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Pillar[5]arene-based Supramolecular Assemblies with Two-Step Sequential Fluorescence Enhancement For Mitochondria-Targeted Cell Imaging

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1. Materials and methods

Materials

All reagents were commercially available and used as supplied without further purification. Water-soluble pillar[5]arene **WP5, 1** and **GM** was synthesized according to literature procedures.[S1,S2,S3] Solvents were either employed as purchased or dried according to procedures described in the literature.

Measurements

NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AV400 spectrometer.

Fluorescence spectroscopy. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell peltier accessory to control temperature.

UV/Vis spectroscopy. UV/Vis spectra and the optical transmittance were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

TEM microscopy. High-resolution Transmission electron microscopy (TEM) images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

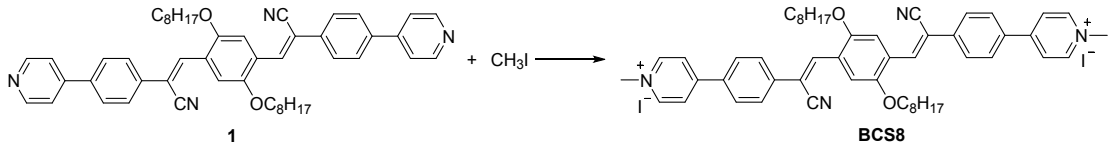
DLS spectroscopy. Solution samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90°. The hydrodynamic diameter (Dh) was determined by DLS experiments at 25°C.

ESI-MS spectroscopy. Electrospray ionization mass spectra (ESI-MS) were measured by Agilent 6520 Q-TOF-MS.

Cytotoxicity experiments. Human cervical cancer cells (HeLa cells) were incubated in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin. HeLa cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37°C in 5% CO_2. Then the cells were incubated with WP5/BCS8/SDBS/TTP ([WP5] = 20 μM, [BCS8] = 10 mM, [SDBS] = 60 μM or 10μM, [TTP] = 1 μM) for 24 h. The relative cellular viability was determined by the MTT assay.

Confocal laser scanning microscopy. HeLa cells were seeded in 6-well plates (5×10^4 cell mL^{-1}, 2 mL per well) for 24 h at 37°C in 5% CO_2. The cells were incubated with the corresponding solution for 4 h. Then the medium was removed, and the cells were washed with phosphate buffer solution for three time. Finally, the cells were subjected to observation by a confocal laser scanning microscope.

2. Synthesis of BCS8



Compound **1** (114 mg, 0.15 mmol) and methyl iodide (85 mg, 0.6 mmol) were dissolved in 50 mL toluene, and the solution was heated to reflux for 24 hours. Then the formed precipitate was filtered and washed by dichloromethane, dried in vacuo, to yield **BCS8** (108mg, 95%) as red powder. ^{1}H NMR (400 MHz, DMSO-D6) δ: 9.07 (d, *J* = 6.8 Hz, 4H, ArH), 8.59 (d, *J* = 6.9 Hz, 4H, ArH), 8.27 (d, *J* = 8.4 Hz, 6H, ArH), 8.00 (d, *J* = 8.5 Hz, 4H, ArH), 7.81 (s, 2H, CH), 4.36 (s, 6H, NCH_3), 4.15 (t, *J* = 6.4 Hz, 4H, OCH_2), 1.85-1.78 (m, 4H, CH_2), 1.48-1.20 (m, 20H, CH_2), 0.77 (t, *J* = 9.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-D6) δ: 153.4, 151.3, 146.2, 139.2, 137.3, 134.6, 129.5, 127.3, 126.3, 124.5, 117.8, 112.9, 112.0, 69.6, 47.7, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.3, 31.6, 29.1, 29.1, 28.8, 25.9, 22.5, 14.4.; IR (KBr) υ: 3708.6, 3034.1, 2925.5, 2208.2, 1736.6, 1638.1, 1577.4, 1496.3, 1425.1, 1363.8, 1289.0, 1251.9, 1209.8, 1019.3, 908.2, 819.1, 702.5, 486.7cm^{-1}; MS (*m/z*): HRMS (ESI) Calcd. for $C_{52}H_{60}IN_4O_2^+$ ($[M\text{-}I^-]^+$): 899.3755, found: 899.3762.

