Electronic Supplementary Information

GSH- and pH-responsive drug delivery system constructed by water-soluble pillar[5]arene and lysine derivative for controllable drug release

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1. General information

All reactions were performed in air atmosphere unless otherwise stated. The commercially available reagents and solvents were either employed as purchased or dried according to procedures described in the literature. Column chromatography was performed with silica gel (200-300 mesh) produced by Qingdao Marine Chemical Factory, Qingdao (China). All yields were given as isolated yields. NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer (or Bruker DPX 400 MHz spectrometer) with internal standard tetramethylsilane (TMS) and solvent signals as internal references at room temperature, and the chemical shifts (δ) were expressed in ppm and J values were given in Hz. Low-resolution electrospray ionization mass spectra (LR-ESI-MS) were obtained on Finnigan Mat TSQ 7000 instruments. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6540Q-TOF LCMS equipped with an electrospray ionization (ESI) probe operating in positive-ion mode with direct infusion. Transmission electron microscope (TEM) investigations were carried out on a JEM-2100 instrument (or JEM 2010 instrument). Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA), using a 200-mW polarized laser source ($\lambda = 514$ nm). The UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 35 UV-Vis Spectrometer. The excitation and emission spectra were recorded on a Hitachi F-7000 Fluorescence Spectrometer. Melting points (M.p.) were determined using a Focus X-4 apparatus (made in China) and were not corrected.

2. Experimental procedure



Scheme S1. The synthesis route of lysine derivative G

1-((10-bromodecyl)oxy)-3,5-dimethylbenzene 1^{S1} : K₂CO₃ (2.07 g, 1.5 mmol) was added to a solution of 3,5-dimethylphenol (0.9 g, 7.4 mmol) and 1,10-dibromodecane (4.4 g, 14.7 mmol) in acetonitrile (80 mL), then the mixture was refluxed for 12 h, after filtrating the precipitation, the solution was evaporated under reduced pressure. DCM (50 mL) was then added to the residue, and the solution was washed by 1N HCl solution (25 mL) and saturated brine (25 mL). Finally, it was dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by silica-gel column chromatograghy (hexane) to afford compound **1** as a colorless oil (1.89 g, 73% yield). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm) = 6.57 (s, 1H, Ar*H*), 6.52 (s, 2H, Ar*H*), 3.91 (t, *J* = 6.5 Hz, 2H, OC*H*₂), 3.39 (t, *J* = 6.9 Hz, 2H, BrC*H*₂), 2.27 (s, 6H, ArC*H*₃), 1.91-1.80 (m, 2H, OCH₂C*H*₂), 1.80-1.69 (m, 2H, BrCH₂C*H*₂), 1.53-1.27 (m, 12H, C*H*₂).



Fig. S1 ¹H NMR spectrum of **1** (400 MHz, CDCl₃, 298 K).

10-(3,5-dimethylphenoxy)decane-1-thiol 2: The solution of **1** (1.89 g, 5.54 mmol) in ethanol (15 mL) and $Na_2S_2O_3 \cdot 5H_2O$ (1.65 g, 6.65 mmol) in water (15 mL) were stirred at refluxed temperature for 2 h, then it was cooled down to room temperature and plenty of white solid was precipitated. After removing the solvent, the precipitation was add to the mixed solution of CHCl₃

(15 mL) and 1N HCl (15 mL), which was refluxed until the solution became clear. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 15 mL), then the combined organic phase was washed by saturated brine (30 mL) and dry over Na₂SO₄, after removing the solvent under vacuum, compound **2** was obtained as a colorless oil (1.39 g, 86% yield). ¹H NMR (300 MHz, CDCl₃, 298 K) δ (ppm) = 6.58 (s, 1H, Ar*H*), 6.53 (s, 2H, Ar*H*), 3.91 (t, J = 6.5 Hz, 2H, OCH₂), 2.52 (q, J = 7.4 Hz, 2H, SHCH₂), 2.28 (s, 6H, ArCH₃), 1.82-1.68 (m, 2H, OCH₂CH₂), 1.68-1.54 (m, 2H, BrCH₂CH₂), 1.52-1.26 (m, 13H, CH₂ and SH). ¹³C NMR (100 MHz, CDCl₃, 298 K) δ (ppm) = 159.3, 139.1, 122.3, 112.45, 67.8, 53.5, 34.1, 29.6, 29.5, 29.4, 29.2, 28.5, 26.2, 24.7, 21.5. HR-ESI-MS: m/z Calcd for C₁₈H₃₁OS [M+H]⁺ 295.2091, found 295.2101.



