Model Membrane Systems
for 2D Monolayer Membrane Coalescence and 3D Bilayer Membrane Fusion

by

Changyuan Wang

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Abstract: Living cell membranes are complicated, and they are difficult to be described in specific numbers and parameters. Thus, we need powerful model systems to understand them from a physics perspective. In particular, we will focus on 3D bilayer membrane fusion and 2D monolayer membrane coalescence. We will describe the mechanisms of these models, the common methods to study those models, their implications for real cells, and their potential applications. Finally, we will briefly mention their further advances in biophysics and bioengineering.

1 Introduction

Living cells are complicated. They are crowded with numerous biological organelles, so when experiments are performed on them, it is difficult to rule out various parameters. Thus, scientists need simple model systems that can mimic cell functions without intricate organelles [6]. Some 3D vesicles and 2D colloidal rafts in aqueous solutions are typical for studying biological membranes. If the model systems are in micron-scale, it is even easy to make movies of their behaviors and phase transition [12].

Membrane fusion, for example, is hard to investigate in living cells. Thus, different artificial vesicles and membranes are introduced for lipid fusion, protein-induced fusion, small ion-induced fusion, and all other kinds of fusion processes. Since the biological systems are simple enough, their mechanical properties and energies can be precisely calculated [12]. This provides a foundation for future application and bioengineering, such as drug delivery.

The formations of model systems are diverse. Some are extracted from real cells, like red blood ghost cells; some are made of other naturally existing biological particles, such as rod-like phages. Some are synthesized by a specific ratio of chemicals under specific conditions. As technology advances, preparation methods are improving accordingly.

2 Giant Unilamellar Vesicles

Giant unilamellar vesicles (GUVs) are a simple model for human cell membranes. They can grow to tens of micrometers in diameter, which is about the scale of human cells [13]. This size makes them easy to be edited, handled, and visualized under optical microscopes [11]. Since they are only composed of a single lipid bilayer, their mechanical and chemical properties can be directly studied without the influence of other cell organelles. Membrane fusion is one of the subfields in which GUV systems work well.

Since GUVs are in micron-scale, simple bright-field microscopy and a camera can easily record their movements and behaviors. The largest challenge is to demonstrate if an event is indeed a membrane fusion. For example, it is hard to distinguish if figure 2(a) is an example of membrane

fusion or bud merging. To understand the difference between membrane fusion and other events, it is necessary to define fusion in the first place.

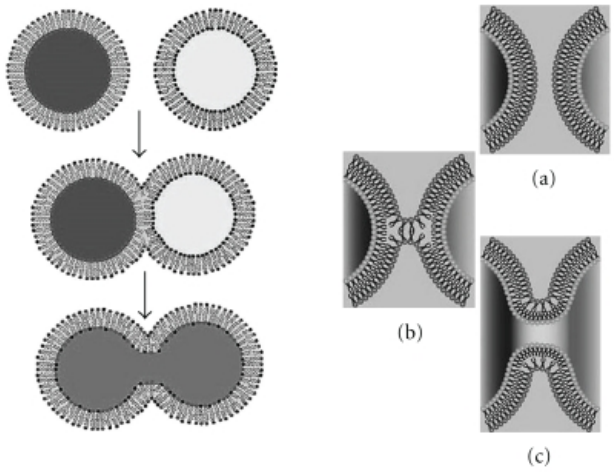

Figure 1: Basic Steps of bilayer membrane fusion. (a) Membrane contact; (b) hemifused state, outer layer lipid mixing; (c) inner layer lipid mixing, pore formation and content mixing. [12].

A commonly accepted definition of fusion involves bilayer membrane components merging, trapped contents mixing, formation of the pore, and limited leakage [2]. Figure 1 gives the fundamental procedures of a bilayer membrane fusion. In contrast, figure 1(b) are buds instead of liposomes. Vesicles need to be individual, isolated chambers that are disconnected from the lipid stack. The two buds in pathway p1 only exhibit the behavior of lipid rearrangement but do not satisfy the other criteria, so this is an example of bud merging.

