Supporting Information

Nanozyme mediated high entropy-driven photothermal enhanced tumor catalytic therapy

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Experimental

Materials

Potassium ferricyanide ($K_3[Fe(CN)_6]$), copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Polyvinylpyrrolidone (PVP-K30) was purchased from Solarbio. Cell-counting kit-8 (CCK-8), DCFH-DA and Annexin V-FITC & PI dual staining kits were acquired from Beyotime (Shanghai, China).

Characterizations

The morphology of HEPBA and energy dispersive spectrometer (EDS) mapping of metal elements were acquired on a JEM-2100F transmission electron microscope (TEM). X-ray diffraction (XRD) pattern of HEPBA was collected from 10° to 80° with a scan rate of 10° per minute on a ShiMADZU XRD-6100 diffractometer. The hydrodynamic particle size of HEPBA was measured via dynamic light scattering (DLS) on Malvern Zetasizer (Nano ZS90).

Photothermal properties

The HEPBA dispersions (1 mL) at various concentrations (0, 25, 50, 100, 200 and 500 μg/mL) were irradiated with an 808 nm NIR laser (Beijing Viasho Technology Co., China) at constant power density of 0.8 W/cm^2 or varied power densities for 10 min. Real-time thermal images were collected with an infrared camera (Fotric 225, USA). To evaluate the photothermal stability, an aqueous dispersion of PB NPs (0.2 mg/mL)

was irradiated by an 808 nm laser (0.8 W·cm^{-2}) for totally five consecutive laser-on/off cycles. Meanwhile, to evaluate the photothermal conversion efficiency (η), the temperature change of HEPBA dispersion (200 μg/mL) was recorded as a function of time under an 808 nm laser irradiation (0.8 W·cm^{-2}) for 600 s. The η was then calculated using Eq. (S1).

$$\eta = \frac{hS(\Delta T_{Max,mix} - \Delta T_{Max,water})}{I(1 - 10^{-A_\lambda})} \quad \text{(S1)}$$

where h is the heat transfer coefficient; S is the surface area of the container, T_{max} is the temperature change of the solution between the maximum steady-state temperature and the ambient equilibrium temperature in the surrounding environment, I is the laser power at 808 nm, A_λ is the absorbance of the HEPBA at 808 nm. The value of hS could be calculated according to Eq. (S2) :

$$hS = \frac{mC_p}{\tau_s} \quad \text{(S2)}$$

where m is the mass of the HEPBA solution; C_p is the heat capacity of the solvent (4.2 J·g^{-1}); τ_s is the sample system time constant, $\tau_s = -\ln\theta$, where θ can be calculated using Eq. (S3) :

$$\theta = \frac{T - T_{surr}}{T_{Max} - T_{surr}} \quad \text{(S3)}$$

Thus, τ_s can be calculated by applying the linear time data from the cooling period versus $-\ln\theta$.

POD-like activity

The POD-like activity of HEPBA was first evaluated by methylene blue (MB) colorimetric assay. The HEPBA (1 mL, 0.2 mg·mL^{-1}) were mixed with 0.4 M H_2O_2, 50

mg·mL^{-1} MB, and the absorbance of MB at 664 nm was detected by UV-vis spectrophotometer at different times. Moreover, the POD-like activity was monitored by a 3,3',5,5'-tetramethylbenzidine (TMB) colorimetric assay. The absorbance at 652 nm of oxidized TMB was monitored. The steady-state kinetic assays were carried out by adding HEPBA (25 μg) into different substances, that is, 200 μL of buffer solution containing various concentration of TMB (0 - 1.0 mM) or H_2O_2 (0 - 12.5 mM). The Michaelis-Menten constants were then obtained by the Lineweaver-Burk double reciprocal equation as follows,

$$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \quad \text{(S4)}$$

where K_m was the Michaelis constant, v was the initial reaction velocity, V_{max} was the maximal reaction velocity and [S] defined as the substrate concentration.

