CS 663: Algorithms in Structural Molecular Biology
Assignment #3

Please email to your TA by 1/30/2013

Molecular Visualization with KiNG

Retrieve PDB file

Go to http://www.rcsb.org, search and download the PDB file 3EZ5.pdb. This is the structure of DNA Polymerase I (pol I) from a thermostable strain of *Bacillus stearothermophilus*. Also download the primary citation for this structure from http://www.ncbi.nlm.nih.gov/pubmed/20152155?dopt=Abstract.

Download KiNG

Go to Resources page on the course website and follow the instructions to download and install KiNG.

About DNA Pol I

DNA polymerases are enzymes that participate in the replication of DNA, an essential step in the cell division process. These molecular machines have a very high fidelity, which an error of 1 in 10^5 bases. The structure of the DNA polymerase can be described best in terms of a human right hand, with the three subdomains as the thumb, fingers and the palm domains. The structure in the PDB file 3EZ5.pdb is the structure of pol I in closed form. (See the primary citation for more details.)

Visualization exercise

Open KiNG and import the file 3EZ5.pdb. This file contains two identical copies of the enzyme pol I along with double-stranded DNA (chains A,B,C constitute one copy, chains D,E,F the other). The palm, thumb and finger domains roughly consist of residues 297-492, 493-645 and 646-876 respectively. Import the two protein chains (A and D) as ribbons, and the four DNA chains as balls and sticks. Color chain A with distinct colors for alpha helices, beta sheets and loops. Color chain D with distinct colors for the palm, thumb, and finger domains. You may have to import the pdb file in parts to be able to do this. Color the bases of the template DNA strand and the primer DNA strand with different colors for both copies. Use the Edit/Draw/Delete option from the Tools menu to change colors. Label the two copies as Pol I copy 1, Pol I copy 2 and put the appropriate chains under each heading, using the Edit hierarchy option under the Edits menu. Select a view of your choice, save the view using the Views menu, giving it an appropriate name.

Zoom in the active site of chain A, which shows the DNA strands up close. From the primary citation for the structure, find out the residues required for the function of the enzyme, and import the side-chains of those residues again in ball and stick format. Color the protein side-chains with a different color than the DNA bases. Save the view. Now, zoom in on protein residue 714. Using the Measure angle and dihedral option from the Tools menu, find out the the closet DNA atom from the protein residue 714. Draw a line between the two atoms using the Edit/Draw/Delete option from the Tools menu. Save the view.

Using the Edit text option in the Edit menu, give a one-line description of the saved views. Save the kinemage as YourName-DNAPolI.kin and email it to your TA by the due date.

MolProbity and All-atom contact analysis

Go to the website http://molprobity.biochem.duke.edu/ and upload the PDB file 3EZ5 on the MolProbity server. Once the file is uploaded, continue to add hydrogens to the file with the No-flips option (the X-ray structure file does not contains hydrogen atoms). Once that is done, continue to Analyze all-atom contacts and geometry. In addition to the default options, turn on the button for van der Waals interactions. Once that is complete, download save the resulting multi-criterion kinemage. After saving your file, log out of MolProbity server.

Open the saved kinemage in KiNG. You will see a stick representation of the protein and DNA chains, with dots for all-atom contacts, which include clashes (atom overlap of more than 0.4 Å), hydrogen bonds and van der Waals interactions between atoms. Turn off the protein side-chains. Turn off the clashes and van der Waals contacts. Zoom in on any alpha helix and observe the characteristic pattern of hydrogen bonding between backbone atoms. Save the view. Repeat the exercise with any beta sheet in the protein. Save the view. Turn the side-chains back on. Turn off the hydrogen bonds and turn on the van der Waals interactions. Zoom in on the DNA strands and observe the stacking interactions between the DNA bases. Save the view.

Turn off the van der Waals and turn on the clashes and hydrogen bonds. Zoom in on the residues essential for enzyme function (the ones which are mentioned in the paper) and observe which of these residues make hydrogen bonds with DNA residues, and which residues clash with the DNA. Save the view. Describe this interaction in brief in the text region of the kinemage (using the edit text option). Save the kinemage and email it to your TA by the due date.