Electronic Supplementary Information (ESI) for

Nanocomposite Liposomes for pH-Controlled Porphyrin Release into Human Prostate Cancer cells

Formation of SiO_2-TTMAPP complex and nanocomposite liposomes

To quantify the number of porphyrin molecules adsorbed onto each primary silica particle, we examined the different ratios of TTMAPP over silica particles ranging from 0 to 8 at pH 9 (Figure S1). The free TTMAPP molecules were separated from silica-attached TTMAPP by centrifugation, and the supernatants were analyzed for the residual TTMAPP spectrophotometrically at its maximum absorption wavelength of 412 nm using an extinction coefficient of 3.6×10^5 M^{-1} cm^{-1}. The primary silica particle concentration, but not the molecular concentration, was used instead and calculated according to equation 1 to better reflect experimental conditions. Figure S1 shows that the amount of unattached TTMAPP reach the plateau at a molar ratio of 3, keep near unchanging until a ratio of 6, and then is doubled its plateau value at a ratio of 8. It is therefore concluded that on average, 4.5 molecules of TTMAPP adsorbed onto each primary silica particle.

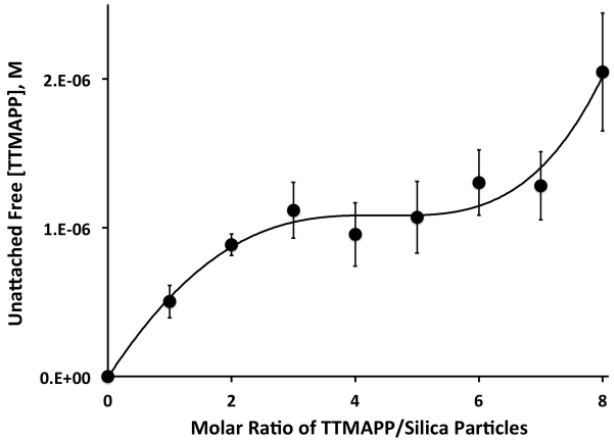

Figure S1. Molar load ratio of TTMAPP over fumed silica primary particles at pH 9 NaOH solutions

The silica-attached TTMAPP particles have a diameter of ~ 10 nm as estimated by the transmission electron microscope (TEM, the left image in Figure 2), which is comparable to the commercially reported size of primary silica nanoparticles (7 nm). This result provides quantitative information of porphyrin adsorption onto fumed silica. The relative amount of silica and TTMAPP were, therefore, controlled within upper plateau region under the experimental conditions. The nanocomposite liposomes are amorphous and formed by encapsulating silica-attached TTMAPP into lipid vesicles of DOPC at weak basic pH, e.g., in diluted NaOH solutions or 0.05 M HEPES buffer at pH 7.4. A loss of *ca.* 20% TTMAPP was found in the formation of liposomes when HEPES buffer solution was used. The molar ratio of TTMAPP over primary silica particle in liposomes was, therefore, estimated to be around 4 (4.5x80%=3.6). The formation of liposomes can be seen in the amorphous coating edge layers over the branched network on TEM image (right image in Figure 2). The nanocomposite liposomes could be sticky and unstable with the formation of larger agglomerates.

Determination of extinction coefficient of TTMAPP

TTMAPP has a strong electronic absorption at 412 nm, which does not change much with the solution pH, as indicated in Table S1. As the analysis of TTMAPP was conducted in weak basic solution, we therefore use 3.6×10^5 $M^{-1} \cdot cm^{-1}$ throughout the paper.

Table S1. Extinction coefficients (ε) of TTMAPP determined at 412 nm against water

ε±S.D., M^{-1} cm^{-1}	***Solutions***
(3.6±0.1) x 10^5	pH 9 NaOH
(3.6±0.1) x 10^5	0.7 M pH 8.0 Tris-HCl buffer
(2.9±0.1) x 10^5	1 M pH 7.3 phosphate buffer
(3.8±0.1) x 10^5	1 M pH 5.4 phosphate buffer
(3.1±0.1) x 10^5	0.7 M pH 5.1 acetic buffer

An example of Beer's Law (A = εbc) plots is given in Figure S3. The slope of the straight line equals to the molar extinction coefficient when using 1-cm cuvette.