Fig. S2 ¹H NMR spectrum of **2** (300 MHz, CDCl₃, 298 K).



Fig. S3 ¹³C NMR spectrum of **2** (100 MHz, CDCl₃, 298 K).

Disulfide pyridine 3: AcOH (0.1 mL) was added to the solution of 1,2-di(pyridin-2-yl)disulfane (0.44 g, 2 mmol) in methanol (5 mL), then compound **2** (0.30 g, 1 mmol) in DCM (5 mL) solution was added dropwise to the above solution over a period of 10 min. The mixture was then stirred for 24 h at 25 °C. After removing the solvent, the residue was dissolved in DCM (15 mL) and washed sequentially by water (10 mL) and saturated brine (10 mL), then dried over Na₂SO₄. After concentrating under vacuum, the residue was purified by silica-gel column chromatography (DCM/MeOH = 200:1, ν/ν) to afford compound **3** as a yellow oil (0.24 g, 60% yield). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm) = 8.45 (dd, *J* = 4.8, 0.8 Hz, 1H, PyH), 7.73 (d, *J* = 8.1 Hz, 1H, PyH), 7.66-7.59 (m, 1H, PyH), 7.06 (m, 1H, PyH), 6.57 (s, 1H, ArH), 6.52 (s, 2H, ArH), 3.91 (t, *J* = 6.5 Hz, 2H, OCH₂), 2.79 (t, *J* = 7.4 Hz, 2H, SCH₂), 2.28 (s, 6H, ArCH₃), 1.80-1.62 (m, 4H, OCH₂CH₂ and SCH₂CH₂), 1.50-1.20 (m, 12H, CH₂). ¹³C NMR (100 MHz, CDCl₃, 298 K) δ (ppm) = 160.8, 159.3, 149.6, 139.2, 137.1, 122.3, 120.6, 119.6, 112.4, 67.8, 39.1, 29.7, 29.6, 29.4, 29.2, 29.0, 28.6, 26.2, 21.6. HR-ESI-MS: m/z Calcd for C₂₃H₃₄NOS₂ [M+H]⁺ 404.2077, found 404.2079.



Fig. S5 13 C NMR spectrum of 3 (100 MHz, CDCl₃, 298 K).

Disulfide amine derivative 4: The mixed methanol solution of **3** (0.73 g, 1.7 mmol) and 2-mercaptoethanaminium chloride (0.13 g, 1.2 mmol) was stirred for 2 h at 25 °C, after removing the solvent, the residue was dissolved in a small amount of methanol (2 mL), which was further added dropwise to plenty of diethyl ether (150 mL) to afford white precipitates, the precipitates were collected by filtration and dried in vacuum, and the target product **4** was obtain as a white powder (0.42 g, 92% yield). M. P. 78-79 °C. ¹H NMR (300 MHz, CD₃OD, 298 K) δ (ppm) = 6.55 (s, 1H, Ar*H*), 6.50 (s, 2H, Ar*H*), 3.91 (t, *J* = 6.4 Hz, 2H, OC*H*₂), 3.28 (t, *J* = 6.6 Hz, 2H, NH₂C*H*₂), 2.92 (t, *J* = 6.7 Hz, 2H, SC*H*₂), 2.76 (t, *J* = 7.3 Hz, 2H, SC*H*₂), 2.24 (s, 6H, ArC*H*₃), 1.81-1.64 (m, 4H, OCH₂C*H*₂ and SCH₂C*H*₂), 1.52-1.29 (m, 12H, C*H*₂). ¹³C NMR (100 MHz, CD₃OD, 298 K) δ (ppm) = 159.2, 138.7, 121.7, 111.9, 67.4, 38.0, 37.8, 33.8, 29.3, 29.2, 29.1, 28.9, 28.8, 28.1, 25.8, 20.2. HR-ESI-MS: m/z Calcd for C₂₀H₃₆NOS₂ [M–CI]⁺ 370.2233, found 370.2236.

