



## Physics of Ion Channels

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**Abstract.** We review the basic physics involved in transport of ions across membrane channels in cells. Electrochemical forces that control the diffusion of ions are discussed both from microscopic and macroscopic perspectives. A case is made for use of Brownian dynamics as the minimal phenomenological model that provides a bridge between experiments and more fundamental theoretical approaches. Application of Brownian and molecular dynamics methods to channels with known molecular structures is discussed.

**Key words:** Brownian dynamics, continuum theories, ion channels, molecular dynamics, permeation models

**Abbreviations:** PB – Poisson-Boltzmann; PNP – Poisson-Nernst-Planck; BD – Brownian dynamics; MD – molecular dynamics

### 1. Introduction

Ion channels are water filled holes in cell membranes that are formed by proteins embedded in the lipid bilayer. They help living organisms to perform a variety of essential tasks from movement to sensation by generating action potentials in nerves, muscles and synapse [1]. To achieve this, a channel needs to conduct thousands of ions when it opens up for a period of few milliseconds. Considering that ion channels are quite narrow (most have a few Å radius), and hence ions have to move in single file, this appears to be quite a remarkable feat. Another outstanding property of biological ion channels, that distinguishes them from being a mere hole in the membrane wall, is their selectivity property. That is, each type of ion channel is designed to conduct a particular ion species. For example, potassium channels can select against sodium ions with a ratio of  $10^4$  to 1. Selectivity is, of course, vital for the normal physiological functioning of the cells because the sodium concentration is much higher outside the cell compared to inside, and vice versa for potassium. Without selectivity, this imbalance in concentrations, which is ultimately responsible for the action potentials, would disappear in a very short time. Both narrowness of ion channels and their selectivity emphasize the importance of ion-channel interactions, and their accurate description should be a primary concern in choosing a permeation model to study their properties.

Channel proteins typically contain thousands of atoms, and once the surrounding lipid and electrolyte are included, the system under consideration becomes too large for a fundamental description. When confronted with such a complex system, the usual response of physicists is to seek a minimal working model that can describe the observed properties of the system reasonably well. If successfully formulated, such a phenomenological model plays a very useful role in further developments in the field because, i) it provides a simple framework for understanding and interpretation of the experimental data, ii) it can be used to make further predictions about the system's behaviour under different conditions, and iii) it provides a natural bridge between the experimental observations and more fundamental theoretical approaches. The last point is important in several respects that require further elaboration. A phenomenological model necessarily contains free parameters that are normally adjusted to obtain the best description of the available data. By deriving these parameters from a more fundamental theory, the status of the model is raised to a more respectable level. Conversely, description of a complex system starting from first principles may be an impossible task, but determination of the parameters used in a coarse-grained model from a more fundamental theory is a much less ambitious job and may be achieved without undue hardship.

The physical picture behind permeation of ions across membrane channels is quite simple. Ions in solution execute a Brownian motion and, under equilibrium conditions, drift around in a completely random manner. However, when there is an electrochemical gradient across the membrane, ions feel an extra force that adds a small drift velocity to their motion, which eventually drives them through the channel (provided it is in an open state). In physical chemistry, properties of electrolyte solutions are described by the continuum theories – the Poisson-Boltzmann (PB) equation for equilibrium situations and the Poisson-Nernst-Planck (PNP) equations for ion transport [2]. During the last two decades, the continuum theories have been increasingly applied to biological systems and have led to enormous strides in understanding their properties [3, 4, 5]. Their application to ion channels have flourished in tandem [6, 7], though questions have been raised recently about their validity in narrow channels [8, 9, 10].

Brownian dynamics (BD) provides a semi-microscopic alternative to the continuum theories, where the motion of ions are followed explicitly using the Langevin equation while the rest of the system is treated in the continuum approximation [11, 12]. Because BD simulations are computationally much more demanding than solving the PB and PNP differential equations, their application to ion channels is more recent and less prevalent. A fully microscopic description of ion channels is provided by molecular dynamics (MD) simulations [13, 14, 15], where the trajectories of all the atoms in the system are followed via the Newton's equation of motion. MD simulations are obviously even more demanding than BD, and we still need a few orders of magnitude increase in computational power in order to make contact with experiments. In classical MD, quantum effects such as induced

polarization, dispersion forces and short range repulsion are taken into account on average by parametrizing the Coulomb and Lennard-Jones potentials between two atoms. Thus for a truly fundamental description of ion channels, one has to consider ab initio MD, which has an even more restricted application domain both in system size and simulation time [12].

Membrane proteins are notoriously difficult to crystallize, and until recently, the molecular structure of biological ion channels were not known. For a long time, the antibiotic peptide gramicidin A, whose structure has been known since 1971 [16], has been used as a surrogate channel model in studying the permeation properties of ion channels [13, 17]. This situation has changed dramatically during the last couple of years with the determination of the crystal structures of the KcsA potassium [18, 19, 20], mechanosensitive MscL [21] and ClC chloride channels [22]. As one would expect, these developments have shifted the focus of theoretical studies from qualitative models to quantitative ones, where the aim is to predict the function of a channel from its underlying molecular structure. Here we discuss the suitability of the permeation models introduced above for this purpose. BD simulations, backed up with MD, is proposed as the most appropriate method. Recent applications of the BD and MD simulations to the gramicidin A, potassium and calcium channels are briefly discussed.

