Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting Information

Precise Anti-Tumor Effect of a Metallopolysaccharide-Based Nanotheranostic: Turning Phototherapy into Programmed Chemotherapy

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Experimental section

1 Enzyme-responsive capability of Cu-NH_2-HA: In order to demonstrate the capability of enzymatic degradation, the Cu-NH_2-HA (the concentration of Cu: 0.33 mg mL^{-1}) was divided into two groups. One group is the blank group, and another was pretreated with 0.187 mg mL^{-1} hyaluronidase (HAase, 150 U mL^{-1}). Each group was incubated at 37 °C for 0, 6, and 12 h, respectively. Then, the mixture was analyzed with GPC method, dynamic light scattering and ^1H NMR.

2 Investigation on photothermal performance: To verify the photothermal conversion capability, sample 1: Cu-NH_2-HA (100 μg mL^{-1} of Cu^{2+} contents), sample 2: NH_2-HA (equal concentration of NH_2-HA in Cu-NH_2-HA), and $CuCl_2$ (100 μg mL^{-1} of Cu^{2+} contents) were prepared. 1.5 mL of these samples were placed in a series of tubes and irradiated by 650 nm laser (1.2 W cm^{-2}), respectively. The entire surface of the samples was adjusted to confirm whether they were covered by the laser spot. Photoinduced temperature change of solutions was recorded for 10 min by using a thermal camera (MAG30, Magnity Electronics, China). Meanwhile, to detect the thermal stability of the Cu-NH_2-HA, its aqueous was cyclically irradiated and cooled for 5 times.

3 PA performance of Cu-NH_2-HA: Cu-NH_2-HA (Cu concentrations of 12.5, 25, 50, 100, and 200 μg mL^{-1}) were separately added into individual plastic tubes, which were immersed at the same depth in Milli-Q water. PA signals for all samples was captured using an inVision 256 real-time multispectral optoacoustic tomography (MSOT) imaging system (iThera Medical GmbH, Neuherberg, Germany) with a 5 MHz

transducer. The PA signal intensity was analyzed by multispectral optoacoustic tomography software.

4 Cell culture: Human cervical cancer (HeLa) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % (v/v) foetal bovine serum (FBS), 1% (v/v) penicillin and streptomycin. Mouse fibrosarcoma (L929) cells and mouse breast cancer (4T1) cells were cultured in normal RPMI-1640 culture medium containing the same additions as DMEM. The cells were incubated under a humidified atmosphere with 5 % CO_2 at 37 °C.

5 Cellular uptake assay: There are three cell lines cultured and medicated in this assay. L929 cells, 4T1 cells, and HeLa cells were firstly incubated in a 6-well cell culture plate 24 h at 37 °C under 5% CO_2. Secondly, these cells were divided into 2 groups as HA pretreated and without HA pretreated. The two groups were both medicated with Cu-NH_2-HA (100 μg mL^{-1} of Cu) for 0, 1, 2, 3, 4, and 5 h. Then, these cells were collected by centrifuge and further crushed with ultrasound to obtain the intracellular fluid. Finally, the analysis of Cu concentration in trace cellular fluids were conducted by inductively coupled plasma mass spectrometry (ICP-MS). Under the same condition, the effect of HAase on the cellular uptake of Cu-NH_2-HA was also performed with the incubation of HAase.

6 *In vitro* cell viabilities of Cu-NH_2-HA and Cu-DETA Complexes: There are three cell lines used as cellular modals in MTT assay. L929 cells were cultured at 37 °C and with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. 4T1 cells and HeLa cells

were cultured at 37 °C with 5% CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were incubated for 24 h at 37 °C under 5% CO_2 in 96-well cell culture plates. The solutions of Cu-NH_2-HA and Cu-DETA complexes with gradient Cu concentrations of 0, 20, 40, 80, 100, and 150 μg mL^{-1} were individually added to the wells. The cells were then incubated for 12 h at 37 °C under 5% CO_2, and finally the cell viability was measured by MTT assay. The cell viability was estimated according to the following equation:

$$\text{Cell viability}[\%] = \left(\frac{\text{ODtreated}}{\text{ODcontrol}}\right) \times 100$$

7 Enzyme-driven Apoptosis cytotoxicity of Cu-NH_2-HA: Apoptosis cytotoxicity was investigated by Annexin V-FITC/PI detection commercial kit. 4T1 cells were seeded in a 6-well culture plate and incubated for 24 h, and then treated with Cu-NH_2-HA in different conditions for 12 h. The cells were harvested by treatment with trypsin. The cells were washed twice by PBS, and then stained with 5 μL PI and 5 μL Annexin V-FITC for at least 15 min at room temperature in the dark. The percentages of necrotic cells, late apoptotic cells, early apoptotic cells and living cells were determined by FCM (Beckton Dickinson, San Jose, CA, USA). Data from 10^6 cells were analyzed for each sample.

