# Electronic Supplementary Information

# Binding of Aromatic Molecules in Fullerene-rich Interior of Fullerene Bilayer Vesicle in Water

Koji Harano, Ricardo Mizoguchi Gorgoll, Eiichi Nakamura*

Department of Chemistry, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

## General

All reactions dealing with air- or moisture-sensitive compounds were carried out in a dry reaction vessel under nitrogen or argon. The water content of the solvent was confirmed with a Karl-Fischer Moisture Titrator (MKC-210, Kyoto Electronic Company) to be less than 100 ppm. Analytical thin-layer chromatography was performed on glass plates coated with 0.25 mm 230-600 mesh silica gel containing a fluorescent indicator (Merck). Gas-liquid chromatography (GLC) analysis was performed on a Shimadzu 14A or 14V machine equipped with glass capillary column HR-1 (0.25-nm i.d. 25 m) or CP-Chirasil-Dex CB (0.25-nm i.d. 25 m). Analysis with high pressure liquid chromatography (HPLC) was performed on JASCO HPLC system equipped

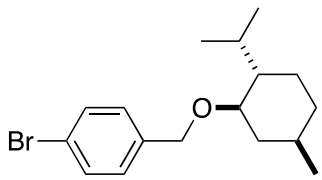
with a Bulcky Prep column (Nacalai Tesque Cosmosil® Buckyprep, 4.6 x 250 mm; column temperature at 40 °C). Flash silica gel column chromatography was performed on silica gel 60N (Kanto, spherical and neutral, 140-325 mesh) as described by Still.[1] Gel permeation column chromatography was performed on a Japan Analytical Industry LC-908 (eluent: chloroform) with JAIGEL 1H and 2H polystyrene columns. Melting points of solid materials were determined on a Mel-Temp capillary melting-point apparatus and were uncorrected. Infrared (IR) spectra were recorded on an ASI Applied Systems React IR1000 equipped with an attenuated total reflection (ATR) instrument or JASCO FT/IR-420 and are reported as wavenumbers ( $\nu$ ) in $cm^{-1}$ with band intensities indicated as s (strong), m (medium), and w (weak). NMR spectra were measured on JEOL ECA-500 spectrometers and reported in parts per million from tetramethylsilane. $^{1}H$ NMR spectra in $CDCl_3$ were referenced internally to tetramethylsilane as a standard, and $^{13}C$ NMR spectra to the solvent resonance. Methyl, methylene, and methyne signals in $^{13}C$ NMR spectra were assigned by DEPT spectra. Routine mass spectra were acquired by atmospheric pressure ionization (APCI) using a quadrupole mass analyzer on Shimadzu QP-8000 or Waters ZQ-S spectrometer, and high resolution spectra by APCI or electrospray ionization (ESI) using a time-of-flight mass analyzer on JEOL JMS-T100LC (AccuTOF) spectrometer with a calibration standard of [60]fullerene. Distilled water was further deionized with Millipore Milli-Q. Dynamic laser light scattering (DLS) study was carried out on a Malvern

Zetasizer Nano ZS machine. Atomic force microscopy (AFM) measurement was performed on a JEOL JSPM-4200 with a silicon cantilever (NSC-35, resonant frequency 120-190 kHz). Scanning transmission electron microscopy (STEM) observations were performed on a JEOL JEM-2100F at 294 K, with a spherical aberration coefficient Cs = 1.0 nm and acceleration voltage of 200 kV, under reduced pressure of $1.0 \times 10^{-5}$ Pa in the sample column. The current density was ca. 0.5 pA. The imaging instrument used was an ultrascan charge-coupled device (CCD) camera (512 × 512 pixels). The UV-Visible spectra were recorded on a JASCO V-570 UV/VIS/NIR Spectrophotometer. Fluorescence spectra were recorded on a HITACHI F-4500 Fluorescence Spectrophotometer.

