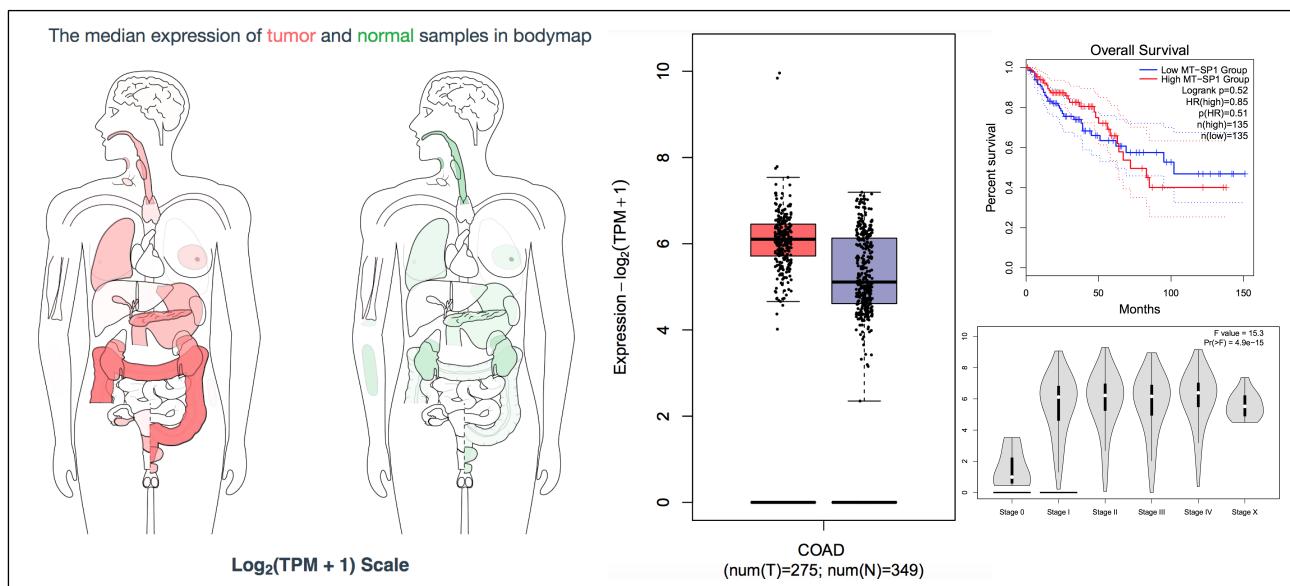


## Analysis of the interaction between MT-SP1 and benzamidine

**MT-SP1** (matriptase/ST14; EC 3.4.21) was first isolated by Shi et al. 1993, as a novel proteinase that was expressed by human breast cancer cells. MT-SP1 is highly expressed in prostate, breast, and colorectal cancers *in vitro* and *in vivo* (Oberst et al., 2001), and inhibition of this enzyme suppresses both primary tumour growth and metastasis in a rat model of prostate and colon cancer (Takeuchi et al., 1999).



This protease has been shown to cleave and activate **hepatocyte growth factor/scattering factor**, and urokinase plasminogen activator, which suggest the function of this protease as an epithelial membrane activator for other proteases and latent growth factors.

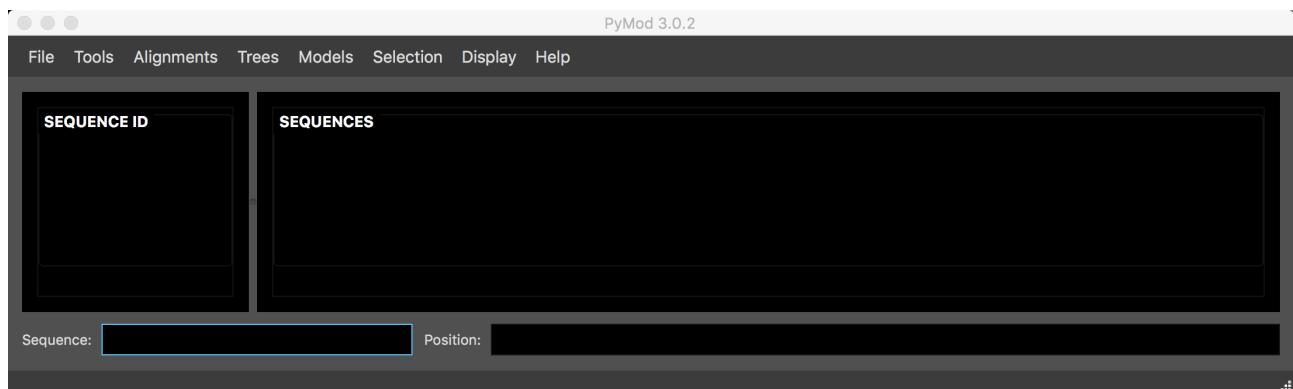
### Project A

Using the three-dimensional model of the catalytic domain of human MT-SP1, obtained by sequence homology, we will study the interaction between this protein and benzamidine, its competitive inhibitor:



A. Modeling the MT-SP1 MACROMOLECULE: Connect to the NCBI site (<https://www.ncbi.nlm.nih.gov>) and download the FASTA sequence of the protein with Accession code: Q9Y5Y6 (ST 14 protein)

1a. Launch the PyMOL program, and from the "Plugins" menu, select "PyMod 3.0". A new window will appear (a second window will ask you to create a new directory, click on OK):



2a. From the "File" menu of this window, import the previously saved sequence Q9Y5Y6 (*File -> sequences and structures -> open from file*)

3a. Left click the sequence name (which turns green), then select "Tools -> Domain Analysis -> hmmscan -> local hmmscan" menu. In the window that appears, select "Pfam-A", click on Submit, and wait for the results. Select the "Trypsin" domain and click on Submit:



4a. Right-click on the name of the sequence, and select "Domains -> Split into Domains". In the window that appears, select "0" flanking residues, and press "Submit".

5a. Select the extracted sequence of the Trypsin domain and select "Tools -> Database Search -> Blast -> local Blast" menu. In the window that appears, select "pdbaa", click on Submit, and wait for the results.

6a. Among the possible templates to use for modeling, select the PDB codes **2Q5** (DESC1, chain A) and Plasminogen **1DDJ** (Chain A) (**or** **7E50** (Chain B)), checking the red box next to the relevant code, and press submit:

Name	E-Value	Identity	Query span	Subject span
pdb 1EAW A Crystal structure of the MTSP1 (matriptase)-BPTI (aprotinin) complex [Homo sapiens] >pdb ...	2.72e-178	236/236 (100.0%)	1-237 (100.0%)	1-237
pdb 3P8F A Chain A, ST14 protein [Homo sapiens] >pdb 3P8G A Chain A, ST14 protein [Homo sapiens] >pdb ...	2.39e-177	235/236 (99.6%)	1-237 (100.0%)	1-237
pdb 3BN9 A Chain A, Membrane-type serine protease 1 [Homo sapiens] >pdb 3BN9 B Chain B, Membrane-typ...	3.62e-177	235/236 (99.6%)	1-237 (100.0%)	1-237
pdb 4IS5 A Crystal Structure of the ligand-free inactive Matriptase [Homo sapiens] >pdb 4ISL A Chain...	5.03e-177	234/236 (99.2%)	1-237 (100.0%)	1-237
pdb 5LYO A Crystal structure of the zymogen matriptase catalytic domain [Homo sapiens] >pdb 5LYO B C...	6.54e-177	234/236 (99.2%)	1-237 (100.0%)	25-261
<input checked="" type="checkbox"/> pdb 1EK8 A Crystal structure of DESC1, a new member of the type II transmembrane serine proteinases ...	3.02e-69	113/222 (50.9%)	1-236 (99.6%)	1-227
pdb 1EKB B The Serine Protease Domain Of Enteropeptidase Bound To Inhibitor Val- Asp-asp-asp-asp-lys...	3.89e-68	103/230 (44.8%)	1-237 (100.0%)	1-232
pdb 4DGJ A Structure of a human enteropeptidase light chain variant [Homo sapiens] >pdb 4DGJ B Struc...	2.63e-66	105/230 (45.7%)	1-237 (100.0%)	1-232
pdb 3W94 A Structure of Oryzias latipes enteropeptidase light chain [Oryzias latipes] >pdb 3W94 B St...	4.51e-64	106/228 (46.5%)	1-236 (99.6%)	1-231
pdb 7MEQ A Chain A, Transmembrane protease serine 2 [Homo sapiens]	1.14e-56	99/225 (44.0%)	1-236 (99.6%)	147-376
pdb 5GVT A Crystal structures of the serine protease domain of murine plasma kallikrein [Mus musculu...	3.03e-54	93/228 (40.8%)	1-236 (99.6%)	1-232
pdb 6A8O A Crystal structures of the serine protease domain of murine plasma kallikrein with peptide...	3.66e-54	93/228 (40.8%)	1-236 (99.6%)	1-232
pdb 6KD5 B Chain B, Transmembrane protease serine 13 [Homo sapiens]	8.59e-54	97/226 (42.9%)	1-236 (99.6%)	1-230
pdb 1O5E H Chain H, Serine protease hepsin [Homo sapiens] >pdb 1O5F H Chain H, Serine protease hepsi...	7.81e-53	98/227 (43.2%)	1-236 (99.6%)	1-239
pdb 6T7P A Human plasmakallikrein protease domain in complex with active site directed inhibitor [Ho...	1.80e-52	95/226 (42.0%)	1-236 (99.6%)	1-232
pdb 3UIR A Crystal structure of the plasmin-textilinin-1 complex [Homo sapiens] >pdb 3UIR B Crystal ...	2.82e-52	101/222 (45.5%)	1-237 (100.0%)	18-242
pdb 1BU1 A Structure of the ternary microplasmin-staphylokinase-microplasmin complex: a proteinase-c...	3.08e-52	101/222 (45.5%)	1-237 (100.0%)	21-245
pdb 6D3X A Highly Potent and Selective Plasmin Inhibitors Based on the Sunflower Trypsin Inhibitor-1...	3.12e-52	101/222 (45.5%)	1-237 (100.0%)	17-241
pdb 601UIA A Structure of plasmin and peptide complex [Homo sapiens] >pdb 601U B Structure of plasmin ...	3.19e-52	101/222 (45.5%)	1-237 (100.0%)	20-244
pdb 5UGD A Protease Inhibitor [Homo sapiens] >pdb 5UGG A Protease Inhibitor [Homo sapiens]	3.27e-52	101/222 (45.5%)	1-237 (100.0%)	22-246
pdb 6D40 A Highly Potent and Selective Plasmin Inhibitors Based on the Sunflower Trypsin Inhibitor-1...	3.53e-52	101/222 (45.5%)	1-237 (100.0%)	19-243
<input checked="" type="checkbox"/> pdb 7E50 B Chain B, Plasminogen [Homo sapiens]	4.31e-52	101/222 (45.5%)	1-237 (100.0%)	26-250
pdb 1DDJ A Chain A, PLASMINOGEN [Homo sapiens] >pdb 1DDJ B Chain B, PLASMINOGEN [Homo sapiens] >pdb ...	6.71e-52	100/222 (45.0%)	1-237 (100.0%)	18-242

