Electronic Supplementary Information (ESI)

An enzyme cascade sensor with resistance to inherent intermediate product by logic-controlled peroxidase mimic catalysis

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Experimental Section

Materials and reagents.

Hydrogen peroxidase (30%); potassium permanganate; sodium chloride; calcium chloride dihydrate; potassium chloride; ammonium chloride; sodium sulfate and potassium phosphate were provided by the Sinopharm Chemical Reagent Co., Ltd (China). Polyvinyl pyrrolidone (PVP) and glucose oxidase (GOx) were purchased from the Sigma-Aldrich Co., Ltd. (USA). Copper (II) chloride dihydrate and 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from Aladdin Reagent Co. (Shanghai, China). Horseradish peroxidase (HRP); glucose; sucrose; maltose; fructose; lactose; urea and creatinine were bought from Sangon Biotech. (China). The G-quadruplex DNA (GGGTTAGGGTTAGGGTTAGGG) was synthesized by the Sangon Biotech. (China). All the reagents were of analytical grade or higher and all solutions were prepared using ultrapure water (18.2 MΩ cm^{-1}) from a Milli-Q water system (Millipore Co., USA).

Apparatus.

The transmission electron microscopy (TEM) image was taken on a JEOL-2100F HRTEM microscope (JEOL, Japan). The XRD pattern was recorded by a Rigaku D/max 2500 diffractometer (Rigaku Co., Japan). The pH values of the solutions were measured by an Ohaus starter 3100 digital pH-meter (Ohaus Co., USA). The UV-vis absorption data were recorded by an Agilent Cary 60 spectrophotometer (Agilent Co., USA) and a Multiskan GO microplate reader (Thermo Fisher Scientific, USA).

Synthesis of copper peroxide (CP) nanodots.

CP nanodots were synthesized according to the previous report.[1] Firstly, a 5 mL solution containing 0.5 g PVP and 0.01 M copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) was magnetically stirred. Then, 5 mL 0.02 M NaOH and 100 μL 30 % H_2O_2 were added successively. After 30 minutes, the CP nanodots was obtained and washed by ultrafiltration. The product was further lyophilized and redissolved in ultrapure water to obtain a 50 mg/mL solution and stored at 4 degree in dark.

Colorimetric detection based on the proposed method.

For the establishment of the glucose standard curve of the reported method, the glucose was firstly incubated with 0.04 mg/mL GOx in 0.2 mM pH 7.4 PBS for 30 min. Then, 100 μL of the above mixture was mixed with G-quadruplex (G_4), CP nanodots, TMB and the final volume is 250 μL (final concentrations: 200 nM G_4, 2 mg/mL CP nanodots, 0.5 mM TMB and different concentrations of glucose). Then, the solutions were further incubated for 120 min. After that, the UV-vis spectra were recorded by the microplate reader.

Colorimetric assay of hydroxyl groups released by CP nanodots decomposition.

According to the previous protocol, [1] $KMnO_4$ was firstly dissolved in 0.1 M H_2SO_4 to obtain a 50 μg/mL solution. After that, 200 μL of the $KMnO_4$ solution was mixed with 20 μL 50 mg/mL CP nanodots or 20 μL 10 mM H_2O_2 separately and incubated for 10 min. After that, the UV-vis spectra were recorded by the microplate reader.

Measurement for the final pH value after glucose incubation.

Typically, the glucose was firstly incubated with 0.04 mg/mL GOx in pH 7.4 PBS for 30 min. Then the mixture was mixed with ultrapure water with the volume ratio of 1:1.5

and the total volume was 5 mL. After further incubation of 120 min, the pH value of the solution was measured by the digital pH meter.

Colorimetric detection based on the GOx-HRP method

Typically, the glucose was incubated with GOx (0.04 mg/mL) for 30 min, and then 100 μL of the mixture was added to 150 μL PBS (50 mM pH 5) which containing 5 ng/mL HRP and 1 mM TMB. After incubated for another 10 min, the absorbance at 652 nm for the above 250 μL reaction systems with different glucose concentrations were recorded.

