SUPPORTING INFORMATION

Synthesis of Disulfide-Based Biodegradable Bridged Silsesquioxane Nanoparticles for Two-Photon Imaging and Therapy of Cancer Cells

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I- EXPERIMENTAL SECTION

Materials. Cetyltrimethylammonium bromide (CTAB) and sodium hydroxide were purchased from Sigma-Aldrich. Absolute ethanol was purchased from Fisher Chemicals. R. Norma Pure. Bis(3-triethoxysilylpropyl)disulfide were purchased from ABCR. (3-azidopropyl)-trimethoxysilane was obtained from a reported procedure by M. Ortega-Muñoz et al (*Adv. Synth. Catal*. **2006**, 348, 2410).

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC 400 spectrometer. Chemical shifts (in δ units, ppm) are referenced to TMS using $CHCl_3$ (δ = 7.26 ppm) and $CDCl_3$ (δ = 77.0 ppm) as the internal standards, respectively, for ^{1}H and ^{13}C NMR spectra. IR spectra were recorded on a Perkin-Elmer 100 FT spectrophotometer. Absorption spectra were recorded on a Hewlett-Packard 8453 spectrophotometer and fluorescence data were collected on a Perkin-Elmer LS55 fluorimeter. Mass spectrometry was carried out at the Laboratoire de Spectrometrie de Masse (Lyon, France) with a Thermo-Finnigan MAT95 apparatus in electronic impact ionization mode. Dynamic light scattering analyses were performed using a Cordouan Technologies DL 135 Particle size analyzer instrument. ^{29}Si and ^{13}C CPMAS solid state NMR sequences were recorded with a VARIAN VNMRS300, using Q8MH8 and adamantane references respectively. TEM analysis performed on a JEOL 1200 EXII instrument. SEM analysis performed on a FEI Quanta FEG 200 instrument.

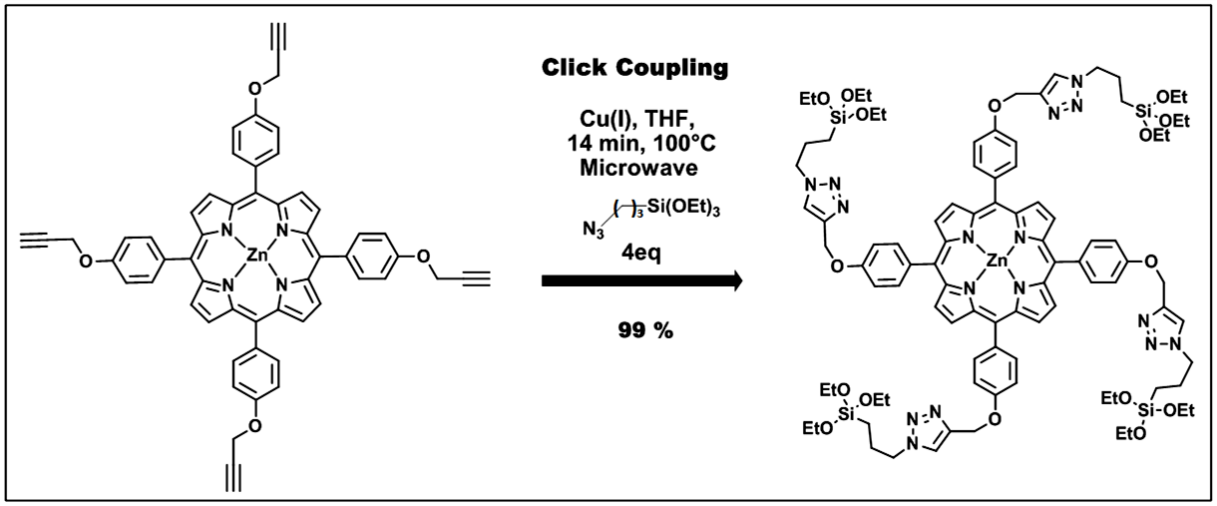
II- PORPHYRIN PRECURSOR SYNTHESIS AND CHARACTERIZATIONS

Propargylation of 5,10,15,20-tetrakis(4-hydroxyphenyl)-porphyrin. 5,10,15,20-Tetrakis(4-hydroxyphenyl)-porphyrin (1.0 g, 1.47 mmol) and propargyl bromide (1.2 mL, 37 eq, 53.6 mmoles) were dissolved in dry N,N-dimethylformamide (DMF) (30 ml) in the presence of potassium carbonate (965 g, 32 eq). The resulting solution was stirred at ambient temperature under an inert atmosphere for night. Then methylene chloride (100 ml) was added and the organic phase was washed with water (2x100 ml), dried with sodium sulfate, filltered and concentrated under vacuum. The residual solution was chromatographed over a silica-gel column

and eluted with CH_2Cl_2 to give titled compound (1.06 g, 87% yield). UV-visible (CH_2Cl_2): λ_{max} (nm) ($\varepsilon\, 10^{-3}$, $mol^{-1}\ dm^3\ cm^{-1}$) 420.5 (482), 56.5 (18), 554 (13.6), 593 (7.5), 648.5 (7.8). ^{1}H NMR (300 MHz, $CDCl_3$) δ -2.77 (s, 2H, NH), 2.69 (s, 4H, H alcyne), 4.96 (s, 8H, CH_2), 7.34 (d, 8H, J = 8.5 Hz, *m*-phenyl), 8.13 (d, 8H, J = 8.5 Hz, *o*-phenyl), 8.86 (s, 8H, pyrrole). ^{13}C NMR (300 MHz, $CDCl_3$) δ 56.35, 76.08, 78.87, 113.31, 119.79, 135.78, 157.64.

