

Problem 1: Transcription factor cascade (adapted from Alon 2.4, 30 points)

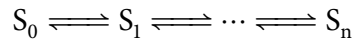
Consider a cascade of three transcriptional activators, $X \rightarrow Y \rightarrow Z$. Protein X is initially present in the cell in its inactive form. At time $t = 0$, X rapidly becomes active and binds the promoter of Y , so that protein Y starts to be produced at a rate β_y . When Y levels exceed a threshold K_y , gene Z begins to be expressed at a rate β_z . All proteins have the same degradation/dilution rate α .

- Using the information above, find an expression for the time derivative of Y . Integrate to find $Y(t)$ given the initial condition $Y(0) = 0$. (Hint: A similar derivation is given in Alon Ch. 2.)
- At what time τ does expression of Z begin?
- What is the concentration of protein Z as a function of time?
- What is the *response time* of the system, i.e., the time between when X is activated and when Z reaches one half of its maximal value?

Problem 2: Multistep Ultrasensitivity (Ingalls 6.8.7, 40 points)

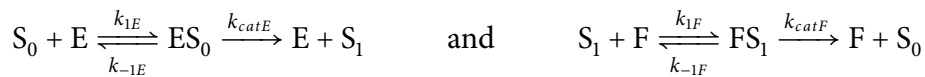
In a 2005 paper, Jeremy Gunawardena presented a straightforward analysis of multistep ultrasensitivity and revealed that it is not well-described by Hill functions¹.

Consider a protein S that undergoes a sequential chain of n phosphorylations, all of which are catalyzed by the same kinase E , but each of which requires a separate collision event. Let S_k denote the protein with k phosphate groups attached. Suppose the phosphatase F acts in a similar multistep manner. The reaction scheme is then



In steady state, the net reaction rate at each step is zero.

- Consider the first phosphorylation-dephosphorylation cycle. Expanding the steps in the catalytic mechanism, we have



Apply a rapid equilibrium assumption to the association reactions ($S_0 + E \leftrightarrow ES_0$ and $S_1 + F \leftrightarrow FS_1$) to describe the reaction rates as

$$k_{catE} [ES_0] = \frac{k_{catE}}{K_{ME}} [S_0] [E] \quad \text{and} \quad k_{catF} [FS_1] = \frac{k_{catF}}{K_{MF}} [S_1] [F]$$

where $K_{ME} = k_{-1E}/k_{1E}$, $K_{MF} = k_{-1F}/k_{1F}$, and $[E]$ and $[F]$ are the concentrations of free kinase and phosphatase.

- Use the fact that at steady state the net phosphorylation-dephosphorylation rate is zero to arrive at the equation

$$\frac{[S_1]}{[S_0]} = \lambda \frac{[E]}{[F]}, \quad \text{where } \lambda = \frac{k_{catE} K_{MF}}{k_{catF} K_{ME}}$$

¹Gunawardena, J. (2005). Multisite protein phosphorylation makes a good threshold but can be a poor switch. *PNAS*

- c) Suppose the kinetic constants are identical for each phosphorylation-dephosphorylation step. In that case, verify that

$$\frac{[S_2]}{[S_1]} = \lambda \frac{[E]}{[F]}, \quad \text{so} \quad \frac{[S_2]}{[S_0]} = \lambda^2 \left(\frac{[E]}{[F]} \right)^2 \quad \text{and more generally} \quad \frac{[S_j]}{[S_0]} = \lambda^j \left(\frac{[E]}{[F]} \right)^j$$

- d) Use the result in part c to write the fraction of protein S that is in the fully phosphorylated form as

$$\frac{[S_n]}{[S_{\text{total}}]} = \frac{(\lambda u)^n}{1 + \lambda u + (\lambda u)^2 + \dots + (\lambda u)^n} \quad (1)$$

where $u = [E]/[F]$ is the ratio of kinase to phosphatase concentrations. Hint: $[S_{\text{total}}] = [S_0] + [S_1] + [S_2] + \dots + [S_n]$.

- e) Use the ratio $[E]/[F]$ to approximate the ratio of total concentrations $[E_{\text{total}}]/[F_{\text{total}}]$, so that equation 1 describes the system's dose-response. Plot this function (vs. u) for various values of n . Take $\lambda = 1$ for simplicity. Use your plots to verify Gunawardena's conclusion that for high values of n , these dose-response curves exhibit a threshold (in this case, at $u = 1$) but their behavior cannot be described as switch-like, as they show near-hyperbolic growth beyond the threshold, regardless of the values of n .

Problem 3: Multistep Ultrasensitivity (Adapted from Ingalls 4.8.3, 30 points)

Consider the general linear system

$$\begin{aligned} \frac{dx}{dt} &= ax + by \\ \frac{dy}{dt} &= cx + dy \end{aligned}$$

Note that the steady state is $(x, y) = (0, 0)$. Choose six sets of parameter values (a , b , c , and d) that yield the following behaviors:

- Stable node (real negative eigenvalues)
- Stable spiral point (complex eigenvalues with negative real part)
- Center (purely imaginary eigenvalues)
- Unstable spiral point (complex eigenvalues with positive real part)
- Unstable node (real positive eigenvalues)
- Saddle point (real eigenvalues of different sign)

In each case, prepare a phase portrait of the system, including a direction field and a few representative trajectories. (Note: Unlike the problem in Ingalls, we will not require that you include the nullclines.) Hint: if either of the off-diagonal entries in the Jacobian matrix are zero, then the eigenvalues are simply the entries on the diagonal.