Theoretical and computational models of ion channels

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Computational studies can make meaningful contributions to our understanding of biological ion channels. A wide variety of methods, at different levels of approximation, can be used. Over the past few years, progress in the experimental determination of three-dimensional structures has given a fresh impetus to the theorists. Noteworthy progress has been made in carefully constructing realistic models of a number of complex biological channels to address important questions about their function.

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Current Opinion in Structural Biology 2002, **12**:182–189

0959-440X/02/$ – see front matter

Abbreviations

Alm	alamethicin
BD	Brownian dynamics
FEP	free energy perturbation
GA	gramicidin A
GCMC	grand canonical Monte Carlo
MD	molecular dynamics
NP	Nernst–Planck
PB	Poisson–Boltzmann
PB-V	PB with voltage
PMF	potential of mean force
PNP	Poisson-Nernst-Planck
TEA	tetraethylammonium

Introduction

Ever since the early days of electrophysiology, theories and modeling of ion channels have contributed to a better understanding and interpretation of experimental data [1]. Although recent progress in the determination of the 3D structures of biological ion channels has provided a wealth of information [2–6,7••,8,9], theoretical considerations are necessary for understanding ion conduction at the atomic level. The combination of atomic-resolution structures with highly sophisticated computational approaches provides a virtual route for interpreting experimental observations and relating a channel structure to its function.

A wide variety of computational approaches, such as molecular dynamics (MD) simulations [10–15,16•,17–19, 20•,21–23,24•,25–30,31•,32,33•,34••,35], continuum electrostatic Poisson–Boltzmann (PB) theory [36,37,38••,39], Brownian dynamics (BD) [40–43,44•,45–47], Poisson-Nernst-Planck (PNP) electrodiffusion theory [48–50] and kinetic rate models [1,51], have helped, and will continue to help, refine our understanding of the molecular determinants of channel function.

It is nearly impossible to cover the entire field of ion channel simulations in the restricted format of a brief review. In the following, some of the most important results that have been obtained during the past few years will be summarized, and the strengths and limitations of various approaches will be highlighted. The review will be concluded by pointing out directions that are likely to become important in the future.

Computational approaches

Arguably, MD provides the most detailed information in theoretical studies of ion channels. The approach consists of constructing an atomic model of a macromolecular system, representing the microscopic forces with a potential function and integrating Newton's classical equation to generate a trajectory. The result is literally a 'simulation' of the dynamical motions of all the atoms as a function of time. With the availability of potential energy functions for proteins and lipids, as well as fast and reliable simulation algorithms, current MD methodologies have reached the point at which one can generate trajectories of very realistic atomic models of complex biological channel membrane systems (for a recent review of simulation methods, see chapters 1–4 in [52]). In recent years, MD simulations with explicit membranes have been used extensively to study an increasingly large number of ion channels: gramicidin A (GA) [11,13,14], alamethicin (Alm) [15,16•], the transmembrane domain of the M2 protein from influenza A virus [17,18], OmpF porin [12,19], the mechanosensitive channel MscL [20•,21] and K^+ channels [22,23,24•,25–30,31•,32,33•,34••,35].

Simple MD trajectories, however, are somewhat limited in their ability to quantitatively characterize complex biomolecular systems. Nonetheless, their scope can be considerably extended by using advanced computational techniques, such as alchemical free energy perturbation (FEP) [23,24•,25,35] and umbrella sampling ([33•,34••]; see chapter 9 in [52]), or by the application of external forces to the system [12,16•,20•]. Alchemical FEP calculations use simulations generated with an altered (unphysical) potential function; thermodynamic integration is then performed to compute the free energy difference between the two end points that correspond to true physical states of the system. Umbrella sampling calculations use simulations generated in the presence of an artificial biasing potential to enhance configurational sampling; the effect of this bias is then removed in post-analysis to compute the unbiased potential of mean force (PMF) of the system [52]. One advantage of these methods is that the individual simulations in FEP and umbrella sampling do not need to communicate with one another and can be generated independently; this leads to the possibility of performing 'coarse-grained' calculations that are massively distributed over a large number of relatively inexpensive computers. Lastly, it is possible to monitor the dynamical motions in the presence of artificial

