Particle-based simulation of conductance of solid-state nanopores and ion channels

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***Abstract*—A three-dimensional numerical simulation technique based on Brownian Dynamics is presented for simulating ion currents flowing through ion channels and solid-state nanopores under various conditions.**
This implementation allows to perform simulations on the μ-seconds time scale in order to obtain information on the conductance of the simulated channels.
Results regarding ion motion in bulk solution and concerning electrostatic calculation along channel axis for a catenary test pore are presented here.
Furthermore, the calculated potential energy profiles and conductance of an open-state configuration of KcsA K^+ channel of *Streptomyces Lividans* bacterium are presented.

I. Introduction

Ion channels are pore-forming proteins that regulate the flow of ions across the membrane in biological cells.
They help the cell to maintain the proper ionic gradient and potential difference with respect to the external environment providing a path for ions exchange across the membrane. Typically they are not simple pores but act as switches as they are characterized by gating mechanisms that determine open and closed states.
Ion channels are fundamental in generating and regulating the electrical activity of cells in the nervous system and moreover malfunctions of ion channels are frequently associated with neurological diseases [1]. It is therefore essential to be able to explain ion channel function at a molecular level.
Solid-state nanopores are nanometer-scale pores located in electrically insulating membranes. They can be adopted as detectors of specific molecules in electrolytic solutions. The detection principle is based on monitoring the variations of the ionic current flowing through the nanopore, caused by the passage of a single molecule [2][3]. Nanopore sensors represent a very promising technology and a fully comprehensive knowledge of their characteristics is needed.
Therefore, permeation of ions from one electrolytic solution to another, through a protein channel or a synthetic pore is a process of considerable importance and realistic analysis of the main dependencies of ion current on the geometrical and compositional characteristics of these structures are highly required.
We present here a particle-based approach which allows to simulate ion motion through membrane pores and to determine their conductance under various conditions.

II. Adopted Model

The understanding of the structure and the function of ion channels and solid-state nanopores needs a comprehensive physical description in terms of theoretical model. This model will help the study of membrane channels describing in details ion motion inside and around the channel, locating potential binding sites, determining crossover velocity and elucidating many other peculiar aspects.
Many approaches have been proposed to model ion motion in electrolytic solutions and through membrane pores. The most interesting and used among these approaches, that model the real system at different levels of abstraction, are Molecular Dynamics (MD), Poisson-Nernst-Planck approach (PNP) and Brownian Dynamics (BD).
MD is a classical low-level approach based on considering pairwise interactions between every atoms in the system and determining their trajectories in time using Newton's equation of motion. It represents a very accurate method but due to its fully-comprehensive nature it is computationally too expensive to obtain information about channel conductance with this approach.
PNP is a high-level of abstraction approach based on electrostatic continuity and mean-field approximation. Ions are not treated explicitly as discrete particles but as continuous charge densities that represent the space-time average of the microscopic ion motion ion. Ion flux inside the system is described by Nernst-Planck equation. The self-consistency of this method is obtained solving iteratively Poisson's equation and Nernst-Planck equation. This approach allows the calculation of conductance but neglects many peculiar aspects of translocation, especially in narrow channels.
Brownian Dynamics approach represents an intermediate level of abstraction between MD and PNP and is the optimum compromise between simulation accuracy and computation time in order to obtain information regarding ion currents flowing through nanometers-scale pores.
BD approach explicitly models only the trajectories of all the ions inside the simulation domain, assuming that the pore and the charges inside the membrane are located at fixed positions during the whole simulation.
In this approach, the presence of water molecules is treated implicitly, considering only its average effect on the ions. The

