Electronic Supplementary Material (ESI) for ChemComm.


Supplementary Information for

# Surface charge engineering of nanosized CuS via acidic amino acid modification enables high peroxidase-mimicking activity at neutral pH for one-pot detection of glucose

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## Experimental Section

### *1. Chemicals and reagents*

Cupric acetate, NaHS, $H_2O_2$ (30%), Congo red, terephthalic acid (TA), glucose (Glu), fructose (Fru), saccharose (Sac), and lactose (Lac) were purchased from Sinopharm Chemical Reagent Co., Ltd. Aspartic acid (Asp), 3,3'5,5'-tetramethylbenzidine (TMB), 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)diammonium salt (ABTS), glutamic acid, cholesterol (Chol), ascorbic acid (AA), dopamine (DA), uric acid (UA), glutathione (GSH), and cysteine (Cys) were provided by Shanghai Aladdin Biochemical Technology Co., Ltd. GOx (100 U/mg, from *Aspergillus niger*) was purchased from Sigma-Aldrich. All chemicals and reagents were of analytical grade and used without further purification. Deionized water was used in the whole study.

### *2. Synthesis of Asp-modified CuS*

Typically, 1.0 g cupric acetate was first dissolved in 20 mL deionized water, and simultaneously 0.05 g Asp was dissolved in another 20 mL deionized water. Then, the two solutions were mixed under a mild stir for 30 min. After that, 0.56 g NaHS dissolved in 10 mL deionized water was dropwise added into the mixture under a wild stir. After reaction for 1 h, the black products were collected by centrifugation and rinsed with adequate deionized water. After dried in a vacuum oven at 50$^{o}$C overnight, Asp-modified CuS was obtained. For comparison, bare CuS was also prepared using the same procedure with no amino acid added.

### *3. Apparatus*

Inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements were carried out on a 725 spectrometer (Agilent Technologies Co., Ltd.). X-ray diffraction (XRD) measurements were performed on a 6100 XRD instrument (Shimadzu) with a Cu Kα radiation. Transmission electron microscopy (TEM) images were obtained by a JEM-2100F microscope (JEOL). Fourier transform infrared (FT-IR) spectra were obtained by a Nicolet Nexus 470 instrument (USA Nicolet Co., Ltd.). X-ray photoelectron spectroscopy (XPS) measurements were carried out on an ESCALAB-MKII spectrometer (Thermo-Fisher Scientific Co., Ltd.). Zeta potential measurements were performed on a Nano ZS90 instrument (UK Malvern Co., Ltd.). All colorimetric experiments were finished on a Cary 8454 ultraviolet-visible (UV-Vis) spectrometer (Agilent Technologies Co., Ltd.). Fluorescence measurements were accomplished using a Cary Eclipse spectrophotometer (Australian Varian Co., Ltd.).

***4. Evaluation of the peroxidase-mimicking activity of Asp-modified CuS in neutral media***

To verify the peroxidase-like activity of Asp-modified CuS under neutral conditions, 100 μL of 0.02 mg/mL Asp-modified CuS, 100 μL of 10 mM $H_2O_2$, and 100 μL of 5 mM TMB (prepared with ethanol) were mixed in 2.7 mL PBS (0.1 M, pH 7.0). The color changes upon time were immediately monitored by UV-Vis measurements.

To uncover the peroxidase-mimicking catalytic path of Asp-modified CuS, dye degradation and hydroxyl radical capture experiments were performed. In the dye

degradation experiment, 100 μL of 0.1 mg/mL Asp-modified CuS and 500 μL of 100 mM $H_2O_2$ were added into 2.4 mL Congo red (20 μM) to initiate the reaction. After incubation at room temperature for 1 h, the color changes were recorded. In the hydroxyl radical capture experiment, TA was employed as a fluorescent probe to track hydroxyl radicals.[1] Typically, 6.7 μg/mL Asp-modified CuS was incubated with 0.25 mM TA and 3.3 mM $H_2O_2$ in 3 mL PBS (0.1 M, pH 7.0) at room temperature for 1 h. The fluorescence measurements were carried out with an excitation wavelength of 315 nm.

