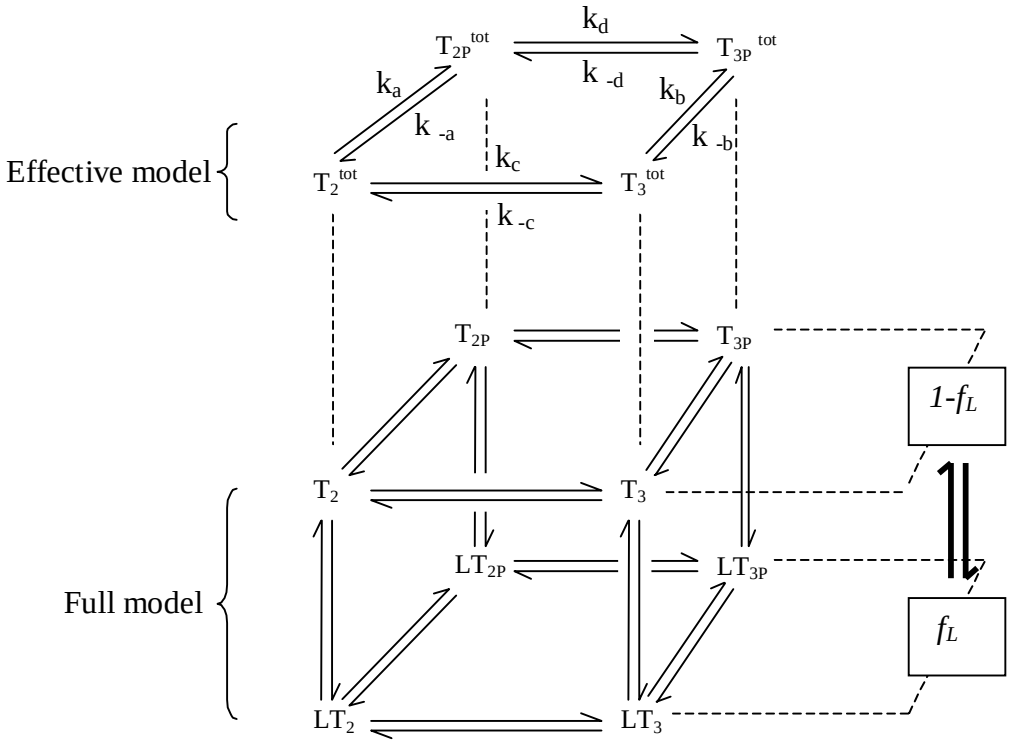
Problem Set 2 **Assigned: 10.06.04**
Due in class **Due: 10.19.04**

1. Biochemistry of the chemotaxis network.



The *E. coli* chemotaxis network is represented here in simplified form. T represents the Tar receptor, and L the ligand or attractant. The receptor can by modified by phosphorylation (subscript P) or methylation (subscripts 2 or 3).

(10) *a.* The Tar receptor binds an extracellular ligand L according to

$L + T \underset{k_L^-}{\overset{k_L^+}{\rightleftharpoons}} LT$, with $K_L = \frac{k_L^+}{k_L^-}$. Calculate the fraction f_L of bound receptor.

(10) *b.* We now assume that the ligand binding reaction is in rapid equilibrium, and only consider total amounts of each modified form of receptor. For example,

$T_{3P}^{\ tot} = T_{3P} + LT_{3P}$, etc. How would you calculate the effective rate constants $k_a,...,k_d$ and $k_{-a},...,k_{-d}$ between these total concentration pools in terms of the rate constants of the original methylation /demethylation and phosphorylation/dephosphorylation reactions?

(10) *c.* The assumed rates in the models of Spiro *et al.* and Barkai *et al.* are shown in the table below. Write down explicitly the effective rate constants for each model, in terms of the symbols listed in the table.

