# 8th Advanced in silico Drug Design workshop 2025

# Molecular Docking Tutorial

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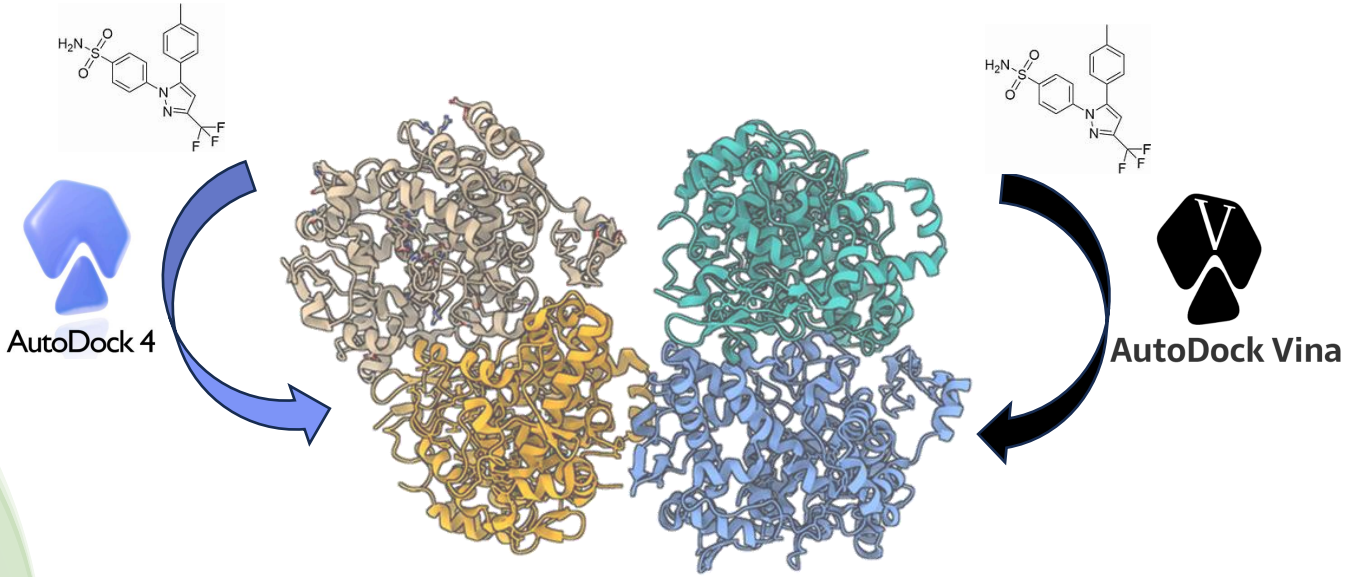
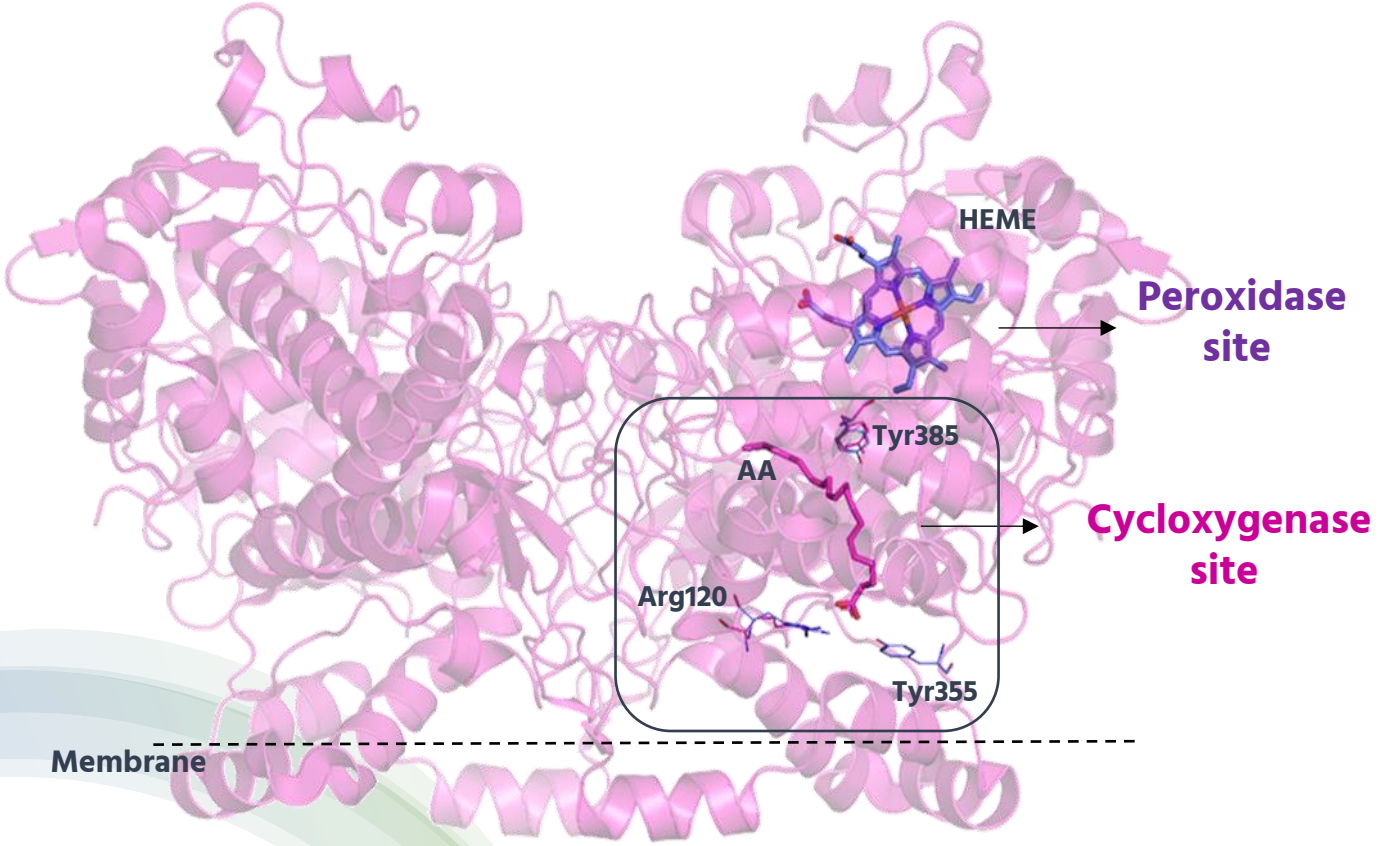


