Eur Biophys J (2002) 31: 283–293
DOI 10.1007/s00249-002-0216-4

REVIEW

Shin-Ho Chung · Serdar Kuyucak

Ion channels: recent progress and prospects

Received: 7 January 2002 / Revised: 12 February 2002 / Accepted: 19 February 2002 / Published online: 16 April 2002

Abstract Determination of the crystal structure of the KcsA potassium channel and its subsequent refinement at 2 Å resolution have stimulated much interest in modelling of ion channels. Here we review the recent developments in ion channels research, focusing especially on the question of structure-function relationships, and discuss how permeation models based on Brownian and molecular dynamics simulations can be used fruitfully in this endeavour.

Keywords Ion channels · Brownian dynamics · Molecular dynamics · Potassium channels

Introduction

Research in ion channels, as in other areas of biology, has long had a strong experimental tradition with relatively little input from theory. The reason for this imbalance between theory and experiment is usually attributed to the complexity of both the biological processes and the underlying molecular structure involved in their execution. Rapid advances in two frontiers, namely determination of the tertiary structures of macromolecules from X-ray diffraction and NMR, and an exponential increase in computational power allowing large-scale simulations of biological processes, give hope that these obstacles will be surmounted in the near future. In this regard, modelling of ion channels has an advantage over other biological processes because their function is rather simple: to allow passive diffusion of selected ions down their electrochemical gradient when the channel is open. There are a host of theories in the physical chemistry of electrolyte solutions that one can use to describe such a process (e.g. Berry et al. 2000). However, lack of structural information on channel forming proteins has hampered efforts for realistic modelling of biological ion channels until recently. Here we stress "biological" because the tertiary structure of the channel forming antibiotic gramicidin A (GA) has been known for a long time (Urry 1971), and much effort has gone into its modelling (see Partenskii and Jordan 1992; Roux and Karplus 1994 for reviews). Unfortunately, the structural and permeation properties of GA appear to be quite different from those of biological channels, and therefore GA is not likely to fulfil the role of a useful stepping stone for them, contrary to earlier expectations.

For these reasons, the appearance of the crystal structure of the KcsA potassium channel at 3.2 Å resolution (Doyle et al. 1998) has been greeted with much enthusiasm in the field. Very recently, MacKinnon's group increased the resolution of the KcsA structure to 2.0 Å, which allowed direct observation of the binding sites of K^+ ions in and near the selectivity filter (Morais-Cabral et al. 2001), as well as the water molecules that are coordinated with these K^+ ions (Zhou et al. 2001). On the physiological front, doubts about the relevance of the bacterial KcsA structure to potassium channels in animals (Meuser et al. 1999) have been largely lifted by careful measurements of its conductance properties (LeMasurier et al. 2001), and by direct substitution of the KcsA filter in Shaker and inward rectifier potassium channels (Lu et al. 2001). A remaining problem for modelling purposes is that the crystal structure of KcsA corresponds to a closed state of the channel and one needs the open-state conformation to calculate its conductance. The conformational changes that occur during the opening of the channel have been investigated using site-directed spin labelling and electron paramagnetic resonance spectroscopy (Perozo et al. 1999; Liu et al. 2001). These studies indicate that during gating the transmembrane helices lining the pore move away from the central

S.-H. Chung (✉) · S. Kuyucak
Department of Theoretical Physics,
Research School of Physical Sciences,
Australian National University, Canberra,
ACT 0200, Australia
E-mail: shin-ho.chung@anu.edu.au
Tel.: +61-2-61252024
Fax: +61-2-62472792

axis on the intracellular side, but the residues forming the selectivity filter and cavity on the extracellular side remain intact. Taken together, these results provide an unprecedented amount of structural and functional information for modelling of a biological ion channel, and will doubtless inspire many studies of the KcsA channel in years to come. Looking beyond the ion channels, the high-resolution structure of Zhou et al. (2001) has the potential to turn the KcsA protein into a microscopic laboratory for testing models used in description of electrolyte solutions, with far-reaching implications for physical chemistry and molecular biology.

Here we review the recent progress in modelling of ion channels. The focus is naturally on potassium channels – specifically the impact of the new structural information on the KcsA protein on functional studies of potassium channels. The tools available from physical chemistry for this purpose are: continuum theories of electrodiffusion, Brownian dynamics (BD) and molecular dynamics (MD) simulations. At present, no single computational method can describe all the functional properties of an ion channel. For example, continuum theories or BD do not distinguish between the monovalent ions. Thus, to understand the selectivity of potassium channels against the sodium ions, one has to appeal to MD simulations. On the other hand, MD is too slow to estimate the conductance of a channel, which is not a problem for the other, more coarse-grained simulation methods. Thus, for a detailed study of the structure-function relationships in ion channels, it is necessary to adapt a multi-pronged or a hierarchical approach to modelling. Below we first discuss the basic tenets of the MD and BD simulation methods that are most appropriate for this purpose. These methods are then applied to modelling of ion channels with a view to predicting their functional properties from the underlying channel structure. Besides the potassium channels we also discuss the calcium channels because of their biological significance and also as a role model for studies of structure-function relationships in other ion channels.

Permeation models

Invention of the patch-clamp technique (Neher and Sakmann 1976) has enabled accurate measurement of *I-V* curves in numerous ion channels during the last two decades. In the absence of structural information on membrane proteins, these results were mostly interpreted using barrier models, where the permeation process is viewed as ions hopping over barriers in the channel (Hille 2001). Because the model parameters in barrier models are determined from fits to physiological data with no direct input from the channel structure, they are not suitable for studying structure-function relationships. The simplest method that allows such a structural input (i.e. calculates the electric forces that drive ions across a channel from its structure) is the continuum theories of electrodiffusion commonly known as the Poisson-Nernst-Planck equations (Eisenberg 1999). Unfortunately, the mean field approximation that forms the basis of the continuum theories was shown to break down in narrow pores with a radius of less than 2 Debye lengths (Corry et al. 2000; Graf et al. 2000; Moy et al. 2000). At the physiological concentration of ~150 mM, this length scale corresponds to 16 Å, which is much larger than typical radii of ion channels. Therefore, despite its attractiveness as a simple method requiring minimal computational effort, continuum theories cannot be used in modelling of ion channels, and one has to resort to alternative methods that represent ions as discrete particles. Here the two options are MD and BD simulations that we discuss briefly below.

