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Dynamic Characteristics of Transient Responses

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Transient responses of signaling molecules are seen in a wide variety of cellular processes that are mediated by distinct molecular mechanisms. Although transient responses might intuitively be thought to depend on the absolute concentration of growth factors or the intensity of stimulation, we here introduce that some transient responses are prompted by temporal rate of increase of stimulation, rather than intensity of stimulation, by three independent mechanisms. These include the Ras system with fast activation and slow inactivation, the ERK-dependent negative feedback loop system, and the receptor degradation system, all of which can be commonly seen in various signaling networks. In addition, we show the distinct transient and steady state characteristics of these systems.

Key words: MAP kinase cascade, signal transduction, kinetic simulation, transient response.

Abbreviations: EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; SOS, Son of sevenless.

Because of the complex nature of signal transduction, it is important to utilize computer simulations of biochemical reactions. However, such studies are still considered preliminary, and most biologists stay away from kinetic simulation. There are several reasons for this: most simulation models thus far reported do not provide what biologists want to know (*e.g.*, predictions); and biologists themselves are not sure what kinetic simulation can provide them, or they do not know how to use it, simply because of lack of experience. Nevertheless, biologists have gotten used to illustrating qualitative relations between signaling molecules as simple cartoons on the basis of their experimental observations. An important purpose of these cartoons is to sort out and summarize ideas about how current networks can explain phenomena of interest. A well-organized cartoon can sometimes be easily converted into a simple kinetic simulation model with appropriate parameters. Such kinetic simulation models can be a useful tool to provide further understanding and even predictions (*1*, *2*). In this review, we discuss how simple models based on simple cartoons can be used to clarify the dynamics of signaling networks by introducing our case study in the modeling of the ERK signaling network in PC12 cells as an example (*3*).

MAPK cascades are highly conserved signal transduction modules that regulate diverse biological processes in all eukaryotes (*4*). Activation of the ERK subgroup of MAPK cascades by growth factors can trigger either cell growth or differentiation (*5*, *6*). It is known that EGF stimulation transiently activates ERK and thus stimulates cell proliferation, whereas NGF stimulation leads to transient and sustained ERK activation and promotes neuronal differentiation in PC12 cells, a well-studied model for proliferation and neuronal differentiation. It has also been reported that the transient ERK activation is mediated by Ras small GTPase (*7*, *8*), whereas the sustained ERK activation is mediated by Rap1 small GTPase (*9*). Importantly, both EGF and NGF stimulations induce a rapid increase of Ras activation, but within a few minutes the Ras activation declines toward basal level despite ongoing growth factor stimulation (*7*, *8*). Thus, EGF and NGF signaling pathways include intrinsic mechanisms that convert the constant growth factor stimulation into the transient Ras activation. Based on the increasing knowledge of molecular mechanisms, several kinetic models of the ERK signaling network have been developed, and the transient versus sustained nature of the ERK activation has been analyzed (*1–3*, *10–14*). In this review, we focus on three distinct mechanisms that produce transient responses to constant stimulation: the Ras model, in which Ras activation is regulated by both stimulation-dependent positive and negative regulators (Fig. 1A); a negative feedback loop model, in which SOS, an activator of Ras, is inhibited by a downstream molecule, ERK (Fig. 1B); and a receptor degradation model, in which receptors are degraded in a phosphorylation-dependent manner (Fig. 1C). We here analyze the dynamics of these mechanisms of transient response using the simple models.

In the Ras model, activation and inactivation of Ras are regulated by a GDP/GTP exchanging factor SOS and by the Ras GTPase-activating protein RasGAP, respectively (*15*). Importantly, both SOS and RasGAP are activated in a growth factor–dependent manner (Fig. 1A) (*16*, *17*). Following the growth factor stimulation, the activated receptor recruits SOS and RasGAP directly or indirectly via adaptor proteins to the plasma membrane,

