Biochemistry 463, Summer II **Your Name:** Key

University of Maryland, College Park **Your SID #:** _____

Biochemistry and Physiology **Prof. Jason Kahn**

Exam I (100 points total) **July 27, 2007**

You have 80 minutes for this exam.

N = 46 + 1

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$\Delta S - \Delta H/T \geq 0$ $\quad pH = -\log([H^+])$ $\quad E = mc^2$

$S = k \ln W$ $\quad \Delta G = \Delta H - T\Delta S$ $\quad pH = pK_a + \log([A^-]/[HA])$

$K_a = [H^+][A^-]/[HA]$ $\quad \Delta G° = -RT \ln K_{eq}$ $\quad e^{i\pi} + 1 = 0$

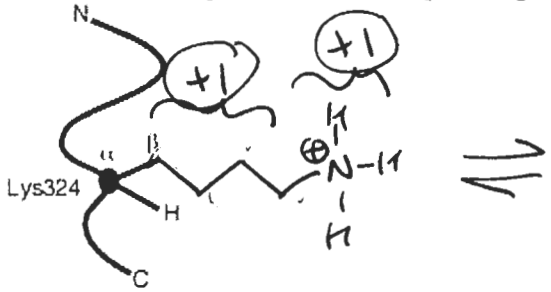
Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. **(20 pts) Acid-base, reactivity, and artistic properties of lysine.**

One of the active site lysines (324) in the enzyme fumarase has a pK_a of 7.1, surprisingly low.

(a; 3 pts) Complete the positively charged lysine side chain on the sketch below and write down the chemical equilibrium corresponding to its deprotonation.



$\rightleftharpoons$

(+1 for any correct eq.)

$Lys\text{-}\overset{\oplus}{N}H_3 + H^+$

(b; 3 pts) It turns out that the active site has a second lysine next to Lys324. The second one has a normal pK_a. How does this help explain the unusual pK_a of Lys324?

The adjacent ⊕ charge destabilizes the proton on Lys-$\overset{\oplus}{N}H_3$ - (+2)

this makes it a stronger acid. (+1)

Score for the page_____

(c; 5 pts) Use the Henderson-Hasselbach equation to calculate the ratio of deprotonated to protonated lysine at both pH 5.9 and at pH 8.1 for Lys324, with its pK_a of 7.1.

$pH = pKa + \log \frac{[Lys\text{-}NH_2]}{[Lys\text{-}NH_3^+]}$ (+2)

$5.9 = 7.1 + \log(\quad)$

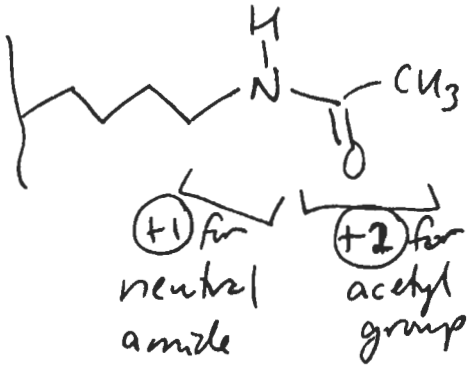
$\frac{[Lys\text{-}NH_2]}{[Lys\text{-}NH_3^+]} = 10^{-1.2}$ (+1)

$= 0.063$ (+1)

$8.1 = 7.1 + \log \frac{[\quad]}{[\quad]}$

$\frac{[Lys\text{-}NH_2]}{[Lys\text{-}NH_3^+]} = 10^{+1} = 10$ (+1)

(d; 5 pts) Because Lys324 has such a low pK_a, it is a much better nucleophile than free Lys. Draw the product of acetylating Lys324. Why is acetyl-lysine important in gene regulation?



Acetylated lysine on histone proteins (in chromatin) is associated with transcriptional activation. (+2)

(e; 4 pts) Write down the name of a molecular visualization program and describe how to select and emphasize one residue using the program.

