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Permeation in ion channels: the interplay of structure and theory

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Combined with high-resolution atomic-level crystal structures of channel forming peptides, theory has become a powerful tool for illuminating factors influencing permeation. Here, advantages and limitations of the more familiar continuum and molecular modeling techniques are briefly outlined. These methods are applied to issues of permeation in two different channel families: gramicidin and K^+ channels. Using structural data, theory provides verifiable atomic-level insights into permeation dynamics, channel conductance and molecular selectivity mechanisms. Not only can theory confirm experimental inference, it can also sometimes provide structural perspectives in advance of experiment.

In the past five years there has been an explosion in the number of high-resolution atomic-level structures of channel-forming peptides and proteins. This review focuses on how the structures of two different channel families – gramicidin [1,2] and bacterial K^+ channels [3–6] – provide insight into permeation. For these channels, with permeation pathways that are self-evident upon structural visualization, there has been fruitful interplay between theory and experiment.

Ion channels are ubiquitous membrane proteins. They are responsible for electrical signaling in muscle, nerve and synapse. Because channel malfunction correlates with a wide range of pathologies, much of neuroscience is devoted to understanding the behavior of these assemblies at the molecular level. Until recently this goal could be addressed only indirectly, by biochemical and electrophysiological means. This changed dramatically with the availability of atomic-level structures. With these as input, modern theoretical tools are sufficiently reliable to allow direct monitoring of molecular details of crucial dynamic aspects of permeation and gating, providing complementary insights to those gleaned from experiment.

Structures – which are low temperature snapshots, often of closed (but permeant-occupied) states – are mines of information. Structure-based theory rationalizes observed channel properties, suggests specific testable hypotheses and generates well-grounded speculations. In this review, two commonly used theoretical methodologies are discussed: molecular dynamics, which describe the detailed interaction of permeating ions with the mobile surroundings of channel protein, lipid and water [7], and continuum techniques, in which the channel assembly is viewed as a rigid framework and water as a structureless solvent [8,9]. The utility of these two approaches will be exemplified by specific applications to the chosen channel families.

Atomic-level theory

Determination of free energy profiles

Given a pore structure, a major goal of ion channel theory is to monitor ion movement through the open channel under conditions mimicking those used in experiments. However, this is presently unattainable because the basic molecular dynamics time step is $\sim$2 fs, whereas typical ionic transits take microseconds and are events that arise only as ultra-rare fluctuations in an equilibrium system. Long computer runs are needed to observe passage of even one ion through an all-atom model comprising channel, lipid and electrolyte [10]; usable statistics minimally requires $\sim$10 such events. Quantitative modeling requires major simplifications, proceeding in hierarchical fashion [11]. One first establishes the free energy profile for ion permeation, accounting for local structural reorientation of the channel protein and pore water (effectively dielectric stabilization [12]; for explicit examples based on different methods, see [13–16]). This averaged energy profile depends only on ionic positions; it determines a mean force governing ionic occupancy of a channel. With a molecular dynamics determination of the local friction hindering ionic motion, conductance can be computed indirectly [17]. Because simulation models typically contain $\sim 10^5$ atoms, they describe just a tiny part of the membrane–protein–water assembly. To compensate, the computational cell is normally replicated periodically, a reliable procedure for uniform systems. Because the dielectric milieu in which a channel protein is embedded is highly non-uniform, this approach less effective, leading to errors in energy barrier estimates that can significantly affect computed conductances [18,19]. Microscopic–mesoscopic hybrid methods avoid 'truncation problems' by surrounding the simulation cell with dielectric continua mimicking electrolyte and lipid [16,20].

Steered molecular dynamics

Energy barriers for ion permeation are often large, and traditional approaches to determining free energy profiles entail a heavy computational load. 'Steered' molecular dynamics, by imposing external forces of a general nature that rapidly drives permeating species

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across the system [21], provides a relatively rapid, although less reliable, estimation procedure because the choice of steering force might influence the permeation pathway and the calculation must be carried out repeatedly to attain high accuracy [22].

