

7.013 Problem Set 2

Friday, February 20,

Problem sets will NOT be accepted late.

Question 1

Your friend, an expert structural biochemist, has managed to solve the crystal structure of Your Favorite Enzyme (YFE) bound to its regulatory subunit (for explanation of x-ray crystallography, see p. 440 of your textbook). Through in vitro biochemical approaches, you have ascertained that the enzyme loses all activity when not bound to its regulatory subunit. YFE catalyzes the following simple chemical reaction:



One of your new goals is to perform site directed mutagenesis and pH dependence experiments with your protein and thus better understand the association between the enzyme and its regulatory subunit. Site-directed mutagenesis involves selectively mutating single amino acid residues of a protein. Your initial experiments concern the following stretch of amino acids:

a) Draw the chemical structure of the side chains of the amino acids in the below figure.

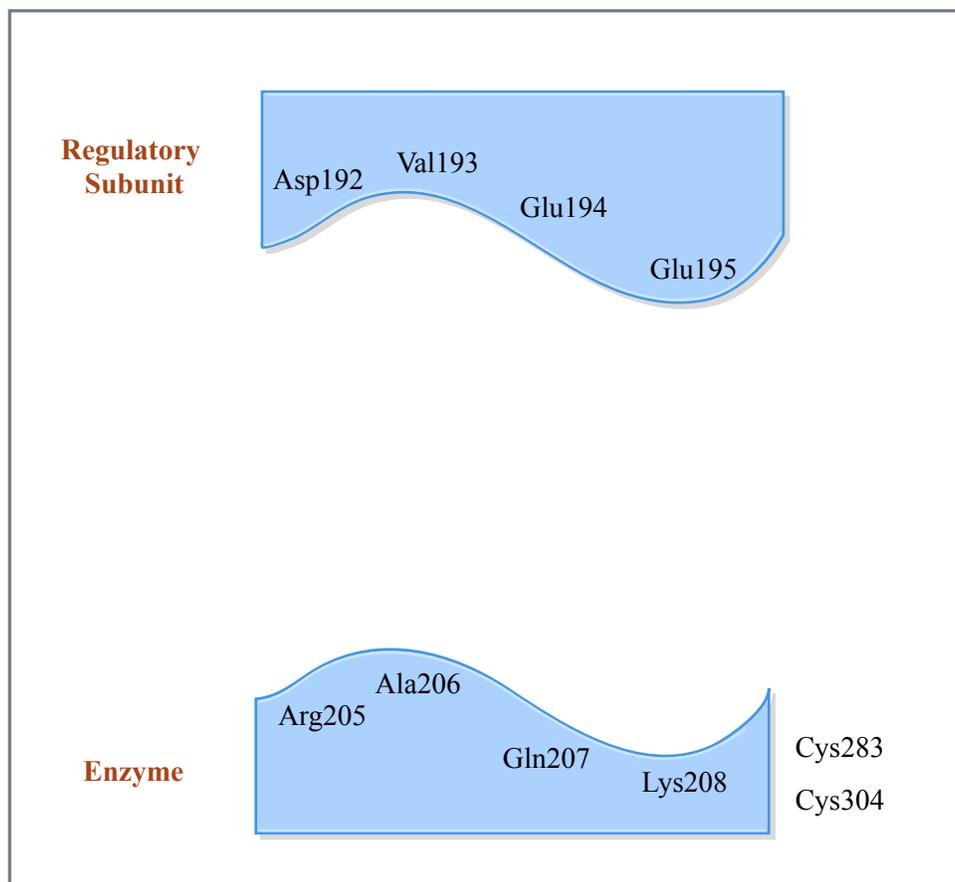


Figure by MIT OCW.

b) For each interaction listed below, identify the strongest type of chemical bond or force expected between each amino acid pair.

Choose from covalent, hydrogen, ionic, or van der Waals.

a. Asp192:Arg205	
b. Val193:Ala206	
c. Glu194:Gln207	
d. Glu195:Lys208	
e. Cys283:Cys304	

c) Describe the expected results of the following mutations on the activity of the enzyme. Rationalize whether and why you will see a *loss of activity* of the enzyme by being specific as to how chemical bonds may be changed.

