Name _____

Chem 352 - Spring 2013 - Exam I

Potentially useful information:

pK_a values for ionizable groups in proteins: (*α-carboxyl*, 3.1; *α-amino*, 8.0; *Asp* & *Glu* side chains, 4.1; *His* side chain, 6.0; *Cys* side chain, 8.3; *Tyr* side chain, 10.9; *Lys* side chain, 10.8; *Arg* side chain, 12.5)

$R = 8.314\ J/(mol \bullet K) = 0.08206\ (L \bullet atm)/(mol \bullet K)$

The questions in this exam all focus on the enzyme phosphofructokinase-1, which is one of the enzymes found in the glycolytic pathway.

1. This enzyme contains two identical subunits comprising 319 amino acid residues each.

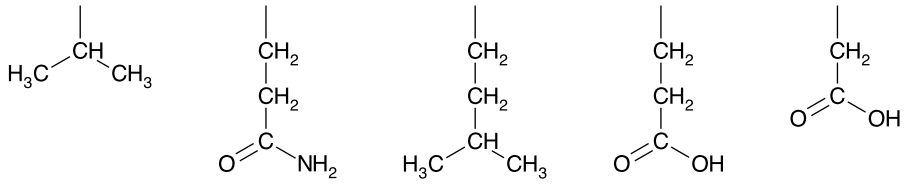
   a. Give an approximate molecular weight for one of these subunits; _____

   b. What does the presence of multiple subunits tell you about the the hierarchical structure of phosphofructokinase? _____
   Explain:

2. The side chains for the amino acids residues from position 200 to 204 in each subunit are shown below, but not necessarily in the same order as is found in phosphofructokinase-1.

   a. Using three-letter codes, identify each of these residues:

   A. _____ B. _____ C. _____ D. _____ E. _____



   b. Which of these is aliphatic? (*Circle all that apply.*) A B C D E
   c. Which of these can hydrogen bond to water? (*Circle all that apply.*) A B C D E
   d. Which of these is aromatic? (*Circle all that apply.*) A B C D E
   e. Which of these is charged at neutral *pH* values? (*Circle all that apply.*) A B C D E
   f. Which of these is hydrophobic? (*Circle all that apply.*) A B C D E
   g. Which of these is basic? (*Circle all that apply.*) A B C D E

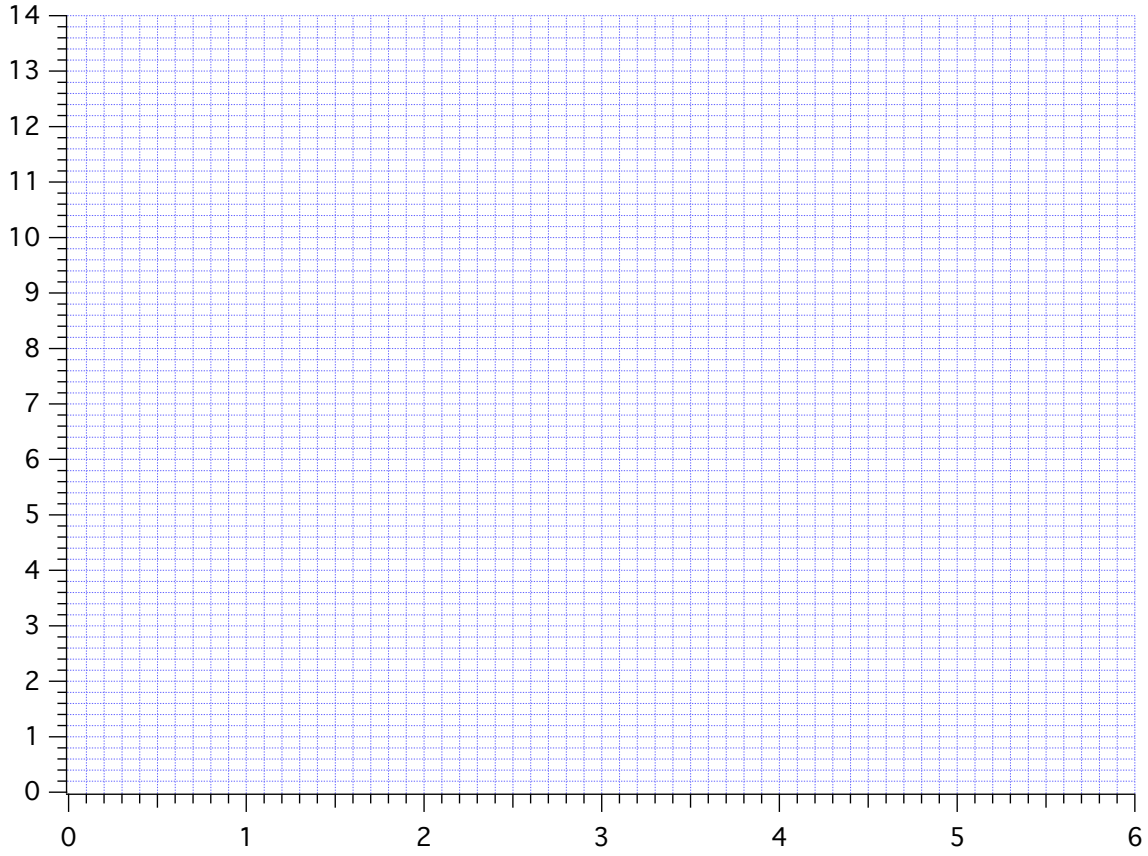
3. The sequence for residues 200 to 204 in each of the phosphofructokinase-1 subunits is Asp-Leu-Val-Gln-Glu. In the space below, draw the chemical structure for this sequence in its predominant charged state at *pH* 7.4. Keep in mind that this sequence is not an isolated pentapeptide, but is part of a larger polypeptide.

