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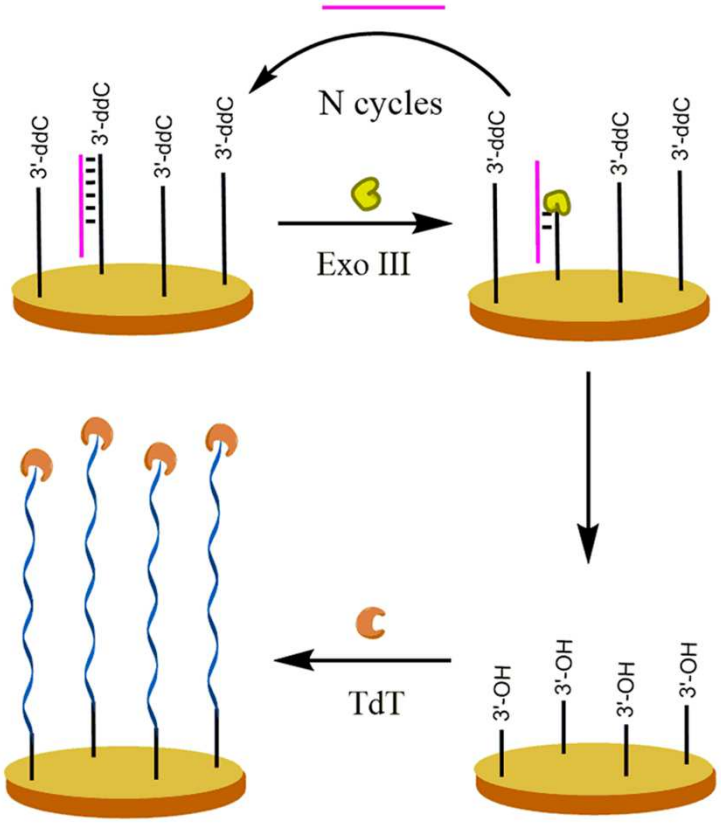
Graphical abstract



Highly sensitive detection of lipopolysaccharide based on collaborative amplification of dual enzymes

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Abstract

In this work, a novel electrochemical biosensor is developed for facile and highly sensitive detection of lipopolysaccharide (LPS) based on collaboration of dual enzymes for multiple-stages signal amplification. Through ingenious design, the specific recognition of target LPS is transformed to the exonuclease III (Exo III)-assisted interface DNA cycling collaborated with the terminal deoxynucleotidyl transferase (TdT)-catalyzed DNA extension, finally inducing significant electrochemical signal concerned with the concentration of LPS. This paper mainly discusses the detection principle, optimization of key factors, and the analytical performance of the biosensor. With the efficient signal amplification, the biosensor shows high sensitivity with a good linearity and a low limit of detection of 1 pg mL^{-1} for LPS. Moreover, the developed biosensor can clearly discriminate LPS from interferents and show high specificity for LPS detection. This biosensor has also been successfully employed to measure LPS in real food samples, suggesting potential opportunity for application in food safety detection.

Keywords: Electrochemical biosensor; Collaborative amplification; Lipopolysaccharide; Food safety

1. Introduction

Lipopolysaccharide (LPS) which is also termed endotoxin has given rise to significant problems for medical and food safety [1, 2]. As a toxic inflammatory stimulator, LPS derived from the outer membrane of Gram-negative bacteria is responsible for a great deal of patients and casualties annually resulted from hemorrhagic colitis, septic shock, organ failure and so on [3-6]. Due to the high activity and toxicity at an extremely low concentration [7], sensitive and specific detection of LPS is greatly important for food safety, clinical diagnosis and therapeutics.

Traditional methods such as limulus amebocyte lysate test and immunoassay have commonly been employed for LPS detection, but they are usually time-consuming, highly vulnerable to experiment context and require cumbersome preparation and detection procedure [8]. Therefore, multiple efforts have been dedicated to the exploitation of alternative assay methods for LPS lately. Natural and artificial ligands with specific cognition and high affinity to LPS provide promising alternatives to seek for accurate and specific detection of LPS [9], thus various biosensors on the basis of these ligands, such as LPS-binding peptides, proteins, functionalized polydiacetylene liposome and pyrene derivatives, have been established for LPS detection [10-12]. However, these biosensors tend to be not stable and specific enough owing to cross-binding or contamination of other interferences coexisting with LPS, while the synthetic biomolecules or probes involved are generally complicated and cost-prohibitive. Thereafter, the aptamer with high

specificity and affinity for LPS is selected successfully, showing particularly attractive applicability, and a number of studies on aptasensors for LPS have been reported [13-15]. Nevertheless, the sensitivity and specificity of these aptasensors are often restrained because of the requirement of signal reporter labels on aptamer probes or without efficient signal amplification. Therefore, it is still an urgent necessity to develop more sensitive, flexible and specific methods for appliable detection of LPS.

