

# Intrinsic Fluctuations, Robustness, and Tunability in Signaling Cycles

Joseph Levine,\* Hao Yuan Kueh,<sup>†</sup> and Leonid Mirny<sup>‡</sup>

\*Computation and Neural Systems Option, California Institute of Technology, Pasadena, California; <sup>†</sup>Graduate Program in Biophysics, Harvard University, Boston, Massachusetts; and <sup>‡</sup>Harvard-MIT Division of Health, Science and Technology, and Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts

**ABSTRACT** Covalent modification cycles (e.g., phosphorylation-dephosphorylation) underlie most cellular signaling and control processes. Low molecular copy number, arising from compartmental segregation and slow diffusion between compartments, potentially renders these cycles vulnerable to intrinsic chemical fluctuations. How can a cell operate reliably in the presence of this inherent stochasticity? How do changes in extrinsic parameters lead to variability of response? Can cells exploit these parameters to tune cycles to different ranges of stimuli? We study the dynamics of an isolated phosphorylation cycle. Our model shows that the cycle transmits information reliably if it is tuned to an optimal parameter range, despite intrinsic fluctuations and even for small input signal amplitudes. At the same time, the cycle is sensitive to changes in the concentration and activity of kinases and phosphatases. This sensitivity can lead to significant cell-to-cell response variability. It also provides a mechanism to tune the cycle to transmit signals in various amplitude ranges. Our results show that signaling cycles possess a surprising combination of robustness and tunability. This combination makes them ubiquitous in eukaryotic signaling, optimizing signaling in the presence of fluctuations using their inherent flexibility. On the other hand, cycles tuned to suppress intrinsic fluctuations can be vulnerable to changes in the number and activity of kinases and phosphatases. Such trade-offs in robustness to intrinsic and extrinsic fluctuations can influence the evolution of signaling cascades, making them the weakest links in cellular circuits.

## INTRODUCTION

Post-translational covalent modification cycles underlie a wide variety of cellular communication and control processes. Enzymes catalyzing these reactions make up a large part of an organism's genome. Humans have >500 protein kinases genes alone, 1.7% of the total genome (1). This emphasizes the importance of studying how cycles both signal reliably (robustness) and adjust to broad ranges of stimuli (tunability).

Covalent modification cycles consist of two reactions: one in which an enzyme catalyzes the addition of a prosthetic group to a substrate (for example phosphorylation by a kinase), and one where another enzyme undoes the modification (dephosphorylation by a phosphatase). Here we use protein phosphorylation terminology, though our results apply to covalent modification in general. Upstream biochemical signals modulate the number of active enzymes (kinases) in the cycle and serve as an input. The activation of kinases leads to change in the number of covalently-modified signaling molecules, which signal downstream and act as an output to the cycle. We assume that the function of the cycle is to generate an output that distinguishes signal from background for the duration of the signal.

This simple architecture has surprisingly rich behavior (2). Two natural limiting cases of the modification kinetics explain this behavior (Fig. 1). The first case (described as unsaturated, first-order or hyperbolic) occurs when catalyzing enzymes are unsaturated with respect to the substrate molecules. This system behaves in steady state as a weakly non-

linear amplifier, yielding graded responses to graded inputs (Fig. 1 *C*, *green*). The second case (saturated, zero-order, or ultrasensitive) occurs when substrate molecules saturate the enzymes. This system behaves like a thresholding digital amplifier in steady state (Fig. 1 *C*, *blue*). Intrinsic fluctuations, arising from stochastic chemical reactions, behave differently in the two cases. Unsaturated cycles are typically noisier, although ultrasensitive cycles have large fluctuations in the high gain region (Fig. 1 *D* and Eqs. 5 and 6) (3).

Biochemical fluctuations may profoundly influence covalent modification cycles (4). The amount of signaling substrate in some systems may be rather low, and low molecular copy number increases the impact of fluctuations (5,6). Yeast, for example, have  $\sim 10^3$  mitogen-activated protein kinase Kss1p and Fus3p molecules per cell (7), while large tissue culture cells have  $\sim 10^4$  (8). Compartmental segregation and slow diffusion (5,6,9–12) potentially makes the effective number of reactants even smaller. For example, it takes >10 h for a protein to diffuse across a 0.5-mm *Xenopus* oocyte (assuming a diffusion coefficient of  $5 \mu\text{m}^2/\text{s}$ ). Reliable signaling must therefore take place despite these fluctuations. Recent work has studied noise in genetic networks (13–17), but protein networks remain relatively unexplored. Fluctuations are both beneficial (causing phenotypic heterogeneity in clonal populations) (18,19) and detrimental (causing unreliable responses to signals).

Here we study a simple phosphorylation cycle subject to a time varying (square pulse) kinase stimulus. Most previous systems level studies focus on biochemical complexities (such as multisite phosphorylation (20,21)) and network properties (such as feedback and cascades (22–25)). We claim simple

Submitted May 12, 2006, and accepted for publication January 16, 2007.

Address reprint requests to Leonid Mirny, Tel.: 617-452-4862; E-mail: leonid@mit.edu.

