Molecular simulation studies of hydrophobic gating in nanopores and ion channels

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Abstract

Gating in channels and nanopores plays a key role in regulating flow of ions across membranes. Molecular simulations provide a 'computational microscope' which enables us to examine the physical nature of gating mechanisms at the level of the single channel molecule. Water enclosed within the confines of a nanoscale pore may exhibit unexpected behaviour. In particular, if the molecular surfaces lining the pore are hydrophobic this promotes de-wetting of the pore. De-wetting is observed as stochastic liquid–vapour transitions within a pore, and may lead to functional closure of a pore to the flow of ions and/or water. Such behaviour was first observed in simulations of simple model nanopores and referred to as 'hydrophobic gating'. Simulations of both the nicotinic acetylcholine receptor and of TWIK-1 potassium channels (the latter alongside experimental studies) suggest hydrophobic gating may occur in some biological ion channels. Current studies are focused on designing hydrophobic gates into biomimetic nanopores.

Introduction

Ion channels and protein (nano)pores play a key role in membrane biology, allowing rapid movement of ions and/or water molecules across cell membranes. The key properties of these membrane protein pores are: permeation (at near diffusion limited rates, i.e. ~1 ns^{-1}); selectivity (e.g. anions compared with cations; or K^+ compared with Na^+); and gating. Structural and biophysical studies have provided detailed insights into the mechanisms underlying these properties. Gating is the switching of a channel between an 'open' state which enables permeation and a 'closed' sate which prevents permeation. Gating may be regulated by ligand binding to a receptor site on a channel protein and/or by changes in the voltage across a cell membrane.

Biomolecular simulations have been used to study the mechanisms of channel permeation, selectivity and gating at the single molecule level *in silico* [1–4]. The structure of a channel or pore protein is computationally embedded in a model of a lipid bilayer, solvated with water and ions, and then MD simulations are used to predict the dynamic behaviour of the channel protein and its interactions with ions, water, and lipid molecules. In this way, it is possible to 'transplant' the crystal structure of a protein (a temporal and spatial average structure determined at a temperature of ~100 K) into a lipid bilayer environment, and to explore its single molecule dynamics under near physiological conditions. This approach has yielded a number of insights into the relationship between channel structures and function.

One possible mechanism for channel and pore closure is 'hydrophobic gating' (also sometimes known as a 'vapour lock' mechanism). In the hydrophobic gating mechanism, the closed state of the channel is not physically occluded, but the central pore is sufficiently hydrophobic and narrow to energetically exclude water molecules and ions (Figure 1A). The aim of the present article is to review simulation studies of hydrophobic gating in simple model systems, to relate this model to recent computational and experimental studies of TWIK-1 potassium channels and to describe how our understanding of hydrophobic gating may be employed in computational design of biomimetic nanopores.

Simple model systems

The concept of hydrophobic gating was developed in simulation studies of simplified models of nanopores [5–7], and in related studies of carbon nanotubes [8]. These demonstrated that a central hydrophobic constriction with a diameter of less than ~9 Å excluded water from a nanopore. In contrast, a diameter of more than ~13 Å would allow water into a pore. Pores with a hydrophobic constriction of intermediate diameter (~10 to ~11 Å) stochastically switched between a wet (i.e. open) and a dry (i.e. closed) state, corresponding to a liquid–vapour transition within the constricted region of the nanopore. More detailed studies demonstrated that the critical diameter at which there was a switch between the wet and dry states depended on the local polarity/hydrophobicity and flexibility of the pore in the region of the constriction [9,10] (Figure 1B). This model of hydrophobic gating recently received experimental support

Key words: membrane, nanopore, channel, gating.

Abbreviations: MD, molecular dynamics; nAChR, nicotinic acetylcholine receptor; PET, polyethylene terephthalate; pLGIC, pentameric ligand gated ion channel.