Submit

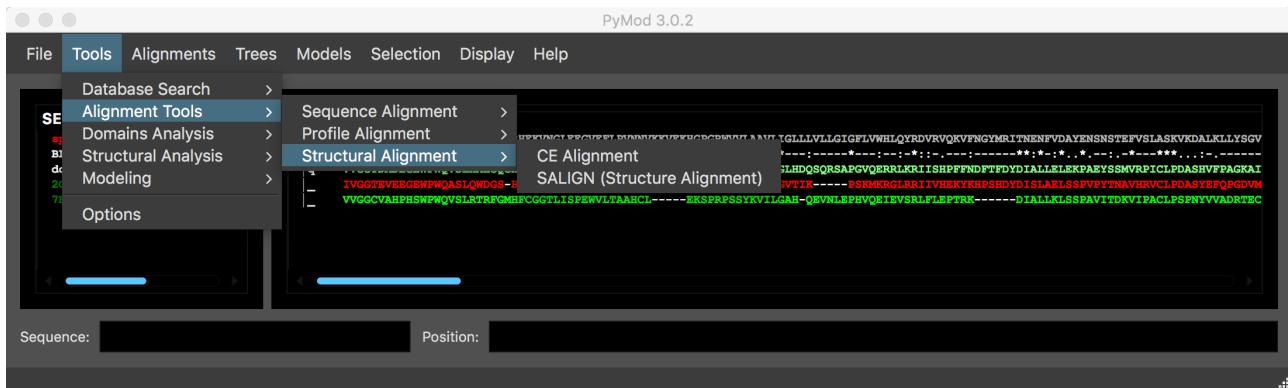
7a. In the window with the PyMod sequences, a cyan square with the sign "-" will appear (cluster of sequences). Pressing the right button on the PDB sequence **2Q5**, select "Structure -> Fetch PDB File".

8a. In the window that appears, select "*Import in PyMod only the structure of the hit sequences fragments*" and click on "Import 3D Structures". A new structure will appear in PyMOL.

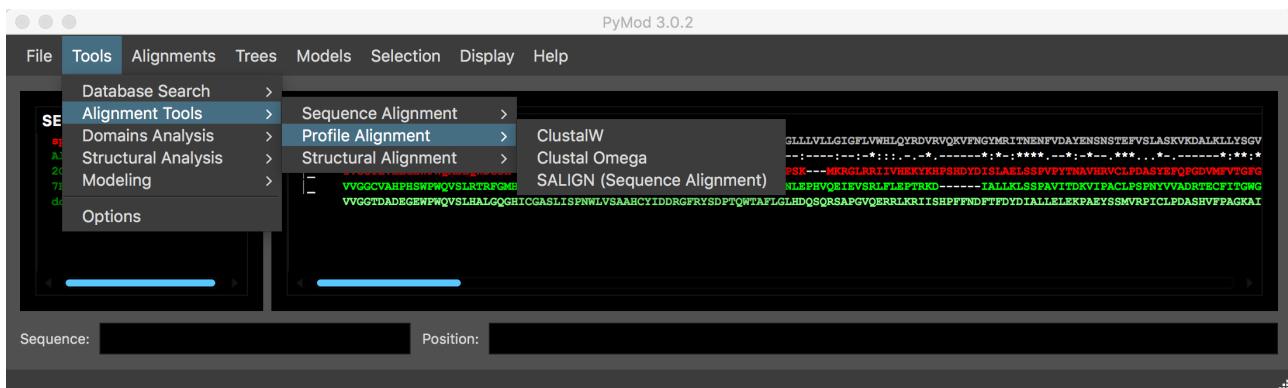


9a. Repeat step 7a and 8a with PDB **1DDJ**.

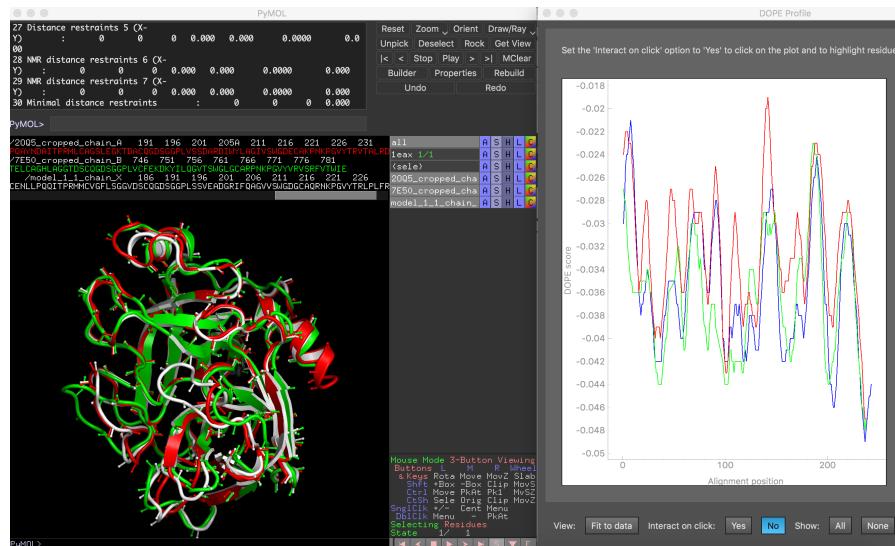
10a. Select both PDB sequences and click on “Tools -> Alignment Tools -> Structural Alignment -> CE Alignment” menu. In the windows that appear, click “Yes”. Note that the two structures have been superposed and the corresponding sequences have been aligned. A window with the corresponding RMSD appears.



11a. Now select the sequence to be modelled and the previously obtained cluster of aligned structures (*Alignment – CE Alignment*), and click on “Tools -> Alignment Tools -> Profile Alignment -> ClustalW”. Click on “Submit” in the window which appears:



12a. Select the sequence you want to model and click on “Tools -> Modeling -> Modeler (Homology Modeling)”. In the window that appears, select all the template by checking the corresponding square. Finally, click on Submit. After a few minutes, the MT-SP1 model will appear on the main PyMOL window, along with an Energy Profile Plot:



13a. Select the modelled sequence, click on “Tools -> Structural Analysis -> Assess with Dope” to pinpoint the regions with less reliability.

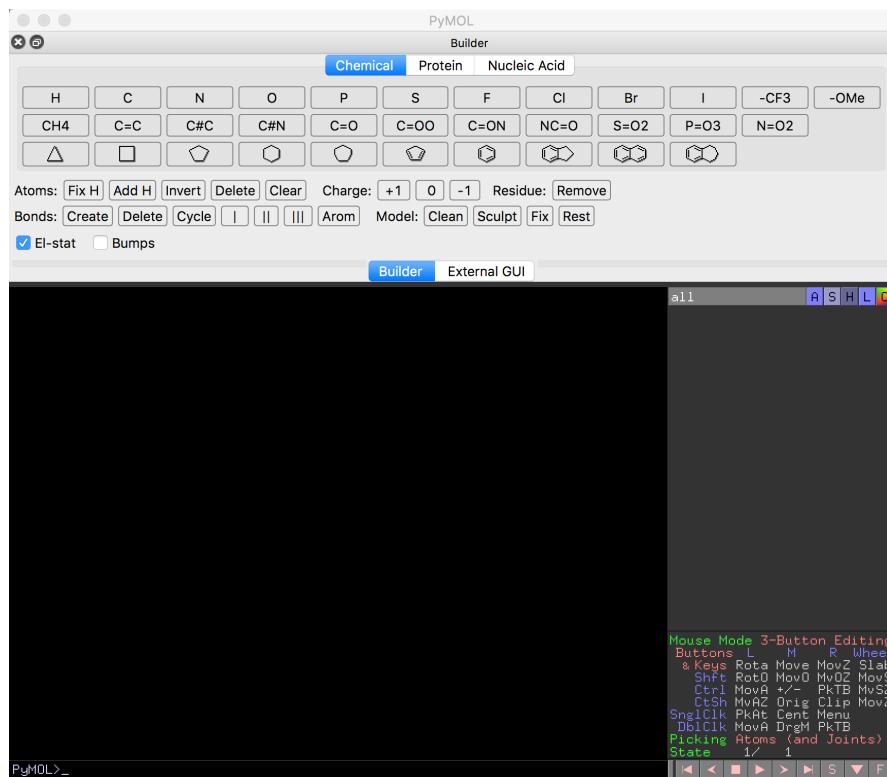
14a. Save your model in PyMOL: “File -> Export Molecule...” from PyMOL menu. Select the model and save as PDB file:



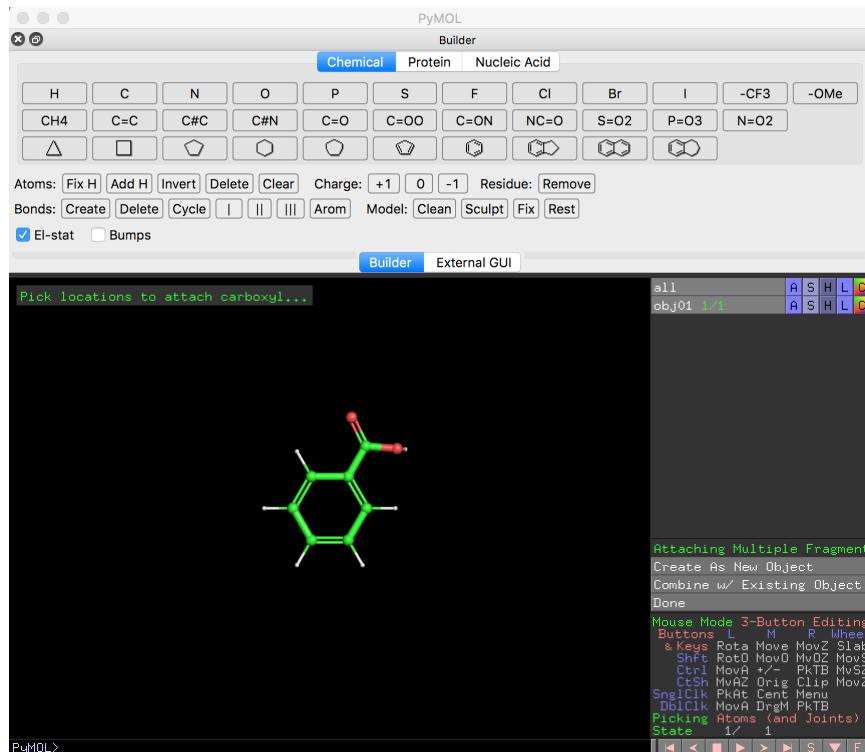
15a. Compare the obtained model with the corresponding crystal structure (PDB: 4IS5) by importing the latter into PyMOL (*File -> Get PDB ...*) (enter the code 4IS5). Discuss the differences between the obtained model and the crystal structure, and the ability of the Dope Score to assess the less reliable regions.

## B. Using PyMOL to build the benzamidine molecule

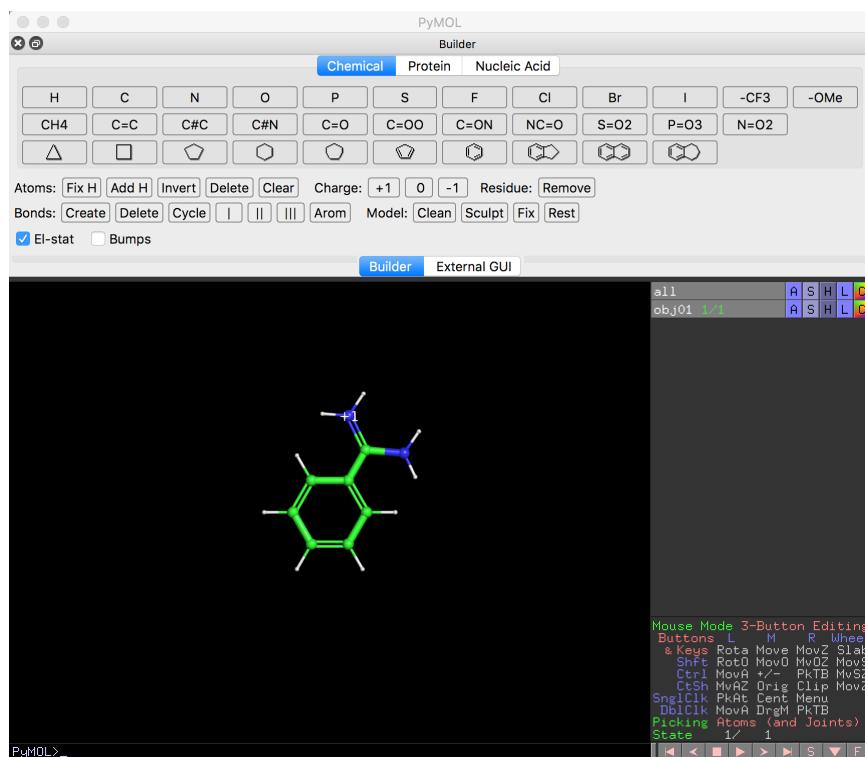
1b. Open the Builder window of PyMOL from the right panel of the external GUI:



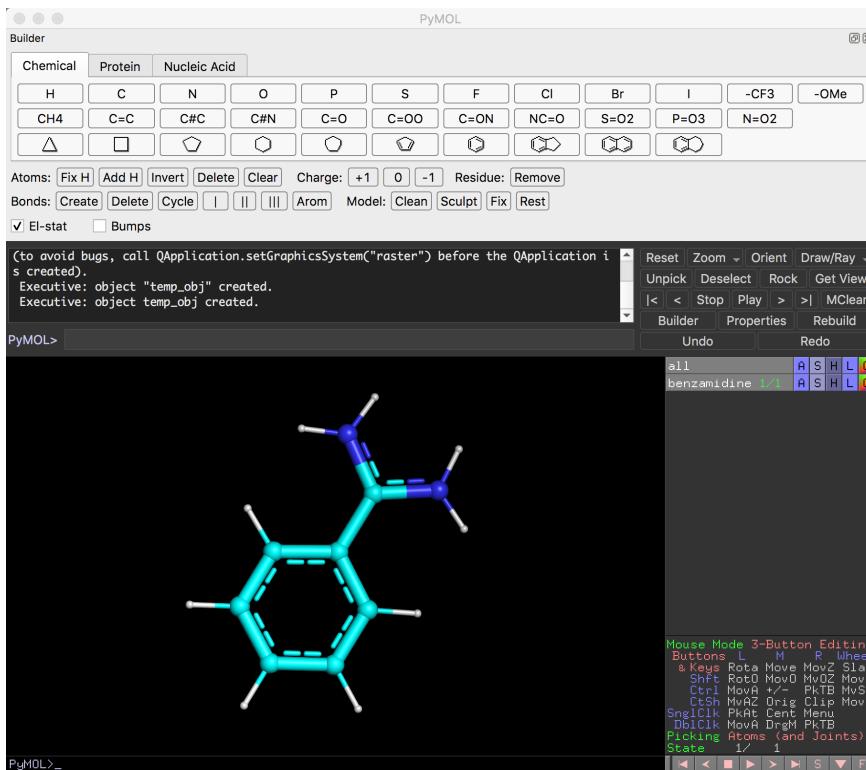
2b. Use the builder to initially build a benzoic acid. Click on the Benzene ring, and then on “create as a new object” on the right menu, then on “Done”. Then, add a carboxyl moiety by clicking on one of the hydrogen atoms of the benzene ring:



3b. Replace the Oxygen atoms with Nitrogen, and add a positive charge on each of them. Click on “N” and then click the Oxygen atoms of the molecule. Finally, click on “+1” and select the N atoms.



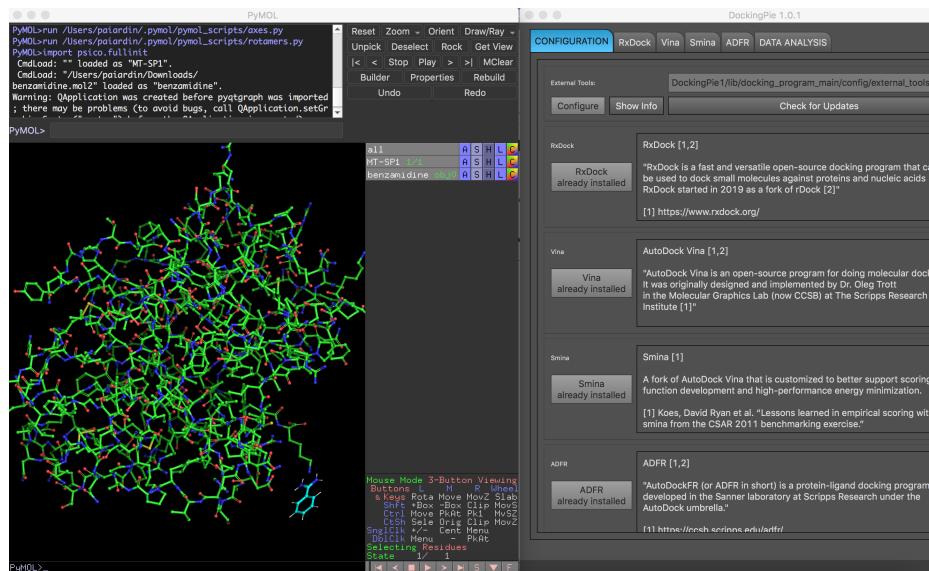
4b. Replace the double bonds of the guanidinium moiety with partial-double bonds. Click on Done. Click on the “A” button of Obj01, then “Clean” to energy minimize the molecule. Save the molecule as a “.mol2” file (*File -> export molecule*):