In virtue of the diverse and unique properties of DNA probe such as high programmability, mechanical flexibility, capacity of specific target recognition [16-18], DNA amplification systems have been effective tools to improve the sensitivity of detection [19, 20]. Among them, enzyme-assisted DNA amplification has received much more interest with the merits of simplicity, high efficiency and sensitivity [21, 22]. Herein, we have developed a novel electrochemical biosensor for facile LPS detection based on collaborative multiple-stages amplification via dual enzymes. The utilized enzymes are exonuclease III (Exo III) which can gradually digest single nucleotide from 3’ flat or hollow end of DNA duplex [23], and terminal deoxynucleotidyl transferase (TdT), a polymerase catalyzing the extension of DNA strand at 3’-hydroxyl terminal without template [24]. Inspired by the fact that the catalytic characteristics of the two enzymes are both closely related with the 3’ terminal of DNA, collaboration of dual enzymes for highly efficient signal amplification may be achieved through ingenious design. With the switch of duplex DNA structure upon target LPS binding as the starting point, the target recognition

can be transformed to released DNA strands that will further trigger Exo III-assisted interface DNA cycling, producing a large number of 3'-hydroxyl accessible for TdT to polymerize long DNA nanowires on the surface of electrode and finally resulting in significant amplification of electrochemical signal. Both Exo III and TdT have no requirement of specific sequence, which may contribute to the design flexibility of the detection strategy. In addition, LPS aptamer here without any modification could firmly bind with LPS and greatly improve the specificity of detection. Moreover, collaboration of the dual enzymes can result in multiple-stages signal amplification to realize highly sensitive detection of LPS. This method for more flexible and specific yet highly sensitive detection of LPS has also been examined in real food stuff, indicating potential practicability for food safety detection in the future.

2. Materials and methods

2.1. Materials and reagents

LPS, bovine serum albumin (BSA), glucose, hemoglobin (Hb), mercaptohexanol (MCH), hexaammineruthenium (III) chloride (RuHex), tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Exonuclease III (Exo III) and terminal deoxynucleotidyl transferase (TdT) were purchased from New England Biolabs, and dNTP was obtained from Takara Biotechnology Co., Ltd. (Dalian, China). All the DNAs (HPLC purified) were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd., and the sequences were as follows.

Aptamer: 5'-CTTCTGCCCGCCTCCTTCCTAGCCGGATCGCGCTGGCCAGATG

ATATAAAGGGTCAGCCCCCCAGGAGACGAGATAGGCGGACACT-3’

Report probe: 5’-GCGATCCGGCTAGGAATTTT-3’

Signal probe: 5’-SH-TTATGCCGGATCGC-ddC-3’

Other chemicals were of analytical grade and utilized as obtained without extra purification. DNA immobilization solution was 10 mM Tris-HCl containing 10 mM TCEP, 1mM EDTA and 100 mM NaCl (pH 7.4). DNA hybridization buffer was 10 mM PBS containing 100 mM NaCl, 5 mM KCl and 2 mM $MgCl_2$ (pH 7.4). Ultrapure water (>18.0 MΩ) for all experiments were prepared from a Millipore-Q system. Milk sample was pretreated by first being centrifuged for 10 min at 10000 rpm to remove the upper fat. After that, 10% (v/v) perchloric acid aqueous solution was added to precipitate protein for several reiterations, then the supernatant was collected and corrected to proper pH. Finally, the supernatant was centrifuged for 5 min at 7000 rpm to be prepared for use.

2.2. Gold electrode treatment and modification

The treatment procedures of gold electrode were nearly the same as previous reports [25]. Firstly, the gold electrode with a diameter of 3.0 mm was polished with alumina slurry of 1.0 μm and 0.3 μm in order, and then ultrasonicated in ethanol and deionized water for 5 min respectively. Subsequently, the electrode was in bath of freshly prepared piranha solution (H_2SO_4: 30% H_2O_2 = 3:1, v/v), followed by washed with deionized water. After that, the electrode was electrochemically cleaned with 0.5 M H_2SO_4 aqueous solution.

Before being immobilized on the electrode, the signal probe was treated with 10

mM TCEP to reduce disulfide bonds. The prepared electrode dried with nitrogen was immerged in DNA immobilization solution containing 2 μM signal probe for 1.5 h at room temperature, and then dipped in 1 mM MCH aqueous solution for 2 h to prohibit nonspecific binding and render the immobilized DNAs in order. Finally, the modified electrode was washed by deionized water and dried with mild nitrogen stream.

2.3. Detection of LPS

For the detection of LPS, LPS aptamer (200 nM) and report probe (200 nM) were firstly mixed and heated to 95 °C for 5 min and gradually cooled to 25 °C for 60 min to form the partial duplex. Then LPS of various concentrations were added and incubated for 30 min at 37 °C to form the aptamer/LPS complex and release the report probe. After that, the signal probe-modified electrode was dipped into the above mixture, followed by incubated with 0.5 U μL^{-1} Exo III (50 μL 1× NEBuffer 4) at room temperature for 30 min. Finally, the electrode was incubated with 1.0 U μL^{-1} TdT (50 μL 1× TdT buffer) and 2 mM dNTP for 90 min at 37 °C, and then gently rinsed with deionized water for the following electrochemical measurements.

