Porphyrin-Functionalized Mesoporous Organosilica Nanoparticles for Two-Photon Imaging of Cancer Cells and Drug Delivery

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SUPPORTING INFORMATION

I-EXPERIMENTAL SECTION

General procedures. Click reaction was performed using a microwave CEM Discover-Explorer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 400 spectrometer. Chemical shifts (in δ units, ppm) are referenced to TMS using CH_2Cl_2 (δ= 5.32 ppm) or $CDCl_3$ (δ= 7.26 ppm) and CD_2Cl_2 (δ=53.6 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. Dynamic Light scattering analyses were performed using a Cordouan Technologies DL 135 Particle size analyzer instrument.^{29}Si and ^{13}C solid state NMR sequences were recorded with a VARIAN VNMRS300, using Q8MH8 and adamantine references respectively. BET analyses were performed using a TRISTAR 3000 gas adsorption analyzer instrument. TEM analyses were performed on a JEOL 1200 EXII instrument.

Materials. Cetyltrimethylammonium bromide (CTAB), sodium hydroxide, pyrrole, 4-(bromomethyl)benzaldehyde, sodium azide and zinc diacetate were purchased from Sigma-Aldrich. Anhydrous ethanol and propionic acid were purchased from Fischer Chemicals. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Methylene chloride and chloroform were distilled over P_2O_5 then CaH_2. Analytical thin-layer chromatography (TLC) was performed on silica gel (Merck, $60F_{254}$). Merck precoated plates

(silica gel 60, 2 mm) were used for preparative thin-layer chromatography. Column chromatography was carried out with silica gel (60 ACC, 15–40 µm, Merck) Bis (3-triethoxysilylpropyl) ethylene was purchased from ABCR. *N, N*-bis (3-triethoxysilyl) propyl) prop-2-yn-1-amine was obtained from a reported procedure by K.Bürglovà et al. (Chem. *Eur. J*. **2014**, 20, 1-13).

II-PORPHYRIN PRECURSOR SYNTHESIS AND CHARACTERIZATIONS

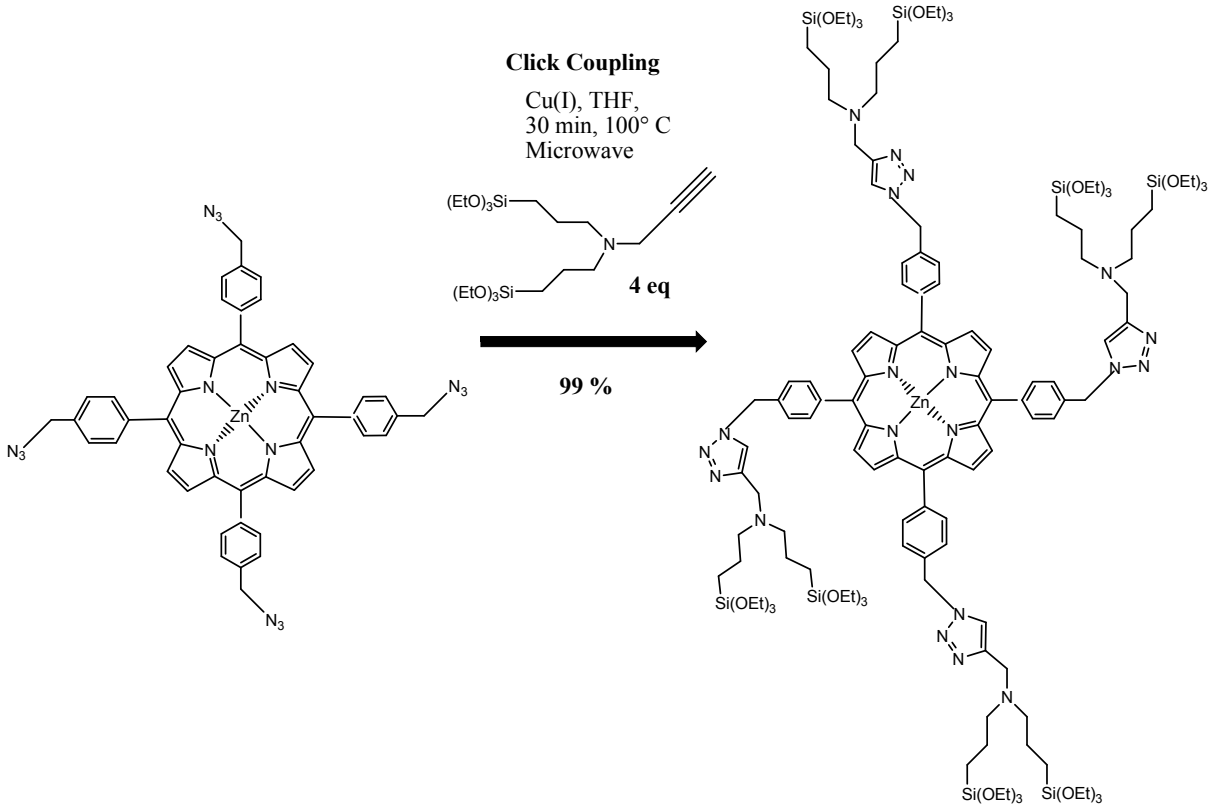
4-(azidomethyl)benzaldehyde. In a typical procedure,[1] 4-(bromomethyl)benzaldehyde was obtained as white solid crystals in 80% yield. Azidation step was performed as following: 4-(bromomethyl)benzaldehyde (1.6 g, 8 mmol) was dissolved in acetone (15 mL), and then poured into a saturated solution of sodium azide in water (5 equivalents). The reaction was activated under microwaves irradiation (300 W, 80 °C, 1 min) until reaction completion checked by TLC. The crude solution was then concentrated under vacuum, and then solubilized in 30 mL of $CHCl_3$, washed with 10 mL of water to remove salts (three times). The organic layer was dried over $MgSO_4$, then evaporated under vacuum to give after purification on chromatoflash ($CHCl_3$ - Petroleum ether) 4-(azidomethyl)benzaldehyde as a pale yellow oil in 95% yield. Spectral data are in accordance with literature.[2]