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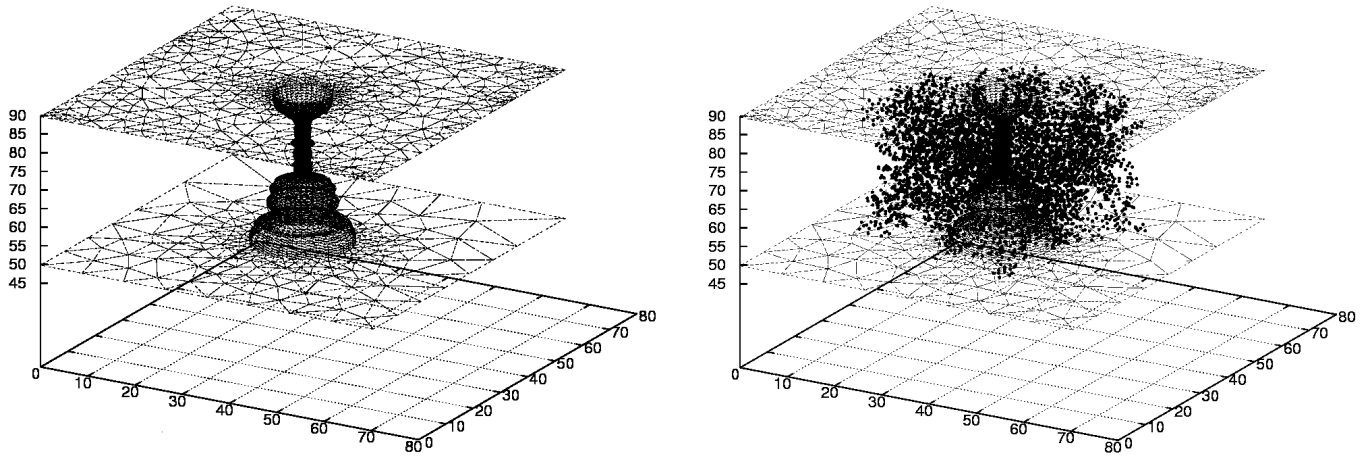

Fig. 1. Surface mesh generation for KcsA K^+ channel. Left figure shows the surface mesh at the membrane-water interface as described in section III. Right figure includes also charge distribution of the protein.

trajectories of ions are described by the Langevin's equation:

$$m_i \frac{d\vec{v}_i}{dt} = -m_i \gamma_i \frac{d\vec{r}_i}{dt} - q_i \vec{E}_i + \vec{F}_{Ri}(t) \tag{1}$$

where m_i, q_i, $\vec{r}_i$ and $\vec{v}_i$ represent the mass, the charge, the position and the velocity of the *i*-th ion respectively. In this formula, the force acting on ion *i* is determined by the total electric field the ion feels ($\vec{E}_i$), by an average frictional force with friction coefficient $m_i\gamma_i$ and by a random force $\vec{F}_R$ that mimics collisions between ions and waters.

For large friction systems, like ions in electrolytic solution, it is possible to neglect the inertial term [4] and determine ion trajectories solving the Brownian Dynamics equation:

$$\frac{d\vec{r}_i}{dt} = -q_i \frac{D_i}{K_B T} \vec{E}_i + \vec{\xi}_i(t) \tag{2}$$

In which D_i is ion diffusion coefficient, K_B is Boltzmann constant, T is the absolute temperature and $\vec{\xi}$ is a random gaussian displacement.

Integrating (2) with finite difference method it is possible to calculate ion position at each time step:

$$\vec{r}_i(t + \Delta t) = \vec{r}_i(t) - q_i \frac{D_i}{K_B T} \vec{E}_i + \vec{X}(6 D_i \Delta t)^{1/2} \tag{3}$$

where $\vec{X}$ is a random vector whose components are chosen from Gaussian distributions centered at zero.

Equation (3) describes ion motion as the result of two distinct forces: an electric force due to the electrostatic interactions with all the charges inside the system and a random one, which mimics the collisions with the surrounding water molecules.

The trajectories of ions in the simulation domain are obtained from eq. (3) in a three-dimensional continuum space for a μ-second of simulated time in order to estimate the channel conductance.

III. Simulation Details

The electric field felt by each ion is determined by all the charges present in the system, therefore we need to solve Poisson's equation in order to determine the force acting on them.

We must now consider that the simulation domain contains two different regions, water and membrane, characterized by their own dielectric constant. A convenient way to solve Poisson's equation in such a domain is to use Levitt's method [5]. This approach finds the solution for an equivalent problem in which all charges are considered in vacuum and induced surface charge densities are placed at the dielectric boundary. The solution of Poisson's equation is found iteratively taking into account the mutual interactions between surface charges.