8 *In vitro* programmed therapeutic effect: The programmed anti-cancer treatment effect was carried out in PI stain assay. HeLa cells were seeded in a 6-well culture plate and incubated for 24 h, and then treated with Cu-NH_2-HA (100 μg mL^{-1} of Cu) under 650 nm laser (1 W cm^{-2}) irradiation for 5 min. After that, these cells were incubated for

different time period, and harvested by treatment with trypsin. The cells were washed twice by PBS, and stained with 5 μL PI for at least 10 min at room temperature in the dark. Fluorescence intensity was measured by flow cytometry with an excitation wavelength at 488 nm.

9 *In vivo* animal model: All animal operations were in accord with institutional animal use and care regulations by Guangxi Normal University Laboratory Animal Centre (No. 202105-002). In this part, Balb/c nude mice were prepared with one-tumor and two-tumor model to reveal the therapeutic efficacy of Cu-NH_2-HA. In one-tumor model, Balb/c nude mice were prepared by subcutaneously injecting a suspension of 5×10^6 4T1 cells in DMEM (100 μL) into subcutaneous layer of mice. As tumors growth with a volume of ~100 mm^3, 4T1 tumor-bearing nude mice were operated for treatment.

In two-tumor model, Balb/c nude mice were subcutaneously injected a suspension of 5×10^6 4T1 cells in DMEM (100 μL) into subcutaneous layer right-side of the mice. 3 days later, the mice were subcutaneously injected a suspension of 5×10^6 4T1 cells in DMEM (100 μL) into subcutaneous layer left-side of the mice. While the left and right tumor growth almost with a volume of ~50 mm^3, the mice were evaluated for programed therapeutic effect.

10 Biodistribution: A total dose of 100 μL of saline solution of Cu-NH_2-HA (500 μg mL^{-1} of Cu) was intravenously injected into the tail veins of 4T1 tumor bearing female Balb/c mice. The tissues (lung, heart, spleen, kidneys, liver, and tumor) were removed at different time intervals (3 h, 6 h, 12 h, 24 h, 48 h, 7 days, and 14 days) after injection. Subsequently, each sample was analysis of Cu content by ICP-MS was carried out.

11 Histological examination: With different treatments for two weeks, the tumors in each group were excised and fixed in 4% formalin solution for histopathological tests. In brief, tumor samples embedding in paraffin blocks were sectioned into 4 μm slices, and we then mounted them onto glass slides for hematoxylin and eosin (H&E) staining and observed their photos using an optical microscope. The histopathological tests for the tissues (tumor, heart, liver, spleen, lung, and kidneys) were carried out according to the similar procedures performed in tumor. All sections were examined under a BioTek CYTATION 5 imaging reader using a 20×objective lens.

12 Biomedical blood assay and blood routine assay: BALB/c mice (male, 5 weeks old) were randomly divided into different groups (n = 3) for each group. Then, the mice were tail I.V. injected of Cu-NH_2-HA (800 μg mL^{-1} of Cu contents, 80 μL). The standard blood assay and blood routine assay, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), blood urea nitrogen (BUN), white blood cells (WBC), red blood cells (RBC), hematocrit neutrophilic (HCT), red blood cell distribution width-coefficient of variation (RDW-CV), platelets (PLT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) were performed and various makers were examined at pretreated, treated for 7 days, and treated for 14 days.

13 *In vivo* infrared thermal imaging and antitumor activity: The tumor-bearing nude mice were divided into 4 groups (n = 5, each group) and treated with different administration: saline (80 μL), saline (80 μL) plus 650 nm irradiation, Cu-NH_2-HA (Cu:

500 μg mL^{-1}, 80 μL), Cu-NH_2-HA + laser (650 nm irradiation, 5 min, 1.2 W cm^{-2}). In group of Cu-NH_2-HA + laser, mice were executed a photothermal therapy after I.V. injection of Cu-NH_2-HA. During the 650 nm laser irradiation, an infrared thermal camera (MAG30, Magnity Electronics, China) was used to monitor the temperature changes of the tumor site. In addition, the data such as tumor sizes and body weights, were collected every other day. The tumor volume (V) was calculated following the equation: $V=ab^2/2$. Where a is the tumor length and b is the tumor width. Relative tumor volume was normalized to its initial size when the administration was initiated.