(+2) Jmol, rasmol, Pymol

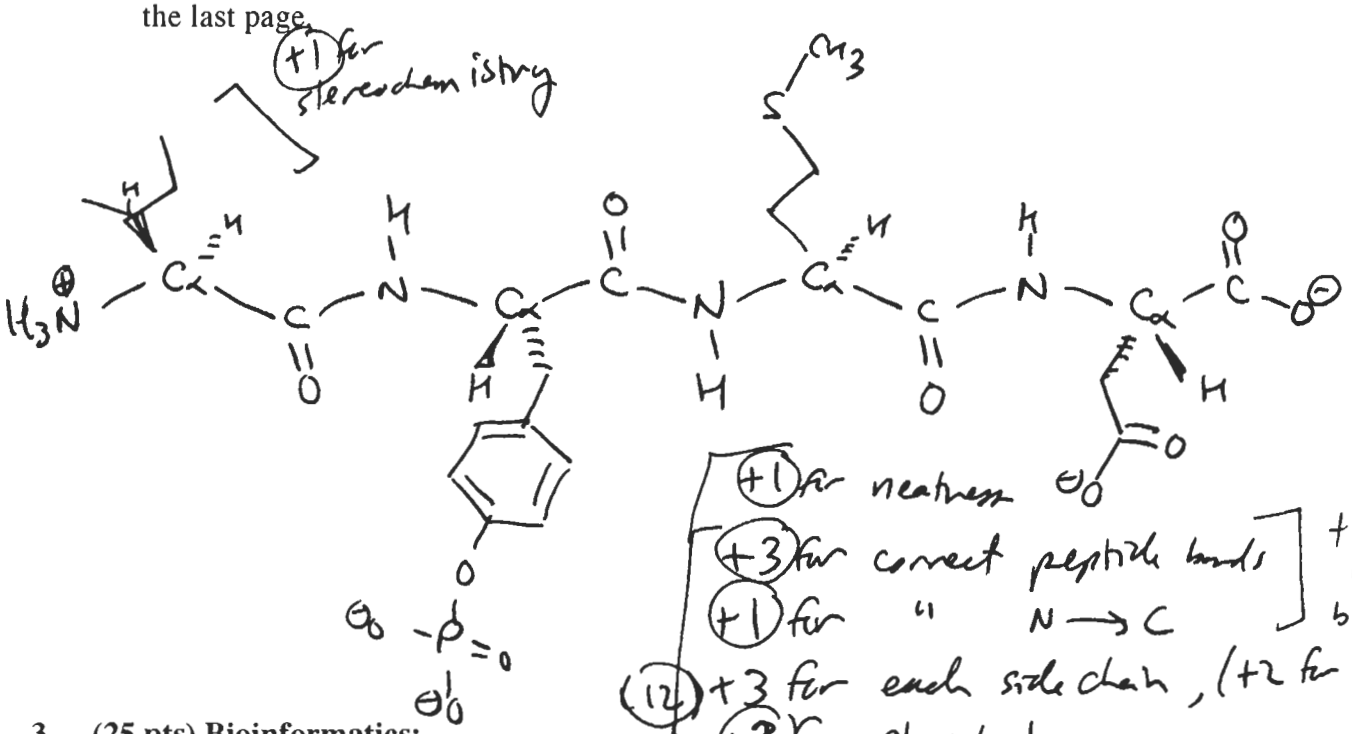
> select (#, #)
> spacefill

+1 each or +2 for reasonable explanation

Score for the page_____

2. (20 pts) Rite of Passage:

Draw the structure of the peptide Ile-pTyr-Met-Asp, including the correct stereochemistry at Cα's and all ionizable groups in their correct protonation states at pH 7. P-tyr = phosphotyrosine, which has pK_a's of ~2 and ~ 5.8, so its charge at pH 7 is –2. If you need more space, the sequence is also on the last page.



3. (25 pts) Bioinformatics:

(a; 12 pts) Briefly describe four steps in a typical bioinformatics/biochemistry "workflow" that a bench biochemist might perform in learning what she can about the likely structure and function of a protein sequence that she has just connected to a function of interest. Don't forget the last and most important step!

