Near-infrared light-triggered drug release from UV-responsive diblock copolymer-coated upconversion nanoparticles with high monodispersity†

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The preparation of a new near-infrared (NIR) light-responsive nanocarrier for controlled drug release is demonstrated. Upconversion nanoparticles (UCNPs) were coated with an amphiphilic diblock copolymer through surface-initiated atom transfer radical polymerization, in which the inner block is hydrophobic, ultraviolet (UV)-sensitive poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) (PNB), and the outer block is hydrophilic poly(methoxy polyethylene glycol monomethacrylate) (POEG). The resulting polymer/UCNP nanocarrier is thermally stable in water over a wide temperature range (5–70 °C) and is uniform in size (120 nm hydrodynamic diameter, polydispersity index <0.1). The diblock copolymer self-assembly on the surface of each UCNP occurs in aqueous solution, which allows encapsulation of antitumor drugs like doxorubicin (DOX) by the hydrophobic “micelle-like” core of PNB surrounding the NIR-sensitive UCNPs. Under 980 nm laser exposure, the UV light emitted by the single UCNP is absorbed by the PNB inner layer, which results in cleavage of o-nitrobenzyl groups and formation of carboxylic acid groups. The increasing hydrophilicity of the diblock copolymer resulting from the NIR light-triggered photochemical reaction can thus disrupt the nanocarrier and leads to the release of DOX molecules. This diblock copolymer self-assembly-based approach to constructing NIR light-responsive nanocarriers of well-defined structures is general and offers possibilities for photocontrolled drug delivery.

Introduction

Photoresponsive polymers have been extensively used to construct nanocarriers for light-controlled drug delivery applications over the past decade or so.1–3 Most photoresponsive polymers require absorption of ultraviolet (UV) light for disruption of their nanocarriers. However, UV light has poor penetration depth through tissues and may damage healthy cells.4,5 In comparison with UV light, near-infrared (NIR) light has minimal phototoxicity and can penetrate deeply in live organisms because of its longer wavelengths in the “biological transparency window” resulting in less absorption and scattering by water and hemoglobin.6–8 Unfortunately, the majority of photoresponsive polymers cannot absorb NIR light to activate a specific photochemical reaction. Upconversion nanoparticles (UCNPs) are inorganic nanocrystals and can absorb NIR light to emit higher energy UV and visible light.9–11 Moreover, they have narrow emission peaks, low toxicity and excellent photostability, making them attractive for biomedical applications.12–15 In recent years, combining UCNPs and UV-sensitive polymers in hybrid nanocarriers has become a widely applied strategy to circumvent the direct UV excitation obstacle.16–23 The basic principle is to use NIR light to excite UCNPs, and the UV light emitted by UCNPs from inside the nanocarrier is then absorbed by the UV-sensitive polymer to carry out the photochemical reaction, leading to nanocarrier disruption and concurrent payload release.

To date, diverse methodologies have been put forward to organize UCNPs and UV-sensitive polymers in constructing nanocarriers for NIR light-triggered drug delivery. Of these, encapsulation of UCNPs in amphiphilic polymer assemblies is the most utilized approach.16,24–29 By employing this method, hydrophobic UCNPs and non-water soluble drugs tend to be entrapped in the hydrophobic core of polymer micelles, while the hydrophilic polymer corona stabilizes the whole nanocarrier in aqueous solution. Despite its simplicity and efficiency, the method often lacks control of UCNP and polymer coassembly in that two or more UCNPs can be loaded in one single micelle, resulting in heterogeneity and a high UCNP content. Recently, a...
method based on ligand exchange was proposed to assemble a UV-sensitive polymer around each UCNP.\(^{30}\) However, the ligand exchange approach may suffer from some shortcomings including the limited thickness of the polymer layer due to the use of a low molecular weight polymer and usually low polymer density because of the steric hindrance effect among polymer chains.\(^{31}\) and also, as a result of low grafting density, quenching of upconversion luminescence (UCL) intensity by the surrounding water molecules.\(^{32}\) Moreover, the combination of UCNPs and UV-sensitive polymers through a silica layer was also investigated. Cerruti et al. reported a NIR light-triggered drug-delivery nanocarrier built up by coating the UCNP with silica which in turn is coated with a UV-cleavable nanogel.\(^{20}\) Despite this interesting progress, it is still challenging and is of fundamental interest to develop novel methodologies for the preparation of UCNP-loaded polymer assemblies of well-controlled architecture and investigate the NIR light-triggered release of payloads.

