BioPharmics™

Version 1.0.5

Surflex™-Dock Plugin for PyMOL User Guide

optibrium™

Contents

1. Introduction

This guide introduces the graphical interface for the Surflex-Dock plugin for PyMOL. For help with other BioPharmics™ features and operations, please refer to the Surflex Manual. For help with PyMOL features and operations, please refer to the PyMOL documentation.
If you have any questions, please feel free to contact support@optibrium.com.

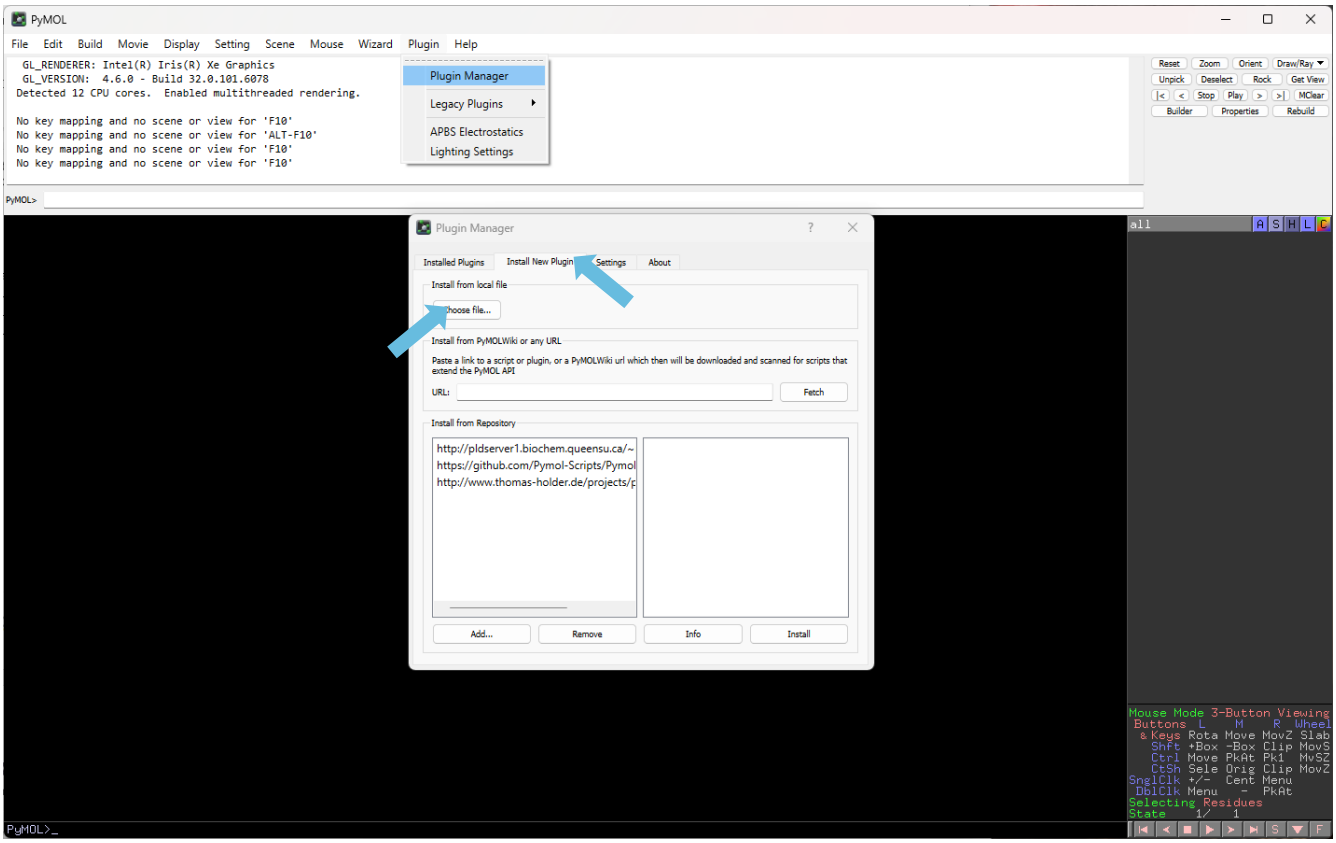
2. How do I... install and run Surflex-Dock in PyMOL

2.1 Pre-requisites

To install the Surflex-Dock plugin for PyMOL, you will need the following:
A working copy of PyMOL version 3.1 or newer, bundled with Python version 3.10.
A license for Surflex-Dock.
The Surflex executables from the Optibrium website from the BioPharmics menu on the left (v5.211 or newer).
The tar.gz file for the Surflex-Dock plugin for PyMOL from the Optibrium website (version 1.0.5 or newer).

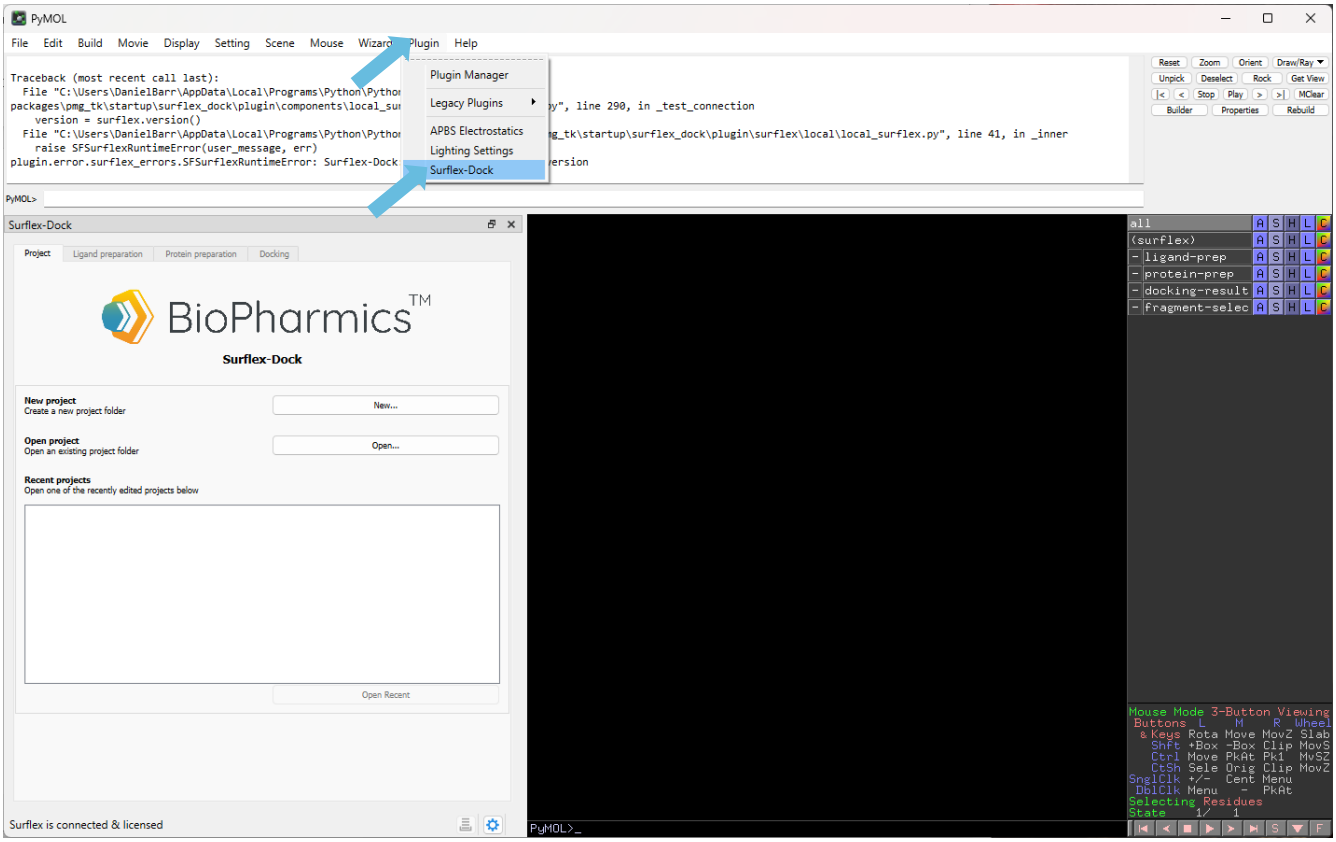
2.2 Installation

To install the Surflex-Dock plugin in PyMOL:
Select **Plugin Manager** under the **Plugin** menu in PyMOL to launch the Plugin Manager.
Select the **Install New Plugin** tab at the top of the **Plugin Manager**.
Select **Choose File**.

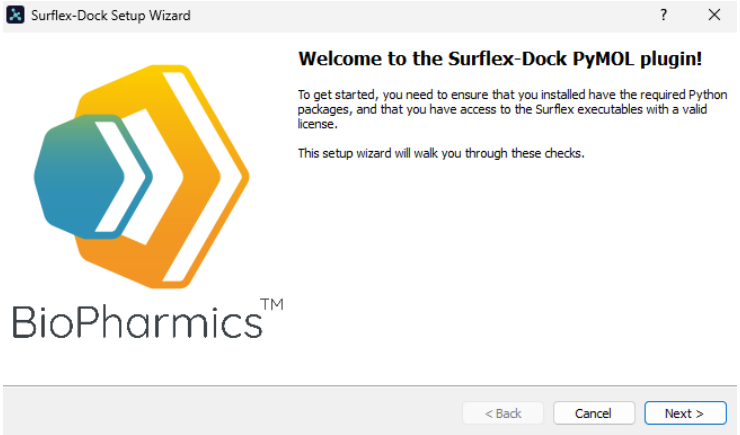


Browse to the location on your computer where you saved the tar.gz file for the Surflex-Dock plugin (you do not need to unzip the archive) and click **Open**.
A pop-up window will ask you to confirm the directory where the plugin will be installed. Keep the default and click **OK**.
A pop-up window will confirm that the plugin has been installed. Click **OK** and close the **Plugin Manager**.

