

# **AIE-Induced Fluorescent Vesicles Containing Amphiphilic Binding Pockets and the FRET Triggered by Host-Guest Chemistry**

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## Experimental section

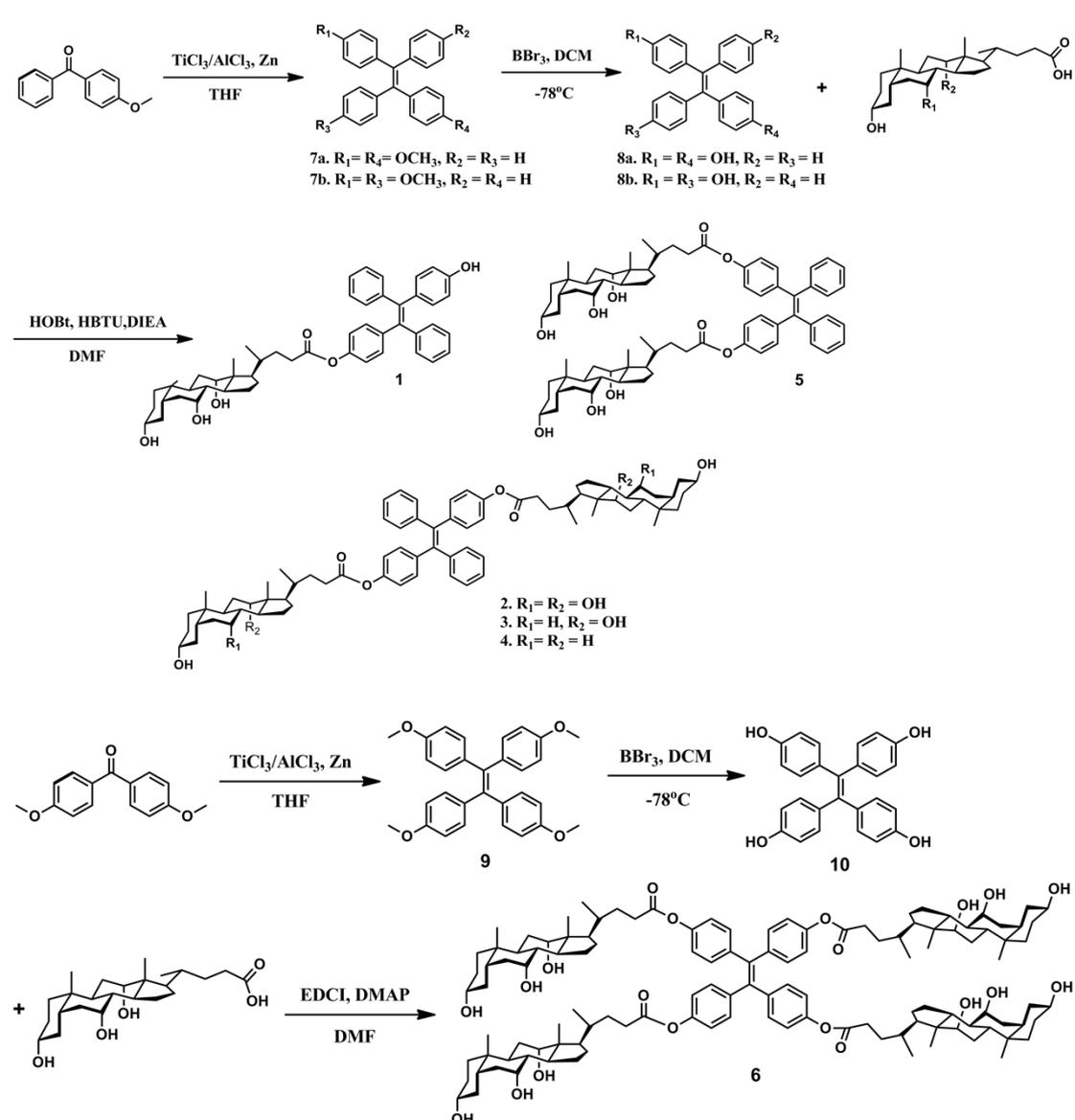
### Materials and Reagents

Cholic acid (CA, 98%), deoxycholic acid (DCA, 98%), lithocholic acid (LCA, 98%), and other chemicals were purchased from Alfa and Acros and used as received without further purification. All the solvents (analytical pure) were purchased from Beijing Chemical Company and purified by standard methods before use.

### Physical Measurements and Instrumentation

$^1\text{H}$  and  $^{13}\text{C}$  NMR were recorded on a JJEOL ECA300 NMR spectrometer. Electrospray ionization mass spectrometry (ESIMS) was measured on a Bruker ESQUIRE-LC spectrometer. TEM images were obtained using JEM 2010 high-resolution transmission electronic microscope at an acceleration voltage of 120 kV. SEM images were obtained using JSM 7401 high-resolution scanning electronic microscope. UV-Vis spectra were collected on PerkinElmer Lambda35 spectrometer. The fluorescence measurements were carried out using a fluorescence spectrometer (Perkin-Elmer, LS55). SAXS and WAXS experiments were performed at the beamline 1W2A of the Beijing Synchrotron Radiation Facility (BSRF) ( $\lambda=1.54\text{\AA}$ ). A Rayonix Mar-165 CCD detector was used to collect the X-ray data.

