

Exam Answers Protein Structure and Function

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.

Three amino acids absorb light in this spectral range: tryptophan (W) with λ_{max} at 280 nm and $\epsilon = 5,000 M^{-1}cm^{-1}$, tyrosine (Y) with λ_{max} at 275 nm and $\epsilon = 1,300 M^{-1}cm^{-1}$ and phenylalanine (F) with a triplet centered at 257 nm, with smaller peaks at 247 and 265 nm and a small $\epsilon = 200 M^{-1}cm^{-1}$ (see lab Exp I, constants taken from Physical Biochemistry from Van Holde). Vasopressin contains Y and F and Oxytocin contains Y. The absorption spectrum of both would be dominated by the absorption of Y at 278 nm, but the spectrum of vasopressin would have an additional small triplet feature approximately 1/6 the height of the Y peak centered at 257 nm.

2. 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? (pKa values taken from Table 3-1 Lehninger, 4th Edition p78)

Vasopressin (pKa)	pH = 1	pH = 7	pH = 9	pH = 11	pH = 14
G ₉ -COOH (2.34)	0	-1	-1	-1	-1
C ₁ --SH (8.18)	0	0	-1	-1	-1
C ₆ --SH (8.18)	0	0	-1	-1	-1
Y ₂ --OH (10.07)	0	0	0	-1	-1
C ₁ -NH ₃ ⁺ (10.28)	+1	+1	+1	0	0
R ₈ --NH ₃ ⁺ (12.48)	+1	+1	+1	+1	0
NET CHRGE (+SH)	+2	+1	-1	-3	-4
NET CHRGE (-SH)	+2	+1	+1	-1	-2

Oxytocin (pKa)	pH = 1	pH = 7	pH = 9	pH = 11	pH = 14
G ₉ -COOH (2.34)	0	-1	-1	-1	-1
C ₁ --SH (8.18)	0	0	-1	-1	-1
C ₆ --SH (8.18)	0	0	-1	-1	-1
Y ₂ --OH (10.07)	0	0	0	-1	-1
C ₁ -NH ₃ ⁺ (10.28)	+1	+1	+1	0	0
NET CHARGE (+SH)	+1	0	-2	-4	-4
NET CHARGE (-SH)	+1	0	0	-2	-2

pI for vasopressin with cysteines included is predicted to be between 7 and 9. Vasopressin is positive at pH 7. The calculated value would be $pI = (8.18+8.18)/2 = 8.18$. Without cysteines (which the next question tells you are bound up in a disulfide bond) the pI for vasopressin is still positive at pH 7, and the pI, predicted to be between 9 and 11, can be calculated to be $pI = (10.07 + 10.28)/2 = 10.18$

pI for oxytocin with cysteines included is predicted to be lower, probably close to pH 7. Oxytocin is in fact, neutral/negative at pH 7. The calculated value is $pI = (2.34 + 8.18 + 8.18)/3 = 6.23$. Without cysteines (bc they are tied up in disulfide bond) the value is $pI = (2.34 + 10.07)/2 = 6.52$

The effect of this difference on function is that the vasopressin is positively charged at pH = 7 while the oxytocin is neutral/negative at pH 7, so they bind to different receptors.

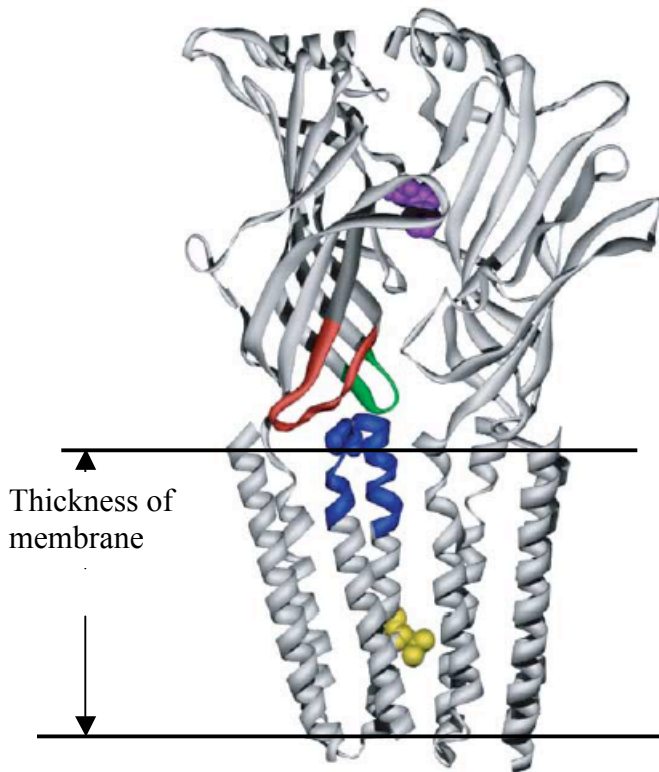
- Both peptides react with reducing agents such as beta mercaptoethanol. What side groups on the peptides are redox active and what does the fact that these groups react with a reducing agent tell you about these side groups?

