

# NIR Photoresponsive Crosslinked Upconverting Nanocarriers Toward Selective Intracellular Drug Release

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**A**n NIR-responsive mesoporous silica coated upconverting nanoparticle (UCNP) conjugate is developed for controllable drug delivery and fluorescence imaging in living cells. In this work, antitumor drug doxorubicin (Dox) molecules are encapsulated within cross-linked photocaged mesoporous silica coated UCNPs. Upon 980 nm light irradiation, Dox could be selectively released through the photocleavage of theo-nitrobenzyl (NB) caged linker by the converted UV emission from UCNPs. This NIR light-responsive nanoparticle conjugate demonstrates high efficiency for the controlled release of the drug in cancer cells. Upon functionalization of the nanocarrier with folic acid (FA), this photocaged FA-conjugated silica-UCNP nanocarrier will also allow targeted intracellular drug delivery and selective fluorescence imaging towards the cell lines with high level expression of folate receptor (FR).

## 1. Introduction

The recent advances in the fabrication and self-assembly of nanostructure carrier systems have gained extensive attention for their potential biomedical applications in drug delivery, disease diagnosis and medical treatment.<sup>[1]</sup> Controlled-release of bioactive molecules at desired location, timing and dosage will be of great importance to guarantee the determinative therapeutic and diagnostic consequence while minimizing the side effects in the process of the treatment.

Currently, a series of controlled release strategies upon external stimulation have been proposed, in which the triggered release has been systematically driven by a simple physical or biochemical perturbation<sup>[2]</sup> such as light, ultrasound, pH, temperature, enzyme or oligonucleotids treatment etc. Among the proposed approaches to selectively control the release of bioactive molecules, the photolysis of photoactivable or 'caged' molecules has been proven to be one of the most elegant strategies, by which the release process can be readily triggered by a beam of light with non-invasive property and high spatiotemporal precision.<sup>[3]</sup> So far, several classes of 'photocaged' systems have been reported to effectively control the release of drug molecules or imaging probes in vitro and in vivo.<sup>[4]</sup> However, most of the existing photocaged systems have to heavily rely on high energy UV or visible light to initiate the active states of caged molecules. The inevitable cellular damage and less tissue-penetration will be the potential limitations for their further biomedical applications in living systems. Therefore, the development of simple and specific photo-responsive systems that enable spatial and temporal control of payload molecule release with limited cellular damage and deeper light penetration will be still highly desirable and the systematic investigation remains the challenge in the field.

Recently, lanthanide-doped upconverting nanoparticles (UCNPs) have received considerable attention in

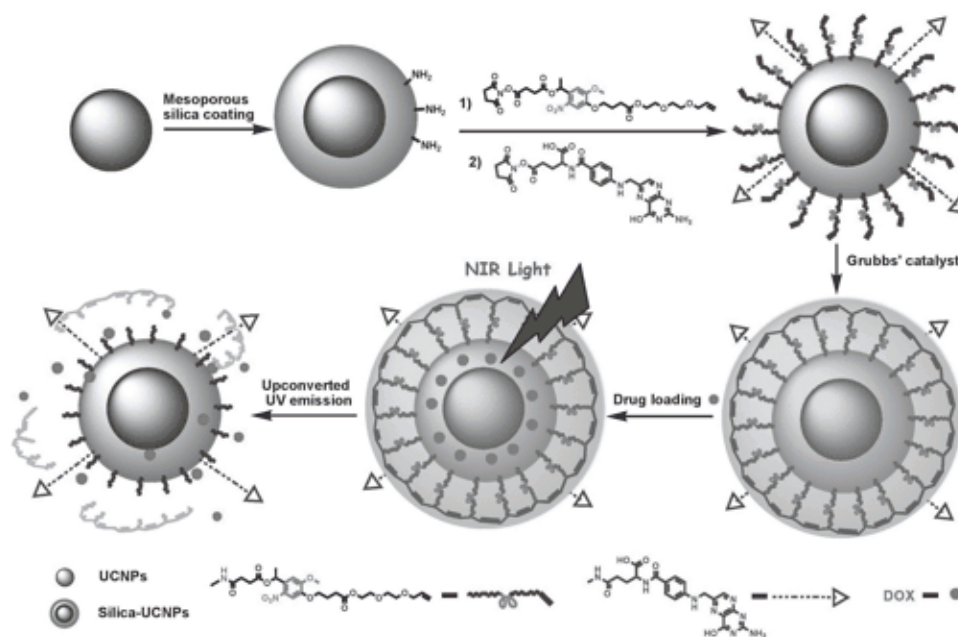
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**Scheme 1.** Experimental design for photo-controlled Dox delivery through photocaged mesoporous silica coated UCNP.

