BROMOCEA code

C. J. F. Solano

Jacobs University Bremen

Outline

Computational methods for studying ion permeation and substrate translocation

Molecular Dynamics (MD) simulations

- Very valuable tool in Biophysics and Biochemistry
- Limitations:
  - High dimensionality of the state space
  - Membrane channels are open systems (fluctuating number of ions)

Grand Canonical Monte Carlo/Brownian Dynamics (GCMC/BD) simulations [1-7]

- Dimensionality of the state space is reduced by projecting out the *uninteresting* degrees of freedom (fast-moving solvent molecules)
- GC ensembles allow the direct evaluation of ion currents

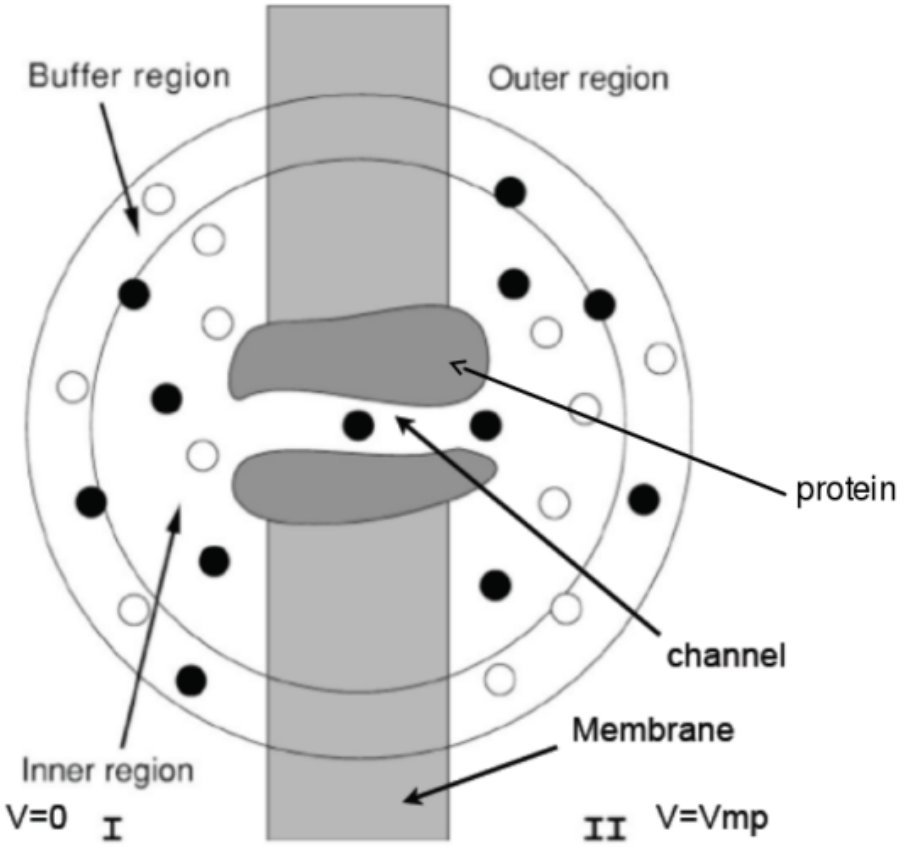
[1] W. Im *et al*, *Biophys. J.* **79**, 788 (2000)
[2] W. Im *et al*, *Biophys. J.* **115**, 4850 (2001)
[3] W. Im *et al*, *J. Mol. Biol.* **322**, 851 (2002)
[4] S. Y. Noskov *et al*, *Biophys. J.* **87**, 2299 (2004)
[5] B. Roux *et al*, *Quart. Rev. Biophys.* **37**, 15 (2004)
[6] B. Egwolf *et al*, *J. Phys. Chem. B* **114**, 2901 (2010)
[7] K. I. Lee *et al*, *Biophys. J.* **100**, 611 (2011)

Outline

General scheme

Only the mobile ions are explicitly simulated, while the influence from the protein, lipid, and water is implicitly taken into account via effective time-invariant potential and dielectric maps