Herein we demonstrate an approach to preparing block copolymer micelles confining one single UCNP inside the core with high monodispersity. Fig. 1a shows a schematic illustration of the nanocarrier design. An amphiphilic diblock copolymer is grown from the UCNP with a hydrophobic, UV-sensitive polymer as the inner block and a hydrophilic polymer as the outer block. Micellization of the diblock copolymer on the UCNP is expected in aqueous solution, resulting in the UCNPs being surrounded by collapsed hydrophobic polymer chains. Upon NIR light excitation, the hydrophobic polymer absorbs the UV light emitted by the UCNP to undergo a photochemical reaction that transforms the hydrophobic block onto the hydrophilic block and thus causing the dissolution of the diblock copolymer with concurrent payload release. Fig. 1b shows the chemical structure of the amphiphilic diblock copolymer and the NIR light-triggered photochemical reaction. More specifically, the synthetic route to our designed nanocarrier has four steps (Fig. S1, ESI†). The first step is the silica coating of the UCNP with amine groups by the co-condensation of silane coupling agents, giving UCNP-NH₂. ATRP initiators, -Br, are then immobilized by the acylation reaction between UCNP-NH₂ and 2-bromoisobutryl bromide. The last two steps are for the growth of an amphiphilic diblock copolymer through ATRP polymerization, the hydrophobic UV-sensitive poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) (PNB) first, followed by the hydrophilic poly[methoxy polyethylene glycol monomethacrylate] (POEG). Upon 980 nm light excitation, the UV upconversion emissions of the UCNPs can be absorbed by the PNB blocks, inducing the photodecay of o-nitrobenzyl (ONB) groups; the resulting carboxylic acid groups on the polymer will increasingly shift PNB toward hydrophilic and thus disrupt the micelle on the UCNP. As shown below, uniform-sized nanocarriers can be obtained, and NIR light-triggered micelle disruption and release of an antitumor drug, tested using encapsulated doxorubicin (DOX), is achievable with a single UCNP in the micelle core.

**Experimental**

**Materials**

All chemicals were of analytical grade and used without further purification except where noted. Chemical reagents were purchased from Sigma-Aldrich. 4,5-Dimethoxy-2-nitrobenzyl methacrylate (NB) and the UCNPs (NaYF₄:Yb/Tm@NaYF₄) were synthesized and characterized by following the procedures reported previously.\(^{16,33}\)

**Synthesis of aminated UCNPs (UCNP-NH₂)**

A silica layer with amine groups was coated on the surface of the UCNP by co-condensation of silane coupling agents in one pot. Typically, a chloroform (CHCl₃) solution of the UCNP (100 mg) was added to an aqueous solution (80 mL) of cetyltrimethylammonium bromide (CTAB, 200 mg). The resulting turbid microemulsion was stirred at 60 °C to remove CHCl₃. Then this solution was heated to 70 °C, and the pH was adjusted to 8–9 using 0.1 M sodium hydroxide (NaOH) solution. Afterwards, a solution composed of 150 µL tetraethyl orthosilicate (TEOS) and 350 µL of anhydrous ethanol (EtOH) was added dropwise to the above solution. After 0.5 h reaction, a mixture of (3-aminopropyl) triethoxysilane (APTES, 250 µL) and EtOH (450 µL) was added. The whole solution was kept at 70 °C for 4 h and stirred overnight at room temperature. Finally, the product of UCNP-NH₂ was isolated by centrifugation with EtOH and redispersed in acetone. This washing procedure was repeated four times to remove the unreacted species, and sample recovery was carried out by lyophilization. The resulting white powder UCNP-NH₂ was used without further purification.

**Synthesis of initiator-coated UCNPs (UCNP-Br)**

The powder of UCNP-NH₂ (100 mg) and triethylamine (TEA, 0.16 mL) were first added to anhydrous toluene (10 mL). Next, this solution was cooled in an ice-water bath. Then 170 µL of 2-bromoisobutyryl bromide was slowly added to the solution. After keeping it in the ice bath for 2 h, the reaction mixture was heated to room temperature and stirred continuously overnight. Afterwards, the UCNP-Br was washed several times with first abundant acetone/water solution (v/v, 1/1) and then with acetone.
Finally, it was precipitated by centrifugation until supernatants became clear and collected by lyophilization.

