High-throughput MD tutorial

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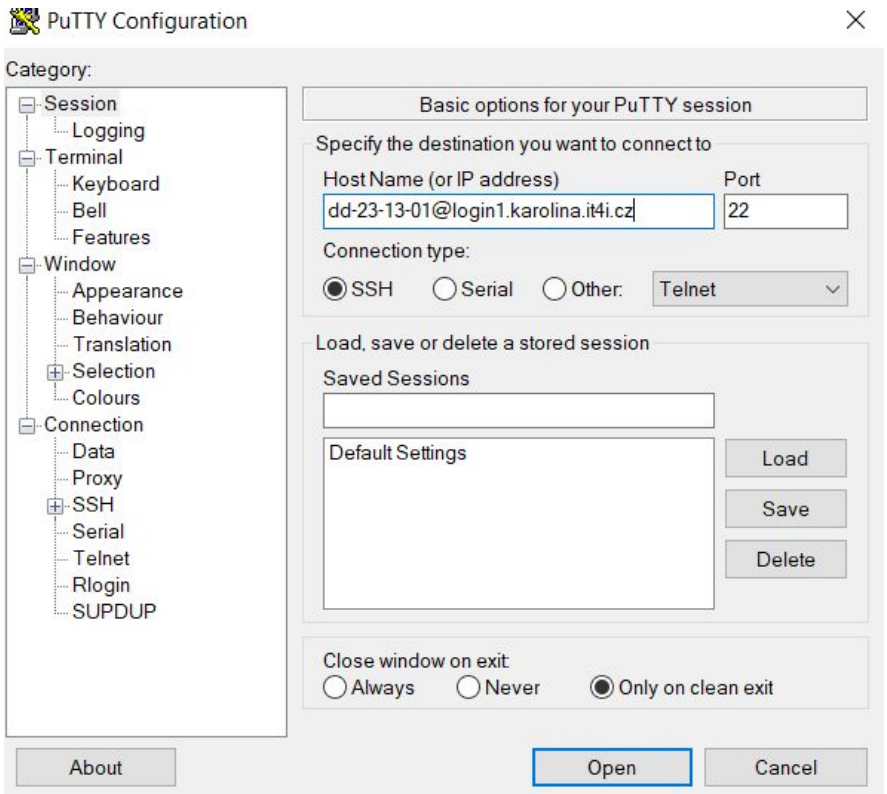
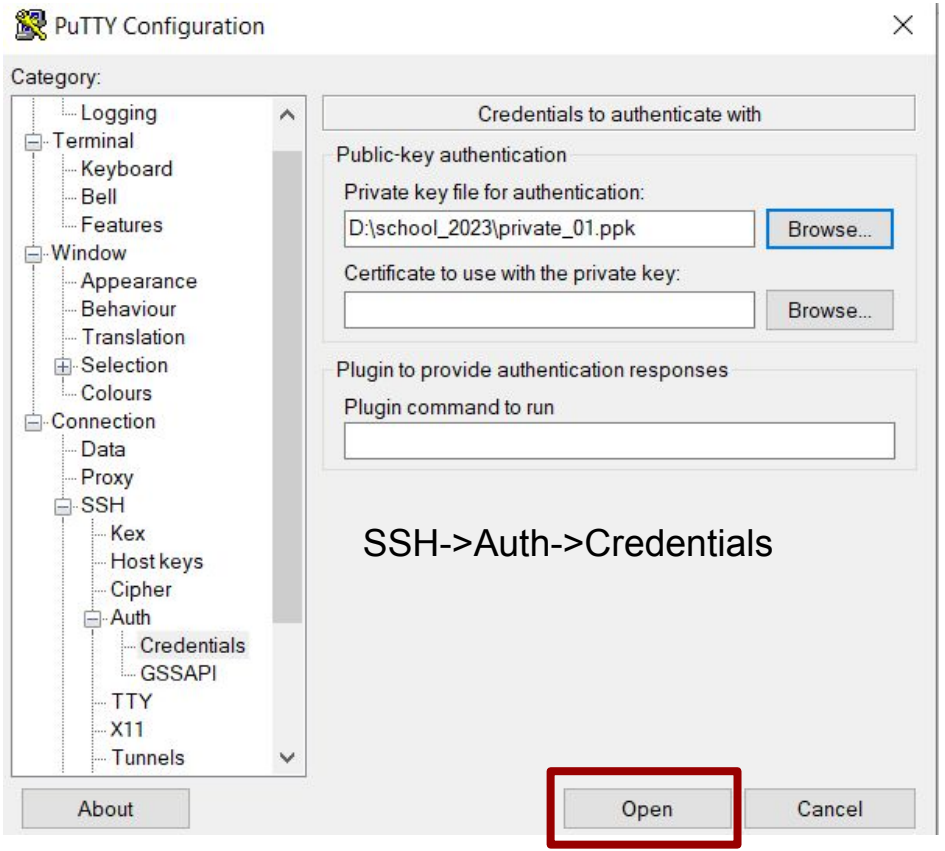
PuTTY


PuTTY Configuration
Category:
Session
Logging
Terminal
Keyboard
Bell
Features
Window
Appearance
Behaviour
Translation
Selection
Colours
Connection
Data
Proxy
SSH
Serial
Telnet
Rlogin
SUPDUP
Basic options for your PuTTY session
Specify the destination you want to connect to
Host Name (or IP address)
Port
dd-23-13-01@login1.karolina.it4i.cz
22
Connection type:
SSH
Serial
Other:
Telnet
Load, save or delete a stored session
Saved Sessions
Default Settings
Load
Save
Delete
Close window on exit:
Always
Never
Only on clean exit
About
Open
Cancel

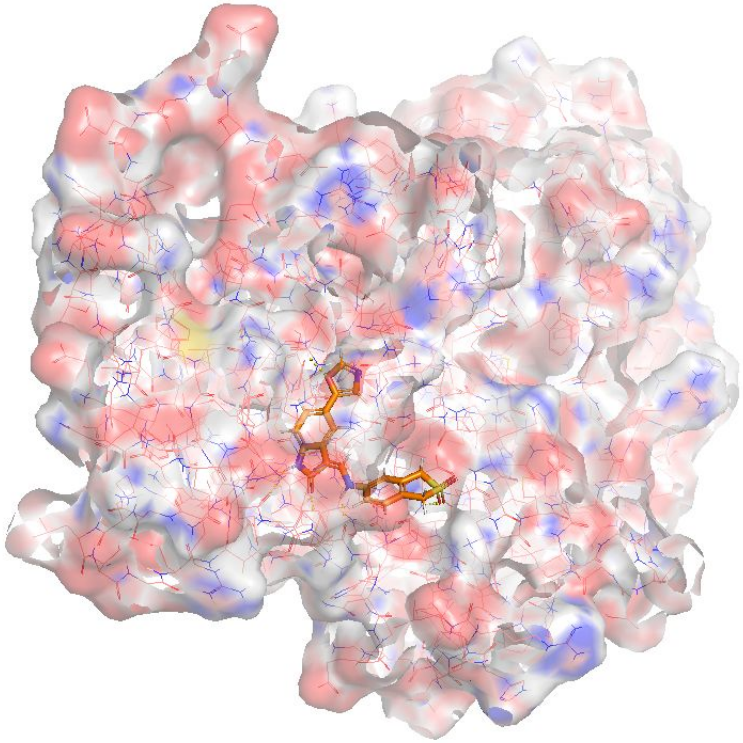

PuTTY Configuration
Category:
Logging
Terminal
Keyboard
Bell
Features
Window
Appearance
Behaviour
Translation
Selection
Colours
Connection
Data
Proxy
SSH
Kex
Host keys
Cipher
Auth
Credentials
GSSAPI
TTY
X11
Tunnels
Credentials to authenticate with
Public-key authentication
Private key file for authentication:
D:\school_2023\private_01.ppk
Browse...
Certificate to use with the private key:
Browse...
Plugin to provide authentication responses
Plugin command to run
SSH->Auth->Credentials
About
Open
Cancel

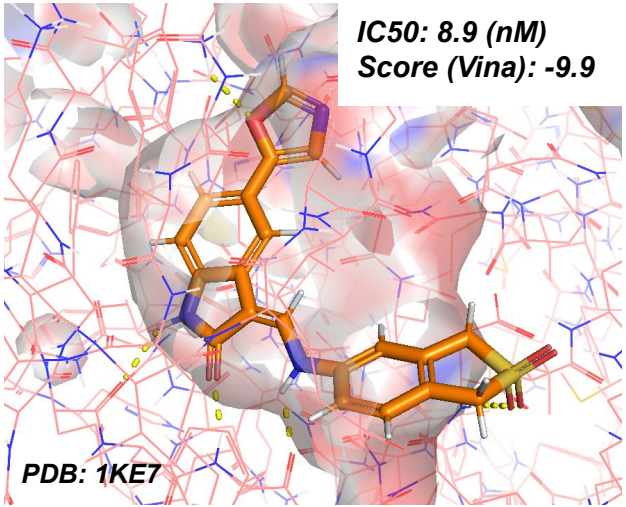
~$ cp ./dd-23-13-01 ~/.ssh/

~$ chmod 600 ~/.ssh/dd-23-13-01

~$ ssh -i ~/.ssh/dd-23-13-01 dd-23-13-01@login1.karolina.it4i.cz

Molecular Docking





Pose – a possible relative orientation of a ligand and a receptor as well as conformation of a ligand and a receptor when they are form complex

Score – the strength of binding of the ligand and the receptor.

Automated scripts for Molecular Docking

https://github.com/ci-lab-cz/docking-scripts

Installation

```
pip install moldock
```

or the latest version

```
pip install git+https://github.com/ci-lab-cz/docking-scripts.git
```

Dependencies

from conda

```
conda install -c conda-forge python=3.9 numpy=1.20 rdkit scipy dask distributed vina
```

