Supplementary Information

Encapsulation of glycosylated porphyrins in silica nanoparticles to enhance efficacy of cancer photodynamic therapy

Experimental section

General methods

Absorption spectra were recorded using a Shimadzu UV-2501-PC. FT-IR spectra were recorded in KBr pellets using GRASEBY SPECAC. The irradiation system used to determine the production of $^1O_2$ and the phototoxicity during *in vitro* experiments was a Lumacare source, model LC-122, consisting on a 250 W halogen lamp coupled to an optical fiber (with a cutoff filter for wavelengths <540 nm). The radiation power was measured with a potentiometer bright Spectra Physics, model 407A and the sensor of the same brand, model 407A-2. The dynamic light scattering (DLS) measurements were taken by Zetasizer Nano ZS from Malvern Instruments. Transmission electron microscopy (TEM) images were obtained using a Hitachi H-9000 transmission electron microscope operatingat an acceleration voltage of 300 kV and JEOL 2200FS transmission electron microscope operating at an acceleration voltage of 200 kV.

All reagents were obtained from commercial sources and were used without further purification steps. Reverse phase column chromatography was carried out on Waters Sep-Pak C18 35 cm^3 cartridges. Analytical thin layer chromatography (TLC) was carried out on pre-coated silica gel sheets (Merck, 60, 0.2 mm).

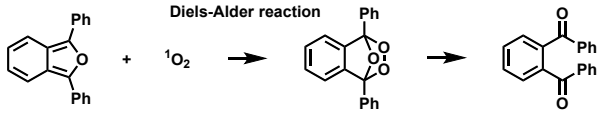
Preparation of NPs

In a 15 mL falcon tube 50 mg of **PS 1** or **PS 2** (29.77 μmol, 4.0 mM) was dissolved in 5.875 mL of EtOH and then 0.375 mL of 25% NH_4OH (2.4 mmol, 0.32 M) was added. The mixture was sonicated for 5 min and 83.7 μL of TEOS (0.375 mmol, 50 mM) in 1.166 mL of EtOH was added. The reaction was incubated for 24 h at 25 °C under continuous agitation (250 rpm) in laboratory incubator shaker (IKA KS 4000 i control) in horizontal position. After that time NPs were isolated by centrifugation (15 mL falcon tubes, 6000 rpm, 30 min) and washed with EtOH. The final NPs were air-dried.

The same method was used to synthetize silica nanoparticles in the absence of PS with the average size of 86.1 ± 10.3 nm (named as **SNP**).

Singlet oxygen generation study

Singlet oxygen ($^1O_2$) was determined by a chemical method using 1,3-diphenylisobenzofuran (DPBF). DPBF has an absorption maximum at 415 nm, thus it is possible to follow the ability of the NPs to generate $^1O_2$ by measuring the DPBF absorption decay, at this wavelength. The solutions were irradiated at RT and under magnetic stirring, with optical fiber (with a cutoff filter for wavelengths <540 nm) at a fluence rate of 10 mW/cm^2.



Scheme SI 1 Reaction of DPBF with $^1O_2$.

PS 1 (0.5 µM), **PS 2** (0.5 µM), **NP 7** (0.131 mg, 12.6 µM of **PS 1**) or **NP 8** (0.022 mg, 1.6 µM of **PS 2**) were placed into 3 mL cuvette which contained solution of DMF:H_2O (9:1, by volume). Then, DPBF (50 µM) in solution of DMF:H_2O was added (total volume in cuvette 3 mL). The breakdown of DPBF was monitored by measuring the decrease in absorbance at 415 nm at pre-established irradiation intervals. The results were expressed by plotting the DPBF depletion against the irradiation time. The depletion of DPBF was calculated as follows: *DPBF depletion* = Abs_1/Abs_0. Abs_0 and Abs_1 are the absorbance values at 415 nm before and after irradiation, respectively.

Stability and photostability of PS-NPs during singlet oxygen generation study

Stability and photostability studies were carried out in the same conditions of irradiation of the singlet oxygen studies. Each point represents the mean of at least three independent experiments, and has a standard deviation lower than 3%. Nanoparticles were stable during singlet oxygen study.

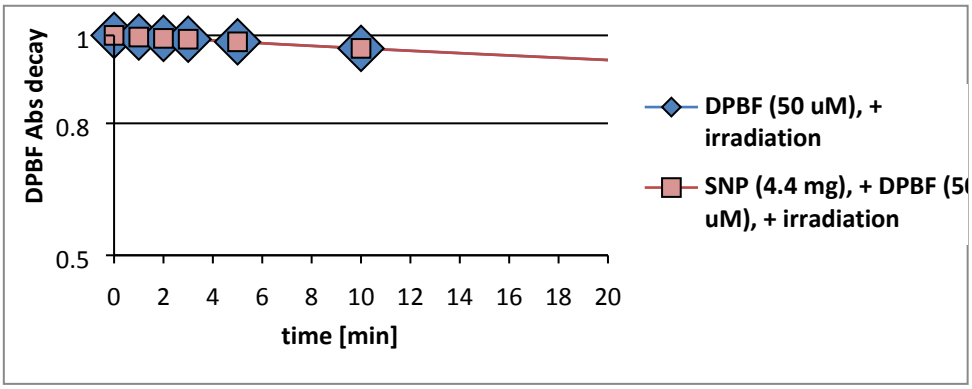


Figure SI 1 Stability of SNPs during singlet oxygen generation study.

