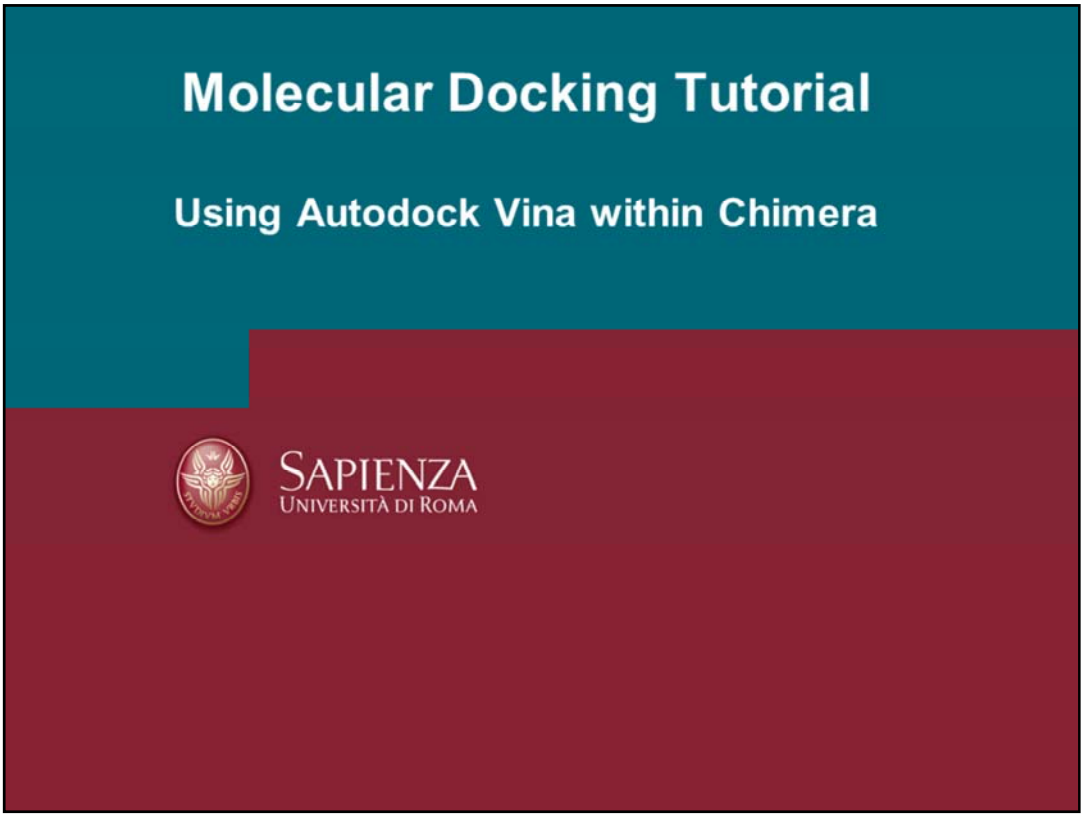
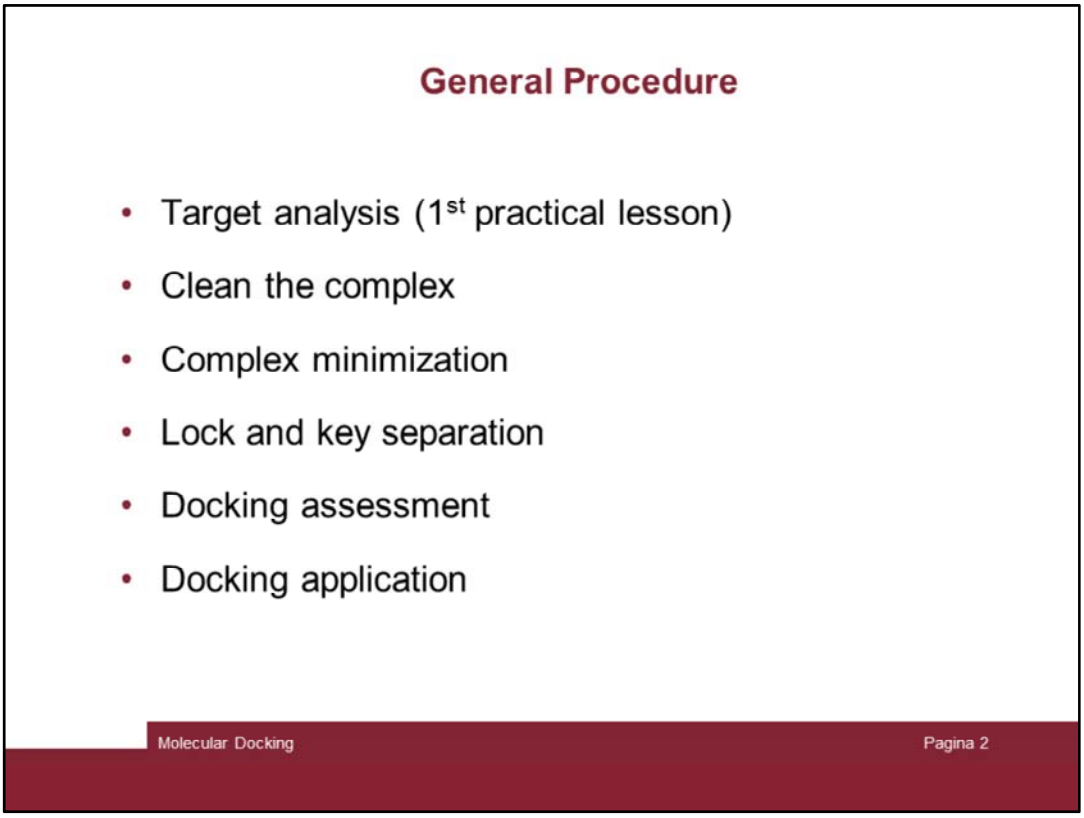


By following this tutorial the user will learn how to perform molecular docking by means of Autodock Vina program.

Docking assessment on a given experimental key/lock complex will be carried out by:

1) Evaluation of ligand docking starting from the experimental ligand conformation (experimental conformation re-docking – ECRD)
2) Evaluation of ligand docking starting from a random generate ligand conformation (random conformation re-docking – RCRD)

Then the binding mode of a molecule reported in an article will be evaluated.



Here is the sequence of minimal operation to set up a docking study.

First the target and its role has to be inspected.

Then a series of actions are to first validate the docking program and apply it

Reference Article

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Phenyl Ether- and Aniline-Containing 2-Aminoquinolines as Potent and Selective Inhibitors of Neuronal Nitric Oxide Synthase

Maris A. Cinelli,[†] Huiying Li,[‡] Anthony V. Pensa,[†] Soosung Kang,[†] Linda J. Roman,[§] Pavel Martásek,[∥,⊥] Thomas L. Poulos,*[,‡] and Richard B. Silverman*[,†]

[†]Department of Chemistry, Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113, United States

[‡]Departments of Molecular Biology and Biochemistry, Pharmaceutical Sciences, and Chemistry, University of California, Irvine, California 92697-3900, United States

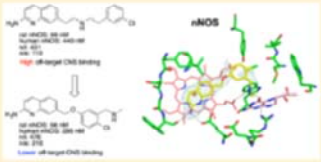
[§]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78384-7760, United States

[∥]Department of Pediatrics, First Faculty of Medicine, Charles University, Prague, Czech Republic

[⊥]BIOCEV, Prague, Czech Republic

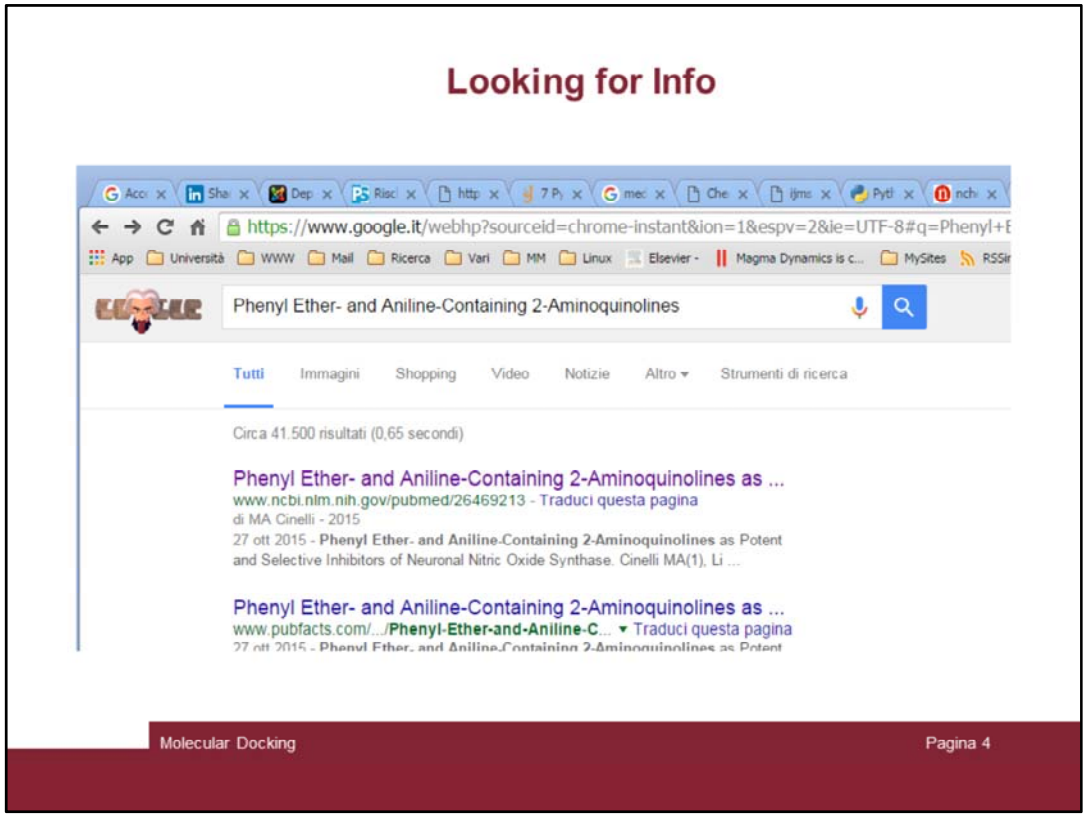
Supporting Information

ABSTRACT: Excess nitric oxide (NO) produced by neuronal nitric oxide synthase (nNOS) is implicated in neurodegenerative disorders. As a result, inhibition of nNOS and reduction of NO levels is desirable therapeutically, but many nNOS inhibitors are poorly bioavailable. Promising members of our previously reported 2-aminoquinoline class of nNOS inhibitors, although orally bioavailable and brain-penetrant, suffer from unfavorable off-target binding to other CNS receptors, and they resemble known promiscuous binders. Rearranged phenyl ether- and aniline-linked 2-aminoquinoline derivatives were therefore designed to (a) disrupt the promiscuous binding pharmacophore and diminish off-target interactions and (b) preserve potency, isoform selectivity, and cell permeability. A series of these compounds was synthesized and tested against purified nNOS, endothelial NOS (eNOS), and inducible NOS (iNOS) enzymes. One compound, 20, displayed high potency, selectivity, and good human nNOS inhibition, and retained some permeability in a Caco-2 assay. Most promisingly, CNS receptor counterscreening revealed that this rearranged scaffold significantly reduces off-target binding.

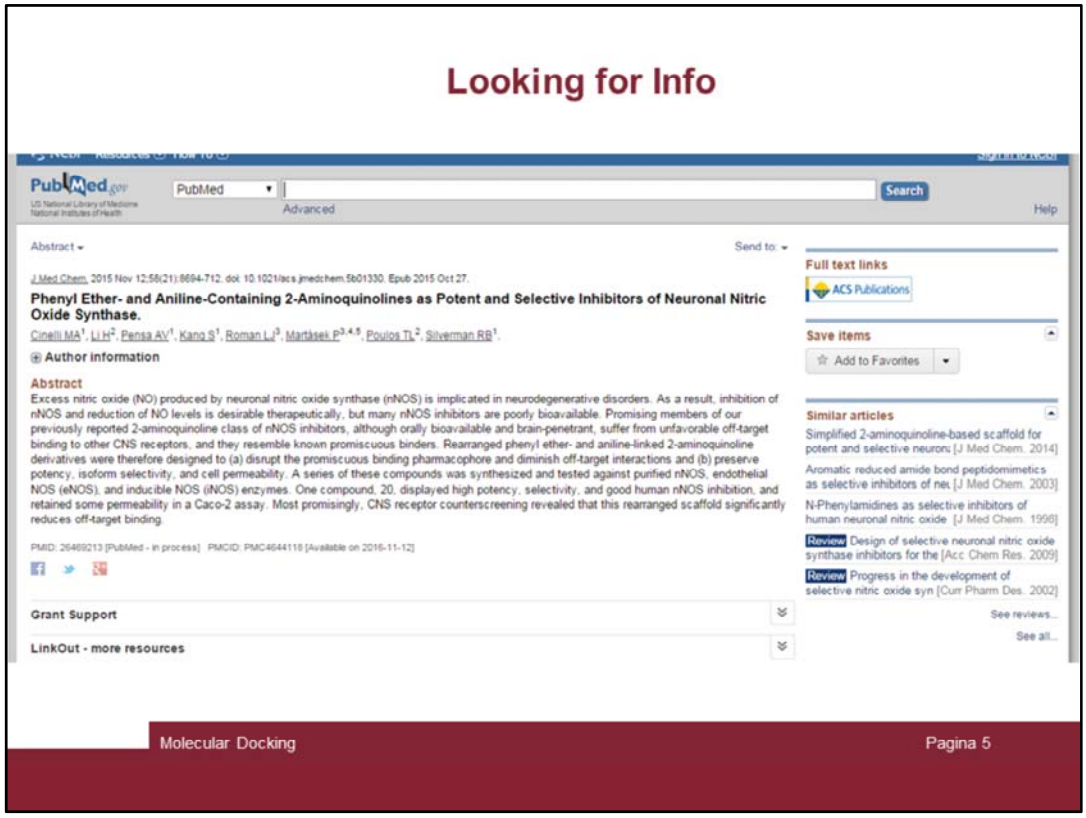


Molecular Docking Pagina 3

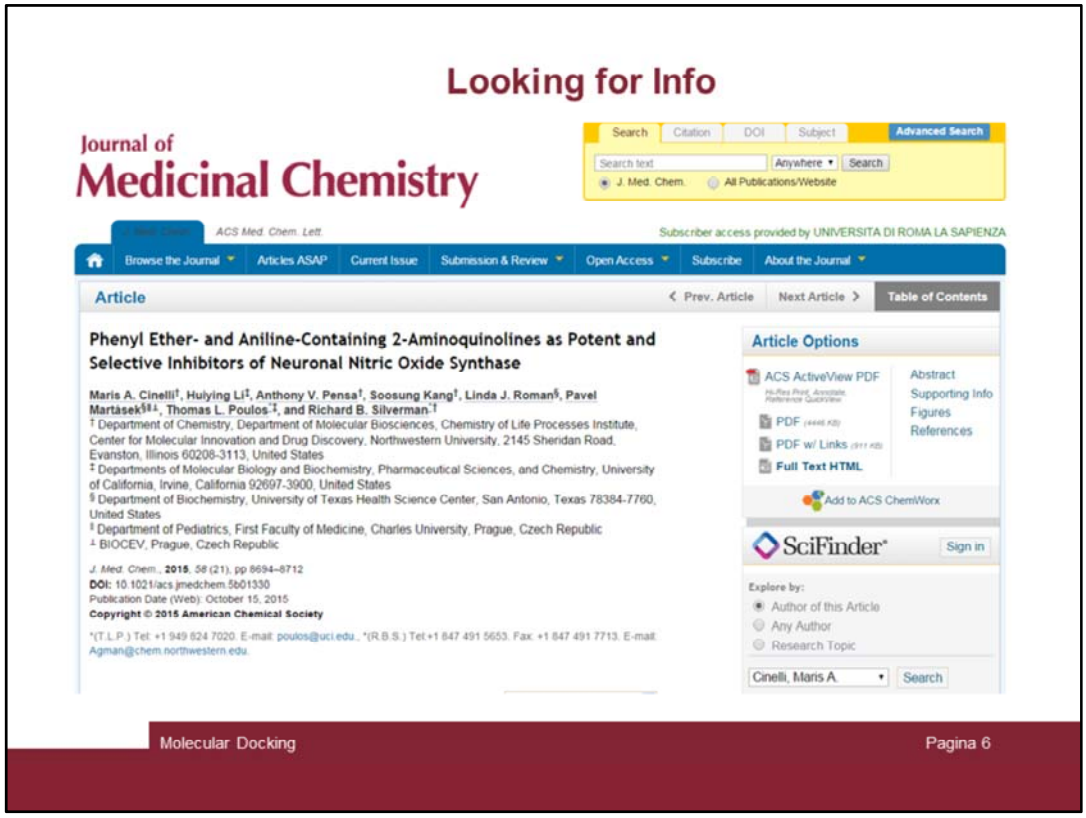
In this tutorial the reference article is here reported, where a series of selective NOS inhibitors are described. Reading the article it seems compound 17 is the most interesting one, so we will focus on that.



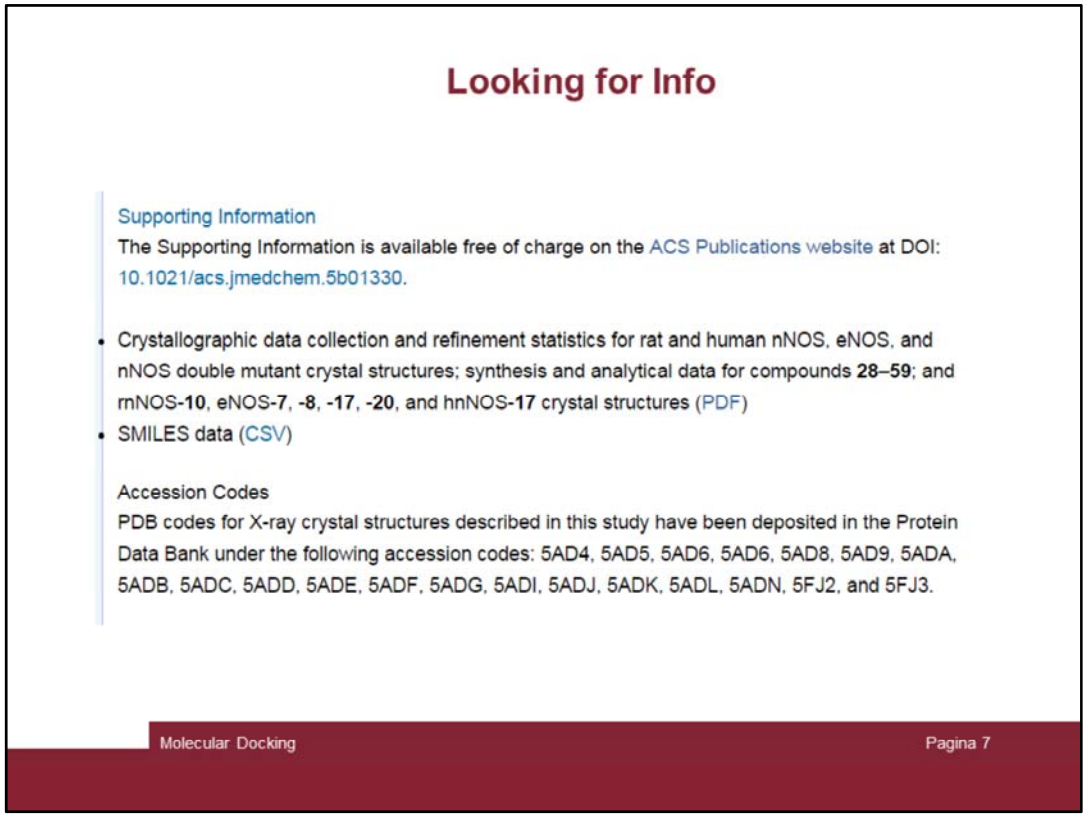
Further info can be gathered in the supporting information included in the article as external files. To get them let's search for the article in the www by googling its title. Then click on the results.



