Biomolecular Structure '99 – Exam 1

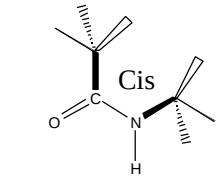
This exam must be worked on **independently**. You are not to discuss any aspect of this exam with ***anyone*** other than Professor Martin. You may use the text, any hand-outs, and the CHIME/Rasmol/SwissPDBViewer help WEB Sites. You should download structure files from our WEB site, where appropriate, and manipulate them in SwissPDBViewer and/or Rasmol, but you may not use other WEB resources or material. Do not begin this exam at the last minute…

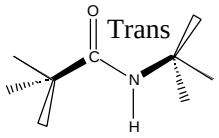
Hint: in several of the questions below, you are asked to describe something with a specific word count limit. Draft your response in a word processor, including everything you can think of that is relevant to the question. Then go back and choose what you believe to be the most important and relevant points and pare the text down to the stated limit. You will be judged on what you choose to present as well as how you present it.

1. We have seen that the peptide bond is planar and can exist in two forms, *cis* and *trans*. Interconversion between *cis* and *trans* forms of the peptide bond is energetically unfavorable (we say there is an energetic barrier to rotation).

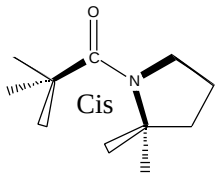
a) (15 points) The amino acid Pro exists about 25% in the *cis* form, while the other amino acids are thought to exist *cis* to only about 0.1%. Briefly propose first why amino acids (including Pro) prefer a *trans* orientation and then explain why Pro tolerates *cis* better than the others.

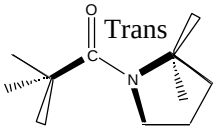
The preference for a *trans* orientation around the C-N bond is most readily explained in terms of sterics. The *trans* configuration above keeps the bulkiest groups farthest away from each other. This is clear for the non-Pro amino acids ("others" at right).





Others

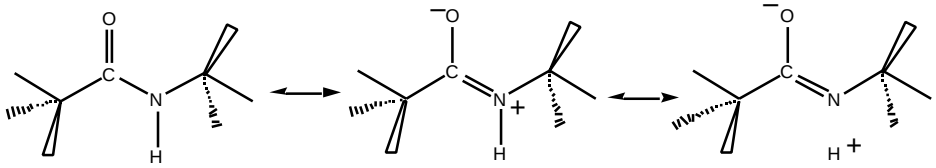




Pro

In Pro, nitrogen has two bonds to carbon, both of which haver unfavorable steric interactions with the chain to the left of the carbonyl above. So the unfavorable interaction described above not decrease, rather a new unfavorable interaction appears, making *trans* **relatively** less favorable.

b) (15 points) It is also observed that the (non-catalyzed) kinetic *activation barrier* for *cis-trans* isomerization is lower for Pro (13 kcal/mol for Pro vs. ≈20 kcal/mol for the others). Propose an explanation. Hint: it does *not* involve sterics).



It is resonance stabilization which limits rotation around the C-N bond, in other words, resonance form(s) impart a partial double bond character to the bond. One of the reasonable resonance forms places the positive charge directly on the amide proton. Pro has no NH proton, so that the equivalent resonance form is much higher in energy. Therefore, the resonance stabilization is less, resulting in a lower bond order for the C-N bond, and a lower barrier to rotation!

c) (10 points) If you were to design an enzyme to catalyze the interconversion between the *cis* and *trans* forms of a peptide, describe a key feature which you would design into the site to facilitate this interconversion.

The best answer to this utilizes the principle outlined in part (b). Anything which **destabilizes** the resonance forms which have double bond character for the C-N bond will lower the barrier to rotation and speed the interconversion. Some suggested placing nonpolar groups near the carbonyl oxgen and the amide proton. Others suggested placing a positive charge near the amide hydrogen or a negative charge near the carbonyl oxygen.

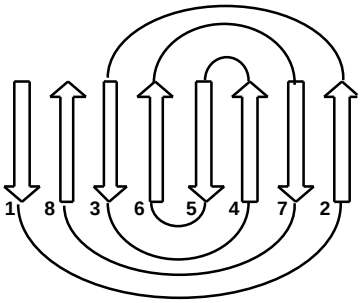
Alternatively, one could design the active site to classically stabilize a form of the peptide bond which is rotated 90° from planar (H-bond donors to the carbonyl oxygen or acceptors to the amide hydrogen). But you must specify that this stabilization is only for the rotated intermediate…

2. Go to the WEB site and download two protein structure files.

Q2Mono.pdb	contains the monomer of protein X with ligand
Q2Dimer.pdb	contains a dimer of the same protein (with ligands)
Q2Unbound.pdb	contains the monomer lacking bound ligand

a) (15 points) Exam **Q2Mono** and describe the structure according to the things we have talked about during the first part of this course, including motifs, what holds components together, etc. (provide as much detail as you can, but limited to 300 words).

The structure contains a beta barrel with a jelly roll (Greek key) motif. The jelly roll is not textbook-simple, but is nevertheless clearly a jelly roll.



On top of one extended sheet lie two interacting α–helices.

The beta barrel is stabilized by a very hydrophobic core. Similarly, the interactions between the barrel and the two helices are primarily hydrophobic, just as we predicted in class. These interactions provide stability for the molecule.

b) (10 points) The sequence from 43-50 is extremely hydrophobic, while the sequence from 76-85 is a mix of hydrophobic and hydrophilic. Explain (100 words or less).

