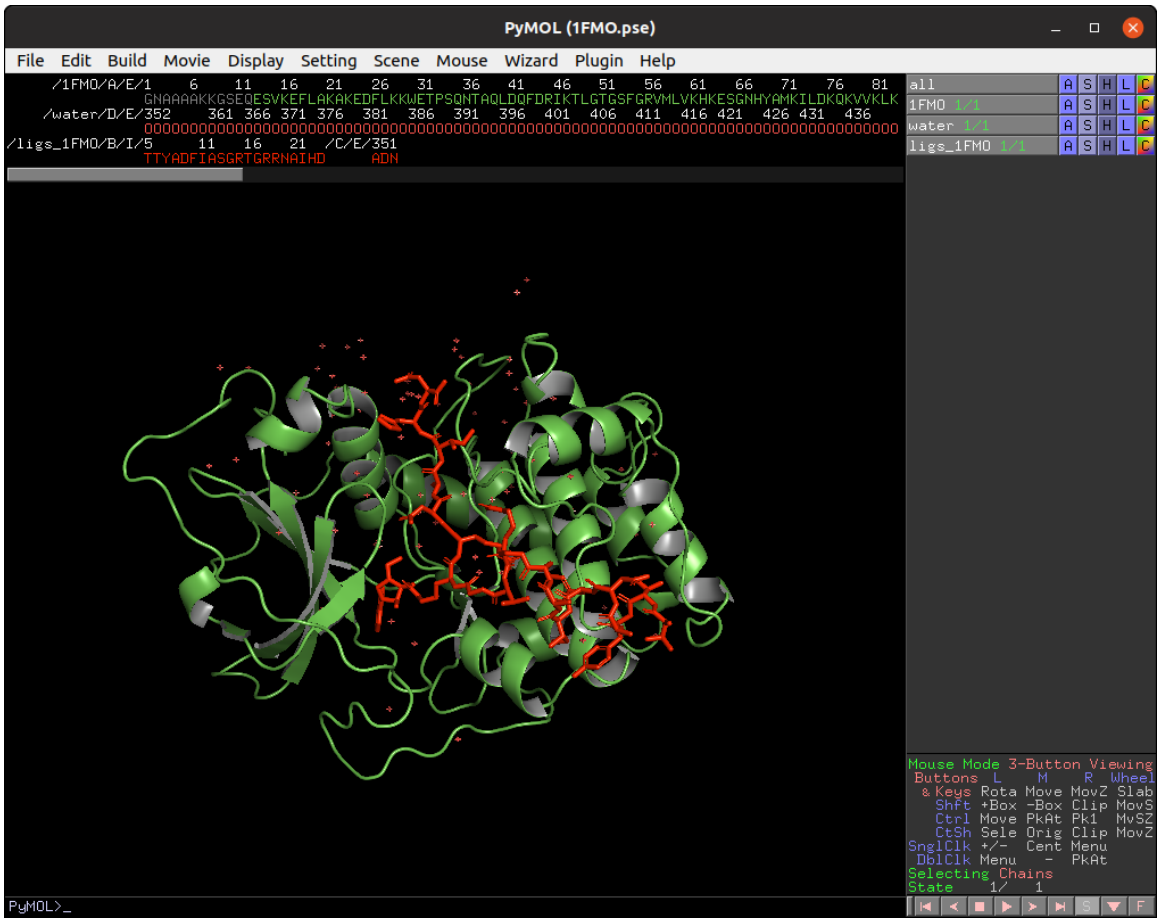
Tutorial

On this tutorial, we are going to demonstrate some features of the parKVFinder, including the graphical user interface (*parKVFinder PyMOL Tools*) and the command-line interface.

All files used on this tutorial can be found under **input** directory, on the **parKVFinder** directory.

parKVFinder PyMOL Tools

First, load **input/1FMO.pse** into PyMOL viewer, which loads two objects in your scene. The **1FMO** is a subunit of a protein kinase A and the **ligs_1FMO** is an adenosine (ADN) and a peptide kinase inhibitor (PKI).