Open up the pubmed page and then click on the ACS Publications icon.

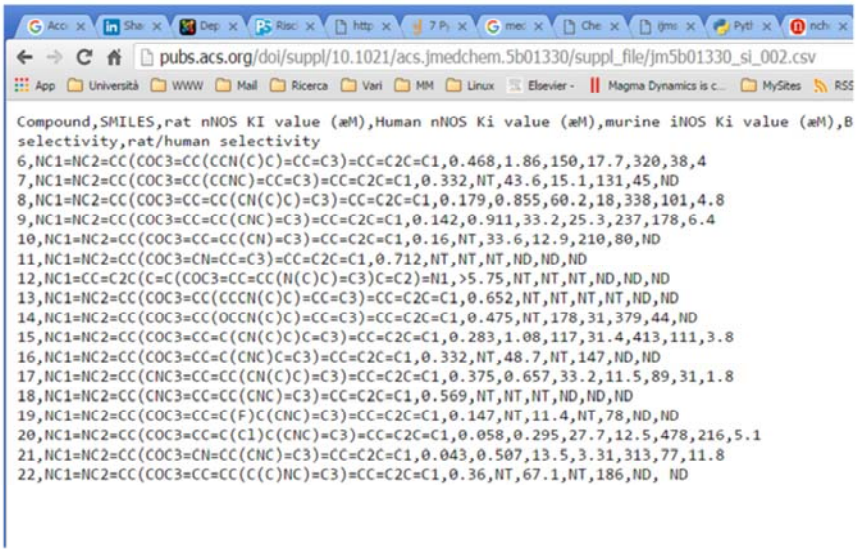


… here is the article directly on the ACS web portal.



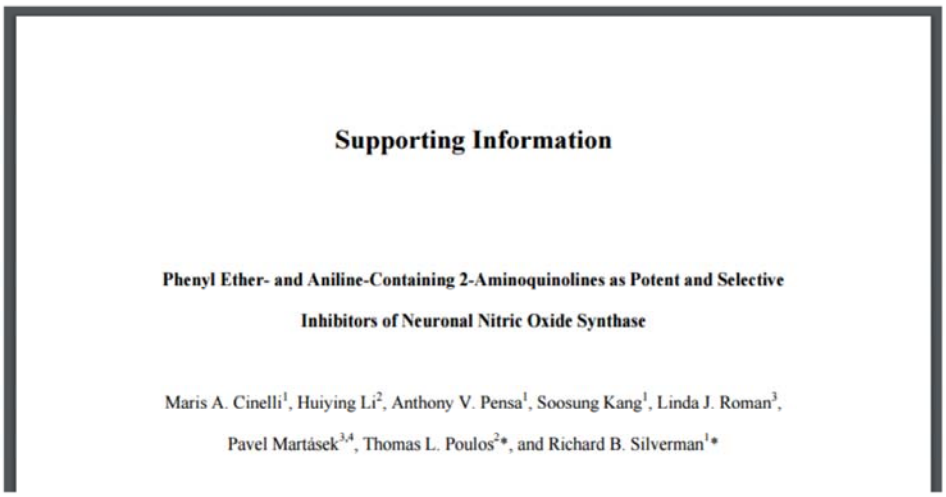
Let’s scroll down it till supporting information appear

Looking for Info



The «csv» file contains the smiles structures and other info

Looking for Info



Supporting Information

Phenyl Ether- and Aniline-Containing 2-Aminoquinolines as Potent and Selective Inhibitors of Neuronal Nitric Oxide Synthase

Maris A. Cinelli[1], Huiying Li[2], Anthony V. Pensa[1], Soosung Kang[1], Linda J. Roman[3], Pavel Martásek[3,4], Thomas L. Poulos[2]*, and Richard B. Silverman[1]*

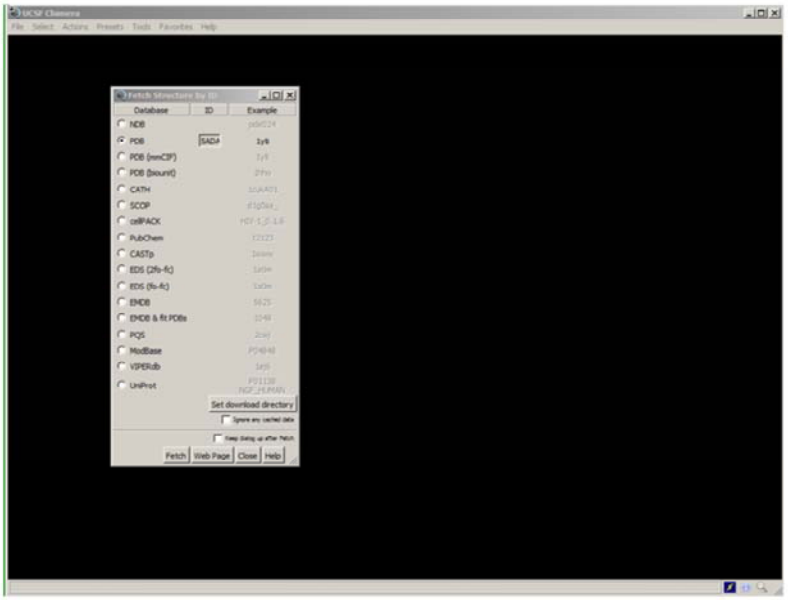
The «pdf» file contains others info

Looking for Info

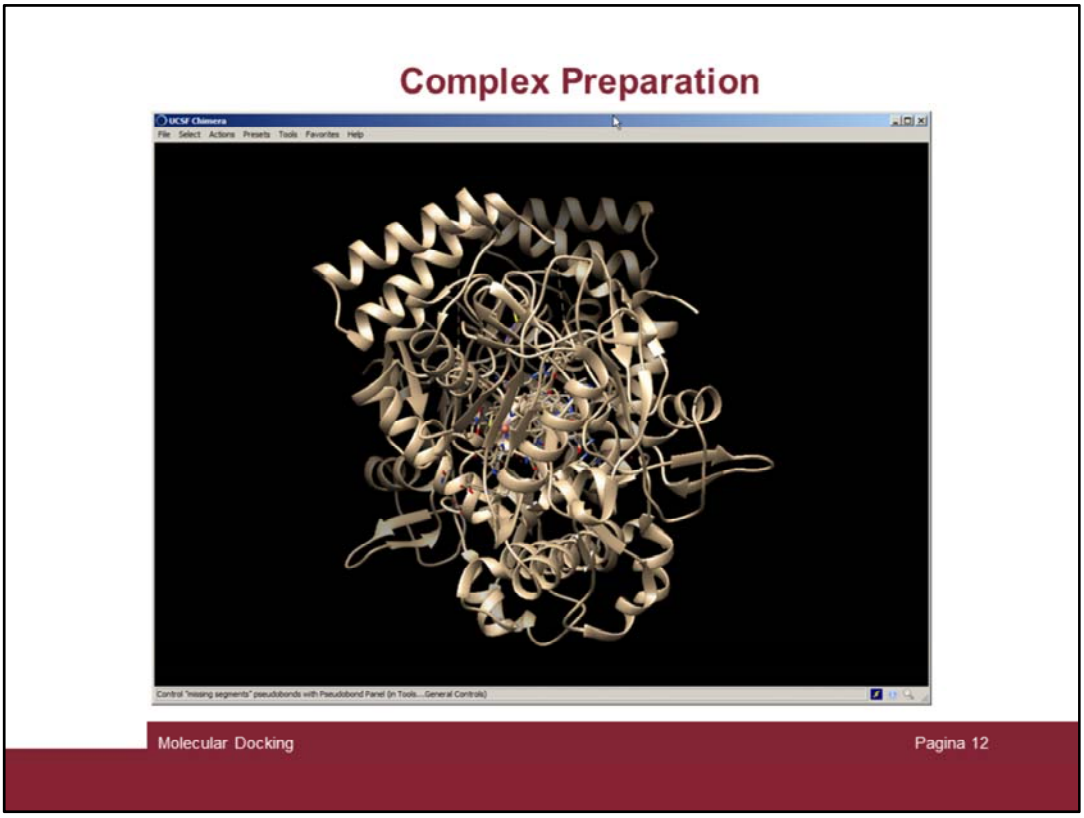
Data set [7]	nNOS-**10**	nNOS-**15**	nNOS-**17**	nNOS-**20**
Data collection				
PDB code	5AD8	5AD9	5ADA	5ADB
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å)	51.9 111.9 164.3	51.7 111.6 164.1	51.9 111.4 164.3	51.7 111.0 165.1
Resolution (Å)	1.91 (1.93-1.91)	2.30 (2.42-2.30)	1.98 (2.05-1.98)	2.05 (2.13-2.05)
Rmerge	0.084 (>1.000)	0.141 (2.111)	0.097 (2.350)	0.126 (3.719)
Rpim	0.047 (>1.000)	0.105 (1.541)	0.066 (1.569)	0.111 (3.276)
CC ½	n/a (0.348)	0.995 (0.360)	0.998 (0.506)	0.997 (0.300)
I / σI	22.3 (1.0)	8.2 (0.8)	10.7 (0.7)	8.0 (0.5)

Scroll it down and stop to the table describing complex with compound 17 (5ADA)

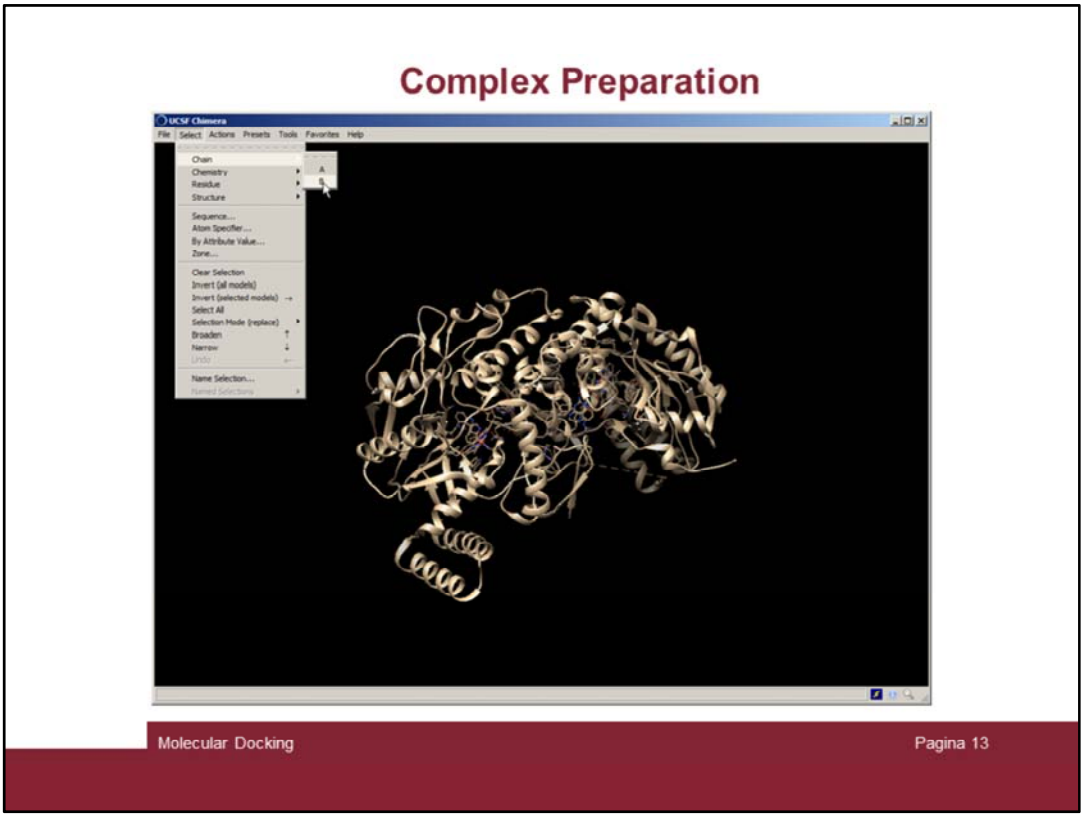
Complex Preparation



Now turn to chimera and fetch the 5ADA pdb file through the File → Fetch

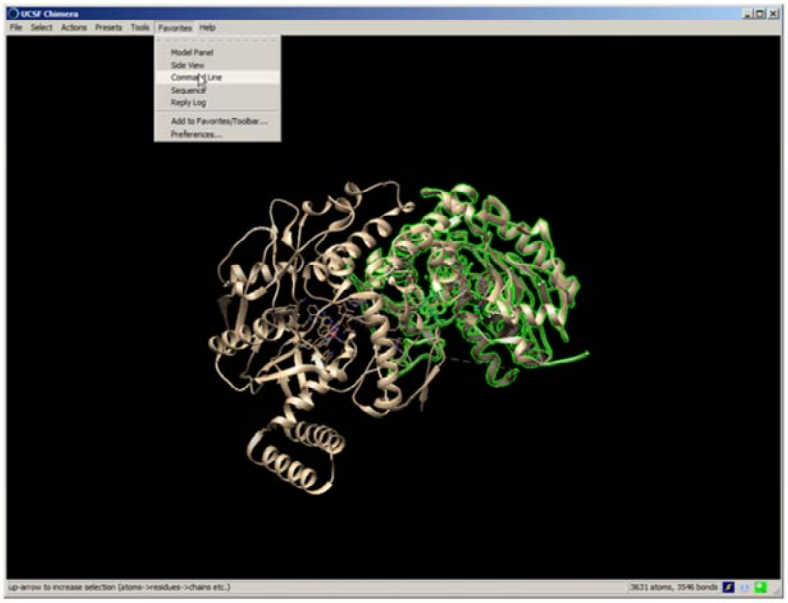


And the **17**/NOS complex is then loaded. Note that therea re two copies of the protein complex, as the NOS act as a dimer (chain A + chain B).



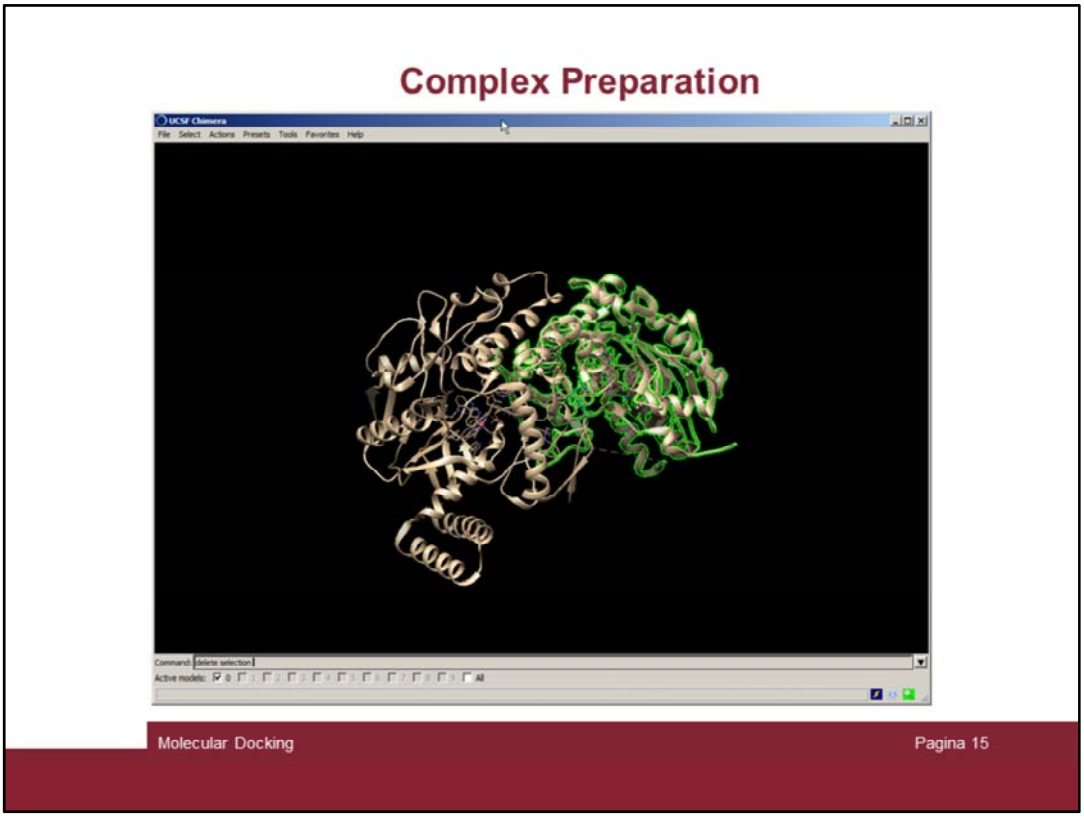
We just need one chain and thus we can delete chain B. To do this first select chain B …

Complex Preparation

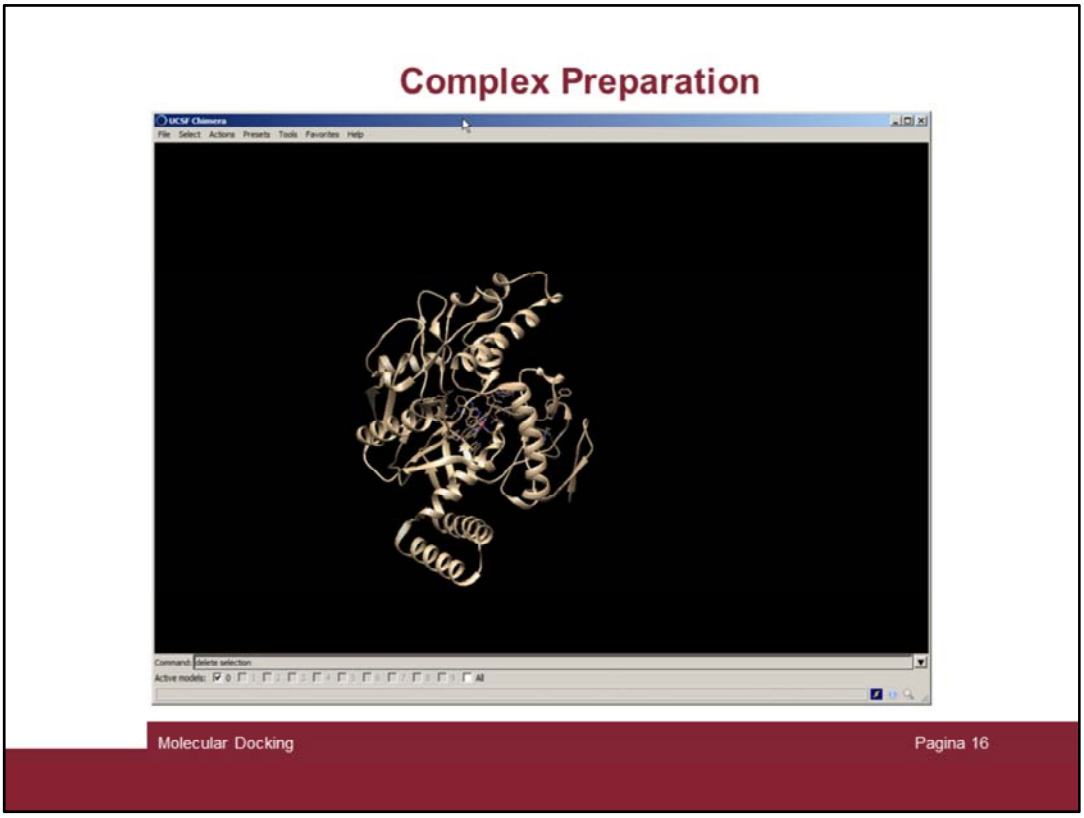


Molecular Docking Pagina 14

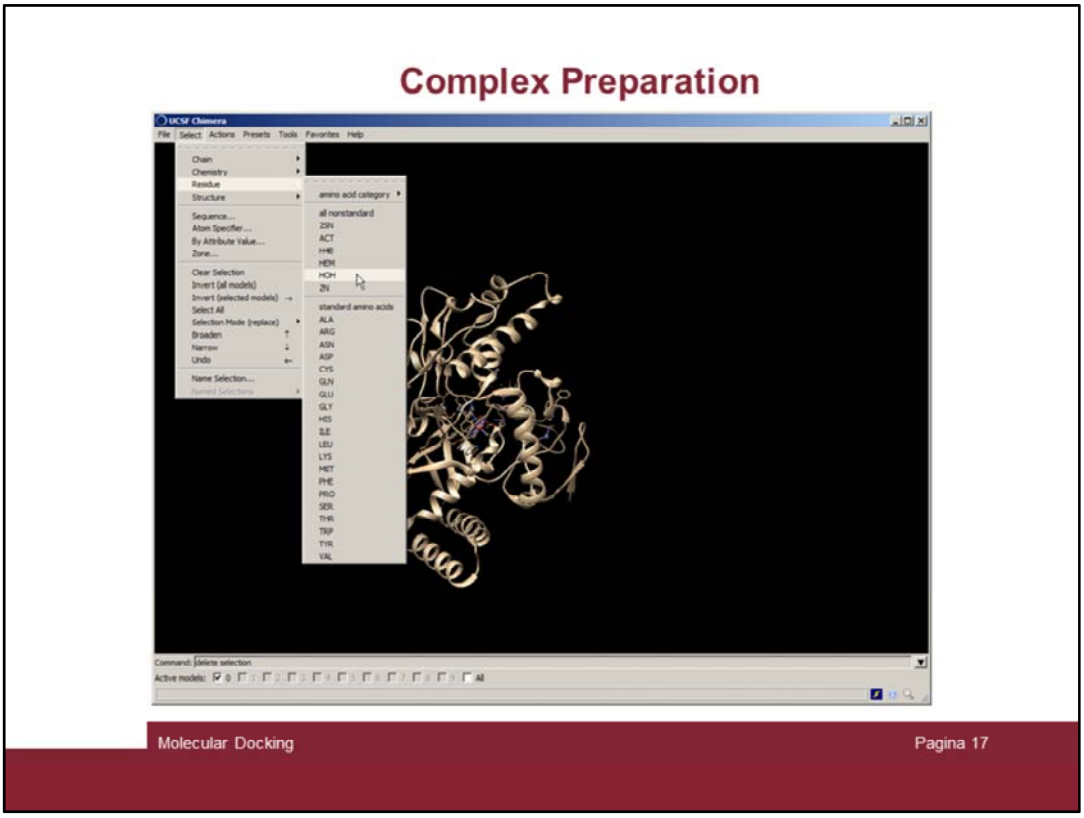
Then open the command line tool …



… and delete the selected chain B by issuing the «delete selection» command (it is also possible to use the «Action → Atoms/Bonds → Delete» command)