external forces that reproduce some aspect of the environment, such as membrane surface tension [20•] or transmembrane voltage [12,16•]. The latter is of particular importance for simulations of ion channels. The simplest approach to introduce a transmembrane voltage in MD consists of applying a constant electric field directed along the axis perpendicular to the membrane, which acts on all the charges in the system [16•] (if the system is simulated with periodic boundary conditions, the electrostatic potential is discontinuous at the boundary of the simulation system, although the electric field and the forces acting on all the charges are continuous). One should note that the significance of an externally applied potential will require further clarification, as it differs from the true physical transmembrane potential arising from the interfacial polarization of electrolytic solution ([37]; this paper provides an in-depth theoretical formulation of the equilibrium properties of selective ion channels, giving explicit and rigorous expressions for the multi-ion PMF and the transmembrane voltage).

Approaches that are simpler and computationally less expensive than all-atom MD are very important tools in studies of ion channels. In particular, macroscopic continuum electrostatic calculations, in which the polar solvent is represented as a structureless dielectric medium, can serve to illustrate fundamental principles of ion permeation in a particularly clear fashion ([38••]; see also chapter 7 in [52]). For example, calculations based on the PB equation are routinely used to determine the pKa of ionizable sidechains in ion channels, for example, OmpF porin [53], Alm [54], M2 [17] and KcsA [38••,39]. Such calculations have also been used to reveal the dominant energetic factors controlling ion occupancy in the cavity of KcsA [36,38••]. The standard PB equation can also be extended to incorporate the influence of the transmembrane voltage (the PB-V [PB with voltage] equation) [37,38••].

BD is an attractive computational approach for simulating the ion permeation process over long timescales without having to treat all the solvent molecules explicitly [40,41]. Recently, the approach has been used in studies of porins [42,43,44•,45] and KcsA [46,47] in particular. BD consists of integrating stochastic equations of motion describing the displacement of the ions; the microscopic ion–ion and ion–channel interactions are represented by some effective potential function [40,41]. This potential corresponds formally to a statistical mechanical multi-ion PMF [37,44•], but it is often approximated by assuming that the solvent is a dielectric continuum and the channel protein is rigid [41–43,44•,45–47]. BD simulations can include the ion displacements in three dimensions [41–43,44•,45,46], but these are sometimes reduced to the 1D movement of ions along the channel axis for the sake of simplicity [40,47]. Ion–ion interactions are incorporated naturally in multi-ion BD [41,44•,45–47], although the influence of concentration and ionic screening has been incorporated at a mean-field level within the PB equation in some cases [42,43]. Current methodological issues in BD include the development of grand canonical Monte Carlo (GCMC) algorithms to implement nonequilibrium boundary conditions [44•] and numerical approaches to approximate the electrostatic reaction field arising from channels with arbitrary geometries [45,46].

PNP is an electrodiffusion theory describing the average ionic fluxes arising from concentration gradients (Fick's law) and electric fields [48–50]. This theory differs from MD and BD because it does not require the explicit simulation of ion movements. The PNP equations in 3D space can be solved numerically using finite-difference relaxation algorithms [48] similar to those used to solve the PB equation (see chapter 7 in [52]) or a spectral elements method [55]. The basic assumption that is made when using PNP is that the electric field is calculated self-consistently from the average ionic charge densities; ion–ion interactions are thus incorporated approximately, at a mean-field level. Furthermore, the channel structure is assumed to be rigid. The PNP theory has been used to describe ion fluxes through the GA channel [48,49,55]. In some applications, the Nernst–Planck (NP) equation is solved for a fixed electrostatic potential, which is not calculated self-consistently [54]. The validity of PNP theory in the context of narrow molecular pores, however, appears to be uncertain [50].

