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Probing microscopic material properties inside simulated membranes through spatially resolved three-dimensional local pressure fields and surface tensions

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ABSTRACT

Cellular lipid membranes are spatially inhomogeneous soft materials. Materials properties such as pressure and surface tension thus show important microscopic-scale variation that is critical to many biological functions. We present a means to calculate pressure and surface tension in a 3D-resolved manner within molecular-dynamics simulations and show how such measurements can yield important insight. We also present the first corrections to local virial and pressure fields to account for the constraints typically used in lipid simulations that otherwise cause problems in highly oriented systems such as bilayers. Based on simulations of an asymmetric bacterial ion channel in a POPC bilayer, we demonstrate how 3D-resolved pressure can probe for both short-range and long-range effects from the protein on the membrane environment. We also show how surface tension is a sensitive metric for inter-leaflet equilibrium and can be used to detect even subtle imbalances between bilayer leaflets in a membrane-protein simulation. Since surface tension is known to modulate the function of many proteins, this effect is an important consideration for predictions of ion channel function. We outline a strategy by which our local pressure measurements, which we make available within a version of the GROMACS simulation package, may be used to design optimally equilibrated membrane-protein simulations.

1. Introduction

Pressure and surface tension are important macroscopic observables of soft materials such as lipid membranes. These properties can be used to explain processes like phase separation, micelle formation, and the structure and assembly of biological membranes. Although pressure and surface tension are ensemble averages, they are not spatially uniform, and local variations often play a critical role in membrane structure. Protein insertion or membrane deformation causes inhomogeneous and often asymmetric perturbation in local pressure, and the surface tension can also undergo substantial local fluctuation; the magnitude of these fluctuations may be important for lipid–protein interactions and membrane shape changes. For instance, antimicrobial peptides are thought to induce lateral pressure anomalies as part of their activity (Liu and DeGrado, 2001; Schibli et al., 2002; Tieleman et al., 2003; Zhao et al., 2002), and mechanosensitive channels are responsive to pressure changes (Gullingsrud and Schulten, 2004; Moe and Blount, 2005; Perozo et al., 2002; Weiss et al., 2003). These quantities are extremely difficult to measure with high spatial resolution via experiment, although macroscopic measurements of their average are feasible.

Due to the magnitude of the fluctuations involved, substantial sampling is required to achieve converged predictions of surface tension via simulation. Until recently, this sampling requirement and the computational power available dictated heavy spatial averaging for computational predictions of local pressure (Lindahl and Edholm, 2000b). Recent advances in computational capacity and parallel simulation technology have enabled much more extensive simulation sampling of membranes on a routine basis.

The ability to measure pressure in a 3D-resolved manner presents several advantages. It enables the evaluation of spatially inhomogeneous phenomena such as the behavior of bilayer lipids around proteins. It also enables the measurement of local fluctuations in a simulated system and the relation of surface tension fluctuations to changes in bilayer structural properties. In more complex lipid mixtures, it enables the measurement of surface tension in regions of composition fluctuation, a quantity believed important to the formation of lipid microdomains or

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cholesterol–lipid complexes (Baumgart et al., 2003; Honerkamp-Smith et al., 2008; Rietveld and Simons, 1998).

However, local pressure itself is not trivial to calculate: even the first calculations on ordered systems (Lindahl and Edholm, 2000b) showed that the *z*-component was not constant, which it should be at equilibrium. Part of this is due to finite molecular size being a natural limit to the resolution, but there have also been methodological issues where bond length (and potentially other) constraints in particular appear to introduce artifacts in non-isotropic systems such as membranes.

Here, we present a means to calculate 3D-resolved *local pressure fields* and surface tension in simulations that is a substantial advance over the traditional 1D profiles previously used by us and others. We also present a new correction to correctly account for constraints in local virials and thus spatially resolved pressure fields. We have integrated this measurement capability into a publicly available special version of the GROMACS molecular simulation software. In this work we employ atomistic representations of lipids and proteins in these simulations to maximize quantitative reproducibility of experimental phenomena, particularly those relating to lipid tail order and protein conformational dynamics. Calculations using an early version of our code without the constraints corrections were previously reported for coarse-grained lipid systems (Ollila et al., 2009). The present implementation has explicitly been designed to support virtually any force field based on pairwise interactions, including atomistic representations, tabulated forms and coarse-grained representations – and it will provide accurate results for systems with constrained bond lengths We calculate the full 3D spatially resolved pressure tensors over arbitrary-size subcells of the triclinic box used to represent the system in GROMACS, enabling local pressure computation in non-rectangular geometries such as hexagonal boxes, truncated octahedra, or rhombic dodecahedra. Local differential pressure along any surface can easily be calculated as the difference between the tangential and normal components of the local pressure, for instance along a vesicle surface or local membrane patch. The surface tension is simply the integral of this quantity across a leaflet or bilayer.