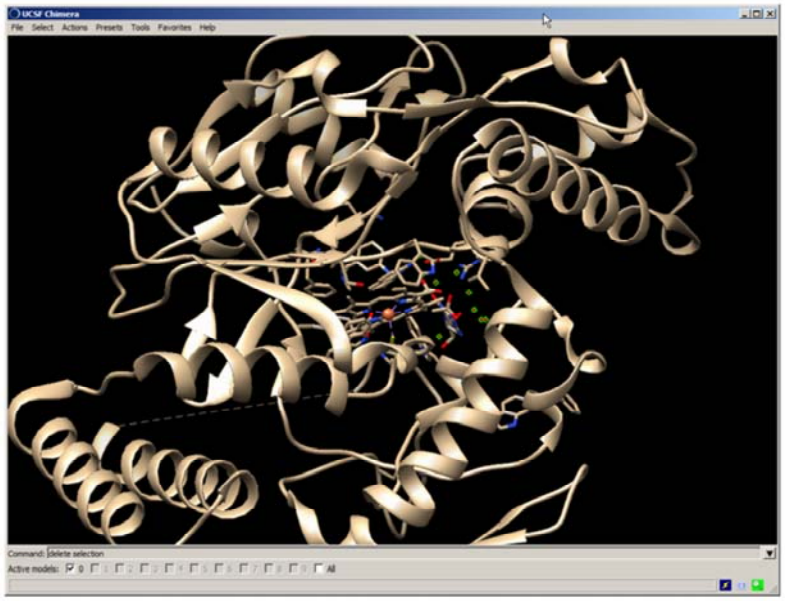
Now we have only one copy of the **17**/NOS complex



To prepare the complex for the geometry relaxation and the subsequent molecular docking runs it is advisable to remove all non standard residue not necessary for the study. Therefore we remove the crystallization water (solvent) and all not interesting ions and small molecules (in this case there is only the solvent to be removed).

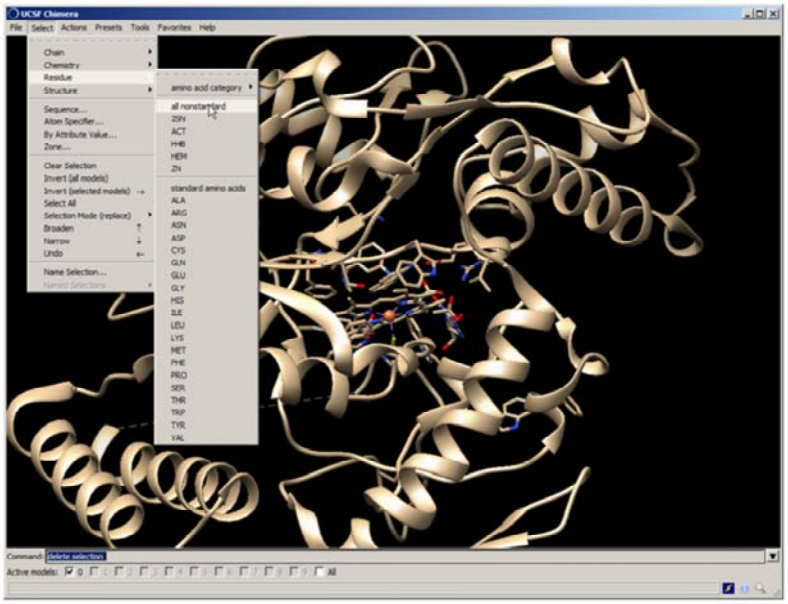
Issue the command “select solvent” in the command line.

Complex Minimization



And the water moleculs should be selected by being highlighted in green.

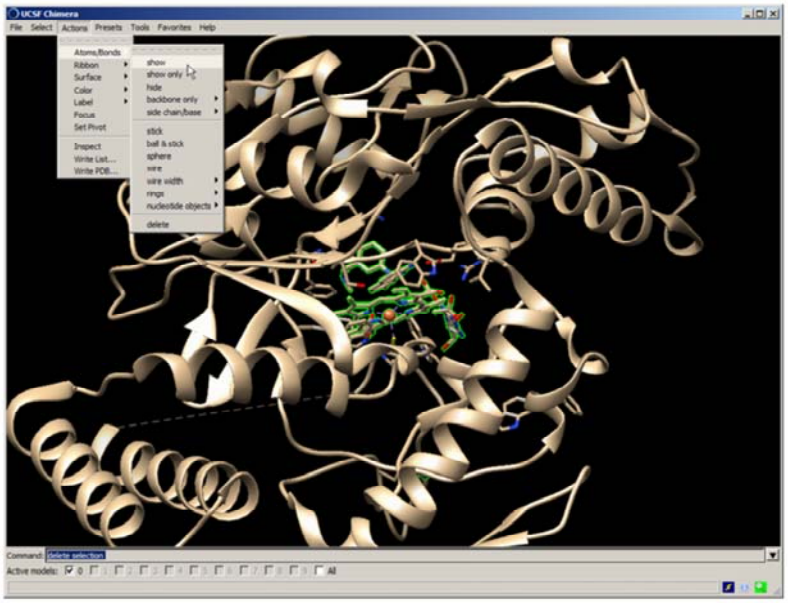
Complex Minimization



Molecular Docking Pagina 19

Then check for all non standard residues by the “Select → all nonstandard” menu

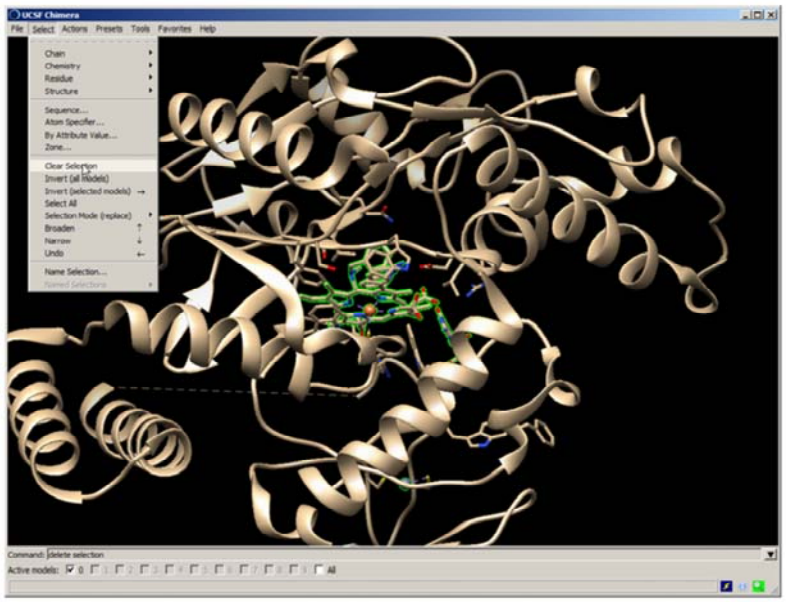
Complex Minimization



Molecular Docking Pagina 20

The nonstandard residues must be displayed ("Action → show")

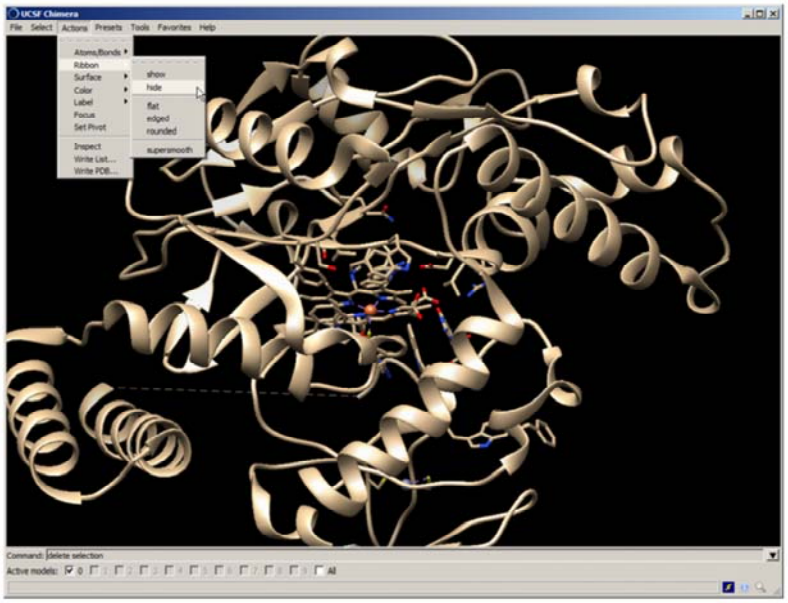
Complex Minimization



Molecular Docking Pagina 21

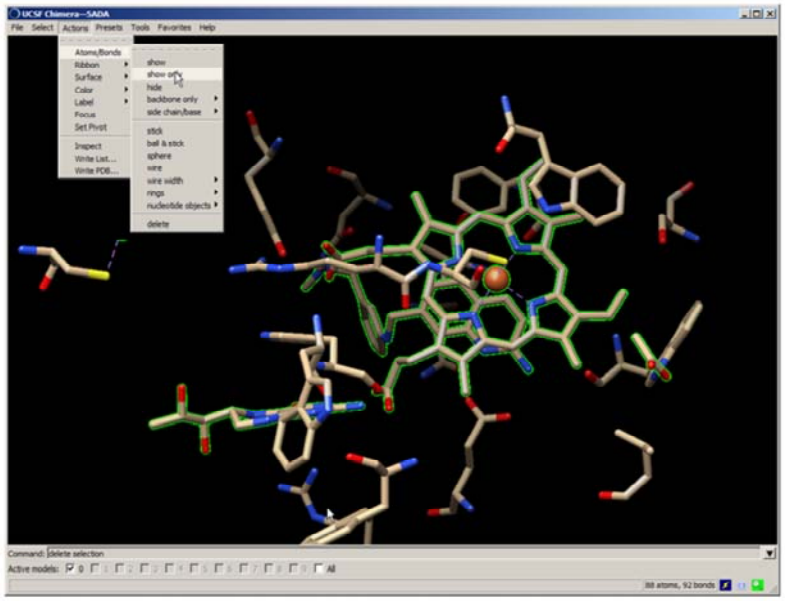
Deselect everything («Select → Clear Selection»)

Complex Minimization



And hide the ribbon

Complex Minimization

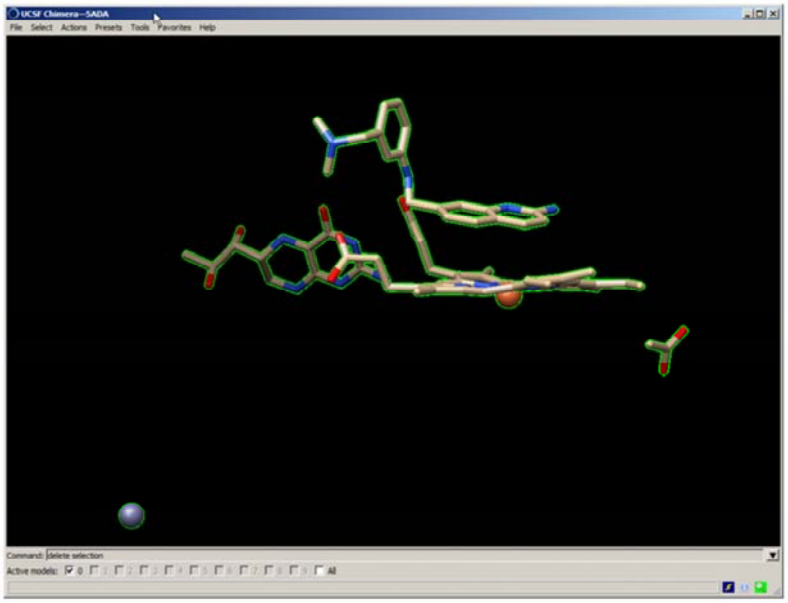


Molecular Docking Pagina 23

Select non standar residue again and focus on that selection

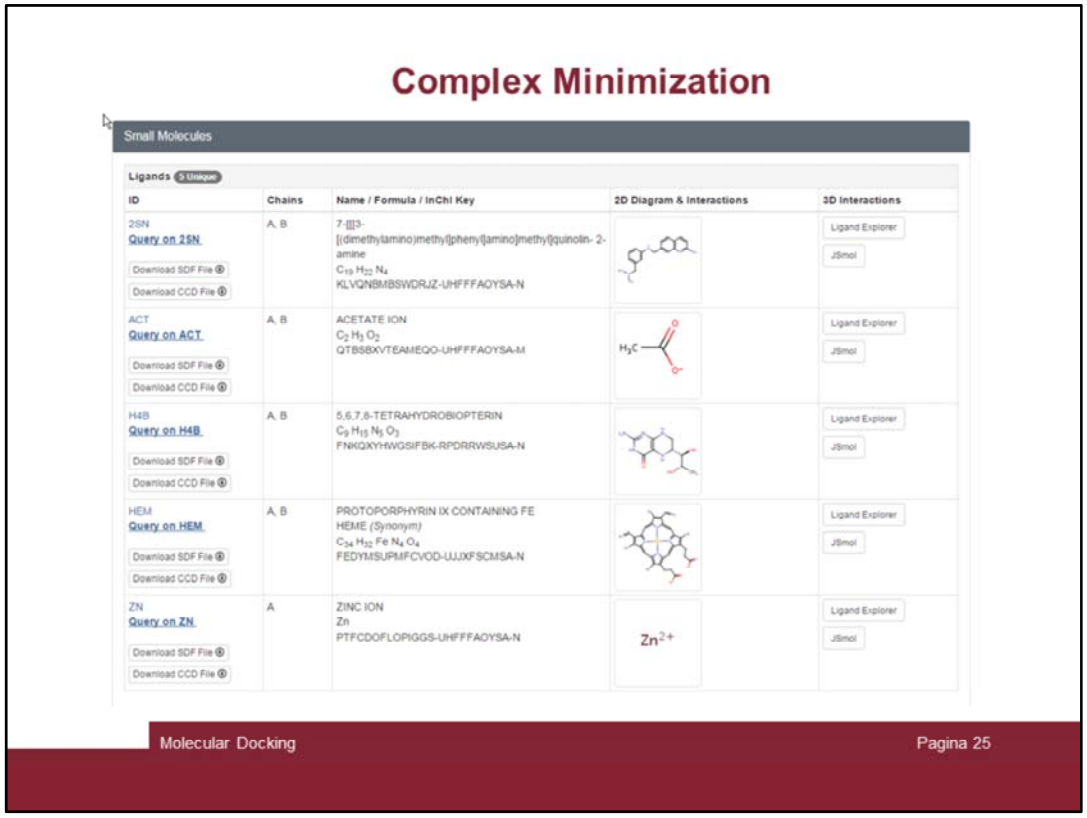
1) "Select → Residue → All nonstandard"
2) "Action → Focus"
3) "Action → Atoms/Bonds → Show only"

Complex Minimization

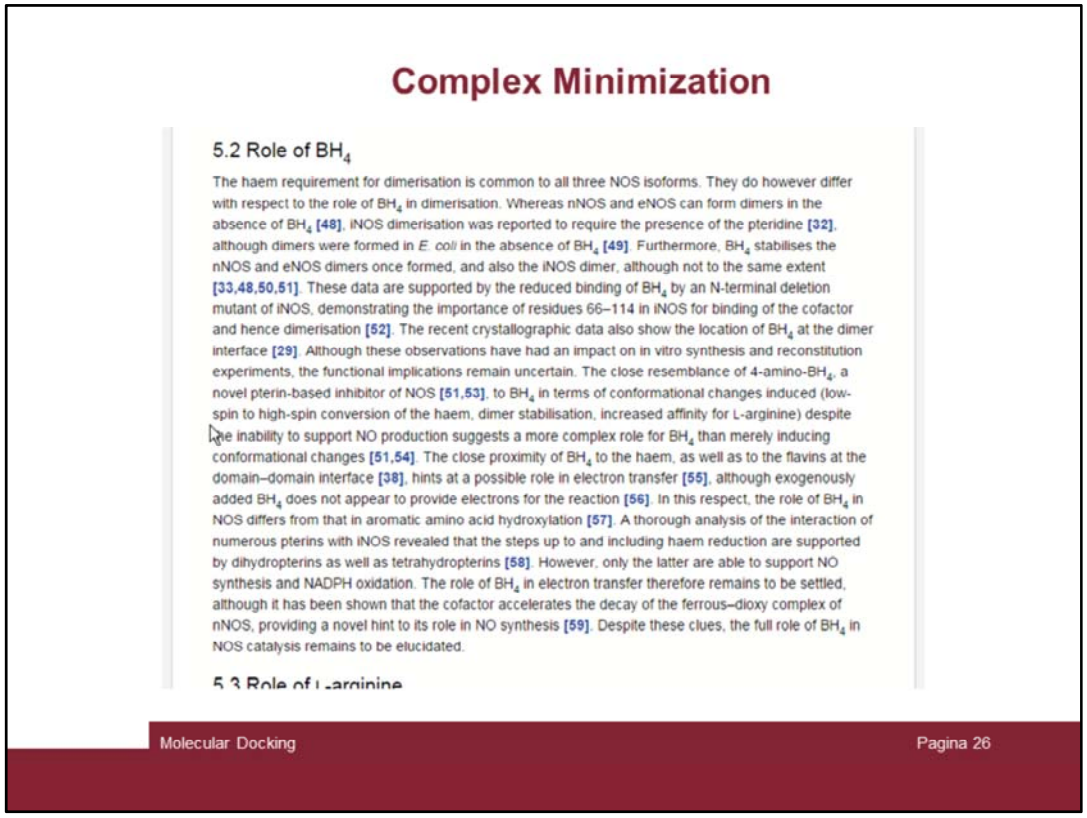


Molecular Docking Pagina 24

There are several nonstandard residues, therefore we have to check on the PDB site for residue information

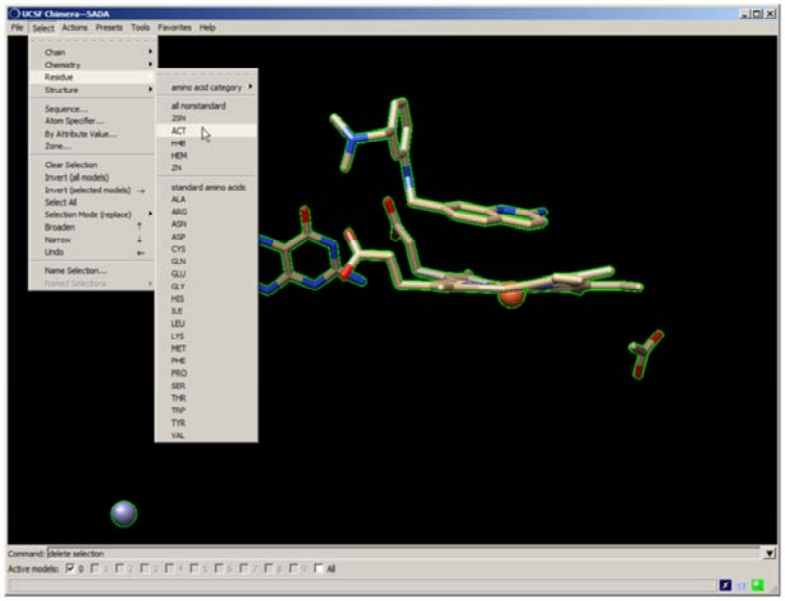


In the PDB site, go to the 5ADA complex and scroll down to the “small Molecules” description section. Among all the small molecules the only not important residue is the acetate ion. Molecule 2SN is the inhibitor we are studying; H4B is a tetrahydrobiopterin acting as a co-factor, HEM is the active co-factor and Zn is a structural ion.



