

## Thermo-Responsive Fluorescent Vesicles Assembled by Fluorescein-Functionalized Pillar[5]arene

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## Electronic Supplementary Information

### *General Methods*

Organic reagents were purchased from Aldrich and used without further purifications. All the solvents and inorganic reagents were commercially available. Me<sub>2</sub>CO was dried with anhydrous MgSO<sub>4</sub> before use. 1,4-Dimethoxypillar[5]arene (Dmpillar),<sup>[S1]</sup> alkynated fluorescein (**S2**),<sup>[S2]</sup> and azido-pillar[5]arene (**S3**)<sup>[S3]</sup> were synthesized according to the literature procedures. Dynamic light scattering (DLS) measurements at variable concentrations were carried out with a Zetasizer Nano ZS instrument from Malvern Instruments Ltd. at 298 K using a 633 nm 'red' laser. The mean hydrodynamic size was

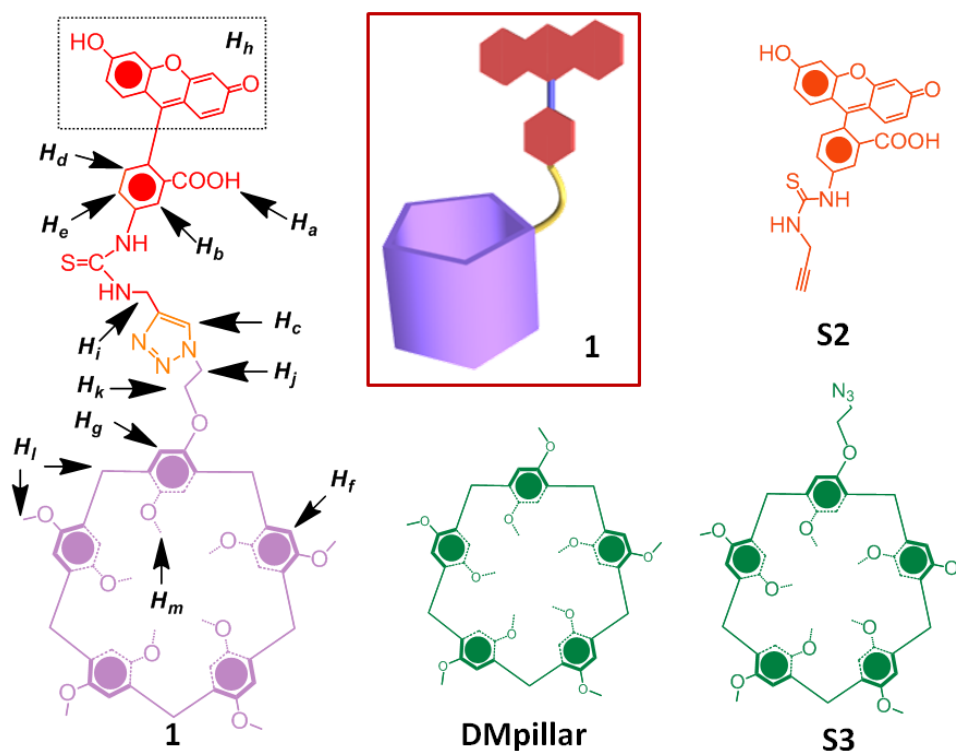
calculated with Zetasizer software. UV-Vis spectra at variable concentrations were recorded with Shimadzu UV-3600 UV-Vis-NIR spectrophotometer at 298 K. The stock solution ( $10^{-4}$  mol·L<sup>-1</sup>) of the compound **1** was prepared with anhydrous Me<sub>2</sub>CO. All the sample solutions for the investigation of the critical assembly concentration were freshly prepared by diluting the stock solution according to literature procedures.<sup>[S4]</sup> The variable temperature emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (Exited at 488 nm).

<sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded at room temperature on Bruker Avance 300 with working frequencies of 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C nuclei. The <sup>1</sup>H COSY NMR and <sup>1</sup>H NOESY NMR spectra were recorded on a BBFO 400 spectrometer. Chemical shifts were reported in ppm relative to the signals corresponding to the residual non-deuterated solvent, and coupling constants were recorded in Hertz (Hz). All <sup>13</sup>C NMR spectra were recorded with simultaneous decoupling of <sup>1</sup>H nuclei. The following abbreviations were used to explain the multiplicities: *s*, singlet; *d*, doublet; *t*, triplet; *b*, broad peaks; *m*, multiplet or overlapping peaks. The electronic spray ionization mass spectra (ESI-MS) were recorded on a ThermoFinnigan LCQ quadrupole ion trap mass spectrometer. The high-resolution MS were performed on a Waters Q-tof Premier MS spectrometer.