- **Inner and buffer regions**: ions treated explicitly and their trajectories calculated from stochastic equations (BD algorithm)
- **Buffer region**: ions concentration maintained in equilibrium with bulk solution using GCMC algorithm
- **Outer region**: ions treated implicitly
- **Protein**: rigid ion-inaccessible low dielectric medium of irregular shape containing a distribution of atomic charges
- **Membrane**: rigid ion-inaccessible low dielectric medium
- **Solvent (water) region**: ion-accessible high dielectric medium and source of random diffusive force



Overdamped Langevin equation and many-body PMF

- **Overdamped Langevin equation**

$$\frac{d\mathbf{r}_i}{dt} = -\frac{D_i(\mathbf{r}_i)}{k_B T}\nabla_i W(\mathbf{r}_1, \ldots, \mathbf{r}_N) + \nabla_i D_i(\mathbf{r}_i) + \zeta_i(t)$$

  - Assumptions: (i) diffusive regime; (ii) hydrodynamics effects are negligible; and (iii) ion self-diffusion constants are position-dependent (along the channel axis)
  - W is the many-body potential of mean force (PMF), $D_i(\mathbf{r}_i)$ is the diffusion constant, and ζ_i the random Gaussian noise representing water collisions with the mobile ions

- **Many-body PMF**

$$W(\mathbf{r}_1, \ldots, \mathbf{r}_N) = \sum_i^N \left\{ q_i \left[\phi_{sf}(\mathbf{r}_i) + \frac{\phi_{rf}(\mathbf{r}_i)}{2} \right] + U_{core}(\mathbf{r}_i) \right\} + \sum_{j>i}^N u_{ij}(r_{ij})$$

  - $\phi_{sf} \equiv$ electrostatic potential from the protein charges and the transmembrane potential
  - $\phi_{rf} \equiv$ reaction field potential arising from the dielectric hetero-junction between solvent, protein, and lipid
  - $U_{core} \equiv$ core-repulsive steric potential from the ion-inaccessible region (protein and lipid)
  - $u_{ij} \equiv$ ion-ion potential including the Lennard-Jones, water-mediated short-range, and dielectric-screened Coulombic electrostatic terms

Limitations

- The treatment of water as a featureless dielectric medium is both a strength and a weakness: it reduces the simulation time by many orders of magnitude as compared to MD simulations but the effects arising from the granularity of the water molecules and their ability to form hydrogen bonds are ignored (relevant in narrow channels)
- Selection of values for the different dielectric constants (water, membrane and protein): the local dielectric constant of water may vary considerably depending on the environment and the protein dielectric constant has a large effect in narrow channels
- **Assumption of a rigid channel**: the motion of polar residues lining the pore may have a significant impact on the ion permeation

Outline

Motivation and description

- **Main goal**: include **explicit atoms** to simulate substrate translocation as well as allows some **flexibility in the pore**
- **Overdamped Langevin equation** can be extended to include explicit atoms without additional modifications
- **PMF** is now a sum over CHARMM [8] internal terms for explicit atoms and nonbonded terms among ions and explicit atoms

$$
\begin{aligned}
W(\mathbf{r}_1,\ldots,\mathbf{r}_N) = & \sum_{bonds} K_b(b-b_0)^2 + \sum_{angles} K_\theta(\theta-\theta_0)^2 \\
& + \sum_{Urey-Bradley} K_S(S-S_0)^2 + \sum_{dihedrals} K_\varphi(1+\cos(n\varphi-\delta)) \\
& + \sum_{impropers} K_\omega(\omega-\omega_0)^2 + \sum_{residues} U_{CMAP} \\
& + \sum_{i}^{N}\left\{ q_i\left[\phi_{sf}(\mathbf{r}_i) + \frac{\phi_{rf}(\mathbf{r}_i)}{2}\right] + U_{core}(\mathbf{r}_i)\right\} + \sum_{j>i}^{N} u_{ij}(r_{ij})
\end{aligned}
$$

[8] B. R. Brook *et al*, *J. Comp. Chem.* **30**, 1545 (2009)