To open the Surflex-Dock plugin:
Select **Surflex-Dock** under the **Plugin** menu in PyMOL.

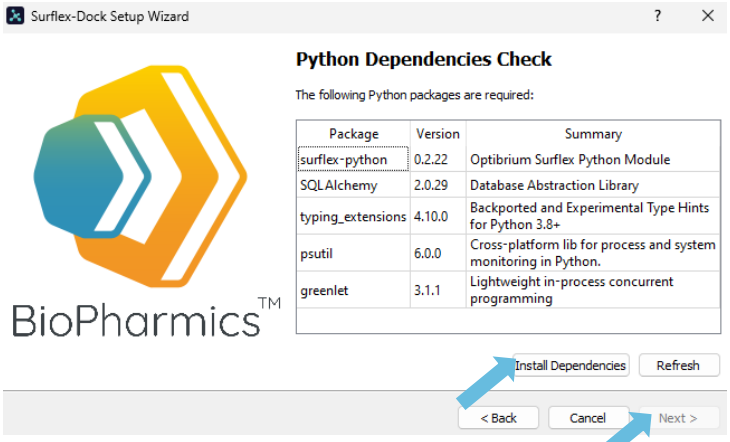


The first time the Surflex-Dock plugin is opened, a wizard will help you configure the plugin to use the Surflex executables and license. You will need to know the location of the Surflex executable files (sf-tools.exe and sf-dock.exe) and the location of your Surflex license (surflex_bin.lic). See Section 2.1 for information about how to obtain the executable and license files.



To configure the Surflex-Dock plugin:
Click **Next** on the Setup Wizard.

The setup wizard first looks for the Python packages required for the plugin and will install them if necessary. Please note that you will need to be connected to the internet during this process. The Python dependencies for the plugin will be shown in the setup wizard and are listed in the file **requirements.txt** inside the tar.gz archive. (**Note:** there is no need to extract the archive before installation; everything can be handled through the wizard.)



Click **Install Dependencies** to have the wizard download and install the required packages.

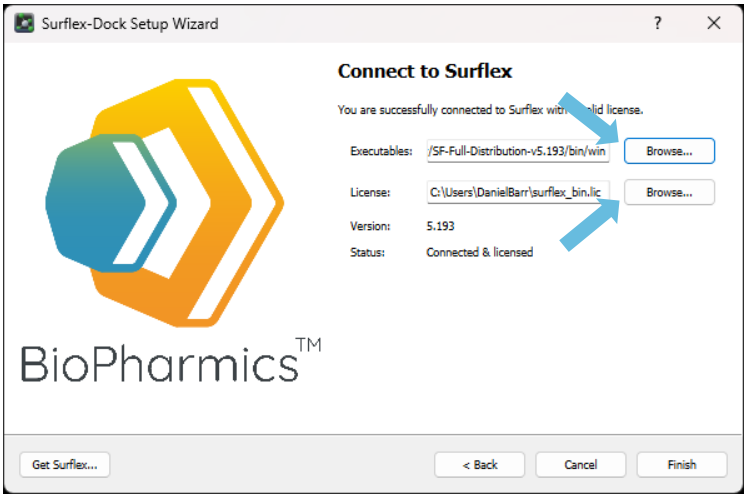
After installing the dependencies, the wizard will prompt you to restart PyMOL so that the changes can take effect. Close and re-open PyMOL to continue.

Select **Surflex-Dock** under the **Plugin** menu in PyMOL again.

Click **Next** on the Setup Wizard. You will see a message that all required Python packages are installed.

Click **Next** to proceed to the Surflex configuration.

The wizard will prompt you to browse to the path containing the Surflex executable files and your Surflex license (see Section 2.1 for information about how to obtain these files).



Click **Browse** next to the **Executables** line of the wizard and navigate to the folder on your computer that contains the Surflex executables (sf-tools.exe and sf-dock.exe) and click **Open**.

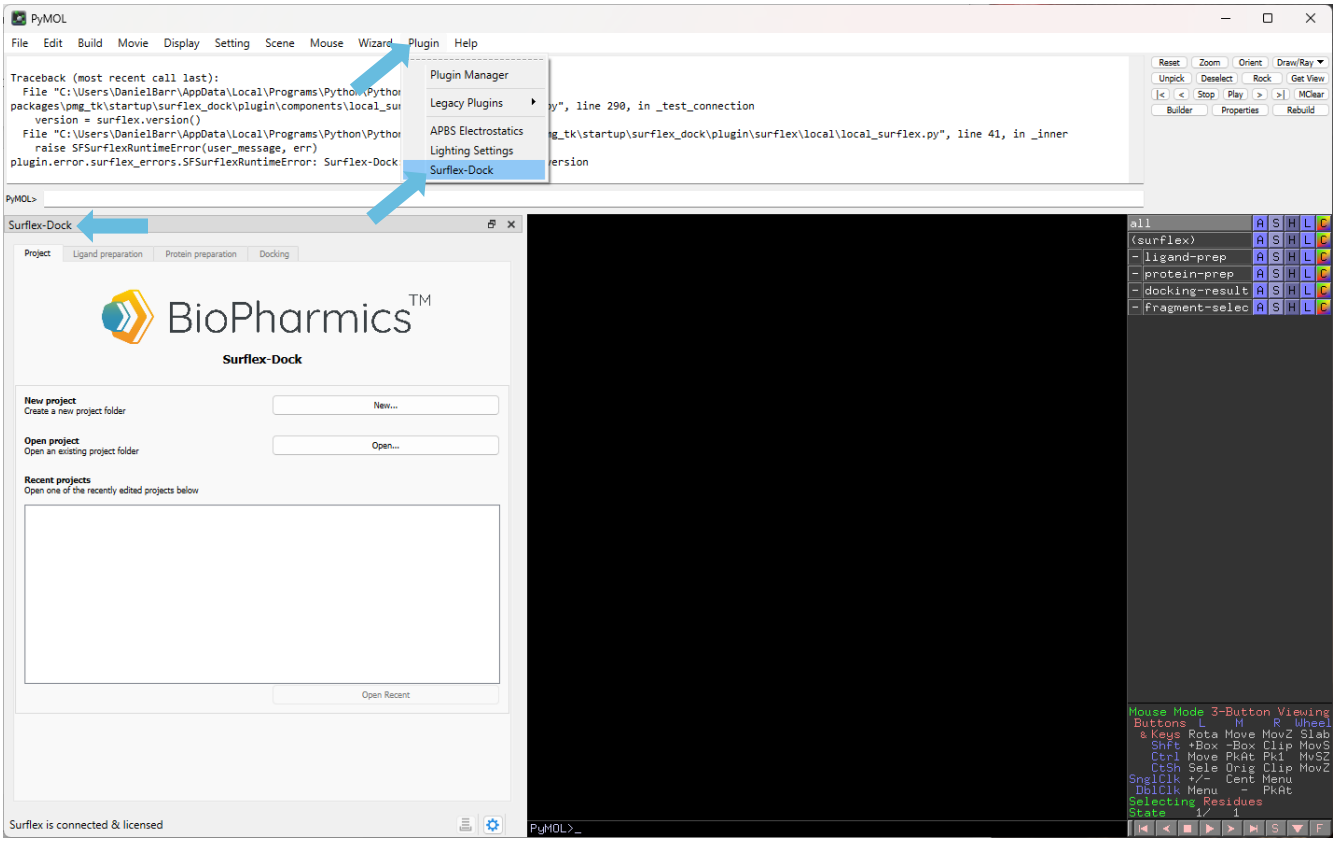
Hint: by default, the Surflex executables are found inside the folder SF-Full-Distribution-vX.XXX inside the bin folder; select the folder in the bin directory that is appropriate for your operating system).

Click **Browse** next to the **License** line of the wizard and navigate to select the Surflex license file (surflex_bin.lic) on your computer and click **Open**.

Click **Finish** to close the Setup Wizard.

2.3 Running the Surflex-Dock plugin for PyMOL

To open the Surflex-Dock plugin in PyMOL:
Select **Surflex-Dock** under the **Plugin** menu in PyMOL.



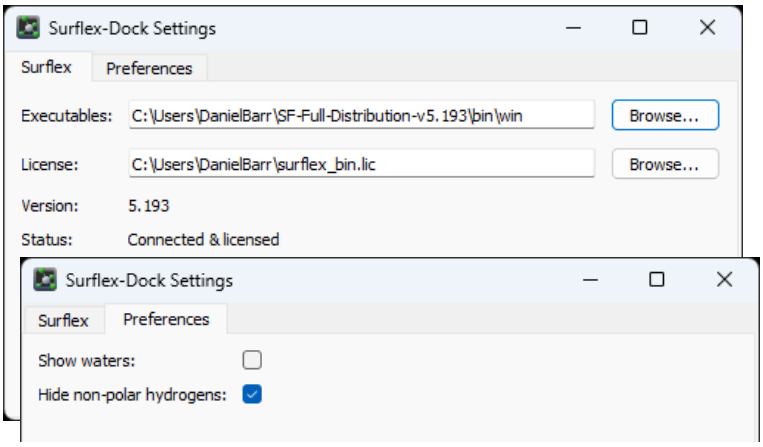
To dock the Surflex-Dock plugin window inside the PyMOL viewer on Windows:
Double-click on the title bar of the Surflex-Dock plugin to dock the plugin.
Click the windows icon  at the top right of the Surflex-Dock plugin to return the plugin to its own window.

