

Electronic Supplementary Information

Smart pH- responsive upconversion nanoparticles for enhanced tumor cellular internalization and near-infrared light-triggered photodynamic therapy

Sheng Wang,^a Lei Zhang,^b Chunhong Dong,^a Lin Su,^a Hanjie Wang*^a and Jin Chang*^a

^a Institute of Nanobiotechnology, School of Materials Science and Engineering, Tianjin University and Tianjin Key Laboratory of Composites and Functional Materials, Tianjin 300072, PR China. E-mail: jinchang@tju.edu.cn (Jin Chang), wanghj@tju.edu.cn (Hanjie Wang)

^b School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, PR China.

EXPERIMENTAL SECTION

Materials.

NaYF₄: Yb, Er (Y: Yb: Er = 78: 20: 2) was synthesized in our lab. Dextran (*M_w* = 10000), Rose Bengal (RB), 4-formylbenzoic acid and adipic dihydrazide (AD) were purchased from Aladdin. Octadecylamine (OA), stearic acid (SA), 6-bromohexanoic acid, folate (FA), bromoacetic acid, 4-(dimethylamino)pyridine (DMAP), poly(ethylene glycol) monomethyl ether (mPEG, *M_n* = 2000), and 9,10-anthracenediylbis-(methylene)dimalonic acid (ABDA) were obtained from Sigma-Aldrich. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were ordered from GL Biochem Ltd. (Shanghai, China). All other chemicals were of reagent grade and were used as received.

Synthesis of dextran-SA (DS), RB-DS, dextran-AD-OA (DAO) and PEG-DAO

The DS was synthesized similarly to the method reported in our previous work.¹ As shown in Scheme S1A, SA (100 mg), DMAP (15 mg) and EDC (100 mg) were dissolved in 20 mL of DMSO. Dextran (200 mg) was dissolved in 20 mL of DMSO. Then mixed the two solutions and stirred at room temperature for 24 h, After completion of the reaction, the solution was dialyzed (MWCO: 3500 Da) against DMSO for 24 h and then pure water for 48 h. Finally, the solution was lyophilized.

The RB-DS was synthesized as shown in Scheme S1B. The Rose Bengal hexanoic acid ester (RB-HA) was first obtained by reacting RB with 6-bromohexanoic acid.² To covalently conjugate RB to DS, 10mL of DMSO solution containing 10 mg of RB-HA, 30 mg of EDC, 5 mg of DMAP and 100 mg of DS was stirred vigorously at room temperature for 24 h. Then the solution was dialyzed (MWCO: 3500 Da) against pure water for 48 h to remove any unreacted RB. And then the solution was lyophilized.

The PEG-DAO was synthesized as shown in Scheme S1C. Dextran was first carboxymethylated using bromoacetic acid to obtain carboxymethyl dextran (CMD).³ Then the DAO was synthesized as follows: CMD (100 mg), EDC (400 mg) and NHS (240 mg) were dissolved in 20 mL of DMSO and the solution was stirred for 30 min to activate the carboxylic acid of CMD. OA (50 mg) was dissolved in 10 mL of DMSO and then added to the solution. The mixture was stirred at room temperature for 6 h. Then, 10 mL of AD solution (5 mg mL⁻¹ in DMSO) was added to the mixture and stirred for an additional 6 h. After reaction completion, the mixture was dialyzed (MWCO: 3500 Da) against ethanol for 24 h and then pure water for 48 h and then lyophilized. Poly(ethylene glycol) with 4-formylbenzoic acid terminal (PEG-CHO) was prepared by reacting mPEG with 4-formylbenzoic acid.⁴ PEG-DAO was prepared as follows:⁵ DAO (100 mg) and PEG-CHO (100 mg) were dissolved in 20 mL of DMSO and reacted for 24 h in dark at room temperature. Then the reaction solution was dialyzed (MWCO: 3500 Da) against pure water for 24 h. The polymer PEG-DAO was finally lyophilized and obtained as white powder.

