Electronic Supplementary Information
for

Multifunctional Supramolecular Vesicles Based on Complex of Ferrocenecarboxylic Acid Capped Pillar[5]arene and Galactose Derivative for Targeted Drug Delivery

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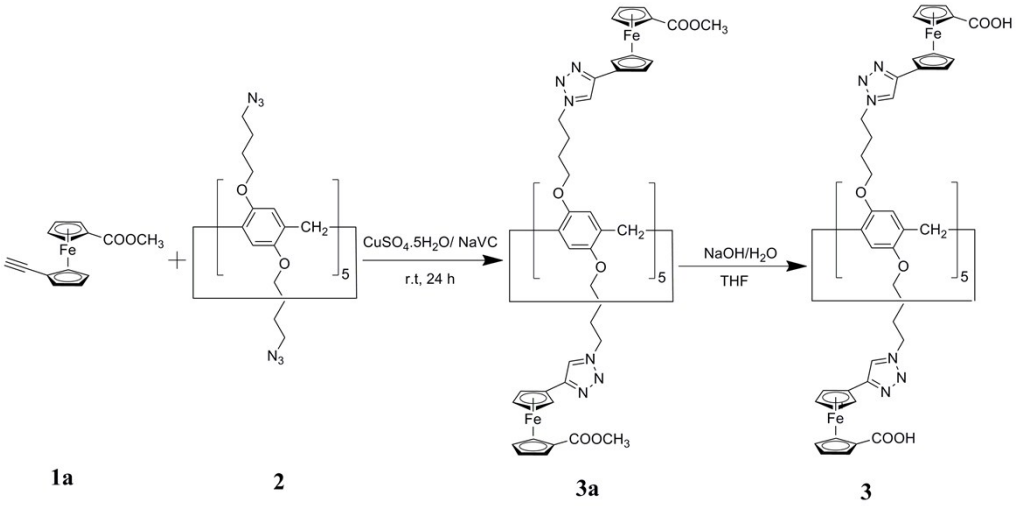
Table of Contents

I. Instrumentation and chemicals

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled. Doxorubicin hydrochloride (DOX) was purchased from Sangon Biotech. ^{1}NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei, respectively. SEM images were obtained using a S-4800 instrument (Hitachi Ltd.) with an accelerating voltage of 10.0 kV. DLS measurements were performed on a Delsa™ Nano system (Beckman Coulter, U.S.A.). UV-vis spectra were recorded with Shimadzu 1750 UV-visible spectrophotometer (Japan) at 298 K. Water surface tension was recorded with BZY-3B surface tension measurer (China). Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37 ^{o}C.

II. Synthesis and characterizations of compound 3 and G



Scheme S1. Synthetic route of compound **3**.

Compound **3a**: Copper sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$, 10 mg, 0.04 mmol] in H_2O (10 mL) was added to **1a** (according to the previously published procedure[1]) (294.8 mg, 1.10 mmol) and **2** (according to the previously published procedure by us[2]) (158.0 mg, 0.10 mmol) in CH_2Cl_2 (10 mL). Sodium ascorbate (20 mg, 0.10 mmol) was then added and the solution was stirred for 24 h at 25 °C. The solvents were removed under vacuum. The remaining solid was disolved in CH_2Cl_2 (50 mL) and washed with H_2O (2 × 25 mL). The organic phase was dried over Na_2SO_4, concentrated under vacuum and subjected to silica gel chromatography (CH_2Cl_2/MeOH, v/v = 100:3) to give **3a** (374.9 mg, 88 %). ^{1}H NMR (500 MHz, $CDCl_3$, ppm) δ 7.68 (s, 10 H), 6.66 (s, 10 H), 4.75 (dd, J = 9.9, 1.2 Hz, 20 H), 4.68 (s, 20 H), 4.41 (t, J = 7.1 Hz, 20 H), 4.32 - 4.29 (m, 40 H), 3.88 - 3.81 (m, 10 H), 3.69 - 3.71 (m, 10 H), 3.65 (s, 10 H), 3.60 (s, 30 H), 2.15 - 2.07 (m, 20 H), 1.77 - 1.74 (m, 20 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm) δ 170.7, 149.8, 144.6, 128.4, 120.1, 115.6, 77.6, , 72.6 , 72.6, 71.3, 70.1, 67.9, 67.8, 51.4, 50.0, 27.3, 26.7. MALDI-TOF-MS: *m/z* calcd for $[M+H]^+$ $C_{220}H_{251}Fe_{10}N_{30}O_{30}$, 4350.2455, found 4350.9743.

