Filamentous Phages as a Model System for Biological Membranes

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Abstract: Living cell membranes are complicated, and it is difficult to measure their physical properties. Thus, we will use colloidal phage membranes as a simple, convenient model system to understand biological membranes from a physics perspective. In particular, we will focus on heterogeneous rafts, their coalescence, their common shapes and shape transitions, and interactions between different rafts. We will also briefly mention their implications for lipid bilayers.

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 [7]. Giant and small unilamellar vesicles (GUVs and SUVs) can solve this problem biologically, but their membranes are still too thin, so the nano-scale membrane mechanics are hard to visualize and quantify. Thus, to better recognize the kinematics and dynamics of biological membranes, it is necessary to construct a simple physics model system.

Colloidal phage membranes are a useful, functional alternative. Researchers have analyzed the thermal fluctuations to attain membranes' lateral compressibility and bending rigidity, which proves that colloidal membranes have similar physical properties to conventional lipid bilayers [7, 1]. Colloidal phage membranes' thickness is on micron-scale, so the membrane transformations and interactions are slow, obvious, and can be visualized. Thus, the pathways, transitions, and movements in such colloids serve as an analogy for living cells.

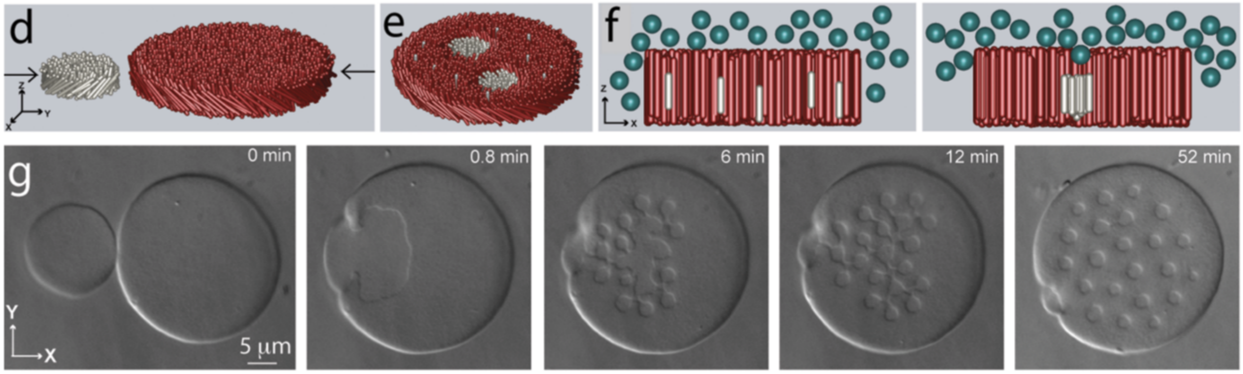

Figure 1: Schematic diagrams and microscopy images of the formation of 2D heterogeneous colloidal membranes. (d) - (f): Schematic illustration of the coalescence of membranes comprised of shorter and longer rods. (g) Lateral association of short right-handed fd-Y21M and long left-handed M13KO7 membranes [12].

Colloidal phage membranes composed of rod-like viruses align laterally, forming a 2D flat membrane. We typically acquire such colloids by self-assembly, which is done in a solution comprised of monodisperse rod-like phages, non-adsorbing dextran polymer, and polyelectrolytes to screen electrostatic repulsion [10]. Tuning the length of phages, the concentration of dextran, and polyelectrolytes will result in different forms of molecular aggregates, and together we can draw a phase diagram, such as (d) in figure 2.

Various phases correspond to distinguished states in living cells. For example, except for homogeneous 2D membranes, heterogeneous rafts also render numerous intriguing observations in recent decades [6, 7]. Experiments show that different lengths of rods with opposite handedness can create the lateral association [12], just as figure 1 reveals. Shorter rods have higher curvatures, so the positions of embedded short rods modify the overall shapes of membranes. These multiple polymorphic shapes resemble the topological transitions in 3D fluid vesicles, providing implications for vesicle fusion, fission, and pore formations [3]. Moreover, the local twist is essential for both lipid and colloidal rafts [7]. Shorter rods induce local twists in a similar way as embedded cholesterol affects lipid bilayers, so the energy and shape change associated with short rods may be relevant to the functions of cholesterol in living cells.

