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ChemE 5440: Advanced Principles of Biomolecular Engineering

Final Exam
Due May 14th, 2019, 4:30 PM

Instructions:

1) Complete all parts for each of the two questions
2) Show all work for full credit
3) Include any code for full credit (print out or post on Git)
4) You must work individually on the final
5) You can consult any textbooks, notes, and scientific literature in preparing your answers
6) You should not consult other people for assistance on the final

1. Allosteric regulation is a fast mechanism cells use to regulate the catalytic activity of metabolic enzymes. For example, Phosphofructokinase (PFK), a key glycolytic enzyme which catalyzes the conversion of $\mathrm{D-fructose\ 6-phosphate}$ (F6P):

$$\mathrm{ATP + D-fructose\ 6-phosphate \longrightarrow ADP + D-fructose\ 1,6-bisphosphate} \quad (1)$$

is strongly activated in the presence of $3' - 5' - \mathrm{AMP}$. Let's build a model of the allosteric regulation of PFK and estimate the model parameters using an experimental dataset for PFK activity ($3' - 5' - \mathrm{AMP}$ versus reaction rate) posted in the *#final_exam-cheme-5440* channel in Slack.

Model: Let the model take the form $\hat{r}_j = r_j v\left(\ldots\right)_j$ where $\hat{r}_j$ denotes the overall rate of the PFK reaction (μM h^{-1}), r_j denotes the *kinetic limit* (μM h^{-1}), i.e., the maximum rate of conversion in the absence of allosteric regulation, and $0 \leq v\left(\ldots\right)_j \leq 1$ (dimensionless) denotes an allosteric regulation function of the form:

$$v\left(\ldots\right)_j = \frac{\sum\limits_{i \in \{X\}} W_i f_i\left(\ldots\right)}{\sum\limits_{j \in \mathcal{C}_j} W_j f_j\left(\ldots\right)} \quad (2)$$

where W_i (dimensionless) denotes the weight of configuration i, while $f_i\left(\cdots\right)$ (dimensionless) is a hill-binding function $f_i = (x/K_i)^{n_i}/(1 + (x/K_i)^{n_i})$ which describes the fraction of bound activator/inhibitor (x) for configuration i; K_i denotes a binding constant (mM), and n_j denotes an order parameter (dimensionless). The summation in the numerator of $v\left(\ldots\right)_j$ is over those configurations that lead to activity, while the summation in the denominator is over all possible regulatory configurations for enzyme j (denoted as $\mathcal{C}_j$). Let the kinetic limit for PFK (E_1, μM) be given by:

$$r_1 = k_{cat} E_1 \left(\frac{F6P}{K_{F6P} + F6P}\right)\left(\frac{ATP}{K_{ATP} + ATP}\right) \quad (3)$$

Assume: (i) the concentration of F6P in the assay equals 0.1 mM and is constant; (ii) the concentration of ATP in the assay equals 2.3 mM and is constant; (iii) the concentration of PFK in the assay equals 0.12 μM and is constant; (iv) K_{F6P} = 0.11 mM and K_{ATP} = 0.42 mM. (v) k_{cat} = 0.4 s^{-1}.

a) Estimate the parameter(s) W_1 (no $3' - 5' - \mathrm{AMP}$) and W_2 (with $3' - 5' - \mathrm{AMP}$)

directly from the dataset. **Note**: this can be done analytically, but need not be.

b) Estimate the binding constants and order parameters for the $3' - 5' - \mathrm{AMP}$ binding function from the dataset.

c) Plot your estimated overall rate (y-axis), and the measured rate (with errorbars), versus the $3' - 5' - \mathrm{AMP}$ concentration (x-axis) on the same plot. Can the proposed model formulation describe the data?

2. Stability analysis of Collins toggle switch (Gardner, T. S., C. R. Cantor, and J. J. Collins, 2000, Nature **403**, 520.)

The behavior of the toggle switch and the conditions of bistability can be understood using the following dimensionless model for the network:

$$\frac{du}{dt} = \frac{\alpha}{1+v^n} - u = f(u,v) \qquad (1)$$

$$\frac{dv}{dt} = \frac{\alpha}{1+u^n} - v = g(u,v) \qquad (2)$$

a) Identify each variable or parameter in the model as one of the following:

i. Concentration of a repressor of gene expression (there are two repressors)
ii. Effective rate of synthesis of repressor; lumped parameter that describes the net effect of RNA polymerase binding, open-complex formation, transcript elongation, transcript termination, repressor binding, ribosome binding and polypeptide elongation
iii. Cooperativity of repression
iv. Degradation rate constant for repressor

b) Plot the nullclines (lines for which f(u,v) = 0 or g(u,v) = 0) for the system for $\alpha = 10$ and $n = 1$ **AND** 2. How many solutions exist for each case? Comment on the influence of the degree of cooperativity.

