

## Minireview

## Ion channel gating: insights via molecular simulations

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**Abstract** Ion channels are gated, i.e. they can switch conformation between a closed and an open state. Molecular dynamics simulations may be used to study the conformational dynamics of ion channels and of simple channel models. Simulations on model nanopores reveal that a narrow ( $<4$  Å) hydrophobic region can form a functionally closed gate in the channel and can be opened by either a small ( $\sim 1$  Å) increase in pore radius or an increase in polarity. Modelling and simulation studies confirm the importance of hydrophobic gating in K channels, and support a model in which hinge-bending of the pore-lining M2 (or S6 in Kv channels) helices underlies channel gating. Simulations of a simple outer membrane protein, OmpA, indicate that a gate may also be formed by interactions of charged side chains within a pore, as is also the case in CIC channels. © 2003 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

**Key words:** Ion channel; Molecular dynamics; Gating; Pore; Outer membrane protein; Nanopore

## 1. Introduction

Ion channels enable rapid ( $\sim 10^7$  ions  $s^{-1}$ ) movement of selected ions through pores in biological membranes. They play a key role in the membrane physiology of excitable and other cells [1]. Ion channels are *gated*, i.e. they can switch between open and closed conformations of the channel, only the former allowing ions to flow. Recent spectacular advances in the structural biology of ion channels (reviewed in [2]) have provided crystallographic snapshots of ion channels in their closed (KcsA [3]; KirBac [4]; nAChR [5]; MscL [6]) and, more rarely, their open (MthK [7]; KvAP [8]; MscS [9]) states. However, protein crystallography yields a time- and space-averaged view of a protein molecule, usually at a temperature of  $\sim 100$  K. In order to fully understand the physico-chemical basis of channel function, some insight into the dynamics of ion channel proteins at  $\sim 300$  K in a membrane environment is required.

Molecular dynamics (MD) simulations have been used for over 25 years to study the dynamics of proteins [10]. Early simulations were limited to rather short time scales and small

proteins. Advances in simulation procedures and computer technology mean that time scales in excess of 10 ns (i.e. equivalent to the mean permeation time of an ion through a channel) are feasible for large systems such as a membrane protein embedded in a lipid bilayer with several thousands of water molecules on either side of the membrane. Such simulations have been used to some effect to study the conformational dynamics of ion channels [11–20] and of related pore-forming proteins, e.g. aquaporins [21,22], and the relationship of the dynamic properties to biological function. The purpose of this brief review is to highlight some of the insights into ion channel gating that have emerged from simulation and modelling studies within our laboratory.

## 2. Model nanopores

In many ion channels the gate seems to be formed by a hydrophobic constriction which is presumed to form a barrier to ion permeation in the closed form of the channel. For example, in the crystal structures of KcsA, KirBac, MscL and the nAChR the closed conformation gate has a radius of 1.3 Å, 0.5 Å, 1.7 Å and 3.1 Å respectively. It is therefore of interest to ask whether a gate has to be completely physically occluded (i.e. radius substantially less than the radius of e.g. a  $Na^+$  ion (0.95 Å) or of a water molecule ( $\sim 1.4$  Å) in order for a gate to be functionally closed. The structure of the nAChR in particular, with a gate radius of  $\sim 3$  Å, suggests that physical occlusion is not essential for a functionally closed state. This being the case, we wish to explore how the functional state of a gate depends upon its geometry (i.e. gate radius) and its polarity. These aspects may be explored via MD simulations of a radically simplified model of a gated ion channel.

In one such model (see Fig. 1A) a cylindrical hydrophobic nanopore of pseudo-atoms surrounds a cavity that opens towards the bulk volume at both ends in a funnel shape, thus creating two symmetric mouth regions. This pore is embedded in a slab of pseudo-atoms that mimics a membrane. The pseudo-atoms have the characteristics of methane molecules. Each atom was held at its initial position by a restraining force such that its RMSD fluctuations were comparable to those seen in full membrane protein simulations. Thus the model nanopore exhibited flexibility and surface roughness comparable to that in the gate region of an ion channel. To generate more hydrophilic pores pseudo-atoms within the pore had partial charges placed on them to generate dipole moments in the gate region of comparable magnitude to those

