Simulation and Analysis of Biochemical Oscillators

The goal of this lab is to explore the dynamics of biochemical oscillators. We will focus on understanding ultra-sensitivity, negative and positive feedback, and the effects of parameter perturbation on oscillator behavior. For your submission, include all MATLAB code, figures generated, and answers to included questions.

Problem 1 (Demo): Stoichiometric sequestration and sensitivity

A) Consider a system of reactions for the following expression:

$$A_f + P_f \rightleftharpoons C$$

where A_f is a free activator, P_f is a free repressor, and C is the activator-repressor complex formed after their binding with dissociation constant $K_d = k_{off}/k_{on}$. The dynamics of A_f, P_f, and C can be written as:

$$\frac{dA_f}{dt} = -k_{on}A_fP_f + k_{off}C$$
$$\frac{dP_f}{dt} = -k_{on}A_fP_f + k_{off}C$$

$$\frac{dC}{dt} = k_{on}A_fP_f - k_{off}C$$

Using the parameters $k_{on} = 0.5\mu M^{-1}s^{-1}$ and $K_d = 0.1\mu M$ write a code that simulates the differential equations above to determine the concentrations of A_f, P_f and C for time-interval $(0, T_{end}), T_{end} = 20$s for initial conditions $A_f = A_0$, $P_f = P_0$ and $C_0 = 0$. Plot the solution for $A_0 = P_0 = 1\mu M$ to ensure the concentrations reach their steady state values by T_{end}.

B) Simulate the model as in part A) with $A_0 = 1\mu M$ and $P_0 = 0{:}\,0.1{:}\,2\mu M$ and plot the final fraction of free activator,

$$f_{end} = A_f(T_{end})/(A_f(T_{end}) + C(T_{end}))$$

as a function of P_0. Compare the result with analytical formula for the fraction of the unbound form

$$f(P, A, K_d) = \frac{\left(A - P - K_d + \sqrt{(A - P - K_d)^2 + 4AK_d}\right)}{2A}$$

where $A = A_f + C, P = P_f + C$ are the total amounts of the two proteins. Note that these are conserved quantities, so $A = A_0$ and $P = P_{f0}$.

C) Plot the results of $f(P, A, K_d)$ as function of $P = 0{:}\,0.01{:}\,2\mu M$ for $A_0 = 1\mu M$ and for 3 different affinities $K_d = 0.001\mu M, 0.1\mu M, 1\mu M$. How does the shape of the curve change with stronger binding?

D) The logarithmic sensitivity is defined as $LG = \frac{dlog(f)}{dlog(P)} = \frac{df}{dP}\frac{P}{f}$. Use the `gradient` function to calculate $\frac{dlog(f)}{dlog(P)}$ for each K_d as in part C and plot LG vs P_0. What does this tell you about the relationship between P and A and the value of K_d required for ultrasensitive responses?

Problem 2: Single Negative Feedback (SNF) Loop

A) Now consider a model in which a protein inhibits its own production by sequestering the activator. As we have seen in problem 1, function $f(P, A, K_d)$ can be used to calculate the proportion of free activator. Let's assume that transcription rate of mRNA (M) is proportional to the fraction of free activator. mRNA is then translated in cytoplasm to produce a cytoplasmic protein (P_c). Nuclear transport converts Pc to P that binds and inhibits the activator, A. This way, protein P inhibits its own transcription generating a single negative feedback loop (SNF) model of a circadian clock. The dynamics of $M, Pc,$ and P are given as

$$\frac{dM}{dt} = \alpha_1 f(P, A, K_d) - \beta_1 M$$
$$\frac{dP_c}{dt} = \alpha_2 M - \beta_2 P_c$$
$$\frac{dP}{dt} = \alpha_3 P_c - \beta_3 P$$

Write a function that can be passed into a built-in ODE solver and takes as input a time, initial conditions for $M, Pc,$ and P, and a vector of parameters $\alpha_{1-3}, \beta_{1-3}, A,$ and K_d. Note that A here is assumed to be constant and is defined by a parameter. Calculate $\frac{dM}{dt}$, $\frac{dP_c}{dt}$, and $\frac{dP}{dt}$ at $t = 0hr$ with initial conditions $M, Pc, P = 1\mu M$ and parameters $\alpha_{1-3} = 0.23hr^{-1}, \beta_{1-3} = 0.165hr^{-1}, A = 0.0659\mu M, K_d = 1 \times 10^{-5}\mu M$.