Negative-stained TEM images was recorded on a JEM 1400 electron microscope (120 kV) equipped with slow scan CCD and using cold cathode field emission as the gun. The samples for negative-stained TEM were prepared by dropping a droplet of the orange sample solution onto a TEM grid (copper grid, 300 meshes, coated with carbon film), and stained with 1% sodium phosphotungstate in H<sub>2</sub>O (about 2 μL) followed by air-drying. SEM images were obtained on a JSM 6340 scanning electron microscope (0.5-30 kV) equipped with Cold cathode FEG as electron source. Fluorescent microscopy and confocal laser scattering microscopy images were recorded by using Nikon confocal microscope.



### Compounds Synthesized



**Scheme S1** The preparation of compound **1**.

**Synthesis of Compound 1:** Compounds **S2** (44.4 mg, 0.1 mmol), **S3** (80.5 mg, 0.1 mmol), tetrakis(acetonitrile) copper(I) hexafluorophosphate (3.7 mg, 0.01 mmol), and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine (TBTA) were dissolved in Me<sub>2</sub>CO (20 mL). The mixture solution was stirred under reflux for 2 d. The solution was then poured into H<sub>2</sub>O (200 mL). The aqueous phase was extracted (3 × 200 mL) with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were dried (Mg<sub>2</sub>SO<sub>4</sub>) and the solvent was removed in vacuum. The mixture was subjected to silica gel column chromatography (SiO<sub>2</sub>, gradient elution from 5% up to 20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford the compound **1** (112.4 mg) in 90% yield. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ = 9.99 (s, 1H), 8.44 (s, 1H), 8.13 (s, 1H), 7.72 (s, 1H), 7.15 (d, *J* = 6, 1H), 6.83-6.74 (m, 8H), 6.66 (d, 2H), 6.60-6.55 (m, 6H), 5.30 (s, 1H), 5.26 (s, 1H), 4.71 (s, 2H), 4.25 (s, 2H), 3.67 (s, 34H), 3.47 (s, 3H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ = 169.2, 159.9, 152.4, 150.8, 150.4, 149.0, 129.6, 128.0, 127.9, 127.5, 124.8, 115.0, 113.8, 113.0, 110.3, 102.6, 83.4, 68.9, 67.3, 56.3, 55.8, 51.0, 50.1, 32.5, 31.1, 30.0, 29.3. HR-MS (ESI): C<sub>70</sub>H<sub>68</sub>N<sub>5</sub>O<sub>15</sub>S calcd for *m/z* = 1250.4433, found *m/z* = 1250.4453 [*M* + H]<sup>+</sup>.

