Supplementary materials

Upconversion Nanoprobes for the Ratiometric Luminescent Sensing of Nitric Oxide

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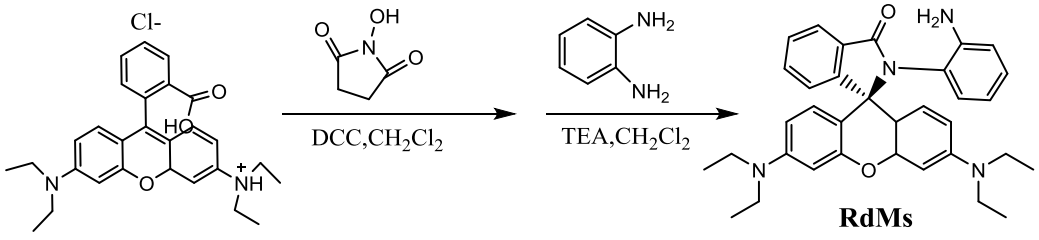
Materials and Methods. Tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES) and hexadecyltrimethyl ammonium bromide (CTAB) were obtained from Sigma–Aldrich (USA). Nitric oxide (NO) was prepared from DEA/NONOate (10 mM stock solution in 0.01 M NaOH). All other reagents were purchased from commercial suppliers and used without further purification. Water used in all experiments was purified via a Milli-Q water system (Millipore, USA). HeLa cells (cervical cancer) and L02 cells (normal human liver cells) were provided by the Biomedical Engineering Center of Hunan University (China). Rat models were provided by the Hunan Provincial Tumor Hospital, Central South University (China). The study was approved by the Ethics Committee of Hunan Provincial Tumor Hospital.

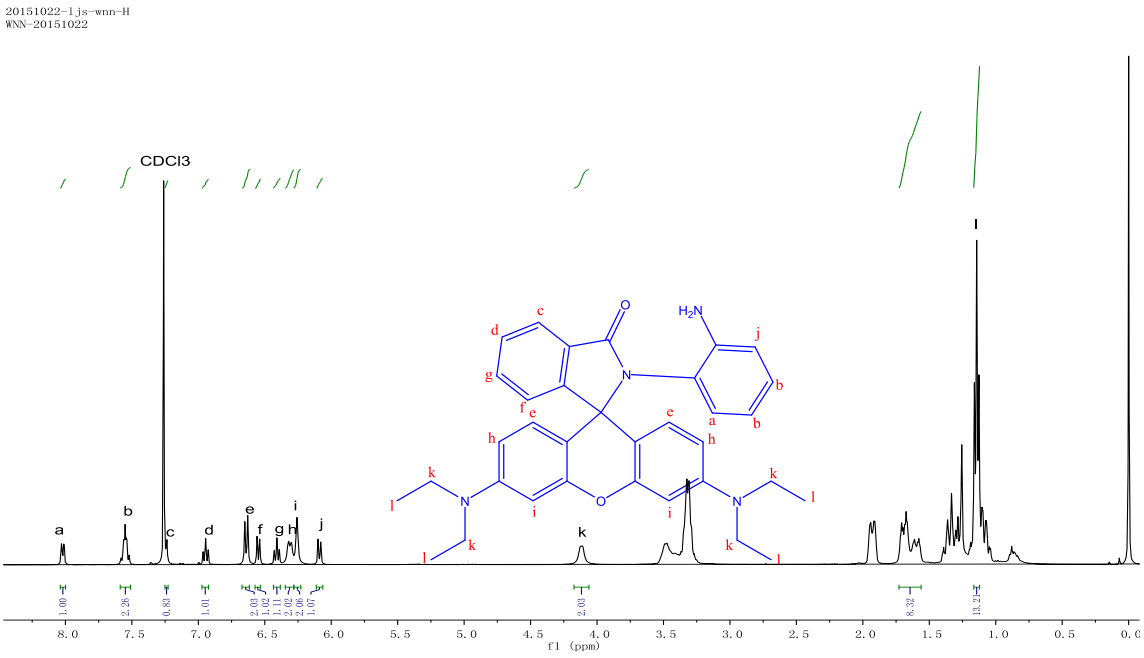
UV-Vis absorption spectra were obtained using a Hitachi U-4100 UV/Vis spectrophotometer (Japan) in quartz cuvettes with 1 cm path length. Transmission electron microscopy (TEM) images were acquired using a JEOL JEM-3010 microscope (Japan). Zeta potential measurements were performed on a Malvern Zetasizer 3000HS (UK). Upconversion luminescence (UCL) spectra were recorded using an external 0−800 mW adjustable continuous wave laser (980 nm, Beijing Hi-Tech Optoelectronic Co., China) as the excitation source, instead of the instrument built-in Xenon source. FT-IR spectra were acquired on a Bomem (Hartmann & Braun, MB-Series) spectrometer. UCL images of cells and tissues were obtained from the 540 channel (490-570 nm) and the 656 channel (630-700 nm) upon excitation at 980 nm using an Nikon TI-E A1 STORM laser scanning confocal microscope (Japan). pH measurements were made using a Mettler-Toledo Delta 320 pH meter (USA).

