Molecular Operating Environment
One fully integrated drug discovery software package

Generously supported by Chemical Computing Group, Montreal, QC, Canada, who provided teaching licences for MOE (Molecular Operating Environment) software package.

Seminar 3

Preparation of a receptor – repetition, docking

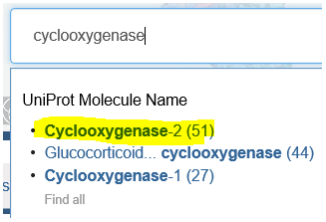
1. **Get the 3D structure of the receptor from the PDB database –** more options:

Search for a structure directly at www.rcsb.org , save it to disk, load into MOE	
Search RCSB PDB database via the **MOE | Open | RCSB PDB** – ideal if you already know the pdb ID.	
Use the internal database of 3D structures contained in the MOE via the **MOE | Open | PDB** -this database contains data of selected structures from the PDB, which have already been partially prepared (some bugs fixed, hydrogens added, excess subunits removed, unimportant water molecules removed, etc.)	

Task 1

Search for cyclooxygenase-2 structure in the PDB database

In the PDB database (www.rcsb.org) locate a suitable structure of cyclooxygenase- 2. Look for the term "cyclooxygenase-2" and filter according to the following criteria: < 2 Å resolution, and presence of naproxen as a ligand.



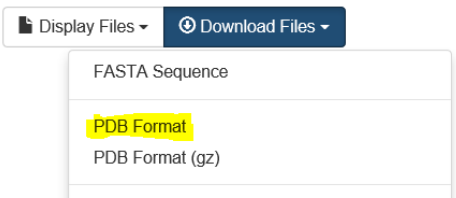
With respect to the chosen PDB structure, complete the following table:

PDB ID	
Source organism	
Year of publishing	
X-ray resolution	
R-Value Work	
R-Value Free	
Ramachandran Outliers	
The number of subunits (strings)	
The number of amino acids of a single string	

Which of the parameters listed above affect the quality of the 3D structure? What are the recommended ranges of values for these protein parameters to be used in molecular modelling?

Which amino acid residues does the ligand of naproxen interact with? Please indicate the specific nature of these interactions.

Download structure in pdb format and save it as PDB_ID.pdb (for PDB_ID supplement specific identifier of the structure – an alphanumeric code with four positions).



Task 2

Preparation of the receptor for docking

1. Open the pdb file that you saved in the previous task.
2. Evaluate the system (**System Manager** or **SEQ**) and suggest the parts of the system, which could/should be removed before starting the corrections/adjustments. Consult with the teacher. In the case of our receptor, it is a homodimer, where the catalytic site is located within each subunit. One subunit can therefore be safely removed and we will continue working with only one subunit. Unimportant water molecules we will deleted later after the 3D optimization of hydrogen bonds. (Remove all strings B, and residues marked as NAG, BOG, CL – these are crystallization artefacts unimportant for docking).
3. Correcting errors using the **MOE | Protein | Structure Preparation.** Evaluation of errors that remain after an automatic correction (type of error, if the position of catalytic place will influence results of docking). *Note*: in the last seminar we used Wizard until you reach the individual types of errors were correcting individual errors or the individual types of errors (**Correct Similar**). In the Structure we have selected choose a particular Preparation error and click on **Correct**, the fix all errors that can be automatically repaired. Advantage: Speed. Disadvantage: I can't affect what method corrects the error (e.g. whether the N and C the end of cappingem will be corrected, or by adding the hub).
4. Adjust structure by **MOE | Compute | Protonate3D**. Application Protonate3D adds hydrogen atoms, the protonation and tautomerism States optimizes amino acid residues and ligands, optimizes the network of hydrogen bridges. Also, the application calculates the partial charge according to the current strength of the field.
5. Selection of ligand – MOE now considered everything except the receptor ligand and solvents (cofactor). For convenience, you should define the ligand. Selection of ligand, followed by command **of the RHS | Ligand | Choose Ligand.** Other "ligands" have become part of the receptor.
6. Try to view into an active space- **the RHS | Siteview**.
7. Save the resulting prepared receptor under the name PDB_ID_prepared.moe.

Task 3

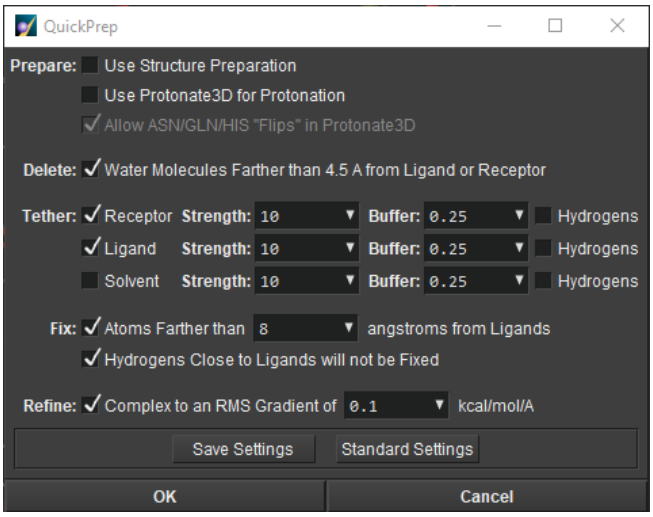
Restrained energy minimization of the ligand-receptor complex

In the process of restrained energy minimization the atoms can be treated as:

a. **free** - can freely move while being minimized
b. **anchored (= tethered) -** limited mobility
c. **fixed –** without the possibility of movement

Energy minimization can be setup and started in **MOE | Compute | Energy | Minimize.** For beginners, however, is easier to use the interface of **RHS | QuickPrep**.

