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COMMUNICATION

NIR light-triggered drug delivery system using core-shell gold nanostars-mesoporous silica nanoparticles by multiphoton absorption photo-dissociation of 2-nitrobenzyl PEG

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Gold nanostars coated with a mesoporous silica shell and functionalized with poly(ethylene glycol) containing photolabile 2-nitrobenzyl moieties are able to release doxorubicin after NIR light irradiation at low power irradiance via a multiphoton absorption photo-dissociation process.

The development of smart delivery systems[1,2] able to release an entrapped payload upon application of specific stimuli has attracted much attention in recent years due to their potential application in biomedicine, in recognition/sensing and in communication protocols.[3,4] Among drug nanocarriers, mesoporous silica (MS) supports are especially appealing because of their unique properties, such as chemical stability, high loading capacity, low cost and biocompatibility. Moreover, MS can be functionalized with (bio)chemical or supramolecular ensembles, which allow the development of stimuli-responsive systems able to release an entrapped cargo in the presence of certain and well-defined physical, chemical or biochemical stimuli.[5]

In this field, gated nanodevices for controlled release of loaded drugs in response of light irradiation has attracted great attention because cargo release can be defined spatially and temporally by fine-tuning the area and time of the light stimulus.[5] A common approach in light-triggered gated MS is the use of photo-labile molecules that have been used for remote activation and release of loaded drugs.[6] However, molecular photo-dissociation involves high energy radiations limiting their use in realistic applications.[7] In order to overcome this shortcoming, multiphoton absorption of near infrared (NIR) radiation is an appealing alternative when using photo-labile molecules.[8] However, photoreactions activated by this process have some drawbacks such as low multiphoton absorbing cross sections of most chromophores and the need of using high laser powers.[9,10] As an alternative, it has been reported that electric field enhancement produced by quantized oscillation of collective electrons in metal nanoparticles allows overcoming the required high irradiances for multiphoton absorption of organic molecules.[11,12] For instance, it has been described that excitation of localized surface plasmons of gold nanostars (AuNSts) in NIR region leads to strong electromagnetic field enhancement taking place at their sharp tips[13] which favour multiphoton absorption in molecules near to the surface of the nanoparticle. However, this concept has been barely studied for drug delivery applications and as far as we know, photo-delivery systems based on NIR light triggered multiphoton molecular dissociation of photo-labile molecules using core-shell AuNSts-MS nanoparticles have not been reported yet. In fact, developing materials with enhanced optical and drug delivery properties[14-17] is a hot topic in nanotechnology.

Based on the above-mentioned facts, we report herein the combination of the well-known properties of MS to design capped materials for drug delivery applications and AuNSts as NIR light sensitizer able to induce multiphoton molecular dissociation. Our design uses an AuNSts core coated with a mesoporous silica shell (AuNSt@mSiO_2). The pores of the AuNSt@mSiO_2 support are loaded with doxorubicin (Dox) and then, the external surface functionalized with a bulky poly(ethylene glycol) (PEG) derivative containing a photo-labile 2-nitrobenzyl linker (**N2** nanoparticles in Scheme 1).[18,19] NIR irradiation of **N2** induces photo-dissociation of 2-nitrobenzyl linker at low power irradiance via a multi-photonic excitation process, with the subsequent detachment of the bulky PEG derivative from the surface of the nanoparticles and release of Dox from the mesoporous shell. Studies with **N2** internalized in HeLa cells demonstrate that NIR irradiation induced a remarkable reduction in cell viability due to a synergistic combination of chemotherapy (via AuNSts-mediated light-

