

## USEFUL CONSTANTS:

**R** (gas constant) =  $8.315 \text{ J}\cdot\text{mol}^{-1}\cdot\text{Kelvin}^{-1} = 8.315 \times 10^{-3} \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{Kelvin}^{-1}$   
**(25 °C) T = 298 K; human physiological temperature (37 °C) T = 310 K.**

<b>Ionizable group in peptides and proteins</b>	<b><i>Approximate</i> ("generic") pK<sub>a</sub> in <u>peptides &amp; proteins</u></b>
-amino	8.0
-carboxyl	3.0
-amino	10.0
guanidino	12.0
thiol	8.5
imidazole	6.5
aromatic hydroxyl	10.0
side chain carboxyl	4.0

1. (8 pts) Consider the phenolic hydroxyl group of a particular Tyr residue in a protein. Suppose the hydroxyl group in the *unfolded* protein in aqueous solution, where the group is exposed to H<sub>2</sub>O, has a pK<sub>a</sub> of 10.0.
- A. (4 pts) If that group is found in a **hydrophobic environment** in the interior of the protein when the protein is folded into its native tertiary structure, would you expect the pK<sub>a</sub> of the phenolic hydroxyl to be higher or lower in the folded protein interior than in H<sub>2</sub>O? Explain your reasoning.

**pK<sub>a</sub> of Tyr-OH would increase (proton would be bound more tightly)**  
**reason: unfavorable to have a charge in hydrophobic environment**

- B. (4 pts) Consider that same phenolic hydroxyl group in the *interior* of the folded, active form of the protein. If there were a charged arginine residue also in the interior, close enough to form a salt bridge (ionic bond) with the ionized phenolate anion, would the presence of the Arg residue increase or decrease the pK<sub>a</sub> of the phenolic hydroxyl group (same hydrophobic environment as in part A)? Explain your reasoning.

**pK<sub>a</sub> of Tyr-OH would go back down**  
**reason: Because if it gets rid of its proton (picks up a - charge), it can form a salt link with the Arg guanidinium<sup>+</sup>, thus "neutralizing" both charges. Ionic bonds are stronger in a hydrophobic environment.**

2. (8 pts) Suppose that the function of an enzyme requires that a specific - amino group act as a **hydrogen bond acceptor**. Assume that pK<sub>a</sub> = 8, the "generic" pK<sub>a</sub> for an -amino group. At pH 7.4, **exactly** what FRACTION (PROPORTION, or express as a PER CENT if you wish) of the protein molecules would have that specific -amino group in the form required for it to act as a **hydrogen bond acceptor**? **Show your work.**

**Answer: For α-amino group to be a hydrogen bond acceptor, it must be in BASE form (base is :NH<sub>2</sub>), not the acid (NH<sub>3</sub><sup>+</sup>) form. Calculate base/acid ratio and convert to FRACTION (or percent) BASE. Start with Henderson-Hasselbalch Equation:**

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}]}{[\text{HA}^+]}$$

$$\text{pH} = \text{pK}_a + \log \left( \frac{[\text{R-NH}_2]}{[\text{R-NH}_3^+]} \right); 7.4 = 8.0 + \log \left( \frac{[\text{R-NH}_2]}{[\text{R-NH}_3^+]} \right)$$

$$\log \left( \frac{[\text{R-NH}_2]}{[\text{R-NH}_3^+]} \right) = \text{pH} - \text{pK}_a = 7.4 - 8.0 = -0.6$$

$$\left( \frac{[\text{R-NH}_2]}{[\text{R-NH}_3^+]} \right) \text{ ratio} = 10^{-0.6} = 0.25 \text{ or } 0.25 \text{ base} / 1 \text{ acid}$$

$$\text{fraction base} = 0.25 / (0.25 + 1) = 0.25 / 1.25$$

$$= \underline{0.20} \text{ (i.e., at pH 7.4, } \underline{20\%} \text{ of total } \alpha\text{-amino group would be in the base form, i.e. the hydrogen bond acceptor form)}$$