**ATRP of NB on UCNP for UCNP@PNB**

The UCNP-Br (125 mg), the NB monomer (655 mg), tris-[2-(dimethylamino)ethyl]amine (Me₂TREN, 37 µL) and DMF (2 mL) were first placed in a 5 mL one-neck flask. Next, 20 mg of Cu(i)Br was added into the solution under argon gas protection. The mixture was then degassed three times using the freeze–pump–thaw procedure and sealed under vacuum. After stirring at room temperature for 10 min, the whole setup was placed in a pre-heated oil bath (90 °C) for 12 h. Afterwards, the reaction was stopped with liquid nitrogen. Finally, the solution was precipitated into methanol once and centrifuged with DMF several times. The light brown UCNP@PNB was collected through lyophilization.

**ATRP of OEG on UCNP@PNB for UCNP@PNB-b-POEG**

In this step, UCNP@PNB was used to initiate polymerization of OEG (M₆, 500 g mol⁻¹) to grow the second block of hydrophilic POEG. The OEG monomer was purified by passing through a column filled with basic alumina to remove the inhibitor. DMF (2.5 mL), UCNP@PNB (200 mg), OEG (2.5 mL) and PMDETA (105 µL) were charged into a one-neck round-bottom flask. Cu(i)Br (28.6 mg) was then added under argon gas protection. The whole solution was degassed three times using the freeze–pump–thaw procedure and sealed under vacuum. The reaction was carried out at 90 °C for 1.5 h. UCNP@PNB-b-POEG was collected by centrifugation (20,000 rpm × 15 min) with DMF three times, dialyzed against deionized (DI) water for three days (3500 MWCO dialysis tubing from Spectra/Por) at room temperature and then recovered by lyophilization.

**Cleavage of grafted diblock copolymer chains**

Hydrofluoric acid (HF, 48 wt%, 0.3 mL) was added to a plastic bottle containing a tetrahydrofuran (THF, 5 mL) solution of UCNP@PNB-b-POEG (20 mg) under constant stirring. (Caution: HF is extraordinarily corrosive, and all operations with aqueous HF should be conducted in a fume hood with suitable personal protective equipment.) After keeping at room temperature for 4 h, this solution was neutralized with NaHCO₃. To completely remove impurities, the supernatant after centrifugation was diazylated against DI water (frequently refreshed) for 24 h. The cleaved polymer was collected by freeze-drying.

**Preparation of DOX-UCNP@PNB-b-POEG**

To load a drug in the diblock copolymer layer on the UCNP, doxorubicin hydrochloride (DOX·HCl, 4 mg) was dissolved in 2.5 mL of DMF and treated with 4 µL of TEA for 4 h. Subsequently, 20 mg of UCNP@PNB-b-POEG was added to this solution, which was then stirred overnight using a magnetic stirring bar in the dark. The resulting solution was dialyzed against Tris buffer solution (pH 7.4) to remove free drug molecules for 3 days. The obtained solution was concentrated by centrifugation and the sample was kept in a refrigerator and used within one month.

**Photolysis of UCNP@PNB-b-POEG under 980 nm excitation**

The aqueous solution of the UCNP@PNB-b-POEG nanocarrier (5 mg mL⁻¹, 0.5 mL) was placed in a dialysis cup (MWCO 3500). By inserting it on top of a quartz cuvette filled with aqueous solution, molecules photocleaved from UCNP@PNB-b-POEG can diffuse through a membrane into the bottom solution in the cuvette. During the irradiation experiment, the whole setup was irradiated by a continuous-wave (CW) 980 nm NIR laser from the top for a number of consecutive cycles of 5 min irradiation followed by 5 min irradiation-off in order to avoid overheating of the solution. Then the absorption spectra of the bottom solution were recorded.

**NIR light-triggered release of drug molecules**

To initiate the release of DOX loaded in UCNP@PNB-b-POEG, the aqueous solution of DOX-UCNP@PNB-b-POEG (3 mg mL⁻¹, 0.4 mL) was placed in the dialysis cup and subjected to 980 nm NIR light irradiation under the same conditions as described above. Likewise, DOX molecules released from the nanocarrier into the solution in the dialysis cup can diffuse into the bottom solution in the cuvette, and the absorption spectra of the bottom solution were recorded every 10 min during the experiment.

