Supporting Information

Stimuli-responsive protoporphyrin IX silica-based nanoparticles for photodynamic therapy *in vitro*

Juan L. Vivero-Escoto[1,2] and Daniel L. Vega[1,2]*

[1]Department of Chemistry, University of North Carolina at Charlotte, Charlotte, NC 28223, USA; [2]The Center for Biomedical Engineering and Science, University of North Carolina at Charlotte, Charlotte, NC 28223, USA. E-mail: jviveroe@uncc.edu

Reagents and Materials. Protoporphyrin-IX, and amino- and mercapto-propyl triethoxysilane which were purchased from Frontier Scientific and Gelest, respectively. The rest of the reagents were purchased from Aldrich and used without further purification.

Synthesis of silyl-functionalized protoporphyrin IX ligand (PpIX-APTES). To synthesize the silyl-functionalized PpIX ligand (Fig. S1), we followed a two steps approach. Firstly, the carboxylic acid groups of PpIX were converted to their corresponding succinimide ester forms (SE-PpIX) by the following procedure: PpIX (1.0 mmol; 562.6 mg), N-hydroxysuccinamide (NHS, 8.0 mmol; 920.8 mg), dimethylamino pyridine (DMAP, 2.0 mmol; 244.3 mg) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 8.0 mmol; 1534 mg) were dissolved in a mixture of dimethylsulfoxide (DMSO):CH_2Cl_2 (50 mL; 1:1 vol.) and stirred under room temperature for 48 h. The succinimide ester derivative of PpIX was purified by precipitation in aqueous solution containing 20% of ethanol. The material was washed several times with the same solution and dried using a lyophilizer. The final product was stored at -20°C. Yield: 734.8 mg (97 %). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 10.12, 10.10, 10.09, 10.05 (4s, 4H, CH), 8.46-8.35 (m, 2H, CH), 6.38 (dd, 2H, CH_2), 6.17 (dd, 2H, CH_2), 4.28 (t, 4H, CH_2), 3.63 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 3.55 (s, 6H, $2CH_3$), 3.15 (t, 4H, CH_2), 2.71 (s, 8H, $4CH_2$). MS (ESI positive ion): m/Z= 757.27 (expected 757.29), 758.29 (expected 758.29), 759.31 (expected 759.29) for $[M+1]^+$, $[M+2]^+$, $[M+3]^+$ respectively. IR = 1736 (ester); 1778 (NHS); 1810 (NHS) cm^{-1}. Finally, APTES-PpIX ligand was obtained by reacting SE-PpIX (0.185 mmol; 140 mg) with aminopropyl triethoxysilane (APTES, 0.444 mmol; 103.8 uL) in the presence of triethylamine (0.444 mmol; 61.9 uL) dissolved in 5 mL of DMSO. The mixture was stirred for 72 h at room temperature. The

silane derivative of PpIX (PpIX-APTES) was purified by precipitation in aqueous solution containing 20% of ethanol. The material was washed several times with the same solution and dried using a lyophilizer. The final product was stored at -20°C. Yield: 149.7 mg (83.5 %). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 10.12, 10.10, 10.09, 10.05 (4s, 4H, CH), 8.46-8.35 (m, 2H, CH), 6.38 (dd, 2H, CH_2), 6.17 (dd, 2H, CH_2), 4.28 (t, 4H, CH_2), 3.63 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 3.55 (s, 6H, $2CH_3$), 3.42 (q, 12H, $6OCH_2$), 3.15 (t, 4H, CH_2), 2.92 (t, 4H, $2CH_2$), 1.21 (m, 4H, $2CH_2$), 0.86 (t, 18H, $6CH_3$), 0.24 (t, 4H, $2SiCH_2$). IR = 2923 (C-H stretch); 1637 (amide); 1066-1102 (Si-O; Si-C) cm^{-1}. MALDI-MS: m/Z= 968.4 (expected 968.5) for [M].

