

Supporting Information

Tuning Aqueous Self-assembly of Porphyrins by Varying the Number of Cationic Side Chains

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1. Synthesis and Characterization

Porphyrin derivative **1**, containing linear side chains with ammonium terminated groups, was synthesized as follows. 4,4',4''-(20-phenylporphyrin-5,10,15-triyl)tribenzoic acid (1-1)¹ (1.0 eq.), 2-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) (5.0 eq.), *N,N*-Diisopropylethylamine (DIPEA) (10.0 eq.) in *N,N*-Dimethylformamide (15 mL) was stirred at room temperature (r.t.) for 1 h, Amine (1-2)² (6.0 eq.) was then added and left to stir for 24 hours in the dark under N₂ atmosphere. After stripping a portion of *N,N*-Dimethylformamide (DMF), the reaction mixture was diluted with dichloromethane (DCM) (20 mL) and then washed successively with satd. Citric Acid (aq.) (35 mL×2), satd. Na₂CO₃ (aq.) (35 mL×2) and water (35 mL×2). The organic phase was evaporated to dryness under reduced pressure after being dried over anhydrous Na₂SO₄. The residue was subjected to column chromatography (SiO₂, CH₂Cl₂/MeOH 40/1 v/v) to allow isolation of products as dark-red solids (1-3).

Concentrated hydrochloric acid (5 mL) was added to the dioxane (10 mL) solution containing the above red solid under vigorous stirring at 0 °C. The mixture was slowly raised to room temperature and stirred in the dark for 24 h. The reaction mixture was dropped into cold acetone (100mL), and the green precipitate was collected by centrifugation. The green solid was redissolved in deionized water (DIW) at pH=2 and reprecipitated in cold acetone (100 mL). The final product is a green solid (yield: 65% in 2 steps), which was dissolved in DIW at a concentration of 2 mM as stock solution. ¹H NMR (600 MHz, CD₃OD, 25 °C) δ (ppm) 8.96 (m, 8H), 8.68 (d, 6H), 8.56 (d, 2H), 8.50 (d, 6H), 8.07 (d, 3H), 3.75 (t, 6H), 3.22 (t, 6H), 2.20 (m, 6H); ¹³C NMR (600MHz, CD₃OD, 25 °C) δ (ppm) 169.40, 142.96, 139.86, 137.36, 134.66,

134.62, 130.42, 130.17, 129.91, 128.12, 126.84, 123.39, 121.36, 121.08, 37.21, 36.72, 27.42. HR-MS (ESI) mass: m/z calculated, for porphyrin **1** $[M + 3H^+]$, 305.8200; Found: $[M + 3H^+]$, 305.8196.

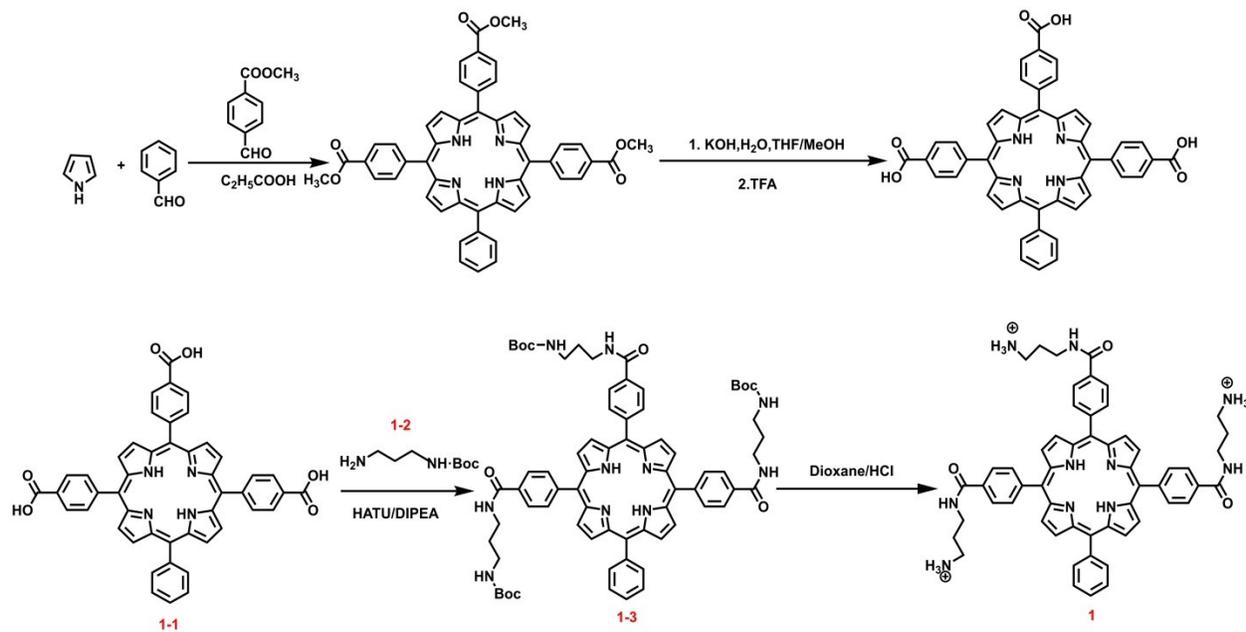


Figure S1 Synthesis scheme of porphyrin derivative **1**.

Porphyrin derivative **2**, containing *para*-side chains with ammonium terminated groups, was synthesized as follows. 4,4'-(porphyrin-5,15-diyl)dibenzoic acid (**2-1**) was synthesized by a modified literature method³. 4,4'-(porphyrin-5,15-diyl)dibenzoic acid (1.0 eq.), 2-(7-Azabenzotriazol-1-yl)-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HATU) (3.0 eq.), *N, N*-Diisopropylethylamine (DIPEA) (6.0 eq.) in *N,N*-Dimethylformamide (DMF) (10 mL) was stirred at room temperature (r.t.) for 1h. Amine (**1-2**) (4.0 eq.) was then added and left to stir for 24 hours in the dark under N_2 atmosphere. After stripping a portion of *N, N*-Dimethylformamide (DMF), the reaction mixture was diluted with dichloromethane (DCM) (10 mL), then washed with saturated citric acid (aq.) (20 mL \times 2), saturated Na_2CO_3 (aq.) (20 mL \times 2) and water (20 mL \times 2), The organic phase was dried on anhydrous Na_2SO_4 and evaporated to dry under reduced pressure. The residue was subjected to column chromatography (SiO_2 , $CH_2Cl_2/MeOH$ 50/1 v/v) allowing isolation of the product as a dark-red solid (**2-2**).

