx \frac{1}{Y_T}\frac{k_2 k_6}{k_1 S k_5} + \frac{k_6^2}{\left(k_1 S + k_2 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}\right)\left(\frac{k_1 S k_5}{k_1 S + k_2} + \frac{k_2 k_6}{k_1 S + k_2}\right)}\frac{k_2}{k_1 S}. \tag{S4.32}$$

Thus, increasing X_T decreases overall noise because X_T increases the denominator terms in the above above formula. Next, we provide exact computation of moments using a semi-analytical approach.

S4–B–d. Accuracy of linear approximation. As mentioned earlier, the moment dynamics in Eq. (S4.11) is not *closed*, as in a lower-order moment depends on a higher-order moment, except for the case of a perfect concerted model. There are several techniques that approximate the moments for such systems, linearization being one of them. We have chosen linearization here because of the analytical tractability it provides. Determining the accuracy of approximations obtained through this technique as well as other moment closure techniques is beyond the scope of this paper and has been dealt elsewhere, e.g., see (7, 8). We rely on validating the approximations using exact computations made possible by employing a slightly different formulation described below.

S4-C. A comment on parameter choice for Fig. 4. When comparing the noise properties, we mathematically control for the mean response. Usually these two quantities are coupled. However, we exploit the fact that we are able to decompose the noise in Y^* into different sources of noise: noise in activation/deactivation of Y^*, time-averaging of noise in X^* and how strongly Y^* is coupled of X^*. Thus, we are able to independently tune time-averaging and the coupling to gain insights into how the noise in X^* transmits to Y^*.

S4-D. Exact moment computation. Our goal here is to compute the first two moments of Y^*. As discussed earlier, a moment of lower order depends upon moments of higher order, resulting in the problem of moment closure. Here, we exploit the fact that X_T is finite to come up with an alternate state space where moment dynamics is closed. The computations follow the formalism proposed in (10). Another closely related method is the method of conditional moments described in (11).

Let us define indicator variables $b_i, i = 0, 1, \dots, X_T$ as

$$b_i = \begin{cases} 1, & X^* = i, \\ 0, & \text{otherwise} . \end{cases} \qquad \text{[S4.33a]}$$

It then follows that

$$\sum_{i=0}^{X_T} b_i = 1, \quad b_i b_j = 0, \, i \neq j, \quad b_i^2 = b_i. \qquad \text{[S4.33b]}$$

We now recast our original model in the new state-space $\begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top$. The transitions (i.e., reactions) and the corresponding transition intensities are as follows.

1. Receptor activation: the transition intensity of a receptor activation event is given by $\sum_{i=0}^{X_T} k_1 b_i (X_T - i)$. Whenever this event occurs, the states reset as

$$\begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top - \sum_{i=0}^{X_T-1} b_i \begin{bmatrix} e_i & Y^* \end{bmatrix}^\top + \sum_{i=0}^{X_T-1} b_i \begin{bmatrix} e_{i+1} & Y^* \end{bmatrix}^\top,$$

   where e_i is a column vector of dimension $X_T + 1$, with all zeros except at the i^{th} position. This reset map simplifies to

$$\begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} 0 & b_0 & b_1 & \dots & b_{X_T-2} & b_{X_T-1} + b_{X_T} & Y^* \end{bmatrix}^\top. \qquad \text{[S4.34a]}$$

2. Receptor deactivation: the transition intensity is given by $\sum_{i=0}^{X_T} b_i k_2 i$, with the map

$$\begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top - \sum_{i=1}^{X_T} b_i \begin{bmatrix} e_i & Y^* \end{bmatrix}^\top + \sum_{i=1}^{X_T} b_i \begin{bmatrix} e_{i-1} & Y^* \end{bmatrix}^\top.$$

   The reset map further simplifies to

$$\begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} b_0 + b_1 & b_2 & b_3 & \dots & b_{X_T} & 0 & Y^* \end{bmatrix}^\top. \qquad \text{[S4.34b]}$$

3. State Y^* to $Y^* + 1$ occurs with transition intensity $\sum_{i=0}^{X_T} k_3 b_i (Y_T - Y^*) + \sum_{i=0}^{X_T} k_5 i b_i (Y_T - Y^*)$ and map

$$\sum_{i=0}^{X_T} b_i \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \sum_{i=0}^{X_T} b_i \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* + 1 \end{bmatrix}^\top,$$

   which results in

$$\begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^* + 1 \end{bmatrix}^\top. \qquad \text{[S4.34c]}$$

4. State Y^* to Y^*-1 occurs with transition intensity $\sum_{i=0}^{X_T} k_4 b_i Y^* + \sum_{i=0}^{X_T} k_6 X_T b_i Y^* - \sum_{i=0}^{X_T} k_6 i b_i Y^*$ and map

$$\sum_{i=0}^{X_T} b_i \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \sum_{i=0}^{X_T} b_i \begin{bmatrix} b_0 & b_1 & \dots & b_{X_T} & Y^*-1 \end{bmatrix}^\top .$$

On simplifying, the above map becomes

$$\begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^* \end{bmatrix}^\top \mapsto \begin{bmatrix} b_0 & b_1 & b_2 & \dots & b_{X_T-1} & b_{X_T} & Y^*-1 \end{bmatrix}^\top . \quad \text{[S4.34d]}$$

We can now write the dynamics of moments of the form $\langle b_i Y^{*m} \rangle$ for $m = 0, 1, 2$. Let us begin with $\langle b_i \rangle$.

$$\frac{d\langle b_0 \rangle}{dt} = -k_1 X_T \langle b_0 \rangle + k_2 \langle b_1 \rangle , \quad \text{[S4.35a]}$$
$$\frac{d\langle b_i \rangle}{dt} = k_1 (X_T - i + 1) \langle b_{i-1} \rangle - k_1 (X_T - i) \langle b_i \rangle + k_2 (i+1) \langle b_{i+1} \rangle - k_2 i \langle b_i \rangle , 1 \le i \le X_T - 1, \quad \text{[S4.35b]}$$
$$\frac{d\langle b_{X_T} \rangle}{dt} = k_1 \langle b_{X_T-1} \rangle - k_2 X_T \langle b_{X_T} \rangle . \quad \text{[S4.35c]}$$

Recalling the definition of b_i, we note that $\langle b_i \rangle$ is same as the probability that $X^* = i$. We have solved these equations in a slightly different notation in Eq. (S4.8). Therefore, the solution to these ODEs is

$$\langle b_i \rangle = \binom{X_T}{i} \left(\frac{k_1}{k_1+k_2} \left(1 - e^{-(k_1+k_2)t}\right) \right)^i \left(1 - \frac{k_1}{k_1+k_2} \left(1 - e^{-(k_1+k_2)t}\right) \right)^{X_T - i} . \quad \text{[S4.36]}$$

Next, we write the dynamics for $\langle b_i Y^* \rangle$.

$$\frac{d\langle b_0 Y^* \rangle}{dt} = -\left(k_1 X_T + k_3 + k_4 + k_6 X_T\right) \langle b_0 Y^* \rangle + k_2 \langle b_1 Y^* \rangle + k_3 Y_T \langle b_0 \rangle , \quad \text{[S4.37a]}$$
$$\begin{aligned} \frac{d\langle b_i Y^* \rangle}{dt} &= k_1 (X_T - i + 1) \langle b_{i-1} Y^* \rangle - \left(k_1 (X_T - i) + k_2 i + k_3 + k_4 + k_5 i + k_6 (X_T - i)\right) \langle b_i Y^* \rangle + k_2 (i+1) \langle b_{i+1} Y^* \rangle \\ &+ k_3 Y_T \langle b_i \rangle + k_5 Y_T i \langle b_i \rangle , 1 \le i \le X_T - 1, \end{aligned} \quad \text{[S4.37b]}$$
$$\frac{d\langle b_{X_T} Y^* \rangle}{dt} = k_1 \langle b_{X_T-1} Y^* \rangle - \left(k_2 X_T + k_3 + k_4 + k_5 X_T\right) \langle b_{X_T} Y^* \rangle + k_3 Y_T \langle b_{X_T} \rangle + k_5 Y_T X_T \langle b_{X_T} \rangle . \quad \text{[S4.37c]}$$

Finally, the ODEs describing the time evolution of $\left\langle b_i Y^{*2} \right\rangle$ are as follows.

$$\frac{d\left\langle b_0 Y^{*2} \right\rangle}{dt} = -k_1 X_T \left\langle b_0 Y^{*2} \right\rangle + k_2 \left\langle b_1 Y^{*2} \right\rangle + k_3 Y_T \langle b_0 \rangle + \left(-k_3 + k_4 + k_6 X_T + 2k_3 Y_T\right) \langle b_0 Y^* \rangle - \left(2k_3 + 2k_4 + 2k_6 X_T\right) \left\langle b_0 Y^{*2} \right\rangle , \quad \text{[S4.38a]}$$
$$\begin{aligned} \frac{d\left\langle b_i Y^{*2} \right\rangle}{dt} &= k_1 (X_T - i + 1) \left\langle b_{i-1} Y^{*2} \right\rangle - k_1 (X_T - i) \left\langle b_i Y^{*2} \right\rangle + k_2 (i+1) \left\langle b_{i+1} Y^{*2} \right\rangle - k_2 i \left\langle b_i Y^{*2} \right\rangle \\ &+ \left(k_3 Y_T + k_5 Y_T i\right) \langle b_i \rangle + \left(-k_3 - k_5 i + k_4 + k_6 X_T - k_6 i + 2k_3 Y_T + 2k_5 Y_T i\right) \langle b_i Y^* \rangle \\ &- \left(2k_3 + 2k_5 i + 2k_4 + 2k_6 X_T - 2k_6 i\right) \left\langle b_i Y^{*2} \right\rangle , \quad 1 \le i \le X_T - 1, \end{aligned} \quad \text{[S4.38b]}$$
$$\begin{aligned} \frac{d\left\langle b_{X_T} Y^{*2} \right\rangle}{dt} &= k_1 \left\langle b_{X_T-1} Y^{*2} \right\rangle - k_2 X_T \left\langle b_{X_T} Y^{*2} \right\rangle + \left(k_3 Y_T + k_5 Y_T X_T\right) \langle b_i \rangle + \left(-k_3 - k_5 X_T + k_4 + 2k_3 Y_T + 2k_5 Y_T X_T\right) \langle b_i Y^* \rangle \\ &- \left(2k_3 + 2k_5 X_T + 2k_4\right) \left\langle b_i Y^{*2} \right\rangle . \end{aligned} \quad \text{[S4.38c]}$$

These ODEs require initial condition to compute transient moments which we discuss below.

Setting initial condition. In absence of stimulus, we have that $\langle b_0 \rangle$=1, because no receptors should be active. All other $\langle b_i \rangle = 0$. Furthermore, $\langle b_i Y^* \rangle = \langle b_i \rangle \langle Y^* \rangle$ and $\left\langle b_i Y^{*2} \right\rangle = \langle b_i \rangle \langle Y^* 2 \rangle$. Therefore the mean and the second moment at time $t = 0$ are given by the first two moments of the Binomial distribution with parameters $\frac{k_3}{k_3+k_4+k_6 X_T}$ and Y_T. Therefore, the initial condition is

$$\langle b_0 Y^* \rangle = \frac{k_3}{k_3 + k_4 + k_6 X_T} Y_T, \quad \left\langle b_0 Y^{*2} \right\rangle = \frac{k_3^2 Y_T^2 + k_3 (k_4 + k_6 X_T) Y_T}{(k_3 + k_4 + k_6 X_T)^2} . \quad \text{[S4.39]}$$

Semi-analytical solution using linear algebra. Let $\mu_0 = \begin{bmatrix}\langle b_0\rangle & \langle b_1\rangle & \dots & \langle b_{X_T}\rangle\end{bmatrix}^\top$ be the collection of the moments of b_i. Then the ODEs can be compactly written as

$$\frac{d\mu_0}{dt} = M_0\mu_0, \tag{S4.40}$$

which has the solution $\mu_0(t) = e^{M_0 t}\mu_0(0)$. We also note that $\sum_i \langle b_i\rangle = 1$ at all times.