Scheme S1: Syntheses of TPE-bile acid conjugates 1-6



## Synthesis

### Synthesis of Tetrakis(2-methoxyphenyl)ethane **7a** and **7b**

A suspension of p-methoxybenzophenone (1.06g, 5.0mmol), 1.34 equiv of  $\text{TiCl}_3/\text{AlCl}_3$  (5.81g, 6.7mmol), and 25 equiv of Zn dust (8.01g, 122.0mmol) in 100mL of dry THF was refluxed for 20 h. The reaction mixture was cooled to room temperature and then filtered. The filtrates were evaporated, and the crude product was separated by automatic high-pressure preparation and purification system (HP Compact) using hexane:  $\text{CH}_2\text{Cl}_2$  as eluent. Product **7a** was isolated in 45% yield (0.45g).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.11-7.06 (m, 10H), 6.93 (t, 4H), 6.64 (t, 4H), 3.74 (s, 6H). MS (TOF)  $m/e$ : 392.2 ( $\text{M}^+$ , calcd 392.1). Single crystal file no.: CCDC 622769. <sup>[1]</sup> Product **7b** was isolated in 45% yield (0.45g).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.11-6.99 (m, 10H), 6.94 (t, 2H), 6.63 (d, 4H), 3.71 (s, 6H). MS (TOF)  $m/e$ : 392.2 ( $\text{M}^+$ , calcd 392.1).

### Synthesis of Tetrakis(2-hydroxyphenyl)ethene **8a**

Into a 100mL flask were added **7a** (1.40g, 3.56mmol) and 20mL of dichloromethane (DCM). The flask was placed in an acetone-dry ice bath at  $-78^\circ\text{C}$ . A solution of boron tribromide (3.59g, 14.3mmol) in 10mL of DCM was added carefully to the mixture under stirring. The resultant mixture was allowed to warm to room temperature and stirred overnight. The reaction product was hydrolyzed by careful shaking with 20mL of water. The organic phase was separated and concentrated by a rotary evaporator. The crude product was purified by recrystallization from THF/methanol to afford a pale-yellow powders **8a** (1.26 g, 97% yield).  $^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$  (TMS, ppm): 9.30 (d, 2H), 7.10-6.92 (m, 10H), 6.73 (t, 4H), 6.50 (d, 4H). MS (TOF)  $m/e$ : 363.1 [ $(\text{M} - \text{H})^+$ , calcd 363.1].

### Synthesis of Tetrakis(2-hydroxyphenyl)ethene **8b**

This compound was synthesized using the same procedure as described for **8a**.

Product **8b** (97 % yield).  $^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$  (TMS, ppm): 7.12-7.02 (m, 10H), 6.88 (t, 4H), 6.56 (t, 4H). MS (TOF)  $m/e$ : 363.1 [ $(\text{M} - \text{H})^+$ , calcd 363.1].

### Synthesis of **2**

To a solution of **8a** (50mg, 0.14mmol) in DMF 20mL was added cholic acid (139mg, 0.34mmol), HOBt (46mg, 0.34mmol), HBTU (130mg, 0.34mmol) and DIEA 0.1mL. The mixture was stirred at room temperature overnight. The solution was evaporated under vacuum. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  and washed with distilled water, brine, dried with  $\text{MgSO}_4$ , purified by silica gel column chromatography using  $\text{CH}_2\text{Cl}_2$ :MeOH=20:1. Product **2** (68 % yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.10 (m, 6H), 7.04-6.98 (m, 8H), 6.83-6.80 (m, 4H), 3.99 (s, 2H, 12-CH), 3.86 (s, 2H, 7-CH), 3.46 (m, 2H, 3-CH), 2.57-1.12 (m, 48H, steroidal skeleton H), 1.01 (d, 6H, 21- $\text{CH}_3$ ), 0.86 (s, 6H, 19- $\text{CH}_3$ ), 0.68 (s, 6H, 18- $\text{CH}_3$ ); MS (TOF)  $m/e$ : 1181.9 [ $(\text{M} + \text{Cl})^-$ , calcd 1181.7].

### Synthesis of **1**

This compound was synthesized using the same procedure as described for **2**.

Product **1** (78 % yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.10-6.97 (m, 12H), 6.89-6.78 (m, 4H), 6.59-6.50 (m, 2H), 4.00 (s, 1H, 12-CH), 3.86 (s, 1H, 7-CH), 3.46 (m, 1H, 3-CH), 2.56-1.12 (m, 24H, steroidal skeleton H), 1.01 (d, 3H, 21- $\text{CH}_3$ ), 0.88 (s, 3H, 19- $\text{CH}_3$ ), 0.69 (s, 3H, 18- $\text{CH}_3$ ); MS (TOF)  $m/e$ : 754.9 [ $\text{M}$ , calcd 754.4].

### Synthesis of **3**

This compound was synthesized using the same procedure as described for **2**.

Product **3** (72 % yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.12-7.08 (m, 6H), 7.04-6.98 (m, 8H), 6.85-6.80 (m, 4H), 3.99 (s, 2H, 12-CH), 3.63 (m, 2H, 3-CH), 2.61-1.00 (m, 50H, steroidal skeleton H), 0.98 (d, 6H, 21- $\text{CH}_3$ ), 0.91 (s, 6H, 19- $\text{CH}_3$ ), 0.68 (s, 6H, 18- $\text{CH}_3$ ); MS (TOF)  $m/e$ : 1137.0 [ $(\text{M} + \text{Na})^+$ , calcd 1136.5].

