Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Boosting laccase-like activity of tetrapeptide capped copper nanoparticles-based nanozymes for colorimetric determination of adrenaline

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Experiments

Materials and chemicals

L-Phe-L-Phe-L-Cys-L-His (FFCH) and L-Cys-L-His (CH) were obtained from Nanjing Peptide Biotech Ltd. (Nanjing, China). L-Cys (C) and L-His (H) were purchased from TCI Shanghai Co. Ltd. (Shanghai, China). Adrenaline (Adr), copper chloride ($CuCl_2$), 2-(*N*-morpholino) ethanesulfonic acid (MES) monohydrate and adrenaline were obtained from Aladdin (Shanghai, China). Laccase (LAC) from *Trametes versicolor* was obtained from Yuanye Biochem (Shanghai) Ltd., *N, N*-dimethylformamide (DMF) and other chemicals were received from Beijing Chemical Works (Beijing, China). All chemical reagents were of analytical grade. The aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA).

Instruments

The ultraviolet-visible (UV-*vis*) absorption spectra were recorded using a TU-1900 UV-vis double-beam spectrometer (Purkinje General, China). A 1.0 mL capacity cuvette with a 1.0 cm path length was used for measuring the UV-vis absorbance.

Scanning electron microscopy (SEM) images were observed using a field emission spectrometer (JSM-7900F, JEOL, Japan).

Fourier transform infrared (FT-IR) spectra were recorded by an FT-IR spectrophotometer (TENSOR-27, Germany).

X-ray photoelectron spectroscopy (XPS) measurements were performed by an ESCALab220i-XL spectrometer (VG Scientific, U.K.).

LAC-like activity of FFCH@CuNPs

The LAC-like catalytic activity of FFCH@CuNPs was surveyed through the oxidation with Adr (30.0 μL,1.0 mg/mL) as the chromogenic LAC substrate by the FFCH@CuNPs catalyst (300.0 μL, 1.0 mg/mL) in MES buffer solution (2.67 mL, 30.0 mM, pH 6.8). The mixture was incubated for 1.0 hour at room temperature, after which the absorbance of the solution was measured using ultraviolet-visible (UV-vis) spectroscopy at a wavelength of 485 nm.

Colorimetric detection of Adr by smart phone

Different concentrations of Adr (1.6, 10.0, 20.0, 30.0, 40.0 μg/mL) were combined with 0.1 mg/mL FFCH@CuNPs in MES buffer solution at room temperature, respectively. After reaction for 1.0 h, FFCH@CuNPs was collected by centrifugation (10,000 rpm, 5.0 min) and Adr concentrations were determined using colored supernatants. First, colorimetric images of the supernatants were taken by a smart phone (iPhone 13 pro max) in an assay accessory of its own design, which was a sun-lit sample stage with a white background. Then, the taken images were investigated through ColorPicker application of the iPhone

13 pro max, which adopts the RGB color model, where R, G, and B represent the color channels of red, green, and blue respectively, and the pixel values of R, G and B stood for P_R, P_G and P_B. Since the supernatant is yellowish-red, the value of $P_R/ (P_R + P_G + P_B)$ was used to distinguish the color difference of the supernatant at different Adr concentrations.

Detection of Adr in saliva

To evaluate the feasibility of the smartphone strategy for Adr detection, the method was used to detect Adr in human saliva. The human saliva samples were pretreated to eliminate interfering proteins. Briefly, 0.1 mL of human saliva sample was taken, diluted with 0.1 mL of ethanol, and mixed for 5.0 min. The mixture was then centrifuged at 10,000 rpm for 10.0 min, and the supernatant was collected and stored at 4 °C for further analysis. To detect Adr, 30.0 μL Adr and FFCH@CuNPs (300.0 μL) were added to 2.67 mL MES buffer at pH 6.8. The reaction solution was incubated at room temperature for 1.0 h. The UV-Vis absorbance of Ador in the reaction solution was measured at 485 nm.

