NAME: SID:

BioE 10

Biomedical Physiology for Engineers

Midterm Exam I

Fall 2010

Name	
SID	

Write your name and SID on the top of each page!
If you need extra space, use the back of the sheet.
No computers or electronic communications devices allowed.
One double-sided sheet of notes allowed.
Please limit all responses to "short answer" questions to 1-2 sentences.

SCORE (for instructors only)

Question 1:		/25
Question 2:		/35
Question 3:		/30
Question 4:		/20
Question 5:		/30
Question 6:		/25
TOTAL		/165

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1. Erkki Ruoslahti and colleagues have shown that the pentapeptide CREKA has the ability to "home" to tumor cells by binding fibrin in tumor-associated blood vessels. This property may be useful for the development of contrast agents and drug delivery vehicles that specifically target tumors.

A. Consider the cysteine residue in CREKA. Calculate the fraction of cysteine residues that are negatively charged at pH 7.4 and 9.0. (10 pts)

B. What will be the <u>most common</u> overall charge on the CREKA pentapeptide at pH 10? Justify your answer, but it is not necessary to do calculations. (10 pts)

C. Suppose you are working at a pharmaceutical company whose goal is to manufacture high-concentration solutions of CREKA. For quality control, you assess the purity of your CREKA solutions by mass spectrometry (MS, measures molecular weight). MS of a pure solution of CREKA would show a single peak of 605 g/mol. However, you notice that when the solution is stored for long periods of time in a refrigerator, you begin to see <u>two</u> peaks by MS, one at 605 g/mol and another at 1210 g/mol. If the peptide is stored this way long enough, the 605 g/mol peak disappears entirely and only the 1210 g/mol peak remains. You

are able to solve this problem and maintain a single 605 g/mol peak by storing the solution in a sealed and refrigerated nitrogen-filled chamber. Provide a plausible explanation for this observation. (5 pts)

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2. The protein phenylalanine hydroxylase (PAH) is necessary to metabolize the amino acid phenylalanine. Genetic deficiency in PAH synthesis leads to the disease phenylketonuria (PKU), an autosomal recessive disease that can cause mental retardation and seizures. Early diagnosis of PKU is of such clinical importance that essentially every newborn in the US is screened for it immediately after birth.

A. Rank these amino acids in order of likelihood to be on the exterior surface of the folded protein: Isoleucine, Threonine, Lysine. Justify your answer. (10 pts)

B. The folding of PAH can be thought of as a reaction in which the native (folded) state (N) is in equilibrium with the unfolded (U) state:

$$U \leftrightarrows N$$

Suppose that at T = 25 ˚C, the entropy change associated with the above folding reaction (ΔS) is -1 kcal/mol·K and the enthalpy change for this reaction (ΔH) is -300 kcal/mol. Calculate (1) the free energy change (ΔG) of protein folding at 25 ˚C, and (2) The ratio of folded PAH molecules to unfolded PAH molecules at 25 ˚C. (10 pts)

C. In principle, PKU could be treated by administering PAH protein (enzyme replacement therapy). Explain in 5 sentences or less how you would use *E. Coli* bacteria to manufacture large quantities of human PAH for this purpose, assuming you have the linear cDNA that encodes human PAH. Your answer should incorporate the following concepts: restriction enzymes, plasmids, transformation, positive selection, and bacterial expansion (growth). (10 pts)

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D. Assume that the frequency of mutant PAH allele in the US population is 0.005 and that the frequency of the "normal" allele is 0.995. If the population of the US is 300 million, estimate the number of people who are heterozygous at the PAH locus but are clinically normal (so-called "carriers"). (5 pts)

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3. Continuing with the enzyme PAH from the previous problem: PAH catalyzes the conversion of phenylalanine to tyrosine.

A. Suppose PAH obeys Michaelis-Menten kinetics with a K_m of 300 μM and a V_{max} of 30 μM Tyrosine/hr. Plot the initial reaction velocity (mM tyrosine formed per hour) versus initial substrate concentration (μM phenylalanine), showing clearly on the plot the location of V_{max} and K_m. (10 pts)

B. For an initial phenylalanine concentration of 150 μM, what would the initial reaction velocity be? (10 pts)

C. Suppose you measure the kinetic parameters of PAH in the presence of a small-molecule inhibitor. If the Km of PAH is now measured to be 450 μM and V_{max} is measured to be 30 μM, what type of inhibitor is this (competitive or allosteric/noncompetitive), and where on the PAH molecule would you predict the inhibitor binds? (10 pts)

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4. Cystic fibrosis (CF) is an autosomal recessive disease that occurs due to deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel expressed in respiratory and pancreatic epithelial cells.

