

BIOCHEMICAL REGULATION

In this chapter we study the ‘modules’ of biochemical regulation. Our treatment follows that of Tyson, *et al.* [1, 2]. These modules are components of biochemical control systems (“systems biology” is the current buzz word). Assembled into a biochemical circuit, they can represent many cellular processes.

Signaling via covalent modification

Many enzymes are regulated by attaching (via a kinase) or removing (via a phosphatase) a

phosphate group: $E \xrightleftharpoons[\text{Phosphatase}]{\text{Kinase}} EP$. Generally, Kinases are very specific, while phosphatases

are not. So the activity of the kinase can be viewed as an input, or stimulus to the covalent ‘switch’, and the amount of phosphorylated enzyme the output, or response. Figure 1a shows the kinetic circuit in the usual informal notation; Figure 1 and the corresponding Madonna Flowchart. We can reduce the system to a single reservoir if we assume that *the total amount of enzyme is constant*, so that we can use as the only variable (reservoir) the amount of phosphorylated enzyme. That is, since $E + EP = E_T$, the above reaction becomes

$E_T - EP \xrightleftharpoons[\text{Phosphatase}]{\text{Kinase}} EP$. Then the diagram in Figure 1a can be abbreviated as shown in Figure

1b.

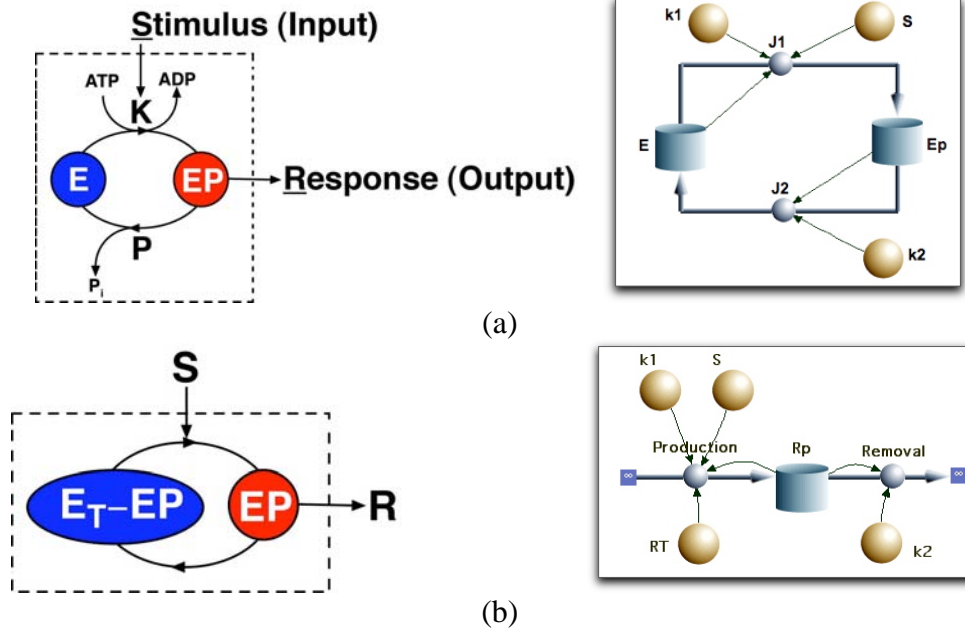
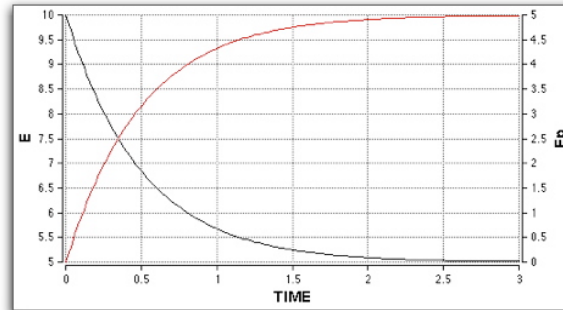


Figure 1. Diagramming covalent modification. (a) Diagram and Madonna Flowchart for the phosphorylation and dephosphorylation of the enzyme E. (b) Reduction of (a) using the constraint $E + E_p = E_T = \text{constant}$.