Fig. S7 ¹³C NMR spectrum of **4** (100 MHz, CD₃OD, 298 K).

2,6-bis((**tert-butoxycarbonyl**)**amino**)**hexanoic acid 5**⁸²: L-lysine (1.02 g, 7.0 mmol) and sodium hydroxide (0.56 g, 14.0 mmol) were dissolved in deionised water (13 mL), then a solution of ditertbutyl dicarbonate (3.82 g, 17.5 mmol) in dry 1,4-dioxane (25 mL) was added dropwise, and the resulting mixture was stirred at 50 °C for 3 h. The mixture was then concentrated to about 6 mL in vacuum, diluted with H₂O (20 mL) and washed with cyclohexane (3 × 25 mL). The pH of the aqueous layer was then adjusted to 3 by using aqueous hydrochloric acid, and the obtained precipitate was extracted with ethyl acetate (3 × 25 mL), the combined organic layer was dried over MgSO₄, after removing the solvent in vacuum, compound **5** was obtained as a yellow oil (1.40 g, 55% yield). ¹H NMR (300 MHz, CDCl₃, 298 K) δ (ppm) = 5.24 (s, 1H, NH), 4.66 (s, 1H, NH), 4.32 (s, 1H, NHCH), 3.14 (s, 2H, NHCH₂), 1.95-1.59 (m, 2H, CHCH₂), 1.57-1.32 (m, 22H, CH₂ and CH₃).

Fig. S8 ¹H NMR spectrum of **4** (300 MHz, CDCl₃, 298 K).

NHS functionalized ester 6⁸³: Compound **5** (0.52 g, 1.5 mmol) and NHS (0.23 g, 2 mmol) were dissolved in anhydrous THF (20 mL) solution, then DCC (0.31 g, 1.5 mmol) was added and the above mixture was stirred at 25 °C for 2 h. After removing the precipitates by filtration, the solution was concentrated under vacuum and purified by silica-gel chromatography (DCM/MeOH = 150:1, v/v) to give compound **6** as a white solid (0.43 g, 65% yield). ¹H NMR (300 MHz, CDCl₃, 298 K) δ (ppm) = 5.17 (s, 1H, NH), 4.67 (s, 2H, NHCH, NH), 3.13 (s, 2H, NHCH₂), 2.84 (s, 4H, O=CCH₂), 2.06-1.78 (m, 2H, CHCH₂), 1.63-1.33 (m, 22H, CH₂ and CH₃).

Fig. S9 ¹H NMR spectrum of **6** (300 MHz, CDCl₃, 298 K).