from pypi

```
pip install meeko
```

Installation of gnina is described at https://github.com/gnina/gnina

Automated scripts for Molecular Docking

https://github.com/ci-lab-cz/docking-scripts

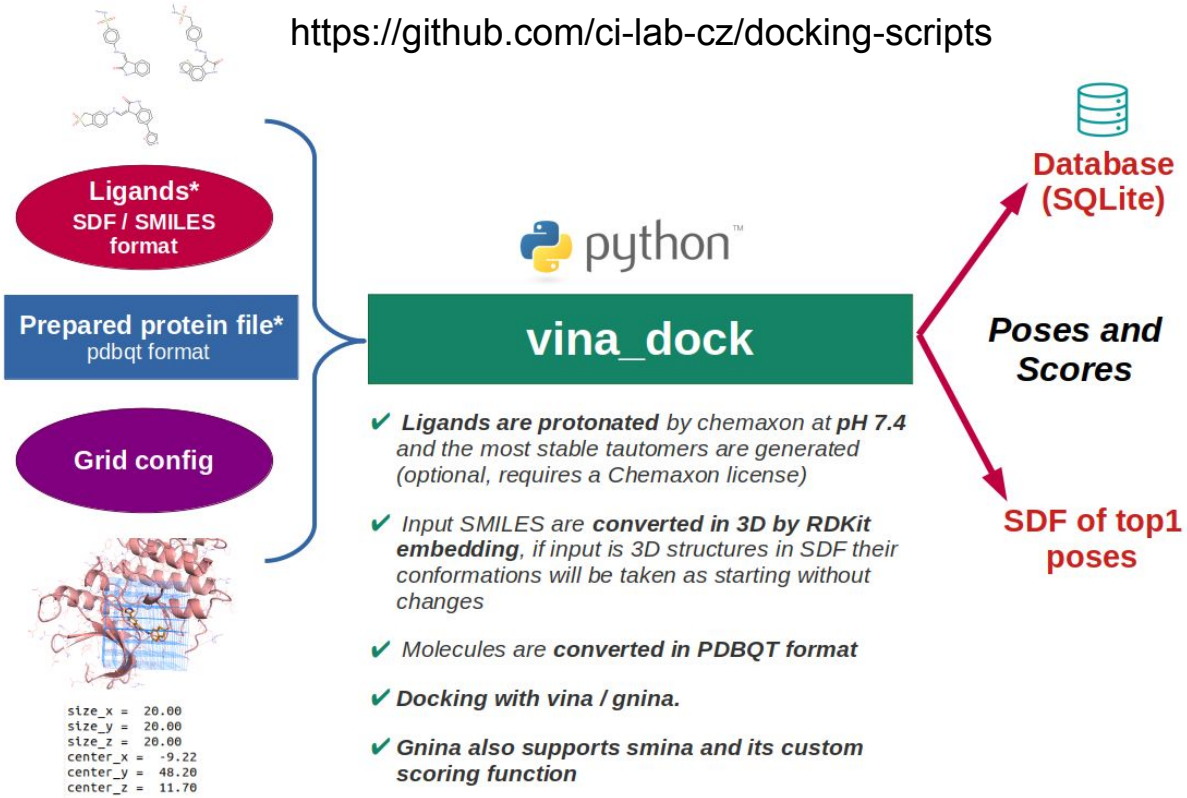


Table: mols

New Record | Delete Record

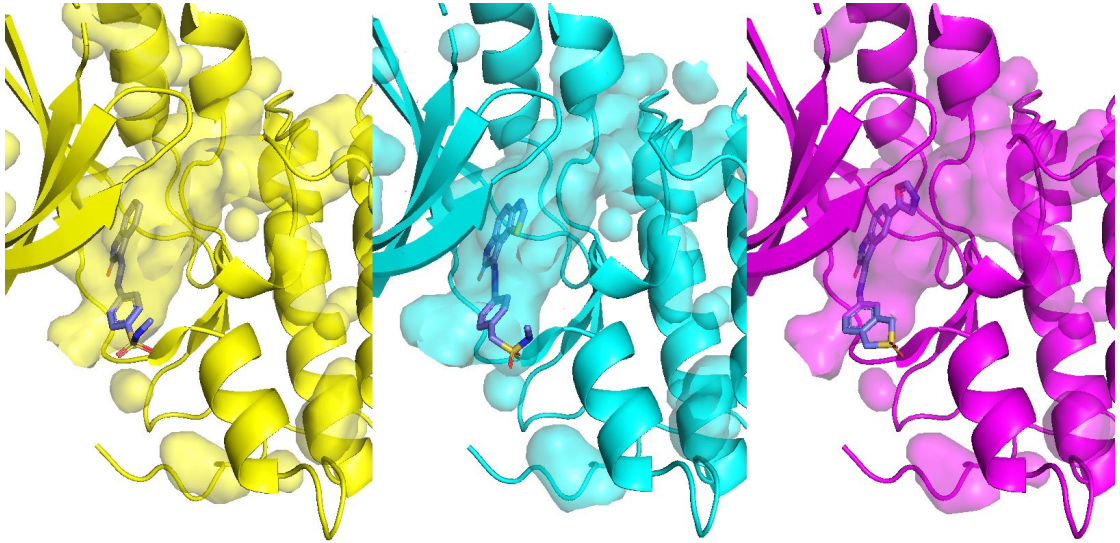
	id	smi	smi_protonated	source_mol_block	source_mol_block_protonated	docking_score	pdb_block	mol_block	time
	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter
1	1BJU_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc(NC(=O)...	1BJU_ligand...	1BJU_ligand...	-6.863	MODEL 1...	1BJU_ligand...	2023-01-23 ...
2	1BJV_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc(NC(=O)...	1BJV_ligand...	1BJV_ligand...	-7.524	MODEL 1...	1BJV_ligand...	2023-01-23 ...
3	1C5Q_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cc2c(I)cccc2...	1C5Q_ligand...	1C5Q_ligand...	-6.111	MODEL 1...	1C5Q_ligan...	2023-01-23 ...
4	1C5S_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cc2ccccc2s1	1C5S_ligand...	1C5S_ligand...	-5.889	MODEL 1...	1C5S_ligan...	2023-01-23 ...
5	1C5T_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cc2cccnc2s1	1C5T_ligand...	1C5T_ligand...	-6.156	MODEL 1...	1C5T_ligand...	2023-01-23 ...
6	1F0T_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc(O)c(CN2...	1F0T_ligand...	1F0T_ligand...	-7.81	MODEL 1...	1F0T_ligand...	2023-01-23 ...
7	1F0U_ligand	COC(=O)C(Cc1cc...	COC(=O)[C@H]...	1F0U_ligand...	1F0U_ligand...	-7.134	MODEL 1...	1F0U_ligand...	2023-01-23 ...
8	1G36_ligand	Cc1nc2ccccc2n1C...	Cc1nc2ccccc2n1Cc1ccc2c(c...	1G36_ligand...	1G36_ligand...	-8.78	MODEL 1...	1G36_ligan...	2023-01-23 ...
9	1GHZ_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc2[nH]c(-...	1GHZ_ligand...	1GHZ_ligand...	-8.197	MODEL 1...	1GHZ_ligan...	2023-01-23 ...
10	1GI1_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc2[nH]c(-...	1GI1_ligand...	1GI1_ligand...	-7.932	MODEL 1...	1GI1_ligand...	2023-01-23 ...
11	1GI6_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1ccc2[nH]c(-...	1GI6_ligand...	1GI6_ligand...	-8.263	MODEL 1...	1GI6_ligand...	2023-01-23 ...
12	1GJ6_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cc2cc(-...	1GJ6_ligand...	1GJ6_ligand...	-8.468	MODEL 1...	1GJ6_ligand...	2023-01-23 ...
13	1K1I_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cccc(C[C@H...	1K1I_ligand...	1K1I_ligand...	-8.946	MODEL 1...	1K1I_ligand...	2023-01-23 ...
14	1K1J_ligand	COC(=O)C1CCN(C...	COC(=O)C1CCN(C(=O)[C@...	1K1J_ligand...	1K1J_ligand...	-7.579	MODEL 1...	1K1J_ligand...	2023-01-23 ...
15	1K1L_ligand	NC(=[NH2+])c1cc...	NC(=[NH2+])c1cccc(C[C@H...	1K1L_ligand...	1K1L_ligand...	-6.695	MODEL 1...	1K1L_ligand...	2023-01-23 ...

Table: setup

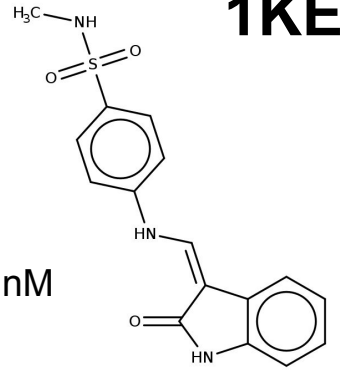
	protonation	protonation_done	protein_pdbqt	protein_setup
	Filter	Filter	Filter	Filter
1	1	1	ATOM 1 ...	size_x = ...

Output SQLite DB

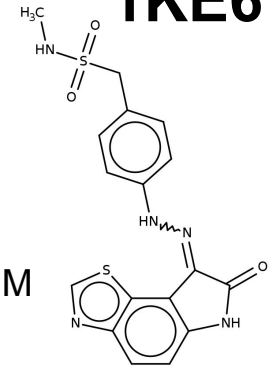
CYCLIN-DEPENDENT KINASE 2 (CDK2)



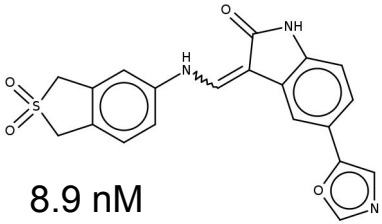
1KE5 **1KE6** **1KE7**



560 nM



5.7 nM



8.9 nM

Automated scripts for Molecular Docking

ssh -i ~/.ssh/dd-23-13-01 dd-23-13-01@login1.karolina.it4i.cz

mkdir docking_tutorial
cd docking_tutorial
cp
/mnt/proj2/dd-23-13/docking_tutorial/{ligands_pH74.smi,protein_prepared.pdbqt,config.txt} .

qsub -A DD-23-13 -v
input=$(pwd)/ligands.smi,protein=$(pwd)/protein_prepared.pdbqt,config=$(pwd)/config.txt
/mnt/proj2/dd-23-13/script_vina.pbs

Prepare docked molecules for MD

```
module load Anaconda3
source activate gmxMMPBSA

python /mnt/proj2/dd-23-13/md-scripts/scripts/sdf2mols.py -i
docking_tutorial/ligands_pH74_protein_prepared_docking_vina_output.sdf -o
docking_tutorial/mols
```

High-throughput MD

Run MD simulation by one command:

ssh -i ~/.ssh/dd-23-13-01 dd-23-13-01@login1.karolina.it4i.cz

```
mkdir md_tutorial
cd md_tutorial
cp /mnt/proj2/dd-23-13/md_tutorial/{protein_prepared.pdb,ligand.mol} .
```

```
qsub -A DD-23-13 -v
lfile=$(pwd)/ligand.mol,pfile=$(pwd)/protein_prepared.pdb,script_path=/mnt/proj2/dd-23-13/md-scripts/scripts
/,wdir=ligand_01ns,mdtime=0.1,gromacs_version='GROMACS/2021.4-foss-2020b-PLUMED-2.7.3'
/mnt/proj2/dd-23-13/md-scripts/Protein-Ligand_MD_PBGBSA/01_complex_preparation_md.pbs
```

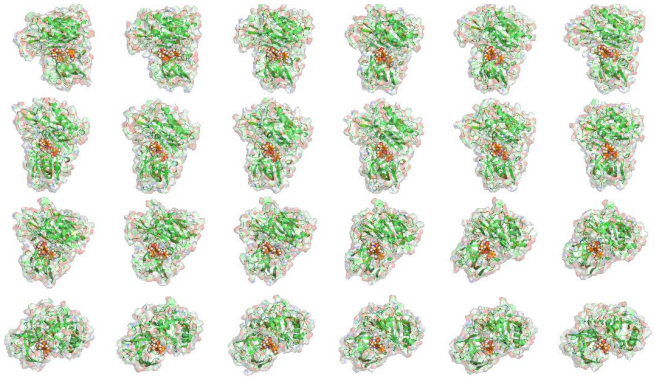
in case of multiple ligands:

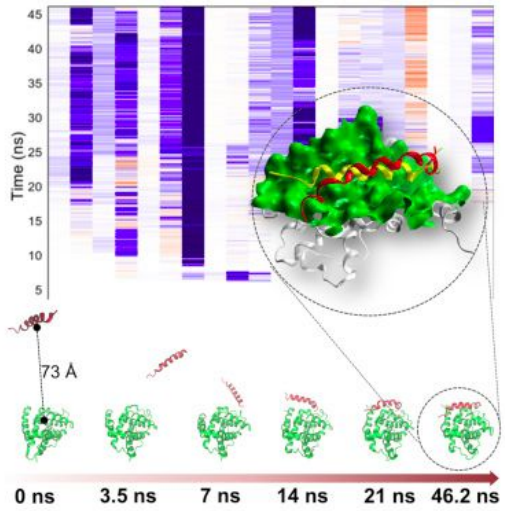
```
 for i in mols/*.mol;do fname=${i##*/}; name=${fname%.*}; echo $i; qsub -A DD-23-13 -v
lfile=$(pwd)/$i,pfile=$(pwd)/protein_prepared.pdb,script_path=/mnt/proj2/dd-23-13/md-scripts/scripts/,wdir=$(
pwd)/$name,mdtime=0.1,gromacs_version='GROMACS/2021.4-foss-2020b-PLUMED-2.7.3'
/mnt/proj2/dd-23-13/md-scripts/Protein-Ligand_MD_PBGBSA/01_complex_preparation_md.pbs;done
```

Molecular dynamics

1. MD simulations **mimic the physical motions of atoms present in the actual environment;**
2. The atoms and molecules are allowed to interact for a fixed period of time, giving **a view of the dynamic "evolution" of the system.**

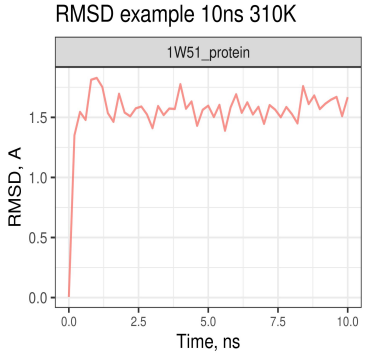
- to explore conformational space
- to explore biological process of molecular recognition
- to investigate ligand pose stability
- to estimate binding affinity of protein-ligand complexes

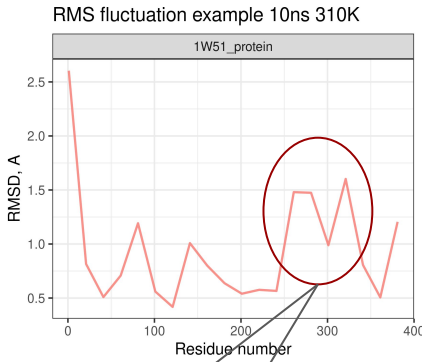




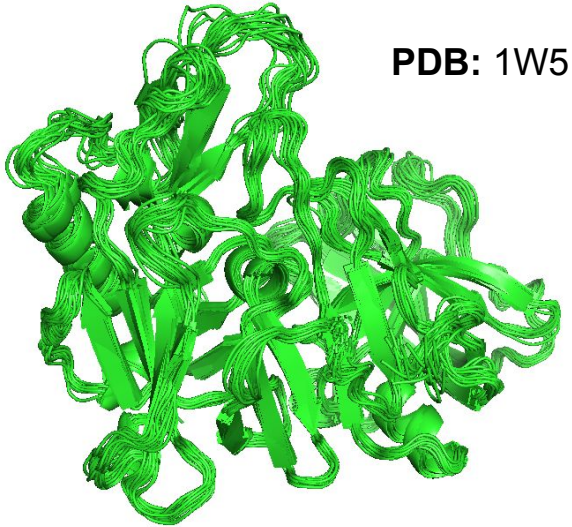
https://doi.org/10.1016/j.str.2017.02.009

What can be done by MD

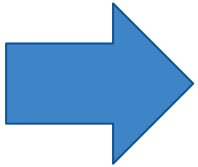




- To explore different conformation of protein
  - To investigate internal-flexibility of protein
  - For practical use we select conformations from the most populated clusters of all conformations

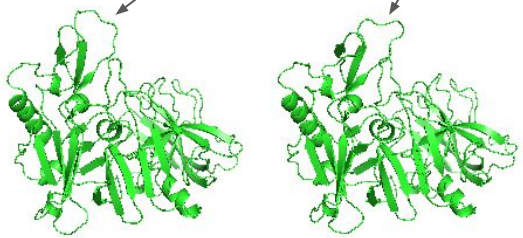


PDB: 1W51



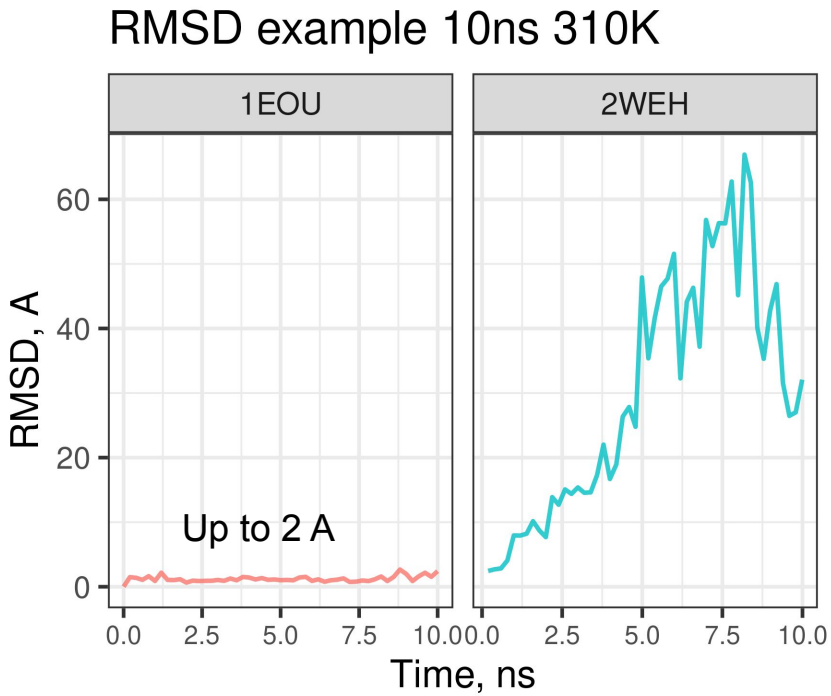
Conformation can be used:

- for further molecular docking study

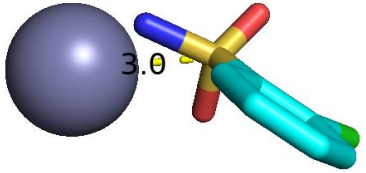


What can be done by MD

- To explore stability of ligand pose

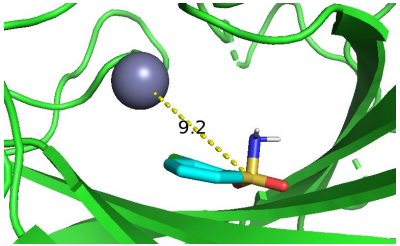


Example of incorrect protonation and pose:

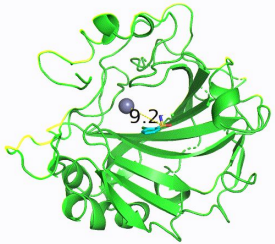


x-ray

PDB: 2WEH



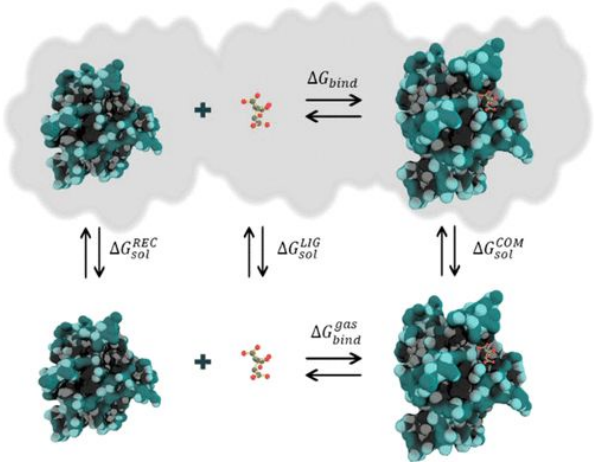
docking



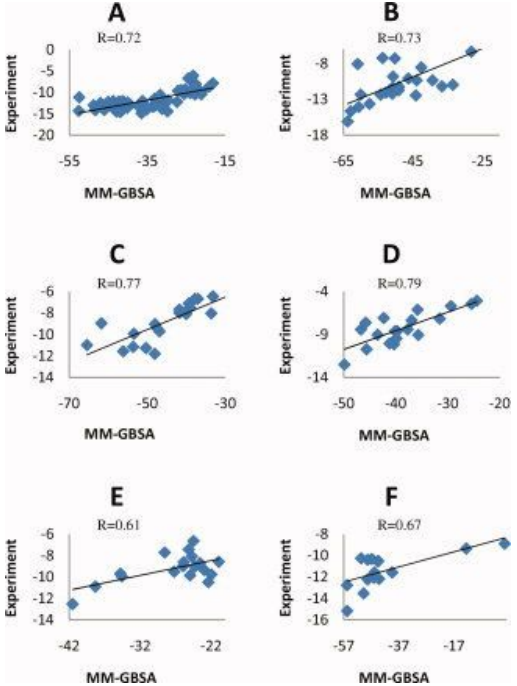
MD 10ns.
Ligand goes out of site

What can be done by MD

- to estimate binding affinity of protein-ligand complexes



*Correlation between **MM-GBSA** predicted and experimental binding free energy.*



Yang T, Wu JC, Yan C, Wang Y, Luo R, Gonzales MB, Dalby KN, Ren P. Virtual screening using molecular simulations. Proteins. 2011 Jun;79(6):1940-51. doi: 10.1002/prot.23018. Epub 2011 Apr 12. PMID: 21491494; PMCID: PMC3092865.

