

Supporting Information for :

Extracellular Electron Transfer Enhanced Electrochemiluminescence

Aptasensor for *Escherichia coli* analysis

Xinyi Zhong,^a Yuan Deng,^a Qiling Yang,^a Sirui Yi,^a Haiyan Qiu,^a Lanlan Chen,^b
Shanwen Hu*^a

a. Department of Health Inspection and Quarantine, School of Public Health, Fujian Medical University, Fuzhou, Fujian, 350122, P.R. China

b. College of Chemistry, Key Laboratory of Analysis and detecting technology, Food Safety MOE, Fuzhou University, Fuzhou 350002, Fujian, P.R. China

Table of contents

Synthesis procedure of cadmium sulfide	2
Table S1 Oligonucleotide sequences in the experiments.....	2
Table S2 PCR Reagent specification	2
Table S3 A comparison of other biosensors.....	3
Figure S1 The Characterization of Cadmium sulfide.....	3
Figure S2 Optimization of GO and CTS ratio on the GCE.....	4
Figure S3 Optimization of the concentration of the aptamer.....	4
Figure.S4 Optimization of the time of aptamer1 capturing <i>E.coli</i>	4
Figure S5 The amplification curves and standard fitted curve of PCR.....	5
References.....	5

Synthesis procedure of cadmium sulfide:

50 mL 0.01 M Cadmium chloride hemi(pentahydrate) was added to the 250 mL three necked bottle, and added the 250 μ L mercaptopropionic acid. Subsequently, the solution was heated and stirred to reflux for reaction with 30 min, and then supplement with 5.5 mL 0.1 M Na₂S when the pH of reaction solution was adjusted to 11. After two hours of heating and stirring, the reaction solution that naturally cooled to room temperature was transferred to a centrifuge tube. An appropriate amount of isopropanol was added and centrifuged at 1000 rpm/min for 10 min, then the supernatant was discarded, repeated the operation three times. Finally, pure water was used as solvent disperse precipitation, and the sample were characterized and refrigerated at 4 °C.

Table S1 Oligonucleotide sequences in the experiment

Oligonucleotides	Sequences
Aptamer-1 ¹	CCGACGCTTATGCCTGCCATCTACAGAGCAGGTGTGACGG-NH ₂
Aptamer-2 ²	NH ₂ -TGAGCCCAAGCCCTGGTATGCGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTTGGCAGGTCTACTTTGGGATC
Anti-apt-1	NH ₂ -TTTTTATCCGTCACACCTGCTCTGTAGATGGCAAGGCATAAGCGTCCGG
Forward primer	GTGCCAGCMGCCGCGGTAA
Reverse primer	CCGTCAATTCMTTTRAGTTT

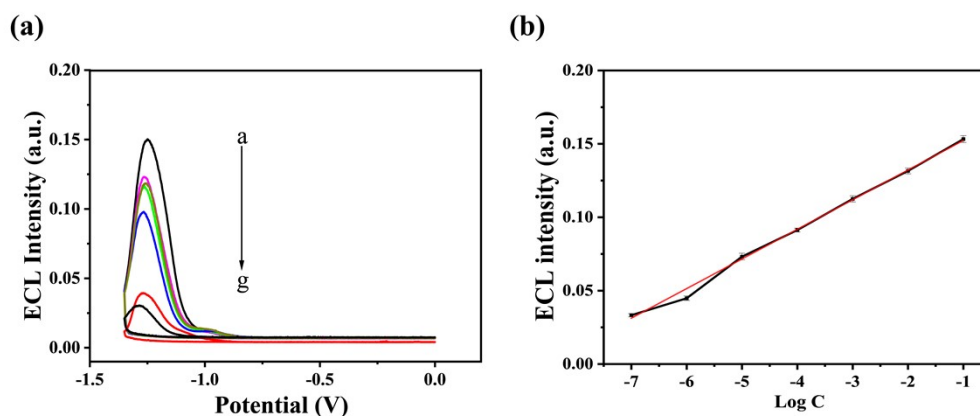
Table S2 PCR Reagent specification*

Reagents	Usage/ μ L
2x SuperReal Premix Plus	10
Forward Primer (10 μ M)	0.6
Reverse Primer (10 μ M)	0.6
ROX Reference Dye II (50 x)	2
DNA templates	2
RNase-free water	4.8
Total	20

*: PCR protocols were 95 °C, 30 s; 95 °C, 5 s; 65 °C, 34 s and cycling 40 times.

