# Membranes and Nonpolar Surfaces

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## Membranes

The function of biomembranes is not restricted to surrounding a cell and its various compartments as passive separation layers. By facilitating transport of molecules from one side to the other, they play also an active role. Membranes are composed of a complex mixture of various lipids and proteins, with the lipids forming a bilayer. Our aim is to understand biomembranes in terms of the self-organization of these constituents. The information about the molecular architecture of membranes accessible experimentally is very limited. Therefore, we use molecular dynamics simulation techniques to computationally model the cooperative processes underlying the mesoscopic properties of membranes. Molecular dynamics simulations based on numerically solving Newton's equations of motion to propagate a system in time and semi-empirical force fields to describe interatomic interactions may provide highly detailed information about the molecular properties of membranes. As a first step, we model membranes as lipid bilayers, see **Figs. 1-4 [1-3]**.

Membrane fusion is a key step for important processes including viral infection and drug delivery. For the first time, we have studied the fusion of lipid vesicles in atomic detail, see **Fig. 1 [1]**. This process involves the formation of a lipidic connection between two opposed bilayers denoted as stalk, (see **Fig. 1a**) and subsequent formation (see **Fig. 1b**) and rupture (see **Fig. 1c**) of a hemifusion diaphragm. This work opens the perspective to investigate a wide range of mesoscopic biological processes at atomic resolution.

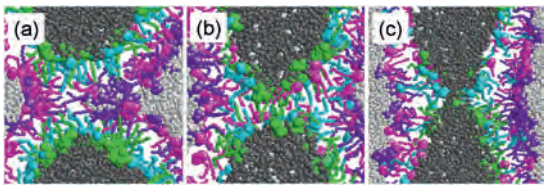


*Fig. 1: Fusion of vesicles composed of dipalmitoyl-phosphateidylcholine (DPPC) and (protonated) palmitic acid (PA) in a molecular dynamics simulation in atomic detail [1]. (a) A stalk as well as a hemifusion diaphragm (b) before and (c) after rupture are depicted. Water and head group atoms are shown as spheres, tails are shown as bonds. Water is depicted in dark or light gray. Colors distinguish between DPPC (purple and green) and PA molecules and the leaflets where the molecules resided initially.*

The free energy of early membrane fusion intermediates has been determined using simulations in conjunction with a coarse grained model, see **Fig. 2 [2]**. We find that the free energy of a stalk is lower than that of a pre-stalk intermediate which involves a single hydrated lipid tail. A peptide known to induce fusion in vitro does not change the free energy of stalks but does lower the free energy of the solvated tail intermediate. These results challenge assumptions of continuum models and support the idea that early fusion kinetics is determined by interbilayer flips of lipid tails.

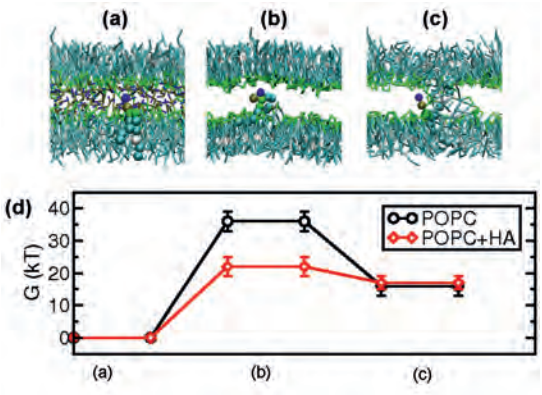


*Fig. 2: Free energy of early membrane intermediates from molecular dynamics simulations in conjunction with a coarse grained model [2]. The system simulated was a palmitoyl-oleoyl-phosphateidylcholine (POPC) bilayer at low hydration fused with its periodic image (a-c). The bilayer at equilibrium (a), the transition state for interbilayer flips of a lipid tail (b), and a stalk (c) are depicted. (d) The free energies in the absence (black) and presence (red) of an influenza hemagglutinin fusion peptide are compared. In (a-c), lipids are shown as sticks and a selected lipid to which a harmonic potential was applied to induce the intermediates on the limited timescale of the simulations is highlighted. In (b,c), the choline and phosphate groups of the other lipids are omitted for clarity.*

Peptides can also induce pores which is the putative mode of action of $\alpha$-helical antimicrobial peptides. We are currently investigating the interaction of the antimicrobial peptide NK-2 with lipid bilayers. Membrane pores, though, can also form in the absence of peptides. In a recent study we have revealed that membrane nanopores, see **Fig. 3a**, correspond to a local free energy minimum, explaining previous data on transmembrane conductance and the metastability of nanopores observed in previous numerical studies **[3]**. In the same set of simulations, the free energy of lipid desorption, see **Fig. 3b**, has been calculated. Our results indicate that the free energies of lipid desorption from a bilayer or from a micelle differ, and that the critical micelle concentration is an inadequate reference for the energetics of lipid desorption from a bilayer unlike commonly assumed.

