Supporting Information

Near-infrared light-mediated LA-UCNPs@SiO$_2$-C/HA@mSiO$_2$-DOX@NB nanocomposite for chemotherapy /PDT/ PTT and imaging

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Materials.

Unless specified, all of the chemicals utilized were analytical grade and utilized without further purification. Yttrium (III) chloride hexahydrate (YCl₃·6H₂O), ytterbium(III) chloride hexahydrate (YbCl₃·6H₂O), thulium(III) chloride hexahydrate (TmCl₃·6H₂O), ammonium fluoride (NH₄F), sodium hydroxide (NaOH), methanol, oleic acid, tetraethylorthosilicate (TEOS), CO-520, Ammonia solution (NH₄OH 25%), 3-(Triethoxysilyl) propyl isocyanate (TPI), hexadecyl trimethyl ammonium bromide (CTAB), (3-aminopropyl) triethoxysilane (APTES), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Lactobionic acid (LA), 1,3-Diphenylisobenzofuran (DPBF), and doxorubicin hydrochloride (DOX) were all purchased from Aladdin. Ethanol absolute, potassium permanganate (KMnO₄), magnesium sulfate anhydrous, cyclohexane, tetrahydrofuran (THF) was dried with molecular sieve and roasted prior to use, ethyl acetate, and dimethyl sulfoxide (DMSO) were all bought from Sinopharm Chemical Reagent Co., Ltd. Hypocrellin A (HA) was purchased from Chengdu Biopurify Phytochemicals Ltd. 1-Octadecene was obtained from Sigma-Aldrich. D-glucose and concentrated hydrochloric acid were purchased from Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd for the synthesis of C-Dots.

Instruments.

Samples were characterized by transmission electron microscopy (TEM Hitachi H-8100) at an accelerating voltage of 20 kV. X-ray diffraction (XRD) is characterized using a Rigaku Ultima IV diffractometer with Cu Ka radiation (40kV, 20 mA). The fluorescence spectra were surveyed by HORIBA FL-3. A Fourier transform infrared (FT-IR) spectroscopy spectrometer (JASCOFT/IR-420) was used to record the infrared spectra of these materials. Zeta potential was carried out on the Brookhaven. Ultraviolet-visible (UV-Vis) spectra were taken on (SHIMADZU UV2550 spectrophotometer). The isotherms of N₂ adsorption/desorption were measured at the temperature of liquid nitrogen using a Micromeritics ASAP 2010M system. The pore size distributions were calculated from the adsorption branches of the N₂ adsorption isotherms using the Barrett-Joyner-Halenda model.
Scheme S1. Synthetic route for the NB linker.
The Synthesis of 2-nitro-1,3-benzenedicarboxylic acid

1,3-dimethyl-2-nitrobenzene (3.95 g, 0.105 mol), water (200 mL) and sodium hydroxide (1.6 g, 0.16 mol) were added into a three neck flask, which was stirred and heated to 95 ºC in oil bath, then KMnO₄ (16.5 g, 0.418 mol) was added in batches over a period of 3 h. The resulting mixture was refluxed for another 20 h, cooled and filtered, the filtrate was acidified with concentrated HCl and the precipitate was collected and dried.

![The 1HNMR spectrum of 2-nitro-1,3-benzenedicarboxylic acid.](image)

**Fig.S1** The 1HNMR spectrum of 2-nitro-1,3-benzenedicarboxylic acid.
The Synthesis of 2-nitro-1,3-benzenedimethanol

A solution of 2-nitro-1,3-benzenedicarboxylic acid (1.6 g, 38 mmol) in 10 mL anhydrous THF (dried with molecular sieve) was added to three neck flask and cooled to 0 ºC in the presence of \( \text{N}_2 \). Besides, 1.0 M borane-tetrahydrofuran (40 mL) was added dropwise over about 1 h with constant pressure drop funnel. The reaction mixture was allowed to warm slowly to room temperature and stirred as well as refluxed for another 48 h. Afterwards, Methanol (8 mL) was added into the reaction system slowly by constant pressure drop funnel, then the mixture was evaporated with a rotary evaporator at 40 ºC. The residue was redissolved in ethyl acetate and washed three to four times with water (100 mL). The organic layer was dried with anhydrous MgSO\(_4\) overnight, the next day the solvent was evaporated once again with a rotary evaporator at 40 ºC. The resulting yellow solid was further purified by silica gel chromatography (hexane:ethyl acetate=1:1) to obtain 2-nitro-1,3-benzenedimethanol.

