Molecular simulations and lipid–protein interactions: potassium channels and other membrane proteins

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Abstract

Molecular dynamics simulations may be used to probe the interactions of membrane proteins with lipids and with detergents at atomic resolution. Examples of such simulations for ion channels and for bacterial outer membrane proteins are described. Comparison of simulations of KcsA (an α-helical bundle) and OmpA (a β-barrel) reveals the importance of two classes of side chains in stabilizing interactions with the head groups of lipid molecules: (i) tryptophan and tyrosine; and (ii) arginine and lysine. Arginine residues interacting with lipid phosphate groups play an important role in stabilizing the voltage-sensor domain of the KvAP channel within a bilayer. Simulations of the bacterial potassium channel KcsA reveal specific interactions of phosphatidylglycerol with an acidic lipid-binding site at the interface between adjacent protein monomers. A combination of molecular modelling and simulation reveals a potential phosphatidylinositol 4,5-bisphosphate-binding site on the surface of Kir6.2.

Introduction

Membrane proteins are of considerable biological importance. They account for approx. 25% of genes [1] and for approx. 50% of drug targets [2], yet they constitute only approx. 0.5% of known structures [3]. Therefore information from methods other than structural biology is of considerable value in maximizing our understanding of this important class of proteins. From a biological perspective, membrane proteins play key roles in a wide range of cellular functions, including signalling across cell membranes, via receptors and ion channels [4]. There are two major classes of membrane protein structure: those formed by bundles of TM (transmembrane) α-helices [5]; and β-barrel proteins from the outer membrane of Gram-negative bacteria [6].

In vivo interactions with lipids help to stabilize the structure of a protein within the membrane and may influence the function of the protein [7]. *In vitro* interactions with detergent molecules are of importance. Detergents are key tools in membrane protein biochemistry [8], as they are used to solubilize membrane proteins prior to purification. Mixed protein/detergent micelles are used in a wide range of experimental studies [9], including membrane protein crystallization, and NMR structure determination [10–12]. It is therefore of some interest to understand the interactions of membrane proteins with detergents, and how these interactions compare with those of lipids.

It is difficult to obtain atomic resolution information on membrane protein interactions with lipids and with detergents. Some information can be obtained from analysis of lipid and/or detergent molecules present within membrane protein crystals [13] and also from spectroscopic studies [14]. MD (molecular dynamics) simulations of membrane proteins [15–17] provide a valuable complement to experimental studies. Earlier simulations provided only limited information on membrane–lipid interactions [18] as simulation times were rather short (~1 ns). However, current simulation times are of the order of 10–100 ns and so enable more reliable analysis of lipid–protein and detergent–protein interactions. Here we review such simulations, focusing on examples from the authors' laboratory, and in particular on ion channels for which the functional significance of lipid–protein interactions is documented.

Key words: KcsA, Kir6.2, lipid–protein interaction, molecular simulation, OmpA, potassium channel.

Abbreviations used: DMPC, dimyristoyl phosphatidylcholine; DPC, dodecylphosphocholine; MD, molecular dynamics; OG, octyl glucoside; PIP_2, phosphatidylinositol 4,5-bisphosphate; POPC, palmitoyl oleoyl phosphatidylcholine; TM, transmembrane; VS, voltage sensor.

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Lipids and detergents

Simulations of membrane proteins in lipid bilayers

MD simulations have enabled comparative analysis of lipid–protein interactions of two membrane proteins: KcsA (an α-helix bundle) embedded in a POPC (palmitoyl oleoyl phosphatidylcholine) bilayer; and OmpA (a β-barrel) in a DMPC (dimyristoyl phosphatidylcholine) bilayer [19,20]. Analysis was focused on interactions suggested to stabilize membrane proteins within a lipid bilayer [21], namely those of amphipathic aromatic side chains (tryptophan and tyrosine), and those of basic (arginine and lysine) side chains. Analysis of H-bonding of tryptophan and tyrosine side chains with lipid head groups revealed that such interactions fluctuated on an approx. 1–5 ns timescale. There were two bands of interacting residues on the surface of KcsA, at either end of the TM domain, whereas there are three such bands on OmpA. Basic side chains formed 'snorkelling' interactions with the phosphates of lipid head groups. Substantial arginine–phosphate interactions were seen for KcsA; for OmpA, the

number of basic–phosphate interactions was smaller and showed more marked fluctuations with respect to time. Basic–phosphate interactions were also explored in simulations of the *Escherichia coli* outer membrane protease OmpT in a DMPC lipid bilayer [22]. This study revealed specific lipid interaction sites on the surface of the OmpT barrel, which correlated with a possible binding site for lipid A suggested on the basis of comparison of X-ray structures of outer membrane proteins.