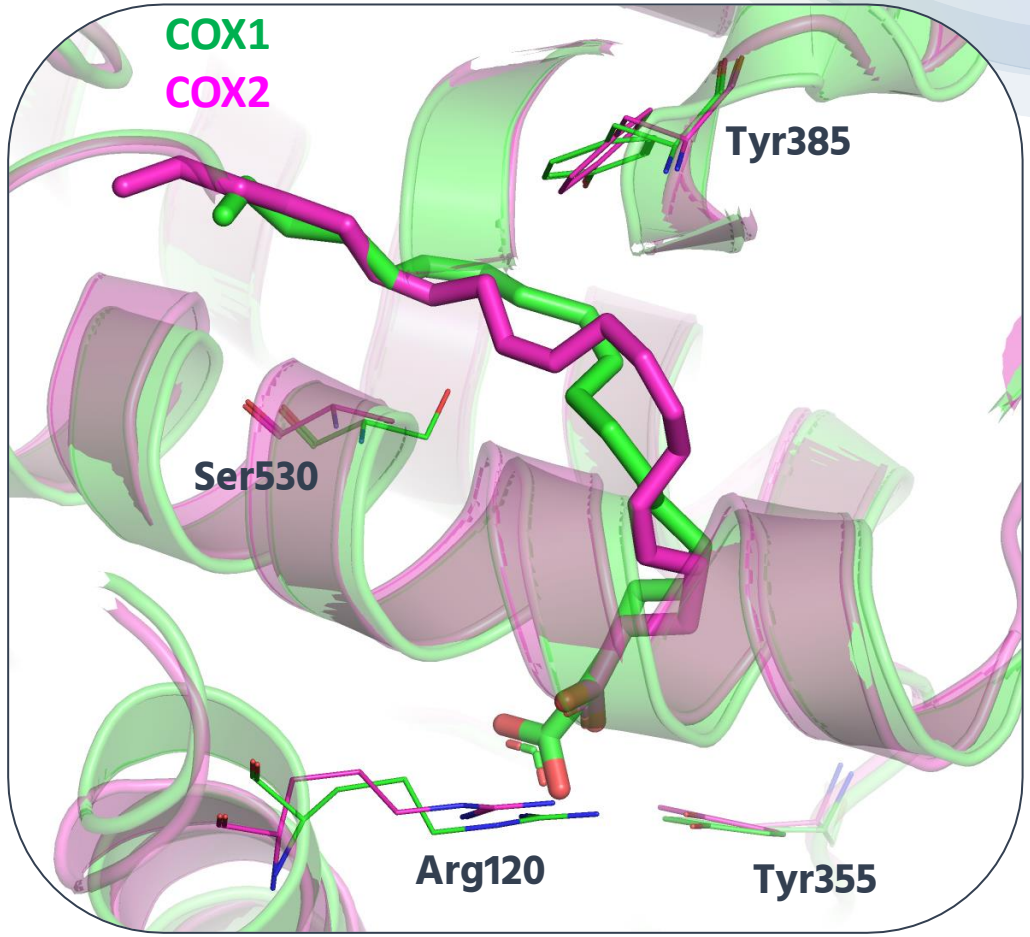

UP Olomouc 27.01. - 31.01.2025

# COXs Mechanism of Action

AutoDock Vina

AutoDock 4



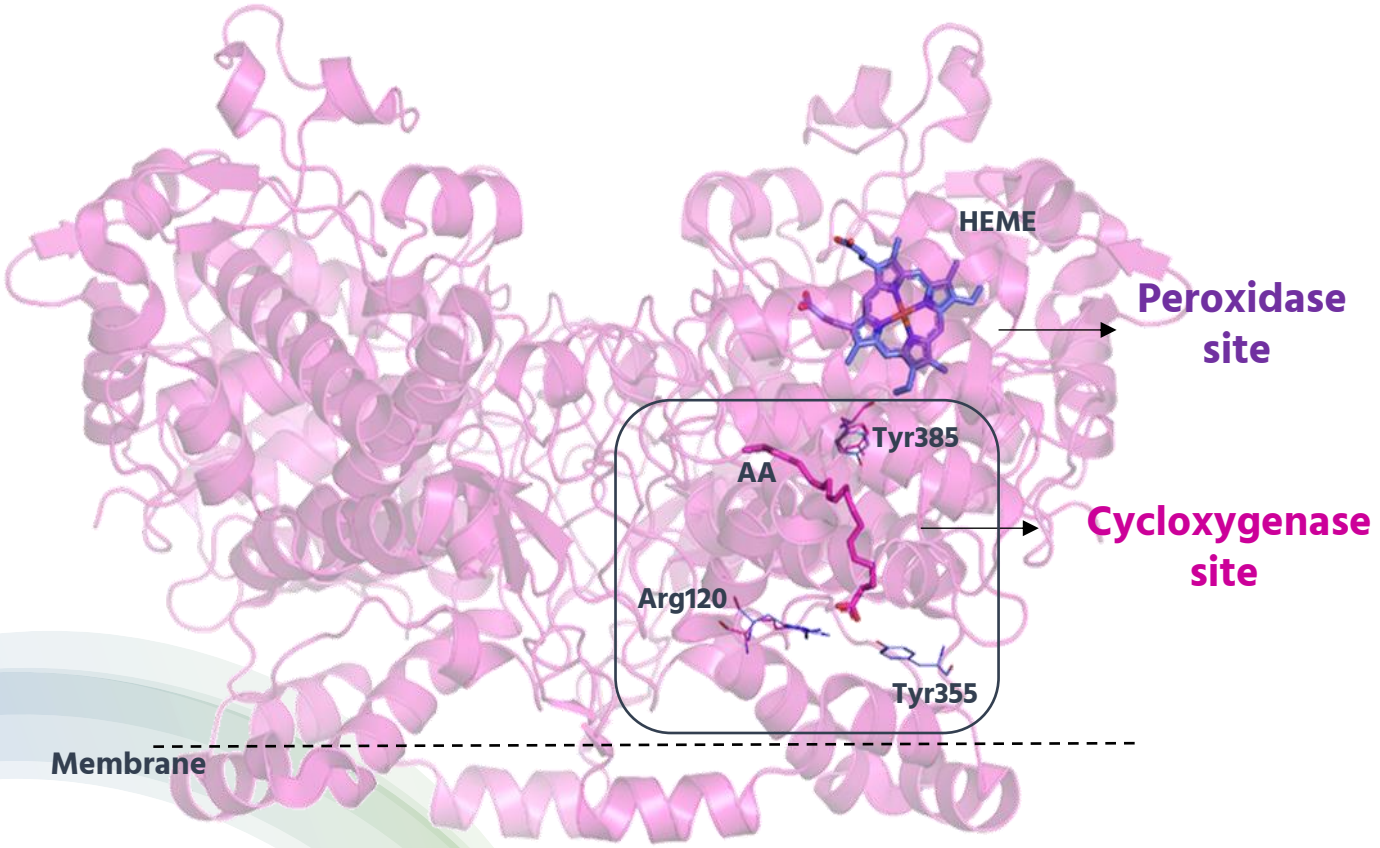



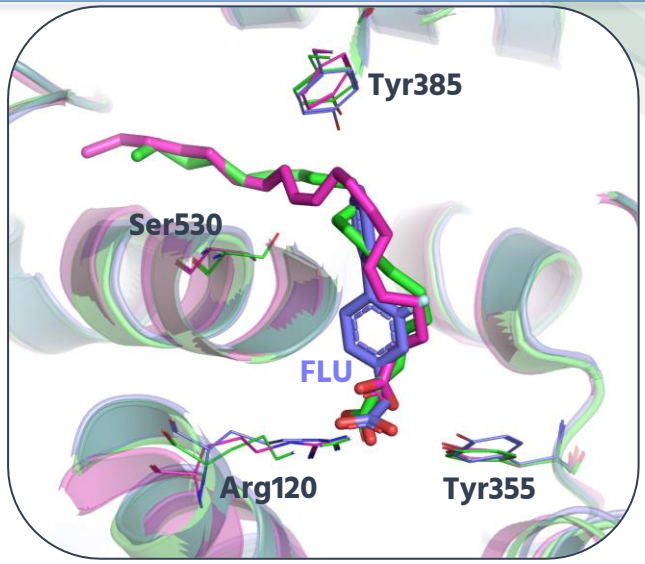

The X-ray structure of COXs complexed with Arachidonic Acid (AA) confirms a L-shaped binding conformation, with the carboxylate moiety of AA binding to **Arg120** and **Tyr355**, while the omega-end positioned in a region termed the top channel in close contact with **Ser530** and **Tyr385**