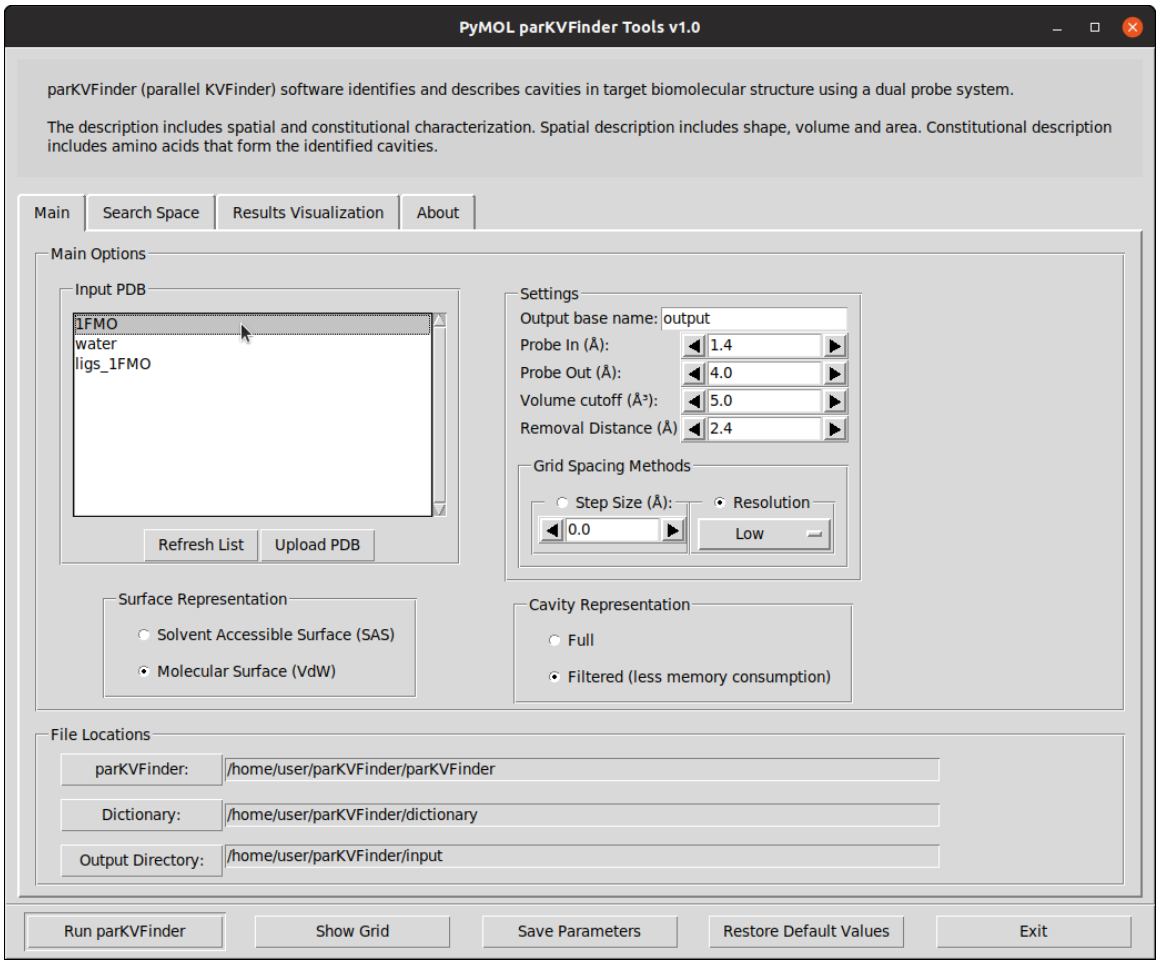


Whole protein detection

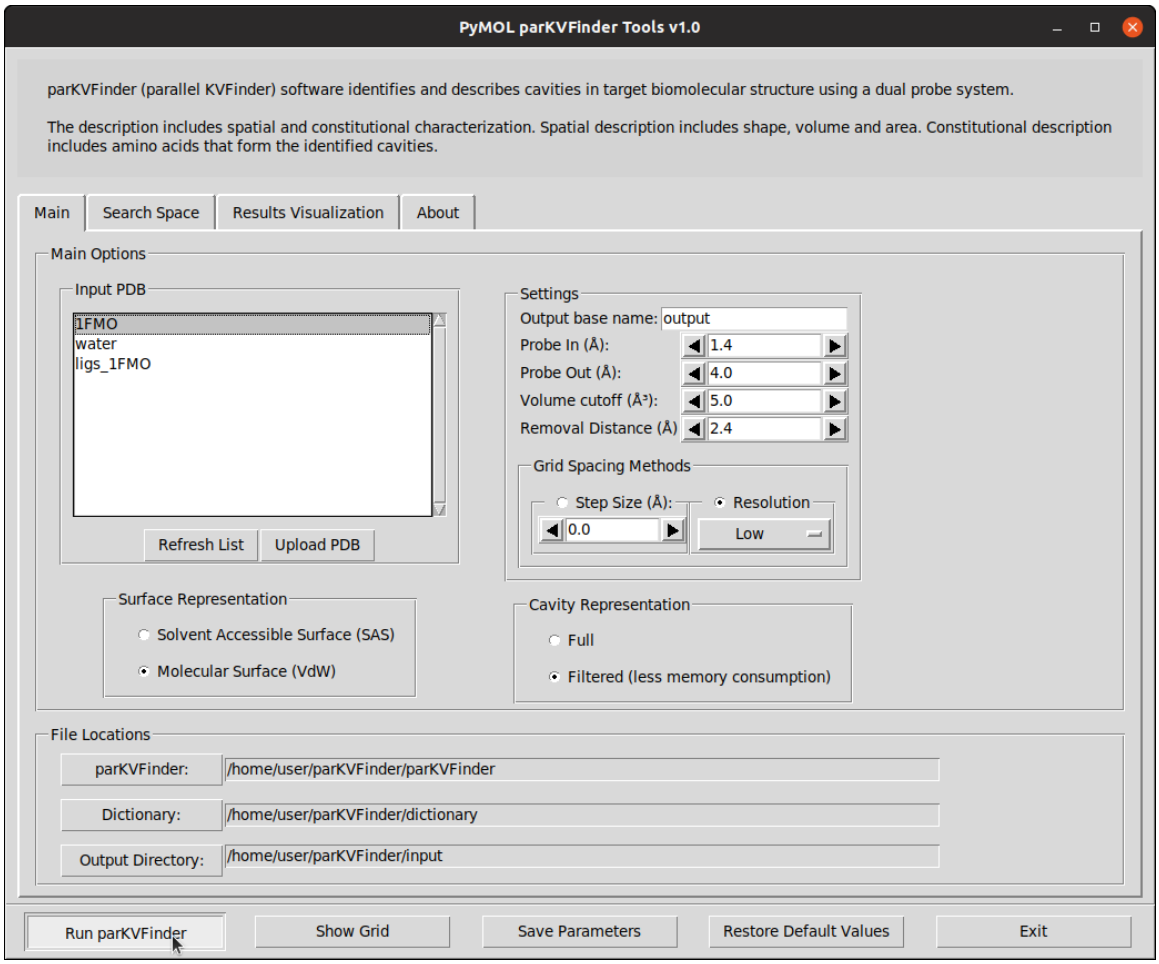
The default parameters are designed to make a simple and fast whole protein detection.

On PyMOL, open **parKVFinder PyMOL Tools** under **Plugin** tab. The objects on the scene will be listed on the **Input PDB** list box, on the **Main** tab. If not, press the **Refresh List**

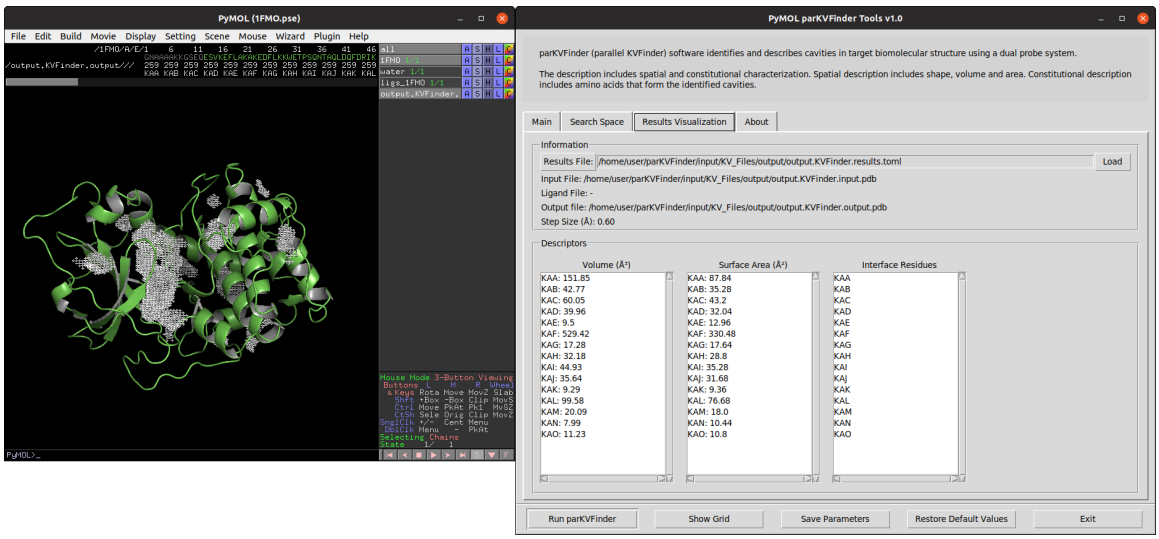
The **Input PDB** selection sets which object will be analyzed by parKVFinder. Select **1FMO** on the list box.



To run parKVFinder with the default parameters, just click **Run parKVFinder** button or press **Enter**.

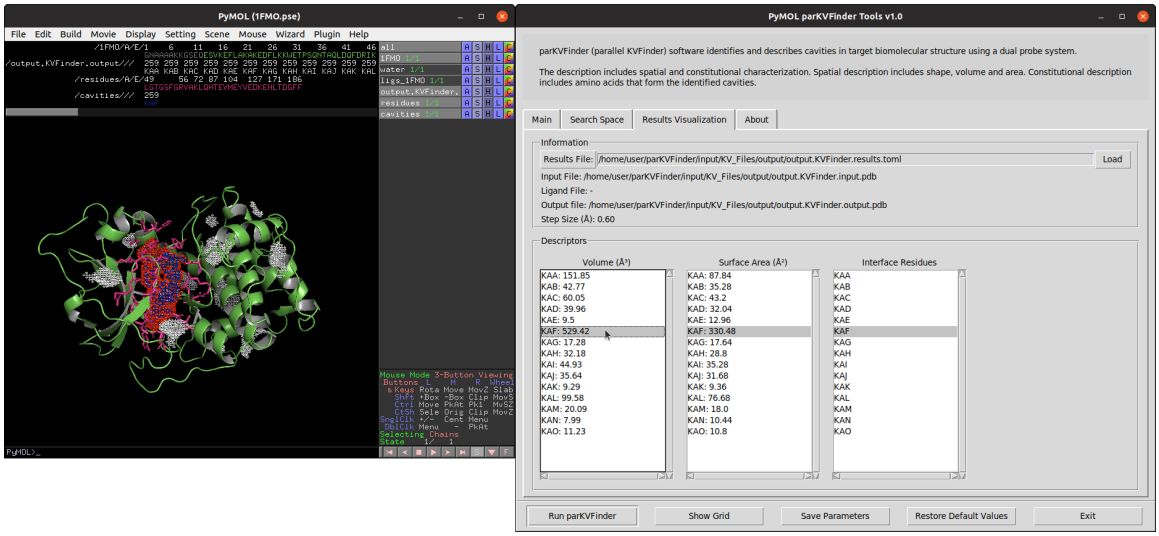


After execution is complete, cavities PDB is loaded into PyMOL viewer as <Output Base Name>.KVFinder.output object and the results file is loaded on the **Results Visualization** tab. In addition, the focus automatically shifts to **Results Visualization** tab.



We can select cavities in the **Volume** or **Surface Area** lists to highlight them on a new object called **cavities**, helping to identify each cavity. Also, we can select cavities in the **Interface Residues** list

to highlight the residues around the cavities on a new object named **residues**.



Changing cavity ceiling

parKVFinder is all about parameter customization. One of parKVFinder's most powerful assets is the ability to manually set the cavity ceiling. parKVFinder works with a double probe system. A smaller probe, called Probe In, and a bigger one, called Probe Out, that defines two molecular surfaces with different molecular accessibility. The space left between these surfaces is considered cavities.

Let's show the effect of varying **Probe Out** and **Removal Distance** on the cavity ceiling.