Spiro model			*Barkai model*		
	L-unbound	*L-bound*		*L-unbound*	*L-bound*
k_a	k_8	0	k_a	*0*	*0*
k_{-a}	k_y	k_y	k_{-a}	k_0	k_0
k_b	$3\ k_8$	$1.1\ k_8$	k_b	k_{p1}	k_{p2}
k_{-b}	k_y	k_y	k_{-b}	k_{-p1}	k_{-p2}
k_c	k_1	k_3	k_c	k	k
k_{-c}	k_{-1}	k_{-1}	k_{-c}	*0*	*0*
k_d	k_1	k_3	k_d	*0*	*0*
k_{-d}	k_{-1}	k_{-1}	k_{-d}	k_m	k_m

Note that some effective rate constants are now functions of L. This is appropriate, since we know for example that the receptors should become less phosphorylated as L increases.

i) In the Spiro model, do k_a/k_{-a} and k_b/k_{-b} increase or decrease with L?

ii) In the Barkai model, we would like k_b to decrease and k_{-b} to increase with L. What does this imply about k_{p1}, k_{p2}, k_{-p1} and k_{-p2}?

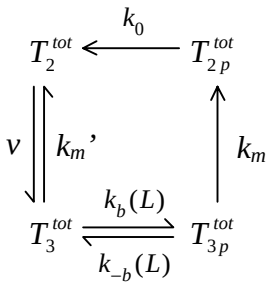
(10) *d.* Spiro model. In steady state, after the slow methylation reactions have had time to equilibrate, let $\alpha(L)$ represent the fraction of receptors that are methylated. Consider now the total concentration of phosphorylated and unphosphorylated receptors. Write out explicitly, in terms of $\alpha(L)$, the effective rates of phosphorylation (k_p) and dephosphorylation (k_{-p}) using

$$k_p = (1-\alpha(L))k_a + \alpha(L)k_b, \qquad k_{-p} = (1-\alpha(L))k_{-a} + \alpha(L)k_{-b}$$

For perfect adaptation to be achieved, the phosphorylated fraction of receptor must be independent of L in steady state. You should have found above that $k_{-p} = k_y$; it is therefore sufficient for perfect adaptation that $k_p = k_p^*$ is a constant.

Set $k_8 = 15\ s^{-1}$; $K_L = 1x10^6\ M^{-1}$; and $k_p^* = 15\ s^{-1}$. Plot k_a and k_b for L = 0, ..., 2 K_L. On the same graph, draw a horizontal line showing the desired k_p^*. Finally, set $k_p = k_p^*$ in the equation above, solve for $\alpha(L)$, and plot this function. This is the magical form of $\alpha(L)$ required for perfect adaptation. The model of Spiro *et al.* is carefully "tuned" in order to achieve this result. We can contrast this situation with the Barkai model in part *f*, which is perfectly adapting but requires no fine tuning.

(10) *e.* Barkai model. Biochemical evidence suggests that the methylation reaction (whose rate constant was written as k in part *c*) operates at saturation with rate v. Show that under this assumption, the entire model reduces to the following reaction scheme:

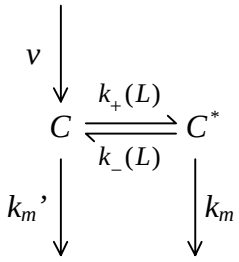


Note that v is a constant *rate* (measured in M s^{-1}) while k_m is a rate constant (measured in s^{-1}). What is the value of k_m' ?

Write down the equation for $\frac{d(T_3^{tot} + T_{3p}^{tot})}{dt}$ and solve for $T_{3p}{}^{tot}$ in steady state. Show that this value is independent of L if and only if $k_m' = 0$. This is the essence of the Barkai model: perfect adaptation is easy to achieve, as long as only the *phosphorylated* receptors are demethylated by the CheB protein.

2. Adaptation and frequency response of the chemotaxis network.

With a slight change of notation, the Barkai model of the chemotaxis network (see Problem 1*f*) can be represented as



Here, v represents the rate of creation of C, the unphosphorylated receptor; C^* is the phosphorylated or active form of the receptor, the actual signal which induces bacterial tumbling; k_m and k_m' are the rate constants of demethylation reactions; and finally, k_+ and k_- are rate constants that represent the effect of ligand binding on the phosphorylation state of the receptor.