Nuclear magnetic resonance spectroscopy (NMR) characterization

^1H NMR spectra was recorded on a JEOLGX 400 D spectrometer operating at 400 MHz using $(\text{CD}_3)_2\text{SO}$ as the solvent and tetramethyl silane as an internal standard at room temperature.

As shown in Figure S1, the intensive signals at $\delta = 4.50\text{-}4.93$ are attributed to dextran; the peaks at $\delta = 1.22, 0.84$ are attributed to SA or OA; the peak at 3.49 ppm ($-\text{OCH}_2\text{CH}_2-$) is attributed to PEG. All of these indicate that DS and PEG-DAO are successfully synthesized.

Self-assembly of UPPLVs

UCNs (NaYF_4) were synthesized in our lab according to a previously published procedure.⁶ The reverse-phase evaporation method was used to transfer UCNs into the aqueous phase. RB-DS (3 mg), FA-DS (5 mg) and PEG-DAO (12 mg) were dissolved in 4 mL of pure water. A 0.4 mL of UCNs solution (2 mg mL^{-1} in dichloromethane) was added to the polymer solution under sonication at 150 W output. Then the organic solvent was evaporated on a rotary evaporator to form the RB-UPPLVs suspension. The preparation of UPPLVs without RB is the same as the method mentioned above except replacing RB-DS with DS.

Physicochemical characterizations of the RB-UPPLVs

The morphology of the RB-UPPLVs was measured by TEM with an operating voltage of 200 kV and bright-field mode. The effective particle diameter of the RB-UPPLVs was determined by DLS. The fluorescence and UV spectrum was used to detect the emission peak strength change of different samples. The PSs loading efficiency (LE) was measured using a UV spectrophotometer, and the content of RB was measured at a wavelength of 549 nm. The LE of the samples was calculated by the following equation.

$$\text{LE (\%)} = \text{amount of RB/total weight of RB-UPPLVs} \times 100 \quad (1)$$