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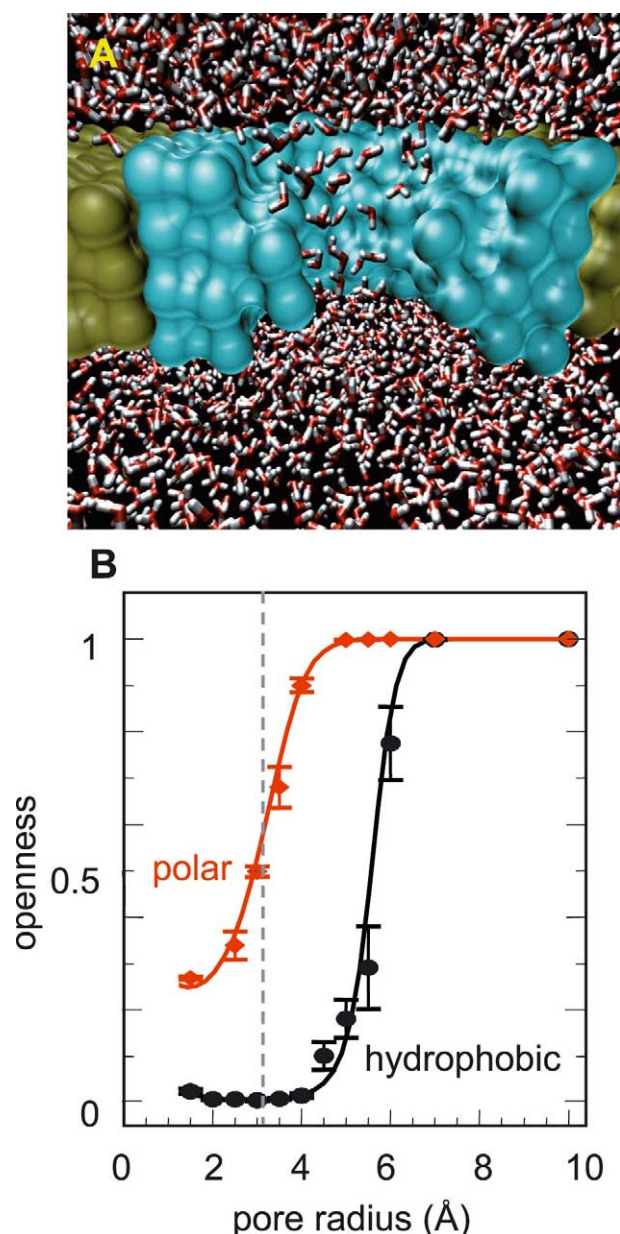


Fig. 1. Hydrophobic gating in a model nanopore [23,24]. A: A simple model of a nanopore (cyan), embedded in a membrane-mimetic slab (gold), with water molecules (red/white) on either side and within the pore. B: Pore openness v. radius for a hydrophobic nanopore (black) compared with a nanopore with a polar lining (red). The simulations ranged from 52 to 164 ns in length. The polar lining was formed by two dipoles of magnitude 1.52 Å aligned parallel to the pore lining. The vertical broken line represents the pore radius in the gating region of the (closed) nAChR.

that would be formed by e.g. a polar amino acid side chain or backbone region (see Fig. 1 caption for details).

As a first approximation to characterising gating we have used simulations to examine the behaviour of water molecules in the model nanopores as a function of pore geometry and polarity [23,24]. Multiple simulations were performed, ranging in duration from ~50 to ~160 ns, corresponding to a total simulation time of ~1.6 μs. The ‘openness’ of a channel model was defined as the fraction of simulation time the pore was filled with water. Thus a channel fully occupied by water throughout a simulation would have an openness of 1,

whereas a channel that remained empty of water would have an openness of 0.

For purely hydrophobic pores gating depends critically on the pore radius (Fig. 1B). The channel (length 16 Å) is closed at small pore radii (<4.5 Å). A hydrophobic pore can be switched from closed to open by increasing its radius by as little as 1 Å. Increasing the hydrophilic character of the pore can also open a small radius pore (Fig. 1B). Thus a closed hydrophobic channel can be opened by a modest increase in either radius and/or the surface charge density of the pore lining. As can be seen by comparison with e.g. the nAChR, these changes in radius and polarity are comparable to those which are thought to occur on gating in ion channel proteins.

In simulations at pore radii ranging from 3.5 Å to 10 Å we have examined the kinetics of oscillations between a liquid-filled and a vapour-filled pore. This behaviour can be explained as capillary evaporation alternating with capillary condensation, driven by pressure fluctuations in the water outside the pore. The free energy difference between the two states is a quadratic function of the radius, as predicted by a simple thermodynamic model (Beckstein and Sansom, in preparation). The free energy landscape shows how a metastable liquid state gradually develops with increasing radius. For radii larger than c. 5.5 Å it becomes the globally stable state and the vapour state vanishes. Thus, we can start to employ simulations to understand the thermodynamic basis of channel function and gating.