Table S3 A comparison of other biosensors

Title	Range of linear (CFU/mL)	LOD (CFU/mL)	ref
Electrochemiluminescence Detection of <i>Escherichia coli</i> O157:H7 Based on a Novel Polydopamine Surface Imprinted Polymer Biosensor	10-10 ⁷	8	3
NaBiF ₄ upconversion nanoparticle-based electrochemiluminescent biosensor for <i>E. coli</i> O157:H7 detection	200-100000	138	4
Electrogenerated Chemiluminescence on Smartphone with Graphene Quantum Dots Nanocomposites for <i>Escherichia Coli</i> Detection	10-10 ⁷	5	5
Electrochemiluminescent detection of <i>Escherichia coli</i> O157:H7 based on Ru(bpy) ₃ (2+)/ZnO nanorod arrays	200-100000	143	6
Electrogenerated chemiluminescence biosensors for the detection of pathogenic bacteria using antimicrobial peptides as capture/signal probes	1.0 × 10 ³ -5.0 × 10 ⁵ (I) 5.0 × 10 ² -5.0 × 10 ⁵ (II)	2.3 × 10 ² (I) 1.2 × 10 ² (II)	7
Potentiometric aptasensing of <i>Escherichia coli</i> based on electrogenerated chemiluminescence as a highly sensitive readout	5-1000	2	8
Electrochemiluminescence detection of <i>Escherichia coli</i> O157:H7 based on mesoporous Ca-doped MgAl ₂ O ₃ -G-SiO ₂ biosensor	10-10 ¹⁰	10	9
This work	10 ² -10 ⁷	10	

**Figure.S1** Characterization of Cadmium sulfide. (a) The ECL intensity of CdS with different dilution ratios. (b) The linear relationship between different dilution ratios of

CdS and ECL intensity (in 0.05 M potassium persulfate, $10\times$ PBS).

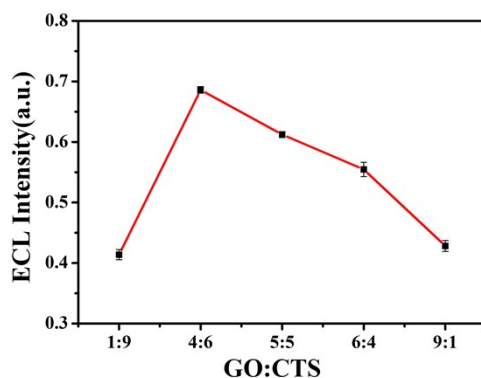


Figure.S2 Optimization of GO and CTS ratio on the GCE. The ECL intensity of biosensor with different molar ratios of graphene oxide and chitosan composites modified on GCE (in 0.05 M potassium persulfate, $10\times$ PBS).

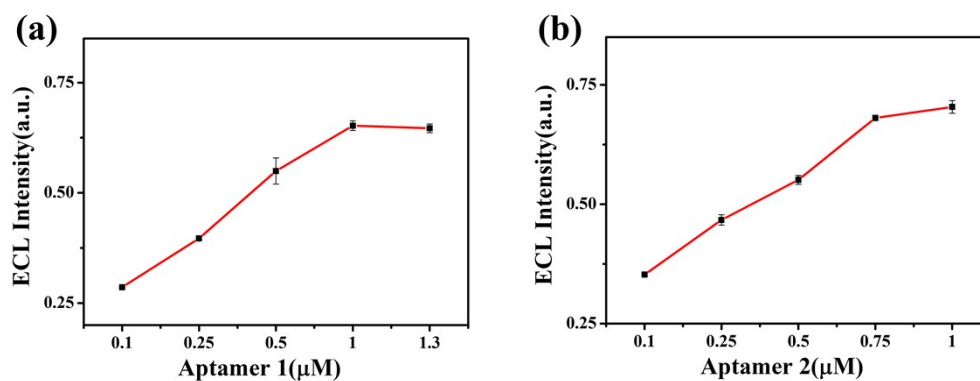


Figure.S3 Optimization of the aptamer concentration. (a) The ECL intensity of biosensor with different aptamer-1 concentrations. (b) The ECL intensity of biosensor with different aptamer-2 concentrations.

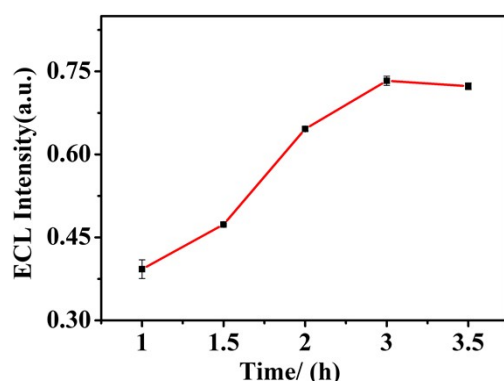


Figure.S4 Optimization of the time of aptamer-1 capturing *E.coli*. The ECL intensity of biosensor at different times of aptamer-2 capturing *E.coli*.

