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Lipid-membrane-incorporated hydrophobic photochromic molecules prepared by the exchange method using cyclodextrins†

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It was found that the exchange method for the preparation of lipid-membrane-incorporated guest molecules was applicable not only to fullerenes but also to other hydrophobic molecules such as azobenzene and stilbene. Advantages of this method are that the long-term stability of lipid-membrane-incorporated azobenzene solution and the maximum ratio of [stilbene]/[lipid] were higher than those prepared by the classical method, which we call the 'premixing method'. Photoisomerisations of these photochromic guest molecules in the lipid membranes maintained the morphology of liposomes.

Introduction

Liposomes are solubilising agents for hydrophobic π-conjugated molecules and have the following three advantages. (i) Liposomes can incorporate a wide variety of hydrophobic compounds into the lipid bilayer.[1] (ii) Various types of liposome can be prepared by selecting and mixing lipids such as phospholipids, aminolipids, and glycolipids, which may confer drug-carrier functionality.[1] (iii) Vesicles can be easily prepared with adjustable size for enhanced permeability and retention (EPR) effects.[2] Therefore, liposomes have attracted considerable attention as materials for drug-delivery systems (DDSs) in which drugs such as anticancer agents are incorporated inside a vesicle or polymers such as DNA are adsorbed outside the vesicle.[3–5] However, researchers are commonly confronted with problems whereby hydrophobic guest molecules cannot be incorporated in lipid membranes at high concentrations and the lipid-membrane-incorporated hydrophobic guest molecules (LMIG) obtained often suffer impaired long-term stability. When LMIG have been prepared by dissolving lipids and guest molecules in organic solvents followed by concentration and extraction with water [which we call the 'premixing method' (Fig. S1A)],[6] hydrophobic molecules tend to form large self-aggregates during the concentration process. It was considered that the aggregates lead to low solubility of hydrophobic molecules and lability of LMIG.

Recently, we demonstrated that lipid-membrane-incorporated fullerenes C_x ($LMIC_x$: x = 60 or 70) can be readily prepared by fullerene exchange from the cavity of γ-cyclodextrin (γ-CDx) to liposomes.[7] In the resulting aqueous solutions of $LMIC_{60}$ and $LMIC_{70}$, the maximum ratio ([fullerene]/[lipids] = 30 mol%) was approximately 14 and 100 times higher than those achieved for $LMIC_{60}$ and $LMIC_{70}$, respectively, prepared by the premixing method. The long-term stabilities of the $LMIC_{60}$ and $LMIC_{70}$ solutions prepared by the exchange method were shown to be much higher than for those prepared by the premixing method. Furthermore, the exchange method promises to enable direct and short-time uptake of guest molecules into living cell membranes.[8] To the best of our knowledge, however, there is no precedent in which the exchange method has been applied to hydrophobic molecules other than fullerenes. Here, we employed azobenzene (**1**), and stilbene (**2**) as hydrophobic photochromic molecules and compared LMIG samples prepared by both methods (Scheme 1 and Fig. S1B). Furthermore, photoisomerisations of these photochromic guest molecules were observed in the lipid membranes.

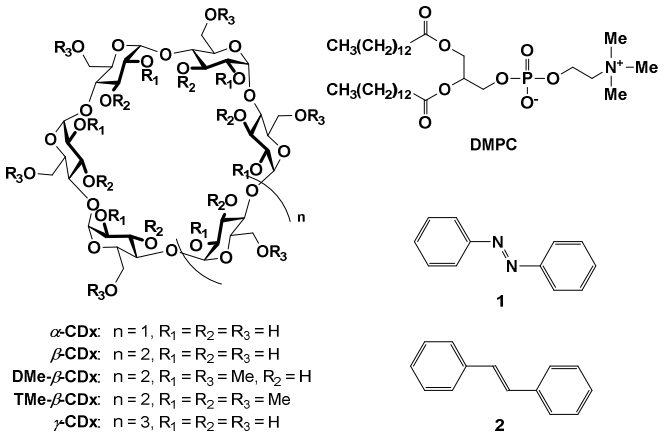
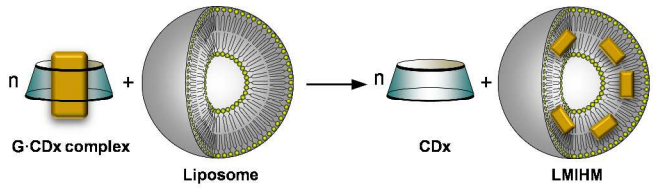


Chart 1 Cyclodextrins, DMPC and compounds **1** and **2**



Scheme 1 Exchange method between the guest (G)•CDx complex and the liposome.

