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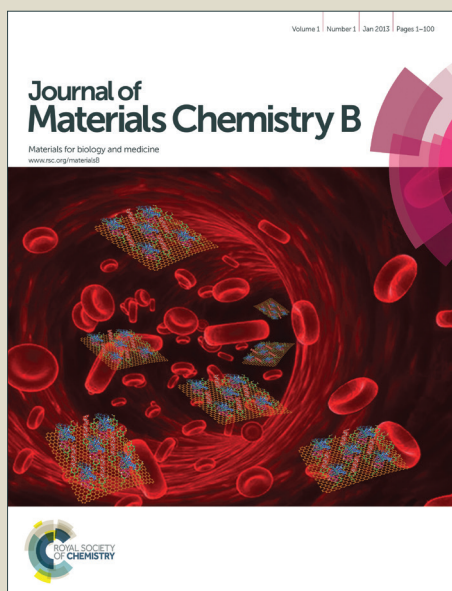
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COMMUNICATION

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

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The synthesis of a zinc porphyrin derivative possessing eight triethoxysilyl groups was performed through a CuAAC-click reaction. This porphyrin was covalently entrapped in ethylene-bridged mesoporous organosilica nanoparticles which efficiently allowed performing doxorubicin delivery and two-photon imaging of breast cancer cells.

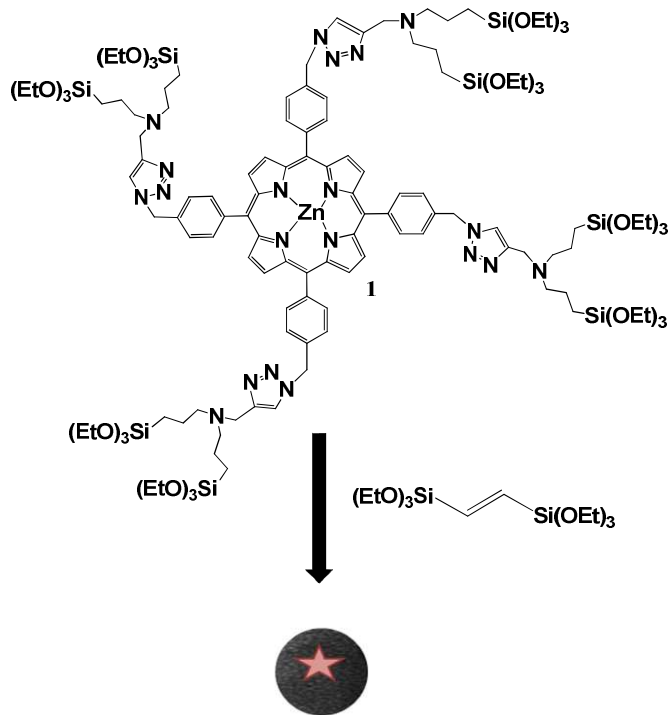
Mesoporous organosilica nanoparticles have very recently attracted much attention for applications in nanomedicine.¹⁻⁸ Such nanoparticles are based on a silsesquioxane framework and an organic fragment which constitutes the structure of the material and which tailors their properties.⁹⁻¹² These nanoparticles are very promising for cancer theranostics due to their low hemolytic properties,¹⁰ high biocompatibility,⁵ and high drug uptake and release capacities. Hollow mesoporous organosilica nanoparticles were prepared for pH-triggered drug and gene delivery, high intensity focused ultrasounds imaging and drug delivery.² Glutathione-triggered biodegradation of mesoporous organosilica nanoparticles was also reported.^{4,8}

Two-photon excitation of nanoparticles has been demonstrated to be a promising strategy for *in-vitro* and *in-vivo* biomedical imaging applications.¹³⁻¹⁶ Indeed, two-photon excitation provides a tri-dimensional spatial resolution with a typical resolution of a micron, while the combination with near-infrared excitation enhances the tissue penetration with a safer treatment.^{13-15, 17-19} We have recently described periodic mesoporous organosilica nanoparticles for two photon-imaging, therapy and drug delivery.⁷ These nanoparticles incorporated a photosensitizer designed for two-photon excitation and allowed two-photon fluorescence imaging of breast cancer cells at 760 nm.

