Molecular switch architecture drives response properties

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Abstract

A wide range of signaling pathways consist of molecular switches, which are proteins that transition between two states—*on* and *off*. Upon perception of a stimulus by its cognate receptor, signal transduction through a molecular switch can occur by one of the following mechanisms: activation, where the transition rate from the *off* state to the *on* state increases; derepression, where the transition rate from the *on* state to the *off* state decreases; and concerted, where both activation and derepression operate simultaneously. Here we use mathematical modeling to compare and contrast these signaling mechanisms in terms of their dose response curves, response times, and abilities to process upstream fluctuations. Our analysis elucidates two general principles. First, activation makes the switch response more sensitive than the receptor response whereas derepression does the opposite. Second, activation operates by speeding up the switch response, as opposed to derepression which slows it down. Consequently, the concerted mechanism is not only able to align the switch dose response with that of the receptor but also to decouple the response time from the stimulus level. Interestingly, activation and derepression perform similarly in terms of processing upstream fluctuations, but better than the concerted mechanism. In addition to these three metrics, we also examine the effect of receptor removal, a common feature of many signaling pathways, on switches governed by activation and derepression. We find that if inactive (active) receptors are preferentially removed then activation (derepression) leads to a sustained response whereas derepression (activation) adapts. In total, we show how a molecular switch architecture governs its response properties. We also discuss biological relevance of these findings.

Introduction

Many molecules involved in intracellular signaling pathways act as molecular switches. These are proteins that can be temporarily modified to transition between two conformations, one corresponding to an *on* (active) state and another to an *off* (inactive) state. Two prominent examples of such switches are proteins that are modified by phosphorylation and dephosphorylation and GTPases that bind nucleotides. For phosphorylation-dephosphorylation cycles, it is common for the covalent addition of a phosphate by a kinase to cause activation of the modified protein. A phosphatase removes the phosphate to turn the protein *off*. In the GTPase cycle, the protein is *on* when bound to Guanosine triphosphate (GTP) and *off* when bound to Guanosine diphosphate (GDP). The transition from the GDP bound state to the GTP bound state requires nucleotide exchange, whereas the transition from GTP bound to GDP bound state is achieved via hydrolysis of the γ phosphate on GTP. The basal rates of nucleotide exchange and hydrolysis are often small. These reaction rates are increased several fold by Guanine Exchange Factors (GEFs) and GTPase Accelerating Proteins (GAPs), respectively [1, 2].

A signaling pathway is often initiated upon recognition of a stimulus by its cognate receptor, which then activates a downstream switch. In principle, a switch may be turned *on* by *at least* two mechanisms: a) by increasing the transition rate from the *off* state to the *on* state, and b) by decreasing the transition rate from the *on* state to the *off* state. We term these mechanisms *activation* and *derepresison*, respectively. Examples of both these mechanisms are found in the GTPase cycle. In animals, signaling through many pathways is initiated by G protein coupled receptors (GPCRs) that respond to a diverse set of external stimuli. These receptors act as GEFs to activate heterotrimeric G proteins [3–6]. Thus, pathway *activation* relies upon increasing the transition rate from the *off* state to the *on* state. There are no GPCRs in plants and other bikonts; the nucleotide exchange occurs spontaneously, without requiring GEF activity [7–9]. G proteins are kept in the *off* state by a repressor such as a GAP or some other protein that holds the self-activating G protein in its inactive state. In this scenario, the presence of a stimulus results in *derepression*, i.e., removal of the repressing activity [10–12]. These two mechanisms for initiating signaling, activation and derepression, are not mutually exclusive. For example, a *concerted* signal initiation whereby both activation and dererpression are used is employed in the GTPase cycle of the yeast mating response pathway [13, 14]. In this example, inactive GPCRs recruit a GAP protein and act to repress, whereas active receptors have GEF activity and act to activate. Thus, perception of a stimulus leads to concerted activation and derepression by increasing GEF activity while decreasing GAP activity.

These three mechanisms are not limited to GTPase cycles. The activation mechanism described here in fact is a simpler abstraction of a linear signaling cascade, a classical framework used to study general properties of signaling pathways [15–19] as well as to model specific signaling pathways [20–22]. While derepression may seem like an unusual mechanism, it occurs in numerous important signaling pathways in plants (e.g., auxin, ethylene, gibberellin, phytochrome), as well as gene regulation [23–27]. Concerted mechanisms are found in bacterial two component systems, wherein the same component acts as kinase and phosphatase [28–35]. Another example of a concerted mechanism is the so-called reciprocal regulation of cell-cycle, where Cdk1 not only phosphorylates its substrate but also inhibits the phosphatase [32].

Mathematical modeling has proven to be a useful tool for un-

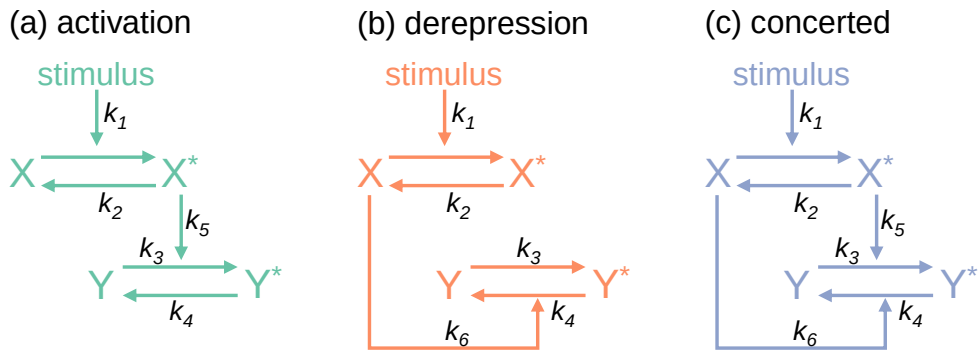

Figure 1: **Mechanisms for signaling through molecular switches**. Presentation of a stimulus activates a receptor ($X \rightarrow X^*$). The reverse reaction causes deactivation of the receptor ($X^* \rightarrow X$). These transitions govern the activity of a molecular switch downstream. (**a**) In the activation mechanism, X^* increases the rate at which the inactive switch (Y) becomes active (Y^*). The opposite reaction $Y^* \rightarrow Y$ has a constant rate. (**b**) In the derepression mechanism, the transition $Y \rightarrow Y^*$ occurs at a constant rate. Activity of the switch is controlled through X: the stimulus decreases X and consequently increases Y^*. (**c**) In the concerted paradigm, both activation and derepression simultaneously control the downstream component.

derstanding the design principles of signaling pathways, and, not surprisingly, mathematical models of activation, derepression and concerted mechanisms have been studied previously. For example, the classical Goldbeter-Koshland model studied zero-order ultrasensitivity of an activation mechanism [15]. Further analyses have examined the effect of receptor numbers [36–38], feedback mechanisms [39, 40], removal of active receptors via endocytosis and degradation [41, 42], etc. Similarly, important properties of the concerted model, such as its ability to do ratiometric signaling [13, 14], to align dose responses at different stages of the signaling pathway [43], as well as its robustness [29, 44] are well-known. The derepression model is relatively less studied. Although there are models of G-signaling in *Arabidopsis thaliana* [45–47], these models have a large number of states and parameters and do not specifically look at system level properties of derepression mechanism.