Reaction path following

Molecular-dynamics-based simulation tools presume that 'reaction coordinates' for ion permeation are known, but these are not always obvious upon structural inspection. Although prokaryotic Cl^{-1} channels are antiporters [23], they share many functional characteristics with their eukaryotic channel cousins. Their X-ray structures exhibit clearly articulated ion binding sites [24,25] but no obvious exit pathways. Bootstrap path mapping Monte Carlo techniques have been developed that permit the ion to find its own way out of the pore and to monitor the energetics of the process [13].

Continuum modeling

Because calculating free energy profiles is computationally costly, the effects of varying external conditions (electrolyte concentration, electric field or lipid composition) are usually ignored. These aspects of the problem can be addressed by greatly simplifying the model system and ignoring molecular detail. This is done implicitly by approximating electrolyte, pore water, peptide and lipid as distinct dielectric continua, separated by sharply delineated, rigid boundaries. Crystal structures determine system geometry: electrical boundaries and the location of important charged features. With this input, current–voltage–concentration profiles can be efficiently computed [8,9,26].

Electrodiffusive modeling

The simplest continuum method is Poisson–Nernst–Planck (PNP) theory which, like the Poisson–Boltzmann description of electrolytes, treats ions as diffuse charge distributions, from which the differential equations of electrodiffusion determine currents [27]. Channel conductivity, like an ordinary resistor, depends inversely on length and directly on area, diffusion coefficient and mean occupancy; it is governed by the mean charge distribution of the protein and the dielectric structure of the system. However, PNP theory has limited applicability. The basic simplification, that ions are diffuse charge clouds, is incorrect in selective channels with constrictions of ionic dimensions, where counterions cannot enter [28,29]. 'Dielectric constants' for pore water and peptide are assigned ad hoc [12,30], with that of water typically between 40 and 80. These approximations suppress molecular detail and roughly account for dielectric shielding – the ion-induced structural reorganization of water and protein. They ensure that there are small energy barriers to permeation but greatly oversimplify reality [31]. Electrodiffusive modeling is best limited to larger radius, porin-like channels, which are only partially polarity selective, such as outer membrane protein F (OmpF). In OmpF, constrictions are short (~4 Å [32]) and, in 1 M electrolyte, the pore can accommodate ~20 ions: these are conditions under which the PNP assumptions are satisfied [33].

Brownian dynamics simulations

An improved continuum approach, better for narrow channels, employs a PNP description of the water–protein–lipid assembly but simulates ions each as a discrete finite-sized entity. Conductance is calculated by monitoring individual ionic transits. In addition to electrodiffusive forces, there is a stochastic component, so that the ion executes Brownian motion [28,29]. With a computational time step of ~1 ps and simulation of few explicit particles, multiple membrane crossings can be observed cheaply compared with molecular dynamics. This approach is also readily adapted to describe multiple occupancy [34–36]. However, systems are still decomposed into sharply delineated, rigidly bounded dielectric domains – assumptions that are least limiting for larger radius pores [12,30,37]. The advantages and disadvantages of these various methods are summarized in Table 1.

Gramicidin

Gramicidin, a 15-residue bacterial peptide with alternating D- and L-amino acids, assembles in lipids as a β-helix and dimerizes, forming an unusually long (~25 Å) and narrow (~5 Å diameter) transmembrane channel, occupied by a single file of approximately eight water molecules. Hydrogen bonds link backbone carbonyl and amide groups creating an environment hospitable to water and univalent cations and inimical to anions. Cations bind near the channel mouth by interaction with aromatic residues of the peptide. Until 1998, the only narrow channel of known structure, gramicidin has been intensely scrutinized both theoretically and experimentally [38,39].

Refining structure

Gramicidin A demonstrates that molecular dynamics is a reliable tool for using structural data, collected in environments very different from those of permeation studies, to establish ambient channel structure. High-resolution NMR studies of gramicidin A in dissimilar lipid surroundings [1,2] differ in two important aspects: the pitch of the peptide backbone and the orientation of Trp9. As interaction with the backbone and the aromatic residues governs permeation energetics in gramicidin A, accurate free energy profiles require a structure representative of gramicidin A in bilayers. When embedded in computational membranes sandwiched between electrolyte reservoirs, and simulated individually for ~100 ns [40], both structures [1,2] converged to a backbone conformation reminiscent of one conformer; Trp9 exhibited spontaneous transitions between the two NMR orientations. Three conclusions emerge: experimental gramicidin A structures are environmentally sensitive; when properly done, long molecular dynamics simulations on channels converge; and the bilayer conformation of a channel can differ notably from those determined experimentally.

