A NUMERICAL SOLVER FOR POISSON AND NERNST-PLANCK EQUATIONS, APPLICATION TO ION CHANNEL CONDUCTION

S. Furini*, F. Zerbetto** and S. Cavalcanti*

* Department of electronics, Computer Science and Systems/University of Bologna, Bologna, Italy
**Department of chemistry "G.Ciamician"/University of Bologna, Bologna, Italy

sfurini@deis.unibo.it

Abstract: Recent results of X-Ray crystallography have provided important information for functional studies of membrane ion channels based on computer simulations. Because of the large number of atoms that constitute the channel proteins, it is prohibitive to approach functional studies using molecular dynamics. To overcome the current computational limit we propose a method based on the Poisson, Nernst, Planck electro-diffusion theory. The basic problem with this approach, is the diffusion coefficients setting. We tested two possibilities: i) space-independent diffusion coefficients, set to experimental values; ii) space-dependent diffusion coefficients, set according to molecular dynamic simulations. A good accordance with the experimental data was obtained with the last setting.

Introduction

Ion channels are protein molecules embedded in the lipid bilayer of the cell membranes. They control the ion fluxes through these membranes playing a central role in several cellular functions, i.e. the cellular excitability [1]. In the last few years, thanks to the structural data provided by X-ray crystallography, it has become possible to analyze the channels at atomic level. In particular, the atomic structures of several bacterial ion channels selectively permeable to potassium ions – KcsA [2], MthK [3], and KvAP [4] - were revealed. The peculiar characteristic of potassium channels is the ability to conduct at a rate close to the diffusion limit (10^8 ions/s) keeping a high selectivity (potassium permeability is 10^4 fold sodium permeability) [1]. The knowledge of the KcsA atomic structure, the first potassium channel crystallized, has permitted to reviles the molecular mechanism underlying these complex channel functions. Molecular dynamic simulations were particularly important for these studies [5-7]. Due to the huge computational resources needed, these dynamic simulations are restricted to the nanosecond time-scale. Ion conduction through an ion channel is a far slower process, millisecond time-scale. Thus, to compute the electrical current through a channel, a simplified mathematical model of ion conduction is needed. Since channel current, so channel conductance, is the main functional characteristic of an ion channel, and it is the easiest to value experimentally, a method to compute currents starting from the molecular structure is nowadays one of the main topic in ion channel studying.

Most of the complexity in ion channel dynamic simulations lies in the high number of water molecules. Consequently, a continuum description of the solvent is the first important step to reduce the computational resources needed. Brownian dynamic simulations are a possible approach based on this idea. In a Brownian dynamic simulation only ions preserved their discrete nature, the solvent is described by diffusion coefficients and stochastic collisions with ions and the effect of the protein by a potential energy function. The potential energy function and the diffusion coefficients are the basic elements of this method, and to avoid arbitrariness in the mathematical model these functions are computed starting from the atomic structure of the channel. A Brownian dynamic simulation based on this approach was tested by Berneche et al. on KcsA [8], getting a good accordance with the experimental data. In this study we present a further simplified approach based on the Poisson, Nernst, Planck (PNP) theory of electro-diffusion, which use a continuum description of the whole system. The limited computational resources needed by this kind of approach, made it the perfect tool to analyze systematically the effects of structural changes on channel functioning. Channel structural changes, due to protein channel mutations, are common in nature, and sometimes connected with diseases . The importance of a mathematical model to analyze these diseases justifies the development of a model of ion conduction as simple as possible.

