FLUORESCENT SUPRAMOLECULAR POLYMERSOMES BASED ON PILLARARENE/PARAQUAT MOLECULAR RECOGNITION FOR PH-CONTROLLED DRUG RELEASE

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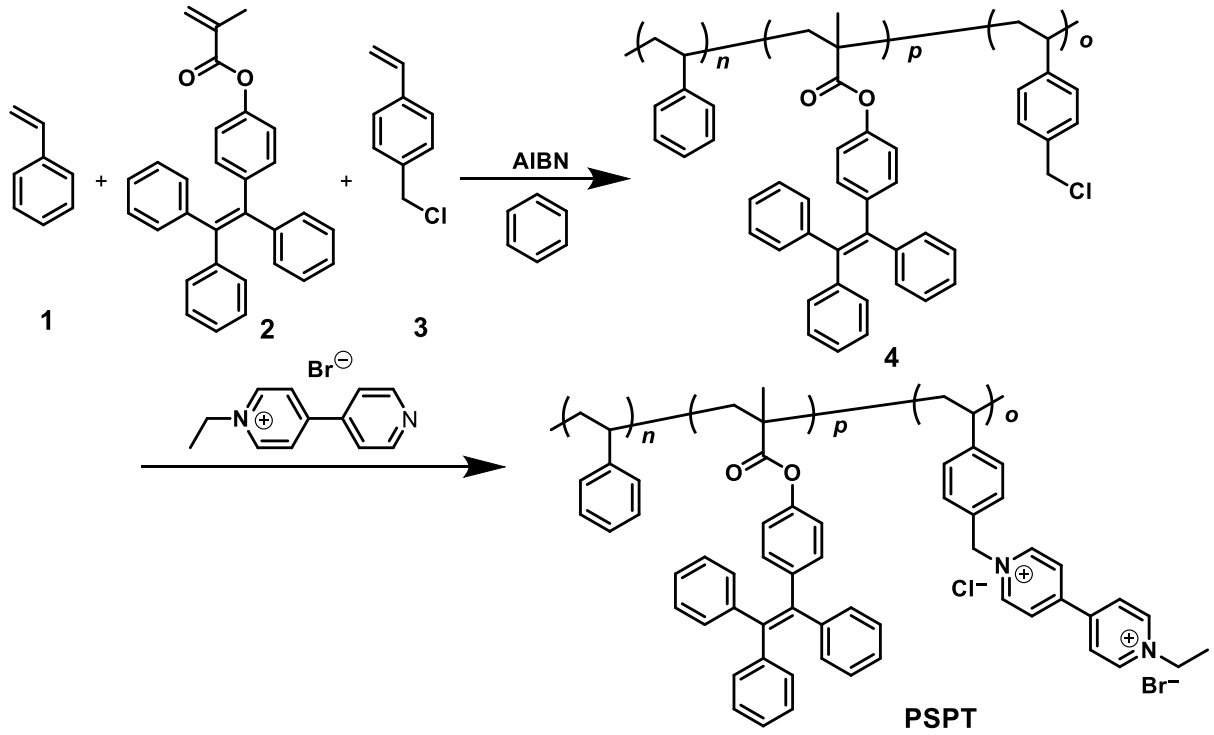
Supporting Information (16 pages)

1. Materials and methods

All reagents were commercially available and used as supplied without further purification. Polymer **4**[S1] was prepared according to a published procedure. Polymer **PSPT** was also prepared according to a published procedure.[S2] NMR spectra were recorded with a Bruker Avance DMX 400 spectrophotometer using the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) with a Waters 1515 pump and Waters 1515 differential refractive index detector (set at 30 ºC). It used a series of three linear Styragel columns (HT2, HT4, and HT5) at an oven temperature of 45 ºC. The eluent was THF at a flow rate of 1.0 mL/min. A series of low polydispersity polystyrene standards was employed for the GPC calibration. Dynamic light scattering (DLS) was carried out on a Malvern Nanosizer S instrument at room temperature. The confocal images were acquired on a confocal scanning laser microscope (CLSM, Radiance 2100, Bio-Rad). Transmission electron microscopy investigations were carried out on a HITACHI HT-7700 instrument.

*2. Synthesis of polymer **PSPT***



Polymer **4** was prepared from styrene (compound **1**), compound **2**, and 4-vinylbenzyl chloride (compound **3**) by free radical polymerization. A mixture of styrene (7.80 mL, 68.1 mmol), compound **2** (950 mg, 2.28 mmol), and 4-vinylbenzyl chloride (348 mg, 2.28 mmol) in 30 mL of benzene was stirred at room temperature. A stream of argon (Ar) was bubbled through for 30 min. In one portion was added 4.90 mg (0.0300 mmol) of azobisisobutyronitrile (AIBN) and the mixture was stirred for 10 min, sealed with a rubber septum, and heated to 70 °C for 24 h. Rapid freezing in liquid nitrogen quenched the polymerization and the solvent was removed under vacuum. The crude product was dissolved in 2 mL of $CHCl_3$ and precipitated into 200 mL of methanol. The precipitated solid was collected by vacuum filtration. This process was repeated three times and the collected polymer was dried in vacuum (2.13 g, 30%). The precipitate was collected by filtration and dried overnight in vacuum to give **4** as white powder ($M_{n,GPC}$ = 12.29 kDa, $M_{w,GPC}$ = 21.14 kDa, PDI = 1.72). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) (ppm): 7.24–6.80 (m, 25H, Ar), 6.78–6.28 (m, 12H, Ar), 4.52 (m, 2H, Ar-CH_2-), 0.94–0.82 (m, 3H, CH_3).