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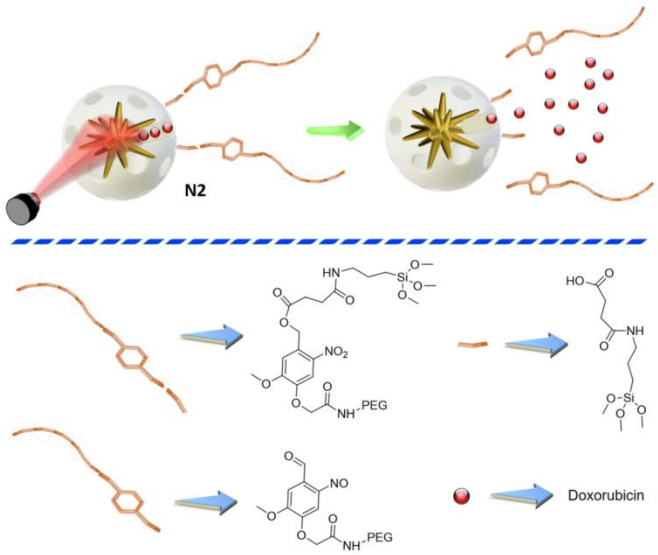
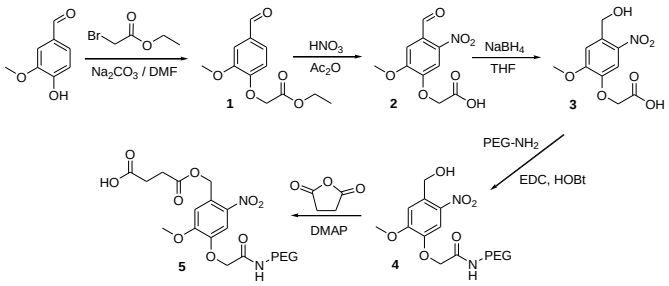
induced uncapping and Dox release) and hyperthermia (due to the presence of the AuNSts and the transformation of light to heat).



Scheme 1 NIR light triggered drug delivery system **N2** based on AuNSt@mSiO$_2$ nanoparticles capped with photo-labile PEG derivative containing a 2-nitrobenzyl linker.



Scheme 2. Synthesis of photolabile PEG derivative **5** bearing a 2-nitrobenzyl linker.

For the preparation of **N2**, the photo-labile molecule **5**, bearing a 2-nitrobenzyl linker with a carboxylate group and a bulky PEG moiety, was synthesized from vanillin (Scheme 2).[20] On the other hand, AuNSts were obtained by the seeded growth method using polyvinylpyrrolidone (PVP) solution in *N*,*N*-dimethylformamide (DMF).[21] This procedure is based on the chemical reduction of $AuCl_4^-$ complexes by DMF and the subsequent deposition of gold atoms in the presence of PVP on spherical gold nanoparticles (AuNPs), acting as seeds. Nanostars with size of 120 nm were synthesized using seeds (AuNPs) of 15 nm. AuNSt@mSiO$_2$-NH$_2$ nanoparticles were obtained by a process in which AuNSts were directly coated with a mesoporous silica shell using a surfactant-templated synthesis and then functionalized with (3-aminopropyl)triethoxysilane.[22,23] The process was carried out under inert atmosphere to avoid gold oxidation and core reshaping in presence of CTAB.[24] Small angle powder X-ray diffraction (PXRD) patterns of AuNSt@mSiO$_2$-NH$_2$ nanoparticles exhibited a peak at 2θ = 2.21°, which could be indexed to (100) diffraction plane of a hexagonal unit cell.[25] This peak also suggested the presence of radial mesopore channels and an ordered mesostructure (Figure S1a).[26] In the wide-angle PXRD pattern, four Au diffraction peaks were clearly discerned at 2θ = 38.15, 44.36, 64.61 and 77.60°, which can be assigned to (111), (200), (220), and (311) reflections of the face-centred cubic gold lattice, respectively.[27] Moreover, N_2 adsorption-desorption isotherms of AuNSt@mSiO$_2$-NH$_2$ nanoparticles showed a typical Type IV curve for mesoporous materials.[28] The average pore size was 2.5 nm, whereas the BET surface area and total pore volume were calculated to be 252 m^2/g and 0.31 cm^3/g, respectively (Figure S1b).[22,27,29]