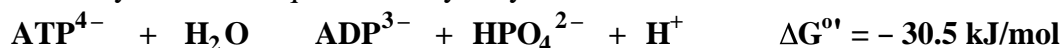
3. (3 pts) Name ONE amino acid whose R group is more hydrophobic than valine. There are several correct answers -- name ONLY ONE (name or abbreviation): Leu or Ile or Phe or Met
4. (3 pts) Which ONE of the amino acids is responsible for MOST of the near ultraviolet (uv) absorbance (280 nm region) of proteins? Tryptophan (Trp)
5. (3 pts) What type of noncovalent bond is mainly responsible for stabilizing secondary structures in proteins? hydrogen bonds

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6. (20 pts) Cells don't "store" large amounts of ATP. Instead of stockpiling ATP, some cells use phosphocreatine (PCr) as a free energy storage compound, and generate more ATP when needed by using phosphocreatine as the phosphoryl group donor. Conversely, when ATP is plentiful they store the extra energy by using ATP to form phosphocreatine. Phosphocreatine is a **phosphoguanidine** compound with a large negative  $G^{\circ}$  for hydrolysis of the P-N bond:



You already know the equation for hydrolysis of ATP:



- A. (4 pts) Assume a temperature of 25°C (298 K) for this entire problem. Write out the coupled reaction for phosphorylation of ADP by phosphocreatine to form ATP. Include charges of the reactants and products (predominant charged forms at pH 7) such that there is charge balance in the reaction, as shown in the equations above, and calculate  $G^{\circ}$  for the coupled reaction. (Show your reasoning.)



Reverse ATP hydrolysis reaction and add the 2  $\Delta G^{\circ}$  values:

$$\Delta G^{\circ} \text{ for overall rxn} = +30.5 \text{ kJ/mol} - 43.0 \text{ kJ/mol} = \underline{\underline{-12.5 \text{ kJ/mol}}}$$

- B. (6 pts) Calculate  $K_{\text{eq}}$  for the coupled reaction. What are its units, and why?

$$\Delta G' = \Delta G^{\circ} + RT \ln\{\text{mass action ratio}\}$$

At equilibrium,

$$\{\text{mass action ratio}\}_{\text{eq}} = K_{\text{eq}}' \text{ and } \Delta G' = 0.$$

$$0 = \Delta G^{\circ} + RT \ln K_{\text{eq}}'$$

$$\Delta G^{\circ} = -RT \ln K_{\text{eq}}' \text{ so } K_{\text{eq}}' = e^{-\Delta G^{\circ}/RT} = e^{-(-12.5 \text{ kJ/mol})/(8.315 \times 10^{-3} \text{ kJ/K}\cdot\text{mol})(298 \text{ K})}$$

$$K_{\text{eq}}' = e^{5.04} = \underline{\underline{155}}$$

For this reaction,  $K_{\text{eq}}$  is unitless;  $K_{\text{eq}} = \{[\text{Cr}][\text{ATP}] / [\text{PCr}][\text{ADP}]\}_{\text{eq}}$   
(The protons can be ignored.)

- C. (6 pts) Suppose that in a neuron, the actual intracellular concentrations are as follows: [ATP] = 2.6 mM, [ADP] = 0.73 mM, [Cr] = 1.0 mM, and [PCr] = 4.7 mM. Calculate the *actual* (physiological) free energy change,  $G'$ , in that neuron at 25°C (298 K) for the phosphorylation of ADP by phosphocreatine to form ATP (reaction in part B). SHOW YOUR WORK AND BE SURE TO STATE UNITS OF ANSWER.

$$\Delta G' = \Delta G^{\circ} + RT \ln\{\text{actual mass action ratio}\}$$

$$\Delta G' = -12.5 \text{ kJ/mol} + 2.4778 \text{ kJ/mol} \ln\{[\text{Cr}][\text{ATP}]/[\text{PCr}][\text{ADP}]\}$$

$$\Delta G' = -12.5 \text{ kJ/mol} + 2.4778 \text{ kJ/mol} \ln\{[1.0 \text{ mM}][2.6 \text{ mM}]/[4.7 \text{ mM}][0.73 \text{ mM}]\}$$

$$\Delta G' = -12.5 \text{ kJ/mol} + 2.4778 \text{ kJ/mol} \ln(0.758) = -12.5 \text{ kJ/mol} + 2.4778 \text{ kJ/mol} (-0.277)$$

