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InsightII: Building and Modifying Peptides, Minimization and Molecular Dynamics Simulations.
Lab 8

Objectives

The objectives of this lab are to learn:

1) building and modifying peptide structures using Biopolymer program
2) setting up and running minimization and molecular dynamics simulations
3) simulations of peptides and proteins

The tutorials recommended for this lab contain a variety of lessons, which include various molecular mechanics simulations. They will demonstrate typical approaches utilized to analyze and simulate peptides.

Outline

Setup

Login into your account on Frodo, where InsightII is set up. (You have modified your .cshrc file earlier, so everything should be set up.)

Open InsightII by typing `i` at the command prompt (`> i`).

Building and Modifying Peptides using Biopolymer

To build up peptides or proteins from amino acid sequences, InsightII uses command **Append**, which is found under **Residue** in the **Biopolymer** module.

Building Peptide Structures

First, enter the **Biopolymer** module by clicking on the MSI logo icon in the top left corner of the screen and selecting this module.

Next, choose **Residue Append**. In the command block, enter the name for the peptide (e.g., pept1) in the **Molecule Name** box, and when the **Residue** option becomes active, choose an amino acid or group from the **Residue Type** list by picking it with the mouse. Sequentially picking residue names will build a peptide from N- to C- terminus in an extended conformation in the graphics workspace. If you wish to build a particular secondary structure conformation, select an appropriate **Motif** setting and continue picking residues. It is also possible to specify the **Phi, Psi** and **Omega** angles (refer to your lecture notes for definitions). However, it might be more convenient to build the structure in an extended conformation first, and then modify this conformation.

The possible conformational motifs are defined as follows:

Motif	Omega	Phi	Psi
Alpha R helix	180.0	-65.0	-40.0
Alpha L Helix	180.0	65.0	-40.0
3-10 Helix	180.0	-60.0	-30.0
Pi Helix	180.0	-30.0	-90.0
Beta Strand	180.0	-120.0	-120.0
Extended	180.0	-180.0	-180.0

Exercise: Build a peptide about 10-15 residues long using **Append** command. You can vary the structural motif if you wish. Examine the effect of adding an OH group as the final residue. What happens if you try to append once you have added this group? Why?

Modifying Peptide Structures

The commands to modify secondary structure of a peptide or a protein are under the **Protein** menu in **Biopolymer**. **Secondary** allows you to alter the conformational motif for chosen residues or for the whole protein. To enter a specific type of turn, select **Turn** from the **Protein** menu, select the type and indicate the initial residue. Alternatively, you can enter values for **Phi, Psi** and **Omega** angles.

The **Rename** command under **Protein** reassigns the residue name for a protein. This can be useful when pieces of proteins have been spliced from different sources.

The **Replace** command under **Residue** can be used to make amino acid mutations and to change a residue from L- to D-, or the opposite. Residues can be deleted using **Delete** command. Explore other commands under **Protein** and **Residue** with **Insight_Help**.

Exercise: Introduce a turn in the peptide you built. Modify secondary structure for ranges of residues; explore secondary structural motifs. After a modification, create a ribbon (**Ribbon** command under **Molecule**) to look at the structure.

Change protonation state of the C- and N-terminal residues (**Cap** under **Protein**). What is this command doing? Change the protonation state of the side chains by specifying various pH's in the **Hydrogen** menu (under **Modify**). Replace various amino acid residues. In the final stage, replace residues number 3 and 8 with Cysteines. Form a disulfide bond between them using **Modify Bond** command.

This peptide will be optimized in the next part of the lab.

Saving Structures

Optimized molecules can be saved using **Molecule Put** menu. File type most frequently used is *Biosym*, but you can also save molecules in other formats, such as Sybyl_Mol2, PDB, CHARMm, etc.

Exercise: Save your peptide after minimization (see below) using Biosym format; you can change the name of the molecule when you create **File Name**. This creates two files: **.car** and **.mdf**, which contain information about the molecule. The name of the saved molecule is the prefix of these two files. For a more detailed description, see the **Discover Files** section below.

