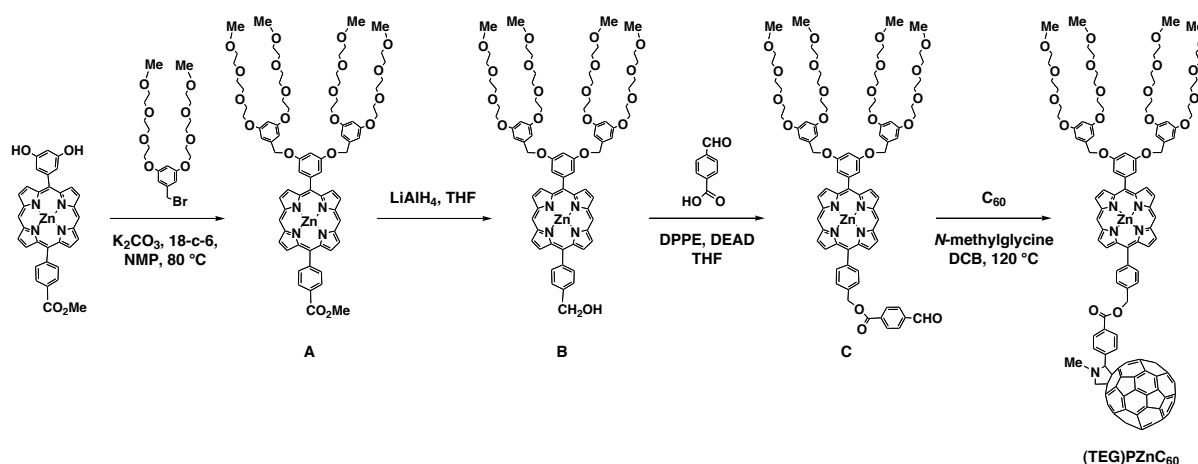


## Supplementary Information

### Self-assembly of a $\pi$ -electronic amphiphile consisting of a zinc porphyrin-fullerene dyad: formation of micro-vesicles with a high stability

Richard Charvet, Dong-Lin Jiang and Takuzo Aida

#### (1) Synthesis



**Compound A:** A mixture of 5-(3',5'-dihydroxyphenyl)-15-(4'-methoxycarbonylphenyl)porphine<sup>1</sup> (42 mg, 0.068 mmol), 3,5-bis(methoxyethoxyethoxyethoxy)benzyl bromide<sup>2</sup> (100 mg, 0.2 mmol), K<sub>2</sub>CO<sub>3</sub> (48 mg, 0.34 mmol) and 18-crown-6 (2.6 mg, 0.014 mmol) in *N*-methylpyrrolidone (5 mL) was heated at 80 °C under argon for 3 days. The reaction mixture was evaporated to dryness, and the residue was subjected to silica gel PTLC using CHCl<sub>3</sub>-MeOH as eluent to isolate the desired compound (A). Yield 90% (90 mg). <sup>1</sup>H NMR ( $\delta$ /ppm, CDCl<sub>3</sub>, 500 MHz, 25 °C): 2.95 (s, 12H, CH<sub>3</sub> in *dendron*-OCH<sub>3</sub>), 3.09 (t, *J* = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.24 (t, *J* = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.37 (t, *J* = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.50 (t, *J* = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>),

3.69 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 3.97 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 4.10 (s, 3H, methyl ester), 5.14 (s, 4H, *dendron*-CH<sub>2</sub>O), 6.44 (d,  $J = 2$  Hz, 2H, *p*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 6.61 (d,  $J = 2$  Hz, 4H, *o*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 7.02 (t,  $J = 2$  Hz, 1H, *p*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.45 (s, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 8.27 (d,  $J = 8$  Hz, 2H, *m*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.41 (d,  $J = 8$  Hz, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.99 (m, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.03 (m, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.30 (m, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.35 (m, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 10.19 (s, 2H, *meso*-H in P<sub>Zn</sub>). MALDI-TOF-MS (dithranol): Calcd. for C<sub>76</sub>H<sub>91</sub>N<sub>4</sub>O<sub>20</sub>Zn [M+H]<sup>+</sup>:  $m/z = 1443.5$ ; Found: 1443.6. UV-vis (THF, 25 °C): 280, 308, 414, 544, 580 nm.

