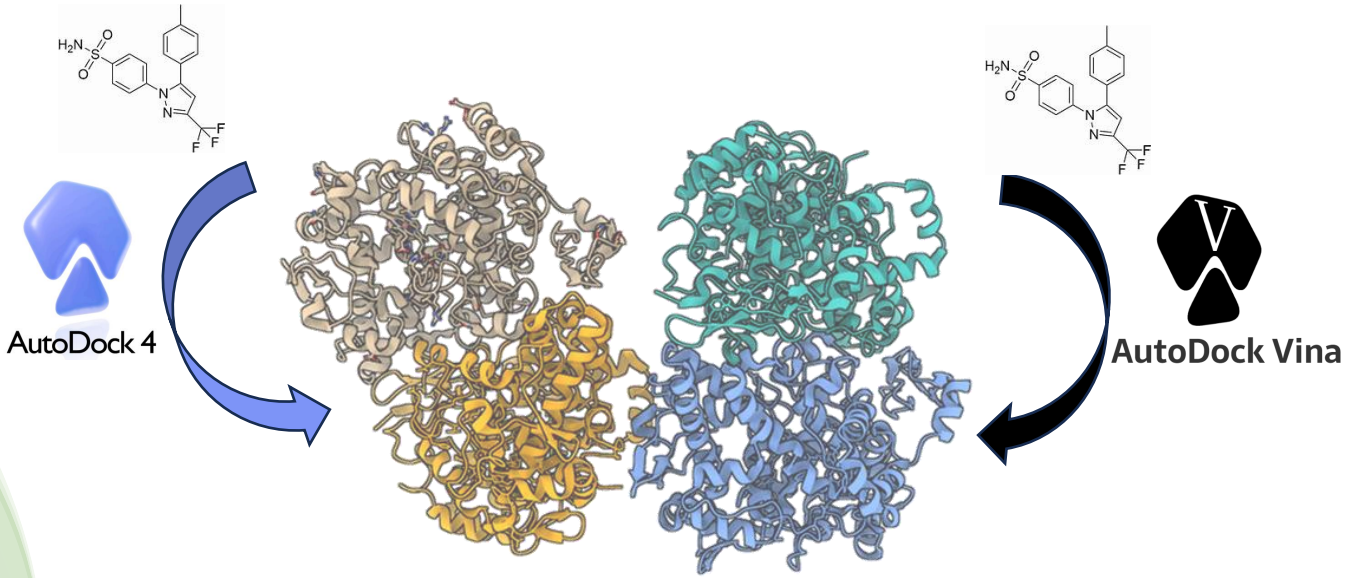
7th Advanced in silico Drug Design workshop/challenge 2024