[NOTE: Your explanation will constitute the majority of points awarded.]

a. Arg205→Ala	
b. Ala206→Leu	
c. Gln207→Asn	
d. Lys208→Arg	
e. Cys283→Ala	

Question 2

The structure of the lipid bilayer does not allow for many polar molecules to pass into the cell. Using genetic approaches, your lab has identified a pair of proteins hypothesized to be involved in transporting two different molecules from outside the cell into the cytoplasm.

Through common biochemical assays, Protein A has been identified as a monomer at physiological concentrations. You decide to analyze the amino acid sequence by constructing a hydropathy plot (which considers the relative hydrophobicity along the primary amino acid sequence—for example, tryptophan would be scored as relatively hydrophobic and serine would be scored as relatively hydrophilic). Your analysis yields the following result.

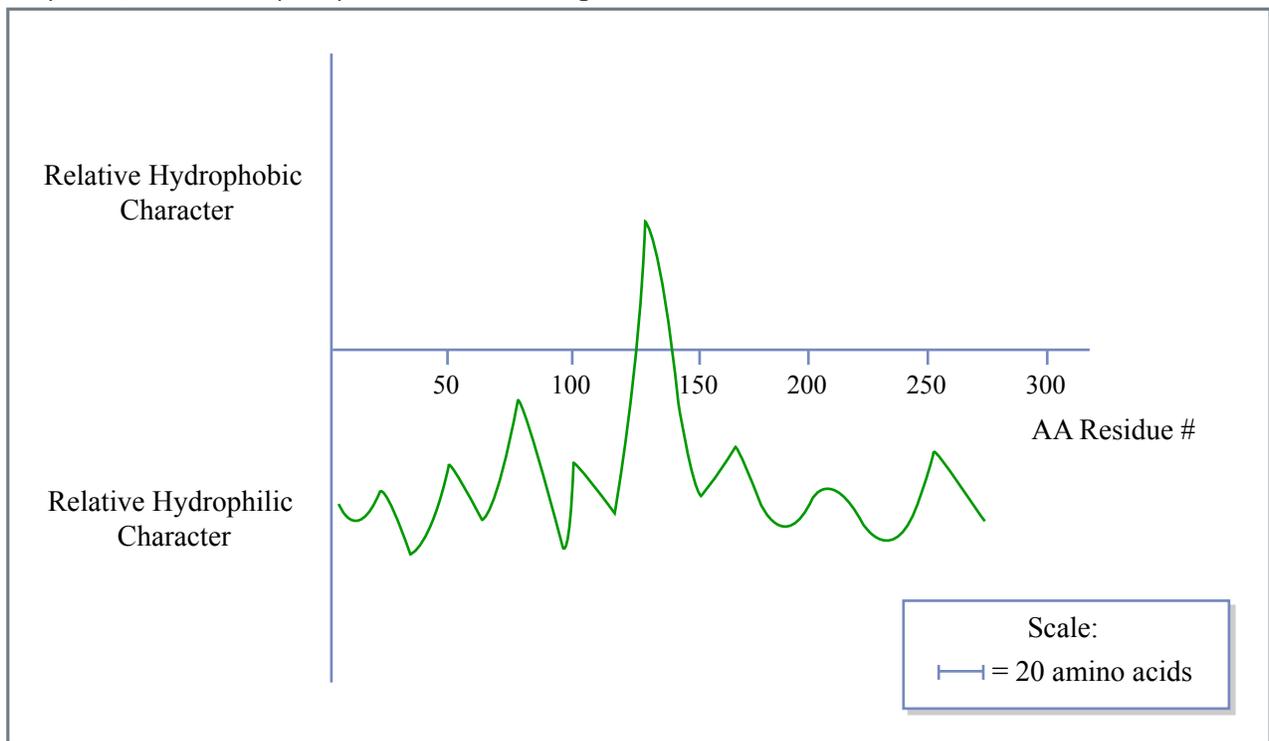


Figure by MIT OCW.