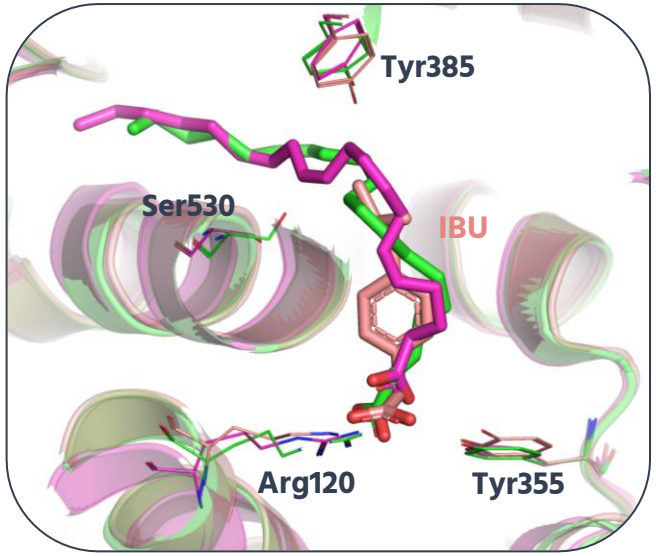
# Mechanism of Action of NSAIDs against COXs

AutoDock Vina

AutoDock 4



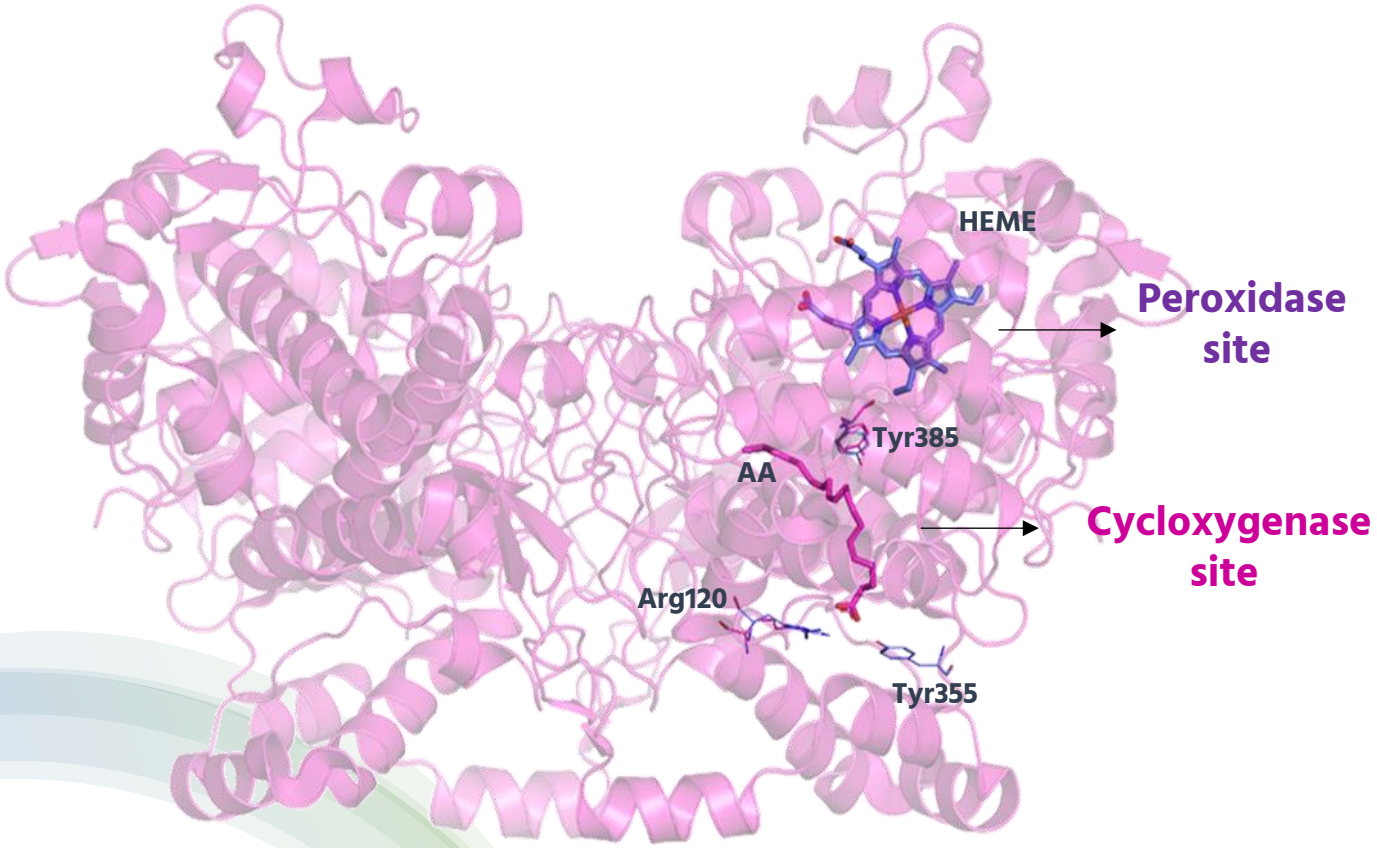



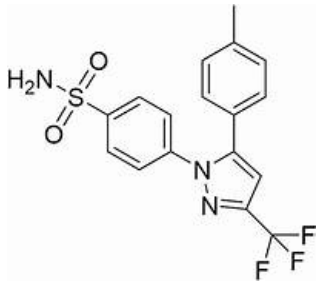

Reversible competitive inhibitors (Ibuprofen and Flurbiprofen) act by interfering with hydrophilic interactions (hydrogen-bonds or salt-bridge) with **Arg120** and **Tyr355** at the entrance of the cyclooxygenase channel.