Singlet oxygen test

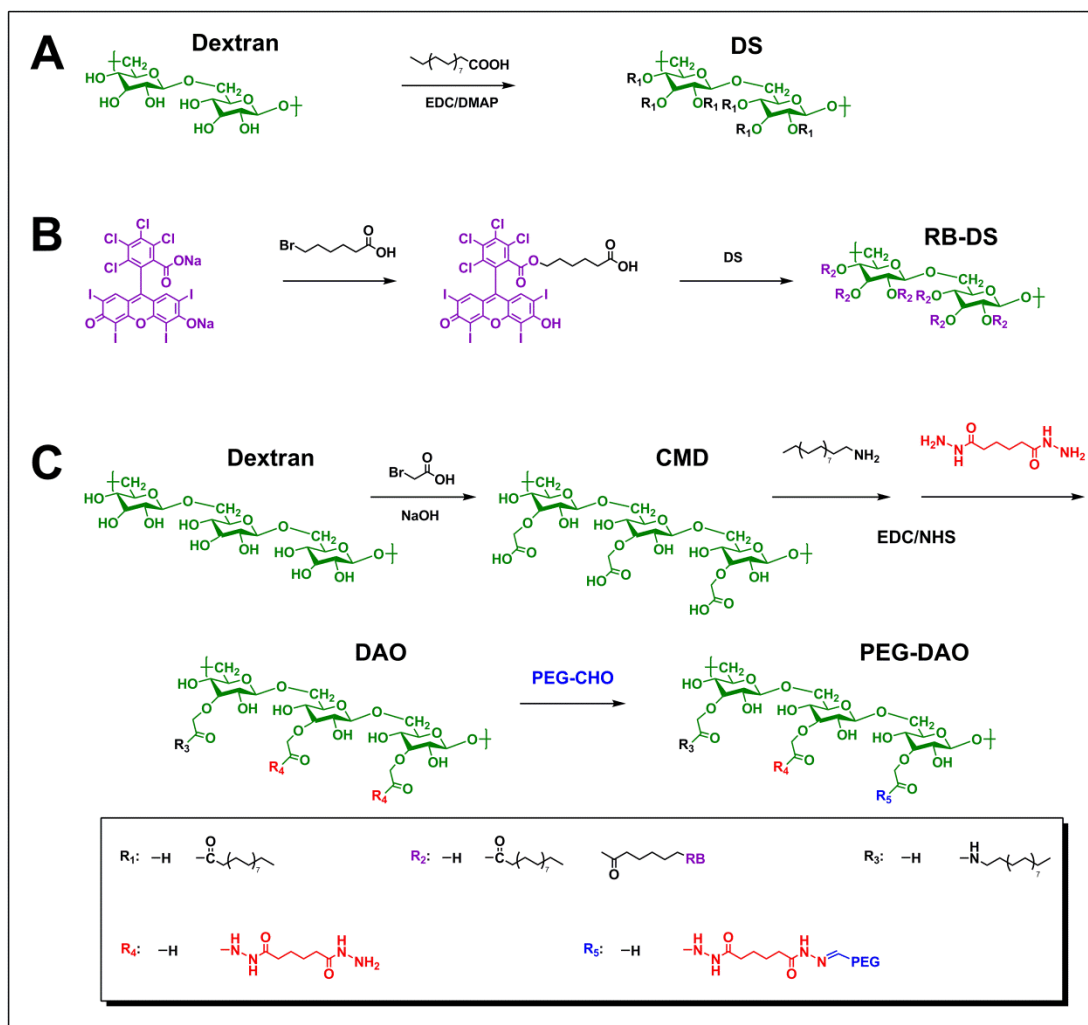
In brief, 0.1 mL of ABDA solution (0.2 mM) was added to 1.5 mL of a sample solution and the solution was irradiated with a 980 nm NIR laser at a power density of 2.5 W cm^{-2} for different times in the dark. The decrease of fluorescence intensity at 430 nm under a 380 nm light excitation was monitored as a function of singlet oxygen production.

Cellular uptake of the RB-UPPLVs

HeLa cells were seeded into a 24-well plate in 500 μL of DMEM with 10% FBS, and incubated at $37 \text{ }^\circ\text{C}$ for 24 h under an atmosphere of 5% CO_2 . Then, pretreated (at pH 5.0 or 7.4 for 3 h) RB-UPPLVs were added to the media and incubated for 2 h. The cells were washed by PBS. Subsequently, the cells were fixed with 4% (w/v) paraformaldehyde aqueous solution and then treated with 4', 6-diamidino-2-phenylindole (DAPI) for 15 min to stain the nucleus. The fluorescence images were observed using an inverted Olympus fluorescence microscope (IX-70).

***In vitro* PDT**

HeLa cells were seeded into a 96-well plate and incubated at $37 \text{ }^\circ\text{C}$ for 24 h in 100 μL of DMEM with 10% FBS under an atmosphere 5% CO_2 . Then 100 μL of samples was added into the wells and incubated for 4 h. After removal of nanoparticles, cells were washed by PBS and transferred into fresh media. After irradiation by the 980 nm NIR laser at power densities of 1.0 W cm^{-2} for 20 min, the cells were incubated for an additional 24 h. The *in vitro* photodynamic effects were assessed by cell viability according to the standard MTT assay as mentioned above.



Scheme S1. Synthesis process of DS (A), RB-DS (B) and PEG-DAO (C).