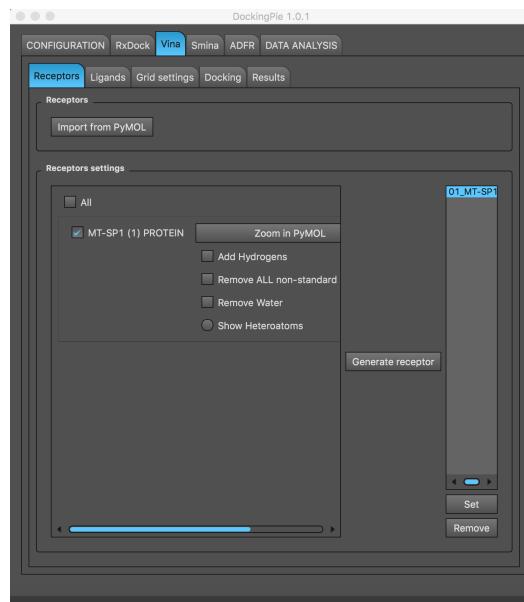
### C. Using the DockingPie plugin of PyMOL to dock the benzamidine molecule into MT-SP1

1c. Launch the PyMOL program, and import the MT-SP1 model and benzamidine (*File -> Open...*). Click on the “A” button beside the object “All” and select “Zoom”.

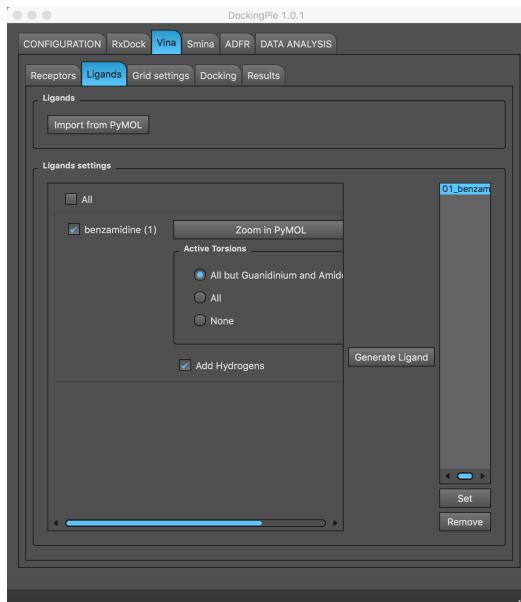
2c. From the "Plugins" menu, select "DockingPie 1.0". A new window will appear:



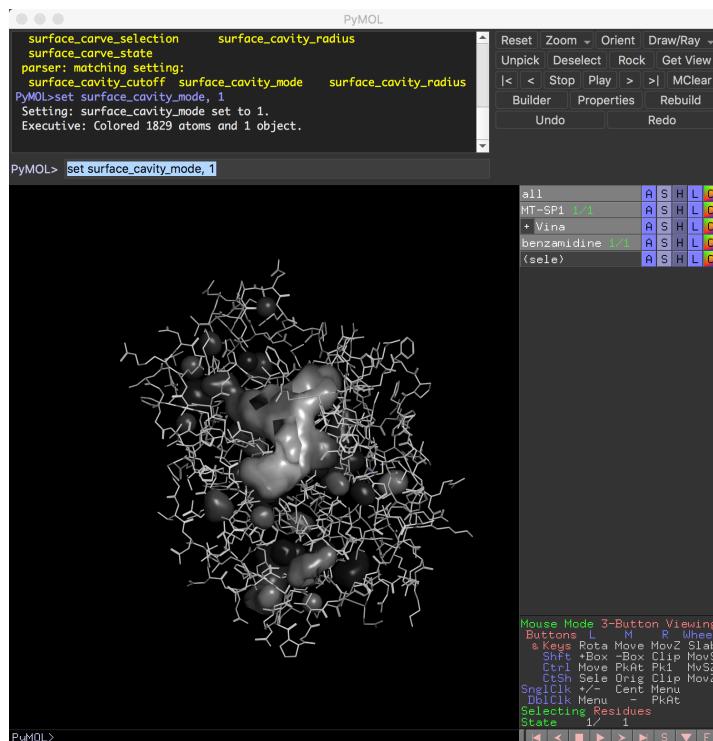
3c. Select the Tab “Vina”, then in the sub-Tab “Receptors”, select “Import from PyMOL” and select the MT-SP1 model. In the “Receptor Settings”, check the button corresponding to the model, and click on “Generate Receptor”. Select the newly generated object on the right column and click “Set”:



4c. Select the sub-Tab “Ligands”, select “Import from PyMOL” and select the benzamidine model. In the “Ligands Settings”, check the button corresponding to the benzamidine, and click on “Generate Ligand”. Select the newly generated object on the right column and click “Set”:



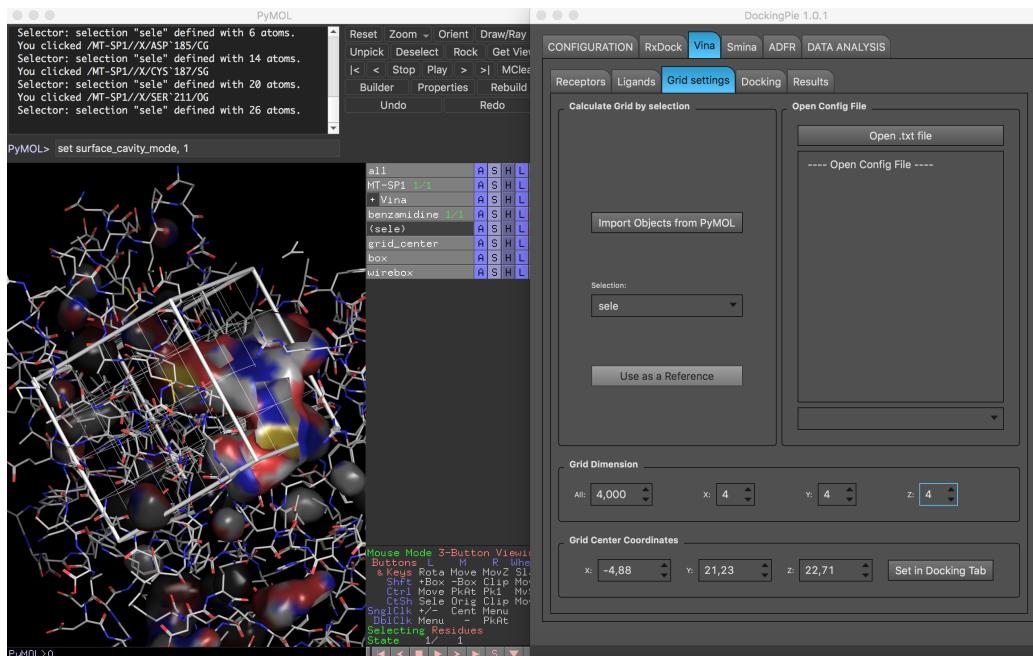
5c. In PyMOL's command input area, type “*set surface\_cavity\_mode, 1*” and press enter. Click on the “S” button beside the MT-SP1 macromolecules, and choose “Surface”. Color the molecule in grey. A cavity of the active site should appear:



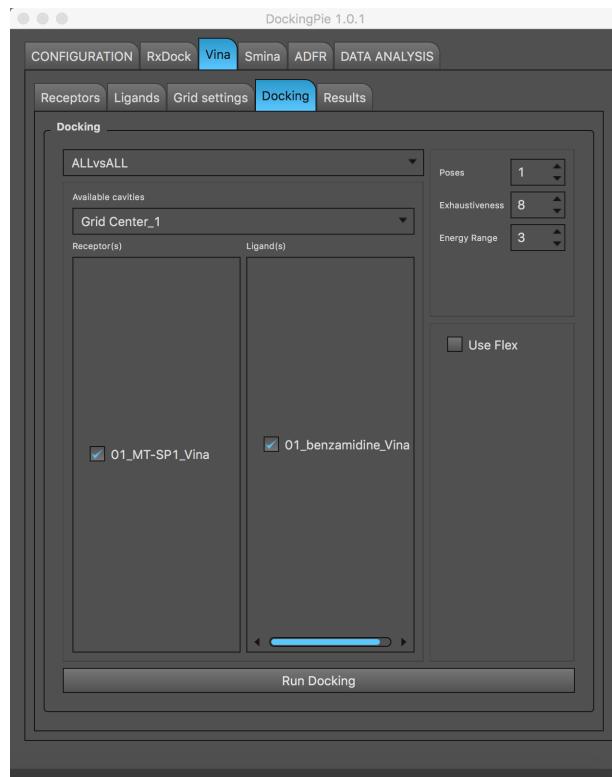
6c. Open the PyMOL sequence viewer. Select the following residues surrounding the cavity:

SER`191  
ASP`185  
CYS`187  
SER`211

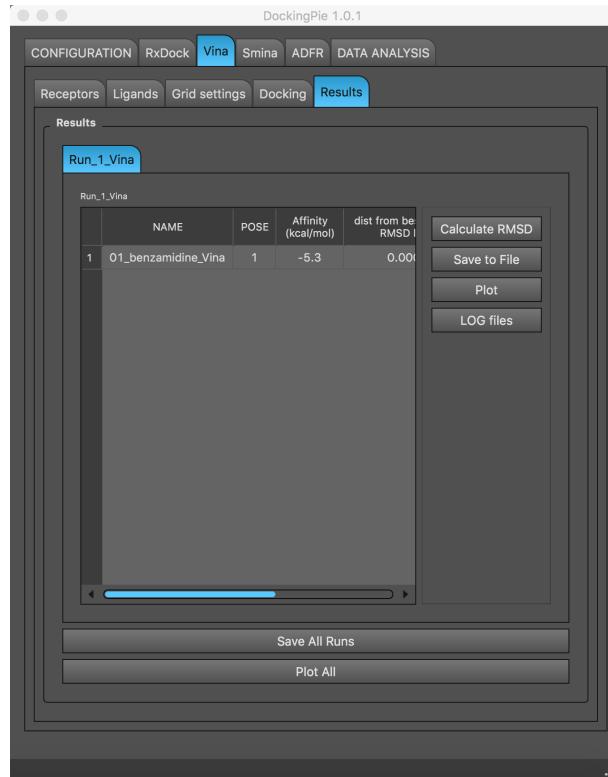
Then, in the “Grid Settings” sub-Tab of DockingPie, click on “Import Objects from PyMOL”, and select “sele” from the “selection” menu. A Grid should appear in PyMOL. Set the grid dimensions to X=4, Y=4, Z=4. Click on “Set in Docking Tab”:



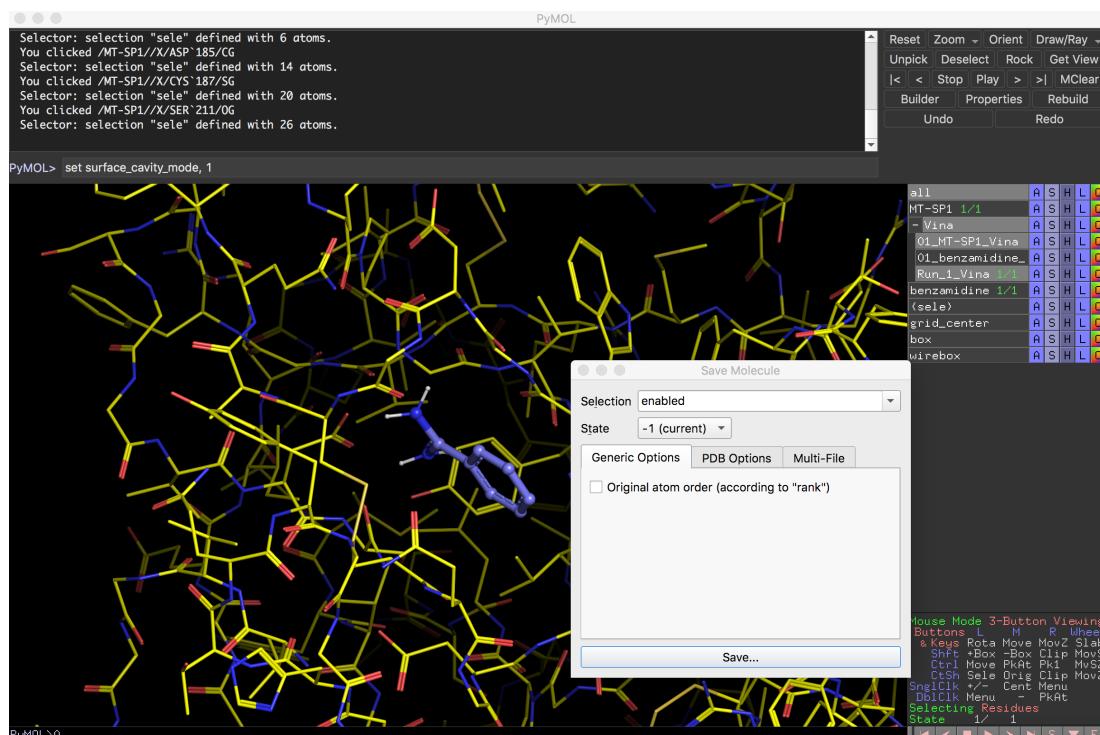
7c. Go to the “Docking” tab and select the ligand and the macromolecule. **Select 10 poses**. Click on “Run Docking”, and then “Start” on the window that appears:



8c. After a few seconds, the docked conformation of Benzamidine should appear in PyMOL. By clicking on the “Results” tab, the corresponding energy should be visualized. Annotate the value:



9c. Save the docking result by deselecting everything except the macromolecule and the ligand in the PyMOL internal GUI, and selecting to “File -> Export Molecule..” in PyMOL, choosing “enabled” as the molecules to save:



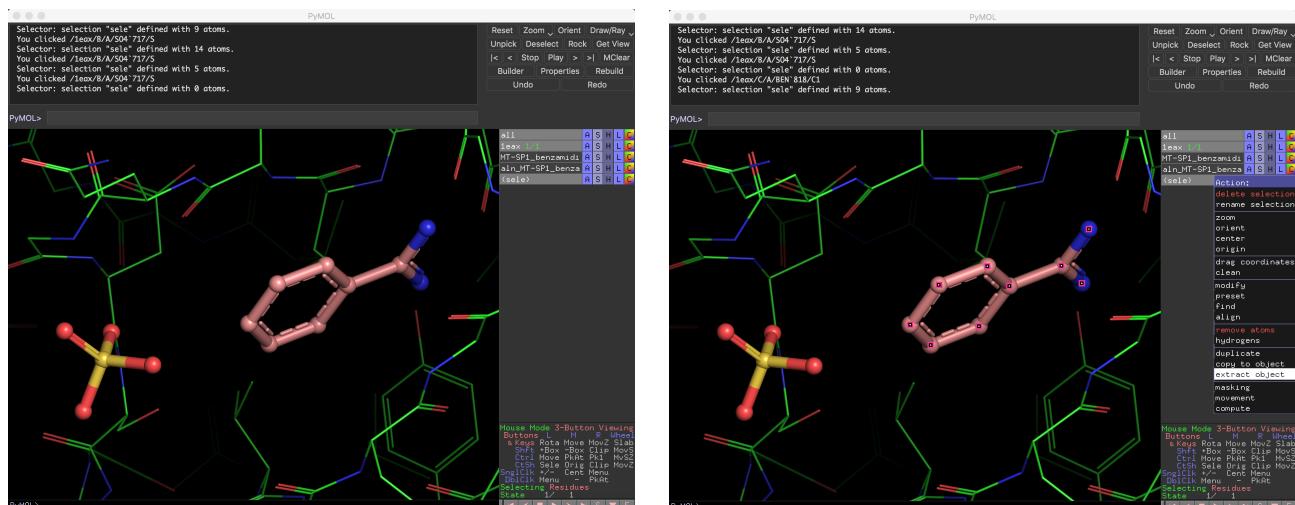
11c. Save as PDB file and assign the name “MT-SP1\_benzamidine\_complex.pdb”

12c. Compare the obtained result with the experimentally determined crystal structure: select “File -> Get PDB...” in PyMOL and type “**1EAX**” as PDB Code. Then, superpose the structures (go to the “A” button of 1EAX and select “Align -> to molecule -> 01\_MT-SP1\_Vina”). Click on “A” of “All” object and select “Zoom”. Discuss the differences between the obtained docked model and the crystal structure, and the ability of the Vina algorithm to predict the correct pose. Try with other docking engines (RXDock, Smina etc..).

#### D. Using PyMOL for rational, structure-based Drug Optimization

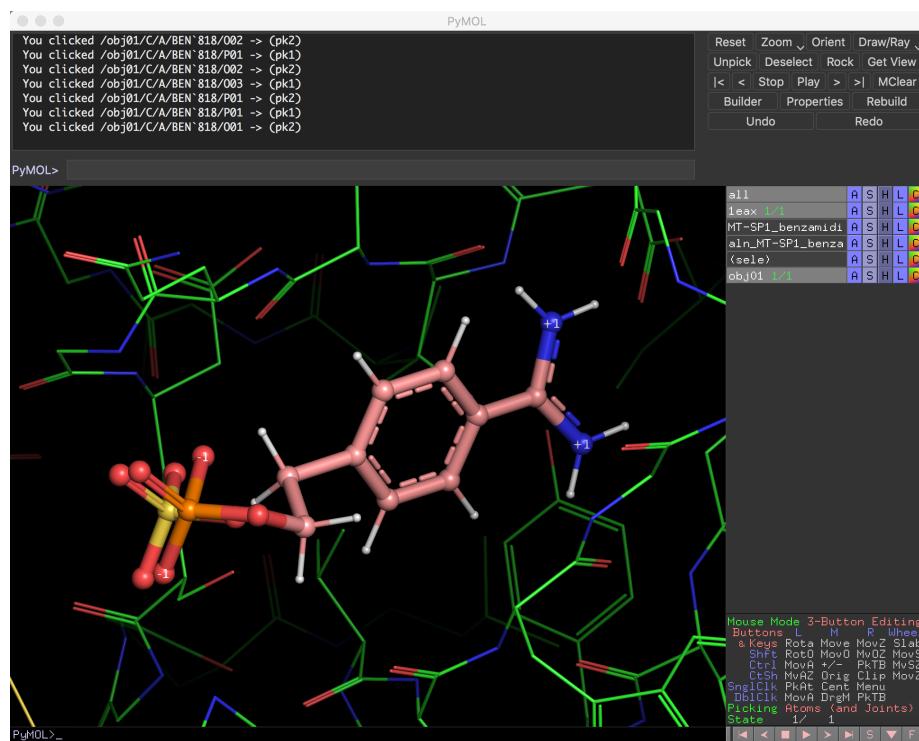
1d. Comparing the experimentally determined crystal structure of MT-SP1 with the obtained model, you’ll have noticed that one of the most striking differences is the presence of a Sulphate ion in the active site (Question: in your opinion, why is this molecule occupying the active site cleft?). We will exploit this information to rationally optimize the benzamidine moiety, in order to obtain a new compound with increased affinity (and potency).

