Supporting Information: Kinetic Model for the Desensitization of G Protein-Coupled Receptor

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Goldbeter-Koshland zero-th order ultrasensitivity and derivation of c_T^* in Eq. 1

The key expression of this study, c_T^* (Eq. 1), is derived from the balance between the GEF and GAP activity-mediated processes. The expression bears the same mathematical structure with that of the phosphorylation-dephosphorylation cycle, formulated by Goldbeter and Koshland, which is known to yield ultrasensitive responses of substrates to the relative amount of two opposing enzymes.[1,2]

Here we derive a general expression equivalent to c_T^* by considering the chemical state

of a receptor catalyzed by phosphatase (P) with concentration P_o and kinase (K) with concentration K_o. When a large number of substrates (receptors) are present, such that the total concentration of phosphorylated (Z_p) and unphosphorylated receptors (Z) is greater than K_o and P_o, i.e., $Z_{tot} \gg K_o, P_o$ with $Z_{tot} = [Z] + [Z_p]$, we can assume that the system is in the Michaelis–Menten (MM) regime. The interconversion of the receptor between Z_p and Z is written as

$$Z_p \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Z. \tag{S1}$$

Z_p is dephosphorylated with the rate

$$r_1 = \frac{k_1 P_o [Z_p]}{K_1 + [Z_p]} = \frac{v_1(1-z)}{J_1 + (1-z)} \tag{S2}$$

where $z = [Z]/Z_{tot}$ with $v_1 = k_1 P_o$, and $J_1 = K_1/Z_{tot}$. Z is phosphorylated with the rate

$$r_2 = \frac{k_2 K_o [Z]}{K_2 + [Z]} = \frac{v_2 z}{J_2 + z} \tag{S3}$$

where $v_2 = k_2 K_o$ and $J_2 = K_2/Z_{tot}$. The concentration of unphosphorylated receptor at steady state, $z^* = [Z]_{ss}/Z_{tot}$, is decided from $r_1(z^*) = r_2(z^*)$,

$$\frac{v_1(1-z^*)}{J_1 + (1-z^*)} = \frac{v_2 z^*}{J_2 + z^*}. \tag{S4}$$

The state of the receptor changes in the range of $0 \leq z^* \leq 1$ in response to the relative rate of dephosphorylation and phosphorylation (or the relative amount of kinase and phosphatase) in the cell ($v_1/v_2 = k_1 P_o / k_2 K_o$). Physically, it is expected that the receptors are in the unphosphorylated state when the rate of dephosphorylation is greater than that of phosphorylation, and vice versa. This switch-like transition of z^* between $z^* \approx 0$ and $z^* \approx 1$ is

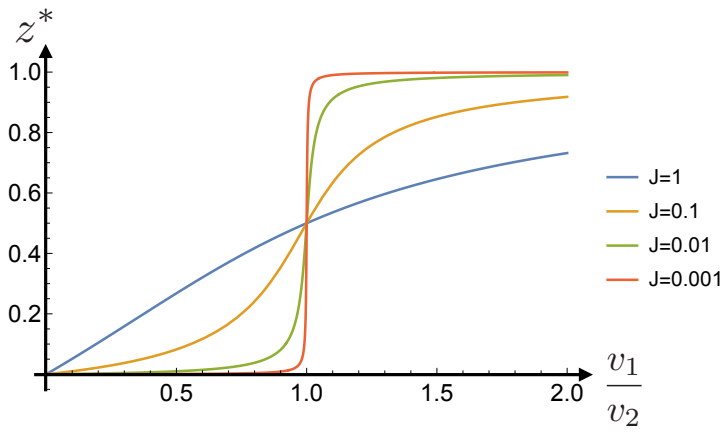

Figure S1: Plots of z^* versus v_1/v_2 for varying J (Eq. S5). This demonstrates the ultrasensitive response of substrate to the two opposing enzymes, especially at small J.

