Molecule-scale controlled-release system based on light-responsive silica nanoparticles†

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We report a molecule-scale controlled-release system based on silica nanoparticles bearing a photoactive *o*-nitrobenzyl bromide linkage, which allows cage and release of drug or biologically active molecules by light.

Controlled-release systems which integrate external stimulation with nanoscale carriers have attracted great attention owing to their potential application in the area of drug delivery.[1] To date, various kinds of stimuli-responsive release strategies have been proposed.[1,2] Light-responsive controlled-release systems possess several advantages, including their non-invasive property, and unique site- and time-specificity.[3,4] Recently, many kinds of light-responsive controlled-released systems have been reported, such as coumarin-modified mesoporous nanoparticles,[3] photolabile gold nanoparticles,[4] and azobenzene-modified nanomaterials.[5] However, the release of guest molecules from these systems is indirect, and involves previous unlocking of a molecular gate and subsequent release of guest molecules. Systems releasing direct, trigger-precise drug doses at a molecular level would be of great value for drug delivery science, pharmaceutics, biomedicine and molecular biology.[6]

Although molecular methods for light-triggered delivery of drug and biologically active molecules have been proposed,[6,7] construction of caged molecules can be costly and involves complicated chemical design, synthesis and purification. Furthermore, the caged molecules are not apt to be further modified with various functions, such as fluorescent, magnetic and targeted functions. A controlled-release system that integrates a molecule-scale light-triggered device with a nanoscale carrier would provide a means for overcoming these challenges.

Herein, we report a molecule-scale controlled-release system based on silica nanoparticles bearing a photoactive *o*-nitrobenzyl bromide linkage, which allows cage and release of drug or biologically active molecules by light. Construction of such light-responsive silica nanoparticles does not involve complicated chemical design, synthesis or purification processes. Furthermore, numerous advantages of silica nanoparticles,[8] including better biocompatibility, ease of functionalization and surface modification, make it feasible for them to be endowed with various functions and to be delivered into living cells.

Silica nanoparticles functionalized with amino and methyl phosphonate groups were synthesized in two steps using a water-in-oil microemulsion system.[9,10] The methyl phosphonate group is necessary to keep the particles well dispersed and at the same time enable amine-based conjugation.[10] Light-responsive silica nanoparticles (structure is shown in Fig. 1) were prepared by covalent conjugation of photoactive *o*-nitrobenzyl bromide molecules with amino groups on the particle surface.

High resolution transmission electron microscopy (TEM) and absorption spectroscopy were used for the characterization of the silica nanoparticles. Fig. 2a shows an example of a TEM image of the silica nanoparticles. The average diameter of the nanoparticles is about 70 nm. Absorption spectra shown in Fig. 2b demonstrate the successful conjugation of *o*-nitrobenzyl bromide molecules on the particle surface (details in the ESI†).

The *o*-nitrobenzyl bromide groups can be used for the direct cage of drug and biologically active molecules which contain carboxylic, phosphate, or hydroxy groups.[7] Drug and biological active molecules can then be irreversibly released by irradiation with light through light-induced photoisomerization of the *o*-nitrobenzyl caged derivatives into *o*-nitrobenzaldehyde (Fig. 3).[6,7]

We performed controlled-release experiments with 5(6)-carboxytetramethylrhodamine (CTMR), which is fluorescent and contains one active carboxylic acid group as a model molecule. CTMR molecules were caged on the surface of silica nanoparticles on the molecule-scale through a direct esterification process between 5(6)-carboxylic groups and surface *o*-nitrobenzyl bromide groups.

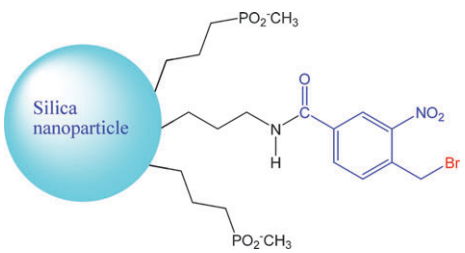

Fig. 1 Structure of light-responsive silica nanoparticles.

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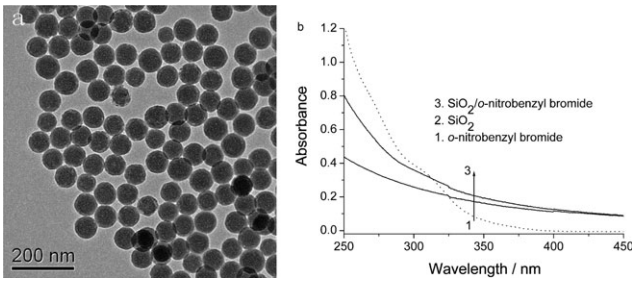

Fig. 2 (a) TEM image of the silica nanoparticles; (b) absorption spectra of an aqueous solution of *o*-nitrobenzyl bromide (line 1), silica nanoparticles (line 2), and silica nanoparticles conjugated with *o*-nitrobenzyl bromide (line 3).