2d. Select the benzamidine moiety, then click on the “A” button beside the “*sele*” object and select “extract object”. A new object called “**obj01**” will be obtained:

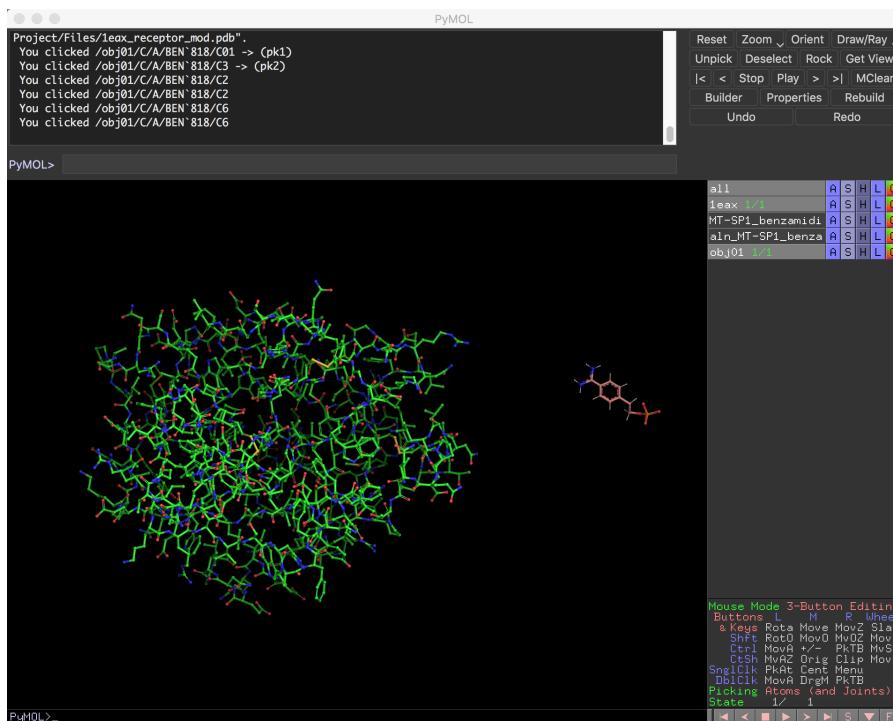


3d. Click on the “A” button beside the “*obj01*” object and select “*hydrogens -> add*”.

4d. Open the Builder window of PyMOL from the right panel of the external GUI. Click on hydrogen atom *in para* position of the guanidium group and replace it with a carbon atom. Add another carbon atom and finally a Phosphate moiety. Try to change the benzamidine structure using the Builder and modifying the dihedral angles of the covalent bonds (to do that, the mouse should be in “Editing mode”, then “Ctrl” (or Cmd on Mac) right-click on the bond and change the angle by moving the mouse), until you obtain a phospho-molecule which mimics the occupancy of the Sulphate ion:

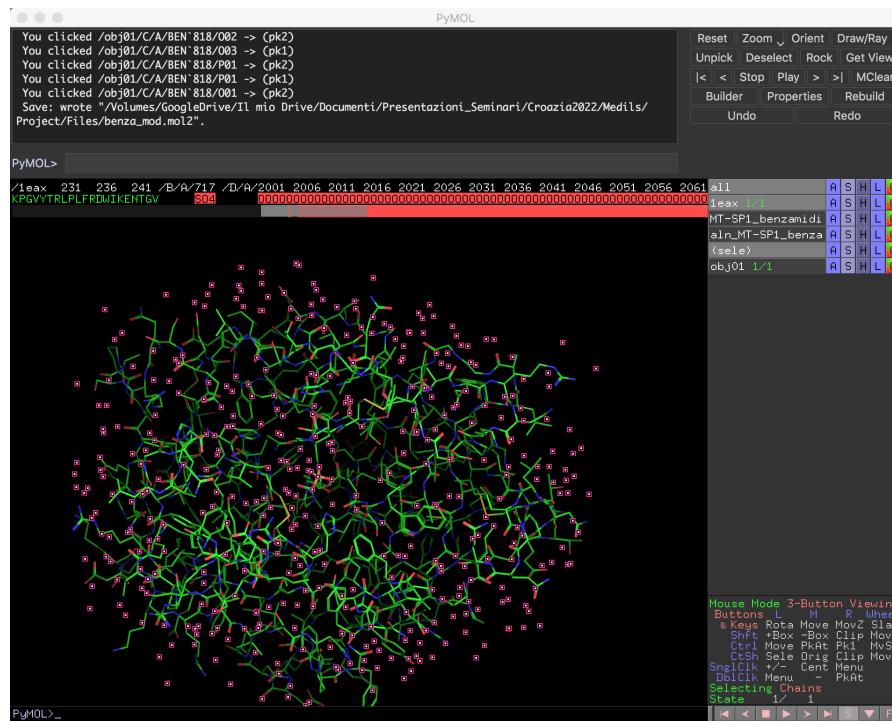


5d. Change the position of the new molecule to put it far from the active site: select “3-Button Editing mode” for the mouse on the lower right part of the internal GUI (*Mouse Mode*), then pressing “Shift” on your keyboard + the central button of your mouse, with the cursor on the new molecule translate it until is far from the active site:



6d. Save as .mol2 file the new molecule and assign the name “benza\_mod.mol2” (*File -> Export molecule -> (select obj01) -> Save as .mol2*).

7d. Since we're going to redock this new molecule in the crystal structure, we'll prepare the macromolecule by removing everything, except the polypeptide chain. Open the Sequence Viewer in PyMOL, and select the SO4 ion and water molecules (put the mouse in "selecting Chains" mode):



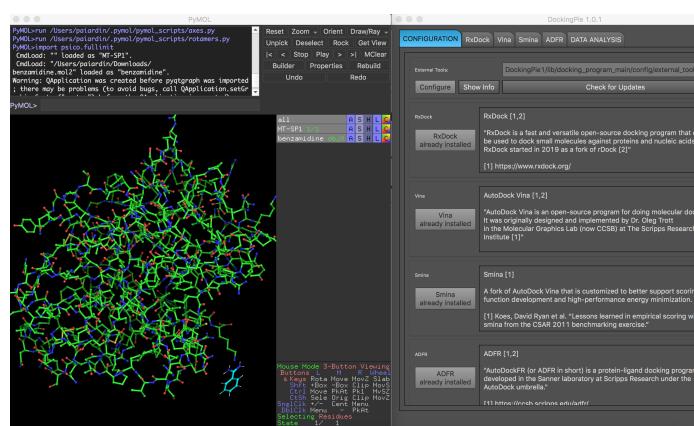
8d. Click on "A" button beside the new selection and select "remove atoms".

9d. Save as .pdb file the new molecule and assign the name "1EAX\_receptor\_mod.pdb" (File -> Export molecule -> (select 1eax) -> Save as .pdb).

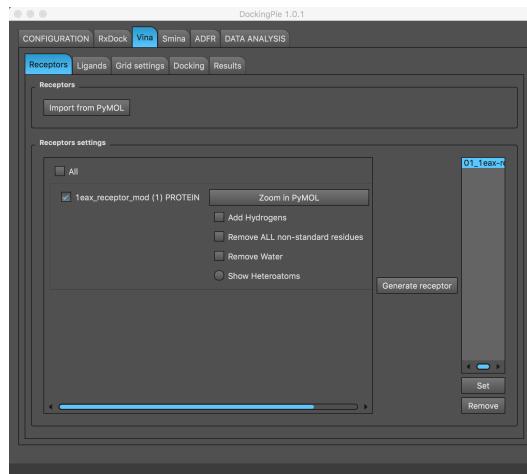
## E. Using the DockingPie plugin of PyMOL to dock the benza\_mod molecule into the crystal structure of MT-SP1

1e. Launch the PyMOL program, and import the 1EAX\_receptor\_mod.pdb and benza\_mod.mol2 (File -> Open...). Click on the "A" button beside the object "All" and select "Zoom".

