

Supporting Information

Mesoporous silica nanoparticle facilitated drug release through cascade photosensitizer activation and cleavage of singlet oxygen sensitive linker

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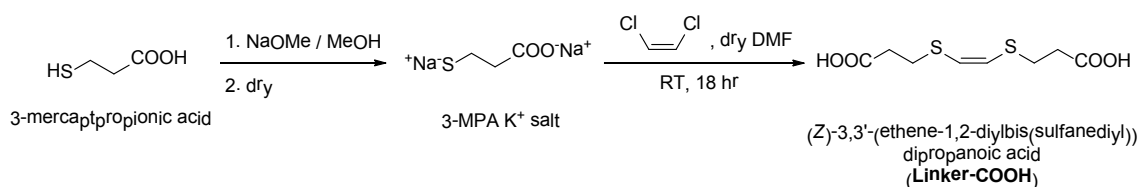
Experimental Section

Reagents: Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane(APTES), 3-mercaptopropionic acid (3-MPA), *cis*-1,2-dichloroethylene, zinc phthalocyanine (ZnPc), fluorescamine, pyrocatechol violet (PV), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), *N*-hydroxysuccinimide (NHS) and 5-(2-aminoethylamino)-1-naphthalenesulfonic acid(EDANS) were purchased from Sigma Aldrich. 30 wt % NaOMe in MeOH was purchased from Acros. Singlet oxygen sensor green[®] (SOSG) reagent was purchased from Molecular Probes (Eugene, OR). All reagents were used as received without further purification. DMF was dried under CaH₂ and distilled. The 525 nm long pass filter was purchased from Edmund optics (Barrington, NJ).

Instrumental methods: TEM image was taken from a transmission electron microscope (JEM-2010, JEOL) and high-resolution TEM image was taken from a transmission electron microscope (JEM-2100, JEOL). FT-IR spectrum was measured from a FT-IR spectrometer (VERTEX70 FT-IR Spectrophotometer, Bruker Optics) using KBr pellet. UV-vis spectra were measured from a UV-vis spectrometer (UV 2550, Shimadzu) and fluorescence spectra were measured from a spectrofluorophotometer (RF-5301 PC, Shimadzu). Hydrodynamic volume was measured from zetasizer (Nano S90, Malvern) at 0.1 mg mL⁻¹ in DW and zeta potential was measured from zetasizer (Nano Z, Malvern) at 0.1 mg mL⁻¹ in DW. Barrett-Joyner-Halenda (BJH) method and Brunauer-Emmett-Teller (BET) method were used to calculate the average pore size distribution and specific surface area of MSN material.

Preparation of MSN: MSN was synthesized following the reported method.¹ Briefly, 100 mg (0.266 mmol) of CTAB was dispersed in 48 mL of water and 0.35 mL of NaOH solution (2 M) was added. The mixture was heated at 80 °C and 500 µL of TEOS was added under vigorous stirring. The mixture was vigorously stirred at 80 °C for additional 2 h. The solution was centrifuged to isolate MSN material, washed with water and methanol and dried under high vacuum for 24 h. For the analysis of MSN by TEM, HR-TEM, FT-IR, UV-vis absorption, fluorescence, DLS, zeta potential, BET and BJH method, a portion of prepared MSN was further treated to eliminate the remaining surfactant from the pore as mentioned in preparation of APMSN.

Preparation of APMSN: Amine-modified MSN (APMSN) was prepared by the method following the literature.¹ As-prepared MSN (25 mg) was dispersed in toluene (2.5 mL) and 35 µL of APTES was added. The reaction suspension was refluxed for 20 h. The solid particles were isolated by centrifuge, washed with toluene, methanol and water and dried under vacuum. The product material was further treated to remove the surfactant in the mesoporous structure. 10 mg of MSN material was dispersed in methanol (1 mL) and concentrated HCl (10 µL) was added. Reaction mixture was heated at 60 °C for 18 h. The mixture was centrifuged to isolate MSN materials, washed with water and methanol and dried under high vacuum for 24 h. The surface amine group was quantified by fluorescamine assay.²



