Supporting Information

Enhancing the efficacy of photodynamic therapy (PDT) *via* water-soluble pillar[5]arene-based supramolecular complexes

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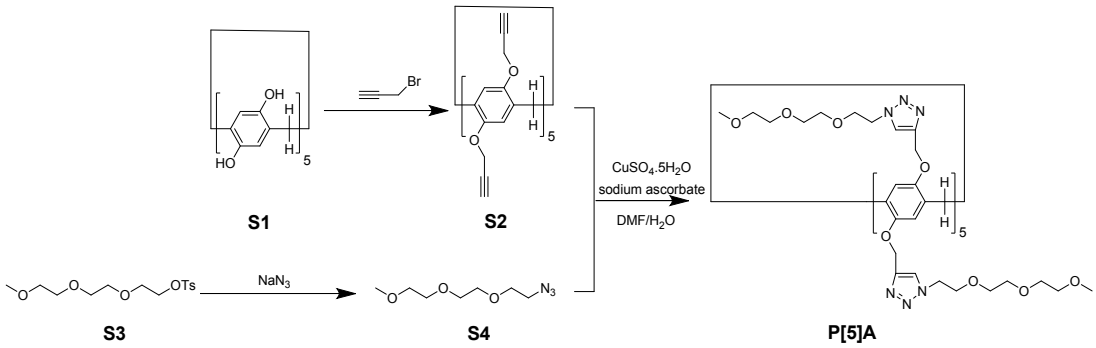
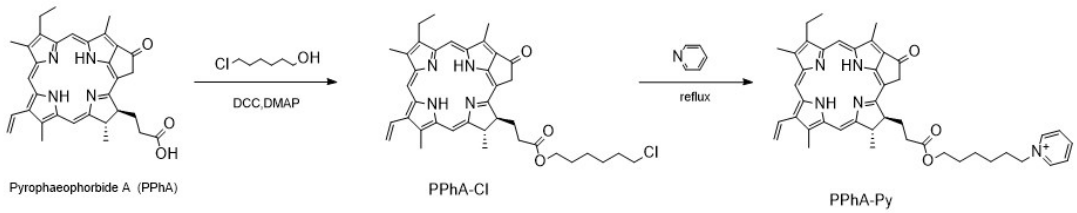
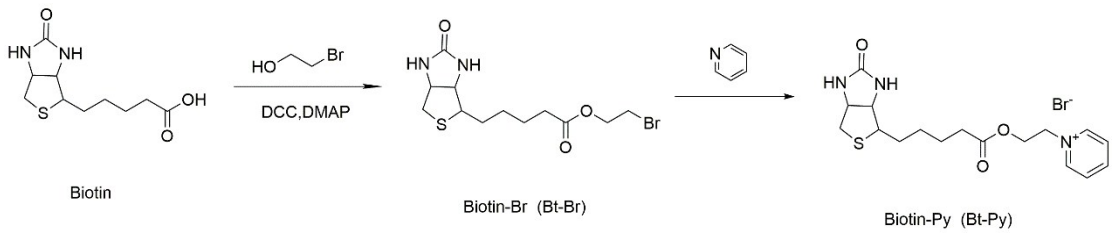


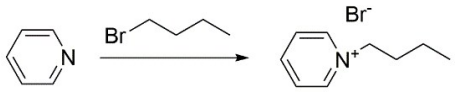
Scheme S1. Synthesis of pillar[5]arene (**P[5]A**)



Scheme S2. Synthesis of pyrophaeophorbide-A-pyridinium (**PPhA-Py**)



Scheme S3. Synthesis of *D*-biotin-pyridinium (**Bt-Py**)



Scheme S4. Synthesis of Guest Model ($\mathbf{G_M}$)

1. Materials and methods

1.1 Materials.

G_M was synthesized according to our previous work[1]. Tetrahydrofuran (THF) was refluxed to remove water over sodium. Dichloromethane (DCM) and *N*, *N*-dimethylformamide (DMF) were dried over calcium hydride and distilled before use. Triethylene glycol, hydroquinone dimethyl, propargyl bromide, 1-bromobutane, 2-bromoethanol, *D*-biotin, 4-dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), boron trifluoride diethyl etherate, *L*-ascorbic acid sodium salt, 6-chlorohexanol, paraformaldehyde and copper(II) sulfate pentahydrate were all purchased from Aladdin Reagents of China and used directly as received. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and 4', 6-diamidino-2-phenylindole (DAPI) were purchased from Beyotime and used as received. Pyrophaeophorbide-A was purchased from Shanghai Xianhui pharmaceutical Co. Ltd and used as received. Other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd (SRC) and purified by standard procedure.

1.2 Methods

^{1}H NMR spectrum was recorded at 400 MHz, using a BRUKER AV400 Spectrophotometer, in D_2O or $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Absorption spectra were determined on a SHIMADZU UV-2550 UV spectrophotometer using in quartz Cuvette with 1 cm beam path length, and fluorescence spectra measurements were performed on a Varian's Cary Eclipse fluorescence spectrophotometer at room temperature with excitation and emission slit width of 10 nm and 5 nm, respectively. MS was performed by using a XEVO G2 TOF. Dynamic light scattering (DLS) measurements were carried out with a BECKMAN COULTER Delasa Nano C particle analyzer. All the measurements were carried out at room temperature. Transmission electron microscopy (TEM) analysis was performed on a JEOL JEM1400 electron microscope operated at 100 kV. Samples for TEM were prepared by dropping the solution onto a carbon-coated copper grid and then dried at room temperature.

