Supporting Information

for

Porphyrin- or phthalocyanine- bridged silsesquioxane theranostic nanoparticles for two-photon imaging, photodynamic therapy or photoacoustic imaging

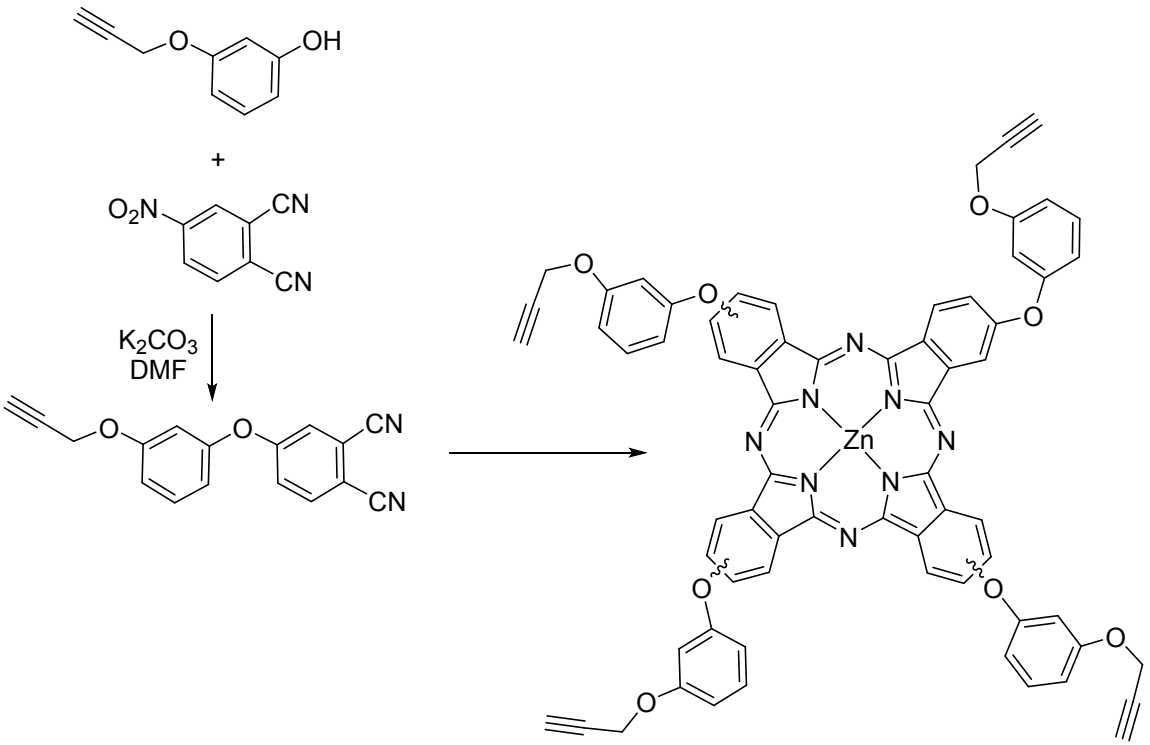
Materials and procedures

Cetyltrimethylammonium bromide (CTAB) and sodium hydroxide were purchased from Sigma-Aldrich. Absolute ethanol was purchased from Fisher Chemicals. R. Norma Pure. (3-azidopropyl)-trimethoxysilane was obtained from a reported procedure by Malvi B, Sarkar BR, Pati D, Mathew R, Ajithkumar TG, Sen Gupta S. "Clickable'" SBA-15 mesoporous materials: synthesis, characterization and their reaction with alkynes.[1] ^{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. Mass spectrometry was carried out at the Laboratoire de Mesures Physiques (Montpellier, France) with a Thermo-Finnigan MAT95 apparatus in MALDI-TOF ionization mode. Dynamic light scattering analysiswere performed using a Cordouan Technologies DL 135 Particle size analyzer instrument. Zeta potential was measured on a Malvern Zetasizer NanoSeries.. ^{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.

4-nitrophthalonitrile (.) was prepared as reported.[2] FT-IR spectra of the neat compounds were recorded using a Perkin Elmer Spectrum 100 FT-IR spectrometer. The mass spectra were recorded on a MALDI (matrix assisted laser desorption ionization) BRUKER Microflex LT. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 500 MHz spectrometer.

