

## Supporting Information

### Imidazole-Modified Porphyrin As a pH-Responsive Sensitizer for Cancer Photodynamic Therapy

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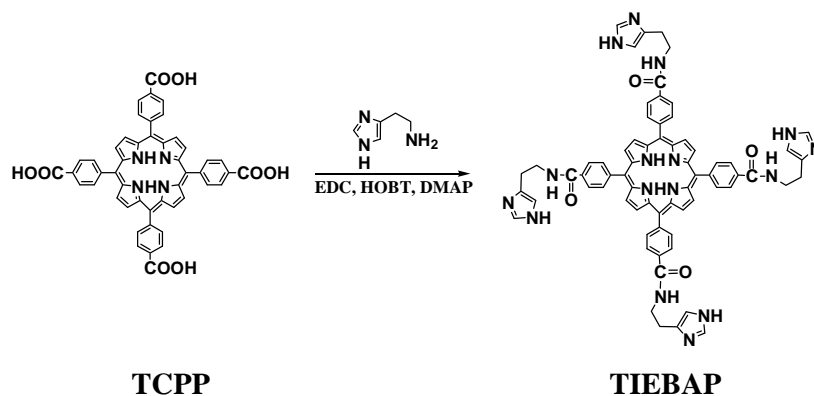
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#### 1. Materials and Instruments

All of the reagents and solvents were obtained commercially and used without further purification. *meso*-Tetra(4-carboxylphenyl) porphine (TCPP, 97%) and *meso*-Tetra(4-sulfonatophenyl)porphine dihydrochloride (TSPP) were purchased from Frontier Scientific, Inc. 1-hydroxybenzotriazole hydrate (HOBT), 4-dimethylamino Pyridine (DMAP, Fluka), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI, Fluka), histamine dihydrochloride, acetic acid, sodium hydroxide, hydrochloric acid, dimethylformamide (DMF) and deuterium oxide (D<sub>2</sub>O, 99% of D) were purchased from Sigma-Aldrich. Deionized water was obtained from a Nanopure Water System (Barnsted System, USA). A Q-switched Nd:YAG laser with pulse duration of 3-4 ns and a maximum energy of 30 mJ at 532 nm (Polaris II, Electro Scientific Industries, Inc.), equipped with a liquid N<sub>2</sub>-cooled germanium photodetector (Applied Detector Corporation), was used for time-resolved <sup>1</sup>O<sub>2</sub> luminescence measurements. Steady-state photooxidation was conducted in oxygen-saturated solutions using a 150 W Xenon lamp (6255 Xenon lamp housed in 66907 Arc Lamp Source, Newport Oriel Instruments) equipped with a monochromator with primary wavelength region 450-2000 nm (77250 1/8 m Monochromator and 77305 Grating, Newport Oriel Instruments). A BioMate 3 UV-visible spectrophotometer (Thermo Scientific) and a Cary 300 UV-vis spectrophotometer (Varian, Inc.) were used for measuring absorbance and spectra. The fluorescence spectra were taken on a FluoroMax-2 spectrofluorometer. ESI MS spectra were obtained on a Finnigan LCQ Duo mass spectroscopy. A 300 MHz Bruker Spectrospin FT-NMR or a Varian Vnmrs 500 MHz NMR were also used for NMR spectra measurements. All of the measurements were carried out at ambient temperature. Samples were shielded from light using aluminum foil when not being irradiated.

#### 2. Synthesis of 5,10,15,20-tetrakis(N-(2-(1H-imidazol-4-yl)ethyl)benzamide)porphyrin (TIEBAP, M.W. = 1163)

The synthesis of TIEBAP was carried out using a modified method according to literature report.<sup>1</sup> Briefly, TCPP (25 mg, 0.032 mmol) was dissolved in 3.0 ml DMF, followed by addition of EDCI (36.5 mg, 0.19 mmol) and wet HOBT (31.4 mg, 0.21 mmol containing at least 11% water). The mixture was stirred for 30 minutes, and then followed by addition of DMAP (44 mg, 0.036 mmol) and histamine which was obtained from histamine dihydrochloride (23.184 mg, 0.126 mmol) pretreated with 2 equivalents of NaOH. The mixture was sonicated for 20 minutes. 6.0 mL of water was added to the above mixture to precipitate out the crude product. The product was then collected by centrifugation. The purification was repeated for one more cycle by re-dissolving the crude product in DMF and adding water to precipitate it out. The final product was dried in a vacuum oven. The yield of TIEBAP such prepared was over 90%. The crude product can be further purified by reverse phase HPLC in needed. This synthetic approach produced a porphyrin sensitizer, in which the four carboxylic acid groups of TCPP were converted into carboxylic amides (Scheme S1).