A concentrated hydrochloric acid solution (5 mL) was added to a dioxane solution containing the red solid obtained above (10 mL) under vigorous stirring at 0 °C. The mixture was slowly warmed to room temperature and stirred at room temperature for 24 hours. The reaction mixture was dropped into cold acetone (100 mL) and the green precipitate was collected by centrifugation. The green solid was redissolved in deionized water (DIW) at pH=2 and reprecipitated in cold acetone (100 mL) to obtain the final product as a green solid. (yield: 54% in 2 steps).

The green solid was adjusted to a concentration of 2mM as stock solution. ^1H NMR (600 MHz, DMSO- d_6 , 25 °C) δ (ppm) 10.71 (s, 2H), 9.73 (d, 4H), 9.09 (d, 4H), 8.41 (d, 4H), 8.36 (d, 4H), 3.59 (t, 4H), 3.05 (t, 4H), 2.03 (m, 4H); ^{13}C NMR (600MHz, DMSO- d_6 , 25 °C) δ (ppm) 166.95, 146.67, 145.37, 143.79, 135.06, 134.14, 133.37, 131.17, 126.68, 118.36, 106.55, 37.38, 36.95, 27.96. HR-MS (ESI) mass: m/z calculated, for porphyrin **2** $[\text{M} + 2\text{H}^+]$, 254.6327; Found: $[\text{M} + 2\text{H}^+]$, 254.6325

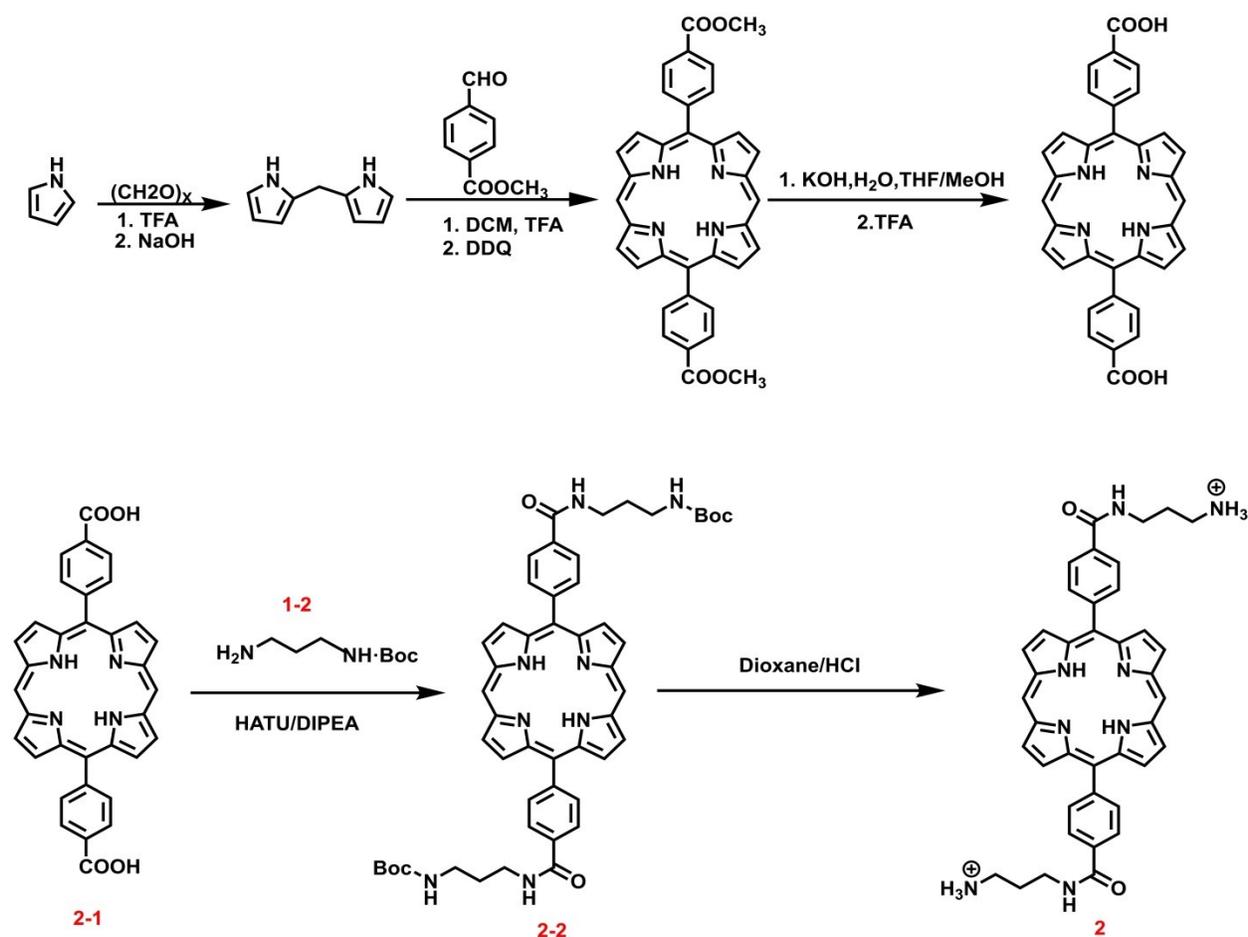


Figure S2 Synthesis scheme of porphyrin derivative **2**.

2. Loading capacity of 2-PPi

The loading capacity is the ratio of (mass of the porphyrin encapsulated in assemblies)/ (mass of the assemblies) $\times 100\%$. In order to ensure the successful assembly of the nanosheets, Porphyrin **2** is assembled in water with 1.0 eq PPi. The ratio of porphyrin/PPi is 1/1, which is also confirmed by TEM and UV-vis spectra. So, we calculated the loading capacity which is $(664 \times 1 / (664 \times 1 + 178 \times 1)) \times 100\% = 78.9\%$.