new

+3 each for any 3

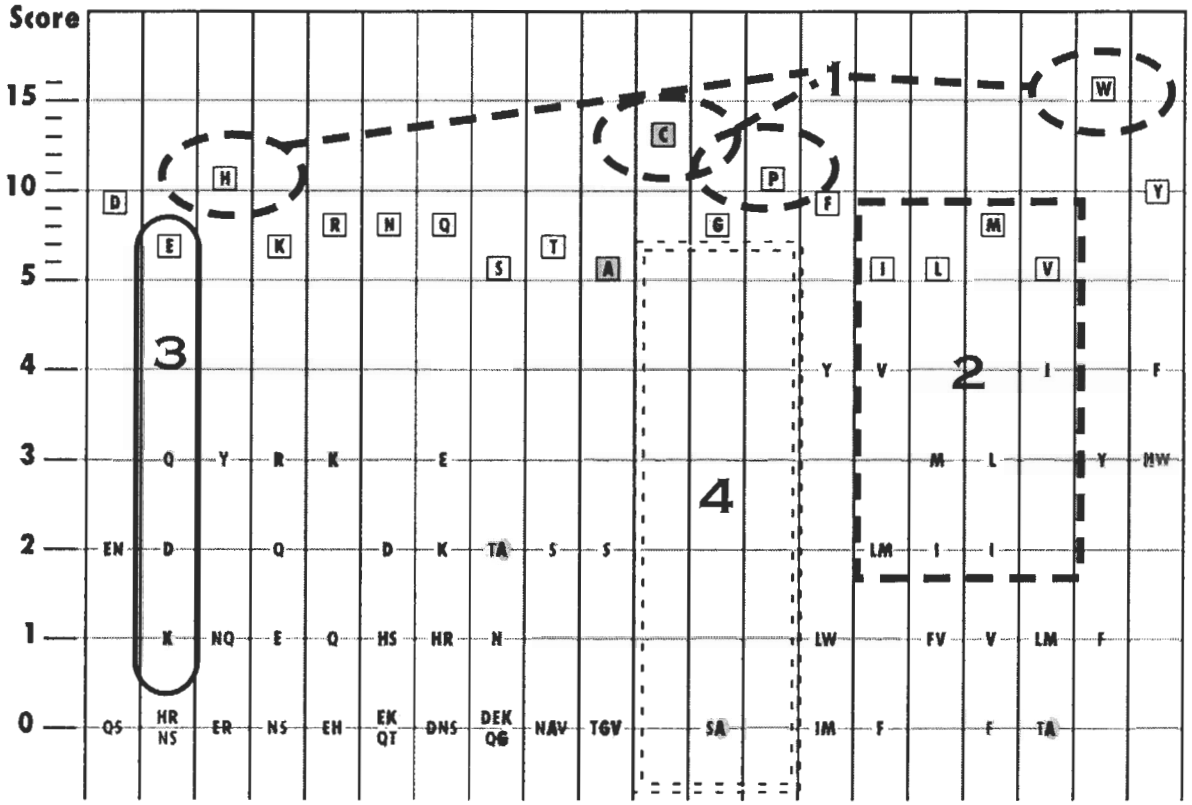
1. Do a BLAST search to identify homologous sequences in the databases.
2. Evaluate the statistical significance of the match
3. Perform PSI-BLAST or other search for family members.
4. Attempt to predict 3-D structure of your protein using any available structures of homologues (threading).
5. Formulate a hypothesis about the function of your protein.

+3

6. Go into the lab and test the hypothesis!

Score for the page_____

(b; 13 pts) Contemplation of the BLOSUM matrix can provide much insight into protein and amino acid properties. Referring to the pictorial version of the top half of the substitution matrix, answer the questions below, whose numbers correspond to the indicated areas on the matrix:



(1) Why are the scores for identical W, C, P, and H residues higher than the scores for other residues? (One answer for all three).

3 These are all rare (+2) amino acids — matching is less likely to be accidental. (+1)

(2) Why are I and V more similar to each other than either is to L, given that L and I are isomers of each other? In general, why do the bulky hydrophobic residues appear to substitute for each other quite readily?

4 - I and V are β-branched (+2), so they fit similarly into sheet/helix structures, as opposed to γ-branched Leu.

- Hydrophobic interactions are non-specific (+2), so these amino acids are more interchangeable than e.g. H-bonding groups.

Score for the page_____

(3) Replacing E with K has a positive similarity score. Why is this initially surprising? Considering where the residues are likely to be located in the protein structure, explain why they do in fact often substitute for each other.

+1 +1 E and K are oppositely charged - seem very different

3 Since they are both likely to be on the surface of the protein and hence solvated, they can both perform the same function of solubilization (or they can switch interactions) +1 +1

(4) What are the special features of C, G, and P that make each of them different from all other amino acids (one feature each).

+1 - C makes disulfides

3 +1 - G is the most flexible

+1 - P is the only cyclic aa, the only one that can make cis peptide bonds.

4. (35 pts) Secondary, tertiary, and miscellaneous structure:

(a; 8 pts) In general, how do chaperones use the free energy available from ATP hydrolysis to improve the success rate of protein folding and avoid aggregation?