Implementation details

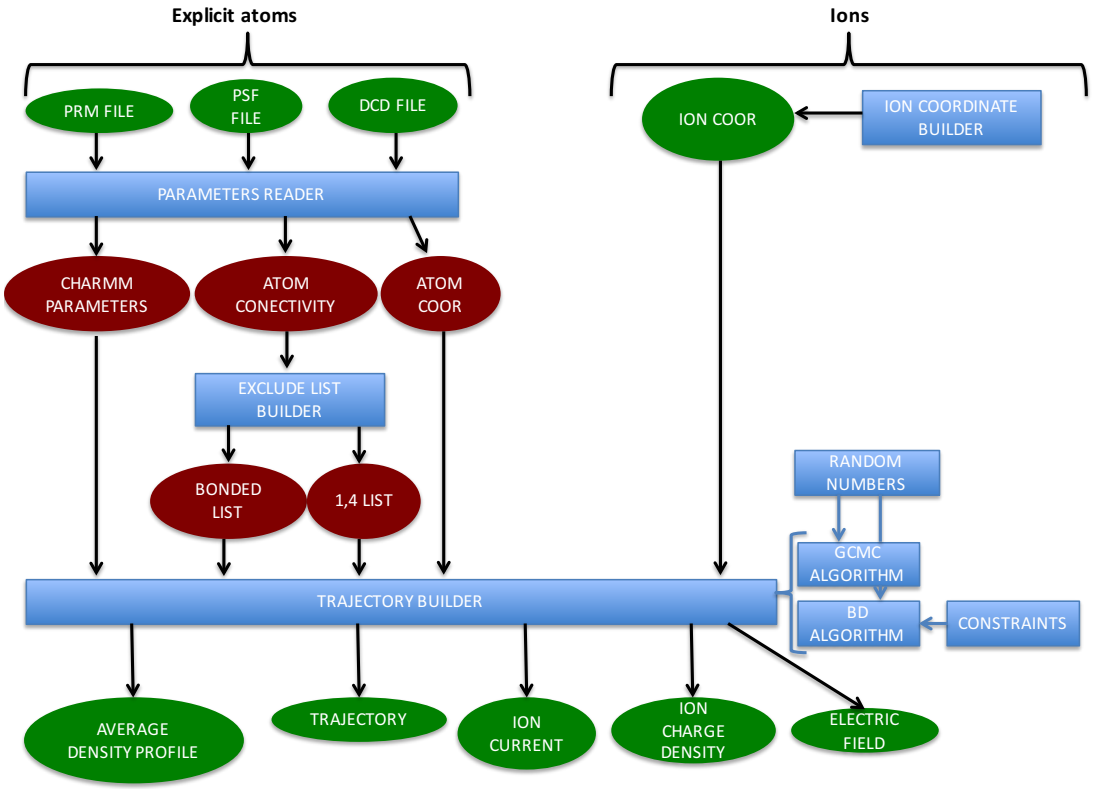
- **CHARMM forces field** for explicit atoms (including backbone torsional correction [9, 10])
- **Distance-dependent dielectric constant** [11] in Coulomb interactions
- **Atom-based force-switching method** [12] for nonbonded forces
- **Alternative core-repulsive potential scheme** where precomputed look-up table is replaced by *on the fly* Lennard-Jones potentials
- **Ermark and McCammon and Second-order stochastic Runge-Kutta algorithms** [13, 14] for BD propagation
- **Bond distance constraints**: SHAKE method [15] has been adapted for using in the BD framework

[9] A. D. MacKerell *et al*, *J. Am. Chem. Soc.* **126**, 698 (2004)
[10] A. D. MacKerell *et al*, *J. Comput. Chem.* **25**, 1400 (2004)
[11] E. L. Mehler *et al*, *Biophys. J.* **75**, 3 (1999)
[12] P. J. Steinbach *et al*, *J. Comp. Chem.* **15**, 667 (1994)
[13] D. L. Ermak *et al*, *J. Chem. Phys.* **69**, 1352 (1978)
[14] D. M. Heyes *et al*, *Mol. Phys.* **98**, 1949 (2000)
[15] J. P. Ryckaert *et al*, *J. Comput. Phys.* **23**, 327 (1977)