Despite these efforts, a systematic comparison of various properties of activation, derepression, and concerted mechanisms of signaling has been lacking. Comparing and contrasting these mechanisms should enable our understanding of why different organisms have chosen different mechanisms as well as instruct design of synthetic switches. To this end, we specifically choose four metrics for the comparison: a) dose response curves, b) response times, c) abilities to suppress or filter stochastic fluctuations in upstream components, and d) effect of receptor removal. The rationale behind choosing to compare dose response curves is that they provide information about the input sensitivity range and the output dynamic range, both of which are of pharmacological importance. We supplement this comparison with response times, which provide information about the dynamics of the signaling activity. The third metric of comparison is motivated from the fact that signaling pathways are subject to inherent stochastic nature of biochemical reactions, further compounded by fluctuations in the number of components [48–53]. Lastly, we study the effect of receptor removal on the response of these signaling mechanisms because many well-studied signaling pathways evince receptor removal [11, 42, 54–56]. To study these properties, we construct both deterministic ordinary differential equation models and stochastic models based on continuous time Markov chains.

Our results show that activation has the following two effects: it makes the switch response more sensitive than that of the receptor, and it speeds up the response in comparison with the basal response time. In contrast, derepression makes the switch response less sensitive than the receptor occupancy and slower than the basal speed. These counteracting behaviors of activation and derepression lead to intermediate sensitivity and intermediate response time for the concerted mechanism. In the special case of a perfect concerted mechanisms (equal activation and repression), the concerted mechanism is able to align the dose-response curve of the pathway with receptor occupancy and also have response time that does not depend upon the stimulus level. The noise comparison reveals that the concerted mechanism is more susceptible to fluctuations than the activation and derepression mechanisms, which perform similarly. Finally, our analysis of the effect of receptor removal highlights another important difference between activation and derepression. Removal of active (inactive) receptors at a faster rate than inactive (active) receptors results in an adaptive response for activation (derepression) and sustained response for derepression (activation). We finally compare our findings with experimental observations, suggesting reasons that might have led biological systems to choose one of these mechanisms over the others.

Model formulation

We consider a two-tier model for each of three mechanisms of signaling through a molecular switch (Fig. 1). The first tier is common for all mechanisms, where an inactive receptor (X) becomes active (X^*) when its corresponding input (stimulus) is presented. The second tier is the molecular switch that transitions between *off* (Y) and *on* (Y^*) states. In the activation mechanism, the transition rate from the *on* state to the *off* state increases as the number of active receptor molecules increases (Fig. 1(a)). In the derepression mechanism, the transition rate from the *off* state to the *on* decreases with decrease in the number of inactive receptor molecules (Fig. 1(b)). In the concerted mechanism, both activation and derepression occur simultaneously (Fig. 1(c)). We model these mechanisms using ordinary differential equations (ODEs), assuming mass-action kinetics. To this end, we denote the time by t, stimulus level by S, the total number of receptors by X_T, and the total number of switches by Y_T. We use X^* and

Y^* to denote the number of active receptors and the number of active switches, respectively. The rate constants are as follows: k_1 is the rate of receptor activation per unit stimulus, k_2 is the rate of receptor deactivation, k_3 is the basal rate of activation of the switch, k_4 is the basal rate of deactivation of the switch, k_5 is the strength of activation of an individual inactive receptor, and k_6 is the strength of repression of an individual active receptor. Thus, the (total) activation strength is $k_5 X_T$ and (total) repression strength is $k_6 X_T$. Lastly, we assume that the total number of each species is conserved, and that each model is in steady state before presentation of the stimulus at $t = 0$.

Note that the concerted mechanism encompasses both activation and derepression mechanisms. Therefore, writing ODEs for the concerted mechanism is sufficient to capture all three mechanisms. The number of active receptors and the number of active switches evolve over time according to the following ODEs:

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

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

The activation and derepression mechanisms represent limiting cases in which $k_6 = 0$ and $k_5 = 0$, respectively. Solving Eq. (1) requires rate constants and initial conditions to be specified. Unless stated otherwise, we assume that initial conditions are given by the pre-stimulus ($S = 0$) steady state:

$$X_0^* = 0, \quad Y_0^* = \frac{k_3}{k_3 + k_4 + k_6 X_T} Y_T. \tag{1c}$$

With the models described by Eq. (1), we next compare the three signaling mechanisms in terms of their dose responses and response times.

Dose responses

We begin our analysis by examining the steady-state dose responses of activation, derepression, and concerted mechanisms. The steady-state solution to Eq. (1) is

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

Here $\overline{X^*}$ and $\overline{Y^*}$ are the number of active receptors and the number of active switches a long time after the stimulus is presented ($t \to \infty$), respectively. Notably, both $\overline{X^*}$ and $\overline{Y^*}$ have the form

$$R = \frac{R_0 \Theta_R + R_\infty S}{\Theta_R + S}, \tag{3}$$

where R_0 is the minimum response corresponding to $S = 0$, R_∞ is the maximum response corresponding to $S \gg \Theta_R$, and Θ_R is the stimulus concentration that produces half-maximal response $\frac{R_0 + R_\infty}{2}$. The dynamic range of the response is given by $R_\infty - R_0$, signifying the maximum the output can change in response to the input. Eq. (3) shows that shapes of dose response curves are same for the three signaling mechanisms. Hence comparison between them can be carried out in terms of R_0, R_∞, and Θ_R.