The OmpA and KcsA simulations permitted analysis of lateral diffusion of lipid molecules. 'Boundary' lipid molecules (i.e. those in contact with the protein surface) diffused at about half the rate of the bulk lipid. For OmpA, the number of boundary DMPC molecules was estimated to be approx. 14. This agrees with an estimate of approx. 11 from spin-label studies of OmpA in DMPG (1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol) bilayers [23]. Overall, these simulations present a dynamic picture of lipid–protein interactions, revealing both non-specific and longer-lived, more specific interactions.

Simulations of membrane proteins in detergent micelles

Simulations in detergent micelles reveal the conformational dynamics of membrane proteins in an environment used for NMR structure determinations [24–28]. Furthermore, membrane proteins are generally crystallized from protein–detergent micelles and many crystal structures reveal bound detergents. It is therefore of interest to understand how detergents interact with membrane proteins, and how they may mimic lipid molecules.

OmpA is one of a small number of membrane proteins whose structure has been solved both by X-ray crystallography [29,30] and by NMR [25,26]. MD simulations have been employed to compare the behaviour of OmpA in a DPC (dodecylphosphocholine) micelle and in a DMPC bilayer [31]. Dynamic fluctuations of the protein structure are approx. 1.5 times greater in the micelle than in the bilayer environment. The enhanced flexibility of the OmpA protein in the micellar environment enables small changes in protein side-chain torsion angles, resulting in the formation of a continuous pore through the centre of the OmpA molecule. This may explain the observed channel formation by OmpA. More recently, extended (50 ns) MD simulations have been used to explore the mechanism of self-assembly of OmpA–detergent micelles, starting with randomly positioned DPC molecules [32]. These latter studies demonstrate that simulations are capable of capturing the spontaneous hydrophobic interactions of detergents with the surface of an integral membrane protein.

Simulations have also been used to compare the interactions of an α-helical membrane protein GlpF (a member of the aquaporin family) with lipid (DMPC) and with detergent OG (octyl glucoside) [33]. OG is widely employed in structural and functional studies of membrane proteins. Greater conformational drift of the extra-membraneous loops of GlpF was seen for the OG simulation than that for the DMPC simulation. The mobility of the TM α-helices was approx. 1.3 times higher in OG than in the DMPC bilayer. Significantly, it was seen that aromatic side chains (tryptophan and tyrosine) and basic side chains (arginine and lysine) play an important role in 'both' protein–detergent and protein–lipid interactions.

MD simulations have helped to reveal the interactions with its membrane environment of the VS (voltage sensor) domain from a bacterial voltage-activated potassium channel, KvAP [34]. There is currently some discussion as to how the VS domain is packed within the intact channel molecule [35] and the implications of this for the mechanism of voltage activation [36]. In particular, it is of interest whether the multiple positively charged Arg residues of the voltage-sensing S4 helix are able to interact favourably with a lipid bilayer, or whether they are buried within the interior of the channel protein. Simulations of the isolated VS domain in a lipid bilayer (POPC/POPG ~ 3:1) have been compared with simulations in a decylmaltoside bilayer (Figure 1; Z.A. Sands, A. Grottesi and M.S.P. Sansom, unpublished work). Analysis of H-bonding reveals that, when within a lipid bilayer, the arginine side chains of the S4 helix of VS are able to form multiple H-bonds with the lipids head groups, especially with the phosphate groups. Multiple H-bonds are also formed to the (uncharged) head groups of decyl maltoside in the micelles, but the average number is somewhat smaller. Thus it would seem that the phosphate groups of the lipid bilayer are important in stabilizing the positively charged side chains of the voltage-sensing S4 helix.