Synthesis of NO-reactive rhodamine B-derived molecules (RdMs).[1]

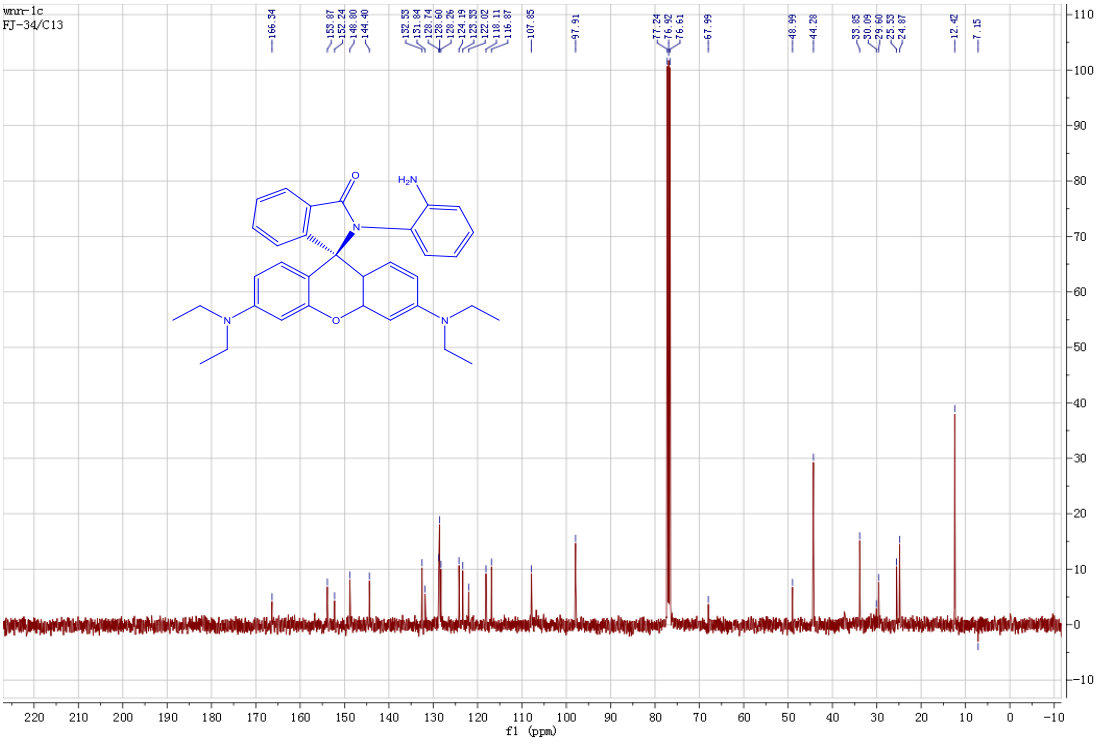


Rhodamine B (250 mg, 0.38 mmol) was dissolved in CH_2Cl_2 (5.0 ml) at room temperature, to which *N*,*N*'-Dicyclohexylcarbodiimide (DCC, 156.6 mg, 0.76 mmol) and 1-hydroxypyrrolidine-2,5-dione (131.1 mg, 1.14 mmol) were added. The mixture was allowed to react for 16 h, and subsequently *O*-diaminobenzene (120 mg, 1.11 mmol) and triethylamine (0.4 mL) were added. After stirring for 3 h, the mixture was concentrated under vacuum and the crude product was purified by silica column chromatography (CH_2Cl_2:CH_3OH = 100:1) to give RdMs as a white powder (85 mg, 46.4% yield). ^{13}C NMR and ^{1}H spectra were recorded on a Bruker DRX-400 spectrometer (Bruker) with TMS employed as an internal standard. All chemical shifts are given in the standard δ notation of parts per million. Mass spectra were measured on LCQ Advantage ion trap mass spectrometer (Thermo Finnigan). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01-8.04 (d,1H), 7.53-7.57 (m, 2H), 7.23-7.26 (m, 1H), 6.95 (d, 1H), 6.64 (d, 2H), 6.55 (d, 1H), 6.41 (t, 1H), 6.30 (d, 2H), 6.26 (s, 2H), 6.09 (d, 1H), 4.1 (s, 2H),1.60-1.67 (m, 8H), 1.14 (t, 12H),.^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 154.0, 152.4, 148.9, 144.5, 132.7, 132.0, 128.9, 128.8, 128.7, 128.4, 124.3, 123.5, 122.2, 118.2, 117.0, 108.0, 106.9, 98.0, 77, 44.4, 12.6. HRMS (ESI) calcd for [M+H^+] 533.3, found 533.3.