Poisson-Nernst-Planck theory

The PNP electro-diffusion theory describes a steady state condition for a system of mobile charges. In membrane channels, the mobile charges are the different ion species in solution, which space distributions are described by the concentrations $C_s(r)$ (subscript s marks the s-th ion specie; r is the space position). Assuming the electric field as the only driving force acting on ions, the steady state flux of the s-th ion specie has the form:

$$J_s(r) = -D_s(r)\nabla C_s(r) - \mu_s(r)C_s(r)z_s e\nabla\psi(r) \quad (1)$$

ISSN: 1727-1983

where $D_s(r)$, $\mu_s(r)$, $z_s(r)$ are respectively diffusion coefficient, mobility and valence of the s-th ion specie; e is the elementary charge and ψ the electrostatic potential. The first term of (1), which is proportional to the concentration gradient, is due to diffusion processes, while the last is produced by the electric field. Since the PNP theory describes a steady state condition, fluxes are time independent, and in virtue of the mass conservation law, the divergence of $J_s(r)$ is zero. This gives the set of differential equations:

$$div\left[D_s(r)\left[\nabla C_s(r) + \frac{ez_s}{k_B T} C_s(r) \nabla \psi(r) \right] \right] = 0 \qquad s = 1,..., N \tag{2}$$

where N is the number of ion species, k_B the Boltzmann's constant and T the absolute temperature. In (2) the Einstein's relation between diffusion coefficient and ion mobility, $\mu / D = k_B T$, was introduced. To complete the mathematical model it is necessary to define how electrostatic potential and ion concentrations are connected. This relation may be defined by the Poisson's equation:

$$div\left[\varepsilon(r)\nabla\psi(r)\right] = -\rho(r) - \sum_{s=1}^{N} ez_s C_s(r) \tag{3}$$

where $\varepsilon(r)$ is the dielectric constant and $\rho(r)$ the charge distribution of the protein atoms, that differently from ion charge distribution is assumed fixed in the space.

In the present study we included in the water solution only two monovalent ion species: one positive ($s = +$) and one negative ($s = -$). Then, the PNP differential equation set (2) and (3) is reduced to:

$$\begin{cases} div\left[D_+(r)\left[\nabla C_+(r) + \frac{e}{k_B T} C_+(r) \nabla \psi(r) \right] \right] = 0 \\ div\left[D_-(r)\left[\nabla C_-(r) - \frac{e}{k_B T} C_-(r) \nabla \psi(r) \right] \right] = 0 \end{cases} \tag{4}$$

$$div\left[\varepsilon(r)\nabla\psi(r)\right] = -\rho(r) - e[C_+(r) - C_-(r)] \tag{5}$$

Once solved these equations, as described in the next section, ion concentrations and electrostatic potential in the space are obtained. Afterwards, the ion fluxes in the channel, and so the electrical current, can be computed by (1).

Method

The differential equations (4) and (5) were numerically solved on a cubic volume formed by 200^3 cubic grid elements (the side of the grid element was 0.5 Å). The cubic volume was divided in three distinct sub-volumes: the ion channel, the membrane and the water solution. The position, the radius and the partial charge of all the atoms made up the ion channel sub-volume. Channel was discretized on the grid by using the discretization algorithm implemented in DELPHI v.4 [9], a well-known Poisson-Boltzmann equation solver. The channel was placed with its geometric centre in the centre of the cube and with the pore axis orthogonal to the upper and lower faces. The extracellular side of the channel pointed to the upper face. The height of cube was chosen double of the channel length along the pore axis (z axis). To separate extra and intra cellular spaces, a sub-volume surrounding the channel and extending between two planes orthogonal to the pore axis was introduced. This volume simulated the lipid bilayer of the cell membrane. The water solution spread all over the volume not occupied by the channel and by the membrane. It was characterized by two space-dependent diffusion coefficients, one for each ionic specie. A specific dielectric constant was assigned to water solution, membrane and channel sub-volumes.

The Poisson's equation (5) was solved in the whole volume, while the mass conservation equations (4) were solved in the water solution only. As boundary conditions for the Poisson's equation we assigned the electrostatic potential on the six faces of cube. The potential was set to 0 on the upper face, whereas the membrane potential value (V_m) to be simulated was applied on the lower face. On the side faces a linear interpolation between 0 and V_m was used. Two different boundary conditions were assigned for the mass conservation equations. On the upper and lower faces the boundary conditions were the ion concentrations to be simulated. On both faces anion and cation concentrations were set equal, to have electrically neutral boundaries. Boundary conditions on the side faces and at the separation surface with channel and membrane were:

$$\begin{aligned} J_+ \cdot \hat{n} &= 0 \\ J_- \cdot \hat{n} &= 0 \end{aligned} \tag{6}$$

where $\hat{n}$ is the surface normal vector. In this way no ion flux was allowed through these surfaces.