In order to determine surface charge densities, it is necessary to define the protein-water interface and to generate a sufficiently dense mesh over the whole boundary. For solid-state nanopores this interface is defined by pore's geometry. In the case of protein ion channels, it is obtained from a charge distribution defined by standard molecular mechanics force fields [6] on the basis of x-ray crystallography data. For any considered channel, for simplicity, a rotational symmetry is adopted.

Once the interface has been determined, the simulator performs a Delaunay triangulation of the whole interface (Fig. 1).

The total electrostatic force acting on an ion is determined by many contributions. Exploiting the superposition principle, the total electric field $\vec{E}_i$ acting on ion *i* is described by the following equation:

$$\vec{E}_i = \vec{E}_{S,i} + \vec{E}_{FC,i} + \vec{E}_{EXT,i} + \sum_{\substack{j=1 \\ i \neq j}}^{N} \vec{E}_{I,ij} \tag{4}$$

where $\vec{E}_{S,i}$ represents the electric field generated by the image charges induced at the membrane-water boundary due to the ion *i* itself. $\vec{E}_{FC,i}$ takes into account the electric fields due to fixed charges inside the membrane and $\vec{E}_{EXT,i}$ is the contribution of the transmembrane potential. The last term in equation (4) describes the electric field generated by all other ions in the system. Notice that the last three terms consider the Coulombic direct interactions and the effects of the induced image charges.

The Brownian Dynamics algorithm requires the calculation of

the electric forces acting on the ions at each time step. This can be achieved by solving Poisson's equation with appropriate boundary conditions. The direct approach is computationally too expensive for the conductance calculation, therefore we adopt the method described and validated by Hoyles [7]. In this approach the electric field due to each charge in the system and to the applied electric field is calculated on a grid of points and the results are stored in lookup tables. During simulations, the total electric field at desired points is calculated by superimposing the pre-calculated elemental contributions provided by each charge in the system and by the applied field, retrieved from the lookup tables. This approach speeds up sensibly computer simulations with minimal loss in accuracy and allows to run simulations long enough to determine the channel conductance.

The grid of points is created on a two-dimensional space exploiting the rotational symmetry of the channel. During the simulations ion trajectories are calculated performing rotations in order to obtain the correct electric fields acting on each ion in a three-dimensional space.

IV. Test of the simulator

The simulation of ion motion, performed according to equation (3), has been tested computing ion current in bulk solution. Obtained currents fit accurately data predicted by the Nernst-Planck model for different values of ionic concentrations and applied potentials (Fig. 2), confirming the correctness of our implementation.

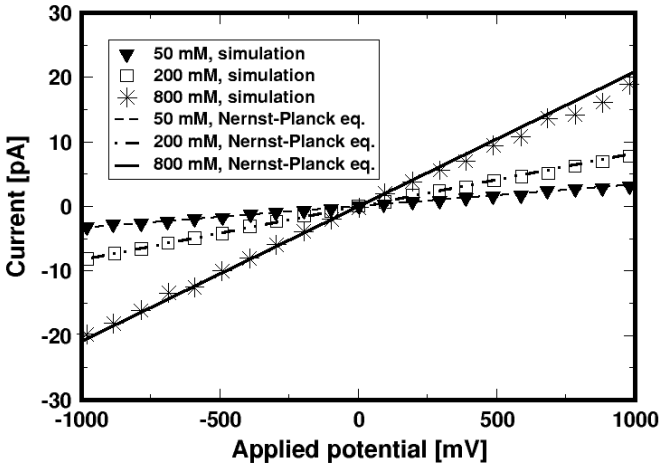

Fig. 2. Comparison between simulations results and theoretical values for the ion motion in a bulk solution at different concentrations. Simulated currents, plotted as a function of the applied potential, well fit those predicted by the Nernst-Planck equation.

Simulations of channels featuring simple geometry have been performed in order to reproduce both electrostatics and conductance results reported in literature. Simulations of a simplified model channel with a cylindrical transmembrane segment joined to catenary vestibules located at each side gave results in good agreement with those obtained by Hoyles both for electrostatic calculation (Fig. 3) and channel conductance (Fig. 4) [7].