A mixture of polymer **4** (1.00 g, 0.0814 mmol) and compound **5**[S3] (1.79 g, 6.75 mmol) in DMF (50 mL) was stirred at 80 °C overnight. The solvent was evaporated and the residue was dissolved in 2.00 mL of chloroform. Then the solution was dropped into cold methanol (100 mL) and the precipitate was collected by filtration; this process was repeated. The solid was dried overnight in a vacuum to give brown powder (0.96 g, 74%). Fig. S3 is the ^{1}H NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **PSPT**.

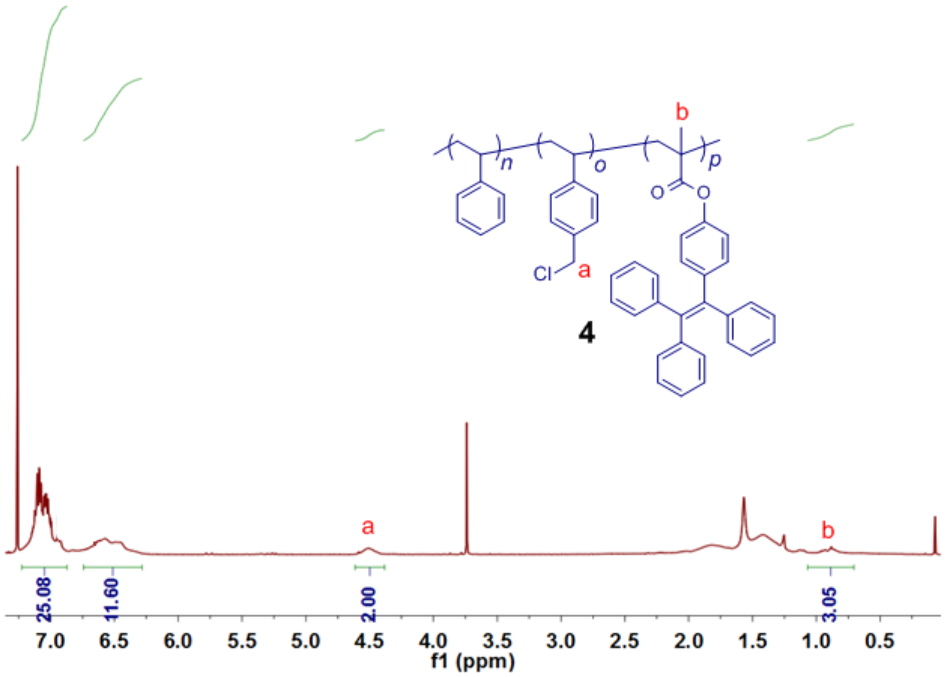
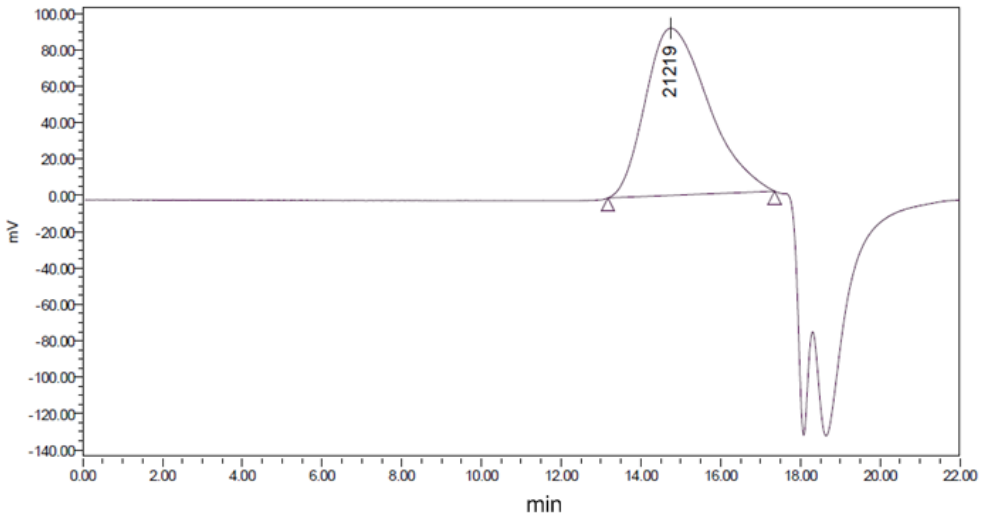


Fig. S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **4**.



M_n	M_w	M_p	M_z	PDI
12288	21138	21219	31782	1.72

Fig. S2. GPC data of **4**.

The ratio of *p/o* was (3.05/3)/(2/2), namely 1.03/1, as calculated based on the integrations of the peaks of H_b and H_a, and *n/o* was 2.72/1 as calculated based on the integrations of peaks of aromatic protons. Therefore, the ratio of *n/o/p* was 2.72/1/1.03 for polymer **4**. According to M_n and the ratio of *n/o/p*, it can be calculated that the values of *n*, *o*, and *p* were 38, 14 and 14, respectively.