Boc-protected disulfide compound 7: Compound **6** (0.43 g, 0.96 mmol) and **4** (0.43 g, 1.06 mmol) were dissolved in anhydrous DMF solution (20 mL), and anhydrous DIPEA (3 mL) was added. Then the mixture was stirred at 25 °C for 12 h. After removing the solvent, DCM (30 mL) was added to dissolve the residue, and the obtained DCM solution was washed sequentially by water (2 × 15 mL) and saturated brine (15 mL), then it was dried over Na₂SO₄ and concentrated under vacuum. The crude product was purified by silica-gel chromatography (DCM/MeOH = 250:1, *v/v*) to give compound **7** as a yellow oil (0.61 g, 91% yield). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm) = 6.58 (s, 1H, Ar*H*), 6.53 (s, 2H, Ar*H*), 6.48 (s, 1H, N*H*), 5.08 (s, 1H, N*H*), 4.58 (s, 1H, N*H*), 4.02 (s, 1H, NHC*H*), 3.91 (t, *J* = 6.5 Hz, 2H, OC*H*₂), 3.63-3.55 (m, 2H, NHC*H*₂), 3.11 (d, *J* = 5.9 Hz, 2H, NHC*H*₂), 2.77 (t, *J* = 6.2 Hz, 2H, SC*H*₂), 2.72-2.65 (m, 2H, SC*H*₂), 2.28 (s, 6H, ArC*H*₃), 1.87-1.59 (m, 6H, C*H*₂), 1.54-1.25 (m, 34H, C*H*₂ and C*H*₃). ¹³C NMR (75 MHz, CDCl₃, 298 K) δ (ppm) = 172.6, 159.1, 156.2, 155.8, 139.0, 122.2, 112.2, 79.7, 78.9, 67.6, 54.39, 40.0, 38.8, 38.4, 37.4, 32.2, 29.61, 29.4, 29.3, 29.2, 29.1, 28.5, 28.4, 28.0, 27.5, 26.0, 22.7, 21.4. HR-ESI-MS: m/z Calcd for C₃₆H₆₄N₃O₆S₂ [M+H]⁺ 698.4232, found 698.4228.

Fig. S11 ¹³C NMR spectrum of **7** (75 MHz, CDCl₃, 298 K).

Guest molecule G: 4N HCl/EA (0.3 mL) was added to the dichloromethane solution of 7 (0.06 g,

0.09 mmol), and the mixture was stirred for at 25 °C 2 h. After removing the solvent, the residue was dissolved in a small amount of anhydrous MeOH (1 mL), which was then added dropwise to plenty of diethyl ether (150 mL), the precipitates was collected by filtration, washed by diethyl ether and dried in vacuum, and guest molecule **G** was obtained as a yellow oil (0.24 g, 70% yield). ¹H NMR (300 MHz, CD₃OD, 298 K) δ (ppm) = 6.55 (s, 1H, Ar*H*), 6.50 (s, 2H, Ar*H*), 3.95-3.86 (m, 3H, OC*H*₂, NH₂C*H*), 3.77-3.41 (m, 3H, N*H* and CHN*H*₂), 3.04-2.80 (m, 6H, NHC*H*₂ and NH₂C*H*₂, SC*H*₂), 2.78-2.67 (m, 2H, SC*H*₂), 2.24 (s, 6H, ArC*H*₃), 2.01-1.81 (m, 2H, N*H*₂), 1.81-1.50 (m, 6H, C*H*₂), 1.48-1.28 (m, 16H, C*H*₂). ¹³C NMR (100 MHz, CD₃OD, 298 K) δ (ppm) = 168.7, 159.2, 138.7, 121.8, 111.9, 67.4, 52.7, 39.0, 38.2, 38.1, 36.8, 36.6, 30.7, 29.2, 29.1, 29.0, 28.9, 28.8, 28.1, 26.7, 25.8, 21.6, 20.2. HR-ESI-MS: m/z Calcd for C₂₆H₄₈N₃O₂S₂ [M–H–2C1]⁺ 498.3183, found 498.3186.

Fig. S12 ¹H NMR spectrum of G (300 MHz, CD₃OD, 298 K).

Fig. S13 ¹³C NMR spectrum of **G** (100 MHz, CD₃OD, 298 K).

