Project 4: Phosphorylation–dephosphorylation

MCSB Bootcamp — Mathematical and Computational Track

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Background and Part I

Many proteins are activated (phosphorylated) by enzymes called kinases, and deactivated (dephosphorylated) by enzymes called phosphatases. Intuitively, the fraction of active protein is determined by the balance of kinases to phosphatases. This balance is maintained by constant activation and inactivation in what is called a *futile cycle*.

a. Sketch a diagram where the horizontal axis is the ratio of kinase to phosphatase (say, in log scale), and the vertical axis is the fraction of activated protein in steady state. Use your intuition to sketch what you think the curve should look like.

   Your answer here.

In mathematics, we call this plot a bifurcation diagram (the steady state as a function of a parameter). In biology, we call this particular plot a dose-response curve (how the output of a system responds to the dose of an input).

Model statement

To activate the protein, the kinase must first bind to the inactive form, then catalyze activation. It may also unbind before it has a chance to catalyze. The same is true for the phosphatase. There are therefore 6 species of molecule. Let $[A]$ be the concentration of A, $[AP]$ be the concentration of the AP complex, and so on. We have defined six populations, with concentrations:

$[A]$	unbound active protein
$[I]$	unbound inactive protein
$[AP]$	complex of active protein and phosphatase
$[P] = [P_{\text{tot}}] - [AP]$	unbound phosphatase
$[IK]$	complex of inactive protein and kinase
$[K] = [K_{\text{tot}}] - [IK]$	unbound kinase

Assume the total concentration of phosphatase $[P_{\text{tot}}]$ and kinase $[K_{\text{tot}}]$ are constant. Therefore, we do not need to track the concentration of P, since it is determined by $[P_{\text{tot}}]$ and $[AP]$. Similarly for K. Therefore, there are 4 concentrations to keep track of.

There are 6 chemical reactions, shown below.

$$\mathrm{I} + \mathrm{K} \underset{k^I_{\text{off}}}{\overset{k^I_{\text{on}}}{\rightleftharpoons}} \mathrm{IK} \xrightarrow{k^A_{\text{cat}}} \mathrm{A} + \mathrm{K}$$

$$\mathrm{A} + \mathrm{P} \underset{k^A_{\text{off}}}{\overset{k^A_{\text{on}}}{\rightleftharpoons}} \mathrm{AP} \xrightarrow{k^I_{\text{cat}}} \mathrm{I} + \mathrm{P}$$

Let's assume reactions are proportional to the concentration of the reactants, so $\mathrm{A} + \mathrm{P} \to \mathrm{AP}$ occurs at rate $k^A_{\text{on}}[P][A] = k^A_{\text{on}}([P_{\text{tot}}]-[AP])[A]$. Therefore, we have defined 6 rate parameters:

$$\begin{aligned} k^A_{\text{on}} &= 10\ \text{s}^{-1}\mu\text{M}^{-1} & \qquad k^I_{\text{on}} &= 10\ \text{s}^{-1}\mu\text{M}^{-1} \\ k^A_{\text{off}} &= 10\ \text{s}^{-1} & \qquad k^I_{\text{off}} &= 10\ \text{s}^{-1} \\ k^A_{\text{cat}} &= 100\ \text{s}^{-1} & \qquad k^I_{\text{cat}} &= 10\ \text{s}^{-1} \end{aligned}$$

Part II

b. Write down 4 ODEs describing the populations of A, I, AP and IK. The first two equations are

$$\begin{aligned} \frac{d[A]}{dt} &= -k^A_{\text{on}}\left([P_{\text{tot}}] - [AP]\right)[A] + k^A_{\text{off}}[AP] + k^A_{\text{cat}}[IK], \\ \frac{d[AP]}{dt} &= +k^A_{\text{on}}\left([P_{\text{tot}}] - [AP]\right)[A] - k^A_{\text{off}}[AP] - k^I_{\text{cat}}[AP]. \end{aligned}$$

Your answer here.

c. Assume that at time $t = 0$, all of the protein is in the inactive, unbound state, and that its concentration is $I_{\text{tot}} = 1\ \mu\text{M}$. What are the appropriate initial conditions for the ODEs?

Your answer here.

d. Write code to simulate this ODE system to steady state. Use the parameters stated above, the initial conditions from the previous question, and $I_{\text{tot}} = 1\ \mu\text{M}$, $K_{\text{tot}} = 1\ \mu\text{M}$ and $P_{\text{tot}} = 1\ \mu\text{M}$.

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# Your code here.
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Part III

e. Do a parameter sweep in K_{tot}, logarithmic from $K_{\text{tot}} = 10^{-3}\ \mu\text{M}$ to $K_{\text{tot}} = 10^{+2}\ \mu\text{M}$. Plot the total amount of activated protein in steady state as a function of K_{tot} (with a log axis in K_{tot}). Does the plot match the intuitive sketch you made in question (a)?

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# Your code here.
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f. Now do the same parameter sweep in K_{tot}, identical to the previous question except $I_{\text{tot}} = 100\ \mu\text{M}$. What changed? In what circumstances might a cell gain an advantage from this kind of dose-response curve (compared to the previous question with $I_{\text{tot}} = 1\ \mu\text{M}$)? In what circumstances might a cell be disadvantaged by a dose-response curve like this (compared to the previous question with $I_{\text{tot}} = 1\ \mu\text{M}$)?[1]

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# Your code here.
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[1] This type of behavior in systems biology is called *ultrasensitivity*. This particular type of ultrasensitivity is called *Goldbeter-Koshland ultrasensitivity*.