

Assignment 2: Introduction to the Insight II Modeling Package and the PDB File Structure and Retrieval

1. **Introduction to Insight II.** If you are not familiar with Insight II you might start by reading the short manual and the tutorial from the web site of the home page of Molecular Simulations (MSI) (www.accelrys.com/insight/index.html). The site URLs may change. The manual also contains a short list of basic UNIX commands and a description of simple text editors. Some of the displays on our computers are slightly different from those described in the tutorial but the differences are not critical. For example, in our version the help window automatically follows the tasks invoked from the pulldown menus, and the dial boxes are located on the left, rather than the right, side of the screen. For more information, refer to the *Insight II User Guide*.
Note: after opening Insight II, ignore the message about detection of the unlicensed mode. Our site does not have license for the Sketch module. Repeated warning messages might occur during the “build in” Insight II Pilot tutorial.
2. **Running Insight II.** Before starting Insight II, you must define the set of environmental variables by running two commands:

```
source /local/msi/cshrc
```

 Alternately, you can insert these lines into your `.cshrc` file and they will be executed by the system automatically every time you log on. In that case, you will only need to specify the command

```
source .cshrc
```

 after editing your `.cshrc` file the first time. When this insertion is complete, you can run Insight II by specifying the command

```
insightII
```

 at the UNIX prompt. Remember, UNIX commands are case sensitive.
Note: NYU staff may have already inserted these commands in your .cshrc file.
3. **PDB Structures.** Check the PDB web site for information about the type of stored 3D structures (i.e., proteins, DNA, RNA, DNA/protein complexes, etc.) and the amount in each group. Report your findings.
4. **Retrieval of PDB Files.** Using the web PDB browser, find coordinate files for the crystal forms II and III of Bovine Pancreatic Trypsin Inhibitor (BPTI) among the many BPTI entries. From the PDB Home page, go to **Searching and Browsing PDB** and then choose **PDB’s web Browser**. You can search by specifying the abbreviation “BPTI” in the Compound window. When the search is completed, records containing BPTI (including its mutated forms) will be displayed at the bottom of the page. Note their ID codes (a number followed by three letters). The two middle letters in this code constitute the name of the subdirectory where the file of

interest resides. For example, the subdirectory name for ID code 1abb is ab and the file name `pdblabb.ent`. Ftp to `ftp.rcsb.org` and login as anonymous (the password instructions will be on the screen). Change directory to `pub/pdb/data/structures/divided/pdb/ab` and get the desired file with the command

```
get pdblabb.ent.z
```

5. **Format of PDB Files.** Read the text in the top of both PDB files and describe the differences in the number of recorded residues, structure resolution, number of solvent molecules, experimental conditions, etc. Attach to the assignment sheet a printout of a few lines, starting with the word ATOM, from a PDB file; mark with arrows and describe the content of each specific format field. (The PDB browser contains information about the format; see also the original paper on PDB files: *J. Mol. Biol.* **112**, 535–542, 1977).
6. **Displaying a Protein in Insight II.** Retrieve from PDB the file of mutated form of BPTI (ID = `7pti`), and start Insight II. From the top menu bar select **Molecule**² and then choose **Get**. Press the PDB button in **Get File Type** and the **User** button specify the directory. Select the file of the `7pti` structure. (Do not press **Heteroatom** button). Execute. The structure of BPTI should now be on the screen. Use **Object**/DepthCue and then **Transform**/Clip for viewing the protein. Change the display (**Molecule**/Display) to **Backbone** only to speed up the response time. Label the mutated residues (**Molecule**/Label). Repeat the operations described above to display the structure of BPTI's crystal form II (keep both structures on the screen). Now, overlay both structures by selecting **Overlay** from **Transform**. In few paragraphs describe the structural differences between both forms of BPTI.
7. **Ramachandran Plots.** To create a file listing with all the dihedral angles ϕ and ψ for a protein, you can use **Protein**/List from the **Biopolymer** module. Select **Dihedrals** and the protein (any of the structures). Press **List_to_file** button, specify the file name, and execute. From the recorded data create a scatter plot (phase diagram), so that each point corresponds to one (ϕ , ψ) value.

²The following notation will be used throughout the homework assignments:

- **Pull-down** corresponds to a menu bar pull-down.
- **Command** corresponds to a command from the pull-down menu.
- **Option** corresponds to an option in the dialog box of a given command.

Summary of Items to Hand in:

- (a) Data with the amount of PDB 3D structures for each category of biomolecules.
- (b) Description of the differences in the informational part of the PDB files for form II and form III of BPTI.
- (c) Explanation of the PDB format for storing atomic coordinates.
- (d) Description of the structural differences between BPTI (form II) and the mutated form (7pti).
- (e) The table with dihedral angles ϕ and ψ listed for BPTI.
- (f) Scatter plot with points (ψ , ϕ) for each residue of BPTI.

Background Reading from Coursepack (Appendix B)

- M. Levitt and A. Warshel, "Computer Simulation of Protein Folding", *Nature* **253**, 694–698 (1975).
- M. Karplus and J. A. McCammon, "The Dynamics of Proteins", *Sci. Amer.* **254**, 42–51 (1986).
- Y. Duan and P. A. Kollman, "Pathways to a Protein Folding Intermediate Observed in a 1-Microsecond Simulation in Aqueous Solution", *Science* **282**, 740–744 (1998).
- H. J. C. Berendsen, "A Glimpse of the Holy Grail", *Science* **282**, 642–643 (1998).
- X. Daura, B. Juan, D. Seebach, W. F. Van Gunsteren, and A. Mark, "Reversible Peptide Folding in Solution by Molecular Dynamics Simulation", *J. Mol. Biol.* **280**, 925–932 (1998).