Zn(II)-5,10,15,20-Tetrakis(4-(azidomethyl)phenyl)porphyrin.
4-(azidomethyl)benzaldehyde (1.1 eq., 377 mg, 2.3 mmol) was dissolved in refluxing propionic acid (75 mL). Then, freshly distilled pyrrole (1 eq., 2.0 mmol, 143 µL) diluted in 15 mL of propionic acid was drop-wised over a period of 30 minutes in a light protected two-neck flask. After removing of solvent, the crude product was purified by chromatography on silica gel (CH_2Cl_2/ Petroleum ether: 7/3) to give 180 mg of tetrakis(4-(azidomethyl)phenyl)porphyrin as a dark purple solid in 37 % yield. Spectral data are in accordance with literature.[3] Tetrakis(4-(azidomethyl)phenyl)porphyrin (180 mg, 0.21 mmol) was solubilized in refluxing THF and Zinc diacetate (5 eq., 1.0 mmol, 200 mg) was added. After 2 h of reaction, THF was removed under vacuum. The crude product was solubilized in chloroform and filtered on silica gel to give Zn(II)-5,10,15,20-Tetrakis(4-(azidomethyl)phenyl)porphyrin as a pale purple solid in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) : 8.91 (s, 8H, $H\beta_{pyrrole}$) ; 8.22 (d, 8H, ^3J = 7.8 Hz, H $_{3,5}$ aryl) ; 7.68 (d, 8H, ^3J = 7.8 Hz, H $_{2,6}$ aryl) ; 4.69 (s, 8H, -CH_2-N_3). FITR(neat KBr) (cm^{-1}) : 2956 (CH aryl), 2850 (CH alkyl), 2098 (N_3). UV-Vis ($CHCl_3$) : λ_{max} nm (ε x10^{-3} L.mol^{-1}.cm^{-1}) 425 (118.5), 555 (6.5), 595 (1.6).

