Electronic Supplementary Information (ESI)

Smart down/upconversion nanomachines integrated with "AND" logic computation and enzyme-free amplification for NIR-II fluorescence-assisted precise and enhanced photodynamic therapy

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EXPERIMENTAL SECTION

Materials. Ce6-DNA was synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China), and other DNA oligonucleotides were provided by Sangon Biotechnology Co., Ltd. (Shanghai, China). The relevant sequences used in this work were listed in Table S1. $NaErF_4$@$NaYF_4$ D/UCNPs were purchased from Xijie Biotechnology Co., Ltd. (Jiangsu, China). Dimethyl sulfoxide (DMSO) and 6 × DNA loading buffer were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), glutathione (GSH), and 2,2,6,6-tetramethylpiperidine (TEMP) were obtained from Aladdin Co., Ltd. (Shanghai, China). Nucleic acid red (NA-Red) dyes, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and calcein-AM were attained from Beyotime Co., Ltd. (Shanghai, China). Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, phosphate-buffered saline (PBS, pH = 7.4), thiazolyl blue tetrazolium bromide (MTT), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM) and 1640 cell culture media, and pyridine iodide (PI) were purchased from Solabao Co., Ltd. (Beijing, China). 1,3-Diphenylisobenzofuran (DPBF) was provided by Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). Mouse 4T1 breast cancer cells and human normal liver HL-7702 cells were obtained from Cobioer Biotechnology Co., Ltd. (Wuhan, China). BALB/c mice (8–10 weeks) were purchased from Hunan SLAC Laboratory Animal Co., Ltd. (Hunan, China), and all related experiments were performed in compliance with the relevant laws or guidelines of China and approved by the Animal Ethical Committee of Guangxi Normal University.

Instrumentation. Ultraviolet-visible (UV-vis) absorption spectra were recorded using a Cary 60 spectrometer (Agilent, USA). The fluorescence spectra were collected with a QuantaMaster 8000 transient steady-state spectrometer (Horiba, Canada). The photographic image of agarose gel was acquired with an Omega 16ic Imaging System (ULTRA-LUM, USA). TEM images were acquired with a FEI Tecnai G2 F-20 transmission electron microscope (FEI Company, Hillsboro, OR, USA) with an accelerating voltage of 200 kV. Fourier-transform infrared (FT-IR) spectra were

attained using a Thermo Electron Nicolet 5700 (Perkin Elmer, USA). Zeta potential analysis was performed with a Nano Zetasizer (Malvern, U.K.). Electron paramagnetic resonance (EPR) spectra were recorded by a Bruker A300 spectrometer (Bruker, USA). Cell viabilities were measured with a microplate reader (BioTek Instruments, USA). Cell imaging was performed on a two-photon laser confocal microscope Leica TCS SP8 DIVE (Leica, Germany). NIR-II fluorescence imaging was conducted with a long-wave optical imaging system (Nuohai Life Science, Shanghai, China) under irradiation with a 980 nm diode CW laser (Changchun New Industries Optoelectronics Tech. Co., Ltd., China).

Preparation of smart D/UCNMs. First, 100 μL of D/UCNPs (25 mM) were mixed with 10 μL of EDC (10 mg/mL) and 10 μL of NHS (10 mg/mL), followed by shaking gently on the oscillator for 1 h to activate the carboxyl group on the surface of D/UCNPs. Then, DNA logic circuits were acquired by thermally annealing amino-modified TP, AP and BHQ3-PP strands, amino-modified SS-DNA and Ce6-DNA strands, respectively, followed by mixing the obtained two kinds of dsDNA probes. Then, the acquired DNA logic circuits were mixed with the activated D/UCNPs and incubated for 24 h. Subsequently, the free DNA probes were removed by centrifugation at 12000 rpm for 12 min. After washing three times with Tris-HCl buffer, smart D/UCNMs (25 mM) were obtained. To prepare D/UCNMs-a, the SS-DNA strand was replaced by the NSS-DNA strand, while BHQ3-PP and AP strands were replaced by the TPP strand for the synthesis of D/UCNMs-b.

