Accurate Description of Antibiotics Translocation through Membrane Pores

Enhanced Sampling Methods for Accurate Free Energy Estimates of Antibiotic Permeation Process through Porins

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In Short

- Antibiotics are transported into bacterial cells via outer-membrane channels called porins.
- A quantitative and accurate description of the translocation process through porins is a prerequisite for understanding how different antibiotics permeate.
- Enhanced sampling methods within the framework of molecular dynamics expedite the collection of data essential for obtaining good estimates of important quantities like free energy. The lack of convergence of these estimates from independent simulations is, however, a concern.
- The present has identified temperature accelerated sliced sampling (TASS) as a suitable approach to study antibiotic permeation that is able to improve sampling along high-dimensional configurational space.
- Applications of the method towards answering some of the critical questions regarding antibiotic permeation via porins will be addressed using the TASS method

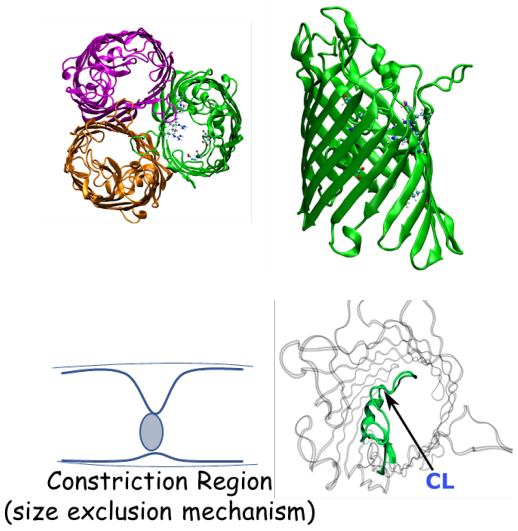

Figure 1: Structure of a typical porin. *The upper panels show top and side views of the OmpF porin. The OmpF is a trimeric complex embedded within the outer membrane. The lower panels depict the constriction loop at the center of the channel that acts as a size-exclusion filter.*

The outer membrane of Gram-negative bacteria acts as an impenetrable barrier against external agents. However, this layer incorporates a number of proteins that allow passive diffusion or specific transport of a range of solutes such as nutrients and ions essential for growth and survival. Among these, some are general diffusion channels called porins that allow the passive transport of a variety of solutes, including antibiotics [1].

Porins are composed of a β-barrel formed by antiparallel β-strands connected by loop segments forming a water-filled pore with a constriction region (CR) with a relatively narrow pore diameter.(Figure 1). The CR acts as a size exclusion filter limiting solutes with molecular weight up to 600 Dalton. Interestingly, the porins OmpF and OmpC (and their orthologues) have been identified as the major routes for antibiotic permeation into Gram-negative bacteria. A number of experimental and computational studies have unraveled details pertaining to factors such as solute charge, mass, polarity, pore diameter, stabilizing interactions at the pore constriction etc. that influence the solute permeation rates through these channels [2]. Recent efforts to towards the design of an efficient antibiotic has also showed the importance of an ionizable amine group on the antibiotic scaffold for efficient permeation [3]. Detailed simulations studies are necessary to rationalize these observations using atomic level description of the permeation mechanism.

The simulations of complex processes such as antibiotic permeation typically suffer from sampling issues. Enhanced sampling methods try to address this issue by applying a potential bias that pushes the system out of these low energy states to the not sufficiently sampled regions (Figure 2) of higher energy or low energy states in a different part of the phase space. Previously, our group has used umbrella sampling and metadynamics to study antibiotic permeation through porins and demonstrated that these methods are unable to achieve good sampling and acceptable convergence in antibiotic-porin simulations. [4,5] An initial aim of the present project was to identify a method that enables effect sampling in these cases. We have recently demonstrated that the temperature accelerated sliced sampling (TASS)

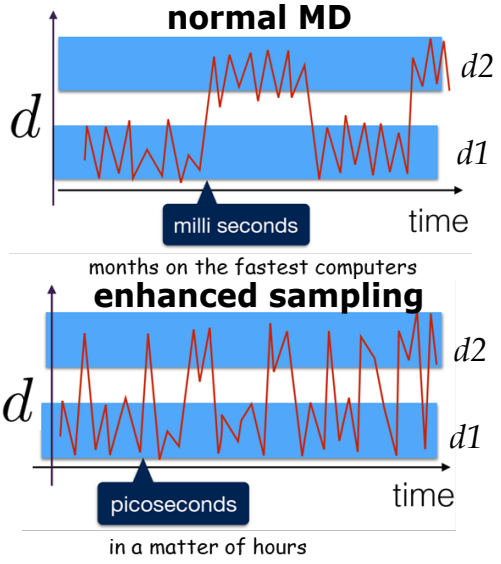

Figure 2: Enhanced sampling schemes improve the rate of transition between states. *This simple scheme depicts two important states d1 and d2 that the system can attain. Depending on the energy barrier between the two states, a normal MD simulation may sample these states only a few times in millisecond long simulations (upper panel). Enhanced sampling methods increase the probability of visiting these states enabling improved statistics for the same computational cost (lower panel).*

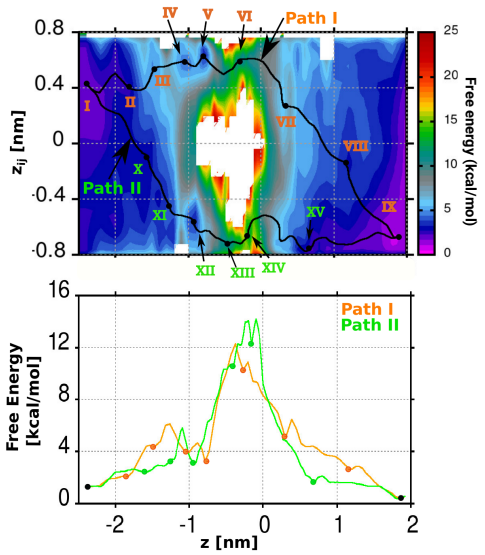

Figure 3: Results from temperature accelerated sliced sampling (TASS) simulations of ciprofloxacin through OmpF. *The upper panel depicts the two-dimensional free energy surface reconstructed from TASS simulations. The lines depict the possible pathways taken by the possible permeation paths and associated antibiotic configurations (in roman numerals). The lower panel depicts the free energy corresponding to the two permeation paths. Adapted with permission from ref ([6]). Copyright 2021 American Chemical Society.*

enables efficient sampling in case of ciprofloxacin permeation through the OmpF channel [6]. We were able to provide accurate estimates of the free energy for permeation and observed good convergence in independent simulation runs (3).

Our next objective to apply TASS to a variety of antibiotics of various complexities to test both the general applicability and limits of the method, and also address important biological questions pertaining to antibiotic permeation. Preliminary studies in this directions have revealed that while the method achieves acceptable sampling and convergence of free energy estimates in most cases, convergence remains an issue in certain specific cases. Future efforts are aimed towards optimizing the simulation setup and TASS methodology to improve the performance in these cases.

WWW

http://www.jacobs-university.de/comp_phys

More Information

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