Supporting Information for

Cu/Au Nanoclusters with peroxidase-like activity for chemiluminescent detection of α–amylase

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Content

1. Experimental Section

1.1 Reagents

α−Amylase, pepsin, and trypsin were provided by Energy Chemical. Chloroauric acid ($HAuCl_4$), luminol, hydrogen peroxide (30%, v/v), glucose oxidase (GOx), Na_2CO_3, $NaHCO_3$, KH_2PO_3, Na_2HPO_4, and $Cu(NO_3)_2$ were purchased from Beijing Chemical Factory (Beijing, China). NaCl, KCl, ascorbic acid, isopropanol, superoxide dismutase (SOD), and Alanine (Ala) were supplied by Energy Chemical (Shanghai, China), Arginine (Arg), Cysteine (Cys), Glutamine (Glu), Glycine (Gly), Phenylalanine (Phe), and Histidine (His) were purchased from Beijing Balinwei Technology Co., Ltd. All chemicals were of analytical grade and had not been further purification.

A 10 mM stock solution of luminol was prepared by dissolving luminol in 0.1 M Na_2CO_3/$NaHCO_3$ buffer solution (pH=10.5) for a week in a dark room and stored at room temperature[1]. The H_2O_2 working solution is obtained by diluting the stock solution with distilled water, and is prepared for daily use.

1.2 Apparatus

The RFL-1 ultra-weak luminescence analyzer (Xi'an Remex Analysis Instrument Co., Ltd, China) was used to record the whole process of the experiment. The absorption spectra of starch and Cu/Au NCs were recorded using a UV-3100 UV VISNIR system (Shimadzu, Japan). Transmission electron microscopy (TEM) images were recorded using an FEI Tecnai G2 S-Twin instrument at an accelerating voltage of

200 kV. EDX spectra of the starch-supported Cu/Au nanoclusters were recorded on a HITACHI SU8020 field emission scanning electron microscope.

1.3 Synthesis of Cu/Au NCs

Starch-supported copper/gold nanoclusters (starch–Cu/Au NCs) were synthesized by a simple one-pot technique in aqueous solution[2, 3]. First, 0.15 ml of 0.1 M $Cu(NO_3)_2$ were dissolved in 10 mL of 1% (w/v) soluble starch with constant shaking at 1500 rpm for 15 min, and then added 0.15 ml of 0.1 M $HAuCl_4$. Next, the mixture was agitated for 15 min at room temperature (the molar ratio of Cu:Au was 1:1). Finally, 0.1 M ascorbic acid was quickly added at room temperature under vigorous stirring. The resulting starch-supported Cu/Au NCs were separated from the remaining free metal ions and unbound starch by centrifugation at 4000 rpm for 10 min. Then, the Cu/Au NCs were washed with distilled water and dried under vacuum at 80 °C for 6 h.