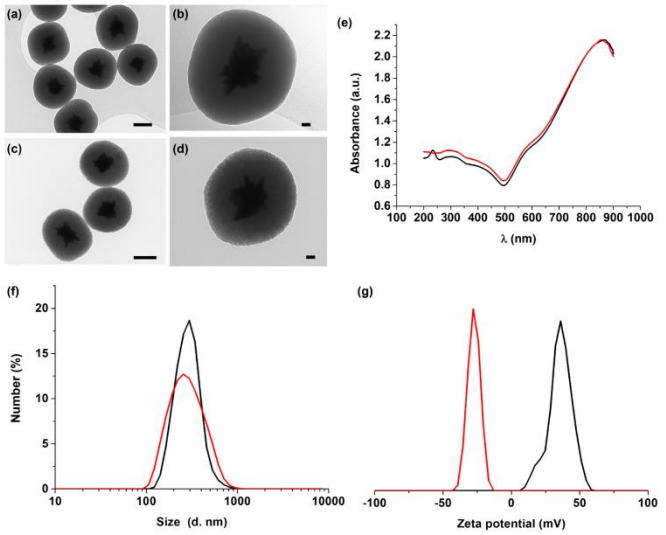


Figure 1 (a,b) TEM images of AuNSt@mSiO$_2$-NH$_2$ and (c,d) **N2** nanoparticles (Scale bars: (a,c) 100 nm; (b,d) 20 nm); (e) Optical extinction spectra of AuNSt@mSiO$_2$-NH$_2$ (black line) and **N2** nanoparticles (red line); (f) Hydrodynamic diameter and (g) zeta potential distributions of AuNSt@mSiO$_2$-NH$_2$ (black line) and **N2** (red line) nanoparticles by DLS.

Once prepared, AuNSt@mSiO$_2$-NH$_2$ nanoparticles were loaded with Dox and the pores were capped by an amidation reaction between the amino groups onto the loaded support and the photo-labile PEG derivative **5** using EDC/NHS to activate the carboxylic acid moiety (**N2** nanoparticles). Bulky PEG was expected to act as gatekeeper preventing drug release from mesoporous shell.[18,19] Aqueous suspensions of **N2** nanoparticles showed a strong LSPR absorption band at 875 nm (see Figure 1e). Besides, hydrodynamic diameter and zeta potential measurements of nanoparticles by DLS (Figures 1f and 1g) showed an average hydrodynamic diameter for AuNSt@mSiO$_2$-NH$_2$ of 296 nm, which increased to 301 nm for **N2** after functionalization of the external surface with the PEG derivative. On the other hand, the zeta potential decreased from +35.7 (for AuNSt@mSiO$_2$-NH$_2$) to -26.5 mV (for **N2**) (Figure 1g). Besides, the organic content in **N2** nanoparticles was calculated to be 15 % by TGA (Figure S3).

After the synthesis and characterization of the hybrid nanocarrier, NIR light-triggered photo-release of doxorubicin from **N2** nanoparticles was studied. For this purpose, an

aqueous **N2** suspension was irradiated at 808 nm using a laser at low power irradiance (1 W cm^{-2})[30] whereas a control sample was kept in the dark. At scheduled times, samples were centrifuged (to remove the nanoparticles) and the emission of Dox released to the solution at 595 nm was measured (λ_{exc}= 490 nm). The obtained results are shown in Figure 2a. As could be seen, a fast and remarkable release was observed (ca. 70% of the total Dox delivered after 60 min) when **N2** nanoparticles were irradiated with NIR light (red curve), whereas in the absence of irradiation the Dox release was lower than 20% (black curve). The observed Dox release upon irradiation of **N2** suspensions with NIR light was ascribed to the AuNSts-mediated photo-cleavage of the 2-nitrobenzyl linker located in the PEG capping ensemble. As a consequence, PEG was detached from the surface with subsequent pore opening and payload release from the mesoporous shell.

