Desmond Tutorial

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D. E. Shaw Research

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April 2012

Preface

Intended Audience

This tutorial is intended for computational chemists using Desmond in setting up and running molecular dynamics simulations. It assumes a broad familiarity with the concepts and techniques of molecular dynamics simulation and the use of the Maestro molecular modeling environment.

Prerequisites

Desmond runs on Intel™-based Linux® systems with Pentium™ 4 or more recent processors; running CentOS 3 (RHEL3) or later. Linux clusters can be networked with either Ethernet™ or Infiniband®.

Viparr requires a recent version of Python; we recommend Version 2.5.1.

This tutorial assumes that someone has prepared the Desmond-Maestro environment for you, either by installing the packages available from Schrödinger LLC or the Academic release of Desmond available from D. E. Shaw Research. Where noted, the procedures described in the tutorial make use of software that is not included in the Academic version. In those cases the software is available from Schrödinger.

About this Guide

This manual contains the following sections:

Chapter 1 describes Desmond and outlines the steps to perform a simulation on a simple protein.

Chapter 2 describes the System Builder, the tool for setting up molecular systems for simulation using Desmond

Chapter 3 describes force field parameter assignment program ***Viparr*** and making changes to the configuration file output by the system builder.

Chapter 4 describes how to run Desmond within the Maestro environment and from the command line.

Chapter 5 describes Free Energy Perturbation and metadynamics simulation using Maestro work- flow.

Chapter 6 describes how to view the results of Desmond simulations in Maestro.

Chapter 7 describes VMD, an alternate workflow available separately from the University of Illinois that can also be used to setup simulation systems and to view trajectories and analyze the results of simulations.

Chapter 8 provides pointers to additional documentation on Desmond-Maestro system.

Release Notes

- Note that Desmond 2.4 and higher internally utilizes a new binary input structure file. These files are called Desmond Molecular System files and have the extension .dms. The .dms file format and related information is documented in the Desmond User's Guide. You do not need to consider the .dms files explicitly with the sole exception of the forceconfig.py setup tool described in "Running Simulations from the Command Line" on page 65.

- Prior to this Desmond release, when MultiSim is used for FEP simulations, MultiSim requires special .mae files for input instead of .cms files. These .mae files are generated by the FEP panels (ligand mutation, protein residue mutation, ring atom mutation, and absolute/total free energy calculation) and include the dual topology structure of the mutations. The FEP MultiSim script will explicitly call the System Builder to prepare the solvated systems and generate the OPLS-AA force field parameters. In the current release, one can load a full system in a .cms file (protein + ligand + membrane + solvent + ions) in the FEP panels, and perform an FEP calculation. This is quite useful when one already has a full system that was previously subjected to MD simulation and now wants to compute the free energy of binding, or similar free energy measure. Of course, if one starts out with only the solute (e.g. protein-ligand complex in vacuum), MultiSim will process this structure including the explicit call to the System Builder to prepare the **.cms** files automatically.

- You will notice that the Maestro toolbars have changed from the prior release to allow for more customization and convenience:
  - The double column main toolbar in prior releases has been split into several smaller toolbars.
  - There is a new "manager" toolbar that has buttons to launch toolbars including Project, Edit, and View. Click the buttons to show or hide the toolbars.
  - You can drag toolbars to any edge of the Workspace, or even place them as free- standing toolbars outside the main window.
  - You can hide toolbar buttons on any toolbar if you do not use them. Right-click the toolbar and choose Customize.
  - Each toolbar can be shown with icons only, with text labels, or text only. Right-click the toolbar and choose the Style menu.

Format Conventions

Command lines appear in a monospaced font:

```
$SCHRODINGER/maestro
```

File names are bolded within text: **equil.cfg**

Menu selections you must make for the tutorial are bolded. For example: Select **Project > Get PDB**.

Screen elements such as panel titles, option names, or button names appear in special text. For example: Click ButtonName.

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1 Desmond Tutorial

Introducing Desmond

Desmond is an advanced classical molecular dynamics simulation system, which has an intuitive graphical user interface integrated in the Maestro molecular modeling environment. Using Desmond, you can perform in silico simulations of small molecules, proteins, nucleic acids, and membrane systems to study the interactions of complex molecular assemblies. With Desmond, you can generate high-quality simulation trajectories of long time scales as well as compute a variety of thermodynamic quantities. Desmond trajectories can be visualized and analyzed using numerous tools in Maestro and VMD.

This tutorial assumes a basic knowledge of molecular mechanics and molecular dynamics. For those users who have not yet used Maestro for molecular modeling, an overview of the Maestro environment is included.

Beyond basic molecular modeling, there are a number of steps that must be performed on a structure to make it viable for simulation with Desmond. Each of these steps mandates an understanding of certain concepts and differing approaches that can be taken.

To enable novices and advanced users alike to process the information in this tutorial, the organization of the tutorial includes:

- A step-by-step example that describes, at a high level, how to perform a simulation on a simple protein.
- In-depth concept sections that cover the detailed information users will need in order to perform a simulation similar to our example.

This tutorial also includes conceptual information and steps for performing free energy perturbation (FEP) calculations. FEP is useful for performing "alchemical" ligand mutations to determine relative binding free energy differences, which is a crucial task for such applications as computational drug design. Moreover, Desmond FEP can be used to compute the absolute solvation free energy of a large variety of solutes as well as the change in free energy associated with protein residue mutation. Another addition to this version of Desmond is improved support for metadynamics simulation setup and analysis.

Steps to Perform Simulation on a Simple Protein

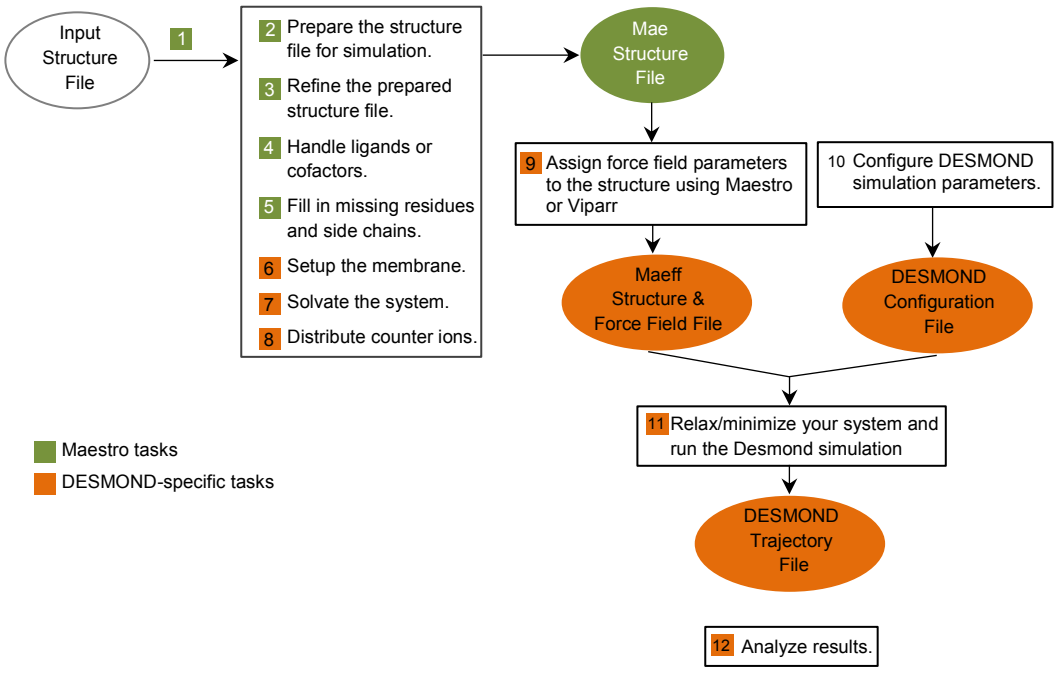
While the example described in this tutorial is a basic simulation on a single protein, the exercise is useful for learning the Maestro-Desmond environment as well as, in general, investigating the molecular properties of different proteins.

Preparing a molecular model for simulation involves these general steps:

1. Import a structure file into Maestro.
2. Prepare the structure file for simulation. During this step, you remove ions and molecules which are, e.g., artifacts of crystallization, set correct bond orders, add hydro- gens, detect disulfide bonds, and fill in missing side chains or whole residues as necessary.
3. Refine the prepared structure file. During this step, you analyze the choices made by Maestro's Protein Preparation Wizard and manually change protonation and tautomeric states of variable residues, and (when present) of ligands and co-factors.
4. If your system is a membrane protein, immerse the protein in the membrane.
5. Generate a solvated system for simulation.
6. Distribute positive or negative counter ions to neutralize the system, and introduce additional ions to set the desired ionic strength (when necessary).
7. Let Maestro assign OPLS-AA force field parameters to the entire molecular system; alternatively, assign force field parameters external to Maestro with Desmond's *Viparr* utility program, which provides access to a variety of widely used force fields.
8. Configure simulation parameters using the Desmond panel in Maestro or edit the Desmond configuration file.
9. Run the simulation from Maestro or from the command line.
10. Analyze your results using the Trajectory Viewer and other analysis tools.

This process is illustrated in Figure 1.1.

Figure 1.1 Simulation process



The rest of this section contains step-by-step procedures for building and running a simulation of a single protein.

Tutorial Steps

1. Start Maestro from your Linux desktop by issuing the following command:

   ```
   $SCHRODINGER/maestro
   ```

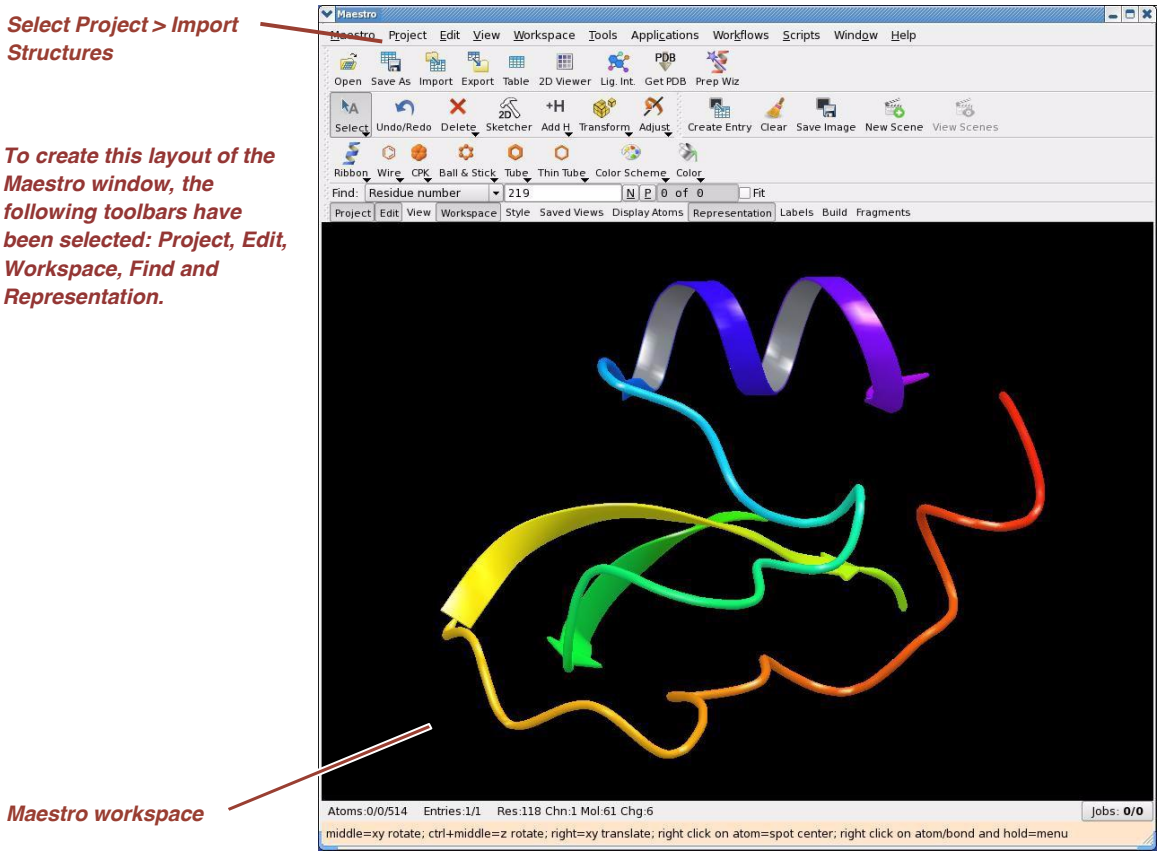
   If you access Maestro through the network (e.g., via VNC), start the program by the command:

   ```
   $SCHRODINGER/maestro -SGL
   ```

   This command will launch Maestro using a software OpenGL graphics library.

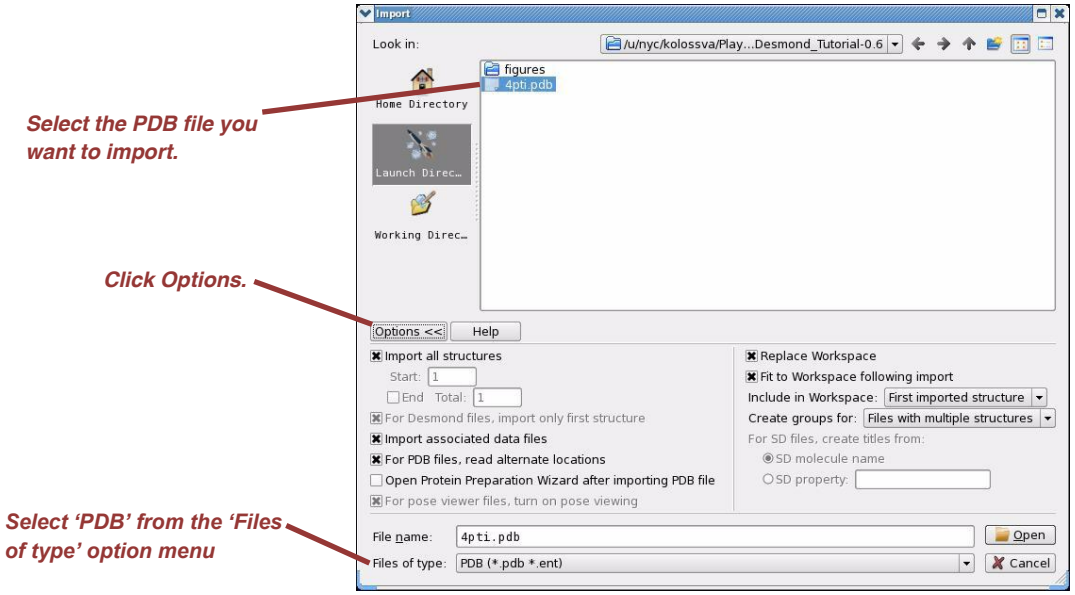
   The Maestro environment appears as shown in Figure 1.2. If you are unfamiliar with Maestro, the Schrödinger Suite or Academic Maestro distributions contain a Maestro Tutorial and User Manual that can be consulted for more detailed information.

Figure 1.2 Maestro main environment



2. Import a protein structure file into your workspace by choosing **Project > Import Structures**. The Import dialog box appears as shown in Figure 1.3.

Figure 1.3 Import dialog box



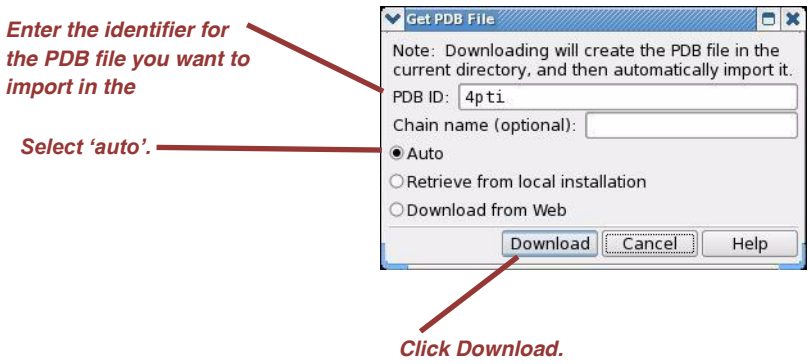
3. Click Options. Set options as desired for the import. Default settings are usually adequate. Click Help to learn about specifics of different options.
4. Choose PDB from the Files of type option menu.
5. Navigate to and select the structure file you will import, and click Open. For this example, we will import the small, proteinase (trypsin) inhibitor protein: 4pti. Therefore, the **4pti.pdb** file has been chosen.

NOTE Maestro supports many common file formats for structural input. Click Help for a list of supported formats.

6. If you want to download the PDB file from the PDB website, choose **Project > Get PDB**.

   The Get PDB File dialog box appears as shown in Figure 1.4.

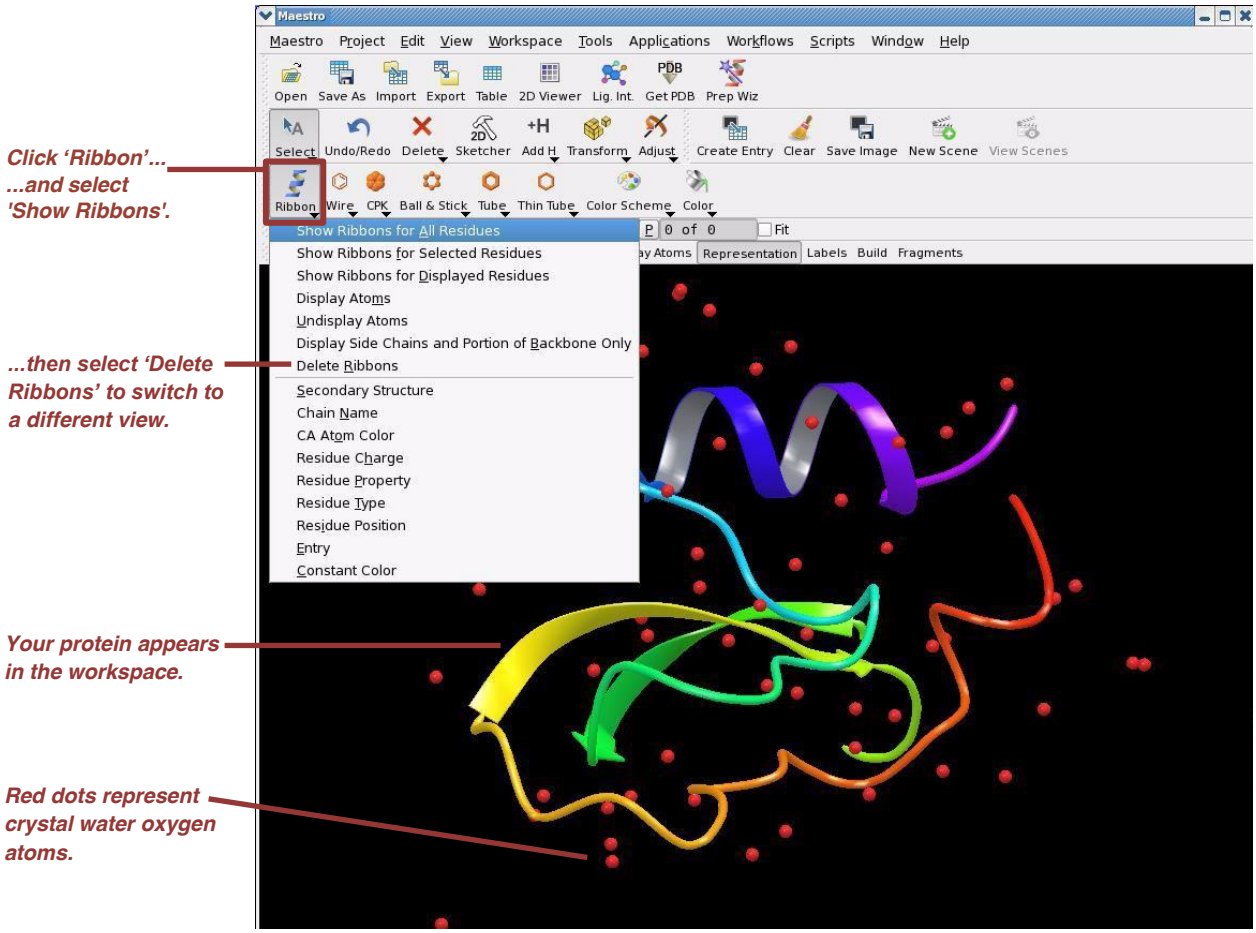
Figure 1.4 Get PDB File dialog box



7. Enter the identifier for the PDB file you want to import into the PDB ID text box.
8. Select Auto to allow Maestro to download the PDB file from the PDB website.
9. Click Download.

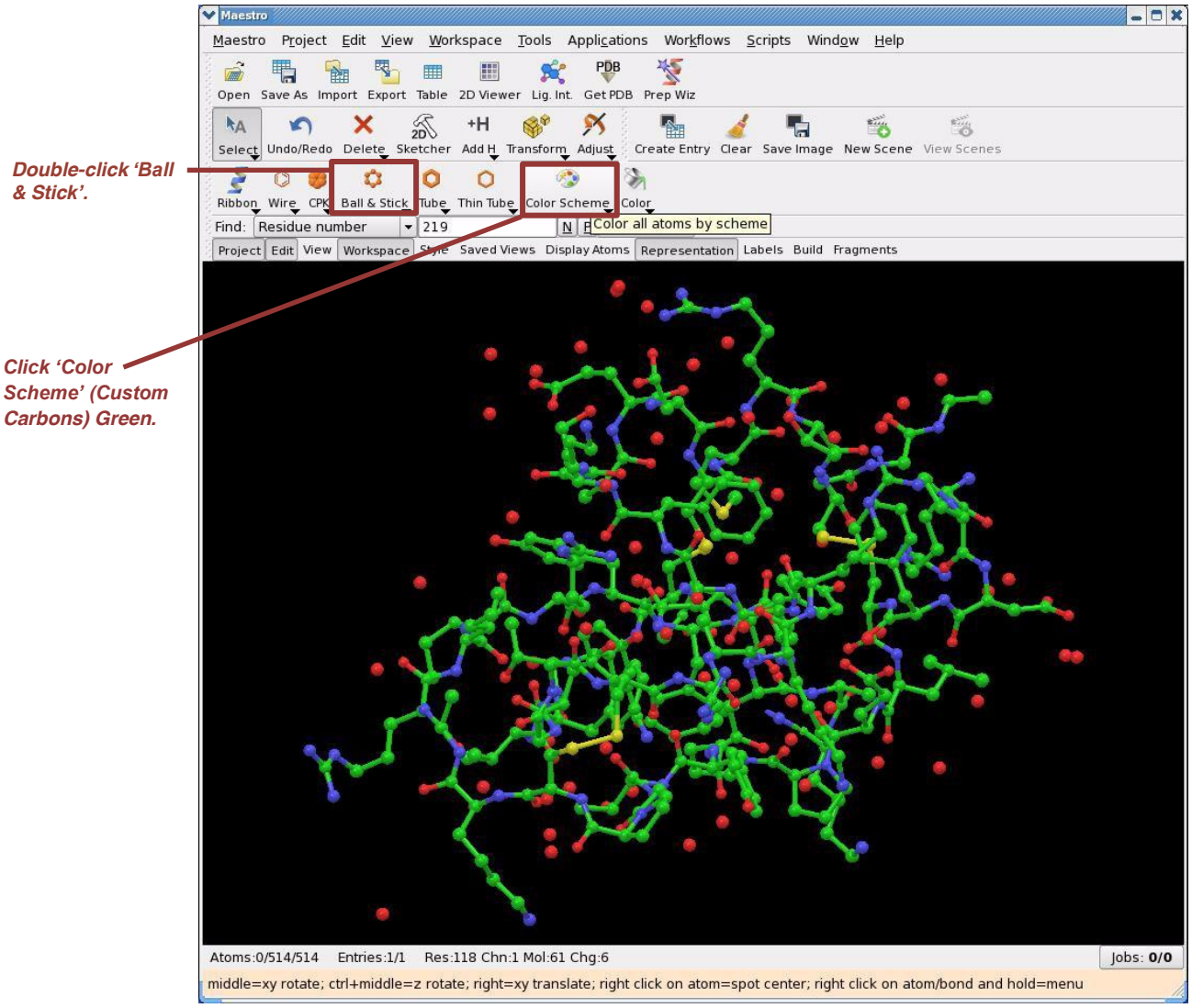
   The protein structure appears in the Maestro workspace as shown in Figure 1.5. The protein structure is displayed using a ribbon representation with colors continuously varying along the rainbow scale from the N terminus toward the C terminus. The red dots show the location of the oxygen atoms of the water molecules present in the X- ray structure.

Figure 1.5 Imported protein structure file



10. Switch from ribbon view to ball-and-stick view by clicking Ribbons and selecting Delete Ribbons from the option menu that appears as shown in Figure 1.5. Next, as shown in Figure 1.6, double-click Ball & Stick and, finally, click Color Scheme to color the carbons in the structure green.

Figure 1.6 Changing from ribbon to ball-and-stick view



Once you have arrived at this point, you are ready to prepare the structure for simulation. Just having a molecular model in the workspace is not enough to perform molecular mechanics/dynamics calculations. You must prepare the protein model so that it corresponds as closely as possible to the biological system you are studying.

From this view, it is clear that there are no hydrogen atoms in the protein structure. Moreover, there might be ill-defined bond orders, protonation states, formal charges, tautomerization states, disulfide bonds, and so on. The red dots shown in the work- space represent crystal waters. All of these issues must be resolved before we can per- form simulation calculations.

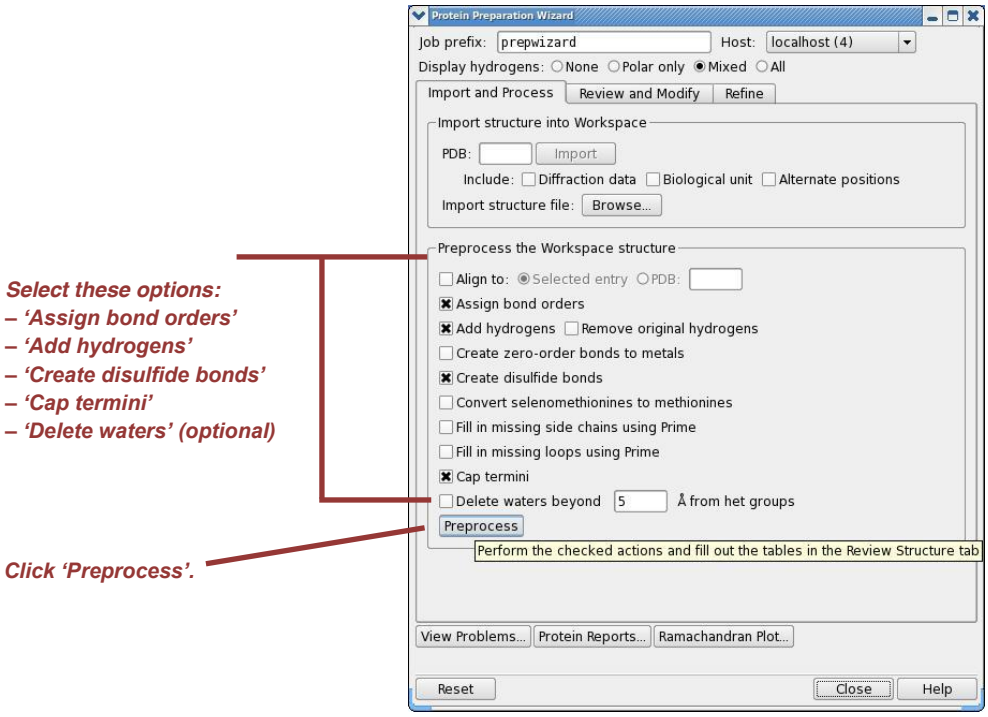
There are two methods of correcting these issues: using Maestro's general purpose molecular structure editor or the Protein Preparation Wizard, which is a very useful automated tool for protein structure preparation. For this tutorial, we will use the Protein Preparation Wizard.

11. Select **Workflows > Protein Preparation Wizard**.

NOTE Note the pop-up warning that Epik and Glide are not available to academic users free of charge, but these tools are generally not necessary to setup Desmond simulation.

The Protein Preparation Wizard panel appears as shown in Figure 1.7.

Figure 1.7 Protein Preparation Wizard panel



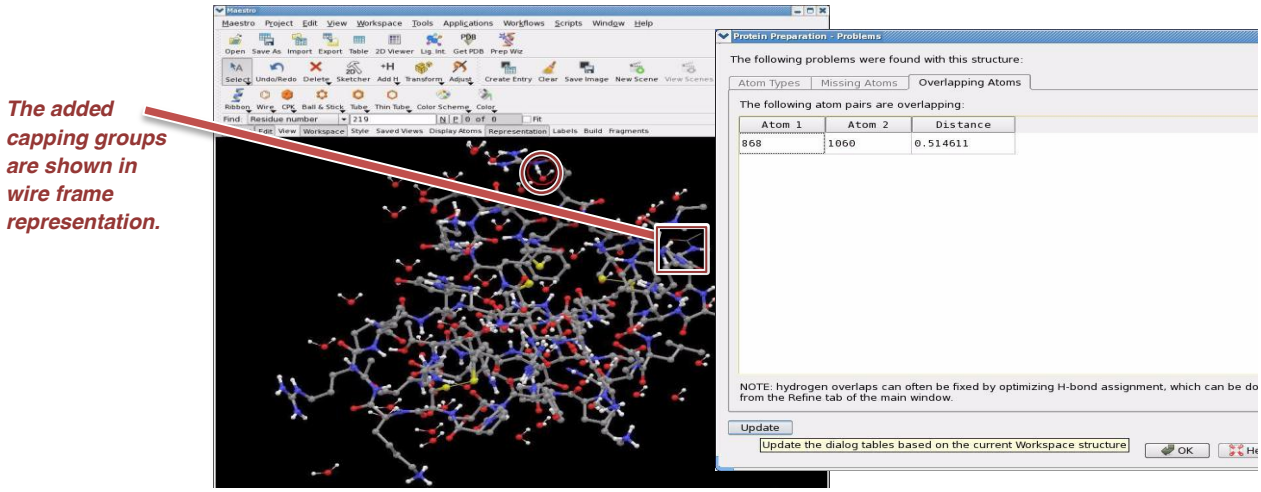
12. In the Preprocess the Workspace structure section, select these options:
    - Assign bond orders
    - Add hydrogens
    - Create disulfide bonds. The 4pti structure has three disulfide bonds, which are all correctly recognized by the Protein Preparation Wizard.
    - Cap termini. Select this option if you want the N or C termini capped. There are many capping groups available; the most common are ACE on the N terminus and NMA on the C terminus.
    - Delete waters. In general, select this option only if you have a reason to remove crystal waters. Set the distance to a small value to remove all water molecules. "Het groups" refers to atoms that are labeled HETATM in a PDB file (anything that is not a protein residue or water).
      Do not delete waters for this Tutorial exercise.

NOTE In most cases crystal waters should not be removed from the interior of the protein molecule, or near the protein surface. These water molecules are crucial structural components of the protein and cannot be adequately reproduced by the algorithm used to solvate the system later on.

13. Click Preprocess to execute the first phase of the protein preparation tasks.

Setup takes only a few seconds to complete. The Protein Preparation Wizard displays the chains, waters, and het groups as shown in Figure 1.8.

Figure 1.8 Protein Preparation Wizard — Preprocessing stage



The Display Hydrogens option in the Protein Preparation Wizard is set to Polar only in Figure 1.7 and, therefore, aliphatic hydrogens are not shown in Figure 1.8. Also note that the added capping groups can be seen in the circled area, shown in wire frame representation.

14. At this point you should have a topologically correct molecular system in the workspace, which can be subjected to molecular mechanics calculations. However, since hydrogen atoms were added by the Protein Preparation Wizard using simple geometric templates, the hydrogen bond network should be optimized.

    For example, as shown in Figure 1.8 in this tutorial exercise, the Protein Preparation Wizard reports a problem with atoms 868 and 1060 being too close (see circled area). Hydrogen atom 868 belongs to ARG53 and hydrogen atom 1060 belongs to HOH153 (one of the crystallographic water molecules).

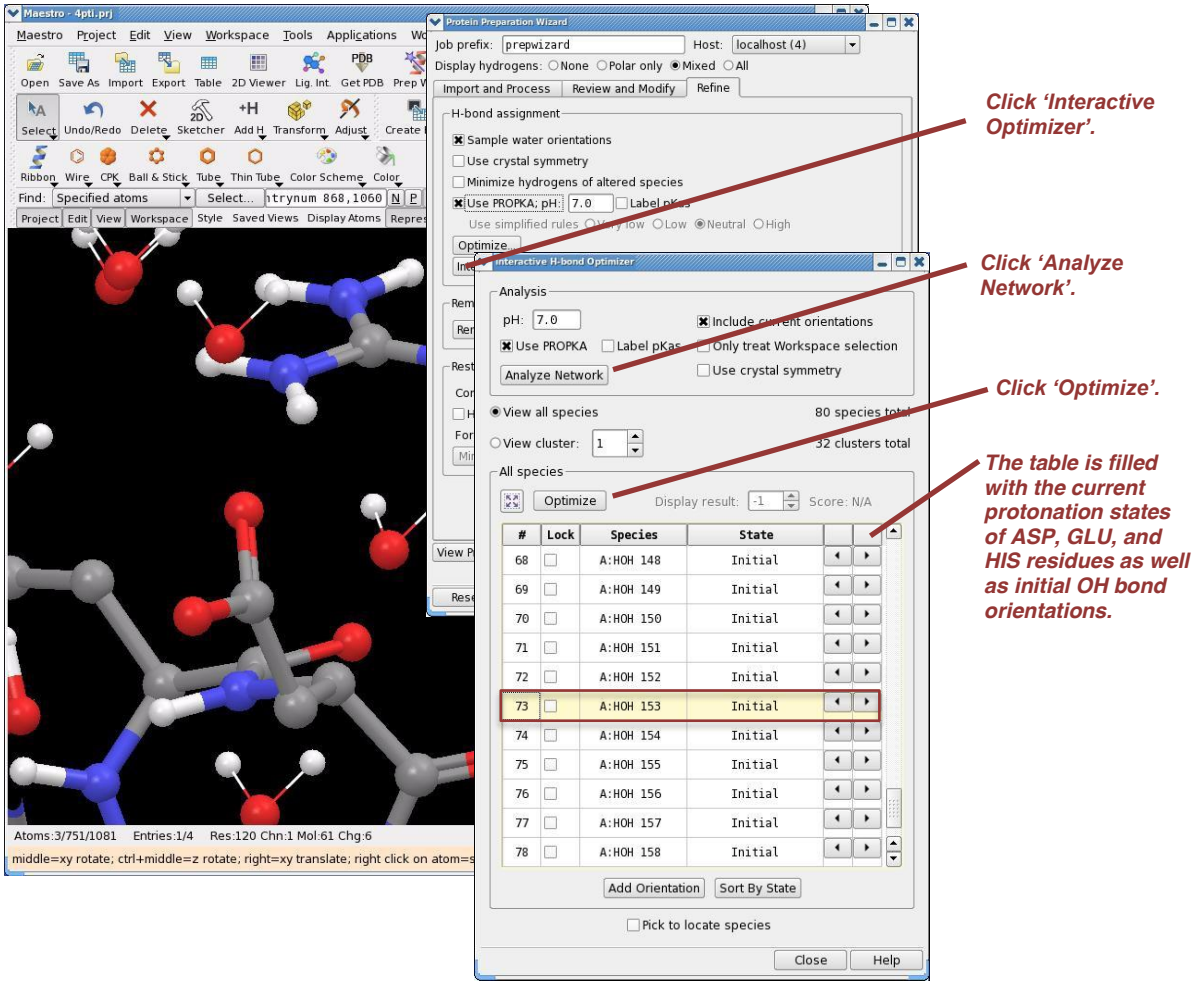
    In general, there are two alternatives for fixing this type of problem. From the H-bond Assignment subpanel when you click the Refine tab:

    – Click Optimize to launch a comprehensive Monte Carlo search: different protonation states of ASN, GLN, and HIS residues are sampled and OH bonds are flipped to optimize hydrogen bond geometry. As part of this procedure you can switch to exhaustive search mode, as well as decide whether the orientation of crystal water molecules should be sampled.

    – Click Interactive Optimizer to launch an interactive tool for hydrogen bond geometry optimization. The interactive optimizer panel is shown in Figure 1.9.

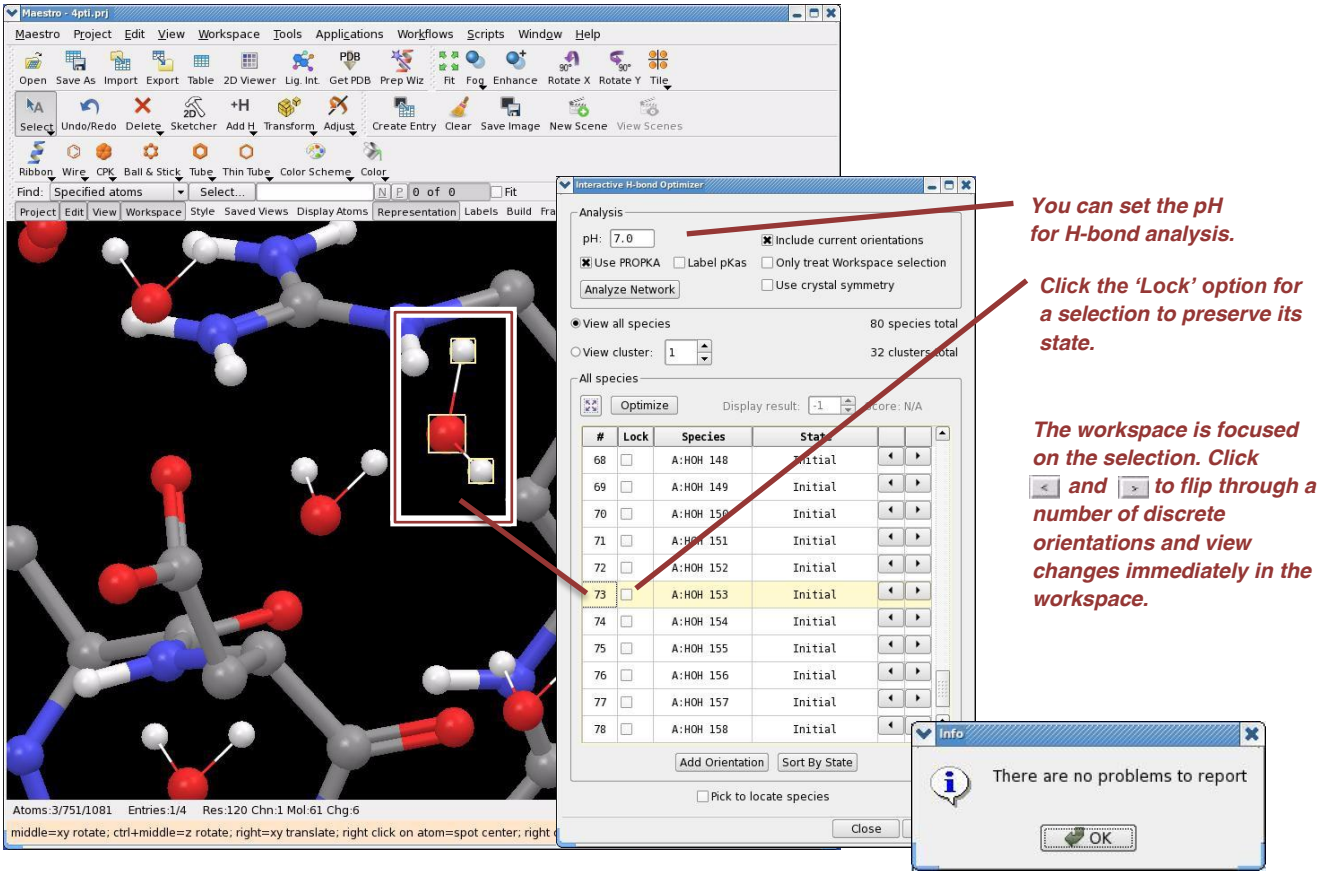
    When you click Analyze Network, the Protein Preparation Wizard fills in the table with the current protonation states of ASP, GLU, and HIS residues as well as initial OH bond orientations. The table shown in Figure 1.9 reflects the current state after clicking Optimize in the Interactive H-bond Optimizer.