Scheme S1. Synthesis of 5,10,15,20-tetrakis(N-(2-(1H-imidazol-4-yl)ethyl)benzamide)porphyrin (TIEBAP)

### 3. Spectra of ESI-MS, <sup>1</sup>H and <sup>13</sup>C NMR

The identity of TIEBAP was confirmed by ESI-MS (Figure S1), <sup>1</sup>H (Figure S2) and <sup>13</sup>C (Figure S3) spectra.

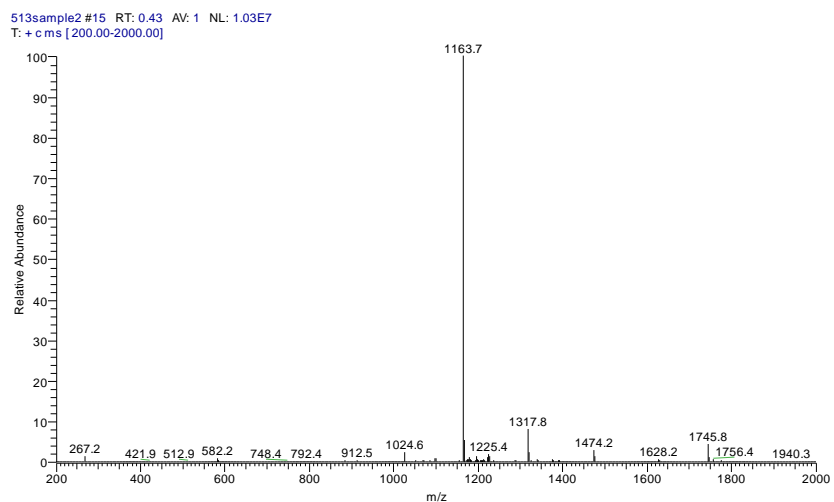


Figure S1. ESI-MS spectrum of TIEBAP in 5% acetic acid water solution

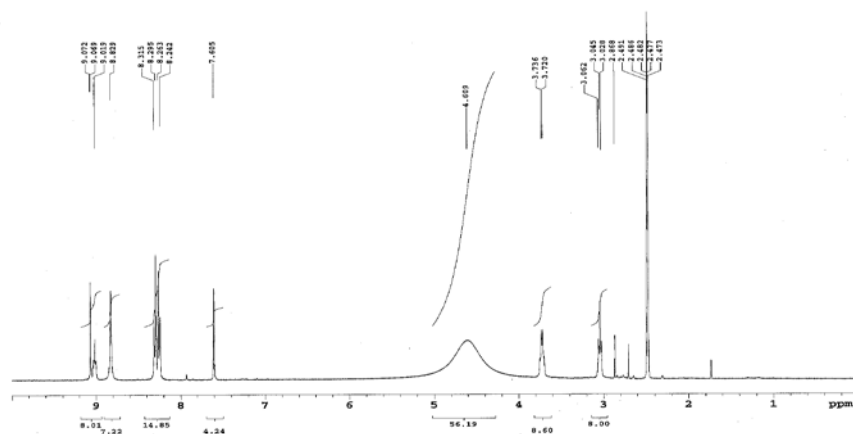


Figure S2.  $^1\text{H}$  NMR spectrum of TIEBAP in deuterium DMSO

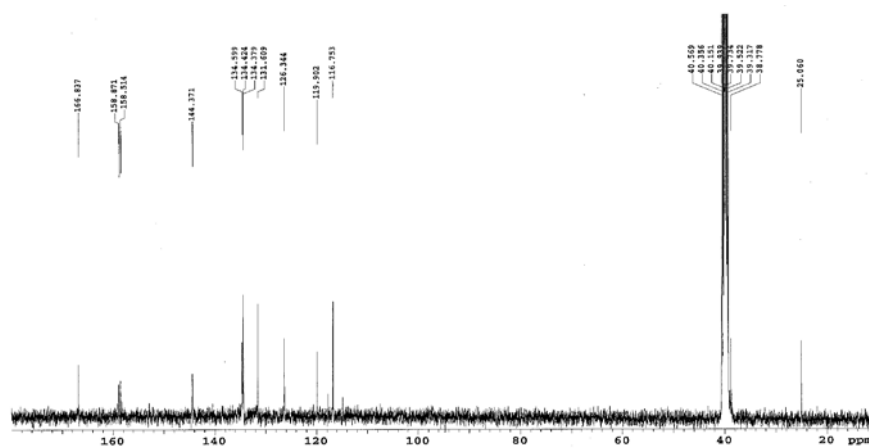


Figure S3.  $^{13}\text{C}$  NMR spectrum of TIEBAP in deuterium DMSO

#### 4. Spectroscopic characterization and TIEBAP aggregation

TCPP is poorly soluble in aqueous solutions of acidic pH. However, its solubility was greatly enhanced upon the formation of TIEBAP due to the protonation of imidazoles. The positive charges at the imidazole moieties could impact the charge distribution of the porphyrin ring only through inductive effects. The porphyrin aggregation can be easily identified by UV-visible spectroscopy, where the Soret band undergoes either a bathochromic (J-type) or hypsochromic (H-type) aggregation shift compared to the porphyrin monomers. The Soret bands of face-to-face dimers and the higher H-type aggregates as well as the higher edge-to-edge J-type aggregates are known to appear at shorter and longer wavelengths, respectively, when compared to the Soret bands of corresponding porphyrin monomers.