a) Based upon the above hydropathy plot, and given that a transmembrane segment is about 20 amino acids long, might you expect Protein A to be embedded in the membrane? If so, show how it would interact with the lipid bilayer below:

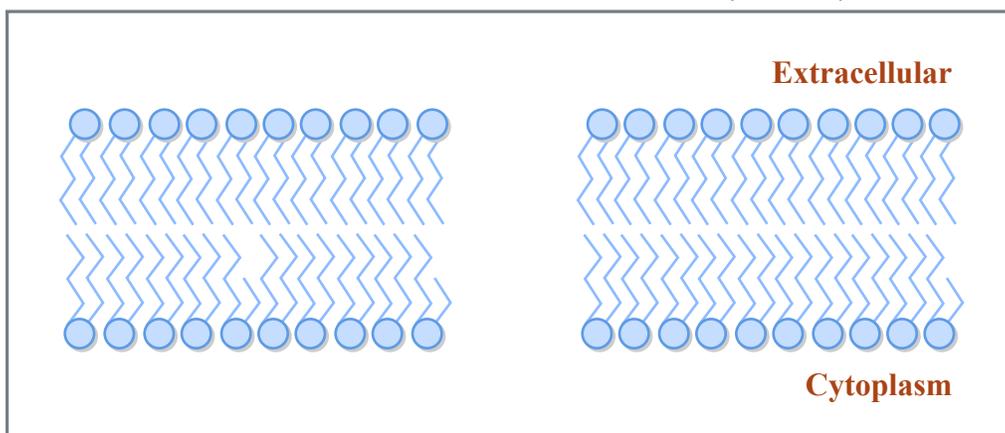


Figure by MIT OCW.

You perform an identical analysis on Protein B and are puzzled by the results. A hydropathy plot does not yield any indication of membrane localization. Thankfully, your pal, an expert biochemist, has characterized Protein B as a homohexamer with strong alpha helical character. Quaternary structural analysis predicts the following arrangement of molecules.

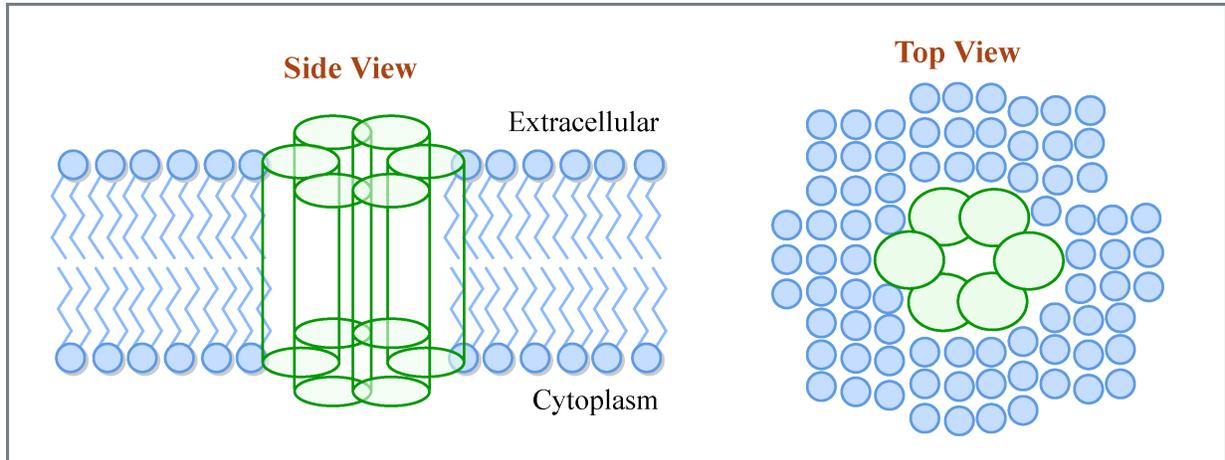


Figure by MIT OCW.

b) Would you expect this protein could serve the role of a channel for the transport of polar molecules? What is your reasoning after hearing of the above structural data.

c) Why is the alpha helical nature of the protein crucial to your assessment of its potential membranous or cytoplasmic localization? Draw a possible orientation of a Protein B hexamer with respect to a lipid bilayer.