At the receptor level, $X_0^* = 0$ and $X_\infty^* = X_T$, notwithstanding the rate parameters. The half-maximal stimulus Θ_{X^*} is equal to $\frac{k_2}{k_1}$, which is the binding affinity of the stimulus with the receptor. The response at the switch level $\overline{Y^*}$ is specified by:

$$Y_0^* = \frac{k_3 Y_T}{k_3 + k_4 + k_6 X_T}, \tag{4a}$$

$$Y_\infty^* = \frac{(k_3 + k_5 X_T) Y_T}{k_3 + k_4 + k_5 X_T}, \tag{4b}$$

$$\Theta_{Y^*} = \Theta_{X^*} \frac{k_3 + k_4 + k_6 X_T}{k_3 + k_4 + k_5 X_T}. \tag{4c}$$

These expressions show that the dose responses at the switch level depend upon the the basal rates as well as activation strength ($k_5 X_T$) and repression strength ($k_6 X_T$). A careful examination of Eq. (4) provides the following insights:

(i) The repression strength $k_6 X_T$ does not affect the maximum response Y_∞^*, but affects the minimum response Y_0^*. In particular, increasing $k_6 X_T$ decreases Y_0^*. The activation strength $k_5 X_T$ increases Y_∞^* and does not affect Y_0^*.

(ii) Relative values of the repression and activation strengths dictate the relationship between the half-maximal stimulus for the switch response (Θ_{Y^*}) vis-á-vis the half-maximal stimulus for the receptor occupancy (Θ_{X^*}). More specifically, $\Theta_{Y^*} < \Theta_{X^*}$ when $k_5 X_T > k_6 X_T$, $\Theta_{Y^*} = \Theta_{X^*}$ when $k_5 X_T = k_6 X_T$, and $\Theta_{Y^*} > \Theta_{X^*}$ when $k_5 X_T < k_6 X_T$. Increasing $k_6 X_T$ increases Θ_{Y^*} while increasing $k_5 X_T$ does the opposite.

Fig. 2 illustrates the aforementioned effects on dose-response curves for the signaling mechanisms considered. Noting that signaling pathways typically show little activity in absence of the stimulus ($Y_0^* \ll Y_T$) and show full activity ($Y_\infty^* \approx Y_T$) if the stimulus is large, it is reasonable to make the following assumptions: $k_3 \ll k_4 + k_6 X_T$ and $k_4 \ll k_3 + k_5 X_T$. With these assumptions in mind, we use the following parameters for Fig. 2: $k_3 = 0$ and $k_6 = 0$ for activation; $k_4 = 0$ and $k_5 = 0$ for derepression; and $k_3 = 0$ and $k_4 = 0$ for concerted. As shown in Fig. 2(a) activation makes the switch response more sensitive to stimulus than the receptor occupancy ($\Theta_{Y^*} < \Theta_{X^*}$). Increasing the activation strength ($k_6 X_T$) increases Y_∞^* and decreases Θ_{Y^*}, increasing the dynamic range (vertical expansion) and sensitivity (leftward shift) of the dose-response curve. The derepression mechanism exhibits an opposite behavior with $\Theta_Y > \Theta_X$. In this scenario, increasing the repression strength increases the dynamic range by decreasing Y_0^* and decreases sensitivity by increasing Θ_{Y^*} (Fig. 2(b)).

Because we ignore the basal rates, changing activation and derepression strengths only influence Θ_{Y^*} in the case of a concerted mechanism. As expected, the switch response is more (less) sensitive than the receptor occupancy if activation (derepression) dominates derepression (activation). There is a perfect alignment of the fractional receptor occupancy curve with the dose response curve of the switch when $k_5 = k_6$ (Fig. 2(c)). Another important property of the concerted model is that it exhibits ratiometric signaling in which the response of Y^* is determined by the ratio of active receptors (X^*)to the total number of receptors (X_T) [13, 14], and the activity of Y^* is independent of the total number of receptors X_T. This may be seen by looking at Y^*

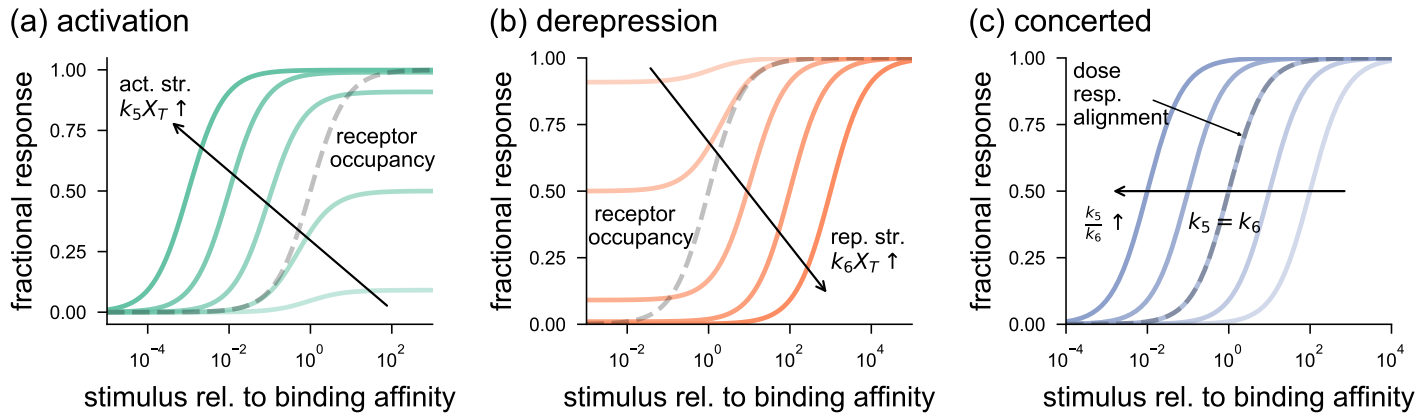

Figure 2: **Dose-response curves for signaling mechanisms through molecular switches**. The response is measured in terms of fraction of active switches Y^*/Y_T as the stimulus level varies. The receptor occupancy curve denotes the fraction of active receptors X^*/X_T. The stimulus is normalized by its binding affinity to the receptor (Θ_{X^*}). **(a)** For the activation mechanism, the half-maximal stimulus (Θ_{Y^*}) of a dose-response curve is less than Θ_{X^*}. Each dose-response curve (solid line) is for a fixed activation strength k_5X_T. Increasing k_5X_T, depicted by the solid arrow, causes an upward expansion and leftward shift in dose-response. For these plots, the following values for parameters were used: $k_3 = 0$, $k_4 = 1$ and $k_6 = 0$. The activation strength (k_5X_T) was varied to take values from $(0.1, 1, 10, 100, 1000)$. **(b)** For the derepression mechanism, the dose-response of the switch for a given repression strength (k_6X_T) has half-maximal stimulus (Θ_Y) greater than Θ_{X^*}. Increasing k_6X_T, shown by the solid arrow, leads to a downward expansion and rightward shift in the dose-response curve. The repression strength k_6X_T takes values from $(0.1, 1, 10, 100, 1000)$. The rest of the parameters were set at $k_3 = 1$, $k_4 = 0$, $k_5 = 0$. **(c)** In the case of concerted mechanism, Θ_{Y^*} may be greater than, equal to, or less than Θ_{X^*}, depending upon the relative values of the activation strength and the derepression strength. Increasing the ratio k_5/k_6, depicted by the solid arrow, shifts the dose response to left. Dose-response alignment ($\Theta_{Y^*} = \Theta_{X^*}$) occurs when $k_5 = k_6$. The parameters used for the plots are $k_3 = 0$ and $k_4 = 0$. The ratio k_5/k_6 was varied over $(0.01, 0.1, 1, 10, 100)$.

