Biochemistry 463, Summer II **Your Name:** Key

University of Maryland, College Park **Your SID #:**

Biochemistry and Physiology **Prof. Jason Kahn**

Exam I (100 points total) **July 22, 2013**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$\Delta S_{system} - \Delta H_{system}/T \geq 0$ $\quad pH = -\log([H^+])$ $\quad E = mc^2$

$S = k\ln W$ $\quad \Delta G = \Delta H - T\Delta S$ $\quad pH = pK_a + \log([A^-]/[HA])$

$K_a = [H^+][A^-]/[HA]$ $\quad \Delta G° = -RT\ln K_{eq}$ $\quad e^{i\pi} + 1 = 0$

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

~

1. (30 pts) Amino acid structure, the peptide bond, and acid-base

(a; 3 pts) Why is histidine frequently found in protein active sites?

It is the only aa with a pKa near neutral pH, so it frequently is involved in proton transfer.

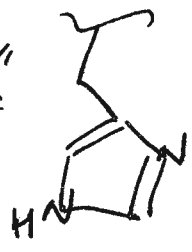
(+3) for either or =

It can chelate metal ions like Fe and Zn

(g; 4 pts) Calculate the ratio between the protonated and deprotonated forms of the histidine side chain at pH 7.2, The protonated form has a pKa of 6.04.

(+1) $pH = pK_a + \log \frac{[A^-]}{[HA]}$

"A^-" =



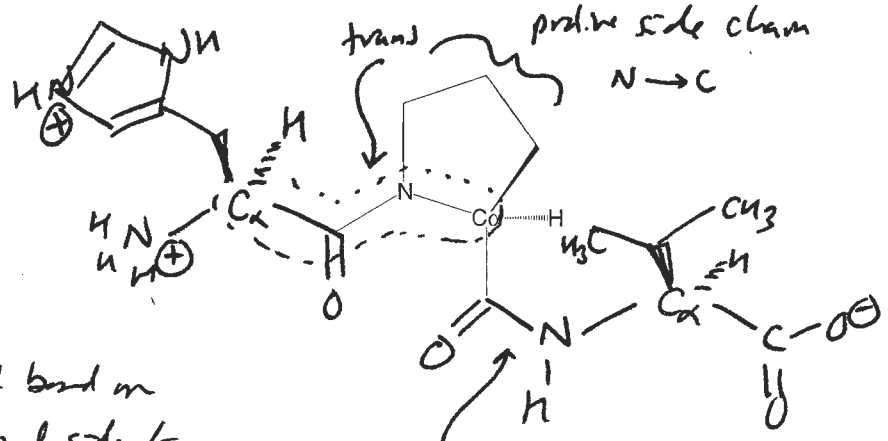
(+1) $\log \frac{[A^-]}{[HA]} = pH - pK_a = 7.2 - 6.04 = 1.16$

