Pillar[5]arene based water-soluble [3]pseudorotaxane with enhanced fluorescence emission for cell imaging and both type I & II photodynamic cancer therapy

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Supporting Information

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1. Materials and methods

Materials

All reagents were commercially available and used as supplied without further purification. 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. For [3]WP5PR, λ_{ex} = 380 nm and λ_{em} = 465 nm.

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.

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

Cytotoxicity experiments. 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. Cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 4 h at 37ºC in 5% CO_2. Then the cells were incubated with different groups for 4 h. The relative cellular viability was determined by the MTT assay.

Confocal laser scanning microscopy. Cells were seeded in 6-well plates (5×10^4 cell mL^{-1}, 2 mL per well) for 24 h at 37ºC 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. The mean fluorescence intensity was analysis by image J.

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.

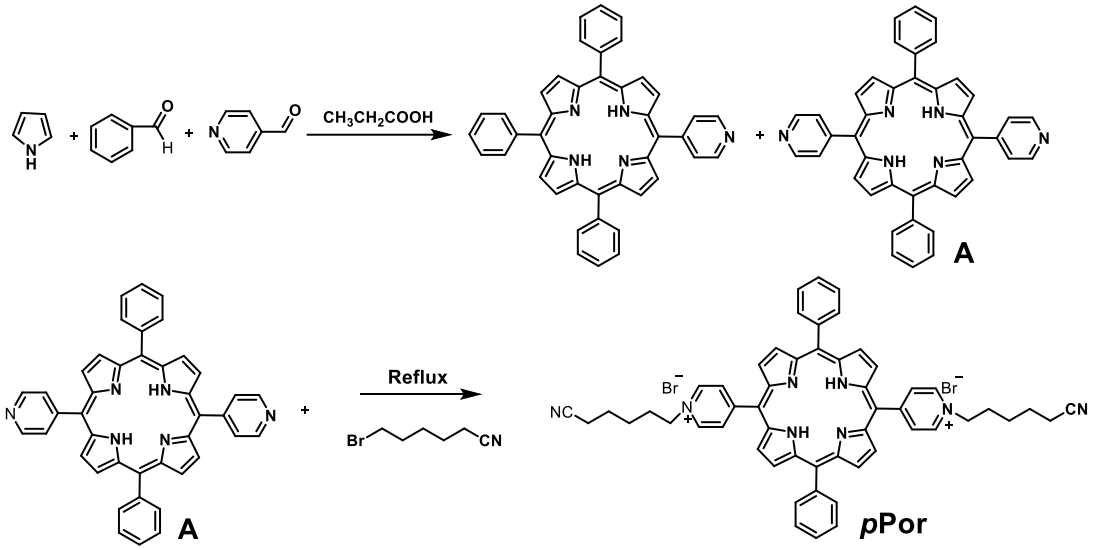
ROS detection. HeLa cells were seeded into 12 mm sterile coverslips in a 6-well plate and maintained for 12 h. The cells were incubated with WP5, pPor, and [3]WP5PR for 4h, then irradiated with 660 nm (1 mM/cm^2) laser for 10 min. Afterward, DCFH-DA/SOSG/APF was added to incubate together with cells and imaged, respectively.

Live-Dead Cell Staining. The same density of HeLa cells (3×10^5 cell mL^{-1}) were distributed into three confocal dishes (35 mm) for 12 h. Then the 2-plate cells were cultured with new DMEM containing [3]WP5PR NPs (70 μM). After 5 h, the cells were subjected to dark or laser irradiation (660 nm, 1.0 mW/cm^2, 10 min). After 48 h, the cells were stained with a calcein AM/propidium iodide mixture for 30 min and washed twice using PBS. The fluorescence images were eventually acquired via a confocal laser scanning microscope.

Flow cytometric analysis. Flow cytometric analysis was performed to detect the cell death. HeLa cells were seeded in a 6-well plate and maintained for 24 h. Then the cells were incubated with PBS, WP5, pPor, [3]WP5PR and the irradiated with 660 nm laser for 10 min, respectively, for 4 h at 37 °C. After that, the cells were washed several times with PBS and analyzed by flow cytometry.

2. **Synthesis of *para*-modified porphyrin (*p*Por)**

Scheme S1. Synthetic route to ***p*Por**.