2.4. Electrochemical measurements

Electrochemical measurements were carried out at room temperature on a CHI 660D electrochemical workstation (CH Instruments, USA) with a traditional three-electrode system including a modified gold electrode, a platinum electrode and a saturated calomel electrode (SCE) as the working electrode, the counter electrode and the reference electrode respectively. Square wave voltammetry (SWV) and chronocoulometry (CC) measurements were conducted in 10 mM Tris-HCl buffer (pH

7.4) containing 50 μM RuHex which was bubbled with and maintained under the nitrogen steam to deoxygenate. Electrochemical impedance spectra (EIS) was recorded in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 1 M KNO_3. SWV was performed with the scan range from 0 to -0.5 V, the frequency of 10 Hz, and the amplitude of 25 mV. For CC measurements, the pulse period was 250 ms. EIS was measured by applying the bias potential of 0.224 V and the amplitude of 5 mV with the frequencies swept from 0.1 Hz to 10 kHz.

3. Results and discussion

3.1. Principle of the strategy

Please insert Scheme 1 here.

The principle of this strategy to detect LPS is schematically illustrated by Scheme 1. The report DNA probe is designed to hybridize with the aptamer of LPS to form a partial duplex. In the existence of LPS, the aptamer-LPS binding destroys the partial duplex and liberate report probe. The released report probe can be captured by the signal probe immobilized on the electrode without free 3'-hydroxyl group (3'-ddC), and the hybridization can form an interfacial duplex with a flat 3' terminus accessible to Exo III. Exo III then catalyzes the stepwise elimination of single nucleotide from the 3'-ddC end of signal probe to expose 3'-hydroxyl, meanwhile release the report probe to bind with another signal probe for a new catalytical cycle. Through the Exo III-assisted interface DNA cycling, a large amount of 3'-hydroxyl can be produced for TdT to initiate the polymerization reaction and generate long DNA nanotails on the surface of electrode, which consequently bind with RuHex to

show a significant electrochemical signal related to the concentration of LPS. Therefore, the multiple-stages signal amplification by collaboration of dual enzymes enables flexible, highly specific and sensitive detection of LPS.

3.2. Verification of the principle

Please insert Fig. 1 here.

Electrochemical techniques including square wave voltammetry (SWV) and chronocoulometry (CC) have been carried out to verify the principle of the designed biosensor. As shown in Fig. 1, the introduction of LPS leads to an evident increase of peak current in SWV measurements, which is consistent with the results of CC, suggesting the LPS-triggered generation of long DNA nanotails on the electrode surface.

Please insert Fig. 2 here.

The stepwise modification and interface properties of electrode have been examined by electrochemical impedance spectroscopy. The Nyquist plot of electrochemical impedance spectra (EIS) comprises a semicircle and a linear portion, which represent the interfacial electron transfer resistance and diffusion-controlled process respectively. As shown in Fig. 2, the bare gold electrode presents a nearly straight line in the EIS (curve a), implying only a diffusion-limited process. An obvious semicircle appears upon the successful immobilization of signal DNA probes that hinder the electron transfer (curve b). Further incubation with the mixture containing target LPS results in the increase of semicircle diameter (curve c), indicating the formation of interfacial duplexes by the hybridization between the

released report probes and immobilized signal probes. After the cleavage reaction of Exo III, the semicircle diameter distinctly decreases (curve d). The final extension effect of TdT leads to significant increase of semicircle diameter due to the blocked electroconductibility by the generated long DNA nanotails (curve e).

3.3. Optimization of experimental conditions

Please insert Fig. 3 here.

Optimization of experimental conditions plays crucial roles in the analytical system, thus it has been conducted to obtain the best assay performance. Firstly, the concentration of report probe as a key factor is optimized, because a very low concentration of it will restrict the signal amplification, whereas an ultrahigh concentration of it will result in a relatively elevated background signal. As presented in Fig. 3A, the variation of CC signal is peak-shape dependent with the concentration of report probe, and the highest signal variation appears at 200 nM, so the optimal concentration of report probe is chosen as 200 nM. The concentration and cleavage time of Exo III are then investigated. As depicted in Fig. 3B, the surface charge increases continuously with the growing concentration of Exo III and almost approaches a plateau at 0.5 U μL^{-1}, thus 0.5 U μL^{-1} Exo III is adopted in the assay experiments. Fig. 3C demonstrates the temporal response of the sensing method with the cleavage time of Exo III, and the optimal time is 30 min according to the maximum surface charge. Moreover, to verify the effect of TdT, the electrochemical responses to different concentrations and polymerization times of TdT are recorded. As shown in Fig. 3D, the CC signal increases gradually along with the rising of TdT

concentration, meanwhile the extension time-dependent change of CC signal is also observed, arriving at steady states at 1.0 U μL^{-1} and 90 min, respectively. Therefore, the concentration of 1.0 U μL^{-1} and the polymerization time of 90 min are sufficient for TdT to produce long DNA nanotails on the surface of electrode.

3.4. Analytical performance of the biosensor

Please insert Fig. 4 here.