First, we should copy the adenosine to a new object using the following PyMOL commands:

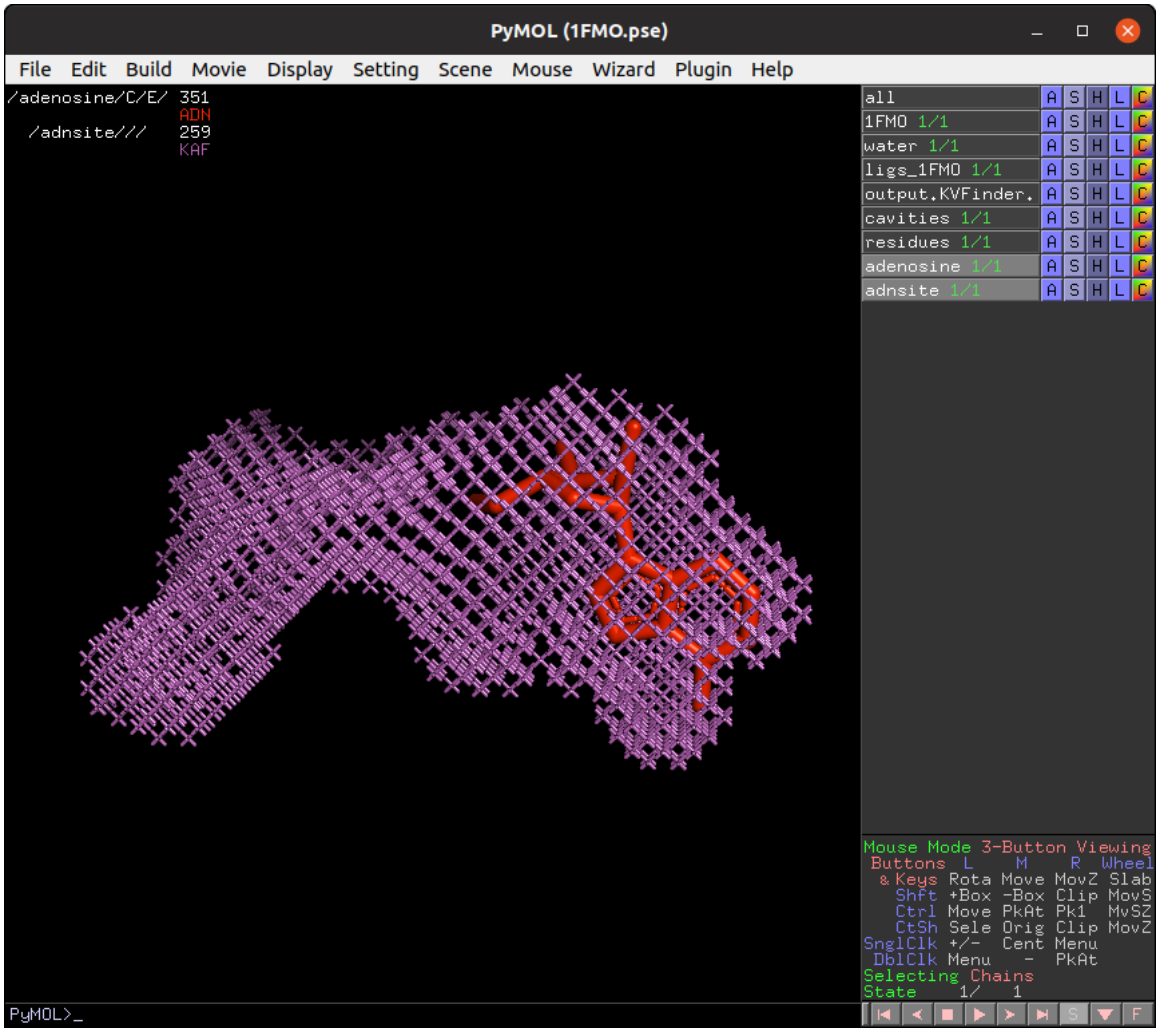
```
# Copy adenosine
select resn ADN
create adenosine, sele
delete sele
```

Also, copy the adenosine cavity (KAF) to a new object (adnsite) to compare the cavity ceiling from the previous execution.

```
# Copy adenosine cavity
select resn KAF and output.KVFinder.output
create adnsite, sele
delete sele
```

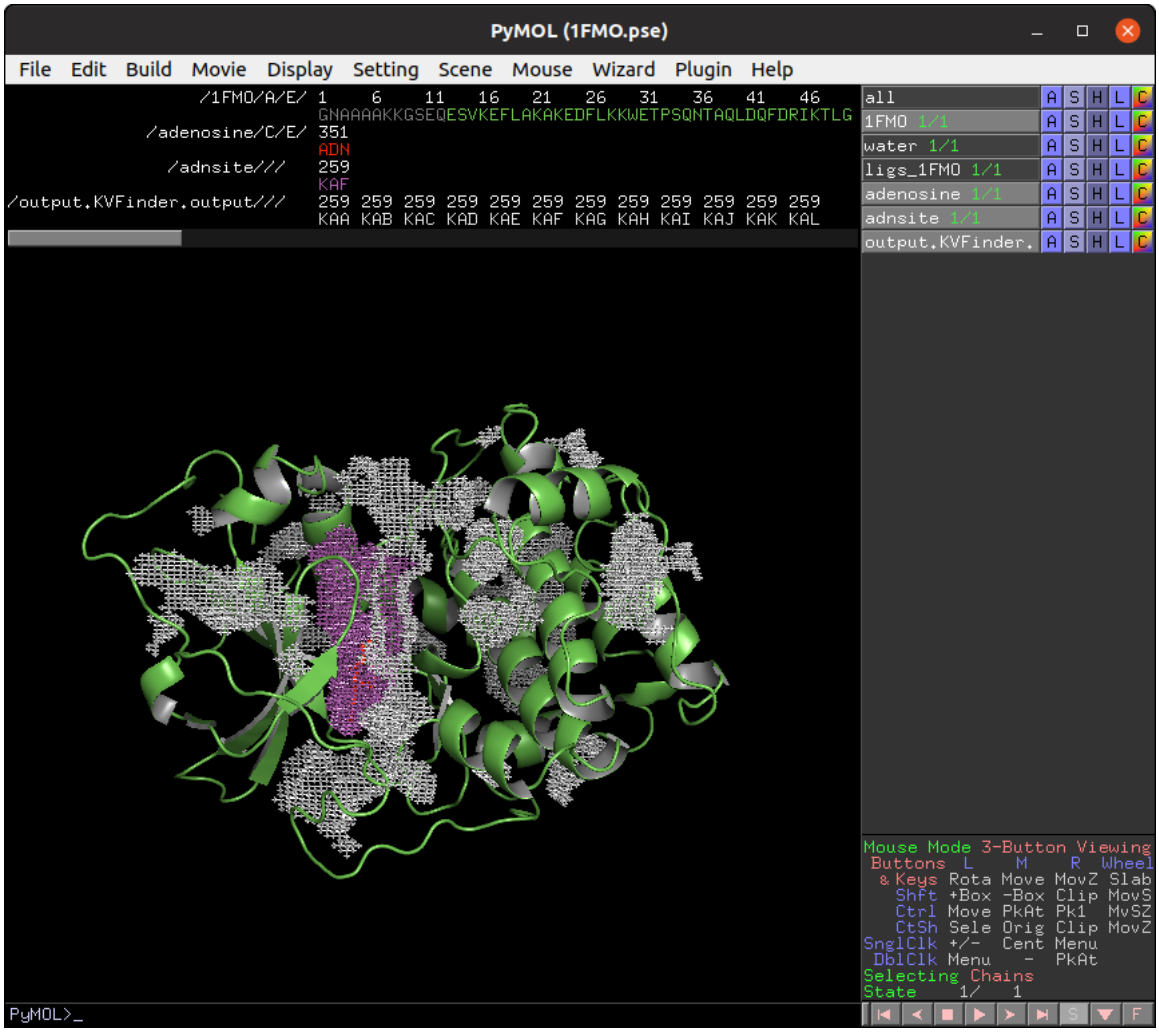
Finally, prepare the new PyMOL scene.

```
# Prepare PyMOL scene
color magenta, adnsite
disable
enable (adnsite, adenosine)
```



Adjusting Probe Out

As mentioned above, adjusting the Probe Out size changes the level of the cavity ceiling. So let's go back on the **Main** tab and change the **Probe Out** size to 8.0 Å. Run parKVFinder again.