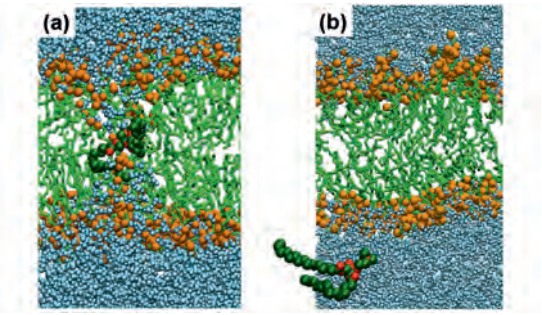


*Fig. 3: The energetics of (a) nanopores and (b) lipid desorption for a DPPC bilayer were determined from atomistic simulations in which a harmonic potential was applied to a selected lipid [3]. Lipid tails are shown as green sticks and headgroups as orange spheres. The lipid subjected to the harmonic potential is highlighted. Water is shown in blue.*

Understanding membranes at physiological conditions requires to investigate their interaction with ions. We are studying the interaction of potassium (K), sodium (Na), and lithium (Li) with zwitterionic phospholipid bilayers, see **Fig. 4(a,b)**. Potassium and sodium are the most abundant cations inside or outside a cell, respectively, whereas lithium intake can be curative or toxic depending on the dosage. We find that all of these cations are adsorbed at the headgroups of a zwitterionic lipid bilayer. The affinity increases in the order K<Na<Li and, thus, increases with decreasing size of the bare ion. These observations agree with results from electrophoresis experiments. In order to facilitate quantitative predictions on the affinity, an improved force field for alkali chlorides in water has been developed **[4]**. For the first time, we have used simulations to study cholesteryl hemisuccinate (CHEMS), an acidic cholesterol ester. CHEMS forms bilayers and is used as a subcomponent of liposomes for drug delivery. For CHEMS in the anionic state, the negative charge of the lipids is found to be almost fully compensated by (sodium) counterions adsorbed at the lipid headgroups, see **Fig. 4c**.

Lipid bilayers may also adsorb cationic peptides as observed for antimicrobial peptides. The antimicrobial peptide NK-2 is not only adsorbed at anionic but also at zwitterionic (phosphateidylethanolamine, PE) lipids while it does not interact with (zwitterionic) phosphateidylcholine (PC) lipids, as indicated from electrophoretic mobilities. We address the question how NK-2 distinguishes between prokaryotic and eukaryotic membranes. The hypothesis that, at physiological conditions, the underlying mechanism is not simply charge complementarity as commonly assumed but more specific molecular interactions, is currently tested.

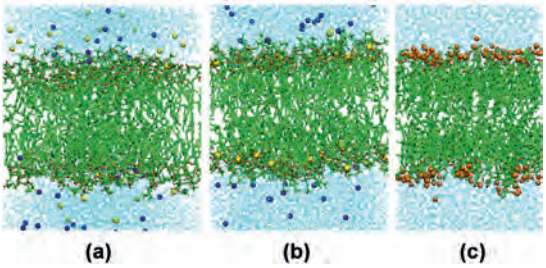


*Fig. 4: Adsorption of ions to lipid bilayers. A POPC bilayer in an aqueous LiCl solution (a) before and (b) after a 100 ns simulation as well as (c) an anionic CHEMS bilayer with Na counterions after a 100 ns simulation are depicted. Lipids are shown as green sticks. Lipid oxygen atoms (red) are indicated. Ions are shown as spheres whose colors distinguish between Li (yellow), Cl (blue), and Na (orange).*

## Electrophoresis of Neutral Particles

Though widely used to assess the surface charge of colloidal particles, electrophoresis experiments may be misleading, as indicated by simulations in our group. For more than a century, electrophoretic mobilities of hydrophobic particles in water have been interpreted in terms of negative surface charges from the adsorption of hydroxide ($OH^-$). In contrast, recent spectroscopic and simulation studies have indicated significant surface affinity for hydronium ($H_3O^+$) but not for hydroxide. In simulations we observe that neutral wax slabs in water in the presence of an electric field but in the absence of ions migrate as though they were negatively charged, see **Fig. 5 [5]**. This work may resolve the controversy between the electrophoretic and spectroscopic studies and supports the view that neat water at hydrophobic surfaces is acidic.

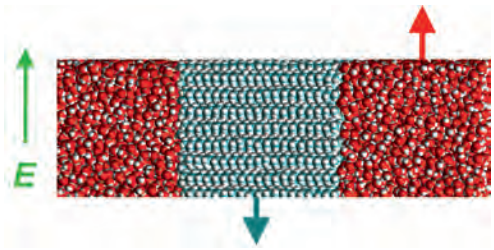


*Fig. 5: Electrophoresis of neutral particles in simulations. A wax slab (hexatriacontane, C36) parallel to an external electric field in water in the absence of ions migrates as if it was negatively charged. Colors distinguish between carbons (cyan), oxygen (red), and hydrogen (white). The direction of the electric field (green) and the motion of wax (cyan) and water (red) are indicated.*

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**References:**

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**[2]** Smirnowa, Y., Marrink, S.J., Lipowsky, R., Knecht, V.: Pre-stalk transition state for membrane fusion includes hydrated lipid tail, in preparation.
**[3]** Grafmüller, A., Lipowsky, R., Knecht, V.: Energetics of membrane nanopores and lipid desorption, submitted.
**[4]** Klasczyk, B., Lipowsky, R., Knecht, V.: Kirkwood-Buff force field for alkali chlorides in water, in preparation.
**[5]** Knecht, V.: Water at hydrophobic surface is acidic: re-interpretation of electrophoretic mobilities.