Cell culture

Cells (NIH3T3, 4T1, and CT26) were cultured in DMEM (KeyGen BioTECH, China) that was supplemented with 10 % standard fetal bovine serum (Corning, USA), penicillin (100 U/mL), and streptomycin (100 U/mL). Cells were maintained at 37 °C with 5 % CO_2 in a humidified condition.

***in vitro* cytotoxicity**

Cells were seeded at a density of 5,000 cells per well in a 96-well plate and incubated overnight. The medium was then replaced with HEPBA at different concentrations (0, 25, 50, 75 or 100 μg/mL). For the photothermal assay, the cells with HEPBA were cultured in a humidified atmosphere containing 5 % CO_2 at 37 °C for 2 h, then irradiated using an 808 nm NIR laser for 4 min at a power density of 0.8 W·cm^{-2} prior to further

culturing for 24 h. Then, cells were washed with PBS and incubated with a mixture of 10 μL CCK-8 and 90 μL medium for 1 h and the absorbance at 450 nm was measured on a microplate reader ((infinite F50, ThermoFisher, USA). The cell viability was calculated according to the equation as follows,

$$cell\ viability = \frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}} \times 100\% \quad \text{(S5)}$$

Hemolysis Assay

Briefly, the erythrocytes from fresh blood were collected through centrifugation at 1,500 r/min and the suspension of erythrocytes (100 μL) was then incubated with HEPBA at different concentrations for 2h at 37 °C. Next, the supernatant was obtained through centrifugation at 10,000 r/min for 10 min. Subsequently, the absorbance of released hemoglobin was monitored on an ultraviolet-visible spectrophotometer at 540 nm and the hemolysis was calculated through the following equation.

$$Hemolysis(\%) = \frac{A - A_n}{A_P - A_n} \times 100\% \quad \text{(S6)}$$

where A, A_n, and A_p were the absorbances of HEPBA, negative control (PBS), and positive control (DI Water), respectively.

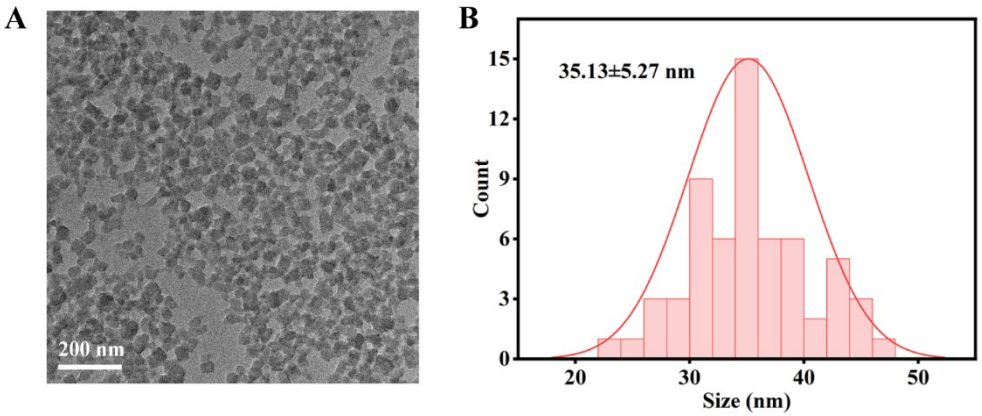


Figure S1. TEM and size distribution of HEPBA.

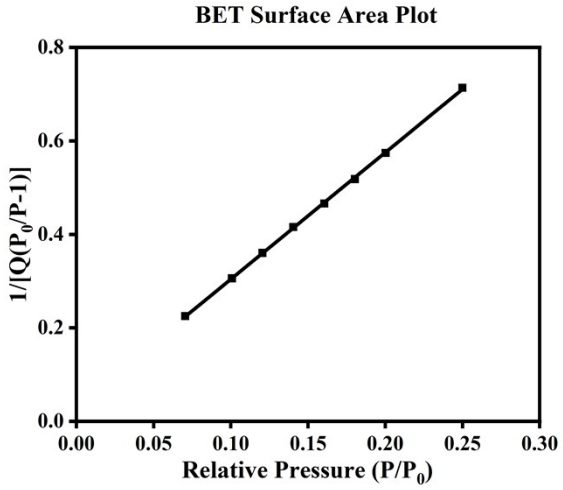
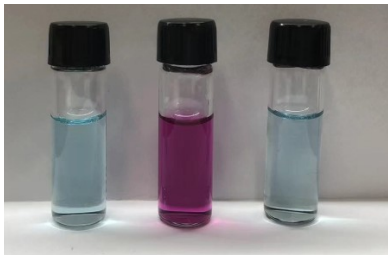


Figure S2. BET surface area plot of HEPBA.



