Facile creation of FRET systems from a pH-responsive AIE fluorescent vesicle

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1. Materials

Octavinyl POSS (98%, Hybrid Plastics), trifluoromethanesulfonic acid (99%, Aldrich), poly(ethylene glycol) methyl ether (PEG-OH, M_n = 350 g/mol, Alfa Aesor), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 99%, Energy Chemical), succinic anhydride (99%, J&K), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%, J&K), 4-phenylazophenol (98%, Alfa Aesar), 1,3-dibromopropane (99%, J&K), thiocarbamide (99%, J&K), 4-(dimethyl-amino)-pyridine (DMAP, 99%, Aldrich), bromotriphenylethylene (98%, Energy Chemical), cysteamine hydrochloride (98%, J&K), tetrakis(triphenylphosphine) palladium (0) (99%, Energy Chemical), 4-formylphenylboronic acid (98%, Energy Chemical), triethylamine (TEA, 99%, Beijing Chemical Works), tetrabutyl ammonium bromide (TBAB, 99%, Energy Chemical), fluorescein isothiocyanate isomer I (FITC, 95%, Energy Chemical), doxorubicin (DOX, 98%, Energy Chemical), sodium hydroxide, potassium carbonate, hydrochloric acid, ethanol, ethyl acetate, petroleum ether, n-hexane and ether (reagent grade, Beijing Chemical Works), tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were purified by stirring over calcium hydride for 24 h followed by distillation. All other reagents were purchased from Sigma-Aldrich and used as received without further purification.

2. Characterizations

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 spectrometer in chloroform-d using tetramethylsilane (TMS) as internal reference. Gel permeation chromatography (GPC) measurements were carried out on GPCmax VE-2001 (Viscotek) equipped with a Viscotek TriSEC Model 302 triple detector array (refractive index detector, viscometer detector, and laser light scattering detector) using two I-3078 Polar Organic Columns. Tetrahydrofuran was used as the eluent at a flow rate of 1.0 mL min^{-1}. Molecular weight (M_n and M_w) and polydispersity indexes (PDI) were obtained using the workstation software equipped with the system by the processing method for dendritic polymers based on a working curve of polystyrene standards. Transmission electron microscopy (TEM) images were obtained on a JEM-2200FS microscope (JEOL, Japan). A 5 μL droplet of solution was dropped onto a copper grid (300 mesh) coated with a carbon film, followed by drying at room temperature. Dynamic light scattering (DLS) spectra were obtained on a commercial laser light scattering spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-τ digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser (λ_0=632.8 nm) was used. All data were averaged over three time measurements. The laser light scattering cell is held in a thermostat index matching vat filled with purified and dust-free toluene, with the temperature controlled to within 0.1 ℃. UV-vis spectra of the samples were measured over different irradiation time intervals on a Hitachi U-3010 spectrometer, and fluorescence measurements were carried out on a Hitachi F4600 photoluminescence spectrometer with a xenon lamp as a light source.

3. Synthesis of AIE monomer, AB$_7$ monomer and tadpole-shaped polymers

Synthesis of AIE monomer, TPE-CHO. [1] Breifly, bromotriphenylethylene (2.01 g, 6 mmol) and 4-formylphenylboronic acid (1.35 g, 9 mmol) were dissolved in the mixture of toluene (40 mL), TBAB (0.19 g, 0.6 mmol) and 1.2 M potassium carbonate aqueous solution (10 mL). The mixture was stirred at room temperature for 0.5 h under Ar gas followed by adding $Pd(PPh_3)_4$ (60 mg, 5.3×10^{-3} mmol) and then heated to 90 ℃ for 24 h. After that the mixture was poured into water and extracted three times with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After moving the solvent under reduced pressure, the residue was chromatographed on a silica gel column with CH_2Cl_2/n-hexane (v/v = 1:2) as an eluent to give faint yellow powder with 90.1% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.02-7.03 (m, 6H), 7.11 (m, 9H), 7.18 (d, 2H), 7.60-7.63 (d, 2H), 9.90 (s, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 126.9, 127.1, 127.7, 127.9, 129.2, 131.3, 134.3, 139.8, 143.0, 150.6, 191.9.

Synthesis of the septvinyl monohydroxyl POSS, (vinyl)$_7$-POSS-OH (AB$_7$ monomer). [2] To a solution of octavinyl POSS (1.9 g, 3 mmol) in 200 mL of fresh distilled CH_2Cl_2, trifluoromethanesulfonic acid (0.27 mL, 3 mmol) was slowly added via syringe at room temperature. After 4 hours, the solution was diluted with CH_2Cl_2 and then washed with saturated aqueous Na_2CO_3 three times. The organic layer was then concentrated by evaporating the excess solvent. Then wet acetone was added into the concentrated solution and the hydrolysis process was performed for 6 h. The crude product was yielded after removing the solvent. The final product was separated chromatographically on silica gel with CH_2Cl_2/hexane (v/v = 2:1) to afford the white solid with 29.1% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 1.62 (s, 1H), 1.22 (t, 2H), 3.82 (t, 2H), 5.85-6.16 (m, 21H). ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 17.3, 58.5, 128.6, 137.2.

