Key

Biochemistry 5700
Fall, 2011
Exam 1

INSTRUCTIONS:

Do not begin until 10:30 AM. The exam contains 13 questions. Please answer questions completely and legibly. Show your work for partial credit. The exam must be turned in by 11:20 AM. Good Luck!!

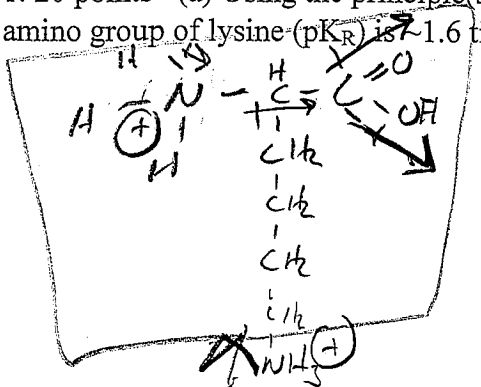
TABLE 3-1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

			pK_a values					
Amino acid	*Abbreviation/ symbol*	M_r	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	*pI*	*Hydropathy index**	*Occurrence in proteins (%)†*
Glycine		75	2.34	9.60				2
Alanine		89	2.34	9.69				8
Proline		115	1.99	10.96				2
Valine		117	2.32	9.62				6
Leucine		131	2.36	9.60				1
Isoleucine		131	2.36	9.68				3
Methionine		149	2.28	9.21				3
Phenylalanine		165	1.83	9.13				9
Tyrosine		181	2.20	9.11	10.07			2
Tryptophan		204	2.38	9.39				4
Serine		105	2.21	9.15				8
Threonine		119	2.11	9.62				9
Cysteine		121	1.96	10.28	8.18			9
Asparagine		132	2.02	8.80				3
Glutamine		146	2.17	9.13				2
Lysine		146	2.18	8.95	10.53		−3.9	5.9
Histidine		155	1.82	9.17	6.00		−3.2	2.3
Arginine		174	2.17	9.04	12.48		−4.5	5.1
Aspartate		133	1.88	9.60	3.65		−3.5	5.3
Glutamate		147	2.19	9.67	4.25		−3.5	6.3

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

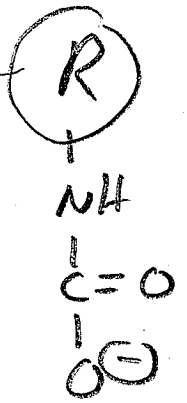
Key

1. 20 points (a) Using the principle(s) discussed in class, explain why the pK_a for the side chain amino group of lysine (pK_R) is ~1.6 times higher than the pKa for the α-amino group.



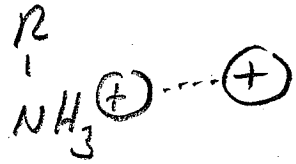
The oxygens on the carboxylic acid group are e⁻ withdrawing. This pulls e⁻ density away from the H atoms bonded to the αN, weakening the bonds, making it easier to remove H+. So the α-amino group is more acidic due to this e⁻ withdrawing effect, & pKa is lower.

(b) Draw the product of the reaction that will occur if CO_2 forms a covalent adduct with the ε-amino group of lysine at pH 7. What type of functional group has been created?