8 +2 - They bind to exposed hydrophobic surfaces.

+2 - ATP hydrolysis powers a conformational change that ejects unfolded protein] release an unfoldase

+2 - The protein then has a chance to refold on its own

+2 - The chaperone cavity isolates the mis/unfolded protein from other copies of itself to prevent aggregation.

Score for the page_____

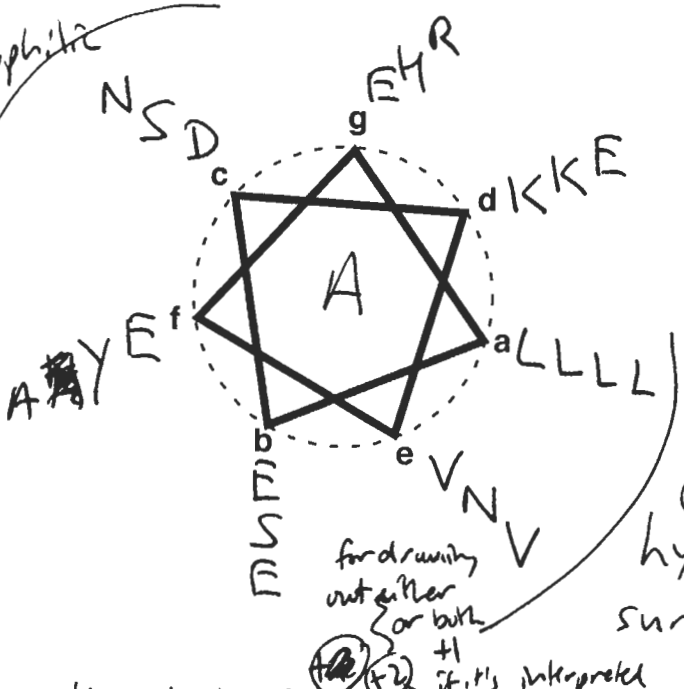
(b; 9 pts) The two sequences below are known to be amphipathic. Which one is part of a beta sheet and which one is an alpha helix? Briefly explain your reasoning. For your convenience seven-pointed stars are sketched below in case you need them.

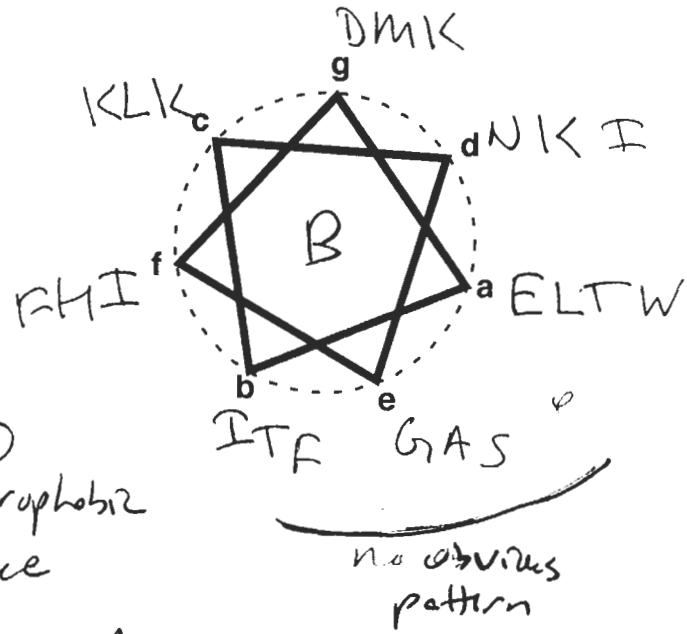
```
                 .(  '  '  '  (  '  (  '  '  (  '
Sequence A:      L  E  D  K  V  E  E  L  S  S  K  N  Y  H  L  E  N  E  V  A  R  L
                 \  \  \  \  |  \  \  \  \  |  \  \  \  \  |  \  \  \  \  |  \  |
                 1  2  3  4  5  6  7  8  9  10    12    14    16    18    20    22
                 (  (  '  (  (  '  (  (  (  '  (  ---> h-philic up
Sequence B:      E  I  K  N  G  I  D  L  T  L  K  A  H  M  T  F  K  I  S  F  K  W
                 |  |  (  |  |  \  \  \  \  |  |  ---> h-phobic down
```

(+1) no obvious pattern

(+1)

hydrophilic





(+1) hydrophobic surface

for drawing out either or both

+1 if it's interpreted

no obvious pattern

- the helical wheel suggests that A is a(n) alpha helix (+2)
- the alternation of polar/nonpolar side chains suggests that B is a β sheet (+2) (+3) (since we are told it's amphipathic)

(of 3) +2 for noting large or branched side chains

(c; 4 pts) Under what conditions is an exothermic ordering reaction thermodynamically favorable? Give an example of an exothermic ordering reaction.