Synthesis of the carboxyl-terminated mPEG, PEG-COOH. PEG-OH (M_n = 350 g/mol , 7 g, 20 mmol) and TEA (1.4 mL, 10 mmol) were dissolved in 40 mL of fresh distilled CH_2Cl_2, and then succinic anhydride (2.2 g, 22 mmol) was added into the solution. After stirring at room temperature for 3 h, the mixture was washed with 2M HCl aqueous solution, saturated NaCl aqueous solution and DI water for three times and dried over anhydrous sodium sulfate. After evaporating the solvent, the residue was dissolved in CH_2Cl_2 and was precipitated in cold ether for three times to afford the white solid with 95.1% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 2.59-2.63 (m, 4H), 3.38 (s, 3H), 4.23 (t, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 29.7, 29.9, 58.7, 63.6, 69.1, 70.6, 72.1, 170.6, 175.8.

Synthesis of tadpole-shaped polymer, PEG-POSS-(vinyl)$_7$. (vinyl)$_7$-POSS-OH (0.3 g, 0.46 mmol), PEG-COOH (0.23 g, 0.5 mmol), EDCI (0.11 g, 0.55 mmol) and DMAP (12 mg, 0.1 mmol) were added to a 50 mL round-bottomed flask equipped with a magnetic stirring bar, followed by the addition of 30 mL of freshly dried CH_2Cl_2 to fully dissolve all the solids. The solution was cooled to 0 ℃ for 15 min and allowed to the room temperature, and then further stirred for another 24 h to complete the reaction. Then, the solution was washed with 2M HCl aqueous solution, saturated NaCl aqueous solution and DI water for three times and dried over anhydrous sodium sulfate. Then the final product was precipitated into n-hexane for several times to remove the excess (vinyl)$_7$-POSS-OH to afford the white powder with 78.7% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 1.19 (t, 2H), 2.62 (m, 4H), 3.38 (s, 3H), 3.83 (t, 2H), 4.26 (t, 2H), 5.85-6.16 (m, 21H). ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 12.4, 28.2, 28.6, 58.3, 60.3, 63.1, 68.2, 71.2, 127.9, 136.4, 173.7.

Synthesis of tadpole-shaped polymer, PEG-POSS-(NH_3Cl)$_7$. PEG-POSS-(vinyl)$_7$ (0.11 g, 0.1 mmol), cysteamine hydrochloride (0.12 g, 1 mmol) and DMPA (25 mg, 0.1 mmol) were dissolved in 10 mL mixture of THF/MeOH (v/v = 1:2). After irradiation under a 365 nm UV lamp at room temperature for 4 h to make sure no vinyl groups existed. The product was precipitated into ether for several times and then purified by ultrafiltration (MWCO 500) and collected after freeze-drying to afford the white powder with 69.5% yield. ^{1}H NMR (MeOD, 400 MHz, ppm): δ 1.04 (t, 14H), 1.19 (t, 2H), 2.62 (m, 4H), 2.66 (t, 14H), 2.81 (t, 14H), 2.96 (t, 14H), 3.38 (s, 3H), 3.83 (t, 2H), 4.26 (t, 2H), 8.28 (s, 21H). ^{13}C NMR (MeOD, 400 MHz, ppm): δ 12.4, 12.7, 25.1, 27.9, 28.2, 28.6, 38.9, 58.3, 60.3, 63.1, 68.2, 71.2, 173.4.

Synthesis of tadpole-shaped polymer, PEG-POSS-(TPE)$_7$. PEG-POSS-(NH_3Cl)$_7$ (0.12 g, 0.06 mmol) and TEA (90 μL, 0.6 mmol) were dissolved in 15 mL of methanol. Then 10 mL of methanol containing TPE-CHO (0.22 g, 0.6 mmol) was dropwise added into the solution under a nitrogen atmosphere. The reaction mixture was degassed three times with nitrogen and kept at room temperature for 12 h to produce yellow precipitates. The crude products were obtained after filtration and washing with cold methanol to afford brilliant yellow powders with 67.3% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 1.04 (t, 14H), 1.15 (t, 2H), 2.62-2.66 (m, 18H), 2.81 (t, 14H), 3.38 (s, 3H), 3.83 (t, 2H), 3.72 (t, 14H), 4.21 (t, 2H), 7.01-7.61 (m, 133H), 8.15 (s, 7H). ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 12.8, 26.4, 29.2, 32.8, 58.9, 60.3, 61.3, 70.5, 126.6, 127.6, 131.5, 132.0, 134.0, 140.3, 141.8, 143.4, 146.6, 162.2, 173.3.