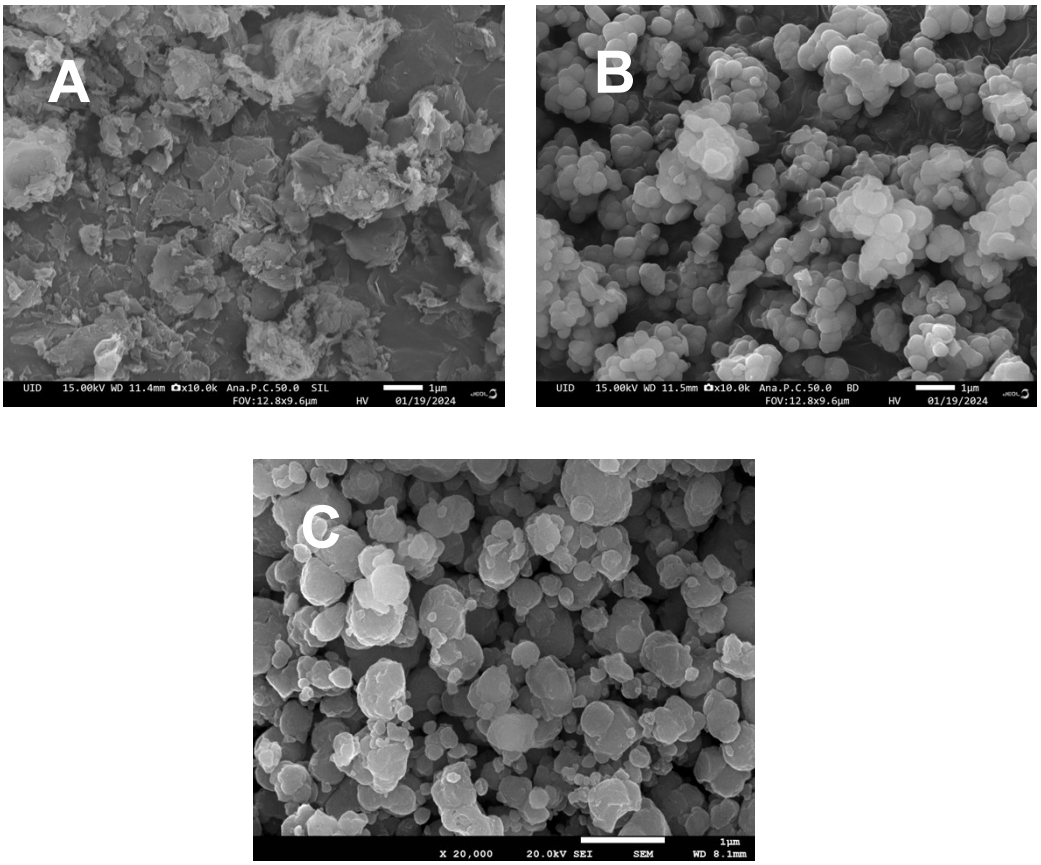


Fig. S1. SEM images of (A) C@CuNPs, H@CuNPs and CH@CuNPs.