when $k_3 = 0$ and $k_4 = 0$:

$$\overline{Y^*} = \frac{SY_T}{S + \frac{k_2 k_6}{k_1 k_5}}. \tag{5}$$

In reality, k_3 and k_4 are likely to be small, but non-zero. Therefore, ratiometric signaling does not hold in a strict sense.

Our theoretical results above show how the dose-response curves behave differently for activation, derepression, and concerted mechanisms. Are some of these behaviors observed in biological systems? One example where the signaling response becomes maximal when only a small fraction of receptors are bound ($\Theta_{Y^*} < \Theta_{X^*}$) is the EGFR–MAPK pathway which elicits a full MAPK response at less than 5% receptor occupancy [57]. Our analysis explains this by an activation mechanism or a concerted mechanism in which the activation strength dominates the repression strength. A contrasting behavior is seen in the ethylene pathway of *Arabidopsis thaliana* in which a loss-of-function mutation of one of the ethylene receptors, *etr1*, shows increased sensitivity to etylene [58]. This points to a derepression mechanism in which decreasing the amount of the receptor lowers the repression strength k_6X_T and shifts the dose response curve to the left. A suggested example of concerted mechanism is the yeast G-signaling pathway, which exhibits both ratiometric signaling [13, 14] and dose response alignment [43].

Response times

Our analysis thus far focused on the steady-state properties of the activation, derepression, and concerted mechanisms. In this section, we study these mechanisms in terms of their response times; that is the time it takes for a signaling output to reach its steady-state. We use the following definition of response time:

$$\mathcal{T}_R = \frac{\int_0^\infty t \left|\overline{R} - R(t)\right| dt}{\int_0^\infty \left|\overline{R} - R(t)\right| dt}, \tag{6}$$

where $R(t)$ is the time-dependent response of the pathway component under consideration and $\overline{R}$ represents its value at steady state [59]. For this definition, $\mathcal{T}_R$ represents the "center of mass" of the response $R(t)$, and is well-defined when $R(0) \neq \overline{R}$. If the response is determined by a single kinetic step, $\mathcal{T}_R$ is reciprocal of the rate constant for that step (section S1, SI). For example, the response time for the receptor is given by

$$\mathcal{T}_{X^*} = \frac{1}{k_1 S + k_2}, \tag{7}$$

which is independent of the initial condition. Eq. (7) shows that the response time decreases if either or both of $k_1 S$ and k_2 increase. Because the response time depends upon the sum $k_1 S + k_2$ and the steady-state receptor occupancy depends upon the ratio $k_1 S/k_2$, these quantities can be tuned independently.

In the absence of stimulus, the response time of the switch follows the same form as Eq. (7):

$$\mathcal{T}_{Y^*}\big|_{S=0} = \frac{1}{k_3 + k_4 + k_6 X_T}. \tag{8}$$

When the stimulus is present, analytic solutions to the integrals in Eq. (6) for the response time of $Y^*(t)$ do not exist, except for a special case of the perfect concerted model $k_5 = k_6$. It is, however, possible to approximate $\mathcal{T}_R$ by linearizing the dynamics Eq. (1) around steady-state:

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_1 S + k_2} + \frac{1}{k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k2}} - \frac{1}{k_1 S + k_2 + k_3 + k_4 + k_6 X_T}. \tag{9}$$

This above equation is exact for the special case when $k_5 = k_6$ (section S2, SI). The first term in Eq. (9) represents the response time of the receptor ($\mathcal{T}_{X^*}$). The second term can be interpreted as the response time of the switch when the receptors are at steady-state, because in that case the switch would be turned *on* at a rate $k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}$ and turned *off* at a rate $k_4 + k_6 X_T - k_6 \frac{k_1 S X_T}{k_1 S + k_2}$.

If the receptor response time is much faster than that of the switch, we expect that the latter does not depend upon the former. Indeed in this limit, Eq. (9) gives

$$\mathcal{T}_{Y^*} \approx \frac{1}{k_3 + k_4 + k_6 X_T + (k_5 - k_6)\frac{k_1 S X_T}{k_1 S + k2}}. \tag{10}$$

Comparing Eq. (10) with the basal response time in Eq. (8) shows that for a given stimulus level, activation speeds up the response in comparison with the basal response. In contrast, derepression slows down the response time and a prefect concerted mechanism does not affect the response time (Fig. 3(a)). In the other limiting case when the receptor timescale is much slower than that of the switch, we expect the receptor dynamics to dictate the response time. Indeed in this case, Eq. (9) reduces to $\mathcal{T}_{Y^*} \approx \mathcal{T}_{X^*}$ such that choice of the mechanism to control the switch has little effect on the response time. Our analytical as well as numerical calculations confirm this behavior (Fig. 3(a)).

Next we examine the scenario where the switch is controlled by varying the stimulus level. Because changing the stimulus affects the response time of the receptor, which in turn affects the response time of the switch, we control for this effect by keeping $k_1 S + k_2$ constant but changing the ratio $k_1 S / k_2$. We find that activation indeed speeds up with increasing stimulus levels, whereas derepression slows down (Fig. 3(b)). Importantly, the response time of the concerted mechanism is independent of the stimulus strength, and, therefore able to respond rapidly over the whole range of stimulus levels. We see that activation is faster than derepression only if the basal response times are equal. Therefore to construct a switch that responds rapidly using derepression, it is necessary for the switch to undergo fast basal cycling. (Fig. 3(b)).

Is there an intuitive explanation to why activation is faster than derepression? The activation model shortens the average lifetime of the *off* state, without affecting the average lifetime of the *on* state. Derepression operates differently; it does not affect the average lifetime of the *off* state, but increases the lifetime of the *on* state. Thus, activation responds faster than derepression. The concerted model decreases the lifetime of the *off* state in addition to increasing the lifetime of the *on state*, its response time lies between those of activation and derepression.