Ion channels: channel-forming peptides

The GA channel was the first ion channel to be simulated with MD [10]. Because the channel formed by this small pentadecapeptide is so well characterized, both structurally [2] and functionally [1], it remains an excellent model system for theoretical studies of ion channels ([11,13]; for a recent review of GA simulations, see [14]). In particular, the GA channel has become an ideal prototypical system for investigating the conduction of protons along a single file of hydrogen-bonded water molecules, a process that is thought to occur via a succession of protonation-dissociation reactions, whereby a hydrogen ion hops from oxygen to oxygen along the water chain according to a Grottus-like mechanism [56,57]. Simulations of proton conduction along such 'proton wire' remain one of the most challenging problems in computational studies of ion channels because of the complexity of the potential energy surface during water dissociation and the difficulty of accounting for the quantum mechanical nature of the light hydrogen nuclei. Quantum mechanical calculations treating the electronic degrees of freedom using density function theory (DFT) have been carried out [58], but useful simulations reaching over sufficiently long timescales must unavoidably rely on empirical potential functions [57,59,60].

Channels formed by small proteins or peptides, such as Alm [15,16•] and M2 [17,18], also represent very interesting model systems. However, their structures in the membrane (α-helical bundles) are not as well characterized experimentally. Alm

is an antimicrobial peptide of 20 residues that forms a stable amphipathic α helix in the membrane [61]. The influenza M2 protein is a simple membrane protein comprising a single transmembrane helix [9]. In lipid bilayers, Alm channels exhibit multiconductance levels, presumably corresponding to helical bundles differing in the number of helical subunits. A proton-permeable channel is formed by four M2 subunits, although the mechanism of proton conduction is still poorly understood. MD has been used to examine the stability of various models of Alm and M2. A hexameric helical bundle of Alm embedded in a fully solvated palmitoyloleoyl phosphatidylcholine (POPC) membrane was shown to be stable for 2 ns [15]. The conductance of the channel was calculated using a combination of PB and NP theories [54]. Models of tetrameric M2 helical bundles were constructed and simulated, though the results are thought to be sensitive to the starting structure and the protonation state of ionizable residues [17]. MD trajectories in a membrane-mimetic environment have suggested two possible conducting states of the ion channel, corresponding to tetramers containing one or two protonated histidines [18]. An interesting aspect of Alm channels is the activation mechanism, which is thought to correspond to the voltage-induced insertion of the helices into the bilayer. To investigate this process, the insertion of Alm at phospholipid/water and octane/water interfaces was simulated under the influence of an applied potential [16•]. Although the peptide did not insert into the bilayer, its insertion into an octane slab in a step-wise fashion via its N terminus was observed, thus providing insight into voltage-driven conformational changes of membrane proteins.

Bacterial porins

Porins from the outer membrane of *Escherichia coli* are macromolecular structures that allow the diffusion of hydrophilic molecules with molecular weight up to 600 Da and exhibit modest ionic selectivity (for a recent review, see [3]). The cation-selective matrixporin (OmpF) is produced under normal conditions, whereas the anion-selective phosphoporin (PhoE) is expressed under limited phosphate conditions. They fold into similar homotrimeric β-barrel structures, each monomer possessing a wide aqueous pore narrowed by long loops at the outer entrance.

MD simulations with explicit ions and solvent molecules [12], and also with a phospholipid bilayer membrane [19] have been carried out to characterize the aqueous pores and investigate the mechanism of ion conduction. The movement of a single Na^+ ion in OmpF was simulated in the presence of a potential of 500 mV [12]; a complete translocation through the pore occurred in 1.3 ns. Tieleman and Berendsen [19] generated a 1 ns MD simulation of an atomic model of the OmpF trimer embedded into a fully solvated palmitoyloleoyl phosphatidylethanolamine (POPE) bilayer membrane (for a total of 65 898 atoms). The simulation revealed the influence of a strong electric field oriented transverse to the pore axis, an aspect of the OmpF pore that was also noted in calculations based on the PB equation [53]. The simulation also provided a wealth of information about the interaction of porin with the surrounding lipids [62].

So far, BD has been the most useful and productive approach to exploring the ion flow through porins [42,43,44•,45]. The valence specificity of OmpF, PhoE and OmpK36, as well as of several OmpF mutants, was examined using one-ion BD simulations [42,43]; a good correlation was achieved between calculated transmission probabilities and experimental ion selectivity. Multi-ion trajectories of a KCl electrolyte bathing OmpF were generated using the GCMC/BD algorithm [44•,45]; the conductance of OmpF calculated from those simulations was in good agreement with experimental estimates.