Molecular dynamics

Conceptually, MD is quite simple; one follows the trajectories of N particles interacting via a many-body potential $(r_1, ..., r_N)$ using Newton's equation of motion:

$$m_i \frac{\mathrm{d}^2 r_i}{\mathrm{d}t^2} = -\nabla_i U(r_1, \ldots, r_N) \qquad i = 1, \ldots, N \tag{1}$$

where m_i and r_i denote the mass and position of the ith particle. From statistical analysis of the trajectory data generated during simulations, one can determine the structural and dynamic properties of a system. The potential functions (or force fields) are clearly the crucial inputs in MD, and the success of MD simulations in representing reality hinges critically on their correct choice. A tremendous amount of work has gone into the development of force fields for biomolecular applications since the early 1980s (see Wang et al. 2001 for a recent review), resulting in some popular simulation packages such as AMBER (Weiner et al. 1984), CHARMM (Brooks et al. 1983) and GROMOS (Hermans et al. 1984). In these programs, the nonbonded interactions between atoms are typically represented by two-body Coulomb and 12-6 Lennard-Jones (LJ) potentials:

$$U_{ij} = \frac{q_i q_j}{r_{ij}} + 4\varepsilon_{ij}\left[\left(\sigma_{ij}/r_{ij}\right)^{12} - \left(\sigma_{ij}/r_{ij}\right)^{6}\right] \tag{2}$$

where q_i denotes the partial charges on atoms, ϵ_{ij} is the depth of the LJ potential at the minimum and σ_{ij} is where the repulsive and attractive terms cancel each other. This pairwise interaction is used for all atoms in the system, including freely moving water molecules and ions in an electrolyte, as well as protein and lipid atoms that are bound to each other by chemical bonds. In order to preserve the bond lengths and angles between atoms in proteins and lipids, harmonic potentials are employed in addition to Eq. (2). The potential parameters are determined empirically from spectroscopic data and by fits to bulk properties. There are also a host of technical problems associated with mimicking a bulk situation with a small system and handling of the

long-range Coulomb interactions. For a discussion of these problems, as well as how physical quantities are determined from statistical analysis of trajectory data, we refer to the textbooks by Allen and Tildesley (1987) and Frenkel and Smit (1996).

Considering the simplicity of the potential functions in current use, MD techniques have been remarkably successful in studies of lipid-protein systems. This suggests that neglected terms in the potential – most notably induced polarization – are incorporated in the two-body terms such that their average effects are represented quite well. To give an example, the dipole moment of a single water molecule is 1.85 D, but in widely used water models such as SPC (Berendsen et al. 1981) and TIP3P (Jorgensen et al. 1983) an inflated moment of $\sim$2.3 D is employed to take into account the induced polarization effects in bulk water. This average treatment of polarization appears to work well as long as one retains the bulk-like environment for water (Tieleman et al. 1997; Wallqvist and Mountain 1999). In application of MD to ion channels, however, there is likely to be problems in this regard because ions move from bulk water into a narrow pore formed by protein molecules with very different polarization characteristics. Firm evidence signalling the failure of the non-polarizable force fields comes from past MD calculations of the potential of mean force in the GA channel: the calculated translocation barriers for monovalent cations are found to be too steep to allow their permeation at the experimentally observed rates (Roux and Karplus 1994). While the importance of polarization has been recognized in some earlier work on GA-like channels (Jordan 1990; Duca and Jordan 1998), polarizable force fields have not been applied to realistic channel models so far. Thus, whether inclusion of the polarization effects in MD simulations of channels will lead to quantitative agreement with the physiological data still remains to be seen.

A second problem in practical applications of MD to ion channels is to do with the simulation time. The rotational motion of water molecules is quite fast, and in order to maintain accuracy, one has to use a very small time step ($\sim$1 fs) in the numerical integration of Eq. (1). Thus an MD simulation lasting a million time steps covers only 1 ns, which is too short to study the conduction of ions in biological channels (a typical channel current of 5 pA corresponds to an average transit time of 32 ns for a single ion). With the doubling of computer speeds every two years, this time limitation will be eventually surmounted. In the mean time, however, one has to use the coarse-grained but much faster BD simulations to calculate the conductance of biological ion channels.

If one identifies the free-energy profiles and conductance of ions as two significant descriptors of ion channels, the foregoing discussion suggests that current applications of MD fail in both respects, and more work needs to be done to turn it into a more reliable and useful method in the study of structure-function relationships. As pointed out above, construction of polarizable force fields for applications of MD to ion channels is one of the first priorities in this regard. A longer-term goal would be to use ab initio MD methods to derive the force field parameters directly from the electronic structure calculations rather than determining them empirically from fits to data (Kuyucak et al. 2001). There has been a great deal of progress in the application of ab initio MD methods since the adaptation of density functional theory (Kohn 1999) to calculate the potentials between atoms on the fly (Car and Parrinello 1985). Initial applications of ab initio MD were in condensed matter physics, though in recent years it has also been used in studies of water, electrolytes and biological molecules. Modelling of ion channels is a natural step in this progression that presents a challenging problem for ab initio MD. As for the time constraint problem, short of waiting for faster computers, one needs to devise methods that will permit faster simulation of the atoms in regions of marginal interest without loss of accuracy for those in regions of focus, which should be a small fraction of the total system for ion permeation.

Notwithstanding the above problems, there are aspects of ion permeation that can still be studied profitably using the currently available MD methods. For example, the selectivity sequences among monovalent cations can be predicted from free-energy perturbation calculations. Because this quantity involves the energy difference for transformation of an ion of one type into another at the same location, inaccuracies in force fields are likely to cancel out, making such predictions more robust compared to the calculation of the free-energy profile of an ion along the permeation pathway. Moreover, since ions with the same valence cannot be distinguished in BD, MD offers the only method for understanding their selectivity sequences. Diffusion coefficients of ions and the dielectric constant of water in the channel are two other local properties that could be estimated from MD. These quantities are required as input parameters in BD simulations that would have to be determined from fits to experimental data otherwise. Thus MD plays a complementary role to BD in many respects, reducing the arbitrariness in the choice of free parameters that so often plagues application of phenomenological models to realistic systems.

Brownian dynamics

Water molecules are ubiquitous in biological systems and their explicit modelling is usually very costly. In a 150 mM salt solution there are 370 water molecules for each ion pair. Thus, an implicit treatment of water would reduce the simulation time of an electrolyte solution by a factor of 10^3 (H_2O is represented by three charge sites in MD). Further, as noted above, the rotational motion of water molecules is very fast, requiring a time step of $\sim$1 fs, whereas one could use a much larger time step of $\sim$100 fs for spherical ions that move only by

overdamped translational diffusion. Together these two factors provide a gain of 10^5 in simulation time, which is sufficient improvement on MD time scales to enable calculation of conductance of an ion channel.

An implicit treatment of water is provided by BD, where one follows the trajectories of N ions in an electrolyte solution using the Langevin equation:

$$m_i \frac{\mathrm{d}v_i}{\mathrm{d}t} = -m_i \gamma_i v_i + R_i + F_i \qquad i = 1, \ldots, N \tag{3}$$

where m_i, v_i and γ_i are the mass, velocity and the friction coefficient of the ith ion. The three force terms on the right-hand side of Eq. (3) correspond to the frictional, random and the systematic forces acting on the ion. The frictional and random forces represent the continual 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 (Reif 1965). Thus the knowledge of the friction coefficient (or the diffusion coefficient D, since $D = kT/m\gamma$ from the Einstein relation) is sufficient to determine these two forces. The random force is sampled from a Gaussian probability distribution with a zero mean, and it is assumed to be Markovian. The systematic forces basically arise from the electric field acting on the ion, and the magnitude of this force is calculated by solving Poisson's equation. In addition, short-range repulsive and hydration forces are added to the right-hand side of Eq. (3) in more refined BD simulations (Corry et al. 2001).