## 2. Permeation Models

In choosing an appropriate permeation model for describing ion transport in channels we need to address two basic physics questions: i) can we represent water, protein and lipid atoms implicitly as continuous dielectric media? ii) can we represent ions in the solution with a continuous charge density? If both answers turn out to be positive, continuum theories, being the simplest, would be the obvious method of choice. A negative answer to both questions, on the other hand, would condemn us to MD with its aforementioned difficulties. There is an ongoing debate about both issues, so one has to be careful in committing a definite answer to either questions at present. Nevertheless the second question has been investigated in some depth recently [8, 9, 10], and the answer appears to be in the negative.

### 2.1. CONTINUUM THEORIES

In order to give some insights into the workings of the continuum theories we briefly introduce the basic formalism for the Poisson, Poisson-Boltzmann and Poisson-Nernst-Planck equations. Fundamental to all charged systems is the Poisson equation

$$\epsilon_0 \nabla \cdot [\epsilon(\mathbf{r}) \nabla \varphi(\mathbf{r})] = -(\rho_{\text{ex}} + \rho_{\text{el}}), \quad (1)$$

whose solution determines the potential  $\varphi$  for a given charge density  $\rho$ . Here we have distinguished between charge densities in the protein  $\rho_{\text{ex}}$  and electrolyte  $\rho_{\text{el}}$ . In

vacuum  $\varepsilon = 1$  but when continuum electrostatics is employed, water is represented by  $\varepsilon_w \sim 80$ , and protein (and lipid), by  $\varepsilon_p \sim 2$ . We remark that these are the typical values used in continuum studies of biomolecules but whether they are appropriate for channels have not been established yet. For a general channel boundary, the Poisson equation can be solved most efficiently using the boundary element method [23, 24]. By solving the Poisson equation for single or multiple ions in the channel, one obtains their potential energy profiles, which can give important insights about their permeation properties. [23, 25]. As a simple illustration, we show the results obtained for a single ion using a schematic cylindrical channel, whose cross section is depicted on top of Figure 1. In the absence of any fixed charges on the protein, the induced charges on the boundary give rise to a dielectric self-energy barrier ( $U_s = 4.6$  kT), which would hinder ion permeation. Fixed charges on the protein generate an additional Coulomb interaction that could cancel this barrier provided they have the opposite sign to that of ion. As shown in Figure 1,  $U_c$  depends on the position of the protein charges, and mouth charges could create a substantial binding site at the pore entrance thereby facilitating ion permeation. In more microscopic terms, the energy barrier arises from the loss of hydration energy because the ion has to shed some of the water molecules in its hydration shells while entering the channel. For ions to permeate across channels at the observed rates, this barrier must be cancelled by attractive ion-protein interactions.

In electrolyte solutions, mobile ions move to minimize the energy of the system and, at equilibrium, distribute themselves according to the Boltzmann factor

$$\rho_{\text{el}}(\mathbf{r}) = \sum_{\nu} z_{\nu} e n_{0\nu} \exp[-z_{\nu} e \varphi(\mathbf{r})/kT], \quad (2)$$

where  $n_{0\nu}$  is the bulk (or reference) number density of ions of species  $\nu$  and  $z_{\nu} e$  is their charge. Substituting  $\rho_{\text{el}}$  in Equation (2) in the Poisson equation (1), one obtains the Poisson-Boltzmann equation for a  $z:z$  electrolyte

$$\varepsilon_0 \nabla \cdot [\varepsilon(\mathbf{r}) \nabla \varphi(\mathbf{r})] = 2ezn_0 \sinh[ze\varphi(\mathbf{r})/kT] - \rho_{\text{ex}}. \quad (3)$$

In this form, the PB equation can only be solved for an infinite plane boundary (Gouy-Chapman theory), which does not have much relevance for ion channels. A more useful example is provided by a central ion in a bulk electrolyte that can be solved by linearizing the PB equation (Debye-Hückel theory)

$$\nabla^2 \varphi = \kappa^2 \varphi, \quad \kappa^{-1} = \sqrt{\frac{\varepsilon_0 \varepsilon kT}{2z^2 e^2 n_0}}. \quad (4)$$

Here  $\kappa^{-1}$  is the Debye screening length and  $\rho_{\text{ex}} = 0$ . Solution of Equation (4) yields the following Coulomb potential around a central ion of diameter  $a$  [2]