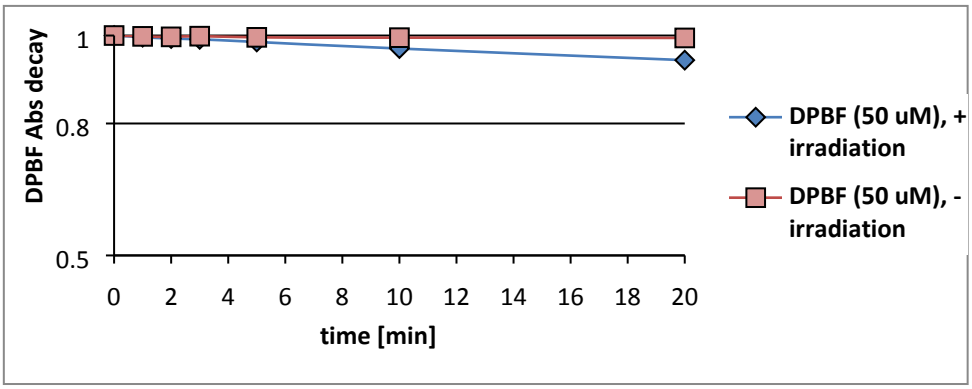


Figure SI 2 Stability of DPBF during singlet oxygen generation study.

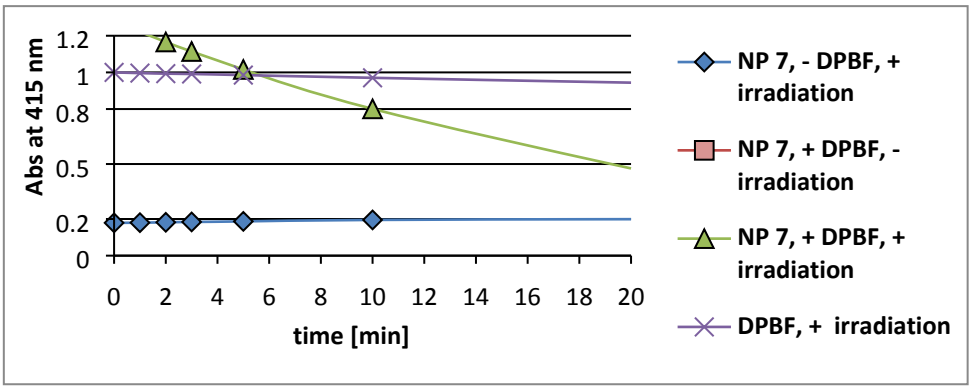


Figure SI 3 Stability of **NP 7** during singlet oxygen generation study.

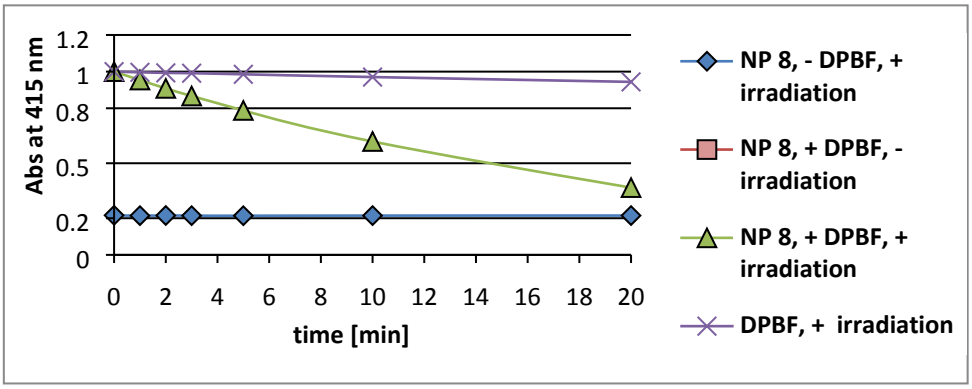


Figure SI 4 Stability of **NP 8** during singlet oxygen generation study.