### Synthesis of **4**

This compound was synthesized using the same procedure as described for **2**.

Product **4** (78 % yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz),  $\delta$  (TMS, ppm): 7.12-7.07 (m, 6H), 7.04-6.98 (m, 8H), 6.85-6.80 (m, 4H), 3.64 (m, 2H, 3-CH), 2.59-1.02 (m, 52H, steroidal skeleton H), 0.96 (d, 6H, 21- $\text{CH}_3$ ), 0.92 (s, 6H, 19- $\text{CH}_3$ ), 0.65 (s, 6H, 18- $\text{CH}_3$ ); MS (TOF)  $m/e$ : 1116.7 [ $(\text{M} + \text{Cl})^-$ , calcd 1116.5].

## Synthesis of 5

This compound was synthesized using the same procedure as described for **2**.

Product **5** (70% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz),  $\delta$  (TMS, ppm): 7.09-7.08 (m, 6H), 7.03-6.98 (m, 8H), 6.82-6.80 (m, 4H), 3.99 (s, 2H, 12-CH), 3.86 (s, 2H, 7-CH), 3.46 (m, 2H, 3-CH), 2.55-1.11 (m, 48H, steroidal skeleton H), 1.01 (d, 6H, 21-CH<sub>3</sub>), 0.88 (s, 6H, 19-CH<sub>3</sub>), 0.69 (s, 6H, 18-CH<sub>3</sub>); MS (TOF) *m/e*: 1168.6 [(M + Na)<sup>+</sup>, calcd 1167.7].

## Synthesis of Tetrakis(4-methoxyphenyl)ethene 9

To a solution of 4,4'-dimethoxybenzophenone (5.00g, 20.6mmol) and zinc powder (6.66 g, 10.7 mmol) in THF 100mL was added dropwise TiCl<sub>4</sub> (7.50mL, 68.4mmol). The mixture was refluxed for 15 h. After cooling to room temperature the mixture was hydrolyzed by addition of H<sub>2</sub>O 100mL. The aqueous layer was extracted with CHCl<sub>3</sub> (3 × 100mL). The combined organic layer was dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> /hexane = 1/1) and further by recrystallization from CH<sub>2</sub>Cl<sub>2</sub>/hexane (2/1) to give **9** as a colorless crystal (3.43 g, 74%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz),  $\delta$  (TMS, ppm): 6.93 (d, 8H), 6.63 (d, 8H), 3.75 (s, 12H). MS (TOF) *m/e*: 452.3 [(M)<sup>+</sup>, calcd 452.5].

## Synthesis of Tetrakis(4-hydroxyphenyl)ethene 10

To a solution of **9** (6.01 g, 13.3mmol) in CH<sub>2</sub>Cl<sub>2</sub> 100mL cooled with an ice-salt bath was added dropwise a 2M CH<sub>2</sub>Cl<sub>2</sub> solution of BBr<sub>3</sub> (37.2mL, 74.4mmol). After removal of the cooling bath, the resulting deep red solution was stirred at room temperature for 12 h and then hydrolyzed by dropwise addition of H<sub>2</sub>O 50mL. The precipitate was collected by filtration and washed with H<sub>2</sub>O. Recrystallization from acetone/H<sub>2</sub>O (1/1) afforded **10** as a colorless crystal (5.26 g, 92%). <sup>1</sup>H NMR (DMSO, 300 MHz),  $\delta$  (TMS, ppm): 9.22 (s, 4H, OH), 6.70 (d, 8H), 6.47 (d, 8H). MS (TOF) *m/e*: 395.5 [(M-1)<sup>-</sup>, calcd 395.4].

## Synthesis of 6

To a solution of **10** (65mg, 0.16mmol) in DMF 20mL was added cholic acid (1.07g, 2.62mmol), EDCI (502mg, 2.62mmol) and DMAP (5.8mg, 0.26mmol). The mixture was stirred at room temperature overnight. The solution was evaporated under vacuum. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and washed with distilled water, brine, dried with MgSO<sub>4</sub>, purified by silica gel column chromatography using CH<sub>2</sub>Cl<sub>2</sub>:MeOH=10:1. Product **6** (28 % yield). <sup>1</sup>H NMR (MeOD:CDCl<sub>3</sub>=2:1, 300 MHz),  $\delta$  (TMS, ppm): 7.05 (d, 8H), 6.82 (d, 8H), 3.97 (s, 4H, 12-CH), 3.82 (s, 4H, 7-CH), 3.34 (m, 4H, 3-CH), 2.65-1.13 (m, 96H, steroidal skeleton H), 1.00 (d, 12H, 21-CH<sub>3</sub>), 0.86 (s, 12H, 19-CH<sub>3</sub>), 0.71 (s, 12H, 18-CH<sub>3</sub>); MS (TOF) *m/e*: 1995.5[(M + Cl)<sup>-</sup>, calcd1995.3].

## Methods

### Procedure of AIE measurement

For the AIE measurement, a stock solution of **1-5** in acetone (0.1mmol) and **6** in methanol (0.1mmol) were prepared. Aliquots of this stock solution were transferred into volumetric flasks (10mL), into which appropriate volumes of acetone and water or methanol and water were added dropwise under vigorous stirring to give 10 $\mu$ M solutions with different water contents (fw = 0–95 vol %). PL spectra were measured immediately after the solutions were prepared.

### Procedure of scanning electron microscope

For the observation of aggregates, a drop of sample suspension was placed on grid and dried by standing at room temperature.