Implementation of the electric forces is trivial in a bulk electrolyte, e.g. maintaining a constant potential difference between two conducting planes leads to a uniform electric field in the system. The situation in ion channels is much more complicated because one has to take into account the effect of the dielectric boundary created by the water-protein interface by solving the Poisson equation with Dirichlet boundary conditions. Because the interaction of ions with the induced charges on the channel wall (self-energy or reaction field) plays a major role in determining their permeation characteristics, an accurate solution of this problem is essential. The channel boundaries have mostly irregular shapes, which rules out analytical approaches using a simplified geometry such as a cylinder. Numerical solution of the Poisson equation can be readily achieved using either the boundary element method (Levitt 1978; Hoyles et al. 1998a) or the finite difference method (Davis and McCammon 1990; Sharp and Honig 1990). However, repeating this procedure at every step of a BD simulation that lasts millions of time steps is not feasible even on a supercomputer. Until recently, this computational bottleneck has prevented application of BD simulations to realistic 3D channel geometries, limiting such studies to schematic 1D channels (Cooper et al. 1985; Jakobsson and Chiu 1987; Bek and Jakobsson 1994). This problem was finally overcome by storing the precalculated values of the electric field and potential in a system of lookup tables, and interpolating the required values from these tables during simulations (Hoyles et al. 1998b). Since then, the lookup-table method has been employed in BD studies of the nicotinic acetylcholine receptor (Chung et al. 1998) and KcsA potassium (Chung et al. 1999, 2002a), calcium (Corry et al. 2001) and GA channels (Edwards et al. 2002). There are also some recent three-dimensional BD simulations of porin channels (Schirmer and Phale 1999; Im et al. 2000). However, the effect of the dielectric boundary on the ions (i.e. the reaction field) is either ignored or grossly simplified in these studies, thereby avoiding the computational bottleneck mentioned above.

Because the Langevin equation (3) is more complicated than that of Newton's, its numerical integration is also more involved. A third-order algorithm that can handle both diffusive and kinetic regimes (van Gunsteren and Berendsen 1982) is most appropriate for application of BD to ion channels as it allows use of short-time steps for tracking the motion of ions inside the channel and longer ones for those in the reservoirs (Chung et al. 1999). Because the forces acting on ions change rapidly in narrow pores, it is important to use short-time steps for correct modelling of ion dynamics in such regions. This is not a problem for the majority of ions in the reservoirs, and use of long-time steps there saves valuable time, enabling longer simulations. For a detailed discussion of BD algorithms and their implementation, we refer to the review articles by Cooper et al. (1985) and Kuyucak et al. (2001).

Another issue is the treatment of the boundary conditions in BD simulations of ion channels, which is almost trivial in comparison to MD. This is because the electric field of an ion in an electrolyte solution is severely dampened compared to the vacuum: dielectric screening due to water reduces it by a factor of 80 and Debye shielding due to counter-ions further attenuates the remaining field. From the Debye-Hückel theory, an ion's field is totally screened out beyond 4 Debye lengths (Berry et al. 2000), and one need not worry about their effects beyond this length scale. For physiological concentrations ($\sim$150 mM), this corresponds to about 30 Å. Thus a simple method is to attach electrolyte-filled reservoirs with dimensions of 30 Å to either side of the channel-lipid complex, and confine the ions in these reservoirs by elastically scattering them off the walls. The electrolyte is assumed to continue beyond the reservoir walls, and the scattering of ions can be construed as simultaneous exit and entry of ions, thereby maintaining their average concentration in the system at all times. The membrane potential can be implemented in a similarly simple manner by applying a uniform electric field across the system. After equilibration, charge separation occurs in the reservoirs, cancelling the applied field there, and the potential drop takes place mainly across the channel. Alternatively, one can use the grand canonical Monte Carlo method that allows fluctuations in ion concentrations and a more realistic implementation of the membrane potential (Im et al. 2000).

Comparison of this more sophisticated method with the simple one shows that, as far as predicting the channel properties is concerned, there is no discernible difference between the two methods (Corry et al. 2002). Thus the simple method with less computational overhead would be preferable in practical applications of BD to ion channels.

Comparisons of BD simulations with the conductance properties of biological ion channels have, so far, not led to any major discrepancies that would signal the failure of the approach. Nevertheless, there are several issues of validation that need to be addressed using a more fundamental theory such as MD. These arise from the treatment of water and protein as continuous dielectric media with a rigid boundary between them. Success of the Born model suggests that use of continuum electrostatics in channels may be justified if the hydration shell of a permeating ion remains intact. This criterion is generally satisfied in biological ion channels, including the narrow selectivity filter regions where protein atoms substitute for water completing the solvation shell. A rigorous justification of this idea and extraction of an effective dielectric constant for channel water require MD simulations, and remain as future problems. A second problem is whether there are any correlations between permeating ions and the motion of protein residues forming the channel. For example, local motions such as flipping of a side-chain may play a role in ion permeation. Again this problem needs to be explored further from MD simulations.

Applications to ion channels

The determination of the crystal structure of the KcsA potassium channel (Doyle et al. 1998) has been a turning point in modelling of biological ion channels. Until then, serious modelling work was carried out only on the GA channel using the MD method, and the results of the modest contact between theory and experiments were not very encouraging. With the appearance of the KcsA structure, most of the theoretical interest has been shifted to the modelling of the KcsA channel. The recent refinement of the KcsA structure at 2 Å resolution (Morais-Cabral et al. 2001; Zhou et al. 2001) will no doubt reinforce this interest. While acknowledging the importance of the KcsA structure, here we would like to advocate a broader approach to the modelling problem that emphasizes structure-function relationships in a variety of channels, not just one with a known structure. The fact remains that there are very diverse ranges of ion channels in nature – even within the potassium channel family – and their modelling will require more ingenuity than solely performing MD simulations using the available crystal structure. In this regard, the coarse-grained BD approach has certain advantages over the microscopic MD method because it is not as sensitive to the details in atomic structure of membrane proteins, and therefore more forgiving against errors one is likely to commit when modelling an ion channel in the absence of its tertiary structure. BD modelling of calcium channels provides a nice illustration of this point and will be discussed in some detail below.