Fig.S2 The 1HNMR spectrum of 2-nitro-1,3-benzenedimethanol.
Synthesis of NB-linker

In a 100 mL three-necked flask, 2-nitro-1,3-benzenedimethanol (1.7977g, Fig. S1 and 2), was dissolved in 36 mL of anhydrous THF in the presence of Ar. And then, 3-(Triethoxysilyl) propyl isocyanate (3.6251g) in 20 ml anhydrous THF was added dropwise with constant pressure drop funnel. Finally, the reaction system was refluxed for another 12 h in oil bath. At the procedure, the reaction flask was quickly covered with aluminum foil to avoid the sunlight.

Synthesis of C-Dots

2.4 g (1 M) of glucose was dissolved in 10 mL of triple distilled water (TDW) and then 10 mL concentrated hydrochloric acid (11.67 M) was added to it. The mixture was sonicated for 2 h. The solution was dried completely in the oven about 80–100 °C, and then the black carbon powder was collected. About 0.8 g of carbon powder was dissolved in 350 mL of TDW and then again sonicated for 10 min. This solution was centrifuged at 6000 rpm and the supernatant was collected for further study.

LA attachment

LA as covalently conjugated onto amine-functionalized UCNPs@SiO₂/C/HA@mSiO₂-DOX@NB linker through the -COOH group by using cross-linking reagents EDC and NHS. In this work, firstly, 100 mg UCNPs@SiO₂-C/HA@mSiO₂-DOX@NB linker was dispersed in 20 mL of toluene and 50 µL of APTES added to the solution. The solution was heated to 50 °C and stirred for 4 h with the flowing of argon to obtain the amino-functional nanocomposites. Secondly, 1 mmol LA was dissolved in 2 mL of DMSO followed by the addition of 1 mmol EDC and 1 mmol NHS. The mixture was then stirred at room temperature for 15 min to activate the carboxylic group of LA. Subsequently, 100 µL of the above solution was added to 5 mL of methanol solution of amino-functionalized UCNPs@SiO₂-C/HA@mSiO₂-DOX@NB linker (60 mg), and the mixture was stirred for 24 h at room temperature. Excess EDC, NHS and LA were removed by repeatedly washing with ethanol.

Detection of singlet oxygen

DPBF was employed as a chemical probe to determine singlet oxygen by measuring the absorption via UV–Vis spectroscopy. Typically, 2 mL of ethanol solution containing DPBF
(10 mmol L\(^{-1}\)) was added to 2 mL of UCNPs@SiO\(_2\)-C/HA solution (0.5 mg mL\(^{-1}\)) and then transferred into a 5 mL cuvette. The solution was irradiated by a 980 nm NIR laser (2.15 W cm\(^{-2}\)) for different time, and then the solution was centrifuged, and the supernatant was collected for UV–Vis absorption.

**Photothermal testing**

To investigate the photothermal effect of UCNPs@SiO\(_2\)-C3/HA3, 2 mL of aqueous solutions (500 µg mL\(^{-1}\)) were irradiated under an NIR laser (980 nm, 2.15 W cm\(^{-2}\)) for 25 min. A digital thermometer was used to measure the temperature during irradiation.

**The modification of FITC on LA-UCNPs@SiO\(_2\)-C3/HA3@mSiO\(_2\)-DOX@NB**

Firstly, 15 mg of FITC and 100 µL of APTES were dissolved to 5 mL of methanol solution, and then the mixture was stirred for 24 h at room temperature for the next step (FITC-APTES). Secondly, 2 mL FITC-APTES solution was added into the above LA-UCNPs@SiO\(_2\)-C3/HA3@mSiO\(_2\)-DOX@NB solution and stirred for another 24 h at room temperature. The FITC-LA-UCNPs@SiO\(_2\)-DOX-C3/HA3@mSiO\(_2\)-DOX@NB was collected by centrifugation at 6000 rpm.