further clarified by rewriting Eq. S4 in the following form with an assumption of $J_1 = J_2 = J$,

$$\frac{v_1}{v_2} = \frac{z^*(J+1-z^*)}{(1-z^*)(J+z^*)} \approx \begin{cases} \frac{\varepsilon(J+1)}{J} & \text{for } v_1/v_2 \ll 1 \\ \frac{(1-\varepsilon)(J+\varepsilon)}{\varepsilon(J+1)} & \text{for } v_1/v_2 \gg 1 \end{cases} \tag{S5}$$

with $\varepsilon \ll 1$, and can more explicitly be demonstrated by plotting z^* as a function of v_1/v_2 (Fig. S1). The first and the second derivatives at the transition point $v_1/v_2 = 1$

$$\left(\frac{dz^*}{d(v_1/v_2)}\right)_{\frac{v_1}{v_2}=1} = \frac{1}{4}\left(1 + \frac{1}{2J}\right) \tag{S6}$$

and

$$\left|\frac{d^2z^*}{d(v_1/v_2)^2}\right|_{\frac{v_1}{v_2}=1} = \frac{1}{4}\left(1 + \frac{1}{2J^2}\right) \tag{S7}$$

indicate that small J value sharpens the transition between $z^* = 0$ and $z^* = 1$.

Solving Eq. S4 for z^* yields the Goldbeter's formula for the zero-th order ultrasensitivity:

$$z^* = \frac{2v_1J_2}{B + \sqrt{B^2 - 4(v_2 - v_1)v_1J_2}} \tag{S8}$$

where $B = v_1 J_2 + v_2 J_1 + v_2 - v_1$. Note that only one of the two solutions from the quadratic equation is physically relevant since $0 \leq z^* \leq 1$. The term inside the square root of Eq. S8

$$\begin{aligned}\Delta &\equiv B^2 - 4(v_2 - v_1)v_1 J_2 \\ &= \underbrace{(J_2+1)^2}_{\equiv a} v_1^2 + \underbrace{(J_1+1)^2}_{\equiv b} v_2^2 + 2\underbrace{\{J_1 J_2 - (J_1 + J_2) - 1\}}_{\equiv c} v_1 v_2 \\ &= a\left(v_1 + \frac{c}{a} v_2\right)^2 + \left(\frac{ab - c^2}{a}\right) v_2^2 \end{aligned} \tag{S9}$$

is positive for all values of v_1, v_2, J_1, $J_2 > 0$, because a, $b > 0$ and $ab - c^2 = 4J_1 J_2 (J_1 + J_2 + 1) > 0$.

Along with the inequalities $\left|\frac{4(v_2 - v_1)v_1 J_2}{B^2}\right| < \left|\frac{4(v_2 - v_1)v_1 J_2}{(v_1 J_2 + v_2 - v_1)^2}\right| \leq \left|\frac{2}{v_1 J_2 (v_2 - v_1)}\right|$, if the system is either in the dephosphorylation or phosphorylation dominant regime ($v_1/v_2 \gg 1$ or $v_1/v_2 \ll 1$), $\left|\frac{4(v_2 - v_1)v_1 J_2}{B^2}\right| \ll 1$ is guaranteed, which simplifies the expression of z^* to

$$z^* \approx \frac{v_1 J_2}{B} = \frac{v_1 J_2}{(J_2 - 1)v_1 + (J_1 + 1)v_2}. \tag{S10}$$

Derivations of $\delta S^\dagger$ and $R_o(c_L; t)$

First, using the condition of $\left|\frac{4(v_{\mathrm{AE}} - 1)}{K_A^f y^2}\right| < \left|\frac{4K_A^f}{(v_{\mathrm{AE}} - 1)}\right| \ll 1$ for $v_{\mathrm{AE}} \ll 1$ and $K_A^f \approx 10^{-2}$ (Table 1), we obtain

$$\delta S \propto 1/y = \left[\kappa_{\mathrm{AE}} \frac{K_E^f + 1}{K_A^f} \frac{\mathrm{RGS}_o}{c_L [R]_{ss}} + \frac{K_A^f - 1}{K_A^f}\right]^{-1} \tag{S11}$$

