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Mannose-targeted Mesoporous Silica Nanoparticles for Photodynamic Therapy.

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Materials and methods

Unless otherwise specified, ACS reagent grade starting materials and solvents were used as received from commercial suppliers without further purification.

Elemental analysis were performed at Service commun de Microanalyses, Université Montpellier 2.

UV spectra were obtained using a Perkin Elmer spectrometer. All measurements were carried out at 298 K, using standard 1 cm path length quartz cuvettes.

Transmission Electron Microscopy (TEM) measurements were carried out with a JEOL 1200 EXII microscope at 100 kV.

Dynamic Light Scattering (DLS) experiments were run using a Malvern spectrogoniometer, Autosizer 4800, with a 50 mW laser source operating at 532 nm. The samples were filtered in absolute EtOH through a 0.8μ m pore size filter.

Specific surface areas were determined by Brunauer-Emmett-Teller (BET) method on a Micromeritics triStar analyser (using 75 points and starting from 0.01 as value for the relative pressure) and the average pore diameters were calculated by the BJH method.

Determination of singlet oxygen quantum yield ($\Phi(^{1}O_{2})$)

Excitation occurred with a Xe-arc, the light was separated in a SPEX 1680, 0.22 μ m double monochromator. The detection at 1270 nm was done through a PTI S/N 1565 monochromator, and the emission was monitored by a liquid nitrogen-cooled Ge-detector model (EO-817L, North Coast Scientific Co). The absorbance of the reference solution (Rose Bengal in EtOH $\Phi_f(^1O_2) = 0.68^1$ and the sample solution (at 418 nm) were set equal (between 0.2 and 0.5) by dilution.

1 DeRosa M.C.; Crutchley R.J. *Coordin. Chem. Rev.* **2002**; *233-234*, 351-371

Solid-state DP (direct polarization) ²⁹Si MAS NMR (pulse time $p1 = 2 \mu s$, delay time D1 = 60s) and CP (cross-polarization) ¹³C MAS NMR (Contact time p15 = 3 ms, pulse time $p3 = 5\mu s$) were recorded using Varian NMRS (400 MHz) spectrometer with a MAS 7.5 mm probe (spinning rate 5 KHz).

Preparation of NP1 :

5 mg of PS **1** (5.44.10⁻³ mmol) were dissolved in 1 mL of absolute EtOH for 15 min. under ultrasounds. 6.7 mg (2.72 10^{-2} mmol) of isocyanatopropyltriethoxysilane and 3.1 mg (2.17.10⁻² mmol) of disopropylethylamine were added. The reaction was stirred at RT for 12h.

686 mg $(1.8.10^{-3} \text{ mol})$ of CTAB were dissolved in 40 mL of NaOH 0.2M at 25°C. The preceding solution was added to this mixture. Then 3.5 mL $(1.57.10^{-2} \text{ mmol})$ of Si(OEt)₄ were added dropwise. After 40 s, the mixture was diluted with 260 mL of deionized H₂O at 25°C. The reaction was stirred for 6 minutes then rapidly neutralized to pH 7 by the addition of 0.2M HCl (about 50 mL). **NP1** were obtained after centrifugation (20 min. 20000 turns/min.). **NP1** were put in suspension in EtOH (ultrasounds) then centrifugated. CTAB was extracted with 30 mL of a solution of EtOH/HCl 12N (4/1) for 2h at 60°C. After centrifugation, the extraction procedure was repeated two times, and then **NP1** were put in suspension in water and centrifugated, until neutral pH.

NP1 (2.5 mg in 5 mL EtOH) were characterized by UV-visible spectra, with $\varepsilon = 295599 \text{ M}^{-1}$. cm⁻¹ for the Soret band. We calculated a loading of 3.5 µmoles of PS per gram of **NP1**

Preparation of NP2 :

250 mg of **NP1** were put in suspension in 6 mL H_2O for 30 min. Then 2.5 mL of EtOH and 391 µl of APTS were added. The suspension was neutralized to pH 6 by addition of HCl 0.2M. The reaction was stirred at RT for 20h, and **NP2** centrifuged for 15 min. at 20000 turns/min. The nanoparticles were washed by EtOH (dispersion with ultrasounds followed by centrifugation). They were dried under vacuum for 16h.

Microanalysis : N 2.07%; C 9.61%; H 3.83%. This corresponds to a loading of 1.5 mmol/g of APTS.

Preparation of NP3 :

100 mg of nanoparticles **NP2** were put in suspension in 5 mL EtOH for 10 min. (Ultrasounds). 59 mg (0.15 mmol) of p-[N-(2-Ethoxy-3,4-dioxocyclobut-1enyl)amino]phenyl- α -D-mannopyranoside were dissolved in 5 mL EtOH-H₂O (50-50). This solution was added dropwise to the suspension of **NP2** in EtOH. 500 µL of Et₃N were added and the suspension was stirred for 18h. After centrifugation (15 min., 20000 turns/min) the nanoparticles were redispersed in water (ultrasounds) and centrifuged again (3 cycles). Then **NP3** were redispersed in EtOH and centrifuged (two cycles). They were dried under vacuum for 16h.

Microanalysis : N 2.28%; C 11.2%; H 4.8%.

Cell culture:

MDA-MB-231 human breast cancer cells (ATCC No HTB-26) were routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 50 μ g/ml gentamycin. For experiments cells were seeded into 96-well plates at 3.10⁴ cells/well in 100 μ l of culture medium and allowed to grow for 24 h in humidified atmosphere at 37°C under 5% CO₂. Cells were then incubated for different times with or without 20 μ g/ml of MSN in the presence or absence of 10 mM mannose. After incubation with MSN, cells were washed twice, maintained in fresh culture medium and then submitted or not to laser irradiation (630-680 nm; 6 mW / cm²) for 40 min. Statistical analysis was performed using the Student's t test to compare paired groups of data. A *P* value of < 0.05 was considered to be statistically significant.

Figures ESI

Figure 1: Uncorrected (blue line) and corrected (green line) UV spectrum of **NP1** in EtOH. The baseline was fitted with mathematical function $y = 165*x^{-1.1592}$ and subtracted to the uncorrected spectrum, in order to neglect the diffusion of the nanoparticles.











Figure 4: DP MAS ²⁹Si NMR of **NP2**

Integration of the signals gave 1.55 mmol of grafting per gram of silica.



Figure 5: CPMAS ¹³C NMR of **NP2** and **NP3** (residual EtOH is still present at the surface of the nanoparticles to avoid aggregation and thus, to facilitate redispersion).



Figure 6 : TEM image of **NP1**.

