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Membrane Disk and Sphere: Controllable Mesoscopic Structures for the Capture and Release of a Targeted Object

Tsutomu Hamada,[†] Ryoko Sugimoto,[†] Mun'delanji C. Vestergaard,[†] Takeshi Nagasaki,[‡] and Masahiro Takagi*[,†]

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan, and Graduate School of Engineering, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

Received May 7, 2010; E-mail: takagi@jaist.ac.jp

Abstract: Design of molecules for self-assembled mesoscopic structures with specific functions is an important and interesting challenge that spans across disciplines such as nanosciences. A closed lipid membrane is a good example of a self-assembled mesostructure. In this study, we developed controllable membrane formation by making a subtle change at the molecular level. We utilized a synthetic photosensitive amphiphile (KAON12) to achieve the photobased molecular manipulation of the opening and closing of membranes through reversible transitions between sphere and disk structures. We found that the mechanism is based on the photoswitching of the membrane line tension, as deduced from the fluctuation of the membrane edge, through the action of KAON12. Furthermore, we demonstrated the controllable capture and release of colloidal particles into and from a membrane sphere. The observation of Brownian motion of the particle confirmed colloidal encapsulation. This successful photomanipulation of mesoscopic membrane structures in a noncontact and reversible manner should lead to a better understanding of the mechanism of membrane self-organization and may see wider application, such as in microreactors and drug-delivery systems.

Introduction

Nature builds from the bottom up. Molecules self-assemble in a predetermined fashion under specific conditions into highly organized supramolecular structures with specific functions. Over the past several decades, the organization of soft matter, such as colloids, polymers, amphiphiles, liquid crystals, and most biological constituents, has been the focus of increasing interest for the manipulation of shape and function at a mesoscopic scale.[1−3] Small perturbations in molecular levels can lead to profound changes in mesoscopic organized structures. It is important to construct or manipulate the assembly of such soft matter in a noncontact and reversible manner so that we can better understand the mechanism of self-organization.

Lipid membranes are a form of self-assembled soft matter, and the constituent molecules are known to form a bilayer interface with hydrophobic tails toward the interior.[4] A closed two-dimensional surface consisting of a lipid bilayer is an essential compartment of living organisms in that it acts as a molecular sieve by preventing the entrance of "unwanted" molecular components while allowing the diffusion of "wanted" molecules. Artificial lipid vesicles are used as biomimetic model membranes because they are controllable[5,6] and enable the elucidation of biological events.[7−9] Specifically, model membrane systems have been exploited for a wide range of applications, including as intrinsic biological interactants,[10,11] biomimetic reactors,[12,13] and drug-delivery systems.[14] Membrane design at a molecular level that would enable the capture and release of desired molecules is an outstanding challenge.[15]

The stability of lipid bilayers is determined by the elastic free energy of the membranes. Within bilayer membranes, edges are disfavored because of the high energy cost of either exposing the hydrophobic lipid chains to water or creating a highly curved rolled edge to hide them. Thus, open membranes with either an edge or a pore have an energy cost per unit length of the exposed edge, i.e., membrane line tension (∼10 pN).[16] For 10-μm-scale membranes, which are the typical size of cells, a closed

[†] Japan Advanced Institute of Science and Technology.
[‡] Osaka City University.

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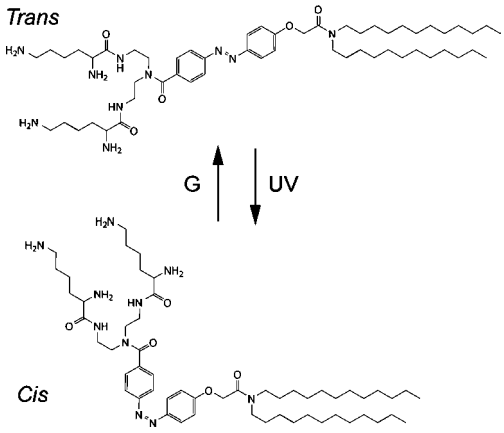
Scheme 1. Structural Formula of the Azobenzene-Containing Amphiphile (KAON12)



membrane without a boundary is energetically more stable than the open form, since the cost of the bending energy to obtain a closed membrane ($\sim 10^{-19}$ J) is much lower than that of the line energy needed to expose the bilayer rim of the open membrane ($\sim 10^{-17}$ J). A decrease in line tension is expected to induce a stable pore within bilayer vesicles.[17,18] Indeed, a membrane pore was experimentally observed in model membrane systems only upon treatment with additives such as surfactants[19,20] or proteins,[21] and this was followed by theoretical analyses.[22−24]