Molecular Docking Tutorial

Dr. Federica Moraca

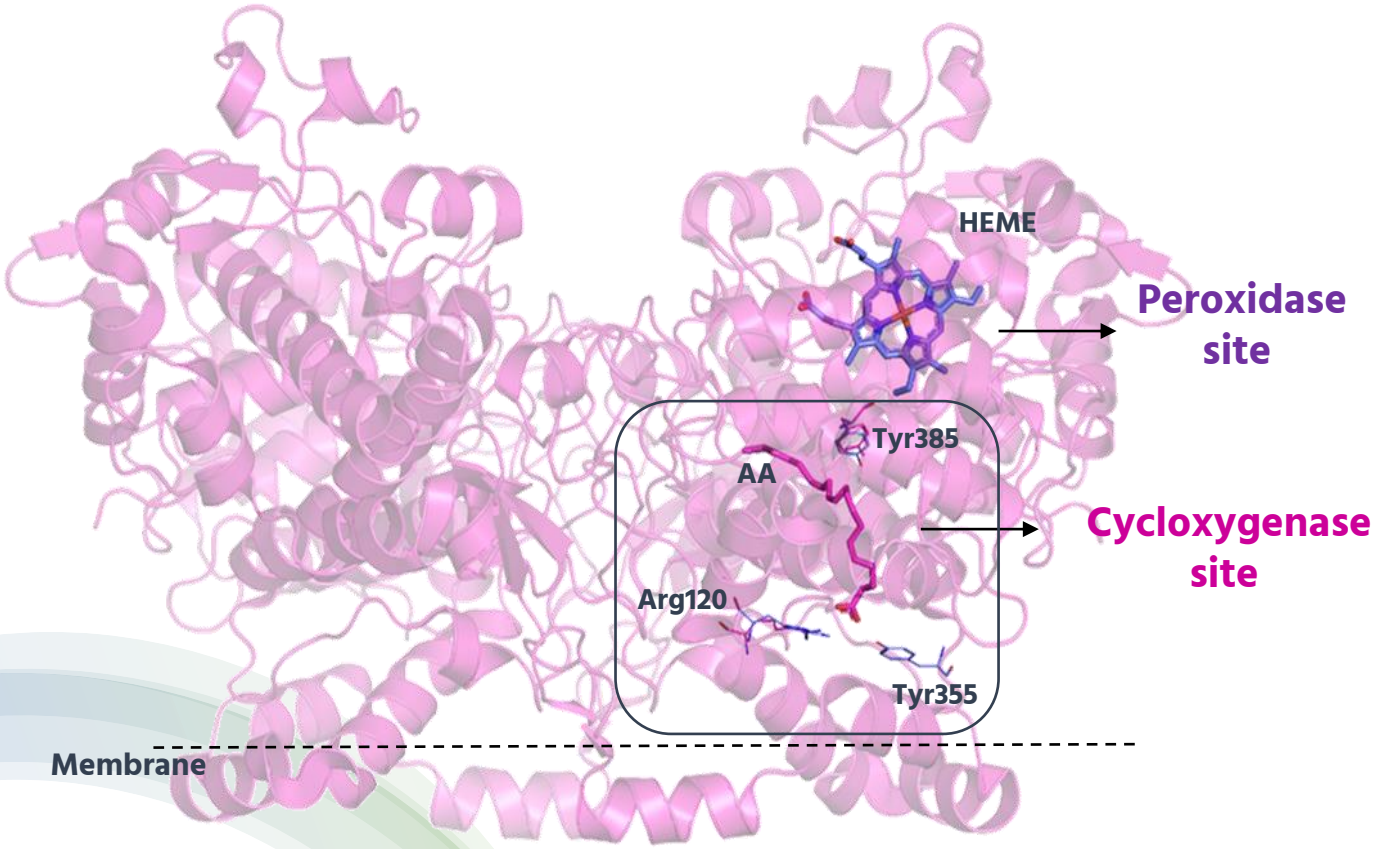


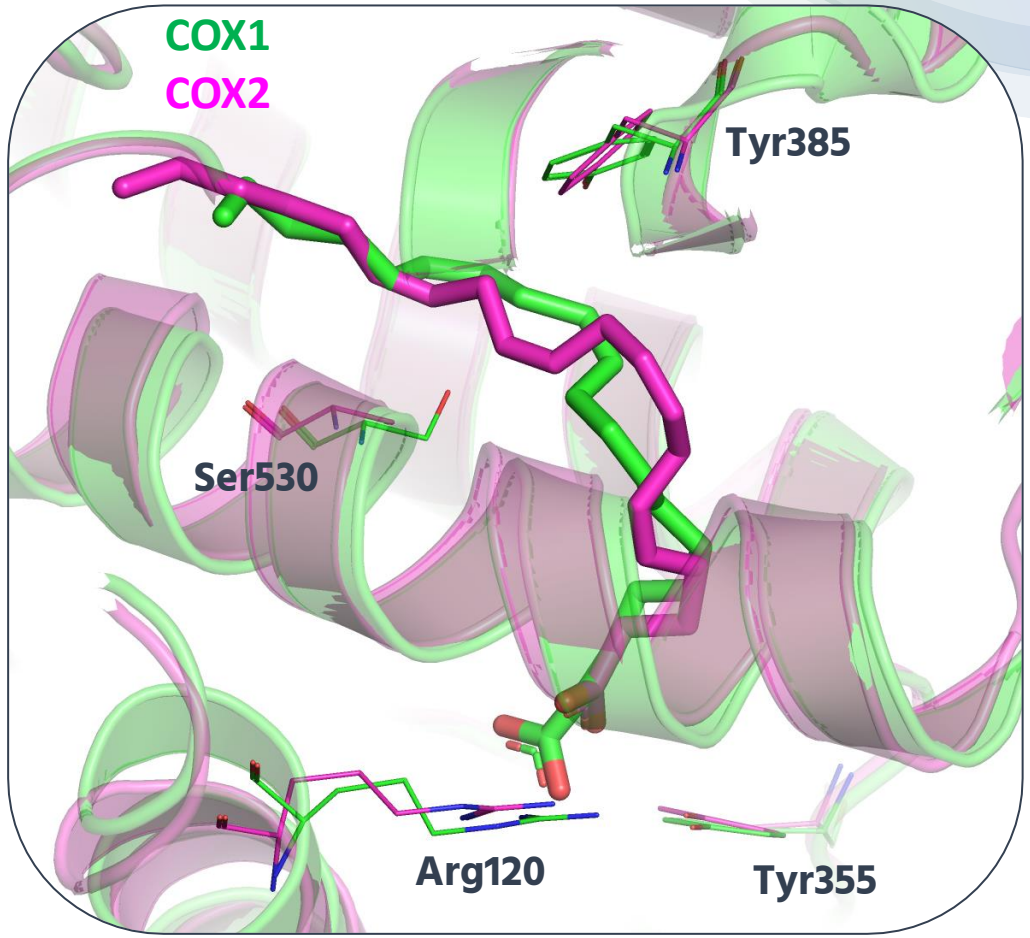
UP Olomouc 29.01. -02.02.2024

COXs Mechanism of Action

AutoDock Vina

AutoDock 4



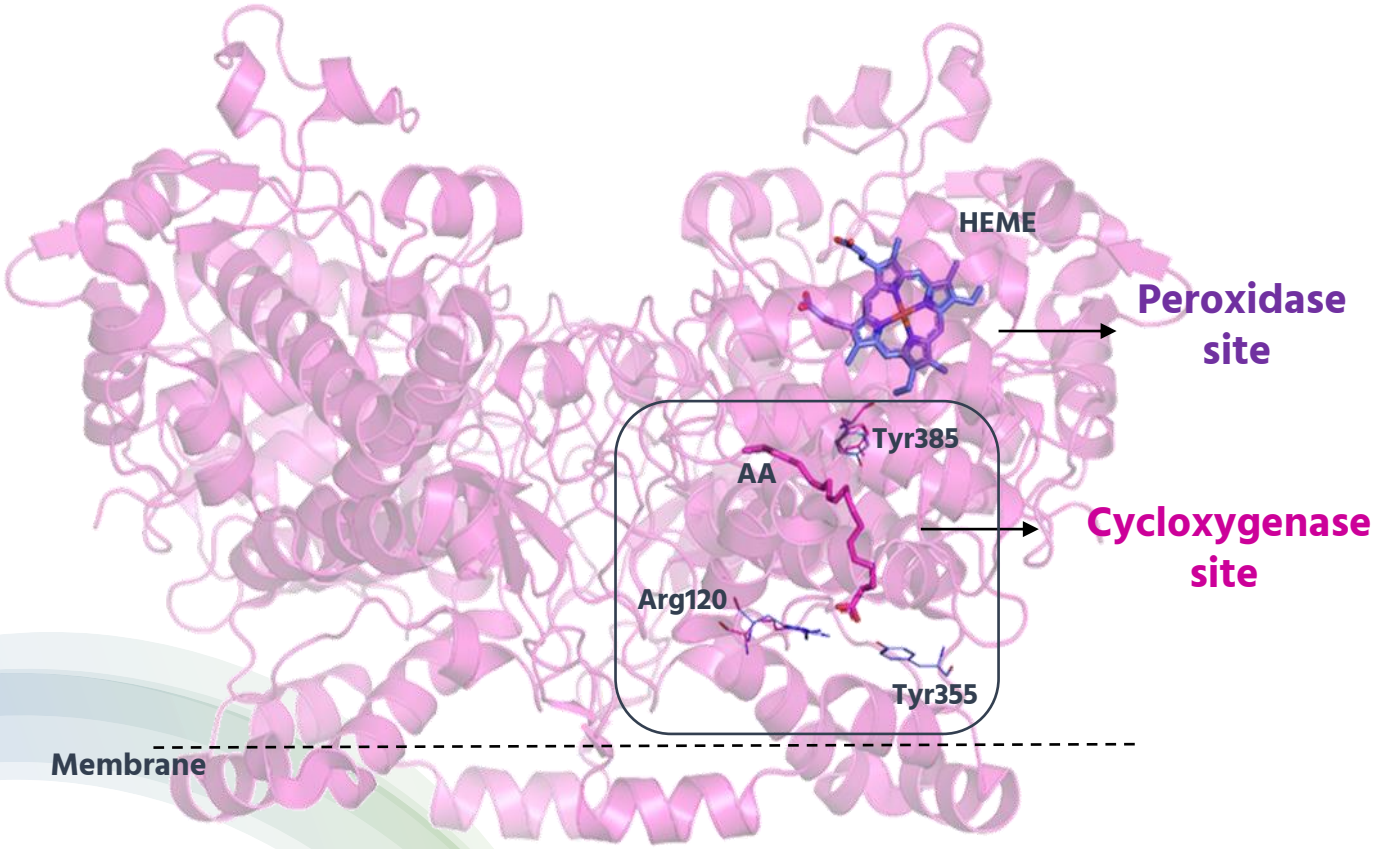


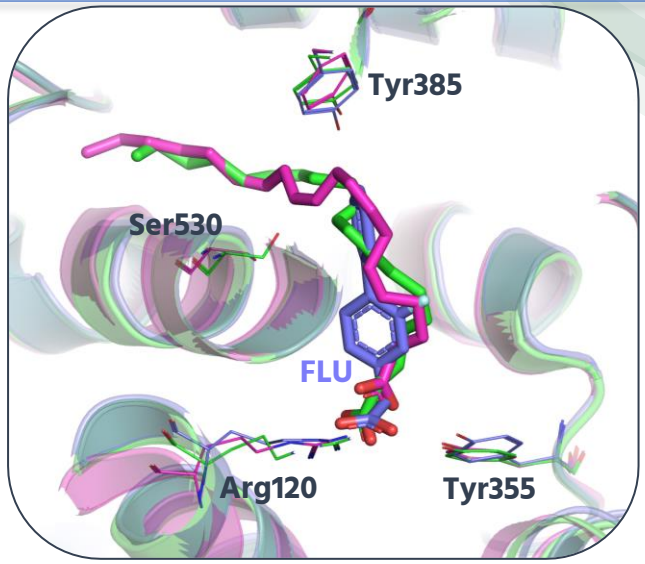
The X-ray structure of COXs complexed with Arachidonic Acid (AA) confirms a L-shaped binding conformation, with the carboxylate moiety of AA binding to **Arg120** and **Tyr355**, while the omega-end positioned in a region termed the top channel in close contact with **Ser530** and **Tyr385**

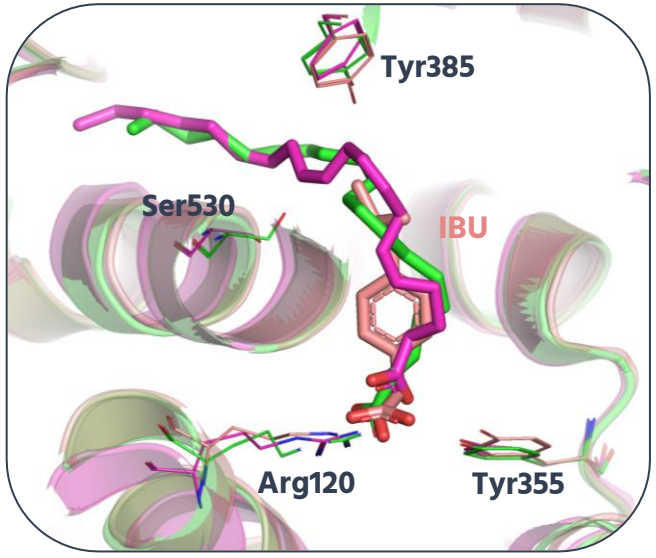
Mechanism of Action of NSAIDs against COXs