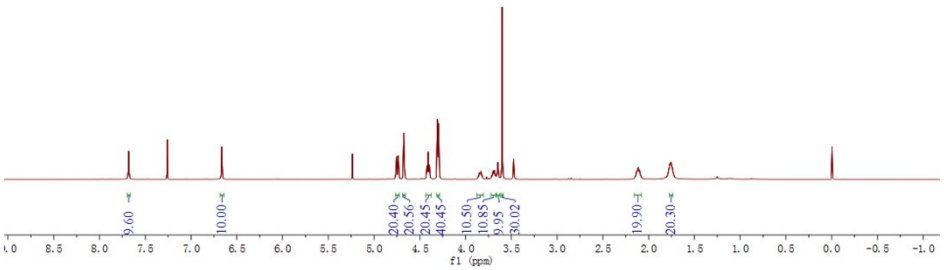


Fig. S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **3a**.

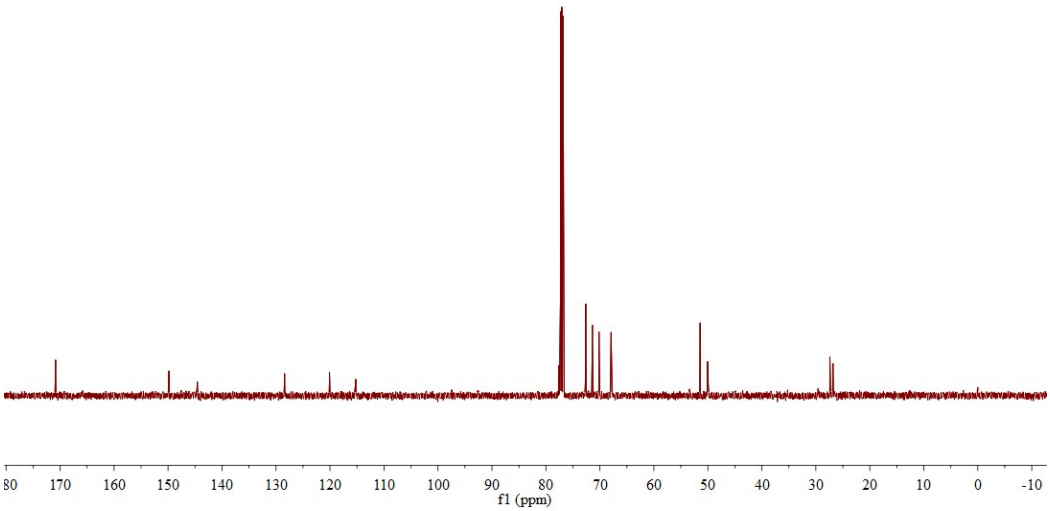


Fig. S2. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **3a**.

Compound **3**: The compound **3a** (228.1 mg, 0.05 mmol) and 50 mL 0.5 M NaOH in 50 mL THF was stirred at 70 °C under nitrogen atmosphere for 24 h. After evaporation of the solvent, the aqueous solution was acidified with 0.1 M HCl. The resulting mixture was filtered to give **3** as yellow solid (209.8 mg, 92 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 12.09 (s, 10 H), 8.16 (s, 10 H), 6.71 (s, 10 H), 4.74 (s, 20 H), 4.52 – 4.55 (m, 20 H), 4.32 – 4.34 (m, 40 H), 3.92 (s, 10 H), 3.77 – 3.38 (m, 40 H), 2.06(s, 20 H), 1.70 (s, 20 H). ^{13}C NMR (125 MHz, DMSO-d6, ppm) δ 170.8, 169.6, 148.9, 143.7, 143.1, 127.9, 120.9, 114.1, 72.3, 70.7, 67.3, 51.0, 49.3, 28.7, 26.7, 26.3. MALDI-TOF-MS: *m*/*z* calcd for $[M+Na]^+$ $C_{210}H_{230}Fe_{10}N_{30}O_{30}Na$, 4234.0835, found 4234.8493.

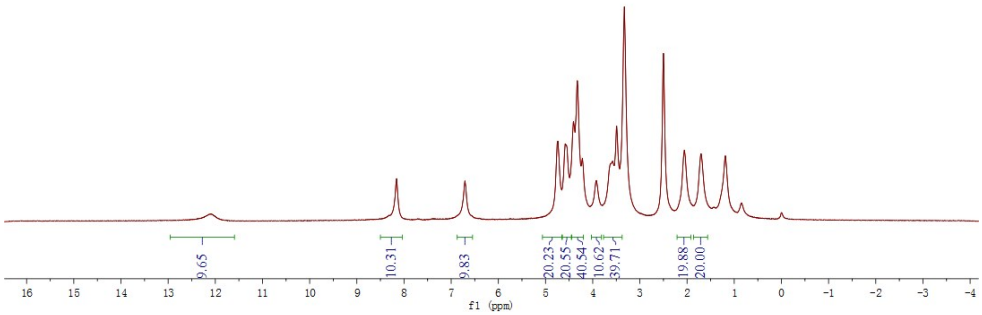


Fig. S3. ^{1}H NMR spectrum (500 MHz, DMSO-d6) of **3**.