5,10,15,20-tetrakis(4-hydroxyphenyl)-porphyrin zinc complex. UV-visible (CH_2Cl_2): λ_{max} (nm) ($\varepsilon\, 10^{-3}$, $mol^{-1}\ dm^3\ cm^{-1}$) 423 (458.2), 551.5 (17.9), 593 (7.7). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (s, 4H, H alcyne), 4.96 (s, 8H, CH_2), 7.34 (d, 8H, J = 8.5 Hz, *m*-phenyl), 8.11 (d, 8H, J = 8.5 Hz, *o*-phenyl), 8.95 (s, 8H, pyrrole).

POR Precursor. A mixture of the tetrapropargyled porphyrine derivative (100 mg, $1.12*10^{-1}$ mmol), bromotris(triphenylphosphine)copper(I) ([$CuBr(PPh_3)_3$], 10 mg, $1*10^{-2}$ mmol), and anhydrous THF (3 mL) was placed in a 10 mL microwave sealable reactor, and (3-azidopropyl)triethoxysilane (150 mg, 0.6 mmol) was added. Then, the tube was flushed with argon and the microwave process was conducted 20 min at 100°C at 200 mW. After evaporation of the solvents, the POR precursor was quantitatively obtained as a purple solid (210 mg, $1.12*10^{-1}$ mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.81 (s, 8H, $\underline{H_{pyrrole}}$), 8.41 (s, 4H, triazole), 88.09 (d, ^{3}J = 8.4 Hz, 8H, H *o*-phenyl), 7.44 (d, ^{3}J = 8.2 Hz, 8H, H *m*-phenyl), 5.43 (s, 8H, N-$\underline{CH_2}$-riazole), 4.45 (t, ^{3}J = 7.0 Hz, 8H, triazole-$\underline{CH_2}$), 3.77 (q, ^{3}J = 7 Hz, 24H, O-$\underline{CH_2}$-CH_3), 1.99 (t, ^{3}J = 7.8 Hz, 8H, triazole-CH_2-$\underline{CH_2}$), 1.16 (t, ^{3}J = 6.9 Hz, 36H, O-CH_2-$\underline{CH_3}$), 0.60 (t, ^{3}J = 7.7 Hz, 8H, $\underline{CH_2}$-Si).^{13}C NMR (400MHz, DMSO): δ 158.8, 149.9, 143.19, 135.5, 131.8, 129, 125, 120, 113.1, 58.2, 53.3, 52.14, 24.3, 18.5, 7.3. ^{29}Si NMR (400MHz, DMSO): δ -46.4. FTIR (neat KBr) ν_{max}/cm^{-1} = 3116, 3032, 2977, 2932, 2882, 1596, 1563, 1502, 1435, 1391, 1346, 1296, 1268, 1240, 1174, 1107, 1068, 996, 945, 840, 789, 712, 684, 533, 422. UV/Vis λ_{max} (EtOH): 426, 561, 604 nm. Emission (EtOH): λ_{max}= 607, 660 nm ($\lambda_{excitation}$= 432 nm). MALDI-TOF: calcd for $C_{92}H_{120}N_{16}O_{16}Si_4Zn$: 1880.690, found 1880.720.



Scheme S1. Design of the POR precursor via click CuAAC coupling.

III- NANOMATERIALS SYNTHESES AND CHARACTERIZATIONS

DIS NPs. A mixture of CTAB (250 mg), distilled water (120 mL), and sodium hydroxide (875 µL, 2 M) was stirred at 80°C during 50 minutes at 700 rpm in a 250 mL three neck round bottom flask. Then, bis(3-triethoxysilylpropyl)disulfide (2.4 mL) was added to the aforementioned solution, and the condensation process was conducted for 2 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 krpm. The sample was sonicated twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$), and washed three times with ethanol, water, and ethanol. Each washing was followed by centrifugation collection of the sample in the same manner. The as-prepared material was dried under vacuum for few hours.

DIS2 NPs. A mixture of CTAB (250 mg), distilled water (120 mL), and sodium hydroxide (875 µL, 2 M) was stirred at 80°C during 50 minutes at 700 rpm in a 250 mL three neck round bottom flask. Then, bis(3-triethoxysilylpropyl)disulfide (2.4 mL) was added to the aforementioned solution along with the two-photon electron donor (177 mg in 1 mL of absolute ethanol), and the condensation process was conducted for 2 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 krpm. The sample was sonicated twice with an alcoholic

solution of ammonium nitrate (6 $g.L^{-1}$), and washed three times with ethanol, water, and ethanol. Each washing was followed by centrifugation collection of the sample in the same manner. The as-prepared material was dried under vacuum for few hours.

