

New and Notable

Medium Matters: Order through Fluctuations?

Atul N. Parikh^{1,2,*}

¹Departments of Biomedical Engineering and Chemical Engineering & Materials Science, University of California, Davis, Davis, California; and ²Centre for Biomimetic Sensor Science, School of Materials Science & Engineering, Nanyang Technological University, Singapore

A fluid lipid bilayer in water fluctuates freely. Even in the absence of specific chemical interactions, a complex interplay of a variety of nonspecific forces—attractive and repulsive, short- and long-ranged—determine the equilibrium separation between these well-hydrated bilayers (1). It also plays critical roles in many biological processes, such as cell adhesion and membrane fusion, in which these surfaces are pushed closer together. Contributing to the interplay are coupled influences of the classical DLVO (named after Derjaguin, Landau, Verwey, and Overbeek, who described forces between small, smooth, and charged surfaces in water) and non-DLVO forces. These forces include a) the omnipresent van der Waals force, which provides a weak attraction in a relatively long-ranged manner, and b) the electrostatic double-layer forces between charged membranes. The non-DLVO forces, contributing to interbilayer interactions, include c) the so-called hydration force (a short-range, exponentially decaying repulsive interaction), which is thought to originate from surface-induced perturbation of water dipoles and its propagation away from the interface through water-water interactions (2), and d) the long-range repulsive Helfrich force, which arises from the

suppression of the free fluctuations of single bilayers, resulting in entropic loss when two membranes come closer together (3). While the first three direct molecular forces (a-c) can be treated independently and additively, the contributions from the fluctuation-induced Helfrich forces (d) are not readily separable, and couple to the other forces in subtle and complex ways.

The situation becomes exacerbated when the membrane's compositional degrees of freedom become involved. Inhomogeneous biological membranes, consisting of mixtures of saturated and unsaturated lipids together with cholesterol, often phase-separate into coexisting phases: a dense phase enriched in saturated lipid and cholesterol designated as the L_o (liquid-ordered) or raft phase and a second, less dense L_d (liquid-disordered) phase consisting predominantly of unsaturated lipid (4). Manifest as nanoscopic or microscopic domains, these coexisting phases are thought to be important biologically, providing the cell membrane with functional hotspots to spatially organize important biological functions, such as molecular recognition, signal transduction, and transport (4,5). The coexisting phases also have differing fluctuation properties, whose superimposition in single membranes and interlamellar ordering across membranes in multibilayers (6,7) further complicate the understanding (and predictions of) relative roles of fundamental surface forces above.

The very ordering of domains in multibilayers (6,7), however, also enables the application of the well-known osmotic stress technique (2), which allows us to then dissect the net intermembrane interactions in terms of fundamental forces. The technique is simple. Consider a solute, excluded from a spatial compartment within an aqueous continuum. It then exerts an osmotic pressure on the compartment by lowering the chemical activity of water on the “outside.” This in turn triggers a withdrawal of water from

the compartment, changing the size, shape, and hydration of the compartment itself (2). This universal osmotic force, in conjunction with accompanying activity of water, can drive conformational change in proteins, gate membrane channels, and mediate enzyme actions, giving the cell a global mechanism for regulating protein activity simply by modulating interactions between the macromolecule, solute, and water at the single molecule level (8).

Applied to multibilayers, the osmotic stress, such as by preferential exclusion of a solute (e.g., poly(ethylene) glycol or dextran) from interbilayer aqueous space, can force individual bilayers together below their equilibrium separation in pure water. This then provides a versatile experimental means to measure and quantify intermembrane forces (2). With increasing solute concentrations, the incipient reequilibration of the multibilayer, with solute-laden water of reduced chemical activity, monotonically reduces the thickness of the interlamellar water layer (d_w) and generates a repulsion or disjoining pressure equal to the osmotic pressure (P) of the solute. Cumulated weight of a significant amount of available data substantiate that this intermembrane repulsion is universal and short-range, growing exponentially ($A\exp(-d_w/\lambda)$) with a decay distance (λ) of $\sim 1-2$ Å, precluding molecular contact between approaching bilayers (2).

At sufficiently high osmotic stresses, the direct molecular forces adequately characterize fundamental forces at play in the limit of short intermembrane distances. At moderate to low osmotic stresses, by contrast, when intermembrane distances are larger—closer to equilibrium separation in pure water—bilayers are better-hydrated and Helfrich forces, due to bending-dominated, thermally excited membrane undulations, come into play (3). Under these

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*Correspondence: anparikh@ucdavis.edu

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conditions, coupling of the bare DLVO forces with Helfrich forces, controlled by bending rigidities (K_c), make predictions of the overall intermembrane interactions difficult. Previously, the contribution of membrane fluctuations to interbilayer repulsion has been theoretically treated in the mean-field limit, where undulatory effects are included in a self-consistent elastic potential (9).

