20.420 Biomolecular Kinetics and Cellular Dynamics
Final Examination
December 17, 2015

Open course notes only, including your own notes, class notes, and homework; no electronic devices permitted.

Please complete **only six problems** of the eight presented. All problems are of equal point value.

Important additional instructions:

- *use a separate blue book for each problem*
- *write your name and the problem number clearly on each booklet*
- *only submit six blue books, for the six problems you wish to have graded; if more than six blue books are submitted, only the first six will be graded*

Problem 1. An ion channel switches between three states: closed, open, and inactivated. Rate constants k_C, k_O, and k_I describe transitions from closed to open, open to inactivated, and inactivated to closed, respectively. Transitions in the other direction do not take place. Please answer the following questions about this system.

a) Write down a diagram illustrating the scenario, along with a set of ordinary differential equations that describe the change in time for each of the three channel states.

b) Determine the concentrations of closed, open, and inactivated forms of the channel in terms of k_C, k_O, k_I, and the total concentration of channels C_T. Show your work.

c) Suppose you wanted to perform a stochastic simulation of 10 channels using the Gillespie algorithm. If all the channels started out in the closed conformation, what would be the initial rate of reactions, and how would you choose the time step till the next reaction (give a formula)?

d) Now suppose you began a Gillespie simulation from approximate equilibrium (keeping in mind that there is no true steady state for a stochastic process). What would be the rate of each reaction? Describe a computational procedure for choosing the next reaction based on these rates.

Problem 2. The predator-prey model describes a situation where prey live off the land with limited food supply, while predators live off of the prey. It can be represented algebraically by:

$$dx/dt = x[x(1 - x) - y]$$

$$dy/dt = y(x - a)$$

where $x \geq 0$ represents the prey population (as a scaled quantity), $y \geq 0$ represents the predator population (also scaled), and $a \geq 0$ is a parameter of the model.

a) Explain why the model makes sense. That is, describe how the rate equations in the model correspond to the elements of the biological situation.

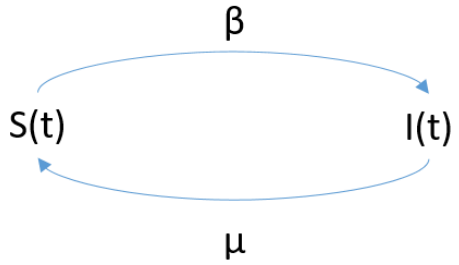
b) Find the fixed points of the model in the biologically relevant quadrant ($x \geq 0, y \geq 0$) for $a < 1$.

c) Characterize the fixed point at (1, 0) by linearizing about it for $a < 1$. What type of fixed point is it?

d) What happens to the predator population for $a > 1$? Rationalize in the context of the model.

Problem 3. A homobivalent antibody binds a soluble homodimeric target protein such that each of the antibody's two Fab domains forms an equivalent interaction with one monomer of the target protein. If the affinity between an isolated Fab and an isolated target monomer is K_d, use an effective concentration approach to derive approximate formulae for the binding constants for formation of the monovalent and bivalent complexes between the full antibody and the full target dimer, K_{d1} and K_{d2}, respectively. Assume that the Fab binding sites range across a distance of zero to r from one another, and that the two cognate binding sites on the target protein also range from zero to r apart. If the antibody is not depleted by binding to the target, what is a formula that describes the total amount of target-bound antibody, as a function of the antibody concentration A.

Problem 4. In this problem, you will analyze a simple model of infectious disease using the methods we used to analyze enzymatic reactions. Our model will consider people in only two states: susceptible to infection (S) and infected (I). The rates for transitions between the states are indicated below:



a) Treat these transformations as if they were chemical reactions and write expressions of the form $nX + mY \rightarrow Z$ for the infectious process by which *I* infects *S* (converting it to *I*), and for the recovery process for the conversion of *I* to *S*

b) Write the rate equations for dS/dt and dI/dt then reduce to a single rate equation for dS/dt in one unknown, *S*. Assume that the total population, *N*, remains constant (no births or deaths).

c) Convert your equation to a dimensionless one:

i. write the units of each term in your answer to the previous question.

ii. write an equation for dimensionless variables *X* and *Y* to replace *S* and *I*. *X* and *Y* should range from zero to one.

iii. One of the constants (and only one) in your equation has units of inverse time. Define the dimensionless variable t^* using the equation $t = t^*/\text{constant}$.

iv. Substitute the dimensionless variables *X*, *Y*, and t^* into your answer to (B).

v. Determine the steady state(s) that is/are possible for *X* and interpret its/their meaning(s) in biological terms.

Problem 5. Consider the peptide sequence QNPTEAELQDMINEVDADGNGT.

a) Explain briefly how Chou and Fasman determined alpha helix and beta sheet propensities of each residue. Using the list of Chou and Fasman-like alpha helical and beta sheet propensities below, translate the peptide sequence into two sequences of Hs, Is, and Bs, one indicating helical propensities and the other indicating sheet propensities. In the key, H denotes a residue with high secondary structure propensity, I denotes a residue with intermediate propensity, and B denotes a structure breaker.

b) Now write down the sequences of peptide residues that could form alpha helix or beta sheet secondary structure. Each type of structure must be a continuous run of four or more residues, consisting of mostly-contiguous Hs terminated by a proline or by one of the following types of sequences BB, IBB, BIB, BBI, or III, where H is a residue with high secondary structure propensity, I is a residue with intermediate propensity, and B is a structure breaker. Which type of secondary structure do you think would be more likely to form and why? What is a spectroscopic technique you could use to probe the conformational preference of the sequence without explicitly determining its structure?

c) Looking again at the list of propensities, you'll see that there are some residues that have both high helical and high sheet preference. Write out their full English names. What is a physicochemical property shared by all of these residues? Pick any two of them and draw their structures.

residue	**A**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**K**	**L**	**M**	**N**	**P**	**Q**	**R**	**S**	**T**	**V**	**W**	**Y**
helix	H	I	I	H	H	B	H	I	I	H	H	B	B	H	I	I	I	H	H	B
sheet	I	H	I	B	H	I	B	H	B	H	H	B	B	H	I	B	H	H	H	H

Problem 6. Kinetics of enzyme inhibition. Consider a setting in which there are two competitive inhibitors of an enzyme, A and B. At most, only one inhibitor can be bound to the enzyme.

a) Beginning with the simple reaction below, derive the Michaelis-Menten form for [ES].