**Materials**

Unless otherwise noted, materials were purchased from Tokyo Kasei Co., Aldrich Inc., and other commercial suppliers and used after appropriate purification before use. Anhydrous ethereal solvents (stabilizer-free) were purchased from WAKO Pure Chemical and purified by a solvent purification system (GlassContous)[2] equipped with columns of activated alumina and supported copper catalyst (Q-5) prior to use. All other solvents were purified by distillation and stored over molecular sieves 4Å. Water enriched in $^{17}O$ (20.0–24.9 atom%) was purchased from Isotec and used as received. **MeH**, **PhH**, **C8H** and **C20H** were prepared by the reported procedures.[3,4]

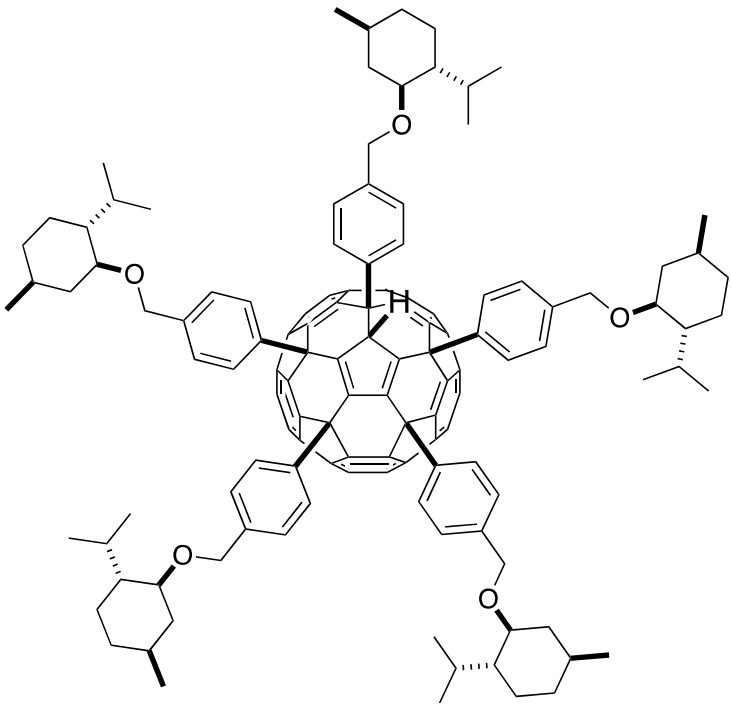
**1-Bromo-4-((L-menthoxy)methyl)benzene**




2.48 g of an oil dispersion of NaH was washed with 30 mL of dry hexane in a 300 mL three neck flask under nitrogen and dried under vacuum to yield a white powder of NaH (1.52 g, 65.2 mmol). After addition of 90 mL of THF to NaH, L-menthol (8.78 g, 56.2 mmol) in THF (45 mL) was added dropwise to the NaH suspension for 10 min at room temperature. 4-Bromobenzyl bromide (9.39 g, 37.5 mmol) in THF (25 mL) was added to the suspension of NaH at 50 °C for 10 min. The reaction mixture was stirred for 1.5 h at reflux temperature and quenched into 50 mL of ice water. The crude was extracted in diethyl ether (100 mL × 3), dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification was performed by column chromatography (silica gel) using hexane/ethyl acetate (95:5) as an eluent to give the product (10.1 g, 31.4 mmol, 83%) as a white crystal. Mp 53.0-54.1 °C; IR (KBr) 2950 (s), 2924 (s), 2866 (s), 1900 (m), 1782 (w), 1636 (m), 1591 (w), 1572 (w), 807 (s); $^1$H NMR (500 MHz, $CDCl_3$) δ 0.71 (d, *J* = 6.9 Hz, 3H), 0.82-1.01 (m, 9H), 1.26-1.40 (m, 2H), 1.61-1.68 (m, 2H), 2.14-2.18 (m, 1H), 2.23-2.29 (m, 1H), 3.16 (dd, *J* = 4.6, 10.6 Hz, 1H), 4.34 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 7.21-7.23 (m, 2H), 7.44-7.64 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 16.08 ($CH_3$), 20.99 ($CH_3$), 22.34 ($CH_3$), 23.24 ($CH_2$), 25.56 (CH), 34.53 ($CH_2$), 40.28 ($CH_2$), 48.30 (CH), 69.62 ($CH_2$), 78.97 (CH),

121.18, 129.40 (CH), 131.36 (CH), 138.21; anal. calcd for $C_{17}H_{25}BrO$: C, 62.77; H: 7.75; found: C, 62.58; H, 7.58.