Nanomaterial synthesis

Resorcinol was first monopropargylated and the resulting phenol derivative was engaged in a nucleophilic substitution on 4-nitrophthalonitrile. The resulting propargylated phthalonitrile was engaged in a cyclotetramerisation reaction in the presence of $Zn(OAc)_2$, the reaction duration being short too avoid degradation or polymerisation of the alkyne moieties.



Scheme S1. Synthesis of tetrapropargylated phthalocyanine PS2

Synthesis of 3-(prop-2-yn-1-yloxy)phenol. Propargyl bromide (62 mmol, 5.6 mL) was added drop by drop to a mixture of resorcinol (25 g, 220 mmol) and dried potassium carbonate (650 mmol, 90 g) in acetone (200 mL). The reaction mixture was refluxed 24 h, filtered and cocnentated. The desired compound was purified on a silica gel column chromatography. Brownish oil (20%, 6.68 g). $C_9H_8O_2$, MW: 148.2 g/mol. FT-IR (cm^{-1}): 3287, 2123, 1594, 1489, 1457, 1372, 1310, 1279, 1222, 1171, 1160, 1078, 1032, 996, 962, 942, 918, 835, 761, 730, 681. ^{1}H NMR (500 MHz, $CDCl_3$) ppm: 7.17 (1H, t, *Ar*CH), 6.58 (1H, dd, *Ar*CH), 6.49-6.52 (2H, m, *Ar*CH), 4.68 (2H, d, CH_2-O), 2.55 (1H, t, CH). ^{13}C NMR (125 MHz, $CDCl_3$) ppm: 158.72 (ArC-

O), 156.62 (ArC-O), 130.50 (ArCH), 109.24 (ArCH), 107.48 (ArCH), 102.89 (ArCH), 78.62 (C), 76.24 (CH), 56.04 (CH_2-O).

Synthesis of propargylated phthalonitrile. 4-nitrophthalonitrile (1.0 g, 5.8 mmol, 1 eq), monopropargylated resorcinol (1.2 g, 8.1 mmol, 1.4 eq.) and potassium carbonate (12.0 g, 87 mmol, 15 eq.) were dissolved in DMF (50 mL) and stirred overnight at room temperature. The reaction mixture was then poured into water and filtered. The resulting crude solid was recovered in dichloromethane, dried over Na_2SO_4, filtered and concentrated. The product was purified over silica gel column (elution gradient, from 1:1 hexane:dichloromethane to pure dichloromethane to 50:1 dichloromethane:ethanol). White solid (60%, 955 mg). $C_{17}H_{10}N_2O_2$, MW: 274.28 g/mol. MALDI-TOF-MS (*m/z*, no matrix): 274.077 $[M]^+$, 294.996 $[M+Na]^+$. FT-IR (cm^{-1}): 3290.8, 3272.5, 3084.5, 2229.9, 1586.5, 1560.9, 1479.1, 1447.1, 1415.2, 1378.5, 1282.2, 1241.3, 1171.0, 1129.1, 1031.0, 976.4, 958.0, 912.0, 882.4, 845.7, 769.8, 711.3, 687.9, 636.2, 553.0. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.73 (d, 1H), 7.38 (t, 1H), 7.30 (d, 2H), 6.94 (d, 1H), 6.71 (s, 2H), 4.71 (s, 2H), 2.56 (s, 1H). ^{13}C NMR ($CDCl_3$, δ, ppm): 161.49, 159.23, 154.51, 135.40, 131.20, 121.56, 117.65, 115.36, 114.95, 113.36, 112.76, 110.01, 109.99, 109.00, 107.71, 77.85, 76.20, 56.04. Anal. calcd. for $C_{17}H_{10}N_2O_2$; C, 74.44; H, 3.68; N, 10.21; found: C, 74.27; H, 3.74; N, 9.91%.