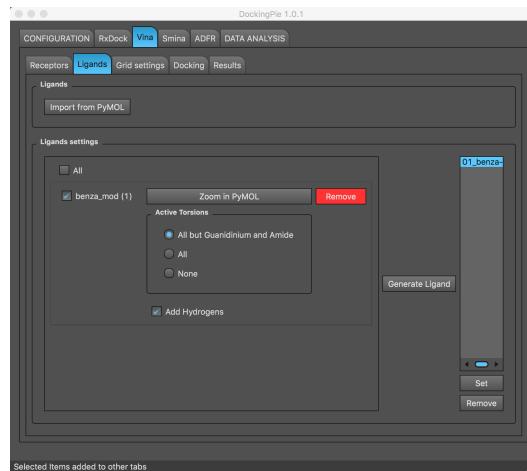
2e. From the "Plugins" menu, select "DockingPie 1.0". A new window will appear:



3e. Select the Tab “Vina”, then in the sub-Tab “Receptors”, select “Import from PyMOL” and select the ***1EAX\_receptor\_mod***. In the “Receptor Settings”, check the button corresponding to the protein, and click on “Generate Receptor”. Select the newly generated object on the right column and click “Set”:



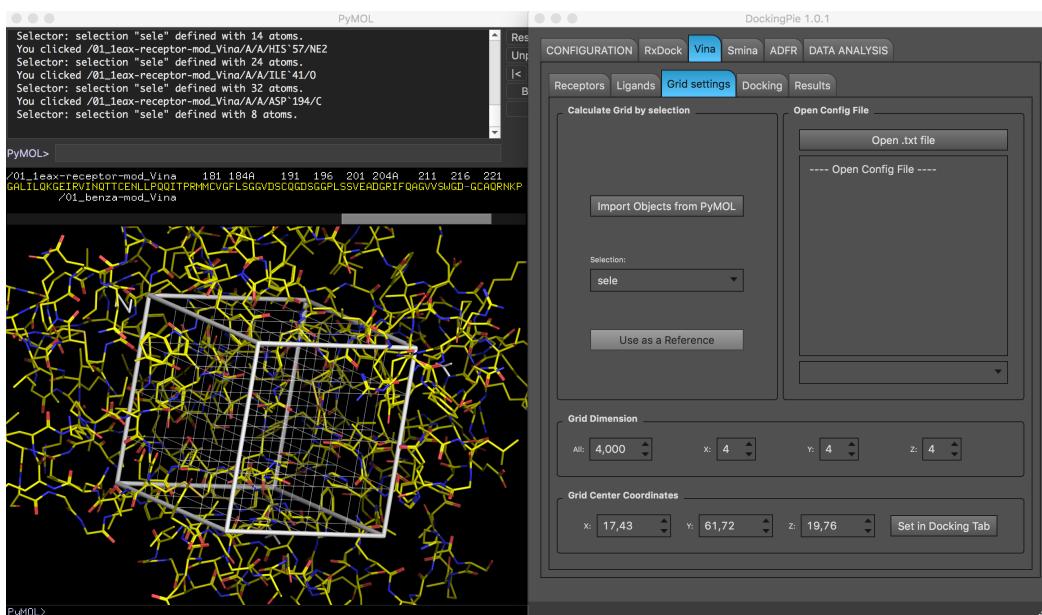
4e. Select the sub-Tab “Ligands”, select “Import from PyMOL” and select the ***benza\_mod*** model. In the “Ligands Settings”, check the button corresponding to the *benza\_mod*, and click on “Generate Ligand”. Select the newly generated object on the right column and click “Set”:



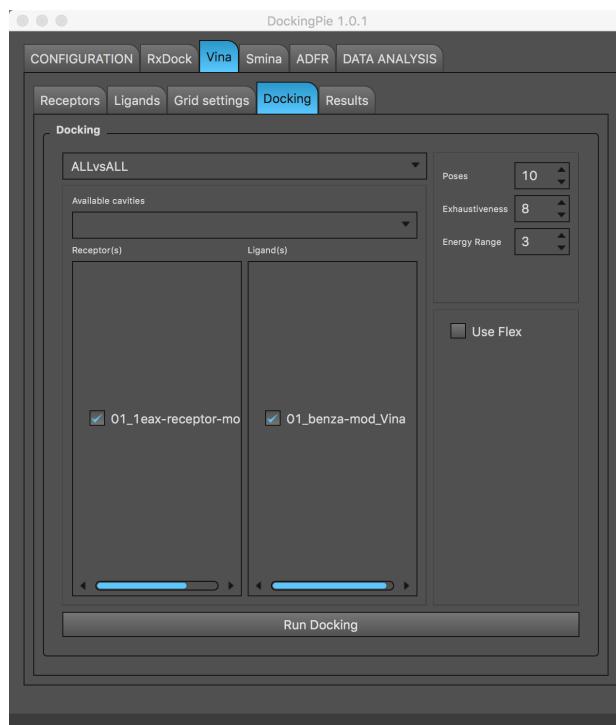
5e. Open the PyMOL sequence viewer. Select the following residues of ***1EAX\_receptor\_mod*** surrounding the cavity:

SER 195  
ASP 189  
HIS 57  
ILE 41

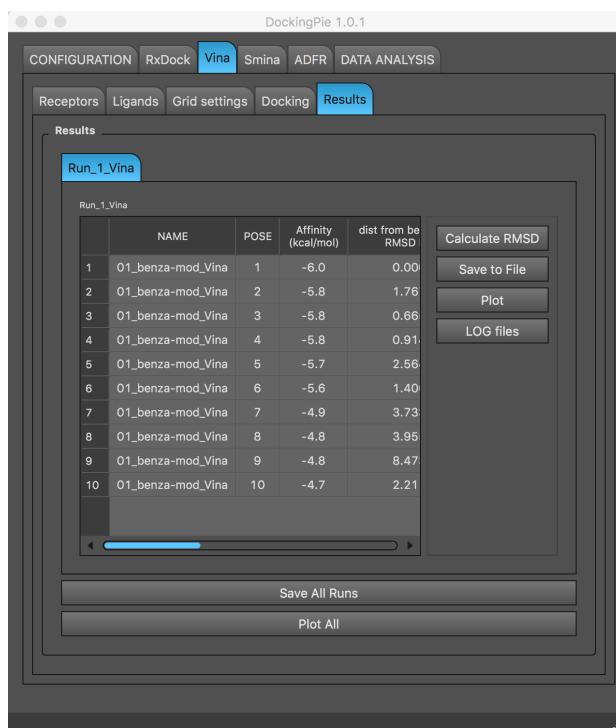
Then, in the “Grid Settings” sub-Tab of DockingPie, click on “Import Objects from PyMOL”, and select “sele” from the “selection” menu. A Grid should appear in PyMOL. Set the grid dimensions to X=4, Y=4, Z=4. Click on “Set in Docking Tab”:



6e. Go to the “Docking” tab and select the ligand and the macromolecule. Select 10 poses. Click on “Run Docking”, and then “Start” on the window that appears:



7e. After a few seconds, the docked conformation of Benza-mod should appear in PyMOL. By clicking on the “Results” tab, the corresponding energy should be visualized. Annotate the value of the pose which better resembles the position of the sulphate ion:



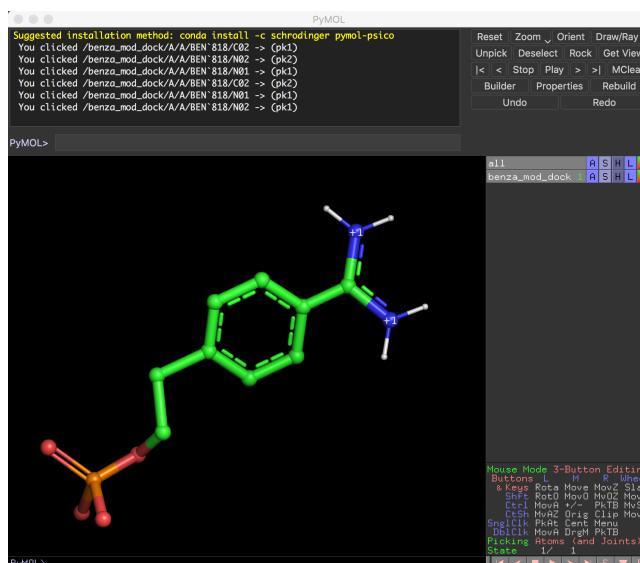
How is the energy compared to the benzamidine? Can you discuss the energy improvements?

8e. Save the docking result by deselecting everything except the macromolecule and the ligand in the PyMOL internal GUI, and selecting to “File -> Export Molecule...” in PyMOL, choosing “enabled” as the molecules to save.

9e. Save as PDB file and assign the name “*1EAX\_benzamod\_complex.pdb*”.

10e. Save the macromolecule alone as a PDB file (name it “*1EAX\_macromolecule.pdb*”)

11e. using the PyMOL builder, modify the benza\_mod as follows and save and the ligand as .mol2 (name it “**benza\_mod\_dock.mol2**”):



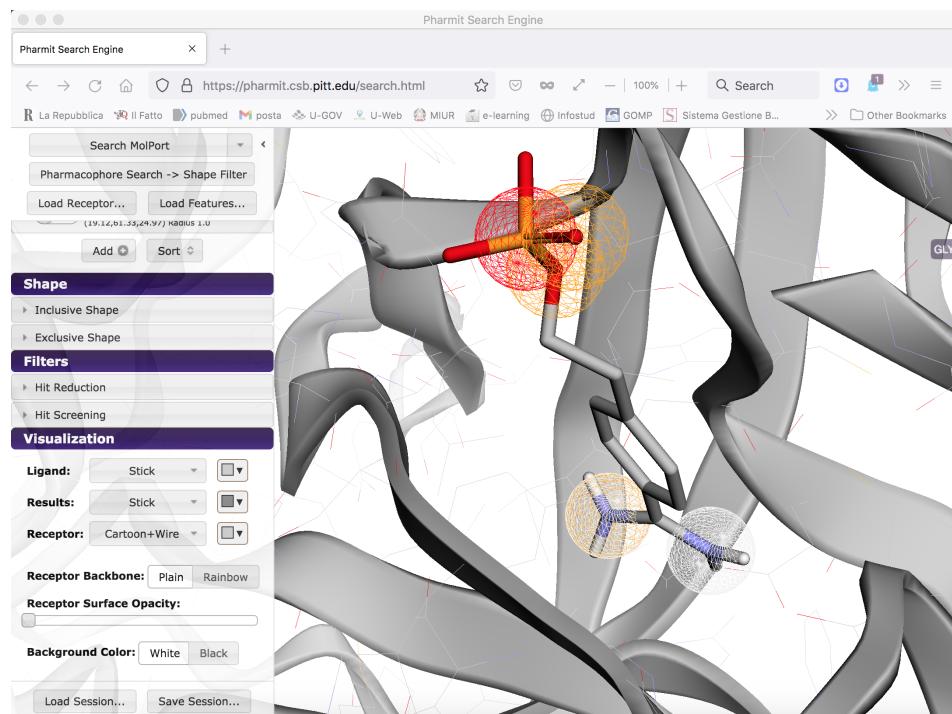
## F. Using the PHARMIT server to carry out a pharmacophore-based Virtual Screening on MT-SP1

1f. Connect to PHARMIT using [Firefox](https://pharmit.csb.pitt.edu) (<https://pharmit.csb.pitt.edu>) and click on “Enter Pharmit Search”.