**6,9,12,15,18-Penta(4-(L-menthoxy)methylphenyl)-1,6,9,15,18-hexahydro(C60-Ih)[5,6]fullerene (MentH)**



34.5 mL of 4-((L-menthoxy)methyl)phenylmagnesium bromide in THF (0.38 M, 13 mmol), was added to a suspension of CuBr•$SMe_2$ (2.77 g, 13.5 mmol) in THF (5 mL) and stirred for 10 min at 35 °C. [60]Fullerene (651 mg, 0.904 mmol) was dissolved in ODCB (40 mL) and added to the organocopper reagent suspension. The reaction mixture was stirred for 1.5 h at 40 °C and quenched with a sat. $NH_4Cl$ aqueous solution (5 mL). After removal of THF and $Me_2S$ under reduced pressure, the reaction mixture was filtered through a pad of silica gel (eluent: hexane, then toluene). The obtained mixture was concentrated under reduced pressure, dissolved in ether, reprecipitated by methanol and collected by filtration through a membrane filter (PTFE, pore size: 0.4 μm). The product was dried under vacuum to yield a red solid (1.75 g, 98%). IR (KBr) 2955 (s), 2922 (s), 2869 (s), 1894 (w), 1797 (w), 1714 (w), 1612 (w), 808 (m); $^1$H NMR (500 MHz, $CDCl_3$) δ 0.67-0.73 (m, 15H), 0.87-0.98 (m, 45H), 1.25-1.36 (m, 10H),

1.58-1.66 (m, 10H), 2.16-2.29 (m, 10H), 3.13-3.20 (m, 5H), 4.35-4.38 (m, 2H), 4.42-4.44 (m, 2H), 4.56-4.58 (m, 2H), 4.62-4.64 (m, 2H), 4.68-4.70 (m, 2H), 5.22 (s, 1H), 7.11-7.12 (m, 2H), 7.17-7.18 (m, 4H), 7.30-7.32 (m, 4H), 7.34-7.36 (m, 2H), 7.54-7.57 (m, 4H), 7.73-7.75 (m, 4H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 14.14, 16.17, 16.24, 16.30, 20.71, 21.02, 22.39, 22.42, 22.62, 22.66, 23.27, 23.38, 25.28, 25.29, 25.52, 25.60, 25.64, 25.66, 31.59, 34.20, 34.54, 34.58, 34.66, 40.27, 40.31, 40.34, 48.25, 48.32, 48.34, 69.85, 69.90, 78.76, 78.87, 78.89, 78.93, 78.99, 127.68, 127.86, 127.88, 127.98, 128.06, 128.13, 128.24, 128.30, 138.05, 138.44, 138.48, 138.66, 138.68, 138.79, 138.83, 143.10, 143.14, 143.69, 143.75, 144.02, 144.12, 144.22, 144.31, 144.33, 144.39, 144.44, 144.50, 144.75, 145.39, 145.44, 145.62, 145.68, 145.78, 145.86, 146.07, 146.25, 146.93, 147.11, 147.21, 147.74, 147.78, 147.88, 148.08, 148.24, 148.39, 148.67, 148.69, 148.74, 151.46, 151.60, 152.18, 152.18, 152.30, 152.60, 152.65, 156.09, 156.21; HRMS (APCI–) calcd for $C_{145}H_{125}O_5^-$ 1945.9527, found 1945.9480.

**Preparation of fullerene vesicle**

The vesicle solutions used for this study were prepared as reported.[4] As an example, **MentK** vesicles were prepared as follows. $^t$BuOK in THF (1.00 M, 52 μL, 52 μmol, 1.5 equiv.) was added to a solution of **MentH** (68.1 mg, 35 μmol) in THF (2.8 mL) and the mixture was stirred under argon. After stirring for 3 h, a portion of the solution of **MentK** (12.5 mM, 0.80 mL, 10 μmol) was slowly injected into ultrapure water (4.2 mL) with stirring at 400 rpm over 1 min using a syringe pump (ISIS Co.) to obtain a vesicle solution of **MentK** (2.0 mM) in

16% THF/water. THF and water were removed by evaporation at ca. 7 kPa. The concentration of **MentK** was determined by UV absorption at 352 nm of wavelength and adjusted to 0.500 mM. DLS analysis showed that the average diameter of a series of vesicles may vary in the range of 20 – 80 nm, depending on the conditions of the preparation, but generally falls in the range of 25–40 nm.