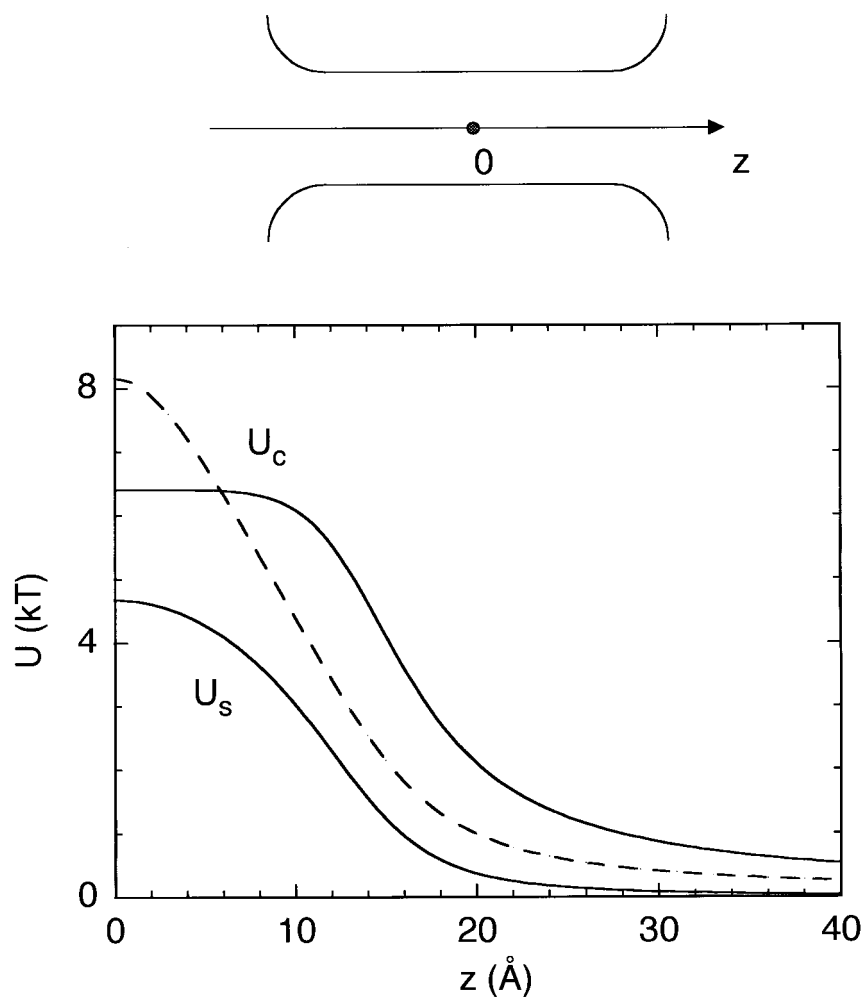
$$\varphi = \frac{ze}{4\pi \varepsilon_0 \varepsilon r} \frac{e^{-\kappa(r-a)}}{(1 + \kappa a)}. \quad (5)$$

We see that the effect of the electrolyte is to suppress the Coulomb potential of an ion by an exponential factor. This happens because the ion attracts the counter ions nearby while repelling the co-ions, which leads to a net counter charge density around it. We stress that this is a collective effect involving many counter and co-ions in a large volume (total screening occurs in a sphere of radius 4 Debye lengths). To give an example, at physiological concentrations (150 mM),  $\kappa^{-1} \sim 8$  Å, and the screening volume with  $r = 32$  Å contains 9 co-ions and 10 counter ions. Both the volume of ion channels and the number of ions in them are only a small fraction of the above figures. This raises questions about the applicability of the PB equation in channels. Indeed detailed tests carried out by comparing the predictions of the PB equation with those of the BD simulations show that the shielding effects are overestimated in narrow channels in the PB formalism [8]. When a test ion is placed inside a bare channel such as shown in Figure 1, counter ions can hardly enter the channel because of the self energy barrier. Thus there is almost no shielding due to counter ions in BD. In contrast, a continuous counter charge density is found around the test ion in the PB solutions, which provides a near perfect shielding for it. Because the self-energy is proportional to charge squared, it is severely underestimated when the charge is distributed around in small quantities as is the case in the PB formalism.

When there are electrochemical forces operating in an electrolyte solution, ions move in the direction of these forces. The flux  $\mathbf{J}_\nu$  of each ion species is described by the Nernst-Planck equation which combines the diffusion due to a concentration gradient with that from a potential gradient

$$\mathbf{J}_\nu = -D_\nu \left( \nabla n_\nu + \frac{z_\nu e n_\nu}{kT} \nabla \varphi \right), \quad (6)$$

where  $n_\nu$  is the number density and  $D_\nu$  is the diffusion coefficient of the ions of species  $\nu$ , and we have used the Einstein relation  $\sigma = (zen/kT)D$  to express the conductivity in terms of the diffusion coefficient. For self-consistency, Equation (6) needs to be solved simultaneously with the Poisson equation (1), and together they form the PNP equations. Because of their non-linear nature, the PNP equations can only be solved numerically [9, 26]. Lack of analytical solutions has made it difficult to obtain an intuitive picture of ion permeation in the PNP approach and delayed recognition of the problems in its application to ion channels. The only difference between the PB and PNP equations is the presence of forces in the latter, otherwise they make the same mean field approximation in the calculation of potentials and concentrations. Thus intuitively one would expect the PNP formalism to suffer from the same problem with the overestimation of shielding effects when it is applied to narrow ion channels. Detailed comparisons of the PNP predictions with BD simulations have indeed confirmed these expectations [9, 10]. Excessive shielding in PNP almost cancels the repulsive dielectric self energy contribution, leading to much larger concentrations and currents in model channels compared to those found from the BD simulations. In practical applications of PNP, this problem is evaded by using diffusion constants for ions that are much smaller than warran-



*Figure 1.* Self and Coulomb energy profiles of an ion with charge  $e$  along the central axis of a cylindrical channel with radius  $4 \text{ \AA}$  and length  $35 \text{ \AA}$ . A cross section of the channel along its central axis is shown at the top. The two Coulomb energy profiles correspond to the charge configurations at the center and at the mouth (solid line) of the channel (dashed line). In the former case, four charges with magnitude  $e/4$  are placed around a ring of radius  $6 \text{ \AA}$  on the  $z = 0$  plane. In the latter case, two of these rings, each with a total charge of  $e$ , are placed near the entrance of the channel at  $z = \pm 12.5 \text{ \AA}$ .

ted. Even then, PNP fails to predict the observed saturation of conductance with increasing concentration because this property is directly related to the presence of energy barriers in a channel.