In order to synthesize ***p*Por**, porphyrin **A** was prepared according to previous report firstly.[S1]

Under N_2 atmosphere, 0.4 mmol (0.246g) porphyrin **A** was added into a 50 mL round-bottled flask, dissolved in 25 mL DMF solution, and refluxed at 115℃. Then 1.2 mmol (0.212g) 6-bromocapronitrile was added into the reaction solution for 24 hours. At the end of the reaction, the solid was precipitated in methanol solution, and finally the product ***p*Por** was obtained through filtration as a purple solid.

***p*Por**: yield, 60%, m.p.262-264℃; ^{1}H NMR (400 MHz, CD_3OD) δ 9.43 (d, J = 6.5 Hz, 6H, ArH), 8.92 (d, J = 6.6 Hz, 8H, CH), 8.14 (d, J = 6.7 Hz, 4H, ArH), 7.84 (t, J = 7.5 Hz, 3H, ArH), 7.77 (t, J = 7.3 Hz, 5H, ArH), 5.02 (d, J = 7.6 Hz, 4H, CH_2), 2.64 (d, J = 7.0 Hz, 4H, CH_2), 2.42 (t, J = 7.6 Hz, 4H, CH_2), 1.94 – 1.90 (m, 4H, CH_2), 1.83 (m, 4H, CH_2); ^{13}C NMR (100 MHz, CD_3OD) δ 143.0, 134.2, 133.0, 126.7, 48.26, 48.08, 48.04, 47.89, 47.83, 47.63, 47.62, 47.40, 47.37, 47.19, 47.13, 46.98; MS (m/z): HRMS (ESI) Calcd. for $C_{54}H_{48}N_8$([M -2Br]$^{2+}$): 404.1995, found: 404.1993.

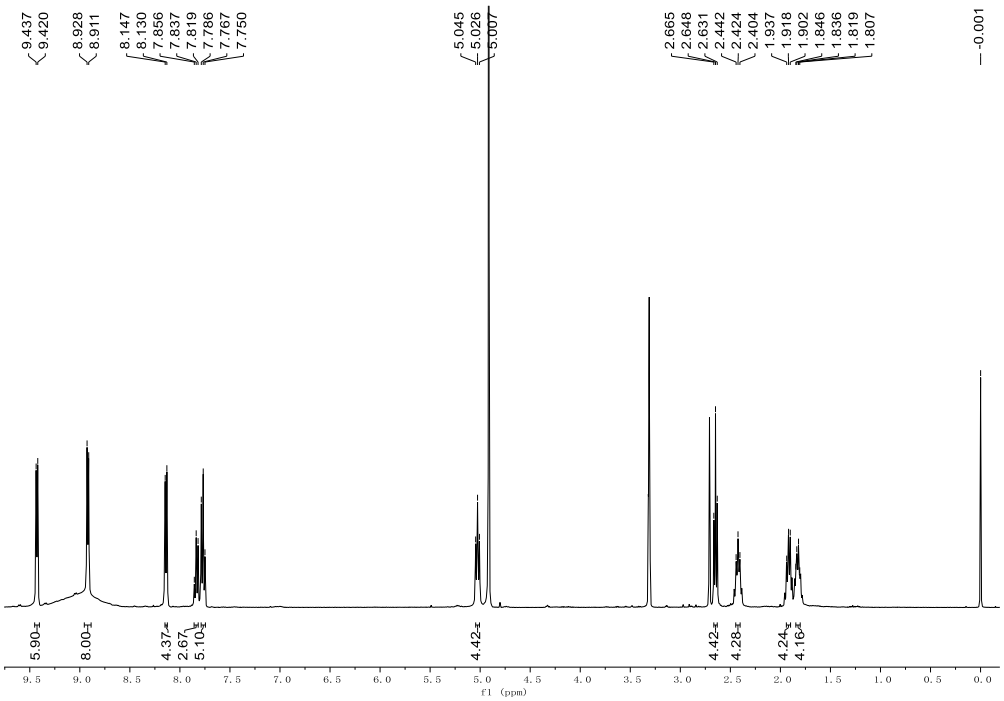


Figure S1. ^{1}HNMR (400 MHz, 298K, CD_3OD) spectrum of ***p*Por**.