Previously, we reported that a synthetic photoresponsive amphiphile (KAON12) can alter the morphology of closed vesicles, such as prolate, oblate, stomatocyte, and budding.[25,26] The azobenzene component serves as a photoresponsive region, and the molecular conformation (trans or cis) can be switched by light irradiation (Scheme 1). In the present study, we used KAON12 to (i) produce stable open membranes in addition to closed membranes, through optimization of the environment in which the vesicles were prepared, and (ii) manipulate reversible transitions between closed and open membranes. This membrane engineering is based on the photoswitching of membrane line tension through the action of KAON12. Further, we demonstrate the controllable capture and release of colloidal particles to and from the membrane by inducing switching between membrane shapes.

Results and Discussion

As described in the Methods (see Supporting Information), the membranes are prepared from a binary system of the photoresponsive molecule KAON12 and a phospholipid, dioleoyl phosphatidylcholine (DOPC), through natural swelling of the lipid film with a Tris-HCl (pH 7.4) buffer solution.[26,27] We observed changes in membrane morphology with a fluorescent microscope at room temperature (the microscopic setup is shown in Figure 1). Figure 2A (left) shows a typical closed spherical membrane structure of the DOPC/KAON12 binary system. Through experimental selection and optimization of the buffer system used, we succeeded in producing open disk-, bowl- and cup-shaped membrane structures (Figure 2A), where the KAON12 isomer is in a trans form. A DOPC bilayer without KAON12 formed only closed structures without a membrane rim. Both open and closed structures were stable over the experimental time scale of approximately 10 min, although some disk-shaped membranes exhibited edge fluctuation. We classified the open membrane structures in terms of the aperture angle θ, i.e., cup with $\theta < \pi/2$, bowl with $\theta > \pi/2$, and disk with $\theta \sim \pi$ (Figure 2B). When the mixing ratio of KAON12 increased to a molar ratio of >35%, cup, bowl, and disk membranes were produced (17.4%, $n > 500$). At a higher KAON12 molar ratio (40%), there was a significant increase in the probability of open membranes (73.1%, $n > 900$), and the disk structure was

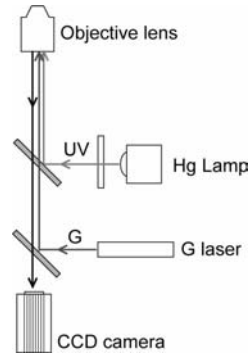


Figure 1. Microscopic setup for the photoirradiation of membranes.

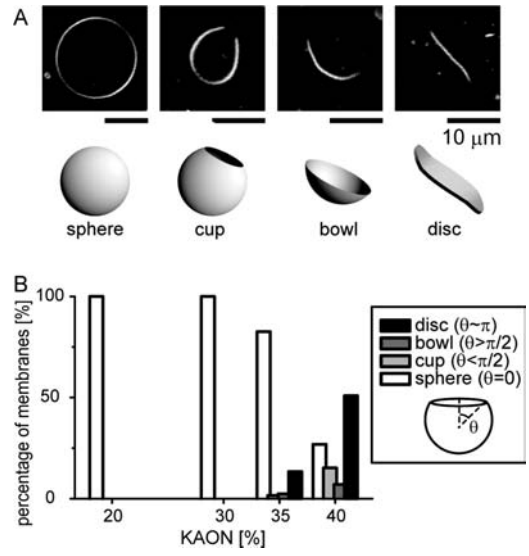


Figure 2. (A) Video microscope images of lipid membranes with sphere, cup, bowl, and disk shapes. (B) Probability of each membrane shape as a function of the KAON12 concentration (molar mixing ratio). The number of observations was $n > 500$.