1. Clean you working area.
2. Open a previously saved receptor PDB_ID_prepared.moe.
3. View the active site (**RHS | SiteView**).
4. Perform **RHS | QuickPrep** according to the following parameters (see figure to the right).
5. Save the resulting ligand-receptor complex as PDB_ID_minim.moe.
6. Observe the ligand-receptor interactions. Generate a 2D diagram showing these



interactions (**RHS | Ligand | Ligand Interactions**). Export as an image.

Note: **The RHS | QuickPrep** can be used for quick fix errors in protein and limited energy minimization. It is therefore a process that, by default, automatically prepares a protein to dock. In our case we **Structure Preparation** and **Protonate3D** made from the **MOE | Protein | Structure**, therefore the Preparation of these elections are not in the **QuickPrep** circled in.

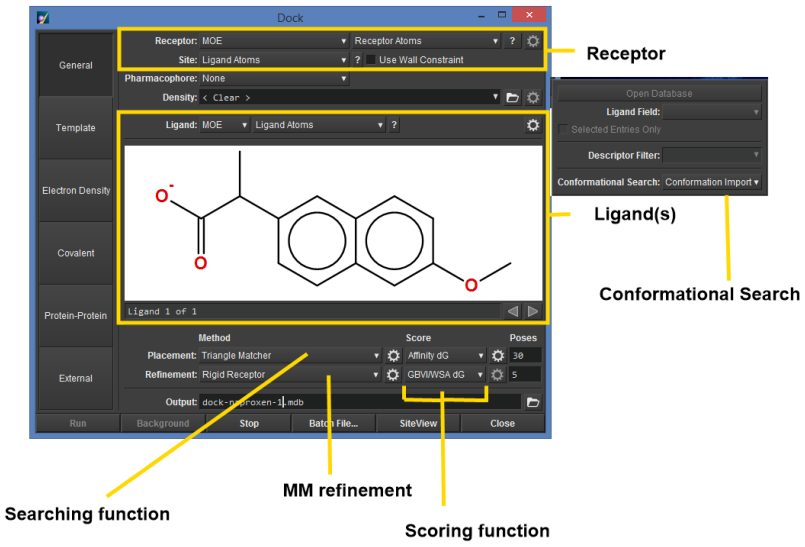
Task 4

Re-docking the original ligand

Redocking the original ligand is used to verify that the settings for the search and scoring function are appropriate for the type of ligand and receptor. Ideally, the docking process should be able to faithfully reproduce the pose of a ligand of the Crystallographic structure. This match is represented as in Å RMSD.

Tip: To monitor the progress docking see active site- **MOE | The RHS | SiteView**

1. Use the settings shown in the following figure. Save the output result of the docking naproxen_redocked_rigid. mdb database. How is the RMSD poses with the best match with the crystallography ligand?



Task 5

Docking of other NSAIDs--rigid docking

Create a new database and place the structure of NSAIDs ibuprofen and diclofenac. Properly prepare ligands in the database for docking. Now dock prepared the database. The entry is not one molecule, but the whole database of ligands. Save the output result of the docking NSAID_docked_rigid .mdb database

Docking settings:

Placement: Triangle Matcher, London dG, 30 poses

Refinement: Rigid Receptor, GBVI/WSA dG, 5 poses

Browse (**Browse**) the resulting database and track interactions with the receptor. Watch the score of individual ligands.

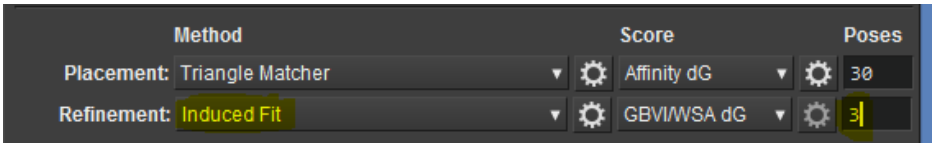
Observe the interactions of the docked ligands with the receptor and compare them with the original ligand (naproxen).

Task 6

Induced-fit docking

Induced-fit docking is docking with the flexibility of the protein (usually only the amino acid residues of the active space of the side). It is a *de facto* process simulation, in which the enzyme and ligand are adapting to each other.

1. Dock selected ligand using induced-fit docking. Settings see figure



Tip: Add input to the database from the previous use of ligands for the task. You can perform the dock only to ligands selected in the database.

2. Since the induced-fit docking does not change only the ligand, but also part of the receptor, results are stored in the output database conformation as ligand and receptor. Watch receptor spatial changes that occurred when the induced-fit docking.