**Compound B:** To a dry THF solution (10 mL) of compound **A** (90 mg, 0.062 mmol) immersed in an ice-bath was progressively added a LiAlH<sub>4</sub> (7 mg, 0.18 mmol) suspension in dry THF (5 mL). After stirring at room temperature for 30 min, the reaction was quenched with 2 drops of water and the solution was evaporated to dryness. The residue was chromatographed on silica gel with CH<sub>2</sub>Cl<sub>2</sub>-MeOH as eluent to isolate the desired compound (**B**) as a pink solid, which was freeze-dried from benzene. Yield 100% (87 mg). <sup>1</sup>H NMR ( $\delta$ /ppm, CDCl<sub>3</sub>, 500 MHz, 25 °C): 2.95 (s, 12H, CH<sub>3</sub> in *dendron*-OCH<sub>3</sub>), 3.09 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.24 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.37 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.50 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.69 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 3.97 (t,  $J = 5$  Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 4.85 (s, 2H, CH<sub>2</sub>OH), 5.13 (s, 4H, *dendron*-CH<sub>2</sub>O), 6.43 (s, 2H, *p*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 6.61 (s, 4H, *o*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 7.02 (s, 1H, *p*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.45 (s, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.62 (d,  $J = 8$  Hz, 2H, *m*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.14 (d,  $J = 8$  Hz, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 9.03 (m, 4H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.32 (m, 4H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 10.17 (s, 2H, *meso*-H in P<sub>Zn</sub>). MALDI-TOF-MS (dithranol): Calcd. for C<sub>75</sub>H<sub>91</sub>N<sub>4</sub>O<sub>19</sub>Zn [M+H]<sup>+</sup>:  $m/z = 1415.5$ ; Found: 1415.4. UV-vis (THF, 25 °C): 278, 310, 392 (sh), 413, 543, 580 nm.

**Compound C:** To a THF solution (0.5 mL) of a mixture of compound **B** (25 mg, 0.018 mmol), 4-formylbenzoic acid (5.5 mg, 0.035 mmol) and diphenylphosphoethane (DPPE, 7 mg, 0.018 mmol) immersed in an ice-bath was progressively added 5.5  $\mu$ L (0.035 mmol) of diethylazodicarboxylate (DEAD) under argon. The solution was then stirred at room temperature until complete disappearance of the starting porphyrin. After quenching by addition of water (1 drop), the reaction mixture was evaporated to dryness, and the residue was subjected to silica gel PTLC using CHCl<sub>3</sub>-MeOH as eluent to isolate the desired compound (**C**) as a red solid, which was freeze-dried from benzene. Yield 83% (22 mg). <sup>1</sup>H

NMR ( $\delta$ /ppm, CDCl<sub>3</sub>, 500 MHz, 25 °C): 2.87 (s, 12H, CH<sub>3</sub> in *dendron*-OCH<sub>3</sub>), 3.00 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.17 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.35 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.50 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.70 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 3.99 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 5.17 (s, 4H, *dendron*-CH<sub>2</sub>O), 5.78 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>), 6.45 (s, 2H, *p*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 6.62 (s, 4H, *o*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 7.03 (s, 1H, *p*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.47 (s, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.86 (d,  $J$  = 8 Hz, 2H, *m*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.04 (d,  $J$  = 8 Hz, *o*-H in HOC-C<sub>6</sub>H<sub>4</sub>), 8.28 (d,  $J$  = 8 Hz, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.41 (d,  $J$  = 8 Hz, *m*-H in HOC-C<sub>6</sub>H<sub>4</sub>), 9.04 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.10 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.35 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.40 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 10.15 (s, 1H, CHO), 10.27 (s, 2H, *meso*-H in P<sub>Zn</sub>). MALDI-TOF-MS (CHCA): Calcd. for C<sub>83</sub>H<sub>95</sub>N<sub>4</sub>O<sub>21</sub>Zn [M+H]<sup>+</sup>:  $m/z$  = 1549.6; Found: 1549.2. UV-vis (THF, 25 °C): 285, 312, 413.5, 543, 580 nm.