The differential equation set was solved by an iterative scheme. The electrostatic potential was first computed solving the Poisson's equation with ion concentrations set to zero. This potential was used to compute ion concentrations by the solution of mass conservation equations, then the new concentrations were used to update the electrostatic potential. This procedure was repeated until a self-consistent solution was found. The convergence was tested by the root mean square deviation between two successive iterations. All the differential equations were solved as described in the Appendix.

ISSN: 1727-1983

KcsA channel

KcsA channel was used to test the algorithm. KcsA is an ion channel selectively permeable to potassium ions of the bacterium *Streptomyces lividans*. It is made by four identical subunits, symmetrically placed around the channel axis. Each subunit consists of 160 amino acids and is characterized by three α helix structures - the outer helix, the pore helix and the inner helix - placed like in figure 1. On the extracellular side the conduction pathway is lined by the carbonyl oxygens of the amino acid sequence TVGYG, one from each subunit. This region, named selectivity filter, is 16 Å long with a mean radius of 1.4 Å and it is widely conserved among different potassium channels. Experimental data and molecular dynamic simulations [5;10;11] have revealed the presence of four ion binding sites inside the selectivity filter, named respectively S1, S2, S3, and S4 (Figure 1). Below the selectivity filter the channel opens in a wide chamber with a mean radius of 6Å connected to the intracellular space by a 15 Å long hydrophobic pore. Both the chamber and the hydrophobic pore are lined by the four inner helixes.

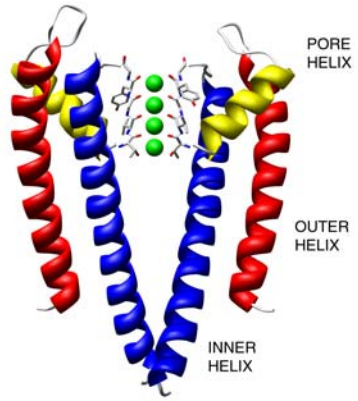

Figure 1: KcsA channel, side view. Only two of the four subunits are shown. Four potassium ions are included in the selectivity filter at the binding sites S1-S4, numbered starting from the top (extracellular side).

The three dimensional atomic coordinates of the KcsA channel were taken from the crystallographic structure determined at 2 Å resolution by Y. Zhou et al. [12] (file 1K4C.pdb of the Protein Data Bank [13]). The atomic coordinates of the first 22 amino acids at the N terminal and of the last 45 at the C terminal were not experimentally determined. Since both C and N terminal are located in the cytoplasm, far from the conduction pathway, these amino acids are not crucial for the present study and therefore, they were not included in the channel model. Side chains with missing atoms were completed using ideal internal coordinates from the AMBER force field [14]. AMBER force field was used to define atomic radii and partial charges too. Since the experimental structure of KcsA corresponds to a closed state [3], we needed to compute a structure of the channel in an opened state. The structure of MthK [15], a potassium channel crystallized in an opened state, was used for this purpose. The only structural difference between KcsA and MthK is the inner helixes orientation. In KcsA these helixes are close together, reducing the hydrophobic pore radius to 0.5 Å, and thus preventing ion fluxes; in MthK an outward movement of the inner helixes, realized by bending around hinge glycines, causes the opening of the channel. The conservation of these hinge amino acids among KcsA, MthK and many potassium channels suggests a common mechanism for channel gating. Thus the opened structure of the KcsA channel was computed minimizing the distance between the inner helixes of KcsA and MthK by a rigid rotation of the intracellular side of the KcsA inner helixes around the hinge glycines.

Parameters definition

The membrane thickness was defined according to the KcsA atomic structure. The channel comes into contact with the membrane by the four outer helixes. These helixes have a hydrophobic segment between the amino acids TRP113 and TRP87. Thus, we used the position of these amino acids to define the thickness of the membrane in our model. The relative dielectric constant was set to 2 in the channel and the membrane and to 80 in the water solution.