To highlight the robustness of Asp-modified CuS, the nanozyme was first incubated in solutions with different pH values or at different temperatures for 2 h, and then its activity was measured under standard conditions.

The steady-state kinetic measurements were carried out by recording the absorbance at 652 nm at a 5 s interval within 1 min. The apparent kinetic parameters were calculated based on the equation $v = V_{max} \times [S]/(K_m + [S])$, where $v$ is the initial velocity, $V_{max}$ is the maximum reaction velocity, $[S]$ is the substrate ($H_2O_2$ or TMB) concentration, and $K_m$ is the Michaelis-Menten constant.

***5. One-pot colorimetric detection of glucose***

For the one-pot colorimetric detection of glucose, 100 μL of glucose with a certain concentration, 50 μL of 50 U/mL GOx, 100 μL of 0.2 mg/mL Asp-modified CuS, 100 μL of 5 mM TMB (prepared with ethanol), and 2.65 mL of 0.1 M PBS (pH 7.0) were mixed together and incubated at room temperature for 20 min. After that, the UV-Vis spectra were recorded.

The glucose levels in human serum samples (these clinical samples were provided by the Affiliated Hospital of Jiangsu University) were detected by our one-pot assay. For each sample, 200 μL of serum, 50 μL of 50 U/mL GOx, 100 μL of 0.2 mg/mL Asp-modified CuS, 100 μL of 5 mM TMB (prepared with ethanol), and 2.55 mL of 0.1 M PBS (pH 7.0) were mixed together and incubated at room temperature for 20 min. Afterwards, the UV-Vis spectra were recorded.

