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Commentary and Perspective

Creation of supramolecular biomembrane by the bottom-up self-assembly: Where material science meets biophysics

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Introduction

The biological membrane consisting of lipid bilayer and associated proteins possesses unique material properties that are critical to the membrane-based biological functions such as signal transduction and energy conversion [1]. The lipid bilayer is a two-dimensional fluid, in which membrane-associated molecules can laterally diffuse and interact with each other. The lipid membrane also acts as a permeation barrier towards water-soluble molecules, forming microscopic / nanoscopic compartments. These unique physicochemical properties of the biological membrane have important implications in material science, and fostered the development of biomimetic materials and systems. On the other hand, novel synthetic materials have a potential to be utilized in the fundamental studies of membrane biophysics. Bottom-up approaches based on the self-assembly of materials are promising to reproduce unique membrane structures and functions, providing insight into the machinery of the biological membrane and enabling a wide range of applications. In this article, we briefly introduce some recent studies to create novel artificial biomembranes using not only conventional phospholipids but also synthetic polymers, nanoparticles, and their hybrids to explore the interface between biophysics and material science. These topics are featured in the corresponding symposium in the 60th Annual Meeting of the Biophysical Society of Japan in 2022 where Drs. Morigaki, Sugihara, Tero, Fijii, Yusa, Yasuhara, and Mr. Masuda delivered invited lectures.

Model Membranes from Synthetic Lipid Bilayers

Model membranes have been developed as a useful tool for reproducing and studying the physicochemical properties of the biological membrane. Studies using model membranes can reveal some fundamental aspects of the biological membrane (Fig. 1). For example, using lipid vesicles and supported lipid bilayers (SLBs), a unique cooperative function was found between two antimicrobial peptides, LL-37 and HNP1 [2]. When they were combined, they kill bacteria more efficiently while minimizing the host damage by suppressing the lysis of mammalian cell membrane. Such a "double cooperativity" may be used in our immune system and may help with developing efficient and safe antimicrobial agents in the future. Lipid vesicles are also utilized as artificial cells in fundamental studies of origins of complexity and living

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systems. In general, lipid vesicles having only the shell of lipid bilayer membrane are more fragile than living cells due to the lack of a cytoskeleton. Such a friability of lipid vesicles poses a challenge when the artificial cells are used as capsules for drug delivery. To amend this instability problem, DNA nanotechnology was used to create a cytoskeleton-like structure in artificial cells, resulting in stabilization of the membrane and enhancing the application of artificial cells [3]. This example highlights versatility of the combination of lipid membrane and designed biopolymer, which can generate robust artificial cells, and may also find applications in drug delivery systems.

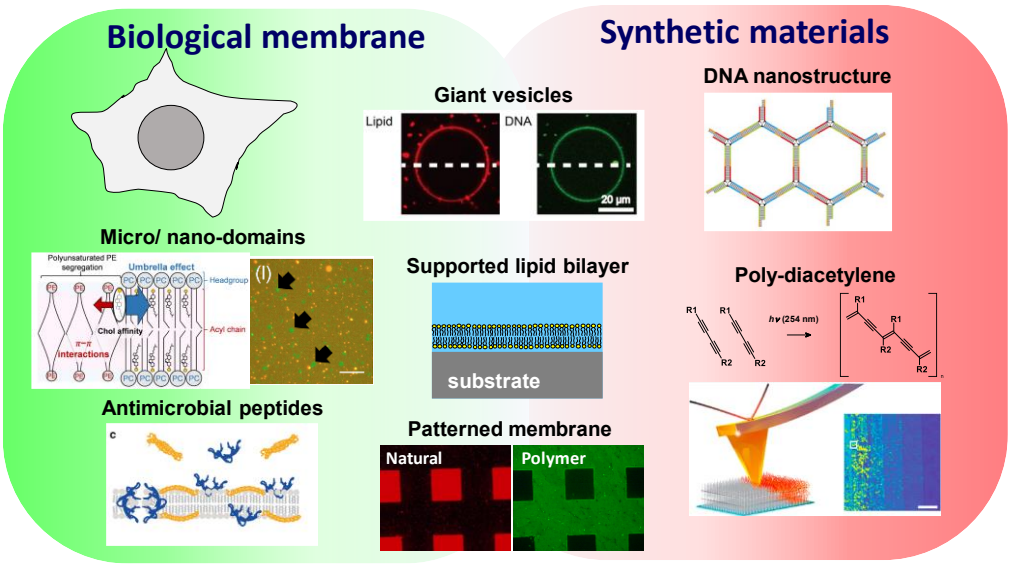

Figure 1 Schematics of the approaches at the interface between membrane biophysics and material sciences. The unique physicochemical properties of the biological membrane have important implications in material sciences, whereas novel synthetic materials have the potential to be utilized in the fundamental studies of membrane biophysics. The images were adapted from Refs. [2], [3], [6] and [13].