200 nm DISP NPs. A mixture of CTAB (125 mg), distilled water (60 mL), and sodium hydroxide (437 µL, 2 M) was stirred at 80°C during 50 minutes at 700 rpm in a 250 mL three neck round bottom flask. Then, bis(3-triethoxysilylpropyl)disulfide (400 µL) was added to the aforementioned solution along with the porphyrin precursor POR (20 mg in 800 µL of absolute ethanol), and the condensation process was conducted for 2 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 krpm. The sample was sonicated twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$), and washed three times with ethanol, water, and ethanol. Each washing was followed by centrifugation collection of the sample in the same manner. The as-prepared material was dried under vacuum for few hours.

50 nm DISP NPs. A mixture of CTAB (125 mg), distilled water (60 mL), and sodium hydroxide (437 µL, 2 M) was stirred at 80°C during 50 minutes at 700 rpm in a 250 mL three neck round bottom flask. Then, bis(3-triethoxysilylpropyl)disulfide (300 µL) was added to the aforementioned solution along with the porphyrin precursor POR (20 mg in 800 µL of absolute ethanol), and the condensation process was conducted for 2 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 krpm. The sample was sonicated twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$), and washed three times with ethanol, water, and ethanol. Each washing was followed by centrifugation collection of the sample in the same manner. The as-prepared material was dried under vacuum for few hours.

Biodegradability studies. A mixture of NPs (1.5 mg), PBS (500 µL), and mercaptoethanol (50 µL) was stirred two days at 37°C in an eppendorf tube. Then, aliquots were taken directly to perform the TEM and DLS analyses.

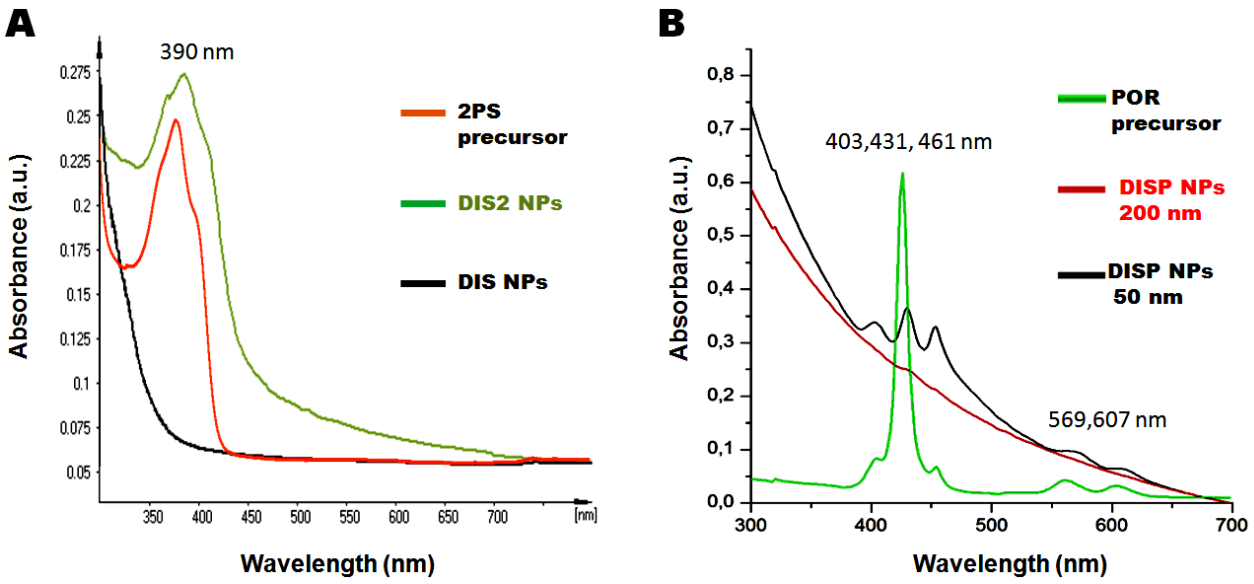


Figure S1. UV-Visible spectra of the 2PS precursor, DIS, and DIS2 NPs (A), and of the POR precursor, 50 nm DISP and 200 nm DISP NPs (B), demonstrating the incorporations of both the 2PS and POR photosensitizers.

