# Problem Set 2

1 Competitive and uncompetitive inhibition (12 points)

a. Reversible enzyme inhibitors can bind enzymes reversibly, and slowing down or halting enzymatic reactions. If an inhibitor occupies the active site of an enzyme and prevents binding of the substrate to the enzyme, we call it a *competitive* inhibitor. In the presence of a competitive inhibitor, the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{max}S_0}{\alpha K_M + S_0}$$

What is α in terms of K_I and I_0? How does a competitive inhibitor affect the maximal turnover rate and the substrate concentration at which half that rate is reached? (Begin with a definition of total enzyme as $[E]_{tot} = [E] + [ES] + [EI]$ and use the derivation of the Michaelis-Menten equation as a guide. Assume S_0, $I_0 \gg [E]_{tot}$).
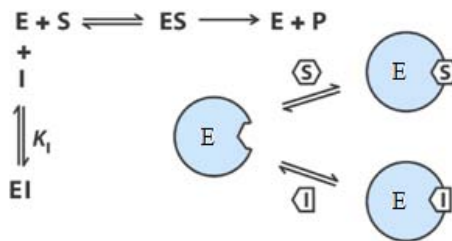

Problem 1a-d Image and problem courtesy of Alexander van Oudenaarden. Used with permission.

b. Unlike a competitive inhibitor, an *uncompetitive* inhibitor binds only to the Enzyme-Substrate (ES) complex, at a site distinct from the substrate active site. In the presence of an uncompetitive inhibitor, the Michaelis-Menten equation is altered to

$$V_0 = \frac{V_{max}S_0}{K_M + \beta S_0}$$

What is β in terms of K_I' and I_0? How does a competitive inhibitor affect the maximal turnover rate and the substrate concentration at which half that rate is reached?
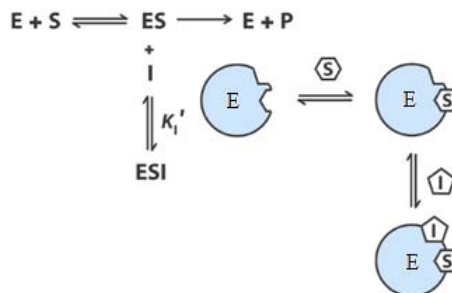
c. A *mixed* inhibitor binds at a site distinct from the substrate active site, but it binds to either ES or the enzyme by itself. Assume detailed balance for the reversible reactions. How is the Michaelis-Menten equation modified in the presence of a mixed inhibitor?
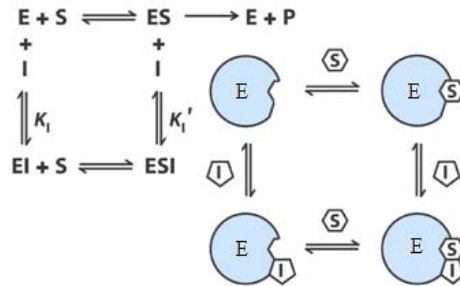
d. From an inspection of the curves below, determine the nature of the inhibition by acetazo-lamide on the carbonic anhydrase reaction, $H_2CO_3 \rightleftharpoons H_2O + CO_2$.
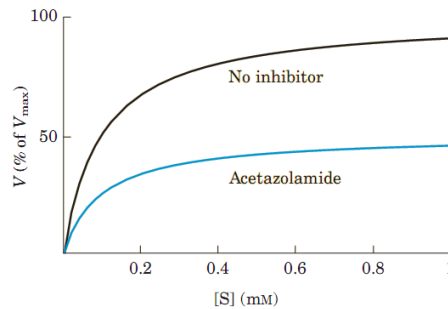
2 Stability analysis of dynamical systems[1] (10 points)

a. For each of the following 1D dynamical systems, identify the fixed points and use a graphical argument ($f(x) = \frac{dx}{dt}$ vs. x) to determine the stability. In addition, plot the potential potential function $V(x)$ ($f(x) = -\frac{dV}{dx}$). r can be positive, zero or negative. Discuss all three scenarios.