We report the use of this technical advance to measure local pressure and surface tension fluctuations within simulations of large fluid bilayers and the local perturbation of bilayer properties by transmembrane proteins. In particular, resolving local pressure beyond simple profiles provides a means to assess long-range effects from membrane perturbations and to guide inter-leaflet membrane equilibration; we present the use of local differential pressure measurements as an efficient and accurate way to direct the construction of membrane-protein simulations with the optimal number of lipids in each leaflet.

2. Theory

Pressure is formally an ensemble property of a system corresponding to the surface integral of the external force. This is not directly accessible in a molecular simulation but can be calculated by using the virial theorem (Hansen and McDonald, 1976):

$$2\mathbf{K} = \Xi \tag{1}$$

where $\mathbf{K}$ is the full average kinetic energy tensor, and Ξ is the virial

$$\Xi = -\sum_{i=1}^{N} \mathbf{r}_i \otimes \mathbf{F}_i \tag{2}$$

where $\mathbf{F}_i$ is the force on particle i located at position $\mathbf{r}_i$. This total virial has an internal component from intra-system forces

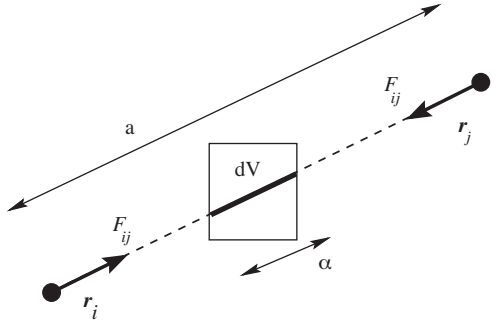

Fig. 1. The configurational part of the local pressure is evaluated as the projection of the pairwise virial on subvolumes (3D grid cells). If the force between particles i and j is $\mathbf{F}_{ij}$, the interaction contribution to the local virial in this cell will be $\Xi_{ij} = -(\alpha/a)\mathbf{r}_{ij} \otimes \mathbf{F}_{ij}$.

(available in the simulation) and an external contribution caused by the normal pressure p_N on the cell surface S,

$$\Xi_{\text{int}} = -\sum_{i=1}^{N} \mathbf{r}_i \otimes \mathbf{F}_i^{\text{int}} \tag{3}$$

$$\Xi_{\text{ext}} = \int_S p_N \mathbf{r} \otimes d\mathbf{S} \tag{4}$$

From this it is possible to solve for the pressure tensor and get an expression that can be evaluated in a simulation

$$\mathbf{p} = 2\frac{\mathbf{K} - \Xi_{\text{int}}}{V} \tag{5}$$

where V is the volume of the system. While formally still an ensemble property, this expression can be extended to microscopic subsystems. The kinetic energy tensor reduces to a sum over the particles present in such a subvolume. In the case of central pair-additive potentials for particle interactions, the internal virial can be written on the Irving–Kirkwood contour form (Irving and Kirkwood, 1950):

$$\Xi_{\text{int}} = -\sum_{i<j} \mathbf{r}_{ij} \otimes \left(-\mathbf{F}_{ij}\right) \int_0^1 \delta\left[\mathbf{R} - \lambda\mathbf{r}_j - (1-\lambda)\mathbf{r}_i\right] d\lambda \tag{6}$$

This amounts to tracing a linear path between each pair of interacting particles, dividing the virial uniformly along the path, and obtaining the total virial as a path integral. This definition of the virial can obviously be made local by limiting the integral to the part of the path that falls in a particular volume (Fig. 1). Unfortunately this approach does not readily extend to lattice summation methods where an interaction cannot be attributed to a specific path between pairs of particles. Sonne et al. (2005) have shown that the alternative Harasima contour makes it possible to calculate the local component from classical Ewald summation, subject to certain assumptions. Due to the high $O(N^2)$ computational complexity this is not a realistic alternative for large simulations, but their tests on smaller reference systems confirmed that the Irving–Kirkwood result for medium to long cut-offs are very close to the Harasima/Ewald result.

2.1. Local virial calculation with bond constraints

The presence of bond constraints can lead to difficulties when calculating the local virial as defined above. Usually, the forces in a system with constraints are determined by first calculating the unconstrained forces as if no constraints were present in the system. During the application of the constraints, a correction to the