(+2) $\frac{[A^-]}{[HA]} = 10^{1.16} = 14.5$ >1 since pH > pK

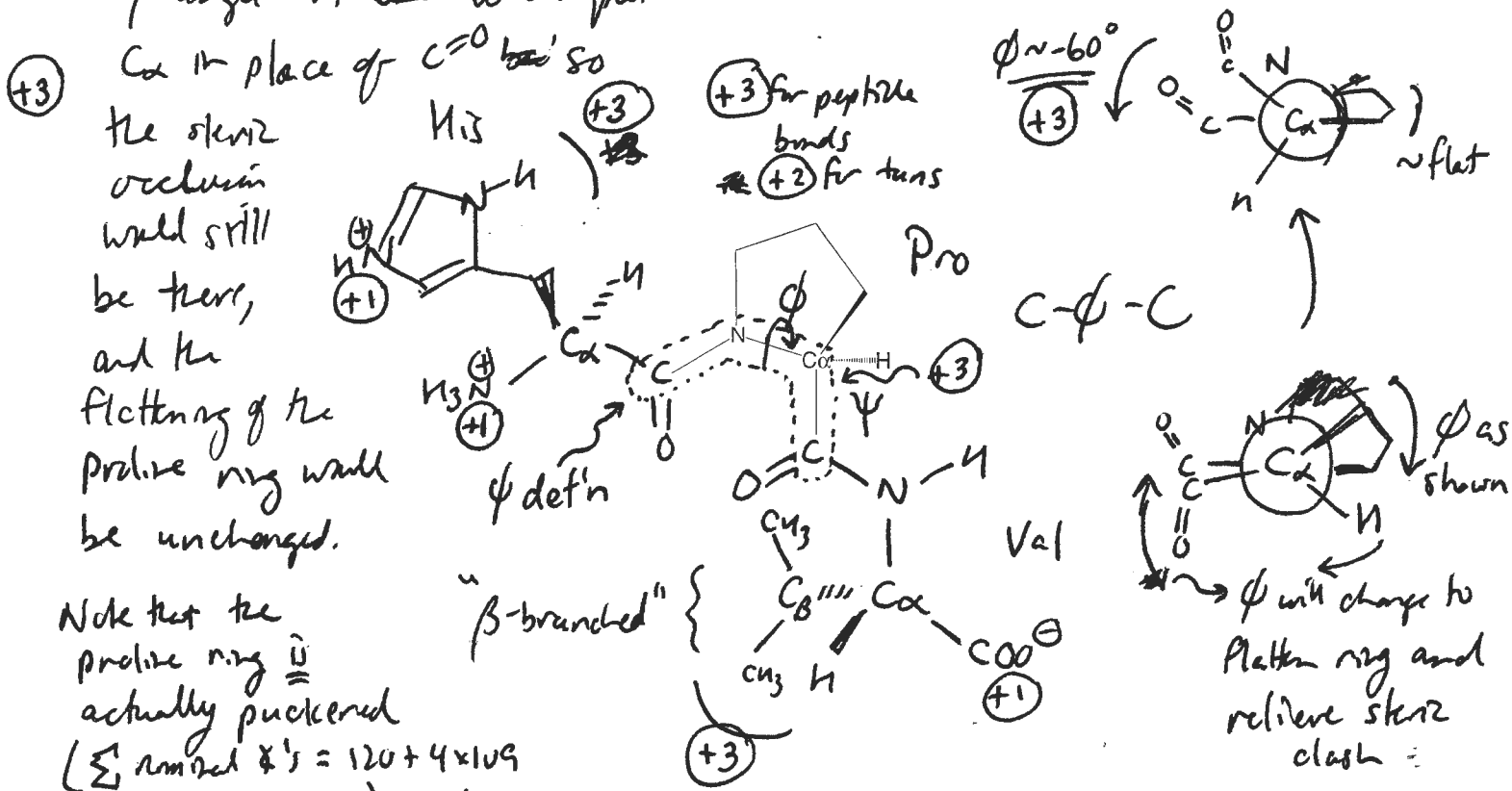
Score for the page 7/7

(b; 14 pts) Draw the tripeptide His-Pro-Val in its predominant ionic form at pH 5, with all of the peptide bonds in the trans conformation. Start from the ring given below. It's there twice in case you need to redraw.

(e; 9 pts) Indicate on your structure the four atoms that define the Φ angle for the proline residue. Assuming that the proline side chain is constrained to be flat, estimate the permitted value of Φ. Would your answer be substantially different if the His-Pro peptide bond were *cis*? Why or why not?



~ A cis peptide bond on the N-terminal side of Pro would not change the φ angle - it would put Cα in place of C=O so the steric occlusion would still be there, and the flattening of the proline ring would be unchanged.



Note that the proline ring is actually puckered ($\sum$ nominal χ's = 120 + 4×109 = 556 > 540), but this doesn't affect the φ angle much.

