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Supporting Information

Dual-Function Colorimetric Platform for Hg^{2+} Detection and Cys/Hcy Discrimination Based on the Peroxidase-Like Activity of CuAu Nanozymes

Kunle Wang, Ya You, Yufei Liu,* Wei Liu*

a School of Pharmacy, Henan Medical University, Xinxiang 453003, P. R. China

* Correspondence authors:

E-mail:liuwei@xxmu.edu.cn

liuyufei19841104@163.com

Experiments

Chemicals

Sodium citrate ($C_6H_5Na_3O_7$),Sodium acetate (NaAc), Hydrogen peroxide (H_2O_2, 30.0%, w/w) was supplied by Aladdin Chemistry Company (Shanghai, China). $NaBH_4$ was obtained from Tianjin Comio Chemical Reagent Co., Ltd. (Tianjin, China). Chloroauric acid tetrahydrate ($HAuCl_4$ $4H_2O$), L-Cys ,Hcy ,$CuSO_4$, $CuCl_2$, $FeCl_2$, $MgCl_2$, $CaCO_3$,other amino acids and other chemicals were purchased from Rhawn Reagent (Shanghai, China). $Hg(NO_3)_2$ was bought from Cyrus Chemical Reagent Co., Ltd. (Foshan, China). Acetic acid was gotten from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjing, China). $AgNO_3$ was purchased from Dongjulong Chemical Technology Co., Ltd. (Tianjin, China). Vitamin C was obtained from Aladdin Chemistry Company (Shanghai, China). Vitamin C, 3, 3′, 5, 5′-tetramethylbenzidine (TMB) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). The water used throughout the study was deionized water (18.2 MΩ cm^{-1}). All chemicals used were analytical reagents.

Apparatus

Ultraviolet-visible (UV-vis) absorption spectra were recorded uing a Shimadzu UV-2700 spectrophotometer (Japan). Zeta potential measurements were carried out with a Zetasizer laser particle analyzer (Zetasizer Nano ZS 90, Malvern, UK). Transmission electron microscopy (TEM) images were obtained using a transmission electron microscope (JEM-F200, Japan electron optics laboratory, Japan) at a voltage of 200 kV. X-ray diffraction (XRD) measurements were performed using a Rigaku SmartLab SE X-ray diffractometer (Japan).

Detection of Hg^{2+}

First, 30μL Hg^{2+}(10-100 μM) is mixed with 150 μL of CuAuNPs. Subsequently, Then, the mixture was added to 2694 μL of NaAc-HAc buffer, 90 μL of H_2O_2 solution, and 36 μL of TMB mixed solution. After thorough mixing, the reaction is incubated for 20 min at room temperature. Finally, the absorbance is measured at 652 nm using a UV spectrophotometer.

Detection of Cys/Hcy

First, 30 μL Cys (0.5-4 mM)or Hcy(0.6-5 mM) solution and 2.7 μL Hg^{2+}solution (1 mM) are sequentially mixed with 150 μL of CuAuNPs. Then, the mixture was added to 2692 μL of NaAc-HAc buffer, 90 μL of H2O2 solution, and 36 μL of TMB mixed solution. After thorough mixing, the reaction is incubated for 20 min at room temperature. Finally, the absorbance is measured at 652 nm using a UV spectrophotometer.

Detection of Hg^{2+} in emollient water

Three batches of emollient water were analyzed.First, sequentially mix 30 μL of the test emollient water with 30 μL Hg^{2+}(10-100 μM) and 150 μL of CuAuNPs. Then, the mixture was added to 2664 μL of NaAc-HAc buffer, 90 μL of H2O2 solution, and 36 μL of TMB mixed solution. After mixing, let it stand for 20 minutes, and finally, measure the absorbance at 652 nm using a UV spectrophotometer.

Detection of Cys/ Hcy in Rat Serum

Specifically, serum samples were mixed with acetonitrile at a volume ratio of 1:3 to precipitate proteins. The supernatant was then collected and subjected to solid-phase extraction (SPE) using GST agarose purification resin (product No. C600327, purchased from Shanghai Sangon Biotech Co., Ltd.) to remove GSH, and the flow-through fraction was collected. After SPE, the GSH removal efficiency was assessed using a reduced glutathione content assay kit (product No. D799613, Sangon Biotech). No GSH was detected following SPE, indicating that its interference was effectively eliminated. Meanwhile, a cysteine content assay kit (product No. D799571, Sangon Biotech) was used to examine the retention of Cys, and the results confirmed that the recovery rate of cysteine during the SPE process ranged from 98.2% to 105.0%. The detection of Hcy was not performed due to the lack of a dedicated Hcy assay reagent. The separated serum sample was stored for subsequent analysis. Following the addition of varying concentrations of Cys/Hcy standards, the mixtures were centrifuged at 10,000 rpm for 10 minutes. Then sequentially mix 30 μL sample, 2.7 μL Hg^{2+}(1 mM), 30 μL Cys (0.5-4 mM) or Hcy(0.6-5 mM) solution with 150 μL of CuAuNPs. Then, the mixture was added to 2662 μL of NaAc-HAc buffer, 90 μL of H_2O_2 solution, and 36 μL of TMB mixed solution. After mixing, let it stand for 20 minutes, and finally, measure the absorbance at 652 nm using a UV spectrophotometer.

