

## Supporting Information

### A novel hypocrellin-based assembly for sonodynamic therapy against glioblastoma

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## Materials and methods

### 1. Synthesis of CTHB

**AETHB** was synthesized according to the previously reported method <sup>[1]</sup>.

**AETHB** (200 mg, 0.342 mmol) and tryptamine (5 g, 31.25 mmol) were dissolved in a mixed solution of 10 mL DMF and 10 mL purified water in a two-neck flask, followed by the addition of NaOH to adjust the pH to 14. The mixture was heated to 100 °C for 12 h in the dark under a nitrogen atmosphere. The reaction solution was poured into 100 mL of purified water and extracted with ethyl acetate (3×100 mL). The organic layer was washed twice with purified water (100 mL) and once with saturated

brine (100 mL), and dried over anhydrous sodium sulfate for 8 h. The solvent was removed by rotary evaporation under reduced pressure to obtain a crude product, which was further purified by silica gel column chromatography (mobile phase: gradient elution of CH<sub>2</sub>Cl<sub>2</sub> / CH<sub>3</sub>OH from 40:1 to 10:1) to obtain the desired product **CTHB** as a blue-black solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 17.22 (s, 1H), 12.89 (s, 1H), 8.12 (s, 1H), 7.70 (s, 1H), 7.39 (s, 1H), 7.15 (m, 3H), 6.58 (s, 1H), 6.31 (s, 1H), 5.19 (s, 1H), 4.14-4.05 (m, 9H), 3.90 (m, 1H), 3.46 (s, 3H), 3.27-3.01 (m, 4H), 2.21 (s, 3H), 1.75 (s, 3H). HR-ESI calcd for [C<sub>41</sub>H<sub>35</sub>N<sub>3</sub>NaO<sub>7</sub>S]<sup>+</sup> m/z: 736.2093 ([M+H]<sup>+</sup>), found: 736.2040.

## 2. Preparation of CTHB NPs

**CTHB** (1 mg) and PEG-PLGA (10 mg, Mw: 1000-1000) were completely dissolved in chloroform (1 mL). Under ice-water bath condition, the mixture was added dropwise using a syringe to the solution of polyvinyl alcohol (PVA) (10 mL in Milli-Q water, 1 %) with continuous ultrasound for 3 min using a microtip probe sonicator (12 W). The resulting emulsion was quickly transferred to a 100 mL round bottom flask and was stirred at room temperature for 8 h to remove chloroform to obtain a clear and transparent solution with magnetic stirring at 500 rpm. The solution was washed for three times using a 100 KDa Amicon Ultra filter (Millipore Corporation) under centrifugation at 3,000 rpm for 10 min to remove PVA and the unloaded **CTHB** and PEG-PLGA to get **CTHB NPs**. The concentration of **CTHB NPs** was calibrated by the standard curve of O.D. value at 640 nm ( $Y=0.0253X-0.0059$ ,  $R^2=0.9999$ ). The concentration of **CTHB NPs** in the text and Supplementary Information refers to the mass concentration of **CTHB**. **CTHB NPs** were stored at 4 °C prior to use.

## 3. Singlet oxygen generation by US irradiation

Under dark condition, 300 μL of TEMP solution was added to the solution of **CTHB NPs** (3 mL, 50 μg mL<sup>-1</sup>) with gentle shake. The mixture was sonicated for 5

min (frequency: 1 MHz, intensity: 0.8 W/cm<sup>2</sup>, duty cycle: 20%) using an ultrasonic physiotherapy apparatus (DJO Chattanooga; model number: 2776; probe size: 1 cm<sup>2</sup>). During the ultrasound, the probe was kept immersed in the solution. ESR experiment was performed to detect singlet oxygen signal. TEMP only solution with the same treatment was used as a control group.

#### **4. Cellular uptake and fluorescence imaging**

U87MG cells were seeded in a 35 mm confocal dish, and the solution of **CTHB NPs** (100  $\mu$ L, 500  $\mu$ g mL<sup>-1</sup>) was added to the culture medium. After the cells were incubated with **CTHB NPs** for 6 h, the cells were carefully washed twice with pre-chilled PBS to remove nanoparticles that did not endocytosed by cells. Fluorescence imaging was acquired by Nikon C1si laser scanning confocal microscope (CLSM).

