Electronic Supplementary Information (ESI)

Mesoporous silica nanobeans dual-functionalized with AIEgens and leaning pillar[6]arene-based supramolecular switches for imaging and stimuli-responsive drug release

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1. Chemicals and instruments

Cetyl trimethyl ammonium chloride (CTAC), sodium hydroxide (NaOH), tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTMS), 3-aminopropyl trimethoxysilane (APTMS), biphenyl ketone, 4-hydroxy biphenyl ketone, 1,4-dibromobutane, potassium carbonate (K_2CO_3), potassium iodide (KI), O-phenylenediamine, mercaptoacetic acid, methyl iodide (CH_3I), glacial acetic acid (CH_3COOH), hydrochloric acid (HCl), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropanol, glutathione (GSH) and doxorubicin (DOX) were purchased from commercial sources and used without further purification. Human liver cells (L02) and hepatic carcinoma cells (HepG2) were gained as gifts from the College of Chemistry and College of Life Science at Jilin University. Fetal bovine serum and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Thermo Fisher Scientific. ^1H NMR spectra were collected on a Bruker AVANCE III 300 MHz NMR spectrometer. ^{13}C NMR spectra were collected on a Bruker-600 MHz NMR spectrometer. Electrospray mass spectrometric analysis (ESI-MS) was performed on a Bruker Agilent1290-microTOF Q II Mass Spectrometer. UV-vis spectra and fluorescence spectra were collected on Japan Shimadzu UV-2550 and Shimadzu FR-5301PC spectrometers, respectively. X-ray diffraction (XRD) measurements were carried out using a PANalytical B.V. Empyrean powder diffractometer. Fourier transform infrared (FT-IR) spectra were recorded on a Vertex 80 V spectrometer. Scanning electron microscope (SEM) images were collected on a HITACHI SU8082 instrument. Transmission electron microscopy (TEM) images were collected on a Tecnai G2 S-Twin F20 instrument at an accelerating voltage of 200 kV. Thermogravimetric analysis (TGA) was performed under an air atmosphere with a heating rate of 10 ºC/min by using a NETZSCH STA499F3 QMS403D thermogravimetric analyzer. Nitrogen-sorption analysis was performed on a Micromeritics

3Flex analyzer. The specific surface area was calculated from the adsorption branch using Brunauer-Emmett-Teller (BET) method.

2. Experimental Section

Preparation of MSNB: CTAC (0.2 g), H_2O (96 mL), and NaOH (0.875 mL, 2M) were mixed in a 250 mL round-bottom flask and the resulting mixture was stirred (500 rpm) at 80 ºC for 30 min under N_2 protection. TEOS (900 μL), MPTMS (70 μL), and APTMS (40 μL) were mixed together, which according to that MPTMS added into TEOS and mixed thoroughly for ca. 30 second, after that APTMS was added into the mixture and mixed thoroughly. The mixture was then added into the previous reaction mixture. After another 2 hours' reaction, a white product was obtained, and purified by centrifugation (10000 rpm, 10 min), and washing with water and ethanol for thrice.

Preparation of MSNS: CTAC (0.2 g), H_2O (96 mL) and NaOH (0.875 mL, 2M) were mixed in a 250 mL round-bottom flask and stirred (500 rpm) at 80 ºC for 30 min under N_2 atmosphere. Then, tetraethylorthosilicate (TEOS, 900 μL) was firstly added into the surfactant solution. Next, after 30 seconds, APTMS (40 μL) was added into the mixture. And finally, after stirring the reaction mixture for 30 seconds, MPTMS (70 μL) was added. After 2 hours reaction, a white product was obtained by centrifugation (10000 rpm, 10 min) and washed with water and ethanol thrice.

Preparation of MSNR: CTAC (0.2 g), H_2O (96 mL) and NaOH (0.875 mL, 2M) were mixed in a 250 mL round-bottom flask and stirred (500 rpm) at 80 ºC for 30 min under N_2 atmosphere. Then, tetraethylorthosilicate (TEOS, 900 μL) was firstly added into the surfactant solution. Next, after 30 seconds, MPTMS (70 μL) was added into the mixture. And finally, after stirring the reaction mixture for

30 seconds, APTMS (40 μL) was added. After 2 hours reaction, a white product was obtained by centrifugation (10000 rpm, 10 min) and washed with water and ethanol for thrice.

Preparation of TPE-OH: 1-(4-Hydroxyphenyl)-1,2,2-triphenylethylene (TPE-OH) was prepared according to literature procedure. In brief, zinc powder (3.1 g, 48 mmol) and $TiCl_4$ (2.7 mL, 24 mmol) were added into anhydrous THF (80 mL) under N_2 atmosphere in ice bath. After being stirred for 15 min, the mixture was warmed to 70 °C and reacted for 2.5 h. The mixture was transferred to an ice bath again and reacted for 15 min. Then, a solution of biphenyl ketone (0.9 g, 4.8 mmol) and 4-hydroxy biphenyl ketone (1.1 g, 5.8 mmol) in THF (30 mL) was slowly added. Afterward, the reaction mixture was refluxed for 24 h, followed by quenching with 10% K_2CO_3 aqueous solution. After filtration, crude product was obtained, and black solid was extracted by CH_2Cl_2. Finally, the pure product was obtained by column chromatography (ethyl acetate/petroleum ether = 1/10, v/v). Yield: 0.4 g, 24.6%. ^{1}H NMR (300 MHz, DMSO-*d6*): 9.36 (s, 1H), 7.15-7.08 (m, 9H), 6.98-6.91 (m, 6H), 6.75-6.72 (m, 2H), 6.51-6.48 (d, 2H).

Preparation of 1-(4-bromobutoxy)-4-(1,2,2-triphenylethenyl)benzene (TPE-Br): TPE-OH (650 mg, 1.9 mmol) and K_2CO_3 (334 mg, 0.74 mmol) and CH_3CN (40 mL) were added into a 100 mL flask. Afterward, 1,4-dibromobutane (0.45 mL, 3.8 mmol) was added into the solution and the reaction mixture was heated at reflux for 72 h. Finally, the product was obtained by column chromatography (ethyl acetate/petroleum ether = 1/5, v/v). Yield: 262.2 mg, 28.5%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.26-7.02 (m, 15H), 6.93-6.91 (m, 2H), 6.63-6.60 (d, 2H), 3.93-3.89 (t, 2H), 3.49-3.45 (t, 2H), 2.04-1.89 (m, 4H).

Preparation of 2-(mercaptomethyl)benzimidazole (BM-SH): O-Phenylenediamine (2.2 g, 20 mmol) and mercaptoacetic acid (2.3 g, 25.0 mmol) were mixed with aqueous HCl solution (30 mL, 4 M), and the

mixture was refluxed under N_2 atmosphere for 6 h. After the reaction, a saturated NaOH solution was gradually added into the mixture, resulting in a solution pH of 8~9. The solid was collected by suction filtration, and washed with deionized water to afford MBM-SH. Yield: 2.3 g, 68.6%. ^{1}H NMR (300 MHz, DMSO-*d6*): 7.49 (m, 2H), 7.15 (m, 2H), 3.92 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*): δ (ppm): 154.13, 122.06, 21.59. HR-MS (ESI): m/z calculated for [M] 164.0403, found m/z $[M+H]^+$ 165.0428, $[M-S-H]^-$ 131.0549.

Preparation of 1,2,3-(trimethyl)benzimidazole iodide (MBM-CH_3): 2-Methylbenzimidazole (264.3 mg, 2 mmol), NaH (72 mg, 3 mmol) and catalytic quantity of KI were added into a flask contains CH_3CN (30 mL). The mixture was stirred under N_2 for 30 min, followed by the injection of CH_3I (1 mL, 16 mmol). The reaction mixture was heated at reflux for 72 h, and a crud product was obtained by rotary evaporation. Then, after being washed with CH_2Cl_2 for 3 times, the product was obtained by filtration. Yield: 540.2 mg, 93.7%. ^{1}H NMR (300 MHz, D_2O): 7.75 (m, 2H), 7.61 (m, 2H), 3.95 (s, 6H), 2.83 (s, 3H).