<sup>2, 3</sup> The higher self-aggregates without a J-type arrangement exhibited a broad band near the wavelength region of the monomer Soret band.<sup>4</sup> TIEBAP carried the characteristic absorption features of a porphyrin, showing a sharp Soret band at 413 nm and Q bands at 521, 558, 592 and 650 nm at pH 5.3; however, a broad Soret band between 410 and 440 nm with a maximum absorption wavelength was centered at 406 nm at pH 8.2 (Figure S4). The Soret band was blue-shifted by 7 nm for a pH change from 5.3 to 8.2. An increase in pH promoted the formation of face-to-face H-type aggregation. The change in the UV-visible spectrum of TIEBAP indicated dissociation of the H-type aggregation of this porphyrin to the monomer upon reducing the pH. In addition, the fluorescence spectra were deeply affected by aggregation, showing a weaker two-band emission spectrum at pH 8.2 but a strong one-band emission spectrum at pH 5.3 (Figure S5). The aggregation is known to reduce the quantum yield and lifetime of the excited triplet states of porphyrins, which also affects the quantum yield of  $^1\text{O}_2$  production ( $\Phi_{\Delta}$ ).<sup>5, 6</sup>

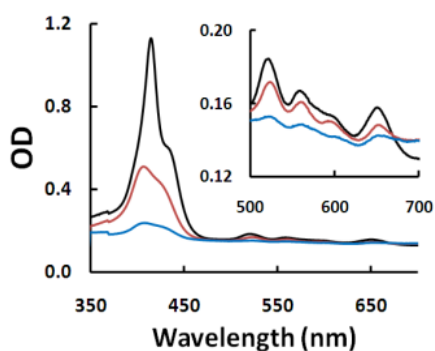


Figure S4. Absorption spectra of  $7.0 \times 10^{-6}$  M TIEBAP in  $\text{H}_2\text{O}$  at pH 5.3 (black), 6.9 (red) and 8.2 (blue)

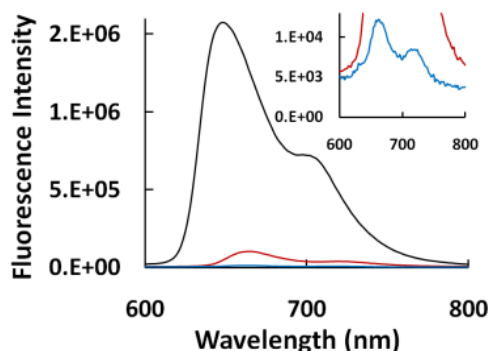


Figure S5. Fluorescence spectra of  $7.0 \times 10^{-6}$  M TIEBAP obtained at an excitation wavelength of 520 nm in  $\text{H}_2\text{O}$  (with 1% methanol) at pH 5.9 (black), 7.1 (red) and 8.2 (blue)

