Supplementary Information

Location of [60]fullerene incorporation in lipid membranes

Atsushi Ikeda,* Kazuya Kiguchi, Tamami Shigematsu, Kazuyuki Nobusawa,
Jun-ichi Kikuchi and Motofusa Akiyama

Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan.

Experimental Section

Materials: γ-CDx was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Dimyristoylphosphatidylcholine (DMPC: **1**) was purchased from NOF Corp. (Tokyo, Japan). 1-Palmitoyl-2-stearoyl-(5-doxyl)-*sn*-glycero-3-phosphocholine (**2**) and 1-palmitoyl-2-stearoyl-(16-doxyl)-*sn*-glycero-3-phosphocholine (**3**) were obtained from Avanti Polar Lipids Inc. (Birmingham, AL). C_{60} (>99.5%) and ^{13}C-enriched (ca. 20–40%) C_{60} were from MER Co. (Tucson, AZ).

Preparation of Liposomes: Appropriate amounts of **1**, **1** + **2** and **1** + **3** were dissolved in chloroform. Each solvent was evaporated under nitrogen gas flow and the residual trace solvent was completely removed *in vacuo*. Hydration of the thin lipid films thus obtained on the wall of a vial was performed above the phase transition temperature with an appropriate amount of water. The unilamellar vesicles were obtained by five freeze-thaw cycles (-195 and 50 °C), followed by 11 times extrusion through 0.05 μm pores using a LiposoFast miniextruder from Avestin above the phase transition temperature. The prepared unilamellar vesicles were uniform in size with diameters of 80–90 nm.

Preparation of $LMIC_{60}$ by fullerene exchange reaction: $LMIC_{60}$ were prepared using an exchange reaction between the liposomes and the C_{60}·γ-CDx complex by heating at 80 °C for 1 h, as described in the previous work.[7a] Final concentrations of the respective components

were [**1**] = 1.00 mM ([C_{60}]/[**1**] = 5, 10 and 20 mol%).

Cryogenic temperature transmission electron microscopy (Cryo-TEM): Cryo-TEM samples were prepared by 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 of water before the sample was introduced. 2–3 μL sample droplets were placed on a microperforated cryo-TEM grid and then soaked up 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 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 Digital Micrographs 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 Digital Micrographs.

Differential Scanning Calorimetry (DSC) Experiments: The phase transition behaviour of the lipid bilayer vesicles was determined with a differential scanning calorimeter (VP-DSC, MicroCal, Inc., Northampton, MA). The concentrations of the respective components were [C_{60}]/[**1**] = 10 mol%. The measurements were performed between 10 and 60 °C at 0.5 °C min^{-1} heating rate. The phase transition temperature (T_m) was evaluated.

^{13}C NMR spectroscopy: ^{13}C NMR spectra were recorded on a JEOL JNM-ECP 600 M spectrometer (150 MHz) in D_2O, and the chemical shifts were expressed with reference to sodium 3-(trimethylsilyl)-1-propanesulphonate as the internal standard. ^{13}C-enriched C_{60} was used to measure all ^{13}C NMR spectra.

Table S1 Chemical shifts (δ) and $\Delta\delta$ of C_{60} carbons in the absence or presence of radical labels.

Liposome	$[C_{60}]/[\mathbf{1}]$ / mol%	δ / ppm	$\Delta\delta$ / ppm[a]
1	10	145.987	—
1-2	10	146.016	+0.029
1-3	10	146.035	+0.048

[a] $\Delta\delta$ denotes the shift from the chemical shift of C_{60} in the absence of **2** or **3** (+ to lower magnetic field, - to higher magnetic field).

Table S2 Phase transition temperatures (T_m) and half-height widths of the transition peak ($\Delta T_{1/2}$) of liposome-**1** and $LMIC_{60}$.

$[C_{60}]/[\mathbf{1}]$ / mol%	T_m / °C	$\Delta T_{1/2}$ / °C
0	24.5	0.5
5	24.7	0.5
10	24.7	0.5
20	24.7	0.5

