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## In Silico Molecular Docking with Ligand Target

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 18, 2023

**Last Modified:** July 29, 2024

**Protocol Integer ID:** 82104

**Keywords:** Molecular Docking, Enzyme-Ligand Interaction, Protein Data Bank, DrugBank, UCSF Chimera, MetaPocket 2.0, AutoDockTools, AutoDock Vina, ProteinsPlus, Bioinformatics, Drug Discovery., Cyclooxygenase, Celecoxib

## Abstract

This study outlines a comprehensive molecular docking protocol aimed at exploring enzyme-ligand interactions, leveraging various bioinformatics tools and databases. The protocol commences with the acquisition of the enzyme's three-dimensional structure from the Protein Data Bank (PDB) and the identification of potential ligands from the DrugBank database. Structural refinement and preparation of the enzyme and ligands are conducted using UCSF Chimera, ensuring proper protonation states and the addition of missing atoms. MetaPocket 2.0 is employed to predict and identify potential binding sites on the enzyme, facilitating the focus of the docking simulations on relevant regions. The prepared structures are then processed using AutoDockTools to generate the necessary input files, including the grid parameter file (GPF) and docking parameter file (DPF). AutoDock Vina is subsequently used to perform the docking simulations, predicting the binding poses and affinities of the ligands within the identified binding sites. The docking results are analyzed and visualized using the ProteinsPlus platform, providing detailed insights into the interaction profiles and enabling the identification of key residues involved in binding.

By integrating these diverse computational tools and databases, the protocol offers a robust framework for studying enzyme-ligand interactions, which is crucial for drug discovery and the understanding of biochemical pathways. The streamlined approach enhances the accuracy and efficiency of docking studies, facilitating the identification of promising therapeutic candidates.

## Materials

### **Computational Tools and Software:**

#### **AutoDockTools (ADT):**

Version: AutoDockTools 1.5.x

Usage: Preparation of the enzyme and ligand files, including the generation of PDBQT files and grid parameter files (GPF).

Download link: [AutoDockTools](#)

#### **AutoDock Vina:**

Version: AutoDock Vina 1.1.x

Usage: Execution of molecular docking simulations to predict binding poses and affinities.

Download link: [AutoDock Vina](#)

#### **UCSF Chimera:**

Version: UCSF Chimera 1.14 or later

Usage: Visualization, preparation, and refinement of the enzyme and ligand structures, including protonation and the addition of missing atoms.

Download link: [UCSF Chimera](#)

#### **MetaPocket 2.0:**

Version: MetaPocket 2.0

Usage: Prediction and identification of potential binding sites on the enzyme.

Access link: [MetaPocket 2.0](#)

#### **ProteinsPlus:**

Platform: ProteinsPlus web server

Usage: Analysis and visualization of docking results, including interaction profiling.

Access link: [ProteinsPlus](#)

#### **Databases:**

#### **Protein Data Bank (PDB):**

Usage: Source of three-dimensional structures of enzymes.

Access link: [PDB](#)

#### **DrugBank:**

Usage: Source of potential ligand molecules, including small molecules and drug-like compounds.

Access link: [DrugBank](#)

#### **Input Files:**

#### **Enzyme Structure:**

Format: PDB file

Source: Downloaded from the PDB database

**Ligand Structures:**

Format: PDB or MOL files

Source: Downloaded from the DrugBank database

**Hardware:**

**Computer System:** Processor: Multi-core CPU (e.g., Intel i7 or higher)

Memory: At least 16 GB RAM

Storage: Minimum 500 GB of free disk space

Operating System: Linux, Windows, or macOS

**Miscellaneous:****Internet Access:**

Required for downloading software, accessing databases, and using web-based tools like MetaPocket 2.0 and ProteinsPlus.

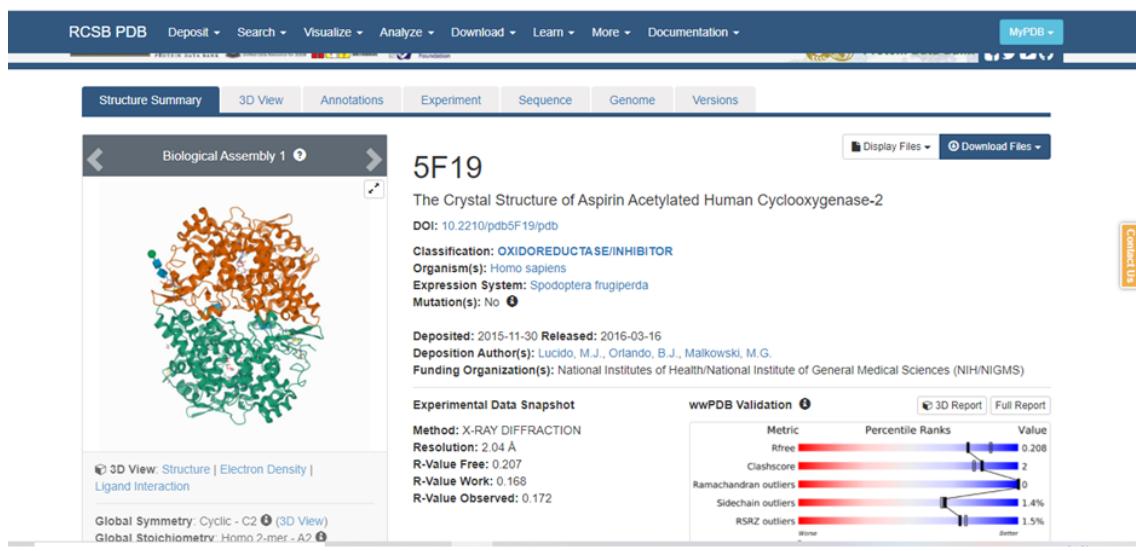
- 1 1) In this protocol we will use as an example of molecular docking, a target protein (receptor) called cyclooxygenase, which is an important enzyme in inflammatory processes. And as a ligand, the anti-inflammatory celecoxib.

## Target receptor search:

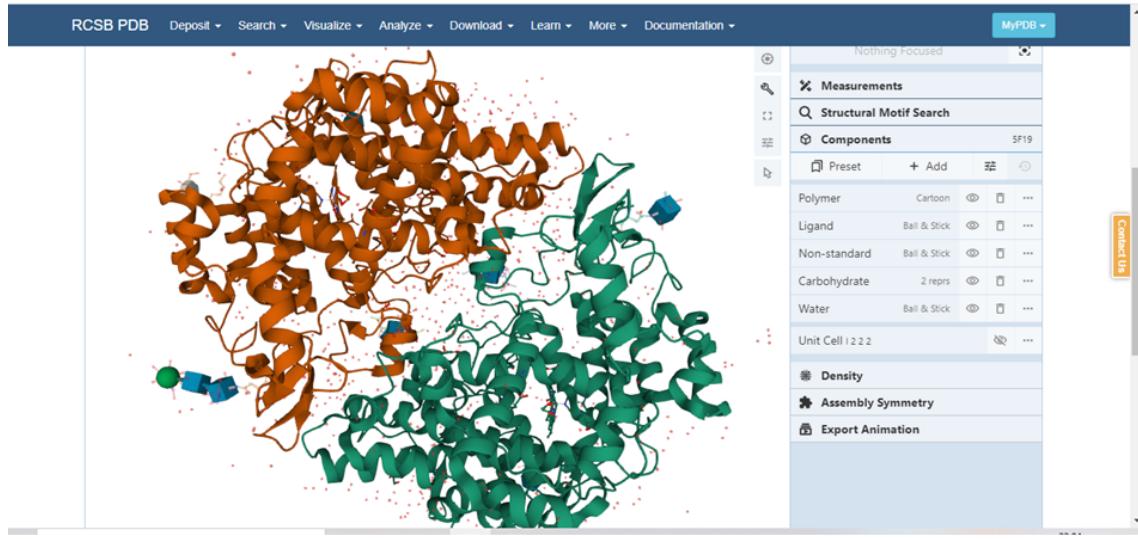
- 2 2) Seeking the three-dimensional (3D) structure of the receiver from experimental data or computational models, here we will use the PDB.

<https://www.rcsb.org/>

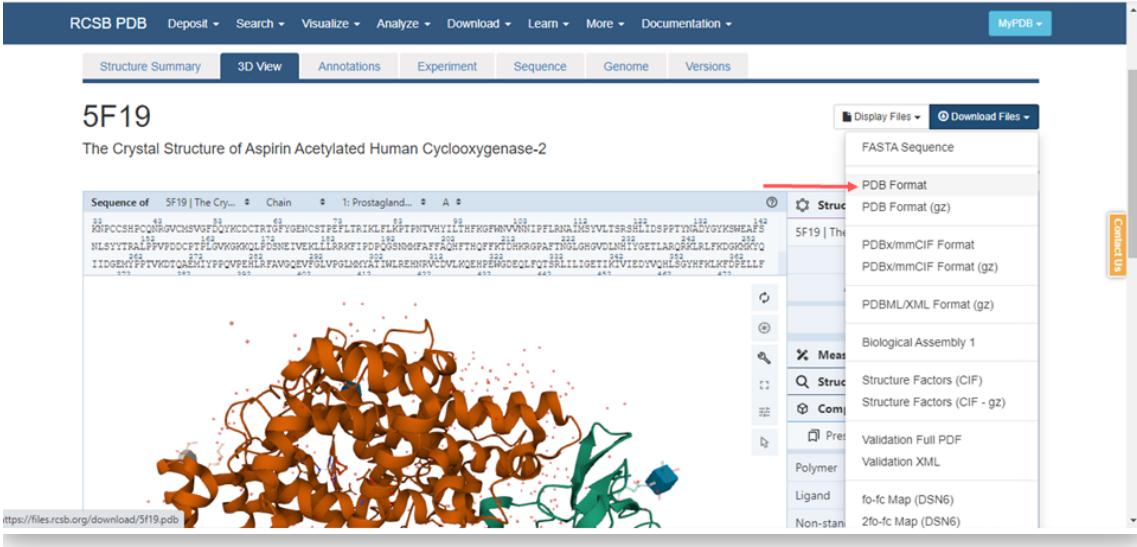
- 3) We search the PDB for the enzyme called cyclooxygenase and choose the one with the best resolution, which in this case was 5F19.



- 4) Go in 3D view.



- 5) On the right tab, under display files, extract the receiver in pdb format and save it in a working folder.