The matrix M_0 is tridiagonal, but its inverse does not exist. This does not affect computation of the transient solution as long as we respect the constraint that all $\langle b_i\rangle$ sum up to one. For steady-state solution, however, we have to solve

$$M_0\mu_0 = 0, \tag{S4.41}$$

which only exhibits a trivial solution $\mu_0 = 0$. To force the summation requirement, we reduce the system such that we get rid of the last equation corresponding to $\langle b_{X_T}\rangle$. We then substitute $\langle b_{X_T}\rangle = 1 - \sum_{i=0}^{X_T-1}\langle b_i\rangle$ wherever we have $\langle b_{X_T}\rangle$. This gives us a reduced system of equation

$$\tilde{M}_0\tilde{\mu_0} + c = 0, \tag{S4.42}$$

which can be straightforwardly solved using standard linear algebra tools.

It is important to note that we already know the transient as well as the stationary solution for these equations - since $\langle b_i\rangle$ are probabilities. However, we present the linear algebra approach for completeness. We will this approach to compute the higher order moments for which analytical solutions are not known.

Let us now solve for the moments $\langle b_i Y^*\rangle$. To this end, we collect all the required moments in μ_1 defined as

$$\mu_1 = \begin{bmatrix}\langle b_0\rangle & \dots & \langle b_{X_T}\rangle & \langle b_0 Y^*\rangle & \dots & \langle b_{X_T} Y^*\rangle .\end{bmatrix} \tag{S4.43}$$

The corresponding ODE system is then

$$\frac{d\mu_1}{dt} = \begin{bmatrix} M_0 & 0 \\ M_{10} & M_{11}\end{bmatrix}\mu_1 \tag{S4.44}$$

As before, we can now compute the solution using matrix exponential. For the moments $\left\langle b_i Y^{*2}\right\rangle$, we can similarly define μ_2

$$\mu_2 = \begin{bmatrix}\langle b_0\rangle & \dots & \langle b_{X_T}\rangle & \langle b_0 Y^*\rangle & \dots & \langle b_{X_T} Y^*\rangle & \left\langle b_0 Y^{*2}\right\rangle & \dots & \left\langle b_{X_T} Y^{*2}\right\rangle .\end{bmatrix} \tag{S4.45}$$

Then we can write the ODE system:

$$\frac{d\mu_2}{dt} = \begin{bmatrix} M_0 & 0 & 0 \\ M_{10} & M_{11} & 0 \\ M_{20} & M_{21} & M_{22}\end{bmatrix}\mu_2. \tag{S4.46}$$

S5. Time-dependent input signals

In this section, we examine how molecular switch architectures process pulsating inputs. To this end, we modify the ODEs for the model as

$$\frac{dX^*}{dt} = k_1 S(t)(X_T - X^*) - k_2 X^*, \tag{S5.1a}$$

$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X^*))Y^*. \tag{S5.1b}$$

We assume that the system is in pre-stimulus steady-state at $t = 0$. Thus the initial condition of the above ODE system is

$$(X^*(0), Y^*(0)) = \left(0, \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}\right). \tag{S5.2}$$

Finally, the pulsating stimulus, $S(t)$, is given by

$$S(t) = \begin{cases} S_p, & n\tau \le t \le n\tau + D\tau, \quad n = 0, 1, 2, 3, \dots \\ 0, & \text{otherwise,}\end{cases} \tag{S5.3}$$

where τ is the period of the pulsating input, S_p is the amplitude, and $D < 1$ is the duty-cycle.

To study the approximate transient solution of the system of ODEs in Eq. (S5.1), we study the solutions to the following two systems:

$$\Xi_H : \begin{cases} \frac{dX^*}{dt} = k_1 S_p (X_T - X^*) - k_2 X^*, \\ \frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X^*))Y^*. \end{cases} \tag{S5.4a}$$

$$\Xi_L : \begin{cases} \frac{dX^*}{dt} = -k_2 X^*, \\ \frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X^*))Y^*. \end{cases} \tag{S5.4b}$$

Here the first system Ξ_H corresponds to the dynamics when the pulse is high (on) and the second system Ξ_L corresponds to the dynamics when the pulse is low (off). We then combine these solutions to obtain the approximate long-term solution of Eq. (S5.1).

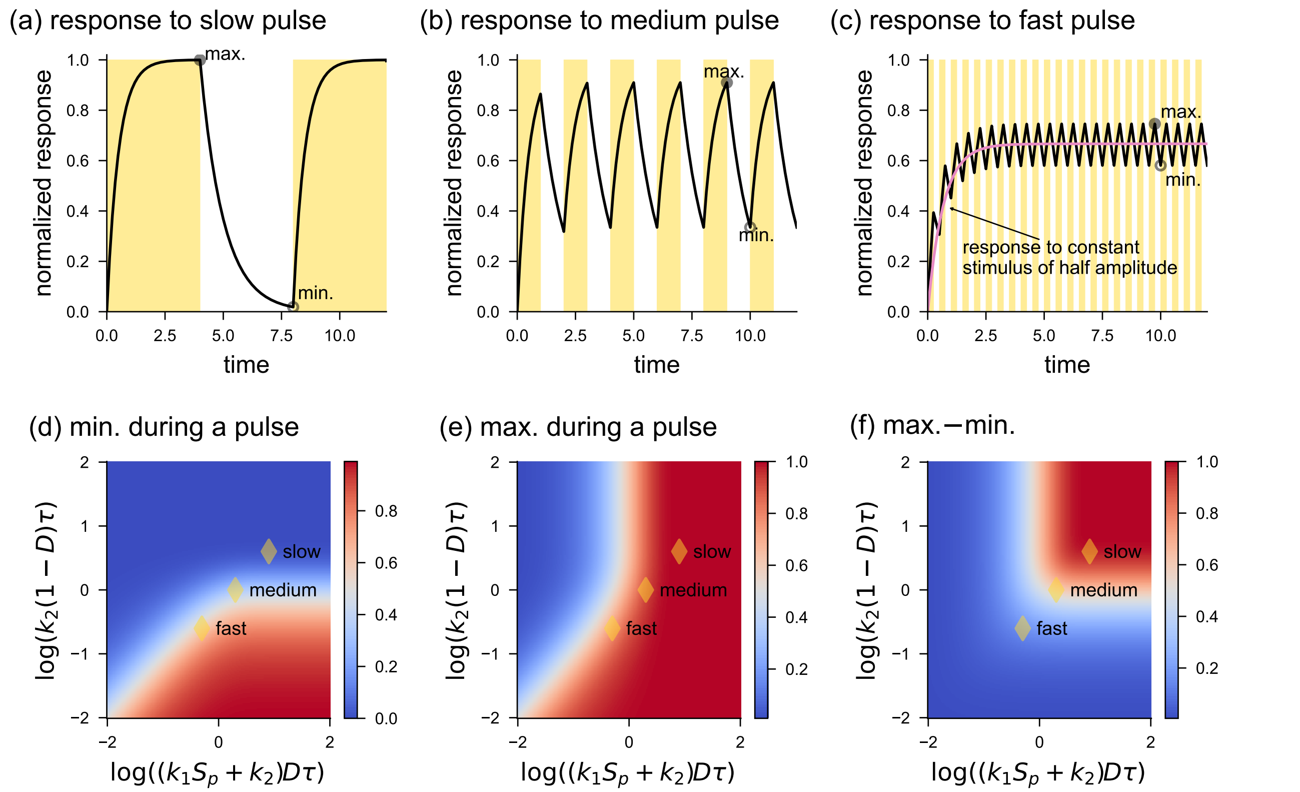

Fig. S5.1. Effect of pulsating input on receptor dynamics. (**a**)–(**c**) show the typical responses of the receptor to pulses of different frequencies. (**d**)–(**f**) illustrate the three features of the receptor response as the normalized on period and the normalized off period of the pulse are varied.

S5-A. Approximate solution when pulse is on. To develop the approximate solution to Ξ_H, we linearize the nonlinearity X^*Y^* around the steady-state solution of Ξ_H as :

$$X^*Y^* \approx \overline{Y^*}_H X^* + \overline{X^*}_H Y^* - \overline{X^*}_H\,\overline{Y^*}_H, \tag{S5.5a}$$

where

$$\overline{X^*}_H = \frac{k_1S_pX_T}{k_1S_p + k_2}, \tag{S5.5b}$$

$$\overline{Y^*}_H = \frac{k_3 + k_5\frac{k_1S_pX_T}{k_1S_p+k_2}}{k_3 + k_4 + k_6X_T + (k_5 - k_6)\frac{k_1S_pX_T}{k_1S_p+k_2}}Y_T. \tag{S5.5c}$$

The resulting linear system of ODEs is given by

$$\frac{dX^*}{dt} = k_1S_pX_T - (k_1S_p + k_2)X^*, \tag{S5.6a}$$

$$\frac{dY^*}{dt} \approx \left(k_3Y_T + (k_5 - k_6)\overline{X^*}_H\,\overline{Y^*}_H\right) + \left(k_5Y_T - (k_5 - k_6)\overline{Y^*}_H\right)X^* - (k_3 + k_4 + k_6X_T + (k_5 - k_6)\overline{X^*}_H)Y^*. \tag{S5.6b}$$

These ODEs are similar to those in Eq. (S2.21). If we use the short-hand notation as $\eta_{0H} = k_1S_pX_T$, $\eta_{1H} = k_1S_p + k_2$, $\xi_{0H} = k_3Y_T + (k_5 - k_6)\overline{X^*}_H\,\overline{Y^*}_H$, $\xi_{1H} = k_5Y_T - (k_5 - k_6)\overline{Y^*}_H$, and $\xi_{2H} = k_3 + k_4 + k_6X_T + (k_5 - k_6)\overline{X^*}_H$, then the solution to Ξ_H is given by

$$X^*(t) = \overline{X^*}_H - \left(\overline{X^*}_H - X^*(0)\right)e^{-\eta_{1H}t}, \tag{S5.7a}$$

$$Y^*(t) \approx \overline{Y^*}_H - \left(\overline{Y^*}_H - Y^*(0)\right)e^{-\xi_{2H}t} + \frac{\xi_{1H}\left(\overline{X^*}_H - X^*(0)\right)}{\eta_{1H} - \xi_{2H}}\left(e^{-\eta_{1H}t} - e^{-\xi_{2H}t}\right). \tag{S5.7b}$$

S5-B. Approximate solution to dynamics when pulse is off. To develop the approximate solution to Ξ_L, we linearize the nonlinearity X^*Y^* around the steady-state solution of Ξ_L as :

$$X^*Y^* \approx \overline{Y^*}_L X^* + \overline{X^*}_L Y^* - \overline{X^*}_L\,\overline{Y^*}_L, \tag{S5.8a}$$

where

$$\overline{X^*}_L = 0, \quad \text{[S5.8b]}$$

$$\overline{Y^*}_L = \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}. \quad \text{[S5.8c]}$$