Results and discussion

Formation of 1 or 2·CDx complexes

To confirm the formation of the complexes of **1** with five kinds of cyclodextrins (CDxs), the UV-vis absorption spectra were measured (Fig. 1).[9] A mixture of α- or DMe-β-CDxs and **1** (black and blue lines in Fig. 1A) exhibited sharp absorption in the 250–400 nm range, indicating that **1** existed in an isolated state within the CDxs' cavities. However, absorbance in the 250–400 nm range was scarcely observed in the mixtures of β-, TMe-β- or γ-CDxs and **1** (red, green and orange lines in Fig. 1A), indicating that these CDxs barely solubilised **1**. Therefore, we used α- and DMe-β-CDxs for the subsequent experiments. As shown in Fig. 1B, absorption peaks around 305 nm clearly indicate that the solubility for the **2**•DMe-β-CDx complex was much higher than those for the other **2**•CDx complexes. Although the trend in solubilities for the **2**•CDx complexes is very similar to that for the **1**•CDx complexes because of their similar structures, α-CDx barely solubilised **2**, compared with **1**.

The concentrations of **1** and **2** in DMe-β-CDx were determined by the absorbances at 322 and 298 nm, respectively, after drying by evaporation and dissolution in ethanol.[10] The concentrations were estimated to be [**1**] = 19.2 and [**2**] = 3.98 mM in the original solutions of the **1** and **2**•DMe-β-CDx complexes.

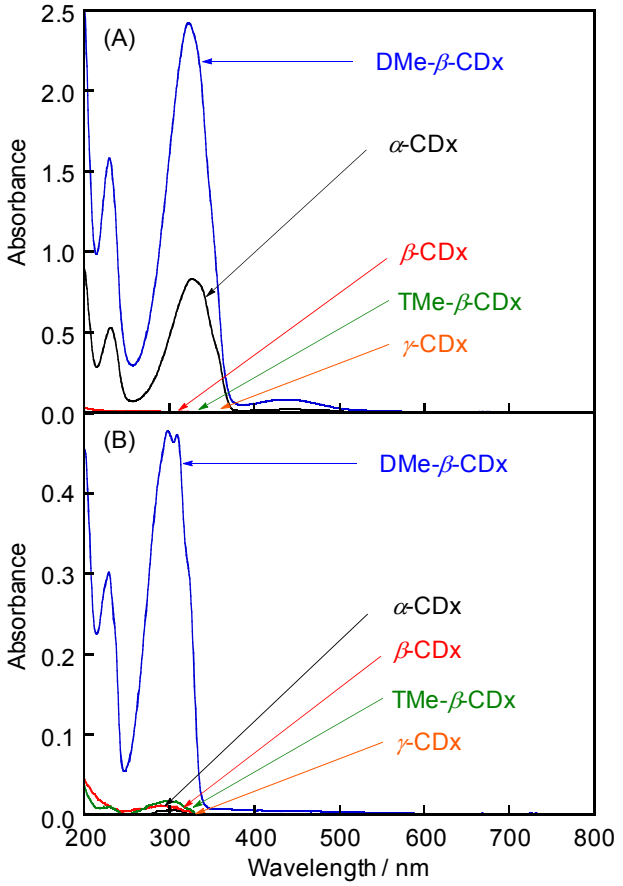


Fig. 1 UV-vis absorption spectra of (A) **1** and (B) **2** complexed with (a) α-CDx (black line), (b) β-CDx (red line), (c) DMe-β-CDx (blue line), (d) TMe-β-CDx (green line) and (e) γ-CDx (orange line). All spectra were measured at 25 °C (1 mm cell). All solutions were diluted 1/10.

Formation of LMI1 and LMI2 by the exchange method

LMI**1** that consisted of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were prepared by the **1**-exchange method from the **1**•CDx complexes into vesicles at 30 °C (Fig. S1B). Immediately after mixing the **1**•α-CDx complex and liposome solutions, a finely dispersed precipitate was observed. Furthermore, a precipitate was also observed in a mixture of α-CDx and liposomes in the absence of **1**. Csempesz *et al.* reported a similar phenomenon for dipalmitoylphosphatidylcholine and α-CDx.[11] Therefore, the precipitate was due to the formation of the complex of α-CDx and DMPC. Consequently, this result indicates that α-CDx is unsuitable for the exchange method. However, no other CDx formed precipitates with DMPC.