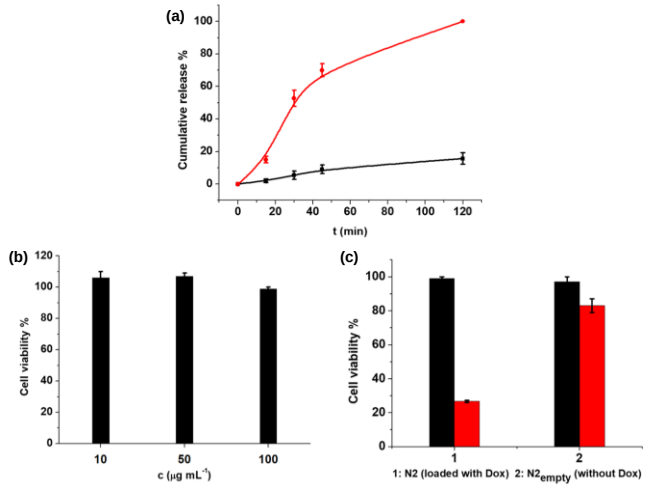


Figure 2 (a) Cumulative release of Dox from **N2** in the presence (red) and absence (black) of NIR light; (b) Cell viability of HeLa cells in the presence of **N2** nanoparticles at different concentrations; (c) Cell viability of HeLa cells in the presence of **N2** and **$N2_{empty}$** nanoparticles at 100 μg mL^{-1} upon 808 nm laser irradiation (red bars) or without any NIR light treatment (black bars).

As a further step, the potential application of **N2** nanoparticles in cells was evaluated. In a first step, the internalization of **N2** nanoparticles in HeLa cells was tested using transmission electron microscopy (TEM) measurements. TEM images showed an efficient nanoparticle uptake after incubation of HeLa cells with a **N2** suspension in DMEM (Figure S4). In a second step, the viability of HeLa cells in the presence of **N2** nanoparticles at different concentrations (10, 50 and 100 μg mL^{-1}, as relevant range recommended for the study of MS toxicity)[31] for 48 h was assessed using WST-1 assay. The obtained results (Figure 2b) demonstrated that **N2** showed no cytotoxicity to HeLa cells up to concentrations of 100 μg mL^{-1}, thus demonstrating that Dox was efficiently entrapped inside the mesoporous silica shell in the nanoparticles.

Once assessed the non-toxicity and the efficient uptake of **N2** nanoparticles by HeLa cells, we carried out *in vitro* NIR light-triggered Dox release studies. In a typical experiment, HeLa cells were incubated with **N2** nanoparticles and then irradiated with a laser at 1 W cm^{-2}. After irradiation, cells were further incubated for 48 h and cell viability was then evaluated. The obtained results are shown in Figure 2c. As could be seen, the viability of HeLa cells treated with **N2** and irradiated with NIR light at low power irradiance (1 W cm^{-2}) decreased to ca. 25%. This could be due to either, the photo-dissociation of 2-nitrobenzyl linker with subsequent PEG detachment and Dox delivery, or hyperthermia. In order to evaluate the independent contribution of both effects, HeLa cells were incubated with **$N2_{empty}$** nanoparticles (similar to **N2** but without payload) under the same conditions as above. As could be seen in Figure 2c, upon NIR light irradiation, HeLa cells viability only decreased to ca. 85%, which is ascribed to hyperthermia. Therefore, cells treated with **N2** nanoparticles and then irradiated with a laser at 1 W cm^{-2} reduced viability by a synergistic combination of hyperthermia (ca. 15%) and chemotherapy (ca. 60%).

