Problem Set No. 5

Due: Thursday, 12/02/10 at the start of class

Objective: To understand regulation and signaling processes in cells, and to develop and apply thermodynamic and kinetic models of them.

Review problems

You should pay special attention to these questions after reading. Note that the answers are given in the back of the book. Formulate your answers fully first and then check them. This can be a significant aid in your understanding of the material, and similar questions may be asked on the final. You do not need to provide written answers in the solutions you hand in.

- ECB 8-6
- ECB 8-8
- ECB 8-10
- ECB 8-13
- ECB 16-10
- ECB 16-13
- ECB 16-17
- ECB 16-20

Problem 1

You are producing recombinant protein "X" in yeast cells. X is synthesized on the rough ER and secreted from the cell. You have a sensitive assay to measure the concentration of active protein in the growth medium in real-time. At the start of the experiment, the concentration of X throughout the cell is negligible. You initiate protein synthesis by addition of a chemical inducer that initiates transcription. Fluorescence experiments suggest that the concentration of X reaches 50% of its steady-state value in the ER in about 10 minutes, and in the Golgi apparatus in about 1 hour. How long do you expect it to take for the rate of secretion of X to the extracellular space to reach 90% of its steady-state value? Consider a two-compartment model of transport for this process (ER→Golgi), based on the one discussed in lecture.

Problem 2

A DNA-binding regulatory protein P is synthesized on free ribosomes in the cytosol. The protein then must fold before it can be transported through the nuclear pore complex into the nucleus, where it binds to DNA. You will develop a simple kinetic model of this process considering the following steps: synthesis, folding, and transport through the pore complex.

Consider first synthesis and folding in the cytosol. Imagine that no P is present initially but that regulatory mechanisms suddenly initiate its expression with constant rate v in molecules/volume/time. Let $[U]$ denote the cytosolic concentration of unfolded protein and $[F]$ the concentration of folded ones. A negligible amount of protein unfolds after folding. The rate coefficient for folding is k_f.

The protein is then transported irreversibly through nuclear pores by binding to a transport protein T. This process is well-described as a Michaelis-Menten-type process,

$$F + T \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} FT \overset{k_2}{\rightarrow} N + T$$

where N indicates the folded regulatory protein now inside the nucleus. This mechanism assumes that the total concentration of T at the nuclear pore, $[T]_0$, does not change with time.

a) What are the major assumptions being made in this model?

b) Write the full reaction mechanism for this process. Then, write expressions for the rate of change of the concentrations $[U], [F], [N], [T], [FT]$.

c) What is the steady-state concentration of unfolded species in the cytosol, $[U]^{SS}$? What is the time-dependence of $[U]^{SS}$ after synthesis begins?

d) Assuming that the reaction with rate constant k_2 is the limiting step, use the quasi-steady-state approximation for the intermediate concentrations $[U], [F], [FT]$. Find expressions for these concentrations and for the rate of delivery of protein to the nucleus of $d[N]/dt$.

e) Using your results in part (d), show that v is bounded by an upper limit in this model. What is the upper limit? Why does this make sense, physically?

f) Instead consider the case in which the concentration $[F]$ cannot be considered at pseudo steady state, but $[U]$ and $[FT]$ still can. Find a differential equation that you could solve numerically to give $[F]$ as a function of time. This equation should only include the known quantities $v, [T]_0$, and the rate coefficients defined above, in addition to the variable being solved for, $[F]$. Indicate an initial condition for this differential equation.

Problem 3

You are using recombinant bacterial cells to produce a particular protein therapeutic P. As a control element, you have engineered them to also encode for a repressor R that prevents transcription of the gene encoding for both P and R (i.e., P and R are expressed simultaneously). This feedback mechanism allows you to control the concentration of produced product within the cell, which might otherwise cause production problems if too high. Consider a highly simplified model in which the following rate expressions describe this process:

- $k_1[S]$ - rate of synthesis of P and R, where $[S]$ is the concentration of the gene encoding for both
- $k_2[P]$ and $k_3[R]$ - rates of degradation of P and R, respectively
- $k_{on}[S][R]$ – rate of binding of repressor to site
- $k_{off}[RS]$ - rate of dissociation of repressor from site
- $K_D = k_{off}/k_{on}$ - dissociation constant for R binding to the regulatory site S
- $[S]_0$ - total concentration of the regulatory site, whether bound or unbound with repressor

a) Develop a reaction model for this system. Write down expressions for the rates of change of concentrations $[P]$, $[R]$, $[S]$, and $[RS]$.

b) Assume that binding and dissociation of R to the regulatory site is fast relative to other processes. Use an appropriate pseudo-steady-state assumption to simplify the rates of change in concentrations of $[R]$ and $[P]$, in terms of the constants above. Be sure to eliminate $[S]$ and $[RS]$ completely from your results.

c) If it turns out that $[R]/K_D \gg 1$ at equilibrium, user your result in part (b) to show that the steady-state concentration of $[P]$ has the following dependence on K_D,

$$[P] \propto K_D^{\frac{1}{2}}$$

d) Estimate the concentration of the DNA regulatory site, $[S]$, for a spherical cell of radius 1 μm.

Problem 4

Insulin is a small protein hormone that regulates the uptake and storage of the energy source glucose. When absent, plasma glucose in the blood stream is not taken up; when it is present, it is with a rate proportional to both the insulin and glucose concentrations. You will develop a very simple model of glucose (G) and insulin (I) interactions in the plasma that describes the behavior of this regulatory mechanism. The following simplified fluxes describe the rates of change of concentrations of the two species:

- The rate of change in insulin concentration in the bloodstream due to its production is proportional to the glucose concentration, $k_1[G]$.
- Insulin is degraded at a rate of $k_2[I]$ molecules/volume/time.
- Glucose is taken up by cells at a rate of $k_3[I][G]$ molecules/volume/time.

a) Write a coupled set of differential equations governing the concentrations $[G]$ and $[I]$ with time.

b) What are the steady-state values of the concentrations? Draw a rough sketch of what you expect the concentration profiles to look like immediately after a meal. That is, plot $[I]$ and $[G]$ as functions of t, with $[I] = 0$ and $[G] = [G]_0$ at time $t = 0$. Your plot need only show qualitative trends—you should not need to solve any differential equations.

c) Assuming the insulin response is very fast, use the quasi-steady-state approximation to simplify these differential equations and solve for $[I]$ and $[G]$ as a function of t for an initial glucose concentration $[G] = [G]_0$ and insulin concentration $[I] = 0$ at $t = 0$.

d) Using your result in part (c), consider the case in which a spike in plasma glucose concentration suddenly occurs as the result of a meal. Find an analytical expression for the time it takes for half of this glucose to be cleared from the blood stream. Does this time depend on the magnitude of the initial spike? Why or why not?

e) Let a basic unit of concentration be defined by the quantity$c = k_1/k_3$. Non-dimensionalize the concentrations according to $[I]^* = [I]/c$ and $[G]^* = [G]/c$. Similarly, nondimensionalize the time by $t^* = k_1 t$. Then, rewrite the differential equations from part (a) using these new non-dimensional variables. You should be left with only one parameter in the differential equations, $k^* \equiv k_2/k_1$.

f) Numerically solve the equations in part (e) using your favorite mathematical software package. Plot $[I]^*$ as a function of t^* for $k^* = 0.1,1.0,2.0$ (three series) and for $[G]_0^* = 1$. Does $k^* = k_2/k_1$ affect the initial or long-time insulin response more strongly?

Problem 5

What was your favorite topic that we discussed this quarter?