Valdés-Tresanco, M.S., Valdés-Tresanco, M.E., Valiente, P.A. and Moreno E. ***gmx_MMPBSA: A New Tool to Perform End-State Free Energy Calculations with GROMACS.*** Journal of Chemical Theory and Computation, 2021 17 (10), 6281-6291. https://pubs.acs.org/doi/10.1021/acs.jctc.1c00645.

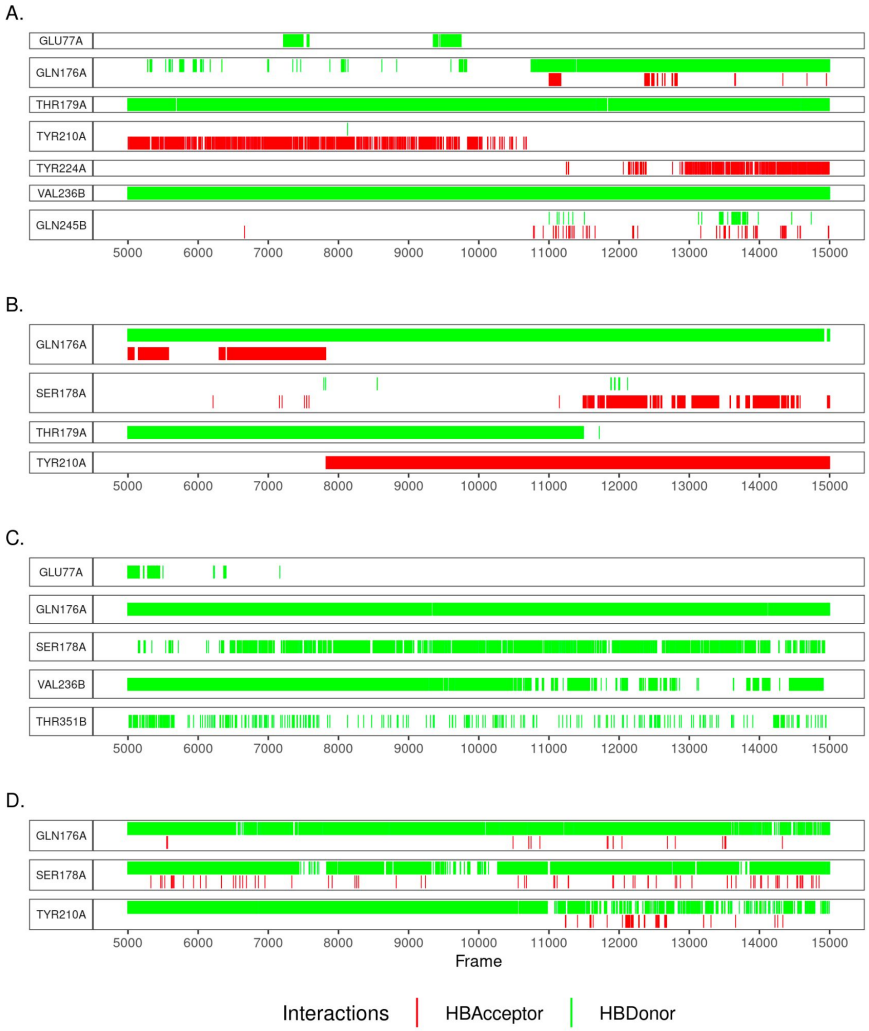
***MMPBSA.py*: An Efficient Program for End-State Free Energy Calculations** Bill R. Miller III, T. Dwight McGee Jr., Jason M. Swails, Nadine Homeyer, Holger Gohlke, and Adrian E. Roitberg Journal of Chemical Theory and Computation **2012** *8* (9), 3314-3321 DOI: 10.1021/ct300418h

What can be done by MD

- to investigate protein-ligand interaction stability

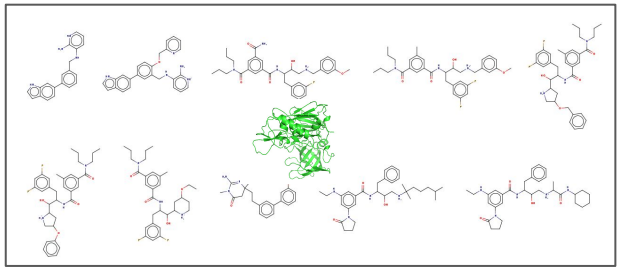
[5]:

ligand	LIG1.G						
protein	TYR38.A		TYR109.A		THR110.A	TRP125.A	
interaction	Hydrophobic	VdWContact	Hydrophobic	VdWContact	Hydrophobic	Hydrophobic	VdWContact
Frame							
0	False	False	True	False	False	True	False
10	False	False	True	True	False	True	False
20	False	False	True	True	False	True	True
30	True	False	True	False	False	True	True
40	False	False	True	False	True	True	True
50	True	False	True	True	False	False	False
60	False	False	True	False	False	False	False
70	False	False	True	True	False	True	False
80	False	False	True	False	False	True	False
90	False	False	False	False	False	True	False



Bouysset, C., Fiorucci, S. ProLIF: a library to encode molecular interactions as fingerprints.
J Cheminform 13, 72 (2021).
https://doi.org/10.1186/s13321-021-00548-6

Aim: to implement an easy to use tool to run whole pipeline of Molecular Dynamics simulation automatically



Perform **High-Throughput Molecular Dynamics**

Rank compounds by:

- Stability of previously obtained pose of ligand
- Calculated Binding Energy
- Stability of protein-ligand contacts

Preprocessing

Structure Preparation
- add missing atoms
- protonation
- etc

Force-field
- Definitions of inter-atomic bonded and non-bonded forces

Simulation box setup
- box size/shape

Solvate system
- add HOH molecules

Neutralize system
- add NA+/CL- ions

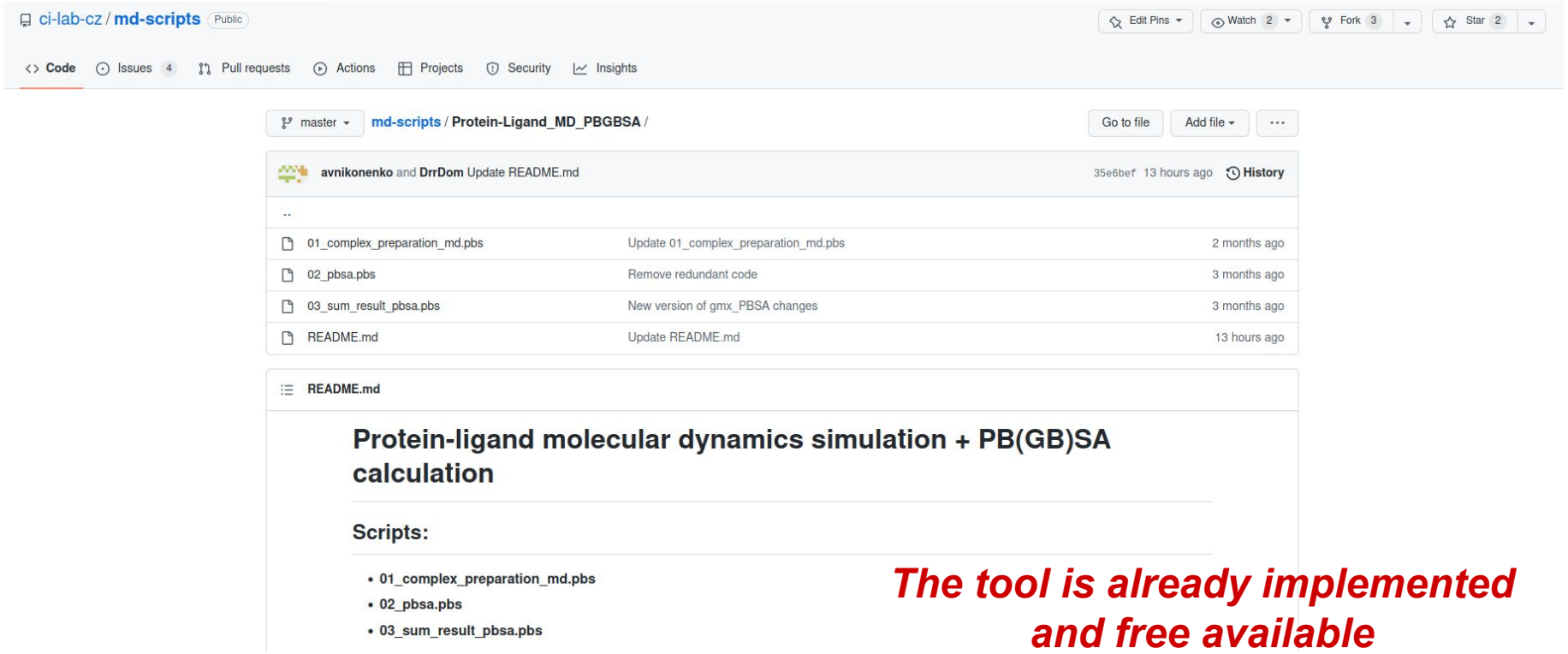
Pre-simulation steps

Energy minimization
- 100 ps

NVT and NPT equilibration
- 1000 ps

Product Simulation

Analysis



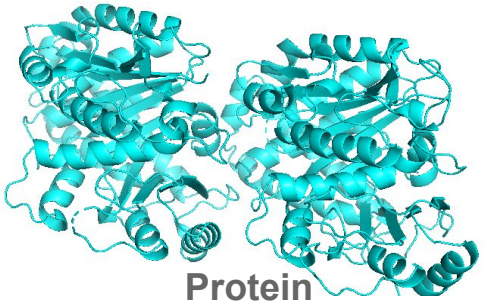
The tool is already implemented and free available

Command: qsub -v lfile=ligand.mol,pfile=protein.pdb,script_path=/scripts,wdir=ligand_protein_1ns,mdtime=1 01_complex_preparation_md.pbs

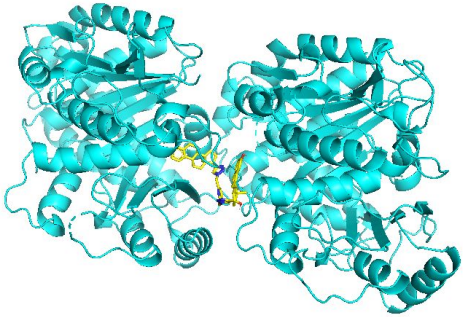
https://github.com/ci-lab-cz/md-scripts/tree/master/Protein-Ligand_MD_PBGBSA

Main features of the tool:

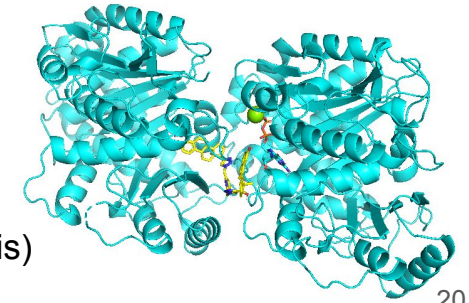
- **User control of simulation time**
  - from 10 ps to 1 μs
- **Default preset optimal parameters to run Molecular Dynamics**
  - can be easily modified
  - useful as teaching source
- **Support of modeling of different molecular systems**
  - protein only, protein-ligand, protein-ligand-cofactor
- **Automatic analysis of simulation:**
  - RMSD plots for both protein and ligand objects
  - Plot of flexibility of each amino acids (RMSF)
- **Support of analysis by additional instruments:**
  - ***ProLIF***: Ligand-Protein interactions (time-dependent function, stability analysis)
  - ***MM(PB)GBSA:*** Calculation of Binding Energy



Protein



Protein-Ligand



Protein-Ligand-Cofactor

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1,000,361 Computed Structure Models (CSM)

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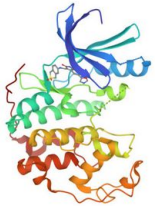
PDB-101 wwPDB EMDataResource Nucleic Acid Database wwPDB Foundation

Structure Summary | 3D View | Annotations | Experiment | Sequence | Genome | Ligands | Versions

Biological Assembly 1



3D View: Structure | 1D-3D View | Electron Density | Validation Report | Ligand Interaction

Global Symmetry: Asymmetric - C1
Global Stoichiometry: Monomer - A1

Find Similar Assemblies

1KE7

Display Files | Download Files

CYCLIN-DEPENDENT KINASE 2 (CDK2) COMPLEXED WITH 3-{[(2,2-DIOXIDO-1,3-DIHYDRO-2-BENZOTHIEN-5-YL)AMINO]METHYLENE}-5-(1,3-OXAZOL-5-YL)-1,3-DIHYDRO-2H-INDOL-2-ONE

PDB DOI: 10.2210/pdb1KE7/pdb

Classification: TRANSFERASE
Organism(s): Homo sapiens
Expression System: Spodoptera frugiperda
Mutation(s): No

Deposited: 2001-11-14 **Released:** 2002-05-14
Deposition Author(s): Bramson, H.N., Corona, J., Davis, S.T., Dickerson, S.H., Edelstein, M., Frye, S.V., Gampe, R.T., Hassell, A.H., Shewchuk, L.M., Kuyper, L.F.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.00 Å
R-Value Free: 0.235
R-Value Work: 0.191

wwPDB Validation | 3D Report | Full Report

Metric	Percentile Ranks	Value
Clashscore		10
Ramachandran outliers		0
Sidechain outliers		0.4%
RSRZ outliers		4.3%

Worse ... Better

Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution

Macromolecules

Find similar proteins by: Sequence (by identity cutoff) | 3D Structure

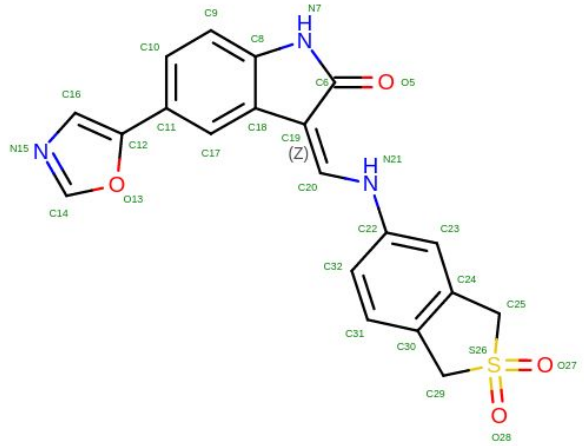
Entity ID: 1

Molecule	Chains	Sequence Length	Organism	Details	Image
Cell division protein kinase 2	A	298	Homo sapiens	**Mutation(s):** 0 **Gene Names:** CDK2, CDKN2 **EC:** 2.7.1.37 (PDB Primary Data), 2.7.11.22 (UniProt)	