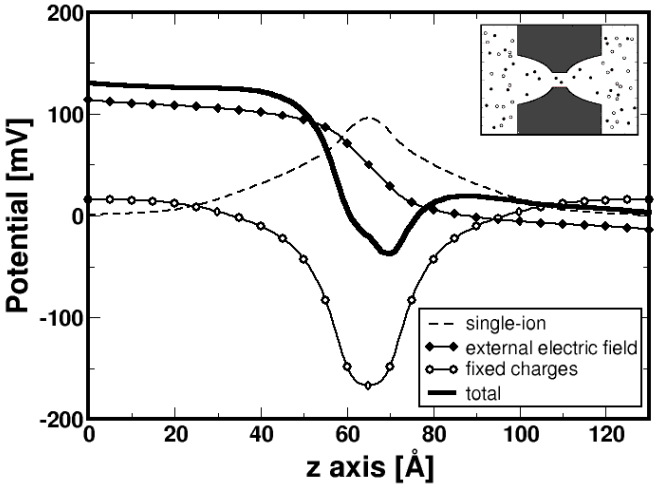

Fig. 3. Electrostatic potential profiles of the catenary channel on the entire simulation domain for an ion that moves along channel axis. Continuous line is the total potential energy as the sum of all the contributions as described by eq. (4). All profiles are generated taking into account image charges. The inset shows a sketch of the simulation domain for the catenary channel.

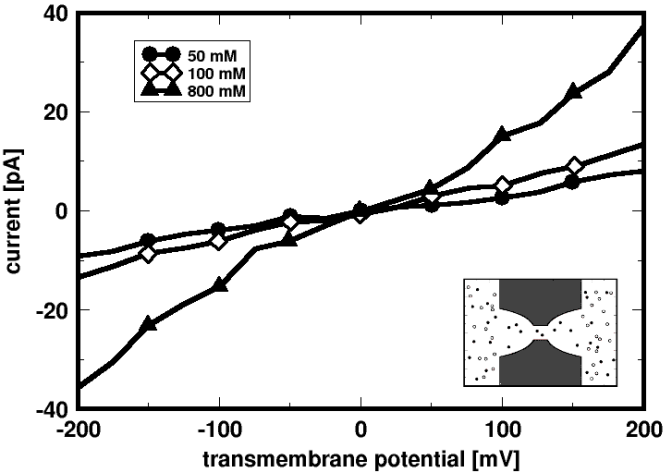

Fig. 4. Currents obtained for the simulated catenary channel as a function of the transmembrane potential at different ionic concentrations of Na^+-Cl^- solution. The inset shows a sketch of the simulation domain.

V. Simulation of the KcsA channel

KcsA is a protein potassium channel of *Streptomyces Lividans* bacterium. It is composed by four identical sub-units positioned determining a central pore (Fig. 5a). The selectivity filter, the narrowest section of the channel placed on the extracellular side of the membrane, has a$\simeq 1.4$ Å radius and can accommodate up to three K^+ ions (Fig. 5b).

Like other potassium channels, KcsA is highly selective and is characterized by a single-file ion transport with a knock-off mechanism inside the selectivity filter. This feature represents the rate-limiting effect for ion flux through the channel.

The simulated channel model has been generated starting from the information deduced from crystallographic analysis. The charges of the selectivity filter region have been shifted

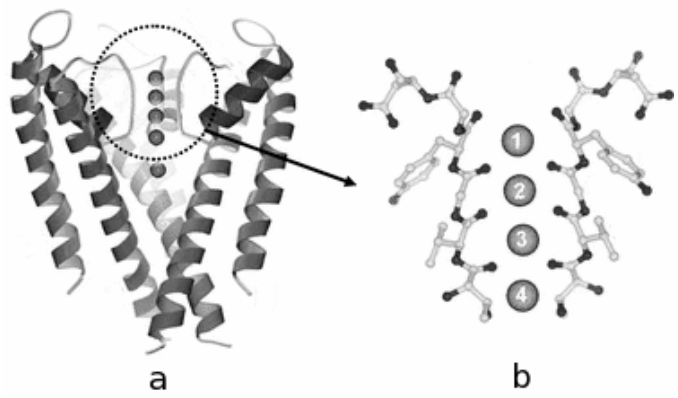

Fig. 5. Structure of KcsA channel (a) and its selectivity filter (b) are shown. Ions are represented as spheres.