3. Supporting Figures

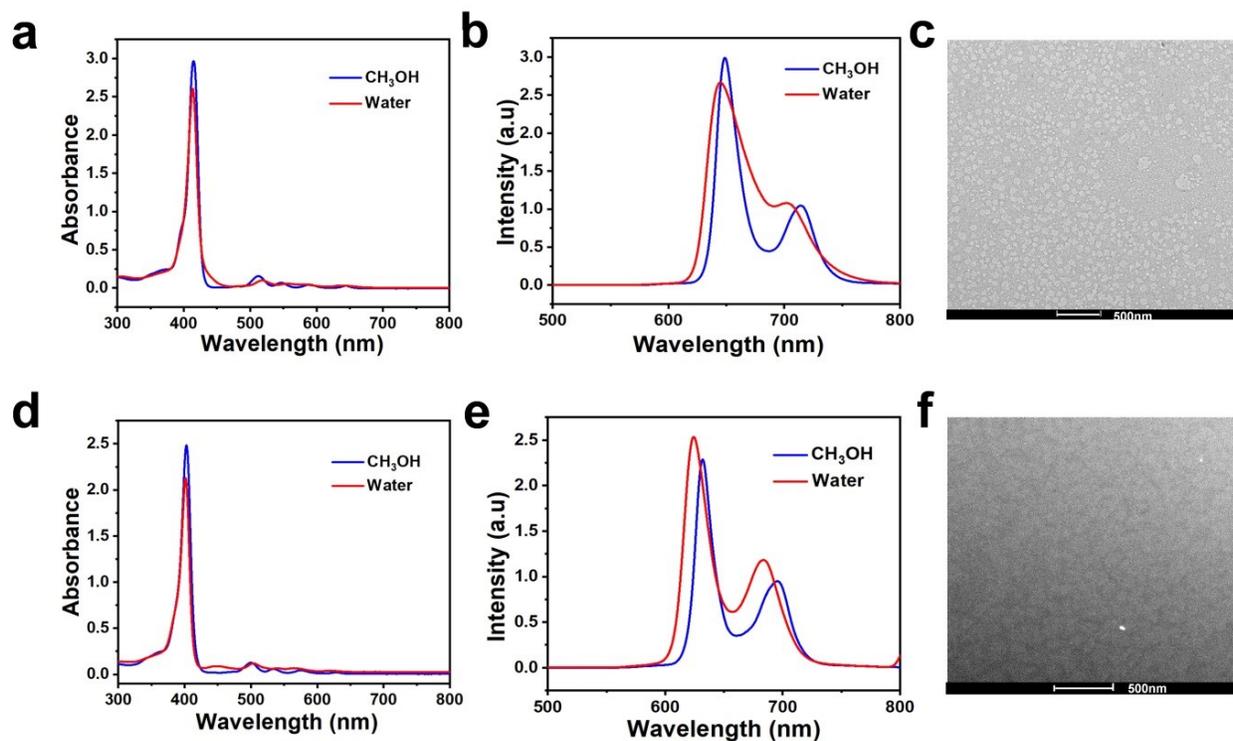


Figure S3 Absorption spectra (a) and Emission spectra (b) of porphyrin **1** in water or CH₃OH. λ_{exc} = 412 nm. TEM images (c) of porphyrin **1** alone in water. Scale bar: 500 nm. Absorption spectra (d) and Emission spectra (e) of porphyrin **2** in water or CH₃OH. λ_{exc} = 402 nm. (d). TEM images (f) of porphyrin **2** alone in water. Scale bar: 500 nm; ($[1]$ = $[2]$ = 10 μ M).