Carbamate

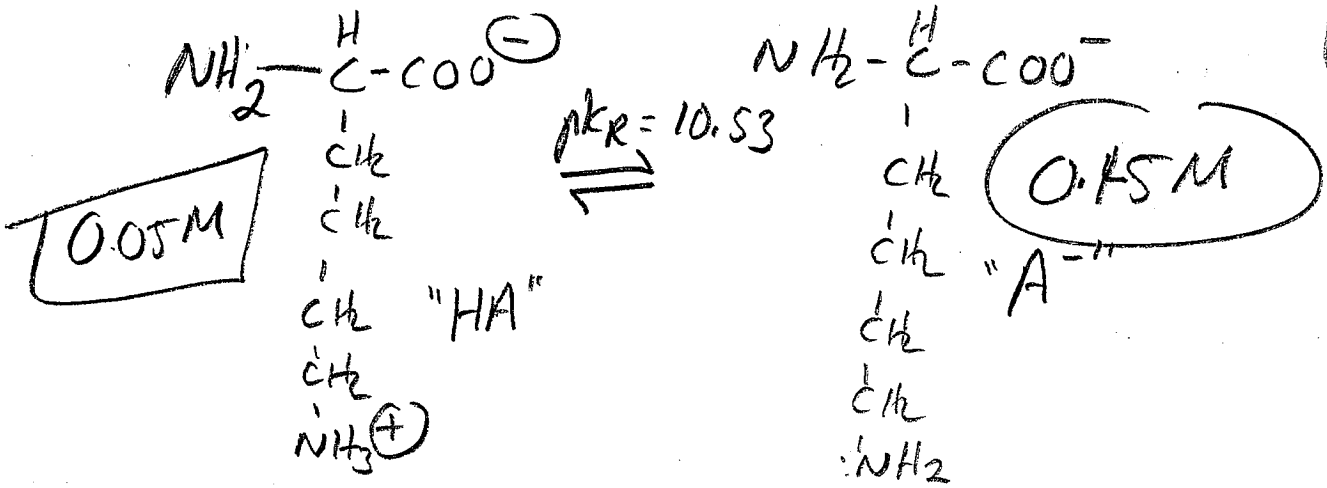
(c) How will the presence of a fixed (i) positive, and (ii) negative charge in close proximity to the ε-amino group affect (i.e. raise, lower, no change) the pK_R value? Briefly explain the basis for each effect.



fixed (+) charge will destabilize protonated amine making it ionize easier to form neutral amine. pKa is lowered.

$R-NH_3^+ \cdots (-)$ fixed (−) charge stabilizes protonated amine making it ionize less readily. pKa is raised

(d) You have a 0.2 M solution of lysine that you are using as a buffer at pH 11. Draw the structures of the two predominant forms of lysine that are in solution at this pH value, and calculate the concentrations of each form (continue on back side of page if needed).



20

1 d (cont)

