

Molecular Docking Tutorial

Using Autodock Vina within Chimera



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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.

General Procedure

- Target analysis (1st practical lesson)
- Clean the complex
- Complex minimization
- Lock and key separation
- Docking assessment
- Docking application

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,^{§,§,1} Thomas L. Poulos,^{¶,‡} and Richard B. Silverman^{†,‡}

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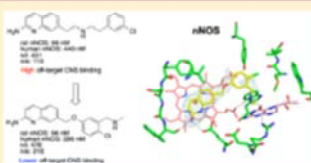
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Supporting Information

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.

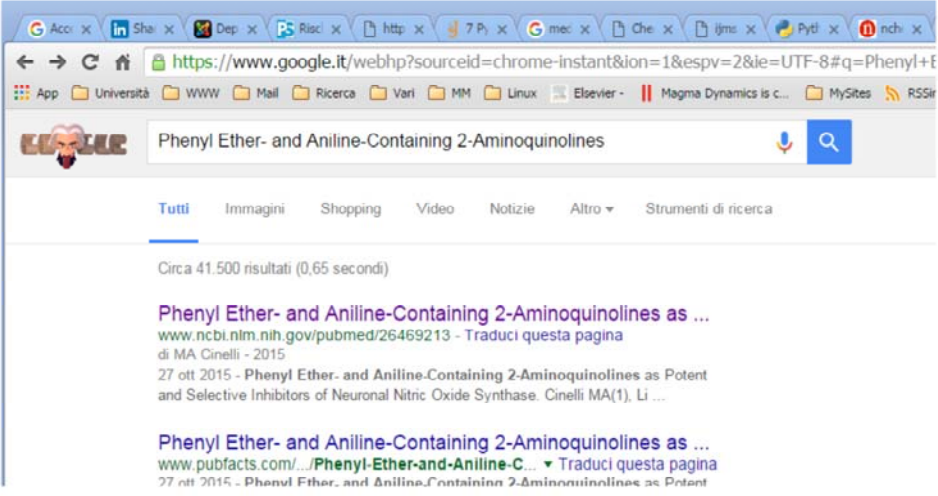


Molecular Docking

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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.

Looking for Info



The screenshot shows a Google search interface. The search bar contains the text "Phenyl Ether- and Aniline-Containing 2-Aminoquinolines". Below the search bar, there are tabs for "Tutti", "Immagini", "Shopping", "Video", "Notizie", "Altro", and "Strumenti di ricerca". The search results show "Circa 41.500 risultati (0,65 secondi)". The first result is from "www.ncbi.nlm.nih.gov/pubmed/26469213" and is titled "Phenyl Ether- and Aniline-Containing 2-Aminoquinolines as ...". The second result is from "www.pubfacts.com" and is titled "Phenyl Ether- and Aniline-Containing 2-Aminoquinolines as ...".

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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.

Looking for Info

The screenshot shows a PubMed article page. At the top, there is a search bar with 'PubMed' entered and a 'Search' button. Below the search bar, the article title is displayed: 'Phenyl Ether- and Aniline-Containing 2-Aminoquinolines as Potent and Selective Inhibitors of Neuronal Nitric Oxide Synthase'. The authors listed are Cinelli MA, Li H, Pensa AV, Kang S, Roman L, Martasek P, Poulos TL, and Silverman RB. The abstract text describes the synthesis and testing of these compounds against purified nNOS, endothelial NOS (eNOS), and inducible NOS (iNOS) enzymes. It mentions that one compound, 20, displayed high potency, selectivity, and good human nNOS inhibition. The article is from J Med Chem, 2015 Nov 12;58(21):8694-712. The page also features a 'Full text links' section with an 'ACS Publications' icon, a 'Save items' section with an 'Add to Favorites' button, and a 'Similar articles' section with several related article titles. At the bottom of the page, there is a dark red banner with the text 'Molecular Docking' on the left and 'Pagina 5' on the right.

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

Looking for Info

Journal of Medicinal Chemistry

ACS Med. Chem. Lett. Subscriber access provided by UNIVERSITA DI ROMA LA SAPIENZA

Search Citation DOI Subject **Advanced Search**

Search text: Anywhere

J. Med. Chem. All Publications/Website

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

Maris A. Cinelli¹, Huiying Li¹, Anthony V. Pensa¹, Soosung Kang¹, Linda J. Roman², Pavel Martasek^{3,4}, Thomas L. Poulos¹, and Richard B. Silverman¹

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Copyright © 2015 American Chemical Society
*(T.L.P.) Tel: +1 949 624 7020. E-mail: poulos@uci.edu. *(R.B.S.) Tel: +1 847 491 5653. Fax: +1 847 491 7713. E-mail: Agman@chem.northwestern.edu.

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Cinelli, Maris A.

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

Looking for Info

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jmedchem.5b01330](https://doi.org/10.1021/acs.jmedchem.5b01330).

- Crystallographic data collection and refinement statistics for rat and human nNOS, eNOS, and nNOS double mutant crystal structures; synthesis and analytical data for compounds 28–59; and mNOS-10, eNOS-7, -8, -17, -20, and hnNOS-17 crystal structures (PDF)
- SMILES data (CSV)

Accession Codes

PDB codes for X-ray crystal structures described in this study have been deposited in the Protein Data Bank under the following accession codes: 5AD4, 5AD5, 5AD6, 5AD6, 5AD8, 5AD9, 5ADA, 5ADB, 5ADC, 5ADD, 5ADE, 5ADF, 5ADG, 5ADI, 5ADJ, 5ADK, 5ADL, 5ADN, 5FJ2, and 5FJ3.

Let's scroll down it till supporting information appear

Looking for Info

```
Compound,SMILES, rat nNOS KI value (aM), Human nNOS Ki value (aM), murine iNOS Ki value (aM), B
selectivity, rat/human selectivity
6, NC1=NC2=CC(COC3=CC(CCN(C)C)=CC=C3)=CC=C2C=C1, 0.468, 1.86, 150, 17.7, 320, 38, 4
7, NC1=NC2=CC(COC3=CC(CCN(C)C)=CC=C3)=CC=C2C=C1, 0.332, NT, 43.6, 15.1, 131, 45, ND
8, NC1=NC2=CC(COC3=CC=CC(CN(C)C)=C3)=CC=C2C=C1, 0.179, 0.855, 60.2, 18, 338, 101, 4.8
9, NC1=NC2=CC(COC3=CC=CC(CNC)=C3)=CC=C2C=C1, 0.142, 0.911, 33.2, 25.3, 237, 178, 6.4
10, NC1=NC2=CC(COC3=CC=CC(CN)=C3)=CC=C2C=C1, 0.16, NT, 33.6, 12.9, 210, 80, ND
11, NC1=NC2=CC(COC3=CN=CC=C3)=CC=C2C=C1, 0.712, NT, NT, NT, ND, ND, ND
12, NC1=CC=C2C(C=C(COC3=CC=CC(N(C)C)=C3)C=C2)=N1, >5.75, NT, NT, NT, ND, ND, ND
13, NC1=NC2=CC(COC3=CC(CCCN(C)C)=CC=C3)=CC=C2C=C1, 0.652, NT, NT, NT, NT, ND, ND
14, NC1=NC2=CC(COC3=CC(OCCN(C)C)=CC=C3)=CC=C2C=C1, 0.475, NT, 178, 31, 379, 44, ND
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16, NC1=NC2=CC(COC3=CC=C(CNC)=C3)=CC=C2C=C1, 0.332, NT, 48.7, NT, 147, ND, ND
17, NC1=NC2=CC(CNC3=CC=CC(CN(C)C)=C3)=CC=C2C=C1, 0.375, 0.657, 33.2, 11.5, 89, 31, 1.8
18, NC1=NC2=CC(CNC3=CC=CC(CNC)=C3)=CC=C2C=C1, 0.569, NT, NT, NT, ND, ND, ND
19, NC1=NC2=CC(COC3=CC=C(F)C(CNC)=C3)=CC=C2C=C1, 0.147, NT, 11.4, NT, 78, ND, ND
20, NC1=NC2=CC(COC3=CC=C(C1)C(CNC)=C3)=CC=C2C=C1, 0.058, 0.295, 27.7, 12.5, 478, 216, 5.1
21, NC1=NC2=CC(COC3=CN=CC(CNC)=C3)=CC=C2C=C1, 0.043, 0.507, 13.5, 3.31, 313, 77, 11.8
22, NC1=NC2=CC(COC3=CC=CC(C(C)NC)=C3)=CC=C2C=C1, 0.36, NT, 67.1, NT, 186, ND, ND
```

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

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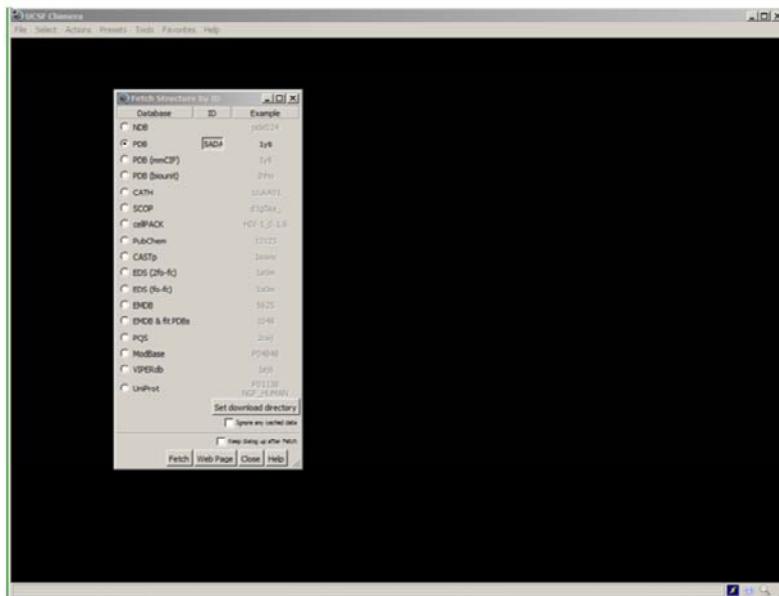
The «pdf» file contains others info

Looking for Info

Data set ^r	nNOS-10	nNOS-15	nNOS-17	nNOS-20
Data collection				
PDB code	5AD8	5AD9	5ADA	5ADB
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell dimensions: <input type="checkbox"/>	51.9 111.9 164.3	51.7 111.6 164.1	51.9 111.4 164.3	51.7 111.0 165.1
a, b, c (Å)				
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)

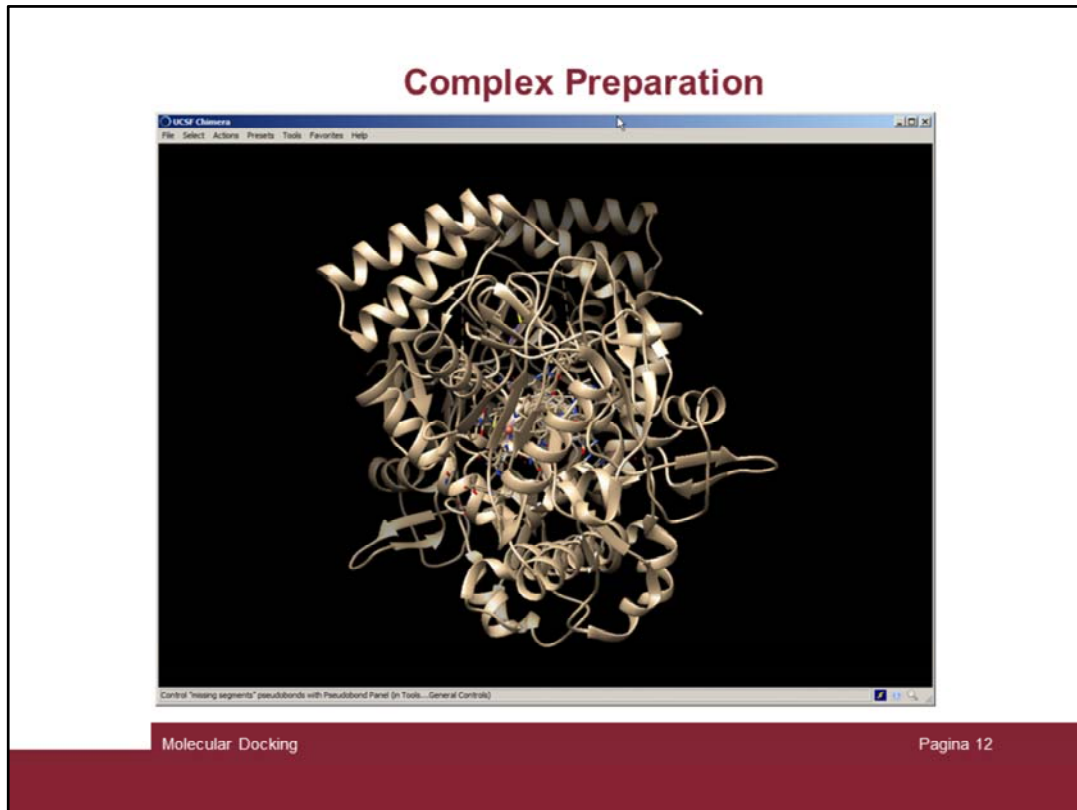
Complex Preparation



Molecular Docking

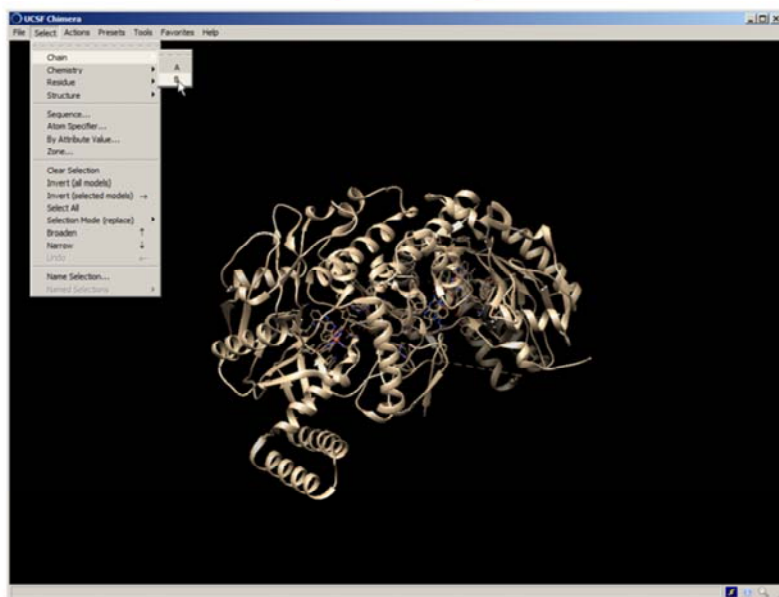
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Now turn to chimera and fetch the 5ADA pdb file through the File → Fetch



