Electronic Supplementary Information

Multivalent mesoporous silica nanoparticles photodelivering nitric oxide with carbon dots as fluorescent reporters

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Contents

1. Materials and methods

All reagents (Sigma-Aldrich) were of high commercial grade and were used without further purification. All solvents used were analytical grade. Temoporfin was purchased from Allorachem SRL.

Nitrogen adsorption-desorption isotherm (Micromeritics Tristar II 3020-Apparatus, Norcross, GA, USA) was used to investigate the porosity of the silica particles. Samples (between 100 and 200 mg) were degassed for 3 hours under nitrogen gas flow at 200°C. For carboamino functionalized silica particles, a temperature of 80°C was chosen to avoid degradation of theorganic molecule. The surface area was calculated by the Brunauer-Emmett-Teller equation in the relative pressure range between 0.05 and 0.2.[1S] The average pore diameters and pore volumes were calculated according to BJH Barrett-Joyner-Halenda (BJH) Adsorption model.

2. Syntheses

Synthesis of MSCD. **MSCD** were prepared according to literature.[2,3S] Briefly, 3.g of $C1_2$Ala was suspended in 600 mL of distilled water at 80°C for 24 h under static conditions. 3 g of the co-structure directing agent, 3-aminopropyl triethoxysilane (APTES) was added to this surfactant solution after 10 min of stirring. 15.5 g of tetraethyl orthosilicate (TEOS) was added and the solution was stirred for another 15 min at 80°C in a closed bottle. The resulting gel was subsequently stored at room temperature (RT) under stirring for 24 h and then at 100°C for 3 days under static conditions. The solid product was filtered and dried at RT and under atmospheric pressure conditions. The obtained as-made solid particles were refluxed with EtOH and HCl 20% w/w (around 5 g of silica in 500 mL of EtOH/HCl mixture) for complete removal of surfactant (evaluated by TGA) leaving carboamino groups on the silica surface to give **MSP-carboamino**. A controlled calcination up to 400ºC was performed with the following temperature steps: 1h from RT to 150ºC, 30 min from 150ºC to 400ºC and final 2h at 400ºC, to yield carbon dots embedded in the silica particles; **MSP-CD**
The samples were rehydrated to reconstitute the native silanol (SiOH) groups using NH_3 solution at pH 8 under sonication for 30 min. APTES was dissolved in 50 mL of toluene and added dropwise to a suspension of silica particles (1.5 g in 250 mL of toluene) at room temperature. The amount of APTES was stoichiometry added to an APTES:SiOH molar ratio of 1:3 depending on the SiOH groups present as determined by thermogravimetric analysis . The reaction mixture was stirred for 3 h at 40ºC. The suspension was then washed with warm toluene, ethanol, acetone and left to dry overnight to obtain solid **MSCD** (Fig. S1).

Characterization of MSCD. Thermogravimetric analysis (TGA) was employed to evaluate and quantify the carboamino functionalization and carbondot amount (Fig. S2). **MSP-carboamino** revealed a two-step decomposition profile (150-300 ºC and 300-400 ºC) which suggest the presence of the carboamino groups linked to the silica backbone (Fig. S2A - red line). The mass weight loss accounted for 14.84 %w/w of carboamino amount on the total sample. After controlled calcination at 400 ºC for 2h (Fig. S2B - red line), a drastic decrease of the mass weight loss indicated partial decomposition of the carboamino groups generating carbondots (**MSP-CD**) as already showed in previous work.[2S] Although small (1.73 %w/w), the amount of carbondots is relevant since as control, a complete calcination (600 ºC for 6h - Fig. S2A - green line) that eliminates all the organic functionalization moieties, displays a completely flat TGA profile with relevant difference in mass weight loss (0.60 %w/w). Of interest, the carboamino functionalization of the MSP-CD restores the decomposition curve with a slightly different shape probably due to the presence of both carboamino and carbondots (Fig. S2A – **MSPCD** black line). TGA analysis determines the subsequent carboamino functionalization of the MSP-carbondots to 10.91 %w/w.