4. Formation and self-assembly of the amphiphilic tadpole-shaped polymer.

PEG-POSS-(TPE)$_7$ (5 mg) was first dissolved in THF (1 mL), which is a good solvent for POSS, TPE and PEG components. Then deionized water (4 mL) was added dropwise into the solution at the rate of 0.05 mL/min via a syringe pump. The colloidal dispersion was further stirred for another 1 h and the temperature was fixed at 25 ℃ during the self-assembling process. The organic solvent (THF) was removed by dialysis (MW cutoff, 1 kDa) against deionized water for 2 days.

5. Determination of pH-triggered destabilization of vesicles.

For determining the pH sensitivity of vesicles, a certain amount of phosphate buffered saline (PBS) with different pH value (pH = 5.0, 5.8, 7.4 and 10.0) was added into 10 mL solution of PEG-POSS-(TPE)$_7$ vesicles. After mildly stirring at 37 ℃ for the appointed time, the fluorescent intensities of vesicles were measured by fluorescence measurements. Concentration in the solvents: 20 μM.

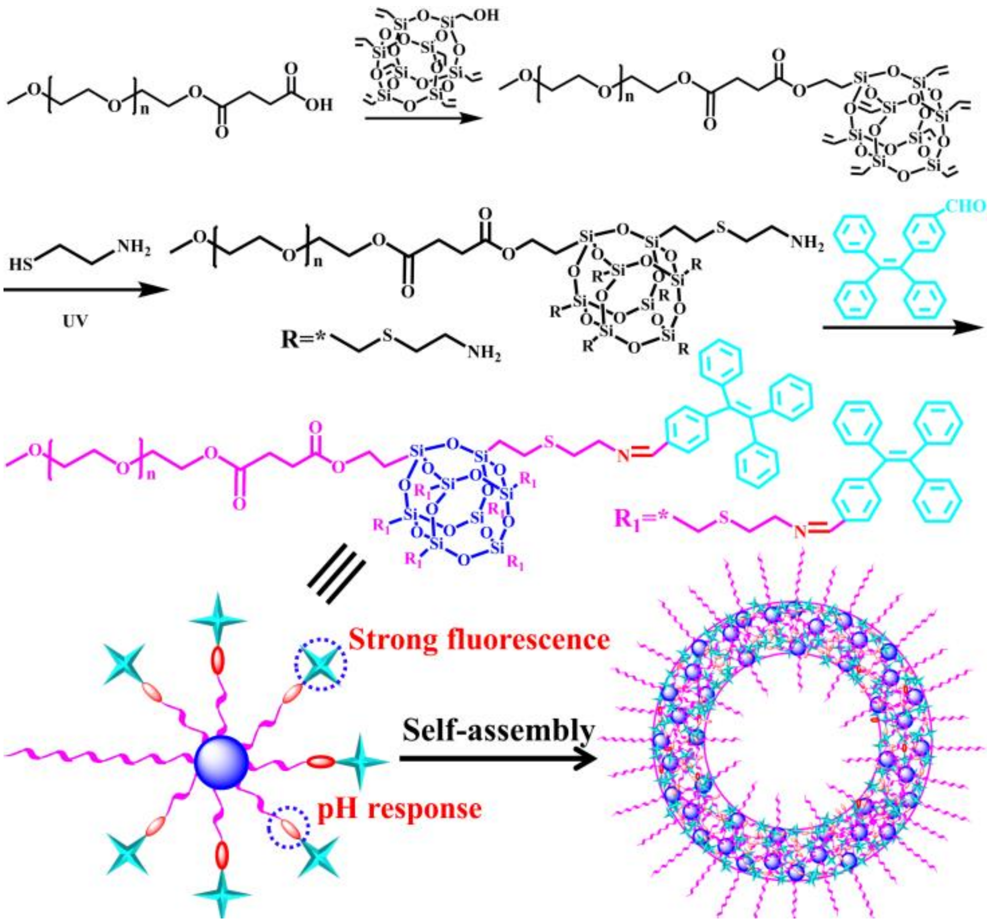
6. Loading of fluorescent dyes into the vesicle.

In order to encapsulate the various probes, vesicular formation was carried out following the same procedure described for the PEG-POSS-(TPE)$_7$ vesicles and using a solution of FITC dyes or DOX molecules in water. The tadpole-shaped polymer PEG-POSS-(TPE)$_7$ was first dissolved in THF and a solution of FITC dyes or DOX molecules was gradually added dropwise into the solution at the rate of 0.05 mL/min via a syringe pump. The colloidal dispersion was further stirred for another 1 h and the temperature was fixed at 25 ℃ during the self-assembling process. The organic solvent (THF) and non-encapsulated fluorescent FITC dyes or DOX molecules were removed by dialysis (MW cutoff, 1 kDa) against deionized water.

References

1. X. Q. Zhang, Z. G. Chi, H. Y. Li, B. J. Xu, X. F. Li, W. Zhou, S. W. Liu, Y. Zhang and J. R. Xu, *Chem. Asian J.*, 2011, **6**, 808.