# Structural differences between COX1 and COX2

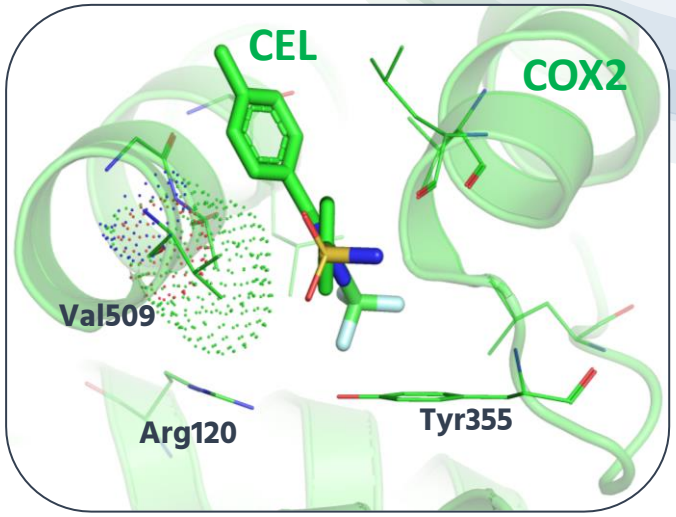
AutoDock Vina

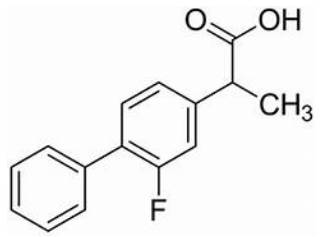
AutoDock 4



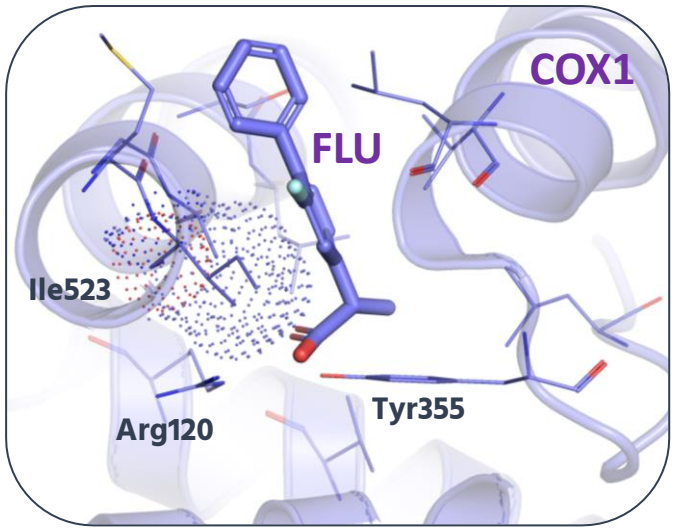



**Celecoxib**
(COX2 selective inhibitor)



**Flurbiprofen**
(COX1/COX2 non selective inhibitor)



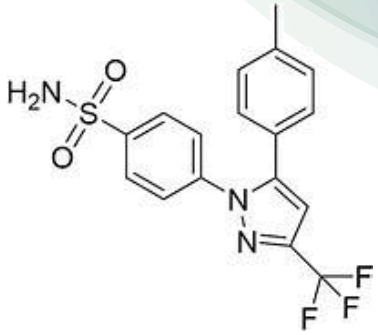
# Molecular Docking Tutorial

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

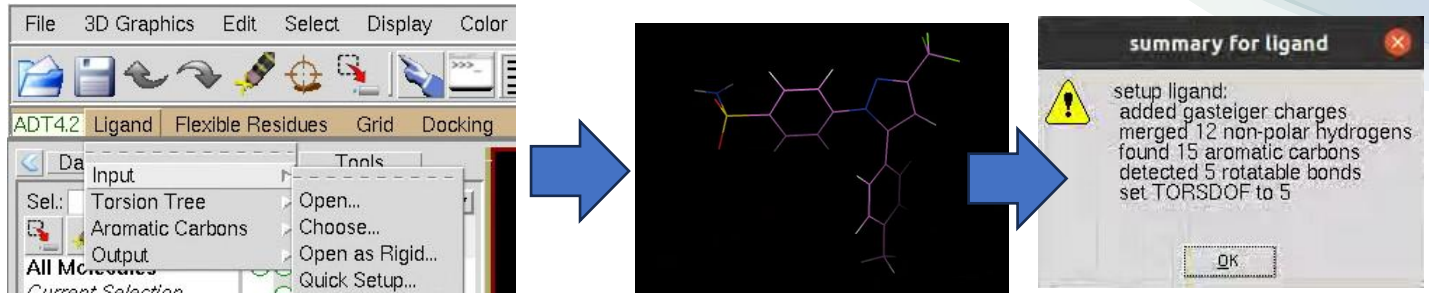
# Molecular Docking Tutorial

## Ligand preparation (GUI of AutoDock Tools)

### 1. Import the Celecoxib (CEL.pdb)

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

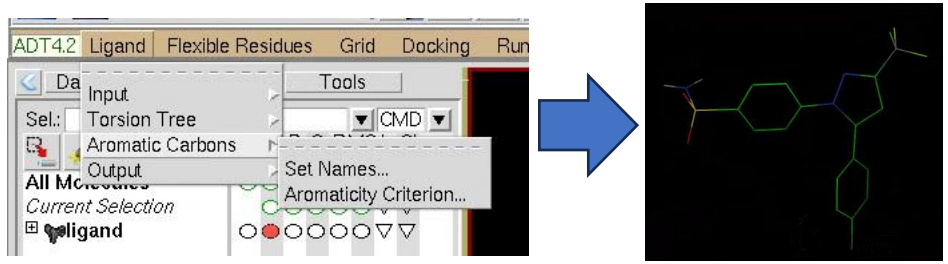
*ligand will be prepared for docking. After clicking the "OK" button, you will see that all the hydrogens atoms are merged to carbon atoms.*