The Crystal Structure of Aspirin Acetylated Human Cyclooxygenase-2

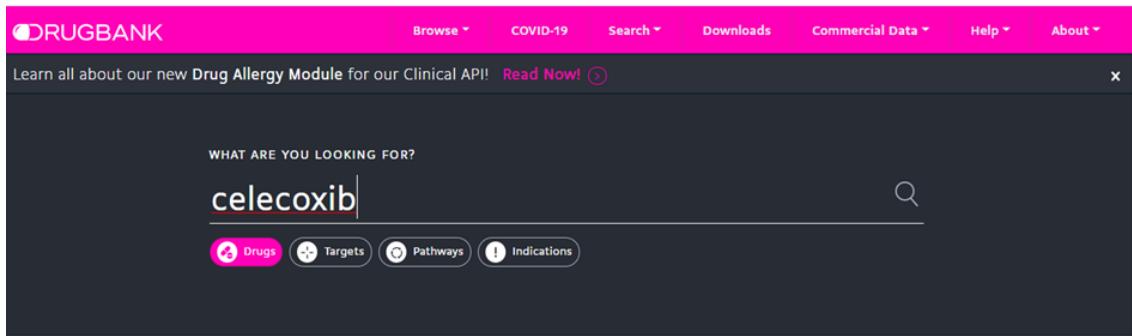
Sequence of 5F19 | The Cry... Chain 1: Prostagland... A

PDB Format  
PDB Format (gz)  
PDBx/mmCIF Format  
PDBx/mmCIF Format (gz)  
PDBML/XML Format (gz)  
Biological Assembly 1  
Structure Factors (CIF)  
Structure Factors (CIF - gz)  
Validation Full PDF  
Validation XML  
fo-fc Map (DSN6)  
2fo-fc Map (DSN6)

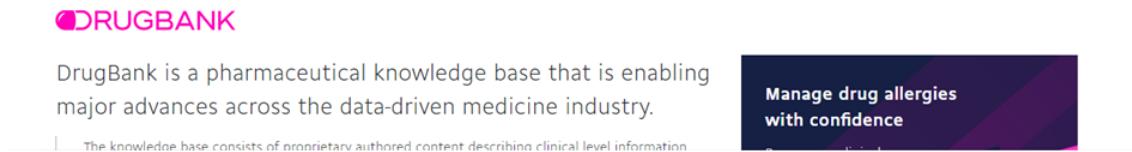
## Target ligand search:

- 3 6) If it is a known chemical structure use the Drugbank for the search, which we will use, if not, use chemical structure design software such as ACD/ChemSketch.  
<https://go.drugbank.com/>

Search for the molecule under the name celecoxib.

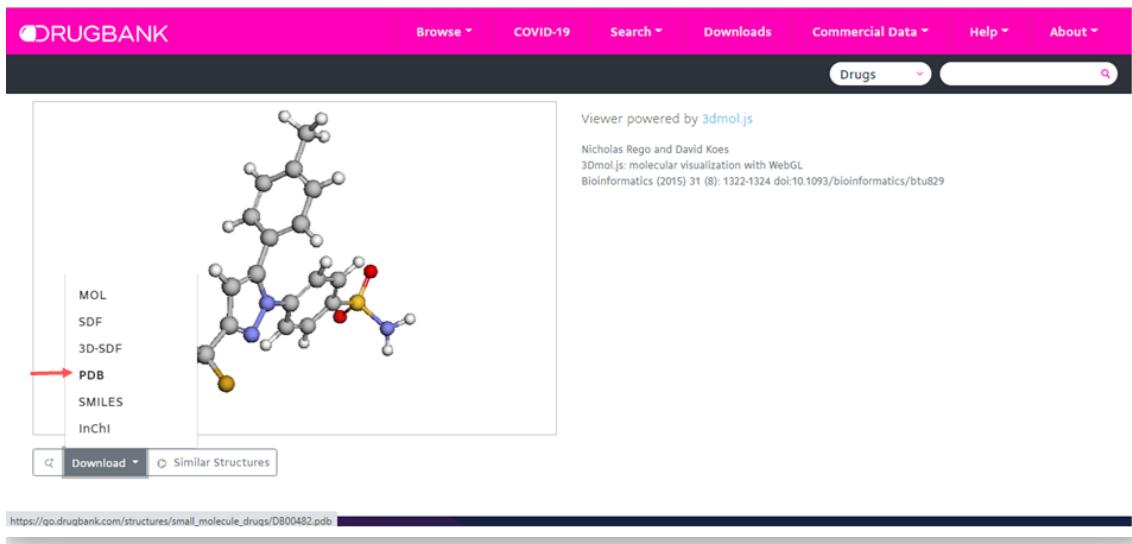


The screenshot shows the DRUGBANK website's search interface. At the top, there is a navigation bar with links for Browse, COVID-19, Search, Downloads, Commercial Data, Help, and About. Below the navigation bar, a banner promotes the new Drug Allergy Module for the Clinical API. The main search bar contains the query 'celecoxib'. Below the search bar are four buttons: Drugs (highlighted in pink), Targets, Pathways, and Indications. The search results page displays the drug information for celecoxib.



The screenshot shows the DRUGBANK homepage. The header features the DRUGBANK logo and navigation links. A prominent banner on the right side of the page reads 'Manage drug allergies with confidence'. Below the banner, a text box states: 'The knowledge base consists of proprietary authored content describing clinical level information'.

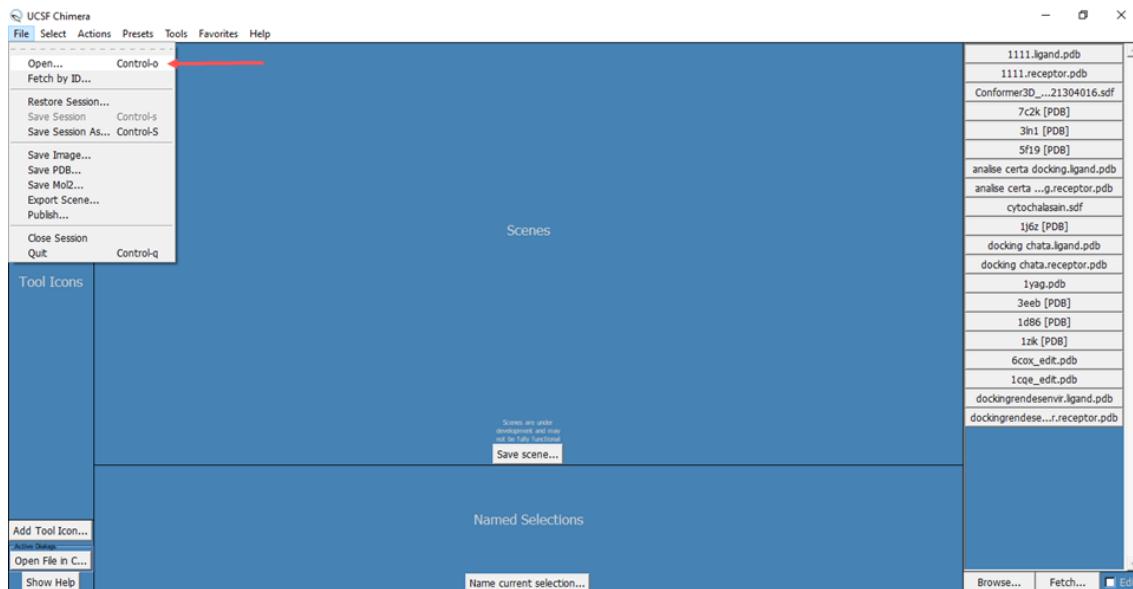
7) In download save the celecoxib molecule in pdb format.



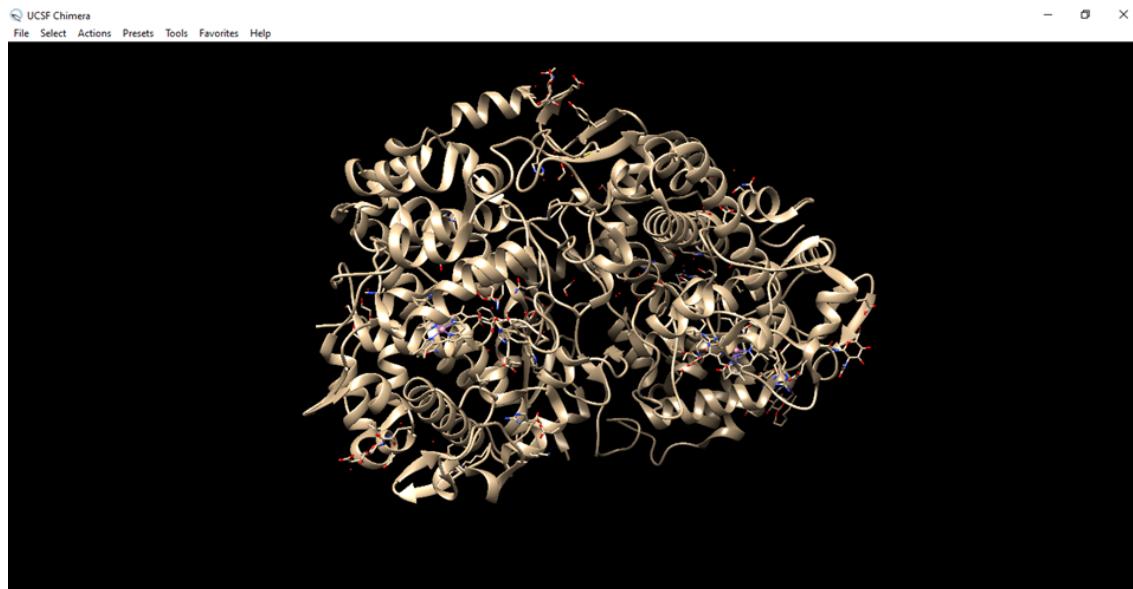
The screenshot shows the DRUGBANK molecular viewer. It displays a 3D ball-and-stick model of the celecoxib molecule. To the left of the molecule, a sidebar lists download formats: MOL, SDF, 3D-SDF, PDB, SMILES, and InChI. An arrow points to the 'PDB' link. Below the sidebar are 'Download' and 'Similar Structures' buttons. The URL https://go.drugbank.com/structures/small\_molecule\_drugs/DB00482.pdb is visible at the bottom of the viewer.