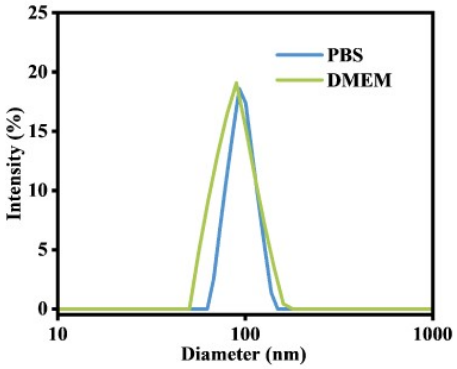


Figure S3. Hydrodynamic size distribution profiles and photo of HEPBA in different media.

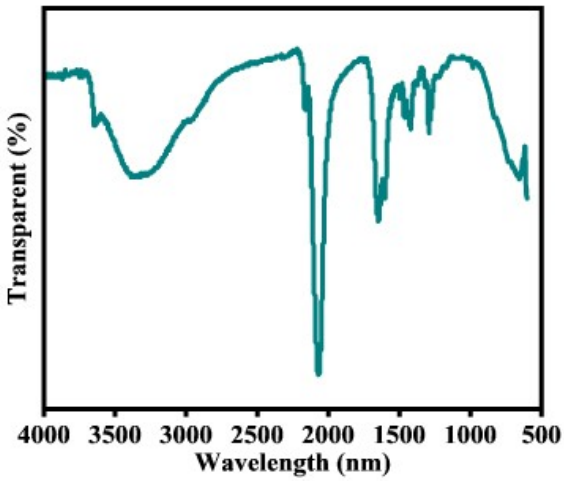


Figure S4. IR spectrum of HEPBA.

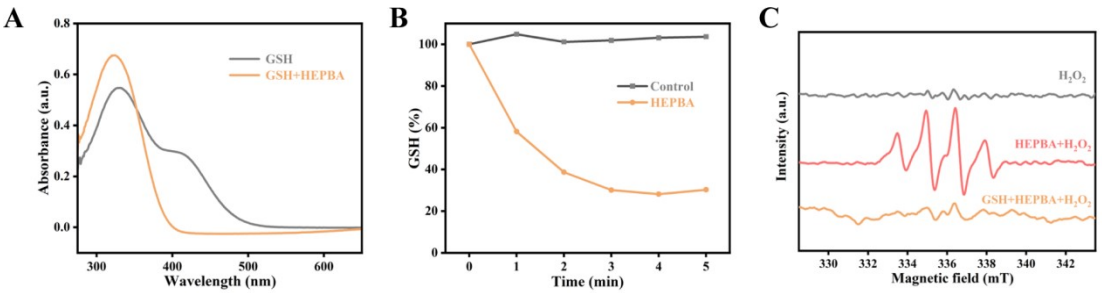


Figure S5. (A-B) UV-vis spectra of GSH under different conditions. (C) ESR spectra of generated ·OH under different conditions.

Table S1 Atomic metal contents of HEPBA measured by ICP.

Atomic	ICP
Fe	56.0%
Mn	13.8%
Co	9.09%
Ni	9.89%
Cu	11.2%

Table S2 kinetic parameters of HEPBA and HRP.

	Substrate	K_m (mM)	v_{max} (10^{-8} M·s^{-1})
HEPBA	TMB	0.0874	9.45
HRP	TMB	0.43	10.0
HEPBA	H_2O_2	2.33	9.19
HRP	H_2O_2	3.70	8.71