2. Syntheses of compounds and characterization

2.1 Synthesis of S2

A flask (100 mL) was charged with a magnetic stirring bar. **S1** (1 g, 1.61 mmol), 3-bromo-1-propyne (2.87 g, 24.15 mmol), K_2CO_3 (3.40 g, 24.15 mmol), and 30 mL DMF were added to the flask under an argon atmosphere. The reaction was carried out in a 60 °C oil bath and stirred for 24 h. Then the resulting mixture was dissolved in dichloromethane and washed with a saturated solution of sodium chloride three times, and the organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography on silica gel by using dichloromethane/petroleum ether (1:1, v/v) as the eluent. The final product was dried under vacuum at room temperature for 24 h (2.48 g, 85.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.82 (s, 10H, Ar*H*), 4.53 (d, 20H, CH≡CC*H*$_2$-), 3.81 (s, 10H, -ArC*H*$_2$Ar-), 2.51 (t, 10H, C*H*≡CCH$_2$-).

2.2 Synthesis of S4

The synthetic procedure was according to the established method[2]. The final product was dried under vacuum 24 h to get a pale yellow liquid in a yield of 85.0 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.70-3.65 (m, 8H), 3.56 (m, 2H), 3.41-3.38 (m, 5H).

2.3 Synthesis of P[5]A

A mixture of **S2** (1 g, 1.0 mmol), **S4** (3.78 g, 20 mmol), $CuSO_4 \cdot 5H_2O$ (0.26 g, 1.0 mmol), and sodium ascorbate (0.59 g, 0.330 mmol) in DMF/H_2O (16 mL/4 mL) was vigorously stirred at room temperature under nitrogen atmosphere. After 24 h, deionized water (20 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (50 mL) three times and the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (silica, dichloromethane/methanol = 40/1, v/v) to give P[5]A (2.31 g, 80%) as a viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.89 (s, 10H), 6.94 (s, 10H), 4.85 (dd, 20H), 4.43 (t, 20H), 3.77 (s, 10H), 3.72 (dd, 20H), 3.50−3.31 (m, 80H), 3.27 (s, 30H).

2.4 Synthesis of PPhA-Cl

Pyropheophorbide-a (106.9 mg, 0.2 mmol), DMAP (36.7 mg, 0.3 mmol), and 6-chlorohexanol (120 mg, 1.0 mmol) were dissolved in anhydrous dichloromethane (15 mL). The flask was cooled to 0 °C in ice water bath. Then DCC (61.9 mg, 0.3 mmol) in 5 mL of anhydrous dichloromethane was added dropwise and stirred for 24 h at room temperature. After filtration, the filtrate was concentrated and further purified by silica gel column chromatography using dichloromethane. After drying two days in a vacuum oven at room temperature, a purple solid was obtained (167.2 mg, yield: 84.7%).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.42 (s, 1H, 5-*H* of pyro), 9.31 (s, 1H, 10-*H* of pyro), 8.48 (s, 1H, 20-*H* of pyro), 7.93 (dd, 1H, –C*H*=CH_2 of pyro), 6.16 (dd, 2H, –CH=C*H*$_2$ of pyro), 5.22 (dd, 2H, 13^2 -C*H*$_2$), 5.05 (d, 2H, -COO-C*H*$_2$-), 4.45 (d, 1H for 18-*H*), 4.29 (s, 1H for 17-*H*), 3.70-3.45 (m, 7H, 8-C*H*$_2$-CH_3, 12-C*H*$_3$, –C*H*$_2$Cl,

), 3.29 (d, 3H, 2-C*H*$_3$), 3.16 (s, 3H, 7-C*H*$_3$), 2.72 (s, 4H, -COO-CH$_2$-C*H*$_2$-, 2×17^2 -*H*), 2.39 (d, 4H, -C*H*$_2$-CH$_2$Cl, 2×17^1 -*H*), 1.74 (d, 5H, 18-C*H*$_3$, -COO-CH$_2$-CH$_2$-C*H*$_2$-), 1.62 (t, 5H, 8-CH$_2$- C*H*$_3$, -C*H*$_2$-CH$_2$-CH$_2$Cl). LRESIMS: *m*/*z* 651.20 [M - H]$^-$ (100%).

2.5 Synthesis of PPhA-Py.

PPhA-Cl (326 mg, 0.5 mmol) was added in pyridine, and then the reaction mixture was refluxed at 110 °C in a sealed flask. After being cooled to room temperature, the unreacted pyridine was removed under vacuum to produce the PPhA-Py with the yield of 96% (420 mg, 0.48 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 9.44-9.21 (m, 4H, 5-*H* and 20-*H* of pyro, 2×*o*-*H* of pyridinium), 8.54 (s, 1H, 10-*H* of pyro), 8.23 (s, 1H, *p*-*H* of pyridinium), 8.02-7.78 (m, 3H, 2×*m*-*H* of pyridinium, –C*H*=CH$_2$ of pyro), 6.20 (dd, 2H, –CH=C*H*$_2$ of pyro), 5.29 (t, 2H, 13^2 -C*H*$_2$), 5.06 (d, 2H, -COO-C*H*$_2$-), 4.82 (s, 2H, -C*H*$_2$-N), 4.48 (d, 1H, 18-*H*), 4.23 (d, 1H, 17-*H*), 3.70-3.55 (m, 5H, 8-C*H*$_2$-CH$_3$, 12-C*H*$_3$), 3.40 (d, 3H, 2-C*H*$_3$), 3.18 (s, 1H, 7-C*H*$_3$), 2.66 (s, 2H, 2×17^2 -*H*), 2.61-2.45 (m, 2H, -C*H*$_2$-C*H*$_2$-N), 2.37-2.16 (m, 4H, 2×17^1-*H*, -COO-CH$_2$-C*H*$_2$-). LRESIMS: *m*/*z* 695.60 [M]$^+$ (100%).

