# **Electronic Supplementary Information**

# Electrochemical biomolecule detection based on the regeneration of high-efficiency cascade catalysis for bifunctional nanozyme

Beibei Kou<sup>1</sup>, Yali Yuan<sup>1</sup>, Ruo Yuan\*, Yaqin Chai\*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China.

\* Corresponding authors at: Tel.: +86 23 68252277, fax: +86 23 68253172.

E-mail addresses: yqchai@swu.edu.cn (Y. Q. Chai), yuanruo@swu.edu.cn (R. Yuan)

# **Table of Contents**

1. Experimental Section	S3
1.1 Materials and Reagents	.S3
1.2 Apparatus	.S4
1.3 Exo I Protection Assay	S4
1.4 Target Cycling Amplification	.S5
1.5 Preparation of the $\beta$ -CD@AuNPs-MWCNTs Nanomaterials	.S5
1.6 Assembly of the Elaborated Sensor	.S6
1.7 Steady-State Kinetic Analysis of $\beta$ -CD@AuNPs as Bifunctional Nanozyn	nes.
2 Results and Discussion	S7
2.1 Mechanism of Target Recycling Amplification	S7
2.2 Characterization of the Designed Aptasensor	.S8
2.3 Optimal Conditions for Aptasensor	.S9
Scheme S1	.S9
Table S2	510
Fig S3	510
Fig S4	511
2.4 Serum Sample Analysis	511

## 1. Experimental Section

#### 1.1 Materials and Reagents.

Carboxylated-magnetic polystyrene microspheres (PSC-COOH) was bought from Tianjin BaseLine ChromTech Research Centre (Tianjin, China). Escherichia coli Exonuclease I (Exo I),  $10 \times$  reaction buffer, horseradish peroxidase (HRP),  $H_2O_2$  (30%, β-cyclodextrin  $(\beta$ -CD), o-phenyldiamine w/w), glucose, (o-PD), N-(3dimethylaminopropyl)-N-ethylcarbodiimide-hydrochloride (EDC) and N-hydroxy succinimide (NHS) were obtained from Sigma-Aldrich Co. Ltd (St. Louis, USA). Chloroauricacid (HAuCl<sub>4</sub>), 4-aminoazobenzene (Azo) and 8-hydroxy-2'deoxyguanosine (8-OHdG, 97%) were got from J&K Scientific Ltd. (Beijing, China). Multiwalled carbon nanotubes (MWCNTs) was acquired from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China) and the carboxylic MWCNTs was prepared according to the literature<sup>1</sup>. All the DNA oligonucleotides that provided by Sangon. Inc. (Shanghai, China) were listed in Table S1.

Table S1. The Information of the Used Oligonucleotides.				
Name	Sequence (from 5' to 3')			
$L_1$	CGT GGA GT <u>G CTA GGG TAT TGA ATG TCG GCC GTA AGT TAG T</u> TG			
	GAG ACA TAG GTT TT- (CH <sub>2</sub> ) <sub>6</sub> -NH <sub>2</sub>			
$L_2$	COOH-CTT TCC TAT GTC TCC AAC TAA CTT ACG G-MB			
$L_3$	CCA CAT ACA TCA TAT TCC GAC ATT CAA TAC CCT A			
Apt	CAT TCA ATA CCC TAG CAC TCC ACG GCG GGC GAT CGG CGG GGG			
	GTG CGT GCG CTC TGT GCC AGG GGG TGG GAC AGA TCA TAT GGG			
	<u>GGT GCT</u>			
fuel	<u>CCT ATG TCT CCA ACT AAC TTA CGG CCG ACA TTC AAT ACC CTA GC</u>			

Phosphated buffered solution (PBS, 0.1 M, pH 7.4) containing 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.1 M NaH<sub>2</sub>PO<sub>4</sub> and 0.1 M KCl was used as working buffer solution for electrochemical detection. 20 mM Tris-HCl buffer (pH 7.4) with 140 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub> was used as a buffer for diluting oligonucleotides.

