Microfluidic Flow Synthesis of Hemin@ZIF-8 Nanozyme with Applications in Cellular Reactive Oxygen Species Sensing and Anti-Cancer Drug Screening

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Supplementary Text

Calculation of mixing efficiency

The mixing efficiency in our microfluidic mixing chip is calculated based on the width of the yellow band area that results from the mixing of red and green fluorescent liquids through Eq. (1):

$$\text{Mixing efficiency } (\eta) = \frac{W_{yellow}}{W_{channel}} \quad (1)$$

where W_{yellow} represents the width of the yellow band area observed in the merged image, indicating the overlap of red and green fluorescent liquids after mixing. $W_{channel}$ denotes the total width of the channel where the mixing occurs. This ratio provides a quantitative measure of the mixing efficiency within the microfluidic device, with the entrance of red fluorescent liquid through inlet 1 and green fluorescent liquid through inlet 2.

The appearance of yellow in the merged image signifies effective mixing of the two liquids. This quantification method directly relates the physical dimensions of the mixed area to the overall channel width, offering a straightforward and intuitive metric for assessing the mixing performance. The calculations of W_{yellow} and $W_{channel}$ leverage image analysis techniques to accurately determine the extent of mixing achieved. This approach ensures that the mixing efficiency metric is grounded in observable, quantifiable changes in the liquid's coloration within the microfluidic chip, reflecting the practical outcomes of the device's design and operation.

Calculation of limit of detection (LOD)

The limit of detection of H_2O_2 was determined using Eq. (2):

$$\text{LOD} = \frac{3\sigma}{K} \quad (2)$$

where K is the slope of the linear part of the calibration curve, and σ is the standard deviation of the blank.

Supplementary Figure

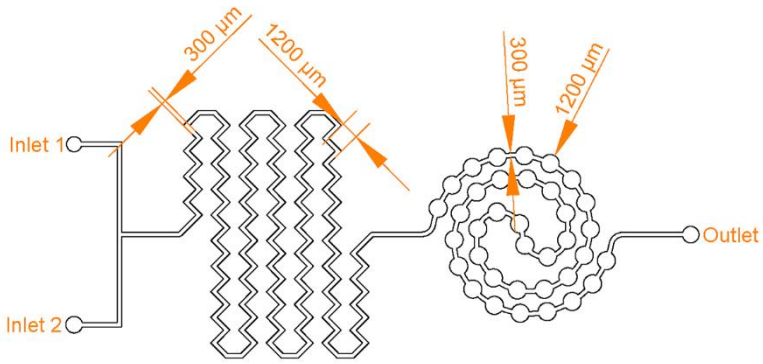

Fig. S1 Design specifications with precise dimensions of the microfluidic mixing chip.

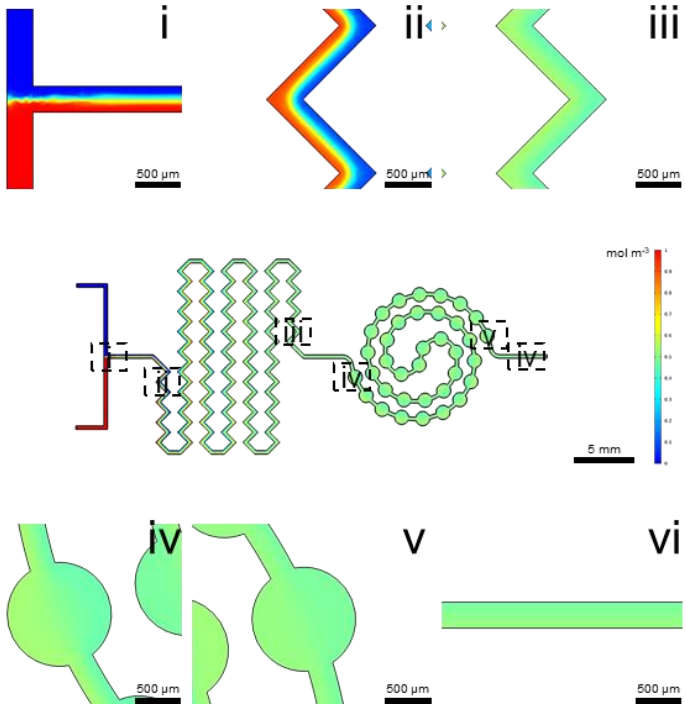

Fig. S2 Simulated concentration field at different locations along the microchannel flow path.