Determination of glucose in the artificial urine.

The artificial urine (AU) was prepared according to the previous report.[2-4] As reported, the concentration of glucose in the urine of healthy people is below 0.8 mM and it is clinically relevant above 2.5 mM. [5-6] For glucose determination, 5.5 mM glucose was spiked (≈100 mg/dL).[7] Before determination, the AU was diluted using ultrapure water and the pH was adjusted to 7.4. After that, the GOx was spiked with the concentration of 0.04 mg/mL and incubated for 30 min. Then, the above GOx-AU mixtures were separately applied for the proposed method and the GOx-HRP method, which both with final volumes of 250 μL and the following experiments are same as those described in the **Colorimetric detection based on the proposed method** and **Colorimetric detection based on the GOx-HRP method** separately. The final glucose concentration in the 250 μL reaction systems were calculated to be ≈ 42.3 μM on account of the sample treatment and detection procedures. For the anti-inherent H_2O_2 experiment, same procedures were performed as above except that 1.2 μM H_2O_2 was spiked in the

250 μL reaction systems of both the proposed method and the GOx-HRP method.

Figures

Fig. S1-S5 Characterization of the synthesized CP nanodots

The synthesized CP nanodots were characterized. As shown in the transmission electron microscope (TEM) image (Fig. S1), the CP nanodots exhibited a spherical morphology with a small diameter of less than 5 nm. In addition, the UV-vis spectrum showed UV-vis absorption below 600 nm (Fig. S2) and the X-ray diffraction (XRD) pattern exhibited a wide diffraction peak at approximately 21.1° (Fig. S3). The MnO_4^- test was applied to confirm the existence of the peroxo groups in the synthesized CP nanodots. As shown in Fig. S4, the colour of the acidic MnO_4^- disappeared when CP nanodots were added. This result could be ascribed to the fact that the CP nanodots quickly dissociated under strongly acidic conditions and generated H_2O_2, which reduced MnO_4^- to colourless Mn^{2+}. All the above characterizations agreed with the original literature for CP nanodot preparation and demonstrated the successful synthesis of CP nanodots.[1] In addition, the pH decrease induced by GOx catalysed glucose oxidation was also confirmed by using methylene red as the pH indicator, which showed an obvious red colour after 30 min of incubation with glucose, while the colour of the blank was still yellow (Fig. S5).

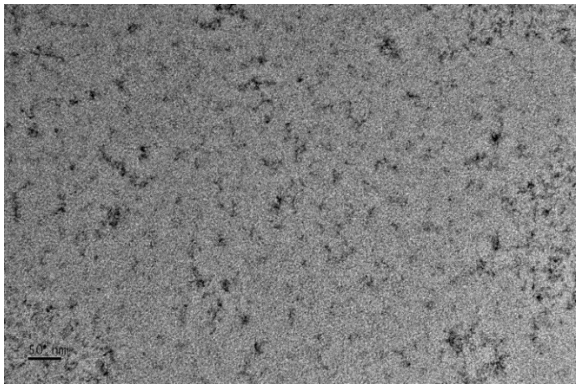

Figure S1 TEM image of the CP nanodots.

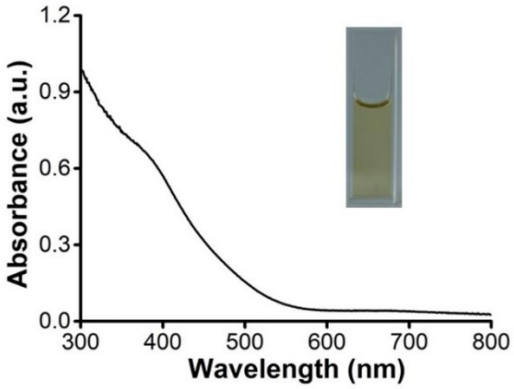

Figure S2 UV–vis absorption spectrum of the synthesized CP nanodots. Inset: digital photo of the synthesized CP nanodots.