2. X. Wang, Y. Y. Yang, P. Y. Gao, D. Li, F. Yang, H. X. Guo, F. J. Xu and D. C. Wu, *Chem. Commun.*, 2014, **50**, 6126.



Scheme S1 Synthetic route of the PEG-POSS-(TPE)$_7$ polymer and schematic illustration of assembled polymeric vesicles.

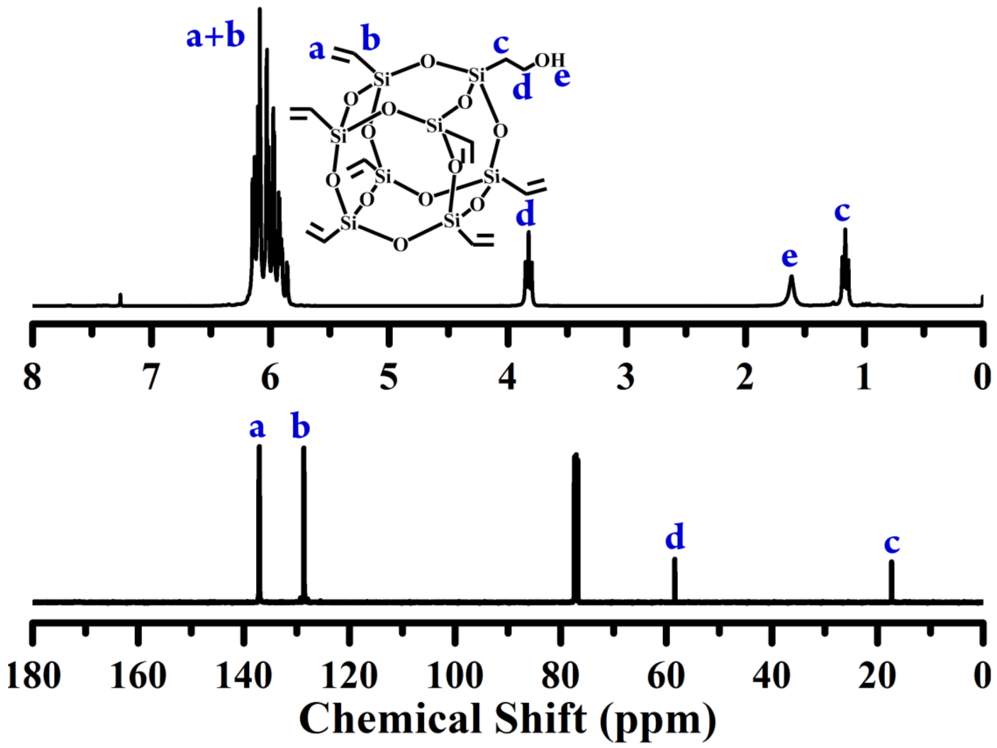


Fig. S1 ^{1}H NMR and ^{13}C NMR spectra of OH-POSS-(vinyl)$_7$ at $CDCl_3$.