Potassium channels

The crystal structure of the KcsA protein revealed three distinct regions in the pore of a potassium channel (Doyle et al. 1998): a narrow selectivity filter facing the extracellular side followed by a water-filled cavity and a hydrophobic pore that extends to the intracellular side. The selectivity filter is ~12 Å long with a ~1.4 Å radius, and is lined up with the carbonyl oxygens of the TVGYG amino acid sequence. Because the channel protein has a four-fold symmetry around the pore axis, these residues form a ring around the pore and each ring contributes four oxygen atoms. The filter is designed to select K^+ ions against Na^+ ions, which have a much higher extracellular concentration (crystal radius of K^+ is 1.33 Å and that of Na^+ is 0.95 Å). The high-resolution structure (Morais-Cabral et al. 2001; Zhou et al. 2001) provides a more clear view of how the potassium channels achieve a high rate of conductance for K^+ ions while retaining their exquisite selectivity. In the crystal structure, K^+ ions are observed to occupy four sites in the filter region with approximately equal probability (see Fig. 1). The first three sites (S1, S2, S3) are located in between the planes defined by the carbonyl oxygens of the Y_{78}, G_{77}, V_{76} and T_{75} residues, whereas S4 lies in between the carbonyl and hydroxyl oxygens of T_{75}. When a K^+ ion is in one these sites, it is solvated by eight oxygens from the neighbouring residues. Another significant observation is the eight-fold coordination of K^+ ions with water molecules in the cavity and the extracellular mouth of the channel (Zhou et al. 2001). These results are interpreted as two K^+ ions permanently occupying either the S1–S3 sites or S2–S4 and oscillating between these two configurations without any significant free

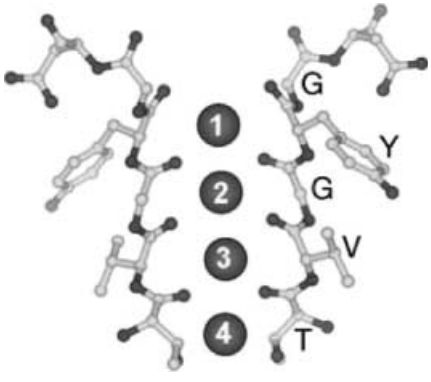

Fig. 1. Structure of the selectivity filter depicting the TVGYG sequence from two of the subunits in the KcsA protein and the ion binding sites S1–S4

energy barriers. The two ions are sandwiched between water molecules, forming a W-K^+-W-K^+-W complex. When a third K^+ ion appears at either end of the filter, this equilibrium configuration is disrupted and the ion-water column moves rapidly until the extra ion is ejected from the filter. The fact that K^+ ions are eight-fold coordinated with oxygen atoms at all sites from entry to exit makes the permeation process energetically very smooth, especially during the critical dehydration and rehydration steps. The carbonyl oxygens of the G_{79} residues point towards the extracellular solution and are too far to contribute directly to the solvation of K^+ ions. Instead, they are likely to be involved in the dehydration step by stripping the water molecules off a K^+ ion that is about to enter the filter from outside (and vice versa for an exiting ion).

The second region in the KcsA structure is the water-filled cavity with a radius of ~5 Å, and four sets of helix dipoles pointing towards its centre. This creates a favourable environment for the presence of a K^+ ion (Roux and MacKinnon 1999), as clearly seen in the crystal structure of KcsA. The cavity is connected to the intracellular side via a long hydrophobic pore formed by four transmembrane helices (TM2) that appears to be designed to minimize the interactions with ions. The radius of this intrapore region is smaller than that of an K^+ ion at places; therefore the crystal structure corresponds to the closed state of the channel. Electron paramagnetic resonance studies with site-directed spin labelling (Perozo et al. 1999; Liu et al. 2001) have shed more light on this region of the KcsA structure by showing that the TM2 helices move away from the channel axis during gating. Thus, in the open state the intrapore radius becomes larger to allow the permeation of K^+ ions.

What are the implications of the KcsA results for other potassium channels? First and foremost, the TVGYG sequence that forms the selectivity filter in the KcsA structure is conserved in all potassium channels (Heginbotham et al. 1992; MacKinnon et al. 1998; Lu et al. 2001). Hence lessons learned about the selective conductance of K^+ ions across the filter from studies of the KcsA structure will be equally applicable to other potassium channels. The intrapore region that controls the gating, on the other hand, appears to exhibit wide variations both in the primary sequence and at the tertiary level. Thus the large variations observed in conductance levels of potassium channels (up to two orders of magnitude), and their saturation properties, must result from differences in the intrapore structure. This suggests a two-pronged approach to modelling of potassium channels. Firstly, by performing MD simulations on the KcsA structure, one can study selective permeation of K^+ ions across the filter region. Secondly, one can model diverse range of potassium channels using BD simulations, by modifying the intrapore region of the KcsA structure as required by various physiological and mutation data. An important requisite in this modelling process is that the BD results for ion dynamics in the selectivity filter should be consistent with the experimental observations and MD results. Below we review the work carried out on these two fronts since the appearance of the KcsA structure in 1998 (see also Roux et al. 2000; Tieleman et al. 2001).

Molecular dynamics studies

Several groups have been involved in MD studies of the KcsA channel so far, and their focus has been mostly on the permeation properties of K^+ ions in the selectivity filter, with varying degrees of complexity in their treatment of the membrane bilayer. In the most sophisticated ones (Berneche and Roux 2000, 2001), the KcsA protein is embedded in a DPPC bilayer and solvated with a 150 mM KCl solution. In a similar study, Shrivastava and Sansom (2000) employed a POPC bilayer with a varying number of K^+ ions. Because explicit simulation of bilayers is computationally expensive, in most MD studies they are represented with octanes (Guidoni et al. 1999, 2000) and nonpolar atoms (Åqvist and Luzhkov 2000; Luzhkov and Åqvist 2000, 2001), or more simply by harmonic constraints applied to the protein atoms (Allen et al. 1999, 2000a; Biggin et al. 2001; Ranatunga et al. 2001). Simplified representations of bilayers enable longer simulations necessary for a detailed study of ion dynamics in the channel. Therefore, it would be worthwhile to find appropriate sets of constraints that can mimic the embedding of the KcsA protein in a lipid bilayer.

The MD simulations performed so far agree with the stable occupation of the channel with three K^+ ions, two in the filter and one in the cavity, as observed in experiments. There is also some evidence that the approach of the K^+ ion in the cavity to the selectivity filter triggers a conduction event, and permeation across the filter occurs through the recycling of ions as $2K^+ \rightarrow 3K^+ \rightarrow 2K^+$ (Åqvist and Luzhkov 2000; Berneche and Roux 2000, 2001). The question of selectivity against Na^+ ions has been addressed in several studies through free-energy perturbation calculations, where a K^+ ion in one of the binding sites is alchemically transformed into a Na^+ ion. The calculated free-energy barriers range from 11 kT (Berneche and Roux 2001) to 8 kT (Allen et al. 2000a) and 5 kT (Luzhkov and Åqvist 2001), which are in rough agreement with the experimental value of ~9 kT extracted from the K^+/Na^+ selectivity ratio of $\sim 10^4$. As stressed before, energy differences are less sensitive to inaccuracies arising from the use of nonpolarizable force fields, making these predictions more reliable.