The resulting linear system of ODEs is given by

$$\frac{dX^*}{dt} = -k_2 X^*, \quad \text{[S5.9a]}$$

$$\frac{dY^*}{dt} \approx k_3 Y_T + \left(k_5 Y_T - (k_5 - k_6)\overline{Y^*}_L\right) X^* - (k_3 + k_4 + k_6 X_T) Y^*. \quad \text{[S5.9b]}$$

These ODEs are similar to those in Eq. (S2.21). Using the notation $\eta_{0L} = 0$, $\eta_{1L} = k_2$, $\xi_{0L} = k_3 Y_T$, $\xi_{1L} = k_5 Y_T - (k_5 - k_6)\overline{Y^*}_L$, and $\xi_{2L} = k_3 + k_4 + k_6 X_T$, the approximate solution Ξ_L is given by

$$X^*(t) = X^*(0) e^{-\eta_{1L} t}, \quad \text{[S5.10a]}$$

$$Y^*(t) \approx \overline{Y^*}_L + \left(Y^*(0) - \overline{Y^*}_L\right) e^{-\xi_{2L} t} - \frac{\xi_{1L} X^*(0)}{\eta_{1L} - \xi_{2L}} \left(e^{-\eta_{1L} t} - e^{-\xi_{2L} t}\right). \quad \text{[S5.10b]}$$

S5-C. Long-term approximate solution to pulsating input. One consequence of using a pulsating stimulus is that for many systems, the effect of initial conditions disappears from the dynamics after an initial 'burn-in' period and the solutions become periodic with the same period as the stimulus. For the ODE system under consideration, this may be more rigorously shown using the Theorem 2 in (12).

To compute the long-term solution, let $(X^*(n\tau), Y^*(n\tau))$ denote the levels of the active receptors and the active switches at the end of the n^{th} period of the pulse. Then using the solutions of Ξ_H and Ξ_L, the dynamics of X^* over the $(n+1)^{th}$ period is

$$X^*(t) = \begin{cases} \overline{X^*}_H - \left(\overline{X^*}_H - X^*(n\tau)\right) e^{-\eta_{1H}(t - n\tau)}, & n\tau \le t \le n\tau + D\tau, \\ \overline{X^*}_H e^{-\eta_{1L}(t - n\tau - D\tau)} - \left(\overline{X^*}_H - X^*(n\tau)\right) e^{-(\eta_{1H} D\tau + \eta_{1L}(t - n\tau - D\tau))}, & n\tau + D\tau \le t \le (n+1)\tau \end{cases} \quad \text{[S5.11a]}$$

Likewise, the dynamics of $Y^*(t)$ is

$$\begin{cases} \overline{Y^*}_H - \left(\overline{Y^*}_H - Y^*(n\tau)\right) e^{-\xi_{2H}(t - n\tau)} + \frac{\xi_{1H}\left(\overline{X^*}_H - X^*(n\tau)\right)}{\eta_{1H} - \xi_{2H}} \left(e^{-\eta_{1H}(t - n\tau)} - e^{-\xi_{2H}(t - n\tau)}\right), & n\tau \le t \le n\tau + D\tau, \\ \overline{Y^*}_L - \frac{\xi_1 X^*(n\tau + D\tau)}{\eta_{1L} - \xi_{2L}} \left(e^{-\eta_{1L}(t - n\tau - D\tau)} - e^{-\xi_{2L}(t - n\tau - D\tau)}\right) + \overline{Y^*}_H e^{-\xi_{2L}(t - n\tau - D\tau)} & \\ - \left(\left(\overline{Y^*}_H - Y^*(n\tau)\right) e^{-\xi_{2H} D\tau} + \frac{\xi_{1H}\left(\overline{X^*}_H - X^*(n\tau)\right)}{\eta_{1H} - \xi_{2H}} \left(e^{-\eta_{1H} D\tau} - e^{-\xi_{2H} D\tau}\right)\right) e^{-\xi_{2L}(t - n\tau - D\tau)} & \\ - \overline{Y^*}_L e^{-\xi_{2L}(t - n\tau - D\tau)}, & n\tau + D\tau \le t \le (n+1)\tau. \end{cases} \quad \text{[S5.11b]}$$

From above equations, we obtain $X^*((n+1)\tau)$ and $Y^*((n+1)\tau)$ as

$$X^*((n+1)\tau) = \overline{X^*}_H e^{-\eta_{1L}(1-D)\tau} - \left(\overline{X^*}_H - X^*(n\tau)\right) e^{-(\eta_{1H} D\tau + \eta_{1L}(1-D)\tau)}, \quad \text{[S5.12a]}$$

$$\begin{aligned} Y^*((n+1)\tau) = {} & \overline{Y^*}_L - \frac{\xi_{1L}\left(\overline{X^*}_H - \left(\overline{X^*}_H - X^*(n\tau)\right) e^{-\eta_{1H} D\tau}\right)}{\eta_{1L} - \xi_{2L}} \left(e^{-\eta_{1L}(1-D)\tau} - e^{-\xi_{2L}(1-D)\tau}\right) + \overline{Y^*}_H e^{-\xi_{2L}(1-D)\tau} \\ & - \left(\left(\overline{Y^*}_H - Y^*(n\tau)\right) e^{-\xi_{2H} D\tau} + \frac{\xi_{1H}\left(\overline{X^*}_H - X^*(n\tau)\right)}{\eta_{1H} - \xi_{2H}} \left(e^{-\eta_{1H} D\tau} - e^{-\xi_{2H} D\tau}\right)\right) e^{-\xi_{2L}(1-D)\tau} \\ & - \overline{Y^*}_L e^{-\xi_{2L}(1-D)\tau}. \end{aligned} \quad \text{[S5.12b]}$$

Let $n \to \infty$, such that the effect of initial conditions disappears. Then, the above dynamics repeats for every period, implying $X^*(n\tau) = X^*((n+1)\tau) = \overline{X^*}_p$ and $Y^*(n\tau) = Y^*((n+1)\tau) = \overline{Y^*}_p$. With this assumption, we obtain the following:

$$\overline{X^*}_p = \overline{X^*}_H \frac{e^{-\eta_{1L}(1-D)\tau} - e^{-(\eta_{1H} D\tau + \eta_{1L}(1-D)\tau)}}{1 - e^{-(\eta_{1H} D\tau + \eta_{1L}(1-D)\tau)}}, \quad \text{[S5.13a]}$$

$$\begin{aligned} \overline{Y^*}_p = {} & \overline{Y^*}_L + \left(\overline{Y^*}_H - \overline{Y^*}_L\right) \frac{e^{-\xi_{2L}(1-D)\tau}\left(1 - e^{-\xi_{2H} D\tau}\right)}{1 - e^{-\xi_{2H} D\tau - \xi_{2L}(1-D)\tau}} \\ & + \overline{X^*}_H \left(\frac{\xi_{1H}}{\eta_{1H} - \xi_{2H}} \frac{e^{-\eta_{1H} D\tau} - e^{-\xi_{2H} D\tau}}{1 - e^{-(\eta_{1H} D\tau + \eta_{1L}(1-D)\tau)}} \frac{e^{-\xi_{2L}(1-D)\tau} - e^{-\eta_{1L}(1-D)\tau - \xi_{2L}(1-D)\tau}}{1 - e^{-\xi_{2H} D\tau - \xi_{2L}(1-D)\tau}}\right) \\ & - \overline{X^*}_H \left(\frac{\xi_{1L}}{\eta_{1L} - \xi_{2L}} \frac{1 - e^{-\eta_{1H} D\tau}}{1 - e^{-(\eta_{1H} D\tau + \eta_{1L}(1-D)\tau)}} \frac{e^{-\eta_{1L}(1-D)\tau} - e^{-\xi_{2L}(1-D)\tau}}{1 - e^{-\xi_{2H} D\tau - \xi_{2L}(1-D)\tau}}\right). \end{aligned} \quad \text{[S5.13b]}$$

The main takeaway from these expressions is that the receptor response at the beginning of a pulse $\overline{X^*}_p$ normalized by the maximum response if the input were constant $(\overline{X^*}_H)$ only depends upon the parameter combinations $\eta_{1L}(1-D)\tau$ and $\eta_{1H} D\tau$.

Here $\eta_{1L}(1-D)\tau$ represents the "off" period of the pulse normalized by the relaxation timescale (or response time) of the receptor while $\eta_{1H}D\tau$ represents the "on" period of the pulse normalized by the response time of the receptor. The expression for $\overline{Y^*}_p$ is more involved, but it may still be seen that the power of the exponential functions contain these same normalized timescales. Two addition timescales $\xi_{2H}D\tau$ and $\xi_{2L}(1-D)\tau$ also appear, which refer to the on and off periods of the pulse with respect to the timescales of the switch.

We note that if the pulse is on for long enough, then the response reaches its steady-state. If the off period of the pulse is long enough too, then the response returns to the basal level. This behavior repeats over subsequent pulses (Fig. S5.1(a). Instead, if the pulse frequency is increased, then the response does not reach the steady-state and pulsates around a lower value (Fig. S5.1(b)). For a pulse of very high frequency, the response approaches to that to a constant stimulus with half amplitude(Fig. S5.1(c)).

We further illustrate the effect of relative timescales on $\overline{X^*}_p$ (minimum response during a pulse) as well as the maximum response during a pulse (computed as $\overline{X^*}_p e^{eta_{1L}(1-D)\tau}$ and the difference between the maximum and the minimum response for the receptor in Fig. S5.1 in Figs. S5.1(d)–(e). It is seen that the minimum response does not return to the basal level if the relative on period of the pulse dominates the relative off period. In this case, the maximum response also approaches the steady-state value to a constant stimulus of same amplitude.

A thorough analysis of the switch dynamics involves four relative timescales and is beyond the scope of this work. However, we note that if the receptor dynamics is fast enough to immediately follow the pulsating input, then the switch dynamics may be understood in the same manner as the results in Fig S5.1. We illustrate this in Fig. S5.2.

S6. Effect of receptor removal

In this section, we include removal of both inactive and active receptors and examine how receptor removal influences the three properties: dose-response, response time, and noise.