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

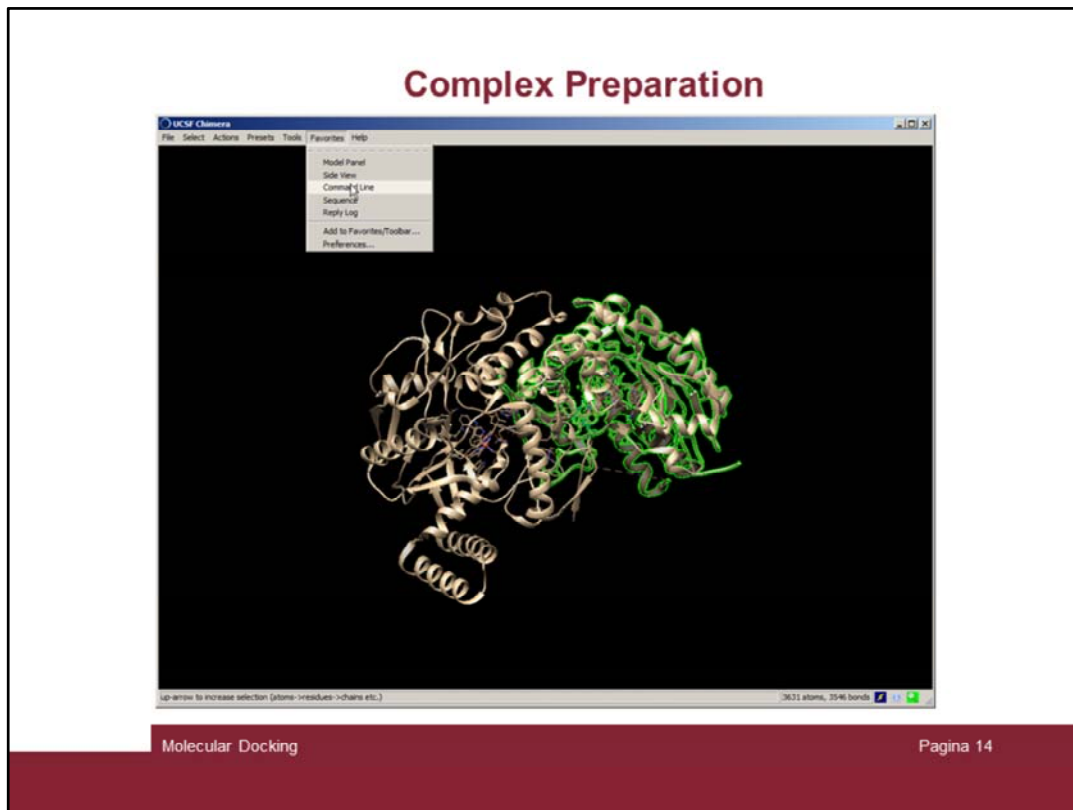
Complex Preparation



Molecular Docking

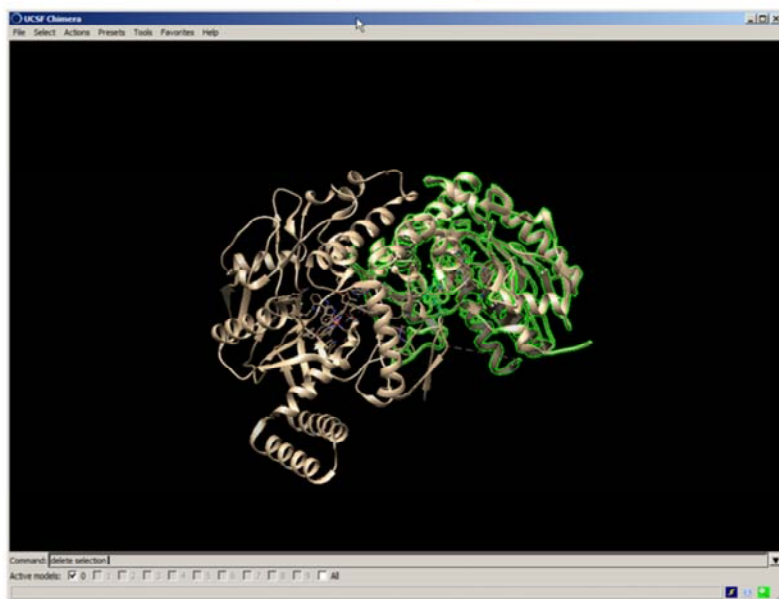
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We just need one chain and thus we can delete chain B. To do this first select chain B ...



Then open the command line tool ...

Complex Preparation

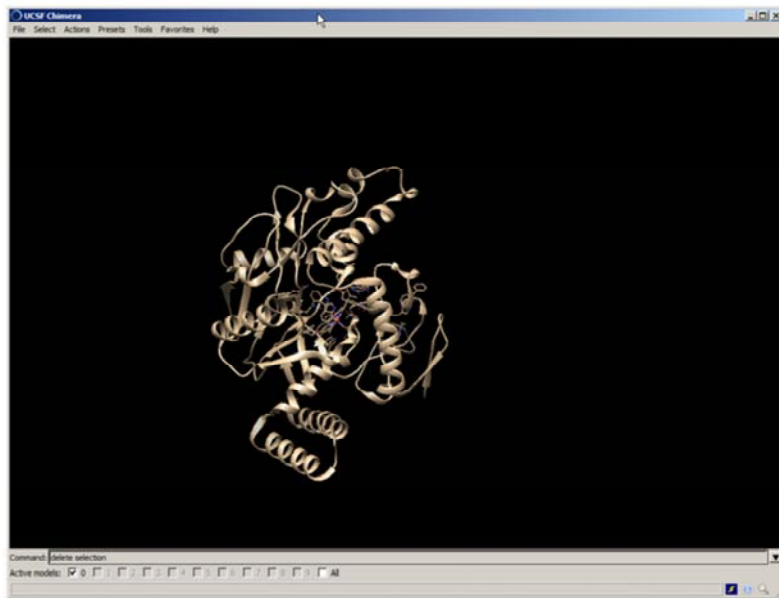


Molecular Docking

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

Complex Preparation

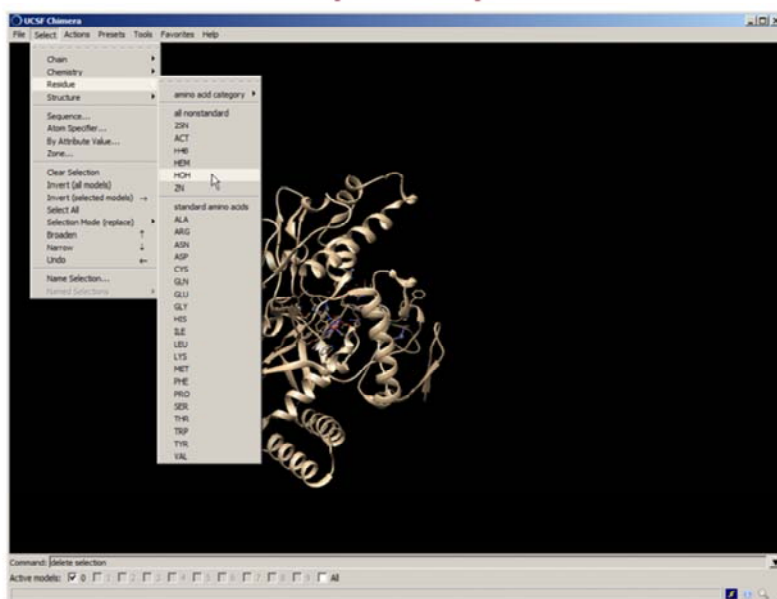


Molecular Docking

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

Complex Preparation

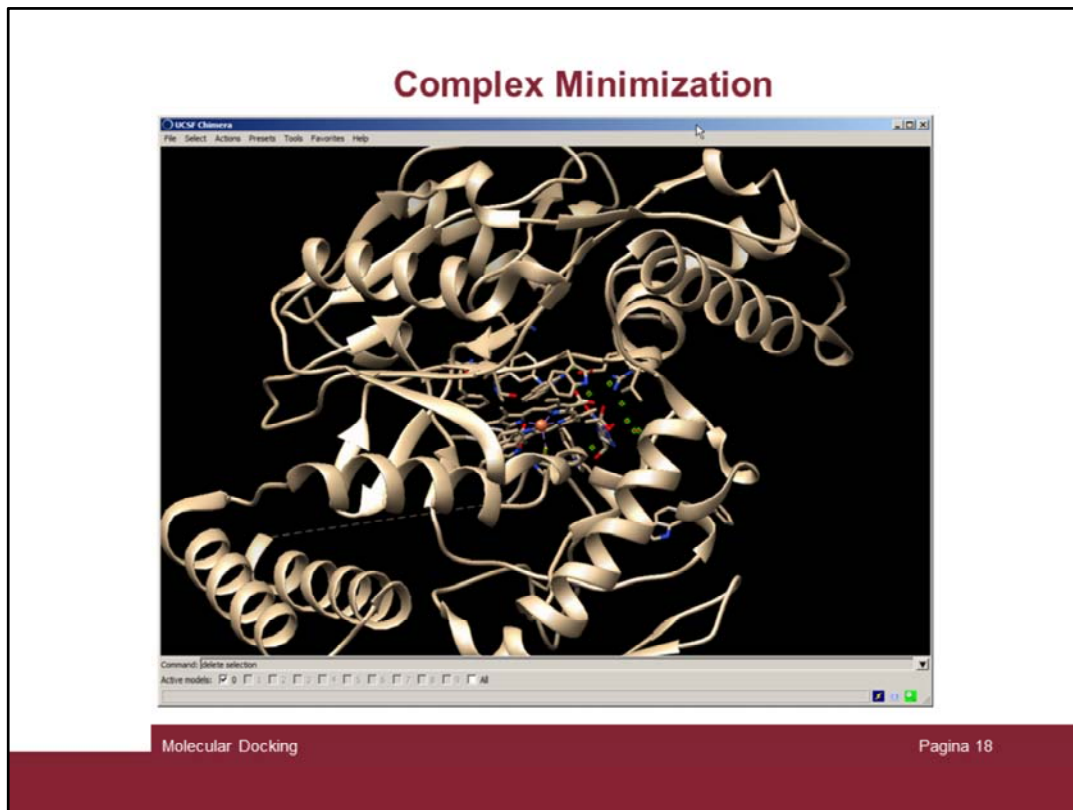


Molecular Docking

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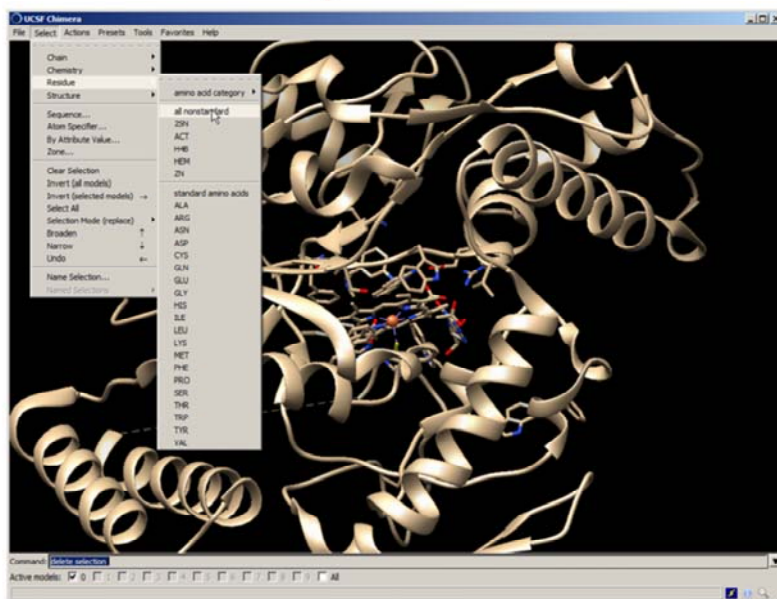
To prepare the complex for the geometry relaxation and the subsequent molecular docking runs it is advisable to remove all non standard residue not necessary for the study. Therefore we remove the crystallization water (solvent) and all not interesting ions and small molecules (in this case there is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

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



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

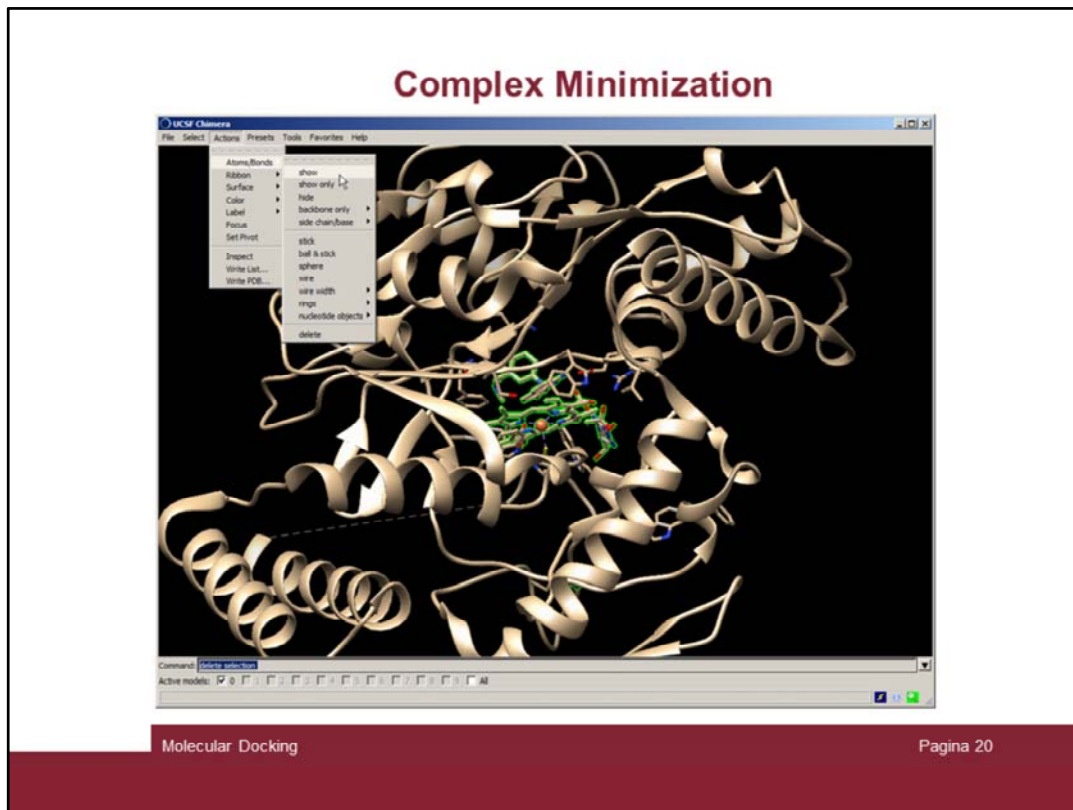
Complex Minimization



Molecular Docking

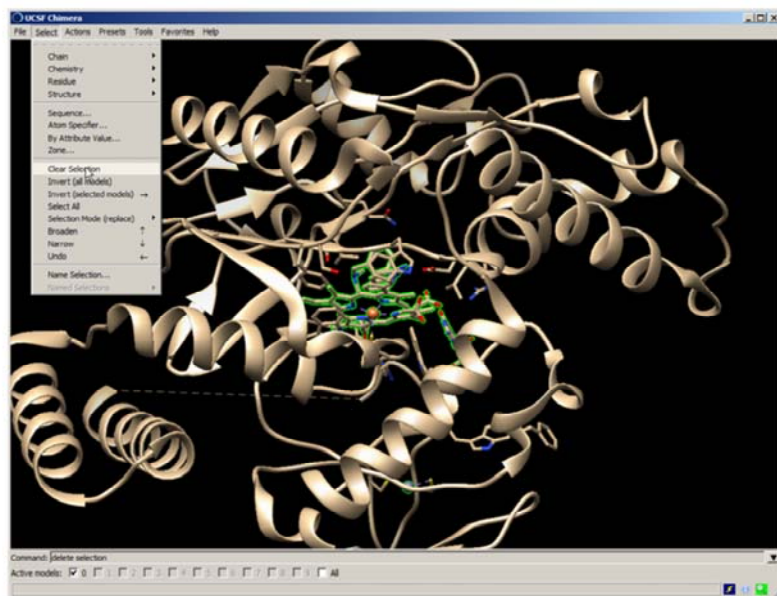
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Then check for all non standard residues by the “Select → all nonstandard” menu