Agarose gel electrophoresis analysis. Typically, 20 μM PP strand (2 μL), 20 μM AP strand (2 μL) and 20 μM TP strand (2 μL) were mixed with 14 μL of Tris-acetate-ethylenediamine buffer. After heating the mixture at 95 ℃ for 10 min and then naturally cooling to room temperature, 2 μM dsDNA probes were obtained. Similarly, 20 μM SS-DNA strand (2 μL) was hybridized with 20 μM Ce6-DNA strand (2 μL, without Ce6 modification) to prepare another dsDNA probes (2 μM). Then, DNA samples were added to 5% agarose gel, respectively. Subsequently, agarose gel was placed in 0.5 × TBE buffer and run for 30 minutes at a constant pressure of 110 V. Finally, the agarose gel was incubated with 100 × SYBR Green I for imaging analysis.

***In vitro* detection of $^1O_2$.** To investigate the "AND" logically gated generation of $^1O_2$, smart D/UCNMs (25 mM) were incubated with 7 mM GSH, 0.2 μM TK1 mRNA, and the mixture of 7 mM GSH and 0.2 μM TK1 mRNA at 37 ℃ for 3 h, respectively. Then, 30 μL of TEMP (10 mg/mL) was added, followed by irradiating with a 980 nm laser with a power of 2.0 W/cm^2 for 20 min, respectively. Finally, the EPR spectra of the resultant solution were recorded, respectively.

In addition, smart D/UCNMs were incubated with 7 mM GSH and 0.2 μM TK1 mRNA at 37 ℃ for 3 h. Then, 90 μL of DPBF (1 mM) was added, followed by irradiating with a 980 nm laser with a power of 2.0 W/cm^2 for 2 min, 5 min, 8 min, 16 min and 32 min, respectively. Finally, the UV–vis absorbance spectra of the resultant solution were collected, respectively.

Detection of intracellular $^1O_2$. In general, 4T1 cells were cultured in 1640 medium containing 25 μL of smart D/UCNMs (25 mM) overnight. After removing the non-internalized medium by washing with PBS for three times, 1 mL of fresh 1640 medium containing 10 μM DCFH-DA was added and incubated for 0.5 h. Subsequently, the cells were irradiated with a 980 nm laser with a power of 2.0 W/cm^2 for 20 min. Finally, the resultant cells were imaged.

Cytotoxicity analysis. Briefly, 4T1 cells and HL-7702 cells were seeded on 96-well plates and cultured in 1640 and DMEM media for 24 h, respectively. Then, 20 μL of smart D/UCNMs (25 mM) were added and continued to incubate overnight. Subsequently, the non-internalized medium was removed by washing with PBS for three times, followed by adding 190 μL of fresh culture medium. After irradiating with a 980 nm laser with a power of 2.0 W/cm^2 for 20 min, the cells were cultured for another 24 h. After adding 10 μL of MTT solution (5 mg/mL), the cells were cultured for 4 h. Finally, the culture medium was replaced by 150 μL of DMSO to completely dissolve the formed formazan crystals, followed by detecting the absorbance intensity of the corresponding samples at 490 nm. The cell viability was calculated as follows: *Cell viability* (%) = ($OD_{treated}/OD_{control}$) × 100%.

For the live/dead staining assay, the cells were treated with 20 μL of smart D/UCNMs (25 mM), followed by irradiating with a 980 nm laser. Then, the cells were

stained with calcein-AM and PI for 20 min, followed by being imaged.