MscL mechanosensitive channel

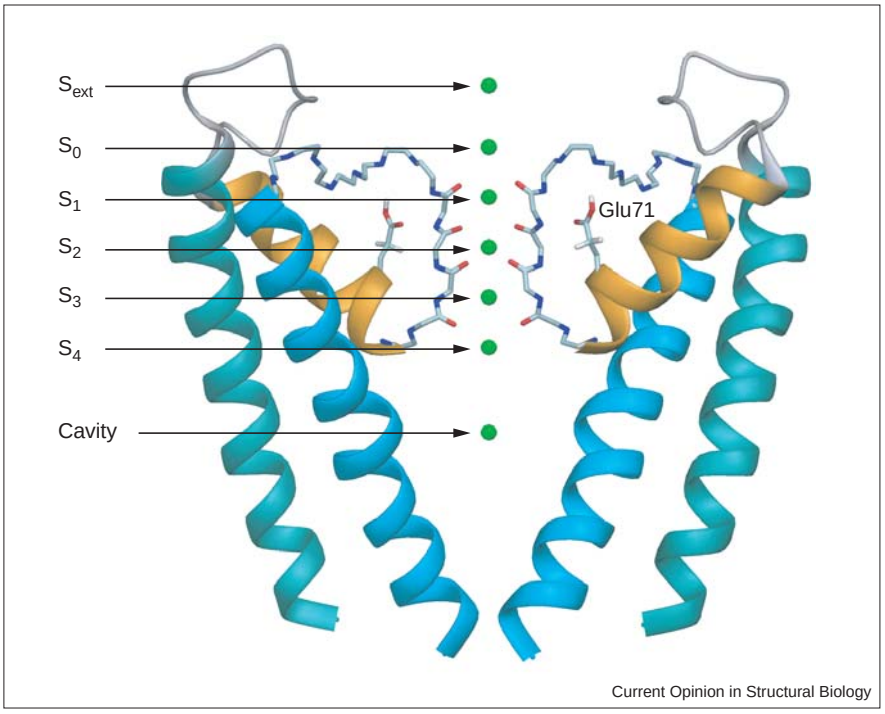
The structure of MscL from *Mycobacterium tuberculosis* has been determined using X-ray crystallography [4]. The MscL channel, a ubiquitous membrane-embedded valve involved in turgor regulation in bacteria, can be gated by tension in the membrane bilayer; in prokaryotes, it plays a crucial role in exocytosis, as well as in the response to osmotic downshock. To understand the molecular mechanisms of tension-dependent channel gating, structural models were developed in which a cytoplasmic gate was formed by a bundle of five N-terminal helices, which was previously unresolved in the crystal structure [63••]. A prediction of the modelization was that, when membrane tension is applied, the transmembrane barrel expands and pulls the gate apart. The models were tested successfully by substituting cysteines for residues predicted to be near each other only in either the closed or open conformations.

The molecular mechanism by which variations in the membrane tension are transduced to the channel structure was investigated using MD simulations of MscL embedded in explicit membranes [20•,21] and using simulations of the bare protein under conditions of constant surface tension (steered MD) [20•]. Under a range of conditions, it was shown that the transmembrane helices tilted considerably as the pore widened, suggesting that membrane thinning and hydrophobic mismatch within the transmembrane helices of MscL might drive gating.

KcsA K^+ channel

In 1998, the 3D structure of the KcsA channel from *Streptomyces lividans* was determined by X-ray crystallography [5]. The main features of the structure are shown in Figure 1. The structure revealed that the pore comprises a wide nonpolar cavity of 8 Å radius on the intracellular side, leading, on the extracellular side, to a narrow pore that is 12 Å long and lined exclusively by backbone carbonyl oxygens [5]. This region of the pore acts as a 'selectivity filter', whereas the wide cavity and the pore helices help overcome the dielectric barrier due to the cell membrane by electrostatically stabilizing a monovalent cation [36]. Even at a relatively fuzzy resolution of 3.4 Å, this is a