Synthesis of redox-responsive silyl-functionalized protoporphyrin IX ligand (PpIX-MPTES). The synthesis the PpIX-MPTES ligand (Fig. S1) was carried out through a two steps approach. First, PDSEA-PpIX was obtained by reacting a DMSO (3.3 mL) solution of SE-PpIX (0.518 mmol; 392 mg) with 2-(pyridine-2-yldisulfanyl) ethanamine hydrochloride[1] (PDSEA*HCl, 1.294 mmol; 288.4 mg) in the presence of triethylamine (1.294 mmol; 180.5 uL). The reaction mixture was stirred at 80 °C for 48 h. The desired product was obtained by filtration after the product precipitated in an aqueous solution containing 20% of ethanol. The material was washed several times with the same solution and dried using a lyophilizer. The final product was stored at -20°C. Yield: 387.6 mg (86 %). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 10.29, 10.22, 10.20, 10.19 (4s, 4H, CH), 8.51-8.56 (m, 2H, CH), 8.47-8.49 (m, 1H, CH), 7.78-7.84 (m, 1H, CH), 7.61-7.63 (m, 1H, CH), 7.28-7.31 (1H, CH), 6.43-6.49 (dd, 2H, CH_2), 6.20-6.24 (dd, 2H, CH_2), 4.27 (bs, 4H, CH_2), 3.61-3.73 (2m, 12H, CH_3), 3.10-3.12 (m, 4H, CH_2), 2.93-3.05 (m, approx. 8H, CH_2) . IR = 1641 (amide) cm^{-1}. The final RR-PpIX silane derivative (MPTES-DS-PpIX) was obtained by reacting PDSEA-PpIX (0.200 mmol; 180 mg) with mercaptopropyl triethoxysilane (MPTES, 0.120 mmol; 128.1 uL) in DMSO (4.5 mL). The mixture was stirred for 36 h at room temperature. PpIX-MPTES was filtered after precipitation in aqueous solution containing 20% of ethanol. The material was washed several times with the same solution and dried using a lyophilizer. The final product was stored at -20°C. Yield: 194.1 mg (84.1 %). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 10.32, 10.23 (2s, 4H, CH), 8.54-8.41 (m, 2H, CH), 6.45-6.39 (dd, 2H, CH_2), 6.19-6.15 (dd, 2H, CH_2), 4.28 (t, 4H, CH_2),3.71-3.64 (q, 4H, OCH_2), 3.63 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 3.55 (s, 6H, $2CH_3$), 3.15 (t, 4H, CH_2), 2.93-3.05 (m, approx. 8H, CH_2), 2.65-2.63 (t, 4H, CH_2), 1.67-1.56 (m, 4H, CH_2), 1.10-1.06 (t, 18H, CH_3), 0.62-0.56 (t, 4H, $2SiCH_2$). IR = 1639 (amide); 1067-1104 (Si-O; Si-C) cm^{-1}.

Quantification of unreacted PpIX-APTES and PpIX-MPTES ligands. The calibration curves of PpIX-APTES and PpIX-MPTES linkers in DMF were determined by UV-vis spectroscopy at the wavelength of 408 nm (soret band). To quantify the amount of unreacted PpIX silane linkers after the grafting protocol, we used the equation from the calibration curves and the following absorbance values: 0.7489 (PpIX-APTES) and 0.6999 (PpIX-MPTES) in a volume of 8 mL.

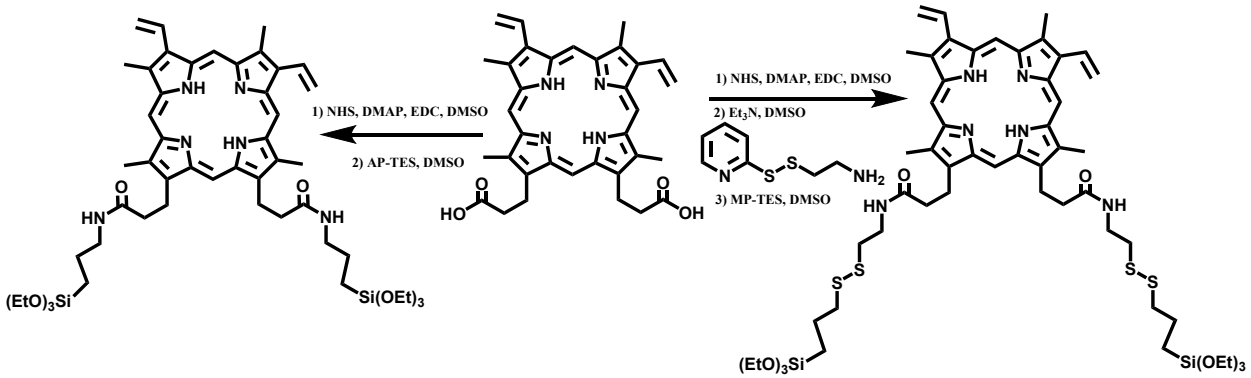

Fig. S1 Schematic representation of the synthesis of PpIX-APTES (left) and PpIX-MPTES (right) ligands.

