20.320 Exam 2
Tuesday November 16th
9:35-10:55

Instructions:

1. Write your name of the front cover of the blue book.

2. Answer all questions in the blue books. This exam paper will not be graded.

3. All questions can be answered in at most a few sentences. We will deduct points for excessively long replies, even if they contain the right answer.

4. State all assumptions for each problem.

5. In order to pace yourself please note that the maximum possible score on this exam is 100.

There are FIVE questions on EIGHT pages.

Question 1   (9 points)

An important transcription factor for cell cycle regulation has been shown to bind the following DNA sequences.

    a.   Compute the probability matrix for the sequences shown below.
           (2 points)

    b.   What is the probability of finding a T in the second position?
           (2 points)

    c.   Based on these training data, what is the probability of finding the sequence ATCGTC in a binding site?
           (5 points)

1	2	3	4	5	6
A	C	C	G	T	A
A	T	C	G	A	A
C	T	C	A	T	C
A	T	G	C	T	A
A	C	C	G	T	A
C	C	G	G	A	A
A	T	C	G	T	A
A	C	T	C	T	A

Question 2   (30 points)

You have been asked to help improve production of an enzyme necessary for synthesis of a pharmaceutical compound. The enzyme has been cloned into a plasmid to be produced in bacteria. After purification, the enzyme is highly active, but the yield is very low. Following examination of the raw bacterial lysate, you determine that the protein is actually being produced at very high levels, but most of it is not soluble. You suspect the problem is connected to protein folding.

A. To understand how the protein folds, you conduct an in vitro experiment by chemically denaturing the protein and then rapidly diluting it into an aqueous solution. The final concentration of the denaturant is so low that it has no effect on folding or structure. This process allows the protein to instantly begin to refold.

   A. Based on analysis of the curve below and making simple and reasonable assumptions about the underlying process, write a rate equation for the formation of folded protein as a function of time when the protein is diluted to a low final concentration.

   B. Draw a reaction coordinate diagram indicating the free energy of the unfolded and folded proteins.
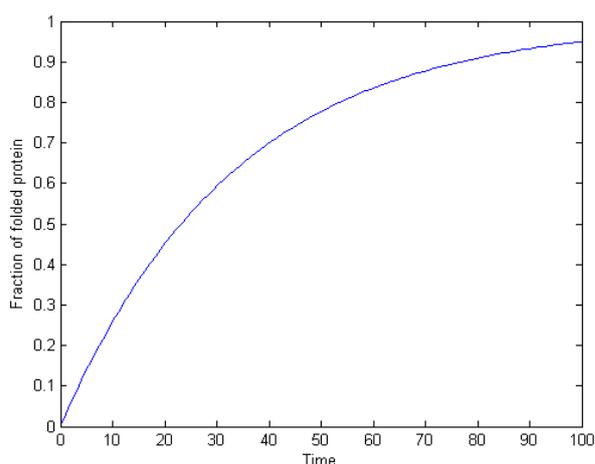
Question 2   (continued)

B. Surprisingly, when the same experiment is performed with the protein present at a higher final concentration, you find that the total yield of folded protein never exceeds 60%. This result occurs despite the fact that you are using the same concentrations of denaturant as before and that you can show that the starting material is completely denatured, as it was in part (a).

  A. Propose an explanation for these observations.

  B. Draw a reaction coordinate diagram similar to that in part (a), but adding the necessary states/transitions to explain the observed behavior.

C. Below we have provided a contact map for the residues in this protein. Identify those residues that are likely to maximally decrease the rate of protein folding.
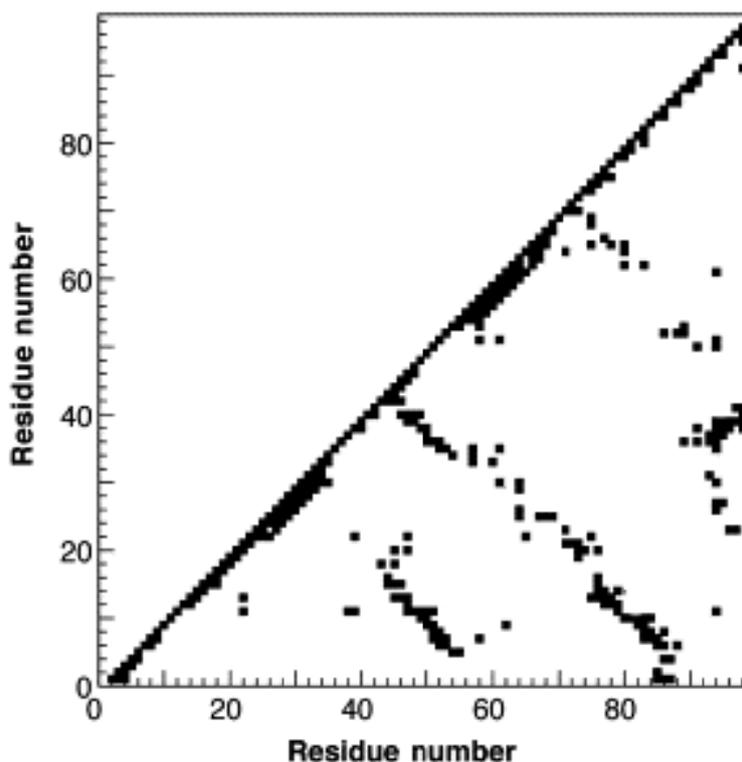
Question 2   (continued)