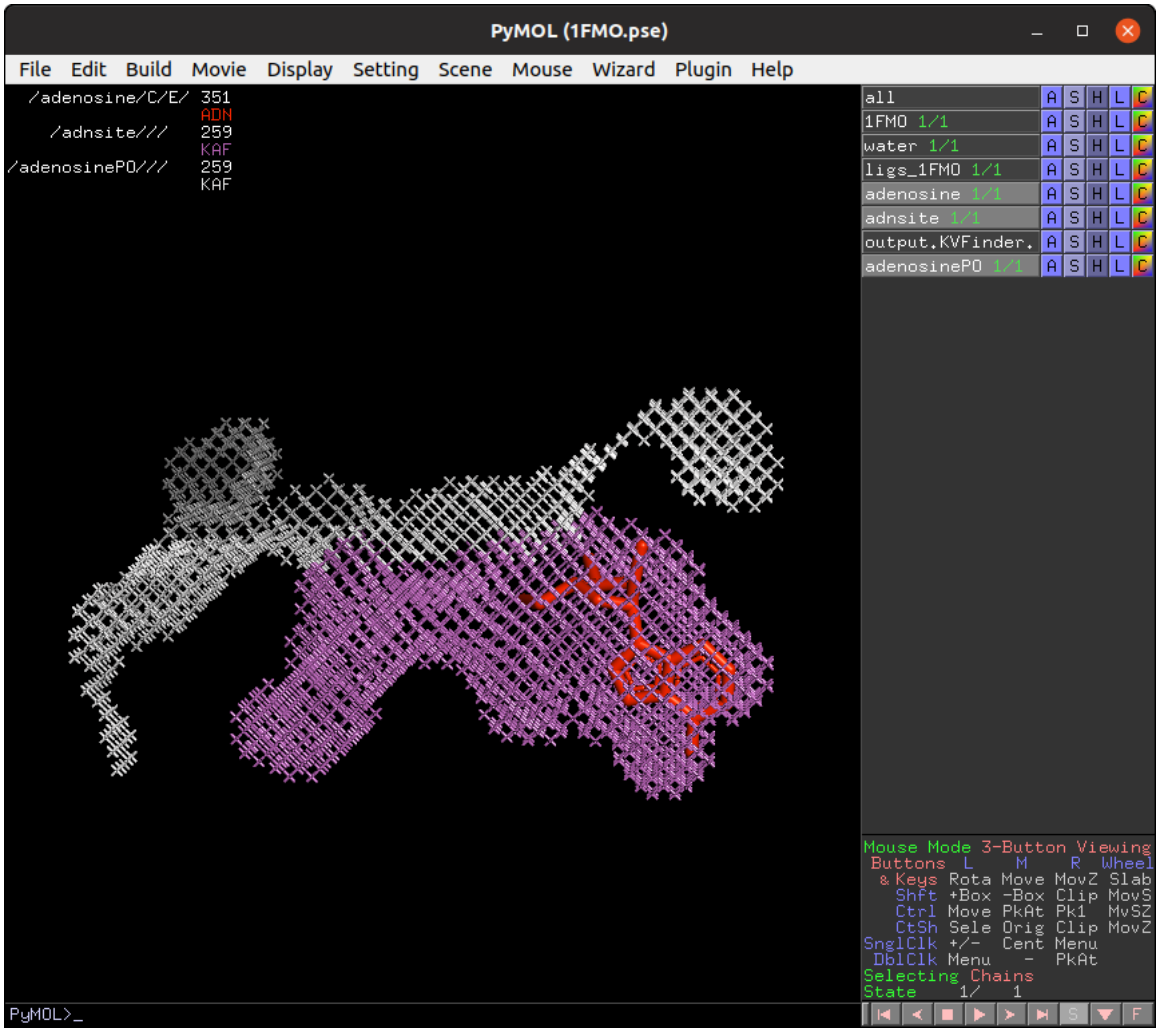


Again, copy the adenosine cavity (KAF) to a new object (adnsitePO).

```
# Copy new adenosine cavity
select resn KAF and output.KVFinder.output
create adnsitePO, sele
delete sele
```

Finally, prepare the PyMOL scene.

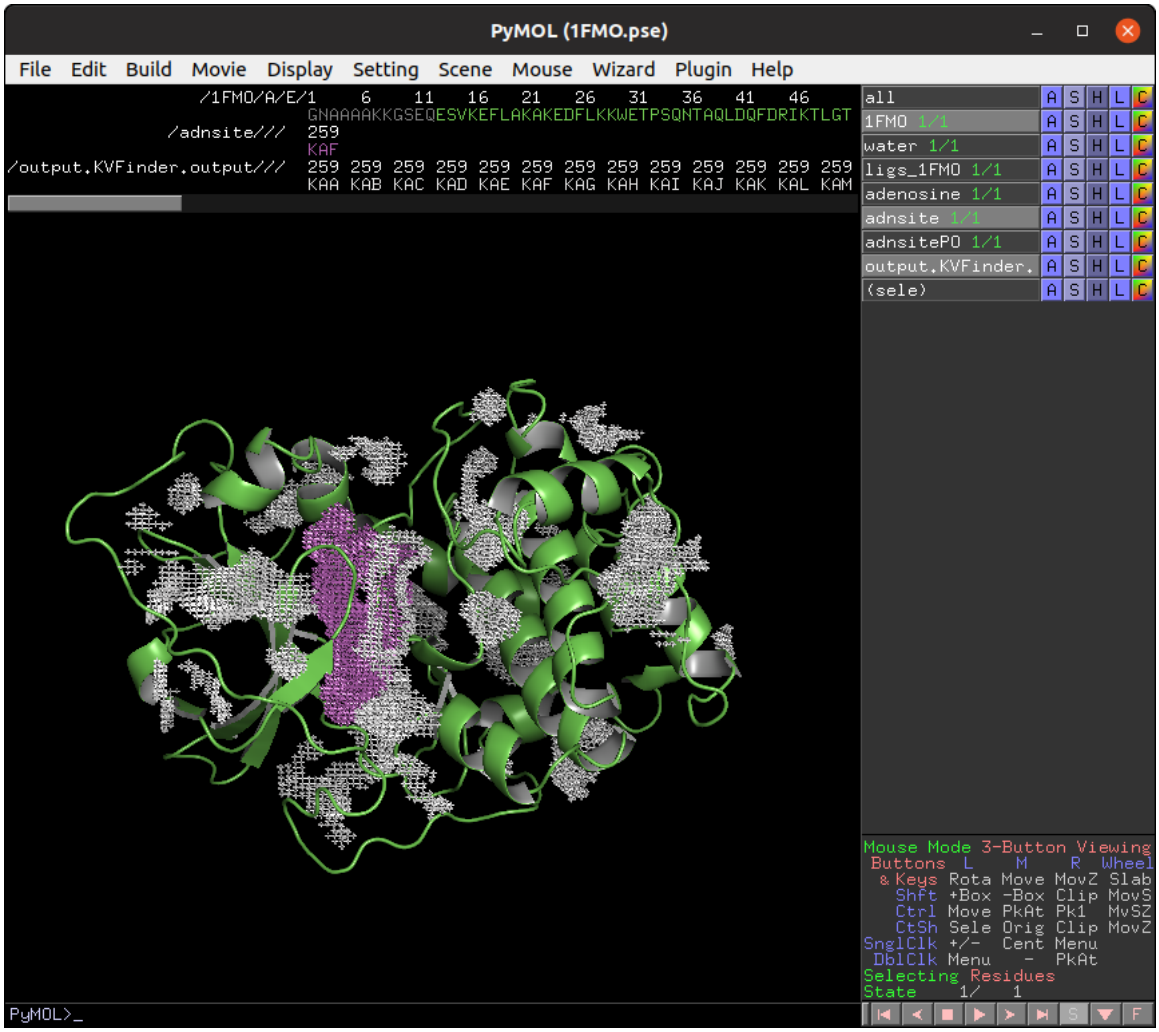
```
# Prepare PyMOL scene
disable
enable (adenosine, adnsite, adnsitePO)
```



Note that the adenosine cavity detected with the 4 Å Probe Out (magenta) has a lower ceiling than that detected with the 8 Å probe (white). Therefore, by increasing the size of the Probe Out, the cavity ceiling is also raised.

Adjusting Removal Distance

Besides adjusting the Probe Out size, we can also adjust the Removal Distance to change the cavity ceiling. So let's go back to the **Main** tab and change the **Removal Distance** to 1.2 Å and the size of **Probe Out** back to 4.0 Å. Run parKVFinder again.