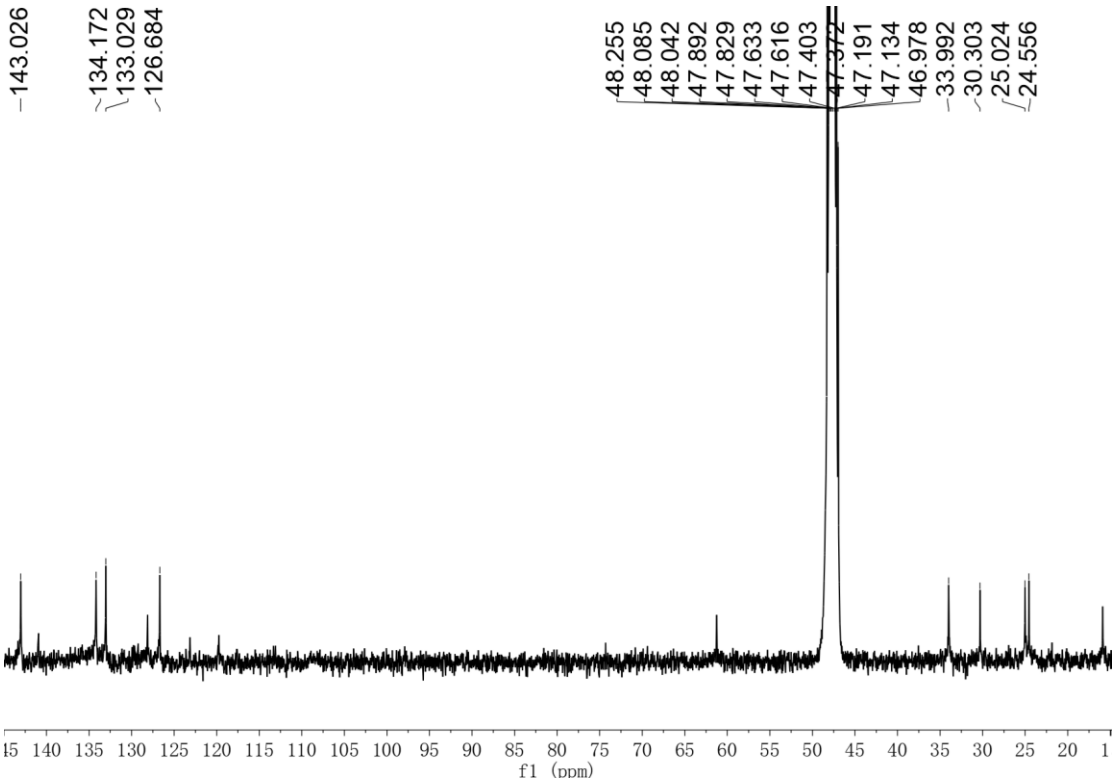


Figure S2. ^{13}C NMR (100 MHz, 298K, CD_3OD) spectrum of ***p*Por**.

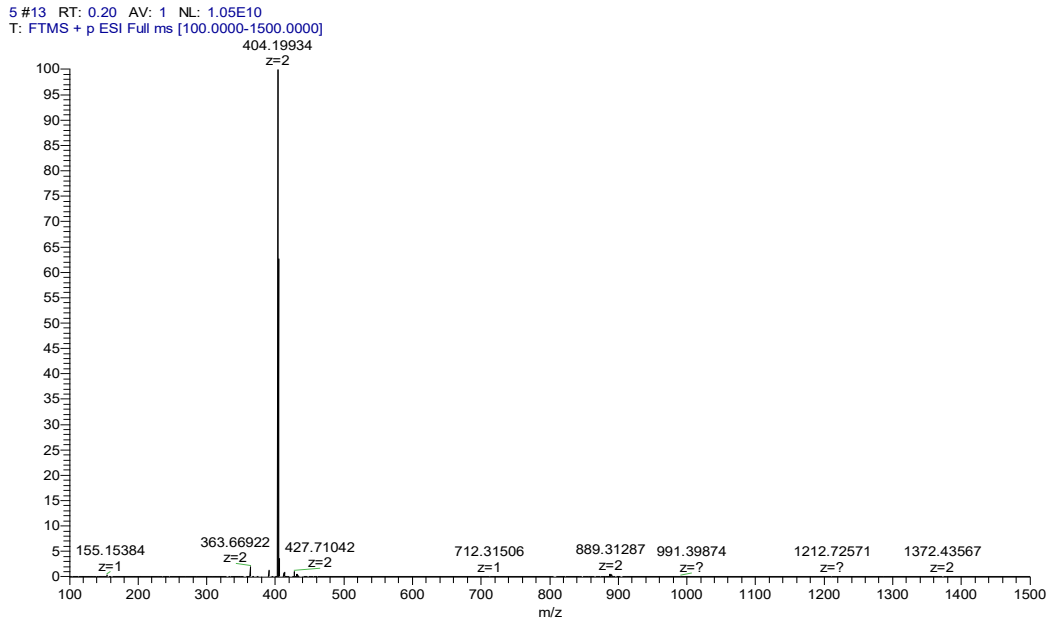
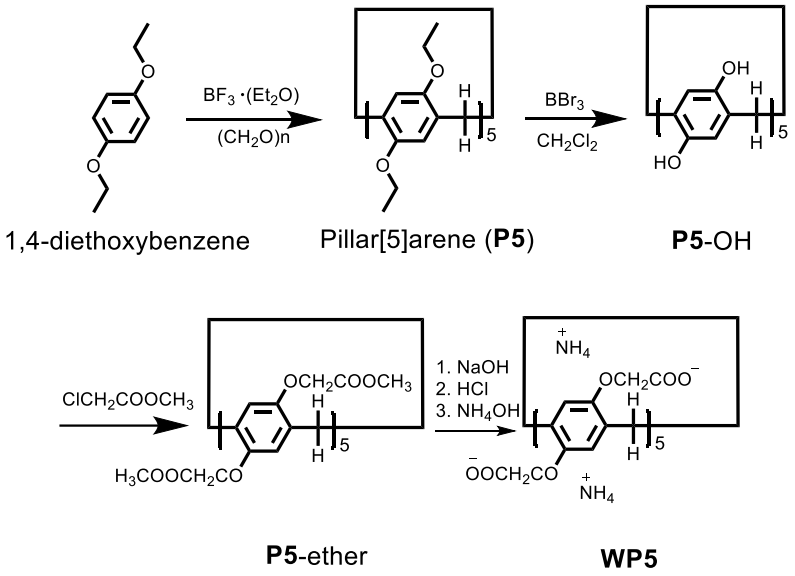