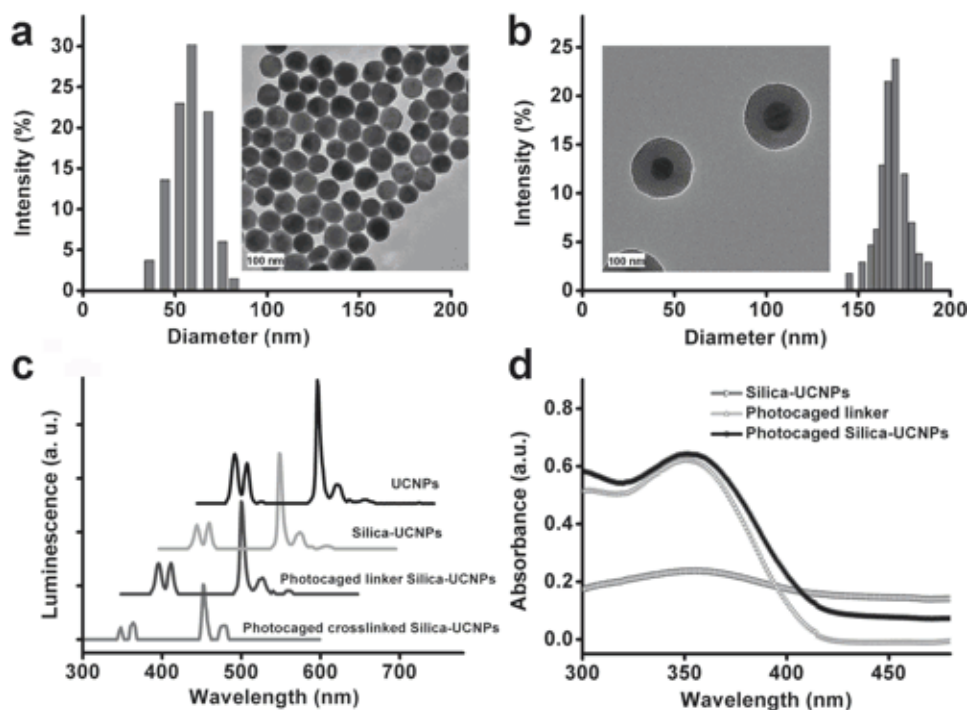
nanobiotechnology and nanomedicine due to their unique photophysical properties.<sup>[5]</sup> Generally, the lower energy NIR light irradiation of lanthanide-doped upconversion nanoparticles would result in the multiplexed emission with the shorter wavelengths ranged from UV to visible region through sequential absorption of multiple photons or energy transfer process. Currently, various kinds of synthetic strategies have been established to supply the unique UCNP with precise control over particle morphology and converted emission wavelength. The subsequent NIR light excitation of these developed UCNP usually exhibit narrow emission and good photostability, which have been extensively applied for biological labeling, molecular imaging and effective therapeutics *in vitro* and *in vivo*.<sup>[6]</sup> Very recently, a few research groups including ours have demonstrated the rational design of remote control of photo-release of imaging probes and payload molecules on the basis of UCNP.<sup>[7]</sup> Herein, we present another simple and specific light-responsive drug delivery nanocarrier by encapsulating antitumor drug Dox within the crosslinked photosensitive linker molecules capped mesoporous silica-coated UCNP. This photo-controlled nanoparticle drug carrier system provides great opportunity for the real-time imaging and selective intracellular drug release with less photo-damage and increased tissue penetration depths in the targeted living system.

## 2. Results and Discussion

**Scheme 1** illustrated the principal design of mesoporous silica coated UCNP nanocarrier for the photo-controlled drug release. In this design, the monodispersed Yb/Tm codoped NaYF<sub>4</sub> @ NaYF<sub>4</sub> core-shell UCNP will be selected as the platform for the further conjugation of antitumor

drug molecules. The lanthanides-doped UCNP will be first coated with silica shell and then coupled to 1-(2-nitrophenyl) ethyl photocaged oligo(ethylene) glycol linker with the vinyl group at the end of the structure.<sup>[8]</sup> The simple intramolecular ring-closing metathesis reaction induced by Grubbs' 2nd generation catalyst and subsequent surface adsorption of antitumor drug molecules, Dox, in the prepared mesoporous silica-UCNP complex would facilitate the formation of crosslinked nanocarriers with Dox encapsulated into the photocaged mesoporous nanostructures. Due to the spectrum overlap between the absorption band of photocaged Dox nanoconjugate and the upconverted emission band of the UCNP in the UV region, NIR light irradiation of UCNP can trigger the cleavage of crosslinked photocaged linker and thus precisely control the targeted drug release from the surface of nanocarriers.