These studies provide a proof of principle of hydrophobic gating, with an increase in either gate radius or polarity leading to opening. This correlates nicely with current gating models for e.g. the nAChR [5,25] and of K channels [26]. However, the nanopore model is radically simplified relative to a real channel structure. We have therefore started simulations of the pore region of the nAChR, based on the recent electron microscopic structure of the pore domain of this protein [5]. Preliminary results (from a 60 ns simulation of water in the nAChR pore domain embedded in a membrane-mimetic slab; Beckstein and Sansom, unpublished data) suggest a hydrophobic gate is present. However, further simulation studies of water and ions in the nAChR pore are needed.

### 3. K channels

The structures of several bacterial K channels are now known (KcsA, MthK, KirBac, KvAP [3,4,7,8,27]) from protein crystallography, and all share a common pore topology. The pore is tetrameric, with four copies of a M1-P-M2 motif (or S5-P-S6 in Kv channels) around the central pore axis. The P loops contribute the extended region of polypeptide chain that contains the TVGYG sequence motif and form the selectivity filter. This filter is the region that interacts closely with permeant ions. It forms a narrow region of the channel that is lined by oxygen atoms and is sufficiently flexible to allow rapid hopping of ions between adjacent binding sites yet sufficiently rigid to allow discrimination between K<sup>+</sup> and Na<sup>+</sup> ions. It has been the subject of numerous simulation studies [11–20] and will not be discussed further here. The interested reader is referred to recent reviews [28–31].

The first K channel structure (of KcsA) seems to correspond to a closed state of the channel. The M2 helices are packed together at their C-termini to form a narrow (radius ~1.3 Å) and hydrophobic intracellular mouth to the channel.