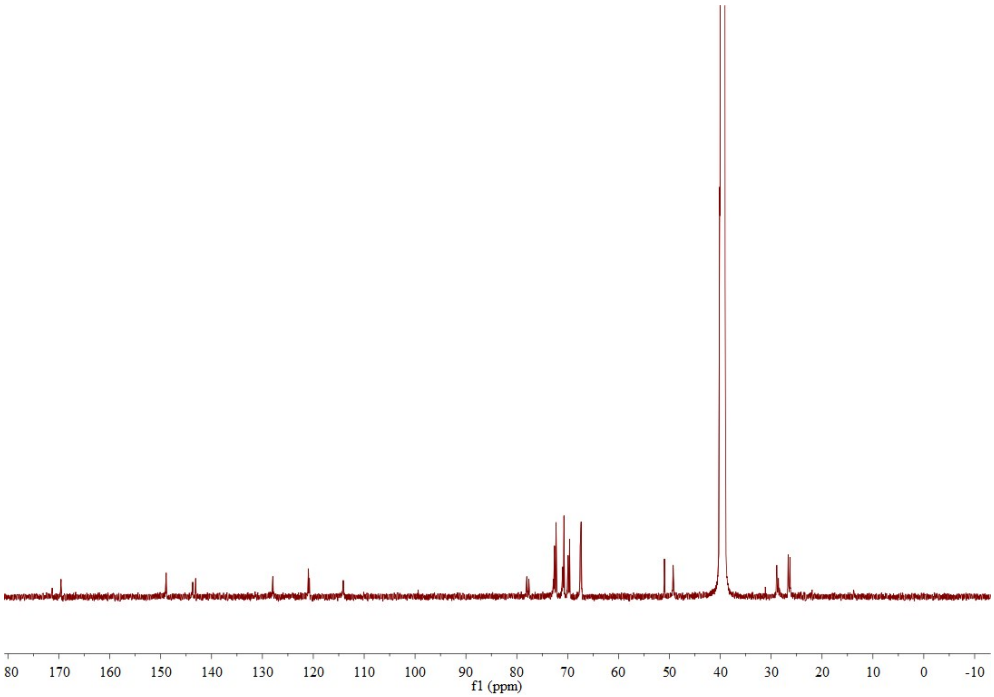
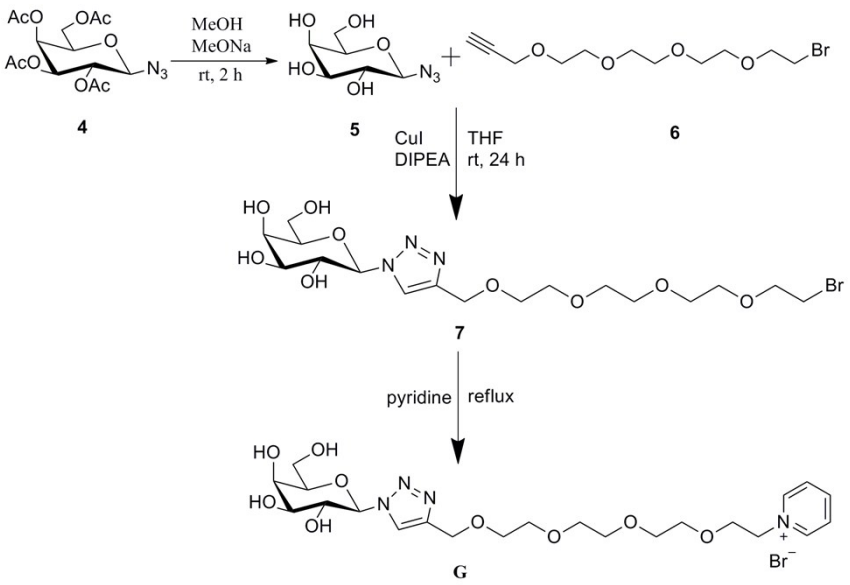


Fig. S4. ^{13}C NMR spectrum (125 MHz, DMSO-d6) of **3**.



Scheme S2. Synthetic route of **G**.

Compound **5**: CH_3ONa (432.1 mg, 8 mmol) was added into a solution of compound **4** (746.6 mg, 2 mmol in 100 mL MeOH). The mixture was stirred at room temperature for 2 h, then neutralized by addition of ion-exchange resin (Amberlite IR 120 H^+) until pH 7, filtered, and the solvent was removed under reduced pressure. The compound **5** was obtained as a colorless oil (389.5 mg, 95 %). ^{1}H NMR (500 MHz, CD_3OD) δ 4.46 - 4.40 (m, 1 H), 3.86 (d, J = 1.8 Hz, 1 H), 3.78 (dd, J = 11.4, 6.9 Hz, 1 H), 3.72 (dd, J = 11.4, 5.1 Hz, 1 H), 3.64 - 3.59 (m, 1 H), 3.53 - 3.42 (m, 2 H).