In the typical experiments, the mesoporous silica coated UCNP complex was synthesized by using sol-gel reaction as reported previously.<sup>[9a]</sup> The dynamic light scattering (DLS) and transmission electron microscope (TEM) image demonstrated that the mesoporous silica coated UCNP exhibited a narrow size distribution of about 170 nm (**Figure 1b**), while the average size of unmodified UCNP itself was about 55 nm (**Figure 1a** and **Figure S1**). The thicker layer of silica shell on the surface of UCNP may enhance the biocompatibility and subsequent drug loading into nanoparticle structures. Both of unmodified and photocaged silica UCNP revealed similar emissions in the UV, visible and NIR spectral regions upon 980 nm laser irradiation (**Figure 1c**), suggesting that modification of UCNP with mesoporous silica shell would not significantly affect the upconversion properties. The mesoporous UCNP were further functionalized with amino groups and subsequently coupled to 1-(2-nitrophenyl) ethyl caged oligo (ethylene) glycol linker which contained vinyl



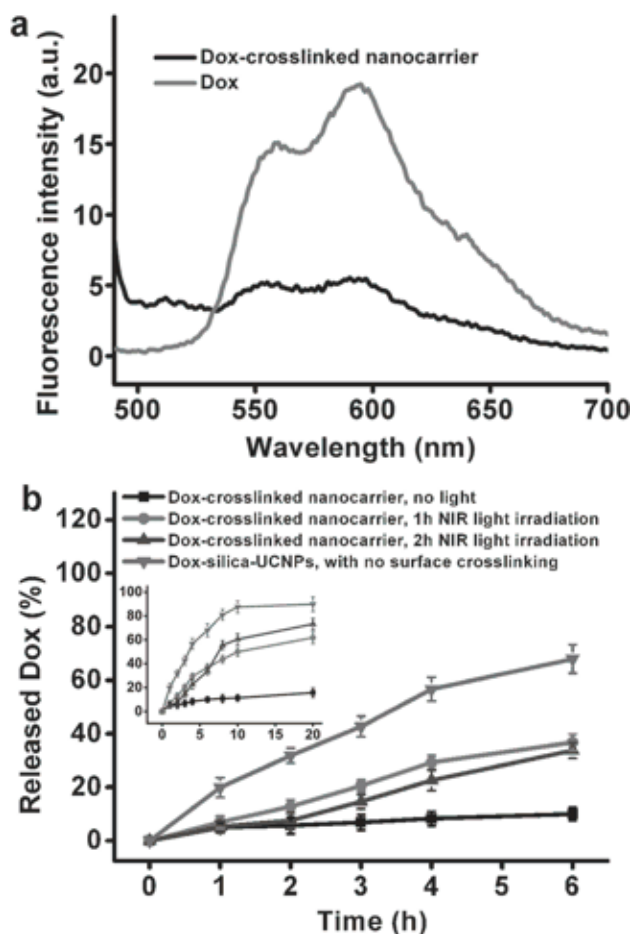
**Figure 1.** a,b) Size distributions of UCNPs and silica-UCNPs, determined by DLS and TEM; The insets are TEM images of UCNPs and mesoporous silica-UCNPs, respectively. c) Luminescence emission spectra of the UCNPs and silica-UCNPs. d) UV-Vis absorption spectra of silica-UCNPs, photocaged linker and photocaged linker functionalized silica-UCNPs (concentration of photocaged linker: 100  $\mu$ M, solvent: DMSO).

group at the end of the linkage. Absorption measurement shown in Figure 1d demonstrated the successful conjugation of  $1.6 \times 10^4$  photocaged linker molecules on the particle surface. Finally, intramolecular polymerization of photocaged linker using a ring-closing metathesis reaction under Grubbs' 2<sup>nd</sup> generation catalyst would result in the formation of the crosslinked nanocarrier which could be loaded with drug molecules for the selective photo-controlled release in the living system. The ability of cross-linked nanocarrier to encapsulate drug molecules was further studied with the commonly used antitumor drug Dox. Typically, the cross-linked nanocarrier was first soaked in a DMSO solution of Dox for 12 h. After removal of unbound drug molecules by centrifugation and repeated washing in PBS (10 mM, pH 7.2), the Dox uptake in the nanocarriers was determined by the difference in UV-Vis absorbance of Dox at  $\sim 480$  nm (Figure S2) and the loading efficiency was thus calculated to be about 2.33% (w/w), which was comparable with the results reported previously.<sup>[9]</sup>