#### **5. Cellular sonodynamic therapy**

U87MG cells were seeded into 12-well plates incubated with different concentrations of **CTHB NPs** at 37 °C with 5% CO<sub>2</sub> for 6 h, and then replaced with fresh culture medium. Before sonodynamic therapy, the ultrasonic physiotherapy apparatus and probe were irradiated with ultraviolet light for 1 h to ensure that the probe was sterile. The probe was kept immersed in the culture medium to ensure that cells could be continuously irradiated by ultrasound. After ultrasound (frequency: 1 MHz, intensity: 0.8 W/cm<sup>2</sup>, duty cycle: 20%) for 60 s, these cells were further incubated for 24 h. MTT assays were performed to evaluate the relative cell viability. The cytotoxicity of **CTHB NPs** was evaluated using a similar parallel procedure without ultrasound irradiation. In addition, U87MG cells were treated with the same sonodynamic treatment for AM / PI contained experiment. These cells were further stained with calcein AM and propidium iodide (PI) for 10 minutes for fluorescence imaging by CLSM.

## **6. Subcutaneous U87MG tumor model and PA imaging in vivo**

All animal experiments comply with the regulations on animal feeding and use stipulated by the China Animal Research Ethics Research Committee. All animal experiments were approved by the Experimental Animal Welfare Ethics Committee of the Institute of Process Engineering, Chinese Academy of Sciences (approval number: IPEAECA2019901). Four-week-old female nude mice weighing approximately 15-20 g were purchased from the Animal Experiment Center, Institute of Process Engineering, Chinese Academy of Sciences. A subcutaneous U87MG tumor model was established by subcutaneously injecting 100  $\mu\text{L}$  of PBS suspension of  $5 \times 10^6$  U87MG cells into the right posterior side of nude mice. When the tumor size was about 100  $\text{mm}^3$ , in vivo PA imaging and sonodynamic therapy experiments were performed.

The solution of **CTHB NPs** (100  $\mu\text{L}$ , 1.0  $\text{mg mL}^{-1}$ ) was injected into the tumor-bearing mice by intravenous injection. At different time points (0, 2, 5, 8, 12, and 24 h), PA images of tumor sites were acquired using the MSOT M128 ultra-high-resolution small animal photoacoustic imaging system.

## **7. Intracranial U87MG tumor model and FL imaging ex vivo**

$2 \times 10^5$  U87MG cells suspended in 5  $\mu\text{L}$  PBS were taken with a micro syringe, and the cell suspension was implanted into the mouse brain using a brain stereotaxic instrument. The injection rate was 1  $\mu\text{L}/\text{min}$  controlled by a micro-injection pump, and the needle was retained for 3 min after the injection. After continued feeding for 10 days, the solution of **CTHB NPs** (100  $\mu\text{L}$ , 1.0  $\text{mg mL}^{-1}$ ) was injected into the mice by intravenous injection. The mice were euthanized 5 h after injection, and main organs and brain were taken for FL imaging.

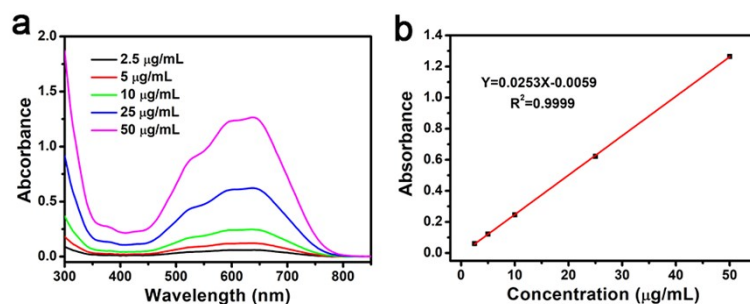
## **8. Subcutaneous U87MG tumor sonodynamic therapy**

The subcutaneous tumor-bearing mice were randomly divided into 4 groups (5

mice in each group): (1) Control group; (2) PBS + ultrasound group; (3) **CTHB NPs** only group (100  $\mu\text{L}$ , 1.0 mg  $\text{mL}^{-1}$ ); (4) **CTHB NPs** + ultrasound group (SDT group). Before sonodynamic therapy, the subcutaneous tumors were evenly covered with a layer of gel to ensure that the probe could closely contact the tumors. After intravenous injection of **CTHB NPs** solution (100  $\mu\text{L}$ , 1.0 mg  $\text{mL}^{-1}$ ) for 5 h, the tumors were treated with ultrasound (frequency: 1 MHz, intensity: 0.8  $\text{W}/\text{cm}^2$ , duty cycle: 20%) for 5 min. After different treatment, the tumor sizes and body weights were measured every two days. Tumor volume ( $V$ ) =  $\text{width}^2 \times \text{length} / 2$ . After 24 h of different treatment, tumors were harvested and stained with H&E for histopathological analysis. On the 14th day, the mice were euthanized and the tumors of mice in each group were collected and weighed.