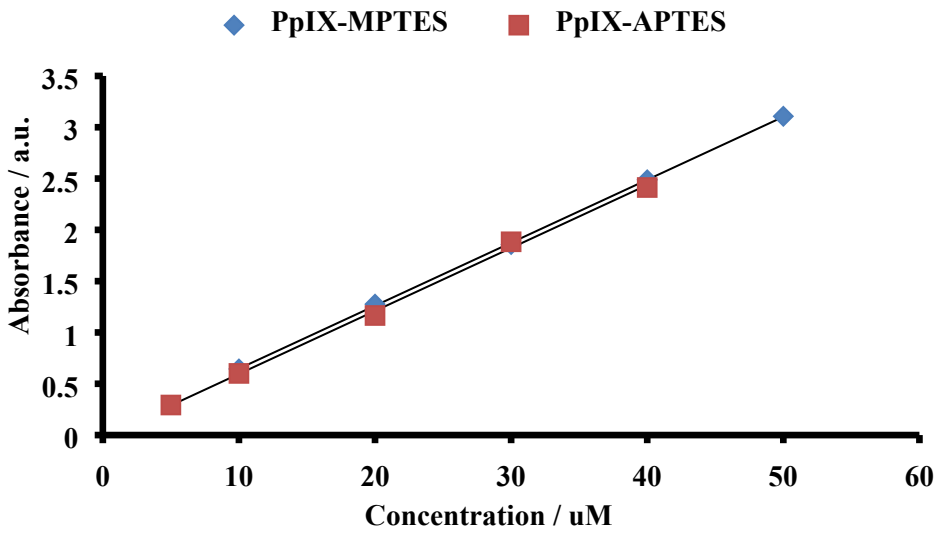

Fig. S2 Calibration curves for PpIX-APTES (red) and PpIX-MPTES (blue) ligands in DMF.

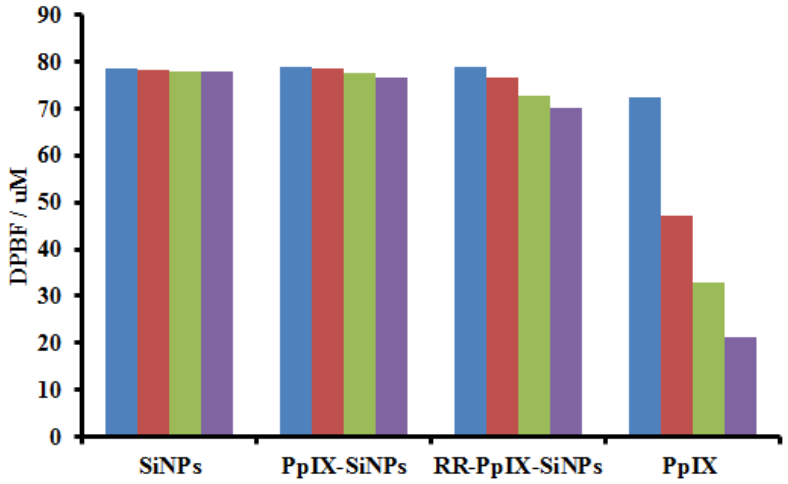

Fig. S3 Concentration of DPBF in the absence of light (blue) and after light exposure for 10 (red), 20 (green) and 30 (purple) seconds.