After encapsulation of Dox drug molecules into nanocarriers, the photo-controlled drug release from the crosslinked mesoporous silica-UCNPs was investigated by analysis of fluorescence of Dox in a PBS buffer solution (10 mM, pH 7.2) in the presence or absence of NIR laser (980 nm) illumination. In general, Dox exhibited a red fluorescence emission at 590 nm with an excitation at 480 nm. The red fluorescence would be easily quenched when the Dox drug molecules were encapsulated or anchored into silica coated UCNPs nanocarriers, mostly attributed to the result of electronic energy transfer<sup>[10]</sup> (Figure 2a). In the absence of NIR laser irradiation, there was very weak fluorescence of Dox

observed in PBS buffer solution, suggesting the stability of the drug loaded silica-UCNPs in the buffer solution and only the very limited drug release observed from the cross-linked nanocarriers. As expected, in the presence of 980 nm light excitation, the solution of photocaged silica-UCNPs conjugate showed the significantly enhanced fluorescence, which could be due to the photo cleavage of caging 1-(2-nitrophenyl) ethyl group done by upconverted UV light from UCNPs. Figure 2b illustrated the correlation of integral fluorescence intensity of the released Dox drug molecules with the duration of NIR light irradiation. More than 75% of Dox release could be detected from silica-UCNPs nanocarriers within 20 hours upon 2 h NIR laser irradiation and even more Dox molecules could be removed from the crosslinked silica-UCNPs nanocarriers when prolonged light excitation was conducted. As a contrast, similar control experiments on the basis of the mesoporous silica-UCNPs nanocarriers but without crosslinked photocaged linker on the surface were also performed to investigate the release of Dox. As shown in Figure 2b, with time increasing, most of drug molecules could be directly released from silica-UCNPs through the process of uncontrolled passive diffusion. These results clearly indicated the fact that the photocaged mesoporous silica-UCNPs could provide a stable and reliable platform toward the controlled release of drug payload molecules upon 980 nm NIR photoactivation (Figure 2 and Figure S3).

Encouraged by the promising results of effective drug release in vitro, we further evaluated the activities of crosslinked silica-UCNPs conjugate as a photo-controlled nanocarrier for the light-responsive drug delivery into the



**Figure 2.** a) Fluorescence spectra of Dox and crosslinked nanocarrier solution with the same concentration ( $5 \mu\text{M}$  in PBS) under 480 nm excitation. b) Percentage of Dox released from crosslinked Dox-nanocarrier with different NIR light irradiation. Control: photocaged linker functionalized silica-UCNPs with Dox loading but no crosslinking on the surface.

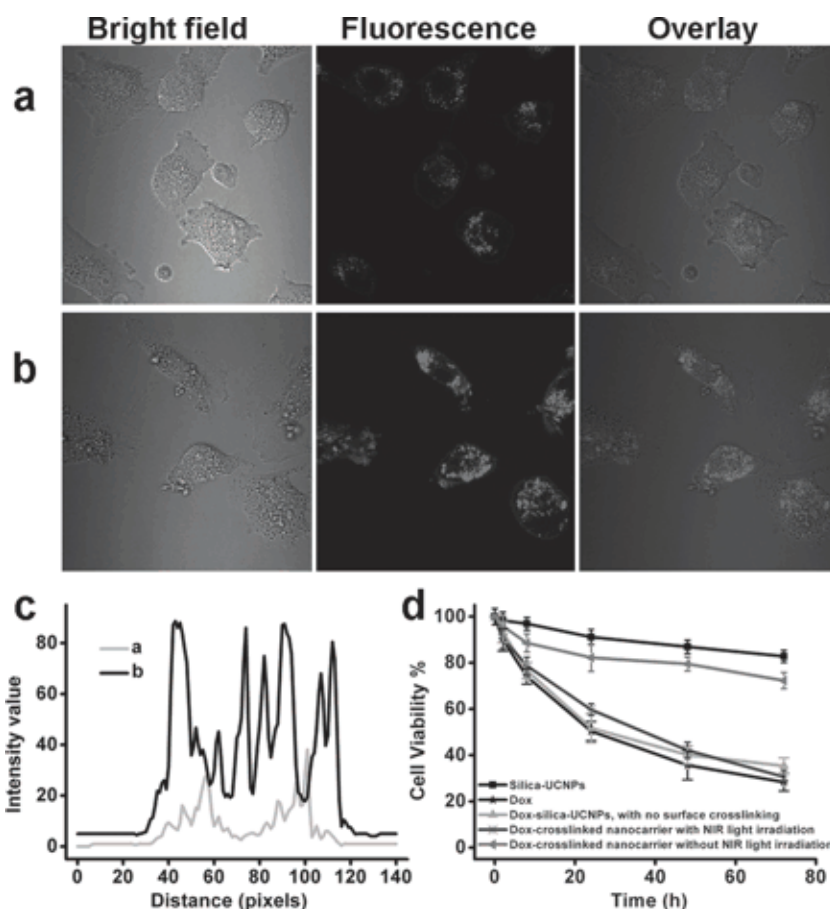
living cells. In this typical study, A-498 tumor cells were chosen to incubate with Dox encapsulated mesoporous silica-UCNPs conjugate ( $5 \mu\text{M}$ ). After 2 h incubation and followed by 2 h of 980 nm light excitation, the fluorescence signals observed in the cell cultures were used to investigate the process of controlled drug release. As shown in **Figure 3a**, before light irradiation, there was weak fluorescence signal detected in A-498 cells, mostly owing to the limited Dox release from the crosslinked silica-UCNPs and the fluorescence of capped Dox in the tiny silica pores was quenched as mentioned previously. But after 980 nm exposure, the crosslinked photocaged linker groups on the surface of nanocarriers were cleaved by the upconverted UV light and such efficient photolysis triggered the release of Dox molecules, which exhibited more significant red fluorescence in the cells when compared to the incubation system with Dox stored in the mesoporous silica-UCNPs but no light irradiation (**Figure 3c**). The significant difference in fluorescence indicated the controlled drug release in living cells upon the effective NIR light excitation.