Figure 1



The structure of the KcsA K^+ channel determined by X-ray crystallography [5]. The channel is formed by four identical subunits disposed symmetrically around a common axis corresponding to the pore (only two of the four monomers are shown for the sake of clarity). The backbone of residues that form the selectivity filter is shown in atomic detail. The inner (light blue), outer (light green) and pore helices (orange) are shown. All the possible K^+ binding sites are indicated by green spheres. The sidechain of Glu71 shown in the figure was not resolved in the X-ray structure. In the initial X-ray structure, K^+ ions were detected in sites S_1, S_3 and S_4 [5]. A spontaneous transition leading to the simultaneous occupancy of sites S_0, S_2 and S_4 was observed in MD [31•]. Other simulations led to the simultaneous occupancy of sites S_2 and S_4 [26,27]. Sites S_0 and S_{ext} were predicted to be local free energy minima by umbrella sampling calculations [34••]. All sites, S_{ext}, S_0, S_1, S_2, S_3 and S_4, were observed in diffraction data at higher resolution [7••].

landmark structure that had a tremendous impact on all subsequent work on ion channels. It is currently the only K^+ channel for which a structure at atomic resolution is available. More recently, higher resolution structures of KcsA were obtained by co-crystallizing the channel with tetrabutylantimony, an electron-dense analog of tetrabutylammonium (TBA), which is a classical intracellular quaternary ammonium blocker of K^+ channels [6], and with a monoclonal Fab antibody fragment [7••]. The latter effort enabled the determination of a very high resolution structure of the channel in high and low K^+ concentrations. The higher resolution structure permitted the detection of energetically favorable sites for K^+ or Rb^+ in the selectivity filter [8].

The availability of an atomic structure of KcsA has triggered a large number of computational studies based on MD [22,23,24•,25–30,31•,32,33•,34••,35], PB [36,38••,39] and BD [46,47]. At the simplest level, continuum electrostatic calculations have provided valuable insight into the function of KcsA. In particular, it was shown that the cavity and the pore helices of the KcsA channel are electrostatically 'tuned' for preferable occupancy by a monovalent cation [36]. Further calculations indicated that the passage of cations through the entrance on the intracellular side, though sterically possible, is unfavorable because of a large dielectric reaction-field energy barrier [38••]. This indicates that the X-ray structure of KcsA corresponds to a closed nonconducting state of the channel. The structure of the open state is not known in atomic detail, though it might be possible to construct approximate models using the information from electron paramagnetic resonance (EPR) data [64]. Calculations based on the PB equation have also been used to determine the protonation state of ionizable residues [38••,39,46,47]. Importantly, the results indicated that the sidechain of Glu71 (a residue located in the vicinity of the selectivity filter that was not resolved in the initial crystallographic structure) is protonated at normal pH [38••,39]. This conclusion was supported by FEP calculations [25,35] and subsequently confirmed by X-ray structures at higher resolution [6,7••]. Lastly, the PB-V equation [37] was used to calculate the variations of the transmembrane voltage along the axis of KcsA [38••]. Suggestively, the results were in much better agreement with the voltage profile (estimated experimentally from Ba^{2+} blockade [65]) when the calculations were based on a model of the open state, rather than on the X-ray structure (closed state) [38••].

Most of the early MD simulations of KcsA were broadly aimed at addressing general questions about the configurations of the ions and water molecules in the selectivity filter [22,23,24•,26,31•]. This was an important issue because many configurations were plausible and consistent with the somewhat limited information available from experiment ([5]; see also Figure 1). Spontaneous transitions between different configurations observed during MD trajectories provided useful information [22,26,31•]. Transitions of two K^+ from sites S_1 and S_3 to sites S_2 and S_4 were observed [26,27]; in addition, one K^+ left the cavity to go into the bulk solution [26]. A concerted transition involving three K^+ occurred during one MD simulation [31•]; the K^+ that was initially in the cavity moved up into site S_4 in the selectivity

filter, while two other K^+ ions located in sites S_1 and S_3 moved simultaneously toward the extracellular side, the outermost K^+ ending up in the external binding site S_0. This was the first reported observation of a K^+ in the S_0 binding site — it was subsequently detected in an X-ray structure at higher resolution [7••]. Interestingly, the amide plane formed by the peptide linkage Val76–Gly77 in the selectivity filter was observed to undergo isomerization transitions, pointing alternately into and away from the pore [31•]. Similar isomerizations have also been observed in other simulations (T Allen, L Guidoni, MS Sansom, personal communication). Although there are some quantitative differences, the isomerization observed in MD simulations clearly anticipated the conformational change now seen in the higher resolution structure at low K^+ concentration [7••].

