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A MARTINI extension for *Pseudomonas aeruginosa* PAO1 lipopolysaccharide

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ABSTRACT

We report a course-grained, large scale simulation of the outer membrane from *Pseudomonas aeruginosa*. Using the MARTINI force field approach of 4-to-1 atom mapping, we simulate an asymmetrically constructed bilayer with over 1100 rough lipopolysaccharide (LPS) and 3100 16:0-18:1-phosphatidylethanolamine. We achieve 90-fold improvement in computational efficiency on a system much larger than reasonable for all-atom simulation. We also compare a coarse-grained LPS/LPS bilayer simulation with known parameters determined from neutron diffraction.

1. Introduction

Pseudomonas aeruginosa is a gram-negative bacterium, often found in soil and plants, which is a known human pathogen. It targets those who are immunodeficient or otherwise compromised, causing infection [1,2]. *P. aeruginosa* has been shown to be non-susceptible to all but a select few antibiotics [3]. This is a characteristic of many gram negative bacteria due to their unique composition and electrostatics of their cell envelope.

Gram negative bacteria have a cell envelope consisting of an inner membrane, periplasm and an outer membrane. The outer membrane is an asymmetric membrane with a large component of the outer leaflet being lipopolysaccharide (LPS) which can cover about 75% of the outer surface [4,5]. The inner leaflet is composed of a mixture of phospholipids, with different headgroup and hydrocarbon tail chain lengths. Analysis of the outer membrane has determined that the most prominent headgroup is phosphoethanolamine (PE), and the most common tails are 18:1 and 16:0 [6,7].

LPS as its name suggests, is a molecule with a mixture of both lipid and sugar components. Many different forms of the LPS molecule exist in nature but all with the similar general form. LPS consists of lipid A which is composed of 5–6 fatty acid acyl tails attached to a disaccharide backbone which create a hydrophobic region which acts as the anchor into the membrane. Attached to the lipid A is the core oligosaccharide which consists of short chain of sugars and has a large amount of negative charge. There is then the O polysaccharide side chain, a chain of repeating sugar chains which vary in length from 1 to 30 units depending on the bacteria and environment [8].

Molecular dynamics (MD) simulations are currently a very powerful tool from observing the dynamics of biological systems. Many simulations provide detail at an atomic resolution using an all-atom (AA) approach. A number of AA LPS simulations have been reported for LPS, including those for *Escherichia coli* [9,10], and *P. aeruginosa* [11–13]. The largest of these simulations incorporate 72 LPS in a single bilayer leaflet.

While AA simulations have proven to be very effective, since a detailed and effective simulation of a cell membrane requires hundreds of thousands of atoms, AA simulations are limited to small system sizes and time scales limiting the sampling of conformal space. Coarse grain (CG) simulations have been created to overcome this limitation. The MARTINI force field has made tremendous progress in providing a useful method for studying the mechanics of biomolecules on both large size and time scales [14]. CG models reduce the internal degrees of freedom of a molecule by averaging out some atomic detail, mapping approximately 4 heavy atoms and their associated hydrogen to 1 pseudo-atom. A key

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feature is the fair reproduction of structural and thermodynamic data [15,16]. The result is a system with much fewer atoms, making it considerably more computationally efficient.

The MARTINI force field has a robust collection of phospholipids and sterols for simulation, and has been extended to both carbohydrates [17] and glycolipids [18], and importantly now includes polarizable water [19]. However, to the best of our knowledge, only a single CG force field for LPS has been developed [20], which has been tested only for non-polarized water and uses DPPE (16:0,16:0 PE) as the base layer, which does not incorporate the effects of the abundant unsaturated lipid tails present in *P. aeruginosa* [6,7]. A model for LPS has been cited as an important missing element of the MARTINI collection [21,15].

A single *P. aeruginosa* cell is pill shaped, approximately 0.5–1 μm wide and 1–5 μm tall. If this cell was coated in LPS, this corresponds to approximately 100,000–1,000,000 LPS molecules. Current attempts to simulate AA LPS systems have been limited to very small systems due to the large size of the LPS molecule [10,13]. Therefore to simulate even the smallest of *P. aeruginosa* bacteria cells, an AA approach would be too computationally intensive to simulate on reasonable timescales.

In this report, we construct a MARTINI compatible LPS model from an AA simulation of the rough LPS from *P. aeruginosa* [13]. This requires construction of new carbohydrates and designing a new AA-to-CG atom mapping. Refining of bond and angle was undertaken to reproduce the calculated dynamics of the AA model. We are able to increase the size of the simulated membrane several fold, while improving computational efficiency. Finally, we compare our results to neutron scattering experiments on the structure of LPS.