S6-A. Model description. As shown in Fig. S6.1, we modify our model by including the production of inactive receptors with rate k_p, removal of inactive receptors with rate k_d, and removal of active receptors of k_d^*. The ODE model for the set up is

$$\frac{dX}{dt} = k_p - k_d X - k_1 S X + k_2 X^* \quad \text{[S6.1a]}$$

$$\frac{dX^*}{dt} = k_1 S X - k_2 X^* - k_d^* X^* \quad \text{[S6.1b]}$$

$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - (k_4 + k_6 X) Y^*. \quad \text{[S6.1c]}$$

We specify the initial condition $(X(0), X^*(0), Y^*(0))$ as the steady-state in the absence of the stimulus:

$$X(0) = \frac{k_p}{k_d}, \quad X^*(0) = 0, \quad Y^*(0) = \frac{k_3}{k_3 + k_4 + \frac{k_6 k_p}{k_d}}. \quad \text{[S6.2]}$$

S6-B. Dose responses. The steady-state numbers of inactive receptors, active receptors, and active switches are computed by setting the derivatives in Eq. (S6.1):

$$\overline{X} = \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}\frac{k_2+k_d^*}{k_1}}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}, \quad \text{[S6.3a]}$$

$$\overline{X^*} = \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*} S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}, \quad \text{[S6.3b]}$$

$$\overline{Y^*} = \frac{k_3 + k_5 \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*} S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}}{k_3 + k_4 + k_5 \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*} S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}} + k_6 \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}\frac{k_2+k_d^*}{k_1}}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}} Y_T = \frac{k_3 + k_5 \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*} S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}\frac{k_3+k_4+k_6\frac{k_p}{k_d}}{k_3+k_4+k_5\frac{k_p}{k_d}\frac{k_d}{k_d^*}}} Y_T. \quad \text{[S6.3c]}$$

Re-arranging terms gives

$$\overline{X} = \frac{X_0 \Theta_X + X_\infty S}{\Theta_X + S} \quad \text{[S6.4a]}$$

$$\overline{X^*} = \frac{X_0^* \Theta_{X^*} + X_\infty^* S}{\Theta_{X^*} + S}, \quad \text{[S6.4b]}$$

$$\overline{Y^*} = \frac{Y_0^* \Theta_{Y^*} + Y_\infty^* S}{\Theta_{Y^*} + S}, \quad \text{[S6.4c]}$$

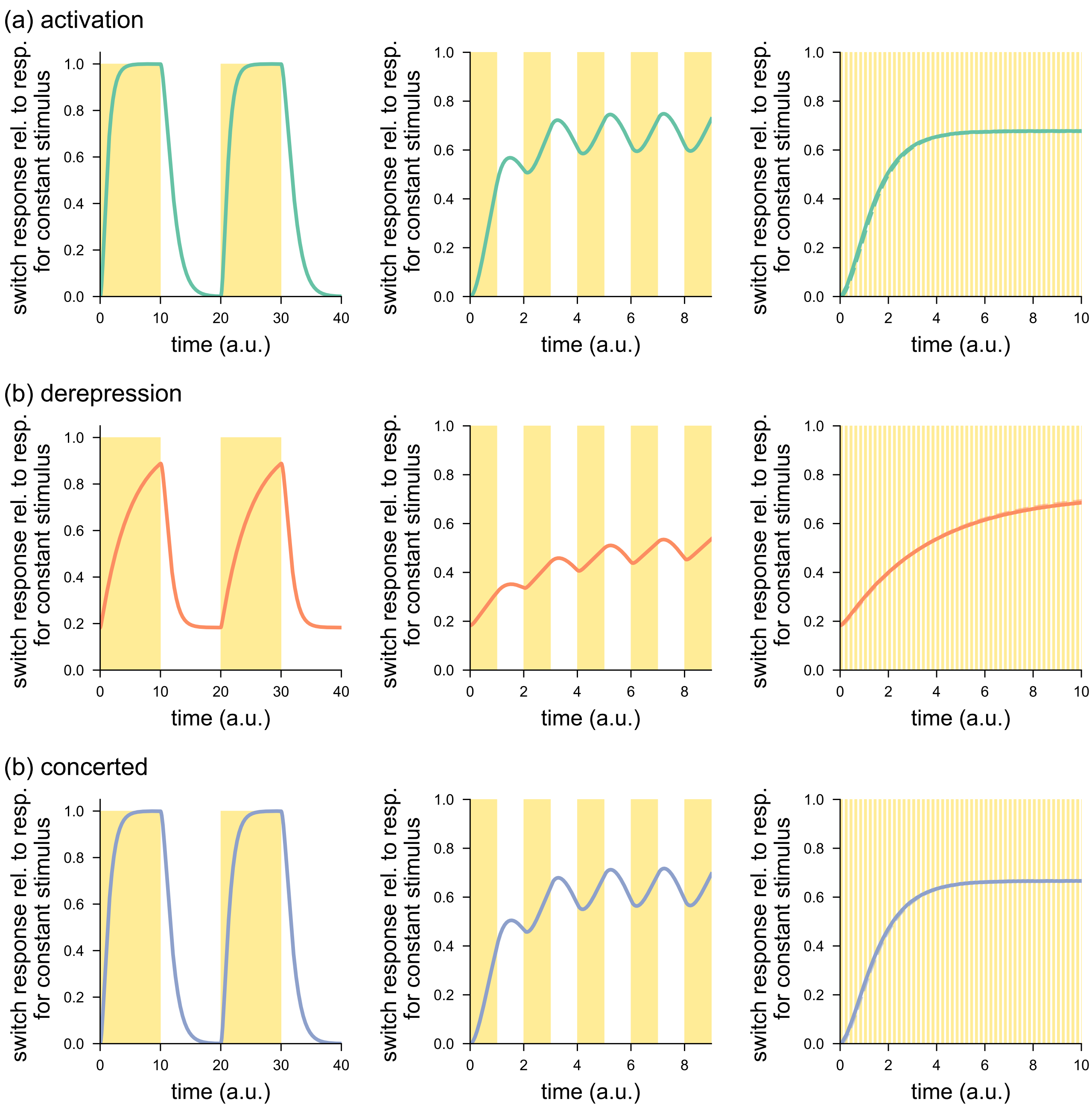

Fig. S5.2. Illustration of switch response to a pulsating input. For each signaling mechanism, we use slow pulses that allows the switch response to attain steady-state when pulse is on and to relax back to basal level when the pulse is off (left column). Likewise, we use pulses of medium frequency (middle column) and high frequency (right column). The responses are qualitatively similar to those of the receptor in Fig. S5.1. The parameters are chosen such that the receptor dynamics is much faster than that of the switch.

where

$$X_0 = \frac{k_p}{k_d}, \quad \text{[S6.4d]}$$

$$X_\infty = 0, \quad \text{[S6.4e]}$$

$$\Theta_X = \frac{k_d}{k_d^*}\frac{k_2 + k_d^*}{k_1}, \quad \text{[S6.4f]}$$

$$X_0^* = 0, \quad \text{[S6.4g]}$$

$$X_\infty^* = \frac{k_d}{k_d^*}\frac{k_p}{k_d}, \quad \text{[S6.4h]}$$

$$\Theta_{X^*} = \Theta_X, \quad \text{[S6.4i]}$$

$$Y_0^* = \frac{k_3 Y_T}{k_3 + k_4 + k_6 \frac{k_p}{k_d}}, \quad \text{[S6.4j]}$$

$$Y_\infty^* = \frac{\left(k_3 + k_5 \frac{k_d}{k_d^*}\frac{k_p}{k_d}\right) Y_T}{k_3 + k_4 + k_5 \frac{k_d}{k_d^*}\frac{k_p}{k_d}}, \quad \text{[S6.4k]}$$

$$\Theta_{Y^*} = \Theta_X \frac{k_3 + k_4 + k_6 \frac{k_p}{k_d}}{k_3 + k_4 + k_5 \frac{k_d}{k_d^*}\frac{k_p}{k_d}}. \quad \text{[S6.4l]}$$

These formulas reveal that except for few scaling operations, the dose-responses exhibit the same behavior as the minimal model.

Finally, we also note that the total number of receptors

$$\overline{X} + \overline{X^*} = \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}\frac{k_2+k_d^*}{k_1} + \frac{k_p}{k_d}\frac{k_d}{k_d^*}S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}, \quad \text{[S6.5]}$$

which equals k_p/k_d if $k_d = k_d^*$, is less than k_p/k_d if $k_d < k_d^*$, and is greater than k_p/k_d if $k_d > k_d^*$.

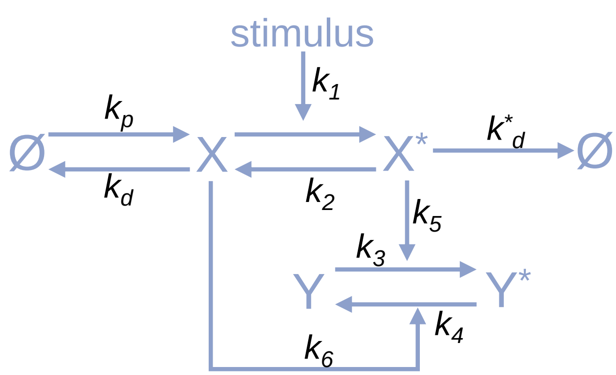

Fig. S6.1. Concerted mechanism with receptor production and degradation.

S6-C. Solution to the receptor dynamics. Our goal here is to examine the effect of receptor removal on different signaling mechanisms. To that end, let us first compute the dynamics at the receptor level.

$$\frac{dX}{dt} = k_p - k_d X - k_1 S X + k_2 X^*, \quad \text{[S6.6a]}$$

$$\frac{dX^*}{dt} = k_1 S X - k_2 X^* - k_d^* X^*. \quad \text{[S6.6b]}$$

Let $\mathcal{X}[\omega]$ and $\mathcal{X}^*[\omega]$ respectively denote the Laplace transforms of $X(t)$ and $X^*(t)$. Taking the initial conditions as $(X(0), X^*(0)) = \left(\frac{k_p}{k_d}, 0\right)$, the Laplace transforms of above ODEs results in the following algebraic equations

$$\omega\mathcal{X}[\omega] - \frac{k_p}{k_d} = \frac{k_p}{\omega} - (k_1 S + k_d)\mathcal{X}[\omega] + k_2\mathcal{X}^*[\omega], \quad \text{[S6.7a]}$$

$$\omega\mathcal{X}^*[\omega] = k_1 S\mathcal{X}[\omega] - (k_2 + k_d^*)\mathcal{X}^*[\omega]. \quad \text{[S6.7b]}$$

The solution to above system of equations is

$$\mathcal{X}[\omega] = \frac{k_p}{k_d} \frac{(\omega + k_d)(\omega + k_2 + k_d^*)}{\omega\left(\omega^2 + (k_1 S + k_2 + k_d + k_d^*)\,\omega + k_1 S k_d^* + k_2 k_d + k_d k_d^*\right)}, \tag{S6.8a}$$

$$\mathcal{X}^*[\omega] = \frac{k_1 S k_p}{k_d} \frac{(\omega + k_d)}{\omega\left(\omega^2 + (k_1 S + k_2 + k_d + k_d^*)\,\omega + k_1 S k_d^* + k_2 k_d + k_d k_d^*\right)}. \tag{S6.8b}$$

Special case when receptor removal rates are equal We first examine the special case when $k_d = k_d^*$ for which the above expressions simplify to

$$\mathcal{X}[\omega] = \frac{k_p}{k_d} \frac{\omega + k_2 + k_d}{\omega\,(\omega + k_1 S + k_2 + k_d)}, \tag{S6.9a}$$

$$\mathcal{X}^*[\omega] = \frac{k_p}{k_d} \frac{k_1 S}{\omega\,(\omega + k_1 S + k_2 + k_d)}. \tag{S6.9b}$$

Taking inverse Laplace transform gives

$$X(t) = \frac{k_p}{k_d} \frac{\frac{k_2 + k_d}{k_1}}{S + \frac{k_2 + k_d}{k_1}} + \left(\frac{k_p}{k_d} - \frac{k_p}{k_d} \frac{\frac{k_2 + k_d}{k_1}}{S + \frac{k_2 + k_d}{k_1}}\right) e^{-(k_1 S + k_2 + k_d)t}, \tag{S6.10a}$$

$$X^*(t) = \frac{k_p}{k_d} \frac{S}{S + \frac{k_2 + k_d}{k_1}} - \frac{k_p}{k_d} \frac{S}{S + \frac{k_2 + k_d}{k_1}} e^{-(k_1 S + k_2 + k_d)t}. \tag{S6.10b}$$