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In keeping with the notion of stimulus-response behavior, let us switch notation and denote the rate of the kinase by **S** (the *stimulus*), and the amount of phosphorylated (active) enzyme by **R** (the *response*).¹ This can be represented by the Flowchart shown in Figure 1b corresponding to the equation

$$\frac{dR}{dt} = k_1(R_T - R) - k_2R$$



(1)

Exercise 1. Construct the Flowchart in Figure 1b and obtain the corresponding equation (1). Plot the solution using $k_1 = 1$, $k_2 = 0.5$, $R(0) = 100$, $R_T = 200$.

Exercise 2. In a separate model, plot the curve dR/dt vs. R . (Hint: **RENAME TIME = R**, $y = (k_1 * S * (R_T - R)) - (k_2 * R)$, plot y vs. R and change the graph labels by clicking on the axes). It should look like Figure 2a.

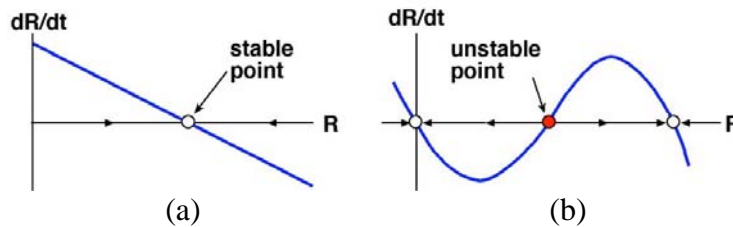


Figure 2. Plotting the rate of a reservoir vs. its content shows whether the equilibrium (Rate = 0) is stable or unstable. (a) The simple linear system in Figure 1b. (c) A nonlinear system; e.g. $dR/dt = aR(R-1)(R-2)$, where a is a tunable constant. This system shows 'switch-like' behavior.

Signal-response behavior

Linear

An enzyme (the response, R) is synthesized at a basal rate k_0 and degraded with a rate constant k_2 . Its production is enhanced by a rate k_1S , where S is a synthetic enzyme. The system can be diagrammed as shown in Figure 3a.

¹ Of course, many enzymes are 'normally on' and are switched off by phosphorylation. However, the modeling proceeds exactly the same.

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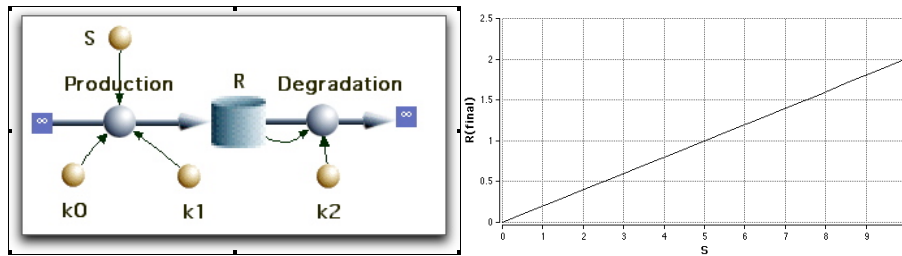


Figure 3. Linear stimulus-response system. (a) Flowchart. (b) Linear response curve: $R(\text{final})$ vs. S .

Exercise 3. Write out the equations for the Flowchart in 'normal' mathematical notation. Find the steady state value of the response, R by setting reservoir rate $dR/dt = 0$. Plot the time behavior and stimulus response behavior (Figure 3b) using the parameter values $k_0 = 0.01$, $k_1 = 1$, $k_2 = 5$, $S = 10$, $R(0) = 1$.

Hyperbolic

The phosphorylation-dephosphorylation system discussed above is re-diagrammed in Figure 4. From the Flowchart, the equation governing the amount of phosphorylated enzyme (i.e. the reservoir, R) is:

$$\frac{dR}{dt} = k_1 S (R_T - R) - k_2 R \quad (2)$$

Exercise 4. Plot the time and response behavior of the system using the parameter values $k_1 = k_2 = 1$, $R_T = 1$, $S = 1$. Solve equation (2) for the steady state of the response, R , and show that the 'Michaelis' constant is k_2/k_1 .