**(TEG)PZnC<sub>60</sub>**: A dry *o*-dichlorobenzene (6 mL) solution of a mixture of compound **C** (9.4 mg, 6  $\mu$ mol), C<sub>60</sub> (13 mg, 18  $\mu$ mol) and *N*-methylglycine (16 mg, 180  $\mu$ mol) was heated at 120 °C under argon until complete disappearance of the starting porphyrin.<sup>3</sup> After cooling, the reaction mixture was chromatographed on silica gel with toluene, toluene-AcOEt (9/1 in vol.) and then CHCl<sub>3</sub>-MeOH as eluents to isolate the desired compound ((TEG)PZnC<sub>60</sub>) as a dark pink solid, which was freeze-dried from benzene. Yield: 77% (10.6 mg). <sup>1</sup>H NMR ( $\delta$ /ppm, CDCl<sub>3</sub>, 500 MHz, 25 °C): 2.81 (s, 3H, NCH<sub>3</sub>), 2.89 (s, 12H, CH<sub>3</sub> in *dendron*-OCH<sub>3</sub>), 3.03 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.18 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.34 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.48 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.68 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 3.95 (t,  $J$  = 5 Hz, 8H, CH<sub>2</sub> in *dendron*-CH<sub>2</sub>CH<sub>2</sub>O), 4.18 (d,  $J$  = 10 Hz, C<sub>60</sub>CH<sub>2</sub>N), 4.91 (d,  $J$  = 10 Hz, C<sub>60</sub>CH<sub>2</sub>N), 4.94 (s, 1H, C<sub>60</sub>CHN), 5.16 (s, 4H, *dendron*-CH<sub>2</sub>O), 5.71 (s, 2H, CH<sub>2</sub> between P<sub>Zn</sub> and C<sub>60</sub>), 6.45 (s, 2H, *p*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 6.61 (s, 4H, *o*-H in *dendron*-C<sub>6</sub>H<sub>3</sub>), 7.03 (s, 1H, *p*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.47 (s, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>3</sub>), 7.84 (d,  $J$  = 8 Hz, 2H, *m*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 7.95 (sh, 2H, *o*-H in C<sub>60</sub>-C<sub>6</sub>H<sub>4</sub>), 8.24 (d,  $J$  = 8 Hz, 2H, *o*-H in P<sub>Zn</sub>-C<sub>6</sub>H<sub>4</sub>), 8.30 (d,  $J$  = 8 Hz, *m*-H in C<sub>60</sub>-C<sub>6</sub>H<sub>4</sub>), 9.02 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.08 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.33 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 9.37 (d,  $J$  = 4 Hz, 2H, pyrrole- $\beta$ -H in P<sub>Zn</sub>), 10.24 (s, 2H, *meso*-H in P<sub>Zn</sub>). MALDI-TOF-MS (CHCA): Calcd. for C<sub>145</sub>H<sub>100</sub>N<sub>5</sub>O<sub>20</sub>Zn [M+H]<sup>+</sup>:  $m/z$  = 2296.6; Found: 2296.7. UV-vis ( $\epsilon$ ; THF, 25 °C):

216 ( $1.8 \times 10^5$ ), 254 ( $1.1 \times 10^5$ ), 310 ( $5.0 \times 10^4$ ), 413 ( $2.9 \times 10^5$ ), 543 ( $2.0 \times 10^4$ ), 580 ( $3.6 \times 10^3$ ), 702 (370) nm.