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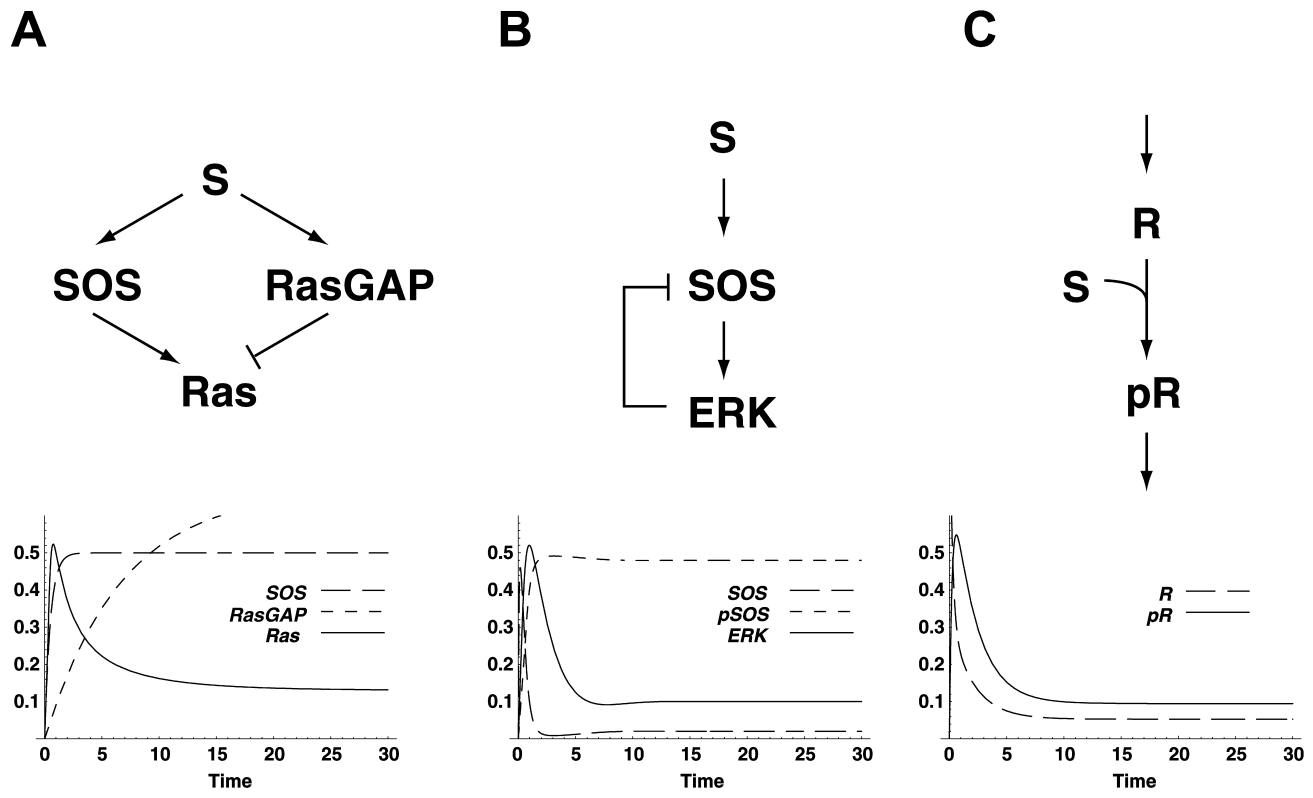

Fig. 1. **Biological representation of simple models.** Upper panels: (A) Ras model, (B) negative feedback model, (C) receptor degradation model. S is an input signal. Arrows and bars denote positive and negative regulations, respectively. Lower panels: time courses of indicated models when $S = 1$ ($t > 0$) was given with certain parameters and initial conditions. Note that the indicated molecules are represented in dimensionless form.

where Ras is located (*17–19*). This mechanism of Ras regulation allows constant stimulation by growth factors to induce transient Ras activation in PC12 cells (*3*). On the basis of this observation, we developed a simple model of Ras activation in which both *SOS* and *RasGAP* activation are dependent on stimulation (Fig. 1A and Eq. A1 in Appendix). When constant stimulation is given with $S = 1$, *SOS* and *RasGAP* activation are induced in a time-dependent manner and reach steady states (Fig. 1A, lower panel). The transient *Ras* activation appears only when *SOS* activation is faster than *RasGAP* activation (*3*). By contrast, transient *Ras* activation disappears when *RasGAP* activation is faster than *SOS* activation (*3*).