Processing upstream fluctuations

The deterministic models used to compare the signaling mechanisms thus far ignore the stochastic nature of biochemical reactions, which becomes relevant when the abundance of receptor and switch proteins are small [48–53, 60, 61]. Therefore, we formulate a stochastic model of the concerted mechanism and analyze the other two mechanisms as its special cases. Our model consists of four reactions: activation of receptor upon recognizing the stimulus, deactivation of receptor, *on* to *off* transition of the molecular switch, and *off* to *on* transition of the molecular switch. The stochastic model is characterized by the probabilistic nature

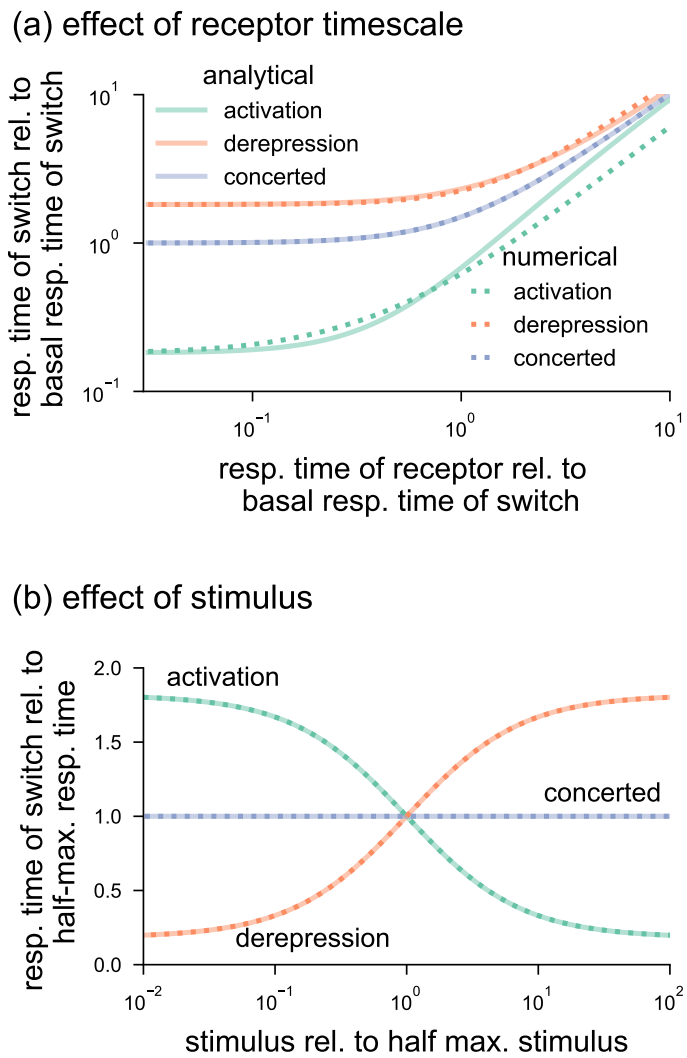

Figure 3: **Response times of molecular switches governed by activation, derepression, and concerted mechanisms**. (**a**) The response time of the switch increases as the response time of the receptor increases. Differences in the signaling mechanisms are more prominent when the receptor response is fast. Activation speeds up the response in comparison with the basal response time whereas dererepssion slows it down. A perfect concerted mechanism does not affect the response time. For each signaling mechanism, the response time is computed using the analytical result in Eq. (9) (solid lines) and numerically validated by using Eq. (6) (dashed lines). To ensure the same basal response and basal response time of the switches across signaling mechanisms, we chose the parameters as $k_3 = 1/9$, $k_4 = 1$, $k_6 = 0$, and $k_5 X_T = 10$ for activation; $k_3 = 1/9$, $k_4 = 0$, $k_5 = 0$, and $k_6 X_T = 1$ for derepression; and $k_3 = 1/9$, $k_4 = 0$ and $k_5 X_T = k_6 X_T = 1$ for concerted. The receptor response time was $k_1 S + k_2$ varied through k_2 while maintaining $k_1 S / k_2 = 1$. (**b**) Increasing the stimulus level, decreases the response time for activation, increases in response time for derepression, and does not affect the response time for the concerted mechanism. The comparison is controlled by setting same response time at half-max. stimulus Θ_{Y^*}. The following parameters were chosen to have same basal response but different basal response times: $k_3 = 1$, $k_4 = 9$, $k_5 X_T = 90$, and $k_6 = 0$ for activation; $k_3 = 10$,$k_4 = 0$,$k_5 = 0$, and $k_6 X_T = 90$ for derepression; and $k_3 = 10$, $k_4 = 0$, $k_5 X_T = k_6 X_T = 90$ for concerted. The receptor occupancy was varied by changing $k_1 S / k_2$ while maintaining the receptor response time $1/(k_1 S + k_2)$, which was chosen to be 100 times faster than the response time of the switches at their respective half maximal stimulus levels.

of each reaction and the discreteness of changes in population counts upon occurrence of a reaction as tabulated in Table 1.

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

Table 1: Transitions and associated rates for the stochastic model.

Our goal is to analyze the noise properties of activation, derepression, and concerted mechanisms. We quantify noise using coefficient of variation squared (CV^2), which is computed by normalizing the variance by mean squared and is a dimensionless quantity. To this end, we use the ODEs that describe the time evolution of the first and second-order moments, and solve them in steady-state to obtain the stationary moments [62, 63] (section S2-E, SI). In particular, moments for the number of active receptors (X^*) are given by

$$\langle X^* \rangle = \frac{k_1 S X_T}{k_1 S + k_2}, CV^2_{X^*} = \frac{\langle X^{*2} \rangle - \langle X^* \rangle^2}{\langle X^* \rangle^2} = \frac{1}{X_T} \frac{k_2}{k_1 S}. \tag{11}$$

Here $\langle . \rangle$ denotes the expected value (average) of its argument. These moments correspond to a binomial distribution with parameters X_T and $\frac{k_1 S}{k_1 S + k_2}$ (section S1-C, SI). The stochastic mean $\langle X^* \rangle$ is same as the steady-state value for X^* in the deterministic model in (1). The coefficient of variation squared increases as the number of receptors (X_T) decreases. Therefore the noise analysis is of importance when X_T is small. In addition, the noise decreases with the ratio $k_1 S / k_2$. Recall that $k_1 S / k_2$ is the stimulus level relative to the binding affinity. Thus the noise diminishes when the stimulus level is much higher than the binding affinity.