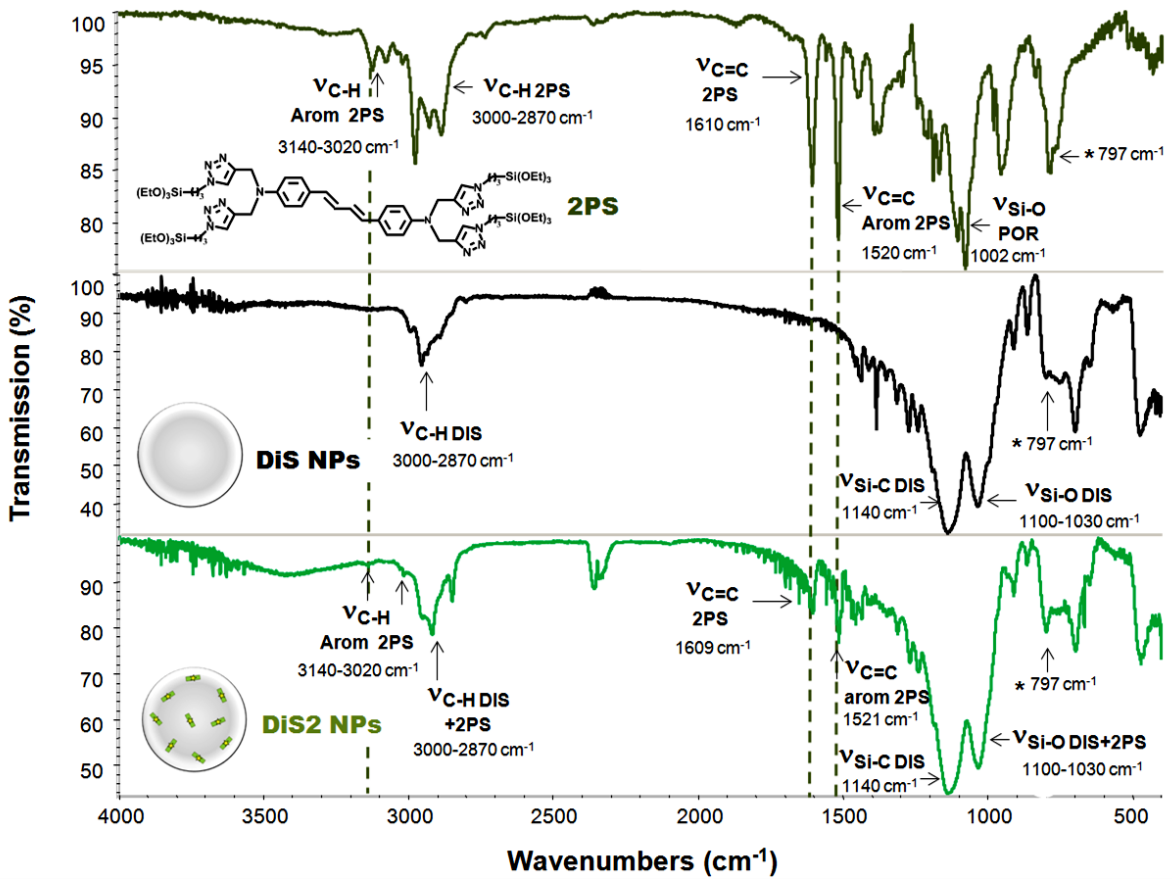


Figure S2. FTIR spectra of the 2PS precursor, DIS, and DIS2 NPs confirming the presence of the DIS and 2PS moieties in the nanomaterials framework. *Out of plan bending of para-disubstituted aromatic rings in the 2PS.