a. On your structure, *label* one example each, for a φ, a ψ and an ω bond.

b. On your structure, *label* one example of a *peptide bond*.

c. On your structure, *circle and label* three different functional groups.

4. *Sketch the titration* curve (*pH vs* Equivalents) for this sequence of amino acids. (Be sure to label the axes.):



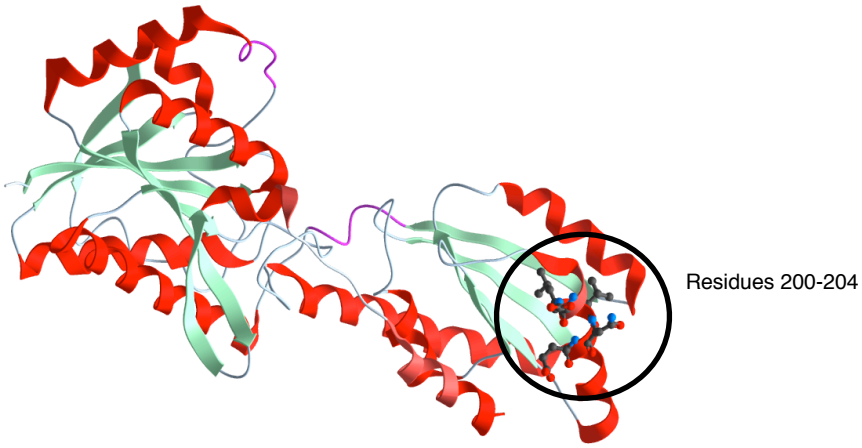
a. What is the *isolelectric pH* for this sequence? _____

b. Is this sequence most likely to be found in the interior or on the surface of the folded protein?

_____

Explain:

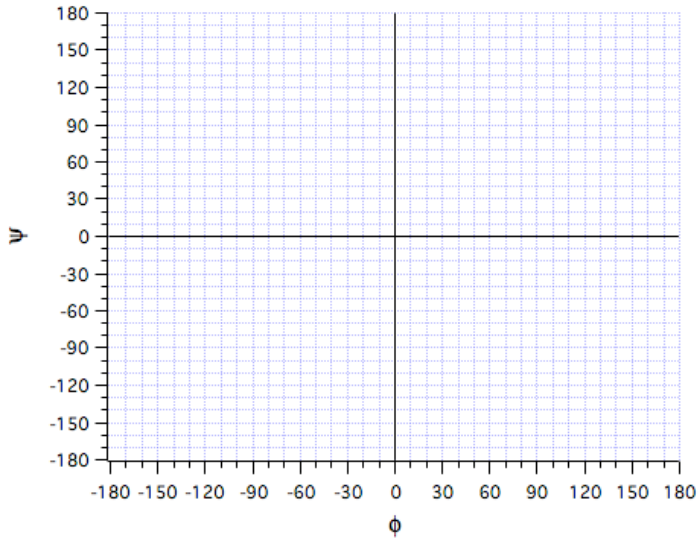
5. Shown below is a ribbon model for the structure of one of the subunits of phosphofructokinase-1, with the location of the pentapeptide you drew circled and labeled.



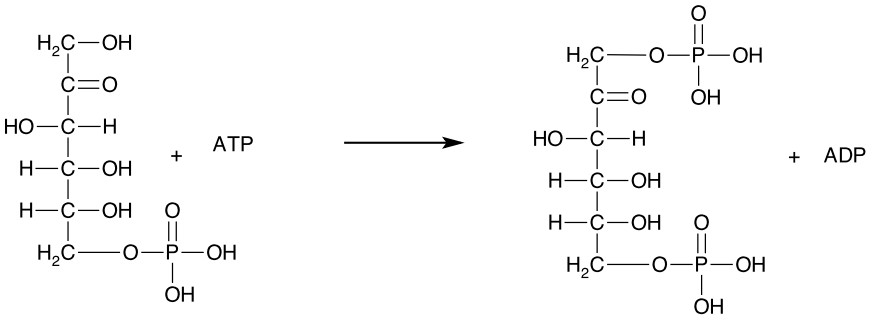
a. What type of secondary structure does the pentapeptide participate in? _____

b. Describe the role that this type of protein secondary structure plays in overall folding of this protein.

c. On the Ramachandran plot shown below, *circle the area* where you expect to find the φ and ψ angle pairs for this pentapeptide sequence within phosphofructokinase-1.