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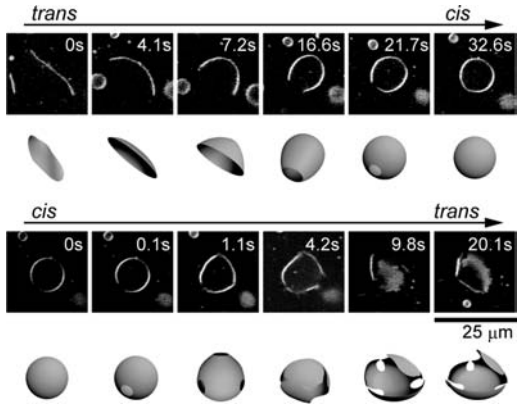

Figure 3. Photoinduced reversible formation of a lipid membrane sphere. Membrane closing (upper) and opening (lower) processes. The time elapsed is shown on each micrograph.

observed most often (69.5% of total open membranes). At a KAON12 concentration above 45%, we could not obtain any micron-sized membranes.

We next investigated the effect of photoirradition on open membrane disks containing ~40% KAON12. The KAON12 isomer can be reversibly switched between trans and cis isomers by light irradiation. When we irradiated the membrane with UV light to induce cis-isomerization of KAON12, the aperture angle of the disk gradually decreased to become a membrane cup and bowl, followed by a sphere (Figure 3 upper and Video S1, Supporting Information). The photoisomerization of KAON12 from the trans- to cis-form transformed the open membrane disk into a closed spherical structure. Interestingly, the membrane sphere reverted to an open membrane by trans-isomerization (i.e., by discontinuing UV irradiation during treatment with green light) (Figure 3 bottom and Video S2, Supporting Information). A single pore was opened at 0.1 s, and an additional two pores were formed at 1.1 s (the number of the total pores was three at that time). Then (after 4.2 s), the size of the pores gradually increased, colliding into a continuous membrane rim, to decrease line energy. The formation of plural pores by trans-isomerization was observed in relatively large-sized membranes, where the diameter of the membrane sphere was above approximately 10 μm, as shown in Figure 3. In contrast, small-sized membranes tended to exhibit opening dynamics with the formation of a single pore. Under our experimental conditions, this membrane system showed high photoresponsiveness (98%, $n > 100$: 72% of membrane disks transformed into spheres and 26% changed into cups) (see Supporting Information).

To determine the change in membrane elastic energy upon photoisomerization, we focused on the thermal motion of the edges of membrane disks. The fluctuation of an edge line is related to the line energy of the membrane, because the line energy is proportional to the length of the exposed edge.[16,19] Since it is difficult to monitor the edge behavior of free-standing membranes in aqueous solution due to thermally induced rotational motion, we observed membrane disks near a glass surface (Figure 4). On the basis of the intensity of fluorescent dyes, we considered that these membrane disks were situated on a first supported bilayer. Notably, the membrane disks near the glass surface did not show closing dynamics upon exposure to UV light, while membrane disks in bulk solution transformed into closed spheres, as shown in Figure 3. This could be attributed to the adhesion energy between interfaces derived