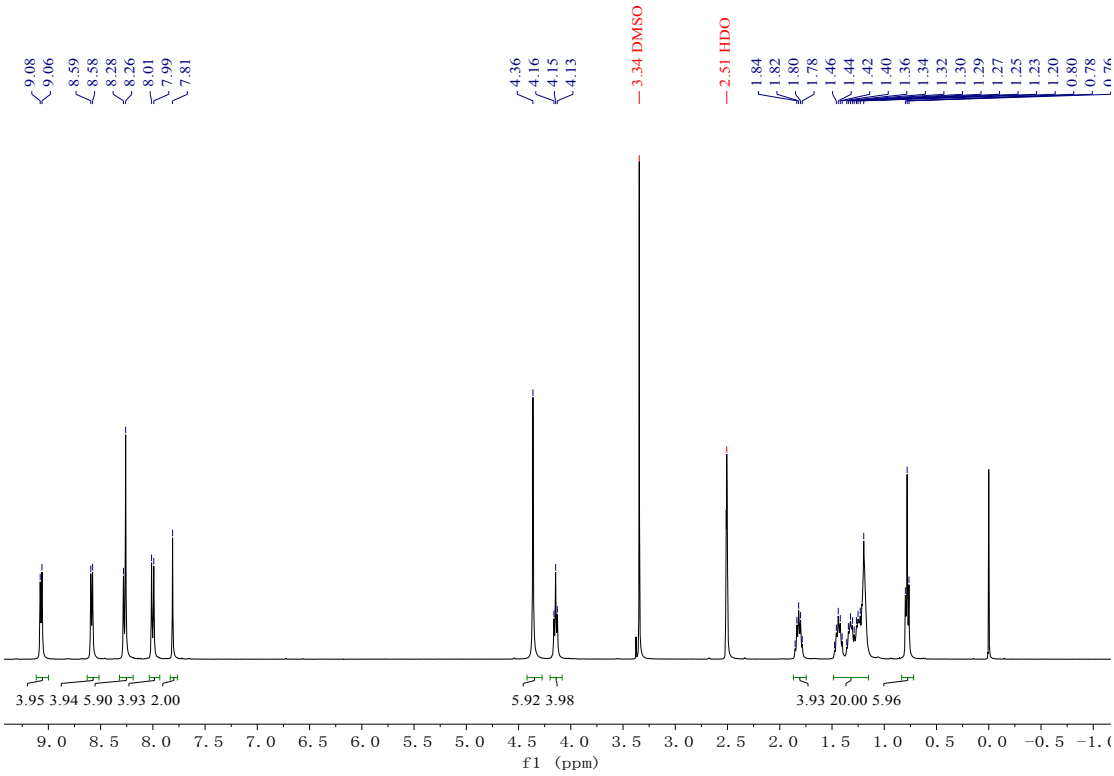


Figure S1. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 293 K) of **BCS8**.

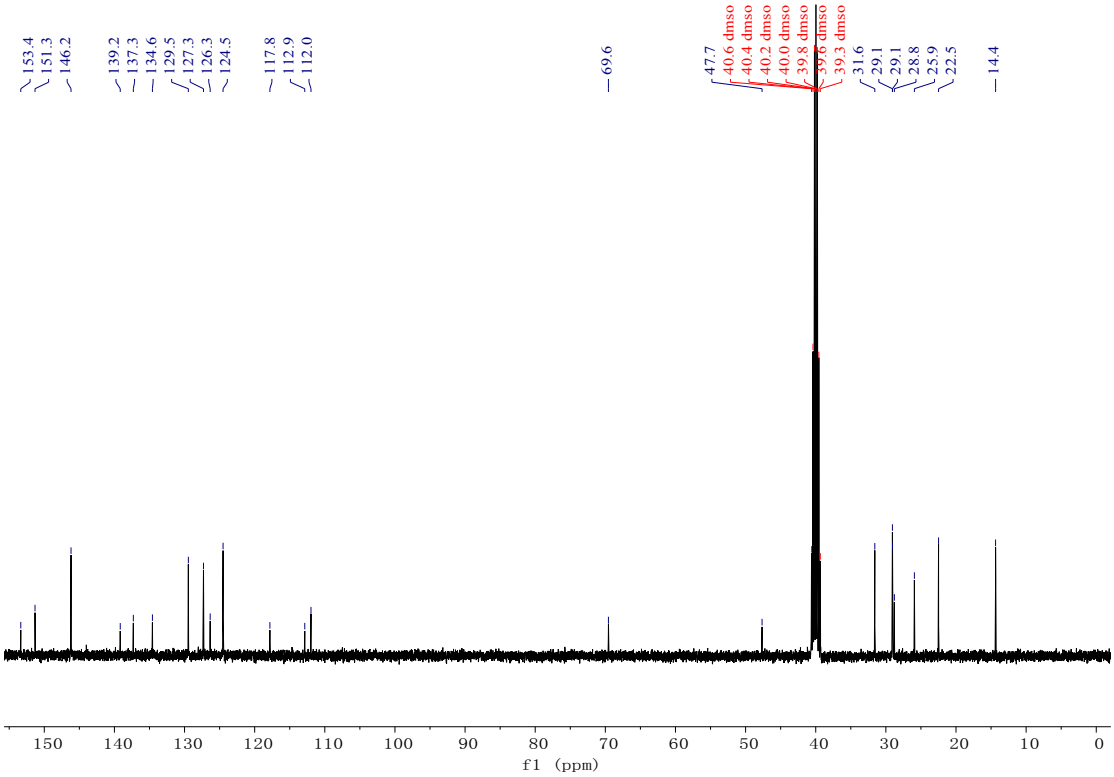


Figure S2. ^{13}C NMR spectrum (DMSO-d_6, room temperature, 100 MHz) of **BCS8**.