To examine the analytical performance of the biosensor, electrochemical responses of the sensor to varying concentrations of target LPS have been explored. With RuHex as the signal molecule, CC technique which can accurately measure the redox charges of RuHex on the electrode surface is employed. Fig. 4 demonstrates the characteristic CC performance to detect different concentrations of LPS. It can be found that the redox charge increases gradually as the abundance of LPS gets higher, and the variation of CC signal is linearly correlated with the logarithmic value of LPS concentration ranging from 2.5 pg mL^{-1} to 1000 pg mL^{-1}. The limit of detection for LPS is evaluated to be 1 pg mL^{-1} based on the 3d/slope criterion. In addition, the repetitive experiments reveal a relative standard deviation below 5.5%, indicating the acceptable reproducibility of this biosensor. Moreover, the developed method shows equivalent or even preferable detection performances compared to some previous reports for LPS detection (Table 1).

Please insert Table 1 here.

Interferents including BSA, RNA, AA, Hb and glucose are utilized to determine the specificity of the biosensor for LPS detection. As demonstrated in Fig. 5, target

LPS and the mixture composed of interferents and LPS are able to induce remarkably enhanced CC signal, while inappreciable signal increasement can be observed for each interferent. These results suggest that the proposed biosensor can clearly discriminate LPS from interferents and show high specificity for LPS detection.

Please insert Fig. 5 here.

LPS in food may potentially threaten human health due to the high toxicity, so it is of great importance to detect LPS from food samples. To examine the practicability of the proposed biosensor, the recovery experiments to assay drinking water samples and milk samples containing LPS have been carried out. LPS standards are respectively added into drinking water samples and milk samples to acquire the final concentrations of 5, 20, 200, 600, and 900 pg mL^{-1}, then the recoveries are measured through the developed biosensor. As shown in Table 2, the LPS recoveries in drinking water samples are calculated to be from 91.8% to 112%, while the recoveries in milk samples are from 90.5% to 109%, all with the acceptable relative errors, indicating satisfactory practicability of the biosensor for LPS detection.

Please insert Table 2 here.

4. Conclusions

In conclusion, through the ingenious design to collaborate Exo III-assisted interface DNA cycling and the TdT-catalyzed DNA extension, a novel electrochemical biosensor is developed for LPS detection. Collaboration of the dual enzymes contribute to the multiple-stages signal amplification to achieve highly sensitive quantitative determination of LPS. Besides, the method shows excellent

selectivity to clearly discriminate LPS from the interferents, meanwhile exhibits satisfactory assay capability in real food samples, providing promising practicability for LPS detection. Furthermore, the unique catalytic features of Exo III and TdT may increase the design flexibility of the detection strategy, which is profitable for converting the method to the detection of other targets and then extending its application.

Acknowledgements

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References

[1] K.J. Kieser, J.C. Kagan, Multi-receptor detection of individual bacterial products by the innate immune system, Nat. Rev. Immunol. 17 (2017) 376-390.

[2] J.D. Sun, P. Zhu, X.M. Wang, J. Ji, J.D. Habimana, J.D. Shao, H.T. Lei, Y.Z. Zhang, X.L. Sun, Cell Based-Green Fluorescent Biosensor Using Cytotoxic Pathway for Bacterial Lipopolysaccharide Recognition, J. Agr. Food Chem. 66 (2018) 6869-6876.

[3] C. Zhang, F. Tian, M.L. Zhang, Z.Q. Zhang, M. Bai, G. Guo, W.J. Zheng, Q. Wang, Y. Shi, L.L. Wang, Endotoxin contamination, a potentially important inflammation factor in water and wastewater: A review, Sci. Total. Environ. 681

(2019) 365-378.

[4] M. Gasparrini, F. Giampieri, T.Y. Forbes-Hernandez, S. Afrin, D. Cianciosi, P. Reboredo-Rodriguez, A. Varela-Lopez, J.J. Zhang, J.L. Quiles, B. Mezzetti, S. Bompadre, M. Battino, Strawberry extracts efficiently counteract inflammatory stress induced by the endotoxin lipopolysaccharide in Human Dermal Fibroblast, Food Chem. Toxicol. 114 (2018) 128-140.

[5] Y. Li, Z.Z. Shi, I. Radauer-Preiml, A. Andosch, E. Casals, U. Luetz-Meindl, M. Cobaleda, Z.M. Lin, M. Jaberi-Douraki, P. Italiani, J. Horejs-Hoeck, M. Himly, N.A. Monteiro-Riviere, A. Duschl, V.F. Puntes, D. Boraschi, Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation, Nanotoxicology 11 (2017) 1157-1175.

[6] J. Zhang, X.Y. Zhu, X.X. Hu, H.W. Liu, J. Li, L.L. Feng, X. Yin, X.B. Zhang, W.H. Tan, Ratiometric Two-Photon Fluorescent Probe for in Vivo Hydrogen Polysulfides Detection and Imaging during Lipopolysaccharide-Induced Acute Organs Injury, Anal. Chem. 88 (2016) 11892-11899.

