Electronic Supplementary Material

Modulation of the enzyme-like activity of CuAsp nanozyme by gallic acid and the selective detection of bisphenol A in infant food packaging

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Chemicals and Instrumentation. $Cu(NO_3)_2 \cdot 3H_2O$, L-Aspartic acid (L-Asp), bisphenol A, L-Glutamic acid (L-Glu), L-Glycine (L-Gly), L-Tyrosine (L-Try), Glucose, Fructose, Sucrose, 3,3′,5,5′-Tetramethylbenzidine (TMB), and N-2-hydroxyethylpiperazine-N-ethane-sulphonicacid (HEPES) were obtained from Macklin Co., Ltd. (Shanghai, China). 2,4-dichlorophenol (2,4-DP) and 4-aminoantipyrine (4-AP) were procured from Aladdin Inc. (Shanghai, China). 2-(N-Morpholino)ethanesulfonic acid monohydrate (MES) and Tris-(hydroxymethyl) aminomethane (Tris-HCl) were procured from Sangon Biotechnology Co., Ltd. (Shanghai, China). Gallic acid (GA), NaCl, KCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, $NaHCO_3$, Na_2CO_3, Na_2SO_4, and CH_3COONa were purchased from Xilong Scientific Co., Ltd. (China). Hydrogen peroxide (H_2O_2, 30%), NaOH， CH_3COOH, $Zn(NO_3)_2$, $CoCl_2$, $NiCl_2$, NaBr, and Benzoic acid were purchased from Beijing Chemical Works. All reagents and chemicals utilized in this work were analytical grades.

Fourier transform infrared (FTIR) spectra were collected on a Shimadzu IRPrestige-21 spectrometer (Japan). X-ray photoelectron spectroscopy (XPS) spectra were obtained using an ESCALAB-250Xi spectrometer (UK). UV-vis spectra and absorbance were determined with a UV-2550 spectrophotometer (Kyoto, Japan). Centrifuge HC-2062 (Anhui, China) was used for centrifugal operations in experiments.

Synthesis of CuAsp. CuAsp was obtained with reference to previous work and slightly optimized.[S1] First, 1 mmol of Asp and 3 mmol of sodium hydroxide were

dissolved in 10 mL of the aqueous solution, and the aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (1.5 mmol, 2 mL) was added to the above mixture at room temperature and the solution was immediately mixed well with a vortex shaker. Then, the mixture was centrifuged at 10,000 r/min for 3 min to remove the supernatant, and the resulting precipitate was washed with ethanol:water (1:1) solution, centrifuged again, and the process was repeated three times. Finally, the obtained CuAsp were dissolved in deionized water and stored in a refrigerator at 4 °C.

Stability comparison of GA-CuAsp and CuAsp.

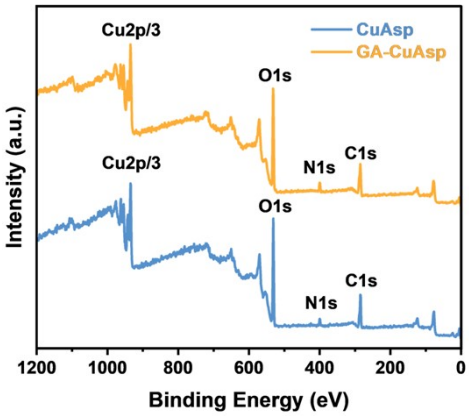
For pH stability. GA-CuAsp and CuAsp were incubated at different pH (3-9) for 6 h to assess the acid-base tolerance of GA-CuAsp and CuAsp, and then the activity was determined by the extent to which they catalysed the oxidation of 2,4-DP. The activities at other pH were compared with the activity at the optimal pH to determine the relative activities at different pH.

For temperature stability. The temperature stability of GA-CuAsp and CuAsp was investigated by exposing them to 25-80 °C for 60 min and then determining their activity by the extent of their catalysed oxidation of 2,4-DP. The activities at other temperatures were compared with those at 25 °C to determine the relative activities at different temperatures.