To dock the Surflex-Dock plugin window inside the PyMOL viewer on Mac:
Click and drag on the title of the Surflex-Dock plugin and drag to the left or right side of the PyMOL display.
Click and drag on the title of the Surflex-Dock plugin and drag it to the centre of the screen.

On Linux, the plugin will always be docked to the main display and cannot be detached to a separate window.

2.4 Surflex-Dock plugin settings

The settings for Surflex-Dock can be changed using the  button found at the bottom-right of the plugin window. In the **Surflex** tab, you can point to new locations for the Surflex executables and/or license file, as needed. In the **Preferences** tab, you can set default visualisation behaviour for showing waters associated with the protein and non-polar hydrogens on ligands.



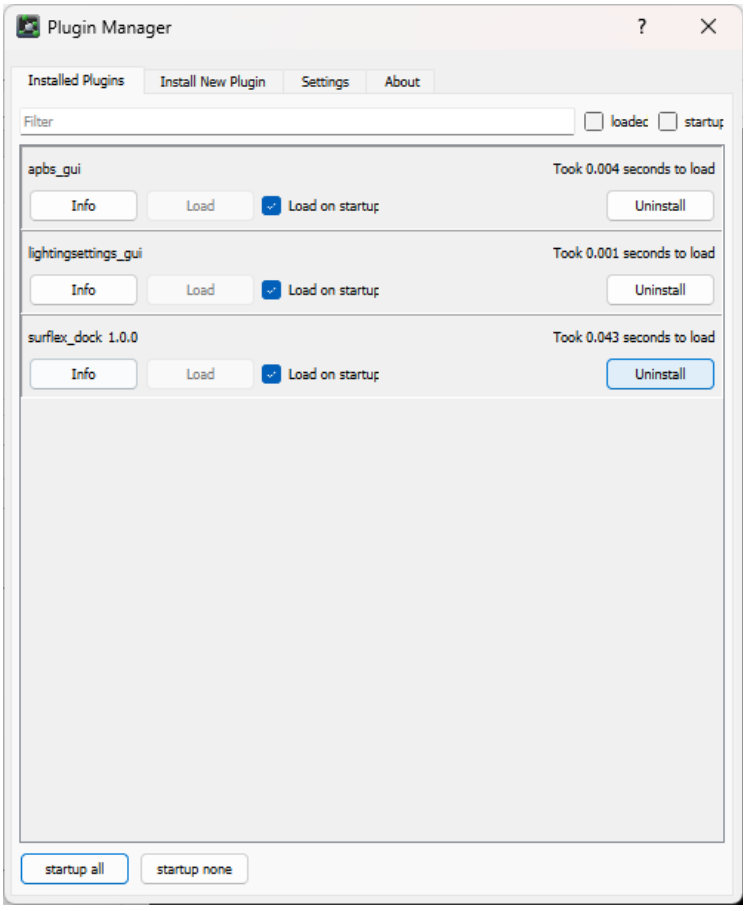
2.5 Uninstalling the Surflex-Dock plugin for PyMOL

To uninstall the Surflex-Dock plugin:

Open the **Plugin Manager** under the **Plugin** menu in PyMOL.

Within the **Installed Plugins** tab of the **Plugin Manager**, click **Uninstall** next to the Surflex-Dock plugin.

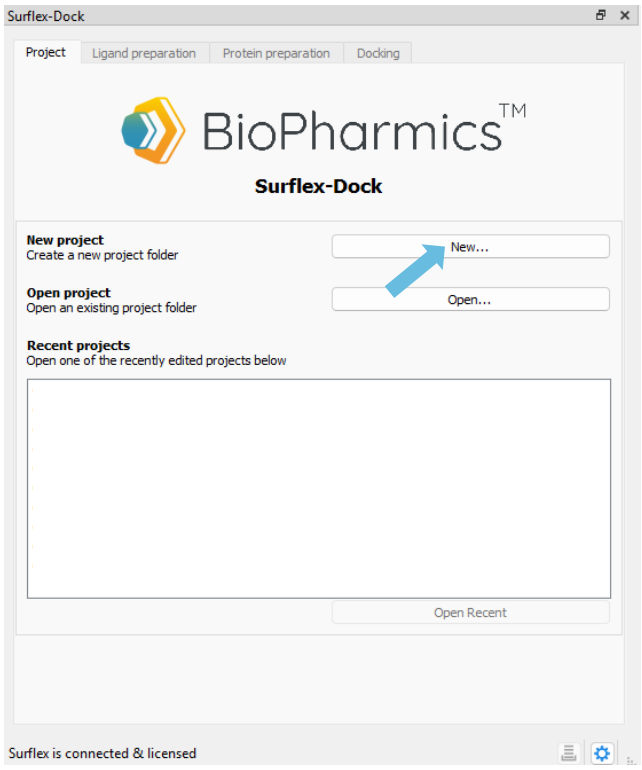
To perform a complete removal of all plugin files and enable a fresh installation, you will also need to delete the configuration file **.pymolpluginsrc.py** in the user's home directory

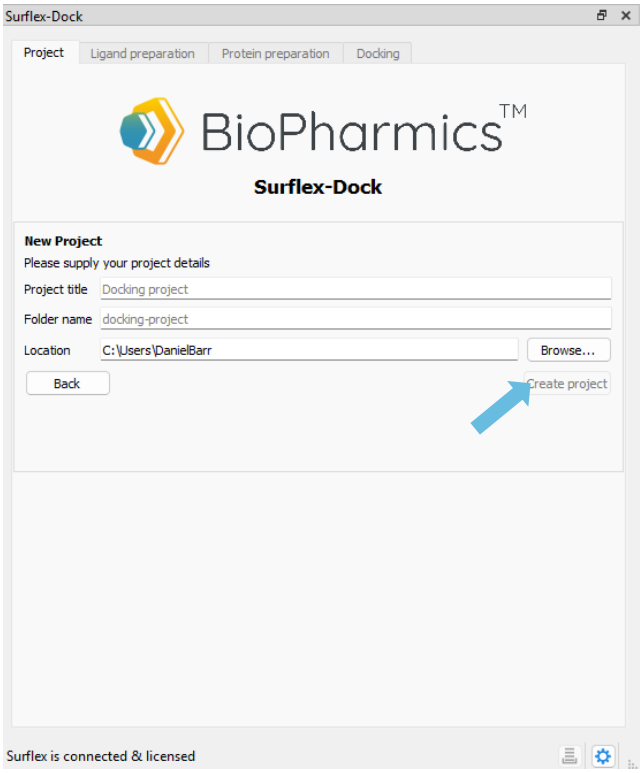


3. How do I... create or open a project

3.1 Creating a docking project

Open the Surflex-Dock plugin (see Section 2.3).
Click **New** in the Surflex-Dock window.
Type a name for your project in the **Project title** box.
In the **Folder name** box, type the name to use for the new directory where project files will be saved; this will create a new directory with that name that will hold all of the project files.
Browse to select the folder where you would like the project directory to be saved in your filesystem and click **Open**.
Click **Create project**.





3.2 Saving a docking project

Surflex-Dock automatically saves your work in the directory specified when you created the project (see Section 3.1). There is no manual control for saving a docking project; all files are saved as you work and will be restored automatically when opening the project. Any custom visualisations made in PyMOL will not be saved as part of the Surflex-Dock project. You can save the PyMOL session separately (see Section 8.3).

3.3 Opening a docking project

To open a Surflex-Dock project:

Open the Surflex-Dock plugin (see Section 2.3).

If you have recently worked on a project, the project directory will appear in the **Recent projects** panel of the Surflex-Dock window.

Click on the recent project you would like to open.

Click **Open Recent** at the bottom of the Surflex-Dock window.

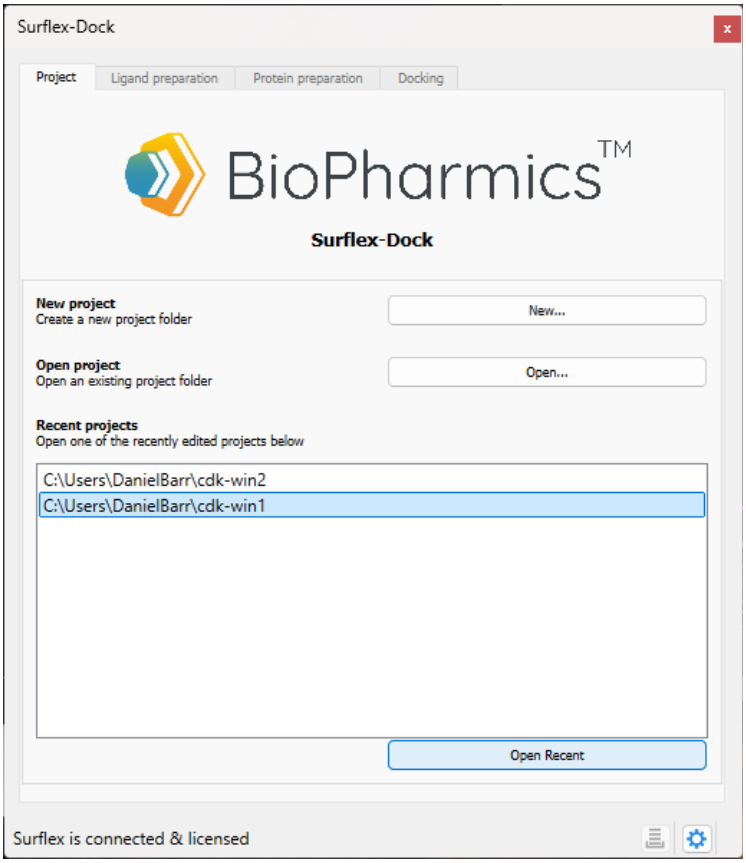
To open a project that does not appear in the **Recent projects** panel of the Surflex-Dock window:

Click **Open** in the Surflex-Dock window.