2.6 Synthesis of Biotin-Br

Biotin-Br was synthesized according to previous literature[3]. *D*-biotin (200 mg, 0.82 mmol), DMAP (330 mg, 1.6 mmol), and 2-bromoethanol (200 mg, 1.6 mmol) were dissolved in anhydrous DMF (10 mL). The flask was cooled to 0 °C in ice water bath. Then DCC (195 mg, 1.6 mmol) in 5 mL of anhydrous DCM was added dropwise and stirred for 24 h at room temperature. The mixture was extracted with DCM and washed with saturated sodium chloride solution three times. The organic layer was dried over anhydrous MgSO$_4$ and concentrated in vacuo. The crude product was purified by silica gel column chromatography using dichloromethane/methanol (50:1, v/v) as eluent and dried under vacuum for 24 h to give *D*-biotin-Br as white solid (165 mg, yield: 57%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 5.38 (d, 1H), 4.98 (s, 1H), 4.56-

4.47 (m, 1H), 4.40 (t, 2H), 4.36-4.29 (m, 1H), 3.53 (t, 2H), 3.17 (ddd, 1H), 2.93 (dd, 1H), 2.74 (d, 1H), 2.40 (t, 2H), 1.77-1.65 (m, 4H), 1.56-1.39 (m, 2H).

2.7 Synthesis of Biotin-Py

Biotin-Py was synthesized according to published work[4]. Biotin-Br (70 mg, 0.2 mmol) was dissolved in pyridine (10 mL), and then the reaction mixture was refluxed at 100 °C for 12 h. After being cooled to room temperature, the solution was precipitated in 200 mL cold ethyl acetate and the precipitate was washed with DCM three times. After drying 24 h under vacuum, the final product was obtained in a yield of 29 % (25 mg). ^{1}H NMR (400 MHz, D_2O) δ ppm: δ 8.94 (d, 2H), 8.64 (t, 1H), 8.15 (t, 2H), 4.97 (t, 2H), 4.68-4.55 (m, 3H), 4.41 (dd , 1H), 3.36-3.21 (m, 1H), 2.99 (dd, 1H), 2.83-2.73 (m, 1H), 2.39 (t, 2H), 1.77-1.40 (m, 4H), 1.37-1.17 (m, 2H).

3. Investigation of the interactions between P[5]A and PPhA-Py.

3.1 Fluorescence titration experiments

To investigate association constant between P[5]A and PPhA-Py, fluorescence titration experiments were performed in aqueous solution, which had a constant concentration of PPhA-Py (1.0×10^{-5} M) and varying concentrations of P[5]A. By a non-linear curve-fitting method, the K_a of P[5]A/PPhA-Py was estimated to be $(1.91 \pm 0.58) \times 10^4$ M^{-1}

The non-linear curve-fittings were based on the following equation[5-7]:

$$\Delta F = (\Delta F_\infty/[G]_0)(0.5[H]_0 + 0.5([G]_0 + 1/K_a) - (0.5([H]_0^2 + (2[H]_0(1/K_a - [G]_0)) + (1/K_a + [G]_0)^2)^{0.5}))$$

Where, ΔF is the fluorescence intensity changes at 685 nm at $[G]_0$; ΔF_∞ is the fluorescence intensity changes at 685 nm when PPhA-Py is completely complexed; $[G]_0$ is the fixed initial concentration of PPhA-Py and $[H]_0$ is the initial concentration of P[5]A.

3.2 UV-*vis* absorption of P[5]A/PPhA-Py complexes.

To further verify the complexation occurred between P[5]A and PPhA-Py, UV-*vis* absorption was performed with a constant concentration of PPhA-Py (1.0×10^{-5} M) and varying concentrations of P[5]A (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 3.0, 4.0, 6.0, 8.0 and 10×10^{-5} M) in water.

4. Critical vesicle concentration (CVC) of the PPhA-Py/P[5]A complexes.

The CVC of P[5]A/PPhA-Py complexes was determined by a fluorescence spectrometer using pyrene as a hydrophobic fluorescent probe. A 5 μL of pyrene solution (1.2 mmol L^{-1}) in acetone was added into a series of volumetric flasks ensuring that the final pyrene concentration was 6.0×10^{-7} mol L^{-1} and then allowed to evaporate at room temperature. P[5]A/PPhA-Py solution was diluted to a series of concentrations ranging from 0.25 to 20 μg/mL by deionized water. The excitation spectra of pyrene with different spherical nanoparticles concentrations were recorded at the excitation wavelength of 335 nm. The CVC was estimated as the cross-point when extrapolating the intensity ratio I_{382}/I_{372} at low and high concentration regions. The CVC of P[5]A/PPhA-Py in aqueous solution is about 5.5×10^{-3} mg/mL.

5. Self-assembly of the P[5]A/PPhA-Py and P[5]A/PPhA-Py/Bt-Py complexes in aqueous solution.

PPhA-Py (2 mg, 2.3 μmol), and P[5]A (15.0 mg, 7 μmol) were dissolved in 0.2 mL of DMF and then stirred and maintained at 25 °C with a water bath for 2 h in the dark. Subsequently, the DMF solution was injected into aqueous solution (9 mL). After stirring for another 2 h, DMF was removed by dialysis (MWCO 12 kDa) against deionized water for 2 days (changing water for 6 times). Finally, the concentration of the assembled solution was 0.135 mg/mL (calculated by PPhA concentration). The

P[5]A/PPhA-Py/Bt-Py assemblies was constructed by mixing P[5]A/PPhA-Py vesicles with a small amount of Bt-Py (0.43 mg, 1 μmol). The mixed solution was strring at room temperature for 24 h. The final concentration was 0.135 mg/mL (calculated by PPhA concentration).

6. Singlet oxygen measurement of PPhA-Py/P[5]A complexes

Singlet oxygen ($^1O_2$) measurement of P[5]A/PPhA-Py complexes with constant concentration of PPhA-Py (1.0×10^{-5} M) and different concentration of P[5]A (0, 1.0, 5.0×10^{-5} M) was performed by using 1,3-diphenylisobenzofuran (DPBF) as a chemical $^1O_2$ probe in a DMF/H_2O (1:100, v/v) mixed solution (1% DMF was used to improve solubility of DPBF)[8,9]. Briefly, 50 μL DPBF (1.5 mg/mL in DMF) was added into 4.95 mL P[5]A/PPhA-Py solution in which the concentration of PPhA-Py and P[5]A were both 1.0×10^{-5} M. The mixed solution was then irradiated with a 660 nm laser light, and the decay of DPBF at 450 nm was monitored every 30 s by UV-*vis* spectrophotometer. Similar procedure was applied to PPhA-Py solution (1.0×10^{-5} M) and P[5]A/PPhA-Py solution (1.0×10^{-5} M/5.0×10^{-5} M, respectively).