Figure 1.9 Protein Preparation Wizard — Interactive H-bond optimizer



Slide the list down to the water section and click entry #73 to select HOH153. The view in the Maestro workspace zooms in and centers on the close contact, as shown in Figure 1.10. Click the Restore View icon next to the Optimize button to zoom out.

Figure 1.10 Protein Preparation Wizard — Interactive H-bond optimizer



Select an item in the table to focus the workspace on the selected residue. For example, selecting item 73 in the table focuses the workspace on HOH153 as shown in Figure 1.10. Originally, this water molecule is in close contact with the HE hydrogen of ARG53. By clicking < and > next to HOH153 in the table you can flip through a pre-defined set of discrete OH orientations and immediately view the result in the workspace. Figure 1.10 shows the workspace when the water molecule is rotated out of the way. Similarly, residue states and OH orientations of other residues can be set manually and, if desired, locked by selecting Lock. At this point we can confirm that the close contact has been eliminated. Click View Problems in the Refine tab (Figure 1.9) and you should see a popup window with the message "There are no problems to report" as shown in Figure 1.10.

Finally, by clicking Optimize you can further optimize the hydrogen bonding network via a computer algorithm. For further information about the Interactive H-bond Optimizer click Help.

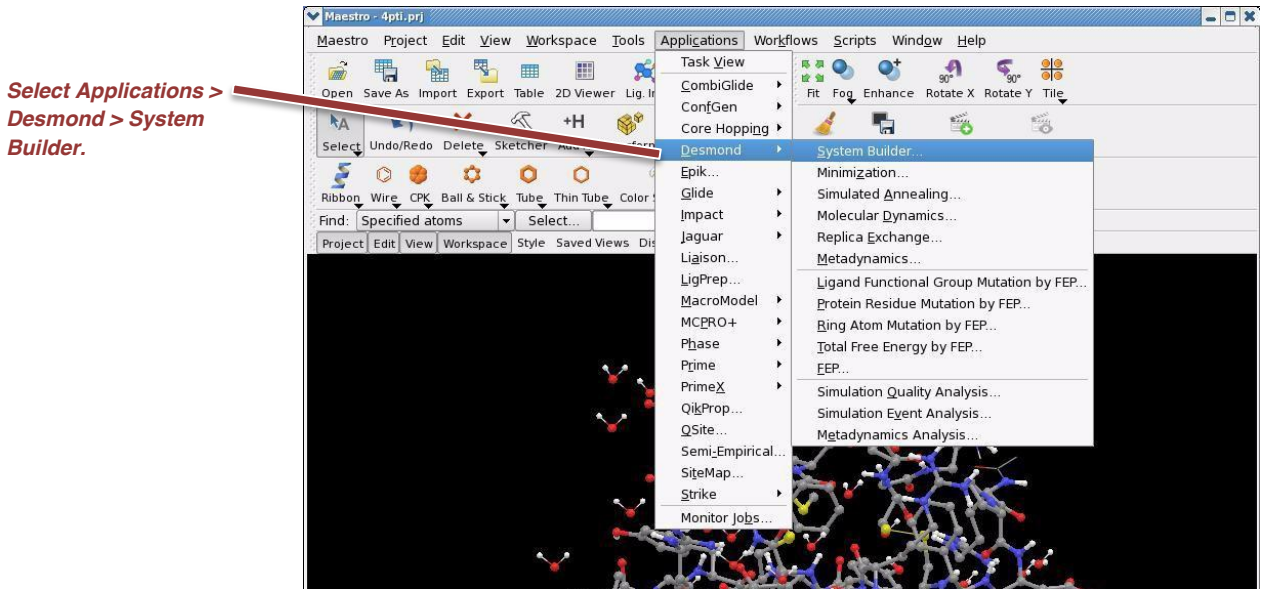
15. Furthermore, provided that you have a Glide license from Schrödinger, the whole protein structure in the workspace can be subjected to constrained refinement by clicking Minimize in the Restrained minimization subpanel on the Refine tab.

NOTE The preferred refinement procedure for MD simulations is to use Desmond's own minimization/relaxation protocol (see below).

NOTE The Protein Preparation Wizard has two options to fill in missing side chains and residues via Prime, provided that you have licensed Schrödinger's Prime application. You only have to select the Fill in missing side chains using Prime option or the Fill in missing loops using Prime option (see Figure 1.7). Note that by selecting either of these options Prime will run with default settings. Consult the Prime documentation for more advanced options including filling in loops in the presence of a membrane bilayer.

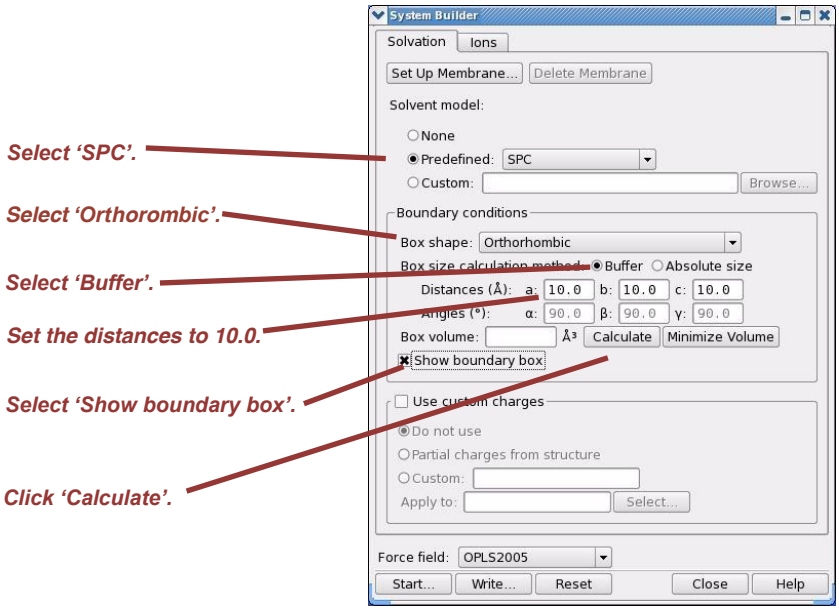
16. The 4pti structure is now ready for preparing a Desmond simulation. Select **Applications > Desmond > System Builder** as shown in Figure 1.11.

Figure 1.11 Launching Desmond System Builder



This launches the Desmond System Builder panel shown in Figure 1.12. The System Builder generates a solvated system that includes the solute (protein, protein complex, protein-ligand complex, protein immersed in a membrane bilayer, etc.) and the solvent water molecules with counter ions.

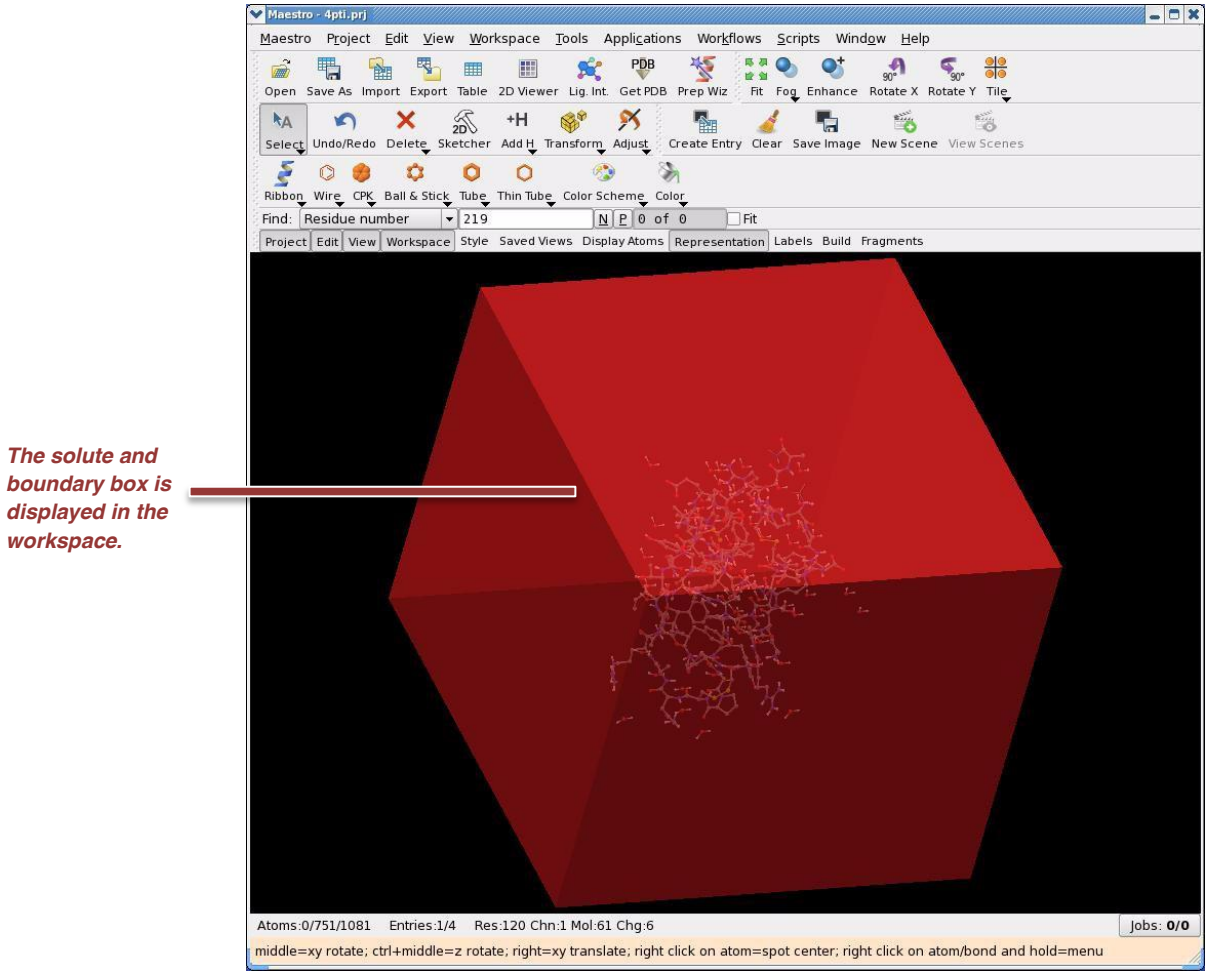
Figure 1.12 Desmond System Builder panel



17. Select SPC from the Predefined option menu.
18. Select Orthorombic from the Box shape option menu.
19. Select Buffer from Box size calculation method.
20. Enter **10.0** in the Distances (Å) box.
21. Select Show boundary box and click Calculate. The System Builder can also be instructed to minimize the volume of the simulation box by aligning the principal axes of the solute along the box vectors or the diagonal. This can save computational time if the solute is not allowed to rotate in the simulation box.

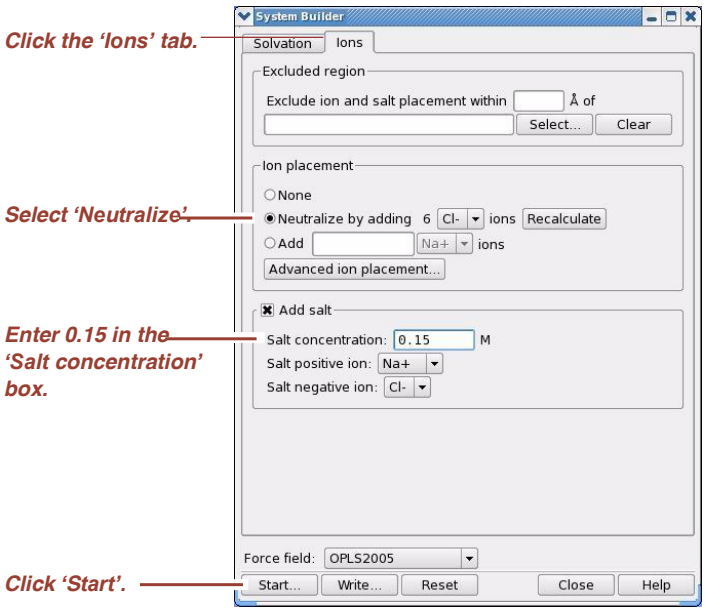
    After setting all these options, the workspace displays the solute and the boundary box as shown in Figure 1.13.

Figure 1.13 Solute and boundary box in the Maestro workspace



22. Click the Ions tab. The Ions panel appears as shown in Figure 1.14.

Figure 1.14 Ions tab in Desmond System Builder



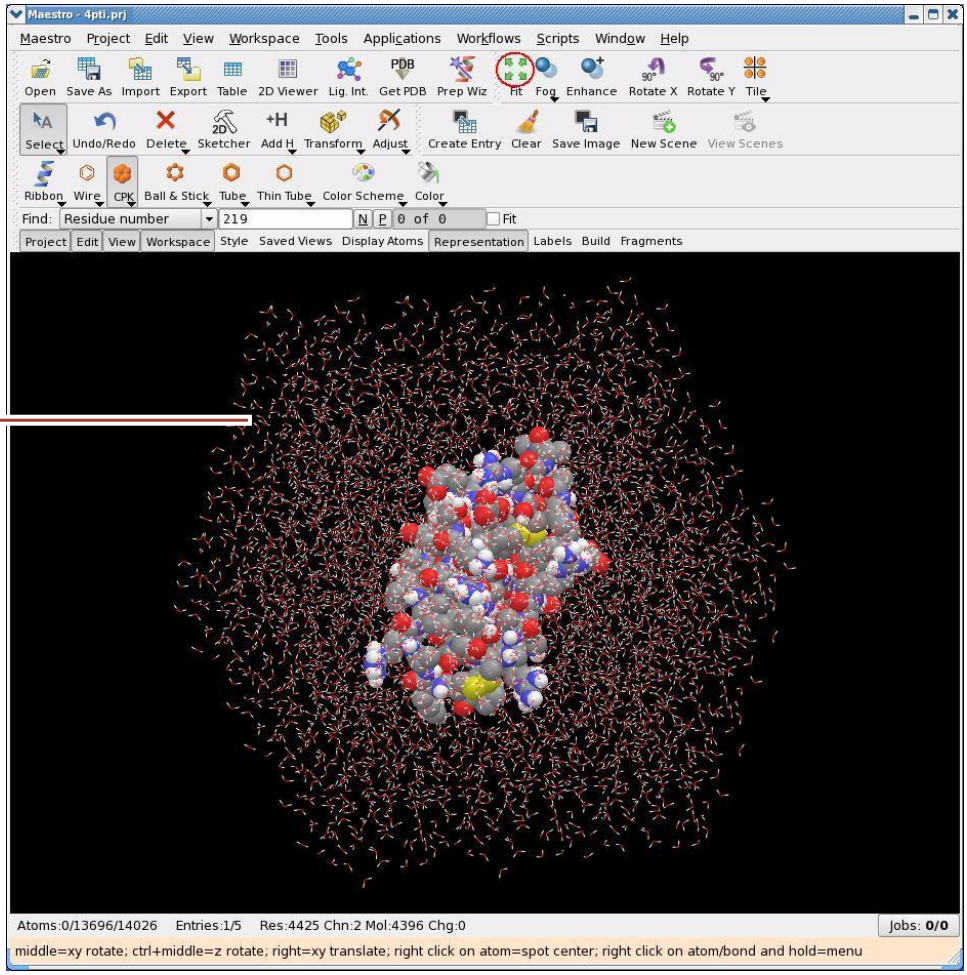
23. For Ion placement, select Neutralize.
24. In the Salt concentration box, enter **0.150**. This will add ions to the simulation box that represent background salt at physiological conditions.

NOTE Membrane systems can also be built with the System Builder, but this is deferred to "Setting Up Membrane Systems" on page 34, which covers membrane setup steps in detail.

25. Click Start to start building the solvated structure. You will need to provide a job name and choose a host on which to run the System Builder job. When complete, the solvated structure in its simulation box appears in the workspace as shown in Figure 1.15. For better clarity the 4pti structure is shown as a CPK model.

NOTE The solvated simulation box will appear off-centered with respect to the red boundary box. This is because the System Builder re-centers the system. To produce the view in Figure 1.15 turn off the Show boundary box option in the System Builder panel (Figure 1.12) and click the Fit to Workspace icon (circled in Figure 1.15).

Figure 1.15 Solvated protein structure in the workspace



For clarity, the protein structure is shown as a CPK model.

System Builder saves the whole simulation system in a composite model system file with the extension **.cms**. Composite model system files are essentially multi-structure Maestro files that are suitable for initiating Desmond simulations.

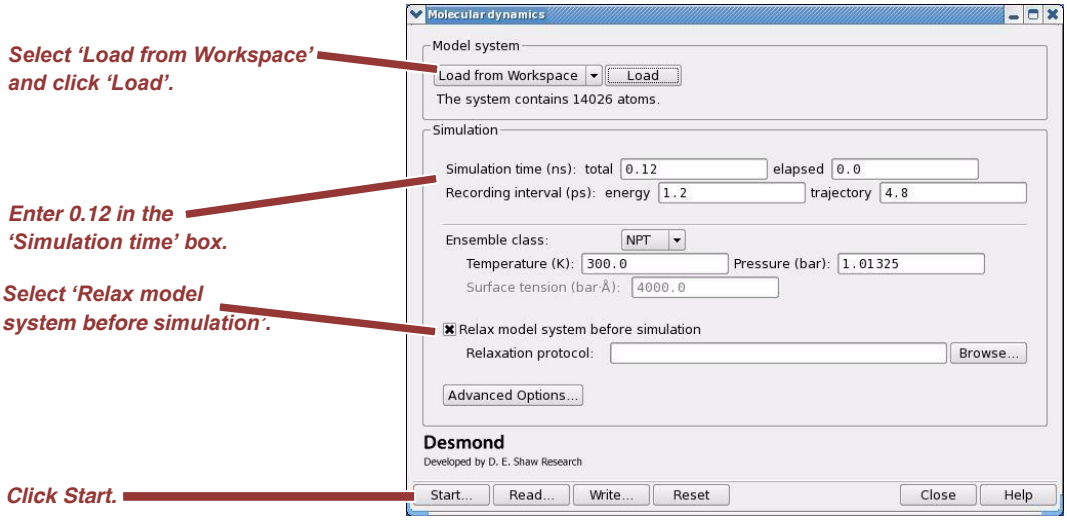
NOTE Maestro automatically assigns the latest OPLS-AA force field parameters available in the Schrödinger Suite to the entire system. If you would rather apply a Desmond provided force field (such as Amber or Charmm force fields, TIP5P water model, etc., you need to process the **.cms** files using the external *Viparr* program (see "Generating Force Field Parameters with Viparr" on page 55).

Now we are ready to perform the Desmond simulation.

Expert users will typically want to start a simulation from the command-line (see "Running Simulations from the Command Line" on page 65). However, for this tutorial we will run it from the Molecular Dynamics panel in Maestro.

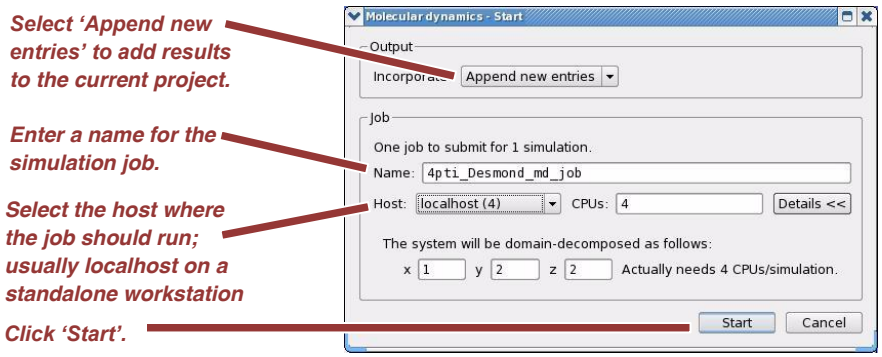
26. Select **Applications > Desmond > Molecular Dynamics**. The Molecular Dynamics panel appears as shown in Figure 1.16.

Figure 1.16 The Molecular Dynamics panel



27. Import the model system into the Molecular Dynamics environment: select either Load from Workspace or Import from file (and select a **.cms** file), and then click Load. The import process may take several minutes for large systems. For this example, select Load from Workspace.
28. In the Simulation time box, set the total simulation time to **0.12** ns.
29. Select Relax model system before simulation. This is a vital step to prepare a molecular system for production-quality MD simulation. Maestro's default relaxation protocol includes two stages of minimization (restrained and unrestrained) followed by four stages of MD runs with gradually diminishing restraints. This is adequate for most simple systems; you may also apply your own relaxation protocols by clicking Browse and selecting a customized command script. For more details see "Running MultiSim jobs from the Command Line" on page 67.
30. Click Advanced Options to set parameters for the simulation. Advanced options are covered in "Specifying Desmond Simulation Parameters" on page 59.
31. Click Start. The Molecular Dynamics-Start dialog box appears as shown in Figure 1.17.

Figure 1.17 The Molecular Dynamics-Start dialog box



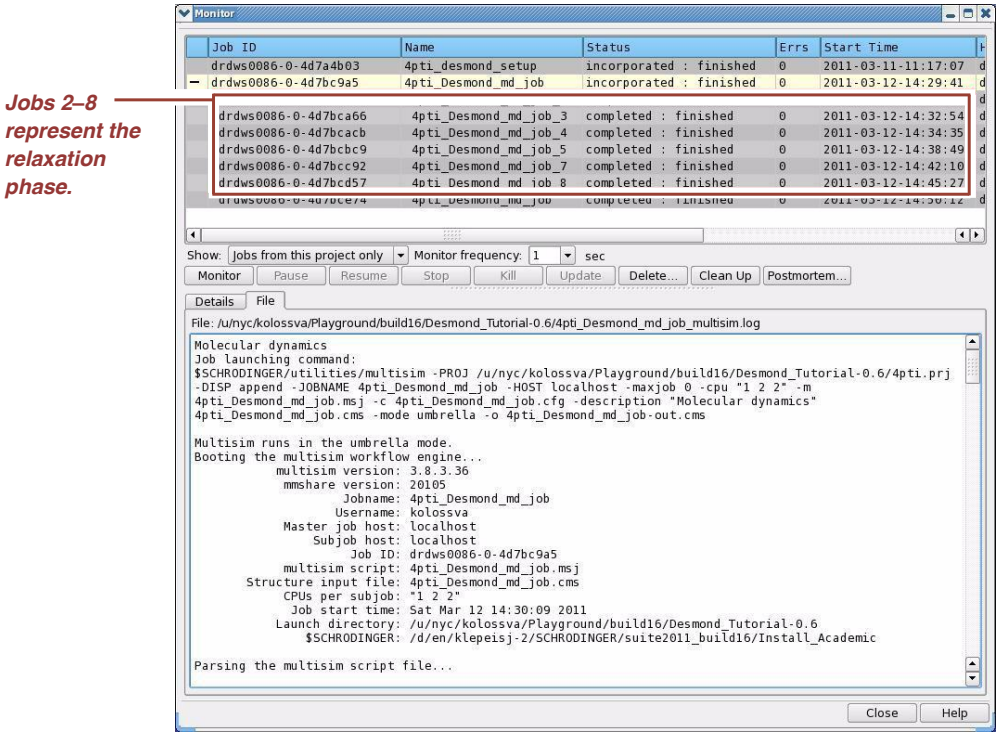
32. Select Append new entries from the Incorporate option menu in the Output area to indicate that results of the Desmond simulation should be added to the current Maestro project.

    In this example the job will run on *localhost*, which is typically a standalone workstation, using 4 CPUs with a domain decomposition (the number of blocks into which the simulation box will be split in the X, Y, and Z directions) of 1x2x2. For large-scale simulations Host is usually a Linux cluster with dozens to hundreds of CPUs.

33. Click Start. The Desmond simulation process begins execution.

    Job progress appears in the Monitor panel similar to that shown in Figure 1.18.

Figure 1.18 The Monitor panel

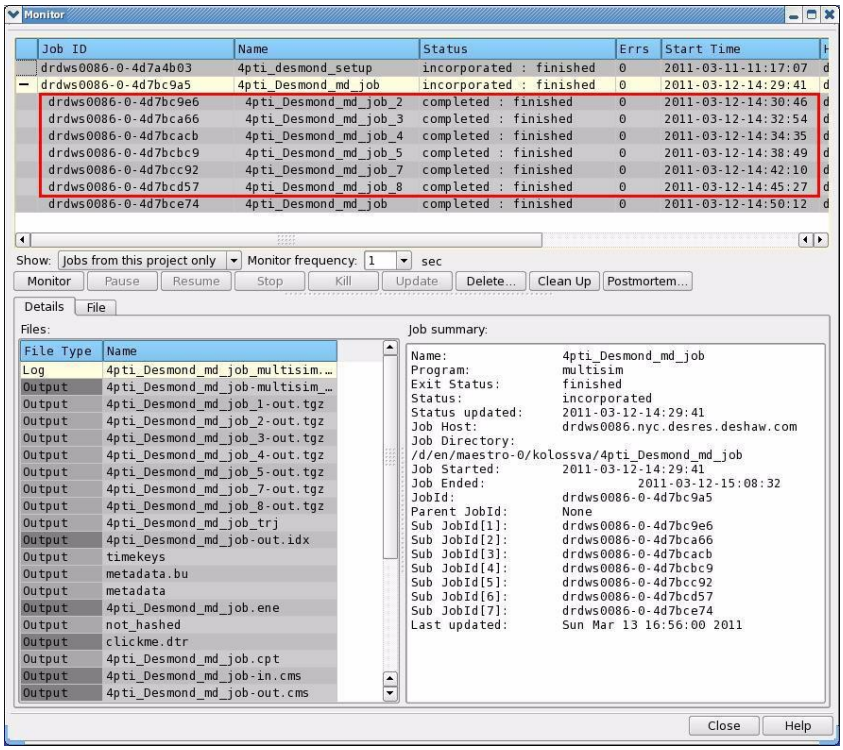


The job (relaxation/equilibration and the production run) will finish in about an hour on 4 CPUs and at that point the Jobs tab in the Monitor panel will display a list similar to that shown in the upper part of Figure 1.18. If you run the job on a smaller number of CPUs you may want to shorten the simulation time accordingly.

Jobs numbered 2–8 represent the relaxation phase (shown in the red rectangle of Figure 1.18). The first job in the list is the master job, and the last job is the production run. There is an additional "solvate pocket" stage (number 6) for systems that require special treatment for explicitly solvating a binding pocket, which is not included in the standard System Builder setup. By default this stage is skipped. The File tab at the bottom of Figure 1.18 shows the log file of the master multisim job. It shows the actual command that is executed and details of the run.

The job summary is shown in the Details tab of the Job Monitor panel shown in Figure 1.19. The most useful information is the list of job IDs required in case a failed job has to be debugged. The results of the simulation are saved in multiple files also listed in the Details tab.

Figure 1.19 List of files in the Monitor panel



In this case the file list includes the following in Table 1.1 below.

Table 1.1 Simulation files

File	Purpose
4.pti_Desmond_md_job.cfg	The Desmond configuration file, which has the simulation parameters for the production run. Note that the actual configuration file read by Desmond is called 4pti_Desmond_md_job-out.cfg, which is subject to post processing the original .cfg file.
4pti_Desmond_md_job.cms	The input structure file for simulation including the force field parameters.
4pti_Desmond_md_job.msj	The "multisim" command script describing the details of the equilibration stages.
4pti_Desmond_md_job_multisim.log	The log file of the master multisim job.
4pti_Desmond_md_job-out.cms	The output structure file (the last frame of the trajectory).
4pti_Desmond_md_job_2-out.tgz **4pti_Desmond_md_job_3-out.tgz**	Compressed tar files containing all the information about the relaxation/equilibration stages.
4pti_Desmond_md_job_4-out.tgz	
4pti_Desmond_md_job_5-out.tgz	
4pti_Desmond_md_job_7-out.tgz	
4pti_Desmond_md_job_8-out.tgz	
4pti_Desmond_md_job.log	The log file of the production simulation.
4pti_Desmond_md_job.ene	The instantaneous energy terms of the production run are stored in the .ene file.

File	Purpose
4pti_Desmond_md_job_trj	The trj file is in fact a directory, which contains the trajectory snapshots in multiple binary files. However, from an analysis point of view this directory can be considered as a single trajectory file associated with an index file .idx and the simbox.dat file containing data about the simulation box.
4pti_Desmond_md_job-out.idx	Index file with the location of the trajectory files.
4pti_Desmond_md_job_simbox.dat	Simulation box size information.
4pti_Desmond_md_job.cpt	A checkpoint file that allows for the bitwise accurate restart of Desmond jobs that crashed for some reason.

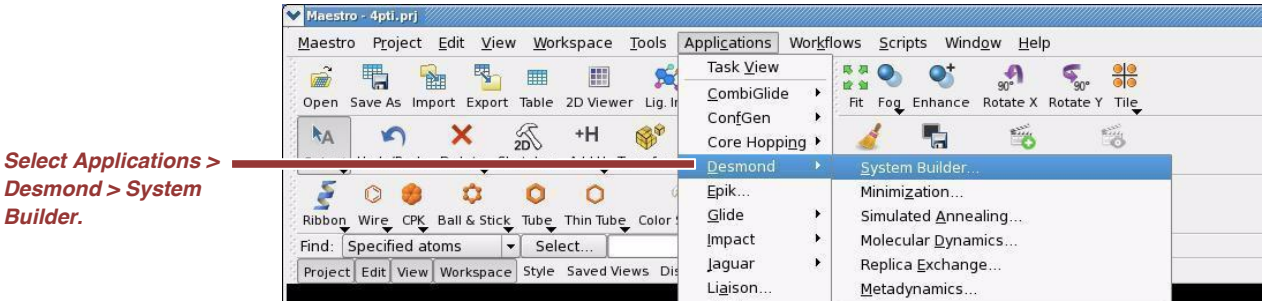
You can find the pertinent documentation in the *Desmond User's Guide* and the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116. Trajectory analysis is covered in "Visualization and Analysis using Maestro" on page 94 and "System Setup and Trajectory Analysis Using VMD" on page 101.

2 Preparing a Desmond Simulation with the System Builder

Overview

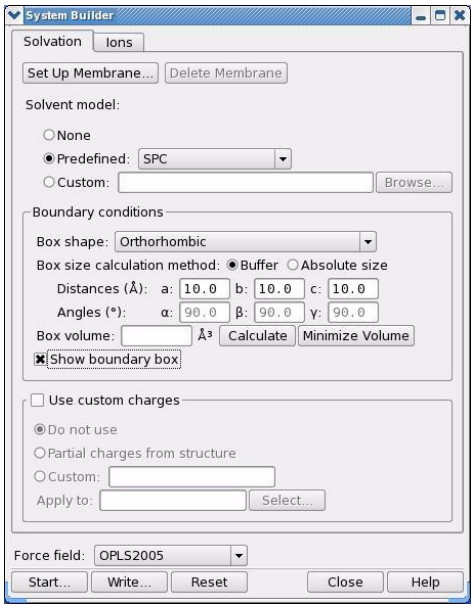
The System Builder is a graphical tool in Maestro that lets you generate a solvated system for Desmond simulations. You can launch the System Builder by selecting **Applications > Desmond > System Builder** as shown in Figure 2.1.

Figure 2.1 Launching Desmond System Builder



The System Builder panel appears as shown in Figure 2.2.

Figure 2.2 System Builder panel



The solvated system generated by System Builder includes the solute (protein, protein complex, protein-ligand complex, or similar systems, or, a protein immersed in a membrane bilayer), solvent, and counter ions. All structural topological information and force field parameters for the solvated system are written to a special Maestro file that is subsequently used for Desmond simulation.

Selecting Solutes and Solvents

The System Builder considers the current contents of the workspace to constitute the solute. Note that some parts of the structure in the workspace may not be displayed, but are still included in the solute.

Supported solvent models in the GUI include SPC, TIP3P, TIP4P, and TIP4PEW water; additionally, *Viparr* allows TIP5P (see "Generating Force Field Parameters with Viparr" on page 55). Organic solvent boxes for DMSO, methanol, and octanol are also available through the System Builder. Furthermore, custom models are also allowed if you can provide the location of a pre-equilibrated box of a different solvent molecule.

Defining the Simulation Box

When defining the simulation box, the goal is to reduce the volume of solvent while ensuring that enough solvent surrounds the solute so that the protein does not `see' a periodic image of itself during simulation. Too much solvent will unduly lengthen the computation.

One way to minimize solvent volume is to select a shape for the simulation box that is similar to the protein structure. The System Builder shown on Figure 2.2 supports

all standard box shapes—cubic, orthorhombic, triclinic, truncated octahedron, and so on. Select the most appropriate shape from the Box shape option menu in the Boundary conditions section of the Solvation tab and click Calculate if you want to compute the box volume. Besides the shape, the box size also depends on how you define the solvent buffer around the solute:

- Apply a buffer distance to each dimension of the simulation box. A typical buffer distance is 10 Å, which is equal to the usual real space Coulombic interaction cutoff for long-range electrostatic calculations.
- Or, set the absolute size of the box.

Next, by clicking Minimize Volume you can instruct the System Builder to minimize the box volume by aligning the principal axes of the solute with the xyz or diagonal vectors of the simulation box—essentially, you can align the protein structure so that it fits inside its simulation box more comfortably. However, this method is only recommended if the solute is not allowed to rotate during MD simulation.

Selecting Show boundary box displays a helpful, translucent graphical representation of the simulation box in the workspace.

NOTE System Builder puts the center of gravity of the solute at the center of the simulation box. As a consequence, fairly non-spherical proteins will appear to shift toward one side of the box, leaving only a thin water buffer on that side. This may not be ideal from a visual perspective, but in terms of periodic boundary conditions, this is perfectly adequate. It is not the distance between the outer surface of the protein and the near face of the simulation box that matters; rather, it is the sum of two such distances with respect to the opposite sides of the protein that is important.

System Builder Output File Format

The System Builder writes the simulation system in a composite model system file with the extension **.cms**. Composite model system files are essentially multi-structure Maestro files that are suitable for initiating Desmond simulations. Typically, the total solvated system is decomposed into five separate sections in the .cms file: protein (solute), counter ions, positive salt ions, negative salt ions, and water molecules. The System Builder also writes Schrödinger OPLS-AA force field parameters in the .cms file. You can find detailed documentation of the **.cms** file format in the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116. Note that Desmond 3.1 uses a different file format called a **.dms** file internally, but in the Desmond/Maestro environment the **.dms** file is transparent to the user, there is no need for it explicitly. The **.dms** file format is documented in the *Desmond User's Guide*.

Adding Custom Charges

In some cases you may want to have specific charges on certain molecules in a system; for example, the ligand molecule. You can specify custom charges in the Use custom charges section of the System Builder. First, you must identify the charge you want to use. You can either select partial charges from a structure or you can identify the column in the Maestro input file that is storing the custom charges. Second, you must specify the subset of atoms

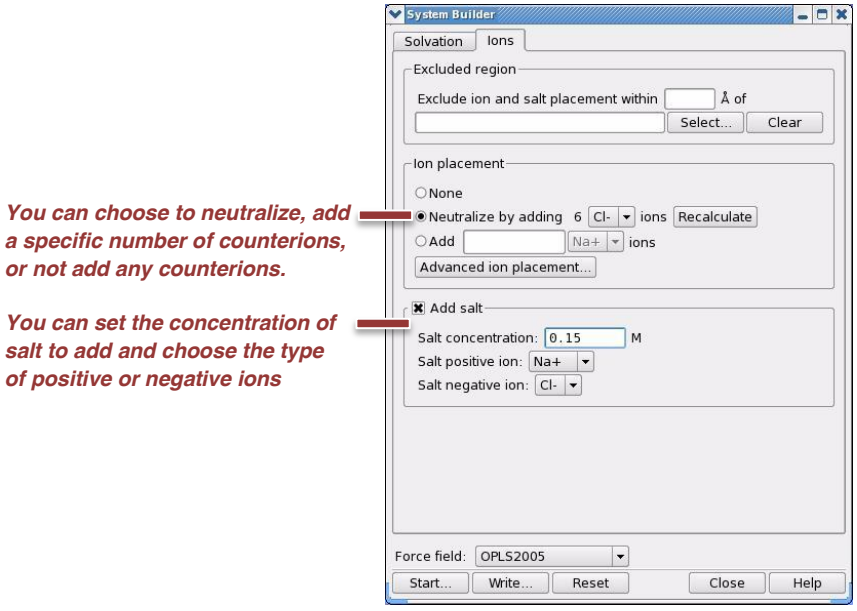
for which the custom charges will be applied using the Select panel. The rest of the atoms will be assigned standard OPLS-AA charges.