6. The reaction catalyzed by phosphofructokinase-1 is shown below:



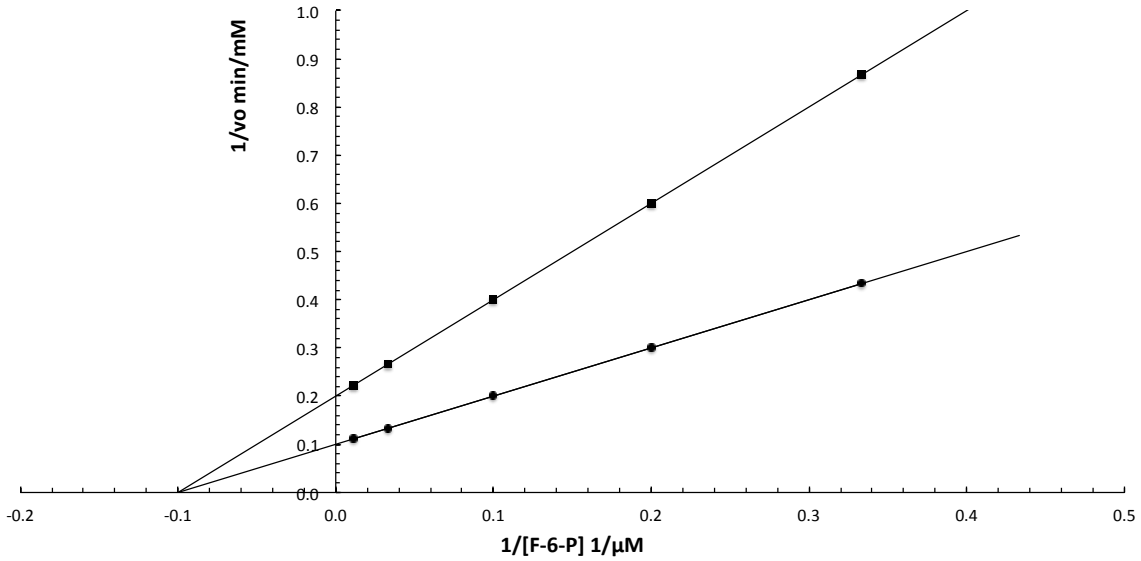
fructose-6-phosphate fructose-1,6-bisphosphate

a. Which of the six classes of enzyme catalyzed reactions does this reaction belong to?

_____

b. The standard free energy change for this reaction is -15.6 kJ/(mol•K). Is this reaction favorable under standard state conditions? _____
Explain:

7. The kinetics of the phosphofructokinase-1 reaction were measured with varying concentrations of fructose-6-phosphate ([F-6-P]) in the presence of saturating levels of ATP. The kinetics were measured using a phosphofructokinase-1 concentration of 1.40 μM, and in the absence and presence of a 10 mM citrate, a known inhibitor of phosphofructokinase-1. The results of these experiments are shown graphically below, where v_o represent the initial reaction rate for a given concentration of fructose-6-phosphate.



a. Base on these data, what is the *turnover number* for phosphofructokinase-1 when saturated with both F-6-P and ATP. _____
Explain:

b. Can the catalytic efficiency of phophofructokinase-1 be improved further, or has this enzyme already attained "catalytic perfection"? _____
Explain:

8. Determine the amino acid sequence of an oligopeptide fragment that was obtained from phosphofructokinase-1 after treatment with the proteolytic enzyme *trypsin*:

- The first amino acid released from an Edman degradation of the intact oligopeptide is **ser**.
- Treatment of the oligopeptide with the proteolytic enzyme *chymotrypsin*, follow by Edman degradation of the resulting fragments, produced the following three fragments:
  asp-gly-tyr-leu-gly-leu-tyr
  ser-ala-leu-thr-glu-gly-leu-glu-val-met-gly-ile-tyr
  glu-asp-arg
- Treatment of the oligopeptide with *cyanogen bromide*, follow by Edman degradation of the resulting fragments, produced the following two fragments:
- **gly-ile-tyr-asp-gly-tyr-leu-gly-leu-tyr-glu-asp-arg**
  ser-ala-leu-thr-glu-gly-leu-glu-val-met