UniProt & NIH Common Fund Data Resources

Find proteins for P24941 *(Homo sapiens)* Explore P24941 Go to UniProtKB: P24941

1KE7: Ligand LS3



Small Molecules

Ligands 1 Unique

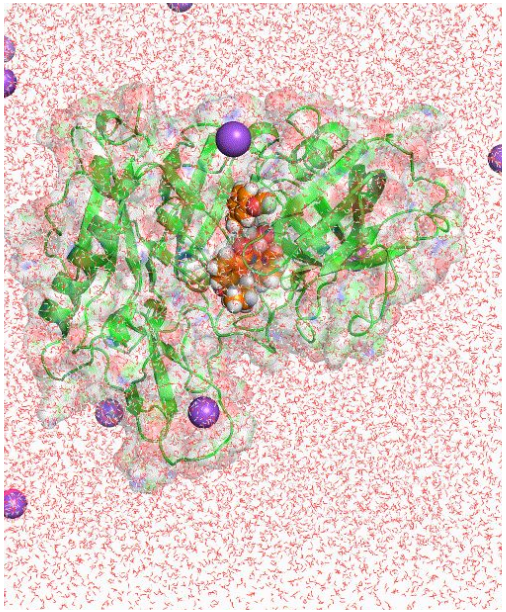
ID	Chains	Name / Formula / InChI Key	2D Diagram	3D Interactions
LS3 Query on LS3	B [auth A]	3-{[(2,2-DIOXIDO-1,3-DIHYDRO-2-BENZOTHIEN-5-YL)AMINO]METHYLENE}-5-(1,3-OXAZOL-5-YL)-1,3-DIHYDRO-2H-INDOL-2-ONE $C_{20}H_{15}N_3O_4S$ FTQYGMLRLRXBPT-IDUWFGFVSA-N		Ligand Interaction

Download Ideal Coordinates CCD File | Download Instance Coordinates

Binding Affinity Annotations

ID	Source	Binding Affinity
LS3	BindingDB: 1KE7	IC50: 8.9 (nM) from 1 assay(s)
	Binding MOAD: 1KE7	IC50: 8.9 (nM) from 1 assay(s)
	PDBBind: 1KE7	IC50: 8.9 (nM) from 1 assay(s)

Classical Molecular Dynamics



Structure Preparation

Preprocessing

Force-field
- Definitions of inter-atomic bonded and no-bonded forces (ligand and protein)

Simulation box setup
- box size/shape

Solvate system
- add HOH molecules

Neutralize system
- add NA+/CL- ions

Pre-simulation steps

Energy minimization
- 100 ps
- to ensure that the system has no steric clashes or inappropriate geometry

NVT and NPT equilibration
- 1000 ps
- equilibrate the solvent and ions around the protein

Product Simulation

Analysis

FAST. FLEXIBLE. FREE.
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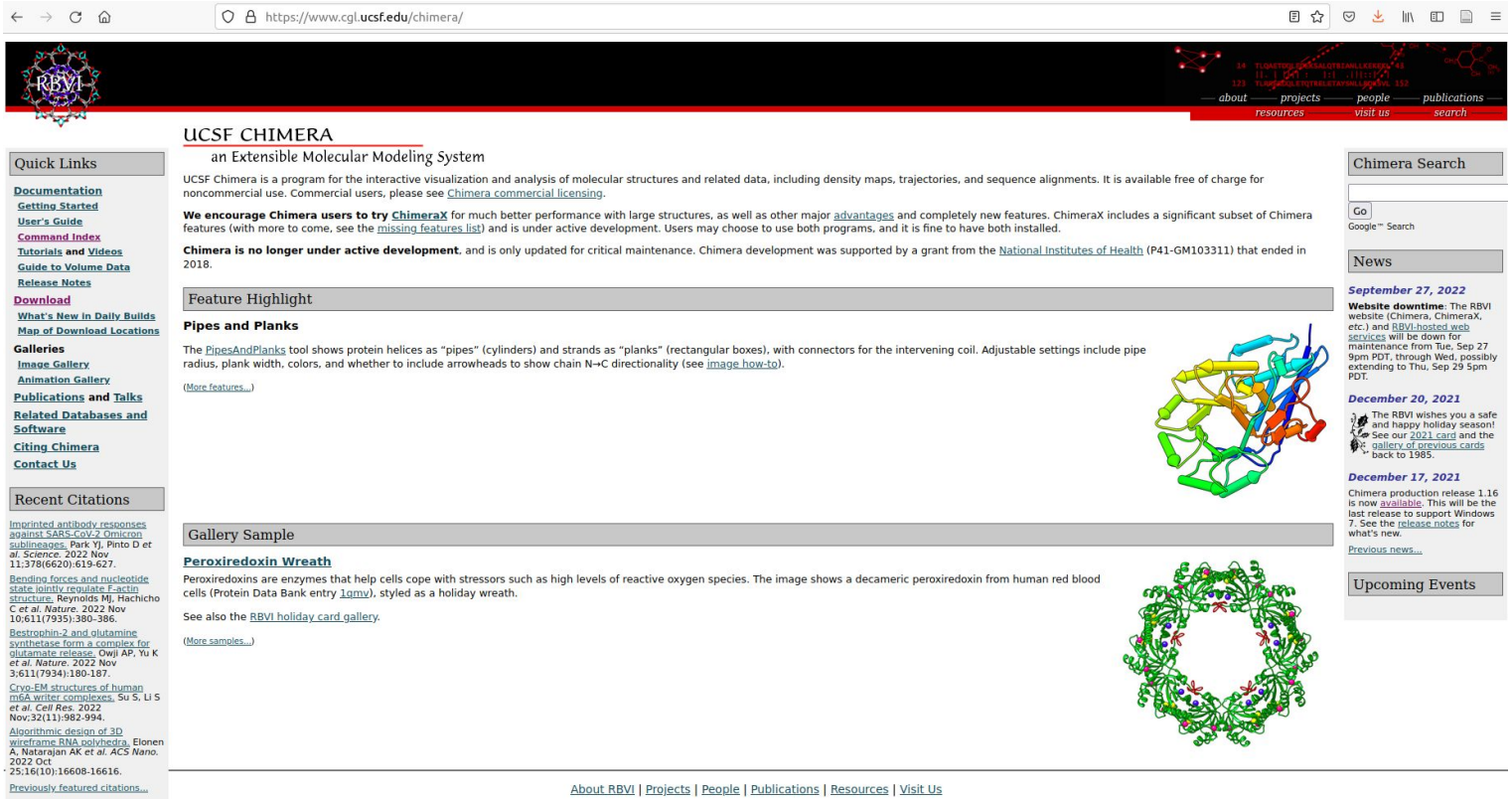
Protein preparation

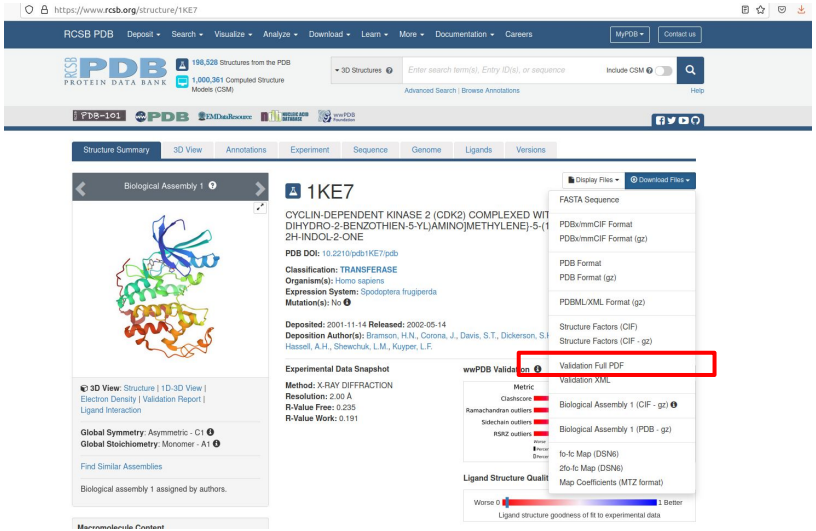


https://www.cgl.ucsf.edu/chimera/

Protein preparation

1. Download structure from PDB (https://www.rcsb.org/) using PDBID

   Download Files -> PDB Format



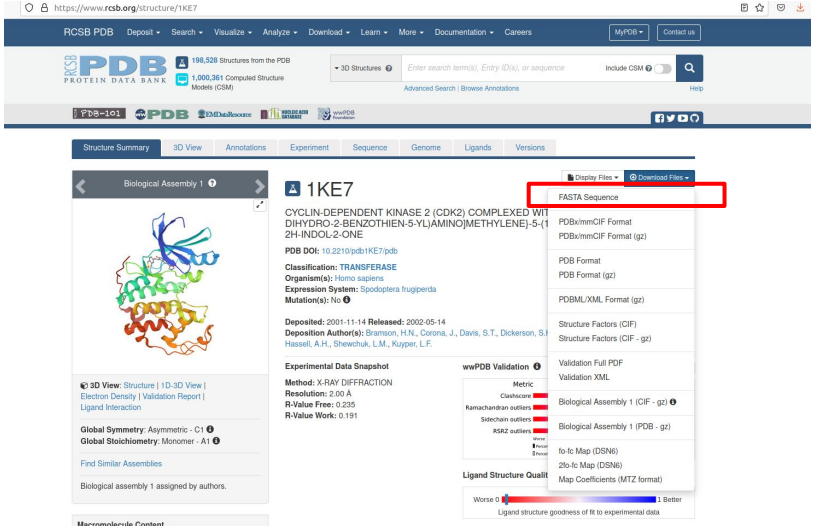
Protein preparation

1. Download structure from PDB (https://www.rcsb.org/) using PDBID

   Download Files -> PDB Format

2. Download sequence from PDB or from UniProt

   Download Files -> Fasta Sequence



Protein preparation

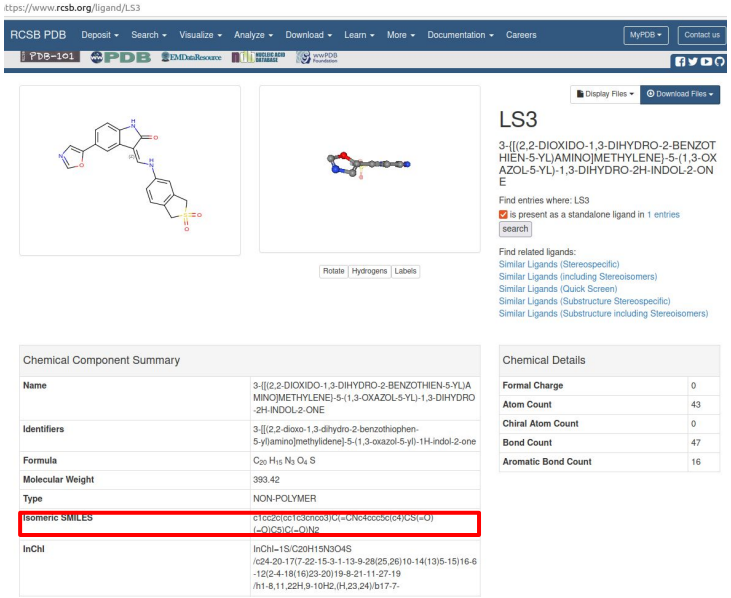
1. Download structure from PDB (https://www.rcsb.org/) using PDBID

   Download Files -> PDB Format

2. Download sequence from PDB or from UniProt

   Download Files -> Fasta Sequence

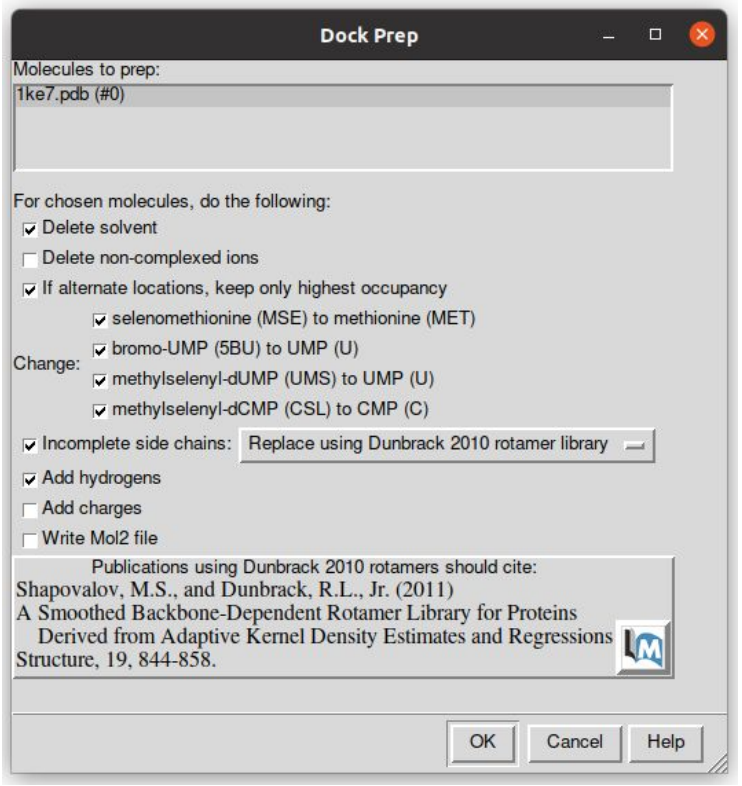
3. *Save smiles of ligand into file (ligand.smi)*



Protein preparation

4. Open Fasta and PDB in **Chimera**
   a. Dock Prepare

Structure Editing -> Dock Prep



Protein preparation

4. Open Fasta and PDB in **Chimera**

   b. fill missing loops by Modeller

      Tools -> Sequence -> Sequence

      Sequence -> Structure -> Modeller (loops/refinement)

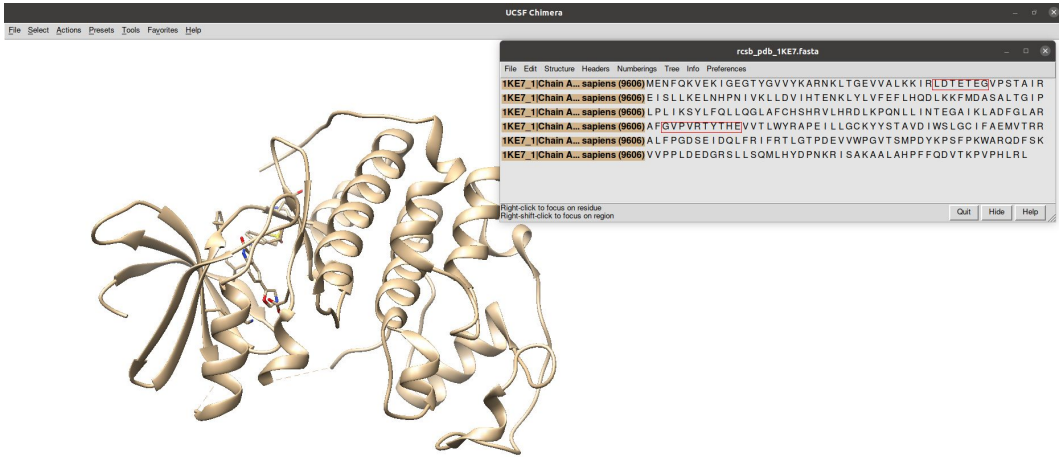
   c. add Hs to selected model

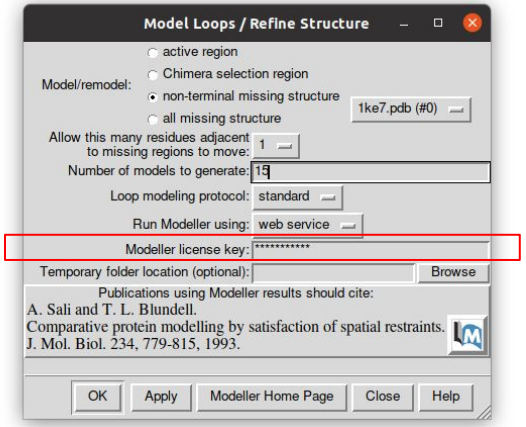
Treatment of Chosen Models

Select atoms | Choose in Model Panel | Hide others

Model	GA341	zDOPE
#1.1	1.00	-1.52
#1.2	1.00	-1.53
#1.3	1.00	-1.45
#1.4	1.00	-1.55
#1.5	1.00	-1.61
#1.6	1.00	-1.60
#1.7	1.00	-1.65
#1.8	1.00	-1.52
#1.9	1.00	-1.50
#1.10	1.00	-1.59
#1.11	1.00	-1.48
#1.12	1.00	-1.55
#1.13	1.00	-1.60
#1.14	1.00	-1.60
#1.15	1.00	-1.49

select the model with the lowest zDOPE





Protein preparation

https://ambermd.org/Questions/HIS.html

AMBER Histidine residues

Histidine (HIS in normal pdb files) is really one of three possible residues:

HID: Histidine with hydrogen on the delta nitrogen

HIE: Histidine with hydrogen on the epsilon nitrogen

HIP: Histidine with hydrogens on both nitrogens; this is positively charged.