Free energy profiles

Molecular dynamics is a powerful tool for refining channel structures. However, its utility for predicting channel

Table 1. Strengths and weaknesses of various modeling methodologies

Approach	Strengths	Weaknesses
Poisson–Nernst – Planck (PNP)	Steady-state solution; simple to apply; yields current–voltage and conductance–concentration profiles; computes currents, does not simulate ionic motion; reliable for wide pores; computationally cheap	Untestable dielectric assumptions (sharp boundaries, artificially large dielectric constants); protein charges are immobile; permeant ions diffuse with associated ion atmospheres; application to narrow pores is unjustified (ion self-energy is ignored); ill-suited to multi-ion channels
Brownian dynamics	Practical for microsecond times; determines current–voltage and conductance-concentration profiles; simulates individual ions; useful for multi-ion channels; best for wide pores	Similar to PNP, but with discrete ions and proper treatment of ionic self energy; ad hoc treatment of inter-pore ion–ion interactions; selectivity is inaccessible because isovalent ions are indistinguishable; problematic for narrow channels; computationally expensive
Molecular dynamics	Practical for nanosecond times; all-atom model of channel, lipid, water and ions; determines free energy profiles and diffusion coefficients; can describe selectivity; reliable for both narrow and wide pores	Computationally complex; even $\sim 10^5$ atoms miniaturizes macroscopic system; inadequate phase space sampling; conductance is indirectly computed; force fields possibly inappropriate for narrow channels; subject to computational artifacts; simulation system is huge compared with the physically interesting region; computationally very expensive

conductance is more limited. Gramicidin A provides a cautionary lesson. Simulations generating free energy profiles for ion translocation yield distressing results [41]. With two standard biomolecular force fields, translocation barriers for K^+ permeation are $\sim$20–25 kT (where k is Boltzmann's constant and T is 300 K), implying conductances $\sim 10^7$-fold too small. Why might this be? Simulations require massive truncations, replicated periodically. However, periodicity introduces artifacts, especially for charged species in systems composed of diverse dielectrics [18], which certainly describes gramicidin A. Recent work suggests these affect the barrier noticeably, reducing, but not eliminating, the discrepancies [19]. The residual errors could reflect force fields designed to describe bulk electrolyte with its open network water structure, vastly different from water ordering in narrow pores. Simulation systems can inadequately describe the transition of an ion from aqueous hydration to channel solvation, a problem that microscopic–mesoscopic hybrid approaches avoid [13,14,16,20]. Finally, sampling the surroundings of an ion might be inadequate to ensure reliable equilibrium statistics.

These problems beg a question. If the gramicidin A simulations are uncertain, why bother computing free energy profiles for other proteins? Gramicidin is probably the most stringent test system. Lipid is within 10 Å of the permeation pathway, much closer than for any physiological channel; dielectric discontinuities are more important here than anywhere else. The permeation pathway is narrow, with the longest known water single file, amplifying the influence of force field inadequacies. Failure to model gramicidin A conductance correctly does not imply that similar problems plague studies of other systems. However, successfully modeling gramicidin would definitively establish procedural reliability.

Channel gating

Channels continually undergo transitions between conformational states, relatively few of which are conductive. This gating crucially influences channel behavior. Gramicidin A is simple enough to study this process in detail [42]. Because the dimeric channel is short, the membrane distorts in response, exerting an axial force pulling the monomers apart. However, initial separation couples monomer rotation with lateral displacement [42], a displacement that is far less costly than separation via rotation–axial coupling (Figure 1) for monomers rotationally displaced by an angle φ. At $\varphi = 0$, both axial (d) and lateral (R) separations are 0; the dimer is most stable, with six inter-monomer hydrogen bonds. In dissociation, the monomers rotate and their junctional hydrogen bonds rupture; carbonyl (and amide) groups of one monomer abut carbonyl (and amide) groups of the other, at a large electrostatic cost. There are two distinct pathways (Figures 1b,c). For $\varphi < 75°$, d is small ($\leq$1 Å) but R is large (increasing to $\geq$3 Å). Beyond 75°, there is bifurcation. On path 1, R grows steadily with φ; the translocational path is disrupted and the channel becomes electrically silent. On path 2, R first increases to $\sim$4 Å, leading to transient channel closure; however, for $\varphi > 75°$ there is a sharp transition. R drops to 0 as d increases abruptly to $\sim$1.6 Å; a continuous aqueous pathway reforms and the channel can again conduct. These conclusions have been independently confirmed experimentally [43].