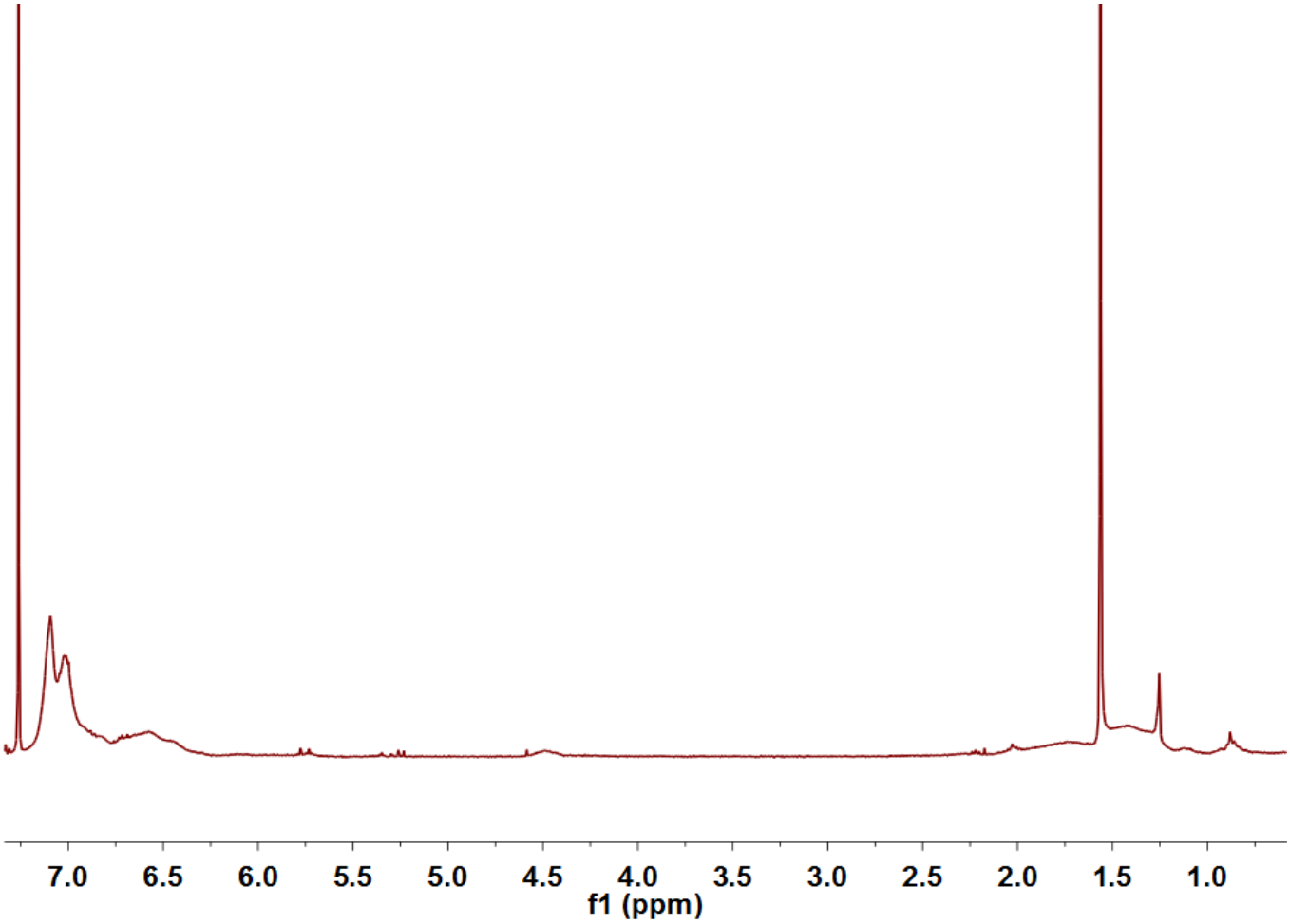
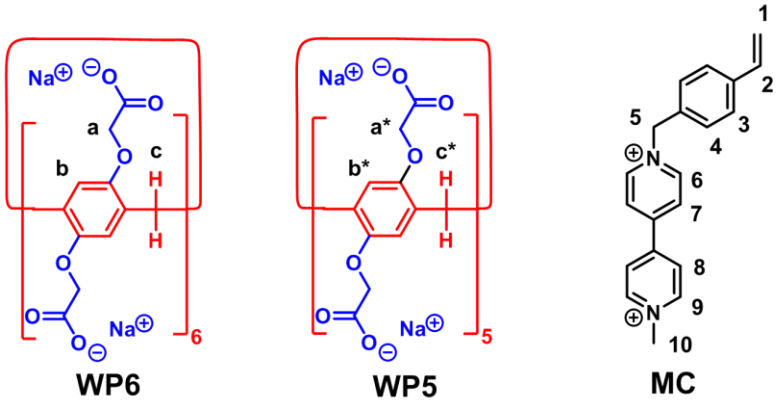


Fig. S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **PSPT**.

3. *ITC experiments of* ***WP5/WP6*** *with* ***MC***



The isothermal titration calorimetry (ITC) experiment provided the association constants (K_a) and the thermodynamic parameters (enthalpy and entropy changes $\Delta H°$ and $\Delta S°$) for the host–guest complexations between **WP5** and **MC** and between **WP6** and **MC**. As shown in Fig. S4, the K_a values of **WP5**⊃**MC** and **WP6**⊃**MC** were calculated to be (8.39 ± 2.68) × 10^4 M^{-1} and (1.12 ± 0.18) × 10^5 M^{-1}, respectively, in 1 : 1 complexation. In addition, the enthalpy and entropy changes were obtained from the ITC data ($\Delta H°$ = –23.43 kJ mol^{-1} and –15.04 kJ mol^{-1} for **WP5**⊃**MC** and **WP6**⊃**MC**, respectively; $\Delta S°$ = 15.7 J mol^{-1} and 46.6 J mol^{-1} for **WP5**⊃**MC** and **WP6**⊃**MC**, respectively), indicating that the complexation was driven by a favorable entropy change with enthalpic assistance.

