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MEMBPLUGIN: studying membrane complexity in VMD

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ABSTRACT

Summary: Computer simulations are giving way to more complex and accurate studies of biological membranes by molecular dynamics (MD) simulations. The analysis of MD trajectories comprises the biophysical characterization of membrane properties or the study of protein–lipid interactions and dynamics. However, there is a lack of automated tools to analyse MD simulations of complex membrane or membrane-protein systems. Here we present MEMBPLUGIN, a plugin for the Visual Molecular Dynamics package that provides algorithms to measure a host of essential biophysical properties in simulated membranes. MEMBPLUGIN features are accessible both through a user-friendly graphical interface and as command-line procedures to be invoked in analysis scripts.

Availability and implementation: MEMBPLUGIN is a VMD extension written in Tcl. Multi-platform source code, documentation and tutorials are freely available at http://membplugin.sourceforge.net.

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1 INTRODUCTION

Although membrane lipids were previously thought to only play a passive role in cellular processes, it is now known that they actively modulate key cell membrane complexes such as lipid rafts (Simons and Gerl, 2010). As a result, some lipids can regulate protein functionality (daCosta *et al.,* 2013; Sadiq *et al.,* 2013) and some are involved in severe diseases (Fabelo *et al.,* 2011). The experimental and computational study of membrane lipids is an emergent field of research (van Meer *et al.,* 2008); thanks to the refinement of lipid force field parameters, modern membrane simulations and analysis tools (Supplementary Table S1) are able to yield accurate biophysical properties close to experimental values (Klauda *et al.,* 2010; Jo *et al.,* 2009) and observables that would otherwise be out of reach of experimental techniques.

Here we describe MEMBPLUGIN, an extension to the free molecular visualization program Visual Molecular Dynamics (VMD) (Humphrey *et al.,* 1996) to study the biophysical properties of membranes and membrane–protein interactions via molecular dynamics (MD) simulations.

2 METHODS AND FEATURES

The first release of MEMBPLUGIN provides five different components for the analysis of planar bilayers, namely, the *Order Parameters*, *Membrane Thickness*, *Lipid Interdigitation*, *Area per Lipid* and *Tilt Angle* tools (Fig. 1A).

(1) The S_{CD} *Order Parameter* tool (Fig. 1B) calculates the carbon–deuterium order parameter, a value typically derived in NMR experiments that reflects the orientational mobility of each C-H bond along the aliphatic lipid tails, and thus membrane fluidity (Vermeer *et al.,* 2007): $S_{CD} = -\frac{1}{2}\langle 3\cos^2\theta - 1\rangle$, where θ is the instantaneous angle between the C-H bond and the bilayer normal.

(2) The *Membrane Thickness* tool measures the distance w between two density peaks along with the location of the middle point between them, z_0, formalized as the first and second central moment of the mass density profile of a chosen atom (i.e. phosphorus) along the membrane normal. In addition, this tool can interpolate the lipid head's positions to compute a 2D thickness map, which provides an average overview of the local membrane deformation along the simulation (Fig. 1C and D).

(3) The *Lipid Interdigitation* tool computes three different acyl chain interdigitation measures, namely, I_ρ, w_ρ and I_C. I_ρ is a correlation-based fraction that evaluates the overlap of the two leaflets' mass distributions along the membrane normal: $I_\rho^2 = 4\int \rho_a(z)\rho_b(z)dz / \int [\rho_a(z) + \rho_b(z)]^2 dz$, where ρ_a and ρ_b represent the mass density profiles for each of the leaflets. The definition ensures that $0 \leq I_\rho \leq 1$ (no to complete mass overlap). w_ρ is defined as the second central moment of $\rho_a\rho_b$, indicates the width of the mass distributions overlap region. Finally, I_C is a coordination-based fraction built by counting the number of atoms that are in contact with the opposite leaflet; contacts are defined geometrically for a chosen set of atoms and cut-off distance (e.g. 4 Å between heavy atoms).