Simple MD simulations alone are insufficient to draw quantitative conclusions about the occupancy of the various binding sites. To address such questions, the free energy of ion configurations is required. Åqvist and Luzhkov [24•] compared systematically the free energy of a large number of ion configurations occupying sites S_1–S_4 using FEP; site S_0, undetected initially [5], was not included in the FEP analysis. They concluded that the selectivity filter was preferably occupied simultaneously by two K^+, located either in S_1 and S_3, or in S_2 and S_4. To fully characterize the ion conduction process, Bernèche and Roux [34••] calculated the complete free energy surface associated with the positions of three K^+ along the axis of the pore using umbrella sampling. Remarkably, the umbrella sampling calculation predicted the existence of two binding sites, S_0 and S_{ext}, located on the extracellular side of the channel [34••]. These binding sites were not detected initially [5], but were observed in diffraction data at higher resolution [7••]. In addition, the umbrella sampling calculations showed that efficient ion conduction involves transitions between two main states with, alternately, two and three K^+ ions occupying the five sites S_0–S_4. The largest free energy barrier to the conduction process was only on the order of 1–3 kcal/mol, implying that the ion conduction process is essentially diffusion limited. Ion–ion repulsion, though shown to be essential for rapid conduction, was seen to act effectively at very short distances.

How both a rapid transport rate for K^+ and a high selectivity against Na^+ can be achieved is a central question concerning the function of all K^+ channels [1]. Issues of ion selectivity in KcsA were addressed both with MD simulations [22,27,30] and with FEP calculations [23,24•,29,34••]. In particular, FEP calculations indicated that sites S_1 and S_2 are naturally selective for K^+ against Na^+ [34••]. An important conclusion from MD simulations is that the microscopic mechanism giving rise to selectivity cannot be represented accurately on the basis of a rigid pore because the observed magnitude of the dynamical fluctuations of the carbonyl oxygens that form the selectivity filter is larger than the difference between the radii of Na^+ and K^+.

The conduction process through KcsA has also been examined using BD simulations [46,47]. Despite the severe limitations of the continuum electrostatic approximation and the assumption of a rigid channel structure, BD simulations confirmed that a multi-ion mechanism, in which the channel is alternately occupied by two or three K^+ ions, is in qualitative accord with the magnitude of the ion flux observed experimentally. However, such BD simulations are unable to capture the influence of the structural dynamical fluctuations of the selectivity filter observed in MD [22,23,24•,26,31•,34••]. The main problem here is not the BD simulation algorithm itself, but the fact that the stochastic trajectories are generated using a continuum electrostatic potential energy surface calculated from a rigid channel structure [46,47]. An alternative strategy might be to perform BD simulations on the multi-ion free energy surface calculated from umbrella sampling [34••], thus combining the strengths of both MD and BD.

The mode of action of classical inhibitors of K^+ channels, such as tetraethylammonium (TEA), has been investigated [32,33•]. The docking of TEA to KcsA showed favorable binding sites on both the intracellular and extracellular sides of the selectivity filter [32]. Umbrella sampling MD calculations with explicit solvent and lipid membrane were used to explore the extracellular blockade of KcsA by TEA [33•]. It was found, in excellent agreement with experiment, that TEA is more stably bound when there are aromatic residues at position 82, located near the extracellular entrance of the channel. TEA binds favorably in extracellular site S_0, which is observed in the umbrella sampling calculation [34••] and in the high-resolution structural data [7••], while two K^+ are located simultaneously in sites S_4 and S_2 (each ion pair being separated by a single water molecule).