Synthesis of carboxylated leaning pillar[6]arene (CLP6): LP6-1 (1.7 mmol, 2.3 g) was prepared according to our previous report and dissolved in THF (100 mL). Then sodium hydroxide solution (20% wt, 50 mL) was added, and the above mixture was heated at reflux for 24 h. Upon cooling the mixture to room temperature and being concentrated to a minimum volume, the residue was acidified with HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried under vacuum to give CLP6 as a white solid (1.8g, 91%). ^{1}H NMR (300 MHz, DMSO-*d6*): 12.90 (s, 9H), 7.04 (s, 4H), 6.97 (s, 8H), 6.67 (s, 4H), 4.52 (s, 8H), 4.51 (s, 8H), 4.12–3.62 (m, 12H).

Synthesis of anionic water soluble carboxylated leaning pillar[6]arene (AWLP6): Carboxylated leaning pillar[6]arene (1 mmol, 1.13 g) and NaOH (8 mmol, 320 mg) were dissolved in deionized H_2O (15 mL). After refluxing the reaction mixture for 12h followed by water removal under vacuum, WLP6 was obtained (1.3g, 99%). ^{1}H NMR (300 MHz, D_2O) δ 7.00 (s, 8H), 6.88 (s, 4H), 6.67 (s, 4H), 4.27 (s, 8H), 4.19 (s, 8H), 3.92 (s, 12H).

Synthesis of MSNB-TPE: MSNB (200 mg), TPE-Br (20 mg), K_2CO_3 (70 mg), and KI (5 mg) were mixed in CH_3CN, and the resulting reaction mixture was stirred for 30 min under N_2 protection. The reaction mixture was heated at reflux for 72 h. The obtained crude product was washed with EtOH and CH_2Cl_2 for three times to afford the pure product.

Synthesis of MSNB-TPE-Py: MSNB-TPE (200 mg) and 2-2'-dithiodipyridine (200 mg) were dispersed in methanol (30 mL). Glacial acetic acid (1 mL) was then added dropwise into the reaction mixture. After being stirred in a N_2 atmosphere for 24 h at room temperature, the reaction mixture was filtrated to give a white product, which was further washed with deionized water and ethanol.

Synthesis of MSNB-TPE-BM: MSNB-TPE-Py (200 mg) and MB-SH (200 mg) were dispersed in methanol (30 mL). Then CH_3COOH (1 mL) was added into the mixture, and the reaction was run in a N_2 atmosphere at room temperature for 24 h. Crude product was collected by centrifugation (10000 rpm, 10 min) which was washed with deionized water and ethanol for three times to give the final product after drying.

Synthesis of MSNB-TPE-MBM: MSNB-TPE-BM (200 mg), K_2CO_3 (70 mg) and CH_3I (1 mL) were mixed with CH_3CN (30 mL). The mixture was heated at 40 ºC for 48 h under N_2 atmosphere. The solid product was collected by filtration, followed by washing with deionized water and ethanol for three times.

DOX loading: Doxorubicin (49 mg, 9×10^{-5} M) and MSNB-TPE-MBM were dispersed in ultrapure water (30 mL). DOX@MSNB-TPE-MBM was obtained after the mixture was stirred at room temperature for 24 h.

Preparation of DF-MSNB and DOX@DF-MSNB: MSNB-TPE-MBM (or DOX@MSNB-TPE-MBM) (30 mg) and WLP6 (10 mg) were added into ultrapure water (15 mL). The resulting mixture was stirred at room temperature for 24 h to afford DF-MSNB or DOX@DF-MSNB.

In-vitro release of DOX@DF-MSNB: The DOX@DF-MSNB (0.5 mg) was loaded into a dialysis bag and placed into a cuvette, after that, PBS solutions (3 mL) with different concentration of GSH (0 mM, 5 mM and 10 mM) or different pH (5.0 and 7.4) were added into the cuvette respectively to active the drug release. The releasing behaviors were monitored by UV-vis spectra, and maximum absorbance of DOX at 488 nm were recorded to calculate the real-time releasing amount according to a previous report.[S1] The DOX@DF-MSNB (0.5 mg) were treated by 20 mM GSH for 5 days to calculate the complete release (λ = 488 nm, A = 0.58).

Electrochemical measurement: Electrochemical measurements of MSNB-TPE-MBM, MSNB-TPE-MBM-GSH, DF-MSNB and DF-MSNB-GSH were carried out at room temperature in a three-electrode cell. Pt foil was used as the counter electrode, and Ag/AgCl (saturated KCl solution) was selected as reference electrode in 1 M KCl solution. Samples (4 mg) were dispersed in mixed solution

(H_2O/C_2H_5OH = 9/10, v/v) containing 50 μL of Nafion solution. After the suspension was placed under ultrasonic treatment for 30 minutes, 30 μL was taken out and coated onto the glassy-carbon electrode (GCE) followed by drying naturally in air. The scanning rate is 100 mV/s.

Cell culture: The human liver cells (L02) and hepatic carcinoma cells (HepG2) were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 4500 mg/L D-glucose, 110 mg/L sodium pyruvate, and L-glutamine. The HepG2 cells were propagated in DMEM containing penicillin, streptomycin, and bovine insulin (0.01 mg/mL). Cells were cultured in a humidified incubator at 37 ºC in 5% CO_2 atmosphere.

Cytotoxicity assay: Cells were seeded in 96-well plates. After incubated with each group of materials for 24 hours, each well was washed with 1 × PBS for three times and treated with 30 μL MTT (5 μg/μL) for 2 h. Resulted products were dissolved in acidic isopropanol, and a Synergy μQuant microtiter plate reader was used to record the absorbance. The experiments were performed in triplicates.

Flow cytometric analysis: HepG2 cells were seeded in 6-well plates and cultured in DMEM medium for 24 hours at first. Then DF-MSNB, DOX and DOX@DF-MSNB which have been disinfected by ethanol (75%) were added into the wells separately and incubated at 37 °C for 24 hour. After incubation, centrifuge to gather the cells and washed with PBS solution for twice. Remove the supernatants and resuspend the cells in binding buffer, after that, using an Annexin V-FITC apoptosis detection kit to determine the cell apoptosis study.

3. Characterizations

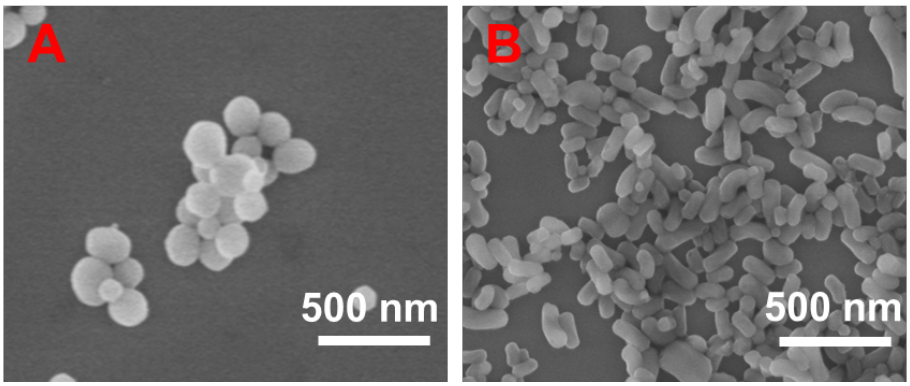

Figure S1. SEM images of (A) sphere-like mesoporous silica nanoparticles that prepared according to co-condensation of TEOS and APTMS and (B) rod-like mesoporous silica nanoparticles that prepared according to co-condensation of TEOS and MPTMS.