BROMOCEA code

- Fortran 90 code implemented as from the BROMOC-D code [16] (> 160 routines)
- Supporting equilibrium and non-equilibrium conditions of ion permeation with a realistic implementation of boundary conditions and transmembrane potential

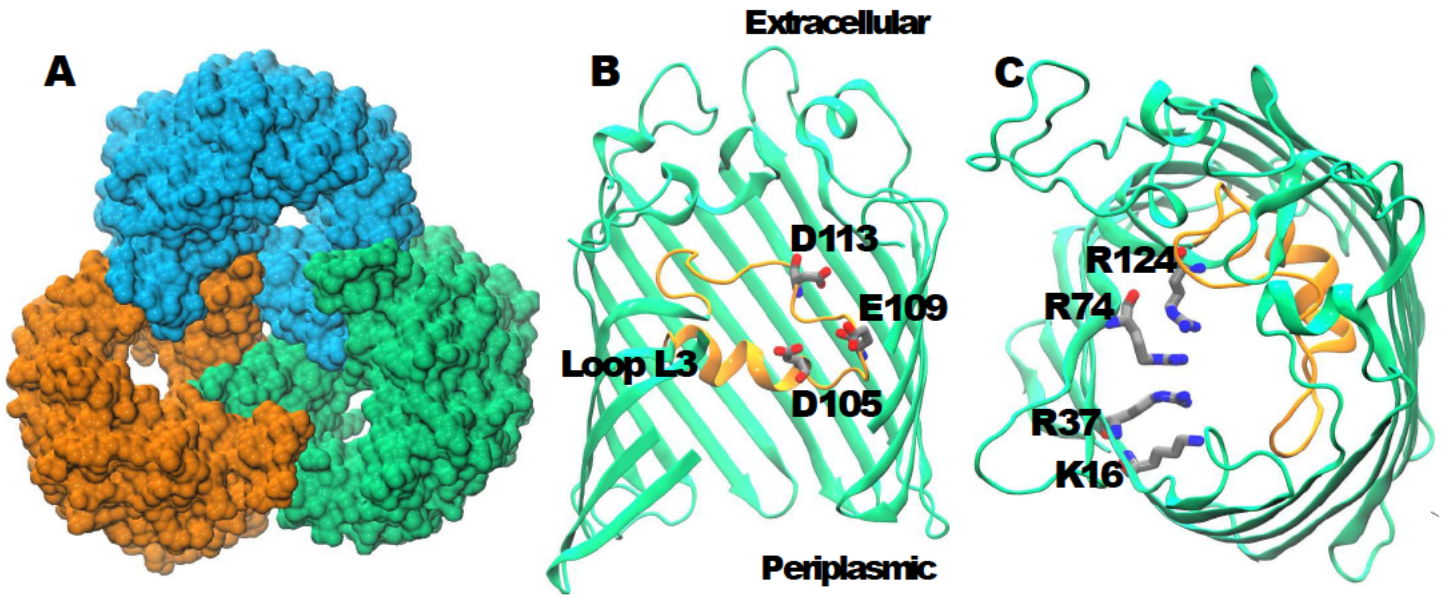
Figure : Diagram depicting the general scheme of the information flow in a BROMOCEA project. Key: green boxes: data files (i.e., input and output files); red boxes: data structures; blue boxes: functionalities/modules



[16] P. M. De Biase *et al*, *J. Chem. Theory Comput.* **8**, 2540 (2012)

Ion permeation across OmpC pore

Figure : (A) Surface representation of the OmpC trimer (PDB ID: 2J1N) from *E. coli* with each monomer formed by 16 β-strands. (B) An OmpC monomer is shown with the L3 loop in yellow leading to a narrow pore together with important negatively charged residues (D105, E109 and D113) shown as sticks. (C) Positively charged residues (K16, R37, R74 and R124) located on the barrel wall, opposite to L3 loop, are shown as sticks



