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Mini-review

Molecular simulations of glycolipids: Towards mammalian cell membrane models

David Shorthouse [1], George Hedger, Heidi Koldsø [2], Mark S.P. Sansom *

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

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ABSTRACT

Glycolipids are key components of mammalian cell membranes, influencing a diverse range of cellular functions. For example, a number of receptor tyrosine kinases, including the epidermal growth factor receptor (EGFR), are allosterically regulated by the glycolipid monosialodihexosylganglioside (GM3). Recent advances in molecular dynamics methods, especially the development of coarse-grained models, have enabled simulations of increasingly complex models of cell membranes. We demonstrate these methodological developments *via* a case study of a coarse-grained model for the ganglioside GM3. This glycolipid is included in simulations of a mixed lipid bilayer model reflecting the compositional complexity of a mammalian cell membrane. The resultant membrane model is used to simulate the interactions of GM3 with the transmembrane domain of the EGFR.

1. Introduction

Glycolipids are essential components of mammalian cell membranes. The content of glycolipids, and specifically of glycosphingolipids, within mammalian cells vary by organelle, cell type, and cell location [1,2]. Glycolipids are implicated in a number of neurological disorders, and also in metabolic disorders such as insulin resistance [3]. Glycolipids may modulate the behaviour of key membrane proteins, including growth factor receptors [4], insulin receptors [5], and some ion channels [6]. Glycolipids are also thought to play a key role in the formation of lipid rafts [7], and thus may influence the dynamics of membrane systems within cells [8]. Despite these important functions, glycolipids are comparatively understudied, in part due to their complex and dynamic nature, and in part reflecting the difficulties associated with gaining high-resolution experimental data. One way to study the dynamic behaviour of these molecules at near atomic resolution is through the use of molecular dynamics (MD) simulations. By using such simulations as a "computational microscope" [9] we are able to obtain information on their single molecule dynamics, interactions with other lipids and with membrane proteins, thus characterising emergent properties and aiding the interpretation of experimental data. Recent developments in molecular simulation enable us to explore ligand binding and/or conformational changes of membrane proteins. In order to extend the biological relevance of such studies we need to be able to simulate *biological* membranes containing complex mixtures of lipids [10,11] crucially including glycolipids.

Whilst a number of atomistic MD simulations have been performed on glycolipid-containing membranes [12,13] the system sizes and timescales required for exploring the dynamic properties of more complex membrane models and to correlate the results of simulations with experimental observations require alternative simulation approaches to be employed. One approach is the use of reduced representation models *via* coarse-grained molecular dynamics (CG-MD) [14,15], enabling simulation of larger systems (e.g. up to 100,000 lipid molecules) for extended (e.g. more than 1 μs) time periods. Here we review the importance of glycolipids in membranes, focussing especially on mammalian cells, and illustrate how CG-MD simulations can be used to explore their interactions with a model membrane protein.

The past decade has seen substantial progress in developing atomistic parameters for the simulation of biologically relevant glycolipids, e.g. ceramides, and exploring the behaviour of simple lipid mixtures which mimic aspects of more complex biological membranes. In particular, it has been shown that the sugar headgroup of galactosylceramide forms hydrogen-bonds to neighbouring lipid molecules, thus slowing lateral diffusion of lipids

* Corresponding author.
E-mail address: mark.sansom@bioch.ox.ac.uk (M.S.P. Sansom).
[1] Current address: MRC Cancer Unit, University of Cambridge, MRC Research Centre, Box 197, Cambridge, CB2 0X1, UK.
[2] Current address: D. E. Shaw Research, 120 W. 45th st., 39th fl., New York, NY 10036, USA.

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in the bilayer [16,17]. Atomistic simulations have also been used to explore the biophysical properties of synthetic glycolipids (alkyl glycosides) in the context of possible industrial applications [18–20]. At the same time, CG-MD methods have been used in large scale simulations of glycolipid-containing membranes. A notable example is provided by a recent simulation of a mixture of galactolipids and phosphatidyl glycerol, modelling thylakoid membranes of cyanobacteria and of plants [21]. This study revealed the emergence nanoscale heterogeneities in these complex bilayers, especially in the case of a model plant thylakoid membrane.