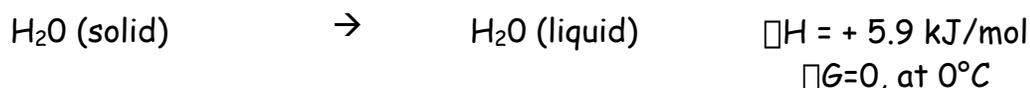
Question 3

a) Draw the tetrahedral cage-like crystalline structure of water in its solid form with unshared electron pairs, etc. Draw at least one water molecule and its nearest neighbors.

b) What type of bonds was made between one water molecule and another in the figure above?

c) What property of molecular water allows for the structure you have drawn?

Consider the following thermodynamic data ($\Delta G = \Delta H - T\Delta S$.)



d) Would you predict that the reaction is favorable at room temperature?

e) Using your knowledge of the solid and liquid forms of water, explain why the ΔH of this reaction is positive? Considering your explanation, why does ice melt?

f) Consider the following observation about tertiary structure of proteins - hydrophobic residues tend to be clustered and often buried deep within the protein. Explain this observation using what you know about the nature of hydrogen bonds.

Question 4

You have recently picked up a research project that was initially undertaken by a former student in your lab. The project involves the biochemical characterization of a protein with a known enzymatic activity. You pull out the student's notebook and see the following graph:

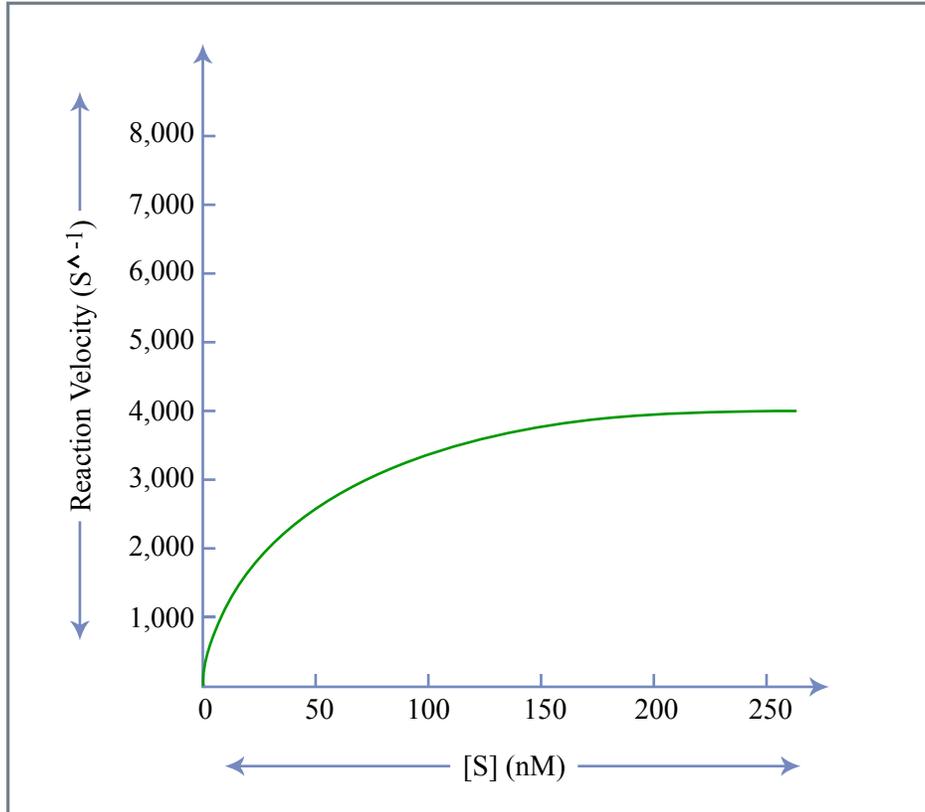


Figure by MIT OCW.