Synthesis of tetrapropargylated phthalocyanine. Propargylated phthalonitrile (1 g, 3.65 mmol, 1 eq.) and zinc acetate (0.67 g, 3.65 mmol, 1 eq.) in dimethylaminoethanol (5 mL) were refluxed under argon for 2 h, then the cooled reaction mixture was poured into water. The resulting precipitate was filtered, and recovered with dichloromethane/ethanol mixture. The product was purified over silica gel column (elution gradient, from dichloromethane to 10:1 dichloromethane:ethanol). Dark-blue powder as isomer mixture (23%, 243 mg). $C_{68}H_{40}N_8O_8Zn$, MW: 1162.50 g/mol. MALDI-TOF-MS (*m/z*, matrix: DHB): 1162.883 $[M]^+$. FT-IR (cm^{-1}): 3284.0, 2928.3, 2120.2, 1588.8, 1475.6, 1394.0, 1361.4, 1335.5, 1311.2, 1259.6, 1215.8, 1170.5, 1088.0, 1034.6, 997.9, 954.4, 841.8, 776.3, 746.3, 684.6, 644.2. ^{1}H NMR (DMSO-d_6, δ, ppm): 9.05 (m, 2H), 8.55 (m, 2H), 7.84-6.78 (m, 26 H), 4.92-4.83 (m, 8H, OCH_2), 3.65-3.57 (m, 4H, OCH_2CH). UV-vis (λ, nm, log ε): THF, 675 (4.82)

Silylation of PS1 precursor

A mixture of the tetrapropargyled porphyrin derivative [26] PS1 (100 mg, 11.2 μmol), bromotris(triphenylphosphine)copper(I) ([CuBr(PPh_3)$_3$], 10 mg, 10 μmol), and anhydrous THF (3 mL) was placed in a 10 mL microwave sealable reactor, and (3-azidopropyl)triethoxysilane (110 mg, 0.43 mmol) was added. Then, the tube was flushed with argon and the microwave process was conducted for 20 min at 100 °C (maximum power 200 W). After evaporation of the solvents, the POR precursor was quantitatively obtained as a purple solid (210 mg, 11.2 μmol). ^{1}H NMR (300 MHz, DMSO-*d_6*): δ (ppm) 8.81 (s, 8H, $\underline{H}_{pyrrole}$), 8.41 (s, 4H, triazole), 8.09 (d, ^{3}J = 8.4 Hz, 8H, $\underline{H}_{Ph}$-pyrrole), 7.44 (d, ^{3}J = 8.2 Hz, 8H, $\underline{H}_{Ph}$-triazole), 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 = 7.0 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 (400 MHz, DMSO-*d_6*): δ (ppm) 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-*d_6*): δ (ppm) -46.4. FTIR (neat KBr) (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} (normalized intensity) (EtOH): 426 (1), 561 (0.065), 604 (0.045) 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.

Silylation of PS2 precursor

A mixture of the tetrapropargyled phthalocyanine derivative PS2 (85 mg, 7.3*10^{-2} 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 (72.2 mg, 0.29 mmol) was added. Then, the tube was flushed with argon and the microwave process was conducted for 35 min at 100°C (maximum power 200 W). After evaporation of the solvents, the PHT precursor was quantitatively obtained as a blue solid (157 mg, 7.3* 10^{-2} mmol). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ (ppm) 8.87-7.40 (m, $\underline{H}_{aromatic}$), 5.27 (d, 8H, O-$\underline{CH_2}$-riazole), 4.32 (t, ^{3}J = 7.0 Hz, 8H, triazole-$\underline{CH_2}$), 3.66 (q, ^{3}J = 7.0 Hz, 24H, O-$\underline{CH_2}$-CH_3), 1.87 (t, ^{3}J = 7.8 Hz, 8H, triazole-CH_2-$\underline{CH_2}$), 1.07 (t, ^{3}J = 7.0 Hz, 36H, O-CH_2-$\underline{CH_3}$), 0.46 (t, ^{3}J = 7.5 Hz, 8H, $\underline{CH_2}$-Si).^{13}C NMR (400MHz, DMSO-*d_6*): δ (ppm) 160.31, 158.7, 158.4, 149.9, 139.8, 133.5, 132.5, 131.9, 131.4, 129.3, 129.0, 124.9, 124.2, 120.9, 120.6, 112.3, 111.3, 111.0, 106.9, 61.9, 58.2, 52.1, 24.3, 18.7, 7.3. ^{29}Si NMR (400MHz, DMSO-*d_6*): δ (ppm) - 46.3.