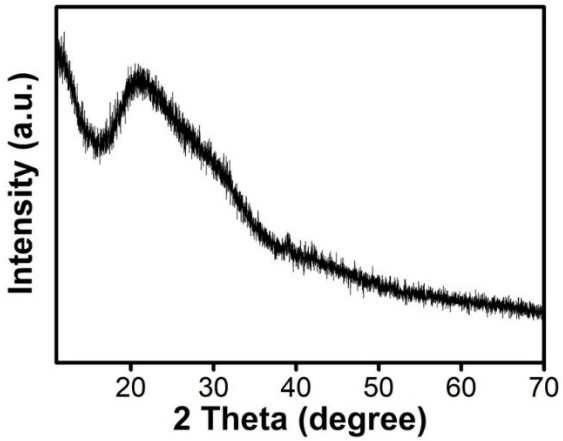

Figure S3 XRD pattern of the synthesized CP nanodots.

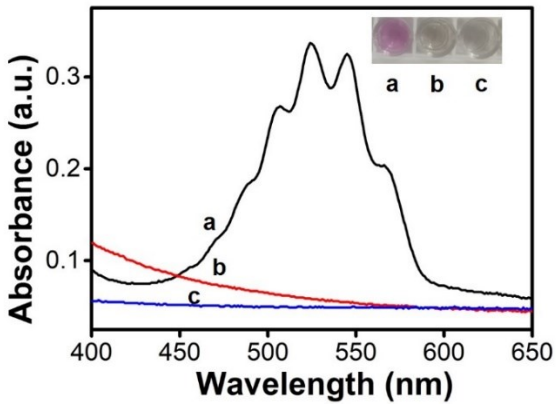

Figure S4 MnO_4^- -based colorimetric test for the peroxo groups in CP nanodots. a: MnO_4^-, b: MnO_4^- + CP nanodots. c: MnO_4^- + H_2O_2.

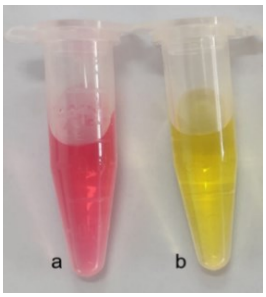

Figure S5 Methyl red test for the pH decrease introduced by the GOx catalyzed glucose oxidation. (0.2 mM pH 7.4 PBS); a: with 1 mM glucose; b: without glucose.

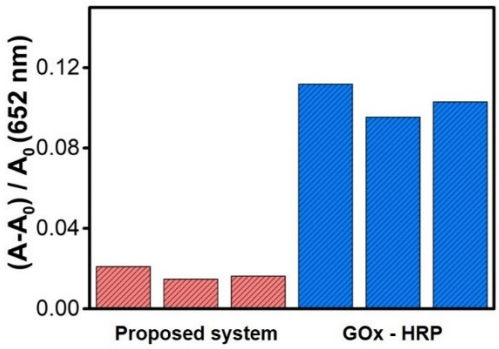

Figure S6. Comparison of the effect of additional H_2O_2 on the proposed "AND" logic-based cascade system (red columns) and the classical enzyme cascade reaction (GOx-HRP, blue columns). A and A_0 are the colorimetric signals of 200 μM glucose with and without additional 20 μM H_2O_2.

Fig. S7-S10 Optimization of the detection parameters

Before the detection of glucose, some experimental parameters, such as the concentrations of GOx, CP nanodots and G_4 were optimized. As shown in Fig. S7, the colorimetric signal gradually increased with increasing GOx concentration below 0.04 mg mL^{-1}, which was chosen as the optimum GOx concentration. In addition, the concentrations of CP nanodots and G_4 were also optimized (Fig. S8 and S9). Then, 2 mg mL^{-1} CP nanodots and 200 nM G_4 were chosen as the optimum concentrations in the final reaction system. The incubation time after adding G_4, CP nanodots and TMB to the glucose-GOx solution was also optimized. As shown in Fig. S10, the colorimetric signal did not show an obvious further increase after 120 min; thus, these conditions were chosen for the experiments.