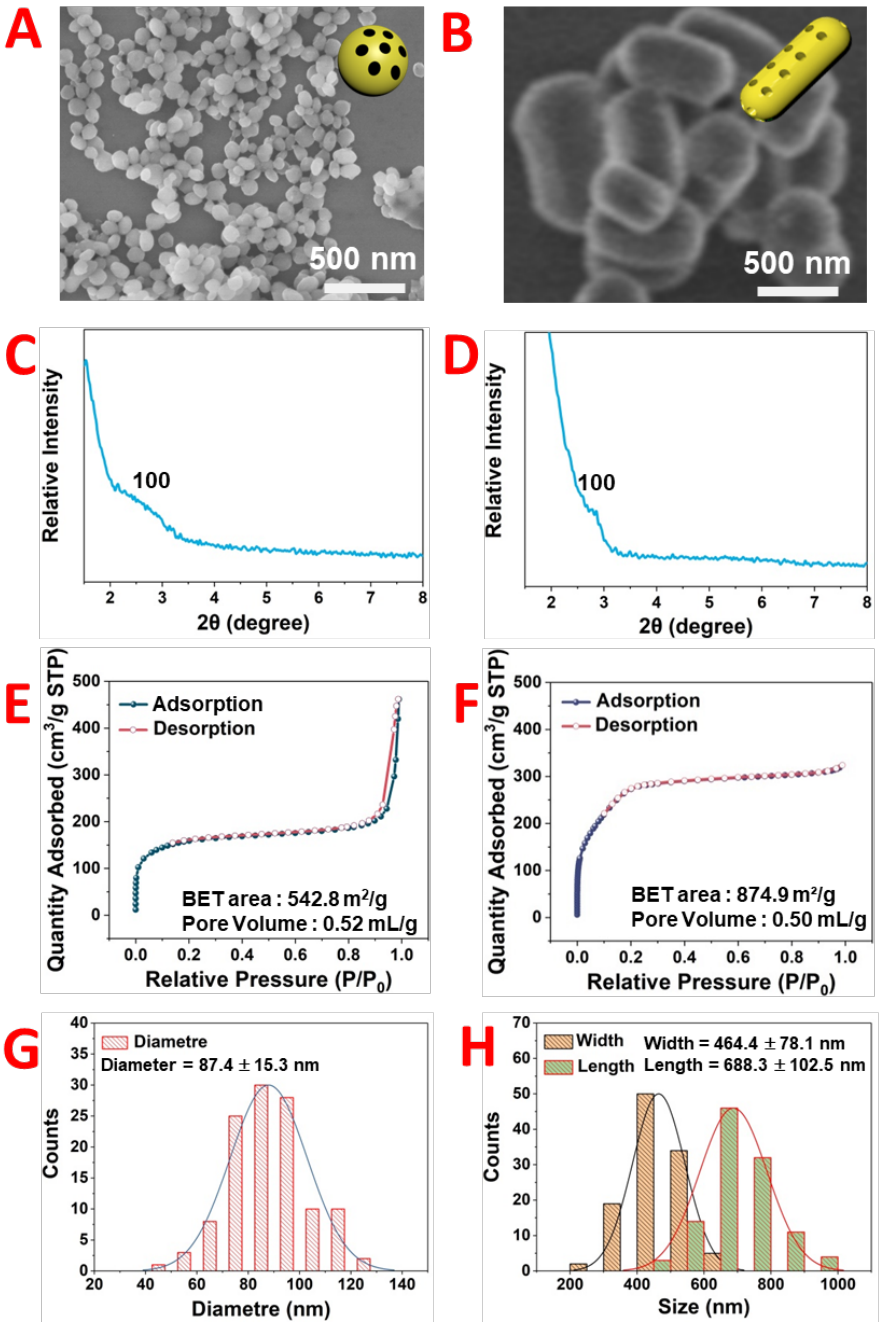

Figure S2. SEM images of (A) MSNS and (B) MSNR; XRD patterns of (C) MSNS and (D) MSNR and N_2 adsorption-desorption isotherms of (E) MSNS and (F) MSNR. Size distribution diagrams of (G) MSNS based on 117 particles and (H) MSNR based on 110 particles.

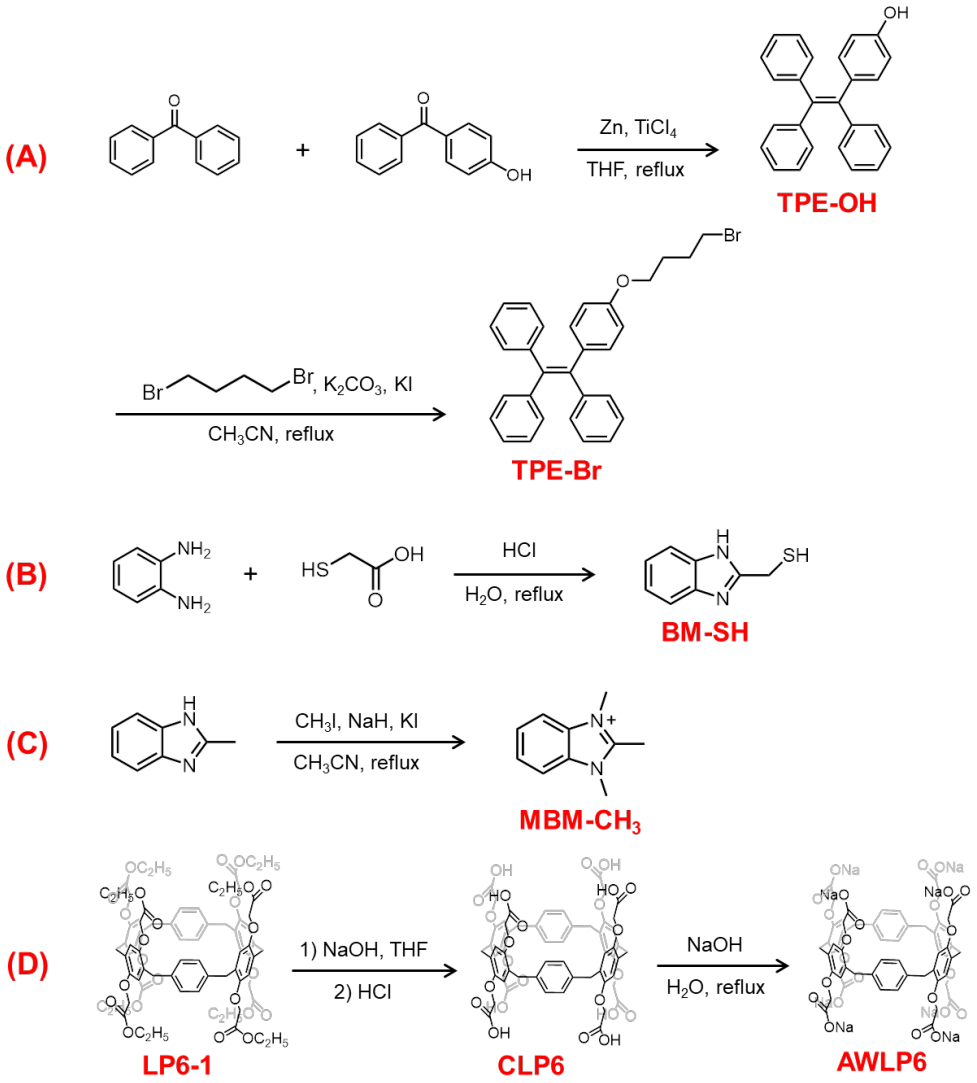

Figure S3. Synthetic routes of (A) TPE-Br, (B) BM-SH, (C) MBM-CH_3, and (D) AWLP6.

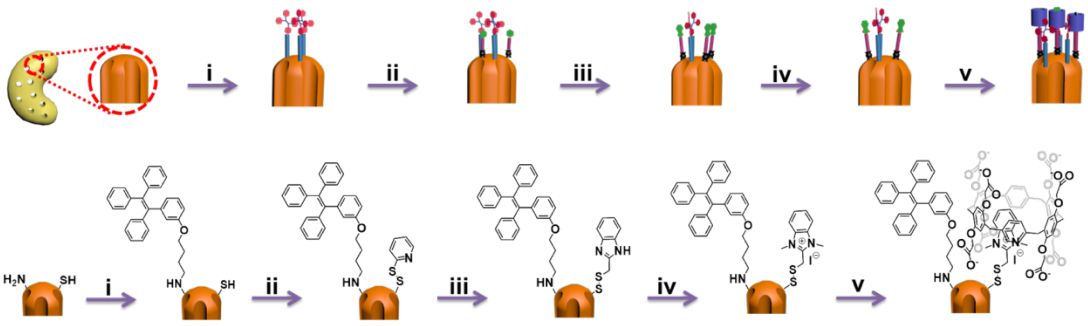

Figure S4. Synthetic route to i) MSNB-TPE, ii) MSNB-TPE-Py, iii) MSNB-TPE-BM, iv) MSNB-TPE-MBM, and v) DF-MSNB.