UV/Vis λ_{max} (relative intensity)(EtOH): 346 (0.43), 611 (0.20), 638 (0.25), 675 (0.75) nm. MALDI-TOF: calcd for $C_{109}H_{124}N_{20}O_{20}Si_4Zn$: 2151.98, found 2151.8.

Synthesis of BSPOR 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 750 rpm in a 250 mL three neck round bottom flask. Then, **POR** (83 mg in a solution of 500 µL of DMSO and 1 mL of absolute ethanol) was added and the stirring continued for 2 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation during 20 minutes at 20 krpm. The sample was then washed three times with a solution of NH_4NO_3 in EtOH (6g/L). Each washing was followed by sonication at 40 ºC and centrifugation collection of the sample. The sample was washed three times with EtOH and collected in the same manner. The as-prepared material was dried under vacuum for few hours. 35.2 mg of product were obtained.

Synthesis of BSPHT NPs

A mixture of CTAB (125 mg), distilled water (60 mL), and sodium hydroxide (437 µL, 2 M) was stirred at 80°C for two hours at 750 rpm in a 250 mL three neck round bottom flask. Then, **PHT** (55 mg in a solution of 2 mL of absolute ethanol) was added and the stirring continued for 24 h. Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and collected by centrifugation for 20 minutes at 20 krpm. The sample was then washed three times with a solution of NH_4NO_3 in EtOH (6g/L). Each washing was followed by sonication at 40 ºC and centrifugation collection of the sample. The sample was washed three times with EtOH and collected in the same manner. The as-prepared material was dried under vacuum for few hours. 27.6 mg of BSPHT NPs were obtained.

Pegylation of BSPHT NPs

4.2 mg of **BSPHT NPs** were suspended in 1 mL EtOH and 3 mL H_2O. 4.2 mg of (6-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-hexyl)trimethoxysilane in 1 mL EtOH were added and the

reaction stirred (500 rpm) at 50°C overnight. The nanoparticles were centrifuged (10 krpm, 7 min.) and washed with EtOH with three cycles of washing-centrifugation.

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 40 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 750 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 µg.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 40 µg.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 750 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 MTT assay was performed and was corrected.

ROS imaging

The detection of intracellular reactive oxygen production (ROS) was realized using DCFDA Cellular ROS Detection Assay Kit (abcam). For this, MCF-7 cells were seeded in a glass bottom microplate and treated with 80 µg mL^{-1} nanoparticles. After 24 h, cells were rinsed and incubated 45 min at 37 °C with DCFDA at 20 µM. Then, cells were rinsed and irradiated or not with a two-photon microscope (3 x 1.57 sec) at 800 nm. Green luminescence traduces the generation of ROS detected at 535 nm.

