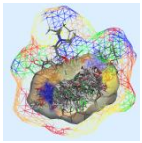

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Computer Lab, Session 6

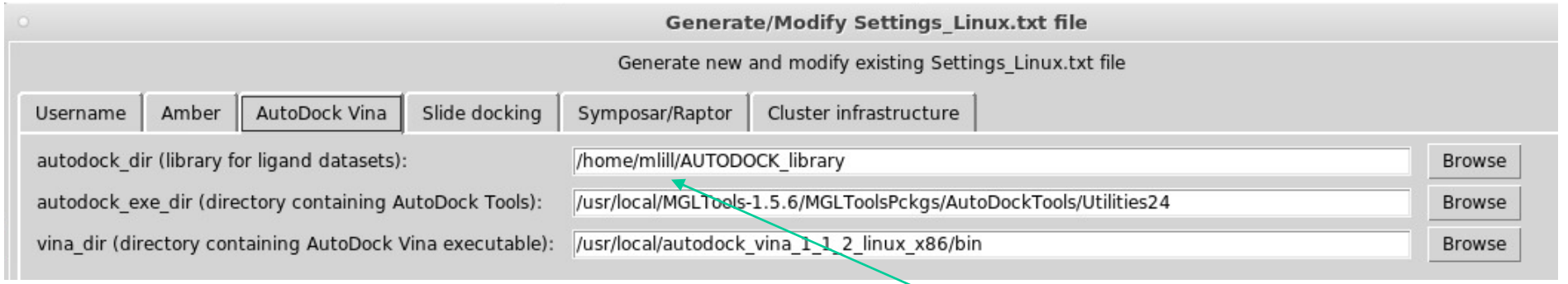
Settings

- Open *.bashrc* file: **gedit ~/.bashrc**
  - Add the following two lines to the end of the file:

    export PATH=/usr/local/MGLTools-1.5.6/bin:$PATH

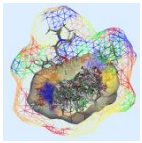
    export PYTHONPATH=/usr/lib64/python2.7/site-packages:$PYTHONPATH

- Open pymol and select **AutoDockVina → Modify Settings_Linux.txt file**
  - Modify the following entries:



  - Press **Save to file**
  - Press **Exit**
  - Exit pymol

Specify own username



Prepare ligand library

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We will dock known thrombin inhibitor into the x-ray structure of thrombin (1mu6).

1. Ligand preparation

We will first set-up the ligand library. We will start with a series of thrombin inhibitors in pdb format:

Download the file *Ligands.tar.gz* from the course website under *Lab6*, and store it in your local *Lab6* subdirectory.

Open a terminal, change into the *Lab6* subdirectory using the **cd** command. Then, unzip and un-tar the file *Ligands.tar.gz* by typing:

tar zxf Ligands.tar.gz

This will produce a folder *Ligands* in the *Lab6* subdirectory which contains the individual pdb files.

Change into the *Ligands* subdirectory, and then open the pdb files in pymol by typing:

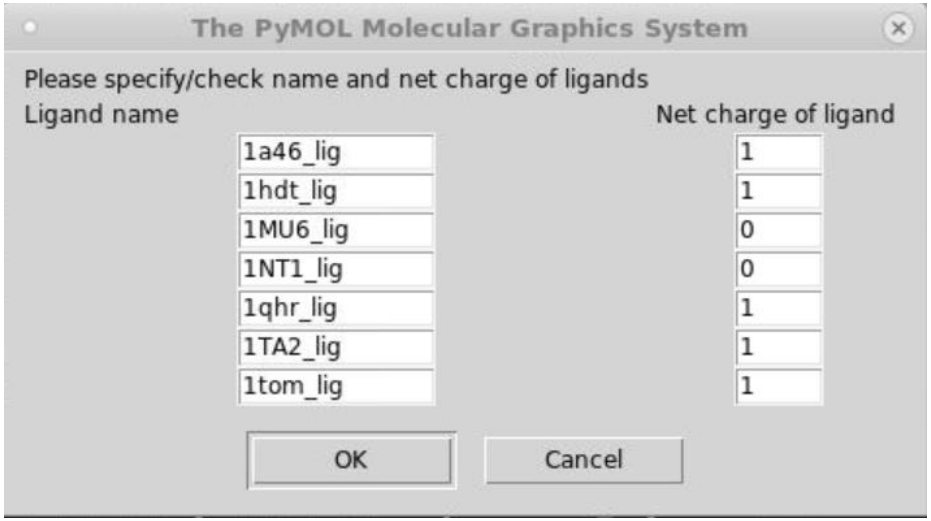
pymol *.pdb

Set-up ligand library for AutoDockVina using Menu item:

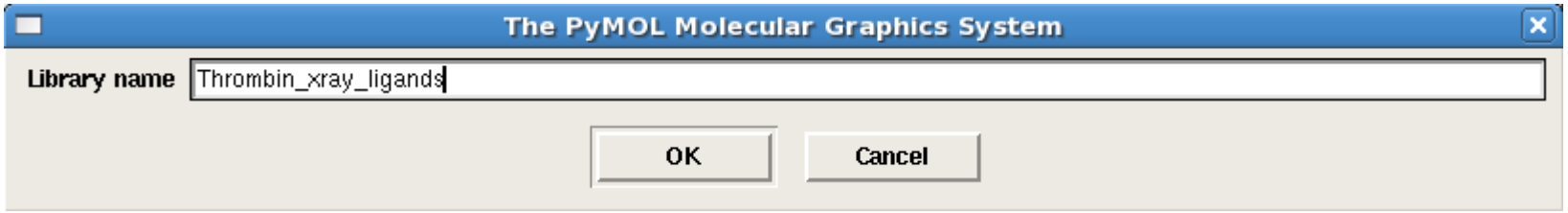
AutoDockVina → Export ligand library

Prepare ligand library

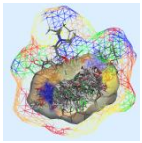
Specify ligand name and net charge of ligand:



Specify library name:



A ligand library will be generated under /home/"username"/AUTODOCK_library



Prepare and run docking

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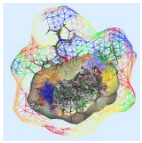
2. Protein preparation and perform docking

We will use the PDB structure 1mu6 as template for docking thrombin inhibitors. You could use the original structure and separate ligand from the protein, by generating two distinct objects in PyMOL. We will use the minimized structure from Lab4 (Lab4_cmp1_final.pse == Lab6_0.pse).

Open Lab6_0.pse in PyMOL and choose **AutoDockVina → Prepare system and start AutoDock** from the menu bar. The following dialog will appear:

Prepare and run docking



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AutoDockVina Plugin

Choose target protein and ligand library for AutoDock simulation

Project definition name

Base directory: /home/mlill/Desktop/CADD_Lab/Lab7 Browse

Project subdirectory: project_xray_ligands

Ligand library

Path to ligand library: Full path to ligand library Search and Import

Protein selection

Protein from file [PDB,MOL2,MOL]

Path to protein file: Full path to protein file Search and Import

File containing multiple protein structures [NMR-PDB]

Path to protein file: Full path to protein file Search and Import

Trajectory from Amber

Path to topology file: Full path to .top file Search and Impor

Path to trajectory file: Full path to .trj file Search and Impor

Protein in current PyMol session

Objects to select from: 1MU6

Protein preparation

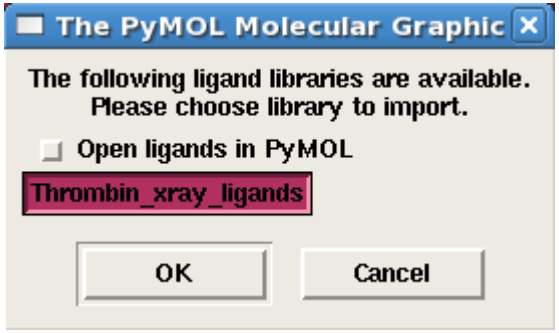
Let autodock change protonation states

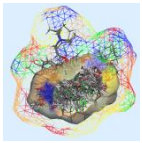
OK Cancel

Prepare and run docking

Define Project subdirectory: project_xray_ligands

Define Ligand library:





Prepare and run docking

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Final dialog:

AutoDockVina Plugin

Choose target protein and ligand library for AutoDock simulation

Project definition name

Base directory: /home/mlill/Desktop/CADD_Lab/Lab7 Browse

Project subdirectory: project_xray_ligands

Ligand library

Path to ligand library: /home/mlill/AUTODOCK_library/Thrombin_xray_ligands Search and Import

Protein selection

Protein from file [PDB,MOL2,MOL]

Path to protein file: Full path to protein file Search and Import

File containing multiple protein structures [NMR-PDB]