2. Methods

2.1. MARTINI parametrization

The coarse graining of LPS was done using the bottom up approach, building on AA simulations, meaning that the AA LPS simulations were mapped into virtual particles at the location of the center of mass of the atom collection they represent. The final decision on the mapping is shown in the schematic of Fig. 1. In many regions, the MARTINI particles are entirely consistent with previous simulations of lipids and saccharides [14]. To provide the highly negative charge of the LPS molecule, eight of the particles were given a −1 charge corresponding to the most electronegative parts of the AA model, and the others were given no charge (see supplementary topology for complete particle assignments).

The bonded parameters for the CG force field are determined from these virtual mapped particles of the AA simulation, and are measured by constructing histograms of the bond length between virtual particles over the entire simulation. The height and peak center of these histograms represent the equilibrium length and spring strength for that bond. The same is done with angles between triplets of virtual atoms in the simulations. Dihedrals however, were omitted as explained in Section 3. The construction of the AA membrane simulations used for parametrization are discussed below.

Most often, the choice of virtual particles fortuitously led to a Gaussian-shaped single peak, but this is not always guaranteed. Restrictions among competing bond/angle/dihedral parameters in the underlying AA force field can distort the histogram shape, or add second, smaller peak values. However, the force field between virtual particles used was always harmonic, and cannot reproduce overly complex distributions. However, the non-Gaussian distributions account for a small fraction of the model, and can still be fit with a Gaussian to capture the majority of its conformational distributions. Thus all recorded histograms were fit to single Gaussian curves using the *fityk* program [22]. If the distribution was split into more than a single peak, the most prominent peak was used.

The height and center of these Gaussian curves were then used as the standard in which to match during a simulation utilizing the newly made CG force field. The strength and center of the harmonic bond and angle potentials were iteratively adjusted until their distribution matched that of the virtual particles from the AA simulation. The first set of iterations were performed on a symmetric LPS bilayer, so that any changes in the per-lipid area was reflected on both sides of the membrane equally, and therefore the

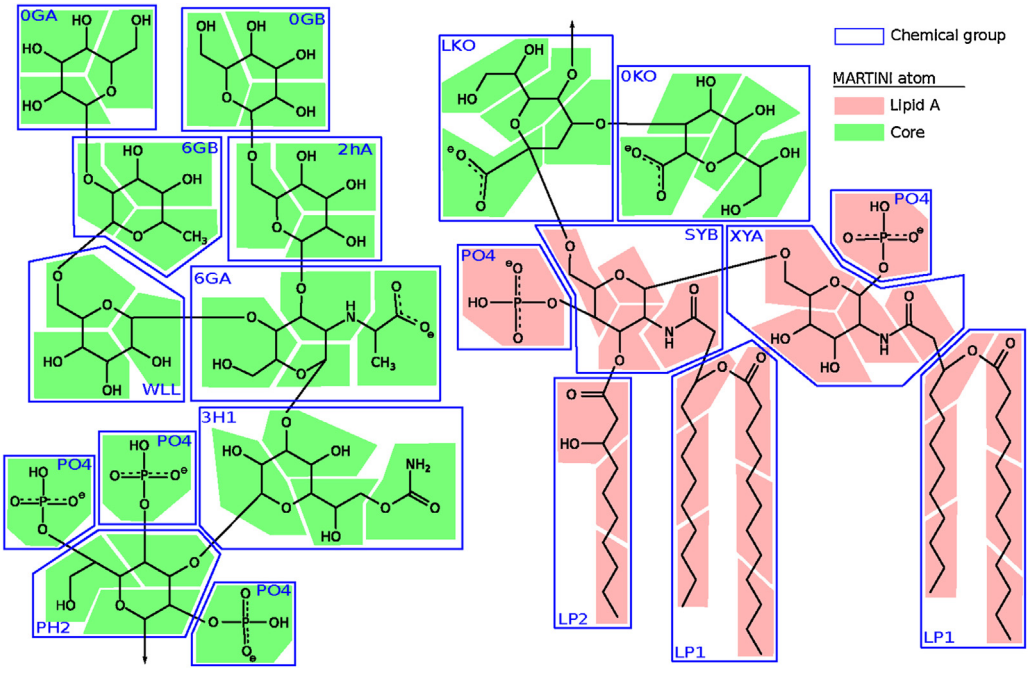

Fig. 1. Schematic of the lipopolysaccharide of *P. aeruginosa* used for simulation. Residues and groups are drawn in boxes, with the naming convention used by Kirschner et al. [13]. Shaded areas indicate coarse grain atom groupings.