For salt concentration stability. The effect of ionic strength on catalytic activity was measured by mixing GA-CuAsp and CuAsp with different concentrations of NaCl (0, 50, 100, 200, 300, 400 and 500 mmol L^{-1}) and then determining the activity based

on the extent to which it catalysed the oxidation of 2,4-DP.

For temporal stability. To investigate the temporal stability of GA-CuAsp and CuAsp, their aqueous solutions were stored at 4 °C for 15 days, and the catalytic reaction experiments were carried out every other day under their respective optimal catalytic conditions. The activity at the first day was considered as 100% for the comparison of time stability.



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83 **Fig. S1** The XPS full spectrum of CuAsp and GA-CuAsp.

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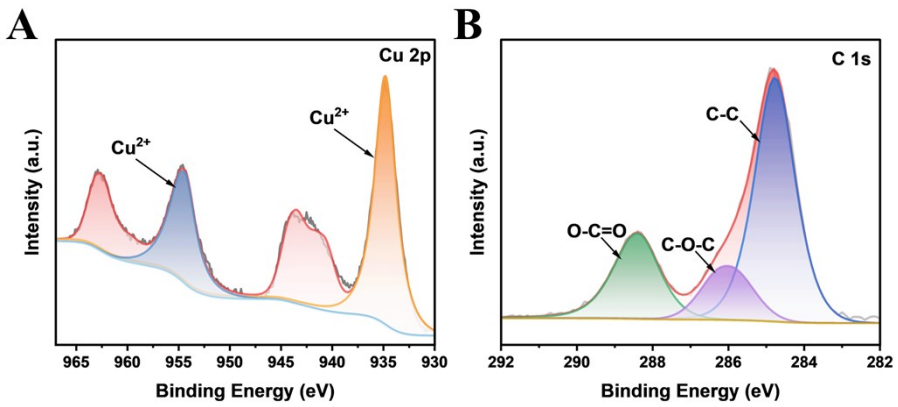


Fig. S2 The XPS spectrum of the CuAsp with the peaks of **(A)** Cu 2p, **(B)** C 1s.

