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)

a) Cysteine: $SH \rightarrow S^- + H^+$ $pKa = 8$

@ pH 7.4: $7.4 = 8 + \log \frac{[A^-]}{[HA]}$

$\log \frac{[S^-]}{[SH]} = 10^{-0.6} = 0.25$ +4

So for every 125 cysteine molecules, 25 are negatively charged or 20%. +1

@ pH 9.0: $9.0 = 8 + \log \frac{[A^-]}{[HA]}$

$\log \frac{[S^-]}{[SH]} = 10^1 = 10$ +4

So for every 11 cysteine molecules, 10 are negatively charged or 91%. +1

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)

b) ionizable groups:

		dominant species @ pH 10	charge
cys: pKa=8	$SH \rightarrow S^- + H^+$	S^-	-1
arg: pKa=12	$NH_2^+ \rightarrow NH + H^+$	NH_2^+	+1
glu: pKa=4	$COOH \rightarrow COO^- + H^+$	COO^-	-1
Lys: pKa=10	$NH_3^+ \rightarrow NH_2 + H^+$	equal amts of $NH_3^+ + NH_2$	0 or +1
ala: pKa = ∅ (nonpolar)	∅	∅	0
carboxy terminus: pKa=2	$COOH \rightarrow COO^- + H^+$	COO^-	-1
amino terminus: pKa=9	$NH_3^+ \rightarrow NH_2 + H^+$	NH_2	+0

So total charge =

$-1 + 1 - 1 + 1 - 1 + 0 = -1$

or

$-1 + 1 - 1 + 0 - 1 + 0 = -2$

answer: -1 or -2

+5 for writing "negative", full points (+10) for summing charges of all contributing groups correctly.

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)

Problem 1c:

We can tell from the MS spectrum that peptides are binding together in groups of 2 (605g/mol goes to 1210g/mol which is double the molecular weight). Therefore, we know that there must be an interaction between the R groups of the peptide and not the termini forming peptide bonds (this would not be limited to the binding of two peptides). SH groups on cysteine molecules form disulfide bonds through the oxidation of sulfhydryl (-SH) groups. Air is a common oxidant that promotes this reaction by oxidizing the -SH. When stored in the presence of nitrogen only, the –SH group is not oxidized and disulfide bonds don't form between the peptides.

Full credit given for mentioning disulfide bonds and the fact that nitrogen prevents this binding reaction from occurring.

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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)

Answer: exterior surface has to be hydrophilic/polar. Lysine has charged R group that ionizes at physiological pH, Threonine has a polar side group (OH) and Isoleucine is non-polar ranking, from most likely to be found on exterior to unlikely would be: lysine, threonine, isoleucine.

- Rubric: 3 points for getting each position right, 1 brownie point if there is some logic in explanation

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)

A. $\Delta G = \Delta H - T\Delta S = \left(-300\frac{kcal}{mol}\right) - (25+273K)\left(-1\frac{kcal}{mol\cdot K}\right) = \mathbf{-2kcal/mol}$

$\frac{[N]}{[U]} \equiv K_{eq}$

$\Delta G = -RTln(K_{eq})$

$K_{eq} = e^{-\frac{\Delta G}{RT}} = e^{-\frac{-2kcal}{0.00199\frac{ckal}{Kmol}\times 298K}} = \mathbf{29.36}$

- Rubric: 5 points for each correct answer, -2 points if there are no units

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)

Answer: insert your linear cDNA of interest into a plasmid using appropriate restriction enzymes, conjugate an antibiotic-resistance gene or a fluorescent gene (depending on desired mode of selection i.e. antibiotic treatment or fluorescent sorting) along with the PAH gene so that they would be co-expressed, insert plasmid into bacteria, transform a bacterial plate with your DNA, antibiotically select so that only bacteria uptaking your

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DNA would survive, grow bacterial culture over night (or longer), lyse bacteria, collect DNA…can then purity and further amplify with PCR

- 2 points for correctly describing each of the concepts.

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)

Answer:

the frequency of carriers should be 2pq = 2*0.005*0.995 = 0.00995. For a population of 300 million, that would correspond to 2,985,000 carriers.