6. Cell culture.

HeLa cells were routinely incubated in Dulbecco's modified Eagle's medium (DMEM) supplied with 50 units/mL (penicillin and streptomycin) and 10% fetal bovine serum (FBS) in a humidified standard atmosphere of 5% CO_2 at 37 °C.

7. Cellular uptake studies.

The cellular uptake experiments were carried out using flow cytometry and confocal laser scanning microscope (CLSM). Flow cytometry was used to set up statistics on the uptake of P[5]A/PPhA-Py vesicles into HeLa cells. The HeLa cells were seeded in six-well flat culture plates with a cell density of 1×10^4 cells per well. After 24 h of culture, P[5]A/PPhA-Py vesicles in DMEM were replaced and treated for 4 h and 24 h, respectively. After the predetermined time, the cells were trypsinized and washed with PBS solution. Then the cells were suspended in PBS for determining the

fluorescence intensity on a BD FACS Calibur flow cytometer.

For CLSM measurement, HeLa cells were cultured in 2 mL culture medium on polylysine-coated glass slides inside 30 mm glass culture dishes and allowed to grow to 50−70% at 37 °C. Afterward, cells (on glass slides) were washed with PBS, and reincubated in DMEM medium containing P[5]A/PPhA-Py vesicles at 37 °C under 5% CO_2 for 24 h. After predetermined time, the culture medium was removed and washed with PBS three times. Subsequently, the 4, 6-diamidino-2-phenylindole (DAPI) was added to stain the nuclei for 3 min. The cells were imaged directly *via* CLSM after washing with PBS three times.

Similar procedure was used to evaluate cell uptake of PPhA-Py aggregates, P[5]A/PPhA-Py/Bt-Py vesicles and free pyropheophorbide-a.

8. *In vitro* dark cytotoxicity

The cytotoxicity of various samples in HeLa cells was determined *via* MTT assay. The cells were seeded in 96-well plates at the cell population of about 5000 cells/well in 200 μL DMEM. After 24 h of incubation in 96-well plates at 37 °C, samples with various concentrations were added to each well. And the samples were then subjected to incubation for 24 h. Then, the wells were incubated for another 4 h with RPMI 1640 medium containing 5 mg mL^{-1} MTT. After discarding the culture medium, 150 μL of DMSO was added to each well to extract the formazan products with gentle agitation for 10 min. The absorbance at 492 nm was measured using a spectrophotometric microplate reader (Thermo Multiskan MK3 spectrometer). The cell viability was calculated as follows: cell viability (%) = $(OD_{test})/(OD_{control}) \times 100$, where OD_{test} is the absorbance in the presence of sample solutions and $OD_{control}$ is the absorbance without treatment.

9. *In vitro* phototoxicity

Similar procedure was used to evaluate the phototoxicity of P[5]A/PPhA-Py vesicles, PPhA-Py assemblies, P[5]A/PPhA-Py/Bt-Py vesicles and free pyropheophorbide-a. After 4 h of incubation, the plate with cells was irradiated with a 660 nm laser light

for 20 min. The cells were then incubated at 37 °C for 24 h, and the cell viability was also estimated using the MTT assay as described above.

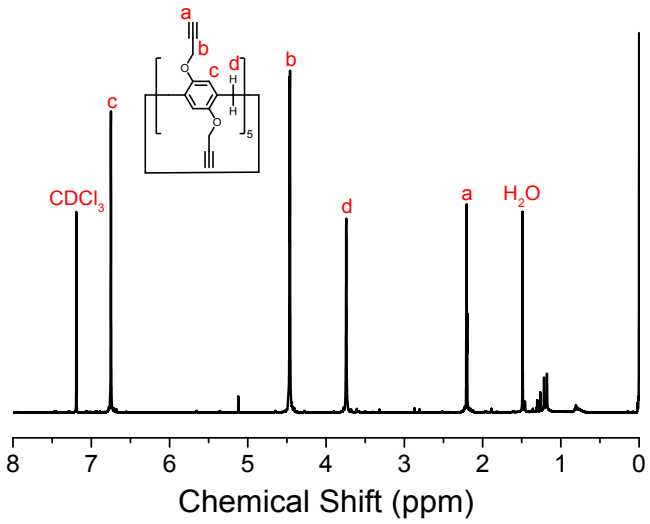


Fig. S1 ^{1}H NMR spectrum of S2.

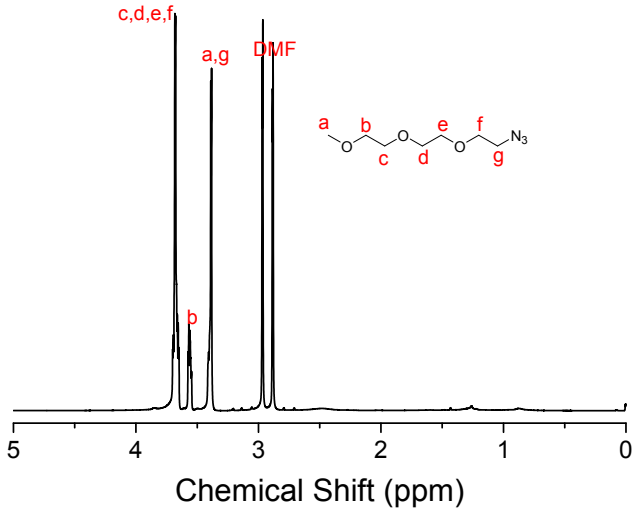


Fig. S2 ^{1}H NMR spectrum of **S4**.

