PrimeX 1.9

Quick Start Guide

Schrödinger Press

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Contents

Document Conventions

In addition to the use of italics for names of documents, the font conventions that are used in this document are summarized in the table below.

Font	Example	Use
Sans serif	Project Table	Names of GUI features, such as panels, menus, menu items, buttons, and labels
Monospace	`$SCHRODINGER/maestro`	File names, directory names, commands, environment variables, command input and output
Italic	*filename*	Text that the user must replace with a value
Sans serif uppercase	CTRL+H	Keyboard keys

Links to other locations in the current document or to other PDF documents are colored like this: Document Conventions.

In descriptions of command syntax, the following UNIX conventions are used: braces { } enclose a choice of required items, square brackets [ ] enclose optional items, and the bar symbol | separates items in a list from which one item must be chosen. Lines of command syntax that wrap should be interpreted as a single command.

File name, path, and environment variable syntax is generally given with the UNIX conventions. To obtain the Windows conventions, replace the forward slash / with the backslash \ in path or directory names, and replace the $ at the beginning of an environment variable with a % at each end. For example, `$SCHRODINGER/maestro` becomes `%SCHRODINGER%\maestro`.

Keyboard references are given in the Windows convention by default, with Mac equivalents in parentheses, for example CTRL+H (⌘H). Where Mac equivalents are not given, COMMAND should be read in place of CTRL. The convention CTRL-H is not used.

In this document, to *type* text means to type the required text in the specified location, and to *enter* text means to type the required text, then press the ENTER key.

References to literature sources are given in square brackets, like this: [10].

Introduction

This tutorial works through the solution of the structure of p38 alpha MAP kinase (also known as mitogen-activated protein kinase 14) complexed with 7-piperidin-4-yl-3,4-dihydroquinolin-2(1H)-one. This serine/threonine kinase has been associated with some critical biological functions, including apoptosis. The starting model comes from pdb entry 1w82, which is the structure of the same protein, in the same space group, but with a different inhibitor and slightly different cell parameters. The structure that is solved has a single amino acid substitution, C162S, compared to the starting model, 1w82.

It is assumed that you have access to a PrimeX 1.9 and Maestro 9.3 installation. If you need to install these products, see the *Installation Guide* for instructions.

This tutorial also assumes that you are familiar with basic Maestro operations. If you need to familiarize yourself with these operations, there are several documents you can use:

- *Maestro User Manual*, for detailed information on using Maestro
- Chapter 3 of the *PrimeX User Manual*, for an introduction to some Maestro features in the context of PrimeX.

PrimeX Tutorial

This tutorial shows how to use the tools available with PrimeX and Maestro for performing an X-ray structure refinement, starting from a model, a sequence, and a set of reflection data. In the early parts of the tutorial, the steps for some actions are given in detail so that you can learn how to perform them. Later in the tutorial, these steps are summarized or condensed.

The job names given in the tutorial are arbitrary, but are chosen to help keep track of the results. The job name for structures incorporated into the Project Table are listed in the table so that you can identify the results of each job.

2.1 Skipping Tutorial Steps

Several of the steps are time-consuming, and you might want to skip over the actual calculations. Structures for various stages of the process have been provided so that you can so do. The structures can be imported as follows.

a. Click the Import button on the Project toolbar.

The Import panel opens.

b. From the Files of type option menu, choose PDB.

c. Select the relevant `ready_for_`*step*`.pdb` file.

The file names have been chosen to reflect the step for which they are intended as input.

d. Ensure that Replace Workspace is selected.

e. Click Import.

The file is imported and the entry is selected and displayed in the Workspace. You may now proceed to the step for which this file is the input.

However, we strongly recommend that you work through the settings, up to the point of starting the job, before skipping the step, so that you get practice in making the settings.

2.2 Preparing for the Exercises

To run the exercises, you need a working directory in which to store the input and output, and you need to copy the input files from the installation into your working directory. This is done automatically in the Tutorials panel, as described below. To copy the input files manually, just unzip the `primex` zip file from the `tutorials` directory of your installation into your working directory.

On Linux, you should first set the `SCHRODINGER` environment variable to the Schrödinger software installation directory, if it is not already set:

csh/tcsh: `setenv SCHRODINGER` *installation-path*

sh/bash/ksh: `export SCHRODINGER=`*installation-path*

If Maestro is not running, start it as follows:

- **Linux:** Enter the following command:

  ```
  $SCHRODINGER/maestro -profile Maestro &
  ```

- **Windows:** Double-click the Maestro icon on the desktop.

  You can also use Start → All Programs → Schrodinger-2012 → Maestro.

- **Mac:** Click the Maestro icon on the dock.

  If it is not on the dock, drag it there from the SchrodingerSuite2012 folder in your Applications folder, or start Maestro from that folder.

Now that Maestro is running, you can start the setup.

1. Choose Help → Tutorials.

   The Tutorials panel opens.

2. Ensure that the Show tutorials by option menu is set to Product, and the option menu below is labeled Product and set to All.

3. Select PrimeX Quick Start Guide in the table.

4. Enter the directory that you want to use for the tutorial in the Copy to text box, or click Browse and navigate to the directory.

   If the directory does not exist, it will be created for you, on confirmation. The default is your current working directory.

5. Click Copy.

   The tutorial files are copied to the specified directory, and a progress dialog box is displayed briefly.

If you used the default directory, the files are now in your current working directory, and you can skip the next two steps. Otherwise, you should set the working directory to the place that your tutorial files were copied to.

6. Choose Project → Change Directory.
7. Navigate to the directory you specified for the tutorial files, and click OK.

You can close the Tutorials panel now, and proceed with the exercises.

2.3 Creating a Maestro Project

When you start Maestro, a scratch project is created. This project must be named in order to keep it for later use. You should therefore create a named Maestro project to save your work, in case you want to complete the exercises at a later time. For more information on Maestro projects, see Chapter 9 of the *Maestro User Manual*.

1. Choose Project → Save As.

   The Save Project dialog box opens. The Look in option menu should contain the current Maestro working directory.

2. In the File name text box, type `primex_tutorial`.
3. Click Save.

This procedure creates a project named `primex_tutorial`. The work that you do during the exercises that follow is automatically saved in this project for later use.

To restart the tutorial from the point you left off, start Maestro and choose Open from the Project menu, then select the project, or choose it from the recent projects at the end of the Project menu.

Alternatively, to open the project on starting Maestro, you can use the following command on Linux

```
$SCHRODINGER/maestro -p primex_tutorial.prj &
```

or double-click on the project on Windows or Mac.

