Electronic supplementary information

Dynamic behavior into giant unilamellar vesicles induced by the uptake of [70]fullerene

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Experimental Section

Materials

γ-CDx was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), dimyristoylphosphatidylcholine (DMPC), and dipalmitoylphosphatidylcholine (DPPC) were obtained from NOF Corp. (Tokyo, Japan). 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (**1**) was obtained from Molecular Probes, Inc. (Eugene, OR). C_{70} (> 99%) was bought from MER Co. (Tucson, AZ, USA).

Preparation of giant unilamellar vesicles (GUVs)

GUVs were fabricated using the electroformation method on indium tin oxide (ITO) electrodes originally designed by Angelova *et al*.[S1,S2] For the electroformation of the vesicles, chloroform solutions containing 1.0 mM of the lipid (i.e., POPC, DMPC, and DPPC) were prepared. Fluorescence microscopy observations were facilitated by the addition of the fluorescent membrane label **1** at a concentration of 0.1 mol %. In general, large amounts of the GUVs with a diameter > 10 μm was obtained under the appropriate conditions (see below). Thus, 10.0 μL of the lipid solution in chloroform was spread in a snakelike pattern without overlap over a 1.5 × 1.5 cm^2 area using a 10 μL Hamilton syringe. Following the deposition of the lipid film onto the ITO-coated glass, the solvent was evaporated by passing a stream of nitrogen gas over the glass and the electroformation chamber was then assembled. The chamber consisted of two ITO-coated coverslips each with a copper wire facing each other with their ITO-coated surfaces. The ITO electrodes were separated by a 3 mm thick polydimethylsiloxane (PDMS) film that was used to seal the chamber. The resulting vesicle electroformation chamber was slowly filled with 450 μL of deionized water. A sinusoidal AC electric field of 10 Hz and 2.0 V (rms) was then applied to the system for 20 min to form

GUVs at temperature greater than that of the phase transition temperature (T_m) using a thermostated observation stage (Linkam 10022/23) (20°C for POPC, 34°C for DMPC and 48°C for DPPC).

Preparation of the C_{70}•γ-CDx complex

C_{70} (5.00 mg, 5.95×10^{-6} mol) and γ-CDx (61.40 mg, 4.73×10^{-5} mol) were placed in an agate capsule with two agate-mixing balls. The mixture was then 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 saline (1.5 mL) to produce a black emulsion. Following a period of centrifugation (18,000 × *g*, 25°C, 20 min), the non-dispersed C_{70} was removed from the solution. The concentration of C_{70} in the C_{70}•γ-CDx complex, as determined by measuring the absorbance of the solution at 380 nm (the molar absorption coefficient for the water-soluble C_{70}•γ-CDx complex is $\varepsilon_{380} = 3.80 \times 10^4$ dm^3 mol^{-1} cm^{-1}),[S3] was found to be 1.66 mM in saline.

Fluorescence microscopy

Microscopic images were acquired using an Olympus IX71 epifluoresence microscope (Tokyo, Japan) equipped with a 60 × objective lens. Fluorescent images were recorded using a Hamamatsu C11440-10C COMS digital camera (Shizuoka, Japan) under the irradiation of an excitation light beam from a mercury lamp through an optical filter (U-MNIBA2, Olympus). The temperature of the sample was maintained using a thermostated observation stage (Linkam 10022/23).

Preparation of C_{70} incorporating small unilamellar vesicles (SUVs)

The appropriate amount of DMPC was dissolved in chloroform (50 mM, 80 μL). The solvent was then evaporated under a stream of nitrogen gas and any residual trace solvent was completely removed *in vacuo*. The thin lipid films obtained in this way on the wall of a vial were hydrated with the appropriate amount of water (1.0 mL) at a temperature greater than

that of T_m. The SUVs were obtained via a series of five freeze-thaw cycles that were conducted at temperatures of -195 and 50°C. The resulting material was then subjected to 11 extrusion processes, where it was passed through 0.05 μm pores using a LiposoFast miniextruder from Avestin at temperature greater than that of T_m. The resulting SUVs were uniform in size, with diameters in the range of 80–100 nm. C_{70} incorporating SUVs were prepared using an exchange reaction between the SUVs and the C_{70}•γ-CDx complex by heating the materials at a temperature of 80 or 30°C for 30 min, as described previously in the literature.[6] The C_{70} incorporating SUVs prepared at 30°C were heated at 80°C for 30 min. The final concentrations of the respective components were [**1**] = 1.00 mM ([C_{70}]/[**1**] = 10 mol%).