## Preparation of the receptor:

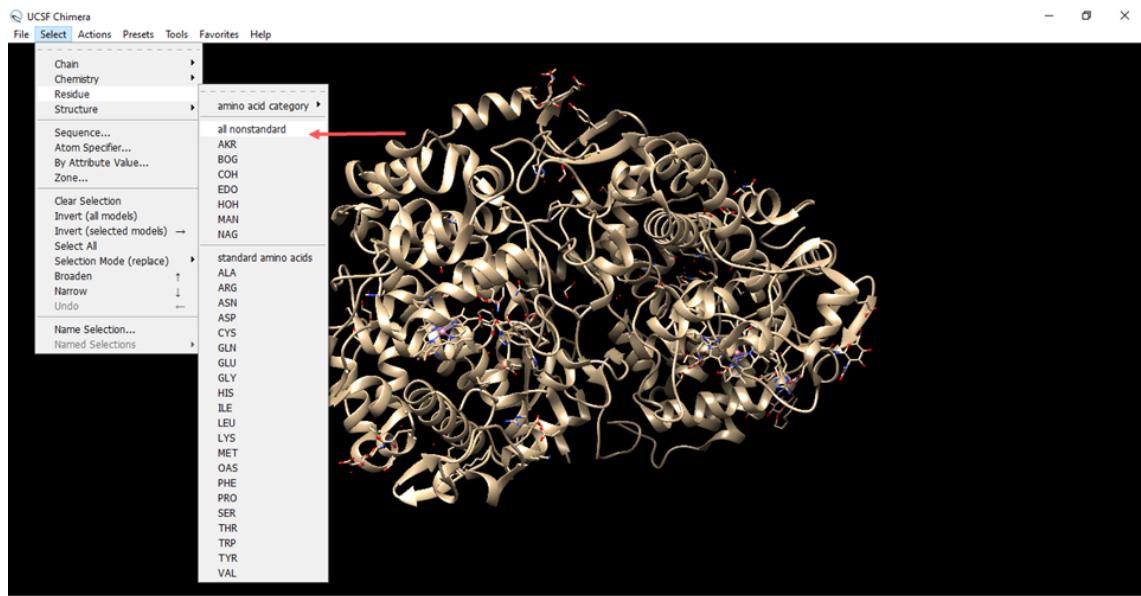
- 4    8) Download the Chimera program from the link:  
<https://www.cgl.ucsf.edu/chimera/download.html>
- 9) Open the figure saved on the computer in Chimera or use the fetch id of 5F19



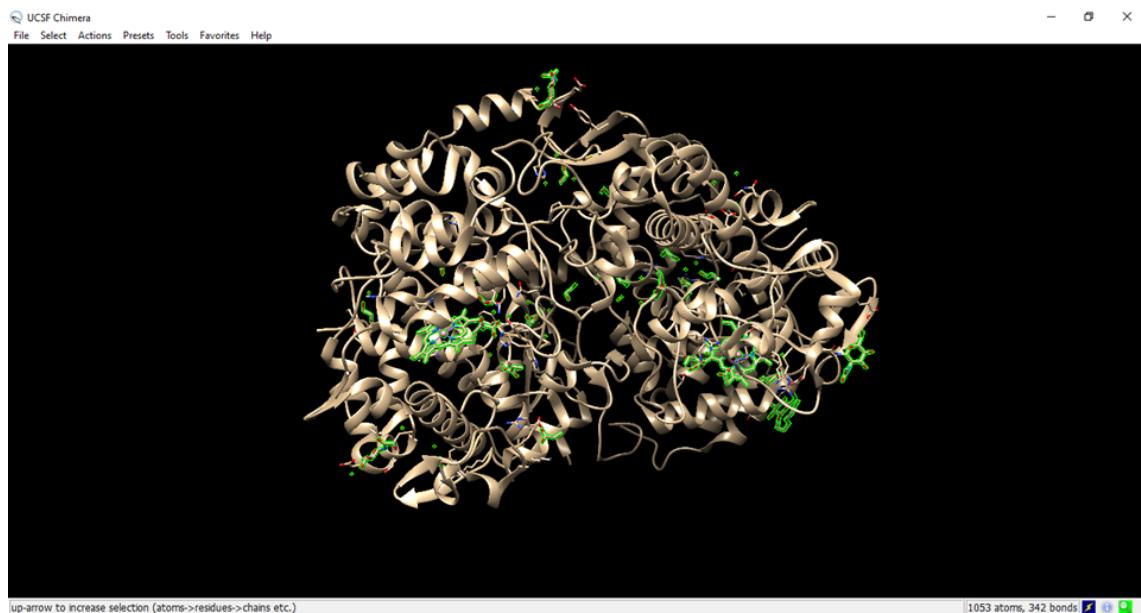
#### 10) Visualization of the cyclooxygenase enzyme (receptor) in Chimera.



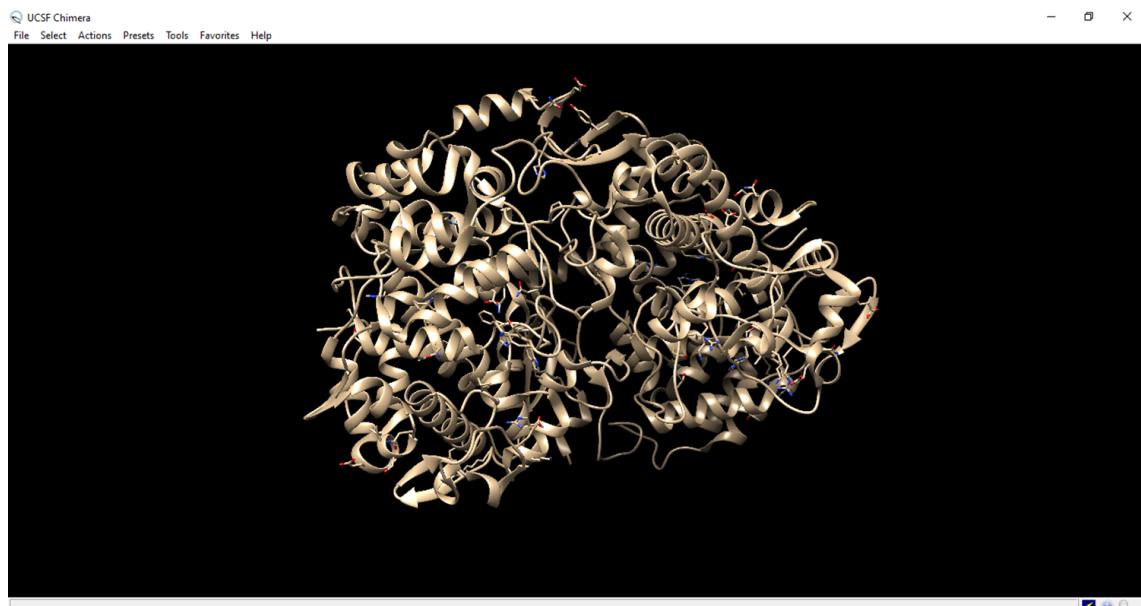
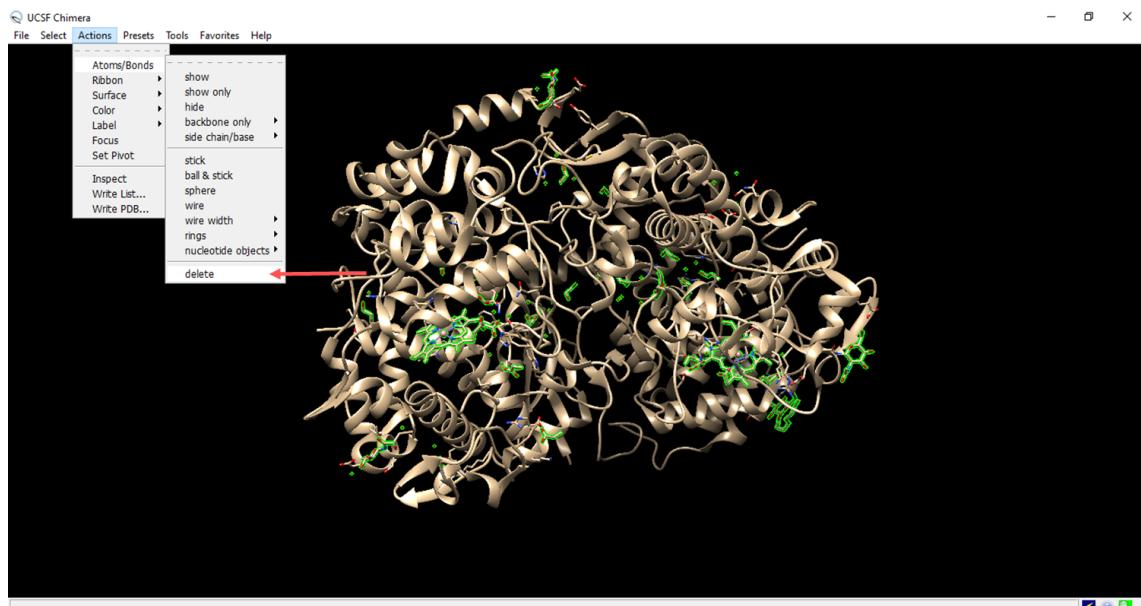
#### 11) Go to select, go to residue and set to all non standard.



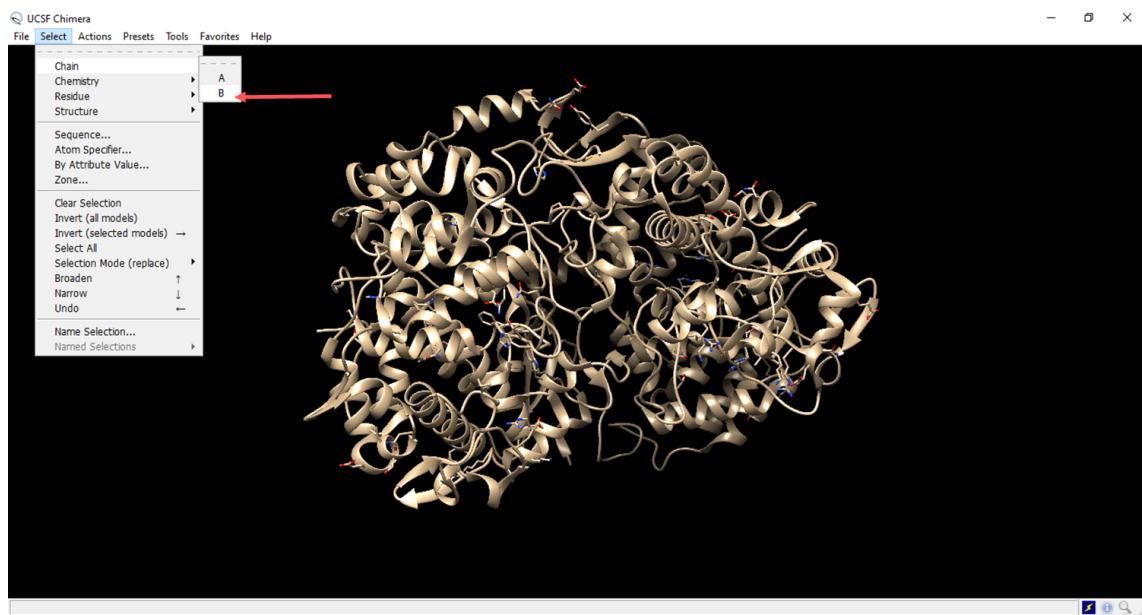
12) Residues that are not from the receiver were marked in green.