### 2. Check for aromatic carbons

Ligand>Aromatic carbons>Set Names

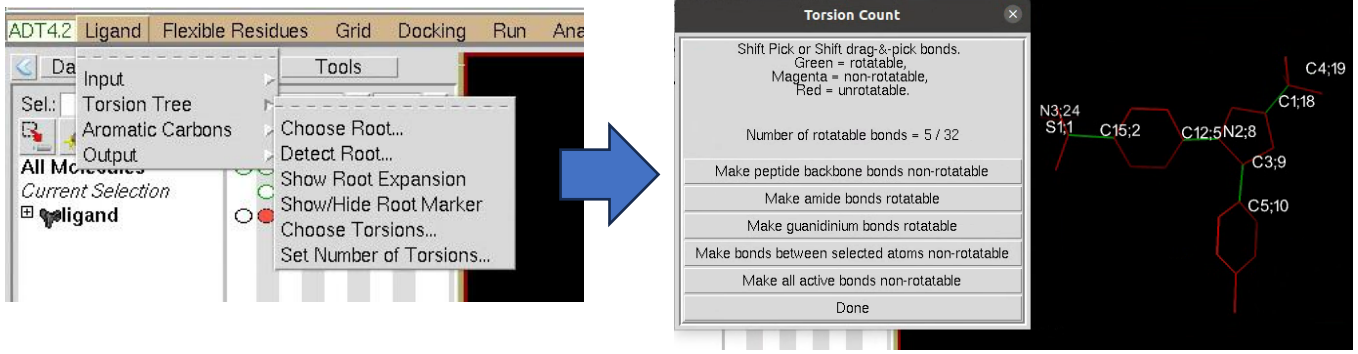
*Aromatic atoms are shown in green*




### 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

## 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**

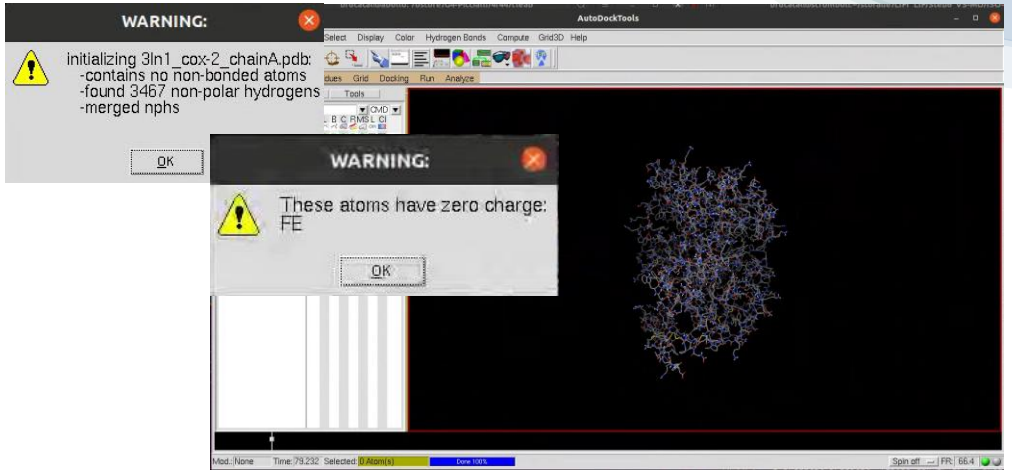
# Molecular Docking Tutorial

## Receptor preparation (GUI of AutoDock Tools)

1. Select protein (only ChainA)

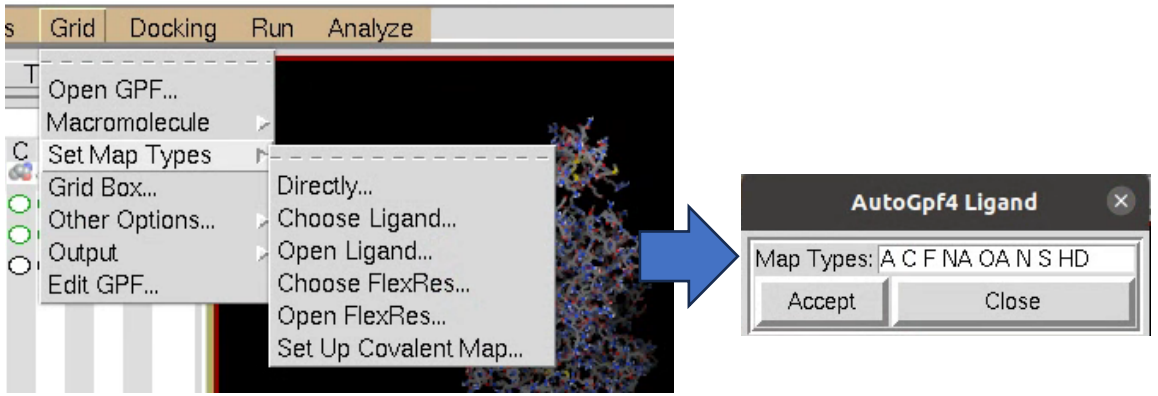
Grid>Macromolecule>Open>cox2.pdb

*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

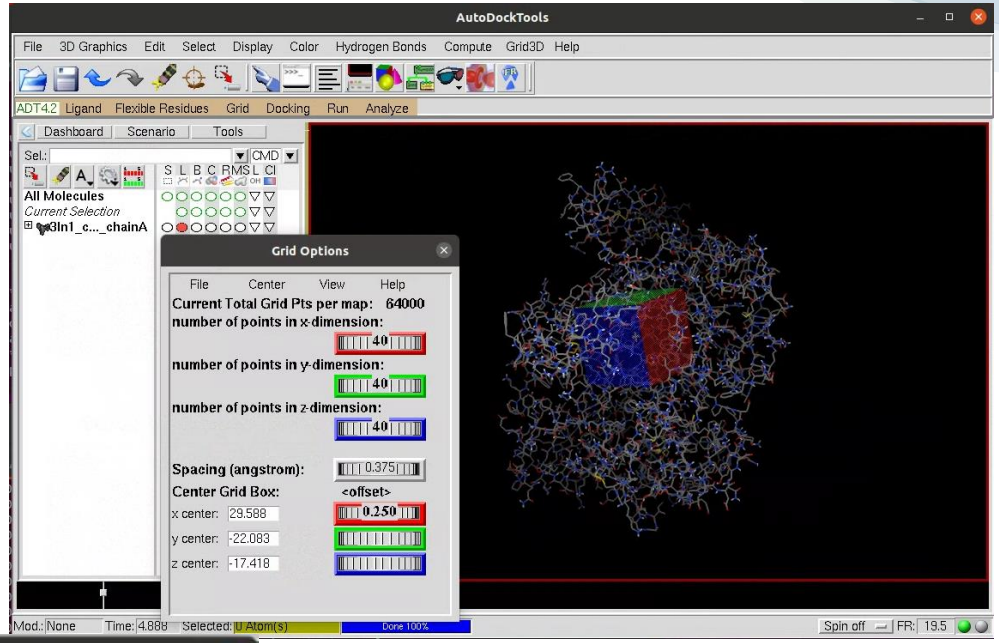
*- A cube with a default size of 40x40x40 will appear.*

*- 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,*

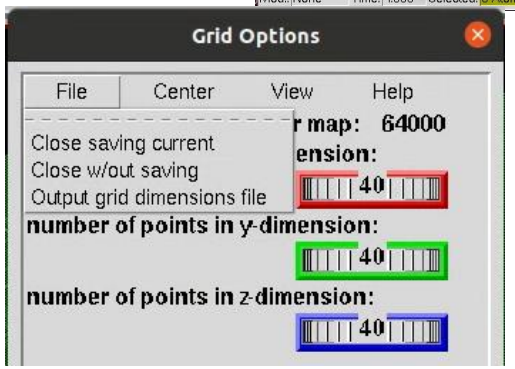
*y center: -22.083,*

*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)

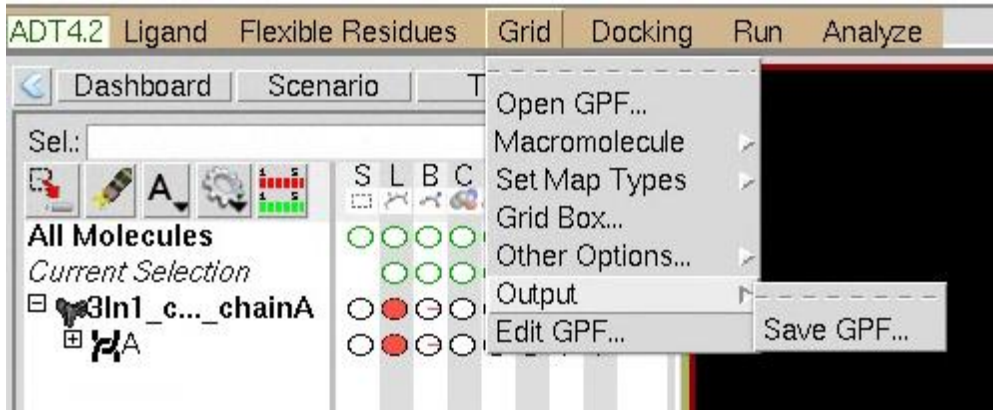
```
HETATM 4482 FE   HEM A 500       5.890  24.568  -1.058  1.00 18.80     2.000 Fe
```