Figures S1-S10

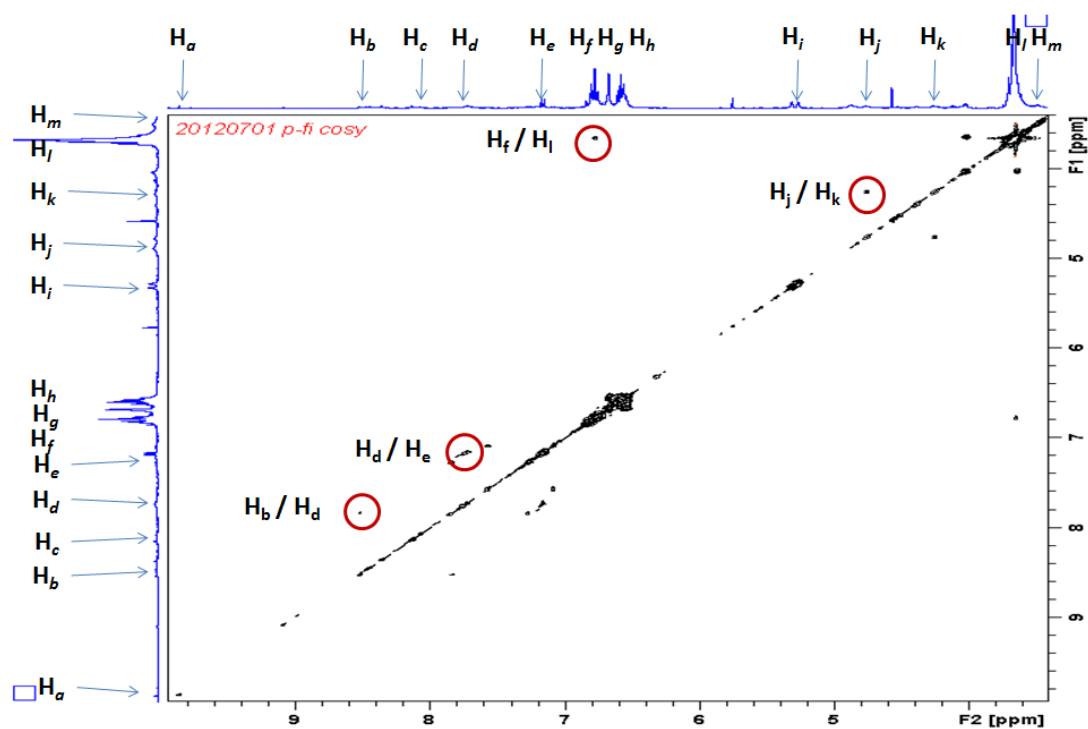


Fig. S1  $^1\text{H}$  COSY NMR spectrum (400 MHz) of compound **1** (0.020 mM) at ambient temperature.

