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University of Toronto
Faculty of Applied Science and Engineering
Division of Engineering Science
Midterm Examination

BME105S – Systems Biology
Thursday February 8, 2007, 4:00 – 5:00 pm

Duration: 60 minutes
Examiners: K. Truong, M. Radisic and W. Stanford

ANSWER ALL QUESTIONS ON THESE SHEETS, USING THE BACK SIDE IF NECESSARY.

1. No calculator and no cellphones are allowed.
2. The number of marks available for each question is indicated in the square brackets []; each portion of a question also shows how many marks are allocated to it.
3. There are two extra blank pages at the end of the test for rough work.

Last Name: Siu
First Name: Kevin
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Tutorial Section: Section 01 (Devin & Emidio)
 Section 02 (Isaac & Elizabeth)
 Section 03 (Elaine & Mark)
 Section 04 (Lorne & Derek)

Total Available Marks:

Question	1	2	3	4	5	6	7	8	Total
Marks Available	2	2	3	3	3	2	1	4	20
Marks Achieved	2	2	2	2	3	2	0.5	4	17.5

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[2] Q1. What types of noncovalent bonds hold together the following solids:

[1] a. Table salt (NaCl), which contains Na^{2+} and Cl^-

ANSWER:

ionic bonding ✓

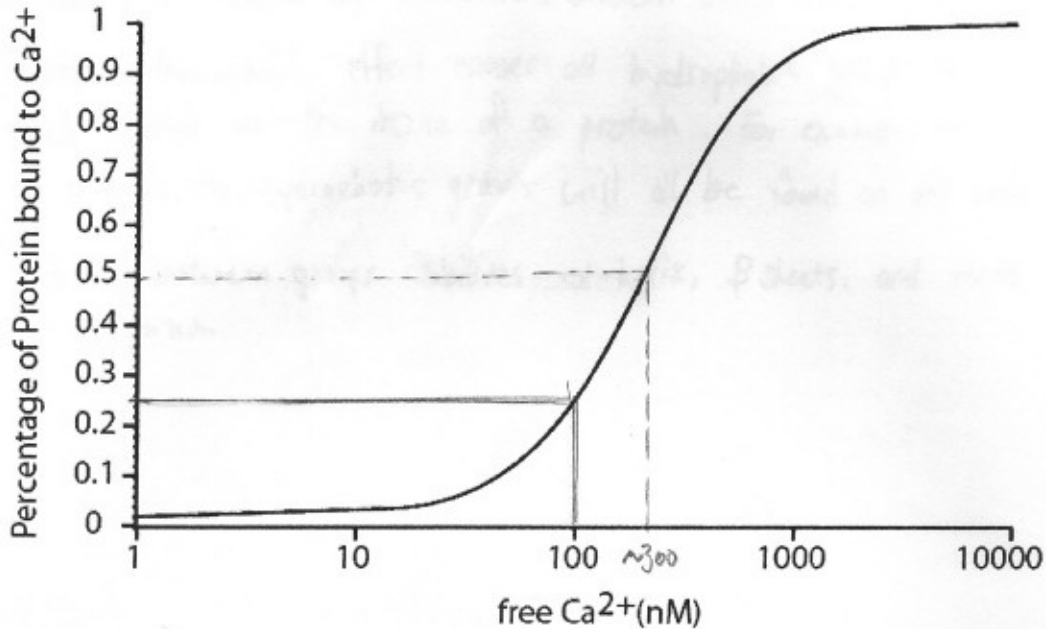


[1] b. Graphite (C), which consists of sheets of covalently bonded carbon atoms

ANSWER:

Van der Waals forces between sheets of carbon atoms. ✓

[2] Q2. A certain protein is known to bind Ca^{2+} . To determine the dissociation constant of this protein to Ca^{2+} , we performed the following experiment. We kept the initial concentration of the protein constant at 1000 nM. Then, we buffered free Ca^{2+} at various concentrations and measured the percentage of protein bound to Ca^{2+} . The below curve was obtained. Estimate the dissociation constant of this protein bound to Ca^{2+} .



ANSWER:

Dissociation constant:

$$K_d = \frac{[\text{Protein}][\text{Ca}^{2+}]}{[\text{Protein}-\text{Ca}^{2+}\text{binding}]}$$

At 100 nM free Ca^{2+} , about 25% of proteins are bound to Ca^{2+} .



$$K_d = \frac{(750 \text{ nM})(100 \text{ nM})}{(250 \text{ nM})}$$

75% is not bound, \therefore 750 nM is dissociated in solution.

2

$$K_d = 300 \text{ nM}$$

[3] Q3. What is the major driving force of protein folding and explain how this relates to the creation of secondary structure?