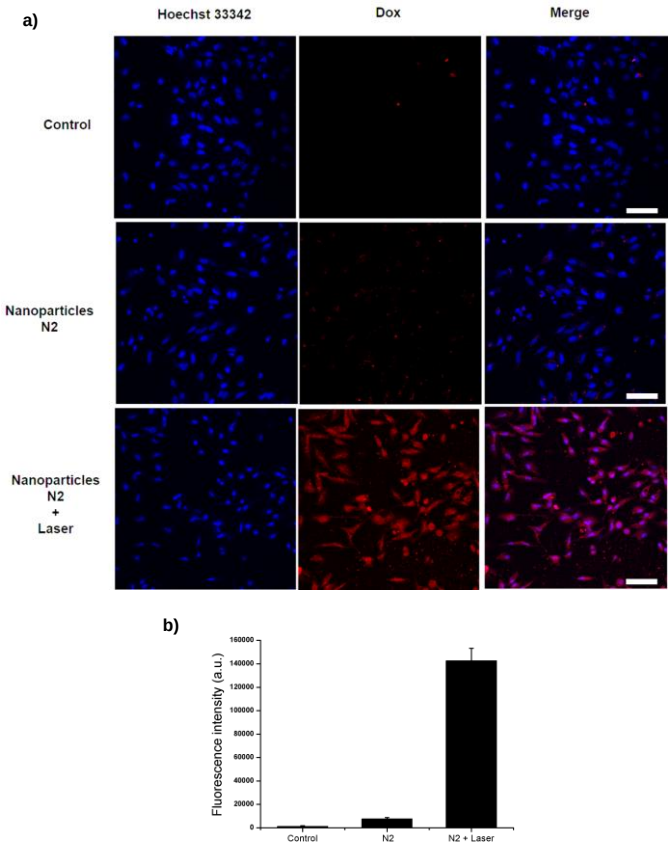


Figure 3 (a) NIR light triggered release of Dox from **N2** nanoparticles in HeLa cells monitored by confocal laser scanning microscopy. From left to right: DNA marker (Hoechst 33342), doxorubicin (Dox) and combined (merge) fluorescence channels. From top to down: control cells (control), non-irradiated cells after incubation with nanoparticles (**N2**), and cell irradiated with NIR light after incubation with nanoparticles (**N2** + Laser). Scale bars: 100 μm. (b) Quantification of the Dox intensity delivered from **N2** nanoparticles by confocal images analysis.

Moreover, NIR light-triggered release of Dox from **N2** nanocarriers in HeLa cells was demonstrated by confocal laser

scanning microscopy. For this purpose, HeLa cells were stained with the DNA-marker dye Hoechst 33342 and the intracellular delivery of Dox after laser irradiation (power density of 1 W cm^{-2}) was monitored. The obtained images are shown in Figure 3a. A negligible Dox fluorescence signal was observed in cells treated with **N2** nanoparticles in the absence of NIR radiation. However, an increase of Dox fluorescence in HeLa cells was found when irradiated with 808 nm NIR light (a marked 19-fold enhancement was found Figure 3b). Control experiments with $\mathbf{N2_{empty}}$ were also carried out and shown in Figure S5. Besides, it was also found that intracellular release of Dox from **N2** nanoparticles could also be triggered by *in situ* irradiation at 633 nm using the confocal laser scanning microscope (Figure S7). These results are consistent with the rupture of the 2-nitrobenzyl linker located in the PEG derivative attached onto the hybrid nanoparticle surface, as consequence of strong electromagnetic field enhancement taking place at their sharp tips in AuNSts that favour multiphoton absorption of the photo-labile 2-nitrobenzyl linker[32] (see additional discussion in the SI). Thus, **N2** nanoparticles can be a suitable platform for NIR light-triggered drug delivery in therapy applications.

In this work a novel drug photo-delivery system based on NIR light triggered photo-cleavage of a 2-nitrobenzyl linker, located in a bulky PEG derivative, using gold nanostars coated with a mesoporous silica shell (AuNSt@mSiO_2) was developed. The prepared nanodevice was able to release an entrapped payload (Dox) upon irradiation with 808 nm light at low power irradiance as a consequence of the multiphoton absorption and dissociation, mediated by the AuNSts, of the photo-labile PEG derivative used as capping ensemble. This is one of the few reported MS nanoparticles able to deliver a cargo by using multiphoton absorption and dissociation of a linker upon NIR light irradiation. Besides, the prepared nanoparticles were non-toxic to HeLa cells. However, a marked decrease in HeLa cells viability (ca. 75%) was observed after **N2** uptake and NIR light irradiation due to a synergic effect of the Dox released and hyperthermia. The methodology employed in this work could be used to obtain other AuNSt@mSiO_2 gated materials, based in the photo-dissociation of certain linkers at low power irradiance via a multi-photonic excitation process, for drug delivery applications.

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Conflicts of interest

There are no conflicts to declare.

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