SLBs are suitable model system to study the two-dimensional complexity of the biological membranes. As a simplified model of the membrane heterogeneity found in the biological membranes (e.g. lipid rafts), phase separation in lipid bilayer has been extensively studied [4]. In a recent study, microdomains of polyunsaturated phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were studied in lipid bilayers comprising monounsaturated PC and cholesterol [5,6]. The number and distribution of double bonds in alkyl chains of polyunsaturated lipids determined the efficiency of domain formation. In addition, the microdomain were found to work as a specific site for membrane fusion, promoting the fusion of proteoliposomes [7]. It suggests that we can construct complex model membranes mimicking the biological membrane by utilizing the purposefully induced phase separations.

One important feature of SLB is the possibility to form complex structures by the microfabrication techniques [8]. A patterned hybrid membrane composed of polymeric and fluid lipid bilayers has been developed using photolithographic polymerization of diacetylene phospholipid [9]. The polymeric bilayer acts as a framework to define the geometry of the fluid bilayers and enhance their stability. The fluid bilayers comprise natural lipids and membrane proteins, and retain the physicochemical properties the biological membrane. The stable framework of polymeric bilayer was also utilized for constructing a nanometric aqueous space on the fluid bilayer region [10]. The nanometric confinement effectively reduced the background noise and enabled sensitive detection of membrane-bound molecules [11].

Polydiacetylene has a conjugated ene-yne backbone that shows unique changes of color by the mechanical stress or structural changes (mechanochromism). It has been extensively applied to polymer/membrane-based biosensors [12]. However, quantitative and anisotropic mechanochromism of polydiacetylene over nanoscale distances remains unaddressed even after 50 years of extensive research. This is because its anisotropic structure on substrates necessitates the application of both vertical and lateral forces (shear forces) to characterize it, whereas atomic force microscopy, which is the usual technique used to investigate nanoscale forces, is only capable of quantifying vertical forces. Recently, this lacuna was addressed by utilizing quantitative friction force microscopy that measures lateral forces [13]. The force-fluorescence correlation at nanoscale obtained in this work can be used to develop quantitative biosensors based on polydiacetylene.

Supramolecular Artificial Biomembranes Formed by Synthetic Materials

A variety of synthetic materials have been developed to fabricate artificial biomembranes that mimic the structure and functions of native cell membranes. Hybridization of a lipid bilayer with synthetic amphiphilic polymers is one promising approach to functionalize membranes. A series of amphiphilic polymethacrylate random copolymers with hydrophilic and hydrophobic side chains were designed to modify a lipid bilayer. Yasuhara *et al.* reported that amphiphilic polymethacrylates with butyl and cationic choline groups on the side chains spontaneously form uniform lipid nanodiscs with a diameter of tens of nanometers through the fragmentation of lipid bilayers (Fig. 2, 1) [14]. The produced nanodiscs can be utilized as a minimal model cell membrane because of their homogeneity and stability in an aqueous solution. The lipid bilayer encompassed in nanodiscs was found to maintain native-membrane-like properties such as gel-to-liquid crystalline phase transition. The nanodiscs have been applied to investigate the interaction of amyloidogenic peptides including amyloid beta (Aβ) [15] and human islet amyloid polypeptide (hIAPP) [14] with a lipid bilayer. In both peptides, the addition of nanodiscs significantly inhibited the formation of amyloid fibrils. This is likely due to the small size of the nanodiscs that limits the number of peptide molecules bound to the same membrane, resulting in the capture of small oligomers of the peptides.

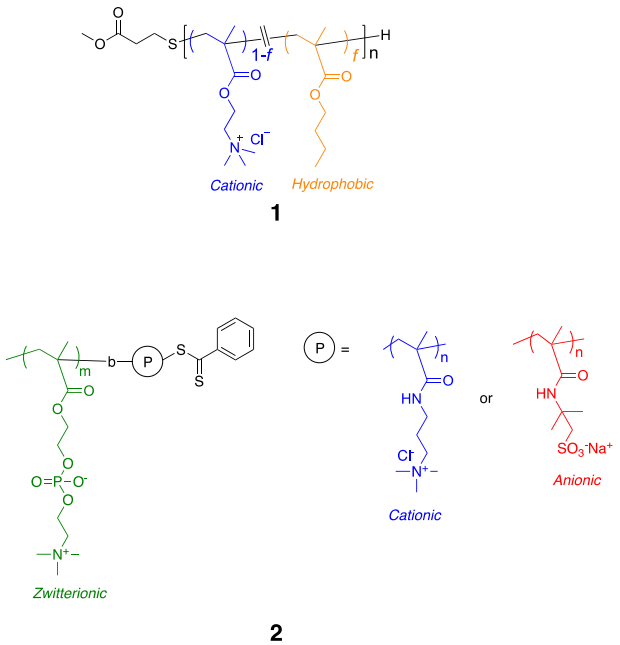

Figure 2 Chemical structures of polymers for the spontaneous formation of nanodiscs (1) [14] and supramolecular assembly via polyion complex formation (2) [19].