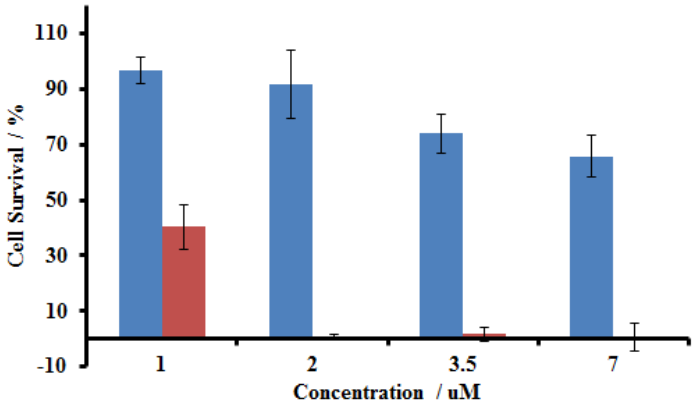

Fig. S4 Cytotoxicity of PpIX in DMF solution in the absence (blue) and presence of light (red). The samples were irradiated (400-700 nm; 170 ± 3 mW/cm^2) for 20 min. The cell survival was measured by MTS assay.

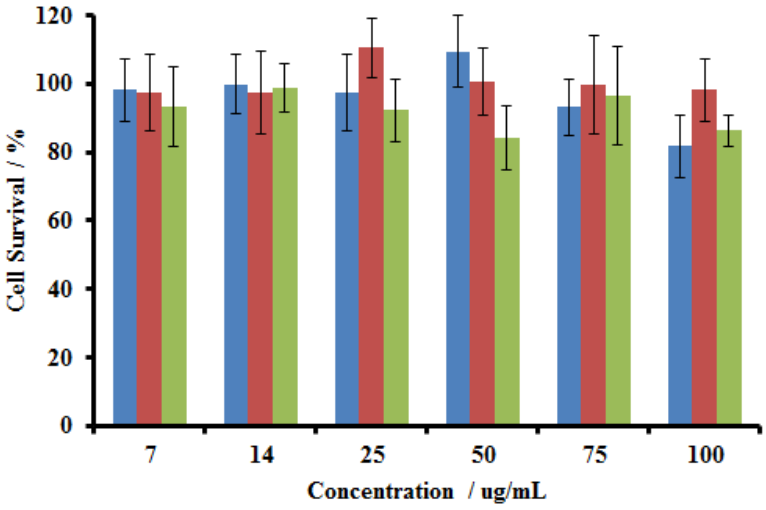

Fig. S5 Cytotoxicity of SiNPs (blue), PpIX-SiNPs (red) and RR-PpIX-SiNPs (green) in the absence of light. The cell survival was measured by MTS assay.

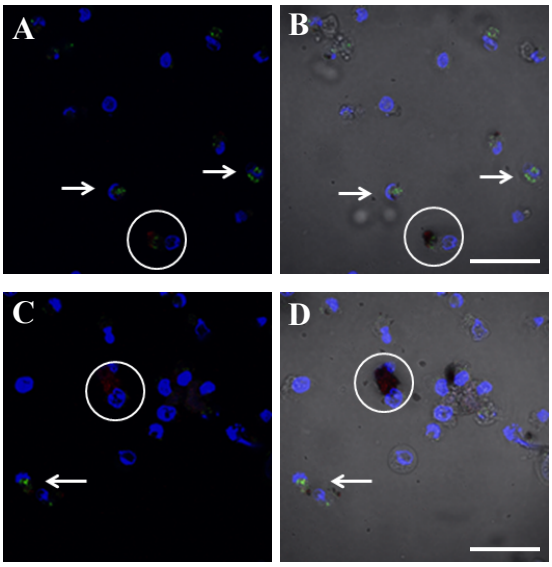

Fig. S6 Confocal fluorescence images of HeLa cells inoculated with RR-PpIX-SiNPs. The overlapped imaged of green (FITC), red (PpIX) and DAPI-stained nuclei (A,C); and the overlapped imaged with the DIC channel (B,D). Scale bars = 30 μm (A-D & I-L). The circles show the release of PpIX molecules and the arrows show the presence of the FITC-labeled RR-PpIX-SiNPs.

1. J. L. Vivero-Escoto, K. M. L. Taylor-Pashow, R. C. Huxford, J. Della Rocca, C. Okoruwa, H. An, W. Lin and W. Lin, *Small*, 2011, **7**, 3519-3528.