Scheme S2. Synthesis of model guest molecule MG

9-(3-bromopropyl)-9H-carbazole 8^{S4}: 60% NaH (0.16 g, 4 mmol) was added to the anhydrous THF (25 mL) solution of 9H-carbazole (0.62 g, 3.7 mmol), then 1,3-dibromopropane (2.01 g, 10 mmol) was added and the mixture was stirred at 25 °C for 12 h. Then, the mixture was poured to plenty of MeOH. After removing the solvent, the residue was dissolved in DCM (25 mL) and water (25 mL), the organic phase was separated and the aqueous phase was extracted by DCM (3 \times 15 mL), then the combined organic phase was washed by saturated brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by silica-gel chromatography (DCM/hexane = 1:1, ν/ν) to give compound **8** as a colorless oil (0.57 g, 52%).

yield). ¹H NMR (300 MHz, CDCl₃, 298 K) δ (ppm) = 8.10 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.53-7.44 (m, 4H, Ar*H*), 7.30-7.19 (m, 2H, Ar*H*), 4.51 (t, *J* = 6.5 Hz, 2H, NC*H*₂), 3.39 (t, *J* = 6.1 Hz, 2H, BrC*H*₂), 2.44 (p, *J* = 6.4 Hz, 2H, NCH₂C*H*₂).

Fig. S14 ¹H NMR spectrum of **8** (300 MHz, CDCl₃, 298 K).

2-(3-(9H-carbazol-9-yl)propyl)isoindoline-1,3-dione 9^{84} : Compound **8** (0.57 g, 2 mmol) and potassium 1,3-dioxoisoindolin-2-ide (0.75 g, 4 mmol) were dissolved in CH₃CN (20 mL), and the mixture was refluxed for 12 h, then it was cooled to room temperature. After removing the precipitates by filtration, the solution was concentrated and purified by silica-gel chromatography (DCM) to give compound 9 as a white solid (0.34 g, 50% yield). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm) = 8.08 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.90-7.79 (m, 2H, Ar*H*), 7.77-7.66 (m, 2H, Ar*H*), 7.53-7.44 (m, 4H, Ar*H*), 7.30-7.19 (m, 2H, Ar*H*), 4.40 (t, *J* = 6.5 Hz, 2H, NC*H*₂), 3.83 (t, *J* = 6.1 Hz, 2H, NphtC*H*₂), 2.36-2.17 (m, 2H, NCH₂C*H*₂).

Fig. S15 ¹H NMR spectrum of **10** (300 MHz, CDCl₃, 298 K).

3-(9H-carbazol-9-yl)propan-1-amine 10^{S5}: Diamid hydrate (2 mL) was added to the stirred solution of **9** (0.33 g, 0.94 mmol) in THF (10 mL), the mixture was refluxed for 4 h. After removing the solvent, the residue was dissolved by DCM (10 mL) and water (10 mL), the organic phase was separated and the aqueous phase was extracted by DCM (3×55 mL), the combined organic phase was washed by saturated brine and dried over Na₂SO₄. After removing the solvent, the target product **10** was obtained as a colorless oil (0.3 g, 90% yield). ¹H NMR (400 MHz, CDCl₃, 298 K) δ (ppm) = 8.10 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.55-7.38 (m, 4H, Ar*H*), 7.27-7.17 (m, 2H, Ar*H*), 4.40 (t, *J* = 6.8 Hz, 2H, NCH₂), 2.73 (t, *J* = 6.9 Hz, 2H, NH₂CH₂), 2.01 (p, *J* = 6.9 Hz, 2H, CH₂).

Fig. S16 ¹H NMR spectrum of **9** (300 MHz, CDCl₃, 298 K).