The nonstandard residues must be displayed (“Action → show”)

Complex Minimization

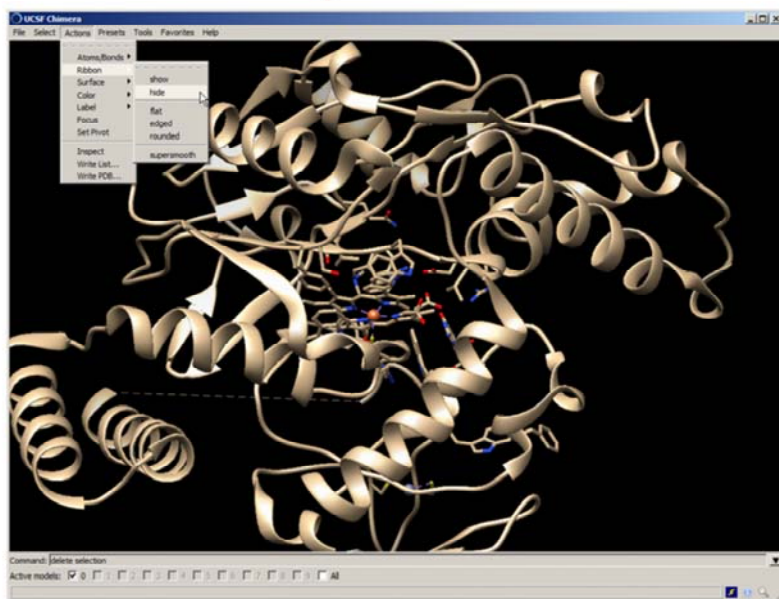


Molecular Docking

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Deselect everything («Select → Clear Selection»)

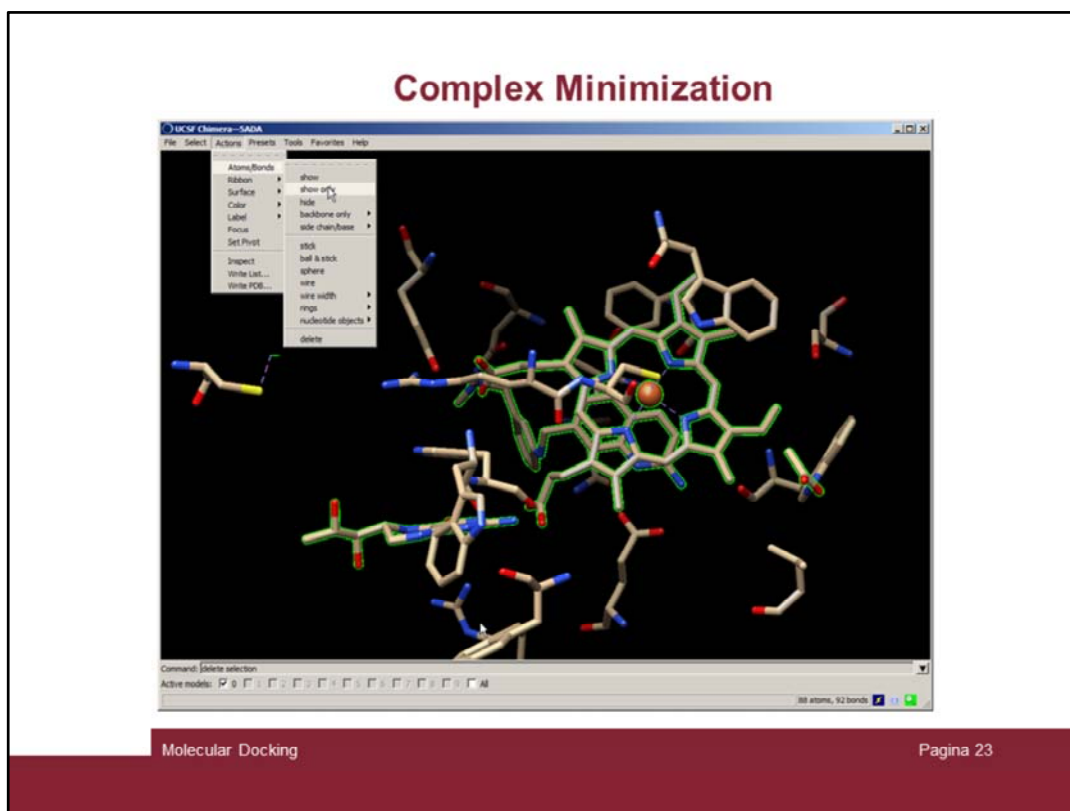
Complex Minimization



Molecular Docking

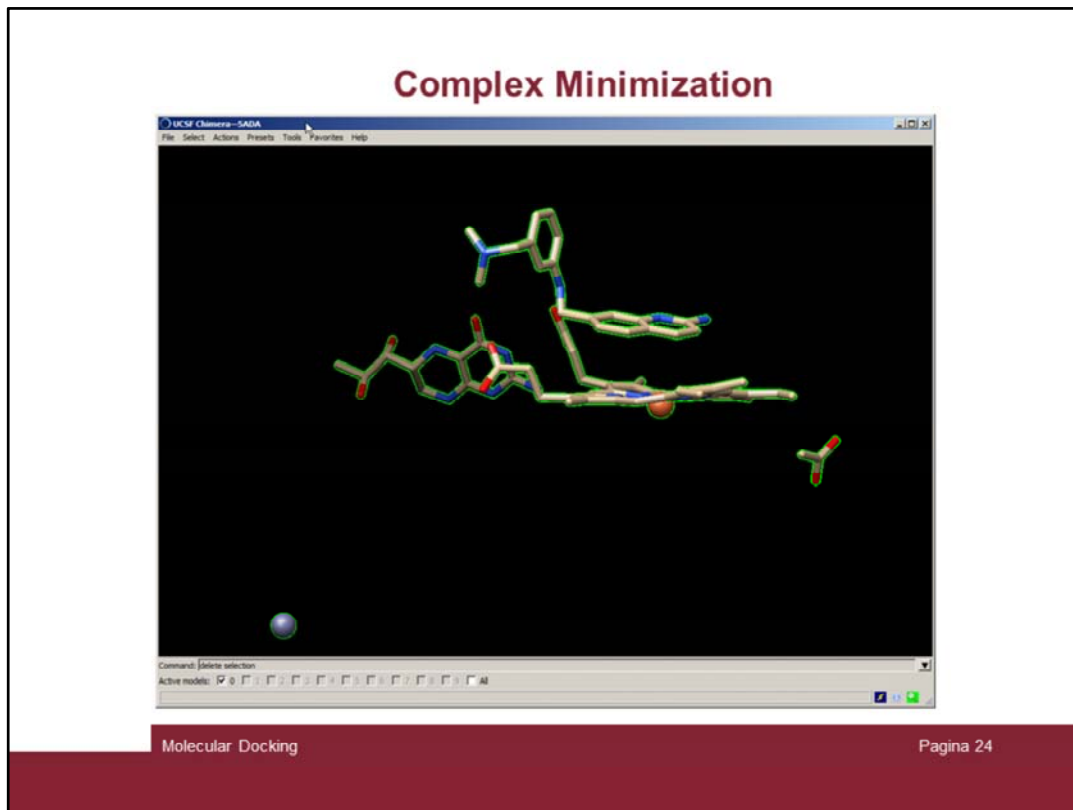
Pagina 22

And hide the ribbon








Select non standar residue again and focus on that selection

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



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

Complex Minimization

Small Molecules				
Ligands 5 Unseen				
ID	Chains	Name / Formula / InChI Key	2D Diagram & Interactions	3D Interactions
2SN Query on 2SN Download SDF File Download CCD File	A, B	7-[[3-[[dimethylamino)methyl]phenyl]amino)methyl]quinolin-2-amine C ₁₉ H ₂₂ N ₄ KLVQNBMBSDRIZ-UHFFFAOYSA-N		Ligand Explorer JSmol
ACT Query on ACT Download SDF File Download CCD File	A, B	ACETATE ION C ₂ H ₃ O ₂ QTBSBXVTEAMEGO-UHFFFAOYSA-M		Ligand Explorer JSmol
H4B Query on H4B Download SDF File Download CCD File	A, B	5,6,7,8-TETRAHYDROBIPTERIN C ₉ H ₁₃ N ₃ O ₂ FNKQXYHWGSIFBK-RPDRRWSUSA-N		Ligand Explorer JSmol
HEM Query on HEM Download SDF File Download CCD File	A, B	PROTOPORPHYRIN IX CONTAINING FE HEME (Synonym) C ₃₄ H ₃₂ FeN ₄ O ₄ FEDYMSUPMFCVOD-UJLXFSCMSA-N		Ligand Explorer JSmol
ZN Query on ZN Download SDF File Download CCD File	A	ZINC ION Zn PTFCDOFLOPIGGS-UHFFFAOYSA-N		Ligand Explorer JSmol

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.

Complex Minimization

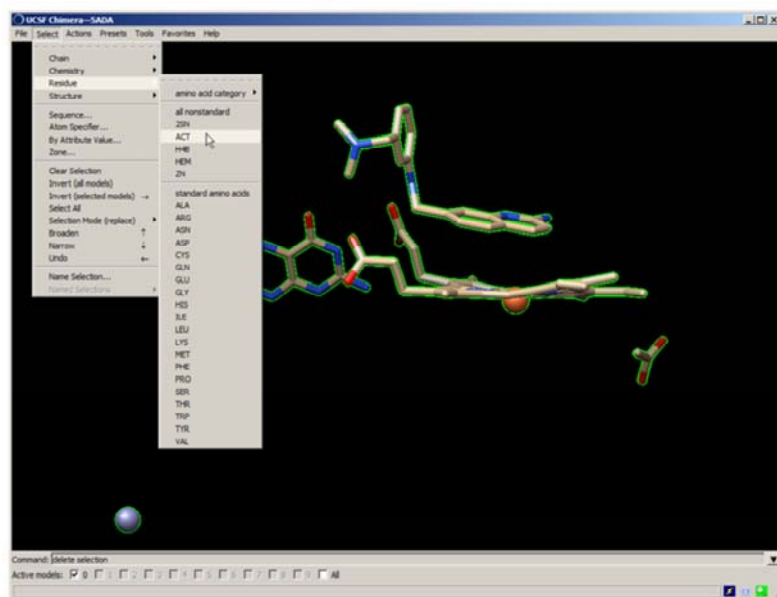
5.2 Role of BH₄

The haem requirement for dimerisation is common to all three NOS isoforms. They do however differ with respect to the role of BH₄ in dimerisation. Whereas nNOS and eNOS can form dimers in the absence of BH₄ [48], iNOS dimerisation was reported to require the presence of the pteridine [32], although dimers were formed in *E. coli* in the absence of BH₄ [49]. Furthermore, BH₄ stabilises the nNOS and eNOS dimers once formed, and also the iNOS dimer, although not to the same extent [33,48,50,51]. These data are supported by the reduced binding of BH₄ by an N-terminal deletion mutant of iNOS, demonstrating the importance of residues 66–114 in iNOS for binding of the cofactor and hence dimerisation [52]. The recent crystallographic data also show the location of BH₄ at the dimer interface [29]. Although these observations have had an impact on in vitro synthesis and reconstitution experiments, the functional implications remain uncertain. The close resemblance of 4-amino-BH₄, a novel pterin-based inhibitor of NOS [51,53], to BH₄ in terms of conformational changes induced (low-spin to high-spin conversion of the haem, dimer stabilisation, increased affinity for L-arginine) despite the inability to support NO production suggests a more complex role for BH₄ than merely inducing conformational changes [51,54]. The close proximity of BH₄ to the haem, as well as to the flavins at the domain–domain interface [38], hints at a possible role in electron transfer [55], although exogenously added BH₄ does not appear to provide electrons for the reaction [56]. In this respect, the role of BH₄ in NOS differs from that in aromatic amino acid hydroxylation [57]. A thorough analysis of the interaction of numerous pterins with iNOS revealed that the steps up to and including haem reduction are supported by dihydropterins as well as tetrahydropterins [58]. However, only the latter are able to support NO synthesis and NADPH oxidation. The role of BH₄ in electron transfer therefore remains to be settled, although it has been shown that the cofactor accelerates the decay of the ferrous–dioxy complex of nNOS, providing a novel hint to its role in NO synthesis [59]. Despite these clues, the full role of BH₄ in NOS catalysis remains to be elucidated.