Nitrogen adsorption-desorption analysis, which measured the porosity features on the materials, showed equivalent changes in surface properties after functionalization (Table S1). Unfunctionalized MSP and MSP-CD shows similar porosity values, as already showed in previous work.[2S] The reduction of surface area and pore volume in **MSP-carboamino** and **MSCD** reveal the carboamino groups. The fact that this reduction is about the 40% in the **MSCD** is due to the functionalization with APTES and toluene that preferentially introduce carboamino moieties on the external surface. In fact, as show in our previous work[4S], the non-selective introduction of carboamino functions on both internal and external surface showed an annihilation of the porosity values.

Therefore, the re-functionalization with amino groups on the MSP-CD is indicated from both the decomposition profile and mass loss between 150-400/500 ºC and the reduction of porosity values. Considering the molecular weight, we estimated to have *ca.* 10^{-3} mol g^{-1} of carboamino groups per total gram of sample, which roughly means 1 carboamino group *per* nm^2 of silica particle, considering the surface area.

Synthesis of MSCD-1. Caesium carbonate (10 % mol) and **MSCD** was add to a solution of 4-chloro-1-nitro-2(trifluoromethyl)benzene (1 eq) in DMSO and the mixture was refluxed overnight. Afterwards the reaction mixture was centrifuged and reactional DMSO removed. Nanoparticles were washed with ethyl acetate (to removed residual 4-chloro-1-nitro-2(trifluoromethyl)benzene) and water (to removed the excess of caesium carbonate). Nanoparticles were dried under vacuum to obtain MSCD-1 as brown powder.

Encapsulation of Temoporfin within MSCD-1. Temoporfin was loaded into **MSCD-1** by incipient wetness impregnation followed by solvent evaporation. A mixture of nanoparticles (1.0 mg.mL-1) and Temoporphin (2.19×10^{-5} mol L^{-1}) was gently stirred in MeOH for two hours. The solvent was removed under vacuum and the nanoparticles were dried overnight in air.

3. Instrumentation

Thermo gravimetric analysis (TGA) was performed to quantify the amount of organic compounds (*i.e.,* carboamino groups or carbon dots). TGA (209, NETZSCH) was operating with a temperature ramp between 20–990 ºC and a heating rate of 20˚C/min with dry air as plug-in gas atmosphere. Dried samples varied from 3 mg to 10 mg. Decomposition temperatures of water/solvent adsorbed of the silica powder was observed from 20–150 ºC; the carboamino groups and carbon dots were considered to decompose within the range of

150-400/500 ºC. The calculation for the final mass of added species was based on the total mass of the dry sample (removing the water/solvent counterpart).

Nitrogen adsorption-desorption isotherm (Micromeritics Tristar II 3020-Apparatus, Norcross, GA, USA) was used to investigate the porosity of the silica particles. Samples (between 100 and 200 mg) were degassed for 3 hours under nitrogen gas flow at 200°C. For carboamino functionalized silica particles, a temperature of 80°C was chosen to avoid degradation of theorganic molecule. The surface area was calculated by the Brunauer-Emmett-Teller (BET) equation in the relative pressure range between 0.05 and 0.2.[1S] The average pore diameters and pore volumes were calculated according to BJH Barrett-Joyner-Halenda (BJH) adsorption model.

Transmission electron microscopy (TEM) experiments were performed with a TEM JEOL JEM-2010 using the bright field in conventional parallel beam (CTEM) mode (BF).

UV-Vis spectra absorption and fluorescence emission spectra were recorded with a JascoV-560 spectrophotometer and a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter, respectively, in air-equilibrated solutions, using either quartz cells with a path length of 1 cm. Fluorescence lifetimes were recorded with the same fluorimeter equipped with a TCSPC Triple Illuminator. The samples were irradiated by a pulsed diode excitation source (Nanoled) at 370 nm. The decays were monitored at 420 nm or at 655 nm, each solution was used to register the prompt signal. The system allowed measurement of fluorescence lifetimes from 200 ps. The multiexponential fit of the fluorescence decay was obtained using the following equation:

$$I(t) = \Sigma\alpha_i \exp(^{-t/\tau i})$$

Absorption spectral changes were monitored by irradiating the sample in a thermostated quartz cell (1 cm path length, 3 mL capacity) under gentle stirring, using a continuum laser with λ_{exc} = 405 nm (*ca.* 100 mW) having a beam diameter of *ca.* 1.5 mm.