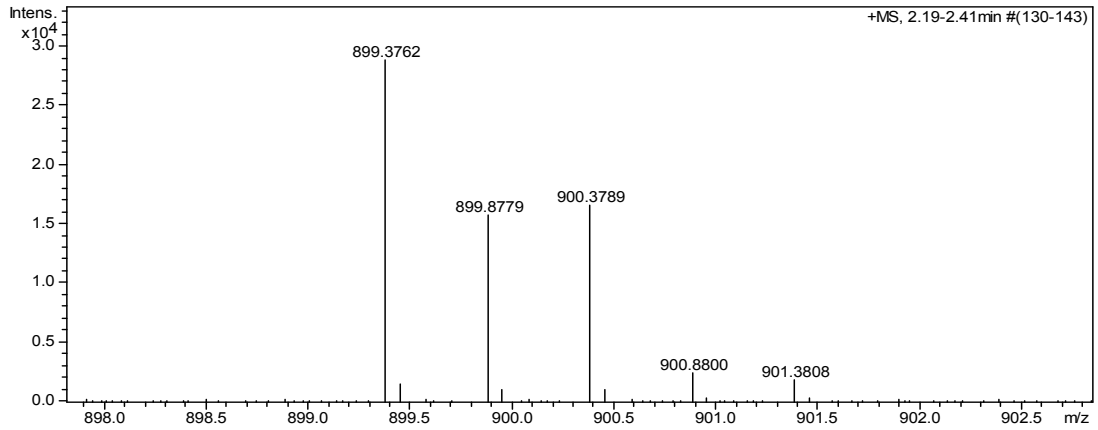


Figure S3. Mass spectra of **BCS8** Calcd. for $C_{52}H_{60}IN_4O_2^+$ ([M-I$^-$]$^+$):899.3755, found: 899.3762.

WP5: ^{1}H NMR (400 MHz, D_2O) δ: 6.62 (s, 10H, ArH), 4.16 (s, 20H, CH_2), 3.74 (s, 10H, CH_2).