- Residues from 104 to 114 in the L3 loop (including negatively charged D105, E109 and D113) undergo thermal fluctuations
- Positively charged residues K16, R37, R74 and R124 also undergo thermal fluctuations

Ion permeation across OmpC pore

Table : Average number of ions in the channel N and average conductance G for 0.3 M KCl at 0.1 V transmembrane potential and 300 K

METHOD	N (K^+)	N (Cl^-)	G [nS]
Original GCMC/BD	3.07±0.01	0.17±0.02	0.56±0.01
Improved GCMC/BD	6.59±0.06	0.79±0.07	0.63±0.04
All-atom applied-field MD	5.95±1.64	1.87±1.06	0.09[1]
Experiment [17]			∼0.41

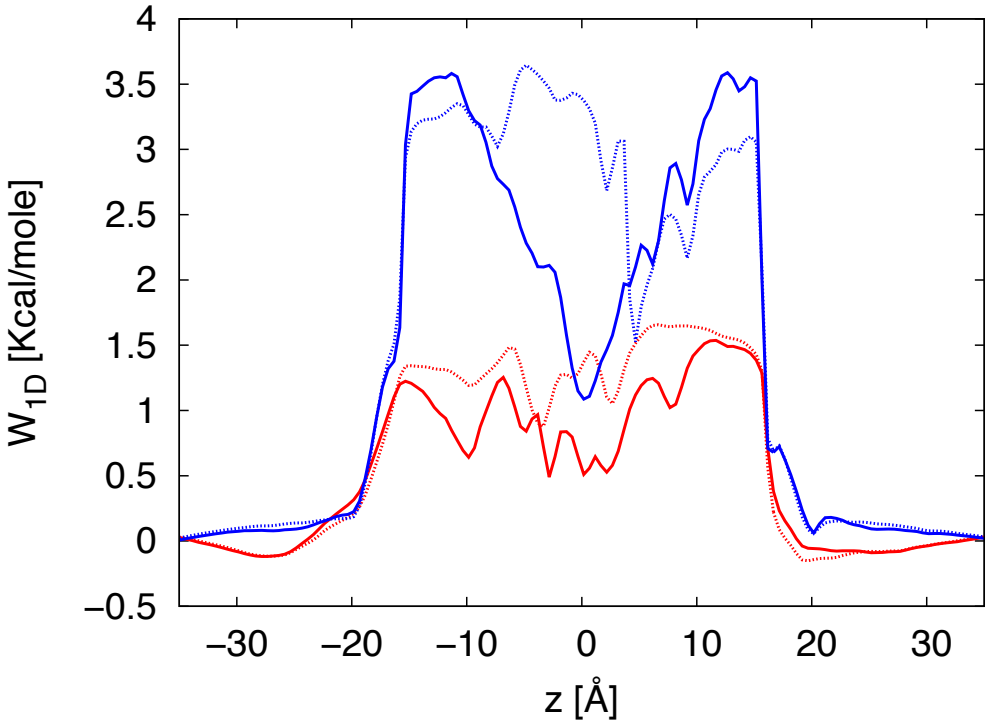
The inclusion of some flexibility in the OmpC constriction zone allows for obtaining an average number of ions inside the channel in agreement with MD simulations and a reasonable conductance estimation

[17] I. Biro *et al*, *Biophys. J.* **97**, 1898 (2009)

[1] The poor conductance estimation is a consequence of the low ion concentration and applied voltage

Ion permeation across OmpC pore

Figure : 1D multi-ion average PMF for 0.3 M KCl solution at equilibirum conditions (i.e., zero transmembrane potential) and 300 K using the original GCMC/BD (dotted lines) and the improved GCMC/BD (solid lines)