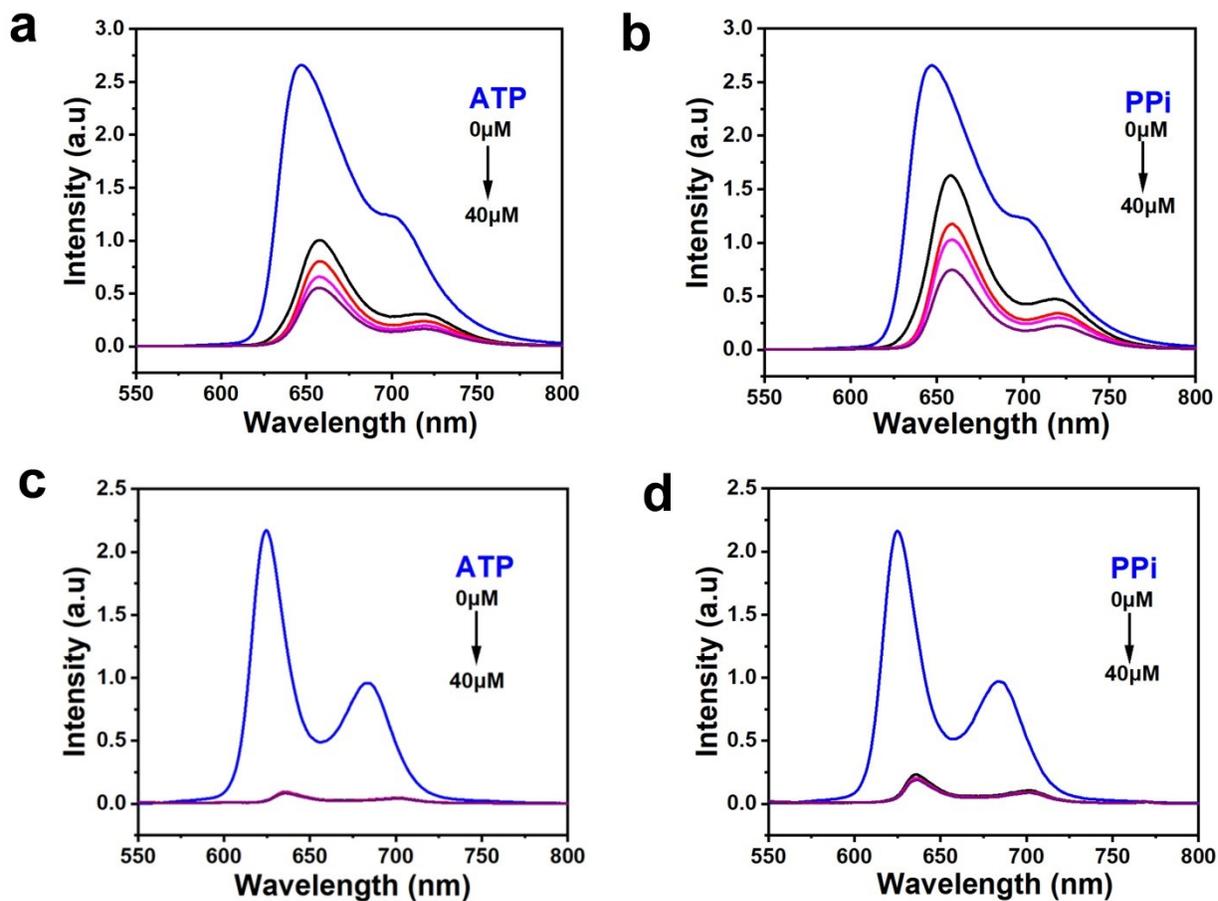


Figure S4 Emission spectra of porphyrin **1** in water with increasing concentrations of ATP (a) or PPI (b). $\lambda_{exc} = 425$ nm. Emission spectra (c) and (d) of porphyrin **2** in water with increasing concentrations of ATP (c) or PPI (d). $\lambda_{exc} = 502$ nm (c) or 512 nm (d). (pH = 7.0; $[1] = [2] = 10 \mu\text{M}$ in all cases).

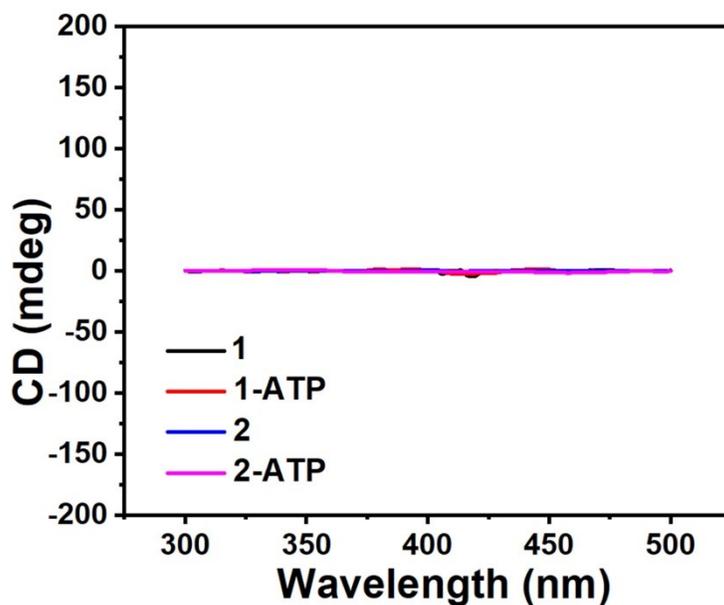


Figure S5 CD spectra of porphyrins **1** and **2** aqueous solutions before and after addition of ATP. ($[1] = 10 \mu\text{M}$; $[\text{ATP}] = 20 \mu\text{M}$; $[2] = 10 \mu\text{M}$; $[\text{ATP}] = 10 \mu\text{M}$).

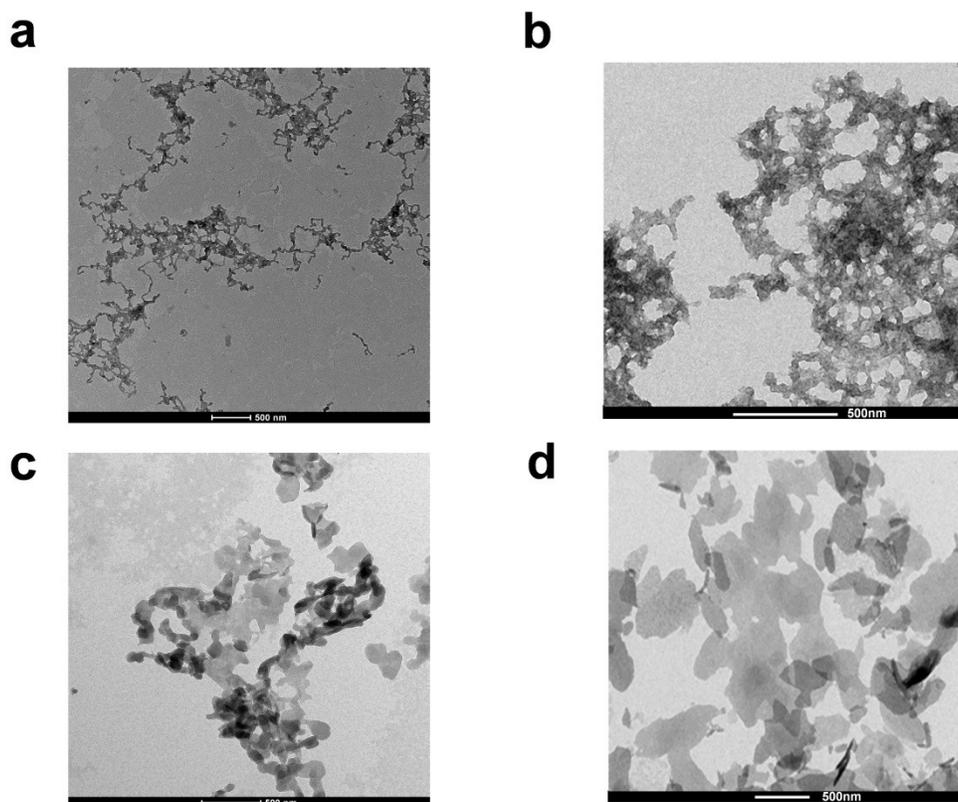


Figure S6 TEM images showing the fibrous nanostructures of the assemblies of porphyrin **1** with 2.0 eq ATP (a) or 3.0 eq PPI (b). TEM images showing the sheet nanostructure of the assemblies of porphyrin **2** with 1.0 eq ATP (c) or PPI (d). Scale bar: 500 nm ($[1] = [2] = 10 \mu\text{M}$).