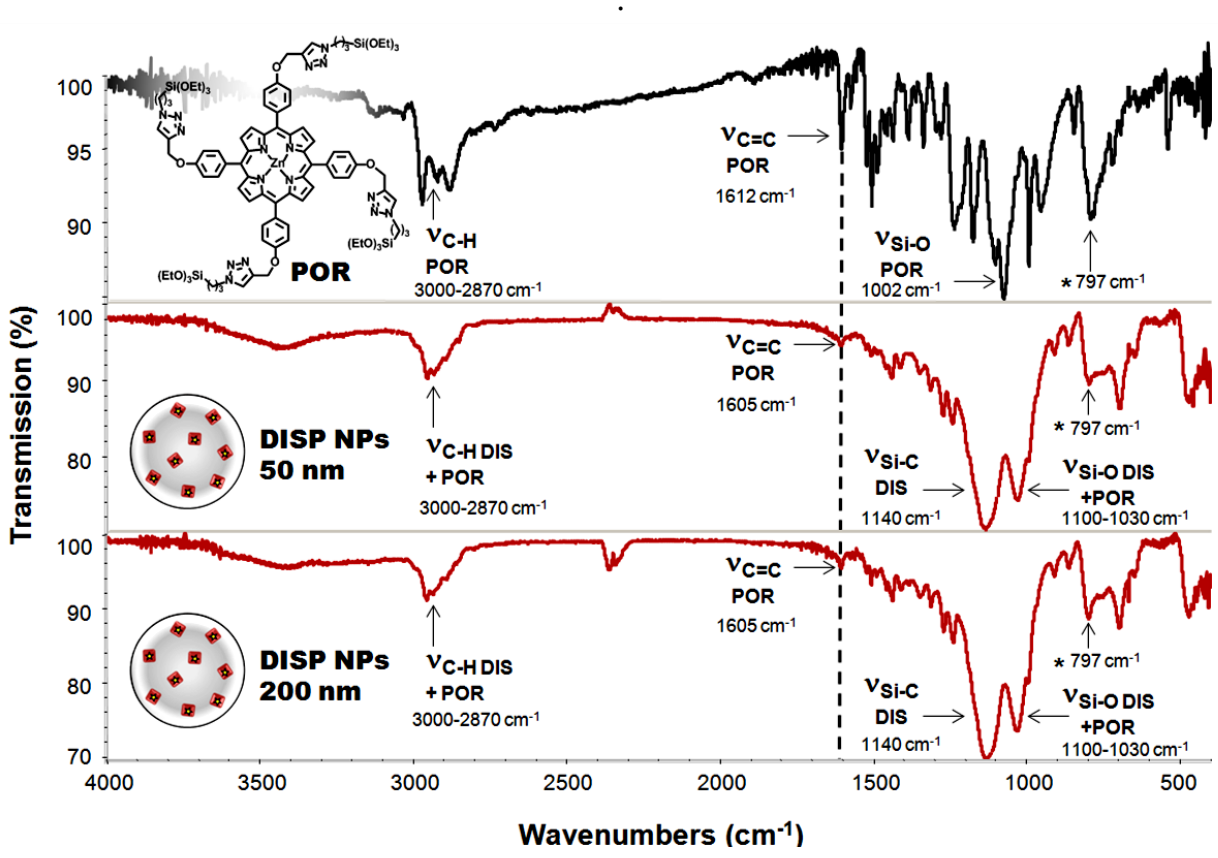


Figure S3. FTIR spectra of the POR precursor, DIS, and DISP NPs confirming the presence of the DIS and POR moieties in the nanomaterials framework.*Out of plan bending of para-disubstituted aromatic rings in the POR.

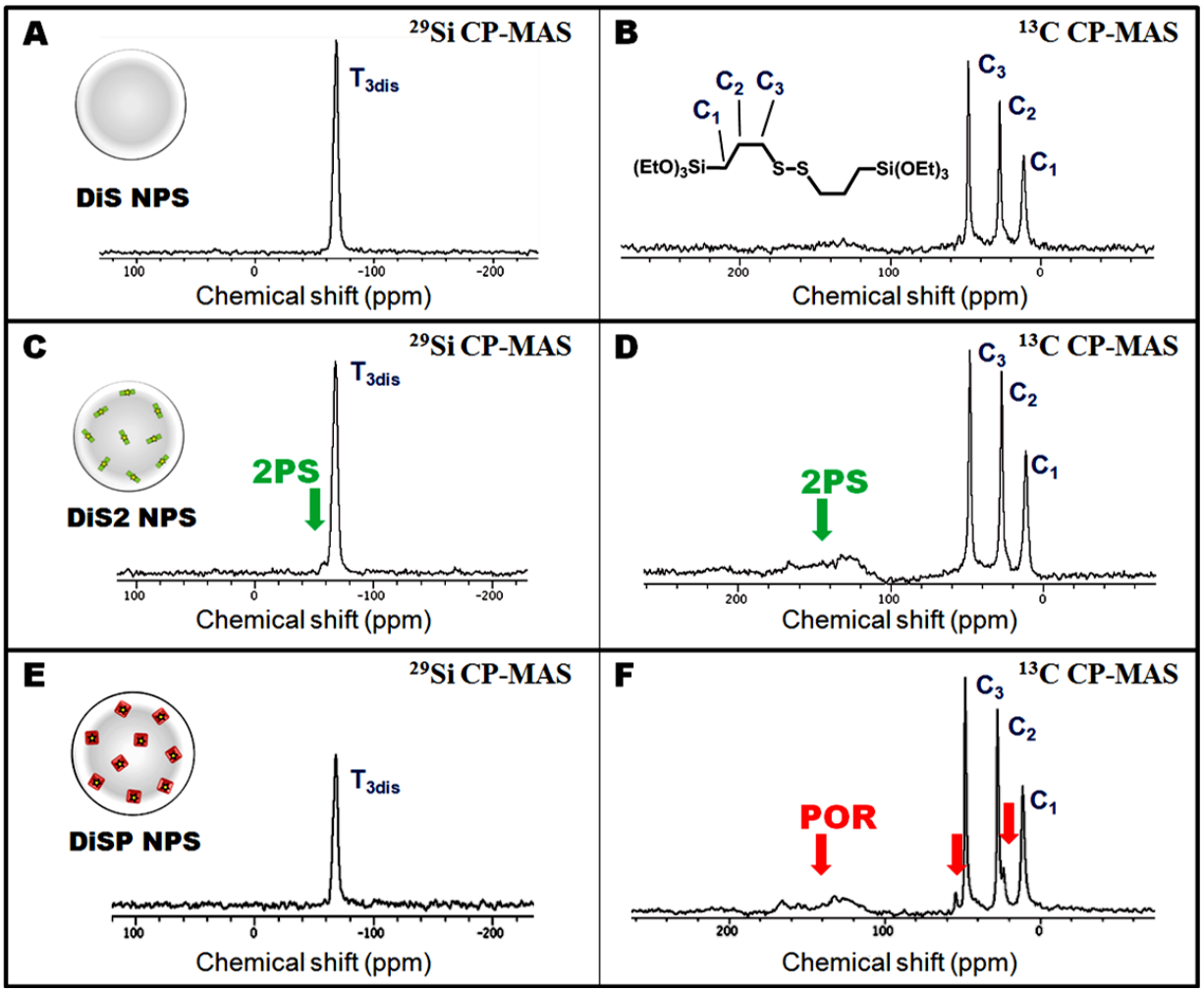


Figure S4. Solid state NMR ^{29}Si and ^{13}C CPMAS spectra of DIS (A-B), DIS2 (C-D), and DISP NPs (E-F).