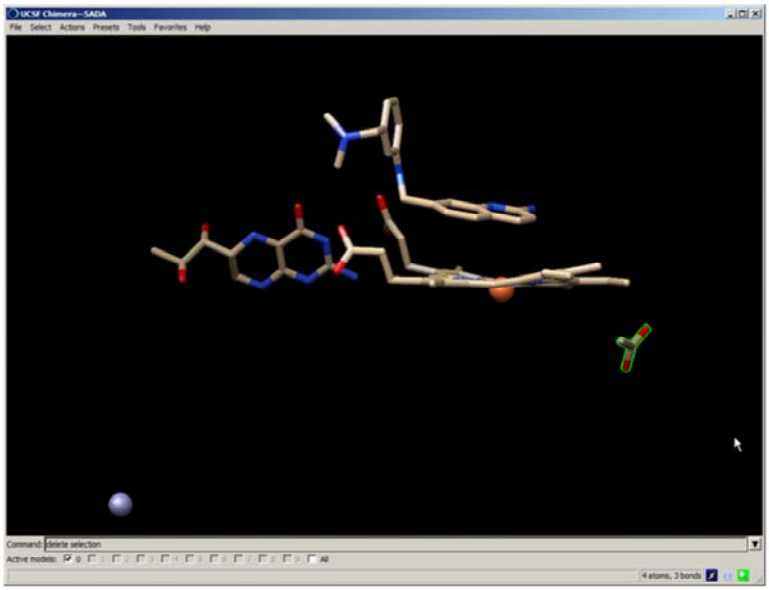
Seeking for tetrahydrobiopterin information it is possible to find information on its role.

Complex Minimization

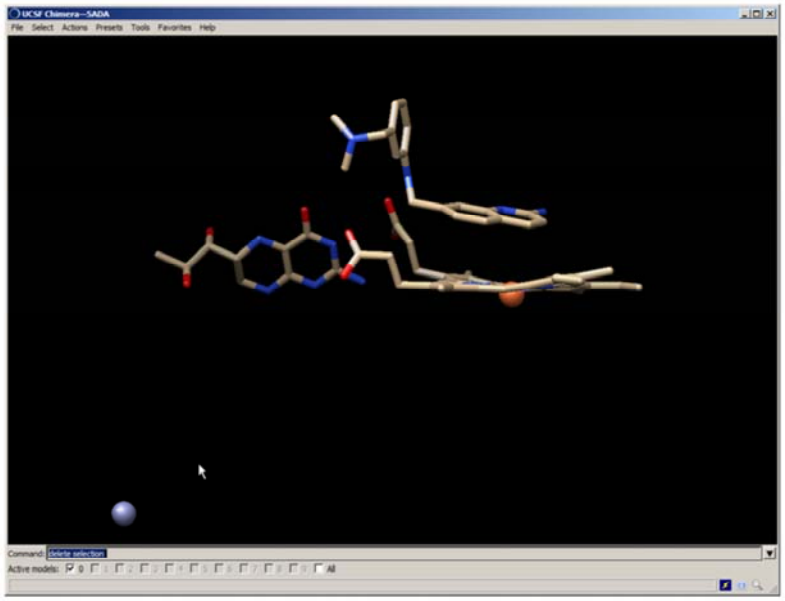


Let's delete the acetate residue: "Select → Residue → ACT"

Complex Minimization



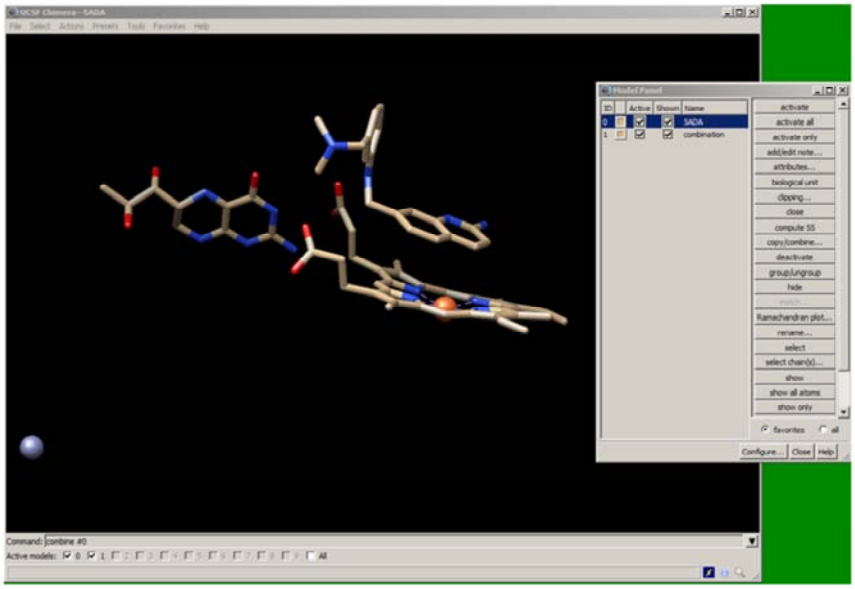
Complex Minimization



Molecular Docking Pagina 29

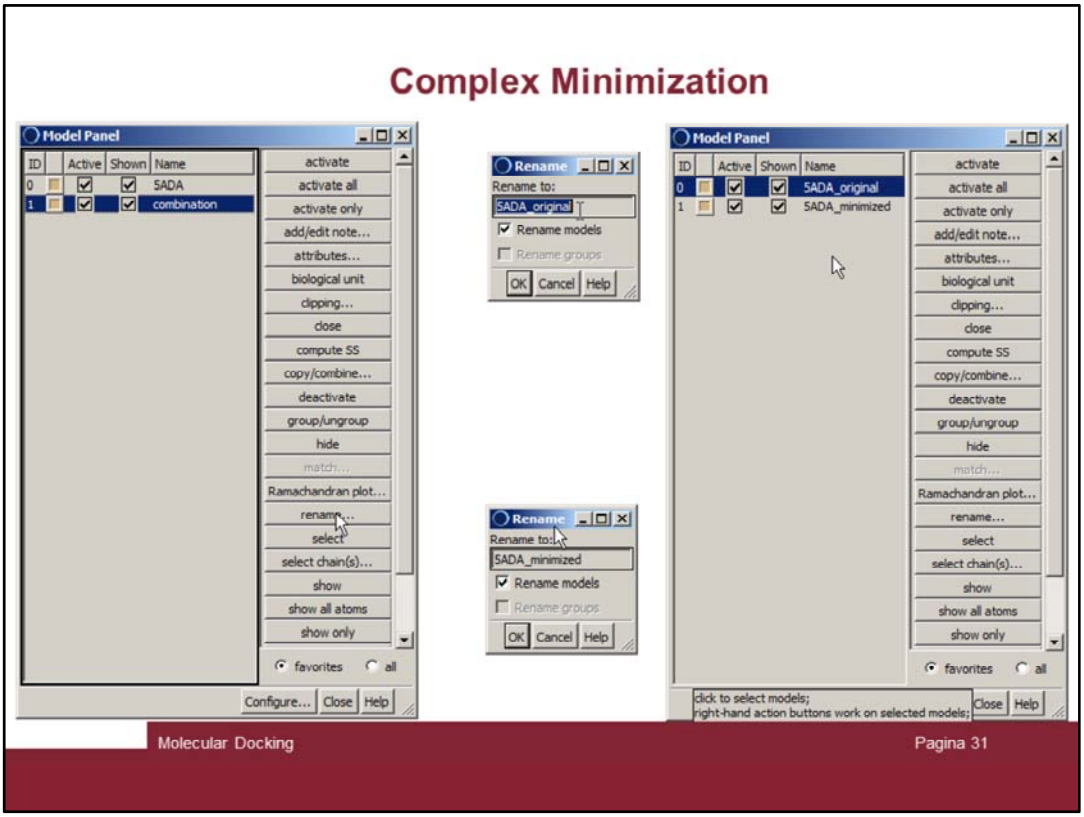
And delete it by issuing the “delete selection” at the command line

Complex Minimization



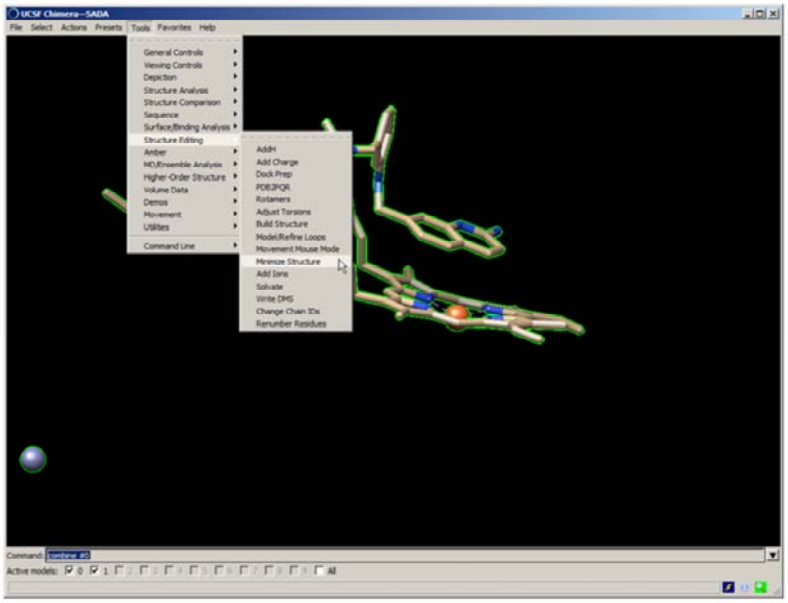
Molecular Docking Pagina 30

Make a copy of the clean complex with the “combine #0” command

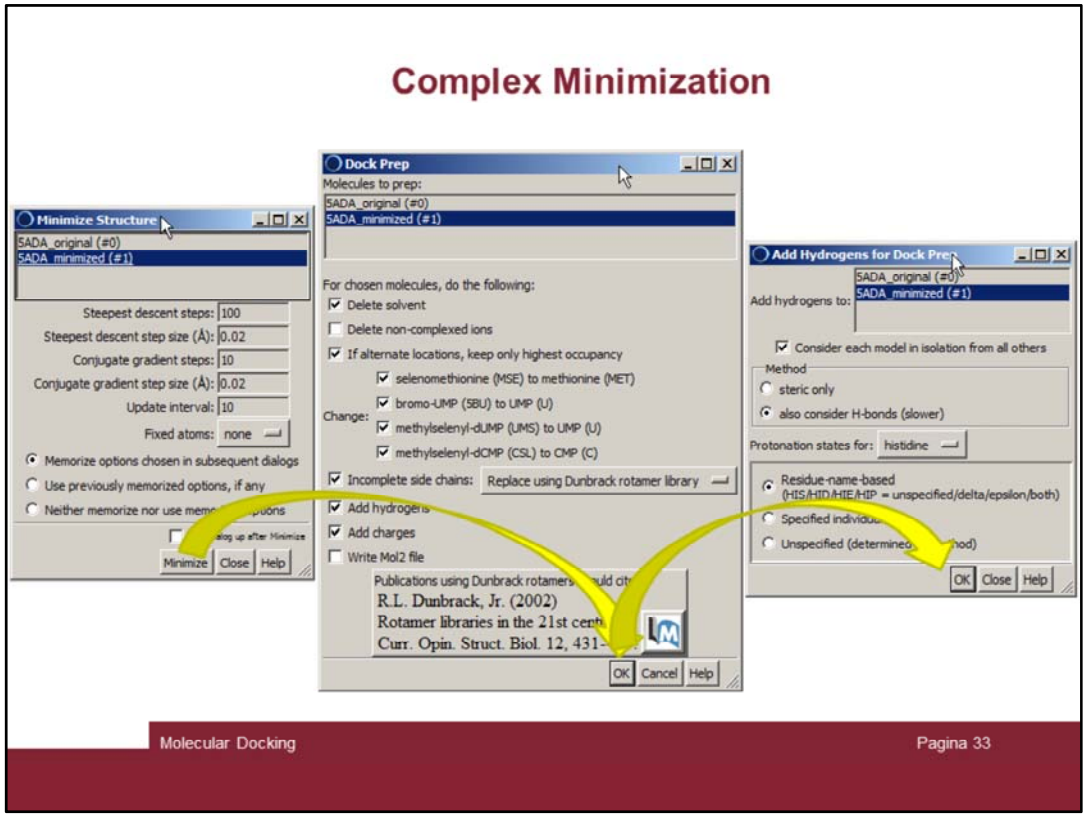


Rename the two complexes as reported in the slide

Complex Minimization

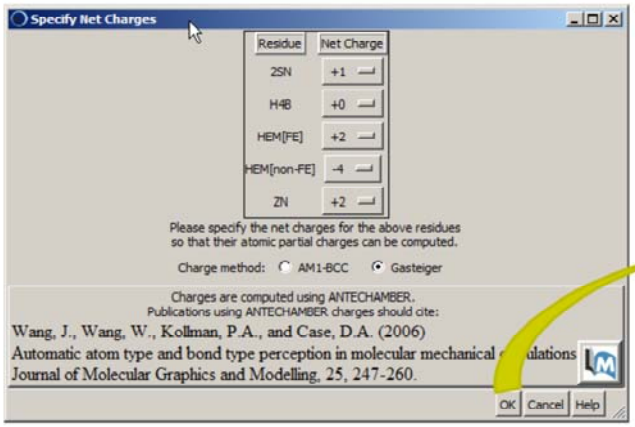


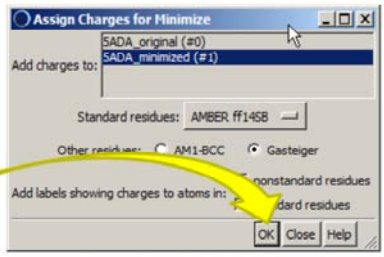
At this point we can try to minimize the complex in area #1 ("Tools → Minimize Structure")



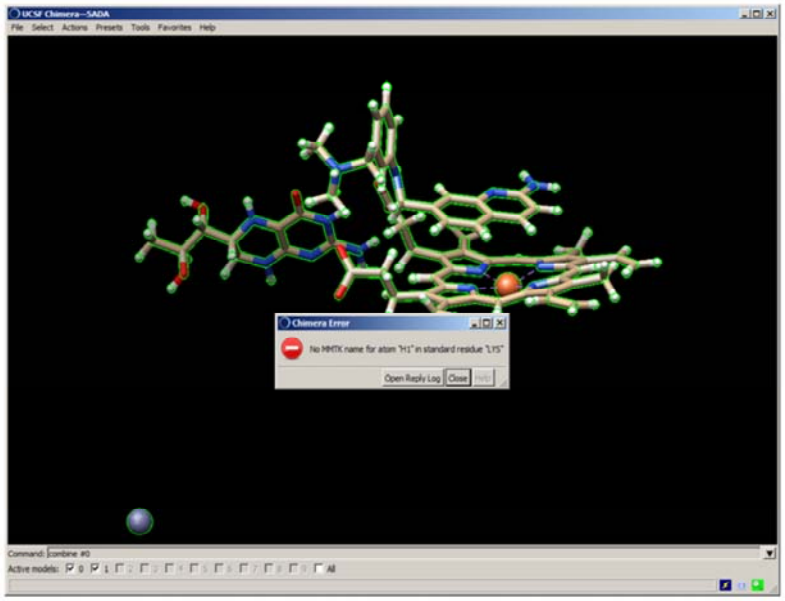
Consecutive windows will pop up

Complex Minimization





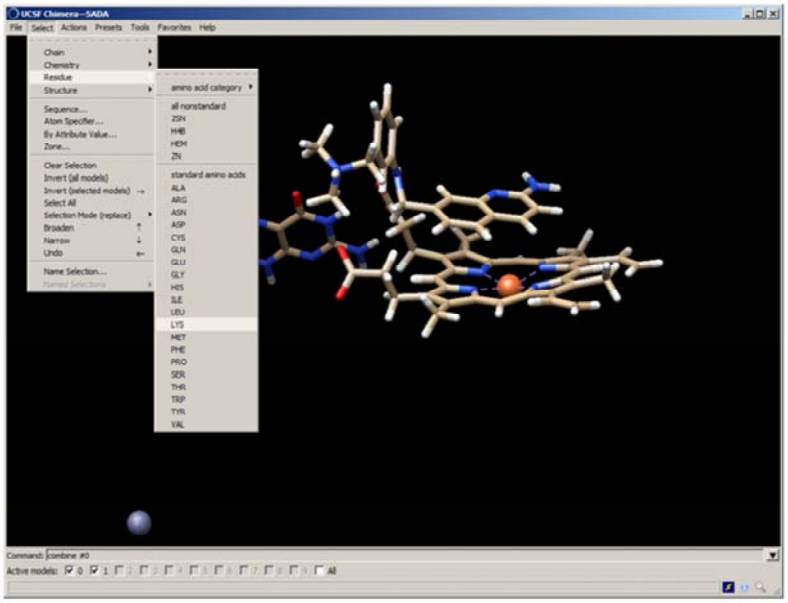
Complex Minimization



Molecular Docking Pagina 35

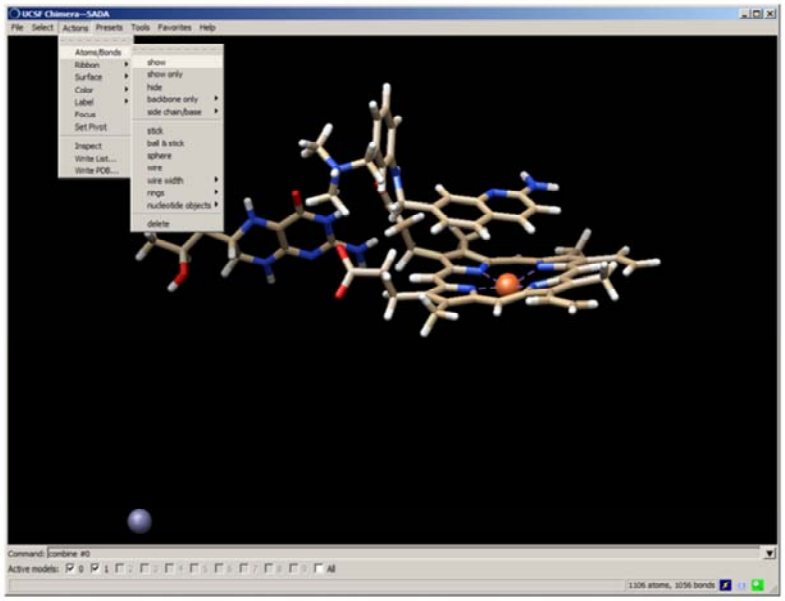
Unfortunately the minimization did not go thru! The program complains about a lysine residue.

