Bioinformatics, 31(17), 2015, 2897–2899
doi: 10.1093/bioinformatics/btv292
Advance Access Publication Date: 7 May 2015
Applications Note

OXFORD

Structural bioinformatics

MemGen: a general web server for the setup of lipid membrane simulation systems

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Associate Editor: Anna Tramontano

Received on March 4, 2015; revised on April 28, 2015; accepted on May 2, 2015

Abstract

Motivation: Molecular dynamics simulations provide atomic insight into the physicochemical characteristics of lipid membranes and hence, a wide range of force field families capable of modelling various lipid types have been developed in recent years. To model membranes in a biologically realistic lipid composition, simulation systems containing multiple different lipids must be assembled.

Results: We present a new web service called MemGen that is capable of setting up simulation systems of heterogenous lipid membranes. MemGen is not restricted to certain lipid force fields or lipid types, but instead builds membranes from uploaded structure files which may contain any kind of amphiphilic molecule. MemGen works with any all-atom or united-atom lipid representation.

Availability and implementation: MemGen is freely available without registration at http://memgen.uni-goettingen.de.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Molecular dynamics (MD) simulations of lipid membranes have come of age. Since the first simulation of a bilayer membrane more than 30 years ago (Van der Ploeg and Berendsen, 1982), MD simulations have been used to study various processes at atomic or near-atomic resolutions such as lipid diffusion, solute permeation, electroporation, undulation and phase transition. In addition, they are required to provide a realistic environment for simulations of membrane proteins. To this end, a range of lipid force fields have been developed which reproduce membrane parameters such as the area per lipid, order parameters and X-ray scattering factors. Atomistic lipid force fields can be separated into united-atom (Berger *et al.*, 1997; Chiu *et al.*, 2009; Piggot *et al.*, 2011; Ulmschneider and Ulmschneider, 2009) and all-atom force fields (Dickson *et al.*, 2012; Jämbeck and Lyubartsev, 2012; Maciejewski *et al.*, 2014; Pastor and MacKerell, Jr., 2011), which differ in the modelling of apolar hydrogen atoms. Hence, force field development is an active field of research, and more force field families covering a wider range of lipid types are expected to be published in the near future.

More recently, because biological membranes contain thousands of different lipid types, the interest in simulations of heterogenous membranes has increased (Róg *et al.*, 2009; Zocher *et al.*, 2013). Setting up such heterogenous membranes can be tedious. Upon building such membranes, atomic overlaps between neighbouring lipids must be strictly avoided. Otherwise, minimizing the energy of the sytem fails, leading to numerical instabilities in the first simulation steps. To our knowledge, two web services have so far been developed to facilitate the setup of membrane simulation systems, namely CHARMM-GUI and Membuilder (Ghahremanpour *et al.*, 2014; Jo *et al.*, 2009). These services allow the user to choose from a list of lipid types and force fields, which are kept on their respective servers. Hence, these services require frequent maintenance upon publication of new lipid types and force fields. In addition, they do not allow the insertion of molecules that are not available server-side.