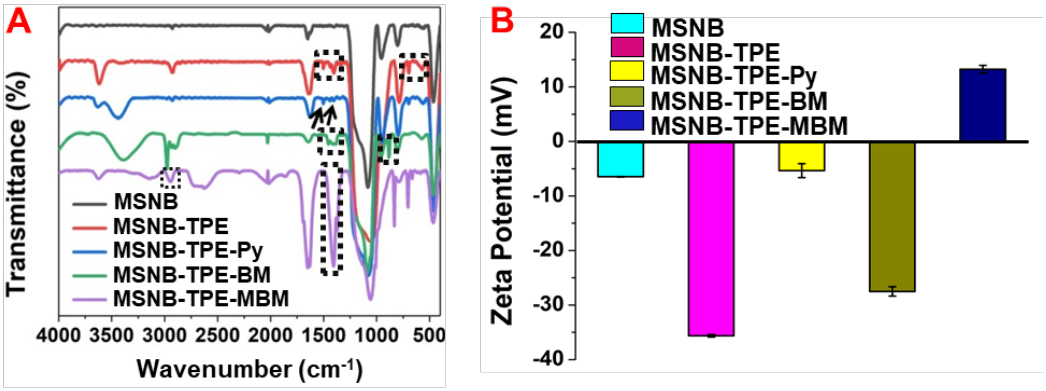

Figure S5. (A) FT-IR spectra and (B) Zeta potentials (in deionized water) of MSNB, MSNB-TPE, MSNB-TPE-Py, MSNB-TPE-BM, and MSNB-TPE-MBM.

In the FT-IR spectra, peaks of C-H bending vibration at 692 cm^{-1}, and C=C antisymmetric stretches at 1508 cm^{-1} and 1404 cm^{-1} confirmed the surface functionalization of MSNB with TPE entity.[S2] Additionally, the successful preparation of MSNB-TPE-Py was confirmed by the appearance of C=N stretching of –Py at 1348 cm^{-1} and 1440 cm^{-1}. New peak of N-H stretching at 887 cm^{-1} of MSNB-TPE-BM (BM = benzimidazole) indicates the existence of BM, and besides, the peak intensity between 1300 cm^{-1} and 1600 cm^{-1} were much stronger than before, ascribing to the vibrations of BM rings.[S1] Finally, the C-N stretching at 1410 cm^{-1} and C-H stretching of $–CH_3$ at 2947 cm^{-1} further proved that $–CH_3$ was successfully connected with N atom of BM and the MSNB-TPE-MBM was successfully prepared. Since the introduction of –SH can result in a more negative zeta potential of nanoparticles,[S3] MSNB exhibited a negative zeta potential of -6.5 ± 0.1 mV due to the excess coverage of –SH groups compared with $–NH_2$ groups, while the zeta potential of MSNB-TPE was measured to be -35.6 ± 0.2 mV, obviously much more negative than that of MSNB, indicating that abundant TPE units were reacted with $–NH_2$ groups on the

surface of MSNB to make the overall system more negatively charged. MSNB-TPE-Py possessed a zeta potential of -5.4 ± 1.3 mV, and the big increase in zeta potential value can be attributed to the covalent attachment of pyridine group via disulfide bond formation. It was demonstrated that materials with lower pKa will get higher zeta potential value under the same condition, and after the further decoration with BM via S-S bond exchange, the zeta potential of MSNB-TPE-BM turned to be more negative (-27.5 ± 0.9 mV) due to the higher pKa of BM (5.5) as compared with Py (5.2).[S4-S6] Finally, because of the formation of benzimidazole salt, the zeta potential of MSNB-TPE-MBM turned to be a positive value of 13.2 ± 0.7 mV. Positively-charged MBM could form inclusion complex with the electron-rich cavity of AWLP6, resulting in a pseudorotaxane-type supramolecular nanovalve (MBM⊂AWLP6), representing the first supramolecular nanovalve based on leaning pillar[6]arenes.

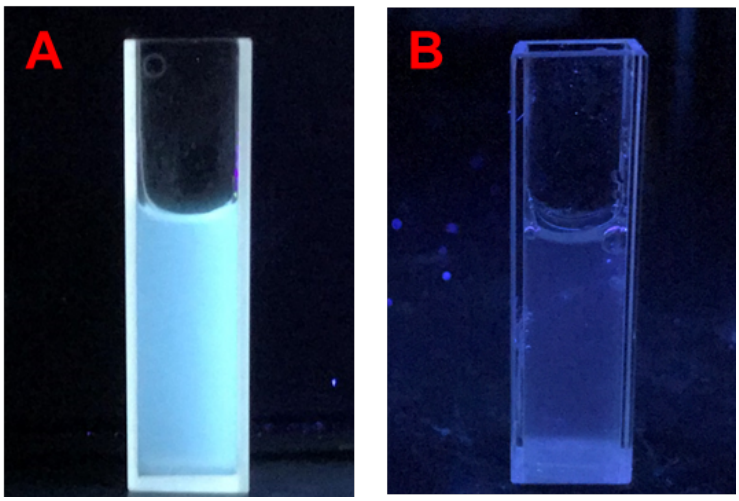

Figure S6. Digital photos of (A) MSNB-TPE and (B) MSNB without TPE decoration in deionized water solution at 365 nm excitation. (Concentration = 0.5 mg/mL).

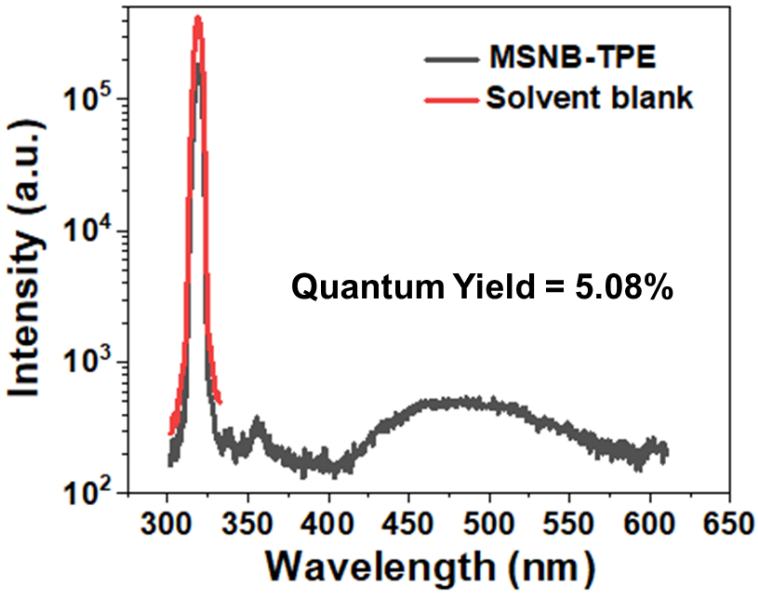

Figure S7. Fluorescence quantum yield of MSB-TPE in deionized water (concentration = 0.25 mg/mL).

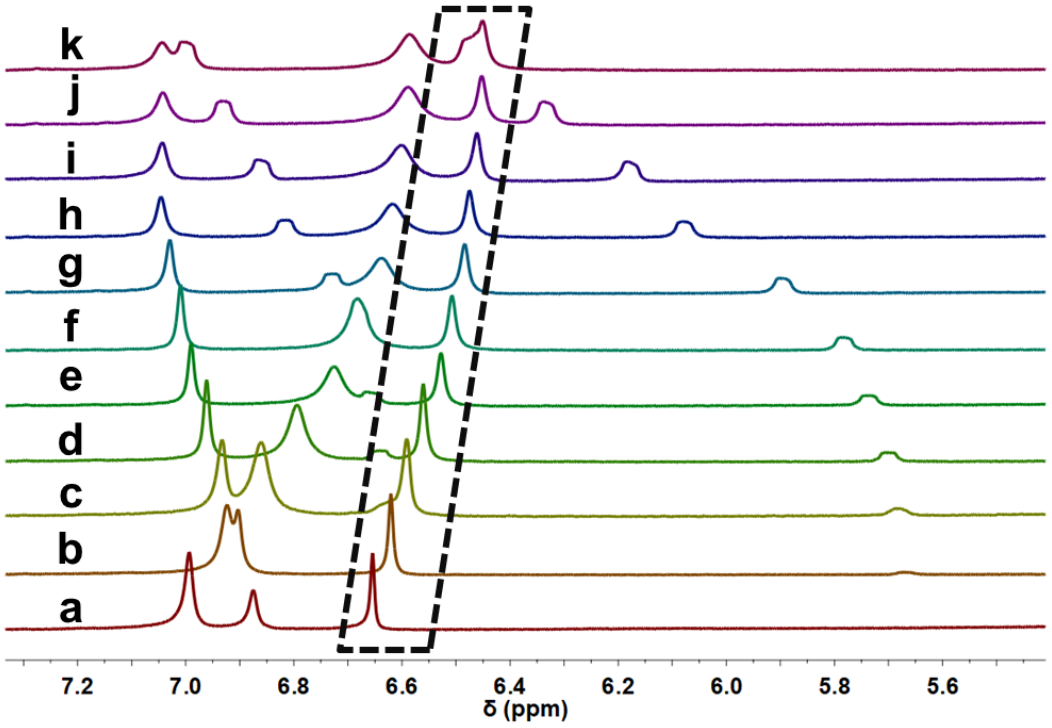

Figure S8. ^{1}H NMR spectra (300 MHz, D_2O, 298 K) of AWLP6 (6.0 mM) upon addition of MBM-CH_3: a) 0 mM, b) 1.2 mM, c) 2.4 mM, d) 3.6 mM, e) 4.8 mM, f) 6.0 mM, g) 7.2 mM, h) 8.4 mM, i) 9.6 mM, j) 10.8 mM, and k) 12.0 mM.