$[HA] + [A^-] = 0.2$

$pH = pKa + \log \frac{[A^-]}{[HA]}$

$11 = 10.53 + \log \frac{[A^-]}{[HA]}$

$0.47 = \log \frac{[A^-]}{[HA]}$; $\frac{[A^-]}{[HA]} = 10^{0.47} = 2.95$

$[A^-] = 2.95[HA]$

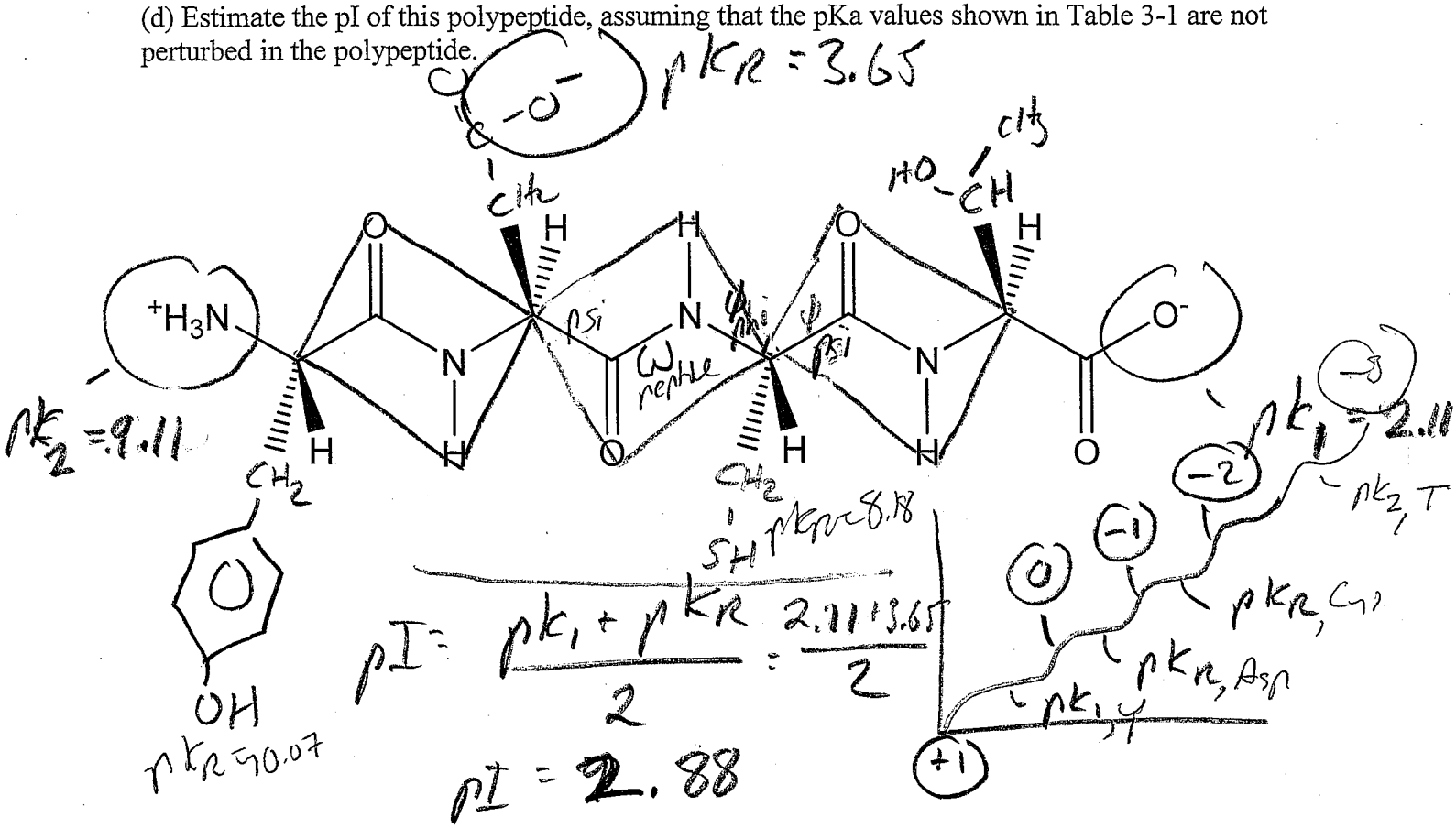
$2.95[HA] + [HA] = 0.2$

$3.95[HA] = 0.2$

$[HA] = 0.051\ M$

$[A^-] = 0.2 - 0.051 = \boxed{0.149\ M}$

2. (12 points) (a) Add the appropriate side chains (using the first "blank" bond provided as a template on each residue as a starting point) to the polypeptide shown below at pH 7.0 to give the sequence: YDCT.
(b) Label the innermost peptide, psi, and phi bonds on the polypeptide.
(c) Draw square units (i.e. four lines connecting four atoms) encompassing the groups that comprise planar units (all four atoms coplanar).
(d) Estimate the pI of this polypeptide, assuming that the pKa values shown in Table 3-1 are not perturbed in the polypeptide.



(3) (**6 points**) Give the 1 and 3 letter codes for an amino acid that matches the indicated property. There may be more than one correct answer, but only name one amino acid for each question.

(a) posttranslationally modified by hydroxylation in collagen	K/Lys or P/Pro
(b) contains two stereocenters	I/Ile or T/Thr
(c) crosslinks chains of alpha-keratin	C/Cys
(d) absorbs significant UV light	W/Trp or F/Phe or Y/Tyr
(e) has a side chain containing a thioether group	M/Met
(f) has a side chain containing a guanidinium group	R/Arg

-4 each

(4) 16 points. You prepare a cell lysate (extract) from a bacterial cell as a source of an enzyme you are interested in studying. Your cell lysate contains 5,000 total mg of protein. You measure the activity of your enzyme in the cell extract and find that 2.0 mg of protein catalyze the conversion of 12 μmol of substrate to product in one minute. You then purify your enzyme to homogeneity using a series of chromatographic columns. Your purified enzyme preparation contains 5.0 total mg of protein. You measure the activity of your purified enzyme and find that 0.0050 mg of protein catalyze the conversion of 20 μmol of substrate to product in one minute. Show your work in answering the following questions.

(a) What is the specific activity of your purified enzyme?