Direct monitoring of NO release in solution was performed by amperometric detection (World Precision Instruments), with a ISO-NO meter, equipped with a data acquisition system, and based on direct amperometric detection of NO with short response time (< 5 s) and sensitivity range 1 nM – 20 μM. The analogue signal was digitalized with a four-channel recording system and transferred to a PC. The sensor was accurately calibrated by mixing standard solutions of $NaNO_2$ with 0.1 M H_2SO_4 and 0.1 M KI according to the reaction:

$$4H^+ + 2I^- + 2NO_2^- \rightarrow 2H_2O + 2NO + I_2$$

Irradiation was performed in a thermostated quartz cell (1 cm path length, 3 mL capacity) using the continuum laser with λ_{exc} = 405 nm. NO measurements were carried out under stirring with the electrode positioned outside the light path in order to avoid NO signal artefacts due to photoelectric interference on the ISO-NO electrode.

4. Figures

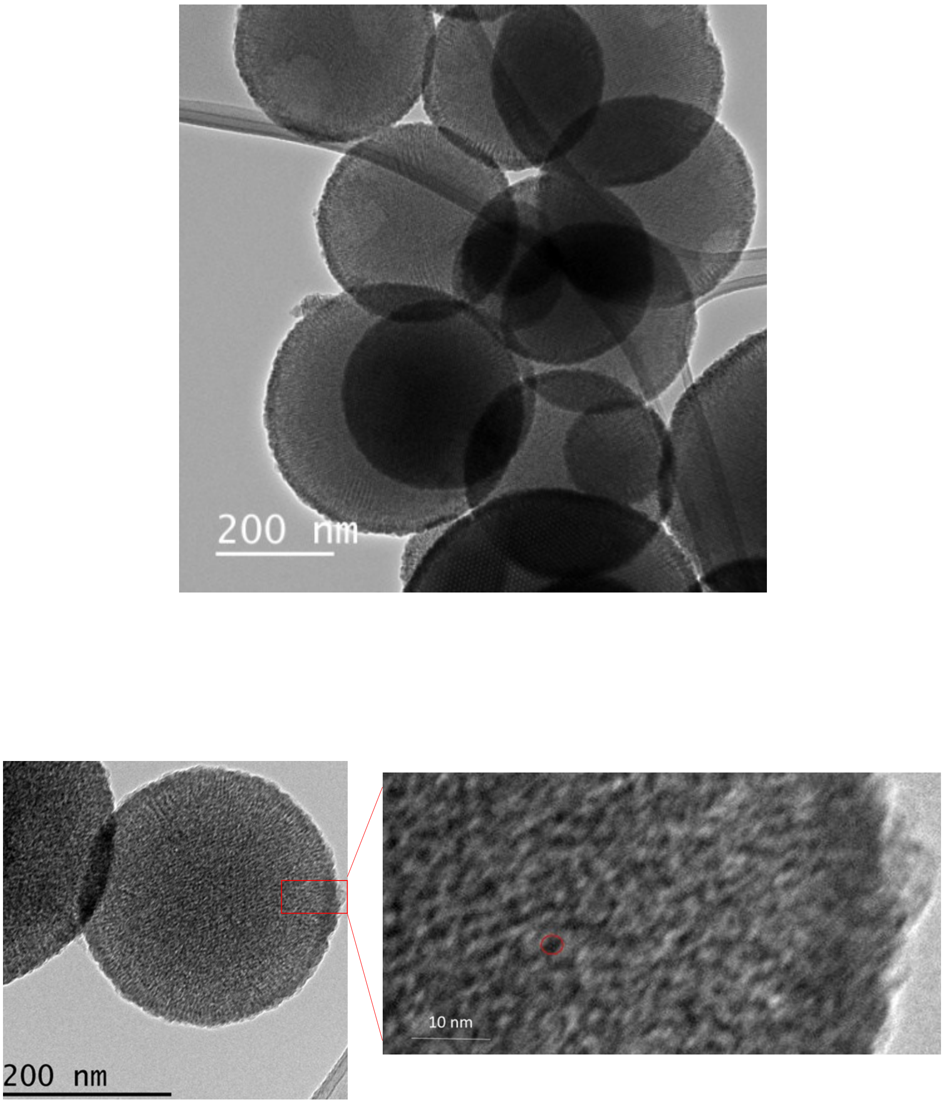

Fig. S1 Representative TEM images of **MSCD**

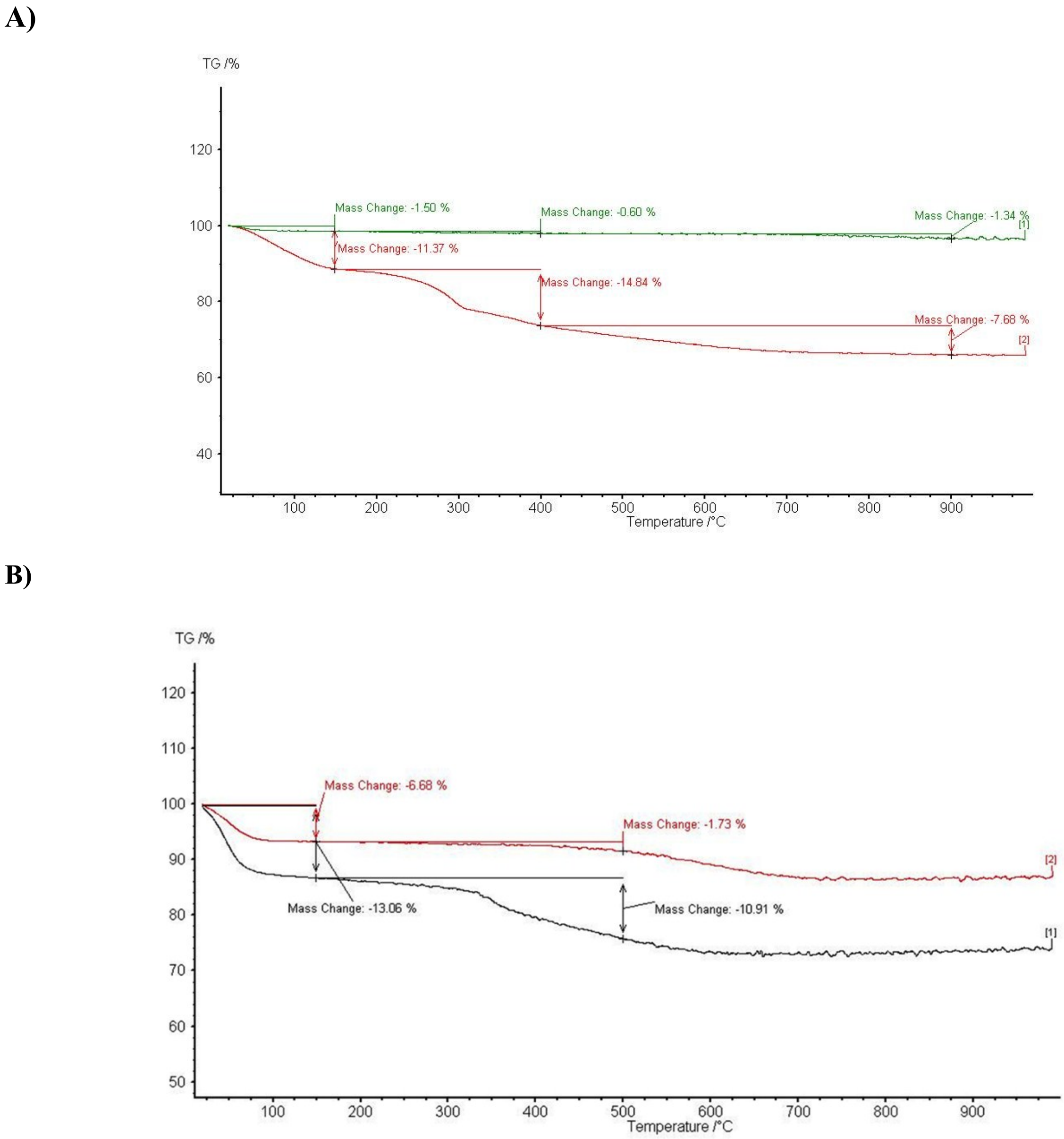

Fig. S2 Thermo gravimetric Analysis (TGA) profile of **A)** MSP-carboamino (red line) and after complete calcination at 600 ºC for 6h (green line) and **B)** MSP-CD (red line) and MSPCD (black line). Decomposition steps and mass weight loss are attributed to water/solvent evaporation (25-150 ºC) and carboamino degradation (150 to 400/500 ºC).