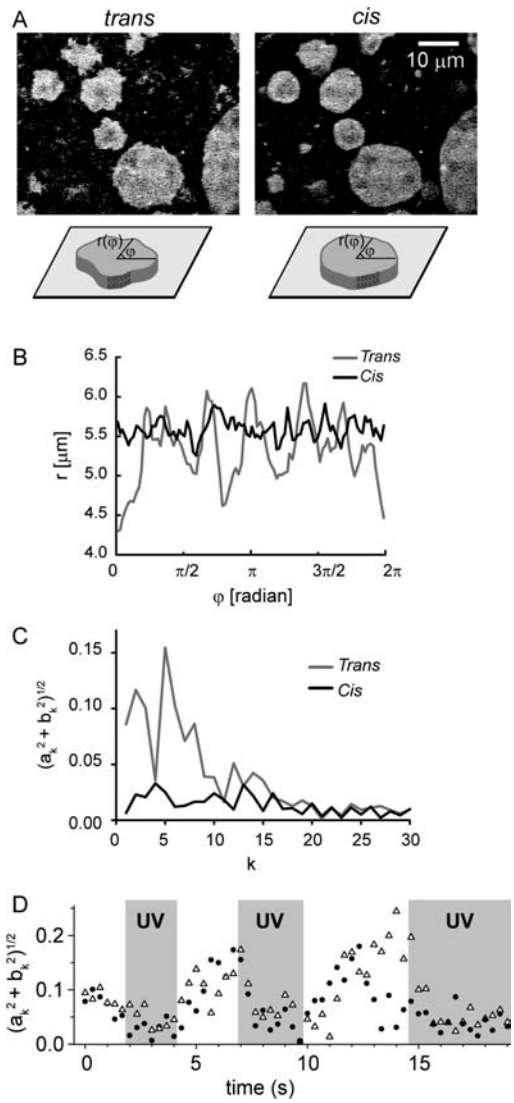

Figure 4. (A) Video microscope images of membrane disks near a glass surface with each isomer. (B) Spatial fluctuation in the radius (r) around the contour. (C) Amplitude $[(a_k^2 + b_k^2)^{1/2}]$ versus wavenumber (k) of edge fluctuation, as obtained by a Fourier analysis of part B. (D) Repetitive photoswitching of the fluctuation behavior. Amplitude $[(a_k^2 + b_k^2)^{1/2}]$ versus time for wavenumber $k = 2$ (ellipsoidal fluctuation) and $k = 3$ (asymmetrical fluctuation) is shown.

from van der Waals forces.[28] We found that the disks showed a marked difference in membrane edge fluctuation between isomers (Figure 4A). To clarify this difference between isomers, we determined the change in the disk radius upon photoisomerization (Figure 4B). Fluctuation of the trans-membrane edge was greater than that of cis-membrane edge, indicating that photoisomerization caused switching of the interfacial line tension of membranes. Next, we calculated the Fourier amplitude of edge fluctuation corresponding to Figure 4B as a function of wavenumber k (Figure 4C). The Fourier expansion of the radii is given by

$$r(\varphi) = r_0\left[1 + \sum_{k=0}^{\infty} a_k \cos(k\varphi) + \sum_{k=0}^{\infty} b_k \sin(k\varphi)\right] \quad (1)$$

where a_k and b_k are the Fourier coefficients. To confirm the dynamics of fluctuation (Video S3, Supporting Information), the amplitudes of the second and third wave numbers are

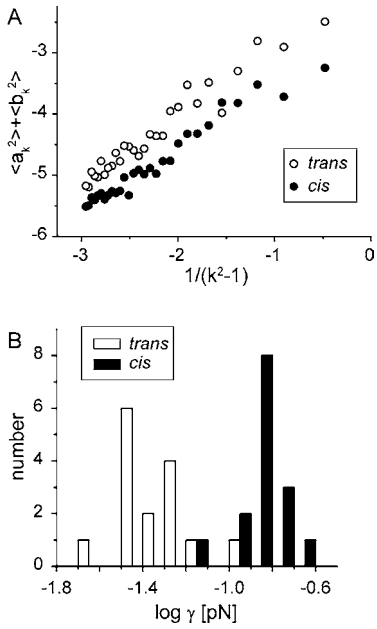

Figure 5. Calculation of disk−membrane line tension from edge fluctuation data. (A) Double-logarithmic plot of the Fourier coefficients against $1/(k^2 - 1)$ of a single disk membrane with each isomer. (B) Distribution of line tension γ of trans- and cis-membranes ($n = 15$).

calculated as a function of time in Figure 4D. The results indicate that the fluctuation was suppressed under UV treatment in a repetitive manner.

