Tension Induced Membrane Fusion

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Fusion of biological membranes is an essential process in many areas of cell biology, ranging from vesicular trafficking and synaptic transmission to cell-cell fusion and viral fusion. Lipid vesicles, which are often used as simplified model systems for the rather complex biological membranes, can also be induced to fuse experimentally by a variety of methods.

Since it is currently not possible to resolve the length and time scales of membrane fusion experimentally, we use a mesoscopic simulation technique, dissipative particle dynamics (DPD), to probe the molecular details and energy barriers of the fusion process **[1-4]**. We focus on the presumably simplest way to induce fusion between a vesicle and a planar bilayer, namely via membrane tension.

The stochastic nature of the process makes it necessary to simulate a large number of fusion attempts in order to obtain reliable fusion statistics and to extract meaningful values for the fusion probability and the average fusion times.

A Molecular Picture of the Fusion Pathway

All successful fusion events follow the same pathway shown in **Fig. 1**. In this fusion pathway, configurations of individual lipids play an important role.

Upon first contact, the vesicle adheres to the planar membrane patch, forming a relatively sharp contact angle. Fusion starts with individual lipids at this 'kink' assuming a splayed tail configuration with one tail inserted in each membrane. This disturbs the local double-bilayer structure and leads to the formation of a disordered membrane domain within the contact zone where the hydrophobic regions of the two bilayers are in direct contact, and which expands following the contact line in a bean-like shape. Finally, within this disordered region, lipids reorder to form a small hemifused patch, which expands for a short time and finally ruptures at the rim to form the fusion pore.

Overall, the fusion process can be decomposed into three sub-processes. (i) Sub-process α corresponds to the first lipid tails moving into the other bilayer, (ii) sub-process β consists of the nucleation of the hemifused patch and (iii) sub-process γ corresponds to the rupture of this patch and formation of the fusion pore.

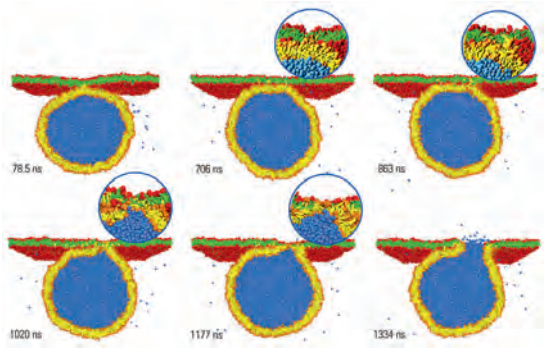

Fig. 1: Fusion of a vesicle to a planar membrane. The vesicle consists of 6869 lipids (orange heads, yellow chains), the planar membrane contains 6911 lipids (red heads, green chains). The water beads originally inside the vesicle are blue, those outside are not shown for clarity. The six snapshots illustrate the development of the fusion event from 78.5 ns after the first contact until the fusion pore opens after 1334 ns. The insets are magnifications of the lipid rearrangements at the contact line.

Fusion Probability and Alternative Pathways

When two tense membranes come into contact they may fuse. Alternatively, tense membranes may rupture or, at lower tensions, either the hemifused patch might expand without rupturing, thereby gaining membrane area and relaxing the membrane tension or the adhering state might remain stable.

Because of these other possibilities, the fusion probability depends strongly on the membrane tension as can be seen in **Fig. 2**. The probability of successful fusion is high for an optimal intermediate tension, but decreases steeply for smaller tensions as adhesion and hemifusion become more favourable. As the tension decreases, the fusion probability seems to vanish before the tensionless membrane state is attained. This would imply that the tension has to exceed a certain threshold value in order to induce fusion **[3, 4]**.

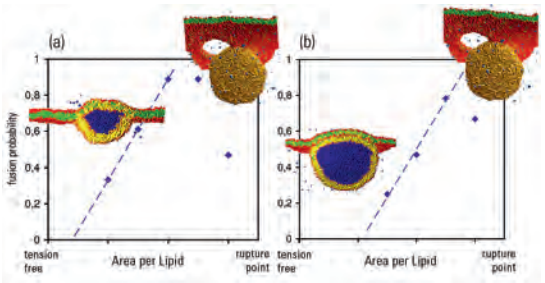

Fig. 2: Fusion probability as a function of molecular area for vesicles with a diameter of (a) 14 nm and (b) 28 nm. In both cases, the fusion probability depends strongly on the tension with a maximum at an optimal value. At higher tensions, fusion becomes less likely because of membrane rupture; at lower tensions, fusion is more and more replaced by hemifusion or adhesion.