Score for the page 23

2. (40 pts) Protein Folding

(a; 9 pts) The thermodynamics of protein folding: What are the two main contributors to ΔS, and what are their signs and the sign of the overall ΔS? What is the sign of ΔH? What is the sign of ΔG for protein folding?

(+1) ΔS of conformational restriction is ⊖ for folding

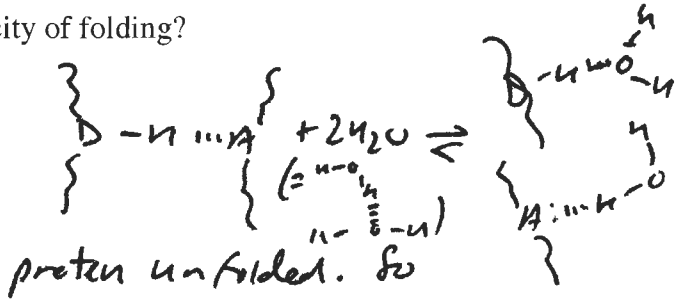
(+1) ΔS of hydrophobic effect is ⊕ for H_2O release upon collapse

(+2) net ΔS for folding is ⊖ (unfavorable)

(+2) ΔH is ⊖ for on noncovalent bond formation

(+3) $\Delta G = \Delta H - T\Delta S$ is typically ⊖ at low T, ⊕ at high T – [in some cases the temperature dependence of the hydrophobic effect itself leads to ⊕ ΔG at cold T = "cold denaturation"]

(b; 6 pts) Explain why H-bonds and electrostatic interactions make contributions to stability that are quantitatively much smaller than the binding energies of the H-bonds and electrostatic contacts seen in proteins. Why are they still important for the specificity of folding?



– ~~P~~An H-bond in a protein ~~is always~~ would be replaced by H-bonds to water if the protein unfolded. So

(+3) the increased stability of the protein reflects the <u>difference</u> in the strength of the protein H-bond vs. the water H-bonds. Similarly, the participants in an ~~ele~~ electrostatic interaction would otherwise be solvated in water.

– However, in a mutant or a misfold, buried protein moieties

(+3) lose the interactions with water but do not gain intramolecular interactions – this is a net loss and dis-favors all ~~pa~~ but native-like structures

Score for the page 15

Here is a proposed mechanism for the GroEl/ES folding machine.

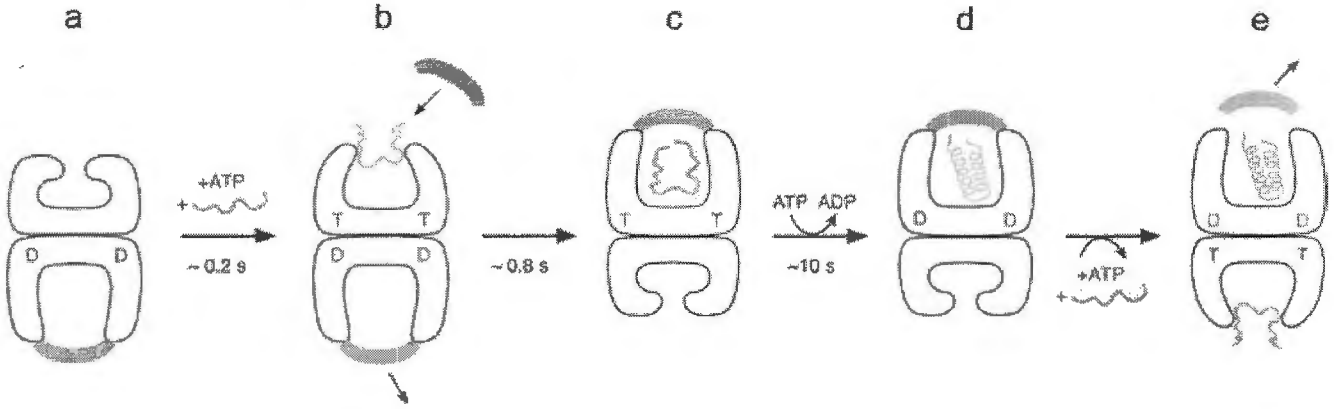


Fig. 2. The polypeptide folding cycle at GroEL. (*a*) The initial polypeptide acceptor state *in vivo* and in a

(c; 6 pts) We listed two related but distinct functions for the chaperonin. What are they; in other words, what does it mean to chaperone the process of protein folding?