## **(2) Measurements**

$^1\text{H}$  NMR spectra were recorded at 298 K on a JEOL model NM-EXCALIBUR, operating at 500 MHz. MALDI-TOF-MS spectra were recorded on an Applied Biosystems model Biospectrometry Workstation™ Voyager-DE™ STR in a reflector mode. Electronic absorption spectra were recorded with a JASCO model V-560 UV/VIS spectrometer and a JASCO model V-570 UV/VIS/NIR spectrometer. Fluorescence measurements were performed with a JASCO model FP-6500 spectrofluorometer. SEM images were obtained with a JEOL model JSM-6700F field emission scanning electron microscope at an accelerating voltage of 5 kV. Silicon 110 was used as substrate and platinum as coating. TEM images were obtained with a JEOL model JEM-2010 transmission electron microscope. One drop of a dilute solution of vesicles was deposited on a collodium surface, left to dry and the observation was performed at room temperature at a voltage of 120 kV. No staining was used. DLS measurements were carried out using an Otsuka model DLS-7000 instrument (Otsuka Electronic Co., Ltd., Japan). Vertically polarized light (488 nm) from an argon ion laser (75 mW) was used as the incident beam. All measurements were performed at  $25.0 \pm 0.1$  °C. Solutions of vesicles were analyzed at least 12 h after preparation.

## **(3) Typical procedure for the preparation of a vesicle solution of (TEG)PZnC<sub>60</sub>**

To a vial containing 3.5 mL of pure water was added dropwise a freshly prepared THF solution (150  $\mu\text{L}$ ) of (TEG)PZnC<sub>60</sub> (0.775 mg) under vigorous stirring. The vial was kept open, and the solution was stirred for 10 min and sonicated in a water bath at room temperature for 30 min. Before analysis, the solution was left standing at room temperature in the dark for at least 4 h.

## **(4) Sulforhodamine-G (SG) encapsulation experiment**

10 mL of pure water containing 2.5 mg of sulforhodamine-G (dye content: 60%) was submitted to ultracentrifugation, and the supernatant solution was recovered and used as an

aqueous stock solution of SG. A freshly prepared THF solution (100  $\mu\text{L}$ ) of **(TEG)PZnC<sub>60</sub>** (0.148 mg) was added dropwise to a vigorously-stirred aqueous solution of SG (2 mL). After sonication for 30 min, the solution was allowed to stand overnight, and then passed through a Sephadex™ G-50 column (4 x 30 cm) using water as eluent. A pale yellow fraction was collected and used for analysis.

#### **(5) A control experiment for sulforhodamine-G encapsulation**

An aqueous solution of SG (1 mL) was added to a 3-h aged vesicle solution, prepared by the addition of a THF solution (100  $\mu\text{L}$ ) of **(TEG)PZnC<sub>60</sub>** (0.073 mg) to water (2 mL). The resulting mixture was stirred at room temperature for 14 h and then passed through a Sephadex™ G-50 column (4 x 30 cm) using water as eluent. A pale yellow fraction was collected, which however did not show any fluorescence characteristic of SG, indicating that SG is not included in the vesicles.

**(6) Fluorescence characteristics of sulforhodamine-G in the presence of a zinc porphyrin reference (compound B, see page S1) and (TEG)PZnC<sub>60</sub>**

To a water/THF (30:70 v/v) solution of sulforhodamine-G was stepwise added a THF solution of **compound B** or (TEG)PZnC<sub>60</sub> (1, 5 and 10 equiv.), and fluorescence emission and excitation spectra were recorded.