This is very simple. Typically, we expect a sheet involved in a barrel to have one hydrophobic side pointing into the barrel (to provide hydrophobic burial) and one hydrophilic side pointing towards solvent (to interact with the solvent). The 76-85 strand follows this expectation, since it's on the surface of the protein. However, the 43-50 outside face of the barrel packs onto the helices and does so via hydrophobic collapse between sheet and helix (hydrophobic) residues.

c) (15 points) Examine **Q2Dimer** and describe the interaction between the two monomers, again with respect to what you have learned so far in class (limit 200 words).

Hint: In Rasmol, use commands like the following:
Select *A and within(5.0,*B) to help see the interface.

A pair of helices from each of the two monomers combine to form what at first looks like a four-helix bundle in the dimer. However, more careful analysis (either visual or by using Rasmol to view interacting amino acids) reveals that the interface is primarily between one helix on each of the monomers. The interaction is more akin to a coiled-coil.

Looking at this interface/core, we find a limited number of hydrophobic amino acid side chains. Specifically, Leu151 and Leu161 form a very nice hydrophobic match, as in the Leu-zippers.

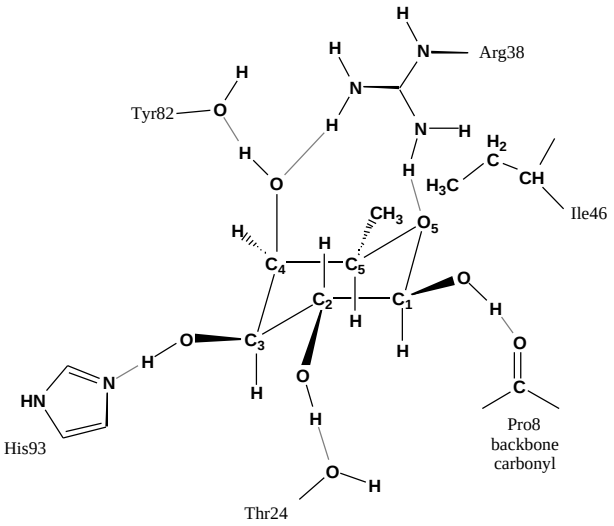
Tyr103 residues H-bond across the interface.

Asn154 H-bonds across the interface with Glu157.

Arg162 H-bonds within a helix with Gln158, to provide "buttressing."

We can also see a water-mediated electrostatic interaction between Glu 157 on the helix and Arg 101 on a loop (there are two identical such interactions).

d) (10 points) Align **Q2Unbound.pdb** with the **Q2Mono.pdb** structure. Describe the interactions between the bound ligand and the protein (limit 200 words).



The following residues H-bond to sugar hydroxyl groups: Thr24, His93, Arg38 (more than one H-bond), and Tyr82. The backbone carbonyl of Pro8 also H-bonds to a sugar hydroxyl. Ile46 has a stable hydrophobic interaction with the 6-methyl group of the sugar.

Looking at the overlayed structures:

Tyr82 and His93 do not move much to accommodate the sugar; they are already positioned to coordinate the sugar.

Thr24 moves a bit.

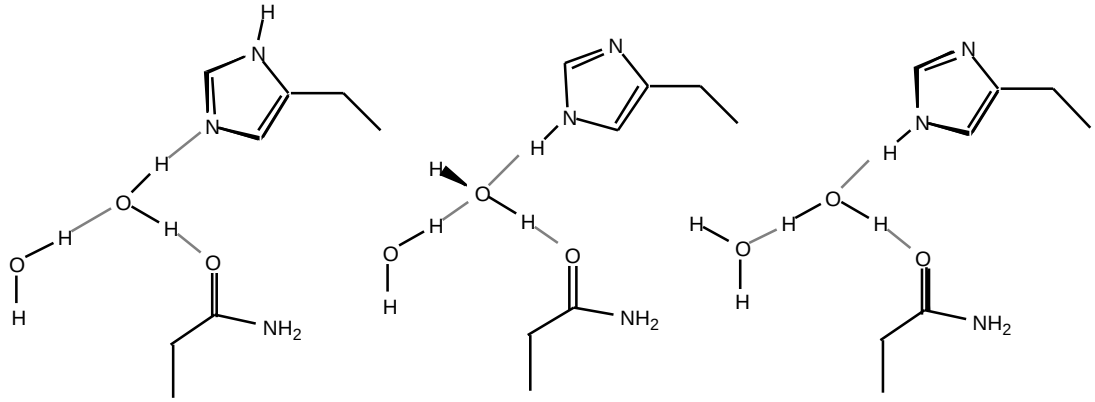
Similarly Ile46 moves toward the sugar somewhat.

Arg38 moves substantially to make two H-bonds to the sugar.

Pro8 seems to be disordered in the unbound structure, but is well-ordered in the bound structure.

e) (10 points) In the structure **Q2Dimer.pdb**, examine carefully water molecule (HOH) 216 (atomno=2859). Describe its interactions (limit to 200 words, but include a drawing (need not be fancy) of the bonding).

This water molecule H-bonds with His129 and Gln158, as well as with another water molecule (210). Any of the pictures below is consistent with the structure.



Extra credit: A colleague has been working on a tutorial for protein structure and would like feedback from faculty and students. The software is available (only) in the CRC under our class directory. For extra credit, take yourself through the tutorial and evaluate it. What features do you like? Did you learn anything you didn't know? Extra extra credit for finding errors!