Boc-protected compound 10: Compound **6** (0.37 g, 0.84 mmol) and **10** (0.30 g, 0.92 mmol) were dissolved in anhydrous DMF solution (10 mL), and the mixture was stirred at 25 °C for 12 h. After removing the solvent, DCM (20 mL) was added to dissolve the residue. The above solution was washed sequentially by water (2 × 15 mL) and saturated brine (15 mL), then it was dried over Na₂SO₄ and concentrated under vacuum. The crude product was purified by silica-gel chromatography (DCM/MeOH = 250:1, ν/ν) to give compound **10** as a yellow oil (0.39 g, 84% yield). M. P. 135-136 °C. ¹H NMR (300 MHz, CDCl₃, 298 K) δ (ppm) = 8.10 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.51-7.38 (m, 4H, Ar*H*), 7.26-7.20 (m, 2H, Ar*H*), 6.12 (s, 1H, N*H*), 4.85 (s, 1H, N*H*), 4.56 (s, 1H, N*H*), 4.38 (t, *J* = 6.8 Hz, 2H, NC*H*₂), 3.89 (s, 1H, NHC*H*), 3.41-3.17 (m, 2H, NHC*H*₂), 3.07 (s, 2H, NHC*H*₂), 2.20-2.01 (m, 2H, CHC*H*₂), 1.85-1.59 (m, 2H, C*H*₂), 1.48-1.23 (m, 22H, C*H*₃ and C*H*₂). ¹³C NMR (100 MHz, CDCl₃, 298 K) δ (ppm) = 172.4, 156.2, 155.9, 140.2, 125.9, 122.6, 120.5, 119.1, 108.6, 80.1, 79.2, 54.4, 40.7, 39.9, 37.5, 31.8, 29.6, 28.7, 28.5, 28.3, 22.6. HR-ESI-MS: m/z Calcd for C₃₁H₄₄N₄O₅Na [M+Na]⁺ 575.3204, found 575.3205.

 $\begin{array}{c} 8.12\\ 8.09\\ 7.50\\ 7.49\\ 7.49\\ 7.49\\ 7.49\\ 7.40\\ 7.21\\ 7.21\\ 7.21\\ 7.21\end{array}$

Fig. S17 1 H NMR spectrum of **10** (400 MHz, CDCl₃, 298 K).

Fig. S18 ¹³C NMR spectrum of **10** (100 MHz, CDCl₃, 298 K).

Model Guest molecule MG: 4N HCl/EA (0.3 mL) was added to the dichloromethane solution of

10 (0.39 g, 0.71 mmol), and the mixture was stirred at 25 °C for 2 h. After removing the solvent, the residue was dissolved in a small amount of anhydrous MeOH (1 mL), which was then added dropwise to plenty of diethyl ether (150 mL), the precipitates was collected by filtration, washed with diethyl ether, and dried in vacuum to give the target guest molecule **MG** as a brown oil (0.25 g, 84% yield). ¹H NMR (300 MHz, CD₃OD, 298 K) δ (ppm) = 8.08 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.54 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.45 (t, *J* = 7.5 Hz, 2H, Ar*H*), 7.20 (t, *J* = 7.4 Hz, 2H, Ar*H*), 4.46 (t, *J* = 7.1 Hz, 2H, NCH₂), 3.87 (t, *J* = 6.6 Hz, 1H, NH₂C*H*), 2.92 (t, *J* = 2.7 Hz, 2H, NHCH₂), 2.20-2.08 (m, 2H, NCH₂CH₂), 1.98-1.78 (m, 2H, CH₂), 1.77-1.61 (m, 2H, CH₂), 1.55-1.39 (m, 2H, CH₂). ¹³C NMR (100 MHz, CD₃OD, 298 K) δ (ppm) = 170.0, 141.6, 126.8, 124.2, 121.2, 120.0, 109.8, 54.2, 41.5, 40.2, 38.7, 32.1, 29.7, 28.1, 23.0. HR-ESI-MS: m/z Calcd for C₂₁H₂₉N₄O [M–H–2C1]⁺ 353.2336, found 353.2340.

Fig. S19 ¹H NMR spectrum of MG (400 MHz, CD₃OD, 298 K).

Fig. S20¹³C NMR spectrum of MG (100 MHz, CD₃OD, 298 K).

3. Job's Plot for WP5⊃MG

Fig. S21 (a) Fluorescence intensity of the mixture of **WP5** and **MG** in water at different molar ratio while $[WP5] + [MG] = 2 \times 10^{-6}$ M. (b) Job's Plot showing 1:1 stoichiometry of the complex between **WP5** and **MG** by plotting the difference fluorescence intensity at 368 nm against the molar fraction of **MG** at an invariant total concentration of 0.002 mM in aqueous solution.