***In vivo* photodynamic therapy.** Suspensions of 4T1 cells (100 μL, about 1×10^7 cells) were subcutaneously injected into the hind limb of each BALB/c mouse. Once the tumor reached 100 mm^3 in volume, these nude mice were randomly divided into two groups (n = 4), in which the tumor tissue was injected with 50 μL of PBS and smart D/UCNMs (25 mM), respectively, followed by irradiating with a 980 nm laser with a power of 2.0 W/ cm^2 for 20 min at 2 h post-injection. During 16 days of treatment, the drug was injected every other day, and the tumor volume and weight of nude mice were recorded. Tumor volume was calculated according to the formula of $½ \times (width)^2 \times length$.

***In vivo and ex vivo* NIR-II fluorescence imaging.** The xenograft 4T1 tumor-bearing mice were intratumorally injected with 50 μL of smart D/UCNMs (25 mM). Then, NIR-II fluorescence imaging of the mice was carried out at different time points (0, 1, 2, 4, 8, 16, and 24 h) under excitation with a 980 nm laser. After injecting the D/UCNMs for 24 h, the mice were sacrificed, and their major organs (heart, liver, spleen, lung, and kidney) were collected for performing *ex vivo* imaging under excitation with a 980 nm laser.

Table S1. DNA sequence used in the experiments

Name	Sequence (5′→3′)
TP	NH_2-TTT TTT GAA ATG GTG GAA AGG AGG GTC CAG GGA GAA CAG AAA C
AP	TTC TCC CTG GAC CCT ATA TCC ATA AA
BHQ3-PP	CCT TTC CAC CAT TTC-BHQ3
PP	CCT TTC CAC CAT TTC
SS-DNA	GTC CAG /iHS-SH/ GGA GAA CTT TTT T-NH_2
Ce6-DNA	GTT CTC CCT GGA CCC TCC TTT CCA CCA TTT C-Ce6
NSS-DNA	GTC CAG GGA GAA CTT TTT T-NH_2
TPP	GTT TCT GTT CTC CCT GGA CCC TCC TTT CCA CCA TTT C
TK1 mRNA mimic	GTT TCT GTT CTC CCT GG
mismatch-4 mimic	CAA ACT GTT CTC CCT GG
miRNA-21 mimic	TAG CTT ATC AGA CTG ATG TTG A
C-myc mRNA mimic	AAA ACA TCA TCA TCC AGG AC
survivin mRNA mimic	AGA GTT CCT GGT GGC GTA GAG ATG

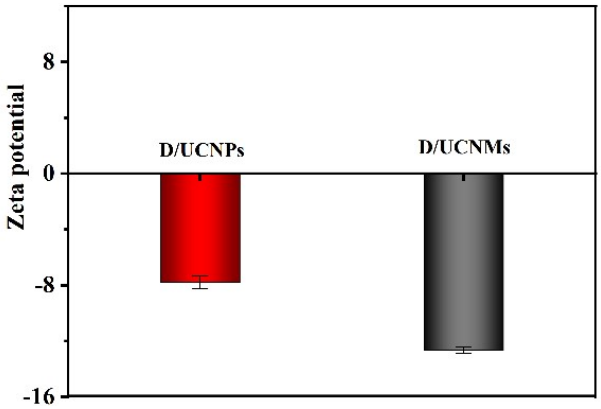


Fig. S1 Zeta potential of D/UCNPs before and after DNA functionalization.

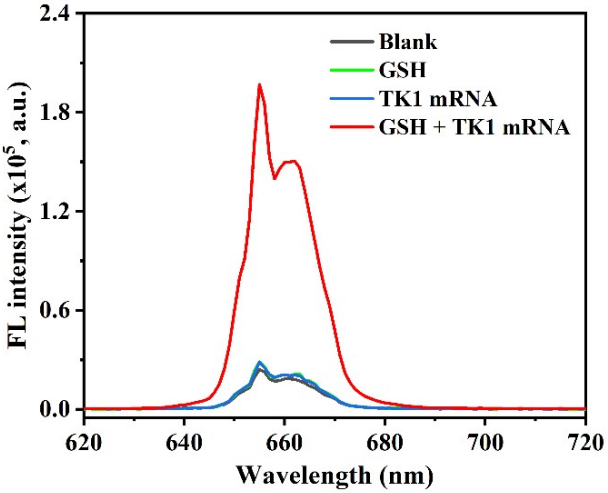


Fig. S2 Fluorescence emission spectra of D/UCNPs after different treatments (The concentrations of GSH and TK1 mRNA were 7 mM and 200 nM, respectively).