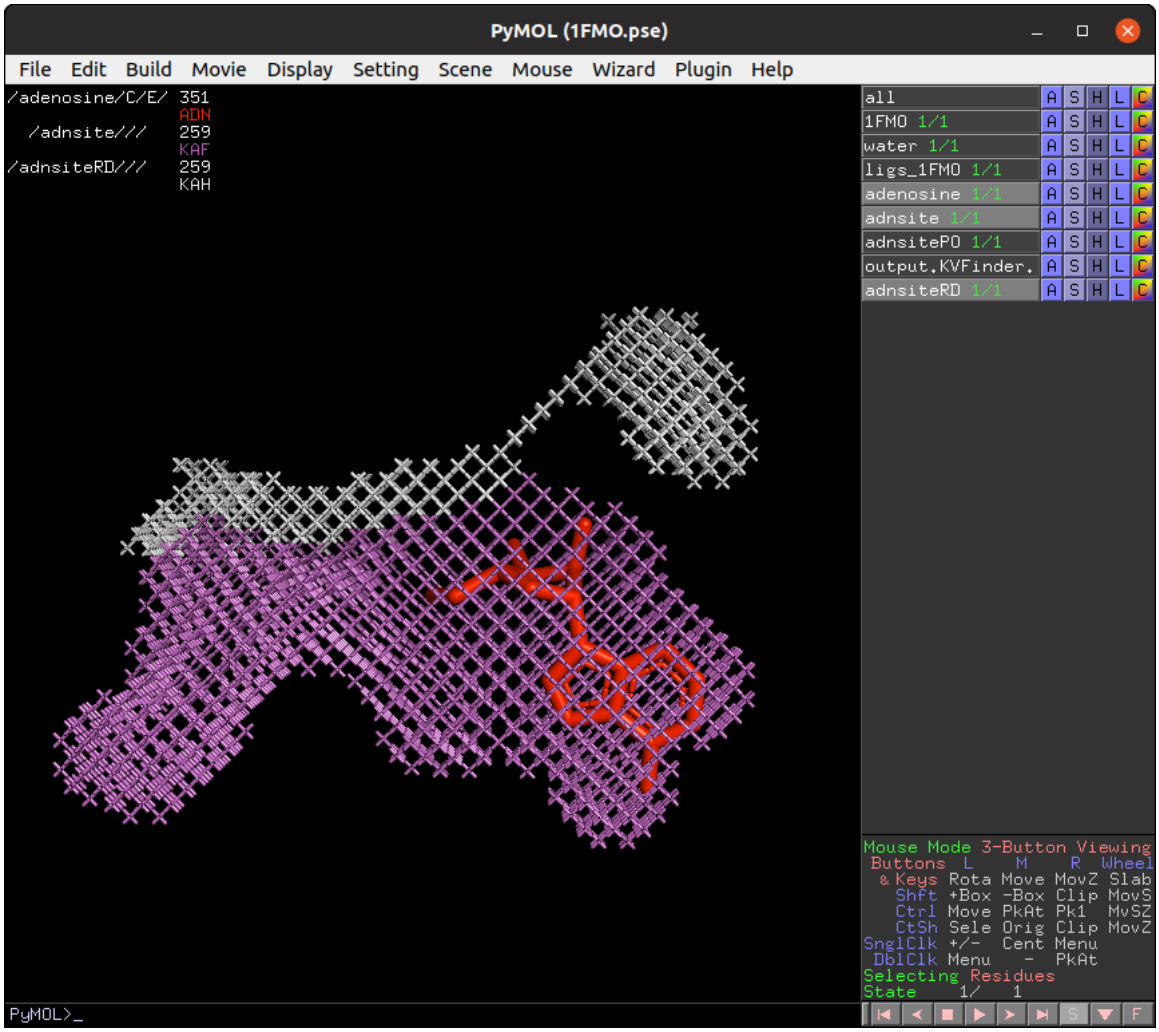


Again, copy the adenosine cavity (KAH) to a new object (adnsiteRD).

```
# Copy new adenosine cavity
select resn KAH and output.KVFinder.output
create adnsiteRD, sele
delete sele
```

Finally, prepare the PyMOL scene.

```
# Prepare PyMOL scene
disable
enable (adenosine, adnsite, adnsiteRD)
```



Note that the adenosine cavity detected with the 2.4 Å Removal Distance (magenta) has a lower ceiling than that detected with the 1.2 Å (white). Therefore, by decreasing the Removal Distance, the cavity ceiling is also raised.

Furthermore, changing the cavity ceiling by varying Probe Out and Removal Distance also affects cavity segregation.

Note: Usually the Removal Distance adjustment is less time consuming than the Probe Out adjustment for similar effects.

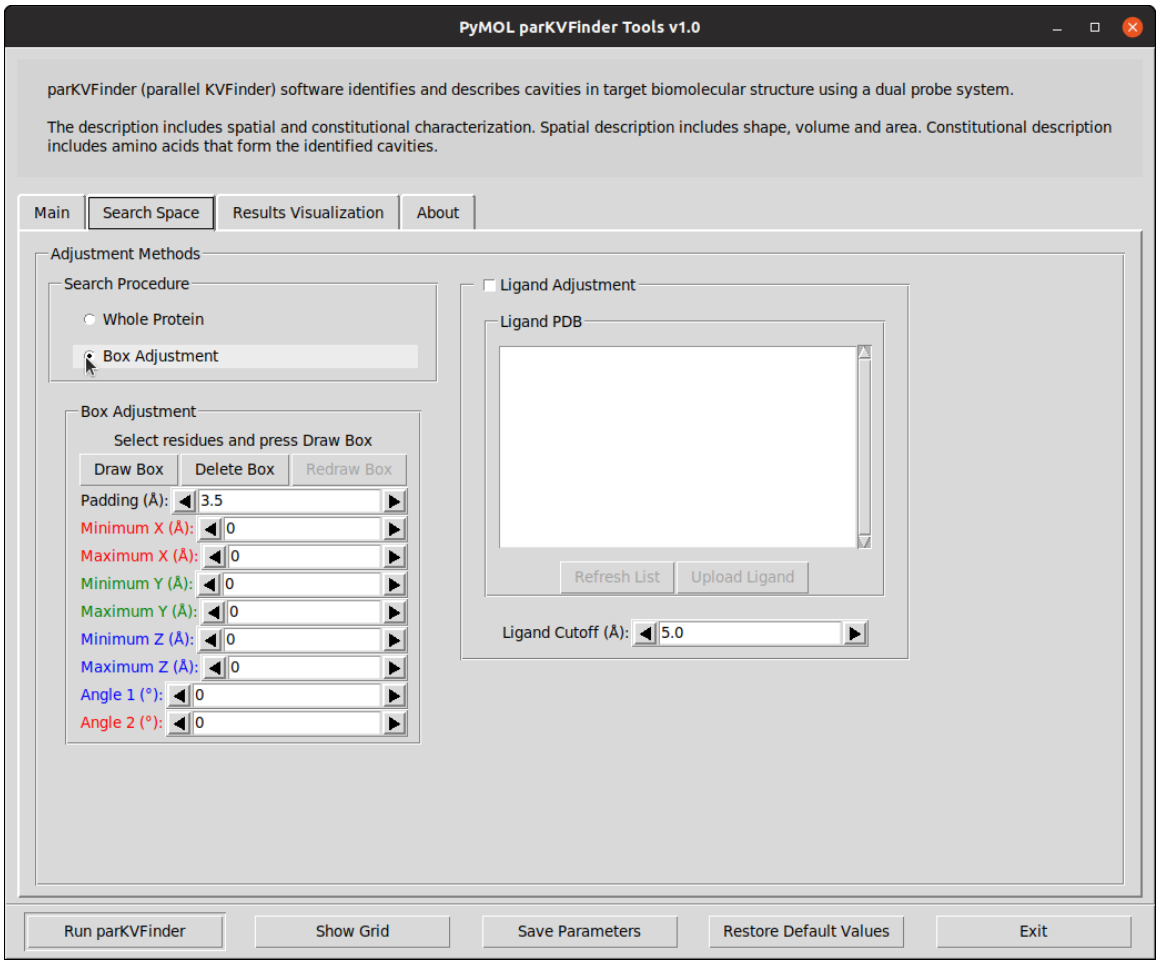
Steered detection

An important feature of parKVFinder is the steered detection of cavities. We continue our tutorial illustrating two distinct methods of cavity segmentation.

Box adjustment mode

Box adjustment mode explores closed regions with a custom box, which can be drawn via the GUI.

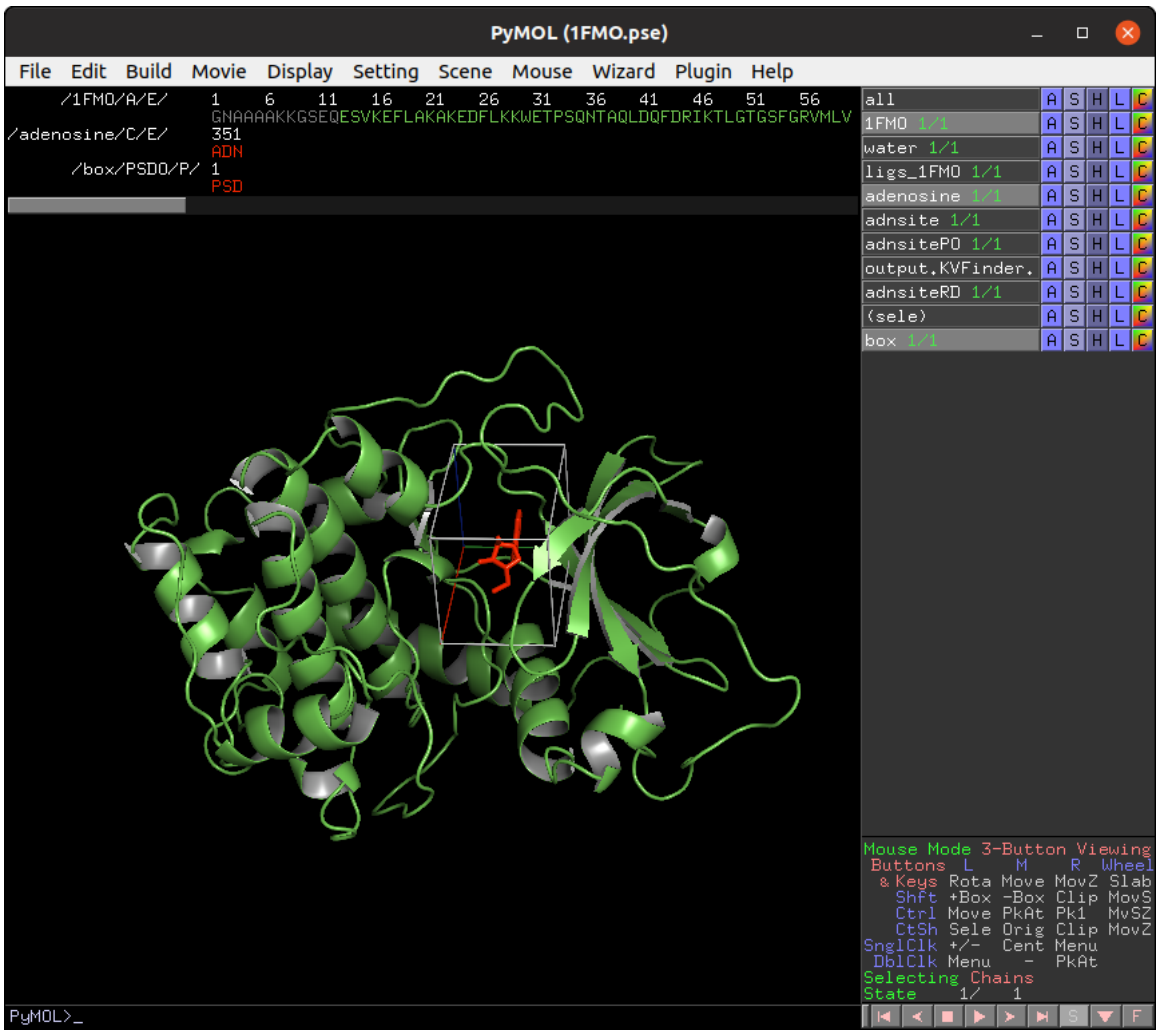
On the **Search Space** tab, select **Box Adjustment** option under **Search Procedure** group. This will enable a **Box Adjustment** frame, which handles the custom box in PyMOL viewer.