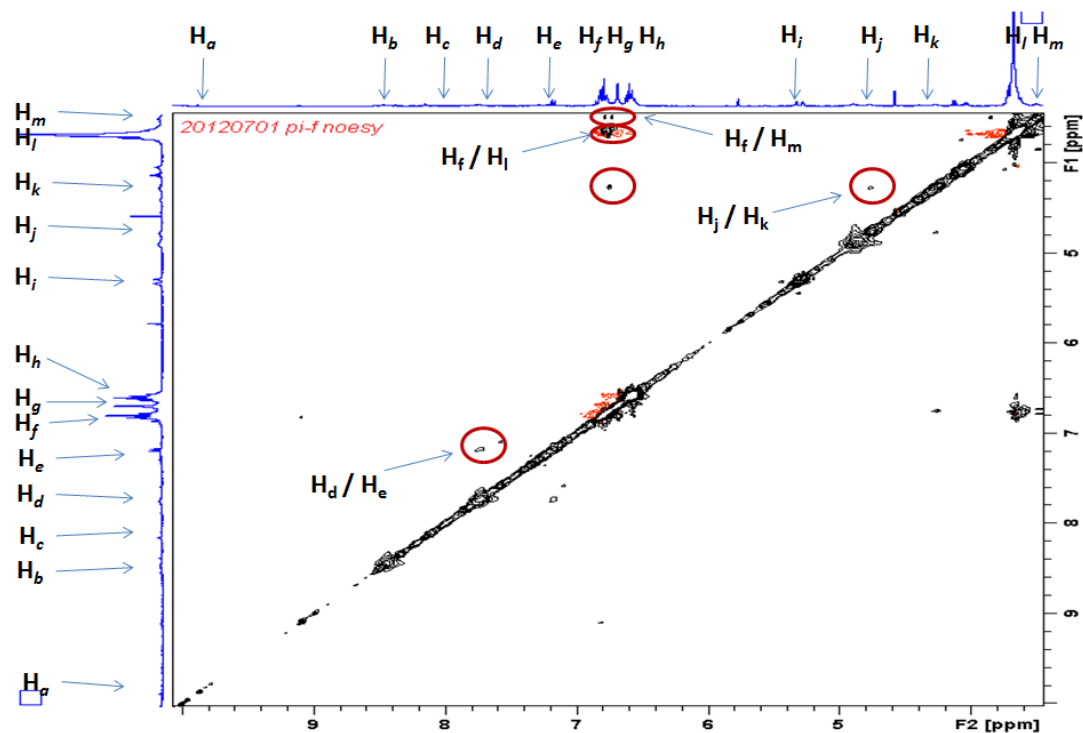
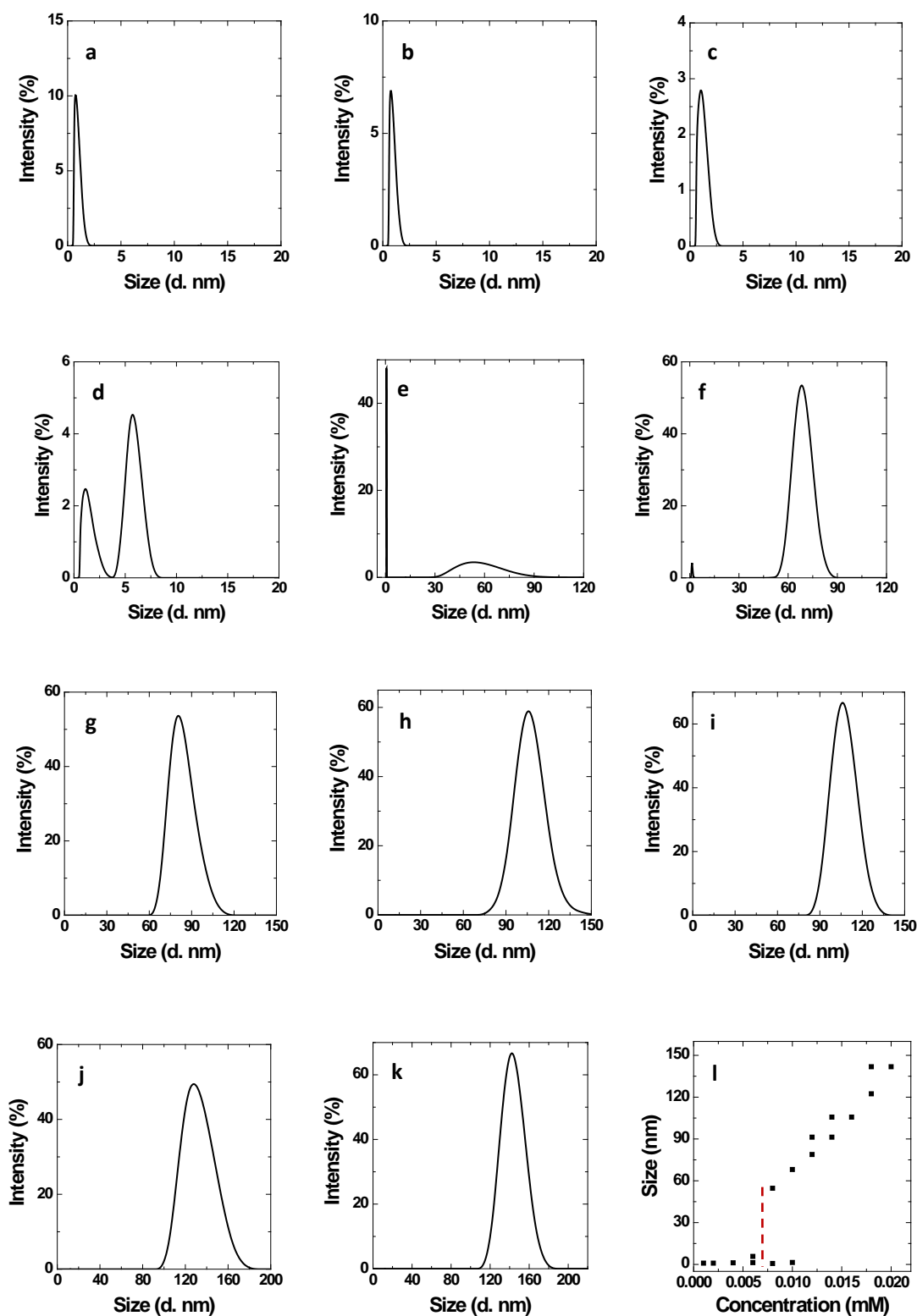
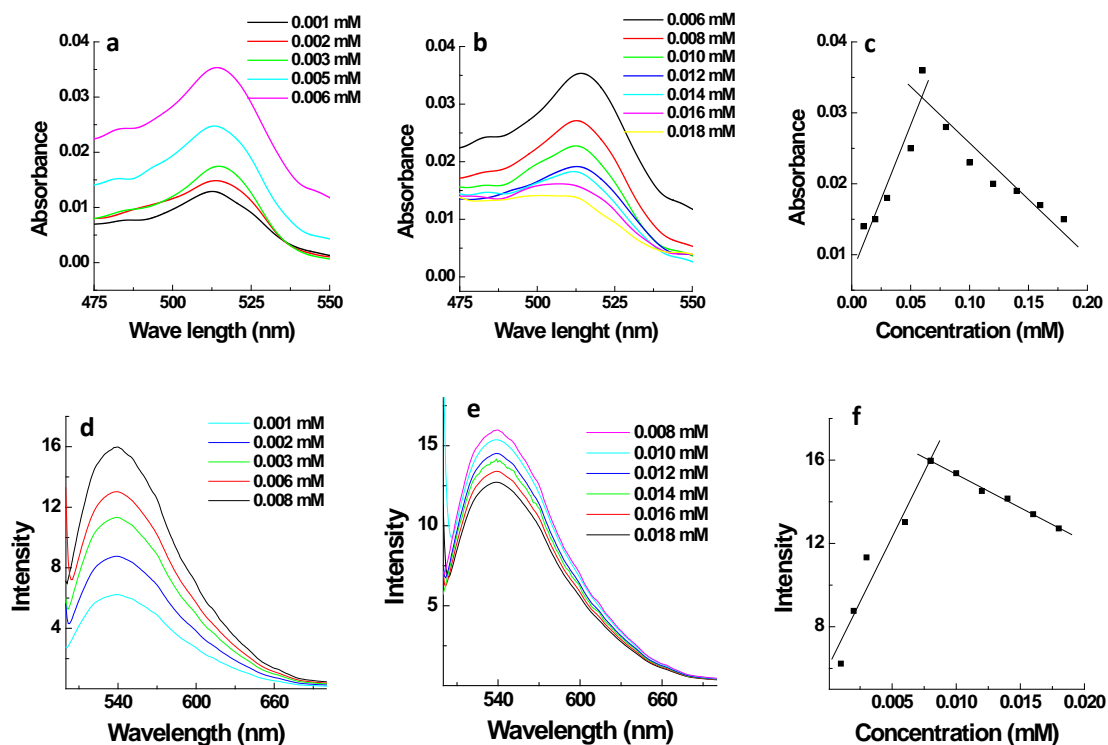