radially in order to obtain a reasonable open-state configuration. Furthermore the protein has been inserted in a realistic representation of the cell membrane featuring 29 Å width. For these simulations realistic values for diffusion coefficients and dielectric constants inside the channel have been adopted [6]. Electrostatic profiles obtained for KcsA K^+ protein channel (Fig. 6) confirm Molecular Dynamics simulations data [6]. Estimated currents, for a wide range of transmembrane potentials and different ionic concentrations, accurately reproduce experimental data (Fig. 7) [8].

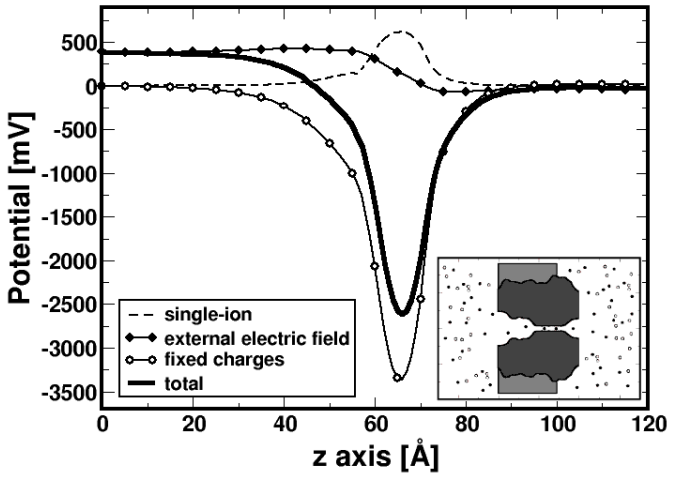

Fig. 6. Electrostatic potential profiles of KcsA channel on the entire simulation domain for an ion that moves along channel axis. Continuous line is the total potential energy as the sum of all the contributes ad described by eq. (4). All profiles are generated taking into account image charges also. The inset shows a sketch of the simulation domain for the KcsA channel.

VI. Conclusion

A numerical simulator for ion transport in biological and synthetic channels has been developed and validated. This simulation procedure allows to investigate how parameters like temperature, applied voltage, and nanopore shape could influence ion translocation dynamics.

Our approach will help the analysis of ion current rectification

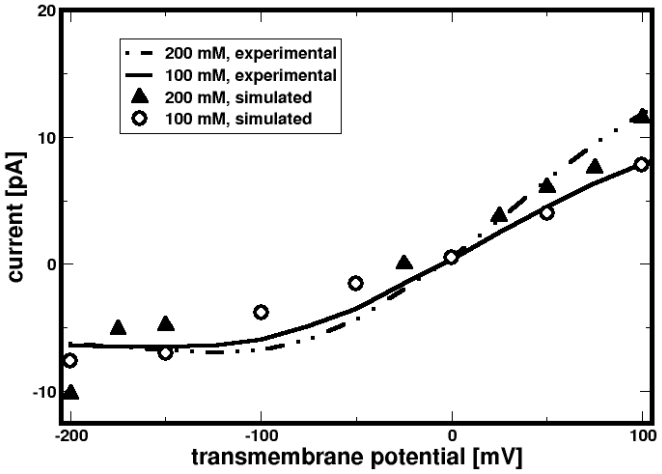

Fig. 7. Comparison between simulations results and experimental data of currents for the considered KcsA channel as a function of transmembrane potential at different ionic concentrations of K^+-Cl^- solution.

mechanism exhibited by particular kinds of nanopores [9], contributing to refine the understanding of nanopore-based sensors.

Moreover our simulator could represent a valid tool for ion channel study. In particular it allows to investigate at the atomic-level the complex dependencies of channel conductance and ion selectivity on electrostatic interactions.

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