2. Characterization and results

2.1 Figure S1

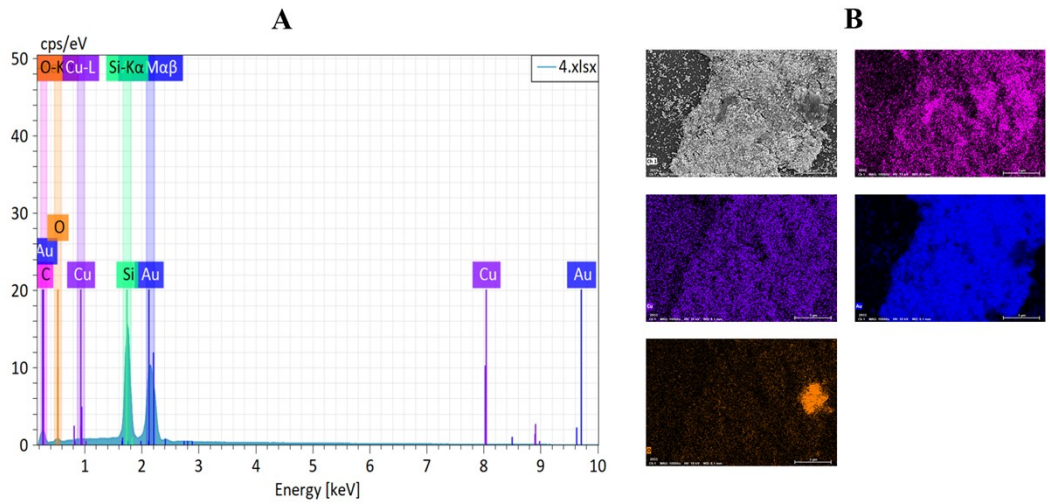

Fig. S1 (A) EDX spectrum of starch–Cu/Au NCs. (The substrate is silicon chip, so it contains silicon element); **(B)** Element mapping: carbon(pink), gold(blue), copper (purple), oxygen (orange).

2.2 Figure S2

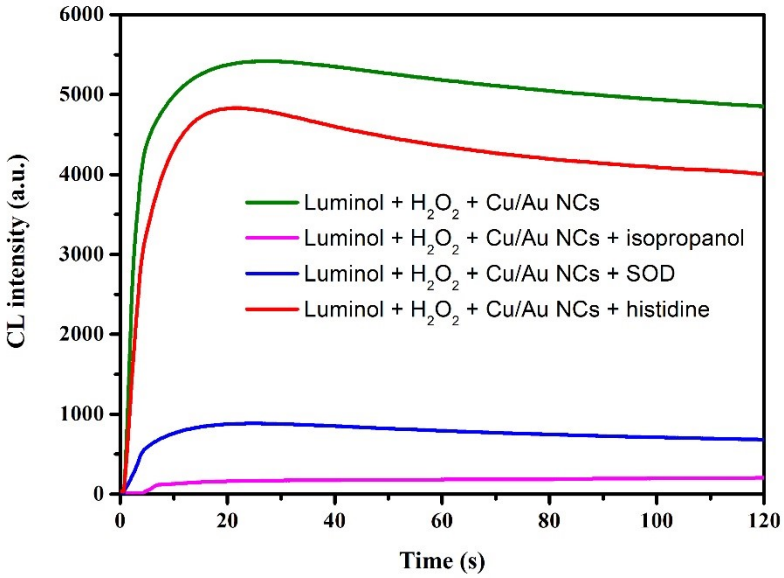

Fig. S2 Influence of different scavengers to the CL system (isopropanol for ·OH, superoxide dismutase (SOD) for $O_2^{\cdot-}$, and histidine for $^1O_2$).

2.3 Figure S3

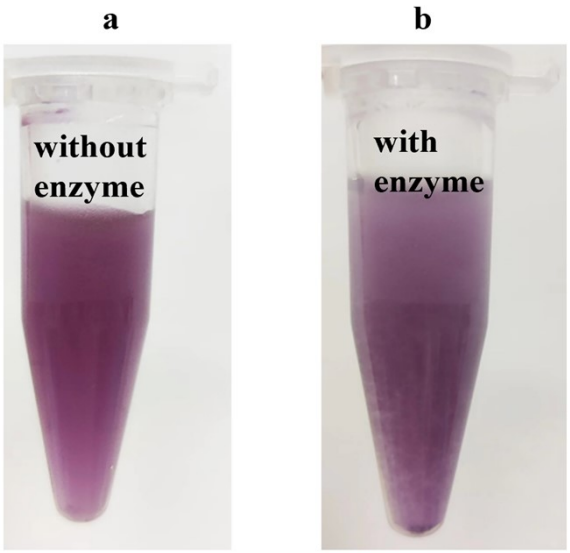

Fig. S3 (a) Starch–Cu/Au NCs; **(b)** Starch–Cu/Au NCs react with α-amylase for ten minutes.