It is up to the user to inspect the environment of each histidine and identify the type that is appropriate.

4. Open Fasta and PDB in Chimera

   d. Set HIS protonated state

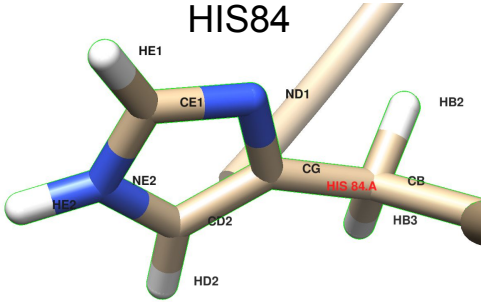
Tools -> General Controls -> Command line

Put in the Command line:

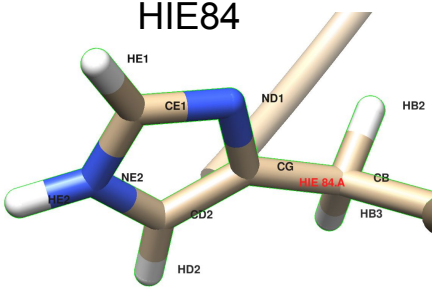
setattr r type HID :HIS@HD1,DD1,TD1,HND
setattr r type HIP :HID@HE2,DE2,TE2
setattr r type HIE :HIS@HE2

check visually active site

HIS84



HIE84



Chimera preparation

4. Open Fasta and PDB in **Chimera**

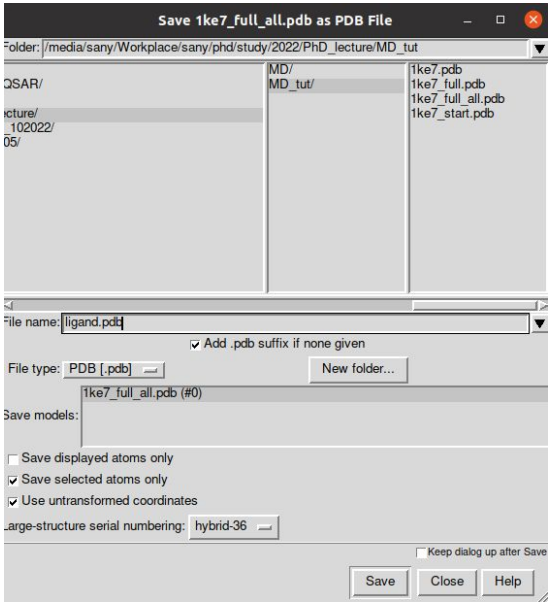
   e. Save protein only (plus ions, metals) and ligand only in *pdb* formats separately

Select -> Residue -> Ligand_id

File -> Save PDB -> Save selected only ligand.pdb

Select -> Invert (all models)

File -> Save PDB -> Save selected only protein_prepared.pdb



Ligand preparation

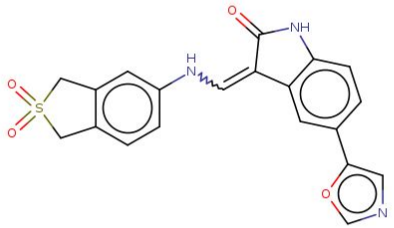
5. Save protonated smiles (pH 7.4) by Marvin into *ligand_74.smi*

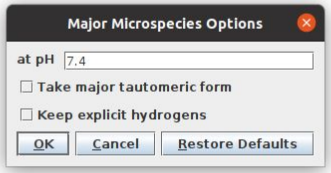
   Open Marvin Sketch

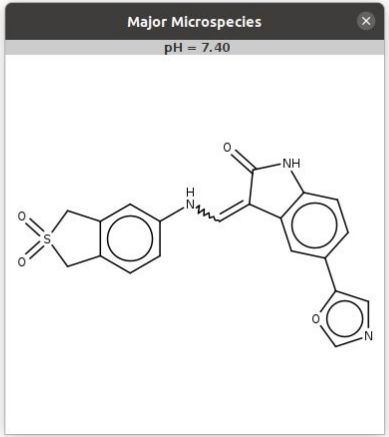
   File -> Open -> ligand.smi

   Calculations -> Protonation -> Major Microspecies -> 7.4 -> Ctrl+L (copy as Smiles)

   Save to ligand_74.smi







Ligand preparation

6. Transform pdb of ligand into mol format (add bonds information from smiles)

   Script https://github.com/ci-lab-cz/md-scripts/blob/master/scripts/pdb2mol.py

   Run in Bash/Shell:

   python md-scripts/scripts/pdb2mol.py -i ligand.pdb --smiles ligand_74.smi -o ligand.mol

Protein Ligand preparation

Input Files for MD:

protein_prepared.pdb

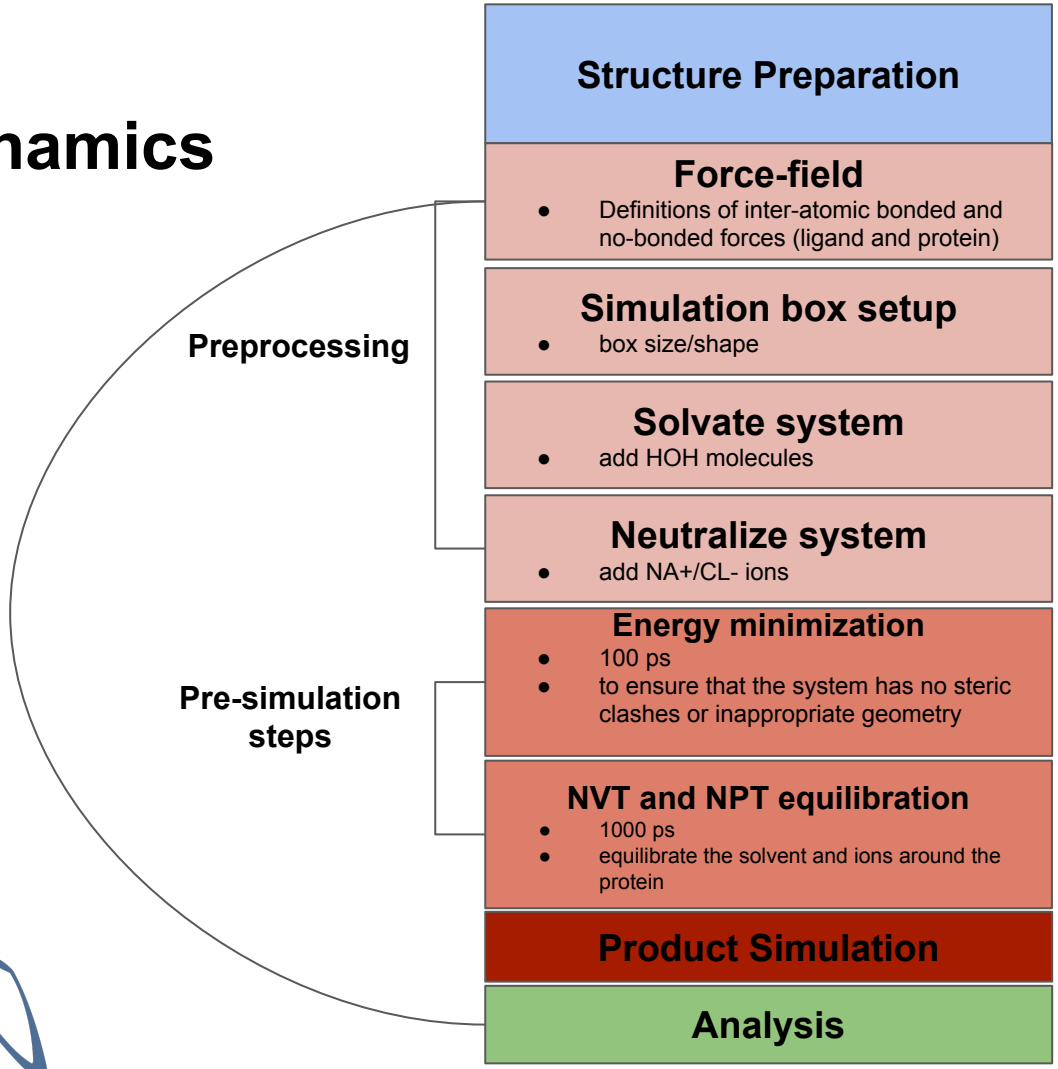
- *no missing non-terminal atoms/residues*
- *removed non-protein residues*
- *added all hydrogens*
- *set HID/HIE/HIP states*

ligand.mol

- *protonated at 7.4 pH*
- *added all hydrogens*

Classical Molecular Dynamics

https://github.com/ci-lab-cz/md-scripts



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Practice: force fields

Prepare the protein topology with pdb2gmx

gmx pdb2gmx

Synopsis

```
gmx pdb2gmx [-f [<.gro/.g96/...>]] [-o [<.gro/.g96/...>]] [-p [<.top>]]
            [-i [<.itp>]] [-n [<.ndx>]] [-q [<.gro/.g96/...>]]
            [-chainsep <enum>] [-merge <enum>] [-ff <string>]
            [-water <enum>] [-[no]inter] [-[no]ss] [-[no]ter]
            [-[no]lys] [-[no]arg] [-[no]asp] [-[no]glu] [-[no]gln]
            [-[no]his] [-angle <real>] [-dist <real>] [-[no]una]
            [-[no]ignh] [-[no]missing] [-[no]v] [-posrefc <real>]
            [-vsite <enum>] [-[no]heavyh] [-[no]deuterate]
            [-[no]chargegrp] [-[no]cmap] [-[no]renum] [-[no]rtpres]
```

Description

`gmx pdb2gmx` reads a .pdb (or .gro) file, reads some database files, adds hydrogens to the molecules and generates coordinates in GROMACS (GROMOS), or optionally .pdb, format and a topology in GROMACS format. These files can subsequently be processed to generate a run input file.

New files:
protein.gro: a molecular structure in Gromos87 format.
topol.top: molecular topology
posre.itp: position restraints

gmx pdb2gmx -f protein.pdb -o protein.gro -water tip3p -ignh <<< “AMBER99SB-ILDN”

https://manual.gromacs.org/documentation/current/onlinehelp/gmx-pdb2gmx.html

Practice: force fields

Prepare the protein topology with pdb2gmx

gmx pdb2gmx

Synopsis

```
gmx pdb2gmx [-f [<.gro/.g96/...>]] [-o [<.gro/.g96/...>]] [-p [<.top>]]
            [-i [<.itp>]] [-n [<.ndx>]] [-q [<.gro/.g96/...>]]
            [-chainsep <enum>] [-merge <enum>] [-ff <string>]
            [-water <enum>] [-[no]inter] [-[no]ss] [-[no]ter]
            [-[no]lys] [-[no]arg] [-[no]asp] [-[no]glu] [-[no]gln]
            [-[no]his] [-angle <real>] [-dist <real>] [-[no]una]
            [-[no]ignh] [-[no]missing] [-[no]v] [-posrefc <real>]
            [-vsite <enum>] [-[no]heavyh] [-[no]deuterate]
            [-[no]chargegrp] [-[no]cmap] [-[no]renum] [-[no]rtpres]
```

pdb2gmx does not work on ligand

Description

`gmx pdb2gmx` reads a .pdb (or .gro) file, reads some database files, adds hydrogens to the molecules and generates coordinates in GROMACS (GROMOS), or optionally .pdb, format and a topology in GROMACS format. These files can subsequently be processed to generate a run input file.

gmx pdb2gmx -f protein.pdb -o protein.gro -water tip3p -ignh <<< “AMBER99SB-ILDN”

https://manual.gromacs.org/documentation/current/onlinehelp/gmx-pdb2gmx.html

Automation tools for ligand topology

AMBER	**Antechamber**	Parametrizes molecules using GAFF
	acpype	A Python interface to Antechamber, writes GROMACS topologies
CHARMM	**CGenFF**	The official CHARMM General Force Field server
GROMOS87/ GROMOS96	**PRODRG 2.5**	An automated server for topology generation
	ATB	A newer server for topology generation, uses GROMOS96 54A7
OPLS-AA	**Topolbuild**	Converts a Tripos .mol2 file into a topology
	TopolGen	A Perl script to convert an all-atom .pdb file to a topology
	LigParGen	A server from the Jorgensen group to produce OPLS topologies

Ligand Force fields

Prepare the ligand topology using external tools

```
antechamber -i ligand.pdb -fi pdb -o lig.mol2 -fo mol2 -c bcc -pf y -s 2
parmchk2 -i lig.mol2 -f mol2 -o lig.frcmod
python pmed_amb2gmx.py -p lig.prmtop -x lig.inpcrd -o UNL
```

Build the topology for the protein-ligand system

Insert a line that says #include lig.itp" into topol.top after the position restraint file is included

Make adjustments is in the [molecules] directive

New files:

lig.mol2 *- file of ligand with charges and bonds*
lig.frcmod *- Parameter modification file*
posre_ligand.itp *- positional restraints*
ligand.gro *- ligand in Gromos87 format*
ligand.inpcrd - *Amber coordinate file of ligand*
ligand.prmtop *- Amber topology file*
ligand.top *- Gromacs topology file*

Changed files: topol.top

Solvation

To perform simulation we should add water molecules to mimic real environment.

There are two steps to defining the box and filling it with solvent:

1) Define the box dimensions using the editconf module
2) Fill the box with water using the solvate module.

We will use a simple cubic box as the unit cell.
First define the box using editconf:

```
gmx editconf -f protein.gro -o protein_newbox.gro -c -d 1.0 -bt cubic
```

a solute-box distance of 1.0 nm will mean that there are at least 2.0 nm between any two periodic images of a protein. This distance should be sufficient for just about any cutoff scheme commonly used in simulations.

New files:
protein_newbox.gro: protein+box system

Solvation

To perform simulation we should add water molecules to mimic real environment.