Polymethacrylate derivatives can also form nanopores, which allows the selective permeation of small molecules, in a lipid bilayer. The interaction of the polymethacrylate derivatives with a lipid bilayer was investigated using giant vesicles, which are cell-sized vesicles enabling *in situ* visualization of membrane dynamics [16]. The addition of the amphiphilic polymethacrylate derivatives with primary ammonium and butyl side chains to giant vesicles induced the release of an entrapped sucrose, a small marker molecule, without any significant morphological perturbations of giant vesicles. In contrast, the large marker (RITC-dextran, 70 kDa) was not released in the same condition, indicating the formation of a pore structure with a definite size of the opening. Since polymethacrylate derivatives with methyl groups as shorter hydrophobic chains did not form pores but induced membrane rupture, the mode of action on the lipid membrane changes depending on the length of the hydrophobic side chains.

Artificial membranes can be produced also solely by synthetic polymers based on the self-assembly of block copolymers. Yusa *et al.* have extensively studied the supramolecular assembly of block copolymers in an aqueous solution mediated by the formation of polyion complex [17-19]. The formation of polyion complex originated in the multi-point electrostatic interactions between polymer chains aids the self-assembly in water that plays a similar role to

the hydrophobic effect in the assembly of lipids. They have designed a series of block polymers consisting of charged (cationic or anionic) and zwitterionic domains (Fig. 2, 2). The polymer was synthesized by a reversible addition fragmentation chain transfer (RAFT) polymerization, which enables precise control of the polymer structure. The topology of the assembly can be modulated by changing the balance in the length of charged and zwitterionic domains. The polymers consisting of a long zwitterionic domain and a relatively shorter charged domain were found to form micelles in which polyion complex was formed in their core [17]. In contrast, the block polymer with a zwitterionic domain shorter than the charged domain assembles into vesicles [18]. Giant polyion complex vesicles in μm scale were also produced by the dialysis of the polymer solution containing a high concentration of sodium chloride as a salt due to the gradual modulation of the electrostatic interaction between charged domains [19].

Artificial membranes for the compartmentalization of an inner space can be fabricated not only by molecules but also using small particles. Liquid marbles are droplets that are stabilized by hydrophobic solid particles adsorbed to an air-liquid interface (Fig. 3A). Liquid marbles can be formed simply by rolling liquid droplets on a dry powder of small solid particles. Fujii *et al.*, promote the engineering of liquid marbles using various hydrophobic particles. Recently, a new type of liquid marbles, so-called polyhedral liquid marbles has been developed (Fig. 3B) [20]. These armored spheres consist of liquid droplets stabilized by hydrophobic hexagonal plates instead of spherical particles used for conventional liquid marbles. Various intriguing forms and shapes of the liquid marbles were fabricated, exhibiting highly ordered crystalline plate arrays. The polyhedral liquid marbles show strong interfacial jamming and are stimuli-responsive. Interestingly, natural creatures were also found to utilize liquid marbles. Some aphids that live in the leaf galls of the host plant are known to fabricate liquid marbles consisting of honeydew and wax particles as an inner liquid and a stabilizer, respectively (Fig. 3C) [21].

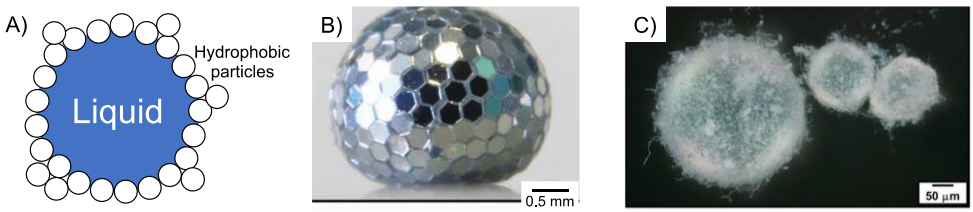

Figure 3 Schematic figure of liquid marble (A) and microscopic pictures of polyhedral liquid marble (B) and liquid marbles fabricated by aphid (C). The images were adapted with permission from Refs. [20] and [21].

Conclusion and Perspective

In this commentary article, we have featured recent progress on the creation of novel biomembrane-mimetic systems that was presented in the symposium at the 60th Annual Meeting of the Biophysical Society of Japan in 2022. Since Bangham firstly reported the spontaneous formation of liposomes by phospholipids in 1965 [22], many researchers actively investigated the development of membrane-forming materials. Lipid-based membrane systems have evolved to achieve structures and properties similar to native biomembranes and have been optimized for biophysical analyses. In particular, our symposium introduced patterned lipid bilayer, phase-separated planner bilayer, mechanoresponsive membrane, lipid nanodisc, and DNA-gel encapsulating vesicles. Additionally, inspired by the self-assembly of lipid molecules in water, various non-lipid materials such as synthetic polymers and particles have been developed to organize biomembrane-like assembly. In near future, these artificial membrane systems are expected to provide a powerful platform for unraveling the mystery of biomembranes in a reconstructive approach.

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