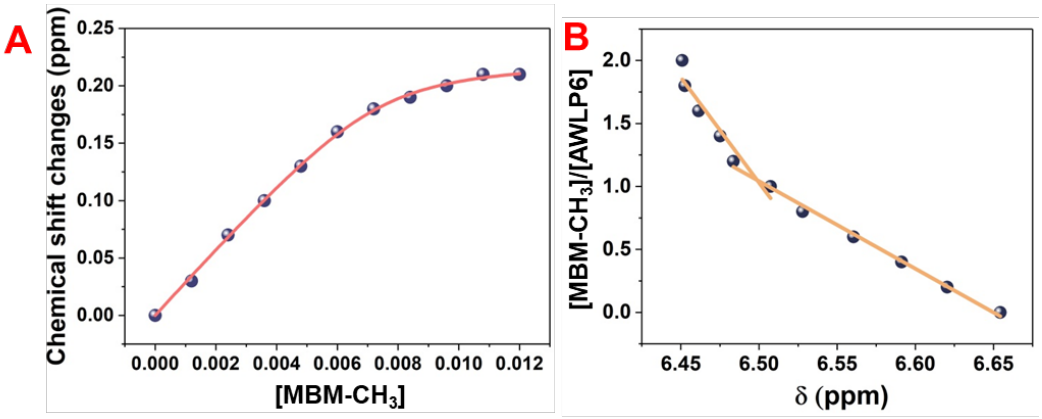

Figure S9. (A) Nonlinear least-squares analysis for the calculation of association constant (K_a). (B) Molar ratio plot for the complexation of AWLP6 and MBM-CH_3, indicating a 1:1 binding stoichiometry.

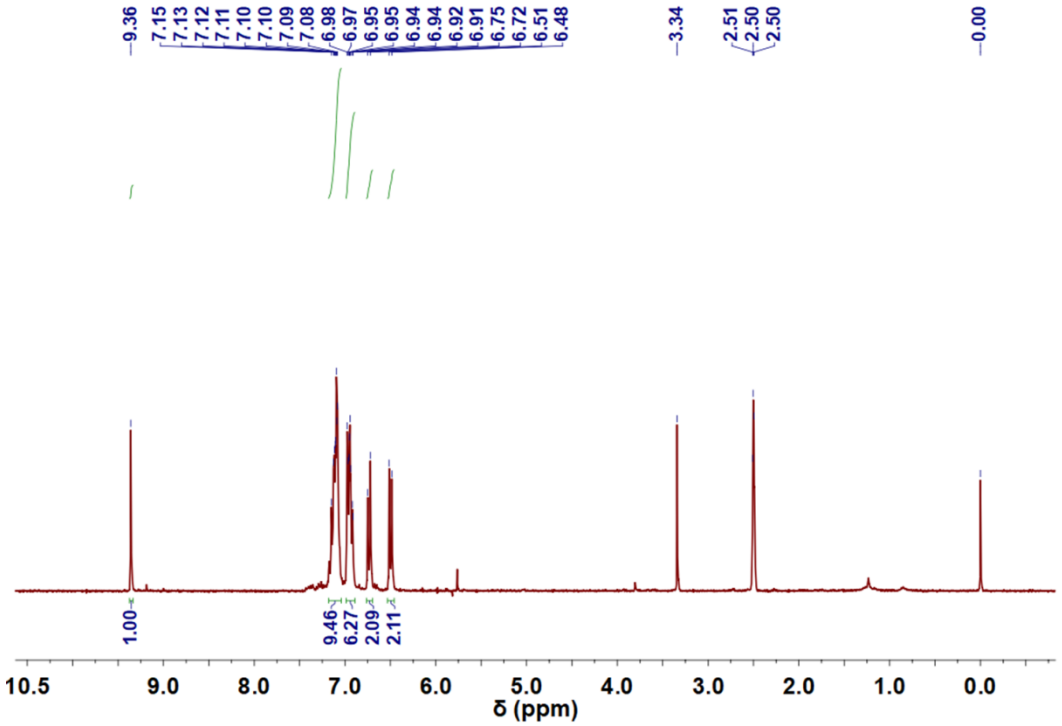

Figure S10. ^{1}H NMR (300 MHz, 298K) spectrum of TPE-OH in DMSO-*d6*.

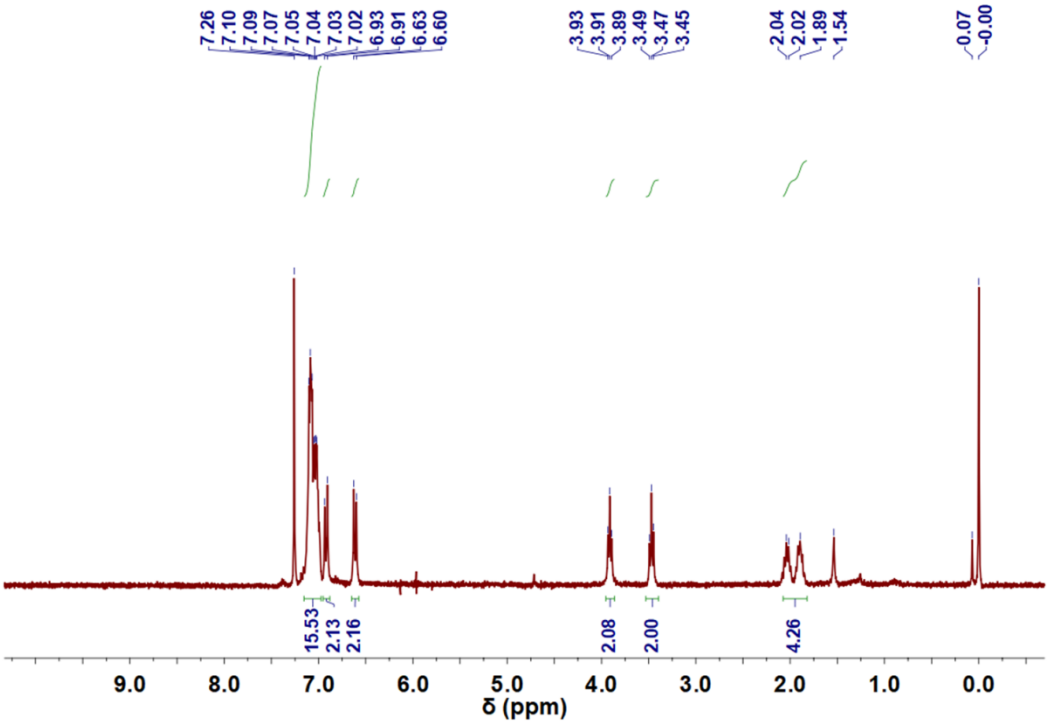

Figure S11. ^{1}H NMR (300 MHz, 298K) spectrum of TPE-Br in $CDCl_3$.