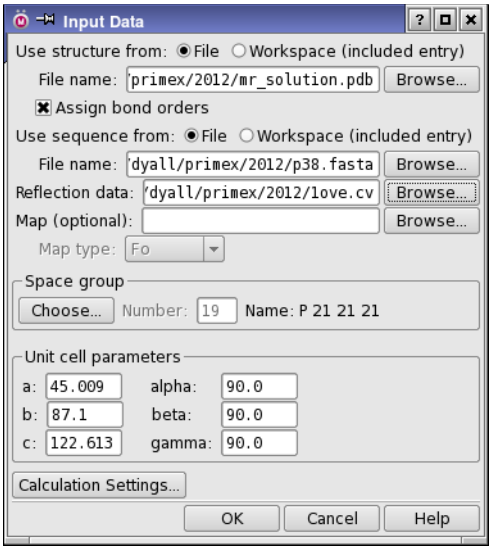

Figure 2.1. The Input Data dialog box.

2.4 Reading the Input Data

The first step is to read the input data, which includes the starting molecular replacement structure, the sequence, and the reflection data.

1. If the PrimeX toolbar is not displayed, choose Applications → PrimeX → Display Toolbar or Window → Toolbars → PrimeX.

   The PrimeX toolbar is a collection of icons that appears in a horizontal bar across the top of the Workspace.

2. Click the Input Data button on the PrimeX toolbar.

   

   The Input Data dialog box opens.

3. Ensure that Assign bond orders is selected.

4. For Use structure from, ensure that File is selected (the default) and click Browse.

   A file selector opens.

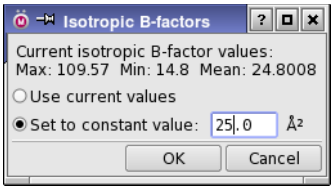

Figure 2.2. The Isotropic B-factors dialog box.

5. Navigate to the `tutorial` directory and select `mr_solution.pdb` from the file list.

   This file is the starting molecular replacement structure from which we will solve our target p38 kinase structure.

6. Click Open.

   The file selector closes, and the Isotropic B-factors dialog box opens.

7. Select Set to constant value, and enter `25` in the text box.

8. Click OK.

   The Isotropic B-factors dialog box closes, and the Input Data dialog box shows the structure file name in the File name text box. The space group and unit cell parameter sections are filled in with data from the structure file. The resolution limits are calculated from the data at the end of the input process.

9. For Use sequence from, ensure that File is selected (the default), and click the Browse button for File name.

   A file selector opens.

10. Select `p38.fasta` from the file list, and click Open.

    The file selector closes and the sequence file name is shown in the File name text box.

11. Click the Browse button for Reflection data.

    A file selector opens.

12. Select `1ove.cv` from the Files list, and click Open.

    The file selector closes, and the Input Data - Test Set dialog box opens. The file you selected already contains a test set for the calculation of R-free, so you do not need to select a test set but can use the current set.

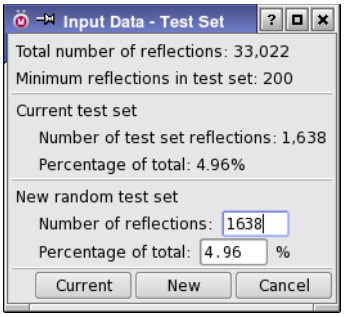

Figure 2.3. The Input Data - Test Set dialog box.

13. Click Current.

    The Input Data - Test Set dialog box closes, and the reflection file name is shown in the Reflection data text box. The space group and unit cell parameter sections are replaced with data from the reflection file. (If the reflection file had contained no cell information, the previous values would have been retained.) There is no map file, so the map specification is skipped.

14. Click Calculation Settings.

    The Calculation Settings dialog box opens. This dialog box can be opened from any of the other panels, and allows you to select various options for the calculations. Here you will check settings for the first stage of the process.

    Note that the option to Include H-atoms in structure output is selected by default. Although you may use PrimeX in the mode where hydrogens are not returned after an operation, the mode whereby hydrogen atom positions are refined in parallel with all other coordinates is recommended.

15. Confirm that the Resolution limits setting is Calculate from data and cell constants.

    The resolution limits have not yet been calculated from the data and the default values of 30Å and 1.0Å are shown for the limits. You can revisit the Calculation Settings panel at any point after the input process, to check the values calculated or to override these values if you wish to exclude either high or low resolution data. Note the other parameters available in this panel. Default values for these parameters are usually appropriate.

16. For Overall B-factor scaling, confirm that Anisotropic is selected.

17. Click OK.

    The Calculation Settings dialog box closes. The input data specification is now complete.

18. Click OK in the Input Data dialog box.

The Input Data dialog box closes. The Project Table panel opens, with the initial structure as the first entry (if you have started this tutorial with a new Maestro session) and many of the icons on the PrimeX toolbar are now active. The structure is included in the Workspace.

2.5 Generating Initial R-Factors

1. From the Task button menu on the PrimeX toolbar, choose R-Factor Calculation. (To display the menu, click and hold on the button.)

   

   The R-Factor Calculation panel is displayed. Since the calculation settings have already been made, there is no need to change them.

2. Click Start.

   The Start dialog box opens. This dialog box allows you to make settings that determine how the job is run and how the results are incorporated into the project.

3. Choose Replace existing entries from the Incorporate option menu.

   The R-factor calculation makes no change in the structure, but just adds the R-factors as properties, so replacing the existing structure does not result in any loss of information. Note also that a job name is automatically generated, which forms the root for all files generated by the job in your default directory.

4. (Optional) Change any other settings in this dialog box for running the job.

5. Click Start.

   This job takes a few minutes or less. When the job finishes, the results are added to the Project Table. Use the horizontal scroll bar to scroll to the values of R and Rfree.

   You might want to enlarge the Project Table panel (by dragging the panel border). You might also want to adjust the column widths, which you can do by choosing Fit to Data from the Columns button menu on the Project Table toolbar.

   

6. Close the R-Factor Calculation panel.

2.6 Rigid-Body Refinement

The rigid-body refinement in this tutorial is performed in two steps. First, the structure as a whole is moved, then the two domains of the protein are reoriented independently.

1. Choose Reciprocal-Space Refinement from the Task button menu on the PrimeX toolbar.

   

   The Reciprocal-Space Refinement panel opens.

2. Choose Rigid bodies from the Refinement method option menu.

   The Rigid bodies tab is displayed.

3. Choose Truncated Newton from the Minimizer option menu.

4. In the Select atoms to define group section, click the All button.

   This selects all the atoms in the structure as the rigid body, so the entire structure will be moved rigidly. The atoms are marked in the Workspace.

5. Click Add.

   A row is added to the Rigid body groups table, identifying the group and the residues that belong to the group. Since there are no residues that are not explicitly included, you do not need to select an option under Atoms not explicitly included in a group.

6. Click Start.

   The Start dialog box opens.

7. Enter “Rigid body refinement, one group” in the Entry Title text box of the Job section.

   The title is used for the result from this calculation, and appears as the entry title in the Project Table. The job is assigned a unique name, which you may also customize if you desire.

   Note that the Incorporate option is Append new entries individually. This calculation generates a new structure. While you could overwrite the starting structure, it is often a good idea to keep structures from the various stages of refinements so that you can follow the progress of refinement and revert back to a previous structure if necessary.

8. Click Start.

The job takes about 5 minutes. After the job finishes, you can proceed to the second step of the rigid-body refinement. First, you need to clear the previous group selections.