To investigate the drug activities of photocaged silica-UCNPs nanocarriers in A-498 cells, we also performed

the cell viability measurements based on the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in **Figure 3d**, incubation of free mesoporous silica-UCNPs with cells could not lead to obvious cell viabilities. Similarly, after incubation of Dox encapsulated mesoporous silica-UCNPs nanocarrier with A-498 cells, there was no significant cytotoxicity observed in the absence of NIR light irradiation of the nanocarriers and only the limited Dox would be removed from the surface of mesoporous silica-UCNPs. However, upon 2 hours light irradiation of drug encapsulated nanocarriers, uncaging of crosslinked surface linkers would result in the controlled release of Dox payload molecules which led to the significant cytotoxicity in the cells. As controls, the similar MTT measurements were also performed by incubation of A-498 cells with free Dox and Dox adsorbed mesoporous silica-UCNPs without crosslinked photocaged linkers on the surface. Both free Dox and Dox loaded nanocarriers without photocaged linker demonstrated the comparable cell death under the same drug concentration. The higher cell viabilities could be observed in both of their cellular incubation when compared to the results in the Dox loaded nanocarrier systems with photocaged linker on the particle surface. There was limited cell viability detected in the photocaged nanocarriers throughout all the time points of cell incubation, and effective antitumor drug activity could be observed only after NIR light irradiation. Moreover, another negative control experiment with only 980 nm laser irradiation but no nanocarriers incubation in the cells indicated no obvious cytotoxicity in A-498 cells (**Figure S4**). These cell viability experiments suggested that this photocaged nanocarrier could work as a reliable platform for the controlled release of drug in living cells and NIR photolysis itself would not cause the notable cell viability.

Furthermore, in order to enhance the selective photo-controlled drug release in the targeted tumor cell lines, we also attach folic acid unit onto the surface of crosslinked mesoporous silica-UCNPs nanocarriers. One major reason to select FA is mostly attributed to its higher binding affinity toward the folate receptor, one tumor-associated protein commonly found on the surfaces of various tumor cell lines with higher level expression while with less expression in normal cells.<sup>[12]</sup> In this study, FA was conjugated to the surface of crosslinked photocaged silica-UCNPs through condensation reaction. The subsequent quantification based on UV-Visible measurements indicated that there were  $\sim 500$  FA molecules loaded on the nanoparticles. The FA functionalized photoactive nanocarriers were finally loaded with Dox molecules under the same condition as compared to the previous nanocarrier system and drug loading percentage determined by UV-Vis absorption measurement was about 2.1% w/w, which is similar to the photocaged nanocarriers without FA ligand conjugation on the surface.