2.4 Figure S4

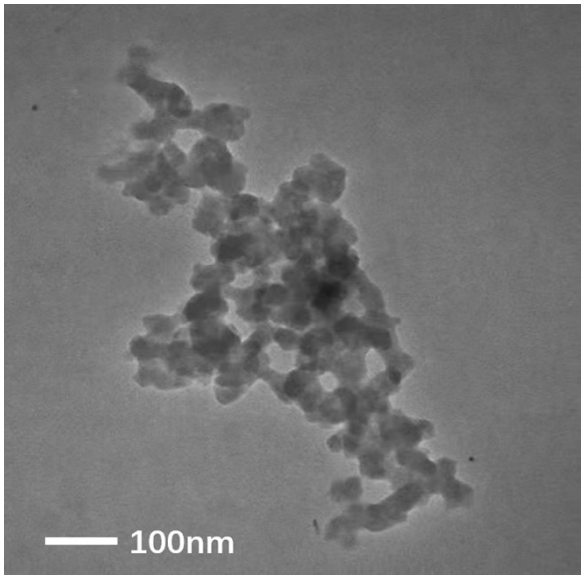

Fig. S4 TEM image of starch–Cu/Au NCs following interaction with α-amylase

2.5 Figure S5-S8

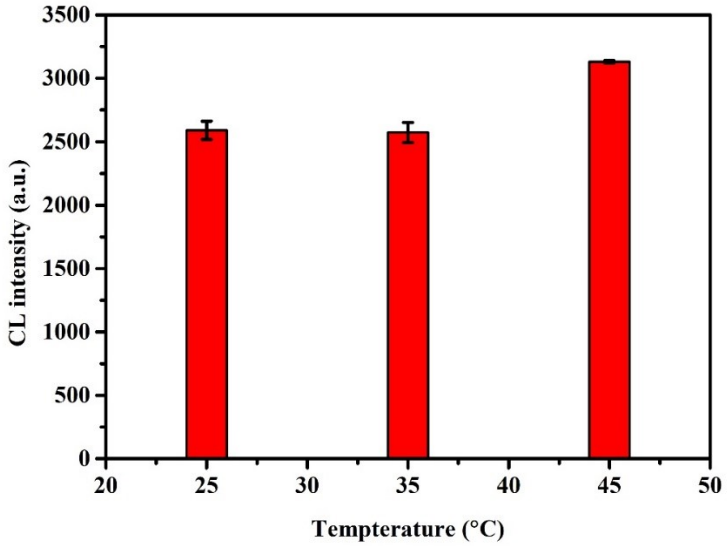

Fig. S5 The effects of different temperature of reaction between α–amylase and Cu/Au NCs on CL intensity (Experimental conditions: Cu/Au NCs solution was mixed with 1 U/mL α–amylase in 10 mM PBS (pH=7.0) and incubated for 10 min at 25, 35, and 45 °C)

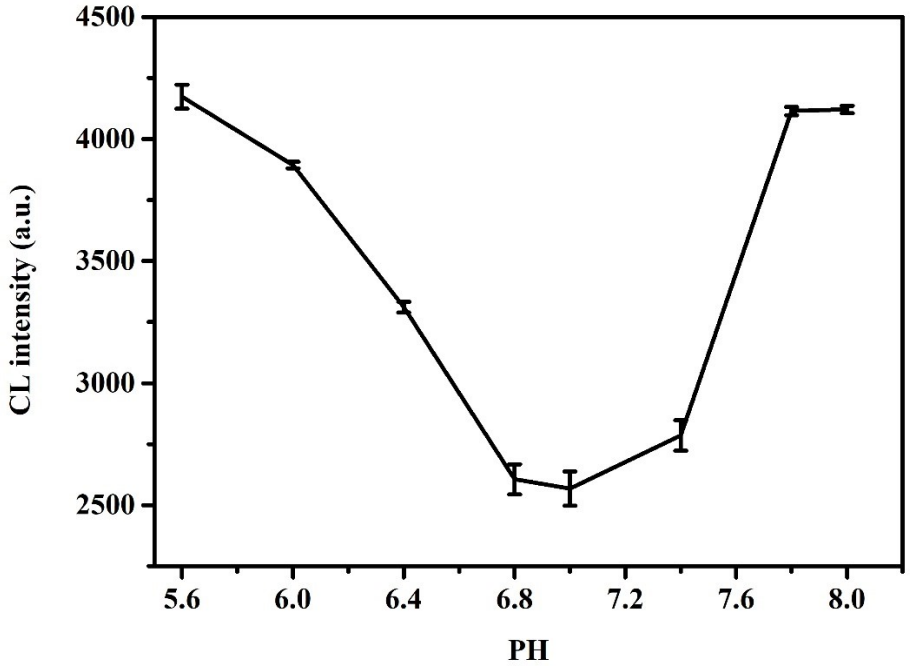

Fig. S6 The effects of different pH values on CL intensity (Experimental conditions: Cu/Au NCs solution was mixed with 1 U/mL α–amylase in 10 mM PBS and incubated for 10 min at room temperature)