In the negative feedback loop model, transient Ras activation is induced by a negative feedback loop as in previous models (Fig. 1B) (*11*). SOS activates a downstream molecule, ERK, through Ras-dependent Raf and MEK activation (*18*). In turn, activated ERK directly or indirectly phosphorylates SOS (*20*). The phosphorylated SOS dissociates from the adaptor protein complex, resulting in a decline of Ras activation (*20*). Therefore, these processes can be regarded as a negative feedback loop. We developed a simple model of this negative feedback loop (Fig. 1B and Eq. A2 in Appendix). Constant stimulation with $S = 1$ activates *SOS*, and this leads to *ERK* activation (Fig. 1B, lower panel). In turn, activated *ERK* inactivates *SOS*, resulting in elevation of *pSOS* (inactive form of *SOS*). Thus, *ERK* is first activated, and then inhibited by the negative feedback loop. *ERK* reaches a steady state with a slight oscillation.

In the receptor degradation model, the phosphorylated receptor undergoes phosphorylation-dependent degradation. Binding of EGF to EGFR triggers receptor autophosphorylation. Phosphorylated EGFR is targeted for ubiquitination and subsequent degradation in the proteasome, resulting in the transient receptor phosphorylation (*21*). On this basis, we developed a simple model of receptor degradation (Fig. 1C and Eq. A3 in Appendix). Constant stimulation with $S = 1$ induces phosphorylation of receptor (R). The phosphorylated receptor (pR) is degraded by an irreversible first-order reaction, resulting in transient receptor activation. Here, pR approaches a nonzero constant where the rate of synthesis and degradation of pR become equivalent (Fig. 1C, lower panel). A transient peak of pR appears only when the degradation rate of pR is greater than the synthesis rate of R.

How can we distinguish these mechanisms of transient responses? If the regulators of such transient responses have been identified, measuring the dynamics of the regulators is the most straightforward way. However, if the molecules involved in such systems have not been fully identified, it is possible still to distinguish which mechanism is likely on the basis of the distinct characteristics described below.

All of the above mechanisms produce transient responses in a dose-dependent manner of stimulation