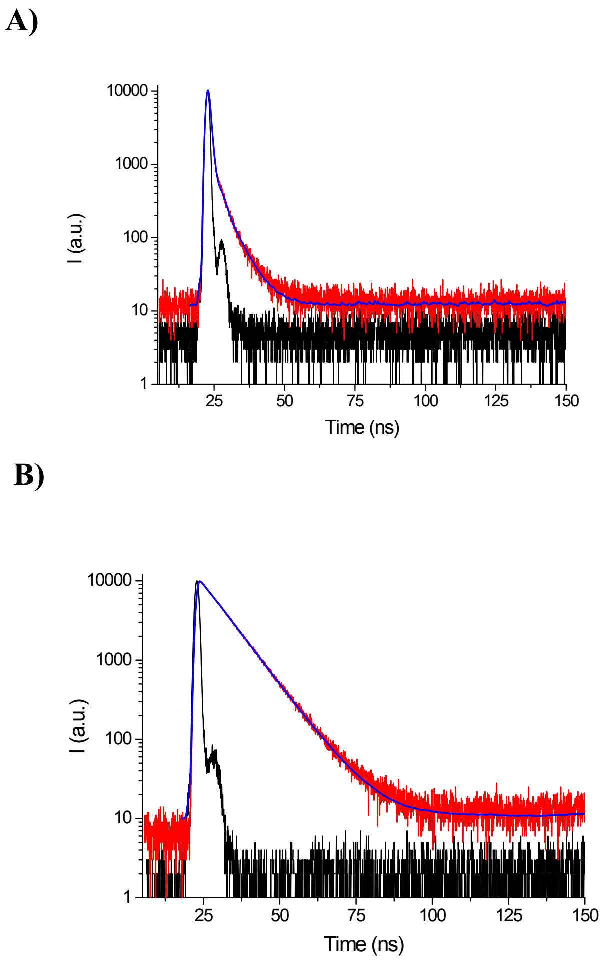

Fig. S3 Luminescence decay profiles and the related fittings (blue lines) at 655 nm of aqueous suspensions of **MSCD-1** (0.1 mg mL^{-1}) after the encapsulation of **4** (**A**) and a methanol solution of **4** (**B**).

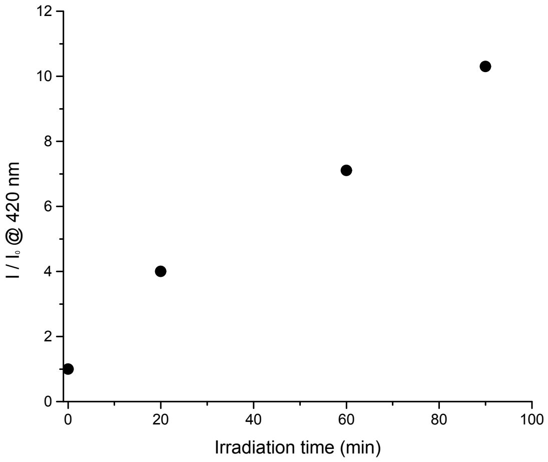

Fig. S4 Correlation of the fluorescence increase observed upon irradiation at 405 nm of aqueous suspensions of **MSCD-1** (0.1 mg mL^{-1}) encapsulating Temoporfin (**4**) (I and I_0 represent the fluorescence intensities after and before each irradiation step irradiation, respectively). Luminescence intensities were recorded with λ_{exc} = 340 nm.

Table S1 Porosity properties of the mesoporous silica by nitrogen adsorption-desorption. Surface areas calculated by Brunauer–Emmett–Teller (BET), pore diameters and pore volumes according to BJH Barrett-Joyner-Halenda (BJH) adsorption model.

	Average pore diameters (nm)	**BET Surface Area (m^2g^{-1})**	**Pore volume (cm^3/g)**
MSP	4.48	931	1.27
MSP-carboamino	4.11	671	0.97
MSP-CD	4.49	961	1.40
MSCD	4.93	593	0.96

5. References

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