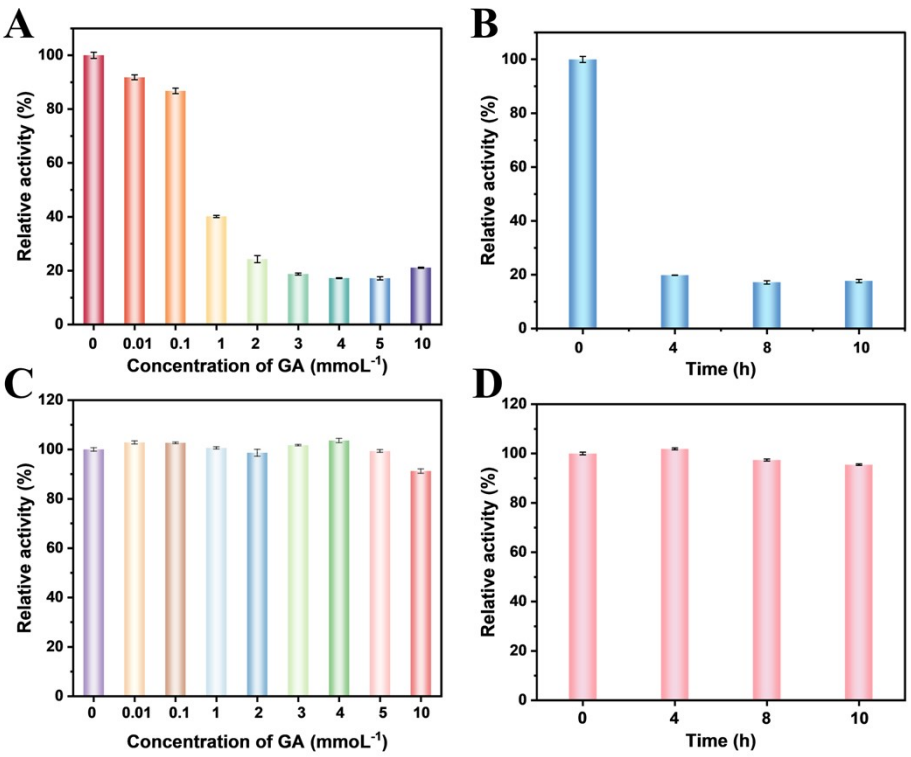
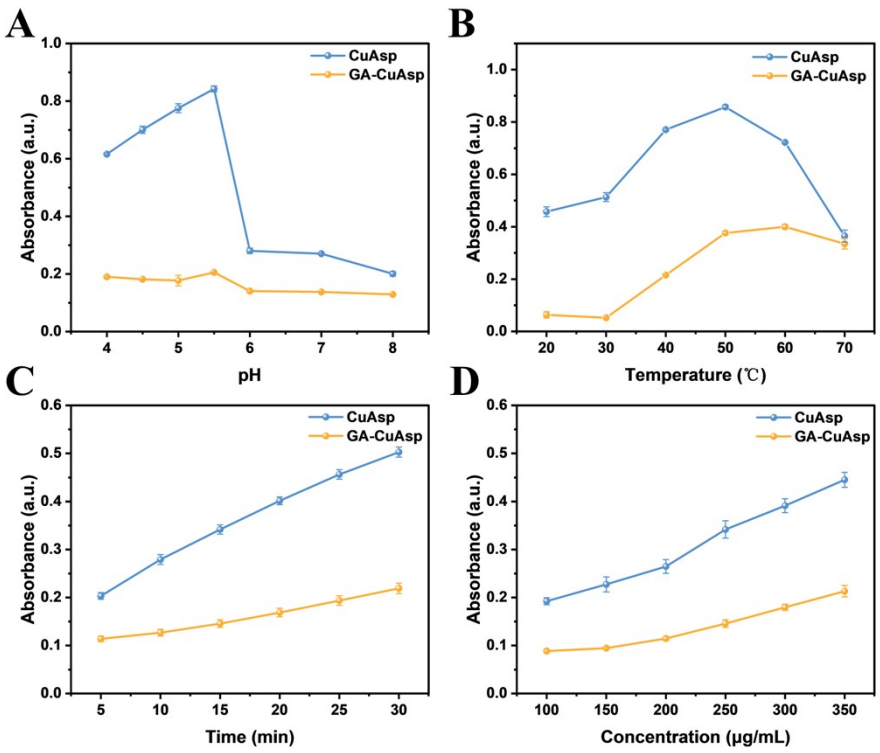


Fig. S3 (A) Effect of different concentrations of GA on the POD-like activity of GA-CuAsp. **(B)** Effect on GA-CuAsp POD-like activity at different times of erosion at the same GA concentration. **(C)** Effect of different concentrations of GA on the Lac-like activity of GA-CuAsp. **(D)** Effect on Lac-like activity of GA-CuAsp at different times of erosion at the same GA concentration.



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112 **Fig. S4** Comparison of POD-like activity reaction conditions between CuAsp and GA-