# 1.2 Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed by a CHI 760E electrochemical workstation (Chenhua Instrument, Shanghai, China) with a conventional three-electrode system including a modified glassy carbon electrode (GCE,  $\phi = 4$  mm), saturated calomel electrode and platinum wire as working, reference, counter electrode, respectively. All DPV measurements were performed in 0.1 M PBS solution (pH 7.4) with the potential from 0.2 to -0.3 V, the step potential of 4 mV and the amplitude of 50 mV. The morphology of nanomaterials was characterized by a Tecnai G2 F30 high-resolution transmission electron microscopy (HRTEM, USA). The sample was irradiated with a table lamp (with a 60 W bulb) and a UV lamp (with wavelength of 365 nm) to accomplish the reversible photoswitching observation. The UV-vis spectra was performed with a UV-2450 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan). 1.3 Exo I Protection Assay.

At first, 30  $\mu$ L of Tris–HCl buffer containing 10  $\mu$ M aptamer probe was incubated with various concentrations of 8-OHdG solutions (37 °C, 1 h). To degrade the unbound aptamer probe, 10  $\mu$ L of 30 U Exo I was added and reacted at 37 °C for 2 h, which was further inactivated by heating the system to 85 °C for 20 min. The obtained product was entitled as 8-OHdG–Apt. 1.4 Target Cycling Amplification.

First, methylene blue and azobenzene-labeled ssDNA  $L_2$  (MB-L<sub>2</sub>-Azo, 10  $\mu$ M) was mixed with ssDNA  $L_1$  (10  $\mu$ M) and ssDNA  $L_3$  (10  $\mu$ M) for 2 h to obtain beacon complex. Afterward, the beacon complex was added into pre-activated PSC-COOH solution to covalently connect  $L_1$  on the PSC-COOH surface with the use of EDC/NHS. Then, 8-OHdG–Apt solution with various concentrations was added and reacted for 2 h, followed by the addition of fuel DNA sequence (10  $\mu$ M), resulting in the release of 8-OHdG-Apt for re-entering cycling amplification. Meanwhile, the supernatant containing numerous released MB-L<sub>2</sub>-Azo was collected by magnetic separation for further modification of the biosensor.

1.5 Preparation of the  $\beta$ -CD@AuNPs-MWCNTs Nanomaterials.

The  $\beta$ -CD@AuNPs-MWCNTs were prepared according to the previous one-pot synthesis procedures with a slight modification<sup>2</sup>. Briefly, 7 mg of functionalized MWCNTs and 500 µL of 0.5% nafion were mixed with 7 mL of ultrapure water and ultrasonicated to obtain a uniform black solution. After that, 1 mL of PBS (0.1 M, pH 7.0), 1 mL of HAuCl<sub>4</sub> (0.01 M) and 2 mL of  $\beta$ -CD (0.01 M) were added into above solution and vigorously stirred at 120 °C for 60 min for the situ generation of  $\beta$ -CD@AuNPs on MWCNTs surface. Ultimately, the obtained  $\beta$ -CD@AuNPs-MWCNTs precipitate was centrifuged and washed three times with ultrapure water, which then re-dispersed in 15 mL water for further use.

1.6 Assembly of the Elaborated Sensor.

First of all, 10  $\mu L$  of  $\beta\text{-}CD@AuNPs\text{-}MWCNTs$  nanomaterials were coated on

S-5

the surface of clean electrode (GCE) and dried in air. Subsequently, 20  $\mu$ L of cycling amplification products (MB-L<sub>2</sub>-Azo) was introduced into this system upon 10 min visible light irradiation. At last, the designed biosensor was determined in 2 mL PBS (pH 7.4) containing glucose to observe the DPV response. The regeneration process of the biosensor was realized under UV irradiation for 10 min.