14 *In vivo* programmed theranostic effect of Cu-NH_2-HA: *In vivo* programmed treatment was carried *via* Balb/c nude mice which bearing with two 4T1 tumors. In the mice model, the right-side tumor was executed for PTT, and the left-side tumor was represented as the non-PTT field for sequential treatment.

Primarily, time-dependent photoacoustic (PA) imaging was captured in 0 h, 3 h, 6 h, 12 h, 24 h and 36 h after tail intravenous injection of Cu-NH_2-HA (100 μL, Cu: 500 μg mL^{-1}). PA imaging was performed by an inVision 256 real-time MSOT imaging systemequipped with a 40 MHz, 256-element linear array transducer on tumors.

Sequentially, mice bearing with two-tumor were divided into 2 groups with saline and Cu-NH_2-HA (100 μL, Cu: 500 μg mL^{-1}), respectively. After tail intravenous injection of saline and Cu-NH_2-HA in 12 h, the right-side tumor was executed under laser irradiation (650 nm irradiation, 5 min, 1.2 W cm^{-2}) in above two groups. Then, the relative tumor volume, time-dependent digital photographs of observation, and harvested tumor were also carried out in this 14-day course.

Supplementary Figures

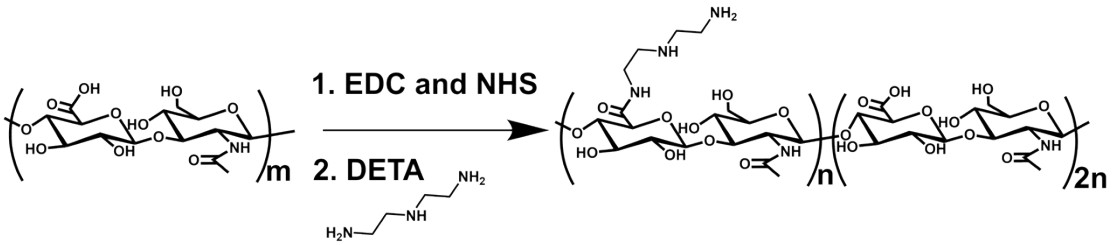

Fig. S1 (A) Schematic diagram showing the steps used to fabricate NH_2-HA.

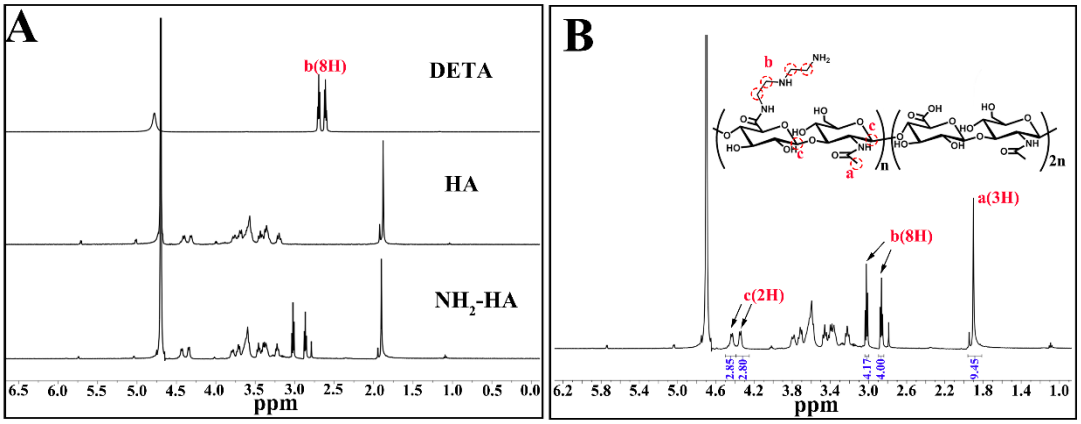

Fig. S2 (A) ^1H NMR spectra of DETA, HA, and NH_2-HA that dispersed in D_2O. (B) ^1H NMR spectrum of NH_2-HA and corresponding chemical structure of NH_2-HA.