113 CuAsp. **(A)** pH. **(B)** Temperature. **(C)** Time. **(D)** Concentration.

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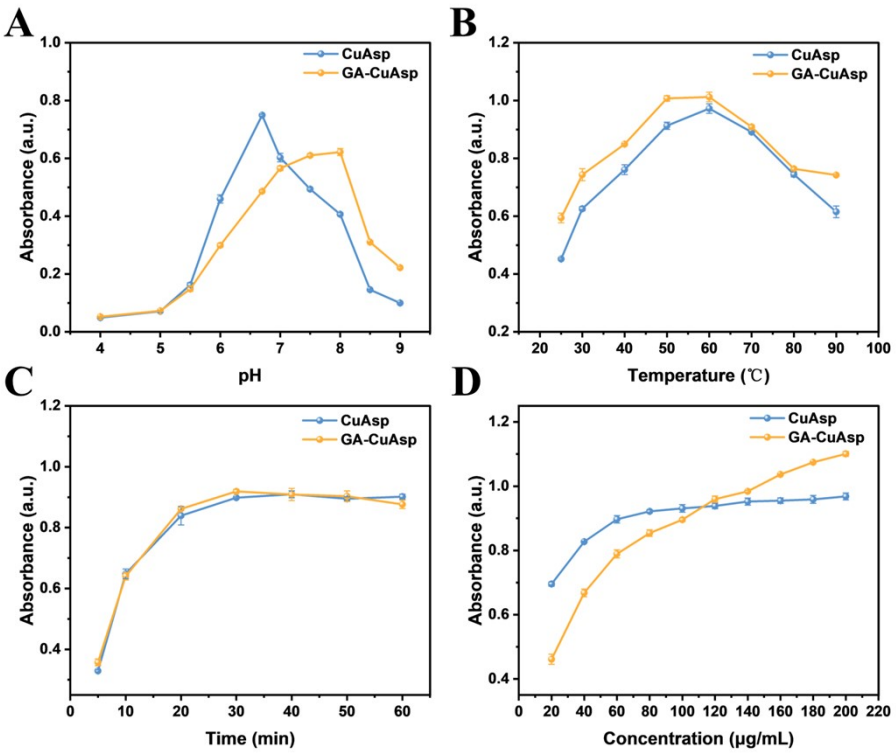
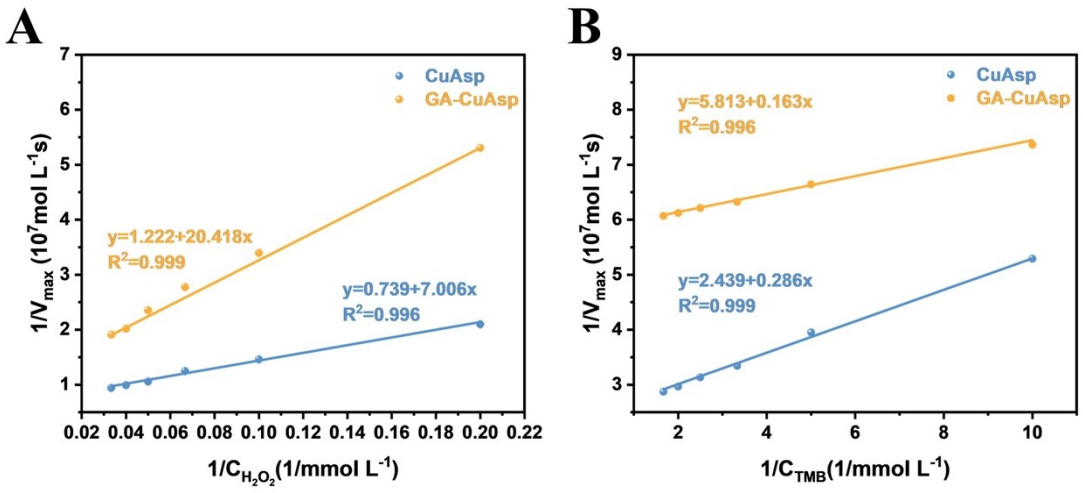


Fig. S5 Comparison of Lac-like activity reaction conditions between CuAsp and GA-CuAsp. **(A)** pH. **(B)** Temperature. **(C)** Time. **(D)** Concentration.



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127 **Fig. S6** Kinetic determination of the POD-like activity of CuAsp and GA-CuAsp.