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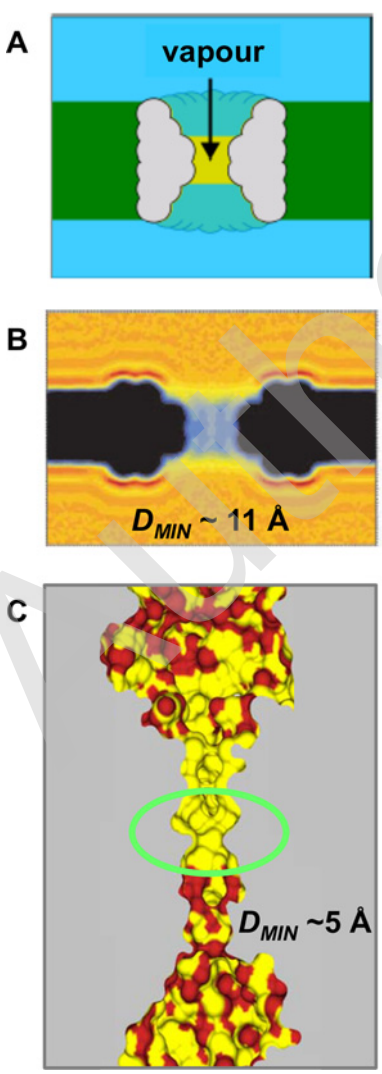
Figure 1 | **(A) Schematic diagram illustrating hydrophobic gating (a 'vapour lock') [17]**

The ion channel/pore (grey) is embedded in a lipid bilayer (green) with water present on either side of the membrane and in both mouths of the pore. The central hydrophobic constriction of the pore excludes water from a de-wetted (vapour) region (in yellow) which forms an energetic barrier to ion and water permeation. (**B**) Water density derived from a simulation of a simplified model of a hydrophobic nanopore [6]. The black area corresponds to a cross-section through the nanopore and the membrane water. Bulk water density is shown in yellow/orange declining to low (~25 % of bulk) water density (blue) in the centre of the 11 Å diameter nanopore. (**C**) Cross-section through the pore of the $5HT_3$ receptor (PDB id 4PIR; [15]). The pore surface is coloured yellow (carbon atoms) and red (polar atoms). The green ring indicates the position of the putative hydrophobic gate (diameter ~5 Å) formed by a ring of L9′ side chains.



from studies of (non-biological) track-etched nanopores in PET membranes [11]. Hydrophobic derivatization of these nanopores resulted in hydrophobic gating. The closed pore could be functionally opened by electro-wetting of the gate, whereby water and ions are driven into the hydrophobic pore by imposing elevated voltages (e.g. >1V) across the membrane.

The model of hydrophobic gating was developed to explore possible gating mechanisms of members of the pLGIC (pentameric ligand-gated ion channel) family of neurotransmitter receptor, which include the nicotinic acetyl choline receptor. These ion channels have a central pore formed by a bundle of five transmembrane M2 helices. At the centre of the pore, there is a constriction formed by consecutive rings of hydrophobic side chains. Simulation studies of a low resolution structure of the nAChR [12], and more recently of higher resolution structures of the bacterial homologue GLIC [13,14] support hydrophobic gating in that a barrier to permeation is formed by the energetic cost of wetting the hydrophobic constriction. Examination of the recent X-ray structure of the related mouse serotonin ($5HT_3$) receptor membrane of the pLGIC family [15] (Figure 1C) reveals the presence of a possible hydrophobic gate with a diameter of ~5 Å.

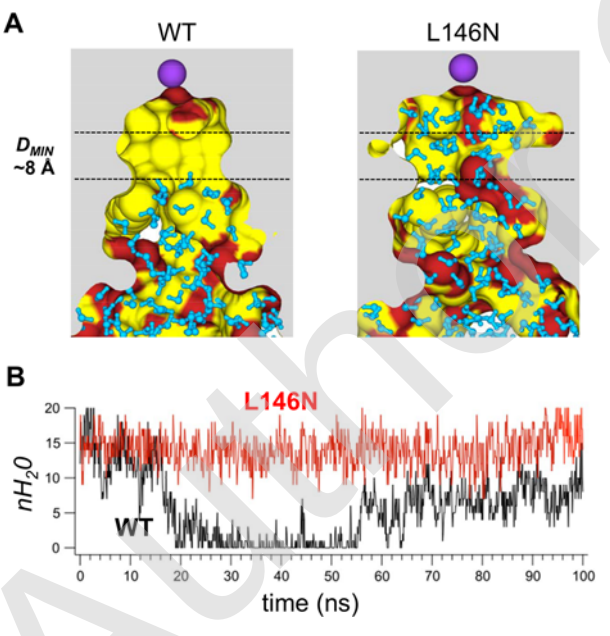
A hydrophobic barrier in the pore of TWIK-1 channels