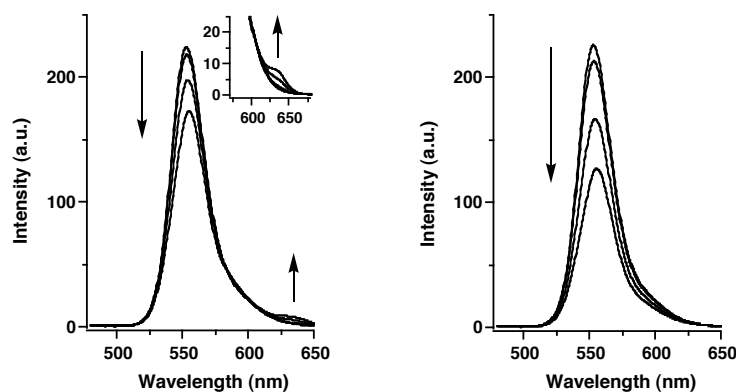


Figure S1. Fluorescence spectral changes of sulforhodamine-G ( $\lambda_{\text{ext}} = 348$  nm,  $abs = 0.016$ ) upon addition of a zinc porphyrin reference (**compound B**; left) and (TEG)PZnC<sub>60</sub> (right) in water/THF (30:70 v/v).

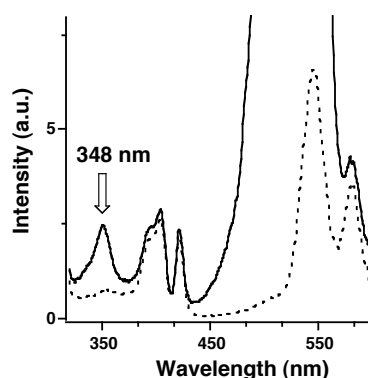


Figure S2. Fluorescence excitation spectra of water/THF (30:70 v/v) solutions of (TEG)PZnC<sub>60</sub> ( $1.1 \times 10^{-5}$  M) in the absence (broken curve) and presence of sulforhodamine-G (0.1 equiv.; solid curve), monitored at 635 nm, an emission band of the zinc porphyrin unit.

### (7) Sulforhodamine-G release experiment upon addition of THF

To an aqueous solution of (TEG)PZnC<sub>60</sub> vesicles containing encapsulated SG (1 mL) was added THF (2 mL) under vigorous stirring.

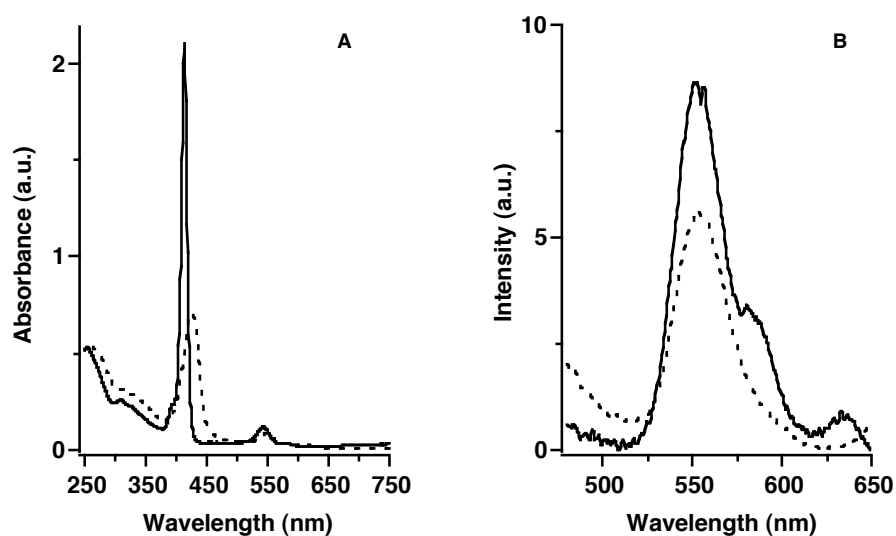


Figure S3. Electronic absorption spectra (A) and fluorescence emission spectra (B) ( $\lambda_{\text{ext}} = 348$  nm) of an aqueous solution of sulforhodamine-G-containing (TEG)PZnC<sub>60</sub> vesicles before (broken curve) and after the addition of THF (solid curve).