Figure S3. HR-MS (ESI) of ***p*Por**. Calcd. for $C_{54}H_{48}N_8$([M -2Br]$^{2+}$): 404.1995, found: 404.1993.

3. Synthesis of **WP5**[S2]

WP5 was prepared according to previous report,[S2] and the ^{1}H NMR was showed in Figure S4.



Scheme S2. Synthesis of water soluble pillar[5]arene (**WP5**).

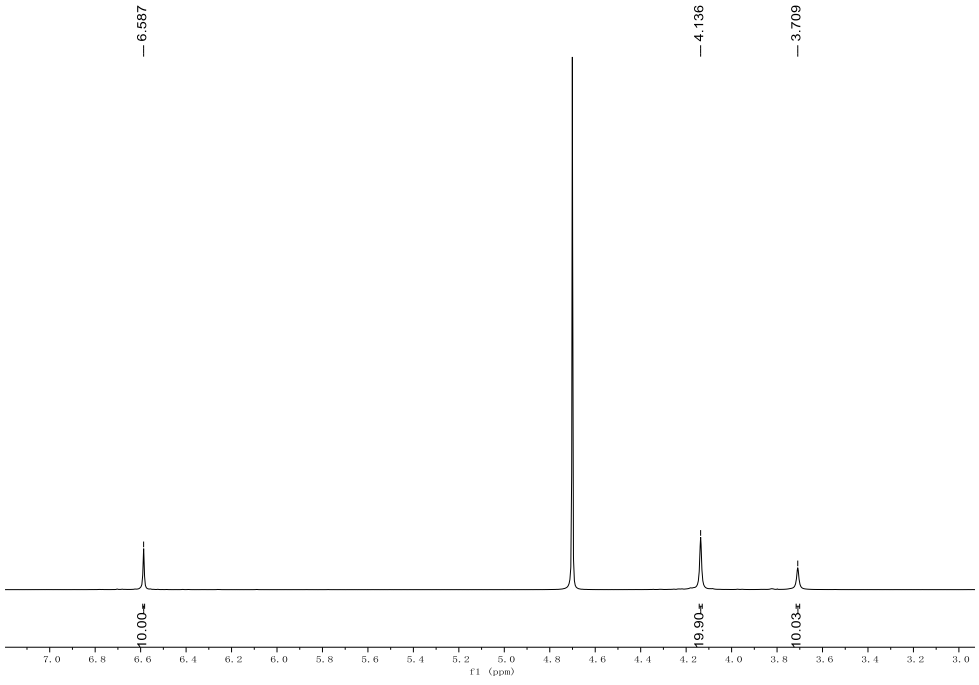


Figure S4. ^{1}H NMR (400 MHz, 298K, D_2O) spectrum of **WP5**.