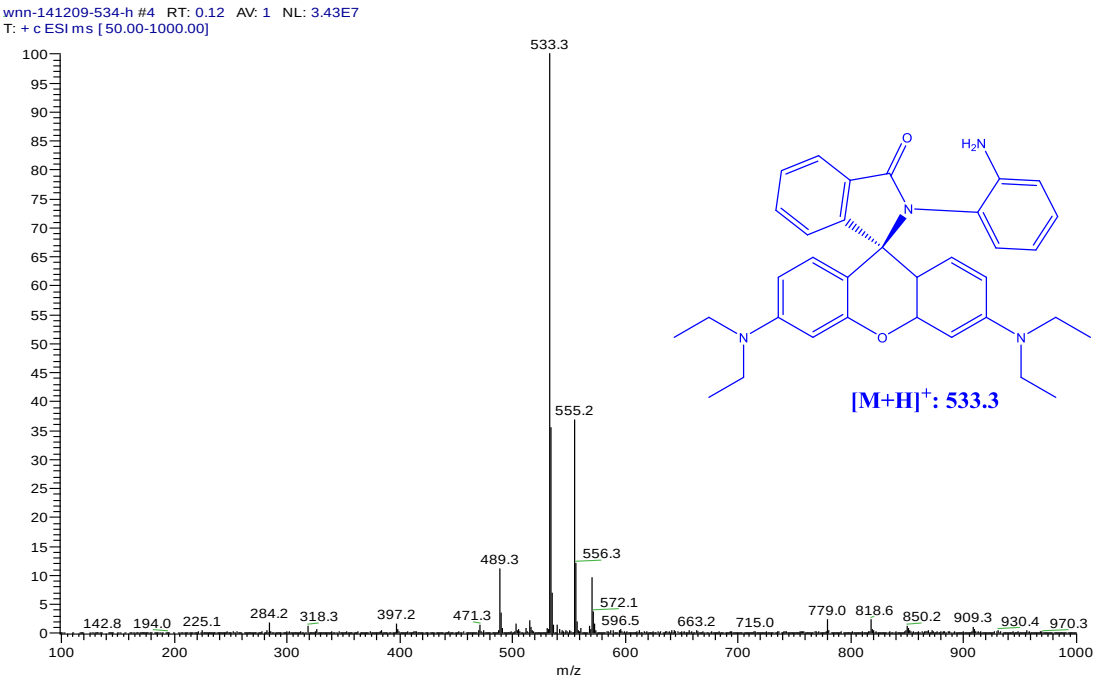
^{1}H NMR of RdMs in $CDCl_3$



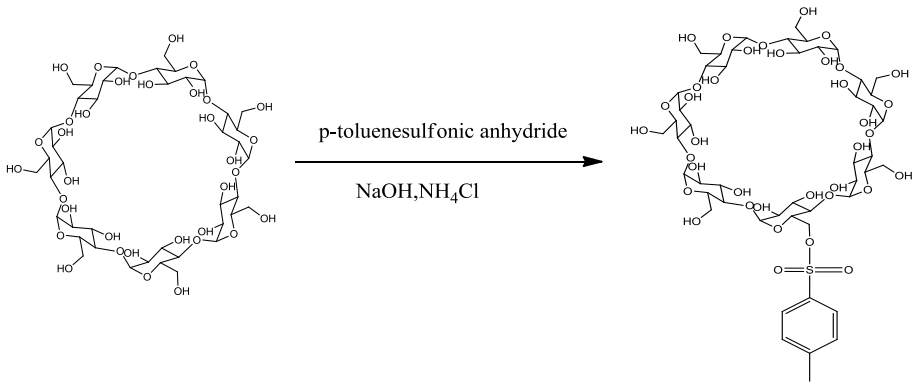
^{13}C NMR of RdMs in $CDCl_3$



ESI-MS of RdMs

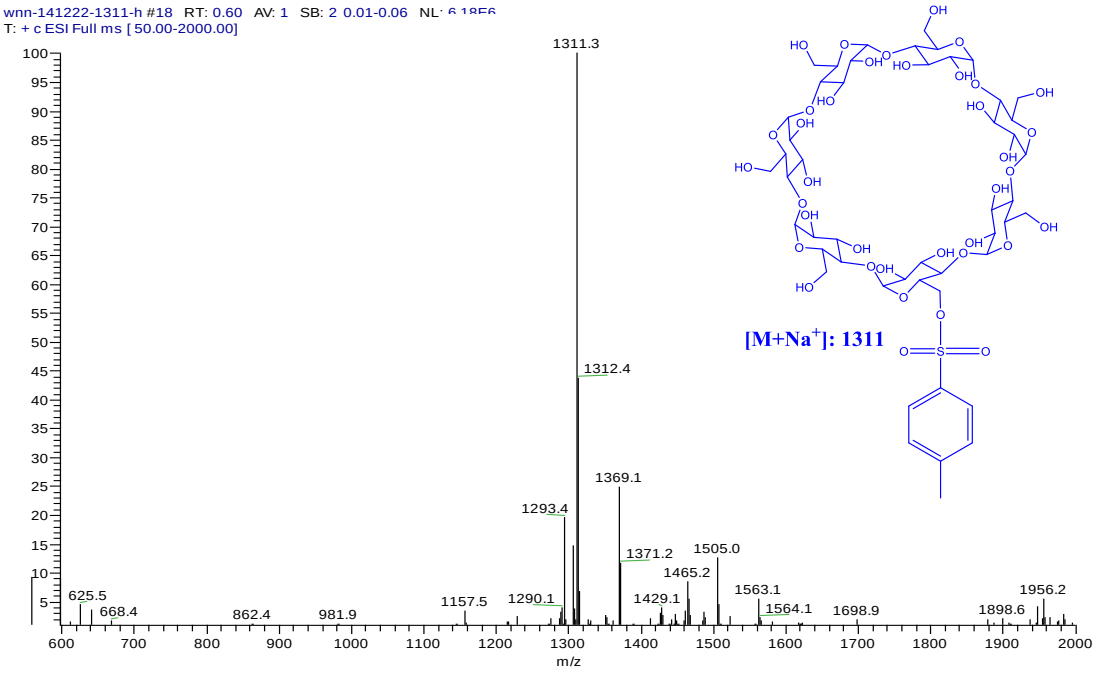


Synthesis of mono-6-deoxy-6-(p-tolylsulfonyl)-β-cyclodextrin (6-OTs-β-CD). [2]



β-CD (11.5 g, 10 mmol) and p-toluenesulfonyl chloride (4.9 g, 15 mmol) was dissolved in 250 mL of water and stirred at room temperature for 3 h. Thereafter, NaOH (5.0 g, 125 mmol) in 50 mL of water was added and the mixture was stirred for 10 min. Unreacted p-toluenesulfonyl chloride was removed by filtration through a sintered glass funnel. The filtrate was brought to pH ~8 by the addition of NH_4Cl (13.4 g), and the filtrate was refrigerated overnight at 4℃. The resulting white precipitate was recovered by filtration, and after drying for 24 h, 8.0 g of pure white solid was afforded (54.2% yield). Mass spectra were measured on LCQ Advantage ion trap mass spectrometer (Thermo Finnigan). HRMS (ESI) calcd for [M+Na^+] 1311, found 1311.1.

ESI-MS of 6-OTs-β-CD



Synthesis of Oleic Acid Capped Upconversion Nanoparticles (UCNPs). UCNPs were prepared by a solvothermal process.[3] 6 mL of oleic acid (OA) and 15 mL of ODE were added to 1 mmol (total amounts) of $LnCl_3$ (Ln: 78 mol % Y + 20 mol %Yb + 2 mol % Er + 0.2 mol % Tm) in a 100 mL three-neck round-bottom flask at room temperature. Next, the reaction solution was heated to 160 ℃ to remove water and oxygen, with vigorous magnetic stirring in the current of nitrogen for 2 h. After the reaction was completed, 10 mL of methanol solution (2.5 mmol NaOH + 0.4 mmol NH_4F) was added dropwise into the solution at room temperature. At this point, the reaction mixture was a turbid solution. The solution was then heated to 50 ℃ for an hour. Next, the reaction solution was further heated to 80 ℃ to remove the remaining water and certain low boiling substances. Finally, the solution was heated to 320℃ under nitrogen, and