Second, a condition of $\left|\frac{4\omega_p (1 + c_L) c_L R_o / K_\pi}{\Gamma^2}\right| \ll 1$, which is valid for $c_L \ll 1$, further approximates Eq. 17 as

$$[R]_{ss} \approx \omega_p R_o / \Gamma. \tag{S12}$$

This simplifies δS to

$$\delta S \propto \left[\kappa_{\mathrm{AE}} \frac{K_E^f + 1}{K_A^f} \frac{\mathrm{RGS}_o \Gamma}{c_L \omega_p R_o} + \frac{K_A^f - 1}{K_A^f} \right]^{-1}. \tag{S13}$$

By rewriting Eq. S13 along with Eq. 18,

$$\begin{aligned} \delta S(c_L; t) &\propto \frac{c_L \omega_p R_o}{\left[\kappa_{\mathrm{AE}} \frac{K_E^f+1}{K_A^f} \mathrm{RGS}_o (1 + c_\beta^{-1} + \omega_p - R_o/K_\pi) + \frac{K_A^f-1}{K_A^f} \omega_p R_o \right] c_L + \kappa_{\mathrm{AE}} \frac{K_E^f+1}{K_A^f} \mathrm{RGS}_o \omega_p} \\ &\propto \frac{c_L \omega_p R_o}{\left[(1 + c_\beta^{-1} + \omega_p) + \left(\frac{K_A^f-1}{K_E^f+1} \frac{\omega_p}{\kappa_{\mathrm{AE}}} \frac{K_\pi}{\mathrm{RGS}_o} - 1 \right) \frac{R_o}{K_\pi} \right] c_L + \omega_p}, \end{aligned} \tag{S14}$$

we obtain the expression of $\delta S^\dagger(c_L; t)$ (Eq. 20).

Next, the evolution equation of R_o (Eq. 3) can be approximated as

$$\begin{aligned} \frac{dR_o}{dt} &\approx -[L R_P^* \cdot \beta A]_{ss} + r_s \\ &\approx -k(c_L) R_o + r_s, \end{aligned} \tag{S15}$$

which, along with the relations $[L R_P^* \cdot \beta A]_{ss} = c_\beta f([R]_{ss}) \approx f(\omega_p R_o/\Gamma)$ and Eq. 6, yields

$$R_o(c_L; t) = R_o^i e^{-k(c_L)t} + \frac{r_s}{k(c_L)} (1 - e^{-k(c_L)t}). \tag{S16}$$

with $k(c_L) = k_d c_L / [(1 + c_\beta^{-1} + \omega_p) c_L + \omega_p]$.

Receptor population, $R_o(c_L; \tau)$

For $c_\beta \gg 1$ and $\omega_p \ll 1$, $R_o(c_L; \tau)$ with $k(c_L) \approx k_d c_L/(c_L + \omega_p)$ (Eq. 24) is approximated at short and long time limits as follows:

(i) For short incubation time, $\tau(=k_d t) \sim \mathcal{O}(1)$,

$$
\begin{aligned}
R_o(c_L;\tau) &\approx R_o^i\left(1-\frac{c_L\tau}{c_L+\omega_p}\right)+(r_s/k_d)\tau+\mathcal{O}(\tau^2) \\
&\approx \begin{cases} R_o^i\left(1-c_L\tau/\omega_p\right)+(r_s/k_d)\tau+\mathcal{O}(\tau^2) & (c_L \ll \omega_p) \\ R_o^i-(R_o^i-r_s/k_d)\tau+\mathcal{O}(\tau^2) & (c_L \gg \omega_p) \end{cases}
\end{aligned} \tag{S17}
$$

(ii) For long incubation time, $\tau \gg 1$,

$$
R_0(c_L;\tau) \approx \frac{r_s(c_L+\omega_p)}{k_d c_L} = \begin{cases} \frac{r_s\omega_p}{k_d c_L} & (c_L \ll \omega_p) \\ \frac{r_s}{k_d} & (c_L \gg \omega_p) \end{cases} \tag{S18}
$$

References

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(2) Qian, H. Phosphorylation Energy Hypothesis: Open Chemical Systems and Their Biological Functions. *Annu. Rev. Phys. Chem.* **2007**, *58*, 113–142.