Characterization of NPs

TEM

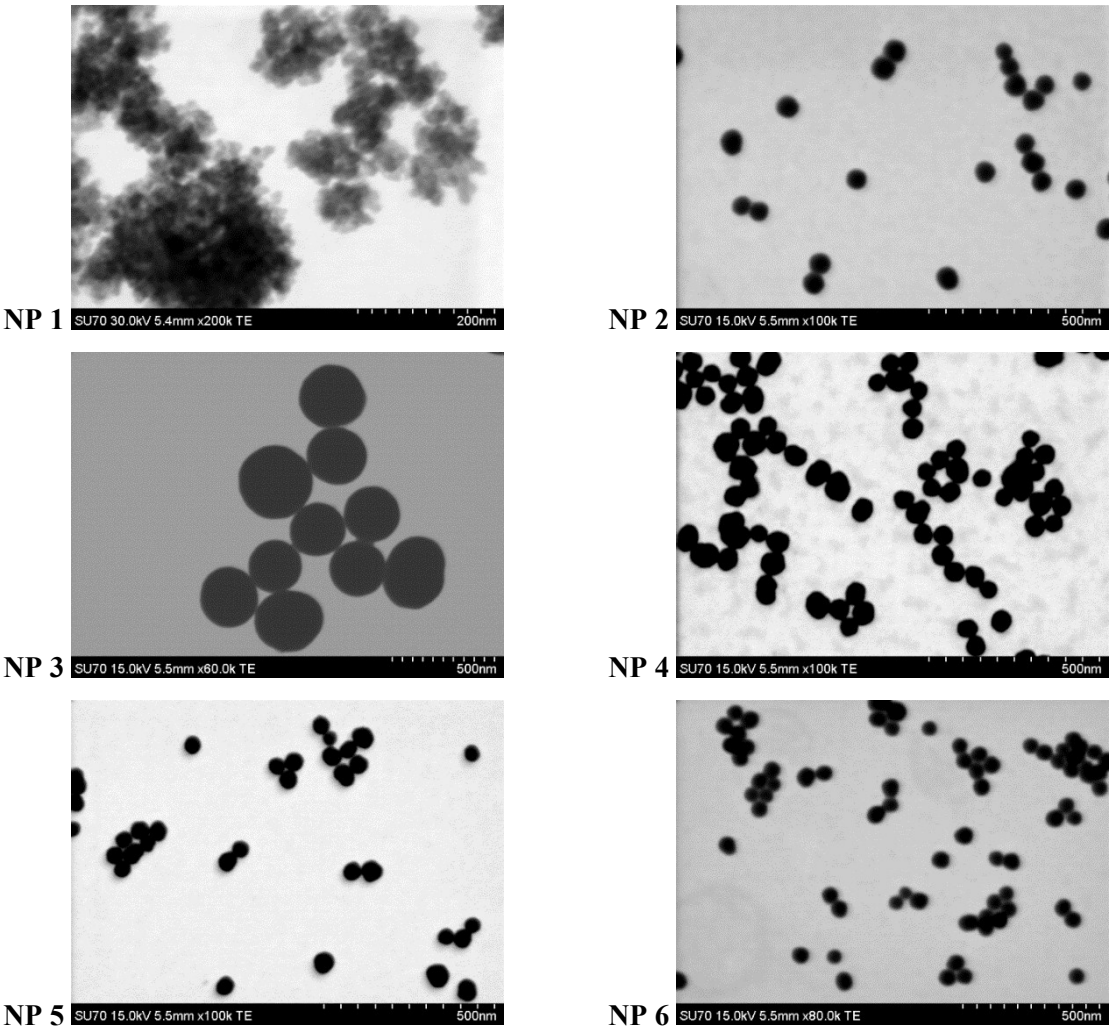


Figure SI 5 TEM images of **NP 1**-**NP 6**.

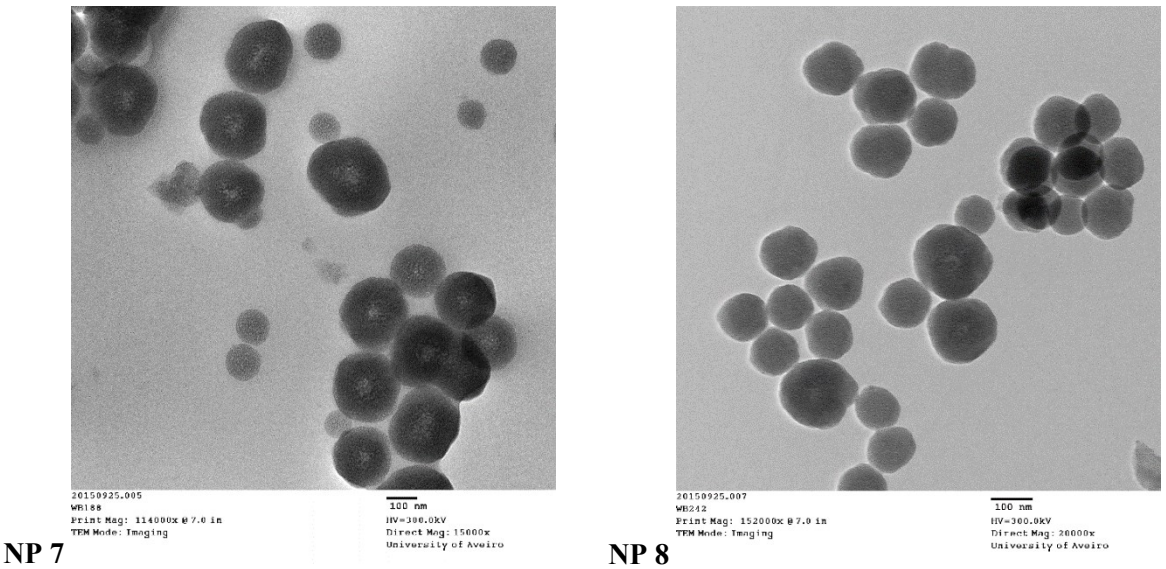


Figure SI 6 TEM images of final **NP 7** and **NP 8**.