4. Host-guest interaction between **WP5** and ***p*Por**

To determine the stoichiometry and association constant between **WP5** and ***p*Por**, Fluorescence titrations were done with solutions which had a constant concentration of ***p*Por** (10 μM) and varying concentrations of **WP5**. By a Stern-Volmer curves, the association constant between the ***p*Por** and **WP5** was calculated. By a mole ratio plot, a 2:1 stoichiometry was obtained; **WP5** was shown to form a 2:1 complex with ***p*Por**.

Subsequently, the formation of **[3]WP5PR** was investigated by 2D Nuclear Overhauser Effect Spectroscopy (NOESY). As shown in Fig. S5 (30.0 mM **WP5** and 15.0 mM ***p*Por** in D_2O/CD_3OD), correlations were observed between protons $H_{\alpha\text{-py}}$ of ***p*Por** and protons H_{bridge} on **WP5**, suggesting that pyridinium groups were threaded into the cavity of **WP5**. Therefore, it was confirmed that when ***p*Por** encountered **WP5**, the alkyl chain of ***p*Por** penetrated through the cavity of **WP5**. The formation of **[3]WP5PR** was mainly driven by multiple electrostatic interactions between the carboxylate anions on **WP5** and the cationic pyridiniums on ***p*Por**, hydrophobic interactions, and π–π stacking interactions.

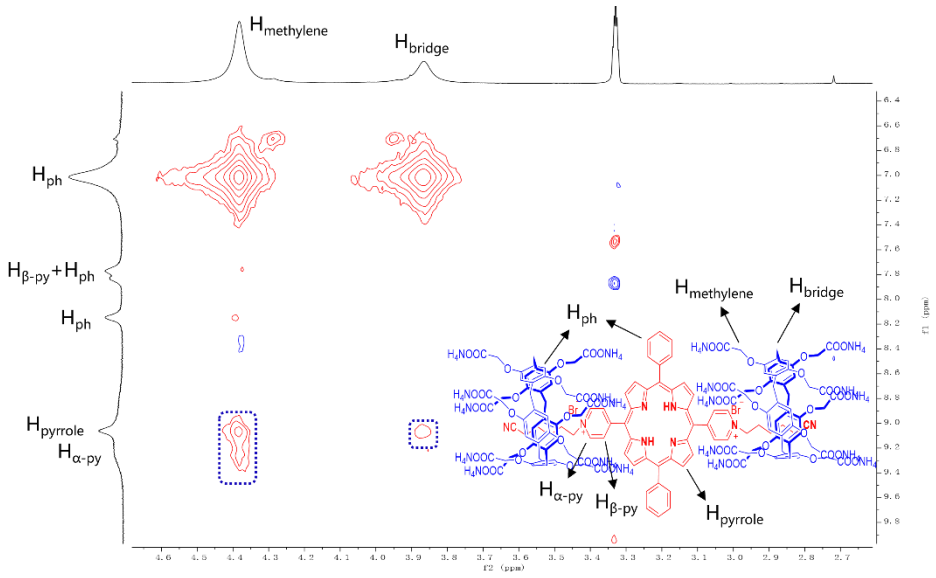


Figure S5. Partial 2D NOESY spectrum of **WP5** and ***p*Por** in CD_3OD + D_2O at 298K.

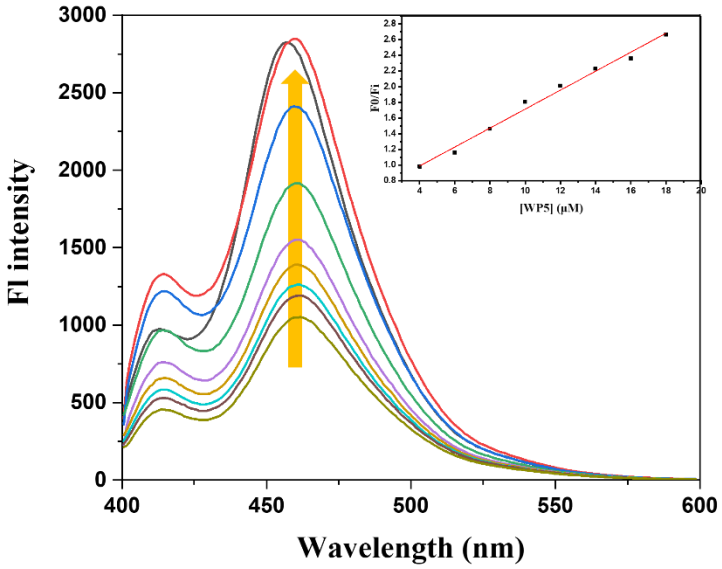


Figure S6. Fluorescence spectra of ***p*Por** in the presence of **WP5** (λ_{ex} = 380 nm). The inset was the Stern-Volmer curves for the **(WP5)$_2$⊃*p*Por** interaction (K = (1.21 ± 0.049) × 10^4 M^{-1}, R^2 = 0.9902). C(***p*Por**) = 10 μM. **WP5** concentrations were 0, 4, 6, 8, 10, 12, 14, 16, 18 μM, respectively.