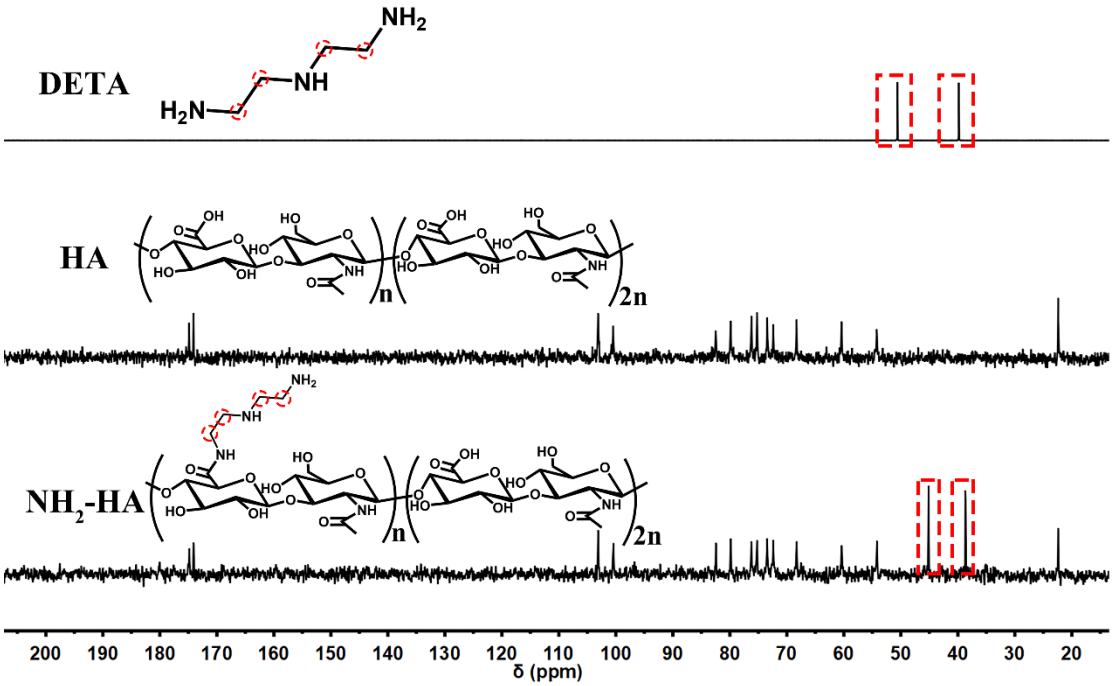

Fig. S3 ^{13}C NMR spectra of DETA, HA, and NH_2-HA (D_2O, 400 MHz).

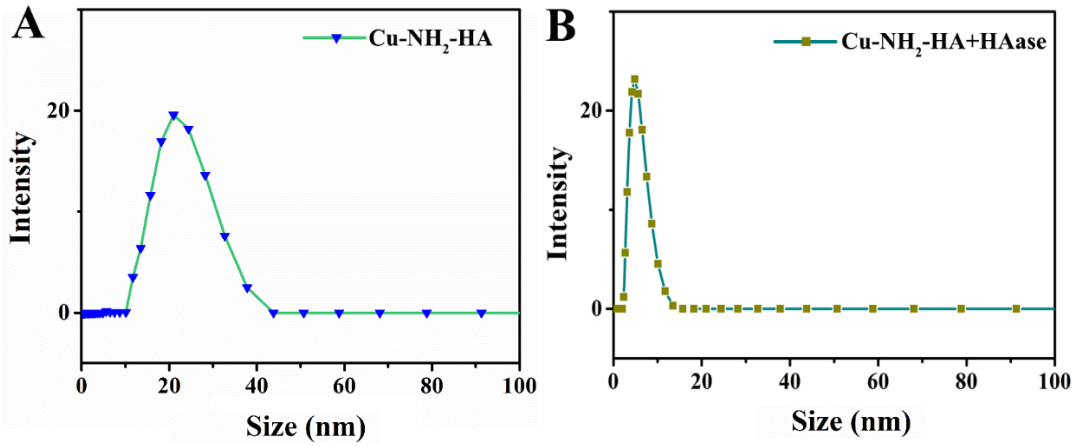

Fig. S4 Hydrodynamic size distributions of Cu-NH_2-HA incubated without (A) and with (B) HAase.