© 2007 by the Biophysical Society

0006-3495/07/06/4473/09 \$2.00

doi: 10.1529/biophysj.106.088856

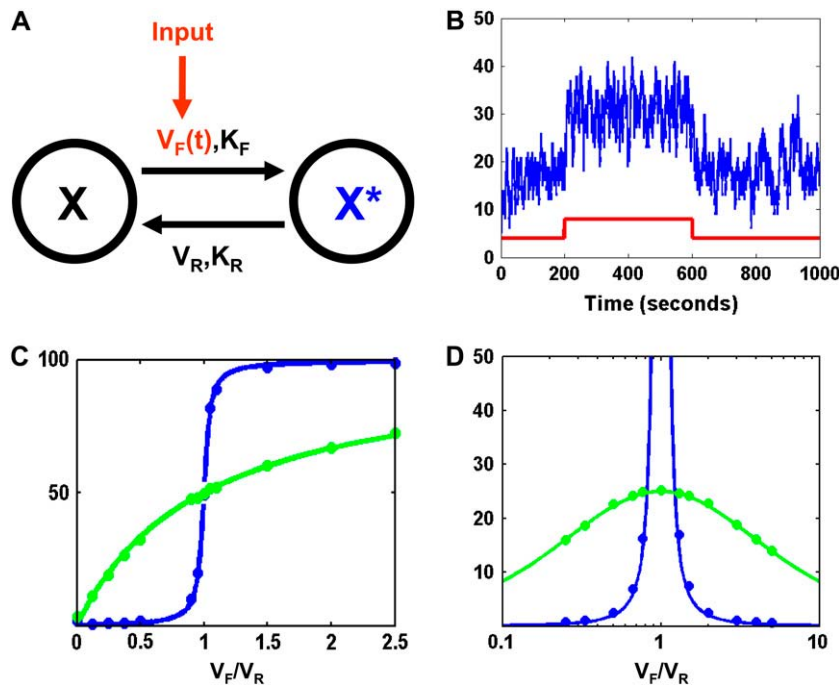


FIGURE 1 Model description. (A) Schematic of our model. (B) The stimulation protocol used in our simulations. The red trace shows the kinase pulse  $V_F(t)$  (not to scale) against background activity. The blue trace shows the number of  $X^*$  molecules in response to this kinase pulse. Phosphatase activity  $V_R$  is held constant. (C) Mean and (D) variance of the steady-state  $X^*$  distribution as a function of the ratio  $V_F/V_R$ , in the hyperbolic (green) and ultrasensitive (blue) limits. Circles represent values from simulations ( $N = 100$ , hyperbolic  $K_M = 5000$ , ultrasensitive  $K_M = 1$ ), curves are Eqs. 5 and 6.

phosphorylation cycles alone have very rich behavior. We address the following:

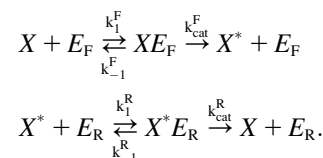
1. How can a signaling cycle work reliably despite intrinsic fluctuations?
2. How do changes in extrinsic parameters lead to variability of response? Can cells use these parameters to tune the cycle to different ranges of stimuli?
3. Which of the two operational regimes (ultrasensitive or hyperbolic) is more beneficial from the point of view of robustness and tunability?

We therefore study the cycle's dynamic behavior and reliability/fidelity of signaling using basic techniques from statistical signal processing and information theory. We argue, in the spirit of Detwiler et al. (26), that cells may use the cycle's tunability to respond to different signaling contexts.

Our model shows that the cycle transmits information reliably in an optimal parameter range, despite intrinsic fluctuations and even for small input amplitudes. The cycle is sensitive to changes in some "soft" extrinsic parameters, such as concentration and activity of kinases and phosphatases. This sensitivity can lead to significant cell-to-cell response variability. It also allows cells to tune the cycle to transmit signals in a given amplitude range. Ultrasensitive cycles are superior to hyperbolic ones in both robustness and tunability. Cycles tuned to suppress intrinsic fluctuations, however, may be vulnerable to changes in the number and activity of kinases and phosphatases (27,28). Hyperbolic cycles, while noisier, require little tuning to transmit a broad range of input amplitudes. This motif's combination of robustness and flexibility makes it potentially suitable for a wide range of signaling tasks inside a cell.

## MODEL AND METHODS

We consider  $N$  molecules of a signaling species  $X$ . Each molecule is either unmodified ( $X$ ) or covalently modified ( $X^*$ ). We ignore effects such as degradation, sequestration, or dilution of the signaling species by enforcing a conservation relation  $X + X^* = N$ . We assume the existence of two enzymes each present with fewer than  $N$  copies:  $E_F$ , which catalyzes the conversion of  $X$  to  $X^*$  and  $E_R$ , which catalyzes the reverse conversion of  $X^*$  to  $X$ . The molecules obey the following reactions:



The assumptions are consistent with minimal substrate sequestration and allow us to collapse the system to a pair of simplified reactions,  $X \rightarrow X^*$  and  $X^* \rightarrow X$ . The rate of creation, or birth, of  $X^*$  from  $X$  is denoted by  $b(X)$ . The rate of destruction, or death, of  $X^*$  is denoted by  $d(X^*)$ . Under our assumptions, these rates follow the Michaelis-Menten approximation with maximal reaction rates  $V_{F/R} = k_{\text{cat}}^{F/R} E_{F/R}$  and Michaelis constants  $K_{F/R} = (k_{-1}^{F/R} + k_{\text{cat}}^{F/R}) / k_1^{F/R}$ , for forward and reverse reactions, respectively. Reaction rates are in units of molecules per second and Michaelis constants are in units of molecules. The net rate of change of  $X^*$  is the difference of the two leading to the dynamics:

$$\frac{dX^*}{dt} = \frac{V_F(N - X^*)}{K_F + N - X^*} - \frac{V_R X^*}{K_R + X^*}. \quad (1)$$

To treat this problem stochastically, we analyze the probability distribution that there are  $n$  molecules of  $X^*$  present at time  $t$ , namely  $p(n|t)$ , for  $0 \leq n \leq N$ . Under the assumption that chemical reactions are well mixed (29), this problem can then be recast into the form of a birth-death Markov process with the birth and death rates given above (30). The time evolution of the probability distribution obeys a master equation,

$$\frac{dp(n)}{dt} = b(n-1)p(n-1) + d(n+1)p(n+1) - (b(n) + d(n))p(n). \quad (2)$$

In the limits of highly unsaturated enzymes ( $K_M \gg N$ ) and highly saturated enzymes ( $K_M \ll 1$ ), the steady-state distribution  $\bar{p}(n)$  can be found in closed form using recursion relations. They are

$$\bar{p}(n)_{\text{hyp}} = \binom{N}{n} \frac{\left(\frac{V_F K_R}{V_R K_F}\right)^n}{\left(1 + \frac{V_F K_R}{V_R K_F}\right)^N}, \quad (3)$$

$$\bar{p}(n)_{\text{ult}} = \left(\frac{V_F}{V_R}\right)^n \left( \frac{1 - \frac{V_F}{V_R}}{1 - \left(\frac{V_F}{V_R}\right)^N} \right). \quad (4)$$

The means and variances of these distributions are given below and are plotted in Fig. 1, *C* and *D*:

$$\langle n \rangle_{\text{hyp}} = \frac{N}{1 + \frac{V_R K_F}{V_F K_R}}, \quad (5a)$$

$$\sigma_{\text{hyp}}^2 = N \frac{\frac{V_R K_F}{V_F K_R}}{\left(1 + \frac{V_R K_F}{V_F K_R}\right)^2}, \quad (5b)$$

$$\langle n \rangle_{\text{ult}} = \frac{\frac{V_F}{V_R}}{\left(1 - \frac{V_F}{V_R}\right)} - (N+1) \frac{\left(\frac{V_F}{V_R}\right)^{N+1}}{1 - \left(\frac{V_F}{V_R}\right)^{N+1}}, \quad (6a)$$

$$\sigma_{\text{ult}}^2 = \frac{\frac{V_F}{V_R}}{\left(1 - \frac{V_F}{V_R}\right)^2} - (N+1)^2 \frac{\left(\frac{V_F}{V_R}\right)^{N+1}}{\left(1 - \left(\frac{V_F}{V_R}\right)^{N+1}\right)^2}. \quad (6b)$$

## Simulations

We simulated our model using a Gillespie algorithm (29) that allows for time-varying input. Obtained distributions and time-series were resampled once per second to prevent oversampling of portions of the time series with faster kinetics.