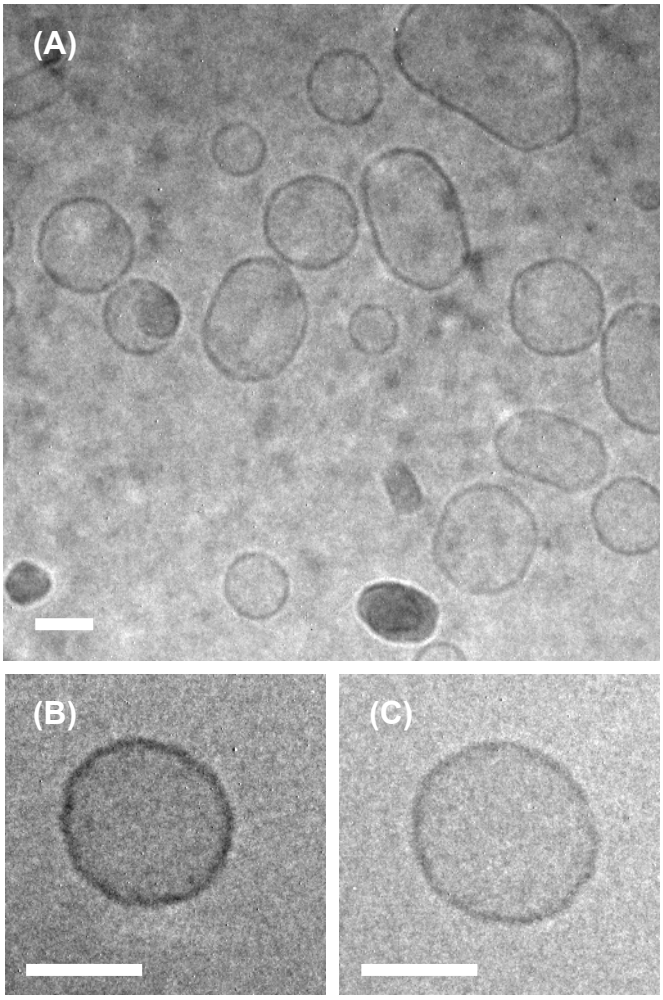

Fig. S1 Cryo-TEM images of liposome-**1** in other areas of the image in Fig.1A (All scale bars show 50 nm).

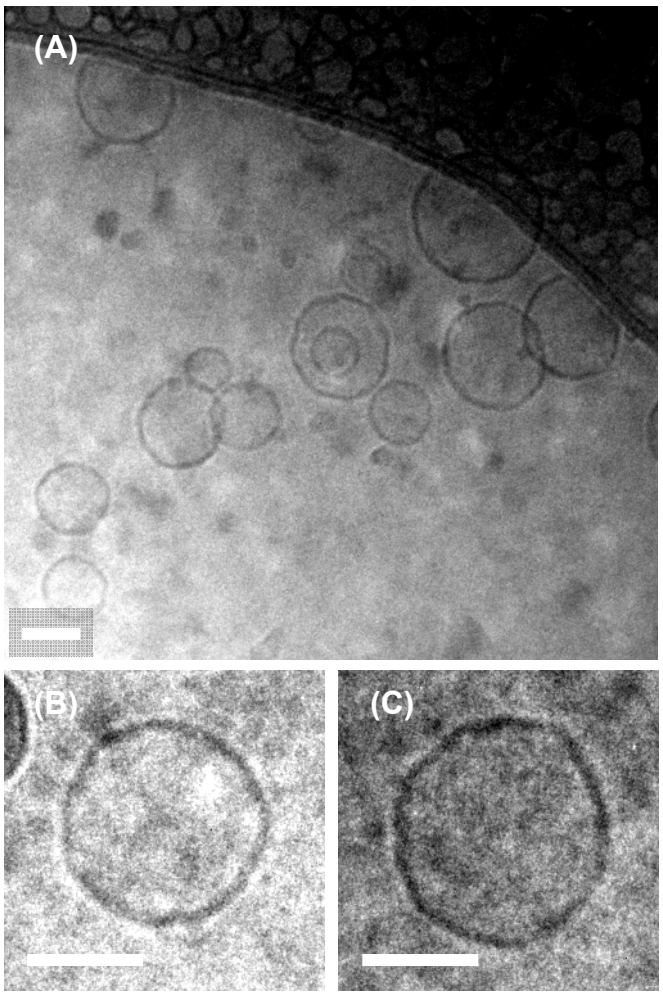

Fig. S2 Cryo-TEM images of $LMIC_{60}$ in other areas of the image in Fig. 1B (All scale bars show 50 nm).

