Stability of Lipids and Lipid Bilayers

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Lipid bilayers belong to the most important structural elements of biological cells. For biological function, a flexible and dynamic internal structure and membrane composition is required. Thus, despite their great inherent stability, the lipid membranes constantly undergo remodeling processes, involving membrane fusion, pore formation and various means of lipid exchange between membranes.

Understanding the molecular mechanisms and energetics that govern such remodeling processes presents a great challenge for both experiments and molecular modeling. This challenge arises from the disparate length and time scales involved. Bilayer membranes have lateral sizes of 100 nm to over 100 µm, yet are only a few nanometers in thickness. The processes of interest take place or are initiated at the scale of one or at most a couple of lipids molecules, i.e. a few nanometers. At the same time, they take place on time scales too fast for high resolution experiments, yet are non-equilibrium processes, and out of reach for most molecular simulations.

Strategies to overcome these difficulties involve either the use of simplified coarse-grained models, with fewer degrees of freedom as used in **[1-3]** to study membrane fusion, or enhanced sampling methods, such as umbrella sampling, which forces the system out of equilibrium, along a chosen reaction coordinate. Here we describe the application of the latter strategy to two membrane related processes.

Energetics of Nano-Pore Formation

Pore formation plays an important role in many cellular processes that require membrane remodeling, as well as for biomedical applications.

Theories of pore formation are often based on classical nucleation theory as a balance between the edge energy and the membrane tension Σ. When the pore radius is close to the molecular scale however, this continuum description is unlikely to hold, because the creation and closure of the hydrophobic pore requires considerable rearrangement of the lipid molecules at the pore edge.

Simulation studies of pore formation are limited to small length and time scales, and thus require artificially large membrane tensions. Alternatively, the free energy required to create a nm sized hydrophilic pore can be calculated with umbrella sampling. Here, the chosen reaction coordinate was the distance z of a certain lipid head group from the bilayer's center of mass.

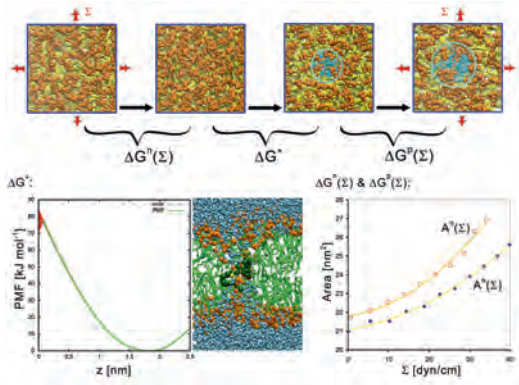

Fig.1: Scheme for calculating the free energy of pore formation as a function of membrane tension in simulated lipid bilayers.

When the headgroup is close to the center of the bilayer a pore forms spontaneously and the corresponding potential of mean force (PMF) can be calculated. This method is however computationally expensive and limited to individual values of membrane tension. A scheme to estimate the pore formation free energy as a function of tension Σ is outlined in **Fig. 1**. In this scheme the process of creating a pore in a bilayer at a given Σ is devided into three steps: 1) reducing the bilayer tension from Σ to zero, 2) creating a nano-pore and 3) stretching the bilayer containing a pore back to Σ.

Using this scheme, the free energy of pore formation has been calculated as a function of Σ. The validity of the results can be tested by comparing the results to a second PMF calculation at a high lateral pressure of -40 bar, which finds the pore formation free energy to be 61.7±3 kJ mol^{-1}. In comparison, the integration scheme leads to a value of 64±4 kJ mol^{-1}, demonstrating, that this method gives reliable results, over a large range of membrane tensions.

The results can be used to estimate the probability of finding a nanopore in a membrane as a function of membrane tension and size. The tensions for which this probability reaches finite values of ~10% correlate well with the order of magnitude of rupture tensions observed for simulated membrane patches and typical vesicle sizes, despite the different timescales. A fit of the pore free energy as a function of Σ for the line tension gives a value of 7.2 pN, which is close to experimental estimates ranging from 8 to 21 pN **[4]**.

As the restrained lipid influences the pathway of pore formation, it is not a priori clear that unrestrained conditions lead to the same intermediate transition states for pore formation. It is not possible to observe the formation of nanopores in unrestrained simulations, but simulations of nanopore closing can give insights to the intermediate con-

formations. The two, very similar closing pathways are shown in **Fig. 2**. The first proceeds via a half-pore, spanning one of the monolayers only and is the same as the one observed in the pore formation pathway of the restrained simulations. In the second case, instead of a half-pore in one monolayer, two smaller hydrophilic indentations are present in both monolayers. A full hydrophobic pore spanning the entire bilayer is never seen.