We remark that the neglect of the dielectric self energy is not the only problem faced by the continuum theories in applications to ion channels. Ion permeation in channels is basically a time dependent process – as an ion moves through the channel, the forces acting on it change. Therefore reduction of this problem to a steady state one is by no means a trivial procedure as assumed in current applications of the PNP equations. These problems are obviously compounded in multi-ion channels, where Coulomb repulsion among ions plays a critical role in the permeation process. In summary, the ion-channel (and ion-ion) interactions are not properly taken into account in the PNP formalism, and hence it does not provide a reliable framework for modeling of ion permeation in narrow channels. Here ‘narrow’ refers to a radius of 5 Å or smaller, which is the case for most biological channels. There are some ion channels with larger radius (e.g. porins), where PNP equations can still be applied profitably.

## 2.2. BROWNIAN DYNAMICS

The above discussion of the continuum theories discourages their use in ion channels. The next simplest permeation model is Brownian dynamics, where trajectories of  $N$  ions in the system are followed using the Langevin equation

$$m_i \frac{d\mathbf{v}_i}{dt} = -m_i \gamma_i \mathbf{v}_i + \mathbf{R}_i + \mathbf{F}_i, \quad i = 1, \dots, N, \quad (7)$$

where  $m_i$ ,  $\mathbf{v}_i$  and  $\gamma_i$  are the mass, velocity and the friction coefficient of the  $i$ th ion. The three force terms on the r.h.s. of Equation (7) correspond to the frictional, random and the total systematic forces acting on the ion. The frictional and random forces represent the incessant collisions of the ion with the surrounding water molecules in an average way. Because they arise from the same source, the two forces are not independent but related through the fluctuation-dissipation theorem [2]. The systematic force on an ion basically arises from the electric field acting on it. Provided continuum electrostatics is valid in channels, the electric field can be determined from the solution of the Poisson equation (1) for a given boundary. In more sophisticated treatments, the Coulomb force between two ions is supplemented with short range and hydration forces that are estimated from MD simulations [12].

The solution of the  $N$  coupled differential equations in (7) is clearly beyond any analytical treatment, leaving computer simulations as the only practical method of solution. A very accurate numerical integration of the Langevin equation can be achieved using a third order algorithm [27]. The real bottle neck in BD simulations is the accurate calculation of the forces acting on ions. Because the Poisson equation itself needs to be solved numerically, repeating this procedure at every time

step would be very time consuming and limit the usefulness of BD simulations. This problem can be circumvented by precalculating the electric potential and field on a grid of points and storing these values in a set of tables [28]. During simulations, the potential and field at desired points are determined by interpolating between the table entries. The simulation system is completed by attaching electrolyte filled reservoirs on either side of a lipid-channel complex. Because the specification of the boundary conditions in reservoirs is rather straightforward [29], we do not dwell on them here.

BD simulations have been applied to a variety of ion channels in recent years (for reviews see [12, 14]), and apart from the gramicidin A channel, the agreement with the experiments has been quite encouraging. Gramicidin A appears to be a special case because it forms a very narrow channel ( $r = 2 \text{ \AA}$ ), where continuum electrostatics fails [30, 31]. Of course, agreement with experiment is not sufficient to ascertain the accuracy of a theoretical model, and we need to justify the parameters employed and the approximations made. The only phenomenological parameter in the Langevin equation (7) is the diffusion coefficient of ions, which can be estimated from MD simulations quite accurately [32, 33]. If one could also calculate the average forces acting on ions from MD simulations, there would be no additional parameters in the model. However, this goal has not been attained yet, and the present applications of BD to channels rely on continuum electrostatics for the calculation of forces. Solution of the Poisson equation requires approximating the protein-water interface with a rigid boundary, and representing the protein and channel water as continuous dielectric media with constants  $\epsilon_p$  and  $\epsilon_w$ , respectively. Apart from gramicidin A, there has been little effort so far to establish the validity of these approximations from microscopic MD simulations. So they remain as important open questions to be investigated in future studies.

### 2.3. MOLECULAR DYNAMICS

Even though BD may provide a successful framework for modeling of ion channels, as stressed above, one still needs to appeal to MD simulations to justify the parameters employed and the approximations invoked in BD. In MD one solves Newton's equation of motion for all the atoms in the system

$$m_i \ddot{\mathbf{r}}_i = -\nabla_i U(\{\mathbf{r}_i\}), \quad (8)$$

and traces their motion via computer simulations. Here  $m_i$  refer to the mass of atoms,  $\mathbf{r}_i$  to their coordinates and  $U(\{\mathbf{r}_i\})$  is the potential function for the system whose gradient specifies the force acting on individual atoms. In most MD force fields, the non-bonded interactions among water molecules and ions in an electrolyte are represented by a pair-wise sum of the Coulomb and Lennard-Jones potentials. The covalent bonds that hold the protein and lipid atoms together are simply described by harmonic potentials. There are two limitations of MD that prevents it from becoming the model of choice in permeation studies. The first

is practical: MD simulations can be run for nanoseconds using present computers whereas many microseconds is required to determine the conductance of a channel. Even if the computer speeds keep increasing at the current rates, it will take decades to reach that level of performance. The second is to do with the force fields employed in the MD simulation packages, whose parameters are determined from fits to the bulk properties of electrolytes. An important omission in this respect is that these force fields do not include the polarization effects explicitly. Polarization interaction constitutes a significant part of the total energy of a water molecule or ion, but because it is expensive to calculate non-additive forces, it is treated approximately by absorbing its effects in the Coulomb and Lennard-Jones potentials. This may be fine in a bulk-like environment but during permeation, ions move from bulk water into a channel which has very different polarization characteristics. As shown below, problems arise when non-polarizable force fields are used in the calculation of free-energy profiles of ions in the gramicidin A channel. Therefore, it is important to develop polarizable force fields in order to obtain more realistic free energies of ions. The computational resources required for this purpose is now within the reach of current parallel and super computers, so this aim may be achieved in near future.