Cysteines oxidized to disulfides react with the reducing agent, b-mercaptoethanol as per the Anfinsen experiment described in class. The fact that the peptides react with this reducing agent tells you that the cysteines have indeed oxidized to form a C1-C6 disulfide, which creates a cyclic peptide of six amino acids

- 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?

Based on the nine amino acids for Vasopressin: $P_{\alpha} = 0.91$, $P_{\beta} = 0.91$ (also since $P_{\alpha} \neq 0.9$ even a turn won't work) predicts a mixed structure, as one sees with the cyclic peptide

Based on the nine amino acids for Oxytocin: $P_{\alpha} = 0.94$, $P_{\beta} = 0.92$ (since $P_{turn} = 1.05 > P_{\beta}$, but $P_{\alpha} \neq 0.9$ even a turn won't work) predicts a mixed structure, as one sees with the cyclic peptide



II Secondary Structure (20 points)

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?

This image shows two four helix bundles in the intramembrane alpha helical domain.

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

The helix dipole, with its positive end at the amino terminal end and its negative end at the carboxy terminal end, will cause two adjacent helices to pack in the antiparallel orientation so as to ameliorate the charge separation. This is particularly important in a membrane.

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.

From the image, you can see 7-8 turns spanning the membrane. Distance = 7.5 turns x 5.4 Å/turn = 40.5 Å

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

When Pro8 isomerizes from trans to cis, it acts as a hinge, adjusting the relative packing of M-N helices from a closed FELIX orientation (20° helical axes separation, +4 ridges in +3 grooves) to more of an open GLOBIN-like fold orientation (50° helical axes separation, +3 ridges in +3 grooves). See figure 5

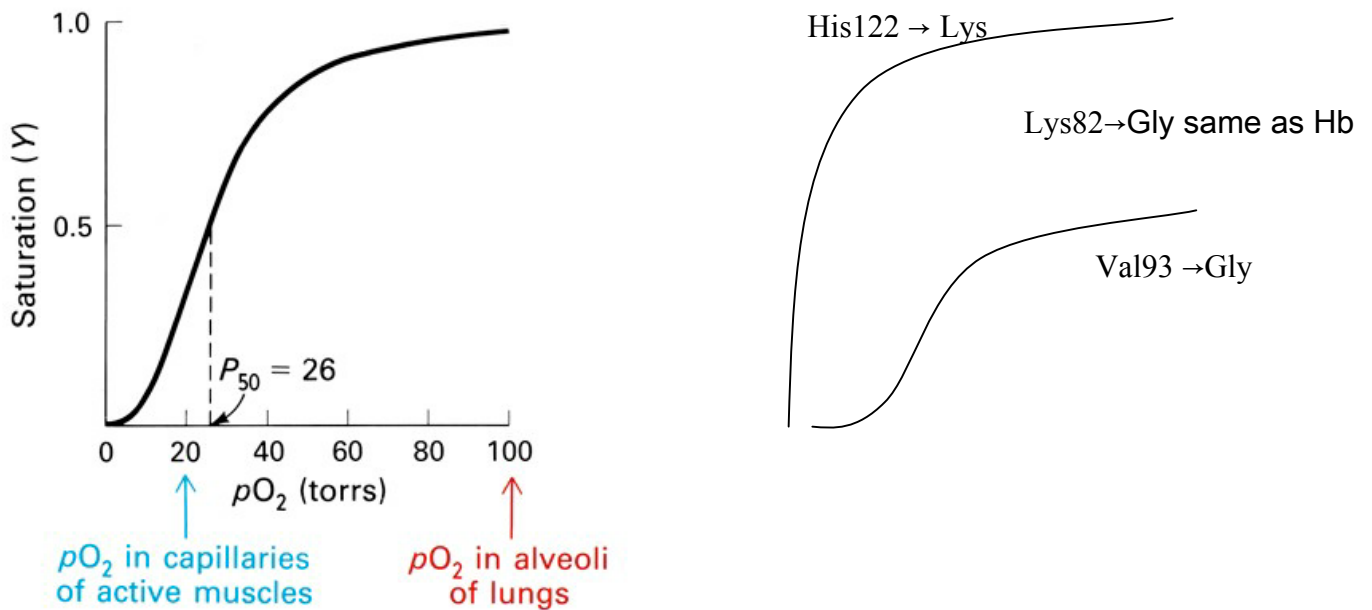
5. How is ligand binding in the extracellular domain communicated to the transmembrane unit? *Loop 7 and loop 2 act together like a caliper to hold pro8 in the trans conformation. Ligand binding (5HT₃ for example) in the extracellular binding domain moves Trp138 (shown in the center of the extracellular domain of this image) shifting the six amino acid β strand, moving loop 7 (the cis loop). This springs open the lock on the Pro8, allowing the trans-cis isomerization, which in turn moves helix N with respect to helix M and allows Leu in center of channel to move out of the way and open the channel.*