As an alternative to a two-photon photosensitizer, we show here that a porphyrin derivative possessing eight trialkoxysilyl groups could be successfully covalently incorporated within ethylene-bridged mesoporous organosilica nanoparticles (EP NPs) and enabled two-photon fluorescence imaging of cancer cells at low laser power. These nanoparticles, when loaded with doxorubicin, induced very efficiently cancer cell killing at low concentration of nanoparticles.

To synthesize mesoporous organosilica nanoparticles containing the zinc-porphyrin moiety, a new sol-gel precursor featuring 8

triethoxysilyl groups was designed. It was obtained in high yield from Zn(II)-5,10,15,20-Tetrakis(4-(azidomethyl)-phenyl)porphyrin **20** through the CuAAC reaction^{21, 22} with *N,N*-bis(3-triethoxysilylpropyl)prop-2-yn-1-amine in mild conditions, under microwave irradiation (ESI Scheme S1). The preparation of the EP NPs (Scheme S1) was carried out in water at 80 °C, using cetyltrimethylammonium bromide as the structure-directing agent under basic catalysis. Bis(triethoxysilyl)ethene (E) and porphyrin **1** were co-condensed under these conditions. After 2 hours, the nanoparticles were centrifuged and the template extracted with ammonium nitrate.



Scheme 1: Synthesis of EP NPs from bistrithoxysilylethene and porphyrin **1**.

The morphology and structure of the EP material were then characterized via various techniques. Transmission electron micrographs (TEM) revealed spherical nanoparticle with rough surfaces and diameter ranging from 200 to 600 nm (Figure 1A). Dynamic light scattering (DLS) confirmed the nano-size of the material with an average hydrodynamic diameter of 460 nm (Figure 1B). Nitrogen adsorption-desorption analysis showed that the NPs are mesoporous, with a type IV isotherm. A very high specific surface area of $1400 \text{ m}^2 \text{ g}^{-1}$, with an average pore diameter of 2.5 nm were deduced using the Brunauer-Emmett-Teller (BET) and Barrett, Joyner et Halenda (BJH) theories (Figure S1). A wormlike porosity was observed by TEM at higher magnification (see image on the right in Figure 1A), consistent with the small angle X-ray diffraction pattern which showed a broad peak at 2.2 degrees (Figure S2).

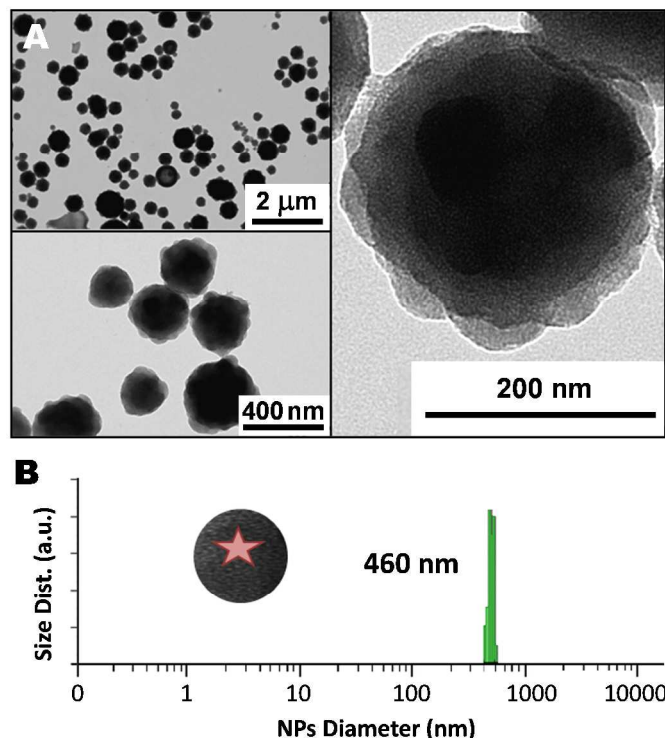


Figure 1: TEM images (A) and DLS size distribution (B) of the EP NPs.