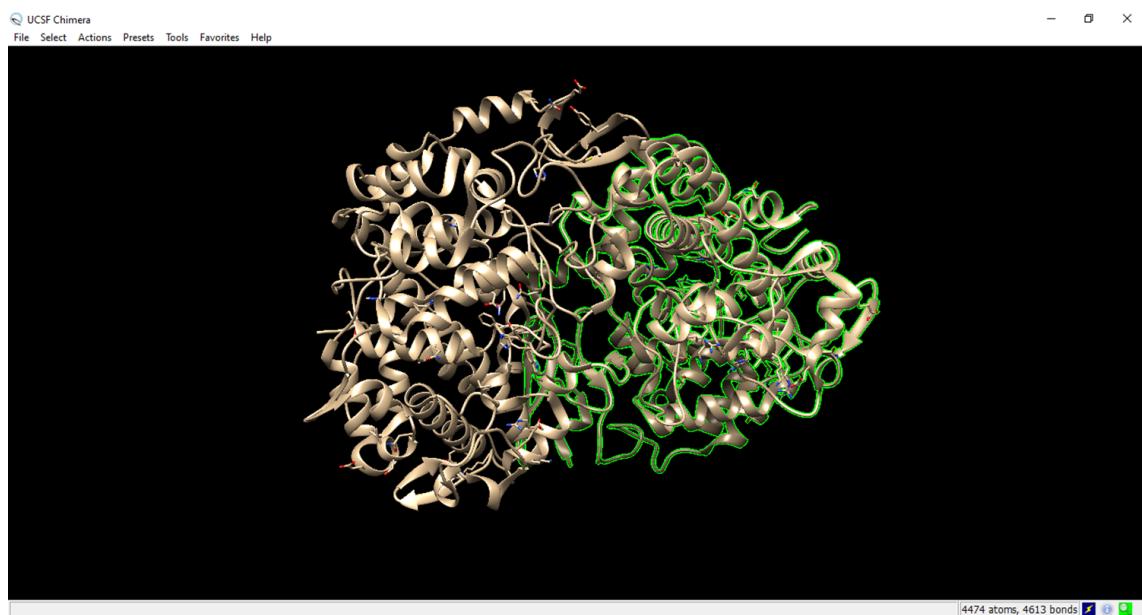
13) Clean the structure

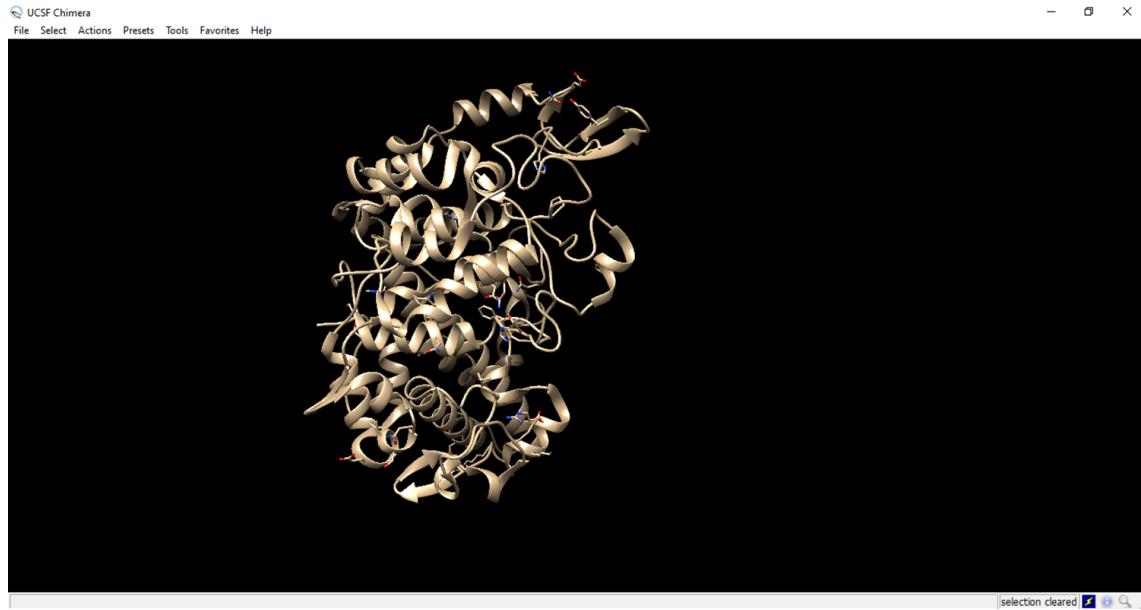
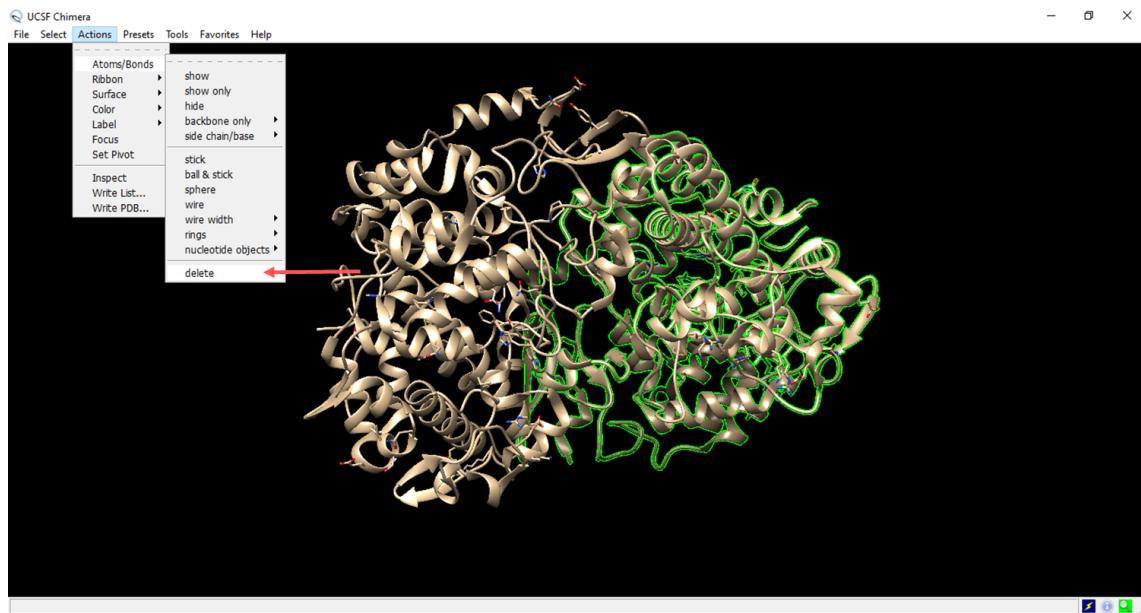


14) Deleting a chain of amino acids to expose the ligand receptor.

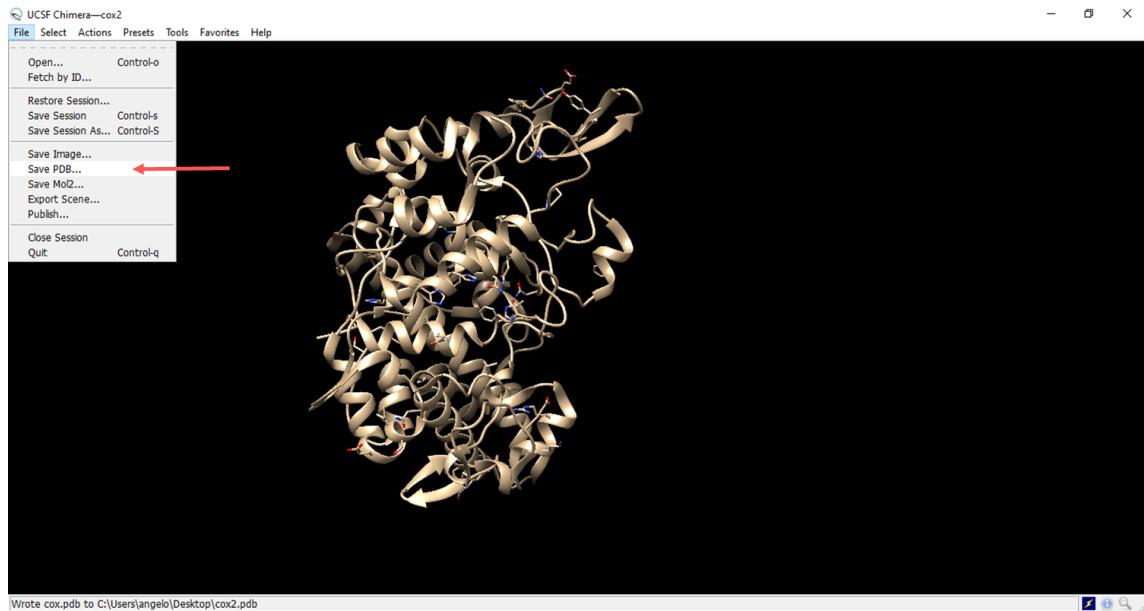


15) The residues to be deleted are marked in green.





