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Exploring Free Energy Profiles Through Ion Channels: Comparison on a Test Case

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Abstract

The calculation of free energy profiles in proteins, and more specifically in ion channels, is a challenge in modern numerical simulations due to convergence problems associates with the electrostatics of the environment and to the modelisation of the fields acting on the permeating ions. The present study is aimed at comparing different simulation techniques, with the purpose of testing their capabilities and limits.

Introduction

The translocation of single ions through cell membranes underlies many important physiological features, such as electrical signaling in neural and muscular systems [1], but despite of a large number of electrophysiological recordings many keypoints are still open questions.

Several steps forward to a deeper knowledge of these problems have been performed after the elucidation of the crystal structure of a bacterial potassium channel, KcsA from *Streptomices Lividans* [2], when it became clear that only microscopic simulations can provide a detailed and quantitative description of many properties of such systems. From atomistic simulations an estimate of teh potential of mean force (PMF) can be obtained and, from it, the energy profiles and barriers ruling the conduction process can be extracted.

Discussion

Different techniques aiming at the reconstruction of the PMF have been applied so far. We here show an application of three methods, namely Steered Molecular Dynamics (SMD) [3], the most widely used Umbrella Sampling Method [4] and finally the recently appeared Metadynamics [5] to a test case, i.e. ions permeating the KcsA potassium channel. The purpose is to establish the degree of reliability and the convergence limitations of the three techniques through their application to the same system.

X-ray investigations, furtherly confirmed by molecular dynamics simulations, show that the conduction process in the KcsA channel involves a short and narrow region of the protein called selectivity filter. Two ions should always reside in this region in a stable conductive situation; at lower potassium concentrations the protein changes it conformation and switcehs to a non-conductive state. The conduction process involves the simultaneous and concerted movement of ions in a single-file, giving origin to a cycle of different occupancy configurations, as indicated in the sketch reported in Fig. 1. Each transition between different configurations is identified by a proper free energy barrier.

In this study we are interested in reconstructing the free energy profile associated to internal transitions, i.e. transitions not involving new ion entries or exits. Our simulative framework is composed by the KcsA atomic structure solved at 2.0 $\mathring{A}$ resolution [6], 8 potassium ions, 24 clorine ions acting as counterions to keep the system electrically neutral, 500 octane molecules mimicking the cell membrane, and 8802 water molecules (SPC model), giving a total of approximately 35000 atoms. In the simulations we adopted the standard GROMACS force field, using the GROMACS [7] package for SMD and US and the ORAC [8] code for metady-

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namics.

Preliminary results for SMD and US simulations are shown in Fig. 2 for a concerted motion of two ions where the energy profile has been reported to a comparable scale with a common reference. From both techniques an energy barrier of approximately $2.5kT$ is found, which is consistent with analogous calculations available in the literature [9]. However the position of the minima obtained from independent implementations of the two methods is different, mainly for the situation after barrier crossing, which is higher for SMD. We address this problem to the intrinsic one-dimensional one-way forced motion of ions in SMD with respect to US. Some minor, but not less important differences due to the reduced set of coordinates used in the two simulations, are also present in trajectory analysis and can affect the final results.

The third proposed technique should be implemented to futher validate the PMF reconstruction. Metadynamics, which is still under investigation at present time and whose results will be discussed at the Workshop, promises to be a more easily converging method and produces an estimate of the PMF without introducing artificial biases on ion dynamics. In fact once a set of appropriate coordinates is chosen, the dynamics is "driven" directly by the free energy profile of the system and it is biased only through the controlled summation of Gaussians centered on the particular trajectory followed. The sampled potential can provide directly quantitative informations on the original structure of the PMF.

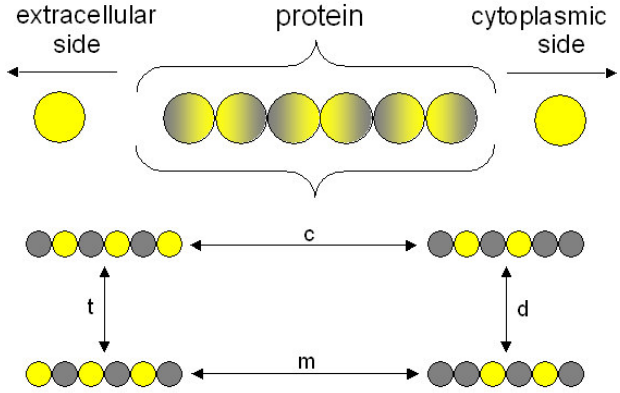

Fig. 1. Conduction cycle inside the channel. Ions move concertedly following a 4-step mechanism like entry (c), double movement (d), triple movement (t) and exit (m). Light grey balls represent potassium ions and dark grey balls stand for water molecules.

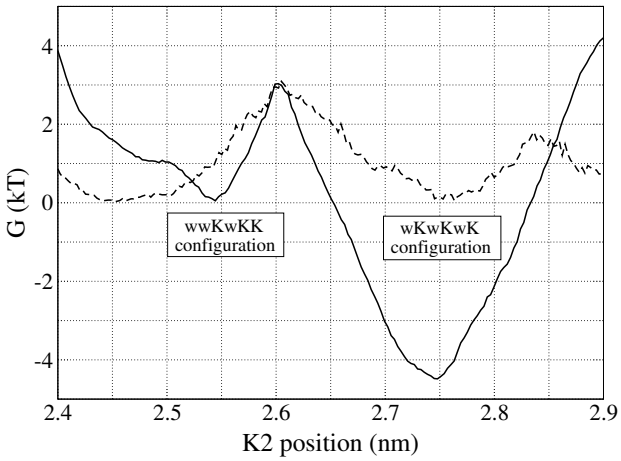

Fig. 2. Reconstructed PMF for internal double movement (wwKwKK)→(wKwKwK) Solid and dashed line refer to US and SMD, respectively.

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