A number of recent reviews discuss simulation studies of glycolipids [13,22–24]. There have also been some studies of the interactions of glycolipids with proteins [22,25]. Here we discuss a CG-MD approach to simulation of glycolipid-containing membranes, illustrating this *via* the ganglioside GM3, and provide an example of modelling protein/glycolipid interactions in a model mammalian cell membrane.

2. Glycolipids and lipid rafts

The lipid raft hypothesis suggests that lipids in complex membranes form micro- (or nano-) domains, and that these play a key role in the dynamic behaviour and organization of membranes in cells [26]. Glycolipids are thought to be a key component of lipid rafts. Thus, when eukaryotic cell membranes are treated with detergent, insoluble membrane patches remain which are highly enriched in glycosphingolipids [27]. It is suggested that these patches are formed by the weak interactions between headgroups of neighbouring glycolipids, leading to their clustering, and the exclusion of other species. The bulky nature of the glycolipid headgroups is such that cholesterol tends to act as molecular "filler" in the gaps between glycolipid molecules. It is suggested that glycolipid-enriched lipid rafts may perform a range of biological functions, including roles in membrane protein sorting, in membrane protein regulation, and in influencing membrane curvature and rigidity [28].

3. Glycolipids as protein modulators – GM3 and growth factor receptors

Glycolipids have been shown to be modulators of protein function. A number of membrane proteins are allosterically regulated by interactions with glycolipids. However, the exact molecular nature of the protein/lipid interactions underlying these regulatory effects is difficult to characterise. Recent studies have indicated that the glycosphingolipid GM3 forms specific interactions with growth factor receptors, such that GM3 inhibits the autophosphorylation of the receptor, and thus inhibits activation [4]. A specific interaction between EGFR and GM3 has been suggested to involve a lysine side chain (Lys618) close to the start of the transmembrane helix of EGFR interacting electrostatically with the anionic N-acetyl neuraminic acid moiety of GM3 [4]. Similarly, glycolipids are implicated in interactions that modulate insulin receptors [3], integrins [29], and nerve growth-factor receptors (NGFR) [30].

4. Atomistic resolution glycolipid simulations

Atomistic force fields for simulating lipids and for simulating carbohydrates have existed for some time [31]. More recently progress has been made in combining these to enable simulations of glycolipids [32]. Thus, atomistic simulations of a bilayer containing large numbers of the glycolipid GM3 have been performed [33]. Related atomistic simulations have been performed on glycolipids such as GM1 [34], and GM3 [12] embedded in model phospholipid bilayers. However, atomistic simulations have yet to address more complex models of biological membranes containing multiple lipid species.

5. Reduced representation glycolipid simulations

As mentioned above, CG-MD methods enable simulation of large bilayer systems on timescales of microseconds or more. A number of different approaches to coarse graining of lipids have been developed [35,36]. Here we focus on the widely employed MARTINI model [15]. The MARTINI model employs an approximately 4:1 mapping between (non-hydrogen) atoms and CG particles. This allows overall structural properties of lipids to be retained, whilst the nature of the lipid/lipid interactions are simplified, and the resultant energy landscape somewhat smoothed, allowing simulation of long timescale behaviour. As a consequence of this degree of simplification, simulations of millions of CG particles on microsecond timescales become practical, thus enabling simulations to address time and length scales directly comparable to those studied experimentally.

The MARTINI force field for simulation of lipids has been well established and widely employed for simpler membrane compositions [37]. Studies of more complex (and biologically relevant) mixed lipid bilayer systems are now becoming possible [38]. We now present a methodology for the development of CG models of glycolipids, and illustrate how we have used these CG models to simulate membranes of complex composition, and to explore protein-membrane interactions.