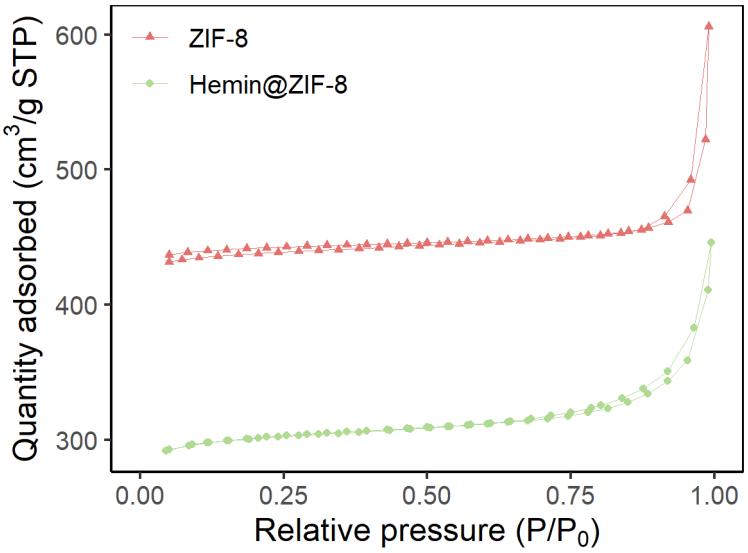

Fig. S3 Nitrogen adsorption-desorption isotherms of ZIF-8 and hemin@ZIF-8.

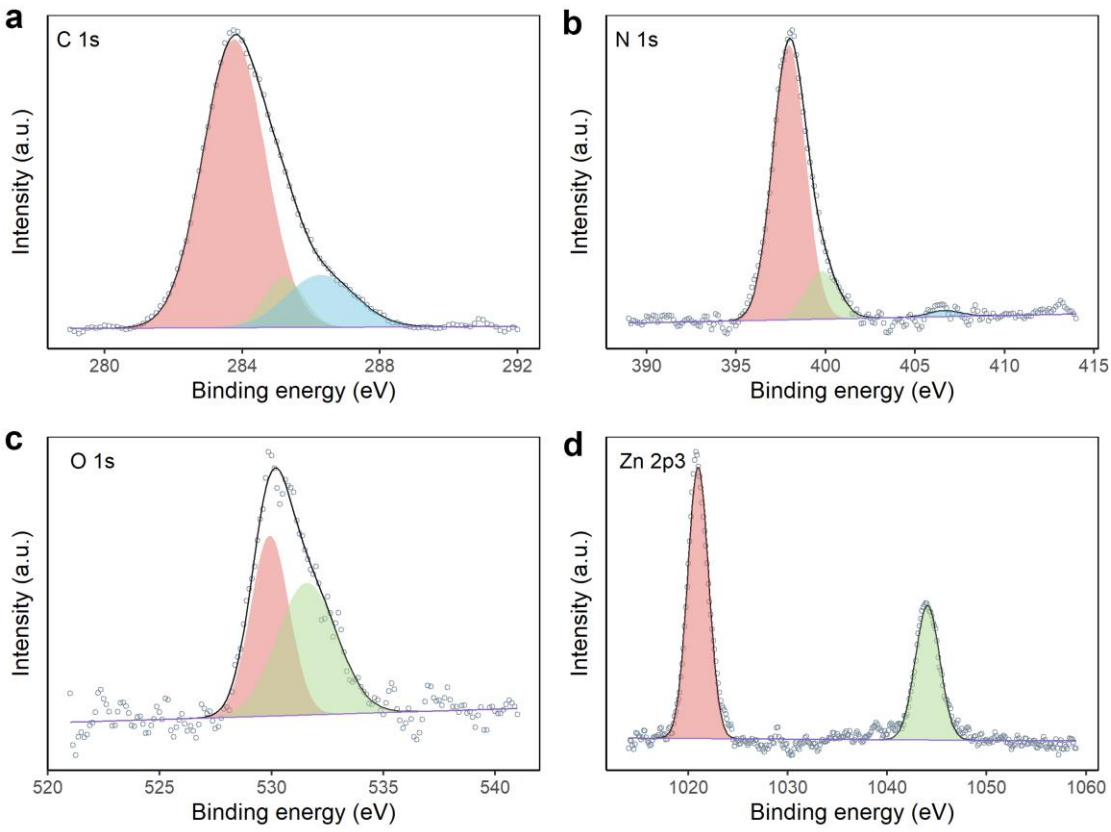

Fig. S4 High-resolution XPS spectra of Hemin@ZIF-8 showing the C1s, N1s, O1s, and Zn 2p3 peaks.