(+3) - Allow repeated cycles of folding ⇄ unfolding to prevent getting trapped in misfolded states on the rugged energy landscape

(+3) - Enforce unimolecular folding to prevent aggregation

(d; 3 pts) What causes a candidate client protein to stick to GroEL?

(+3) A misfolded protein will frequently show patches of hydrophobic surface which bind non specifically to the hydrophobic patch inside the groEL cavity.

(e; 5 pts) In the c-> d step, the protein is released from binding and is allowed to refold on its own. We called the cavity a particular kind of cage, Name it and describe its function.

(+3) The Anfinsen cage - provides a hydrophilic surface that repels what should be the insides of the substrate protein, and enforces unimolecular folding. (+2) as above

(f; 3 pts) The client protein may need to be unfolded and allowed to refold many times. Why does a cyclic process like this require ATP hydrolysis? [If it didn't use an external energy source, what would happen?]

(+3) One of the five states above must be the thermodynamically most stable, and the differences in energy of are large w.r.t. KT = thermal energy. A cyclic process would get stuck in one state.

[And going around in a directional way always requires free energy input]

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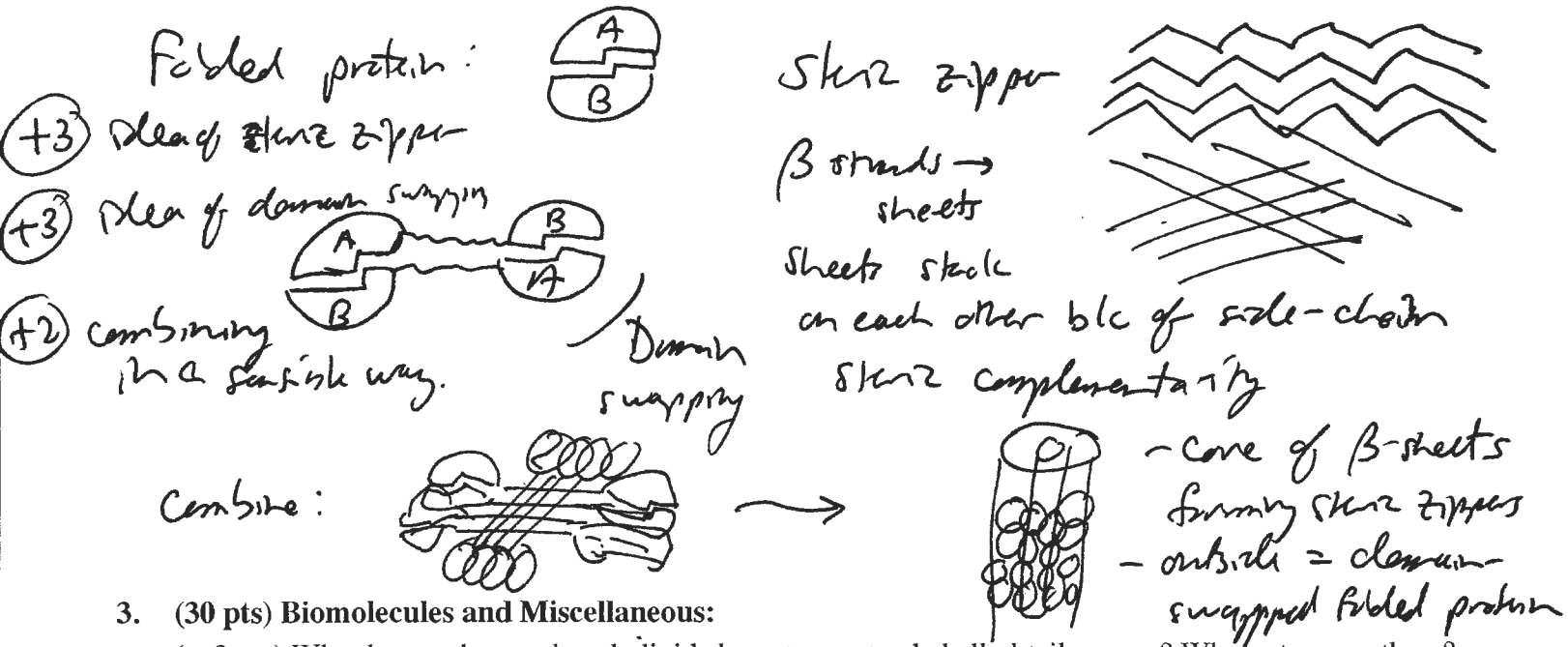
(e; 8 pts) Sketch the model that protein aggregation can occur **through** a combination of steric zipper (=stacked β sheet) formation and domain swapping.