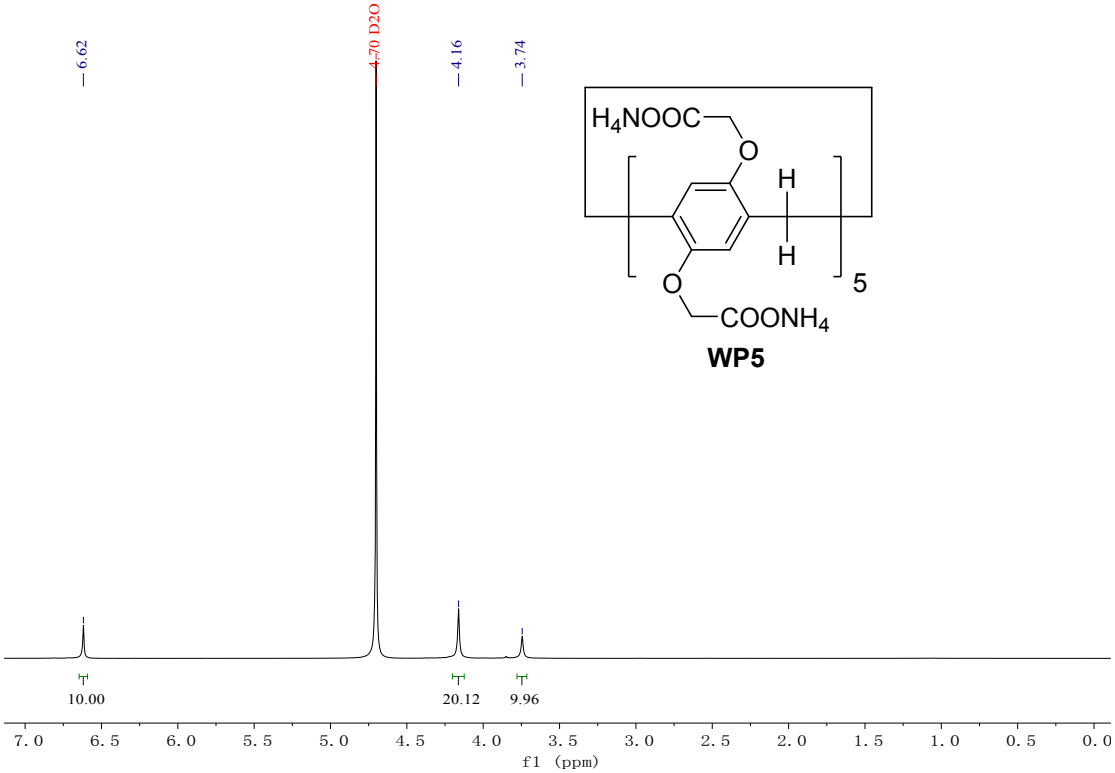


Figure S4. ^{1}H NMR spectrum (400 MHz, D_2O, 293 K) of **WP5**.

G_M: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.57 (d, J = 5.9 Hz, 2H, ArH), 8.09 (d, J = 5.4 Hz, 2H, ArH), 7.76 (d, J = 6.9 Hz, 2H, ArH), 7.53-7.46 (m, 3H, ArH), 4.21 (s, 3H, NCH_3).

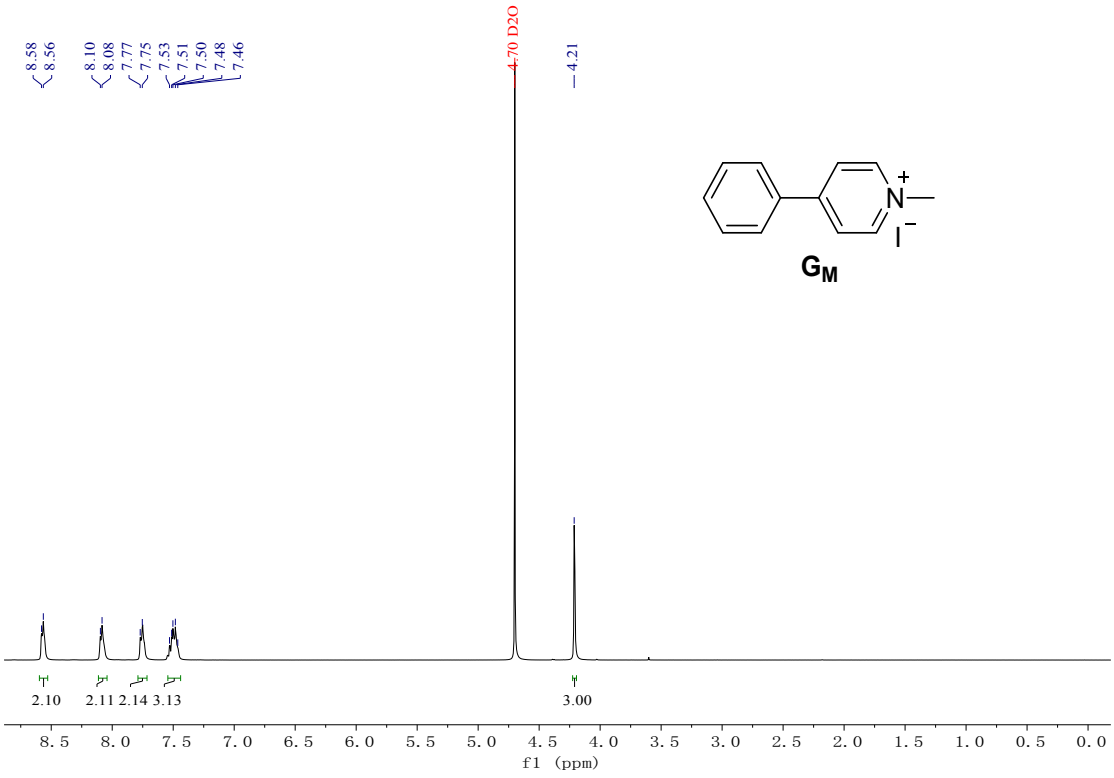


Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O, 293 K) of **G_M**.