- 5 points for correct answer, 3 points for partially correct answer

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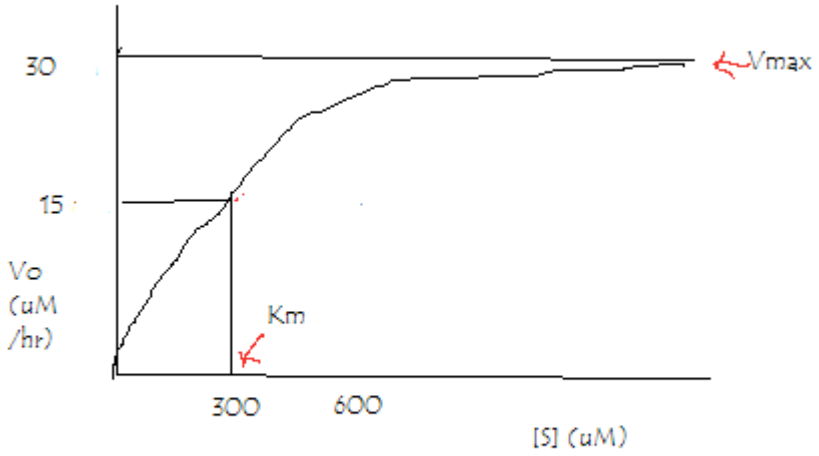
3. Continuing with the enzyme PAH from the previous problem: PAH catalyzes the conversion of phenylalanine to tyrosine.

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)



Points for graph picture, labeling 1) Vmax 2) Km, numbers, units

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

Vo = (Vmax*S)/(Km+S) → (30 uM tyr/hr*150uM)/(300uM+150uM) → **10 tyr/hr**

Points for: Equation (+5, but -3 if missing but plugged in numbers correctly into eq), +5 final answer and units

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)

Competitive inhibitor as Km increases and Vmax stays the same. This inhibitor would bind to the active site of the enzyme, the location where the original substrate was supposed to bind.

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Points for: competitive inhibitor and binding to active site (or any other word used to describe the location).

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)

$$1480\ amino\ acids \times \left[\frac{3\ bp}{1\ amino\ acid}\right] = 4440\ coding\ bps$$

$$\frac{4440\ coding\ bps}{170{,}000\ total\ bps} \times 100\% = 2.61\%$$

+3 for 2.61%, if left out 3bp/aa but other work is reasonable +1/3, if off by a factor of 10 or 100 +2/3, if no % as units +2/3

Possible functions for non-coding DNA:

- genetic "switches" that do not encode proteins, but do regulate when and where genes are expressed
- determine the expression levels of various genes
- determine where transcription factors attach
- features essential to chromosome structure, centromere function and homolog recognition in meiosis
- determine how much of a particular protein gets generated.
- Introns
- Stability
- Structure
- Complementary strand

+1 for each, total of 2.

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)

5' AAC ACC AAA GAT GAT 3'

+5 if correct, if backwards +2/5

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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)

Two acceptable versions:

a. Version 1

i. Normal:

ATC	ATC	TTT	GGT	GTT
Isoleucine	Isoleucine	Phenylalanine	Glycine	Valine

ii. Mutant:

ATC	ATT	GGT	GTT
Isoleucine	Isoleucine	Glycine	Valine

b. Version 2

i. Normal:

AAC	ACC	AAA	GAT	GAT
Asparagine	Threonine	Lysine	Aspartic acid	Aspartic acid

ii. Mutant:

AAC	ACC	AAT	GAT
Asparagine	Threonine	Asparagine	Aspartic acid

+1 for each correct aa, +1 if removed correct bps for mutant, +2/5 if only codons, if backwards either +1/5 or+2/5 depending on the issue

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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)

Answer: 3'-TAG GCC ATG GAT CCT AAG GTG CCA ATG-5'

- 5 points for correct direction (5'→3' to 3'→5'), 5 points for correct sequence (-2 points for a mostly correct sequence)

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 <u>mass</u> 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)

5 cycles, 660 g/mol base pair

$100\ DNA\ molecules \times 2^5 = \times\ 3{,}200\ DNA\ molecules$

Strand had 27 base pairs

$$3{,}200\ DNA\ molecules \times 27 \frac{base\ pairs}{DNA\ molecule} \times \frac{1mol}{6.022 \times 10^{23} bp} \times \frac{660g}{mol}$$

$$= \mathbf{9.47 \times 10^{-17} g}$$

- 10 points for a fully correct answer, 5 points for partially correct answer, - 2 points for lack of units

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

Answer:

- alternate splicing (removing all the introns and join all the exons together or chopping off certain intron/exons and keeping whatever is necessary
- rna degradation (RNA interference (RNAi) pathway, small interfering RNAs = siRNAi, microRNA = miRNAs, small hairpin RNAs = shRNAs)
- the above two processing mechanisms were explicitly described in lecture, another acceptable mechanisms: 5' 7-methylguanosine cap addition, mRNA editing (with an early stop codon, for instance), polyadenylation at 3' end (linking polyA tail to mRNA, protecting mRNA from degradation by exonucleases), etc.