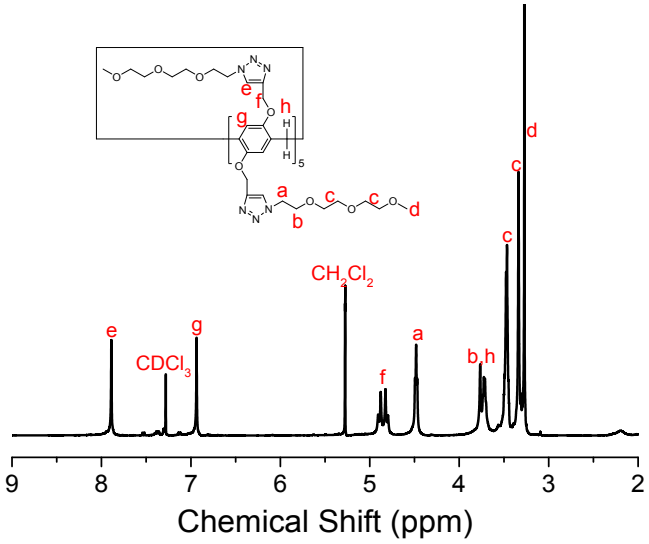


Fig. S3 ^{1}H NMR spectrum of **P[5]A**.

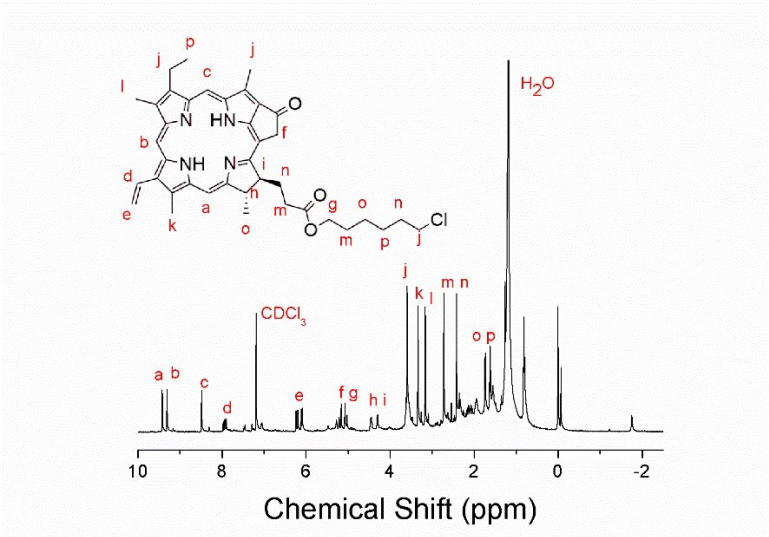


Fig. S4 ^{1}H NMR spectrum of **PPhA-Cl**.

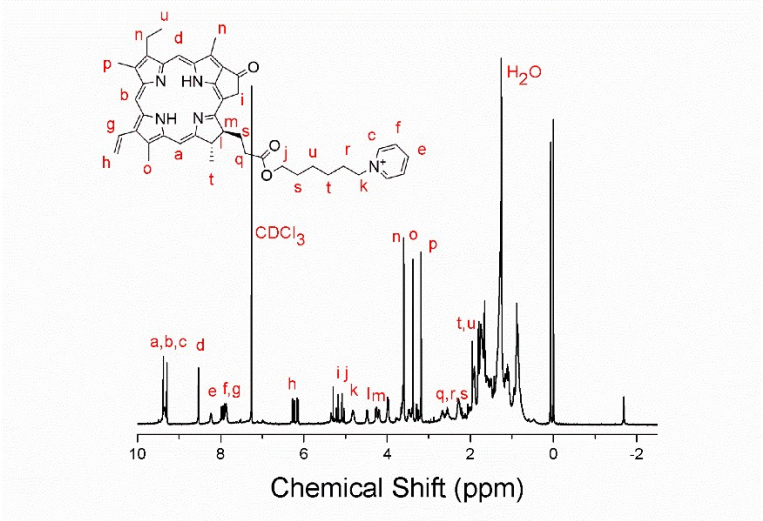


Fig. S5 ^{1}H NMR spectrum of **PPhA-Py**.

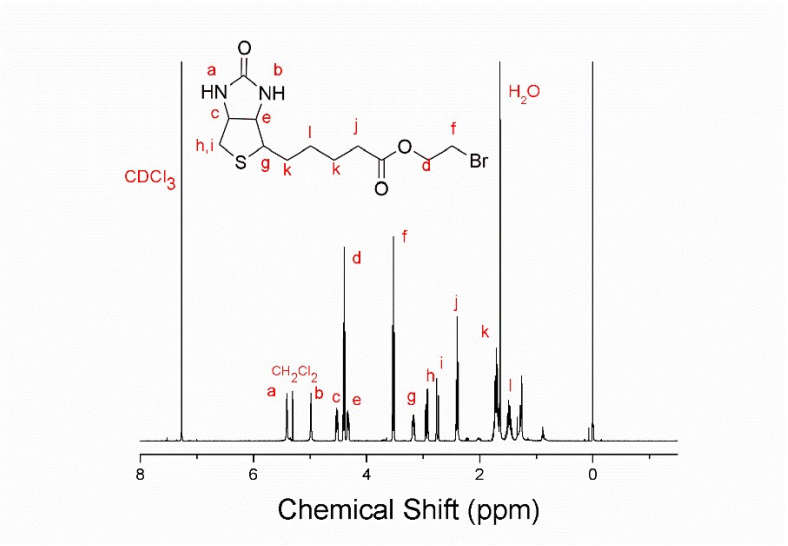


Fig. S6 ^{1}H NMR spectrum of **biotin-Br**.