Diffusion of ions and water in the KcsA channel has been studied by Allen et al. (1999, 2000a, 2000b) and Biggin et al. (2001). The main finding from these studies is that the diffusion coefficient of K^+ ions is suppressed down to about 10% of the bulk value in the filter region but remains relatively high (> 50% of the bulk value) in the rest of the channel. These results provide inputs for the BD simulations as discussed below. Motions of

individual ions in the channel have also been discussed in some MD studies of KcsA. However, such single-event studies have little meaning statistically, and cannot be used to draw conclusions about permeation dynamics.

Brownian dynamics studies

BD simulations of the KcsA channel have been carried out by Chung et al. (1999, 2002a, 2002b) and Allen and Chung (2001) in three dimensions and by Mashl et al. (2001) in one dimension. The restriction of the ions' motion to the pore axis saves a great deal of computational time but the effect of this approximation on the permeation properties of ions has not been studied so far. In the first BD study, Chung et al. (1999) employed a simplified pore shape and represented the charge residues on carbonyl groups in the selectivity filter and in the inner and outer mouths with dipoles, whose strengths were optimized for maximum conductance. The selectivity filter was occupied by two K^+ ions on average and a third K^+ ion was required to initiate conductance, consistent with experiments and MD results. The results for *I-V* curves and saturation of conductance were in reasonable agreement with the physiological data. This was followed by a more sophisticated study that included all the experimentally determined channel protein in the model structure (Chung et al. 2002a). Several open-state configurations of the KcsA channel were constructed from the (closed) crystal structure via MD simulations. The refined study yielded similar results and confirmed the permeation mechanism found in the earlier study.

An intuitive illustration of the permeation mechanism is provided by the multi-ion potential profiles obtained by minimizing the energies of ions resident in the channel while a test ion is brought in the channel in small steps (Fig. 2). This corresponds to the electrostatic work done on the test ion, and since there are no counter ions in the pore region, it should give a fairly good account of the dynamic behaviour of the ions in the channel during BD simulations. For a single K^+ ion there is a very deep well (67 kT) that will permanently bind it to the selectivity filter (Fig. 2A). The potential profile of a second ion in the presence of the first one is again attractive, although the well depth is reduced by about half (Fig. 2B). A third K^+ ion is still attracted to the channel from the intracellular side but now it faces a barrier of several kT high. Once it goes over this barrier through thermal fluctuations, it moves rapidly under the potential gradient towards the selectivity filter and destabilizes the equilibrium of the two resident ions there. From this point on, the three ions move more or less in tandem to the right until the right-most one is expelled from the channel, leaving again two K^+ ions in the filter. Analysis of the BD simulations indicate that permeation dynamics in the filter region is dominated by Coulomb repulsion during a conduction event, and despite the large suppression of the diffusion coefficient as found in

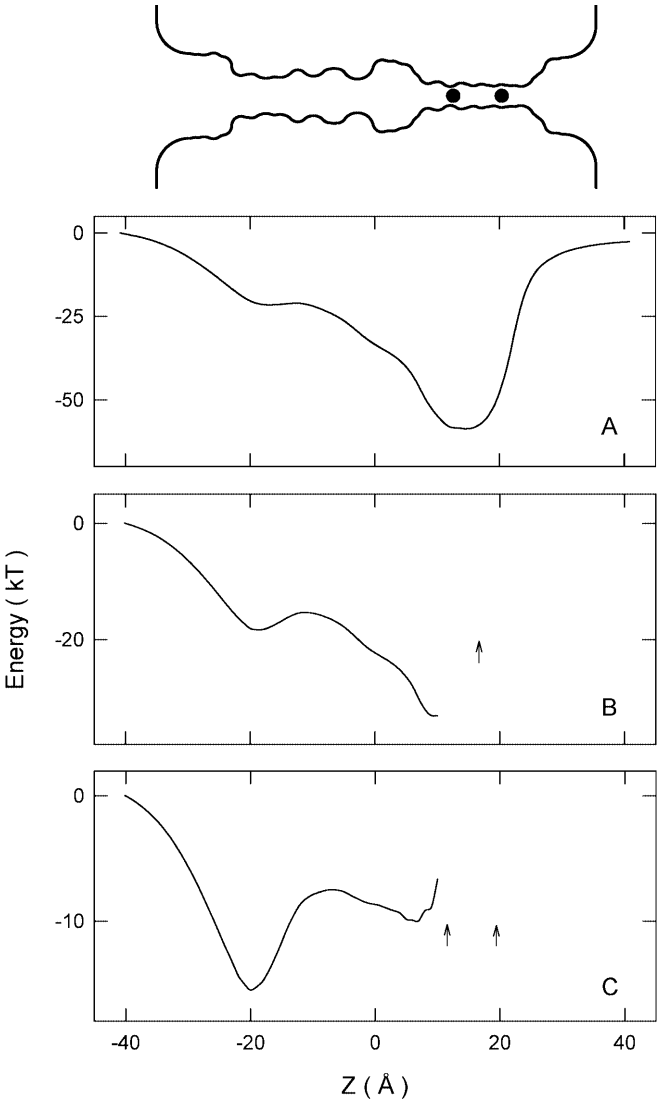

Fig. 2. Potential energy profiles of a K^+ ion traversing the KcsA channel under an applied field of 10^7 V/m when there are 0 (**A**), 1 (**B**) and 2 (**C**) resident ions in the channel. The dielectric constants used in the solution of Poisson's equation are 60 for channel water and 2 for the protein. The electric field is in the z direction, driving ions from inside the cell (*left*) to outside (*right*). The *upward arrows* indicate the location of the resident ions when the test ion is at the centre of the channel. The schematic channel in the *inset* shows the positions of the ions in case **C**

MD simulations, this is actually the fastest step in permeation.

This result is very significant because it shows that the selectivity property is decoupled from the other physiological properties of the channel, in that the filter rapidly recycles K^+ ions and therefore it is not the main rate-limiting step in the overall permeation process. As stressed above, the selectivity filter is conserved in all potassium channels, and had it played an important role in their conductance and saturation properties, one would not be able to explain the diversity of these quantities. In the present situation, one can start modelling other potassium channels using the KcsA

structure as a template. A first step in this direction was taken by Chung et al. (2002b), who constructed a simplified model of KcsA that reproduced the calculated properties of the atomic-detail model. They found that changing the radius of the intrapore region from 2 to 5 Å in the simplified model increased the channel conductance by two orders of magnitude, sufficient to explain the range observed in nature. This gives hope that individual potassium channels can be modelled using BD by taking into account available structural and physiological data. In this respect, homology and MD modelling of different potassium channels based on the KcsA structure could provide valuable clues (Capener et al. 2000; Shrivastava et al. 2000). Another important source of information is site-directed mutagenesis, which can help to identify the charge residues that influence the permeation characteristics of a channel (e.g. Thompson et al. 2000; Kubo and Murata 2001). The recent modelling of calcium channels provides an apt illustration of this point, that we next discuss.