4. Investigation of the binding constant between WP5 and MG

To determine the binding constant, ¹H NMR titration experiments were carried out in aqueous solution containing constant concentration of **MG** (3×10^{-3} M) and varying concentration of **WP5** in pD = 7.2 D₂O solution by using the same methods as that of reported by Li.^{S6} It was found that

in the different pH solutions of **WP5** and **MG**, with the addition of **WP5**, the protons of **MG** would exchange with that of **WP5**, so to determine the binding constant we should maintain the pD of the solution. Chemical shift changes of H_c of the guest **MG** upon the addition of **WP5** were measured and the binding constant was calculated from Eq.1.

 $\Delta \delta = (\Delta \delta_{\infty} / [G]_0) (0.5[H]_0 + 0.5([G]_0 + 1/K_a) - (0.5([H]_0^2 + (2[H]_0 (1/K_a - [G]_0)) + (1/K_a + [G]_0)^2)^{0.5}) Eq.1$

Where $\Delta\delta$ is the chemical shift change of H_c of the guest at [H]₀, $\Delta\delta_{\infty}$ is the chemical shift change of H_{δ} when the guest is completely complexed, [G]₀ is the fixed initial concentration of the guest, and [H]₀ is the varying concentrations of the host. By non-linear fitting the spectrum data with Eq.1, the binding constant K_a was determined to be (1.87 ± 0.38) × 10³ M⁻¹.

Figure S22. (a) Partial ¹H NMR spectra (300 MHz, D₂O, 298K) of **MG** at a concentration of 3.0 mM upon the addition of **WP5**: (1) 0 mM, (2) 0.3 mM, (3) 0.6 mM, (4) 0.9 mM, (5) 1.2 mM, (6) 1.8 mM, (7) 2.4 mM, (8) 3.0 mM, (9) 3.6 mM, (10) 4.2 mM, and (11) 4.8 mM., (b) the chemical shift changes of H_c of **MG** (3.0 mM) upon the addition of **WP5** (0-4.8 mM). The red solid line was obtained from the nonlinear curve-fitting.

5. Determination of the best molar ratio of WP5 and G leading to

aggregation

Based on the fact that when micelles or other nanoparticles was existed in the testing system, pyrene will be preferentially solubilized in the hydrophobic region of the nanoparticles, resulting a loss of fluorescence intensity. The fluorescence intensity of pyrene in solution with a fixed concentration of **G** and increasing concentration of **WP5** was then recorded to determine the best molar ratio of the supramolecular aggregates constructed by **WP5** and **G**. It was found that upon gradual addition of **WP5**, the fluorescent intensity of pyrene at 373 nm underwent a sharp decrease to the minimum at a **[G]/[WP5]** ratio of 5:1, and then an inverse increase upon further

addition of **WP5**. This decrease of the fluorescent intensity of pyrene indicated the formation of higher-order complexes between **G** and **WP5**, whereas it underwent disassembly upon further addition of **WP5**, generating a simple 1:1 inclusion complex. Thus, the best molar ratio 5:1 ([**G**]/[**WP5**]) for the formation of supramolecular aggregates was observed at the inflection point.

Fig. S23 (a) Fluorescence emission spectra of pyrene in aqueous solutions of **G** (0.04 mM) by increasing the concentration of **WP5** from 0 to 0.015 mM at 25 °C. (b) Dependence of the relative fluorescence intensity of pyrene on **WP5** concentration with a fixed concentration of **G** (0.04 mM) at 25 °C ([pyrene] = 0.001 mM).

6. Critical aggregation concentration (CAC) determination of

Fig. S24 The concentration-dependent conductivity of **G** in the presence of **WP5** ([WP5]/[G] = 1/5).

7. The pH responsibility of supramolecular vesicles formed by WP5⊃G

Fig. S25 (a) TEM image of **WP5** \supset **G** aggregates after the solution pH was adjusted to 5.0, (b) Tyndall effect of **WP5** \supset **G** aggregates (right: the solution of **WP5** \supset **G** aggregates (pH = 7.0), left: after the solution pH was adjusted to 5.0).