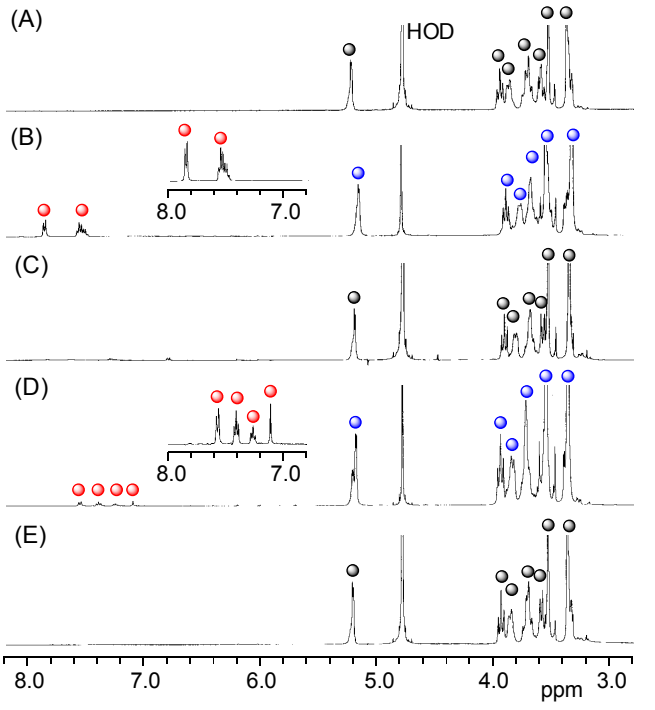


Fig. 2 ^{1}H NMR spectra of (A) DMe-β-CDx, (B) the **1**•DMe-β-CDx complex, (C) LMI**1**, (D) the **2**•DMe-β-CDx complex and (E) LMI**2** in D_2O at 25 °C (●: free DMe-β-CDx, ●: **1** or **2** and ●: DMe-β-CDx in the **1**•DMe-β-CDx or **2**•DMe-β-CDx complex). The insets show the region of 6.8–8.0 ppm. [**1** or **2**]/[DMPC] = 10.0 mol%.

The ^{1}H NMR spectra of the **1**•DMe-*β*-CDx complex before mixing with DMPC-liposome showed that peaks assignable to **1** and DMe-*β*-CDx in the complex appeared at δ = 7.48–7.61 and 7.86 ppm, and 3.90 and 5.18 ppm, respectively (red and blue circles in Fig. 2B). As shown in Figs. 2C and S2, the ^{1}H NMR spectra were taken at 25 °C after mixing the **1**•DMe-*β*-CDx complex and DMPC-liposome at 30 °C for 1 h ([**1**]/[DMPC] = 10 mol% in D_2O). Fig. 2C shows that the peaks coalesced between free and complexed DMe-*β*-CDx (blue circles in Fig. 2B) shifted to the same position as free DMe-*β*-CDx (black circles in Fig. 2A and 2C) and the peaks at δ = 7.48–7.61 and 7.86 ppm (red circles in Fig. 2B), which are assignable to **1** in the **1**•DMe-*β*-CDx complex, disappeared. These results and the absence of precipitate, clearly indicate that all of **1** released from the DMe-*β*-CDx cavities was transferred into the lipid membranes to yield vesicle-incorporated **1**.

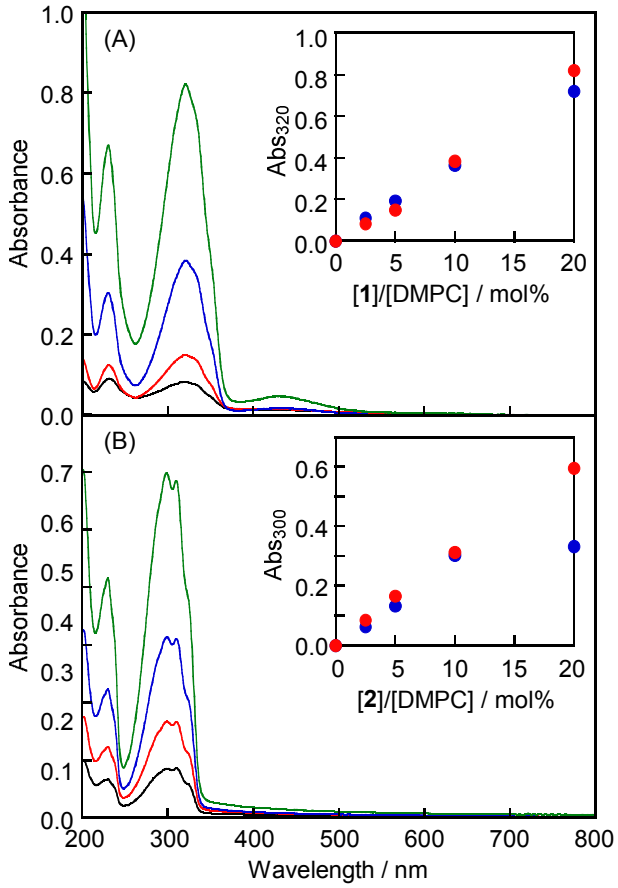


Fig. 3 UV-vis absorption spectra of (A) LMI**1** and (B) LMI**2** prepared by the exchange method. [**1** or **2**]/[DMPC] = (a) 2.5 (black line), (b) 5.0 (red line), (c) 10.0 (blue line) and (d) 20.0 (green line) mol% ([**1**] = 0.4 mM, [**2**] = 0.2 mM). (Inset) The absorbance (Abs) at λ_{max} versus [**1**]/[DMPC] in LMI**1** prepared by the exchange (red) and premixing (blue) methods. The values of λ_{max} were 320 and 300 nm for LMI**1** and LMI**2**, respectively. All absorption spectra were obtained by subtracting the light scattering of DMPC liposomes and were measured at 25 °C (1 mm cell).