1.7 Steady-State Kinetic Analysis of  $\beta$ -CD@AuNPs as Bifunctional Nanozymes.

Kinetic measurements were carried out by monitoring the absorbance change at 417 nm on a UV-Vis spectrophotometer. To investigate the kinetic mechanism of the  $\beta$ -CD@AuNPs with GOx-like activity, steady-state kinetic parameter was evaluated by changing the concentration of glucose. In detail, 150 µL of PBS (0.1 M, pH 7.4), 150 µL of the purified  $\beta$ -CD@AuNPs and different concentrations of glucose (5-180 mM) were mixed, which then was diluted to 1.5 mL with the PBS buffer and reacted thoroughly for 30 min. After centrifugation, 1 mL of the supernatant, 12.5 µL of HRP (0.05 mg·mL<sup>-1</sup>) and 125 µL of o-PD (5 mM) were continuously added into 500 µL of PBS buffer solution for the UV-vis measurements.

To investigate the kinetic mechanism of the HRP-like activity of the  $\beta$ -CD@AuNPs, steady-state kinetic parameters for the HRP-mimicking color reaction were determined by changing the concentration of o-PD and H<sub>2</sub>O<sub>2</sub>, respectively. First, the concentration of H<sub>2</sub>O<sub>2</sub> was fixed as 16 mM and the concentration of o-PD was changed (0.1-1.2 mM). After that, 75 µL of purified  $\beta$ -CD@AuNPs, 250 µL of H<sub>2</sub>O<sub>2</sub> and various concentrations of o-PD were added in 1.0 mL PBS solution (pH 7.4). The mixture solutions were reacted for 20 min and then used for absorbance measurement

at wavelength 417 nm. Under the same conditions, the experiments were carried out by changing the concentrations of H<sub>2</sub>O<sub>2</sub> (1-160 mM) with the concentration of o-PD fixed at 0.8 mM. The Michaelis-Menten constant ( $K_m$ ) and maximum initial velocity ( $V_{max}$ ) was calculated based on a Lineweaver-Burk plot:  $1/V = K_m/V_{max}$  ( $1/[S] + 1/K_m$ ), where [S] was the concentration of the substrate, V was the initial velocity.

## 2 Results and Discussion

## 2.1 Mechanism of Target Recycling Amplification

As displayed in Scheme 1, we designed five DNA strands, ssDNA  $L_{1}$ , methylene blue and azobenzene-labeled ssDNA  $L_2$  (MB-L<sub>2</sub>-Azo), ssDNA  $L_3$ , Apt and fuel, respectively. Apt was used to identify the target 8-OHdG. MB-L<sub>2</sub>-Azo was mixed with ssDNA  $L_1$  and ssDNA  $L_3$  to obtain beacon complex. In the absence of target 8-OHdG, Apt was digested by exonuclease I (Exo I), the downstream reactions were thus inhibited. However, in the presence of target 8-OHdG, the binding between target and Apt protected the Apt from degradation by Exo I. The resulting 8-OHdG– Apt complexes could trigger the strand displacement reaction and made the  $L_3$ sequence release from beacon complex. Then fuel sequence compelled 8-OHdG–Apt complexes release for re-entering cycling amplification, which synchronously released MB-L<sub>2</sub>-Azo and collected by magnetic separation for further modification of the biosensor.

#### 2.2 Characterization of the Designed Aptasensor

To confirm the stepwise modification of the aptasensor, CV measurements were performed in 2 mL PBS (pH 7.4) containing 5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> and 0.1 M KCl. As depicted in Fig. S1A, compared with the bare GCE (curve a), the  $\beta$ -CD@AuNPs-MWCNTs nanomaterials-modified electrode showed enhanced redox peak (curve b) owing to the excellent conductivity of  $\beta$ -CD@AuNPs and MWCNTs. Subsequently, the redox peak declined (curve c) when the target cycling amplification products (MB-L<sub>2</sub>-Azo) was introduced onto the above modified electrode via host-guest recognition between  $\beta$ -CD and Azo, which could be attributed to the hindrance of DNA strands to electron transfer. We also studied the contribution of MB-L<sub>2</sub>-Azo to the enzyme cascade amplification. As depicted in Fig. S1B, the presence of 4.0 mM MB-L<sub>2</sub>-Azo led to a significant enhancement in current response compared with the absence of MB-L<sub>2</sub>-Azo, confirming that the DPV signal was produced by MB-L<sub>2</sub>-Azo.



