Using enzymatic reaction to enhance photodynamic therapy effect of porphyrin dityrosine phosphates

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Supporting Information

General.
Commercially available reagents were used without further purification. Acid phosphatase: 1 U corresponds to the amount of enzyme which hydrolyzes 1 μmol of 4-nitrophenyl phosphate per minute at pH 6.0 and 37 ^{o}C. ^{1}H NMR and ^{31}P NMR spectra were obtained on a 300 MHz Varian XL-300 using DMSO-d_6 as the solvent. Mass-spectra were measured on a Finnigan TSQ7000 System. Emission spectra were recorded on a Perkin-Elmer LS-55 luminance spectrometer. Cell images were taken on a Nikon Edipse TE2000-U confocal microscope with epifluorescence and a phase contrast system; MTT results were recorded on an IEMS Analyzer (Lab-system, Type 1401).

Syntheses and characterizations.
Protoporphyrin dityrosine phosphate (PpIX-DTP, **2**): After 56.3 mg of protoporphyrin (**1**) (0.1 mmol) and 83.4 mg of o-benzotriazol-1-yl-N, N, N', N'-tetramethyluronium hexafluorophosphate (HBTU, 0.22 mmol) were dissolved in 5 mL of DMF and stirred at room temperature for 2 hours, 57.4 mg of tyrosine phosphate (0.22 mmol) and 0.19 mL of N, N-diisopropylethylamine (DIEA, 1.1 mmol) were added to the reaction mixture. The resulting reaction solution was stirred at room temperature overnight. After about 20 hours, 20 mL of water was added to the reaction mixture and the pH was adjusted to around 1 by 1N HCl. The resulting solid was obtained by filtration and washed by acid water for 2 times. After dried in a freeze drier, 48 mg (yield: 46 %) of the title product was obtained and used to do further biological measurements. ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm): 10.22 (q, 4H), 8.44-8.60 (m, 2H), 7.24-7.30 (d, 4H), 6.95-7.03 (d, 4H), 6.42-6.55 (d, 2H), 6.24-6.32 (d, 2H), 4.34-4.42 (m, 4H), 4.20-4.28 (m, 4H), 3.60-3.80 (s, 12H), 3.20-3.25 (m, 4H), and 3.12-3.28 (m, 4H). ^{31}P NMR: δ (ppm): -6.3178. MS: calc. M^+ = 1048.3, obsvd. $(M+1)^+$ = 1049.4.

Protoporphyrin dityrosine (PpIX-DT, **3**): Compound **3** was synthesized with the yield of 50 % according to method described above. ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm): 10.30 (q, 4H), 8.56-8.65 (m, 2H), 7.10-7.20 (d, 4H), 6.89-6.88 (d, 4H), 6.44-6.54 (d, 2H), 6.20-6.30 (d, 2H), 4.30-4.45 (m, 4H), 4.18-4.30 (m, 4H), 3.55-3.80 (s, 12H), 3.22-3.30 (m, 4H), and 3.10-3.30 (m, 4H). MS: calc. M^+ = 888.4, obsvd. $(M+Na)^+$ = 911.8.

Protoporphyrin bisphosphonate (PpIX-BP, **5**): After 56.3 mg of protoporphyrin (**1**) (0.1 mmol) and 83.4 mg of HBTU (0.22 mmol) were dissolved in 5 mL of DMF and stirred at room temperature for 2 hours, 72.8 mg of tetraethyl-3-amino-propane-1,1-bisphosphonate (0.22 mmol) and 0.19 mL of DIEA (1.1 mmol) were added to the reaction mixture. The resulting reaction solution was stirred at room temperature overnight. Then the solution was

extracted with water and chloroform three times. The organic layer was collected and dried with sodium sulphate. After filtration, the solvent was removed in vacuum to yield intermediate (**4**). Compound **4** was further purified by flash silica column with yield of 40%. Afterward, the pure compound **4** was treated with 3 equiv. of bromotrimethylsilane (TMSBr) with stirring over 14 h. The product **5** was readily prepared by treatment in acetone with slightly excess of water for 30 minutes, and then dried in vacuum after removing the solvents. MS of **5**: calc. M^{+} = 964, obsvd. M^{2+} = 483.06.

Cytotoxicity test.

HeLa cells were seeded into a 96-well plate at a concentration of $3x10^3$cells/well in 100 μL MEM medium with 10% FBS. Compounds **2**, **3** and **5** at 100, 50, and 25 μM, respectively, were added when cells were plated. Then, the cell cultures were incubated for 24 hours at 37 ^{o}C and 5% CO_2. For dark cell survival assay, MTT assays were carried out to measure the proliferation of Hela cells. For photocytotoxicity assay, the photosensitizer-contained medium was replaced by fresh medium. Cells were then exposed to light emitted from a 400-watt tungsten lamp with a heat isolation filter and an ORIEL filter (model 59490) to remove emission wavelength short than 480 nm. The irradiation light was at an intensity of 5 mW/cm^2. Cells were then incubated at 37℃ in a CO_2 incubator for 24 h. The optical density (OD) of the dissolved formazan crystals was measured at wavelengths of 570 and 690 nm. The percentage of cell viability was calculated using the following equation: viability (%) = $OD_{\text{treatment group}} \times 100/OD_{\text{control group}}$, where $OD = OD_{570\text{-}690\ nm}$.

In vitro conversion of 2 to 3 in water.