### Procedure of transmission electron microscopy

For the observation of aggregates, a drop of sample suspension was placed on 400-mesh formvar copper grids coated with carbon. About 2 min after the deposition, the grid was tapped with filter paper to remove surface water.

## Preparation of unloaded and loaded vesicles

Compound **2** (2.8mg,  $2.45 \times 10^{-3}$  mmol) was dissolved in acetone (2.5mL). A portion of the above acetone solution (0.35mL) was diluted to 2.10mL with acetone. Deionized water (1.4mL) was added dropwise into the diluted acetone solution to induce the unloaded vesicle formation. Compound **5** (2.8mg,  $2.45 \times 10^{-3}$  mmol) was dissolved in acetone (2.5mL). A portion of the above acetone solution (0.35mL) was diluted to 1.75mL with acetone. Deionized water (1.75mL) was added dropwise into the diluted acetone solution to induce the unloaded vesicle formation. Then the vesicle suspension was placed in a dialysis bag (molecular weight cutoff =8000), dialysed against deionized water for 24 hours to remove acetone. The deionized water was refreshed every 2h. The final vesicle samples were diluted to 25ml with deionized water for fluorescence measurements.

Compound **2** (2.8mg,  $2.45 \times 10^{-3}$  mmol) and 0.5 $\mu$ equ Nap were dissolved in acetone (2.5mL). A portion of the above acetone solution (0.35mL) was diluted to 2.10mL with acetone. Deionized water (1.4mL) was added dropwise into the diluted acetone solution to induce the loaded vesicle formation. Then the vesicle suspension was placed in a dialysis bag (molecular weight cutoff =8000), dialysed against deionized water to remove acetone and free donor molecules. The deionized water was refreshed several times until no ultraviolet signals of donor chromophores were detected in the exchanged water. The final vesicle samples were diluted to 25ml with deionized water for fluorescence measurements.

## Procedure of ThT and GFP fluorescence measurement

To carry out the ThT fluorescence measurement, 3mL vesicular **V2** aqueous solution was transferred into a glass cuvette. 100 $\mu$ L ThT solution ( $1.0 \times 10^{-4}$  mol/L in water) was added into glass cuvette. After 6h incubation, the fluorescence of the solution was measured. To carry out the GFP fluorescence measurement, 3mL vesicular **V2** aqueous solution was transferred into a glass cuvette. 50 $\mu$ L GFP solution ( $1.0 \times 10^{-3}$  mol/L in methanol) was added into glass cuvette. After 30 minutes incubation, the fluorescence of the solution was measured.

## Procedure of FRET measurement

To carry out the FRET measurement, 2.5mL vesicle aqueous solution was transferred into a glass cuvette. An aliquot of 50 $\mu$ L dye solution ( $1.0 \times 10^{-4}$  mol/L in methanol) was added into glass cuvette. After 3 minutes incubation, the fluorescence of the solution was measured.

The FRET efficiencies were calculated using the donor emission intensities in the presence and the absence of the acceptor molecules using following equation. FRET efficiency (E) =  $[1 - (I_{DA}/I_D)] * 100\%$  where  $I_{DA}$  and  $I_D$  are donor emission intensities in the presence and the absence of the acceptor molecules.

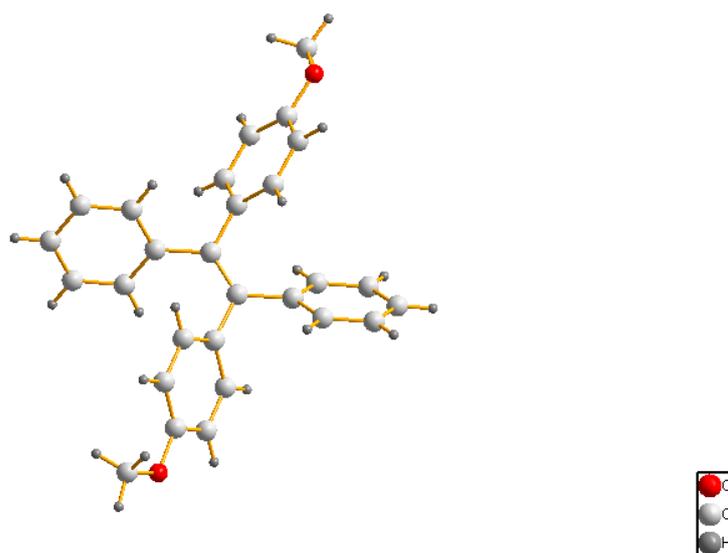


Figure S1 .X-ray crystal structures of **7a**

Figure S2. <sup>1</sup>H NMR spectra of 7a (solid line) and 7b (dash line)