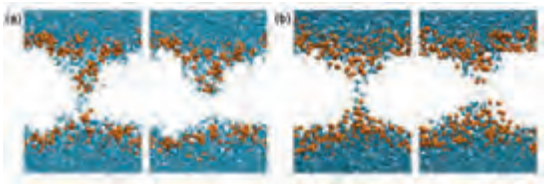

Fig 2: Two possible intermediate states observed in pore closing simulations. (a) a 'half-pore' spanning one monolayer (b) two smaller hydrophilic indentations, one in eachmonolayer.

Lipid Exchange and Local Geometry

Similar PMF calculations, restraining the lipid head-group at a range of distances outside the bilayer membrane can be used to estimate the desorption free energy **[5]**.

For a tension-free membrane the desorption free energy is found to be 63±2 kJ mol^{-1}. Using the same protocol to determine the free energy change upon desorption of a lipid from a tense bilayer with a lateral pressure of -40 bar, we find a desorption free energy of 80±2 kJ mol^{-1}, 17 kJ mol^{-1} larger than for the desorption from the relaxed bilayer. To understand this difference, the different contributions to the free energy change have to be considered. The major contribution will be the energy cost of exposing the hydrophobic tails to the water. However, when the lipid is pulled into solution, its tails, which are extended within a conical region in the bilayer, become disordered with random orientations, as in **Fig. 3c**, increasing their conformational entropy. This represents a favorable contribution to lipid desorption and partially compensates the hydrophobic interactions. In the tense bilayer, the lipids are more disordered and therefore gain less entropy in solution. To investigate this effect of the local structure further, the free energy of desorption of a lipid from a spherical micelle has also been calculated. Due to the high local curvature the lipid tails in the micelle are even more disordered than in the tense bilayer. As expected, the desorption free energy for the micelle is also higher than for the tension-free bilayer, with 73±1.3 kJ mol^{-1}.

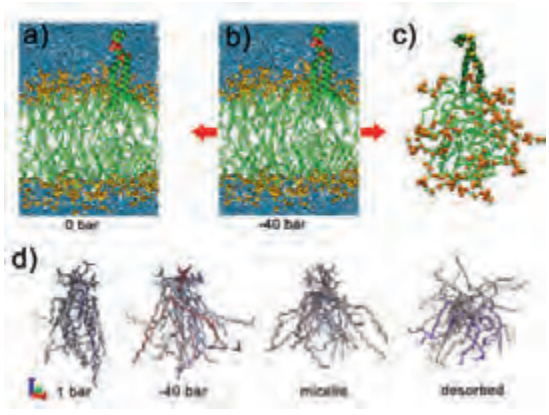

Fig.3: Three different aggregates, for which the desorption free energy has been calculated (a-c). (d) The conformational space of individual lipids within the three aggregates and in solution.

For a more quantitative assessment, the average conformational entropy of lipid tails in the different aggregates has been estimated with the quasi-harmonic (QH) approximation. The results are summarized in Table 1. In the tense membrane, the change in conformational entropy of 14±5 kJ mol^{-1} provides a quantitative explanation for the difference in desorption free energy. The difference in conformational entropy in the spherical micelle, on the other hand, with 55±5 kJ mol^{-1} is significantly larger than that in the desorption free energies, which only differ by ~10 kJ mol^{-1}. Therefore, the large lipid entropy change is to a large part compensated by other free energy contributions. This result is consistent with the observation, that the number of tail–water contacts for a lipid chain in the micelle is approximately 5 times as high as in the membrane aggregates, leading to a reduced contribution of water–chain interactions.

What has become clear from studying these three systems is that the local structure has a strong influence on the free energy of lipids in the aggregates.

	ΔG^{des} (kJ/mol)	$\Delta\Delta G^{des}$ (kJ/mol)	$T\Delta\Delta S$ (kJ/mol)
Bilayer P_{II} =0 bar	63 ±2	0	0
Bilayer P_{II} =40 bar	80 ±2	17 ±3	14±5
Micelle	73 ±1.3	10 ±3	55 ±5

Table.1: The free energy for lipid desorption, ΔG^{des} for the transfer of a DPPC lipid from an aggregate into water, the difference from the bilayer at zero tension, $\Delta\Delta G^{des}$ and the change in lipid entropy in the aggregates relative to the bilayer at zero tension, $-T\Delta\Delta S$

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