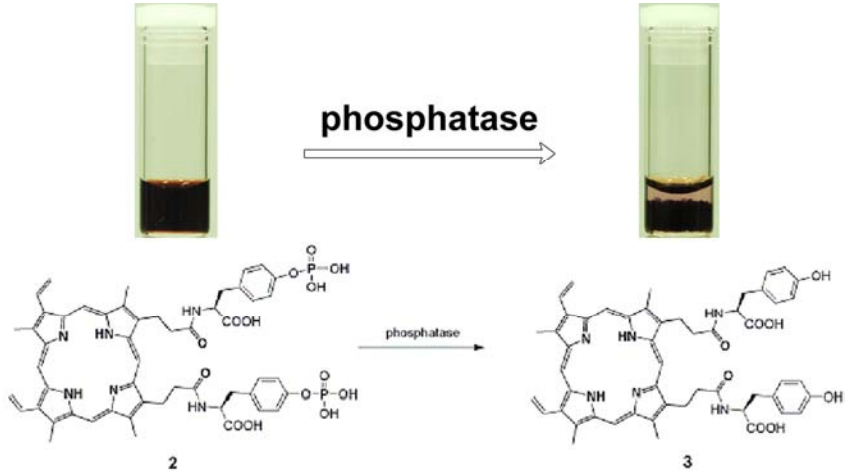

Fig. S1. *In vitro* dephosphorylation of **2** ([**2**]= 1 mg/mL, total volume = 0.5 mL) by acid phosphatase (10 μL of 50 U/mL) to form hydrophobic **3** at room temperature and pH 4.8.

Location of 2 inside cells.

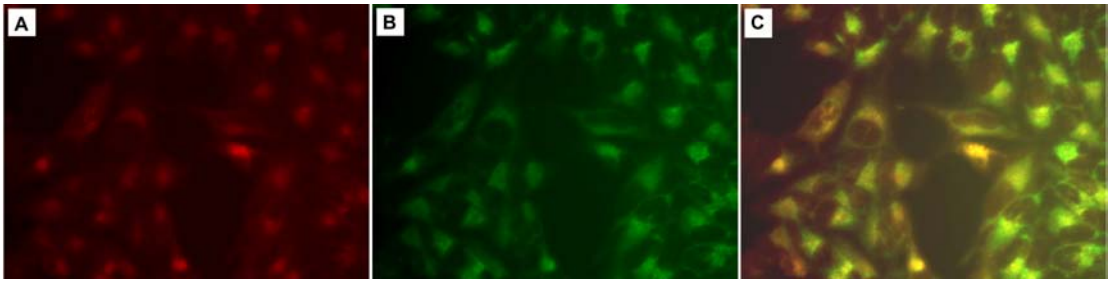

Fig. S2. Hela cells incubated with **2** at concentration of 25 μM and Mitotracker® Green at 100 nM. (A) fluorescence image of cells with **2**; (B) fluorescence image of cells with Mitotracker® Green; (C) merged image of image A &B.

Dark cytotoxicity and phototoxicity of 3.

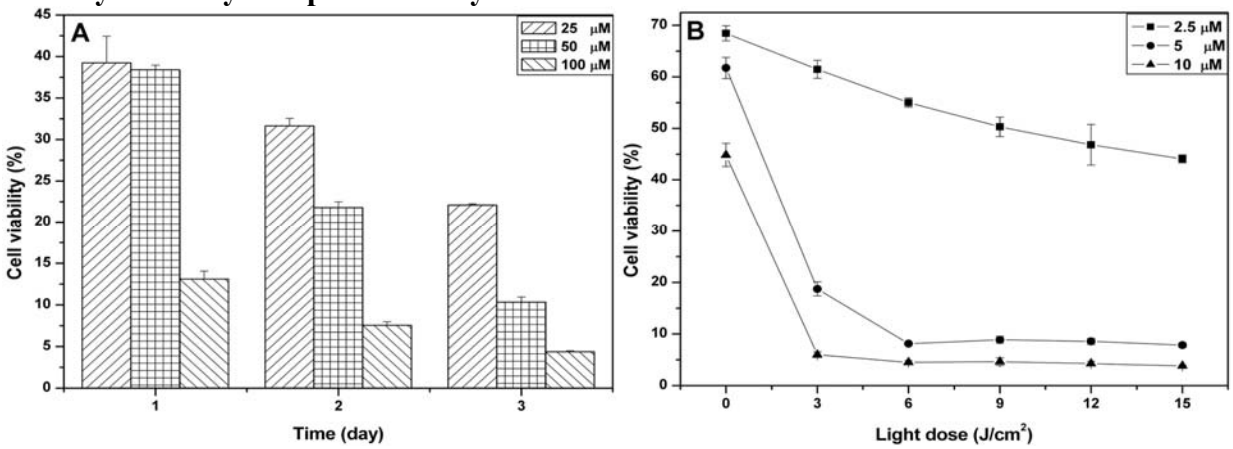

Fig. S3. (A) MTT assay of **3** on Hela cell; (B) PDT effect of **3** on Hela cell.

The dark toxicities of **2** and **3** can be explained qualitatively. As shown in Fig. S3, **3** shows IC_{50} of 8.5 μM to Hela cells in dark at day 1; the hydrophilic compound, **2**, however, is more difficult to penetrate the hydrophobic bilayer membrane of the cells, to buildup certain amount of **3** inside the cells, and to show dark toxicity. Therefore, compound **3** shows higher dark toxicity than that of **2**.