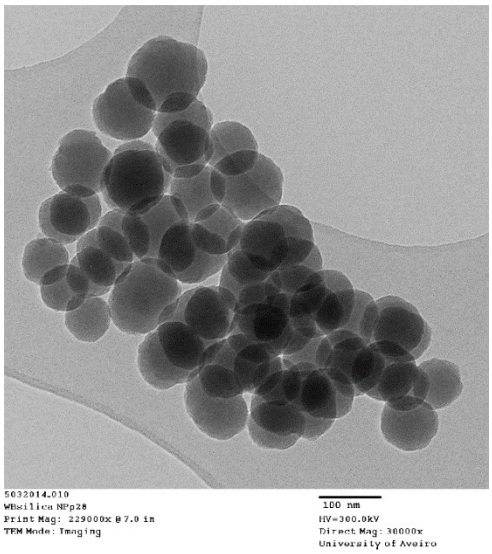


Figure SI 7 TEM image of SNPs without PS.

DLS

		Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm): 276.5	Peak 1:	233.5	100.0	33.42
PdI: 0.271	Peak 2:	0.000	0.0	0.000
Intercept: 0.953	Peak 3:	0.000	0.0	0.000
Result quality Good				

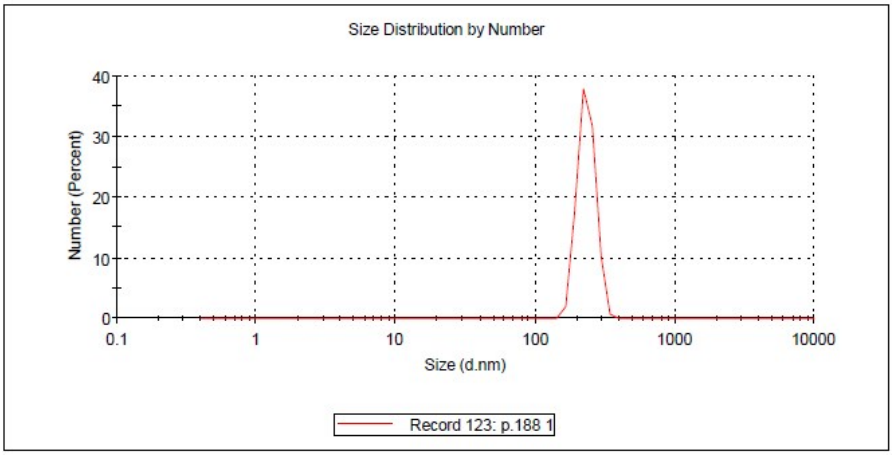


Figure SI 8 DLS size distribution of **NP 7** in water.

		Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm): 159.6	Peak 1:	133.2	100.0	26.32
PdI: 0.214	Peak 2:	0.000	0.0	0.000
Intercept: 0.876	Peak 3:	0.000	0.0	0.000
Result quality Good				

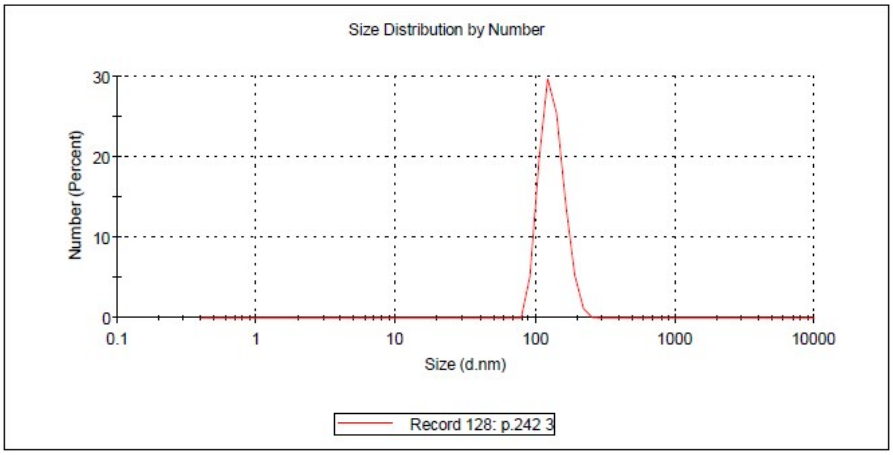
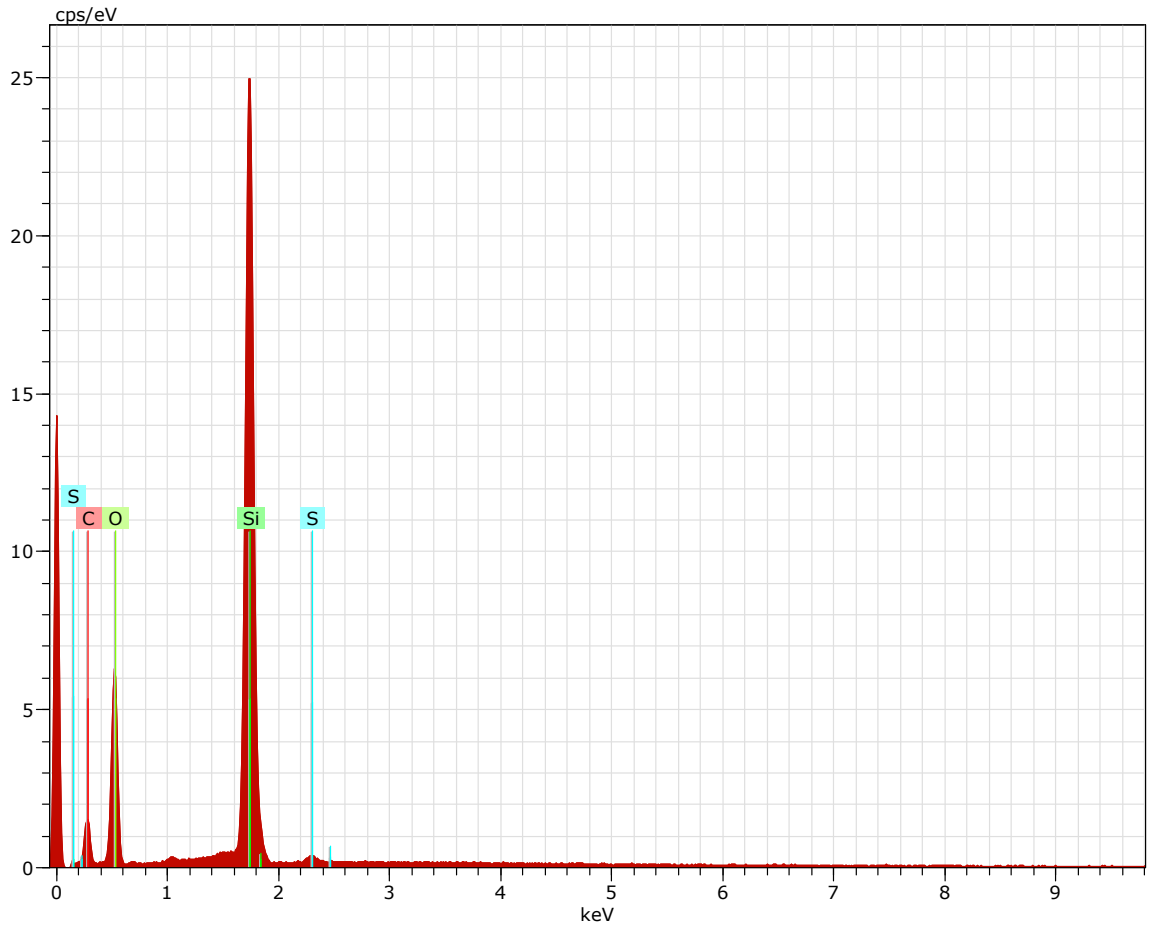