To differentiate fusion from other events, we can use different colors of fluorescence to label different lipids. For instance, we can label the pre-prepared GUVs as red and label the lipid stack as green. If a red GUV merges with a bud from the stack, the red color will diffuse and contaminate the green substrate in the 3-hours experiment. To better image the samples, we can do a z-scanning in confocal microscopy and build a 3D image [13].

The standardized and repeatable approaches of preparing GUVs are also a reason for them to be a widely appreciated model. In recent years, numerous mature techniques of growing GUVs have been emerging, such as immersing small unilamellar vesicles and letting them swell in aqueous solution for about 5-20 minutes [9]. This method is relatively quick and easy.

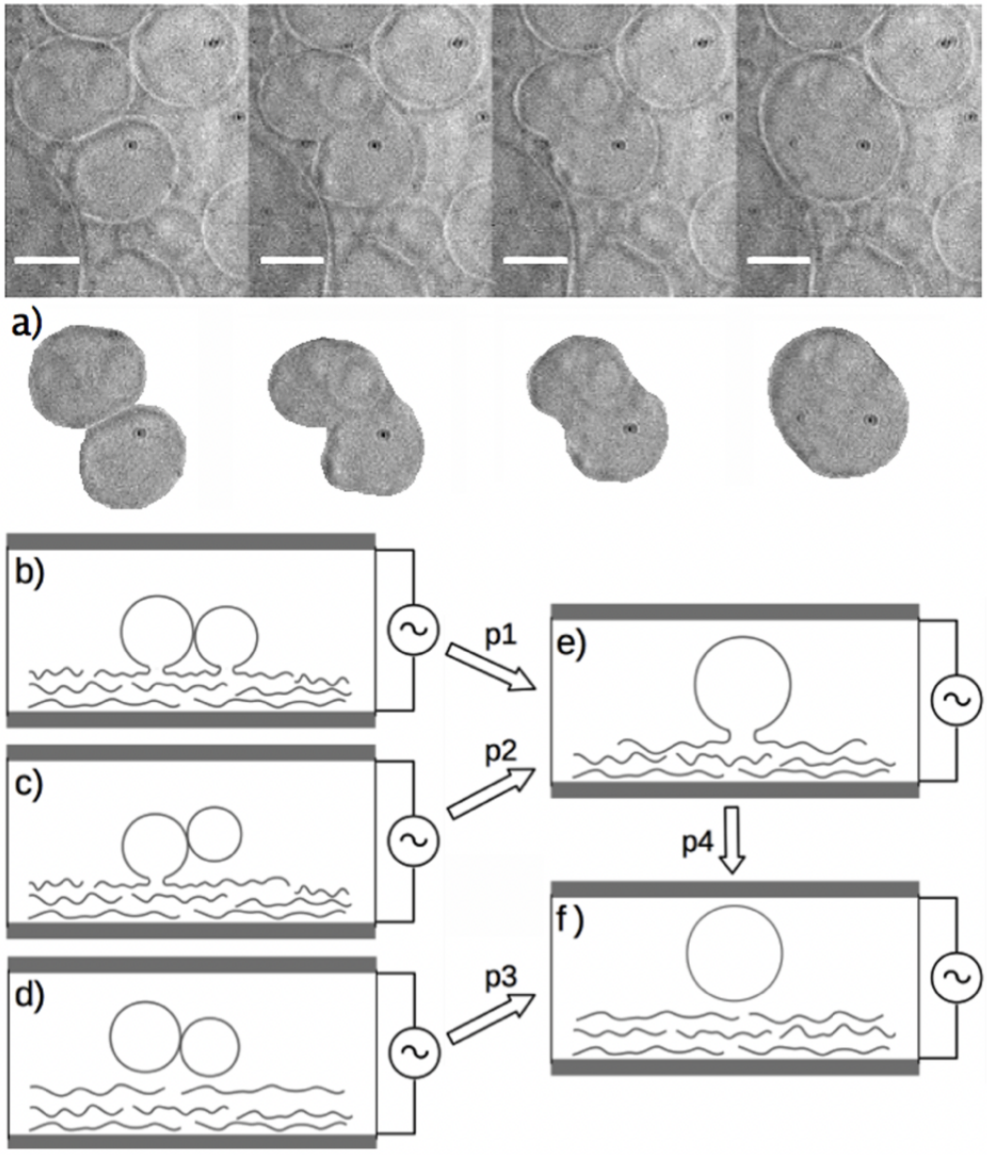

Figure 2: (a) Upper part: phase contrast images exhibiting a typical vesicle fusion during electroformation. The scale bars are 10 μm, and the time interval between two consecutive images is 0.73 s. Lower Part: two fusing membranes extracted by hand from the upper images for better visualization. (b) - (f) are schematic diagrams showing the states of buds and membranes. Pathway p1 is bud merging. Pathway p2 and p3 are membrane fusion. Pathway p4 is GUV detachment from the underlying lipid stack [13].

3 Small Unilamellar Vesicles

Some other protein and lipid fusion steps are in much smaller scales, such as virus-cell fusion, virus-mediated cell-cell fusion, and egg fertilization. For these nanoscale systems, we need a smaller liposome model, like small unilamellar vesicles (SUVs) [3]. SUVs are typically below 100nm, and 10 - 50 nm is a common size. Because of their sizes, they move much more quickly than GUVs, which makes their details even harder to be tracked under traditional microscopy.

Like bud merging in GUV experiments, aggregation is an event similar to membrane fusion in SUV experiments, and they need to be recognized and discarded. Fluorescence imaging is still a typical method. After labeling SUVs, they may stack on each other and form aggregation, which may look like a huge merged vesicle. The way to identify it is to use the excimer to monomer (E/M) ratio. For different sizes of liposomes, they have different E/M ratios, and the spectra of excimers and monomers are distinct. Thus, by measuring the wavelength spectrum, the event of merging can be confirmed and the rate of merging can be calculated [3].