Closed-form expressions for the moments are not available for Y^* owing to the nonlinear term $X^* Y^*$ in reaction rates, except for the special case of a perfect concerted model ($k_5 = k_6$). We approximate the mean response and the noise by considering a linearized system around the steady-state

$$\langle Y^* \rangle \approx \frac{k_3 + k_5 \frac{k_1 S}{k_1 S + k_2} X_T}{k_3 + k_4 + k_6 \frac{k_2}{k_1 S + k_2} X_T} Y_T, \tag{12a}$$

$$CV^2_{Y^*} \approx \underbrace{\frac{1}{Y_T} \frac{k_4 + k_6 X_T \frac{k_2}{k_1 S + k_2}}{k_3 + k_5 X_T \frac{k_1 S}{k_1 S + k_2}}}_{\text{contribution from act./deact. of } Y^*} + \underbrace{\frac{k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}}{k_1 S + k_2 + k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}}}_{\text{time-averaging}} \times \underbrace{\frac{\left(\frac{k_1 S X_T}{k_1 S + k_2}\right)^2 (k_4 k_5 + k_6(k_3 + k_5 X_T))^2}{\left(k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}\right)^2 \left(k_3 + k_4 + k_5 \frac{k_1 S X_T}{k_1 S + k_2} + k_6 \frac{k_2 X_T}{k_1 S + k_2}\right)^2}}_{\text{coupling}} CV^2_{X^*}. \tag{12b}$$

We validate these approximations using exact semi-analytical approach based on [64] (section S2-E, SI). The formula for $CV^2_{Y^*}$ above is written in terms of various sources of noise, as previously done for gene regulation models [65, 66]. Specifically, the noise in the signaling activity of the switch arises from two sources: activation/deactivation reactions of the switch, and noise in the number of active receptors, which is processed via time-averaging and coupling.

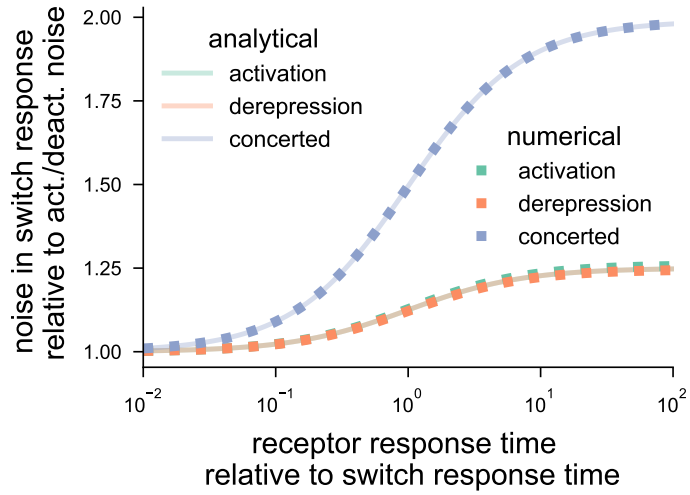

Figure 4: **Noise in the number of active switch molecules**. The concerted model has a higher noise than activation and derepression, which perform similar. The difference is negligible when receptor dynamics is fast, and is more prominent when receptor is slow. Noise is quantified using coefficient of variation squared. The analytical result is computed using Eq. (12), which is validated numerically. To control for the effects of variations in the mean number of switches and the differences in timescales, the following parameters were chosen: $k_3 = 0$, $k_4 = 1$, $k_5 = 0.02$, and $k_6 = 0$ for activation; $k_3 = 1$, $k_4 = 0$, $k_5 = 0$, and $k_6 = 0.02$ for derepression; and $k_3 = 0$, $k_4 = 0$, $k_5 = k_6 = 0.02$ for concerted. In addition, X_T and $Y_T = 100$ were taken to be 100 each. The receptor response time ($1/(k_1 S + k_2)$) was varied through k_2 while maintaining the receptor occupancy by $k_1 S / k_2 = 1$.

The contribution from activation/deactivation of the swich has a similar form as $CV^2_{X^*}$ in Eq. (11). Accordingly, the contribution of this term decreases with increase in Y_T or increase in the ratio of the total activation rate with total deactivation rate. This ratio increases if the activation strength increases or the repression strength decreases. We note that changing these parameters also affect the time-averaging as well as the coupling term. One possibility through which only the contribution from $CV^2_{X^*}$ to overall $CV^2_{Y^*}$ is affected is to change $k_1 S + k_2$ while maintaining the ratio $k_1 S / k_2$. In such a scenario, all other terms remain constant except for the time-averaging term. In the limiting case where receptor dynamics is very fast, the contribution from $CV^2_{X^*}$ to $CV^2_{Y^*}$ becomes negligible due to efficient time-averaging of fluctuations in X^*. One more interesting limiting case is that of ratiometric signaling. Our deterministic analysis shows that for a concerted mechanism without basal rates ($k_3 = 0$ and $k_4 = 0$), the steady-state response ($\overline{Y^*}$) does not depend upon the total number of receptors (X_T). It is easy to see that this assertion does not hold true for $CV^2_{Y^*}$, which still depends upon X_T through the time-averaging term and $CV^2_{X^*}$.

Next, we compare the noise properties of activation, derepression, and concerted mechanisms. To mathematically control the comparison, we assume that the receptor dynamics is

same across the three strategies. In addition, we maintain the same average rate at which switch turns *on* from the *off* state, i.e., $k_3 + k_5 \frac{k_1 S X_T}{k_1 S + k_2}$, and the same average rate at which the switch turns *off* from the *on* state, i.e., $k_4 + k_6 \frac{k_2 X_T}{k_1 S + k_2}$. These assumptions ensure that the difference in the noise properties, if any, is solely due to the architecture of the molecular switch and not dependent on the parameters. With this setup, we vary the timescale of the receptor, while keeping the same ratio $k_1 S / k_2$. As shown in Fig. 4, the noise properties of these signaling mechanisms are similar when the receptor timescale is fast. This is expected because the dominant contribution in $CV^2_{Y^*}$ comes from its own activation and deactivation. However, when the receptor timescale is slower than that of the switch, the overall noise increases regardless of the signaling mechanism and the noise performance of the concerted mechanism becomes worse than the other two mechanisms.

The observation that activation and derepression both have similar noise and their concerted action has higher noise is surprising in light of our analyses of dose-response and response time. In terms of those properties, activation and derepression counteract to enable intermediate response for the concerted mechanism. Intuitively, this occurs because in the concerted mechanism, fluctuations in the upstream component affect both transitions $Y \rightarrow Y^*$ and $Y^* \rightarrow Y$. In case of activation and derepression, however, only one of these transitions is coupled with the upstream component. As a result, concerted mechanism has a worse noise performance.