Calcium channels

Calcium channels are as common as potassium channels and have many similar properties, e.g. they are extremely selective against Na^+ ions and exploit a multi-ion Coulomb repulsion mechanism to achieve a high throughput of Ca^{2+} ions. The fact that Ca^{2+} and Na^+ ions have similar radii but different charges indicates that, unlike potassium channels, selectivity must be based on the latter. Another difference of the selectivity property from the potassium channels is that it is contingent upon the presence of Ca^{2+} ions in the channel; in their absence, Na^+ ions conduct at an even faster rate than Ca^{2+}. These observations suggest that one may be able to explain the selectivity of calcium channels within the BD framework without having to appeal to MD. Since the tertiary structure of the calcium channels are not known, this indeed appears to be the only way to study the structure-function relationships in this important class of channels.

A first attempt to model L-type calcium channels using BD was made by Corry et al. (2001), who used the available information on the structure and conductance properties to construct a model channel consisting of inner and outer vestibules and a selectivity filter (see the inset in Fig. 3). The selectivity filter is the most important part of the model and requires a careful design in order to reproduce the observed properties of calcium channels. Two critical elements, namely its size and charges on its walls, are determined from the experimental data. The radius is set to 2.8 Å from the size of tetramethylammonium – the largest permeable ion (McCleskey and Almers 1985) – and the mutation data indicate the presence of four negatively charged glutamate residues in the filter region (Yang et al. 1993). BD simulations performed with this model have been very successful in replicating many physiological properties

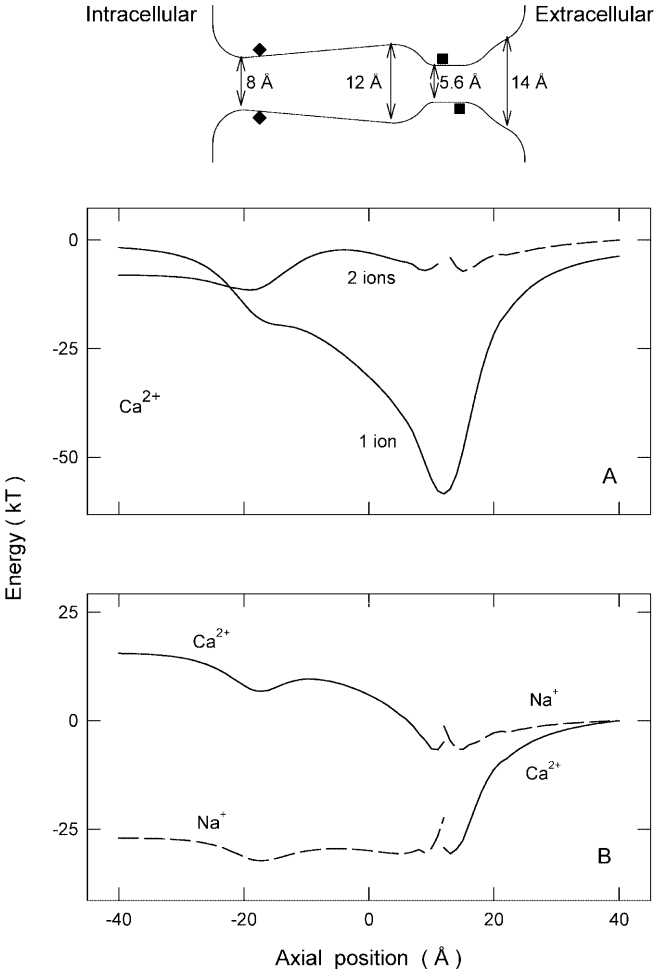

Fig. 3A, B. Shape of a model calcium channel and locations of charge residues (*inset*). Potential energy profiles for one and two Ca^{2+} ions are shown in **A**, and the profile for the mixed system is shown in **B**. The parameters are as in Fig. 2 except that the electric field is reversed to the inward direction (*right to left*)

of L-type calcium channels. These include current-voltage curves, saturation of conductance with concentration, selectivity against Na^+ ions, the anomalous mole fraction effect, attenuation of calcium current by external sodium ions, and the effect of mutating glutamate residues on blocking the sodium current (Corry et al. 2001).

In Fig. 3 we present multi-ion potential profiles for Ca^{2+} and mixed Ca^{2+} and Na^+ ions in the channel that illustrate the modus operandi of calcium channels. As shown in Fig. 3A, a single Ca^{2+} ion is deeply bound (58 kT) in the selectivity filter, and a second Ca^{2+} ion is easily attracted to the channel from the right (extracellular side). 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. Thus a single Ca^{2+} ion is in a waiting state and entry of a second Ca^{2+} ion triggers a conduction event. Profiles for Na^+ ions are similar except permeation involves three Na^+ ions just as in the case of potassium channels. The mechanism of selectivity

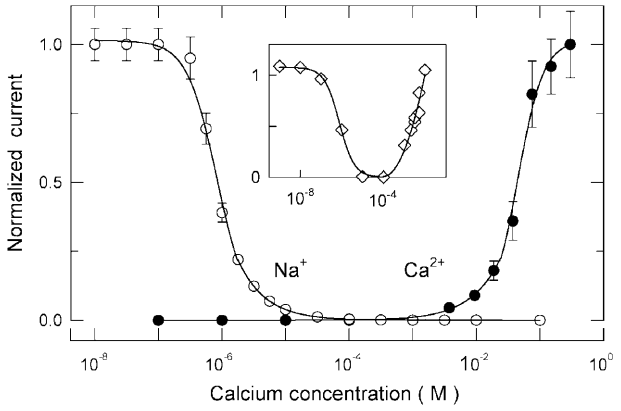

Fig. 4. Mole fraction effect. Ca^{2+} (*filled circles*) and Na^+ (*open circles*) current passing through the channel under an applied potential of –200 mV. Current, normalized by the maximum value of each, is plotted against the Ca^{2+} concentrations while keeping the Na^+ concentration fixed at 150 mM. Experimental results from Almers et al. (1984) are shown in the inset

is explained in Fig. 3B. When a Na^+ ion is resident in the filter, a Ca^{2+} ion is attracted to the filter and expels the Na^+ ion from the channel upon entry. A similar result is obtained when there are two Na^+ ions in the filter. In the reverse case of a Ca^{2+} ion in the filter, although a Na^+ ion is still attracted, it is unable to push the Ca^{2+} ion over the large barrier of 16 kT. Thus, once a Ca^{2+} ion enters the channel, Na^+ ions cannot push it out, and only another 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.

Of the many properties of the calcium channels, the most interesting one is no doubt the anomalous mole fraction effect, so-called because the channel current vanishes at a certain range of Ca^{2+} concentrations in the presence of a fixed 150 mM Na^+, as shown in the inset of Fig. 4. The BD results shown in Fig. 4 indicate that the rapid drop and subsequent vanishing of the channel current is due to blocking of the Na^+ current by Ca^{2+} ions. Once the Ca^{2+} concentration is high enough to allow two Ca^{2+} ions in the filter, the channel starts conducting again but now Ca^{2+} ions instead of Na^+. The level of agreement between theory and experiment obtained from BD studies of calcium channels is substantial and should encourage further applications of the BD method to modelling of other ion channels.