### 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
```

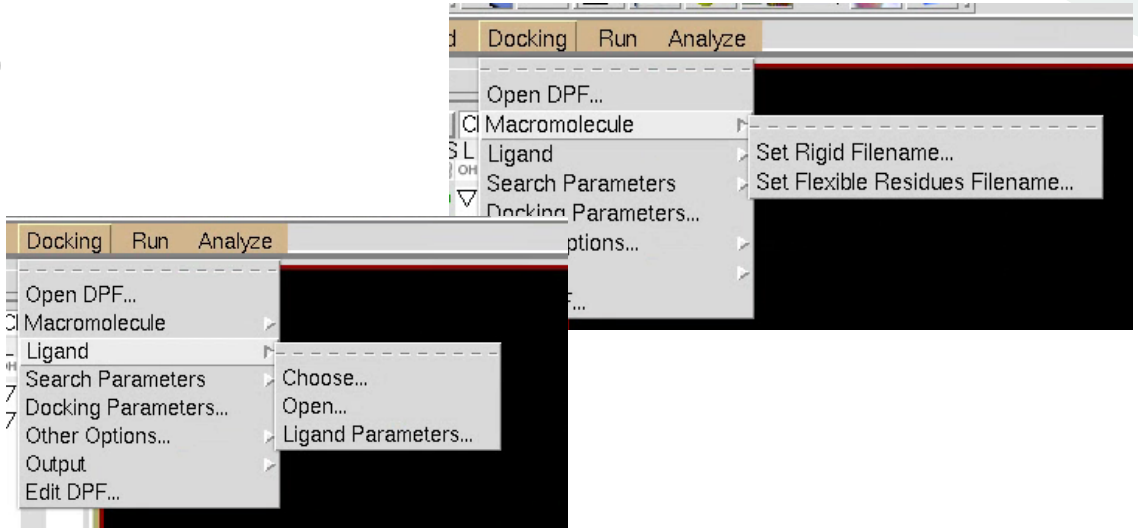
Repeat the same procedure for COX1…

# Molecular Docking Tutorial

## Preparing the Docking Parameter File (DPF)

### 1. From the ADT GUI import receptor (PDBQT)

Docking>Macromolecule>Set Rigid Filename>cox2.pdbqt

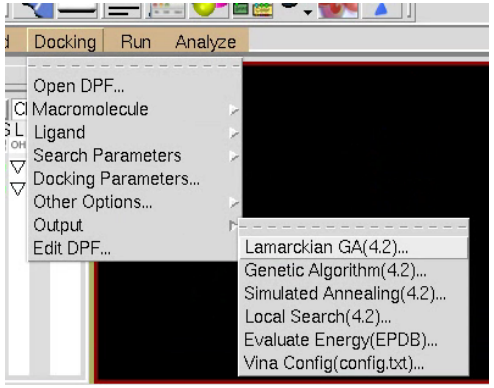



### 2. From the ADT GUI import ligand (PDBQT)

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
```

