Mastering Membrane Fusion

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Membrane fusion is an exciting but relatively complex phenomenon. In real cells it involves the participation of a number of so called fusogenic proteins who are thought to perform the role of a recognition system that brings two membranes together, perturbs the lipid bilayers, and eventually assists the lipid mixing. The fusion process is of significant importance as it is involved in vital cellular functions like import of food stuff and export of waste (endo- and exocytosis), fertilization, signaling in nerve cells and others. Experimental tools for the controlled fusion of membranes should be essential in order to improve and optimize fusion applications like drug delivery, artificial fertilization and gene transfer.

Thus, achieving control on fusion has been the driving force for initiating experiments on model membranes. The most popular bilayer system on which fusion was studied are solutions of small unilamellar vesicles (LUVs) of ~100nm in size. However, in such systems the fusion efficiency is set rather indirectly, the measured properties are determined by the bulk solution (and not by individual vesicle pairs) and thus represent averages over a large number of vesicles. In addition, due to the small size, the behavior of LUVs may be governed by membrane tension and high curvature effects. In contrast, applying optical microscopy to follow interactions between giant unilamellar vesicles (GUVs) gives access to direct observation of fusion events. The GUV size (~10μm) brings the model systems up to the level where the membrane dimensions are of cell-size. In the last decade, several powerful tools such as micropipette aspiration have been developed to allow the experimental manipulation of GUVs. Combining optical microscopy with micropipettes is a rather promising route for studying and achieving control over membrane fusion.

Recently, the investigation of two types of fusion-inducing mechanisms in GUVs was initiated in our lab. In one of them, the inter-membrane interaction is triggered by metal ions forming complexes between functionalized molecules in the bilayers. In the second approach, we apply strong electric pulses to vesicles in contact. In both cases fusion is induced. Using high speed digital imaging we follow the evolution of the fused membranes with unprecedented time resolution of about 50μs. Fusion dynamics as reported in the literature has been limited, so far, to time resolution of about 1ms. For the first time, we were able to observe the opening of the fusion pore with high temporal resolution.

Fusion of Functionalized Membranes:
The membranes are functionalized with synthetic fusogenic molecules **[1]** which can form a complex with multivalent ions. The fusogenic molecules have a lipid-like structure with hydrophilic headgroup containing a specific ligand. The ligands form coordination complexes with metal ions in 2:1 ratio. When the complexes are formed between ligands from opposing membranes, the expected event is fusion. This fusion scenario was probed in our lab **[2]** by means of manipulating two vesicles with micropipettes. A third micropipette was used to locally inject a solution of multivalent ions. The ions were observed to induce adhesion between the two vesicles, which was followed by fusion. **Fig. 1** is a simplified cartoon of the possible fusion mechanism occurring at molecular level. The resolution of optical microscopy (~0.5μm) limits the observation to the micrometer scale and the molecular events cannot be revealed. Thus it is not clear how many complexes are involved in the fusion event.

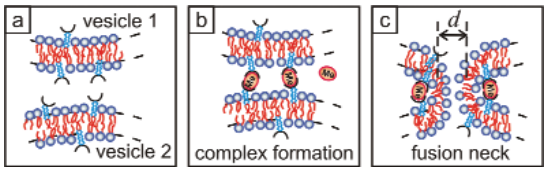

Fig. 1: Possible steps in the fusion of functionalized membranes: (a) two functionalized lipid vesicles are brought into contact; (b) a solution of multivalent ions is locally injected in the contact area leading to the formation of inter-membrane complexes; (c) the opening of the fusion neck is initiated.

Electrofusion:
When exposed to weak AC field, vesicles align in the direction of the field. This can bring two vesicles into contact. The subsequent application of DC pulses leads to charging of the membrane. This creates transmembrane potentials which are enhanced at the vesicle poles (facing the electrodes). The corresponding compression of the membrane effectively induces tension. DC pulses can lead to perforation of the membrane in two cases **[3]**: (i) when the transmembrane potential exceeds some critical value (~1V); or (ii) when the total membrane tension approaches the lysis tension of the membrane (~5dyn/cm). When poration is induced in the contact area of two vesicles, fusion is expected to occur. **Fig. 2** illustrates a possible mechanism of electrofusion of two bilayers in contact.

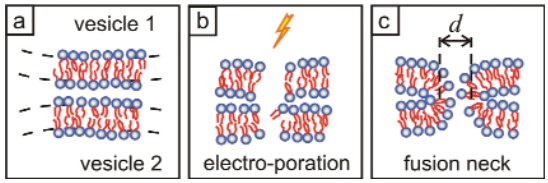

Fig. 2: Possible steps in electrofusion: (a) two lipid vesicles are brought into contact and aligned using AC field; (b) a short electric pulse is applied causing membrane poration; (c) the lipids from the opposing bilayers mix initiating the opening of the fusion neck.

In the experiments, the vesicles are placed between two electrodes and then observed with phase contrast microscopy. One example of a fusion event observed with a fast digital camera and phase contrast microscopy is presented in **Fig. 3**. The two vesicles were aligned by an AC field applied prior to the DC pulse. Time t=0 corresponds to the beginning of the pulse. A closer look at the micrograph sequence (not all of the acquired snapshots are displayed) shows that fusion has already occurred within the first 50µs. Using intensity profile image analysis, we are able to follow the evolution of the opening of the fusion neck diameter, *d*, (see F**ig. 1c** and **Fig. 2c**). The experiments extend over five orders of magnitude in time, ranging from microseconds to seconds. Two characteristic times are revealed, presumably corresponding to two different processes: molecular rearrangement of the lipid bilayers related with relaxation of the edge curvature of the fusion pore (t~1ms), and hydrodynamics of mixing of the fluid contents of the fusing vesicles.

In certain cases, when electrofusion is induced, multiple fusion events are observed (the example in **Fig. 3** illustrates one of them). The reason for this behavior is that the fusing vesicles have porated at several places. This leads to reclosure of the membrane inside the product vesicle and to formation of smaller internalized vesicles (one can see this in **Fig. 3** at t=10s where the internal vesicles appear as brighter spots inside the resulting vesicle; the brighter gray values are due to refractive index mismatch of the vesicle contents).

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.

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References:

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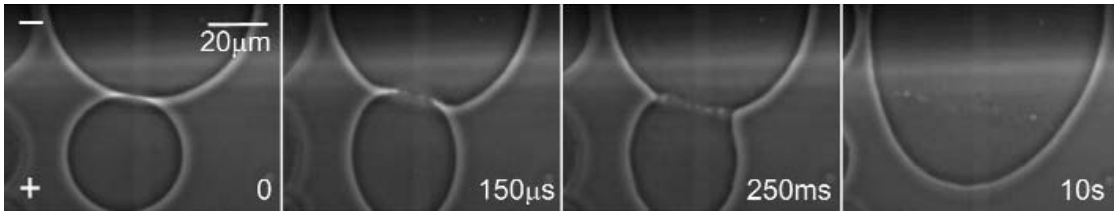

Fig. 3: Snapshot sequences of electrofusion of a vesicle couple. The time is indicated on each snapshot. The electrodes polarity is indicated with a plus (+) and a minus (-) sign on the first snapshots. The image acquisition rate was 30,000fps. The applied pulse has a strength of 90V and a duration of 150µs.