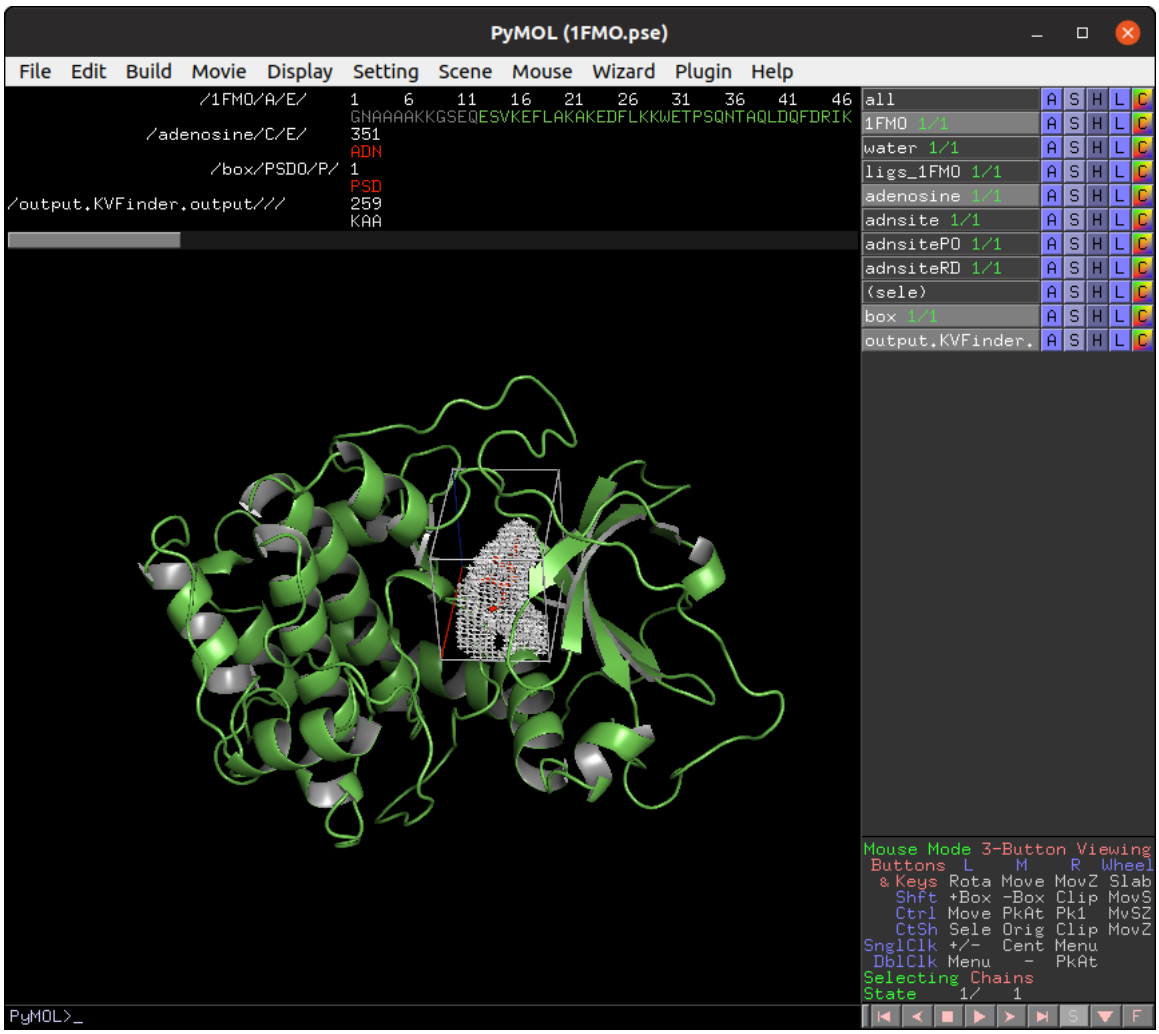
The custom box is drawn based on the (sele) object in the PyMOL viewer.

Then, select the adenosine ligand. This can be made on the PyMOL viewer by clicking on the ligand structure or using `select resn ADN` PyMOL command.

Click on **Draw Box** Button. This will create a custom box that limits the search space. It is fully customizable, but we will not change it for now.



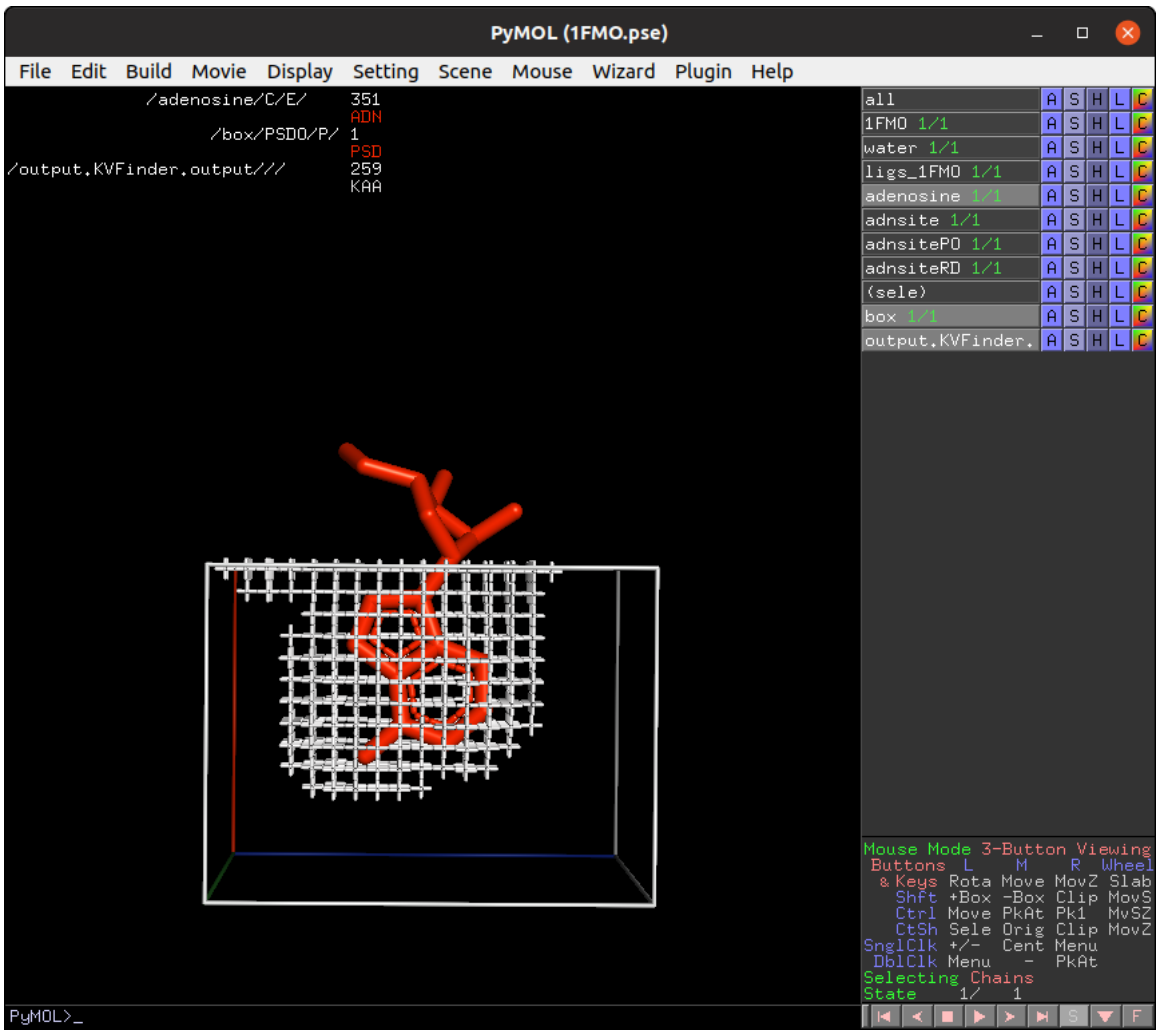
On the **Main** tab, change **Removal Distance** back to 2.4 Å and rerun parKVFinder.