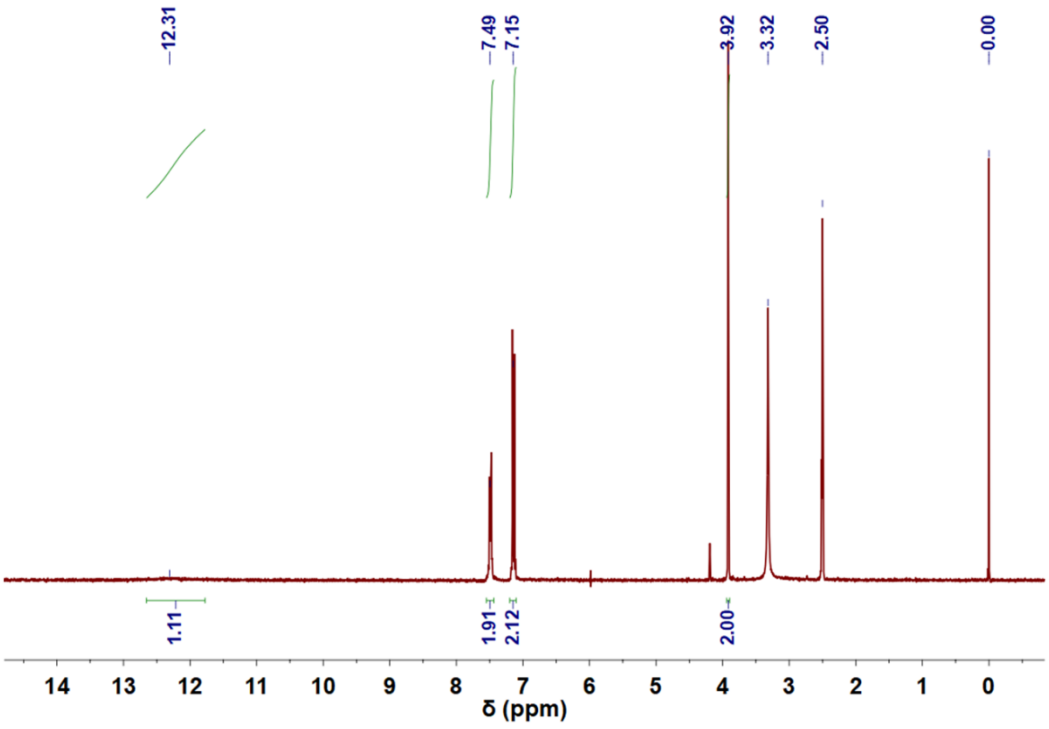

Figure S12. ^{1}H NMR (300 MHz, 298K) spectrum of BM-SH in DMSO-*d6*.

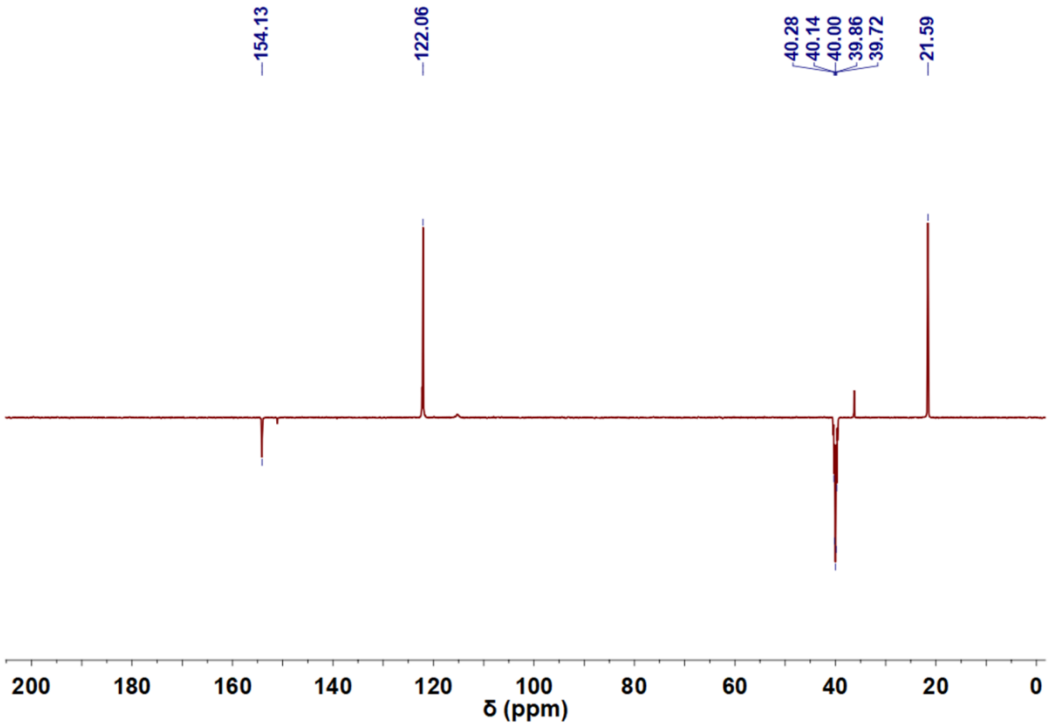

Figure S13. DEPTQ ^{13}C NMR (151 MHz, 298K) spectrum of BM-SH in DMSO-*d6*.

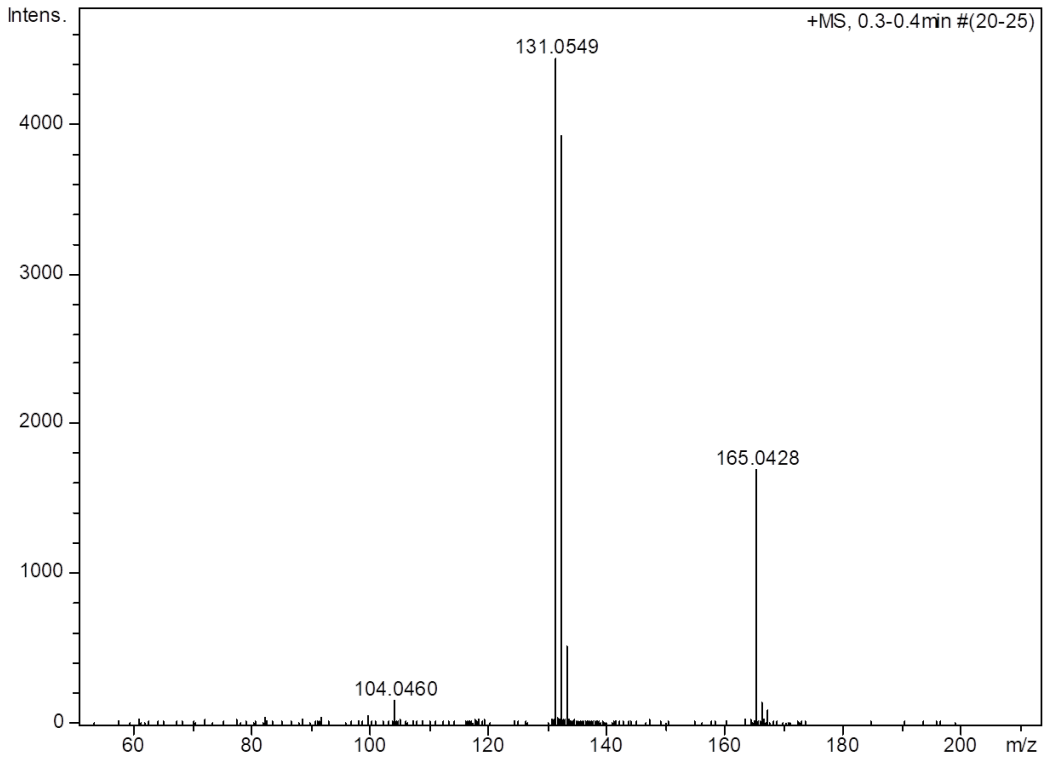

Figure S14. HR-MS (ESI) spectrum of BM-SH.