**$^1$H NMR measurement of MentK vesicle**

$^t$BuOK in THF (1.0 M, 19 μL, 19 μmol) was added to a solution of **MentK** (24.4 mg, 12.5 μmol) in THF (1.0 mL) and the mixture was stirred under nitrogen. During the mixing, the reaction mixture became a dark transparent solution. After 3 h, a portion of the solution of **MentK** (12.5 mM, 0.8 mL, 10 μmol) was slowly injected into $D_2O$ (4.2 mL) with stirring at 400 rpm over 1 min using a syringe pump (ISIS Co.) to obtain a vesicle solution of **MentK** (2.0 mM) in 16% THF/$D_2O$. THF was removed by evaporation at ca 7 kPa. The concentration of **MentK** was determined by UV absorption at 352 nm of wavelength and adjusted to 2.00 mM. The $^1$H NMR of the $D_2O$ solution of **MentK** vesicle was measured on JEOL ECA-500 at 20 °C.

**Preparation of liposomes**

Egg lecithin (132 mg, 0.180 mmol) was dissolved in $CHCl_3$/MeOH (1:1, 1.30 mL) and exposed to Ar flow until an orange film was obtained. The film was

then dried under vacuum overnight and hydrated by MilliQ (2.00 mL). In order to reduce the size of the particles, the solution was exposed to probe sonicator for 1 h.

**DLS analysis**

Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS instrument equipped with a He–Ne laser operating at 4 mW power and 633 nm wavelength, and a computer-controlled correlator, at a 173° accumulation angle. Measurements were carried out in a polystyrene or glass cuvette. The sample was equilibrated for 5 min at the set temperature each time. The data were processed by Dispersion Technology software version 5.10 to give *Z*-average particle size and polydispersity index by cumulant analysis, and the particle size distribution by CONTIN analysis.[5]

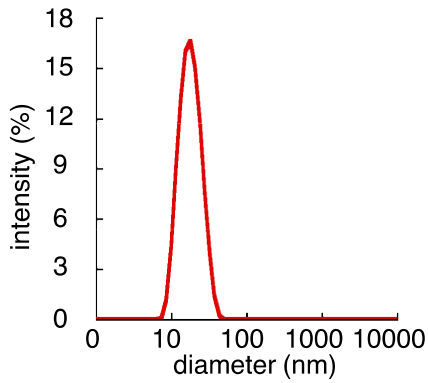



**Figure S1.** Size distribution of **MentK** vesicle.

**Determination of permeability coefficient by NMR relaxation time measurement.**

The permeability coefficient ($P$) was determined by the NMR method established in the studies of lipid vesicles.[6] Aqueous solutions of $MnCl_2$ (0.50 mM, 200 μL) and the fullerene vesicles (200 μL) were mixed to give vesicle solutions containing 0.25 mM $MnCl_2$. The average hydrodynamic radius ($R_h$) of the vesicles was determined by DLS analysis, and the radius did not change (±13.2%) from 10 to 80 °C. Aqueous solutions of the fullerene vesicles in $^{17}O$-enriched water were prepared in the same manner on a smaller scale using the same vesicle solutions (20 μL) and an aqueous solution of $MnCl_2$ (0.50 mM, 20 μL) in water containing 20 atom% of $^{17}O$. The sample solution was transferred to a capillary NMR tube (Nihon Seimitsu Kagaku N-502B, tube diameter 2 mm). The NMR probe temperature was calibrated using methanol (10–30 °C) and ethylene glycol (40–80 °C) before the measurement.[7] We measured the transverse relaxation time of the vesicle solution from to 80 °C in steps of 10 °C. The sample was equilibrated for 5 min at the set temperature each time, and the relaxation time was recorded three times at that temperature. The transverse relaxation time ($T_2$) of $^{17}O$ was recorded three times without spinning the sample tube. The transverse relaxation time of the interior water ($T_{2i}$) was measured, and, as a reference, the transverse relaxation time of blank water ($T_{2ref}$) was also measured at each temperature. The permeability coefficient ($P$) is obtained from the following equation:

$$P = \frac{R}{3}\left(\frac{1}{T_{2i}} - \frac{1}{T_{2ref}}\right) \qquad (1)$$

where $R$ is the radius of the vesicles obtained from the DLS analysis at each temperature.