Another way to save structures, regardless whether they are correct or not, is to save files as folders. Go to **File** menu in the **Viewer**, choose **Save_Folder.** Leaving a * in the *Save Object* box ensures that all objects present on the screen, even those blanked, will be saved. Choose **Folder Name** and execute. This creates a **.psv** file, which is a binary file (you can't read it with the `more` command) that saves all objects present visualized as they are on the screen. This is

useful when you work on the project and have to stop. It saves your work exactly at the point you left it. Moreover, you can create final pictures of your structures, e.g., a ribbon view of your protein, and save it for making pictures later, etc. You can open the file using **Restore_Folder,** and the structure appears on the screen exactly as you saved it.

Note: **.psv** files are usually quite large, more so if they contain some sophisticated visual displays, such as ribbons or CPK. Thus, it is necessary to remove these files as soon as possible (use Unix shell for that, `remove` command).

Exercise: Retrieve lysozyme molecule using **Molecule Get** command. Create and color ribbon, display active site residues (use **Subset** menu), etc. Make it as nice a picture as you like. Save the structure with **Save_Folder**, then remove all objects using **Object Delete**. Make sure no objects are present. Now, restore the folder. When you are done with this exercise, delete all objects and the **.psv** file you created.

Running Discover Simulations

A general strategy for setting up Discover simulations was given in the previous manual (page 11). Before any run, **always check Hydrogens** and **fix Potentials**. You can change atom charge, hybridization or potential if needed, and check it by labeling (under **Molecule Label**).

Note: You can choose a forcefield you want to use with the **Forcefield** button (on the left). The default is cvff.

The procedure for running minimization with **Discover** has been explained in the previous manual.

Exercise: Run a minimization on the peptide you constructed. Use 100 iterations, steepest descent gradient, no cross, no morse, till the derivative reaches 0.1. Run it interactively and observe the peptide.

Discover Files

When the **Discover** job has completed, you will see a minimized structure on your screen. There is also a number of files created before or during the run. These files are found in the directory from which you have run **Discover**. They will have a file prefix which matches the name of the object that was minimized, along with a job number. Here are some of the files:

.car The initial coordinate file for the structure. It contains the atom name and number, x, y, and z coordinates, the residues name and number to which the atom belongs, and the potential type and charge for each atom in the unminimized structure. This file is written

automatically when a **Discover** job is started.

.mdf The molecular data file, supplements the **.car** file in describing a structure. It contains atom names, potential types, group and residue names, partial charges, switching atom, out-of-plane, and free energy flags, information about number of bonds and bond orders. Also written automatically before a **Discover** run.

.inp The command file, which contains all of the instructions for running the simulation. It can be set up from **InsightII** by using the options in the **Discover** module, or it can be created or edited at the Unix level.

.cor Similar to **.car** file. Contains the same information but for the minimized structure.

.prm Summarizes the parameter assignments made and identifies any parameters which were assigned automatically.

.out The output file, contains a log of the entire **Discover** run. Records summaries of all steps performed in the simulation, including the energies and derivatives before and after the simulation.

Exercise: Open a Unix shell, and use the command `more` to examine **.car, .mdf, .inp, .prm** and **.out** files created during the minimization of your peptide.

Running a Background Discover Job

Molecular mechanics calculations can take a long time, and that time increases with the size of the molecule. Therefore, it is better to set up a **Discover** run and execute it non-interactively. This allows the graphics to be used for truly interactive work.

Discover requires the files **.mdf, .car,** and **.inp** to initiate a run. To create these files, set up a **Discover** run as described earlier, but when filling the **Run** parameter box, choose *Command File* as **Computation Mode**. Other parameters should stay the same.

Make sure that all three files needed are in the proper directory. When running a **background discover job**, start the process by typing the command `discover` at the Unix prompt (> `discover`). You will be prompted to supply the file prefix and answer a few questions. When you use cvff forcefield, all the questions will be satisfied with the default (hit "Return" button). Use this option for any future Discover calculations, except when specified otherwise.