Adding Ions

Click the Ions tab in the System Builder panel as shown in Figure 2.3 to add ions to your system. By default the System Builder automatically neutralizes the solute. For example, if the solute has a net charge of +N, System Builder will randomly select N residues on the surface of the protein with a positive formal charge and, respectively, place a negative counterion in the vicinity of the selected residue. For negatively charged solutes, positive counterions will be similarly positioned. Click Advanced ion placement to place counterions in a more sophisticated manner; this procedure is illustrated in "Setting Up Membrane Systems" on page 34 where membrane setup is discussed using a concrete example. You can also define an excluded region in the Select panel where neither counterions nor salt ions are allowed.

- An arbitrary number of counterions can be added; for example, if there is a reason not to neutralize the system (refer to the special note in "Setting Up Membrane Systems" on page 34). Conversely, you may choose not to place any counterions.
- Background salt can be added to the system by specifying the salt concentration in the Add salt section in the Ions tab. The salt ions will be randomly distributed in the entire solvent volume of the simulation box excluding, of course, the volume occupied by the solute and if present, the lipid bilayer.
- The type of positive or negative ions to use can be specified in the Ions tab.

Figure 2.3 Ions tab in Desmond System Builderpanel



Generating the Solvated System

At this point, you can generate the solvated system by clicking Start in the System Builder panel. After this task has completed, the solvated system will appear in the workspace (see Figure 1.15 on page 25) and the resulting system, which is ready for Desmond simulation will be written to a **.cms** file (see "System Builder Output File Format" on page 32**)**.

NOTE System Builder automatically assigns the latest OPLS-AA force field parameters available in the Schrödinger Suite to the entire system. If you would rather apply a Desmond provided force field (Amber or Charmm force fields, TIP5P water model, etc.), you need to process the **.cms** file using the external *Viparr* program (see "Generating Force Field Parameters with Viparr " on page 55).

You can also generate the solvated system from the command line. You may decide to use this method if you want to manually edit the input file to produce an effect that cannot directly be generated by the System Builder.

To generate the solvated system from the command line:

1. Click Write in the System Builder panel to write the job files to disk.

   There are two input files:

   — **my_setup.mae**. The Maestro structure file of the solute, which serves as the input for system setup.

   — **my_setup.csb**. The command file, which can be hand-edited for custom setup cases. For detailed documentation of the **.csb** file see the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

   And a single output file (besides a log file):

   — **my_setup-out.cms**. The Maestro structure file of the entire simulation system including OPLS-AA force field parameters. For details on the **.cms** file see the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

2. Execute the following command at the command line:

   ```
   $ $SCHRODINGER/run $SCHRODINGER/utilities/system_builder
   my_setup.csb
   ```

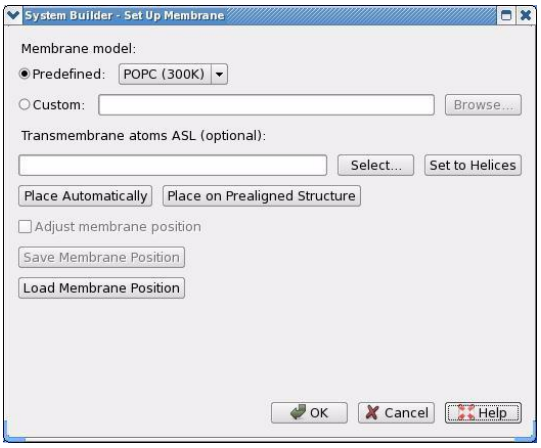
   where `my_setup` is the file name given to "Write".

NOTE Run the `system_builder` script with the `-help` command argument instead of the **.csb** file to see advanced options.

Setting Up Membrane Systems

The Setup Membrane panel as shown in Figure 2.4 can be used to embed a membrane protein structure in a membrane bilayer. In this procedure a template consisting of an equilibrated membrane—including the accompanying water—is used to generate a large enough region to encompass the protein. Using the Setup Membrane panel, you can position the protein within the membrane using a semi-automated procedure.

Figure 2.4 Membrane setup in the System Builder

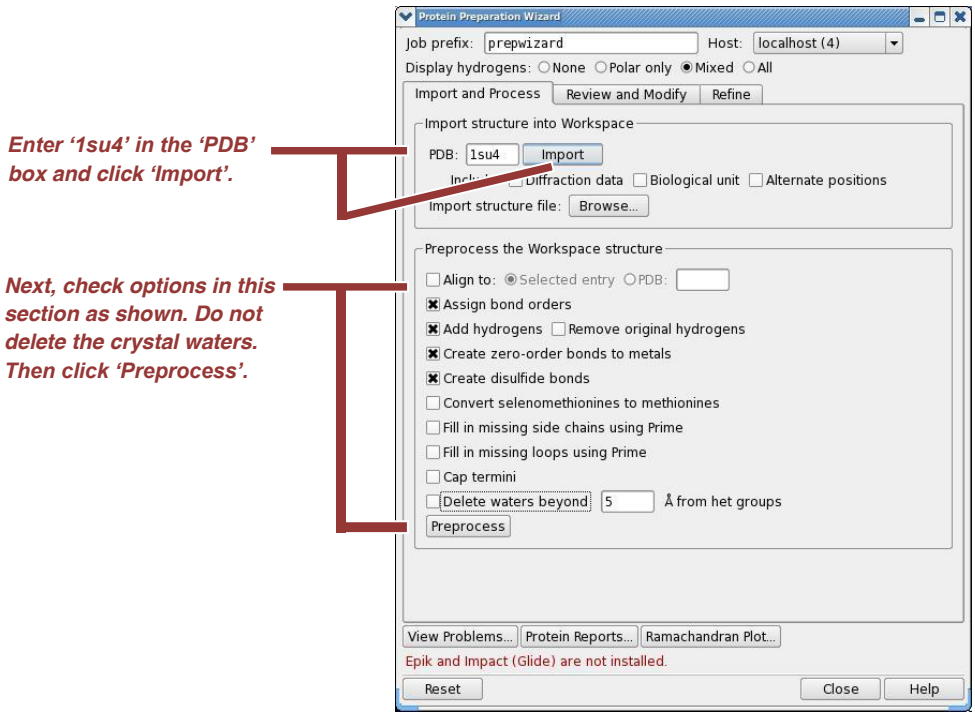


As a real-life example we can consider the calcium ATPase protein, 1su4. Follow the procedure below to prepare this protein for membrane simulation in Desmond.

To setup the 1su4 membrane protein system for Desmond simulation:

1. Open the Protein Preparation Wizard by selecting **Workflows > Protein Preparation Wizard**. Type "**1su4**" in the PDB box and click Import as shown in Figure 2.5.
2. When the structure appears in the Maestro workspace, set the preprocessing options as shown in Figure 2.5; do not delete the crystal waters as they are integral to the protein structure. Click Preprocess. The tables will be filled in by the Protein Preparation Wizard.
3. Click the **Review and Modify** tab, select the sodium ion in the Het table (the focus of the workspace view will center and zoom on the Na ion) and then click Delete. The sodium ion is an artifact of the crystallization process; only the Ca ions are integral parts of the system.
4. You may want to experiment with H-bond assignment as described in Figure 1.10, but it is not essential for the current membrane setup exercise. Click Close to exit from the Protein Preparation Wizard panel.

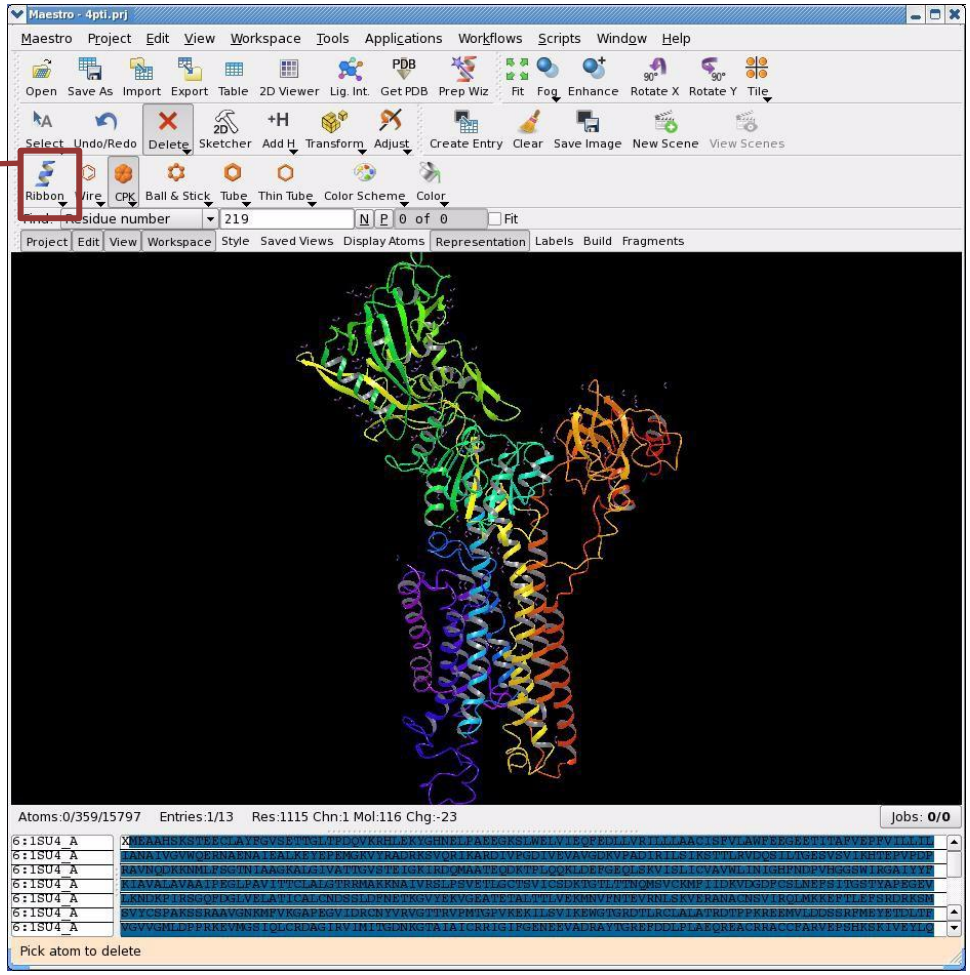
Figure 2.5 Preprocessing the 1su4 structure



5. Change the view to ribbon view in the workspace and orient the protein similar to what can be seen in Figure 2.6, which is the standard way of looking at membrane proteins (with the transmembrane bundle aligned along the vertical axis).

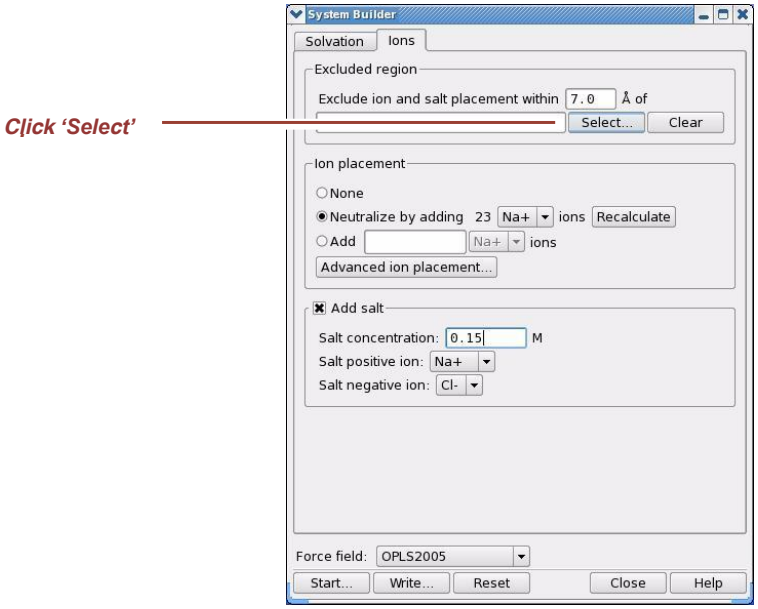
Figure 2.6 The 1su4 structure in standard orientation

Click 'Ribbon' and select 'Show Ribbons for All Residues' from the option menu.



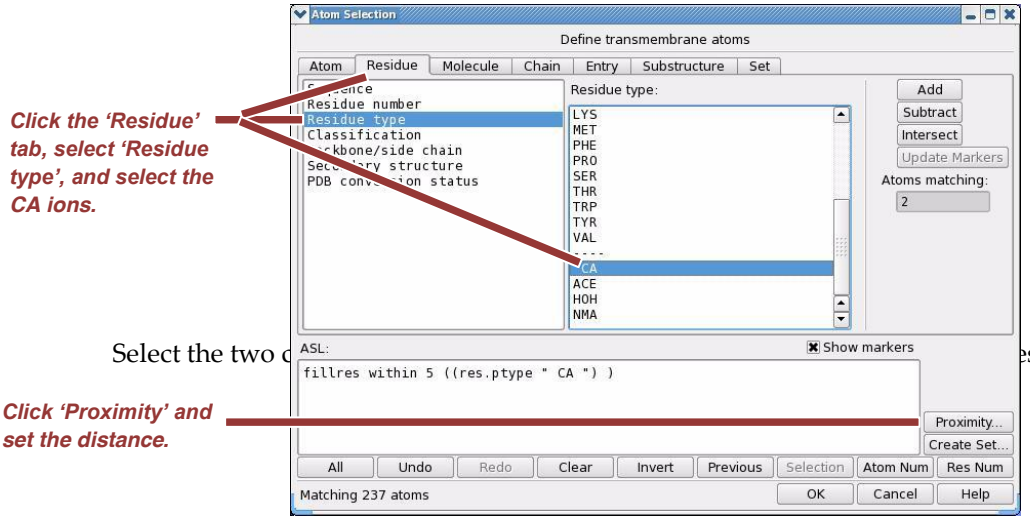
6. Launch the System Builder by selecting **Applications > Desmond > System Builder** and click the Ions tab. The panel appears as shown in Figure 2.7.

Figure 2.7 The Ions tab in the System Builder panel



7. In this tutorial exercise we do not want to place counterions in the vicinity of the calcium ions. This can be achieved the following way. Click Select in the Excluded region section. The Atom Selection panel opens as shown in Figure 2.8.

Figure 2.8 Selecting the excluded region

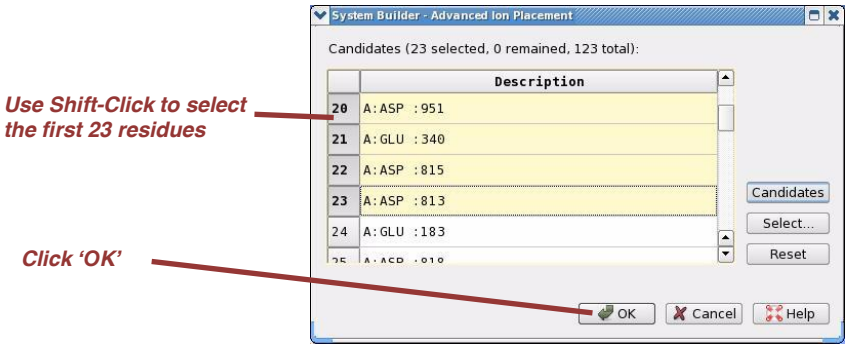


8. Select the two calcium ions by "residue type" and click Proximity to select all residues within 5 Å. The resulting ASL expression at the bottom of the selection panel defines the excluded region. Click OK.

NOTE The Excluded region section in the Ions tab in Figure 2.7 also has an option to define a region around the selected atoms, but the command in the ASL section (`fillres within 5 ((res.ptype "CA"))`) shown in Figure 2.8 takes precedence.

9. Click Advanced ion placement in the Ion placement section of the Ion tab shown in Figure 2.7 to open the dialog box shown in Figure 2.9. Click Candidates. A list of all negatively charged residues, which lie outside of the excluded region appear in the table as shown in Figure 2.9.

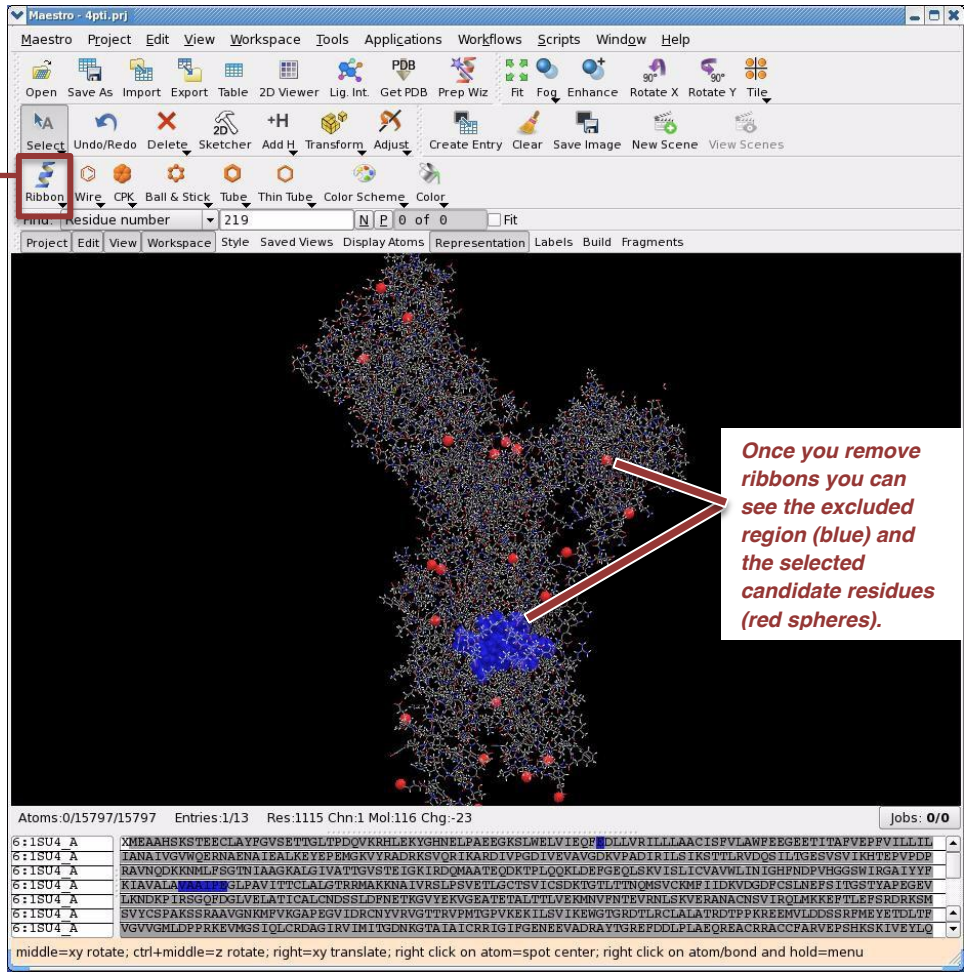
Figure 2.9 Placement of the counterions



Note that the 1su4 structure has a net total charge of –23 after being processed with the Protein Preparation Wizard. The candidate residues are listed in the table in no particular order; therefore, if you select the first 23 residues, a good spacial distribution of the positive counterions should result. Click OK.

10. Temporarily turn off the ribbon view so you can see the visual feedback of candidate selection in the workspace. The 1su4 structure should appear similar to the workspace in Figure 2.10.

Figure 2.10 Visual feedback of ion placement



The blue "blob" shows the excluded region and the selected candidate residues are highlighted by red spheres. The positive counterions will be placed close to the red spheres, which mark the atoms within the residues that have the largest-magnitude partial charge. Also note that the residues that constitute the excluded region are highlighted in blue in the sequence viewer.

In many cases, you may want to place only a few counterions at particular locations and distribute the rest of the charges in a truly random fashion. Follow the steps below to achieve this goal.

a. Set the Solvent model to None (Figure 2.2 on page 30) and instead of neutralizing, explicitly add the number of counterions to the system that you want to place at particular locations (Select Add in Figure 2.3 on page 33).

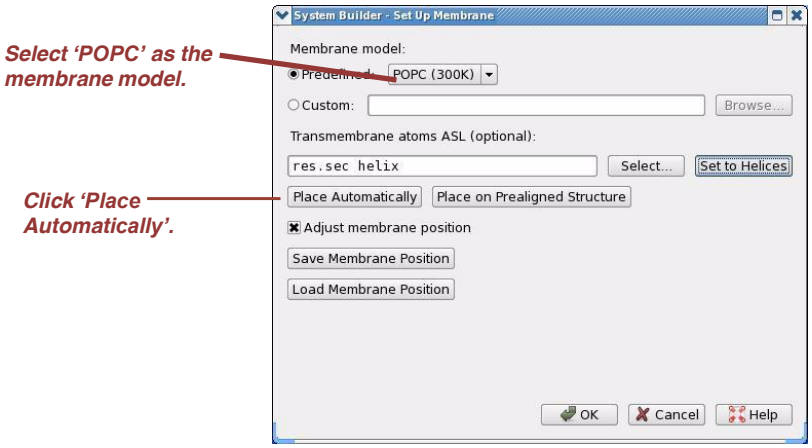
b. Apply the "advanced ion placement" procedure above to place this subset of counterions in the desired locations.

c. Run the System Builder to generate this intermediate system with no solvent and a remaining net total charge.

d. Import the resulting .cms file back to Maestro and re-run the System Builder with the appropriate solvent and the "neutralize" option, with no further application of the "advanced ion placement" procedure.

5. After setting the Solvation and Ions options, click Setup Membrane in the Solvation tab of the System Builder. The Membrane Setup dialog box appears as shown in Figure 2.11.

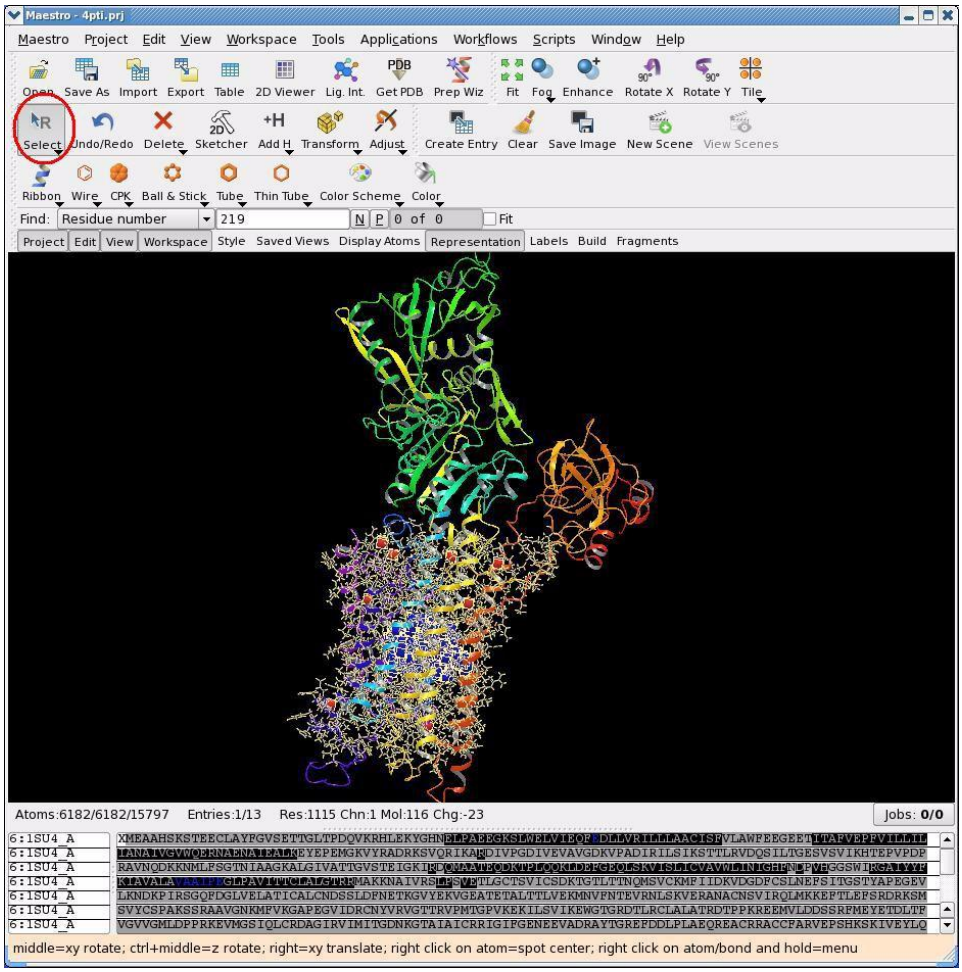
Figure 2.11 Set Up Membrane dialog box



6. Set the membrane model to POPC. Other membrane models include DPPC, POPE, and DMPC. The temperature in parentheses shows the temperature at which the membrane model was equilibrated. (Note that even though the panel suggests it, custom lipid models are currently not supported.) If at this point you click Set to Helices and then Place Automatically, the System Builder will attempt to position the membrane bilayer at a reasonable location based on the information found in the HELIX section of the 1su4 PDB file. If such information is not present in your PDB file, try Tools > Assign Secondary Structure in Maestro. As you will see, however, this placement will not be satisfactory in this case. By specifying the set of transmembrane atoms more accurately, you can expect a highly improved auto placement.

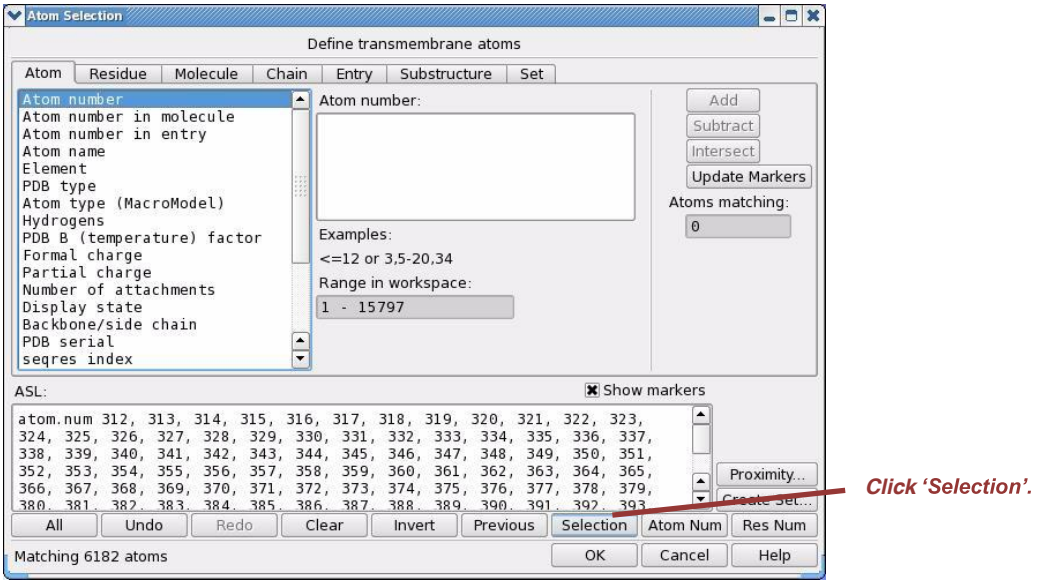
7. Selection of transmembrane residues can be done a number of different ways using the Select panel. In this tutorial exercise let's assume that we do not know the relevant residue numbers (that we could simply add in as an ASL expression), and try to make an approximate selection by hand. In order to do this, change the Select tool in the Edit toolbar (circled in Figure 2.12) to 'R' (residue selection) and select a rectangular area with the cursor around the transmembrane helices of 1su4 as shown in Figure 2.12.

Figure 2.12 Selecting transmembrane residues



8. The selected residues will be highlighted in yellow color. After making the selection in the workspace click Select in the Set Up Membrane dialog box to open the Atom Selection dialog box and click Selection as shown in Figure 2.13.

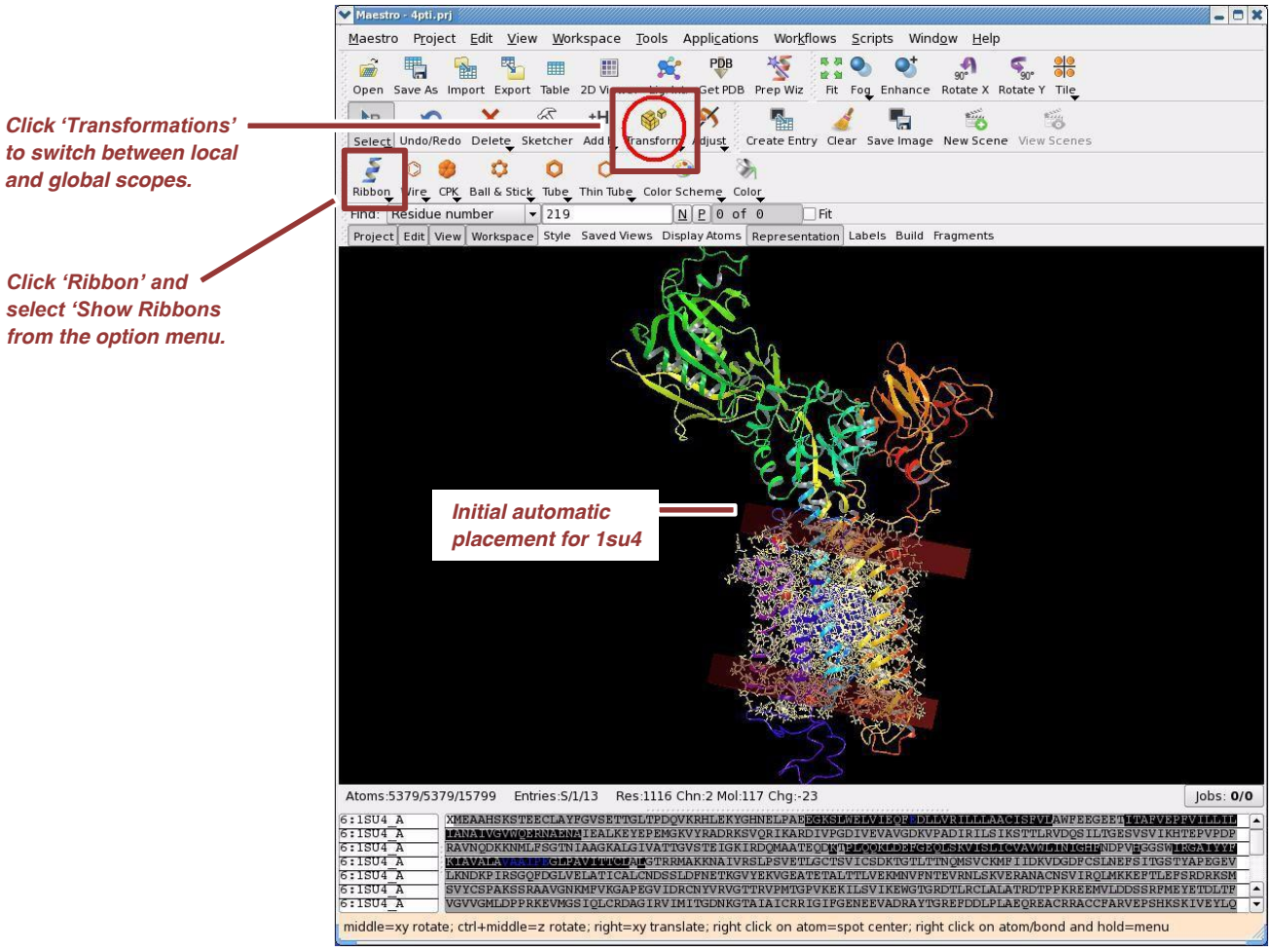
Figure 2.13 Importing the selection into the Atom Selection dialog box



9. When the ASL area is filled, click OK. At this point the ASL area will be filled with the selection in the Set Up Membrane dialog box. Click Place Automatically as shown in Figure 2.11 on page 41 and the resulting membrane placement will appear in the workspace as shown in Figure 2.14. As you can see, the membrane positioning needs minor adjustment.

NOTE Switch back to ribbon view for the remainder of this membrane setup exercise.

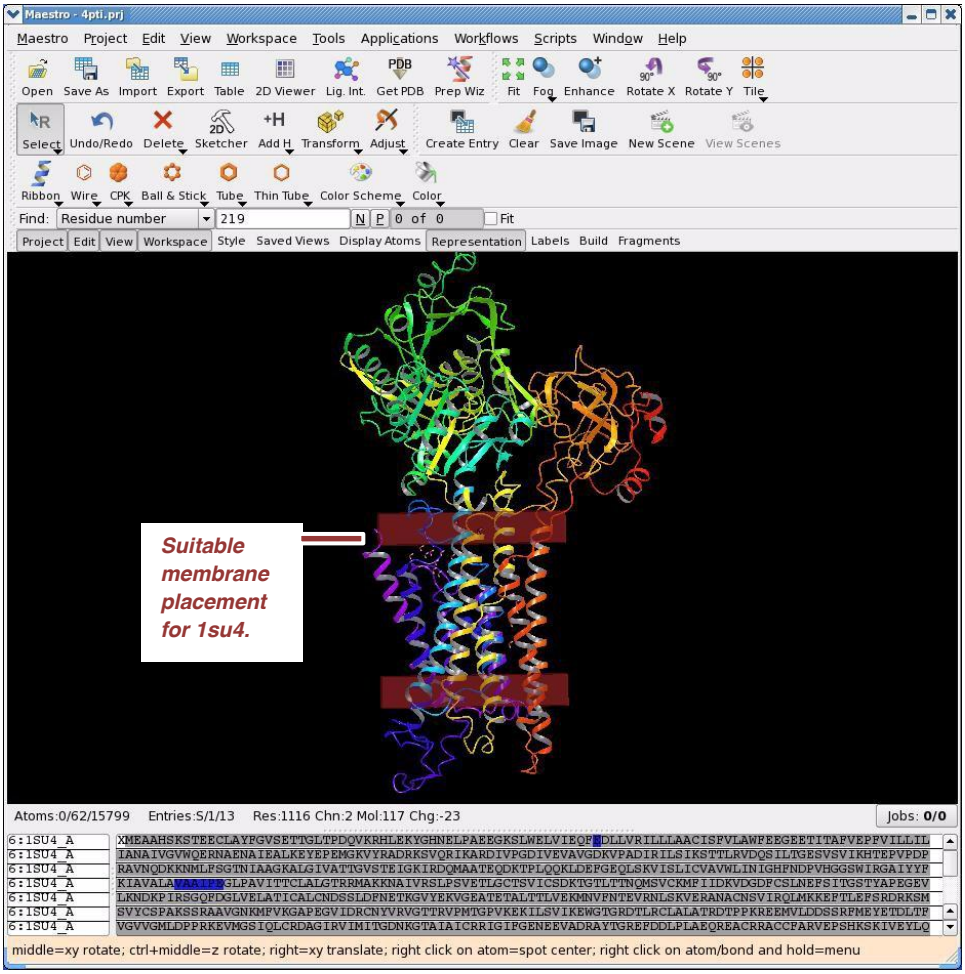
Figure 2.14 Initial automatic membrane placement for 1su4



10. Select Adjust membrane position in the Set Up Membrane dialog box as shown in Figure 2.11 on page 41. At this point you should be able to translate and rotate the membrane model in the workspace, relative to the protein structure. Moving the membrane relative to the protein structure is termed the local scope for transformations.

    However, for a better view you will probably want to translate/rotate the system as a whole as well. This is called the global scope and you can switch back and forth between the local and the global scopes by repeatedly clicking Transformations (called out in Figure 2.14). With only a few translations and rotations, switching between the local and global scopes, you should be able to place the membrane in a suitable starting position. After applying manual transformations you should place the membrane similar to that shown in Figure 2.15.

Figure 2.15 Adjusted position of the membrane for 1su4



NOTE You may want to position two or more different membrane proteins (e.g., for mutagenesis studies) in a bilayer in exactly the same way. Of course, this would be virtually impossible to do, trying to reproduce the same manual adjustments. The Set Up Membrane dialog box in the System Builder provides a convenient way to automate this procedure. Any time you are satisfied with the membrane placement, the net translation/rotation transformation matrix can be saved in the project entry by simply clicking Save Membrane Position in the Set Up Membrane dialog box (see Figure 2.11 on page 41). Then, for any subsequent protein, you can simply click Load Membrane Position to position the entry according to the saved transformation matrix. This way, all proteins will be placed in the membrane in exactly the same position and orientation.

11. Finally, click OK in the Set Up Membrane dialog box and click Start in the System Builder panel to start the membrane system setup. For 1su4, the setup procedure should take 6–8 minutes. The resulting simulation system is shown in Figure 2.16 on page 46. For better visualization, the protein is shown as a CPK model and the lipid bilayer is highlighted in green.

NOTE The visible gap at the top and bottom of the lipid layer is the gap between the water layer that is part of the POPC model and the extra water layer, which was added to the system to satisfy the boundary conditions. This is expected since the system is put

together from two different, pre-equilibrated boxes of a solvated lipid bilayer and plain solvent, respectively. The gap should go away after a relatively short equilibration period. However, membrane systems should be equilibrated for an extended period of time to resolve the large hole that was carved out of the lipid bilayer to accommodate the transmembrane part of the protein. The hole is shown for the 1su4 structure in Figure 2.17.

NOTE The protein in the simulation box in Figure 2.16 is positioned so that the head of the protein is shifted to the top of the simulation box. This is the result of a visual artifact discussed in "Defining the Simulation Box" on page 31. System Builder puts the center of gravity of the solute at the center of the simulation box. As a consequence, fairly non-spherical systems will appear to shift toward one side of the simulation box. Membrane systems are extreme cases resulting in the view on Figure 2.16. This may not be ideal from a visual perspective, but in terms of periodic boundary conditions, this is perfectly adequate.