POR 1. A mixture of the tetraazido porphyrine derivative (20 mg, 2.2*10^{-2} mmol), bromotris (triphenylphosphine)copper(I) ([$CuBr(PPh_3)_3$], 4 mg, 4. 4*10^{-3} mmol), and anhydrous THF (3 mL) was placed in a 10 mL microwave sealable reactor, and *N,N*-bis(3-triethoxysilyl)propyl)prop-2-yn-1-amine (41.4 mg, 8.92*10^{-2}mmol) was added. Then, the tube

[1] Lin, W.; Peng, D.; Wang, B.; Long, L.; Guo, C.; Yuan, J. *Eur. J. Org. Chem.* **2008**, *5*, 793-796.
[2] Barbe, J.-M.; Canard, G.; Brande`s, S.; Guilard, R. *Eur. J. Org. Chem*. **2005**, *21*, 4601-4611.
[3] Eggenspiller, A. ; Michelin, C. ; Desbois, N. Richard, P. ; Barbe, J.-M.; Denat, F. ; Licona, C. ; Gaiddon, C. ; Sayeh, A. ; Choquet, P. ; Gros, C. P. *Eur. J. Org. Chem.* **2013**, *29*, 6629-6643.

was flushed with argon and the microwave process was conducted for 30 min at 100 °C (maximum power 200 W). After evaporation of the solvent, the POR precursor was quantitavely obtained as a purple solid (59.8 mg, $2.2*10^{-1}$mmol)**.** ^{1}H NMR (300 MHz, CD_2Cl_2): δ (ppm) 8.79 (s, 8H, $H\beta_{pyrrole}$), 8.14(d, ^{3}J = 7.8 Hz, 8H, $H_{3,5}$ aryl), 7.39 (d, ^{3}J = 7.6 Hz, 8H, $H_{2,6}$ aryl), 6.96 (s, 4H, triazole), 5.32 (s, 8H, aryl-CH_2-triazole), 3.78 (q, ^{3}J = 6.4 Hz, 48H, O-CH_2-CH_3), 3.36 (s, 8H, N-CH_2-triazole), 2.43 (t,16H, ^{3}J = 6 Hz, $(CH_2$-CH_2-$CH_2)_2$N-CH_2-triazole), 1.45 (m,16H, CH_2-CH_2-CH_2-Si), 1.18 (t, ^{3}J = 7.0 Hz, 72H, O-CH_2-CH_3), 0.59 (t, ^{3}J = 7.4 Hz, 16H, CH_2-Si). ^{13}C NMR (400 MHz, CD_2Cl_2): δ 149.9, 135.8, 131.7, 128.5, 128.3, 125.4, 79.2, 71.9, 67.8 58.1, 56.4, 41.6, 34.1, 29.6, 25.5, 20.4, 7.7. ^{29}Si NMR (400 MHz, CD_2Cl_2): δ -45.1. 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 (EtOH) $\lambda_{max,}$ nm : 424, 559, 598 .



Scheme S1. Design of the POR 1 via click CuAAC coupling.

III-NANOMATERIAL SYNTHESIS AND CHARACTERIZATIONS

EP NPs. A mixture of CTAB (125 mg), distilled water (60 mL), and sodium hydroxide (437 µl, 2 M) was stirred at 80 °C for 50 minutes at 750 rpm in a 250 mL three -neck round bottom flask. Then, 1,2 bis(triethoxysilyl)ethylene (350 µL, 0.92 mmol) was added to the aforementioned solution along with the two-photon photosensitizer (33 mg, 0.012 mmol, 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 20 minutes at 30 Krpm. The sample was then washed three times with a solution of NH_4NO_3 in EtOH (6 g/ L), 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.

DOX loading of the NPs. A mixture of NPs (10 mg), DOX (3.2 mg), and deionized water (1 mL) was prepared in an eppendorf tube, sonicated for 5 min in a bath at 45 °C, and 30 µL of hydrochloric acid (0.02M) were added to reach pH 5.5. Then, the solution was stirred overnight at 25 °C. The solution was neutralized with aliquots of sodium hydroxide and stirred for 30 minutes. Finally, the NPs were collected by centrifugation during 5 min at 10 000 rpm. The sample was washed five times with water, and dried for few hours under vacuum. The loading capacities were deduced by the titration of doxorubicin in the supernatant fractions.