**AFM analysis**

AFM measurement was conducted by using JEOL JSPM-4200 with a silicon cantilever (NSC-35, resonant frequency 120-190 kHz). Samples were deposited on a substrate (5 × 5 $mm^2$) in aliquot of 2 μm under air. After drying the sample by blowing air for 10 sec and under reduced pressure (5 × $10^{-2}$ Pa), the AFM image was obtained by AC mode measurement (Figure S2).

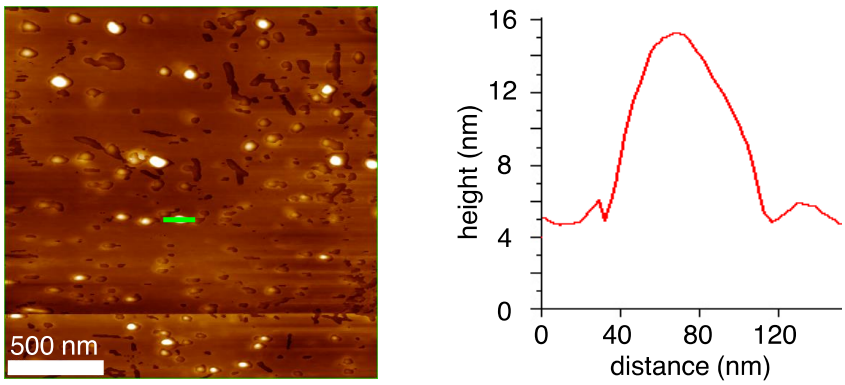



**Figure S2.** AFM image of **MentK** vesicle on mica and cross-sectional profile along the light-green line.

**STEM measurement**

STEM measurement was conducted on a JEOL JEM-2100F at 294 K with a spherical aberration coefficient Cs = 1.0 nm at an acceleration voltage of 200 kV

under reduced pressure of $1.0 \times 10^{-5}$ Pa in the sample column. The current density is ca. 0.5 pA·cm$^{-2}$. The imaging instrument used was an ultrascan charge-coupled device (CCD) camera (512 × 512 pixels). 1 μM of **MentK** vesicle (2 μL) was deposited on a transmission electron microscopy (TEM) copper mesh coated with carbon film (Super Ultra High Resoluton Carbon film, thickness < 6 nm, Oken Shoji Co., Ltd.), then dried under reduced pressure ($4 \times 10^{-2}$ Pa) at room temperature for 18 h.