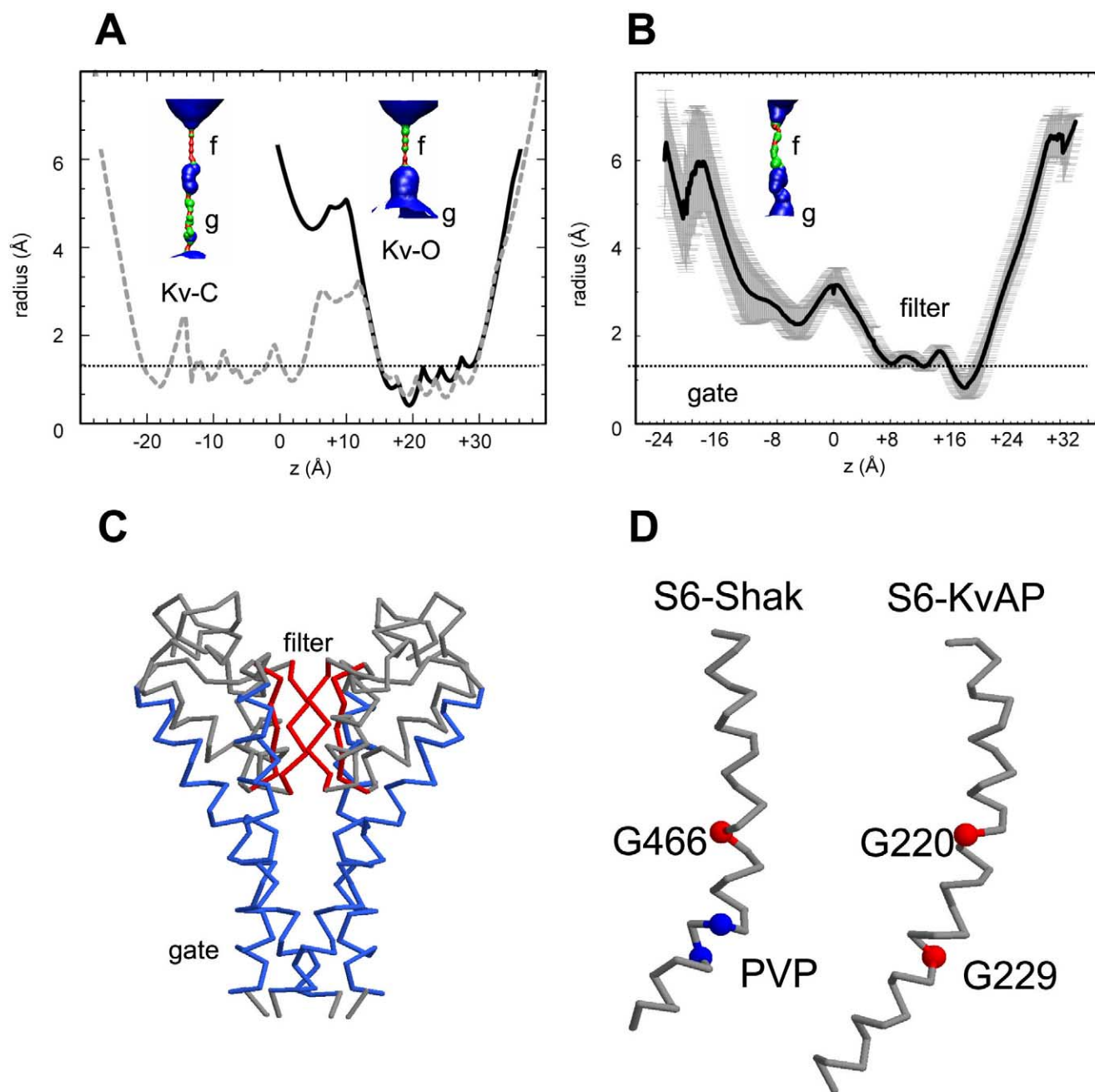


Fig. 2. Gating in Kv (Shaker) channel models. A: Pore radius profiles (calculated using HOLE [66]) for Shaker Kv pore models in the closed (grey, broken line) and open (black, solid line) conformations. The insets show the pore-lining surface for the closed (Kv-C) and open (Kv-O) models, based on KcsA and KvAP respectively. B: Pore radius profile averaged (solid black line  $\pm$  S.D.) over the duration of a 5 ns simulation of a Shaker Kv (S5-P-S6)<sub>4</sub> model embedded in an octane slab [43]. The locations of the presumed intracellular gate and filter regions are indicated. C: Shaker Kv pore model in the closed state (based on KcsA). Only the P loop and S6 helices are shown for clarity. The filter is shown in red and the S6 helices in blue. D: Comparison of the conformations of the S6 helix from a model of the Shaker pore domain in an open state (i.e. at the end of the 5 ns simulation in B) and from the crystal structure of KvAP. The  $\alpha$  atoms of the conserved glycine residues (G466 in Shaker; G220 in KvAP) and of the prolines of the PVP motif (in Shaker) or the corresponding glycine (G229) in KvAP are shown as spheres.

A number of computational approaches [32–34] have indicated that this region forms a significant energy barrier to ion permeation and thus might be considered to be the gate region of the channel.

A combination of spectroscopic [35–37] and simulation [18,38] studies suggested that movement of the M2 helices to widen the intracellular mouth of the pore might correspond to the gating mechanism of KcsA. Steered MD simulations in

which the M2 helices in the gate region were slowly forced apart [39] supported this hypothesis. The latter simulations also suggested that the M2 helices might not move entirely as rigid bodies, but might bend slightly during the opening of the pore.

The crystal structure of a Ca-activated K channel (MthK) captured in its open state confirmed these proposals [7,26]. In particular, the structure of MthK revealed the importance of a