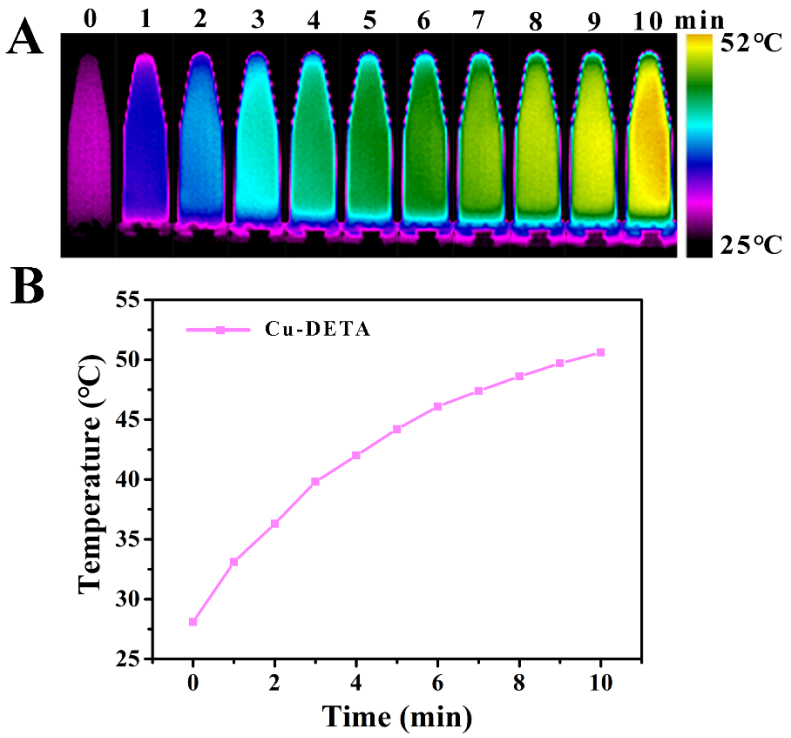

Fig. S5 (A) Photothermal imaging pictures and (B) photothermal heating curve of Cu(II)-coordination centers (Cu-DETA, 1.2 W cm^{-2}, 650-nm laser) for 10 min, [Cu] = 100 μg mL^{-1}.

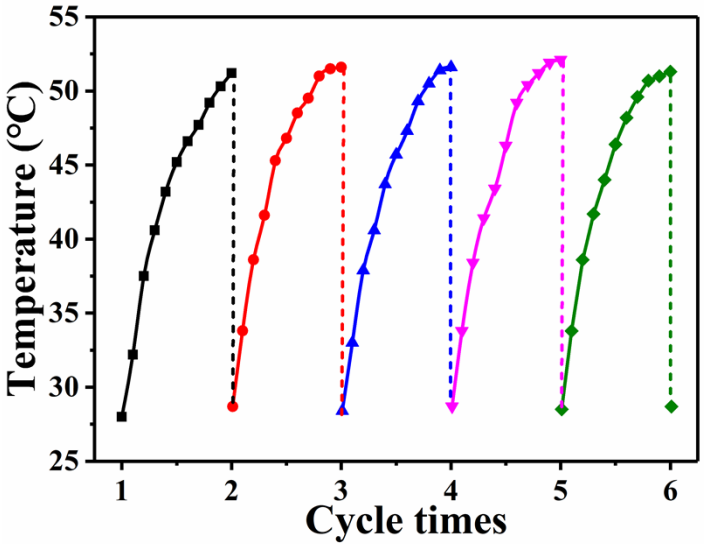

Fig. S6 Five cycles of the photothermal test of Cu-NH_2-HA (1.2 W cm^{-2}, 650-nm laser) for 10 min, [Cu] = 100 μg mL^{-1}.

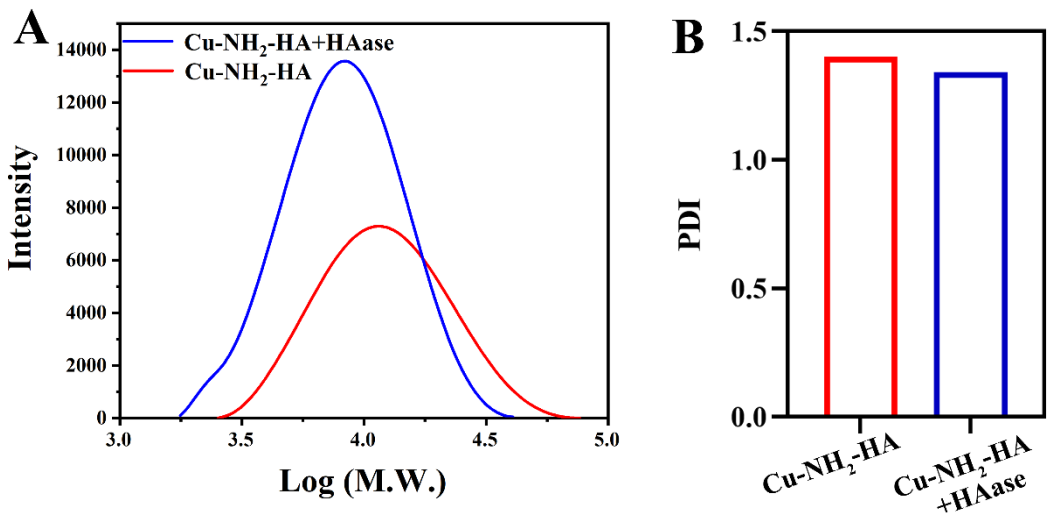

Fig. S7 Molecular weight distribution curve (A) and PDI (B) of Cu-NH_2-HA before and after treatment with HAase for 12 h.