Fig. S1 (A) CVs of (a) bare GCE; (b)  $\beta$ -CD@AuNPs-MWCNTs /GCE and (c) MB-L<sub>2</sub>-Azo/ $\beta$ -CD@AuNPs-MWCNTs/GCE, (B) signal changes of the DPV peak without and with MB-L<sub>2</sub>-Azo on the electrode. Detection solution: 2 mL PBS (pH 7.4) solution with 4 mM glucose.

#### 2.3 Optimal Conditions for Aptasensor.

The glucose concentrations in detection solution greatly influenced signal

amplification of bifunctional nanozyme, and thus the corresponding DPV signals toward glucose with different concentrations was assessed. From Fig. S2, we could see that the current enhanced with increasing of glucose concentration and reached to a constant value after 4 mM. Therefore, 4 mM was regarded as the optimal concentration of glucose.



Fig. S2 Optimization of glucose concentration in the detection system.



Scheme S1. Schematic illustration of bifunctional nanozyme-catalyzed cascade reaction by using o-PD as the chromogenic substrate.

Table S2.	Comparison	of Sensing P	erformances of	Various	Methods	for 8	-OHdG	Detection
-----------	------------	--------------	----------------	---------	---------	-------	-------	-----------

Analytical method	<b>Detection limit</b>	Linear range	Ref.
-------------------	------------------------	--------------	------





**Fig. S3** (A) DPVs of the modified electrode before (b) and after (a) interaction with target cycle amplification products (c) the regeneration of efficient nanozymes cascade amplification. (B) Regeneration cycles of efficient nanozymes cascade amplification under alternating UV/visible light. Error bars: standard deviation (SD), n = 3.



**Fig. S4** (A) Selectivity of the aptasensor toward blank, 100 nM Cu<sup>2+</sup>, 100 nM Al<sup>3+</sup>, 100 nM UA, 100 nM AA, 100 nM guanine, 100 nM guanosine, and 1 nM 8-OHdG. (B) Reproducibility of the aptasensor in three parallel intra-assays and interassays (8-OHdG concentration:1 nM).

2.4 Serum Sample Analysis.

To further investigate the practical application of the proposed aptasensor, the recovery tests for various concentrations of 8-OHdG were diluted by 50-fold diluted human serum samples. In view of the results from Table S3, the recoveries was ranged from 97.5% to 102% with RSD from 1.6% to 2.8%, suggesting that the sensing system was prospective for target detection in real samples.

Sample number	Added/pM	Found/pM	Recovery/%	RSD/%
1	1.00	1.01	101	1.6
2	10.0	10.2	102	2.5
3	1.00×10 <sup>2</sup>	97.5	97.5	2.8
4	1.00×10 <sup>3</sup>	989	98.9	1.8

**Table S3**. Recovery Results of 8-OHdG Added in 50% Human Serum Samples (n = 3).

#### References

- 1 Y. Y. Chang, Y. Zhuo, Y. Q. Chai, R. Yuan, Anal. Chem., 2017, 89, 8266-8272.
- 2 Y. Zhao, Y. C. Huang, H. Zhu, Q. Q. Zhu, Y. S. Xia, J. Am. Chem. Soc., 2016, 138, 16645-16654.
- 3 Y. J. Liu, M. Wei, L. Q. Zhang, Y. J. Zhang, W. Wei, L. H. Yin, Y. P. Pu, S. Q. Liu, Anal.

- Chem., 2016, 88, 6509-6514.
- 4 M. Z. H. Khana, X. Q. Liua, Y. F. Tanga, X. H. Liu, *Biosens. Bioelectron.*, 2018, **117**, 508-514.
- 5 Z. P. Guo, X. H. Liu, Y. L. Liu, G. F. Wu, X. Q. Lu, *Biosens. Bioelectron.*, 2016, 86, 671-676.
- 6 J. N. Hao, Y. S. Li, Adv. Funct. Mater., 2019. DOI: 10.1002/adfm.201903058.
- G. Ammanath, U. H. Yildiz, A. Palaniappan, B. Liedberg, ACS Appl. Mater. Interfaces, 2018, 10, 7730-7736.