D. A mutagenesis screen has identified a number of mutations that improve the final yield of protein. These mutations are also observed to increase the rate of folding even at dilute solution. The mutations were mapped to the amino acid sequence and found to all cluster in a small region of the protein, which is shown below.

Original sequence:	G	A	L	A	N	L	A	V	I	I
Improved sequence:	P	R	L	A	D	L	A	R	K	I

Predict the effect of these mutations on the structure and rationalize why they have the observed effect on the final yield of protein. Please keep your answer brief. Excessively long answers will not be graded.

END OF QUESTION 2

Question 3   (20 points)

P53 is an important regulator of the cell cycle. In its activated form it promotes the transcription of several cell-cycle arrest genes. Mdm2 is an E3 ubiquitin ligase, responsible for regulating p53 protein expression. In this problem we consider their interaction in a protein network. We consider here a simplified model: p53 is constitutively expressed at rate α_0 and Mdm2 is constitutively expressed at rate β_0. Both of these proteins are degraded with first-order rate constant γ_0. The E3-ubiquitin ligase activity of Mdm2 is activated by phosphorylation by the serine/threonine kinase Akt. You can assume this phosphorylation occurs at a constant activation rate k_1. Mdm2 can also be dephosphorylated at constant rate k_{-1} by a constitutively active phosphatase (PP1). To simplify this problem, assume that the concentrations of the phosphatase (PP1) and the kinase (Akt) are constant. In its phosphorylated form, Mdm2 can bind to p53 (k_2, k_{-2}) and target it to degradation by the proteasome in a ubiquitin mediated process, releasing phosphorylated Mdm2. You can lump this degradation process using the degradation rate constant k_{deg}.

a) Draw a schematic of the system
b) Give the differential equations underlying this system
c) What is the steady-state value of p53 in the absence of Akt?
d) How do you suspect the presence of Akt will change this steady-state? (One sentence answer)
e) P53 acts as a transcription factor of many genes, including activating transcription of *mdm2*. What type of regulatory motif is represented by the transcriptional effects of p53 on *mdm2*?
f) A scientist has developed a competitive antibody that specifically recognizes the activated phosphorylated form of Mdm2. How would you change your differential equation for d[Mdm2-P]/dt given in b)? You do not need to rewrite the full equation, just give the additional terms. Use k_{on} and k_{off} for the antibody binding kinetic rate constants.

Question 4   (20 points)

Consider two mutually regulating transcription factors as depicted below:
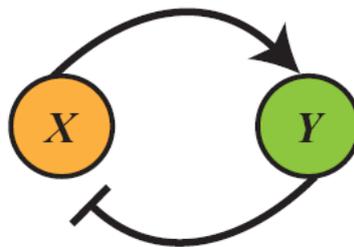
1) Write down a set of ODEs describing the dynamics of this system. **Clearly state all assumptions.**

2) Linearize the system about its critical points neglecting basal rates of synthesis and using the following parameter values: $\beta_y=5/4$; $\beta_x=1$; $K_{D,y}=2$; $\alpha_x=\alpha_y=1$. **Show all work**.

   **Hint:** _You may assume that the physiological concentration of X is always much smaller than the dissociation constant for the interaction of X and the promoter of Y._

3) Analyze the stability of all critical points. Will the system be stable or unstable, and oscillatory or non-oscillatory?

Question 5: (21 points)

Short Answers. Note: You should be able to answer each these questions with three or fewer sentences.

EXCESSIVELY LONG ANSWERS WILL NOT BE GRADED.

A. Why are there some regions of the Ramanchandran plot that are unoccupied?

B. What is the rationale behind the use of foldamers compared to natural proteins?

C. Give two ways in which protein misfolding can contribute to disease

D. The DeltaG of hydrogen bond formation is -6Kcal/mol. How does this compare to the DeltaG of protein folding? Why is there such a difference or lack of?

E. What are the two conditions necessary for oscillations and why?

F. Is the following feedforward motif coherent or incoherent?
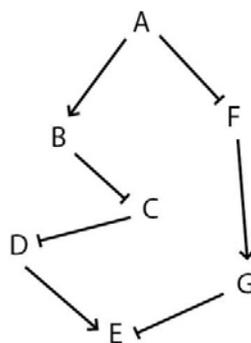
G. In antibody-mediated tumor treatment, is a higher affinity (lower KD) always desired? Why?

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