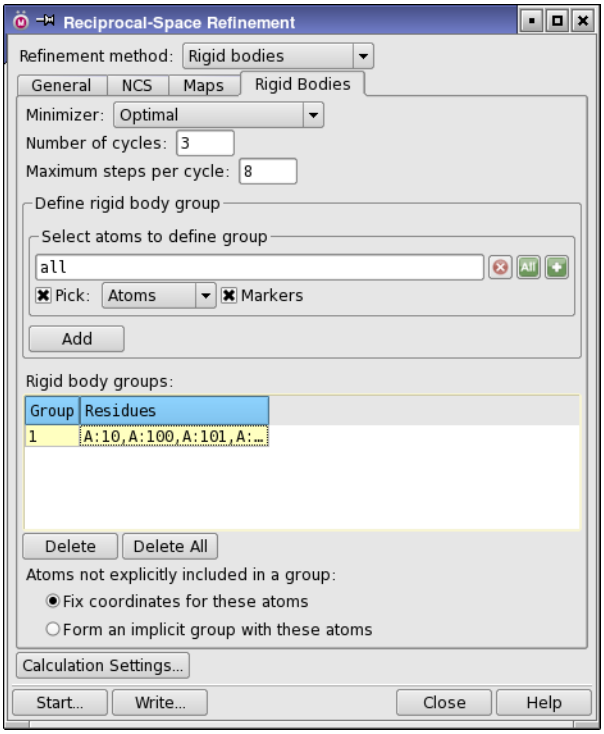

Figure 2.4. The Rigid Bodies tab in the Reciprocal-Space Refinement panel.

9. Click Delete All in the Rigid Bodies tab of the Reciprocal-Space Refinement panel.

   The group is deleted from the Rigid body groups table.

10. Click the delete button in the Select atoms to define group section.

    

    The ASL text box is cleared.

11. Enter `8` in the Number of cycles text box.

12. Orient the Workspace structure (middle mouse button or CTRL + middle mouse button) so that the narrow waist between the two domains of the protein is vertical or horizontal.

13. Ensure that Pick is selected in the Select atoms to define group tools, and choose Residues from the Pick option menu.

14. Drag out a box around one half of the structure to include one of the two protein domains.

15. Click Add.

    A row is added to the Rigid body groups table, identifying the group and the residues that belong to the group.

16. Under Atoms not explicitly included in a group, select Form an implicit group with these atoms.

    The residues in the other domain are now in a group, and the two domains will be oriented independently in the rigid-body refinement job.

17. Click Start.

    The Start dialog box opens.

18. Enter "Rigid body refinement, two groups" in the Entry title text box of the Job section.

19. Click Start.

    The job takes a few minutes.

20. When the job finishes, display the structures in row 2 and row 3 alternately, to see how the two domains have been shifted in the rigid-body refinement.

    To simplify the view, remove hydrogen atoms from the display by selecting All hydrogens from the Undisplay button menu on the Display Atoms toolbar.

    

21. Examine the R-factors in the Project Table, to determine whether further rigid-body refinement is necessary.

In general, rigid body refinement should progress to the point where the R-factor is less than 0.49. For this system it is possible for the R-factor to go down to about 0.36. If necessary, select another subset and refine the two domains again.

2.7 Simulated Annealing Refinement

1. In the Reciprocal-Space Refinement panel (which should be still open), choose Simulated Annealing from the Refinement method option menu.

   The Simulated Annealing tab is displayed.

2. Ensure that the text in the text box of the Select atoms to minimize section is `all`. If not, click the All button.

3. For Energy model, ensure that Approximate is selected (the default).

4. Change the number of cooling steps to 250.

   Shortening the refinement is done here in the interests of a quicker turnaround. The results from this short refinement are adequate, although a longer cooling phase might reduce the R-factors somewhat more.

5. In the General tab under X-ray term weight, select Constant weight and ensure that the value in the text box is `0.5`.

The weight of the X-ray terms is primarily judged by the bond angle RMSD and bond length RMSD, which measure the deviation of the structure from expected geometry. While the targets for these values vary among crystallographers, values less than 2.5 degrees and 0.015Å, respectively, are considered reasonable. Decreasing this weight decreases the RMSD values, while only modestly impacting the R-factors. Weights of less than 0.1 are rarely necessary, but will vary depending on how the X-ray reflection data was processed before refinement. For reflection data on an absolute scale, a weight of 0.5 is usually optimal.

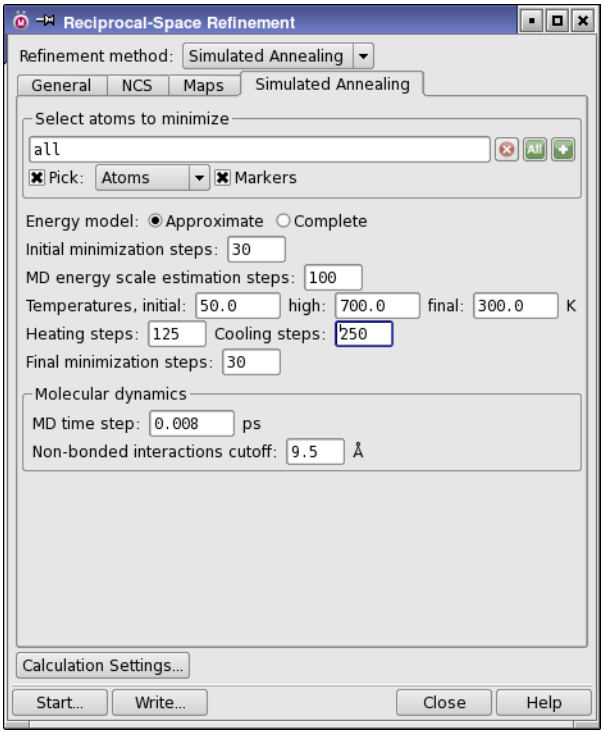

Figure 2.5. The Simulated Annealing tab in the Reciprocal-Space Refinement panel.

So far, no map has been generated. At the end of this refinement, we will generate a map.

6. In the Maps tab, for Coefficients select 2Fo-Fc.

   You can leave the remaining options at their defaults.

7. Click Start.

   The Start dialog box opens.

8. Enter "SA, cooling steps 250, otherwise default" in the Entry title text box of the Job section.

9. Click Start.

   This job takes about 30 minutes on a 2 GHz processor. You can follow the R and R-free values in the Monitor panel, which you can open from the Applications menu or by clicking the Jobs button in the status bar of the main window. They are the last two numbers in each group of numbers that are displayed.

   When the job finishes, the new structure is displayed in the Workspace with the map. Only a part of the map is displayed. You can change the display volume by spot-centering on an atom (right-click the atom).

2.8 Mutating Amino Acids and Placing Side Chains

In the next step, the model structure will be mutated to match the reference sequence, and side chains will be placed.