maintained at this temperature for 2 h. After the heating, 8 mL of cyclohexane was poured into the solution at room temperature. The resulting mixture was centrifugally separated (10000 rpm, 5 min at 20 ℃) and was washed three times with ethanol. The obtained UCNPs were redispersed in 10 mL of cyclohexane before use.

Preparation of the Upconversion Nanoconjugate. For mesoporous silica coating,[4,5] 2 mL cyclohexane solution containing 5 $mg \cdot mL^{-1}$ of UCNPs was first mixed with 0.1 g CTAB and 20 mL water. The above mixture was then stirred vigorously at room temperature to allow cyclohexane to evaporate, resulting in a clear solution (UCNPs is about 0.5 $mg \cdot mL^{-1}$). Then, 10 mL of the aqueous CTAB-stabilized UCNP solution was added to a mixture of 20 mL of water, 3mL of ethanol, and 150 μL of 0.2 M NaOH solution. The mixture was heated to 30℃ under stirring, to which 150 μL TEOS was added dropwise, and the reaction mixture was allowed to stir for 48 h. For amine functionalization, 32 μL of (3-aminopropyl) triethoxysilane (APTES) was added to the above reaction mixture, which was allowed to stir for another 24 h. The product was centrifuged and washed with ethanol three times. Surfactants were removed via a fast and efficient ion-exchange method and the final amine-terminated UCNP@MSN nanoparticles were transferred into 50 mL ethanol containing 0.3 g of NH_4NO_3, and were kept at 45 ℃ for 24 h. To form the βCD-capped RdMs layer, 10 mg RdMs was added to 5 mL of the amine-functionalized UCNP@MSN ethanol solution (2 $mg \cdot mL^{-1}$). The mixture was stirred for 24 h at room temperature.

Thereafter, the particles were washed several times with ethanol and deionized water through iterative dispersion/precipitation cycles. The product was then dispersed in ethanol containing 6-OTs-β-CD (90 mg). The mixture was allowed to react for 24 h at 45 ℃ under nitrogen atmosphere to yield the final UCNP@RdMMSN@βCD upconversion nanoconjugates.

