Biochimica et Biophysica Acta 1858 (2016) 1772–1777

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

# A kinetic model for molecular diffusion through pores☆

Tommaso D'Agostino, Samuele Salis, Matteo Ceccarelli *

*Department of Physics, University of Cagliari, Italy*

ARTICLE INFO

*Article history:*
Received 31 October 2015
Received in revised form 18 December 2015
Accepted 7 January 2016
Available online 12 January 2016

*Keywords:*
Porins
Molecular dynamics
Kinetics
Transport

ABSTRACT

The number of pathogens developing multiple drug resistance is ever increasing. The impact on healthcare systems is huge and the need for novel antibiotics as well a new way to develop them is urgent, especially against Gram-negative bacteria. The first defense of these bacteria is the outer membrane, where unspecific protein channels (porins) modulate nutrients passive diffusion. Also polar antibiotics enter through this path and down-regulation and/or mutation of porins are very common in drug resistant strains. Our inability to come up with novel effective antibiotics mostly relies upon the insufficient comprehension of the key molecular features enabling better penetration through porins.

Molecular dynamics simulations offer an extraordinary tool in the study of the dynamics of biological systems; however, one of the major drawbacks of this method is that its use is currently restricted to study time scales of the order of microsecond. Enhanced sampling methods like Metadynamics have been recently used to investigate the diffusion of antibiotics through bacterial porins. The main limitation is that dynamical properties cannot be estimated because of the different potential that the systems under study are experiencing. Recently, the scope of Metadynamics has been extended. By applying an a posteriori analysis one can obtain rates of transitions and rate-limiting steps of the process under study, directly comparable with kinetic data extracted from electrophysiology experiments. In this work, we apply this method to the study of the permeability of *Escherichia coli*'s OmpF with respect to Meropenem, finding good agreement with the residence time obtained analyzing experimental current noise. This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.



## 1. Introduction

Bacterial resistance is a phenomenon that inhibits the capacity of any drug to be effective against pathogens through diverse mechanisms. It is based upon the consideration that, when a patient is under pharmacologic treatment, most of the bacteria will be killed and the most resistant mutants will be able to prosper and reproduce because of the lack of competitors for food and nutrient resources. Gram-Negative bacteria show a distinct resistance mechanism due to the presence of the additional outer membrane that prevents the permeation of any molecules with sufficiently high rate. This obstruction is particularly severe for small polar molecules, for which diffusion depends strongly on porin expression [1,2].

Outer membrane porins are today believed to be the main pathway for polar antibiotics to reach internal targets [3]. Bacteria can develop resistance by changing the rate with which these compounds permeate the OM, either by modulating the expression of porins, or by selecting mutations of key residues that alter the permeability of the porins themselves [4]. The discovery of new effective polar antibiotics passes through the determination of the mechanism controlling translocation through porins.

A clear representation of the process, showing both the rate-limiting steps of the translocation and the most important interactions that a given molecule experiences during the passage through a single porin, is still missing. This is due to the absence of a direct and robust method to assess permeation by quantifying the flux [5]. Current methods for determining the permeability of porins to different compounds, such as liposome swelling assays [6,7] and electrophysiology [8], rely on indirect measurements of kinetics parameters, with some limitations on their use. Simulation methods, thanks to the very high resolution in time and space, have the capability to investigate the properties that are necessary to improve the passage of molecules through porins and eventually to rationalize the mechanism behind translocation. Though restricted to the range of microseconds, a few orders of magnitude less than biological time, enhanced sampling methods like Metadynamics have been successfully used [7,9,10]. These methods employ a biasing potential that speeds up the exploration of the configurational space, altering the kinetics of the system. In order to compare simulation results directly with experiments, a kinetic model needs to be introduced with an a posteriori analysis of trajectories.

☆ This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.

* Corresponding author at: Department of Physics, University of Cagliari, Cittadella Universitaria di Monserrato, S.P. Monserrato-Sestu Km 0.700 I-09042 Monserrato (CA), Italy.

http://dx.doi.org/10.1016/j.bbamem.2016.01.004
0005-2736/

Some recent methods (e.g. [11–13]) critically depend on the calculated free energy barrier [14] and on the estimation of the diffusion constant for the translocating molecule. On the other hand, Tiwary and Parrinello proposed a new method in which Metadynamics is combined with a reweighting procedure able to get a set of estimated real times for each transition. If the simulated times, after the a posteriori statistical test of Kolmogorov–Smirnov (KS) [15], follow a Poissonian distribution, it is possible to assess the goodness of the obtained set of times. With this approach we do not have (i) to guess on the transmission coefficient and (ii) to use the estimated free energy barrier encountered in the transition to calculate transition times, reducing the otherwise critical dependence on the barrier itself.

The reliability of the method was tested to investigate unbinding of compounds from an enzyme [16], where the co-complex structure was taken as starting point. Here we show how to extend this method to the permeation of polar molecules through porins, where in general the co-complex is either unknown or not unique and might not represent the rate-limiting step of the process. The few co-complexes of porins/antibiotics obtained so far by soaking have shown distinct binding sites for compounds with different total charge. In addition, simulations showed that the permeation doesn't rely on the determined binding site, since for ampicillin the permeation rate through OmpF increases upon destruction of the binding site by point mutations [17].