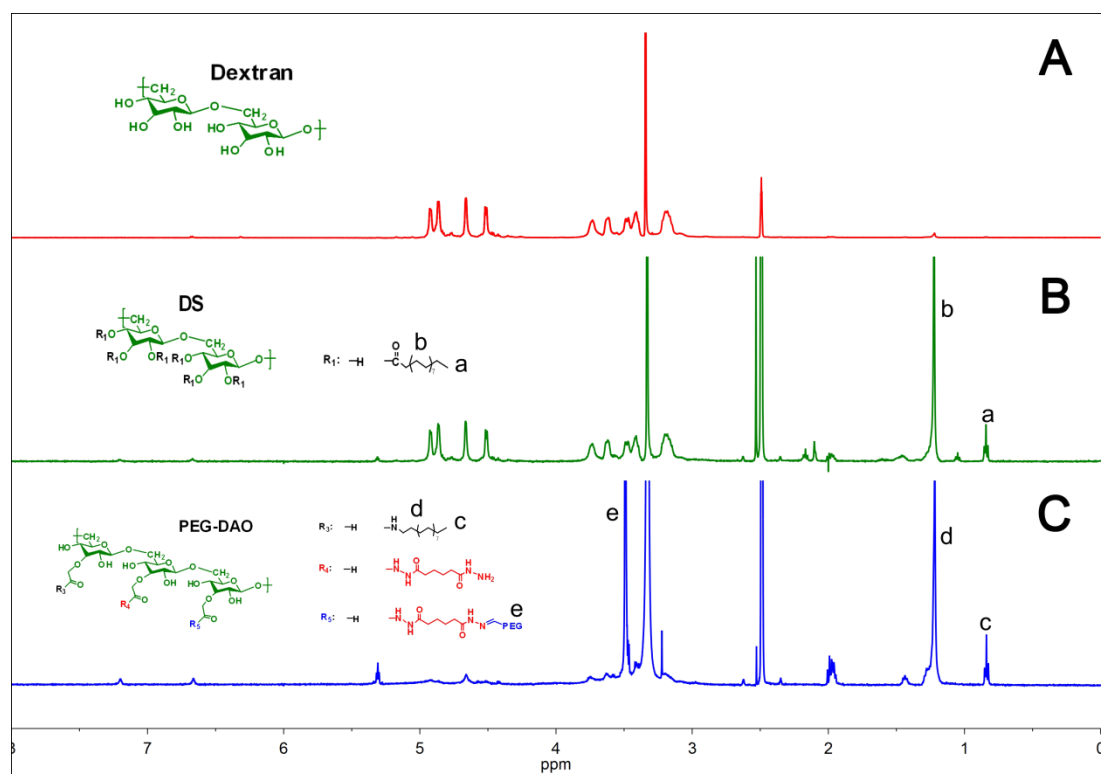


Figure S1. ¹H NMR spectra of dextran (A), DS (B) and PEG-DAO (C).

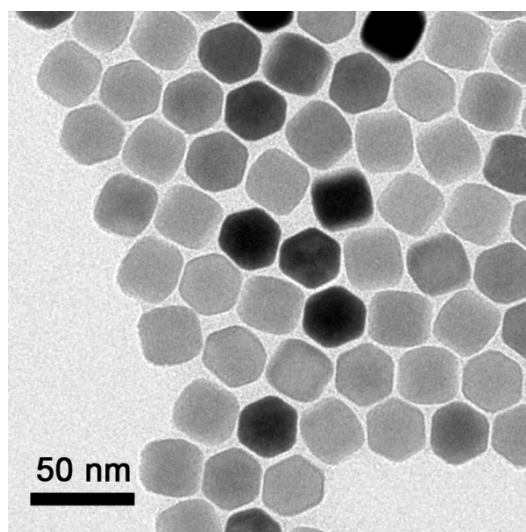


Figure S2. TEM image of the UCNs.