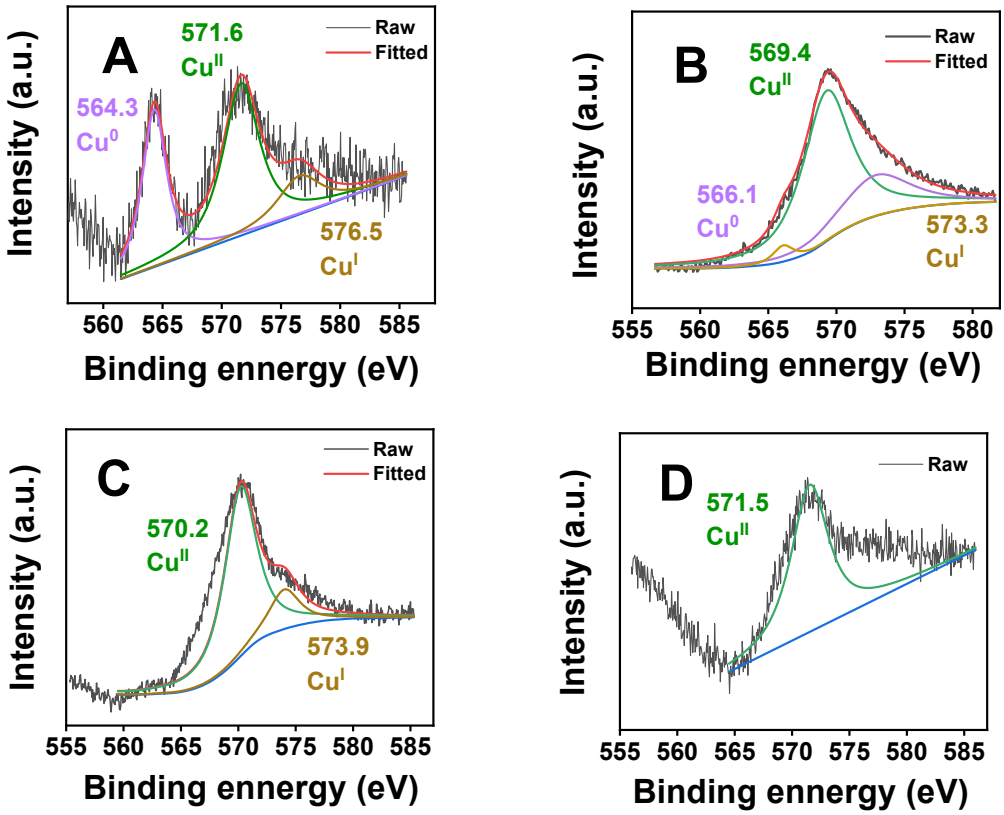


Fig. S2. (A) Cu LMM Auger spectrum of FFCH@CuNPs nanozymes; Cu LMM Auger spectra of (B) CH@CuNPs, (C) C@CuNPs and (D) H@CuNPs; (blue is the baseline, green is the peaks of Cu^{2+}, brown is the peaks of Cu^{+}, purple is the peaks of Cu^{0}. These peaks were obtained by means of XPS peak-differentiating-imitating analysis).

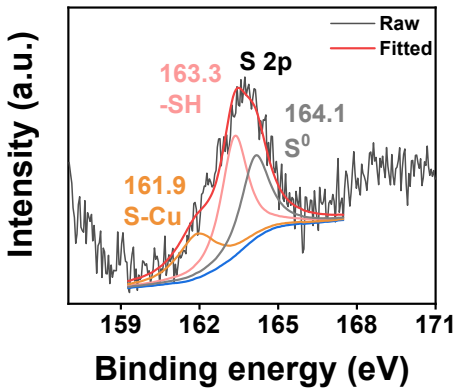


Fig. S3. S 2p XPS spectrum of FFCH@CuNPs nanozymes (pink is the peaks of -SH, gray is the peaks of S^{0} and the orange is the peaks of S-Cu. These peaks were obtained by means of XPS peak-differentiating-imitating analysis).

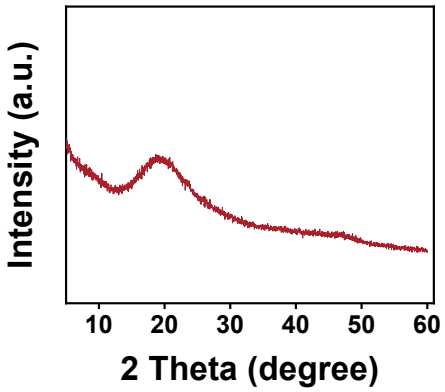


Fig. S4. XRD patterns of FFCH@CuNPs nanozyme.