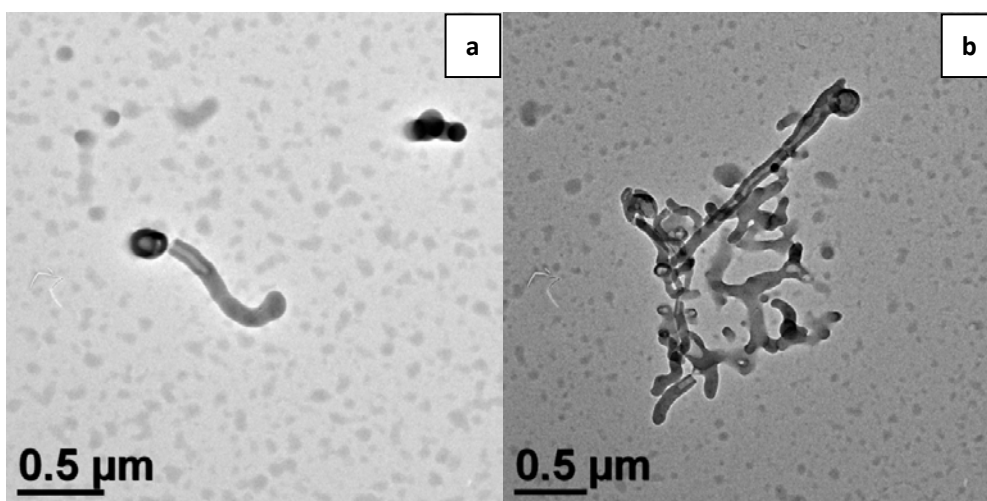
Fig. S2  $^1\text{H}$  NOESY NMR spectrum (400 MHz) of compound **1** (0.020 mM) at ambient temperature.