DOX wt (%)	20

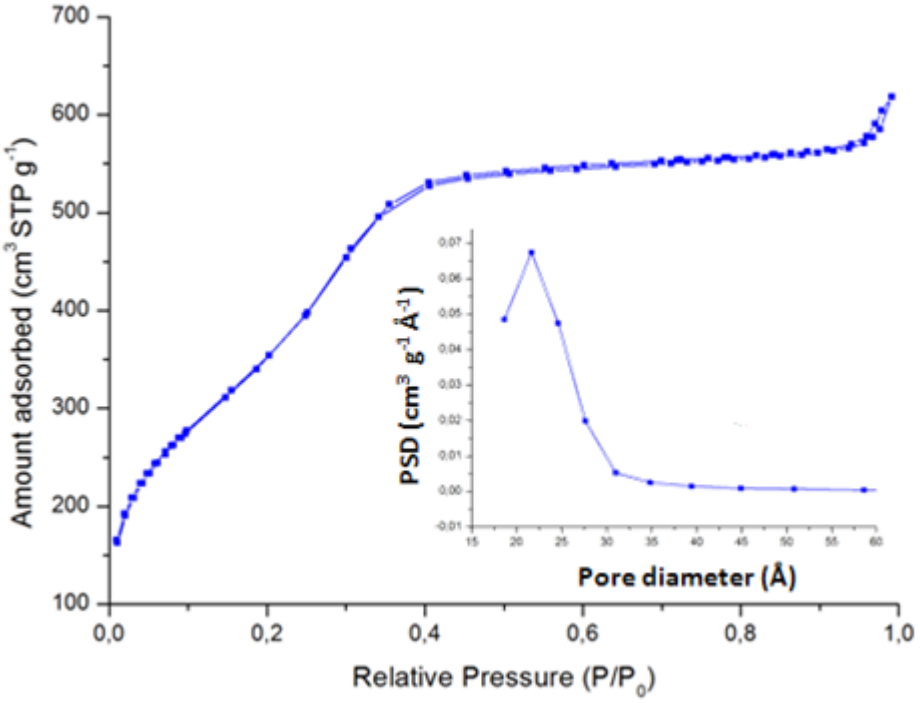


Figure S1. N_2-adsorption-desorption isotherm and pore size distribution of EP NPs.

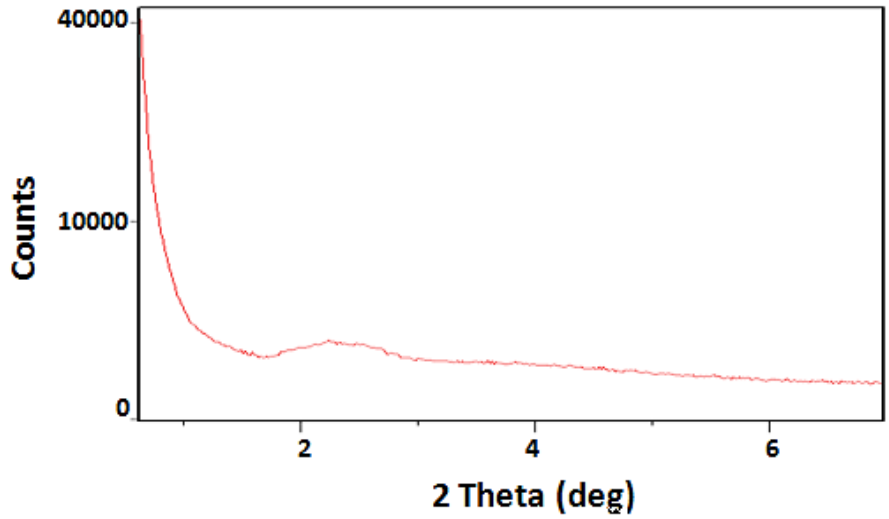


Figure S2. Small angle X-ray diffraction pattern of EP NPs (Cu k_{α}).

