Unveiling Membrane Fusion

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Membrane fusion is an essential and ubiquitous cellular process. It is involved, for example, in cellular secretion via exocytosis, signalling between nerve cells, and virus infection. In both the life sciences and bioengineering, controlled membrane fusion has many possible applications, such as drug delivery, gene transfer, chemical microreactors, or synthesis of nanomaterials. While previous studies have explored many of the steps involved in membrane fusion, the efforts to fully understand the dynamics of membrane fusion have been stymied by the speed with which this process occurs.

Recently, our lab has succeeded in the development of two independent methods of initiating the fusion process in a controlled manner. This, in turn enabled us to observe the subsequent fusion dynamics, using phase contrast microscopy and a fast digital camera, with a temporal resolution in the microsecond range **[1]**. This time resolution is unprecedented, as direct observations of fusion in the literature access only times larger than several milliseconds.

The fusion process was observed on giant unilamellar vesicles (~ several tens of micrometers in diameter). In the first protocol **[2]**, the vesicles were functionalized with synthetic fusion-triggering molecules (β-diketonate ligands). Then, two of these liposomes were aspirated into two glass micropipettes. Membrane fusion was subsequently induced by the local addition of ions that form a complex between two fusogenic molecules embedded in the opposing membranes; see **Fig. 1**.

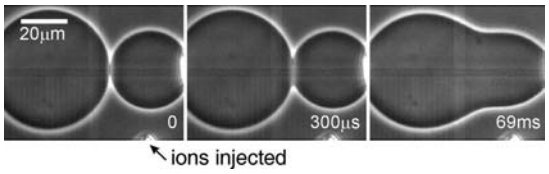

Fig. 1: Snapshots from the fusion of two functionalized vesicles held by micropipettes (only the right pipette tip is visible on the snapshots). A third pipette (bottom right corner) is used to inject a small volume (few tens of nanoliters) of solution of $EuCl_3$ which triggers the fusion. The time after the beginning of the fusion process is indicated in the lower right corner.

In the second protocol, two lipid vesicles were brought into contact by weak alternating electric fields. The AC field served to line up the vesicles along the direction of the field. Thus, while the micropipettes were used to manipulate the vesicles in the first protocol, the AC field was the manipulation tool in the second one. Once close contact was established, membrane fusion was induced by exposing the vesicles to a strong electric pulse. Such a pulse leads to the formation of membrane pores **[3]** in the opposing membranes, which subsequently fuse in order to dispose of the edges of the pores. In the presence of salt in the vesicle exterior, the vesicles deform to acquire cylindrical shapes with round caps **[4]**. In the absence of salt, this curious deformation is not observed, and multiple fusion necks are formed in contrast to the no-salt case where a single fusion neck is formed; see **Fig. 2**.

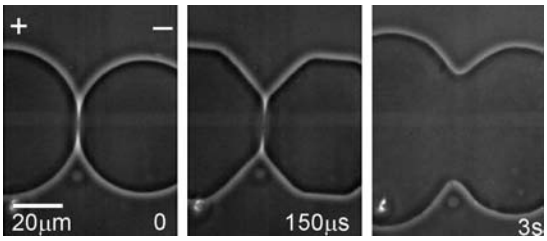

Fig. 2: Snapshot series from the electrofusion of two vesicles. The polarity of the electrodes is indicated with a plus (+) or a minus (-) sign on the first snapshot. The amplitude of the pulse was 150 V (3 kV/cm), and its duration was 150 μs. The starting time t = 0 corresponds to the beginning of the pulse. The image acquisition rate was 20 000 frames per second. The external vesicle solution contained 1 mM NaCl, which causes the flattening of the vesicle membrane and induce cylindrical deformation [4].