**Supplementary Figures**

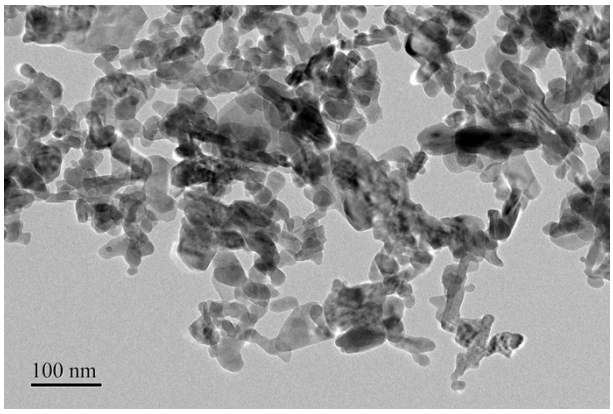


**Figure S1.** TEM image of bare CuS.

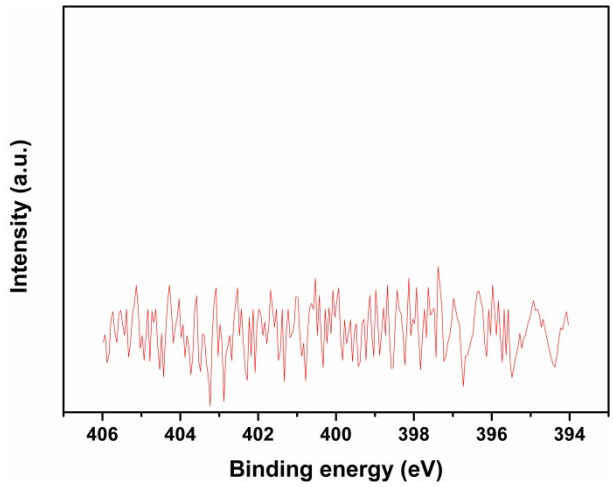


**Figure S2.** Fine N 1s XPS of bare CuS.

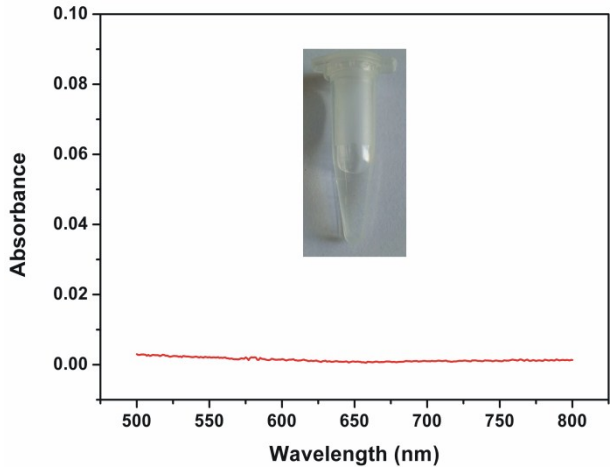


**Figure S3.** 6.7 μg/mL Asp-modified CuS has a negligible color background.

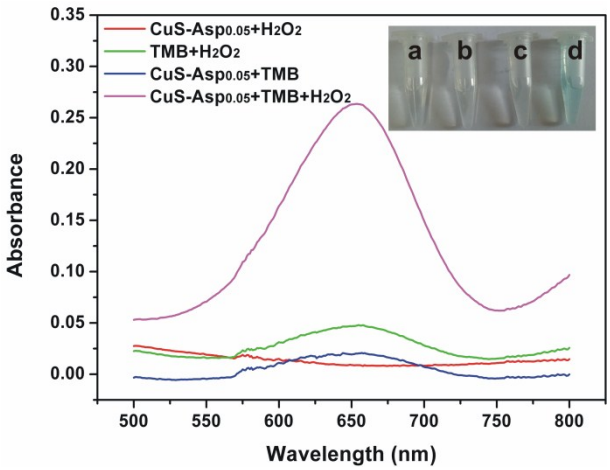


**Figure S4.** Different combinations of CuS-$Asp_{0.05}$, $H_2O_2$, and TMB in 0.1 M PBS (pH 7.0). The inset shows the corresponding photograph (a─TMB+$H_2O_2$, b─CuS-$Asp_{0.05}$+TMB, c─CuS-$Asp_{0.05}$+$H_2O_2$, d─CuS-$Asp_{0.05}$+$H_2O_2$+TMB).

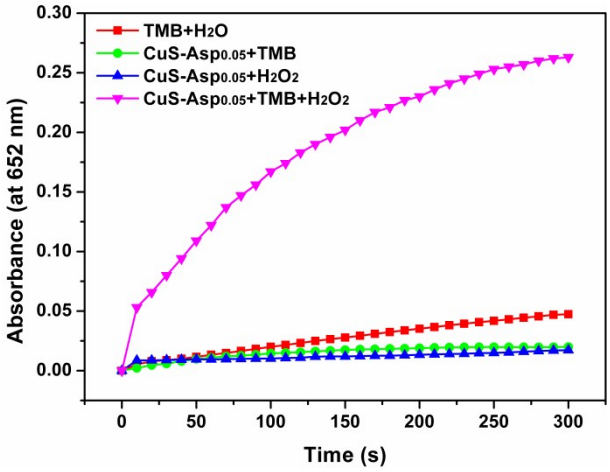


**Figure S5.** Time-dependent color changes of different combinations of CuS-$Asp_{0.05}$, $H_2O_2$, and TMB in 0.1 M PBS (pH 7.0).