Table S1. Photosensitizers weight percent determination in the NPs.

Sample	**NPs N wt% [a]**	**NPs 2PS or POR wt% [b]**
DIS2 NPs	5.88	28
50 nm DISP NPs	1.62	10
200 nm DISP NPs	2.25	14

[a] Elemental analysis by combustion measurements of the NPs.
[b] Determination based on the nitrogen wt% in the 2PS and POR molecules.

IV-CHARACTERIZATION OF THE DEGRADED NANOMATERIALS

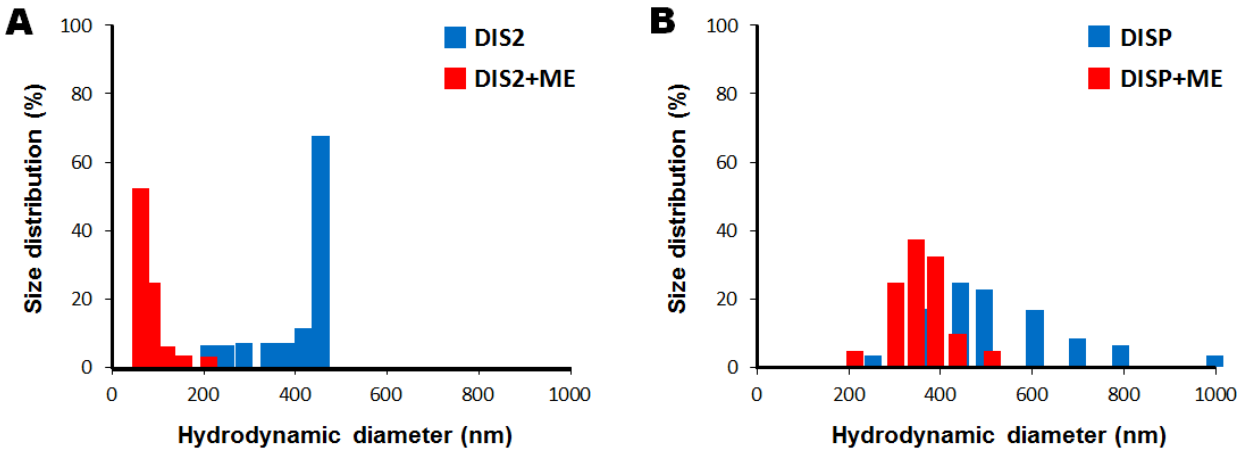


Figure S5. DLS size distributions of DIS2 (A) and DISP NPs (B) before and after mercaptoethanol addition in near-physiological conditions for 48 h, consistent with the expected decrease of particle sizes. The initial large sizes of BS NPs arise from: (a) aggregates of NPs, and (b) the hydration layer around particles.

