

Name: _____

Amino acid	pK _{as}
glycine	2.35; 9.78
alanine	2.35; 9.87
valine	2.29; 9.74
leucine	2.33; 9.74
isoleucine	2.32; 9.76
methionine	2.13; 9.28
proline	1.95; 10.64
phenylalanine	2.20; 9.31
tryptophan	2.46; 9.41
serine	2.19; 9.21
threonine	2.09; 9.10
asparagine	2.14; 8.72
glutamine	2.17; 9.13
tyrosine	2.20; 9.21
cysteine	1.92; 10.70
lysine	2.16; 9.06; 10.54
arginine	1.82; 8.99; 12.48
histidine	1.80; 9.33; 6.04
aspartic acid	1.99; 9.90; 3.90
glutamic acid	2.10; 9.47; 4.07

$$F_{\text{unfolded}} = \frac{Abs - Y_F}{Y_U - Y_F}$$

1.) Tris is a commonly used buffer in biochemistry. Write explicit directions on how to prepare 500 mL of 40 mM Tris at pH 8.00. You will start with two jars of solid Tris. One jar contains the acid form; one jar contains the free base form. Do not add anything besides the two forms of solid Tris and water to prepare your solution.

- The pK_A of Tris is 8.30
- Tris-HCl (acid form) is 157.60 g/mole
- Tris base (base form) is 121.14 g/mole

2a.) Draw the chemical structure for the peptide with sequence KRCHEKAD.

2b.) Indicate each peptide bond on your drawing.

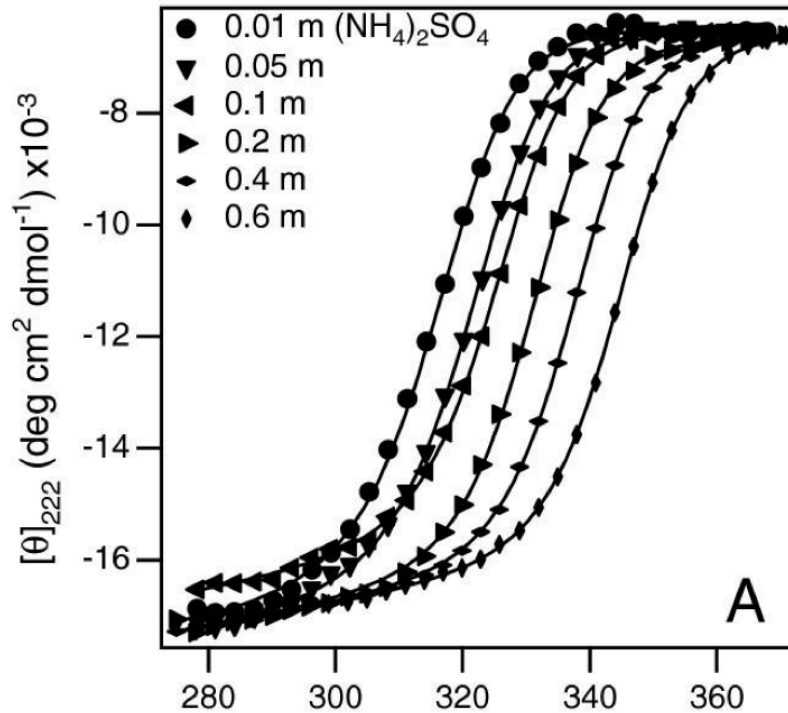
2c.) Indicate each Φ angle on your drawing.

2d.) Indicate each Ψ angle on your drawing.

2e.) What is the most probable bond angle across the peptide bond? Explain your answer.

2f.) What is the isoelectric point of this peptide? (show your work)

3.) Circular dichroism (CD) can be used to detect protein secondary structures. The molar ellipticity of 222 nm light ($[\theta]_{222}$) becomes more negative as the amount of alpha-helices in a protein solution increases. This plot shows molar ellipticity vs. temperature in Kelvin for the DNA binding domain of *lac* repressor.



3a.) If you didn't drink milk for lunch, the *E. coli* within your gut are currently using *lac* repressor to shut down their system for metabolizing lactose. What fraction of the *lac* repressor DNA binding domains is folded at 37 C (310 K)? Assume the ammonium sulfate concentration is negligible (0.01 m). Show your work.

3b.) The melting temperature (T_m) is defined as the temperature at which the fraction of folded proteins is equal to the fraction of unfolded proteins. Determine the T_m at each different ammonium sulfate concentration.

$$T_m^{0.01m} =$$

$$T_m^{0.05m} =$$

$$T_m^{0.1m} =$$

$$T_m^{0.2m} =$$

$$T_m^{0.4m} =$$

$$T_m^{0.6m} =$$

3c.) Does ammonium sulfate increase or decrease the T_m ?

3d.) Does ammonium sulfate increase or decrease the stability of a protein fold?

3e.) Articulate a hypothesis as to how you think ammonium sulfate may exact this effect at the molecular level.