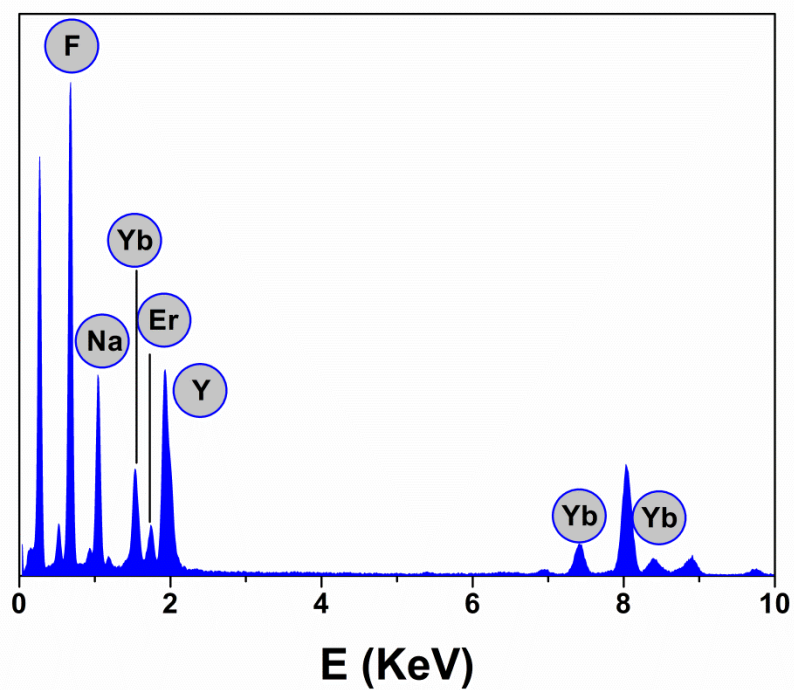
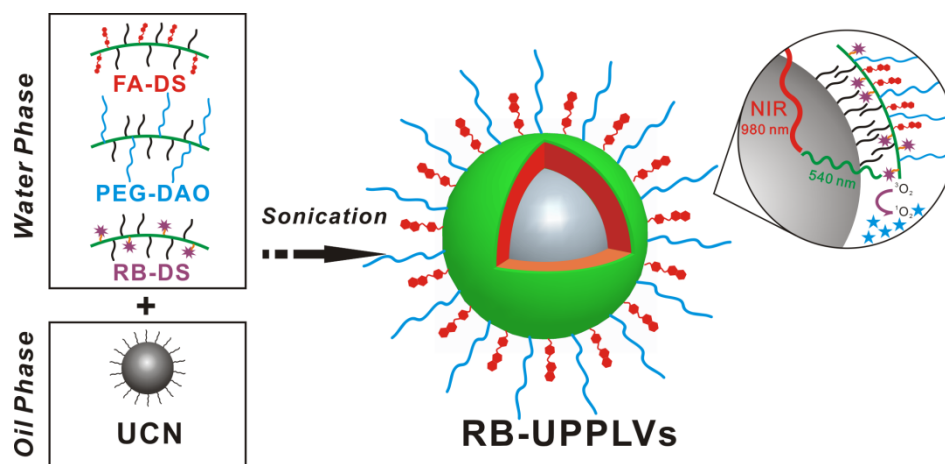


Figure S3. TEM-associated EDX spectrum of the UCNs.



Scheme S2. Preparation process of the RB-UPPLVs.