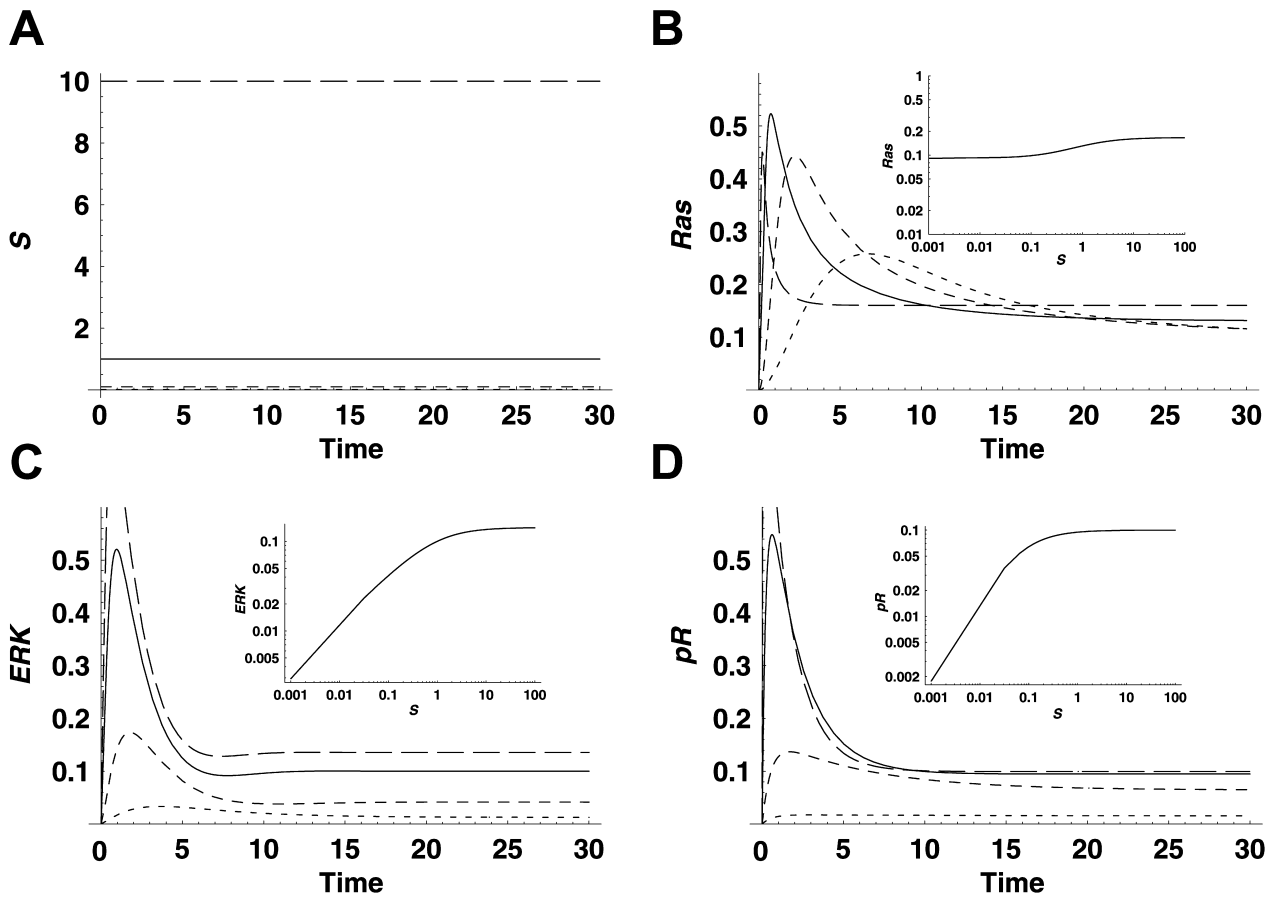

Fig. 2. **Responses to constant stimulation.** (A) Constant stimulation. Dashed line, solid line, short dashed line and dotted line indicate S = 10, 1, 0.1 and 0.01, respectively. (B) *Ras* activation. (C) *ERK* activation. (D) *pR* activation. (B–D) Insets show dose-response curves of indicated molecules at steady state.

(Fig. 2). Here, we use constant stimulation, termed step stimulation. Although the transient responses in all models look similar, the Ras model exhibits different characteristics from the other two models. The peak time of the transient *Ras* activation becomes faster as the intensity of stimulation increases (Fig. 2B), compared to the other models. In addition, *Ras* activation at steady state is relatively insensitive to the intensity of stimulation (Fig. 2B), whereas the steady states of *ERK* activation and *pR* are sensitive to the intensity of stimulation (Fig. 2, C and D). The dose-response curves in each model clearly highlight the different dependencies on the intensity of stimulation (Fig. 2, insets). The activation levels at steady state of the negative feedback model and the receptor-degradation model depend on S, whereas that of the Ras model is almost independent of S, when S is small (Fig. 2, B–D, insets). Under such conditions, transient *Ras* activation is always induced in response to multiple stepwise increases of stimulation without changing the steady state levels. Because the steady state level of *Ras* becomes a constant irrespective of the intensity of stimulation when the amplitude of *SOS* and *RasGAP* in response to the stimulation are always equal, such systems are termed perfect adaptation systems. They can be also found in the robust adaptation of chemotaxis of *E. coli* (*22*, *23*).

Under physiological conditions, concentrations of growth factors are likely to increase gradually rather than in a stepwise manner. Therefore, we assume gradually increasing stimulation, termed ramp stimulation, and examine the response in each model. The ramp stimulation is given by $S = 1 - e^{-rt}$ (Fig. 3A), where the initial velocity of S is equal to r. Note that S approaches 1 as t approaches infinity, irrespective of r. As the rate increases, the peak amplitude increases and the peak times become faster in each model (Fig. 3, B–D). Thus, the transient response in each model appears to depend on the temporal rate of stimulation, rather than the absolute concentration of stimulation. However, there are some differences between the models. First, the peak amplitude of the negative feedback model falls below the steady state level (Fig. 3C), whereas those of the other two models always exceed the steady state levels (Fig. 3, B and D). Also, the peak time of the negative feedback model is less sensitive to the rate than those of the other models. Furthermore, the peak times of the Ras model and the negative feedback model both show similar transition patterns in response to step and ramp stimulation, whereas that of the receptor degradation model is more sensitive to the rate than to the intensity of stimulation.