Ion channel modulation by specific interactions with lipids

It is evident that specific interactions of lipids with ion channels are of significance to channel stability and/or biological activity. Such interactions may be probed by carefully designed simulations.

KcsA and acidic lipids

A number of experiments implicate acidic (i.e. negatively charged) head groups of phospholipid in the structural integrity and function of the bacterial potassium channel KcsA [37]. The electron density in the crystal structure of KcsA reveals a lipid-binding site, but only a fragment of a lipid molecule is present in the co-ordinates. Significantly, the presence of negatively charged lipids is required for ion conduction through the KcsA potassium channel, suggesting that binding of lipid to KcsA is important for channel function [38]. Spectroscopic studies also support a specific acidic lipid-binding site at the interface between two monomers in the tetrameric channel structure [39].

A 20 ns MD simulation in a mixed lipid bilayer (POPE/POPG ~ 3:1) has been used to explore the interactions of KcsA with acidic phospholipids [40]. Analysis of the conformational drift of KcsA relative to a simulation in POPC [41] suggested a degree of stabilization of the protein structure by the mixed lipid bilayer. Analysis of protein–lipid

Figure 1 | Simulation of membrane proteins in a (A) lipid bilayer or (B) detergent micelle

In both cases, the simulation is of the VS domain from the bacterial voltage-gated potassium channel KvAP (Z.A. Sands, A. Grotlesi and M.S.P. Sansom, unpublished work). The VS is shown as a Cα ribbon with the basic (blue) and amphipathic aromatic (tryptophan and tyrosine; in purple) residues in space-filling format. (**A**) The lipid bilayer (a mixture of POPC and POPG) is shown in grey, with the phosphorus atoms of the head groups as orange spheres. The water (w), interfacial (i) and hydrophobic core (h) regions are labelled. (**B**) The detergent micelle is formed by decyl maltoside molecules, shown in grey with the oxygen atoms of the glycosidic head group as red spheres.