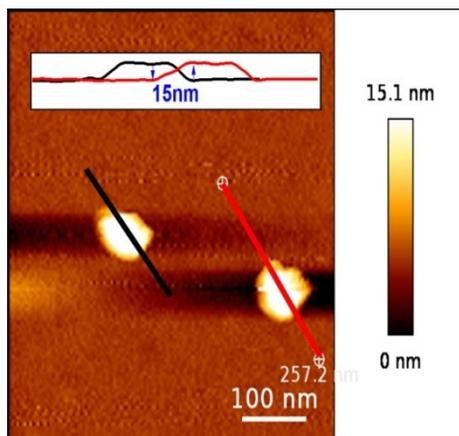


Figure S7 AFM images of 2-PPi. Scale bar: 100 nm ($[2] = 10 \mu\text{M}$; $[\text{PPi}] = 10 \mu\text{M}$).

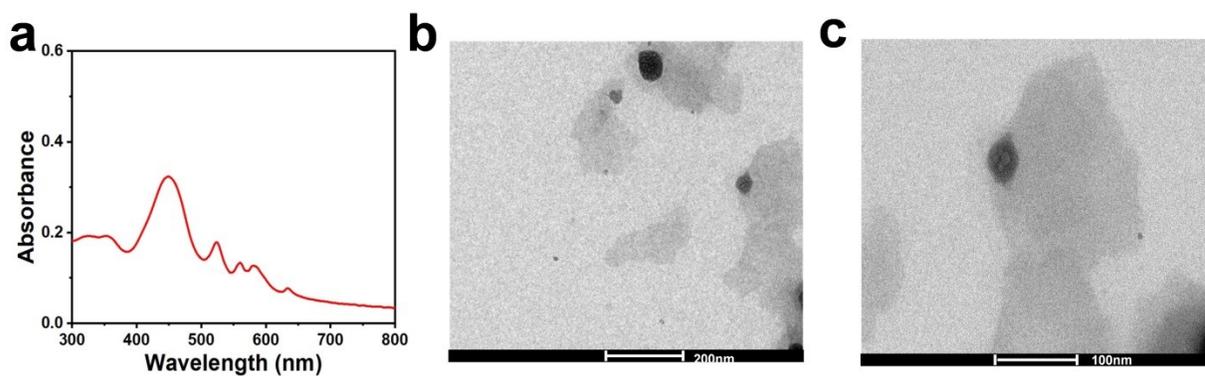


Figure S8 The Absorption spectra (a) of 2-PPi in MEM (minimum essential medium). TEM images (b and c) showing the nanosheet structures of 2-PPi in MEM; ($[2\text{-PPi}] = 10 \mu\text{M}$).

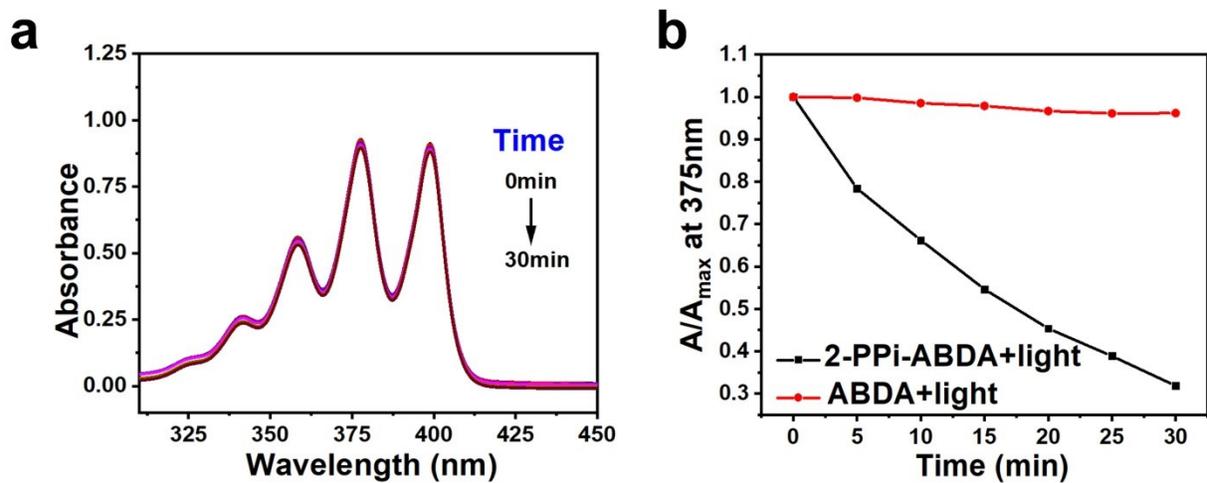


Figure S9 The Absorption spectra (a) of ROS probe (ABDA) in water (measured every 5 min) with increasing the time of light irradiation. Comparison of the decay rate (b) of ABDA absorbance under light irradiation, with or without 2-PPi. ($[2\text{-PPi}] = 10 \mu\text{M}$; $[\text{ABDA}] = 100 \mu\text{M}$; Light power: 50 mW cm^{-2} at 630 nm for 30 min)

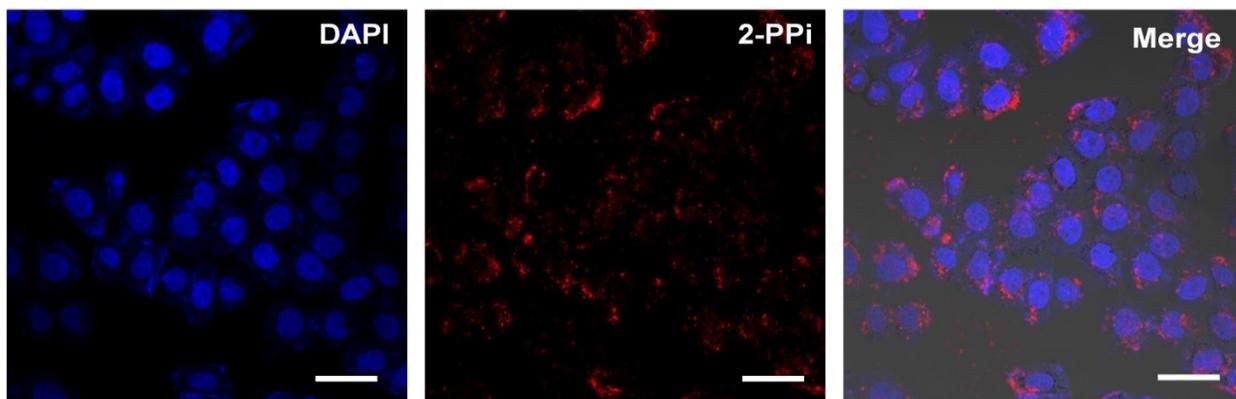


Figure S10 CLSM images of HeLa cells treated with 2-PPi for 12 h. Scale bar: $50 \mu\text{m}$; ($[2\text{-PPi}] = 10 \mu\text{M}$)