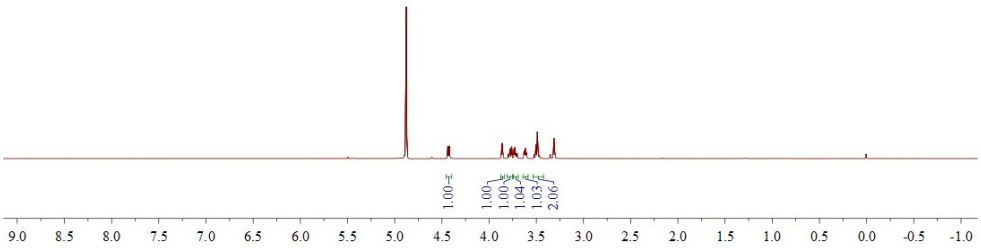


Fig. S5 ^{1}H NMR spectrum (500 MHz, CD_3OD) of **5**.

Compound **7**: The synthesis of **6** was performed according to the previously published procedure.[3] A mixture of **5** (41.1 mg, 0.2 mmol), **6** (50.2 mg, 0.2 mmol), CuI (18.6 mg, 0.1 mmol) and DIPEA (0.68 mL, 3.9 mmol) was dissolved in 5 mL THF. The mixture was stirred at room temperature under nitrogen atmosphere for 24 h. The mixture was concentrated under reduced pressure. Then 20 mL DCM was added into the crude solid. The mixture was washed with water (3 × 10 mL) and saturated NaCl (3 × 10 mL). The organic phase was dried with $MgSO_4$ and was concentrated under reduced

pressure. The crude product was purified by flash column chromatography (eluent: petroleum ether/ ethyl acetate = 2:1) to give **7** as a yellow oil (90.1 mg, 90 %). ^{1}H NMR (500 MHz, CD_3OD, ppm) δ 8.23 (s, 1 H), 5.57 (d, J = 9.1 Hz, 1 H), 4.67 (s, 2 H), 4.14 (t, J = 9.2 Hz, 1 H), 3.98 (s, 1 H), 3.83 (d, J = 5.7 Hz, 1 H), 3.79 (d, J = 5.7 Hz, 1 H), 3.77 (d, J = 5.7 Hz, 1 H), 3.76 - 3.72 (m, 2 H), 3.72 (s, 1 H), 3.71 - 3.61 (m, 13 H), 3.50 (t, J = 5.9 Hz, 1 H), 3.35 (s, 1 H). HRMS: m/z calcd for $[M+Na]^+$ $C_{17}H_{30}BrN_3O_9Na$, 522.1165, found 522.1057.

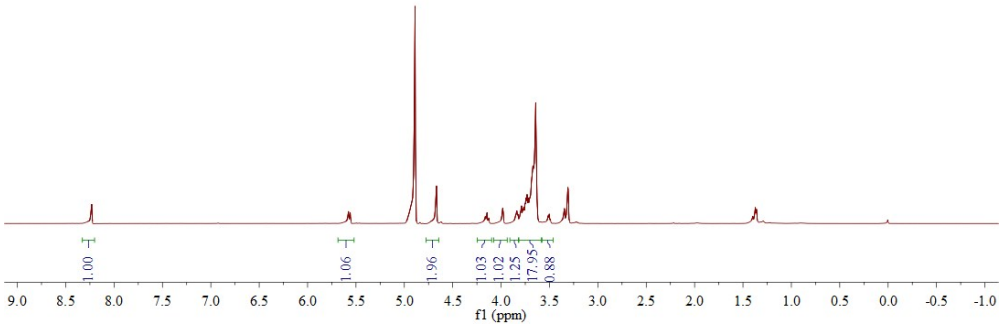


Fig. S6 ^{1}H NMR spectrum (500 MHz, CD_3OD) of **7**.