AutoDock Vina

AutoDock 4





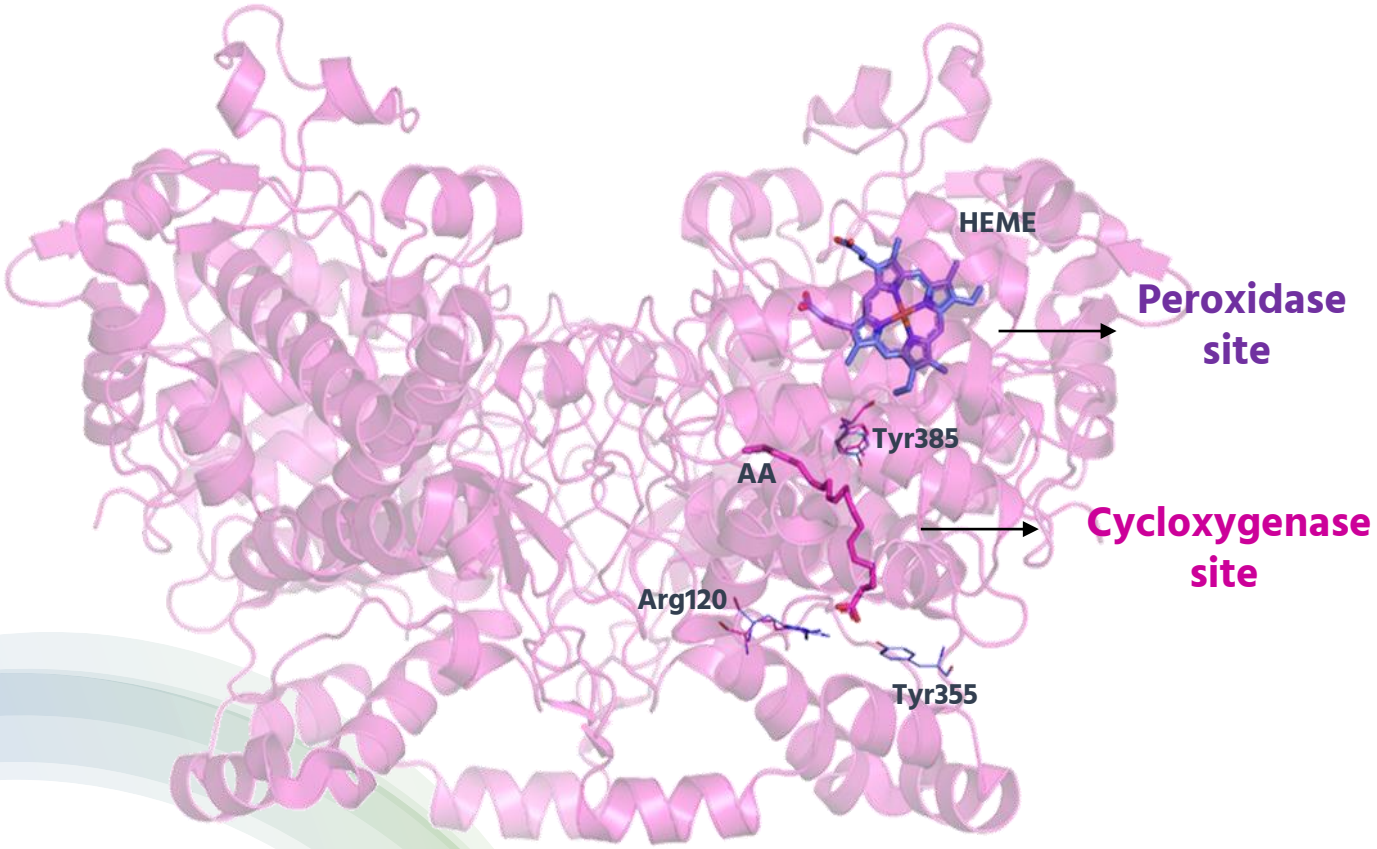


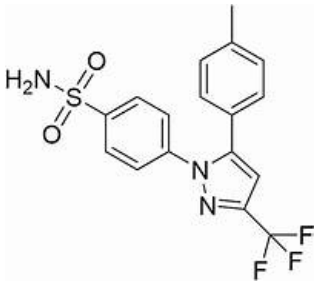
Reversible competitive inhibitors (Ibuprofen and Flurbiprofen) act by interfering with hydrophilic interactions (hydrogen-bonds or salt-bridge) with **Arg120** and **Tyr355** at the entrance of the cyclooxygenase channel.

Structural differences between COX1 and COX2

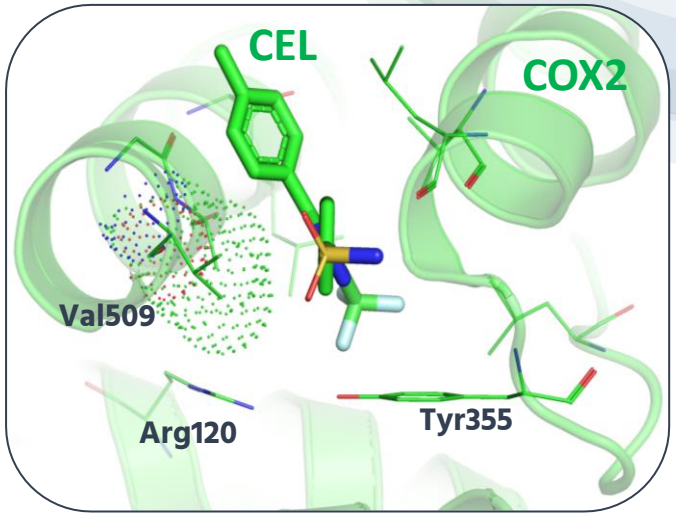
AutoDock Vina

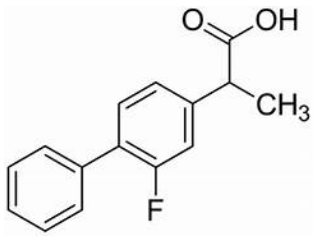
AutoDock 4



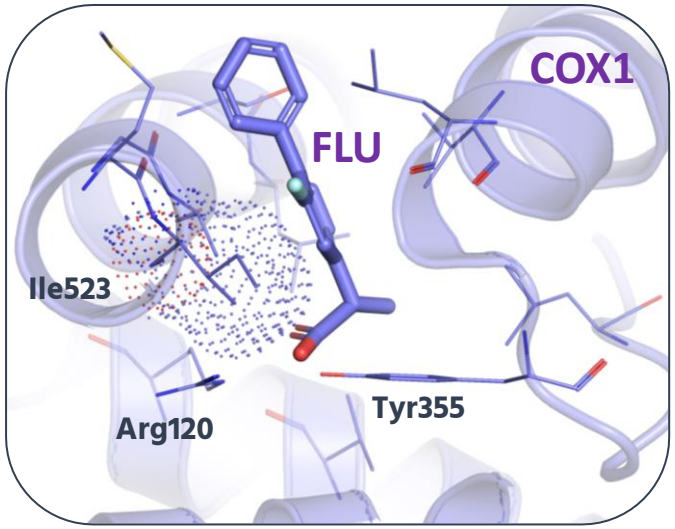


Celecoxib
(COX2 selective inhibitor)





Flurbiprofen
(COX1/COX2 non selective inhibitor)