Complex Minimization

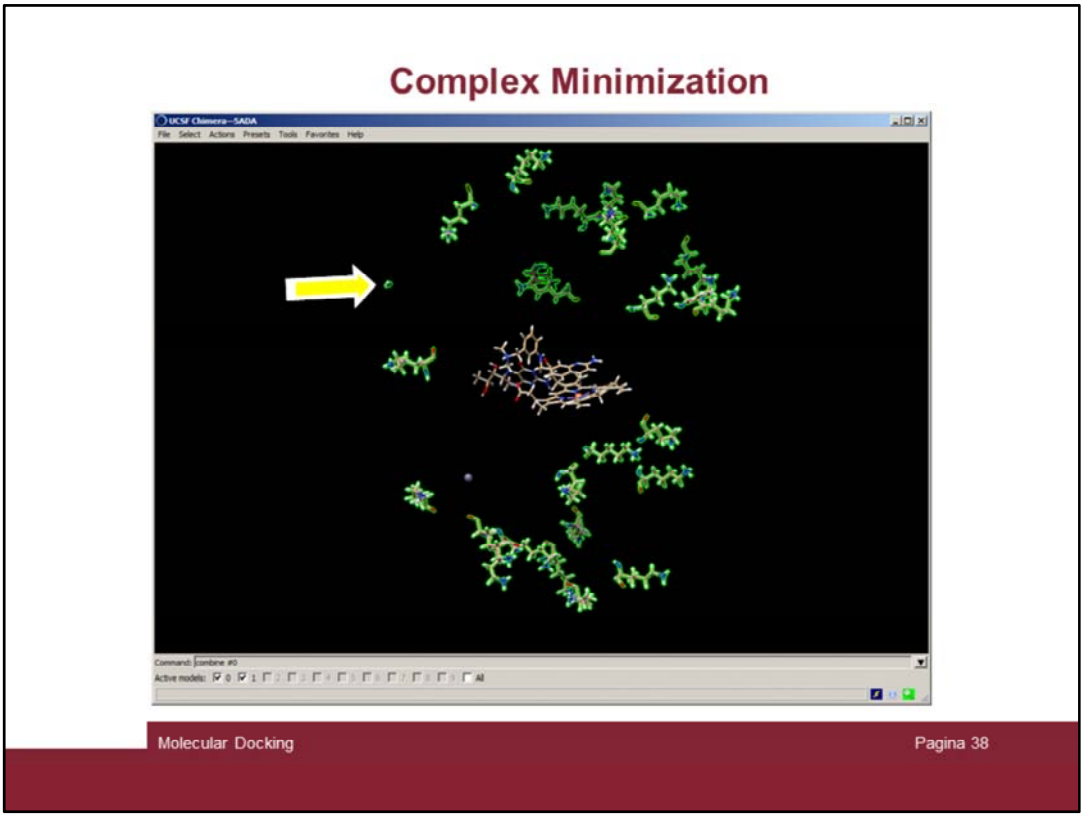


To check we can display all the lysine residues ("select → Residue → Lys")

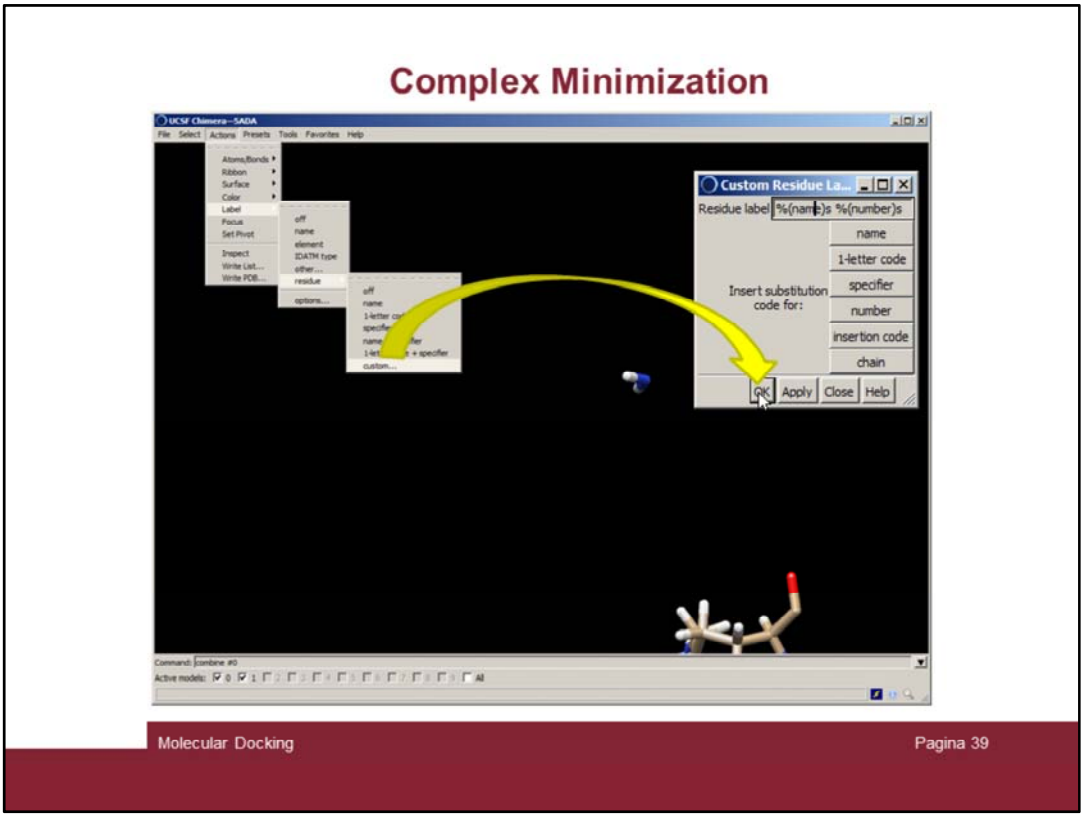
Complex Minimization



… “Actions → show”

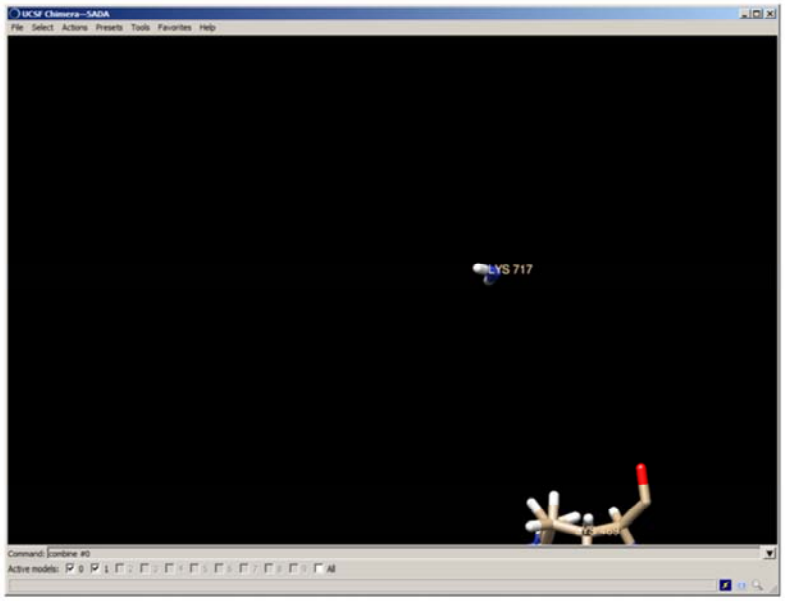


And zoom out to see all the lysines. In this slide we can se there is a smaller lysine residues. Most of the time this is the last residue of the protein sequence that normally is not complete



Unselect everything and zoom in to that residues and label all the residues ("Actions → Label → residue")

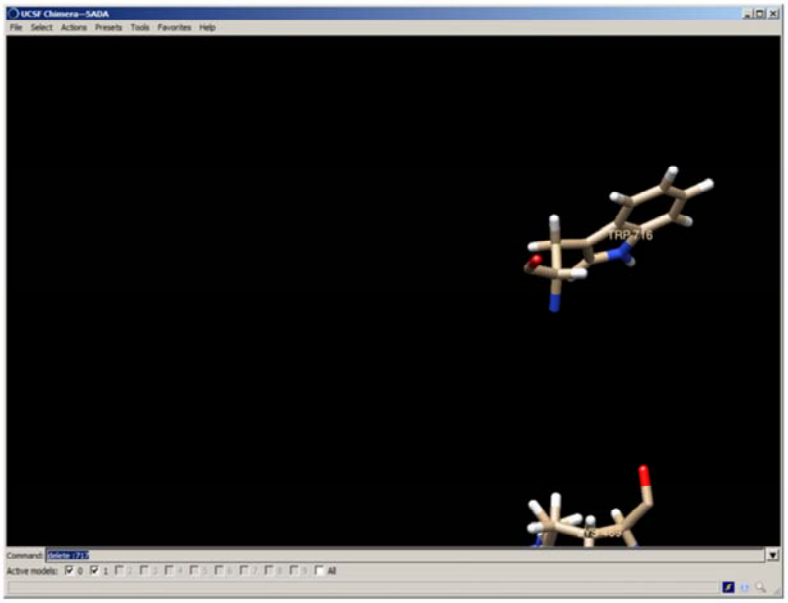
Complex Minimization



Molecular Docking Pagina 40

And the “LYS 717” label will appear.

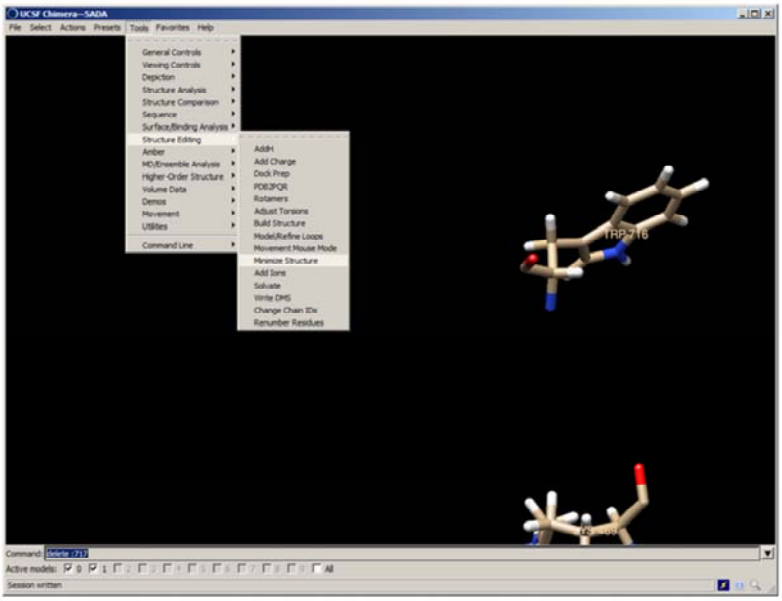
Complex Minimization



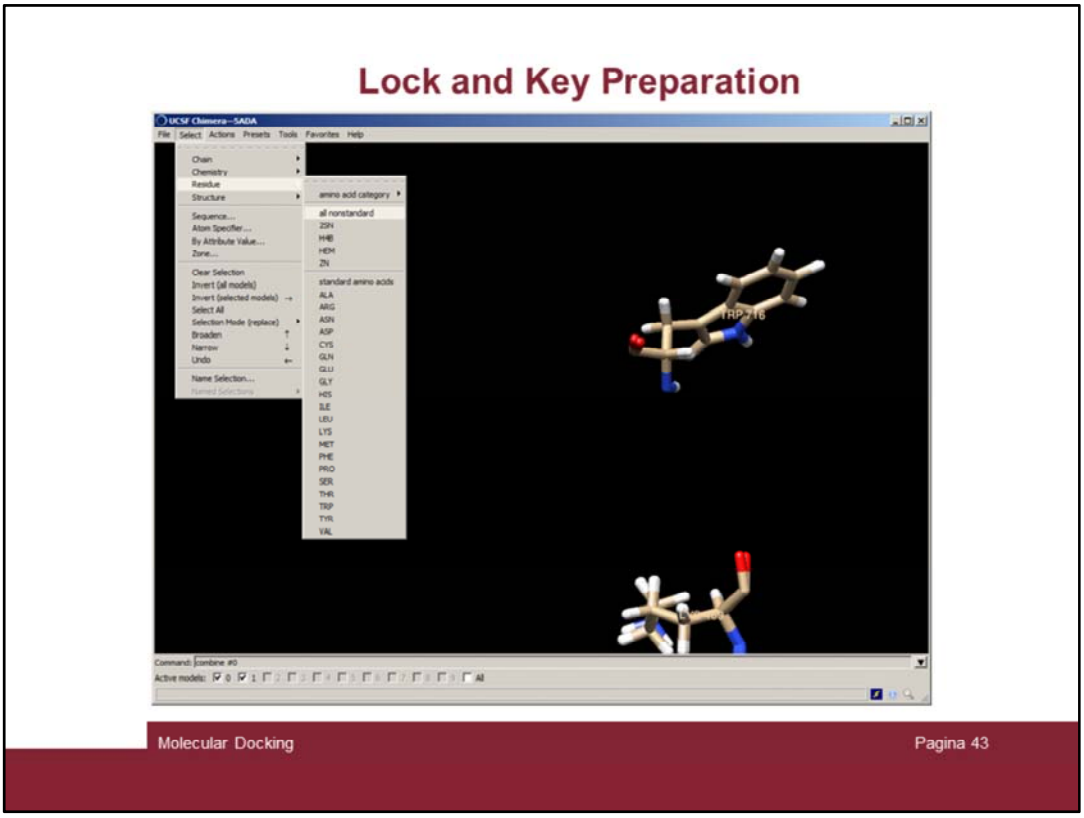
Molecular Docking Pagina 41

Delete residue 717 by issuing the “delete :717” command

Complex Minimization

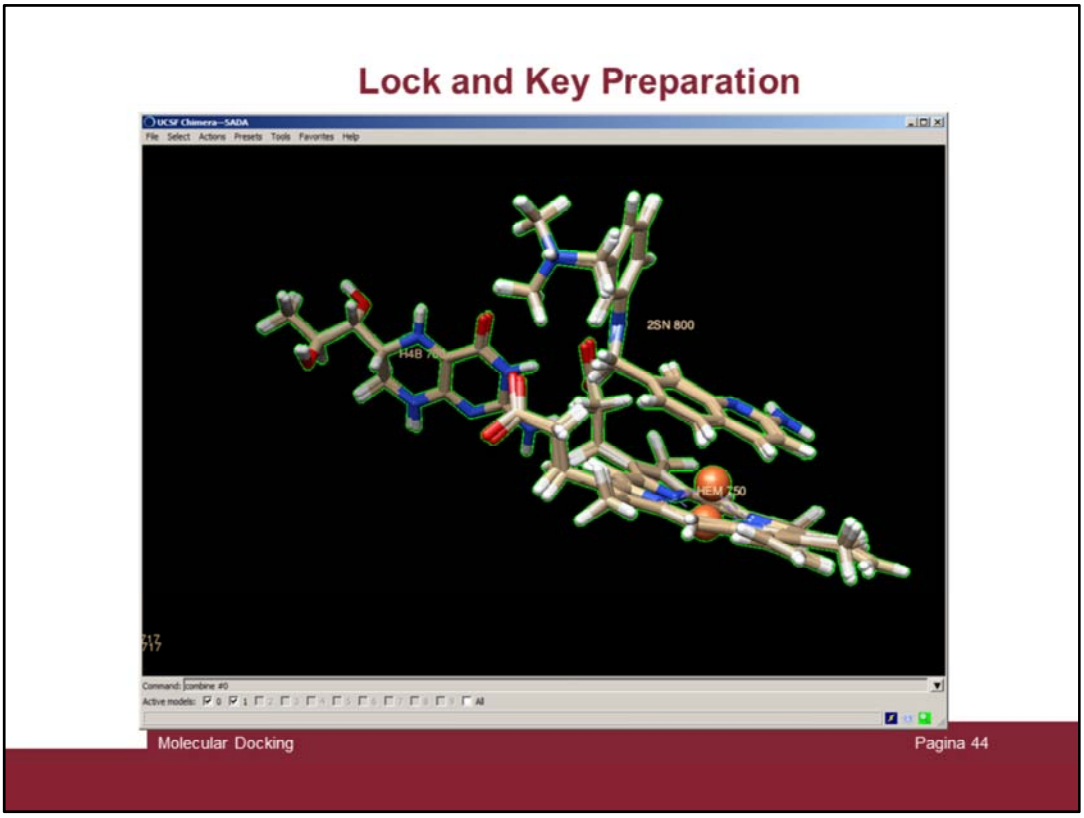


And try again to minimize “Structure Editing → Minimize Structure”

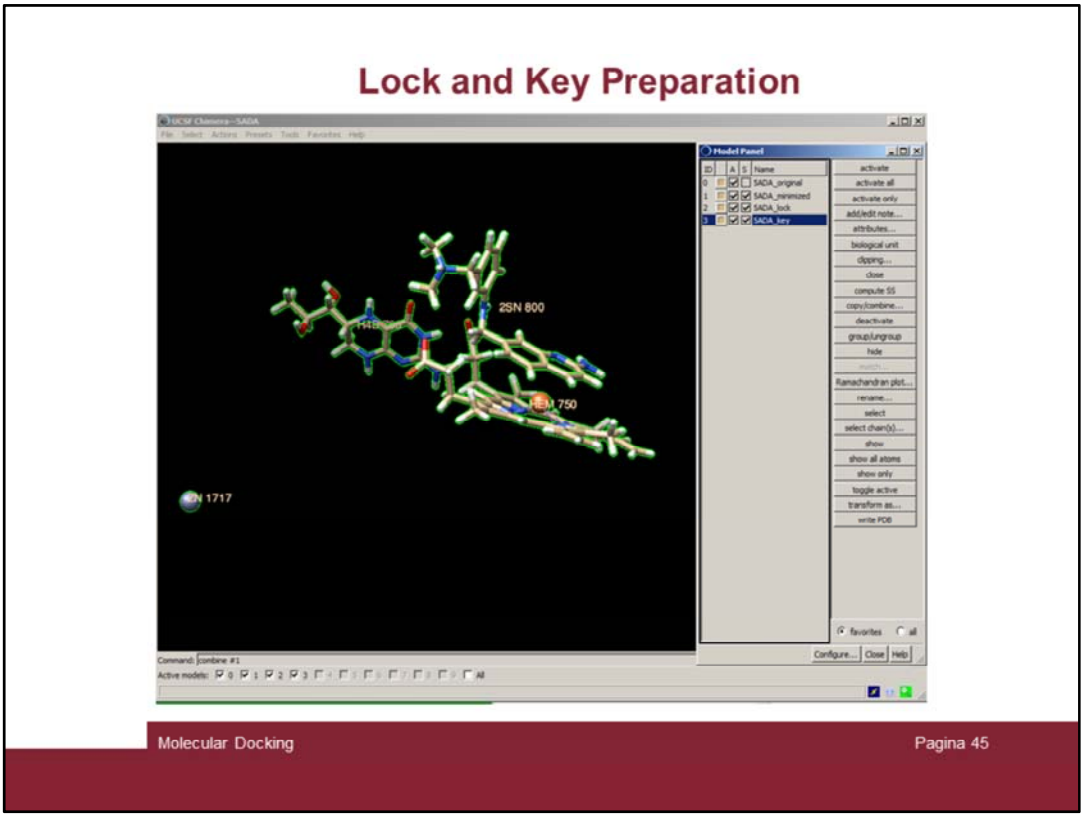


The minimization starts and it will take some minutes to finish.