4

$$\frac{20 \text{ umol/min}}{0.0050 \text{ mg}} = 4000 \text{ U/mg}$$

4 (b) What is the % recovery of enzyme activity in going from the crude extract to purified enzyme?

extract: $\frac{12 \text{ U}}{2 \text{ mg}} = \frac{6 \text{ U}}{\text{mg}}$ $\frac{6 \text{ U}}{\text{mg}} \times 5000 \text{ mg} = 30{,}000 \text{ U}$

pure $4000 \text{ U/mg} \times 5 \text{ mg} = 20{,}000 \text{ U}$ $\frac{20{,}000}{30{,}000} \times 100 = 67\%$

4 (c) What overall fold-purification did you achieve in purifying your enzyme?

$$\frac{4000}{6} = 667$$

4 (d) About what percentage of the total protein in your cell extract did your enzyme of interest constitute?

$$\frac{1}{\text{fold}_{\text{pure}}} \times 100 = \frac{1}{667} \times 100 = 0.15\%$$

16

5.(a) (4 points) Your purified protein runs as a single band on **nondenaturing** PAGE. When run on **denaturing SDS**-PAGE, your protein separates into three different bands on the gel. Based on the migration distances of the three bands relative to molecular weight standards, your three subunits have apparent molecular masses of 80,000 (α), 40,000 (β), and 10,000 (γ) daltons. The relative staining intensities of the three bands on your gel, determined by laser densitometry, are **120:100:10** for the **α:β:γ** subunits (these are arbitrary units; a staining intensity number of 100 is twice as intense as a number of 50).
Use this information to deduce the subunit ratio of your newly-isolated protein.

$\alpha_3\beta_5\gamma_2$

4

$\frac{\text{ST}}{M_r}$: $\frac{120}{80} = 1.5$; $\frac{100}{40} = 2.5$; $\frac{10}{10} = 1$

$2(\alpha_{1.5}\ \beta_{2.5}\ \gamma_1) = \alpha_3\ \beta_5\ \gamma_2$

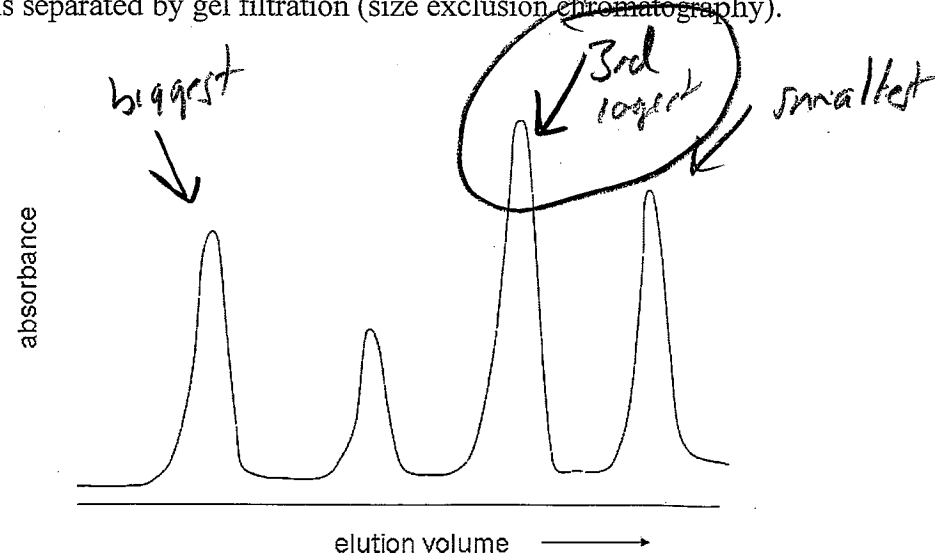
(b) (4 points) The answer you provided above is for the enzyme "protomer"- the lowest whole number ratio of subunits. Briefly explain what additional experiment(s) you would perform to determine the actual number of each subunit present in the true protein multimer.

4 Use size exclusion chromatography with molecular weight standards to determine the native molecular weight. This will be some multiplicate of the protomer, which has M_r = 45,000

e.g. If M_r native is 900,000, then there are 2 protomers in the multimeric unit & it is $\alpha_6\beta_{10}\gamma_4$

(6) (**3 points**) A mixture of 4 proteins is separated by gel filtration (size exclusion chromatography). Indicate with an arrow the peak that corresponds to the protein that is the third largest of the four proteins.