[7] E.C. Dullah, C.M. Ongkudon, Current trends in endotoxin detection and analysis of endotoxin-protein interactions, Crit. Rev. Biotechnol. 37 (2017) 251-261.

[8] S.K. Lim, P. Chen, F.L. Lee, S. Moochha, B. Liedberg, Peptide-Assembled Graphene Oxide as a Fluorescent Turn-On Sensor for Lipopolysaccharide (Endotoxin) Detection, Anal. Chem. 87 (2015) 9408-9412.

[9] X.L. Liu, Z.C. Yang, W.B. Xu, Y.M. Chu, J. Yang, Y.C. Yan, Y. Hu, Y. Wang, J.L.

Hua, Fine tuning of pyridinium-functionalized dibenzo[a,c]phenazine near-infrared AIE fluorescent biosensors for the detection of lipopolysaccharide, bacterial imaging and photodynamic antibacterial therapy, J. Mater. Chem. C. 7 (2019) 12509-12517.

[10] A. Oda, D. Kato, K. Yoshioka, M. Tanaka, T. Kamata, M. Todokoro, O. Niwa, Fluorinated Nanocarbon Film Electrode Capable of Signal Amplification for Lipopolysaccharide Detection, Electrochim. Acta 197 (2016) 152-158.

[11] T. Liu, L.Q. Gao, J. Zhao, Y. Cao, Y.G. Tang, P. Miao, A polymyxin B-silver nanoparticle colloidal system and the application of lipopolysaccharide analysis, Analyst 143 (2018) 1053-1058.

[12] Y.Y. Tang, A. Kang, X.T. Yang, L.Y. Hu, Y.Y. Tang, S. Li, Y.C. Xie, Q.Q. Miao, Y. Pan, D. Zhu, A robust OFF-ON fluorescent biosensor for detection and clearance of bacterial endotoxin by specific peptide based aggregation induced emission, Sensor Actuat. B-Chem. 304 (2020).

[13] S.E. Kim, W. Su, M. Cho, Y. Lee, W.S. Choe, Harnessing aptamers for electrochemical detection of endotoxin, Anal. Biochem. 424 (2012) 12-20.

[14] L.X. Wen, J.J. Lv, L. Chen, S.B. Li, X.J. Mou, Y. Xu, A fluorescent probe composed of quantum dot labeled aptamer and graphene oxide for the determination of the lipopolysaccharide endotoxin, Microchim. Acta 186 (2019).

[15] L. Ma, N.N. Sun, Y.Y. Meng, C.H. Tu, X.Q. Cao, Y.C. Wei, L.Q. Chu, A.P. Diao, Harnessing the affinity of magnetic nanoparticles toward dye-labeled DNA and developing it as an universal aptasensor revealed by lipopolysaccharide detection,

Anal. Chim. Acta. 1036 (2018) 107-114.

[16] M. Zeraati, D.B. Langley, P. Schofield, A.L. Moye, R. Rouet, W.E. Hughes, T.M. Bryan, M.E. Dinger, D. Christ, I-motif DNA structures are formed in the nuclei of human cells, Nat. Chem. 10 (2018) 631-637.

[17] L. Liu, Y. You, K. Zhou, B.Y. Guo, Z. Cao, Y.L. Zhao, H.C. Wu, A Dual-Response DNA Probe for Simultaneously Monitoring Enzymatic Activity and Environmental pH Using a Nanopore, Angew. Chem. Int. Edit. 58 (2019) 14929-14934.

[18] N.D.M. Sani, L.Y. Heng, R.S.P.M. Marugan, N.F. Rajab, Electrochemical DNA biosensor for potential carcinogen detection in food sample, Food Chem. 269 (2018) 503-510.

[19] M.X. You, Y.F. Lyu, D. Han, L.P. Qiu, Q.L. Liu, T. Chen, C.S. Wu, L. Peng, L.Q. Zhang, G. Bao, W.H. Tan, DNA probes for monitoring dynamic and transient molecular encounters on live cell membranes, Nat. Nanotechnol. 12 (2017) 453-459.

[20] K.W. Ren, Y.F. Xu, Y. Liu, M. Yang, H.X. Ju, A Responsive "Nano String Light" for Highly Efficient mRNA Imaging in Living Cells via Accelerated DNA Cascade Reaction, Acs Nano 12 (2018) 263-271.

[21] W.H. Zhou, L. Hu, L.M. Ying, Z. Zhao, P.K. Chu, X.F. Yu, A CRISPR-Cas9-triggered strand displacement amplification method for ultrasensitive DNA detection, Nat. Commun. 9 (2018).

[22] C. Liu, X.L. Cui, B.X. Zhao, P. Narkhede, Y.W. Gao, J. Liu, X.Y. Dou, Q. Dai,

L.S. Zhang, C. He, DNA 5-Methylcytosine-Specific Amplification and Sequencing, J. Am. Chem. Soc. 142 (2020) 4539-4543.

[23] X.Y. Hua, E.F. Yang, W.T. Yang, R. Yuan, W.J. Xu, LAMP-generated H+ ions-induced dimer i-motif as signal transducer for ultrasensitive electrochemical detection of DNA, Chem. Commun. 55 (2019) 12463-12466.