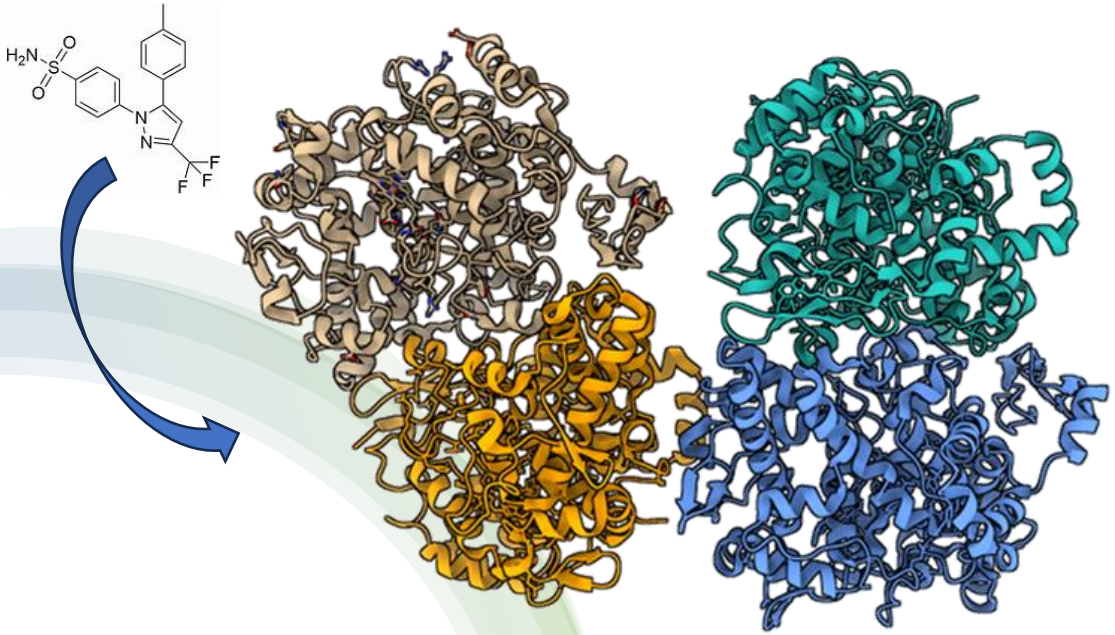
Repeat the same procedure for COX1…

# Molecular Docking Tutorial

## RUN AutoDock4

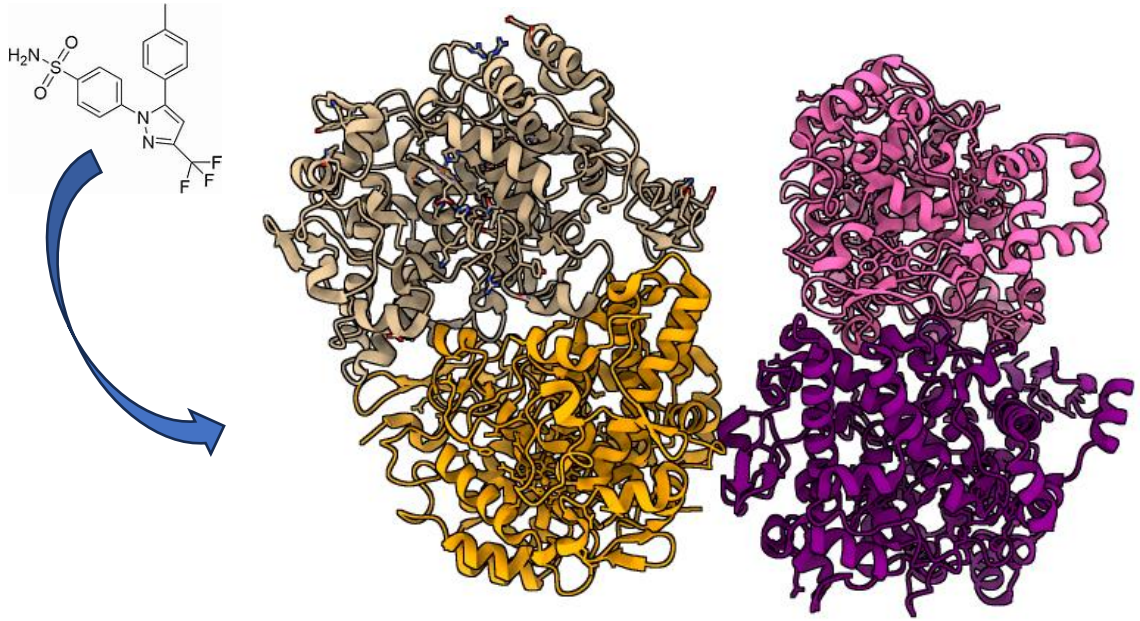
### CEL vs COX2

```
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*

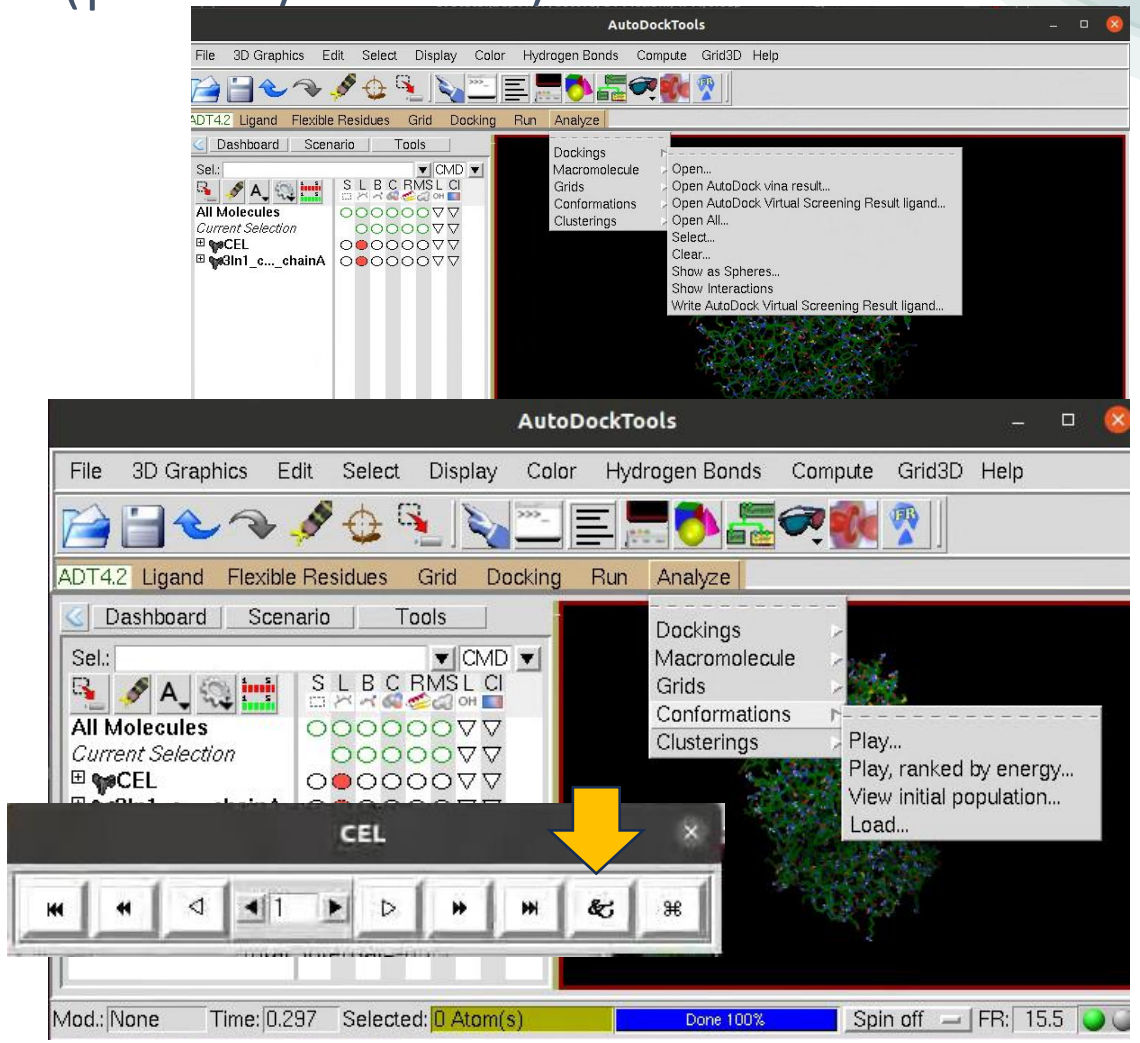
3. Visualize docking conformations

Analyze>Conformations>Play Ranked by Energy

4. Write all the conformations in pdbqt file

Click on the indicated icon>Write all

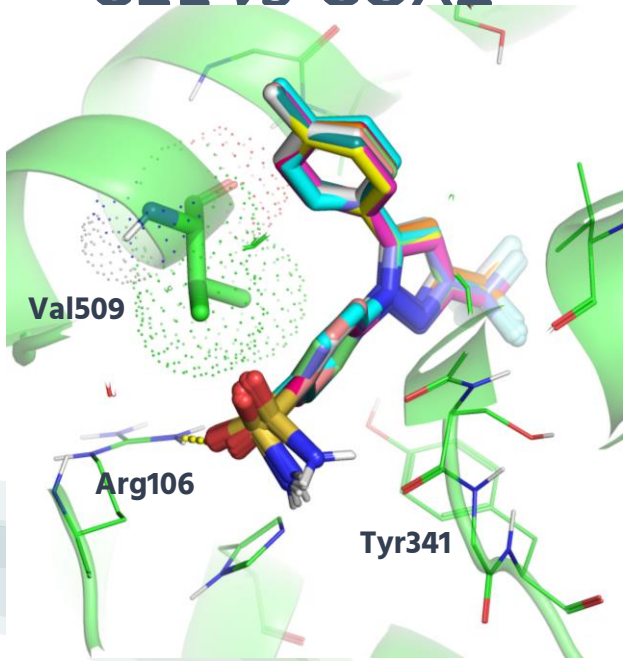
*Docking conformations will be write ranked by energy.*
*«Conf0.pdbqt» has the best energy value*



# Molecular Docking Tutorial

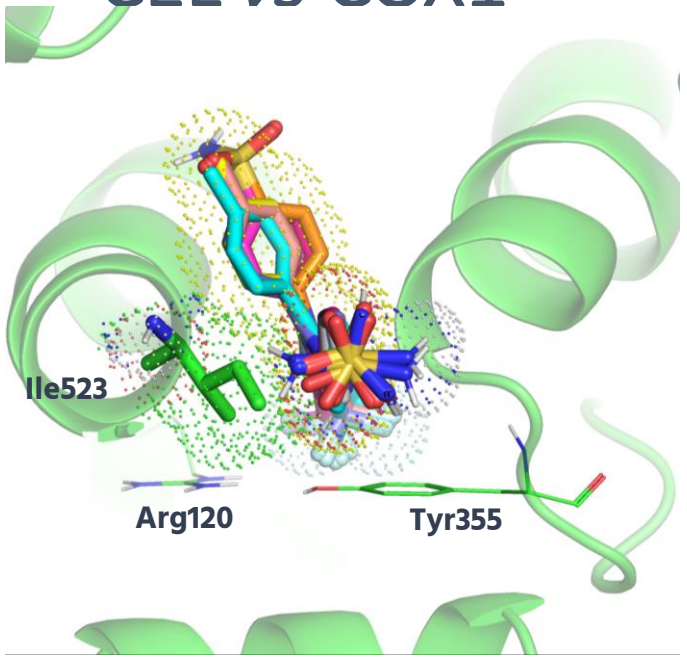
## Docking analysis with PyMOL

### CEL *vs* COX2



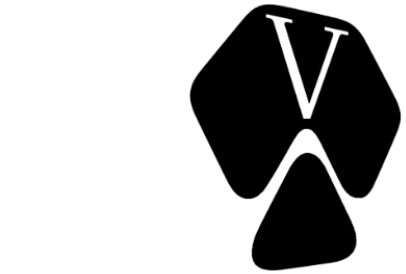

All the poses converge to one unique binding mode with a binding affinity score of -10.41 kcal/mol. The sulfonamide group directly interact with hydrogen-bonds with **Arg106**

### 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**

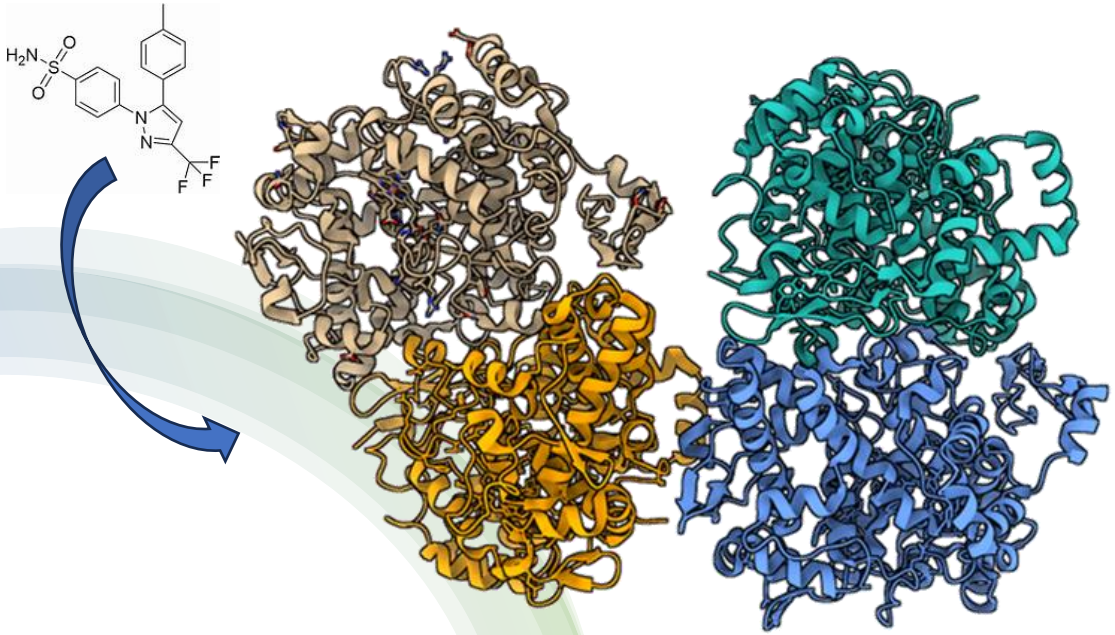
# Molecular Docking Tutorial

AutoDock Vina

## RUN AutoDock Vina

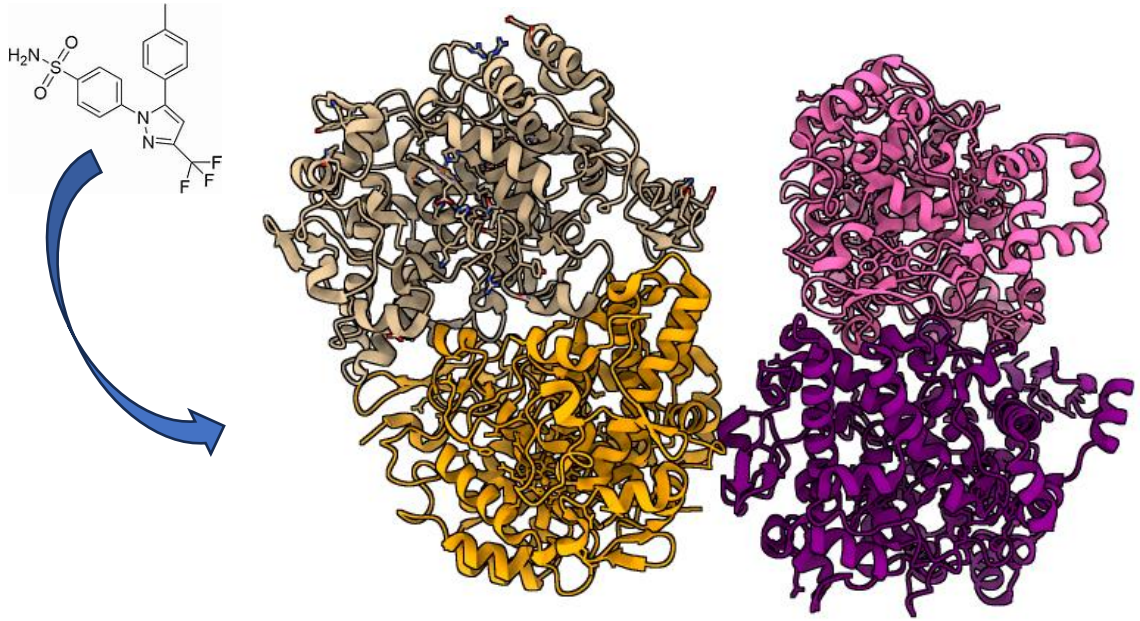
### CEL vs COX2