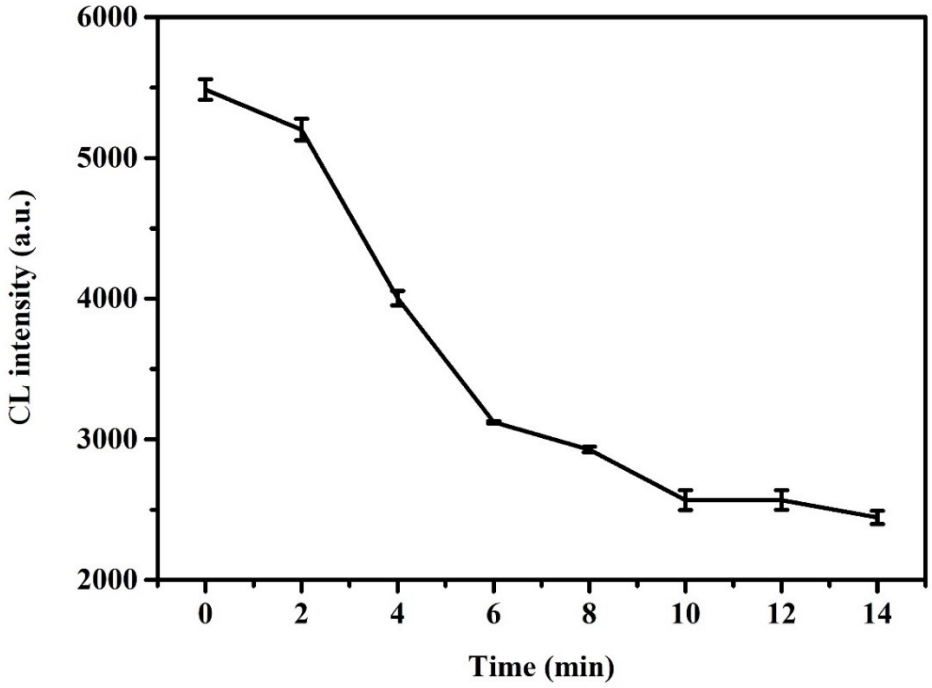

Fig. S7 The effects of different time of reaction between α−amylase and Cu/Au NCs on CL intensity (Experimental conditions: Cu/Au NCs solution was mixed with 1 U/mL α−amylase in 10 mM PBS (pH=7.0) and incubated at room temperature)