Figure SI 9 DLS size distribution of **NP 8** in water.

EDS

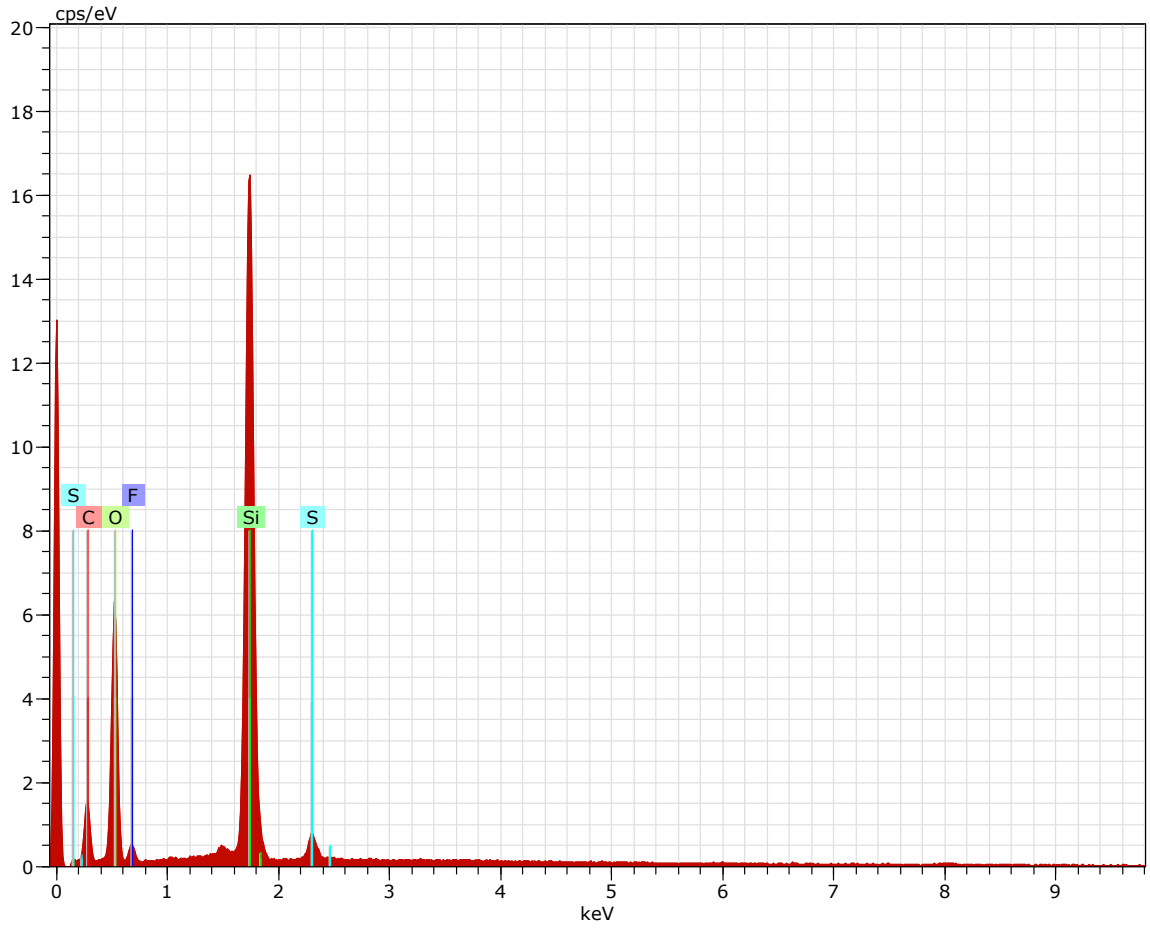


NP 7.spx Date:27-08-2015 18:14:01 HV:30.0kV Puls th.:4.70kcps

Spectrum: NP 7.spx

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	41.70	49.39	50.21	15.75
Carbon	K-series	22.35	26.47	35.85	10.29
Silicon	K-series	20.01	23.70	13.72	2.75
Sulfur	K-series	0.37	0.43	0.22	0.13
	Total:	84.42	100.00	100.00	

Figure SI 10 EDS of **NP 7**.



NP 8.spx Date:27-08-2015 18:10:23 HV:30.0kV Puls th.:3.83kcps

Spectrum: NP 8.spx

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	56.15	49.49	49.70	20.76
Carbon	K-series	29.40	25.91	34.67	13.04