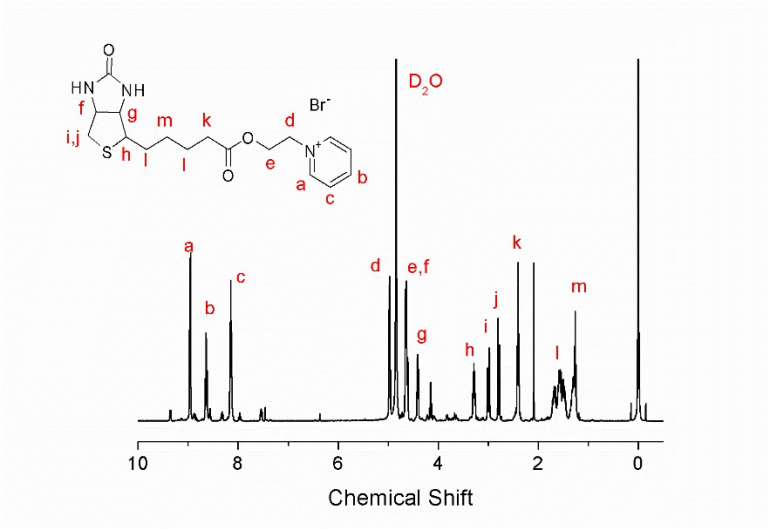


Fig. S7 ^{1}H NMR spectrum of **biotin-Py**.

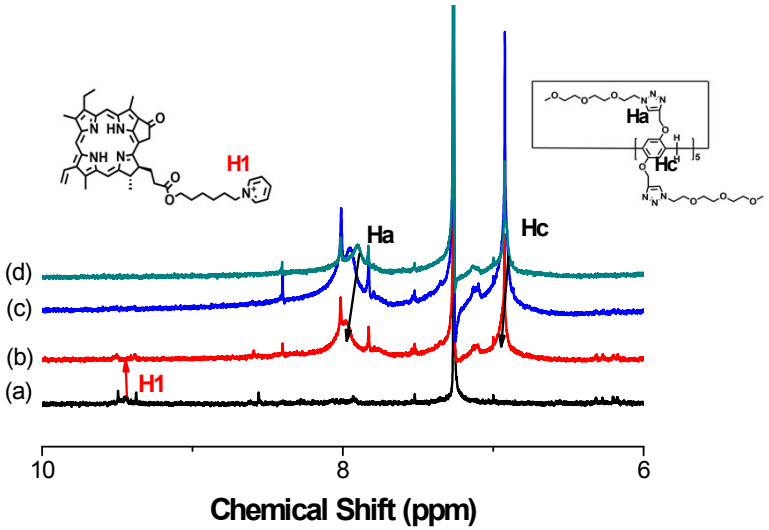


Fig. S8 Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of PPhA-Py at a constant concentration of 2 mM with different concentrations of P[5]A: (a) 0 mM, (b) 2 mM, (c) 4 mM and (d) individual P[5]A.

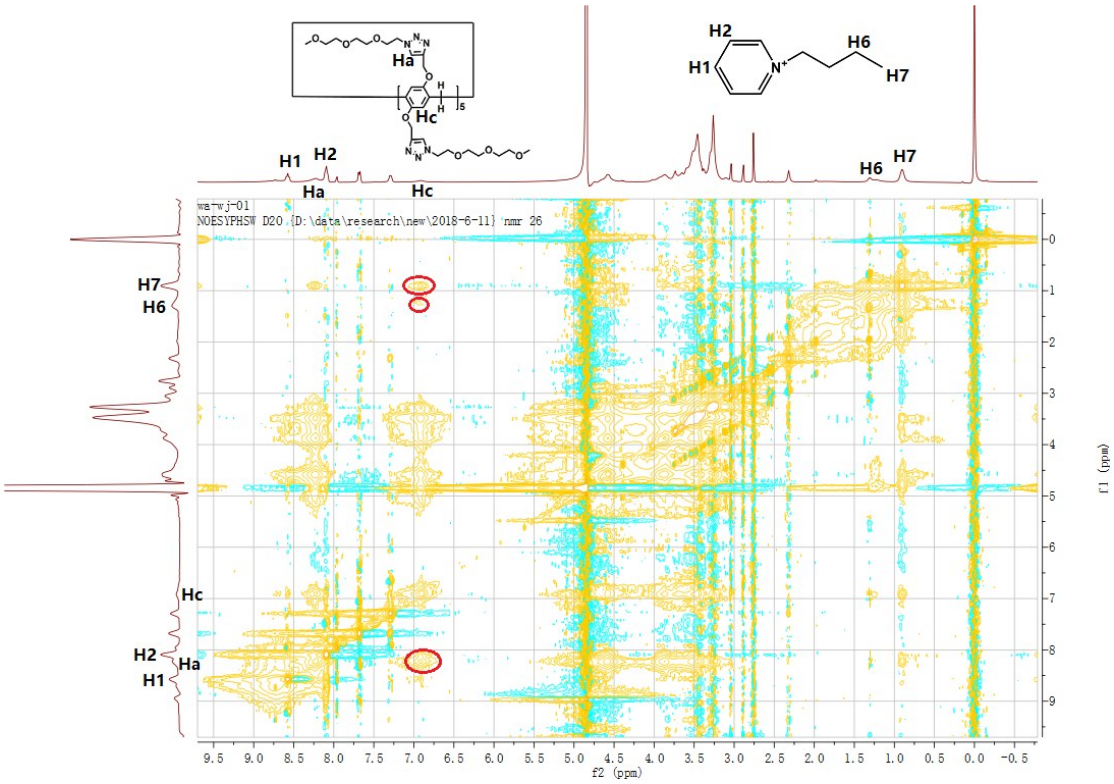


Fig. S9 2D NOESY spectrum of P[5]A⊃GM (400 MHz, D_2O, 298 K), P[5]A = 30 mM, GM = 30 mM.