In the process of cellular studies, HeLa cell lines were chosen as our main target due to the high expression of folic acid receptor in the cells. As a negative control, NIH/3T3 cells were used as there is no significant expression of folate receptor reported in this cell structure.<sup>[13]</sup> Both HeLa and NIH/3T3 cells were cultured and then incubated with

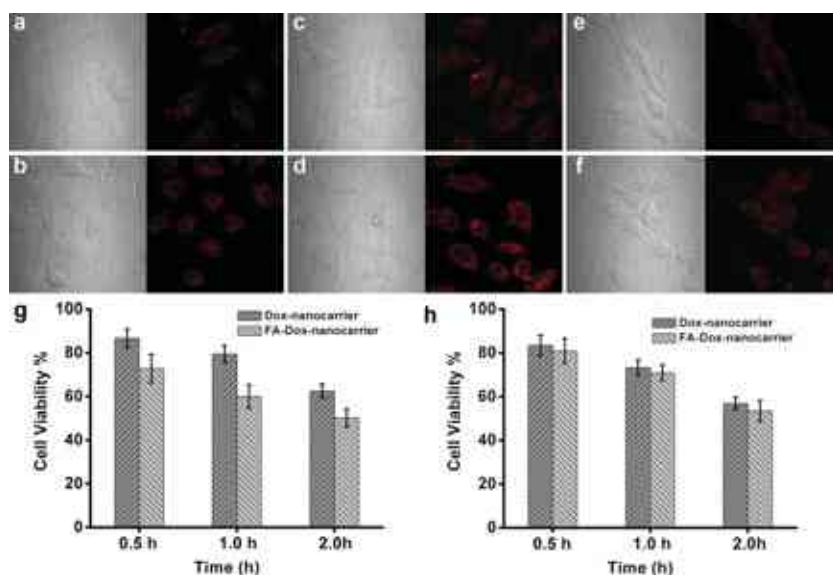


**Figure 3.** Bright field and confocal microscopy images of A-498 cell line. Cells incubated with photocaged nanocarrier for 2 h, a) before NIR light irradiation; b) after 2 h NIR light irradiation. c) Fluorescence intensity of cellular incubation before NIR irradiation and after NIR irradiation (measured by Image J).<sup>[11]</sup> d) Cell viability assay of A-498 cells upon the treatment with different nanocarriers.

FA-Dox-nanocarriers in Dulbecco's modified eagle medium (DMEM) at 37 °C to investigate the effective live cell fluorescent imaging and antitumor drug activities. Similar cell incubation with the Dox-nanocarriers but no FA affinity ligand on particle surface was also conducted, which was used as another negative control to further evaluate the cellular selectivity towards imaging and drug release. As shown in **Figure 4**, significant red fluorescence signals could be observed into the HeLa cells upon the short incubation with photocaged FA-Dox-nanocarrier and subsequent NIR light irradiation (Figure 4d). The similar intracellular incubation and following 980 nm laser irradiation of HeLa cells with photocaged Dox-nanocarrier but no FA ligand on the surface showed some fluorescence signals, however, the fluorescence was weaker than the HeLa cells incubated with FA-Dox-nanocarrier (Figure 4b). These results suggested that FA target units on the surface of photocaged silica UCNPs nanocarriers can work as affinity ligand to selectively direct the photo-controlled drug release in the folate receptor-rich tumor cell lines. The weak fluorescence signals were also found in the control experiment with the incubation of FA-Dox-nanocarrier in NIH/3T3 cells (Figure 4e). There was no significant cellular uptake enhanced by the specific recognition between FA and folate receptor in NIH/3T3 cells and

such limited fluorescence observed could be mostly contributed by the nonspecific binding of released Dox from nanocarriers upon NIR laser photolysis (Figure 4f).

Apart from the imaging results to investigate the photo-controlled drug release in living cells, the targeted intracellular antitumor activities were also studied in HeLa and NIH/3T3 cells by using two photocaged mesoporous nanocarriers with and without FA ligand, respectively. As shown in Figure 4g and h, cellular incubation and subsequent photolysis of photocaged nanocarrier without FA ligand in both HeLa and NIH/3T3 cells would lead to the cell viabilities in the cells, indicating the similar nonspecific cellular uptake of nanocarriers and photo-controlled Dox release in these two cells. Compare to the photocaged nanocarrier without FA units, the intracellular incubation and following light irradiation by using FA-Dox nanocarrier exhibited more significant cytotoxicity in the folic acid receptor-rich HeLa cells (Figure 4g), which was consistent with the results obtained in the imaging measurement. In the cell studies by using NIH/3T3 cell lines, introduction of both FA-Dox-nanocarrier and Dox-nanocarrier with no FA conjugation would not indicate the different cell viability even after NIR laser irradiation. Unlike the higher drug activities obtained in the HeLa cells with FA-Dox-nanocarriers, there were less antitumor activities