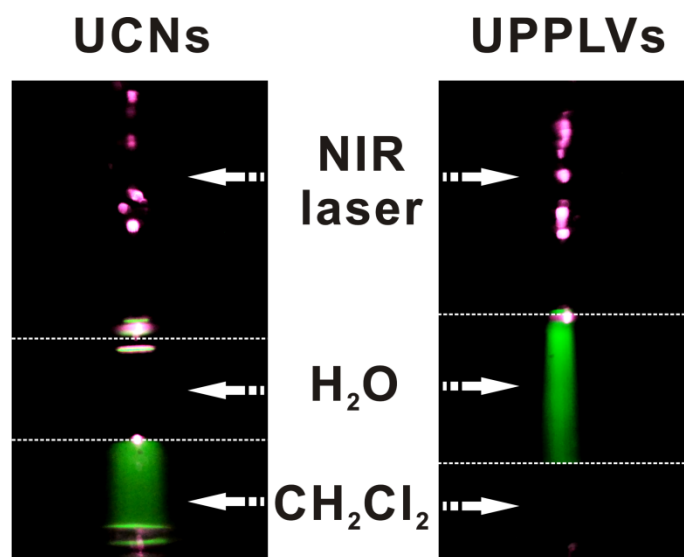


Figure S4. A photograph of hydrophobic UCNs dispersed in dichloromethane (left) and hydrophilic UPPLVs dispersed in water (right). The green fluorescence is emitted by upconversion nanoparticles under the excitation of 980 nm NIR light.

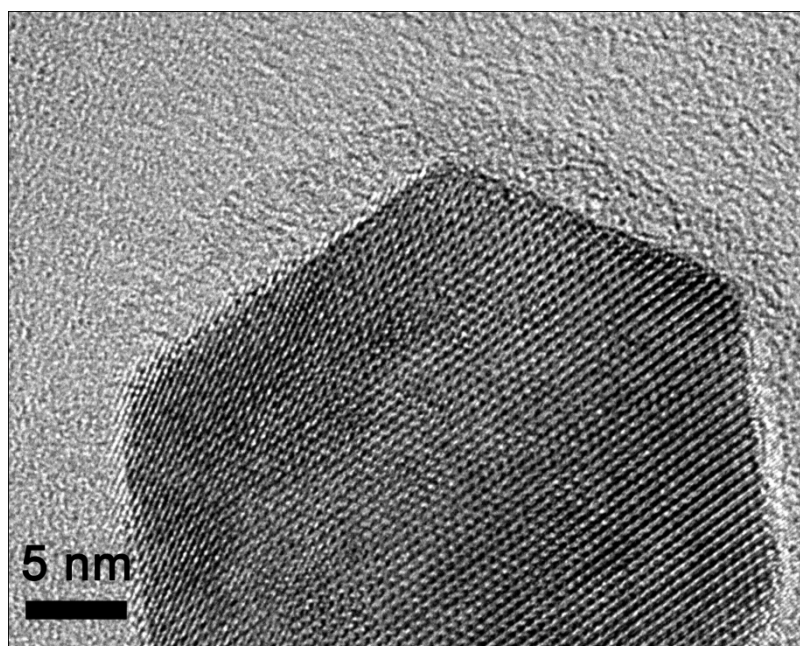


Figure S5. A HRTEM image of the RB-UPPLVs, which shows the core-shell structure of the RB-UPPLVs.

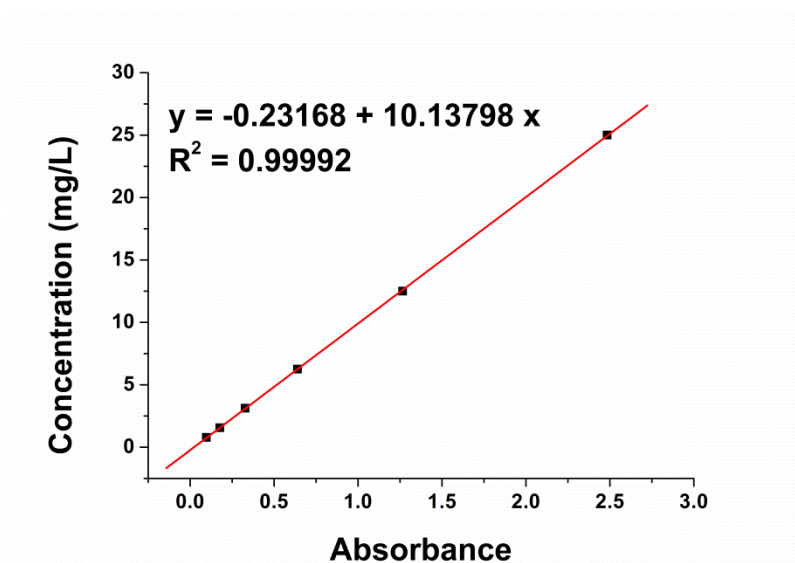


Figure S6. The concentration (mg/L) of RB as a function of the absorbance at a wavelength of 549 nm.