As shown in Fig. S6, the fluorescence of ***p*Por** was enhanced with the gradual addition of **WP5** and the association constant (K) was calculated to be (1.21 ± 0.049) × 10^4 M^{-1}.

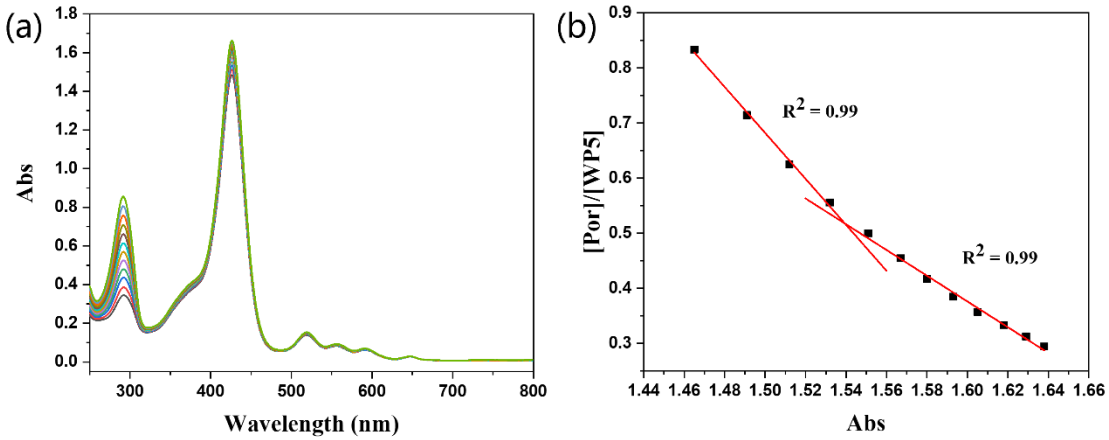


Figure S7. **(a)** UV titration of ***p*Por** in methanol solution with increasing **WP5** concentration. **(b)** Plot the work of the **(WP5)$_2$⊃*p*Por** complex by plotting the UV absorbance at 424 nm. Working diagram of composite **(WP5)$_2$⊃*p*Por** showing a stoichiometric ratio of 2:1 between **WP5** and ***p*Por**.

The mole ratio plot based on the UV titration experiments indicated that **WP5** and ***p*Por** had a 2 : 1 stoichiometry in **[3]WP5PR** (Fig. S7, ESI†). Therefore, according to the above experiments, we can confirm that **[3]WP5PR** is automatically formed when ***p*Por** and **WP5** were mixed in water.

5. ROS generation ability

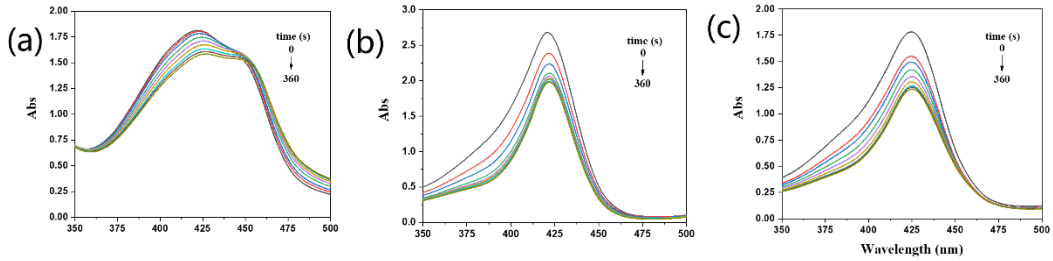


Figure S8. Time-dependent UV-vis spectra of **(a) DPBF** and **(b)** with ***p*Por (c)** with **[3]WP5PR** after irradiation with 660 nm light (1 mW cm^{-2}) in aqueous solution.