B) Simulate the dynamics of M, P_c, and P over the time interval $(0, 96hr)$ with initial conditions $M, Pc, P = 1\mu M$ and parameters $\alpha_{1-3} = 0.23hr^{-1}, \beta_{1-3} = 0.165hr^{-1}, A = 0.0659\mu M,$ and $K_d = 1 \times 10^{-5}, 1 \times 10^{-4}, 1 \times 10^{-3}\mu M$. Plot M, P_c, and P vs t for each $K_d$value.

C) Based on your observations, how does K_d influence the dynamics of M? What does this tell you about the role of repressor-activator binding in generating oscillations?

Problem 3: Adding a second feedback loop

We now know that stoichiometry of components and binding is critical for sustained oscillations. Are there additional mechanisms that we can implement to ensure more balanced stoichiometry between components? To test this, we will now build off the SNF model by adding a second feedback loop. In this model, the concentration of the activator, A, is no longer constant. Instead, the production of A is repressed by an additional protein, R

$$\frac{dA}{dt} = \frac{\gamma_2}{R} - \delta_2 A$$

The production of R is in turn activated by A when it is not sequestered by P. We can use $f(P, A, K_d)$ in the production term:

$$\frac{dR}{dt} = \gamma_1 f(P, A, K_d) - \delta_1 R$$

A) Using the single-negative feedback loop model as a starting point, add a second negative feedback loop by adding $\frac{dR}{dt}$ and $\frac{dA}{dt}$ to your previous model to make a negative-negative feedback (NNF) loop structure. Note that A is no longer a constant parameter and is determined by $\frac{dA}{dt}$. Calculate $\frac{dR}{dt}$ and $\frac{dA}{dt}$ at $t = 0hr$, with initial conditions for M, P_c, P, R, and $A = 0.1$ and parameters $\alpha_{1-3}, \beta_{1-3}, \gamma_1 = 1, \gamma_2 = 0.0043, \delta_{1-2} = 0.2$, and $K_d = 1 \times 10^{-5}$.

B) Simulate the dynamics of M, P_c, and P, R, and A, using the negative-negative loop structure from part A), for the time-interval $(0, 24hr)$, and the same initial conditions and parameters as part 3A, (M, P_c, P, R, and $A = 0.1$ and parameters $\alpha_{1-3}, \beta_{1-3}, \gamma_1 = 1, \gamma_2 = 0.0043, \delta_{1-2} = 0.2, K_d = 1 \times 10^{-5}$). Plot M, P_c, P, vs t and R, A vs. t on separate figures.

C) Simulate and plot the dynamics of M using the single-negative feedback structure for the time-interval $(0, 24hr)$, with initial conditions $M, Pc, P = 1$ and parameters $\alpha_{1-3}, \beta_{1-3} = 1, A = 0.0659, K_d = 1 \times 10^{-5}$. Add the dynamics of M in the negative-negative feedback loop structure from part B) to this plot for comparison.

D) Repeat part B) and C) after increasing the rates α_{1-3} by 2X. Plot M $v.t$ for SNF and NNF loops after changing the production rates. Based on your results here and 3C, which structure is more sensitive to perturbations?

E) What if you were to add a positive feedback loop instead of negative feedback? For positive feedback, the dynamics of R will stay the same. However, for A, we instead have

$$\frac{dA}{dt} = \gamma_2 R - \delta_2 A$$

Modify your expression for $\frac{dA}{dt}$ in your previous model accordingly to make a positive-negative feedback (PNF) loop structure. Calculate $\frac{dA}{dt}$ at $t = 0$, with initial conditions for M, P_c, P, R, and $A = 0.1$ and parameters $\alpha_{1-3}, \beta_{1-3}, \gamma_1 = 1, \gamma_2 = 0.0395, \delta_{1-2} = 0.2$, and $K_d = 1 \times 10^{-5}$.

F) Simulate the dynamics of M, P_c, and P, R, and A, using the positive-negative loop structure from part 4E, from $t = [0\ 24]$, and with initial conditions for M, P_c, P, R, and $A = 0.1$ and parameters $\alpha_{1-3}, \beta_{1-3}, \gamma_1 = 1, \gamma_2 = 0.0395, \delta_{1-2} = 0.2$, and $K_d = 1 \times 10^{-5}$. Plot M, P_c, P, vs t and R, A vs. t on separate figures.

G) On one figure, plot M $v.t$ for the SNF loop from part 3C and M $v.t$ for the PNF loop from part 4F.

H) Repeat part 4F and 4G after increasing the rates α_{1-3} by 2X. Plot M $v.t$ for SNF and PNF loops after changing the production rates. Based on your results, which structure is more sensitive to perturbations?