The diffusion coefficients were assigned considering three distinct regions: the channel outside (CO) corresponding to $z > 16$ Å and $z < -15$ Å, the selectivity filter (SF) spanning from $z = 0$ Å to $z = 16$ Å and the intracellular chamber (IC) from the intracellular channel mouth, $z = -15$ Å, to the intracellular end of the SF, $z = 0$ Å. Outside of the channel the diffusion coefficients were set respectively to $D_+ = 1.96 \cdot 10^{-9}\ \mathrm{m}^2/s$ and $D_- = 2.03 \cdot 10^{-9}\ \mathrm{m}^2/s$, according to the experimental values of free-diffusion in water solution for potassium and chloride ions. The mean radius of SF and IC is respectively 1.4 Å and 6 Å. In these areas ions and water molecules interact with the channel, causing a change in the diffusion processes and consequently in diffusion coefficient values. Molecular dynamic simulations predict a potassium diffusion coefficient reduced to 10% with respect to the CO value in SF and to 50% in IC [5;16]. We compared experimental and computed currents with two different choices of the diffusion coefficients: i) Diffusion coefficients set according to the reduction computed by molecular dynamics; ii) diffusion coefficients set to the experimental values for free-diffusion in the whole system (CO, SF and IC).

Results

Computed currents overestimate the experimental data [17] if the experimental diffusion coefficients in free solution are used in the whole system (Figure 2 and 3). Setting the membrane potential to 25 mV and both the extracellular and intracellular ion concentrations to

ISSN: 1727-1983

100 mM, the computed current is 4 times the experimental value. The approximation gets worst rising the ion concentrations and the membrane potential. A possible approach to reproduce the experimental data is to use the diffusion coefficients as fitting parameters. Computed currents reproduce quite well the experimental data with a reduction of both the diffusion coefficients to 25% in the whole system (data not shown). However, a similar reduction of the diffusion coefficients is not justifiable. Otherwise, inside the channel, diffusion processes take place differently and it is reasonable to use different values for the diffusion coefficients. The usage of the diffusion coefficients computed by molecular dynamics [5;16] causes an improvement in the accordance between simulated and experimental data (Figure 2 and 3). Experimental data are yet overestimated, but the overestimation is widely reduced. The root mean square deviation (RMSD) drops from 16 pA, with experimental diffusion coefficients, to 6 pA when is used a 10% and 50% reduction of the diffusion coefficients respectively in SF and IC. Overestimation is high at high membrane potential, but drops when the membrane potential falls in the physiological range. The RMSD is just 1.8 pA when the membrane potential is 25 mV and the ion concentrations range from 20 to 800 mM (Figure 4).

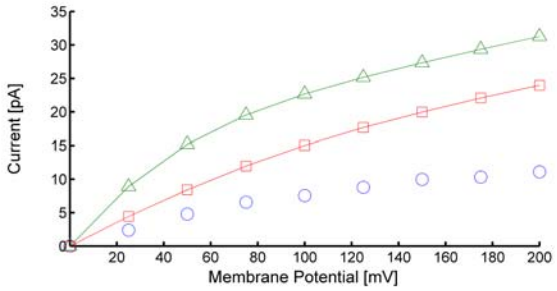

Figure 2: Current-Voltage characteristic. Experimental data (o). Computed currents with diffusion coefficients: set to free diffusion values in the whole system (triangles); reduced to 10% in SF and to 50% in IC (square).

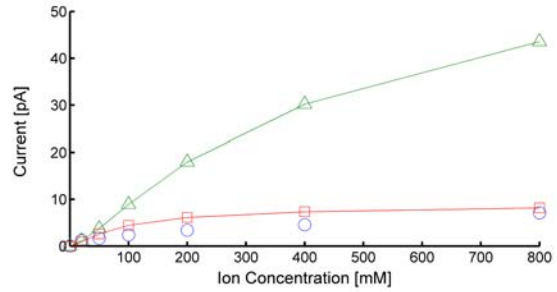

Figure 3 Current-Concentration characteristic. The same symbols of figure 3 are used.

