

Sample Questions Dr. Wimley Biochem 601. These are from past exams.

1) For the reaction $A \leftrightarrow B$, ΔG° is -3407 J/mol at 25°C (298 K). What concentration of $[B]$ will you have if you add 0.14M $[A]$ to a test tube and let the system come to equilibrium.

$A \leftrightarrow B$ so $K = [B]/[A]$

$\Delta G^\circ = -RT\ln(K)$ at equilibrium and $K = e^{\Delta G^\circ/(-RT)}$ so $K = 3.956$

if $[B]/[A] = 3.96$ the fraction of A is 0.202 and the fraction of B is 0.798

**Since the total concentration is 0.14 M, the concentration of A is 0.028M
The concentration of B is 0.112 M**

2) Name 5 types of non covalent interactions that contribute to biomolecule structure. Give a one-sentence description of each.

Hydrogen Bonding – An electrostatic interaction that occurs between polar (partially charged) atoms and involves a hydrogen atom bridge to a lone electron pair.

Electrostatic/ionic – Ionic interactions that occur between atoms that have a net charge. Can be attractive (opposite charges) or repulsive (like charges).

Van der Waals – Induced dipole interaction that occurs when two atomic groups are in close proximity

Dipole – Electrostatic interactions that occur between atomic groups that are dipolar due to a partial charge separation.

Hydrophobic effect – An unfavorable interaction between non-polar groups and water that favors the segregation of nonpolar groups into environments that are not exposed to water.

3A) Draw the structure of the tripeptide DFT at pH 7 (4 pts)

3B) Estimate the isoelectric point (pI) of this peptide to ± 0.5 units (2 pts)

This peptide has three ionizable groups, the Aspartate (D) sidechain (pKa~4), the carboxy terminus (pKa~3.5) and the Amino terminus (pKa ~9). The peptide at its pI will have its two carboxyl groups about 50% ionized, so they equal the fully ionized amino terminal group. This the pI will be around the pKa of the two carboxyls, or ~3.7

4) A folded protein has a hydrophobic core in which the side chain of amino acid 20 is in contact with the sidechain of amino acid 40. They are completely surrounded by nonpolar (hydrophobic) sidechains. You make a series of mutants where you change the residues at position 20 and at position 40. For each of the three pairs of mutants, decide whether the folded form of the protein in column B should be MORE stable or LESS stable than the one in column A (6 pts)

Protein A	Protein B	Is Protein B More or Less stable than Protein A ?
Valine20 Leucine40	Alanine20 Alanine40	Less stable, because the nonpolar sidechains in B are smaller than in A
Lysine20 Aspartate40	Lysine20 Lysine40	Less stable because a +/- salt bridge has been replaced by two positive charges
Phenylalanine40 Isoleucine40	Leucine20 Glutamine40	Less stable because a hydrophobic residue (I) has been replaced by a polar residue (Q)

5) You incubate a folded globular protein with a non-specific protease (a protein which can cleave a peptide chain equally well at points in a sequence). Which of the following types of secondary structure is most likely to be cleaved by the protease: α -helices, β -sheets or reverse turns. In one or two sentences explain your answer. (5 pts)

Reverse turns are most likely to be cleaved because they are exposed on the surface of a typical folded protein.

6) (6pts total) How many amino acids are found in the repeating unit of a α -helical coiled-coil protein Seven

Name a protein that contains a long α -helical coiled-coil:

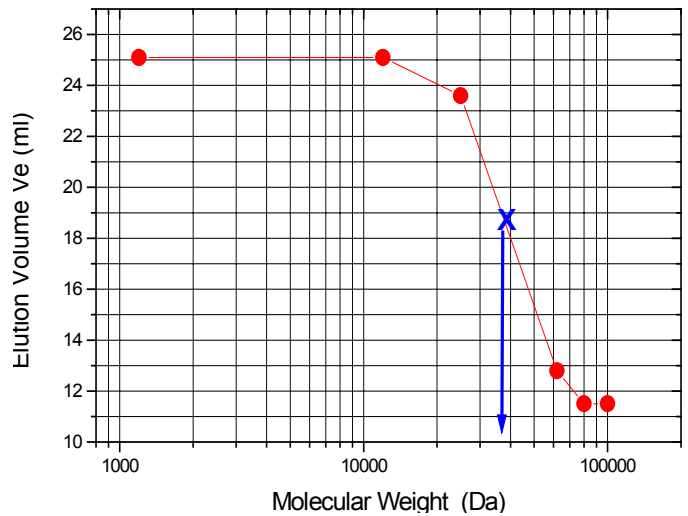
Myosin, Tropomyosin, alpha keratin

Name a fibrous protein that does not contain a long α -helical coiled coil_

collagen, f-actin, beta keratin

7) You collect the following data for proteins of known molecular weight eluting from a molecular sieve (gel filtration) column. Use the graph if you think it will be helpful. (6 pts)