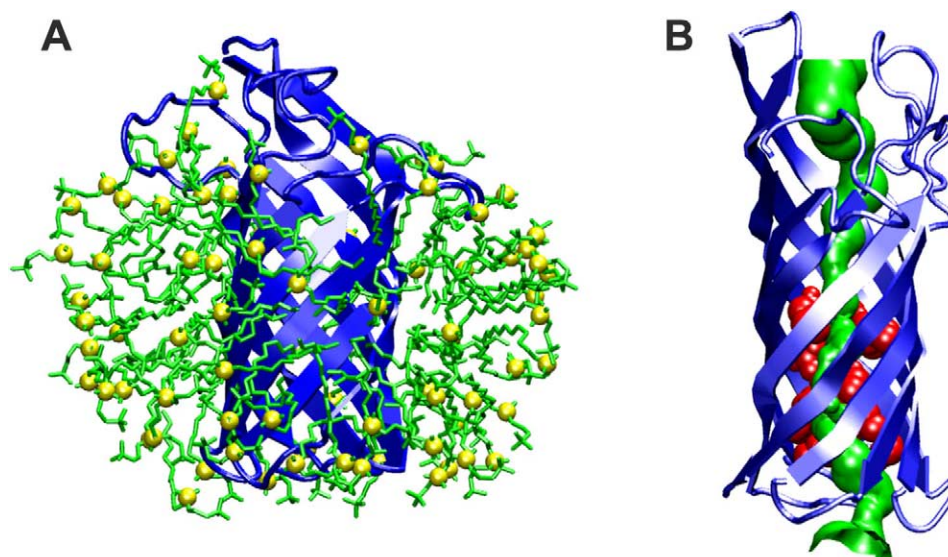


Fig. 3. Salt bridge-mediated gating in OmpA. A: OmpA (blue) in a DPC (green/yellow) micelle structure from a 10 ns MD simulation [57]. B: Pore-lining diagram of an open state of OmpA extracted from the simulation of a OmpA/DPC micelle. The green surface represents the lining of the pore, and the red side chains are those responsible for constriction/gating of the pore.

conserved glycine residue midway along the M2 helix as a potential molecular hinge. In the closed state of the channel (exemplified by KcsA) the M2 helix is relatively undistorted whereas in the open state (as in MthK) the M2 helix is kinked in the region of the conserved glycine. It is of interest that molecular hinges within a pore-lining  $\alpha$ -helix had been proposed as gating mechanism for nAChR [5,25] and for the S6 helix of Kv channels [40,41] (see below).

Homology modelling and simulations have been used to compare a model of the transmembrane domain of KcsA in an open state with the same domain of KcsA in a closed state [34]. Born energy calculations suggested that the intracellular hydrophobic gate in the closed state provides a barrier of height  $\sim 5$  kT to monovalent cation permeation whilst in the open state the barrier was absent. MD simulations (10 ns duration in an octane slab – a simple membrane mimetic) revealed that the closed and open state models were of comparable conformational stability. Substantial conformational fluctuations were observed in the intracellular gate region during both the closed state and the open state simulations. In the open state simulation rapid (i.e. within  $\sim 5$  ns) exit of all three  $K^+$  ions initially within the pore occurred through the intracellular mouth. The M2 helix was observed to undergo kink and swivel motions about the molecular hinge formed by residue G99.

The structure of a bacterial Kv channel (KvAP) also apparently in an open state [8] reveals the S6 helix (the Kv homologue of M2) to be kinked so as to open the intracellular gate. The situation in Kv channels from higher organisms may be a little more complex. These contain a highly conserved PVP motif a few residues C-terminal to the conserved glycine. A number of simulation [42,43] and structural bioinformatics [44] studies have indicated that proline-containing motifs can form dynamic hinges in transmembrane helices, and experimental studies [45] indicate that the S6 helix may be kinked in the Kv channel in the vicinity of the PVP motif. We have therefore used model building and simulation studies to investigate the conformational dynamics of the S6 helix in

Kv channel models and the possible consequences for channel gating.

First, let us consider the pore dimensions for Kv channel (i.e. S5-P-S6) models built in a closed state (i.e. using KcsA as a template) and in an open state (using KvAP as a template). Comparison of pore radius profiles for the two models (Fig. 2A) shows that in the closed state the pore radius is less than 1.3 Å (the radius of a  $K^+$  ion) in the gate region, and lined by hydrophobic side chains. In contrast, this region has a radius  $> 6$  Å in the open state and so will not present a barrier to ion permeation.

MD simulations have been used to explore the conformational dynamics of the S6 helix hinge in models of a fragment of a Kv channel, namely an (S5-P-S6)<sub>4</sub> tetramer. The latter is a model of the complete pore-forming domain of a Kv channel. The model was embedded in an octane slab (a simple membrane mimetic) and 5 ns duration MD simulations were performed. Averaging the pore radius profile over the duration of the simulation (Fig. 2B) shows that the gate region opens to some extent, and the final structure from the simulation seems to correspond to a (partially) open state of the channel. This change in channel state during the simulation correlates with indications that the PVP motif forms a molecular hinge. The conformational dynamics of S6 are modulated by the remainder of protein relative to simulations of isolated S6 helices [41] but it remains flexible. These simulation results suggest a channel gating model in which S6 bends in the vicinity of the PVP motif in addition to the region around the conserved glycine (G466) that is N-terminal to the PVP motif. Thus, K channel gating may depend on a complex nanoswitch with three rigid helical sections linked by two molecular hinges.