Spectrophotometric Measurements. Both UV/vis absorption and upconversion luminescence measurements of RdMs (5 μM, 10% ethanol) or UCNP@RdMMSN@βCD nanoconjugates (300 $\mu g \cdot mL^{-1}$) were conducted in PBS (10 mM, pH 7.4) at room temperature. A stock solution of DEA/NONOate (10 mM) was prepared in 0.01 M NaOH solution. Following the additions of different concentration of DEA/NONOate solution, the luminescence intensities were recorded at excitation wavelength of 980 nm with emission wavelength in the range of 400-900 nm, power intensity: 1.66 A.

Cytotoxicity and Cell Imaging. Hela cells were cultured using high-glucose Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% fetal bovine serum (GIBCO) and 1% penicillin-streptomycin (10,000 U/mL, 10,000 $\mu g\ mL^{-1}$, Invitrogen). Liver cells were grown in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), and 100 U/mL penicillin. Cultures were maintained at 37℃ in a humidified atmosphere containing 5% CO_2. The cytotoxic effect of UCNP@RdMMSN@βCD with or without treating NO was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide (MTT) assay following standard protocols.[6] For confocal imaging, cells were incubated with UCNP@MSN@βCD for 0 h, 0.5 h, 1 h, 2 h, and 3 h before being washed three times with PBS. Z-scanning confocal imaging in Hela cells were observed with a Nikon TI-E A1 STORM laser scanning confocal microscope at excitation wavelength of 980 nm, with power intensity: 0.5 W. Immediately prior to the intracellular localization imaging experiments, cells were incubated with UCNP@MSN@βCD for 1.5 h, then treated with the nucleus-staining dye Hochest(1.0 μM), and LysoTracker® Green DND (1.0 μM) for 30min in the incubator at 37 ℃ with 5 % CO_2, and were then rinsed three times with PBS.

Tissue Imaging. The liver tissues from Sprague-Dawley rats were cryosectioned at -20 ℃ into slices of 1.0 mm thick, and stained with 300 μg·mL^{-1} UCNP@RdMMSN@βCD in the absence or presence of NO (200 μM) for 3 h in 10% goat serum-containing PBS at 37 ℃. After washing with PBS to remove the remaining nanoparticles, UCL imaging and Z-scan UCL imaging accumulated along the Z-direction at a depth of 0−700 μm of treated tissues were recorded using a Nikon TI-E A1 STORM laser scanning confocal microscope at excitation wavelength of 980 nm, with power intensity: 0.5 W. The UCL emission was collected at the 540 channel (490-570 nm) and 656 channel (630-700 nm).

NO Analysis in HIRI mouse model. To explore the protective mechanism of Oct on HIRI, UCNP@RdMMSN@βCD upconversion nanoconjugates were used to monitor NO level in the serum and tissue samples of rat models. All Sprague-Dawley rats were fasted for 12 h before the experiment but were given access to water. Nine Sprague-Dawley rats were randomly divided into 3 equal groups: the sham-operated group (Blank), the IR group (IR), and the IR + octreotide pretreatment group (Oct-IR). Each rat was anesthetized with an intraperitoneal injection of 10% chloral hydrate (30 mg·kg^{-1}). Thirty minutes before laparotomy, the Oct-IR group rats received an injection of octreotide intraperitoneally (20 μg·kg^{-1}, Jilin Province A-Think Pharmaceutical Co., Ltd., Jilin, China). Control (Blank) and IR group rats received the same volume isotonic saline. All rats underwent a laparotomy. HIRI models were set up in the IR and OCTIR groups by using the Pringle's method,[7] which consists of ischemia for 0.5 h followed by reperfusion for 4 h. All rats were sacrificed, and the plasma from portalvein blood samples and the liver tissue slices (1-2 mm) were collected. Plasma (300 μL) was mixed with 600 μL nanoprobes (450 μg·mL^{-1}), and the mixture was incubated at 37 ℃ for 1 h, before being subjected to luminescence measurements. Liver tissue slices were immersed in a PBS solution containing the nanoprobe (300 μg·mL^{-1}) and 10% goat serum at 37 ℃ for 1 h, before luminescence imaging.

Additional Figures

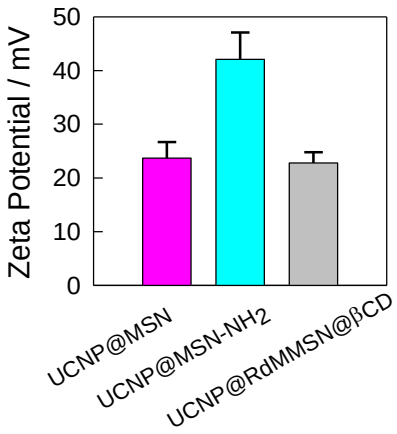


Figure S1. Zeta potential of UCNP@MSN, UCNP@MSN-NH_2 and UCNP@RdMMSN@βCD.

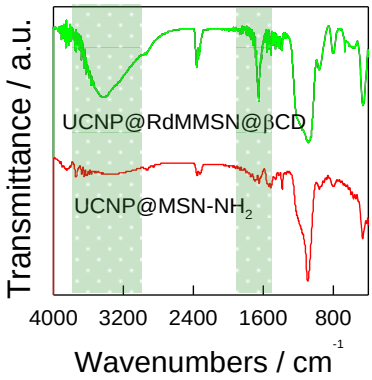


Figure S2. FTIR spectra of the UCNP@MSN-NH_2 nanoparticles (red) and the UCNP@RdMMSN@βCD nanoparticles (green).