1. Choose Mutate Model to Sequence from the Task button menu on the PrimeX toolbar.

   

   The Mutate Model to Sequence panel opens. You will see the one substitution highlighted in the model (target) sequence.

2. Click Mutate to Match Sequence.

   After a few seconds, the Place Side Chains button becomes available, upon completion of all amino acid substitutions.

3. Click Place Side Chains.

   The Real-Space Refinement panel opens with Predict Side Chains chosen from the Method option menu. The mutated residues are already selected.

   Note that multiple side chains can be selected and placed in a single run.

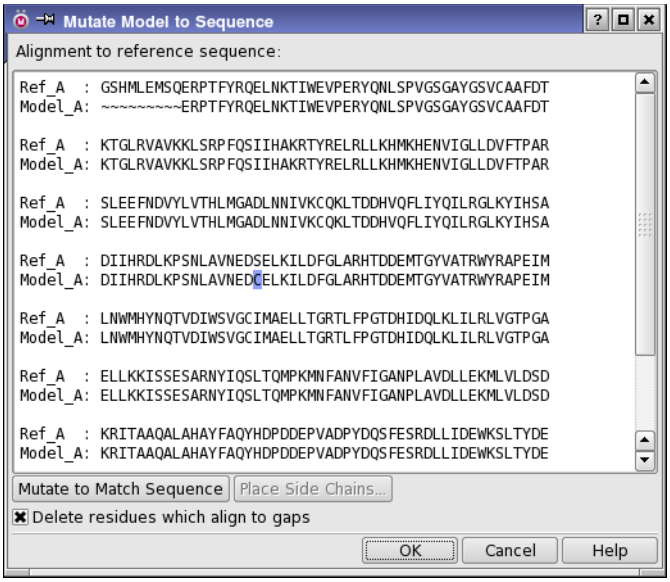

Figure 2.6. The Mutate Model to Sequence dialog box.

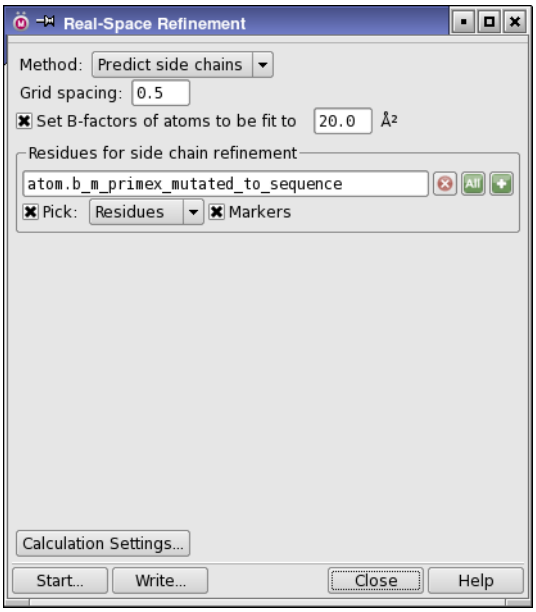

Figure 2.7. The Real-Space Refinement panel showing side-chain prediction controls.

4. Click Start.

   The Start dialog box opens.

5. Enter “Mutate, place side chains” in the Entry title text box of the Job section.

6. Click Start.

   The job takes a few minutes. When it finishes, a new entry is added to the Project Table, and the structure is displayed in the Workspace with a new map.

2.9 Reciprocal-Space Minimization

1. Choose Reciprocal-Space Refinement from the Task button menu on the PrimeX toolbar.

   

   The Reciprocal-Space Refinement panel opens.

2. Choose Minimization from the Refinement method option menu.

   The Minimization tab is displayed.

3. Ensure that Optimal is chosen from the Minimizer option menu.

   The quasi-Newton minimizer is usually the most effective for this type of operation. With this choice, the program uses the quasi-Newton minimizer as the optimal one, unless high gradients make the more stable conjugate gradient minimizer a better choice. The remaining default settings are appropriate for this job.

4. Enter 8 in the Number of cycles text box.

   There is no need to change the settings in the other tabs.

5. Click Start.

   The Start dialog box opens.

6. Enter “Minimization xyz” in the Entry title text box of the Job section.

7. Click Start.

   The job takes a few minutes. When it finishes, a new entry is added to the Project Table, and the structure is displayed in the Workspace with a new map.

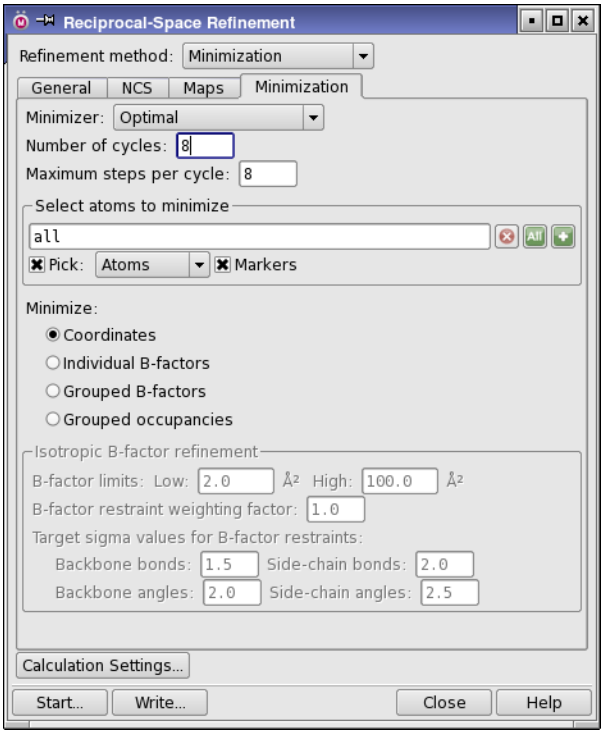

Figure 2.8. The Minimization tab in the Reciprocal-Space Refinement panel.

2.10 Examining the Density Fit

1. Click the Density Fit button on the PrimeX toolbar.

   

   The Density Fit panel opens. After a short delay, the table is filled with real-space R-factors for each residue and for the side chain and backbone of each residue. Low values indicate the best fit.

2. Click the Backbone Fit column heading twice.

   The values are sorted from highest to lowest. Some of the worst fits are found for residues around 31–36, 117–122, 170–174, and 353–354, but your results may vary depending on the success of your refinement to this point.