Cryogenic temperature transmission electron microscopy (Cryo-TEM)

The cryo-TEM samples were prepared using a universal cryofixation and cryopreparation system (Leica EM CPC, Wetzlar, Germany). To prevent the evaporation of water from the sample, the isolation chamber was humidified to near saturation levels prior to the introduction of sample. Sample droplets (2–3 μL) were placed on a microperforated cryo-TEM grid and subsequently absorbed by a filter paper, resulting in the formation of thin liquid films of 10–300 nm in thickness that freely spanned the micropores in a carbon-coated lacelike polymer layer supported by a metal mesh grid. Following a minimum holding time of 30 s, the sample grid assembly was rapidly vitrified with liquid ethane at its melting temperature (-163 to -170°C). The holding time was specifically selected to relax any possible flow deformations resulting from the blotting process. The vitreous specimen was held under liquid nitrogen until it was loaded into a cryogenic sample holder (Gatan 626.DH). The imaging was performed with a JEOL JEM-3100 FEF system (Tokyo, Japan) operating at 300 kV. The use of a minimal dose system (MDS) was facilitated by the electron radiation sensitivity of the sample being probed. The images were recorded on a Gatan 794 multiscan digital camera (Pleasanton, CA, USA) and processed with version 3.8.1 of Digital Micrographs. The optical density gradients in the background, which are normally ramp-shaped, were digitally corrected using a custom-made subroutine compatible with Digital Micrographs.

References

S1 M. Angelova and D. Dimitrov, *Faraday Discuss. Chem. Soc.,* 1986, **81**, 303–311.

S2 G. Staneva, M. I. Angelova and K. Koumanov, *Chem. Phys. Lipids,* 2004, **129**, 53–62.

S3 K. Komatsu, K. Fujiwara, Y. Murata and T. Braun, *J. Chem. Soc., Perkin Trans. 1,* 1999, 2963–2966.

S4 L.A. Bagatolli, T. Parasassi and E. Gratton, *Chem. Phys. Lipids*, 2000, **105**, 135–147.

Movie S1 Time-dependent shape transitions of the GUVs consisting of POPC following the addition of the C_{70}•γ-CDx complex solution at ambient temperature (×4 play). The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image. The movie corresponds to the images provided in **Fig. 1a-d** of the main text.

