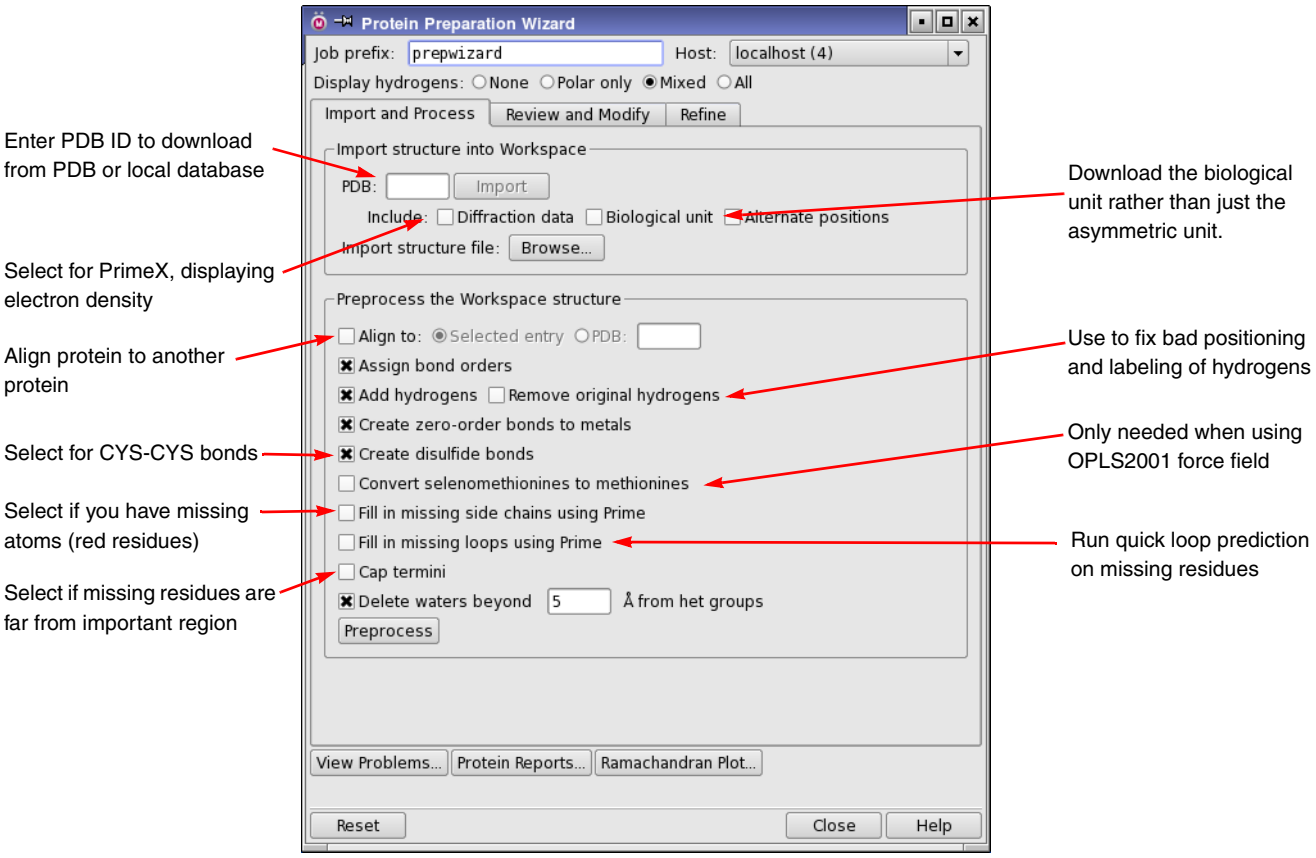
Protein Preparation

This sheet summarizes basic protein preparation with the Protein Preparation Wizard. More information on protein preparation is given in the *Protein Preparation Guide*. You can open the Protein Preparation Wizard from the Workflows menu or Tasks menu or with the toolbar button.



Preprocess:

1. Enter the PDB ID of your proten in the PDB text box and click Import.
2. Examine the structure in the Workspace for the following structural issues (colored atoms).
   - Red residues have missing atoms. Select Fill in missing side chains using Prime.
   - Light blue residues have adjacent missing residues (missing loops). Do one of the following:
     - Select Cap termini if the missing residues are not important for the modeling task, especially for long loops.
     - Select Fill in missing loops using Prime. Follow up with a full Prime loop prediction if the loop is important. Good for short loops (up to 5 residues).
     - Select neither, but do a full Prime loop prediction (or use PrimeX with the diffraction data, if available).
   - Green residues have alternate positions. If you want to use the alternate, select the residues, right-click and choose Switch Alternate Positions. If you want both, duplicate the protein and prepare both alternates.
   - Dark blue residues have mistyped atoms. Fix these in the Atom Properties tab of the Build panel before proceeding.
3. If you want to align the protein to another protein, select Align to, and specify the other protein.

4. If the structure has hydrogens, select Remove original hydrogens to fix problems like bad placement or bad labels.
5. Select Create disulfide bonds (unless you don't want disulfide bonds).
6. If you want to keep selenium atoms, deselect Convert selenomethionines to methionines. Conversion is only necessary if you want to use the OPLS_2001 force field for modeling.
7. Set a range for keeping structural waters, or deselect Delete waters beyond to keep all waters.
8. Click Preprocess.

Fix structural problems:

After preprocessing, the Protein Preparation - Problems dialog box opens if there are unfixed problems.

- If there are mistyped atoms, fix them in the Build panel (Edit > Build > Atom Properties)
- Overlapping hydrogens are mostly fixed by H-bond optimization and terminal flips, or by minimization. Check again at the end of the preparation.
- Fix missing atoms by capping, side-chain prediction or loop prediction, if you didn't do this the first time around. You can do this by running the Preprocess step again with different options selected.

Review and modify:

In the Review and Modify tab, examine the structure and delete parts you don't want to use for modeling.

1. Delete unwanted chains, waters, and het groups, by selecting them in the tables and clicking Delete.
2. Click Generate States to run Epik for ionization and tautomeric states of the het groups (ligand). Include metal-binding states if the het group is bonded to a metal.
3. Examine the states and select the state that you think is most reasonable. This step is important for optimizing the H-bond network.

Optimize the H-bond network:

Optimize orientation of polar hydrogens, flip terminal amides and histidines, adjust protein protonation states.

1. Select Exhaustive sampling for a more thorough but longer optimization.
2. Select a pH range option for protonation or deprotonation of residues.
3. Click Optimize.

Run restrained minimization

Minimize the structure with harmonic restraints on the heavy atoms, to remove strain.

1. Select Hydrogens only if you don't want to allow the heavy atoms to move at all.
2. Click Minimize to make settings and start the minimization job.

Check the results:

After the entire process is done, you should carefully check your results:

- Open the Problems panel (View Problems) to check for any remaining errors.
- Use the Protein Reports panel to check for other possible problems.
- Use the Ramachandran Plot panel to locate residues with unusual dihedrals.