K^+ channels

The X-ray structure of the KcsA peptide established the architecture of the permeation pathway in K^+-channels [3]. The glycine residues of the strictly conserved Gly-Tyr-Gly-Val-Thr sequence allow an unusual fold, with backbone carbonyl groups lining a narrow pore; this opens out into a mid-channel aqueous cavity. Although folded differently, the pore, lined with backbone carbonyl groups, is reminiscent of gramicidin. Both the cavity and the four single-file sites of the pore (labeled 'cavity' and S_1–S_4 in Figure 2) can solvate cations [3]. Intermediate water molecules separate ions in the filter. The structure provides clear links to function. The binding pockets, if sufficiently rigid, account for K^+–Na^+ discrimination. Conduction proceeds concertedly. As K^+ permeates from the cytoplasm, the cavity ion moves toward the filter with

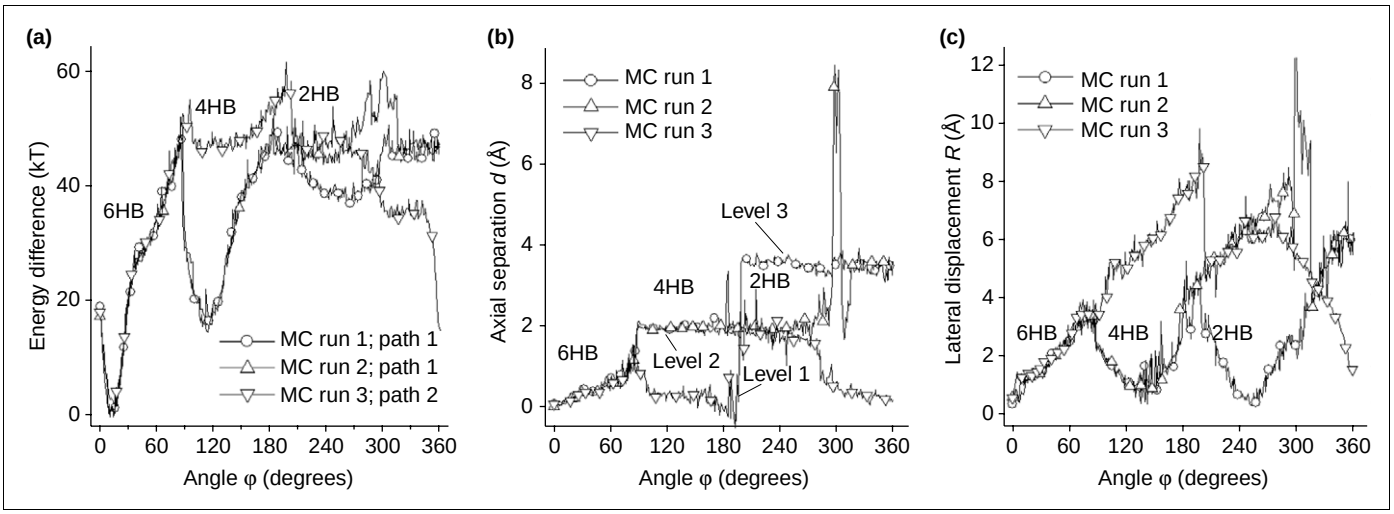

Figure 1. Properties of typical gramicidin A dimer dissociation pathways as functions of the angular coordinate, φ. Two paths are indicated: (i) formation of a four hydrogen bond (HB) state [Monte Carlo (MC) runs 1 and 2]; (ii) direct monomer dissociation from the 6HB state by radial displacement (MC run 3). The three panels present profiles in **(a)** energy; **(b)** axial displacement, *d*, where Levels 1, 2 and 3 correspond to axially separated gramicidin A monomers in the 6HB, 4HB and 2HB states, respectively; and **(c)** lateral displacement, *R*. Reproduced, with permission, from Ref. [42] © (2004) The Biophysical Society.

its resident K^+–water–K^+ complex, pushing the complex further along until its outer ion drops off; the cavity ion then enters the filter and the process begins anew.