## Signal/noise ratio

The steady-state signal/noise ratio (SNR) of a signal with high and low values is defined as

$$SNR = \frac{\mu_{\text{HIGH}} - \mu_{\text{LOW}}}{\sqrt{\sigma_{\text{HIGH}}^2 + \sigma_{\text{LOW}}^2}}. \quad (7)$$

The values  $\mu_A$  and  $\sigma_A^2$  are the mean and variance, respectively, of the steady-state  $X^*$  distribution when  $V_F$  is at level *A*. Systems were allowed to relax to steady state for 20,000 s and the  $X^*$  time series for 30,000 s after this was used as the steady-state distribution.

## Mutual information

Mutual information, a relation between an input signal distribution  $X$  and an output signal distribution  $Y$ , quantifies how much one learns about one dis-

tribution by knowing the other (31). The mutual information between  $X$  and  $Y$  is given by the expression

$$I_{X,Y} = \sum_{x \in X, y \in Y} p(x, y) \log_2 \left( \frac{p(x, y)}{p(x)p(y)} \right). \quad (8)$$

We identify  $X(t)$  with the kinase input  $V_F(t)$  and  $Y(t)$  with the distribution of  $X^*(t)$ . By integrating over  $t$ , one can obtain the joint distribution  $p(X, Y)$ . The kinase pulse, after an initial period in the low state allowing the system to relax to a steady state, takes on two values (high and then low) for equal amounts of time. From an information theoretic standpoint, this represents a fair coin containing one bit of information. The output distribution  $X^*$ , taking possible values from 0 to  $N$ , was divided into 10 equally spaced bins to simulate discreteness of a biological detector. Different bin sizes and numbers did not change our qualitative results.

## RESULTS

We model a population of signaling proteins being briefly phosphorylated by a kinase pulse above background activity and constant phosphatase activity (Fig. 1).

Can a cycle be tuned to minimize intrinsic fluctuations? The cycle possesses three readily adjustable parameters: forward phosphorylation velocity  $[V_F] = k_{\text{cat}}^F [E_F]$ ; reverse dephosphorylation velocity  $[V_R] = k_{\text{cat}}^R [E_R]$ ; and substrate molecule number  $N$ . Cells can change the “soft” parameters  $[V_F]$  and  $[V_R]$  in real time by changing local enzyme concentrations  $[E]$ . The other two reaction parameters  $K_F$  and  $K_R$  represent less tunable (“hard”) binding parameters that may be changed by protein mutations on a much larger timescale. All parameters ( $k_{\text{cat}}$ ,  $K_m$ ,  $[E_R]$ ,  $[E_F]$ , and  $N$ ) can also be changed by covalent modification (32), making the cycle a highly tunable part of cellular circuitry.

## Effects of intrinsic fluctuations

The first way to fight intrinsic fluctuations would be to increase the number of substrate molecules in the cycle. Signal/noise ratio (SNR, Eq. 7), the mean signal level divided by fluctuation magnitude, measures a noisy signal’s fidelity. Random process steady-state SNRs typically grow with their size. As the number of substrate molecules increases, the system transitions from the hyperbolic to the ultrasensitive regime. The cycle’s SNR increases as  $\sim \sqrt{N}$  in the hyperbolic regime and transitions smoothly to an  $\sim N$  increase in the ultrasensitive regime (Eqs. 5a, 5b, 6a, and 6b and Fig. 2 *A*). Thus, increasing  $N$  indeed seems to increase signaling fidelity.

This approach incurs a penalty in the cycle dynamics. Fig. 2 *B* plots the time for a cycle to reach steady state given a step input of kinase, starting with no substrate phosphorylated. The cycle’s architecture causes switching time to increase slightly sublinearly with  $N$  in the unsaturated regime and linearly in the ultrasensitive regime. When unsaturated, catalytic velocities increase with the number of substrate molecules, compensating somewhat for the greater amount of substrate. When saturated, each substrate conversion takes constant time, and total switching time increases linearly with  $N$ .

Mutual information provides another way to quantify the performance of a signaling pathway. This information

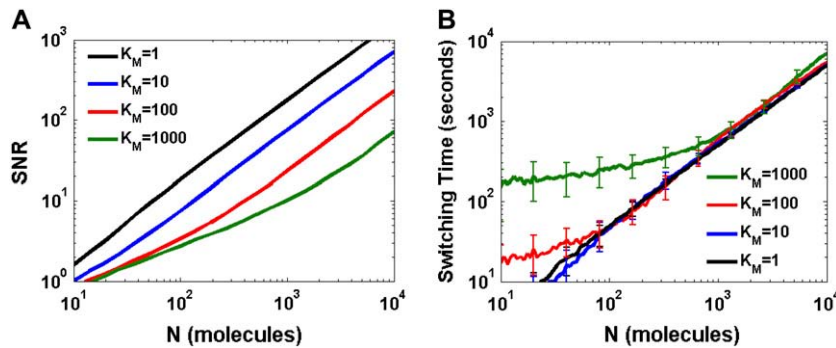


FIGURE 2 Noise rejection and switching time dependence on substrate amount,  $N$ . Colors represent different Michaelis constants; blue,  $K_M = 1$ ; green,  $K_M = 10$ ; and red,  $K_M = 100$ . (A) Steady-state SNR. Kinase and phosphatase rates (molecules/s) were  $V_{F,Low} = 4$ ,  $V_{F,High} = 8$ ,  $V_R = 6$ . (B) Mean switching time and standard deviation (error bars) in response to step input. The cycle was initialized at  $X^* = 0$  and given a step in kinase from  $V_{F,Low} = 0$  to  $V_{F,High} = 8$ , with phosphatase values at  $V_R = 6$ . The system was allowed to relax to a numerically determined steady state and the first crossing point was chosen as the switching time. Panels A and B are plotted on the same abscissa scale for comparison. All curves are averaged over 100 trials.

theoretic quantity (Eq. 8) measures how much a communication channel's output reduces uncertainty about the input. The input pulse with equal high and low times contains one bit of information. The mutual information transmitted by the channel thus takes a maximum value of one bit when the output allows the high and low states to be distinguished and a minimum value of zero bits when recognizing the original input signal in the distorted output is impossible. These maximal and minimal values are convenient for comparing signaling fidelity under different conditions. Mutual information reduced if the duration of the output pulse is shorter than duration of the activating input.