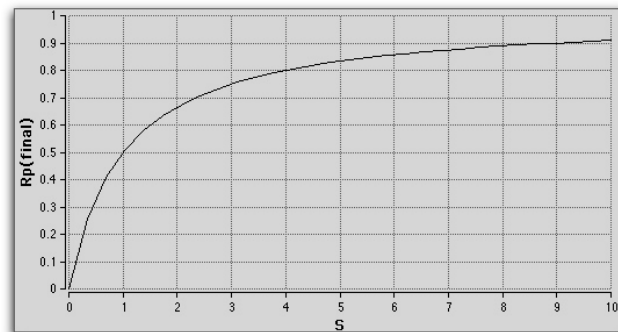
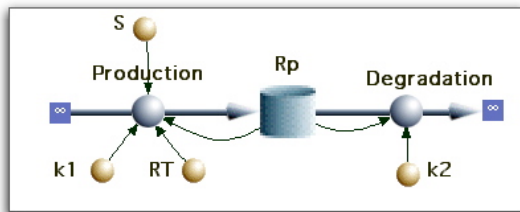


Figure 4. Hyperbolic system. (a) Flowchart for MM. (b). Graph of (a)

Zero-order ultrasensitivity

In the above two examples the kinetics were governed by mass-action rate laws. A dramatically different behavior is obtained if the rates are governed by Michaelis-Menten kinetics. Figure 5 shows the Flowchart for the phosphorylation-dephosphorylation system when the kinase and phosphatase obey MM kinetics. The stimulus-response behavior is sigmoidal, and very sharp (corresponding to a Hill coefficient of > 10). This behavior, first discovered by Goldbeter and Koshland [3] has been used to model a variety of biochemical systems [4, 5].

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Exercise 5. Reproduce the Flowchart, write the equations in mathematical notation, and plot the signal-response curve for the system in Figure 5. Use the parameters: $R_T = 3$, $k_1 = k_2 = 1$, $K_{m1} = 0.05$, $K_{m2} = 0.05$, where the K_m 's are the Michaelis constants for phosphorylation and dephosphorylation, respectively.

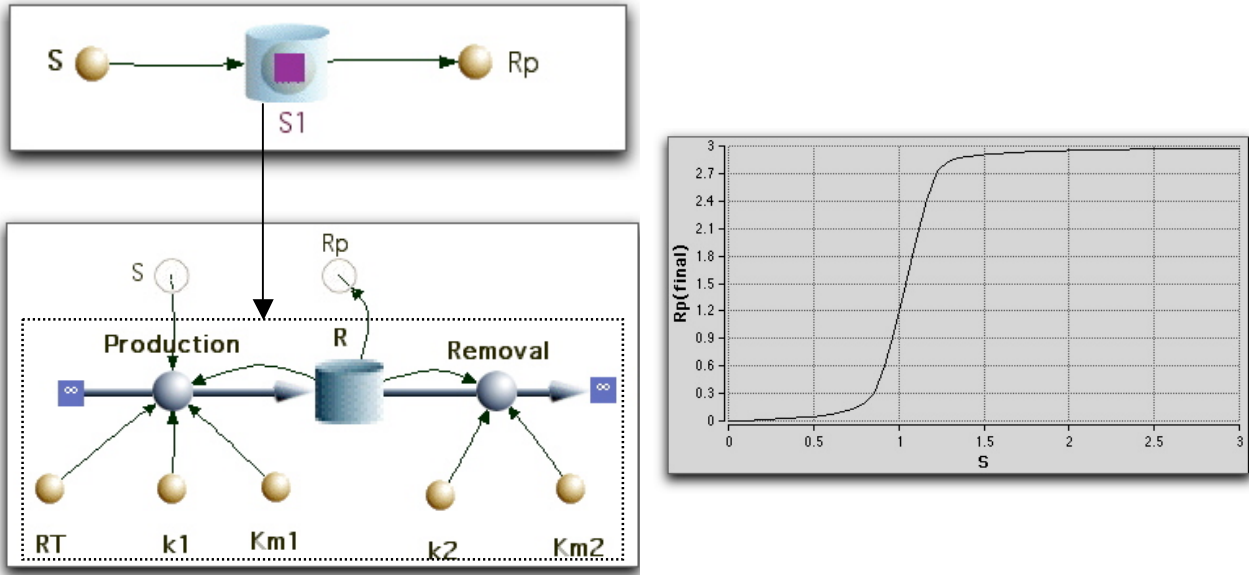


Figure 5. (a) Flowchart for signal response system governed by Michaelis-Menten kinetics. The bottom panel shows the contents of the submodel obtained by grouping the model icons except for the stimulus, S , and the function R_p giving the value of the reservoir, R (dashed line: ---). (b) The sigmoidal signal-response curve that resembles a high order of cooperativity.