Fluorescence spectra shown in Fig. 4a indicate that CTMR molecules can be effectively caged by *o*-nitrobenzyl bromide group functionalized silica nanoparticles. A negligible fluorescence signal from silica nanoparticles without *o*-nitrobenzyl bromide groups excludes the possibility of non-specific interactions of CTMR with the particle surface. The inset of Fig. 4a shows the structure of the *o*-nitrobenzyl caged CTMR. According to the fluorometric studies of the CTMR conjugated silica nanoparticles, and assuming the density of silica nanoparticles is equal to pure silica,[9] we have estimated that about 240 CTMR molecules were capable of binding to each nanoparticle.

To monitor the release process of the caged CTMR, we then quantified the CTMR that was released by photolysis of the light-responsive silica nanoparticles. After irradiation of an aqueous solution of the caged CTMR with UV light with wavelengths longer than 310 nm, the photoproduct, CTMR, was isolated through centrifugation, and then diluted with deionized water for fluorescence measurements. Control experiments were carried out by monitoring the fluorescence of the diluted solutions which were isolated from samples kept in dark conditions. Fig. 4b gives the normalized fluorescence emission spectra of the released CTMR after different durations of exposure to UV light. These fluorescence emission spectra were normalized by subtracting the fluorescence signals obtained from the control experiments in order to minimize the negative fluorescence signals from the incomplete centrifugation process.

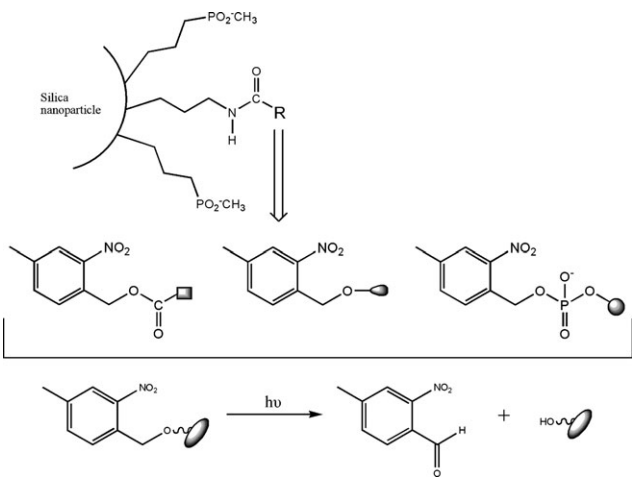

Fig. 3 Chemical structure of various types of *o*-nitrobenzyl caged drug and biologically active molecules (top), and generalized photochemical reaction of an *o*-nitrobenzyl caged derivative (bottom).

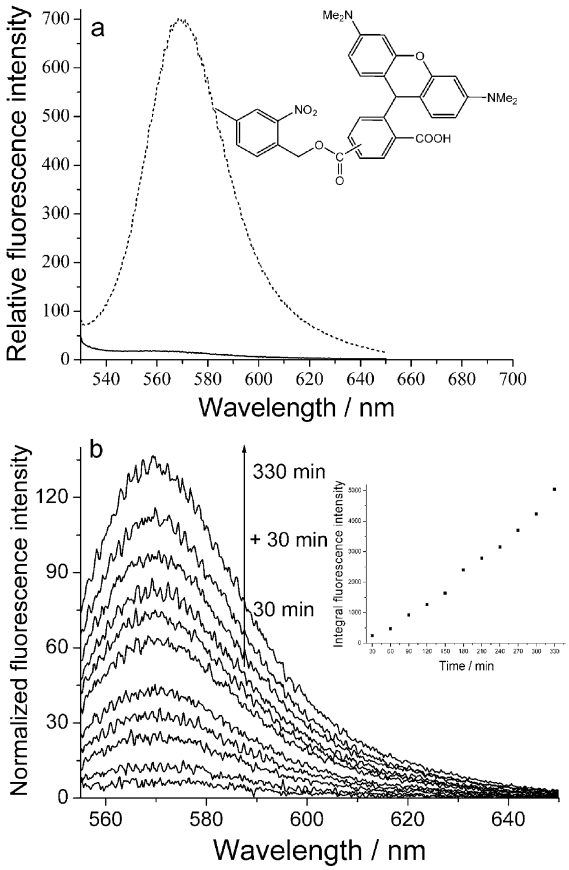

Fig. 4 (a) Fluorescence spectra of CTMR on the surface of silica nanoparticles with (···) and without (—) *o*-nitrobenzyl bromide groups; (b) normalized fluorescence emission spectra of the released CTMR after different durations of exposure to UV light. Inset: correlation of integral fluorescence intensity of the released CTMR with the duration of exposure to light.