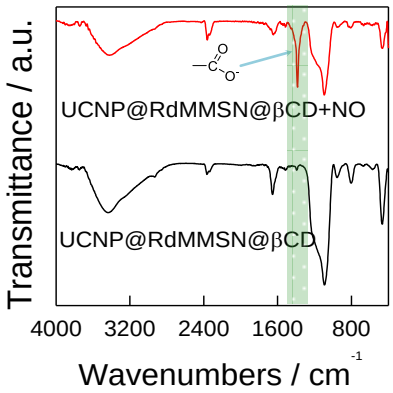


Figure S3. FTIR spectra of the UCNP@RdMMSN@βCD nanoparticles before (black) and after (red) NO treatment.

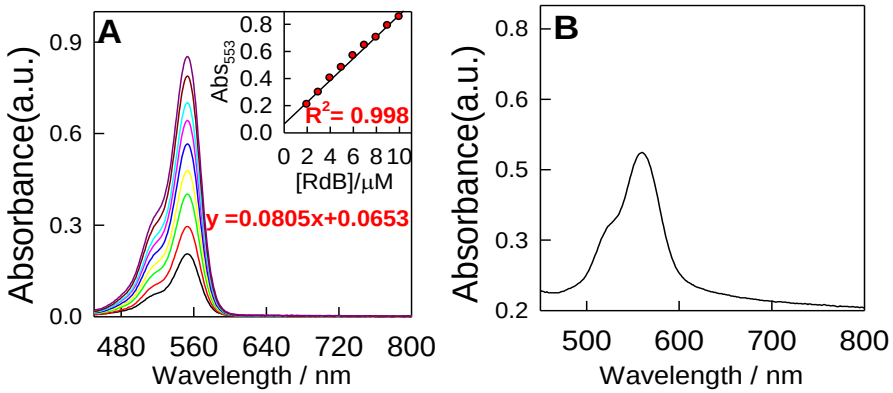


Figure S4. (A) Standard curve for determining RdB concentration from an unknown sample. UV-vis spectra are recorded in PBS buffer with 10% ethanol (pH 7.4). (B) Absorption spectrum of UCNP@RdMMSN@βCD nanosensors (25 $\mu g \cdot mL^{-1}$) in test buffer (PBS with 10% ethanol, pH 7.4) after incubating with excess NO.

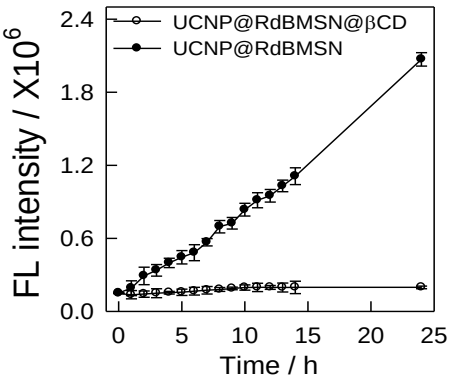


Figure S5. RdB-leaching tests of the nanoconjugates with or without βCD modification. To measure leakage of rhodamine B (RdB), particles were dispersed in 1 mL of buffer solution, sealed in a dialysis tube with a molecular weight cutoff of 8000-14000 Da, and dialyzed against water. The luminescence intensity of fluids outside of the dialysis tube was collected and measured at different time points.

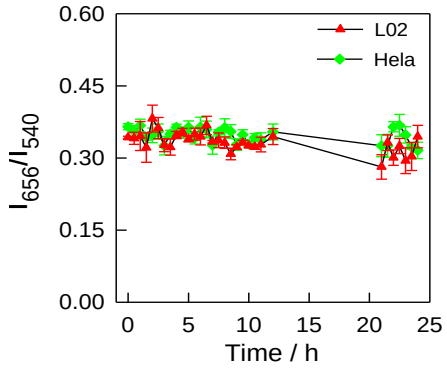


Figure S6. The ratio I_{656}/I_{540} of UCNP@RdMMSN@βCD as a function of incubation time in liver and HeLa cell extracts. Cell extracts are prepared as follows: 5×10^6 cells were harvested with trypsin treatment and centrifuged at 1500 g for 2 min. Then, cells were washed 3 times with 10 mL of cold PBS, centrifuged and resuspended in 0.5 mL of ice-cold cell lysis buffer (Cell Signaling) on ice for 5 min. Cells were pulse-sonicated on ice 5 times for 5 s each. Then, the centrifuged extracts at 15,000 g for 20 min at 4 ℃ and supernatants were collected. Last, the suspension is purged using bubbling nitrogen.