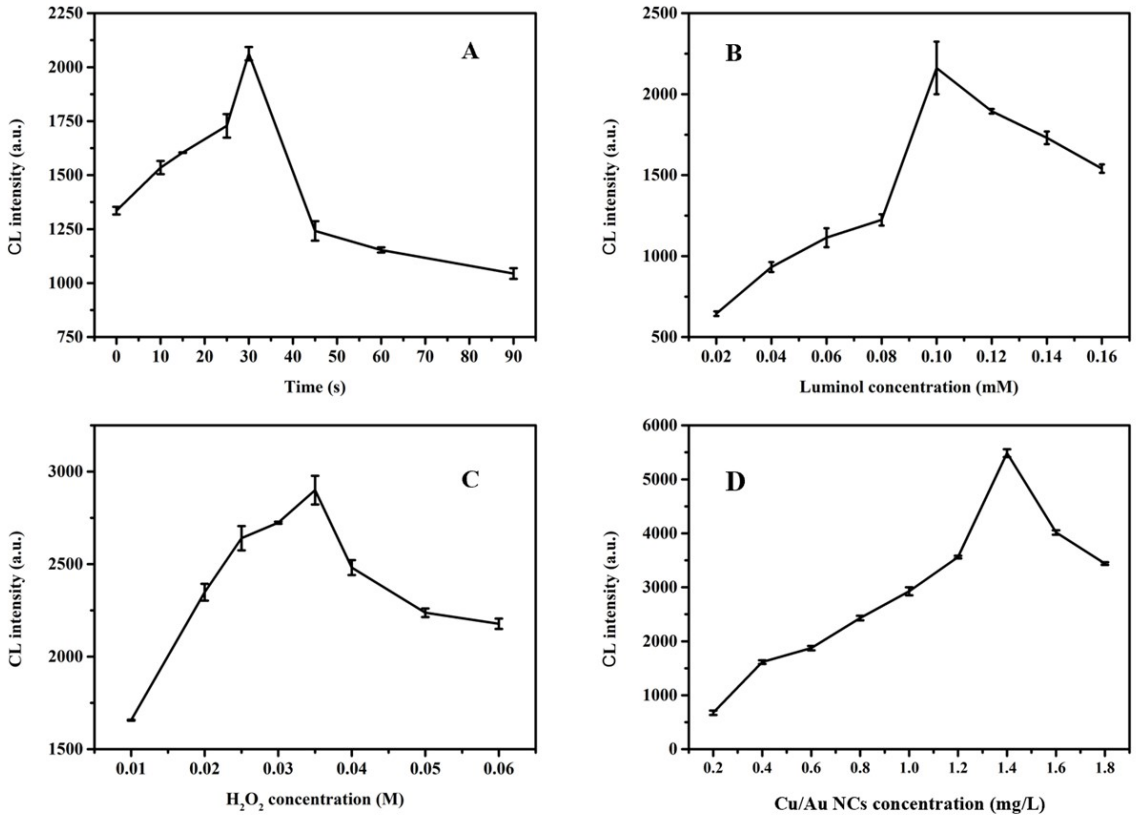

Fig. S8 (A) Effects of different reaction times on experiments **(**Experimental conditions: 0.1 mM luminol, 0.05 M H_2O_2, 1 mg L^{-1} Cu/Au NCs); **(B)** Effects of different luminol concentrations on experiments (Experimental conditions: Cu/Au NCs were mixed with H_2O_2 and placed in a dark room for 0.5 h, 0.05 M H_2O_2, 1 mg L^{-1} Cu/Au NCs); **(C)** Effects of different H_2O_2 concentrations on experiments (Experimental conditions: Cu/Au NCs were mixed with H_2O_2 and placed in a dark room for 0.5 h, 0.1 mM luminol,1 mg L^{-1} Cu/Au NCs); **(D)** Effects of different Cu/Au NCs concentrations on experiments (Experimental conditions: Cu/Au NCs were mixed with H_2O_2 and placed in a dark room for 0.5 h, 0.1 mM luminol,0.035 M H_2O_2)

3. Table

Table S1 Analytical results by this system for real serum sample (n = 3)

Sample	Found in sample (U/mL±SD)	Added (U/mL)	Total found (U/mL±SD)	Recovery rate (%)	RSD (%)
Bovine serum	0.091±0.016	0.2	0.288±0.018	98.5	6.3
		1.0	1.106±0.008	101.5	0.8
		1.5	1.596±0.010	100.3	0.6
		2.0	2.074±0.017	99.2	0.8
		4.0	3.975±0.112	97.1	2.8
		5.0	5.149±0.057	101.2	1.1
		6.0	6.059±0.033	99.5	0.5

4. References

1. C. Vakh, A. Kuzmin, A. Sadetskaya, P. Bogdanova, M. Voznesenskiy, O. Osmolovskaya and A. Bulatov, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020, **237**, 118382.
2. Z. Dehghani, J. Mohammadnejad and M. Hosseini, *Analytical and Bioanalytical Chemistry*, 2019, **411**, 3621-3629.
3. N. V. Suramwar, S. R. Thakare and N. T. Khaty, *Arabian Journal of Chemistry*, 2016, **9**, S1807-S1812.