Effect of receptor removal

Our models of signaling mechanisms in Fig. 1 assume conservation of numbers of receptor molecules (X_T) and of switch molecules (Y_T). These assumptions do not hold in case of some signaling pathways where stimulus-mediated removal of receptors occurs. Reported examples of such phenomena include GPCRs [54], EGFR [42], AMPA-type glutamate receptors [55], the receptor-like kinase FLS2 [56] and regulator of G-signaling (RGS) in *Arabidopsis thaliana* [11]. On the one hand, removal of active receptors is proposed to be a mechanism for desensitizing the response to a sustained stimulus [67, 68], and consequently enabling signaling over a broad range [41,42]. On the other hand, phosphorylation and subsequent removal of RGS, which is both a receptor candidate and a GAP, is proposed to result in sustained activation of signaling in *Arabidopsis thaliana* [9, 11, 45]. With an aim to explain these seemingly opposite behaviors of signaling pathways, we ask whether the signaling mechanisms, particularly activation and derepression, behave differently upon removal of receptors. To answer this, we reformulate the models in Fig. 1 by including production of inactive receptors (X) at a rate k_p, removal of inactive receptors with rate k_d, and removal of active receptors with rate k_d^*. This model is simpler than those showing a broad range [41] or relative sensing [42], but is capable of adaptation [68] which is what we focus on.

Inclusion of receptor removal results in the following modification of the ODE system in Eq (1)

$$\frac{dX}{dt} = k_p - k_d X - k_1 S X + k_2 X^*, \tag{13a}$$

$$\frac{dX^*}{dt} = k_1 S X - k_2 X^* - k_d^* X^*, \tag{13b}$$

$$\frac{dY^*}{dt} = \left(k_3 + k_5 X^*\right)\left(Y_T - Y^*\right) - \left(k_4 + k_6 X\right) Y^*. \tag{13c}$$

The initial conditions are: $X(0) = \frac{k_p}{k_d}$, $X^*(0) = 0$, and $Y^*(0) = \frac{k_3 Y_T}{k_3 + k_4 + k_6 k_p / k_d}$. As before, setting $k_6 = 0$ and $k_5 = 0$, respectively, result in ODEs for the activation and derepression mechanisms.

An important distinction between the ODEs in Eq. (1) and the ODEs in Eq. (13) is that the receptor dynamics only has one timescale $1/(k_1 S + k_2)$ in the former but two timescales in the latter (S3-B, SI). The interplay between these two timescales allows $X(t)$ and $X^*(t)$ to transiently respond to a stimulus with at the fast timescale, followed by an eventual return towards their respective pre-stimulus levels at the slow timescale. Note that the switch response in the activation mechanism depends upon the active receptors X^*. Thus if X^* increases and returns towards its basal level, Y^* is also expected to follow the same dynamics. Likewise, if a derepression mechanism governs the switch then a decreases in X would lead to increase in Y^*. Further, if X returns towards its basal level, Y^* should also follow this trend. Such a behavior is referred to as adaptation [68, 69].

What are appropriate parameter regimes where the switch response $Y^*(t)$ in Eq (13) adapts to a sustained stimulus? Our analysis shows that adaptation by X^* occurs when $k_d^* > k_d$, i.e., active receptors are removed at a faster rate than inactive receptors. In contrast, adaptation by X happens when $k_d^* < k_d$ (S3-B, SI). We note that all other parameters also affect the response properties, but the relative rates of receptor removal are the most important determinants of adaptive behavior. We illustrate these results in Fig. 5. To further bolster our observations, we also examine the scenarios where the inactive receptors are preferentially removed for an activation mechanism and active receptors are preferentially removed for a derepression mechanism. Interestingly, in both these cases, the response sustains and does not adapt. These results thus provide another set of differences between activation and derepression mechanisms. It is also plausible controlling the relative rates of receptor removal is a deliberate mechanism to control the downstream response.

Discussion

Molecular switches are important components in signaling pathways. A switch transitions between *on* and *off* states and signaling through it occurs by increasing its *on*-to-*off* transition rate (activation), or by decreasing its *off*-to-*on* transition rate (derepression), or by simultaneously doing both (concerted). In this work, we studied these signaling mechanisms in terms of their dose-responses, response times, and ability to process upstream fluctuations. We further examined how the effect of removal of receptors depends upon the signaling mechanism governing the switch. Below we enumerate the key differences in the properties of the signaling response that depend upon how the switch is controlled.

- Both activation and derepression mis-align the signaling activity with the receptor occupancy. In particular, activation

reduces the stimulus level required for half-maximal signaling activity in comparison with the binding affinity of the receptor ($\Theta_{Y^*} < \Theta_X$). Derepression leads to $\Theta_{Y^*} > \Theta_X$. The dose response align ($\Theta_{Y^*} = \Theta_X$) for a perfect concerted mechanism which corresponds to equal strengths of activation and repression (Fig. 2).

- A concerted mechanism is capable of ratiometric signaling, where the signaling output only depends upon fractional receptor occupancy and not on the total number of receptors.
- Presentation of a stimulus speeds up the response for an activation mechanism, whereas it slows down the response for a derepression mechanism. The response time does not change for a perfect concerted mechanism (Fig. 3).
- Activation and derepression mechanisms have similar noise in the signaling activity. Concerted mechanism has a higher noise (Fig. 4).
- Preferential removal of active (inactive) receptors leads to an adaptive response for activation (derepression) mechanism and a sustained response for derepression (activation) mechanism (Fig. 5).

These results suggest several trade-offs in the properties of response that depend upon the underlying signaling mechanism. For instance, activation enables sensitive and rapid response, but at the cost of misaligned dose response with receptor occupancy, resulting in poor information fidelity [70]. Derepression also leads to misalignment between dose response and receptor occupancy, albeit the response is less sensitive and slow. The concerted mechanism has a better information fidelity, but it comes at the cost of a poor control over the speed of response and a higher susceptibility to upstream fluctuations. It is worth noting that additional layers of regulation such as feedback and feedforward loops may compensate for some of the shortcomings of a signaling mechanism. One such example is a negative feedback mechanism, which aligns dose response for a signaling pathway operating through activation [43, 70, 71].