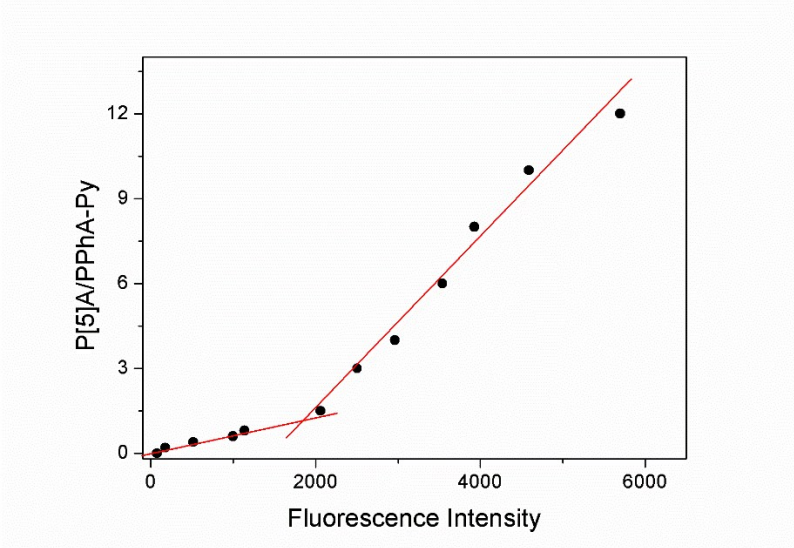


Fig. S10 Molar ratio plot for P[5]A and PPhA-Py, indicating a 1:1 stoichiometry.

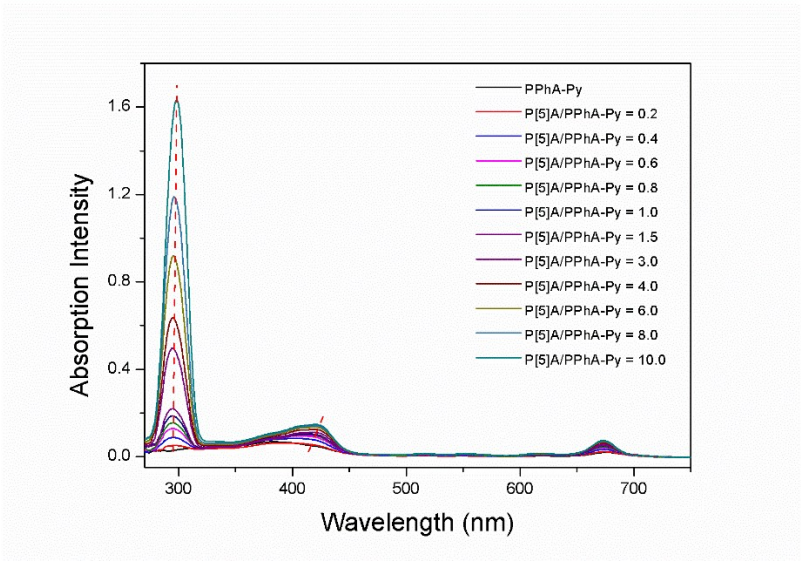


Fig. S11 UV-*vis* absorption spectra of PPhA-Py (1.0×10^{-5} M) in aqueous solution with different concentrations of P[5]A: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 3.0, 4.0, 6.0, 8.0 and 10×10^{-5} M.

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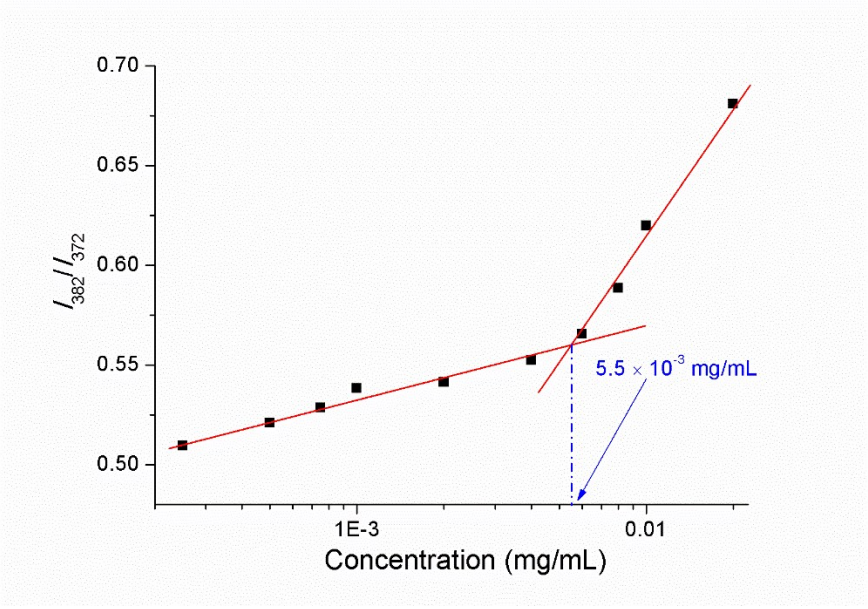


Fig. S12 Plot of the I_{382}/I_{372} ratio with different concentrations of PPhA-Py/P[5]A vesicles.