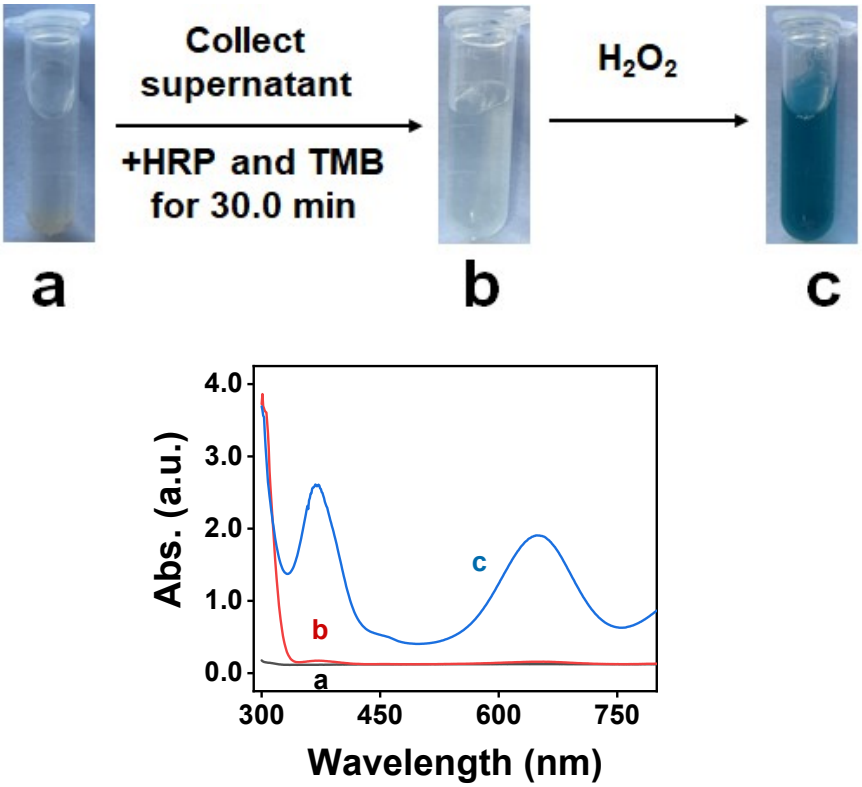


Fig. S5. Photos of (a) supernatant of 2,4-DP catalyzed by FFCH@CuNPs for 1.0 h, (b) supernatant with TMB and HRP reacted for 30.0 min and (c) after adding H_2O_2. The corresponding absorbance at 414 nm and 650 nm as shown above.

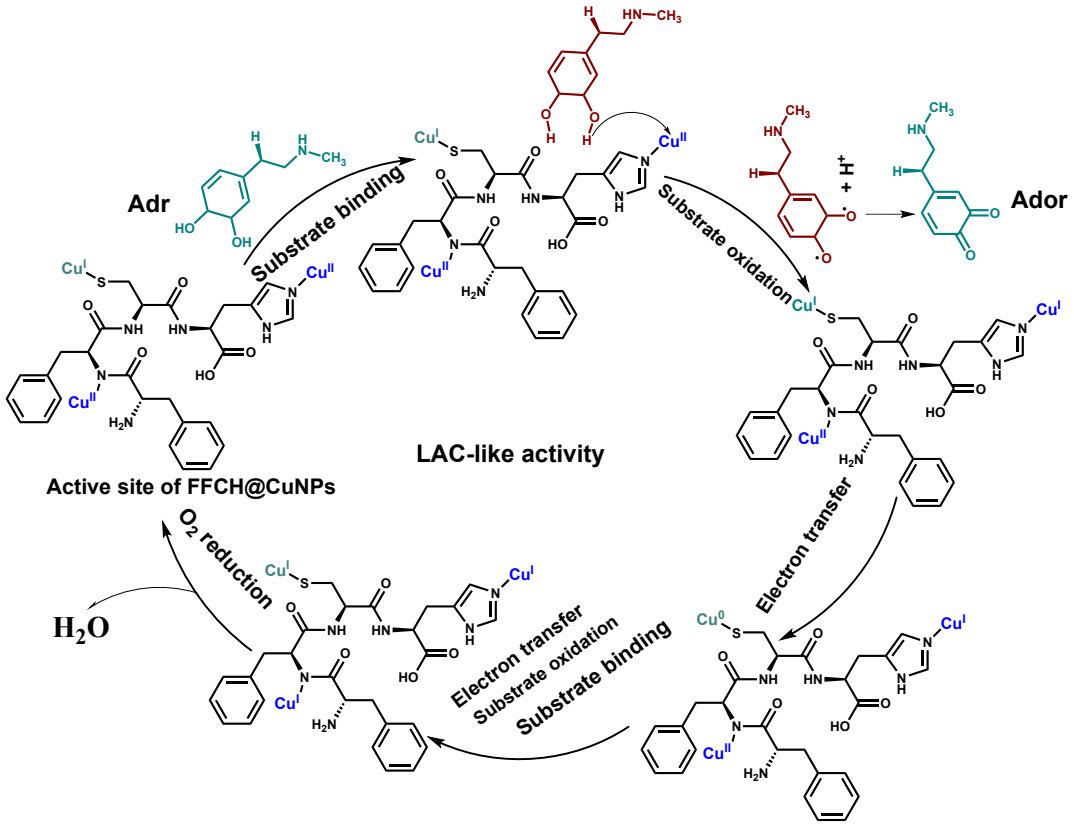


Fig. S6. Schematic of possible catalytic mechanism involving the FFCH@CuNPs nanozymes.