By applying capillary wave theory, we analyzed edge fluctuation to obtain the value of interfacial line tension.[29,30] On the basis of the model presented by Stottrup et al.,[29] the relationship between the Fourier coefficients and wavenumber and line tension can be expressed as

$$\langle a_k^{\,2}\rangle + \langle b_k^{\,2}\rangle = \frac{2k_BT}{\pi r_0 \gamma}\,\frac{1}{k^2 - 1} \qquad (2)$$

where k_B is the Boltzmann constant and T is the absolute temperature. Figure 5A shows the relationship between the Fourier coefficient and wavenumber of a single membrane disk for each isomer, where the Fourier coefficient was averaged over 30 time-sequence images. The linear slope of the fitted data gives us the line tensions for the membrane as eq 2. Figure 5B shows the distribution of line tension with each isomer deduced from the aforementioned analysis for 15 membranes. The average line tension of trans-membranes ($\gamma_{trans} = 5.0 \pm 2.2 \times 10^{-2}$ pN) was smaller than that of cis-membranes ($\gamma_{cis} = 1.5 \pm 0.4 \times 10^{-1}$ pN). The dispersion is probably due to a wide variation in the mixed fraction of KAON12 during the vesicle preparation, as previously reported.[26] By taking these experimentally obtained values of the membrane line tension into account, we consider the stability of membrane shapes for a phenomenological discussion. The total free energy of a membrane is given by the sum of the bending and line energies[23]

$$F = \int \left[\frac{\kappa}{2}(c_1 + c_2 - c_0)^2 + \kappa' c_1 c_2\right] dA + \gamma \int dl \qquad (3)$$

where dA is the area element of the membrane surface, dl is the line element along the membrane pore, c_1 and c_2 are the two principal curvatures, c_0 is the spontaneous curvature, κ is the bending modulus, and κ' is the Gaussian bending modulus. Now we consider a membrane with the aperture angle θ. Under the assumption that $c_0 = 0$ and $\kappa \sim \kappa'$,[31] the free energy F of the membrane with a surface area of $4\pi R_0^2$ (R_0 is the radius of sphere when $\theta = 0$) is expressed as a function of θ:

$$F = 6\pi\kappa(1 + \cos\theta) + 2\pi\gamma R_0 \sin\theta\left(\frac{2}{1 + \cos\theta}\right)^{1/2} \qquad (4)$$

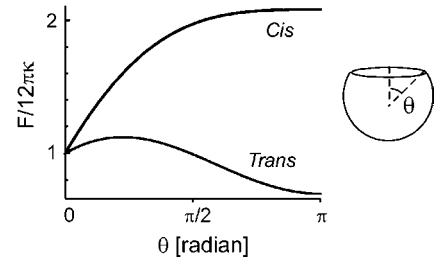

Figure 6. Elastic free energies of trans- and cis-membranes as a function of the aperture angle θ.

Figure 6 shows the free energies of trans- and cis-membranes with $\gamma_{trans} = 5 \times 10^{-2}$ pN, $\gamma_{cis} = 1.5 \times 10^{-1}$ pN, $R_0 = 5$ μm, $\kappa = 1.2 \times 10^{-19}$ J.[31] We assumed that the bending modulus of the membrane is essentially constant for photoisomerization and nearly equal to that of conventional phospholipid membranes. While the free energy of a sphere with $\theta = 0$ is a constant value, the free energy of an open membrane changes between isomers. Photoisomerization switches the stable membrane shape: trans-membranes preferably have open disk structure ($\theta = \pi$), while cis-membranes tend to show closed spherical structure ($\theta = 0$). Thus, the photoswitching of membrane line tension may be the main mechanism that underlies the closing and opening events of DOPC/KAON12 membranes. Critical line tension is also estimated to be $\gamma_c = 7.2 \times 10^{-2}$ pN by simply comparing the free energies between sphere ($12\pi\kappa$) and disk ($4\pi\gamma R_0$). The deviation of γ_{trans} ($5.0 \pm 2.2 \times 10^{-2}$ pN) approaches γ_c (7.2×10^{-2} pN), which indicates that some trans-membranes do not have open structures. This corresponds to the probability of free-standing open membranes (73.1%), as shown in Figure 2B. Here we discussed the phenomenological model with the experimentally obtained line tensions of each isomer. Although the relevance of free energy in molecular conformation is beyond the scope of this paper, a possible factor contributing to the change in line tension is the bulky structure of the cis isomer. Line energy is an energy cost along the membrane rim by either exposing the hydrophobic lipid chains to water or creating a highly curved rolled edge to hide them. Cis-isomerization expands the surface area per molecule within bilayer membranes,[25,26] which indicates that the oil−water contact region between the hydrophobic tails of the membrane molecules and the aqueous phase increases. Thus, cis-membranes have higher line energy than trans-membranes. It is also reported that, using small unilamellar vesicles, cis-isomerization of KAON12 induced membrane fusion to increase the size of vesicles,[32] which implies that cis-membranes tend to lower curvature surface, probably because of the bulky conformation of the cis isomer. In other words, cis-membranes