III. Hb and Mb Cooperativity and Allosterity (15 points)

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 allosterity?
Lys(82) → Gly (slide 21-DBG binding in beta)	Y	Y	Y	Y
His(E7) → Gly (slide 3 same as Mb)	Y	N	N	N
His(F8) → Ala (slide 3, same as Mb)	N	N	N	N
Val(93) → Gly (slide 12 labelled offending residue)	Y	Y	N	Y
His(122) → Lys (slide 20)	Y	Y	Y	N

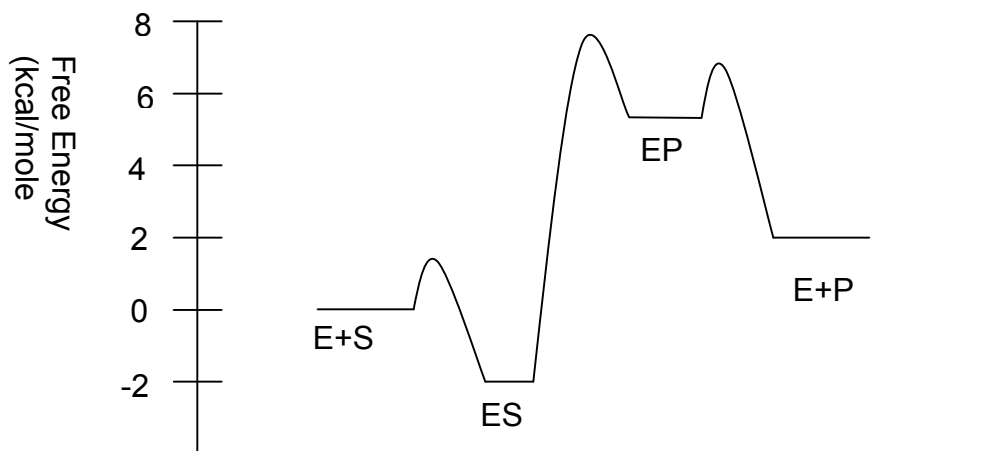
PART B: If you have answered Yes in part A to the mutant having the capacity to bind oxygen reversibly, then predict how this mutation will perturb the oxygen binding curve below (sketch a new curve or curves on the drawing below and label with the name of the mutant). Normal Hb binding to oxygen is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



How Hb transports oxygen

IV Enzyme functions (25 points)

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.



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 $k_{\text{cat}} = k_2$.

ANS: $3.89 \times 10^5 \text{ s}^{-1}$

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

ANS: $2.57 \times 10^{-6} \text{ s}$ or about 3 us

c) Calculate K_m .

ANS: $3.37 \times 10^{-2} \text{ M}$

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

The deeper the pit for ES, the harder the climb back out over the activation barrier!