Fig. 3 A illustrates the impact of large  $N$  on signaling by plotting the mutual information transmitted by the cycle for the pulse input. Strikingly, the performance of the cycle as an information transmitting channel has a maximum at some optimal value of  $N$ , while dropping at smaller or larger number of substrate molecules. At low  $N$ , the cycle's intrinsic fluctuations distort pulse transmission by causing the high and low outputs to overlap and blur (Fig. 3 B). As  $N$  increases and we enter saturation, the mutual information approaches its maximal value of one, indicating a cleaner transmission of the pulse (Fig. 3 C). Finally, as  $N$  increases, further the mutual information decreases again due to slow response. This sluggish behavior at large  $N$  distorts the output leading to drop in information loss (Fig. 3, D and E). The system's optimal performance for a particular range of  $N$  is similar to system size resonance (33,34).

Our model shows that a cycle works reliably when substrate amount lies in an optimal range, despite intrinsic fluctuations. A small number of substrate molecules leads to fluctuations. Excess substrate slows down the response, leading to a highly distorted or completely lost signal. The range of substrate that provides reliable signal transduction depends on the amplitude and duration of the pulse (C. Gomez-Urbe, G. Verghese, and L. A. Mirny, unpublished). The cycle probably tolerates substrate number fluctuations within the optimal range.

### Effects of extrinsic parameters on the signaling performance

How sensitive is the signaling cycle to extrinsic variations in kinases and phosphatases? To study this we measure the

information transmitted by the cycle as a function of the number of kinase molecules  $V_F$  and phosphatase molecules  $V_R$ , with an optimal  $N$  and balanced enzymatic saturations ( $K_M = K_F = K_R$ ). The input pulse has background level  $V_{F,Low}$  and signal level  $V_{F,High}$  (Fig. 1). Fig. 4 shows information transmitted by a cycle as a function of  $V_R$  in response to two pulses with different background and signal levels.

An ultrasensitive cycle (Fig. 4 B) separates signal from background and transmits up to one bit of information. Cells must tune  $V_R$  to  $V_{F,Low} < V_R < V_{F,High}$  so high inputs produce high response and low inputs produce low response. As  $V_R$  leaves this range, signaling quality diminishes rapidly. The range of optimal  $V_R$  values is broader for signals strongly above background and narrower for weaker ones. Cycles tuned to one amplitude range respond poorly to signals far outside this range.

Hyperbolic cycles require less tuning and signal well in a broad range of  $V_R$  (Fig. 4 C). Signaling quality depends strongly on the background kinase activity  $V_{F,Low}$ . Signaling is somewhat erratic for signals on smaller background (Fig. 4 C, blue), but more so for signals on large backgrounds (Fig. 4 C, red).

Gradient sensing is an example of signaling on varying background levels (28,36–39). Here, cycles signal changes in a stimulant's concentration or gradient regardless of its background concentration. Hyperbolic cycles perform this task poorly, while ultrasensitive cycles need tuning.

This required tuning makes ultrasensitive cycles sensitive to extrinsic kinase and phosphatase fluctuations. Such fluctuations can be aggravated by compartmentalization and lead to cell-to-cell variation in response to external stimuli. The tuning requirement also makes signaling cycles vulnerable to mutations affecting the activity or concentration of kinases and phosphatases (27,28,40). Thus, ultrasensitive cycles minimize the effect of intrinsic fluctuations but remain sensitive to variations in extrinsic parameters.

Hyperbolic cycles are robust to extrinsic fluctuations but generate significant intrinsic noise and lose more information. The range of workable  $V_R$  levels increases as the cycle becomes less saturated.

Cycles are more robust to Michaelis constant changes. Fig. 5 A plots mutual information for varying saturation in