Figure 2.16 Final simulation system for 1su4

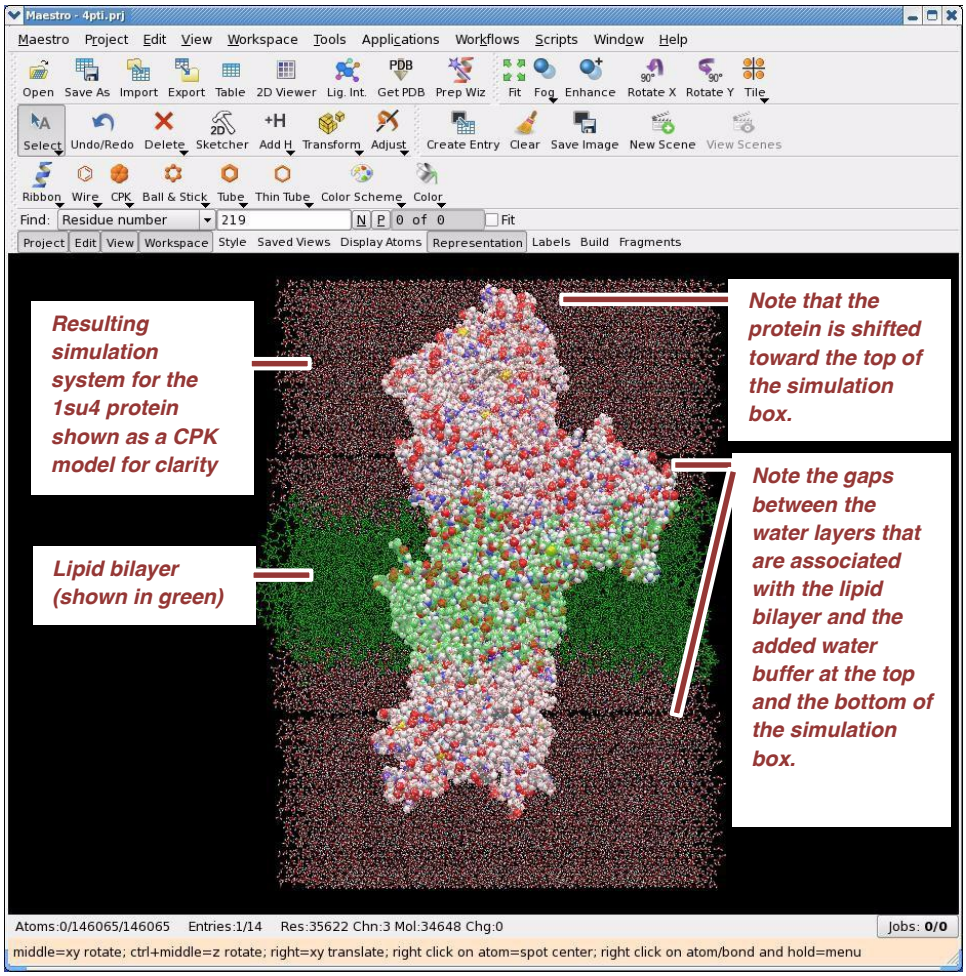
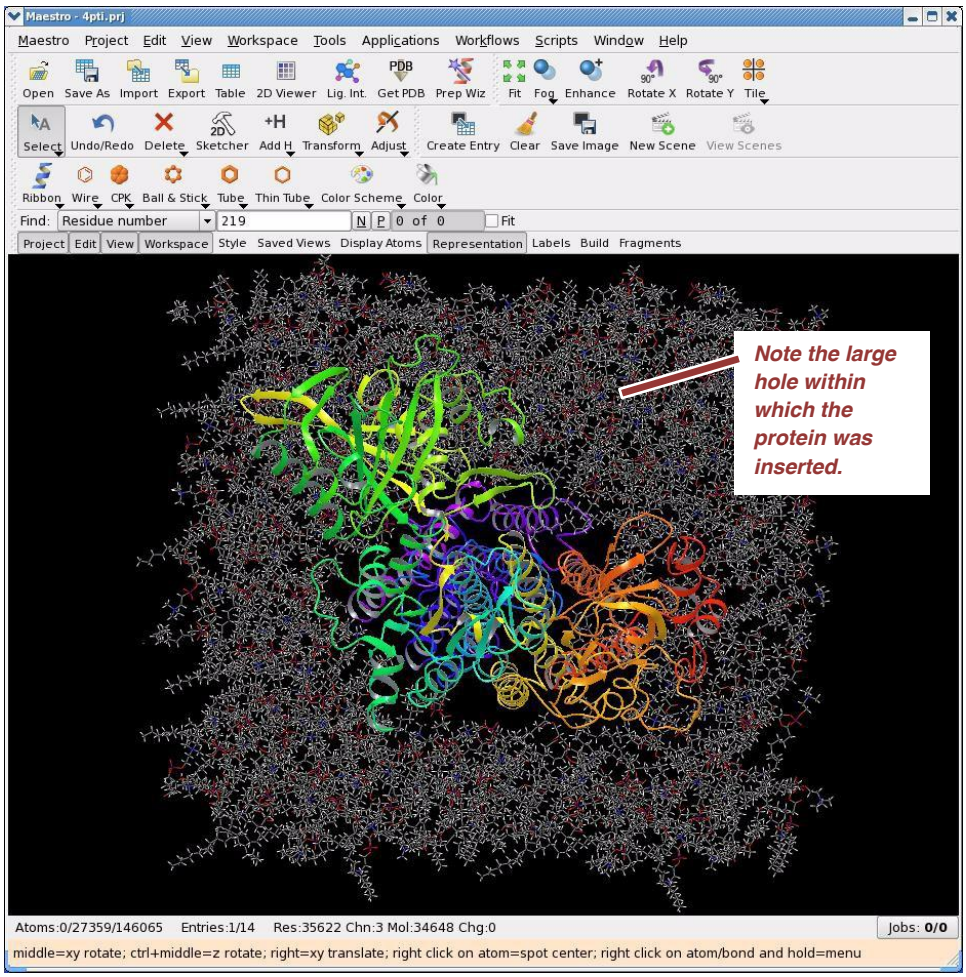


Figure 2.17 Transmembrane hole in the POPC bilayer for 1su4



12. More experienced users may want to run the System Builder job from the command line. Instead of clicking Start, click Write to write a command script file to disk that can be executed from the command line.

    a. Click Write in the System Builder panel to write the job files to disk.

    There are two input files:

    – **my_setup.mae**. This is the Maestro structure file of the solute (1su4 protein, calcium ions, crystal water molecules, neutralizing ions, and counterions), which serves as the input for system setup.

    – **my_setup.csb**. This is the command file, which can be hand-edited for custom setup cases. For detailed documentation of the .csb file see the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

    And a single output file (besides a log file):

    – **my_setup-out.cms**. This is the Maestro structure file of the entire membrane simulation system including OPLS-AA force field parameters. For detailed documentation of the **.cms** file see the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

b. Execute the following command at the command line:

```
$ $SCHRODINGER/run $SCHRODINGER/utilities/system_builder
my_setup.csb
```

where `my_setup` is the file name given to "Write".

NOTE You can automatically perform setup of a GPCR membrane system by running this script from the command line:

```
$SCHRODINGER/run mold_gpcr_membrane.py
```

Use the -help option for usage. **mold_gpcr_membrane.py** takes an input GPCR structure in **.mae** format and aligns it to a pre-equilibrated protein-membrane system from a list or a user-defined template.

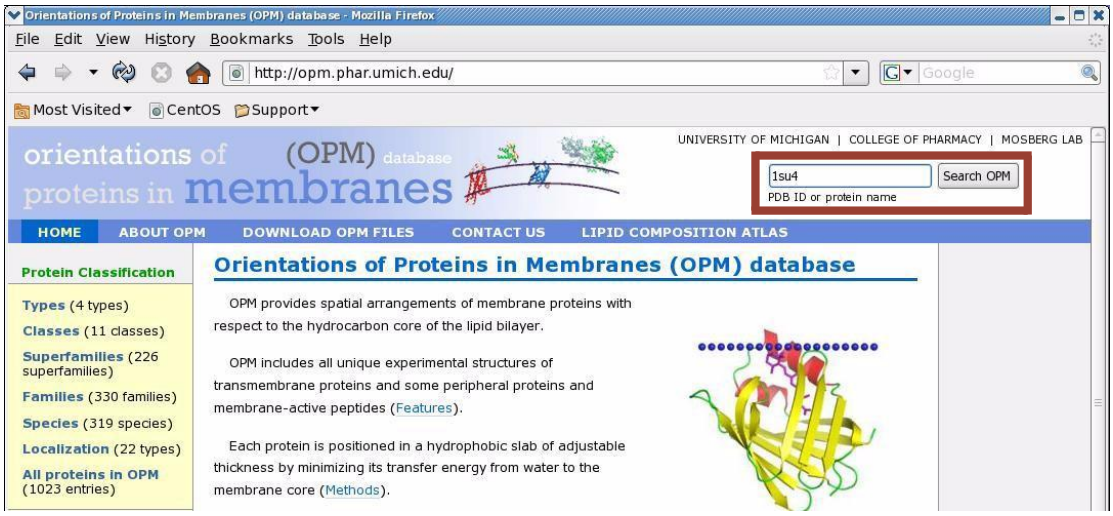
Importing Membrane Placement from the OPM Database

In many cases you can find a high-quality membrane placement already present in the OPM (Orientations of Proteins in Membranes) database at the University of Michigan. Using the System Builder you can import OPM placements as well as other pre-aligned placements. In this section, we will import the OPM placement for 1su4.

To import a membrane placement from the OPM database:

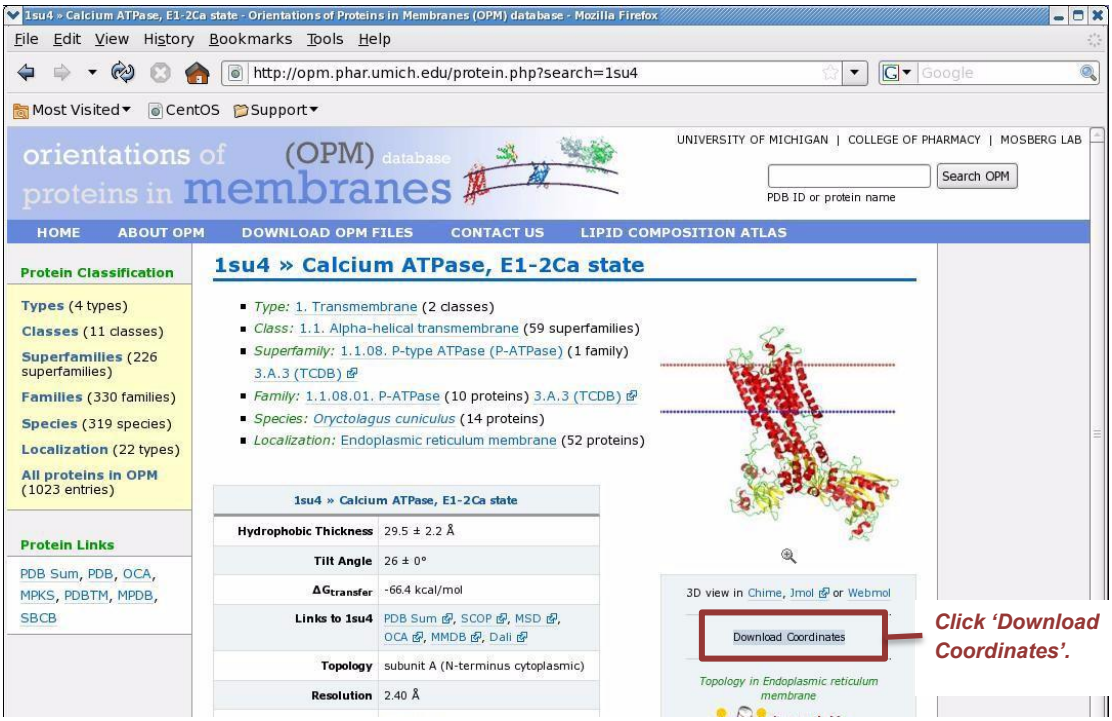
1. Search the OPM database by typing **1su4** in the Search OPM box as shown in Figure 2.18.

Figure 2.18 The OPM Home page



2. Click Download Coordinates on the search result page as shown in Figure 2.19. In the file browser, give the name **1su4_opm.pdb** and save the file in your directory.

Figure 2.19 Downloading the OPM pre-aligned coordinates

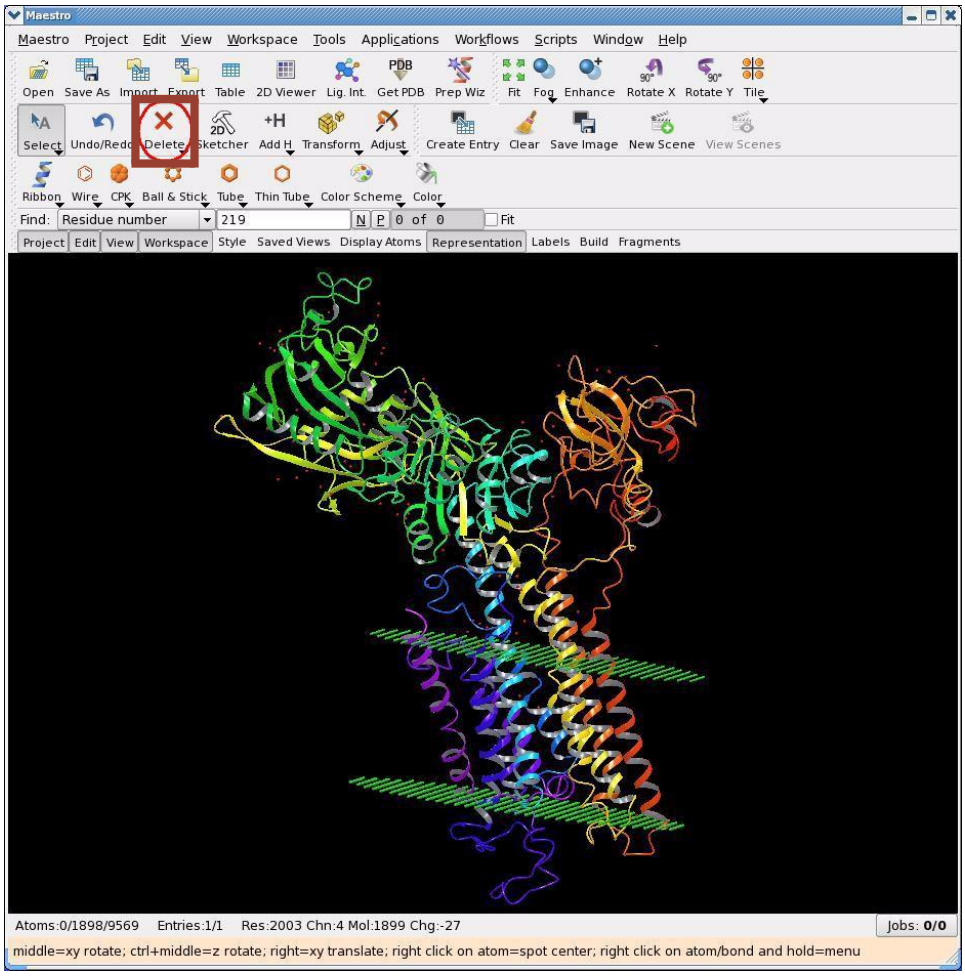


3.

4. Maestro will issue a warning, but ignore it. As you can see on Figure 2.20, the reason for the warning message is the presence of two sets of dummy atoms in the PDB structure shown as layers of colored disks in the workspace. These dummy atoms designate the positions of the lipid head groups in the OPM database.

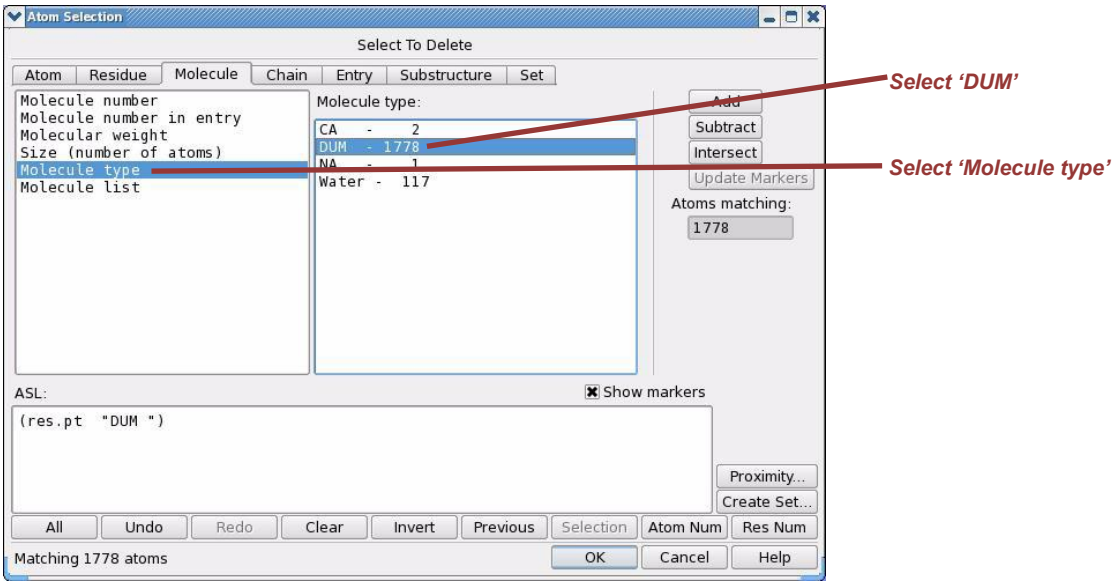
   Note that the system is shown in a similar orientation that we used in the previous exercise for better comparison, not the official OPM orientation shown in Figure 2.19.

Figure 2.20 Importing the OPM PDB file in the workspace



3. Delete the dummy atoms by clicking and holding the red 'X' icon in the Workspace toolbar and launching the Selection panel.
4. In the Selection panel click the Molecule tab.
5. Select Molecule type in the left panel and then DUM in the right panel as shown in Figure 2.21.

Figure 2.21 Selecting the dummy atoms for deletion



6. Click Add and then OK, and both disks will disappear from the workspace.

NOTE These atoms are not necessary for building the membrane. The protein in **1su4_opm.pdb** is placed in a coordinate system such that the membrane should be aligned at +/- *d*/2 along the Z axis where *d* is the distance between the two sets of head groups in the bilayer. For 1su4 the value of *d* is 29.6 Å.

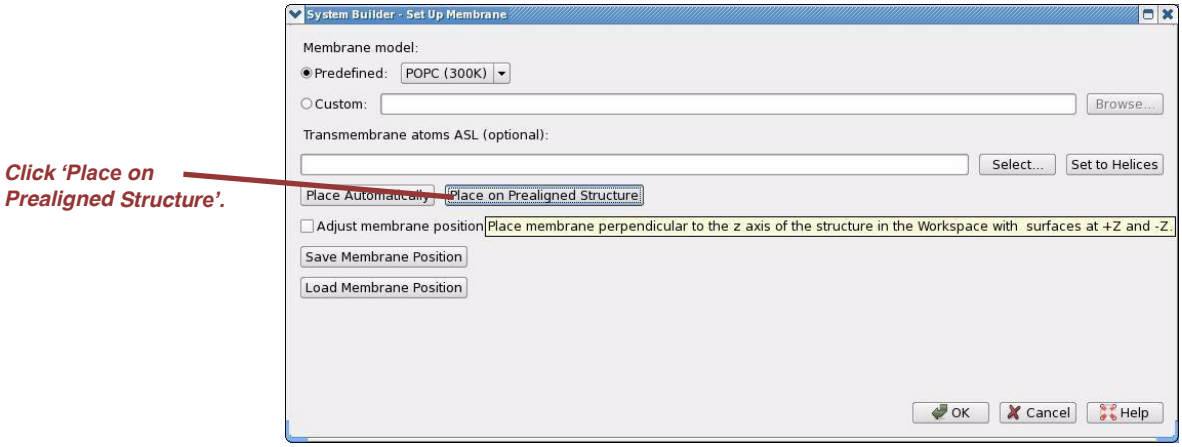
7. Preprocess the remaining system with the Protein Preparation Wizard exactly as was done in the previous exercise shown in Figure 2.5 on page 36. Do not forget to delete the sodium ion and ignore or cancel any warning messages.

NOTE The total charge of the system is -27 as opposed to -23 because the OPM based PDB file does not have the correct +2 charge assigned to the Ca ions (there are two of them). The charges can be added in Maestro using the "Increment formal charge" button in the Build menu. Toggle this button on and then click twice both Ca ions in the Workspace to bump up their charge to +2.

8. Launch the System Builder by selecting **Applications > Desmond > System Builder** and set the solvation and ion placement options.

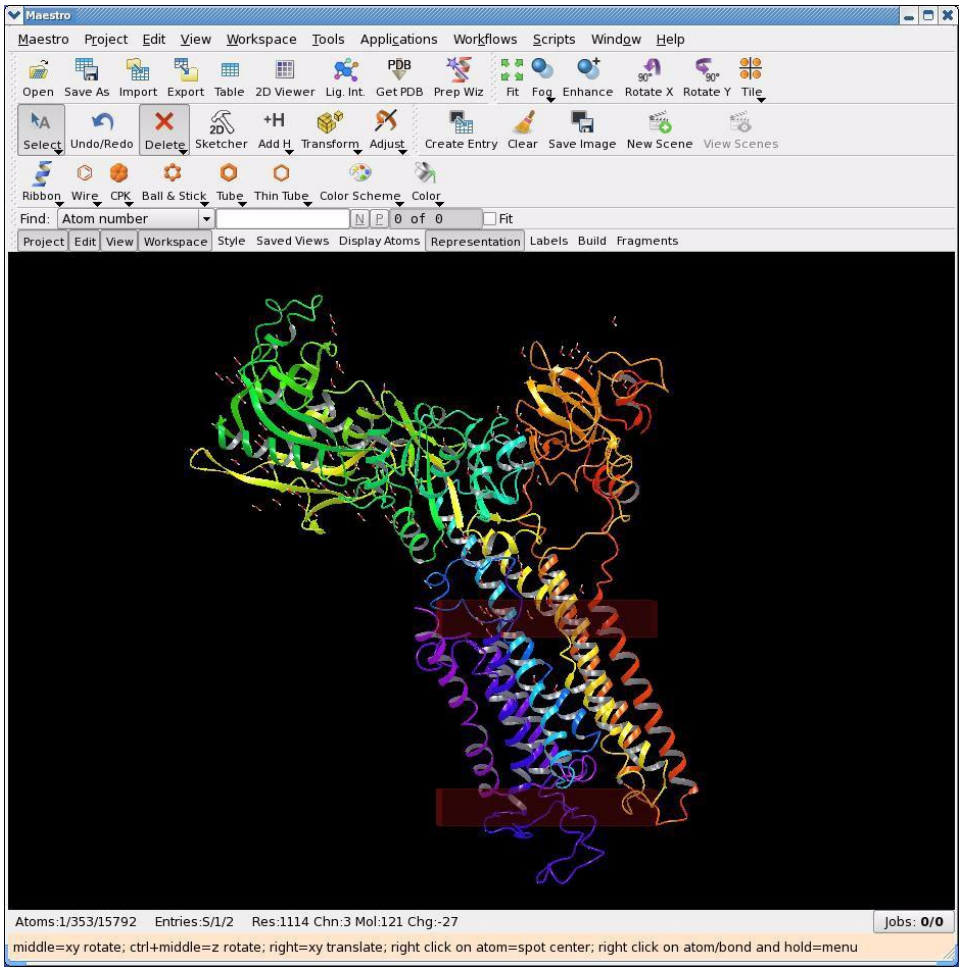
9. Open the Set Up Membrane dialog box. Click Place on Prealigned Structure as shown in Figure 2.22.

Figure 2.22 Set Up Membrane dialog box



The imported membrane placement is displayed in Figure 2.23.

Figure 2.23 OPM pre-aligned membrane shown in the workspace



As you can see the placement is quite different from that shown in Figure 2.14 and Figure 2.15. Note the different angle between the transmembrane helices, the plane of the membrane, and the vertical shift between the two membranes.

10. Click OK in the Set Up Membrane dialog box.
11. Click Start in the System Builder panel to generate the membrane.

    The full system is shown in Figure 2.24 and Figure 2.25 for comparison with Figure 2.16 on page 46 and Figure 2.17 on page 47. It is clear from the comparison of Figure 2.17 and Figure 2.25 that the OPM placement results in a much better quality immersion of the protein in the lipid bilayer with much less void left in the channel.

Figure 2.24 Full membrane simulation system for 1su4

Note that the visual artifact discussed with respect to Figure 2.16 on page 46 is even more pronounced here. The protein seems to extend into the vacuum. Nonetheless, because of the periodic boundary conditions, this is correct. Basically, it is a periodic image of the protruding part of the protein at the bottom of the simulation box where there is plenty of water that is used in the computation.

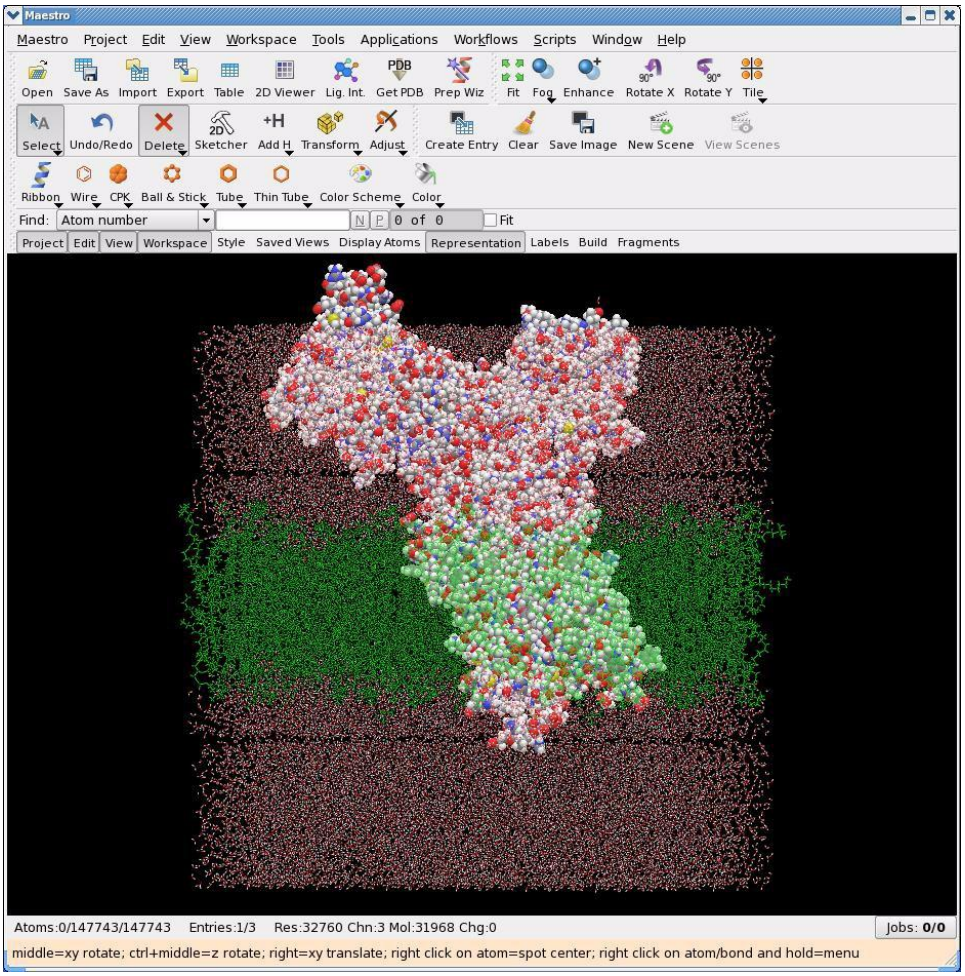
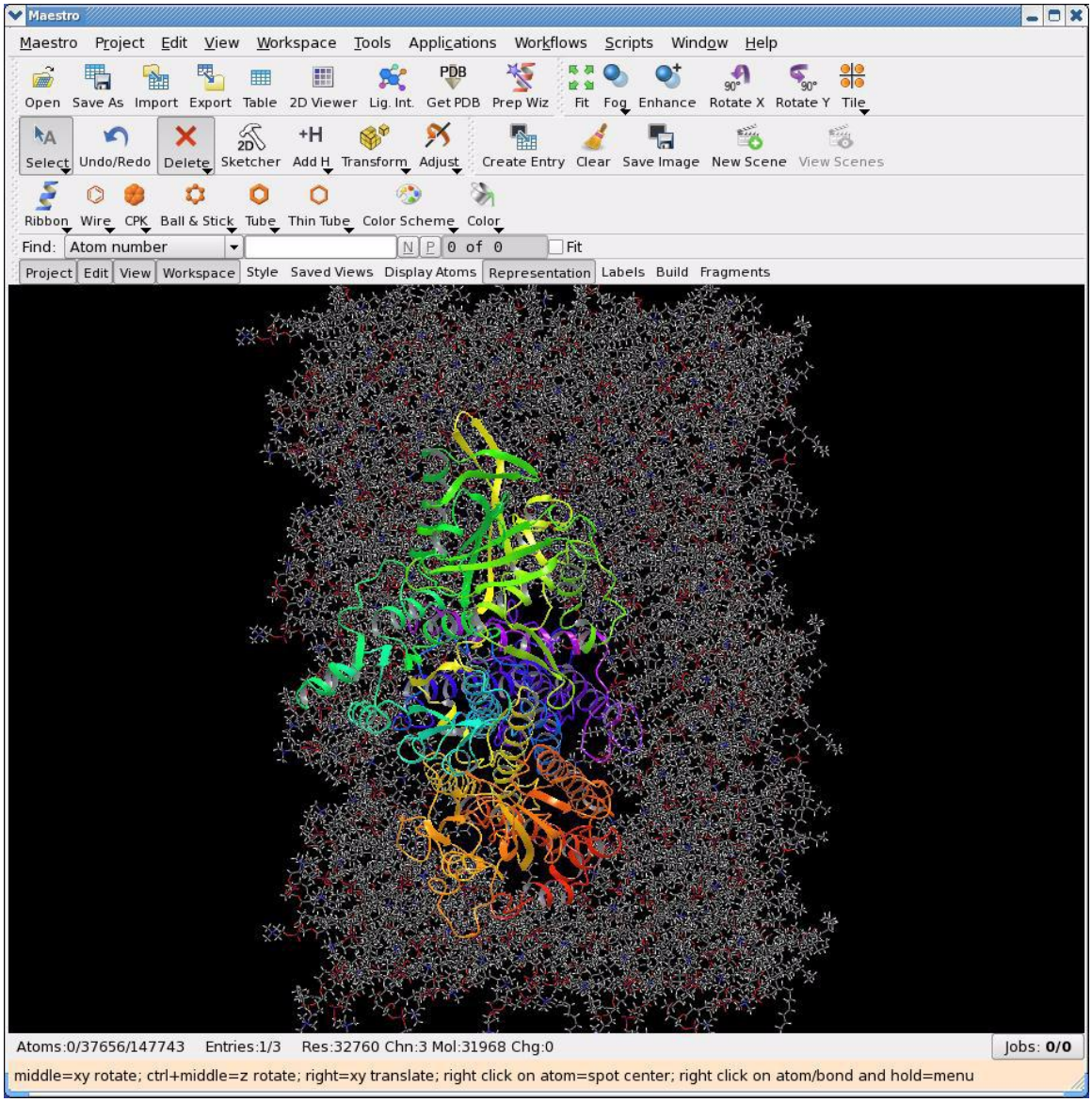


Figure 2.25 OPM transmembrane hold in the POPC bilayer for 1su4



3 Finishing Preparations for Desmond Simulation

Overview

There are a few more tasks that must take place before a simulation can be run:

- The System Builder will always generate OPLS-AA force field parameters for the simulation system it builds and the parameters will be included in the **.cms** output file. However, you may want to assign different force field parameters in case the Schrödinger provided OPLS-AA parameters are not adequate for a particular system. You can assign alternative force field parameters for your system using a companion program to Desmond called *Viparr*.
- You will also have to specify Desmond run-time simulation parameters.

These topics are addressed in the following sections along with instructions for converting Amber Molecular Dynamics simulation input files to Desmond **.cms** files.

Generating Force Field Parameters with Viparr

Viparr is a Python script that reads a **.cms** (or **.mae**) file and writes another **.cms** file, which has all the force field parameters required to run a Desmond simulation. *Viparr* can assign force field parameters from a variety of well-established force fields and water models. *Viparr* force field assignment is template based. This means that unlike force field servers, e.g., Schrödinger's OPLS-AA server that can generate parameters for various atom types derived automatically from the molecule's topological structure, *Viparr* is designed to find templates (such as an entire residue) in **.cms** files and assign the appropriate force field parameters by looking at a template database associated with a particular force field.
The resulting parameters should therefore be identical with those of the published, authentic force field.

NOTE When *Viparr* begins it wipes out all previous force field information from the input **.cms** file. Therefore, it is efficient to build the simulation system using System Builder, and then perform post-processing on the **.cms** file with *Viparr* to assign the desired force field parameters.

The *Viparr* script is called **viparr.py** and basic help on its usage can be obtained via the command line (output for version 1.8.1):

The command line invocation of the *Viparr* script is

```
$ $SCHRODINGER/run -FROM desmond viparr.py
```

but it is referred to as **viparr.py** below.

```
$ viparr.py --help
usage:
    viparr.py [options] mae_file maeff_outfile

Description:
    Viparr is a force field parameter assignment program.
    -f and -d are used to specify force fields; the order of force fields
    is important: earlier ones take precedence over later ones.

Simple example:
    viparr.py -f charmm27 -f spc mae_file maeff_outfile

More complicated example:
    viparr.py -d me/myfiles/myff -f charmm27 -f spc mae_file
    maeff_outfile

Available built-in force fields:
    amber03
    amber94
    amber96
    amber99
    amber99SB
    amber99SB-ILDN
    amber99bsc0
    amber99bsc0xol
    cgenff_base_v2b6
    cgenff_base_v2b7
    charmm22nocmap
    charmm22star
    charmm27
    charmm32
    charmm36_lipids
    charmm36_nucleicacids
    oplsaa_impact_2001
    oplsaa_impact_2005
    oplsaa_ions_Jensen_2006
    spc
    spce
    tip3p
    tip3p_charmm
    tip4p
    tip4p2005
    tip4pew
    tip5p

    Options:
    --version   show program's version number and exit
```

```
-h, --help   show this help message and exit
-cCTNUM      run Viparr for one CT block only, e.g., for the
             first, use "-c 1"
-fFFNAME     built-in force field name; several can be listed,
             each preceded by its own -f
-dFORCEFIELD user-provided force field directory; several can be
             listed, each preceded by its own -d
-mMERGE_DIR path to user-defined force field directory that is to be
             merged with previously specified force field; several
             can be listed, each preceded by its own -m
-nFFIO_NAME force field name to put into output file
-pPLUGINDIR override for plugins directory (advanced usage)
-x           do not print header info (for testing purposes)
-v           verbose output
```

The built-in force fields include the latest versions of Amber, CHARMM, and OPLS-AA families of fixed-charge force fields. *Viparr* also provides the parameters for all SPC and TIP families of water models. A simulation system, or *chemical system*, is described using a number of structures called *connection tables* that reside in *CT blocks* in the **.cms** file. These CT blocks are designated by the `f_m_ct{}` structure and are composed of a number of chains, which in turn are composed of a number of residues; for our purposes, residues may be something other than amino acids (for example, water molecules or ions). *Viparr* matches residues in the chemical system to templates in the force fields.

Viparr uses atomic numbers and bond structure to match residues to templates. Thus if there are non-standard atom or residue PDB names in the **.cms** file, there is no need to modify them to match the names used in the force field. You can freely modify atom and residue names for historic, idiosyncratic, or any other purposes. In particular, *Viparr* will identify the N- and C-terminal versions of the residues correctly, as well as protonated/deprotonated versions of a residue—even if they are not identified as such in the **.cms** file.

Viparr uses the atom ordering in the **.cms** file and does not alter this ordering when creating the output file. Residue numbering is also left unchanged, and can begin with any integer (including negative integers); numbering does not even need to be contiguous, which is typical for many PDB files. Residues with different chain names can have the same residue number. To aid in diagnosing problems with the input **.cms** file, messages involving residues have the form: `< "chain_name", residue_number > (residue_name)` and is usually preceded by a structure number (CT number).

Viparr outputs a compressed force field representation when all the residues in a connection table are the same. For a connection table that only contains water molecules, this means that force field parameters are output only once.

For the 1su4 example used in "Preparing a Desmond simulation with the System Builder" on page 30, invocation of *Viparr* is very simple:

```
$ viparr.py -f charmm27 -f spc 1su4_desmond.cms 1su4_desmond_out.cms
```

where **1su4_desmond.cms** is the output file from the Desmond Panel. The CHARMM27 force field has built-in parameters for the protein, calcium, and the POPC lipid model. The resulting **1su4_desmond_out.cms** structure file along with the **1su4_desmond.cfg** configuration file that can be generated using the **Applications > Desmond > Molecular Dynamics** panel can now be used to run Desmond simulations (see "Specifying Desmond Simulation Parameters" on page 59).

This simple example already shows a very useful feature of *Viparr*; it can be used to specify multiple force fields. In this case, each residue in the chemical system matches a template in exactly one of the specified force fields. This means that the protein residues and the two calcium atoms will be assigned CHARMM27 parameters whereas the water molecules get SPC parameters. The command line invocation for assigning the same protein parameters, but a different water model of TIP4P, appears similar to this:

```
$ viparr.py -f charmm27 -f tip4p 1su4_desmond.cms 1su4_desmond_out.cms
```

You can also use multiple force fields to combine components of two or more force fields. In this case, residues in the chemical system match templates in more than one of the specified force fields. In this operation mode, all matching force fields are applied. For example, one force field provides the angle parameters for a set of residues, while another force field provides the dihedral parameters for the same residues. The force field components should be disjoint so there is no conflict with the parameters that are assigned to each component.

You may also use multiple force fields when parameters assigned to a particular residue by one force field override parameters assigned by another force field. This situation is similar to combining force fields; residues in the chemical system match templates in more than one of the specified force fields and all matching force fields are applied. However, in this case two or more force fields provide parameters for the same term; for example, two force fields provide parameters for the angle between atoms 1, 2, and 3 causing a conflict. The conflict is resolved by allowing the first force field (that matches the residue) to take precedence. The order is the order in which the force fields were specified on the command line.

In all multiple force field assignment cases, *Viparr* prints an error message and aborts if a bond exists between two residues that are not matched by the same force field.

The selected force fields should also have consistent van der Waals combining rules. Otherwise, *Viparr* will abort with an error message.

Viparr will print the following warning and error messages when matching residues to templates:

- If any residue matches more than one template in a force field, then *Viparr* exits with an error. (No *Viparr* force field should contain identical templates.)
- If any residue name is matched to a force field template with a different name, a message is printed. A maximum of 5 messages are printed per residue-template name pair.
- *Viparr* will check that all residues are matched by one of the selected force fields. If there are any unmatched residues, *Viparr* will print all unmatched residues and exit with an error. A maximum of 5 messages are printed per unmatched residue name.
- If any residue is matched by more than one of the selected force fields, *Viparr* will print a warning message. The user should be cautious and be responsible for any intended use of multiple force fields with multiple matches.

Besides the `-f` option, there are two more basic options. The `-c` option allows the user to assign parameters for a particular structure (CT block) in the input **.cms** file and the `-n` option provides a means to include a user-defined comment in the output **.cms** file for annotation purposes.