+1 for either or both

(+1) $\Delta H < 0$

(+1) $\Delta S < 0$

$\Delta G = \Delta H - T\Delta S$

ok if implicit

(+1) ice crystallizing from water

folded development

protein folding

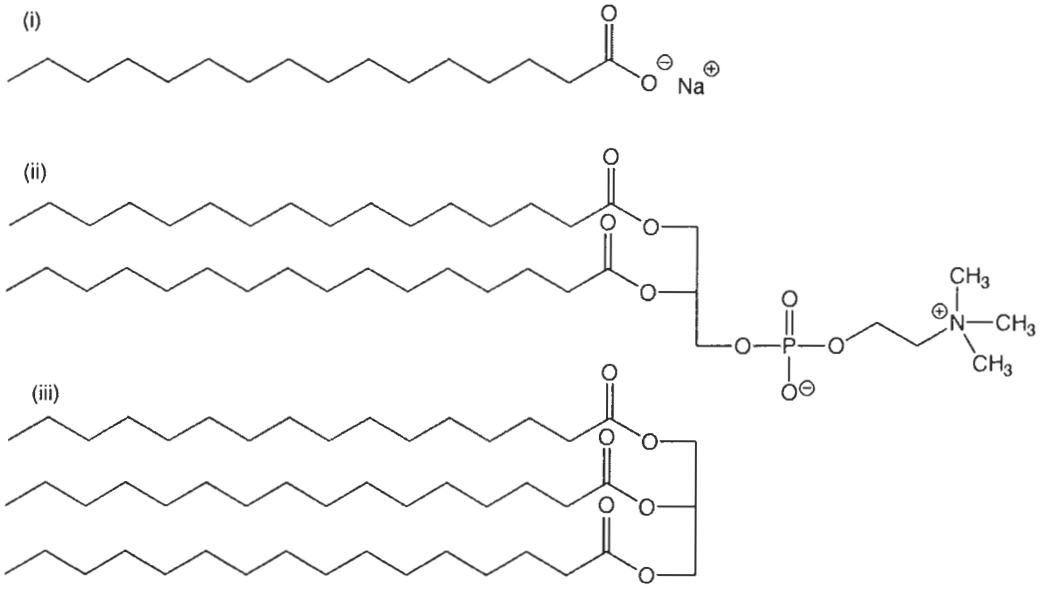
DNA hybridization

ΔG is $\ominus$ at low T, unfavorable at high T

(+2)

Score for the page_____

(d; 9 pts) Explain why molecule (i) below forms a micelle, molecule (ii) forms a bilayer, and molecule (iii) forms a globule. Briefly describe two biological functions for membranes.



(+2 for identifying hydrophobic effect; +2 for identifying that the molecule's shape dictates micelle vs. membrane)

i. is an alkyl chain with a polar head group –

triangular / conical → packs into a sphere

the head groups don't face in!!

ii. is a phospholipid with a rectangular / cylindrical shape –

packs into a sheet –

2 lipid chains / ... / ...

head group vs. 1.

iii. is much more hydrophobic – not amphipathic, so it excludes water completely.

etc.

(+2 each for any two)

1. Creation of concentration gradients for energy transduction, signalling
2. Communication with the outside world
3. Protection / preservation of the cell's contents.

Score for the page_____

[Sequence from question 2: Ile-P-Tyr-Met-Asp]

(e; 5 pts) How does the steric zipper model provide a quite general and yet individualized failure mode for proteins?

(+3) – many different short peptides can form steric zippers – clear that the model can explain many examples of protein mis folding

(+2) – but the zipper is sequence-specific and there are many possible β sheet topologies – therefore there is limited "cross talk" among proteins.

Most people did not know that this question referred to the β sheet model presented on overheads.

Page	Score
1	/6
2	/14
3	/32
4	/7
5	/14
6	/13
7	/9
Total	/100

Page 8 >

Score for the page /15