1. $\frac{dx}{dt} = rx - x^2$
2. $\frac{dx}{dt} = rx - x^3$

b. (Strogatz 6.4) Consider the following 2D dynamical systems (two species competing for the same limited resource, for example, "rabbits" vs. "sheep"), where $x, y \succeq 0$. Find the fixed points, investigate their stability, draw the nullclines, and sketch plausible phase portraits. Indicate the basins of attraction of any stable fixed points.

1. $\frac{dx}{dt} = x(3 - x - y), \frac{dy}{dt} = y(2 - x - y)$
2. $\frac{dx}{dt} = x(3 - 2x - y), \frac{dy}{dt} = y(2 - x - y)$

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[1]Chapter 2 & Chapter 6, "Nonlinear dynamics and chaos" by Steven Strogatz.

3 Positive feedback and bistability (12 points)

a. Suppose the protein X is a transcriptional activator, and a promoter D which is activated by the binding of X. If the downstream gene happens to code for X itself, the resulting positive feedback can lead to bistability. We will consider an scenario such as that described in problem 3 of the first problem set in which there are two binding sites and binding is strongly cooperative.
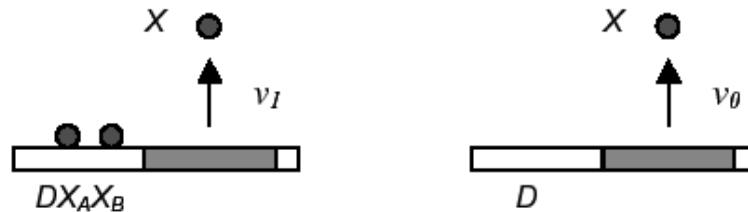
Problem by Alexander van Oudenaarden. OCW 8.591J Systems Biology, Fall 2004, Problem Set 1, 3.

1. Let ν_1 be the rate of expression from DNA bound by two X molecules (DX_AX_B), and $\nu_0 < \nu_1$ the rate of expression from free DNA (D); note that these rates of expression are assumed to have units of concentration per unit time. Use the results of problems 2 and 3 of the first problem set to show for an organism that grows at rate γ, where the degradation of X is negligible compared to dilution, the time-evolution of the concentration $x \equiv [X]$ may be written in the form:

$$\frac{dx}{dt} = \frac{\nu_0 + \nu_1 K_1 K_2 x^2}{1 + K_1 K_2 x^2} - \gamma x$$

where K_i $(i = 1, 2)$ denote *association* constants. (Note that in the first problem set Ks stand for *dissociation* constants)

2. Steady state solutions occur at those values of at which the rate of generation, $f(x) = \frac{\nu_0 + \nu_1 K_1 K_2 x^2}{1 + K_1 K_2 x^2}$, and degradation, $g(x) = \gamma x$, are equal. How many steady states can be in this system? Sketch 5 qualitatively different positions of the function $f(x)$ relative to the function $g(x)$. Label those values of x for which $f(x) > g(x)$ with a rightward arrow, and those for which $f(x) < g(x)$ with a leftward arrow. Which solutions are stable?

b. For two genes, positive feedback can arise from mutual inhibition. Consider a toggle switch modeled by the equations (Box 1, T.S.Gardner *et al, Nature* 2000)

$$\frac{du}{dt} = \frac{\alpha}{1 + v^2} - u$$
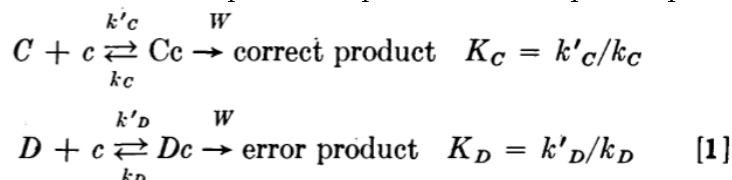
$$\frac{dv}{dt} = \frac{\alpha}{1 + u^2} - v$$

Which values of α give the system bistability? Justify your answer.

4 Kinetic Proofreading

Many cellular processes, such as transcription of a gene, translation of mRNA, or even the recognition of an antibody by a T-cell require high fidelity. During translation, for example, the ribosomes

are attempting to perform their enzymatic function on a particular substrate, but many other substrates in the cell look similar so it is difficult to perform the enzymatic function accurately. Yet, translation errors occur at typical rates as low as $\sim 10^{-4}$. In this problem, we will look at a mechanism that is employed to achieve such high accuracy. This was first suggested by J. J. Hopfield (see ref at end).