Because of the time limitation, the use of MD in channels is restricted to calculation of free energy profiles of ions and some other quantities related to permeation. For example, it can be used to study the ordering of water molecules, diffusion coefficients, and flexibility of protein atoms. In this respect, it plays a complimentary role to the BD simulations by providing the required input parameters such as the diffusion coefficients of ions and the effective dielectric constants of protein and channel water. Also, size based selectivity of ions cannot be described in BD, so the free-energy perturbation calculations from MD simulations are required to explain this property.

### 3. Applications to Ion Channels

The primary observable of an ion channel is its conductance. Because the channel current ( $I$ ) depends on both the applied voltage ( $V$ ) and bath concentrations ( $C$ ), the conductance data are typically presented in the form  $I-V$  and  $I-C$  curves. In the physiological range of 100 mV, the  $I-V$  curves are usually linear with a conductance in the range of 10–100 pS, though superlinearities are observed at higher voltages sometimes. Model studies with BD simulations suggest that these non-linearities may result from the presence of residual energy barriers in a channel [31]. The  $I-C$  curves, on the other hand, exhibit saturation even in the physiological range of 150 mM. This behaviour is described by the Michaelis-Menten function (Hille 2001)

$$I = \frac{I_{\max}}{1 + C_s/C}, \quad (9)$$

where  $I_{\max}$  is the maximum conductance and  $C_s$  is the saturation concentration at half-maximum. The measured  $C_s$  values range from 10 mM for Ca channels to about 300 mM in gramicidin A. An intuitive explanation of saturation follows from the fact that ions move across channels in single file and may need to overcome energy barriers. Thus, regardless of the bath concentrations, there is a minimal transport time for each ion to cross a channel, which translates to a maximal possible current for a given voltage. The ability of a model to reproduce  $I-C$  curves is much more important than fitting  $I-V$  curves, which can be achieved, for example, by simply adjusting the diffusion coefficient of ions. In contrast, there is no easy way of achieving saturation in a permeation model other than describing ion dynamics in the channel correctly.

### 3.1. GRAMICIDIN A CHANNEL

In membranes, the dimer of the antibiotic peptide gramicidin A (GA) forms a cylindrical channel with length 25 Å and radius 2 Å, that selectively conducts monovalent cations, binds divalent cations, and rejects anions. Its physiological properties are characterized by linear  $I-V$  curves and relatively large half-saturation concentrations. These observations point to lack of substantial barriers within the channel. In addition, NMR studies indicate well established binding sites near the pore entrances. This wealth of functional data has been matched with an atomic resolution structure since 1971 [16]. For these reasons, the GA channel have played a prominent role in development of permeation models in ion channels. There is an extensive literature on modeling of the GA channel which can be traced from the review articles [13, 17, 34]. While the appearance of the structure of the KcsA potassium and other channels has reduced the appeal of GA somewhat, it still remains one of the simplest and most well known channels, and in this respect, it can play an important role in development and testing of the permeation models. In the following, we stress this aspect of GA.

As noted earlier, continuum electrostatics fails in the GA channel [31]. This is not surprising because water molecules form a single-file column in the channel as dictated by its geometry. In the absence of a reliable force, one can use the inverse method to construct a potential of mean force (PMF) of ions that successfully reproduces the known properties of GA when employed in BD simulations. Such a PMF has been constructed for potassium ions [31], and as shown in Figure 2, it has two binding sites at the channel entrances and a central barrier of about 5 kT. The energy wells are required to explain the observed binding of cations at the channel mouths and the barrier is essential for reproducing the saturation of the conductance.

The PMF in Figure 2 distills the observed properties of the GA channel, so it can be directly used to assess the accuracy of the PMF profiles calculated from MD simulations. To date, all such MD calculations have led to central energy barriers that are too high to allow ion permeation through the GA channel at the observed