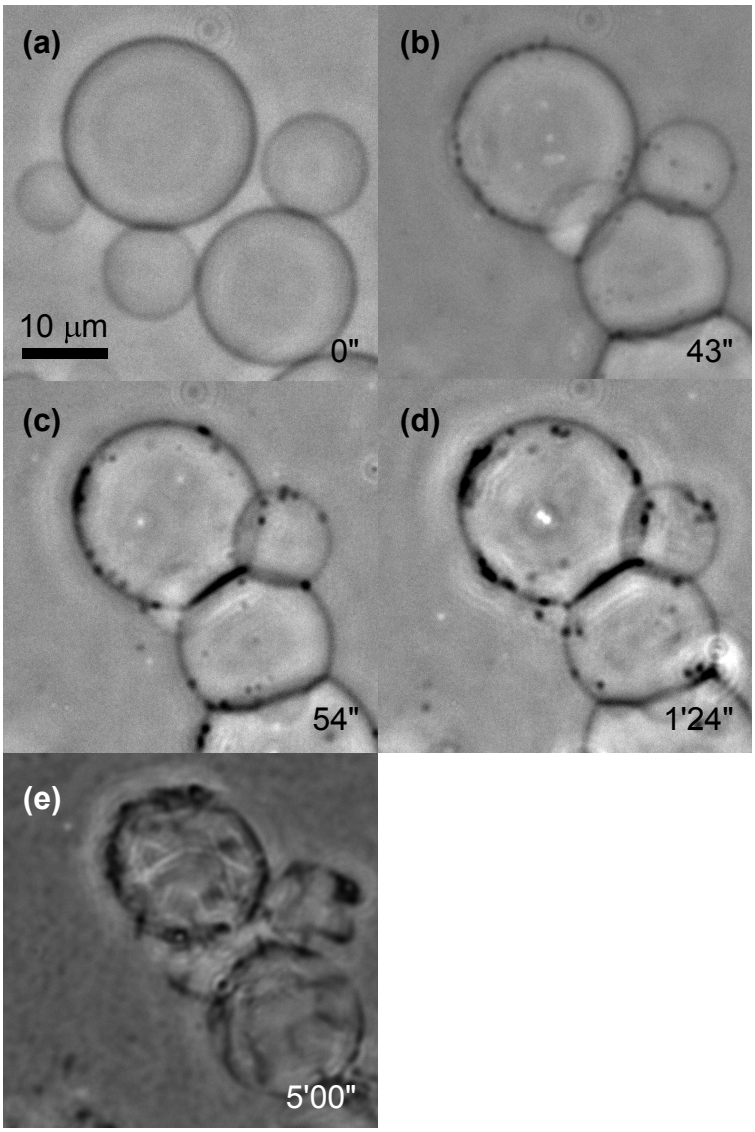

Fig. S1 Time-dependent shape transitions of the GUVs consisting of POPC following the addition of the C_{70}•γ-CDx complex solution at ambient temperature. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image.

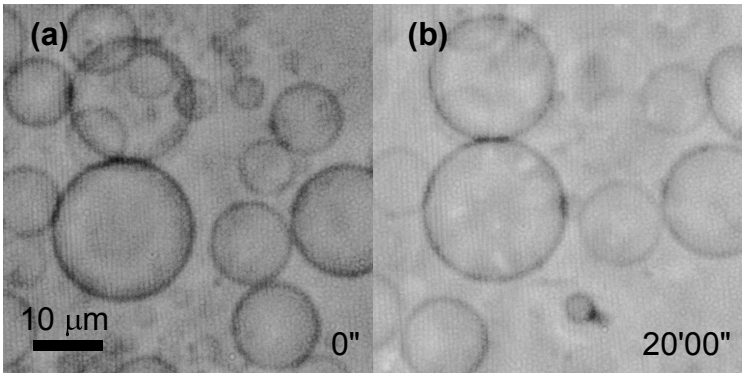

Fig. S2 Time-dependent shape transitions of the GUVs consisting of POPC following the addition of the γ-CDx (1 mM) solution at ambient temperature. The time elapsed following the starting injection of the γ-CDx solution through the micropipette has been indicated for each image.

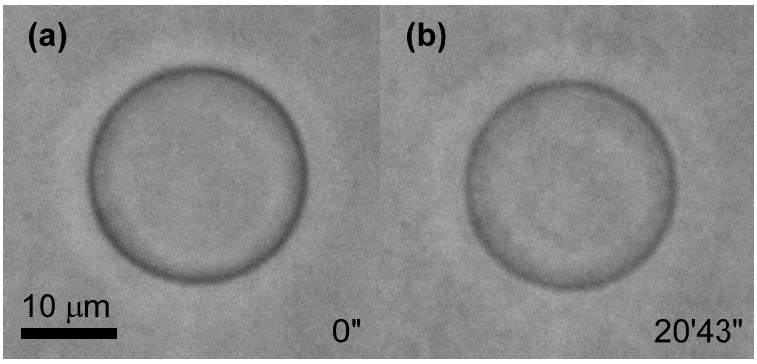

Fig. S3 Time-dependent shape transitions of the GUVs consisting of POPC following the addition of the C_{60}•γ-CDx complex solution at ambient temperature. The time elapsed following the starting injection of the C_{60}•γ-CDx complex solution through the micropipette has been indicated for each image.