**Characterization**

The morphology of the surface coated UCNP was examined using a Hitachi H-7500 transmission electron microscope (TEM) at an accelerating voltage of 80 kV. TEM specimens were prepared by adding dilute sample solution (~10 µL) dropwise onto carbon-coated copper grids while allowing the solvent to evaporate completely and then stained with phosphotungstic acid (PTA). Infrared spectra were recorded on a Bomem FTIR spectrometer (ABB MB104PH) using the diffuse reflection mode. Thermogravimetric analysis (TGA) was performed in an argon atmosphere at a heating rate of 10 °C min⁻¹ from room temperature to 700 °C (SETSYS, TG-DTA 1600). Size exclusion chromatography (SEC) measurements were performed on a Waters system equipped with a photodiode array detector (PDA 996) and a refractive index detector (RI 410). THF was used as the eluent at an elution rate of 1 mL min⁻¹, while polystyrene standards were used for calibration. Dynamic light scattering (DLS) was carried out on a Malvern Zetasizer Nano ZS ZEN3600 system with a helium-neon laser (wavelength, λ = 633 nm) in a quartz cuvette. To investigate the effect of temperature on the size of nanoparticles, samples were kept for 15 min at each temperature before the measurement. All measurements were carried out at a scattering angle of 173°. The photolysis of o-nitrobenzyl groups, as well as the release of drug molecules, was monitored by recording the UV-vis spectra on a Varian 50 Bio UV-vis spectrophotometer. The upconversion luminescence (UCL) spectra of as-synthesized nanoparticles in solution in a quartz cuvette with 10 mm path length were recorded using a double-monochromator Fluorolog 2 instrument from Spex. A power-adjustable 980 nm laser diode (MDL-H-980 nm-4 W, Changchun New Industries Optoelectronics Tech. Co., Ltd) was employed as the upconversion pump source for the UCL measurement as well...
as for the NIR irradiation experiments. The cleavage of o-nitrobenzyl groups from the PNB-b-POEG grafted UCNP with or without loaded DOX upon UV light irradiation was conducted utilizing an OmniCure® Series 1000 UV lamp with a 365 nm filter (approximately 1 mW cm⁻²). In this experiment, the emission spectra of DOX ($\lambda_{ex} = 480 \text{ nm}$) from UCNP@PNB-b-POEG were recorded immediately on a Varian Cary Eclipse fluorescence spectrophotometer after each irradiation period. For the control test, the solution free of UV irradiation was kept in the dark and the emission spectra were taken at the same time points.

Results and discussion

1. Characterization of UCNP@PNB-b-POEG

The NaYF₄:18%Yb/0.5%Tm@NaYF₄ (UCNP) was synthesized using a thermal decomposition method according to our previously reported work. As shown in Fig. 2a, these uniform nanocrystals have a rod-like shape with an average dimension of 34.7 ($L$) × 22.1 ($W$) nm. Then a silica layer with amine groups was coated onto the surface of the UCNP (UCNP-NH₂) by the co-condensation of TEOS and APTES through a one-pot method. It should be mentioned that the residual surfactant (CTAB) was retained for the next steps of modification. Afterwards, ATRP initiators were anchored on its surface to obtain UCNP-Br through the acylation reaction between UCNP-NH₂ and N-bromoisobutyryl bromide. To determine whether the silica layer was coated on the UCNP to form a core–shell structure and the diblock copolymer was grown from the silica layer surface after ATRP, TEM measurements were performed. The core–shell structure of UCNP-NH₂ was first revealed by the TEM observation (Fig. S2, ESI†). The TEM specimens of UCNP-Br were stained with PTA for comparing the difference before and after grafting the diblock copolymer under the same conditions. As shown in Fig. 2b, the centered UCNP is surrounded by a light-gray ring as the outmost layer (SiOₓ–Br) with an average thickness of 6 nm. Thus, the average size of UCNP-Br is 47 × 34 nm before further functionalization. Afterwards, UV-sensitive PNB and hydrophilic POEG polymers were grown by surface-initiated ATRP, successively. Negative staining TEM images in Fig. 2c and d show that the outmost layers are different from that shown in Fig. 2b and the core–shell–shell structured nanoparticles are observed. Moreover, the length of the polymer layer increased from ~30 nm for PNB to ~42 nm for PNB-b-POEG. Therefore, the average size of UCNP@PNB-b-POEG is estimated to be 120 nm (dry state) assuming that it is spherical. More TEM images are provided in the ESI† (Fig. S3–S5).