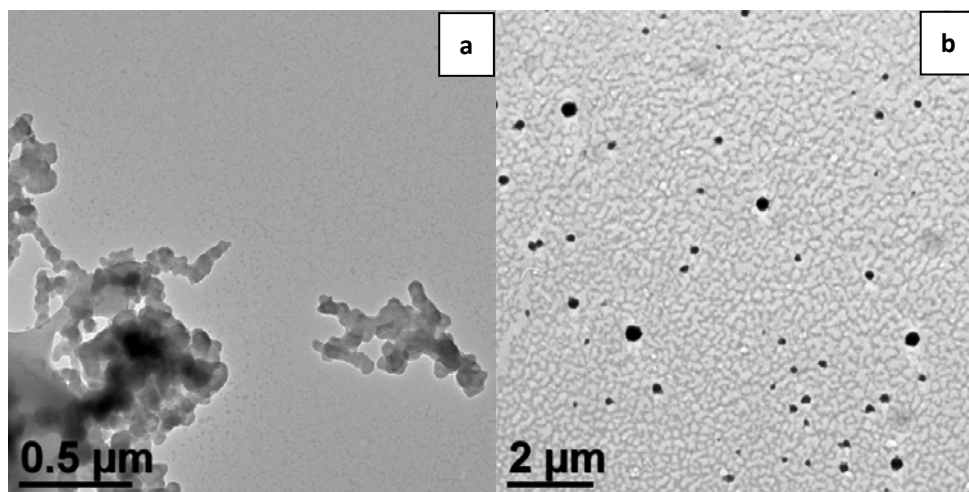
**Fig. S3** DLS size distributions of compound **1** (*a*, 0.001 mM; *b*, 0.002 mM; *c*, 0.004 mM; *d*, 0.006 mM; *e*, 0.008 mM; *f*, 0.010 mM; *g*, 0.012 mM; *h*, 0.014 mM; *i*, 0.016 mM; *j*, 0.018 mM; *k*, 0.020 mM) in Me<sub>2</sub>CO. Mean size changes of the assemblies in Me<sub>2</sub>CO under variable concentrations (figure *l*, from 0.0001 to 0.020 mM) detected by DLS.



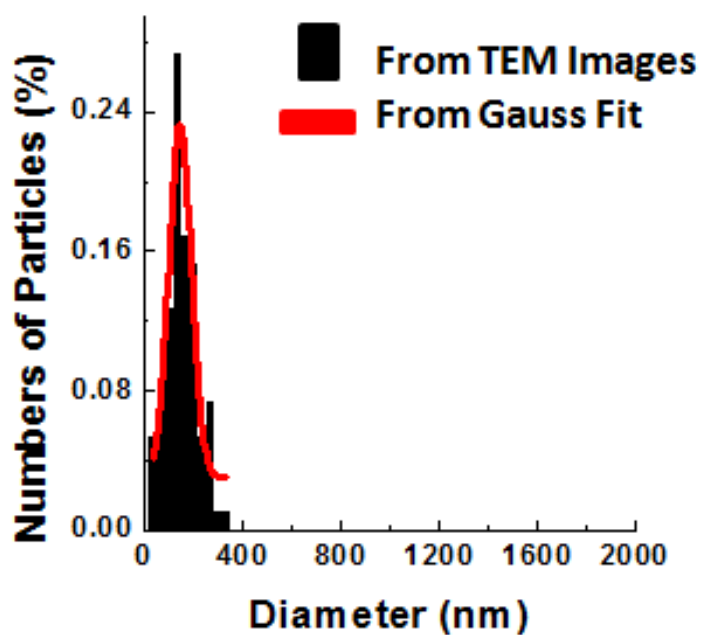
**Fig. S4** The increase (a) and decrease (b) for the UV-Vis absorption of compound **1** in  $\text{Me}_2\text{CO}$  under variable concentrations. Critical assembly concentration (CAC) of compound **1** in  $\text{Me}_2\text{CO}$  was calculated by detecting the maximum absorption band in the UV-Vis spectra (c). The intensity changes of fluorescence upon various concentrations (d, e). The CAC of compound **1** in  $\text{Me}_2\text{CO}$  was calculated by detecting the maximum emission band in the fluorescence measurements (f).



**Fig. S5** Micro-morphologies of compound **1** in saturated  $\text{MeOH}$  by negative stained TEM: a few hollow spheres (a) and irregular blocks (b).