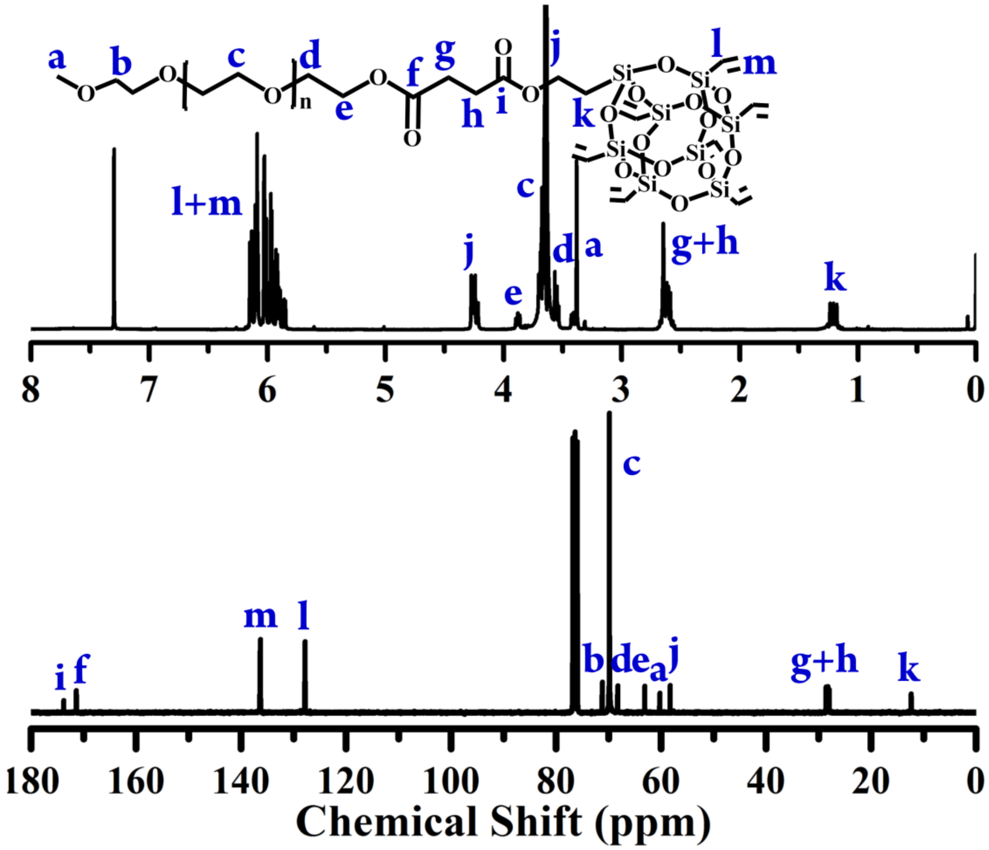


Fig. S2 ^{1}H NMR and ^{13}C NMR spectra of PEG-POSS-(vinyl)$_7$ at $CDCl_3$.

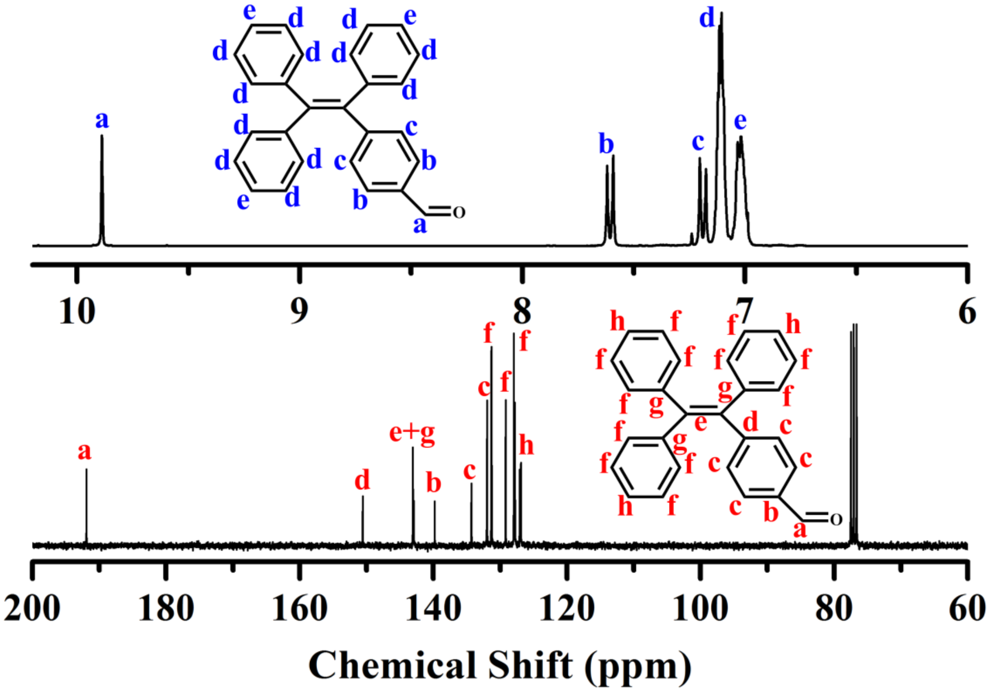


Fig. S3 ^{1}H NMR and ^{13}C NMR spectra of TPE-CHO at $CDCl_3$.