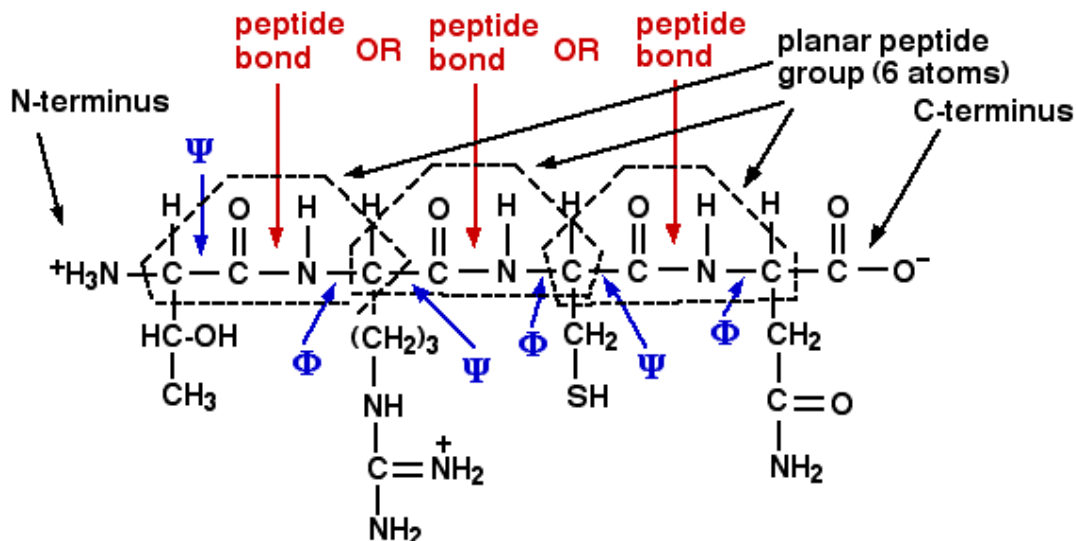
$$\Delta G' = -12.5 \text{ kJ/mol} + (-0.69 \text{ kJ/mol}) = \underline{\underline{-13.2 \text{ kJ/mol}}}$$

- D. (4 pts) Under the conditions in the neuron (part C) would the reaction proceed forward (phosphoryl transfer from PCr to ADP) or in reverse (phosphoryl transfer from ATP to Cr)? **Explain.** (If you were unable to do the calculation in part C, state how you would have determined the reaction direction.)

The reaction would proceed forward (phosphoryl transfer from PCr to ADP) because  $\Delta G < 0$ .

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7. (20 points) The structure of a tetrapeptide is shown below in the form that would predominate at pH 7.0.



- A. (6 pts) What is the sequence of the peptide? (Use full names, 3-letter, or 1-letter abbreviations.)

Thr - Arg - Cys - Asn (or TRCN)  
(or Threonine-Arginine-Cysteine-Asparagine)

- B. (4 pts) Label the **N-terminal** and **C-terminal** ends of the peptide, indicating which is which.

- C. (6 pts) With labeled arrows (  $\uparrow$  ), indicate

1) one peptide bond (label only one) (There are 3 choices.)

2) bonds whose rotations change the  $\phi$  (phi) and  $\psi$  (psi) angles

Label only 1 bond as  $\phi$  (phi) and 1 bond as  $\psi$  (psi). (There are several correct choices.)

- D. (4 pts) Circle 6 atoms that are coplanar (all 6 atoms in the same plane). Circle only ONE group of 6 atoms; you have several correct choices. **Do NOT include any R group (side chain) atoms.** (There are 3 choices.)