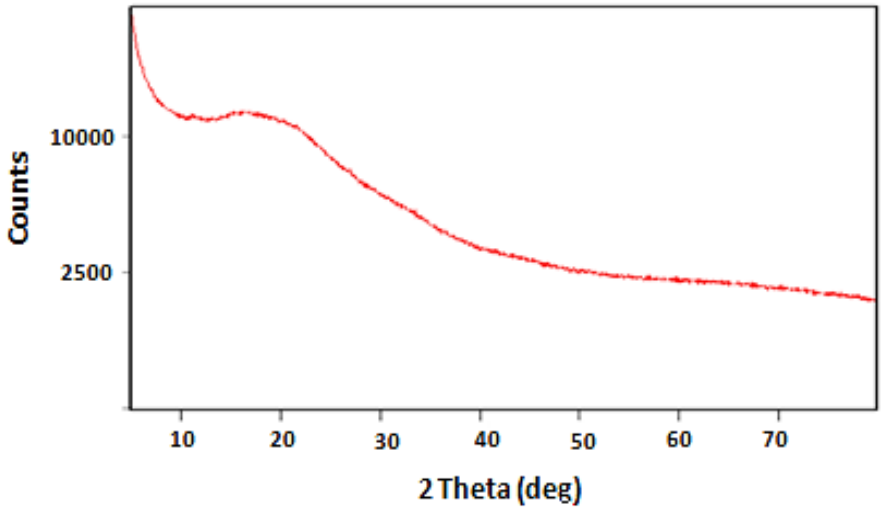


Figure S3. Wide angle X-ray diffraction pattern of EP NPs (Cu k_{α}).