**Apoptosis Detection by AM**

To visualize the cell apoptosis, 1 × 10\(^5\) HePG2 cells were seeded on a 6-well plate and cultured overnight in a humidified incubator at 37 °C with 5% CO\(_2\). Then, cells were treated with LA-UCNPs@SiO\(_2\)-C3/HA3@mSiO\(_2\)-DOX@NB2 (500 µg mL\(^{-1}\)) for 4 h. Then, cells were irradiated with NIR laser light (980 nm, 1 W cm\(^{-2}\)) for 0-20 min. Cells were incubated for another 4 h in fresh culture medium before being collected. Finally, cells were stained with AM for analysis.
**Fig. S3** The TEM images of (A) UCNPs@SiO$_2$, (B) UCNPs@SiO$_2$-C1/HA1, (C) UCNPs@SiO$_2$-C2/HA2

**Fig. S4** Low-angle XRD patterns of samples
Fig. S5 (A) Nitrogen adsorption-desorption isotherms and (B) pore size distribution for UCNPs@SiO$_2$-C3/HA3@mSiO$_2$, UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB1, UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2, and UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB3.

The porous structures of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$ and UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NBs were further studied by N$_2$ adsorption-desorption (Fig. S11). Mesoporous structures derived from the mSiO$_2$ shell that are verified by the typical IV adsorption isotherm and H4 hysteresis loop in middle relative pressure region as shown in Fig. S11A of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$. And the hysteresis loop at high relative pressure region implies the void structure derived from the accumulation of these nanoparticles. Moreover, the absorption amounts of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NBs decrease remarkably due to the drug loading and NB-linker modification. Here, NB silane coupling agent was exploited as the “gate” to seal the drug molecules inside the mesopore, so that with increasing the NB linker, the DOX loading efficiency aggrandizes from 5.24±0.04 % (UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB1) to 9.73±0.05 % (UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB3) as present in Table S1. In addition, the DOX loading and organic modification also makes the surface area and pore volume decrease from 563 m$^2$ g$^{-1}$ and 0.64 cm$^3$ g$^{-1}$ of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$ to 130 m$^2$ g$^{-1}$ and 0.53 cm$^3$ g$^{-1}$ of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB1, 95 m$^2$ g$^{-1}$ and 0.35 cm$^3$ g$^{-1}$ of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2, 59 m$^2$ g$^{-1}$ and 0.23 cm$^3$ g$^{-1}$ of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB3, respectively (Table S1).
Table S1 The porous parameters of the samples

<table>
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<tr>
<th>Sample</th>
<th>Surface area (m² g⁻¹)</th>
<th>Pore volume (cm³ g⁻¹)</th>
<th>Pore size (nm)</th>
<th>Loading efficiency (%)</th>
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<tr>
<td>UCNPs@SiO₂-C3/HA3@mSiO₂</td>
<td>563</td>
<td>0.64</td>
<td>2.35</td>
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<tr>
<td>UCNPs@SiO₂-C3/HA3@mSiO₂-DOX@NB1</td>
<td>130</td>
<td>0.53</td>
<td>2.20</td>
<td>5.24±0.04</td>
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<tr>
<td>UCNPs@SiO₂-C3/HA3@mSiO₂-DOX@NB2</td>
<td>95</td>
<td>0.35</td>
<td>2.10</td>
<td>8.60±0.03</td>
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<tr>
<td>UCNPs@SiO₂-C3/HA3@mSiO₂-DOX@NB3</td>
<td>59</td>
<td>0.23</td>
<td>2.00</td>
<td>9.73±0.05</td>
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</tbody>
</table>

Fig. S6 The UV/Vis spectrum of the samples.
Fig. S7 (A) The XPS spectrum of UCNPs@SiO$_2$-C3. (B) The high resolution XPS spectra of C1s.