6. Cell imaging

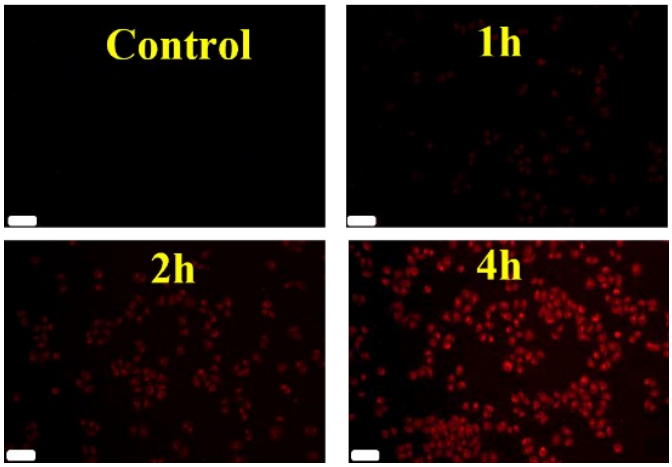


Figure S9. CLSM images of HeLa cells incubated **[3]WP5PR** for 0h, 1h, 2h and 4h.

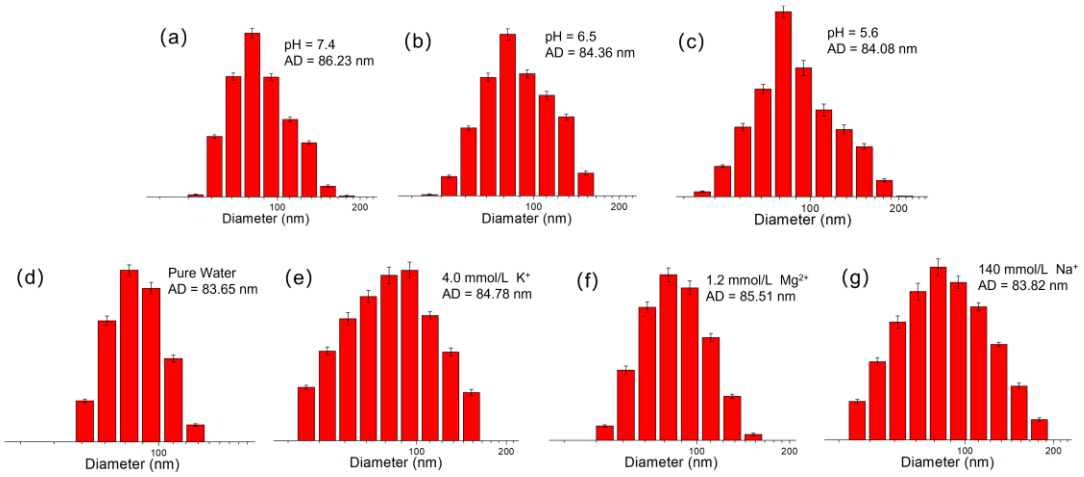


Figure S10. DLS studies of [3]WP5PR-based nanoparticles under different conditions.

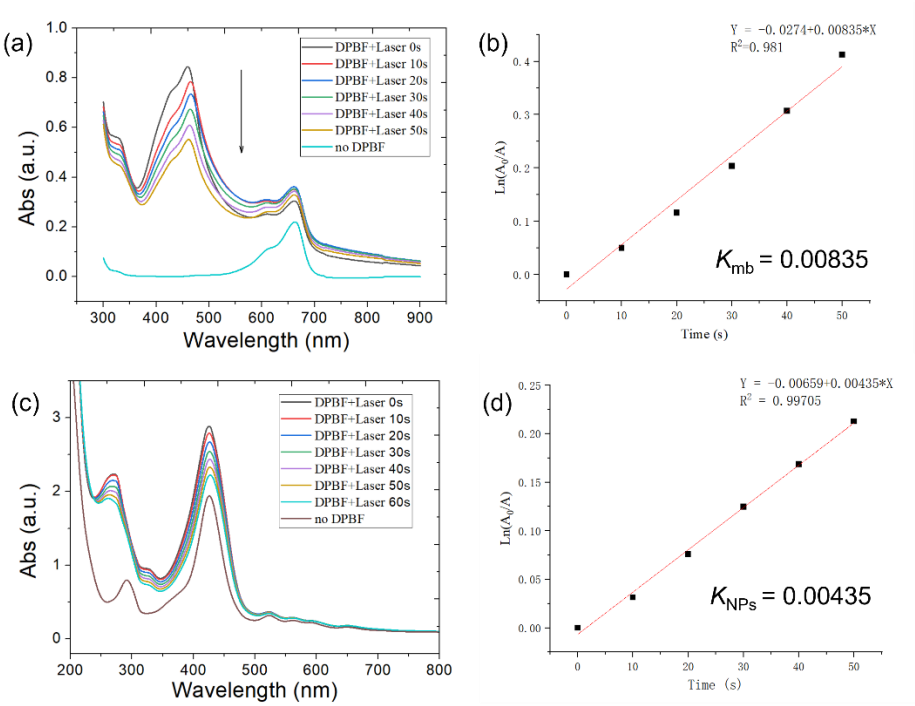


Figure S11. Time-dependent UV-vis spectra of (a) MB with DPBF after irradiation, (b) [3]WP5PR with with DPBF after irradiation.