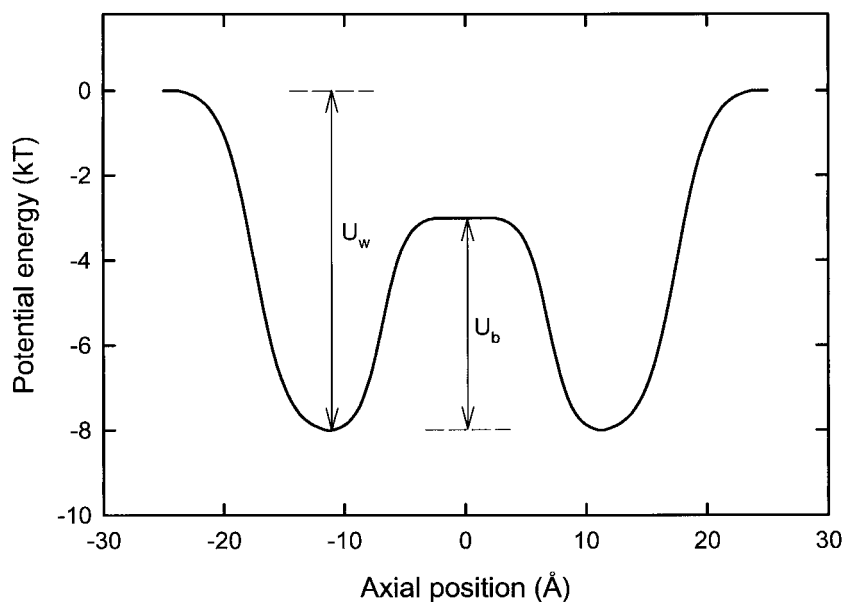


Figure 2. The potential profile of a potassium ion in the GA channel constructed using the inverse method. The well depth  $U_w$  and barrier height  $U_b$  are indicated by the arrows. The curved parts in the profile are produced using a Fermi function of the form  $1/\{1 + \exp[\pm(z - z_0)/d]\}$ . The profile shown with  $U_w = 8 kT$  and  $U_b = 5 kT$  gives the best description of the physiological data for potassium ions.

rates. Two recent examples obtained using the CHARMM and GROMACS force fields are shown in Figure 3 to illustrate this point: both PMFs have barrier heights above 20 kT, which would result in suppression of the channel conductance by more than a factor of million. As pointed out above, a possible explanation of the failure of these force fields is their neglect of the polarization effects. While the importance of polarization has been recognized in earlier studies of the GA channel [35], it is yet to be seen whether its inclusion will solve the problem with the PMF pointed out in Figures 2 and 3. If the lack of polarization is found to be responsible for the observed discrepancy, this will naturally have implications for all ion channels.

### 3.2. POTASSIUM CHANNELS

The determination of the crystal structure of the KcsA potassium channel [18] has given a new impetus to modeling of ion channels. While KcsA is a bacterial potassium channel, quite different from those found in animals in many details (e.g. gating), two of its main features are expected to be preserved in all potassium channels, namely, the narrow selectivity filter with a 1.5 Å radius that holds two

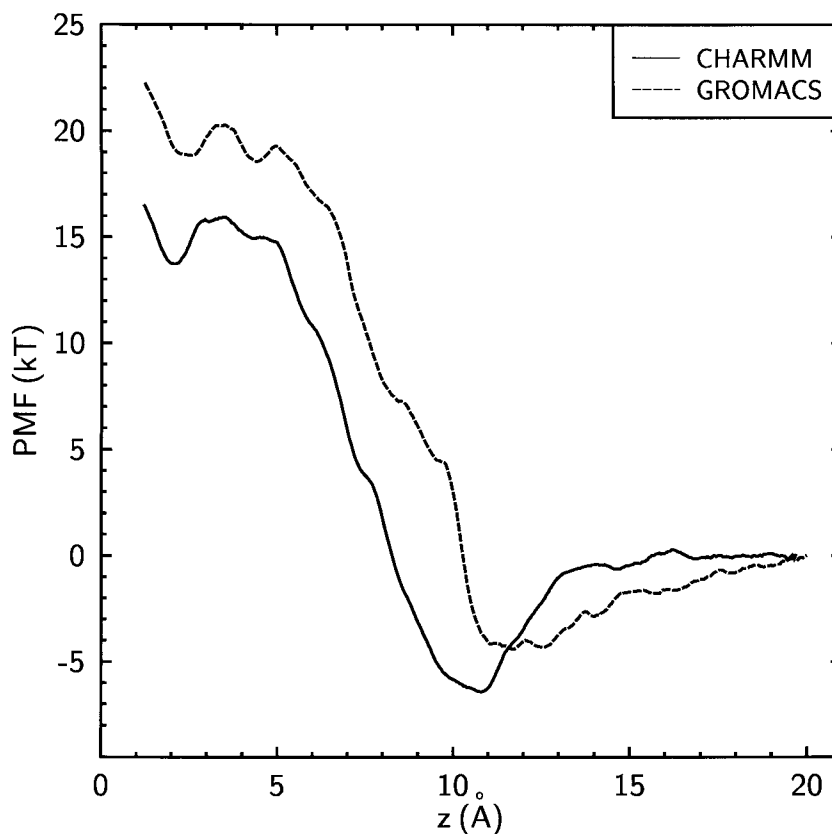


Figure 3. PMF profiles of a potassium ion along the central axis of the GA channel calculated with the CHARMM and GROMACS force fields. The PMFs are calculated using the umbrella sampling technique and the weighed histogram analysis method.

$K^+$  ions, and a water filled cavity that follows it. The role of the filter is to select the  $K^+$  ions against  $Na^+$  and the cavity helps in reducing the self-energy barrier of ions. Here we present a brief overview of the model studies of the KcsA channel, and refer to reviews [12, 14, 15] and original articles on the applications of the BD [36, 37, 38] and MD methods [39, 40, 41, 42, 43].