To be noted, when SUVs stack on each other in proper positions and directions, they usually fuse spontaneously [3]. Thus, in hours of experiment, it is obvious that which are aggregations or merged membranes.

Aside from confocal and fluorescence microscopy, fluorescence assays can also monitor nanoscale vesicles. The assays include both the lipid and the content mixing. For lipid rearrangement, Forster Resonance Energy Transfer (FRET) is applied. After tagging SUVs with both the donor and acceptor fluorescent probes, when two SUVs merge, the distance between the donor-acceptor pair will increase. Since FRET efficiency is inversely proportional to the sixth power of the donor-acceptor distance, a small change in distance will result in a huge effect on energy transfer efficiency. Thus, as the distance increases, FRET efficiency rapidly decreases, and the monitored data can be used to calculate the percentage of lipid mixing as a function of time [12].

For trapped content mixing, two sets of vesicles are injected with different chemicals. For example, one set of SUVs is encapsulated with ANTS and another set is encapsulated with DPX. Since DPX serves as the fluorescent quencher of ANTS, as fusion successfully happens, DPX will meet ANTS, and a decrease in fluorescence intensity will reveal the extent of content mixing. Other types of chemicals have also been used, but the general idea remains the same [**ffusion_def_image**].

Since lipid mixing is the initial stage while content mixing is the final stage of membrane fusion, as the start and finish are properly monitored, the completion of the fusion process is demonstrated.

4 2D Homogeneous Colloidal Membrane

If rod-like phages are placed in a solution of a certain concentration of salt and dextran, phages will assemble by themselves and form 2D planar colloidal membranes [6]. If the curvature is altered, the membrane may close into a vesicle. 2D planar membranes are more widely explored because they open structures with an exposed edge, which renders a great configuration for studying elasticity of membrane sheets [5].