Similarly, after mixing the **2**•DMe-*β*-CDx complex and DMPC-liposomes at 30 °C for 1 h, the peaks of free and complexed DMe-*β*-CDx (blue circles in Fig 2D) coalesced and shifted to the same position as that of free DMe-*β*-CDx at 25 °C (black circles in Fig. 2A and 2E), while the four peaks in the δ = 7.0–7.6 ppm region (red circles in Fig. 2D), which are assignable to **2** in the **2**•DMe-*β*-CDx complex, disappeared in the same manner as **1** (Fig. S3). These results clearly indicate that all **2**, also released from the DMe-*β*-CDx cavities, were transferred into the lipid membranes.

This conclusion was further supported by the UV-vis absorption spectra. The absorbance of LMI**1** was very similar to those of the **1**•DMe-*β*-CDx complex and bare **1** in chloroform (Fig. 3A). Consequently, **1** in LMI**1** obtained by the exchange method existed in an isolated (i.e., disaggregated) state. LMI**1** obtained by the exchange method had very similar absorbance at 320 nm as those obtained by the premixing method as described previously (Fig. 3A inset),[12] indicating that LMI**1** can also be prepared by the exchange method. Furthermore, because the absorbance at λ_{max} (Abs_{320}) versus [**1**]/[DMPC] in LMI**1** gave a linear plot, in the liposomes in the range of [**1**]/[DMPC] = 0–20 mol%, the microenvironments of **1** in liposomes are identical over this range (Fig. 3A inset). In analogy to LMI**1**, the absorbance of LMI**2** prepared by both methods, below 10 mol%, lay on an identical line (Fig. 3B inset). However, the absorbance of LMI**2** prepared by the premixing method was saturated above 10 mol% (Figs. 3B inset and S4),[13] whereas that prepared by the exchange method at 30 °C increased linearly up to 20 mol%. This result indicates that the maximum ratio obtained by the exchange method was higher than that obtained by the premixing method.

Morphology of LMI1 and LMI2 prepared by both methods

The hydrodynamic diameters (D_{hy}) were measured with a dynamic light scattering spectrophotometer (DLS). The average D_{hy} values in both LMI**1** and LMI**2** prepared by the exchange method were 70–90 nm and the fluctuation of the values was ±5 nm (Fig. S5A and S5B and Table S1). Similar average D_{hy} values were observed in both LMI**1** and LMI**2** prepared by the premixing method (Fig. S5C and S5D and Table S2). These results indicate that the presence of **1** and **2** in the lipid membranes hardly affected the liposome diameters by both methods. Furthermore, similar morphologies were observed in LMI**1** prepared by both methods, as cryo-TEM images show in Fig. 4(A)–(D). Although cryo-TEM images of LMI**2** prepared by both methods partially showed that lipid membranes tend to have planar structures [Fig. 4(E)–(H)], the reason is not yet clear.[14]