**Figure 4.** a–f): Confocal microscopy and bright field images of different cell lines. HeLa cells incubated with photoactive nanocarriers without FA a) before and b) after NIR light irradiation; HeLa cell line incubated with FA-Dox-nanocarriers c) before and d) after NIR light irradiation; NIH/3T3 cells incubated with FA-Dox-nanocarriers e) before and f) after NIR light irradiation. Time course of the cell viability of g) HeLa cells and h) NIH/3T3 cells treated with Dox-nanocarriers and FA-Dox-nanocarriers, respectively; all the samples were carried out with 980 nm laser irradiation and incubated for another 20 h incubation before MTT assay. The concentration of Dox was 5  $\mu\text{M}$  in all samples.

observed in NIH/3T3 cells when the cells were treated with FA-Dox-nanocarriers and followed by 980 nm laser irradiation. The low cell viability was mostly owing to the less cellular uptake of FA-Dox-UCNPs nanocarriers caused by the limited folic acid receptor expression in the NIH/3T3 cells. The targeted intracellular antitumor activities and imaging results unequivocally showed that FA-Dox-silica UCNPs may serve as an effective platform to selectively photo-control the drug release into the folate receptor over-expressed tumor cell lines without significant cellular damage caused by the NIR light irradiation. Moreover, the longer wavelength of NIR light applied here may also allow the deeper tissue penetration which could significantly benefit the further biomedical studies in the living systems with minimum unwanted side effects.

### 3. Conclusion

In this work, a NIR light-responsive crosslinked mesoporous silica-UCNPs drug delivery conjugate has been designed and synthesized. By capping the mesoporous silica-UCNPs with the crosslinked *o*-nitrobenzyl photoactivatable linker, the modified silica-UCNPs complex can serve as photocaged nanocarriers to encapsulate the payload molecules within the mesopores. After loading with antitumor drug molecules of Dox and followed by NIR light irradiation, the crosslinked *o*-nitrobenzyl photoactive linker on the particle surface could be efficiently cleaved by the converted UV light from UCNPs, which could effectively trigger the photo-controlled drug release into the living cells. Moreover, upon the functionalization of the photocaged nanocarriers with FA units,

the selective drug delivery can be easily achieved in the targeted tumor cell lines in which folate receptor has been highly expressed. This novel and effective drug loaded photocaged nanocarrier may demonstrate new possibility for the selective cell imaging and controlled drug release in the living system with less photo damage and deeper light penetration. Such promising applications greatly encourage us to conduct the targeted tumor imaging and drug delivery in vivo, which is currently under the process in the animal models.

### 4. Experimental Section

**Preparation of Photocaged Crosslinked Nanocarriers:** To a solution of photocaged linker (10 mg, 0.019 mmol) in *N,N*-dimethylformamide (DMF) (800  $\mu\text{L}$ ) was added *N*-hydroxysuccinimide (HOSU) (2.61 mg, 0.023 mmol) with an ice/water bath. Then 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (4.35 mg, 0.023 mmol) was added into the mixture and allowed to stir for 20 hours (Scheme S1, Supporting Information). The generated photocaged linker-

NHS ester was used in the next step synthesis without further purification.

Mesoporous silica-UCNP-NH<sub>2</sub> (20 mg) was suspended in DMF (400  $\mu\text{L}$ ), then the photocaged linker-NHS ester (1.2 mL, 20 mM) and *N,N*-diisopropylethylamine (DIPEA) (8  $\mu\text{L}$ ) was added. After overnight reaction, the photocaged linker functionalized mesoporous silica-UCNPs were collected by centrifugation and washed with DMF for three times. After re-suspension of surface functionalized silica-UCNPs together with Grubbs' 2<sup>nd</sup> generation catalyst (2 mg) in DCM (400  $\mu\text{L}$ ), the mixture was allowed to stir for 24 hours in dark condition under room temperature. Finally, the crosslinked mesoporous silica-UCNPs were obtained and washed with DCM and ethanol, respectively. The loading efficiency of crosslinked photocaged linker on the surface of mesoporous silica-UCNPs was estimated based on the absorbance of 350 nm. A calibration curve determined by the known concentration of photocaged linkage group was used as a standard.

**Preparation of FA Target Crosslinked Nanocarriers:** Folic acid (1 mg, 2.2  $\mu\text{mol}$ ) was mixed with HOSU (0.35 mg, 3  $\mu\text{mol}$ ) and EDC (0.45 mg, 2.35  $\mu\text{mol}$ ) in DMSO at room temperature. After 12 hours reaction, the surface crosslinked photocaged silica-UCNPs (10 mg) in DMSO were added and the reaction mixture was stirred for 12 h at room temperature in the dark. Finally, the folic acid target crosslinked nanocarrier was collected by centrifugation and washed three times in DMSO. The as-prepared FA target crosslinked nanocarrier was used for further investigation.