3. **Host-guest study of WP5⊃G_M**

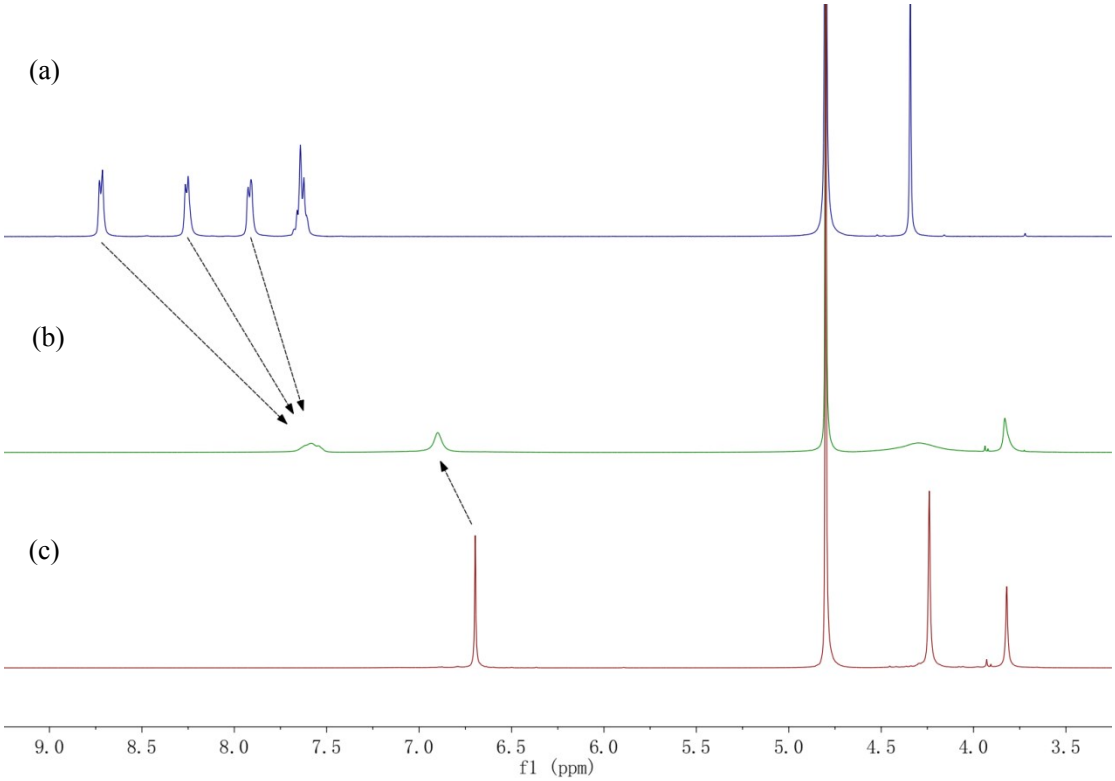


Figure S6. ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of (a) **G_M** (10.0 mM), (b) **G_M**+**WP5** ([**G_M**] = 10.0 mM, [**WP5**] =10.0 mM), and (c) **WP5** (10.0 mM)