Scheme S1 Synthesis of Linker-COOH

Synthesis of linker-COOH: Linker-COOH was synthesised by the modified method of Baugh *et al.*³ To a solution of 30 wt % NaOMe in MeOH (7.34 mL, 39.14 mmol) and 3-MPA (1.7 mL, 19.57 mmol) was added while stirring at 0 °C. Methanol was evaporated and the salt was dried *in vacuo*. The disodium salt of 3-MPA was dispersed in dry DMF (10 mL) and the solution of *cis*-1,2-dichloroethylene (781 µL, 10.3 mmol) in EtOH (500 µL) was added dropwise. Reaction mixture was stirred at RT for 18 h. The solution was diluted with water (50 mL) and acidified to pH 3 with 1 M KHSO₄. This mixture was washed with EtOAc (3 x 100 mL) and the combined organic layers were washed with water (2 x 100 mL) and brine (100 mL), dried with dry Na₂SO₄ and concentrated. Crude product was washed with diethyl ether to obtain the white powder and recrystallized from EtOAc / Hexane and dried under vacuum. (Yield = 64 %)

^1H NMR (300 MHz, d_4 -MeOD): δ 6.19 (s, 2H), 2.93 (t, 4H), 2.60 (t, 4H)

Preparation of Pc@AP: ZnPc was loaded into the pores of APMSN in DMSO following the literature.⁴ 25 mg of APMSN was soaked into the 5 mL of 0.5 mg mL⁻¹ ZnPc solution in DMSO and stirred for 24 h at dark condition. MSN material was obtained by centrifugation, washed by PBS and water and dried under vacuum for 24 h to obtain Pc@AP. Loaded ZnPc was quantified by UV-vis absorbance at 672 nm in DMSO.

Preparation of AP-L: APMSN (50 mg) was dispersed in DMSO (5 mL) and 71 mg of Linker-COOH (0.300 mmol), 290 mg of EDC-HCl (1.50 mmol) and 170 mg of NHS (1.50 mmol) were added into the suspension. The mixture was stirred at RT for 24 h and centrifuged to collect the MSN material. The obtained material was washed with DMSO, water and methanol and dried under vacuum for 24 h. The surface conjugated linker-COOH was quantified indirectly by quantifying the remaining amine group by fluorescamine method and PV-Ni²⁺ back titration method.^{2, 5}

Preparation of AP-E: EDANS was conjugated with surface carboxylic acid of AP-L. 5 mg of AP-L was dispersed in 1 mL of DMSO and 250 μL of 100 mM EDANS in 0.5 mg/mL ZnPc/DMSO solution, 24 mg of EDC-HCl (0.125 mmol) and 14 mg of NHS (0.125 mmol) was added into the suspension and stirred at room temperature in dark condition for 24 h. Reaction suspension was centrifuged to isolate the MSN material and washed with DMSO, water and methanol and dried under vacuum for 24 h. Conjugated EDANS was quantified by fluorescence at 445 nm (EDANS) in DMSO.

Preparation of Pc@AP-E: EDANS was conjugated with surface carboxylic acid of AP-L while ZnPc was been loading into the porous structure of the mesoporous silica according to the literature.⁴ 5 mg of AP-L was dispersed in 1 mL of 0.5 mg mL⁻¹ ZnPc in DMSO and stirred at RT with dark condition for 24 h. Subsequently, 250 μL of 100 mM EDANS in 0.5 mg/mL ZnPc/DMSO solution, 24 mg of EDC-HCl (0.125 mmol) and 14 mg of NHS (0.125 mmol) was added into the suspension and stirred at same condition for additional 12 h. Reaction suspension was centrifuged to isolate the MSN material and washed carefully with 0.5 mg mL⁻¹ ZnPc in DMSO, 10 % DMSO in PBS and water and dried under vacuum for 24 h. Loaded ZnPc and conjugated EDANS was quantified by UV-vis absorbance at 672 nm (ZnPc) and fluorescence at 445 nm (EDANS) in DMSO, respectively.