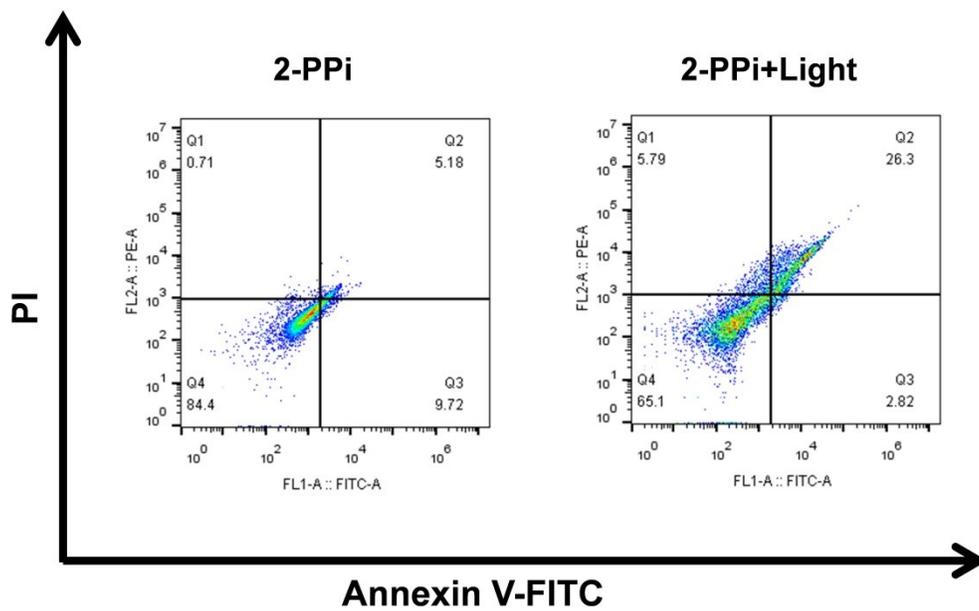


Figure S11 Flow cytometry assays of Annexin V-FITC and PI-stained HeLa cells after exposure to different treatments ([2-PPi] = 10 μ M).

NMR Spectra of Porphyrin 1 (^1H NMR, ^{13}C NMR)

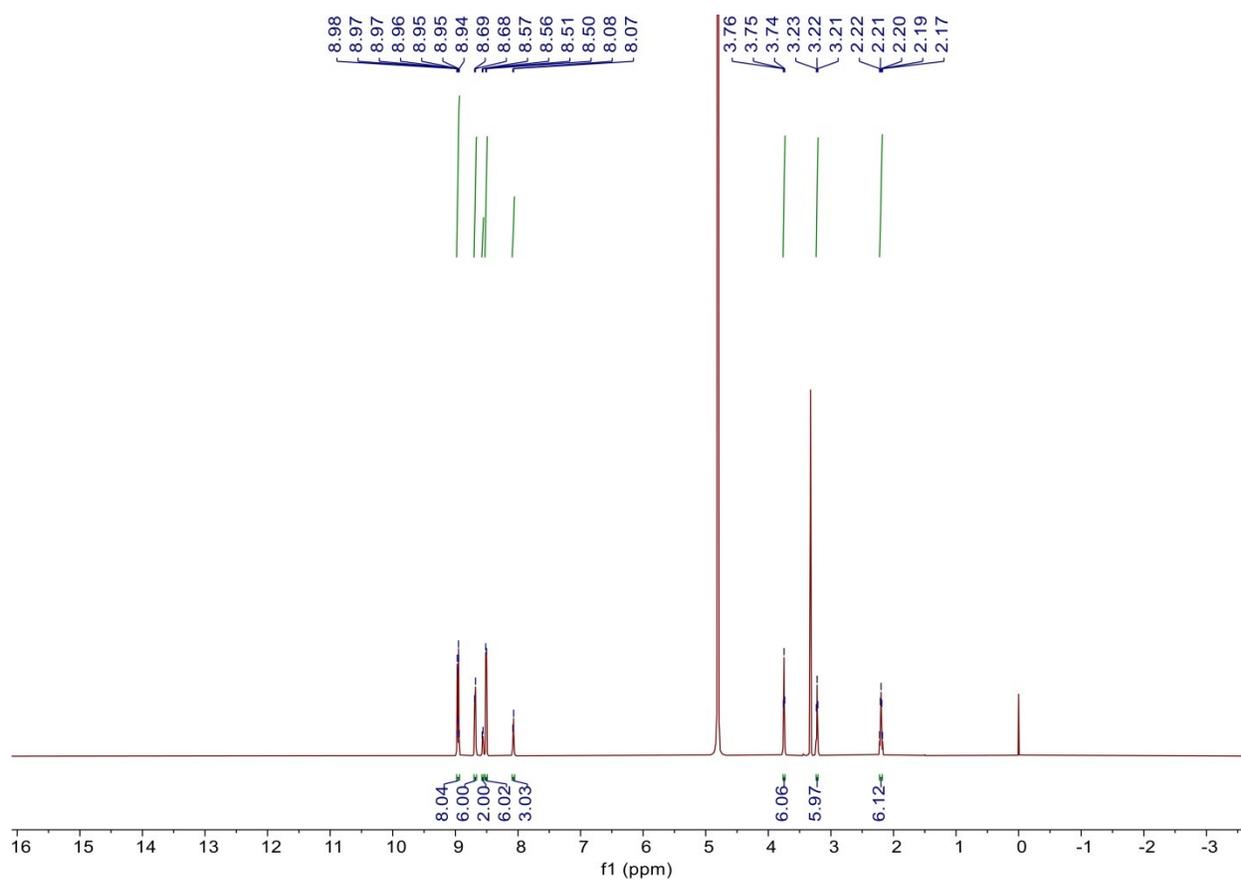


Figure S12 ^1H -NMR of porphyrin 1 in CD_3OD .