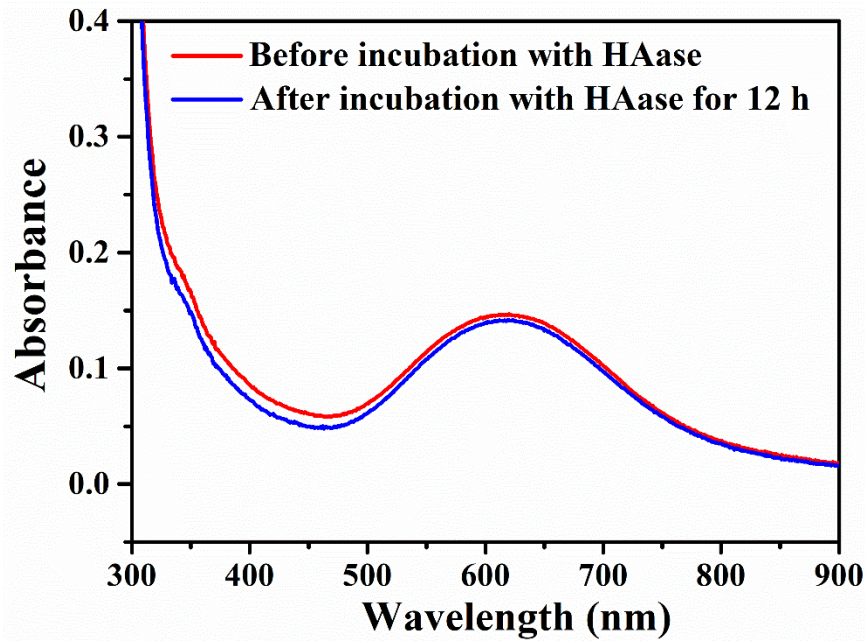

Fig. S8 UV-Vis-NIR spectra of Cu-NH_2-HA before and after incubation with HAase.

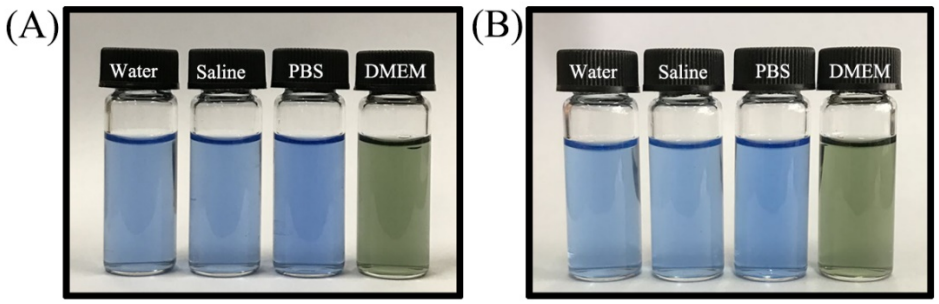

Fig. S9 Photographs of Cu-NH_2-HA dispersed in various physiological solutions (water, saline, PBS, and DMEM) with different time: 0 day (A) and 7 days (B).

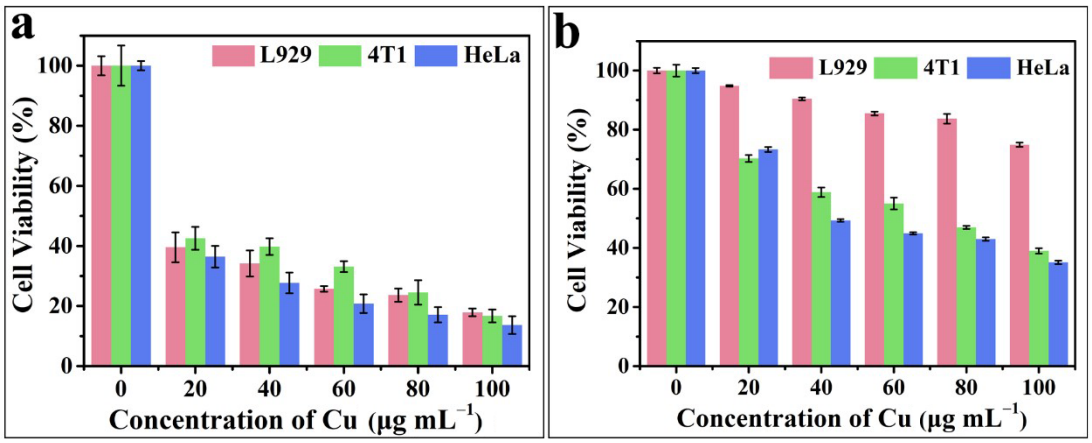

Fig. S10 Concentration-dependent dark cytotoxicities on L929, 4T1, and HeLa cells after incubated for 12 h: Cu-DETA (A) and Cu-NH_2-HA (B).

