Molecular Dynamics simulations of polypeptides in water solution under physiological conditions. Application to fragments of SARS-CoV 3CL protease.

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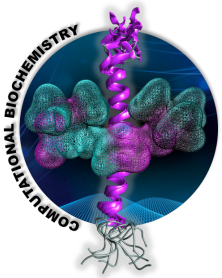

Computational Biochemistry

Master in Theoretical Chemistry and Computational Modelling

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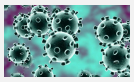
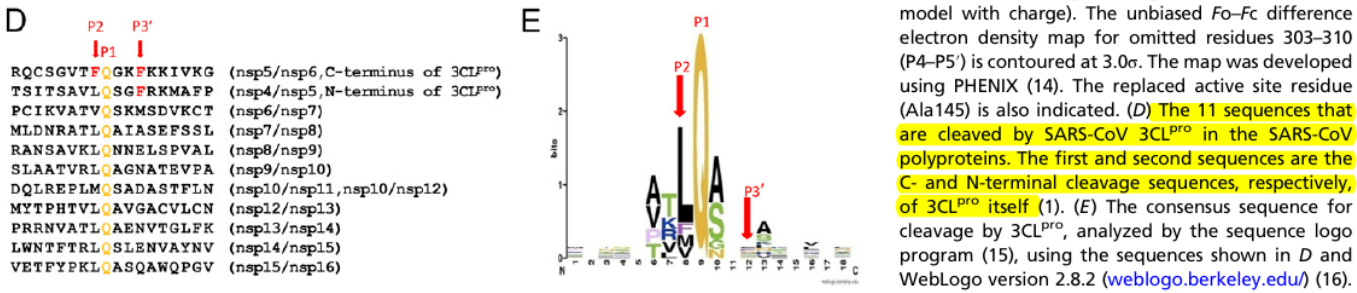


System

- Fragment of the sequence that are cleaved by SARS-CoV 3CL^{pro} (3C-like protease) in the SARS-CoV prolyproteins



model with charge). The unbiased *Fo–Fc* difference electron density map for omitted residues 303–310 (P4–P5′) is contoured at 3.0σ. The map was developed using PHENIX (14). The replaced active site residue (Ala145) is also indicated. (*D*) The 11 sequences that are cleaved by SARS-CoV 3CL^{pro} in the SARS-CoV polyproteins. The first and second sequences are the C- and N-terminal cleavage sequences, respectively, of 3CL^{pro} itself (1). (*E*) The consensus sequence for cleavage by 3CL^{pro}, analyzed by the sequence logo program (15), using the sequences shown in *D* and WebLogo version 2.8.2 (weblogo.berkeley.edu/) (16).

T. Muramatsu et al, PNAS 113, 12997 (2016)

- Fragment KIVKG
  - K ⇒ Lysine (lys)
  - I ⇒ Isoleucine (ile)
  - V ⇒ Valine (val)
  - G ⇒ Glycine (gly)
- Convention: write the sequence from N- (left) to C-terminus (right).
- Terminal groups: ACE (CH_3CO-) and NME (-$NHCH_3$)

PyMOL

Working directory

```
> mkdir pymol
> cd pymol
> cp ../for-students/pymol/6lyr.pdb .
```

Pymol

```
> pymol 6lyr.pdb &
```

Modify the style

```
PyMOL > as lines
PyMOL > as spheres
PyMOL > as ribbon
PyMOL > as cartoon
PyMOL > as sticks
PyMOL > as surface
```

PyMOL

Working directory

```
> mkdir 1-build
> cd 1-build
> cp ../for-students/pdb-file-charmm27/3clpro.pdb .
```

Pymol

```
> pymol 3clpro.pdb &
```

Review the sequence: Display, Sequence or Display, Sequence mode. Residue Names

Protein Data Bank (PDB)

Through an internet information portal and downloadable data archive, the PDB provides access to 3D structure data for large biological molecules (proteins, DNA, and RNA). https://www.rcsb.org