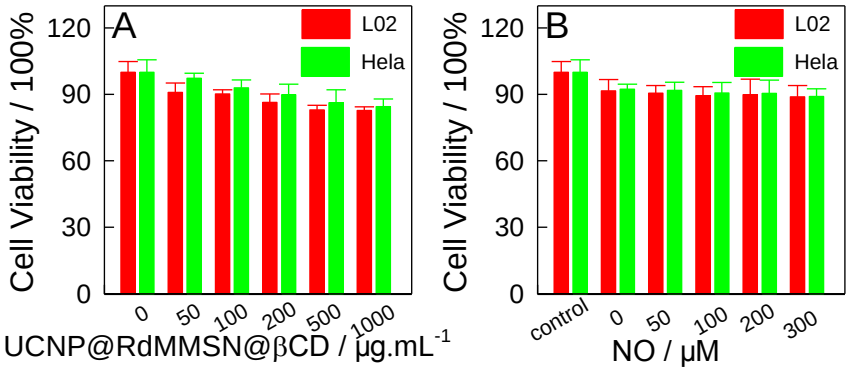


Figure S7. MTT cytotoxicity assay of liver cells (L02) and Hela cells treated with different concentrations of UCNP@RdMMSN@βCD (A) or NO-treated UCNP@RdMMSN@βCD(B) for 48 h in fresh medium.

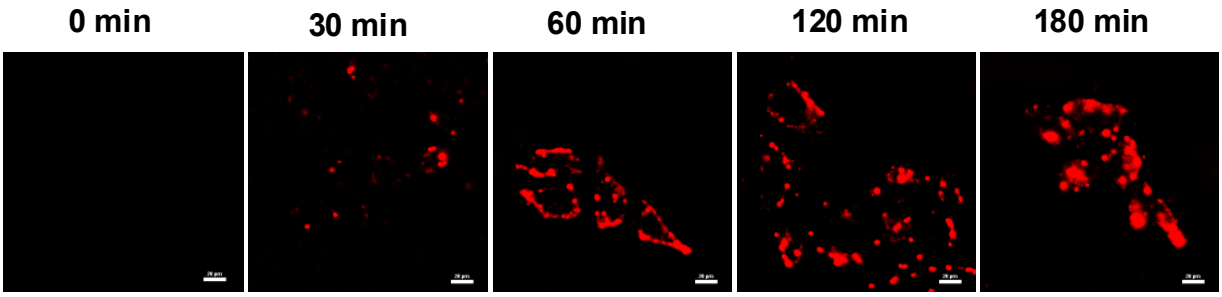


Figure S8. Luminescence images of HeLa cells treated with 300 μg·mL^{-1} UCNP@MSN@βCD for different times. λ_{ex} = 980 nm; λ_{em} = 630-700 nm. Scale bar: 20 μm.

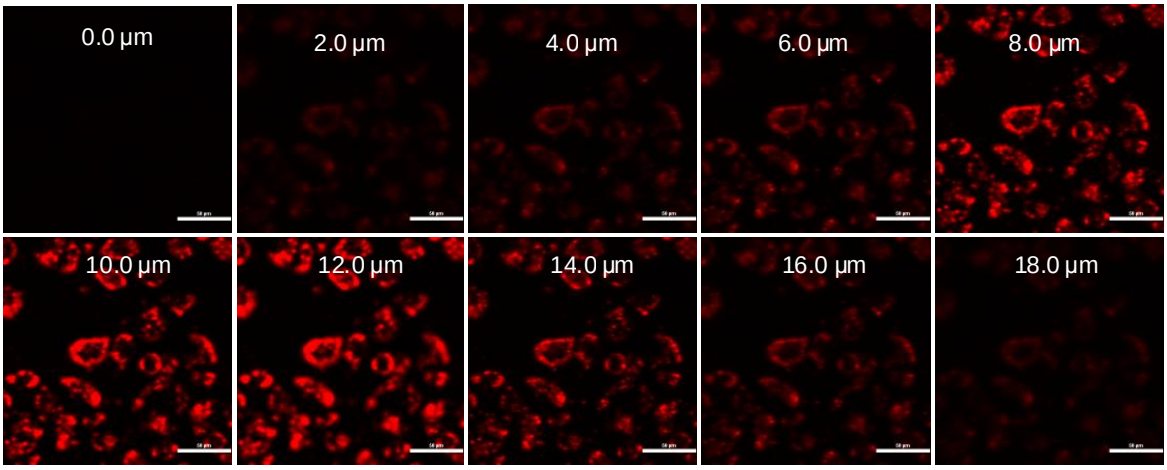


Figure S9. Confocal images of Z-direction slices of HeLa cells treated with UCNP@MSN@βCD (300 μg·mL^{-1}). λ_{ex} = 980 nm; λ_{em} = 630-700 nm. Scale bar: 50 μm.