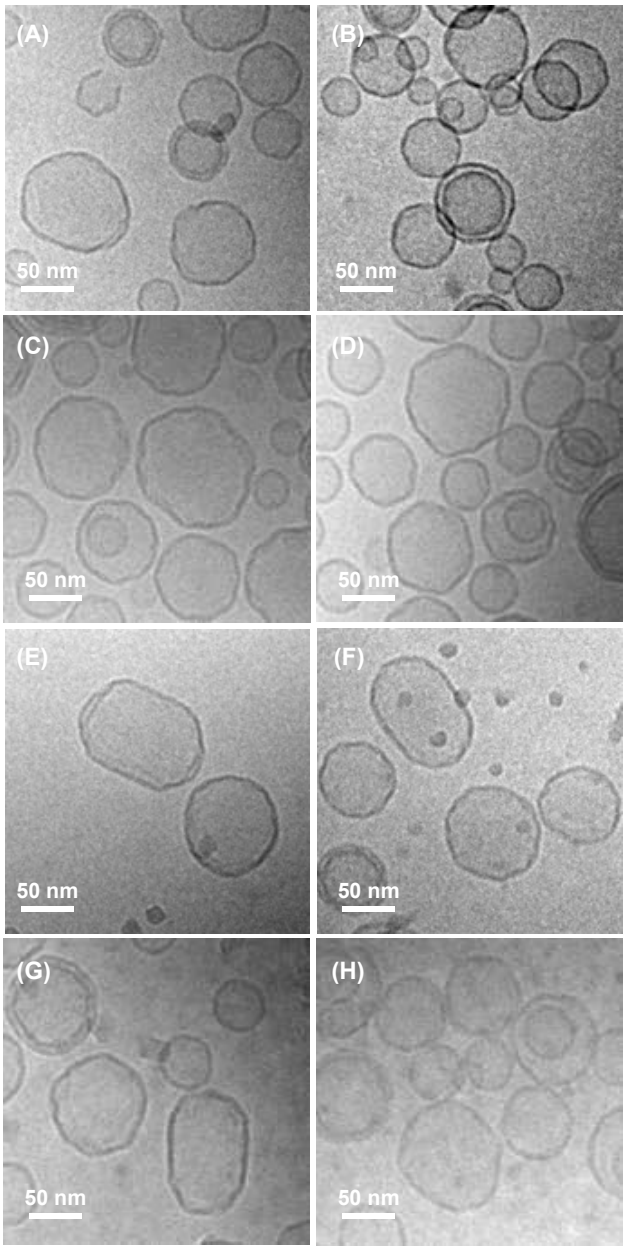


Fig. 4 Cryo-TEM images of (A) and (B) LMI**1** prepared by the exchange method, (C) and (D) LMI**1** prepared by the premixing method, (E) and (F) LMI**2** prepared by the exchange method and (G) and (H) LMI**2** prepared by the premixing method. [**1** or **2**]/[DMPC] = 10 mol%. These pairs were in other areas of the image.

Effect of the presence of 1 and 2 on phase transition temperature

DSC was used to observe whether the photoisomerisations of **1** and **2** changed the phase transition temperature (T_m) of the unilamellar liposomes.[15] The T_m of LMI**1** and LMI**2** underwent small changes compared with that of DMPC liposomes (23.2 °C) (Fig. 5, LMI**1** (red line): T_m = 22.9, LMI**2** (green line): 23.1 °C, [**1** or **2**]/[DMPC] = 10 mol%). These results suggest that **1** and **2** scarcely perturb packing of alkyl chains in DMPC.

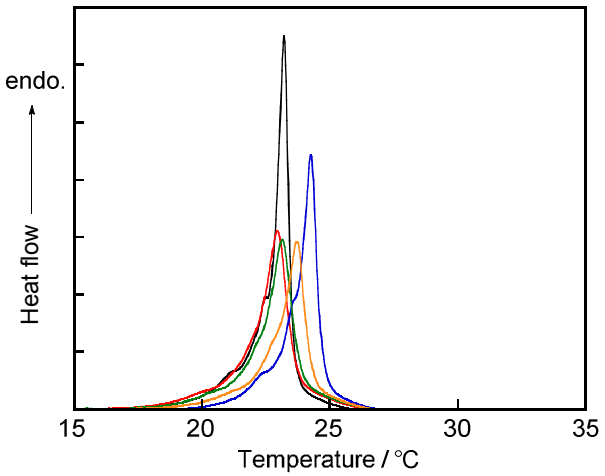


Fig. 5 DSC curves of DMPC (black), LMI**1** before (red) and after (blue) photoirradiation (330 < λ < 380 nm) and LMI**2** before (green) and after (orange) photoirradiation (λ = 365 nm). [**1** or **2**]/[DMPC] = 10 mol%. [DMPC] = 4.0 mM.

Stabilities of LMI1 and LMI2 prepared by the exchange and premixing methods

The LMI**1** solution prepared by the exchange method was more stable than that prepared by the premixing method (Fig. 6A). Using the exchange method, over 97% of LMI**1** remained in aqueous solution after 30 days and no precipitation was exhibited for at least 1 month at room temperature (Fig. 6A red bar). Using the premixing method, only half of **1** in LMI**1** was soluble in water and a slight precipitation was observed after 30 days at room temperature (Fig. 6A blue bar). In contrast, the LMI**2** solutions prepared by both methods had similar stabilities after 7 and 30 days (Fig. 6B). Although the decreases in absorbance were confirmed after 30 days, the amount of precipitate for LMI**2** was too small to be observed by the naked eye.

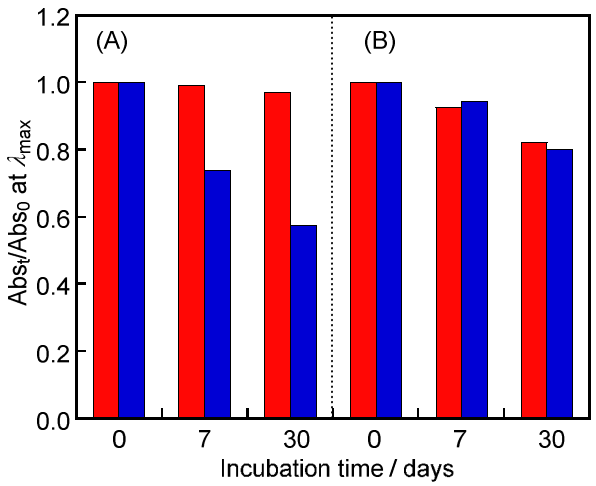


Fig. 6 Change in the absorption at λ_{max} of (A) LMI**1** and (B) LMI**2** prepared by the exchange method (red bar) and the premixing method (blue bar) with incubation times 0, 7 and 30 days. The values of λ_{max} were 320 nm for LMI**1** and 300 nm for LMI**2**. [**1** or **2**]/[DMPC] =10.0 mol%.