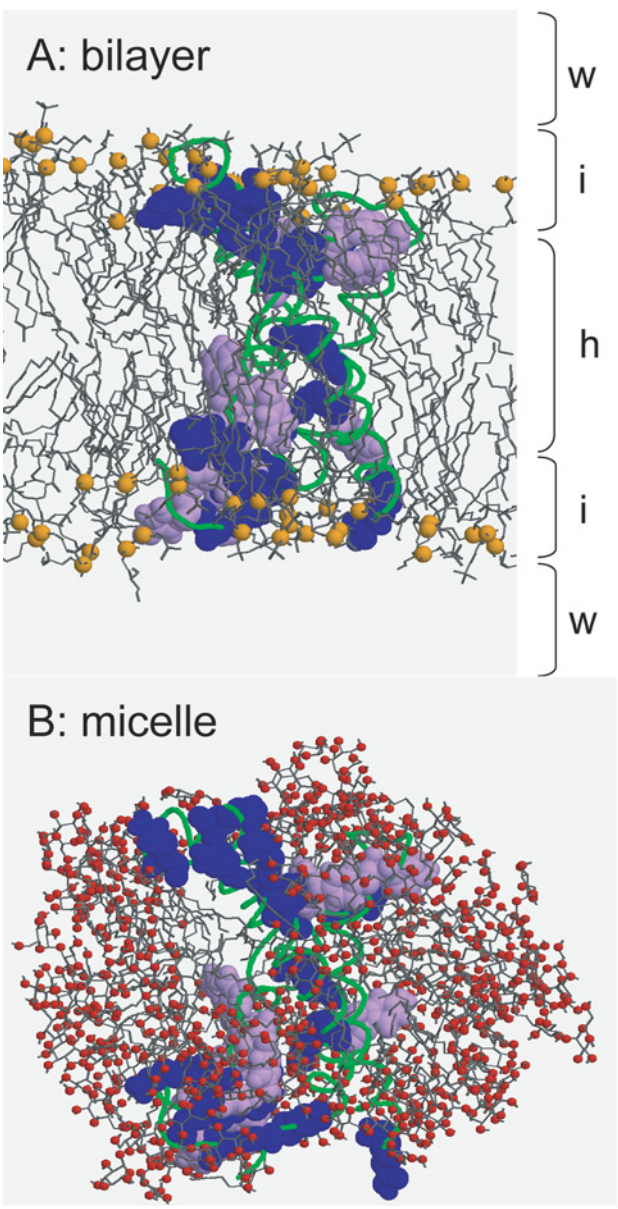
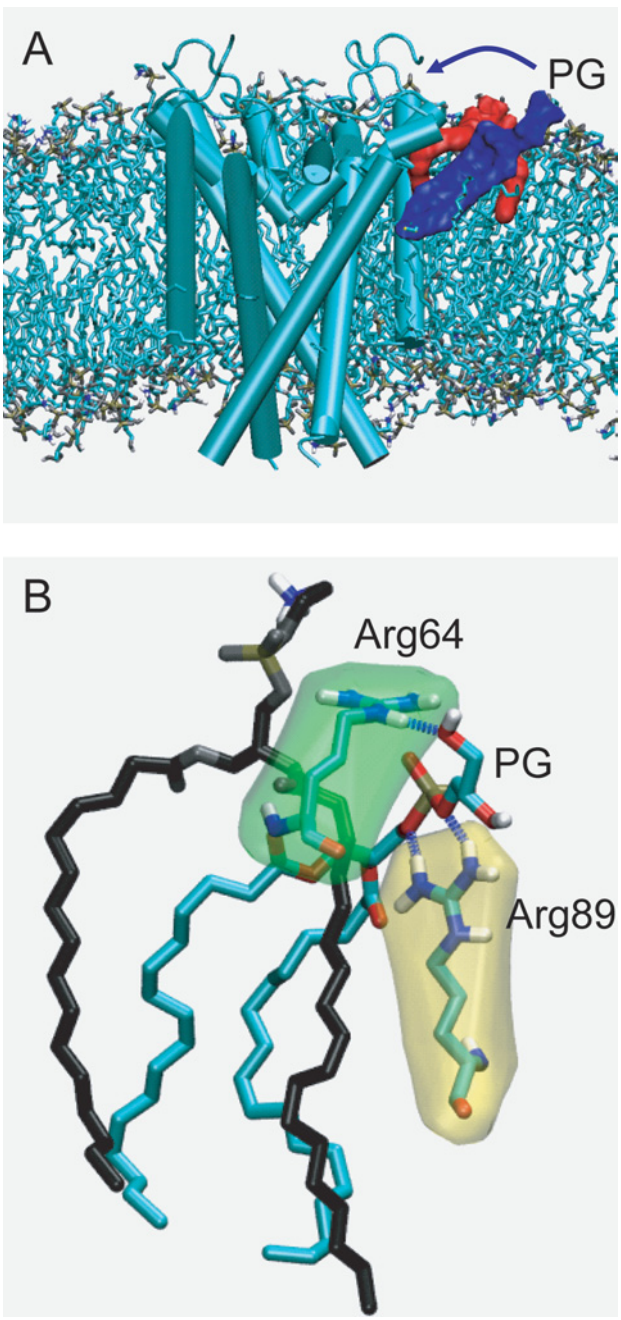