#### 4. OmpA

Not all channels share a similar architecture and not all gates are based upon hydrophobic gating. We have studied a different class of pores in the case of the simple bacterial

outer membrane protein OmpA (Fig. 3). The biological role of OmpA in the outer membrane of *Escherichia coli* remains a little unclear. It has a structural role linking the outer membrane to the periplasmic peptidoglycan and the extracellular loops are involved in recognition events underlying e.g. bacterial conjugation and pathogenesis. Furthermore, a number of studies suggest that OmpA and closely related proteins [46–51] can form ion-permeable pores when inserted in lipid bilayers, and that these pores switch between closed and open states (on a relatively long time scale, i.e.  $\sim 100$  ms).

The structure of OmpA has been determined by both protein crystallography [52,53] and nuclear magnetic resonance (NMR) of OmpA/detergent micelles [54,55]. The crystal structure (at 1.65 Å resolution) seems to correspond to a closed state of the channel, as the potential pore through the centre of the eight-stranded  $\beta$ -barrel fold is interrupted in a number of places by side chain–side chain interactions within the barrel. The NMR structure has been suggested to reveal a more flexible state of OmpA.

In order to explore possible gating mechanisms of the OmpA channel molecular modelling and MD simulations were used to explore the conformational dynamics of the protein, in particular the possibility of transient formation of a central pore [56]. These studies suggested a gating mechanism involving isomerisation of Arg138, a side chain in the barrel interior which in the X-ray structure forms part of a pore-blocking salt bridge, such that it formed alternate salt bridges with Glu52 (closed state) and Glu128 (open state). A simulation in which Arg138 formed a salt bridge with Glu128 resulted in a functionally open pore, with a predicted conductance similar to experimental measurements.

To further explore the conformational dynamics of OmpA multiple 10 ns duration MD simulations were run to compare the behaviour of OmpA in a detergent micelle and in a phospholipid bilayer [57]. The dynamic fluctuations of the protein structure were  $\sim 1.5$  times greater in the micelle environment than in the lipid bilayer. As a consequence of the enhanced flexibility of the OmpA protein in the micellar environment, side chain torsion angle changes resulted in spontaneous formation of a continuous pore through the centre of the OmpA molecule. These side chain conformational changes may be coupled to changes in extracellular loop conformations (Hall, Bond, Biggin and Sansom, unpublished data) and thus could explain the experimentally observed channel formation by OmpA. We suggest that similar conformational changes may occur when the protein is present in the (more viscous) lipid bilayer environment, but on a substantially longer time scale.

Recently we have extended our simulations to other small outer membrane proteins such as OmpX [55] and OpcA [58]. In both of these cases, open transbilayer pores can be formed by dynamic changes in conformation of the loops on the extracellular surface of the protein (Bond, Cox and Sansom, unpublished data). This suggests that pore formation by the small outer membrane proteins may be more common than has been previously assumed, and that such channels may be gated by changes in the interaction of loops with extracellular components.

## 5. Conclusions

Simulation studies have provided proof of principle of hydrophobic gating, revealing that such a gate may be opened

either by expansion and/or by an increase in the polarity of the side chains located in the narrowest region of the gate. Such a gating mechanism is compatible with the structure of the (presumed) gates in a number of channels, including K channels, the nAChR and the mechanosensitive channel MscL. Further simulation studies are needed to establish the energetic cost of opening a hydrophobic gate, and the change in free energy profile for a permeant ion on switching from a closed to an open state in simple nanopore models.

A combination of simulation and modelling studies have provided an insight into the mechanics of gating in K channels. In particular, such simulations suggest that multiple molecular hinges may be present in the pore-gating S6 helices of Kv channels. Further simulations of hinged transmembrane helices are needed, in order to understand the energetics of helix bending and to enable us to construct more coarse-grained ('mechanical') models of ion channel in order to understand how pore-gating is coupled to conformational changes elsewhere in the protein.

Modelling and simulation studies of a 'simple' bacterial outer membrane protein (OmpA) suggest that channel gates need not always be hydrophobic. In OmpA the gate seems to be formed by a salt bridge that blocks the channel. This shows some similarities to the gating mechanism of CIC chloride channels [59] where a pore-blocking glutamate side chain changes conformation during gating.

So far simulation studies have focussed on the gate per se, rather than the mechanism of coupling conformational changes elsewhere in the channel protein (changes induced by e.g. ligand binding or a change in transmembrane voltage) to the gating event. Analysis of the mechanism of such coupling will prove challenging. However, studies on e.g. glutamate receptors suggest that a combination of protein crystallography [60–63] with molecular simulations and related computational studies [64,65] will be central to understanding such events that lie at the heart of the physiological function of ion channels.

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