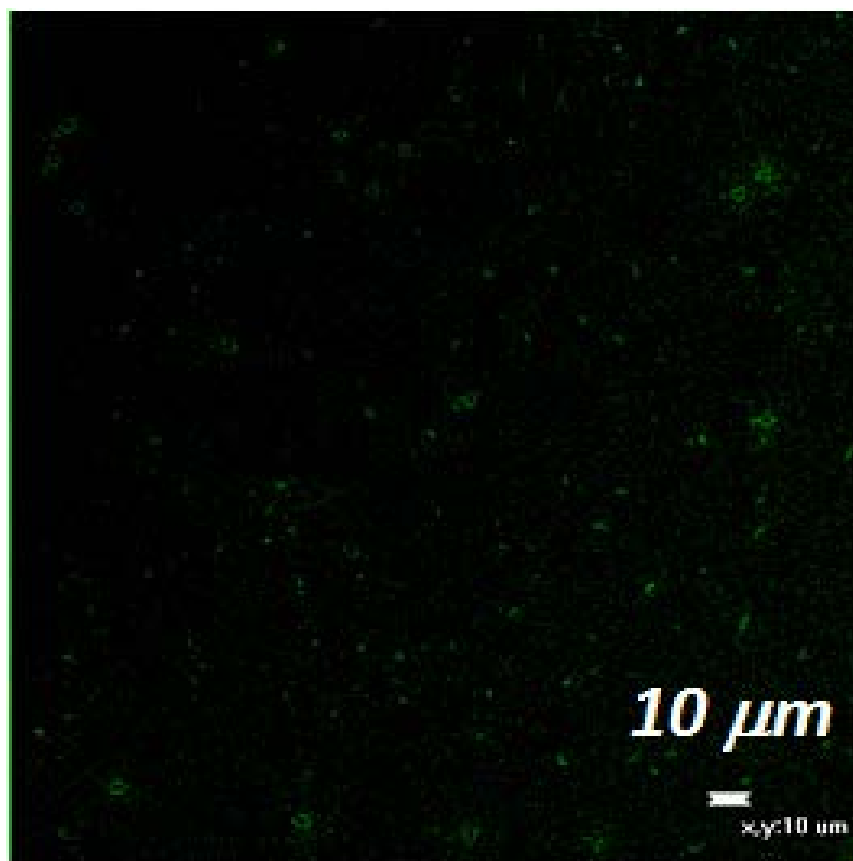


**Fig. S6** Micro-morphologies of compound **1** in saturated MeCN by negative stained TEM: a few irregular shapes (a) and solid particles (b).

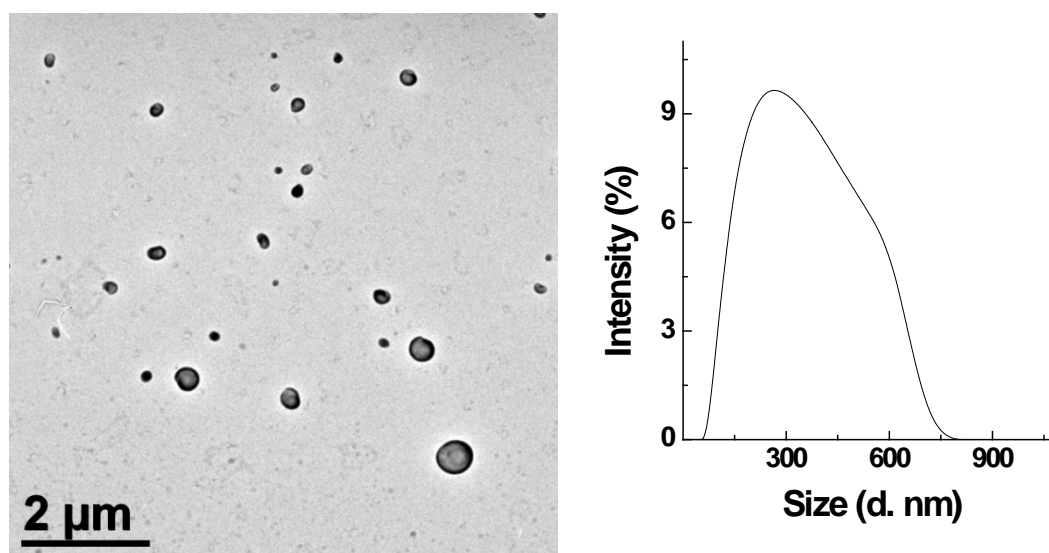


**Fig. S7** Size distributions of the vesicles assembled from compound **1** in Me<sub>2</sub>CO (0.020 mM) at room temperature, determined by TEM images.