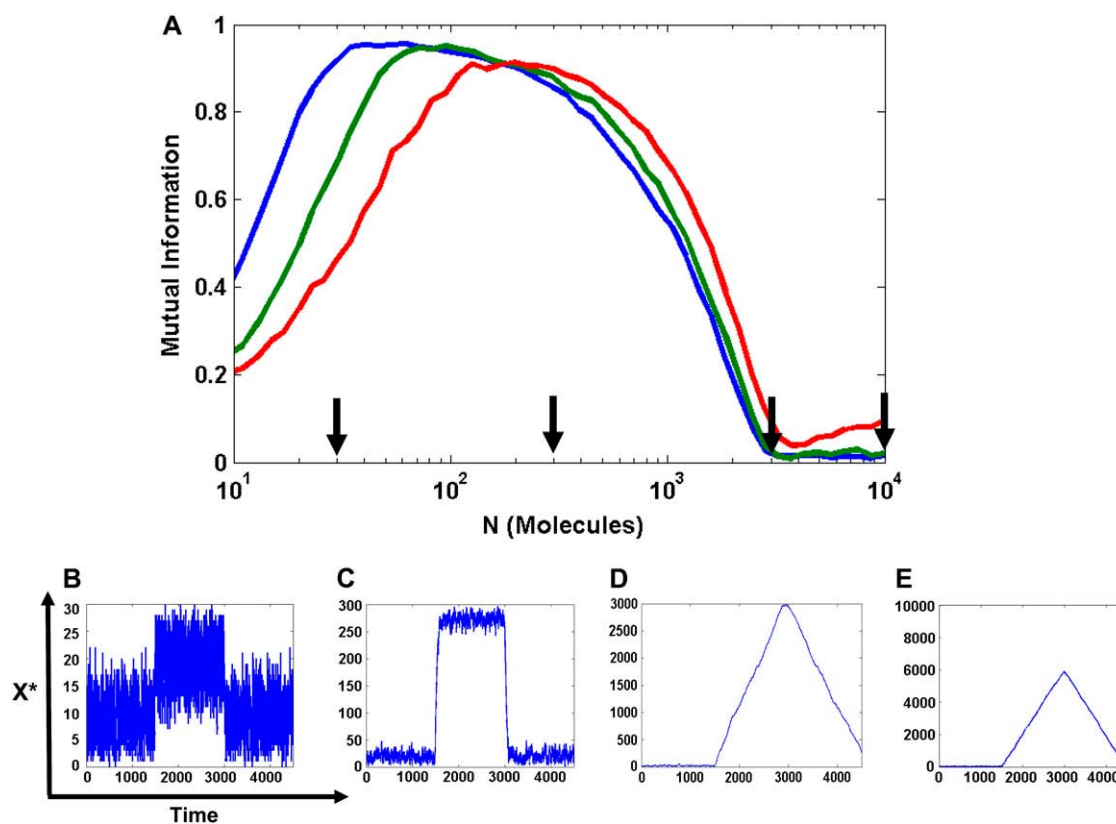


FIGURE 3 Dynamic signaling quality as a function of substrate amount,  $N$ . Colors are as in Fig. 2. (A) Mutual information between the input and the output. The cycle was initially equilibrated for 1500 s at  $V_{F,Low}$  and then given a 1500 s  $V_{F,High}$  pulse before returning to  $V_{F,Low}$  for another 1500 s. Kinase and phosphatase parameters are identical to those in Fig. 2 *a*. Curves are averaged over 100 trials. (B–E) Representative time traces for  $N = 30$  (B),  $N = 300$  (C),  $N = 3000$  (D), and  $N = 10,000$  (E), shown by arrows in panel A.

forward and backward enzymes. The cycle functions optimally with both pathways similarly saturated. Precise matching is not necessary. This is fortunate since Michaelis parameters are not easily tuned, being set by enzyme-substrate binding affinity and changed by competitive inhibitors or mutations. This suggests that competitive inhibition of cycle enzymes is a poor way of controlling them. Noncompetitive or allosteric inhibition and covalent modification can alter  $V_F$  or  $V_R$  and have more profound effect on the signaling.

How does a cycle respond to simultaneous changes in  $N$ ,  $V_R$ , and  $K_m$ ? Fig. 5, *B* and *C*, plots mutual information as a function of  $V_R$  and  $K_m$  for two different values of  $N$ . Increasing  $K_m$  causes a transition from the ultrasensitive to the hyperbolic regime. Low  $K_m$  cycles signal well in a tight  $V_R$  range (Fig. 4 *B*). As  $K_m$  increases, the cycle grows more robust to mistuned  $V_R$  while maximal mutual information decreases (Fig. 4 *C*). The fan-shaped phase space volume is typical for various values of  $N$ . Increasing  $N$  increases both the volume of workable phase space and the maximal mutual information of that volume (Fig. 5, *B* and *C*) until the system encounters the slowdown at high  $N$  explained before. An intermediate  $K_m$  regime is robust to intrinsic noise and has a broader working parameter range.

## DISCUSSION

Despite their simplicity, covalent modification cycles exhibit rich behaviors with several implications for the functioning and evolution of signaling networks. Simple covalent cycles function well in a limited range of kinetic parameters. This range determines the cycle's inherent flexibilities, fragilities, and robustness.

The amount of signaling substrate,  $N$ , influences both the effect of fluctuations and the response speed. These competing constraints lead to an optimal number of substrate molecules to minimize noise effects in dynamic signaling, suggesting that cells control the amount of substrate. This optimal number of substrate molecules is reminiscent of system size resonance (33,34,41), although the optimum system size in covalent signaling arises from the tradeoff between noise and dynamics, not through enhancement of the signal by a critical value of intrinsic noise. A recent study of Morishita et al. (42) used a different measure of signal reliability, and came to the same conclusion of an optimal number of substrate molecules required for reliable signaling.

Substrate overexpression potentially mistunes a cycle, and this mistuning may propagate through an entire cascade.



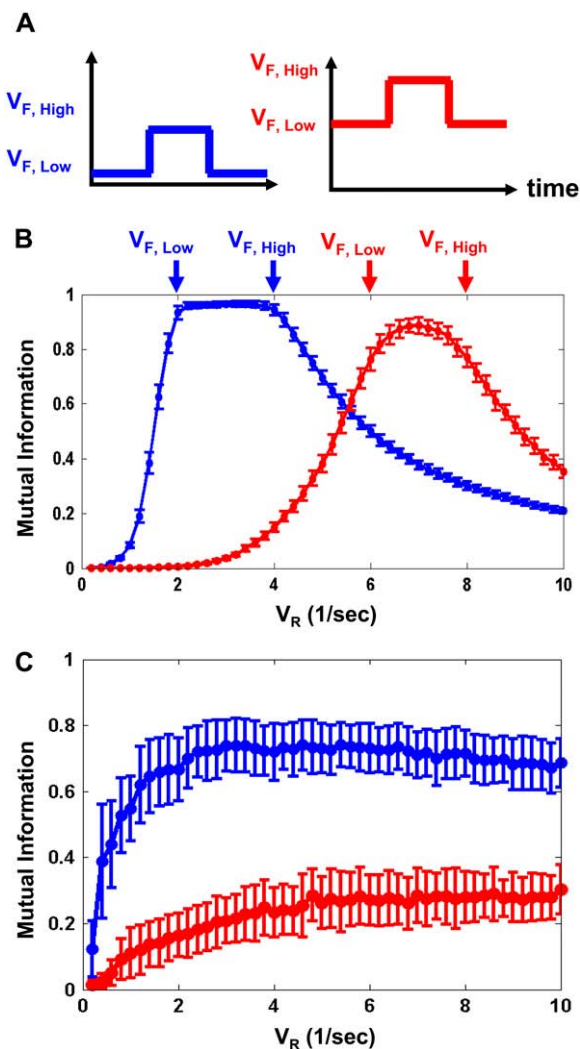


FIGURE 4 Signaling quality dependence on kinase and phosphatase balance. (A) Two input stimuli with different background  $V_{F,Low}$  and  $V_{F,High}$  signal levels (molecules/s). Blue,  $V_{F,Low} = 2$ ;  $V_{F,High} = 4$ . Red,  $V_{F,Low} = 6$ ;  $V_{F,High} = 8$  (shown by arrows on B). (B) Ultrasensitive cycle response to two stimuli as a function of  $V_R$  (for  $K_M = 5$ ). (C) Hyperbolic cycle response as a function of  $V_R$  ( $K_M = 1000$ ). Panels B and C plot mutual information between input and output. Pulse times are the same as in Fig. 3, and  $N = 100$ . Curves represent means, and error bars represent standard deviations, of mutual information over 100 trials.