Silicon	K-series	20.37	17.95	10.27	2.80
Fluorine	K-series	6.66	5.87	4.97	3.67
Sulfur	K-series	0.88	0.77	0.39	0.19
	Total:	113.47	100.00	100.00	

Figure SI 11 EDS of **NP 8**.

UV-Vis

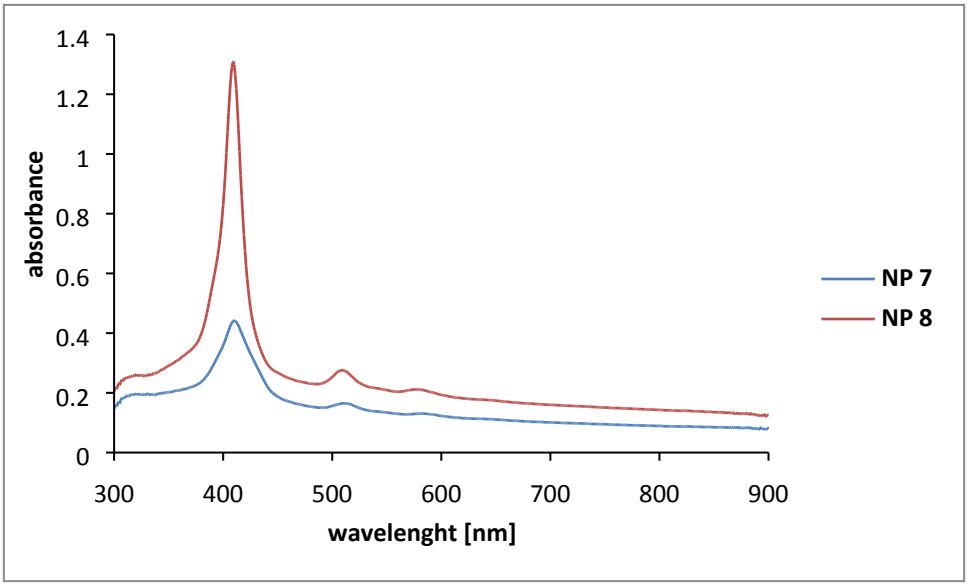


Figure SI 12 UV-Vis spectra of **NP 7** and **NP 8**. UV-Vis absorption spectra were collected after dispersion of 0.535 mg of **NP 7** in 3mL of distilled water and 0.510 mg of **NP 8** in 3 mL of distilled water.

FT-IR

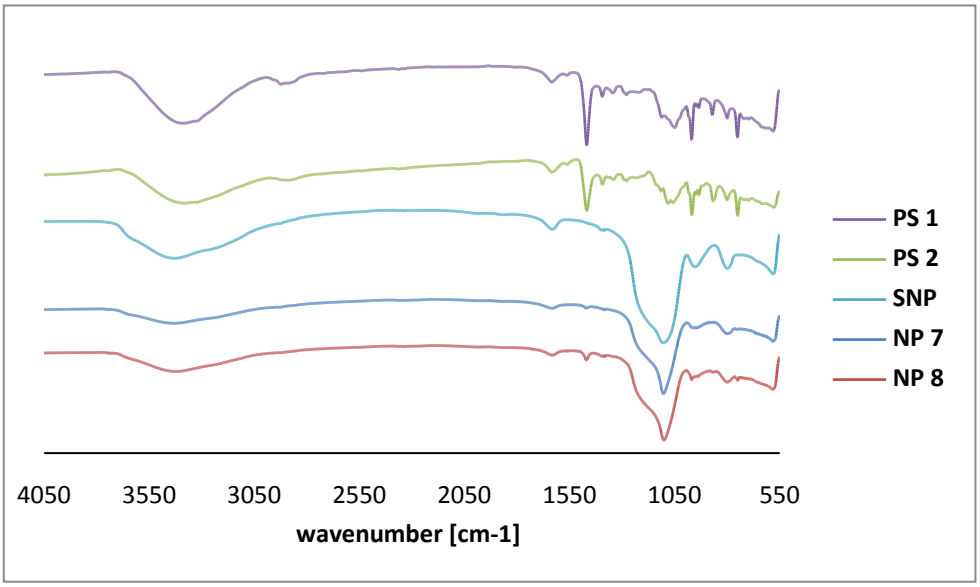


Figure SI 13 FT-IR spectra of **PS 1**, **PS 2**, **NP 7**, **NP 8** and **SNP**. FT-IR spectra were recorded in KBr pellets.