Because the crystal structure of KcsA corresponds to its closed state, a first hurdle in model studies is to find its open state. This can be achieved by a variety of means but such a process necessarily introduces some arbitrariness in the results. Thus at this stage, model studies are useful for understanding the permeation mechanism in the potassium channel but claims of precise agreement with data should be taken with a pinch of salt. In BD simulations of the KcsA channel, it is found that the selectivity filter is always occupied with two  $K^+$  ions in agreement with the observations [18, 19]. When there is an applied voltage pushing the ions

outside the cell, the presence of a third ion in the cavity destabilizes the equilibrium of the two resident ions in the filter. The three ions move more or less in tandem towards the extracellular region until the one nearest to the bulk is expelled from the channel, leaving again two  $K^+$  ions in the filter. This permeation mechanism appears to be quite robust as very different parametrizations of the channel have given similar results.

The permeation mechanism delineated by the potential energy profiles and the BD simulations sheds much light into the central paradox in operation of ion channels (i.e. large conductance vs. selectivity), and how nature has solved this problem. The selectivity filter is very narrow to enable it to differentiate between potassium and sodium ions, and it has a very deep binding site. On the basis of these two factors, one would intuitively expect ions' crossing of the filter to be the rate-limiting step in the permeation process. In fact, Coulomb repulsion in the three-ion system causes it to be unstable and thereby making this the fastest step in permeation. A related puzzle is the large variations (nearly two orders of magnitude) observed in the conductance levels of various potassium channels. Clearly one could not explain such a diversity, had the filter been the rate limiting step because it is presumed to be conserved. Study of multi-ion potential profiles show that the energy barrier in KcsA can be reduced substantially by increasing the radius of the inner mouth of the channel by a few Å, which leads to an exponential increase in conductance [44]. Thus the large conductance variations in potassium channels can be explained by changes in the radius of the intracellular mouth while keeping the selectivity filter on the opposite side intact.

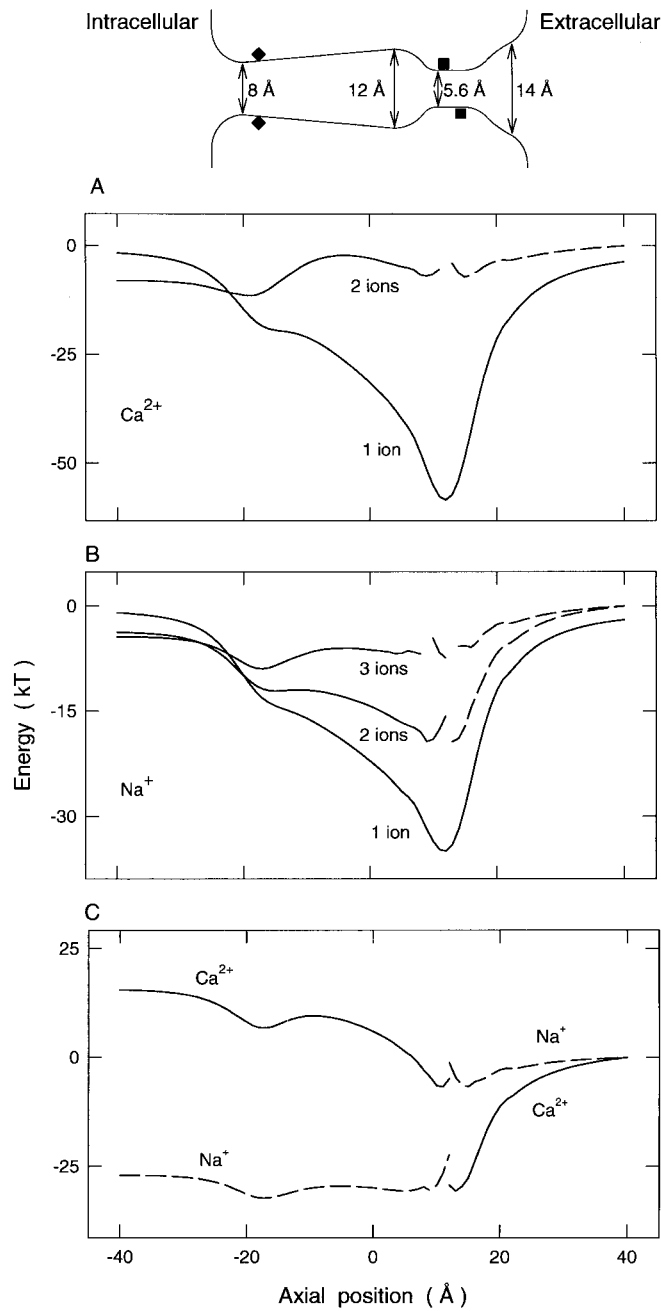
Many MD studies of the KcsA channel have been performed since the appearance of its structure. Most of them focus on free-energy calculations of  $K^+$  ions in the selectivity filter. There is a general agreement among the MD simulations that the KcsA protein can hold three  $K^+$  ions in a stable conformation, two in the filter and one in the central cavity, as observed in the x-ray structure [18, 19]. A study of the PMF of the  $K^+$  ions in the selectivity filter reveals that the ions face only a few kT energy barriers, and hence can freely diffuse in that region [45]. However, in view of the uncertainties associated with the non-polarizable force fields employed in these studies, the absolute values of the calculated free energies has to be interpreted with caution. The free energy differences between  $K^+$  and  $Na^+$  ions in the selectivity filter have also been calculated. The predicted relative energy barriers ( $\sim 8$  kT) can explain the observed selectivity margin of the channel. Such energy differences are expected to be less sensitive to potential defects in the force fields, and hence the results may be more robust.