Conclusions and outlook

The field of ion channels has entered into a phase of rapid development in the last few years, fuelled by the recent progress in determination of the crystal structure of the KcsA potassium channel and computational methods that can relate the function of a channel to its structure. Here we gave a brief review of the two computer simulation methods – molecular and Brownian dynamics – which will play a prominent role in future studies of ion channels. It is pointed out that the force fields used in current applications of MD are derived under bulk conditions, that is, for globular proteins embedded in an electrolyte solution. An ion channel, on the other hand, can be construed as a small volume of electrolyte solution embedded in a pore formed by the channel protein. Thus, the force fields currently employed in MD studies are not expected to give reliable results when applied to ion channels. This is underscored by the inability of these force fields to account for the potential of mean force in the gramicidin A channel. Before investing heavily in MD studies of the KcsA channel, it is desirable that new, polarizable force fields are developed for applications of MD to ion channels. Because of its simple structure, gramicidin A presumably offers the best channel model for this purpose.

Success of the BD simulations in explaining the physiological properties of the potassium and calcium channels indicates that this method is eminently suitable for studying the structure-function relationships in biological ion channels. As the modelling of calcium channel shows, a detailed tertiary structure is not essential for this purpose: knowledge of the gross shape of the channel and the approximate locations of the charged residues in the channel wall are sufficient. This confers an additional advantage to BD over MD because atomic-detail structural information, necessary for the latter method, is lacking for most channels at present. The difficulty of crystallizing the membrane proteins suggests that this situation is not likely to change in the foreseeable future. Thus, deducing the structural information on a channel from the available functional data using BD simulations is likely to be the main approach in channel studies for some time. While this method is not as straightforward as applying MD to a given structure, and will require more ingenuity, the payoff, in terms of understanding the operation of a multitude of channels, is certainly worth the effort.

Acknowledgements This work is supported in part by grants from the Australian Research Council and the National Health & Medical Research Council of Australia. We thank our collaborators Toby Allen, Ben Corry and Matthew Hoyles, who have contributed to the BD and MD studies of channels reported in this review.

References

Allen MP, Tildesley DJ (1987) Computer simulation of liquids. Oxford University Press, Oxford

Allen TW, Chung SH (2001) Brownian dynamics study of an open-state KcsA potassium channel. Biochim Biophys Acta 1515:83–91

Allen TW, Kuyucak S, Chung SH (1999) Molecular dynamics study of the KcsA potassium channel. Biophys J 77:2502–2516

Allen TW, Kuyucak S, Chung SH (2000a) The potassium channel: structure, selectivity and diffusion. J Chem Phys 112:8191–8204

Allen TW, Kuyucak S, Chung SH (2000b) Molecular dynamics estimates of ion diffusion in model hydrophobic and the KcsA potassium channels. Biophys Chem 86:1–14

Almers W, McCleskey EW, Palade PT (1984) A non-selective cation conductance in frog muscle membrane blocked by micromolar external calcium ions. J Physiol 353:565–583

Åqvist J, Luzhkov V (2000) Ion permeation mechanism of the potassium channel. Nature 404:881–884

Bek S, Jakobsson E (1994) Brownian dynamics study of a multiply-occupied cation channel: application to understanding permeation in potassium channel. Biophys J 66:1028–1038

Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J (1981) Interaction models of water in relation to protein hydration. In: Pullman B (ed) Intermolecular forces. Reidel, Dordrecht, pp 331–342

Berneche S, Roux B (2000) Molecular dynamics of the KcsA K^+ channel in a bilayer membrane. Biophys J 78:2900–2917

Berneche S, Roux B (2001) Energetics of ion conduction through the K^+ channel. Nature 414:73–77

Berry RS, Rice SA, Ross J (2000) Physical chemistry, 2nd edn. Oxford University Press, New York

Biggin PC, Smith GR, Shrivastava I, Choe S, Sansom MSP (2001) Potassium and sodium ions in a potassium channel studied by molecular dynamics simulations. Biochim Biophys Acta 1510:1–9

Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M (1983) CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. J Comput Chem 4:187–217

Capener CE, Shrivastava IH, Ranatunga KM, Forrest LR, Smith GR, Sansom MSP (2000) Homology modelling and molecular dynamcis simulation of an inward rectifier potassium channel. Biophys J 78:2929–2942

Car R, Parrinello M (1985) Unified approach for molecular dynamics and density-functional theory. Phys Rev Lett 55:2471–2474

Chung SH, Hoyles M, Allen TW, Kuyucak S (1998) Study of ionic currents across a model membrane channel using Brownian dynamics. Biophys J 75:793–809

Chung SH, Allen TW, Hoyles M, Kuyucak S (1999) Permeation of ions across the potassium channel: Brownian dynamics studies. Biophys J 77:2517–2533

Chung SH, Allen TW, Kuyucak S (2002a) Conducting-state properties of the KcsA potassium channel from molecular and Brownian dynamics simulations. Biophys J 82:628–645

Chung SH, Allen TW, Kuyucak S (2002b) Modelling diverse range of potassium channels with Brownian dynamics. Biophys J 82:(in press)

Cooper KE, Jakobsson E, Wolynes P (1985) The theory of ion transport through membrane channels. Prog Biophys Mol Biol 46:51–96

Corry B, Kuyucak S, Chung SH (2000) Tests of continuum theories as models of ion channels: II. Poisson-Nernst-Planck theory versus Brownian dynamics. Biophys J 78:2364–2381

Corry B, Allen TW, Kuyucak S, Chung SH (2001) Mechanisms of permeation and selectivity in calcium channels. Biophys J 80:195–214

Corry B, Hoyles M, Allen TW, Walker M, Kuyucak S, Chung SH (2002) Reservoir boundaries in Brownian dynamics simulations of ion channels. Biophys J 82:1975–1984

Davis ME, McCammon JA (1990) Electrostatics in biomolecular structure and dynamics. Chem Rev 90:509–521

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Duca KA, Jordan PC (1998) Comparison of selectively polarizable force fields for ion-water-peptide interactions: ion translocation in a gramicidin-like channel. J Phys Chem 102:9127–9138

Edwards S, Corry B, Kuyucak S, Chung SH (2002) Ion permeation in the gramicidin a channel: continuum electrostatics and beyond. Biophys J 82:(in press)

Eisenberg RS (1999) From structure to function in open ionic channels. J Membr Biol 171:1–24

Frenkel D, Smit B (1996) Understanding molecular simulation: from algorithms to applications. Academic Press, San Diego

Graf PA, Nitzan M, Kurnikova G, Coalson RD (2000) A dynamic lattice Monte Carlo model of ion transport in inhomogeneous dielectric environments: method and implementation. J Phys Chem B 104:12324–12338

Guidoni L, Torre V, Carloni P (1999) Potassium and sodium binding to the outer mouth of the K^+ channel. Biochemistry 38:8599–8604

Guidoni L, Torre V, Carloni P (2000) Water and potassium dynamics inside the KcsA K^+ channel. FEBS Lett 477:37–42