In vitro assays

Cells culture

Human bladder cancer cell lines UM-UC-3 and HT-1376 derived from high-grade transitional cell carcinoma were obtained from the American Type Culture Collection (ATCC®, Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma) supplemented with 2 $g.L^{-1}$ sodium bicarbonate (Sigma), 2 mM L-glutamine (Sigma), 10% (v/v) of heat-inactivated Fetal Bovine Serum (FBS; Life Technologies, Carlsbad, CA, USA) and antibiotic/antimicotic containing 100 units.mL-1 penicillin, 100 µg.mL-1 streptomycin and 0.25 µg.mL-1 amphotericin B (Sigma). UM-UC-3 and HT-1376 cells were seeded at a density of 1.5 x 104 in 96-well culture plates (Orange Scientific, Braine-l'Alleud, Belgium). 24 hours after plating, cells were overnight incubated with different concentrations of NPs (0-0.010 mg/mL in medium) in the dark.

Cellular uptake of NPs

After incubation with NPs in the dark, UM-UC-3 and HT-1376 cells were washed with PBS buffer and mechanically scrapped in 1% (m/v) sodium dodecyl sulfate (SDS; Sigma) in PBS buffer at pH 7.0. NPs intracellular concentration was determined by spectrofluorimetry using a microplate reader (Synergy HT, Biotek, Winooski, VT, USA) with the excitation filter (set at 360±40 nm) and emission filter (645±40 nm). Results were normalized for protein concentration (determined by bicinchoninic acid reagent; Pierce, Rockford, IL, USA).

Microscopic evaluation

UM-UC-3 and HT-1376 bladder cancer cells were left to grown on coated glass coverslips with poly-L-lysine (Sigma). After 24 h cells were incubated with 0.010 mg/mL of NPs overnight, at 37 ºC. Then, cells were fixed with 4% paraformaldehyde (PFA; Merck, Darmstadt, Germany) for 10 min at RT. In the end, the samples were rinsed in PBS, and mounted in VectaSHIELD mounting medium containing 4´,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, CA, Burlingame) for visualization under a confocal microscope (LSM 510, Carl Zeiss, Gottingen, Germany). For DAPI detection, specimen was excited at 405 nm and light emitted was collected between 430-500 nm.

PDT treatments on cells

Photodynamic irradiation was carried out in fresh culture medium, in the absence of NPs, covering UM-UC-3 and HT-1376 cell monolayers with RPMI medium and exposing them to white light delivered by the illumination system LC-122 LumaCare at 12 mW/cm^{-2} for 40 min. As a control, sham-irradiated cells were used. These cells were kept in the dark for the same durations and under the same conditions as the irradiated cells. In all trials, triplicate wells were settled under each experimental condition, and each experiment was repeated at least three times.

MTT assay

MTT assay was used to determine cell metabolic activity after NPs incubation in the dark, irradiation, or both after 24 h. This colorimetric assay is measuring the ability of bladder cancer cells to reduce yellow 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT, Sigma), to a purple formazan on a microplate reader (Synergy HT). The results are expressed in percentage of control (i.e. optical density of formazan from cells not exposed to NPs).

Redox quenching studies

Photodynamic treatment was performed with cell monolayers covered with culture medium containing 50 nM of redox quenchers (sodium azide, L-histidine and L-cysteine from Sigma) just after NPs uptake. 24 h after PDT, the effect of quenchers on cell viability was evaluated using MTT viability assay.

PSs release form NPs in the biological media

In 2 mL eppendorf, 0.5 mg of **NP 7** (bearing 0.145 µmol of **PS 1**) or 0.5 mg of **NP 8** (bearing 0.108 µmol of **PS 2**) were dispersed in 1 mL of PBS buffer or RPMI medium. The dispersion was placed in laboratory incubator shaker (IKA KS4000) and kept at 37 °C without agitation for 4 h and 18 h. After 4 h and 18 h, 0.250 mL of the mixture was mixed with 1.250 mL of EtOH and nanoparticles were centrifuged (13300 RPM, RT, 1 min). Then, 1 mL of supernatant was mixed with 2 mL of EtOH and UV-Vis was measured. The release of PS form NPs was calculated as follows: *release of PS form NPs = mol of release PS from NPs / mol of PS in NPs.*

Compering the behavior of nanoparticles after 4 h and 18 h incubations, slow release of PS form the silica matrix was observed. Higher release in case of **NP 8** was noted then in **NP 7**. However, during singlet oxygen study nanoparticles were stable under conditions used in the study (**Figure SI 3** and **Figure SI 4**).