PDB

PDB file

```
> less 3clpro.pdb
```

- ATOM
- Atom number
- Atom class (C is carbon atom in carbonyl groups in the backbone chain, CA is an alpha carbon atom,....)
- Residue name (three letter code)
- Residue number (from N terminal into C terminal)
- (x,y,z) coordinates in Amstrongs
- Ocuppancy. (X-ray)
- Temperature factor. (X-ray)
- Element name (4 characters): 1-2 Chemical Symbol

MD package

Paths

Add the following line to your .bashrc file

```
source /usr/local/gromacs/bin/GMXRC.bash
```

GROningen MAchine for Chemical Simulations

```
> gmx -version
```

Copyright (c) 1991-2000, University of Groningen, The Netherlands.
Copyright (c) 2001-2017, The GROMACS development team at:
Uppsala University, Stockholm University and the Royal Institute of Technology, Sweden.
check out http://www.gromacs.org for more information.

Force fields in Gromacs

Force fields (ff)

```
> ls -lita $GROMACS_DIR/share/gromacs/top |grep ff
```

AMBER (94, 99, 99sb, 99sb-ildn, 99, 03, GS)
CHARMM27
GROMOS(96) (43a1/2/3,53a5,53a6), Modif GROMOS87
OPLS-AA

Contributions:
http://www.gromacs.org/Downloads/User_contributions/Force_fields

Force fields in Gromacs

ff organization

```
> ls $GROMACS_DIR/share/gromacs/top/charmm27.ff
```

`forcefield.doc`	FF documentation
	Force Field parameters
`forcefield.itp`	Includes the whole FF topology (`less` and see)
`ffnonbonded.itp` `ffnanonbonded.itp`	Non bonded terms (VdW)
`ffbonded.itp` `ffnabonded.itp`	Bonded terms (bonds, angles, dihedrals)
`gb.itp`	Implicit solvation parameters
`cmap.itp`	CHARMM27 specific parameters
`aminoacids.rtp`	Residue Topology Database
also for `dna` and `rna` (depends on the force field)	

Gromacs modules

General syntax

`> gmx program_name -flags` (single precission)
`> gmx_d program_name -flags` (double precission)

`> gmx pdb2gmx -h`

Options to specify input files:

`-f` [<.gro/.g96/...>] (eiwit.pdb) Structure file: gro g96 pdb brk ent esp tpr

Options to specify output files:

`-o` [<.gro/.g96/...>] (conf.gro) Structure file: gro g96 pdb brk ent esp
`-p` [<.top>] (topol.top) Topology file

Other options:

`-[no]ter` (no) Interactive termini selection, instead of charged (default)

Topology

Create topology

```
> gmx pdb2gmx -f 3clpro.pdb -o 3clpro.gro -p 3clpro.top -ter
```

Select the Force Field:

```
8: CHARMM27 all-atom force field (CHARM22 plus CMAP for proteins)
```

Select the Water Model:

```
1: TIP3P TIP 3-point, recommended
```

Select start terminus type for ACE-1:

```
2: None
```

Select end terminus type for CT3-7

```
4: None
```

```
You have successfully generated a topology from: 3clpro.pdb.
The Charmm27 force field and the tip3p water model are used.
```

Topology

Files

```
> less 3clpro.gro
```

Free text
Number of atoms
Residue Number, Residue name, atom class, atom number, (x,y,z) coordinates in nm

```
> less 3clpro.top
```

Data requiered to evaluate the potential energy function: charges, bonded atoms, angles, dihedrals,...

Periodic boundary conditions

Unit cell

```
> gmx editconf -f 3clpro.gro -o 3clpro-box.gro -bt cubic -box 3.0
3.0 3.0
```

-bt <enum> (triclinic) Box type for -box and -d: triclinic, cubic,...
-box <vector> (0 0 0) Box vector lengths (a,b,c)

Solvent molecules

```
> gmx solvate -cp 3clpro-box.gro -cs -o 3clpro-box-solv.gro
-p 3clpro.top
```

```
Output configuration contains 2648 atoms in 857 residues
Volume : 27 (nm^ 3)
Density : 981.765 (g/l)
Number of SOL molecules: 850
```