These solutions monotonically reach their respective steady-states. Furthermore, the response time for each solution may be computed straightforwardly as

$$\mathcal{T}_X = \mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2 + k_d}. \tag{S6.11}$$

Unequal receptor removal rates We first define the following parameters

$$\zeta = \frac{k_1 S + k_2 + k_d + k_d^*}{2\sqrt{(k_1 S + k_d)(k_2 + k_d^*) - k_1 S k_2}}, \tag{S6.12a}$$

$$\kappa = \sqrt{(k_1 S + k_d)(k_2 + k_d^*) - k_1 S k_2}. \tag{S6.12b}$$

The roots of the term $\omega^2 + 2\zeta\kappa\omega + \kappa^2$ are

$$\omega_{1,2} = \kappa(-\zeta \pm \sqrt{\zeta^2 - 1}). \tag{S6.13a}$$

The following usual relations hold for ω_1 and ω_2:

$$\omega_1 + \omega_2 = -2\kappa\zeta, \tag{S6.13b}$$

$$\omega_1 \omega_2 = \kappa^2, \tag{S6.13c}$$

$$\omega_1 - \omega_2 = 2\kappa\sqrt{\zeta^2 - 1}. \tag{S6.13d}$$

Then, the transient solution for active receptors is given by

$$X^*(t) = \frac{k_1 k_p S}{\kappa^2} + c_1^* e^{\omega_1 t} + c_2^* e^{\omega_2 t}. \tag{S6.14a}$$

Here the terms c_1^* and c_2^* are

$$c_1^* = \frac{k_1 S}{2\kappa\sqrt{\zeta^2 - 1}} \left(\frac{k_p}{k_d} + \frac{k_p \omega_2}{\kappa^2}\right), c_2^* = -\frac{k_1 S}{2\kappa\sqrt{\zeta^2 - 1}} \left(\frac{k_p}{k_d} + \frac{k_p \omega_1}{\kappa^2}\right). \tag{S6.14b}$$

Using the solution of $X^*(t)$, $X(t)$ can also be computed as follows.

$$X(t) = \frac{k_p(k_2 + k_d^*)}{\kappa^2} + \left(\frac{c_1^* \omega_1 + c_1^*(k_2 + k_d^*)}{k_1 S}\right) e^{\omega_1 t} + \left(\frac{c_2^* \omega_2 + c_2^*(k_2 + k_d^*)}{k_1 S}\right) e^{\omega_2 t}. \tag{S6.14c}$$

Having determined these solutions, we next show that $\zeta > 1$, which implies that the roots $\omega_{1,2}$ are real. To see this, we look at ζ^2

$$\zeta^2 = \frac{(k_1 S + k_2 + k_d + k_d^*)^2}{4\,(k_2 k_d + k_d k_d^* + k_1 k_d^* S)} \tag{S6.15a}$$

$$= \frac{(k_1 S + k_d)^2 + (k_2 + k_d^*)^2 + 2(k_1 S + k_d)(k_2 + k_d^*)}{4\left((k_1 S + k_d)(k_2 + k_d^*) - k_1 S k_2\right)}. \tag{S6.15b}$$

This implies that

$$(k_1S + k_d)^2 + (k_2 + k_d^*)^2 + (2 - 4\zeta^2)(k_1S + k_d)(k_2 + k_d^*) + 4\zeta^2 k_1 S k_2 = 0 \quad \text{[S6.15c]}$$

$$\implies ((k_1S + k_d) - (k_2 + k_d^*))^2 + (4 - 4\zeta^2)(k_1S + k_d)(k_2 + k_d^*) + 4\zeta^2 k_1 S k_2 = 0. \quad \text{[S6.15d]}$$

Because all terms in the above equation are positive, except may be for $4 - 4\zeta^2$, a real solution for ζ exists only if $4 - 4\zeta^2 < 0$. Therefore, $\zeta > 1$. Consequently, the roots ω_1 and ω_2 defined in Eq. (S6.13a) are negative and satisfy

$$\omega_2 < \omega_1 < 0, \quad |\omega_1| < |\omega_2|. \quad \text{[S6.16]}$$

Examining the transient solutions $X(t)$ and $X^*(t)$ in Eq. (S6.14) shows that the both X and X^* have two timescales for relaxing to their respective steady-states, determined by ω_1 and ω_2. Because $|\omega_2| > |\omega_1|$, we refer to the timescale set by ω_2 as fast timescale and the one set by ω_1 as the slow timescale. The parameter ζ controls the difference between the magnitudes of ω_1 and ω_2. One consequence of these two timescales is that $X(t)$ now starts from the initial condition $X(t) = k_p/k_d$ and may reach its steady-state non-monotonically. Likewise, $X^*(t)$ starts from $X^*(0) = 0$ and may reach its steady-state non-monotonically.

Our results in the case of $k_d = k_d^*$ show that in that case, $X(t)$ and $X^*(t)$ are monotonic. Therefore, we ask whether a difference between k_d and k_d^* is enough to enable the non-monotonic behaviors. Note that $dX^*/dt > 0$ right after $t = 0$, the non-monotonocity would be a maximum. Assuming that $X^*(t)$ attains a maximum at a time $t_{\max}$, we must have that $t_{\max}$ is a solution to

$$\frac{dX^*}{dt} = 0, \quad \text{[S6.17]}$$

which implies

$$t_{\max} = \frac{1}{\omega_2 - \omega_1} \log\left(-\frac{c_1^*\omega_1}{c_2^*\omega_2}\right). \quad \text{[S6.18]}$$

Because $\omega_2 - \omega_1 < 0$, $t_{\max} > 0$ requires that the argument of the logarithmic function above is positive but less than 1. With some algebraic manipulation, this requirement simplifies to

$$0 < \frac{k_1S + k_2 + k_d^* - k_d - \sqrt{(k_1S + k_2 + k_d^* - k_d)^2 - 4k_1S(k_d^* - k_d)}}{k_1S + k_2 + k_d^* - k_d + \sqrt{(k_1S + k_2 + k_d^* - k_d)^2 - 4k_1S(k_d^* - k_d)}} \leq 1. \quad \text{[S6.19]}$$

The above inequality is satisfied when $k_d^* > k_d$. That is, $X^*(t)$ attains a maximum at this time. To repeatthe same analysis for $X(t)$, it is easy to see that $X(t)$ decreases in right after $t = 0$, so the non-monotonocity would be a minimum. Let this occur at time $t_{\min}$, which is solution to

$$\frac{dX}{dt} = 0, \quad \text{[S6.20]}$$

giving the inequality

$$0 < \frac{k_1S + k_2 + k_d - k_d^* - \sqrt{(k_1S + k_2 + k_d - k_d^*)^2 - 4k_2(k_d - k_d^*)}}{k_1S + k_2 + k_d - k_d^* + \sqrt{(k_1S + k_2 + k_d - k_d^*)^2 - 4k_2(k_d - k_d^*)}} \leq 1. \quad \text{[S6.21]}$$

This inequality is satisfied when $k_d > k_d^*$.

Thus our analysis shows that the difference between k_d and k_d^* gives rise to non-monotonicity. In particular, when $k_d > k_d^*$, then we have that $X(t)$ first undershoots and then increases to its steady-state. Likewise when $k_d^* > k_d$, then $X^*(t)$ first overshoots and then decrease to its steady-state.

S6-D. Response times. We compute the 'approximate' response time using the frequency domain approach. To this end, the Laplace transforms of the error signals, $E_X = \overline{X} - X$ and $E_{X^*} = \overline{X^*} - X^*$, are

$$\mathcal{E}_X[\omega] = \frac{\overline{X}}{\omega} - \mathcal{X}[\omega] = \frac{k_p(k_2 + k_d^*)}{\kappa^2}\frac{2\zeta\kappa\omega + \kappa^2}{\omega^2 + 2\zeta\kappa\omega + \kappa^2} - \frac{\frac{k_p}{k_d}(\omega + k_2 + k_d^*) + k_p}{\omega^2 + 2\zeta\kappa\omega + \kappa^2}$$
$$= \frac{k_p}{k_d\kappa^2}\frac{\left(2\zeta\kappa(k_2k_d + k_dk_d^*) - \kappa^2\left(k_2 + k_d + k_d^*\right)\right) + (k_dk_d^* + k_2k_d - \kappa^2)\omega}{(\omega^2 + 2\zeta\kappa\omega + \kappa^2)} \quad \text{[S6.22]}$$

$$\mathcal{E}_{X^*}[\omega] = \frac{\overline{X^*}}{\omega} - \mathcal{X}^*[\omega] = \frac{k_1k_pS}{\kappa^2\omega} - \left(\frac{\frac{k_1k_pS}{k_d}}{\omega^2 + 2\zeta\kappa\omega + \kappa^2} + \frac{k_1k_pS}{\omega(\omega^2 + 2\zeta\kappa\omega + \kappa^2)}\right) = \frac{k_1k_pS}{k_d\kappa^2}\frac{(\omega + 2\zeta\kappa)k_d - \kappa^2}{(\omega^2 + 2\zeta\kappa\omega + \kappa^2)}. \quad \text{[S6.23]}$$

The response times for these are given by

$$\mathcal{T}_X = \frac{2\zeta}{\kappa} + \frac{k_2(k_d + k_d^*) - \kappa^2}{(k_2 + k_d + k_d^*)\kappa^2 - 2\zeta\kappa(k_2k_d + k_dk_d^*)} = \frac{k_1S + k_2 + k_d + k_d^*}{(k_1S + k_d)(k_2 + k_d^*) - k_1Sk_2} - \frac{k_d^*}{k_2(k_d^* - k_d) + k_d^{*2}}, \quad \text{[S6.24]}$$

$$\mathcal{T}_{X^*} = \frac{2\zeta}{\kappa} + \frac{k_d}{\kappa^2 - 2k_d\zeta\kappa} = \frac{k_1S + k_2 + k_d + k_d^*}{(k_1S + k_d)(k_2 + k_d^*) - k_1Sk_2} + \frac{k_d}{k_1S(k_d^* - k_d) - k_d^2}. \quad \text{[S6.25]}$$

In the special case where $k_d = k_d^*$, we have that

$$\mathcal{T}_X = \mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2 + k_d}. \quad \text{[S6.26]}$$

Next, we compute the response time for the switch. The differential equation that governs Y^* in Eq. (S6.1) consists of two nonlinear terms, XY^* and X^*Y^*. Linearizing around steady-state solution $(\overline{X}, \overline{X^*}, \overline{Y^*})$ leads to

$$XY^* \approx \overline{Y^*}X + \overline{X}Y^* - \overline{X}\,\overline{Y^*}, \quad \text{[S6.27a]}$$

$$X^*Y^* \approx \overline{Y^*}X^* + \overline{X^*}Y^* - \overline{X^*}\,\overline{Y^*}, \quad \text{[S6.27b]}$$

where

$$\overline{X} = \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}\frac{k_2+k_d^*}{k_1}}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}, \quad \text{[S6.28]}$$

$$\overline{X^*} = \frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}S}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}, \quad \text{[S6.29]}$$

$$\overline{Y^*} = \frac{k_3 + k_5\frac{\frac{k_p}{k_d}\frac{k_d}{k_d^*}S}{S+\frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}}}{S + \frac{k_2+k_d^*}{k_1}\frac{k_d}{k_d^*}\frac{k_3+k_4+k_6\frac{k_p}{k_d}}{k_3+k_4+k_5\frac{k_p}{k_d}\frac{k_d}{k_d^*}}}Y_T. \quad \text{[S6.30]}$$

