

Sample Exam I – Protein Structure

1. Primary Structure and amino acid chemistry

The peptide hormones vasopressin (ADH) and oxytocin each contain only nine amino acids. Vasopressin is an antidiuretic: even at low doses it controls the resorption of water by the distal tubules of the kidneys and regulates the osmotic content of blood. At high doses it can affect blood pressure. Oxytocin stimulates contraction of uterine smooth muscle. It is secreted during labor to effect delivery of the fetus. Oxytocin is therapeutically delivered to accelerate contractions in a labor that is not progressing. The primary sequences of the two peptides are shown below.

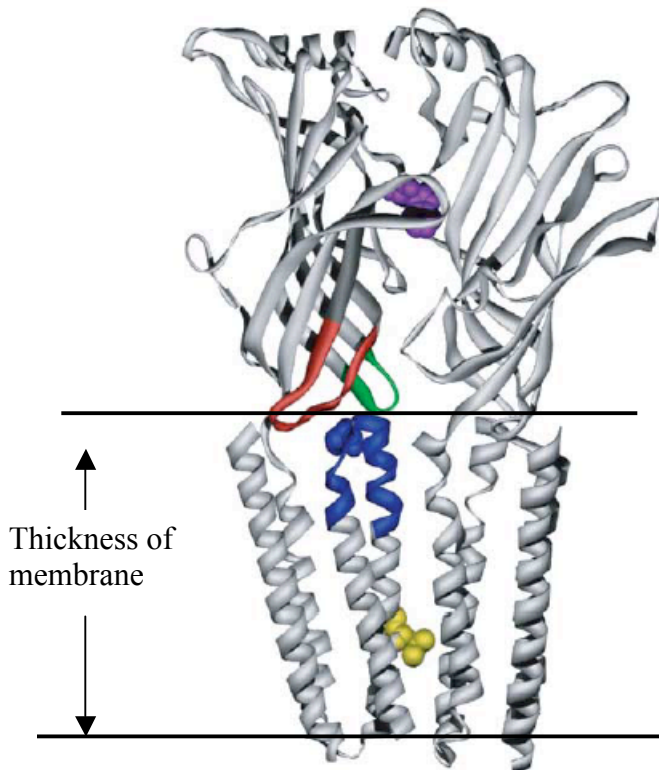
Vasopressin: CYFQNCPRG

Oxytocin: CYIQNCPLG

1. Explain how you can distinguish these two peptide hormones by their UV visible spectra in the 250-300 nm range.
2. Both peptides react with reducing agents such as beta mercaptoethanol (BME). What particular side group reacts with this reducing agent, and what does the fact that these peptides both react with BME tell you about the side groups and therefore this peptide?
3. Calculate the overall charge of the peptides at pH 1, 7, 9, 11 and 14. Are the peptides positively or negatively charged at pH 7? Between which pH values in this range are the peptides neutral? Calculate the pI of each peptide. How might the differences affect their function?
4. Use the Chou and Fassman rules to calculate the probabilities for this nonapeptide to form a section of alpha helix, beta strand, or loop. Use either vasopressin or oxytocin. What is your conclusion?

II Secondary Structure

Shown here is a 5HT₃ receptor protein (Nature 438, 2005 p248-252)



1. What structural motif discussed in class is exhibited by the alpha helical domain?

2. Many of these helices appear to be antiparallel. How might the helix dipole play a role in assembling this molecule?

3. The alpha domain is described as being transmembrane. Calculate the thickness of a membrane spanned by this structural unit given your knowledge of the regular dimensions of an alpha helix.

4. Explain how the isomerization of Pro8 (shown at the top of membrane) affects the packing of these two helices.

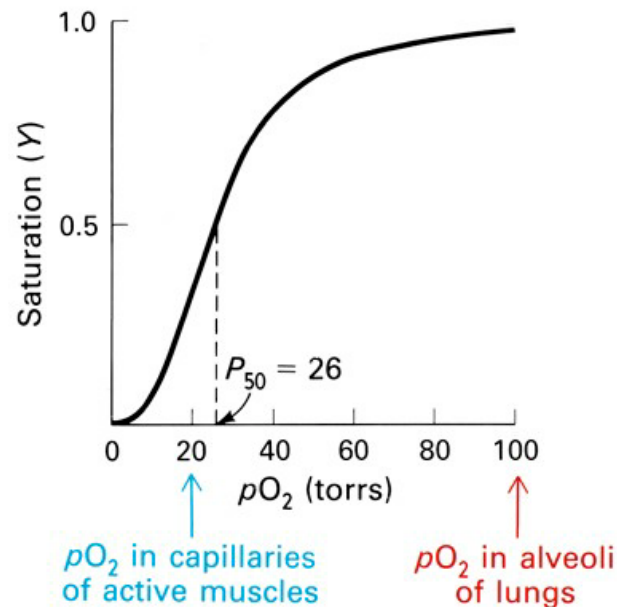
5. How is ligand binding in the extracellular domain communicated to the transmembrane unit? (use back to answer and keep answer short)

III. Hb and Mb Cooperativity and Allostery

PART A: Answer the questions posed in the column headings for a Hb made up of mutant alpha subunits as indicated. The mutations made are mutations in just the hemoglobin **alpha** subunit. The slide index in parentheses after the mutation is where you can find that or a similar residue discussed in L 13.