- 5 points per each correct description of processing step

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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)

Full credit: The 2'-OH in ribose is much more chemically reactive (i.e. less stable) than the 2'-H in deoxyribose.

Half credit given to:

- *Double stranded nature of DNA makes it more stable*
- *High degree of compacting and smaller grooves than RNA makes DNA less accessible to enzymes and other molecules that can attach and degrade it.*

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

A 510(k) is a premarket notification for devices regulated as Class I and Class II devices that must be filed with the FDA. It is only applicable to devices that demonstrate substantial equivalence to other Class I or Class I legally marketed devices. Other devices (Class III or not similar to existing devices) must file a PMA (Premarket Approval)

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

- *Self-renewal: the ability to go through numerous cycles of cell division while maintaining the undifferentiated state*
- *Potency: the capacity to differentiate into specialized cell types*

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

- *The peptide bond has a 'partial double bond character' due to resonance structures*
- *Unpaired electrons are somewhere between the N atom and forming a double bond with the C atom in a peptide bond. Since double/triple bonds give no rotational freedom, the bond is therefore planar.*

E. Define protein secondary structure. (5 pts)

- *Secondary structures are structures defined by hydrogen bonding between backbone amino acids and carbonyl groups (full credit)*
- *Examples of structures include: alpha helix and beta sheet (half credit)*