ANSWER:

Protein folding is caused by interactions between amino acid groups

- Hydrophilic-hydrophobic effect causes all hydrophobic groups to stick together on the inside of a protein. For example, in an α -helix, the hydrophobic groups will all be found on one side
- H-bonding between groups stabilizes α -helix, β sheets, and turns in a protein.

2

[3] Q4. The purification of a specific protein from a heterogeneous mixture of proteins is of great importance to the bioengineer. You have a mixture containing only these five proteins. You have no antibodies available that recognize these proteins. Devise a scheme for separating them; justify your proposed method.

Protein	Molecular weight (kDa)	Description
Phosphorylase <i>a</i>	97.4	This enzyme is involved in glycogen degradation. Phosphorylase <i>a</i> is identical to phosphorylase <i>b</i> , but a serine residue has a phosphate group attached to it, making it the active form of the enzyme.
Phosphorylase <i>b</i>	97.4	Phosphorylase <i>b</i> is identical to phosphorylase <i>a</i> , but it has no phosphate group present on a serine residue; it is the inactive form of the enzyme.
Catalase	57.5	This protein is responsible for the decomposition of hydrogen peroxide.
Histone 2A	14.5	This protein is rich in basic amino acids and has a net positive charge at neutral pH; it has a strong affinity for DNA.
Lysozyme	14.3	This enzyme hydrolyses certain polysaccharides.

ANSWER:

- First separate the proteins by mass using a gel.
 - The middle protein is the Catalase, while there are two on each "side" of the mass spectrum which are not separated (Phosphorylases in one "group" and Histone/Lysozyme in 2nd)
- To separate the Histone 2A and Lysozyme, electrophoresis can be used to separate by charge, since it has a more positive charge than Lysozyme at neutral pH.
- Then, to separate the Phosphorylases, the sample can be phosphorylated to determine which has the phosphate group attached to it.

[3] Q5. You determine that a particular peptide has no secondary structure and has a molecular weight of 2510 daltons. After treatment with chymotrypsin, the peptide is cleaved into three fragments with molecular weights of 395, 1020 and 1095. Note: Chymotrypsin is a protein that cleaves peptides at the carboxyl side of tyrosine, tryptophan and phenylalanine. In contrast, after treatment with trypsin, the peptide is cleaved into two fragments with molecular weights of 790 and 1720. Note: Trypsin is a protein that cleaves peptides at the carboxyl side of arginine and lysine.

[1] a. If the average weight of an amino acid is about 100 daltons, approximately how many amino acid residues are present in the peptide?

ANSWER: ~ 25 residues.

[2] b. The amino acid composition of the peptide was determined and shown in the table below. Given this data, which amino acid may occur at the carboxyl end of the peptide?

Amino acid	%	Amino acid	%
Ala	8.1	Leu	17.0
Arg	0	Lys	8.0
Asn	4.2	Met	0
Asp	3.9	Phe	3.8
Cys	3.8	Pro	0
Gln	3.9	Ser	3.4
Glu	7.8	Thr	3.5
Gly	8.4	Trp	0
His	4.0	Tyr	3.4
Ile	4.1	Val	12.7

ANSWER:

• From chymotrypsin, the peptide is cleaved at Tyr & Phe since there is no Trp. Since it is cut into three fragments, two cuts are made. And since the % of Tyr and Phe are roughly equal, there must have been one of each, making two cuts in the middle of the chain.

• From trypsin, the peptide is cleaved at Lys only since there is no Arg. The trypsin make only one cut, since there are two fragments. But Lys is 8% of the peptide, about twice that of Tyr or Phe. Assuming the masses of these amino acids are roughly equal, then there must be two Lys residues in the peptide.

- Since Trypsin made only one cut, one of the Lys residues must have already been on the C-end of the peptide

\therefore Lys is likely on the carboxyl side of the peptide.

[2] Q6. What two requirements are necessary for DNA to be genetic material?

ANSWER:

1. Must be able to replicate to carry genetic information to daughter cells
2. Must contain information to produce proteins for cellular functions.

2
2

[1] Q7. The amino acid sequences of a yeast protein and human protein having the same function are found to be 60% identical. However, the corresponding DNA sequences are only 45% identical. Account for this differing degree of identity.

ANSWER:

The corresponding DNA may have a different number of introns, or different position of introns, which may cause a discrepancy in the DNA sequences but not in the amino acid sequences since any differences in introns are cut out anyway.

0.5
2/1

[4] Q8. Meselson and Stahl used experiments with ^{14}N and ^{15}N isotopes to determine that DNA replication was semi-conservative (i.e. one strand from the parent and one from the daughter). What result would they have obtained if DNA replication was conservative (i.e. the parental double helix stayed together)? Give the expected distribution of DNA molecules after 1.0 and 2.0 generations for conservative replication.

ANSWER:

