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Molecular dynamics simulations of potassium channels

Carmen Domene*

Physical and Theoretical Chemistry Laboratory,
University of Oxford,
Oxford, OX1 3QZ, UK

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Abstract: Despite the complexity of ion-channels, MD simulations based on realistic all-atom models have become a powerful technique for providing accurate descriptions of the structure and dynamics of these systems, complementing and reinforcing experimental work. Successful multidisciplinary collaborations, progress in the experimental determination of three-dimensional structures of membrane proteins together with new algorithms for molecular simulations and the increasing speed and availability of supercomputers, have made possible a considerable progress in this area of biophysics. This review aims at highlighting some of the work in the area of potassium channels and molecular dynamics simulations where numerous fundamental questions about the structure, function, folding and dynamics of these systems remain as yet unresolved challenges.

Keywords: Membrane proteins, ion channels, potassium channels, lipids, molecular dynamics simulations, selectivity, gating

1 Biological membranes

Biological membranes are composed principally of a lipid bilayer, which *in vivo* is a complex mixture of many different species of lipids, and membrane proteins. Such membranes, which are typically $30-40$ Å thick, enable cells to create an internal environment separate from the outside world, and also may provide different compartments within the cell. Protein molecules may be embedded within the lipid bilayer or bound to one or other surface. The lipid head group region of a bilayer provides a polar area in which both lipid headgroups and water molecules are present, with both of which, a membrane protein may

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* E-mail: carmen.domene@chem.ox.ac.uk

interact. The central hydrophobic region of the bilayer creates a barrier to permeation of ions and polar solute molecules in the absence of specific channels and transporters. These lipid assemblies exhibit very complex structural and dynamic properties: individual motions of lipids may occur on a time scale of tens to hundreds picoseconds while lateral diffusion might take tens of nanoseconds. More complex biomembrane environments are present in for example the outer membranes of Gram negative bacteria. These membranes have an inner leaflet composed mainly of phospholipid, but an outer leaflet containing large, complex lipopolysaccharide molecules. In general, there is usually a high concentration of K^+ ions inside the cell and a high concentration of Na^+ ions outside. A voltage difference across the membrane is established known as the membrane potential.

2 Membrane proteins

Membrane proteins constitute $20-30\%$ of genes in most organisms [1] so there may be about 10,000 membrane proteins encoded in the human genome. They play key roles in regulation of cellular activities; the function of membrane proteins is essential for a wide range of physiological events such as neurotransmitter transport, adhesion, regulation and nerve impulse transmission. Two major classes of biomedical important membrane proteins are ion channels and solute transporters, both of which facilitate the movement of ions or other polar molecules across the impermeable core of the bilayer.

From a medical point of view, membrane protein and ion channel disfunctions in particular, can cause diseases in many tissues affecting muscles, kidney, heart and bones [2]. This group of diseases has been termed channelophathies [3]. Elucidation of several dominantly inherited epilepsies caused by mutations of ions channels are among some recent important advances in neurology [4] and genetic alterations of various ion channels produce heritable cardiac arrhythmia that predispose affected individuals to sudden deaths [5]. However, despite the progress made recently, there are still major difficulties before these diseases are fully understood. Consequently, interest in membrane proteins and ion channels in particular, as pharmaceutical drug targets continues to grow. Seven years ago, membrane proteins represented $\sim 50\%$ of the molecular targets of drug therapy with ion channels contributing $\sim 5\%$ to the figure [6]. Membrane proteins represent more than two-thirds of the known protein targets for drugs [7].

Despite the large number of genes encoding membrane proteins, the number of membrane protein structures presently known at high resolution is rather small, just about 0.2% of them. Only over a hundred membrane proteins have been determined among which are monotopic membrane proteins (7), transmembrane proteins such as bacterial rhodopsins (5), G protein-coupled receptors (1), beta-barrel membrane proteins (31), ion channels (12), multi-drug efflux transporters (2), H^+/Cl^- (1), amino acid secondary (3) or ATP binding cassette transporters (5) photosynthetic reaction centers, light-harvesting complexes and photosystems, respiratory proteins (7) and oxidases (5). For a detailed classification of all the 3D structures of membrane proteins see: http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html or http://blanco.biomol.uci.edu/

Membrane_Proteins_xtal.html. Such an expansion in the number of membrane proteins is due to the fact that technological advances have allowed structural biology to reach a position where the elucidation of the three-dimensional structure of relevant proteins on a large scale appears possible. At the same time high-throughput approaches are emerging [8–10] to characterize membrane proteins and analyze their topology, issues of significant interest for drug discovery. Unfortunately, the expression and crystallization of integral membrane proteins, such as ion channels, is still fraught with difficulties. Many membrane proteins are naturally expressed in quantities much smaller than those required for structural studies and production of protein crystals suitable for high-resolution X-ray diffraction is problematic due to the amphiphilic character of the membrane protein surface and the requirement for maintaining the membrane environment. Several methodologies have been developed to circumvent these problems and proteins are stabilized with detergent micelles or co-crystallise with particular antibody fragments. The former provides crystals of limited resolution and the latter despite being able to yield crystals that diffract at high resolution is likely to yield deformed structures due to the interactions with the antibody fragment.

Due to these inherent experimental difficulties in obtaining structural data for membrane proteins, it is essential that we maximize our understanding of the structural/functional relationships of those membrane proteins for which we have experimental structures. Computational approaches have proven to be useful and have become a standard tool for investigations of membrane proteins [11–27]. Moreover, a combination of molecular modeling and simulation helps us to extrapolate from the structure of prokaryotic membrane proteins to the structure and dynamics of their human homologues, which may also aid in experimental structure determination. Ultimately, simulation offers the prospect of understanding the effects of mutations on physiological function in a variety of disease states, and aiding in the discovery and design of drugs to help combat such diseases.

3 Ion channels: families and topologies

Ion channels are a large and biomedically important family of integral membrane proteins [28]. They govern the electrical properties of the membranes of excitable cells such as neurons or muscle, although channels are also found in the membranes of non-excitable cells and a wide range of organisms from viruses to plants. Ion channels form pores in biological membranes and each time the channel opens, thousands of millions of ions diffuse down their electrochemical gradient across the membrane ($\sim 10^8$ ions/s). In contrast, protein pumps, for example, use energy from light or adenosine triphosphate (ATP) to transport ions and other solutes across the membranes.

Channels do not stay open all of the time. Instead, they are ‘gated’ by either the binding of small molecules to the channel protein or by changes in voltage across the membrane. Channel gating is believed to operate via conformational changes that induce structural changes in the pore which alter the energetic profile of the ion permeation pathway.

Traditionally, channels are classified according to the type of ion for which the channel is selective, e.g. K^+ channels, Na^+ channels, Cl^- channels. They are also classified according to the factors that control the gating of the channel, e.g. ligand-gated ion channels, voltage-gated ion channels. Voltage-gated are made up of one or more pore-forming subunits which determine the ion selectivity and a number of accessory subunits which can play important roles in modulation of channel function and inactivation. These channels open and close in response to changes in the electrical potential difference across the membrane. In contrast, ligand-gated channels open in response to extracellular agonist and are classified in (i) the nicotinic receptor superfamily, (ii) the glutamate receptor family and (iii) the ionotropic ATP receptors. Besides these factors, calcium, pH, phosphorylation and lipids may regulate ion channels.

4 Potassium channels

The K+ channels are perhaps the most intensively studied group of ion channel [29, 30]. The major families are presented in Figure 1: (i) Kv channels, which are activated by a change in transmembrane voltage (ii) Kir, inward rectifier channels, which have a higher conductance for K^+ ions moving into the cell than outwards; and (iii) TWIK and related channels, which contain two copies of the selectivity filter motif in one polypeptide chain. Therefore, two of these polypeptide chains are required to form the channel.

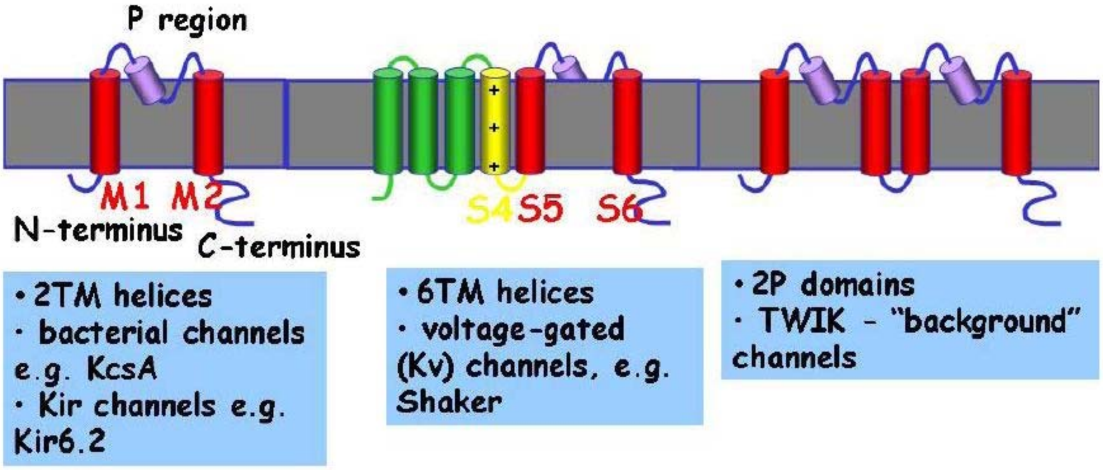

Fig. 1 Schematic diagrams showing the topology of one transmembrane (TM) subunit of each of the K^+ channel families. Left diagram represents the simplest core TM domain, made up of two TM helices (M1 and M2) plus a re-entrant pore loop containing a short pore helix plus an extended loop that contains the filter motif. Middle diagram represents topology of one subunit containing six TM helices. S5 and S6 correspond to M1 and M2 and S4 is the voltage sensor. Right diagram represents the TWIK and related channels, which contain two copies of the selectivity filter motif in one polypeptide chain. Therefore, two of these polypeptide chains are required to form the channel.

In general, ion channels are multimeric proteins and although some are homomultimers, most are assembled from distinct subunits coded for by different genes. When the channels are formed by identical subunits, these are called α subunits. If there are other subunits which may modulate their function or be essential for the function of the channel, they are classified as β and γ subunits such as in the case of the neuronal nicotinic acetylcholine receptors. This is an important aspect to be taken into account when considering the effect of mutations which may confer a loss or a gain in channel function. For example, dominant negative effects can reduce channel function down to a quarter of that of the wild type with dimeric channels while the effect can be much stronger with tetrameric channels [2].

At present, there are examples of resolved structures for most of the categories in which ion channels have been classified (See Table 1, Figure 2). There are Kv channel, Kir channels, Cl^- channel, mechano-sensitive channels, Ca^{2+}-gated potassium channel, open and closed structures. These few structures however, provide a good source of information to get insight into the two main properties of ion channels, their gating and selectivity mechanisms.

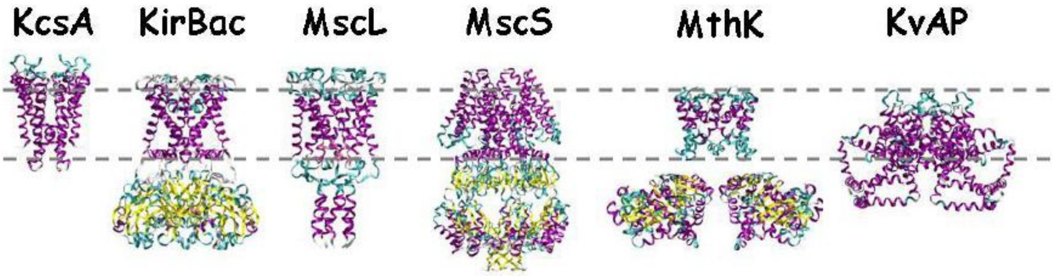

Fig. 2 Schematic diagrams of the folds of several ion channels represented as ribbons: KcsA, KirBac1.1, MscL, MscS, MthK, and KvAP. The horizontal lines show the approximate location of the bilayer with the intracellular domains at the bottom. The diagram was generated using VMD [36].

All potassium channels share the same core topology and structure (Figure 1). The channel-forming core is composed of two transmembrane helices, TM, (M1 and M2 or S5 and S6 in Kv channels) separated by a re-entrant loop made of a short pore helix (P) plus a more extended region of polypeptide that forms the selectivity filter (F). K^+ channels differ in the presence or absence of additional TM helices, and of additional non-membrane domains and subunits that control their gating. Kv channels for example, are composed by four subunits and each subunit consists of six α helices (S1-S6) with both amino and carboxyl termini on the intracellular side of the membrane. The first four TM helices (S1-S4) form the voltage-sensing domain whereas the last two (S5-S6) form the pore domain. The selectivity filter is formed by the residues corresponding to the signature sequence TVGYG common to all potassium channels. The conformation adopted by this motif in the tetrameric channel is essential to the permeation and selectivity mechanism of the channel.

Table 1 Summary of some of the structures of ion channels available at present. For a complete description visit: http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html or http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html.

Channel	PDB	Resolution	Species	Function
KcsA	1BL8 1K4C 1K4D	3.2 Å 2.0 Å 2.0 Å	*Streptomyces lividans*	H^+ gated
NaK	2AHY 2AHZ	2.4 Å 2.8 Å	*Bacillus cereus*	Non-selective tetrameric cation channel
MthK	1LNQ	3.3 Å	*Methanobacterium* thermoautotrophicum	Ca^{+2} gated
KvAP	1ORQ (Full-length channel) 1ORS (Voltage sensor domain)	3.2 Å 1.9 Å	*Aeropyrum pernix*	Voltage-gated potassium channel
Kv1.2	2A79	2.9 Å	*Rattus norvegicus* (expressed in *Pichia pastoris*)	Voltage-gated Potassium channel
KirBac1.1	1P7B	3.65 Å	*Burkholderia pseudomallei*	Inward-Rectifier Potassium channel
KirBac3.1	1XL4 (Intermediate State 1) 1XL6 (Intermediate State 2)	2.60 Å 2.85 Å	Magnetospirillum magnetotacticum	Inward-Rectifier Potassium channel
Kir IC domain	1N9P	1.8 Å	*Mus musculus*	Inward Rectifying Potassium Channel IC domain of Kir3.1
nAChR	1OED	4.0 Å	*Torpedo marmorata*	Acetylcholine Receptor
AChBP	1UX2	2.20 Å	*Lymnaea stagnalis*	Acetylcholine binding protein
MscL	1MSL	3.5 Å	*Mycobacterium tuberculosis*	Large Conductance Mechanosensitive channel
MscS	1MXM	3.9 Å	*Escherichia c*	Small Conductance Mechanosensitive channel
FluM2	1MP6 1NYJ	NMR	*Influenza A*	M2 H+ channel
ClC	1KPL 1KPK 1OTS	3.0 Å 3.5 Å 2.51 Å	*Salmonella typhimurium* Eschericia coli Eschericia coli	Chloride Channel

The glycine residues in the filter sequence are absolutely conserved in all K^+ channels. Mutation of either glycine of the GYG motif results in loss of K^+ selectivity [31]. Recently, it was demonstrated experimentally [32, 33] that the first glycine in the selectivity filter is a surrogate D-amino acid which is used to fulfil specific dihedral angle requirements. To create the binding sites where potassium ions reside, the peptide main chain has to adopt an unusual conformation and the dihedral angles of the TVGY sequence alternate between the left-handed and the right-handed α-helical regions of the Ramachandran plot. Glycine absolute conservation was explained as a result of glycine being the only natural amino acid that can be found easily in the left-handed α-helical region of the Ramachandran plot and which can play this role due to its achiral nature.

5 Molecular Dynamic Simulations of Ion Channels

Molecular dynamics (MD) simulation is one of the most useful tools for the study of dynamical processes in biological systems, which in many cases complements experimental work. MD simulations complemented by a number of other specific computational techniques offer a unique way to relate the structure and function of these biological systems. Since the first simulation of a protein about three decades ago, progress towards more realistic representation of solvent and membrane, larger systems and longer timescales has been made [34]. There have been a vast number of simulation studies of potassium channels since the first KcsA structure was published. Many of these studies have been reviewed several times in past years [11, 14, 15, 20, 23, 35, 37–41].

This computational approach consists in monitoring the evolution in time of the system of interest where the interactions between atoms are described by simple empirical potential functions. Forces are then calculated as gradients of the potential energy and by integrating many times Newton's law of motion, velocities and trajectories of the system are generated. Equilibrium quantities are then calculated by averaging over a trajectory of sufficient length which would have sampled a representative ensemble of the state of the system. Although the basic idea behind this technique appears to be rather simple, in practice there are many complications and one has to be careful at setting the initial conditions, analysing in a systematic way the influence of simulation protocols to ensure reliability, choosing appropriate algorithms or storing and analysing the huge amount of data generated.

In practice, the success of this technique relies also on the effective formulation of the potential energy function and suitable integrating algorithms. The potential energy function includes terms representing interactions between atoms that are covalently bonded to one another and also terms for electrostatics and van der Waals interactions between non-bonded atoms. Bonded terms include covalent bond-stretching, angle bending and dihedrals which are modelled by harmonic and sinusoidal functions.

Currently, there are many force-fields available in the context of biomolecular simulations such as GROMOS [42], OPLS [43], CHARMM [44], AMBER [45] which differ in the parameters used for the atomic interactions (charges and Lennard-Jones radii) and the explicit form of the potential. A great deal of variability exists in the functional forms of the potential energy and in the numerical values of the parameters but in general, all the interactions are calculated between pairs of atoms neglecting the many-body nature of the interactions. This many-body nature refers to the fact that the motion of every single atom influences and depends on the motion of the surrounding atoms which would require coupled equations to describe the dynamics of the system.

Transferability of the functional form and parameters is an important feature of a force-field, that is, the same set of parameters should be capable of describing related systems. In the context of ion channel simulations, they usually do not allow for polarization effects [35], which is a possible limitation shared with a number of other studies of ion selectivity [46, 47] and permeation [48]. However, although some sensitivity is sometimes reported, a consensus picture emerges from many different studies. It remains likely that electronic polarization could play an effect during movement of the ions in the channel [49, 50] and this will merit further study. However, in the absence of a fully developed polarisable force field for ions, water, protein and lipids, the current studies have proved to delineate some of the main features of ion channels. Nevertheless, it is expected to see future research directed at quantum mechanics/molecular mechanics (QM/MM) and *ab initio* simulation methods, with the advent of linear-scaling quantum mechanical methods. Some of these *ab initio* techniques have already been applied in K-channel studies [50–55]. These studies have focused on studying the physical origins of the barrier-less knock-on diffusion mechanism taking into account ion polarization [54] and calculation of electrostatic potentials and binding energies and the influence of polarization effects [51].

Due to computing limitations, the first simulations were carried out *in vacuo* and later water was modelled implicitly so that bulk solvent effects were included via a dielectric constant in the electrostatic energy term. Nowadays, because of the increasing timescales accessible to MD and computer power, explicit water molecules around the protein and counterions and lipid bilayers are easily afforded enabling the description of the complexity of membrane systems. Alternatively, there is the option of using an octane slab to mimic the membrane. In cases where conformational changes are thought to be important for the purpose of the study, the slab of octane molecules provides a membrane-mimetic environment of lower viscosity facilitating a greater sampling of the conformational space. What still remains a challenge is to extend the simulation studies to more complex and biologically realistic lipid mixtures if one bears in mind that a number of ion channels exhibit functionally important interactions with lipid molecules [56].

When setting up the simulation system, to overcome the problem of surface effects and to ensure that the system does not have an abrupt limit with vacuum, periodic boundary conditions are imposed [57]. The system is replicated throughout space to form an infinite lattice and it is this periodicity which introduces artifacts (that are smaller in large systems) [58, 59]. The issue is how large these artifacts are and whether they are enhanced by the inclusion of long range electrostatics [60]. In the particular case of membrane simulations and ion channels, it destroys the driving force for steady-state non-equilibrium ion flow as an ion concentration gradient cannot be modeled; the intracellular and extracellular milieux would not be distinguished due to the periodic boundary conditions. This could be however easily done by having two membranes (though the size of the system increases considerably and so the computational expense) or by implementing slab geometric boundary conditions [61].

The most time-consuming part of the simulation is the calculation of the non-bonded interactions. The importance of accurately accounting for long-range interactions in molecular dynamics simulations has been an area of active research for a long time as it is a crucial issue in computational modeling [59, 60, 62–70]. It is essential therefore, to develop reliable and efficient methods to deal with these interactions as well as to use them with care and insight. The Coulombic energy decays very slowly ($\sim 1/r$) with the interparticle distances and the vast majority of the simulation time is devoted to the calculation of these interactions. Therefore, to speed up the calculations several approximations are usually considered. Two of the most common ones are the lattice sum methods such as particle mesh Ewald (PME) and the truncation of the long-range interactions with the use of cut-offs [57].

In recent literature, studies dealing with the importance of accurately treating these interactions are reported in the context of structure and stability of membranes or membranes with inserted peptides and several groups have described artifacts linked with the choice of electrostatic treatment in biological applications [59, 66, 71–73]. It is interesting to observe, that some controversy emerges from these studies and that there is an ongoing debate concerning possible artifacts arising from the use of these methods.

The simulation temperature can be controlled in several ways [57]. In most protein simulations the system is coupled to an external bath or ‘thermostat’ with which it can exchange energy. Use of the Berendsen and the Nosé-Hoover thermostats are the most popular ways of controlling the temperature. The latest is said to yield a perfect canonical distribution. Pressure is controlled in a similar manner and it has been shown that hydrogen-bonding patterns may change drastically with pressure [74].

At present, one of the real challenges is the development of approaches that allow the exploration of longer time scales. Simulation times are generally too short to yield proper sampling of conformational changes of biomolecules and it is difficult to extract functionally relevant motions. Recently, there have been a number of exciting developments aimed at extending both the time- and length-scales accessible to biophysical simulations (See [75] and references within). One of these methods is the so-called metadynamics technique [76] which has been successfully applied to a range of biological problems some of which are related to membrane protein biophysics. Metadynamics has been used to describe the reaction path and a mechanism of passage of ampicillin through OmpF [77] and in another application the chloride ion translocation through the ion transporter was explored and free energy surfaces of the process were described [78]. As the passage of a single ion across a channel takes about a microsecond and currently, only tens of nanoseconds simulations can be routinely performed, it is envisaged that application of techniques such as metadynamics will proof rather helpful in the study of these systems, especially in issues concerning gating mechanisms.

In a recent review Chung and Corry [25] gave an overview of three different theoretical approaches commonly used to understand the dynamics of ion permeation across channels. Besides molecular dynamics simulations, described before, stochastic dynamics [79–81] and continuum theories [82–86] have been widely used. The reader is referred to this review for further details.

6 Structural changes during ion channel gating

One important aspect of the function of potassium channels is their ability to undergo conformational transitions in response to electrical signals or ligand binding. In particular, voltage-gated ion channels open and close to produce electrical signals that spread rapidly over the entire plasma membrane, the so-called action or membrane potential, which is essential for life. Despite the remarkable progress in the understanding of selectivity and permeability, which is described later on, characterising the processes involved in gating remains a challenge. The time scale of gating is too long to enable this process to be studied directly by MD and structural details remain scarce.

The KcsA channelThe KcsA channel, the prototype of K-channels, is opened by low pH and in the original experiment the crystals were grown under conditions where the predominant conformation should be the closed one. This and some other experimental evidence suggest that the open state of the channel must be different from the one seen in the crystal structure. In the structure, the M2 helices are tilted with respect to the membrane normal and each other, forming a bundle. This tightly packed bundle of helices leave only a narrow hole for ion and water penetration from the cytoplasm indicating that this represents a closed state of the channel. A number of studies on various K^+ channels also support a conformational change at the inner mouth upon channel gating. Site-directed spin labelling and electron paramagnetic resonance spectroscopy reported changes in the structure of KcsA upon lowering of pH [87–89]. Blocking experiments with tetraalkylammonium ions on Kv channels and KcsA suggest that these large cations can enter the central cavity of the channel via the intracellular mouth when the channel is open [90–93]. However, the radii of these organic cations are larger than the dimension of the intracellular mouth of the crystallographic structure. And in addition, experiments using cyclic nucleotide-gating channels also support the idea of conformational changes in the tightly packed bundle of helices upon opening. Nevertheless, the mechanism whereby a lowering of intracellular pH leads to such change in conformation remains unknown.

Electron paramagnetic resonance spectroscopy (EPR) with site-directed spin labelling showed that during gating the M2 transmembrane helix undergoes a rotational motion that provides a wider opening at the intracellular end of the channel and a bending of the M2 helices at a conserved glycine position. Simulation studies combined with principal component analysis (PCA) [94] have enabled to test the hinge-bending hypothesis and different procedures have been used to displace the intracellular gate formed by the M2 bundle aiming for an open model. In contrast, large mammalian voltage-gated potassium channels are thought to have another bending site further down towards the intracellular opening at the conserved PXP sequence motif.

Several groups have modelled open states of the channel. Allen and Chung [95] performed Brownian Dynamics (BD) simulations based on an open state model and Mashl *et al.* [96] carried also BD of an open system they modelled by rotating the M2 helices by 20° which widen the intracellular mouth of the protein. Biggin and Sansom [97] attempted to generate open-state models by steered simulations. The approach they followed can be described as placing a balloon inside the channel and gradually inflating it. The balloon is a van der Waals sphere whose parameters change by a small amount at each time step and the balloon is only seen by the protein, not the water or the ions. Unfortunately, the resulting structures from the process were conformationally unstable though after a rather long relaxation process, they were thought to relax into a new state which had a larger pore radius at the intracellular mouth. A number of other computational approaches have indicated that this tight bundle forms a significant energy barrier to ion permeation and therefore, it might be considered to be the gate region of the channel. Steered MD simulations in which the M2 helices in the gate region were slowly forced apart also supported this hypothesis and suggested that the M2 helices might not move entirely as rigid bodies. In contrast, the M2 helices might bend slightly during the opening of the pore. Subsequently, determination of the open structures of MthK [98], a calcium-gated channel, and KvAP [99, 100], a voltage-gated channel, confirmed some of the above proposals and provided new insights into channel gating.

Homology modelling and simulations have been used to compare a model of the transmembrane domain of KcsA in a closed state with the same domain of an open model based on the MthK structure [101]. Both models were of comparable stability and the M2 helix was observed to undergo kink and swivel motions about the molecular hinge at G99. Born energy calculations suggested that the intracellular hydrophobic gate in the closed state has a barrier to monovalent cations which corresponds to about 5kT whereas in the open state there was not a barrier.

The MthK channel is a Ca^{2+}-activated K^+ channel captured in its open state. The resolution of the crystals is just good enough to describe the pore backbone but not the positions of the sidechains. However, the structure suggests that the functional MthK is associated with an octameric assembly of RCK domains and subunits. The channel is a tetramer with identical transmembrane architecture of that of KcsA and two regulators of K^+ conductance (RCK) domains. In the case of MthK, these α/β folds associated with binding regulatory nucleotides or other ligands, bind Ca^{2+} ions and it has been suggested that the RCK domains pull on M2 when Ca^{2+} ions bind. The inner helices of MthK bend away from each other with the C-terminal end of the helices lying between the outer helices of adjacent monomers. The bending is located at a glycine residue, Gly83, which is the potential hinge point and which sequence alignment indicates it is highly conserved in K^+ channels. The linker polypeptide between the carboxyl terminus of M2 and the amino terminus of the RCK domains is not visible in the crystal

The nature of voltage sensing by voltage-activated ion channels is a key problem in membrane protein structural biology. The structure of the integral KvAP bacterial channel assembly was crystallographically determined to moderate resolution with a high resolution structure of the isolated voltage sensor [99, 102, 103]. It is composed of six transmembrane helical segments and each monomer consists of a pore domain and a voltage sensor. At present the scientific community is divided concerning views on the conformation and orientation of the voltage sensor upon membrane depolarisation [104]. Nevertheless, there is the consensus that the voltage sensor of Kv channels forms an independent domain. At least four competing models for the orientation of the voltage sensor domain relative to the pore domain have been proposed: the helical screw model [105], the transporter model [106], the paddle model [102] and the twisted S4 model [107]. Later on, the high resolution structure of a voltage sensor domain in a mammalian channel, Kv1.2, was solved. Kv1.2 is similar to the isolated KvAP voltage sensor and packs rather loosely to the pore domain [108, 109]. It is thought that the voltage sensor domain move in response to changes in voltage across the membrane bilayer but the exact nature and extent of such motions is still undetermined. It is likely then, that simulations [110–112] in combination with experimental data will be able to provide information on the location of the voltage sensor relative to the membrane and a complete structural understanding of the conformational changes during the transition from the closed to the open state in Kv channels.

The structures of two bacterial mechanosensitive channels, MscS [113] and MscL [114], have also been determined providing another excellent set of systems to obtain a coherent picture of molecular events which take place during gating. Although these are not potassium selective channels, they conduct potassium and their gating mechanisms clearly illustrate a concurrent gating process in α-helical channels that complements what is now known from studies of K^+ channels [115]. A brief overview of their mechanism of action will be given next.

Mechanosensitive ion channels convert mechanical forces exerted on the surrounding membranes into electrical or biochemical signals [116–118]. Despite the functional similarity of MscS and MscL, their architectures are very different and there is not any sequence homology between them. MscS exhibites a slight preference for the conductance of anions relative to cations (~ 3 Cl^- ions for 2 K^+ ions) [119].

MscL is a large-conductance channel which corresponds to the closed state and whose open state has been also characterised by electron paramagnetic resonance spectroscopy. It serves as a safety valve protecting bacterial cells against hypoosmotic shock. It has been studied extensively using a variety of methods at a functional and structural level. Rees and co-workers [114] solved the structure which is composed by five identical subunits arranged around a central pore. Each of the subunits consists of two TM helices plus a small intracellular domain at the carboxyl terminus. The gate has been proposed to be formed by two rings of hydrophobic side chains that present an energetic barrier to ion permeation likewise in other K channels. MscL is gated by the application of tension to the lipid bilayer [12, 121, 122], and its gating is not dependent on any other protein or cellular structure [123]. At a critical value for the tension, MscL opens and this open channel is not ion-selective and can pass relatively large organic ions. Many studies [124–128] have aimed at elucidating how tension of the surrounding lipid bilayer causes the major conformational change in the structure leading to its opening. A two-stage gating model was proposed and tested experimentally and computationally. To gain insight into the effect of the lipid membrane composition and geometry on MscL structure, Meyer etl al. [129] conducted a molecular simulation study. During the course of the simulation a spontaneous restructuring of the periplasmic loops occurred, leading to interactions between one of the loops and phospholipid headgroups. Previous experimental studies of the role of the loops agree with the observation that opening starts with a restructuring of the periplasmic loop, suggesting an effect of the curved bilayer. Unfortunately, due to limited resources, only one simulation of the large system was performed. However, the results obtained suggested that through the geometry and composition of the bilayer, the protein structure can be affected even on short timescales.

The small conductance channel, MscS, is also sensitive to changes in the transmembrane voltage and the crystal structure, which includes a voltage sensing element, seems to represent the open state. The structure is a homoheptamer and the central pore runs down it. The TM domain of each subunit contains three α-helices and a large cytoplasmic domain composed mainly of β-sheets. It was suggested that small amino acids such as glycine and alanine at every fourth position in regions that participate in helix-helix packing, may be implicated in the conformational rearrangements which might take place under different lateral pressures from the surrounding membrane during gating [130, 131]. The position of these residues is conserved in the MscS family of proteins and it is essential for these channels to function. Recently, in an effort to relate electrophysiological measurements on MscS and properties of the MscS crystal conformation, the response of the channel to voltage and pressure as determined by patch-clamp experiments, as well as MscS electrostatics and transport properties as determined through all-atom molecular dynamics simulations were reported [132]. The experiments reveal that MscS is a slightly anion-selective channel with a conductance of approximately 1 ns, activated by pressure and inactivated in a voltage-dependent manner. On the other hand, the simulations, covering over 200 ns and including biasing electrostatic potentials, show that MscS restrained to the crystal conformation exhibits low conductance; unrestrained it increases the channel radius upon application of a large electrostatic bias and exhibits then ion conduction that matches experimentally determined conductances.

More recently, the structure of KirBac1.1 potassium channel in an open state has been modeled using as starting point the structure of KirBac1.1 in its closed conformation (Figure 3) in order to study the gating mechanism in inwardly rectifying potassium channels. The open model [133, 134] was refined by comparing it to projection maps of successive structures generated in the modeling process with the projection map of the open KirBac3.1 crystal until cross-correlation values were experimentally acceptable [133]. The homology identity over 287 residues between KirBac1.1 and KirBac3.1 is approximately 40%, thus the assumption that the overall structural folds were similar was made. To test the validity of the open state model, molecular dynamic simulations of the model in octane were carried out. The conformational change responsible for gating seemed to involve concerted and/or sequential hinge-bending motions about conserved glycines (Gly134 and Gly143) and other flexible elements in the M2 helix (Gly 137). Overall, the open model was stable and will be useful in the study of potential gating and block mechanisms in this family of potassium channels. As pointed out by [135] the availability of models of KirBac3.1 in the closed and open states provides a unique opportunity to examine the importance of microscopic factors affecting channel function using computational methods.

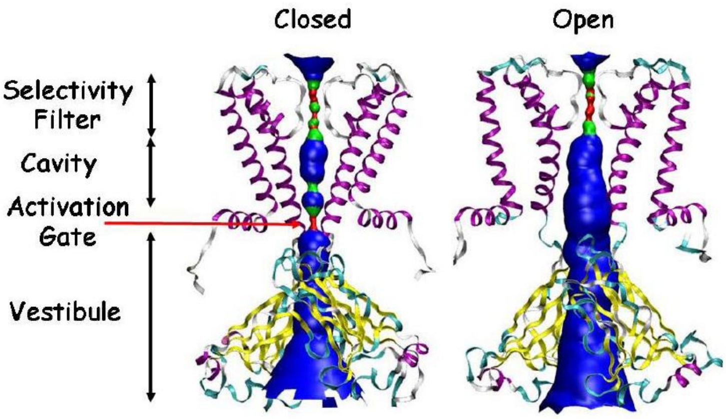

Fig. 3 Models of the closed and open conformers of KirBac1.1 represented as ribbons. Only two of the four subunits are shown for clarity. For each structure, the pore-lining surface is shown. Volume colored in red indicates that there is not enough space for a water molecule to pass, green where one or two water molecules could fit and blue represents the volume were many water molecules can fit. Molecular graphics images were prepared using VMD [36].

To conclude, it is important to be aware of the limitations of simulation approaches regarding gating. Current available time scales are insufficient to sample the gating process *per se*, which takes place on a micro to millisecond timescale. However, MD simulations in combination with principal component analysis (PCA), also known as essential dynamics, seem to be able to capture some of the early stages of the gating process, whereby local short timescale structural rearrangements may lead to larger conformational changes. The combination of these techniques can be illustrated with the analysis of the motion of the pore-lining M2 helices in a simulation of the transmembrane domain of the KirBac1.1 channel [136]. It was found that a major element of the motion of the M2 helices has to do with the bending movement about a molecular hinge at a central glycine residue (Gly134). This motion about the glycine residue was then decomposed into a helix kink term and a swivel term and it appeared that the M2 bundle may behave dynamically as a dimer-of-dimers.

7 Potassium Permeability and Selectivity

Structural studies in conjunction with computational studies have provided profound insight into ion conduction mechanisms. The very first crystal structure of a potassium channel, that of the bacterial channel protein KcsA [137], provided us with the first image of a potassium channel and its interactions with ions at atomic resolution. Most of the early MD simulations of KcsA were aimed at addressing how channels are able to achieve a fast throughput while at the same time remain highly selective and about the configurations of ions and water molecules in the selectivity filter [48, 138–142]. Traditionally, the ability of these channels to conduct ions at nearly the diffusion limit has been described in terms of a concerted multi-ion transition, the "knock-on" mechanism, in which ion channel attraction and ion channel repulsion lay compensating effects and ions move simultaneously in a single file through the narrow pore of the protein.

The KcsA structure solved at a resolution of 3.2 Å and the one which followed [143] at a higher resolution (2.0 Å) revealed the presence of multiple dehydrated K^+ ions coordinated by main chain carbonyl groups of the selectivity filter (Figure 3). Four ion-binding sites within the selectivity (S1 to S4) and a single site in the central cavity were identified in the initial structure. The first three sites are made of exclusively main-chain carbonyl oxygen atoms. The fourth site, closest to the intracellular side, is made of four main-chain carbonyl oxygen atoms and four threonine side-chain hydroxyl oxygen atoms. The central water-filled cavity holds a K^+ ion coordinated by eight water molecules, ready to enter the selectivity filter which is also thought to be stabilised at that particular position by the negative dipole of the protein helices. Two extra sites at the extracellular side of the channel were observed in the higher resolution structure: SEXT and S0. SEXT is likely to be surrounded by bulk water and is on the extracellular side of S0. Site 0 is formed by four oxygen atoms from the carbonyls of residue Gly79 and bulk waters. At high concentration of K^+ ions, all four sites S1-S4 are equally occupied on average. Rapid switching between configurations of ions in sites S1/S3 and S2/S4 was proposed and later predicted by simulation studies where a relatively flat energy landscape was revealed [48]. As a K^+ ion enters the filter from the bulk solution where it is solvated by water molecules, this hydration shell is replaced by oxygen atoms of the backbone carbonyl groups and threonine sidechains at site S4. Therefore, the coordination number of the K^+ ions in the selectivity filter is made up of eight oxygen atoms from the protein, arranged at the corners of a cube with the ion at its centre.

Detailed calculations of the energetics of ion permeation through the selectivity filter have provided a picture of the mechanism of permeation in good agreement with experimental results and encouragingly, newer simulations using the high-resolution structure supported a number of conclusions derived from simulations based on the lower resolution structure.

Selectivity sequences for permanent ions through KcsA have been determined by symmetrical solution conductance and by reversal potentials under bi-ionic or mixed-ion conditions [144]. These and other experiments [145] suggest that Rb^+ is permeant (albeit with an approximately fourfold lower conductance than K^+ at 200 mM) and is also a strong promoter of channel opening. Cs^+ behaves primarily as a blocker, having an ionic conductance two orders-of-magnitude lower than that of K^+ [146]. Free energy calculations have also predicted, in terms of transition state theory, a permeability ratio for K^+ over Na^+ of $100 - 1000$ whereas that over Rb^+ was only around 20, in agreement with experimental values [48].

It is of particular interest to probe some aspects of ion conduction and selectivity by comparing the behavior of the alkali cation series. K^+ ions permeate readily through K^+ channels. These channels exclude the smaller alkali metal cations Li^+ (radius 0.60 Å)

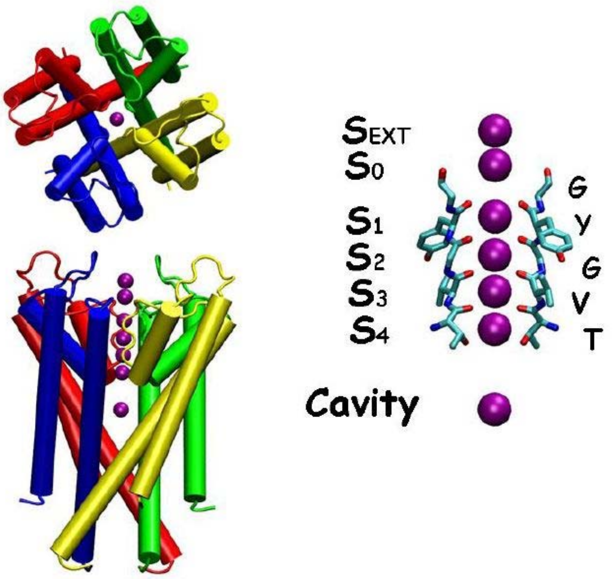

Fig. 4 Schematic diagrams showing the topology of KcsA with the transmembrane helices represented by cylinders and K+ ions by purple spheres. Top left figure shows the arrangement of all four subunits looking from the extracellular side directly down through the pore. Bottom left figure shows a cross-sectional view through the channel. Right figure shows and schematic structure of the cation binding sites in the selectivity filter of KcsA channel, TVGYG motif. Only two subunits are shown for clarity. The extracellular side is on the top and the intracellular side is at the bottom. Binding sites are labelled SEXT, S0-S4 and cavity.

and Na^+ (0.95 Å) but allow permeation of the larger members of the series Rb^+ (1.48 Å) and Cs^+ (1.69 Å). In fact, Rb^+ is nearly a perfect K^+ (1.33 Å) analog because its size and permeability characteristics are very similar to those of K^+ and its permeation rates are usually comparable to those for K^+. In classical terms, both Rb^+ and Cs^+ have a lower permeability due to steric repulsion and Li^+ and Na^+ are not fully stabilized in the selectivity filter because of their small size. Some other divalent ions such as Ba2+ block the channel. One recent computational study considered the behaviour of the selectivity filter of the high-resolution KcsA structure in the presence of different cations (Na^+, K^+, Rb^+, Cs^+) [138].

In simulations where the lower resolution KcsA model was used [138], a major difference with previous ones was the inclusion of water molecules which were buried between the back of the selectivity filter and the remainder of the protein in the X-ray structure. These water molecules H-bond to the amide nitrogen of G^{79} and participate in a hydrogen bond network between residues G^{79} and D^{80} and they can be considered 'structural waters' as they showed comparable mobility to the surrounding protein and did not exchange with water molecules within the filter or the central cavity. Simulation data also showed that the two most readily permeant ions, K^+ and Rb^+, had similar interactions with the selectivity filter. In contrast, Na^+ ions tended to distort the filter by binding to a ring of four carbonyl oxygens and the larger Cs^+ ions resulted in a small degree of expansion of the filter relative to the X-ray structure. Cs^+ ions also appeared to interact differently with the gate region of the channel, showing some tendency to bind within a predominantly hydrophobic pocket.

Recently, experimental studies were described in the literature where the absolute ion occupancy of the selectivity filter of KcsA was explored by crystallizing KcsA in Tl^+ instead of K^+ [147]. Tl^+ was used because it permeates these channels, it has a similar radius to that of K^+ and it offers distinct crystallographic advantages such as a strong diffraction signal and a certain anomalous signal at particular wavelengths. By comparing the electron density peaks of K^+, Rb^+ and Cs^+ to those of Tl^+ and using Tl^+ occupancy as a reference, the occupancies of K^+, Rb^+ and Cs^+ were estimated. For K^+, Rb^+ and Cs^+ the total number of ions contained within four sites in the selectivity filter at a given time is about two and that at low concentrations the number of ions drops to one. At high concentrations, the protein structures in Rb^+ and Cs^+ were very similar to the high-K^+ or Tl^+ except that there were only three major occupied positions in the selectivity filter instead of four. The authors suggested that the permeating cations serve as "counter-charges" to the partial negative charges of the carbonyl oxygens of the protein and therefore, they are required to maintain the structural stability of the filter. The filter structure was found to change as a function of the concentration and ultimately, a lack of these ”counter-charges” results in conformational changes in the selectivity filter as already described in simulation studies.

Mutations of the threonine residue to cysteine in the selectivity filter were also carried out [148] to characterize its influence on the conduction of the channel and the distribution of ions in the selectivity filter. In the wild-type channel, configurations S1/S3 and S2/S4 are nearly energetically equivalent as described before and the effect of the mutation is to shift the equilibrium distribution from S2/S4 to S1/S3. However, the mutation was found to influence not only the conduction but also the total occupancy of K^+ at sites S2 and S4 without much change at sites S1 and S3. In contrast, it did not affect the conduction of Rb^+ but this result can be understood if one considers that configurations S1/S3 and S2/S4 are not as energetically similar for Rb^+ as they are for K^+.

Calculations aimed at exploring the basis of selectivity are becoming increasingly more sophisticated and informative. Novel experimental data is also becoming available for nonselective cationic channels, such as the NaK channel [149], which apparently is able to conduct both K^+ and Na^+. Theoretical studies comparing the features of such systems would be more than welcomed. The reader is referred to a recent scholarly article by Noskov and Roux [150].

8 Free Energy Calculations

Classical molecular dynamics simulations alone are not sufficient to provide quantitative conclusions about the fine balance between the energies of ion-protein, ion-water interactions and the helix dipoles of the protein or about the occupancy of the various binding sites of the channel because the average time required for permeation of an ion is typically much longer than the times that can be afforded currently with MD. Therefore, energetic problems associated with ion permeation have been addressed by means of free energy perturbation (FEP) [47] and potential of mean force (PMF) [48] approaches which are two of the many powerful computational techniques available to quantitatively characterize biomolecular systems. Both methods involve extensive sampling of the configurational space of the system but the main difference is that the free energy is evaluated using unphysical paths in FEP while the system moves along a physical path in the PMF approach. Besides, the potential surfaces of the initial and final states of the model system are connected via a set of intermediate mapping potentials in the FEP protocol whereas in the PMF protocol, the probability function is directly evaluated for the system along selected reaction coordinates and an umbrella-sampling method is used where a special biasing potential guides the system along the reaction coordinates providing better sampling of the relevant configuration. Later, the bias introduced by the potential is removed after analysis to characterize the unbiased free energy surface of the system.

Bernèche and Roux performed a set of rigorous potential of mean force (PMF) calculations, arriving at similar conclusions to those of Åqvist and Luzhkov using FEP. These calculations were able to reproduce the four cation-binding sites located in the selectivity filter and they also anticipated the existence of two additional sites (S0 and SEXT) located at the extracellular sides, which were later confirmed by the high-resolution KcsA structure. The most stable configuration of the filter was found to be that with sites S2 and S4 occupied by ions and S1 and S3 occupied by water molecules. Later, the high-resolution structure confirmed what was already established by the free energy calculations. The fourth binding site showed a slight preference for sodium and the authors [151] concluded that this result seemed to be in agreement with the experimental observation that Na^+ can block KcsA from the inner side at low concentrations of this ion.

The largest free energy barrier to the conduction process was found to be of the order of $1-5$ kcal/mol, somehow surprising given the large hydration energy of K^+ ions. These values infer that the ion conduction process is essentially diffusion limited. Ion-ion repulsion was found essential for rapid conduction though acting only at very short distances. Site 2 (S2) was found to provide the strongest stabilisation of all sites while S3 was considerable less favourable.

There is only one discrepancy between both groups [152]. Åqvist and Luzhkov reported that the channel has a considerable affinity for accommodating an ion in the internal cavity. In contrast, the PMF surface infers that there is no affinity for an ion in the cavity. This means that the configuration where ions are occupying S2, S4 and there is another ion in bulk water in the extracellular side of the membrane is found to have, using the PMF approach, a lower energy than a situation where ions were at S2 and S4 and in the cavity at the same time because the cavity affinity of an S2/S4 configuration was not considered. Åqvist and Luzhkov mentioned that it is unlikely that the permeation mechanism would involve states with no ions in the cavity as suggested by the PMF study and they elegantly demonstrated the role of the internal cavity ions in the knock-on mechanism.

Later, Koskov *et al.* [153, 154]. suggested that selectivity may result from the electrostatic interactions between the carbonyl ligands of the amino acids lining the selectivity filter and the permeating ion. However, Treptow and Tarek [155] have performed free energy calculations on KcsA and Kv1.2 and have reported that clearly the immediate environment of the K^+ ions provided by the carbonyls lining the selectivity filter of potassium channels does not control alone the K^+ translocation between the up and down microstates. They summarize by saying that to a large extent, the electrostatic environment monitors this turnover, influencing the conductive properties of potassium channels.

Hellgren *et al.* [156] have recently reported simulation data where the configuration of ions in the selectivity filter does not correspond to the usual S1/S3 or S2/S4. There are many differences between the set-ups of these simulations and those reported by [157] working with the same channel. Some of the differences are the number of water molecules in the cavity of the channel, minor differences in the protonation states of the channel transmembranes, the force fields employed for protein, water and particularly for ions etc. The the force field parameters for ions is possibly the main reason for such a discrepancy. However, it is certainly judicious the authors' suggestion [156] that many more extensive simulations would be necessary to fully describe all the possible configurations of ions in this family of K channels.

Schulten *et al.* [158] have also reported movies of the overall conduction of K^+ ions through Kv1.2 by applying a series of three different electric fields (voltage bias). As suggested in previous studies, the conduction is described as concerted in the selectivity filter even if the crystal structure of the channel is in its open conformation. Permeation involves $2-3$ ions residing mainly at the sites identified previously by crystallography and modeling. Nevertheless, these simulations revealed, the jumps of ions between these sites and they identify the sequence of multi-ion configurations involved in permeation.

9 Filter Flexibility

Originally, it was proposed that the selectivity filter of KcsA was 'constrained' in an optimal geometry so that a dehydrated K^+ ion fitted with proper coordination and K^+ ions at close proximity in the selectivity filter repelled each other, overcoming the strong interaction between ion and protein and allowing rapid conduction [137]. However, the high resolution structure determined at low concentration of K^+ revealed that there was a degree of flexibility in the selectivity filter and changes in the filter conformation.

Simulations once again correlate well with structural studies and have made an important contribution to the analysis of the conformational dynamics of the selectivity filter. For instance, simulations based on the lower-resolution KcsA structure were able to describe these distortions and simulations based on the higher-resolution KcsA structure showed that when the filter became occupied by a number of water molecules instead of ions, it distorted substantially and the filter conformation was not maintained in the absence of cations. MD predicted the conformational change seen in the high resolution structure at low concentration. These distortions were described as likely to be involved in lowering the permeation activation energy barriers between adjacent sites at short timescales. At longer timescales, the distortions were pointed out to be possibly related to the rapid closures of the filter that appears to be independent of the activation of K^+ channels, a mechanism known as C-type inactivation.

Not only is there crystallographic and computational evidence in support of the flexibility of the filter but there is also electrophysiological evidence that suggests that the selectivity filter may act as another gate. A number of mutations in the vicinity of the filter region of Kir6.2 and backbone mutations of KirBac2.1 have been interpreted in terms of local changes in filter conformation related to fast gating. This phenomenon is known as C-type inactivation and is also common in voltage-gated channels. Differences between the conformation of the tyrosine side chains of the GYG motif of the selectivity filter and the P-helix conformation and sequence between the KcsA structure and KirBac1.1 can be observed. The hydrogen bond formed by the tyrosine of the selectivity filter and a tryptophan in the P-helix which seems to stabilize the filter in the KcsA structure is absent from KirBac1.1. In contrast to the crystal structure of KcsA, in the X-Ray structure of KirBac1.1, the carbonyl oxygens of residue G112 do not point directly towards the center of the pore but changes in the conformation of the carbonyls of the filter are observed in simulations. However, likewise in KcsA simulations, MD simulations of KirBac1.1 [157] provided evidence of filter flexibility and observation of occasional peptide carbonyls flipping from being directed towards the center of the pore. Two reasons were suggested to justify this effect: the carbonyl flipping seemed to correlate with the concerted translocation of the ions through the channel and/or it was due to relaxation of the filter structure. It was also possible to observe that in simulations of KirBac1.1 in the absence of ions, three of the four chains were distorted such that the valine carbonyl oxygen was directed away from the pore. Due to the fact that this behavior of the selectivity filter has been observed in a variety of simulations of different channels and homology models, it was suggested that such small changes in backbone conformation may be an inherit property of K-channel filters and that a nonfunctional filter conformation may be induced either by a transient or prolonged absence of ions from the filter or promoted by mutations in the vicinity of it.

More recently, Roux et al presented the analysis of some MD simulations of KcsA and they showed that the carbonyl groups lining the pore are indeed dynamic and it was proposed that their intrinsic electrostatic properties control ion selectivity and that selectivity for K^+ seems to emerge as a consequence of the flexible filter [153]. However, ion passage along the selectivity filter takes place at rates six to seven orders of magnitude slower than atomic motion, that is bond stretch or dihedral motion. Therefore, permeating ions are likely to see an average position of the selectivity filter atoms and it is difficult to reconcile the idea that atomic fluctuations could play a key role in the selectivity or conductivity of the channel [25]. Structural flexibility that alters the average positions of the protein atoms may play a more prominent role than the one attributed so far. Recently, a question has been posed on whether or not a gate at the selectivity filter of potassium channels exist [159]. Using free energy molecular dynamics simulations, it was shown that the reorientation of two peptide linkages in the selectivity filter of the KcsA channel could lead to a stable non-conducting conformational state. Two microscopic factors were described to influence the transition toward such a conformational state: the occupancy of one specific cation binding site in the selectivity filter (S2) and the strength of the intersubunit interactions involving the GYG signature sequence. The authors describe that such an 'inactivated' state is not caused by a physical constriction of the pore and that the structural rearrangement involves only one of the four subunits at a time.

10 Homology Modelling and mammalian potassium channels

From a physiological perspective, it would be more interesting to perform simulations of mammalian K^+ channels in complex membrane environments but due to the lack of eukaryotic structures other routes ought to be pursued. Given the high sequence identity among K^+ channels, current studies could be extended by using crystallographic coordinates of the already solved structures as templates in combination with sequence alignments between the template and the target for constructing comparative models to aid in the interpretation of experiments [160]. An optimal sequence alignment is essential for the success of the homology modelling with percentage of sequence identity between the template and the model superior or equal to 40%. Verification and refinement by comparison with experimental data (mutagenesis studies mainly) lead to accurate homology models. There have been several studies with homology models described in the literature of inward rectifier channels (Kirs) [161], Kv channels, especially the Shaker Kv channel [162], or those who belong to the superfamily of the ligand-gated ion channels [163] such as the nicotinic acetylcholine receptor (nAChR) complementing experimental work or as pure theoretical exercises. Such models have been used to examine the relationship between structure and function in mammalian channels, in studies of channel gating and to aid the design of drugs that selectively block these channels, providing some understanding of human membrane protein on the basis of bacterial homologues.

11 Protein/Lipid interactions

Another important aspect of potassium channel biophysics is the description of protein/lipid interactions. Interactions with the membrane lipids are essential to the structure and stability of ion channels and membrane proteins in general [164]. Building on earlier simulation studies of pure lipid bilayers (reviewed in e.g. [165, 166]), MD simulations have been extended to increasingly complex membrane proteins [167–170]. These simulations are able to provide insights into the nature of the interactions between membrane proteins and their lipid environment [168, 171–174] and analysis of those crystal structures of membrane proteins that contain lipids provides a detailed structural perspective on lipid/protein interactions [56, 175]. A number of experimental studies have also revealed the importance of bound lipid molecules for the stability and function of some membrane proteins [176–180] For example, in the case of the K^+ channel KcsA, acidic phospholipids appear to bind to specific (non-annular) sites at which they play a role in refolding and possibly in function [181–183].

Examples of recent computational work aiming to understand these interactions are the comparative studies [184, 185] of simulations of two integral membrane proteins, representing the two main classes of such protein an α-helical membrane protein (KcsA) vs a β-barrel protein (OmpA) or the comparison of the behaviour of a membrane protein in a lipid bilayer and a detergent micelle environment [186]. These studies have revealed for example the importance of amphipathic aromatic residues (Trp and Tyr) at membrane/water interfaces [187, 188]. They have also revealed the importance of 'snorkelling' interactions of basic sidechains with the phosphate groups of lipids indicating that this mechanism is likely to be crucial for a range of membrane proteins and can involve both lysine and arginine sidechains. More recently, molecular dynamics simulations have been used to unmask details of specific interactions of anionic phospholipids with intersubunit binding sites on the surface of the bacterial potassium channel KcsA [189]. Crystallographic data on a diacyl glycerol fragment at this site were used to model phosphatidylethanolamine (PE), or phosphatidylglycerol (PG), or phosphatidic acid (PA) at the intersubunit binding sites. H-bond analysis revealed that in terms of lipid-protein interactions PA $>$ PG $>>$ PE and revealed how anionic lipids (PG and PA) bind to a site provided by two key arginine residues (R(64) and R(89)) at the interface between adjacent subunits. The phosphate oxygen atoms of bound PG formed H-bonds to the guanidinium group of R(89), whereas the terminal glycerol H-bonded to R(64). In some other study [190], the role of the transmembrane aromatic residues of the KirBac1.1 potassium channel was proposed as a means of directing and stabilising the conformational changes during conformational transitions between the open and closed models, e.g., the gating of the channel. These aromatics residues shifts from an even distribution in the closed state toward the membrane/solute interface in the open state model. Recently, a comparative study of inwardly rectifying potassium channels has been reported [191] where analysis of lipid-protein interactions emphasizes the key role of the slide helix of these channels, which lies approximately parallel to the bilayer-water interface and forms a link between the transmembrane and intracellular domains of the channel.

Experimental work showed that the functional properties of the mechanosensitive channel of large conductance (MscL) are affected by variations in lipid composition [192–194]. MD has also been used to investigate the effects of such lipid compositions on the protein at a molecular level. Two sets of simulations were performed. In the first, trajectories using lipids with different headgroups (phosphatidylcholine and phosphatidylethanolamine) were compared. Protein-lipid interactions were clearly altered by the headgroup changes, leading to conformational differences in the C-terminal region of MscL. In the second set of simulations, lipid tails were gradually shortened, thinning the membrane over a molecular dynamics trajectory. These simulations showed evidence of hydrophobic matching between MscL and the lipid membrane, as previously proposed. For all simulations, protein-lipid interaction energies in the second transmembrane region were correlated to mutagenic data, emphasizing the importance of lipid interactions for proper MscL function.

To date, we do not have yet that many answers regarding many aspects of the interplay between proteins and lipid molecules but it is certain that MD simulations are able to reveal both specific and non-specific interactions of membrane protein components with their lipid bilayer environment. More structural, physiological and computational studies are required to reveal new principles concerning processes such as membrane fusion [195] or the implications and effects of lipid composition in protein/lipid interactions in the modulation of the gating equilibrium. Computational studies of protein-lipid interactions in the K-channel family have started to emerge using atomistic simulations [196] which help in obtaining a detailed understanding of lipid-protein interactions at atomic resolution. However, coarse grain simulations will certainly also place a central role. These studies are extremely important, since only limited data on lipid-protein interactions might be obtained from structural data. The reader is referred to [197–199] and references within for a detailed description of this topic.

12 Future Prospects

Despite the first MD simulations of biological systems being performed as long ago as the late seventies, it is only in the last decade that accurate force-fields and treatment of electrostatic interactions have been available, sufficient computational resources have lead to adequate sampling of the thermal fluctuations of realistic atomistic models and the characterisation of the free energy landscape governing events such as permeation. Nevertheless, at present, the greatest limitation of molecular dynamics is the computational power requirements leading to sampling issues and limitations on the description of long time scale events. In addition, direct estimation of conductance is not possible and therefore direct comparison with experimental data is impeded. However, with the continuous increases in high-computing technology and the sequencing of the genomes of many organisms, biomolecular modelling and computational approaches are a key bridge between the atomic level pictures provided by experimental techniques such as X-Ray crystallography, NMR or EM and the dynamics and mechanisms underlying important cellular processes which lead to their function.

Nevertheless, there has not yet been a quantitatively successful description of ion permeation that predicts electrophysiological properties without the need of arbitrary parameters which rely on experimental data. Progress in understanding more complex systems is envisaged to be obtained by combining multiple computational methods in a coordinated way with data provided by a numerous variety of experimental approaches. It seems likely that comparisons between multiple MD simulations of related systems will become of increasing biological importance, suggesting a need for databases, which are already starting to emerge, in which to store the results of simulation studies in an accessible form.

To date, most simulations studies have been of single bacterial potassium channels in simple model membranes. From a structural and physiological perspective, the major challenge lies in determining high-resolution structures of a wider range of mammalian channel families; one would like to perform simulations of models of mammalian K channels in complex membrane environments. It would also be interesting to move from simple models of channel structure to systems where many models are integrated improving the realism of the biological environment present in the simulation and where details of protein structure, dynamics and energetics could be analysed in a wider context.

Although there have been considerable advances in this field, much remains to be done and from a computational point of view, one of the major challenges is to extend the method to longer time and length scales in order to be able to simulate processes such as gating, calculate free energies of different channel states and their interconnecting activation barriers and study the thermodynamics of blocker binding.

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