[24] L. Wang, Y.H. Pan, Y.F. Liu, Z.W. Sun, Y. Huang, J.L. Li, J. Yang, Y. Xiang, G.X. Li, Fabrication of an Aptamer-Coated Liposome Complex for the Detection and Profiling of Exosomes Based on Terminal Deoxynucleotidyl Transferase-Mediated Signal Amplification, ACS Appl. Mater. Interfaces 12 (2020) 322-329.

[25] Y. Huang, J. Zheng, L. Wang, X.G. Duan, Y.S. Wang, Y. Xiang, G.X. Li, Sensitive detection of chloramphenicol based on Ag-DNAzyme-mediated signal amplification modulated by DNA/metal ion interaction, Biosens. Bioelectron. 127 (2019) 45-49.

[26] W. Su, M. Lin, H. Lee, M. Cho, W.S. Choe, Y. Lee, Determination of endotoxin through an aptamer-based impedance biosensor, Biosens. Bioelectron. 32 (2012) 32-36.

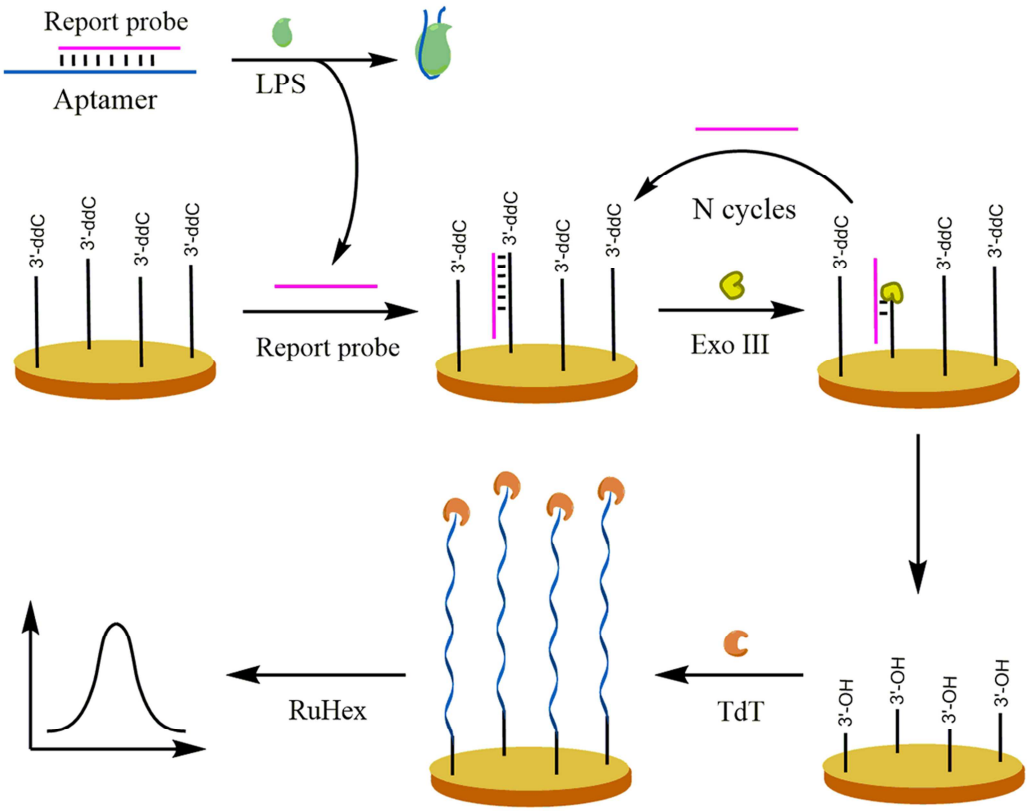
[27] M.Y. Zuo, L.J. Chen, H. Jiang, L. Tan, Z.F. Luo, Y.M. Wang, Detecting endotoxin with a flow cytometry-based magnetic aptasensor, Anal. Biochem. 466 (2014) 38-43.

[28] W.T. Xu, J.J. Tian, X.L. Shao, L.J. Zhu, K.L. Huang, Y.B. Luo, A rapid and visual aptasensor for Lipopolysaccharides detection based on the bulb-like triplex

turn-on switch coupled with HCR-HRP nanostructures, Biosens. Bioelectron. 89 (2017) 795-801.

[29] L.T. Zeng, J.S. Wu, Q. Dai, W.M. Liu, P.F. Wang, C.S. Lee, Sensing of Bacterial Endotoxin in Aqueous Solution by Supramolecular Assembly of Pyrene Derivative, Org. Lett. 12 (2010) 4014-4017.

Figures and Captions



Scheme 1. Schematic representation of the proposed electrochemical strategy for LPS detection. Not drawn to scale.