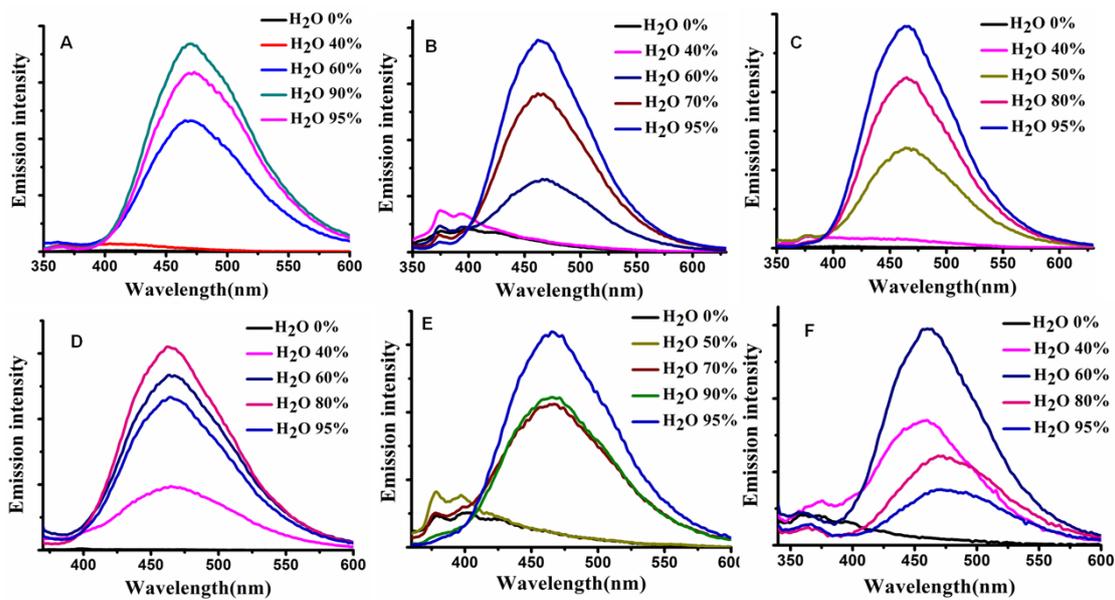
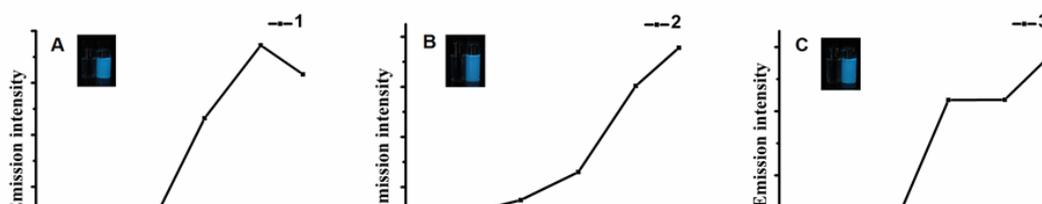
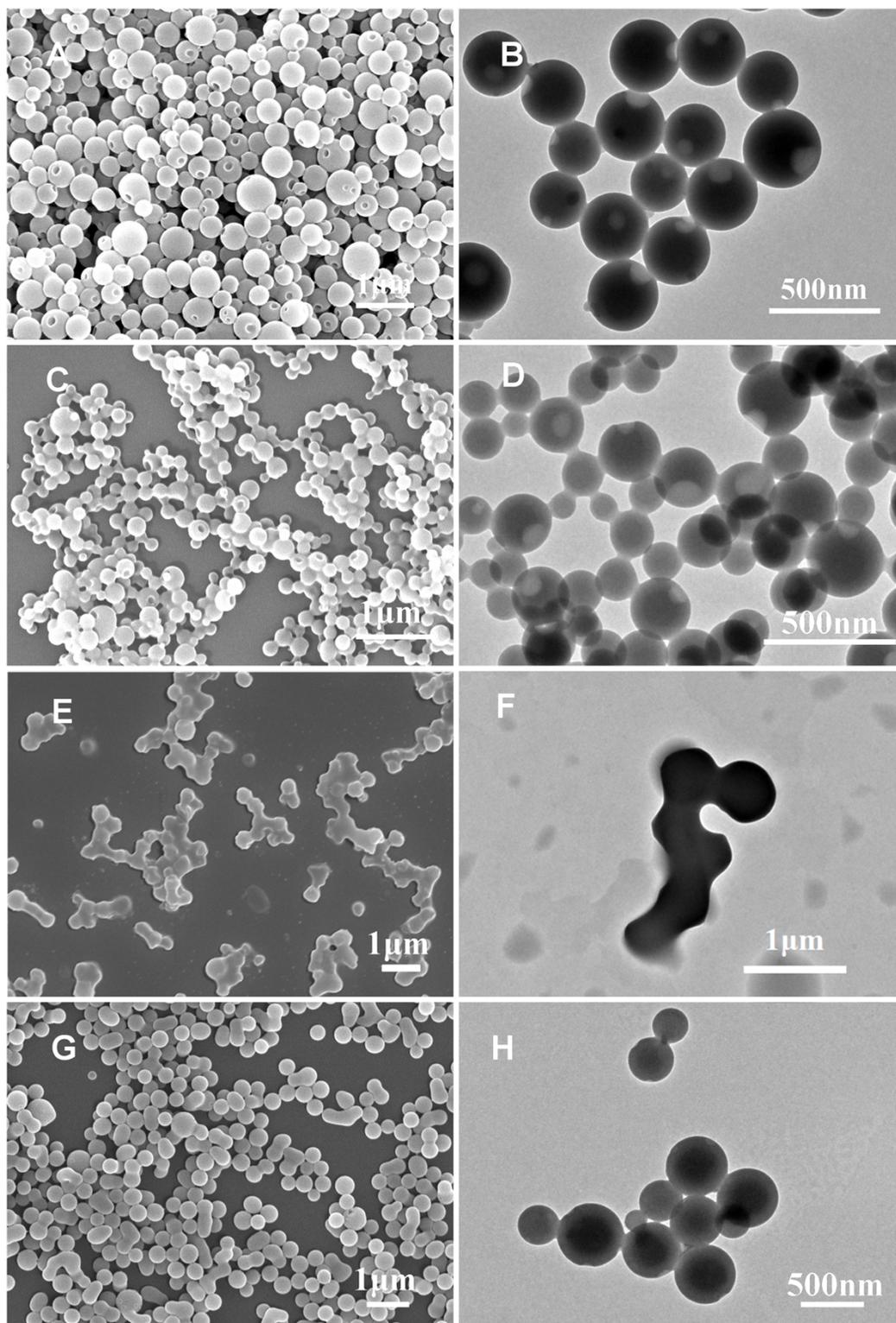