6. Developing glycolipid parameters

The first step in the development of a CG model of a glycolipid was to convert from an atomistic structure to an equivalent CG representation. In this process, using the approximate 4:1 mapping scheme on which MARTINI is based, the carbohydrate rings of the glycan headgroup of the lipid are represented as triangles of three CG particles, with the addition of a central massless particle which enables each sugar ring to be flexibly linked to its neighbour. This is illustrated for the simple ganglioside GM3 in Fig. 1, where the atomistic structure is shown alongside the CG model. Parameters for the CG models were then generated through comparison to atomistic simulations [10]. Parameters were adjusted so that inter-ring distances and angles in CG simulations of a single GM3 molecule matched the equivalent distances within atomistic simulations [31].

7. Setting up of complex systems

The initial step in the setup of complex membrane systems is achieved *via* the process of self-assembly of a POPC bilayer, either as a pure membrane or with proteins embedded. In a self-assembly system, lipid molecules are randomly placed in a solvated box of appropriate size, and molecular dynamics are applied. The properties of the lipid molecules are such that (as in biology), they will self-assemble into bilayers. This method works well for symmetrical membranes, as generally the lipid species will become evenly distributed across both sides of the bilayer. Asymmetric membranes however, are more complicated to produce. A recently developed method [10] allows us to obtain an asymmetric complex membrane based on a preformed POPC membrane. In short, the desired composition is obtained by exchanging random POPC molecules within each leaflet either by renaming, or alignment of bulkier lipids (such as GM3). The system is then energy minimized, and water and ions added before molecular dynamics simulations are run. An example of a small section from a mammalian plasma membrane model can be seen in Fig. 2. Each lipid species headgroup is coloured separately, with tails all shown in white. Using this method,

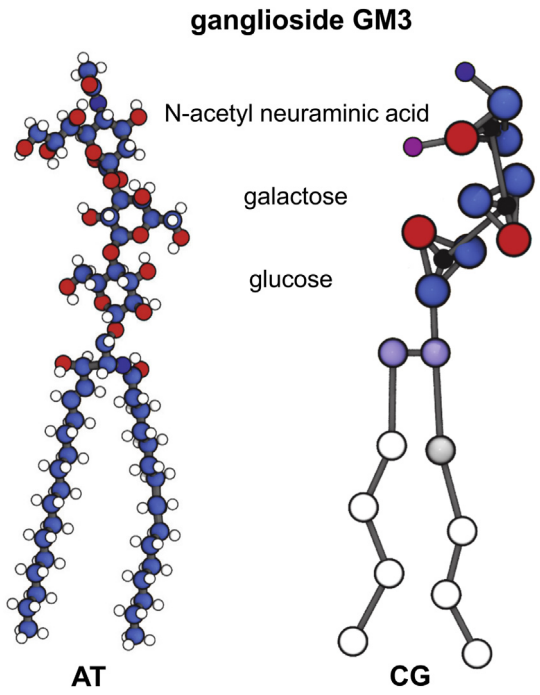

Fig. 1. The monosialodihexosylganglioside GM3, shown as atomistic (AT) and coarse-grained (CG) structures. The AT structure was generated using the *tleap* subprogram of AMBER [47], using the GLYCAM force field [31,32]. The CG structure shows the three particle rings used to model each carbohydrate of the glycan headgroup, along with the "rotational node" particles in the centre of each ring (black). Colours represent different MARTINI [15] particle types within the headgroup: blue = slightly polar particles; red = polar/charged particles; black = massless particles defining the centres of the rings.

complex, asymmetrical, and hence more biologically relevant membrane systems can be quickly set up and simulated.

8. Current applications of CG-MD to complex membrane systems

Simulations of model, mixed lipid bilayers that approximate biological membranes have provided insights into the lipid/lipid interactions that underlie their dynamic and structural complexity. For example, simulations of a model mammalian plasma membrane [10] have revealed that a number of lipid species, notably the glycolipid GM3, cluster together by forming self-associating networks and that the formation of such nanoscale clusters correlates with spontaneous membrane curvature. Simulations of the membrane envelope of the influenza A virion has shown that glycolipids have a stabilizing effect on the biophysical properties of the membranes [39], and in particular that the bulky glycolipid headgroups interact so as to reduce mobility of both lipids and proteins within the viral membrane, thus contributing to the structural robustness of the virion to changes in environmental conditions.