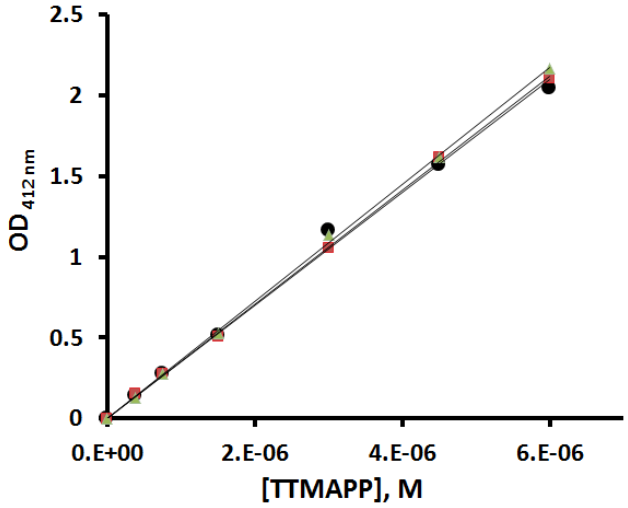

Figure S2. Beer's Law plot in 0.7 M pH 8.0 Tris-HCl buffer solution

Stability of Silica-TTMAPP-DOPC at different pH

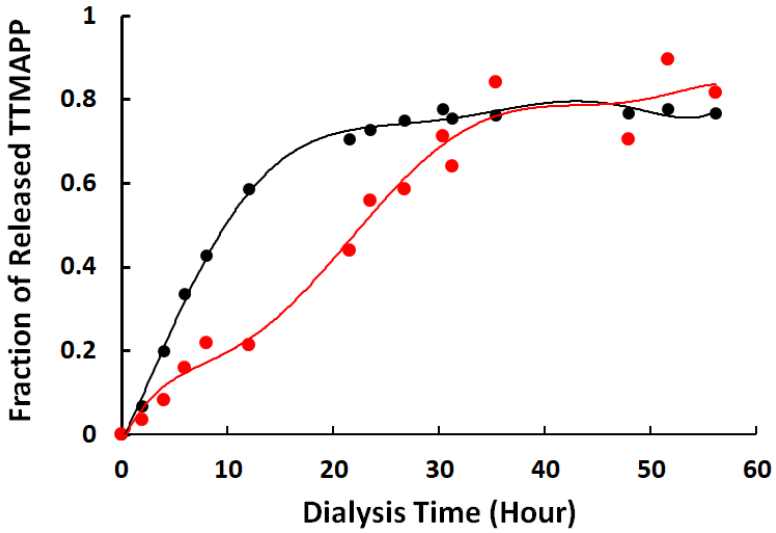

Figure S3. TTMAPP release from liposomes as a function of dialysis time at pH 5.6 (black line) and pH 7.5 (red line)

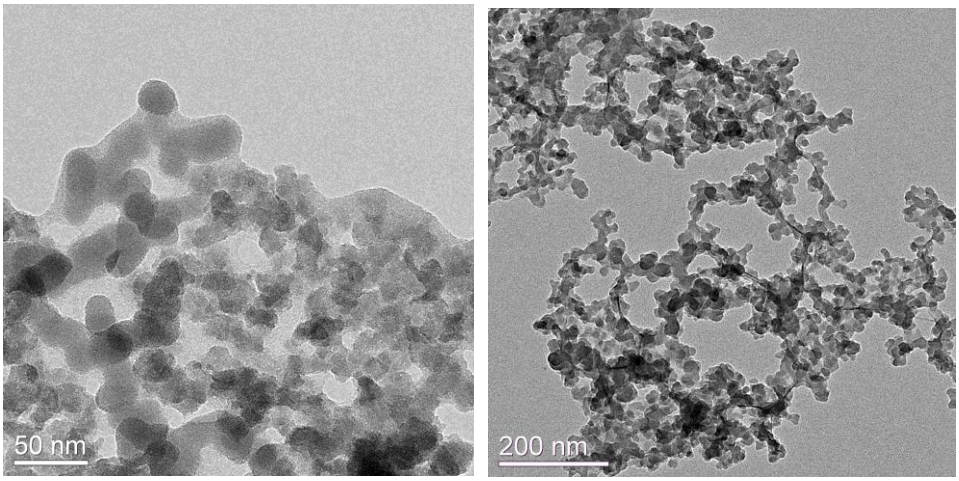

Figure S4. Additional TEM images of SiO2-TTMAPP-DOPC at pH 8-9

Determination of $^1O_2$ quantum yield in D2O upon irradiation of TTMAPP at 532 nm

To determine the quantum yield of $^1O_2$ by free TTMAPP, the absorbance of TTMAPP samples and a reference of TSPP was ranged from 0.01 to 0.8 at an excitation wavelength of 532. The initial $^1O_2$ intensities on its decay curves were extrapolated to time zero for each measurement. The quantum yield of $^1O_2$ production (φ_Δ) was calculated according to the following equation:

$$\frac{\phi_{\Delta,TTMAPP}}{\phi_{\Delta,TSPP}} = \frac{Slope_{TTMAPP}}{Slope_{TSPP}}$$

Here $\varphi_{\Delta,TTMAPP}$ and $\varphi_{\Delta,TSPP}$ (= 0.63)[1] are the quantum yields for the sample TTMAPP and the reference TSPP, respectively. $Slope_{TTMAPP}$ and $Slope_{TSPP}$ represent the slopes derived from linear response of $^1O_2$ signals as a functional of absorbance at 532 nm for TTMAPP and TSPP, respectively. The effect of $^1O_2$ production as a function of absorbance at excitation wavelength of 532 nm is shown in Figure S5.