Browse to the project folder that you would like to open.

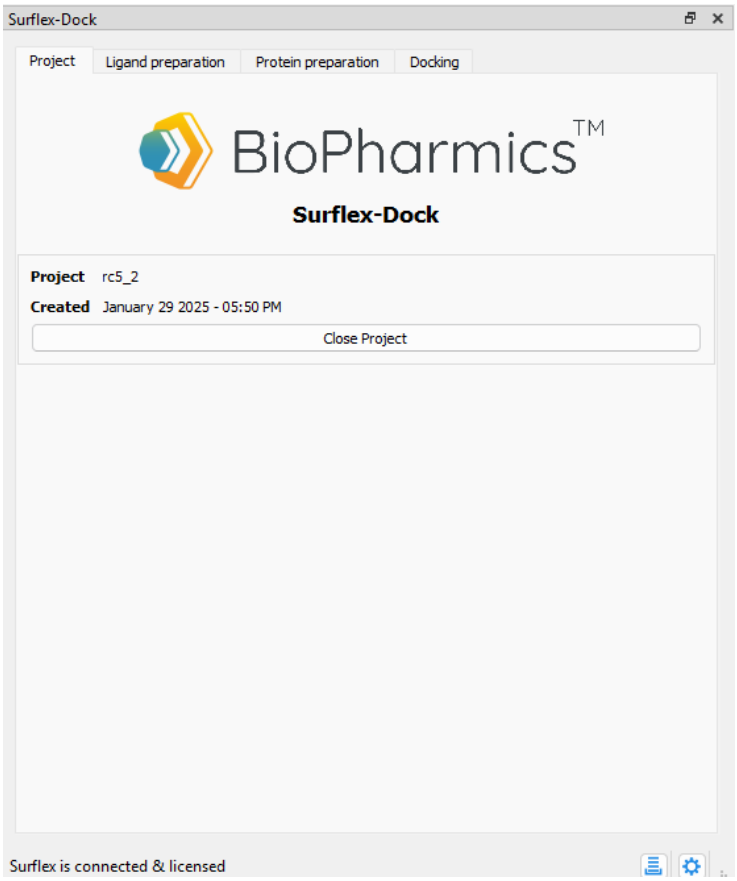
Click on the project folder in your file system to select the project.

Click **Open**.



3.4 Closing a project

When finished with a project, you can close the project and open another project.
Click on the **Project** tab of the plugin.
Click **Close Project**.



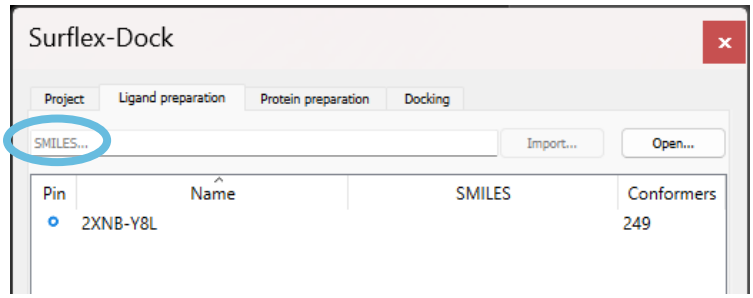
4. How do I... import and export data

4.1 Importing ligands

To prepare a ligand for docking, switch to the **Ligand preparation** tab in the plugin.

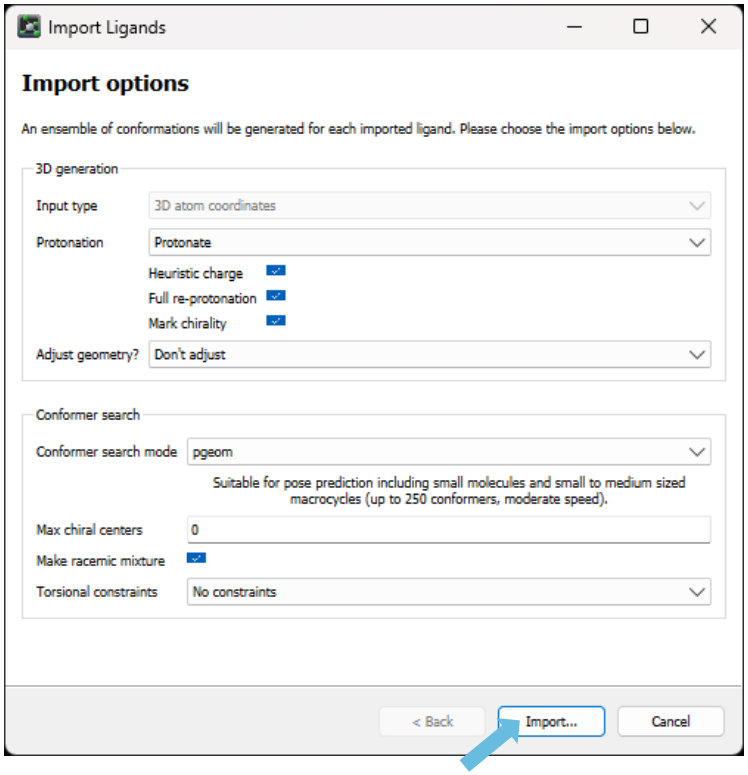
Click on the **Ligand preparation** tab in the plugin.

Click **Open** to import the ligand(s) in MOL2 or SDF format or as a list of SMILES strings.



Note: If using a text file in SMILES format for input, put one SMILES string per line, with the compound name or identifier separated by a space after the SMILES string.

To add a ligand manually, paste the SMILES string for the ligand into the **SMILES...** box in the plugin and click **Import**. You can paste multiple SMILES strings into the import box, separated by commas, and they will automatically be named lig, lig_2, lig_3, etc.



Regardless of the input format, 3D coordinates will be generated for each ligand. You can control the behaviour of protonation, charge manipulation, enumeration of chiral centres, and geometry optimisation. The behaviour of these functions is described in Section 2.3 of the Surflex Manual.

For each ligand, a conformation search is performed using parameter selection schemes, available from the pull-down menu in the **Conformer search** area of the **Import Ligands** wizard. The behaviour of these parameter schemes, with additional options for handling chiral centres and torsional constraints (e.g., from an NMR experiment), is described in Section 2.4 of the Surflex Manual.

When you have finished selecting your import options, click **Import**.

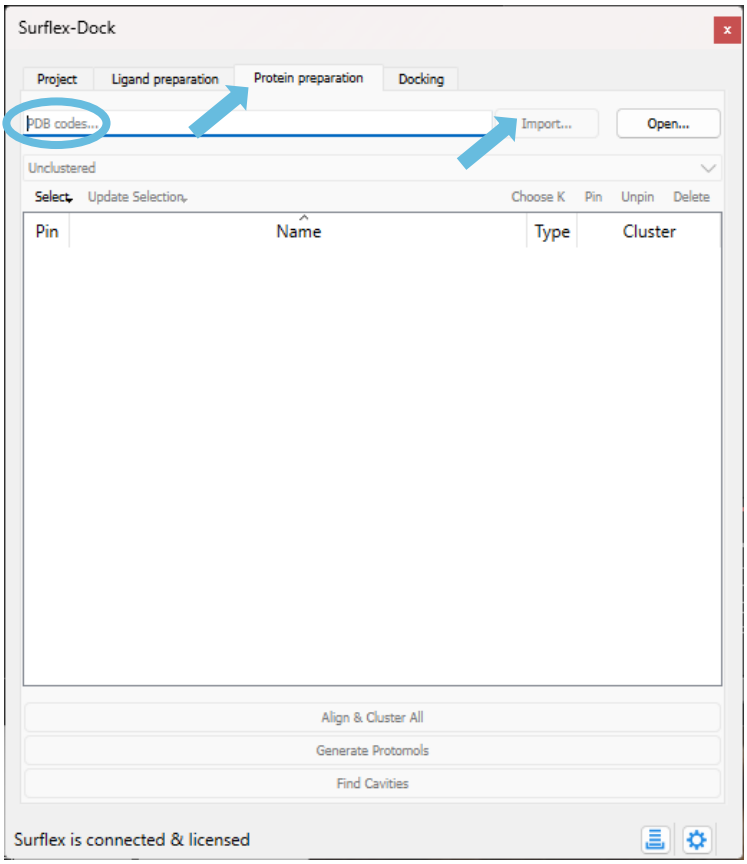
4.2 Importing protein structures

To import and prepare protein structures for docking, switch to the **Protein preparation** tab in the plugin.

Click on the **Protein preparation** tab in the plugin.

Type or paste a comma-separated list of PDB codes and click **Import** to download and import the protein(s) directly from the Protein Data Bank.

To add a protein from a local file, click **Open** and browse to select the file(s) to import.