The C-Dots of this complex have been characterized by X-ray photoelectron. The XPS spectrum of UCNPs@SiO$_2$-C3 (Fig. S7A) shows the peak at 285, 532, 103, 158, and 698 eV, which is attributed to C1s, O1s, Si2p, Y3d, and F1s, respectively. Fig. S7B reveals the high resolution XPS spectra of C1s. The peaks at 284.3, 285.4, 287.0, and 288.2 eV suggest the C-C, C-C/H, C-O-C, and C=O group of C-Dots.
**Fig. S8** Fluorescence spectra at various excitation wavelengths. (A) Dox, (B) C-Dots, (C) HA.
The corresponding upconversion luminescence (UCL) spectra under NIR laser excitation (980 nm) were recorded as shown in Fig. S9. The different intensity emission peaks at about 344, 360, 450, 475 and 808 nm are assigned to the $^1I_6 \rightarrow ^3F_4$, $^1D_2 \rightarrow ^3H_6$, $^1D_2 \rightarrow ^3F_4$, $^1G_4 \rightarrow ^3H_6$ and $^3H_4 \rightarrow ^3H_6$ transition from Tm$^{3+}$, respectively. After the growth of NaYF$_4$ shell, NaYF$_4$:Yb, Tm@NaYF$_4$ exhibits enhanced fluorescence emission. And the decreased fluorescence of UCNPs@SiO$_2$-C3/HA3 is ascribed to the light-scattering effect on both emission and incident light by the silica layer. Furthermore, the emission at 450-500 nm exhibits the more inhibition owing to the blue light absorption of HA.
**Fig. S10** Absorption spectra changes of DPBF treated with UCNPs@SiO$_2$-C3/HA3 after 980 nm irradiation for different times.

DPBF was employed as a chemical probe to evaluate ROS generation by the UV−Vis absorption spectroscopy because DPBF can react with ROS irreversibly to cause the decrease of its characteristic absorption. The typical absorbance of DPBF at 420 nm decreases obviously because of the efficient generation of ROS from UCNPs@SiO2-C3/HA3 illuminated by 980 nm NIR light.
To further study the NIR-triggered release behavior, the DOX release from UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2 in the dark and then with NIR irradiation was recorded as shown in Fig. S11A. When the solution of UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2 was placed under dark condition for the first 330 min, just few leakages can be detected (below 10.23±0.03 %). After that, the NIR irradiation was introduced and the release is enhanced obviously. In order to further testify the role of NB “gate” on the controlled release, the release behaviors of the nanocomposite without NB “gate” (UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX) were investigated as shown in Fig. S11B. DOX release more freely from UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX with and without NIR irradiation, but there is not significant difference between the release performances of them. Both of them can reach about 90.26±0.42 % DOX release after 240 min. With NB “gate”, UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2 reveals the excellent controlled release triggered by NIR. Based on the above investigation, it is the sensitive NB “gate” to insure the NIR-controlled DOX release that is significant for the operable DOX chemotherapy.
The direct evidence for the UV sensitive NB linker is present in Fig. S12. Based on the above equation, NB linker could be broken under UV irradiation, and Fig S12 shows the UV-Vis spectra of NB linker (25 µL, 0.20 M) dissolved in PH 6.5 PBS (3 mL) treated by UV irradiation for different time. The increased absorbance at about 300–350 nm owing to the 2-nitro-1,3-benzenedialdehyde fall off from NB linker. And the inset photo exhibits the color change for NB linker solution before and after the UV irradiation.
**Fig. S13** DCF fluorescence images in HePG2 cells treated with LA-UCNPs@SiO$_2$-C3/HA3 under NIR irradiation for (A) 0 min, (B) 5 min, (C) 10 min, and (D) 20 min. Scale bar: (100 µm).

Intracellular ROS generation was detected using DCFH-DA as indicator, which is oxidized to form a green fluorescent substance (2’7’-dichlofluorescein, DCF) inside cells (Fig. S13). After excited by 980 nm light, HePG2 cells shows enhanced green fluorescence with the increasing the irradiation time owing to the improved ROS amount. On the contrast, without 980 nm irradiation there is no remarked green fluorescence (Fig. S13). Above high intracellular ROS generation capability make LA-UCNPs@SiO$_2$-C3/HA3 as promising candidate as the PDT agent.
**Fig. S14** Fluorescence images of calcein-AM (green, live cells) costained HePG2 cells after laser irradiation for (A) control, (B) 0 min, (C) 10 min, (D) 20 min. scale bar: (100 µm).

To further visualize cell survival treated by LA-UCNPs@SiO$_2$-C3/HA3@mSiO$_2$-DOX@NB2, the HePG2 cells are stained with calcein acetoxymethyl ester (calcein-AM), which is a typical dye for living cells. From Fig. S14, with prolonging the irradiation time, the green fluorescence derived from AM is inhibited due to the decreased cell viability. Based on the above investigation, the synergistic effect of chemotherapy, PTT and PDT induce the enhanced cell cytotoxicity.