Let c be a recognition site, to which two substrates can bind, C, the correct and D, the wrong one. The correct complex then produces the expected product:

$$C + c \underset{k_C}{\overset{k'_C}{\rightleftarrows}} Cc \overset{W}{\rightarrow} \text{correct product} \quad K_C = k'_C/k_C$$

$$D + c \underset{k_D}{\overset{k'_D}{\rightleftarrows}} Dc \overset{W}{\rightarrow} \text{error product} \quad K_D = k'_D/k_D \qquad [1]$$
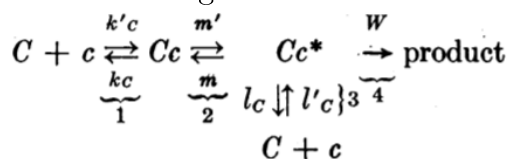
The rate of product formation from the complex (W) is determined by the strength of the covalent bonds between, for example, two amino acids, and is approximately the same for both the products.

Fidelity is quantified in terms of the error fraction f, which is the ratio of rate of production of wrong product to correct product.

a. Assuming that the substrate concentrations in the cell are mainteined at a constant (and equal) value for C, D, what is the error fraction? In the limit of $W \ll k_c$, the error fraction is minimized. What is this minimum value, f_0? (Note that being in the same medium, the concentration of the recognition sites, c, is the same for both reactions)

Now consider adding an additional intermediate state of the complex in the above reaction:

$$C + c \underset{\underset{1}{k_c}}{\overset{k'_c}{\rightleftarrows}} Cc \underset{\underset{2}{m}}{\overset{m'}{\rightleftarrows}} \quad Cc^* \overset{W}{\rightarrow} \text{product}$$
$$l_c \updownarrow l'_c \}_3 \quad^4$$
$$C + c$$

b. What is the error rate in this process? Detailed balance puts a restriction on the values that m, m' can take. What is this constraint? It turns out that under this constraint, $f \geq f_0$, so this might not seem like a good method to increase fidelity.

Next assume steps 2, 3 in the above reaction are one way steps, i.e. $m = 0, l'_c = 0$. The reaction steps can be made strongly directed like this by coupling them with other strongly driven reactions, such as ATP hydrolysis, so that they are nearly one way.

c. If $m' < k_c$, so that you can assume reaction 1 is in quasi-equilibrium by itself, and with $m = 0, l'_c = 0$, show that the error fraction now is $\approx f_0^2$. (The ratio of off rates for the original and modified complex is the same, since it involves the same substrate falling off.)

Adding multiple steps like this can greatly increase the specificity of the reaction. With n one way steps, the error fraction can be lowered to f_0^n. However, at each of these steps, the cell has to consume energy (for ATP hydrolysis), so this is a costly function. Another mechanism involves introducing a delay between complex formation and product precipitation. Since the dissociation rate of the worng substrate is higher, this delay allows more time for the wrong substrate to fall off. See Uri Alon proofreadign chapter for details.

Ref: Kinetic Proofreading: A New Mechanism for Reducing Errors in Biosynthetic Processes Requiring High Specificity, J. J. Hopfield, PNAS 71(10), 4135-4139

5 Circadian Clocks (30 points)

Several protein expression levels in plant and animal cells go through a daily cycle, driven by exposure to sunlight during the day and darkness at night. However, even in complete darkness, these expression levels oscillate with an intrinsic period of about 24 hours. The systems which drive these oscillations are known as circadian clocks. At the heart of most of these systems is a pair of transcriptionally regulated proteins: an activator (X) and an inhibitor (Y). In this problem set, we will see how such a simple system can be made to generate oscillations. We consider two possible system architectures (arrows represent activation, blunt ends represent inhibition):
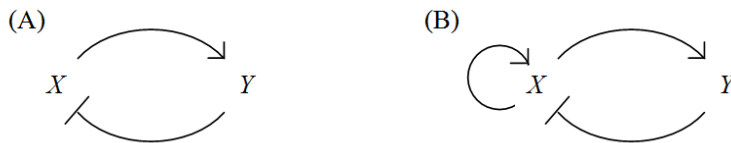
The corresponding dynamical equations are (using $x = [X]$ and $y = [Y]$):