On the previous step we have defined a box, then we should fill it with solvent (water). Solvation is accomplished using solvate command:

gmx solvate -cp protein_newbox.gro -cs spc216.gro -o protein_solv.gro -p topol.top

-cp - the configuration of the protein (-cp)

-cs - the configuration of the solvent (-cs)

spc216.gro is a generic equilibrated 3-point solvent model (suitable for SPC, SPC/E, or TIP3P water, since they are all three-point water models)

New files:
protein_solv.gro: *protein + solvent system*
Changed files:
topol.top

Adding ions

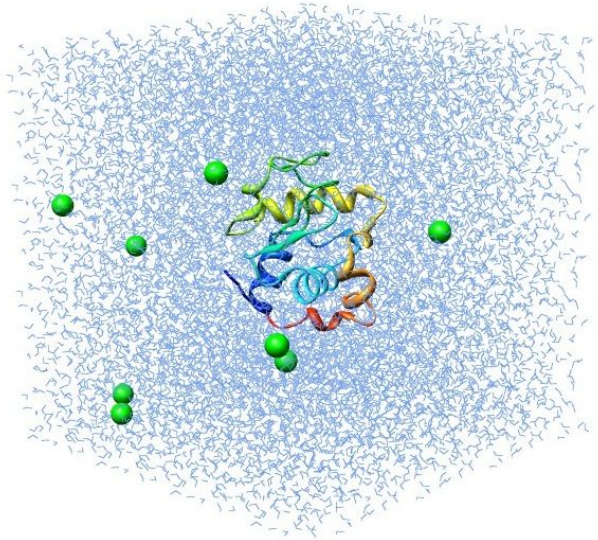
We need to add ions of CL- or NA+ to neutralize our protein:

```
gmx grompp -f ions.mdp -c protein_solv.gro -p topol.top -o ions.tpr
```

```
gmx genion -s ions.tpr -o protein_solv_ions.gro -p topol.top -pname NA -nname CL -neutral
```

New files:
protein_solv_ions.gro: *protein + solvent + ions system*
ions.tpr: *portable binary run input file. This file contains the starting structure, the molecular topology and all the simulation parameters.*

Changed files: topol.top



Solvated protein with ions

Energy minimization

Before we can begin dynamics, we must ensure that the system has no steric clashes or inappropriate geometry (equilibrium state). **The structure is relaxed through a process called energy minimization (EM)**.

Prepare files:

```
gmx grompp -f minim.mdp -c protein_solv_ions.gro -p topol.top -o  em.tpr
```

Run the minimization:

```
gmx mdrun -v -deffnm em –s em.tpr
```

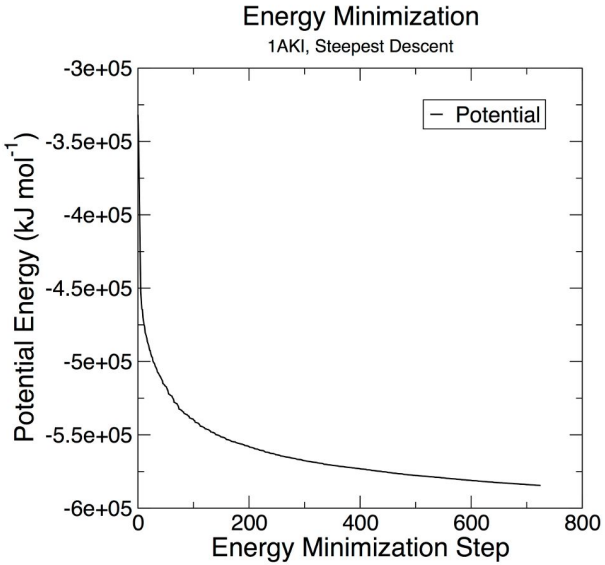
New files:
em.log: ASCII-text log file of the EM process
em.edr: Binary energy file
em.tpr: Contains the starting structure, the molecular topology and all the simulation parameters.
em.trr: Binary full-precision trajectory
em.gro: Energy-minimized structure

Energy minimization

How to do an analysis:

The em.edr file contains all of the energy terms that GROMACS collects during EM. You can analyze any .edr file using the GROMACS energy module:

gmx energy -f em.edr -o potential.xvg << "Potential"



Equilibration

Energy Minimization ensured that we have a reasonable starting structure, but to begin real dynamics, we must equilibrate the solvent and ions around the protein.

Why do we need equilibration?

If we were to attempt unrestrained dynamics at this point, the system may collapse.

The reason is that the solvent is mostly optimized within itself, and not necessarily with the solute. It needs to be brought to the temperature we wish to simulate and establish the proper orientation around the solute (the protein). After we achieved the correct temperature (based on kinetic energies), we will apply pressure to the system until it reaches the proper density.

Controlling the system

Thermodynamic system has a number of state variables which describe its macroscopic state such as

- Particle number, volume, temperature, pressure, total energy

They are not all independent, but connected by equations of state

Example: Ideal gas of non-interacting point particles

$$p = p(V,T) \qquad p \cdot V = N \cdot k_B \cdot T$$
$$E = E(V,T) \qquad p = \frac{N}{V} k_B T$$
$$E = \frac{3}{2} N k_B T$$

https://web.mst.edu/~vojtat/class_5403/MolecularDynamics.pdf

Restrain the system

To equilibrate the solvent and ions around the protein we need to apply position restraints on the heavy atoms of the protein.

The purpose of **posre.itp** is to apply a position restraining force on the heavy atoms of the protein (anything that is not a hydrogen). Movement is permitted, but only after overcoming a substantial energy penalty.

NVT equilibration

Canonical ensemble (NVT)

- Particle number N
- Volume V
- Temperature T

} External parameters

- Total energy E
- Pressure P

} Observables to be calculated

In MD simulation: some state variables are external parameters, others are observables to be calculated

Requires a **thermostat**, an algorithm that adds and removes energy to keep the temperature constant

- Velocity rescaling based on equipartition theorem

$$\langle E_{kin} \rangle = \frac{1}{2} \sum_i m_i \langle \vec{v}_i^{\,2} \rangle = \frac{3}{2} N k_B T$$

- Berendsen thermostat, Anderson thermostat

https://web.mst.edu/~vojtat/class_5403/MolecularDynamics.pdf

NVT equilibration

to run NVT equlibration:

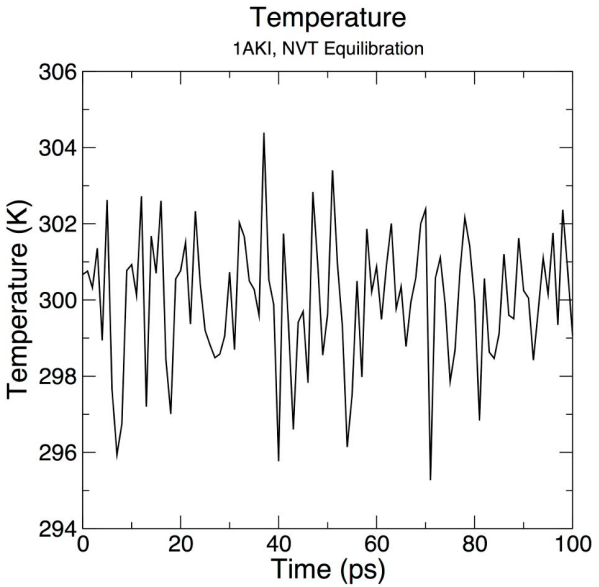
gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr

gmx mdrun -deffnm nvt -s nvt.tpr

An analysis:

gmx energy -f nvt.edr -o temperature.xvg <<< “Temperature”

New files:
nvt.log: *ASCII-text log file of the equilibration process*
nvt.edr: *Binary energy file*
nvt.trr: *Binary full-precision trajectory*
nvt.gro: *NVT-minimized structure*



NPT equilibration

Isothermal–isobaric ensemble (NPT)

- Particle number N
- Pressure P
- Temperature T

} External parameters

- Total energy E
- Volume V

} Observables to be calculated

Requires a **barostat** in addition to the thermostat, an algorithm that changes volume to keep the pressure constant

https://web.mst.edu/~vojtat/class_5403/MolecularDynamics.pdf

NPT equilibration

Note that we are now including the -t flag to include the checkpoint file from the NVT equilibration

The coordinate file (-c) is the final output of the NVT simulation.

to run NPT equlibration:

gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p topol.top -o npt.tpr gmx mdrun -deffnm npt -s npt.tpr

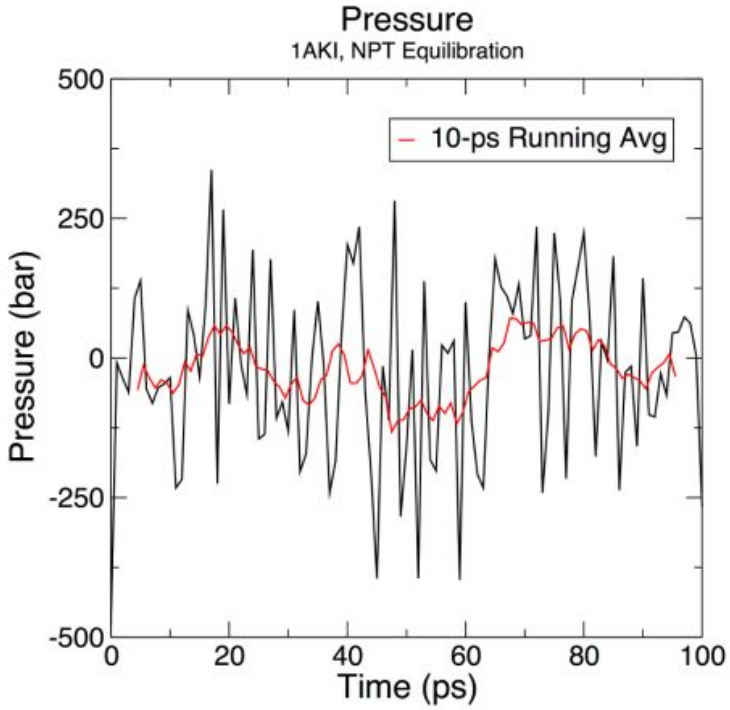
An analysis:

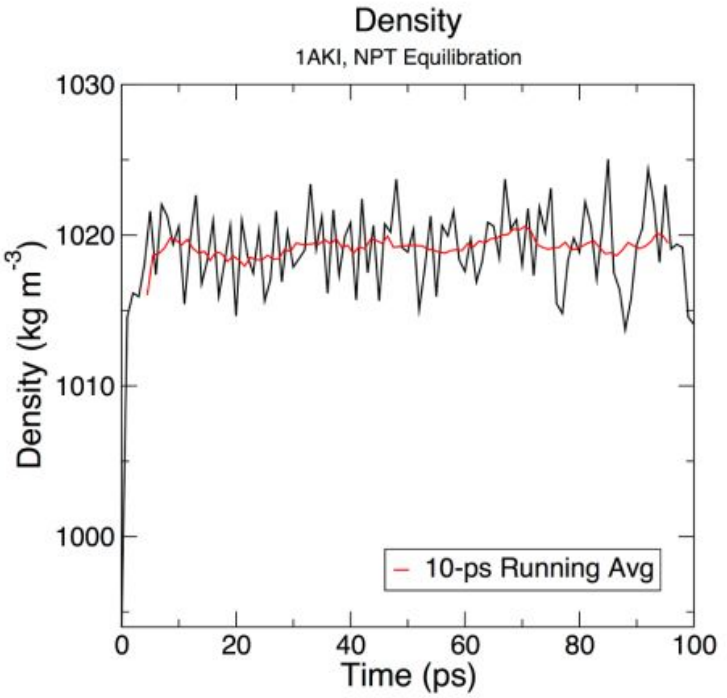
gmx energy -f npt.edr -o pressure.xvg <<< ”Pressure”

gmx energy -f npt.edr -o density.xvg <<< “Density”

New files:
nvt.log: *ASCII-text log file of the equilibration process*
nvt.edr: *Binary energy file*
nvt.trr: *Binary full-precision trajectory*
nvt.gro: *NVT-minimized structure*

NPT equilibration



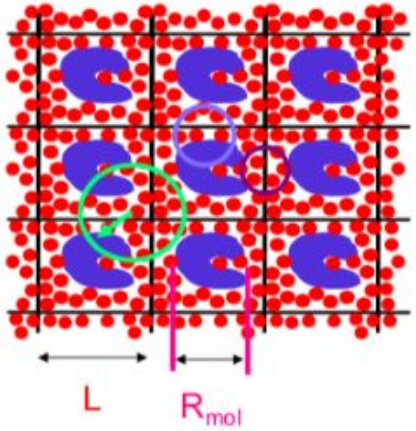


In our tool we run 1000 ps NPT equilibration by default

Periodic Boundary Conditions

Periodic boundary conditions (PBCs) are a set of boundary conditions which are often chosen for approximating a large (infinite) system by using a small part called a unit cell.

- PBCs are often used in computer simulations and mathematical models.
- The topology of two-dimensional PBC is equal to that of a world map of some video games; the geometry of the unit cell satisfies perfect two-dimensional tiling, and when an object passes through one side of the unit cell, it reappears on the opposite side with the same velocity.