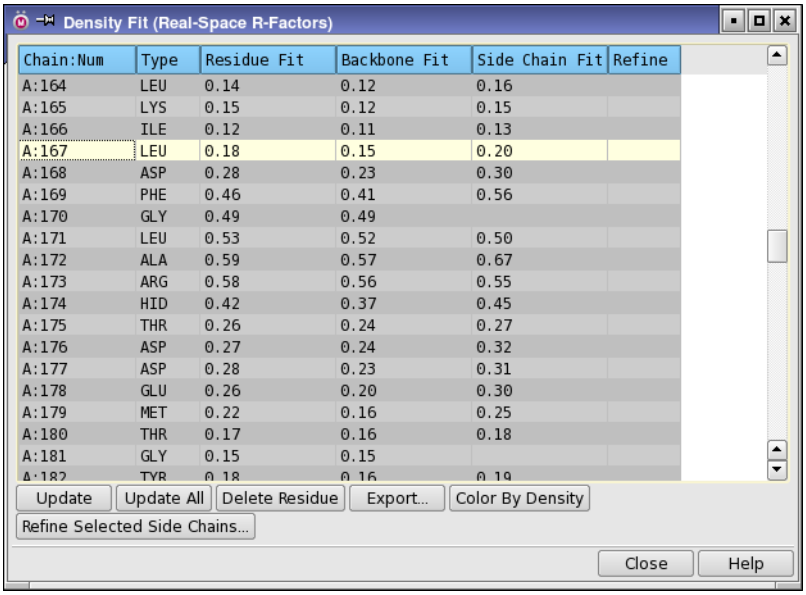

Figure 2.9. The Density Fit panel.

3. Click the Chain:Num column heading.

   The values are sorted by residue number again.

4. Scroll down to center the table around residues 168–174.

   These residues have higher values for the backbone fit, and we should consider rebuilding this segment of the chain.

5. Click residue 167 in the table.

   The Workspace view zooms in to this residue, which clearly fits the density well.

6. Click residue 175 in the table.

   This residue also has a good fit. Residues 167 and 175 can therefore provide reliable anchor points for a loop refinement, which will be done in the next section. Similar considerations suggest that the polypeptide segment containing residues 118 to 123 also requires loop refinement.

7. Close the Density Fit panel.

2.11 Loop Refinement

In this step, several of the loops are refined, including the one identified in the previous section.

1. Choose Real-Space Refinement from the Task button menu on the PrimeX toolbar.

   

   The Real-Space Refinement panel opens.

2. Choose Refine loops from the Method option menu.

   A table of loops is displayed in the center of the panel. These are regions lacking secondary structure, which are most likely to need to be rebuilt. The loops are identified by a label in the Feature column, and a starting (Res1) and ending (Res2) residue number. Loops are defined from the alpha carbon of Res1 to the alpha carbon of Res2.

3. Scroll down to the loop that includes residues 118 to 123, and click the label.

   The row is highlighted in yellow, and the residues are marked in the Workspace. The loop information is entered automatically in the Start and End text boxes below the loop table. This loop is on the outer part of the protein. The actual label may vary, depending on how Maestro classified the residues into loops, which depends on the results obtained so far.

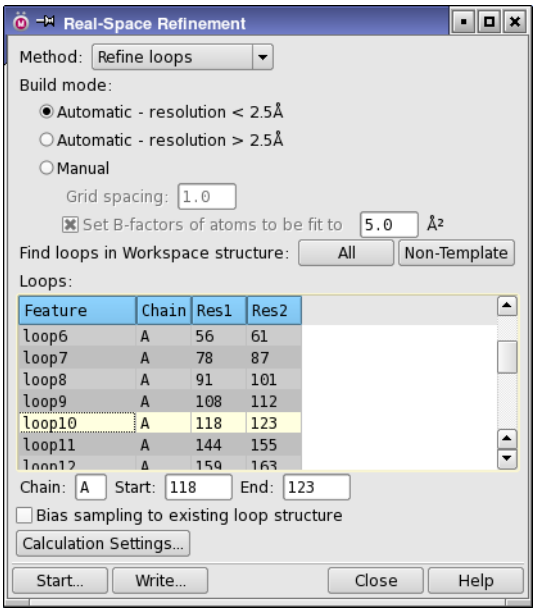

Figure 2.10. The Real-Space Refinement panel showing loop refinement controls.

4. Under Build mode, ensure that Automatic - resolution <2.5Å is selected.

   Different methods are suggested for low-resolution structures and moderate- to high-resolution structures. The cutoff between the two is not a hard and fast rule. Some poorly resolved loops in high-resolution structures might occasionally benefit from the low-resolution method, and clearly defined loops in lower-resolution structures might sometimes be productively built with the high-resolution setting.

5. Start the job with the entry title "Build loop 118-123".

   This job takes about 5 minutes. Usually only one structure is returned for this loop fitting calculation.

To compare the new loop to the original structure, the new entry will be fixed in the Workspace, and the original structure also displayed. The atoms that are not in the loops will be hidden. The atoms in the entries will also be colored so that they can be distinguished.

6. In the Project Table, select the new loop by clicking on its row.
7. Choose Entry → Fix in the Project Table panel.

   A padlock icon appears in the In column for the new loop, and the entry is displayed in the Workspace.

8. Click the In column for the entry immediately above the new loop to display the structure that was the input for building the loop from residue 118 to residue 123.

   This entry is included in the Workspace as well, along with its map. This map needs to be undisplayed—only the map from the loop refinement is needed at this point.

9. Right-click and hold on the S button in the Title column for the for the entry that was the input to loop building, and choose Hide all surfaces from the menu that appears.

   The maps are undisplayed.

10. From the Color Scheme button menu on the Representation toolbar, choose Element (Entry Carbons).

    

    The carbon atoms of each entry are colored with a different color. The sequences in the main sequence viewer are also colored. The structure before loop refinement is colored a light blue (cyan).

11. From the Display Only button menu on the Display Atoms toolbar, choose Select.

    

    The Atom Selection dialog box opens.

12. In the Residue tab, select Residue number from the list, and enter `117-123` in the Residue number text box.

13. Click Add, then click OK.

    The Atom Selection dialog box closes, and only residues 117 to 123 are displayed in the Workspace.

14. Click the Fit button on the Workspace toolbar.

    

15. Right-click on one of the atoms in the center of the loop.

    The view (including the map) is centered on this atom.

    Note that the refined loop is in a different location from the original loop, and fits the density much better.

16. From the Display Only button menu on the Display Atoms toolbar, choose All.

    All atoms are redisplayed. You can also change the color scheme back to Element.

    At this point, the fixed entry should still be selected in the Project Table, and be the only selected entry. (Selected entries are highlighted yellow in the Project Table.)

17. In the Project Table panel, choose Entry → Exclude.

    The predicted loop structure is no longer displayed in the Workspace.

After performing the loop refinement, a reciprocal-space minimization should be performed.

18. Include the predicted loop in the Workspace (click its In column in the Project Table).

19. Follow the instructions in Section 2.9 on page 16 to perform a minimization.

The next task is to refine the loop that was examined in the previous section, using the results of the minimization just performed.

20. Choose Real-Space Refinement from the Task button menu on the PrimeX toolbar.

    

    The Real-Space Refinement panel opens.

21. Choose Refine loops from the Method option menu.
22. Ensure that the loop to be refined is specified by Chain: `A`, Start: `167` and End: `175`.

    These are the residues identified above as good "anchors". The remaining residues in this loop, 175–183, have much better real-space R-factors than residues 168–174. Reducing the size of the loop also makes the calculation faster.

23. Under Build mode, ensure that Automatic - resolution > 2.5Å is selected.
24. Start the job with an appropriate entry title.