Biochemical feedback systems

There are many kinds of feedback in biochemical systems, some direct, some indirect. Here we give several examples of positive and negative feedback modules. For example, the informal diagram in Figure 6 is a model by B. Goodwin for an oscillating genetic circuit [6]. From the diagram one can write the equations governing the system.

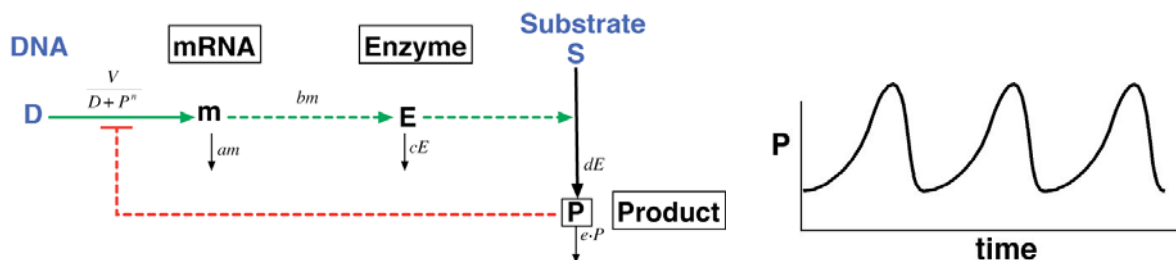


Figure 6. The Goodwin genetic oscillator. Solid lines are flows, dashed lines are signals (green = enhancers, red = inhibitors). The variable quantities (reservoirs) are black, and the parameters are blue.

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Exercise 6. See if you can convert the diagram in Figure 9 to equations, and a Berkeley Madonna Flowchart.

Direct autocatalysis

The simplest positive feedback is pure autocatalysis whereby a protein, A, catalyzes its own production. The Flowchart in Figure 7a shows this feedback. The production flow has the form $dA/dt = kA^n$. The graph in Figure 7b shows the explosive growth of A for $n = 1$.

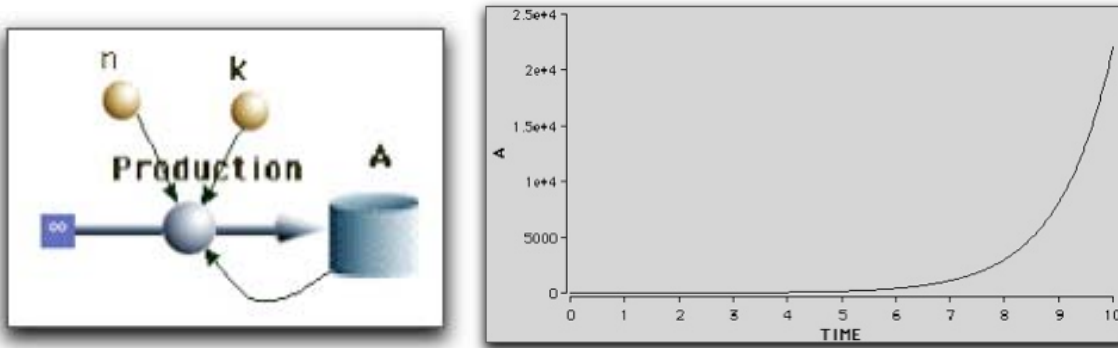


Figure 7. Simple autocatalysis. (a) Flowchart. (b) $A(t)$ for $k = 1$, $n = 1$, $A(0) = 1$, Production = kA^n .

Exercise 7. Simulate the Flowchart using the parameters in the figure caption of Figure 7. For $n \geq 2$, use the STIFF solver (Rosenbrock) and a slider for n and STOPTIME. You will have to reduce the STOPTIME for $n \geq 2$ considerably because the growth rate of A becomes *much* faster!