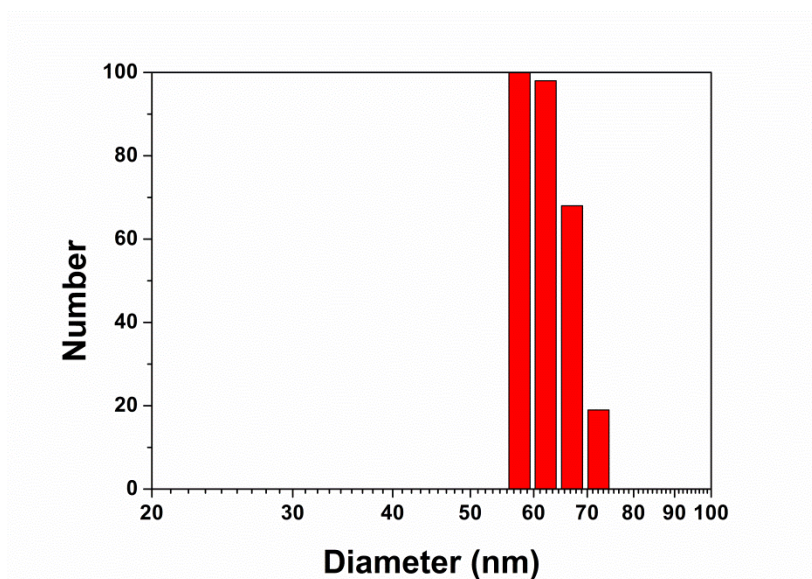


Figure S7. Effective particle diameter of the RB-UPPLVs.

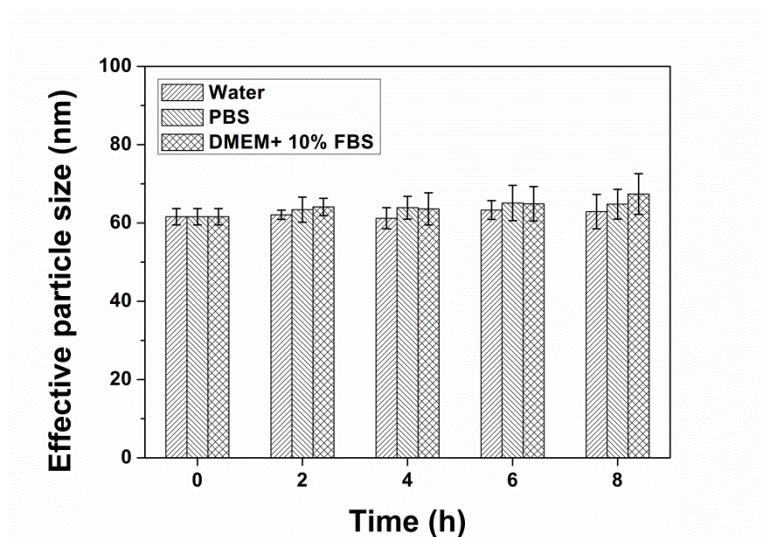


Figure S8. Colloidal stability of freshly prepared RB-UPPLVs in water, PBS and DMEM + 10% FBS.