Viparr also supports user-defined, special force fields (the `-d`, `-m`, and `-p` options), but that is beyond the scope of this tutorial. Please refer to the *Desmond User's Guide* for information.

Adding Constraints

NOTE While the System Builder automatically adds all the necessary constraints to the **.cms** file, *Viparr* does not. If you process a **.mae** or **.cms** file with *Viparr* you will have to add the constraints in a separate step by running the following command:

```
$ $SCHRODINGER/run -FROM desmond build_constraints.py input.mae
output.mae
```

where **input.mae** is typically the Maestro file with *Viparr* parameters and **output.mae** will include the constraints. The command `$SCHRODINGER/run -FROM desmond build_constraints.py -help` will list more options.

Importing a Simulation System from the Amber Molecular Dynamics Package

If you have developed simulation systems using Amber Molecular Dynamics package (http://ambermd.org), you can import these systems into the Desmond environment. Desmond includes a command-line script for converting an Amber **prmtop** file and associated **crd** file into a Desmond **cms** file. The script is called **amber_prm2cms** and can be launched from the command line as follows:

```
$ $SCHRODINGER/run -FROM mmshare amber_prm2cms.py -p my_amber_system.prm
-c my_amber_system.crd -o output.cms
```

The two input files should be standard Amber **prmtop** and **crd** files, respectively, and the output file is the Desmond **cms** file. Since Desmond reads the force field parameters directly from the **cms** file, it is guaranteed that Desmond will apply the exact same force field, in one-to-one agreement with the Amber **prmtop** file.

NOTE While the resulting `output.cms` file requires no additional treatment for Desmond simulations (and constraints will be automatically included), analysis tools available in Maestro do require reformatting before use. **Applications->Desmond->Model System Regeneration…** is a new option in Desmond that allows one to reformat such a system by dissecting it into separate blocks in the **cms** file including the solute, solvent, ligand, ions, etc., respectively, allowing for proper use with analysis tools.

Specifying Desmond Simulation Parameters

Before it can perform simulations, Desmond needs certain simulation parameters to be set. These parameters can be set in two ways:

- Using one of the Desmond applications in Maestro.
- Editing the Desmond configuration file directly.

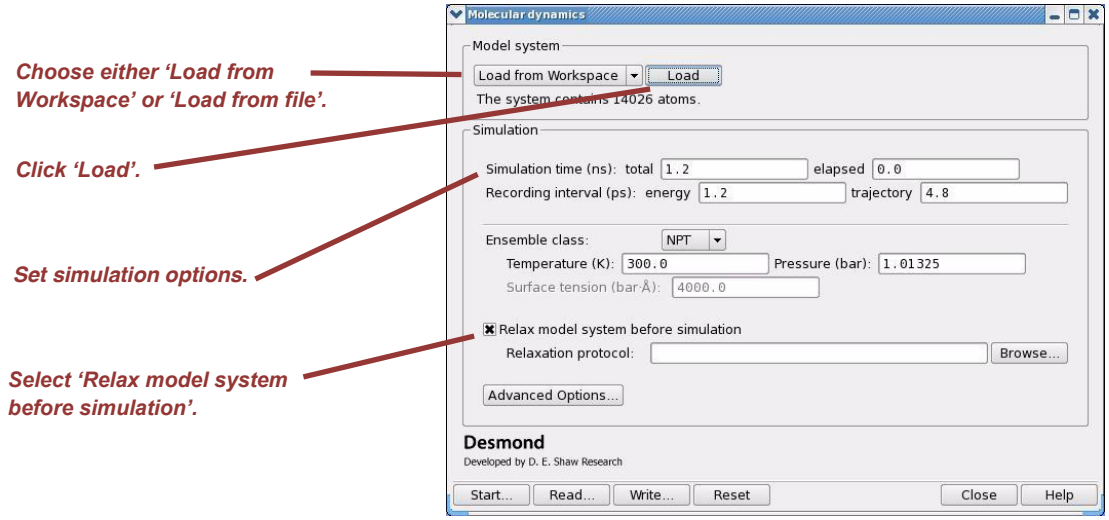
The following sections provide more details on each option.

Using Desmond applications in Maestro

There are several Desmond applications available in Maestro from the **Applications > Desmond** menu. The applications include minimization, molecular dynamics, simulated annealing, free energy perturbation (FEP), replica exchange, and metadynamics. In this tutorial we will focus on molecular dynamics and FEP, and give a simple example of metadynamics. Minimization is typically used in system relaxation, which will be covered. Simulated annealing and replica exchange require very similar setup to molecular dynamics and are both documented in the *Desmond User's Guide* and in the *Schrödinger Desmond User Manual*.

The Molecular Dynamics panel appears as shown in Figure 3.1.

Figure 3.1 Setting up a Desmond simulation



Import the model system into the Molecular Dynamics panel by clicking Load: select either Load from the Workspace or Import from file (and select a **.cms** file), and then click Load. The import process may take a few seconds for large systems.

The basic settings shown in Figure 3.1 include:

- **Simulation options.** Include simulation time, simulation intervals (time intervals by which different energy terms are recorded and trajectory snapshots are saved), and ensemble class (NVE, NVT, NPT, NPAT, and NPγT).
- **Model relaxation.** Select Relax the model system before simulation. You can either use a default protocol (which includes a series of pre-defined minimizations and molecular dynamics executions to relax the system before the production simulation starts) or you can import your own relaxation protocol. The stage-wise relaxation/equilibration tasks can be defined in a so-called **multisim** script file, which allows for running multiple simulations. The syntax of multisim **.msj** file is documented in the *Schrödinger Desmond User Manual*.

Relaxation and equilibration of membrane systems requires extra care to prevent water molecules from diffusing into initial voids between the membrane protein and the lipid bilayer as shown in Figure 2.17 on page 47 and Figure 2.25 on page 54. The Desmond/Maestro distribution includes a special script that can be used to generate a custom relaxation protocol that you can then import to the Molecular Dynamics panel. Run the following command from the command line to generate a suitable relaxation protocol for the OPM-placed 1su4 system in "Importing Membrane Placement from the OPM Database" on page 48.

```
$SCHRODINGER/run -FROM mmshare relax_membrane.py -t 300 -i
1su4_opm_Desmond_setup-out.cms
```

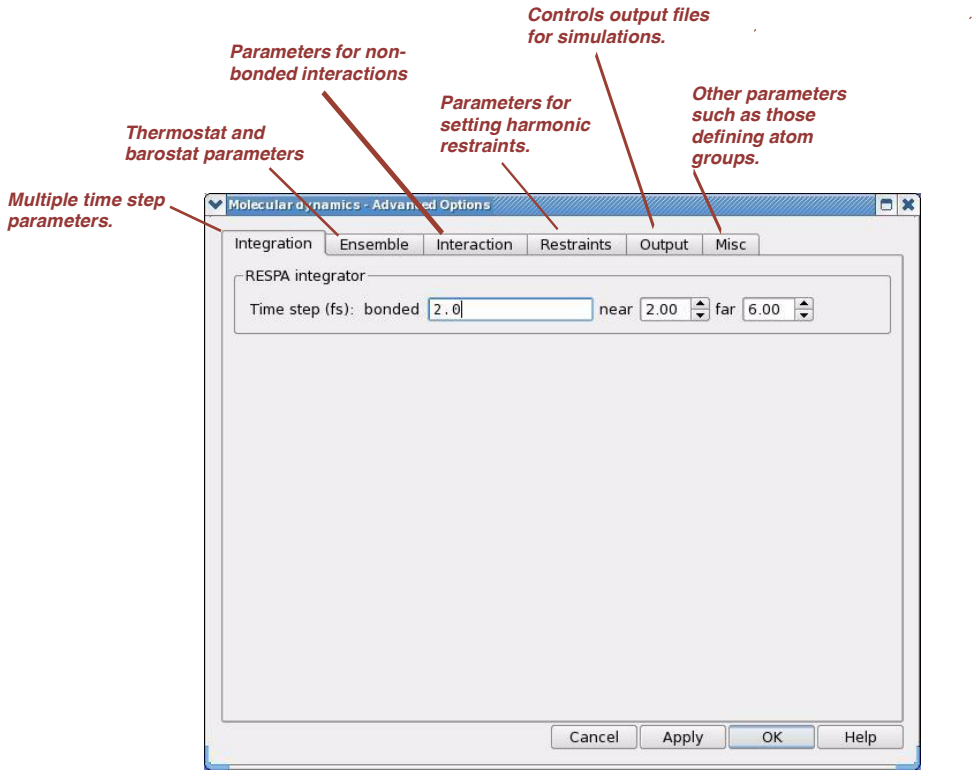
You can directly import the resulting **relax.msj** file as a suitable custom protocol in the Molecular Dynamics panel, but you can also manually edit the multisim script beforehand to modify or introduce additional relaxation stages.

When you click Advanced Options, the Advanced Options dialog box appears as shown in Figure 3.2.

Options in the Integration tab for the MD task include the multiple time step (RESPA) schedule and constraints.

Some parameters are interdependent; for example, spinboxes for setting the time step schedule. If you set the innermost time step and hit **Return** the midrange and longrange time steps are adjusted automatically. Of course, you can always manually edit the values or use the spinbox up/down arrows to adjust the values.

Figure 3.2 Advanced Options for simulation job



The Advanced Options dialog box has several tabs:

- Ensemble—Contains thermostat and barostat parameters.
- Interaction—Contains parameters for computing non-bonded interactions.
- Restraint—Provides a table in which multiple sets of atoms can be subjected to harmonic restraint with a user-defined force constant. Atom selection can be directly input as an ASL expression or you can click Select to bring up the Atom Selection dialog box.
- Output—Includes the names of various Desmond output files, and allows you to specify how often these files are updated during the simulation. For example, different energy terms are recorded in the energy sequence file in user-defined intervals. Snapshots of the simulation are periodically saved in the trajectory file and the entire simulation state is also saved at user-defined intervals in a checkpoint file. Desmond simulations can be restarted from checkpoint files with bitwise accuracy.

NOTE The Output tab has a very useful option. If Glue close solute molecules together is set, the Desmond trajectory file will be constructed in such a way that receptor ligand complexes, DNA strands or any other multi-molecule structures always stay together for visualization purposes even if their primary images separate due to the periodic boundary conditions. In earlier versions of Desmond this separation (which, of course, does not affect the simulation itself) caused unsatisfactory visual artifacts in trajectory viewing.

- **Misc**—Provides control over miscellaneous parameters, including the definition of atom groups. Atom group definitions are documented in the *Desmond User's Guide* listed in "Documentation Resources" on page 116. Atom groups include thermostat groups, energy groups (associated with Desmond's vrun application), frozen atoms, etc. Note that atom groups are assigned in the **.cms** structure file.

NOTE A `frozen' atom group means a set of atoms that move together during the entire MD simulation as a rigid body. In Desmond, unfrozen atoms cannot be connected to frozen atoms.

For example, an entire molecule can be frozen (during equilibration), but one cannot freeze only the heavy atoms, or just certain residues. In that case, restraints should be used. Also note that frozen atoms cannot be used with NPT simulations, because the Virial will be incorrect. The other atom group termed *energy* is used to define parts of the system whose interaction energies can be monitored during a simulation by application of the "energy groups" plugin, which is documented in the *Desmond User's Guide*. Atom groups can be selected the same way as restraint groups, using ASL syntax or the Atom Selection dialog box.

The Molecular Dynamics panel is filled in automatically with default parameter values. To set your own default values so they are automatically presented in the Molecular Dynamics panel, set the desired values in any of the tabs and save it by clicking Write. Then, copy the resulting **.cfg** file to **~/.schrodinger/desmond31/desmond_default.cfg**. The new default values take effect the next time you open the Molecular Dynamics panel.

Detailed documentation of the Molecular Dynamics run-time parameter settings can be found in the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

Once you have set values for relevant parameters, you are ready to execute a simulation.

Editing the Desmond Conguration File Directly

The Desmond conguration file syntax is described in detail in the *Desmond User's Guide* (see "Documentation Resources" on page 116). You can edit the Desmond configuration file directly. It can be useful to edit existing configuration files that were used in other projects. You can always write out a configuration file and use it as a template by clicking Write in the Molecular Dynamics panel.

4 Running Desmond Simulations

Overview

You can run a Desmond simulation from the Molecular Dynamics panel or the command line. Before running a simulation you should select Relax the model system before simulation in the Molecular Dynamics panel. As discussed earlier, one should perform structure relaxation to prepare a molecular system for production-quality MD simulation. Maestro's built-in relaxation protocol includes two stages of minimization (restrained and unrestrained) followed by four stages of short MD runs with gradually diminishing restraints and increasing temperature.

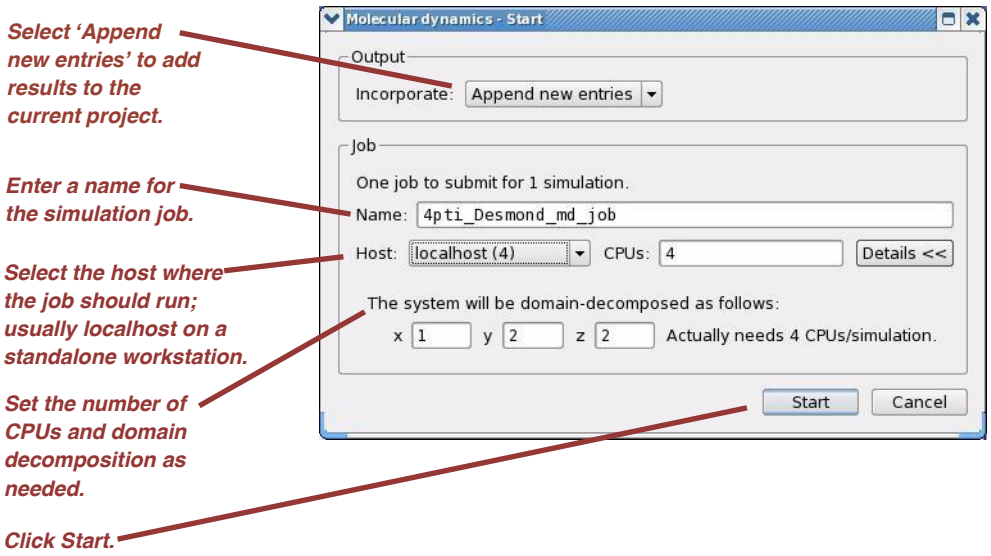
The relaxation parameters may also require edits. The relaxation protocol is written to a **.msj** command file (multisim job) which can be run from the command line (see "Running MultiSim jobs from the Command Line" on page 67). You can manually adjust the relaxation parameters by hand-editing the **.msj** file.

NOTE If you decide to run the simulation from the Molecular Dynamics panel, you will be limited to use the built-in OPLS-AA force field. You can use *Viparr* to generate other than OPLS- AA parameters, but currently, if you run the simulation using *Viparr* you will need to run it from the command line.

Running Simulations from the Molecular Dynamics Panel

1. Click Start at the bottom of the Molecular Dynamics panel to launch Desmond simulation jobs directly from Maestro. The Molecular Dynamics-Start dialog box appears as shown in Figure 4.1.

Figure 4.1 Running a Desmond simulation



2. Select Append new entries from the Incorporate option menu in the Output area to indicate that results of the Desmond simulation should be added to the current Maestro project.
3. In this example, the job will run on localhost, which is typically a standalone workstation, using 4 CPUs with a domain decomposition (the number of blocks into which the simulation box will be split in the X, Y, and Z directions) of 1x2x2. For large-scale simulations Host is usually a Linux cluster with dozens to hundreds of CPUs.
4. Click Start. The Desmond simulation process begins.

Running Simulations from the Command Line

Given a valid **.cms** structure file and corresponding **.cfg** conguration file, the command line syntax for running Desmond jobs is as follows.

```
$SCHRODINGER/desmond -HOST <hostname> -exec <desmond_task> -P <cpu>
-c <jobname>.cfg -in <jobname>.cms
```

In case a job has to be restarted from a checkpoint file, replace the `-c` and `-in` options with `-restore` and the name of the checkpoint file:

```
$SCHRODINGER/desmond -HOST <hostname> -exec <desmond_task> -P <cpu>
-restore <jobname>.cpt
```

where:

- `<desmond task>` can be either `minimize` to run a simple minimization, `mdsim` (`remd` for replica exchange job) to run an MD simulation (including FEP jobs described in "Preparing Free Energy Perturbation and Metadynamics" on page 69), or `vrun` to run the vrun trajectory analysis tool (see the *Desmond User's Guide* for information about vrun). Note that vrun is the application used by the Schrödinger Simulation Event Analysis tool, which can be accessed from the Applications > Desmond menu.
- `<jobname>` is the name of the job.
- `<hostname>` is the name of the compute host, which can also be the name of a queue on a cluster.

- `<cpu>` species the number of CPUs you want each Desmond simulation subjob to use. This should be 1 for running the serial version of Desmond (for example, to quickly test on your workstation whether your job will start at all), or a power of 2. The number of CPUs requested for a restore job must be the same number of CPUs used with the job that saved the checkpoint file. You can restart a Desmond MD simulation job from the Molecular Dynamics panel as well as shown in Figure 3.1 on page 60. Select Import from file and browse to select the checkpoint file. Also note that the restore mechanism can readily be used for continuing a completed simulation if you simply want to extend the trajectory, but make sure to specify the total simulation time of the extended simulation, not the additional time. For example, if you want to extend a 100 ns simulation by another 100 ns, simulation time should be set to 200 ns.

NOTE Advanced users should consider the following:

- The Desmond driver in the Maestro environment tries automatically optimizing certain configuration parameters and, therefore, the actual **.cfg** file (called ***your_jobname*-out.cfg**) that is used by Desmond itself may not be identical to the original **.cfg** file that you prepared. The differences can be safely ignored most of the time, but in some cases you may want to run Desmond with the exact parameters given in the **.cfg** file. To turn off internal optimization, launch Desmond from the command line with the `-noopt` argument:

  ```
  $SCHRODINGER/desmond -noopt ...
  ```

- By default Desmond applications are run in single precision. Use the `-dp` option to switch to double precision. This is only recommended for debugging purposes because of the performance penalty caused from using double precision.

  There is a particularly useful command-line script that can automatically generate reasonable Desmond configuration settings to facilitate efficient force computations including non-power-of-two Fourier grids. The script is called **forceconfig** and can be launched as follows:

  ```
  $SCHRODINGER/run -FROM desmond forceconfig.py <input.dms>
  ```

  input.dms is a binary file that is generated automatically prior to any Desmond run and is normally invisible to the user. However, for the purpose of using **forceconfig.py** one needs to generate **input.dms** explicitly using the following command:

  ```
  $SCHRODINGER/run -FROM desmond mae2dms input.cms input.dms
  ```

  where **input.cms** can be the output structure file of System Builder or Viparr.

NOTE **(For expert users)** The **.dms** file format is the native format for Desmond and the only format supported in the standalone Desmond source distribution. If you have a **.dms** file, there is a reverse script called **dms2mae** that can be used the same way to convert a **.dms** file to **.cms** format.

  The **forceconfig** script analyzes the system and outputs the recommended Desmond configuration settings. The output can be copied and pasted into a custom Desmond configuration file for use with the simulation system at hand. Run the script with the `-help` option to see optional command-line arguments.

- While a Desmond job is running, all files related to the job are continuously updated in a temporary directory; at job completion (or if the job crashes), files are copied back to the working directory.

The location of this temporary directory is:
tmpdir/username/jobname

where:
- **tmpdir** is specified in the **$SCHRODINGER/schrodinger.hosts** file
- **username** is the username under which the job was submitted
- **jobname** is the name of the Desmond job

Use the `-LOCAL` option to keep all job files in your working directory all the time. Detailed documentation of several more options for running Desmond on the command line can be found in the Schrödinger Desmond User Manual listed in "Documentation Resources" on page 116 and the `-help` option gives a quick reference with examples.

NOTE Although running Desmond from the command line as described in this section is fully functional, the preferred way of running even single jobs is via the MultiSim utility.

Running MultiSim Jobs from the Command Line

It is often necessary to run multiple jobs, either sequentially or simultaneously, to complete a particular computational task. The two most common cases are system relaxation and FEP simulations. System relaxation was discussed on Figure 1.16 on page 26 and FEP simulations are introduced in "Preparing Free Energy Perturbation and Metadynamics" on page 69.

Multiple jobs are handled by the MultiSim facility. MultiSim reads a **.msj** command script file and runs multiple simulations defined within it. For instance, to apply a custom relaxation protocol the following command can be used:

```
$SCHRODINGER/utilities/multisim -JOBNAME <jobname> -maxjob <maxjob>
-cpu <cpu> -HOST localhost -SUBHOST <hostname> -i <jobname>.cms
-m <jobname>.msj -o <jobname>-out.cms
```

where:

- `<jobname>` is the name of the job.
- `<maxjob>` is the maximum number of subjobs (Desmond simulation jobs of any kind) that can be submitted to a host or a queue simultaneously. A value of zero means that there is no limit.
- `<cpu>` is the number of CPUs per subjob, which can also be specified as, e.g., "2 2 2" (note the quotes) indicating 8 CPUs with a spatial decomposition of 2x2x2.
- `-HOST localhost` means that the master job runs on your local workstation.
- `<hostname>` given with the `-SUBHOST` option is the name of the host or queue where the simulation jobs will be running.
- `-c <config_file>` is an optional argument used to set non-default Desmond configuration parameters.
- `-mode umbrella` is the default option when a MultiSim job is run on a cluster. MultiSim jobs include a cascade of consecutive subjobs, each of which requires a new CPU allocation; depending on the availability of cluster resources, this can involve a significant time lag. The `-mode umbrella` option instructs the cluster job submission system to recycle allocated CPUs, thus providing better job turnaround time.

The input files include a **.cms** file, which is the output file of the System Builder, and the **.msj** command script file, which defines the custom relaxation protocol. The custom-relaxed system will be saved in the **<jobname>-out.cms** file.

The **.msj** syntax as well as a complete list of MultiSim options can be found in the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116 and the `-help` option can be used for quick reference.

NOTE The MultiSim facility has a very useful restore mechanism. If a MultiSim job fails (for example, an FEP job may involve hundreds of individual Desmond subjobs), it can be restarted from a MultiSim checkpoint file so that only incomplete jobs (crashed or not yet run) will be re-run.

NOTE The MultiSim **.msj** file syntax provides an elegant and powerful alternative to Desmond **.cfg** files. The user can fully configure Desmond, separately for each MultiSim stage, inside the **.msj** script itself. The corresponding **.cfg** files will be generated automatically for each subjob. Also note that configuration settings in the **.msj** file take precedence over settings in **.cfg** file(s).

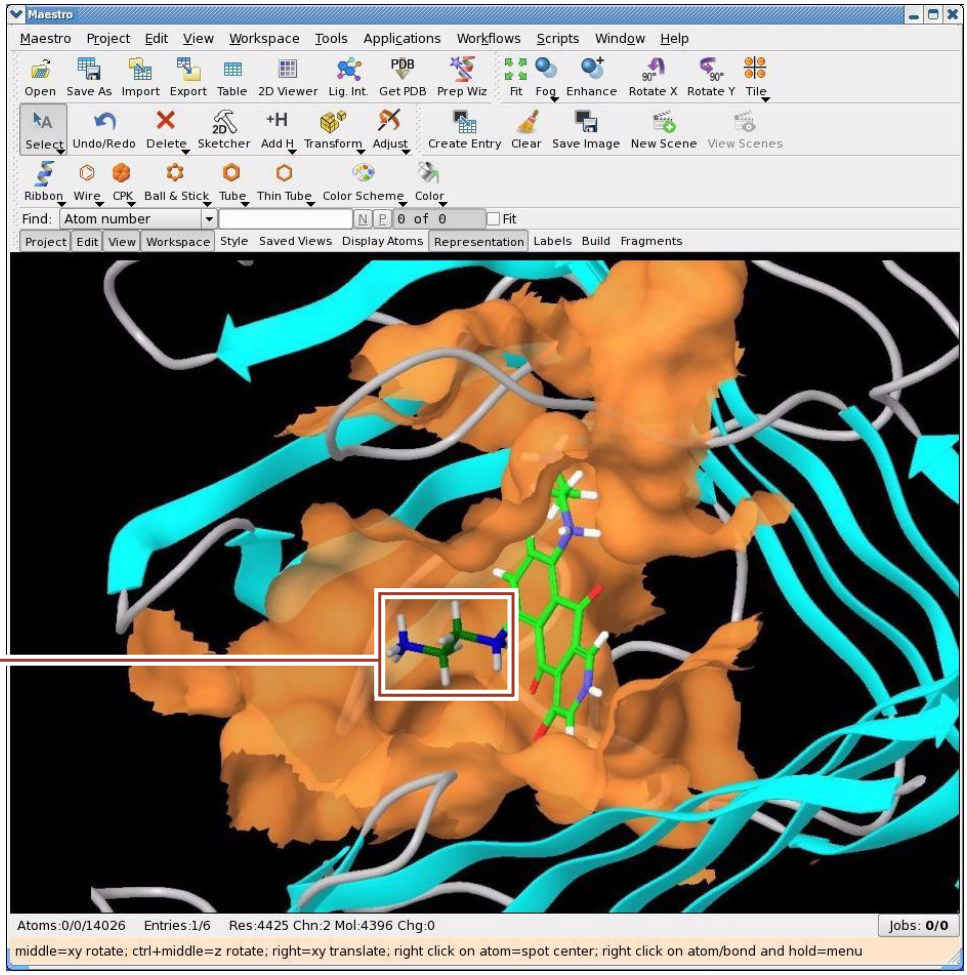
5 Preparing Free Energy Perturbation and Metadynamics

Overview

You can use Desmond to run free energy perturbation (FEP) calculations using the dual topology approach to perform ligand and amino acid residue mutation, annihilation (absolute free energy calculation), and single-atom mutation in a ring structure. From Maestro, you can visually specify the fixed and variable parts of a molecule that are subjected to FEP calculations and then store the atom correspondence map (which defines dual topologies) in the Maestro **.mae** file.

Ligand mutation—the most common FEP calculation—is illustrated in Figure 5.1 using a typical scenario. The figure shows a graphical representation of the soluble form of the human urokinase plasminogen activator receptor (sUPAR, PDB code 2fd6) and the ZINC-01538934 ligand. The protein structure has been processed using the Protein Preparation Wizard as described in "Tutorial Steps" on page 12, and the ligand was docked using Schrödinger's Glide application as part of a virtual screening experiment on the ZINC database (http://zinc.docking.org/). The ligand is rendered in a tube representation in the sUPAR active site with green carbon atoms and ball-and-stick representation.

Figure 5.1 FEP Example—Ligand mutation

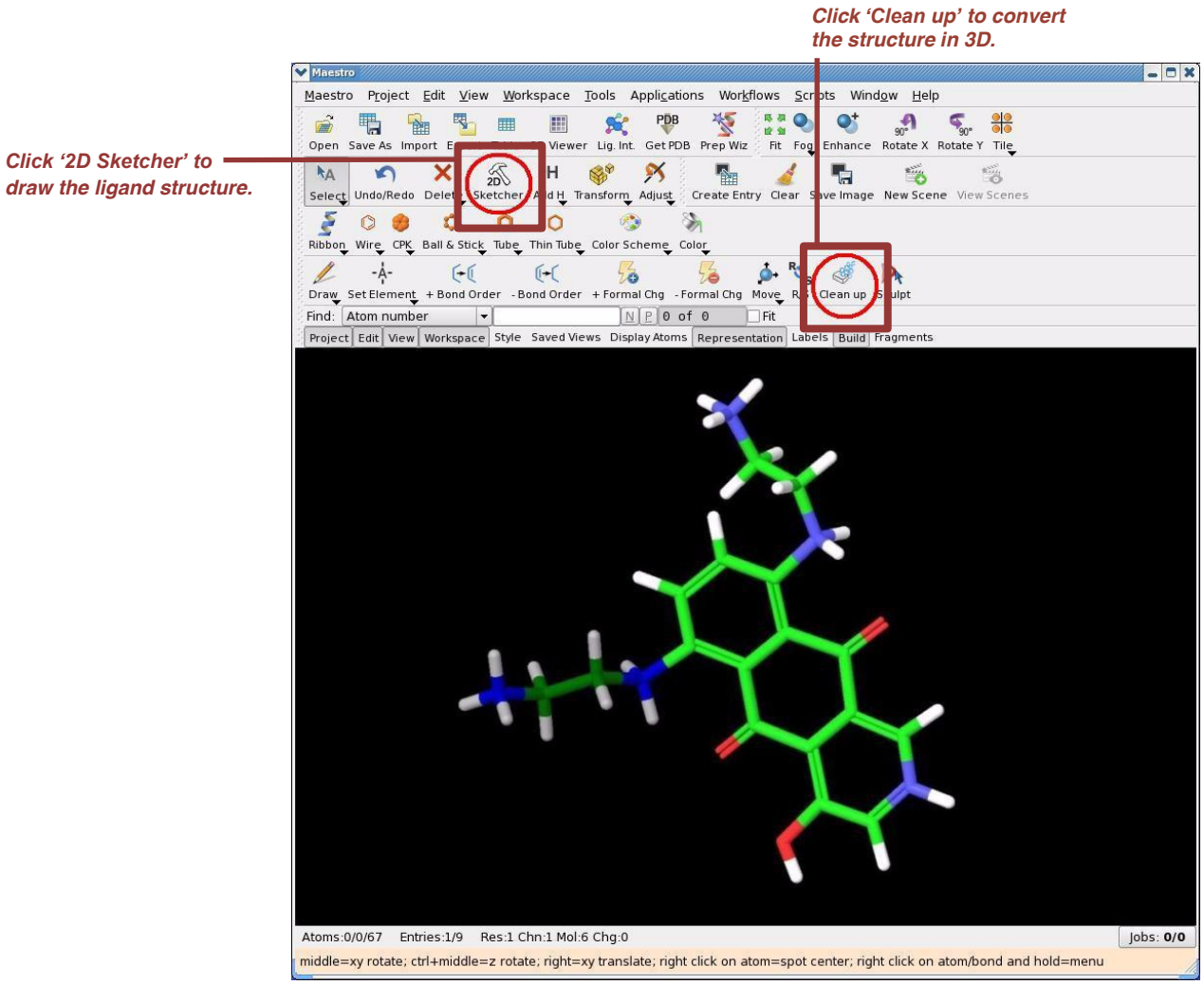


This side chain will be mutated to replace the NH3+ with a methyl group.

As shown in Figure 5.1, contact residues in the active site are displayed with a matching molecular surface to emphasize the 3D layout of the active site. One of the two –NH2 — CH2 — CH2 — NH3+ side chains is colored darker (see at the center of the workspace in Figure 5.1); this is the side chain where the terminal NH3+ group will be mutated to a methyl by FEP.

Note that the setup in Figure 5.1 is only an illustration showing a common real-life example for FEP calculations; however, you will not need to perform any docking calculations for this tutorial exercise. Instead, just build the ligand structure using the 2D sketcher tool (un-check convert to 3D) and then click Clean up geometry as shown in Figure 5.2. (2D sketcher is available in the **Edit** tab and Clean Up is available in the **Build** tab in the Workspace menu.)

Figure 5.2 The ZINC-01538934 ligand structure



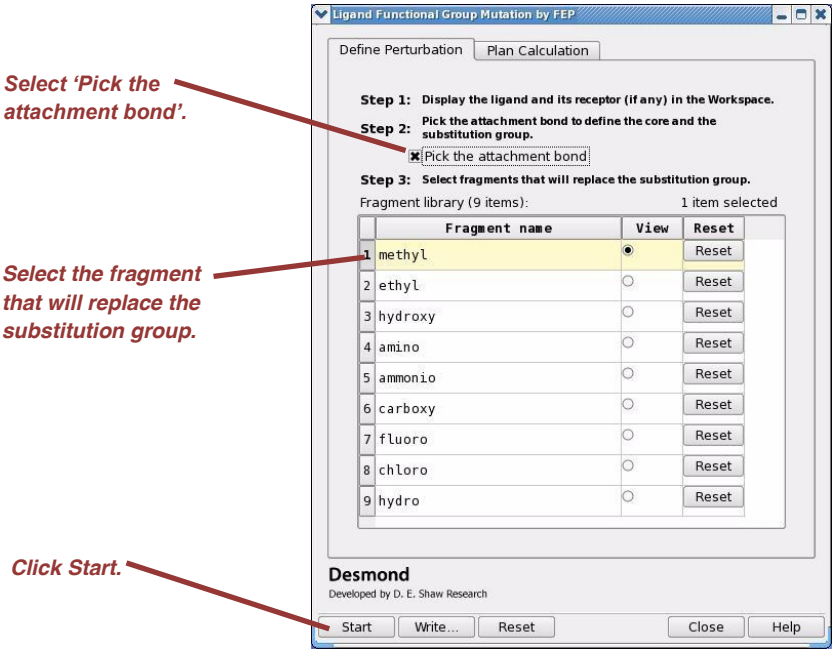
Setting Up an FEP Calculation

To setup a Free Energy Perturbation calculation for ligand mutation:

1. Select **Applications > Desmond > Ligand Functional Group Mutation by FEP**.

   The Ligand Functional Group Mutation by FEP panel appears as shown in Figure 5.3.

Figure 5.3 Ligand Functional Group Mutation by FEP panel

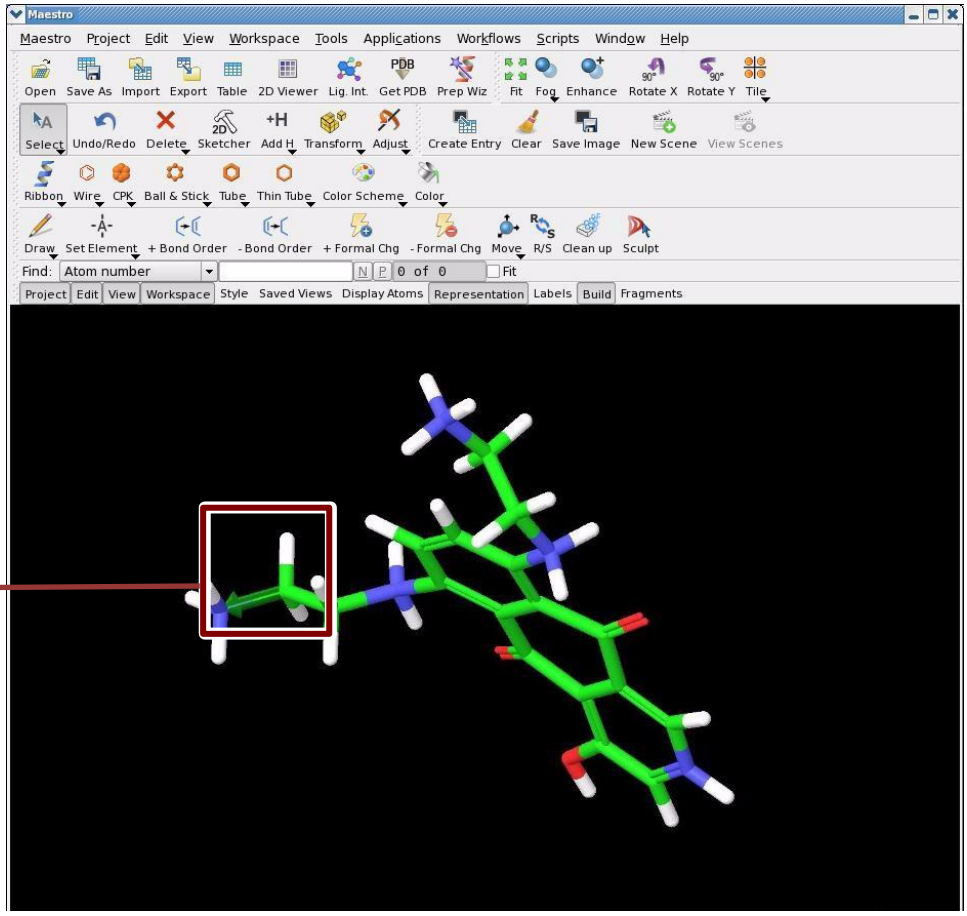


2. Select 'Pick the attachment bond' (Step 2 in Figure 5.3).

   When you define a structural perturbation for FEP, the ligand molecule is separated into two parts: a fixed part (called the *core*) and a variable part (called the *substitution group*). The substitution group will be mutated during simulation, and the core will remain intact. As shown in Figure 5.4, the core and the substitution groups are connected by a single bond termed the *attachment bond* (green arrow), which has to be picked by hand. Currently, the attachment bond can only be a single, covalent bond in the molecule.

NOTE When selecting the attachment bond, you determine the direction of the arrow by which half of the attachment bond you select. The attachment bond arrow should point from the core to the substitution group.

Figure 5.4 Defining the mutation



The attachment bond is shown as a green arrow in the workspace. The arrow should point from the core to the substitution group.

3. Define the mutation to be performed by selecting the fragment(s) that will replace the substitution group from the Fragment Library. For this example, select the methyl row from the list of fragments. The selected row is highlighted in yellow as shown in Figure 5.3.

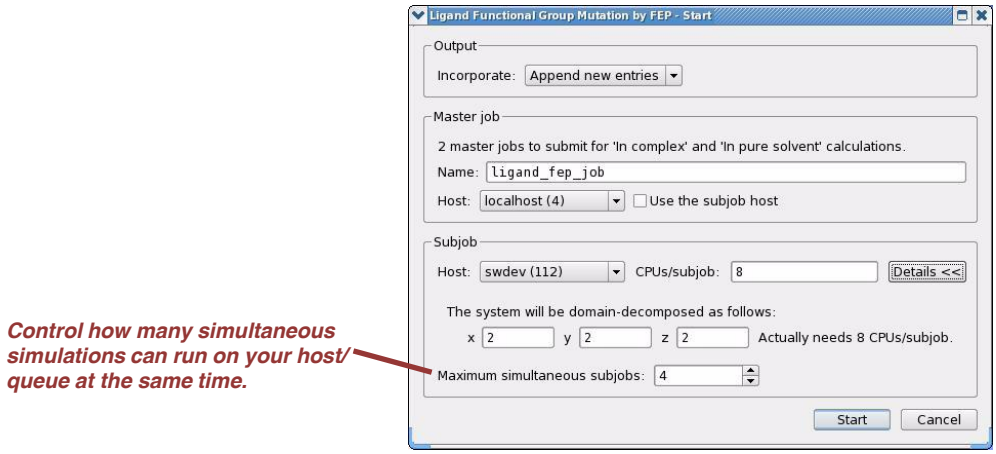
   You can apply multiple mutants at a time by using CTRL-click and SHIFT-click to select the mutants from the Fragment Library. When you select multiple fragments, the FEP ligand mutation application creates different systems for each mutant, and runs multiple thermodynamic cycles to compute, respectively, the binding free energy difference between the reference ligand and each of the selected mutants, with respect to the receptor.