(A)

$$\frac{dx}{dt} = v_x + k_x \frac{A_1}{A_1 + y} - \gamma_x x$$
$$\frac{dy}{dt} = v_y + k_y \frac{x}{A_2 + x} - \gamma_y y$$

(B)

$$\frac{dx}{dt} = v_x + k_x \frac{x^2}{(A_3)^2 + x^2} \frac{A_1}{A_1 + y} - \gamma_x x$$
$$\frac{dy}{dt} = v_y + k_y \frac{x}{A_2 + x} - \gamma_y y$$

a. Identify the parameters corresponding to basal transcription rate, maximal transcription rate, and degradation rate.

b. We have assumed the following: for both (A) and (B), the X promoter is inactivated by the binding of a single molecule of Y, and the Y promoter is activated by the binding of a single molecule of X. In addition, for (B), the X promoter is activated by the cooperative binding of two molecules of X. The various fractions that appear in the equations represent the activity of promoters. Explain what each of these fractions means in the context of gene regulation.

c. Normalization of units. Assume that $A_2 \gg x$. Redefine variables and show that by choosing units properly the previous equations can be written in the form

(A)

$$\frac{d\bar{x}}{d\bar{t}} = \bar{\gamma}_x(\bar{v}_x + \bar{k}_x\frac{1}{1+\bar{y}} - \bar{x})$$

$$\frac{d\bar{y}}{d\bar{t}} = \bar{v}_y + \bar{k}_y\bar{x} - \bar{y}$$

(B)

$$\frac{d\bar{x}}{d\bar{t}} = \bar{\gamma}_x(\bar{v}_x + \bar{k}_x\frac{\bar{x}^2}{1+\bar{x}^2}\frac{1}{1+\bar{y}} - \bar{x})$$

$$\frac{d\bar{y}}{d\bar{t}} = \bar{v}_y + \bar{k}_y\bar{x} - \bar{y}$$

From now on we will work with the simplified equations, dropping the bars on the variable and paramter symbols. These equations are of the general form

$$\frac{dx}{dt} = f(x, y)$$

$$\frac{dy}{dt} = g(x, y)$$

Let (x_0, y_0) be a fixed point of the system $(f(x_0, y_0) = 0, g(x_0, y_0) = 0)$ and recall that if we define

$$A = \left. \begin{pmatrix} \frac{\partial f}{\partial x} & \frac{\partial f}{\partial y} \\ \frac{\partial g}{\partial x} & \frac{\partial g}{\partial y} \end{pmatrix} \right|_{x_0, y_0}$$

fixed points of this two dimensional system are stable if and only if $Tr(A) < 0$ and $Det(A) > 0$.

d. **COMPUTATION** For systems (A) and (B) separately, assume $v_x = 0.1$, $v_y = 0.0$, $k_x = 4.0$, $k_y = 2.0$ and $\gamma_x = 10$ and plot the nullclines $f(x, y) = 0$ and $g(x, y) = 0$ on the y vs. x phase plane. Draw the vector field indicating the direction of motion in different regions of the plane and, based on the graphs, comment on the stability of the fixed point.

e. For system (A), prove that the system will always converge to a stable fixed point.

f. For system (B),

1. Assume $v_x = 0.1$, $v_y = 0.0$, $k_x = 4.0$, $k_y = 2.0$ and let γ_x be a variable parameter. Find the fixed point of the system. If this fixed point becomes unstable oscillations will arise. By numerically analyzing $Tr(A)$ and $Det(A)$ as a function of γ_x write down the conditions on γ_x under which the system is oscillatory. What does this condition mean in terms of the timescale of X and Y?

2. **COMPUTATION** Solve the equations numerically using the parameters given in (f1). Choose two different values of γ_x, in one case the system shows sustained oscillations, while in the other case the system approaches a stable fixed point.

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3. **COMPUTATION** By varying γ_x, we can tune the period of the oscillator. How much does the amplitude of oscillations change? What would you expect in the case of a repressilator? Interpret your results and comment on the tunability[1] of an oscillator.

4. System (B) is a network motif found in many biological oscillatory systems (Uri Alon's book, Chapter 6.5). Look up in the literature for an example.