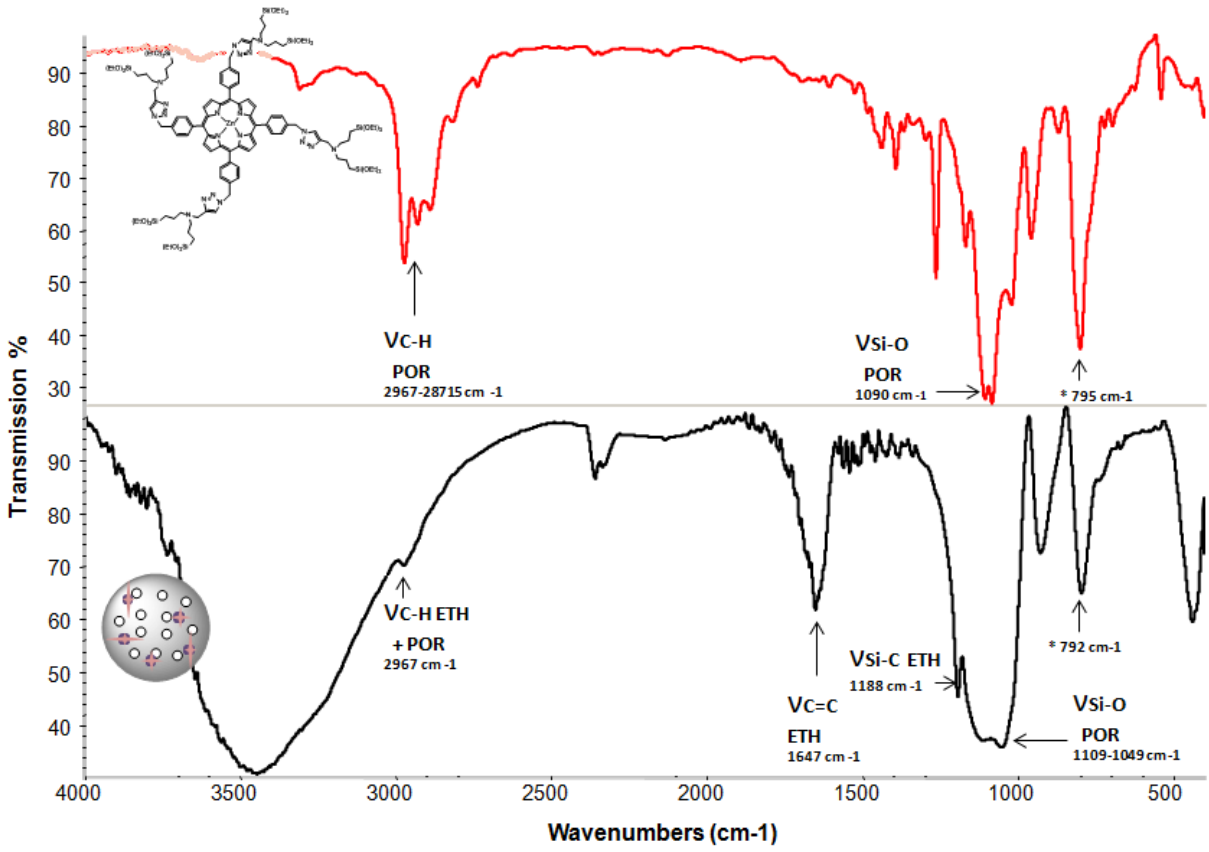


Figure S4. FTIR spectra of the POR 1, and EP NPs confirming the presence of the ETH and POR moieties in the nanomaterials framework. * Out of plan bending of *para*-disubstituted aromatic rings in the POR.

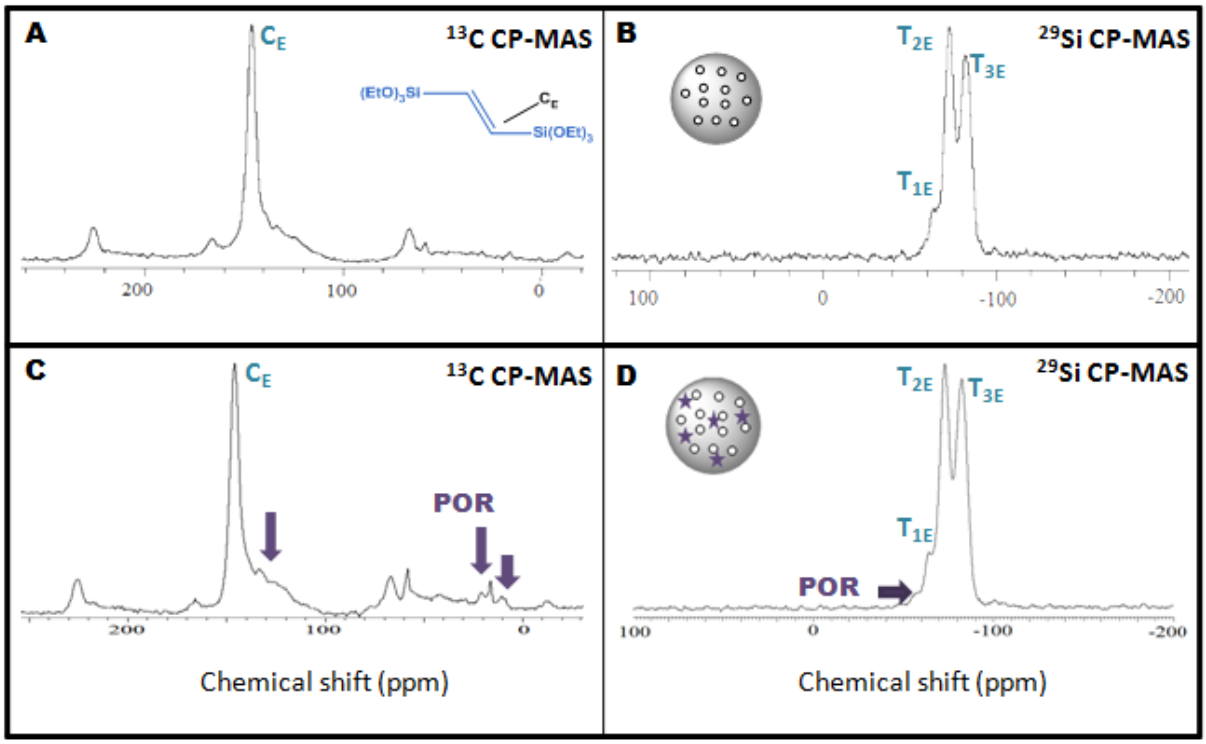


Figure S5. ^{29}Si and ^{13}C Solid state CPMAS NMR spectra of E NPs (**A**-**B**) and EP NPs (**C**-**D**).