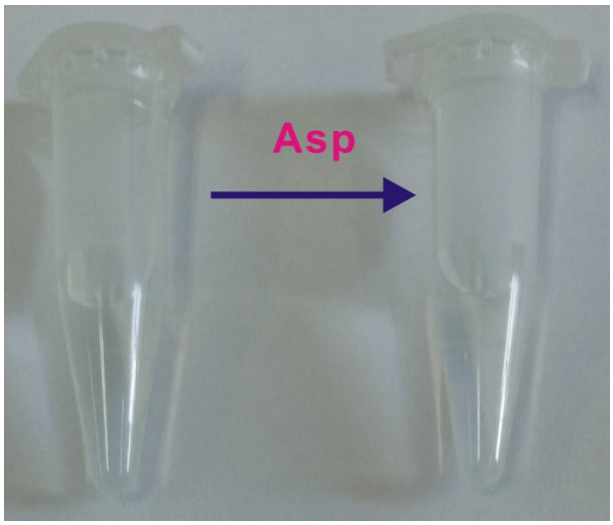


**Figure S6.** The Asp modifier cannot trigger the TMB color reaction in the presence of $H_2O_2$.

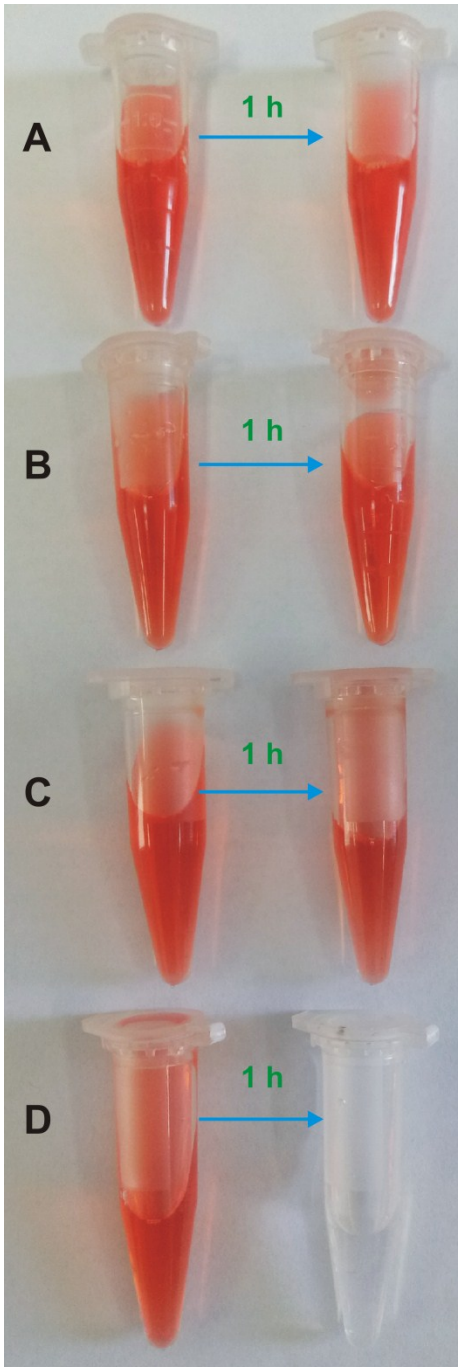


**Figure S7.** Degradation of Congo red in different systems (A─None, B─CuS-$Asp_{0.05}$, C─$H_2O_2$, D─CuS-$Asp_{0.05}$+$H_2O_2$).