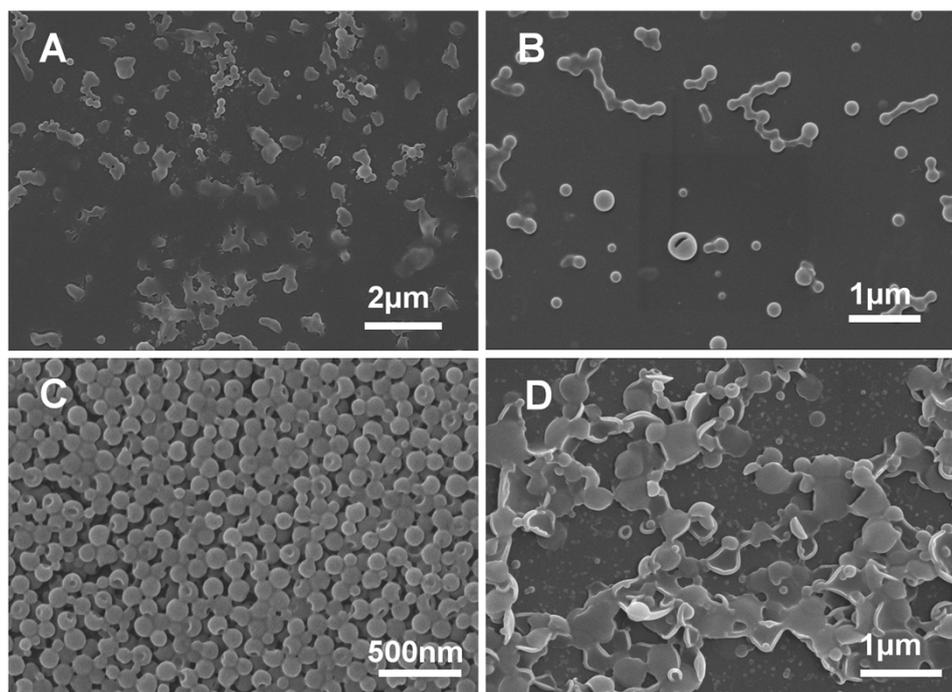
Figure S3. PL spectra of 1 (A), 2 (B), 3 (C), 4 (D), 5 (E) in the acetone /water mixtures and 6 (F) in the methanol /water mixtures with different fractions of water (fw),  $\lambda_{ex} = 325$  nm. Concentration in the solvent mixtures: 10  $\mu$ M.



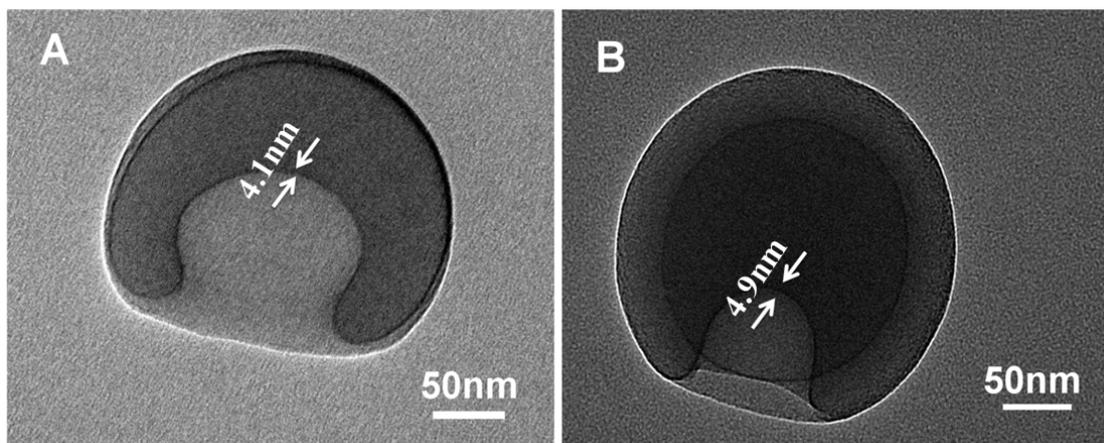
**Figure S4.** Plots of fluorescence quantum yields of **1** (A), **2** (B), **3** (C), **4** (D), **5** (E) vs solvent compositions of acetone /water mixtures and **6** (F) vs solvent compositions of methanol /water mixtures. Concentration in the solvent mixtures: 10 $\mu$ M.  $\lambda_{\text{ex}}$  = 325 nm. Photographs are compounds in water/acetone or water/methanol 90% mixtures under illumination of a handheld UV lam.