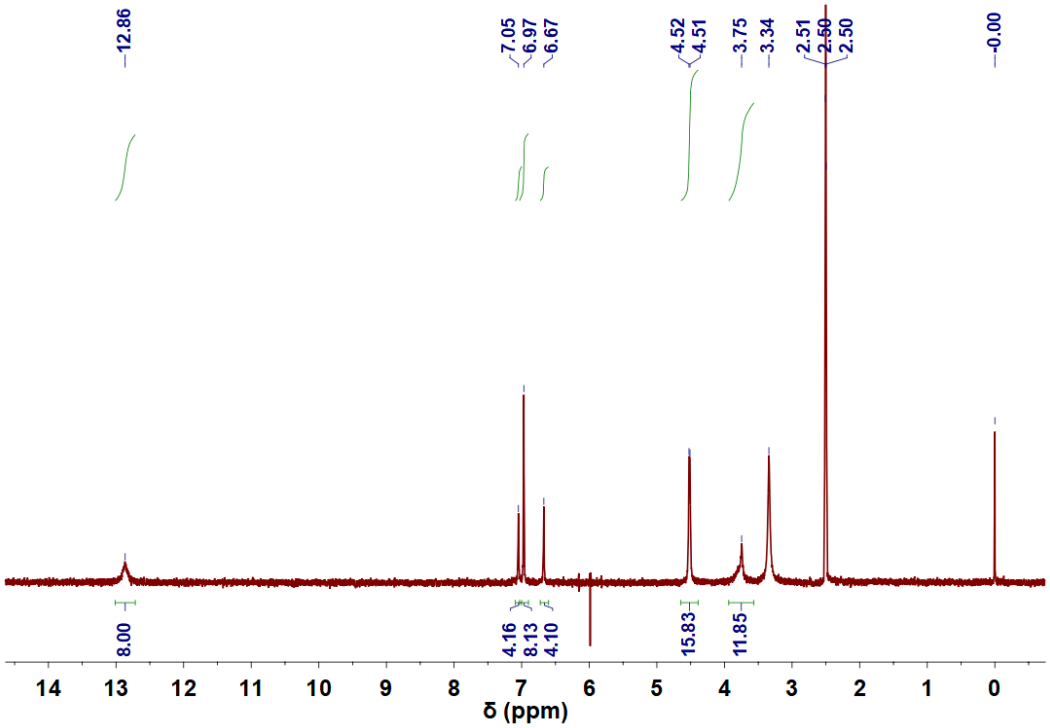

Figure S15. ^{1}H NMR (300 MHz, 298K) spectrum of CLP6 in DMSO-*d6*.

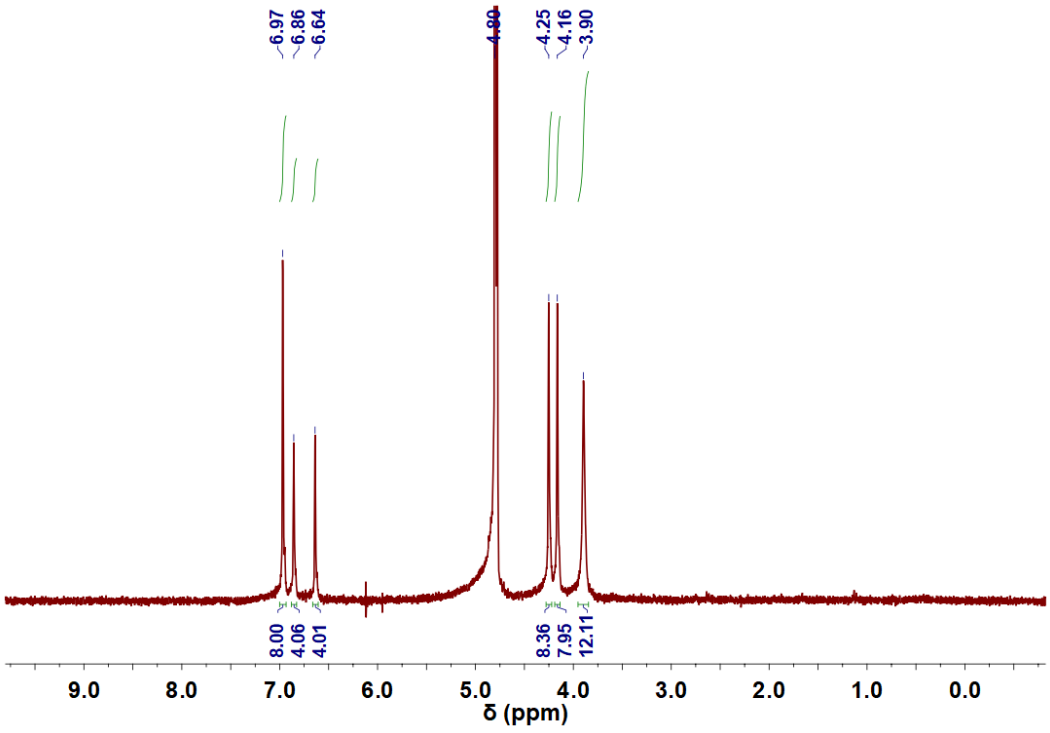

Figure S16. ^{1}H NMR (300 MHz, 298K) spectrum of AWLP6 in D_2O.

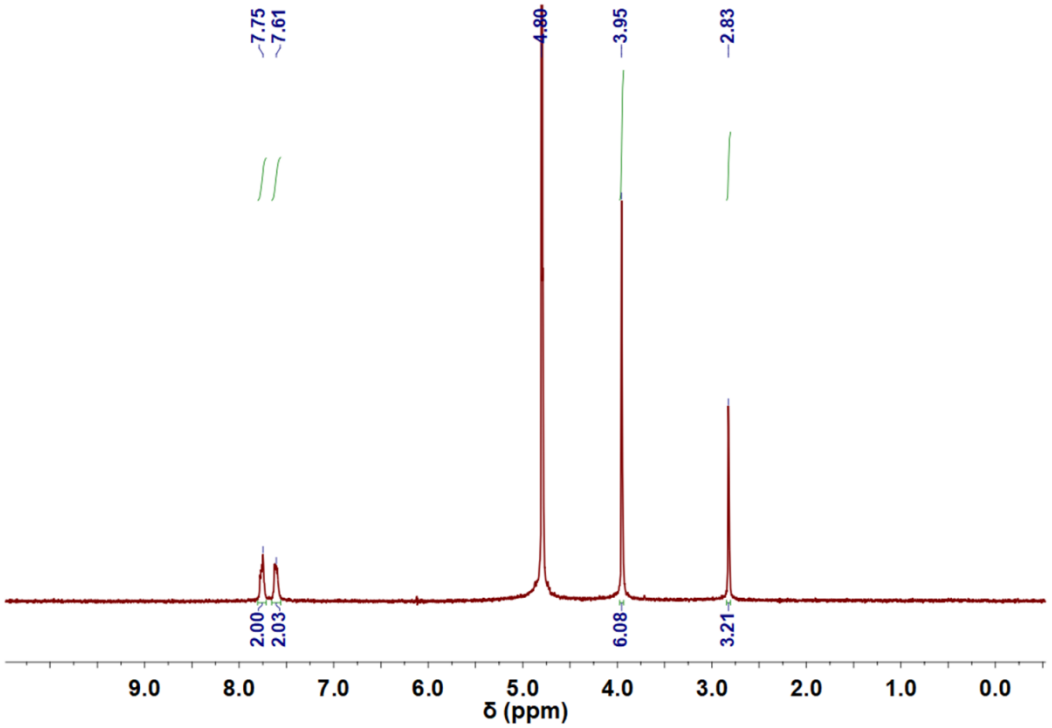

Figure S17. ^{1}H NMR (300 MHz, 298K) spectrum of MBM-CH_3 in D_2O.

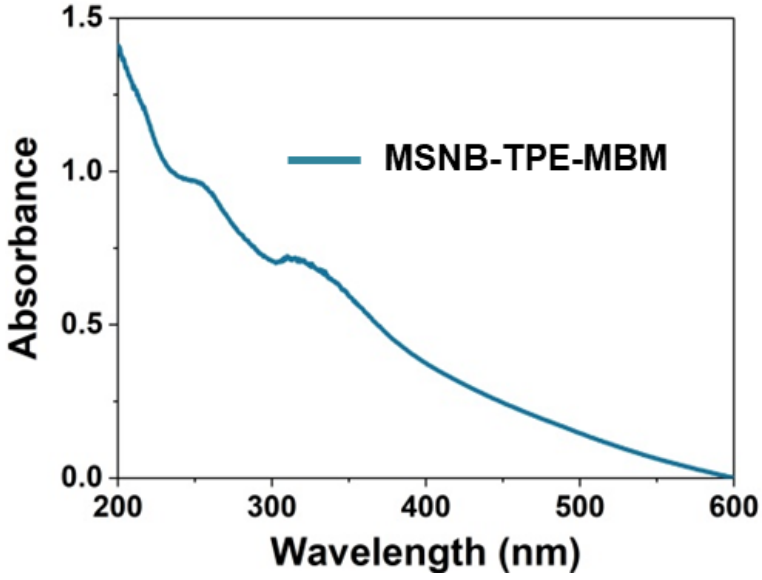

Figure S18. UV-vis spectrum of MSNB-TPE-MBM.