To calculate the grafting density of polymer chains and initiation efficient, thermogravimetric analysis was performed. As shown in Fig. S6 (ESI†), three main regions of weight loss were found (i) the loss of moisture ($T < 80 \text{ °C}$), (ii) the decomposition of residual CTAB ($80–150 \text{ °C}$), and (iii) organic species (APTES/initiators/polymers) plus co-condensation of the silica matrix ($T > 250 \text{ °C}$). For simplicity, we assume that no dehydration of silica takes place at 250–450 °C and the initiation efficiency of PNB-Br is 100%. In addition, the molecular weight of PNB-b-POEG ($M_a 10.9 \times 10^5 \text{ g mol}^{-1}$, PDI 1.48) was obtained from the SEC (Fig. S7, ESI†) by the etching silica layer with HF. Based on the above information, the estimated initiator and polymer grafting densities (ESI†) are approximately 4.4 and 0.26 chains per nm², respectively. Notably, the initiator grafting density of UCNP-Br (4.4 chains per nm² or 0.34 mmol g⁻¹) is a relatively high value. Furthermore, the initiation efficiency is around 6%. This relatively low value is likely to be caused by the large bulky groups of the monomers.

FTIR spectroscopy also confirmed the successive steps of UCNP surface coating (Fig. 3). With the organic ligand of oleic acid (OA) on the nanoparticle surface, the IR spectrum of the UCNP shows characteristic bands at 2926 (–CH₃ stretching), 2853 (–CH₂– stretching), 1447 (C–H bending) and 1556 cm⁻¹ (COO– asymmetric stretching) of OA molecules (Fig. 3a). In comparison with the FTIR spectrum of the UCNP, new characteristic bands at 1034 (Si–O stretching), 1511 (N–H bending) and 1636 cm⁻¹ (N–H shear bending) appear in the spectrum of UCNP-NH₂ (Fig. 3b). It is worth noting that there is a little CTAB left in UCNP-NH₂ as revealed by the C–H stretching band at 3000–2800 cm⁻¹. For UCNP-Br, new absorption bands appear at 1535 (C–N in NH–O=C stretching), 1703 (C=O stretching) and 3319 cm⁻¹ (stretching vibration of secondary amide N–H), which confirms the immobilization of initiator groups (Fig. 3c). After growth of the first polymer block of PNB, the spectrum of UCNP@PNB displays the presence of nitro groups by 1333 (symmetric vibration) and 1524 cm⁻¹ (asymmetric vibration) (Fig. 3d). In addition, the characteristic band at 1732 cm⁻¹ assigned to the ester carbonyl stretch of the methacrylate also proves the formation of PNB. After the second ATRP for POEG, the characteristic peaks of UCNP@PNB-b-POEG at 2876 (C–H stretching), 1728 (ester carbonyl stretching) and 1100 cm⁻¹ (C–O–C stretching) are enhanced due to overlapping of the absorptions of the two polymers, indicating that...
the second POEG block had grown from the UCNP@PNB surface (Fig. 3e).

To reveal whether or not the grafted diblock copolymer can absorb the emitted UV light of the UCNP, extinction and upconversion luminescence (UCL) spectra are shown in Fig. 4. The inset of Fig. 4 shows a picture of an aqueous solution of UCNP@PNB-b-POEG upon 980 nm excitation. The emitted visible light from the UCNP is readily observed where the laser beam passes through the solution (the scattering of the main emission at 450 nm gives the apparent blue color of the solution). Also shown are the UCL spectra of neat UCNP, UCNP@PNB and UCNP@PNB-b-POEG, together with the absorption spectrum of UCNP@PNB-b-POEG. The intensity of the UV light emitted by the UCNP with respect to visible light (475 nm) emission is significantly reduced for the UCNP functionalized with UV-sensitive PNB. Since the SiO₂–Br layer has no influence on the upconversion emissions (Fig. S8, ESI†), the decreased UV light emission is mainly attributed to the absorption of the PNB layer. As confirmed by the UV-vis absorption spectra, o-nitrobenzyl groups in the grafted polymers show a strong absorption at ~350 nm, which overlaps with the emission of the UCNP in the UV region.

As mentioned above, it is of interest to prepare nanocarriers with high monodispersity. In the present case, it is difficult to assess from TEM images of UCNP@PNB-b-POEG that show apparent formation of aggregates. Therefore, DLS measurements were carried out to measure polydispersity of the designed nanocarrier. Fig. 5a shows the mean hydrodynamic diameter ($D_h$) and polydispersity index (PDI) for UCNP@PNB-b-POEG in water over a wide temperature range, indicating an almost constant size of 130 nm and a PDI around 0.07. This extremely low PDI (<0.1) confirms the near monodispersity of UCNP@PNB-b-POEG. Thus, the aggregates in TEM images (Fig. 2) are caused by the evaporation process during the preparation of the sample. By combining the DLS and TEM results, it is safe to say that each single UCNP is covered by the amphiphilic diblock copolymer and that the dispersion in water is thermally stable.