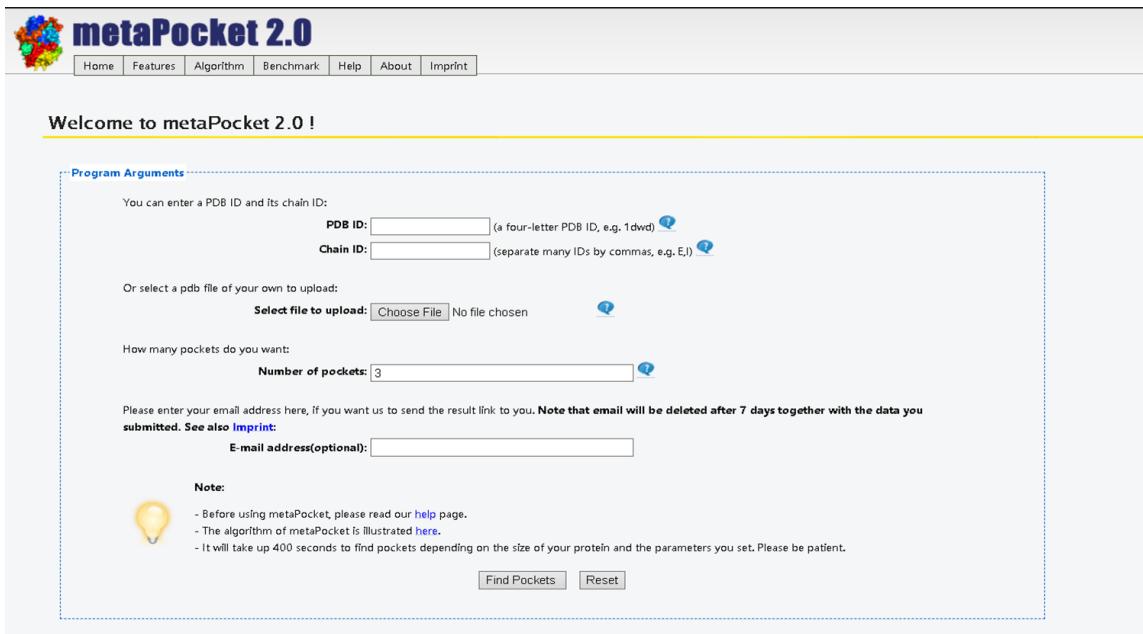
16) Save the file in pdb format.



17) MetaPocket 2.0 is an online tool used to predict binding sites on proteins.

<https://metapocket.embl.org/>

Provide the structure of the prepared target protein in PDB (Protein Data Bank) format.



The screenshot shows the metaPocket 2.0 web interface. At the top, there is a navigation bar with links for Home, Features, Algorithm, Benchmark, Help, About, and Imprint. Below the navigation bar, a header says 'Welcome to metaPocket 2.0 !'. The main form area has a title 'Program Arguments'. It contains fields for entering a PDB ID and Chain ID, both with explanatory text and help icons. There is also a section for uploading a PDB file. Below these, there is a field for specifying the number of pockets (set to 3). Further down, there is a note about email submission and a field for an optional email address. At the bottom, there is a 'Note' section with a lightbulb icon and a list of instructions, followed by 'Find Pockets' and 'Reset' buttons.

18) Select item 4 to choose the best results



ATOM	6	C3	SFW	2	33.801	33.990	16.100	2	1.63
ATOM	9	C3	FFX	3	19.610	46.166	24.370	1	3.62
ATOM	10	C3	PAS	3	19.600	49.508	17.780	1	2.72
TER									
ATOM	1	C3	MPF	1	8.428	43.774	24.140	3	19.60
ATOM	2	C3	MPF	2	31.324	36.717	14.645	5	6.98
ATOM	3	C3	MPF	3	19.605	47.037	21.075	2	6.34

3. Result Download HELP

Result files of MetaPocket:

Download MetaPocket result files:

1. The protein file you submitted (PDB format).
2. MetaPocket result file of top 3 pocket sites (PDB format).
3. MetaPocket result file of all the predict pocket sites (PDB format).
4. MetaPocket result file of grid points from top 3 result clusters (PDB format). ←
5. MetaPocket result file of grid points from all the result clusters (PDB format).
6. A python script to visualize the protein structure and binding sites using PyMOL.

(\*\*Note: Please make sure to download file "1." and "2." to the same folder before to run this script.)

7. All the surface atoms of the protein you submit (PDB format).

8. All the surface residues of the protein you submit (PDB format).

Result files of individual predictors:

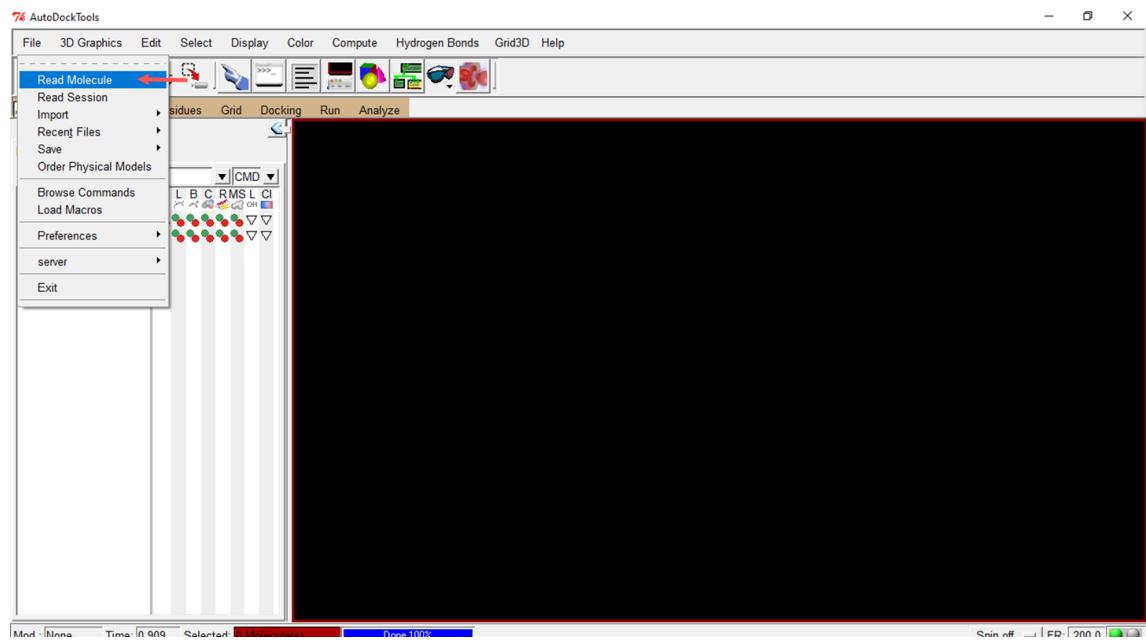
PASS11: Clusters points (PDB format). Binding sites points (PDB format).  
LIGSITEcys: Cluster points (PDB format). Binding sites points (PDB format).  
Fpocket: Clusters points (PDB format). Binding sites points (PDB format).  
SURFNET: Clusters points (PDB format). Binding sites points (PDB format).  
GHECOM: Clusters points (PDB format). Binding sites points (PDB format).  
ConCavity: Clusters points (PDB format). Binding sites points (PDB format).