Molecular weight 100,000	$V_e = 11.5$ ml
Molecular weight 80,000	$V_e = 11.5$ ml
Molecular weight 62,000	$V_e = 12.8$ ml
Molecular weight 25,000	$V_e = 23.6$ ml
Molecular Weight 12,000	$V_e = 25.1$ ml
Molecular Weight 12000	$V_e = 25.1$ ml



What is the void volume V_o ?

The void volume (V_o) is the volume at which all the excluded molecules elute, 11.5 ml

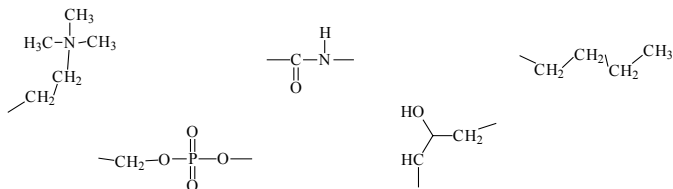
What is the included volume V_i ?

The total volume (V_t) is the volume at which all the maximally retained molecules elute (25.1 ml). The included volume (V_i) is the difference between V_o and V_t or 13.6 ml.

Within ± 2500 Da, estimate the molecular weight of a protein that elutes at 18.7 ml?

I drew an "X" at the spot where the elution volume of the unknown crosses the curve. The elution volume is proportional to molecular weight, so the molecular weight corresponding to an elution volume of 18.7 can be read from the graph as shown by the arrow. The unknown molecular weight is ~38,000

8) You have purified a single lipid species from human cells. Using mass spectrometry you obtain the following fragments from that pure lipid (6 pts)



Based on these fragments, identify the original lipid species from the list below. **Circle the correct answer.**

- 1) sphingomyelin
- 2) ganglioside
- 3) phosphatidylethanolamine
- 4) progesterone
- 5) cardiolipin
- 6) phosphatidylcholine

Sphingomyelin is the only lipid discussed in class that has all of these chemical groups.

9) Briefly explain why there is an essential glycine residue in the loop region of all Helix-loop-Helix or EF hand motifs. In your explanation, state the property of glycine makes it essential in that position? (5 pts)

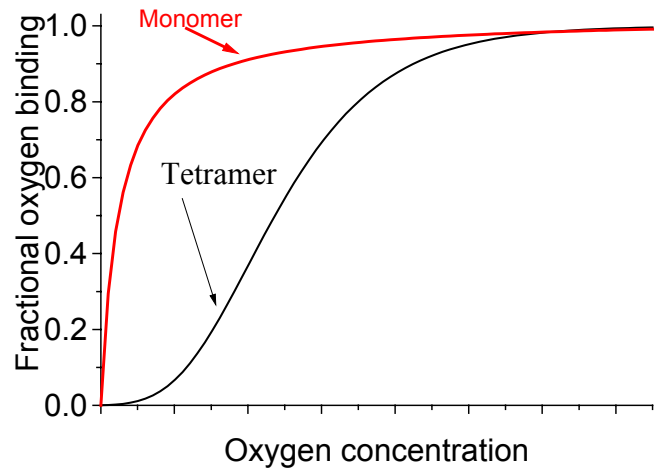
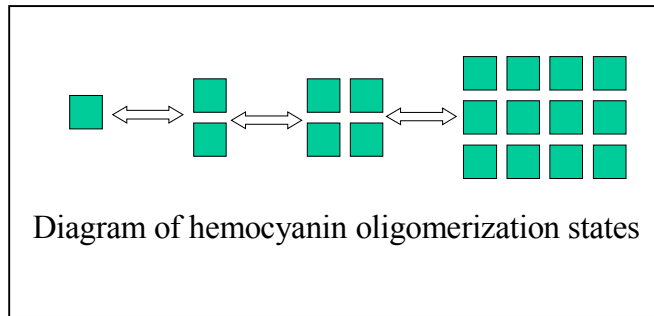
The loop glycine is essential because only glycine has the conformational flexibility around its alpha carbon to allow the required bend in the chain in the calcium binding loop.