2f. Click on “Load Receptor...” on the left column and choose “**1EAX\_macromolecule.pdb**”.

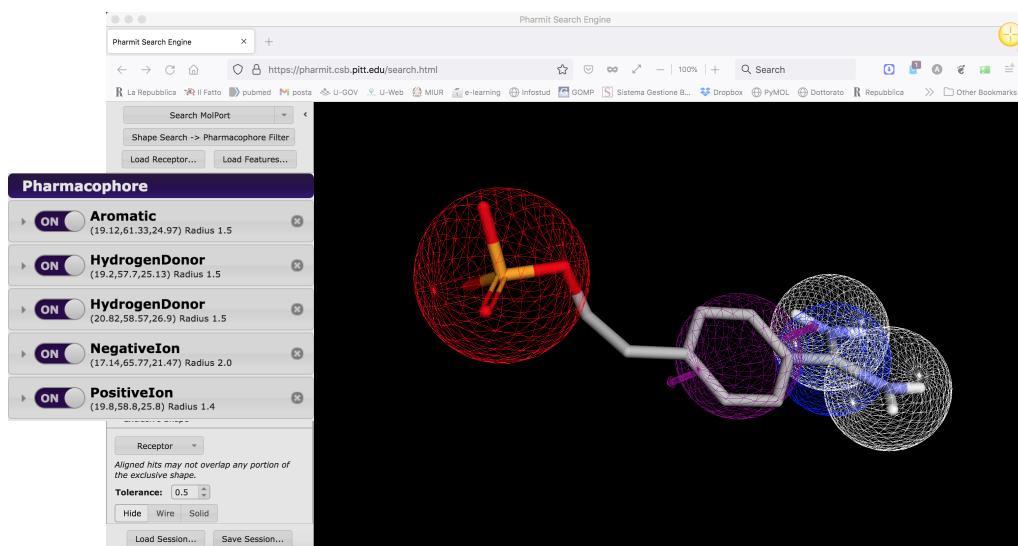
3f. Click on “Load features...” on the left column and choose “**benza\_mod\_dock.mol2**”:

4f. Set the “Receptor Surface Opacity” bar to the minimum value:

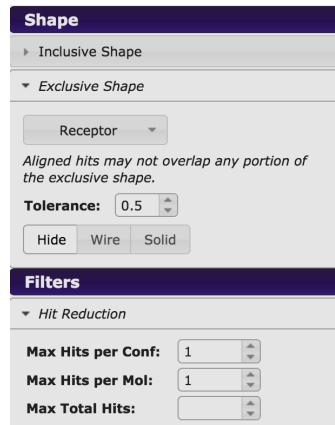


5f. Set “background color” to black.

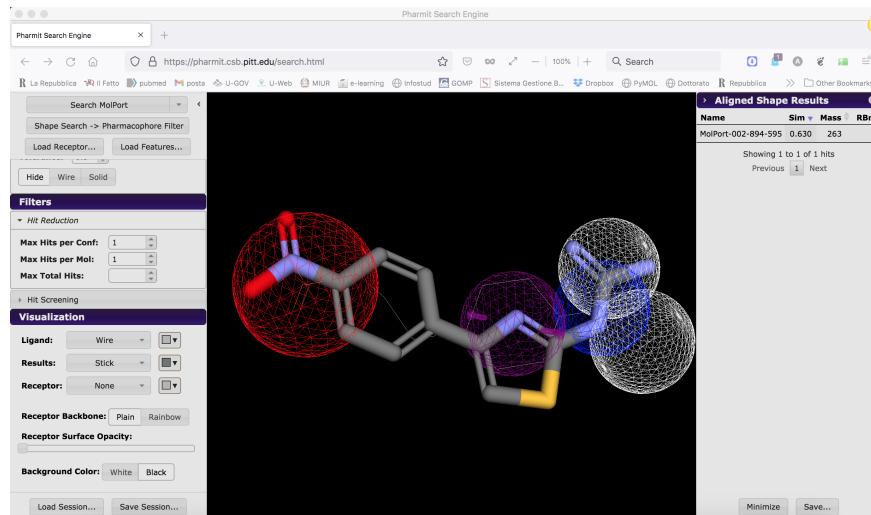
6f. Set the following pharmacophore map:



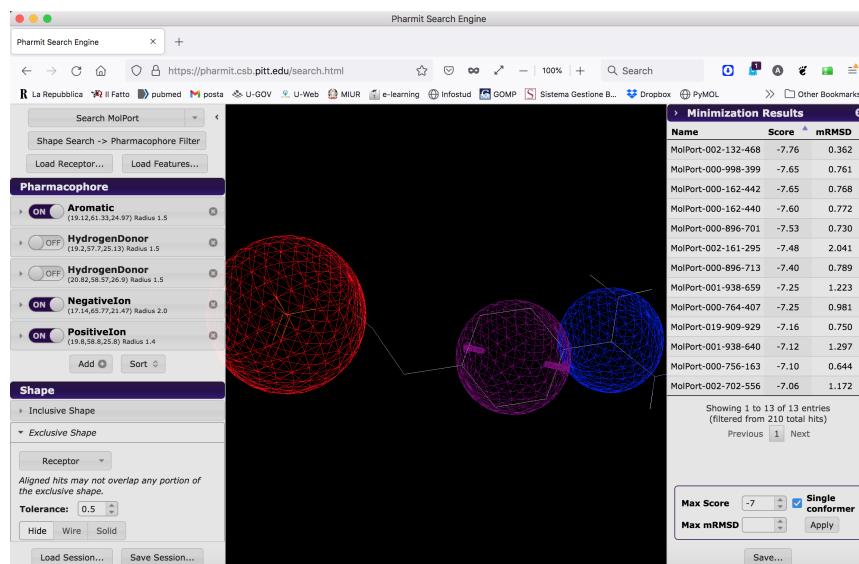
7f. Set the following values:



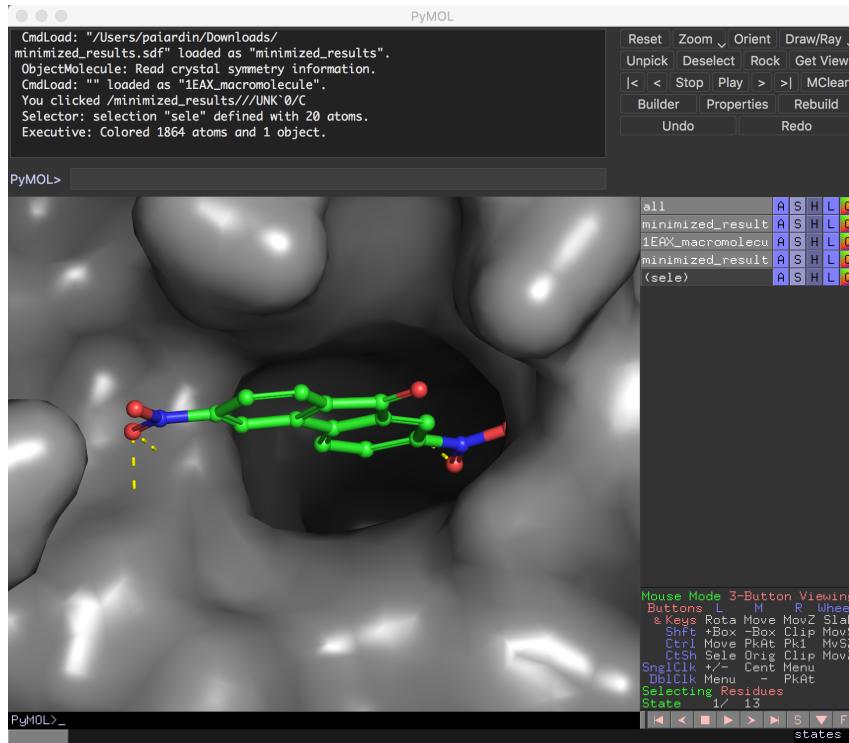
8f. Click on "Search MolPort":



9f. Try to repeat the search by removing the PH-constraints of the Hydrogen-bond donors. Minimize the obtained results and keep only the Results with Score < -7.0 (single conformer):



10f. Try different settings, and each time save the obtained results (Save...) as .sdf files. Explore the obtained results in PyMOL (by using the movie buttons on the bottom part of the internal GUI):



11f. Split the sdf in single states (Action -> State -> Split)

12f. As a final exercise, repeat the Dockings with DockingPie and rank the obtained compounds. Discuss the obtained results.