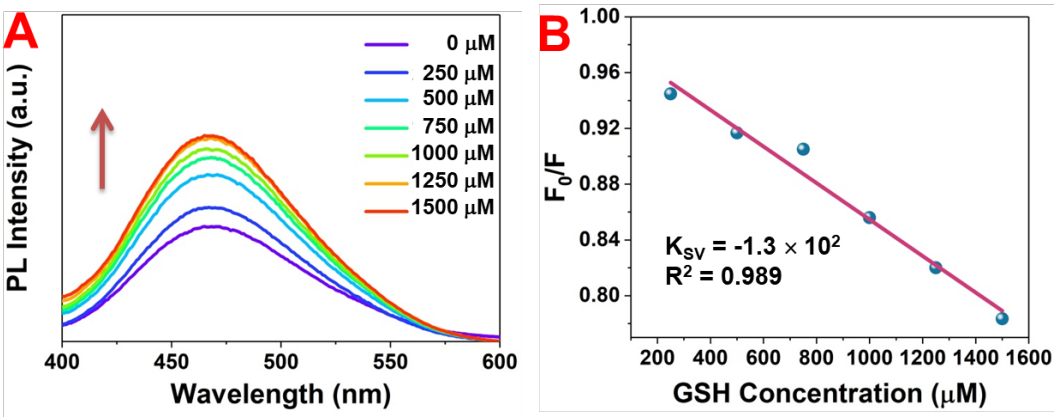

Figure S19. (A) Fluorescence emission intensity increases with the increasing concentration of GSH in deionized water, and (B) the corresponding relationships between emission intensity of MSNB-TPE-MBM and concentrations of GSH (0.25 mg/mL; λ_{ex} = 317 nm, λ_{em} = 475 nm; slit widths: ex = 5 nm, em = 5 nm).

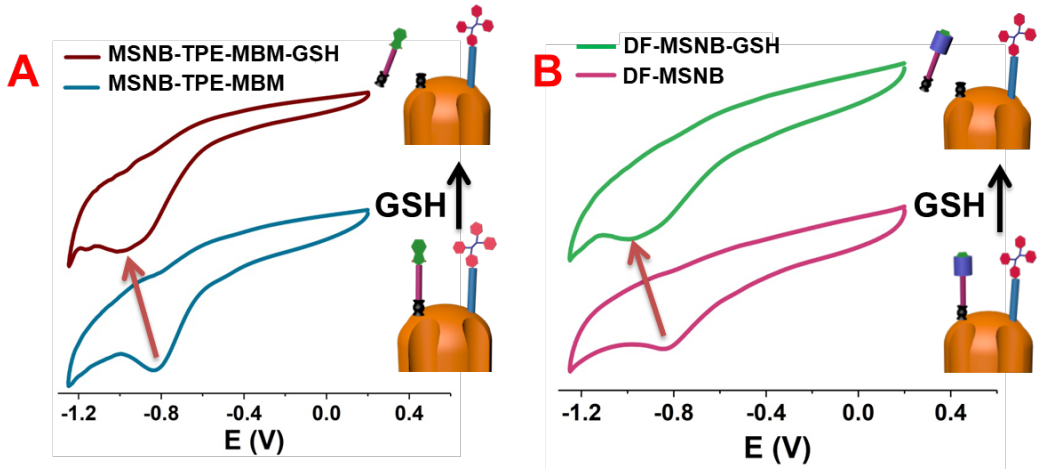

Figure S20. Cyclic voltammograms of (A) MSNB-TPE-MBM and (B) DF-MSNB before and after treatment with 20 mM of GSH. Insets show the corresponding schematic presentation of the surface changes of MSNB after GSH cleavage of the disulfide bonds. (Concentration is 0.25 mg/mL, MSNB-TPE-MBM and DF-MSNB after treatment with 20 mM GSH have been labeled as MSNB-TPE-MBM-GSH and DF-MSNB-GSH, respectively).

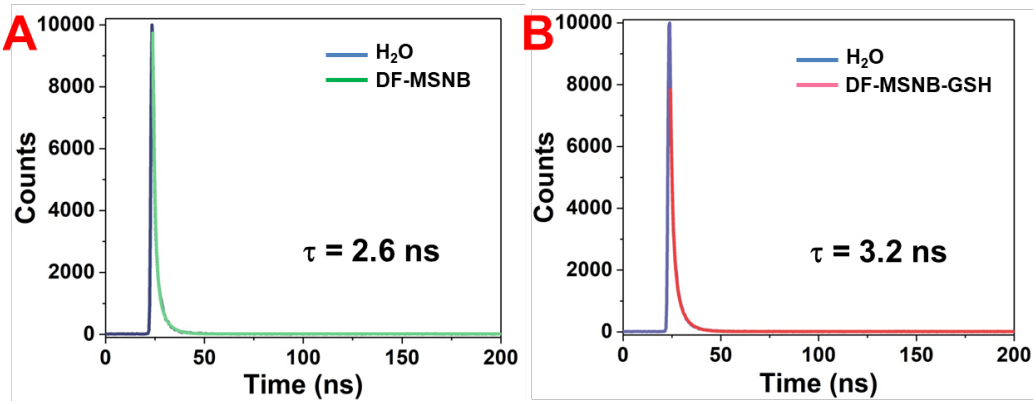

Figure S21. Time-resolved fluorescence decay curves of (A) DF-MSNB and (B) DF-MSNB-GSH resulting from the treatment of DF-MSNB with 20 mM of GSH.

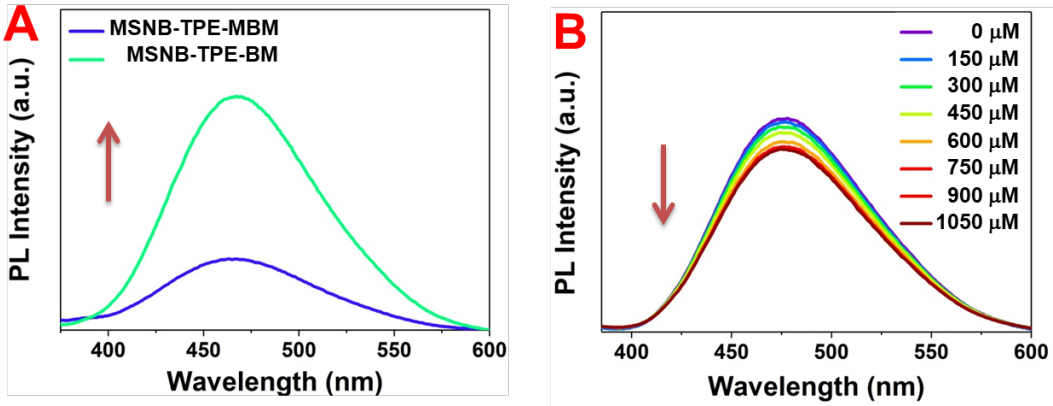

Figure S22. (A) Fluorescence spectra of MSNB-TPE-MBM and MSNB-TPE-BM (0.5 mg/mL; λ_{ex} = 317 nm, λ_{em} = 475 nm; slit widths: ex = 5 nm, em = 3 nm). (B) Fluorescence quenching of TPE-Br (200 μM) with increasing concentration of MBM-CH_3 (0-1050 μM) in deionized water.

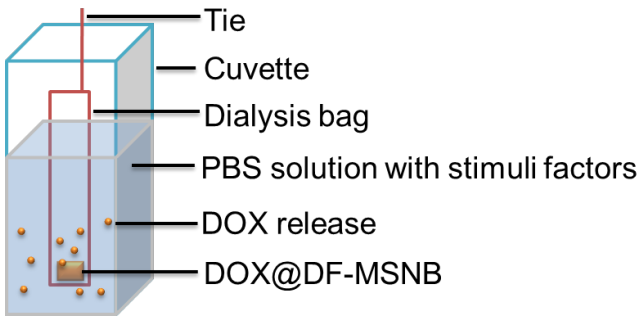

Figure S23. Schematic illustration of in-vitro release experiments of DOX@DF-MSNB.

4. References

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