The length of phages ranges from 100 nm to a few microns, but the fluid sheets they formed exhibit similar properties. The membranes are all liquid-like monolayers lying in the x-y plane; they are composed of chiral or achiral rods aligning in the z-direction. For example, in figure 3, researchers use *fd* wild-type (*wt*) viruses with contour length of 880 nm. They separate them into left-handed and right-handed membranes and test their merging behaviors. Figure 3(B) shows that either a π-wall or an array of pores form at the coalescence edges when two same-handedness membranes fuse. Twisted bridges are built up along the proximal locations of fusion. In contrast, (C) reveals that when the membranes with opposite chirality merge. The rods on the edge align and become the interior of the membranes. Slowly the adjoining neck widens, and two cusp defects appear [5].

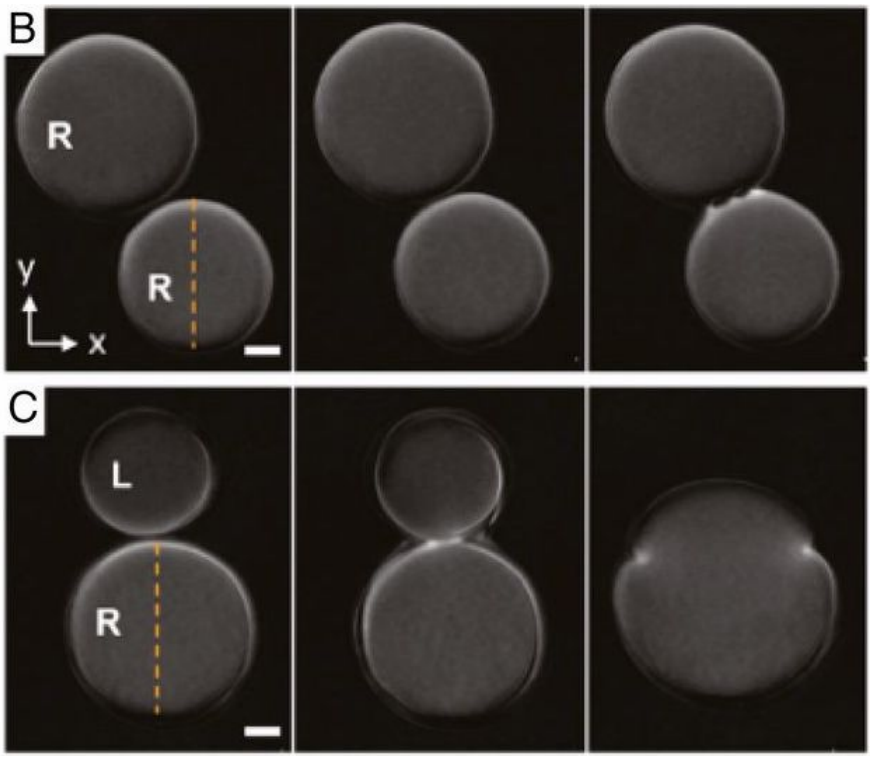

Figure 3: Lateral coalescence of membranes with opposite chirality results in different edges. (B) The coalescence of two right-handed membranes leads to the pore formation. (C) Coalescence of a left-handed membrane with a right-handed membrane produces two cusps that separate the left-handed edge section from the right-handed one. Scale bars represent 4 μm [5].

2D lipid membranes are extensively studied because they are the fundamental building blocks of biological membranes [8]. Since lipid rafts are typically in nanoscales, it is hard to visualize them under optical microscopes or handle them in experiments [6]. Phage membranes have similar properties to living cell membranes, but they can grow to a few microns. Their sizes ensure that their behaviors are visible.

As researchers pay close attention to the fusion of phage rafts, the insights may render more opportunities for understanding the merging and splitting of living cells. The modification made on phage membranes may even provide ideas for applying bioengineering to living cells.