In conclusion, these three models describe mechanisms to detect the temporal rate of stimulation, leading to

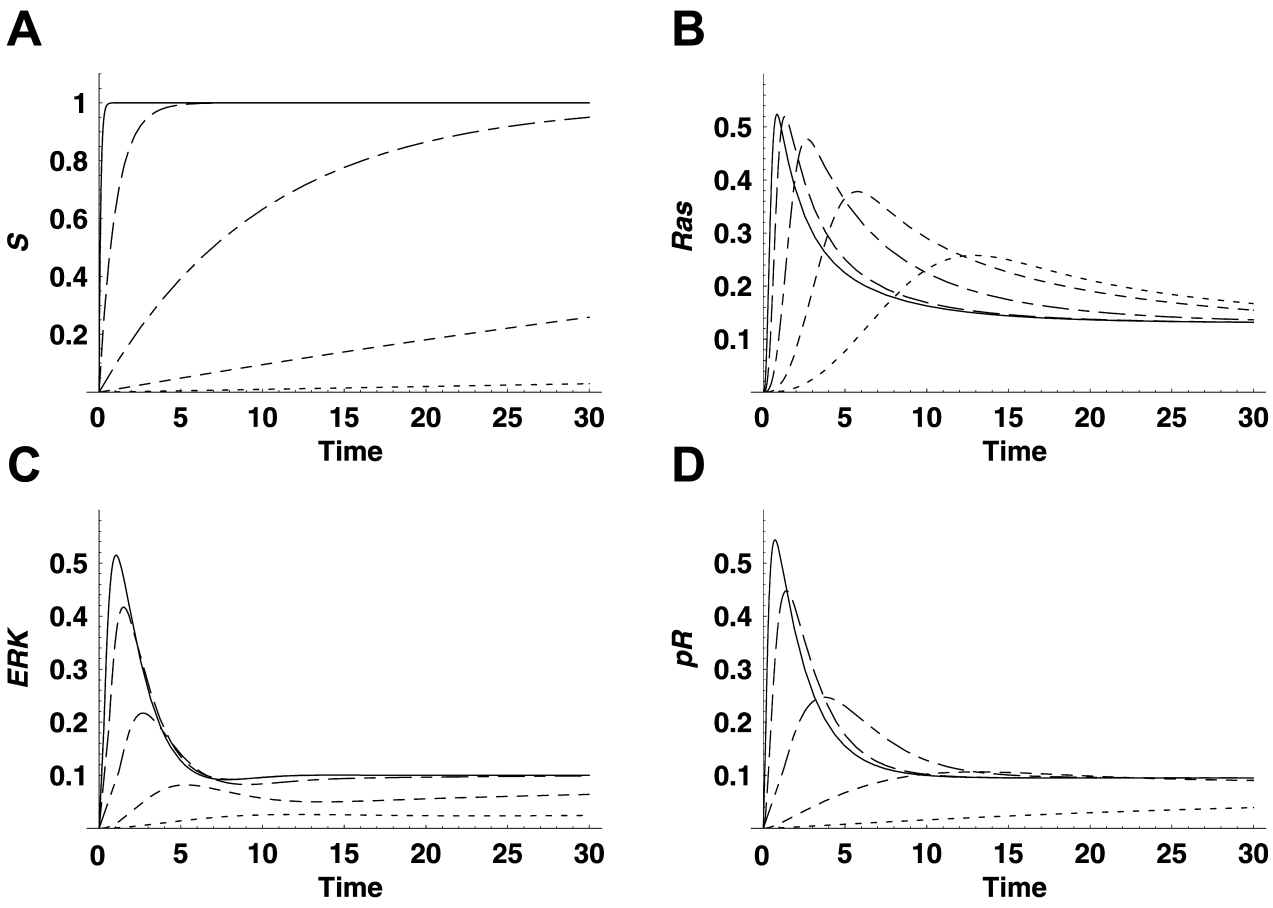

Fig. 3. **Responses to ramp stimulation.** (A) Ramp stimulation was given by $S = 1 - e^{-rt}$: Solid line, dashed line, dashed and dotted line, short dashed line and dotted line indicate r = 10, 1, 0.1, 0.01 and 0.001, respectively. (B) *Ras* activation. (C) *ERK* activation. (D) *pR* activation.

transient activation of downstream molecules. It might be physiologically preferable to detect the temporal rate of stimulation, rather than the intensity of stimulation (*22*), as a provision against an emergency, such as a rapid change in the environment. Although all these three models can respond to stepwise increase in stimulation, resulting in transient responses at each step (data not shown), the steady state levels of the Ras model is the most robust to changes in the intensity of stimulation. By contrast, another type of transient response can be seen in a well-known model of action potential, the Hodgkin-Huxley model (*24*). In this case, a positive feedback loop of voltage-sensitive channels can sense the membrane potential and trigger an action potential when the potential exceeds a threshold. Such systems that sense the intensity of stimulation show distinct characteristics from these three models.