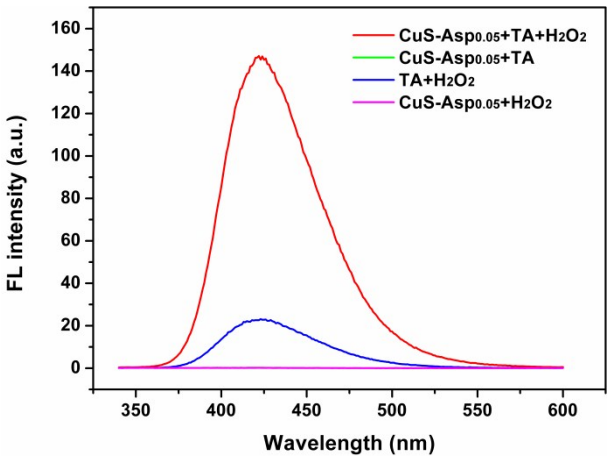


**Figure S8.** Capture of hydroxyl radicals by TA.

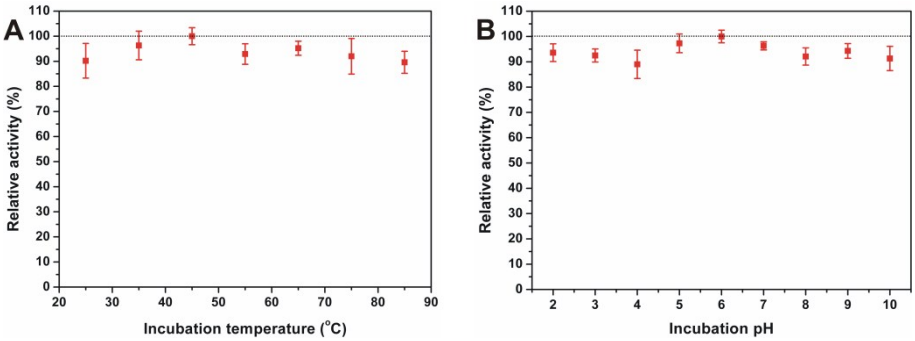


**Figure S9.** Robustness of the CuS-$Asp_{0.05}$ nanozyme against (A) harsh temperature and (B) pH.

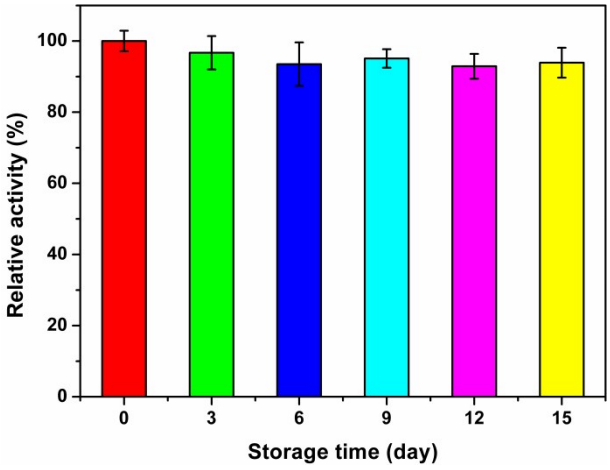


**Figure S10.** Stability of the CuS-$Asp_{0.05}$ activity upon storage time.

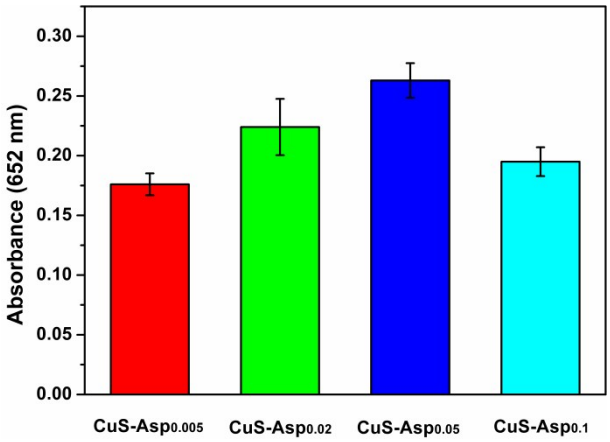


**Figure S11.** CuS-$Asp_{0.05}$ exhibits the highest peroxidase-mimicking catalytic activity among these prepared Asp-modified CuS materials.