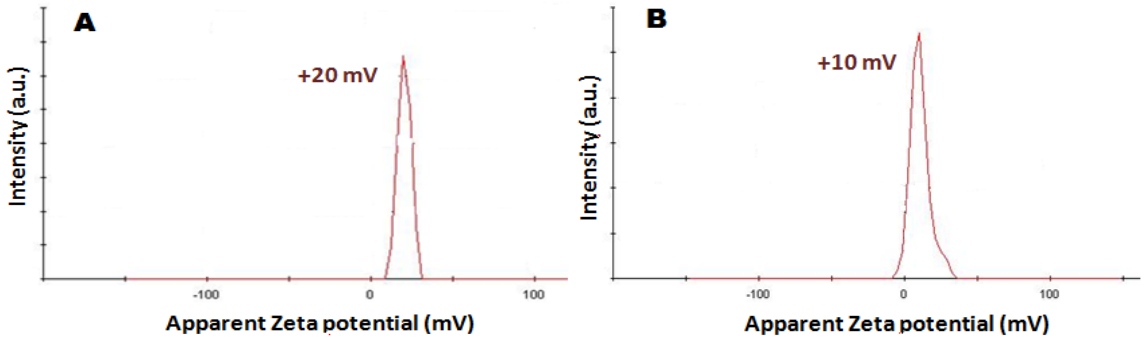


Figure S6. Zeta potential of EP NPs at PH 5.5 (**A**) and PH 7.4 (**B**).

Table S1. Photosensitizer weight percent determination in the EP NPs.

NPs N wt% [a]	NPs POR wt% [b]
1.55	11

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

IV-TWO-PHOTON IN-VITRO STUDIES

TP-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 LSM 780 LIVE (Carl Zeiss, Le Pecq, France), at 750 or 800 nm with a slice depth (Z stack) of 0.62 µm.

V-UNLOADED NPS IN-VITRO CONTROL

Cytotoxicity for 72 h. MCF-7 cells were incubated with increasing concentrations of EP NPs (from 5 to 200 µg.mL^{-1}). After 72 h treatment, a MTT assay was performed and data are mean ± SD of 3 experiments.

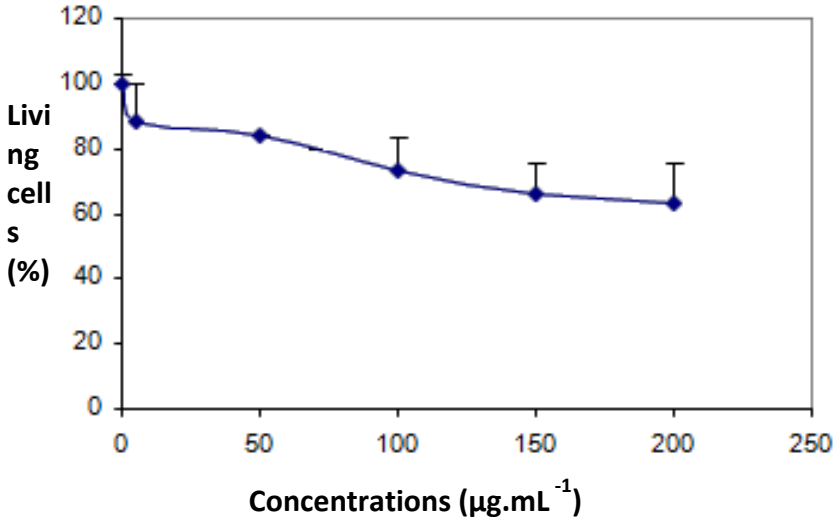


Figure S7. Cytotoxicity of unloaded EP NPs in MCF-7 cells after 72 h of incubation.

VI - DRUG DELIVERY EXPERIMENTS

MCF-7 cells were seeded into 96-well plates at 2000 cells per well in 200 µL culture medium and allowed to grow for 24 h. Increasing concentrations of EP NPs (from 0.01 to 10 $\mu g.mL^{-1}$) dispersed in sterile water, were incubated in culture medium of MCF-7 cells during 72 h and a MTT assay was performed to evaluate the toxicity. Briefly, cells were incubated for 4 h with 0.5 $mg.mL^{-1}$ of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Promega) in media. The MTT/media solution was then removed and the precipitated crystals were dissolved in EtOH/DMSO (1:1). The solution absorbance was read at 540 nm.