Further control experiments from samples exposed to periods in light and dark conditions showed no detectable release of CTMR in the period in dark conditions (Fig. 5), indicating that no other release processes occur under dark conditions and that liberation is temporally and spatially restricted to regions to which light is applied. The inset of Fig. 4b shows the correlation of integral fluorescence intensity of the released CTMR with the duration of exposure to light. These pilot studies of our controlled-release system demonstrate its ability in the precise control of molecule liberation on the molecule-scale. This system can also be extended to the cage and release of other drug or biologically active molecules. In addition, different from existing molecular methods for molecule liberation, our system does not involve the release of the formed *o*-nitrobenzaldehyde side-product under photolysis which can be harmful in biological systems.

Silica nanoparticles were apt to be endowed with various functions. Here we prepared light-responsive silica nanoparticles doped with a fluorescent dye,[9] tris(2,2′-bipyridyl)

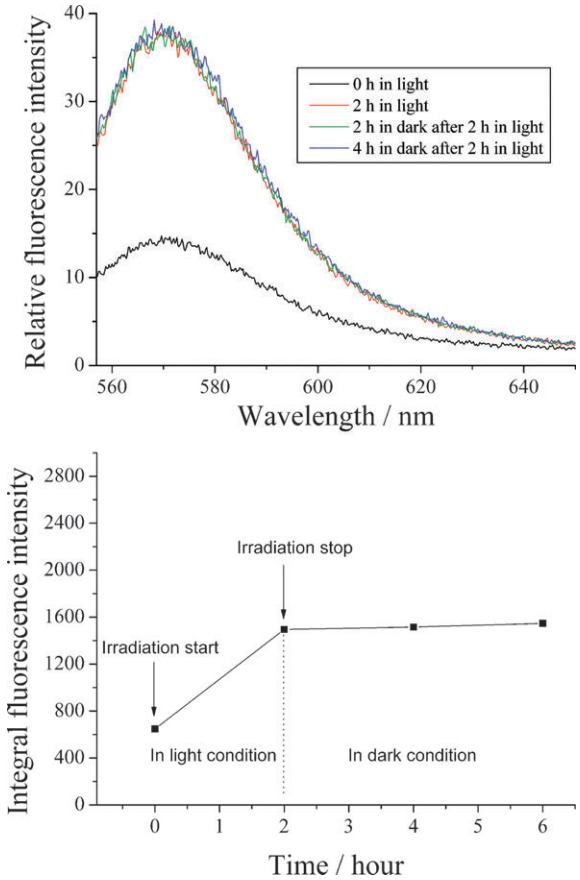

Fig. 5 Fluorescence emission spectra of released CTMR after periods in light and dark conditions.

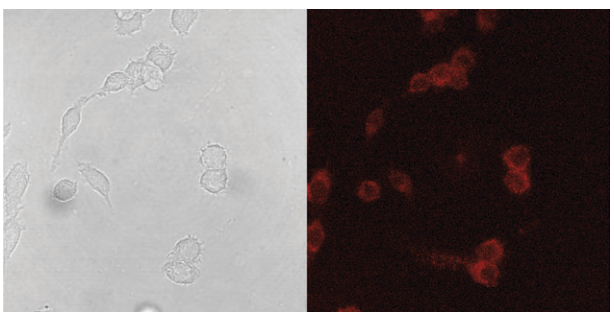

Fig. 6 Bright bright-field (left) and confocal fluorescence (right) microscopy images of the incubated RAW 264.7 cells.

dichlororuthenium(II) (RuBpy). The fluorescent silica nanoparticles were then used for the cage of an analgesic and anti-inflammatory drug, ibuprofen, which contains one carboxylic group. The drug-carrying silica nanoparticles were incubated with RAW 264.7 cells for 24 h on plate and then washed with phosphate buffered saline (PBS) to remove the non-internalized nanoparticles. Fig. 6 shows the bright-field and fluorescence microscopy images of the incubated cells. As can be seen, significant fluorescence was observed inside the cells, indicating that drug-carrying silica nanoparticles functionalized with fluorescent properties can be optically monitored inside cells with confocal fluorescence microscopy. Silica nanoparticles with fluorescent function will also provide a promising platform to optically monitor intracellular trafficking and real-time drug action.[11]

In conclusion, we have developed a new molecule-scale controlled-release system based on light-responsive silica nanoparticles which can be used for direct cage and release of drug and biologically active molecules which contain carboxylic, phosphate, or hydroxyl groups. The results represent a proof-of-concept for the cage and release of CTMR and can be extended to other drug and biologically active molecules. In addition, different from existing molecular methods for molecule liberation, numerous advantages of silica nanoparticles allow our system to be endowed with various functions,[12] which will provide promising platforms for multifunctional applications in drug delivery science, biomedicine and molecular biology.

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