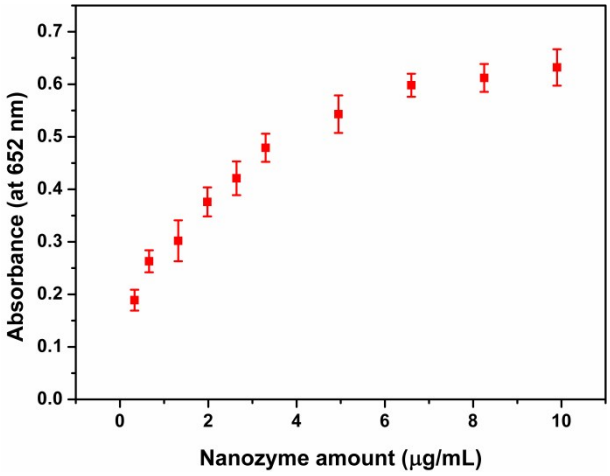


**Figure S12.** Effect of the CuS-$Asp_{0.05}$ amount used on the TMB color reaction.

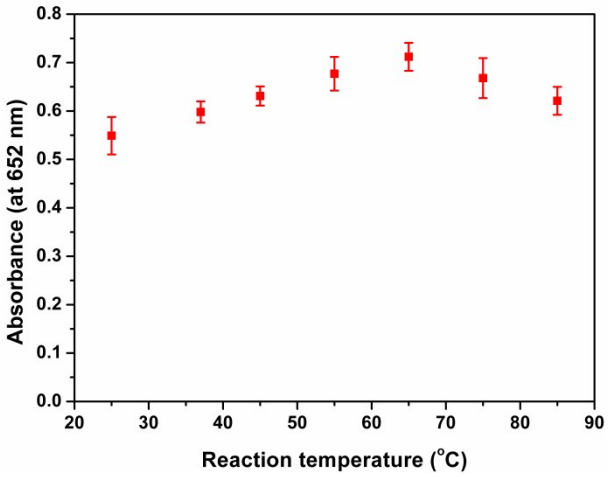


**Figure S13.** Effect of the reaction temperature on the TMB color reaction catalyzed

by CuS-Asp$_{0.05}$.

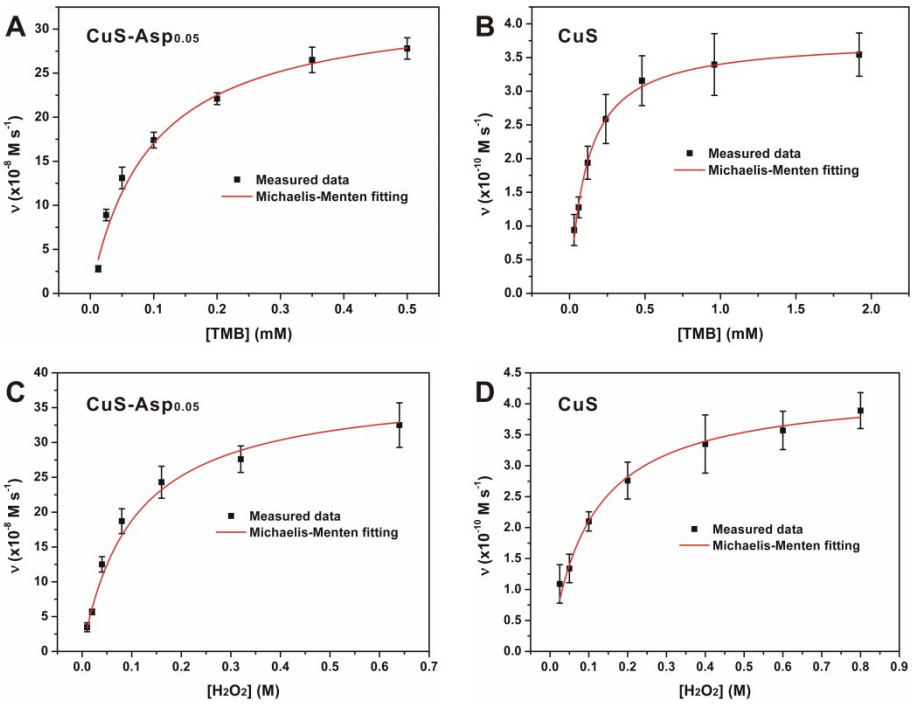


**Figure S14.** Steady-state kinetic measurements of (A and C) CuS-Asp$_{0.05}$ and (B and D) bare CuS toward (A and B) TMB and (C and D) $H_2O_2$ in neutral media.