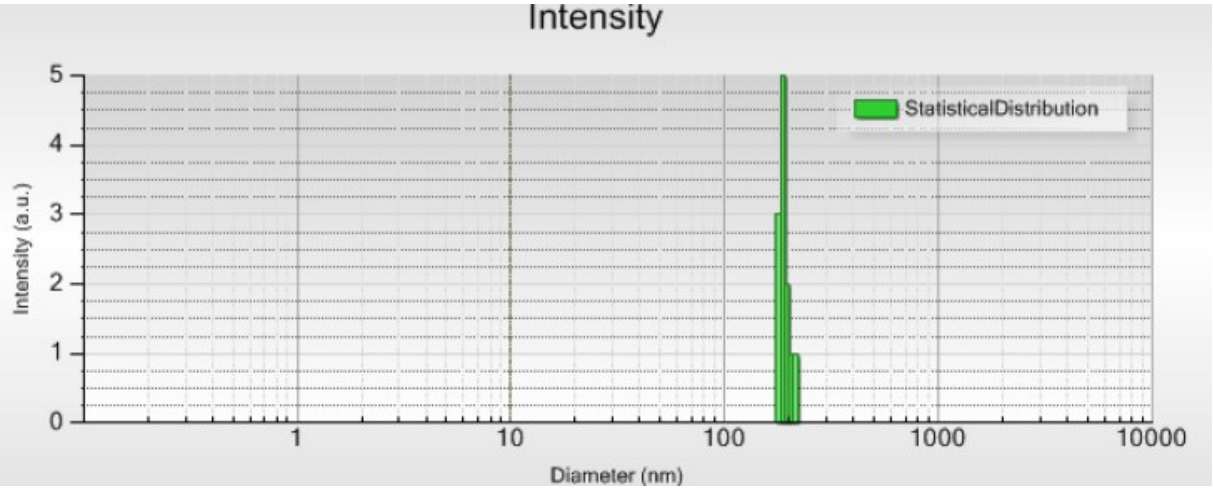
Photoacoustic imaging

PAI was performed on the VevoLAZR system (Visualsonics, Fujifilm) using the LZ201 transducer (256 elements linear array; 9-18 MHz) allowing a 100 µm axial resolution. The laser is tunable between 680 and 970 nm and acquisitions can be made continuously with 1 nm steps providing a photoacoustic spectrum.

Photoacoustic spectra of BSPHT and BSPHTPEG NPs (2 mg/mL in PBS) were first acquired in phantoms in order to evaluate their potential photoacoustic contrast and to determine the best wavelength to be used for *in vivo* imaging in mice.

Six weeks old NMRI nude mice (Janvier, Le genest saint isle, France) were anesthetized (air/isofluran 4% for induction and 2.5% thereafter) and were placed in the imaging set up. An US gel (Parker Laboratories Inc., USA) was applied on the mouse and the transducer was placed upon the carotid artery. Color Doppler imaging was performed to precisely locate the carotid and to define the Region Of Interest (ROI) to be used on the photoacoustic images for studying the blood pharmacokinetic of the NPs. Real time PAI was performed at 700 nm and started 2 min before BSPHT or BSPHTPEG NPs (200 µL at 2 mg/mL in PBS) were injected via the tail vein. The transducer was also moved alternatively to the liver and the kidney before, and 1, 3, 5 and 24 hours after the NPs injection and B mode (ultrasound) and multispectral PA (680-970 nm) was acquired simultaneously on a 30x30 mm^2 slice. The *in vivo* acquisitions were analysed by spectral unmixing using the NPs spectra obtained previously in phantoms.

A



B

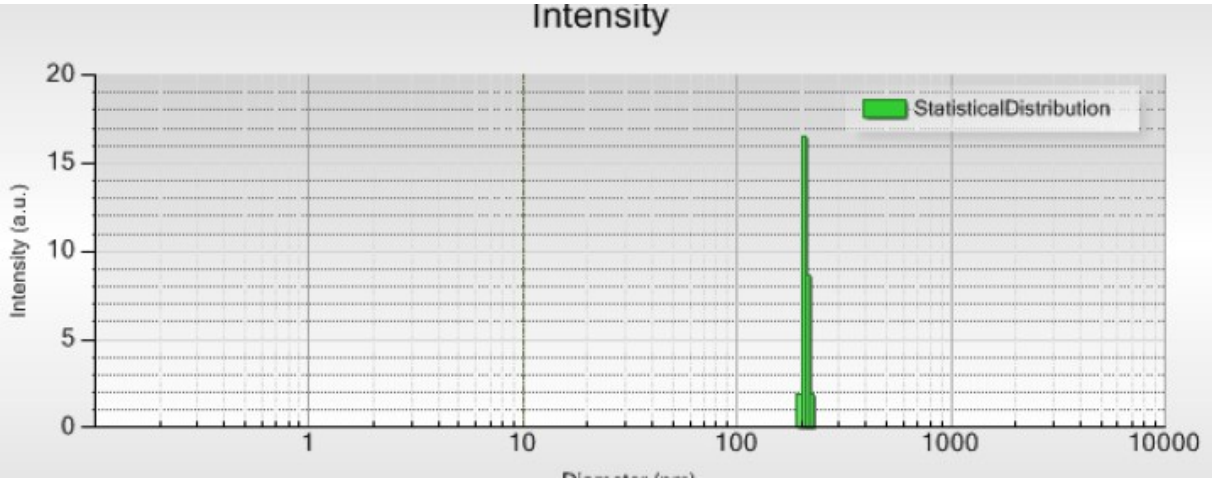


Fig. S1. Dynamic Light scattering of **BSPOR** (A) and **BSPHT** (B)