## 19) Choose the first one that has the best result

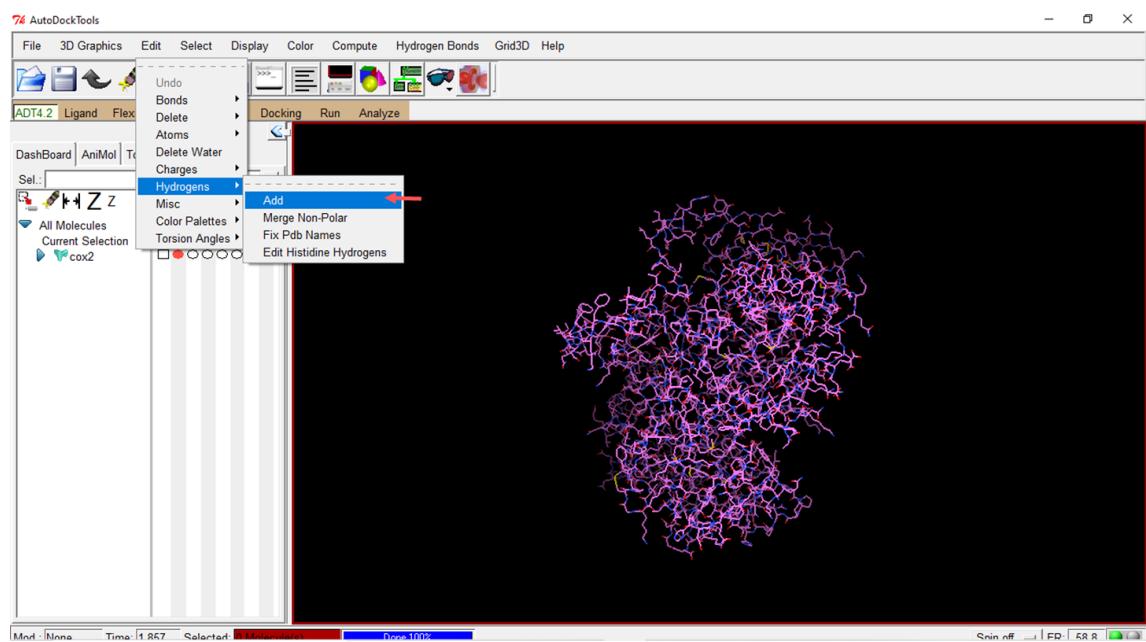


## 20) Tools to prepare structures, such as AutoDockTools

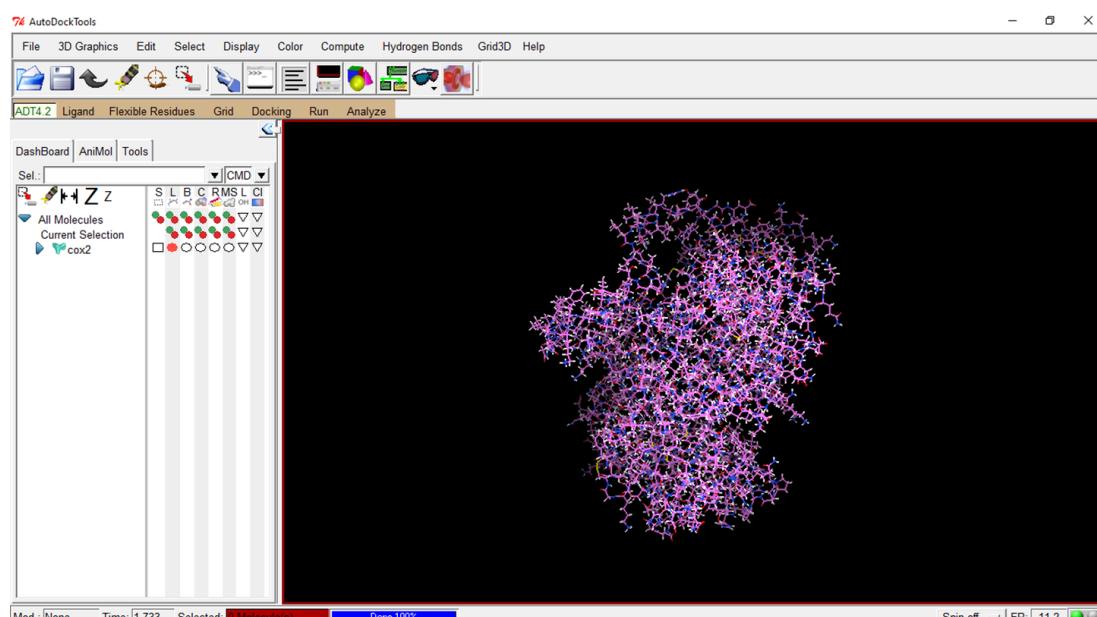
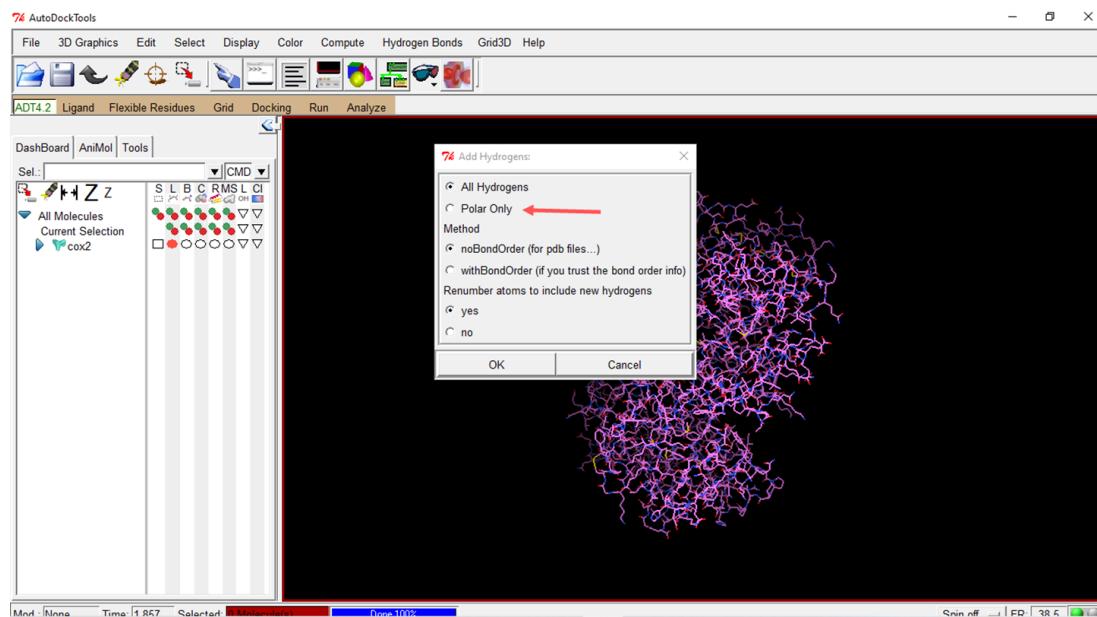
Download: <https://autodocksuite.scripps.edu/adt/>



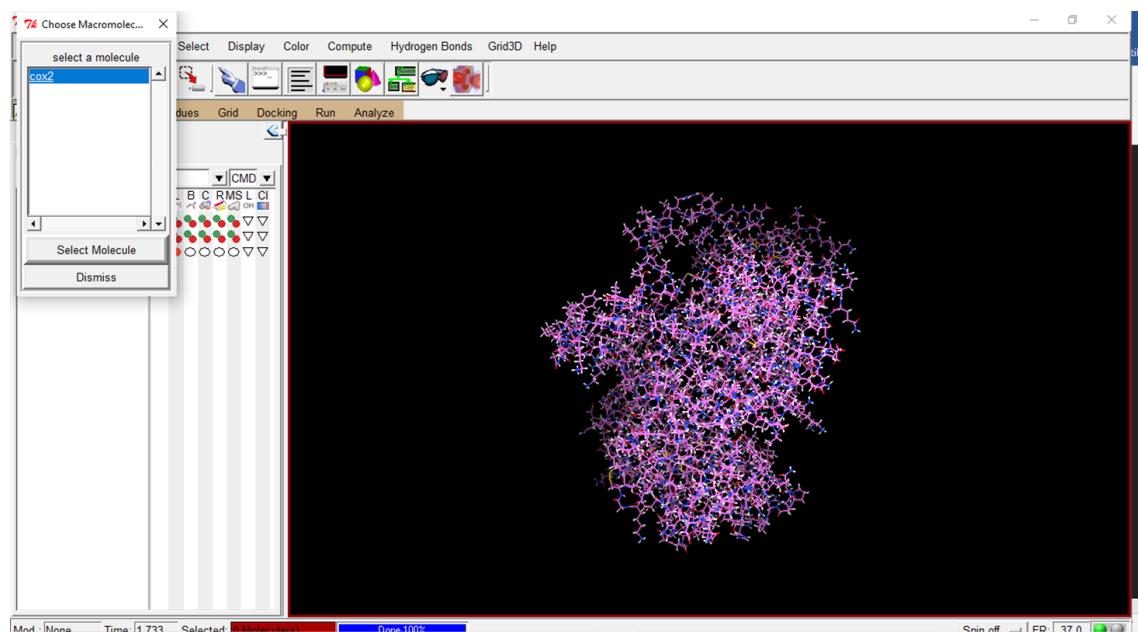
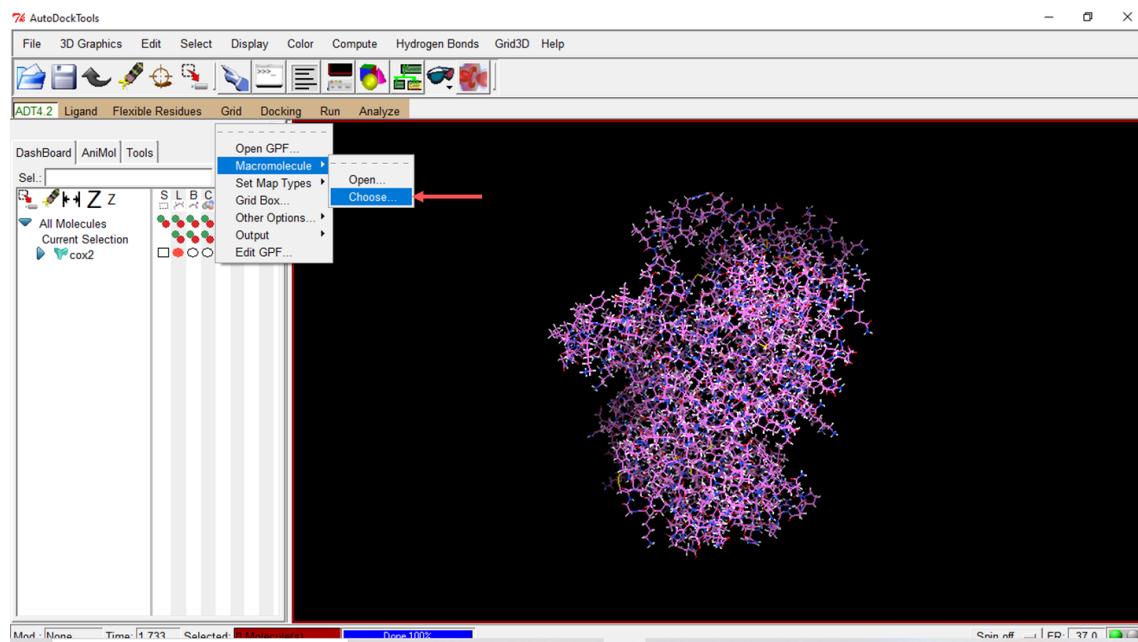
## 21) Add hydrogens to the molecule