128 Lineweaver-Burk plot of reaction rate versus **(A)** H_2O_2 concentrations and **(B)** TMB

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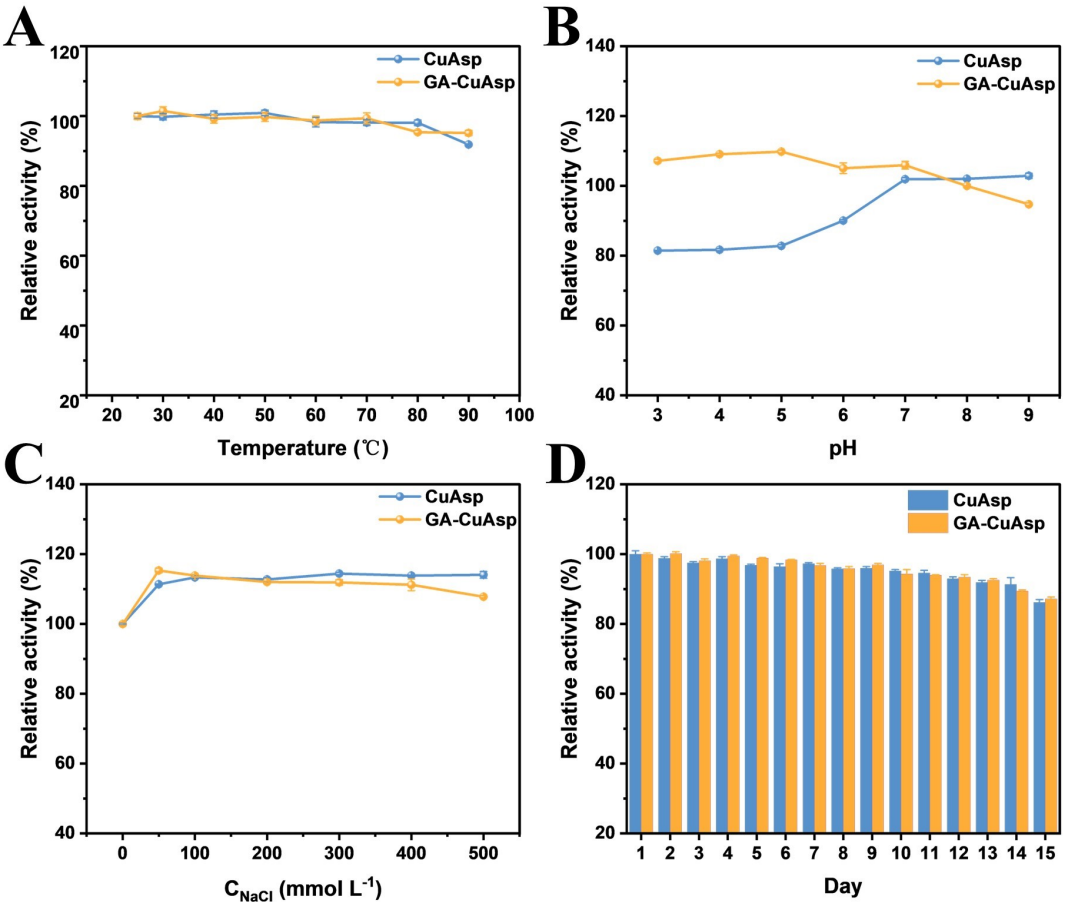
Table S1. Comparison of kinetic parameters of laccase-like activity of GA-CuAsp with other catalysts.

Catalysts	K_m (mmol L^{-1})	V_{max} (μmol L^{-1} min^{-1})	Reference
CH-Cu	0.42	7.3	[S2]
Bpy-Cu	0.19	1.48	[S3]
MI-Bpy-Cu	0.11	7.72	[S4]
Cu-Cys NLs	0.14	1.44	[S5]
Laccase	0.147	12.22	[S6]
CuAsp	0.235	23.41	This work
GA-CuAsp	0.066	12.75	This work



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151 **Fig. S7** Comparison of the stability of CuAsp and GA-CuAsp. **(A)** Temperature. **(B)**

152 pH. **(C)** NaCl. **(D)** Day. Date are shown as mean ± SD (n = 3).