Substituting these in Eq. (S6.1) yields

$$\frac{dX}{dt} = k_p - k_d X - k_1 S X + k_2 X^* \quad \text{[S6.31a]}$$

$$\frac{dX^*}{dt} = k_1 S X - k_2 X^* - k_d^* X^* \quad \text{[S6.31b]}$$

$$\frac{dY^*}{dt} = k_3 Y_T + k_5 Y_T X^* - (k_3 + k_4) Y^* - k_5\left(\overline{Y^*}X^* + \overline{X^*}Y^* - \overline{X^*}\,\overline{Y^*}\right) - k_6\left(\overline{Y^*}X + \overline{X}Y^* - \overline{X}\,\overline{Y^*}\right). \quad \text{[S6.31c]}$$

The last equation above can be rearranged as

$$\frac{dY^*}{dt} = \left(k_3 Y_T + k_5\overline{X^*}\,\overline{Y^*} + k_6\overline{X}\,\overline{Y^*}\right) + k_5\left(Y_T - \overline{Y^*}\right)X^* - k_6\overline{Y^*}X - \left(k_3 + k_4 + k_5\overline{X^*} + k_6\overline{X}\right)Y^*, \quad \text{[S6.31d]}$$

which has the same form as the two-tier linear system with two inputs in Eq. (S2.28) with $\xi_0 = k_3 Y_T + k_5\overline{X^*}\,\overline{Y^*} + k_6\overline{X}\,\overline{Y^*}$, $\xi_{11} = -k_6 Y^*$, $\xi_{12} = k_5(Y_T - Y^*)$, and $\xi_2 = k_3 + k_4 + k_5\overline{X^*} + k_6\overline{X}$. Using Eq. (S2.33d), we can write the response time as

$$\mathcal{T}_{Y^*} = \frac{1}{\xi_2} + \frac{\xi_{11}\mathcal{T}_X\mathcal{E}_X[0] + \xi_{12}\mathcal{T}_{X^*}\mathcal{E}_{X^*}[0]}{\overline{Y^*} - Y(0) + \xi_{11}\mathcal{E}_X[0] + \xi_{12}\mathcal{E}_{X^*}[0]}. \quad \text{[S6.32]}$$

We compute $\mathcal{E}_X[0]$ from Eq. (S6.22) and $\mathcal{E}_{X^*}[0]$ from Eq. (S6.23)

$$\mathcal{E}_0[\omega] = \frac{k_p}{k_d\kappa^2}\frac{\left(2\zeta\kappa(k_2k_d + k_dk_d^*) - \kappa^2\left(k_2 + k_d + k_d^*\right)\right)}{\kappa^2} \quad \text{[S6.33]}$$

$$\mathcal{E}_{X^*}[0] = \frac{k_1k_pS}{k_d\kappa^2}\frac{2\zeta\kappa k_d - \kappa^2}{\kappa^2}. \quad \text{[S6.34]}$$

S7. Receptor kinetic proofreading

Let us consider a scenario in which the active form of the receptor undergoes n states as in $X_1^*, X_2^*, \ldots, X_n^*$. We assume that the ligand binding occurs at a rate $k_1 S$ whereas ligand unbinding from all the states occurs at the rate k_2. The forward transition through the states occurs through the rate k_f (Fig. S7.1).

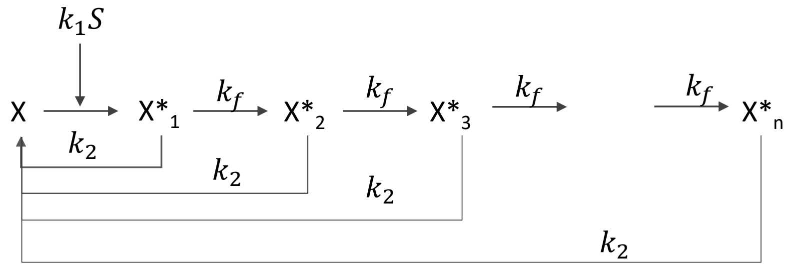

Fig. S7.1. A scheme for receptor kinetic proofreading

The ODE for this model are given by

$$\frac{dX_1^*}{dt} = k_1 S\left(X_T - \sum_{i=1}^{n} X_i^*\right) - k_2 X_1^* - k_f X_1^* \quad \text{[S7.1a]}$$

$$\frac{dX_2^*}{dt} = k_f X_1^* - k_2 X_2^* - k_f X_2^* \quad \text{[S7.1b]}$$

$$\frac{dX_3^*}{dt} = k_f X_2^* - k_2 X_3^* - k_f X_3^* \quad \text{[S7.1c]}$$

$$\vdots \qquad \vdots \quad \text{[S7.1d]}$$

$$\frac{dX_n^*}{dt} = k_f X_{n-1}^* - k_2 X_n^*. \quad \text{[S7.1e]}$$

Let us first compute the steady-state solution. Let us denote $\epsilon = \frac{k_f}{k_f + k_2}$. We start by computing the solutions X_2^* onward. We note that

$$X_2^* = \epsilon X_1^* \quad \text{[S7.2a]}$$

$$X_3^* = \epsilon X_2^* = \epsilon^2 X_1^* \quad \text{[S7.2b]}$$

$$\vdots \qquad \vdots \quad \text{[S7.2c]}$$

$$X_{n-1}^* = \epsilon X_{n-2}^* = \epsilon^{n-2} X_1^*. \quad \text{[S7.2d]}$$

First, we compute the steady-state for X_n^* in terms of X_{n-1}^*:

$$X_n^* = \frac{k_f}{k_2} X_{n-1}^*. \quad \text{[S7.3]}$$

Substituting these gives

$$\overline{X_1^*} = \frac{k_1 S X_T}{k_1 S\left(1 + \epsilon + \epsilon^2 + \ldots + \epsilon^{n-2} + \frac{\epsilon^{n-1}}{1-\epsilon}\right) + k_2 + k_f} \quad \text{[S7.4]}$$

$$= \frac{k_1 S X_T}{\frac{k_1 S}{1-\epsilon} + k_2 + k_f} \quad \text{[S7.5]}$$

$$= \frac{k_1\left(1 - \frac{k_f}{k_2 + k_f}\right) S X_T}{k_1 S + (k_2 + k_f)\left(1 - \frac{k_f}{k_2 + k_f}\right)} \quad \text{[S7.6]}$$

$$= \frac{\frac{k_2}{k_2 + k_f} S X_T}{S + \frac{k_2}{k_1}} \quad \text{[S7.7]}$$

Rest of the steady-states may be computed as below.

$$\overline{X_2^*} = \frac{k_2 k_f}{(k_2+k_f)^2}\frac{SX_T}{S+\frac{k_2}{k_1}} \quad \text{[S7.8]}$$

$$\overline{X_3^*} = \frac{k_2 k_f^2}{(k_2+k_f)^3}\frac{SX_T}{S+\frac{k_2}{k_1}} \quad \text{[S7.9]}$$

$$\ldots \qquad \ldots \quad \text{[S7.10]}$$

$$\overline{X_{n-1}^*} = \frac{k_2 k_f^{n-2}}{(k_2+k_f)^{n-1}}\frac{SX_T}{S+\frac{k_2}{k_1}} \quad \text{[S7.11]}$$

$$\overline{X_n^*} = \frac{k_f^{n-1}}{(k_2+k_f)^{n-1}}\frac{SX_T}{S+\frac{k_2}{k_1}}. \quad \text{[S7.12]}$$

The total amount of receptors that are ligated is given by the sum $\sum_{i=1}^{n}\overline{X_i^*}$. We compute it below.

$$\sum_{i=1}^{n}\overline{X_i^*} = \frac{SX_T}{S+\frac{k_2}{k_1}}, \quad \text{[S7.13]}$$

which is independent of k_f.

An interesting point to note is that if only X_n^* is signaling competent, then having intermediate steps only affects the overall response in terms of amplitude, but not its shape. Thus, our dose-response curves generalize to the scenario when there is kinetic proofreading.

We also note that the ratio of the signaling competent complex with the sum of all ligated complexes is

$$\frac{X_n^*}{\sum_{i=1}^{n}\overline{X_i^*}} = \frac{k_f^{n-1}}{(k_2+k_f)^{n-1}}, \quad \text{[S7.14]}$$

which is consistent with previous results (13–15).

S7-A. Dose-response of the switch. We consider the following dynamics through which the receptor affects the switch.

$$\frac{dY^*}{dt} = (k_3 + k_5 X_n^*)(Y_T - Y^*) - (k_4 + k_6(X_T - X_n^*))Y^*. \quad \text{[S7.15]}$$

Here we assume that all receptor states are capable of repressing the downstream switch, except X_n^* which is the only competent state for activating the downstream switch (section SI). Moreover, all receptors are assumed to be unligated before the arrival of the stimulus. Therefore, the initial conditions are $X_i^* = 0\ \forall i \in \{1,2,\ldots,n\}$ and $Y^* = \frac{k_3 Y_T}{k_3+k_4+k_6 X_T}$.

The steady-state response for the switch is then given by

$$\overline{Y^*} = \frac{\frac{k_2 k_3}{k_1(k_3+k_4+k_5' X_T)} + \frac{k_3 + k_5' X_T}{k_3+k_4+k_5' X_T}S}{\frac{k_2(k_3+k_4+k_6 X_T)}{k_1(k_3+k_4+k_5' X_T)} + S}Y_T, \quad \text{[S7.16]}$$

where $k_5' = \frac{k_5 k_f^{n-1}}{(k_2+k_f)^{n-1}}$. Thus, all properties of the dose-response curve are similar to that of the two-tier model in Fig. 1 in the main text, except for activation strength being $k_5' X_T$.