a) Describe what is going on in this experiment. Estimate a v_{\max} and K_m and write these on the graph above.

b) Why does the curve approach a maximum?

c) On the same graph, draw the corresponding curve if twice as much enzyme was added initially in the experiment. From your new data, estimate a v_{\max} and K_m and write these next to your new data.

d) On the coordinates below, draw a simple reaction profile for an energetically favorable one-step reaction with a high activation energy. Label the reactants and products.

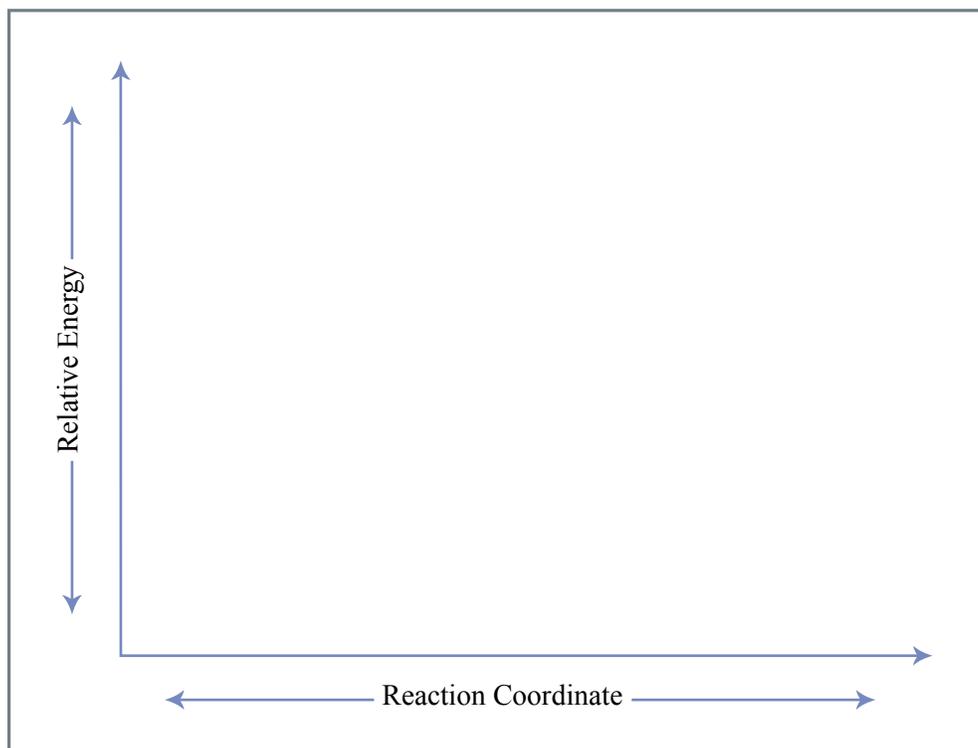


Figure by MIT OCW.

e) Now draw the profile for the catalyzed reaction on the **same** graph with a dashed line. Finally, comment on the changes that the addition of enzyme imposes on the following:

Choose from "Increases" or "Decreases" or "No Change".

A. Free energy of products	
B. Overall free energy change (ΔG)	
C. Activation Energy of forward reaction	
D. Activation Energy of reverse reaction	
E. Rate of forward reaction	
F. Rate of reverse reaction	

Question 5

a) Nucleotides are the building blocks for DNA polymers. Draw the below dNTPs and indicate clearly the 5' end and the 3'-hydroxyl.

Deoxyadenosine 5'-triphosphate (dATP)

Deoxycytosine 5'-triphosphate (dCTP)

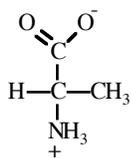
Melting of double stranded DNA to single strands is an important technique in molecular biology. Your favorite computer program predicts the below melting temperatures for the corresponding oligonucleotide sequences you plan to use for a technique called PCR (polymerase chain reaction). In this process we will learn about later in the semester, the oligonucleotides will anneal to complementary DNA.