Specific interactions of membrane proteins with lipids may also be probed using CG-MD [40–43]. For example, this has been applied to the interactions of PIP_2 with the transmembrane (TM) helix dimer of the EGFR [44]. We can also use this approach to probe the interactions of GM3 with the EGFR, which as noted above is of functional importance in allosteric regulation of the receptor. An NMR-derived structure (PDB id: 2M20) of the dimeric TM domain of the EGFR receptor (consisting of residues 618–645) was simulated in an asymmetric lipid bilayer model, representative of the lipid composition of a mammalian plasma membrane. Thus the lipid composition of the outer (i.e. extracellular) leaflet of the bilayer was PC:PE:Sph:GM3:cholesterol = 40:10:15:10:25, and for the inner leaflet was PC:PE:Sph:GM3:cholesterol = 10:40:15:10:25 [10]. A CG-MD simulation of a 15 × 15 nm bilayer containing 700 lipid molecules

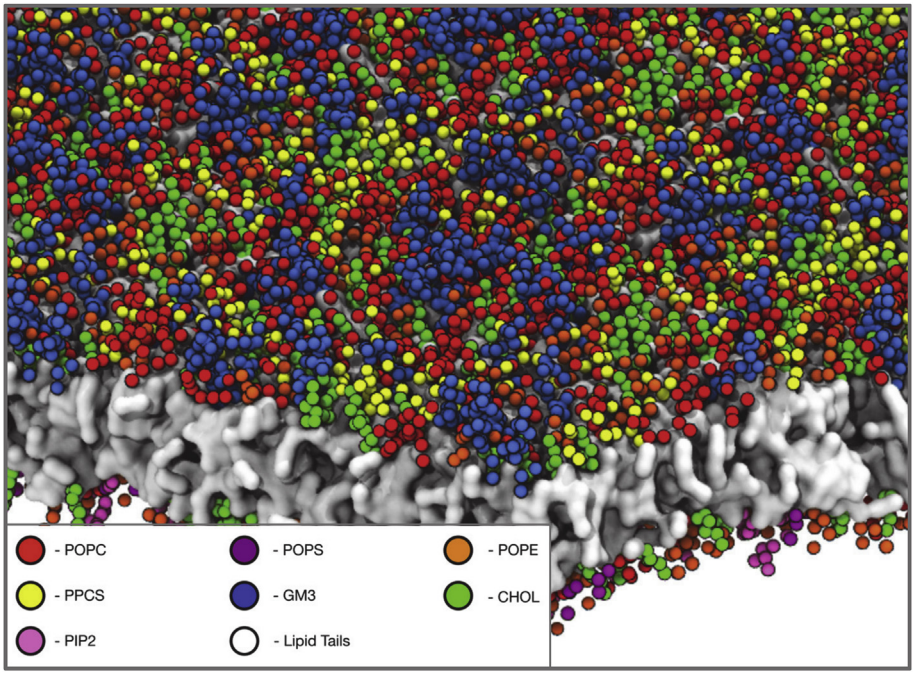

Fig. 2. Snapshot from a CG-MD simulation of a model of the lipid bilayer component of a mammalian cell membrane, with the glycolipid GM3 shown in blue. The system is 15 × 15 nm in size, and contains 700 lipids.