10) Below is a partial sequence of a protein known to span the membrane with a single membrane-spanning α -helix. Circle the segment that spans the membrane. (5 pts)

STTGVAMHTS TSSSVTKSYI SSQTNDTHKR DTYAATPRAH EVSEISVRTV
 YPPEEETGER VQLAHHFSEP EITLIIFGVM AGVIGTILLI SYGIRRLIKK
 SPSPDKPLPS PDTDVPLSSV EIENPETSQ

Look for a part of the sequence containing 19-25 residues that are mostly hydrophobic and contain no charged amino acids. There is only one such segment.

11) The oxygen carrying protein found in the blood of crustaceans, mollusks and insects is called hemocyanin. Depending on the solution conditions, hemocyanin can change its oligomerization state from monomer to dimer, tetramer and to large oligomers. The oxygen binding curve is shown for the tetramer. **Draw the curve** that you would expect for a monomeric protein (5 pts)



This system is analogous to myoglobin/hemoglobin where the tetrameric hemoglobin has cooperative (sigmoidal) oxygen binding. The monomeric myoglobin has hyperbolic oxygen binding. Recall that cooperativity requires structural interaction between subunits, so the monomer can not be cooperative.

12) What percentage of histidine's sidechain is positively charged at pH 5.5? Assume the sidechain is exposed to water.

Start with Henderson-Hasselbach: $\text{pH} = \text{pK}_a + \log(\text{A}/\text{AH})$

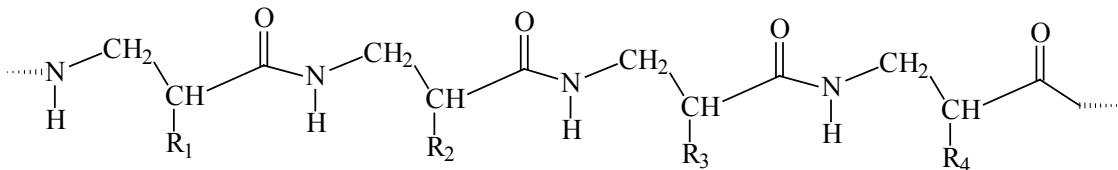
$$5.5 = 6.5 + \log(\text{A}/\text{AH}) \quad \text{so} \quad \text{A}/\text{AH} = 10^{-1} = 0.1$$

This is the RATIO of A (the deprotonated form) to AH, the protonated form

The fraction deprotonated is $0.1/(0.1+1) = 0.091$ or 9.1% (This is uncharged)

Thus the fraction positively charged is 0.909 or 90.9%

13) You have synthesized a polymer with the chemical structure below using the same 20 R-group sidechains as found in proteins. Would you expect this polymer to be more likely, or less likely than a polypeptide to fold into elements of secondary structure? Explain your answer. (6 pts)



The first thing you should notice is that this polymer has an extra CH₂ group in the backbone. This increases conformational flexibility and makes this polymer much less likely to fold into secondary structure. For this question, I accepted ANY answer that correctly used any of the principles I discussed in my lectures.

14) Imagine that you are writing a computer program to search a protein sequence database for coiled-coil proteins. Write one repeating patterns you could have the program search for. (5 pts)

Use the following codes

X-Any residue

H-hydrophobic

B-Basic (+ charged)

A-Acidic (- charged)

P- Polar

C-Cysteine

G-Glycine

Pr-Proline

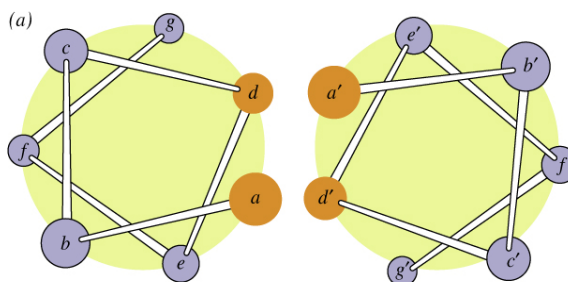


Figure 6-14a. The coiled coil of α keratin.
[After McLachlan, A.D. and Stewart, M., J. Mol. Biol. 98, 295 (1975).]
Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

As an example of using the codes: If you wanted to search for a pattern that had four hydrophobic amino acids, a Gly-Pro pair and then three basic amino acids, you would write: HHHHGPBBB

The coiled-coil contains a heptad repeat (7 residues). Generally a and d are hydrophobic (often one is alanine) while e and g are often of opposite charge. I accepted any heptad repeat that had a pattern like: HXXHBXA or HXXHAXB