2 Controlling Shapes and Topologies

Since cells and cell transformations involve multiple geometries, controlling the shapes and topologies of colloidal membranes to match comparable lipid bilayers is crucial. Figure 3 explains that the number fractions, embedded positions, and orientations all modify the curvature of flat membranes. Thus, fusing different lengths of rods is a method to change shapes[13].

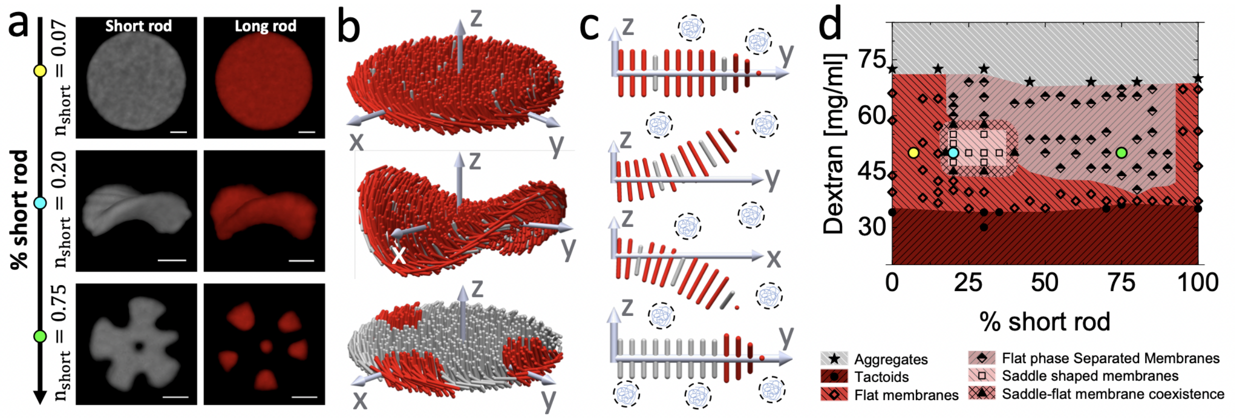

Figure 2: Real and schematic diagrams of two-component colloidal membranes. (a) Heterogeneous colloidal membranes composed of long (red) and short (gray) rods. As the number fraction of short rods increases, 2D flat disk gradually changes into saddle surfaces, and at even higher fraction, phase separation occurs. Scale bar, 2 μm; (b-c) Schematics corresponding to different n_{short} fractions. The positions and orientations of rods influence the shapes of membranes; (d) Phase diagram of binary rods mixtures. [13].

After we match the shapes, the quantities that are difficult to measure in lipid bilayers can in turn be accessed in colloidal membranes. For example, Gaussian curvature is key to estimating membrane curvature generation and modulation such as endocytosis and exocytosis, cell differentiation, and cell motility [13, 11, 8]. Integrating the whole surface to get Gaussian modulus is challenging for lipid bilayers, but it is doable for colloids. Gaussian modulus is correlated to membrane stability. Doping different length of rods change the stability of membranes, which also makes Gaussian modulus computable [13].

Colloidal membranes, traditional lipid bilayers, and many other membrane-based systems are characterized by the same continuum energy [13]. Then deriving such Gaussian modulus also helps us understand general membranes in a broader context.

3 Shape Transitions and its Implications

Diverse shapes transform into each other naturally or when they are exposed to external stimuli. Some processes are reversible and some are not, and these procedures can be matched to reversible and irreversible activities in living cells. Figure 3 is a clear example.