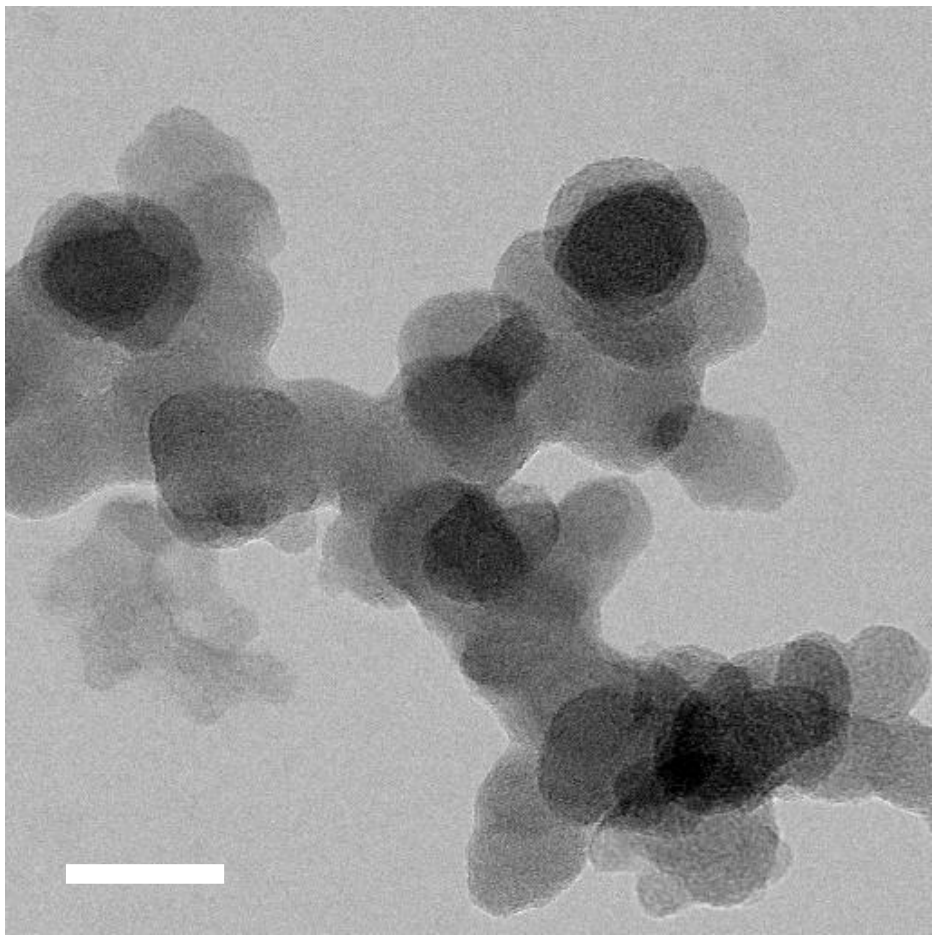


**Figure S3.** STEM image of **MentK** vesicle on a carbon film without staining. Scale bar is 50 nm.

**SEM measurement**

SEM measurement was conducted on a FEI Magellan 400L. An aqueous solution of the vesicles (0.5 mM, 0.2 mL) was placed on an indium-tin oxide (ITO)/glass substrate cleaned by UV/ozone treatment just before use, and was spin-coated (1500 rpm) for 30 s. After drying under reduced pressure ($5 \times 10^{-2}$ Pa) for 10 min, the ITO substrate was subjected to the SEM observation at an acceleration voltage of 1.5–2 kV under a vacuum of $5 \times 10^{-5}$ Pa without any conductive coatings.

Unlike **PhK** and **C20K** vesicles that maintain their spherical shape on a solid substrate, the **MentK** vesicles almost entirely collapsed on the ITO surface (Figure S4), suggesting that the bulky menthyloxy groups reduces structural integrity of the vesicle to make the bilayer flexible.

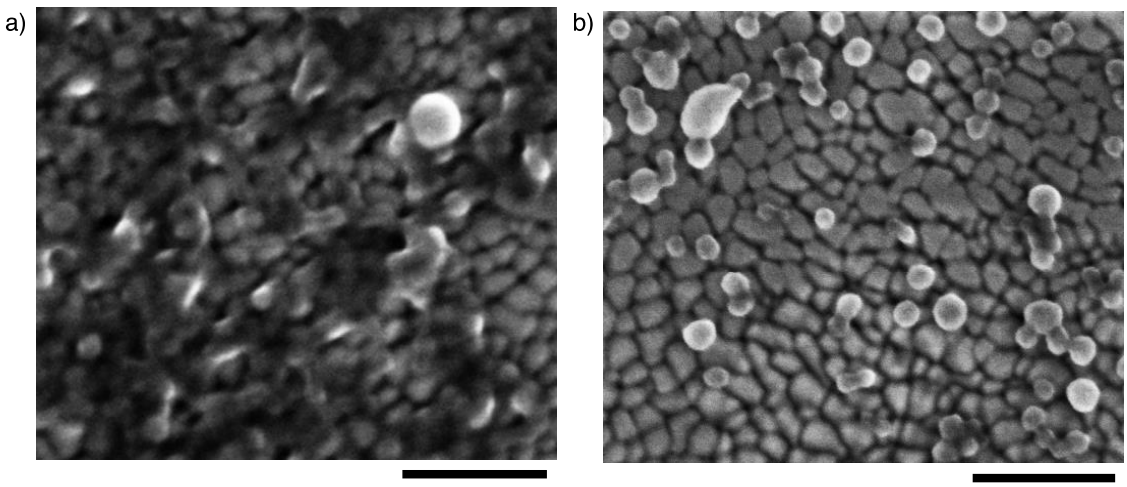



**Figure S4.** SEM image of **MentK** (a) and **PhK** (b) vesicles on ITO. Scale bar is 100 nm. Figure S4a was taken at acceleration voltage of 2 kV and current

intensity of 25 pA and Figure S4b was taken at acceleration voltage of 1.5 kV and current intensity of 6.3 pA