Recent combined simulation and experimental studies have demonstrated the presence of a hydrophobic barrier to ion permeation within the pore of TWIK-1 channels, a member of the K2P family of potassium channels [16]. In the TWIK-1 channel, there is a hydrophobic cavity, of ~8 Å diameter, deep within the pore, sited immediately below (i.e. cytoplasmic to) the selectivity filter (Figure 2A). This cavity is continuous with the open cytoplasmic mouth of the channel. In simulations of the wild-type (WT) TWIK-1 channel, this hydrophobic cavity exhibits stochastic wetting/de-wetting such that the average state of the cavity is incompletely solvated (i.e. 'dry'). This was shown to be true regardless of the exact forcefield and protein restraint conditions used within the simulations, i.e. the results are robust to variations in the details of the calculations. The presence of this hydrophobic barrier region within the TWIK-1 pore correlates with a very low experimental conductivity for membranes containing TWIK-1 channels. However, if one introduces polar side chains (e.g. L146N) into the cavity, then water remains stably in this region throughout a 0.1 μs simulation. Significantly, this correlates with a substantial (~10-fold) increase in channel activity. This functional change is also seen for different patterns of polar mutations (e.g. L146S, L146T, L146N and L146D) which lead to permanent wetting of this region of the channel pore [16] (Figure 2). Furthermore, calculation of the free energy profile for a K^+ ion moved along the pore axis through this region confirm that the WT channel has a substantive energetic barrier to K^+ permeation in the vicinity of the hydrophobic gate, and that this barrier is reduced by the L146N mutation.

This result may be relevant to other potassium channels which also have a hydrophobic lining to their central cavity [17]. For example long (microsecond-to-millisecond)

Figure 2 | A hydrophobic barrier to permeation in the TWIK-1 potassium channel [16]

(**A**) Snapshots from simulations of the TWIK-1 channel (using the OPLS forecefield), showing a cross-section of the pore extending from a potassium ion at the base of the selectivity filter (top; purple sphere) to the cytoplasmic mouth of the channel (bottom) filled with water molecules (cyan). The pore lining protein and lipid atoms are coloured using the same yellow (hydrophobic) compared with red (polar) scheme as in Figure 1C. The dotted horizontal lines indicate the hydrophobic 'gate' region of the cavity, which has a diameter of ∼8 Å. In the simulation of the WT channel, this cavity is devoid of water molecules (i.e. dry), whereas in the higher conductance L146N mutant this cavity is filled with waters (i.e. wet). (**B**) The number of waters present in the hydrophobic gate region for the WT (black) and L146N (red) Twik-1 channel. Stochastic de-wetting of the WT pore can be seen, whereas the L146N pore remains wet throughout the simulation.



simulations of the Kv channel have shown that the hydrophobic lining of the inner pore cavity may promote a hydrophobic collapse of the pore [18]. A de-wetting step was concurrent with pore closure.

Designing a hydrophobic gate in a β-barrel nanopore

Having established that hydrophobic gating is not only seen *in silico*, but also *in vitro* and *in vivo*, it is of interest to test (and potentially exploit) our understanding by attempting to design a hydrophobic gate into a novel nanopore. This requires a 'blank' nanopore template into which to design a gate. Nanopores based on the β-barrel architecture present in bacterial outer membrane proteins and related proteins are ideal for this purpose [19]. We therefore generated a model of a 14 stranded antiparallel β-barrel as a template, based on the pore domain of the toxin α-haemolysin which has been used in a pore-based biosensors [20]. Using this template, we have modelled a series pores, modifying the shape (i.e. diameter profile) and lining (i.e. hydrophobicity compared with hydrophilicity) of the pore. Starting with an hourglass-shaped pore lined by rings of hydrophilic side chains (which formed an open pore of minimum diameter ∼9 Å), we engineered rings of hydrophobic side chains into the central pore constriction (Figure 3A) [21]. Such a constriction indeed formed a hydrophobic gate and impeded the flow of water molecules or ions through the pore during simulations. It was also possible to fine-tune the height of the central energetic barrier height by modifying via the number of rings of leucine side chains which formed the central constriction. Thus a pore with three consecutive leucine (L) rings exhibits substantive energetic barriers to water, cation and anion permeation (Figure 3B). If the hydrophobic constriction is less extended then the barrier height to e.g. water of chloride ion permeation is lowered (from 24 kJ/mol in LLL to 9 kJ/mol in LL for water, and from 46 kJ/mol in LLL to 20 kJ/mol in LL for chloride), and stochastic wetting/dewetting may be seen (Figure 3C). Interestingly, in the putative hydrophobic gate of the $5HT_3$ receptor (see above), there are also two rings of hydrophobic side chains, L9′ and V13′.