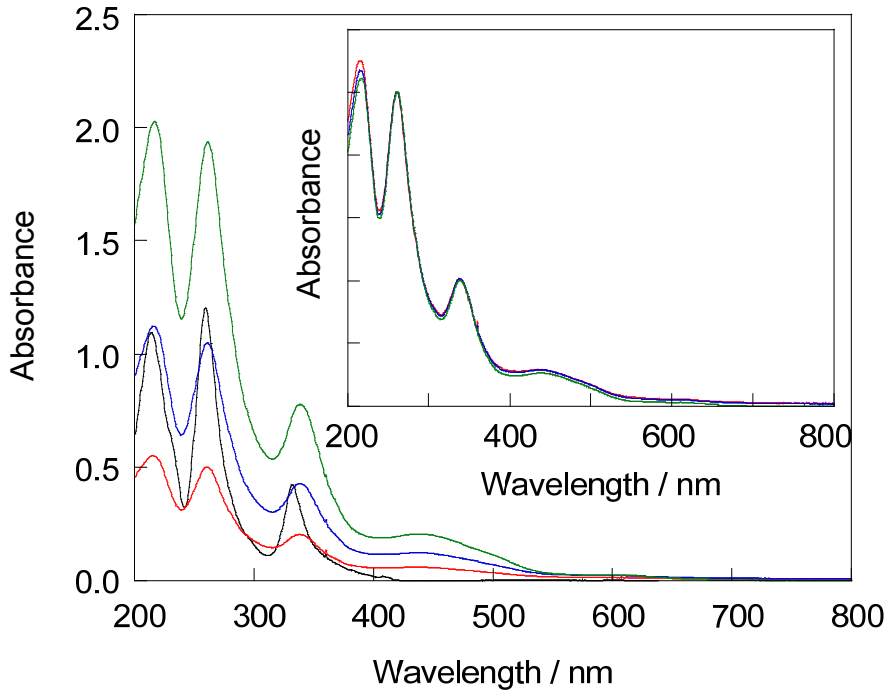

Fig. S3 UV-vis spectral changes of a) the C_{60}•γ-CDx complex (black line) and $LMIC_{60}$ {[C_{60}]/[**1**] = 5 (red line), 10 (blue line) and 20 mol% (green line)} (1 mm cell). Inset: The absorption in each sample normalised to the C_{60} concentrations.