5 2D Heterogeneous Colloidal Membrane

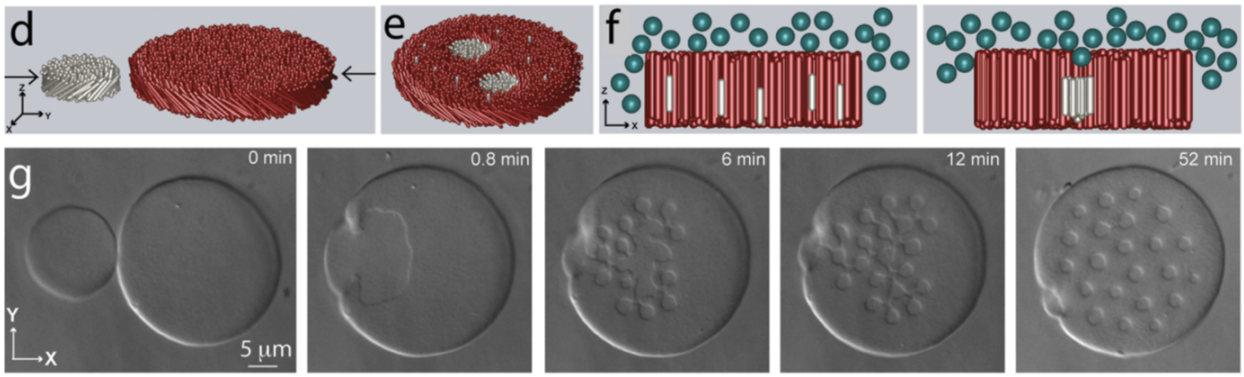

Figure 4: Schematic diagrams and microscopy images of the formation of 2D heterogeneous colloidal membranes. (d) - (f): Schematic illustration of the coalescence of membranes comprised of shorter and longer rods. (g) Lateral association of short right-handed fd-Y21M and long left-handed M13KO7 membranes [10].

Figure 4 shows the self-assembly of membranes composed of different lengths and chirality of rods. Each monodisperse raft contains approximately 20,000 virus particles [6]. fd-Y21M is about 10 μm; M13K07 is about 13 μm. Their lateral coalescence works well. If the difference of lengths of two types of rods is too significant, a heterogeneous membrane may fail to form.

Self-assembly of such membranes makes the whole coalescence process easy. After two membranes are mixed, the sample can sit overnight, and the lateral association is spontaneous. Since phage rafts are large, their movements are slow enough to be recorded by a camera.

In a broader context, both homogeneous and heterogeneous coalescence help demonstrate the importance of long-range repulsive interactions in chiral inclusion in colloidal membrane systems. The size of rafts also enables researchers to test the effective distance of repulsive interaction, which is about a few microns. Moreover, the local twist is essential for both lipid and colloidal rafts. This implies the necessity of cholesterol because it induces the local twist [6]. Cholesterol is largely unexplored in biology and biophysics fields, and this implication may intrigue more studies in the future.

For both homogeneous and heterogeneous colloidal membranes, 2D-LC-PolScope can visualize the details of lateral coalescence. 3D-LC-PolScope can record the twisted angle of rods and

check their chirality [4]. The experiments are easy to repeat and replicate due to the standard microscopy methods.

6 Conclusion and Perspectives

GUVs, SUVs, homogeneous and heterogeneous colloidal membrane systems are all model systems for precisely understanding real cells and their phase transformations. Actually, they also have numerous applications. For example, unilamellar vesicles, especially GUVs, are widely applied in targeted drug delivery systems. They can serve as spherical chambers taking cargos, and the lipid bilayers can easily merge with human membranes. SUVs can also take hydrophilic drugs inside the sack and hydrophobic drugs on lipid layers if the drug particles are small enough [3].

There are also other model systems that focus on membranes specifically, like ghost red blood cell, which is a red blood cell without a nucleus. The ghost cells directly come from the real red blood cells that experienced hemolysis, which is a process to destroy their nuclei [1]. These empty cell envelopes are even more popular in natural drug delivery fields because compared to artificial chemicals, they have limited side effects.

There are also specific events among membranes that are still under research, such as fusion of phospholipid vesicles with planar phospholipid bilayer membranes [7]. The surface energy and energies for edges and curvatures for colloidal membranes are also essential because they provide information about the conditions where membranes can close up into a vesicle automatically. These model systems can not only answer questions in natural biological systems but also offer implications for bioengineering.

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