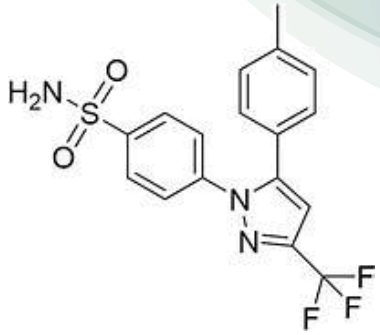
Molecular Docking Tutorial

AutoDock Vina

AutoDock 4

TASK:

Perform Molecular Docking calculations of Celecoxib against both the COX1 and COX2 isoforms, in order to understand the molecular basis of its COX2 selectivity



Celecoxib
(COX2 selective inhibitor)

1. **MGLTools (GUI of AutoDock Tools)**
2. **AutoDock4 and AutoDock Vina docking engines**
3. **PyMOL (visualization results)**

Molecular Docking Tutorial

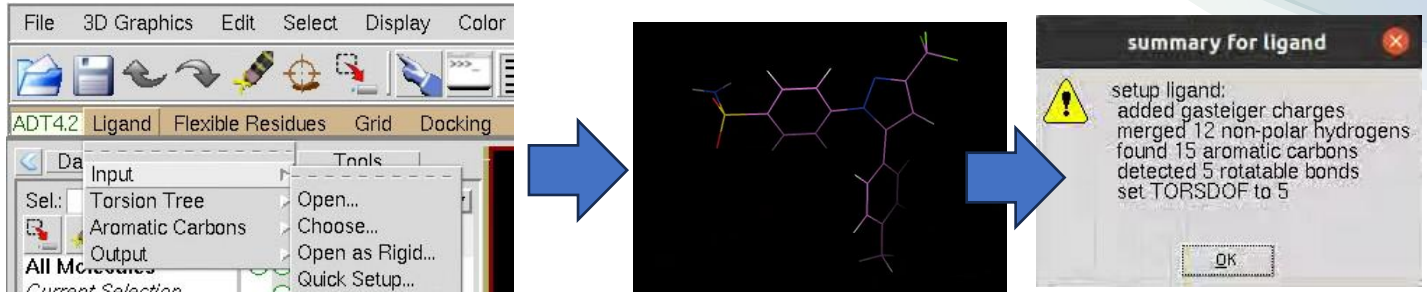
Molecular Docking Tutorial

Ligand preparation (GUI of AutoDock Tools)

1. Import the Celecoxib (CEL.pdb)

Ligand>Input>Open>*.pdb>CEL.pdb

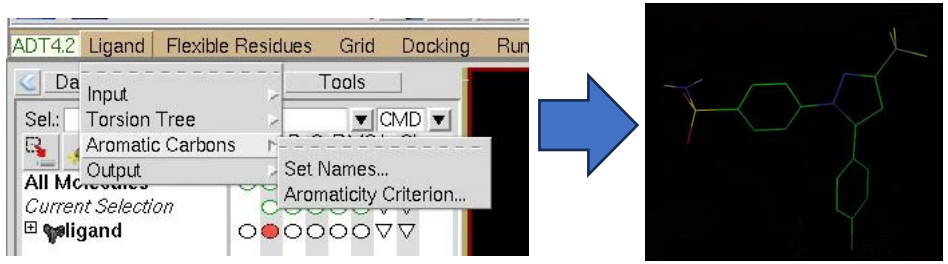
ligand will be prepared for docking. After clicking the "OK" button, you will see that all the hydrogens atoms are merged to carbon atoms.



2. Check for aromatic carbons

Ligand>Aromatic carbons>Set Names

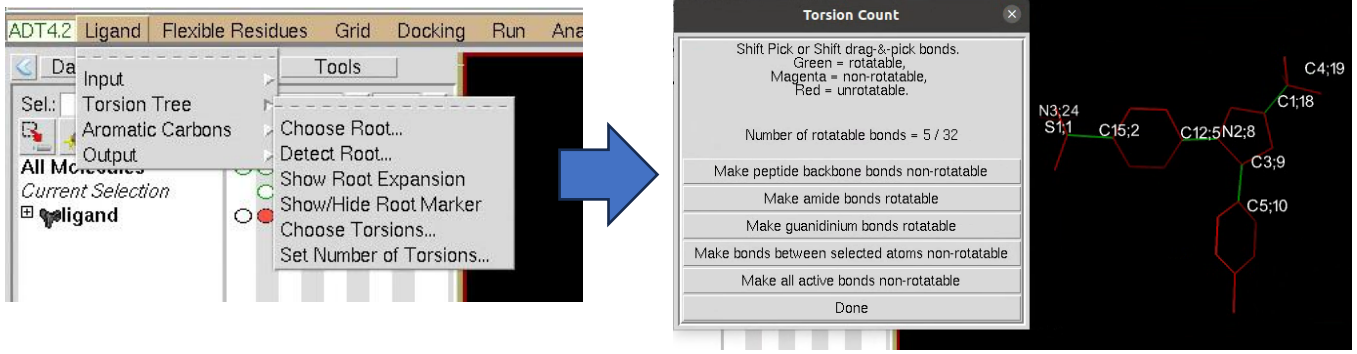
Aromatic atoms are shown in green



3. Check rotatable torsions

Ligand>Torsion Tree>Choose Torsion

Rotatable bonds are shown in green, unrotatable in red. Celecoxib has 5 rotatable bonds



4. Save pdbqt file

Ligand>Output>Save as> *.pdbqt>CEL.pdbqt

Molecular Docking Tutorial

AutoDock 4

Let's have a look on the CEL.pdbqt file

```
REMARK  5 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK    1  A    between atoms: S1_1  and  C15_2
REMARK    2  A    between atoms: S1_1  and  N3_24
REMARK    3  A    between atoms: C12_5  and  N2_8
REMARK    4  A    between atoms: C3_9  and  C5_10
REMARK    5  A    between atoms: C1_18  and  C4_19
ROOT                                  Coor x   Coor y   Coor z    Occ. B-factor   Charges Types
ATOM      1  S1  CEL A 682      25.931 -21.467 -17.155  1.00 43.78     0.256 S
ATOM      2  O2  CEL A 682      25.772 -20.039 -17.291  1.00 45.34    -0.201 OA
ATOM      3  O1  CEL A 682      25.436 -22.106 -15.949  1.00 45.63    -0.201 OA
ENDROOT
BRANCH   1   4
ATOM      4  C15 CEL A 682      27.679 -21.706 -17.131  1.00 41.41     0.079 A
ATOM      5  C14 CEL A 682      28.218 -22.829 -16.556  1.00 39.70     0.027 A
ATOM      6  C13 CEL A 682      29.584 -22.964 -16.543  1.00 39.68     0.033 A
ATOM      7  C12 CEL A 682      30.341 -21.967 -17.109  1.00 39.92     0.059 A
ATOM      8  C17 CEL A 682      29.796 -20.853 -17.697  1.00 40.26     0.033 A
ATOM      9  C16 CEL A 682      28.434 -20.714 -17.707  1.00 40.98     0.027 A
BRANCH   7  10
ATOM     10  N2  CEL A 682      31.724 -22.006 -17.132  1.00 40.07    -0.233 N
ATOM     11  C3  CEL A 682      32.622 -22.574 -16.260  1.00 40.53     0.071 A
ATOM     12  C2  CEL A 682      33.843 -22.337 -16.837  1.00 41.52     0.070 A
TORSDOF 5
```