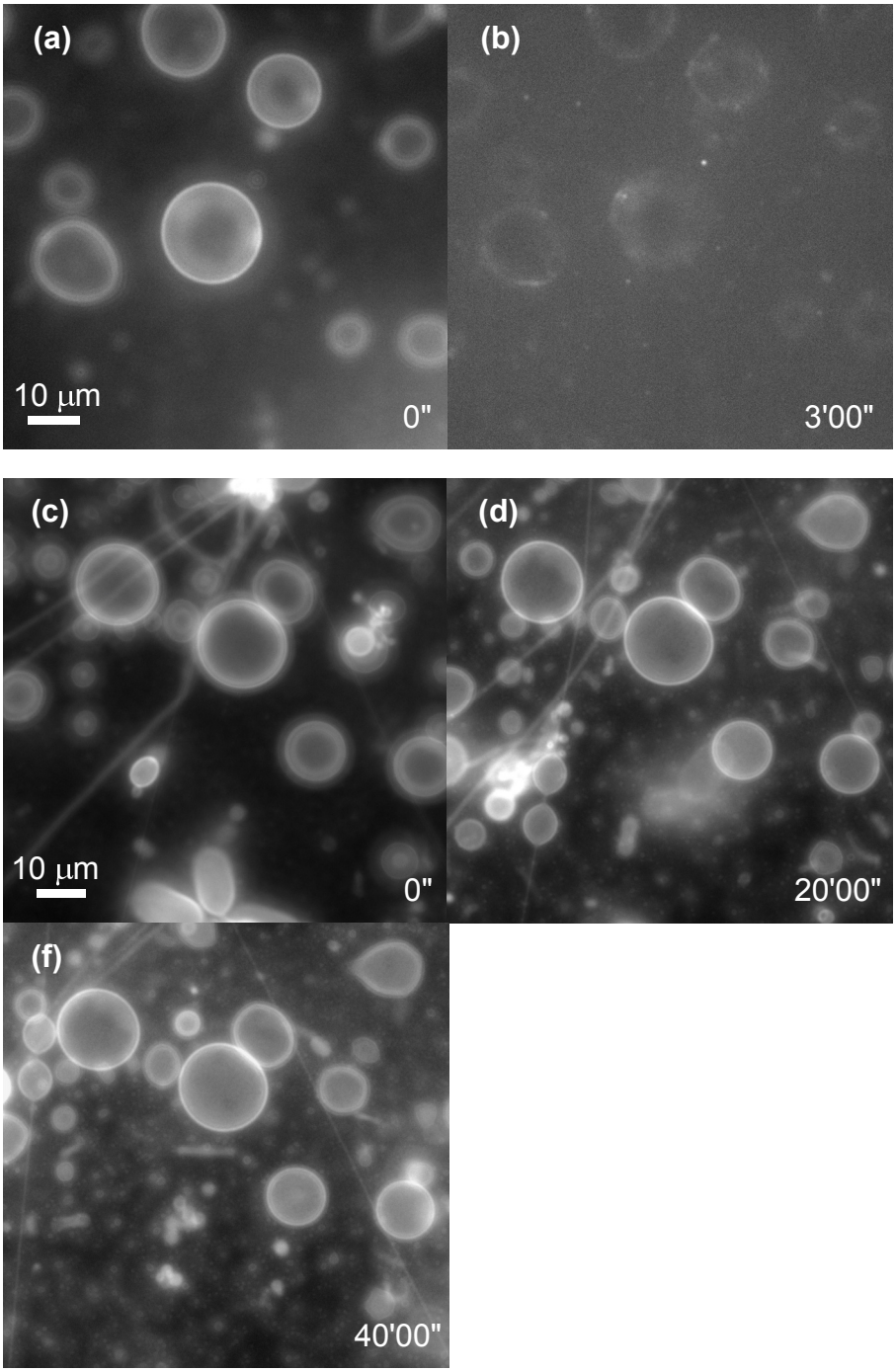

Fig. S4 Time-dependent fluorescent microscopy images of the GUVs consisting of POPC and **1** by the addition of solutions of (a) and (b) the C_{70}•γ-CDx and (c)-(f) the C_{60}•γ-CDx complexes at ambient temperature. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution or the C_{60}•γ-CDx complex solution through the micropipette has been indicated for each image. Thin wires in (c), (d) and (e) show lipid tubes coexisting with the GUVs.[S4]

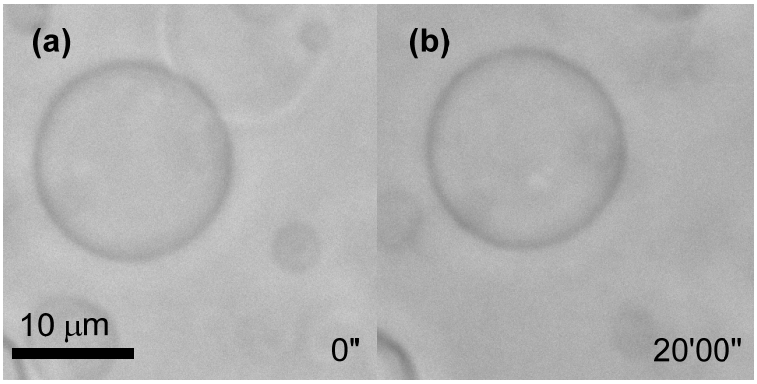

Fig. S5 Time-dependent shape transitions of the GUVs consisting of DPPC following the addition of the C_{70}•γ-CDx complex solution at ambient temperature. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image.