Compound **G**: The compound **7** (57.9 mg, 0.1 mmol) was dissolved in 3 mL pyridine. The mixture was reflux under nitrogen atmosphere for 12 h, then concentrated under reduced pressure. The crude product was purified by flash column chromatography (eluent: DCM/ MeOH = 20:1) to give **G** as a yellow oil (36 mg, 58 %). ^{1}H NMR (500 MHz, CD_3OD, ppm) δ 8.95 (d, J = 5.7 Hz, 2 H), 8.53 (t, J = 7.8 Hz, 1 H), 8.20 (s, 1 H), 8.07 (t, J = 6.9 Hz, 2 H), 5.59 (t, J = 13.4 Hz, 1 H), 4.72 (d d, J = 9.7, 4.8 Hz, 2 H), 4.60 (s, 2 H), 4.16 (t, J = 9.3 Hz, 1 H), 4.01 (d, J = 2.8 Hz, 1 H), 3.99 – 3.94 (m, 2 H), 3.88 (t, J = 5.8 Hz, 1 H), 3.77 (d, J = 4.9 Hz, 2 H), 3. 70 -3. 66 (m, 6 H), 3.62 (d d, J = 7.4, 5.1 Hz, 4 H), 3.56 (d d, J = 5.5, 2.6 Hz, 2 H), 3.54 – 3.51 (m, 2 H). ^{13}C NMR (125 MHz, CD_3OD, ppm) δ 145.8, 145.5, 145.0, 128.1, 123.1, 89.1, 79.0, 74.3, 70.5, 70.5, 70.5, 70.0, 69.5, 69.1, 63.8, 61.6. HRMS: m/z calcd for $[M-Br]^+$ $C_{22}H_{35}N_4O_9$, 499.2587, found 499.2407.

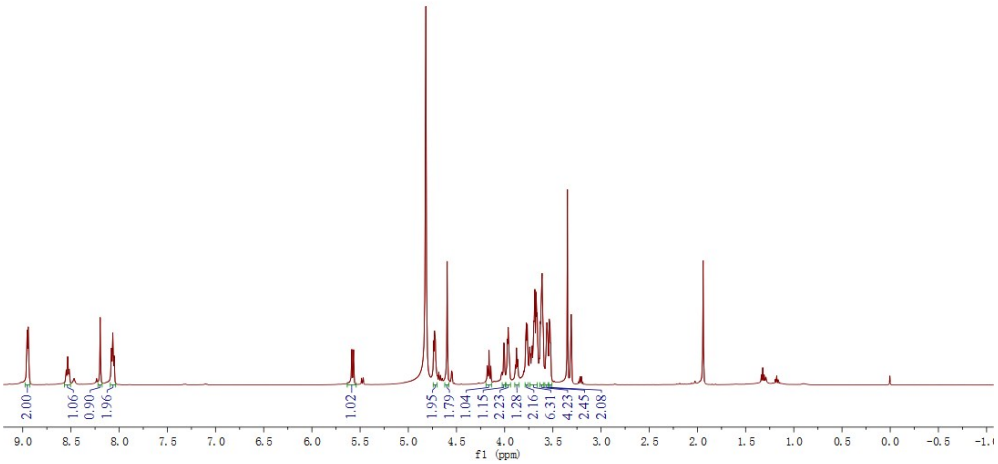


Fig. S7. ^{1}H NMR spectrum (500 MHz, CD_3OD) of **G**.

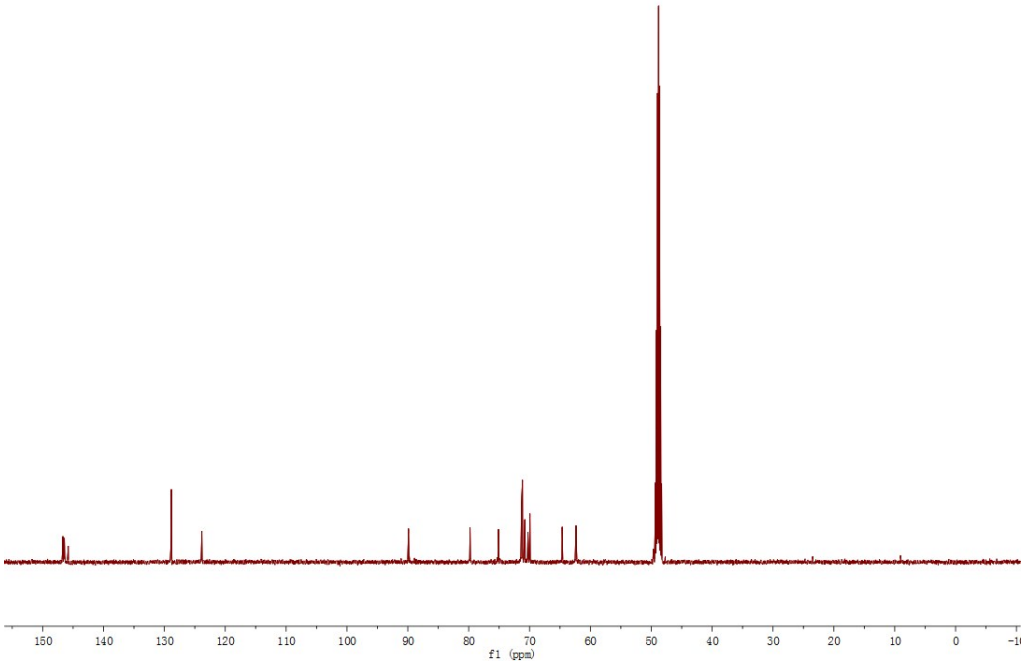


Fig. S8. ^{13}C NMR spectrum (125 MHz, CD_3OD) of **G**.