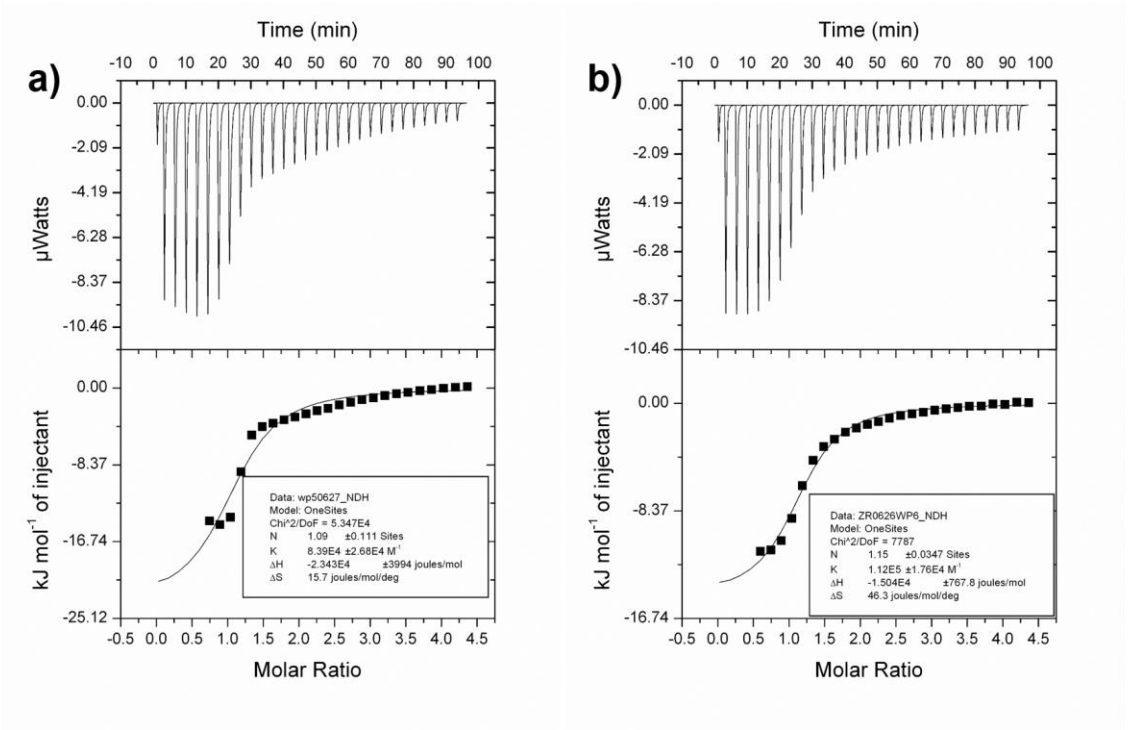


Fig. S4. a) Microcalorimetric titration of **WP5** with **MC** in water at 298 K. (top) Raw ITC data for 24 sequential injections (2.00 mL per injection) of a **MC** solution (2.00 mM) into a **WP5** solution (0.100 mM). (bottom) Net reaction heat obtained from the integration of the calorimetric traces. b) Microcalorimetric titration of **WP6** with **MC** in water at 298 K. (top) Raw ITC data for 24 sequential

injections (2.00 mL per injection) of a **MC** solution (2.00 mM) into a **WP6** solution (0.100 mM). (bottom) Net reaction heat obtained from the integration of the calorimetric traces.

4. *^{1}H NMR spectra of **WP5/WP6** with **MC***

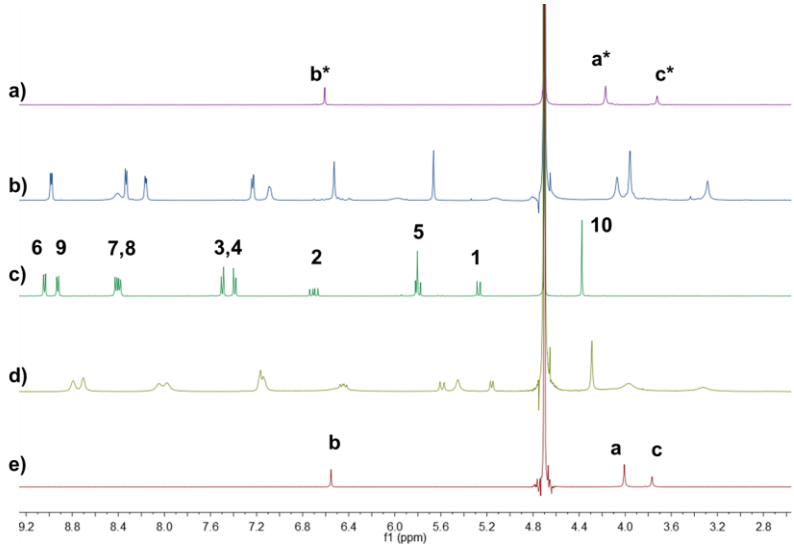


Fig. S5. Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K): a) 1.00 mM **WP5**; b) 1.00 mM **WP5** + 1.00 mM **MC**; c) 1.00 mM **MC**; d) 1.00 mM **WP6** + 1.00 mM **MC**; e) 1.00 mM **WP6**.