8. DLS result and ζ -potential of MTZ-loaded vesicles

Fig. S26 DLS results of WP5 + G aggregates after MTZ loaded. [WP5] = 0.029 mM, [G] = 0.072 mM, and [MTZ] = 0.024 mM. The average diameter is 210 nm.

Fig. S27 The zeta potential of WP5 + G aggregates after MTZ loaded. [WP5] = 0.029 mM, [G] = 0.072 mM, and [MTZ] = 0.024 mM.

9. Release of MTZ from MTZ-loaded vesicles under acidic condition

Fig. S28 UV-Vis spectra of blank vesicles, MTZ-loaded vesicles, and MTZ-loaded vesicles in pH = 5.0 solution.

10. Time-dependent UV-Vis spectra of MTZ-loaded vesicles under

different stimuli

Fig. S29 Time-dependent UV-Vis spectra of MTZ-loaded vesicles (4 mL MTZ-loaded vesicular solution was diluted to 5 mL) in (a) pure water (pH = 7.0), (b) aqueous solution containing GSH (1 mM, pH = 7.0), (c) aqueous solution containing GSH (4 mM, pH = 7.0), (d) aqueous solution containing GSH (10 mM, pH = 7.0), and (e) aqueous solution containing GSH (4 mM, pH = 5.0).

11. Images of living HepG2 cells in different incubated media

The relative *in vitro* cytotoxicities of MTZ, blank vesicles, and MTZ-loaded vesicles against HepG2 cell were assessed using MTT assay. Briefly, the cells were seeded in 96-well plates at a density of 10⁴ cells per well in 200 µL complete DMEM containing 10% fetal bovine serum, supplemented with 50 U mL⁻¹ penicillin and 50 U mL⁻¹ streptomycin, and cultured in 5% CO₂ at 37 °C for 24 h. Then the cells were exposed to serial dilutions of MTZ, blank vesicles ([G] = 5 µM and [WP5] = 2 µM), or MTZ-loaded vesicles ([G] = 5 µM and [WP5] = 2 µM), and further incubated for 24 h, 48 h, and 72 h, respectively. Then, the cells were washed and replenished with fresh culture medium, and further incubated for 2 h. Subsequently, 20 µL of MTT solution was added into each cell and incubated for another 4 h. After that, the medium containing MTT was removed and dimethyl sulfoxide (DMSO, 150 µL) was added to each well to dissolve the MTT formazan crystals. Finally, the plates were shaken for 10 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader (BioTek ELx808). Untreated cells in media were used as the blank control. All experiments were carried out with six replicates. The

cytotoxicity was expressed as the percentage of the cell viability as compared with the blank control.

Fig. S30 Images of living HepG2 cancer cells (a) incubated with blank vesicles (b), free MTZ (c), and MTZ-loaded vesicles (d) after incubating for 1d, 2d and 3d respectively.

12. References

- S1. G. Yu, M. Xue, Z. Zhang, J. Li, C. Han and F. Huang, J. Am. Chem. Soc., 2012, 134, 13248-13251.
- S2. S. J. Milner, A. Seve, A. M. Snelling, G. H. Thomas, K. G. Kerr, A. Routledge and A.-K. Duhme-Klair, Org. Biomol. Chem., 2013, 11, 3461-3468.
- S3. P. Sun, J. Wang, X. Yao, Y. Peng, X. Tu, P. Du, Z. Zheng and X. Wang, ACS Appl. Mater. Interfaces, 2014, 6, 12495-12504.
- S4. E. Hattemer, R. Zentel, E. Mecher and K. Meerholz, Macromolecules, 2000, 33, 1972-1977.
- S5. E. S. Schaffert, G. Höfner and K. T. Wanner, Bioorg. Med. Chem., 2011, 19, 6492-6504.
- S6. C. Li, J. Ma, L. Zhao, Y. Zhang, Y. Yu, X. Shu, J. Li and X. Jia, *Chem. Commun.*, 2013, 49, 1924-1926.