The composition and photophysical properties of the two-photon-sensitive nanomaterial were then investigated. First, the successful incorporation of the porphyrin fragments was clearly seen from the UV-Vis spectrum of EP NPs with the band at 430 nm (see Figure 2). Elemental analysis of nitrogen was used to determine the porphyrin content in the NPs, which was 0.04 mmol/g (ESI). The wide angle X-ray diffraction pattern only showed a broad peak at 20° , corresponding to non-regular repetitions within the siloxane framework (Figure S3), without any sharp peak that would evidence regular repetition of organics within the structure. The presence of only R-SiO₃ environments was checked by ²⁹Si CP-MAS NMR (Figure S5). Fourier transform infrared (FTIR) and ¹³C nuclear magnetic resonance (NMR) spectroscopies (see Figures S4 and S5) confirmed the presence of the ethylene and the porphyrin bridges. It is noteworthy that the zeta potential was positive (Figure S6 ESI), due to the protonation of the tertiary amines at pH 7.4 and 5.5. Additionally, as shown by the UV-Vis spectrum (Figure 2), the porphyrin moiety was clearly aggregated inside the nanoparticles. Indeed, the Soret band was enlarged and Q bands shifted to the red which suggests the presence of *J* aggregates.²³ Porphyrins usually

have a low two-photon absorption cross-section but *J* aggregation of porphyrin derivatives is known to increase the two-photon absorption cross-section.²⁴

Consequently, the two-photon imaging capacity of the EP NPs was assessed in cancer cells. The cytotoxicity of the nanoparticles on MCF-7 breast cancer cells was measured with the MTT assay, and the percentage of living cells was high, up to a concentration of $50 \mu\text{g mL}^{-1}$ (see Figure S7). Then, the nanomaterial was incubated for 20 h in MCF-7 breast cancer cells. The cellular uptake was determined via two-photon fluorescence imaging (TPEF). The laser excitation was performed with a Carl Zeiss two-photon confocal microscope at low power (2.8% of the input power) at different wavelengths. The cell membranes were stained with Cell Mask 15 min before the imaging experiments. EP NPs were successfully endocytosed by cancer cells as shown by TPEF at 750 and 800 nm (see Figure 3A and B respectively). Thus, these findings demonstrate the suitability of porphyrin-functionalized mesoporous organosilica nanoparticles for two-photon imaging.

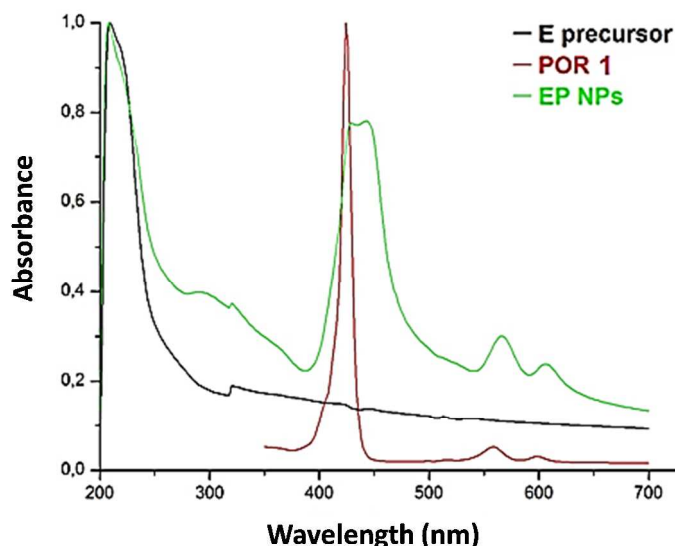


Figure 2: UV-Vis spectra of POR 1, bis(triethoxysilyl)ethylene (E) and EP NPs in EtOH.