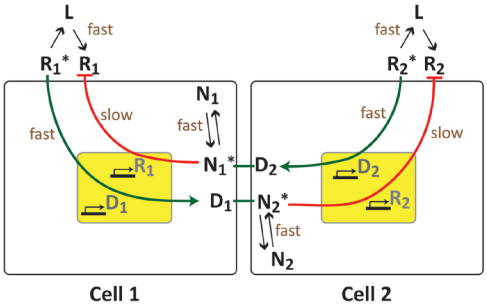
c) To gain intuition for this system, generate a vector steamline plot in *u,v*-space for $\alpha = 10$ and $n = 1$ **and** 2. (eg. in Matlab, "StreamPlot[{f(u,v), g(u,v)}, {u,v}] or in matplotlib, streamplot(f(u,v), g(u,v), u, v)). In words, describe what the vector with components, f(u,v), g(u,v) indicates? On your graph, identify the steady states on the plot and assess the character of each steady state (stable or unstable?). What is the influence of the degree of cooperativity?

d) Build the Jacobian for the system (for arbitrary α and n) at its steady states and write down the stability criterion. (You can leave your expression in terms of u_s and v_s, the concentrations of u and v at steady-state.) Use the stability criterion to explain the influence of the degree of cooperativity and the rate of synthesis on the stability of the center steady state (i.e., the one with $u = v$).

e) Find the numerical eigenvalues for the center steady state for $\alpha = 10$ and $n = 1$ and 2. How does the change in cooperativity affect the system?

f) Now we will use what we have learned about stability of coupled inhibition in the Collins toggle switch to consider patterning via intercellular signaling under the control of a growth factor, *L*. The particular case we consider models so called "juxtacrine" signaling in which signals are only communicated between adjacent cells via membrane-

bound pairs of receptors. A common example of this mechanism is mediated by Notch (*N*) and Delta (*D*) (see Alberts, pp. 893-895). We consider a case inspired by vascular patterning in which it is thought that the activated receptor (*R*) to Vascular Endothelial Growth Factor (VEGF = *L*) up-regulates the expression of Delta when activated by binding VEGF (activated receptor = R^* = *R*-*L*). In turn, the binding of Delta to Notch in a neighboring cell down-regulates expression of the receptor. If this coupled set of reactions becomes unstable, the VEGF-R rises in one cell and drops in the neighboring cell; the cell with high VEGF-R then becomes a "tip cell" that attempts to form a vessel sprout.[1] This scenario is depicted in the diagram for a two-cell model of the patterning process.



$$\frac{dR_i^*}{dt} = k_f L R_i - k_r R_i^* \quad (3)$$

$$\frac{dN_i^*}{dt} = k_f^{ND} N_i D_j - k_r^{ND} N_i^* \quad (4)$$

$$\frac{dD_i}{dt} = k_D R_i^* - \gamma_D D_i \quad (5)$$

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + N_i^{*n}} - \gamma_R R_i \quad (6)$$

The species balances (3)-(6) are the same for both cells (e.g., $i = 1$ and $j = 2$, or visa versa). Notch is assumed to be present in excess such that N_i = const.

f-1) Assume fast equilibrium for the processes in (3)-(5) to find the "toggle switch" of coupled inhibition between the two cells. You should obtain two equations of the form:

$$\frac{dR_1}{dt} = f(R_1, R_2)$$

$$\frac{dR_2}{dt} = g(R_1, R_2)$$

f-2) Non-dimensionalize your equations in f-1 to find the form above for the Collins toggle switch (eqs 1, 2). Non-dimensionalize by subbing in the following non-dimensional variables, $u = R_1/K$, $v = R_2/K$, $\tau = \gamma_R t$. Comment on the influence that the concentration of ligand has on the stability of the uniform state. What else could you manipulate to drive the system toward the instability?

[1] Jakobsson, Lars, et al. "Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting." Nature cell biology 12.10 (2010): 943-953.