Increasing  $V_F$  and  $V_R$  would compensate for this effect, at a cost increasing the number of kinases and phosphatases. These in turn play the role of  $N$  for upstream steps of a cascade. In cases such as membrane receptors responding to a small number of external ligands, upregulating receptors would not increase  $V_F$ . Other mechanisms, such as allosteric interactions between receptors (43) or positive feedback, might increase  $V_F$  and speed up the cycle.

Experiments controlling the amount of signaling substrate (e.g., by inducible expression) can test our prediction of optimal concentration of substrates needed for reliable signaling. Spectroscopic studies of the spatial distribution of

signaling substrates would also shed light on this phenomenon (44,45). On evolutionary timescales, changes in substrate gene dosage might mistune a cycle (46). Comparative genomics can help verify this prediction by correlating the frequency of gene duplications with the rate of evolution of kinases and phosphatases.

Cycles are also sensitive to the number of kinases and phosphatases, and to changes in their binding and rate constants. While the number of molecules can be regulated by the cell and is subject to random fluctuations (“soft” parameters), binding and rate constants of the enzymes cannot be easily tuned in real time but are subject to changes on the evolutionary timescales (“hard” parameters). This has three main implications.

First, extrinsic fluctuations in the number of enzymes may reduce the fidelity of signaling. Tuned ultrasensitive cycles with weak input are particularly sensitive, especially for weak signals on large backgrounds in situations like chemotaxis (36–38,47). Such fluctuations may lead to noise and cell-to-cell variability that, in turn, can be harmful, or may provide a diversity of responses and increase population-average survival and fitness (18,19). Sensitivity to exact tuning also leaves cycles vulnerable to kinase and phosphatase mutations. Numerous somatic mutations in kinases (28) and phosphatases have been detected in cancer cells (27) and pathogenic infections, like anthrax (40). These mutations often have moderate (less than a factor of two) effects on  $k_{cat}$  and  $K_m$  (27), while causing a profound physiological effect. These mutations may cause excessive signaling or lack of response to stimuli, especially in fragile parameter regimes. Our results predict that the physiological effects of changes in  $k_{cat}$  can be more profound than perturbations of  $K_m$ .

Second, sensitivity to extrinsic parameters provides an easy way to tune a signaling cycle to transmit signals of various amplitudes and background levels (see Fig. 4 A). This requires changing expression, degradation, modification or internalization of either kinases or phosphatases, possibly through feedback. This allows a cycle to adapt to a broad range of background amplitudes, similar to adaptation observed in bacterial chemotaxis (36,38). Although feedback is often associated with bistability (23,48–50) or noise suppression (22,51), we wish to highlight that feedback can keep signaling cycles tuned and adapt them to a range of stimuli amplitudes and background levels.

Third, easy tuning of signaling cycles to transmit signals of various amplitudes allows rapid evolution of signaling networks. Duplication and tuning of part of an existing cascade can produce a new pathway. Tuning a new cycle away from the optimal regime of the ancestral one may minimize the cross-talk between cascades. This remarkable tunability can make signaling cascades ubiquitous elements of cell circuitry.

Our model has several limitations and provides several directions for further research. We ignore multiple phosphorylation (20,21) and consider only the simplest signaling

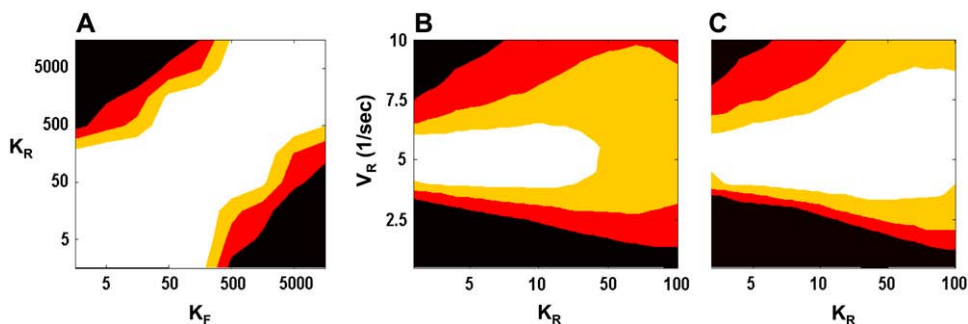


FIGURE 5 Cycle phase space. (A) Effect of kinase ( $K_F$ ) and phosphatase ( $K_R$ ) Michaelis constants on mutual information. (B,C) Combined effect of varying  $K_M$  and  $V_R$  on the mutual information for  $N = 100$  (B) and  $N = 200$  (C). Contours highlight regimes of reliable signaling (black,  $I = 0$ –0.25; red, 0.25–0.50; yellow, 0.5–0.75; and white, 0.75–1.0).

cycle undergoing a single phosphorylation event. Our model assumes Michaelis-Menten kinetics, a good approximation for systems like the phosphorylation of Fus3p by Ste7p in yeast, but unreasonable for systems like the phosphorylation of ERK2 MAP kinase by Mek-1 in *Xenopus* oocytes (8). We do not model spatial effects explicitly (52,53), although we consider them implicitly by studying sensitivity to the external parameters  $N$ ,  $V_R$ , and  $V_F$ . Spatial heterogeneity (11), scaffolding (54,55), and compartmentalization (9,12,56,57) can lead to significant fluctuations in the number of active kinases, phosphatases, and their substrates. These fluctuations lead to mistuning and significant cell-to-cell variability in signaling predicted by our model and discussed above.

Here we studied the cycle's response to a square pulse of kinase activity. Three observations motivate our choice of a square pulse. First, response to a square pulse input is a sensitive engineering probe for both dynamic and steady-state behavior of the system. Second, single cell time-lapse microscopy has recently revealed pulsatile and oscillatory dynamics inherent in major signaling networks such as the DNA damage response in both prokaryotes (58) and eukaryotes (59), and the NF- $\kappa$ B network (60). More examples will undoubtedly emerge as single cell techniques improve and expand. Third, extracellular inputs to signaling networks potentially take this form. For example, a receptor subject to spontaneous background activation may undergo rapid activation by a higher ligand concentration (or high affinity ligand), followed by a rapid inactivation (by internalization, or drop in the concentration of an activator).

We aim to understand the complexities, strengths, and weaknesses of this simple system, which provides a baseline for understanding the more complex networks often found in signaling pathways. More complex signaling networks probably evolved to improve performance of the simple cycle and to convey additional functionality. Cascades of moderate-gain phosphorylation cycles often appear, such as the canonical MAP kinase cascade, which shows net high gain (61). This topology has also been implicated in noise suppression (24). Feedback networks of kinase systems exhibit bistable behavior, as in *Xenopus* p42 MAPK/Cdc42 (23). Scaling up (62) our stochastic analysis to networks may prove challenging, although theoretical and experimental work in this spirit has begun in the context of gene expression networks. (14,17,56,63). We hope analyses of this type will

produce a more systematic and quantitative understanding of biological signaling pathways.