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8. (15 pts) Answer the following questions about this peptide: **Glu-Lys-Trp-Phe-Leu-Arg-Val**
- A. (5 pts) List all the **ionizable** functional groups on the peptide with their "generic"  $pK_a$  values, in order of  $pK_a$ , from lowest to highest. Give names or abbreviated names of functional groups, not just names or abbreviations of amino acids. (A table of generic  $pK_a$ s for functional groups in peptides and proteins is on cover sheet of exam.)

name of functional group	generic $pK_a$	(part B) <i>approx.</i> charge on group at pH 1.5 (no calcul'n)	(part C) <i>approx.</i> charge on group at pH 5.2 (no calcul'n)
$\alpha$ -carboxyl COOH	~3	0	-1
Glu-R-carboxyl COOH	~4	0	-1
$\alpha$ -amino $NH_3^+$	~8	+1	+1
Lys $\epsilon$ -amino $NH_3^+$	~10	+1	+1
Arg-guanidinium <sup>+</sup>	~12	+1	+1

- B. (5 pts) List the **approximate** charge at pH 1.5 (the charge on the **predominant** form) for each individual functional group in the third column on the table above, and **calculate the isoelectric point (pI) of the peptide**. (Show where you got your answer.) (**charges in column above**)

**pI = the pH where net charge is exactly zero, i.e. the pH exactly halfway between the 2  $pK_a$  values "surrounding" pH region where net charge is zero. Net charge at low pH (say, pH 1.5) would be +3, so must add 3 equivalents of  $OH^-$  to dissociate 3 molar equivalents of protons to get to net charge of zero on peptide. pI (pH after addition of exactly 3 equivalents of  $OH^-$ ) is halfway between  $pK_a$  of  $\alpha$ -amino and  $\epsilon$ -amino:**

$$pI = (8 + 10)/2 = \underline{9}$$

- C. (5 pts) List the **approximate** charge at pH 5.2 (the charge on the **predominant** form) for each individual functional group in the last column on the table above, and find the **net charge on the whole peptide at pH 5.2?** (Show where you got your answer.) (**charges in column above**)

**Sum of individual charges at pH 7.3 = (-1 - 1 + 1 + 1 + 1) = ~+1**

9. (9 pts) Suppose that a protein can bind either a small molecule inhibitor **I** or its natural ligand **[L]**. Results of 2 experiments in which the fractional saturation  $\theta$  was measured as a function of the concentration of the inhibitor **[I]** and as a function of the concentration of ligand **[L]** are plotted on the graph below.

- A. (5 pts) Determine **from the graph** (not by a calculation) the dissociation equilibrium constants ( $K_d$  values) for the **P•I** complex and for the **P•L** complex. **Show on the graph where your answers came from, and include units of the  $K_d$  values if they have units.**

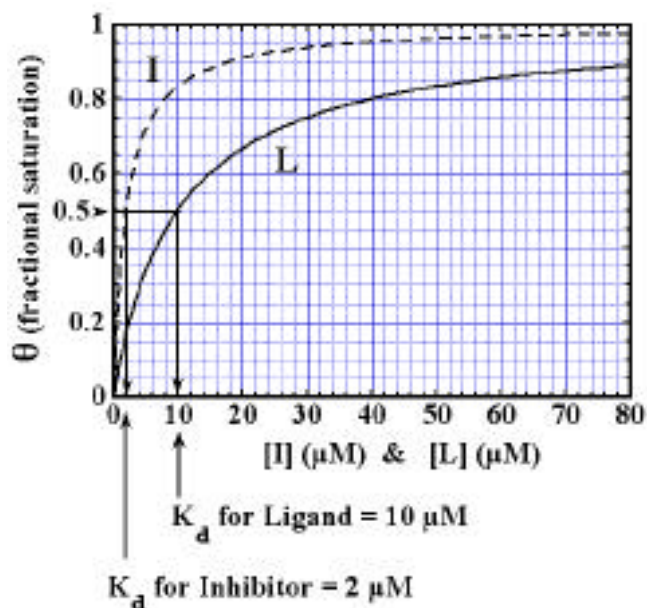
See graph.