III. The preparation and characterization of the vesicles

4.12 mg (1 μmol) of pillar[5]arene **3**, 24 μL NaOH (0.08 g/mL), 4 μL H_2O_2 (30%) were dissolved in 1 mL distilled water and stirred for 30min. Then mixed 0.51mg (1 μmol) **G** with it. After ultrasonic for 30 minutes and staying overnight, **FACP5G** was obtained, which were characterized by SEM and DLS.

IV. DOX loading and release of cationic vesicles

4.12 mg (1 μmol) of pillar[5]arene **3**, 24 μL NaOH (0.08 g/mL), 4 μL H_2O_2 (30%) were dissolved in 1 mL distilled water and stirred 30 min. Then mixed 0.51 mg (1 μmol) **G** and 0.58 mg (1 μmol) DOX with it. After ultrasonic for 30 minutes and staying overnight, DOX-loaded vesicles were purified by dialysis (molecular weight cutoff 8000-14000) in distilled water until the water outside the dialysis tube exhibited negligible DOX fluorescence. The amount of unloaded DOX in the dialysate was quantitatively measured by UV-vis spectrophotometry at 490 nm.

The DOX encapsulation was calculated by the following equations: [4]

encapsulation efficiency (%) = ($m_{DOX\text{-}loaded}/m_{DOX}$) 100 (1)

$m_{DOX\text{-}loaded}$ and m_{DOX} are mass of DOX encapsulated in vesicles and DOX added, respectively.

The DOX release from DOX-loaded vesicles was studied at different pH buffer solutions, with or without GSH (10 mM). 1 mL of DOX-loaded vesicles in a dialysis bag was added into 10 mL of corresponding release medium at room temperature. At specified time intervals, 0.1 mL of the release medium was taken out for measuring the DOX released DOX concentration with an Epoch microplate spectrophotometer (Biotek). The concentration of DOX was determined by measurement of absorbance at 490 nm using a standard absorbance vs. concentration curve constructed for DOX in the corresponding release medium.

V. Cell culture and cell viability

MCF-7 (human breast cancer cell) and 293T (human embryonic kidney T) cells were cultured in DMEM medium containing 10% FBS, 1% penicillin/streptomycin (complete DMEM) in a humidified atmosphere with 100% humidity and 5% CO_2 at 37 °C.

The relative cytotoxicities of unloaded vesicles, DOX and DOX-loaded vesicles were evaluated in vitro by MTT assay, respectively. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μL complete DMEM and grew for 24 h at 37 °C. Subsequently, cells were incubated with unloaded vesicles, DOX, and DOX-loaded vesicles at different concentrations for 24 h, 48 h, or 72 h. The cells were washed and the fresh medium containing MTT was added into each plate. The cells were incubated for another 4 h. After that, the medium containing MTT was removed, dimethyl sulfoxide (100 μL) was added to each well, and the plates were gently shaken for 10 min to dissolve the formazan crystals. Finally, the absorbance at 490 nm was recorded with a microplate reader.

VI. In vitro transfection assay

MCF-7 cells were seeded in 6-well plates for 24 h. The plates were washed with PBS and replaced with fresh medium. The cells were incubated with DOX and DOX-loaded vesicles for 6 h. The medium was then removed and washed three times with PBS. Add 0.5 mL trypsin and collected in a centrifuge tube. The liquid supernatant was abandoned, the cells were washed with PBS and examined by flow cytometry. Untreated cells were used as negative control.

VII. Confocal laser scanning microscopy (CLSM)

MCF-7 cells were seeded in 20 mm plastic bottomed μ-dishes for 24 h. The medium was replaced with a fresh one and then incubated with DOX-loaded vesicles for 6 h. The dishes were washed with PBS three times and fixed with 4.0%

paraformaldehyde at room temperature for 15 min. After washing with PBS, the cells were all stained with DAPI for 5 min. Finally, the cells were washed with PBS and then observed under a confocal fluorescence microscope.