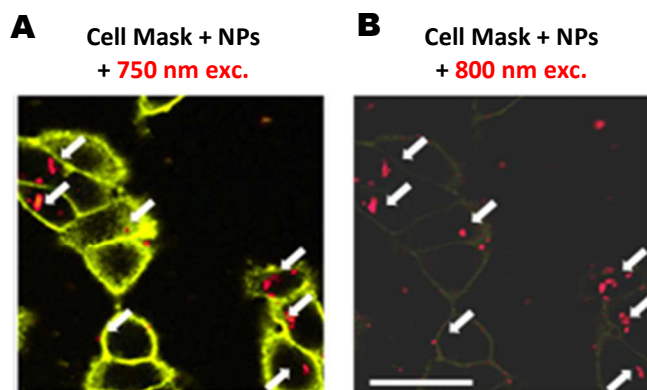


Figure 3: Two-photon fluorescence imaging of MCF-7 breast cancer cells. Cell membranes were stained with Cell Mask TM Orange Plasma Membrane, EP NPs were incubated and irradiated at 750 (A) and 800 nm (B). Arrows indicate NPs in cells. Scale bar 10 μm .

Owing to their high porosity, the nanoparticles were then applied as drug nano-carriers. EP NPs were loaded with doxorubicin at pH 5.5⁴

and the drug loading was 20 wt %. The release of doxorubicin has been studied with similar systems and was pH dependent.^{4,7} Therefore EP NPs were incubated with MCF-7 for 72 h and found to be remarkably efficient to cause cell death as only 25% of cancer cells survived at a concentration of EP NPs of only 1 $\mu\text{g.mL}^{-1}$ (see Figure 4).

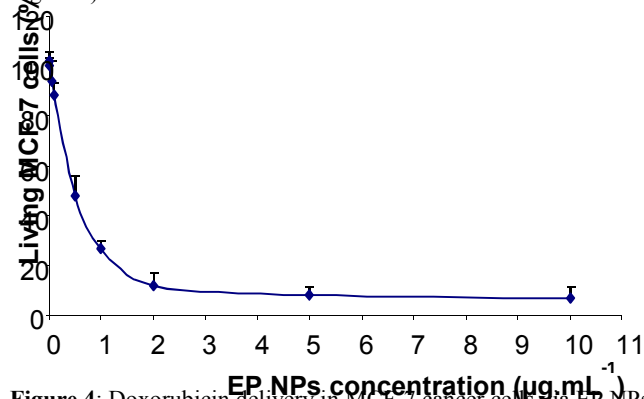


Figure 4: Doxorubicin delivery in MCF-7 cancer cells via EP NPs

Conclusions

We have prepared an octa-trialkoxysilane-substituted Zn porphyrin which was successfully covalently incorporated in ethylene-bridged mesoporous organosilica nanoparticles. The nanoscaled particles displayed a very high specific surface area, enabling drug loading and release. Moreover, the aggregation of the porphyrin derivative within the mesopores enabled potent two-photon fluorescence imaging of breast cancer cells. Furthermore, the doxorubicin-loaded organosilica nanoparticles were very efficient to induce cancer cell killing showing promising potential for theranostic applications.

Notes and references

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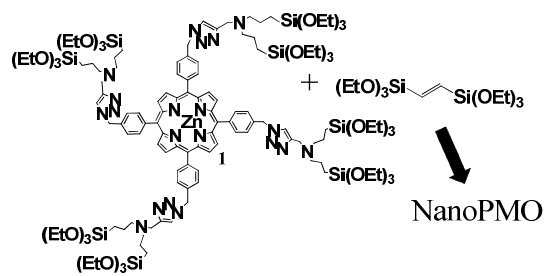
† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [Experimental details of porphyrin **1** and EP NPs syntheses, X-ray diffraction patterns, solid-state NMR, BET, Zeta potential, Cytotoxicity of EP NPs,

experimental details on two-photon imaging and doxorubicin delivery]. See DOI: 10.1039/c000000x/

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The synthesis of porphyrin-functionalized ethylene-based Mesoporous Organosilica Nanoparticles was performed.