S7-B. Response times of receptor states. Taking Laplace transform of the system of equations, we obtain:

$$\omega\mathcal{X}^*{}_1[\omega] = \frac{k_1 S X_T}{\omega} - k_1 S\sum_{i=1}^{n}\mathcal{X}^*{}_i[\omega] - k_2\mathcal{X}^*{}_1[\omega] - k_f\mathcal{X}^*{}_1[\omega] \quad \text{[S7.17]}$$

$$\omega\mathcal{X}^*{}_2[\omega] = k_f\mathcal{X}^*{}_1[\omega] - k_2\mathcal{X}^*{}_2[\omega] - k_f\mathcal{X}^*{}_2[\omega] \quad \text{[S7.18]}$$

$$\omega\mathcal{X}^*{}_3[\omega] = k_f\mathcal{X}^*{}_2[\omega] - k_2\mathcal{X}^*{}_3[\omega] - k_f\mathcal{X}^*{}_3[\omega] \quad \text{[S7.19]}$$

$$\vdots \qquad \vdots \quad \text{[S7.20]}$$

$$\omega\mathcal{X}^*{}_n[\omega] = k_f\mathcal{X}^*{}_{n-1}[\omega] - k_2\mathcal{X}^*{}_n[\omega] \quad \text{[S7.21]}$$

[S7.22]

Here we have assumed that there are no active receptors before arrival of the stimulus. We can solve the algebraic equations iteratively. To this end, let $\epsilon_w = \frac{k_f}{\omega+k_2+k_f}$. Then, we have that

$$\mathcal{X}^*{}_2[\omega] = \epsilon_w \mathcal{X}^*{}_1[\omega] \tag{S7.23}$$
$$\mathcal{X}^*{}_3[\omega] = \epsilon_w \mathcal{X}^*{}_2[\omega] = \epsilon_w^2 \mathcal{X}^*{}_1[\omega] \tag{S7.24}$$
$$\vdots \qquad \vdots \tag{S7.25}$$
$$\mathcal{X}^*{}_{n-1} = \epsilon_w \mathcal{X}^*{}_{n-2} = \epsilon^{n-2} \mathcal{X}^*{}_1. \tag{S7.26}$$

Moreover, $\mathcal{X}^*{}_n = \frac{k_f}{\omega+k_f}\mathcal{X}^*{}_{n-1}$. Solving for $\mathcal{X}_1[\omega]$

$$\mathcal{X}_1[\omega] = \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)}. \tag{S7.27}$$

The rest of term are

$$\mathcal{X}^*{}_2[\omega] = \frac{k_f}{\omega + k_2 + k_f} \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)} \tag{S7.28}$$
$$\mathcal{X}^*{}_3[\omega] = \left(\frac{k_f}{\omega + k_2 + k_f}\right)^2 \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)} \tag{S7.29}$$
$$\vdots \qquad \vdots \tag{S7.30}$$
$$\mathcal{X}^*{}_{n-1} = \left(\frac{k_f}{\omega + k_2 + k_f}\right)^{n-2} \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)} \tag{S7.31}$$

Finally $\mathcal{X}^*{}_n[\omega]$ is

$$\mathcal{X}^*{}_n[\omega] = \frac{k_f}{\omega + k_2} \left(\frac{k_f}{\omega + k_2 + k_f}\right)^{n-2} \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)} \tag{S7.32}$$

To compute the response time of X_n^*, we find the error signal as

$$\mathcal{E}_{X_n^*}[\omega] = \frac{\overline{X_n^*}}{\omega} - \mathcal{X}^*{}_n[\omega] \tag{S7.33}$$
$$= \frac{k_f^{n-1}}{(k_2 + k_f)^{n-1}} \frac{k_1 S X_T}{k_1 S + k_2} \frac{1}{\omega} - \frac{k_f}{\omega + k_2} \left(\frac{k_f}{\omega + k_2 + k_f}\right)^{n-2} \frac{k_1 S X_T}{\omega} \frac{\omega + k_2}{(\omega + k_2 + k_f)(\omega + k_1 S + k_2)} \tag{S7.34}$$
$$= \frac{k_f^{n-1}}{(k_2 + k_f)^{n-1}} \frac{k_1 S X_T}{k_1 S + k_2} \left(\frac{k_1 S + k_2}{\omega + k_1 S + k_2} + \frac{1}{\omega + k_1 S + k_2} \sum_{i=0}^{n-2} \frac{(k_2 + k_f)^i}{(\omega + k_2 + k_f)^{i+1}}\right) \tag{S7.35}$$

Note that here we have assumed $n \geq 2$. The case of $n = 1$ is same as the toy-model, so we do not consider it here. We can now compute the response time of $\mathcal{X}^*{}_n$ as

$$\mathcal{T}_{X_n^*} = -\left.\frac{d \log\left(\mathcal{E}_{X_n^*}[\omega]\right)}{d\omega}\right|_{\omega=0} = \frac{1}{k_1 S + k_2} + \frac{n}{2} \frac{1}{k_2 + k_f} \frac{(n-1)(k_1 S + k_2)}{(k_2 + k_f) + (n-1)(k_1 S + k_2)}. \tag{S7.36}$$

S7-C. Response time for switch. The differential equation that governs Y^* consists of the nonlinear term, $X_n^* Y^*$. Linearizing around steady-state solution $(\overline{X^*}_n, \overline{Y^*})$ leads to

$$X_n^* Y^* \approx \overline{Y^*} X_n^* + \overline{X^*}_n Y^* - \overline{X^*}_n \, \overline{Y^*}. \tag{S7.37}$$

This results in dynamics that resembles the one studied in the section S2-B. The response time is thus given by

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_3 + k_4 + k_6 X_T + (k_5' - k_6)\frac{k_1 S X_T}{k_1 S + k2}} + \frac{1}{k_1 S + k_2} \times \frac{k_3 + k_4 + k_6 X_T + (k_5' - k_6)\frac{k_1 S X_T}{k_1 S + k2}}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T + (k_5' - k_6)\frac{k_1 S X_T}{k_1 S + k2}}. \tag{S7.38}$$

S8. Alternating activation and derepression

In this section, we consider signaling cascades consisting of alternating activation and derepression based switches. The first cascade is shown in Fig. S8.1(a). It is built upon the activation mechanism of Fig. 1(a) in the main text, where the receptor activates a downstream switch ($Y \rightleftarrows Y^*$). We add a downstream switch ($Z \rightleftarrows Z^*$) which is derepressed. The second cascade, shown in Fig. S8.1(b), is a modification of the derepression mechanism of Fig. 1(b) in the sense that a downstream component is now activated by the derepressed switch.

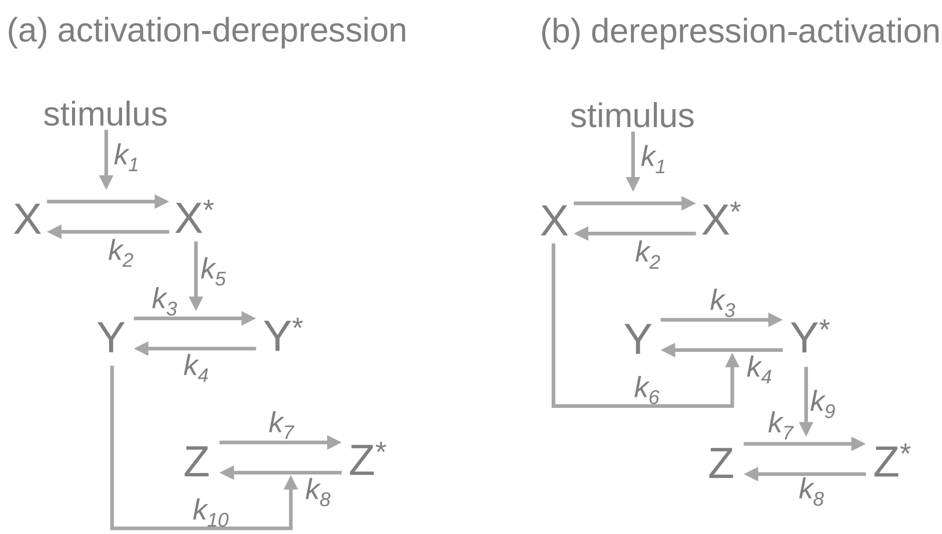

Fig. S8.1. Three tier cascades with alternating activation and derepression mechanisms

S8-A. Activation followed by derepression. The ODEs that govern the dynamics of this cascade are

$$\frac{dX^*}{dt} = k_1 S X_T - (k_1 S + k_2) X^* \qquad \text{[S8.1a]}$$

$$\frac{dY^*}{dt} = (k_3 + k_5 X^*)(Y_T - Y^*) - k_4 Y^* \qquad \text{[S8.1b]}$$

$$\frac{dZ^*}{dt} = k_7 (Z_T - Z^*) - (k_8 + k_{10}(Y_T - Y^*)) Z^* \qquad \text{[S8.1c]}$$

We obtain the steady-states by setting each of the derivatives to zero. We express each of the steady-states in a similar form as that of Eq. (3) in the main text

$$R = \frac{R_0 \Theta_R + R_\infty S}{\Theta_R + S}. \qquad \text{[S8.2]}$$

For example, steady-state of X^* is specified by

$$X_0^* = 0, \qquad \text{[S8.3a]}$$

$$X_\infty^* = X_T, \qquad \text{[S8.3b]}$$

$$\Theta_{X^*} = \frac{k_2}{k_1}. \qquad \text{[S8.3c]}$$

The steady-state of Y^* is specified by

$$Y_0^* = \frac{k_3}{k_3 + k_4} Y_T, \qquad \text{[S8.4a]}$$

$$Y_\infty^* = \frac{k_3 + k_5 X_T}{k_3 + k_4 + k_5 X_T} Y_T, \qquad \text{[S8.4b]}$$

$$\Theta_{Y^*} = \Theta_{X^*} \frac{k_3 + k_4}{k_3 + k_4 + k_5 X_T} < \Theta_{X^*}. \qquad \text{[S8.4c]}$$

As expected, activation caused the dose-response of $\overline{Y^*}$ to shift towards left in comparison with that of $\overline{X^*}$, i.e., $\Theta_{Y^*} < \Theta_{X^*}$. Finally, the steady-state of Z^* is specified by

$$Z_0^* = \frac{k_7 Z_T}{k_7 + k_8 + k_{10} \frac{k_4 Y_T}{k_3 + k_4}}, \qquad \text{[S8.5a]}$$

$$Z_\infty^* = \frac{k_7 Z_T}{k_7 + k_8 + k_{10} \frac{k_4 Y_T}{k_3 + k_4 + k_5 X_T}}, \qquad \text{[S8.5b]}$$

$$\Theta_{Z^*} = \Theta_{Y^*} \frac{k_7 + k_8 + k_{10} \frac{k_4 Y_T}{k_3 + k_4}}{k_7 + k_8 + k_{10} \frac{k_4 Y_T}{k_3 + k_4 + k_5 X_T}} > \Theta_{Y^*}. \qquad \text{[S8.5c]}$$

We observe that $\Theta_Z > \Theta_{Y^*}$. This means that the derepression layer has an opposite effect of activation and shifts the dose-response back towards right.

S8-B. Derepression followed by activation. The ODEs that govern the dynamics of this cascade are

$$\frac{dX^*}{dt} = k_1 S X_T - (k_1 S + k_2) X^* \qquad \text{[S8.6a]}$$

$$\frac{dY^*}{dt} = k_3 (Y_T - Y^*) - (k_4 + k_6 (X_T - X^*)) Y^* \qquad \text{[S8.6b]}$$

$$\frac{dZ^*}{dt} = (k_7 + k_9 Y^*)(Z_T - Z^*) - k_8 Z^* \qquad \text{[S8.6c]}$$

For this model, the steady-state of $\overline{X^*}$ has the same specification as Eq. (S8.3). The steady-state $\overline{Y^*}$ is prescribed by

$$Y_0^* = \frac{k_3}{k_3 + k_4 + k_6 X_T} Y_T, \qquad \text{[S8.7a]}$$

$$Y_\infty^* = \frac{k_3}{k_3 + k_4} Y_T, \qquad \text{[S8.7b]}$$

$$\Theta_{Y^*} = \Theta_X \frac{k_3 + k_4 + k_6 X_T}{k_3 + k_4} > \Theta_{X^*}. \qquad \text{[S8.7c]}$$

Because $\Theta_{Y^*} > \Theta_{X^*}$, the dose response of Y^* is towards the right to that of X^*. This results from the fact that this switch is governed by a derepression mechanism. We now look at the parameters specifying $\overline{Z^*}$:

$$Z_0^* = \frac{k_7 + k_9 \frac{k_3 Y_T}{k_3+k_4+k_6 X_T}}{k_7 + k_8 + k_9 \frac{k_3 Y_T}{k_3+k_4+k_6 X_T}} Z_T, \tag{S8.8a}$$

$$Z_\infty^* = \frac{k_7 + k_9 \frac{k_3 Y_T}{k_3+k_4}}{k_7 + k_8 + k_9 \frac{k_3 Y_T}{k_3+k_4}} Z_T, \tag{S8.8b}$$

$$\Theta_{Z^*} = \Theta_{Y^*} \frac{k_7 + k_8 + k_9 \frac{k_3 Y_T}{k_3+k_4+k_6 X_T}}{k_7 + k_8 + k_9 \frac{k_3 Y_T}{k_3+k_4}} < \Theta_{Y^*}. \tag{S8.8c}$$

We see that $\Theta_{Z^*} < \Theta_{Y^*}$. So, the dose-response of Z^* is towards the left of Y^*, which implies that activation of the third layer counteracts the shifting caused of derepression of the second layer. It is important to point out that the effects of these mechanisms on Z_0^* and Z_∞^* are different. A systematic analysis of these effects on alternating cascades will be carried out in a future work.