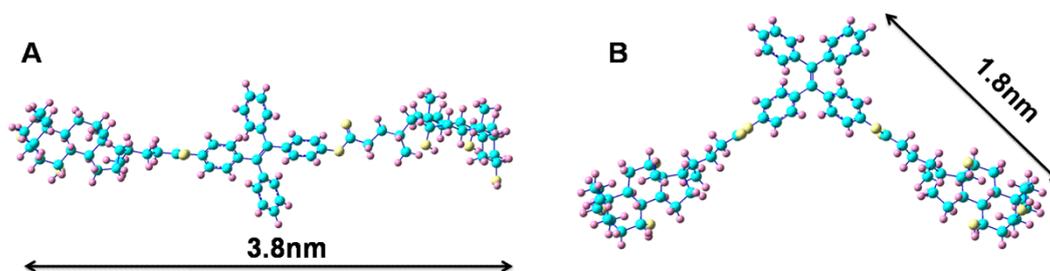
**Figure S5.** SEM microphotographs of the self-organized structures of (A) **1** at fw = 50%, (C) **3** at fw = 40%, (E) **4** at fw = 30% formed in the water /acetone mixtures, (G) **6** at fw = 30% formed in the water /methanol mixtures. TEM microphotographs of the self-organized structures of (B) **1** at fw = 50%, (D) **3** at fw = 40%, (F) **4** at fw = 30% formed in the water /acetone mixtures, (H) **6** at fw = 30% formed in the water /methanol mixtures.



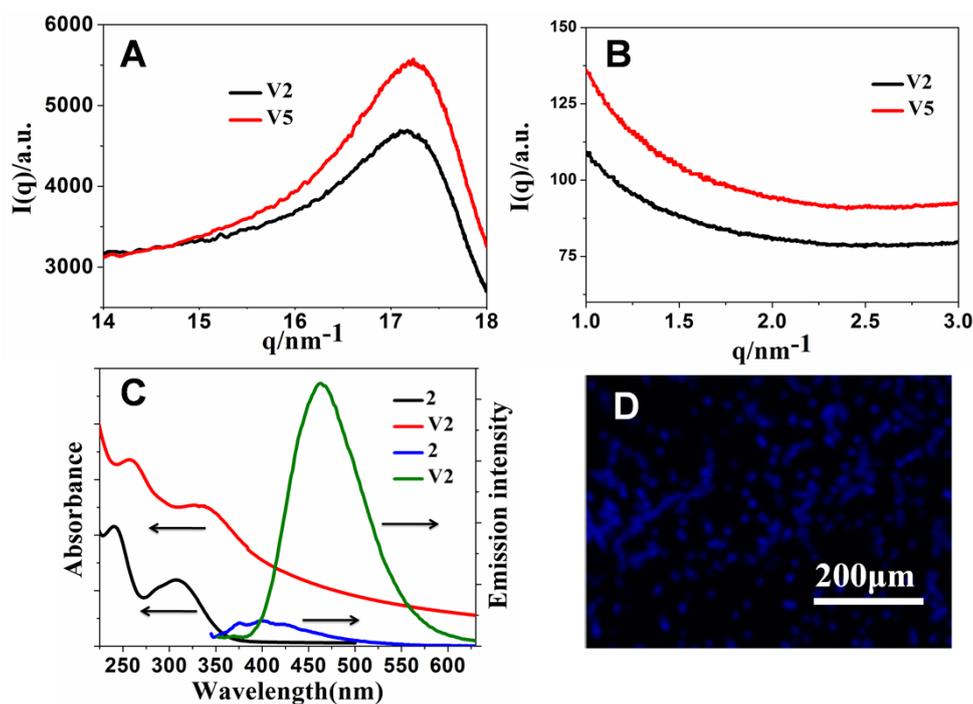
**Figure S6.** SEM microphotographs of the self-organized structures of **5** formed in the water /acetone mixtures (A) at fw = 10%, (B) at fw = 30%, (C) at fw = 50%, (D) at fw = 90%.



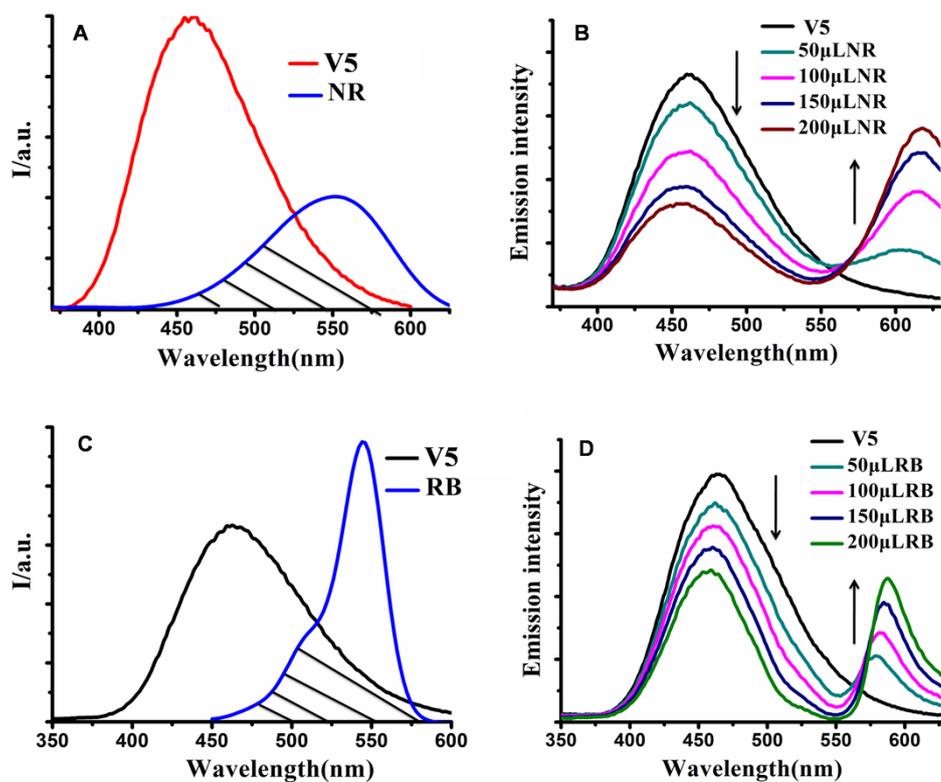
**Figure S7.** TEM microphotographs of the self-organized structures formed in the water /acetone mixtures (A) **2** at fw = 40%, (B) **5** at fw = 50%.