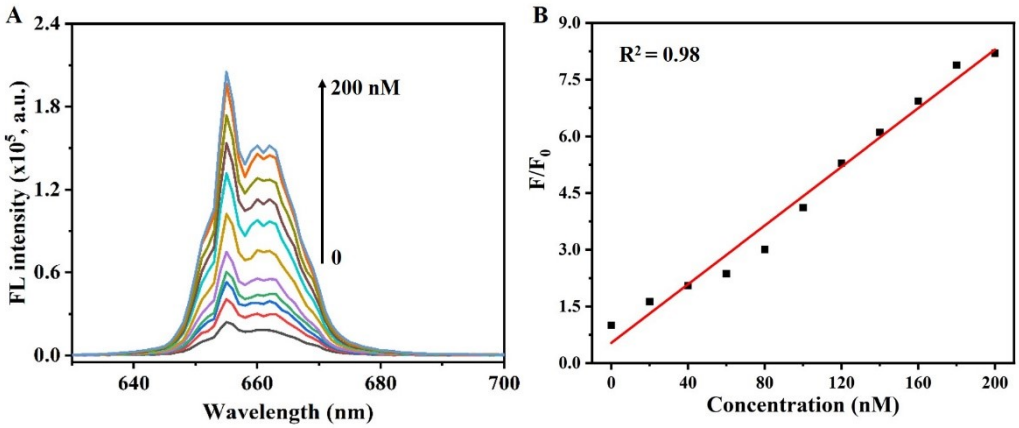
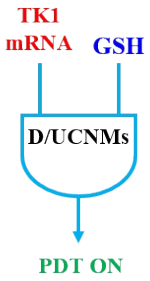


Fig. S3 (A) The effect of TK1 mRNA concentration on the fluorescence intensity of smart D/UCNMs in the presence of 7 mM GSH. (B) The corresponding linear relationship between TK1 mRNA concentration and F/F_0 value (F_0 and F representing the upconversion fluorescence intensity of D/UCNPs before and after treatments with different concentrations of TK1 mRNA).



Input	TK1 mRNA	0	1	0	1
	GSH	0	0	1	1
Output		0	0	0	1

Fig. S4 The truth table of smart D/UCNMs for "AND" logically gated PDT.

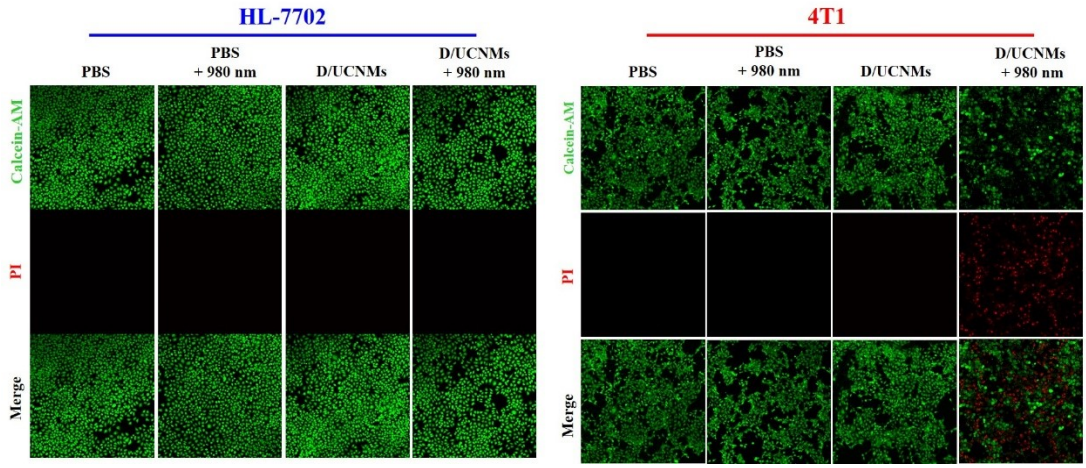


Fig. S5 Fluorescence live/dead stain micrographs of HL-7702 cells and 4T1 cells after different treatments.