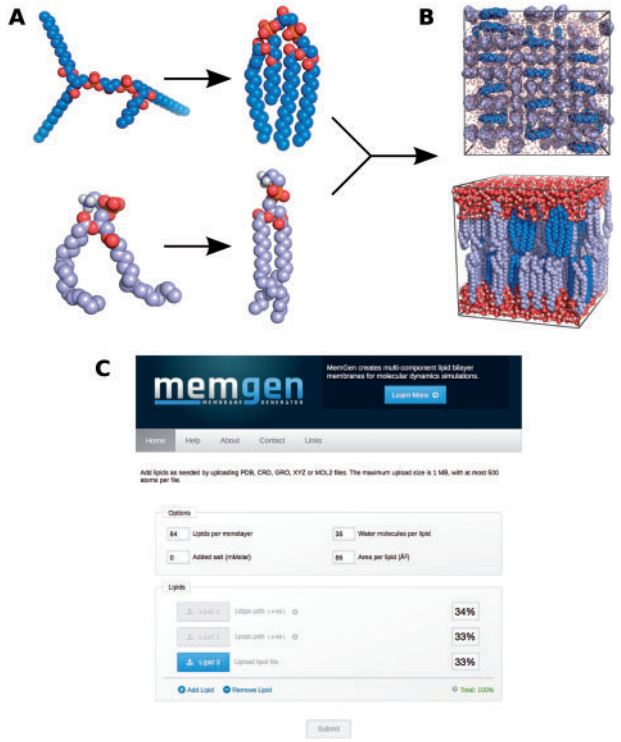

Fig. 1. Setup of a membrane simulation system by MemGen. (**A**) A compact representation of the lipids is created, allowing the setup of a membrane without atomic overlaps. (**B**) Membrane simulation system containing cardiolipin (dark blue), POPE (light blue) and water (red/white). Top: top view; bottom: side view. (**C**) Screenshot of MemGen

Following an alternative approach, we present the new web server MemGen that automatically sets up lipid membrane simulation systems. The server is not restricted to certain force fields, lipid types, or specific MD simulation software. The user uploads one or more lipid structure files (http://memgen.uni-goettingen.de). Alternatively, any amphiphilic molecule such as an alcohol or detergent may be uploaded. Subsequently, the server generates a compact representation of each lipid that is aligned along the *z*-axis (Fig. 1A), to avoid atomic overlaps in the assembled membrane. After building the membrane with the requested molar fractions of each lipid, the server hydrates the membrane with a requested number of water molecules per lipid. Eventually, counter ions and, optionally, sodium chloride is added (Fig. 1B). The final structure is provided for download in PDB format, which can be processed by any MD software.

2 Methods

In order to generate a compact representation of an uploaded lipid, a force field topology of the lipids is required. To this end, MemGen builds a GAFF topology of each lipid using ACPYPE, which builds upon the Antechamber software (da Silva and Vranken, 2012; Wang *et al.*, 2004, 2006). Because GAFF requires an all-atom representation, hydrogen atoms are added with Open Babel if the user uploads a united-atom lipid (O'Boyle *et al.*, 2011). By analysing the bond structure of the lipid, MemGen detects the terminal atoms of the lipid tails, as well as a central head group atom. Subsequently, using the Gromacs simulation software and applying a combination of simulated annealing, constant-force pulling on the head group and tail atoms, as well as position-restraining potentials, a highly compact representation of the lipid is created (Fig. 1A) (Pronk *et al.*, 2013). Hydrogen atoms added by Open Babel are removed.

A lipid monolayer is generated by placing the compact representations of the lipids on a square grid, and two such monolayers are assembled to build the bilayer (Fig. 1B). To release some strain from the highly ordered lipids, a 1.2-picosecond simulation is conducted at 300 Kelvin with position-restraining potentials on the terminal tail atoms. The requested number of water molecules per lipid are added. The charge of the lipid is determined by counting the number of amine, phosphate and carboxyl groups. Accordingly, counter ions are added to neutralize the simulation cell. If requested, additional salt is added.

3 Discussion

We tested MemGen using lipids from a wide range of all-atom and united-atom force fields, including various sterol, detergent and alcohol molecules (see Supporting Discussion). Tested lipids contained between one and six lipid tails and different head group types. The energy minization and equilibration of the systems were numerically stable. An example is shown in supplementary Figure S1 in the supporting material. It is important to note that MemGen provides a highly ordered and hence, unphysical initial configuration, which requires careful equilibration for at least 10 nanoseconds. MemGen does not provide or maintain lipid force field files. These must be taken from the force field developers' web sites, or from repositories such as LipidBook (Domański *et al.*, 2010).

By design, MemGen does not generate asymmetric bilayers with differing compositions of the two monolayers. Because the areas per lipid are not additive in lipid mixtures, it is difficult to predict the equilibrated membrane area of some lipid mixtures. However, since membranes are nearly incompressible, any area mismatch between the two monolayers results in large lateral pressures. To set up such asymmetric membranes, the user may first equilibrate two symmetric systems, removing an appropriate number of lipids manually to achieve the same equilibrium area, and subsequently merge one leaflet from each simulation (Gurtovenko and Vattulainen, 2008).

Funding

This project was supported by the Deutsche Forschungsgemeinschaft (HU 1971/1-1).

Conflict of Interest: none declared.

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