Fusion Time Distribution and Energy Barriers

The tension determines not only the success rates, but also the time scale of fusion. At lower tensions (i) the fusion times become larger and (ii) the fusion time distributions become broader. From the statistics of the fusion time together with separate simulations in which one lipid was pulled into a splayed conformation, the energy barriers for the fusion observed in these simulations could be estimated **[2, 3]**.

Fig. 3 shows the average fusion time as a function of the molecular area. It appears to grow exponentially with decreasing tension. This exponential growth of the fusion times together with the decreasing fusion probability makes it exceedingly difficult to determine the time scale of fusion from computer simulations as the tensionless state is approached.

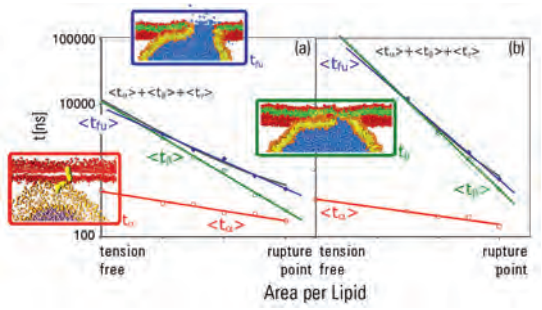

Fig. 3: The average duration of the tension dependent sub-processes α (red circles) and β (green diamonds) displayed together with the average fusion time (blue diamonds) as a function of the area per molecule for vesicles with a diameter of (a) 14 nm and (b) 28 nm. Both times depend exponentially on the area per molecule. The three insets show the final states of the sub-processes.

The average duration of the sub-processes α and β, both decrease exponentially with increasing tension, as shown in **Fig. 3**. This implies that the corresponding energy barriers should depend linearly on the membrane tension. The timescale of sub-process γ on the other hand, is found to vary between 150 ns and 300 ns, independent of both tension and vesicle size.

Since the sub-process α involves the movement of single lipids, relative to their surroundings, it is accessible to direct simulation. In two adhering membranes, a single lipid tail was pulled slowly with a harmonic potential from its original position into the other bilayer, so that the lipid assumes a splayed conformation as observed in the fusion simulations. The average work required for this process in 20 independent simulations was found to be $9 \pm 2\,k_BT$. This value constitutes an upper bound for the energy barrier and should correspond to the barrier itself for very slow pulling. Using the Jarzynski relation on this data leads to a similar barrier height of $8\,k_BT$.

The energy barrier for this process is provided by the (partially) hydrated polar head groups of the proximal monolayers. In the coarse-grained simulations it is implemented via one specific interaction parameter. Simulation enforcing a splayed lipid conformation, have shown, that the height of the barrier is primarily governed by the value of this parameter. Thus the barrier size can be tuned in such a way that it is consistent with available reference data such as the hydration energy of one hydrocarbon chain.

Using the information obtained in these simulations in the fits to the other timescales, the energy barriers corresponding to the other sub-processes for a tension free membrane can be estimated to be $10-15\,k_BT$ and $8\,k_BT$. At low tensions the total fusion time is dominated by the timescale of hemifusion. Thus the simulation statistics suggest that the main energy barrier for fusion of tensionless membranes is of the order of $10-15\,k_BT$ **[2,3]**.

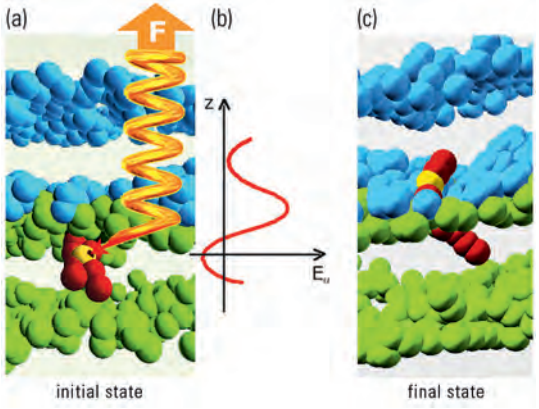

Fig. 4 Simulations of enforced lipid flips used to measure the corresponding energy barrier (a) From two adhering bilayers (head beads blue/green, tail beads omitted for clarity) a single lipid is selected (orange heads, yellow/red tails) and a force F arising from a slowly moving harmonic potential is applied to one of its tail beads (red), until the tail has flipped to the other bilayer, so that the lipid has assumed a splayed configuration with one tail inserted in each bilayer as shown in (c). (b) Energy landscape for the bead as a function of the displacement z of the yellow bead. It has a high barrier in the centre corresponding to the repulsive head groups and increases to the sides reflecting displacement of the head group into the hydrophobic region.

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