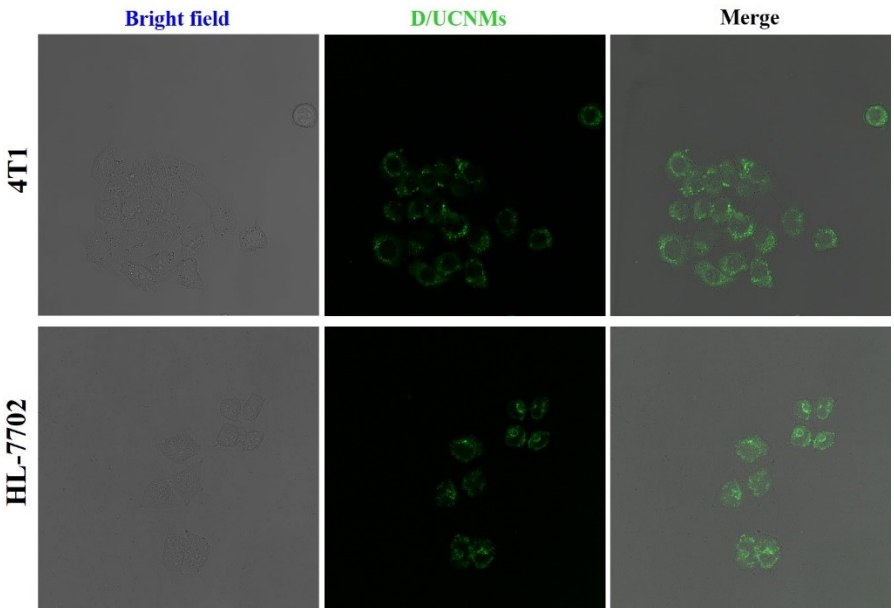


Fig. S6 Fluorescence imaging characterization of the uptake of smart D/UCNMs into HL-7702 cells and 4T1 cells.

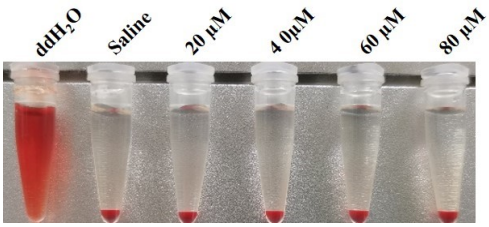


Fig. S7 Photographs of the blood cells after incubating with different concentrations of D/UCNMs at 37 °C for 2 h. Water and saline were used as positive and negative controls, respectively.

Fig. S8 NIR-II fluorescence imaging of D/UCNPs solution before and after the addition of the mixture of 10 mM GSH and 1 uM TK1 mRNA.

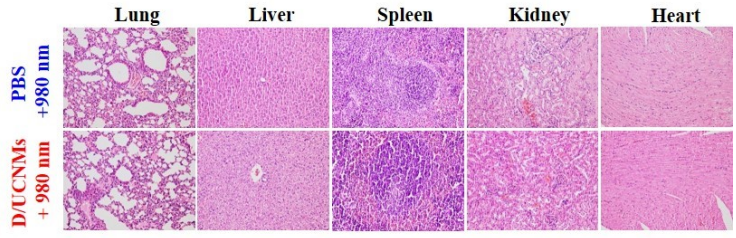


Fig. S9 H&E staining images of major organs collected from the mice after different treatments for 16 days.