A. The CFTR gene consists of approximately 170,000 base pairs and encodes a protein of 1480 amino acids. Estimate the percentage of base pairs in the CFTR gene involved in encoding an amino acid. Name two functions/categories for the non-coding DNA. (5 pts)

B. A portion of the sequence of the coding strand corresponding to normal CFTR is:

5'-ATC ATC TTT GGT GTT-3'

Write the nucleotide sequence of the complementary (noncoding) strand for the sequence given above, with the 5' end of that sequence on the left and the 3' end on the right (per the usual convention). (5 pts)

C. The underlined nucleotides (CTT) are deleted in the most common form of CF. Assuming the A at the 5' end of this sequence is the first base in a codon, write the peptide sequence encoded by (1) the normal (wild-type) CFTR sequence and (2) the deletion mutant. (10 pts)

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5. Consider the following mRNA sequence:

5'-AUC CGG UAC CUA GGA UUC CAC GGU UAC-3'

A. Write the cDNA sequence that would result from reverse transcription of this mRNA sequence. (10 pts)

B. Suppose you then used DNA polymerase to synthesize the complementary strand to the sequence produced in A. You then amplify the resulting double-stranded DNA molecule through 5 cycles of PCR. If you start with 100 molecules of DNA prior to the first round of amplification, what mass of DNA will you have at the end of 5 cycles of PCR amplification? Assume the molecular weight of one base pair is 660 g/mol. (10 pts)

C. mRNA undergoes several key processing steps prior to export from the nucleus. Name two (2) of these processing steps. (10 pts)

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6. Provide short (1-2 sentence) answers to the following questions.

A. DNA can be recovered from remains of biological tissue that is hundreds (or even thousands) of years old, yet RNA will degrade in a matter of hours to days at room temperature. Provide a chemical rationale for this difference in stability. (5 pts)