    This job takes about 10 minutes. Several structures are returned from loop fitting and are selected in the Project Table. There is some variation between the loop predictions, but all cover a different region of space from the original. You can use the display techniques in Step 6 through Step 16 to view the fit of the loop predictions to the map. To select multiple entries in the Project Table use control-click.

After performing the loop refinement, a reciprocal-space minimization should be performed.

25. Include one of the loop predictions in the Workspace.

    The first loop has the lowest Ref Score, and thus by this measure has the best combined fitting to the electron density with a low-energy conformation. However, the other returned structures are worth considering. Note that the regions where the models have the most divergence indicate where the electron density is the most ambiguous.

26. Follow the instructions in Section 2.9 on page 16 with an appropriate entry title to perform a minimization, using a constant weight of 0.5.

Finally, the loop spanned by residues 30–38 should be deleted. The electron density is not yet accurate enough to place these residues with Loop Refinement. Before you delete the residues, the entry will be duplicated so that the results of the minimization are preserved.

27. Ensure that the new loop entry with the lowest Ref Score is selected, and type CTRL+D (⌘D).

    The entry is duplicated, selected, and included in the Workspace.

28. Change the title for the duplicated entry to indicate its origin.
29. From the Delete button menu on the Edit toolbar, choose Select.

    

    The Atom Selection dialog box opens.
30. In the Residue tab, choose Residue number from the list.
31. Enter `30-38` in the Residue number text box.
32. Click Add, then click OK.

    The Atom Selection dialog box closes, and the residues are deleted from the structure.

2.12 Optimizing Hydrogen-Bonding Networks

At the moderate resolution of most protein crystal structures, it is difficult or impossible to determine from X-ray data alone which orientation the terminal groups in residues like His, Gln, and Asn should take. However, the most likely orientation can be deduced from optimizing the number and quality of hydrogen bonds among adjacent hydrogen-bonding groups. Similarly, polar hydrogens may be oriented in alternate ways to optimize hydrogen-bonding interactions. In this step, you will use a tool to assign these orientations to optimize the hydrogen bonding.

1. Choose Optimize H-bond Networks from the Task button menu on the PrimeX toolbar.

   

2. Ensure that Include current orientations is selected in the Analysis section, and click Analyze Network.

   The analysis may take a minute.
3. When the table is populated, ensure that View all species is selected, and click Optimize.

   This task rotates the chi2 (chi3 for Gln) angle on these residues 180 degrees if a better hydrogen-bond network is possible or it is energetically favorable. The rotated residues are marked with the label Flip in the Workspace.
4. Zoom in on each residue that has been flipped, and check that the orientation is what you expect.

   You can do this by selecting residues in the table. Many of these residues are on the surface of the protein, and are not hydrogen-bonded.

To display hydrogen atoms, choose All Hydrogens from the Also Display button menu on the Display Atoms toolbar.

If you want to examine the hydrogen bonds in the protein, Choose Intra H-bonds from the H-Bonds button menu on the Labels toolbar, and click on an atom in the protein.

To hide them again, choose Delete H-bonds from the same button menu.

If you want to hide the hydrogen atoms, choose All Hydrogens from the Undisplay button menu on the Display Atoms toolbar.

Likewise, you can hide nonpolar hydrogens by choosing Nonpolar Hydrogens.

5. When you have finished inspecting the residues, close the Interactive H-bond Network Optimizer panel, and run a reciprocal-space coordinate minimization with an appropriate entry title, following the instructions in Section 2.9 on page 16 using a constant weight of 0.5.

2.13 B-Factor Refinement

1. Open the Reciprocal-Space Refinement panel (if it is not already open).
2. Choose Minimization from the Refinement method option menu.

   The Minimization tab is displayed.
3. Set the number of cycles to 8, and ensure that the maximum steps per cycle is 8.
4. Under Minimize, select Individual B-factors.
5. In the Isotropic B-factor refinement section, enter `1.0` in the B-factor restraint weighting factor text box.
6. In the General tab, ensure that Use current values is selected under Isotropic B-factor values, and that the X-ray term weight setting is Constant weight with a value of `0.5`.

   With this combination of weights for B-factor restraints and the X-ray term, the restraint for the B-factors will be moderately strong. This setting is appropriate for the middle of a moderate-resolution structure refinement, such as the one in this tutorial.

7. Start the job with an appropriate entry title.

2.14 Ligand Placement

At this point, the ligand can be placed. First, the ligand is imported and its structure corrected.

1. Click the Import button on the Project toolbar.

   

   The Import panel opens.

2. From the Files of type option menu, choose Common or PDB.
3. Select `358.pdb`.
4. If the import options are not visible, click Options.
5. Ensure that Replace Workspace is selected.
6. Click Open.

   If a warning is posted about conversion errors, ignore it, because the structure will be fixed in the next step. The file is imported and the entry is selected and displayed in the Workspace.

7. From the Tools menu, choose Assign Bond Orders.

   Bond orders are assigned, and are shown in the Workspace. Many PDB files do not have information on bond orders, so these must be assigned for input to calculations that use force fields.

Next, a difference map is created for the protein.

8. Display the output structure from the B-factor refinement in the Workspace (click its In column in the Project Table).
9. Click the Create Map button on the PrimeX toolbar.

   

   The Create Map panel opens.

10. For Coefficients, select Fo-Fc and 2Fo-Fc.

    You can leave the remaining options at their defaults.

11. Start the job (any name will do).

    When the job finishes, the Manage Surfaces panel is displayed with two maps: the 2Fo-Fc map generated earlier, and the Fo-Fc map.

12. In the Manage Surfaces panel, click the V column for the Fo-Fc map.

    The 2Fo-Fc map is undisplayed, and only the Fo-Fc map is shown.

Next, the display will be centered on residue 167.

13. Choose Edit → Find or type CTRL+F (⌘F) in the main window.

    The Find toolbar is displayed.

14. From the Find option menu, select Residue number.

15. Enter `A:167` in the Residue number text box.

16. Click N.

    The display is centered on residue 167 and zoomed in.

17. In the Manage Surfaces panel, check the box next to the Display at most text box and change the value to 30, then press ENTER.

18. Choose Ligand/Solvent Placement from the Task toolbar button on the PrimeX toolbar.

    

    The Ligand/Solvent Placement panel opens. A progress box may be displayed for the detection of density "blobs", which are used to place the ligand.

19. Under Use ligands from, select Selected entries.

20. In the Project Table, select the entry with the title 358.

    The structure of the protein should still be displayed in the Workspace, but the ligand is not displayed because it is not included, only selected.

21. Ensure that Refine poses is selected.

22. Select the first blob listed in the Density blobs table.

    The density may appear as a few large disconnected fragments of electron density, depending on the contour level used for the map. However, it is sufficient to select the largest central electron density volume.