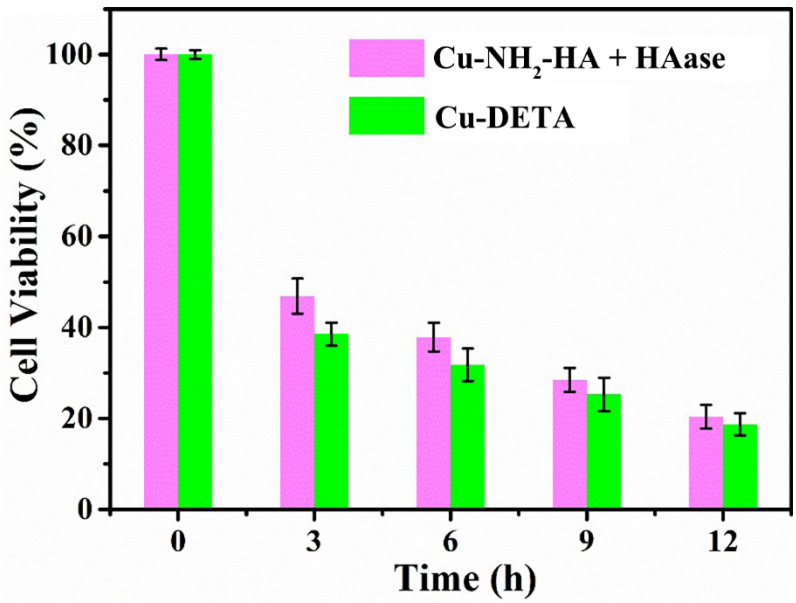

Fig. S11 Time-dependent dark cytotoxicity of 4T1 cells incubated with Cu-NH_2-HA and Cu-DETA. The Cu concentration was 100 μg mL^{-1}. Before incubation with 4T1 cells, Cu-NH_2-HA was pretreated by HAase for 6 h.

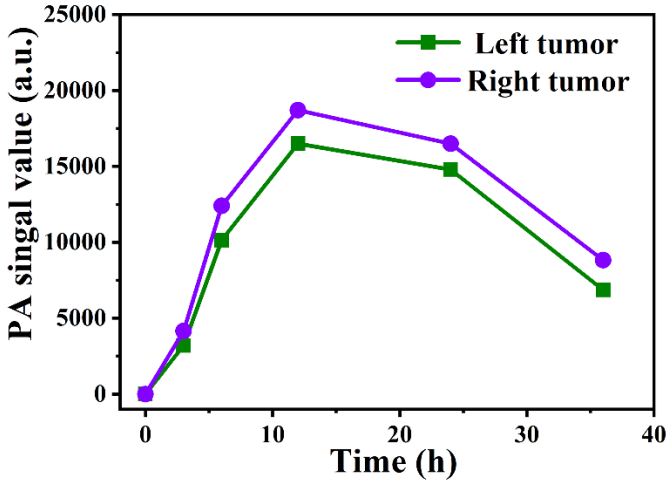

Fig. S12 Quantitative analysis of PA signal *in vivo*.