VIII. Fig. S9-S14

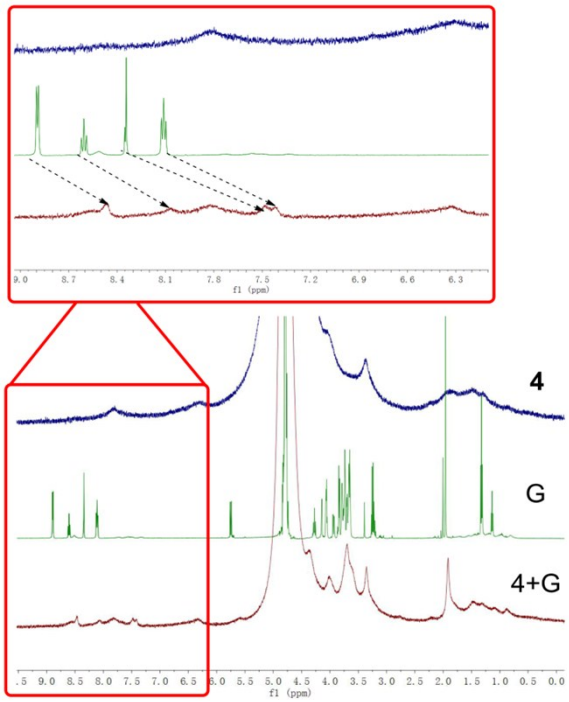


Fig. S9. Partial ^{1}H NMR spectra of **FACP5** (blue line), **G** (green line) and the mixture of **FACP5** and **G** (1:1))(red line) in D_2O.

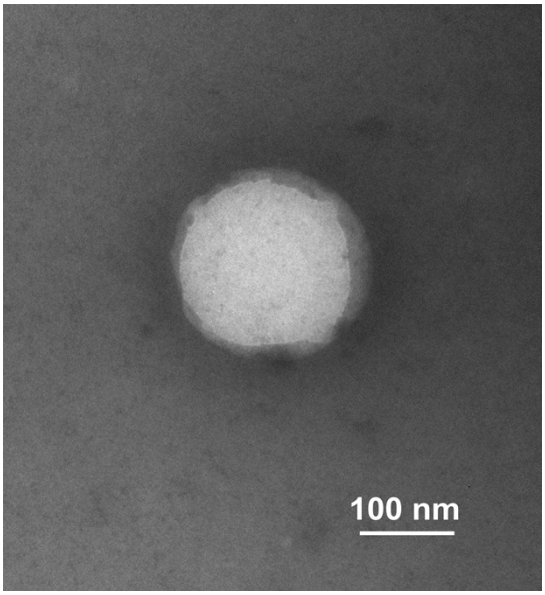


Fig. S10. TEM image of the supramolecular vesicles based on the complexation between **FACP5** and **G**.

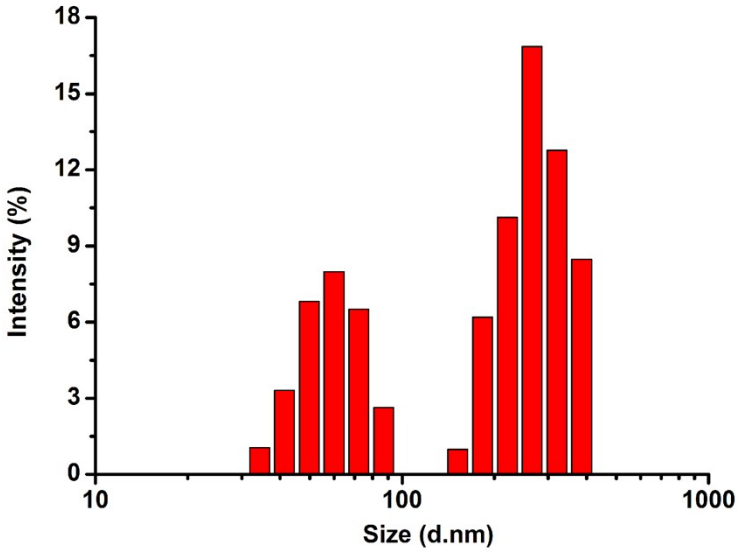


Fig. S11. DLS histogram of the vesicles formed from **FACP5G.**

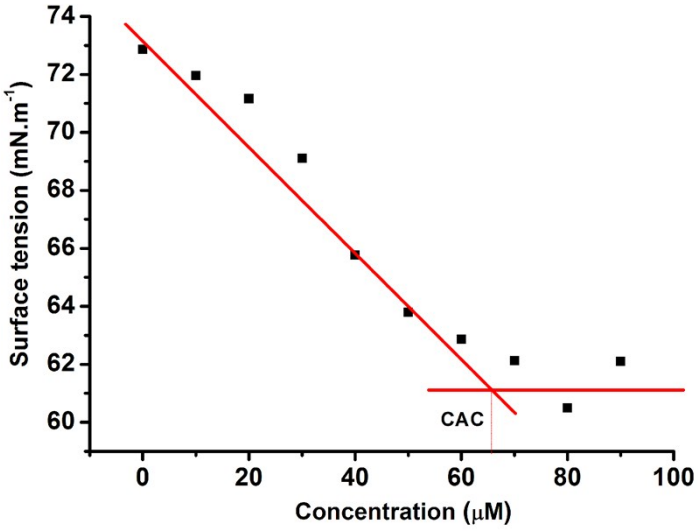


Fig. S12. Surface tension of water vs. the concentration of **FACP5G**. There are two linear segments in the curve and a sudden decrease of the slope, implying that the CAC is approximately 66 μM in water.