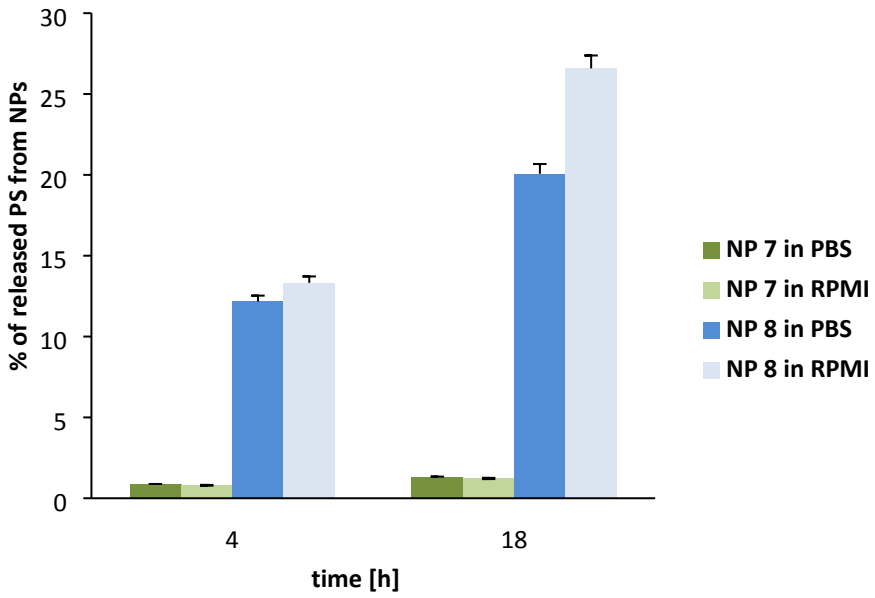


Figure SI 14 PSs release form **NP 7** and **NP 8** in the biological media. Each point represents the mean of at least three independent experiments, and has a standard deviation lower than 3%.

Dark toxicity studies of PSs and NPs

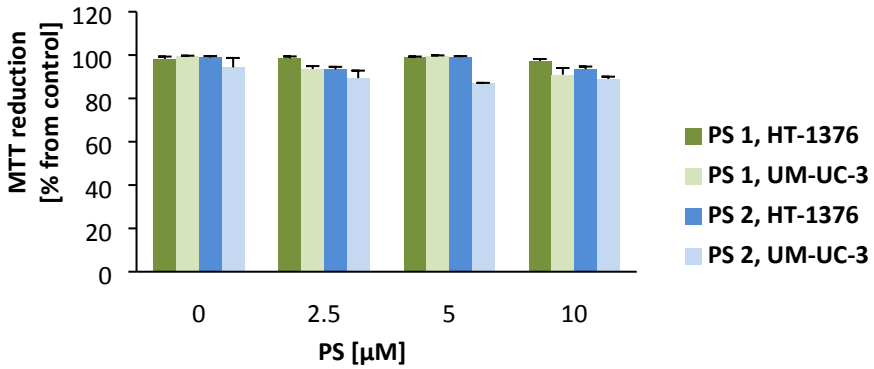


Figure SI 15 Non-dark toxicity of **PS 1** and **PS 2** (0-10 µM in PBS) determined 24 h after treatment using the MTT assay. The percentage of cytotoxicity was calculated relatively to control cells (cells incubated with PBS). Data are means ± s.e.m. of at least three independent experiments performed in triplicates.

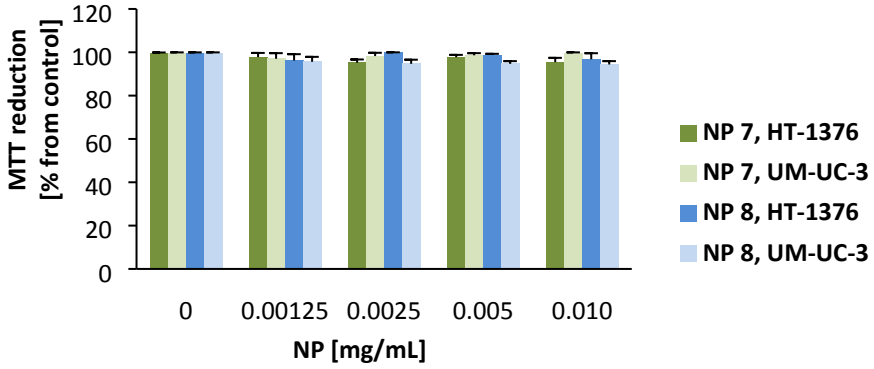


Figure SI 16 Non-dark toxicity of **NP 7** (0-0.010 mg/mL, 0-2.89 µM in medium) and **NP 8** (0-0.010 mg/mL, 0-2.15 µM in medium) determined 24 h after treatment using the MTT assay. The percentage of cytotoxicity was calculated relatively to control cells (cells incubated with RPMI medium). Data are means ± s.e.m. of at least three independent experiments performed in triplicates.

Data analysis

Statistical data analysis was carried out using Excel's Analysis ToolPak (Student's t test). The values are represented as mean ± s.e.m. Level of significance was set at values $*P<0.05$, $**P<0.01$ and $***P<0.001$.