Detection of singlet oxygen: Generation of singlet oxygen from Pc@AP was measured by the

fluorescence at 530 nm from reacted product of SOSG and singlet oxygen. ${}^6\text{Pc@AP}$ was dispersed in PBS to final concentration of 2 mg mL^{-1} and SOSG was added into the suspension to final concentration of $1 \text{ }\mu\text{M}$. Suspension was irradiated by long-pass filtered ($> 525 \text{ nm}$) halogen lamp (250 W) with final intensity of 50 mW cm^{-2} . Fluorescence of reacted SOSG ($\lambda_{\text{ex}} = 504 \text{ nm}$) was measured at different time scales (0, 10, 20, 30, 60 min). Control experiment was operated under dark condition (60 min).

Serum stability of Pc@AP : Nonspecific leakage of loaded photosensitizer was monitored by UV-vis spectrometry. 1 mg mL^{-1} suspension of Pc@AP in 10 % serum / DMEM solution was stirred at RT with light irradiation of long-wavelength light by halogen lamp ($> 525 \text{ nm}$, 100 mW cm^{-2}). $200 \text{ }\mu\text{L}$ of suspension was collected and centrifuged at each time scale. Obtained Pc@AP was re-suspended in DMSO to leach out ZnPc from the pore and centrifuged. UV-vis absorbance at 625 nm was monitored to monitor the remaining amount of ZnPc in Pc@AP .

Photo-responsive release of Pc@AP-E : Pc@AP-E was dispersed in 10 % DMSO / PBS to final concentration of 1 mg mL^{-1} and the model drug release was measured by fluorescence emission of EDANS ($\lambda_{\text{ex}} = 340 \text{ nm}$) at 483 nm. For the light condition, the suspension was stirred while being irradiated by long-pass filtered ($> 525 \text{ nm}$) halogen lamp (250 W) to final intensity of 100 mW cm^{-2} with water jacket to control the temperature. For the dark condition, the suspension was stirred at dark. $200 \text{ }\mu\text{L}$ of suspension was collected centrifuged and the fluorescence of the supernatant was monitored at each time scale. For the light induced release, suspension of Pc@AP-E or AP-E was stirred at dark for 1 h, then irradiated at same condition immediately and the release was monitored by fluorescence of EDANS.

Supplementary figures

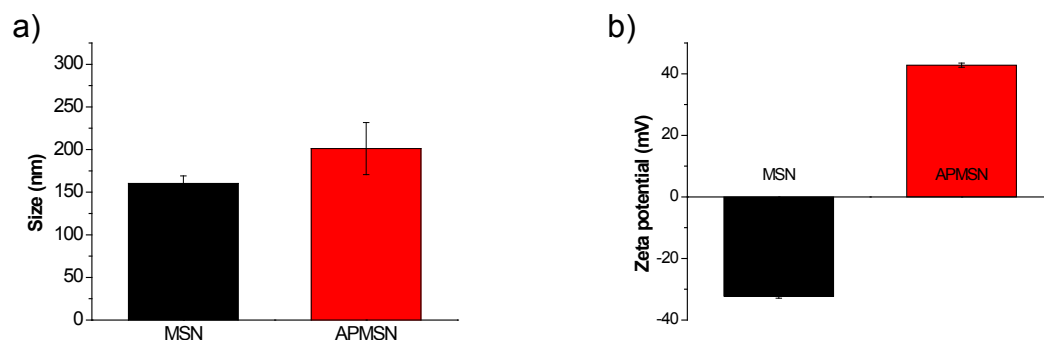


Fig. S1 (a) Hydrodynamic size calculated from dynamic light scattering (DLS) and (b) zeta potential of MSN (black) and APMSN (red).