5.3 Role of L-arginine

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

Complex Minimization

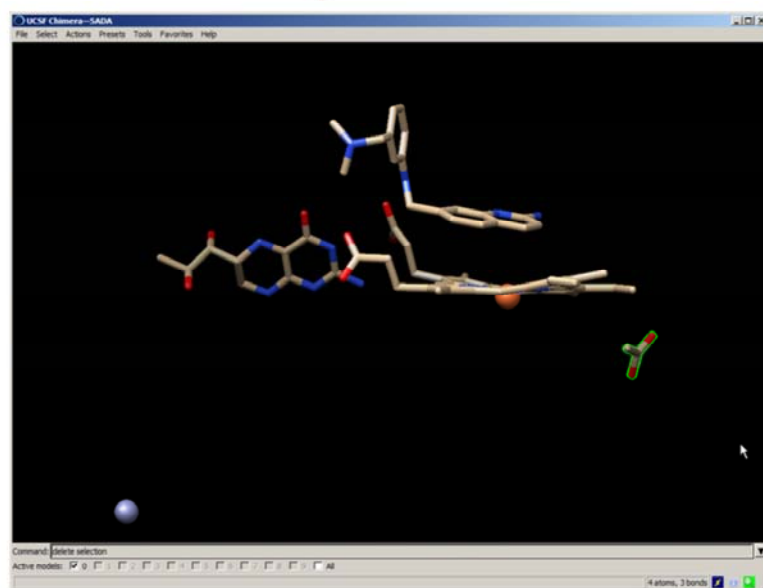


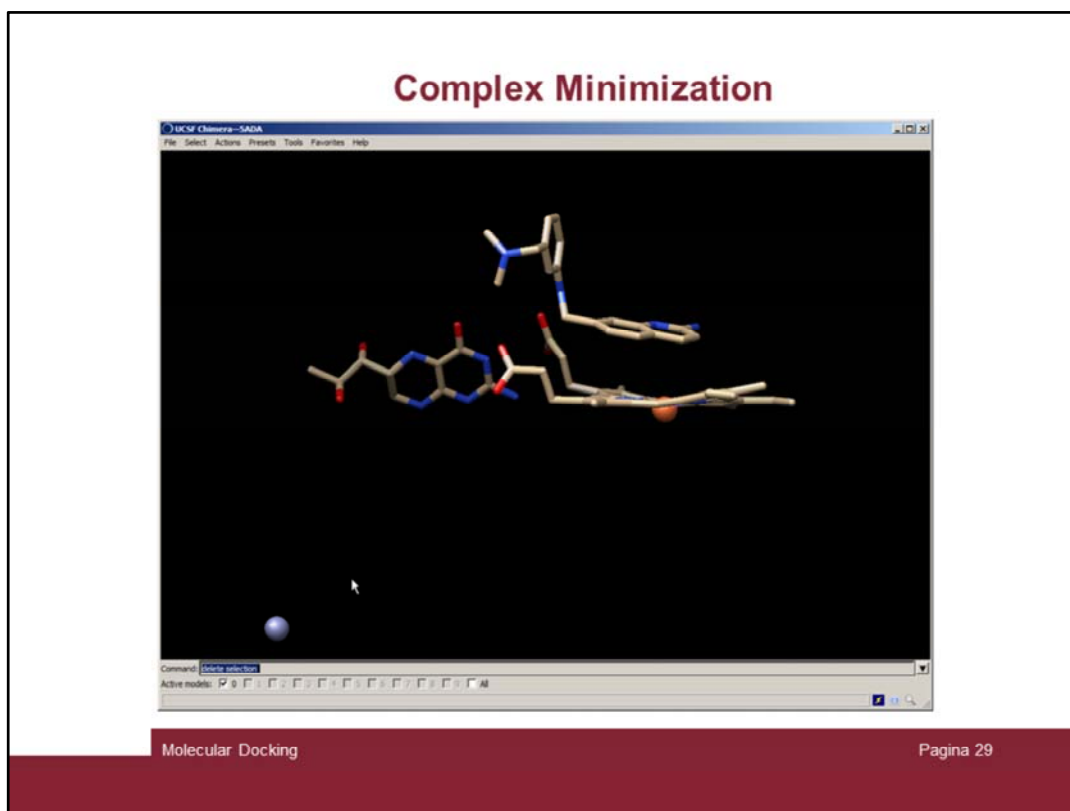
Molecular Docking

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

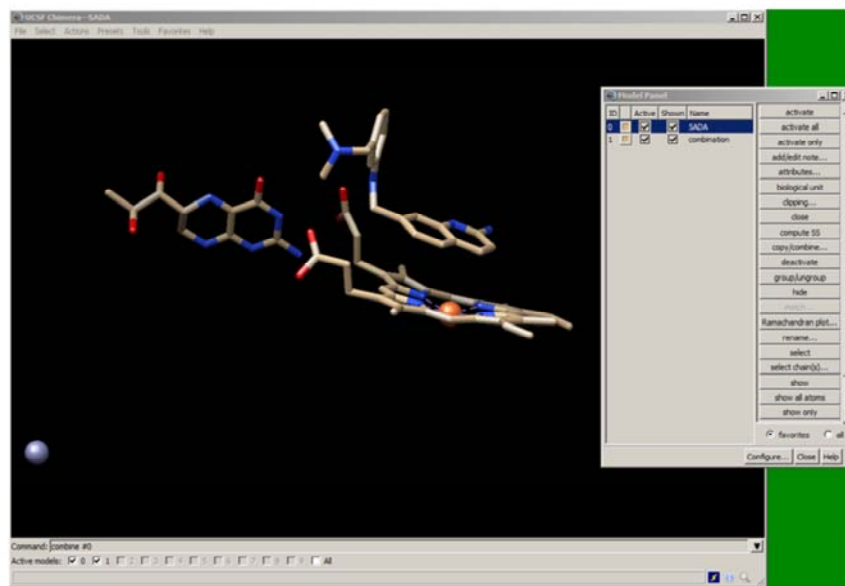
Complex Minimization





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

Complex Minimization



Molecular Docking

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

Complex Minimization

The screenshot illustrates the process of renaming models in the Molecular Docking software. It shows two instances of the 'Model Panel' window and two 'Rename' dialog boxes.

Model Panel (Left):

ID	Active	Shown	Name
0	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	SADA
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	combination

Rename Dialog (Top):

Rename to: SADA_original
 Rename models
 Rename groups

Rename Dialog (Bottom):

Rename to: SADA_minimized
 Rename models
 Rename groups

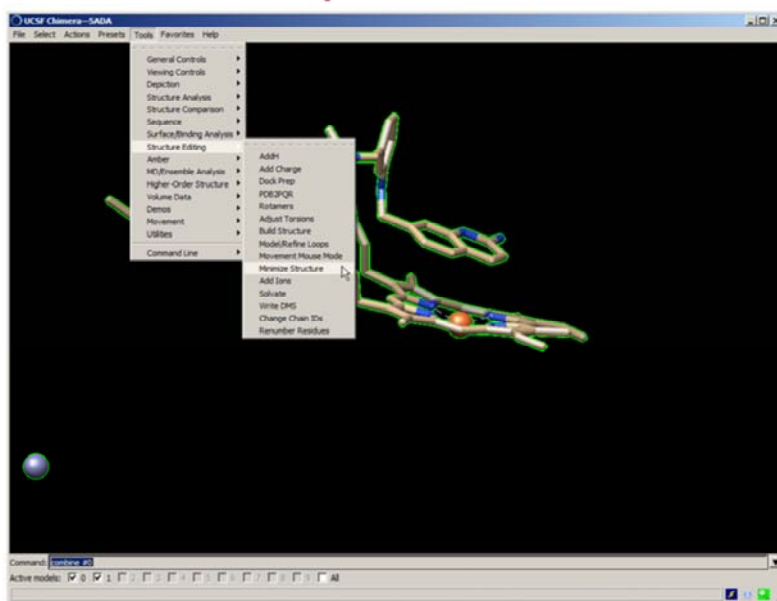
Model Panel (Right):

ID	Active	Shown	Name
0	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	SADA_original
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	SADA_minimized

The bottom of the slide features a dark red banner with the text 'Molecular Docking' on the left and 'Pagina 31' on the right.

Rename the two complexes as reported in the slide

Complex Minimization

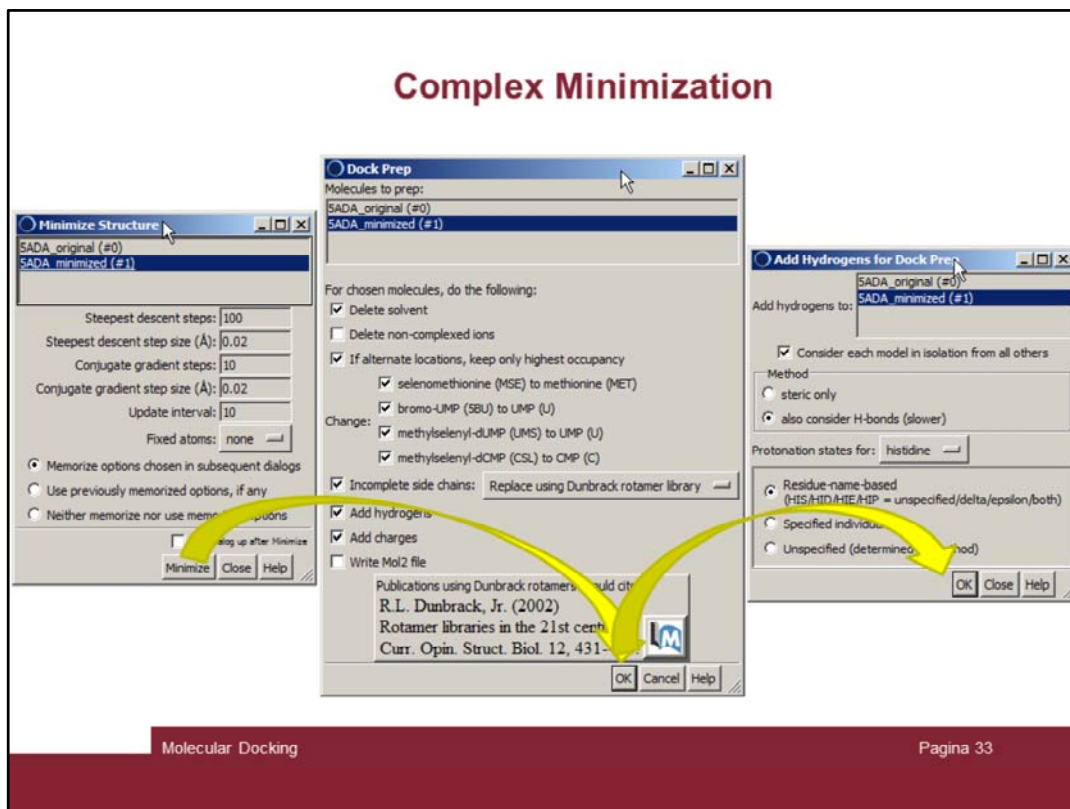


Molecular Docking

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

Complex Minimization



Consecutive windows will pop up

Complex Minimization

Specify Net Charges

Residue	Net Charge
ZSN	+1
H4B	+0
HEM[FE]	+2
HEM[non-FE]	-4
ZN	+2

Please specify the net charges for the above residues so that their atomic partial charges can be computed.

Charge method: AM1-BCC Gasteiger

Charges are computed using ANTECHAMBER.
Publications using ANTECHAMBER charges should cite:
Wang, J., Wang, W., Kollman, P.A., and Case, D.A. (2006)
Automatic atom type and bond type perception in molecular mechanical simulations
Journal of Molecular Graphics and Modelling, 25, 247-260.

OK Cancel Help

Assign Charges for Minimize

Add charges to: SADA_original (#0)
SADA_minimized (#1)