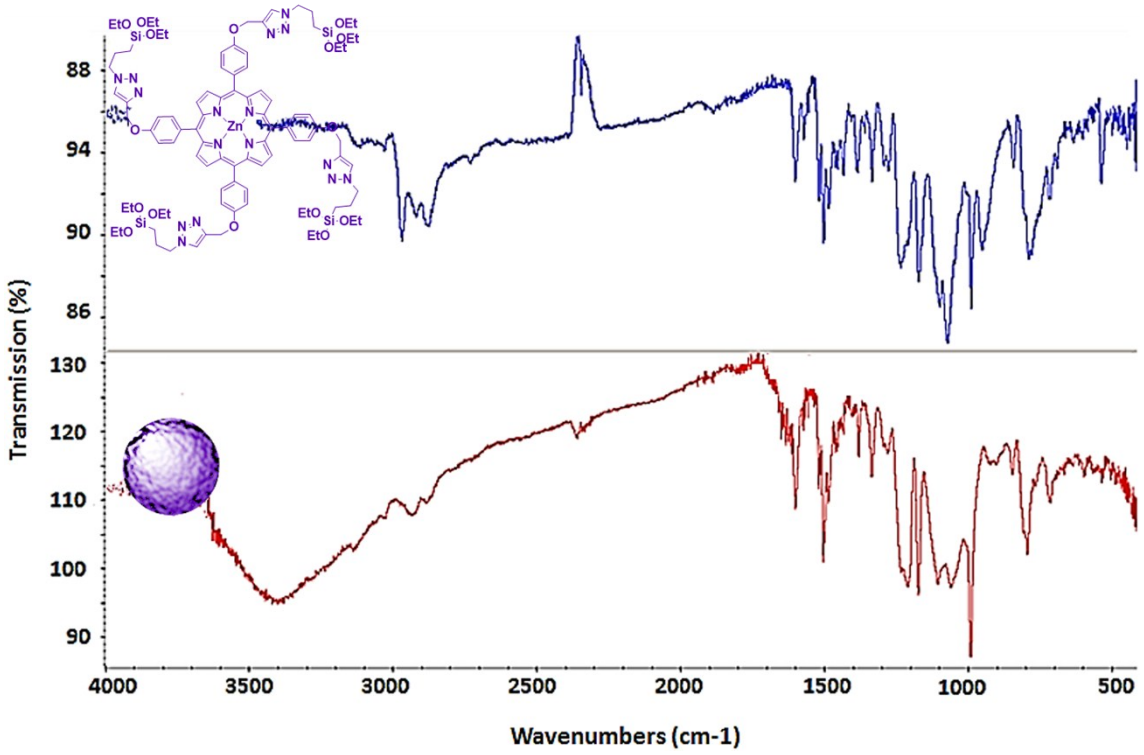


Fig. S2. FTIR spectra of POR and BSPOR NPs.