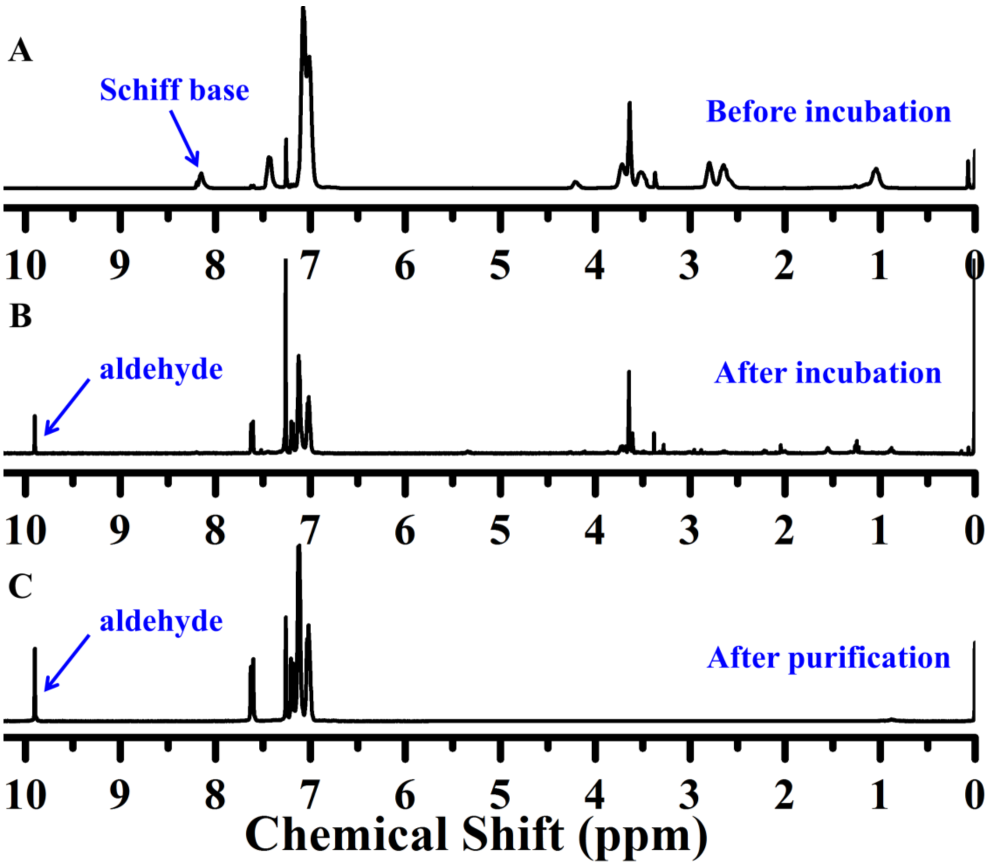


Fig. S4 ^{1}H NMR spectra of the PEG-POSS-(TPE)$_7$ polymer before (A) and after (B) incubation in acidic condition, and (C) after purification of (B) at $CDCl_3$.

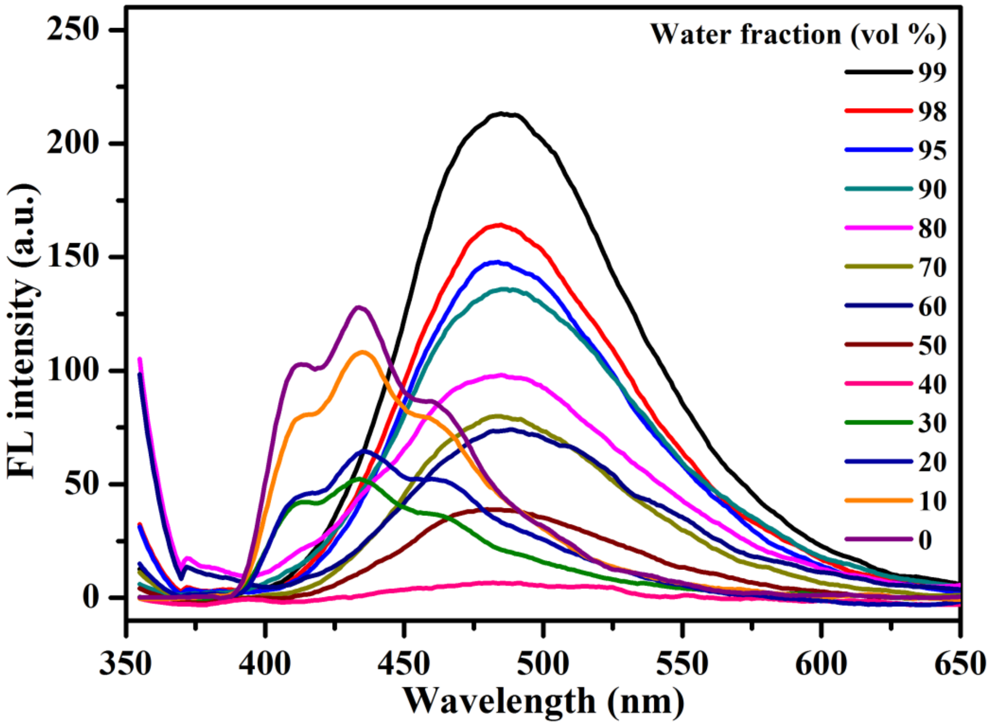


Fig. S5 Fluorescence spectra of TPE-CHO in THF/water mixtures excited by 330 nm.

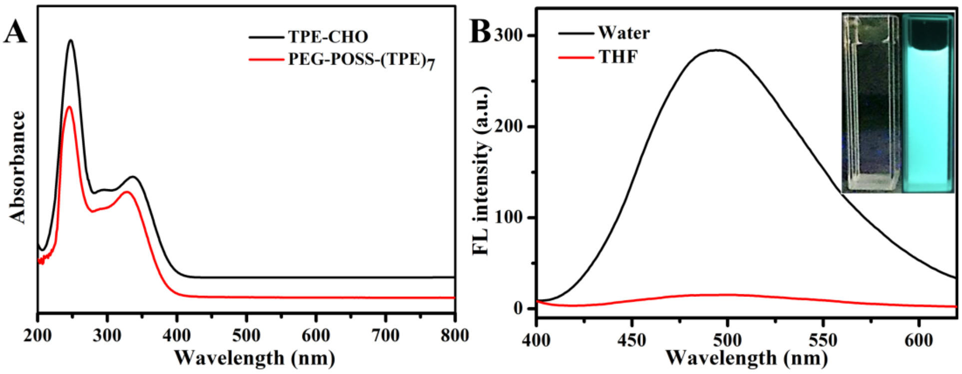


Fig. S6 (A) UV-vis spectra of TPE-CHO molecule and PEG-POSS-$(TPE)_7$ polymer in THF. (B) Fluorescence spectra of PEG-POSS-$(TPE)_7$ in water and THF excited by 330 nm.