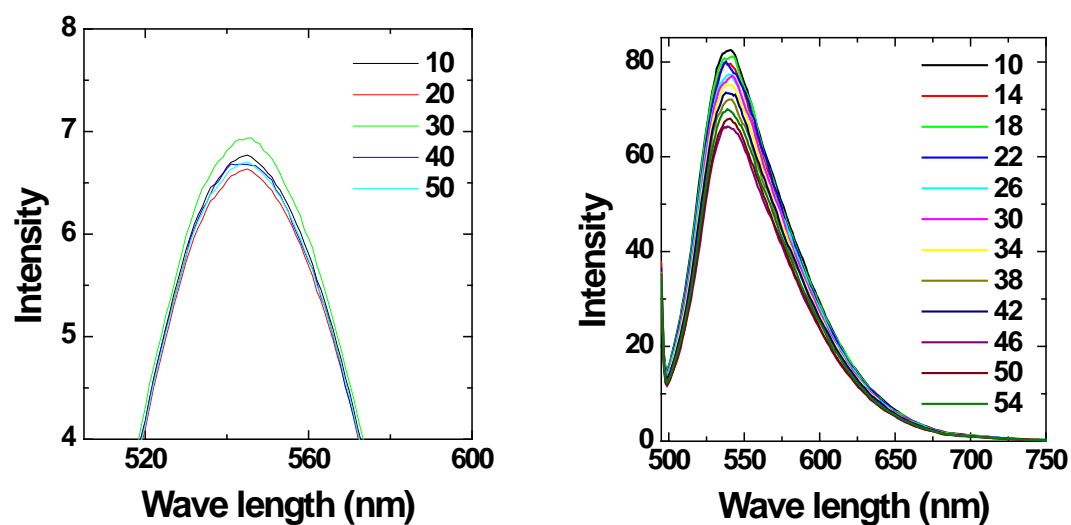




**Fig. S8** Confocal laser scanning microscopy image of the vesicles assembled from compound **1** in Me<sub>2</sub>CO (0.020 mM) at room temperature, scale bar = 10 μm.



**Fig. S9** Negative stained TEM image (left) of compound **1** in Me<sub>2</sub>CO (0.020 mM) after heating the sample to 35 °C in a sealed vial. DLS size distributions (right) of compound **1** (0.020 mM) in Me<sub>2</sub>CO at 35 °C in a sealed vial.



**Fig. S10** Variable temperature emission spectra (excited at 488 nm) of compound **1** in dilute Me<sub>2</sub>CO (left, 0.001 mM, 10-50 °C) and compound **S2** in saturated Me<sub>2</sub>CO (right, 10-54 °C).

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### References

- [S1] T. Ogoshi, S. Kanai, S. Fujinami, T. Yamagishi and Y. Nakamoto, *J. Am. Chem. Soc.*, 2008, **130**, 5022.
- [S2] (a) M. Ikeda, J. Minari, N. Shimada, M. Numata, K. Sakurai and S. Shinkai, *Org. Biomol. Chem.*, 2007, **5**, 2219; (b) P. Manikwar, T. Zimmerman, F. J. Blanco, T. D. Williams and T. J. Siahhan, *Bioconjugate Chem.*, 2011, **22**, 1330; (c) L. Albertazzi, M. Fernandez-Villamarin, R. Riguera and E. Fernandez-Megia, *Bioconjugate Chem.*, 2012, **23**, 1059.
- [S3] N. L. Strutt, R. S. Forgan, J. S. Spruell, Y. Y. Botros and J. F. Stoddart, *J. Am. Chem. Soc.*, 2011, **133**, 5668.
- [S4] (a) P. Mukerjee, M. J. Gumkowski, C. C. Chan and R. Sharma, *J. Phys. Chem.*, 1990, **94**, 8832; (b) B. W. Liu, Y. Chen, B. E. Song and Y. Liu, *Chem. Commun.*, 2011, **47**, 441.