In summary, we show that signaling cycles can reliably transmit information despite intrinsic fluctuations. An optimal number of substrate molecules balances speed of response with noise rejection. A trade-off exists between robustness to intrinsic and extrinsic fluctuations. In the ultrasensitive regime, the cycle is robust to intrinsic fluctuations but requires tuning of the kinase/phosphatase ratio. Fluctuations in this ratio lead to cell-to-cell variability of the signaling response. In contrast, a cycle in the hyperbolic (unsaturated) regime suffers from strong intrinsic fluctuations while being robust to changes in extrinsic parameters. Such a cycle requires no tuning but demonstrates poor performance. An intermediate regime shows the best of both worlds, being robust to both intrinsic noise and variations in extrinsic parameters. One can imagine that, depending on the spectrum of stimuli and requirements for more or less noisy response, an organism can choose to operate different signaling pathways in different regimes.

## REFERENCES

- Manning, G., D. B. Whyte, R. Martinez, T. Hunter, and S. Sudarsanam. 2002. The protein kinase complement of the human genome. *Science*. 298:1912–1934.
- Goldbeter, A., and D. E. Koshland, Jr. 1981. An amplified sensitivity arising from covalent modification in biological systems. *Proc. Natl. Acad. Sci. USA*. 78:6840–6844.
- Paulsson, J., O. G. Berg, and M. Ehrenberg. 2000. Stochastic focusing: fluctuation-enhanced sensitivity of intracellular regulation. *Proc. Natl. Acad. Sci. USA*. 97:7148–7153.
- Berg, O. G., J. Paulsson, and M. Ehrenberg. 2000. Fluctuations in repressor control: thermodynamic constraints on stochastic focusing. *Biophys. J.* 79:2944–2953.
- Bialek, W. 2000. Stability and noise in biochemical switches. cond-mat/0005235: <http://www.arxiv.org/abs/cond-mat/0005235>.
- Bialek, W., and S. Setayeshgar. 2003. Physical limits to biochemical signaling. physics/0301001: <http://arxiv.org/abs/physics/0301001>.
- Bardwell, L., J. G. Cook, E. C. Chang, B. R. Cairns, and J. Thormer. 1996. Signaling in the yeast pheromone response pathway: specific and high-affinity interaction of the mitogen-activated protein (MAP) kinases Kss1 and Fus3 with the upstream MAP kinase kinase Ste7. *Mol. Cell. Biol.* 16:3637–3650.
- Ferrell, J. E., Jr. 1996. Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem. Sci.* 21:460–466.