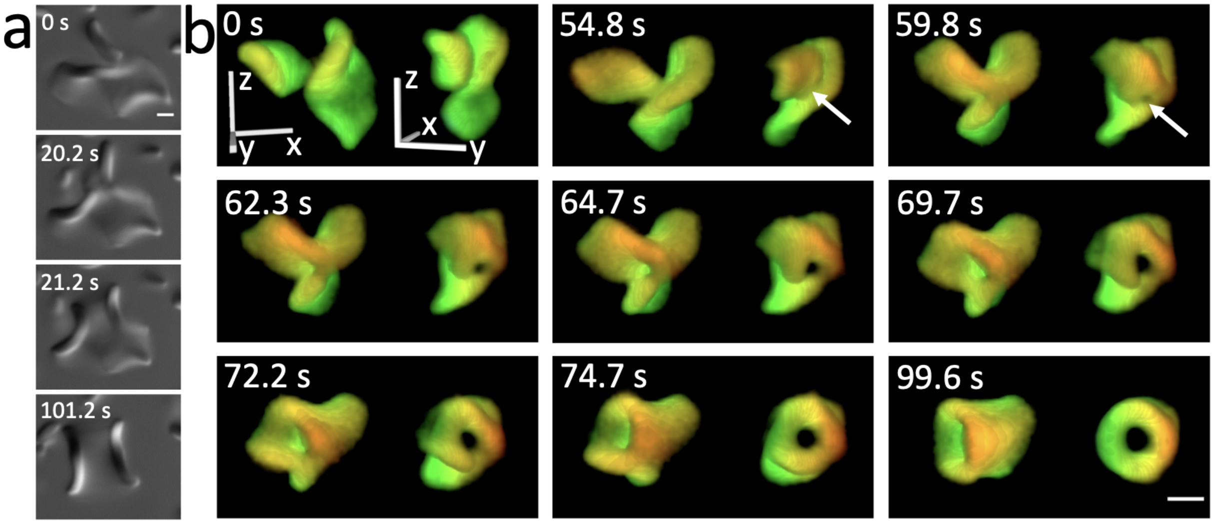

Figure 3: (a) Catenoid-shaped membrane formed by the coalescence of two saddle-shaped surfaces under DIC microscopy; (b) 3D false-colored images of this coalescence process. It records the intermediate steps as real time visualization under fluorescent microscopy. The long rods are labeled with fluorescent, so they are visible when we perform z-scanning under an optical microscope. Scale bars, 2 μm. [13].

First, a 2D disk transforms into a saddle by embedding shorter rods into the background of longer rods. If two saddle membranes contact each other under some specific orientations, they will fuse into a catenoid. Catenoid is not the endpoint. By merging with more saddle surfaces, it grows into tri-noid, four-noid, and other topologically distinct structures with more genera. Thus, in theory, any topological structure can be realized under careful treatment, and we achieve the goal of controlling the shapes and topology of heterogeneous colloidal membranes. In this case, phage colloids can potentially mimic any topological configurations of biological membranes by altering the fractions of short rods and applying them to merge.

For both lipid bilayers and colloidal monolayers, the number and appearance of singularities imply membrane-based transformations, such as fusion and fission. However, the lipid bilayers are too thin, so their dynamic interactions are too quick to be captured. Also, the nano-scale singularities in cell membranes are too small to be visualized. Colloidal phage membranes, in contrast, exhibit micron-scale pores that can be recorded under an optical microscope. The coalescence of two surfaces also takes minutes to complete, so the interim stages are easy to be tracked and analyzed in software. For example, the pores in figure 3 are obvious, and their energies and Gaussian curvatures can be calculated from the shapes.