Ligand verification is an important preparation step performed by Surflex-Dock during protein import. In published structures, it sometimes happens that the coordinates identified for the ligand in the X-ray structure do not match the SMILES for the ligand (some atoms may be missing or unable to be assigned in the X-ray density maps). During protein preparation, Surflex-Dock checks each ligand structure from the PDB against its SMILES and will display "quality ligand" in cases where the structure and SMILES match and "ligand" otherwise. It is recommended to use only qualified ligands to guide docking experiments.

4.3 Exporting docking results

Surflex-Dock automatically saves all your work in the directory specified when you created the project (see Section 3.1). There is no manual control for saving or exporting results from a docking project.

Docking results are saved in the project working directory (see Section 3.1) in MOL2 format with files named:

`<ligand>_docking_results<i>.mol2`

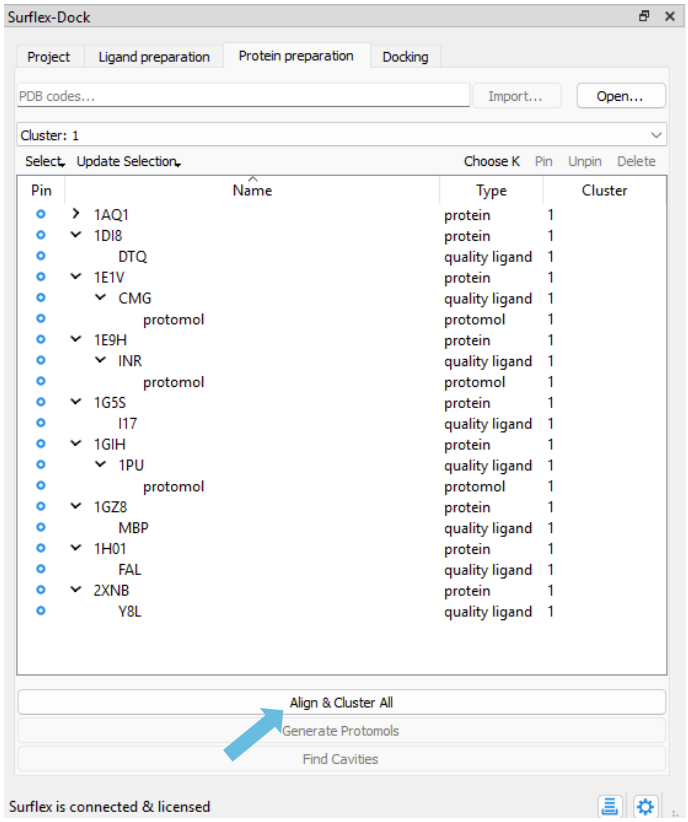
These files can be opened in StarDrop to give one pose per line, along with the pose number, pose family and probability, and the docking score (in units of pKd).

Docking results can be exported to a text or spreadsheet editor (e.g. MS Excel) using Ctrl+C and Ctrl+V.

5. How do I... prepare proteins for docking

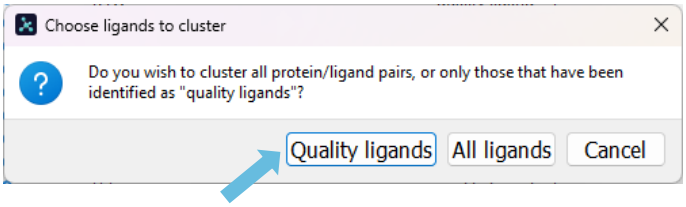
Protein import (see Section 4.2) will load the 3D conformation of the protein and associated ligand, if available. Surflex-Dock will attempt to verify that the ligand structure matches the SMILES string provided with the structure. Additionally, the protein and ligands will be prepared for subsequent docking, including the identification of appropriate protonation states.

5.1 Aligning and clustering protein structures



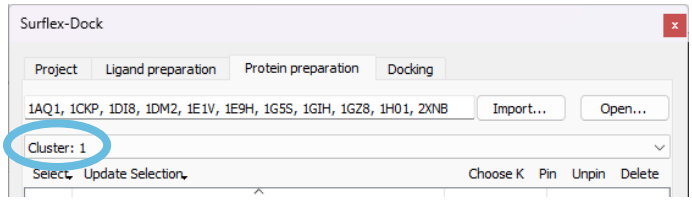
For proteins with associated ligands, it is necessary to align and cluster the proteins to prepare for docking.
Click on the **Protein preparation** tab.
Click **Align & Cluster All**.
Note: Only proteins with associated ligands can be clustered.

The protein conformations are aligned using the electrostatic and steric representations of the binding pocket surface, defined by the volume around the bound ligand. This differs from conventional alignments that use the protein backbone; this binding site alignment provides a better template to guide docking experiments and 3D design.
Note: Proteins within an identified cluster will be aligned to one another. Different clusters will not be aligned to one another and should be visualised separately.



It is recommended whenever possible to use only **quality ligands** for clustering, as other ligands can have significant errors in them that can skew docking results (see Section 4.2).

After the clustering job is finished, the cluster with the most proteins will be displayed automatically.
Click **Cluster 1** to open a drop-down menu and view other clusters.

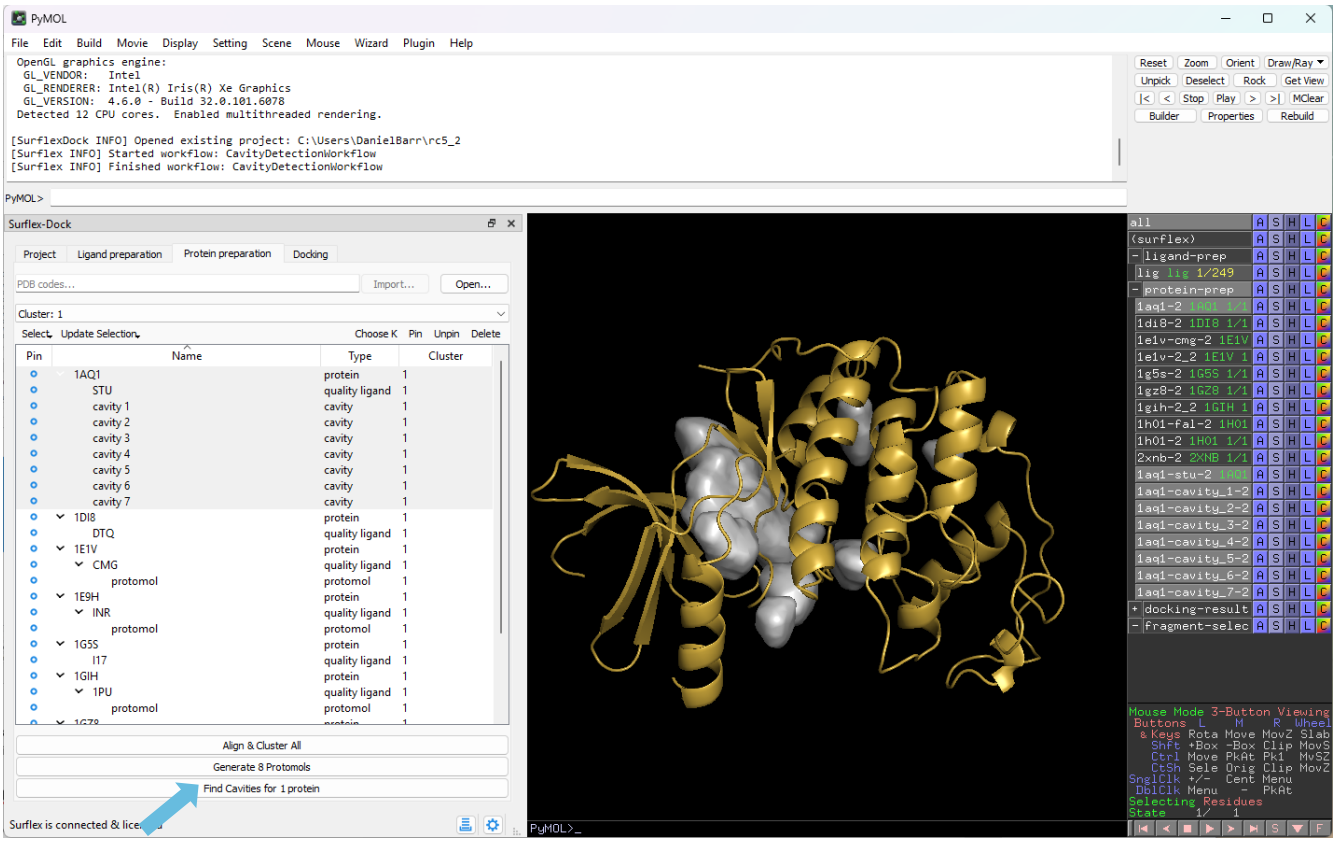


5.2 Detecting cavities in protein structures

For proteins without associated ligands, docking can be performed into cavities in the protein surface.
Click on the **Protein preparation** tab.
Select the proteins for cavity detection.

- Use Ctrl+click and/or Shift+click to select specific proteins.
- Click **Select** above the protein list and choose **Proteins** to easily select all proteins.

Click **Find Cavities**.



Note: Proteins without ligands will not be aligned during cavity detection. To visualise the cavities for multiple homologous proteins, it is recommended to align the protein structures before performing the cavity detection.