Information about ligand active (A) torsions

Atom description

Torsion definitions

Number of active torsion

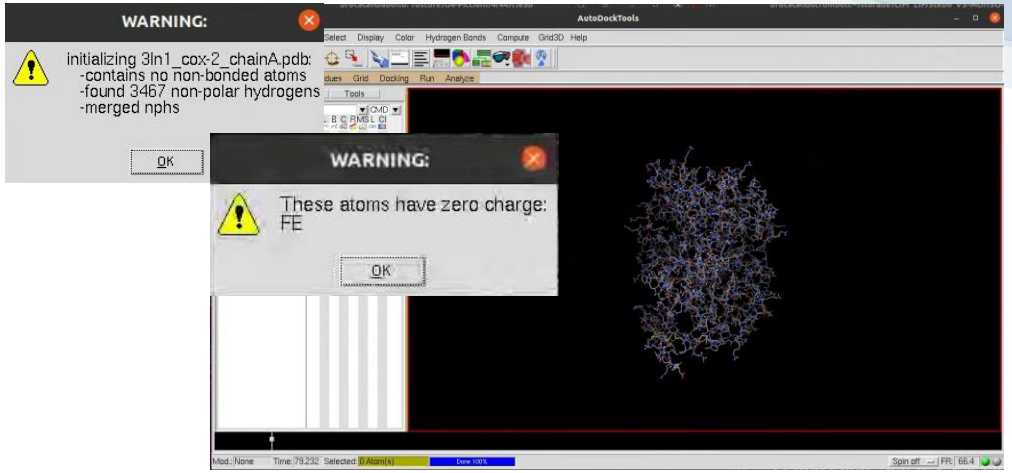
Molecular Docking Tutorial

Receptor preparation (GUI of AutoDock Tools)

1. Select protein (only ChainA)

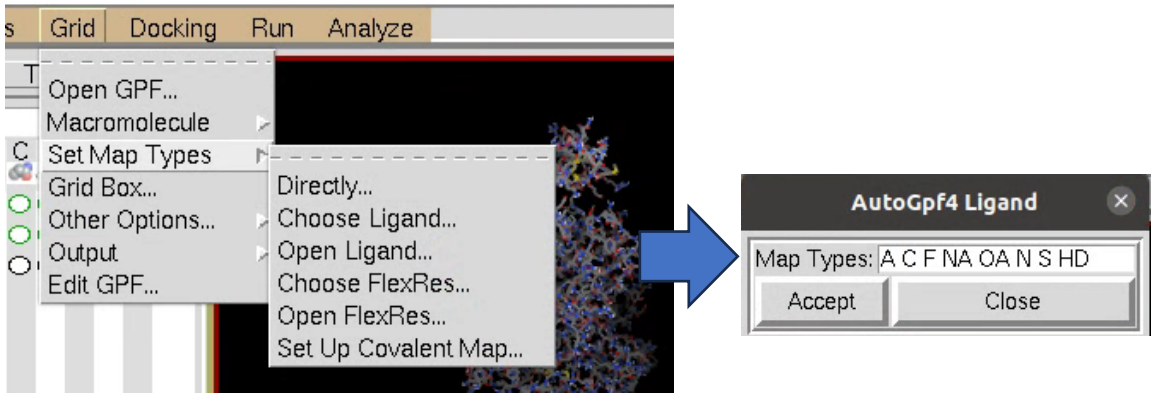
Grid>Macromolecule>Open>cox2.pdb

protein will be prepared for docking (nonpolar hydrogens merged with carbons, charges assigned)



2. Set the Celecoxib Map Types

Grid>Set Map Types>Directly (A C F NA OA N S HD)



Molecular Docking Tutorial

COX2 preparation (GUI of AutoDock Tools)

3. Locate the Grid Box on the ciclooxigenase site

Grid>Grid Box

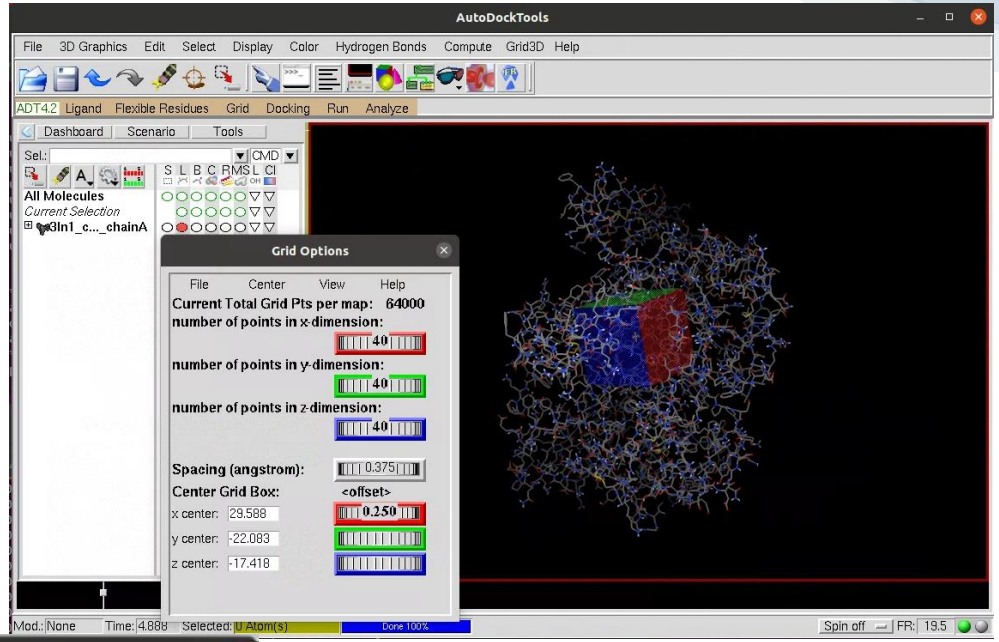
- A cube with a default size of 40x40x40 will appear.

- Adjust the box coordinates so it will cover active site (at the center of CEL), but not much more. For a good centering of the grid box we suggest to manually modify to

x center: 29.588,

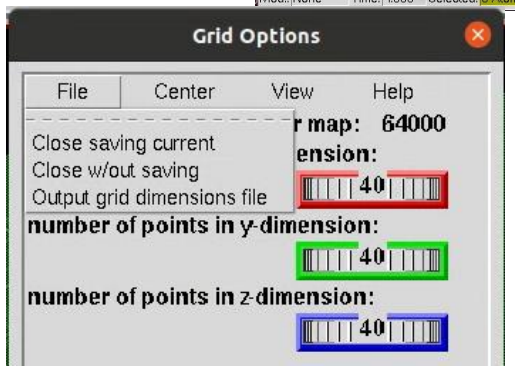
y center: -22.083,

z center: -17.418



4. Save the Grid Box Parameter

From the Grid Options window: File> Close saving current



Molecular Docking Tutorial

COX2 preparation (GUI of AutoDock Tools)

5. Manually edit protein PDBQT to charge iron (+2)

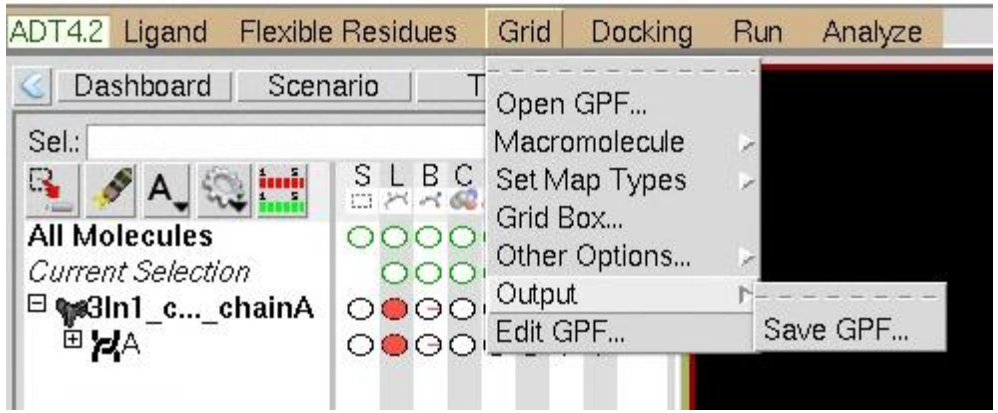
```
HETATM 4482 FE   HEM A 500       5.890  24.568  -1.058  1.00 18.80     2.000 Fe
```

6. Save the Grid Parameter File (GPF)

Grid>Output>Save GPF>cox-2.gpf

Alternatively you can run the following command

```
prepare_gpf4.py -l CEL.pdbqt -r cox2.pdbqt –o cox-2.gpf
```