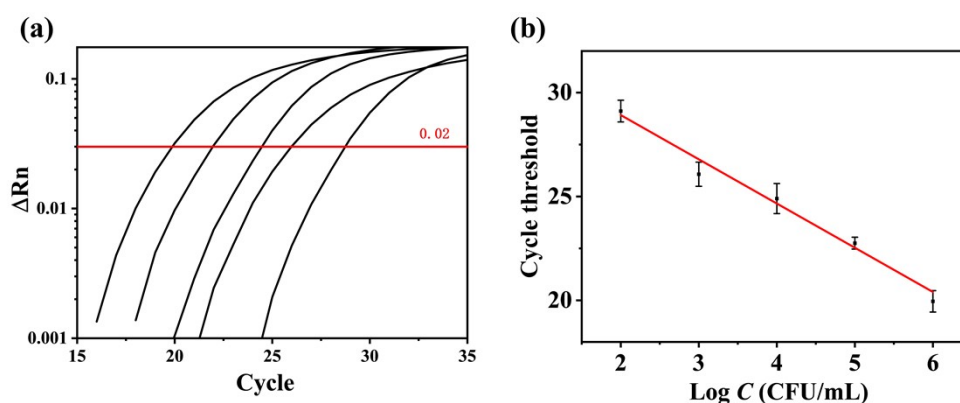


Figure.S5 The amplification curves and standard fitted curve of PCR. (a) The black lines are standard samples (10^2 , 10^3 , 10^4 , 10^5 , 10^6 CFU/mL). (b) The logarithmic calibration curve of the standard samples with different concentrations.

References

1. Khang, J.; Kim, D.; Chung, K. W.; Lee, J. H., Chemiluminescent aptasensor capable of rapidly quantifying Escherichia Coli O157:H7. *Talanta* **2016**, 147, 177-183.
2. Zou, Y.; Duan, N.; Wu, S.; Shen, M.; Wang, Z., Selection, Identification, and Binding Mechanism Studies of an ssDNA Aptamer Targeted to Different Stages of E-coli O157:H7. *J. Agric. Food Chem.* **2018**, 66, (22), 5677-5682.
3. S. Chen, X. Chen, L. Zhang, J. Gao and Q. Ma, Electrochemiluminescence Detection of Escherichia coli O157:H7 Based on a Novel Polydopamine Surface Imprinted Polymer Biosensor, *ACS Appl. Mater. Interfaces*, **2017**, 9, 5430-5436.
4. D. Liu, X. Lv, C. Zhao, J. Li, J. Huang, L. Weng, L. He and S. Liu, NaBiF(4) upconversion nanoparticle-based electrochemiluminescent biosensor for E. coli O157 : H7 detection, *RSC Adv.*, **2022**, 12, 30174-30180.
5. S. Li, J. L. Liu, Z. T. Chen, Y. L. Lu, S. S. Low, L. H. Zhu, C. Cheng, Y. He, Q. M. Chen, B. Su and

- Q. J. Liu, Electrogenerated chemiluminescence on smartphone with graphene quantum dots nanocomposites for Escherichia Coli detection, *Sens. Actuators, B*, **2019**, 297, 126811.
6. D. Liu, T. Li, W. Huang, Z. Ma, W. Zhang, R. Zhang, H. Yan, B. Yang and S. Liu, Electrochemiluminescent detection of Escherichia coli O157:H7 based on Ru(bpy)₃(²⁺)/ZnO nanorod arrays, *Nanotechnology*, **2019**, 30, 025501.
7. Z. J. Li, H. Y. Yang, L. J. Sun, H. L. Qi, Q. Gao and C. X. Zhang, Electrogenerated chemiluminescence biosensors for the detection of pathogenic bacteria using antimicrobial peptides as capture/signal probes, *Sens. Actuators, B*, **2015**, 210, 468-474.
8. X. Q. Gao, T. J. Jiang and W. Qin, Potentiometric aptasensing of Escherichia coli based on electrogenerated chemiluminescence as a highly sensitive readout, *Biosens. Bioelectron.*, **2022**, 200, 113923.
9. Z. D. Meng, C. H. Jung and W. C. Oh, Electrochemiluminescence detection of Escherichia coli O157:H7 based on mesoporous Ca-doped MgAl₂O₃-G-SiO₂ biosensor, *New J. Chem.*, **2022**, 46, 20120-20128.