Molecular Dynamics Simulations

To perform dynamics, enter **Discover** module and select **Dynamics** under **Parameters.** In the parameter block, choose the following parameters:

List: Off
Equilibration: 100
Steps: 1000
History: 10
Charges: On
Temperature: 300
Time Step: 1.0
Constant Pressure: Off

This defines a molecular dynamics simulation in which the target temperature of 300 K is reached in 100 femtoseconds (equilibration), since the time step is 1 fs. Steps define how many iterations are to be performed after the system is equilibrated (*resume* stage): here, 1 ps. The history option determines at what interval dynamic structures are written to a file (called **.his**).

Exercise: Set up molecular dynamics simulations on your peptide using parameters described above. Go to the **Run** parameter box, choose *Interactive* as *Computation Mode.* Remember to set *Run Minimization* to *Off*, and *Run Dynamics* to *On.*
Examine the input and output files generated by this calculation.

Constraints and Restraints

Atoms or residues can be fixed or restrained prior to running minimization or molecular dynamics simulations. This allows us to carry out a variety of modeling strategies to address specific objectives. Moreover, introducing constraints or fixing atoms limits simulation time, which may be of importance when working with large molecules, such as proteins.

In the **Discover** module, go to the **Constraint** pulldown. **Fix** parameter box allows you to fix various elements of the molecule, starting with atoms up to the whole molecule. **GenericDis** menu allows you to add restraints on distances between atoms. Explore other commands under **Constraints**, such as **TemplateForce, Tether** and others using **Insight_Help**.

Exercise: Set up a minimization or dynamics of you peptide, in an interactive mode, using **Fix**. First, adjust hydrogens and potentials. Go to **Fix** parameter box, choose residues to fix.

Note: If you run a simulation on a small number of residues, it might be easier to fix the whole molecule first, and then delete residues of interest. To do that, choose the following parameters:
Color Constraint: Off or On
Activation: Add
Atoms_to_Fix: All
Constraint_Level: Molecule
Fix_Spec: click on the molecule or type its name
Now, choose parameters as follows:
Activation: Delete

Atoms_to_Fix: All
Constraint_Level: Monomer
Fix_Spec: click on the reside or type its name

You can do that for several residues. When you are done, set *Activation* to *List*. This will give you a list of fixed residues or atoms. Make sure that everything is correct.
Now, you can go to **Parameters** to set up minimization conditions and then **Run** it.

Analyzing Simulations

The data can be analyzed with the **Analysis** module, which provides options for creating various types of graphs. For this exercise, we are going to analyze the dynamics data obtained earlier.

Exercise: Make sure no objects are present on the screen. Now, read in the molecule to be analyzed, i.e., the peptide on which you ran dynamics. Use **Molecule Get**, set *Get File Type* to *Archive*, fill *File Name* parameter box by clicking on the appropriate **.car** file. Make sure that the retrieved molecule is the peptide right before dynamics. Now, you can retrieve a dynamic trajectory, which is contained in the **.his** file.

To read in a trajectory, go to **Analysis** module, choose **Trajectory Get.** Choose *Trajectory File* (**.his**) from the *Files* parameter box. Make sure the prefix of the **.his** file is the same as the prefix of the **.car** file you have just read in. The *Trajectory Object* box will be filled automatically. Other parameters: set *Load Velocities* on, for other parameters use defaults.

Now, create a graph. Go to **Construct_Graph** under **Trajectory**. Click on *New Graph*, and construct a graph in which the X-axis represents time, Y-axis - energy (you can use total). When you are done, click on *End Graph*. The graph should appear on the screen.
You can also plot other variables, such as Temperature vs. Time, Distance vs. Time, etc.

You can view the simulation with the **Animate** command under **Trajectory.** Simply go to this pulldown, click on the molecule as the *Animate Object Name*, make sure the trajectory file is correct, and execute. The molecule will start to move. Changes in the energy are shown on the graph and displayed directly on the screen. To stop the simulation, choose **Unanimate** under **Trajectory**.

What was the energy of the peptide before dynamics, and what is it at time of 1 ps?

Another useful command under **Trajectory** is **Conformation**, which allows you to get an average dynamic structure. Explore other features of this command with **Insight_Help**. You can

also modify the graph using the **Graph** pulldown.