### (8) Sulforhodamine-G release experiment upon addition of Triton X-100

To an aqueous solution of **(TEG)PZnC<sub>60</sub>** vesicles was stepwise added an aqueous solution of Triton X-100 (0.5 wt%) under vigorous stirring. Spectroscopic analyses were performed 20 min after every addition. Fluorescence spectra, upon excitation of SG at 348 nm, were recorded.

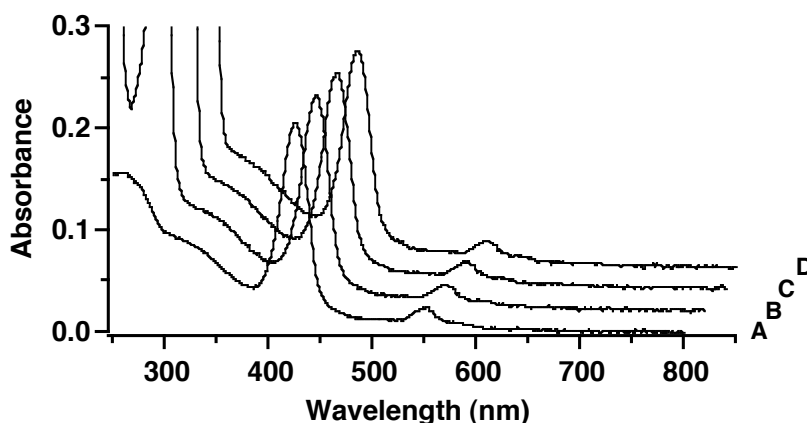


Figure S4. Electronic absorption spectra of an aqueous solution of sulforhodamine-G-containing **(TEG)PZnC<sub>60</sub>** vesicles, before (**A**) and after the addition of 0.1 (**B**), 0.3 (**C**) and 0.5 mL (**D**) of an aqueous solution of Triton X-100 (0.5 wt%).

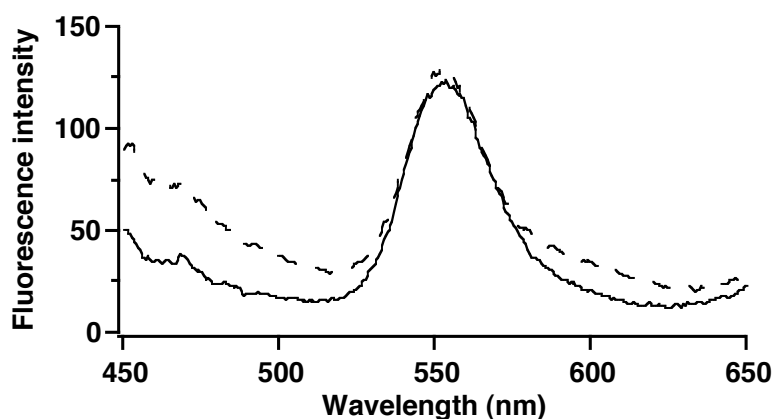


Figure S5. Fluorescence emission spectra of sulforhodamine-G-containing **(TEG)PZnC<sub>60</sub>** vesicles, before (solid curve) and after the addition of 0.5 mL of an aqueous solution of Triton X-100 (0.5 wt%) (broken curve).