With two transmembrane α helices per subunit (the outer and inner helices), the basic topology of KcsA is similar to that of the family of inward rectifiers, although it has a high sequence similarity to all known K^+ channels [5]. Given the high sequence identity among K^+ channels, it is tempting to extend the current studies to other K^+ channels by using the crystallographic structure of KcsA as a template for constructing comparative atomic models. Achieving meaningful results with such comparative modelization techniques relies on the sequence alignment between the template and the target structures (see chapter 14 in [52]). In practice, assessing the validity of the sequence alignment is difficult. Efforts to construct a comparative model of an inwardly rectifying K^+ channel (Kir) provide a good illustration of the problems encountered with this approach [28]. A particular sequence alignment was used and, according to a number of criteria, the model seemed plausible and reasonable. However, a chimera of Kir that incorporated the pore domain of KcsA but assumed a different sequence alignment was shown experimentally to retain the main functional features of inward rectification [66••], suggesting that this might be the correct alignment.

Conclusions

The availability of crystallographic structures at high resolution has permitted a critical test of the accuracy of computational approaches in studies of ion channels. In the case of Alm and M2, computations based on MD and PB have helped to assess the structural stability of various models of α-helical bundle structures [15,16•,17,18]. In the case of porins, computations based on PB and BD have started to uncover the microscopic origin of the charge specificity of these channels [42,43]. In the case of MscL, MD simulations with applied surface tension [20•] and modelization [63••] have started to provide important clues concerning the large conformational change involved in the mechanosensitive gating mechanism. Finally, in the case of the KcsA K^+ channel, it is particularly encouraging to note that the results from the calculations have been very consistent with the information emerging from higher resolution structural data. Calculations based on PB [38••,39], as well as on FEP [25,35], suggested that the sidechain of Glu71 should be protonated at normal pH, a result that was essentially confirmed later by the crystallographic structures determined at higher resolution [6,7••]. Furthermore, the isomerization of the peptide linkage between Val76–Gly77, initially observed in MD [31•], appears to be closely related to a conformational change seen at low K^+ concentration [7••]. Lastly, the S_0 and S_{ext} extracellular K^+ binding sites, predicted by umbrella sampling calculations [34••], were observed in diffraction data at higher resolution [7••]. These successful predictions, in advance of the fact, reinforce significantly the confidence in computational studies of ion channels based on atomic models.

In the near future, one may expect that MD simulations of ion channels based on atomic models will continue to grow in scope and complexity. Atomic systems of 50 000 to 100 000 atoms with explicit solvent and lipid membrane will be routinely simulated for hundreds of nanoseconds. Nonetheless, such all-atom MD trajectories will still be too short to simulate ion permeation explicitly for most ion channels. Computational techniques based on FEP [23,24•,25,34••,35] and umbrella sampling [33•,34••] should play an increasingly important role in the quantitative characterization of ion channels. In order to compute ionic fluxes, it shall be possible to extend the results from MD to the appropriate timescales by using the multi-ion PMF, obtained from umbrella sampling MD calculations, for generating long BD trajectories. Kinetic rate models might also provide a framework for incorporating the PMF calculated from MD [51].

In addressing questions about ion selectivity, it will be important to critically examine the potential functions used to generate MD simulations [67]. Current biomolecular force fields are based on pairwise additive nonbonded interatomic potential energy functions and effects due to induced electronic polarization are neglected [68]. MD simulations can lead to meaningful results as long as they are based upon potential functions that are well calibrated and parameterized to reproduce experimental solvation free energies of ions [34••]. Nonetheless, the situation is expected to be more difficult in the case of small cations such as Na^+ or divalent ions such as Ca^{2+}. Addressing detailed questions about ion selectivity, which results from a delicate balance of very large interactions, will definitely require more efforts to develop accurate polarizable force fields [68].

Update

Recently, the structures of two related transmembrane proteins, the bacterial glycerol facilitator (GlpF) [69] and human aquaporin-1 (AQP1) [70,71], have been determined to high resolution using X-ray crystallography. Detailed MD simulations carried out on the basis of these structures have helped to elucidate the mechanism of water permeation through these highly selective membrane channels [72••]. In particular, the conserved fingerprint motif (asparagine-proline-alanine, NPA) is proposed to function as a proton filter, thus preventing the formation of an efficient proton wire [57].

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