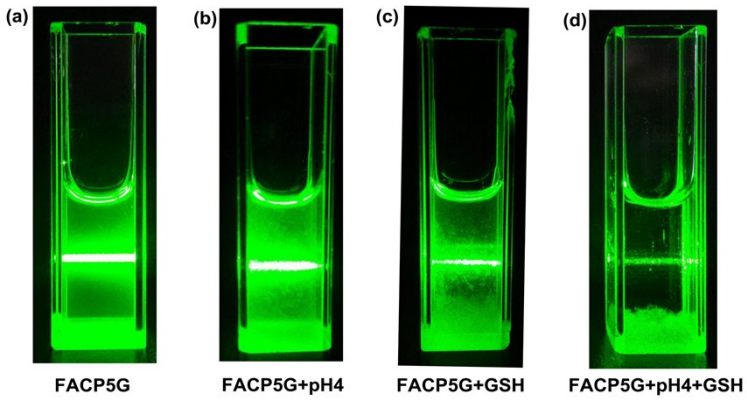


Fig. S13. (a) Tyndall effect of **FACP5G** aggregation (left); (b) Tyndall effect of **FACP5G** vesicles in the acid environment (pH 4); (c) Tyndall effect of **FACP5G** vesicles in the presence of GSH (10 mM); (d) Tyndall effect of of **FACP5G** vesicles in the presence of GSH and acid environment.

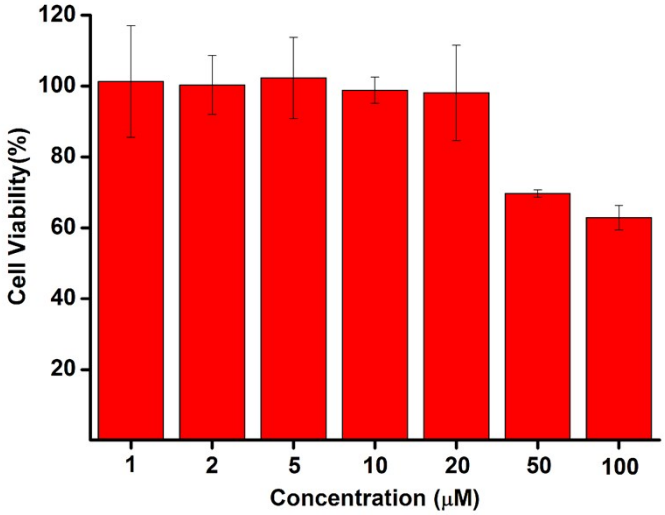


Fig. S14. Cell viability measured by methyl thiazole tetrazolium cell survival (MTT) assay after being treated with different concentrations of **FACP5G** for 24 h of 293T cells

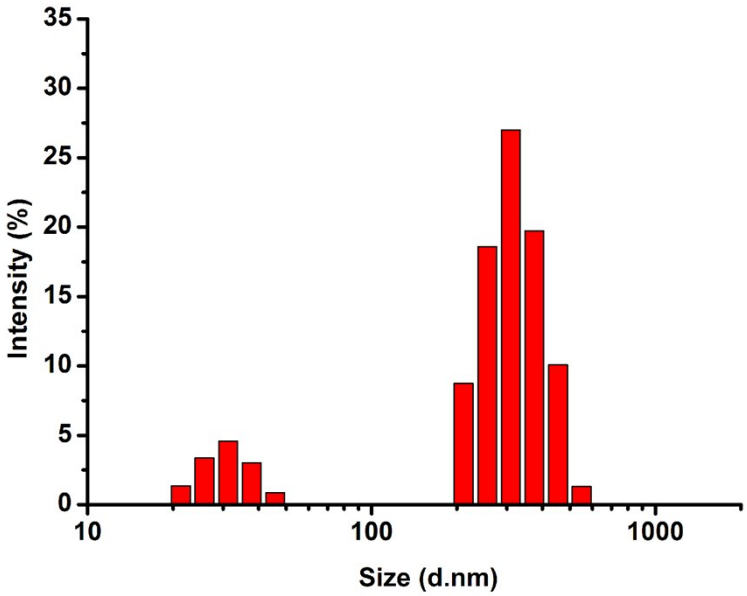


Fig. S15. DLS histogram of DOX-loaded **FACP5G** vesicles.

IX. References

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