To investigate the NIR light-triggered drug release, the loading of an antitumor drug, doxorubicin (DOX), by UCNP@PNB-b-POEG was first examined. DLS measurements shown in Fig. 5b indicate that the average $D_h$ of the DOX-loaded NP is slightly larger than that without DOX encapsulation, from 130 to 140 nm. The inset pictures (Fig. 5c) show aqueous solutions of UCNP@PNB-b-POEG before and after DOX encapsulation. While the transparent solution of unloaded nanoparticles has a light-yellow color, the solution of DOX-UCNP@PNB-b-POEG becomes light-pink colored. The UV-vis spectra of DOX and the two solutions of nanoparticles are also shown. At first, the DOX molecules possess a strong absorption band in the 475–500 nm region, while DOX free UCNP@PNB-b-POEG has no absorption in the same region. By contrast, the nanoparticle solution after DOX encapsulation shows the absorption of DOX and the absorption band displays a slight (~5 nm) red-shift, which may be attributed to the π–π stacking interaction among drug molecules or between DOX and benzyl rings in PNB blocks within the micellar core.49 These results show clearly that the drug molecules are loaded in the diblock copolymer (likely in the hydrophobic PBN inner layer) surrounding the single-UCNP core. The pink color of the DOX-UCNP@PNB-b-POEG solution is caused by the absorption of loaded DOX.

2. Light-induced disruption of UCNP@PNB-b-POEG

The photochemical reactions of ONB-containing polymers activated by UV light (365 nm) are well known.39,41,42 In the present study, we also first applied UV light to induce the photocleavage reaction. As shown in Fig. 6, under UV light exposure of low intensity, the absorption band at 355 nm decreases continuously over irradiation time, indicating the removal of o-nitrobenzyl groups from UCNP@PNB-b-POEG. In addition, an isosbestic point appears at 380 nm when the 355 nm continuously decreases, implying the existence of two distinct absorbing species in equilibrium with each other at a given UV irradiation time.
we further went on to investigate the photocleavage of ONB groups initiated by a continuous-wave 980 nm diode laser. Owing to the small beam diameter (3 × 3 mm) of the NIR light laser, it is hard to collect the UV-vis spectra using a large volume of the nanocarrier solution like that in the UV irradiation experiment. While using a small volume, the overheating problem caused by 980 nm light is difficult to be avoided. Therefore, a setup was utilized to monitor the photocleavage reaction. As shown in Fig. 7a, a dialysis cup containing an aqueous solution of UCNP@PNB-b-POEG (0.5 mL) was immersed into water in a quartz cuvette. Once the photoreaction occurs, the cleaved molecules of low molecular weight can diffuse into the underneath solution through a dialysis membrane (MWCO 3500) for equilibrium. By tracking the UV-vis spectra of the solution in the cuvette, cleaved chromophores can be observed. Since the methacrylic acid groups formed after the cleavage of ONB from UCNP@PNB-b-POEG are pH sensitive, which may affect the stability of the nanocarrier. By tracking the UV-vis spectra of the solution in the cuvette, cleaved chromophores can be observed. Since the methacrylic acid groups formed after the cleavage of ONB from UCNP@PNB-b-POEG are pH sensitive, which may affect the stability of the nanocarrier, the photocleavage reaction of PNB in H₂O shows the hydrodynamic diameter (Dₜ) of the nanoparticles before (left) and after (right) DOX encapsulation.

Apparently, the peak at 355 nm decreases by about 17% after 90 min of low-intensity UV exposure.

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Fig. 6 Absorption spectra of UCNP@PNB-b-POEG (0.05 mg mL⁻¹, 1 mL) recorded after various time periods of 365 nm UV irradiation (1 mW cm⁻²), showing the photocleavage reaction of PNB in H₂O.

The solution in the dialysis cup was irradiated by a NIR laser from the top for a number of consecutive cycles of 5 min NIR irradiation-on followed by 5 min irradiation-off to avoid overheating of the solution. The absorption spectra of the solution in the cuvette were recorded every 1 h during the irradiation test. In Fig. 7b, the collected UV-vis spectra during the reaction are presented. When the sample was kept in the dark, the absorption in the 300–550 nm region was absent for over 15 h, indicating that there were no cleaved molecules in the solution. By contrast, the absorption band in the 300–425 nm region was observed after 1 h of 980 nm irradiation and increased in intensity with increasing the irradiation time, indicating the presence of the photocleaved nitroso compound in solution. To better reveal the effect of NIR light irradiation on the cleavage reaction, Fig. 7c plots the absorbance at 375 nm (in Fig. 7b) as a function of time. Upon switching on the 980 nm laser, the slopes of the curve in the 0–2, 3.5–5.5 and 18.5–20.5 h regions are larger than those in 2–3.5, 5.5–7 and 20.5–22 h regions during which the laser is off. These results confirm that the UV light emitted by the UCNP upon NIR irradiation can be absorbed by PNB blocks in UCNP@PNB-b-POEG to initiate the photocleavage of ONB groups and thus disrupt the nanocarrier.