Trans-cis photoisomerisation in liposomes

The *trans*-to-*cis* isomerisation of **1** was confirmed by a significant decrease in the absorption maximum at 320 nm (*trans*-**1**) and an increase in the absorption maximum at 445 nm (*cis*-**1**) by photoirradiation of UV light ($330 < \lambda < 380$ nm using glass filters) (Fig. 7A). In contrast, the *cis*-to-*trans* isomerisation was observed by photoirradiation of visible light ($\lambda > 400$ nm). This photoisomerisation could be repeated reversibly three times (Fig. 7A inset) and no precipitate was observed after the photoisomerisations. The results suggest that the photoisomerisation of **1** occurs within the lipid membrane. However, *trans*-to-*cis* isomerisation of **2** is irreversible because of the production of phenanthrene from *cis*-**2** as mentioned in previous papers (Fig. 7B).[16] After the process of the *trans*-to-*cis* and *cis*-to-*trans* isomerisation, the ratio of *trans*-**2**, *cis*-**2** and phenanthrene composition is determined by ^{1}H NMR spectra (Fig. S6) as follows: [*trans*-**2**]:[*cis*-**2**]:[phenanthrene] = < 5:> 90:< 5 (mol/mol/mol) after the photoirradiation of $\lambda = 365$ nm

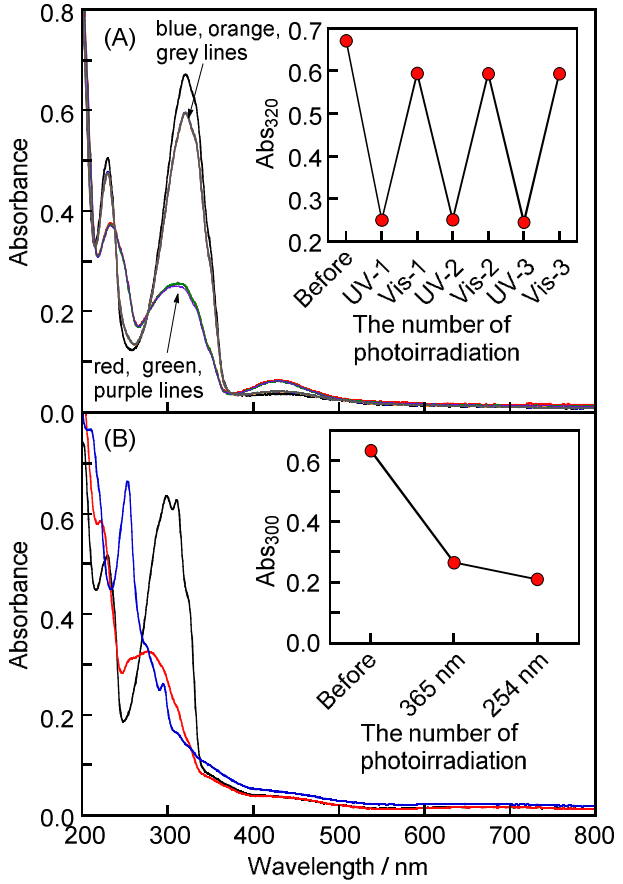


Fig. 7 UV-vis absorption spectral change of (A) LMI**1** before and after the photoirradiation of UV light ($330 < \lambda < 380$ nm; *trans*-to-*cis*; red, green and purple line) or visible light ($\lambda > 400$ nm; *cis*-to-*trans*; blue, orange and grey line) and (B) LMI**2** before (black line) and after the photoirradiation of $\lambda = 365$ nm (*trans*-to-*cis*; red line) or $\lambda = 254$ nm (*cis*-to-*trans*; blue line) ([**1**] = [**2**] = 0.4 mM, [**1** or **2**]/[DMPC] = 10 mol%). All absorption spectra were obtained by subtracting the light scattering of DMPC liposomes and were measured at 25 °C (1 mm cell). (Inset) The absorbance (Abs) at 320 or 300 nm versus the number of photoirradiation in LMI**1** or LMI**2**.

Table 1 Isomer distribution (%) before and after photoirradiation

Photo-irradiation	**1**		Photo-irradiation	**2**	
Phenanthrene	*Trans*/%	*Cis*/%		*Trans*/%	*Cis*/%
Before	87	13	Before	100	0	0
UV	22	78	Vis	< 5	> 90	< 5
Vis	76	24	UV	< 1	< 1	> 99

and [*trans*-**2**]:[*cis*-**2**]:[phenanthrene] = <1:<1:>99 (mol/mol/mol) after photoirradiation of $\lambda = 254$ nm (Table 1).

Morphology of LMI1 and LMI2 after photoirradiation

As shown in Fig. S7 and Table S3, the average D_{hy} after the photoirradiation in both LMI**1**(*cis*) and LMI**2**(*cis*) were also in 70-80 nm range. Furthermore, similar morphologies were observed in LMI**1** (*trans*) and (*cis*) or LMI**2** (*trans*) and (*cis*) as shown by cryo-TEM images in Figs. 4 and 8 before and after photoirradiation. These results indicate that photoisomerisations of **1** and **2** did not bring about disruption or fusion of liposomes.