Conclusions

In this paper we have attempted to relate structure and function of ion channels, making use of the KcsA experimental data. To this end we developed a numerical solver of the PNP equations. In ion channel simulation studies, an approach based on the PNP electro-diffusion theory is complementary to an approach based on molecular dynamics. The last can reveal the atomic details about ion channel functioning, while the former, giving up the atomic detail, provide a way to compute ion currents. To simplify the mathematical model of ion conduction, the PNP theory describes the ion distributions with continuum functions. Despite this simplifying assumption the computed currents are in good accordance with the experimental data. It is important to highlight that the accordance between simulated and experimental data was obtained with diffusion coefficient values matching the ones computed by molecular dynamic simulations. Therefore these coefficients must not to be interpreted as fitting parameters. Instead, they connect the microscopic description provided by molecular dynamics to the macroscopic of the PNP theory.

The accordance with the experimental data is good especially with membrane potential in the physiological range. At higher values an overestimation of the current was obtained, probably due to saturation effects not introduced in the model. Even if an improvement of the mathematical model in this direction is desirable, the present approximation provides a good tool to analyze the effects of structural changes on the channel functioning.

Appendix: Numerical Method

The numerical procedure used to solve the mass conservation equations (4) is presented here. For sake of clarity we will refer to a two-dimensional case, generalization to the three-dimensional case is immediate. The same numerical procedure was used to solve the Poisson's equation (5). The ion flux in the x direction between the grid elements $[i-1, j]$and $[i, j]$ was expressed as:

$$J^x_{i-1} = -\overline{D}_{i,i-1}\left[\frac{C_{i,j} - C_{i-1,j}}{h} + \frac{ze}{k_B T}\overline{C}_{i,i-1}\frac{\psi_{i,j} - \psi_{i-1,j}}{h}\right] \qquad (7)$$

that is the lattice version of equation (1) (h is the grid step, $\overline{D}_{i,i-1}$ and $\overline{C}_{i,i-1}$ are respectively the median diffusion coefficient and ion concentration between the grid elements $[i-1, j]$ and $[i, j]$). The ion fluxes J^x_{i+1} (in the x direction between the elements $[i, j]$ and $[i+1, j]$), J^y_{j-1} and J^y_{j+1} were expressed consistently. According to the mass conservation law, net steady state flux through any grid element is zero, that is:

ISSN: 1727-1983

$$J_{i-1}^{x} - J_{i+1}^{x} + J_{j-1}^{y} - J_{j+1}^{y} = 0 \quad (8)$$

Replacing in this equation the fluxes expressed as in (7) it is possible to write:

$$C_{i,j} = \frac{\sum_k C_k \frac{D_{i,j} + D_k}{2}\left[1 - \frac{ez}{2k_B T}\left(\psi_{i,j} - \psi_k\right)\right]}{\sum_k \frac{D_{i,j} + D_k}{2}\left[1 + \frac{ez}{2k_B T}\left(\psi_{i,j} - \psi_k\right)\right]} \quad (9)$$

where k is an index that rounds over the four surrounding grid elements ($k = [i-1, j], [\mathrm{i}+1, \mathrm{j}], [i, j-1], [\mathrm{i}, \mathrm{j}+1]$). Equation (9) was used in an iterative scheme based on the successive over relaxation techniques to find the solution of the mass conservation equation [18]. At the first step ion concentration in each grid element is set randomly, these concentrations are then updated, according to:

$$C_{i,j}^{n} = (1-w)C_{i,j}^{n-1} + wC_{i,j} \quad (10)$$

where $C_{i,j}^{n}$ and $C_{i,j}^{n-1}$ are respectively the concentration computed at the step n and $n-1$, and $C_{i,j}$ is obtained by (9). Setting correctly the weight w, the number of iterations to reach the solution drops appreciably. To test the convergence to solution the root mean square distance between two successive iterations is used, the procedure is stopped when the root mean square distance falls below 10^{-3}.

References

[1] Hille B., Ionic channels of excitable membranes, Sinauer Associates, Sunderland, MA ed. 1992.