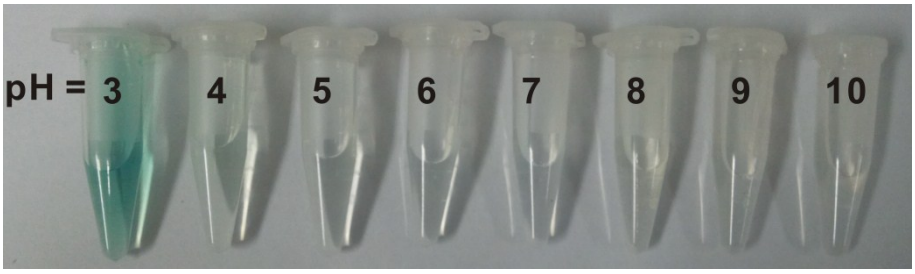


**Figure S15.** The ABTS color reaction catalyzed by CuS-Asp$_{0.05}$ in buffers with different pH values.

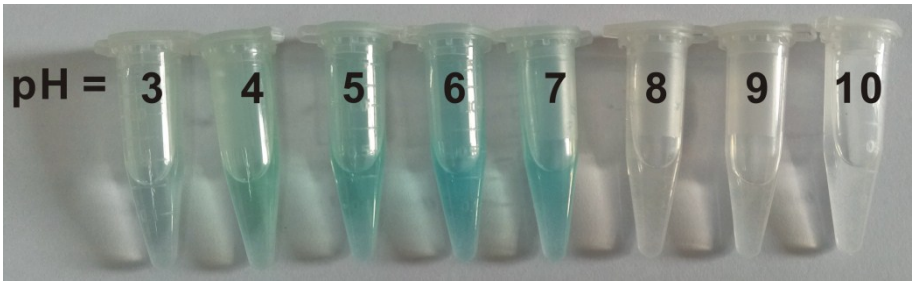


**Figure S16.** The TMB color reaction catalyzed by glutamic acid-modified CuS in buffers with different pH values.

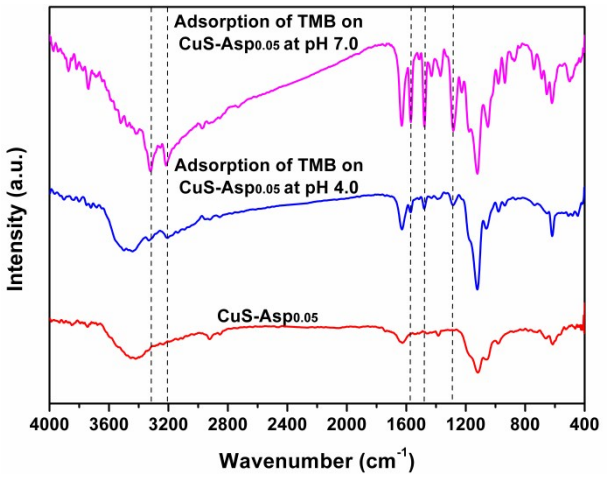


**Figure S17.** FT-IR spectra of CuS-$Asp_{0.05}$ before and after adsorption of TMB at different pH values. The absorption signals labeled with dashed line are attributed to the TMB substrate adsorbed on the nanozyme.

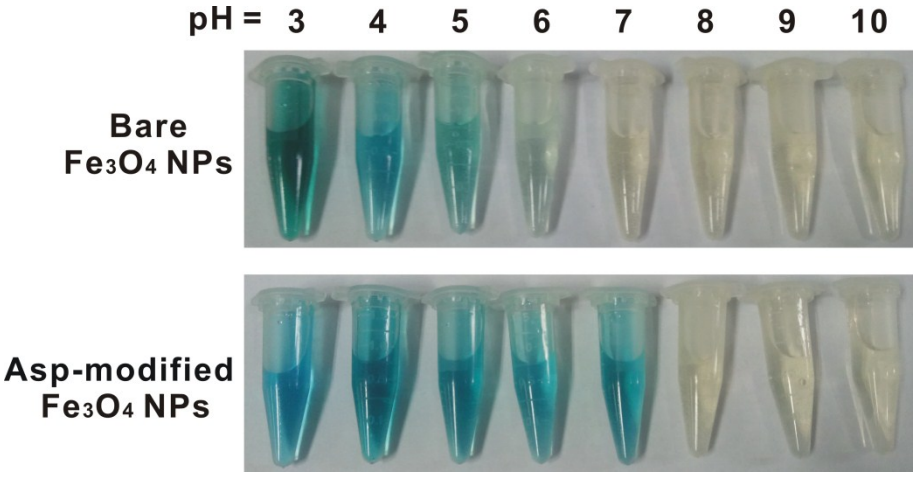


**Figure S18.** The TMB color reaction catalyzed by bare $Fe_3O_4$ NPs and Asp-modified

$Fe_3O_4$ NPs in buffers with different pH values.