Effect of photoisomerisation for phase transition temperature

By DSC measurements, the T_m change for LMI**1**(*cis*) was slightly larger than that for LMI**2**(*cis*) (LMI**1**(*cis*): T_m = 24.3, LMI**2**(*cis*): 23.7 °C) (Fig. 5 blue and orange lines). The result suggests that the *cis* isomer of **1** perturbs the packing of alkyl chains in DMPC compared with the *cis* isomers of **2**. In contrast, the half-height widths of the transition peaks ($\Delta T_{1/2}$) of LMI**1**(*cis*) and LMI**2**(*cis*) are smaller than those of LMI**1**(*trans*) and LMI**2**(*trans*), respectively [DMPC liposome: $\Delta T_{1/2}$ = 0.5, LMI**1**(*trans*): 1.2, LMI**1**(*cis*): 0.7, LMI**2**(*trans*): 1.1 and LMI**2**(*cis*): 1.0 °C] (Fig. 5). These results suggest that the *cis* isomers of **1** and **2** interact with the alkyl chains of DMPC less than with the *trans* isomers of **1** and **2**.

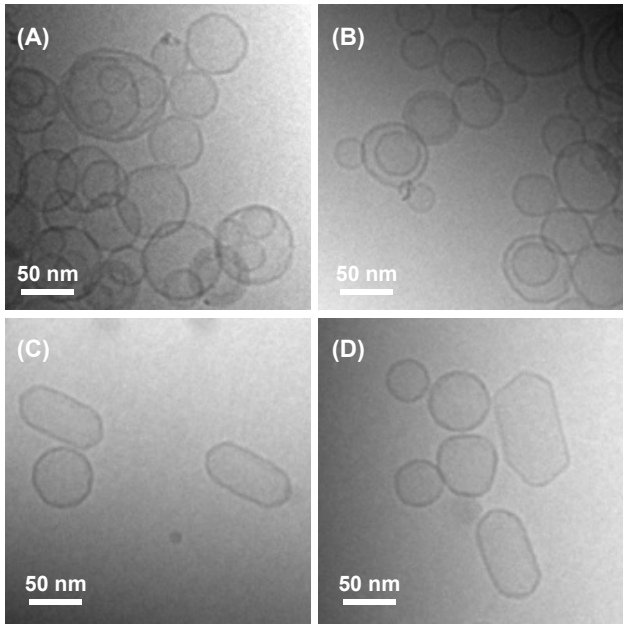


Fig. 8 Cryo-TEM images of (A) and (B) LMI**1**(*cis*) and (C) and (D) LMI**2**(*cis*) prepared by the exchange method. [**1** or **2**]/[DMPC] = 10 mol%. These pairs are in other areas of the image.

Experimental

Materials. Azobenzene (**1**), stilbene (**2**), α-CDx, β-CDx, γ-CDx, and TMe-β-CDx were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). DMe-β-CDx was purchased from Sigma-Aldrich Chemical Co., Inc. (St. Louis, MO, USA). Dimyristoylphosphatidylcholine (DMPC) was obtained from NOF Corp. (Tokyo, Japan).

Preparation of the 1 and 2•CDx Complexes. **1** or **2** (2.74×10^{-5} mol) and CDx (5.48×10^{-5} mol) were placed in an agate capsule with two agate-mixing balls. The mixture was mixed vigorously at 30 Hz for 20 min using a high-speed vibration mill (MM 200; Retsch Co., Ltd., Haan, Germany). The solid mixture was suspended in water or D_2O (1.5 mL) to produce a black emulsion. After centrifugation (18000 × g, 25 °C, 20 min), the non-dispersed **1** or **2** was removed from the solution. The concentrations of **1** and **2** in the **1** and **2**•DMe-β-CDx complex, determined by measuring the absorbances of the solution at 322 and 298 nm (the molar absorption coefficient for the water-soluble **1** and **2**•DMe-β-CDx complex are $\varepsilon_{322} = 1.24 \times 10^4$ and $\varepsilon_{298} = 1.20 \times 10^4$ dm^3 mol^{-1} cm^{-1}, respectively), were 19.2 and 3.98 mM in water.