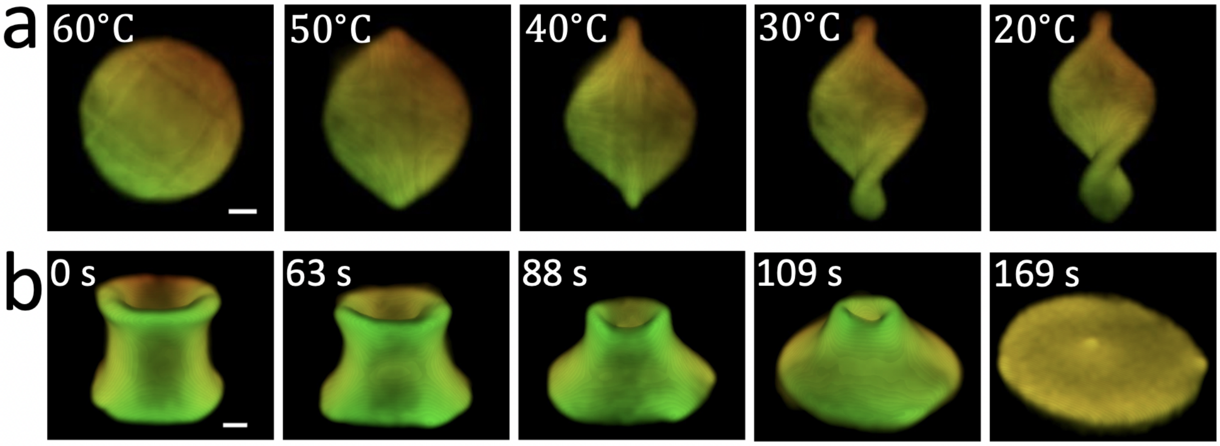

Figure 4: 3D false-color images showing the geometry and topology changes of colloidal membranes when exposed to stimuli. (a) Disk-to-saddle and disk-to-helicoid transformations as temperature decreases; (b) Catenoid-to-disk transformation as time elapses when raising the temperature up to 70 °C. Scale bars, 2 μm. [13].

Phase transitions also occur dramatically when colloidal membranes are exposed to external stimuli. Topology alters the stability of membranes, so when colloids undergo a macroscopic parameter change, like temperature, the rods reorganize. For example, topologically distinct geometry displays different transformations when they undergo the same temperature elevation. At 60 °C, saddle membranes rearrange into flat surfaces, but catenoids exhibits almost no change. This is the same physics as decreasing the concentration of surrounding polymers, like Dextran, because both methods reduce the osmotic pressure between the concentration of rods and enveloping polymers. Such conversions are reversible.

Previously, the technique of designing stimuli-responsive materials with alternative 3D topologies is not fully developed in biology. The most advanced one is elastic sheets that react to macroscopic stimuli [14, 9]. The shape transitions described above have not been done before, and they may be applied to improve the functionality of model membrane systems. It may also help simple models imitate real membranes more and assist scientists to investigate cells more deeply. For instance, reversible reconfiguration is common in living systems. Application in bioengineering can be another usage.

4 Raft-Raft Interaction

Once individual heterogeneous membranes can be designed and obtained, naturally the next step is to understand and quantify raft-raft interactions. Experiments tell us rafts with the same chirality have long-ranged repulsions, while opposite chirality attracts [4]. Raft thickness, local tilt, and concentration of surrounding polymers also affect the magnitude of forces.

The underlying principles have not been fully unveiled, but they might be closely related to living cell membranes. The reason is for traditional lipid bilayers, higher-order configurations are achieved by membrane-mediated interactions. Colloidal rods are in the same case. The hierarchical configuration can imitate the complexity of biological membranes by tuning the type of phages and handedness. Rearrangements like saddle surfaces becoming catenoid at particular contact angles can also be analogous to second-order structure formations in living cells [13]. By changing the lengths and chirality of rods, the composition and orientation of colloids adjust correspondingly, giving us various forms of assembly.

5 Conclusion and Perspectives

Heterogeneous rafts render implications for curvature changes, phase and shape transitions, splitting and merging events, vesicle fusion, fission, pore formation, membrane-membrane interactions, and higher-order structure formations in living cells. They are also a potential stimuli-responsive material for bioengineers. Since they are large, thick, simple, and change slowly, they are relatively easy to be quantitatively analyzed and be assigned with specific formulas, such as some equation of states [2].

Thus, colloidal membranes are useful for both understanding the fundamental pathways and structures of biological cells and designing artificial cells. Such colloids can mimic lipid bilayers even more precisely. Experiments have been successfully achieved lateral coalescence of bilayers of short rods in the background of long rods [5]. If we label the ends of phages of hydrophobic and hydrophilic molecules, they can potentially form a phage bilayer. In that case, physical and even biological properties can be even more precisely described, and colloidal membranes will become a more powerful model for soft matter physics.

References

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