9. Bhalla, U. S. 2004. Signaling in small subcellular volumes. II. Stochastic and diffusion effects on synaptic network properties. *Biophys. J.* 87:745–753.
10. Elowitz, M. B., M. G. Surette, P. E. Wolf, J. B. Stock, and S. Leibler. 1999. Protein mobility in the cytoplasm of *Escherichia coli*. *J. Bacteriol.* 181:197–203.
11. Kholodenko, B. N., G. C. Brown, and J. B. Hoek. 2000. Diffusion control of protein phosphorylation in signal transduction pathways. *Biochem. J.* 350:901–907.
12. Kholodenko, B. N. 2003. Four-dimensional organization of protein kinase signaling cascades: the roles of diffusion, endocytosis and molecular motors. *J. Exp. Biol.* 206:2073–2082.
13. Elowitz, M. B., A. J. Levine, E. D. Siggia, and P. S. Swain. 2002. Stochastic gene expression in a single cell. *Science*. 297:1183–1186.
14. Pedraza, J. M., and A. van Oudenaarden. 2005. Noise propagation in gene networks. *Science*. 307:1965–1969.
15. Thattai, M., and A. van Oudenaarden. 2001. Intrinsic noise in gene regulatory networks. *Proc. Natl. Acad. Sci. USA*. 98:8614–8619.
16. Raser, J. M., and E. K. O'Shea. 2004. Control of stochasticity in eukaryotic gene expression. *Science*. 304:1811–1814.
17. Rosenfeld, N., J. W. Young, U. Alon, P. S. Swain, and M. B. Elowitz. 2005. Gene regulation at the single-cell level. *Science*. 307:1962–1965.
18. Kirschner, M., and J. Gerhart. 1998. Evolvability. *Proc. Natl. Acad. Sci. USA*. 95:8420–8427.
19. Thattai, M., and A. van Oudenaarden. 2004. Stochastic gene expression in fluctuating environments. *Genetics*. 167:523–530.
20. Swain, P. S., and E. D. Siggia. 2002. The role of proofreading in signal transduction specificity. *Biophys. J.* 82:2928–2933.
21. Ferrell, J. E., Jr., and R. R. Bhatt. 1997. Mechanistic studies of the dual phosphorylation of mitogen-activated protein kinase. *J. Biol. Chem.* 272:19008–19016.
22. Becskei, A., and L. Serrano. 2000. Engineering stability in gene networks by autoregulation. *Nature*. 405:590–593.
23. Xiong, W., and J. E. Ferrell, Jr. 2003. A positive-feedback-based bistable “memory module” that governs a cell fate decision. *Nature*. 426:460–465.
24. Thattai, M., and A. van Oudenaarden. 2002. Attenuation of noise in ultrasensitive signaling cascades. *Biophys. J.* 82:2943–2950.
25. Heinrich, R., B. G. Neel, and T. A. Rapoport. 2002. Mathematical models of protein kinase signal transduction. *Mol. Cell*. 9:957–970.
26. Detwiler, P. B., S. Ramanathan, A. Sengupta, and B. I. Shraiman. 2000. Engineering aspects of enzymatic signal transduction: photoreceptors in the retina. *Biophys. J.* 79:2801–2817.
27. Wang, Z., D. Shen, D. W. Parsons, A. Bardelli, J. Sager, S. Szabo, J. Ptak, N. Silliman, B. A. Peters, M. S. van der Heijden, G. Parmigiani, H. Yan, T. L. Wang, G. Riggins, S. M. Powell, J. K. Willson, S. Markowitz, K. W. Kinzler, B. Vogelstein, and V. E. Velculescu. 2004. Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science*. 304:1164–1166.
28. Bardelli, A., D. W. Parsons, N. Silliman, J. Ptak, S. Szabo, S. Saha, S. Markowitz, J. K. Willson, G. Parmigiani, K. W. Kinzler, B. Vogelstein, and V. E. Velculescu. 2003. Mutational analysis of the tyrosine kinome in colorectal cancers. *Science*. 300:949.
29. Gillespie, D. T. 1977. Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.* 81:2340–2361.
30. Rao, C. V., and A. Arkin. 2003. Stochastic chemical kinetics and the quasi-steady-state assumption: application to Gillespie algorithm. *J. Chem. Phys.* 118:4999–5010.
31. Cover, T. M., and J. A. Thomas. 1991. Elements of Information Theory. Wiley, New York.
32. Gerhart, J. C., and M. W. Kirschner. 1997. Cells, Embryos and Evolution. Blackwell Science, Boston.
33. Jung, P., and J. Shuai. 2001. Optimal sizes of ion channel clusters. *Europhys. Lett.* 56:29–35.
34. Schmid, G., I. Goychuk, and P. Hanggi. 2001. Stochastic resonance as a collective property of ion channel assemblies. *Europhys. Lett.* 56:22–28.
35. Reference deleted in proof.
36. Berg, H. C. 2004. *E. coli* in Motion. Springer, New York.
37. Bray, D. 2002. Bacterial chemotaxis and the question of gain. *Proc. Natl. Acad. Sci. USA*. 99:7–9.
38. Barkai, N., and S. Leibler. 1997. Robustness in simple biochemical networks. *Nature*. 387:913–917.
39. Alon, U., M. G. Surette, N. Barkai, and S. Leibler. 1999. Robustness in bacterial chemotaxis. *Nature*. 397:168–171.
40. Bardwell, A. J., M. Abdollahi, and L. Bardwell. 2004. Anthrax lethal factor-cleavage products of MAPK (mitogen-activated protein kinase) kinases exhibit reduced binding to their cognate MAPKs. *Biochem. J.* 378:569–577.
41. Pikovsky, A., A. Zaikin, and M. A. de la Casa. 2002. System size resonance in coupled noisy systems and in the Ising model. *Phys. Rev. Lett.* 88:050601.
42. Morishita, Y., T. J. Kobayashi, and K. Aihara. 2006. An optimal number of molecules for signal amplification and discrimination in a chemical cascade. *Biophys. J.* 91:2072–2081.
43. Bray, D., and T. Duke. 2004. Conformational spread: the propagation of allosteric states in large multiprotein complexes. *Annu. Rev. Biophys. Biomol. Struct.* 33:53–73.
44. Isalan, M., C. Lemerle, and L. Serrano. 2005. Engineering gene networks to emulate *Drosophila* embryonic pattern formation. *PLoS Biol.* 3:e64.
45. Vaknin, A., and H. C. Berg. 2004. Single-cell FRET imaging of phosphatase activity in the *Escherichia coli* chemotaxis system. *Proc. Natl. Acad. Sci. USA*. 101:17072–17077.
46. Veitia, R. A. 2005. Gene dosage balance: deletions, duplications and dominance. *Trends Genet.* 21:33–35.
47. Levchenko, A., and P. A. Iglesias. 2002. Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils. *Biophys. J.* 82:50–63.
48. Angeli, D., J. E. Ferrell, Jr., and E. D. Sontag. 2004. Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *Proc. Natl. Acad. Sci. USA*. 101:1822–1827.
49. Ferrell, J. E., and W. Xiong. 2001. Bistability in cell signaling: How to make continuous processes discontinuous, and reversible processes irreversible. *Chaos*. 11:227–236.
50. Ozbudak, E. M., M. Thattai, H. N. Lim, B. I. Shraiman, and A. Van Oudenaarden. 2004. Multistability in the lactose utilization network of *Escherichia coli*. *Nature*. 427:737–740.
51. Becskei, A., B. Seraphin, and L. Serrano. 2001. Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. *EMBO J.* 20:2528–2535.
52. Shimizu, T. S., S. V. Aksenov, and D. Bray. 2003. A spatially extended stochastic model of the bacterial chemotaxis signaling pathway. *J. Mol. Biol.* 329:291–309.
53. Shvartsman, S. Y. 2002. Shooting from the hip: spatial control of signal release by intracellular waves. *Proc. Natl. Acad. Sci. USA*. 99:9087–9089.
54. Levchenko, A., J. Bruck, and P. W. Sternberg. 2000. Scaffold proteins may biphasically affect the levels of mitogen-activated protein kinase signaling and reduce its threshold properties. *Proc. Natl. Acad. Sci. USA*. 97:5818–5823.
55. Ferrell, J. E., Jr. 2000. What do scaffold proteins really do? *Sci. STKE*. 2000:PE1.
56. Lemerle, C., B. Di Ventura, and L. Serrano. 2005. Space as the final frontier in stochastic simulations of biological systems. *FEBS Lett.* 579:1789–1794.
57. Bhalla, U. S. 2004. Signaling in small subcellular volumes. I. Stochastic and diffusion effects on individual pathways. *Biophys. J.* 87:733–744.



58. Friedman, N., S. Vardi, M. Ronen, U. Alon, and J. Stavans. 2005. Precise temporal modulation in the response of the SOS DNA repair network in individual bacteria. *PLoS Biol.* 3:e238.
59. Lahav, G., N. Rosenfeld, A. Sigal, N. Geva-Zatorsky, A. J. Levine, M. B. Elowitz, and U. Alon. 2004. Dynamics of the p53-Mdm2 feedback loop in individual cells. *Nat. Genet.* 36:147–150.
60. Nelson, D. E., A. E. C. Ihekweaba, M. Elliott, J. R. Johnson, C. A. Gibney, B. E. Foreman, G. Nelson, V. See, C. A. Horton, D. G. Spiller, S. W. Edwards, H. P. McDowell, J. F. Unitt, E. Sullivan, R. Grimley, N. Benson, D. S. Broomhead, D. B. Kell, and M. R. H. White. 2004. Oscillations in NF- $\kappa$ B signaling control the dynamics of gene expression. *Science*. 306:704–708.
61. Huang, C. Y., and J. E. Ferrell, Jr. 1996. Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. USA*. 93:10078–10083.
62. Sauro, H. M., and B. N. Kholodenko. 2004. Quantitative analysis of signaling networks. *Prog. Biophys. Mol. Biol.* 86:5–43.
63. Paulsson, J. 2004. Summing up the noise in gene networks. *Nature*. 427:415–418.