One of the advantages using step and ramp stimulation is that we can directly compare *in vivo* dynamics in intact cells with *in silico* dynamics, allowing us more careful interpretation of *in vivo* dynamics. Because such stimulation patterns sometimes reveal distinct characteristics of alternative models that show similar responses, we can predict suitable stimulation patterns using simple models, with which we can distinguish alternative models by further *in vivo* dynamics measurements.

A detailed kinetic simulation model requires parameters such as K_d and K_{cat}, which should be measured experimentally. Kinetic parameters measured *in vitro* do not necessarily reflect the *in vivo* kinetics of intact cells. In addition, it is often difficult to determine expression levels and subcellular localization of molecules. Therefore, unknown parameters need to be assumed or estimated on the basis of *in vivo* dynamics. This is the most difficult part in developing convincing models, and the reason why many biologists hesitate to develop models. Here, we show that even simple models with minimal parameters can exhibit similar responses to those observed in experiments, and are useful to extract distinct and essential characteristics, which can be further tested by experiment. Thus, even simple models based on cartoons can be used to understand systems behaviors and to design suitable experiments to distinguish alternative models.

APPENDIX: Formulas for the simple models

The simple models shown in Fig. 1 can be described by the following differential equations.

In the Ras model (Fig. 1A), the reactions are described as follows:

$$\frac{dSOS}{dt} = k_1 S\,(1 - SOS) - k_2\,SOS$$
$$\frac{dRasGAP}{dt} = k_3 S\,(1 - RasGAP) - k_4\,RasGAP$$
$$\frac{dRas}{dt} = k_5\,SOS\,(1 - Ras) - k_6\,RasGAP\,Ras \qquad \text{(A1)}$$

where SOS, $RasGAP$ and Ras denote active fractions of the molecules, and S denotes stimulation. Kinetic parameters are set as follows: $k_1 = 1$, $k_2 = 1$, $k_3 = 0.1$, $k_4 = 0.05$, $k_5 = 10$ and $k_6 = 50$, and all initial conditions are set to zero.

In the negative feedback loop model (Fig. 1B), the reactions are described as follows:

$$\frac{dSOS}{dt} = k_1 S\,iSOS - k_2\,SOS - k_3\,ERK\,SOS + k_4\,pSOS$$
$$\frac{dpSOS}{dt} = k_3\,ERK\,SOS - k_4\,pSOS + k_1 S\,piSOS - \mathrm{k}_2\,pSOS$$
$$\frac{dERK}{dt} = k_5\,SOS - k_6\,ERK$$
$$\frac{dpiSOS}{dt} = k_3\,ERK\,iSOS - k_4\,piSOS - k_1 S\,piSOS + k_2\,pSOS \qquad \text{(A2)}$$

where $iSOS$, SOS, $piSOS$ and $pSOS$ denote inactive SOS, SOS-S complex, phosphorylated SOS and phosphorylated SOS-S complex, respectively. The total concentration of SOS is conserved: $iSOS + SOS + piSOS + pSOS = 1$. ERK and S denote activated ERK and stimulation, respectively. Kinetic parameters are set as follows: $k_1 = 10$, $k_2 = 10$, $k_3 = 4.8$, $k_4 = 0.02$, $k_5 = 2.5$ and $k_6 = 0.5$, and all initial conditions except $iSOS$ are set to zero.

In the receptor degradation model (Fig. 1C), the reactions are described as follows:

$$\frac{dpR}{dt} = k_1 S\,R - k_2\,pR - k_3\,pR$$
$$\frac{dR}{dt} = k_5 - k_6\,R - k_1 S\,R + k_2\,pR \qquad \text{(A3)}$$

where R, and pR denote active fractions of the molecules, and S denotes stimulation. Kinetic parameters are set as follows: $k_1 = 3$, $k_2 = 1$, $k_3 = 1/3$, $k_4 = 0$, $k_5 = 1/15$ and $k_6 = 1/15$, and initial conditions of R and pR are set to zero and one, respectively.

Note that the rate of an enzymatic reaction is approximated by the product of K_{cat}, enzyme concentration and substrate concentration for simplicity. In addition, a series of reaction steps is approximated by a single reaction, provided that the cascade is straightforward.

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