$\Phi_{NPs} = \Phi_{mb} * (K_{NPs}/K_{mb}) * (A_{mb}/A_{NPs})$, A is the absorbance at 660nm.
So, $\Phi_{NPs} = 0.751\Phi_{mb}$.

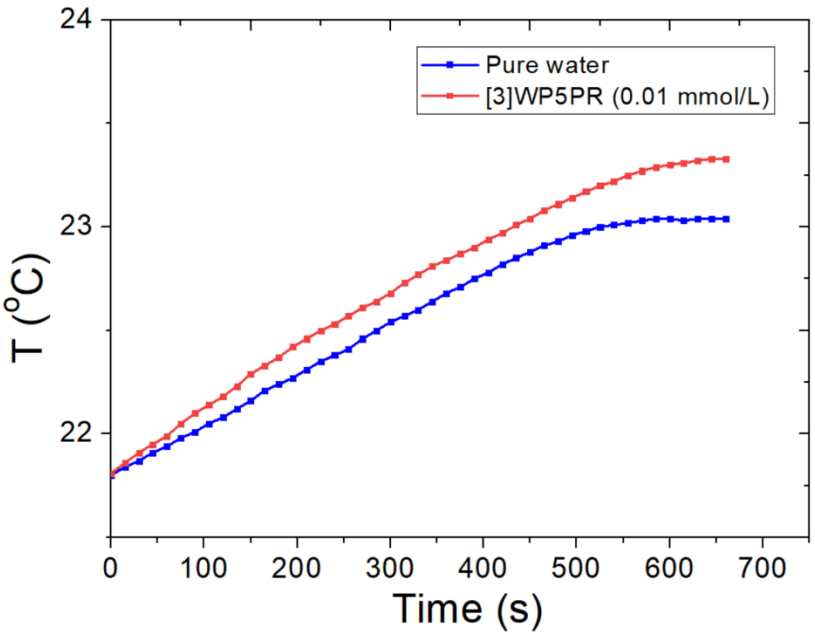


Figure S12. Temperature increasement of pure water and **[3]WP5PR** solution (0.01 mM) with 660 nm laser irradiation (1 mW/cm^2).

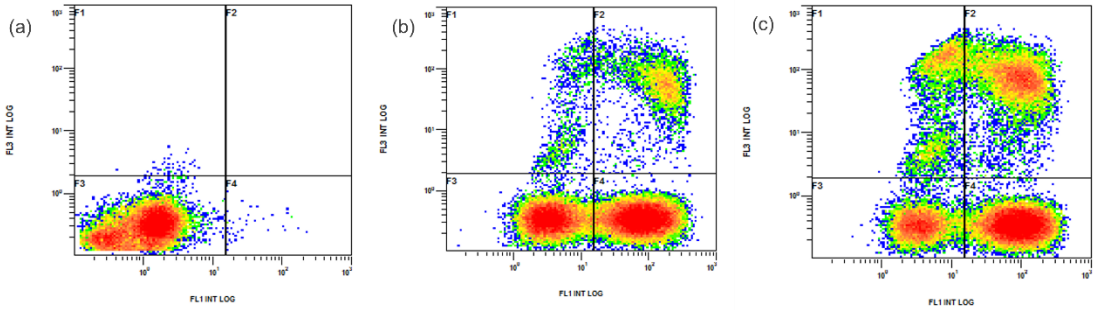


Figure S13. Flow cytometry experiments of (a) **[3]WP5PR**, (b) **pPor** + Laser, and (c) **[3]WP5PR** + Laser. [C] = 70 μM.

References

S1. Yang Wang, Chenwei Wang, Renhua Long, Yufeng Cao, Dongli Fan, Moupan Cen, Leyu Cao, Yanmei Chen, Yong Yao, *Chem. Commun.*, **2019**, *55*, 5167-5170

S2. Tomoki Ogoshi, Masayoshi Hashizume, Tada-aki Yamagishi and Yoshiaki Nakamoto, *Chem. Commun.*, **2010,** *46*, 3708-3710.