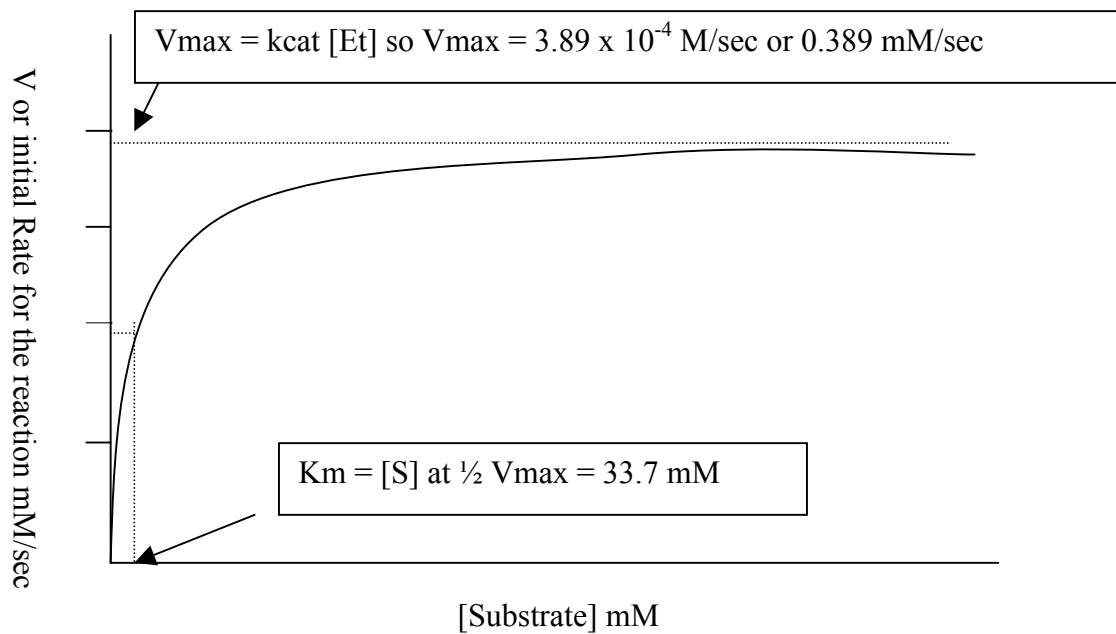
e) Calculate k_{cat}/K_m and comment on the efficiency of this enzyme compared to a bimolecular reaction with a second order rate constant of $10^8 \text{ M}^{-1}\text{s}^{-1}$.

ANS: $1.15 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ or 1/10 times diffusion controlled which is still pretty fast.

f) Calculate K_{eq} .

ANS: 3.37×10^{-2}

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 (15 points)

Use this figure to answer the questions below.

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

For a process to be spontaneous, ΔG° must be < 0 . At a single T , the entropic and enthalpic terms both contribute to the overall free energy: $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$

As the temperature rises, the ΔG° for folding becomes positive because [despite the increasingly favorable enthalpic contributions to folding (ΔH becomes more negative at higher temperature)] the entropic term becomes more negative and overwhelms the enthalpic contribution as $-T\Delta S$. This means that despite the fact that better bonds are made, the solvent is too intrinsically disordered at a higher temperature to make the hydrophobic effect (liberation of solvent clathrate cages as hydrophobic side groups are buried) count for much.

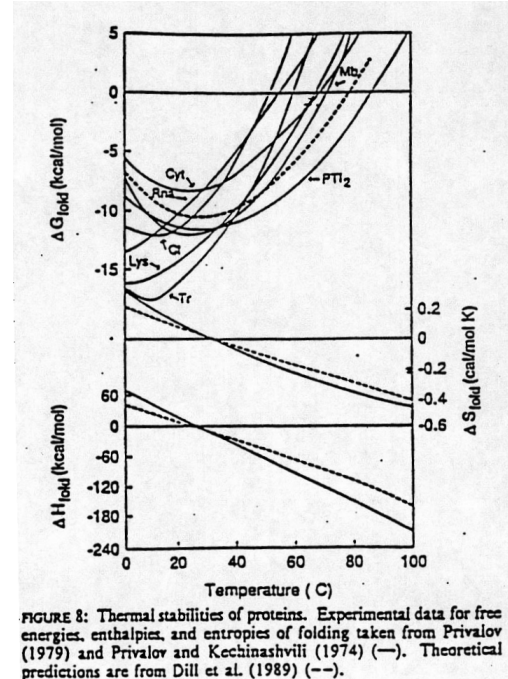


FIGURE 8: Thermal stabilities of proteins. Experimental data for free energies, enthalpies, and entropies of folding taken from Privalov (1979) and Privalov and Kechinashvili (1974) (—). Theoretical predictions are from Dill et al. (1989) (---).

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

Proteins have lowered stability at low temperatures because the enthalpic term actually becomes positive (ΔH becomes positive), meaning that the bonds between the carbonyl oxygens and amide hydrogens are better with the solvent than with each other. The entropic term actually favors folding at lower temperatures (ΔS becomes positive).

3. Why are proteins stable at all?

As the figure shows, protein folding is a fine balance between large enthalpies and large entropies, and the area in which the free energy for folding is negative is a fairly narrow region. Both enthalpy and entropy are temperature dependent, both getting more negative at high temperatures. This leads to a narrow thermal window. If the temperature gets too low, entropy favors folding the protein, but if the temperature goes too low, the enthalpy can lead to cold denaturation. If the temperature gets too high, the enthalpy favors the folded protein, but if the temperature gets too high, the entropy can lead to heat denaturation. And so it goes.