NOTE The short list of substitution groups is not a limitation, it is a practical warning. Any mutation involving more than only a very few atoms is subject to very large uncertainty in the calculated Delta G values and it is virtually impossible to achieve converged results in a feasible simulation time. Nonetheless, you can create arbitrary groups rather than selecting mutations from the Fragment Library. See "Creating a Custom Fragment Group" on page 79 for details.

4. Click Start. FEP simulation starts execution.

NOTE Desmond uses Bennett's acceptance ratio method for FEP calculations, which means that numerous independent simulations will be run for a single FEP calculation. Since the independent simulations can be run simultaneously, you should be careful as to how many simulations can be allowed to run on your host/queue at the same time. You can control this parameter in the Start dialog box by setting the maximum number of subjobs as shown in Figure 5.5. The value zero means no limit. In this example the master/multisim job will run on localhost (local workstation) whereas the subjobs (the individual Desmond FEP simulations) will run on a cluster via a submission queue called *swdev*, which provides a total of 112 CPU cores. Out of the 112 only 32 cores will be used simultaneously; 4 FEP simulations running at the same time, each utilizing 8 cores. Note that the total number of FEP simulations is 24 by default: 12 lambda windows for both mutating the ligand in the enzyme-ligand complex and mutating it in pure solvent, respectively, moreover each FEP simulation involves an independent relaxation protocol. FEP simulations are computationally very expensive.

Figure 5.5 Ligand Functional Group Mutation by FEP - Start dialog box



5. Alternatively, click Write. A total of four files are written:
   - **Two Maestro files: my-fep-job_lig.mae** and **my-fep-job_cmp.mae**. The first file contains the original ligand and one or multiple mutants. The second file contains the receptor and the ligand structures. Note that both files are regular Maestro files with no force field information included at this point. The only information about the FEP setup in these files is the definition of the attachment bond.
   - **Two MultiSim command script files: my-fep-job_solvent.msj** and **my-fep-job_complex.msj**. The first script defines a series of simulations involving the ligands in the solvent and the second script defines corresponding simulations involving the ligand-receptor complexes.

     Both **.msj** scripts can be run from the command line as described in "Running MultiSim jobs from the Command Line" on page 67.

NOTE Mutants do not need to be stoichiometric equivalents as in this example. Atoms that have no match in the mutant (or vice versa) will be annihilated into, or grown out of, dummy atoms during the course of the FEP simulation in accordance with the double topology technique.

NOTE For more realistic simulations, you will need to adjust the conformation of the mutant. See "Adjusting the Conformation of the Mutant" on page 85 for details.

NOTE This tutorial does not go into details about the configuration settings for FEP simulations, see the *Desmond User's Guide* and the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116 for a full account of this topic.

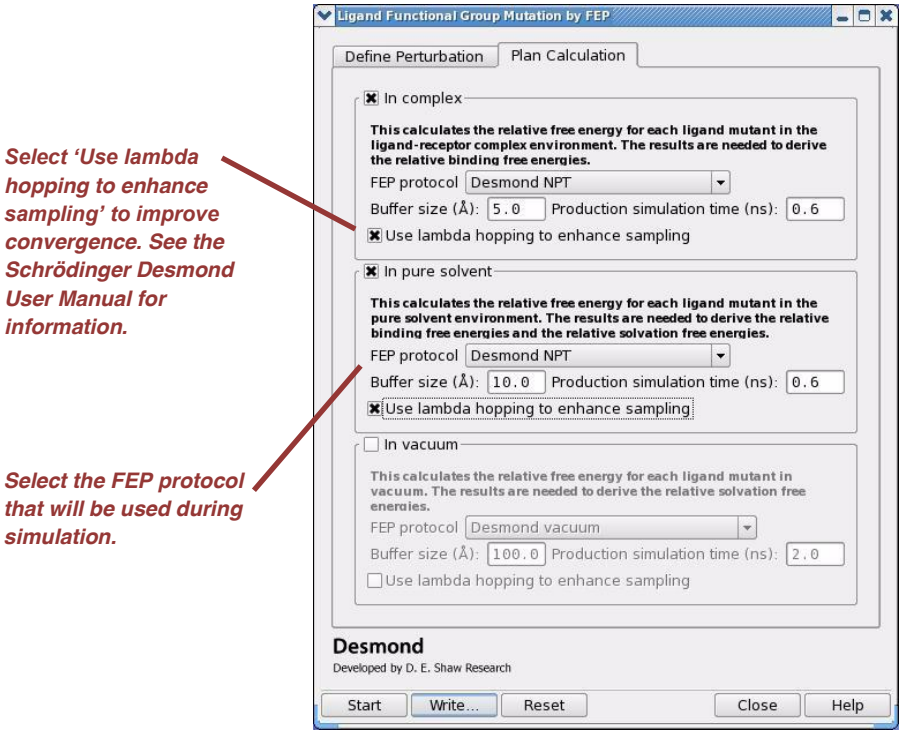
6. There are several ways to run the FEP simulation:

– Write your own Desmond configuration file and run the FEP simulation from the command line. See "Running FEP Simulations from the Command Line" on page 78 for instructions to run the FEP simulation from the command line. This is the most flexible, but least automated approach.

– Use Maestro to generate any non-default Desmond configuration file and run the FEP simulation from the command line. See "Using Maestro To Generate A Desmond FEP Configuration File" on page 76 for instructions on using Maestro to generate the configuration file. See "Running FEP Simulations from the Command Line" on page 78 for instructions to run the FEP simulation from the command line.

– Run the FEP simulation directly from the Ligand Functional Group Mutation by FEP panel by clicking Start. The workflow defined in the **.msj** files is automatically executed. This workflow has a short description shown under the Plan Calculation tab shown in Figure 5.6; essentially, system setup with System Builder is automatically performed, and all required Desmond simulations (NPT or NVT) are run with reasonable default settings, but user-defined workflows can also be imported. This option is the least flexible, but most convenient solution.

Figure 5.6 FEP workflow control



NOTE Lambda hopping is a form of replica exchange in which coordinate exchanges of systems simulated in adjacent lambda windows are attempted periodically. For mutations involving conformational flexibility, lambda hopping can improve convergence. For details see the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

NOTE All of these options use the Schrödinger OPLS-AA force field server. Of course, you can use *Viparr* to generate force field parameters for other force fields, however, *Viparr* has limited coverage of the chemical space of organic compounds, which means that for most ligand mutations OPLS-AA is currently the only viable force field.

7. Compute the binding free energy difference between two different ligand molecules. Regardless of which method was used in step 6, two output **.mae** files have been generated: ***jobname*_solvent-out.mae** and ***jobname*_complex-out.mae**. The difference in free energy between the ligand molecules in pure solvent (`dG_solvent`) and the difference when bound to the receptor (`dG_complex`) are recorded, respectively, in these **-out.mae** files.

   Import the **.mae** files into Maestro, open the Project Table, and locate the `dG` values at the far end of the table. The relative binding free energy of `ddG_binding` is equal to the difference between `dG_complex` and `dG_solvent`: `dG_complex - dG_solvent`.

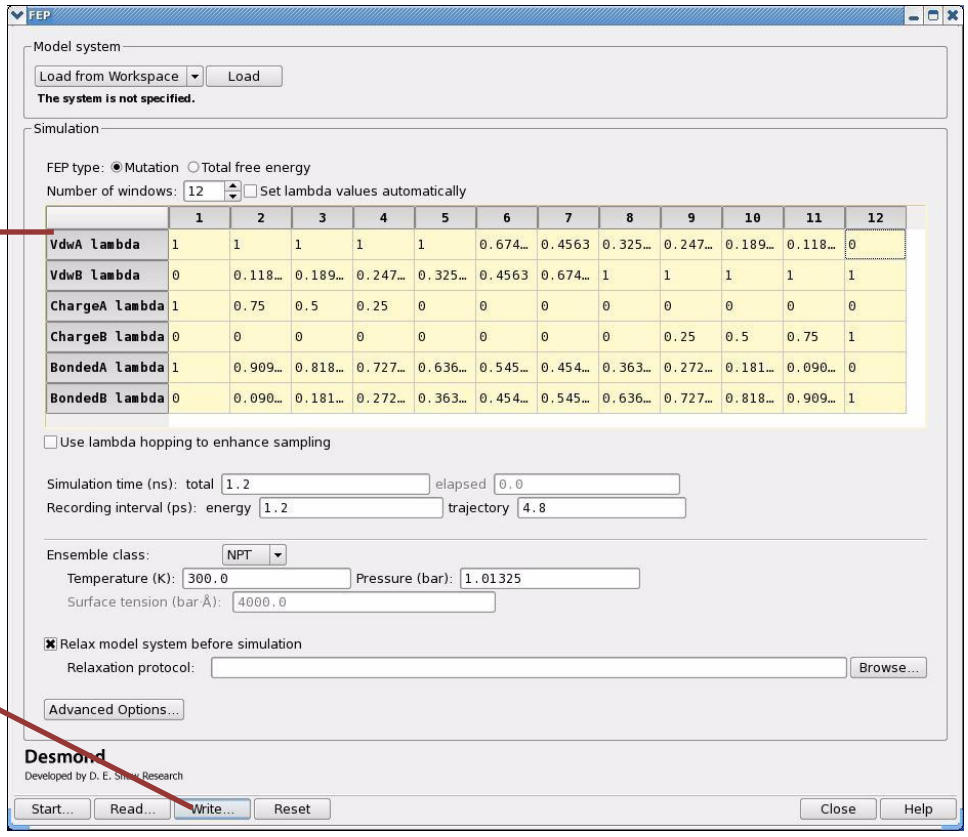
   If `ddG_binding` is negative, the mutation resulted in better binding; otherwise if `ddG_binding` is positive, the original ligand molecule had more favorable binding.

Using Maestro to Generate a Desmond FEP Configuration File

To create a Desmond conguration file for FEP:

1. Select **Applications > Desmond > FEP**. The FEP panel appears as shown in Figure 5.7.

Figure 5.7 Setting FEP parameters from the FEP panel



The FEP panel is similar to the Molecular Dynamics panel and has the same functionality, but typically it is only used to write out a custom Desmond FEP configuration file. If, however, you should load a structure into this panel, the structure must have already been prepared for FEP simulations.

The Simulation section of the panel allows you to set a number of FEP related parameters. For details see the *Desmond User's Guide* and the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

2. Select the λ windows that should be included in FEP simulation.

NOTE FEP simulations involve many separate calculations with different values of λ and it is quite possible that some of them will crash at the first try. When this happens, you should rerun the FEP simulation only for the failed λ windows. The GUI lets you select the failed λ windows for a re-run. However, we **strongly recommend** that you run all FEP jobs from their respective FEP panels (such as the Ligand Functional Group Mutation by FEP panel), or from the command line with input files generated by these windows. Doing so allows the FEP simulation to run via the MultiSim system, which has a built-in automatic recovery mechanism; with this recovery mechanism, you can restart a failed job from the MultiSim checkpoint file.

3. Write out the Desmond configuration file by clicking Write.

Running FEP Simulations from the Command Line

FEP simulations can be run from the command line using a **.msj** command script file in exactly the same way as described in "Running MultiSim jobs from the Command Line" on page 67:

```
$SCHRODINGER/utilities/multisim -JOBNAME <jobname> -maxjob <maxjob>
-cpu <cpu> -HOST localhost -SUBHOST <hostname> -i <jobname>.mae
-m <jobname>.msj -o <jobname>-out.mae
```

where:

- `<jobname>` is the name of the job.
- `<maxjob>` is the maximum number of subjobs that can be submitted to a host or a queue simultaneously. A value of zero means that there is no limit.
- `<cpu>` is the number of CPUs per subjob, which can also be specified as, e.g., "2 2 2" (note the quotes) indicating 8 CPUs with a spatial decomposition of 2x2x2.
- `-HOST localhost` means that the master job runs on your local workstation whereas
- `<hostname>` given with the `-SUBHOST` option is the name of the host or queue where the simulation jobs will be running.

NOTE **Prior to this Desmond release**, when MultiSim is used for FEP simulations, MultiSim requires special **.mae** files for input instead of .**cms** files. These **.mae** files are generated by the FEP panels (ligand mutation, protein residue mutation, ring atom mutation, and absolute/total free energy calculation) and include the dual topology structure of the mutations. The FEP MultiSim script will explicitly call the System Builder to prepare the solvated systems and generate the OPLS-AA force field parameters.

In the current release, one can load a full system in a .cms file (protein + ligand + membrane + solvent + ions) in the FEP panels, and perform an FEP calculation. This is quite useful when one already has a full system that was previously subjected to MD simulation and now wants to compute the free energy of binding, or similar free energy measure. Of course, if one starts out with only the solute (e.g. protein-ligand complex in vacuum), MultiSim will process this structure including the explicit call to the System Builder to prepare the **.cms** files automatically.

To apply non-default FEP λ parameters or to change the number of Lambda windows, add the '`-c config_file`' option to the command syntax where `config_file` is a Desmond FEP conguration file that was generated by the FEP panels as shown in Figure 5.7 on page 76.

Note that this conguration file will be used throughout the **.msj** workflow to make sure that all minimization and MD runs use the same λ settings for consistency. Also note that FEP parameters like any other Desmond configuration setting can always be defined directly in the MultiSim script and those values set in the **.msj** file take precedence. In particular, you can cut-and-paste the FEP block from the **.cfg** file defining the custom lambda schedule inside the `desmond{}` block below in the stock **.msj** file that is generated by the ligand/residue/ring-atom FEP panels.

```
task {
   task = "desmond:auto"
   set_family = {
      desmond = {
```

```
            checkpt.wall_interval= 7200.0
            checkpt.write_last_step = no
            fep = {
             lambda = {

                ...
              }}
        }
    }
}
```

For details see the *Desmond User's Guide* and the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

NOTE There is a Python script available, which can be used directly to compute the free energy difference associated with a FEP job (the script is run by default as part of an MultiSim workflow). The following script will process the "energy" file output of the different λ runs and compute the free energy:

```
$SCHRODINGER/run -FROM desmond bennett.py
```

Use the `-help` option to learn about the use of this script and consult the *Desmond User's Manual* listed in "Documentation Resources" on page 116 for more details.

Creating a Custom Fragment Group

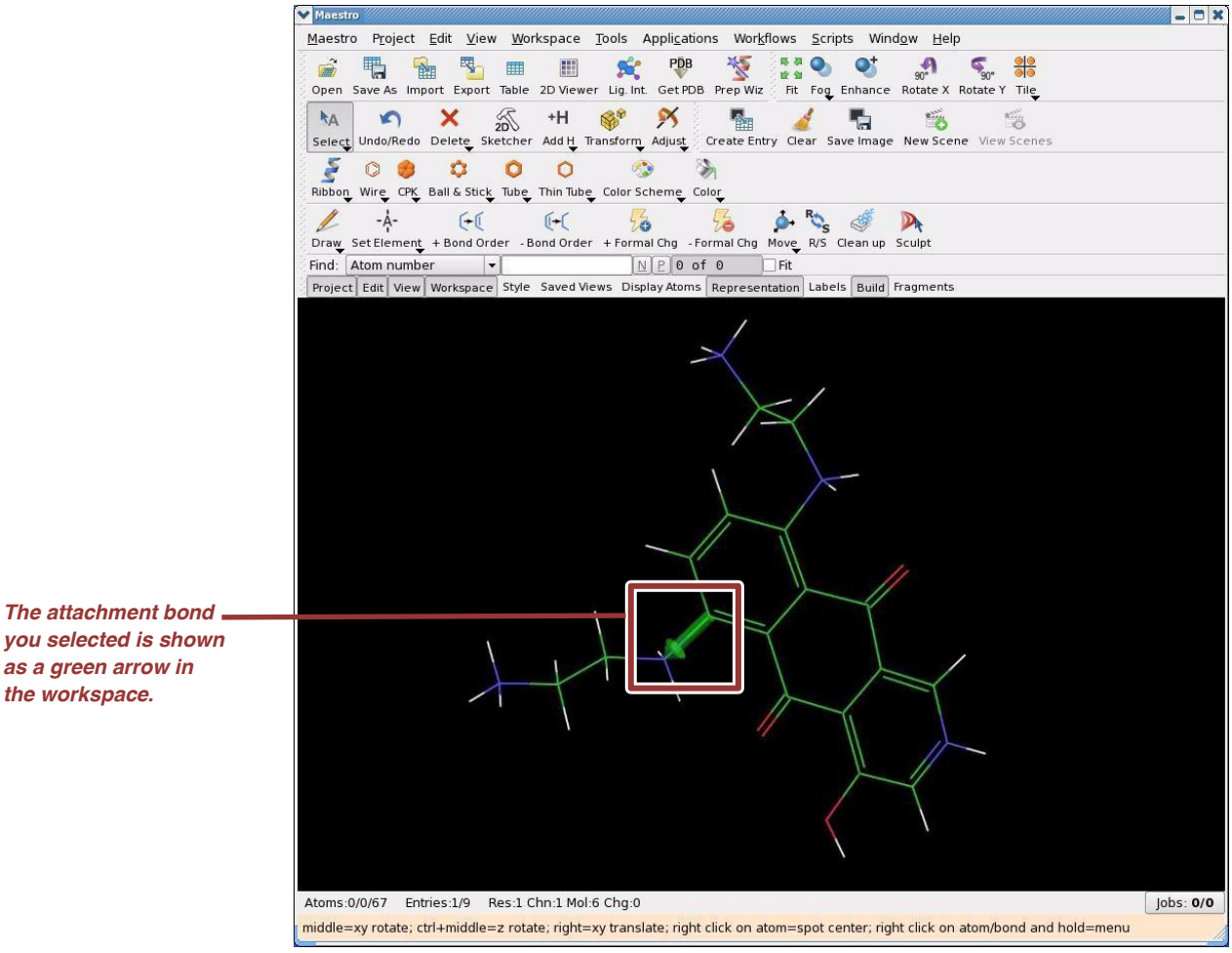
If the Fragment Library does not have the mutant structure you want to use, you can edit any pre-defined substitution group manually using the general Build/Edit tools in Maestro. The only caveat is that the customized substitution group will retain the name of the fragment that you modified. However, the FEP simulation will use the modified fragment.

Use caution with custom fragments. Any mutation that involves more than a few atoms is likely subject to very large uncertainties in the free energy differences computed between different lambda windows. Try decomposing a large mutation into several small mutations and run multiple MultiSim FEP simulations. It will take longer, but results will be more reliable.

For this exercise let's make a larger mutation in the ligand molecule and turn the whole $^{+}$ $–NH_2^+ — CH_2 — CH_2 — NH_3$ side chain into a `butyl` group. The current view of the workspace should be similar to that shown in Figure 5.4 on page 73.

1. Click Pick the attachment bond in the Ligand Functional Group Mutation by FEP panel (see Figure 5.3 on page 72) and click OK when asked to confirm that you are reselecting the bond. This time choose the attachment bond shown in Figure 5.8. For clarity, the view is changed to wire frame representation.

Figure 5.8 Picking the attachment bond for creating a butyl side chain



2. Select the methyl fragment on the Define Perturbation tab and select the View option as shown in Figure 5.9. The view in the workspace will change to that shown in Figure 5.10.

Figure 5.9 Selecting the methyl group as the base for the butyl substitution group

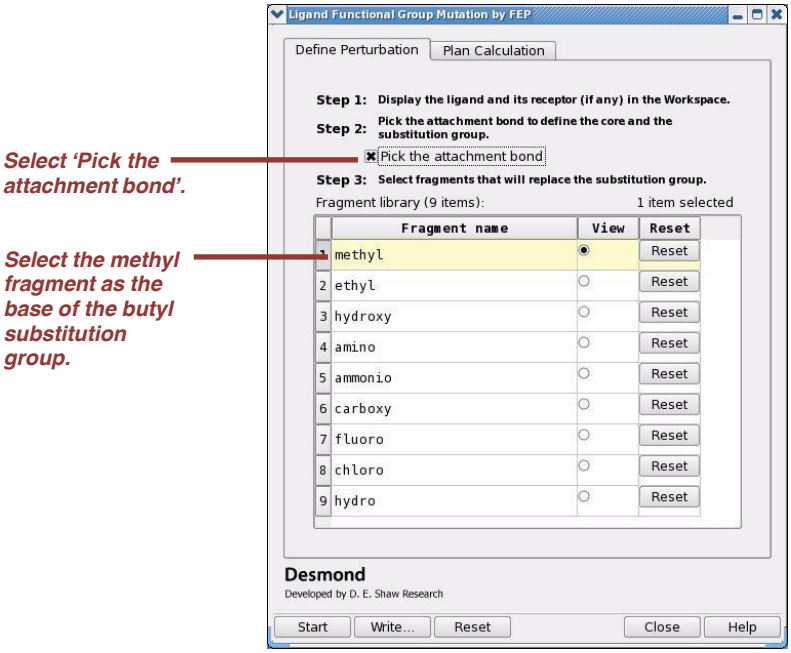
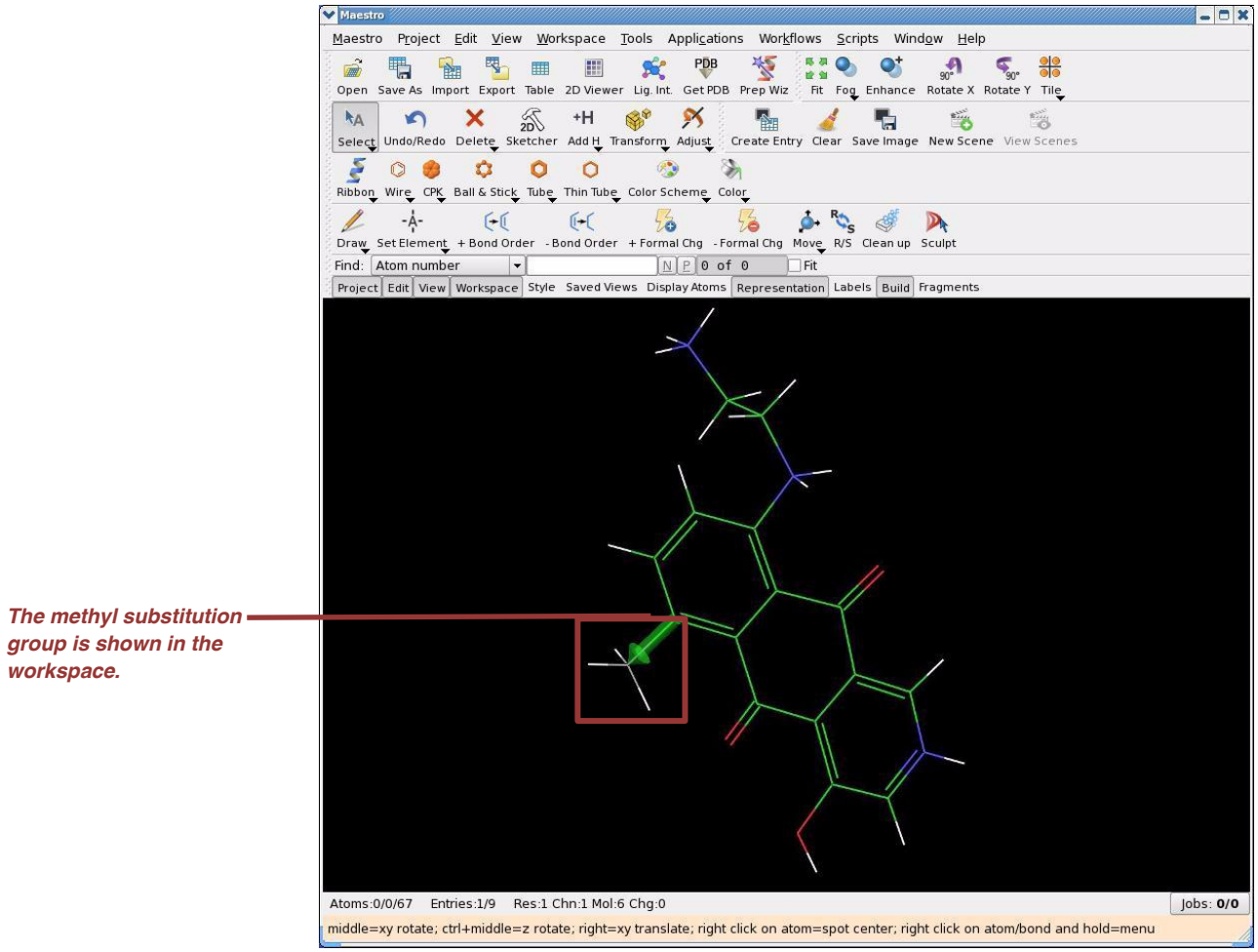


Figure 5.10 The intermediate methyl substitution group shown in the workspace



3. Open the fragment library by selecting **Edit > Build > Fragments**. The panel opens with the Fragments option menu set to Diverse Fragments as shown in Figure 5.11. Select Grow, select Pick and select Bonds. Then select the grow bond with the mouse as shown by the bright green arrow in Figure 5.12.

Figure 5.11 The Build panel

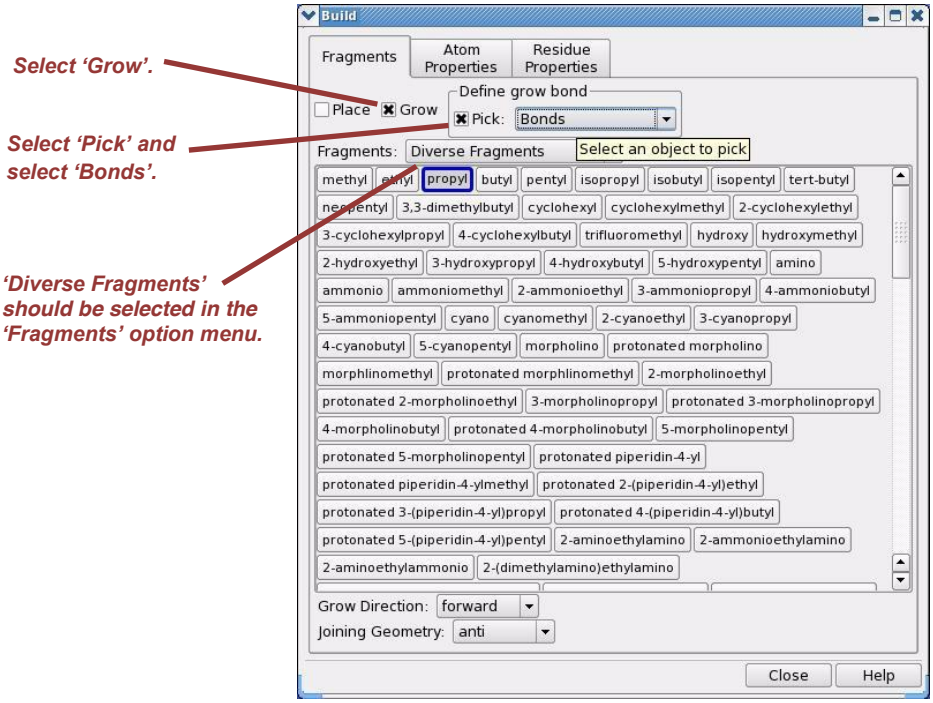
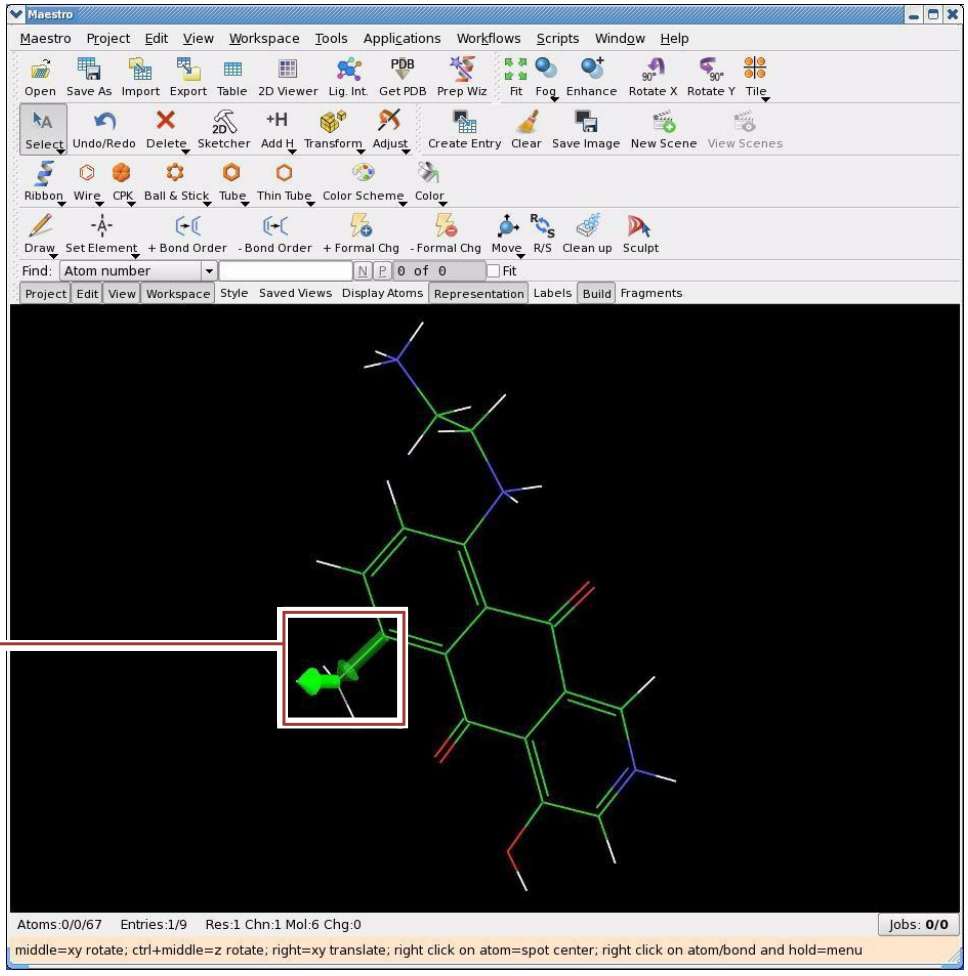


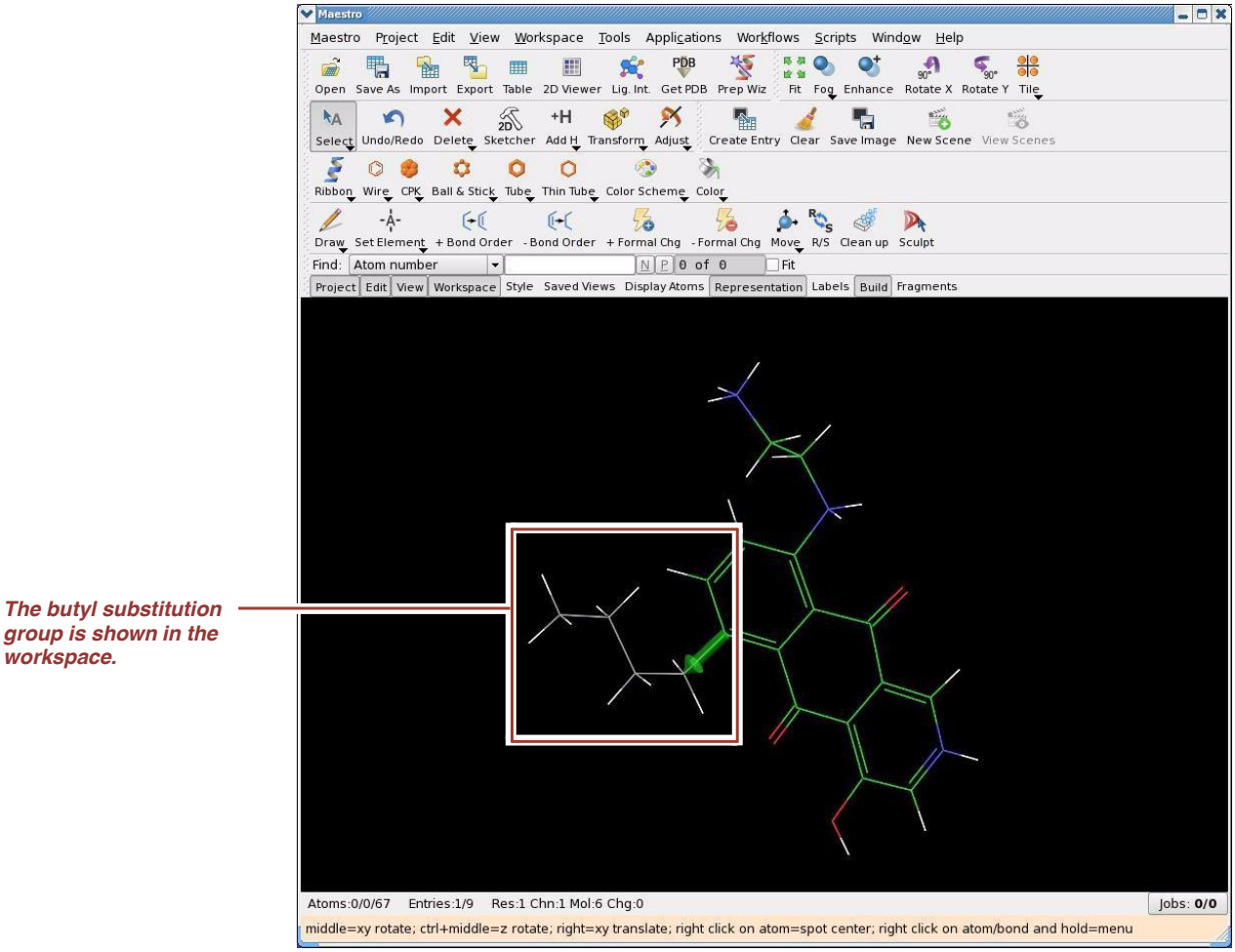
Figure 5.12 Selecting the grow bond



Use the cursor to select the grow bond in the workspace.

4. Select propyl from the fragment list in the Fragments tab of the Build panel as shown in Figure 5.11 on page 83. The resulting side chain is a `butyl` group as shown in Figure 5.13. Close the Build panel.

Figure 5.13 The butyl substitution group shown in the workspace

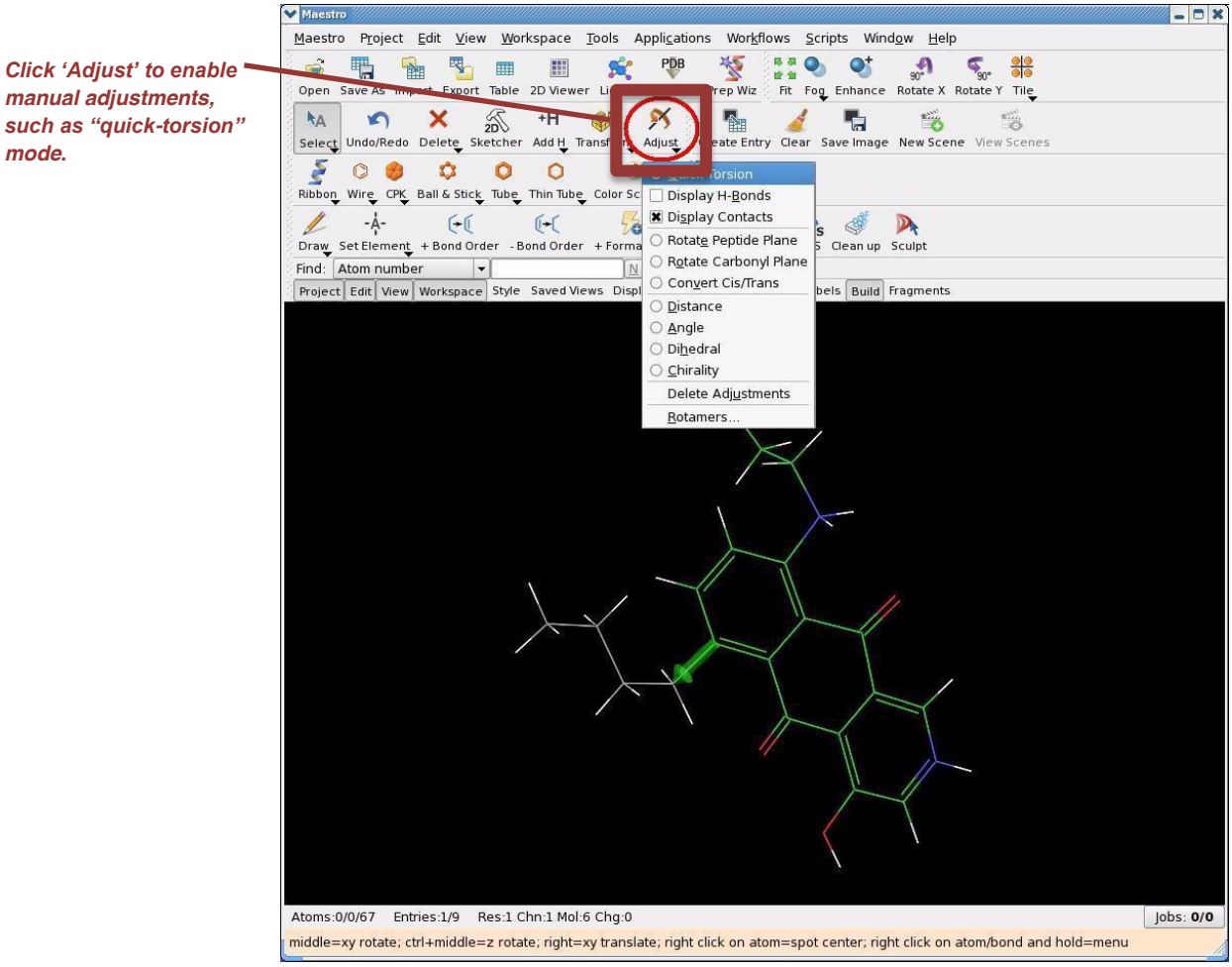


Adjusting the Conformation of the Mutant

The initial conformation of the mutant must be as close as possible to that of the original ligand for FEP simulations. Using Maestro, you can make manual adjustments to the conformation of small molecules.