Select again all nonstandard residues

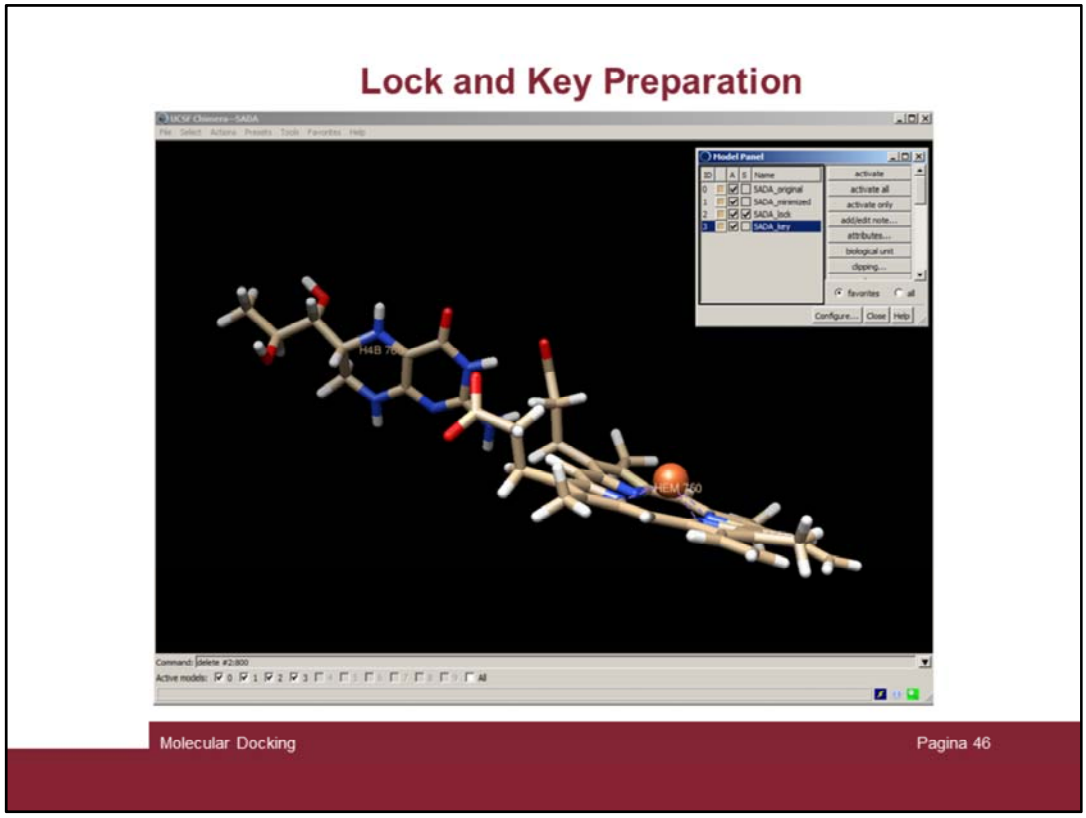


And focus on those. In this slide it is possible to observe that small movements occurred either in the ligand 2SN and the cofactors H4B and HEM. Note the Fe ion! This is actually an error due to the fact that molecular mechanics calculations are not very good in handle heavy metals.

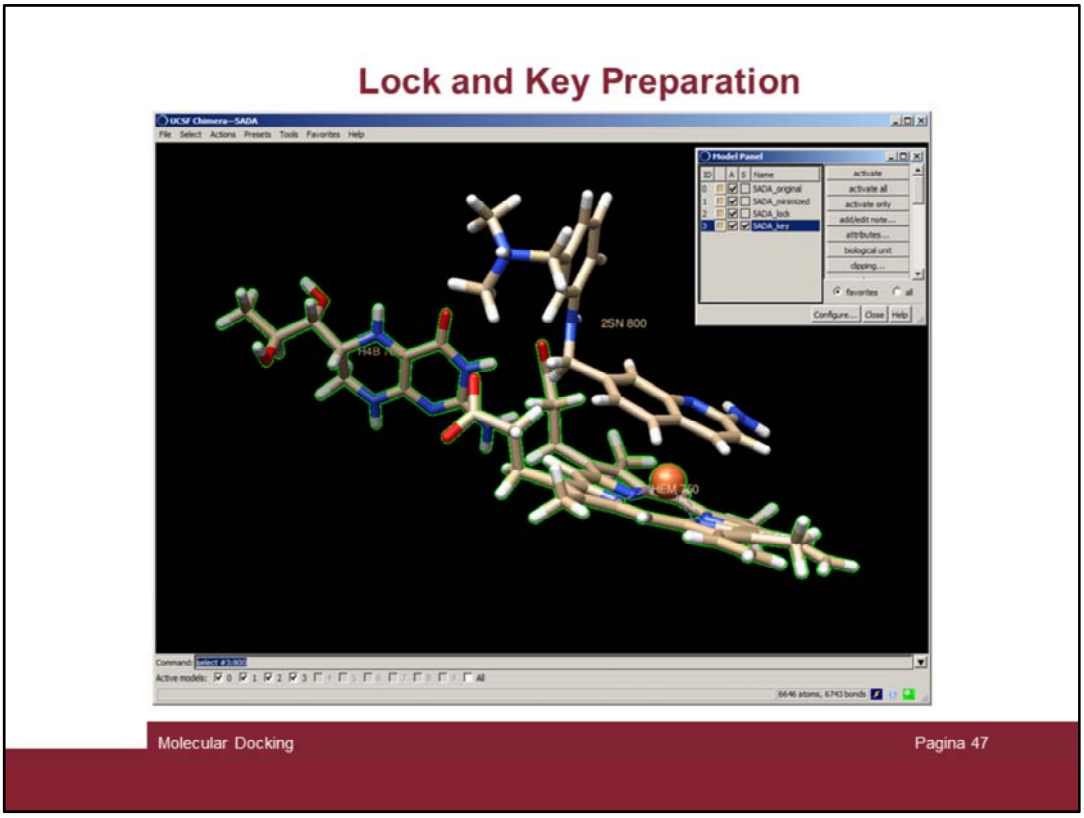


Move on to prepare lock and key.

Issue the command “combine #1” twice and rename areas #2 and #3 as in the slide.



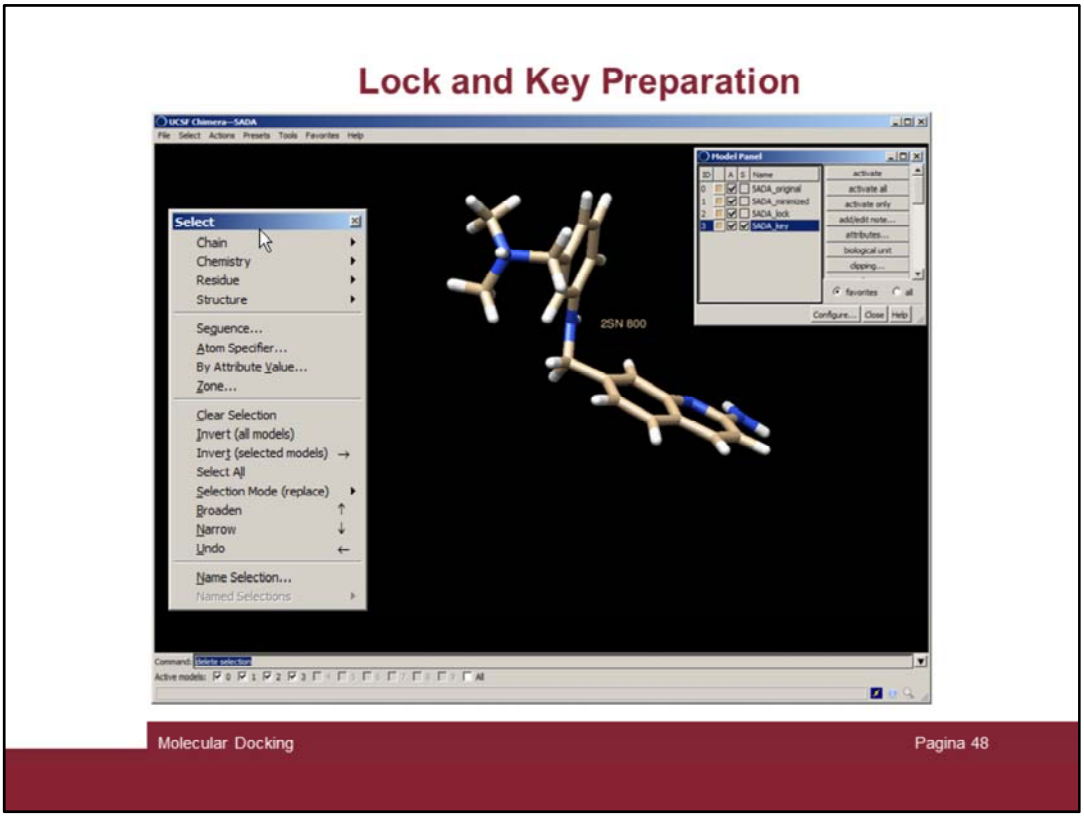
Delete residue 800 (the ligand) form area #2



Then make the reverse in area #3:

1) Issue the “select #3:800” command

2)



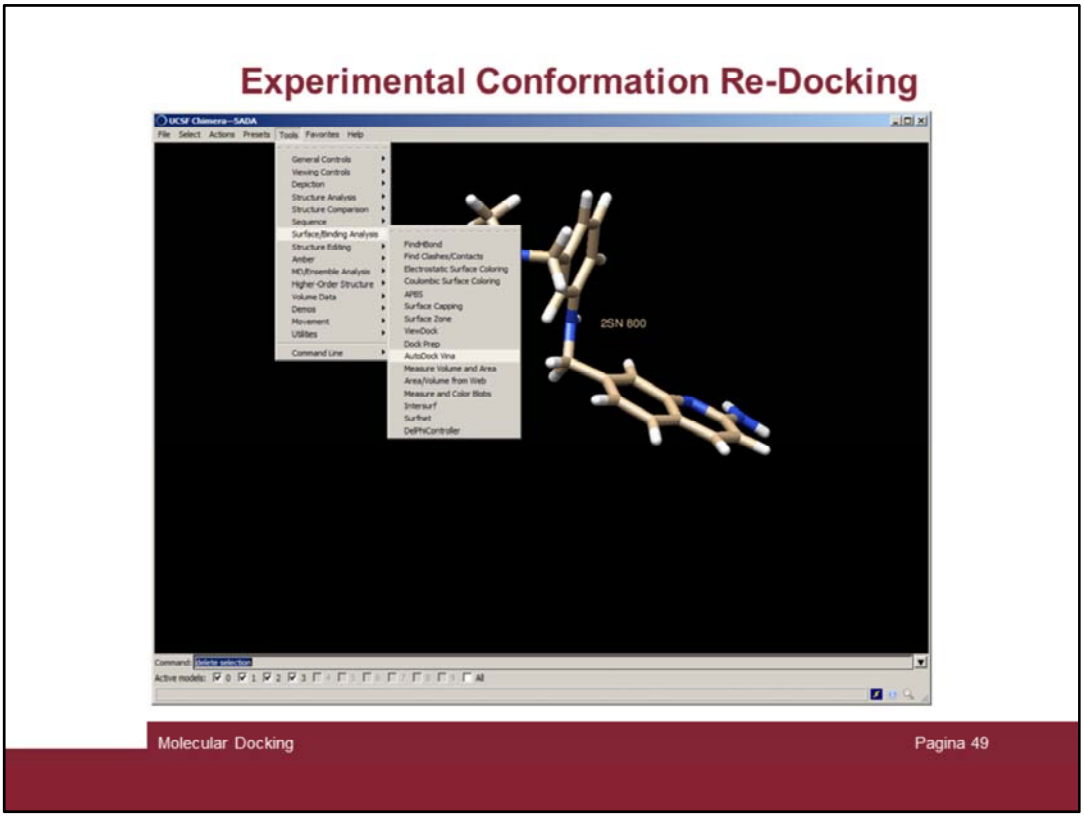
Then make the reverse in area #3:

1) Issue the “select #3:800” command

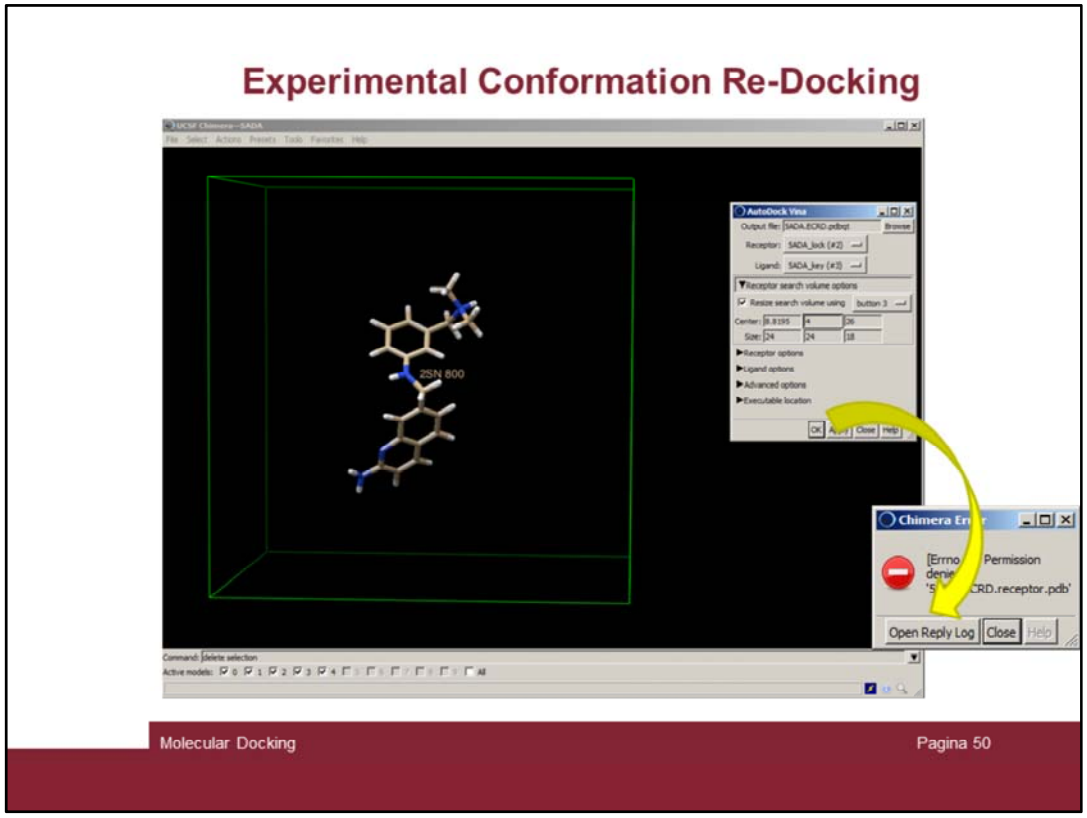
2) “Select → Invert (selected models)→”

3) Issue the “delete selection” command

Now we have in areas #2 and #3 the isolated lock and key, respectively



Let's try to perform a ECRD using Autodock Vina ("Structure/Binding Analysis → Autodock Vina")

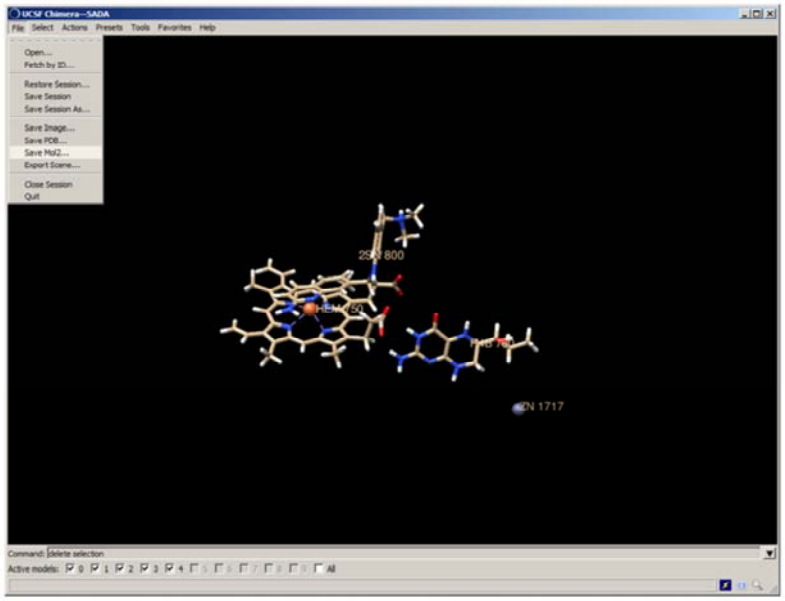


Set the parameters similar to shown in the slide and click OK.

Again there are problems. The program complains.