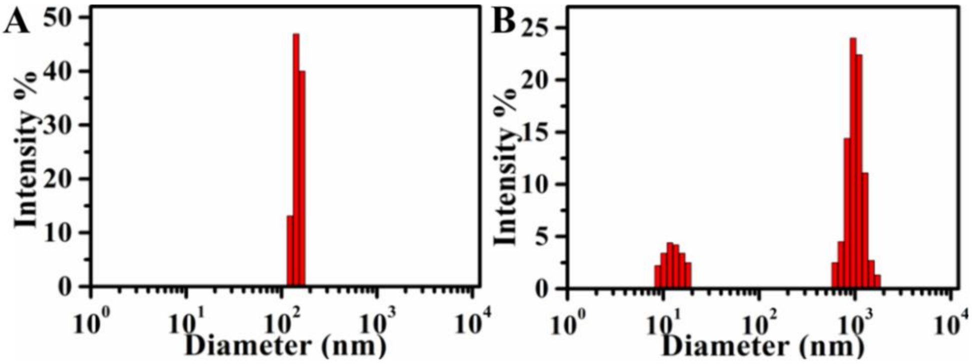


Fig. S7 DLS profiles of PEG-POSS-$(TPE)_7$ vesicles before (A) and after (B) incubation at pH 5.0 PBS solution for 4 h.

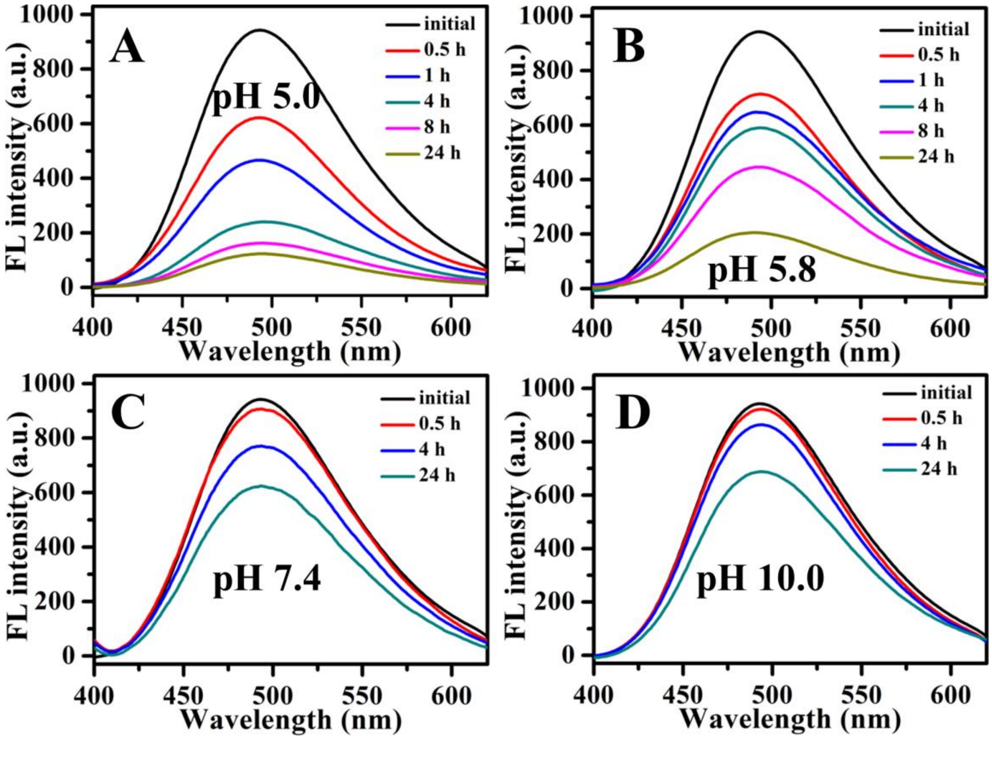


Fig. S8 Change of fluorescence intensity of PEG-POSS-$(TPE)_7$ vesicles in response to (A) pH 5.0, (B) pH 5.8, (C) pH 7.4, and (D) pH 10.0 in PBS solutions at 37 ℃ for various treated time.

