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Lipid interaction sites on channels, transporters and receptors: Recent insights from molecular dynamics simulations☆

George Hedger, Mark S.P. Sansom *

Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

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ABSTRACT

Lipid molecules are able to selectively interact with specific sites on integral membrane proteins, and modulate their structure and function. Identification and characterization of these sites are of importance for our understanding of the molecular basis of membrane protein function and stability, and may facilitate the design of lipid-like drug molecules. Molecular dynamics simulations provide a powerful tool for the identification of these sites, complementing advances in membrane protein structural biology and biophysics. We describe recent notable biomolecular simulation studies which have identified lipid interaction sites on a range of different membrane proteins. The sites identified in these simulation studies agree well with those identified by complementary experimental techniques. This demonstrates the power of the molecular dynamics approach in the prediction and characterization of lipid interaction sites on integral membrane proteins.
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1. Introduction

Cells are separated from their environment and compartmentalized by membranes. These barriers are composed of lipid bilayers (with the various lipid species distributed asymmetrically between the two leaflets of the bilayer), into which proteins are embedded. Parallel advances in lipidomics [1] and in the structural biology of membrane proteins [2,3] over the past decade have revealed some of the complexities of the composition of cell membranes. Thus, the structures of ca. 1500 membrane proteins have been determined (http://blanco.biomol.uci.edu/mpstruc/) as have those of ca. 40,000 lipid species (http://www.lipidmaps.org/data/structure/) [4]. The lipidome of membranes varies according to cell age, metabolic state, stage in cell cycle, organelle, and spatial location, resulting in a complex protein–lipid interactome. In addition to providing a bilayer environment, it is increasingly appreciated that the function of embedded proteins can be modulated by interactions with this complex lipid mixture [5–12]. Of particular interest, an emerging feature present within the protein–lipid interactome is that certain lipid molecules can selectively bind to specific sites on integral membrane proteins, and modulate both their structure and their function [13].

As recently reviewed [14], we now possess over 100 structures of membrane proteins containing electron density interpreted as bound lipid molecules. Structural identification of specific lipid binding sites aids our mechanistic understanding of lipid modulation of protein function, such as in the case of Kir2.2 and PIP_2 [15]. Identification of sites of allosteric modulation on proteins is also of interest for the assessment of protein druggability [16]. The majority of membrane protein structures containing bound lipid molecules were solved using X-ray crystallography. In many cases such structures have been obtained from crystals grown in the presence of detergent. It is likely that the lipids observed represent a biased sample of tight binding lipids, and in some cases the molecular identity of the observed electron density corresponding to detergent and/or lipid may be uncertain. This may change as more membrane protein structures are determined using crystals obtained from lipidic cubic phases [17] which better approximate a native membrane environment.

Molecular dynamics simulations allow membrane protein structures to be computationally re-embedded into lipid bilayers, and their dynamic interactions with surrounding lipid molecules to be characterized [18]. A number of recent simulation studies probing lipid interactions have identified specific lipid binding sites. These sites show good agreement with those identified from a range of structural studies. A number of other, presumably weaker, binding sites can also be resolved. While these weaker sites may not always be observed by X-ray crystallography, there are a number of other biophysical techniques which allow

Abbreviations: MD, molecular dynamics; CG, coarse-grained; NMR, nuclear magnetic resonance; ESR, electron spin resonance; EM, electron microscopy; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; PIP_2, phosphatidylinositol-4,5-bisphosphate; Kir, inward rectifying potassium ion channel; nAChR, nicotinic acetylcholine receptor; $GABA_AR$, γ-aminobutyric acid receptor; GPCR, G-protein coupled receptor; β_2AR, β_2-adrenergic receptor; ANT/AAC, ADP/ATP carrier; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase.

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* Corresponding author.
E-mail address: mark.sansom@bioch.ox.ac.uk (M.S.P. Sansom).

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us to probe lipid interactions with membrane proteins, including e.g. fluorescence spectroscopy [19], EPR [20], NMR [21], and mass spectrometry [13]. These techniques provide further points of reference for simulation studies membrane protein–lipid interactions.

Within this review article we survey recent simulation studies which identify lipid interaction sites on membrane proteins. We focus on specific binding of lipids to defined sites on membrane proteins. We also focus on channels, receptors, and transporters, for which there are functional and structural data on the biological importance of lipid–protein interactions. Overall we find molecular dynamics simulations to have strong predictive power and to be well-suited for identification of these sites. Additionally the simulation approach provides a means for further characterization of the identified sites, for instance by estimations of lipid binding affinities [22–24], as well as enabling functional insight into mechanisms of lipid modulation [25–27].

1.1. Lipid modulation of membrane protein function

The functions of a range of membrane proteins are known to be modulated by their lipid environment, including potassium channels [6,7], receptor tyrosine kinases (RTKs) [8], G-protein coupled receptors (GPCRs) [9,10], solute transporters such as BetP [5] and the ADP/ATP carrier [28], redox proteins such as cytochrome c oxidase [29], and certain P-type ATPases [30]. Such lipid modulation can influence several different aspects of protein function, including effects on the activity of a membrane protein, modulating protein–protein interactions, and altering cellular localization by sequestering a protein to spatially defined regions of a membrane. In certain cases, a lipid may represent a native ligand for the protein rather than an allosteric modulator, as is the case for the sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) group of lipid-activated GPCRs [31].