7. Run AutoGrid

```
autogrid4 -p cox-2.gpf -l cox2.glg
```

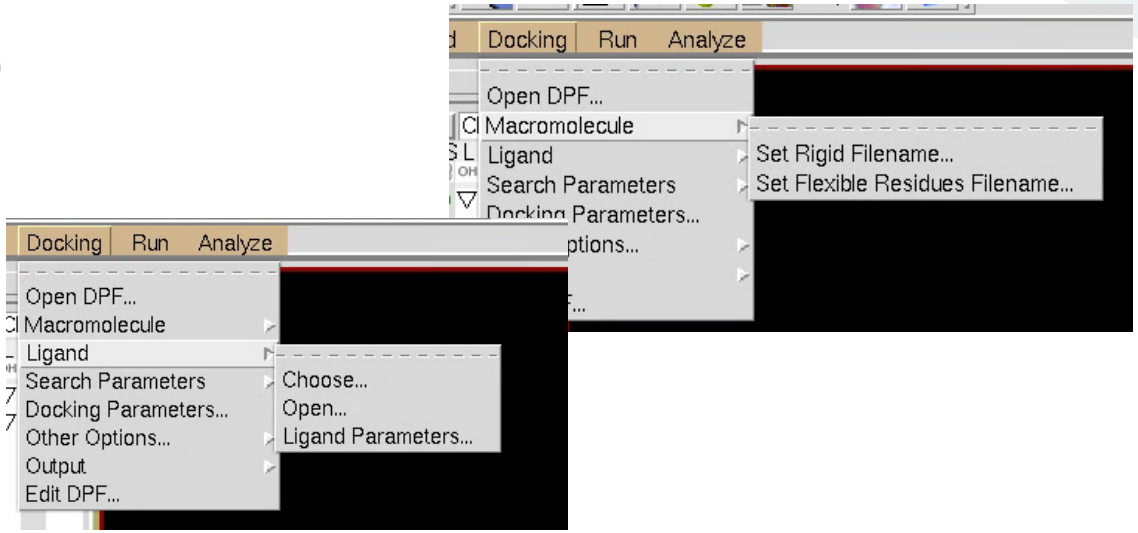
Repeat the same procedure for COX1…

Molecular Docking Tutorial

Preparing the Docking Parameter File (DPF)

1. From the ADT GUI import receptor (PDBQT)

Docking>Macromolecule>Set Rigid Filename>cox2.pdbqt

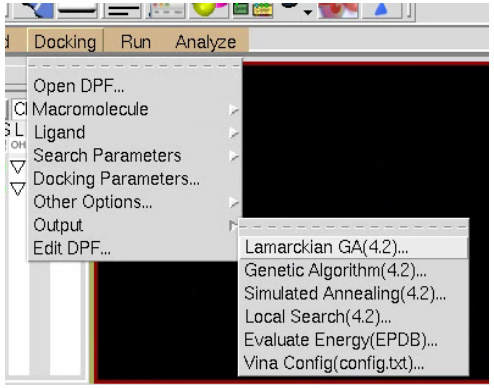


2. From the ADT GUI import ligand (PDBQT)

Docking>Ligand>Open>CEL.pdbqt

3. Save the Docking Parameter File (DPF)

Docking>Output>Lamarchian GA>docking-cox2.dpf



Alternatively you can run the following command

```
prepare_dpf4.py -l CEL.pdbqt -r cox-2.pdbqt –o docking-cox2.dpf
```

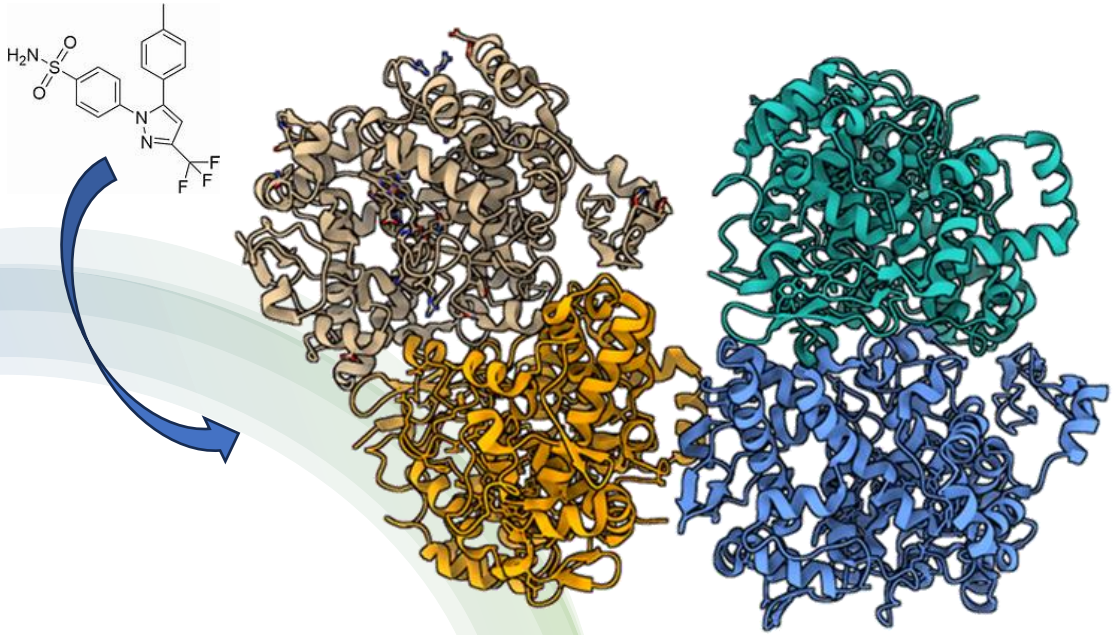
Repeat the same procedure for COX1…

Molecular Docking Tutorial

RUN AutoDock4

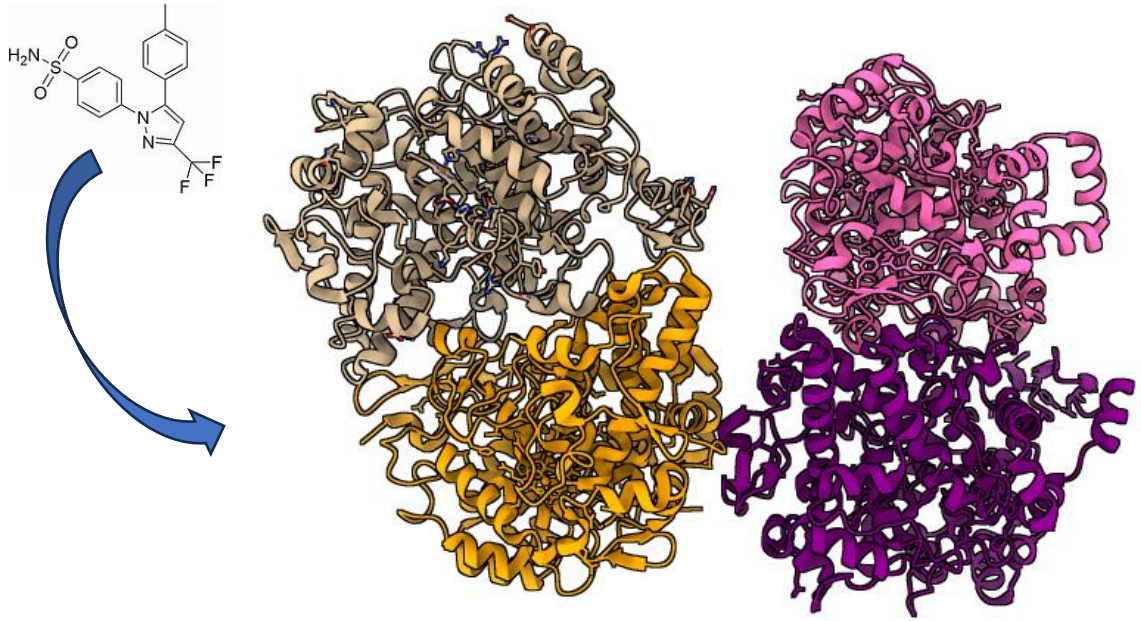
CEL vs COX2

```
Autodock4 -p docking-cox2.dpf -l docking-cox2.dlg
```