**Dox Loading on Crosslinked Nanocarrier/FA-Crosslinked Nanocarriers:** 200  $\mu\text{L}$  of Dox in DMSO (10 mg/mL) solution was mixed with 6.7 mg photocaged crosslinked nanocarrier or FA-crosslinked nanocarrier. The mixture was allowed to stir for 24 h in the dark. After the excessive Dox was removed by centrifugation at 10 000

rpm and washed with PBS buffer solutions for three times, the final products (Dox loaded nanocarrier with or without folic acid on the surface) was dispersed in PBS for the subsequent in vitro and cellular experiments. During the process of drug loading, the supernatant and washed solutions were collected and the loading efficiency was determined by measuring the UV absorbance of Dox at 480 nm (Figure S2, Supporting Information).

**Dox Release from Photocaged Nanocarriers by 980 nm Laser Irradiation:** The stock solutions (20  $\mu$ L, 25  $\mu$ M, in 10 mM PBS) of as-prepared drug loaded silica-UCNPs nanocarriers were transferred to 1.5 mL Eppendorf tube and then the solutions were illuminated with 980 nm laser at different time intervals (1–3 h). After light irradiation, the samples were diluted to 5  $\mu$ M and the amount of released Dox in the supernatant solution was monitored by using a fluorescence spectrophotometer with an excitation wavelength of 480 nm.

**Cell Culture:** A-498 cell line was purchased from the American-type culture collection (ATCC Cat No.: HTB-44) and maintained in DMEM medium containing 10% FBS (Invitrogen, Burlington, Canada). HeLa cells (ATCC No.: CCL-2) were cultured in RPMI-1640 medium with 10% FBS. NIH/3T3 cells (ATCC No.: CRL-1658) were cultured in DMEM medium with 10% FBS. All of the cell lines were cultured under humidified atmosphere of 5% CO<sub>2</sub> at 37 °C.

**Cellular Imaging:** A-498 cells (20  $\times$  10<sup>4</sup>) were placed in a 35-mm diameter  $\mu$ -dish plastic-bottom (ibidi GmbH, Germany) and cultured in medium for one day before the imaging measurement. After washing the cells twice with DMEM medium and followed by the treatment of DMEM medium (1 mL) containing 5  $\mu$ M of photocaged drug nanocarriers, the cells were further incubated for 2 h at 37 °C with 5% CO<sub>2</sub>. Then the cells were illuminated with 980 nm laser for 2 h (with 5 min break for each 30 min exposure). Upon light irradiation, cells were washed by Hank's balanced salt solution (HBSS) for three times. The fluorescence imaging measurements were conducted under a confocal fluorescence microscope (Nikon, Eclipse TE2000-E) with an excitation filter (535/50 nm) and an emission filter (610/75 nm).

For the FA target drug release experiments, HeLa and NIH/3T3 cell lines were seeded in 35-mm diameter  $\mu$ -dish plastic-bottom and cultured for 24 h in medium with density of 20  $\times$  10<sup>4</sup> per dish. After the cells were washed twice with culture medium, the cells were incubated with FA-Dox silica-UCNPs nanocarrier (5  $\mu$ M) for half an hour. Then the cells were irradiated with 980 nm light for 2 h (with 5 min break after each 30 min light excitation). After washing with medium and HBSS buffer solutions, the cells were ready for fluorescent imaging measurement.

**Cytotoxicity Assay:** The in vitro intracellular cytotoxicity was measured using a standard methyl thiazolyltetrazolium (MTT) assay. A-498 cells were seeded in a 96-wells plates with a density of 1  $\times$  10<sup>4</sup> per well in DMEM medium. After 24 h, the cells were treated with 5  $\mu$ M of photocaged drug nanocarriers in DMEM medium and then were further incubated for 2 h. Upon the appropriate light irradiation of cell samples and followed by addition of MTT solution (1.0 mg mL<sup>-1</sup>), the cell viability was evaluated by MTT assay as previously described.<sup>[14]</sup>

For FA targeted drug release experiments, HeLa and NIH/3T3 cells were cultured in 96-wells plates with a density of 1  $\times$  10<sup>4</sup> per well in medium for 24 h. The cells was washed with fresh medium and incubated with FA-crosslinked Dox mesoporous silica-UCNPs for half an hour. After incubation, the cells were washed with fresh

medium and irradiated with 980 nm laser for 2 h. Then the cells were cultured for 20 h at 37 °C with 5% CO<sub>2</sub> for the further MTT assays.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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