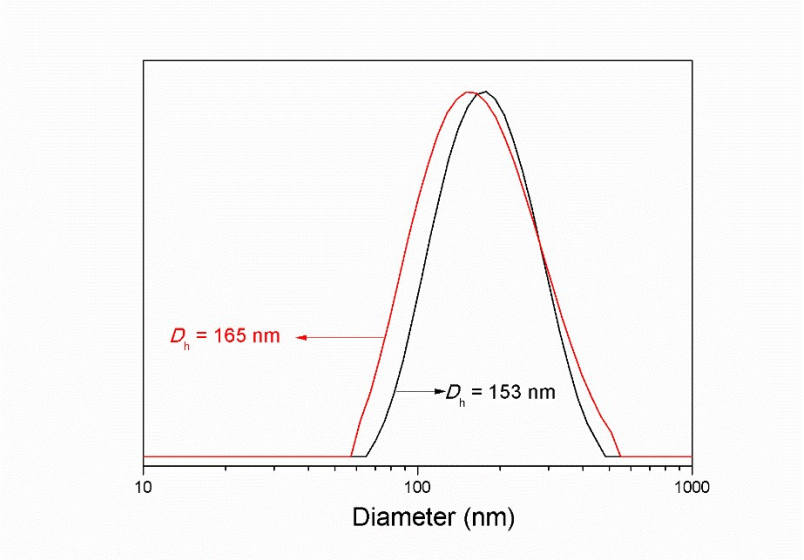
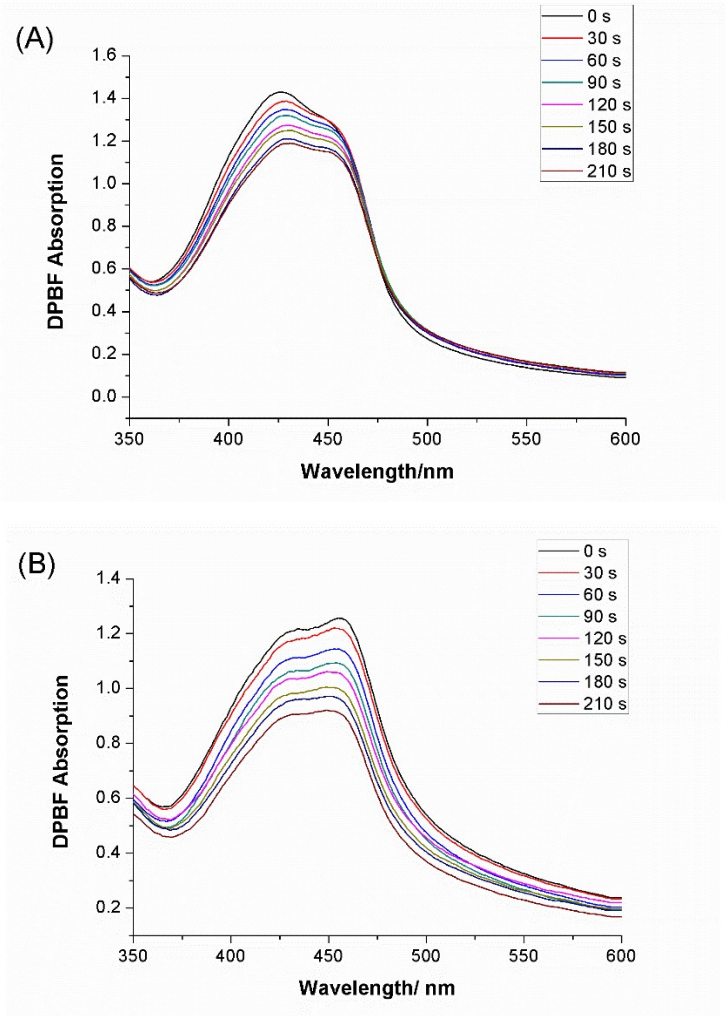


Fig.S13 DLS results of PPhA-Py (black line) and P[5]A/PPhA-Py complexes (red line).



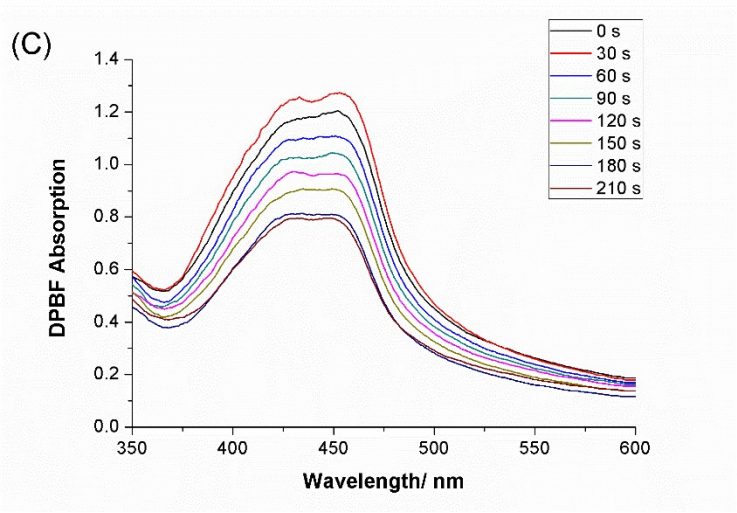


Fig. S14 UV-*vis* spectra of DPBF in the solution of P[5]A:PPhA = 0:1 (A), P[5]A:PPhA = 1:1 (B) and P[5]A:PPhA = 5:1 (C) under a 660 nm laser light, respectively.

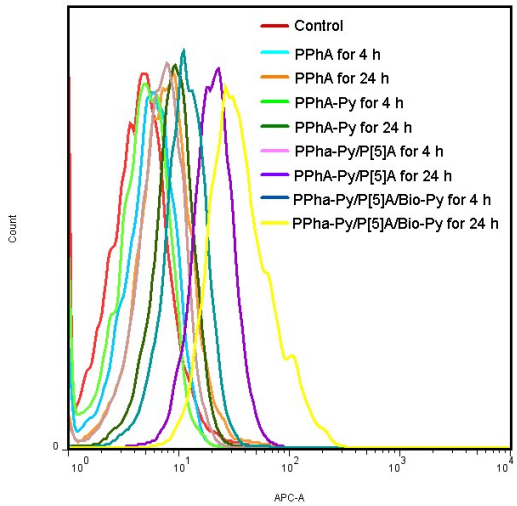


Fig. S15 The cellular uptake of free PPhA, PPhA-Py assemblies, PPhA-Py/P[5]A and PPhA-Py/P[5]A/Bt-Py supramolecular vesicles at 4 h and 24 h, respectively.

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