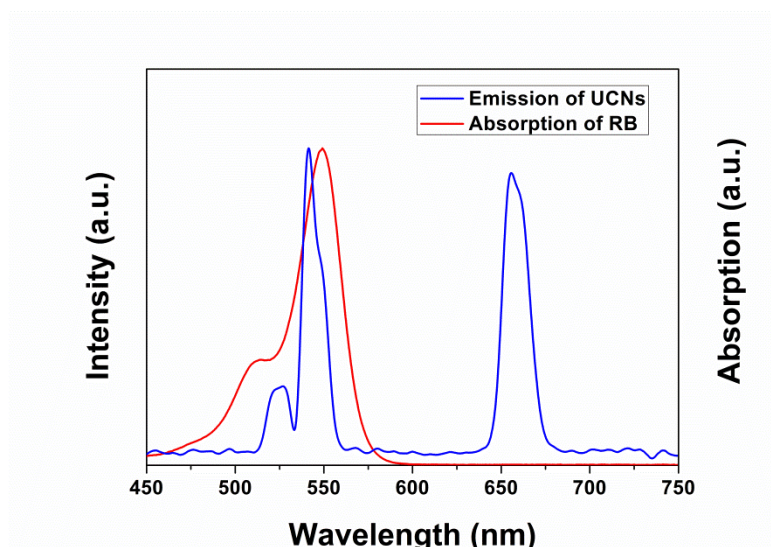


Figure S9. Spectral overlap between the upconversion emission spectrum of the UCNs and the absorption spectrum of the RB.

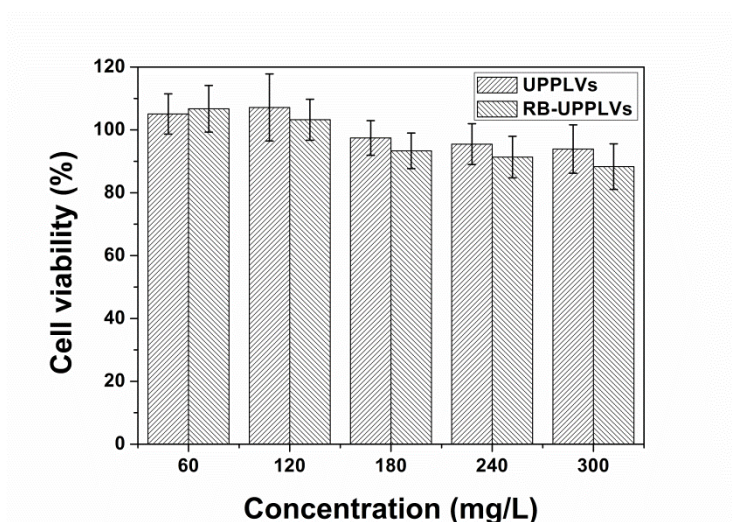


Figure S10. Cytotoxicity of UPPLVs and RB-UPPLVs against HeLa cells following 24 h of incubation.

References

1. S. Wang, D. Zeng, J. Niu, H. Wang, L. Wang, Q. Li, C. Li, H. Song, J. Chang and L. Zhang, *Journal of Materials Chemistry B*, 2014, **2**, 877.
2. N. Sugita, K. I. Kawabata, K. Sasaki, I. Sakata and S. I. Umemura, *Bioconjugate chemistry*, 2007, **18**, 866.
3. K. M. McLean, G. Johnson, R. C. Chatelier, G. J. Beumer, J. G. Steele and H. J. Griesser, *Colloids and surfaces B: biointerfaces*, 2000, **18**, 221.
4. L. Zhu, D. Wang, X. Wei, X. Zhu, J. Li, C. Tu, Y. Su, J. Wu, B. Zhu and D. Yan, *Journal of Controlled Release*, 2013, **169**, 228.
5. X. Duan, J. Xiao, Q. Yin, Z. Zhang, H. Yu, S. Mao and Y. Li, *ACS nano*, 2013, **7**, 5858.
6. S. Sun, H. Zeng, D. B. Robinson, S. Raoux, P. M. Rice, S. X. Wang and G. Li, *Journal of the American Chemical Society*, 2004, **126**, 273.