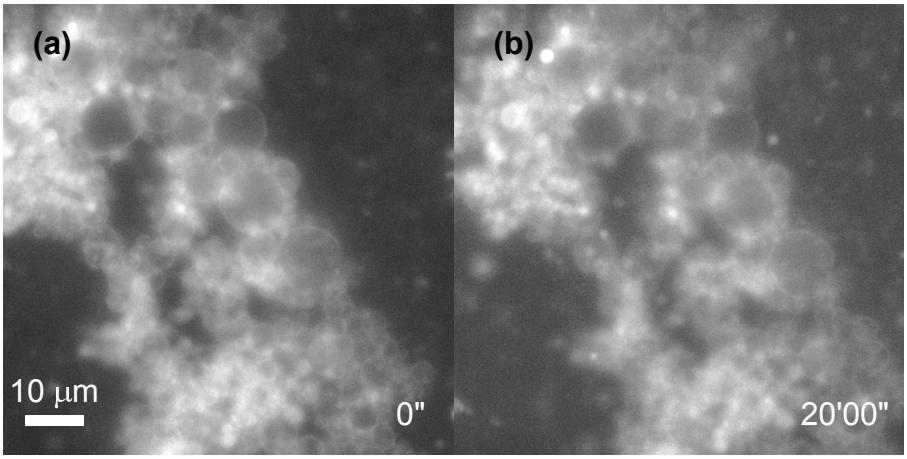

Fig. S6 Time-dependent fluorescent microscopy images of the GUVs consisting of DPPC and **1** by the addition of the C_{70}•γ-CDx complex solution at ambient temperature. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image.

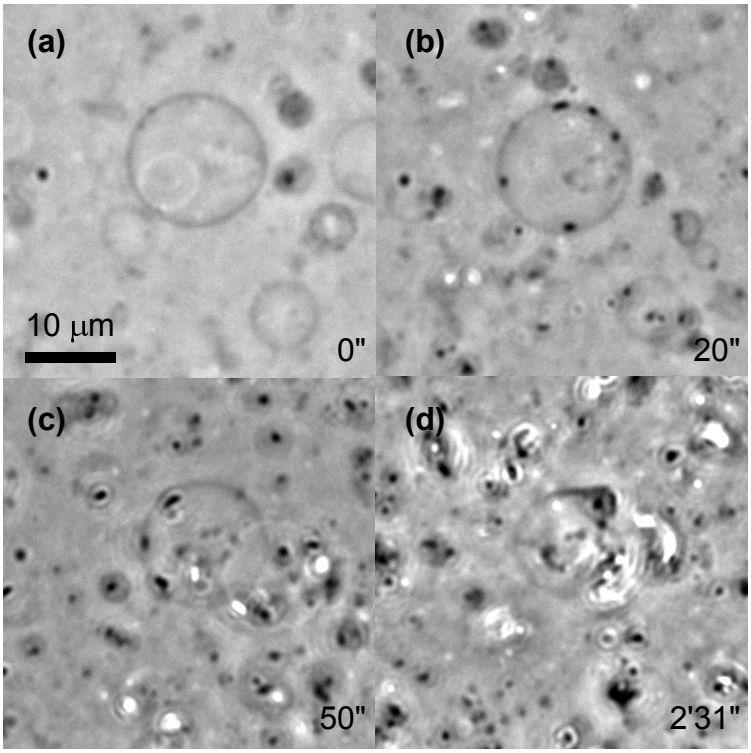

Fig. S7 Time-dependent shape transitions of the GUVs consisting of DMPC following the addition of the C_{70}•γ-CDx complex solution at 37°C. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image.