At 25°C water density is 0.997 kg/l

Periodic boundary conditions

Final result

```
> less 3clpro-box-solv.gro
```

contains now the water solvent molecules

```
> gmx editconf -f 3clpro-box-solv.gro -o 3clpro-box-solv.pdb
> pymol 3clpro-box-solv.pdb
```

Input files

Working directory

```
> cd ..
> mkdir 2-equilibration
> cd 2-equilibration
> cp ../1-build/3clpro-box-solv.gro .
> cp ../1-build/3clpro.top .
```

Input files

.mdp file

```
> cp ../for-students/equilibration-mdp/equiNVT.mdp .
> less equiNVT.mdp
integrator = md ; leap-frog integrator
dt = 0.0005 ; 0.5 fs
nsteps = 8000 ; 4 ps
nstxout = 200 ; Save coordinates every 0.1 ps
Tcoupl = v-rescale
gen_vel = yes ; Should be the first equilibration
gen_temp = 298.0 ; Temperature to generate corresponding
Maxwell distribution
gen_seed = 12345678 ; Random seed
```

Neutralization

.tpr file

```
gmx grompp -f equiNVT.mdp -c 3clpro-box-solv.gro -p
3clpro.top -o 3clpro-a.tpr -maxwarn 1

NOTE 1 [file 3clpro.top, line 975]:
System has non-zero total charge: 2.000000
```

Neutralization

Neutralization

```
> gmx genion -s 3clpro-a.tpr -p 3clpro.top -o 3clpro.gro -nn 2
```

-nn <int> (0) Number of negative ions

```
Select group: SOL
Replacing 2 solute molecules in topology file (3clpro.top) by 0
NA and 2 CL ions.
```

3clpro.gro now includes 2 Cl- atoms

```
> less 3clpro.gro
```

Now the system is neutral

```
> gmx grompp -f equiNVT.mdp -c 3clpro.gro -p 3clpro.top -o
3clpro.tpr
```

Check

```
> gmx editconf -f 3clpro.gro -o 3clpro.pdb
> pymol 3clpro.pdb
```

Equilibration

Equilibration run

```
> gmx mdrun -deffnm 3clpro -c 3clpro.g96 -ntomp 24
```

`-deffnm` <string> Set the default filename for all file options
`-nt` <int> (0) Total number of threads to start (0 is guess)

Output:

`> less 3clpro.g96` (final positions and velocities)
`> less 3clpro.log` (information)

Production run

Working directory

```
> cd ..
> mkdir 3-run
> cd 3-run
> cp ../2-equilibration/3clpro.top .
> cp ../for-students/g96-equilibrated/3clpro.g96 . (400 ps)
> cp ../for-students/run-mdp/runNVT.mdp .
```

runNVT.mdp

```
> less runNVT.mdp

nsteps = 4000 ; 2 ps
nstxout = 2 ; Save coordinates every 1 fs
gen_vel = no ; Should be the first equilibration
```

Production run

Run

```
> gmx grompp -f runNVT.mdp -c 3clpro.g96 -p 3clpro.top -o
3clpro.tpr
> gmx mdrun -deffnm 3clpro -c 3clpro.g96 -ntomp 24
```

Output files:

`3clpro.trr` (coordinates, velocities, ...)

`3clpro.tpr` (Structure+mass)

Production run

Cartoons

```
> gmx traj -f 3clpro.trr -s 3clpro.tpr -oxt cartoon.pdb
```

Select: `0` (System)
Output file: `cartoon.pdb`

```
> pymol cartoon.pdb
```

Play (lower right corner)

Roving_detail shows details of the environment centered on the screen

Display | Roving Detail | Hydrogen Bonds

```
> gmx traj -f 3clpro.trr -s 3clpro.tpr -oxt cartoon.pdb
```

Select: `1` (Protein)
Output file: `cartoon.pdb`

```
> pymol cartoon.pdb
```

Play (lower right corner)

