BP205 Midterm

Due Date: 2-26-2009, 5pm

Name:_____

Start time: _____

End time: _____

Instructions: Take this exam in one continuous 4 hour period. No collaboration is allowed. All class notes, problem sets, readings, and textbooks may be consulted in taking the exam, but the internet should not be used. A scientific calculator may be used.

1 Biphasic Regulation of transcription

A protein, P, both positively and negatively regulates its own production through binding sites on its promoter. We will model its production using the Shea-Ackers formulation of promoter kinetics:

$$\frac{dP}{dt} = \alpha \frac{K_1 P}{1 + P_1 P + K_2 P} - \gamma P \tag{1}$$

where α represents the basal production rate of P in $mole/sec$, K_1 is the equilibrium association constant for the site of positive regulation in $1/mole$, K_2 is the equilibrium association constant for the site of negative regulation in $1/mole$, and γ is the decay rate of P in $1/sec$.

a. Write a de-dimensionalized form of equation (1). You should be able to reduce the system to two de-dimensionalized parameters.

b. Determine the values of the de-dimensionalized variable at which the system is at steady state.

c. Determine the conditions under which these steady states will be stable.

d. Does the system undergo any bifurcations as the parameters that you defined in part a. vary? If so, at what value of these parameters does the bifurcation occur?

2 Regulation of transcription: mRNA and protein

In this problem we will simulate transcription with a model that considers both protein and mRNA. Consider a protein P that positively regulates the production of its corresponding mRNA, M. We will model the system with this simple set of equations:

$$\frac{dM}{dt} = c + k_m * P - \gamma_m * M \tag{2}$$

$$\frac{dP}{dt} = k_p * M - \gamma_p * P \tag{3}$$

where c is the basal production rate of M in $mole/sec$, k_m gives the proprotionality between P and the increase that it causes in production of M in $1/sec$, k_p is the rate at which P is produced from a single M transcript in $1/sec$, γ_m is the rate at which M degrades in $1/sec$, and γ_p is the rate at which P degrades in $1/sec$.

a. Solve for M and P at the steady state of this system.

b. Write out the Jacobian of this system at this steady state.

c. Compute the determinant and trace of the Jacobian as a function of the parameters of the system.

d. Under what circumstances will the system's steady state be unstable? Give a qualitative explanation for why the steady state is unstable under these circumstances.

e. Under what conditions will the system have a steady state that is approached as a spiral in M-P space? Can you qualitatively describe why this set of parameters might lead to a steady state that is approached in this way?

3. Two-component signal transduction

The EnvZ/OmpR system in *E. coli* is a two-component signal transduction system used to regulate expression of genes in response to extracellular osmolarity. *EnvZ*, the sensor component, is a membrane bound protein that autophosphorylates in response to extracellular osmolarity. *EnvZ* transfers this phosphate to *OmpR*, the response regulator component, which is a transcription factor for several genes. One (simplified) model for this system is shown below:

$$A.\quad EnvZ + ATP \rightleftharpoons EnvZ \cdot ATP \xrightarrow{k_1(s)} EnvZ_P + ADP$$

$$B.\quad EnvZ_P + OmpR \rightleftharpoons EnvZ_P \cdot OmpR \xrightarrow{k_2} EnvZ + OmpR_P$$

$$C.\quad EnvZ \cdot ATP + OmpR_P \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} EnvZ \cdot ATP \cdot OmpR_P \xrightarrow{k_4} EnvZ \cdot ATP + OmpR + P_i$$

where $EnvZ_P$ and $OmpR_P$ are the phosphorylated species of *EnvZ* and *OmpR*, and $k_1(s)$ is a kinetic rate of autophosphorylation that depends on the strength of the extracellular osmolarity, *s*. Thus, we can think about this system as taking the input $k_1(s)$ and converting it to the output transcription factor, $OmpR_P$.

1. What is happening in reaction C? Is ATP energetically participating or does it have another role?

The function that describes the steady-state relationship between output $OmpR_P$ and input $k_1(s)$ is known as the dose response. One way to get an expression for the dose response is to write out all seven differential equations that describe the reactions A-C. Instead, we can take a shortcut:

2. Write the differential equation that describes the rate of change of the intermediate complex $EnvZ \cdot ATP \cdot OmpR_P$.

3. Write expressions for (a) the flux of ATP into the system and (b) the flux of inorganic phosphate (P_i) out of the system.

4. At steady state, the flux of the intermediate complex $EnvZ \cdot ATP \cdot OmpR_P$ will be constant. Use this fact and your expressions in part 2-3 to write down the system's dose response (the concentration of output $OmpR_P$ as a function of input $k_1(s)$). You should find that the dose response depends only on rate constants, and not concentrations. How might this be a useful feature for a signal transduction system?

4. Bacterial Metabolism and Sensing

A spherical bacterium is suspended in water and takes up oxygen in the water in order to survive. The quantity of water is very large compared to the bacteria, so its oxygen consumption will not affect the bulk oxygen level, c_0. Assume that the oxygen is consumed instantaneously and that at the cell's surface, the concentration of oxygen is zero.

1. What is the full concentration profile of oxygen with respect to the distance from the bacterium? What is the max number of molecules the bacterium can consume?

2. We can estimate a bacterium's metabolic activity as its rate of oxygen consumption per unit mass. Using $c_0 = 0.2$ mol-m^{-3} and the diffusion constant for oxygen in water $D_O = 2.1 \times 10^{-5}$ $cm^2 s^{-1}$, what is the max metabolic activity of a bacterium of some radius Z? Report any assumptions that you make.

3. The metabolic activity of a bacterium is actually about 0.015 mole-$kg^{-1}s^{-1}$. What limit does this put on the size of a bacterium? What are some ways a species might go beyond this limit?

4. Bacteria release small molecules in order to signal to other bacterium nearby (quorum sensing). Suppose that at time 0, the bacterium releases some number M of small molecules. The molecules will spread by diffusion and at some time later will be sensed by a nearby bacterium. Assume the molecule does not degrade and that the two bacteria do not move. Show that from the perspective of the nearby bacterium, the concentration of small molecule will appear to increase over time, reach a global maximum, then decrease forever.