Now, let's customize the box parameters to segment the binding site of our target protein.

Each axis is associated with one color (red with X, green with Y and blue with Z). The adjustment is made by the arrows or directly setting the value in the entry on the **Search Space** tab in the **Box Adjustment** group. We can also adjust the box angles by the same procedure. After altering the values, just click on **Redraw Box** button to redraw the box object using the new values.

Then, on the **Search Space** tab, reduce **Maximum X** to 1.0 Å and click **Redraw Box**. Rerun parKVFinder.



Lastly, click on **Delete Box** button to delete the custom box.

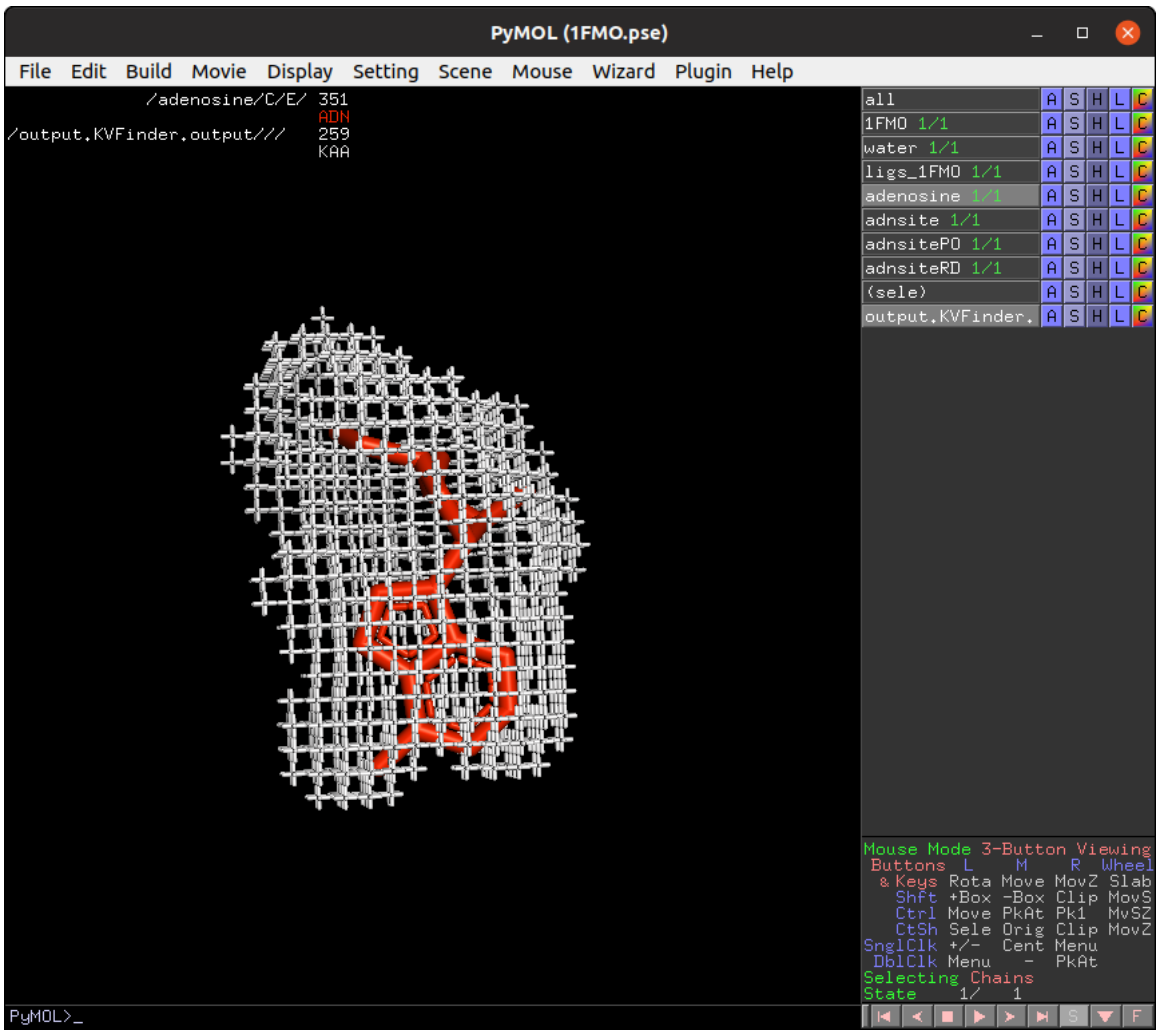
Ligand adjustment mode

A last feature is to limit the search around a structure. In this last example, let's do a whole protein prospection again, but limiting the search space around ligands.

First, on the **Search Space** tab, select **Whole Protein** option under **Search Procedure** group. This will disable the previous enabled **Box Adjustment** frame.

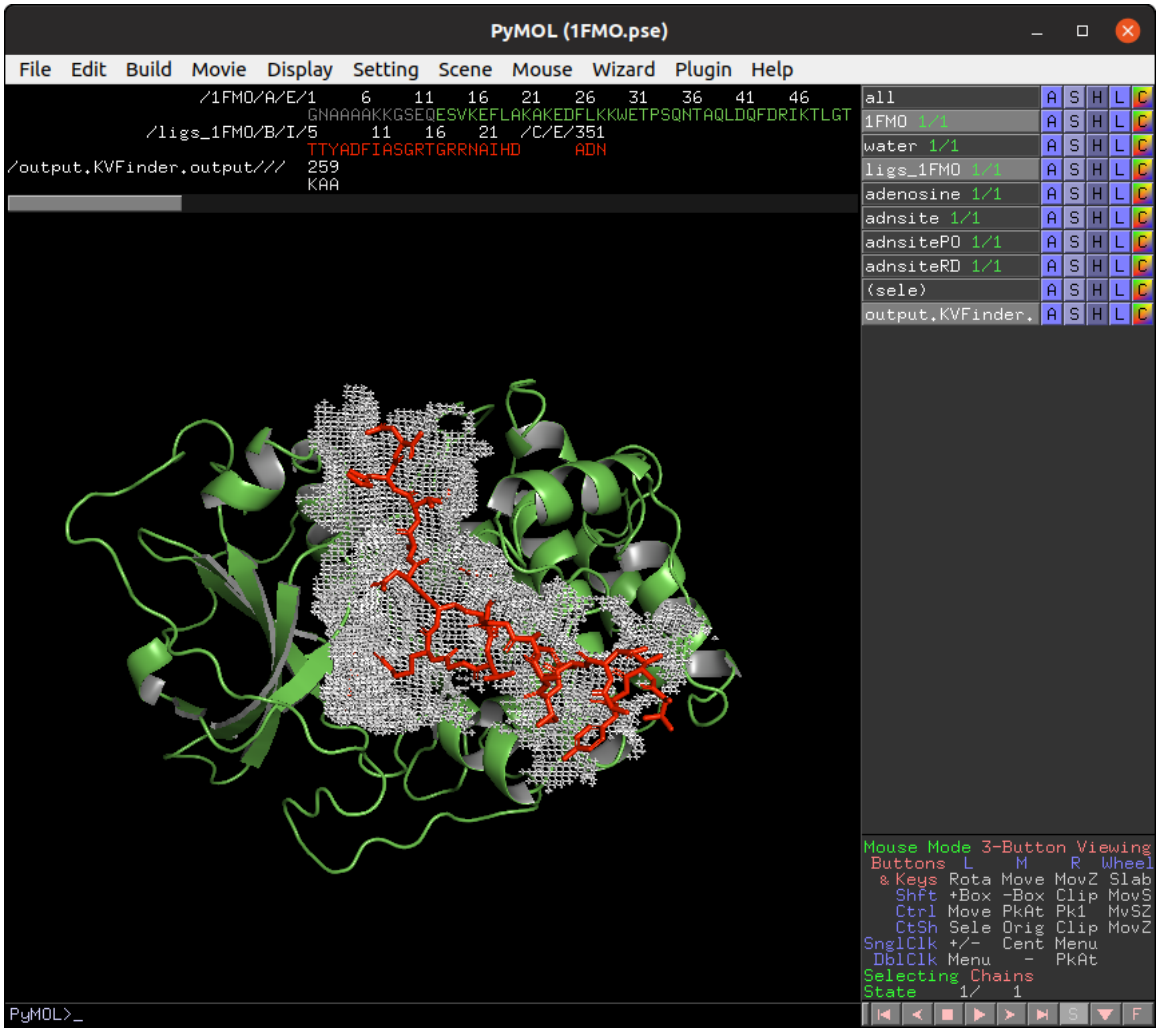
Still on the **Search Space** tab, click on the check button **Ligand Adjustment**. This will enable the buttons **Refresh List** and **Upload Ligand**.