Despite being similar in terms of misaligned dose response, there is a key difference between activation and derepression. The former operates as an 'eager' system which is sensitive to small receptor occupancy and speeds up the response upon receiving a stimulus. The latter works as a 'conservative' system which ignores small receptor occupancy as if waiting for additional evidence before committing to a response. The response time also slows down as the stimulus increases. Activation seems fit for purposes where the cost of a false negative is much higher than a false positive. For example, adrenaline response to an imminent danger should be sensitive and fast because cost of a false positive is small but a false negative is high. In contrast, derepression is appropriate for scenarios where cost of a false positive is much higher than a false negative. Based on the observation the derepression based signaling is found in many pathways in plants and we speculate that it happens because plants have to continually balance their resources between growth and immunity [72, 73]. For example, plants would perhaps ignore growth of a low level of pathogenic bacteria before allocating resources to fight them. Another possible scenario where derepression may be used is irreversible cell-fate decisions.

Given that activation and derepression shift dose-response to opposite directions, a natural question to ask is whether dose-response alignment can occur in a signaling cascade where activation and derepression do not operate in a concerted fashion, but operate sequentially? To explore this possibility, we constructed a three-tier model where the response Y^* in Fig. 1(a) led to derepression of a downstream component. Our analysis showed that indeed the response of the downstream component was better aligned with the dose response than Y^*. We also analyzed an alternate mechanism where derepression was followed by activation by modifying Fig. 1(b). As expected, the dose response of the downstream component aligned with the dose response better than that of Y^* (section S2-F, SI).

Until now, we have discussed the drawback of having misalignment between dose response and receptor occupancy. It is plausible that it offers some advantages too. Consider a scenario where active receptors are preferentially removed, resulting in adaptation of the signaling response (Fig. 5). Recent work has shown that it is possible to exploit this to perform relative sensing (fold-change detection) if the receptor removal is a multi-step process [42]. Alternatively, a negative feedback may also result in adaptive response and thereby a fold-change detection [59]. A key feature of fold-change detection is that the dose response curve shifts towards right each time the system adapts [59, 74]. Our results suggest that a relative sensing property may be implemented with a derepression mechanism if the receptor removal operates on inactive receptors. We speculate that a negative feedback operating on inactive receptors would also yield the same effect. It is of course possible to shift the dose-response of a concerted mechanism too as seen in Fig. 2. However, this would come at the cost of misaligned dose response. How do cells balance between being responsive to a large range of stimulus as well as transmitting information with high fidelity is an interesting problem in itself.

Whereas removal for active receptors for an activation mechanism results in an adaptive response, removal of inactive receptors manifests as a sustained response. Derepression also generates such distinct profiles: adaptive response when inactive receptors are removed and sustained response when active receptors are removed. Given that many important switches, e.g., G proteins, process signals from a large number of receptors, such distinct profiles of the signaling response may be used to distinguish signals for a downstream response. To our knowledge, active receptors are often removed as compared to inactive receptors, however this does not rule out the possibility that for receptors their inactive forms are preferentially removed.

A caveat of the models of signaling mechanisms considered herein is that they are based on mass action kinetics and therefore cannot capture saturation effects. Traditionally, signaling pathways are modeled using Michelis-Menten kinetics. While both exact and approximate analytical results are key ingredients of this work, it is usually not possible to derive those with Michelist-Menten kinetics. Nonetheless, whether our results hold when a switch operates in saturating regime needs to be established in a future work. Another direction of future work would be to extend such analysis to include feedback and feedforward regulation. The noise analysis in our work is limited to intrinsic noise which arises due to randomness in activation and inactivation of different components. Incorporating the effect of cell-to-

cell variability in the number of components, which is presumed to be a major source of overall noise at least in yeast pheromone pathway [75], would be another direction of exploration. Finally, we note that similar mechanisms of regulating cellular responses may exist in other contexts, e.g., gene regulation, metabolic pathways. It would be worthwhile to extend our analysis to these and develop a unifying theory.

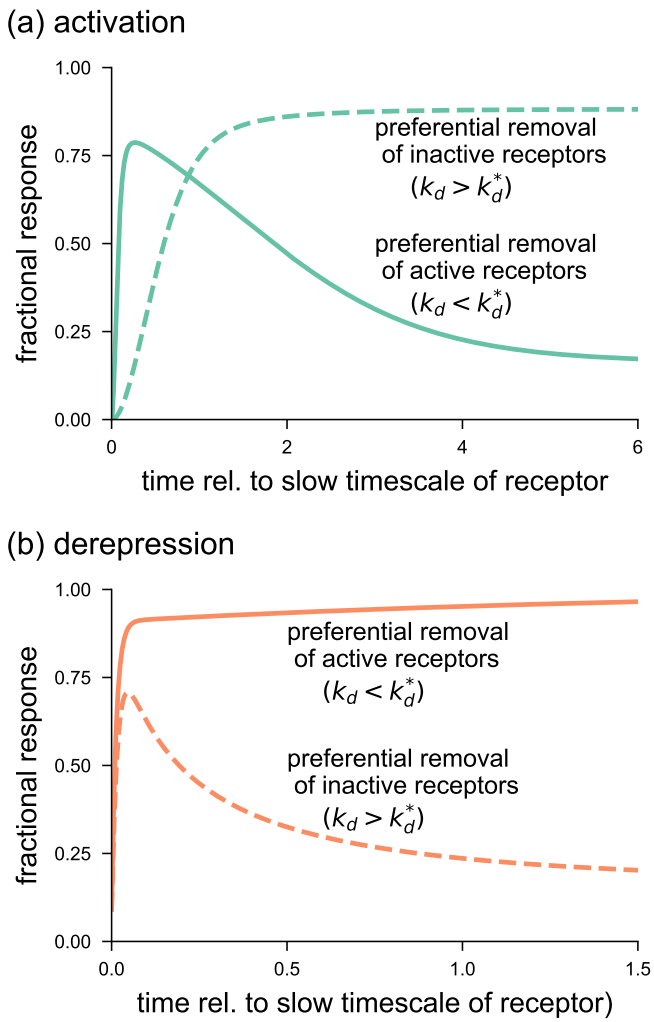

Figure 5: **Effect of receptor removal on responses of activation and derepression mechanisms**. The response is measured in terms of fraction of active receptors (Y^*/Y_T) over time, which is normalized to the slow timescale of the receptor (see section S4, SI). (a) For an activation mechanism, the switch response adapts, i.e., returns towards basal response after a transient, if the rate of removal of active receptors (k_d^*) is higher than that of inactive receptors (k_d). In contrast, if inactive receptors are removed at a faster rate, then the response sustains.(b) For derepression mechanism, preferential removal of X results in adaptation. In contrast, preferential removal of X^* causes sustain response.

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