Modeling channel conductance

The earliest simulations showed that the detailed picture is more complex [14]. Analyses of conduction energetics [15,44] provided new insight [45], identifying two more cation-binding sites localized in the solvent just beyond the exit of the single file (S_0 and S_{ext} in Figure 2), one before independent confirmation by X-ray [4] and computational [46] analyses. The study of Bernèche and Roux [44] provided other surprises (Figure 2). There are two distinct low-energy pathways on the free energy surface, differing by ~1 kcal mol^{-1} and corresponding to different dynamic ion–water complexes. In one, the ions are always separated by interstitial water. The lower energy assembly is a surprise. Two cations make direct contact in configuration (Figure 2b), where electrostatic repulsion > ~80 kcal mol^{-1} and the inter-ion force is equivalent to a transmembrane potential of ~30 V. This enormous driving force propels the K^+-water-K^+ assembly (Figure 2c) through the filter, forming a three-ion K^+-water-K^+-water-K^+ complex (Figure 2d). The filter shuttles between double and triple occupancy, as observed experimentally [4,47]. The filter free energy profile, along with a molecular-dynamic-generated diffusion coefficient, was used to indirectly determine conductance (Figure 3) [48]; the agreement with experiment is encouraging [49]. However, there are still caveats. The transition of the ion from bulk hydration to channel solvation was not fully monitored, so the free energy difference between the doubly and triply occupied pore was empirically adjusted to replicate proper pore occupancy. In addition, theory predicted super-ohmic behavior [48], not the sub-ohmic current–voltage profiles observed [49].

Molecular basis of selectivity

Selectivity is also more complex than structural inspection would suggest. The hydration free energy of Na^+ is ~15 kcal mol^{-1} – larger than that of K^+. Were binding pockets truly rigid and totally unable to adjust to Na^+, the energy penalty for Na^+ occupancy of the filter would be ~10 kcal mol^{-1} per ion. Na^+ could never enter; the selectivity ratio would be miniscule, far less than its typical value of ~10^{-4} [50]. However, theory suggests that K^+–Na^+ permeation free energy differs by 3–6 kcal mol^{-1} [20,44,51–53]. Simulation indicates the filter backbone is not strictly rigid, with atomic rms motions as large as ~1 Å [44]. Thus, the filter might deform substantially [54] to accommodate Na^+, consistent with C-type inactivation where the channel is electrically altered, becoming Na^+-selective and non-conducting [55]. In fact, ions are required for channel stability [47,56], again suggestive of deformability. Instead of an absolute prohibition, the deformational energy penalty might be moderate. The binding cavities of the channel must shrink by ~0.4 Å to perfectly accommodate Na^+; based on 1 Å backbone rms motions of the selectivity filter backbone atoms [44], the associated energy would be ~0.25 kT, greatly reducing the bias against Na^+ occupancy.

Assigning protonation states

The KcsA peptide has numerous ionizable residues. Most lie at or near the peptide–water interface, and would be ionized at neutral pH. But the protonation state of Glu71 is not obvious from structural inspection because, even though embedded in the peptide, it can orient so that it interacts strongly with Asp80 [15]; the two acidic groups might share one proton and interact cooperatively. Structural studies, confirming this speculation, suggest they are linked by a short hydrogen bond [4] – ideal conditions for proton shuttling. Moreover, as the residues are near the selectivity filter, their joint protonation state might be sensitive to pore occupancy. Continuum dielectric methods provide estimates of how residue pKs are affected by their environment [57]. There are some surprises. For an ion-free filter, Glu71 would be protonated and Asp80 ionized; however, as such filters deform easily [58], this configuration seems inaccessible [4,47]. In the multiply

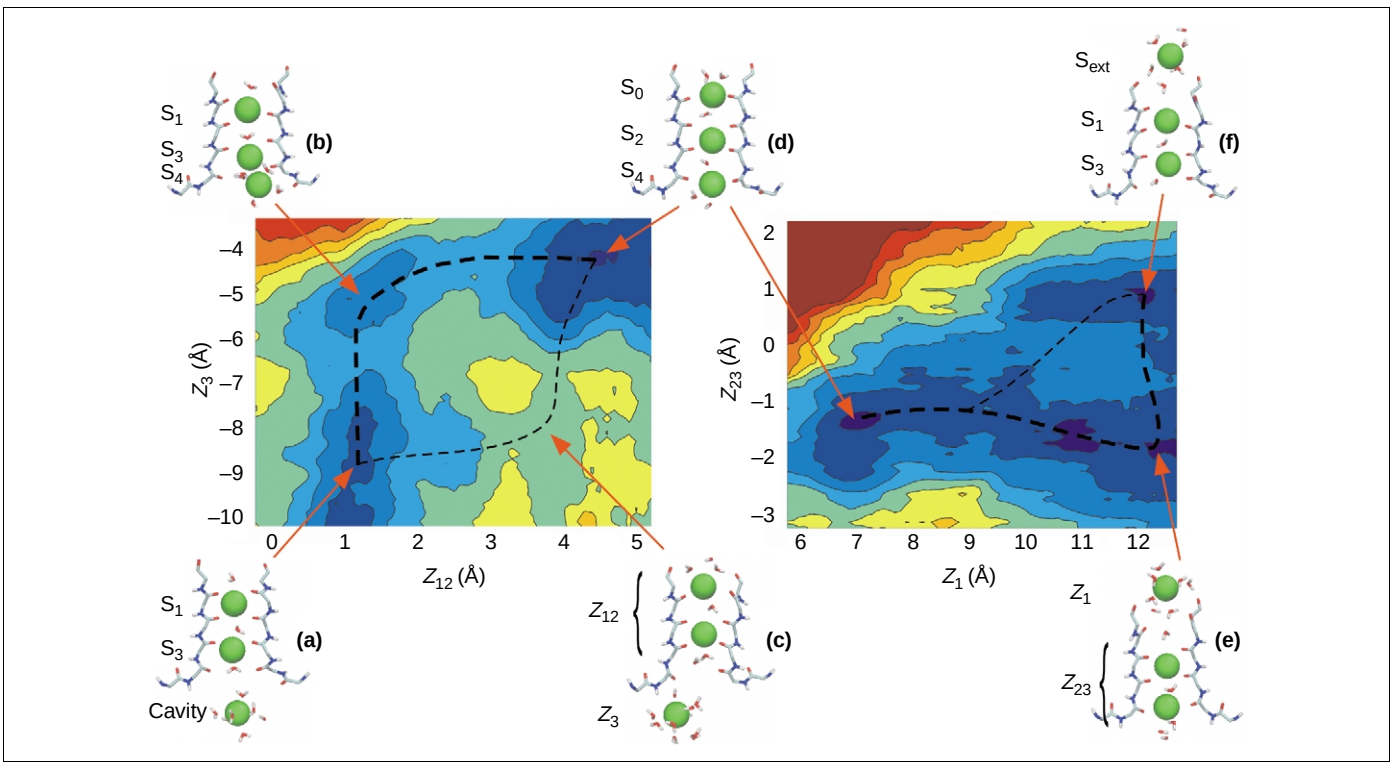

Figure 2. Topographic free energy maps of ion conduction in the KcsA K^+ channel selectivity filter with color contours, separated by 1 kcal mol^{-1}. Ionic positions Z_1, Z_2 and Z_3, (numbered from the outermost ion) are relative to the center of mass of the Thr75-Val76-Gly77-Tyr78 backbone atoms. The notation S_n, S_{ext} and 'cavity' enumerates the seven ion binding sites in KcsA: S_1 to S_4 are the sites in the selectivity filter itself; 'cavity' is the site in the water-filled mid-membrane cavity of the channel. S_0 and S_{ext} refer to binding sites in the exterior mouth of the channel [4]. (c) and (e) define reduced reaction coordinates $\{Z_{12},Z_3\}$ and $\{Z_1,Z_{23}\}$ with Z_{ij} being the center of mass of atoms i and j. The low-energy pathway (thick broken line) follows configuration (a)-(b)-(d)-(f); the secondary pathway, (a)-(c)-(d)-(f) (thin broken line), with an ~1 kcal mol^{-1} larger barrier, is also possible. Reproduced, with permission, from Ref. [44] © (2001) Nature Publishing Group (http://www.nature.com/).

occupied channel, the proton associates with Asp80. However, such calculations are limited by their dielectric assumptions which, like continuum modeling generally, assign high permittivity to water in the pore. Modeling using a microscopic–mesoscopic perspective, with very different dielectric assumptions, argues that the proton can never be more than partially transferred to the Glu71 because otherwise the electrical potential in the filter would become very negative, with an over-strong tendency for barium block [53].

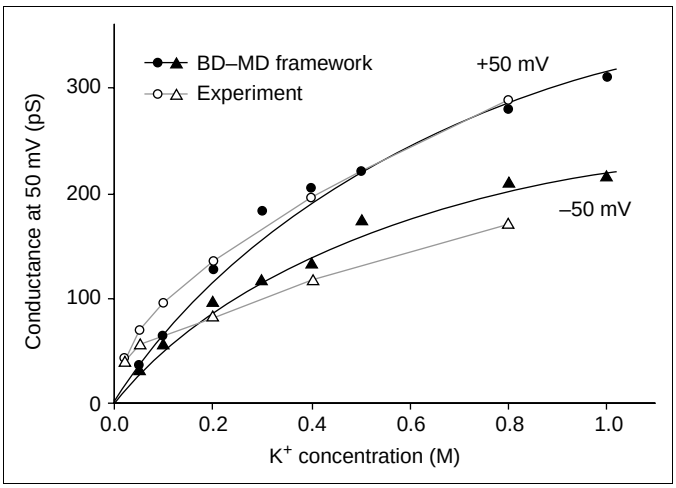

Figure 3. Computed [48] and experimental [49] conductances of the KcsA channel at ±50 mV as functions of K^+ concentration for symmetrical electrolytes. At positive voltages, external solutions are at higher potential; the data provide evidence of slight rectification, replicated by theory. Theoretical curves were generated using a Brownian dynamics–molecular dynamics (BD–MD) framework. Molecular dynamics was used to establish the free energy surface of Figure 2 and the diffusion coefficient profile; these data were used as input for Brownian dynamics simulations of the stochastic motion of the translocating K^+ [48]. Reproduced, with permission, from Ref. [48] © (2003) National Academy of Sciences, USA.

Variability of K^+ channel conductance

All K^+ channels are similar in selectivity but they exhibit a spectacularly large conductance spread, from a 2 pS delayed rectifier to the nearly 300 pS maxi-K channel [50]. What could account for such variety? Brownian dynamics studies based on the KcsA template provide a speculative answer [35]. Comparing KcsA [3] with the MthK channel [5] clearly indicates that, unlike the more-or-less rigid filter, the inner mouth is very flexible. Based on this observation, Chung *et al.* [35] computed currents for model systems as functions of inner pore radius. As this radius increased from 2 Å to 5 Å, channel conductance grew nearly 300-fold, from ~0.7 pS to ~200 pS. This is not a simple cross-sectional effect; even corrected for cation size, no more than a 30-fold increase is to be expected.

What remains to be done

Where are the open theoretical challenges? Simulations have not yet reproduced electrical behavior in the simplest system, gramicidin A; there is a need for a reliable polarizable force field. The basis for selectivity in K^+ channels is still disputed; molecular level methods for computing ion currents are needed. In ClC Cl^{-1} channels, gating and permeation are inextricably coupled, and the permeation pathway is not obvious; there is a need for

methods that reliably model gating and other slow conformational rearrangements. Large-scale simulations are computationally extremely expensive; there is a need for methods that integrate macroscopic continuum approaches with atomistic simulations if simulation is to become a desktop tool. Membrane proteins are environmentally sensitive and could functionally depend on the behavior of both lipid and electrolyte; there is a need for a simulation methodology permitting two-way feedback between macroscopic fluctuations and behavior on microscopic time scales.

Concluding remarks

Using high-resolution structural data, modern theoretical methods can illuminate permeation. They provide a practical way to use structure to describe membrane-bound ion channels reliably. Theory can illuminate the atomic bases for selectivity, permeation and gating, and, under favorable circumstances, can provide new structural insights independent of, and in advance of, experiment.

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