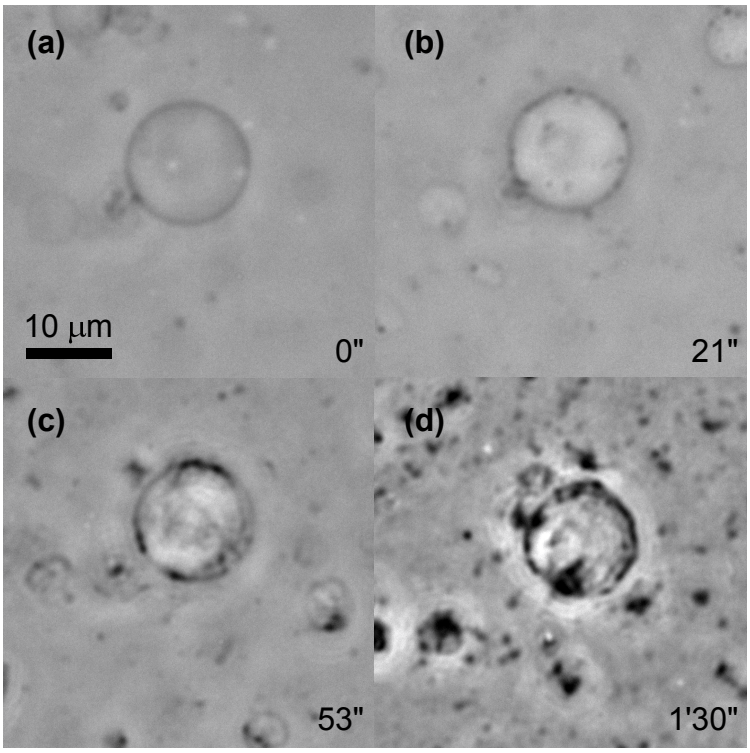

Fig. S8 Time-dependent shape transitions of the GUVs consisting of DPPC following the addition of the C_{70}•γ-CDx complex solution at 44°C. The time elapsed following the starting injection of the C_{70}•γ-CDx complex solution through the micropipette has been indicated for each image.

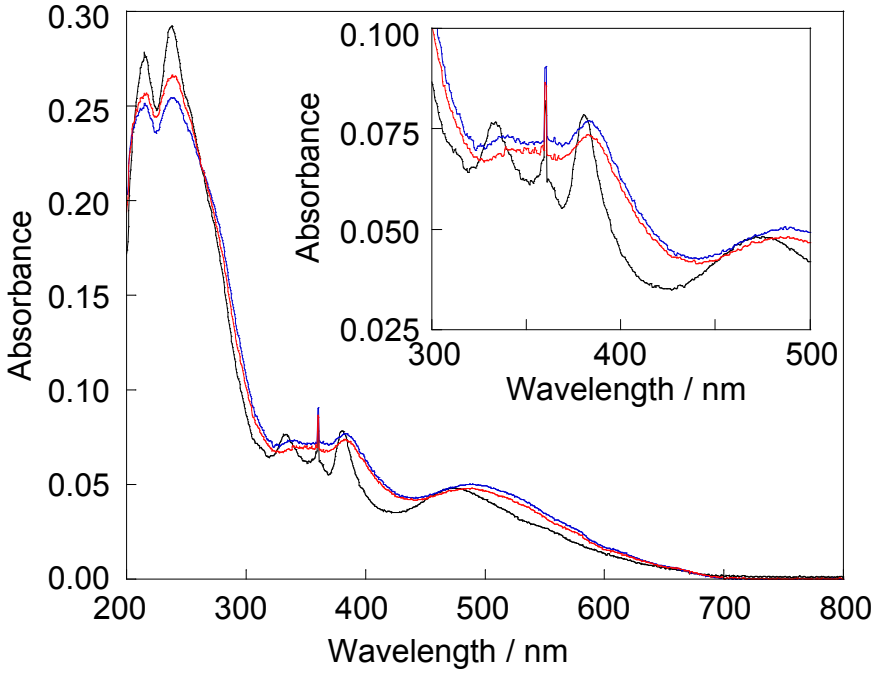

Fig. S9 UV-vis absorption spectra of the C_{70}•γ-CDx complex (black line), the C_{70}-incorporating SUVs prepared by the C_{70}-exchange reactions performed at 30°C (blue line) and 80°C (red line) ([C_{70}] = 0.01 mM, [DMPC] = 0.10 mM, 1 mm cell). The inset shows the 300–500 nm region of the spectrum.