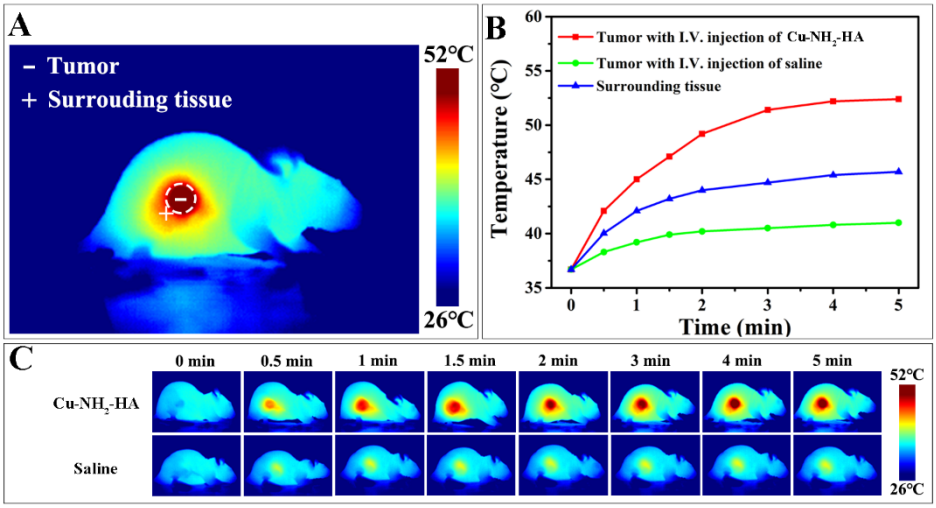

Fig. S13 (A) The whole-body photothermal image of a 4T1 tumor-bearing mouse after I.V. injection of Cu-NH_2-HA solution (80 μL, 500 μg mL^{-1} of Cu) exposed to a 650 nm laser (1.2 W cm^{-2}) for 5 min. The white circle represents the irradiation area. Points – and + indicate the location of the tumor and surrounding tissue, respectively. (B) Photothermal images of the tumor in the 4T1 tumor-bearing mouse after I.V. injection of Cu-NH_2-HA solution or saline at different time periods (0–5 min). (C) The corresponding time-dependent photothermal curves of the samples shown in (A) and (B).

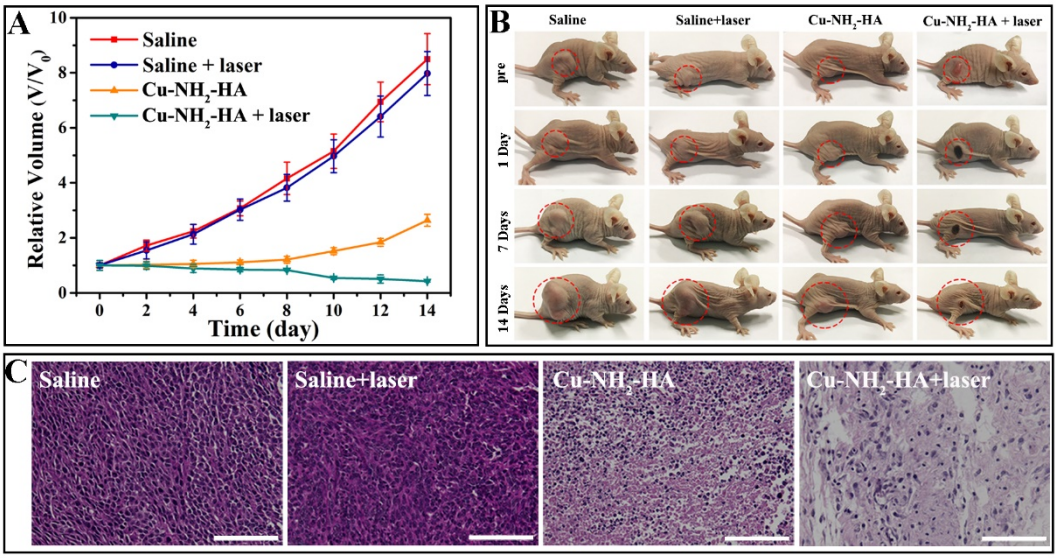

Fig. S14 One 4T1-tumor bearing mice model: (A) Tumor growth profiles after different treatments on 4T1 tumor-bearing mice. (B) Digital photographs before and after different treatments during 14 days. (C) The corresponding histological H&E staining assay of the harvested 4T1 tumors after various treatments, scale bar: 100 μm.

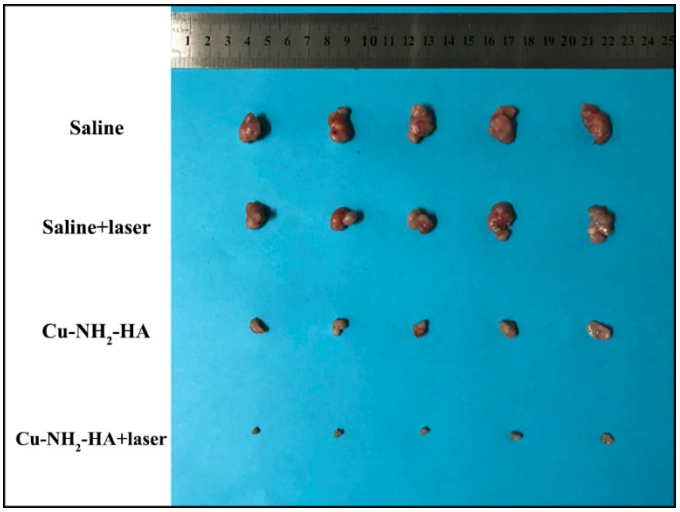

Fig. S15 One 4T1-tumor bearing mice model: digital photographs of harvested tumors after different treatments for 14 days.