Required
(no atom sees another one twice):

$L > 2R_c$ $R_c < ½L$

Preferred
(protein does not see a copy of itself)

$L > R_c + R_{mol}$ $R_c < L - R_{mol}$

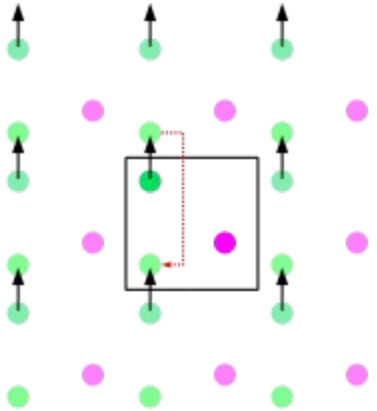
Even better
(no solvent sees two proteins)

$L > 2R_c + R_{mol}$ $R_c < ½(L - R_{mol})$

<nu mbe r>

- Cubic:

Rectangular:



Production MD

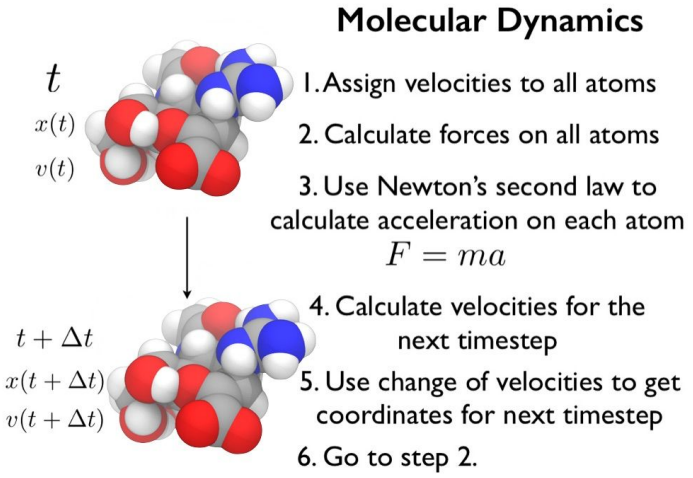
the system is now well-equilibrated at the desired temperature and pressure. We are now ready to release the position restraints and run production MD

We will run MD simulation

gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md_0_1.tpr

gmx mdrun -deffnm md_0_1 -s md_0_1.tpr

New files:
md_0_1.tpr: portable binary run input file. It contains the starting structure of the simulation, the molecular topology and all the simulation parameters.
md_0_1.log: *ASCII-text log file of the equilibration process*
md_0_1.cpt: *portable checkpoint file. The complete state of the simulation is stored in the checkpoint file, including extended thermostat/barostat variables, random number states and NMR time averaged data*
md_0_1.edr*: Binary energy file*
md_0_1.xtc: *Binary full-precision trajectory*
md_0_1.gro*:* starting structure of the simulation in



Analysis of calculated MD simulation

Remove PBC:

```
gmx trjconv -s md_out.tpr -f md_out.xtc -pbc nojump -o md_out_noj_noPBC.xtc <<<
"System"
```

Center system:

```
gmx trjconv -s md_out.tpr -f md_out_noj_noPBC.xtc -o md_centermolsnoPBC.xtc -pbc mol
-center -n index.ndx <<< "Protein_UNL System"
```

Alignment of all frames (Remove rotations and translations):

```
gmx trjconv -s md_out.tpr -f md_centermolsnoPBC.xtc -fit rot+trans -o md_fit.xtc -n index.ndx
<<< "Protein_UNL System"
```

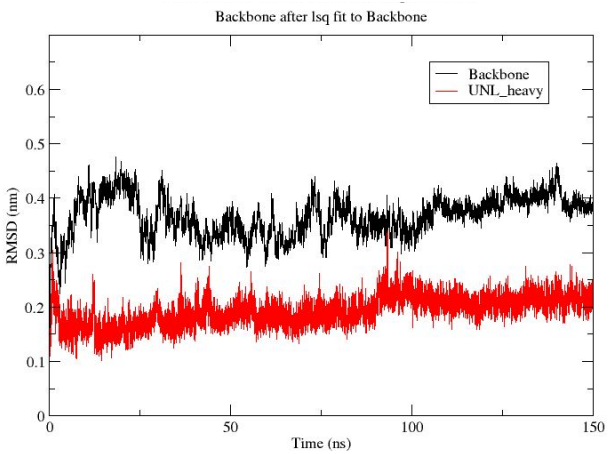
Analysis of calculated MD simulation

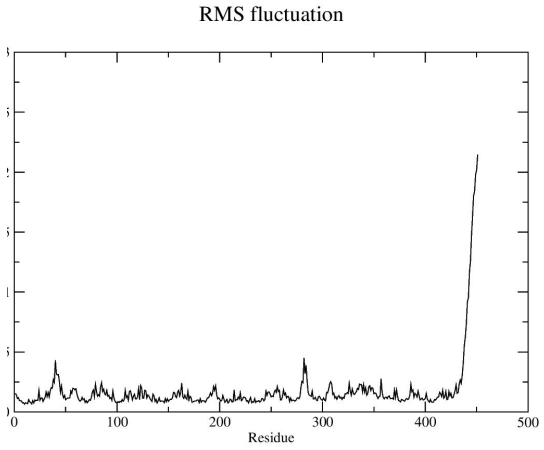
RMSD:

gmx rms -s md_0_1.tpr -f md_0_1_noPBC.xtc -o rmsd.xvg -tu ns

RMSF:

gmx rmsf -s md_0_1.tpr -f md_0_1_noPBC.xtc -o rmsf.xvg -oq rmsf.pdb -res





xmgrace

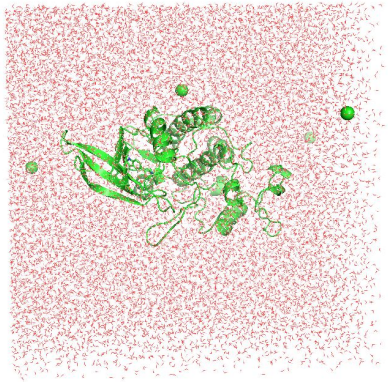
MD simulation by one command:

Output files:

md_out.tpr: portable binary run input file. It contains the starting structure of the simulation, the molecular topology and all the simulation parameters.
md*_out.*log: *ASCII-text log file of the equilibration process*
md*_out.*cpt: *portable checkpoint file. The complete state of the simulation is stored in the checkpoint file, including extended thermostat/barostat variables, random number states and NMR time averaged data*
***md*_out.*edr*:** *Binary energy file*
md*_out.*xtc: *Binary full-precision trajectory*
***md*_out.*gro*:** starting structure of the simulation

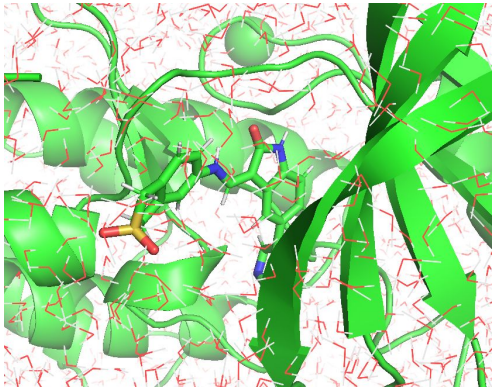
md_fit.xtc: *fitted trajectory (removed PBC and the rotation and translation, centered) to use for rmsd and energy calculation analysis*
md_short_forcheck.xtc: *fitted short trajectory (each 100 step is skipped)*
frame.pdb: a frame from the trajectory to provide topology

MD trajectory analysis files:
rmsd.xvg - rmsd of Backbone of protein
rmsd_UNL.xvg - rmsd of the heavy atoms of ligand
gyrate.xvg - radius of gyration of the protein
rmsf.xvg - root mean square fluctuation of each amino acids



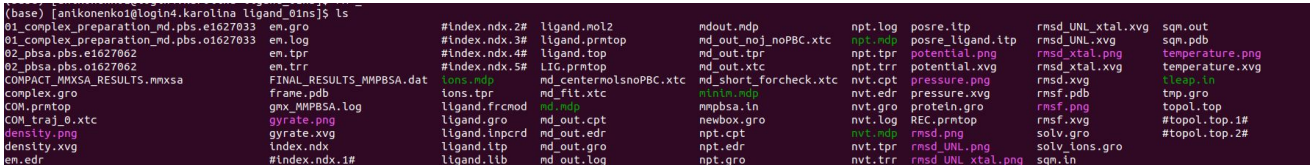
Check your own MD trajectory

frame.pdb - a frame from the trajectory to provide topology
md_fit.xtc - your fitted MD trajectory



Analysis of calculated MD simulation

working dir: */home/user/MD_tutorial*



Run on it4i cluster:
Visualize plots by xmgrace:
module load grace
cd ligand_01ns/
*for i in *.xvg; do gracebat -hdevice PNG $i;done*

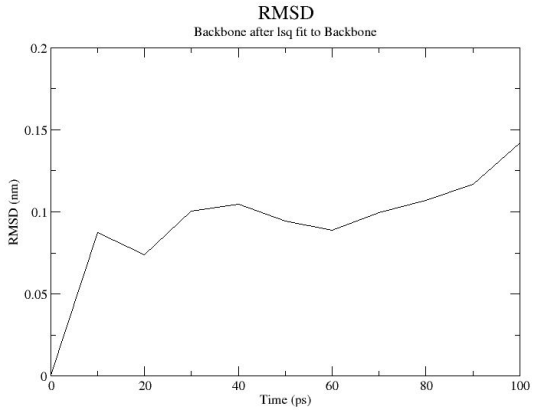
Run analysis on your own computer:

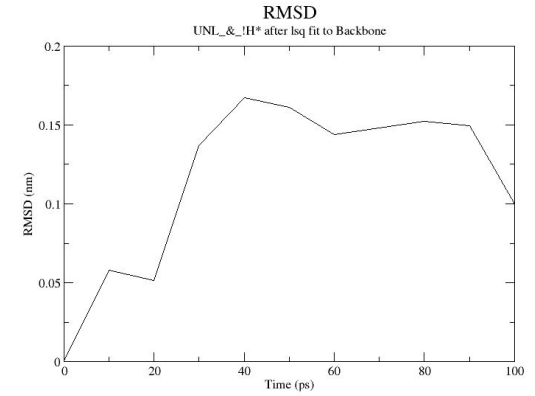
*scp -I path/to/your/private/key dd-23-13-XX@login1.karolina.it4i.cz:md_tutorial/ligand_01ns/{frame.pdb,md_fit.xtc, *.png} .*

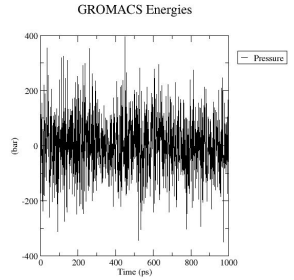
Analysis of the calculated MD simulation

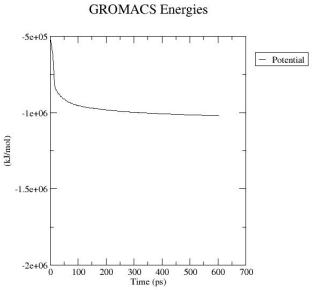
```
# This file was created Mon Nov 28 18:33:21 2022
# Created by:
#         :-) GROMACS - gmx rms, 2021.4-EasyBuild-4.5.1-PLUMED-2.7.3 (-:
#
# Executable:   /apps/all/GROMACS/2021.4-foss-2020b-PLUMED-2.7.3/bin/gmx
# Data prefix:  /apps/all/GROMACS/2021.4-foss-2020b-PLUMED-2.7.3
# Working dir:  /mnt/proj1/dd-22-84/MD_tutorial/ligand_01ns
# Command line:
#   gmx rms -s md_out.tpr -f md_fit.xtc -o rmsd.xvg -n index.ndx -tu ps
# gmx rms is part of G R O M A C S:
#
# God Rules Over Mankind, Animals, Cosmos and Such
#
@    title "RMSD"
@    xaxis  label "Time (ps)"
@    yaxis  label "RMSD (nm)"
@TYPE xy
@ subtitle "Backbone after lsq fit to Backbone"
    0.0000000    0.0004955
   10.0000000    0.0872569
   20.0000000    0.0738152
   30.0000000    0.1004452
   40.0000000    0.1048534
   50.0000000    0.0945487
   60.0000000    0.0887801
   70.0000000    0.0993429
   80.0000000    0.1069565
   90.0000000    0.1168087
  100.0000000    0.1420962
```

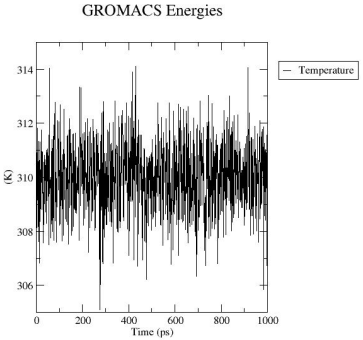
```
# This file was created Mon Nov 28 18:33:21 2022
# Created by:
#         :-) GROMACS - gmx rms, 2021.4-EasyBuild-4.5.1-PLUMED-2.7.3 (-:
#
# Executable:   /apps/all/GROMACS/2021.4-foss-2020b-PLUMED-2.7.3/bin/gmx
# Data prefix:  /apps/all/GROMACS/2021.4-foss-2020b-PLUMED-2.7.3
# Working dir:  /mnt/proj1/dd-22-84/MD_tutorial/ligand_01ns
# Command line:
#   gmx rms -s md_out.tpr -f md_fit.xtc -o rmsd_UNL.xvg -n index.ndx -tu ps
# gmx rms is part of G R O M A C S:
#
# God Rules Over Mankind, Animals, Cosmos and Such
#
@    title "RMSD"
@    xaxis  label "Time (ps)"
@    yaxis  label "RMSD (nm)"
@TYPE xy
@ subtitle "UNL_&_!H* after lsq fit to Backbone"
    0.0000000    0.0005219
   10.0000000    0.0578194
   20.0000000    0.0515443
   30.0000000    0.1366709
   40.0000000    0.1673483
   50.0000000    0.1613055
   60.0000000    0.1436562
   70.0000000    0.1480425
   80.0000000    0.1522363
   90.0000000    0.1496438
  100.0000000    0.0998629
```











MMPBSA / MMGBSA

End-state free energy calculations
with GROMACS files

```
 GNU nano 2.3.1                                File: md-scripts/scripts/mmpbsa.in

Sample input file for PB/GB calculation
#This input file is meant to show only that gmx_MMPBSA works. Although, we tried to use the input files as recommended in the
#Amber manual, some parameters have been changed to perform more expensive calculations in a reasonable amount of time. Feel free to change the parameters
#according to what is better for your system.
&general
sys_name="PB_GB_IE",
#startframe=1, interval=25, verbose=2, PBRadii=3,
startframe=1, interval=1, verbose=2, PBRadii=3,
interaction_entropy=1, ie_segment=25, temperature=310
/
&gb
igb=5, saltcon=0.150,
/
&pb
istrng=0.15, fillratio=4.0, radiopt=0, indi=1, exdi=80.0
/
```

1) You can change **ie_segment** from 25 to 100

   nano /mnt/proj2/dd-23-13/*md-scripts/scripts/mmpbsa.in*

working dir: */home/user/md_tutorial*

2) run script for energy calculation:

qsub -A DD-22-13 -v tpr=md_out.tpr,xtc=md_fit.xtc,script_path=/mnt/proj2/dd-23-13/md-scripts/scripts/,wdir=ligand_01ns/,NP=11 /mnt/proj2/dd-23-13/md-scripts/Protein-Ligand_MD_PBGBSA/02_pbsa.pbs

3) After calculation will be finished

   cat ligand_01ns/FINAL_RESULTS_MMPBSA.dat

For multiple molecules you can run other script to collect MM(P/G)BSA energies from all directories

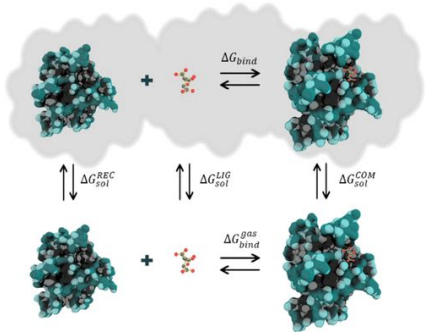
wdir=$(pwd)/md_tutorial bash 03_sum_result_pbsa.pbs

```
Sum_Result_GENERALIZED_BORN.csv  Sum_Result_IE.csv  Sum_Result_POISSON_BOLTZMANN.csv
```

```
(miniconda3)[anikonenko1@login2.karolina dd-23-13]$ cat md_tutorial/Sum_Result_*
fname,deltaG,SD
1ke5_ligand,-30.6897,4.1740
1ke6_ligand,-23.3582,2.7462
1ke7_ligand,-19.3363,4.8091
fname,IE,SD,SEM
1ke5_ligand,3.8787,2.5746,1.4865
1ke6_ligand,9.3748,0.0504,0.0291
1ke7_ligand,12.6552,0.0505,0.0291
```

What can be done by MD

- to estimate binding affinity of protein-ligand complexes
  - Molecular mechanics Poisson–Boltzmann surface area (**MM/PBSA**)
  - Molecular mechanics generalized Born surface area (**MM/GBSA**)



In the MM/PBSA or MM/GBSA approach, the free energy for binding of the ligand (L) to the protein receptor (R) to form the complex (RL),

$$\Delta G_{\text{bind}} = G_{\text{RL}} - G_{\text{R}} - G_{\text{L}} \quad (4)$$

can be decomposed into contributions of different interactions and expressed as (58)

$$\Delta G_{\text{bind}} = \Delta H - T\Delta S = \Delta E_{\text{MM}} + \Delta G_{\text{sol}} - T\Delta S \quad (5)$$

in which

$$\Delta E_{\text{MM}} = \Delta E_{\text{int}} + \Delta E_{\text{ele}} + \Delta E_{\text{vdW}} \quad (6)$$

$$\Delta G_{\text{sol}} = \Delta G_{\text{PB/GB}} + \Delta G_{\text{SA}} \quad (7)$$

$$\Delta G_{\text{SA}} = \gamma \cdot \text{SASA} + b \quad (8)$$

Valdés-Tresanco, M.S., Valdés-Tresanco, M.E., Valiente, P.A. and Moreno E. ***gmx_MMPBSA: A New Tool to Perform End-State Free Energy Calculations with GROMACS.*** Journal of Chemical Theory and Computation, 2021 17 (10), 6281-6291. https://pubs.acs.org/doi/10.1021/acs.jctc.1c00645.
***MMPBSA.py*: An Efficient Program for End-State Free Energy Calculations** Bill R. Miller III, T. Dwight McGee Jr., Jason M. Swails, Nadine Homeyer, Holger Gohlke, and Adrian E. Roitberg Journal of Chemical Theory and Computation **2012** *8* (9), 3314-3321 DOI: 10.1021/ct300418h

What can be done by MD

- to estimate binding affinity of protein-ligand complexes

Total $G_{Binding}$=

- **Gas-phase molecular mechanics energy ΔE_{MM}:**
  - changes in the **internal energies** ΔE_{int} (bond, angle, and dihedral energies)
  - **electrostatic energies** ΔE_{ele}
  - **van der Waals energies** ΔE_{vdW}
- **sum of the electrostatic solvation energy G_{sol}**
  - **The polar contribution** is calculated using either the PB or GB model (ΔGPB/GB). Where the GB method gives an analytical expression for the polar solvation energy and is thus much faster than the PB method.
  - **nonpolar energy** is usually estimated using the solvent-accessible surface area (**SASA**)
- The change in **conformational entropy** **$-T\Delta S$**
  - is usually calculated by normal-mode analysis (or Interaction entropy) on a set of conformational snapshots taken from MD simulations.