3



(7) (**4 points**) Properly name the following levels of protein structure.

a) A regular, recurring arrangement in space of adjacent amino acid residues in a polypeptide chain	secondary
b) spatial relationship between all amino acids in polypeptide; the 3-D structure of the polypeptide	tertiary
c) A simple combination of a few 2° elements with a specific geometric arrangement	motif
d) a polypeptide chain or part of a polypeptide chain that can independently fold into a stable tertiary structure	domain

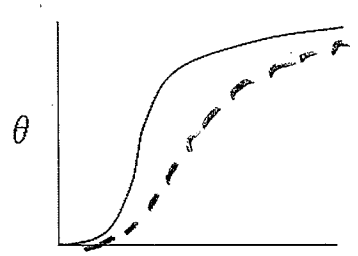
(8) (10 points)

(a) In an alpha helix, an intrastrand hydrogen bond is formed between residue "n" and residue _____.	n + 4
(b)What is the approximate orientation (e.g., perpendicular, parallel, antiparallel, etc) of adjacent α-helices in myoglobin?	perpendicular
(c) What is the approximate orientation (see (b) above) of adjacent α-helices in hemerythrin, a protein built around the four helix bundle motif?	anti parallel
(d) What is the approximate orientation (see (b) above) of adjacent β-strands in a protein built around the α/β barrel motif?	parallel
(e) In a 3 strand beta sheet, how many interchain hydrogen bonds are formed, on average, per amino acid residue on an outside strand and (ii) the inner strand ?	1 + 2 > 0.0 pr
(f) In a 3 strand beta sheet, how many interchain hydrogen bonds are formed, on average, per amino acid residue on a the inner strand ?	2
(g) An alpha helix containing polar residues on one side and nonpolar residues on the other is called _____.	amphipathic
(h) What chromatographic technique can be applied to separate enzymes on the basis of differences in net surface charge?	ion exchange
(i) What chromatographic technique can be applied to purify an enzyme that binds a particular ligand with high specificity?	affinity
(j) What amino acid residue is most commonly associated with formation of a cis peptide bond in a polypeptide?	Pro

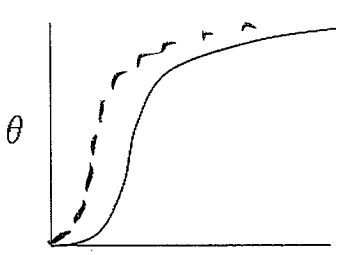
9. (3 points) Fill in the blanks correctly: The collagen helix is ___L___ handed, and ___3___ strands interwine in a ___R___ -handed direction to make a collagen fiber. 3

10. (3 points) Fill in the blanks correctly: The alpha keratin helix is ___R___ handed, and ___2___ strands interwine in a ___L___ -handed direction to make a coiled coil. 3

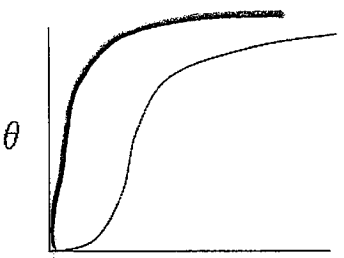
11 (**5 points**) Add a second curve to each of the oxygen dissociation curves for hemoglobin to indicate what effect the indicated change has on the curve. 5



(a) increase $[CO_2]$

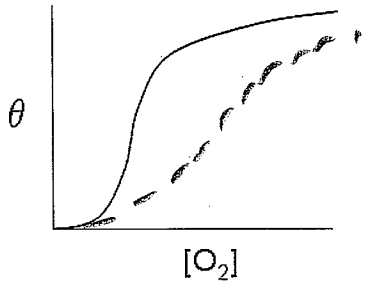


(b) increase pH



(c) completely remove DPG

(d) dissociate subunits and isolate α monomers



(e) increase [DPG]

12. (b) (4 points) Fill in the blanks: The binding of O_2 to heme changes the molecular geometry of the heme from ___Square pyramidal___ to ___octahedral___. The oxidation state of iron in deoxyheme is ___Fe^{2+}___, the oxidation state in oxyheme is ___2+___. O_2 binds to heme on the side closest to the ___distal___ histidine. Deoxy Hb is also referred to as the ___T___ form. Oxy Hb is also referred to as the ___R___ form. 4

15

This is what a really good answer to option 1 looks like.
see next pages for student answers to options 2 and 3

13 8. (6 points) Short essay- answer **any one** of the following questions:

Option 1: Explain the molecular basis for the disease sickle cell anemia, and the physiological consequences resulting from this.