5.3 Selecting ligands and generating protomols for docking

Automatically selecting ligands for ensemble docking

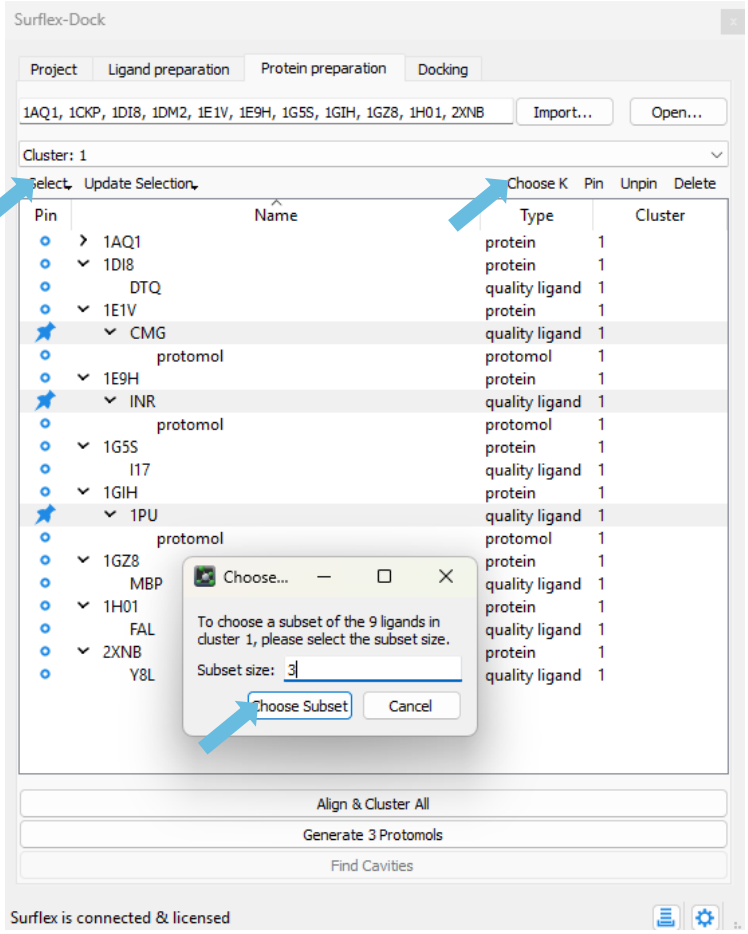
It is often desirable to dock into multiple structures in a single experiment, i.e. ensemble docking to account for the dynamic flexibility of the binding pocket and avoid being led astray by the peculiarities of a single pose. Because the time required for docking increases with the number of protein conformations used, it is often convenient to choose a representative subset of structures to use for ensemble docking. While it is tempting to choose the most diverse structures, this can allow outlier variants to dominate the docking. Instead, it is recommended to choose the central exemplars of the most distinct clusters identified during alignment (see Section 5.1). In Surflex-Dock, this is implemented as the choose_k function (see Section 3.3 of the Surflex Manual).

Surflex-Dock can choose an optimal subset of ligands that have been aligned and clustered to use for docking.

Click **Choose K** in the Protein Preparation window

Enter the size of the desired subset.

Click **Choose Subset**.



Manually selecting ligands for ensemble docking

You can manually select the ligands or cavities which you want to use for docking.

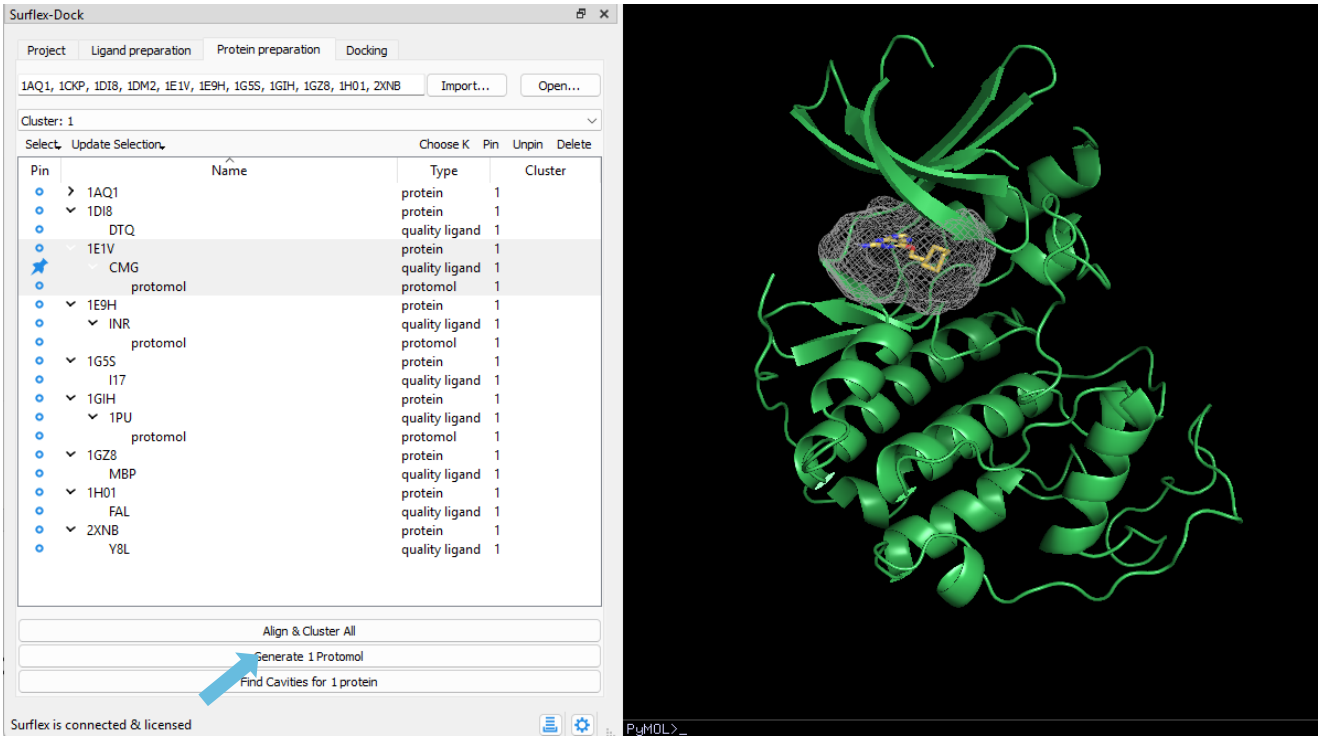
Use Ctrl+click and/or Shift+click to select specific ligands or cavities.

Click **Select** above the protein list to easily select all ligands or cavities.

Generating protomols

The 3D shape and properties of the binding site are defined as a protomol (see Section 3.4 of the Surflex Manual). The docking algorithm will dock ligands into these protomols. To generate a protomol for a ligand binding site:
Select the ligand(s) or cavities for which you want to generate the protomol(s).
Click **Generate Protomols**.

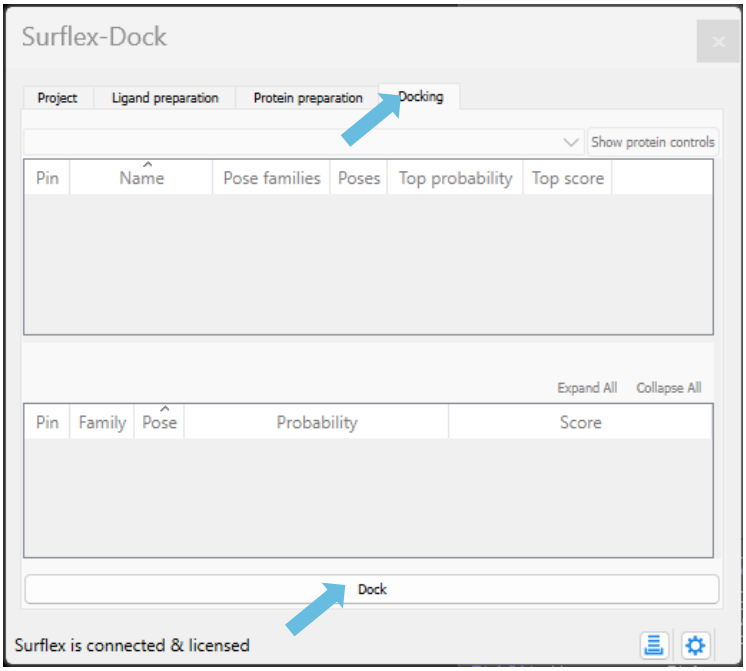
Note: docking results may be different for protomols generated separately (e.g. selecting one ligand at a time to generate the protomol) or together (e.g. selecting multiple ligands at once to generate protomols) because the union of the surfaces of all selected ligands is used for protomol generation.



6. How do I... perform a docking experiment

6.1 Docking ligands into protomols

To perform a docking experiment:



Change to the **Docking** tab in the plugin.
Click **Dock** at the bottom of the plugin window to open the Docking wizard.
Select the protomols (grouped by cluster, see Section 5.3) to dock against and click **Next**.
Select the known ligands to use as docking templates, and for pose family identification. Use Ctrl+click or Shift+click to select multiple ligands.
Click **Next**.

Note: It is recommended to use only qualified ligands for docking and pose identification (see Section 4.2).

Select which ligands to dock. Use Ctrl+click or Shift+click to select multiple ligands.
Click **Next**.
Choose the desired docking mode (see Section 3.7 of the Surflex Manual for a description of the available docking modes and their intended uses).
Set any desired constraints (see Section 7).
Click **Dock**.

6.2 Viewing docking results

Multiple docking runs in the same project can be accessed using the pull-down menu at the top-left of the **Docking** tab, organised by the name of the protein-ligand pair or protein cavity used to generate the protomol. The top panel of the **Docking** tab displays the docked ligands with a summary of the docking results, including the name of the ligand, the number of distinct pose families identified, the number of unique poses, the probability of the most likely pose family being correct and the top docking score (in units of pKd). The bottom panel of the **Docking** tab shows the pose families and unique poses for each docked ligand, including the family number, pose number, probability of that docking family being correct, and the docking score for each pose (in units of pKd).