Analysis

Working directory

```
> cd ..
> mkdir 4-analysis
> cd 4-analysis
> cp ../3-run/3clpro.tpr .
> cp ../3-run/3clpro.g96 .
> cp ../3-run/3clpro.trr . (coordinates)
> cp ../3-run/3clpro.edr . (energies)
```

Analysis

Bond distances (I)

Create distances.ndx file

```
> cat distances.ndx
[CO-lys-2]
27 28
```

```
gmx distance -f 3clpro.trr -s 3clpro.tpr -n distances.ndx
-oall -xvg none
```

`-xvg` <enum> (xmgrace) Plot formatting: none, xmgrace, xmgr

`-oall` [<.xvg>] (dist.xvg) (Optional) All distances as function of time

```
Select group: 0
Ctrl+d
```

Analysis

Bond distances (II)

Output:

```
CO-lys-2:
Number of samples: 2001
Average distance: 0.12314 nm
Standard deviation: 0.00191 nm
```

```
> less dist.xvg
```

Plot: `> gnuplot`

```
gnuplot> plot 'dist.xvg' with linespoints
```

Analysis

Bond distances (III)

```
> cat distances.ndx
[CO-lys-2]
27 28
[CACB-ILE-3]
31 33
```

```
gmx distance -f 3clpro.trr -s 3clpro.tpr -n distances.ndx
-oall -xvg none
Select group: 1
Ctrl+d
```

Analysis

Bond distances (IV)

Output:

```
CACB-ILE-3:
Number of samples: 2001
Average distance: 0.15340 nm
Standard deviation: 0.00342 nm
```

Plot: `> gnuplot`

```
gnuplot> plot 'dist.xvg' with linespoints
```

Analysis

Bond angles

```
> cat distances.ndx
[CA-C-O-lys-2]
9 27 28
gmx angle -f 3clpro.trr -n distances.ndx -ov -xvg none
Select group: 2 (Ctrl+d)
```

Output:

```
< angle > = 122.146
< angle^2 > = 14932.4
Std. Dev. = 3.57043
```

Plot:

```
> gnuplot
gnuplot> plot 'angaver.xvg' with linespoints
```

Analysis

Dihedral angles

```
> gmx rama -f 3clpro.trr -s 3clpro.tpr -xvg none
> less rama.xvg
> grep LYS-2 rama.xvg | awk '{print $1}' | cat -n >
phi-lys-2.dat
> grep LYS-2 rama.xvg | awk '{print $2}' | cat -n >
psi-lys-2.dat
> wc -l phi-lys-2.dat
2001 phi-lys-2.dat
```

Plot:

```
gnuplot
gnuplot> plot 'phi-lys-2.dat' with linespoints
gnuplot> plot 'psi-lys-2.dat' with linespoints
```

Analysis

Temperature

```
> gmx traj -f 3clpro.trr -s 3clpro.tpr -xvg none -ot
Select a group: 0
```

Plot:

```
gnuplot
gnuplot> plot 'temp.xvg' with linespoints
```

Analysis

Energy

```
> gmx energy -f 3clpro.edr -s 3clpro.tpr -xvg none
Select a group: 15 (double enter)
Energy        Average   Err.Est.   RMSD Tot-Drift
-------------------------------------------------
Total Energy -25421.9   44         150.922 110.342 (kJ/mol)
```

An error estimate of the average is given based on a block averages over 5 blocks using the full-precision averages.

Drift is calculated by performing a least-squares fit of the data to a straight line. The reported total drift is the difference of the fit at the first and last point.

Plot:

```
gnuplot
gnuplot> plot 'energy.xvg' with linespoints
```

Analysis

Kinetic Energy

```
> gmx energy -f 3clpro.edr -s 3clpro.tpr -xvg none
Select a group: 14 (Kinetic energy) (double enter)
Energy        Average   Err.Est.   RMSD Tot-Drift
-------------------------------------------------------
Kinetic En. 9758.48     39       157.374 35.7389 (kJ/mol)
```

Test:

$$2644 \text{ atoms} \times \frac{3}{2} \underbrace{k_B T}_{2.478 \text{kJ/mol}} = 9827 \text{ kJ/mol}$$