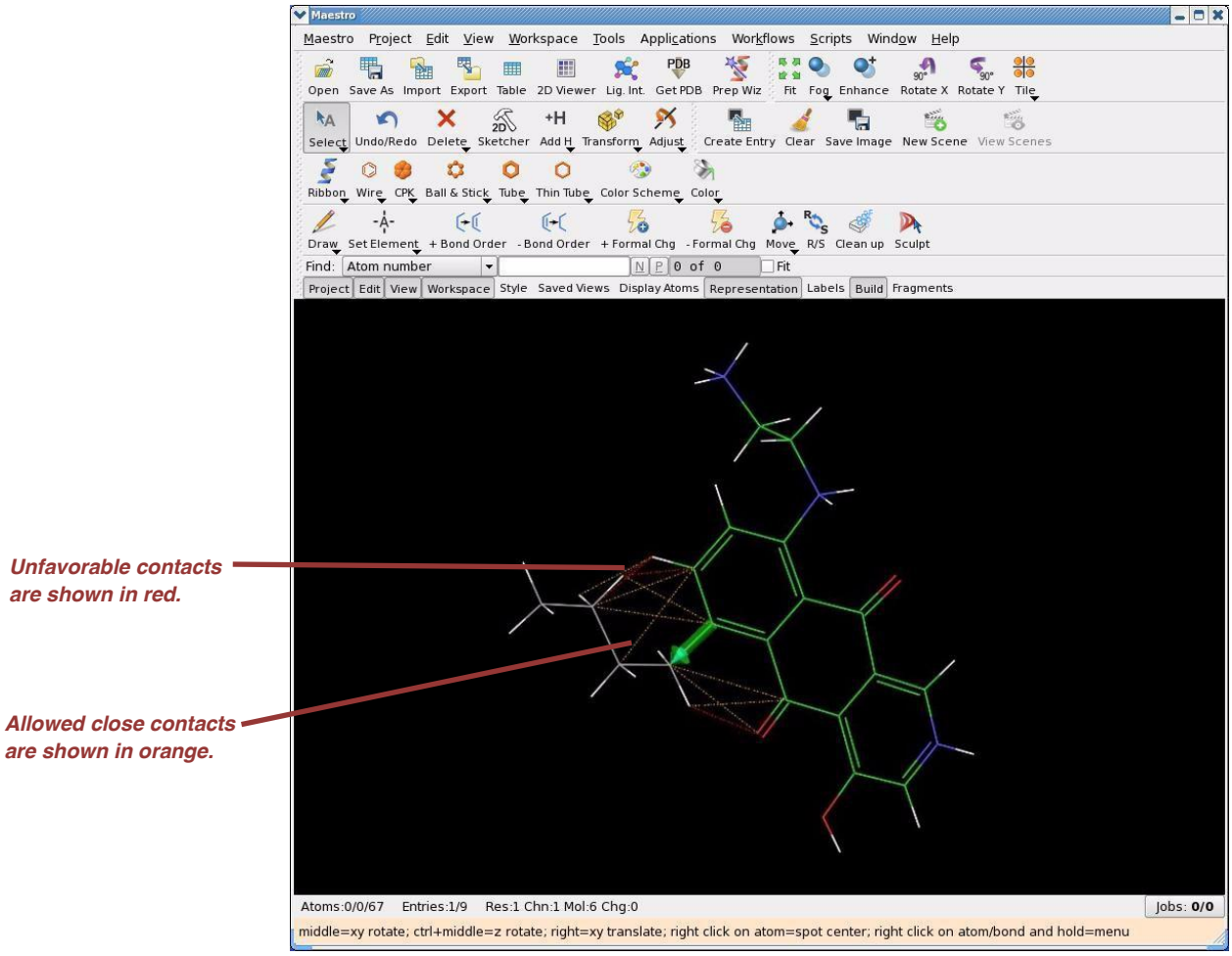
1. Click Maestro's Adjust tool as shown in Figure 5.14 to turn on the manual adjustment option. Toggle this tool to switch between manual adjustment mode and global translation/rotation mode.

Figure 5.14 Manual adjustment of the substitution group conformation



2. Click the attachment bond (shown in green) to display the non-bonded contacts between the `butyl` fragment and the ligand core. If you don't see any contacts shown in the workspace, select Display Contacts in the Adjust tool and also make sure that the Quick Torsion option is set. Click and hold the left mouse button to bring up the Adjust tool's option menu. As shown in Figure 5.15, there are numerous allowed close contacts (displayed in orange) and a few unfavorable contacts (displayed in red).

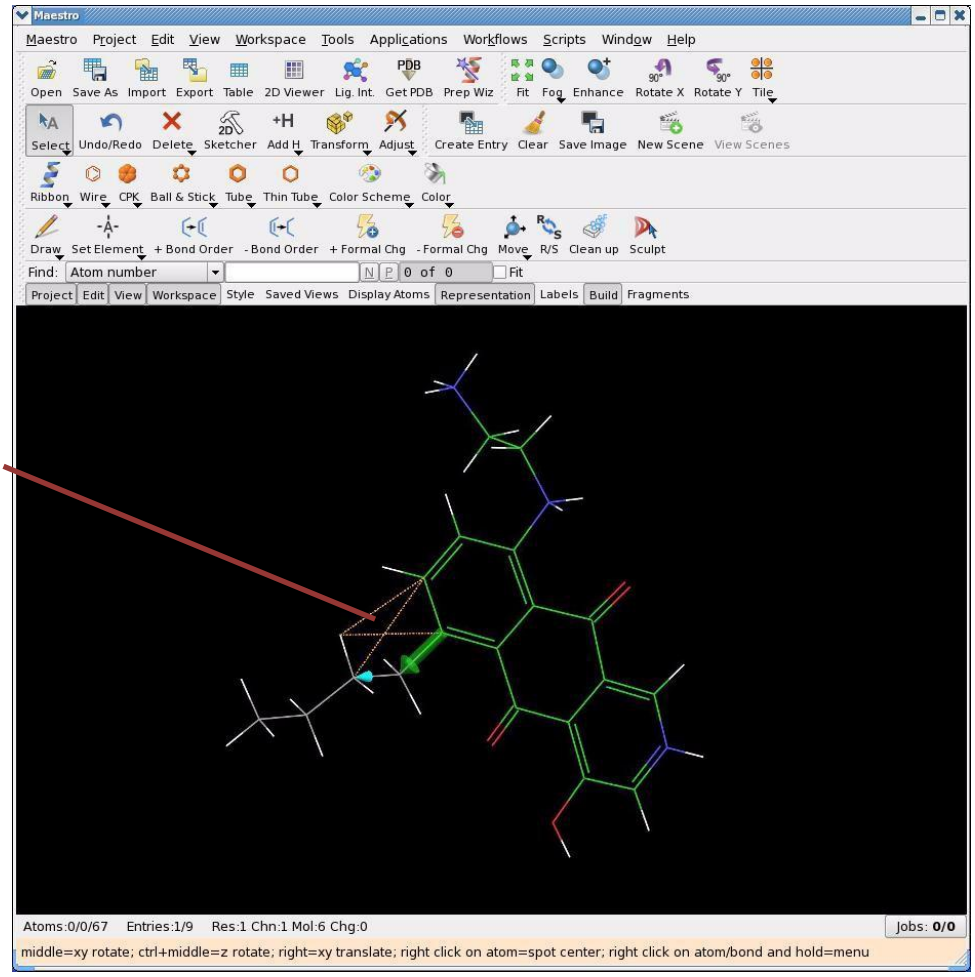
Figure 5.15 Displaying the non-bonded contacts



3. To arrive at a suitable conformation of the substitution group, you must adjust the torsion angle (and often, multiple angles). You can alter the torsion angle manually by holding down the left mouse button and moving the mouse horizontally, or by rotating the mouse wheel. Try relaxing the unfavorable contacts with rotations about different bonds in the `butyl` side chain. You should arrive to a conformation similar to that shown in Figure 5.16. As you can see in Figure 5.17 the altered `butyl` conformation is now fairly close to that of the original –NH2+ — CH2 — CH2 — NH3+ group.

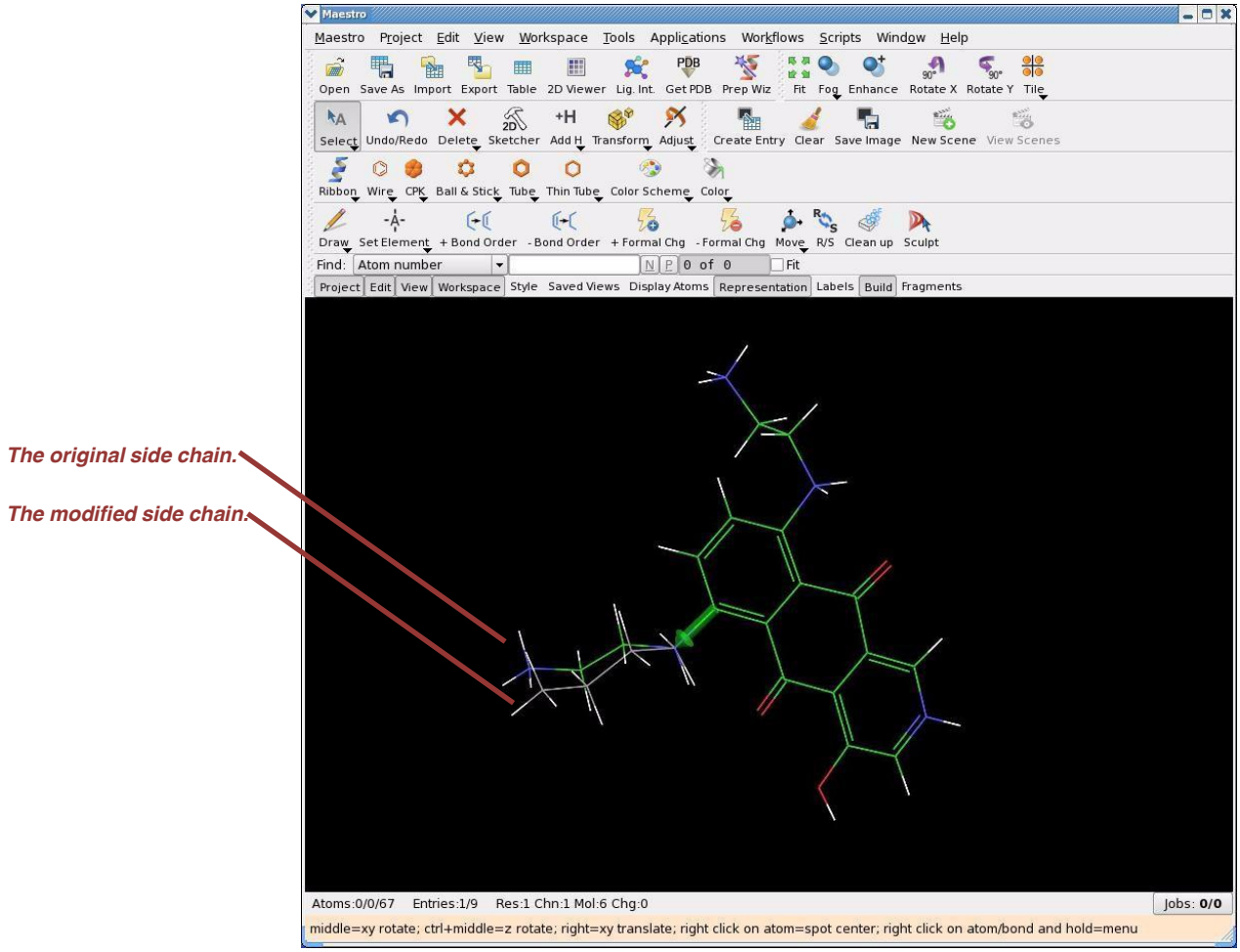
To restore the default methyl substitution group, you can click the 'Reset' column in the Ligand Functional Group Mutation by FEP panel.

Figure 5.16 Unfavorable contacts removed



Only allowed close contacts remain. The unfavorable contacts have been removed.

Figure 5.17 Butyl group superposed with original side chain



Other Types of Mutations

You can also perform these mutations from the **Applications > Desmond** menu:

- **Protein residue mutation** can be used for two different types of calculation. With one calculation, the difference in free energy is computed for a ligand bound to a protein versus the same ligand bound to a mutated form of the same receptor with amino acid residue mutations applied to the protein. With the other calculation, the difference in free energy is computed for a protein versus a mutated protein, without a ligand. Only single point mutations can be applied in a single FEP calculation. To consider multiple mutations, you must run multiple FEP calculations. However, you can always setup multiple single-point mutations in a single FEP run, for example, to compare the effect on binding free energy of different single-point mutations.
- **Ring atom mutation** can be used to replace one atom with another in a ring structure.

- **Absolute or total free energy calculation** can be used to compute absolute solvation free energies by annihilating a whole solute molecule.

Setup for these types of mutations is straightforward and very similar to ligand mutation setup. Please consult Chapter 4 and Appendix B of the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116 for details about FEP setup and the syntax for programming **.msj** scripts, respectively.

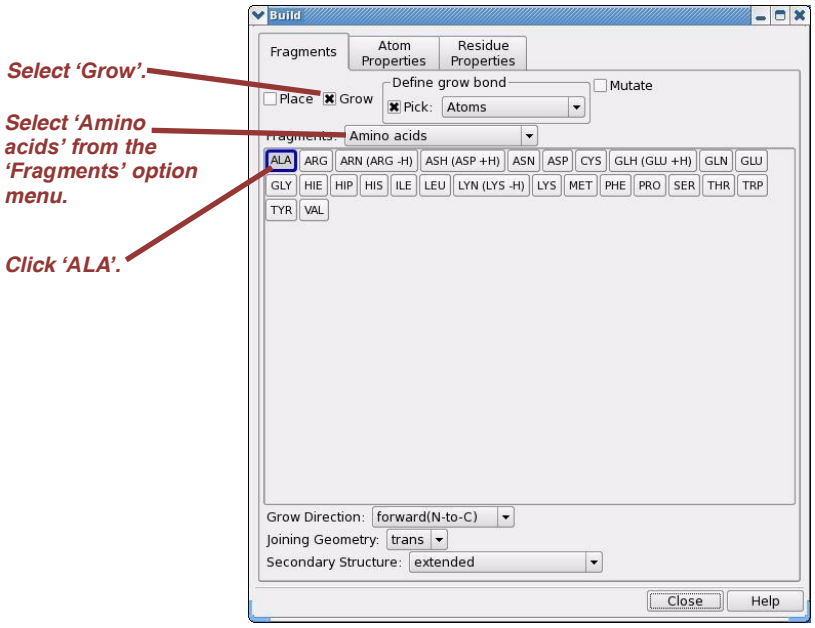
Aside: Metadynamics

Metadynamics is a different kind of free energy perturbation method, which enhances sampling of the underlying free energy space by biasing against previously visited values of user-specified collective variables. The biasing is achieved by periodically dropping repulsive kernels of Gaussian shape at the current location of the simulation in the phase space of the collective variables. This history-dependent potential encourages the system to explore new values of the collective variables, and the accumulation of potential allows the system to cross potential barriers much more quickly than would occur in standard dynamics. Metadynamics is fully documented in the *Desmond User's Guide* listed in "Documentation Resources" on page 116.

In this Tutorial we present a simple working example to get familiarized with metadynamics. The example involves generating the well-known 2-dimensional free energy surface of alanine dipeptide with respect to its phi and psi dihedral angles. Follow these steps to generate and plot the free energy surface.

1. Build alanine dipeptide in Maestro.
    a. Open the fragment library panel by selecting **Edit > Build > Fragments**.
    b. On the Fragments tab of the Build panel, select Grow, select Amino acids from the Fragments option menu, and click ALA in the table as shown in Figure 5.18.
    c. Close the Build panel. The alanine dipeptide molecule appears in the Maestro Workspace. (Alanine dipeptide is in fact a single alanine molecule with capping groups ACE on the N-terminus and NMA on the C-terminus.)

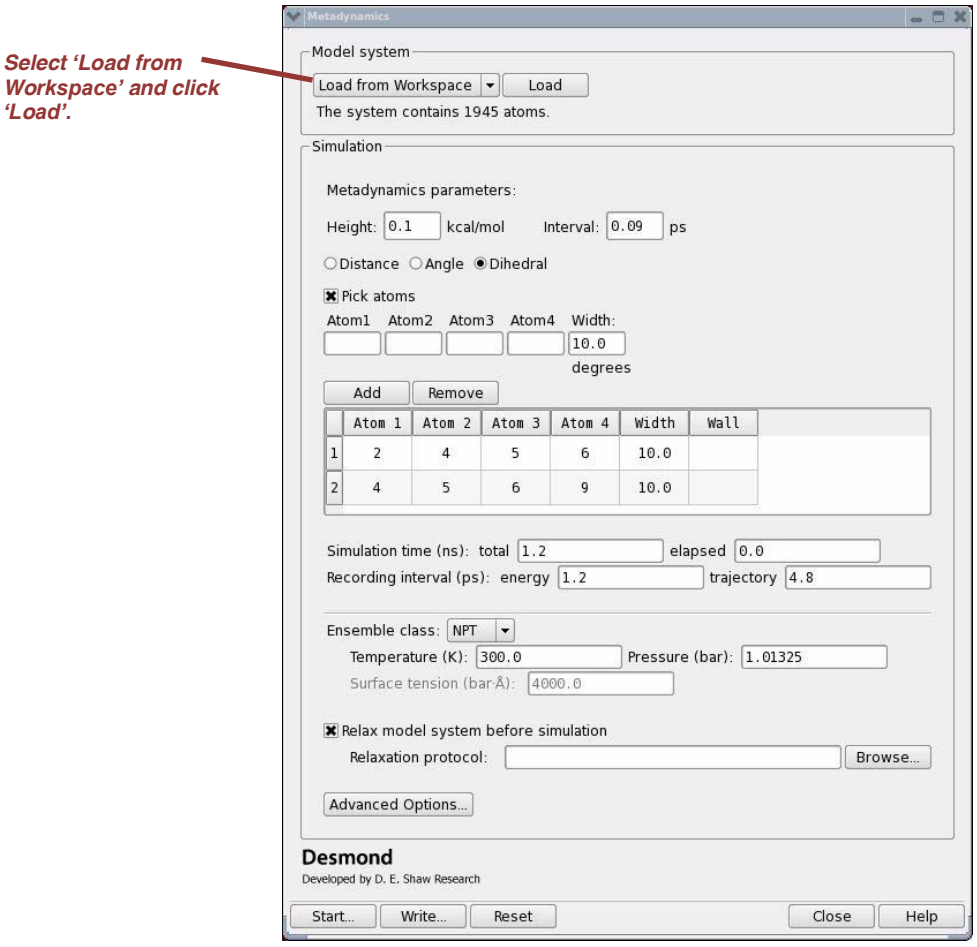
Figure 5.18 Fragment library Build panel



2. Clean up the geometry by selecting **Edit > Build > Clean Up Geometry**.
3. Build a solvated Desmond simulation box with the System Builder as described in step 16 on page 21.
4. Open the metadynamics panel by selecting **Applications > Desmond > Metadynamics**. The Metadynamics panel appears as shown in Figure 5.19.

   Metadynamics setup is virtually identical to molecular dynamics setup except for defining the collective variables and the height and shape of the Gaussian kernel potential.

Figure 5.19 Metadynamics panel



5. In the Metadynamics panel, select Load from Workspace and click Load to import the solvated system from the Workspace.
6. Hide the water molecules by selecting **Workspace > Display/UnDisplay Atoms > Undisplay: Waters**.
7. Click Dihedral and Pick atoms and select the phi and psi angles in the Workspace by clicking the four consecutive backbone atoms, respectively. Click Add and the selected dihedrals appear in the table.

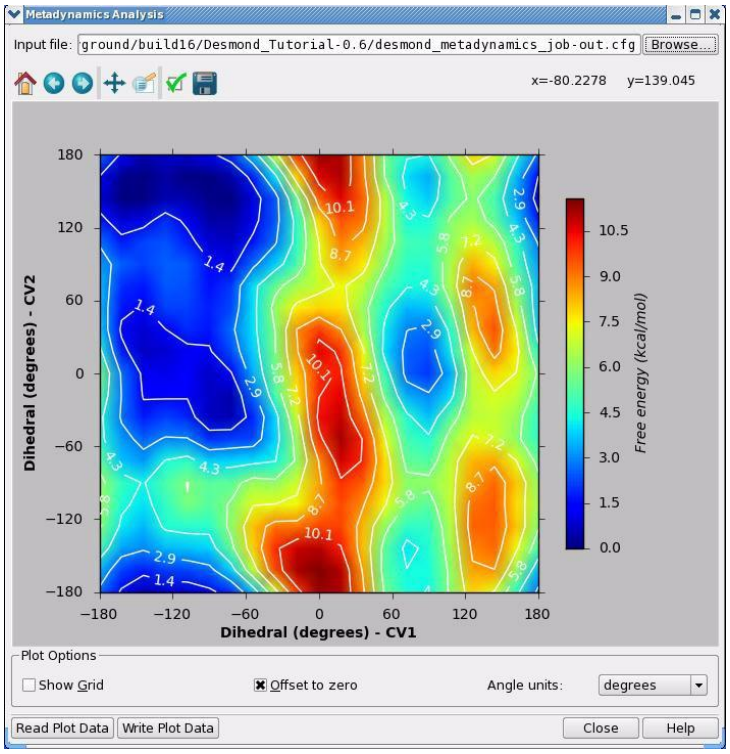
NOTE You can also change the height and width of the Gaussian kernel and the frequency of dropping the kernel in phase space; however, the default values should suffice.

NOTE The rest of setup is identical to running a molecular dynamics job. Note, however that this metadynamics simulation should run for 1.2 ns to achieve convergence.

NOTE Metadynamics is also supported via MultiSim. More complicated collective variables (via generic ASL expressions) can be set using MultiSim and command line execution.

8. When the job finishes, select **Applications > Desmond > Metadynamics Analysis**. The Metadynamics Analysis panel opens. Click Browse and open the **desmond_metadynamics_job-out.cfg** file (or the corresponding **-out.cfg** file if you provided a custom name for the job). Reading the data will take a few minutes. Then the free energy contour plot appears as shown in Figure 5.20.

Figure 5.20 Metadynamics Analysis panel



Consult the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116 for details on manipulating the plot and exporting the free energy data.

6 Visualization and Analysis Using Maestro

Overview

Maestro provides three basic visualization and analysis tools. Maestro's Trajectory Player is similar to the ePlayer found in the Project Table, but it is specifically designed to nicely animate Desmond trajectories much faster than the ePlayer could, and it has a number of specific visualization options.

Maestro can also perform basic quality analysis using the Simulation Quality Analysis panel and detailed geometric and energetic analysis of the trajectory can be carried out using the Simulation Event Analysis tool.

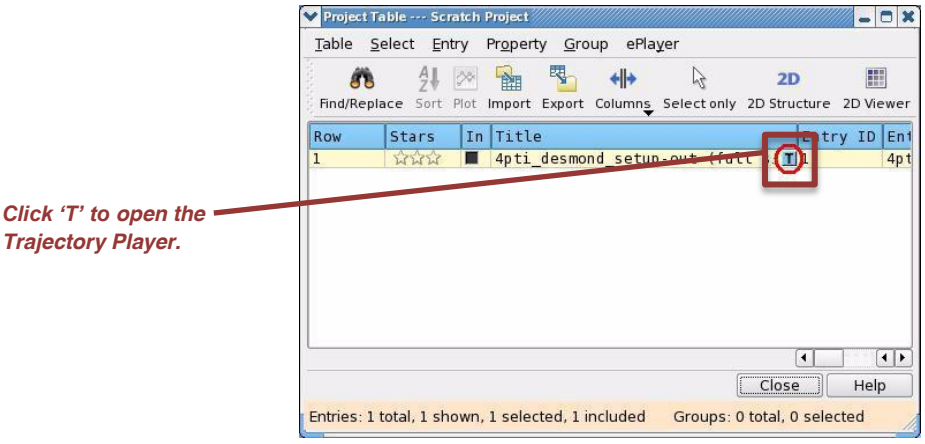
The following sections cover the basics of these tools.

Animating Desmond Trajectories with the Trajectory Player

Assuming that you have completed the first exercise in this tutorial in "Tutorial Steps" on page 12 you should have a completed Desmond simulation of the 4pti structure in your working directory with the base name of **desmond_job** by default (unless you gave it a different name).

1. Select **Project > Import Structures**: Read in the **desmond_job-out.cms** file. You will have to set the file format selector in the Import panel to Desmond to display Desmond (**.cms**) files in the file browser window.
2. Select **Project > Show Table**: Open the Project Table. The Trajectory Player can be launched from the Project Table. Note the blue **T** in the Title column in the Project Table, as shown in Figure 6.1.

Figure 6.1 Launching the Trajectory Player



3. Click **T** to open the Trajectory Player.

   The Trajectory Player is shown in Figure 6.2 and the associated Maestro workspace view can be seen in Figure 6.3.

   The Trajectory Player has two sets of options for frame control and display properties; the best way to learn about these options is to experiment with them. The Trajectory Player is fully documented in the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

NOTE In most cases you do not want the entire system to be displayed when, for example, visually inspecting the conformational changes of a protein molecule along the trajectory. The Trajectory Player recognizes Maestro's current display settings, which means that you can select/deselect any set of atoms for display using the Workspace > Display menu options and you can also render the atoms in any way you want. The Trajectory Player will generate a smooth animation with your current display settings and you can even save it in an MPEG movie file for incorporation in any presentation material. You can also save selected snapshots of the trajectory in Maestro format.

NOTE Options to center the trajectory view in the Trajectory Player include:

- Center molecules in the Display section of the Trajectory panel (under Positioning) allows you to post-process a trajectory such that the part of the system defined in the ASL text box will be centered in the simulation box. This option is very useful when the solute appears to jump in and out of the primary simulation box, which can be a visual artifact caused by the application of periodic boundary conditions. The Center molecules option is a post-processing option that only affects visualization and leaves the actual trajectory intact. Note that the Center molecules option solves the same visual artifact issues during simulation that the Glue close solute molecules together option solves; the Glue close solute molecules together option can be found in the Output tab of the Advanced Options dialog box for the Molecular Dynamics panel. See Figure 3.2 on page 61.
- `mdsim.trajectory.center` is an option that can also be used to center the trajectory in the simulation box; this option can be set in the Desmond **.cfg** configuration file. The `mdsim.trajectory.center` option is performed at run-time, resulting in a centered trajectory file. Refer to the *Desmond User's Guide* for information.

- Additional trajectory manipulation options are available from the command line, as shown next. The first utility can be used to concatenate multiple trajectories and the second utility to extract selected frames of trajectory into a series of output structure files. Use the `-help` option for more details.

```
SCHRODINGER/run -FROM desmond manipulate_trj.py
$SCHRODINGER/run -FROM desmond trajectory_extract_frame.py
```

Figure 6.2 The Trajectory Player

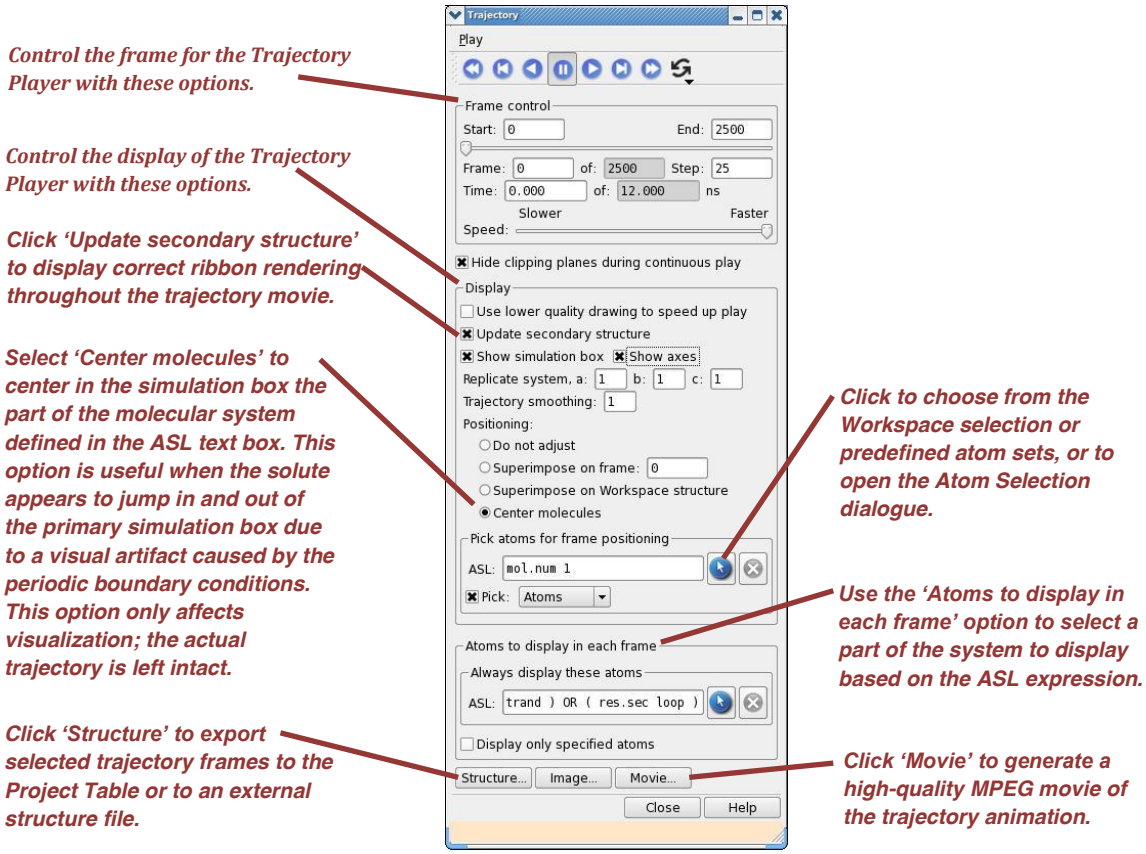
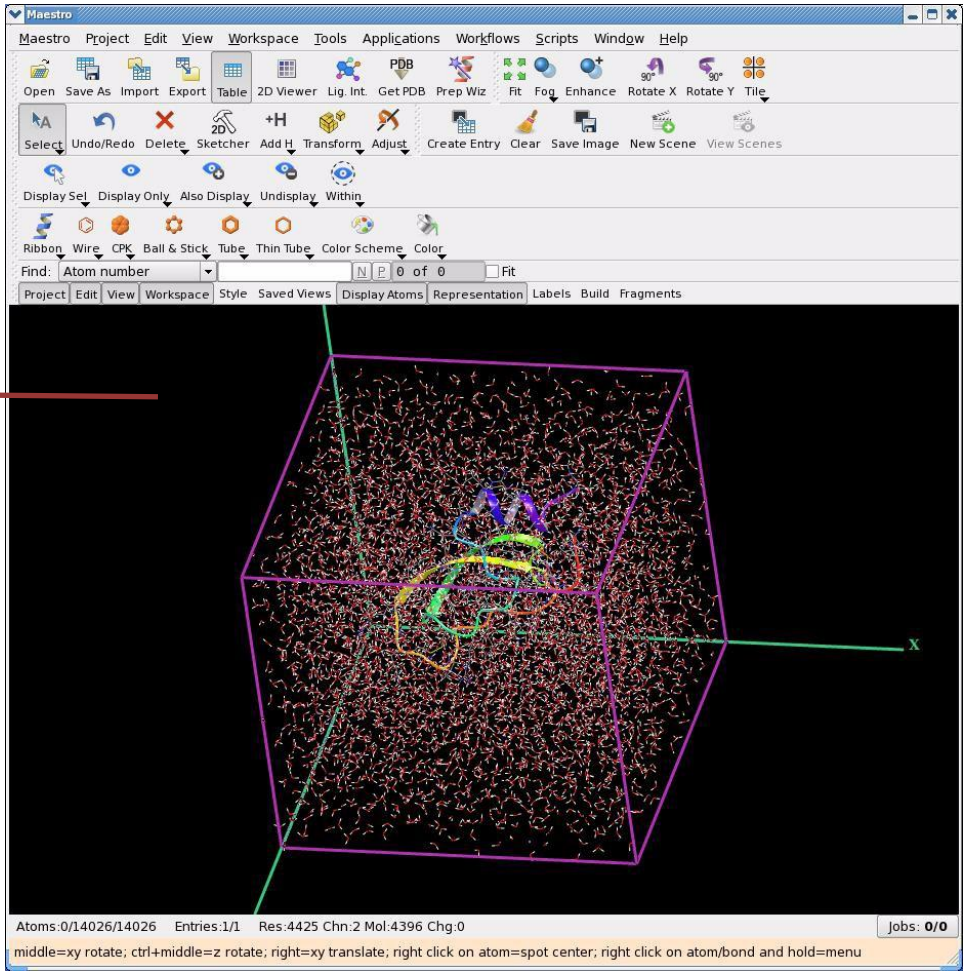


Figure 6.3 Workspace view for trajectory visualization

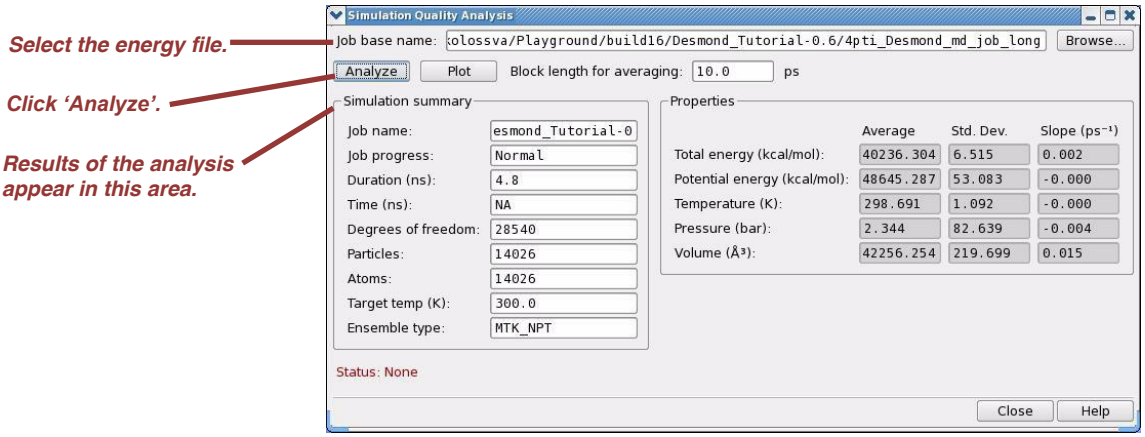


Workspace view of the Trajectory Player.

Performing Simulation Quality Analysis

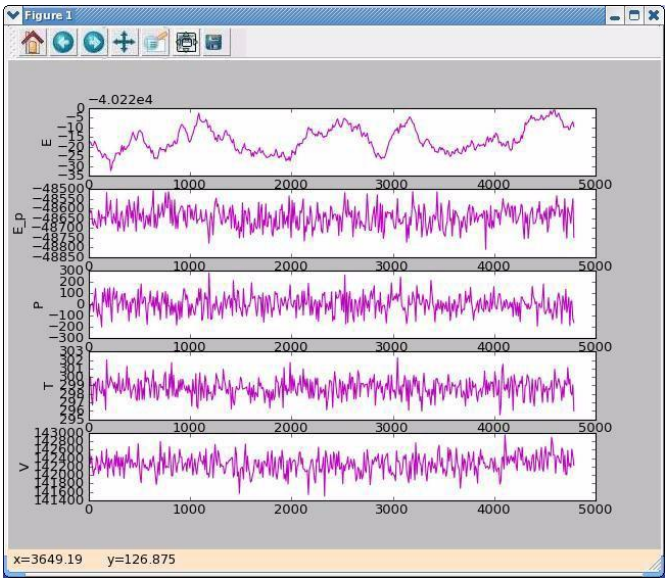
Maestro can also perform basic quality analysis. Launch this tool from the **Applications > Desmond > Simulation Quality Analysis**. The quality analysis panel appears as shown in Figure 6.4.

Figure 6.4 Simulation Quality Analysis panel



Click Browse and select the "energy" file **desmond_job.ene**, then click Analyze. You should see similar output to that shown in Figure 6.4. The table displays useful information about simulation parameters and show the statistical properties of basic thermodynamic quantities based on block averaging. Click Plot to display an interactive graphical representation of the data table as shown in Figure 6.5.

Figure 6.5 Interactive Simulation Quality Analysis Plot



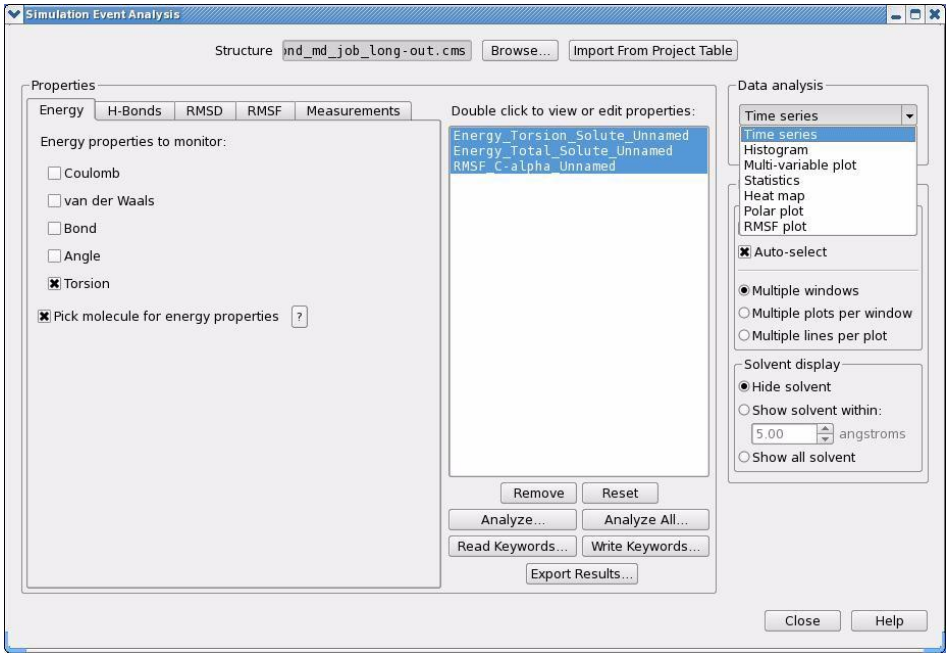
You can find more details about simulation quality analysis including plotting options in the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116.

Performing Simulation Event Analysis

The *Simulation Event Analysis* tool (SEA) can be accessed from the Applications > Desmond menu. SEA is a sophisticated analysis tool described fully in *Chapter 5.3* of the *Schrödinger Desmond User Manual* listed in "Documentation Resources" on page 116. Here we only intend to give a flavor of using SEA.