Writing in this issue of the *Biophysical Journal*, Kollmitzer et al. (10) report an elegant study, which quantifies bending rigidities and intermembrane domain forces in situ for a phase-separated, ternary multibilayer system. By combining 1) osmotic stress small-angle x-ray scattering experiments, 2) Monte Carlo simulations with input parameters, namely K_c , λ , and A optimized against experiments, and 3) ab initio determination of van der Waals interactions (i.e., the Hamaker constant (H)), the authors circumvent the need for mean-field approximations. They quantitatively dissect total osmotic pressure (P) in terms of individual contributions from bare DLVO components, namely, hydration repulsion $A\exp(-d_w/\lambda)$ and van der Waals interactions $-H/6\pi d_w^3$, as well as the contribution from undulation interactions obtained by subtracting from the total pressure and fit to the exponential fall-off $A_{\text{und}}\exp(-d_w/\lambda_{\text{und}})$ for each of the two coexisting phases.

For study, the authors use a phase-separating ternary mixture consisting of DOPC (dioleoyl phosphatidylcholine), DSPC (distearoyl phosphatidylcholine), and Chol (cholesterol) at the molar ratio of 0.42:0.37:0.21. The multibilayer formed from this mixture is known to phase-separate into coexisting L_o and L_d phases, producing domains (11).

An important feature of phase-separating membrane multilayers is that the lateral intralayer phase separation couples with the interlayer smectic ordering, producing long-range alignment of these phase-separated domains, resulting in distinct Bragg

peaks (6,7). The full q -range small-angle x-ray scattering analysis of the shapes and positions of the Bragg peaks isolates individual contributions from each of the two coexisting phases, yielding both average bilayer periodicity (d) and mean-square fluctuations of the membrane spacing (Δ^2). Their Monte Carlo simulations—performed for a stack of eight bilayers of size 700×700 Å, discretized on a square $N \times N$ lattice (N varied from 6 to 32)—allows them to deduce spatially and temporally averaged intermembrane separation (d_w) and time-averaged fluctuations. These are subsequently fit to the corresponding experimental data to optimize the interaction parameters, namely the strength and range of hydration interactions as well as the bending rigidity characterizing the thermal undulations. For ab initio determination of van der Waals attraction, they calculate the H value using hydrocarbon multilayers for a range of bilayer heights (d_B , 45–60 Å) and interbilayer separations (d_w , 5–30 Å) in water.

They find that the bending rigidities of the L_o phase domains ($120 \pm 20 \times 10^{-21}$ J) are approximately three times higher than the L_d phase domains ($44 \pm 10 \times 10^{-21}$ J), with correspondingly higher fluctuations for the latter—as expected. Comparing relative contributions from individual components, they find noteworthy trends. The ab initio calculations of the constant H reveal only a small difference (3%), suggesting that the van der Waals attractions are comparable for L_o - L_o and L_d - L_d domains. At high osmotic stresses and small interbilayer separations the hydration forces are also comparable for the two phases, and, as expected, represent the dominant contribution. This is consistent with the high cost of removal of water from the hydration sites of polar headgroups for closely apposing bilayers (2). As the osmotic stress is reduced and bilayer separation increased, they find that the contribution from the membrane fluctuations begins to dominate for both the L_o and the L_d phases.

The crossover from hydration to fluctuation-dominated repulsion occurs at much smaller interbilayer separations for the L_d phase, albeit with a shorter decay length, which characterizes the range of interactions. This then suggests the preponderance of fluctuations over a wide range of bilayer separations for the structurally more disordered and laterally more fluid L_d phase domains. This work by Kollmitzer et al. (10) thus appears to better complete a quantitative description of fundamental surface forces that govern interactions between fluctuating membrane surfaces.

These findings—quantifying relative strengths and ranges of repulsive forces due to hydration and fluctuations, as well as van der Waals attraction—provide a solid starting point toward developing a quantitative model of how the balance of forces translates into lowering of the free energy that drives the long-range positional correlations between aligned domains of like phases in membrane multilayers (6).

As the authors aptly speculate, these findings might also offer a physical basis for appreciating how domain-domain interactions might facilitate segregation, patterning, and modulate binding constants of membrane-embedded receptors and ligands such as in intermembrane junctions characterizing T-cell adhesion during immune response (12,13). More generally, they illustrate how solvent-mediated fundamental forces might beget order in interacting membranes, further reminding us of how the properties and activities of the medium, i.e., water, matter in guiding biomolecular organization.

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