Autocatalysis by inhibition of destruction

The same autocatalytic step can be achieved by inhibiting the destruction or removal of a protein, as shown in Figure 8a.

Exercise 8. Simulate the system in Figure 8a using the parameters in the figure caption.

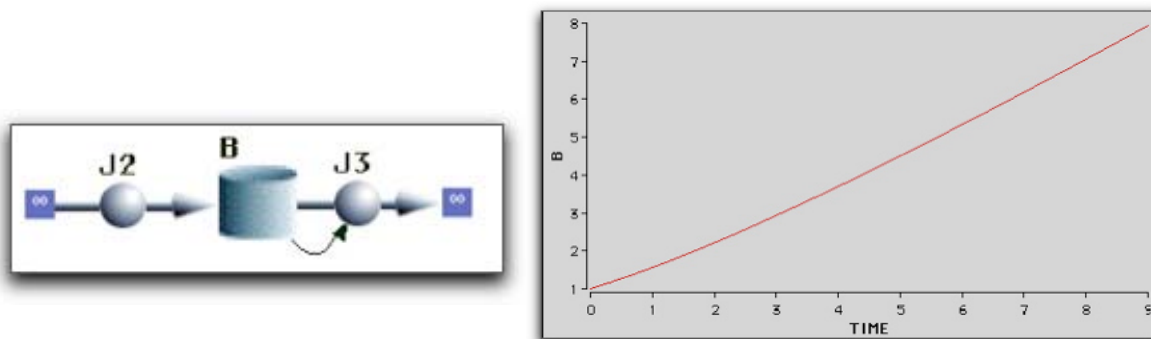


Figure 8. Autocatalysis by removal inhibition. (a) Flowchart. (b) Plot of B vs. time using production ($J2$) = 1, removal ($J3$) = $1/(1 + B)$, $B(0) = 1$.

Indirect autocatalysis

Autocatalysis can arise when each of two chemicals enhance the production of the other (Figure 9a). Autocatalysis can also arise by *negative* feedback via mutual inhibition of production, or by a mixed positive and negative feedback (Figure 9b).

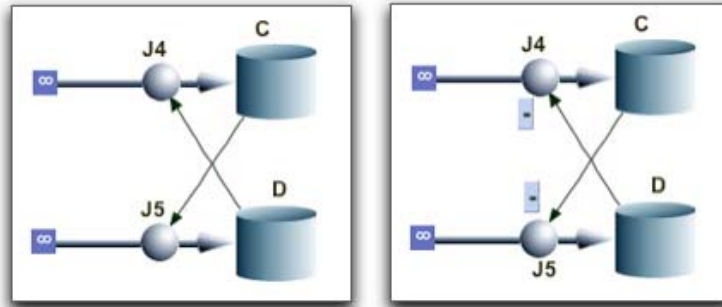


Figure 9. Indirect autocatalysis. (a) Positive feedback via mutual enhancement of production: $J4 = 2D$, $J5 = C$. (b) Autocatalysis can also arise by *negative* feedback via mutual inhibition of production: $J4 = 1/(1 + 2D)$, $J5 = 1/(1 + C)$. Madonna has no direct way to indicate whether the feedback enhances or inhibits the flow, but minus signs created by the Text tool can be placed adjacent to the parameter arrow to denote inhibition. Finally, autocatalysis can be achieved by a mixed positive ($J5 = C$) and negative ($J4 = D/(1 + D)$) feedback.

Exercise 9. Simulate the unstable (unbounded increase) in C and D for the three types of feedback: positive, negative, and mixed. You can set all constants equal to unity and use the form $1/(1 + X)$ or $X/(1 + X)$ as the inhibitory feedback functions. Make a plot showing the difference between those two forms.

Adaptation

Many—if not most—cellular sensory systems exhibit the property of *adaptation*: The activity of the response returns to its ‘basal’ level despite changes in the stimulus. An example is the run-tumble frequency of *E. coli*. Raising the uniform level of a chemoattractant causes a transient increase in tumble frequency that soon settles back to its original steady state before the attractant was added.

A circuit for implementing perfect adaptation is shown in Figure 10a. This combines the simple linear response module with a signal pathway through an intermediate, X.