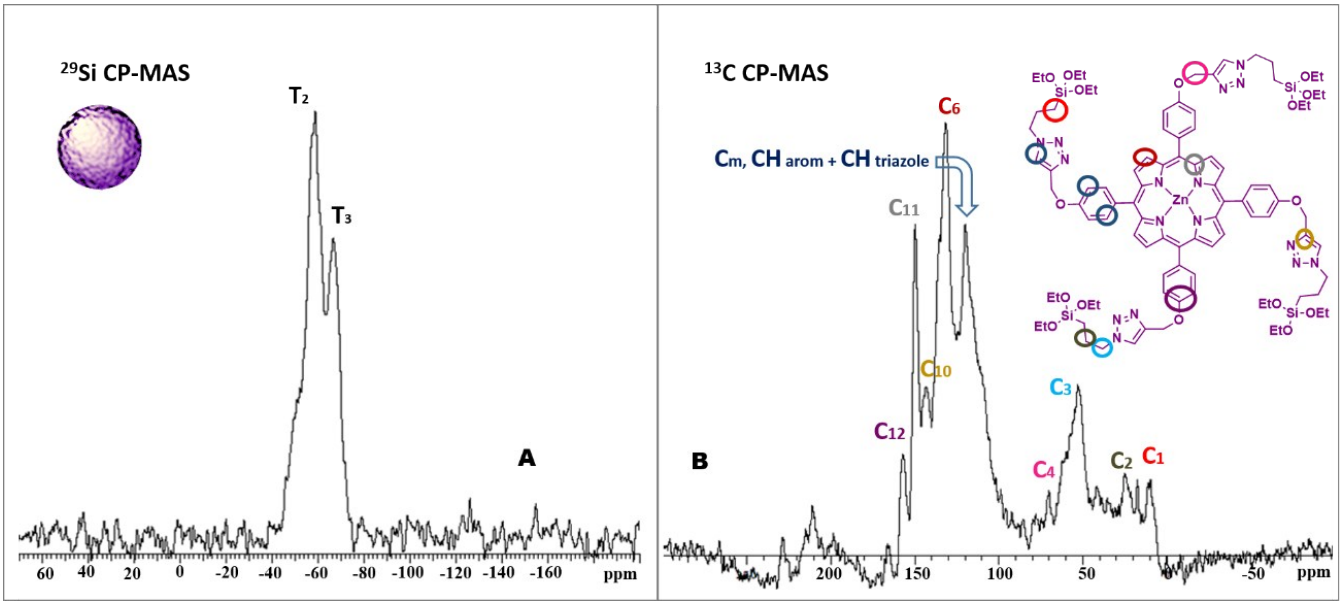


Fig. S3. A) ^{29}Si solid state NMR. B) ^{13}C solid state NMR.

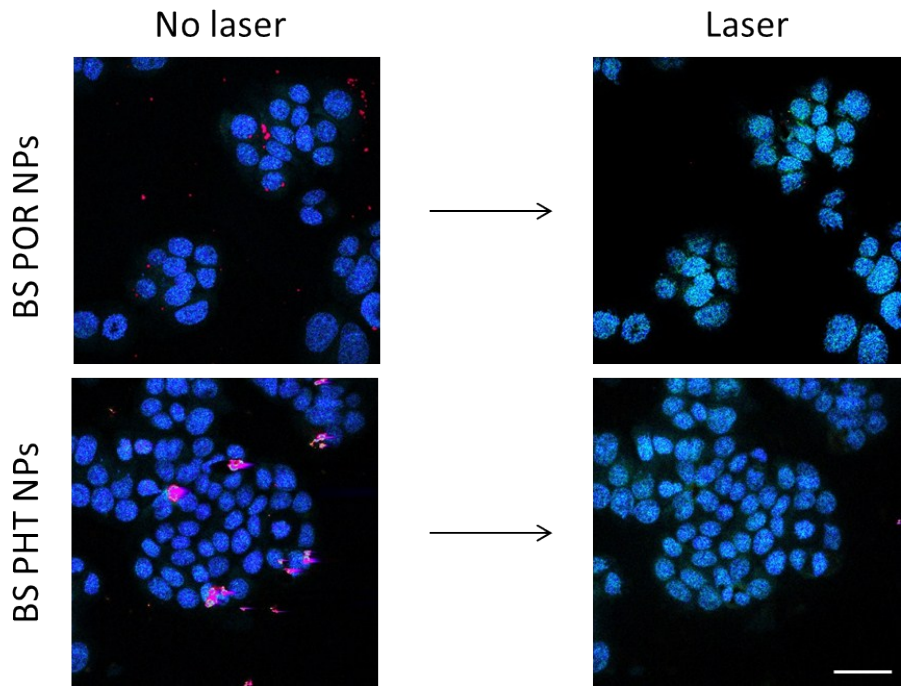


Fig S4 : ***In vitro*** **evidence for photo bleaching of BS POR NPs and BS PHT NPs.** MCF7 living cells were incubated with 40 µg.mL^{-1} of BS PHT NPs or BS POR NPs. Nuclei were stained with Hoescht. Cells and NPs (red/pink) were imaged before (No laser) and after irradiation (Laser) with an excitation at 800 nm for 2.53 sec (power 100%, laser power input 4300 mW). Scale bar is 10 µm (magnification x 40).