To view the docking results:

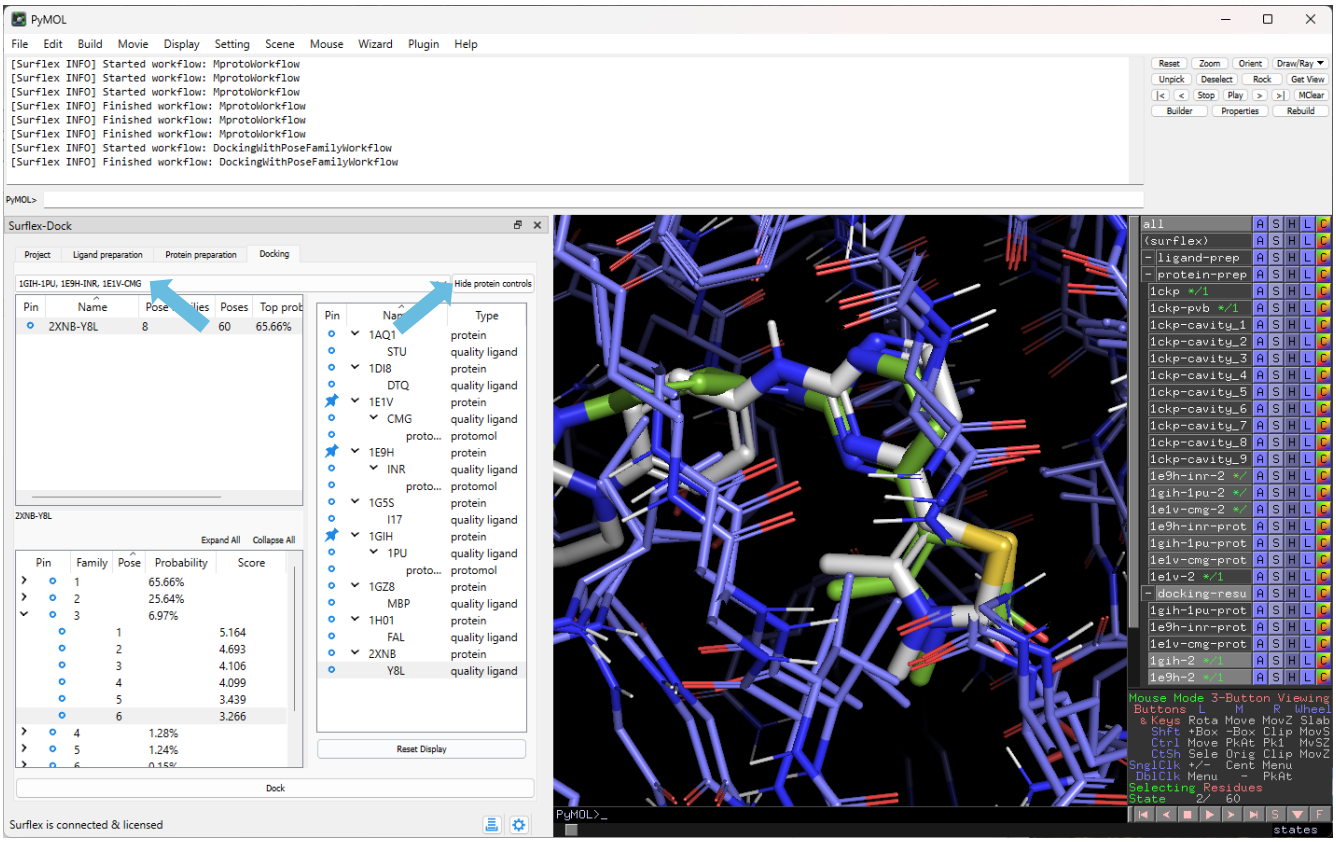
Select the docking experiment for which you wish to view the results from the drop-down menu at the top left of the plugin.

Select the docked ligand from the list in the top panel of the **Docking** tab to view the docked poses and pose families for that ligand.

Select the pose family or individual pose in the bottom panel of the docking tab to explore the docked poses.

Toggle the **Pin** button to the left of each pose to keep poses of interest displayed in the viewing window.

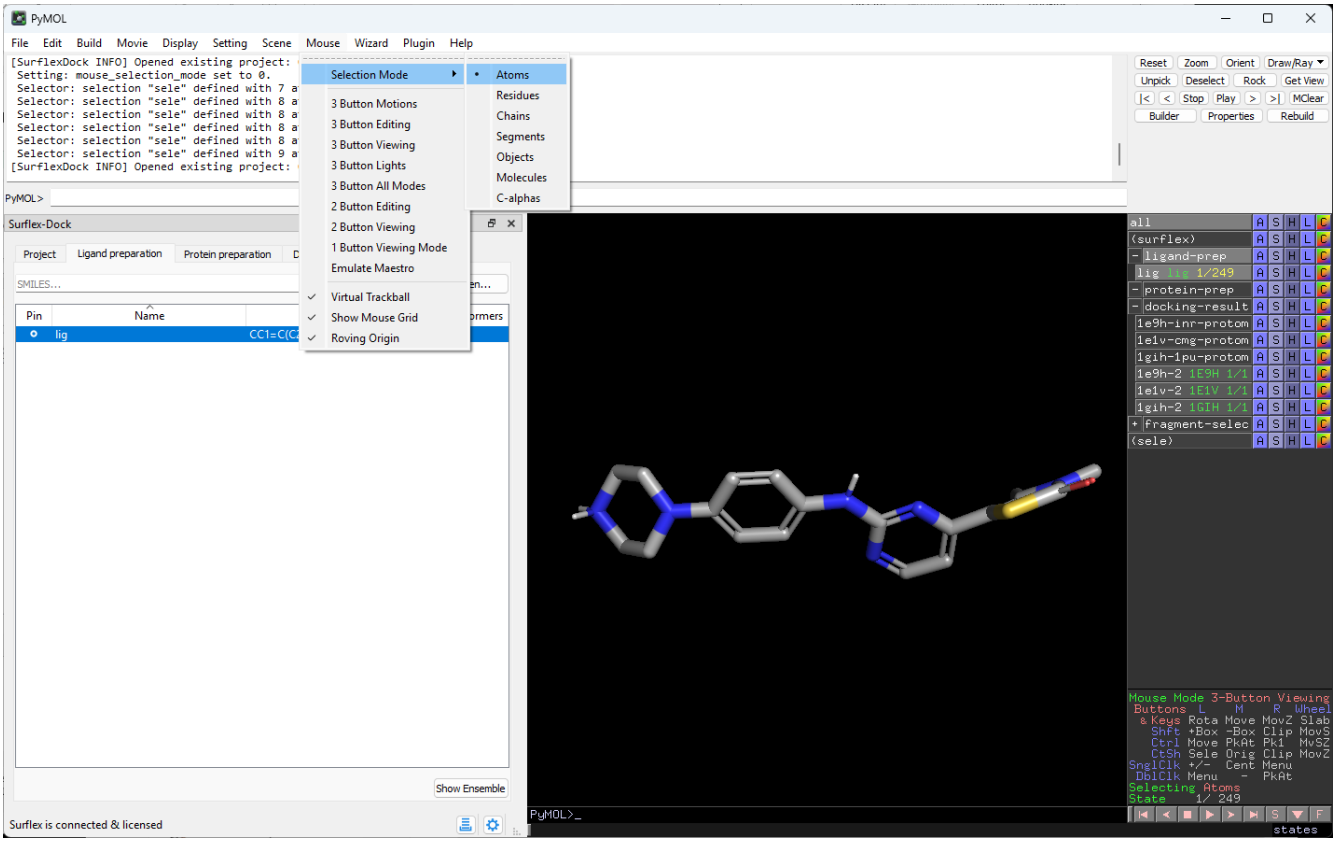
At any time, you can display protein structures, cavities, known ligands, and/or protomols along with the docking poses using the **Show/Hide protein controls** button.



7. How do I... use constraints

In some cases, a user has knowledge about the likely conformation of a substructure within a molecule (e.g., from detailed energetic calculations) or about a molecule's likely bound configuration. In such cases, it may be desirable to make use of constraints on molecular pose during conformer generation and/or docking. Details concerning the use of torsional or positional constraints can be found in Section 2.6 of the Surflex Manual.

To use constraints during conformer generation or docking, you will first need to import the template ligand as described in Section 4.1.
Choose **Atoms** as the **Selection mode** under the **Mouse** menu in PyMOL.