Determination of association constant for WP5⊃BCS8

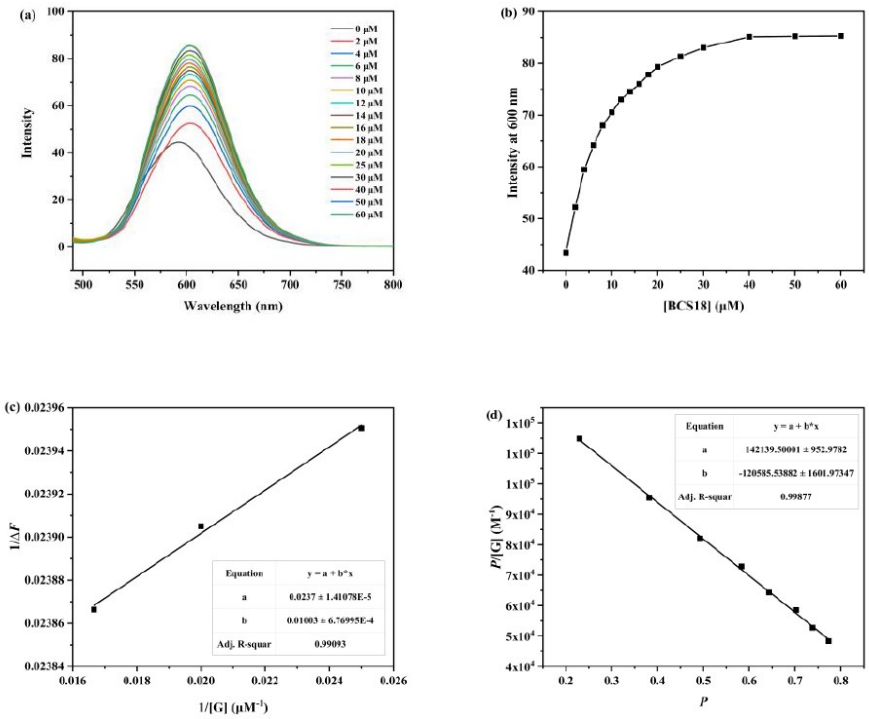


Figure S7. (a) Fluorescence spectra of **BCS8** (10 μM) in water upon addition of different concentrations of **WP5**. (b) Maximum intensity at 600nm of **BCS8/WP5** with different ratios. (c) Benesi-Hildebrand plot for complexation of **BCS8**with **WP5**. Δ_0, the difference in *F*values of **BCS8**in the uncomplexedand fully complexed species, was determined as the y-intercept of a plot of $\Delta = F - Fu$ versus $1/[\mathbf{BSC8}]_0$in the high initial concentration range of **WP5**; $\Delta_0 = 1/0.0237 = 42.19$. (d) *p* = fraction of pillararene units bound. $P = \Delta/\Delta_0$; Δ is the observed chemical shift change relative to uncomplexed species. The linear nature of this plot demonstrated that the complexation between **WP5** and **BSC8** was statistical, that is, the two 1-methylpyridin-1-iumbinding sitesbehavedindependently. From the intercept and the slope of the Scatchard plot, the averageassociation constant (K_{av}) was determined to be $1.31\times10^6 M^{-1}$for **WP5⊃BSC8**.Since K_1/K_2 = 4:1 far statistical systems (K_1= [**WP5⊃BSC8**]/{[**WP5**][**BSC8**]} and K_2 = [**WP5⊃BSC82**]/{[**WP5⊃BSC8**][**BSC8**]}, $K_1$and K_2were calculated to be $2.10 \times 10^6 M^{-1}$and $5.25 \times 10^5 M^{-1}$, respectively.

Job for WP5⊃BCS8

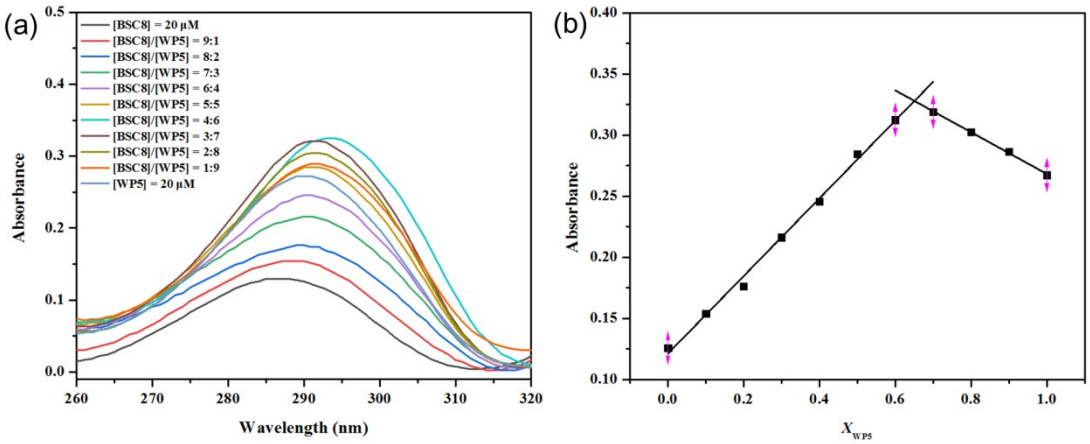


Figure S8. UV-Vis absorption spectra of complex **WP5⊃BCS8** with different molar ratios in water while [**WP5**] + [$\mathbf{G_M}$] = 10 μM. (b) Job of the complex **WP5⊃BCS8** showing a 2:1 stoichiometry between **WP5** and **BCS8** by plotting the absorbance differences at 293 nm (a characteristic absorption peak of **WP5**)against the mole fraction of **BCS8**.

4 *Self-assembly of* BCS8

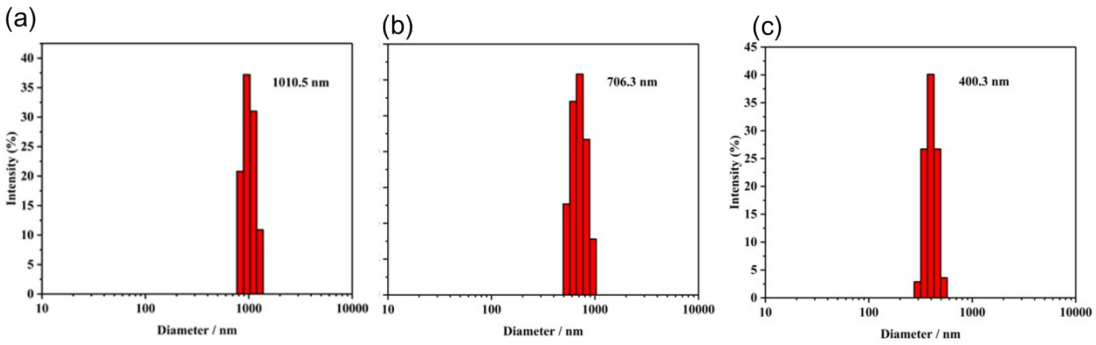


***Figure S9*. DLS** study of **BCS8** (10 μM) in DMSO/H_2O mixed solution. (a) Pure water, (b) DMO/H_2O = 1:9, (c) DMSO/H_2O = 2:8.