With either method, ligand mediated fusion or electrofusion, the process was recorded using a fast digital camera with an acquisition rate of 20 000 frames per second, corresponding to a temporal resolution of 50 microseconds. This constitutes a 1000-fold improvement compared to other direct-observation microscopy reports on fusion. The direct imaging provided by the two fusion protocols and the fast acquisition speed confirmed that the fusion process is extremely fast, and offered some insight into the dynamics of the process. The improved temporal resolution suggests that for the formation of a fusion neck, the cell needs only a few hundred nanoseconds. Within 50 microseconds, the fusion neck connecting the two vesicles was observed to have already reached a diameter of a few micrometers **[1]**. This suggests that the opening of the fusion pore occurs with an expansion velocity of a few centimeters per second. The experimental data could be extrapolated to shorter times covered by simulation studies performed in our department. The latter nicely support the conjecture that fusion times are on the order of 200 nanoseconds.

Having demonstrated the potential of the method for controlling and imaging membrane fusion, we applied it to a slightly more sophisticated system. Namely, we fused two vesicles whose membranes were composed of different lipids (Dioleoylphosphocholine and Sphingomyelin) and cholesterol. At a certain temperature, these lipids form fluid phases, also known as liquid ordered and liquid disordered. These phases are immiscible and the liquid ordered phase, which is stabilized by cholesterol, is thought to mimic rafts in cell membranes.

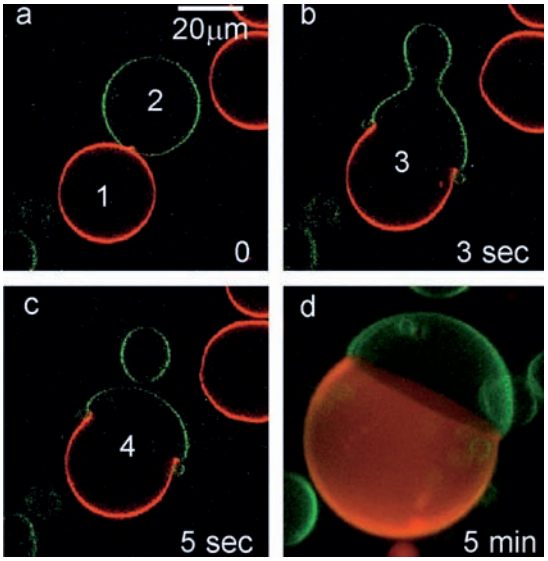

Fig. 3: Creating a multidomain vesicle by electrofusion of two vesicles of different composition as observed with fluorescence microscopy. The images (a-c) are acquired with confocal microscopy scans nearly at the equatorial plane of the fusing vesicles. (a) Vesicle 1 is composed of Dioleoylphosphocholine:Cholesterol (8:2) and labeled with the fluorescent dye DiI-C_{18} (red). Vesicle 2 is made of Sphingomyelin:Cholesterol (7:3) and labeled with the fluorescent dye perylene (green). (b) The two vesicles were subjected to an electric pulse of strength 300 V (6 kV/cm) and duration 300 μs. Vesicles 1 and 2 have fused to form vesicle 3. (c) Right after the fusion, the Sphingomyelin:Cholesterol part (green) begins to bud forming a small daughter vesicle. (d) A three-dimensional image projection of vesicle 4.

When two such vesicles are forced to fuse, the resulting vesicle contains two or more domains. We used the electrofusion protocol to form these multidomain vesicles **[5]**. The fusion products were explored using confocal microscopy, see **Fig. 3**. Having the tool to form these domains on vesicles in a controlled fashion would allow us to study their stability at various conditions like temperature and membrane tension (PhD project of Natalya Bezlyepkina).

In conclusion, we have achieved controlled fusion induced by two approaches: ligand mediated fusion and electrofusion. The tools available in our lab have allowed us to reach unprecedented time resolution of the fusion process. Being able to control fusion, we used our approach to form multidomain vesicles and study the stability of the domains. Currently we apply the electrofusion of giant vesicles as a tool to create microreactors with very small volumes (postdoctoral project of Peng Yang). The vesicles used in the present study were only tens of microns in size. Fusing two of these vesicles of different content would be equivalent to performing a reaction in a tiny volume of some picoliters, which would be advantageous for synthesis of nanomaterials. Furthermore, vesicles as microscopic vessels loaded with polymer solutions can be used to study phase separation in confined systems (PhD project of Yanhong Li), which mimics microcompartmentation in cells.

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References:

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