The Simulation Event Analysis panel is shown in Figure 6.6. When the SEA panel opens, the Trajectory Player panel opens automatically and the Workspace view changes to only show the solute in wireframe representation. Keep the Trajectory Player panel open for the full duration of the analysis.

Figure 6.6 Simulation Event Analysis panel



SEA can perform two basic types of analysis based on the Desmond trajectory: energetic analysis and geometric analysis.

- Energetic analysis is carried out using a Desmond application called *vrun*. vrun applies single point Desmond calculation on each trajectory frame separately. In the context of SEA, vrun can be used to monitor a wide range of inter-group and intra-group energetic terms between pre-defined atom groups. A typical example is the interaction energy between a ligand and the active site residues of an enzyme. For details about vrun consult the *Desmond User's Guide* listed in "Documentation Resources" on page 116.
- Geometric analysis is used to monitor torsion angles, distances, and so forth, throughout the simulation.

To begin analysis, click Browse next to Structure to select the **desmond_job-out.cms** file. Then, determine the analysis to perform, and the atoms to which to apply the analysis, from the Properties area, which has multiple tabs.

The tasks you can perform appear in the middle of the Simulation Event Analysis panel. Click Analyze to run the analysis.

NOTE When performing energetic analysis, vrun is executed and job progress is displayed in the Job Monitor panel.

After analysis tasks have been completed, there are a number of post-processing options listed under Data Analysis that can be performed. For example, click Export Results to export results for further analysis in external spreadsheet programs.

In this example, a number of quantities will be monitored along the trajectory including the total energy and torsional energy of the 4pti protein molecule, and the RMS fluctuation of the C-alpha atoms.

Trajectory analysis and sophisticated visualization and analysis tools can be applied to Desmond trajectories utilizing the VMD visualization package, which is covered in the next section.

7 System Setup and Trajectory Analysis Using VMD

Overview

VMD is primarily a molecular visualization program, but it is a natural environment for analysis with its strong support for atom selections, the ability to efficiently animate trajectories that contain thousands of snapshots, and support for Tcl and Python scripting languages. The current release version of VMD 1.9.1 available at the VMD website (**http://www.ks.uiuc.edu/Research/vmd/**) has the built-in capability of writing Maestro files and reading Desmond trajectory files. The former is useful for preparing your simulation system in VMD for running Desmond simulations and the latter allows for using VMD's sophisticated tools to analyze Desmond trajectories.

This section assumes a Unix/Linux environment and basic familiarity with VMD. If you're just getting started with VMD, a number of excellent tutorials already exist and can be found on the VMD home page:

http://www.ks.uiuc.edu/Research/vmd/current/docs.html#tutorials

The VMD Python Interface

The Python interface can be accessed from either the console (the terminal from which you launched VMD), or from an Idle window that runs inside of VMD (preferred). To initiate the Python interface in the console, simply type `gopython` at the `vmd>` prompt. To launch the built-in Idle window, you'll need to set your `PYTHONPATH` properly as described in the Desmond installation instructions.

The console command to launch the Idle window is

```
gopython -command "import vmdidle; vmdidle.start()"
```

You may wish to copy this command into your `.vmdrc` so that you open this window every time you launch VMD. Once you have a Python prompt at either the console or the Idle window, you're ready to start using the VMD Python interface.

In Python, classes and functions are organized into modules. Some modules are provided by the Python interpreter when you launch Python, while others are discovered at runtime and loaded from separate files. When you access Python from within VMD, the VMD executable provides several built-in modules for loading data into VMD, as well as querying and modifying VMD state. The most important of these are the `molecule` module, the `atomsel` module, and the `vmdnumpy` module. Use the built in `help()` command to get the most up-to-date documentation for these modules.

Loading and Viewing Trajectories

Thanks to its file plugin architecture, VMD can load many different kinds of files. To simplify the discussion, we're going to assume that you're working with only two kinds of files:

- **Structure files: .mae** or **.cms** files created by Maestro or Desmond. These contain topology, mass, charge and possibly force field information about a single molecular system, including any solvent or ions that might be present. They also contain a single snapshot of the positions of the atoms and of any virtual sites in that system, and optional velocities.

  You can read either of these files with VMD selecting the Maestro file type, **mae**.
- **Trajectory files: .dtr** files created by Desmond. Although we will always refer to a trajectory as a file, the on-disk representation of a dtr is in fact a directory containing metadata files as well as frame files holding the actual snapshots. Resist the temptation to think of the frame files as being somehow independent of the rest of tragic- tory; they are not, and renaming or moving them around will cause issues.

  There are two file types in VMD associated with Desmond trajectories. The default, **`dtr`**, loads just the positions. A second type, **`dtrv`**, loads positions as well as velocities, if they are present in the frames.

NOTE When referring to a Desmond trajectory file, there is a special file called **clickme.dtr** that should be used. **clickme.dtr** resides in the same subdirectory where all the other trajectory file pieces are written.

Knowing the file type will be necessary for loading structure and trajectory files from either the command line or the scripting interface. Both structure files and trajectory files can be loaded in three different ways, with varying degrees of flexibility and ease of use.

Loading Files from the Command Line

If you are comfortable using the shell and the command line, loading files into VMD using command line arguments is the easiest way to go. The syntax is:

```
vmd -mae structure.cms -dtr trajectory_subdir/clickme.dtr
```

Launching VMD in this fashion will create a new molecule, initialize its structure from the `cms` file, create an initial set of coordinates from that same file, and finally append all the snapshots from the `dtr` file to that molecule.

Multiple structure and trajectory files can be loaded into separate molecules using combinations of the `-m` and `-f` options. The effect of `-m` is to cause a new molecule to be created for each subsequent file. The `-f` option causes subsequent files to be loaded into the same molecule. For example:

```
vmd -m -mae mol1.mae mol2.mae -f mol3.mae -dtr trajectory_subdir/
clickme.dtr
```

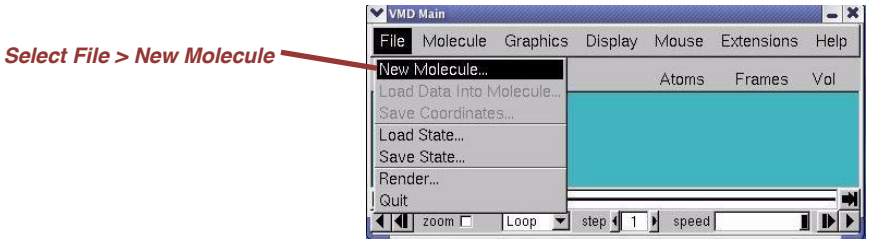
In this example, three molecules will be created. The first molecule will take data from **mol1.mae**, the second will take data from **mol2.mae**, and the third will take data from both **mol3.mae** and the trajectory file referred to by `trajectory_subdir/clickme.dtr`. Think of `-m` for loading multiple molecules, while `-f` is for loading multiple files.

Note that the command line loads all the snapshots from all the specified files before giving control back to the user. If your trajectories are so large that not all snapshots will fit into memory, you'll need to use either the graphical user interface or the scripting interface to load a subset of snapshots into memory instead.

Loading Files from the GUI

VMD's File menu lets you load **.cms** and **.dtr** files using a graphical user interface (GUI). Select **File > New Molecule** in the Main menu (Figure 7.1). Click Browse, navigate to your structure file, and click OK or press **Enter** to select the file. You should now see your file in the Filename field, and underneath the filename the file type chooser should show Maestro File as its selected item (Figure 7.2). When the file and its type are to your satisfaction, click Load to proceed.

Figure 7.1 Loading files from the GUI



Once you've loaded a molecule, you'll notice that the molecule chooser at the top of the Molecule File Browser now points to your newly created molecule. If you create more new molecules using different **.cms** files, select New Molecule from the molecule chooser and repeat the steps above. Otherwise, to append snapshots to this molecule from a trajectory, click Browse again and navigate to the location of the **.dtr** file you wish to load. Since **.dtr** trajectories are stored as directories rather than ordinary files, clicking on a DTR trajectory will take you inside of a directory (Figure 7.3). You should see a file called **clickme.dtr**. Click on it, and VMD will figure out that you wanted to load the frames within the directory containing the **clickme.dtr** file.

Figure 7.2 Loading the Maestro file

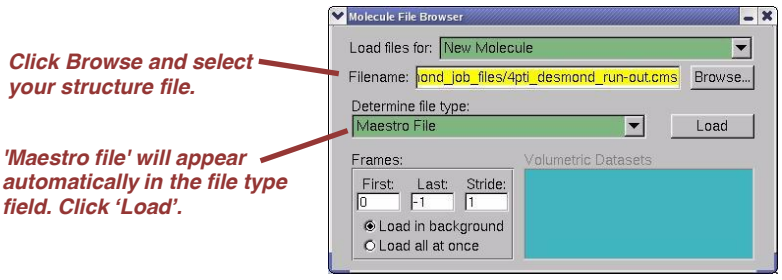
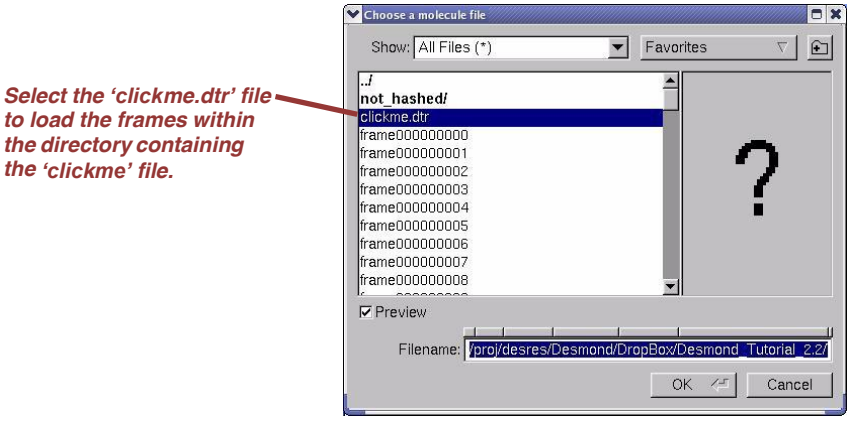


Figure 7.3 Loading trajectory data

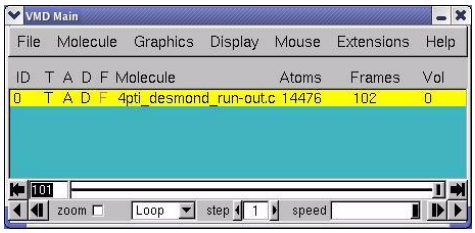


At this point, you may wish to limit the number of snapshots that are loaded from the trajectory. In the Molecule File Browser (Figure 7.2) the file type should automatically be reset to Desmond Trajectory and the Frames box underneath should now be active. Type in values for the snapshots you want to load. The default First value of 0 corresponds to the first snapshot in the trajectory; the default Last value of −1 corresponds to the last snapshot; and a Stride of 1 means load every snapshot in the selected range. Choosing a Stride of 10 would load every 10th snapshot from the trajectory.

You also have the choice of Load in background, which is slower but keeps VMD responsive during the load and gives you the option of canceling the load before it completes, and Load all at once, which is quite a bit faster but leaves VMD in an unresponsive state until the load has completed. Use Load all at once whenever you're reasonably sure that all the frames you're about to load will fit in memory.

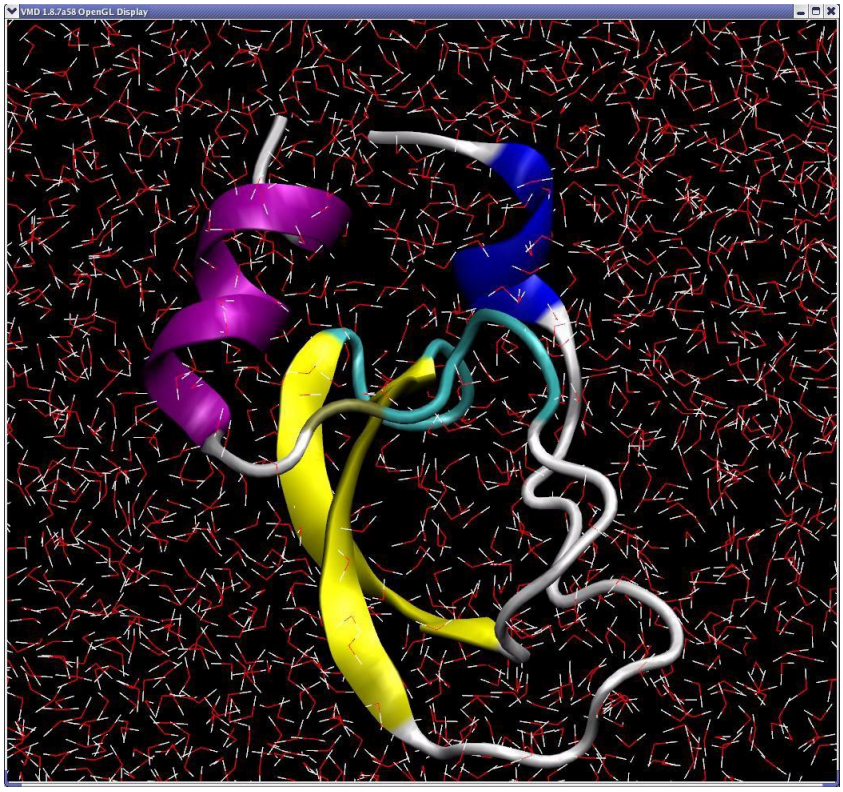
After loading the frames, you should see similar molecular information in the VMD Main window shown in Figure 7.4. At the bottom of the window you can see the controls of the VMD Trajectory Player quite similar to those of the Maestro Trajectory Player in Figure 6.2 on page 96.

Figure 7.4 VMD Trajectory Player



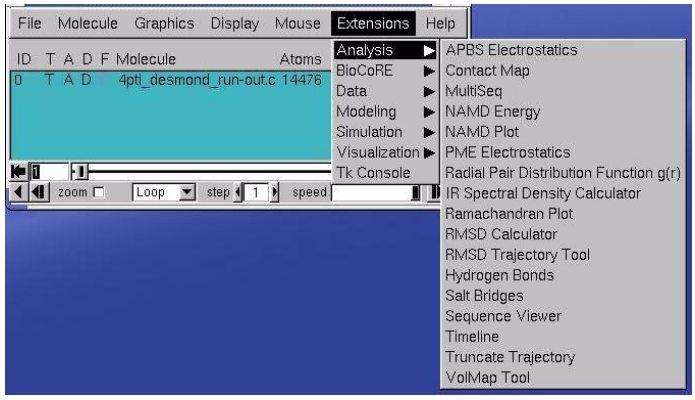
The trajectory movie can be seen in the VMD OpenGL Display window shown in Figure 7.5. Note that VMD can handle much larger trajectories than Maestro.

Figure 7.5 VMD OpenGL Display



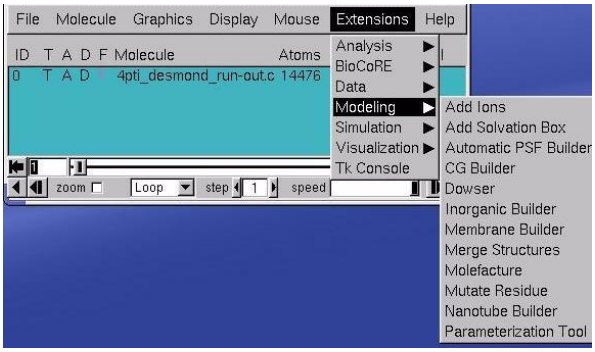
VMD provides a number of trajectory analysis tools available from the **Extensions > Analysis** menu in the Main window shown in Figure 7.6.

Figure 7.6 VMD Analysis tools



You can also setup simulation systems in VMD using tools from the **Extensions > Modeling** menu (Figure 7.7).

Figure 7.7 VMD Modeling tools



The general workflow of setting up a molecular system for Desmond simulation in VMD is as follows.

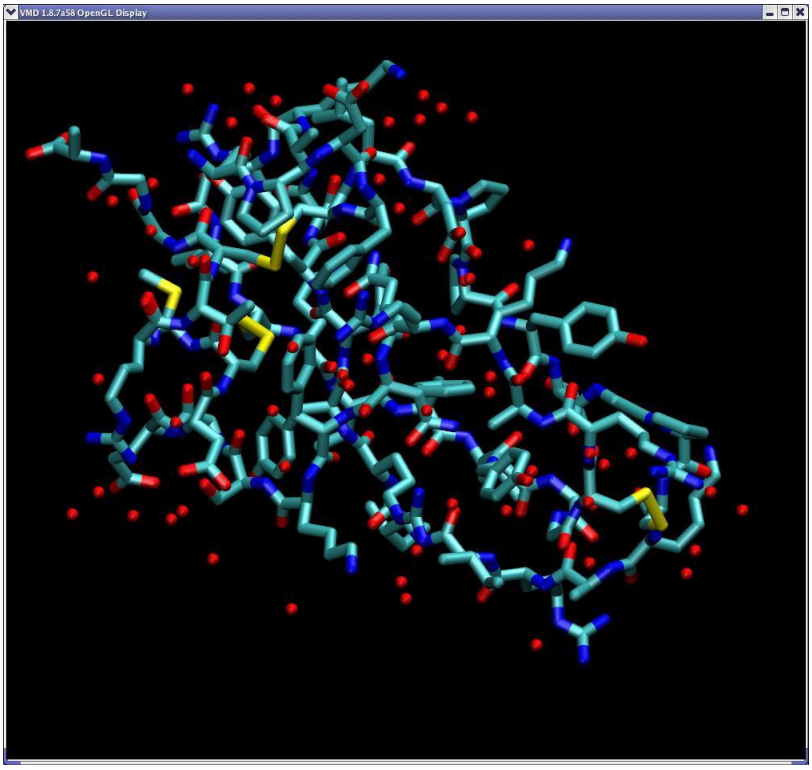
1. Load a molecule from any source that VMD can read; for example, load a protein structure from a PDB file.
2. Use the modeling tools in VMD shown in Figure 7.7 to generate the simulation system. The tools provide similar functionality to that of the Protein Preparation Wizard and the Desmond System Builder in Maestro (see Figure 1.6 on page 16 and Figure 2.2 on page 30). The main building tool in VMD is the popular Automatic PSF Builder.
3. When you are satisfied with your setup save the system in a Maestro file. Make sure that your system is selected in the VMD Main window (click on it if it isn't highlighted in yellow) and export a Maestro file by selecting **File > Save Coordinates** and choosing the **mae** file type.
4. Use *Viparr* and the `build_constraints` script as described in "Finishing Preparations for Desmond Simulation" on page 55 to add force field parameters and constraints to complete the setup for running a Desmond simulation with a molecular system generated in VMD.

As an exercise, try building a simulation system with the 4pti structure that we used in "Desmond Tutorial" on page 10 to introduce Desmond setup in Maestro.

Building a solvated protein system for Desmond simulation:

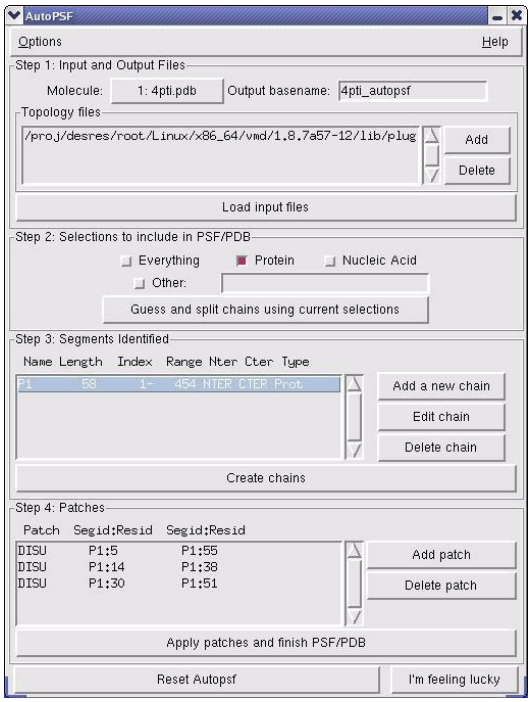
1. Load the 4pti protein structure from the PDB file in your working directory using the procedure depicted in Figure 7.1 on page 103 and Figure 7.2 on page 104, but using the **pdb** file type. Alternatively, start VMD from the command line with the '`vmd 4pti`' command. The structure should appear in the VMD Display window as shown in Figure 7.8. The protein structure will be displayed in a wire frame representation; to show the structure as depicted in Figure 7.8, change the drawing method to "licorice" in the Graphical Representations window for better picture quality.

Figure 7.8 Loading 4pti.pdb into VMD



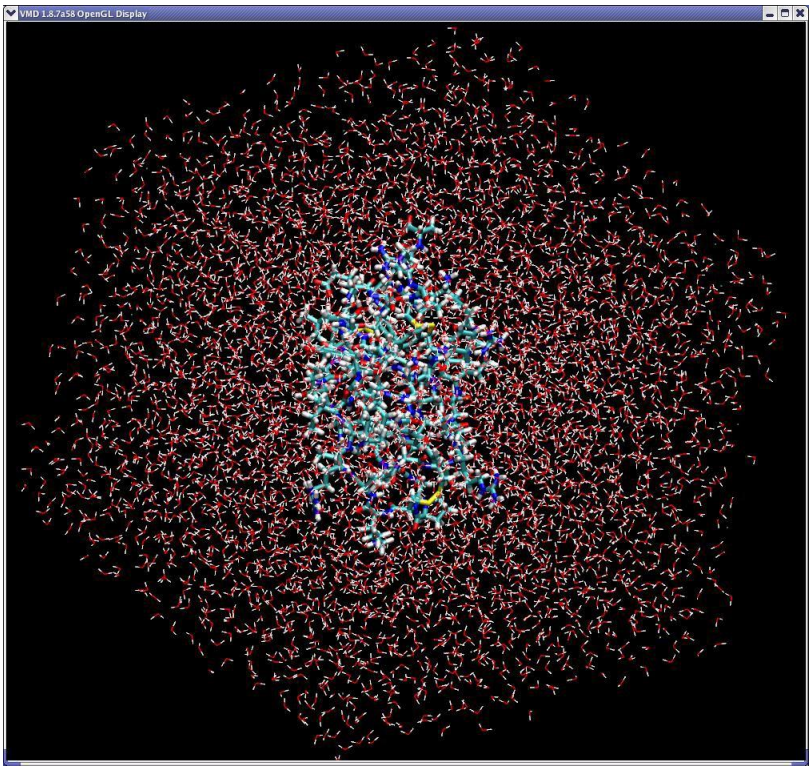
2. Select **Extensions > Modeling > Automatic PSF Builder** from the menu shown in Figure 7.7. The AutoPSF window appears as shown in Figure 7.9. Under Options, select the Add solvation box and Add neutralizing ions options.

Figure 7.9 VMD AutoPSF window



3. Click I'm feeling lucky at the lower right corner of the AutoPSF window to complete the automatic setup. Default settings in Step 1 through Step 4 should work, if you want to change any parameter, please consult the VMD documentation. The solvated protein should appear in the VMD Display as shown in Figure 7.10.

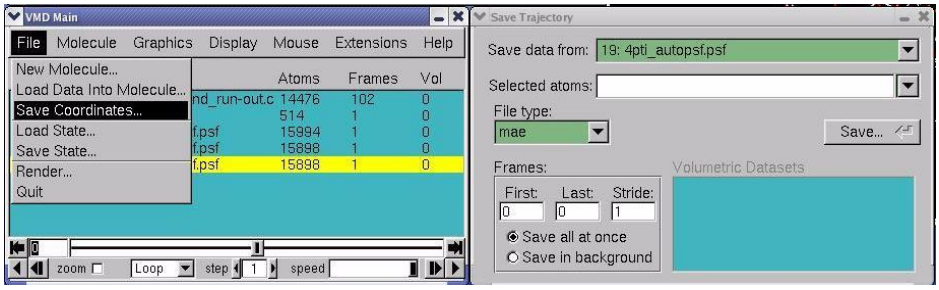
Figure 7.10 The solvated 4pti structure in VMD



4. Make sure that the last molecule in the VMD Main window is selected and select **File > Save Coordinates** as shown in Figure 7.11. Set the file type to **mae** and click Save. Give a name to the Maestro output file in the file browser and click OK. You can also write out the Maestro file by issuing the following command from the VMD text window:

   ```
   vmd > animate write mae my_output_file.mae
   ```

Figure 7.11 Saving the 4pti system in Maestro format



5. Process the Maestro file with *Viparr* and the `build_constraints` script from the command line to add force field parameters and constraints. Now you are ready to run a Desmond simulation. You can either import the simulation system into the Desmond Molecular Dynamics panel in Maestro, or, you can run a Desmond simulation from the command line with this file and an appropriate Desmond configuration file as described in "Running Desmond simulations" on page 64.

Loading files from the scripting interface

Files can also be loaded into VMD using the Tcl or Python scripting interface. We'll confine ourselves here to the Python interface, although you may find using the Tcl interface with VMD TkCon window more convenient for simple tasks.

Type the command `gopython` in the VMD text window to start the Python interface. Load the molecule module by typing `import molecule` at the Python prompt (>>>). To load a structure file and all frames of a trajectory file (henceforth we use the short- hand **trajectory.dtr** as the generic name of a trajectory file instead of **trajectory_subdir/clickme.dtr**), enter the following commands:

```
import molecule
molid = molecule.read(molid=-1, filetype='mae',
filename='structure.cms')
molecule.read(molid, filetype='dtr', filename='trajectory.dtr', wait-
for=-1)
```

The −1 argument to `molecule.read` indicates that a new molecule should be created for the file. We specify filetype and filename to load the structure. Finally, we use the `molid` returned by the first read command as the first argument to the second read command so that data from the trajectory gets appended to the original structure file. The `waitfor=-1` means to load all frames in the trajectory before returning from the call. This corresponds to the 'Load all at once' option in the File menu.

You can also specify a range of frames to be loaded:

```
molecule.read(molid, 'dtr', 'trajectory.dtr',
beg=5, end=55, skip=10, waitfor=-1)
```

Assuming there were at least 55 snapshots in `trajectory.dtr`, this command would load snapshots 5, 15, 25, 35, 45, and 55 into the molecule with the given `molid`.

Getting Information about Snapshots

Data in VMD is organized into *Molecules*, which represent entire molecular systems as well any number of snapshots associated with those systems. As molecules are loaded, they are assigned an integer key called a `molid`. Exactly one molecule at any given time is tagged as the `top` molecule; unless you set the top molecule yourself, this will always be the most recently loaded molecule.

Each molecule in VMD has a fixed number of atoms. To each atom, VMD associates various attributes such as name, type, mass, charge, etc. This data is usually obtained from a structure file, but missing values not supplied by the structure file are guessed, and the stored values can be overridden using scripting commands as described below.

The snapshots associated with each molecule are referred to as frames. Each frame holds one set of coordinates for each atom in the molecule, as well as unit cell dimensions for that frame. Velocities can also be stored if the trajectory file supplies them and the user requests it using the appropriate file type. Table 7.1 summarizes the functions in the molecule module for querying the high level structure of molecules in VMD.

In the rest of this section, we outline the Python interface provided by VMD to extract atom and frame information from a molecule

Atom selections

An atom selection consists of all the atoms in a particular molecule and frame that satisfy some predicate, or set of conditions. We often refer to the predicate as the selection, but it should be kept in mind that the set of atoms satisfying the predicate could be different for different frames. Atom selections are used in the GUI to select which atoms to draw with each style. The same selection language is used in the scripting interface to specify a predicate for an atom selection object.

Atom selection objects are created in the VMD Python interface using the `atomsel` module:

```
# Import the atomsel type
from atomsel import
atomsel
# Create a selection corresponding to all atoms in the top molecule,
# pointing to the current
frame. all = atomsel()
```

Table 7.1 Summary of functions from VMD built-in molecule

Module function	Result
new(name)	molid of newly created molecule
listall()	list of available molid values
numatoms(m)	number of atoms in molecule m
numframes(m)	number of frames (snapshots) in mole-
get_top()	molid of top molecule; -1 if none
set_top(m)	make molecule m the top molecule
get_frame(m)	get current frame of molecule m
set_frame(m, frame)	set current frame for molecule m to frame
get_periodic(m=-1, frame=-1)	Dictionary of periodic cell data; default top molecule, current timestep
set_periodic(m=-1, frame=-1,)	Set periodic cell keywords a, b, c, alpha, beta, gamma
get_physical_time(m=-1, frame=-1)	time value associated with timestep; default top molecule, current timestep
read(molid, filetype, filename, ...)	load molecule files
write(molid, filetype, filename, ...)	Save molecule files

```
# Select just the atoms named 'CA' (i.e., the alpha carbons), in the
# top molecule in the current
frame ca = atomsel(selection='name
CA')

# Select the protein atoms in frame 10 of the top molecule; if the
# current frame changes, the selection will still point to the same
# frame.
pro10 = atomsel('protein', frame=10)

# Select the waters near the protein from molecules 0 and 1.
wat0 = atomsel('water and same residue as (within 5 of protein)', molid=0)
wat1 = atomsel('water and same residue as (within 5 of protein)', molid=1)
```

As illustrated above, the `atomsel` type takes three arguments, all of which are optional:

- `selection`; default `all`. This is the selection predicate. The selection language is described in full in the *VMD User's Guide*.
- `molid`; default `-1`. The `molid` is the molecule associated with the selection, and never changes. The default `molid` of `-1` means the top molecule at the time that the selection was created. If the top molecule happens to change after the selection is created, for instance, when a new molecule is loaded, the `molid` does not change. If a molecule gets deleted from VMD's workspace, all associated atom selections are invalidated.
- `frame`; default `-1`. The `frame` is the index of the snapshot referenced by the selection. The default value of `-1` means that the selection should reference whatever the current frame happens to be. A `frame` value of 0 or higher means the selection always refers to the same frame, even if the current frame changes. If the frame referenced by selection does not exist, then the last frame in the molecule is used.

You can get and set the frame attribute in the usual Python way:

```
n1 = len(wat0)   # number of atoms in the selection
f = wat0.frame   # returns -1
wat0.frame = 20  # makes wat0 reference frame 20
n2 = len(wat0)   # will be equal to n1!
wat0.update()    # recompute the selection based on frame 20
n3 = len(wat0)   # might be different from n1
```

Note, however, that changing the frame does *not* cause the selection to be recomputed. Only the `update()` method changes the set of atoms referenced by an existing selection. However, changing the frame *does* change the result of calculations that depend on the coordinates, such as `center()` and `rmsd()`. The reason things are done this way is because one might want to select a set of atoms based on a distance criterion, such as the `wat0` selection in the example above, and then see how the positions of those atoms change over time. There would be no way to do this if changing the frame automatically updated the selection.

With an atom selection object in hand, you can extract data about a molecule for the atoms in the selection. Continuing from the first example:

```
# Get the number of alpha carbons
num_ca = len(ca)

# get the residue name of each alpha carbon
resnames = ca.get('resname')

# Set the 'type' attribute of all atoms to 'X'
all.set('type', 'X')

# Compute the center of mass the protein atoms in frame 10
mass = pro10.get('mass')
com = pro10.center(mass)

# Align pro10 to the corresponding atoms in frame 0, and compute
# the RMSD relative to frame 0.
pro0 = atomsel('protein', frame=0)
m = pro10.fit(pro0, weight=mass)
pro10.move(m)
rms = pro10.fit(pro0)
```

Snapshots

Time-varying data in VMD includes positions, velocities, and periodic cell parameters. The atom selection interface can be used to extract positions and velocities from a given frame in the same way as other attributes:

```
# get the x, y, and z coordinates for the atoms in wat1 as Python
lists x = wat1.get('x')
y = wat1.get('y')
z = wat1.get('z')
# get the z component of the velocity
vz = wat1.get('vz')
```

However, this method comes with significant overhead if a large number of snapshots are to be processed, and the Python lists returned by the `get()` are not as efficient or convenient to work with as NumPy arrays.

For this reason, VMD implements a built-in module called `vmdnumpy` that returns positions and velocities as NumPy arrays, rather than lists. The syntax is

```
vmdnumpy.positions(molid=-1, frame=-1) # Return reference to coordinates
vmdnumpy.velocities(molid=-1, frame=-1) # Return reference to velocities
```

The arrays returned by `positions()` and `velocities()` are zero-copy references to the snapshot data stored in VMD. They will be sized as N x 3 arrays, where N is the number of atoms in the molecule. The x, y, and z components of the position or velocity are stored in columns 0, 1, and 2 of the respective arrays. If a frame holds no velocities, then the `velocities()` method will return `None`. The `molid` argument defaults to the top molecule, just like atom selections, and the frame defaults to the current frame at the time the array is returned. Since the arrays reference snapshot data without making a copy, they must not be used after the frame to which they refer is deleted; any attempt to do so will probably result in a program crash. However, accessing positions with `vmdnumpy` is far more efficient than extracting coordinates from atom selections. The most efficient way to extract a subset of the coordinates corresponding to a selection is to use the atom indices as a slice argument to the positions array:

```
pos = vmdnumpy.positions() # all positions in top molecule's
                           current frame
inds = ca.get('index')   # index of all CA atoms
ca_pos = pos[inds]       # M x 3 array, where M = len(ca)
```

As summarized in Table 7.1, the periodic cell data is returned by `molecule.get_periodic()` as a dictionary whose keys are `a`, `b`, `c`, `alpha`, `beta`, and `gamma`, corresponding to the lengths of the three unit cell shift vectors and the angle between `b` and `c`, `a` and `c`, and `a` and `b`, respectively. To change the unit cell by hand, use `molecule.set_periodic()` with the same keyword arguments as the dictionary to change the desired attributes.

Centering trajectories

To generate a continuous trajectory view with the protein centered in the primary simulation box, load your trajectory into VMD and try either of the following commands from VMD's Tk console (**Extensions > Tk Console**):

```
pbc wrap -centersel protein -center com -compound res -all

pbc wrap -center bb -centersel protein -sel "not protein" -compound
res -all
```

You may have to superimpose all frames of the trajectory onto a selected frame in order to center the view in the main VMD graphics window. To do so, select **VMD > Extensions > Analysis > RMSD Trajectory Tool**, and select the Align option. Note that the transformed trajectory can be written to disk for further analysis.

Writing Structures and Trajectories

Modified structures or trajectories can be written back out to disk for further analysis or as input to simulation preparation. There are two distinct methods for writing out coordinates. The first method for writing coordinates and structure is the `molecule.write` command:

```
molecule.write(molid, 'dtr', 'trajectory.dtr', waitfor=-1)
```

The `molid`, filetype, and filename are required parameters. The `waitfor` option indicates how many frames should be written before returning from the command; you will almost always want to use -1. You can also specify a range of frames to be written, just like the `molecule.read` command:

```
molecule.write(molid, 'dtr', 'trajectory.dtr', beg=100, end=-1, skip=10,
waitfor=-1)
```

This command would write every 10th frame starting from frame 100. Coordinates can also be written using an atom selection:

```
sel = atomsel('name CA')
sel.frame = 45
sel.write('mae', 'ca45.mae')
```

This command would create write a Maestro file containing just the CA atoms whose coordinates were taken from frame 45.

Analyzing Whole Trajectories

We can now give a nontrivial trajectory analysis example that illustrates the use of `atomsel`, `vmdnumpy`, and `molecule`. The script shown in Figure 7.12 runs through all the frames in a molecule, aligns each frame to the first frame, and computes the aligned RMSD for each frame and the averaged position of the alpha carbons. To use this script, it's necessary to load the reference structure and any trajectories to be processed into VMD, using any of the methods outlined in "Loading and Viewing Trajectories" on page 102.

Once that's done, you can launch the script as described in "The VMD Python Interface" on page 101.

Figure 7.12 Analysis script example

```
from atomsel import atomsel
import molecule, vmdnumpy
import numpy

avg = None                # will hold averaged coordinates
all = atomsel()           # all atoms in current frame
ref = atomsel('name CA', frame=0) # reference frame is frame 0
sel = atomsel('name CA')  # alpha carbons in current frame
inds = sel.get('index')   # indexes of alpha carbons don't change
mass = sel.get('mass')    # masses don't change, either
rms = [0.]                # will hold RMSD to reference frame

def processFrame():
    global avg
    all.move( sel.fit(ref, mass) ) # Align current frame w/ref frame
    rms.append( sel.rmsd(ref, mass) ) # Append new RMSD value
    # Get positions of CA atoms
    pos = vmdnumpy.positions()
    ca_pos = pos[inds]

    # Accumulate positions into average
    if avg is None: avg = ca_pos[:] # make a copy of ca_pos
    else: avg += ca_pos # add this frame's contribution

# Loop over frames
n = molecule.numframes(molecule.get_top()
for i in range(1,n ):
    molecule.set_frame(molecule.get_top(), i)
    processFrame()

# Scale the average
avg /= n
```

8 Documentation Resources

Detailed Desmond documentation can be found in the D. E. Shaw Research *Desmond User's Guide*.

The Desmond user community website provides an interactive user forum for exchanging Desmond related information: **http://groups.google.com/group/desmond-md-users/**

You must register with the group, but this is done by simply applying for group membership on the site. Once you are a member, you can search the forum and post comments, as well as browse the Desmond MD Users site, which provides additional Desmond resources, such as FAQs, sample simulations, and information about Desmond work- shops: **https://sites.google.com/site/desmondmdusers/**

Be sure to check the "Known Issues" file already included with the Desmond/Maestro distribution for undocumented issues and problems with the current release.

Schrödinger also provides an extensive searchable knowledgebase with numerous Desmond-related articles: **http://schrodinger.com/kb**

Schrödinger's Desmond documentation includes the *Quick Start Guide* and the *Desmond User Manual*:

```
$SCHRODINGER/docs/desmond/des31_quick_start.pdf
```

and

```
$SCHRODINGER/docs/desmond/des31_user_manual.pdf
```

The documentation is also available by choosing Help > Manuals Index in Maestro. Maestro documentation can be found online at **www.schrodinger.com** and context specific.-
online help can be brought up in any Maestro panel, which has a Help button. The complete Maestro documentation set includes:

```
$SCHRODINGER/docs/maestro/mae93_command_reference.pdf,
$SCHRODINGER/docs/maestro/mae93_tutorial.pdf,
$SCHRODINGER/docs/maestro/mae93_user_manual.pdf.
```

The full ASL (atom specification language) documentation can be found in:

```
$SCHRODINGER/docs/maestro/mae93_help/misc/asl.html
```