After exposure to NIR light, the rest of the solution in the dialysis cup was taken out for further analysis. This solution was first dialysed against two litres of deionized water (pH ~ 7.0 and frequent change) for eight hours to remove any residual photocleaved molecules. Its UV-vis spectrum was then recorded and compared to the initial spectrum before the NIR light irradiation experiment on the UCNP@PNB-b-POEG solution. As can be noticed from Fig. 7d, the occurrence of photocleavage of ONB groups under 980 nm laser irradiation is indicated by the decreased absorption of ONB remained in the nanocarrier, with the absorbance at 350 nm decreased by about 16%. Fig. 7e shows the hydrodynamic diameter (Dₜ) of the nanoparticles before and after the 980 nm laser exposure. They swell from 128 (PDI 0.07) to 139 nm (PDI 0.126), indicating that NIR light induced the disruption of the nanocarrier due to shifting hydrophobic–hydrophilic balance in PNB chains. Indeed, as ONB groups are cleaved upon irradiation, carboxylic acid groups are formed and the PNB chains assume a more hydrophilic balance in PNB chains. Indeed, as ONB groups are cleaved upon irradiation, carboxylic acid groups are formed.
(ionized at pH 7), which makes the diblock copolymer chains more hydrophilic and adopt a more extended chain conformation, resulting in an increase in the hydrodynamic diameter (Fig. 7e). Moreover, the FTIR spectra shown in Fig. 7f for UCNP@PNB–b–POEG before and after 980 nm irradiation also show the effect of the photocleavage reaction. There is a broad characteristic band in the 2750–3300 cm\(^{-1}\) region, which is attributed to O–H stretching of the carboxylic acid groups. This is also consistent with the decrease in band intensity at 1524, 1728 and 2980 cm\(^{-1}\), implying diminishing carbonyl and nitro groups in UCNP@PNB–b–POEG after 980 nm irradiation. All these results indicate the occurrence of the photolysis reaction under 980 nm excitation, resulting in the conversion of carbonyl groups into carboxylic acid groups.

## 3. Photoinduced drug release from UCNP@PNB–b–POEG

After confirming the disruption of the UCNP@PNB–b–POEG using a 980 nm laser, the antitumor drug DOX was loaded in the nanocarrier and the NIR light-triggered drug release was investigated with DOX-UCNP@PNB–b–POEG in the same Tris buffer to keep pH constant during the process. The choice of DOX was made considering its excellent stability in Tris buffer solution\(^{43}\) and its widespread use in in vitro and in vivo studies.\(^{44,46}\) Likewise, DOX release upon direct low-intensity UV light irradiation. Under 365 nm irradiation, the absorbance at 350 nm gradually decreases to about 80%, indicating the occurrence of photocleavage reactions. However, the absorption band of DOX (475–500 nm) remains essentially unchanged in the meantime, which indicates the photostability of DOX under UV irradiation. As for the fluorescence spectra, there is a weak emission band in the 550–600 nm region before irradiation, indicating that the fluorescence of DOX is quenched after encapsulating into the nanocarrier. By contrast, after 2 h of UV exposure the fluorescence intensity increases by 30%. To better observe the change during the whole period of the experiment, Fig. 8c shows the plot of emission intensity at 585 nm vs. UV exposure time. The intensity gradually increases with the increasing irradiation time, while it remains constant in the control test where no UV was applied to the nanocarrier solution. These results confirm that UV light induced disruption of DOX-UCNP@PNB–b–POEG can lead to the changing micro-environment of DOX molecules. In the beginning, the fluorescence intensity of DOX is significantly quenched by the hydrophobic region of micelles, which is consistent with a previous report.\(^{47}\) Upon 365 nm irradiation, the hydrophobic region turns increasingly into hydrophilic due to the photocleavage reaction, which gives rise to the dissolution of DOX into the aqueous solution and thus enhances the emission intensity.