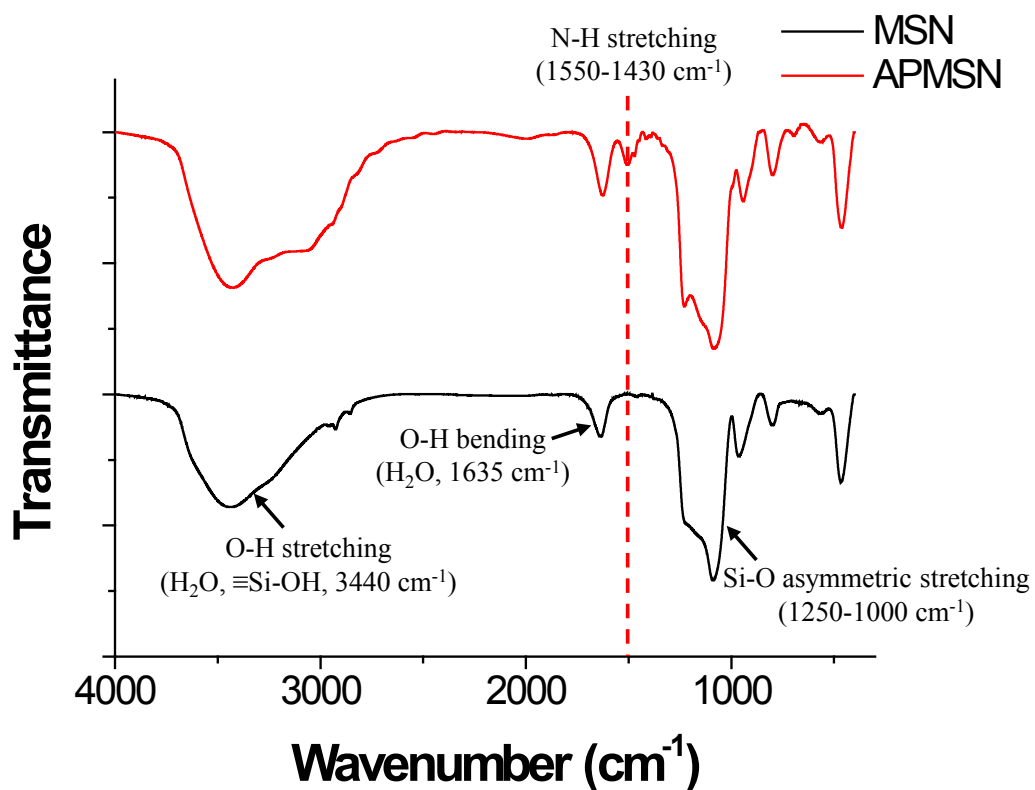


Fig. S2 IR spectra of MSN (black) and APMSN (red).

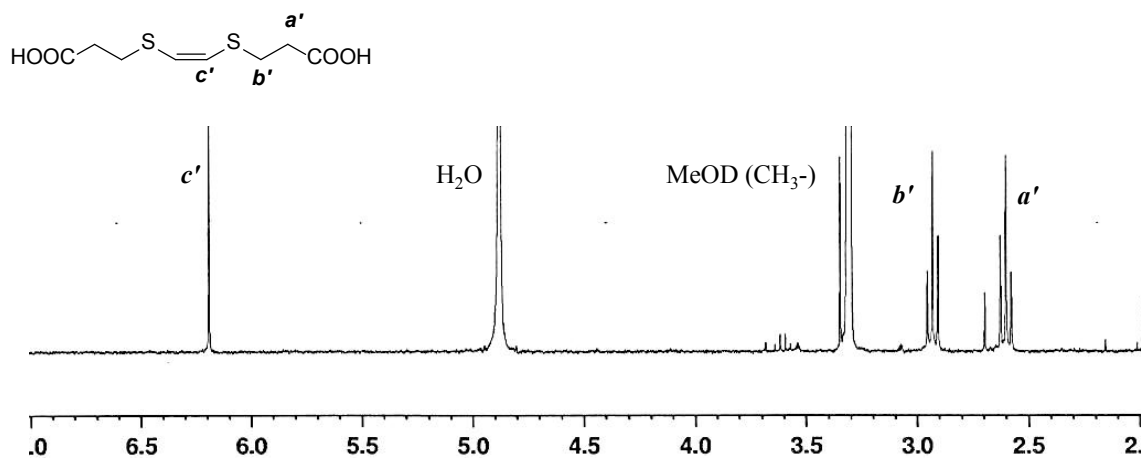


Fig. S3 ¹H NMR spectrum of Linker-COOH.