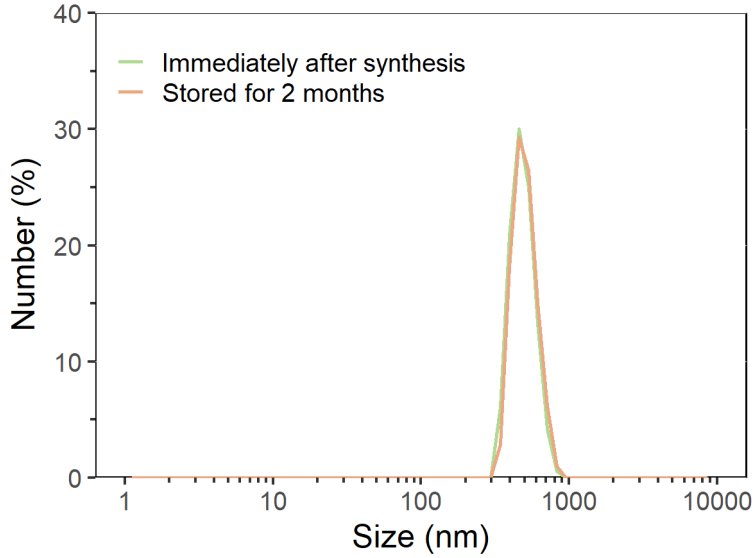

Fig. S5 DLS size distribution analysis of hemin@ZIF-8 immediately after synthesis and after storage for 30 days.

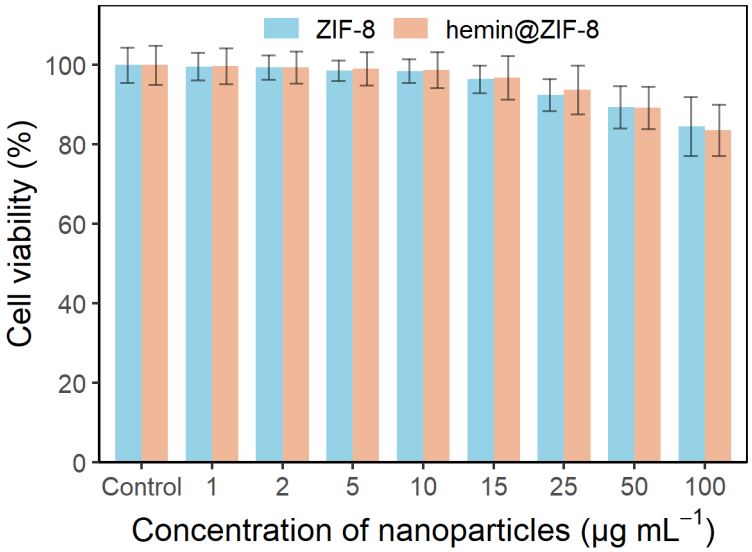

Fig. S6 Cell viability in culture media supplemented with different concentrations of hemin@ZIF-8.

Supplementary Table

Table S1 Calculation of recovery for H_2O_2 detection.

Theoretical concentration (μM)	Experimental concentration (μM)	Recovery (%)
1	1.178	117.8
2	2.602	130.1
5	4.724	94.5

Table S2 Calculation of recovery for cell identification.

Theoretical concentration (cell mL^{-1})	Experimental concentration (cell mL^{-1})	Recovery (%)
20	19	95
50	35	70
100	133	133
200	258	129
500	478	95.6

Supplementary Movie

Movie S1 Simulation of flow field formation in the designed microfluidic chip.

Movie S2 Simulation of fluid mixing in the designed microfluidic chip.