## (9) Additional data

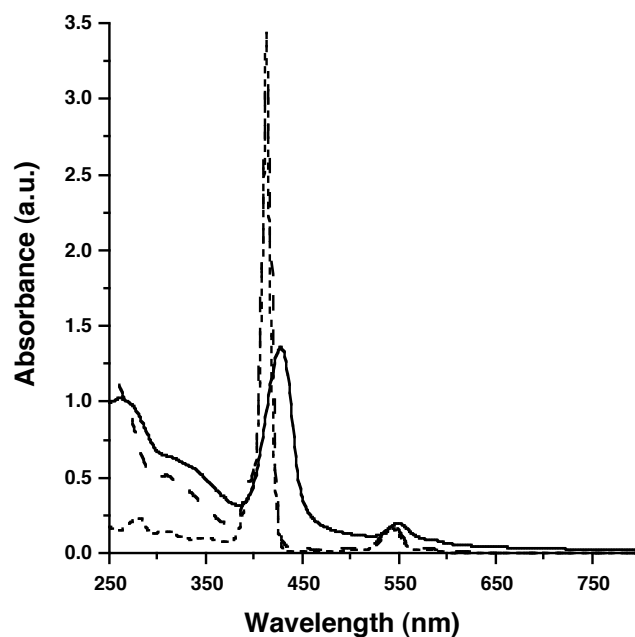


Figure S6. Electronic absorption spectra of **compound B** (see page S1) in THF (---), and **(TEG)PZnC<sub>60</sub>** in THF (— · —) and water (solid curve) ( $5.0 \times 10^{-2}$  mM).

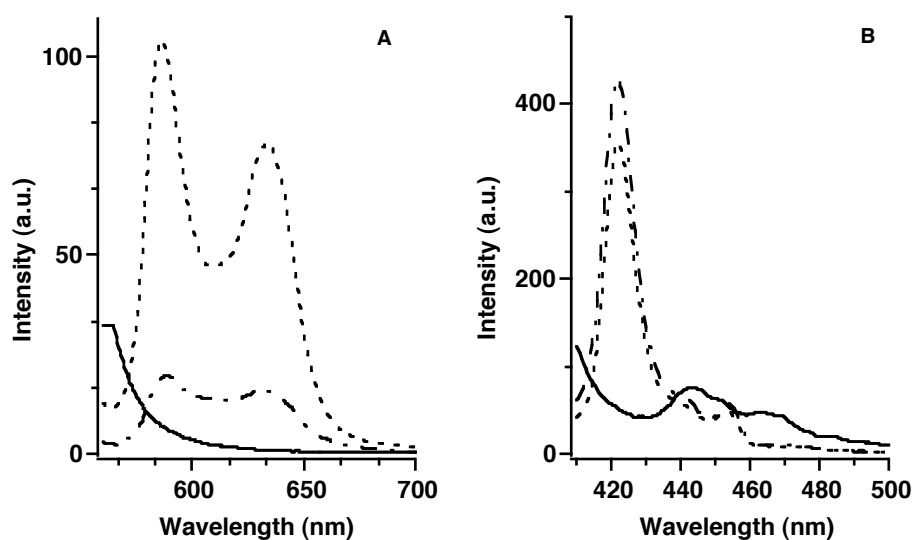


Figure S7.  $S_1$  (A;  $\lambda_{\text{ext}} = 543$  nm,  $abs = 0.02$ ) and  $S_2$  (B;  $\lambda_{\text{ext}} = 400$  nm,  $abs = 0.2$ ) emission spectra of **compound B** in THF (---), and **(TEG)PZnC<sub>60</sub>** in THF (broken curve) and water (solid curve).

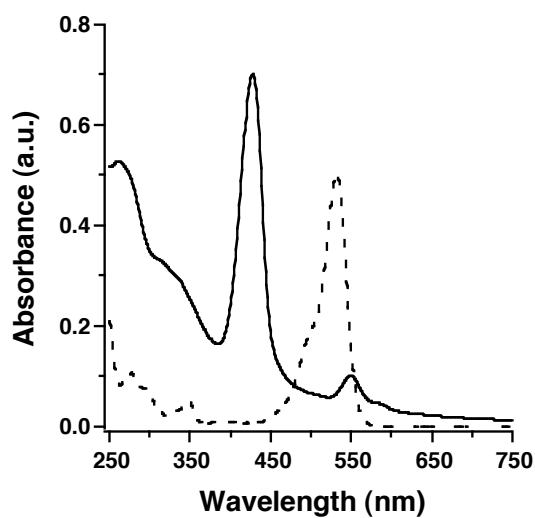


Figure S8. Electronic absorption spectra of aqueous solutions of **(TEG)PZnC<sub>60</sub>** vesicles (solid curve) and sulforhodamine-G (broken curve).