Oligonucleotide sequence	Melting temperature (T_m)
TGTAACCATTCATATTCATGAC	50.2° C
AAGAGCGCTTGGTGCTTGCATT	62.1° C

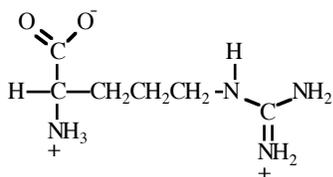
b) Explain a possible reason for the disparity in melting temperature.

c) What is the chemical basis for this disparity?

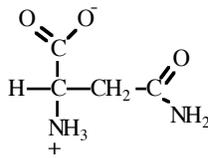
STRUCTURES OF AMINO ACIDS at pH 7.0



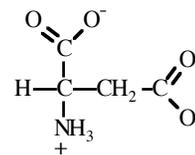
ALANINE
(ala)



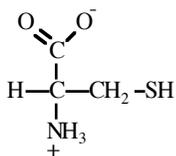
ARGININE
(arg)



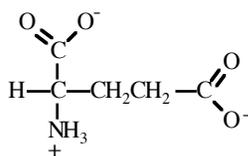
ASPARAGINE
(asn)



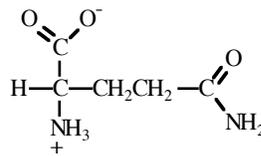
ASPARTIC ACID
(asp)



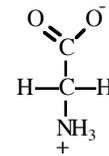
CYSTEINE
(cys)



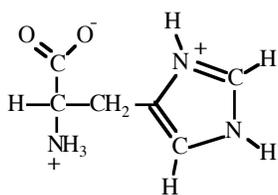
GLUTAMIC ACID
(glu)



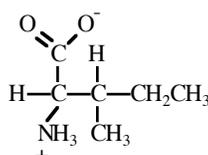
GLUTAMINE
(gln)



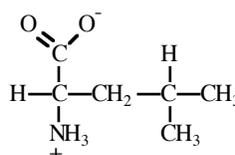
GLYCINE
(gly)



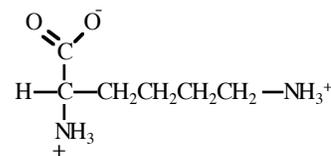
HISTIDINE
(his)



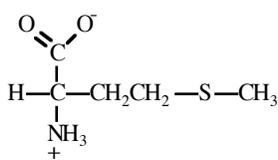
ISOLEUCINE
(ile)



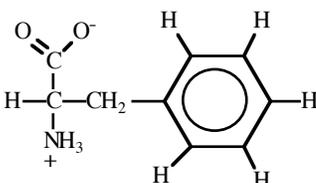
LEUCINE
(leu)



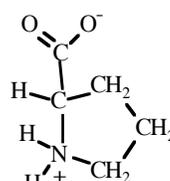
LYSINE
(lys)



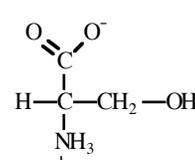
METHIONINE
(met)



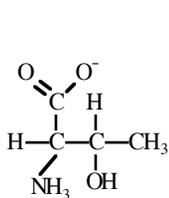
PHENYLALANINE
(phe)



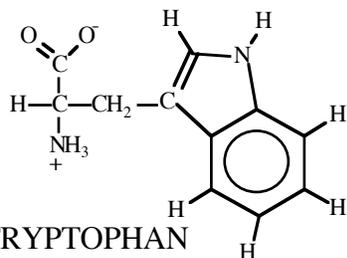
PROLINE
(pro)



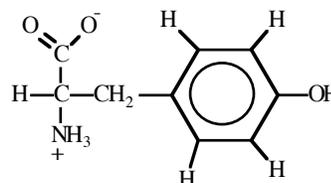
SERINE
(ser)



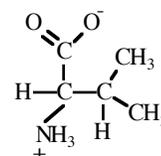
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)