Set $\alpha_+ = \frac{\partial k_+}{\partial L} < 0$, and $\alpha_- = \frac{\partial k_-}{\partial L} > 0$. This ensures that a sudden increase of ligand concentration causes a drop in the phosphorylated fraction of the receptor.

(10) *a.* Write down the equations for dC/dt and dC^*/dt. Solve for the steady state concentrations C_{ss} and C^*_{ss}. Under what conditions will C^*_{ss} be independent of L?

(10) *b.* Set $\delta C = C - C_{ss}$, $\delta C^* = C^* - C^*_{ss}$. Derive the linearized equations representing fluctuations from steady state, driven by fluctuations $\delta L(t)$ of the ligand concentration. You should obtain

$$\frac{d}{dt}\begin{bmatrix}\delta C \\ \delta C^*\end{bmatrix} = \begin{bmatrix}-(k_+ + k_m') & +k_- \\ +k_+ & -(k_- + k_m)\end{bmatrix}\begin{bmatrix}\delta C \\ \delta C^*\end{bmatrix} + (\alpha_- C^*_{ss} - \alpha_+ C_{ss})\delta L\begin{bmatrix}1 \\ -1\end{bmatrix}.$$

(10) *c.* Assume for now that $\delta L = 0$, $k_m' = 0$, and $k_m = 0$. Calculate the eigenvectors and eigenvalues of the above matrix. You will find that one of the eigenvalues is zero. Recalculate this eigenvalue to first order in k_m.

On a graph of δC vs. δC^*, plot the eigenvectors and note the slow and fast eigenvalues. Sketch a few typical timecourses for various initial values of $\{\delta C, \delta C^*\}$. This initial perturbation might arise if the system had first reached steady state for one value of L, but that value was abruptly changed. Sketch out such an event, showing a step increase in L at time $t = 0$, and the subsequent evolution of C, C^*, and C_T as functions of time.

(10) *d.* Now assume that δL, δC, $\delta C^* \sim e^{i\omega t}$. This corresponds to Fourier transforming the equation above.

Calculate the *transfer function* $T(\omega) = \left| \frac{\delta C^*(\omega)}{\delta L(\omega)} \right|$.

Claiming that perfect adaptation holds corresponds to claiming that $T(\omega)$ has no dc component ($T(\omega = 0) = 0$). Show that this is true only if $k_m' = 0$. Assume from now on that $k_m' = 0$.

(i) What is the behavior of $T(\omega)$ as $\omega \to 0$?

(ii) What is the behavior of $T(\omega)$ as $\omega \to \infty$?

(iii) Calculate the value ω^* at which $T(\omega)$ is maximized.

(iv) Make a sketch of $T(\omega)$, indicating all the important regimes.

(10) *e.* From this sketch, it should be clear that the chemotaxis network serves as a bandpass filter: variations of L slower than the demethylation rate k_m are suppressed by the adaptation property of the network; fast fluctuations of L are suppressed because C^* cannot respond any faster than the phosphorylation rate.

(i) Suppose $f_{out}(t) = df_{in}(t)/dt$. Calculate $T_{diff}(\omega) = |f_{out}(\omega)/f_{in}(\omega)|$. This is the transfer function of a differentiator. For what values of ω does the chemotaxis network serve as a differentiator?

(ii) The network most efficiently transmits signals at the frequency ω^* calculated in part *d*(iii). What is the value of ω^*, assuming $k_m \sim 0.01\ s^{-1}$ and $k_+ \sim 10\ s^{-1}$?

(iii) It is said that "a cell compares the attractant concentration at any given time to that 4 seconds ago", generating a tumble if it registers a decrease or a run if it registers an increase. That is, only by *differentiating* the input does the cell manage to swim up an attractant gradient. Is the timescale of 4 seconds consistent with your answer from the part (ii)?