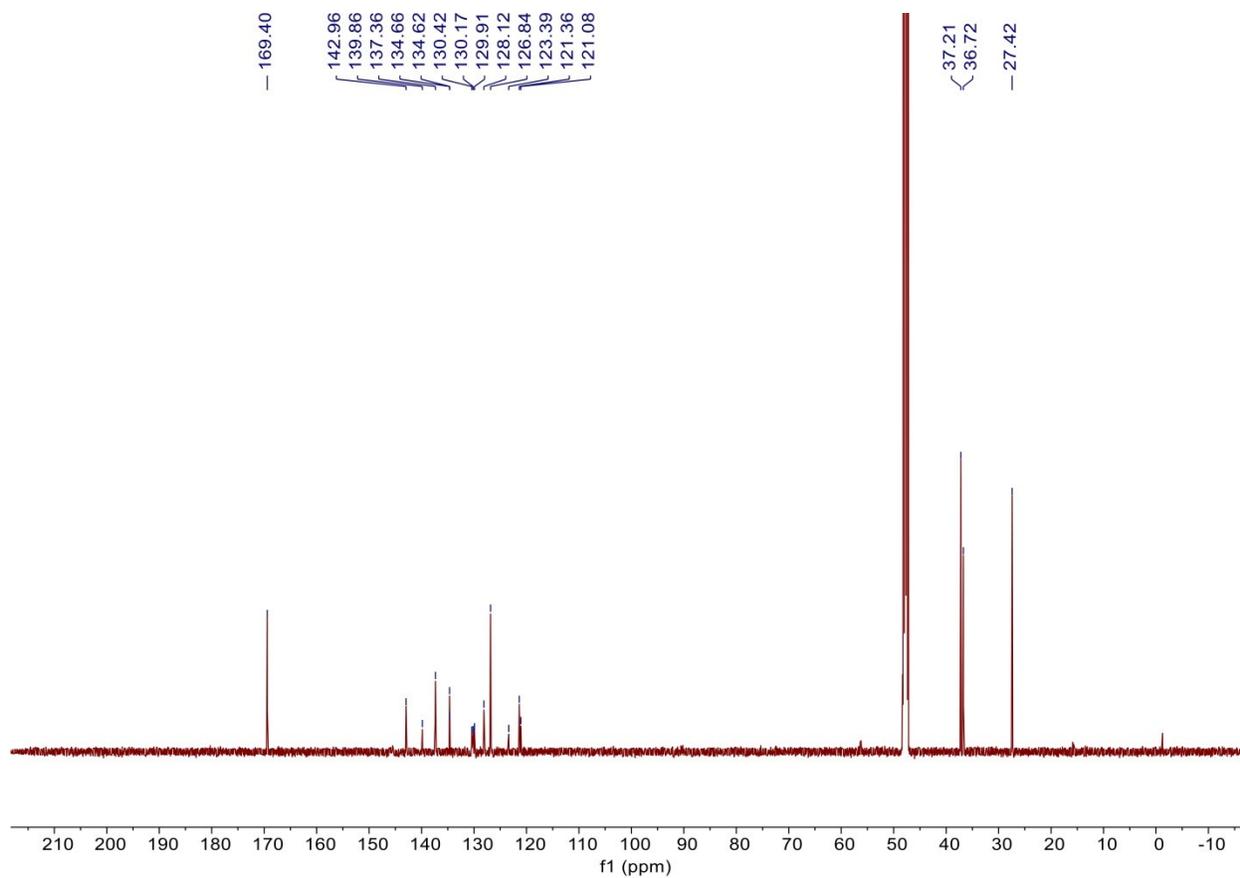


Figure S13 ^{13}C -NMR of porphyrin **1** in CD_3OD .

NMR Spectra of Porphyrin 2 (^1H NMR, ^{13}C NMR)