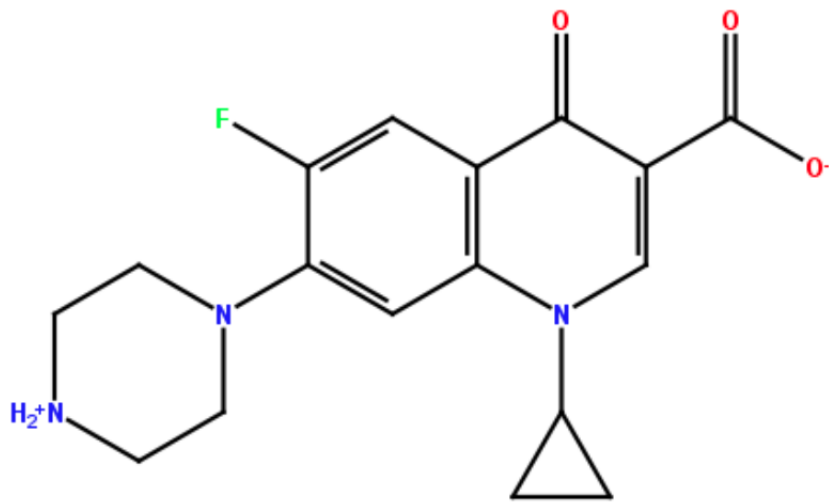
The ion PMFs are sensitive to the inclusion of some pore flexibility in the GCMC/BD framework

Ciprofloxacin transport across OmpC pore

Simulation procedure

- $T = 300$ K; Transmembrane potential$= 0, \pm 0.1$ V; Time step$=$ 5 fs; 1 GCMC step at every BD step; Force switching method
- Distance-dependent dielectric constants for Coulomb interactions among explicit atoms; SHAKE bond constraints
- 10 independent runs
- Initial configuration: ciprofloxacin $\sim$ 10 Å away from the constriction zone (extracellular side)
- Two types of simulations: (i) **without ions** (SWHI); (ii) **0.3 M KCl solution** (SWI)

Figure : Ciproloxacin forces field parameterization methodology with ffTK [18] (VMD plugin)

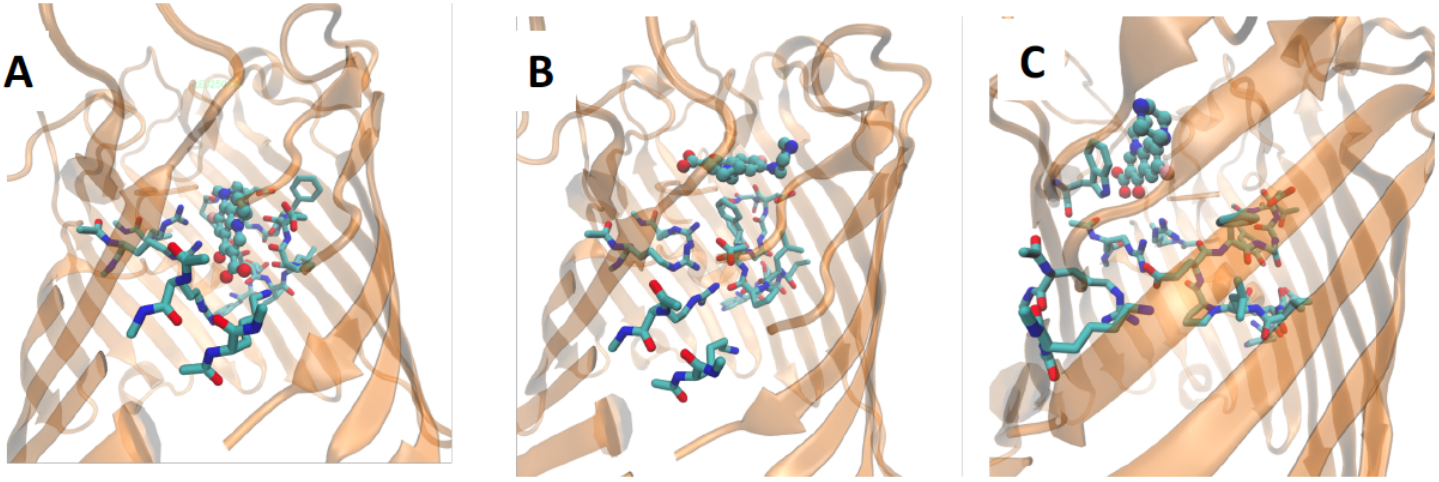


[18] C. G. Mayne *et al*, *J. Comput. Chem.* **34**, 2757 (2013)