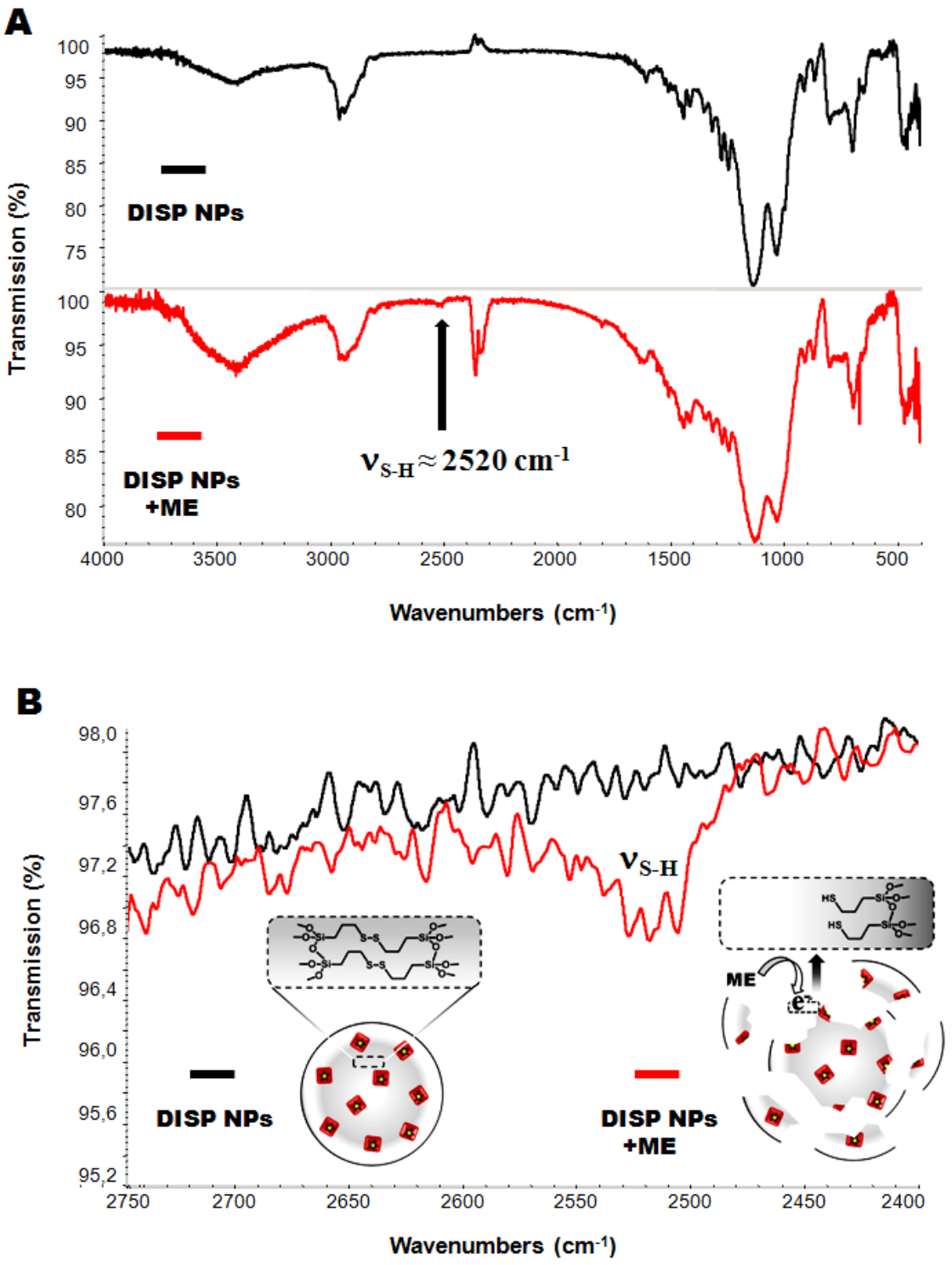


Figure S6. FTIR spectra of DISP NPs before and after mercaptoethanol addition (A), confirming the degradation of the disulfide framework with the appearance of the thiol stretching band (B).

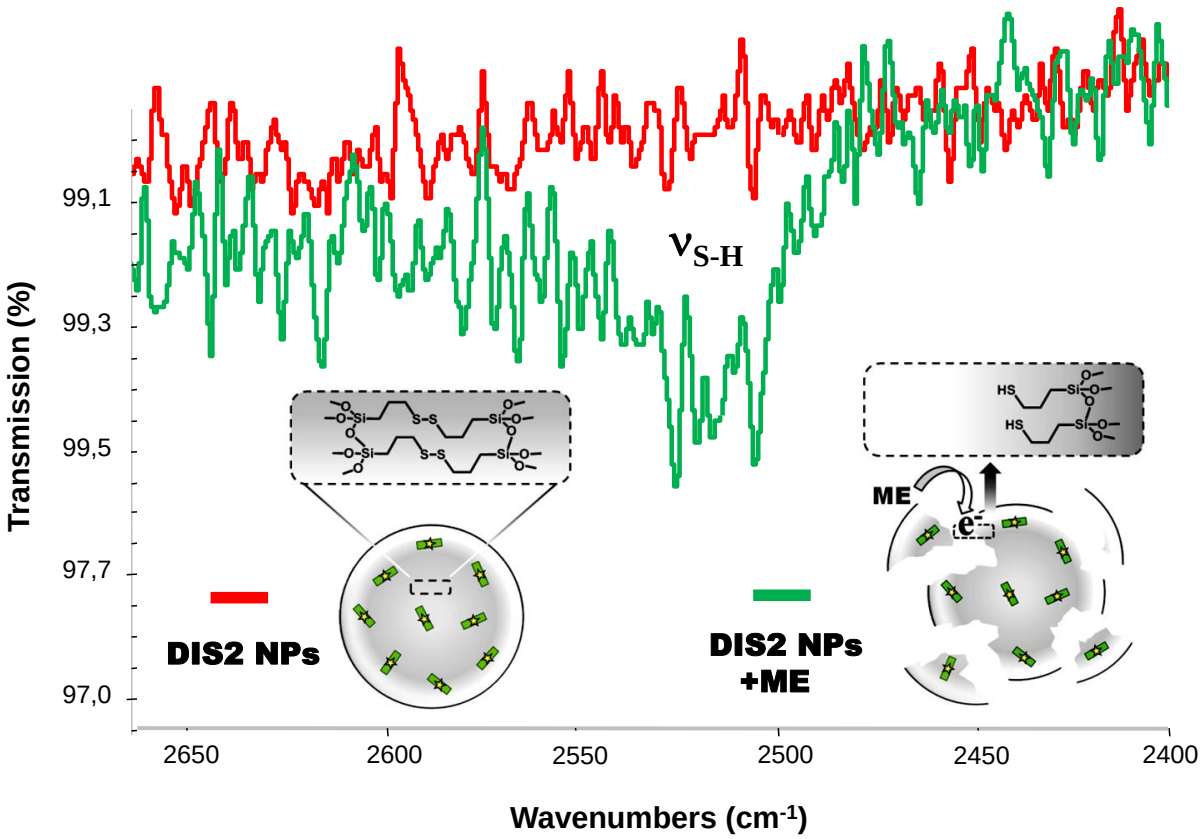


Figure S7. FTIR spectra from 2650 to 2400 cm^{-1} of DIS2 NPs before and after mercaptoethanol addition confirming the degradation of the disulfide framework with the appearance of the thiol stretching band (B).