Path to protein file: Full path to protein file Search and Import

Trajectory from Amber

Path to topology file: Full path to .top file Search and Impor

Path to trajectory file: Full path to .trj file Search and Impor

Protein in current PyMol session

Objects to select from: 1MU6

Protein preparation

Let autodock change protonation states

OK Cancel

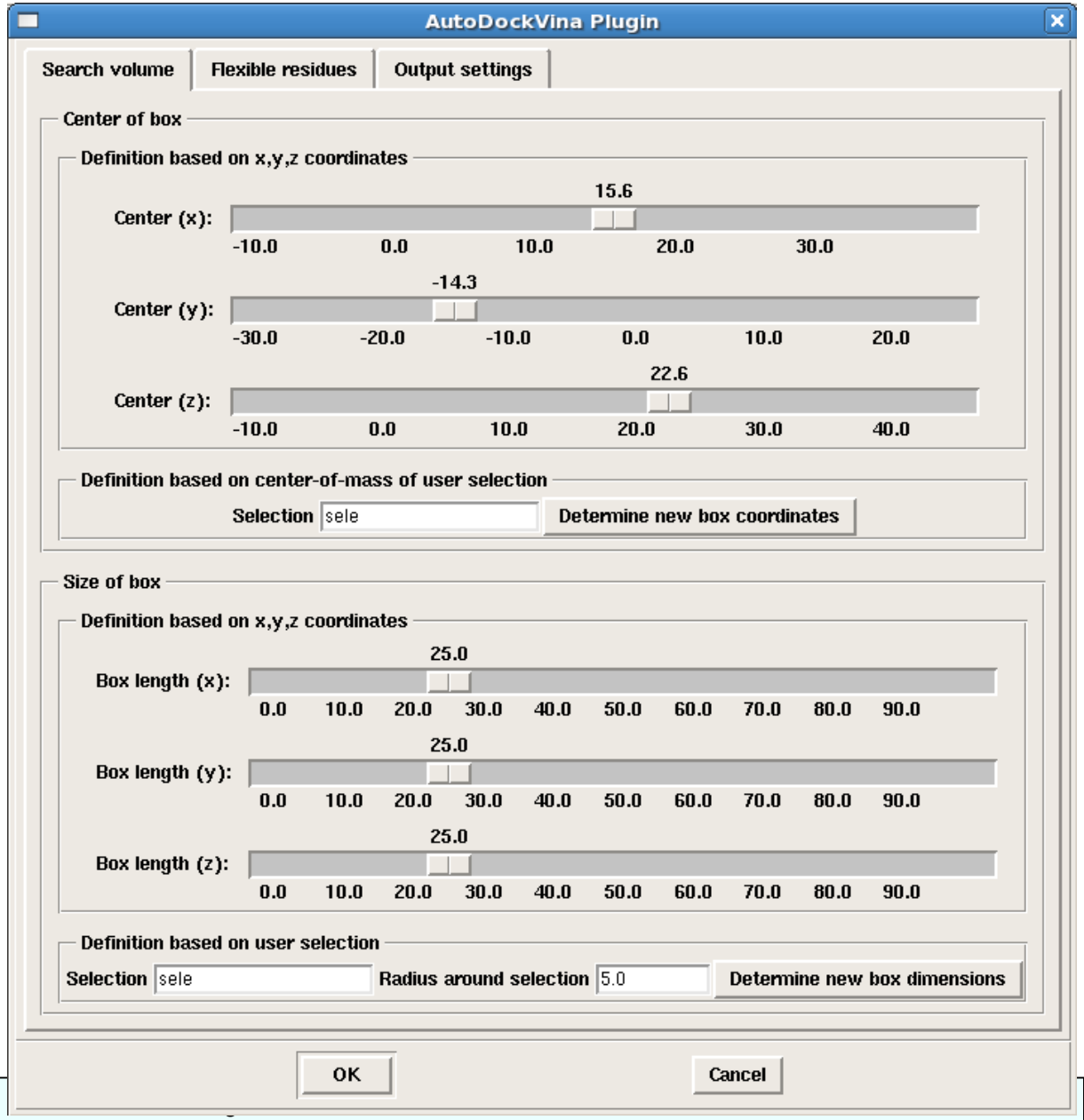
Run docking

Next, define settings for AutoDock Vina simulations:

Define size of search volume covering the binding pocket

Tip: Show x-ray ligand only and define box to cover space of ligand plus extra space OR simply select ligand (→ (sele)) and **Determine new box coordinates** and **Determine new box dimensions** based on this selection.

Use default settings for sub-dialogs “Flexible residues” and “Output settings”