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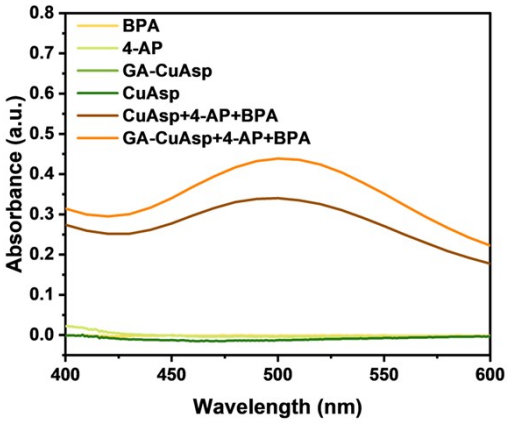
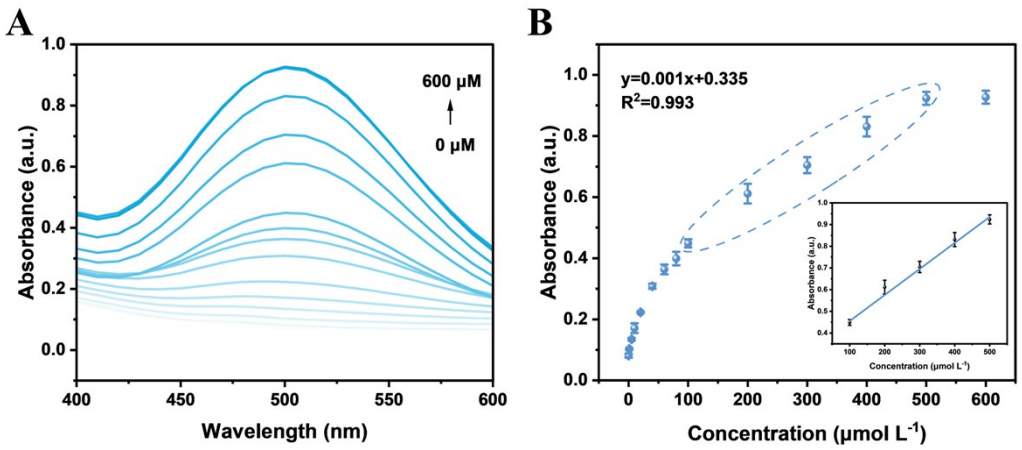


Fig. S8 Feasibility analysis of BPA.



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176 **Fig. S9 (A)** UV-Vis spectra based on CuAsp for the detection of different

177 concentrations of BPA. **(B)** Detection of different concentrations of BPA based on

178 CuAsp and the corresponding standard curves. Inset: linear relationship between

179 absorbance and BPA concentration (100-500 μmol L^{-1}). Date are shown as mean ± SD

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192 **Table S2.** Comparison of performance of different methods for bisphenol A detection.

Sensor	Analyzed samples	Linear range (μmol L^{-1})	LOD (μmol L^{-1})	References
CDs@Eu-AMP	Water	0.10-100	0.02	[S7]
PDMS@SNCM/ITO	Wine	1.00-100	0.23	[S8]
$Mo_2Ti_2AlC_3$/MWCNT	Milk pack, Plastic bottle	0.01-8.50	2.7×10^{-3}	[S9]
TYR-TiO_2-MWCNTs-PDDA-Nafion/GE	Plastic bag for rice	0.28-45.05	0.066	[S10]
COF/GCE	Bottles	0.10-50	0.02	[S11]
RGO-Ag/PLL/ GCE	Drinking water	1.00-80	0.54	[S12]
CuAsp	--	100-500	41.40	This work
GA-CuAsp	Water, Infant food packaging	0-100	0.75	This work

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202 **Supplemental references**

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