5. *pH-responsive ^{1}H NMR spectra of* ***WP5/WP6*** *with* ***MC***

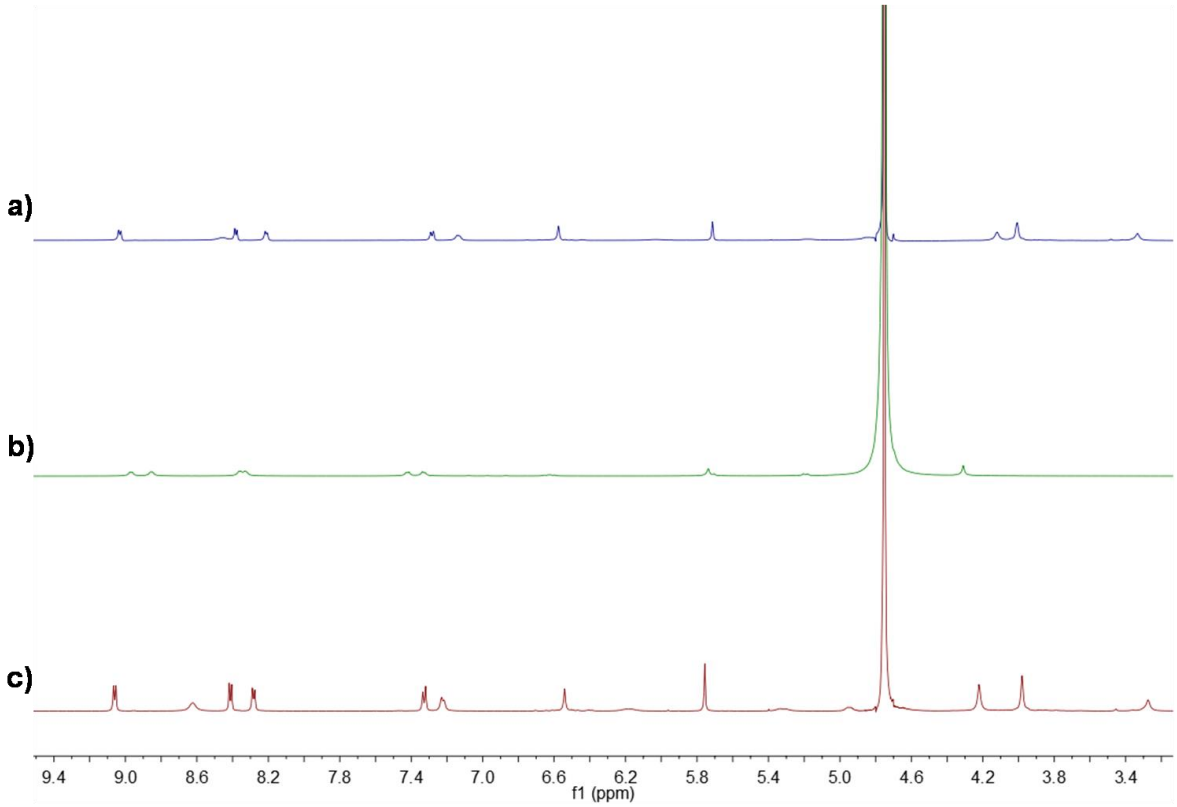


Fig. S6. Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K): a) 1.00 mM **WP5** + 1.00 mM **MC**; b) after addtion of a drop of DCl to a); c) after neutralization with NaOD to b).

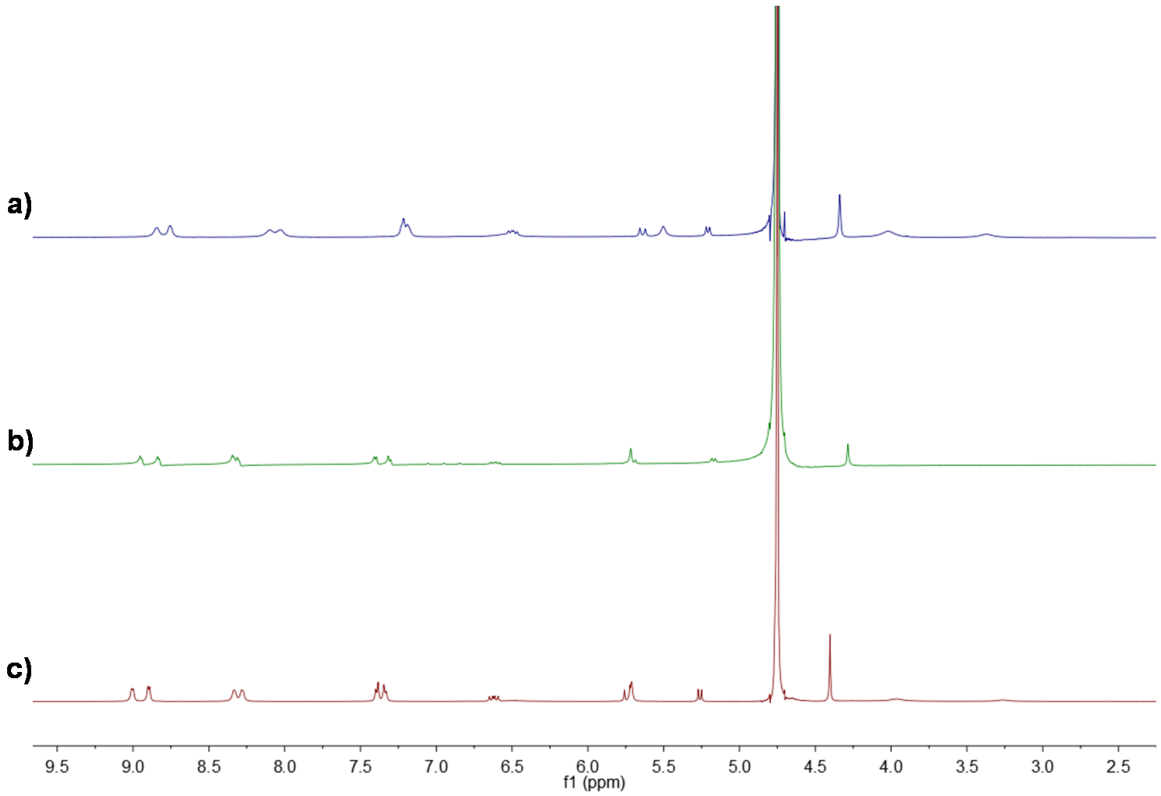


Fig. S7. Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K): a) 1.00 mM **WP6** + 1.00 mM **MC**; b) after addtion of a drop of DCl to a); c) after neutralization with NaOD to b).

6*. DLS results of* ***PR5****/****PR6*** *in water*

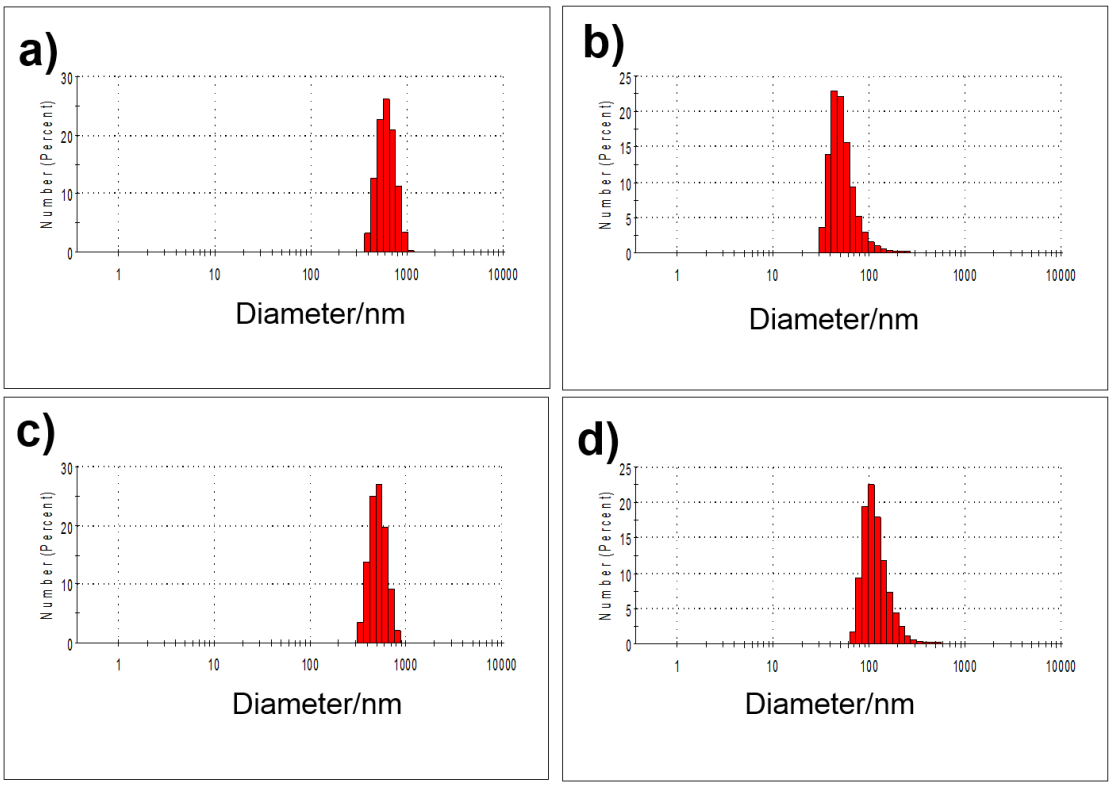


Fig. S8. a) DLS of **PR5** (5.00×10^{-4} M) in water after adding HCl to adjust the solution pH to 5.5; b) DLS of **PR5** (5.00×10^{-4} M) in water after adding NaOH to adjust the solution pH to 7.4; c) DLS of **PR6** (5.00×10^{-4} M) in water after adding HCl to adjust the solution pH to 5.5; d) DLS of **PR6** (5.00×10^{-4} M) in water after adding NaOH to adjust the solution pH to 7.4.

