Electronic supplementary information for

Phthalocyanine-based Mesoporous Organosilica Nanoparticles:
NIR photodynamic efficiency and siRNA photochemical internalization

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Fig. S1 Structure of the tetra-propargylphthalocyanine previously used to prepare phthalocyanine-bridged silsesquioxane nanoparticles

**Synthesis**

**Materials and methods.** 4,5-Dichlorophthalonitrile $^1$ and monopropargylated resorcinol $^2$ were prepared as previously described. Cetyltrimethylammonium bromide (CTAB, 95%), THF, sodium hydroxide and ammonium nitrate (NH$_4$NO$_3$), were purchased from Sigma-Aldrich. Azidopropyltriethoxysilane was purchased from Abcr GmbH&Co. TEM analysis was performed on a JEOL 1200 EXII instrument. HRTEM analyses were performed on a JEOL 2200 FS, operated at 200 kV. Images were recorded near Scherzer defocus on a 4kx4k CCD camera (Gatan US4000). Dynamic light scattering analyses were performed using a Cordouan Technologies DL 135 Particle size analyzer instrument. $^1$H NMR and $^{13}$C spectra were recorded on a Bruker AC 400 or on a Varian 500 MHz spectrometer and were calibrated to TMS on the basis of the relative chemical shift ($\delta$ in ppm) of the residual non-deuterated solvent as an internal standard. FT-IR spectra were recorded on a Perkin-Elmer 100 FT spectrophotometer. UV-vis absorption spectra were recorded on a Hewlett-Packard 8453 spectrophotometer. Zeta potential were performed with a Malvern Instrument.

**Synthesis of phthalonitrile 3.** 4,5-dichlorophthalonitrile 1 (5.08 mmol, 1 g), monopropargylated resorcinol 2 (15.2 mmol, 3 equiv.) and K$_2$CO$_3$ (100 mmol, 14 g) were
stirred in dry DMF (10 mL) at room temperature during three days until disappearance of 1 (monitored by TLC). Reaction mixture was then poured into water and extracted by dichloromethane. The organic phase was dried on Na$_2$SO$_4$ and concentrated. The crude product was purified on a silica gel column chromatography eluted by dichloromethane / hexane (1/1), yielding white crystalline solid. 990 mg (45%). C$_{26}$H$_{16}$N$_2$O$_4$, MW 420.4. FT-IR (cm$^{-1}$): 3288, 3047, 2232, 2122, 1595, 1578, 1500, 1482, 1453, 1398, 1376, 1286, 1260, 1201, 1129, 1078, 1030, 997, 942, 918, 859, 765, 750, 683. $^1$H NMR (DMSO-$d_6$, δ, ppm): 7.82 (1H, s, ArCH), 7.35-7.37 (1H, t, ArCH), 6.85-6.87 (1H, d, ArCH), 6.76 (1H, s, ArCH), 6.70-6.72 (1H, d, ArCH), 4.81 (2H, s, CH$_2$-O), 3.57 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ, ppm): 159.16 (ArC-O), 156.29 (ArC-O), 151.25 (ArC-O), 131.29 (ArCH), 125.65 (ArCH), 115.81 (CN), 112.06 (ArC-C), 111.61 (ArCH), 111.42 (ArCH), 106.01 (ArCH), 79.38 (C), 78.80 (CH), 56.23 (CH$_2$-O).

Fig. S2 FT-IR spectrum of 3
Synthesis of phthalocyanine 4. Phthalonitrile 3 (235 mg, 0.56 mmol) and Zn(OAc)$_2$ (50 mg, 0.28 mmol) were added to hot dimethylaminoethanol (10 mL) and refluxed during one hour. The cooled reaction mixture was poured into water and filtrated. The resulting crude solid was purified a silica gel column chromatography eluted first by dichloromethane then by dichloromethane/ethanol (100/1), yielding phthalocyanine 4 as a dark-blue powder. 56 mg
(22%). $C_{104}H_{64}N_8O_{16}Zn$, MW 1747.2. FT-IR (cm$^{-1}$): 3292, 3055, 1586, 1484, 1445, 1397, 1263, 1176, 1133, 1091, 1028, 941, 733. MALDI-TOF-MS (m/z, matrix: DHB): 1747.875 [M$^+$.]

$^1$H NMR (CDCl$_3$, δ, ppm): 7.41 (8 H), 7.33 (8 H), 6.86 (8 H), 6.73 (16 H), 4.71 (s, 16 H) 2.59 (s, 8 H). $^{13}$C NMR (DMSO-$d_6$, δ, ppm): 159.13, 158.40, 149.12, 134.07, 130.86, 114.09, 110.94, 110.45, 105.59, 79.52, 78.83, 56.16. UV-vis (CHCl$_3$) log ε (nm) 356 (4.69), 686 (4.97).