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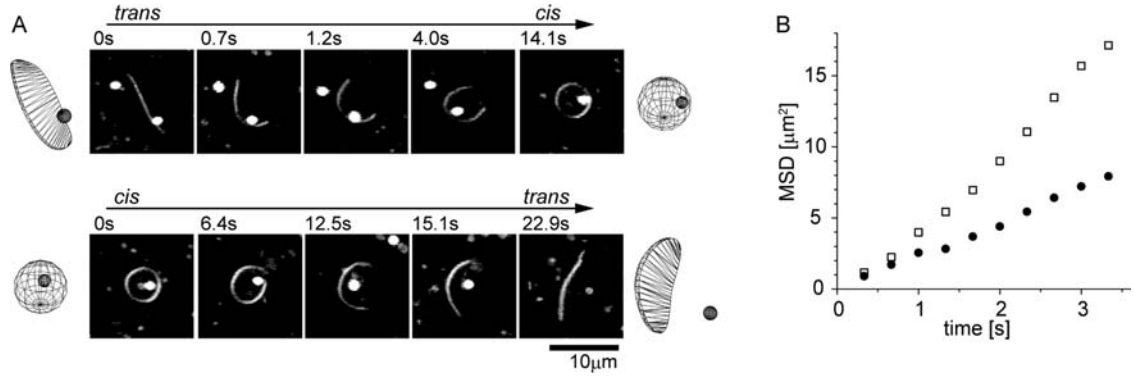

Figure 7. Reversible control of the capture and release of a colloidal particle with the membrane. (A) Video microscope images. The time elapsed is shown on each micrograph. Schematics of the starting and final images for each dynamics are shown. (B) Mean square displacement (MSD) of free (open squares) and encapsulated (filled circles) particles. MSD is defined as $\langle[\mathbf{R}(t) - \mathbf{R}(t_0)]^2\rangle$, where $\mathbf{R} = (R_x, R_y)$ is a two-dimensional vector that indicates the spatial position of the particle.

disfavor highly curved molecular arrangement at the membrane edge, which may lead to high line tension. Further theoretical studies on the effect of molecular conformation on membrane mechanics are awaited.

We hypothesized that this membrane system could be exploited for the controlled encapsulation and release of a target object, such as in vesicular transport. Therefore, we demonstrated the reversible capture and release of a colloidal particle (0.5 μm) into and from the membrane. During the transition from the open to closed dynamics upon UV irradiation, a colloidal particle was captured into the closed vesicular space (Figure 7A upper). Release of the particle from the membrane sphere by trans-isomerization is shown in lower part of Figure 7A (Video S4, Supporting Information). Diffusion of the particle was suppressed while it was encapsulated in the closed membrane space. The analysis of Brownian motion of the particle clearly shows the effect of the confined membrane space. Figure 7B shows a plot of the mean square displacement (MSD) of the free (open square) and encapsulated (filled circle) particles against time. Because the size of a membrane sphere is virtually unchanged before and after one cycle of photoirradiation (see the Supporting Information), we concluded that there is no loss of membrane materials during the opening and closing events.

Conclusion

In summary, we succeeded in controlling the membrane to give sphere, cup, bowl, and disk shapes, by the photoswitching of membrane line tension. This membrane system enables the capture and release of targeted objects. Light is an efficient tool for manipulating soft matter, because the required energy can be instantaneously applied without contact and without damage to the chemical composition of the medium. These findings provide an example of engineered self-assembled membrane structures for a specific function.

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Supporting Information Available: Experimental section, photoinduced formation of membrane spheres in a large microscopic field, sizes of membrane spheres before and after one opening−closing event, videos of membrane dynamics corresponding to Figures 3, 4, and 7. This information is available free of charge via the Internet at http://pubs.acs.org/.

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