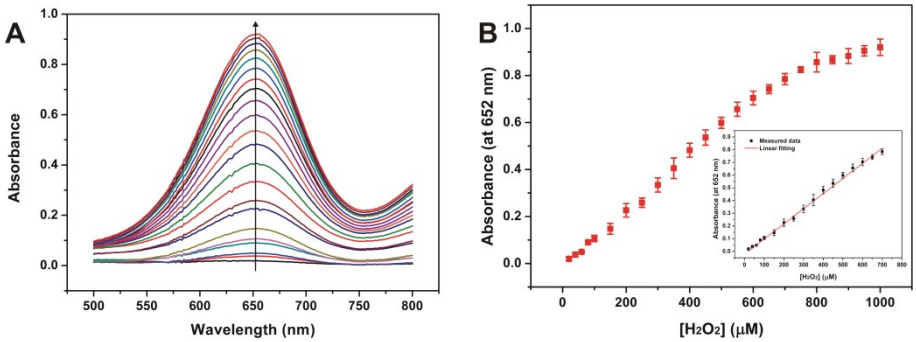


**Figure S19.** (A) displays the UV-Vis spectra of the CuS-$Asp_{0.05}$+$H_2O_2$+TMB system with different concentrations of $H_2O_2$. (B) shows the relationship between the $H_2O_2$ level and the absorbance at 652 nm.

**Supplementary Tables**

**Table S1.** Four Asp-modified CuS materials prepared in our work.

| Material | Amount of Asp used for modification (g) | Amount of Asp detected by ICP-OES (%) |
|---|---|---|
| Bare CuS | — | — |
| CuS-Asp$_{0.005}$ | 0.005 | 1.27 |
| CuS-Asp$_{0.02}$ | 0.02 | 5.32 |
| CuS-Asp$_{0.05}$ | 0.05 | 9.17 |
| CuS-Asp$_{0.1}$ | 0.1 | 11.09 |

**Table S2.** Comparison of apparent kinetic parameters of CuS-$Asp_{0.05}$ and bare CuS toward TMB and $H_2O_2$ in neutral media and natural HRP at pH 4.0.

| Nanozyme | Substrate | $K_m$ (mM) | $V_{max}$ ($10^{-8}$ M/s) |
|---|---|---|---|
| Bare CuS | TMB | 0.11 | 0.038 |
| | $H_2O_2$ | 101.8 | 0.043 |
| CuS-$Asp_{0.05}$ | TMB | 0.09 | 33.1 |
| | $H_2O_2$ | 103.2 | 38.2 |
| Natural HRP[2] | TMB | 0.38 | 10.8 |
| | $H_2O_2$ | 4.5 | 9.7 |

**Table S3.** Performance comparison of our one-pot colorimetric assay with previously reported assays for glucose detection.

| Peroxidase mimic | Linear range (mM) | LOD (μM) | Ref. |
|---|---|---|---|
| $Fe_3O_4$ MNPs | 0.05~1 | 30 | 3 |
| MNP/NG | Up to 18 | 57.9 | 4 |
| $WSe_2$ nanosheets | 0.01~0.06 | 10 | 5 |
| $Cu_{0.89}Zn_{0.11}O$ | 0.025~0.5 | 1.5 | 6 |
| GO-COOH | 0.001~0.02 | 1 | 7 |
| $CoPW_{11}O_{39}$ | 0.033~0.5 | 23.4 | 8 |
| $CuS\text{-}Asp_{0.05}$ | 0.025~0.6 | 4.9 | This work |

**Table S4.** Reliability of our one-pot assay in sensing glucose in clinical serum samples.

| Sample | Detected (mM) | Clinical data (mM) | Relative error (%) |
|---|---|---|---|
| 1# | 5.57±0.23 | 5.73 | -2.8 |
| 2# | 4.32±0.11 | 4.26 | 1.4 |
| 3# | 8.59±0.34 | 8.74 | -1.7 |

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