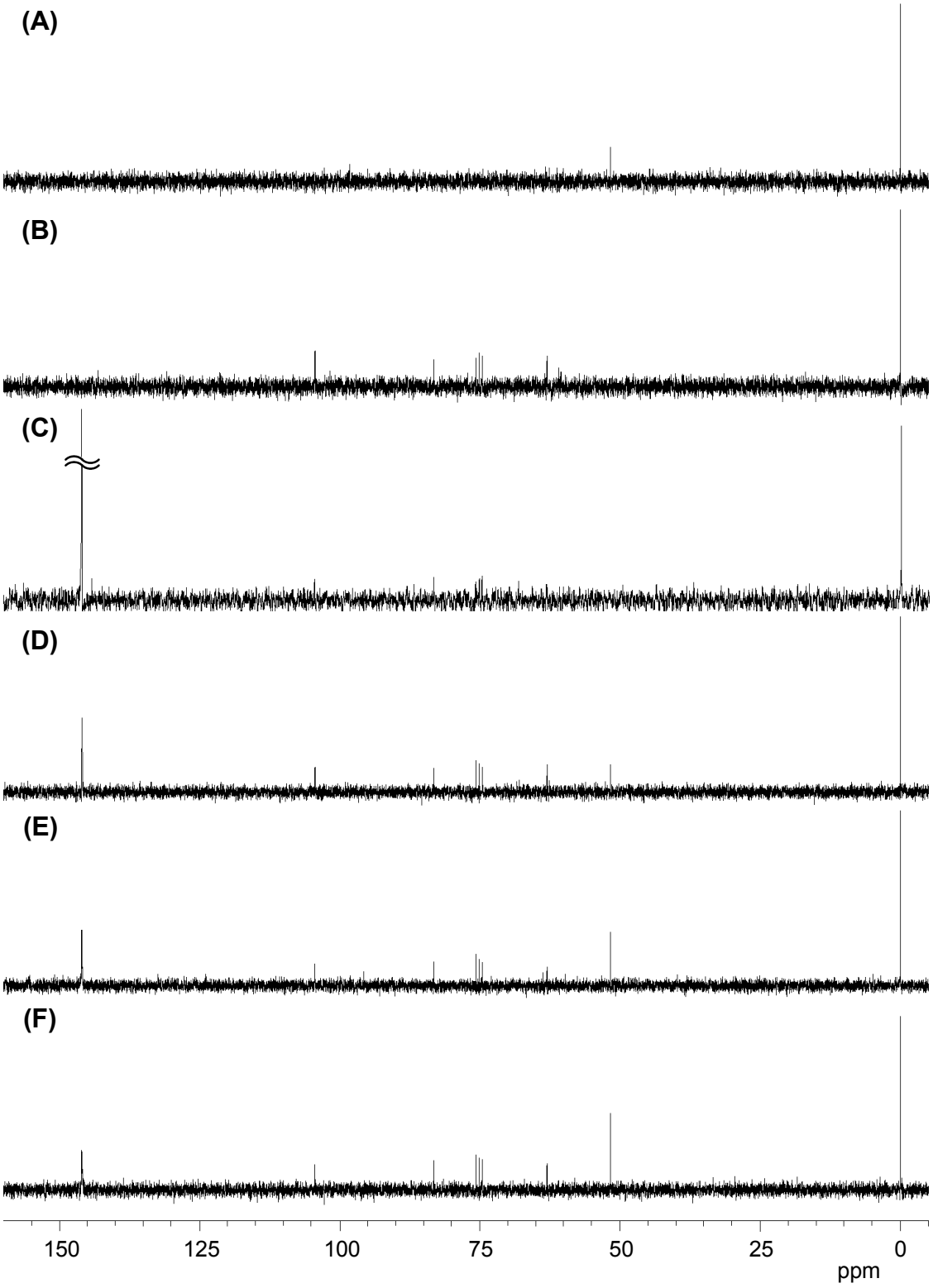

Fig. S4 ^{13}C NMR spectra of (A) liposome-**1**, (B) γ-CDx, (C) $LMIC_{60}$ and $LMIC_{60}$ in the presence of (D) **2** and (E) **3** at 25 °C: 150 MHz, D_2O, [**1**] = [**1** + **2**] = [**1** + **3**] = 1.0 mM, [**2**] = [**3**] = 0.10 mM, [C_{60}] = 0.10 mM. The peak intensities were normalised with respect to the value of sodium 3-(trimethylsilyl)-1-propanesulphonate (5.8 mM).

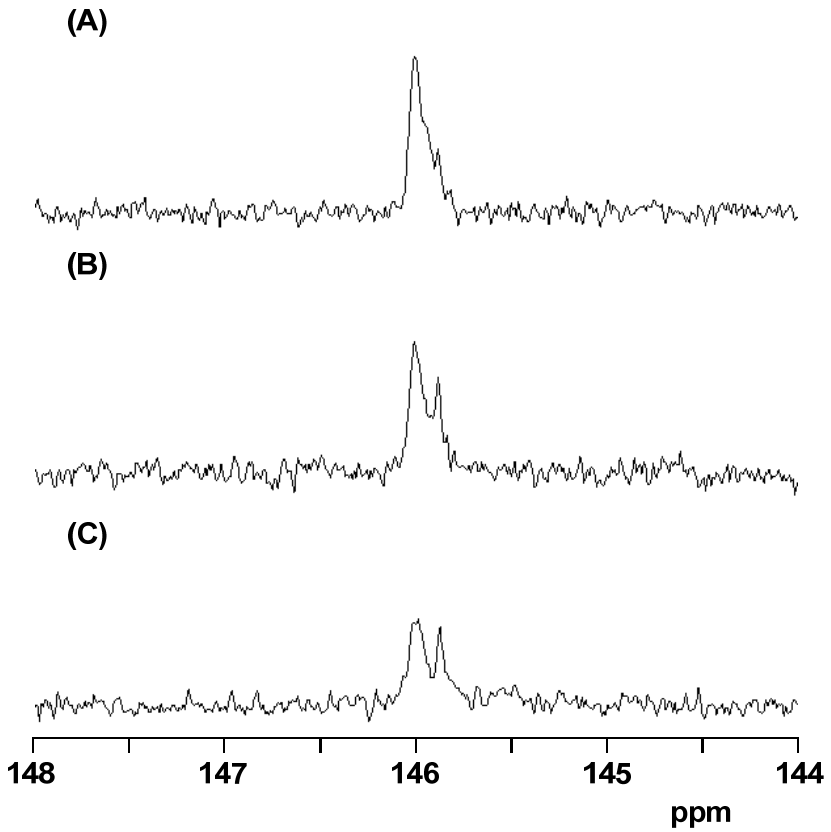

Fig. S5 Partial ^{13}C NMR spectra of (A) $LMIC_{60}$ and $LMIC_{60}$ in the presence of (B) **2** and (C) **3** at 25 °C: 150 MHz, D_2O, [**1**] = 1.0 mM, [**2**] = [**3**] = 0.10 mM, [C_{60}] = 0.10 mM. The peak intensities were normalised with respect to the value of sodium 3-(trimethylsilyl)-1-propanesulphonate (5.8 mM).