CEL vs COX1

```
Autodock4 -p docking-cox1.dpf -l docking-cox1.dlg
```



This will take around 4-5 minutes…

Molecular Docking Tutorial

Docking analysis (poses/scores)

1. Open the .dlg file

Analyze>Dockings>Open>file.dlg

2. Open the receptor (PDBQT)

Analyze>Macromolecule>Open>receptor.pdbqt

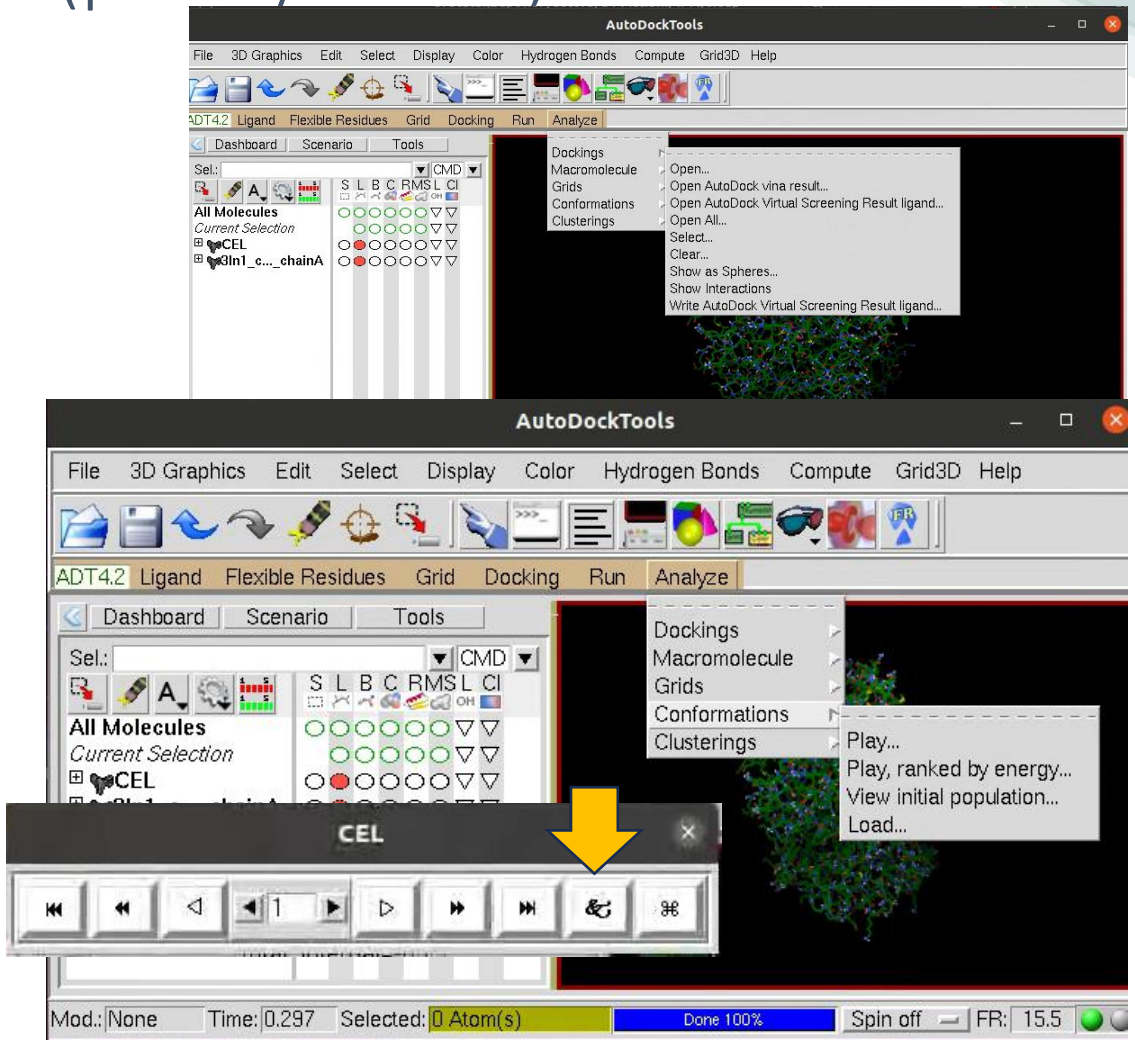
3. Visualize docking conformations

Analyze>Conformations>Play Ranked by Energy

4. Write all the conformations in pdbqt file

Click on the indicated icon>Write all

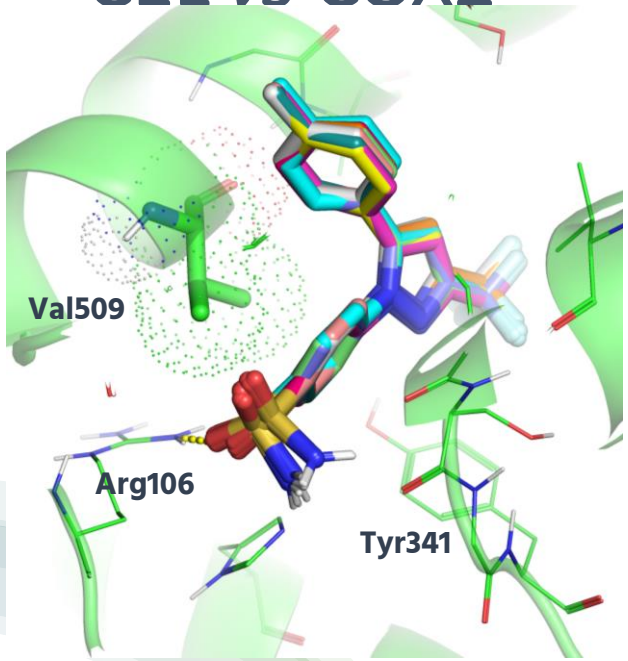
Docking conformations will be write ranked by energy.
«Conf0.pdbqt» has the best energy value



Molecular Docking Tutorial

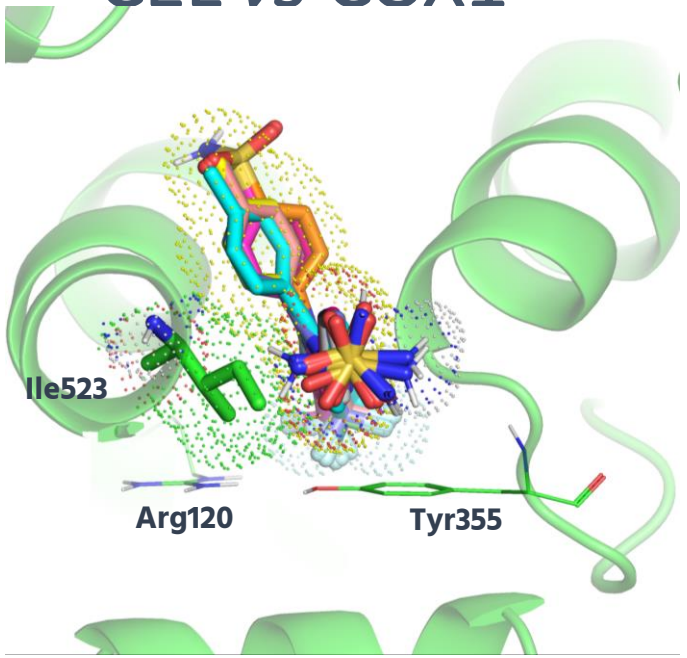
Docking analysis with PyMOL

CEL *vs* COX2



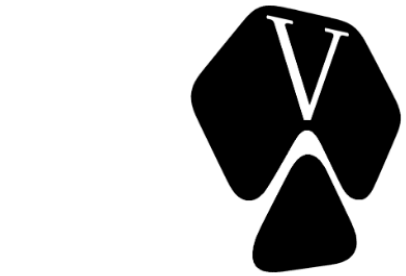
All the poses converge to one unique binding mode with a binding affinity score of -10.41 kcal/mol. The sulfonamide group directly interact with hydrogen-bonds with **Arg106**