To study the photostability of NPs in cells, a two photon excitation was realized on cells incubated with NPs and monitored by confocal microscope in real time. MCF7 living cells were incubated with 40 µg.mL^{-1} of nanoparticles. Cells were stained with Hoescht (blue) for the visualization of nuclei. The confocal images were obtained on living cells at magnification x40. Images indicated as ''No laser '' were realized with an excitation at 800 nm with a moderate laser power (6% of the input laser power input 4300 mW). Then, in order to induce photo bleaching of NPs (red/pink), samples were submitted to a maximal irradiation at 800 nm for 2.53 sec (power 100%). Finally, new images (referred as ''Laser'') were realized with the same moderate power used as before.

This experiment demonstrates the loss of luminescence of NPs after the excitation at 100% of the laser power (power used for PDT). In contrast, the low laser power used for imaging did not induce a fast decrease of the luminescence (data not shown).

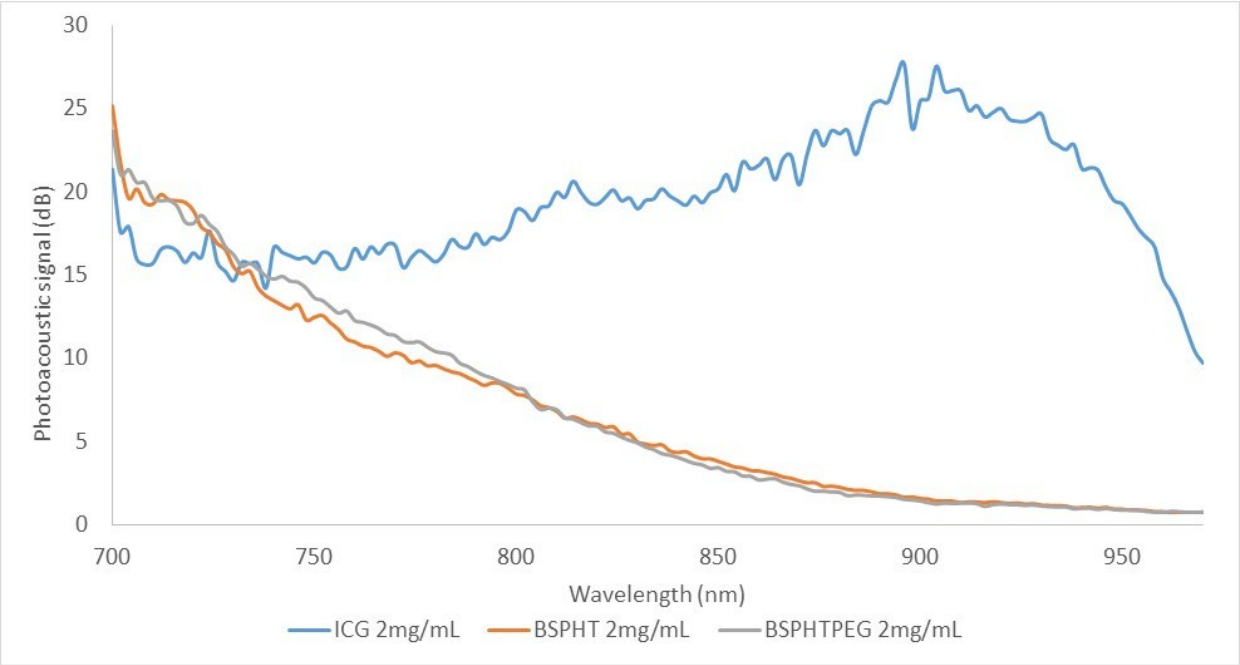


Fig. S5 *In vitro* photoacoustic signal of BSPHT and BSPHTPEG nanoparticles and Indocyanine green (ICG). The PA signal was measured from a dispersion of 2 mg/mL of nanoparticles or a 2 mg/mL solution of ICG in PBS and EtOH.

References

[1] *J. Mater. Chem.* **2009**, *19,* 1409-1416.

[2] J. G. Young and W. Onyebuagu. Synthesis and Characterization of di-disubstituted Phthalocyanines. *J. Org. Chem.* **1990**, *55*, 2155-2159