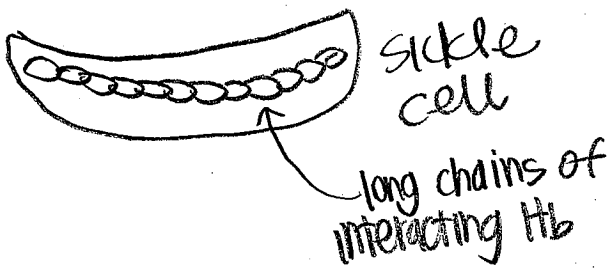
Option 2: Explain how different protein sequences can result in proteins assuming the same overall structure

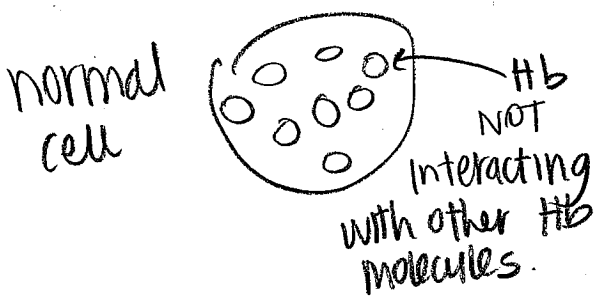
Option 3: Explain the driving forces that result in a globular protein folding into the proper 3-dimensional shape.

Sickle cell anemia results from a surface mutation on the β subunit of hemoglobin - this radical surface mutation changes its ability to interact with other hemoglobin molecules because the mutated residue is hydrophobic and is able to interact with a hydrophobic pocket on other Hemoglobin molecules.

used to be a lysine, now its hydrophobic

- This causes the hemoglobin molecules to aggregate together in long chains. The chain structure makes a different shape in Red Blood cells, forming a sickle shape, which subsequently makes it difficult for RBCs to move throughout the capillaries.





This is what a really good answer to option 2 looks like

13 8. (6 points) Short essay- answer **any one** of the following questions:

Option 1: Explain the molecular basis for the disease sickle cell anemia, and the physiological consequences resulting from this.

Option 2: Explain how different protein sequences can result in proteins assuming the same overall structure

Option 3: Explain the driving forces that result in a globular protein folding into the proper 3-dimensional shape.

2

There are some sites in a polypeptide chain that are highly conserved (identical) in the chains of similar proteins, some sites that are conserved (amino acids must be similar- replace an aromatic AA with another aromatic AA or a polar charged AA with another charged polar AA), and some sites that are not conserved. Because of the properties of the AAs in the sites that are highly and moderately conserved, the polypeptide will form similar intra- and interchain bonds and attractions, resulting in similar folding and structures between the proteins. ~~[illegible]~~

6

This is another example of what a good answer for option 2 looks like

13 8. (6 points) Short essay- answer **any one** of the following questions:

Option 1: Explain the molecular basis for the disease sickle cell anemia, and the physiological consequences resulting from this.

Option 2: Explain how different protein sequences can result in proteins assuming the same overall structure

Option 3: Explain the driving forces that result in a globular protein folding into the proper 3-dimensional shape.

Often if a protein has a change of one amino acid for another the change will not alter shape if the amino acids have similar properties. For example if a protein needs an amphipatic α helix to have proper shape & folding we could sub. a Valine for a leucine on the hydrophobic side w/o much change in structure & overall structure would be retained.

In short A.A. w/similar properties can often be switched w/o changing overall structure

If however very different Amino Acids are switched it can result in dramatic change, e.g. switch aspartate w/lysine change in charge length & branching = Probably totally new conformation

6

This is what a really good answer to option 3 looks like

13 9. (6 points) Short essay- answer **any one** of the following questions:
Option 1: Explain the molecular basis for the disease sickle cell anemia, and the physiological consequences resulting from this.
Option 2: Explain how different protein sequences can result in proteins assuming the same overall structure
Option 3: Explain the driving forces that result in a globular protein folding into the proper 3-dimensional shape.

3: H-bond: Intrachain H-bond can stabilize the α-~~helix~~ helices in globular protein, ~~there~~ other H-bond between chains or solvent may also present

Salt bridge: Salt bridge between ⊕ and ⊖ side ~~chains~~ can stabilize the structure of ~~it~~ the hydrophilic region of protein.

Ion-dipole: Ion-dipole interaction between ⊕ or ⊖ AA residues and water can happened on the protein surface

hydrophobic ~~effect~~ effect: the most important force in folding of globular protein. Nonpolar AAs can aggregate in the nonpolor interior of protein. This aggregation ~~is~~ is due to the increase entropy of water.

6