23. Start the job with an appropriate entry title.

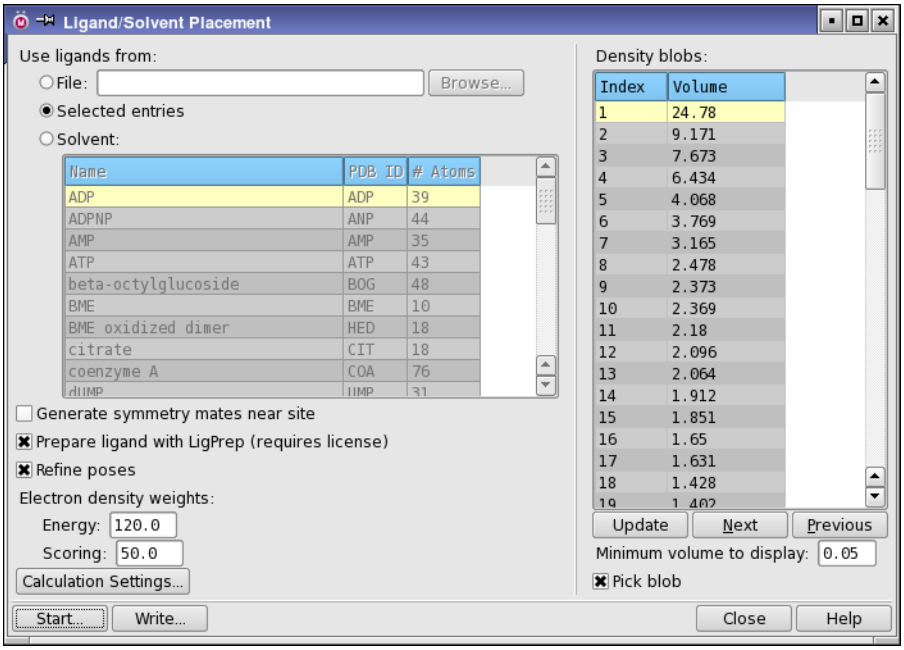

Figure 2.11. The Ligand/Solvent Placement panel.

This job can take several minutes to run. The job generates conformers of the ligand that are rotated and translated to best fit the electron density and the model. These "poses" are then ranked.

Ligand placement, using the Refine Poses option, returns four properties used for scoring the poses to the Project Table. The score named glide gscore is based on the Glide computation of the chemical complementarity of the ligand pose with the protein (the GlideScore), and should be negative. A more negative number indicates a better fit. The score named Ligand R is the real-space R-factor for the fit to the electron density. This is the primary score by which the poses are ranked. The Ligand Coverage score is the portion of atoms covered by electron density from an Fo-Fc map contoured at 3 sigma. The Ligand Score is a composite of Ligand R and Ligand Coverage.

The next steps demonstrate the examination of the ligand poses that were returned, and how well they fit the density. Normally, you would choose the best fit to the electron density, and you can base your decision on the scores in the Project Table. Thus, for this tutorial, selection of the first returned structure for the next task is satisfactory. If you wish to skip the examination of the density fit, include the first returned structure in the Workspace and continue to Step 32. However, you might decide on the basis of the examination that some other pose is better.

24. Select the first returned structure in the Project Table.

    This is the structure that has the map associated with it, which will be used for comparing the ligands in the next few steps.

25. From the Entry menu, choose Fix.

    The entry, with its map, is fixed in the Workspace and will not be undisplayed when you include other entries. Thus, you will be comparing the ligand poses for the first entry with all the others.

26. Include all of the returned structures in the Workspace (click the In column for the first, shift-click the last).

27. From the Undisplay button menu on the Display Atoms toolbar, choose Protein.

    

    The ligands should now be the only molecules displayed in the Workspace.

28. From the Color Scheme button menu on the Representation toolbar, choose Entry.

    

29. Right-click on a ligand atom, then zoom in so you can see it clearly.

30. Include the ligands in the Workspace one by one, and examine their fit to the density.

31. Select the fixed entry in the Project Table, and choose Entry → Unfix.

    The entry is no longer fixed.

32. Include the structure that you want to use for the next stage in the Workspace.

    If not all the atoms are displayed, choose All from the Display Only menu button on the Display Atoms toolbar.

    

33. Run a reciprocal-space coordinate minimization with an appropriate entry title, following the instructions in Section 2.9 on page 16.

34. Run a B-factor refinement with an appropriate entry title, following the instructions in Section 2.13 on page 24.

35. Repeat the coordinate minimization with an appropriate entry title.

2.15 Rebuilding a Loop

At this point, the loop that was deleted in an earlier step can be built back in. The loop in question is from residues 28 to 40. The region around residues 28–40 will have clearer electron density now, but correct building of this loop is still difficult.

1. Choose Real-Space Refinement from the Select a task button menu on the PrimeX toolbar.

   

   The Real-Space Refinement panel opens.

2. Choose Refine loops from the Method option menu.

   The loop in question does not appear in the Loops table, because this table is determined from the structure in the Workspace, and the residues of interest are missing. However, you can change the values in the Start and End text boxes for any of the existing loops to define the desired loop.

3. Click the label for loop1.

   The row is highlighted in yellow, and the residues are marked in the Workspace.

4. Change the Start and End values to `28` and `40`.

5. Under Build mode, select Automatic - resolution > 2.5Å.

   Although this structure has better resolution than 2.5Å, the poorly defined electron density for this loop is better fit using the lower resolution setting. Cases where this tactic will be productive are hard to predict. Switching to the alternate automatic method is the first thing to try when the initial setting does not work well.

6. Start the job with an appropriate entry title.

   This job takes a few minutes. Several structures are returned from loop fitting and are selected in the Project Table. These loops are ranked by a scoring function labeled Ref Score (refinement score). There is often not much difference between the loops that can be distinguished from the electron density, so choosing the first is usually fine.

7. When the job finishes, run a reciprocal-space coordinate minimization with an appropriate entry title, following the instructions in Section 2.9 on page 16 with a constant weight of 0.5.

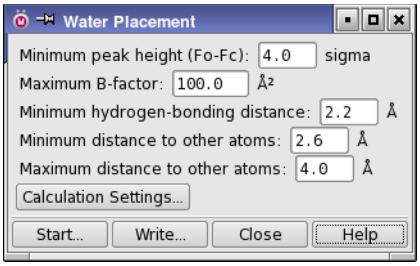

Figure 2.12. The Water Placement panel.

2.16 Adding Waters

Now that the protein and ligand have been fairly well refined, the next step is to add waters.

1. Choose Water Placement from the Select a task button menu on the PrimeX toolbar.

   

   The Water Placement panel opens. The defaults can be used for this job, so no changes in the settings are needed.

2. Start the job with an appropriate entry title.
3. Run a reciprocal-space coordinate minimization, following the instructions in Section 2.9 on page 16.
4. Run a B-factor refinement with a B-factor restraint weighting factor of 0.2, following the instructions in Section 2.13 on page 24.

2.17 Assessing the Results

The published structure that you have solved is 1OVE. To compare the structures you can import `1OVE.pdb` from the `tutorial` directory. Although you will find some differences, the bulk of the model refinement is finished at this point, and this is the end of the tutorial.