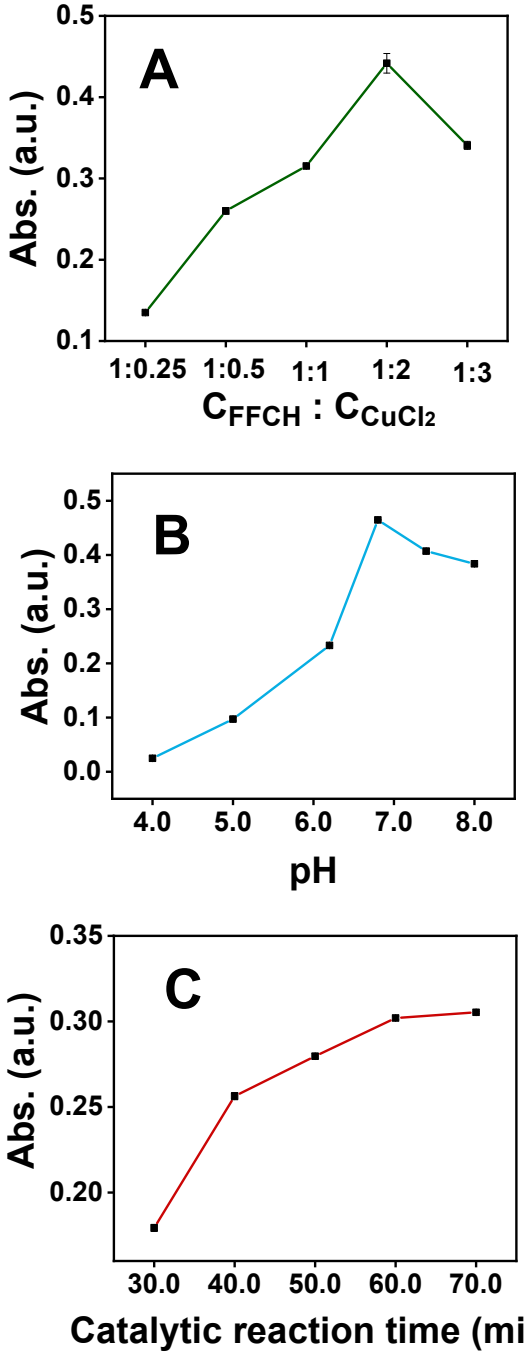
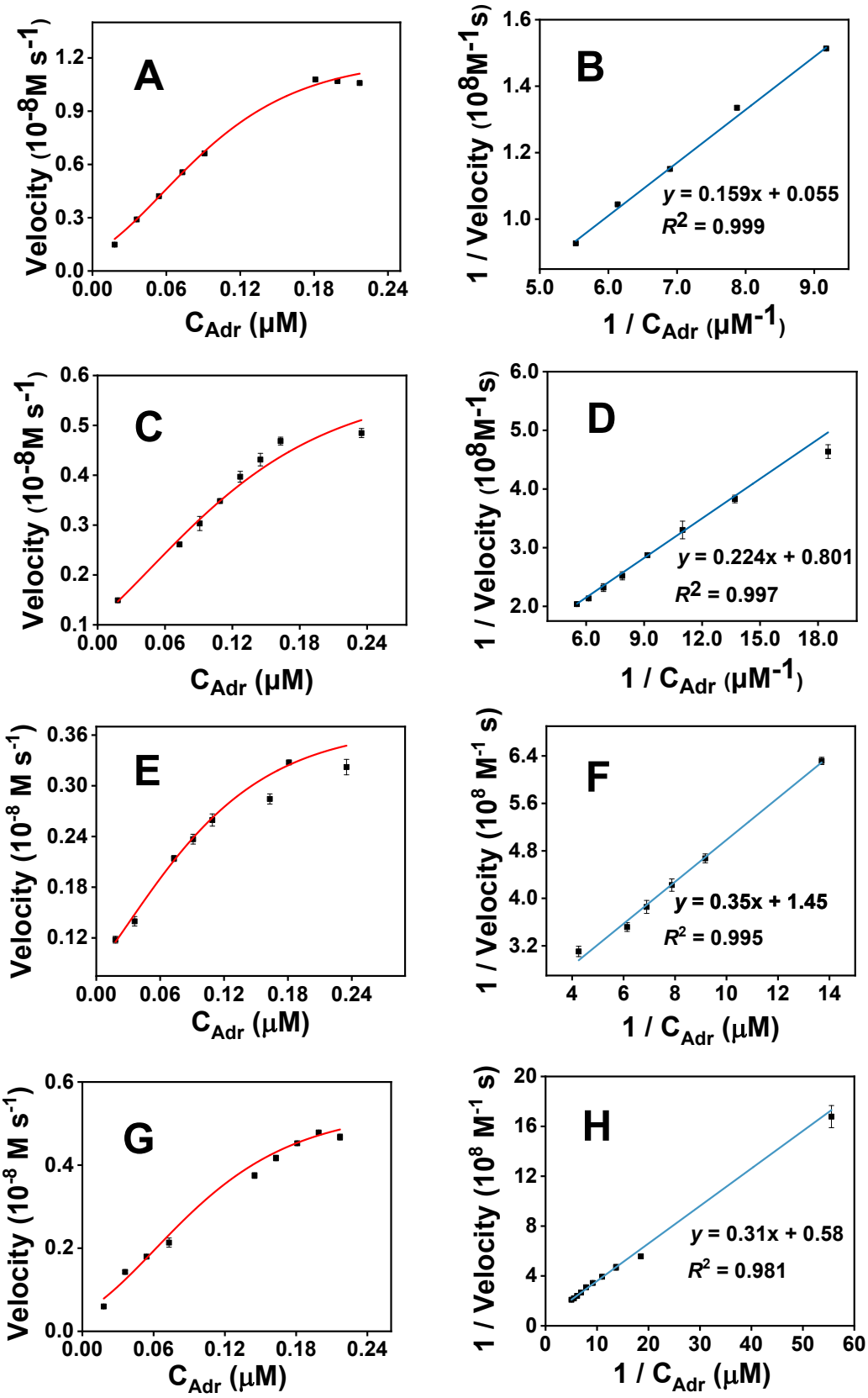