B. In the world of medical device development, what is a 510(k)? (5 pts)

C. What are the two defining properties that make a stem cell a stem cell? (5 pts)

D. Why is the peptide bond described as "planar"? (5 pts)

E. Define protein secondary structure. (5 pts)

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STRUCTURE AND BIOCHEMISTRY OF AMINO ACIDS

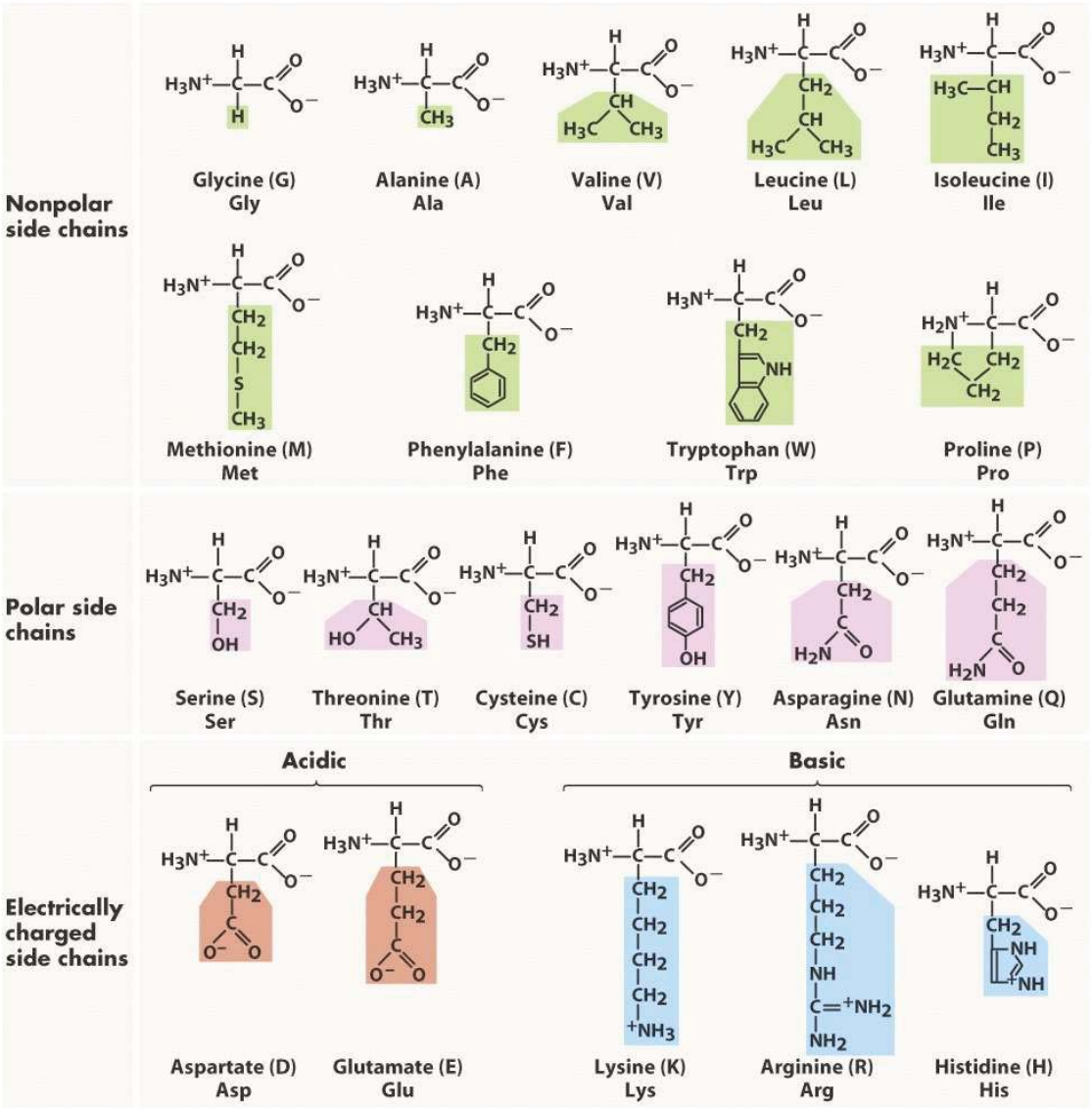

Figure 3-5 Biological Science, 2/e

Approximate relevant pKa's:

Termini:
Carboxy terminus: 2
Amino terminus: 9

Side chains:
Arg: 12
Asp, Glu: 4
Cys: 8
His: 6
Tyr, Lys: 10

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THE GENETIC CODE

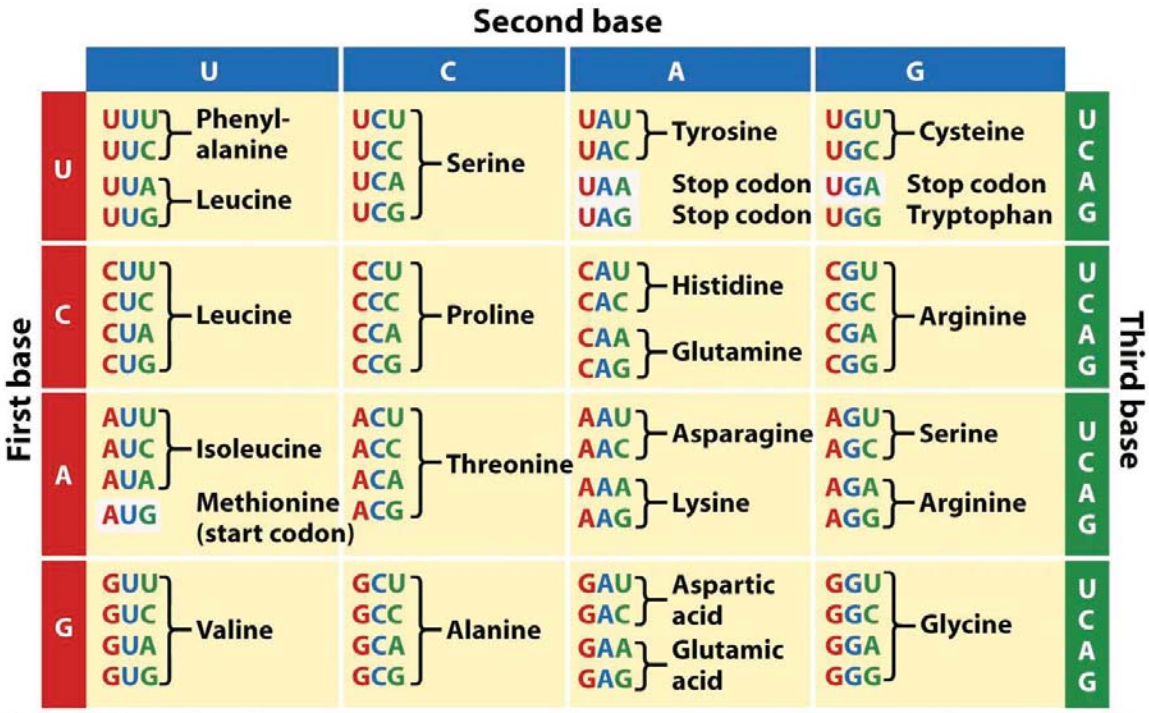

Figure 15-8 Biological Science, 2/e