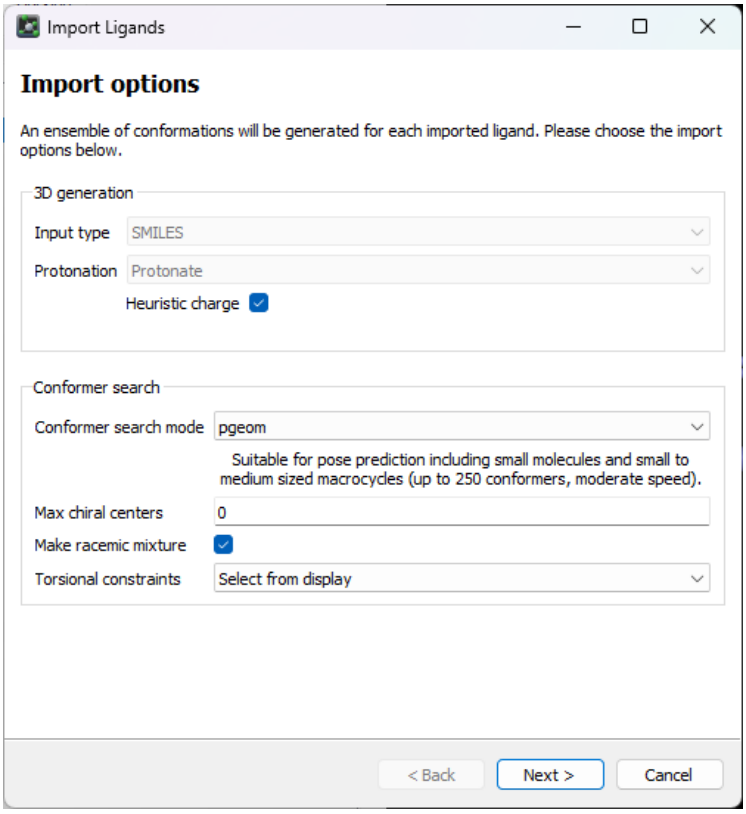


Import your ligand(s) using the SMILES box or the **Open** file dialog.

Click on the pull-down menu next to **Torsional Constraints**.

Select whether you will upload a constraints file or manually select constraints on the display. If you choose to read constraints from a file, **Browse** to select and upload that file.

Click **Next**.



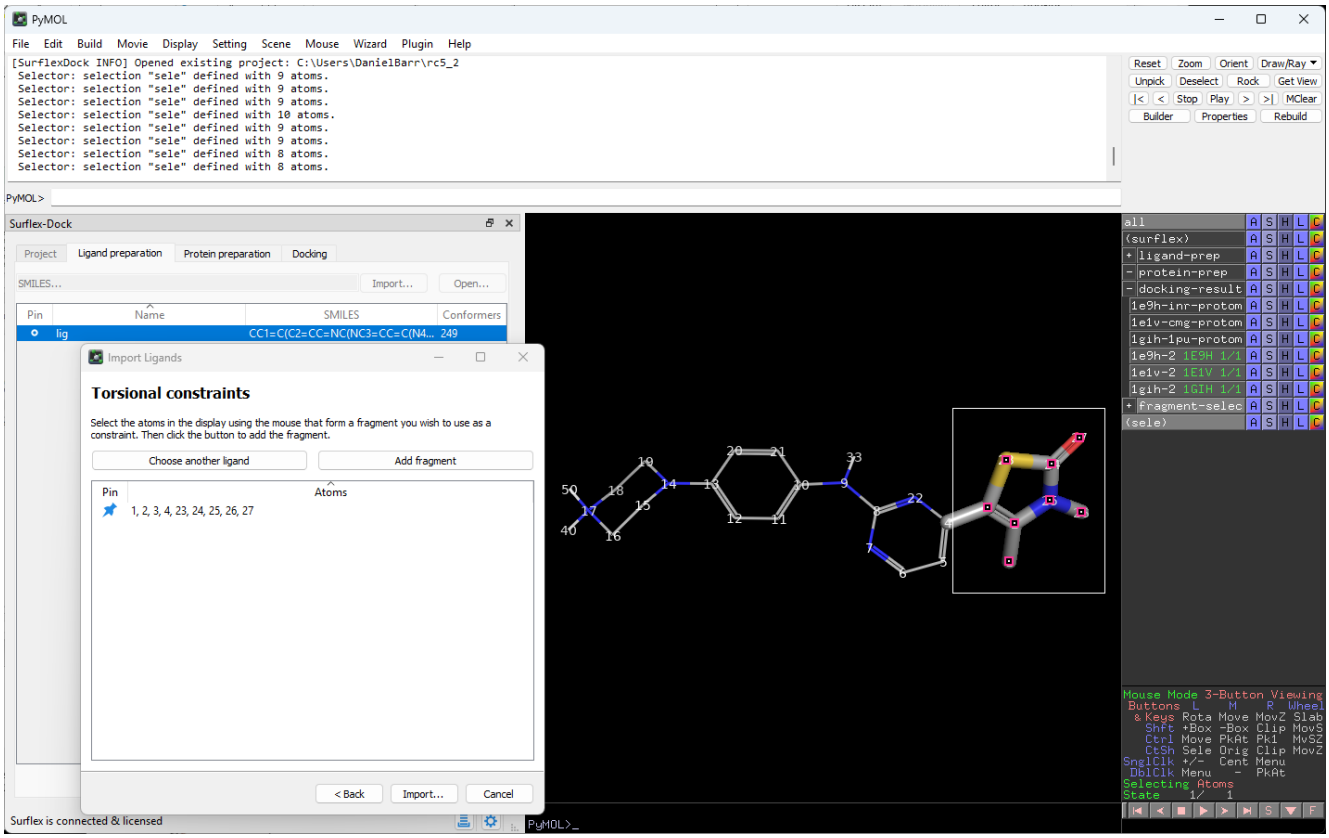
Select the template ligand from the list of ligands in the **Ligand preparation** tab. The template ligand will be displayed with atoms numbered in the PyMOL display

Click **Choose ligand.**

- Hold the **Shift** key and click with your mouse to create a box around the atoms you want to use to define the constraint.

Click **Add Fragment**.

Click **Import** to import your ligand(s) using the torsional constraints.

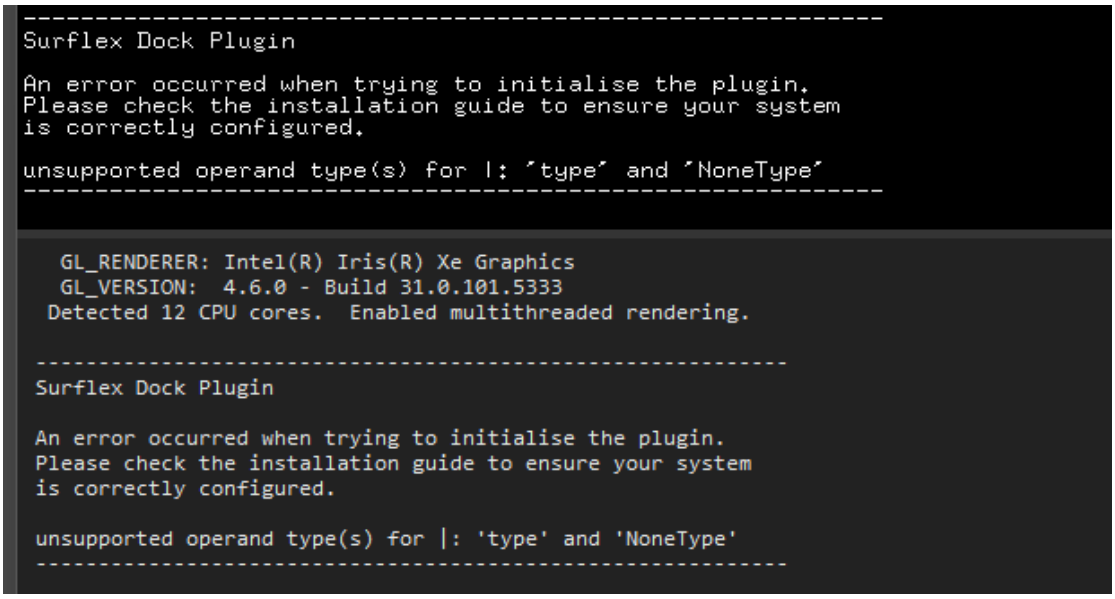


8. How do I... troubleshoot problems

8.1 Installation problems

Issue: The Surflex-Dock plugin is not functioning or is not loading in my PDB files.
Check the Surflex.exe file is saved locally on the machine and not on a cloud-based drive (eg., Onedrive).

Issue: The Surflex-Dock plugin doesn't start, showing the error message "An error occurred when trying to initialise the plugin".



This can happen if you update PyMOL using the option **Help->Check for Updates**, but it starts using an unsupported Python version. Use the following commands to check the Python version PyMOL is using:

```
import sys
print(sys.version)
```

Then, ensure that you only have Python 3.10 installed by uninstalling any other Python version and re-install PyMOL.

8.2 PyMOL crashes

PyMOL crashes are occasionally observed when using the Surflex-Dock plugin. We have done our best to investigate and mitigate these events; because the crash arises from the underlying PyMOL code and not the plugin itself, it is not always straightforward to diagnose or resolve. If you experience crashes, please send the crash report and log files for the PyMOL session to support@optibrium.com and we will be happy to investigate.

8.3 Saving and restoring a PyMOL session

It is not recommended to alter the visualisations in PyMOL while using the plugin; the plugin will override any visualisations made in PyMOL. After running all calculations, you can select or pin the structures of interest in the plugin to load them in the PyMOL visualisation state. Note that only the structures that are selected or pinned in the plugin will be available to view in the PyMOL interface. After selecting all structures of interest, you can close the Surflex-Dock plugin and customise the visualisation using the selection and display features in PyMOL.
Any custom visualisations made in PyMOL will not be saved as part of the Surflex-Dock project. It is recommended to only use the Surflex-Dock visualisation tools while performing docking calculations. When you have completed the calculations, you can close the plugin and use PyMOL to make other visualisations. You can save and open PyMOL sessions under the **File** menu in PyMOL.
You can also create visualisations in PyMOL (or another favourite visualisation program) by loading the output files from the Surflex-Dock plugin calculations.