### 5. Quantum yield of $^1\text{O}_2$ production ( $\Phi_\Delta$ )

$\Phi_\Delta$  was determined by a time-resolved laser technique as previously described in which the  $^1\text{O}_2$  phosphorescence at 1270 nm was monitored.<sup>7,8</sup> The data points of the initial  $\sim 5$   $\mu\text{s}$  were not used due to electronic interference signals from the detector and the initial  $^1\text{O}_2$  intensity was extrapolated to time zero. The intensity of the pulses at 532 nm was adjusted within the range from 20 mJ to 30 mJ. The  $\Phi_\Delta$  of TIEBAP was determined in the aerated 50 mM pH 5.1 acetic acid/acetate  $\text{D}_2\text{O}$  buffer solutions by comparing with a reference sensitizer of TSPP in  $\text{D}_2\text{O}$  using the known value of  $\Phi_{\Delta, \text{TSPP}}$  0.63.<sup>9</sup>  $\Phi_\Delta$  was calculated according eq. 1. The absorbance of TIEBAP and reference TSPP was controlled within the range between 0.05 and 0.5.

$$\frac{\Phi_{\Delta, \text{TIEBAP}}}{\Phi_{\Delta, \text{TSPP}}} = \frac{S_{\text{TIEBAP}}}{S_{\text{TSPP}}} \quad (1)$$

Here,  $\Phi_{\Delta, \text{TIEBAP}}$  and  $\Phi_{\Delta, \text{TSPP}}$  are the  $\Phi_\Delta$  from TIEBAP and reference TSPP, respectively, and  $S_{\text{TIEBAP}}$  and  $S_{\text{TSPP}}$  represent the slopes obtained from the plot of initial intensity of  $^1\text{O}_2$  via the absorbance at an excitation wavelength of 532 nm for TIEBAP and reference TSPP, respectively. The relative  $\Phi_\Delta$  of TIEBAP was determined to be  $0.53 \pm 0.01$ , which is consistent with the  $\Phi_\Delta$  of TCPP (0.53) in weak alkaline solutions.<sup>10</sup> An example of  $\Phi_\Delta$  measurements is shown in Figure S6. The system errors in extrapolation of  $^1\text{O}_2$  intensity to time zero may exist from different sample media, which results in simulated lines in Figure S6 not passing through the origin. Those deviations do not affect the slope calculations and can be corrected by parallelly shifting points of  $^1\text{O}_2$  intensity at various OD along y-axis.

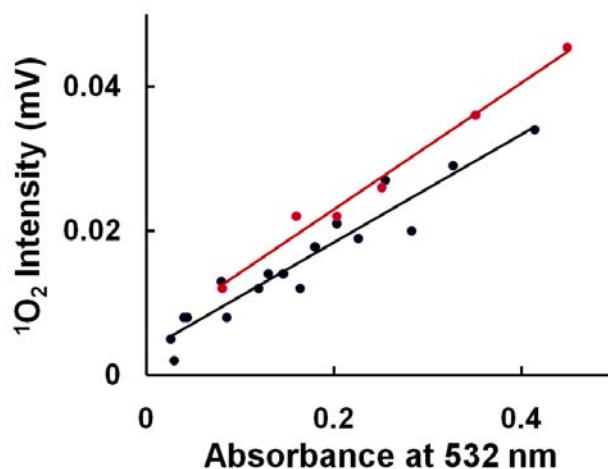


Figure S6. Initial intensity of <sup>1</sup>O<sub>2</sub> production vs. absorbance for TIEBAP (black dots) and reference TSP (red dots) at 532 nm. Solid lines are theoretical simulation using linear least-square fitting method.

#### 6. Effect of pH on initial <sup>1</sup>O<sub>2</sub> intensity

Figure S7 indicates pH-dependent photosensitization. The initial intensity of <sup>1</sup>O<sub>2</sub> production increases with a decrease of pH. The maximum <sup>1</sup>O<sub>2</sub> production by protonated TIEBAP was observed in acidic solutions. Sensitizer deactivation occurs at alkaline pH due to TIEBAP aggregation and the quenching of triplet states and <sup>1</sup>O<sub>2</sub>.