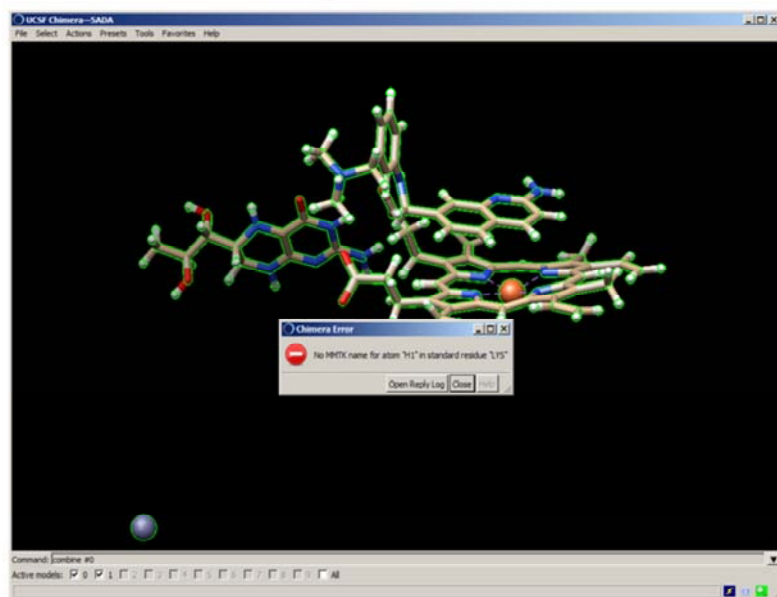
Standard residues: AMBER ff14SB

Other residues: AM1-BCC Gasteiger

Add labels showing charges to atoms in: nonstandard residues
 standard residues

OK Close Help

Complex Minimization

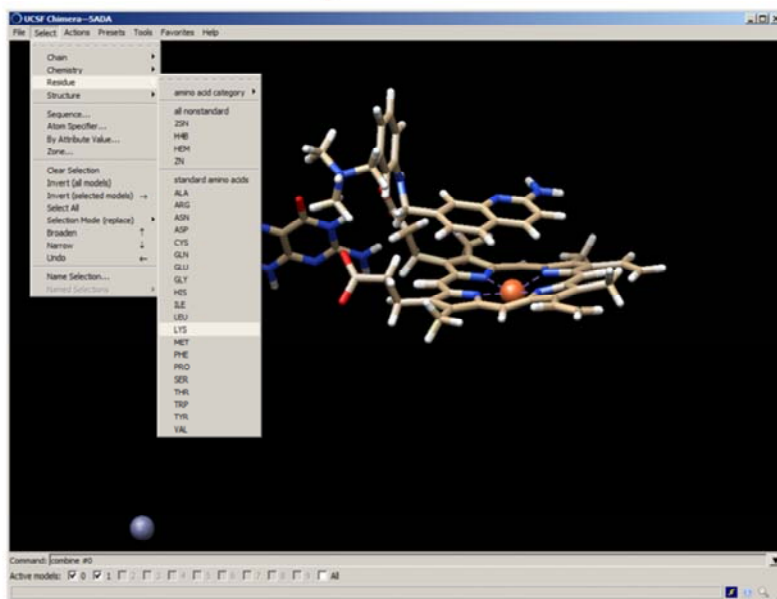


Molecular Docking

Pagina 35

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

Complex Minimization

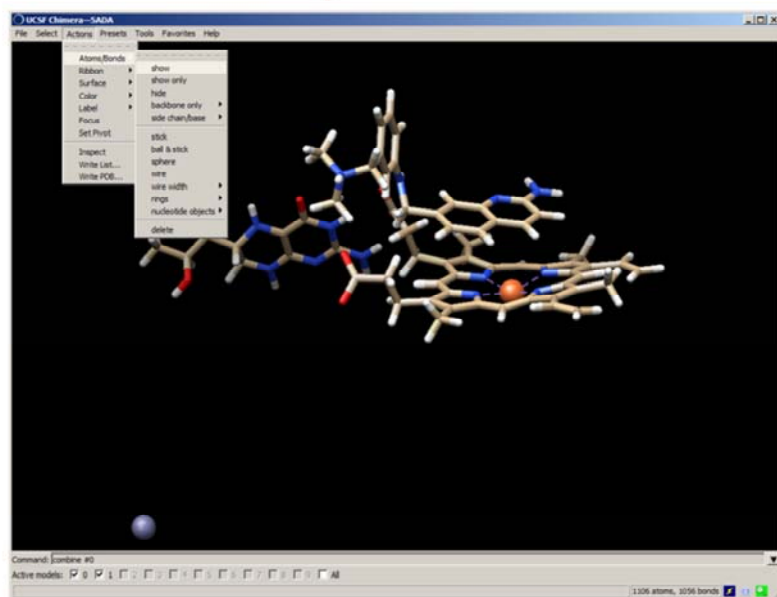


Molecular Docking

Página 36

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

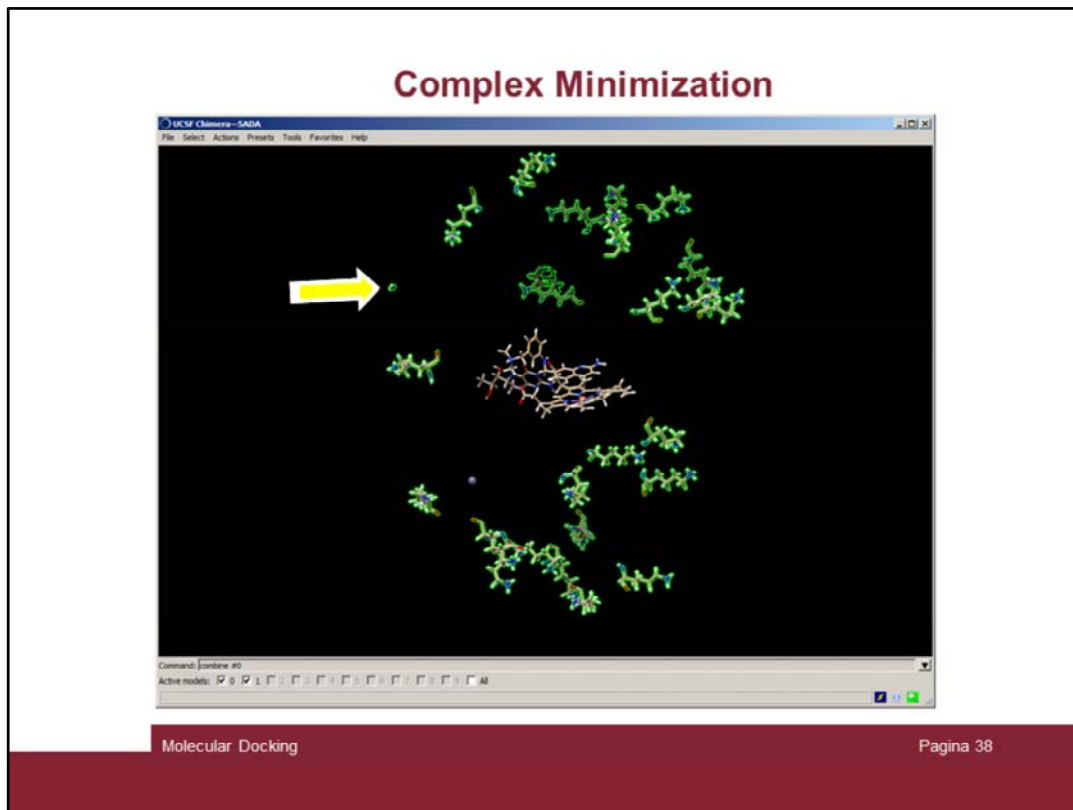
Complex Minimization



Molecular Docking

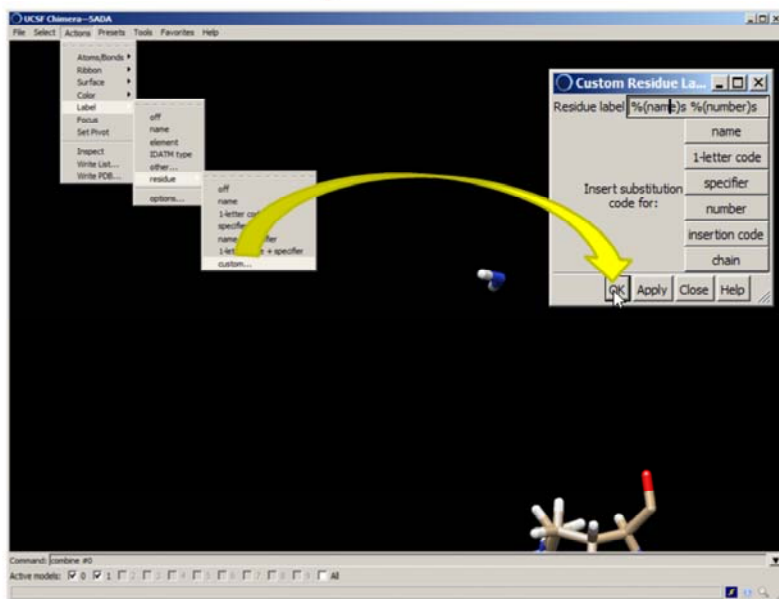
Pagina 37

... "Actions → show"



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

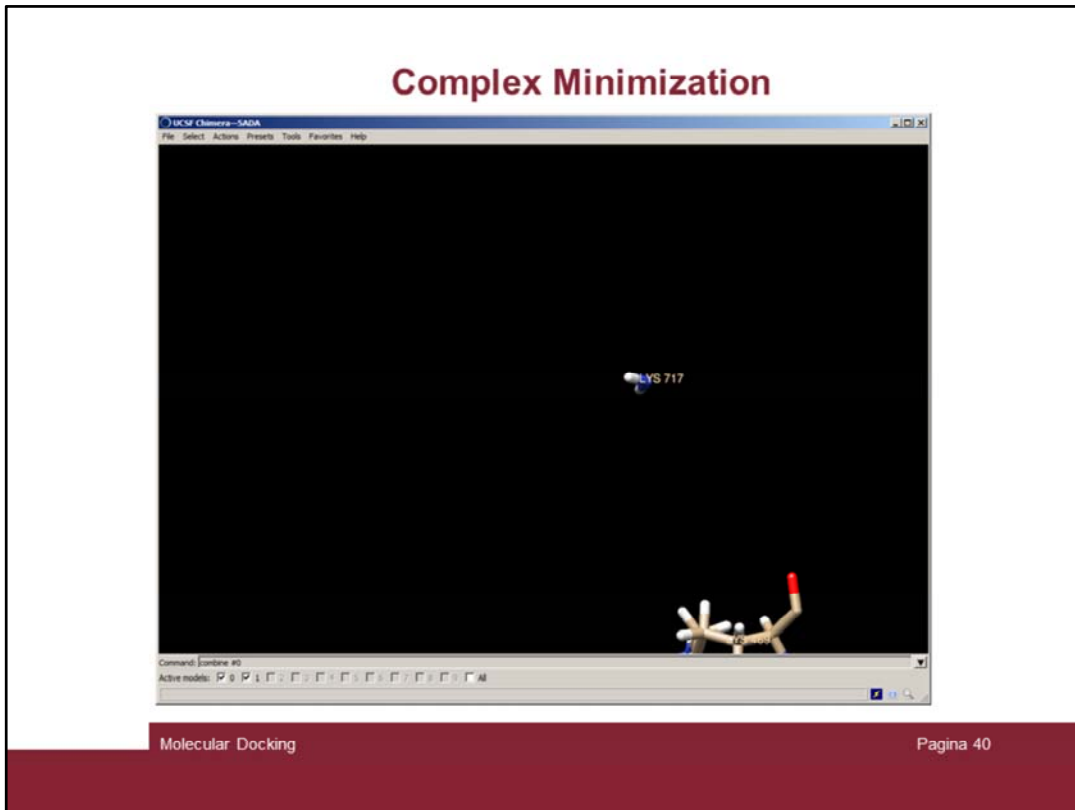
Complex Minimization



Molecular Docking

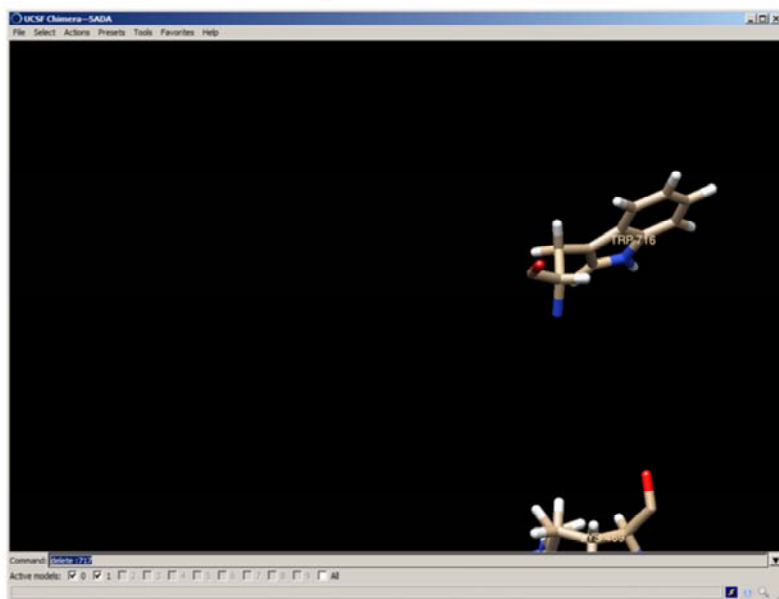
Página 39

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



And the "LYS 717" label will appear.

Complex Minimization

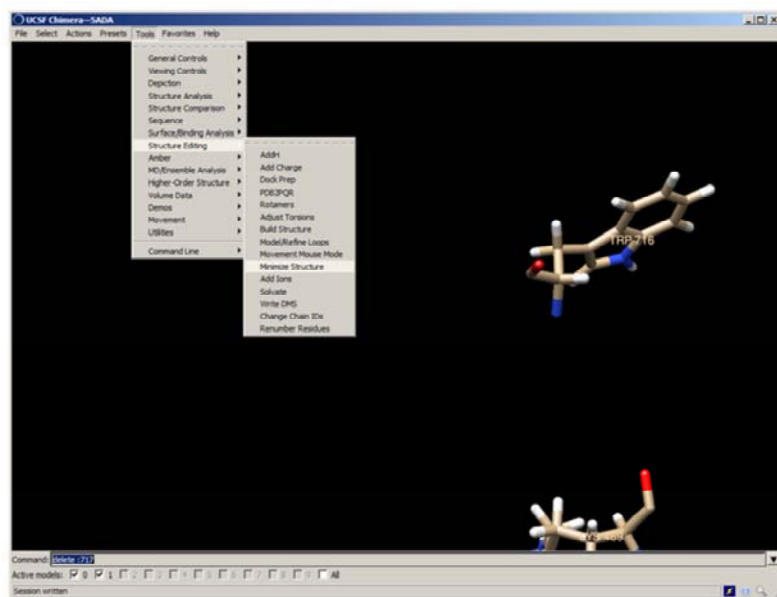


Molecular Docking

Página 41

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

Complex Minimization

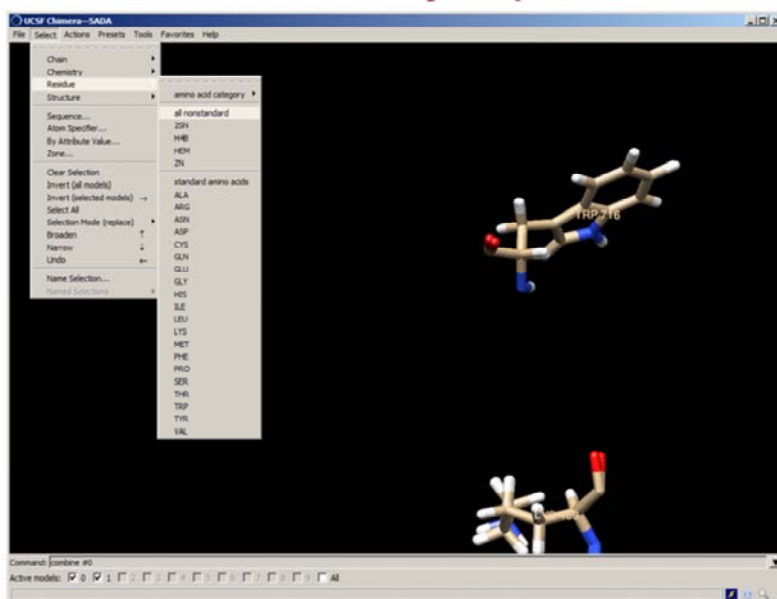


Molecular Docking

Pagina 42

And try again to minimize "Structure Editing → Minimize Structure"