In the MM/PBSA or MM/GBSA approach, the free energy for binding of the ligand (L) to the protein receptor (R) to form the complex (RL),

$$\Delta G_{bind} = G_{RL} - G_R - G_L \quad (4)$$

can be decomposed into contributions of different interactions and expressed as (58)

$$\Delta G_{bind} = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{sol} - T\Delta S \quad (5)$$

in which

$$\Delta E_{MM} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdW} \quad (6)$$

$$\Delta G_{sol} = \Delta G_{PB/GB} + \Delta G_{SA} \quad (7)$$

$$\Delta G_{SA} = \gamma \cdot SASA + b \quad (8)$$

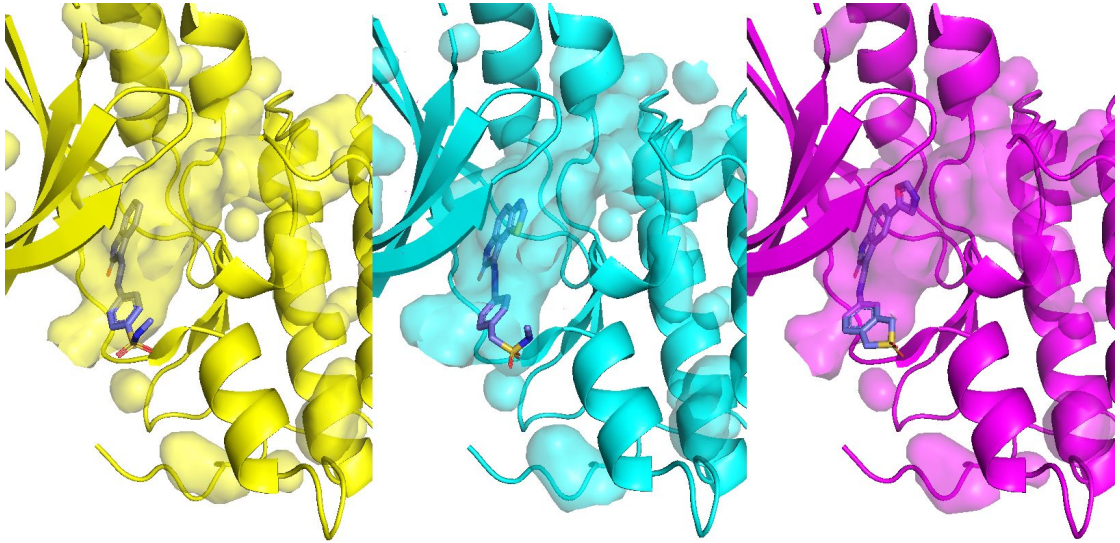
```
EDISPER              -1407.08         18.20         18.20          5.49          5.49

GGAS                 -3184.42        101.56         74.86         30.62         22.57
GSOLV                -2452.28         57.05         52.79         17.20         15.92

TOTAL                -5636.70        116.49         31.95         35.12          9.63

--------------------------------------------------------------------------------
--------------------------------------------------------------------------------
Ligand:
Energy Component       Average     SD(Prop.)          SD   SEM(Prop.)         SEM
--------------------------------------------------------------------------------
BOND                     11.40          2.28          2.28          0.69          0.69
ANGLE                    45.46          2.79          2.79          0.84          0.84
DIHED                    23.24          2.06          2.06          0.62          0.62
VDWAALS                  -3.31          0.62          0.62          0.19          0.19
EEL                      95.11          0.99          0.99          0.30          0.30
1-4 VDW                   7.97          0.54          0.54          0.16          0.16
1-4 EEL                -225.15          1.58          1.58          0.48          0.48
EPB                     -34.82          0.85          0.85          0.26          0.26
ENPOLAR                  40.24          0.17          0.17          0.05          0.05
EDISPER                 -42.92          0.18          0.18          0.06          0.06

GGAS                    -45.28          4.62          3.37          1.39
GSOLV                   -37.50          0.89          0.95          0.27

TOTAL                   -82.78          4.71          2.96          1.42

--------------------------------------------------------------------------------
--------------------------------------------------------------------------------
Delta (Complex - Receptor - Ligand):
Energy Component       Average     SD(Prop.)          SD   SEM(Prop.)         SEM
--------------------------------------------------------------------------------
ΔBOND                    -0.00          2.08          0.00          0.63          0.00
ΔANGLE                   -0.00          2.09          0.00          0.63          0.00
ΔDIHED                    0.00          1.45          0.00          0.44          0.00
ΔVDWAALS                -46.46          0.60          2.50          0.18          0.75
ΔEEL                    -41.29          0.26          7.77          0.08          2.34
Δ1-4 VDW                 -0.00          0.30          0.00          0.09          0.00
Δ1-4 EEL                  0.00          1.33          0.00          0.40          0.00
ΔEPB                     64.17          0.71          4.26          0.22          1.28
ΔENPOLAR                -32.09          0.07          0.75          0.02          0.23
ΔEDISPER                 57.51          0.07          0.85          0.02          0.26

ΔGGAS                   -87.75          0.66          7.62          0.20          2.30
ΔGSOLV                   89.59          0.72          4.38          0.22          1.32

ΔTOTAL                    1.84          0.98          6.93          0.29          2.09
--------------------------------------------------------------------------------
--------------------------------------------------------------------------------
Using Interaction Entropy Approximation:
ΔG binding =      4.51 +/-     6.93
--------------------------------------------------------------------------------
--------------------------------------------------------------------------------
```

GENERALIZED BORN:
POISSON BOLTZMANN:

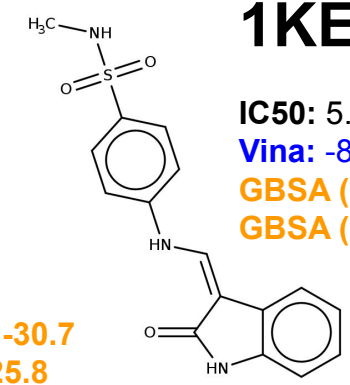
```
(base) [anikonenko1@login4.karolina MD_tutorial]$ grep 'G binding =' ligand_01ns/FINAL_RESULTS_MMPBSA.dat
ΔG binding =     -36.09 +/-     4.58
ΔG binding =       4.51 +/-     6.93
```

MMPBSA Energy and **MMGBSA Energy** cannot be compared within the different methods. But you can rank your molecules by energies obtained from each method separately.

CYCLIN-DEPENDENT KINASE 2 (CDK2)



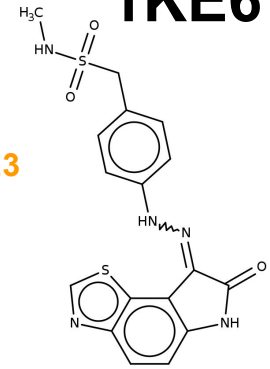
1KE5



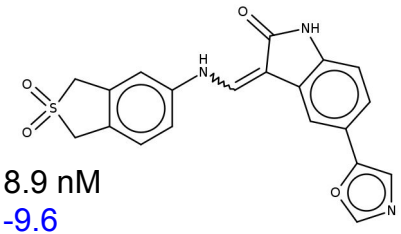
IC50: 560 nM
Vina: -8.7
GBSA (0.1 ns): -30.7
GBSA (1 ns): -25.8

1KE6

IC50: 5.7 nM
Vina: -8.9
GBSA (0.1ns): -23.3
GBSA (1ns): -21.5

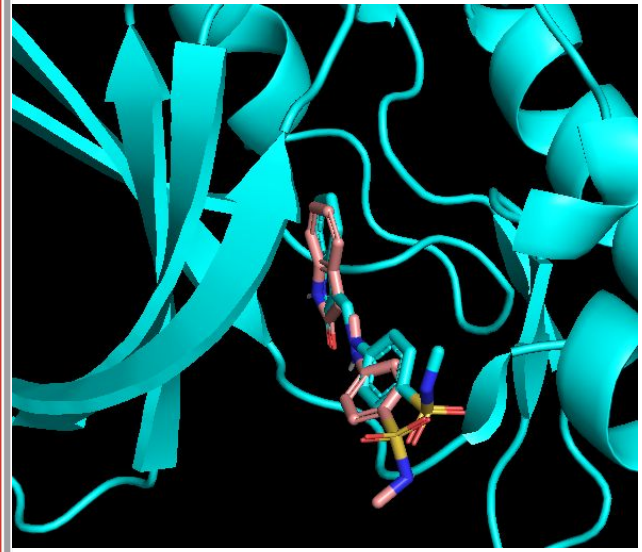


1KE7

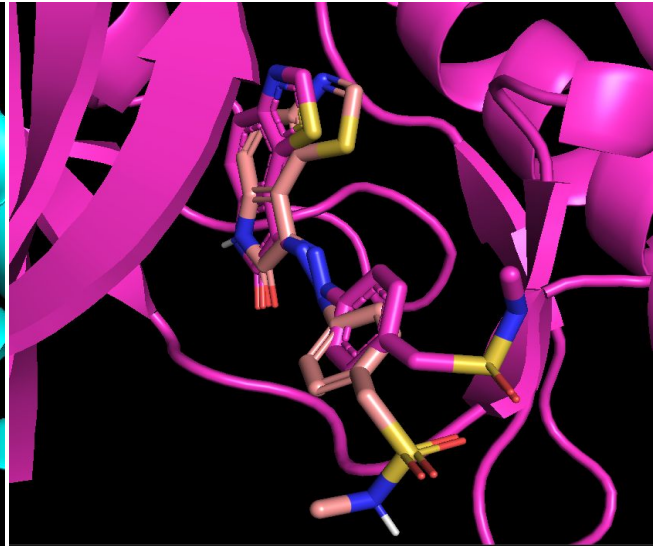


IC50: 8.9 nM
Vina: -9.6
GBSA (0.1ns): -19.3
GBSA (1ns): -27.1

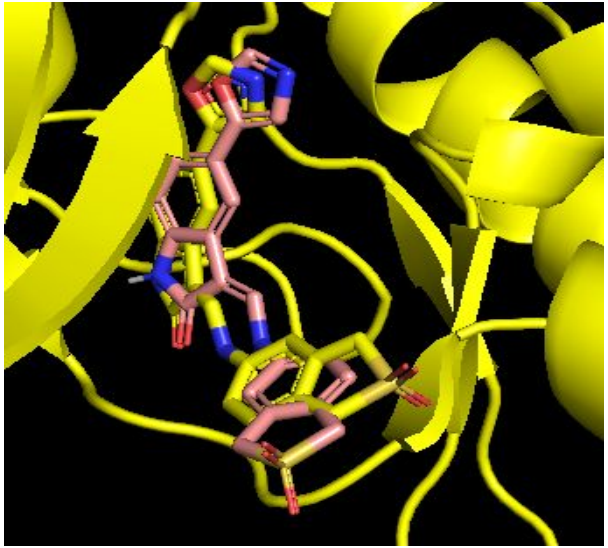
Docking best pose



1KE5

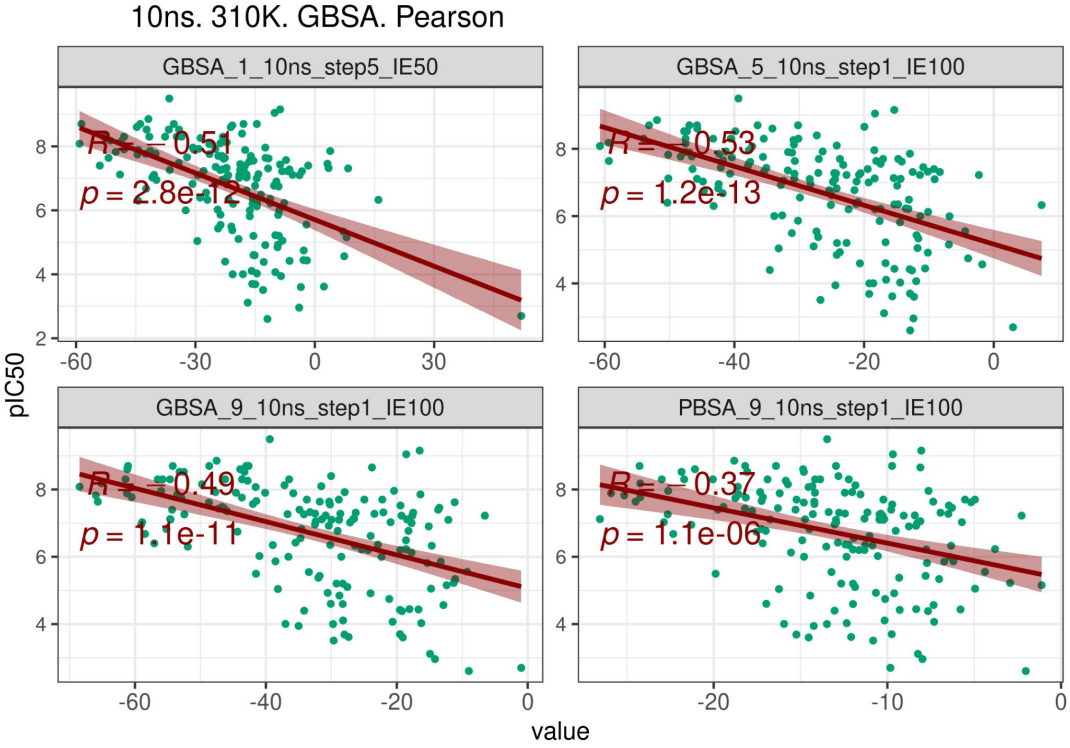


1KE6



1KE7

Beta Secretase



Thank you for your attention!