Click the **Refresh List** button to display all objects in the scene in the **Ligand PDB** list box. Select the adenosine on the list box and reduce **Ligand Cutoff** to 3.0 Å. Run parKVFinder again.



Now, let's shift focus to the two ligands (adenosine and PKI) in the ligs_1FMO object.

On the **Search Space** tab, select the ligs_1FMO on the **Ligand PDB** list box and increase **Ligand Cutoff** back to 5.0 Å. Back on the **Main** tab, increase **Probe Out** to 10.0 Å and reduce **Removal Distance** to 0.0 Å. Run parKVFinder again.



Command line interface

parKVFinder has a command-line interface, which can be useful for molecular dynamics and high-throughput analysis. It also handles the same parameters available in parKVFinder PyMOL Tools, except for box rotations in box adjustment mode.

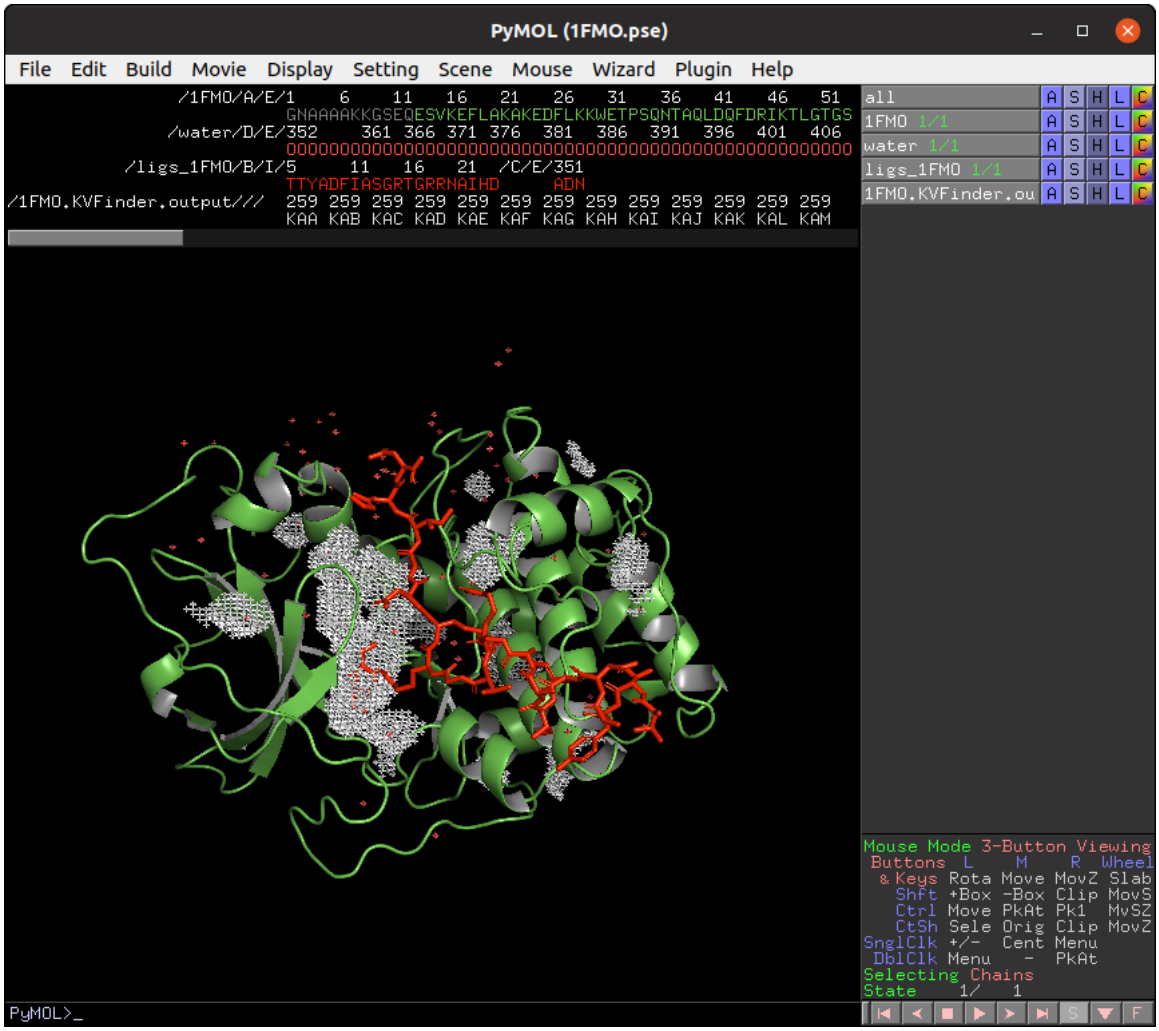
```
/home/user/parKVFinder$ parKVFinder
parKVFinder (parallel KVFinder) software identifies and describes cavities in
target biomolecular structure using a dual probe system.

The description includes spatial and constitutional characterization. Spatial
description includes shape, volume and area. Constitutional description includes
amino acids that form the identified cavities.

Usage: parKVFinder PDB [options],
    where PDB is a path to a target PDB file.

Options:
  -h, --help
      Display this help message.
  -v, --version
```

```
      Display parKVFinder version.
  --verbose
      Print extra information to stdout.
```

Standard execution of the command line interface only requires the PDB file path of the target protein. So let's repeat the execution of 1FMO protein with default parameters.

```
/home/user/parKVFinder$ parKVFinder$ parKVFinder input/1FMO.pdb
[PID 8504] Running parKVFinder for: /home/user/parKVFinder/input/1FMO.pdb
done!
Elapsed time: 0.78 seconds
```

To view the cavities, the cavities PDB file must be loaded into PyMOL.



There a set of options for customizing parKVFinder's command line execution. These options are displayed in a help menu with their default values when applicable.

```
/home/user/parKVFinder$ parKVFinder$ parKVFinder -h
================================================================================
============================== parKVFinder help menu ===========================
parKVFinder (parallel KVFinder) software identifies and describes cavities in
target biomolecular structure using a dual probe system.
```

```
The description includes spatial and constitutional characterization. Spatial
description includes shape, volume and area. Constitutional description includes
amino acids that form the identified cavities.

Usage: parKVFinder PDB [options],
    where PDB is a path to a target PDB file.

Options:
  -h, --help
      Display this help message.
  -v, --version
      Display parKVFinder version.
  --verbose
      Print extra information to stdout.

General options:
  -p, --parameters  [<.toml>]
      Define path to parameters file.
  -d, --dictionary  [<dictionary>]
      Define path to a custom dictionary file.
  -r, --resolution  <enum>      (Low)
      Define resolution mode. Options include: Off, Low, Medium and High.
  -s, --step        <real>      (0.0)
      Define step size (grid spacing).
  -i, --probe_in    <real>      (1.4)
      Define probe in size.
  -o, --probe_out   <real>      (4.0)
      Define probe out size.
  --volume_cutoff   <real>      (5.0)
      Define cavities volume filter.
  --removal_distance    <real>      (2.4)
      Define removal distance when comparing probes surfaces.
  -t, --template            (paramters.toml)
      Create a parameter file template with defined parameters in current
      working directory.

Box adjustment options:
  -B, --box
      Define a search box mode where parKVFinder will detect cavities.
  --custom_box      [<file>]
      Define a custom search box based on a file containing the minimum and
      maximum cartesian values of each axis in angstrom.
  --residues_box    [<file>]
      Automatically set a search box based a file containing a tab-separated
      list of residues.
  --padding     <real>      (3.5)
      Define residues box padding. Adds a length in each box direction.

Surface options:
  -S, --surface     <enum>      (VdW)
      Define a surface representation. Options include: SAS and VdW. SAS
      specifies solvent accessible surface. VdW specifies van der Waals
      molecular surface.
```

```
Ligand options:
  -L, --ligand      [<.pdb>]
      Define path to ligand PDB file.
  --ligand_cutoff   <real>      (5.0)
      Define ligand radius distance cutoff.

================================================================================
================================================================================
```