[2] Doyle, D. A., Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R., "The Structure of the Potassium Channel: Molecular Basis of K+ Conduction and Selectivity," Science, vol. 280, no. 5360, pp. 69-77, Apr.1998.

[3] Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R., "The open pore conformation of potassium channels," Nature, vol. 417, no. 6888, pp. 523-526, May2002.

[4] Jiang, Y., Lee, A., Chen, J., Ruta, V., Cadene, M., Chait, B. T., and MacKinnon, R., "X-ray structure of a voltage-dependent K+ channel," Nature, vol. 423, no. 6935, pp. 33-41, May2003.

[5] Allen, T. W., Kuyucak, S., and Chung, S. H., "Molecular Dynamics Study of the KcsA Potassium Channel," Biophysical Journal, vol. 77, no. 5, pp. 2502-2516, Nov.1999.

[6] Berneche, S. and Roux, B., "Molecular Dynamics of the KcsA K+ Channel in a Bilayer Membrane," Biophysical Journal, vol. 78, no. 6, pp. 2900-2917, June2000.

[7] Shrivastava, I. H. and Sansom, M. S. P., "Simulations of Ion Permeation Through a Potassium Channel: Molecular Dynamics of KcsA in a Phospholipid Bilayer," Biophysical Journal, vol. 78, no. 2, pp. 557-570, Feb.2000.

[8] Berneche, S. and Roux, B., "A microscopic view of ion conduction through the K+ channel," Proceedings of the National Academy of Sciences, vol. 100, no. 15, pp. 8644-8648, July2003.

[9] W.Rocchia, S.Sridharan, A.Nicholls, E.Alexov, A.Chiabrera, and B.Honig, "Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: Applications to the molecular systems and geometric objects," Journal of Computational Chemistry, vol. 23, no. 1, pp. 128-137, 2002.

[10] Zhou, Y. and MacKinnon, R., "The Occupancy of Ions in the K+ Selectivity Filter: Charge Balance and Coupling of Ion Binding to a Protein Conformational Change Underlie High Conduction Rates," Journal of Molecular Biology, vol. 333, no. 5, pp. 965-975, Nov.2003.

[11] Berneche, S. and Roux, B., "Molecular Dynamics of the KcsA K+ Channel in a Bilayer Membrane," Biophysical Journal, vol. 78, no. 6, pp. 2900-2917, June2000.

[12] Zhou, Y., Morais-Cabral, J. H., Kaufman, A., and MacKinnon, R., "Chemistry of ion coordination and hydration revealed by a K+ channel-Fab complex at 2.0[thinsp][angst] resolution," Nature, vol. 414, no. 6859, pp. 43-48, Nov.2001.

[13] Protein Data Bank, Internet site address: http://rcbs.org 2005.

[14] D.A.Case, T.A.Darden, T.E.Cheatham, C.L.Simmerling, J.Wang, R.E.Duke, R.Luo, K.M.Merz, B.Wang, D.A.Pearlman, M.Crowley, S.Brozell, V.Tsui, H.Gohlke, J.Mongan, V.Hornak, G.Cui, P.Beroza, C.Shafmeister, J.W.Caldwell, W.S.Ross, and P.A.Kolmann, AMBER 8 University of California, San Francisco: 2004.

[15] Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R., "Crystal structure and mechanism of a calcium-gated potassium channel," Nature, vol. 417, no. 6888, pp. 515-522, May2002.

ISSN: 1727-1983

[16] Allen, T. W., Kuyucak, S., and Chung, S. H., "Molecular dynamics estimates of ion diffusion in model hydrophobic and KcsA potassium channels," Biophysical Chemistry, vol. 86, no. 1, pp. 1-14, July2000.

[17] LeMasurier, M., Heginbotham, L., and Miller, C., "KcsA: It's a Potassium Channel," The Journal of General Physiology, vol. 118, no. 3, pp. 303-314, Aug.2001.

[18] Young, D. M., Iterative solution of large linear systems, Academic press: New York and London ed. 1971.

ISSN: 1727-1983