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<p>INIT Rp = 0.1</p> <p>$Rp_Production = k5*YP*(RT-RP)/(Km5+RT-RP)$</p> <p>$RP_Removal = k6*RP/(Km6+RP)$</p> <p>$Yp_Production = k3*X*(YT-YP)/(Km3+YT-YP)$</p> <p>$Yp_Removal = k4*YP/(Km4+YP)$</p> <p>$J1 = k0 + k1*S$</p> <p>$J2 = k2*X + k2p*RP*X$</p>	<p>$Km6 = 0.01$</p> <p>$YT = 1$</p> <p>$k3 = 0.1$</p> <p>$Km3 = 0.01$</p> <p>$k4 = 0.2$</p> <p>$Km4 = 0.01$</p> <p>$k0 = 0$</p> <p>$k1 = 1$</p> <p>$S = 2$</p> <p>$k2 = 0.01$</p> <p>$k2p = 10$</p>
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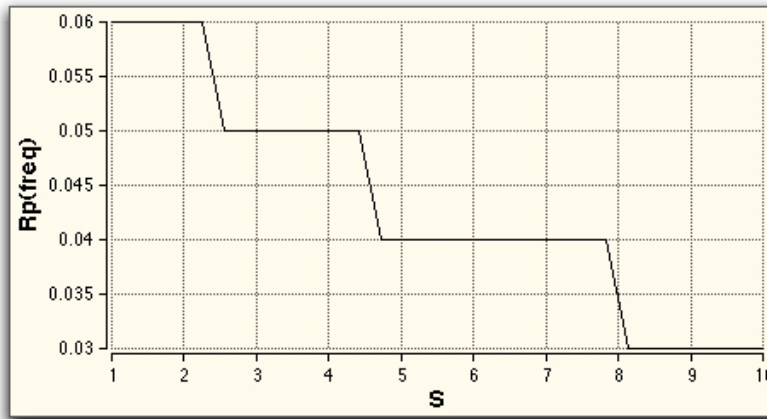


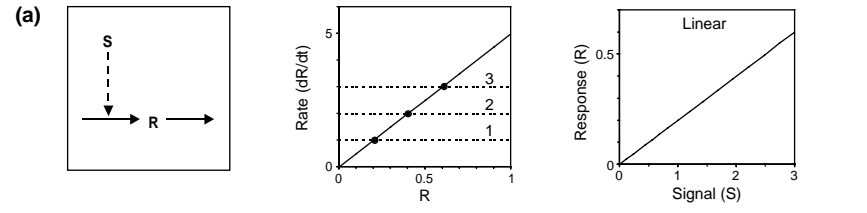
Figure 11. Negative feedback oscillator. (a) Flowchart with the functions hidden. (b) Equation window. (c) Response (Rp) vs. Stimulus (S).

References

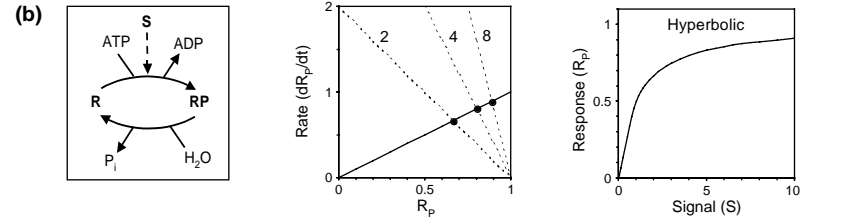
1. Tyson, J., Chen, K., and Novak, B. (2003). Sniffers, buzzers, toggles, and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol.* 15, 221-231.
2. Fall, C.P., Marland, E., Tyson, J., and Wagner, J. eds. (2002). *Computational Cell Biology*, Volume 20 (New York: Springer-Verlag).
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4. Ferrell, J. (1996). Tripping the Switch Fantastic: How a Protein Kinase Cascade Can Convert Graded Inputs into Switch-Like Outputs. *Trends in Biochemical Science* 21, 460-466.
5. Goldbeter, A. (1996). *Biochemical Oscillations and Cellular Rhythms* (Cambridge: Cambridge University Press).
6. Murray, J.D. (2002). *Mathematical biology*, 3rd Edition (New York: Springer).