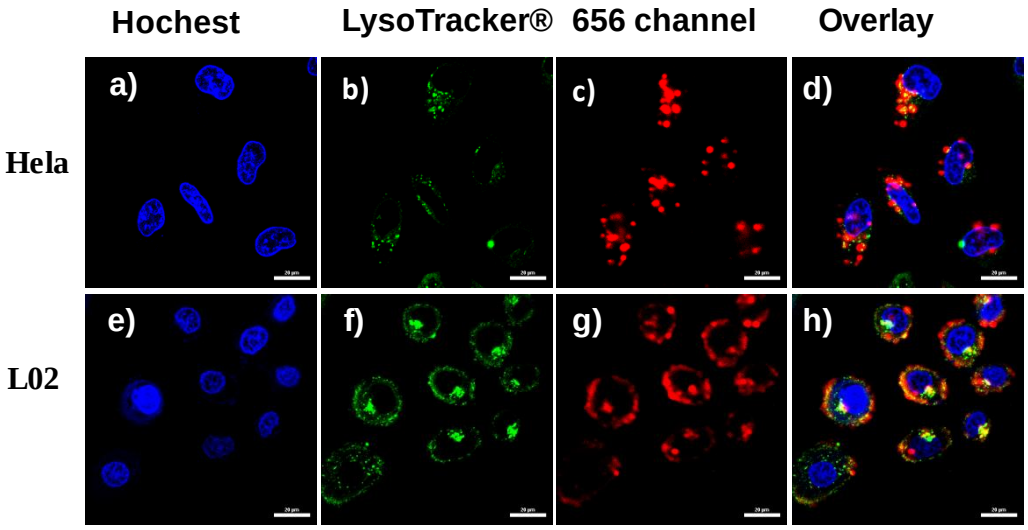


Figure S10. Luminescence images of cells treated with UCNP@MSN@βCD (300 μg·mL^{-1}), (the nucleus dye Hochest, and LysoTracker® Green DND was co-incubated with cells); Scale bar: 20 μm.

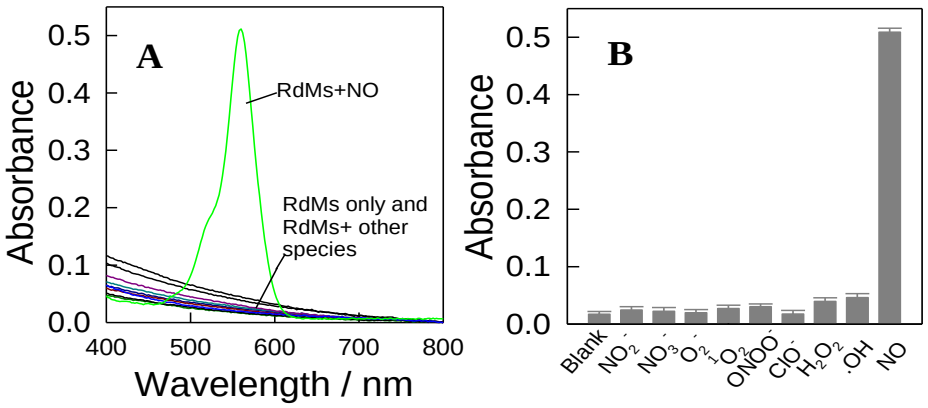


Figure S11. (A) Absorption spectra of RdMs (5.0 μM, 10% ethanol, pH 7.4) in the presence of selected species (100 μM for ROS and RNS, 30 μM for NO). (B) Absorption intensity at 556 nm in the presence of different selected species. The magnitudes of the error bars were calculated from three independent experiments.

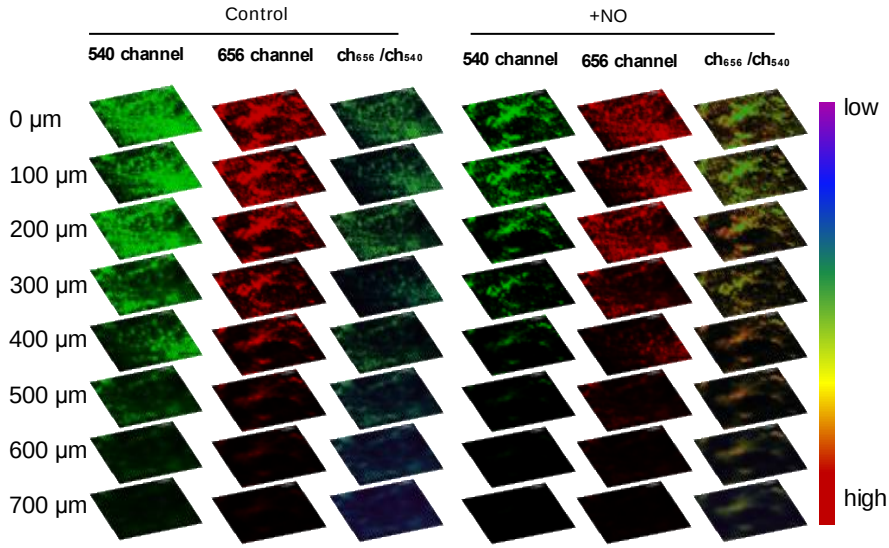


Figure S12. Confocal Z-scan of a cryosectioned rat liver slice incubated with 300 $\mu g \cdot mL^{-1}$ UCNP@RdMMSN@βCD nanoconjugate in the presence/absence of 0.2 mM DEA NONOate for 3 h. The 540 nm channel (λ_{em} = 490-570 nm) and the 656 nm channel (λ_{em} = 630-700 nm) are recorded upon excitation at 980 nm with power intensity: 0.5 W. The ch_{650}/ch_{550} channel shows luminescence ratio images resulting from the 540 and 656 nm channels, which is displayed in pseudocolor.

References

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