Our approach consists of consecutive steps: (i) perform a long Metadynamics run to identify in the conformational space the most stable porins/antibiotic complexes (minima in the reconstructed free energy), (ii) perform many short Metadynamics runs from the most stable conformations to obtain the rates of transitions among them, and (iii) apply Tiwary's method to obtain the characteristic time of first escape from the most stable conformations, tested with the KS method, and eventually create a kinetic model of the permeation.

The question whether the translocation process through porins takes place thanks to the existence of either a single or multiple binding sites, that are rate limiting structures, is central to the problem of designing antibiotics with enhanced permeation. Recently we introduced a new method to analyze those electrophysiology current traces that show a simple decrease in the average current upon antibiotics addition, without any time-resolved jump that would allow estimating kinetic parameters [18]. We applied this method to investigate the diffusion of Meropenem through *Escherichia coli* OmpF. The measured residence time, a few microseconds only, suggests the existence of a binding site with a very low affinity. The approach proposed here is applied to this example and, as we will see, we can distinguish between two possible reactive paths of diffusion identifying the real rate limiting steps. This approach offers a unique insight in the peculiarities that determine the evolution of the process at very short time scales, overcoming the intrinsic limitations of both experimental and computational methods [19]. The approach is general enough to be extended to other channels, such as the VDAC pore located in the outer membrane of mitochondria and involved in transport of substrates [20].

## 2. Methods

### 2.1. Force field, software used

All the simulations were run using ACEMD software [21]. The interactions were calculated using the AMBER 99SB-ILDN force field [22] for the protein, tip3p for water [23] and GAFF for lipids and substrates [24]. The parameters used for Meropenem are available in [25]. Metadynamics simulations were performed with PLUMED plugin, version 1.3 [26] and visualization and plotting of molecular interactions, as well as measurement of the most stable interactions, were done with VMD software [27].

### 2.2. Equilibration

The protein (PDB accession code: 2OMF) was inserted into a pre-equilibrated POPC bilayer. Based on PropKa results [28], residue E296 was protonated. Lipids located within 3.0 Å from the trimer were deleted (the final membrane was composed by 259 POPCs), and the membrane was relaxed through small, successive NVT simulations with increasing temperature. The position of the protein's $C_\alpha$, as well as the z-coordinate of the phosphorous heads of the lipids and hence the width of the OM, were restrained. After the lipids were packed around the protein, 17,372 water molecules were added and through successive NVT and NPT simulations for up to 1 ns the system reached the final dimensions of $107.9 \times 107.9 \times 86.7$ Å. To make sure that the system had reached a stable conformation, a 400 ns NVT simulation was run and the average accessible radius was calculated (Supplemental Fig. 1).

### 2.3. Metadynamics

To enhance the exploration of the phase space, we followed the scheme reported in [26]. Two collective variables (CVs) of the system under study were selected and their fluctuations over time were increased by adding a history-dependent potential, reducing the probability of obtaining a conformation already seen during the simulation (Supplemental Fig. 2).

CV1 or Zcm (vertical axis of the FESs): projection along the axis of diffusion (that is, the direction perpendicular to the plane of the membrane) of the distance between the center of masses of protein and antibiotic.

CV2 (horizontal axis of the FESs): orientation of the substrate, as given by the normalized projection, on the axis of diffusion, of the distance between the positive ($NH_2+$) and negative ($CO_2$-) groups of the antibiotic. A positive value corresponds to the antibiotic pointing toward the periplasmic space with its positive group.

The main Free Energy Surface (FES) was obtained with the simultaneous exploration of 4 walkers (Fig. 1A) [29]. Following the well-tempered scheme [30], we added a Gaussian potential every 2 ps with initial height of 1.2 Kcal/mol and modulated by a secondary temperature of 5000 K. On the other hand, in single-shot simulations, under the hypothesis that the saddle zone of the transition should not be considerably biased by the hills depositions, we lowered the initial height of the hills to 0.1 Kcal/mol with a secondary temperature of 3000 K and the inverse deposition rate to 5 ps. From now on, we will refer to this particular set of parameters as *infrequently biased* condition. To calculate the times that unbiased simulations would have required for obtaining the same results, the method expressed in [16,31] was used and the acceleration factor caused by the deposition of the external potential was calculated. We then applied an exponential fit of the single transitions [15,31] to obtain the characteristic, real time of the processes.

## 3. Results

We reconstructed the free energy with respect to the two collective variables using a 2 μs long Metadynamics run in order to identify the most probable conformations. After 1.5 μs, we didn't observe any substantial change in the free energy surface and in the location of most stable states (Fig. 1A). The region located in the range of Zcm [−5:2] Å shows on average high free energy: we see a central prohibited region and two minima, labeled L (left) and R (right), corresponding to Meropenem translocating with its principal axis parallel to the direction of diffusion, respectively with the negative and positive group pointing down. This is the constriction region, where steric hindrance plays an important role in the permeation by not giving to Meropenem enough space to reorient. On the other hand, the presence of three minima, one just above the constriction region, C, and the other