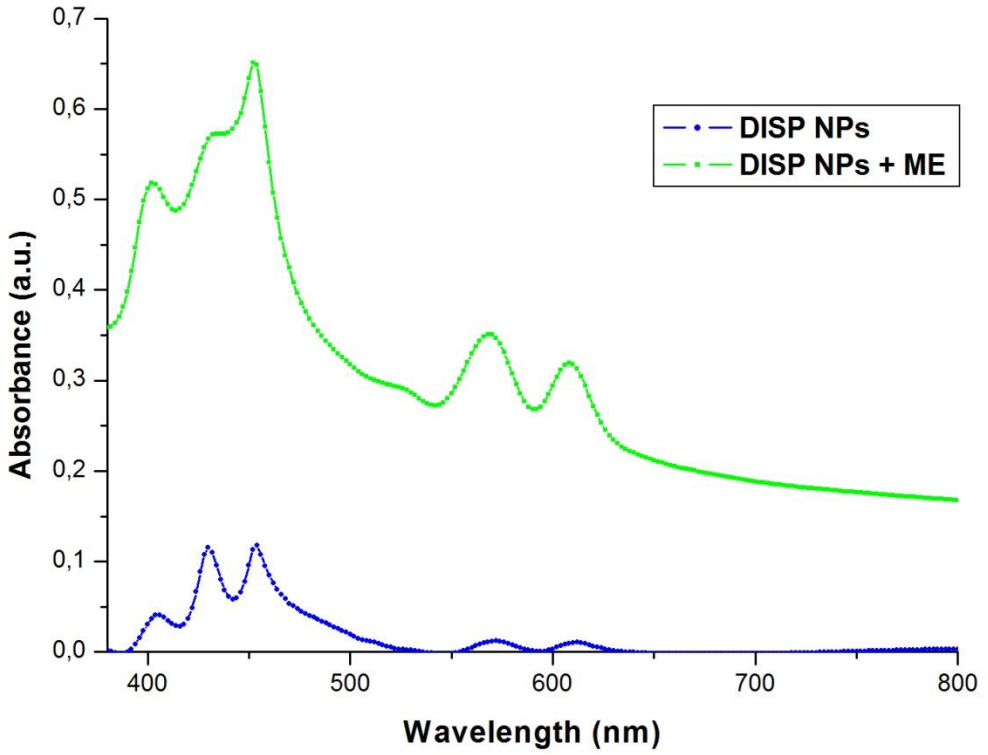


Figure S8. UV-Vis spectra of DISP NPs before and after mercaptoethanol treatment More J aggregates are seen.

V- TWO-PHOTON IN-VITRO STUDIES

Two-photon fluorescence imaging. The day prior to the experiment, MCF-7 cells were seeded onto bottom glass dishes (World Precision Instrument, Stevenage, UK) at a density of 10^6 cells.cm^{-2}. Adherent cells were then washed once and incubated in 1 mL culture medium containing NPs at a concentration of 80 µg.mL^{-1} for 20 h. Fifteen min before the end of incubation, cells were loaded with Cell Mask^{TM} Orange Plasma Membrane Stains (Invitrogen, Cergy Pontoise, France) for membrane staining at a final concentration of 5 µg.mL^{-1}. Before visualization, cells were washed gently with phenol red-free DMEM. Cells were then scanned

with a LSM 780 LIVE confocal microscope (Carl Zeiss, Le Pecq, France), at 760 or 800 nm with a slice depth (Z stack) of 0.62 µm.

TPE-therapy. MCF-7 human breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 50 $\mu g.mL^{-1}$ gentamycin. All cells were allowed to grow in humidified atmosphere at 37°C under 5% CO_2. For *in vitro* phototoxicity, MCF-7 cells were seeded into a 384 multiwell glass-bottomed plate (thickness 0.17 mm), with a black polystyrene frame, 2000 cells per well in 50 µL of culture medium, and allowed to grow for 24 h. NPs were then dispersed under ultrasounds in PBS at a concentration of 1 $mg.mL^{-1}$ and cells were then incubated for 20 h with or without nanoparticles at a final concentration of 50 $\mu g.mL^{-1}$ in supplemented DMEM. After incubation with NPs, cells were washed twice, maintained in fresh culture medium, and then submitted (or not) to laser irradiation; with the Carl Zeiss Microscope LSM 780 LIVE confocal microscope (laser power input 3W). Half of the well was irradiated at 760 or 800 nm by three scans of 1.57 s duration in 4 different areas of the well. The laser beam was focused by a microscope objective lens (Carl Zeiss 10x , NA 0.4). After 2 days, the MTS assay was performed and was corrected.

Cytotoxicity studies. MCF-7 cells were incubated with increasing concentrations (from 2.5 to 125 $\mu g.mL^{-1}$) of DIS2 or DISP NPs. Three days after treatment, a MTT test was performed to quantified the toxicity of NPs in absence of photo-stimulation"