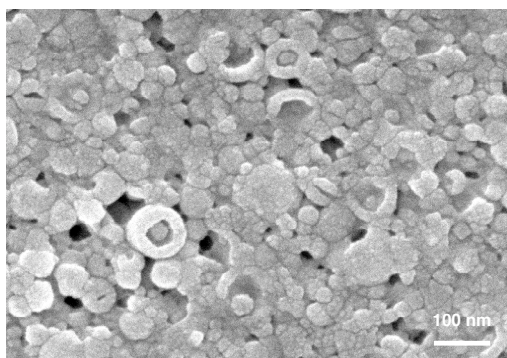


Figure S9. FE-SEM micrograph of **(TEG)PZnC<sub>60</sub>** vesicles prepared in water/THF (85:15 v/v) .

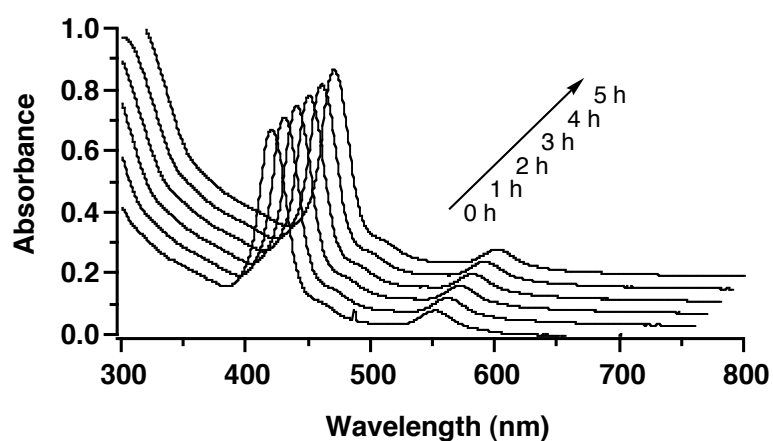


Figure S10. Electronic absorption spectra of an aqueous solution of **(TEG)PZnC<sub>60</sub>** vesicles ( $8.0 \times 10^{-6}$  M) during heating at 70 °C for 5 h.

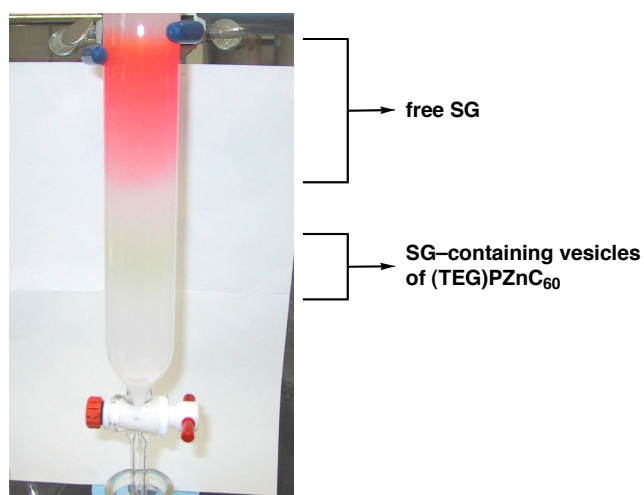


Figure S11. Size-exclusion chromatography on Sephadex™ G-50 of an aqueous solution of **(TEG)PZnC<sub>60</sub>** vesicles, prepared in the presence of sulforhodamine-G (SG). A picture of the chromatographic event using water as eluent.

## **Supporting references**

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- 2 M. J. Hannon, P. C. Mayers and P. C. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1881.
- 3 M. Maggini, G. Scorrano and M. Prato, *J. Am. Chem. Soc.*, 1993, **115**, 9798.