To further prove the release of DOX from DOX-UCNP@PNB–b–POEG under low-intensity UV irradiation, the setup with a dialysis cup immersed in water in a cuvette (Fig. 7a) was utilized. The fluorescence emission of DOX was indeed observed in the
solution underneath the dialysis cup containing the DOX-UCNP@PNB-b-POEG solution subjected to UV irradiation, indicating the release of DOX molecules from the nanocarrier and diffusion through the membrane into the solution. The amount of DOX released was monitored by recording the fluorescence emission intensity at 585 nm of the bottom solution; the results are shown in Fig. 8d. Over the 16 h period prior to UV irradiation, only a little DOX was detected. However, when the solution was exposed to 365 nm UV of 1 mW cm$^{-2}$ for 15 min, the release amount showed a threefold increase in the following 16 h.

Finally, the release of DOX molecules triggered by NIR light irradiation was investigated using the same setup. The DOX-UCNP@PNB-b-POEG solution in the dialysis cup was exposed to the 980 nm laser from the top. The absorption spectra of the bottom solution were taken every 10 min, i.e., after each cycle of 5 min irradiation followed by 5 min irradiation off. The UV-vis spectra collected in the process are presented in Fig. 9a. Without NIR light illumination, after 2 h of immersion of the dialysis cup with water in the cuvette, the bottom solution basically has no absorption in the 300–700 nm region, indicating little DOX diffusion from the top solution of DOX-UCNP@PNB-b-POEG across the membrane and thus the DOX encapsulation stability. However, after NIR irradiation of the top solution, absorption bands of DOX in the 450–550 nm region are detected, indicating the release of DOX molecules from DOX-UCNP@PNB-b-POEG as a result of NIR induced photocleavage of ONB groups on the PNB blocks. Shown in Fig. 9b is the plot of absorbance at 497 nm as a function of time, and the change in the amount of DOX molecules in the bottom solution is better revealed. Before 980 nm laser irradiation, the absorbance increases slightly up to 120 min. After switching on the 980 nm laser, the absorbance increases significantly in the 120–240 min period, indicating the NIR light-induced disruption of DOX-UCNP@PNB-b-POEG and the concomitant drug release. Then, after switching off the 980 nm laser during the period of 240–300 min, no further increase in absorbance is observed; instead, there appears to be a slight decrease, implying some kind of equilibrium of DOX molecules across the solution. Upon switching on the NIR light again in the 300–420 min period, the absorbance starts to increase again, indicating resumed nanocarrier disruption and drug release. However, the increase is smaller than in the first NIR light period. This is understandable, because under continuous exposure to 980 nm laser, the nanocarrier disruption and DOX release should eventually reach a stationary state with no further change observed. All these results confirm that the release of DOX molecules from UCNP@PNB-b-POEG can be activated by NIR light.

**Conclusions**

In this work, a bottom-up strategy has been put forward to prepare a NIR light-responsive nanocarrier with a high degree of monodispersity (PDI < 0.1) based on combining a UCNP...
with a photoresponsive polymer. We showed that coating a thin silica layer (~6 nm) on the surface of the UCNP facilitates the incorporation of ATRP initiators through covalent bonding, which allows the use of surface-initiated controlled radical polymerization to grow an amphiphilic diblock copolymer comprising PNB (a UV-sensitive hydrophobic polymer) and POEG (a hydrophilic polymer). The rationally designed UCNP@PNB-Pb-POEG was investigated as a new NIR light-responsive nanocarrier for controlled drug delivery. In aqueous solution, each UCNP is covered by a hydrophobic PNB inner layer and a water-soluble POEG corona ensuring the stable dispersion of the nanoparticles. The nanocarrier, in a sense, is like a self-assembled diblock copolymer micelle whose hydrophobic core contains one single UCNP. Our study found that despite the single UCNP at the center of each diblock copolymer assembly, upon 980 nm NIR light irradiation, UCNP-emitted UV light is absorbed by the UV-labile PNB, resulting in photocleavage of o-nitrobenzyl groups and, consequently, making the PNB block increasingly hydrophilic due to the formation of carboxylic acid. This photoreaction under NIR light excitation was found to shift the hydrophobic–hydrophilic balance of the diblock copolymer assembly on the UCNP surface, which gives rise to disruption of the nanocarrier and the concomitant release of encapsulated DOX molecules. In all, we have demonstrated, for the first time to our knowledge, an approach to preparing a NIR light-triggered drug release nanocarrier using an UCNP-assisted photolysis reaction in diblock copolymer self-assembly surrounding the UCNP. This general method offers new possibilities for further development and exploitation for applications of UCNP/polymer hybrid materials of controlled structures.

Conflicts of interest
There are no conflicts to declare.

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Notes and references