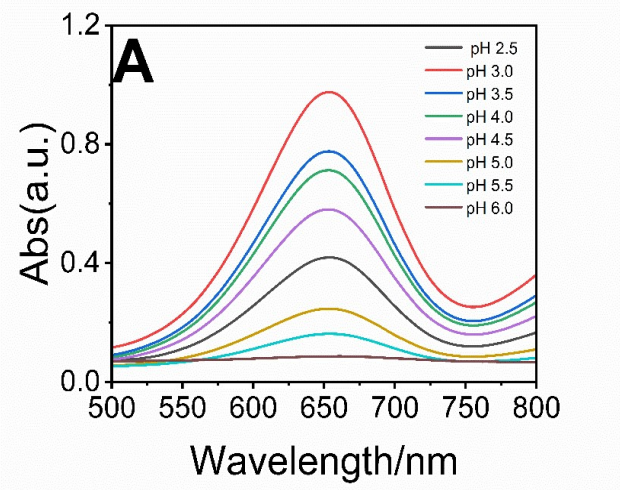
A
pH 2.5
pH 3.0
pH 3.5
pH 4.0
pH 4.5
pH 5.0
pH 5.5
pH 6.0
Abs(a.u.)
1.2
0.8
0.4
0.0
500
550
600
650
700
750
800
Wavelength/nm

Table S1 Comparison of linear ranges and LODs for the detection of Hg^{2+}, Cys, and Hcy with previously reported nanozymes.

Nanomaterials	Test substance	Linear range	LOD	Recoveries (%)	RSD(%)	Ref.
GTP	Hg^{2+}	50-10000 nM	38 nM	100.6-114.7	＜9.6	1
Fe_3S_4		50-2000 nM	26 nM	90-110	＜5.0	2
AgNPs-CMs		2-833 nM 2500-40000 nM	1.1 nM	95.65-106.56	＜7.84	3
CeO_2–MIL (Fe)		0-6 nM	2 nM	98.7-101.2	/	4
CuAuNPs		100-900 nM	10 nM	90.4-110.2	＜6.7	**This work**
GTP	L-Cys	0.1-20 μM	0.094 μM	90.4-98.3	＜2.7	1
Fe-CDs		1-60 μM	0.29 μM	90.7-98.62	＜3.3	5
BSA-$Cu_3(PO_4)_2$ HNFs		10-200 μM	3.5 μM	93.3-103.6	＜6.99	6
Ficin-CuNFs		0.5-30 μM	0.1 μM	100.55-105.34	＜1.12	7
CuAuNPs		5-40 μM	2.5 μM	99.4-108.1	＜6.2	**This work**
Fe-CDs	Hcy	1-60 μM	1.41 μM	98.67-99.87	＜3.3	5
BSA-$Cu_3(PO_4)_2$ HNFs		10-200 μM	7.7 μM	91.9-107	＜6.33	6
Ficin-CuNFs		0.5-30 μM	0.3 μM	97.72-106.67	＜0.76	7
CuAuNPs		6-50 μM	2.5 μM	95.0-104.1	＜6.4	**This work**

Table S2 Inter-day and intra day precision for the detection of Hg^{2+}, Cys and Hcy

Samples	Added	Inter-Day (RSD, %; n=5)	Intra-Day (RSD, %; n=5)
CuAuNPs-Hg^{2+}	300 nM	5.3	2.6
	500 nM	7.7	3.4
	700 nM	10.8	8.6
CuAuNPs-Hg^{2+}-Cys	15 μM	8.4	8.6
	25 μM	1.3	1.1
	35 μM	1.2	0.5
CuAuNPs-Hg^{2+}-Hcy	15 μM	5.2	1.3
	30 μM	2.9	0.8
	45 μM	0.6	0.9

Table S3 Detection of Hg^{2+} in emollient water(n=3)

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD(%)
1	0.2	0.21	105.2	4.0
	0.6	0.56	93.9	2.9
	0.9	0.91	101.3	1.2
2	0.2	0.21	104.2	3.8
	0.6	0.58	97.1	2.8
	0.9	0.89	98.8	1.7
3	0.2	0.23	114.0	3.9
	0.6	0.59	98.3	6.7
	0.9	0.88	98.0	2.4

Table S4 Detection of Cys in Rat Serum(n=3)

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD(%)
1	15	16.21	108.1	6.2
	25	24.85	99.4	1.2
	35	35.98	102.8	2.4
2	15	15.06	100.4	1.1
	25	25.72	102.9	5.5
	35	36.15	103.3	3.9
3	15	15.16	101.1	1.1
	25	25.32	101.3	2.6
	35	35.43	101.2	1.2

Table S5 Detection of Hcy in Rat Serum(n=3)

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD(%)
	15	15.31	102.1	1.9
1	30	28.50	95.0	4.3
	45	47.08	104.6	2.0
	15	15.88	105.9	6.4
2	30	29.91	99.7	2.3
	45	45.80	101.8	2.6
	15	14.92	99.4	0.2
3	30	31.23	104.1	2.3
	45	46.07	102.4	3.1

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