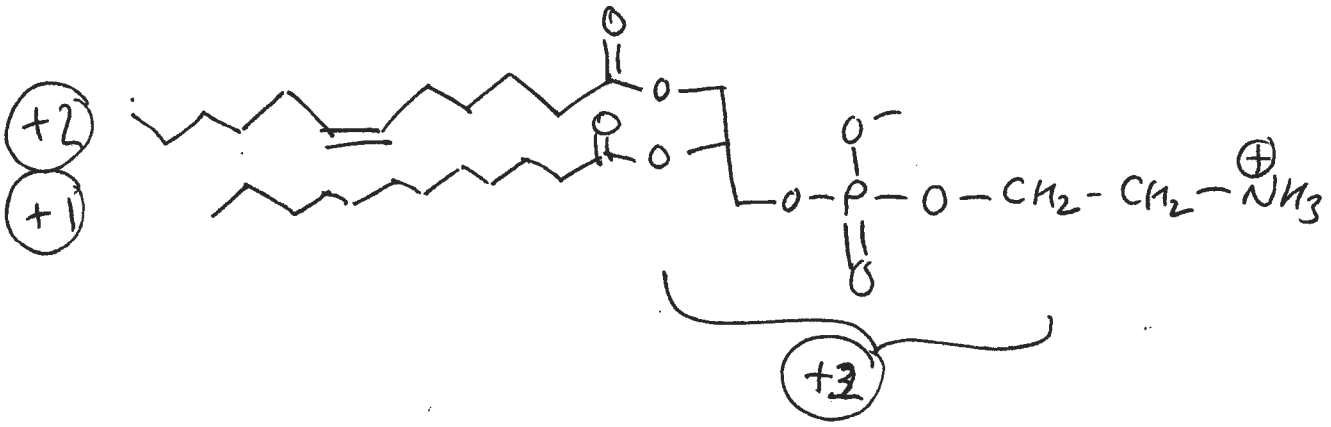
3. (30 pts) Biomolecules and Miscellaneous:

(a; 3 pts) Why do membrane phospholipids have two extended alkyl tail groups? Why not one or three?

(+2) One → micelles b/c the head groups are wider than one tail

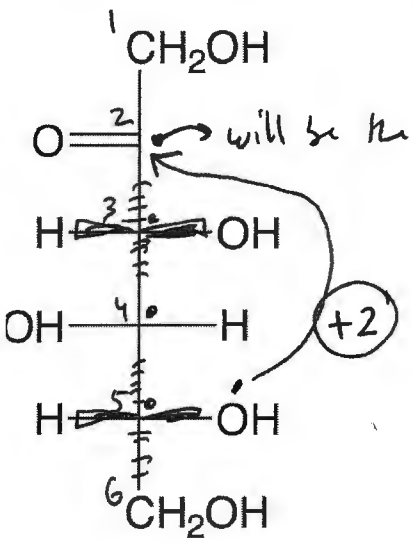
(+1) Three → presumably would be less stable as a bilayer due to exposed hydrophobic surface

(c; 6 pts) Draw a phosphatidylethanolamine (ethanolamine = $-OCH_2CH_2NH_3+$) with one saturated R group and one monounsaturated R group with a cis double bond.