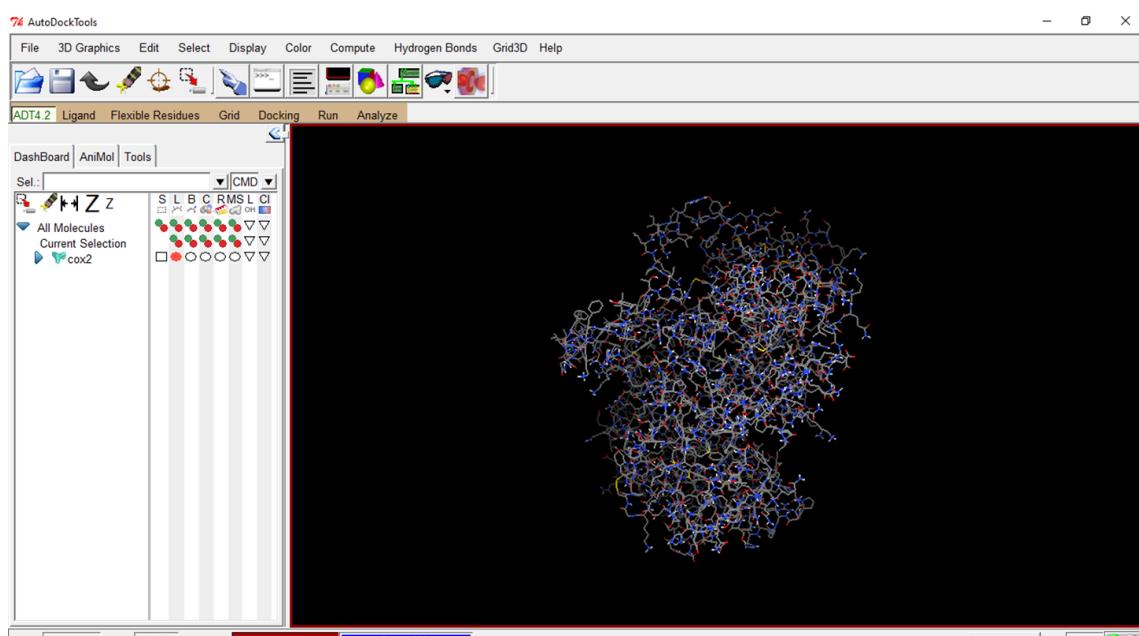
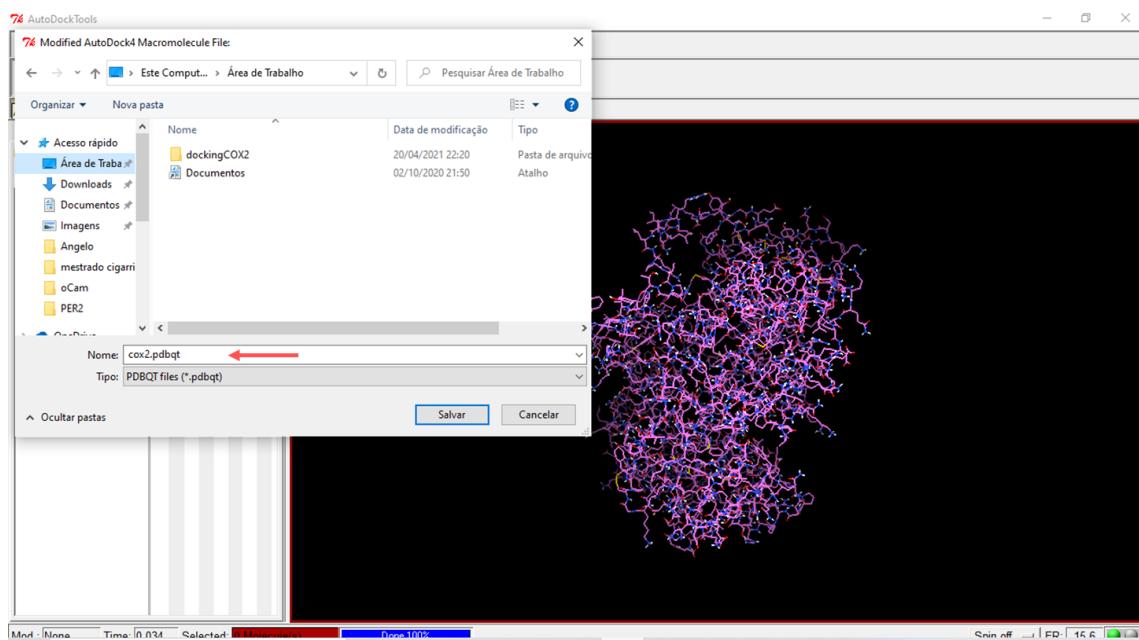


## 22) Using the "polar only" option means that only polar hydrogens are considered when preparing the molecules.

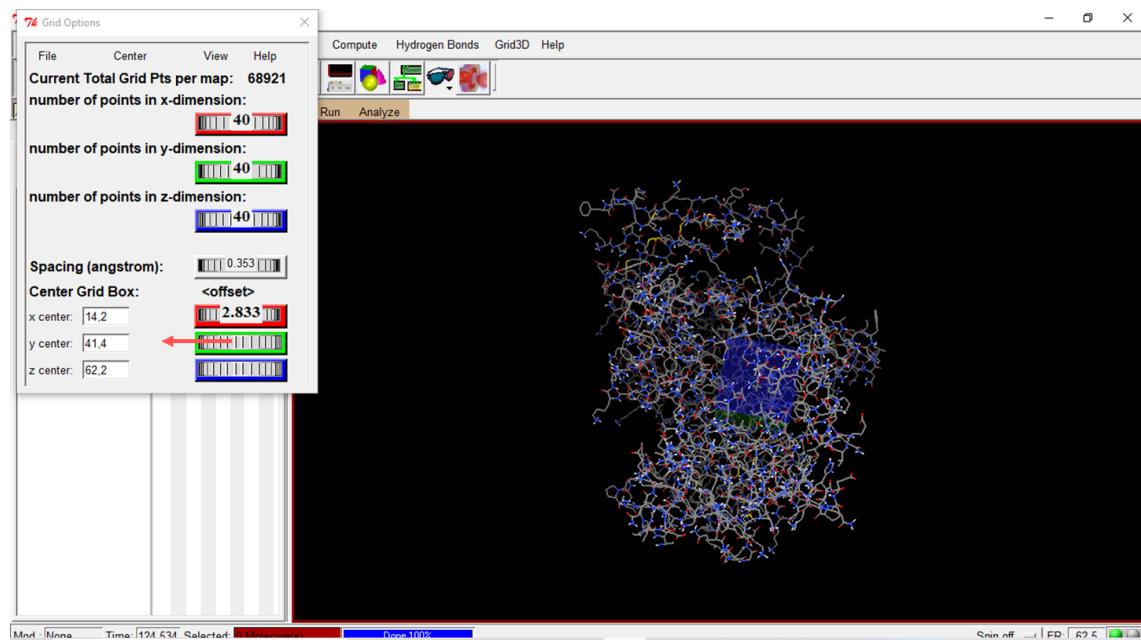


23) Choose the prepared macromolecule to save

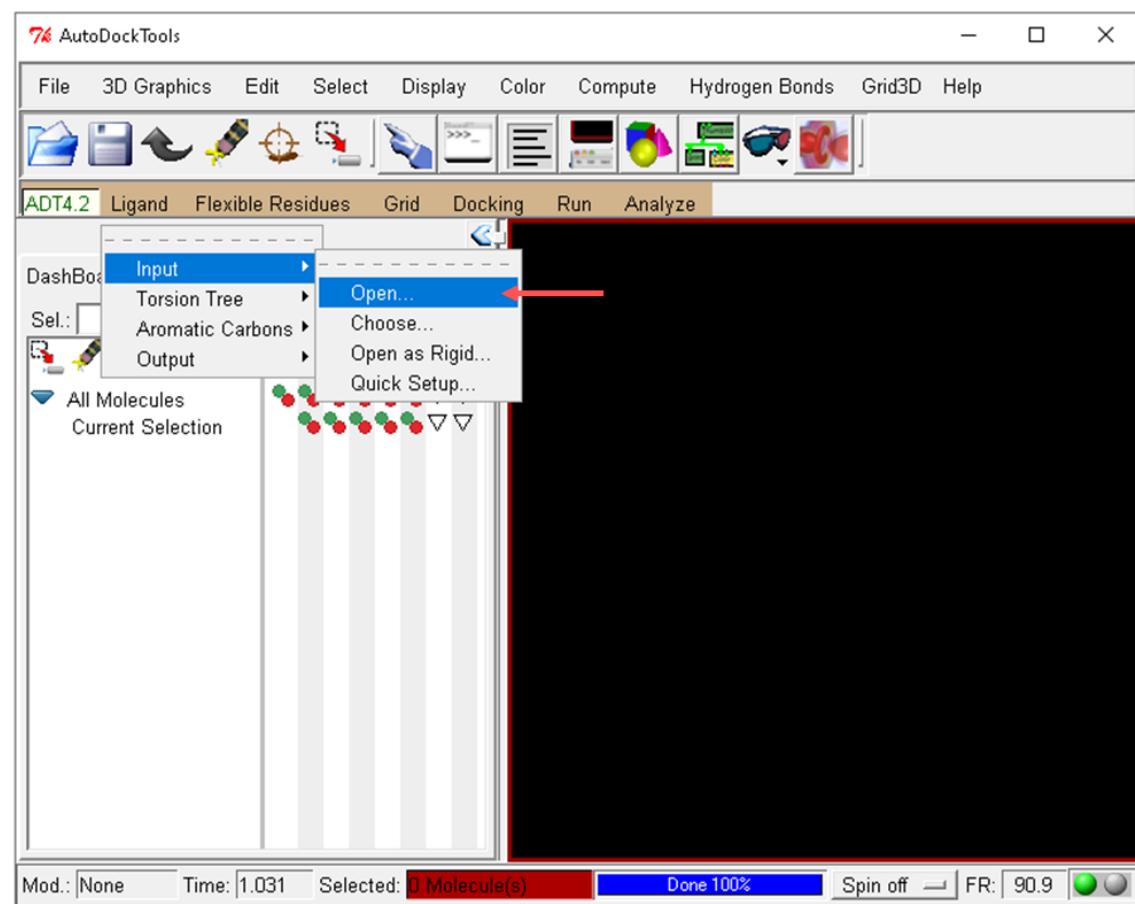




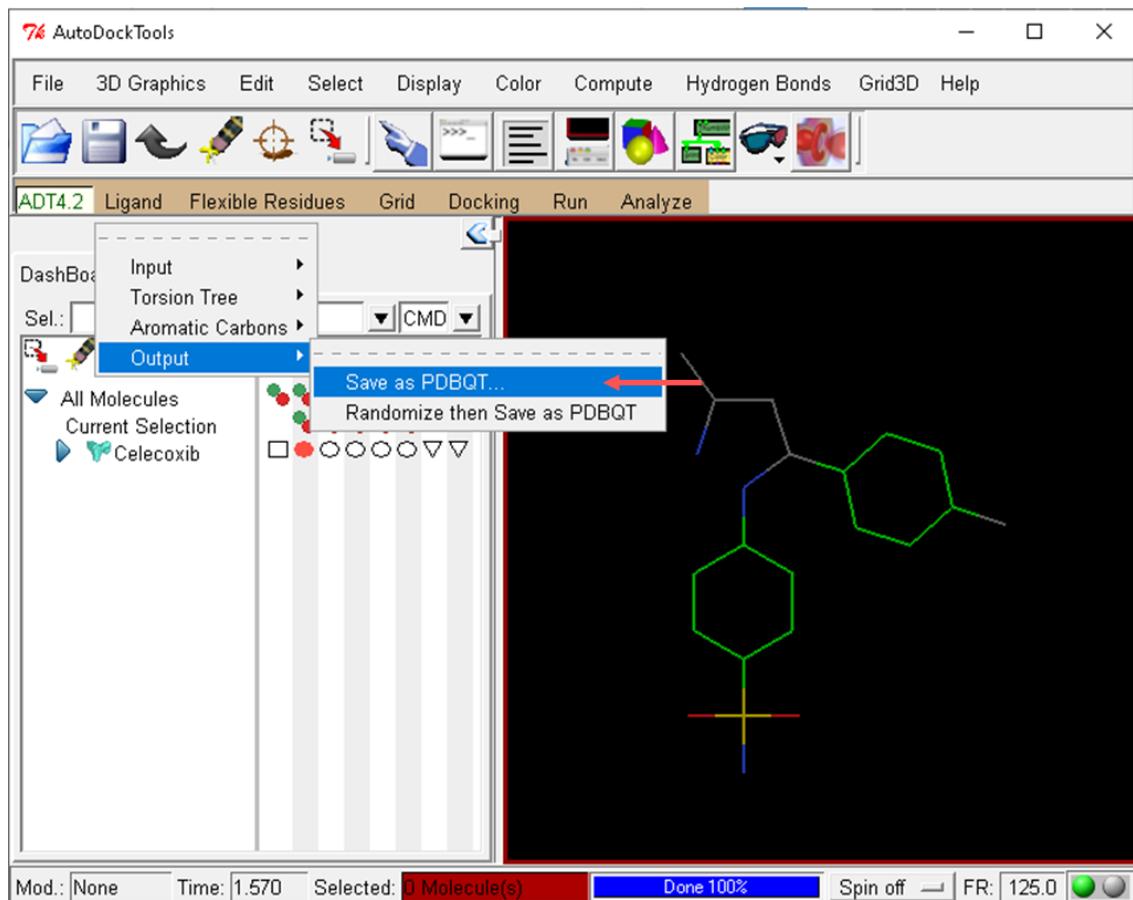
24) Configure the Grid Box with the parameters provided by metaPocket 2.0