In a number of cases structural, biophysical and functional assays have been combined to provide a detailed picture of lipid modulation. For example, this is the case for eukaryotic inward rectifying potassium ion (Kir) channels. Functional assays revealed Kir channels to be dependent on the presence of the anionic lipid phosphatidyl inositol-4,5-bisphosphate (PIP_2) for activation. Subsequently, simulation studies [25,26] and crystal structures [15] revealed four specific PIP_2 binding sites and enabled the mechanism of PIP_2 channel modulation to be structurally rationalized (see Section 2.1.2). In other cases careful biochemical analysis has revealed a functional dependence on certain lipid species, but the mechanism of modulation remains unclear. This is the case for the epidermal growth factor receptor (EGFR/ErbB1) which is a single-pass transmembrane receptor known to be modulated by an array of lipids, including PIP species and the glycolipid GM3 [8]. However exactly how these lipid species control receptor activity remains unclear, with proposals including an influence of receptor dimerization propensity, direct conformational stabilization and orientation effects, and larger scale lipid-induced clustering of the receptor.

1.2. Lipid interaction sites on integral membrane proteins

Lipids interact with membrane proteins via multiple modes. The presence of integral membrane proteins may induce formation of a lipid "annulus" around the protein. Due to interactions with the protein, lipids within this annulus exhibit decreased motional freedom compared to their non-interacting bulk counterparts, and are detectable by EPR [32,33]. This immobilizing effect of the protein may extend beyond the first shell of directly interacting annular lipids, leading to further outer shells with a lesser extent of lipid immobilization, as suggested by MD simulations [34,35]. In addition certain lipid species may bind to specific sites on the membrane protein surface — often described as "non-annular" lipids. Binding may be driven by formation of physicochemical interactions between the lipid and protein surface, as well as by complementary geometry, for instance "slotting" of lipid molecules into "grooves" on the protein surface [36] or binding at the interface between subunits [37]. Binding sites may tightly coordinate the lipid [15], or act to cause weaker and more dynamic localization [38]. Efforts have been made to describe general features of lipid binding sites and sequence interaction motifs, such as for cholesterol [39] and cardiolipin [40].

1.3. Biomolecular simulation approaches for lipid binding site identification

Molecular dynamics (MD) simulations provide a powerful tool to characterize the dynamics and interactions of membrane proteins with surrounding lipid molecules [18]. However, the computational cost of the simulations is such that length scales beyond microseconds are not currently readily accessible [41], especially for extended systems containing multiple membrane proteins. This has prompted the development of more approximate coarse-grained (CG) representations of membrane lipids and proteins in MD simulations [42,43] in which groups of atoms are represented as single particles (Fig. 1). Reducing the number of particles in the system reduces the computational demand involved in running the simulation and thus allows access to longer time and length scales, with the caveat that the level of approximation made in a given CG model has to be matched to the underlying biological interactions being probed. CG simulations can thus allow significantly enhanced lipid exploration of the protein surface and candidate binding sites, while sacrificing the finer detail of lipid–protein interactions. These approximations may be reconciled to some degree by conversion of the endpoint of a CG system back to atomistic detail [44,45] and subsequently running an atomistic simulation to assess the validity of the CG system arrangement, a so called (serial) multiscale modelling approach [46]. The MARTINI CG force field has been most widely applied in the field of protein–lipid interactions. We note that CG simulations may now extend to hundreds of microseconds [23], and contain many hundreds of protein molecules [47], while atomistic simulations of individual proteins may reach tens of microseconds duration using high performance computing resources [48].

The structure of a membrane protein used as initial input for MD simulations may be from X-ray diffraction, cryoelectron microscopy, or NMR. If the 3D structure of the protein is not known experimentally, in some cases a model may be built by modelling [35]. The membrane protein is then embedded into a lipid bilayer. This may be achieved either by self-assembly simulations [49] in which short simulations are run to allow the spontaneous formation of a bilayer around an integral membrane protein, or by a number of methods which insert a membrane protein into a pre-assembled bilayer [50–52]. Advances in lipid parameterization [53], along with a growing appreciation of the

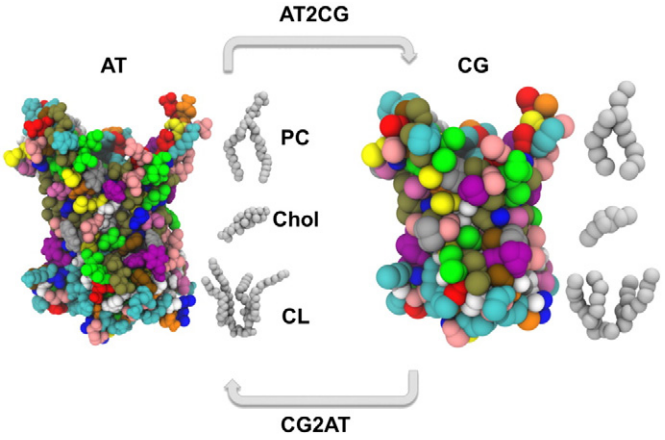

Fig. 1. Schematic of a multiscale approach to modelling and simulation of lipid interactions with an integral membrane protein. The ADP/ATP carrier (ANT1/AAC1; PDB: 1OKC [36]) is depicted as spheres colored by residue type, at both the atomistic (left) and CG (right) scale. Phosphatidylcholine (PC), cholesterol (Chol), and cardiolipin (CL) molecules are shown as grey spheres.