Preparation of lipid membrane-incorporated guest molecules (LMI1 and LMI2) by the exchange method. DMPC (9.76 mg, 1.44×10^{-5} mol) in chloroform (1 mL) was dried using a rotary evaporator at 40 °C. Water (4.0 mL) was added to the mixture and shaken with a vortex mixer for 5 min. To change from multilamellar to unilamellar vesicles and to obtain a narrow size distribution, the solution was repeatedly frozen and thawed three times and extruded eleven times (LiposoFast-Basic; Avestin Inc., Ottawa, Canada) with two stacked polycarbonate membranes, pore size 50 nm. The solution was diluted with water to a final concentration of 8.0 and 4.0 mM lipids for **1** and **2**, respectively. An aqueous solution of the **1**•DMe-β-CDx complex (x mL, [**1**] = 1.44 mM), liposomes (1.0 mL, [DMPC] = 4.00 mM) and water (1.0−x mL) was heated at 30 °C for 1 h in a 5-mL glass vial. Values of the [**1**]/[DMPC] ratio were changed from 2.5 to 20 mol%. LMI**2** ([**2**]/[DMPC] = 2.5–20 mol%) was also prepared by mixing water solutions of the lipids (1.0 mL, [DMPC] = 2.00 mM), the **2**•γ-CDx complex (x mL, [**2**] = 0.72 mM) and water (1.0–x mL) at 30 °C for 1 h in a 5 mL glass vial.

Photoisomerisation of 1 and 2 in the liposomes. The photoirradiation for LMI**1** was performed using a xenon lamp (SX-UID500X, 500 W; Ushio Inc., Tokyo, Japan) equipped with colour glass filter ($330 < \lambda < 380$ nm, UTVAF-50S-33U, Shigma Koki Co. Ltd., Tokyo, Japan) or (> 420 nm, SCF-50S-42L, Shigma Koki Co. Ltd., Tokyo, Japan). The light was cooled by passing it through a water filter. However, the photoirradiation for LMI**2** was performed using two convenient UV lamps (SLUV-6, 6 W; As One. Corp., Tokyo, Japan). Water solutions of LMI**1** and LMI**2** were exposed to light for 30 min at 25 °C. After photoirradiation, to determine of the stoichiometries of **1** and **2**, an equivalent of DMSO-d_6 were added to the water solutions of LMI**1** and LMI**2** to destroy the liposomes. The stoichiometries of **1** and **2** were calculated from the integral intensity ratio of the ^{1}H NMR spectra.

UV–vis Absorption Spectra. UV–vis spectra were recorded using a UV-3600PC spectrophotometer (Shimadzu Corp., Kyoto, Japan). All experiments were performed at 25 °C and a 1 mm cell was used.

^{1}H NMR spectroscopy. ^{1}H NMR data were recorded on a Varian 400-MR (400 MHz) spectrometer (Varian Associates, Inc.; Palo Alto, CA, USA).

Cryogenic temperature transmission electron microscopy (Cryo-TEM). Cryo-TEM samples were prepared using a universal cryofixation and cryopreparation system (Leica EM CPC, Wetzlar, Germany). To prevent water evaporation from the sample, the isolated chamber was humidified to near saturation before the sample was introduced. 2–3 μL sample droplets were placed on a microperforated cryo-TEM grid and then absorbed by a filter paper, resulting in the formation of thin liquid films of 10–300 nm thickness freely spanning the micropores in a carbon-coated lacelike polymer layer supported by a metal mesh grid. After a minimum 30 s holding time, the sample grid assembly was rapidly vitrified with liquid ethane at its melting temperature (−163–−170 °C). The holding time was adopted to relax any possible flow deformation that may have resulted from the blotting process. The vitreous specimen was kept under liquid nitrogen until it was loaded into a cryogenic sample holder (Gatan 626.DH). Imaging was performed with a JEOL JEM-3100 FEF instrument operating at 300 kV (Tokyo, Japan). The use of a minimal dose system (MDS) was necessitated by the electron radiation sensitivity of the sample probed. Images were recorded on a Gatan 794 multiscan digital camera and processed with DigitalMicrographs software, version 3.8.1. The optical density gradients in the background, which are normally ramp-shaped were digitally corrected using a custom-made subroutine compatible with DigitalMicrographs.

Dynamic Light Scattering (DLS). The hydrodynamic diameter of LMI**1** or **2** was measured on an instrument for electrophoretic light scattering with a laser Doppler system (Zetasizer Nano ZS, Malvern Instruments Ltd., Malvern, UK).

Conclusions

This study demonstrated that LMI**1** and LMI**2** can be prepared by the exchange method with a high ratio of [**1** or **2**]/[DMPC] (20 mol%). The results indicate that the exchange method was applicable to guest molecules and a CDx derivative other than fullerenes and γ-CDx. Over the range of these ratios, **1** in LMI**1** and **2** in LMI**2** obtained by the exchange method existed in an isolated state. The long-term stability of the LMI**1** solution prepared by the exchange method was much higher than that prepared by the premixing method. Furthermore, the maximum ratio (20 mol%) of LMI**2** obtained by the exchange method was higher than that obtained by the premixing method. The *trans*-to-*cis* isomerisations of **1** were repeated reversibly three times by photoirradiation in the lipid membranes. LMI**1** and LMI**2** were stable upon photoisomerisation and their morphologies

were unchanged. We believe that the exchange method will open the door to enable liposomal pharmaceutical preparation.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, schematic representation of the premixing and exchange methods, ^{1}H NMR spectra and UV-vis absorption spectra. See DOI: 10.1039/b000000x/

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