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_{cat}}{\rightarrow} E + P$$

Indicate in your derivation the point at which you use the quasi-steady-state assumption. (Recall that you will need to derive a rate equation for the species that is assumed to be at steady, and that your final equation for [ES] should contain only rate constants, [S] and E_{total}.

b) Modify the enzymatic reaction above to include the two inhibitors by rederiving the Michaelis-Menten form for [ES] in the presence of the inhibitors. Use K_A and K_B for the equilibrium binding constants of the inhibitors to the enzyme

c) Write an equation for the amount of free enzyme, [E], as a function of the other components of the system that is analogous to the one you used in your derivation above.

d) Write an equation for corresponding to the steady-state assumption of part (a) and use your answer from part (C) to simplify it. The answer should be an equation for [ES] containing only rate/equilibrium constants, [S], [A], [B] and E_{total}.

e) Draw a sketch for how the rate of production formation varies with [S] when there is no inhibitor, and in the presence of medium and high concentrations of A. Indicate what constitutes a useful scale for determining a “high” concentration of A.

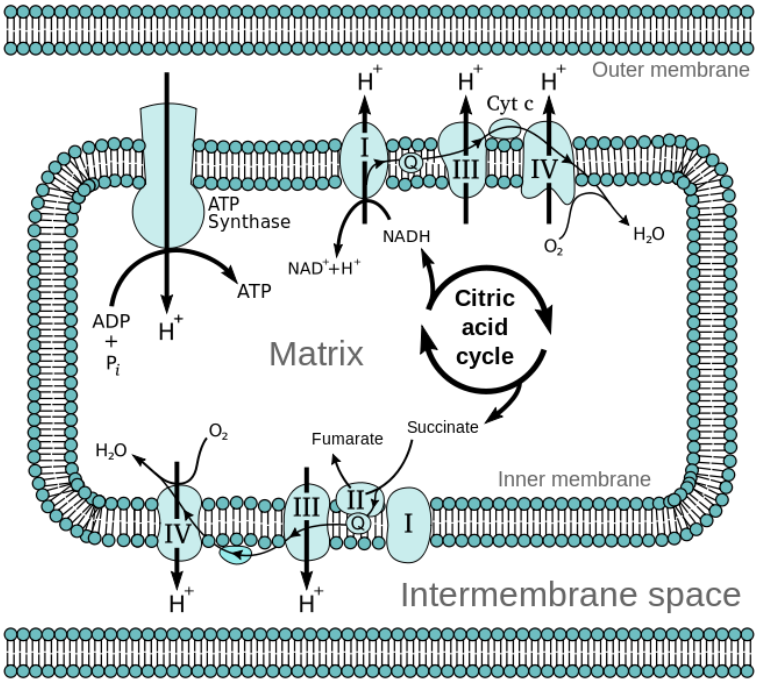
Problem 7. An SPR biosensor experiment is performed with an immobilized protein and flowing soluble ligand. The observed rate constant during the association phase, and the signal output at equilibrium, are given as a function of ligand concentration in the table below.

$[L]_0$ (nM)	k_{obs} (s^{-1})	RU_{eq}
0.14	0.00177	1.63
0.84	0.00186	9.34
2.1	0.00201	21.5
5.60	0.00245	47.1
14.0	0.00350	82.5
35.0	0.00612	118
84.0	0.0122	141
210	0.0280	155
560	0.0718	161
1400	0.177	163

a) Sketch graphs of $\log(k_{obs})$ and RU_{eq} *vs.* $\log([L]_0)$, and use these to determine K_d, k_{on}, and k_{off}. Are these data self-consistent?

b) In a separate experiment, 2 nM of the soluble protein and 0.1 nM ligand are mixed. At equilibrium, what fraction of ligand is complexed with the protein? At what time following mixing will 95% of this equilibrium value be attained?

Problem 8. FBA of chemiosmosis. You may (or may not) recall that mitochondria generate a strong proton gradient across their inner membrane, which is then used to generate ATP as shown below.



a) A full model of mitochondrial metabolism would require more than two hundred reactions. Don't worry, we won't ask you to write those. Rather, write equations for the following reactions, assuming 1:1 stoichiometry for all species:

i. generation of ATP

ii. consumption of NADH (don't forget the generation of the electron)

iii. reduction of O_2 to form water (use the notation H_{in} and H_{out} for the two locations of protons)

b) There are several genetic disorders in which mitochondrial function is impaired. Explain how you could use FBA to explore how ATP production is affected by loss of specific enzymes.

c) Very briefly explain what flux variability analysis is.

d) Some genetic variants alter the catalytic properties of enzymes (k_{cat} and K_m) without inactivating them. Many of these genetic variants have no phenotype of their own. How could you use flux variability analysis to identify pairs of mutations that would have a severe phenotype together but no phenotype on their own?