7**. The critical aggregation concentrations of **PR5** and **PR6

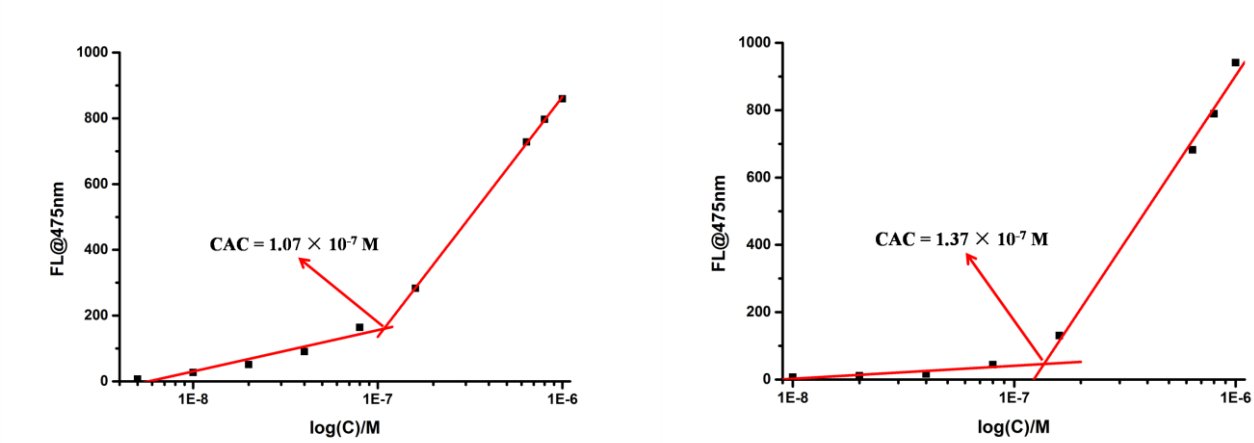


Fig. S9. Plots of the fluorescence (FL) intensity at 475 nm *vs* the concentration of a) **PR5** and b) **PR6**.

8. *Fluorescence change of DOX/**PR6** and drug release curves of DOX-loaded **PR6** under different pH values*

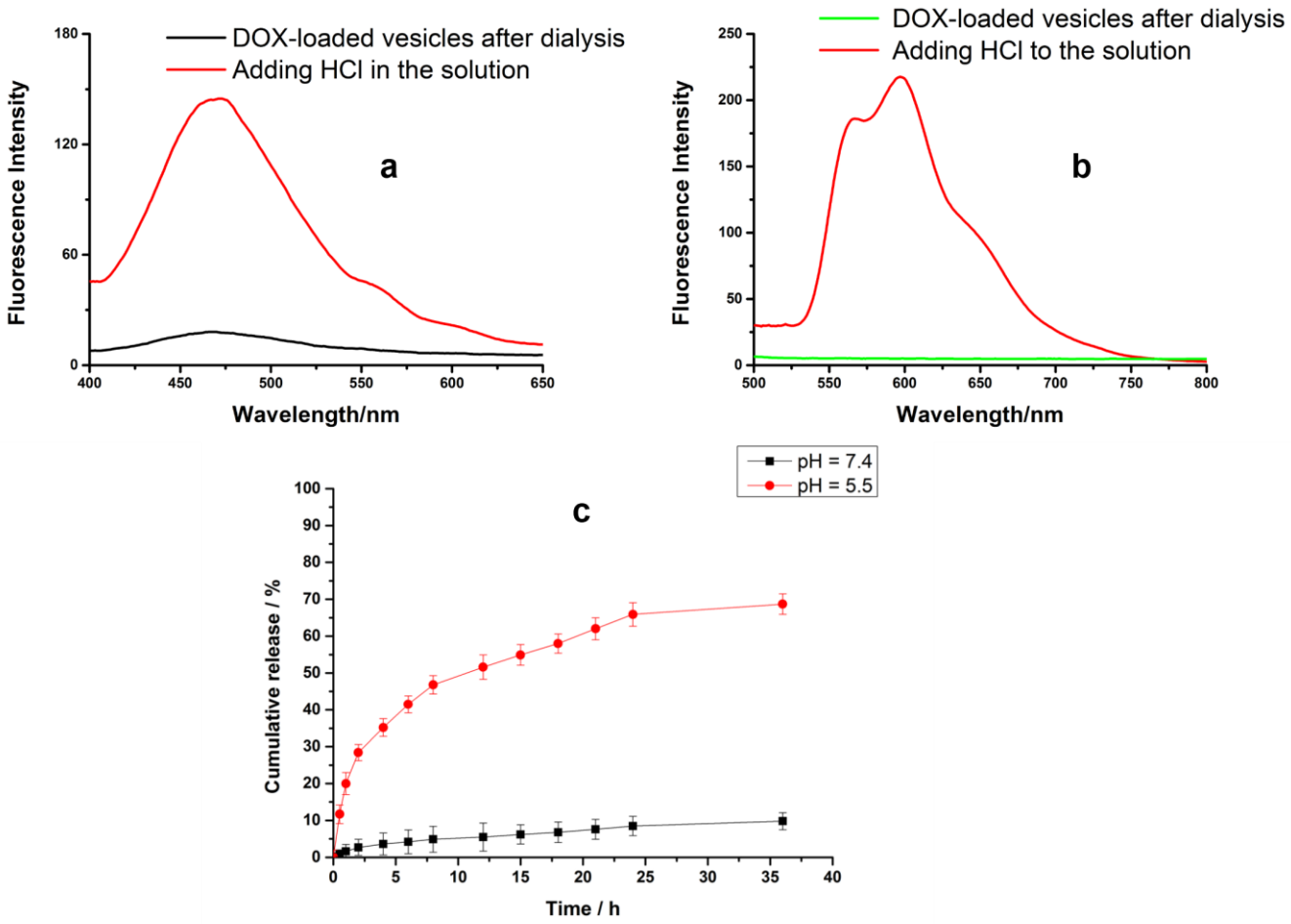


Fig. S10. a) Fluorescence change of **PR6** (excitation wavelength is 350 nm) before/after adding HCl to the DOX-loaded **PR6** vesicle solution; b) fluorescence change of DOX (excitation wavelength is 485 nm) before/after adding HCl to the DOX-loaded **PR6** vesicle solution; c) drug release curves of DOX-loaded **PR6** under different pH values.

***9**. DOX encapsulation experiments*

DOX loading experiments: DOX-loaded vesicles were prepared by adding a certain amount of DOX into a freshly prepared aqueous solution of either **PR5** or **PR6** (2.5×10^{-4} M for both). The ultimate concentrations of DOX, **PR5** and **PR6** were 0.05, 0.25 and 0.25 mM, respectively. And then the prepared DOX-loaded vesicles were purified by dialysis (molecular weight cutoff = 3500) in distilled water for several times until the water outside the dialysis tube exhibited negligible DOX fluorescence. As a result, DOX was successfully loaded into the vesicles constructed from **PR5** and **PR6**. The DOX encapsulation and loading efficiency were calculated by the following equations:[S4]

$$\text{Encapsulation Efficiency (\%)} = (m_{\text{DOX-loaded}} / m_{\text{DOX}}) \times 100$$

$$\text{Loading content (\%)} = (m_{\text{DOX-loaded}} / m_{\text{DOX-loading nanoparticles}}) \times 100$$

$m_{\text{DOX-loaded,}}$ and m_{DOX} are mass of DOX encapsulated in vesicles and mass of DOX added, respectively, $m_{\text{DOX-loading nanoparticles}}$ is mass of nanoparticles. The mass of DOX was measured by a UV spectrophotometer at 490 nm and calculated as relative to a standard calibration curve in the concentrations from 5.00×10^{-3} to 2.50×10^{-2} mM in water.

The encapsulation efficiency of DOX-loaded **PR5** and **PR6** were calculated to be 31.2% and 33.1%, respectively, and the loading contents of DOX-loaded **PR5** and **PR6** were calculated to be 11.2% and 12.6%, respectively.

10. *TEM of the DOX-loaded* ***PR5/PR6***

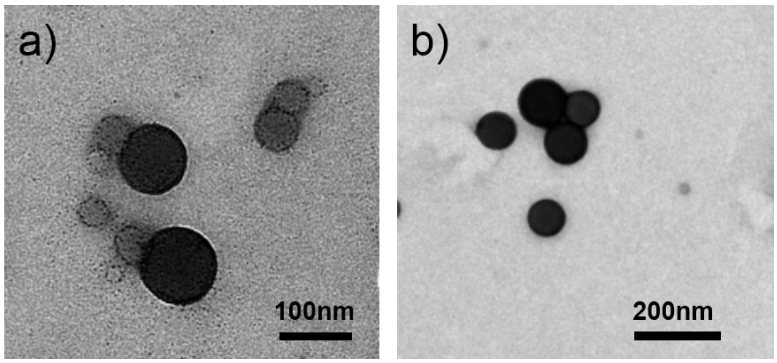


Figure S11. TEM images of DOX-loaded a) **PR5**; b) **PR6**.

11. *The mechanism of the FRET induced fluorescence quenching*

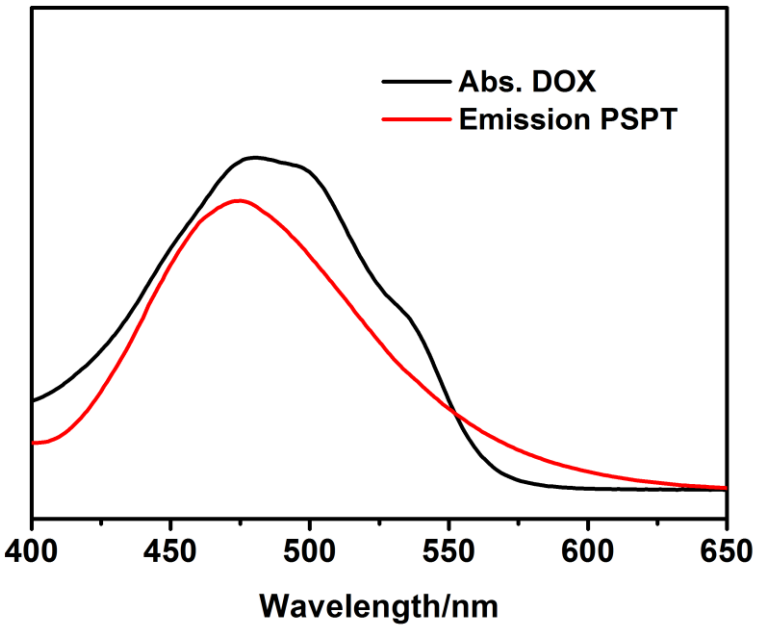


Fig. S12. UV−vis spectrum of DOX and fluorescence spectrum of **PSPT**.

There was an overlap in the emission spectrum of **PSPT** and the absorption spectrum of DOX, hence the emitted fluorescence from **PSPT** could be absorbed by DOX.

References

S1. Ji, X.; Zhu, K.; Yan, X.; Ma, Y.; Li, J.; Hu, B.; Yu, Y.; Huang, F. pH-Responsive supramolecular polymerization in aqueous media driven by electrostatic attraction-enhanced crown ether-based molecular recognition. *Macromol. Rapid Commun.* **2012**, 33, 1197−1202.

S2. Ji, X.; Chen, J.; Xue, M. A miscible and adaptive poly(methyl acrylate)/polystyrene blend formed by multiple-responsive host-guest interactions. *Macromol. Chem. Phys.* **2014**, 215, 536−543.

S3. Winslow, L. N.; Rillema, D. P.; Welch, J. H.; Singh, P. A rhenium (I) bipyrimidine tricarbonyl complex containing methyl viologen as the 6th ligand - NMR and structural results. *Inorg. Chem.* **1989**, 28, 1596−1599.

S4. Wang, K.; Guo, D.-S.; Wang, X.; Liu, Y. Multistimuli responsive supramolecular vesicles based on the recognition of p-sulfonatocalixarene and its controllable release of doxorubicin. *ACS Nano* **2011**, 5, 2880−2894.