Figure 1

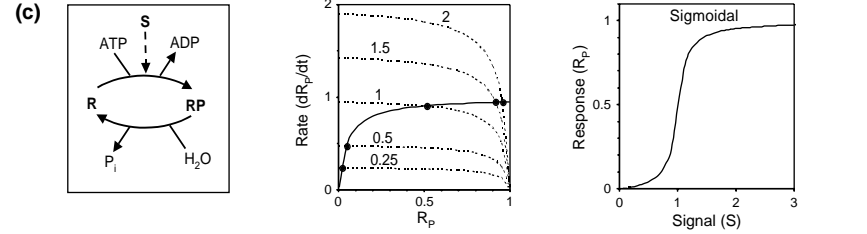
(a) *Linear Response*



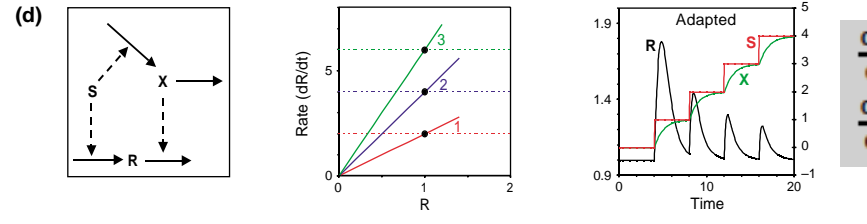
(b) *Hyperbolic Response*



(c) *Sigmoidal Response*



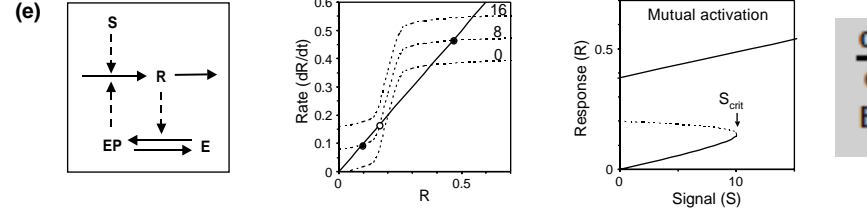
(d) *Perfect Adaptation*



$$\frac{dR}{dt} = k_1 S - k_2 X \cdot R \quad R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$

$$\frac{dX}{dt} = k_3 S - k_4 X \quad X_{ss} = \frac{k_3 S}{k_4}$$

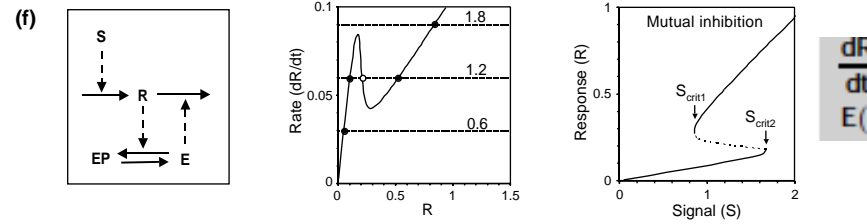
(e) *Mutual Activation*



$$\frac{dR}{dt} = k_0 E_p(R) + k_1 S - k_2 X \cdot R$$

$$E_p(R) = G(k_3 R, k_4, J_3, J_4)$$

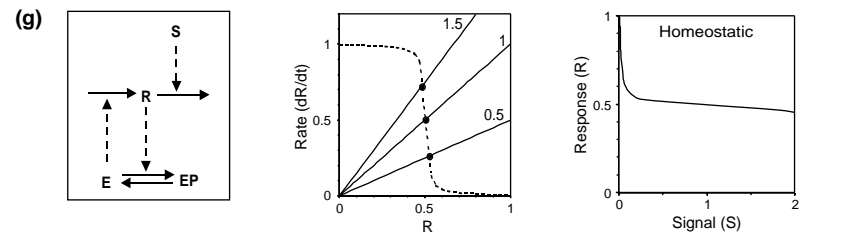
(f) *Mutual Inhibition*



$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R - k_2' E(R) \cdot R$$

$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

(g) *Negative Feedback Homeostasis*



$$\frac{dR}{dt} = k_0 E(R) - k_2 S \cdot R$$

$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

Current Opinion in Cell Biology

Figure 2