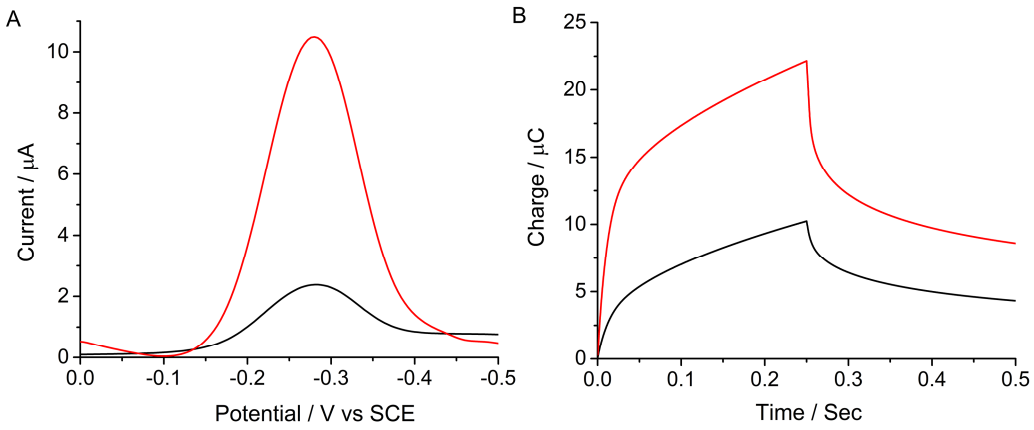


Fig. 1. Electrochemical signals to confirm the assay principle. (A) Square wave voltammograms and (B) Chronocoulometry curves obtained in the presence of 1000 pg mL^{-1} LPS (red line) and the absence of LPS (black line).

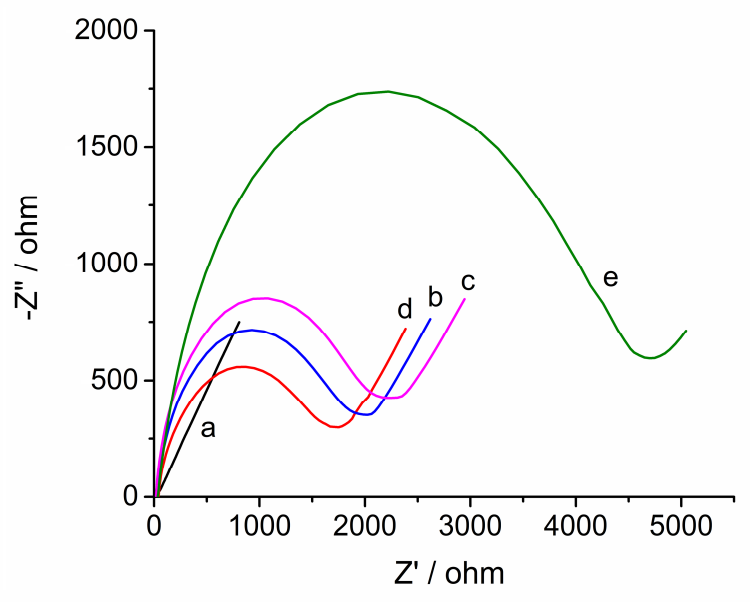


Fig. 2. EIS measurements corresponding to the stepwise treatment of electrode, (a) the bare gold electrode, (b) the electrode modified with signal probe, (c) the modified electrode incubated with the mixture containing LPS, (d) after the cleavage reaction of 0.5 U μL^{-1} Exo III, (e) further treatment with 1.0 U μL^{-1} TdT.

