SB241 **Homework 2B** **Dave McKay/ Chris Garcia**

Due: Monday 20 October 2003

This is the second of two installments of homework on protein structure.

Protein coordinate files in pdb format can be downloaded from the course web site for:

--ras protein with GMPPNP (a GTP analog) bound: **ras-gmppnp.pdb**
--ras protein with GDP bound: **ras-gdp-on-gmppnp.pdb** (this molecule has been superimposed on the GMPPNP structure of ras, and the header has been spliced into the file; consequently, the unit cell and space group information is no longer relevant).
--a fragment of the B-subunit of the F1 ATPase: **F1-B-frag.pdb** (please note that this is a fragment of one subunit; it will simplify your life immensely over looking at the entire molecule)

This part of the assignment should help you feel comfortable with (a) looking at two structures of a protein and recognizing their conformational differences; (b) finding useful information (e.g. resolution of structure determination; B-factor of particular part of the molecule) from the pdb file; (c) looking at detailed interactions of a ligand (GDP or GMPPNP in this case) with the protein and how the interactions change going from GMPPNP to GDP.

Paper that is included with this exercise:

Milburn, M. V., Tong, L., deVos, A. M., Brunger, A., Yamaizumi, Z., Nishimura, S., and Kim, S. H., Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins, *Science, 247*, 939-945. (1990).

First, try loading the two ras structure files. This can be done with Swiss PDB Viewer, for example. (If you are using Swiss PDB Viewer, then in order to see solvent molecules, you will need to go to the "Prefs" menu, then to "Loading protein…", and deselect "Ignore solvent (WAT SOL HOH)".) Look at the headers on the two pdb files. (In Swiss PDB Viewer, this can be done by clicking the little text page icon at the lower left of the toolbar or by using the menu "File" -> "Open text file…").

1. What is the resolution of each of the structures?

2. First, locate the nucleotide (hint: it's bound in the P-loop). This will be the reference point for much of this exercise. Also, find the second Walker motif, $D_{57}XXX$ on the beta strand next to the P-loop motif. Now, look for the most obvious changes in the backbone between GDP-bound and GMPPNP-bound. Referencing the paper by Milburn et al., you should be able to find the "Switch I" and "Switch II" regions. Give a succinct description of the main differences in overall/backbone conformation, as you see them. Are there one or two residues that you would pick as being particularly important for the change, in the sense that there are major differences in their conformations between the two structures?

3. Now, look at the Mg-nucleotide complexes. Mg^{2+} generally has "octahedral" coordination, which is to say, it likes ligands at ±x, ±y and ±z in an orthogonal coordinate system at ~ 2Å distance. What are the Mg^{2+} ligands in the MgGMPPNP structure, and what are they in the MgGDP structure (draw a rough sketch of them). Are both structures at sufficient resolution to see the local water molecules clearly?

Now, looking at the F1 ATPase domain fragment,

4. Overall topology: Find the nucleotide, the P-loop, and the second Walker motif, $D_{269}Dxx$. Is the topology of the placement of these two motifs in the □/□ structure similar to that found in ras protein, or that of RecA?

5. Look at the environment of the Mg^{2+} ion in the MgAMPPNP complex. What ligands (at ~2 Å) do you see in this structure? Are there any solvent molecules (the answer is no, and it is not because your instructor forgot to keep them in the file). Why are you not seeing solvent molecules in this structure (Hint: look at the header of the pdb file).

6. In the Walker B motif, what is the distance between the $C_{\square}$s of residues 269 and 270, and how does this compare to the standard value of 3.9 Å? Can you see what is unusual about this peptide bond? (Its unusual feature is observed in many, but not all, ATP and GTP binding proteins of the Walker motif class).

7. B-factors. These are recorded in the pdb file; on each line with an atomic coordinate, they follow the x,y,z and occupancy values:

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                                      x      y       z   occ. B-factor
ATOM      9  O   ALA Z   2       4.142   2.061   2.497  1.00 20.00
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They also pop up with the coordinates when you click on an atom in Swiss PDB Viewer.

By coloring a molecule by B-factor, you can locate the regions that have the highest B-factors, i.e. the highest uncertainty in the structure, For the F1 ATPase fragment, and also for one of the ras structures, find a region of high B-factor, and find (either by clicking on the atoms or by scanning the pdb file) the numerical values. In ballpark figures, how do they compare for the two structures?

Chris Garcia Problem: On the course web site there are two PDB files of protein-protein complexes. In fact these complexes are of a single receptor bound to two different ligands using the same receptor binding site. Your homework problem deals with understanding the different and shared binding modes the receptor uses to bind to two different ligands:

a. Download the PDB files and examine them in your swiss-PDB program. In particular zoom in on the "interface" or contact region between receptor and ligand. Describe your initial observation of the two interfaces, compare and contrast.

b. Go to the "protein interaction server" (http://www.biochem.ucl.ac.uk/bsm/PP/server/) and calculate the various values describing your two protein interfaces. List them here. What differences do you see in the results. can you find a hotspot ? (clue, each of the two molecules in the complex have different chain ID's to input into the program).

c. Based on the equations I showed in class relating heat capacity to buried surface area, calculate an estimated Cp from the output of the interaction server. What does this value suggest as the driving force for association ?

d. Based on the previous analysis, can you make an educated structural argument for why one receptor is able to bind to two chemically unique ligand surfaces ?