In the course of the tutorial, a number of shortcuts have been taken in the interests of presenting the entire workflow. In particular, the termini still have large B factors, and the loops that were refined previously are in need of further refinement. Below are some suggestions for examining the structure and locating regions for further refinement:

- Choose Atom PDB B factor from the Color Scheme toolbar button menu.

  

This scheme uses a blue-white-red color ramp for the temperature, with blue at the low end and red at the high end. The color scheme is also applied to the residues in the sequence viewer. You can identify the residues by pausing the pointer over a residue in the sequence viewer or over an atom in the Workspace.

- Use the Density Fit panel to display the density fits, and choose regions for loop building or manual adjustment based on this property.
- The RMSD bond angle is still high, even though the RMSD bond length has a reasonable value. This suggests that the structure may still have errors that require adjustment. Use the Protein Reports tool to find regions of the structure with large bond angle deviations from expected values.

Getting Help

Information about Schrödinger software is available in two main places:

- The `docs` folder (directory) of your software installation, which contains HTML and PDF documentation. Index pages are available in this folder.
- The Schrödinger web site, http://www.schrodinger.com/, particularly the Support Center, http://www.schrodinger.com/supportcenter, and the Knowledge Base, http://www.schrodinger.com/kb.

Finding Information in Maestro

Maestro provides access to nearly all the information available on Schrödinger software.

To get information:

- Pause the pointer over a GUI feature (button, menu item, menu, ...). In the main window, information is displayed in the Auto-Help text box, which is located at the foot of the main window, or in a tooltip. In other panels, information is displayed in a tooltip.

  If the tooltip does not appear within a second, check that Show tooltips is selected under General → Appearance in the Preferences panel, which you can open with CTRL+, (⌘,). Not all features have tooltips.
- Click the Help button in a panel or press F1 for information about a panel or the tab that is displayed in a panel. The help topic is displayed in your browser.
- Choose Help → Online Help or press CTRL+H (⌘H) to open the default help topic in your browser.
- When help is displayed in your browser, use the navigation links or search the help in the side bar.
- Choose Help → Manuals Index, to open a PDF file that has links to all the PDF documents. Click a link to open the document.
- Choose Help → Search Manuals to search the manuals. The search tab in Adobe Reader opens, and you can search across all the PDF documents. You must have Adobe Reader installed to use this feature.

For information on:

- Problems and solutions: choose Help → Knowledge Base or Help → Known Issues → *product*.
- Software updates: choose Maestro → Check for Updates.
- New software features: choose Help → New Features.
- Scripts available for download: choose Scripts → Update.
- Python scripting: choose Help → Python Module Overview.
- Utility programs: choose Help → About Utilities.
- Keyboard shortcuts: choose Help → Keyboard Shortcuts.
- Installation and licensing: see the *Installation Guide*.
- Running and managing jobs: see the *Job Control Guide*.
- Using Maestro: see the *Maestro User Manual*.
- Maestro commands: see the *Maestro Command Reference Manual*.

Contacting Technical Support

If you have questions that are not answered from any of the above sources, contact Schrödinger using the information below.

E-mail: help@schrodinger.com
USPS: Schrödinger, 101 SW Main Street, Suite 1300, Portland, OR 97204
Phone: (503) 299-1150
Fax: (503) 299-4532
WWW: http://www.schrodinger.com
FTP: ftp://ftp.schrodinger.com

Generally, e-mail correspondence is best because you can send machine output, if necessary. When sending e-mail messages, please include the following information:

- All relevant user input and machine output
- PrimeX purchaser (company, research institution, or individual)
- Primary PrimeX user
- Installation, licensing, and machine information as described below.

Gathering Information for Technical Support

This section describes how to gather the required machine, licensing, and installation information, and any other job-related or failure-related information, to send to technical support.

For general enquiries or problems:

1. Open the Diagnostics panel.
   - **Maestro:** Help → Diagnostics
   - **Windows:** Start → All Programs → Schrodinger-2012 → Diagnostics
   - **Mac:** Applications → Schrodinger2012 → Diagnostics
   - **Command line:** `$SCHRODINGER/diagnostics`
2. When the diagnostics have run, click Technical Support.

   A dialog box opens, with instructions. You can highlight and copy the name of the file.
3. Attach the file specified in the dialog box to your e-mail message.

If your job failed:

1. Open the Monitor panel in Maestro.

   Use Applications → Monitor Jobs or Tasks → Monitor Jobs.
2. Select the failed job in the table, and click Postmortem.

   The Postmortem panel opens.
3. If your data is not sensitive and you can send it, select Include structures and deselect Automatically obfuscate path names.
4. Click Create.

   An archive file is created in your working directory, and an information dialog box with the name of the file opens. You can highlight and copy the name of the file.
5. Attach the file specified in the dialog box to your e-mail message.
6. Copy and paste any log messages from the window used to start Maestro (or the job) into the email message,or attach them as a file.
   - **Windows:** Right-click in the window and choose Select All, then press ENTER to copy the text.
   - **Mac:** Start the Console application (Applications → Utilities), filter on the application that you used to start the job (Maestro, BioLuminate, Elements), copy the text.

If Maestro failed:

1. Open the Diagnostics panel.

   - **Windows:** Start → All Programs → Schrodinger-2012 → Diagnostics
   - **Mac:** Applications → Schrodinger2012 → Diagnostics
   - **Linux/command line:** `$SCHRODINGER/diagnostics`

2. When the diagnostics have run, click Technical Support.

   A dialog box opens, with instructions. You can highlight and copy the name of the file.

3. Attach the file specified in the dialog box to your e-mail message.

4. Attach the file `maestro_error.txt` to your e-mail message.

   This file should be in the following location:

   - **Windows:** `%LOCALAPPDATA%\Schrodinger\appcrash`
     (Choose Start → Run and paste this location into the Open text box.)
   - **Mac:** `Documents/Schrodinger`
   - **Linux:** Maestro's working directory specified in the dialog box (the location is given in the terminal window).

5. On Windows, also attach the file `maestro.EXE.dmp`, which is in the same location as `maestro_error.txt`.

120 West 45th Street
17th Floor
New York, NY 10036

101 SW Main Street
Suite 1300
Portland, OR 97204

245 First Street
Riverview II, 18th Floor
Cambridge, MA 02142

8910 University Center Lane
Suite 270
San Diego, CA 92122

155 Gibbs St
Suite 430
Rockville, MD 20850-0353

Dynamostraße 13
D-68165 Mannheim
Germany

Zeppelinstraße 73
D-81669 München
Germany

Potsdamer Platz 11
D-10785 Berlin
Germany

Quatro House
Frimley Road
Camberley GU16 7ER
United Kingdom

8F Pacific Century Place
1-11-1 Marunouchi
Chiyoda-ku, Tokyo 100-6208
Japan

No. 102, 4th Block
3rd Main Road, 3rd Stage
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Bangalore 560079, India

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