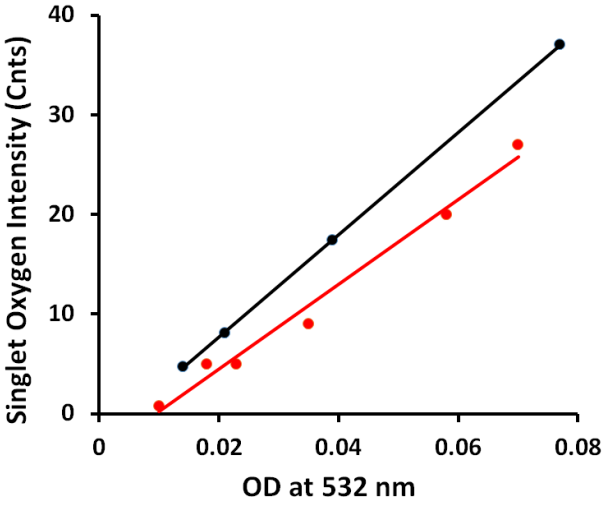

Figure S5. Effect of $^1O_2$ production as a function of absorbance at excitation wavelength of 532 nm upon irradiation of TTMAPP (black line) and TSPP (red line), dots: experimental results and lines: linear fitting

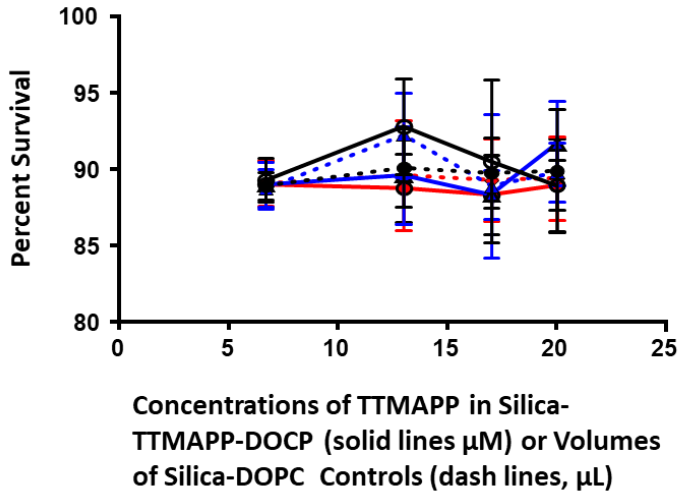

Figure S6. Dark controls of the cytotoxicity of Silica-TTMAPP-DOPC on DU145 cells incubated at pH 7.4 (solid black lines), pH 6.3 (solid blue line) and pH 5.4 (solid red line) as a function of TTMAPP concentrations. The corresponding colored dash lines represent controls with same amount of silica and DOPC in the absence of TTMAPP. The data represents the plot of mean cell viability ± standard deviation with n = 4.

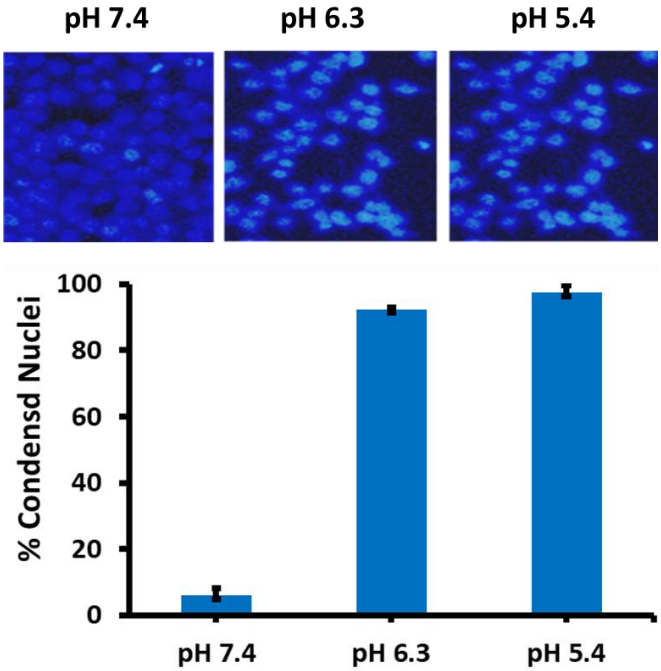

Figure S7. Detection of DU145 cell death by DAPI staining assay upon cell treatments with Silica-TTMAP-DOPC containing 13 µM TTMAPP at pH 7.4, pH 6.3 and pH 5.4. Cells were counted in 4 fields in each treatment. Values are presented as means and SD of percent cells with condensed chromatin. Means differ significantly between pH 7.4 and pH 5.4, and between pH 7.4 and pH 6.3.

(1) Charles Tanielian; Christian Wolff; Esch, M. Singlet Oxygen Production in Water: Aggregation and Charge-Transfer Effects. *J. Phys. Chem.* **1996,** *100* (16), 6555-6560.