5. *Self-assembly of WP5⊃*BCS8

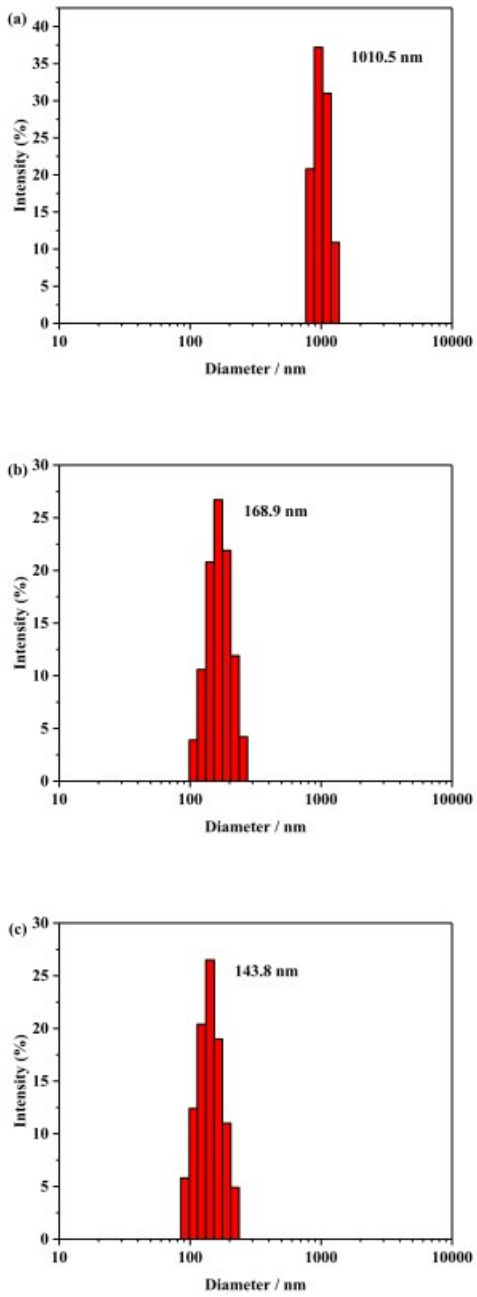


***Figure S10*. DLS** study **BCS8**, (b) **WP5/BCS8** and (c) **WP5/BCS8/SDBS** self-assembly in water. [**BCS8]** = [**SDBS]** = 1/2[**WP5]** = 10 μM,