Heginbotham L, Abramson T, MacKinnon R (1992) A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels. Science 258:1152–1155

Hermans J, Berendsen HJC, van Gunsteren WF, Postma JPM (1984) A consistent empirical potential for water-protein interactions. Biopolymers 23:1513–1518

Hille B (2001) Ionic channels of excitable membranes, 3rd edn. Sinauer, Sunderland, Mass

Hoyles M, Kuyucak S, Chung SH (1998a) Solutions of Poisson's equation in channel-like geometries. Comput Phys Commun 115:45–68

Hoyles M, Kuyucak S, Chung SH (1998b) Computer simulation of ion conductance in membrane channels. Phys Rev E 58:3654–3661

Im W, Seefeld S, Roux B (2000) A grand canonical Monte Carlo-Brownian dynamics algorithm for simulating ion channels. Biophys J 79:788–801

Jakobsson E, Chiu SW (1987) Stochastic theory of ion movement in channels with single-ion occupancy. Biophys J 52:33–45

Jordan PC (1990) Ion-water and ion-polypeptide correlations in a gramicidin-like channel. Biophys J 58:1133–1156

Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML (1983) Comparison of simple potential functions for simulation of liquid water. J Chem Phys 79:926–935

Kohn W (1999) Nobel lecture: electronic structure of matter-wave functions and density functionals. Rev Mod Phys 71:1253–1266

Kubo Y, Murata Y (2001) Control of rectification and permeation by two distinct sites after the second transmembrane region in Kir2.1 K^+ channels. J Physiol (London) 531:645–660

Kuyucak S, Andersen OS, Chung SH (2001) Models of permeation in ion channels. Rep Prog Phys 64:1427–1472

LeMasurier M, Heginbotham L, Miller C (2001) KscA: it's a potassium channel. J Gen Physiol 118:303–313

Levitt DG (1978) Electrostatic calculations for an ion channel. I. Energy and potential profiles and interactions between ions. Biophys J 22:209–219

Liu YS, Sompornpisut P, Perozo E (2001) Structure of the KcsA channel intracellular gate in the open state. Nat Struct Biol 8:883–887

Lu Z, Klem AM, Ramu Y (2001) Ion conduction pore is conserved among potassium channels. Nature 413:809–813

Luzhkov VB, Åqvist J (2000) A computational study of ion binding and protonation states in the KcsA potassium channel. Biochim Biophys Acta 1481:360–370

Luzhkov VB, Åqvist J (2001) K^+/Na^+ selectivity of the KcsA potassium channel from microscopic free energy perturbation calculations. Biochim Biophys Acta 1548:194–202

MacKinnon R, Cohen SL, Kuo A, Lee A, Chait BT (1998) Structural conservation in prokaryotic and eukaryotic potassium channels. Nature 280:106–109

Mashl RJ, Tang Y, Schnitzer J, Jakobsson E (2001) Hierarchical approach to predicting permeation in ion channels. Biophys J 81:2473–2483

McCleskey EW, Almers W (1985) The Ca channel in skeletal muscle is a large pore. Proc Natl Acad Sci USA 82:7149–7153

Meuser D, Splitt H, Wagner R, Schrempf H (1999) Exploring the open pore of the potassium channel from *Streptomyces lividans*. FEBS Lett 462:447–452

Morais-Cabral JH, Zhou Y, MacKinnon R (2001) Energetic optimization of ion conduction rate by the K^+ selectivity filter. Nature 414:37–42

Moy G, Corry B, Kuyucak S, Chung SH (2000) Tests of continuum theories as models of ion channels. I. Poisson-Boltzmann theory versus Brownian dynamics. Biophys J 78:2349–2363

Neher E, Sakmann B (1976) Single-channel currents recorded from membrane of denervated frog muscle fibers. Nature 260:799–802

Partenskii MB, Jordan PC (1992) Theoretical perspectives on ion-channel electrostatics: continuum and microscopic approaches. Q Rev Biophys 25:477–510

Perozo E, Cortes DM, Cuello LG (1999) Structural rearrangements underlying K^+-channel activation gating. Science 285:73–78

Ranatunga KM, Shrivastava IH, Smith GR, Sansom MSP (2001) Side-chain ionization states in a potassium channel. Biophys J 80:1210–1219

Reif F (1965) Fundamentals of statistical and thermal physics. McGraw-Hill, New York

Roux B, Karplus M (1994) Molecular dynamics simulations of the gramicidin channel. Annu Rev Biophys Biomol Struct 23:731–761

Roux B, MacKinnon R (1999) The cavity and pore helices in the KcsA K^+ channel: electrostatic stabilization of monovalent cations. Science 285:100–102

Roux B, Berneche S, Im W (2000) Ion channels, permeation, and electrostatics: insight into the function of KcsA. Biochemistry 39:13295–13306

Schirmer T, Phale PS (1999) Brownian dynamics simulation of ion flow through porin channels. J Mol Biol 294:1159–1167

Sharp KA, Honig B (1990) Electrostatic interactions in macromolecules: theory and applications. Annu Rev Biophys Biophys Chem 19:301–332

Shrivastava IH, Sansom MSP (2000) Simulation of ion permeation through a potassium channel: molecular dynamics of KcsA in a phospholipid bilayer. Biophys J 78:557–570

Shrivastava IH, Capener CE, Forrest LR, Sansom MSP (2000) Structure and dynamics of K channel pore-lining helices: a comparative simulation study. Biophys J 78:79–92

Thompson GA, Leyland ML, Ashmole I, Sutcliffe MJ, Stanfield PR (2000) Residues beyond the selectivity filter of the K^+ channel Kir2.1 regulate permeation and block by external Rb^+ and Cs^+. J Physiol (London) 526:231–240

Tieleman DP, Marrink SJ, Berendsen HJC (1997) A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems. Biochim Biophys Acta 1331:235–270

Tieleman DP, Biggin PC, Smith GR, Sansom MSP (2001) Simulation approaches to ion channel structure-function relationships. Q Rev Biophys 34:473–561

Urry DW (1971) The gramicidin A transmembrane channel: a proposed π_{LD} helix. Proc Natl Acad Sci USA 68:672–676

van Gunsteren WF, Berendsen HJC (1982) Algorithms for Brownian dynamics. Mol Phys 45:637–647

Wallqvist A, Mountain RD (1999) Molecular models of water: derivation and description. Rev Comput Chem 13:183–247

Wang W, Donini O, Reyes CN, Kollman PA (2001) Biomolecular simulations. Annu Rev Biophys Biomol Struct 30:211–243

Weiner SJ, Kollman PA, Case DA, Singh UC, Ghio C, Alagona G, Profeta S, Weiner P (1984) A new force field for molecular mechanical simulation of nucleic acids and proteins. J Am Chem Soc 106:765–784

Yang J, Ellinor PT, Sather WA, Zhang JF, Tsien RW (1993) Molecular determinants of Ca^{2+} selectivity and ion permeation in L-type Ca^{2+} channels. Nature 366:158–161

Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001) Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution. Nature 414:43–48