Lock and Key Preparation

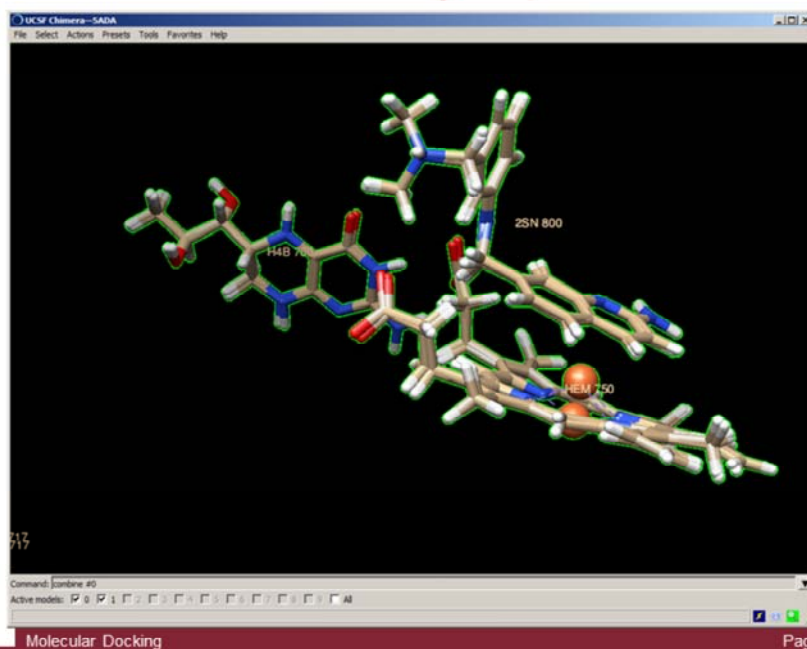


Molecular Docking

Pagina 43

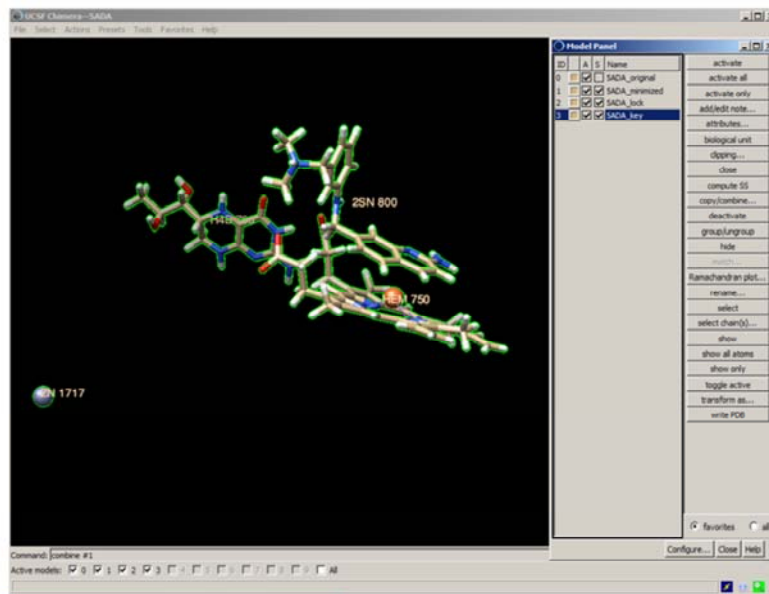
The minimization starts and it will take some minutes to finish.
Select again all nonstandard residues

Lock and Key Preparation



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.

Lock and Key Preparation



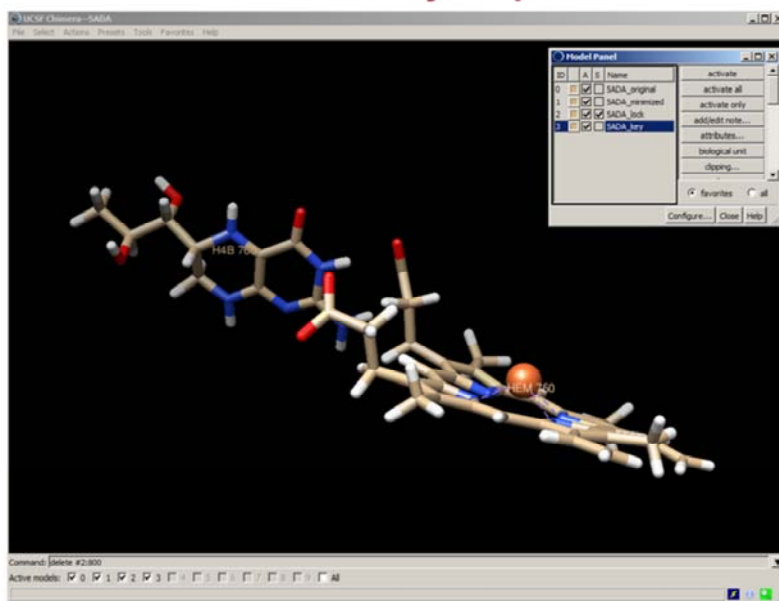
Molecular Docking

Pagina 45

Move on to prepare lock and key.

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

Lock and Key Preparation

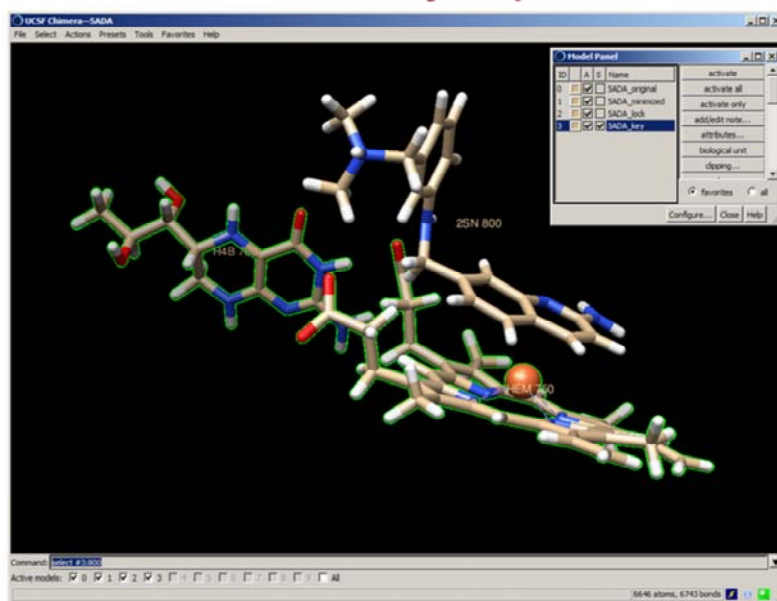


Molecular Docking

Pagina 46

Delete residue 800 (the ligand) form area #2

Lock and Key Preparation



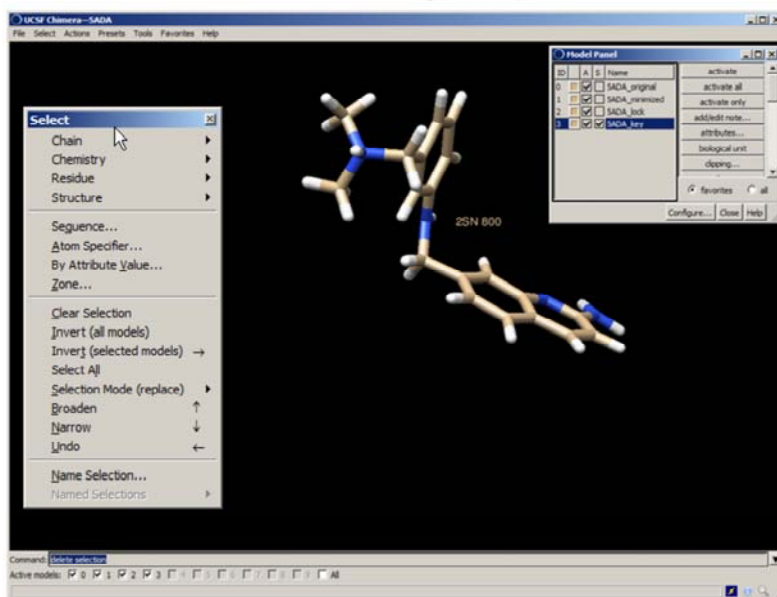
Molecular Docking

Pagina 47

Then make the reverse in area #3:

- 1) Issue the "select #3:800" command
- 2)

Lock and Key Preparation



Molecular Docking

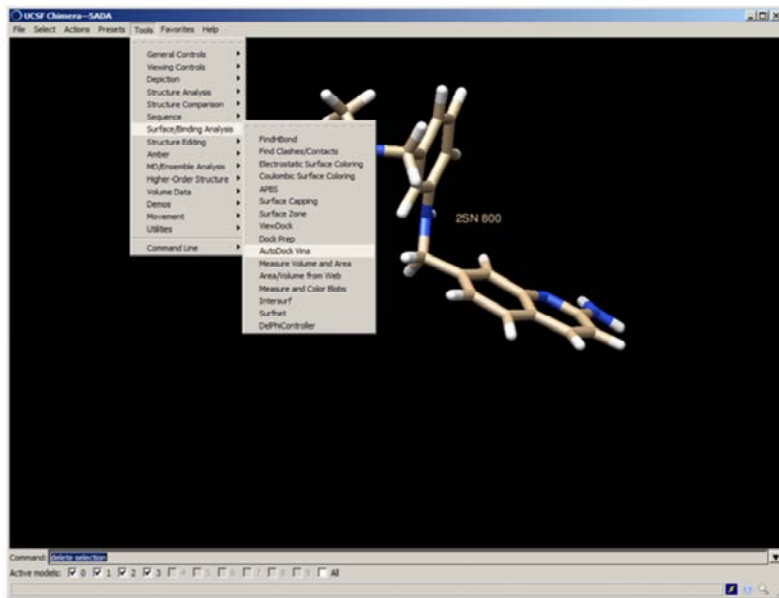
Pagina 48

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

Experimental Conformation Re-Docking

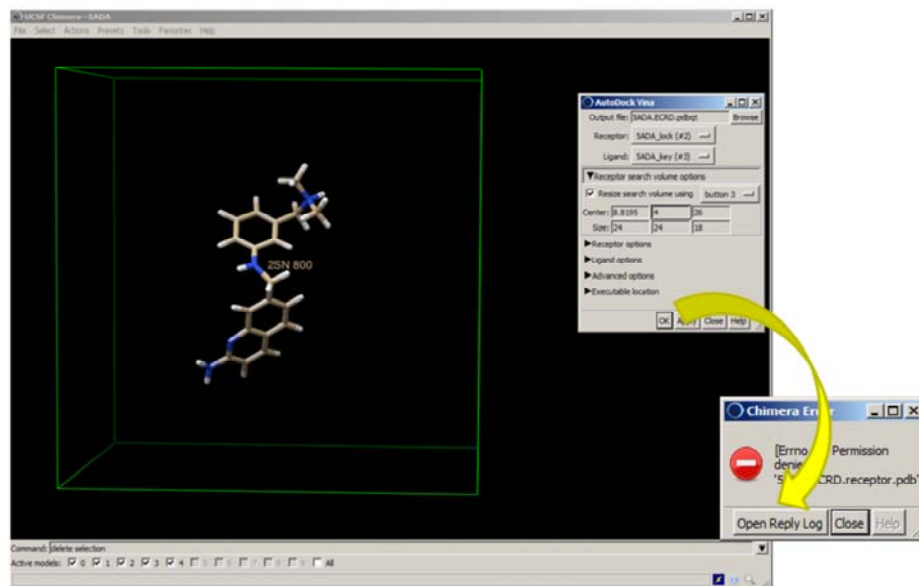


Molecular Docking

Pagina 49

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

Experimental Conformation Re-Docking



Molecular Docking

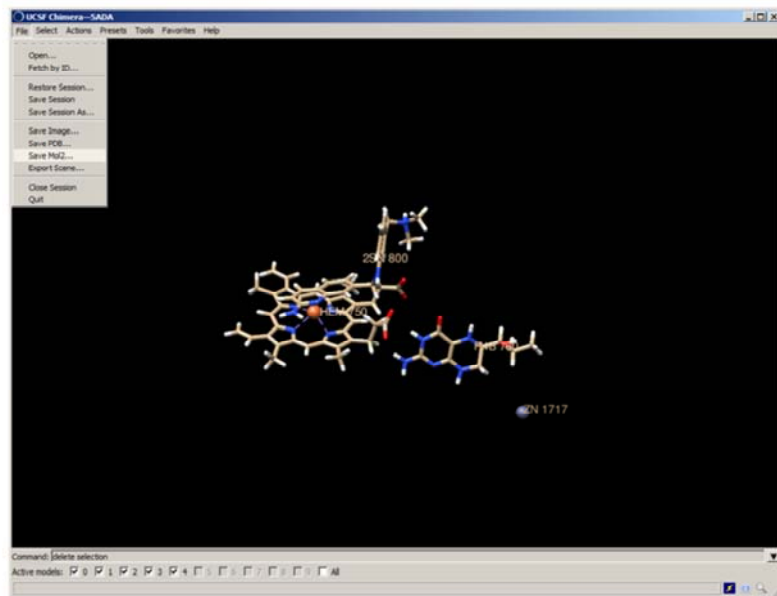
Pagina 50

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

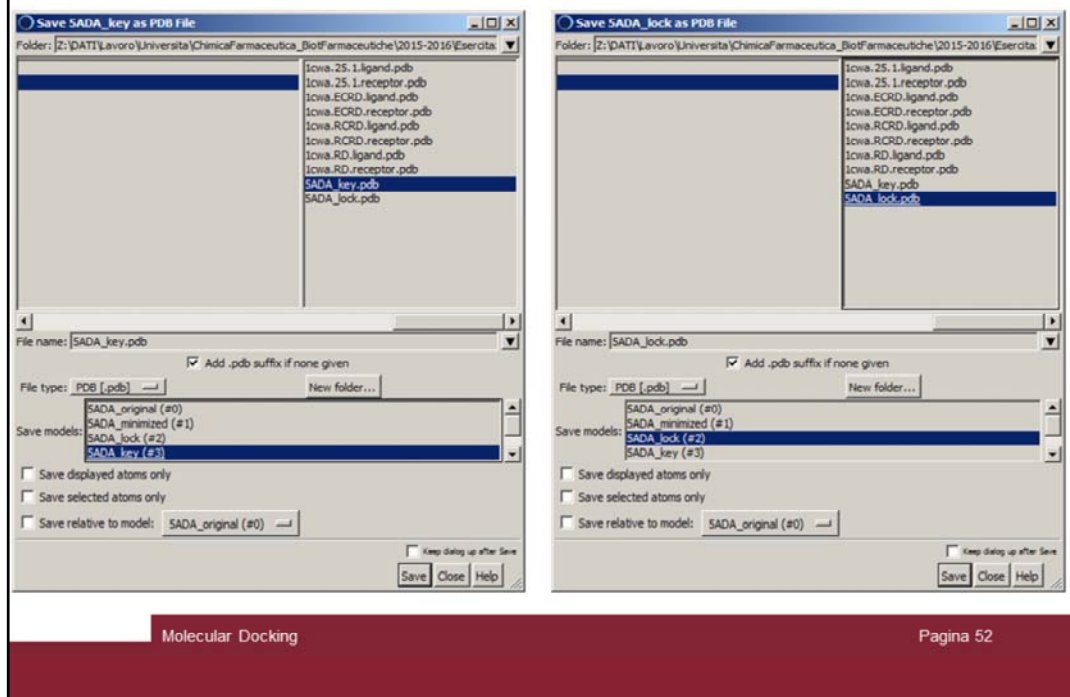


Molecular Docking

Página 51

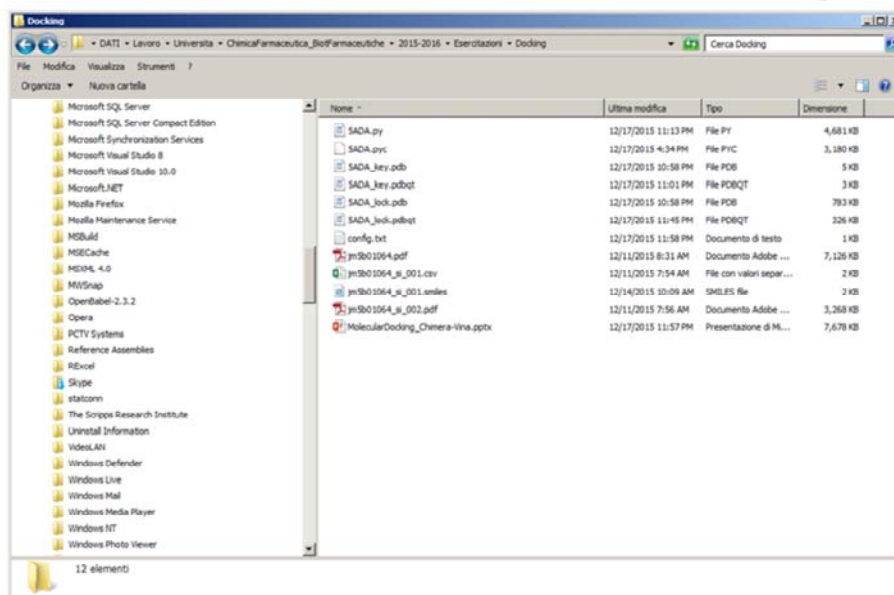
To workaround we can make the docking using the DOS terminal, but we need to save the molecules.