Ciprofloxacin transport across OmpC pore

SWHI simulations (without ions)

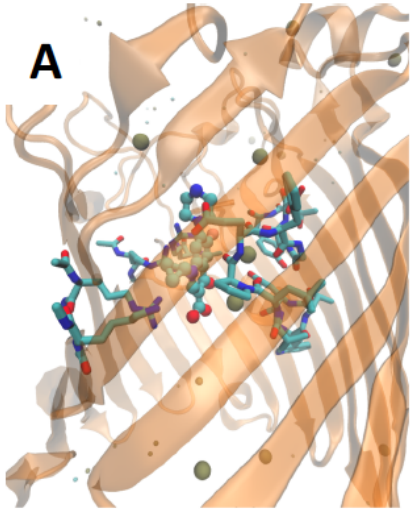
- Ciprofloxacin always approaches to the constriction zone
- All dipole orientations are allowable at the mouth of the constriction zone (Fig. B)
- Strong feedback between Ciprofloxacin and flexible L3 loop
- Ciprofloxacin goes deeper inside the constriction zone when the COO^- group is ahead (Fig. A)
- π-stacking between Ciprofloxacin and residue 72 (Fig. C)
- No influences of different voltages on the ciprofloxacin transport

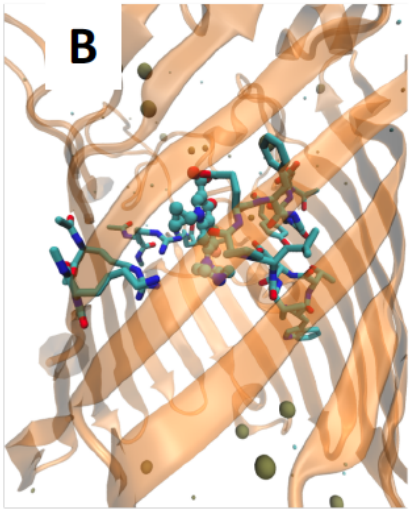


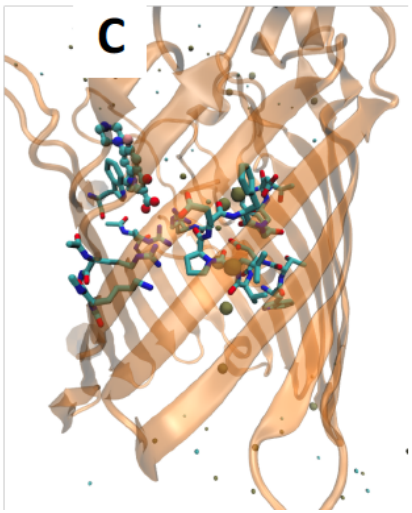
Ciprofloxacin transport across OmpC pore

SWI simulations (0.3 M KCl)

- Proportion of simulation in which Ciprofloxacin approaches to the constriction zone: 60% ($V_{mp} = 0$), 70% ($V_{mp} = 0.1\,V$), 50% ($V_{mp} = -0.1\,V$)
- Different preferred orientation: COO^- group ahead ($V_{mp} = 0, 0.1\,V$); $H2N^+$ group ahead ($V_{mp} = -0.1\,V$)
- Ciprofloxacin goes deeper inside the constriction zone mainly when the COO^- group is ahead (Fig. A). Only 1 case in which the $H2N^+$ group is ahead inside the constriction zone (Fig. B)
- Long time and stable π-stacking between Ciprofloxacin and residue 72 (Fig. C)







Future work

- Gain extra computational efficiency: (i) multiple time step method [19]; (ii) OpenMP parallelization
- Procedure for extracting self-diffusion constants from all-atom MD simulations
- Inclusion of hydrodynamics effects among particles
- Inclusion of polarization in the PMF(atom dipole polarizability model)
- Techniques for enhanced sampling (umbrella sampling, metadynamics)