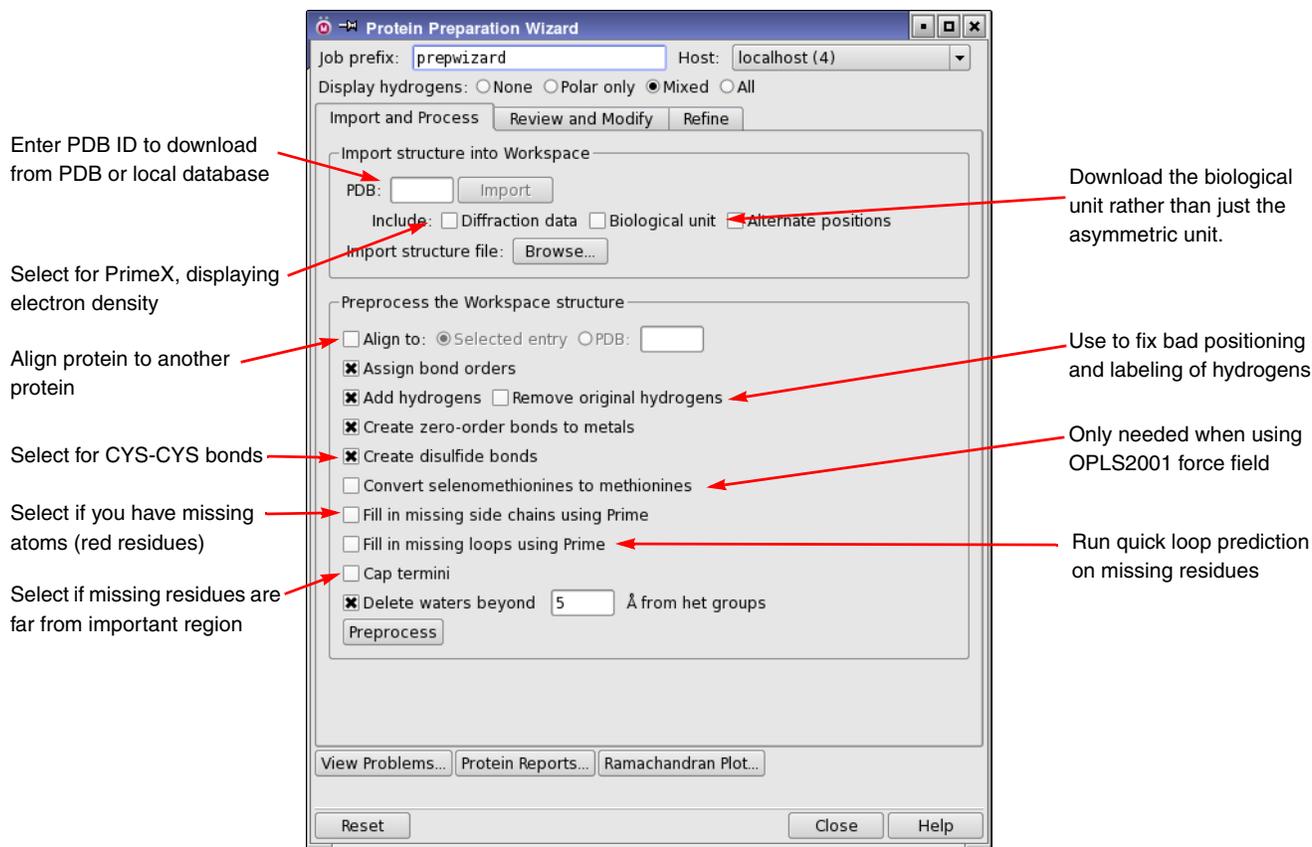


# Protein Preparation

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 protein 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.