Experimental Conformation Re-Docking



Save both the lock and the key into pdb files

Experimental Conformation Re-Docking



Check you have the files

Experimental Conformation Re-Docking

```
Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking>"C:\Program Files (x86)\OpenBabel-2.3.2\babel.exe" -xrcp 5ADA_lock.pdb 5ADA_lock.pdbqt  
Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking>cd Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking
```

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:

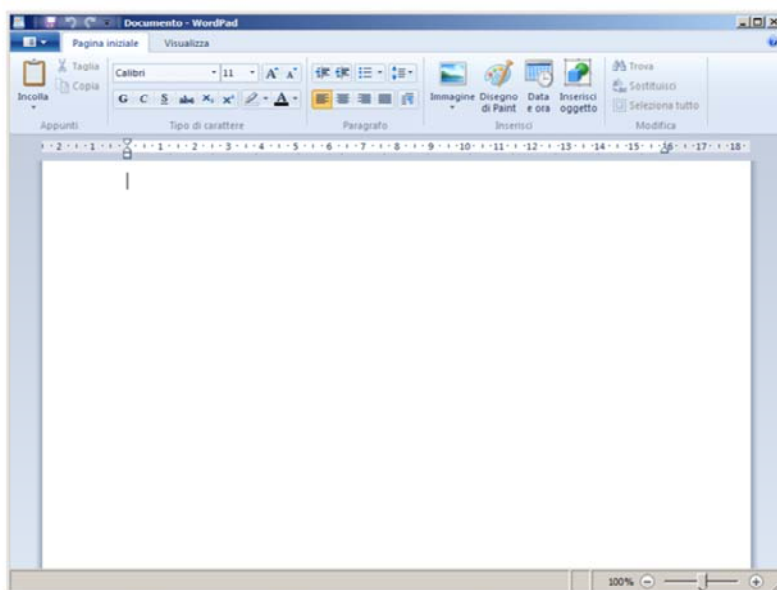
```
"C:\Program Files (x86)\OpenBabel-2.3.2\obabel.exe" -xp -ipdb 5ADA_key.pdb -opdbqt 5ADA_key.pdbqt
```

Experimental Conformation Re-Docking

 SADA.py	12/17/2015 11:13 PM	File PY	4,681 KB
 SADA.pyc	12/17/2015 4:34 PM	File PYC	3,180 KB
 SADA_key.pdb	12/17/2015 10:58 PM	File PDB	5 KB
 SADA_key.pdbqt	12/17/2015 11:01 PM	File PDBQT	3 KB
 SADA_lock.pdb	12/17/2015 10:58 PM	File PDB	783 KB
 SADA_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

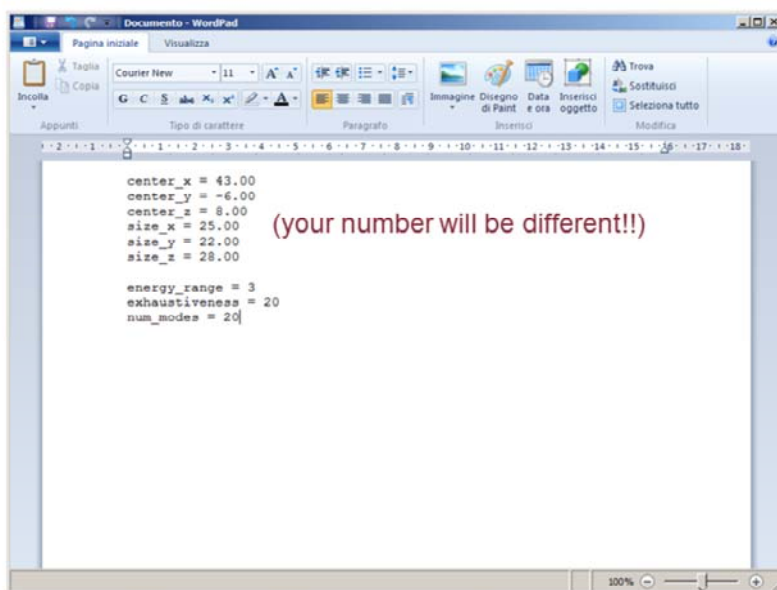
Check al the file are present

Experimental Conformation Re-Docking



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

Experimental Conformation Re-Docking



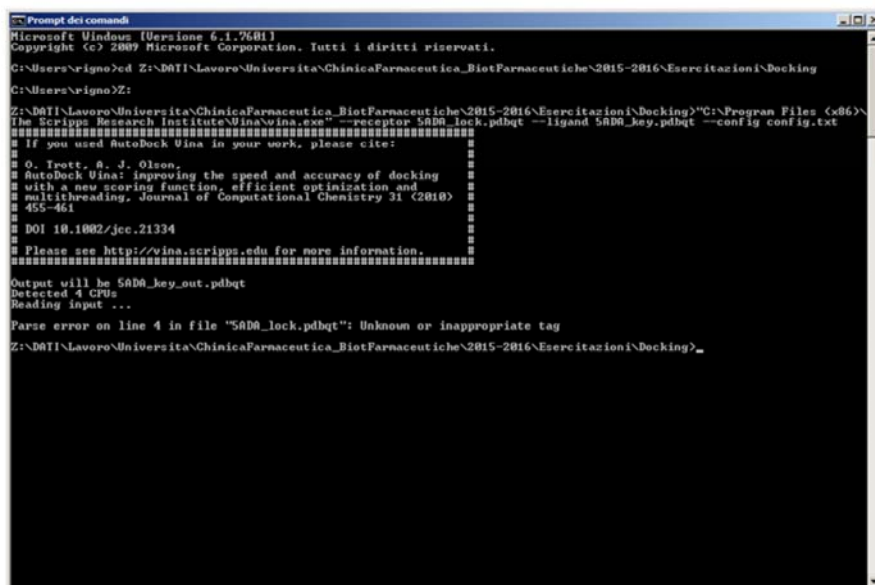
```
center_x = 43.00
center_y = -6.00
center_z = 8.00
size_x = 25.00
size_y = 22.00
size_z = 28.00

energy_range = 3
exhaustiveness = 20
num_modes = 20
```

(your number will be different!!)

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

Experimental Conformation Re-Docking



```
Prompt dei comandi
Microsoft Windows [Versione 6.1.7601]
Copyright (c) 2009 Microsoft Corporation. Tutti i diritti riservati.

C:\Users\Rigno>cd Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking
C:\Users\Rigno>Z:
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
#####
# If you used AutoDock Vina in your work, please cite:
#####
# O. Trott, A. J. Olson,
# AutoDock Vina: improving the speed and accuracy of docking
# with a new scoring function, efficient optimization and
# multithreading. Journal of Computational Chemistry 31 (2010)
# 455-461
# DOI 10.1002/jcc.21334
# Please see http://vina.scripps.edu for more information.
#####
Output will be 5ADA_key_out.pdbqt
Detected 4 CPUs.
Reading input ...

Parse error on line 4 in file "5ADA_lock.pdbqt": Unknown or inappropriate tag
Z:\DATI\Lavoro\Universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\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

The screenshot shows a WordPad window titled 'SADA_lock.pdbqt - WordPad'. The text content is as follows:

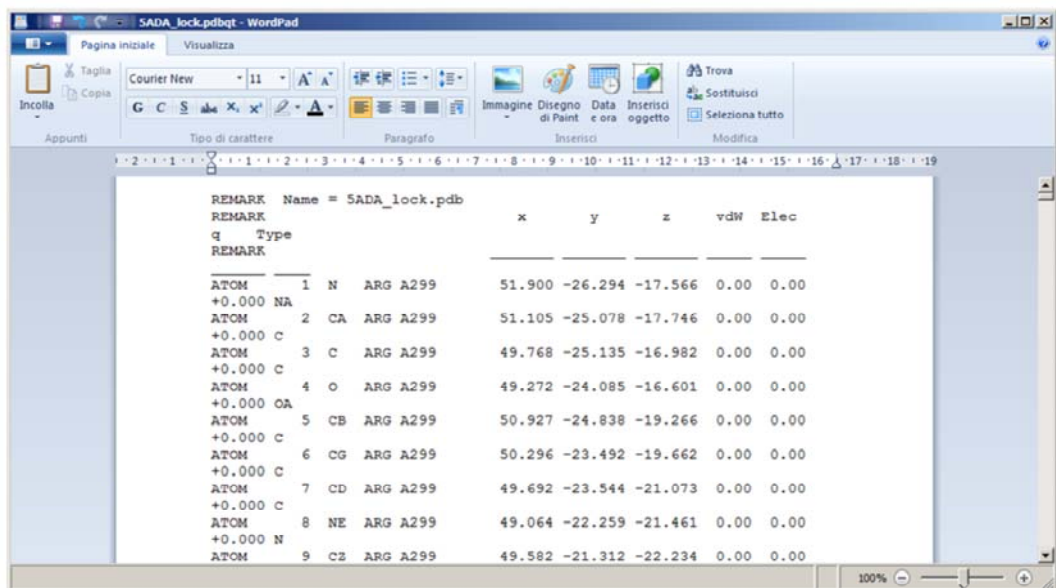
```
REMARK Name = SADA_lock.pdb
REMARK
q Type
REMARK
ROOT
ATOM 1 N ARG A299 51.900 -26.294 -17.566 0.00 0.00
+0.000 NA
ATOM 2 CA ARG A299 51.105 -25.078 -17.746 0.00 0.00
+0.000 C
ATOM 3 C ARG A299 49.768 -25.135 -16.982 0.00 0.00
+0.000 C
ATOM 4 O ARG A299 49.272 -24.085 -16.601 0.00 0.00
+0.000 OA
ATOM 5 CB ARG A299 50.927 -24.838 -19.266 0.00 0.00
+0.000 C
ATOM 6 CG ARG A299 50.296 -23.492 -19.662 0.00 0.00
+0.000 C
ATOM 7 CD ARG A299 49.692 -23.544 -21.073 0.00 0.00
+0.000 C
ATOM 8 NE ARG A299 49.064 -22.259 -21.461 0.00 0.00
+0.000 N
```

A red arrow points to the 'ROOT' line.

Open and fix it!

First remove the line containing the "ROOT" word at the beginning of the file

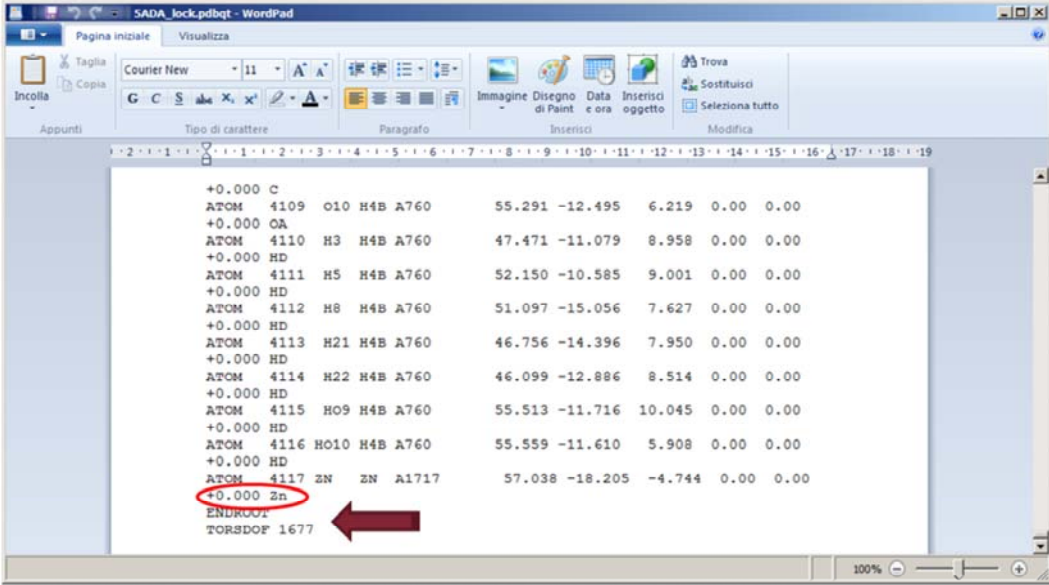
Experimental Conformation Re-Docking



The screenshot shows a WordPad window titled "SADA_lock.pdbqt - WordPad". The window contains a table of atomic coordinates and properties. The table has columns for atom ID, element, residue name, residue ID, and coordinates (x, y, z), along with van der Waals (vdW) and electrostatic (Elec) radii. The data is as follows:

Atom ID	Element	Residue Name	Residue ID	x	y	z	vdW	Elec
1	N	ARG	A299	51.900	-26.294	-17.566	0.00	0.00
2	CA	ARG	A299	51.105	-25.078	-17.746	0.00	0.00
3	C	ARG	A299	49.768	-25.135	-16.982	0.00	0.00
4	O	ARG	A299	49.272	-24.085	-16.601	0.00	0.00
5	CB	ARG	A299	50.927	-24.838	-19.266	0.00	0.00
6	CG	ARG	A299	50.296	-23.492	-19.662	0.00	0.00
7	CD	ARG	A299	49.692	-23.544	-21.073	0.00	0.00
8	NE	ARG	A299	49.064	-22.259	-21.461	0.00	0.00
9	CS	ARG	A299	49.582	-21.312	-22.234	0.00	0.00

Experimental Conformation Re-Docking



The screenshot shows a WordPad window titled 'SADA_lock.pdbqt - WordPad'. The text content is as follows:

```
+0.000 C
ATOM 4109 O10 H4B A760 55.291 -12.495 6.219 0.00 0.00
+0.000 CA
ATOM 4110 H3 H4B A760 47.471 -11.079 8.958 0.00 0.00
+0.000 HD
ATOM 4111 H5 H4B A760 52.150 -10.585 9.001 0.00 0.00
+0.000 HD
ATOM 4112 H8 H4B A760 51.097 -15.056 7.627 0.00 0.00
+0.000 HD
ATOM 4113 H21 H4B A760 46.756 -14.396 7.950 0.00 0.00
+0.000 HD
ATOM 4114 H22 H4B A760 46.099 -12.886 8.514 0.00 0.00
+0.000 HD
ATOM 4115 HO9 H4B A760 55.513 -11.716 10.045 0.00 0.00
+0.000 HD
ATOM 4116 HO10 H4B A760 55.559 -11.610 5.908 0.00 0.00
+0.000 HD
ATOM 4117 ZN ZN A1717 57.038 -18.205 -4.744 0.00 0.00
+0.000 Zn
ENDROOT
TORSDOF 1677
```

The line '+0.000 Zn' is circled in red, and a red arrow points to it. The bottom of the window shows 'Molecular Docking' on the left and 'Pagina 61' on the right.

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

Experimental Conformation Re-Docking

The screenshot shows a WordPad window titled 'SADA_lock.pdbqt - WordPad'. The text inside is a list of atoms and their coordinates, with the last line circled in red:

Charge	Atom	X	Y	Z	Occupancy	Displacement	Occupancy	Displacement
+0.000	C							
+0.000	ATOM 4109	O10	H4B	A760	55.291	-12.495	6.219	0.00 0.00
+0.000	ATOM 4110	H3	H4B	A760	47.471	-11.079	8.958	0.00 0.00
+0.000	ATOM 4111	H5	H4B	A760	52.150	-10.585	9.001	0.00 0.00
+0.000	ATOM 4112	H8	H4B	A760	51.097	-15.056	7.627	0.00 0.00
+0.000	ATOM 4113	H21	H4B	A760	46.756	-14.396	7.950	0.00 0.00
+0.000	ATOM 4114	H22	H4B	A760	46.099	-12.886	8.514	0.00 0.00
+0.000	ATOM 4115	H09	H4B	A760	55.513	-11.716	10.045	0.00 0.00
+0.000	ATOM 4116	H010	H4B	A760	55.559	-11.610	5.908	0.00 0.00
+2.000	ATOM 4117	Zn	Zn	A1717	57.038	-18.205	-4.744	0.00 0.00

Assume a charge of 2.0 for the Zn

Experimental Conformation Re-Docking

The screenshot shows a WordPad window titled 'SADA_lock.pdbqt - WordPad'. The text inside is a list of atoms with their coordinates. The atom 'ATOM 4092 FE' is circled in red, and a red arrow points to it from the right. The text '+3.000 Fe' is also circled in red.

+0.000	N								
ATOM	4090	NC	HEM	A750	37.991	-9.080	3.301	0.00	0.00
+0.000	N								
ATOM	4091	ND	HEM	A750	40.750	-9.387	3.246	0.00	0.00
+0.000	N								
ATOM	4092	FE	HEM	A750	39.502	-9.487	5.082	0.00	0.00
+3.000	Fe								
ATOM	4093	N1	H4B	A760	49.164	-13.702	8.126	0.00	0.00
+0.000	NA								
ATOM	4094	C2	H4B	A760	48.129	-12.937	8.348	0.00	0.00
+0.000	C								
ATOM	4095	N2	H4B	A760	46.880	-13.397	8.115	0.00	0.00
+0.000	NA								
ATOM	4096	N3	H4B	A760	48.305	-11.630	8.788	0.00	0.00
+0.000	N								
ATOM	4097	C4	H4B	A760	49.518	-11.036	8.938	0.00	0.00
+0.000	C								
ATOM	4098	O4	H4B	A760	49.642	-9.932	9.382	0.00	0.00
+0.000	OA								
ATOM	4099	C4A	H4B	A760	50.642	-11.959	8.563	0.00	0.00
+0.000	C								
ATOM	4100	C8A	H4B	A760	50.382	-13.239	8.164	0.00	0.00

Fix also the charge on the Fe by setting to 3.0

Experimental Conformation Re-Docking

```
Z:\DAT1\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
#####
# If you used AutoDock Vina in your work, please cite: #
# #
# O. Trott, A. J. Olson, #
# AutoDock Vina: improving the speed and accuracy of docking #
# with a new scoring function, efficient optimization and #
# multithreading, Journal of Computational Chemistry 31 (2010) #
# 455-461 #
# DOI 10.1002/jcc.21334 #
# Please see http://vina.scripps.edu for more information. #
#####
Output will be 5ADa_key_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1635671968
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
#####
```

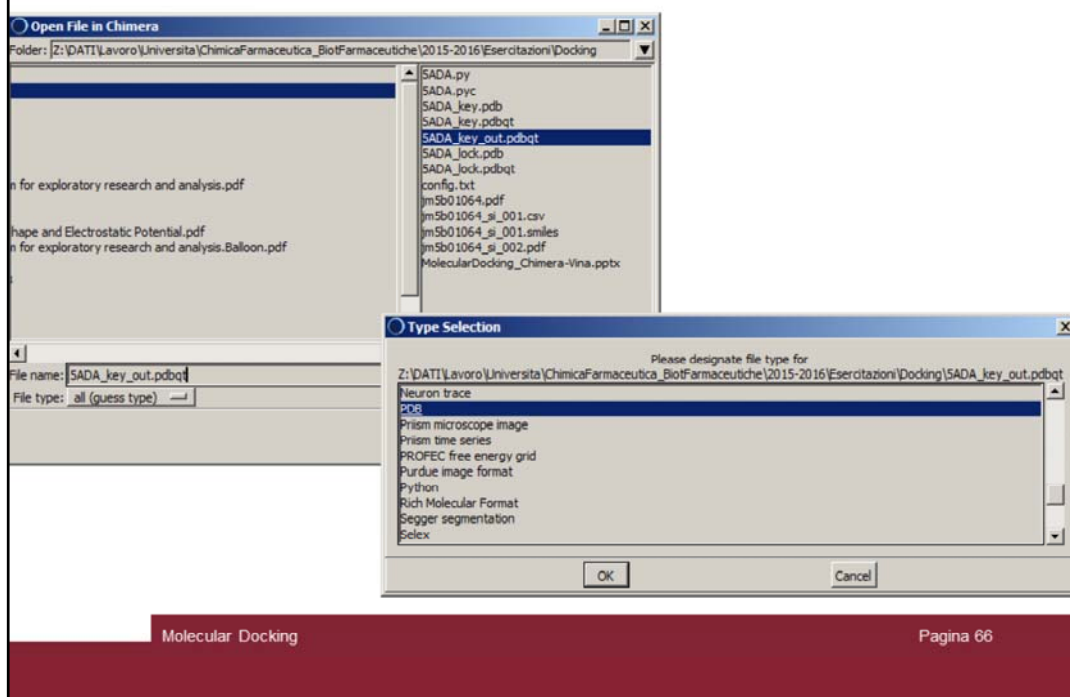
Launch the docking and now it should go.

Experimental Conformation Re-Docking

```
Prompt dei comandi
Z:\DIT\lavoro\universita\ChimicaFarmaceutica_BiotFarmaceutiche\2015-2016\Esercitazioni\Docking>"C:\Program Files (x86)\
The Scripps Research Institute\ vina.exe" --receptor 5a00_dock.pdbqt --ligand 5a00_key.pdbqt --config config.txt
=====
# If you used AutoDock Vina in your work, please cite:
#
# O. Trott, A. J. Olson,
# AutoDock Vina: Improving the speed and accuracy of docking
# with a new scoring function, efficient optimization and
# multithreading, Journal of Computational Chemistry 31 (2010)
# 455-461
# DOI 10.1002/jcc.21334
# Please see http://vina.scripps.edu for more information.
=====
Output will be 5a00_key_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1635671968
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|
done.
Refining results ... done.
mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|-----|-----|-----|
1      | -9.6      | 0.0000    | 0.0000
2      | -9.2      | 2.074    | 3.772
3      | -8.5      | 2.292    | 3.625
4      | -8.4      | 1.427    | 1.952
5      | -8.4      | 1.783    | 2.378
6      | -8.2      | 2.035    | 2.365
7      | -8.0      | 2.280    | 8.074
8      | -7.7      | 11.734   | 14.198
9      | -7.6      | 3.698    | 8.533
10     | -7.6      | 3.849    | 8.249
11     | -7.4      | 2.557    | 7.524
12     | -7.3      | 2.516    | 8.396
13     | -7.3      | 2.597    | 8.364
14     | -7.3      | 12.176   | 14.248
15     | -7.3      | 12.017   | 15.264
16     | -7.3      | 3.315    | 8.177
17     | -7.0      | 5.885    | 7.588
18     | -7.0      | 3.231    | 7.639
19     | -6.8      | 2.376    | 7.445
Writing output ... done.
```

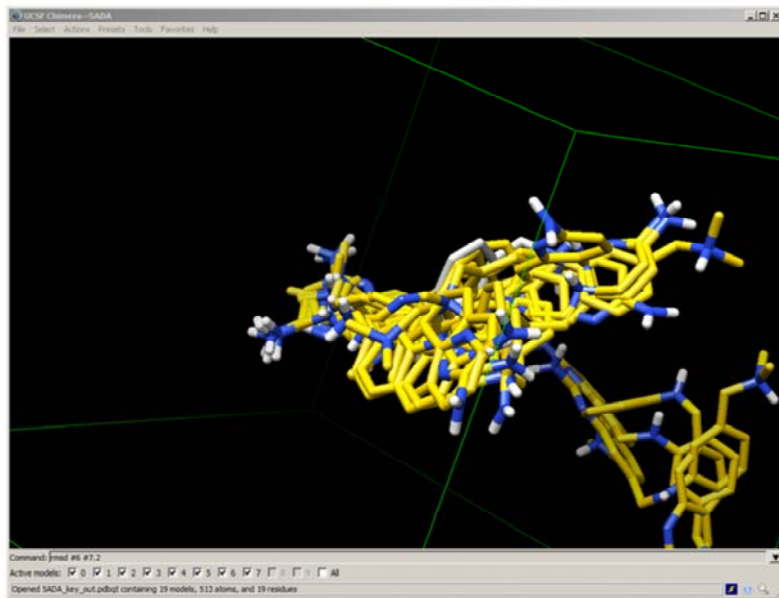
When it will be over

Experimental Conformation Re-Docking



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

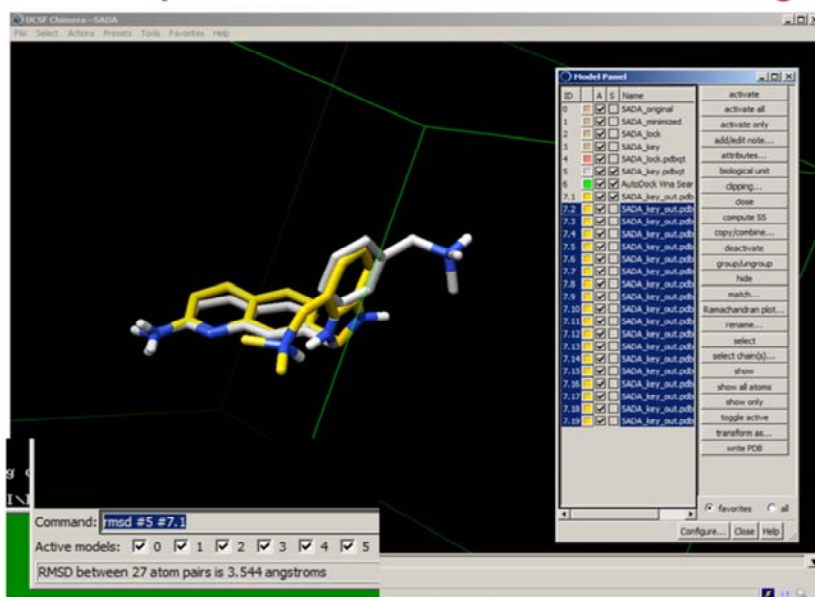


Molecular Docking

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The docked conformations will appear in the chimera windows.

Experimental Conformation Re-Docking



Molecular Docking

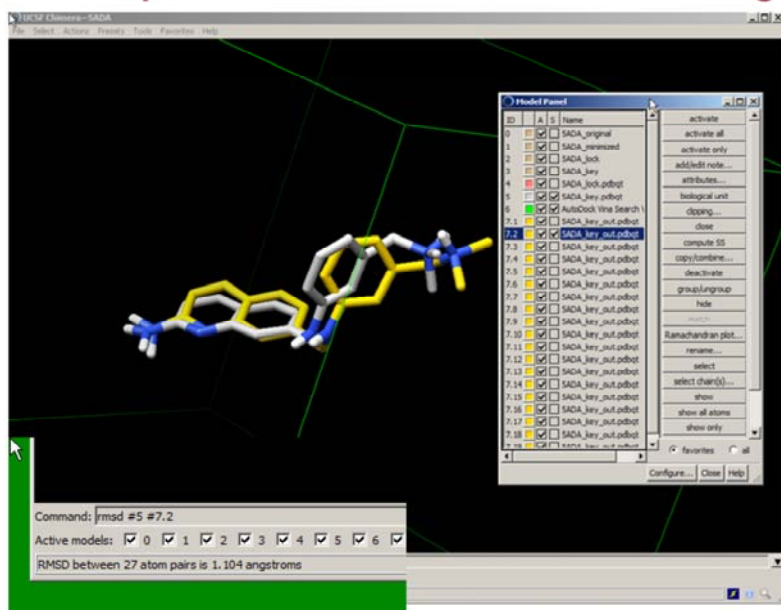
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Ungroup them and hide all conformations except the first (7.1 here).

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.

Experimental Conformation Re-Docking



Molecular Docking

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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