```
vina –-config config.txt
```



### CEL vs COX1

```
vina –-config config.txt
```



This will take around 1-2 minutes…

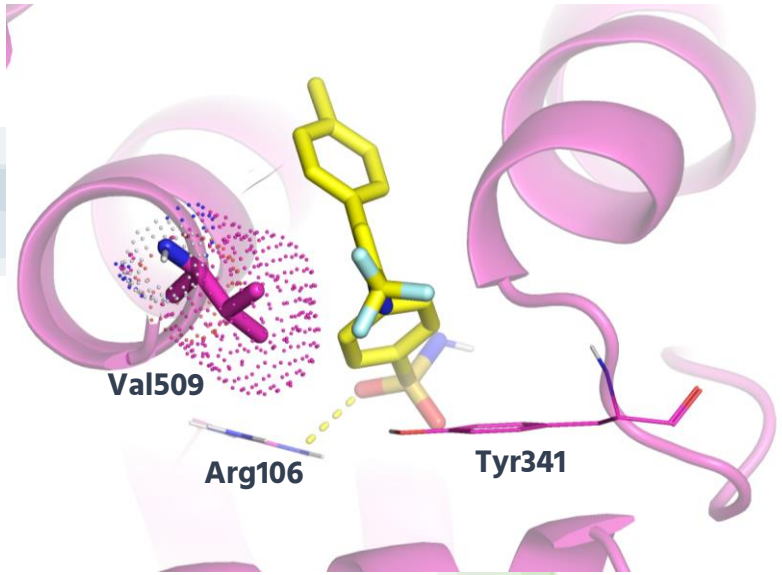
AutoDock Vina

# Molecular Docking Tutorial

## 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