Conclusions

Simulation studies have been used to demonstrate the presence of hydrophobic barriers to ion and water permeation in model nanopores and channels, and in a number of cases, the presence of such barriers is confirmed by ion current measurements and by structural data. It is now possible to use our understanding of hydrophobic gating to design barriers into biomimetic nanopores. It will be important to further test such designs, both computationally and experimentally. One key property of a hydrophobic gate is that it may be overcome by electro-wetting, as has been shown computationally for simplified models of nanopores [22] and as is seen in experimental studies of hydrophobic nanopores in PET membranes [11] (see above). It will therefore be of considerable importance to further examine the role of electro-wetting in ion channels and designed nanopores containing possible hydrophobic gates. Furthermore, the evolutionary role of hydrophobic gating in biological channels should be considered.

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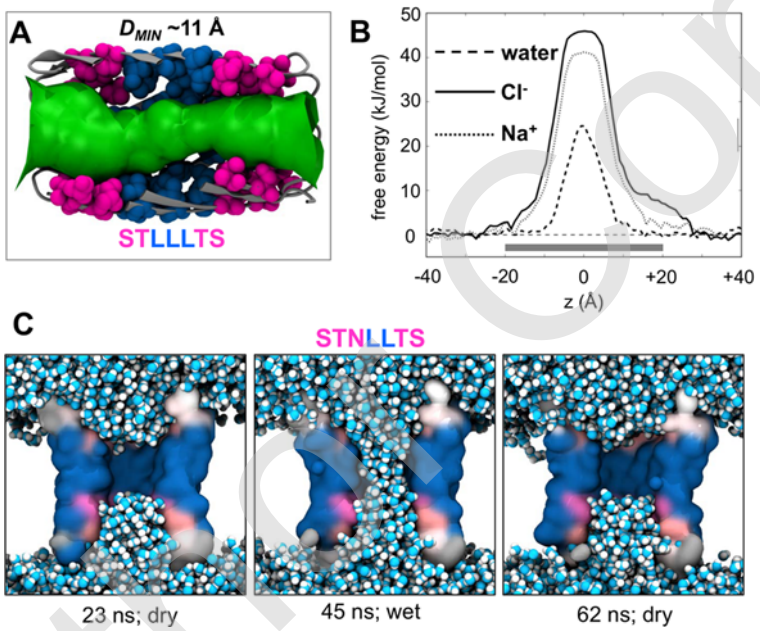
Figure 3 | Design of a hydrophobic gate in a β-barrel nanopore [21]

(**A**) A hydrophobic gate (formed by three rings of leucine (L) residues) within a 14-stranded β-barrel pore protein. The green surface shows the hourglass shape of the pore, with a central constriction of diameter ~11 Å. The pore is lined by rings of hydrophilic (S, T – pink) and hydrophobic (L – blue) side chains. (**B**) Potential of mean force (PMF; i.e. free energy) profiles for movement of a water molecule or ion along the axis (z) of the STLLLTS pore shown in (**A**) embedded in a phospholipid bilayer. The approximate extent of the bilayer and pore is indicated by the horizontal grey bar. A significant energetic barrier is seen in the central construction region for all three species. (**C**) Stochastic wetting and drying in a similar a β-barrel nanopore, but containing only two rings of leucine (L) residue in the central constriction. The lipid bilayer is omitted for clarity and water molecules are shown in cyan/white.



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