Domain Formation in Model Membranes Induced by Electrofusion of Giant Vesicles

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Lipid segregation in cell membranes is believed to play a fundamental role in biological processes, such as membrane signaling, trafficking, and sorting of membrane components. The current understanding is that cell membranes are not homogeneous in composition. It has been proposed that some membrane proteins are segregated from each other by their preferential partitioning into regions of different lipid domains (also referred to as rafts) within a continuous cellular membrane [1]. These domains were distinguished according to their phase state as liquid-ordered and liquid-disordered. Our study focuses on characterizing the properties, the stability and the formation dynamics of such domains in lipid bilayers.

We investigate lipid domains in model membranes using confocal laser scanning microscopy. As a model system, we use giant unilamellar vesicles (GUVs) prepared from lipid mixtures containing the "classical" raft components" [2, 3]: cholesterol, dioleoylphosphatidylcholine and sphingomyelin. When GUVs are prepared from a multicomponent lipid mixture, the composition of the different vesicles in a batch can vary drastically depending on their individual history. In order to obtain a vesicle with a well-defined composition, we fuse two vesicles with "simpler" lipid composition (single- or two-component) as proposed previously [4]. Two vesicles of different composition are subjected to a strong electric pulse and fuse [5, 6]. The resulting vesicles were observed over a certain period of time after fusion and their 3D images were recorded to study the domain formation and dynamics. Using vesicle couples of different sizes and starting compositions, we access various points in the Gibbs triangle composition diagram.

One example for creating a multidomain vesicle in this way is given in Fig. 1. The initial composition of the resulting domains in the vesicle right after fusion corresponds to the compositions of the starting vesicles. Knowing the initial area of the domains, we can calculate the exact composition of the final fused vesicle. Over time, the lipids in the domains of the fused vesicle redistribute to reach equilibrium. This redistribution is expressed in change in the area of the domains. Knowing the area of the domains and the exact initial composition, as well as using literature data for the area per molecule in each of the two lipid phases, we attempt to determine the location of the tie lines in the coexistence region of liquid ordered and liquid disordered phases. To access the exact domain areas, we use confocal stacks from which a digital 3D image of the vesicle is constructed with home-developed software for image analysis (Fig. 1e presents an example of such a vesicle).

Finally, using vesicle electrofusion, we hope to bring about the current understanding on formation and dynamics of domains in multicomponent membranes.

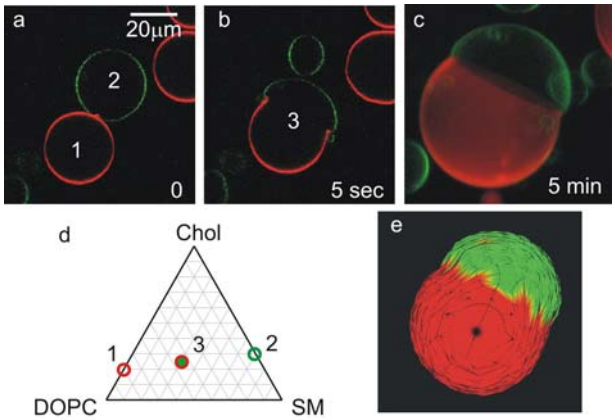

Figure 1. Example for creating a multidomain vesicle by electrofusion of two vesicles of different composition as observed with confocal microscopy. The images (a, b) are acquired with confocal microscopy scans nearly at the equatorial plane of the fusing vesicles. (a) Vesicle 1 is composed of DOPC:Chol (8:2) and labelled with DiI-C18 (*red*). Vesicle 2 is made of SM:Chol (7:3) and labeled with perylene (*green*). (b) The two vesicles were subjected to an electric pulse (6 kV/cm, duration 300 μs) and fused to form vesicle 3. Right after the fusion, the SM-Chol membrane part (*green*) begins to bud forming a small daughter vesicle. (c) A three-dimensional image projection of vesicle 3. (d) The numbered circles in the Gibbs diagram indicate the approximate composition of the vesicles marked with the same numbers as in the microscopy images. (e) Digitalized image of vesicle 3 reconstructed from confocal sections.

References

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