**Loading of prodan in fullerene vesicles**

Loading of prodan for this study was done as reported:[8] An aqueous solution of prodan (1.25 mL, 20 μM, 2.5% methanol) was added to an aqueous solution of $R_5C_{60}K$ (1.25 mL, 20 μM) and stirred for 12 hours. The sample was submitted for photoluminescence analysis ($\lambda_{ex}$ = 366 nm).

**Loading of aromatic molecules in fullerene vesicles**

The aromatic compound (ca. 1 mg) was added to 2.00 mL of a 0.50 mM aqueous solution of fullerene vesicle. The suspension was vigorous stirred for 12 h and left to rest for 24 h. The remaining solid was removed by centrifugation (TOMY MC-150) of the supernatant at 5000 rpm for 30 min twice. 0.350 mL of the supernatant was collected and the aromatic compound was extensively extracted from the vesicle solution with dichloromethane. The amount of aromatic molecule was determined by UV absorption at the wavelength of 250 nm for biphenyl, 283 nm for *p*-terphenyl, and 295 nm for phenanthrene. *Trans*-decalin was loaded by a similar procedure: *trans*-decalin (100 μL) was added to 4.50 mL of a 0.50 mM aqueous solution of fullerene vesicle. The mixture was stirred overnight for 12 h and left to rest for 24 h, which resulted in

complete phase separation of the aqueous and organic layers. 0.500 mL of the aqueous phase was collected and *trans*-decalin was extracted from the vesicle solution with hexane. The amount of naphthalene and *trans*-decalin was determined by GC using *n*-dodecane as an internal standard. The number of molecules incorporated per fullerene monomer was calculated after consideration of the amount of compound solubilized in water, which was determined by a control experiment carried out in parallel to the main experiment.

**Table S1.** Ratio of hydrophobic molecules incorporated per fullerene monomer.

| vesicle | incorporation ratio | |
|---|---|---|
| | naphthalene | *trans*-decalin |
| **MeK** | 0.30 | 0.11 |
| **PhK** | 0.34 | 0.17 |
| **C8K** | 0.19 | 0.48 |
| **C20K** | 0.27 | 0.38 |
| **MentK** | 0.90 | 0.47 |

**Table S2.** Ratio of aromatic molecules incorporated per fullerene monomer.

| vesicle | incorporation ratio | | |
|---|---|---|---|
| | phenanthrene | biphenyl | *p*-terphenyl |
| **MeK** | 0.09 ± 0.01 | 0.012 ± 0.005 | 0.003 ± 0.002 |
| **PhK** | 0.13 ± 0.01 | 0.03 ± 0.01 | 0.009 ± 0.002 |
| **C8K** | 0.22 ± 0.05 | 0.06 ± 0.03 | 0.03 ± 0.01 |
| **C20K** | 0.26 ± 0.01 | 0.05 ± 0.03 | 0.011 ± 0.004 |
| **MentK** | 0.32 ± 0.02 | 0.12 ± 0.04 | 0.025 ± 0.004 |
| Reference (liposome) | 0.003 ± 0.002 | 0.0020 ± 0.0009 | –0.002 ± 0.005 |

**Table S3.** Change of the size (%) of the vesicle after incorporation of the aromatic molecules determined by DLS at 25 °C.

| vesicle | size increase (%) | | |
|---|---|---|---|
| | phenanthrene | biphenyl | *p*-terphenyl |
| **MeK** | –14.30 ± 0.04 | 1 ± 3 | –15.60 ± 0.03 |
| **PhK** | 12.25 ± 0.05 | 22.7 ± 0.1 | 11.1 ± 0.1 |
| **C8K** | 7.3 ± 0.4 | 17.2 ± 0.2 | 9.4 ±0.3 |

| | | | |
|---|---|---|---|
| **C20K** | –1 ± 2 | 6.6 ± 0.2 | –2.6 ± 0.6 |
| **MentK** | 20.50 ± 0.06 | 35.10 ± 0.04 | 29.57 ±0.07 |

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