Fig. S7. Dependence of the laccase-like activity of FFCH@CuNPs on (A) concentration ratio of FFCH to $CuCl_2$; (B) buffer pH and (C) catalytic reaction time.


A
Velocity (10-8M s-1)
CAdr (μM)
B
1 / Velocity (108M-1s)
y = 0.159x + 0.055
R2 = 0.999
1 / CAdr (μM-1)
C
Velocity (10-8M s-1)
CAdr (μM)
D
1 / Velocity (108M-1s)
y = 0.224x + 0.801
R2 = 0.997
1 / CAdr (μM-1)
E
Velocity (10-8 M s-1)
CAdr (μM)
F
1 / Velocity (108 M-1 s)
y = 0.35x + 1.45
R2 = 0.995
1 / CAdr (μM)
G
Velocity (10-8 M s-1)
CAdr (μM)
H
1 / Velocity (108 M-1 s)
y = 0.31x + 0.58
R2 = 0.981
1 / CAdr (μM)

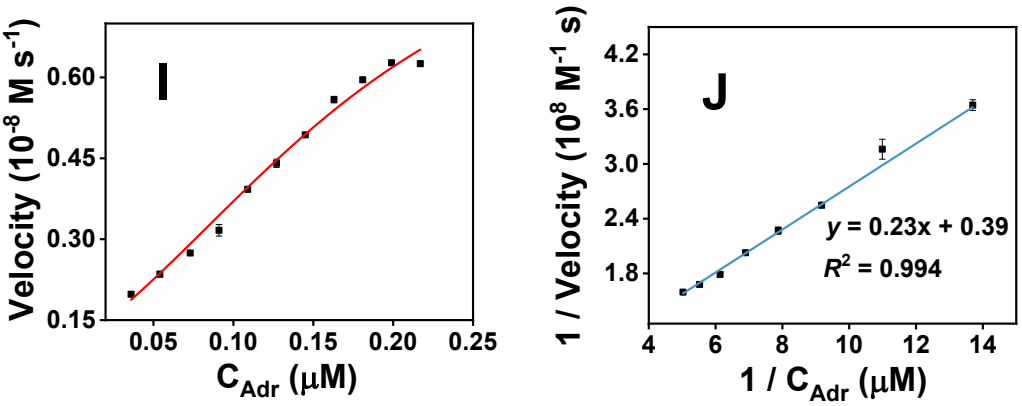


Fig. S8. Steady-state kinetics of FFCH@CuNPs nanozymes (A), LAC (C), C@CuNPs (E), H@CuNPs (G) and CH@CuNPs (I) in the oxidation reaction of Adr to Ador. Lineweaver-Burk plot for FFCH@CuNPs nanozymes (B), LAC (D), C@CuNPs (F), H@CuNPs (H) and CH@CuNPs (J) oxidizing Adr at room temperature.

Table S1 Comparison LAC-like activity of FCH@CuNPs with the reported nanozymes

Catalysts	K_m (mM)	V_{max} (10^{-8} M s^{-1})	Ref.
CA@CuNPs	0.12	13.0	X. Xu, *et al.,* *Catal. Sci. Technol.,* 2021, **11**, 3402–3410
ATP@CuNPs	0.21	3.7	H. Huang, *et al.,* *J. Mater. Chem. B,* 2019, **7**, 6508–6514.
C@CuNPs	0.14	2.4	M. Guan, *et al.,* *Front. Chem. Sci. Eng.,* 2020, **15**, 310–318.
CH@CuNPs	0.42	12.2	J. Wang, *et al.,* *Appl.Catal., B,* 2019, **254**, 452–462.
FFCH@CuNPs	2.89	18.2	**This work**