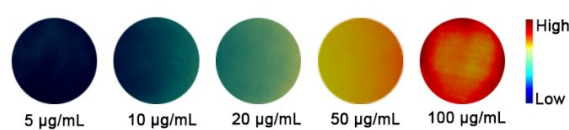
### **9. Intracranial U87MG tumor sonodynamic therapy**

The tumor-bearing mice were randomly divided into 3 groups (4 mice in each group): (1) PBS + ultrasound group; (2) **CTHB NPs** only group (100  $\mu\text{L}$ , 1.0 mg  $\text{mL}^{-1}$ ); (3) **CTHB NPs** + ultrasound group (SDT group). Before sonodynamic therapy, the top of mice's head was evenly covered with a layer of gel to ensure that the probe could closely contact the mice's head. After intravenous injection of **CTHB NPs** solution (100  $\mu\text{L}$ , 1.0 mg  $\text{mL}^{-1}$ ) for 5 h, the brain tumors were treated with ultrasound (frequency: 1 MHz, intensity: 0.8  $\text{W}/\text{cm}^2$ , duty cycle: 20%) for 5 min. The body weights of mice were measured every two days with different treatment, and the survival rates were counted. On the 10th day, the brains from each group were taken for H&E staining and a panoramic scan.

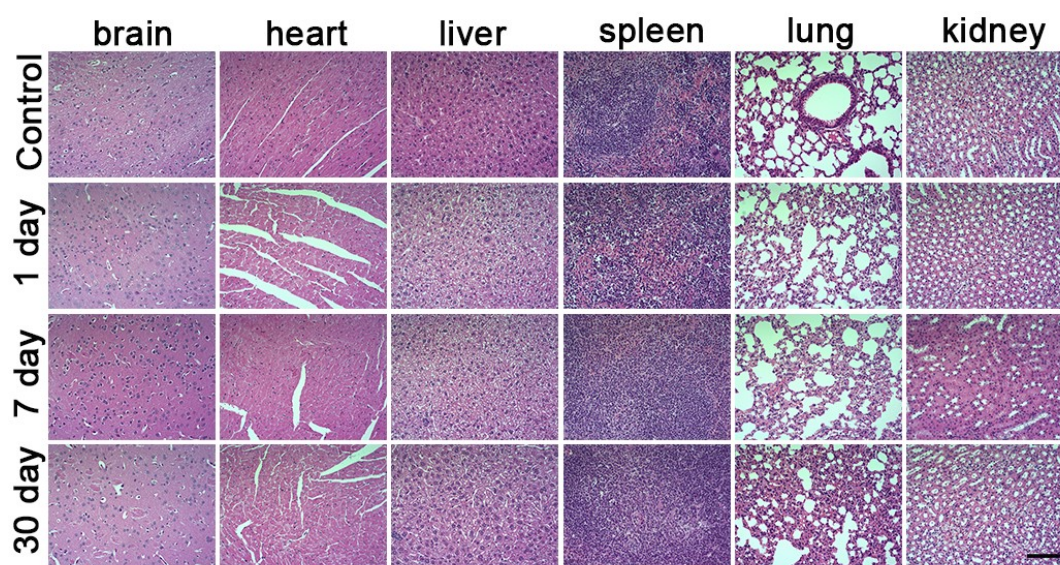


**Figure S1.** (a) UV-vis absorption spectrum of **CTHB** in different concentrations. (b)

Absorption calibration curve of **CTHB** at 640 nm.



**Figure S2.** In vitro PA images of **CTHB** NPs with different concentrations (5, 10, 20, 50, and 100  $\mu\text{g mL}^{-1}$ , respectively).



**Figure S3.** H&E staining images of the brain, heart, liver, spleen, lung, and kidneys in mice on 1st, 7th, and 30th day post-i.v. injection of **CTHB** NPs. Normal mice served as the control group. Scale bar: 100  $\mu\text{m}$ .

## REFERENCES

- 1 C. Zhang, J. Wu, W. Liu, X. Zheng and P. Wang, *ACS Appl. Mater. Interfaces*, 2019, **11**, 44989-44998.