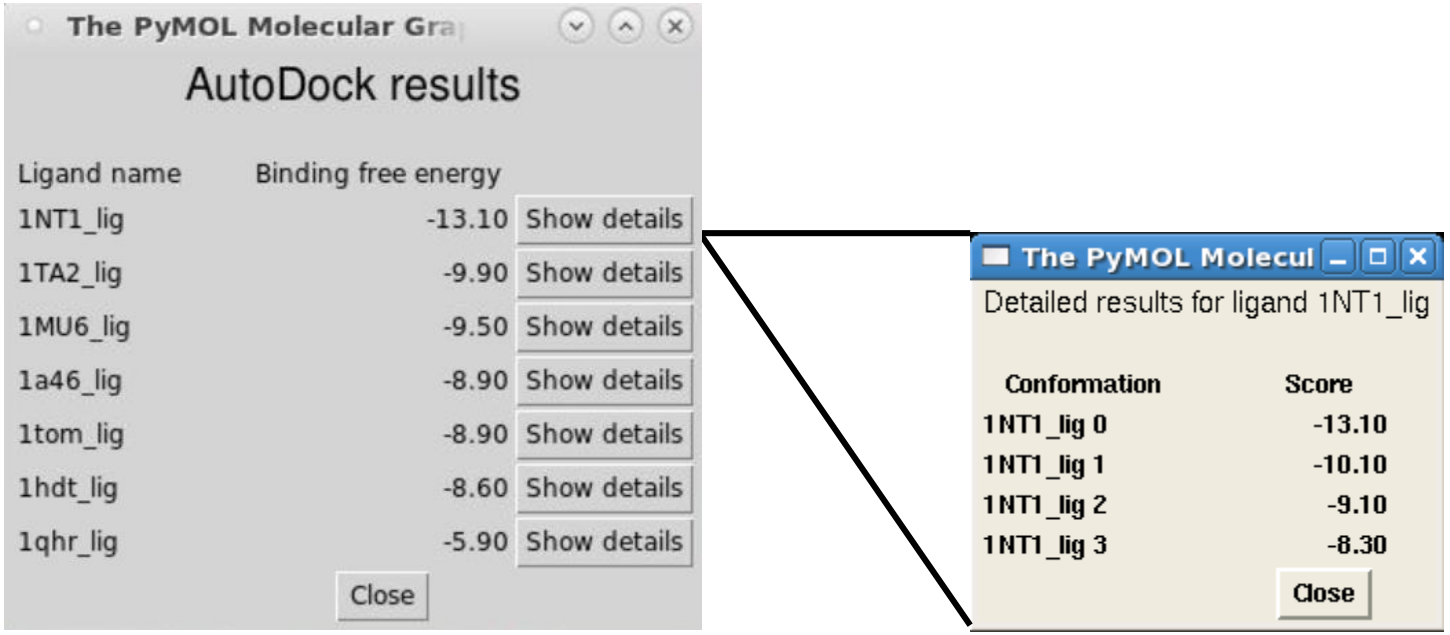
Analyze results

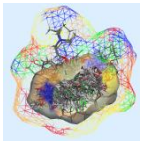
3. Analysis

The simulation will take about 20-30min. You can check if the simulation has been completed and import the results with **AutoDockVina →Import Results**. Select *Monitor.aut* from the folder *project_xray_ligands_client*.

All existing objects will be deleted and the solutions will be read into PyMOL. The different states in PyMOL show the ligand configurations. You can use the same buttons and menu items as for the MD simulation analysis to toggle between the states or show all states at the same time.

Also, a dialog will show the predicted affinities for each ligand:





Analyze results

Please, compare visually the top binding pose with the native ligand configuration. The native poses are contained in the Ligands_xray.tar.gz file you can download from the course website.

Untar the file: **tar –zxf Ligand_xray.tar.gz**

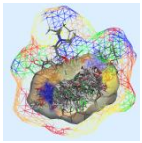
Open the native poses in PyMOL:

1MU6_ligand.pdb, 1NT1_ligamd.pdb, 1TA2_ligand.pdb, 1a46_ligand.pdb, 1hdt_ligand.pdb, 1qhr_ligand.pdb, 1tom_ligand.pdb

To compute RMSD between docked and native poses, e.g. for 1MU6 ligand:

Open new terminal

- **module load anaconda**
- **cd Lab6**
- **python /usr/local/rmsdoe.py -ref Ligands_xray/1MU6_ligand.mol2 -in project_xray_ligands_client/out/1MU6_lig/1MU6_lig.mol2 -v -overlay false**



Different protein structure

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4. Alternative ligand structure for macrocycle (1NT1)

In the previous docking simulation the x-ray conformation of the macrocyclic structure of the co-crystallized ligand in 1NT1 was used.

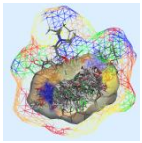
We will now repeat the simulations using the lowest energy conformation in solvent obtained by conformational search.

Please repeat steps 1.-3. with the following changes:

a. Download and untar file *AlternativeMacrocycles.tar.gz* containing the ligand conformations (x-ray, lowest energy conformation in solvent).

b. Prepare ligand library using pdb files in *AlternativeMacrocycles*. Use library name *1NT1.*

c. Perform docking simulation using *1NT1* ligand library and *project_1NT1* as project subdirectory.



Different protein structure

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5. Alternative protein structure for docking

To study the influence of protein structure on docking performance, we will repeat the docking simulations under 1.-3. but

a. Using *Lab6_1.pse* instead of *Lab6_0.pse* (X-ray structure: 1hdt).

b. Specify *project_xray_ligands_1hdt* as project subdirectory.