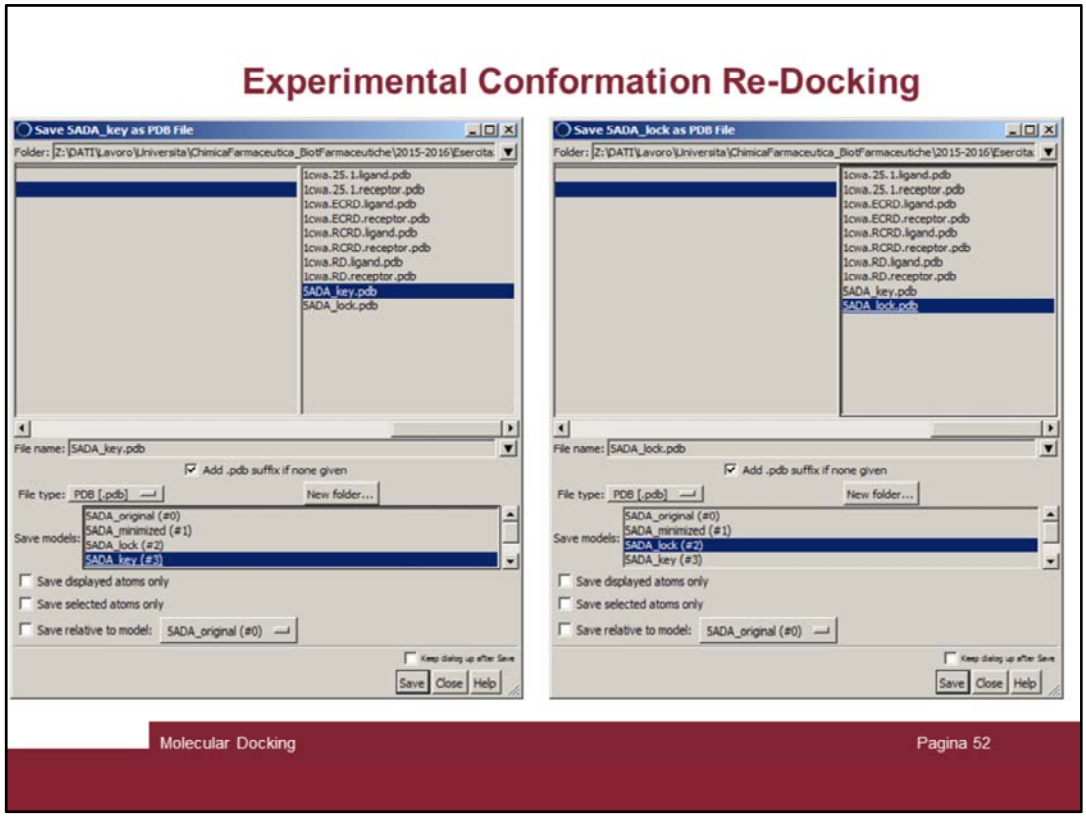
Very likely the problem is the fact that there are nonstandard residues embedded in the lock (H4B, HEM, Zn)

Experimental Conformation Re-Docking



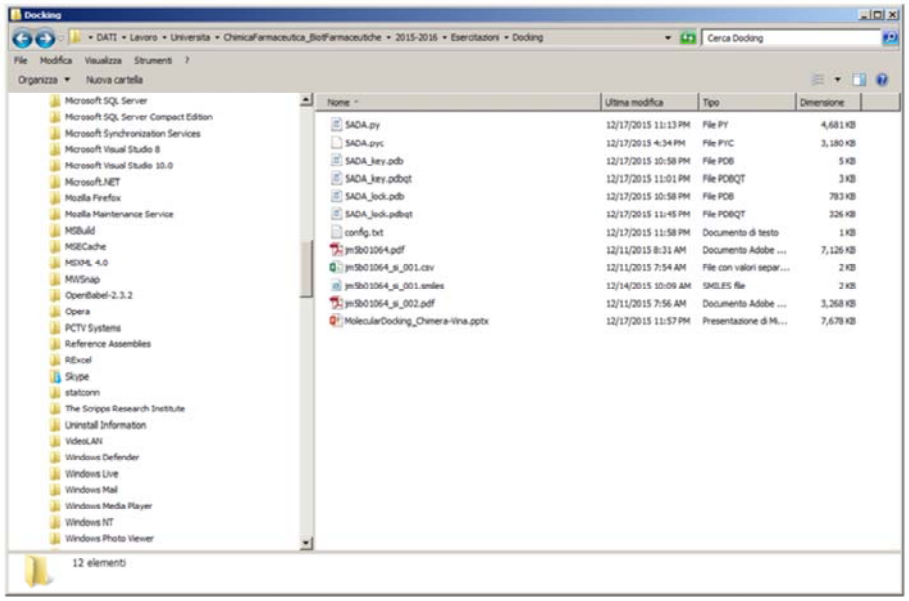
Molecular Docking Pagina 51

To workaround we can make the docking using the DOS terminal, but we need to save the molecules.

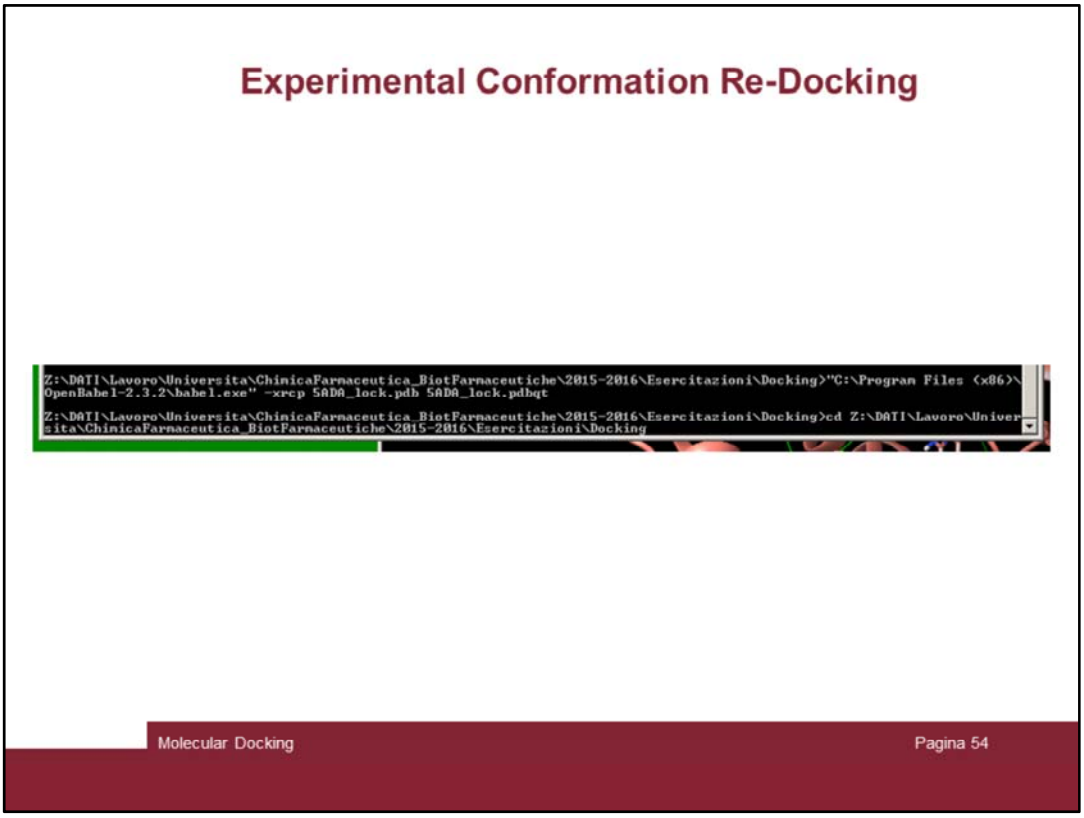


Save both the lock and the key into pdb files

Experimental Conformation Re-Docking



Check you have the files



Then use babel to convert the two molecules by issuing the following command.

1) First move in the correct path

cd Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking

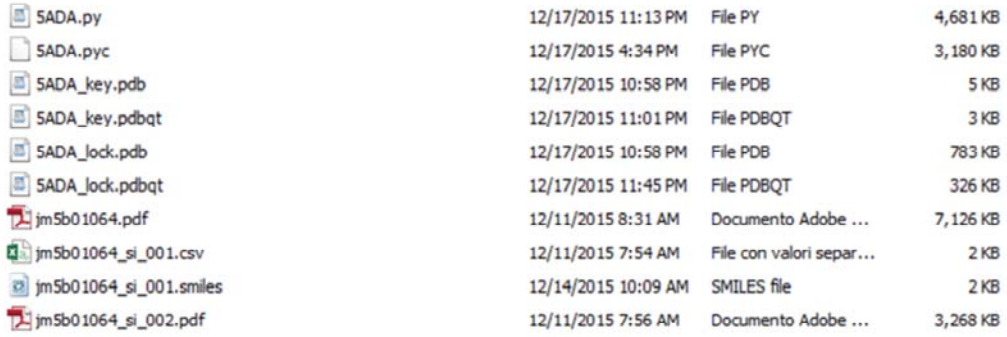
2) Convert the lock (this will take time!!):

"C:\Program Files (x86)\OpenBabel-2.3.2\babel.exe" -xrcp 5ADA_lock.pdb 5ADA_lock.pdbqt

3) Convert the key:

"C:\Program Files (x86)\OpenBabel-2.3.2\obabel.exe" -xp -ipdb 5ADA_key.pdb -opdbqt 5ADA_key.pdbqt

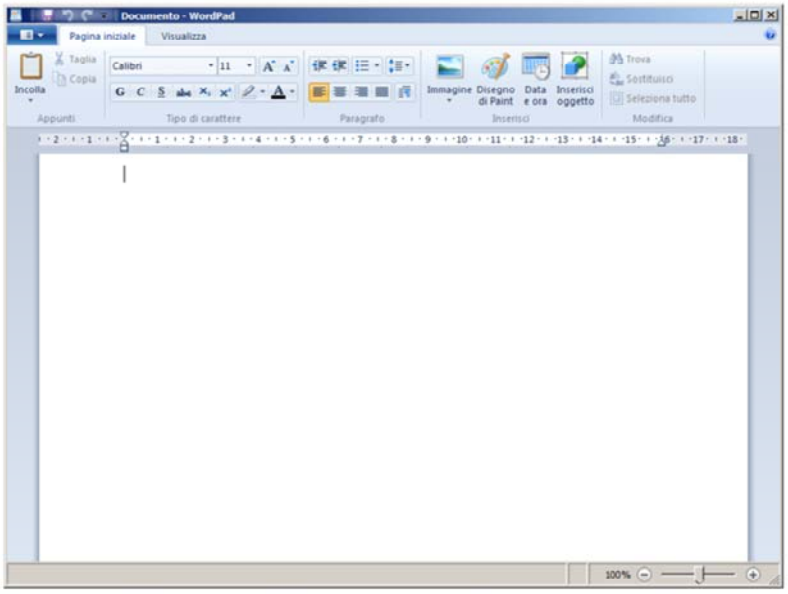
Experimental Conformation Re-Docking



5ADA.py	12/17/2015 11:13 PM	File PY	4,681 KB
5ADA.pyc	12/17/2015 4:34 PM	File PYC	3,180 KB
5ADA_key.pdb	12/17/2015 10:58 PM	File PDB	5 KB
5ADA_key.pdbqt	12/17/2015 11:01 PM	File PDBQT	3 KB
5ADA_lock.pdb	12/17/2015 10:58 PM	File PDB	783 KB
5ADA_lock.pdbqt	12/17/2015 11:45 PM	File PDBQT	326 KB
jm5b01064.pdf	12/11/2015 8:31 AM	Documento Adobe ...	7,126 KB
jm5b01064_si_001.csv	12/11/2015 7:54 AM	File con valori separ...	2 KB
jm5b01064_si_001.smiles	12/14/2015 10:09 AM	SMILES file	2 KB
jm5b01064_si_002.pdf	12/11/2015 7:56 AM	Documento Adobe ...	3,268 KB

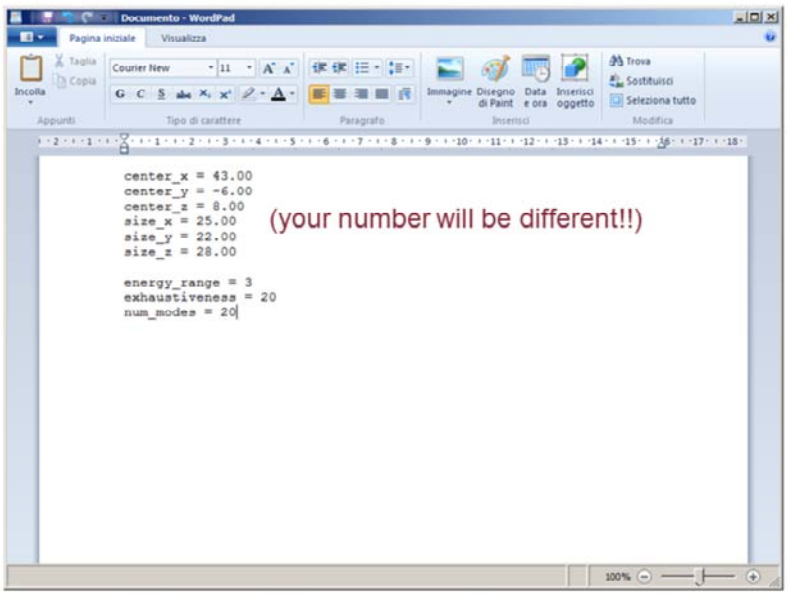
Check al the file are present

Experimental Conformation Re-Docking



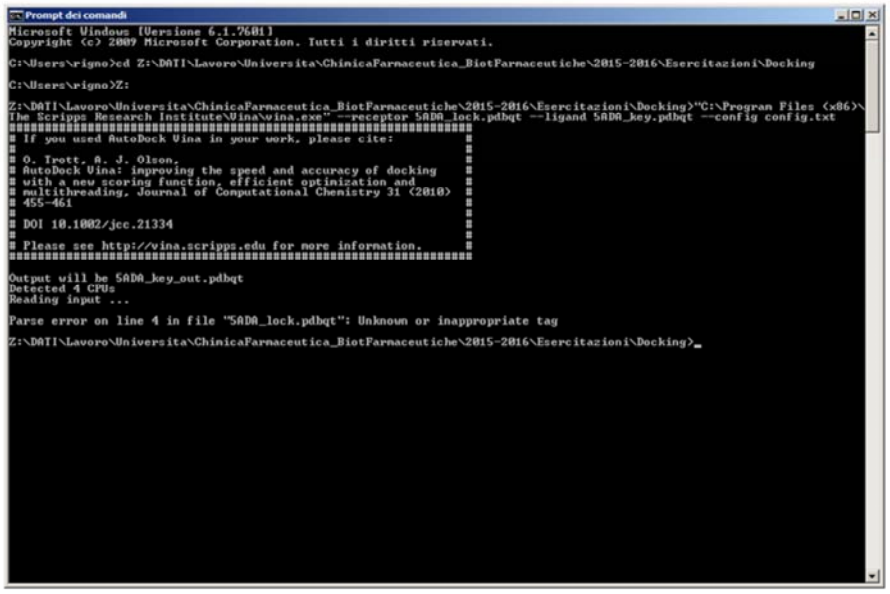
Then prepare a config file for Autodock Vina, Open the wordpad program

Experimental Conformation Re-Docking



Insert the correct info (your number will be different!!)

Experimental Conformation Re-Docking

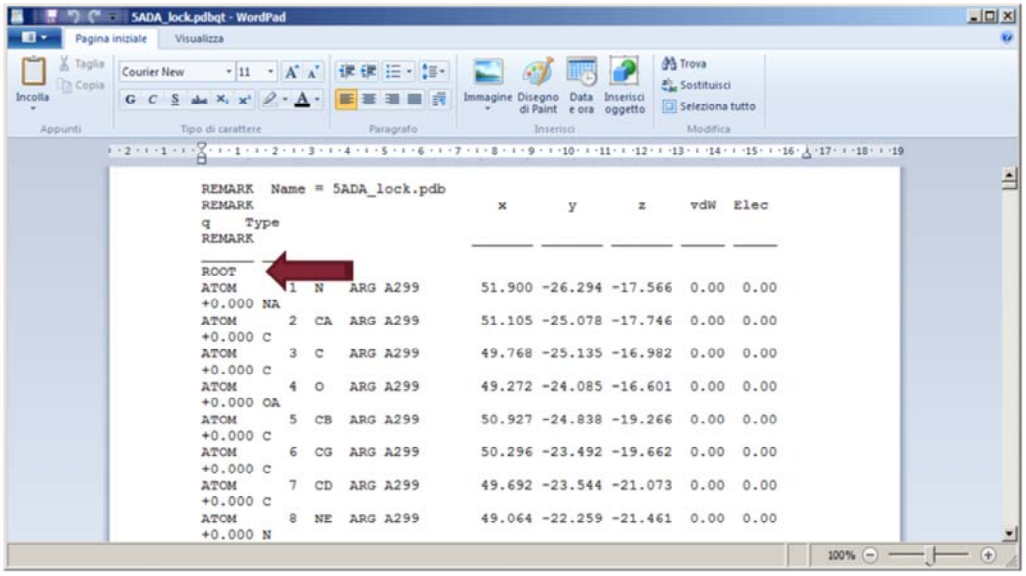


Try to run the docking in the DOS terminal by issuing the following command:

Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor 5ADA_lock.pdbqt --ligand 5ADA_key.pdbqt --config config.txt

The program stops outputting an error is in the lock file

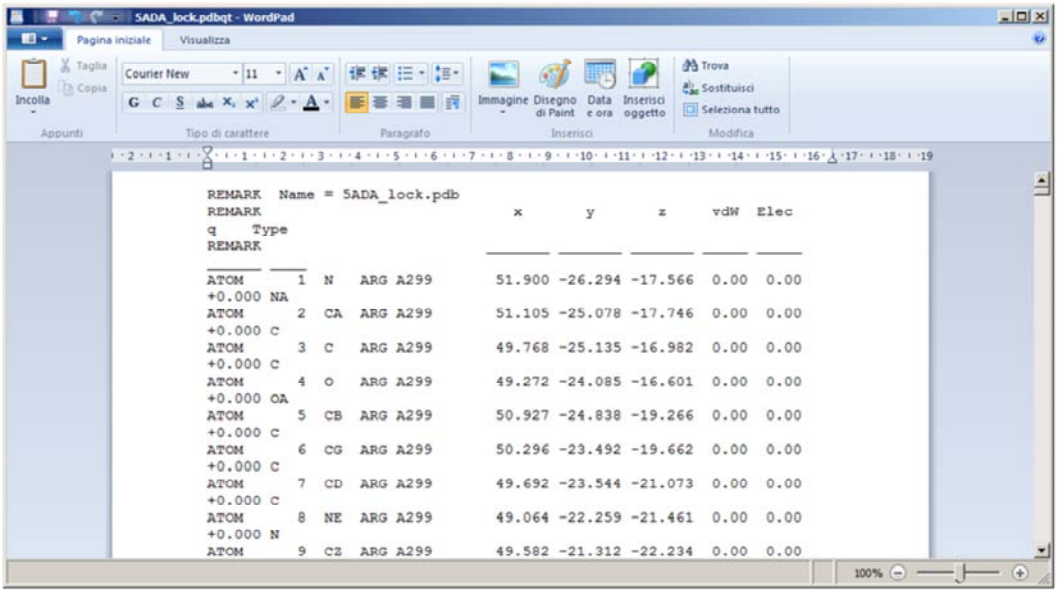
Experimental Conformation Re-Docking

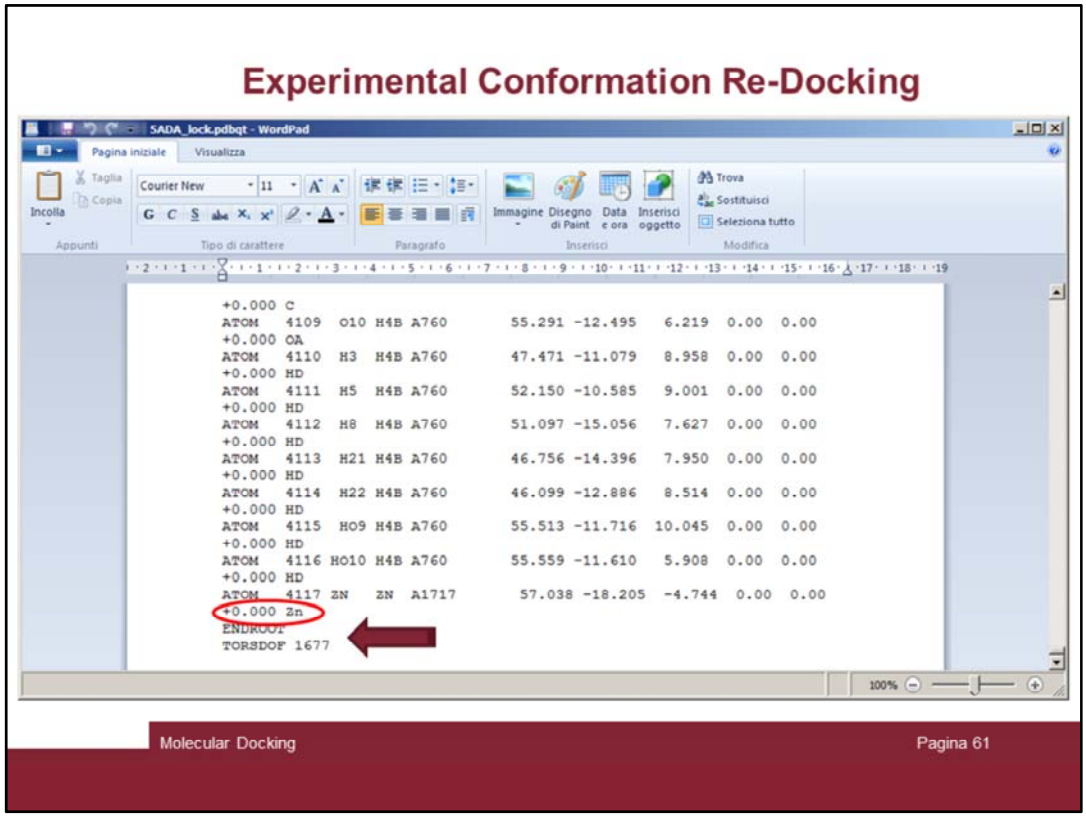


Open and fix it!