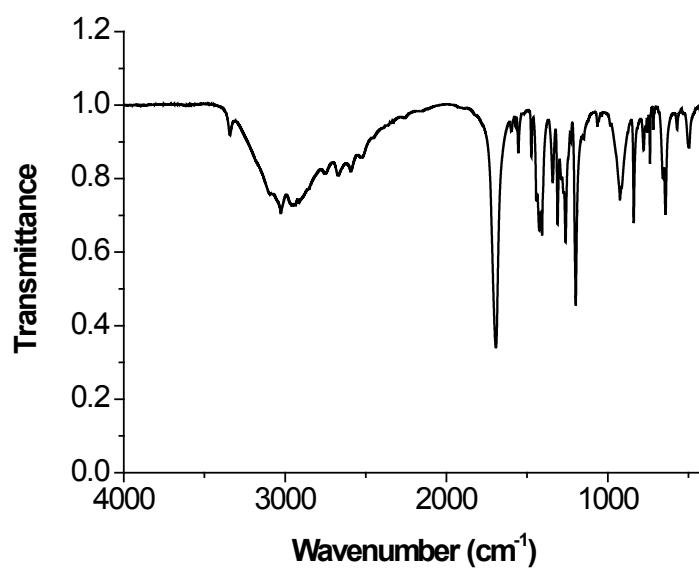


Fig. S4 IR spectrum of Linker-COOH.

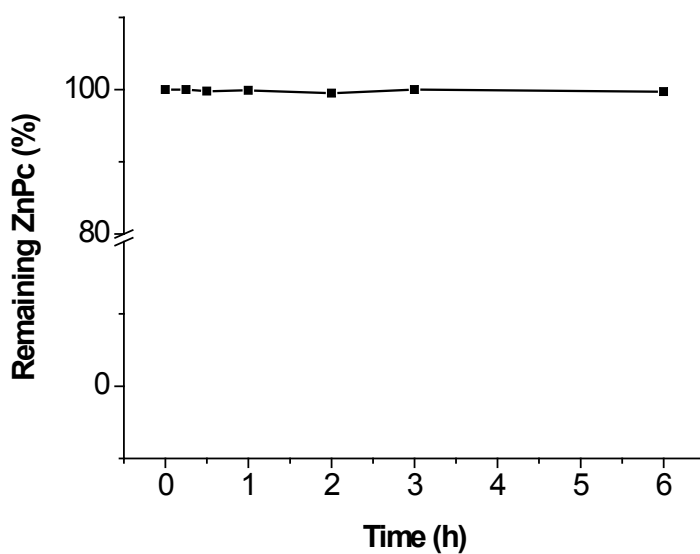


Fig. S5 Remaining ZnPc in Pc@AP incubated in 10 % serum / DMEM with light irradiation.

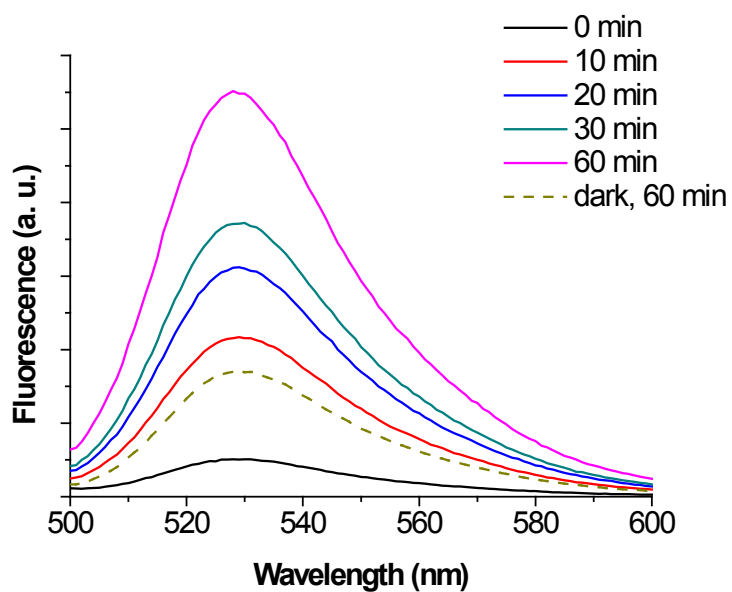


Fig. S6 Generation of singlet oxygen from ZnPc@APMSN by light irradiation (100 mW cm^{-2}).

References

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