### 3.3. CALCIUM CHANNELS

Calcium channels are as common in excitable cells as potassium channels and share similar properties, that is, they are extremely selective (margin for Ca/Na is  $10^3$ ) and yet conduct at the picoampere level [46]. But there is also a crucial difference:

selectivity is based on charge and not size. Radius of the  $\text{Ca}^{2+}$  ion (0.99 Å) is only slightly larger than that of  $\text{Na}^+$  while the pore radius is estimated to be about 2.8 Å [47]. Thus selectivity of calcium channels can be understood at the BD level without having to appeal to MD. An intriguing feature of this selectivity against  $\text{Na}^+$  ions is that it is contingent upon the presence of  $\text{Ca}^{2+}$  ions. In their absence,  $\text{Na}^+$  ions conduct at an even faster rate than  $\text{Ca}^{2+}$ . Physiological properties of calcium channels are well known but the corresponding information on the structural side is rather scarce, i.e. their tertiary structures have not been determined from crystallography yet. This discourages any attempts to model calcium channels using MD simulations because they are quite sensitive to structural details, and it would be very difficult to get sensible results out of MD in such circumstances. Fortunately, structural requirements for BD simulations are much less demanding – an approximate shape of the channel and positions of the partial charges in the protein are all one needs to model its functional properties. Such an attempt has recently been made in a model study of L-type calcium channel [48]. We remark that a similar study was carried out using the PNP formalism earlier [49]. But in view of the general criticism of the continuum theories presented above, reliability of these results are questionable.

The shape of the calcium channel used in the model study is shown in the inset of Figure 4. It is inspired by the KcsA structure but modified to take into account a variety of physiological data. Four glutamate residues are known to play an essential role in the channel conductivity and selectivity [50], and these are represented by four negative charges in the narrow selectivity filter (indicated by squares in the figure). The only other charge residues required to make the channel conduct are the set of four dipoles placed on the intracellular mouth (diamonds in the figure). Multi-ion potential profiles give an intuitive understanding of the permeation mechanism in the calcium channel as shown in Figure 4. It is seen that a single  $\text{Ca}^{2+}$  ion would be deeply bound in the selectivity filter (A). A second  $\text{Ca}^{2+}$  ion is attracted to the channel from the extracellular side, and the two ions can coexist in the filter region in a semi-stable equilibrium, until the resident ion on the left climbs over the barrier of 5 kT via thermal fluctuations and exits the channel. A similar picture is obtained for the  $\text{Na}^+$  ions (B), except three of them can coexist in the filter and the final barrier to permeation is only 1 kT, which explains why the sodium ions conduct faster. Selectivity of the calcium channel can be understood by constructing multi-ion profiles with mixed set of ions (C). When a  $\text{Na}^+$  ion is resident in the filter, a  $\text{Ca}^{2+}$  ion is attracted to the filter and expels the  $\text{Na}^+$  ion from the channel upon entry. A similar result is obtained when there are two  $\text{Na}^+$  ions in the filter. In the reverse case of a  $\text{Ca}^{2+}$  ion in the filter, though a  $\text{Na}^+$  ion is still attracted, it is unable to push the  $\text{Ca}^{2+}$  ion over the large barrier of 16 kT. Thus once a  $\text{Ca}^{2+}$  ion enters the channel,  $\text{Na}^+$  ions cannot push it out, only another  $\text{Ca}^{2+}$  ion can achieve that feat. This gives a simple explanation of the selectivity mechanism in calcium channels in terms of the electrostatic interactions of ions, which is in conformity with the insights gathered from the rate theory models.



*Figure 4.* Shape of a model calcium channel and locations of charge residues (inset). Potential energy profiles for 1 and 2  $\text{Ca}^{2+}$  ions (A), and 1, 2 and 3  $\text{Na}^{+}$  ions (B). The profiles for the mixed system is shown in (C). The dielectric constants used in the solution of the Poisson equation are 60 for channel water and 2 for the protein. The electric field is in the  $z$  direction driving ions from outside the cell (right) to inside (left).

Many physiological properties of calcium channels have been explained using this simple model in BD simulations [48]. For example, the  $I-V$  and  $I-C$  curves are found to be in good agreement with the experimental observations. Other, more exotic properties that are described by BD include the anomalous mole fraction effect, blocking of calcium current by external sodium, and how the mutation of the glutamate residues in the filter changes the mole fraction effect.

#### 4. Conclusions

It is argued that Brownian dynamics currently provides the best alternative for studying structure-function relations in ion channels. Continuum theories are ruled out because they ignore the dielectric self-energy which is responsible for many properties such as saturation of conductance through creation of energy barriers. Molecular dynamics, although more fundamental than BD, is unable to calculate the key property of channels (i.e. conductance), hence it is of limited use for the above purpose.

Studies of potassium and calcium channels using continuum electrostatics and BD demonstrate that permeation mechanism involves multi-ions, and Coulomb repulsion among the ions plays an essential role in explaining the central paradox of ion channels, that is, the fast permeation of ions across a binding site (i.e. the selectivity filter). Comparisons of BD simulation results to experimental observations are very encouraging for future applications of this method as they indicate that basic properties of ion channels can be understood using a simplified model in the absence of a detailed tertiary structure. Of course, much more work is needed to justify the use continuum electrostatics in ion channels, and to develop reliable force fields for MD applications.

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