(4) The *Area per Lipid* tool (Fig. 1E) calculates both the total area per lipid and the area per lipid of each lipid species of

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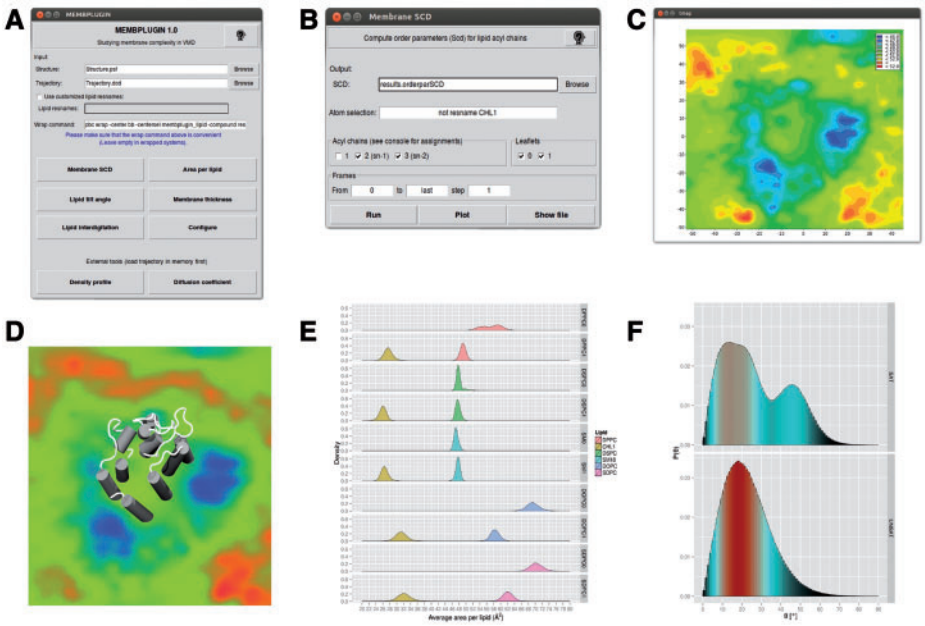

Fig. 1. Views from a sample MEMBPLUGIN-based analysis session. (**A**) The tool manager. (**B**) S_{CD} order parameter tool GUI. (**C**) A 2D local thickness map of a lipid bilayer in a membrane-protein simulation. (**D**) Average volumetric map of the local membrane thickness around the receptor in the same simulation. (**E**) Area per lipid distributions: results for pure (labels ending in 0) and 33% cholesterol binary mixtures (labels ending in 1). DPPC, DSPC, SM18, DOPC, SDPC and CHL1 stand for $diC_{16:0}$, $diC_{18:0}$, C_{18}-sphingomyelin, $diC_{18:2}$, $C_{22:6}$-$C_{18:0}$ and cholesterol, respectively. (**F**) Cholesterol tilt angle distributions for two different membrane systems: UNSAT, rich in unsaturates and SAT, rich in saturates. The R ggplot2 library (Wickham, 2009) was used to render plots (E) and (F) out of MEMBPLUGIN's output

the membrane under analysis. To this end, it uses a user-customizable selection of one key atom (e.g. sterols) or a triad of atoms (e.g. phospho- or sphingolipids). The *x* and *y* coordinates of the former set of points are projected onto a plane delimited by the simulation box, which is subsequently divided into polygons through a Voronoi diagram using the *qvoronoi* program from the Qhull package (Barber *et al.*, 1996). Thereby, the area of each polygon is calculated.

(5) Finally, the *Tilt Angle* tool (Fig. 1F) computes the angle θ between any user-defined vector (i.e. two atoms per lipid species) and the membrane normal.

MEMBPLUGIN is used within VMD either in a mouse-driven interactive fashion, or procedurally in user-written scripts. (i) *Interactively*: after installation, the plugin becomes available in VMD's *Analysis* menu. MEMBPLUGIN's manager provides access to the analysis tools (Fig. 1A) and the corresponding tool-specific settings (e.g. the set of atoms making up the lipids for the system at hand). (ii) *Procedurally*: consistent with VMD's framework, each tool can be invoked as a Tcl command. Therefore, scripts have access to the analytical features that enable construction of complex workflows in a high-level language.

3 CONCLUSION

MEMBPLUGIN automates the analysis of simulated lipid bilayers through the calculation and visualization of a variety of biophysical parameters commonly used in membrane and membrane-protein simulations. It is worth noting that this first release of MEMBPLUGIN provides convenient access to both standard (e.g. order parameter, thickness maps) and novel algorithms (e.g. measures of interdigitation). Despite the fact that MD results are limited by the accuracy of simulations and thus must be interpreted with caution, especially in large heterogeneous systems, they can provide good estimations of parameters otherwise not measurable in experiments.

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