CEL *vs* COX1



Of the 10 poses, 7 are oriented with the sulfonamide moiety toward **Arg120** and **Tyr355**, while 3 poses are in opposite orientation. Nonetheless, the presence of **Ile523** hampers Celecoxib to directly interact with **Arg120** and **Tyr355**. The binding affinity is also less stable (-7.62 Kcal/mol)

Molecular Docking Tutorial



AutoDock Vina

Molecular Docking Tutorial

Docking parameter file (config.txt)

Remember:
AutoDock Vina internally precalculates the grid maps. **You don't have to run AutoGrid4.**

Protein
Ligand

Output file name

Box center

Box size

Search exhaustiveness

Number of docking poses

```
receptor = cox2.pdbqt
ligand   = CEL.pdbqt

out      = vina_results.pdbqt
log      = vina_results.log

center_x =  29.588
center_y = -22.083
center_z = -17.418

size_x   = 40
size_y   = 40
size_z   = 40

exhaustiveness = 8

num_modes      = 10
```

For an optimal comparison we will use the same grid coordinates and sizes as AutoDock4

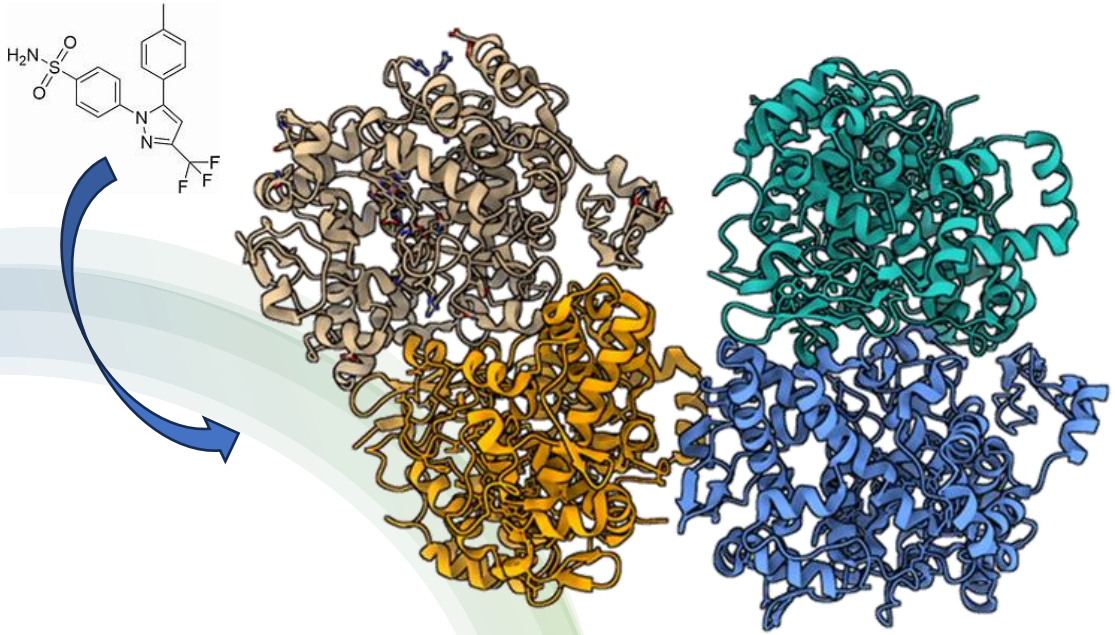
Molecular Docking Tutorial

AutoDock Vina

RUN AutoDock Vina

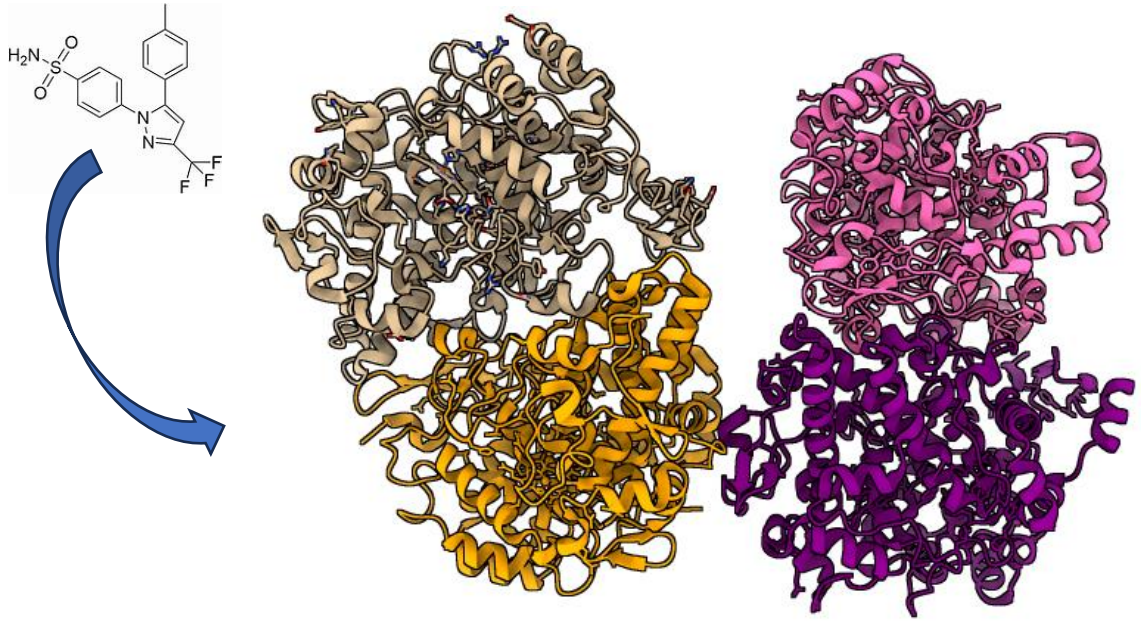
CEL vs COX2

```
vina –-config config.txt
```



CEL vs COX1

```
vina –-config config.txt
```



This will take around 1-2 minutes…

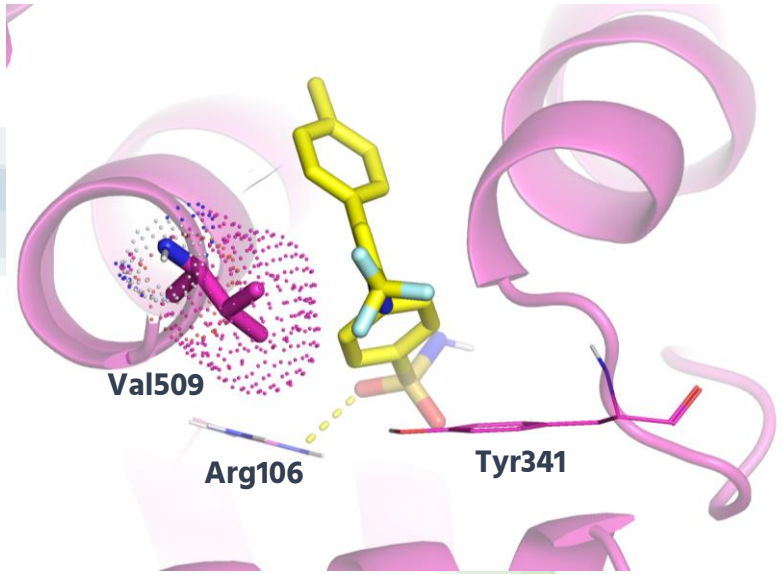
Molecular Docking Tutorial

AutoDock Vina

Docking analysis with PyMOL

CEL vs COX2

1. Open the vina_results_cox2.pdbqt file and split
   Action>State>Split
2. Open the cox2.pdbqt file



CEL vs COX1

1. Open the vina_results_cox1.pdbqt file
   Action>State>Split
2. Open the cox1.pdbqt file