## 25) Select the ligand

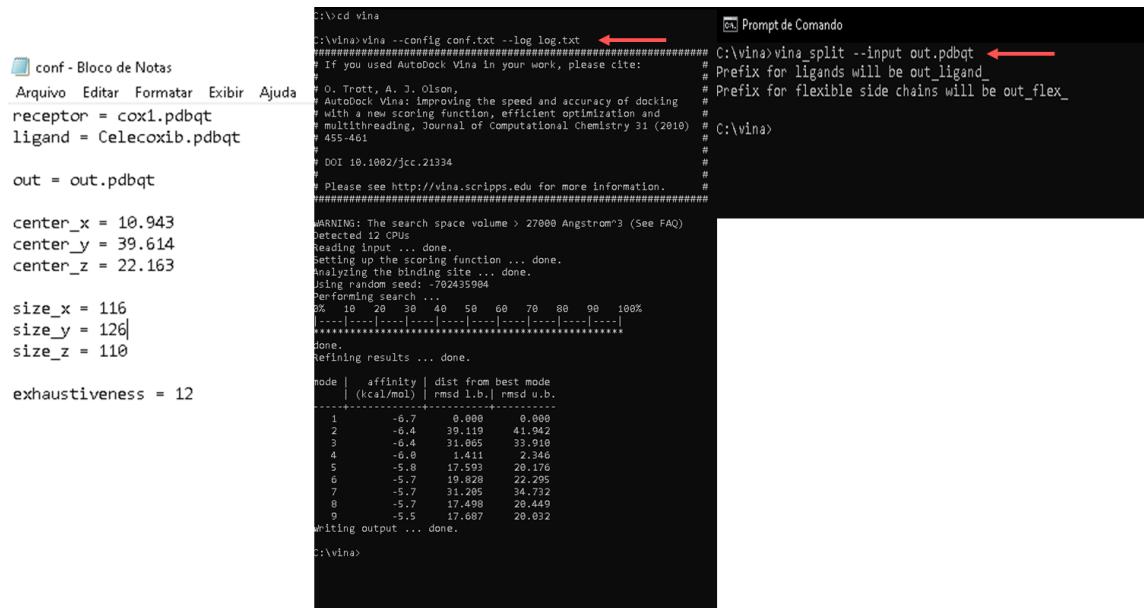


## 26) Save the celecoxib molecule in PDBQT format



27) Autodock Vina is molecular docking software and is generally used via the command line. The basic command to run Autodock Vina follows the following format:

```
vina --receptor <arquivo receptor.pdbqt> --ligand <arquivo  
ligante.pdbqt> --out <arquivo_saida.pdbqt>
```



```

C:\>cd vina
C:\vina>vina --config conf.txt --log log.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.
#
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -702435904
Performing search ...
  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
[----|-----|-----|-----|-----|-----|-----|-----|-----]
done.
Refining results ... done.

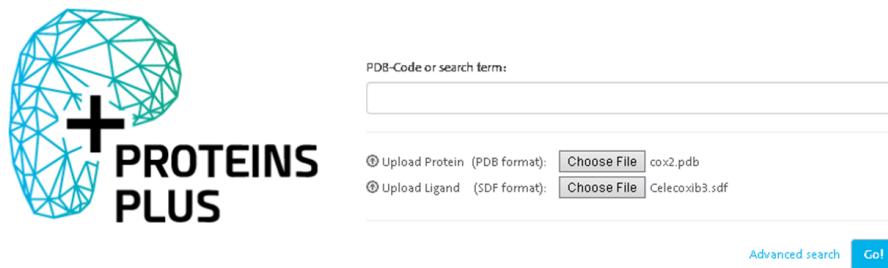
mode | affinity | dist from best mode
     | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----+-----+-----+-----+
  1   -0.7   9.886   0.000
  2   -6.4   39.119  41.942
  3   -6.4   31.065  33.918
  4   -6.0   14.411  2.346
  5   -5.8   17.593  28.176
  6   -5.7   19.828  22.295
  7   -5.7   31.205  34.732
  8   -5.7   17.498  28.449
  9   -5.5   17.687  28.032

Writing output ... done.

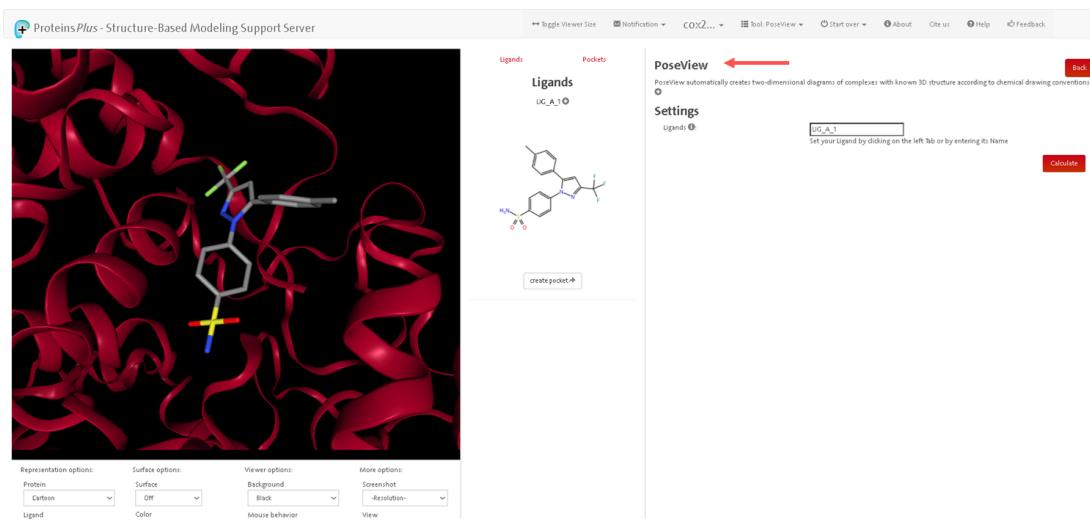
C:\vina>

```

28) Protein Plus The platform offers a series of integrated tools for protein and ligand analysis and visualization <https://proteins.plus/>  
Choose the prepared files and click go.

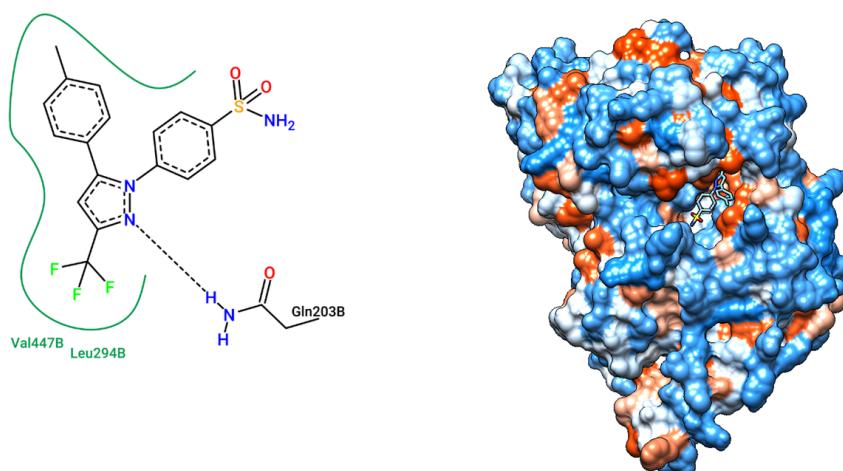


29) After loading the file and configuring the parameters, start the analysis by clicking the appropriate button (for example, "Submit" or "Run PoseView").  
The tool will process the protein-ligand complex and generate a diagram of the interactions.



The screenshot shows the ProteinsPlus interface. On the left, a 3D ribbon model of a protein is shown with a green stick model of a ligand bound to it. The interface has several tabs at the top: 'Ligands' (selected), 'Pockets', 'PoseView' (highlighted with a red arrow), 'COX2...', 'Tool: PoseView', 'Start over', 'About', 'Cite us', 'Help', and 'Feedback'. Below the tabs, there are sections for 'Ligands' (listing 'LG\_A\_1') and 'Settings' (with a 'Create pocket' button). A 2D chemical structure of the ligand 'LG\_A\_1' is displayed in the PoseView section.

30) After the analysis is completed, the tool will generate an interaction diagram. This diagram illustrates hydrogen bonds, hydrophobic interactions, and other important interactions between the protein and ligand.



## Protocol references

1. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* 2009;30(16):2785-2791.
2. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31(2):455-461.
3. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des.* 2011;7(2):146-157.
4. Cavasotto CN, Orry AJ, Murgolo NJ, Czarniecki MF, Kocsi SA, Hawes BE. Docking and high throughput docking: successes and pitfalls. *J Chem Inf Model.* 2009;49(4):1079-1093.
5. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov.* 2004;3(11):935-949.
6. Shoichet BK. Virtual screening of chemical libraries. *Nature.* 2004;432(7019):862-865.