Problem Set 1   *Assigned:*   *09.21.04*
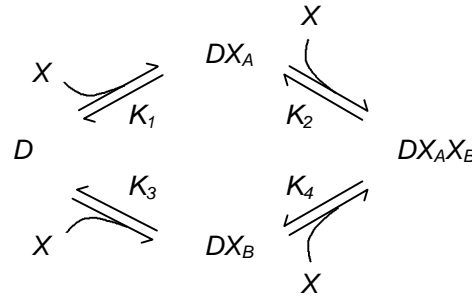*Due in class*   *Due:*   *10.05.04*

1.   *Binding kinetics, detailed balance and cooperativity.*

D is a DNA segment containing two sites, A and B, to which the protein X can bind in any order.

(10)   *a.*   In the figure, the K_i represent association constants in inverse concentration units. According to detailed balance, the individual binding reactions are in equilibrium. Find two different expressions for the equilibrium ratio $[DX_AX_B]/[D][X]^2$, corresponding to the two possible binding paths. Can all four association constants be independently specified?

(10)   *b.*   The total DNA concentration is $[D_{tot}] = [D]+[DX_A]+[DX_B]+[DX_AX_B]$. If $K_2 \gg K_1$ and $K_4 \gg K_3$, show that the singly-bound forms $[DX_A]$ and $[DX_B]$ form a negligible fraction of this total. This corresponds to a cooperative system in which the second binding is highly encouraged once the first has already occurred.

(10)   *c.*   Assuming $[D_{tot}] \approx [D]+[DX_AX_B]$, find the bound fraction $f = [DX_AX_B]/[D_{tot}]$ in terms of $[X]$ and the association constants.

2.   *Dilution of proteins due to cell growth.*

A single bacterial cell at time $t = 0$ has volume V_0. After a time interval T_D, the doubling time, the cell grows and divides into two cells, each of volume V_0; after another interval T_D, there are four cells, and so on.

(10)   *a.*   Show that the combined volume of cells at time t may be written as $V(t) = V_0\, e^{\gamma t}$. Find γ in terms of T_D.

(10)   *b.*   The protein X is created at some rate $k(t)$, so the total number of molecules of X satisfies $dn_x/dt = k(t)$. Show that the concentration $[X] = n_x/V$ satisfies

$$\frac{d}{dt}[X] = \frac{k(t)}{V} - \gamma[X].$$

Discuss the origin of the decay term.

3.   *Positive feedback and bistability*

Suppose the protein *X* be a transcriptional activator, and *D* a promoter 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.
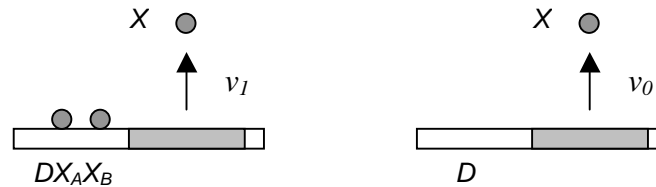
(10)   *a.*   Let v_1 be the rate of expression from bound DNA (DX_AX_B), and $v_0 < v_1$ the rate of expression from free DNA (*D*). Use the results of Problems 1 and 2 to show that the time-evolution of the concentration $x \equiv [X]$ may be written in the following form:

$$\frac{dx}{dt} = \underbrace{\frac{v_0 + v_1 K_1 K_2 x^2}{1 + K_1 K_2 x^2}}_{f(x)} - \underbrace{\gamma \cdot x}_{g(x)}$$

(30)   *b.*   Steady state solutions occur at those values of *x* for which the rates of generation (*f(x)*) and degradation (*g(x)*) are equal. Set $\gamma = 1$ and $K_1 K_2 = 1$, and use the accompanying MATLAB file ps1.m to explore the intersections of *f(x)* and *g(x)* as the parameters v_0 and v_1 are varied. The figures (i) through (v) show schematically the types of behaviors that can occur. Plot an example each of types (i), (iii) and (v), indicating the parameter values that generated them. 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?

(i)   (ii)   (iii)   (iv)   (v)
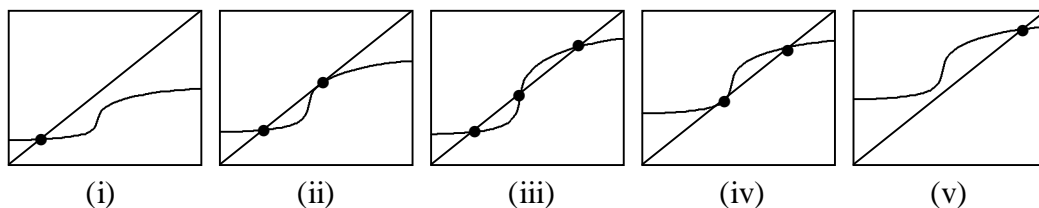
(10)   *c.*   The boundary between bistability and monostability is given by parameters for which the system has precisely two fixed points (types (ii) and (iv)). Setting $\gamma = 1$ and $K_1 K_2 = 1$, rewrite the condition $f(x) = g(x)$ as a cubic equation of the form $x^3 + c_2 x^2 + c_1 x + c_0 = 0$. Any cubic can always be factorised as $(x - a_1)(x - a_2)(x - a_3)$. What are the conditions on the roots that would lead to type (ii) or type (iv) behavior? Apply this condition and compare coefficients to obtain parametrized equations for v_1 and v_0. Show on a graph of v_1 vs. v_0 the region over which the system is bistable.