Hb alpha chain mutation	Does this subunit bind heme Fe?	Does this subunit bind O ₂ reversibly?	Does the α ₂ β ₂ tetramer show cooperativity?	Does the α ₂ β ₂ tetramer show allostery?
Lys(82) → Gly (slide 21-DBG binding in beta)				
His(E7) → Gly (slide 3 same as Mb)				
His(F8) → Ala (slide 3, same as Mb)				
Val(93) → Gly (slide 12 labelled offending residue)				
His(122) → Lys (slide 20)				

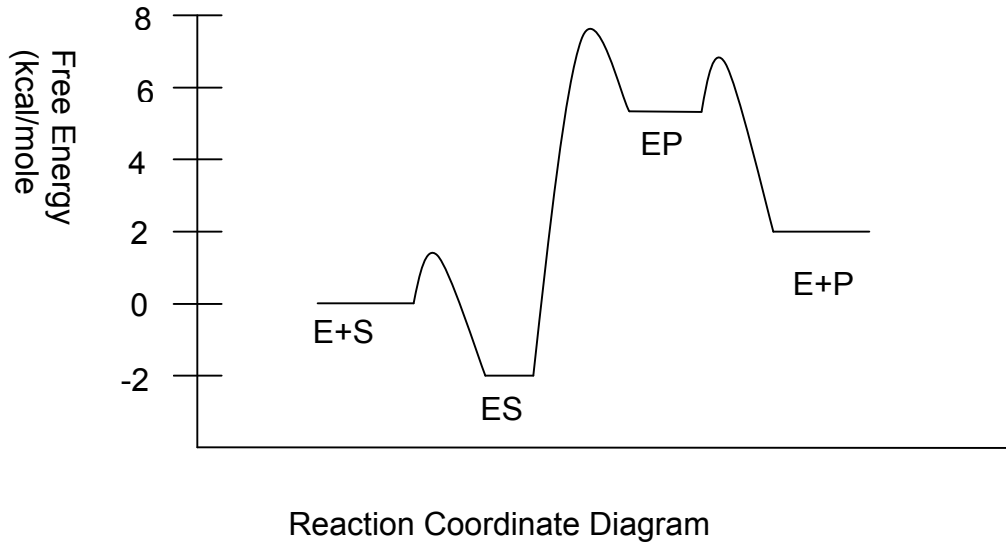
PART B: If you have answered Yes in part A to the mutant having the capacity to bind oxygen reversibly, then predict how this mutation will perturb the oxygen binding curve below (sketch a new curve or curves on the drawing below and label with the name of the mutant). Normal Hb binding to oxygen is shown for you to use as a marker.



How Hb transports oxygen

IV Enzyme functions

Proline Isomerase is an enzyme which catalyzes the conversion from trans to cis proline. PI allows proteins to reach their thermodynamic minimum more quickly. Reconstruct the kinetic and thermodynamic constants from the following reaction coordinate diagram.



Thermodynamic and kinetic constants should be calculated at $t = 25.0^\circ \text{C}$ where $RT = (1.98 \text{ cal/mol K}) \times 298 \text{ K} = 0.59 \text{ kcal/mole}$. Please include units!

a) Calculate $k_{\text{cat}} = k_2$.

b) How long does the substrate “live” on the enzyme?

c) Calculate K_m .

d) Why doesn't the enzyme evolve so as to bind the substrate more tightly?

e) Calculate k_{cat}/K_m and comment on the efficiency of this enzyme compared to a bimolecular reaction with a second order rate constant of $10^8 \text{ M}^{-1}\text{s}^{-1}$.

f) Calculate K_{eq} .

g) Draw a Michaelis Menten curve for this enzyme as precisely as possible using the constants above at a concentration of 10 nM enzyme.

V. Thermodynamics of Protein Folding

Use this figure to answer the questions below.

1. Why is it that most proteins denature at high temperature?

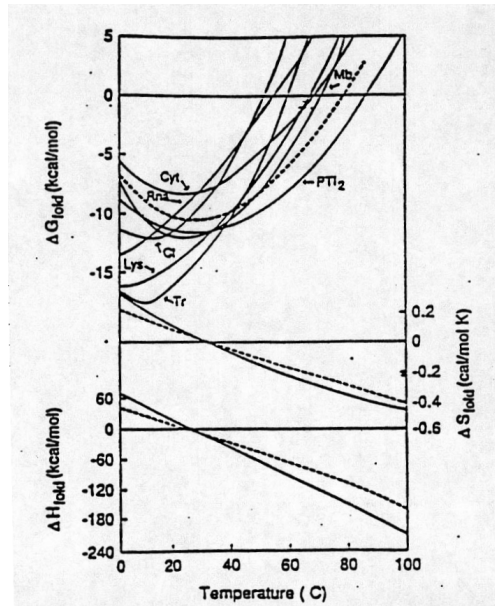


FIGURE 8: Thermal stabilities of proteins. Experimental data for free energies, enthalpies, and entropies of folding taken from Privalov (1979) and Privalov and Kechinashvili (1974) (—). Theoretical predictions are from Dill et al. (1989) (---).

2. Why do proteins have lower stability at temperatures below about 20°C?

3. Why are proteins stable at all?