![Fig. S5 FT-IR spectrum of 4](image1)

![Fig. S6 MALDI-TOF-MS spectrum of 4 (DHB)](image2)
Fig. S7 $^1$H NMR spectrum of 4 (CDCl$_3$)

Fig. S8 $^{13}$C NMR spectrum of 4 (DMSO-$d_6$)
Synthesis of phthalocyanine 5. Phthalocyanine 4 (43.00 mg, 0.0246 mmol), BrCu(PPh₃)₃ (3.87 mg, 0.0042 mmol) azidopropyltriethoxysilane (48.04 mg, 0.197 mmol) were dissolved in THF (1.5 mL) under argon. The mixture was heated to 100°C under microwave irradiation at 200 W for 30 min (3x 10 min). THF was then evaporated. C₁₇₆H₂₃₂N₃₂O₄₀Si₈Zn, MW 3726.04. FT-IR (ν cm⁻¹, KBr pellet): 3148, 3071, 2978, 2925, 2881, 2096 (residue), 1597, 1491, 1445, 1397, 1267, 1180, 1133, 1079, 1035, 963, 788. MALDI-TOF-MS (m/z, matrix: DCTB): 3727.290 [M+H]+. ¹H NMR (DMSO-d₆, δ, ppm): 8.23 (s, 8 H), 7.65 (m, 8 H), 7.63 (d, ³J = 4 Hz, 8H), 7.47 (s, 8 H), 7.01 (s, 8 H) 6.96 (d, ³J = 4 Hz, 8 H), 5.24 (s, 16 H), 4.31 (d, ³J = 8 Hz, 16 H), 3.69 (q, ³J = 8 Hz, 48 H), 1.83 (m, 16 H) 1.12 (t, ³J = 8 Hz, 72 H), 047 (m, 16 H). ¹³C NMR (DMSO-d₆, δ, ppm): 160.11, 159.19, 158.58, 149.74, 142.87, 133.58, 132.76, 124.95, 114.44, 110.98, 110.60, 105.51, 58.17, 56.50, 52.06, 25.60, 18.65, 7.18. ²⁹Si NMR (DMSO-d₆, δ, ppm): -47.

Synthesis of PHT-PMO. Cetyltrimethylammonium bromide (79.5 mg), NaOH (2 M, 279.2 µL) were heated to 80°C for 2 h in H₂O (40 mL). Phthalocyanine 5 (43.00 mg, 0.0246 mmol) in EtOH (1.5 mL) was then added. The mixture was heated to 80°C for 28 h at 750 rpm. The reaction was then centrifuged for 20 min (20000 rpm) and the collected nanoparticles were suspended in a solution of NH₄NO₃ (6 g·L⁻¹ in 95% EtOH) under ultrasounds at 35°C for 30 min. After centrifugation, the extraction was repeated two times, and the nanoparticles washed with H₂O and EtOH. After drying under vacuum, 34.4 mg of nanoparticles were obtained. FT-IR (ν cm⁻¹, KBr pellet): 3484, 3148, 3071, 2930, 2857, 2096 (residue), 1602, 1481, 1442, 1394, 1273, 1132, 1079, 1025, 943, 880, 769.
Fig. S10 A, B HRTEM of PHT-PMO at different magnifications showing the sheet structure of the walls.
Biological experiments

**Cell culture.** Human breast cancer cells MCF-7 (purchased from the main global bioresource center: the American Type Culture Collection - ATCC) were cultured in DMEM supplemented with 10% fetal bovine serum and 50 μg.mL\(^{-1}\) gentamycin and allowed to grow in humidified atmosphere at 37 °C under 5 % CO\(_2\).

**Cytotoxicity measurement.** For cytotoxicity analysis, MCF-7 cells were seeded into a 96-well plate, 1000 cells per well in 200 μL of culture medium, and allowed to grow for 24 h. Then cells were treated with increasing concentrations of PHT-PMO (from 1 to 100 μg.mL\(^{-1}\)) and after 3 days, a MTT assay was performed as previously described\(^{34}\). Briefly, cells were incubated in the presence of 0.5 mg.mL\(^{-1}\) MTT during 4 h to determine mitochondrial enzyme activity. Then, MTT precipitates were dissolved in 150 μL of an ethanol/DMSO (1:1) solution and absorbance was read at 540 nm.

![Fig. S11 Cytotoxic study of PHT-PMO on MCF-7 cells treated with increased concentrations of nanoparticles during 72 h in the absence of light (dark toxicity). Data are mean ± standard deviation of 3 experiments.](image)

**Light excitation for PDT.** MCF-7 cancer cells were seeded into 96-well plates at a concentration of 1000 cells per well in 100 μL of culture medium and allowed to grow for 24 h. Then, cells were incubated 24 h, with or without increasing concentrations of PHT-PMO (10, 25, 50 μg.mL\(^{-1}\)). After incubation, cells were submitted, or not, to laser irradiation at 405
nm (18.75 J.cm\(^{-2}\) for 10 min) or 650 nm (11.25 J.cm\(^{-2}\) for 20 min). Two days after irradiation, MTT assay was performed to evaluate the phototoxicity of \textit{PHT-PMO}.