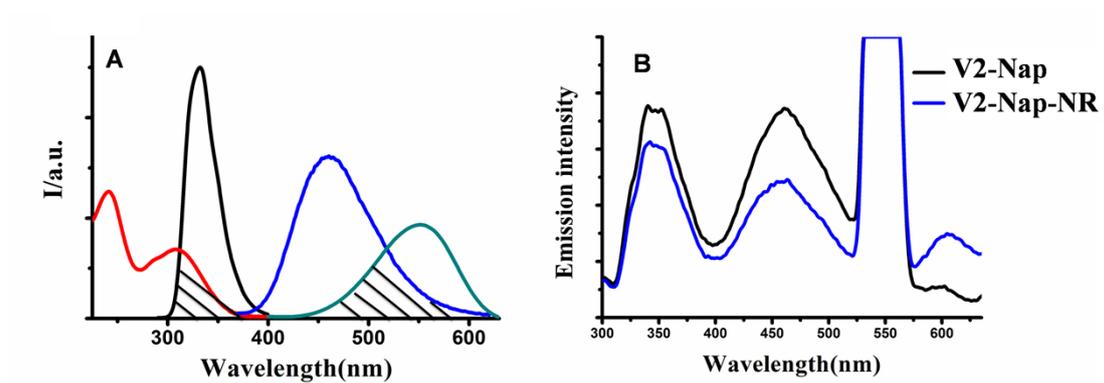
**Figure S8.** B3LYP/6-31- G\*optimized structure of compound **2** (A) and **5** (B)



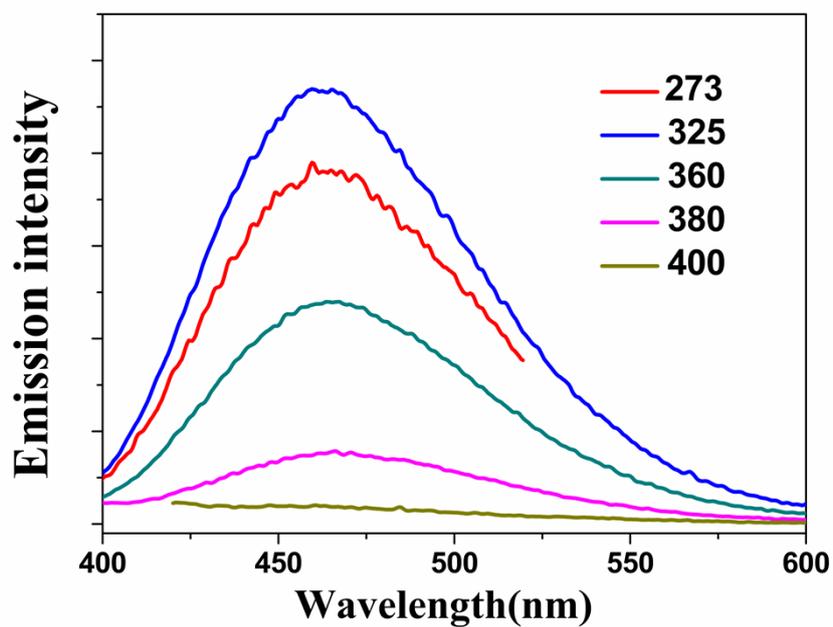
**Figure S9.** WAXS (A) and SAXS (B) patterns of the vesicles V2 and V5; (C) Comparison of the absorption and fluorescence spectra of compound 2 and the formed vesicle V2; (D) Confocal laser scanning microscopy image of the vesicles V2,  $\lambda_{ex} = 405$  nm.



**Figure S10.** Absorption and emission spectra of NR and vesicular V5 (A), RB and vesicular V5 (C), Fluorescence spectra of the vesicular V5 encapsulating NR (B) and RB (D). NR and RB is  $10^{-4}M$  in MeOH,  $\lambda_{ex} = 325$  nm



**Figure S11.** (A) Emission spectra of naphthalene (black), vesicular V2 (blue), absorption spectra of vesicular V2 (red), NR (green); (B) Fluorescence spectra of naphthalene-loaded vesicles V2-Nap and 50 $\mu$ L NR; NR is 10<sup>-4</sup>M in MeOH,  $\lambda_{\text{exc}}$  = 273 nm



**Figure S12.** Emission spectra of vesicular V2 at different excitation wavelength

- [1] H. Tong, Y. Hong, Y. Q. Dong, M. Ha lussler, Z. Li, Jacky W. Y. Lam, Y. P. Dong and B. Z. Tang, *J. Phys. Chem. B.*, 2007, **111**, 11817.