First remove the line containing the “ROOT” word at the beginning of the file

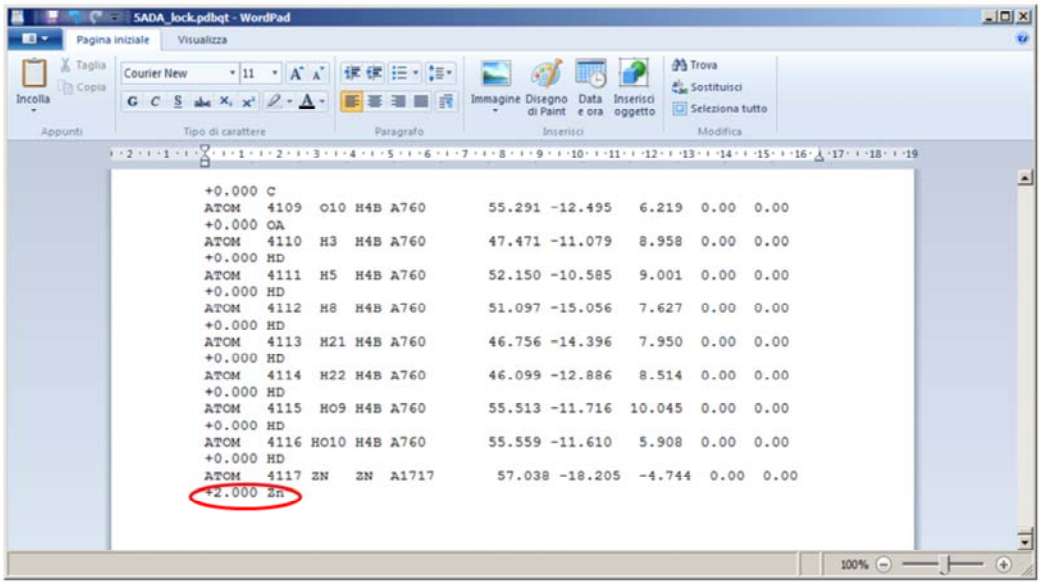
Experimental Conformation Re-Docking



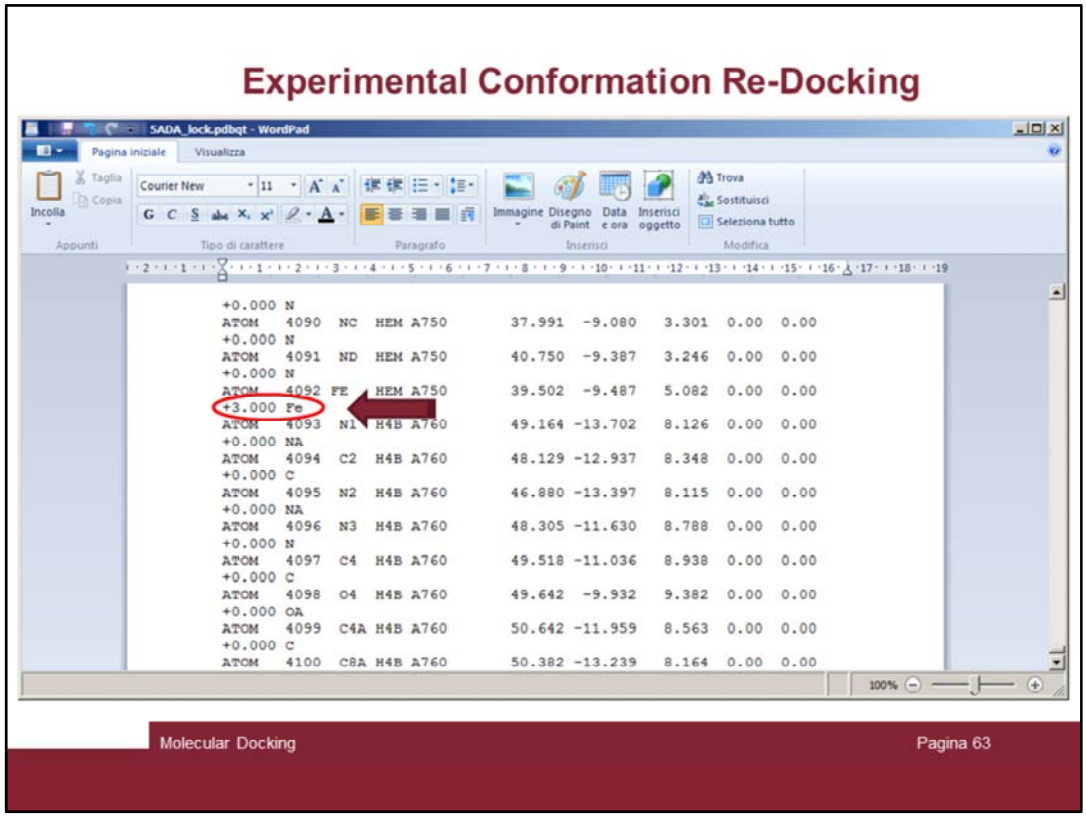


Then remove the ENDROOT and TORSDOF containing lines and correct the charge on the Zn atom!

Experimental Conformation Re-Docking

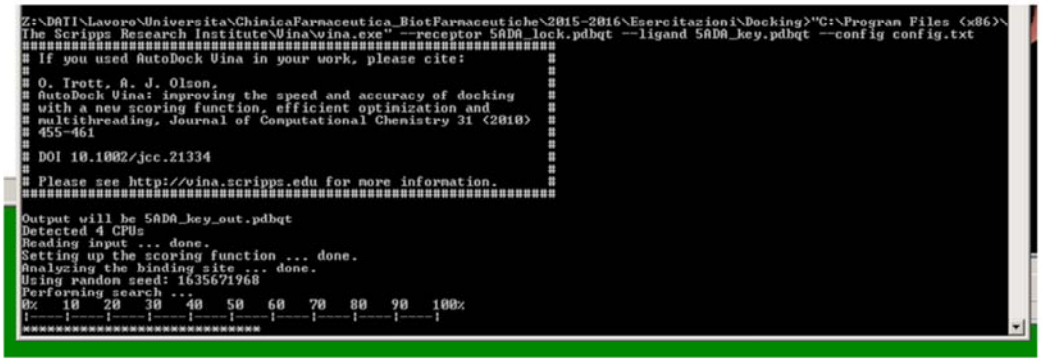


Assume a charge of 2.0 for the Zn



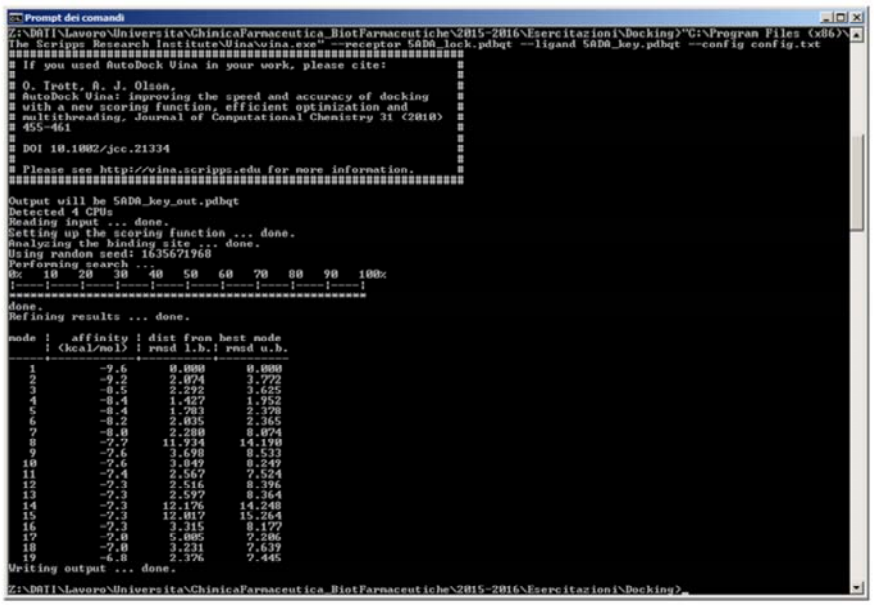
Fix also the charge on the Fe by setting to 3.0

Experimental Conformation Re-Docking

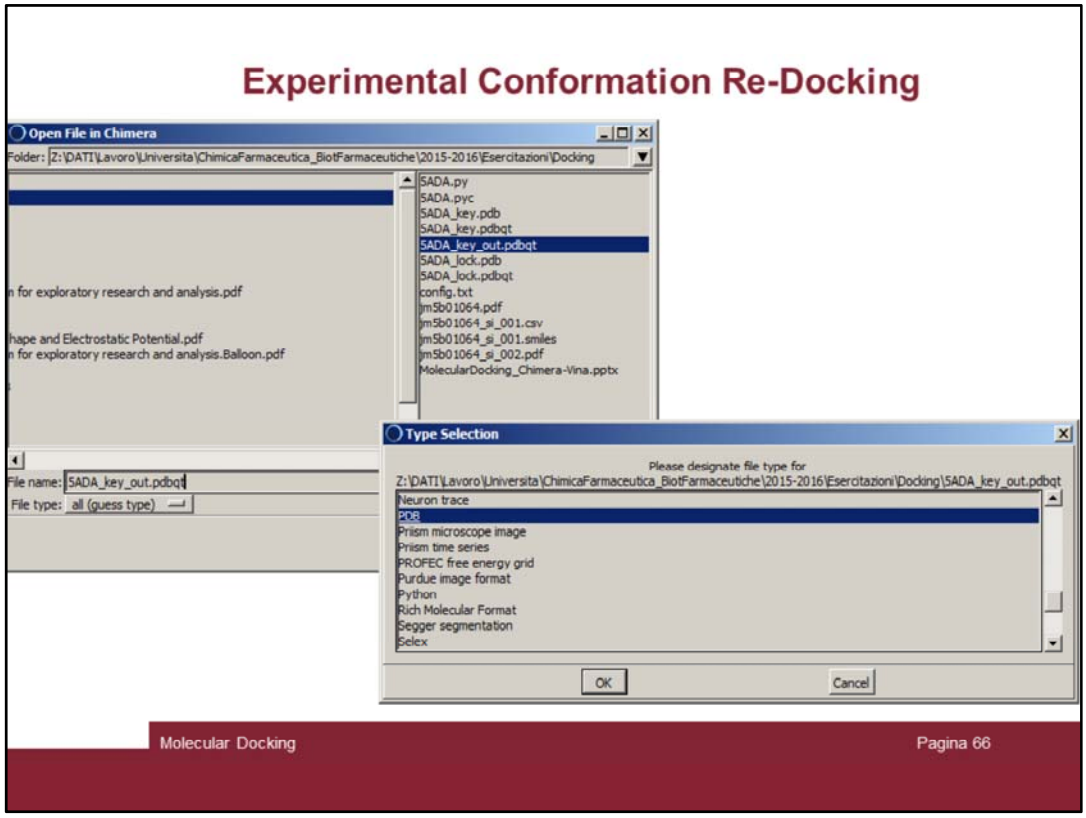


Launch the docking and now it should go.

Experimental Conformation Re-Docking

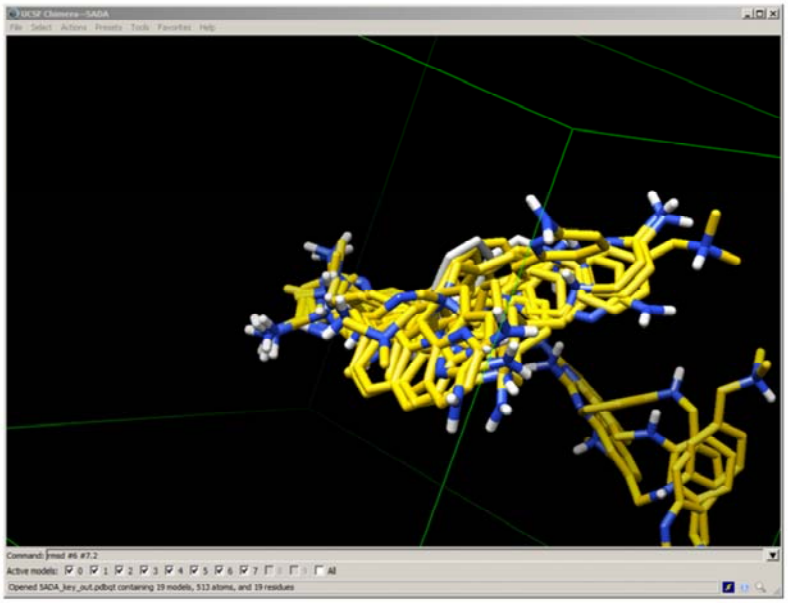


When it will be over



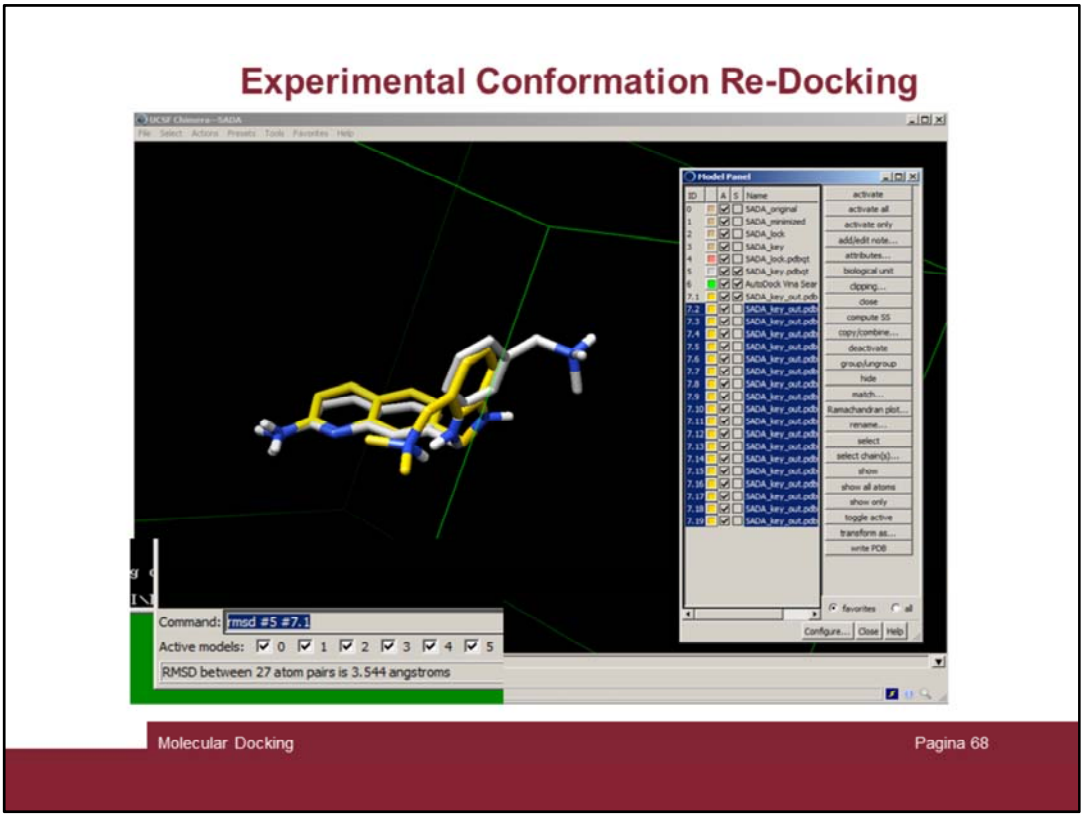
Read in chimera the output file (5ADA_key_out.pdbqt) and instruct the program to consider the file as a normal PDB one

Experimental Conformation Re-Docking



Molecular Docking Pagina 67

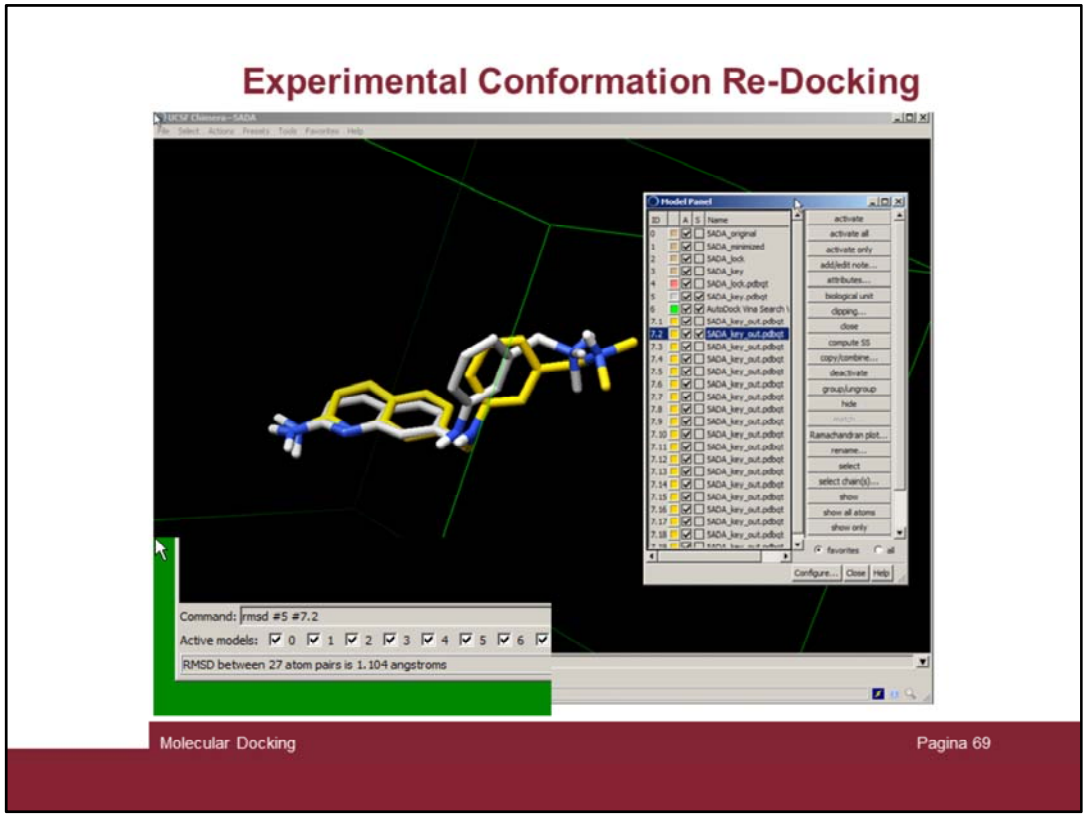
The docked conformations will appear in the chimera windows.



Ungroup them and hide all conformations except the first (7.1 here).

As you can see the yellow conformation (re-docked) is well superimposed on the experimental one. It is also possible to calculate the RMSD value by issuing the “rmsd #5 #7.1” command.

We have an RMSD value of 3.5, it is not very good but actually the trimethyl amino methyl side chain have space to move.



Indeed the second conformation is much better with an RMSD value of just 1.1! And it is only 0.4 kcal/mol away from conformation 1 (see slide 65)

Exercise

1. Make a random conformation of compound 17 using balloon, starting from its smiles (slide 8)
2. Perform a random conformation re-docking (RCRD)
3. Prepare a different molecule and study its binding mode