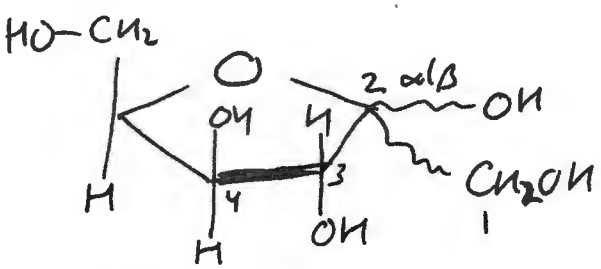
Score for the page 17

(e; 6 pts) Here is the Fischer projection of D-sorbose. Indicate which hydroxyl attacks the ketone to make the furanose form of the ring, and draw the Haworth projection of the furanose ring. Indicate the anomeric carbon stereochemistry with a squiggle.



D-Sorbose

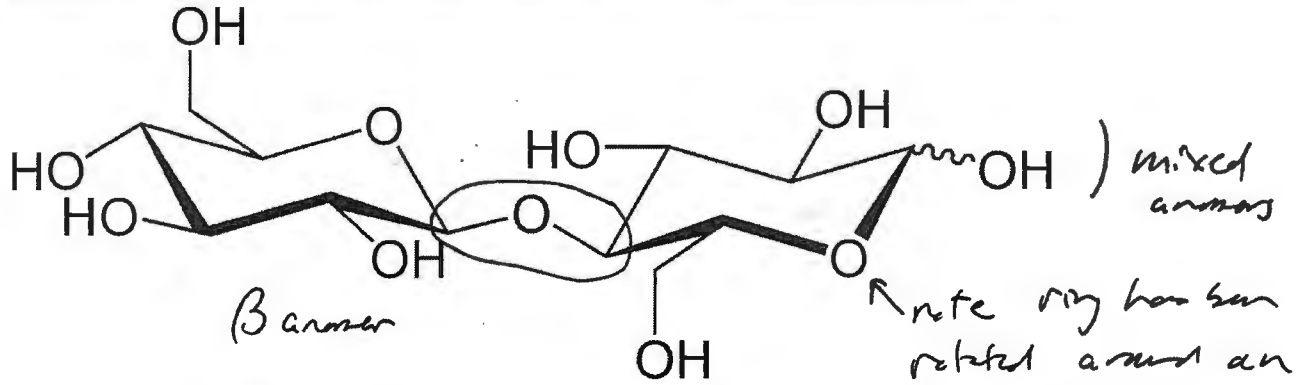
furanose → 5 membered ring including O



(+2) for Haworth of furanose

(+2) for correct

Here is the structure of cellobiose, a disaccharide derived from cellulose.



(3 pts) Circle and name the linkage between the two glucose moieties.

β(1→4) glycosidic bond

(+2) (+1)

Score for the page 9

(12 pts) We discussed several ligands for Hemoglobin, including CO_2, H^+, and Cl^-. Explain why it makes sense in terms of physiology for each of them to decrease the binding affinity of Hb for O_2.

(+4) CO_2 - ↑CO_2 indicates more active metabolism — means more O_2 is needed. Also an indirect indicator of pH, and a waste product that must be transported out of the body.

(+4) H^+ - ↓pH is another indicator of ↑ metabolic load must also be transported, partially on Hb.

(+4) Cl^- - flood into RBC as HCO_3^- floods out - again, ↑Cl^- is an indirect sensor for ↑ metabolism.

Page	Score
1	/7
2	/23
3	/15
4	/17
5	/17
6	/9
7	/12
Total	**/100**

Score for the page 12