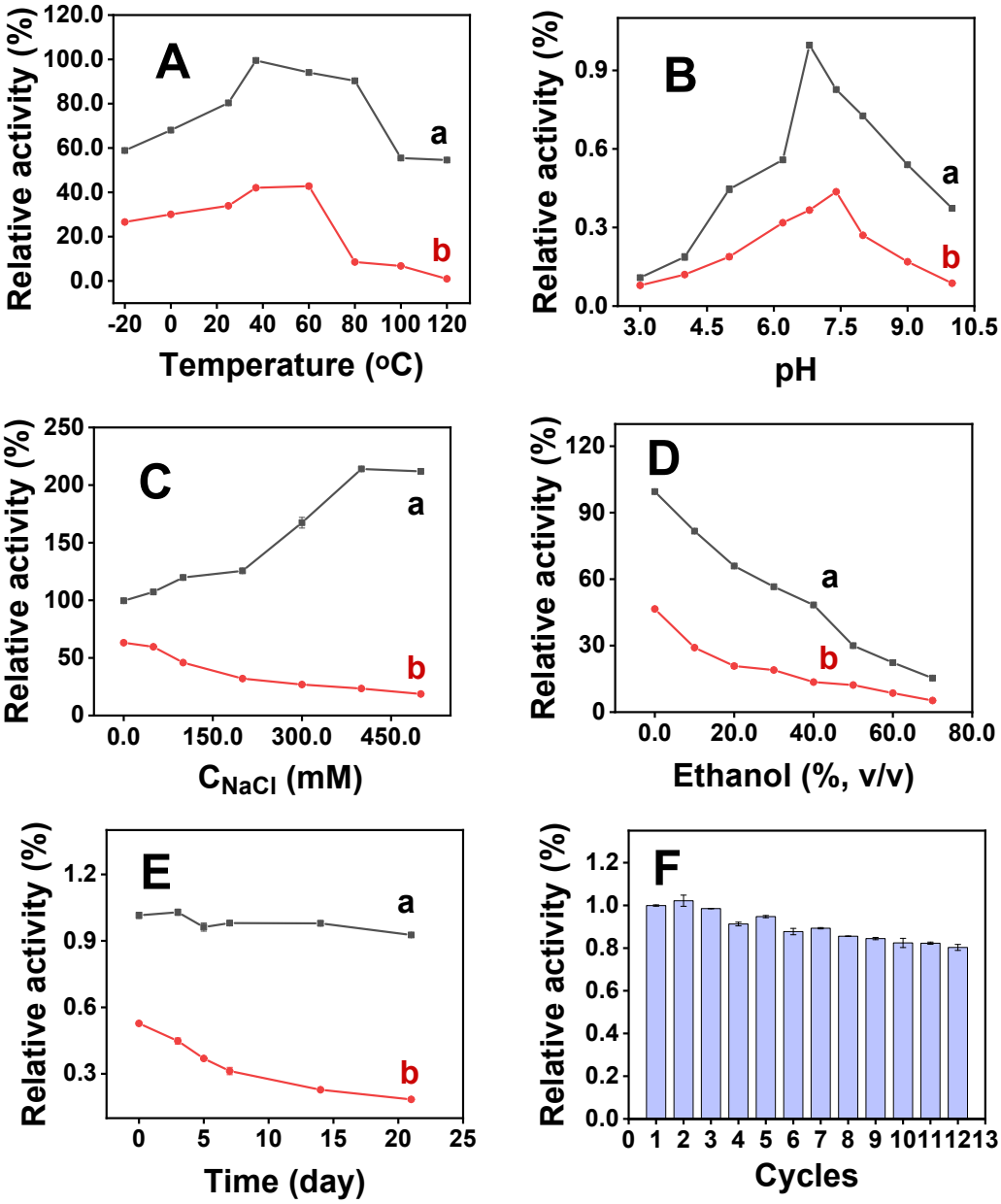


Fig. S9. Stability of FFCH@CuNPs nanozymes (a) and LAC (b) at different (A) temperature; (B) pH; (C) NaCl concentration; (D) content of ethanol; (E) storage time in water. (F) Relative activity of FFCH@CuNPs nanozymes in the chromogenic reaction during the recycling and reuse process.

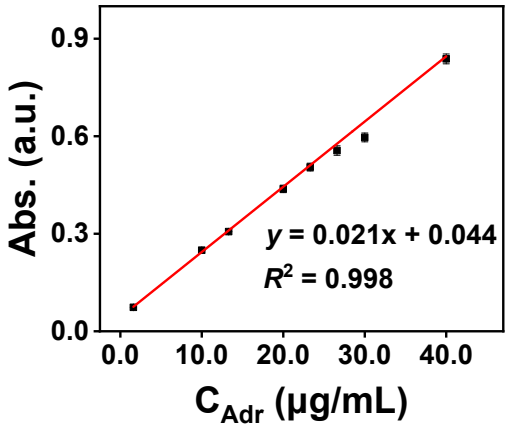


Fig. S10. Absorbance at 485 nm different concentration of Adr catalyzed by FFCH@CuNPs for 1.0 h.

Table S2 Comparison of linear range and LOD for detection of Adr upon nanozymes

Nanozymes	Linear range (μg/mL)	LOD (μg/mL)	Reference
GMP@CuNPs	-	0.41	H. Liang, et. al., ACS Appl. Mater. Inter., 2017, **9**, 1352–1360.
CH@CuNPs	5.0-50.0	0.31	J. Wang, et. al., Appl. Catal., B, 2019, **254**, 452–462
GNFs	5.0-25.0	0.30	X. Z. Xu, et. al., Anal. Chim. Acta. 2015, **879**, 97-103
CTN1	0.82-16.47	5.60	H. Ma, et. al., Colloids Surf., A, 2021, **613**, 126105
FFCH@CuNPs	1.6-40.0	1.10	**This work**

Table S3 Recovery of the proposed assay*

Saliva Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
	10.0	10.4	104.0	3.2
1	20.0	20.6	103.0	3.7
	30.0	30.3	101.0	1.2
	10.0	10.6	106.0	2.0
2	20.0	19.7	98.5	2.3
	30.0	30.4	101.3	3.2
	10.0	10.5	105.0	1.4
3	20.0	19.8	99.0	4.3
	30.0	29.9	99.7	3.3

* Blank controlled saliva was used for recovery study (n=3).