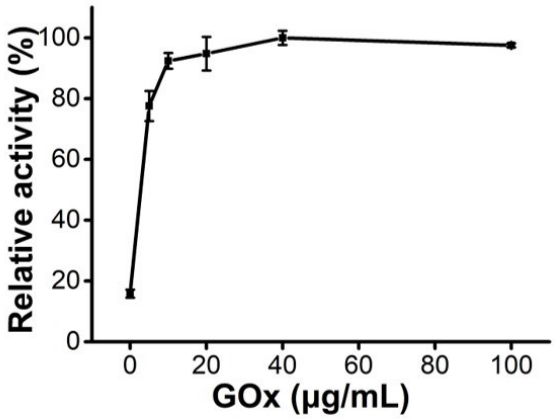

Figure S7 Optimization for the concentration of GOx.

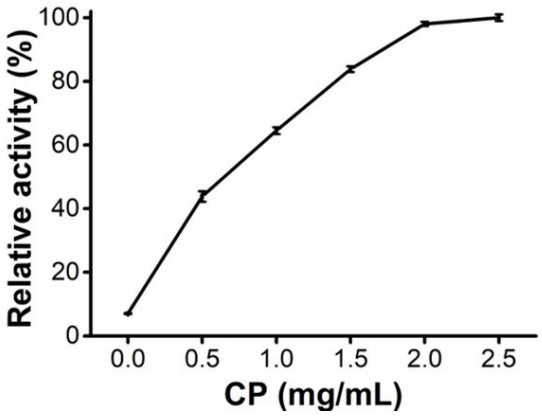

Figure S8 Optimization for the concentration of CP nanodots.

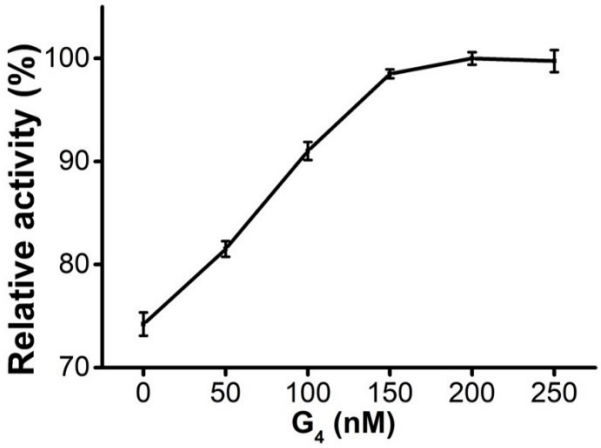

Figure S9 Optimization for the concentration of G_4 DNA.

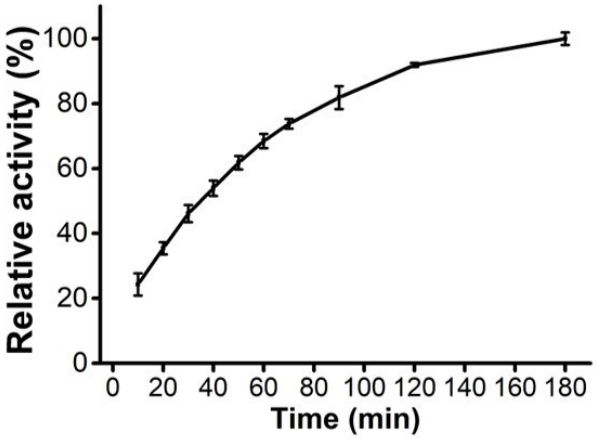

Figure S10 Optimization for the reaction time after the addition of G_4, CP nanodots and TMB to the GOx-glucose.

Tables

Table S1 Comparison of the proposed strategy with some recently reported colorimetric glucose sensors.

Method	Linear range	LOD	Reference
Colorimetric	0 - 0.8 mM	11.4 μM	8
	0.1 - 0.5 mM	0.03 mM	9
	100 - 1000 μM	2.31 μM	10
	10 - 300 μM	9.2 μM	11
	1 - 100 μM	4.12 μM	12
	8 - 140 μM	2.67 μM	13
	20 -1000 μM	8.34 μM	This work

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