Simulation studies of the interactions between membrane proteins and detergents

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Abstract
Interactions between membrane proteins and detergents are important in biophysical and structural studies and are also biologically relevant in the context of folding and transport. Despite a paucity of high-resolution data on protein–detergent interactions, novel methods and increased computational power enable simulations to provide a means of understanding such interactions in detail. Simulations have been used to compare the effect of lipid or detergent on the structure and dynamics of membrane proteins. Moreover, some of the longest and most complex simulations to date have been used to observe the spontaneous formation of membrane protein–detergent micelles. Common mechanistic steps in the micelle self-assembly process were identified for both α-helical and β-barrel membrane proteins, and a simple kinetic mechanism was proposed. Recently, simplified (i.e. coarse-grained) models have been utilized to follow long timescale transitions in membrane protein–detergent assemblies.

Introduction
Despite the biological importance of membrane proteins, only limited structural studies have been possible because of the difficulties of expression, purification and refolding. In particular, the inhomogeneous nature of the membrane environment has necessitated the use of detergent micelles to solubilize membrane proteins. Detergent molecules sequester the non-polar regions of the protein away from water. Mixed protein–detergent micelles are used in crystallization for X-ray studies, or for solution NMR. Thus detergents play a key role in membrane protein biochemistry [1] and also provide models of relevance to biological processes such as folding and transport [2]. It is therefore important that we understand the interactions of membrane proteins with detergents. However, only limited information is available on these interactions [3]. MD (molecular dynamics) simulations of membrane proteins [4] provide a method for analysing both static and dynamic aspects of interactions of lipids and detergents with membrane proteins in atomistic detail.

Membrane protein–detergent micelles
Recently, improved methods and computational power have enabled us to carry out the simulation of complex membrane protein–detergent systems on timescales ranging from 10 to 100 ns. Our studies have focused on the eight-stranded β-barrel domain of OmpA, a bacterial outer membrane protein whose crystal structure is known [5]. Early simulations [6] focused on the nature of pore formation by this protein. More recently, folding studies [7] and NMR structure determination in DPC (dodecylphosphocholine) [8] or dihexanoylphosphatidylcholine [9] detergent micelles have recommended OmpA as a target for simulation studies in a range of environments. To this end, we performed a comparative MD study of OmpA in a DMPC (dimyristoylphosphatidylcholine) bilayer and in a DPC detergent micelle [10] (Figure 1A). The detergent molecules, which were initially packed around the β-barrel in a toroidal manner, formed a stable ellipsoidal geometry consistent with NMR data [11]. Nevertheless, the micelle appeared to create a highly dynamic environment around OmpA, resulting in peptide backbone fluctuations of approximately twice the magnitude of those observed in a lipid bilayer, in agreement with site-directed spin-labelling experiments [12]. Moreover, a gradient of flexibility was observed along the axis of the β-barrel, as seen in heteronuclear NOE NMR measurements [8]. Such flexibility may have consequences for pore formation by OmpA [10]. Subsequently, the simulated behaviour of OmpA in detergent micelles seems to be similar for other membrane proteins, including both other β-barrel outer membrane proteins (K. Cox, PJ. Bond and M.S.P. Sansom, unpublished work) and α-helical membrane proteins such as GlpF [13].

Micelle self-assembly
In addition to equilibrium simulations based on preformed detergent–protein micelles, it is of interest to investigate their self-assembly, particularly given the complex phase behaviour of detergents. Thus, following recent MD studies of the self-assembly of pure detergent micelles [14], we have demonstrated the spontaneous formation of OmpA–DPC detergent micelles [15] from a system containing OmpA in a detergent solution (Figure 1B). We were able to describe a detailed mechanism with three distinct phases for the process...
of self-assembly. The first phase involved rapid formation of small detergent micelles and patches of protein–detergent interaction extending for a few nanoseconds, driven primarily by the thermodynamic tendency to bury the hydrophobic surfaces of DPC tails and the OmpA transmembrane region. The second phase was characterized by fusion of pure detergent micelles with one another and with detergents on the protein surface, driven via strong electrostatic interactions between DPC head groups, resulting in adsorption of most of the detergent molecules to the protein. Significantly, monomeric detergent did not, in general, adsorb to the protein surface, which is consistent with the observation that in vitro folding of OmpA N-terminal domain into micelles depended on the presence of preformed pure micelles [16]. Finally, in the third phase, of over tens of nanoseconds, the loosely packed OmpA–DPC mixed micelle equilibrated, with the soft hydrophobic forces involved in packing of detergent tails against one another and the protein surface dominating, resulting in the gradual optimization of the complex energetic balance between protein, detergent and solvent (Figure 1C).

It should be noted that we have observed a similar mechanism of micelle formation for a simple model α-helical membrane protein [15], namely the dimer of the GpA (glycoporphin A) transmembrane helix. The structure of GpA in DPC micelles has been determined using solution NMR [17], as well as by solid-state NMR in lipid bilayers [18]. Other simulation studies have reported spontaneous detergent assembly around GpA [19], as well as around the simple β-barrel OmpX [20]. The kinetics of association of DPC around OmpA and around GpA in our simulations could be characterized by measuring the time-dependent \( R_g \) (radius of gyration) for detergent molecules as each micelle is formed (Figure 2). For each protein, this process may be fitted by a single exponential function, the time constant of which seems to scale with the surface area of the protein, even when the simulations are extended to 100 ns. On this basis, the mechanism of formation of a detergent–protein micelle may be approximated by a simple stochastic model, involving random diffusion and adsorption of detergent molecules to the protein surface [15].
Towards longer time-scales

In addition to the mixed protein–detergent micelles formed during the self-assembly simulations, a relatively stable pure detergent mini-micelle or ‘globule’ was also present that remained stable in solution for 100 ns (Figure 1C). Encouragingly, the final form of the self-assembled protein–detergent micelle closely resembled its preformed counterpart. We have suggested that, for much longer time-scales, the globule may act as a ‘buffer’, enabling detergent molecules to exchange between the ‘globule’ and the main protein–detergent micelle. To test this further, we have recently developed a CG (coarse-grained) model of these systems (P.J. Bond and M.S.P. Sansom, unpublished work), in which groups of several atoms are treated as single particles and simplified potential functions are used to enhance the observed simulation time-scales [21]. In particular, we have extended the semiquantitative CG simulations of lipids performed by Marrink et al. [22] to also enable simulation of proteins. Preliminary CG simulations reveal similar mechanisms (for tens of nanoseconds) of protein–detergent self-assembly to their atomistic counterparts. Moreover, for longer time-scales (i.e. hundreds of nanoseconds), the CG MD simulations reveal the eventual fusion of the ‘globule’ with the main protein–detergent micelle, as predicted previously from our atomistic systems. The resulting aggregate structure with all detergents integrated (Figure 1D) is in excellent agreement with the atomistic counterparts. We have demonstrated the spontaneous formation of detergent micelles around α-helical and β-barrel proteins and shown that this process occurs via a common mechanism, the kinetics of which can be approximated by a simple diffusive, stochastic adsorption model. Finally, preliminary studies have revealed that many of the features of atomistic self-assembly simulations can be captured via simplified CG models. This provides proof-of-principle that longer time-scales are accessible for membrane–protein simulations, thus making possible ‘brute force’ simulations of large-scale dynamic transitions in protein–detergent assemblies and, by extension, protein–lipid assemblies.

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References


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