6 *Host-guest study of **WP5⊃HDPP***

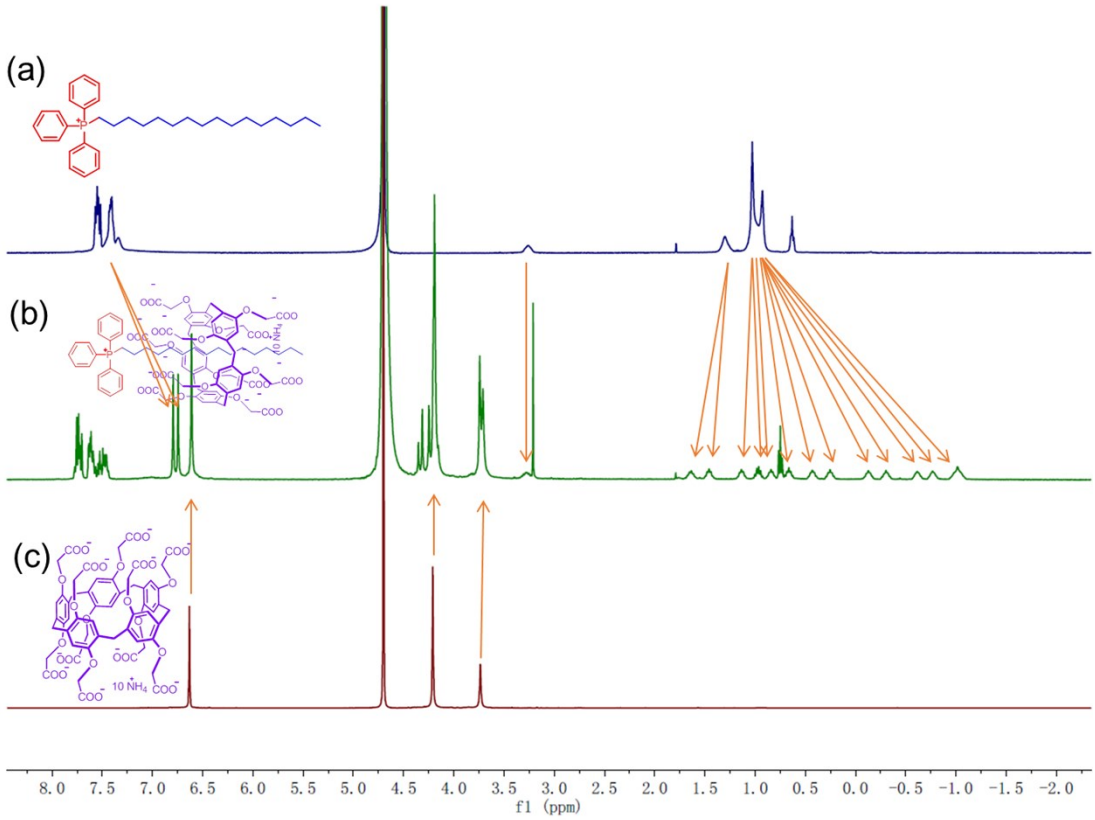


Figure S11. ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of (a) **HDPP** (10.0 mM), (b) **HDPP** +**WP5** ([**HDPP**] = 10.0 mM, [**WP5**] =10.0 mM), and (c) **WP5** (10.0 mM).

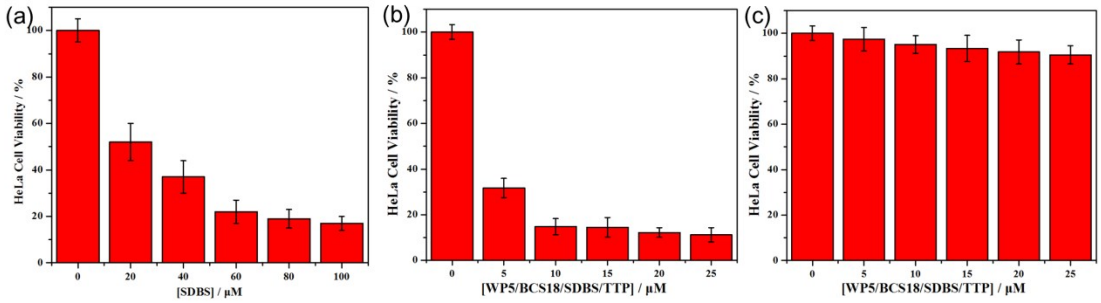


Figure S12. Viabilities of the HeLa cells determined by MTT assay after incubation with various concentrations of **SDBS** and **WP5/BCS8/SDBS/TTP** for 24 h. (a) [**SDBS**]. (b) [**BCS8**] = 1/2 [**WP5**] = 1/6 [**SDBS**] = 10 [**TTP**]; (c) [**BCS8**] = 1/2 [**WP5**] = [**SDBS**] = 10 [**TTP**].

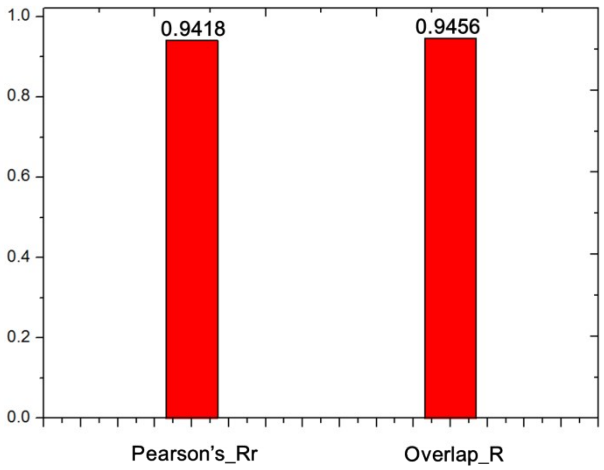


Figure S13. Pearson's_Rr and overlap coefficient between the Mito-Tracker Green and our **WP5/BCS8/SDBS/HDPP** nanoparticles calculated by "Image J".

References:

S1. T. Ogoshi, M. Hashizume, T.-a. Yamagishi, and Y. Nakamoto, *Chem. Commun.*, 2010, *46*, 3708-3710.

S2. Y. Yao, R. Zhao, Y. Shi, Y. Cai, J. Chen, S. Sun, W. Zhang, and R. Tang, *Chem. Commun.*, 2018, *54*, 8068-8071.

S3. D. Jun, M. Paar, J. Binder, J. Marek, M. Pohanka, P. Stodulka, and K. Kuca, *Lett. Org. Chem.*, 2009, *6*, 500-503.