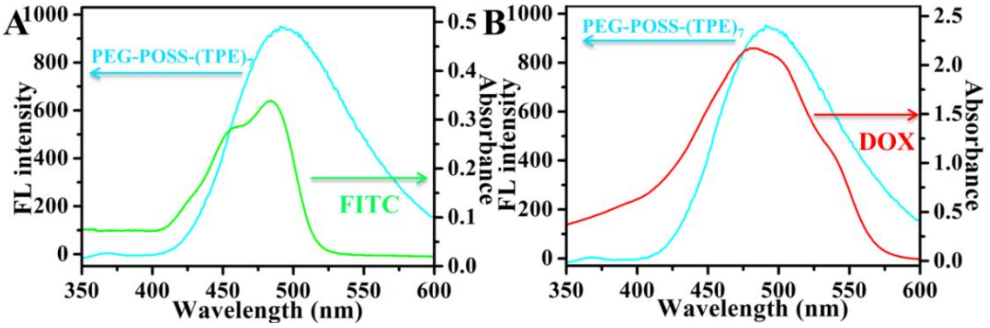
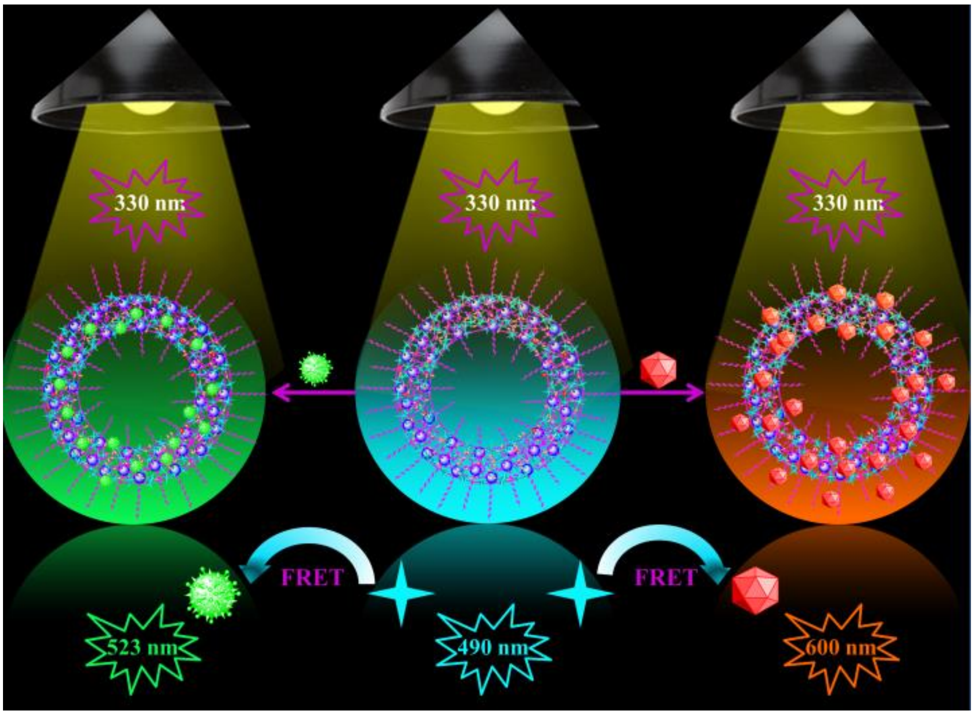


Fig. S9 Absorption and emission spectra of (A) PEG-POSS-(TPE)$_7$ (cyan) and FITC (green) couple, and (B) PEG-POSS-(TPE)$_7$ (cyan) and DOX (red) couple.



Scheme S2. Schematic illustration of energy transfer from TPE donor to FITC or DOX acceptor at the wall of PEG-POSS-(TPE)$_7$ vesicle.

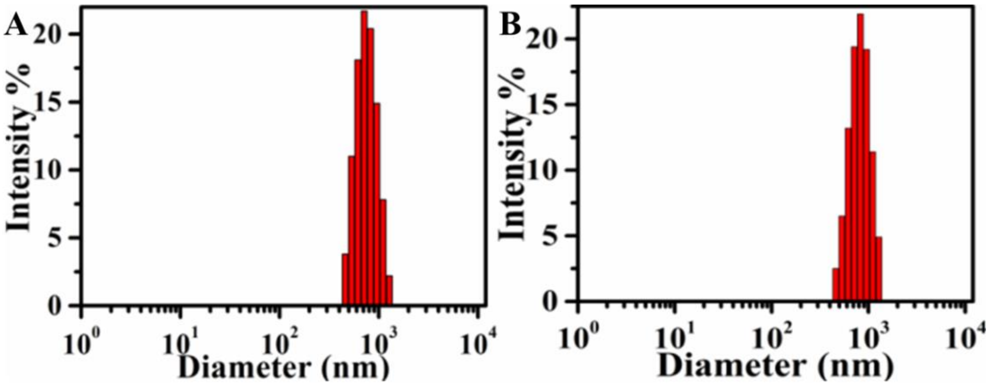


Fig. S10 DLS profiles of (A) PEG-POSS-(TPE)$_7$@FITC and (b) PEG-POSS-(TPE)$_7$@DOX aggregates.