Tutorials

Biopolymer Tutorials

There are several Biopolymer tutorials available. From Online Tutorials, select Biopolymer tutorials. Choose the following lessons to practice:

- Lesson 1, 2, 3, 5, 6, 7, and 8.

Note: At the end of lesson 6, do not remove your molecule (do not execute line 11). Close the pilot and print the figure using Export Plot command, as described in the Appendix.

Other Tutorials

To practice additional aspects of Discover, select Discover Tutorials, lessons 3 and 4.

From InsightII tutorials, choose Application 4, and 8 through 12. Additionally, run through the Analysis Module and Graph Pulldown tutorials.

Appendix: Printing from InsightII

You can print figures from InsightII using **Export Plot** or **Export Image** commands.

Export Plot

This allows you to print figures as line drawings and stereo. Follow the directions below, assuming your molecule and other elements of your figure have been prepared.

To print figures:

1) Go to **File** pulldown, **click-and-drag** to highlight the **Export Plot** command, and let go of the mouse button. At this point, a parameter block appear.

2) make the following choices:

Printer Type:	postscript
Plot to Printer:	on (highlighted in yellow)
Printer Name:	preview (**click** on the printer name in the Parameter Box)

Choose *Landscape* or *Banner* orientation for the figure

Plot Scale:	1.0 (you can also choose other scale)
Gray Scale:	off (unless you want a black&white picture)
Line Width:	5 (that's the width recommended but you can experiment with other choices)
Save Device File:	on
Output File Name:	Figure1.ps (or some other file name; remember to keep **.ps** at the end, since that designates a file format, postscript)

After the file name has been assigned, you can go back to *Print Command.* Parameter box on the right should now contain a correct command line that includes a proper file name.

3) **Click** on *Execute* button to execute command. Close the parameter box by **clicking** on the *Cancel* button.

4) The figure will be now constructed and displayed on the screen using the Adobe Postscript Previewer. This allows you to see and evaluate the figure before it is printed.

Note: you can access a previewer any time from a Unix shell.
At the command prompt type:

```
> xpsview
```

This will open Adobe Previewer. Choose the file to view (must be in the postscript format, of course).

5) Print the figure from the Unix shell.

```
> lp filename.ps
```

To print stereo figures:

1) Prepare stereo view of your molecule on the screen. Go to **Session** pulldown, then **Stereo**.

2) Choose the following parameters:

Stereo Mode:	on
Stereo Angle:	3.5
Stereo Type:	Side By Side
Stereo Separation:	0.4 (or 0.5)

3) Execute

4) Go to **Export Plot** command, as described above. Fill the parameter box in a similar manner but with the following exceptions:

Exceptions:

Stereo:	on
Stereo Angle:	3.5
Stereo Separation:	0.4 (or 0.5)
Stereo Scale:	1.0

Other parameters should be the same as before.

5) Execute, preview and print the figure, as described above.

Export Image

This command allows to create detailed pictures that reflects exactly the image present on the screen. Thus, before printing, make sure that the you have a desired image on the screen. Moreover, it is frequently desirable to change the background from black to white.

Changing background:

1) Go to **Session** pulldown, then **Environment**.

2) In the parameter block, choose:
Background: on
Background Color: choose white from the color palette

3) Execute

It may be necessary to adjust colors or labels in the image on the screen.

To print figures:

1) Go to **File** pulldown, then **Export Image**

2) Choose the following parameters:

Image Format:	postscript
Color Space:	RGB
Image Size:	Current Window
Specify Dest Size:	By Scale
Scale:	1.0
Image Quality:	72 dpi (for some figures, you may choose 300 dpi for better resolution; 600 dpi is usually not recommended for our printers)
Landscape:	on (gives better figures; off - for banner orientation)
Print Image:	on
Printer Name:	preview
Save Device File:	on
Output File Name:	Figure1.ps (or anyname.ps)

Go back to *Print Command* now, click on the command line in the *Printer Comnd List.* It should now appear in the proper box.

3) Execute, preview and print the figure, as described above.