**ROS production.** The detection of intracellular reactive oxygen production (ROS) was realized using DCFDA Cellular ROS Detection Assay Kit (abcam). For ROS measurement, MCF-7 cells were seeded as for PDT experiments and incubated 24 h with \textit{PHT-PMO} (25 μg.mL\(^{-1}\)). 45 min before irradiation, cells were incubated at 37°C with DCFDA (2,7-dichlorofluorescein diacetate) at 20 μM, submitted to laser irradiation and then washed two times with culture medium. Pictures were performed on fluorescence microscope under a 535 nm wavelength excitation. Green luminescence traduces the generation of ROS.

**NIR excitation for PDT.** MCF-7 cancer cells were seeded into a 384 multiwell glass-bottom plate (thickness 0.17 mm), with a black polystyrene frame, 500 cells per well in 50 μL of culture medium, and allowed to grow for 24 h. Then, cells were incubated 24 h, with or without \textit{PHT-PMO} (20 μg.mL\(^{-1}\)). After incubation, cells were submitted, or not, to laser irradiation with the Carl Zeiss Microscope LSM 780 (laser power input 3W). Half of the well was irradiated at 760 nm or 810 nm or 1080 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/0.3 EC Plan-Neofluar). The scan size does not allow irradiating more areas without overlapping. After 2 days, the MTT assay was performed as previously described and was corrected according to the following formula: Abs control -2 x (Abs control - Abs \textit{PHT-PMO}).\textsuperscript{S4}

**Amination of \textit{PHT-PMO} (PHT-PMO-NH\(_2\)).** \textit{PHT-PMO} (20 mg) were put in suspension in toluene (2 mL) and sonicated. Then, (3-aminopropyl)triethoxysilane (APTES, 31 μL) were added. The reaction was stirred at 80°C for 16 h. Then, the sample was gathered in Eppendorf tubes and collected by centrifugation during 15 minutes at 14 krpm and washed three times with EtOH. Nanoparticles suspended in EtOH, sonicated and centrifuged 10 min at 10 krpm. Finally, \textit{PHT-PMO-NH\(_2\)} were dried under vacuum for a few hours. 23 mg of product were obtained. The amination was verified by the zeta potential measurement by using a Nano ZS apparatus (Malvern).

**Complexation with siRNA.** Various ratios of \textit{PHT-PMO-NH\(_2\)} and siRNA were mixed in a total volume of 18 μl and incubated 15 min at 37°C to induce the complexation of siRNA and \textit{PHT-PMO-NH\(_2\)}. Then, samples were added with xylene blue (6 μL) and deposited on agarose gel (2,5%). Samples are submitted to migration (45 min; 50 V). The retard in the migration of siRNA demonstrated the complexation with \textit{PHT-PMO-NH\(_2\)}. Staining by GelRed in the experimental conditions described by the manufacturer (FluoProbes\textsuperscript{®}).
**Photo-induced siRNA delivery.** The day prior to transfection, $2.10^9$ cells were seeded into a 384 multiwell glass-bottom plate (Proteigene, France). **PHT-PMO-NH$_2$** at 1.25 mg mL$^{-1}$ in ultrapure water were sonicated for 30 min before use. siRNA at 1 µg µL$^{-1}$ in ultrapure water were prepared at siRNA/pSiNP ratio 1:30 (µL:µL). The mixtures freshly prepared were incubated for 15 min at 37 °C for pairing. Then, cells were incubated for 20 h with 40 µg mL$^{-1}$ **PHT-PMO-NH$_2$** paired with siRNA. After incubation, the cells were submitted (or not) to laser irradiation using the Carl Zeiss Microscope (laser power input 3 W). Half of the well was irradiated at 810 nm by three scans of 1.57 s duration in four different areas of the well. No supplementary scan can be performed without overlapping. A microscope objective lens (Carl Zeiss 10-fold magnification/objective 0.3 EC Plan-Neofluar) is used to focus the laser beam. After 2 days transfection, luciferase activity was assessed by addition into the culture medium of luciferin ($10^{-3}$ M, final concentration) purchased from Promega (France). Living cell luminescence was measured 10 min after by a multilabel plate reader (Wallac1420, PerkinElmer, USA) for 5 s. Results were corrected according to the following formula: $\text{Lum}_{\text{non-irradiated}} - 2(\text{Lum}_{\text{non-irradiated}} - \text{Lum}_{\text{irradiated}})$, where Lum is the luminescence emitted. Values are expressed as a percentage of luciferase activity compared to non-irradiated well (set as 100 %).

**Statistical analysis.** Statistical analysis was performed using the Student’s test to compare paired groups of data. A p-value <0.05 was considered to be statistically significant.

**PCI assay.** MCF-7 cells were seeded on 96-well plate glass-bottom (Proteigene, France). One day after seeding, cells were incubated for 24 h with **PHT-PMO-NH$_2$** at 25 µg.mL$^{-1}$ and then irradiated at 405 nm, 5 min (LSM 780, Carl Zeiss 10-fold magnification/objective 0.3 EC Plan-Neofluar, laser power: 12%). One hour after irradiation, cultures were incubated 15 min at 37°C with 0.5 µM acridine orange. Then, cells were imaged on the same microscope under blue light excitation ($\lambda_{\text{exc}} = 490$ nm), images were collected from 550 nm to 650 nm.

**References**