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STRUCTURE AND BIOCHEMISTRY OF AMINO ACIDS

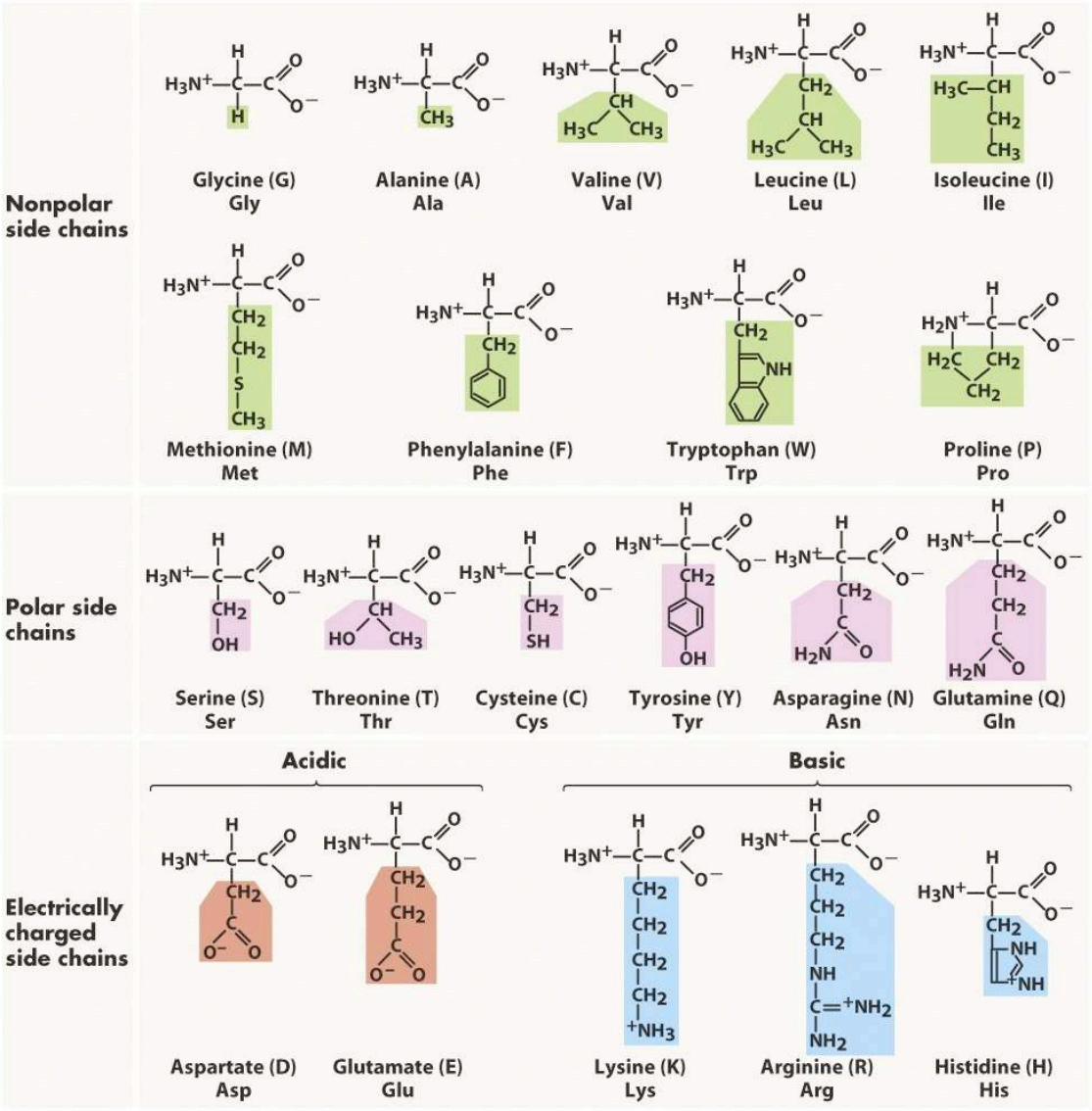

Figure 3-5 Biological Science, 2/e

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

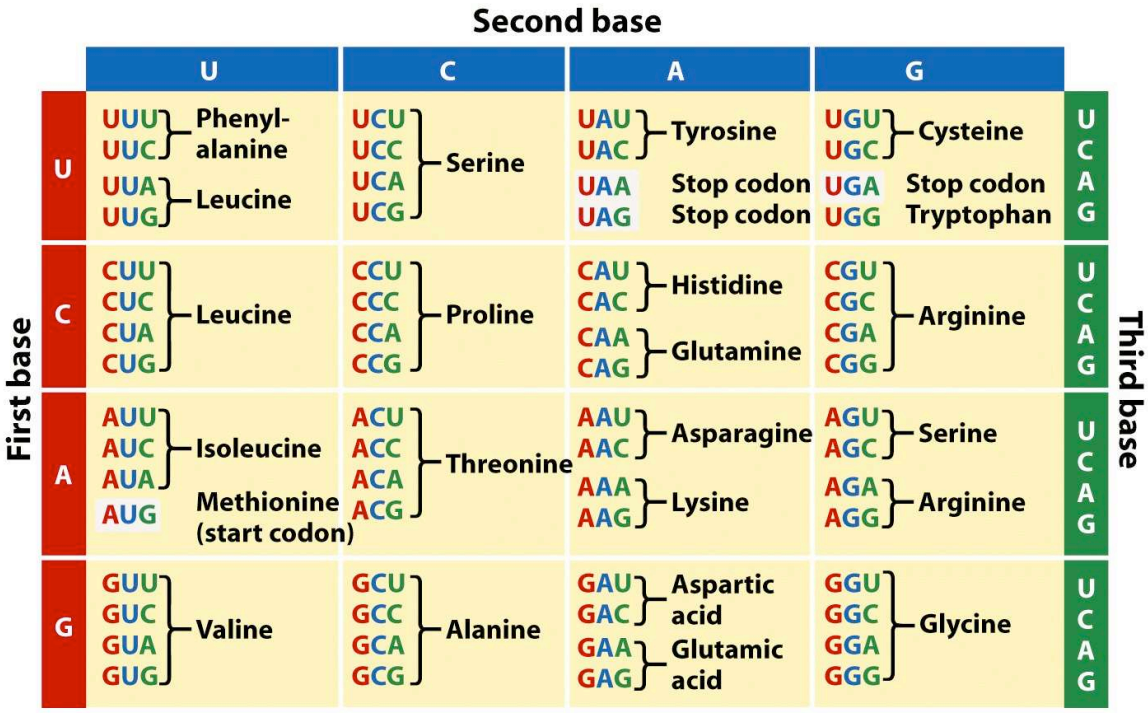

Figure 15-8 Biological Science, 2/e