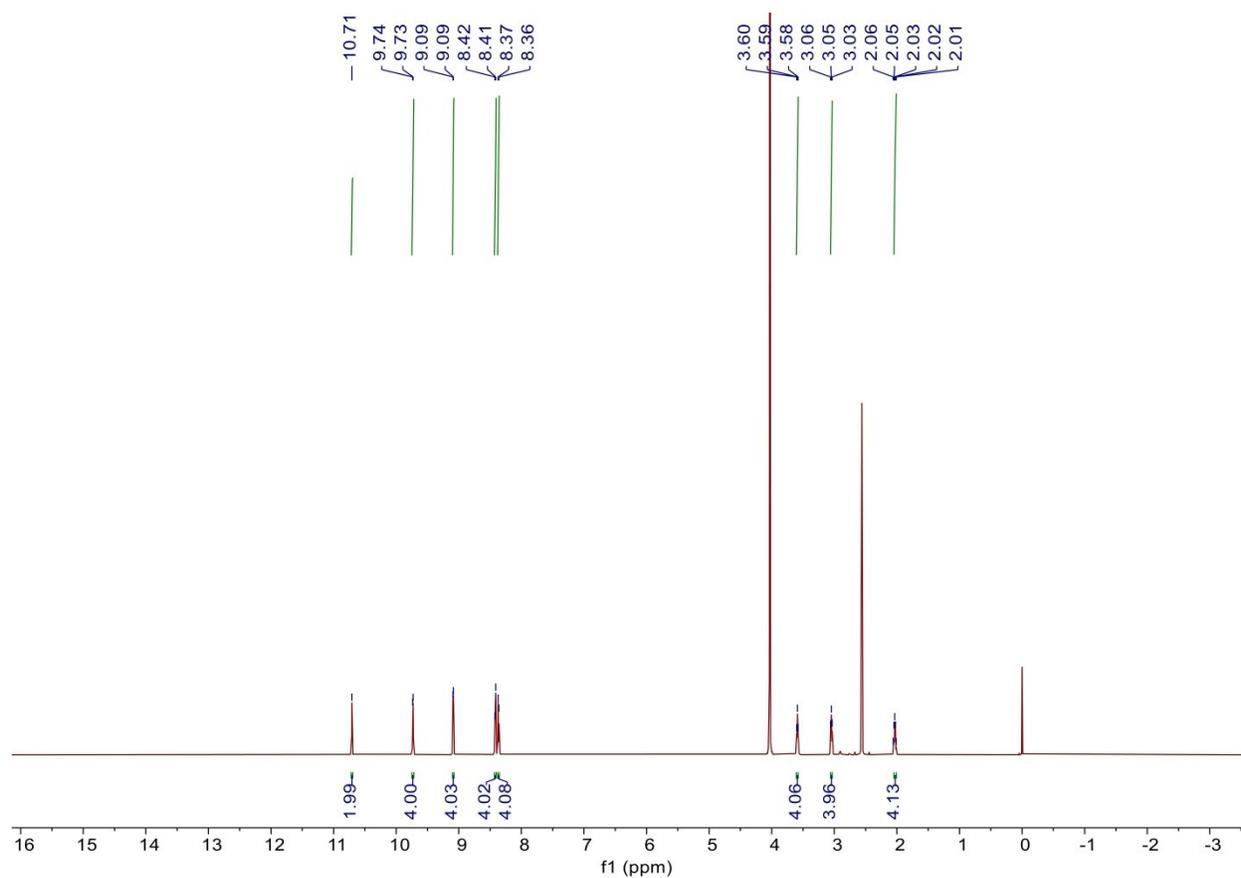


Figure S14 ^1H -NMR of porphyrin 2 in $\text{DMSO-}d_6$ and D_2O .

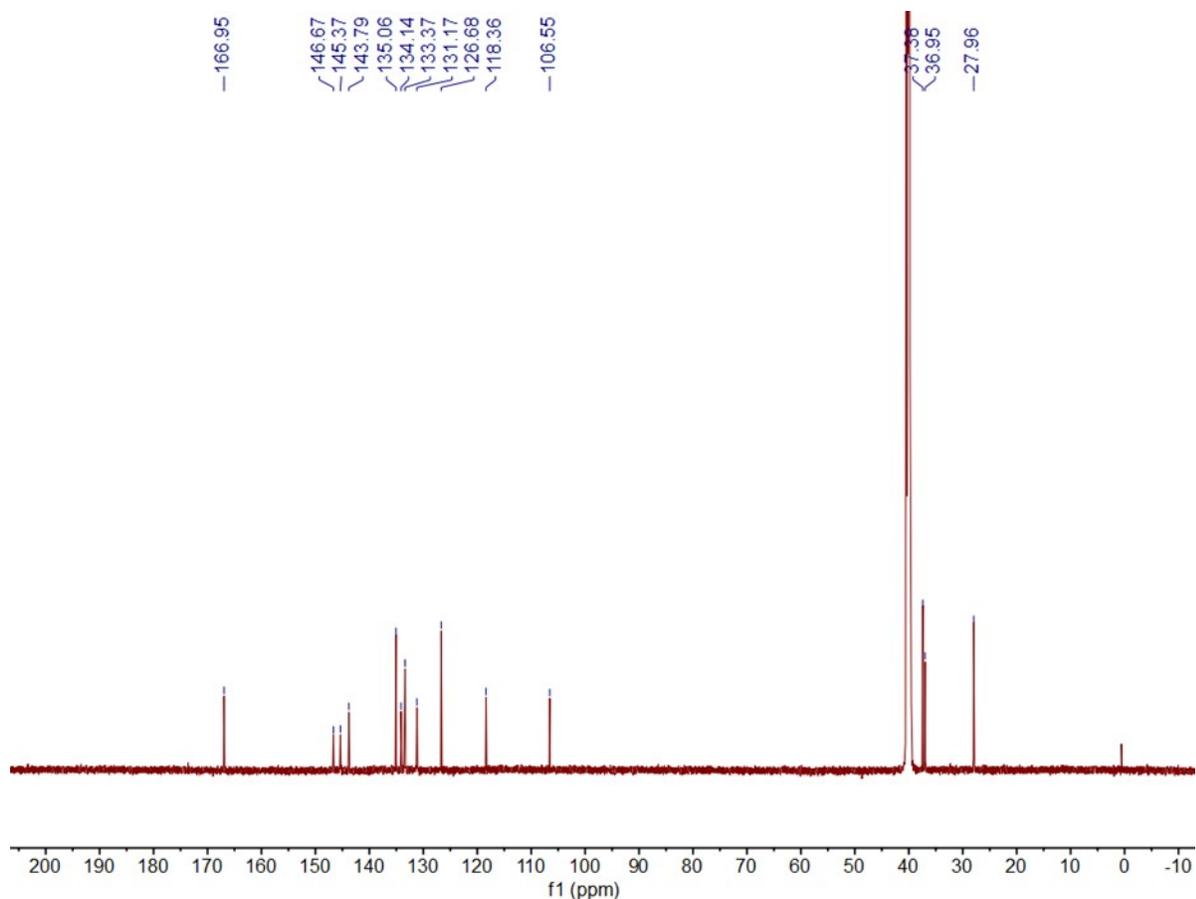


Figure S15 ^{13}C -NMR of porphyrin **2** in $\text{DMSO-}d_6$ and D_2O .

References

1. Zhao, X.; Yuan, L.; Zhang, Z. Q.; Wang, Y. S.; Yu, Q.; Li, J., Synthetic Methodology for the Fabrication of Porous Porphyrin Materials with Metal-Organic-Polymer Aerogels. *Inorg. Chem.* **2016**, *55* (11), 5287-5296.
2. Amirbekyan, K.; Duchemin, N.; Benedetti, E.; Joseph, R.; Colon, A.; Markarian, S. A.; Bethge, L.; Vonhoff, S.; Klusmann, S.; Cossy, J.; Vasseur, J.-J.; Arseniyadis, S.; Smietana, M., Design, Synthesis, and Binding Affinity Evaluation of Hoechst 33258 Derivatives for the Development of Sequence-Specific DNA-Based Asymmetric Catalysts. *ACS Catal.* **2016**, *6* (5), 3096-3105.
3. Lu, K.; He, C.; Lin, W., Nanoscale metal-organic framework for highly effective photodynamic therapy of resistant head and neck cancer. *J. Am. Chem. Soc.* **2014**, *136* (48), 16712-16715.