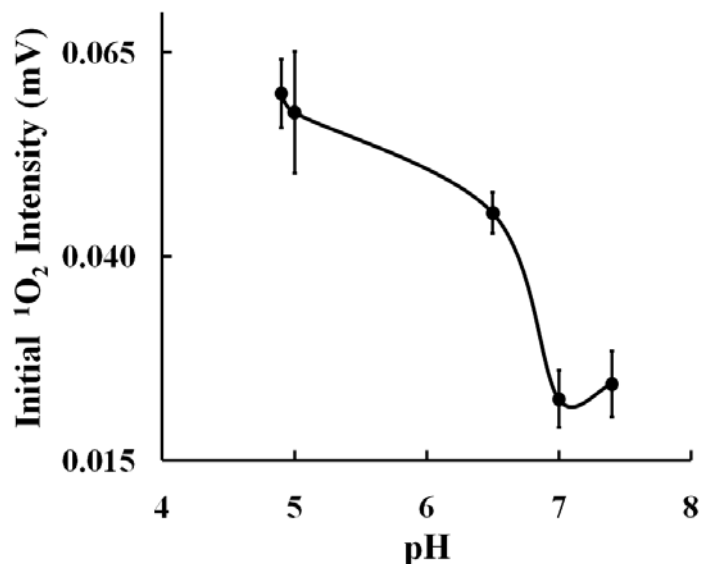


Figure S7. Initial intensity of <sup>1</sup>O<sub>2</sub> production vs. pH upon irradiation of  $7.0 \times 10^{-6}$  M TIEBAP solution (with 1% methanol in D<sub>2</sub>O) at 532 nm

## 7. Photostability of TIEBAP

The photostability of TIEBAP in 0.1 M pH 5.6 acetate buffer solution (with 10% DMF) was monitored by UV/Visible spectrophotometer. Measurements were carried out at room temperature under visible irradiation at 520 nm for up to 130 minutes. Our results revealed that the porphyrin rings in TIEBAP exhibited fair photostability in weak acidic solutions, showing 20%, 30% and 40% decrease at a maximum absorption wavelength of 415 nm for 50, 90 and 130 minute irradiation, respectively (Figure S8). The quenching of  $^1\text{O}_2$  by imidazole moieties should include both physical and chemical reactions. However, the kinetics of porphyrin decomposition through the quenching of  $^1\text{O}_2$  by imidazole moieties requires further investigations.

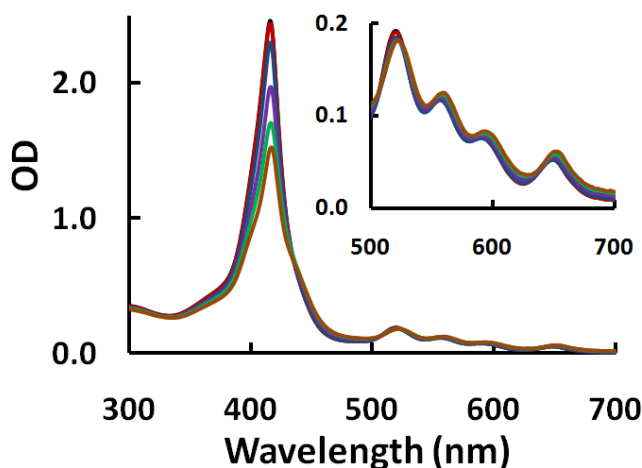


Figure S8. Photostability of  $1.5 \times 10^{-5}$  M TIEBAP in  $\text{O}_2$ -saturated pH 5.6 acetate buffer solution (0.10 M with 10 % DMF) upon irradiation at 520 nm for 0 minute (black line), 2 minutes (red line), 10 minutes (blue line), 50 minutes (purple line), 90 minutes (green line) and 130 minutes (orange line)

## 8. Cell Viability by *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay.

The breast cancer cells were grown in McCoy's 5a medium supplemented with 10% FBS and antibiotics (10 IU/mL penicillin) in 75-cm<sup>2</sup> tissue culture flasks at 37°C in a 5%  $\text{CO}_2$ /95% air humidified incubator. Before the experiments, the cells were re-suspended at a concentration of  $1 \times 10^6$  cell/mL in PBS buffer medium. An MTT assay measures cell viability to evaluate the effects of TIEBAP based phototherapy on cancer cells. The cancer cells were seeded in 96-well plates (well diameter 6.4 mm) at a density of 10,000 cells/well and allowed to attach for 24 h at 37°C in a 5%  $\text{CO}_2$  incubator before treatment with TIEBAP. Cell viability was determined 1 hour after

visible light irradiation (at an average intensity of 7.3 mW/cm<sup>2</sup>) of TIEBAP using the MTT cell proliferation assay kit (ATCC 30-1010k). MTT is a yellow dye that is taken up by viable cells and reduced to an insoluble purple-colored formazan salt by enzymes in the endo-plasmic reticulum, cytosol, and mitochondria; the amount of chromophores produced is proportional to the number of viable cells. Following incubation in the presence or absence of TIEBAP, 50 µL of a 5-mg/mL MTT solution was added to each well and incubated for 30 minutes. The cells were centrifuged, and the suspension was discarded. 200 µL of DMSO was added to each well and incubated for 10 min. The absorbance was read at 540 nm using a Multiskan Ascent Plate Reader with the Ascent software (Labsystems).

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