$K_d$  for Inhibitor = 2  $\mu M$

$K_d$  for Ligand = 10  $\mu M$

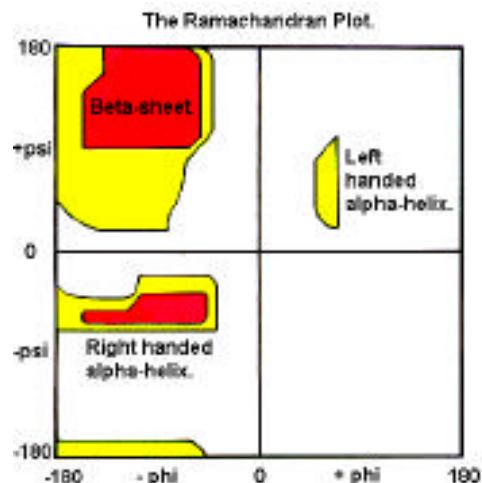
- B. (4 pts) Which of the small molecules binds more tightly to the protein, the **Inhibitor** or the **Ligand**? Justify your answer.

**Inhibitor binds more tightly (has the lower  $K_d$  value).**



10. (11 points)

Here is a Ramachandran diagram for "allowable" ( $\Phi$ ,  $\Psi$ ) angles for Ala residues in proteins.



The following table gives values for the phi ( $\Phi$ ) & psi ( $\Psi$ ) angles of residues 24 to 54 of a protein:

res. #	amino acid	( $^{\circ}$ )	( $^{\circ}$ )	res. #	amino acid	( $^{\circ}$ )	( $^{\circ}$ )
24		-60	147	40		-70	-18
25		-49	-32	41		-89	-36
26	Gly	-67	-34	42		-137	142
27		-58	-49	43		-142	140
28		-60	-42	44		-141	129
29		-57	-46	45		-135	132
30		-62	-44	46		-102	83
31		-61	-48	47	Pro	-60	-33
32		-59	-51	48	Gly	-91	-4
33		-60	-44	49	Ser	-45	122
34		-63	-43	50		-137	138
35		-61	-51	51		-136	142
36	Pro	-55	-8	52		-143	135
37		68	27	53		-138	133
38		79	6	54	xxx?	67	-179
39		-90	109				

**Hints:** Right-handed  $\alpha$ -helices have  $\Phi$ ,  $\Psi$  angles of about  $-57^{\circ}$ ,  $-47^{\circ}$ .  
 Parallel  $\beta$ -sheets have  $\Phi$ ,  $\Psi$  angles of about  $-119^{\circ}$ ,  $+113^{\circ}$ .  
 Antiparallel  $\beta$ -sheets have  $\Phi$ ,  $\Psi$  angles of about  $-139^{\circ}$ ,  $+135^{\circ}$ .  
 Reverse turns: (Type I  $\beta$  bend):  $\Phi_2, \Psi_2$  (2nd residue in turn) =  $-60^{\circ}$ ,  $-30^{\circ}$ ;  
 $\Phi_3, \Psi_3$  (3rd residue in turn) =  $-90^{\circ}$ ,  $0^{\circ}$ .  
 Pro has a limited range for  $\Phi$ , about  $-60^{\circ} \pm 25^{\circ}$ .

- A. (3 pts) What is the probable secondary structure of residues 27-35?  
**Most likely a right-handed  $\alpha$ -helix ( $\Phi$ ,  $\Psi$  angles all similar, and all close to theoretical values for right-handed  $\alpha$ -helix, which are  $-57^{\circ}$ ,  $-47^{\circ}$ )**
- B. (5 pts) What are the probable secondary structures of residues 42-53?  
**Most likely a 2-stranded antiparallel  $\beta$  sheet, with a Type I turn between the 2 strands. Residues 42-45 have close to theoretical antiparallel  $\beta$  structure angles ( $-139^{\circ}$ ,  $+135^{\circ}$ ), the Pro-Gly sequence would seem to be residues 2 & 3 of a Type I turn, and residues 50-53 again have theoretical antiparallel  $\beta$  structure angles.**
- C. (3 pts) What is the probable identity of residue 54? (Explain.)  
**GLY; because no non-Gly residue can adopt those values of the  $\Phi$ ,  $\Psi$  angles.**

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