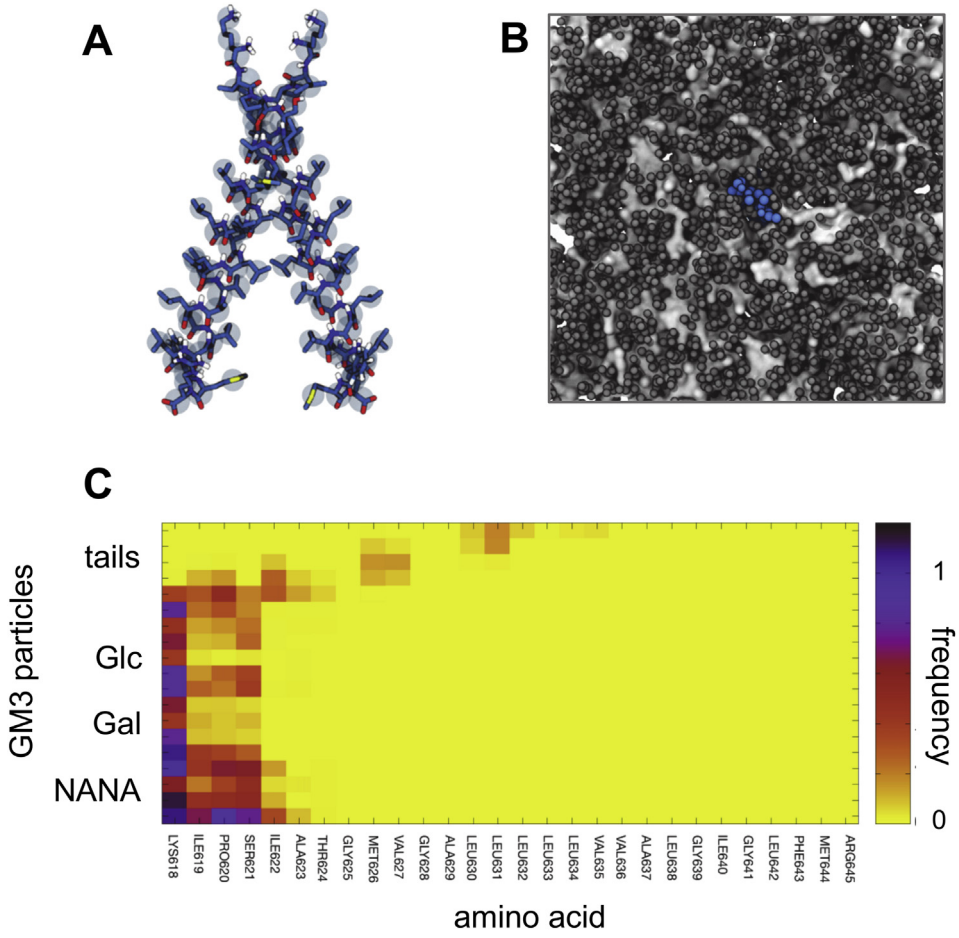

Fig. 3. Analysis of protein/lipid interactions using CG-MD simulations of a membrane protein in a model mammalian cell membrane. (A) Structure of the transmembrane helix dimer of the EGF receptor, a membrane protein known to interact with the glycolipid GM3. The NMR determined structure (PDB id 2M20) is shown in conventional 'bonds' format and the derived CG model as transparent grey spheres. (B) Lipid bilayer (grey) containing the TM helix dimer (blue), taken from a 2 μs duration CG-MD simulation. (C) Fractional interactions (on a yellow to blue heat map) of the CG particles within GM3 (vertical axis) along the length of the TM helix (horizontal axis).

of duration 2 μs was performed, and the interactions of lipids with the EGFR TM domain were analysed (Fig. 3). The results indicate persistent interactions between the residues at the N-termini of the TM helices and GM3, and in particular of residue Lys618 which has been implicated by mutational studies as playing a key role in EGFR/GM3 interactions [4]. Having identified the key role of this residue in interactions between the EGFR TM domain and glycolipids, it will now be possible to employ more extensive simulation studies, both CG and atomistic, to characterise in detail the nature of the protein/glycolipid interactions (Koldsø et al., ms in preparation) and the free energy landscape underlying these interactions (Hedger et al., ms. in preparation).

9. Conclusions

Recent advances in CG methodologies, and in particular the development of a CG model for simple glycolipid species, have extended simulation studies to models of mixed lipid bilayers whose composition reflects the complexities of biological membranes. This in turn allows us to probe the dynamic interactions between membrane proteins and glycolipids, enabling us to better understand e.g. the structural basis of regulation of EGFR and related receptors by gangliosides such as GM3. Current developments [45] are extending the available repertoire of lipids for which CG models are available. This will allow the simulation of more complex and biologically realistic membranes, providing further insights into how the interplay of lipid/lipid and lipid/protein interactions determine the structural and dynamic properties of complex and crowded [46] biological membranes.

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