Figure 2 | Simulation of the KcsA TM domain in a POPE–POPG bilayer

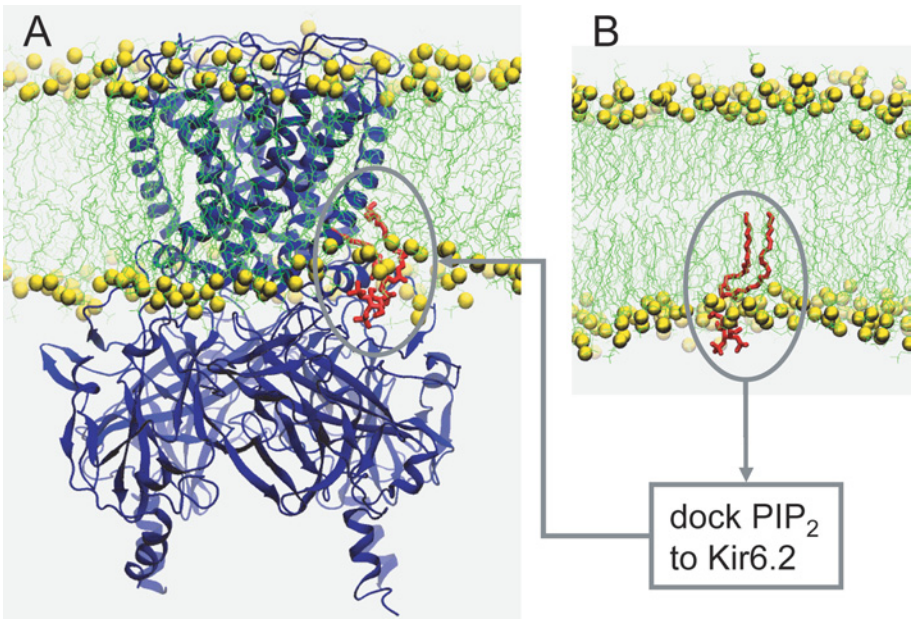
(**A**) The KcsA molecule is shown embedded in a lipid bilayer, after 10 ns of simulation. Two adjacent lipid molecules are shown in space-filling format, with the POPG head group labelled. (**B**) A close-up from the same simulation system (after 27 ns) showing the H-bonding interactions of a POPG molecule (the same one shown in **A** in space-filling format) with two arginines residues (Arg^{43} and Arg^{68} of two adjacent subunits of the KcsA tetramer). A POPE molecule (in dark grey) is also shown for comparison. Data taken from [40].



interactions revealed an increase with time in the number of interactions with acidic (POPG) head groups. In particular, a POPG molecule from the bilayer (Figure 2A) during the course of the simulation binds to the specific subunit–subunit interfacial site on KcsA, where it forms H-bonds with the side chains of Arg^{64} and Arg^{89} (Figure 2B). These are the two arginine residues that appear, on the basis of the lipid fragment

Figure 3 | **Simulation of Kir6.2 and PIP_2**

(**A**) Model of a complex of Kir6.2 channel (blue) and PIP_2 (red) embedded in a lipid bilayer (POPC in green, with phosphorus atoms as yellow spheres). This model was derived by docking a PIP_2 molecule taken from a simulation of PIP_2 in a POPC bilayer, as shown in (**B**) (S. Haider and M.S.P. Sansom, unpublished work).



built into the crystal structure, to form the lipid-biding site. Thus simulations confirmed a specific lipid–protein interaction of functional significance.

Inward rectifier potassium channels and PIP_2 (phosphatidylinositol 4,5-bisphosphate)

The inward rectifier (Kir) class of K channels regulate cell excitability by stabilizing the membrane potential close to the $[K^+]$equilibrium potential, and are involved in K^+ transport across membranes. Kir6.2 is the pore-forming component of the ATP-sensitive potassium channel, which regulates insulin release from pancreatic β-cells [42]. There is evidence that Kir6.2 and related Kir channels are regulated by a number of modulators. In particular, PIP_2 is essential for maintenance of the activity of all Kir channels [43]. The structure of the Kir6.2 channel has been modelled, using a bacterial Kir homologue (KirBac1.1) as a template for the TM domain, and the Kir3.1 intracellular domain as a template for the homologous domain of Kir6.2 [44]. This model structure has enabled simulation studies of Kir6.2–PIP_2 interactions (S. Haider, F. Ashcroft and M.S.P. Sansom, unpublished work; Figure 3).

MD simulations of PIP_2 in a POPC lipid bilayer were used to sample possible conformations of PIP_2 in a membrane environment. These PIP_2 models were subsequently used in a docking study of PIP_2 bound to Kir6.2. The model of the Kir6.2–PIP_2 complex was refined by an MD simulation in a POPC bilayer. Significantly, the binding site on the channel surface is such that the PIP_2 molecule is not greatly perturbed from its preferred location within a lipid bilayer. This model and simulation permit a more detailed characterization of the nature of Kir–PIP_2 interactions.

Conclusions and future directions

It is evident that MD simulations are able to reveal both specific and non-specific interactions of membrane protein components with their lipid bilayer environment. Future simulations will extend such studies to a wider range of membrane proteins, including those peripheral membrane proteins that form strong interactions with specific lipids [45]. This will help us to arrive a detailed understanding of lipid–protein interactions at atomic resolution.

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