S8-C. Response time for a representative three-tier system. Let us consider the following representative system of ordinary differential equations

$$\frac{dR_1}{dt} = \eta_0 - \eta_1 R_1, \tag{S8.9a}$$

$$\frac{dR_2}{dt} = \xi_0 + \xi_1 R_1 - \xi_2 R_2, \tag{S8.9b}$$

$$\frac{dR_3}{dt} = \nu_0 + \nu_1 R_2 - \nu_2 R_3, \tag{S8.9c}$$

with initial condition

$$(R_1(0), R_2(0), R_3(0)) = \left(0, \frac{\xi_0}{\xi_2}, \frac{\nu_0 + \nu_1 \frac{\xi_0}{\xi_2}}{\nu_2}\right). \tag{S8.9d}$$

Taking Laplace transforms, we obtain the following

$$\mathcal{R}_1[\omega] = \frac{\eta_0}{\eta_1}\left(\frac{1}{\omega} - \frac{1}{\omega+\eta_1}\right) - \frac{R_1(0)}{\omega+\eta_1}, \tag{S8.10a}$$

$$\mathcal{R}_2[\omega] = \frac{\xi_0}{\xi_2}\left(\frac{1}{\omega} - \frac{1}{\omega+\xi_2}\right) + \frac{\eta_0\xi_1}{\eta_1\xi_2}\left(\frac{1}{\omega} - \frac{\omega+\eta_1+\xi_2}{(\omega+\eta_1)(\omega+\xi_2)}\right) + \frac{\xi_1 R_1(0)}{(\omega+\eta_1)(\omega+\xi_2)} + \frac{R_2(0)}{\omega+\xi_2}, \tag{S8.10b}$$

$$\mathcal{R}_3[\omega] = \frac{\nu_0}{\nu_2}\left(\frac{1}{\omega} - \frac{1}{\omega+\nu_2}\right) + \frac{\nu_1}{\omega+\nu_2}\mathcal{R}_2[\omega] + \frac{R_3(0)}{\omega+\nu_2}, \tag{S8.10c}$$

where $\mathcal{R}_1[\omega]$ and $\mathcal{R}_2[\omega]$ are same as those in Eq. (S2.22).

The response times for $\mathcal{R}_1[\omega]$ and $\mathcal{R}_2[\omega]$ are same as those in Eq. (S2.26). The response time for $\mathcal{R}_3[\omega]$ may be computed in the same manner by defining an error signal $E_{R_3} = \overline{R_3} - R_3$. Its Laplace transform is then given by

$$\mathcal{E}_{R_3}[\omega] = \frac{\nu_0 + \nu_1\overline{R_2} - R_3(0)}{\omega+\nu_2} + \frac{\nu_1}{\omega+\nu_2}\mathcal{E}_{R_2}[\omega]. \tag{S8.11}$$

With some algebraic manipulation, the response time may be computed as

$$\mathcal{T}_{R_3} = -\left.\frac{d\log\left(\mathcal{E}_{R3}[\omega]\right)}{d\omega}\right|_{\omega=0} = \frac{1}{\nu_2} + \frac{\mathcal{T}_{R_2}}{1 + \frac{\eta_1\xi_2}{(\eta+\xi_2)\nu_2}}. \tag{S8.12a}$$

S8-D. Response time for activation followed by derepression. To compute the response time, we linearize the dynamics of Y^* and Z^* around $(\overline{X^*}, \overline{Y^*}, \overline{Z^*})$. With this linearization, we have that $\eta_0 = k_1 S X_T, \eta_1 = k_1 S + k_2$, $\xi_0 = k_3 Y_T + k_5 \overline{X^*}\,\overline{Y^*}$, $\xi_1 = k_5(Y_T - \overline{Y^*})$, $\xi_2 = k_3 + k_4 + k_5\overline{X^*}$, $\nu_0 = k_7 Z_T - k_{10}\overline{Y^* Z^*}$, $\nu_1 = k_{10}\overline{Z^*}$, and $\nu_2 = k_7 + k_8 + k_{10}(Y_T - \overline{Y^*})$. With these parameters, it is straightforward to see that $1/\nu_2$ is an increasing function of S, which is expected as derepression is operating at Z^*. Our analysis of response time of two-tier cascade has already shown that $\mathcal{T}_{R_2}$ is a decreasing function of S for this activation at Y^*. The time-averaging term is upper-bounded by 1. Therefore, the response time varies less strongly with stimulus as it is sum of two functions, one increasing and another decreasing.

S8-E. Response time for derepression followed by activation. With the same arguments as above, we have that $1/\nu_2$ decreases with the stimulus, whereas $\mathcal{T}_{R_2}$ increases with stimulus. Thus, the response time varies less strongly with stimulus.

S9. Noise analysis for general upstream kinetics

Our analysis of the stochastic two-tier model in the section S4 assumed that the total number of receptors are conserved. Thus, dynamics of the statistical moments of active receptors, X^*, are sufficient to describe the dynamics of X and X^*. In practice, X and X^* may be more complicated, as the receptor dynamics may involve receptor removal (section S6) or kinetic proofreading (section S7), etc. In a more general scenario, we can generalize such a model such that X and X^* are two correlated stochastic processes that do not depend of Y^*.

Our goal here is to understand how such a generalization affects the abilities of activation, derepression, and concerted mechanisms to process upstream fluctuations. Let $(X_{det}, X^*_{det}, Y^*_{det})$ be the steady-state solution of the deterministic description of the model. Linearizing the transition rates of $Y \to Y^*$ and $Y^* \to Y$ around $(X_{det}, X^*_{det}, Y^*_{det})$ gives

$$(k_3 + k_5 X^*)(Y_T - Y^*) \approx (k_3 + k_5 X^*_{det})(Y_T - Y^*_{det}) + k_5(Y_T - Y^*_{det})\left(X^* - X^*_{det}\right) - (k_3 + k_5 X^*_{det})\left(Y^* - Y^*_{det}\right) \quad \text{[S9.1a]}$$

$$(k_4 + k_6 X)Y^* \approx (k_4 + k_6 X_{det})Y^*_{det} + k_6 Y^*_{det}(X - X_{det}) + (k_4 + k_6 X_{det}))(Y^* - Y^*_{det}) \quad \text{[S9.1b]}$$

With these linearized rates, we have that $\langle Y^* - Y^*_{det}\rangle = 0$, giving the following in steady-state:

$$(k_3 + k_5 X^*_{det})(Y_T - Y^*_{det}) - (k_4 + k_6 X_{det})Y^*_{det} = 0, \quad \text{[S9.2]}$$

where we have also used $\langle X - X_{det}\rangle = 0$ and $\langle X^* - X^*_{det}\rangle = 0$.

Further, the second centered moment (i.e., variance) of Y^* in steady-state satisfies

$$\frac{d\left\langle (Y^* - Y^*_{det})^2\right\rangle}{dt} \approx$$
$$\left\langle \left((k_3 + k_5 X^*_{det})(Y_T - Y^*_{det}) + k_5(Y_T - Y^*_{det})\left(X^* - X^*_{det}\right) - (k_3 + k_5 X^*_{det})\left(Y^* - Y^*_{det}\right)\right)\left(1 + 2Y^* - 2Y^*_{det}\right)\right\rangle$$
$$\left\langle \left((k_4 + k_6 X_{det})Y^*_{det} + k_6 Y^*_{det}(X - X_{det}) + (k_4 + k_6 X_{det}))(Y^* - Y^*_{det})\right)\left(1 - 2Y^* + 2Y^*_{det}\right)\right\rangle. \quad \text{[S9.3a]}$$

Using the fact the centered means are zero for X, X^* and Y^*, we get

$$\frac{d\left\langle (Y^* - Y^*_{det})^2\right\rangle}{dt} \approx (k_3 + k_5 X^*_{det})(Y_T - Y^*_{det}) + (k_4 + k_6 X_{det})Y^*_{det} + 2k_5(Y_T - Y^*_{det})\left\langle \left(X^* - X^*_{det}\right)\left(Y^* - Y^*_{det}\right)\right\rangle$$
$$- 2k_6 Y^*_{det}\left\langle (X - X_{det})\left(Y^* - Y^*_{det}\right)\right\rangle - 2\left(k_3 + k_4 + k_5 X^*_{det} + k_6 X_{det}\right)\left\langle (Y^* - Y^*_{det})^2\right\rangle. \quad \text{[S9.3b]}$$

Thus, we obtain the steady-state variance

$$\left\langle (Y^* - Y^*_{det})^2\right\rangle \approx \frac{(k_3 + k_5 X^*_{det})(Y_T - Y^*_{det}) + (k_4 + k_6 X_{det})Y^*_{det}}{2\left(k_3 + k_4 + k_5 X^*_{det} + k_6 X_{det}\right)} + \frac{k_5(Y_T - Y^*_{det})\left\langle \left(X^* - X^*_{det}\right)\left(Y^* - Y^*_{det}\right)\right\rangle}{k_3 + k_4 + k_5 X^*_{det} + k_6 X_{det}}$$
$$- \frac{k_6 Y^*_{det}\left\langle (X - X_{det})\left(Y^* - Y^*_{det}\right)\right\rangle}{k_3 + k_4 + k_5 X^*_{det} + k_6 X_{det}}. \quad \text{[S9.3c]}$$

Here $\left\langle \left(X^* - X^*_{det}\right)\left(Y^* - Y^*_{det}\right)\right\rangle$ is the correlation between X^* and Y^*, which is positive as increase in X^* increases Y^*. Moreover, $\left\langle (X - X_{det})\left(Y^* - Y^*_{det}\right)\right\rangle$ is the correlation between X and Y^*, which is negative as increase in X implies decrease in Y^*. A simple example to see this is the different signs of these correlations is the two-tier model where $X_{det} + X^*_{det} = X_T$, yielding

$$\left\langle (X - X_{det})\left(Y^* - Y^*_{det}\right)\right\rangle = -\left\langle \left(X^* - X^*_{det}\right)\left(Y^* - Y^*_{det}\right)\right\rangle. \quad \text{[S9.3d]}$$

We can now use Eq. (S9.3c) to show that activation and derepression mechanisms have less noise than a concerted mechanism. Note that noise, defined by the coefficient of variation, equals the variance over squared of mean. As long as we control of the mean across different signaling mechanisms, Eq. (S9.3c) is enough to compare their noise properties. To keep the same forward and backward transition rates of the switch, we keep $k_3 + k_5 X^*_{det}$ and $k_4 + k_6 X_{det}$ across the signaling mechanisms. In that case, it is straightforward to see that the variance decreases for activation ($k_6 = 0$) and for derepression ($k_5 = 0$).

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