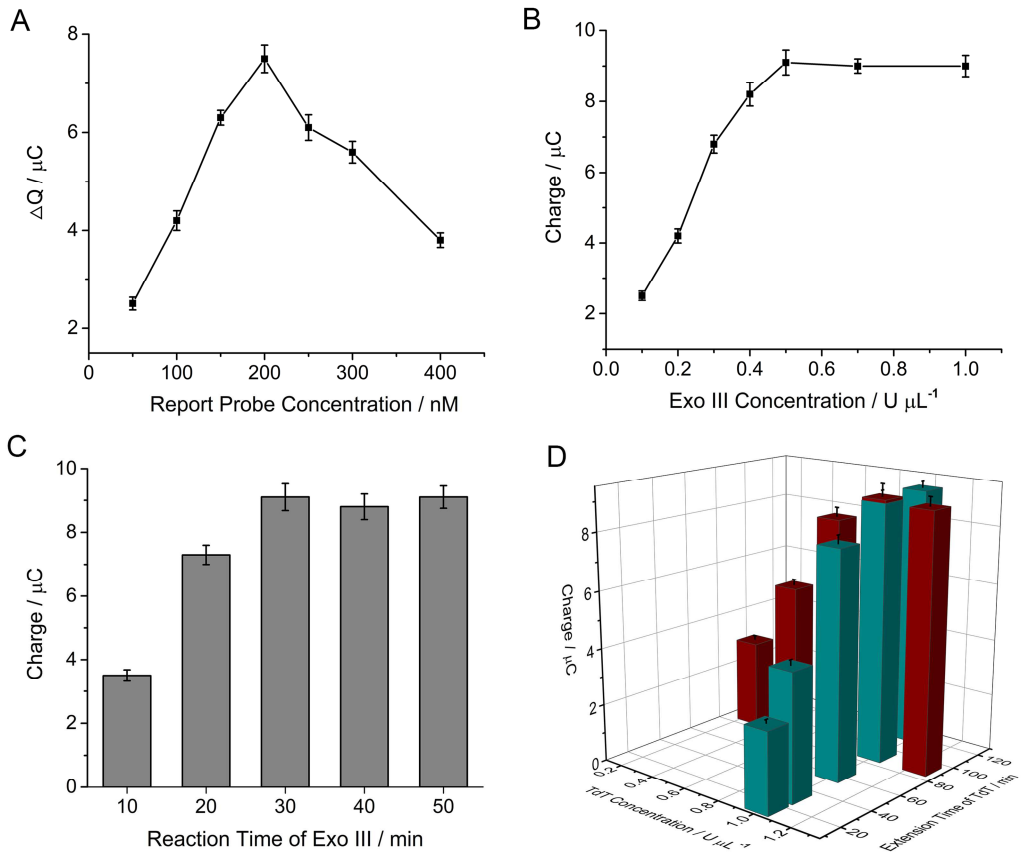


Fig. 3. Optimization of key experimental conditions. (A) Relationship between the variation of redox charge (△Q) with the concentration of report probe. (B) Variance of the redox charge with the concentration of Exo III. (C) Variance of the redox charge with the cleavage time of Exo III. (D) Variance of the redox charge with the concentration and polymerization time of TdT. Error bars show standard deviations of three independent measurements.

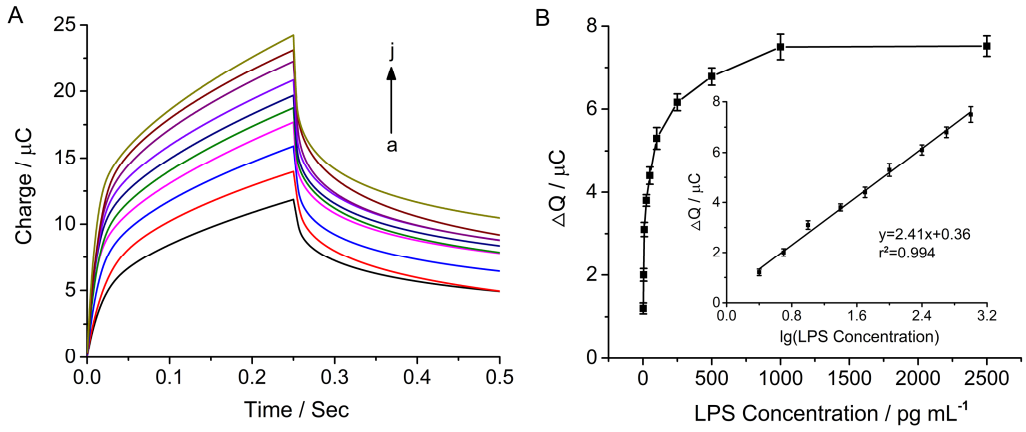


Fig. 4. (A) Chronocoulometry curves corresponding to different concentrations of target LPS, a ~ j (pg mL^{-1}): 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500. (B) Calibration curve based on variation of redox charge (△Q) versus LPS concentration, inset displays the linear relationship of charge variation versus logarithm of LPS concentration. Error bars show standard deviations of three independent measurements.

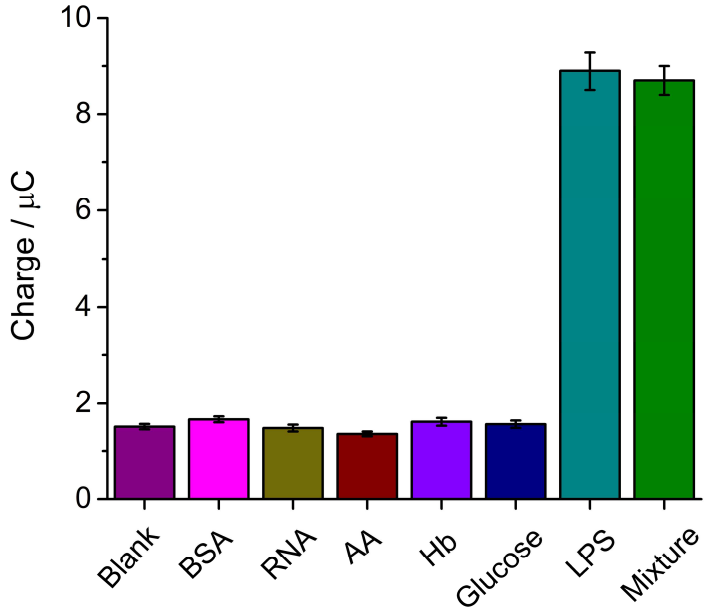


Fig. 5. Electrochemical response of the developed biosensor to interferents and target LPS to examine the detection specificity. Blank control: 10 mM PBS (pH 7.4). Concentrations of all the interferents and LPS are 1000 pg mL^{-1}. Error bars show standard deviations of three independent measurements.

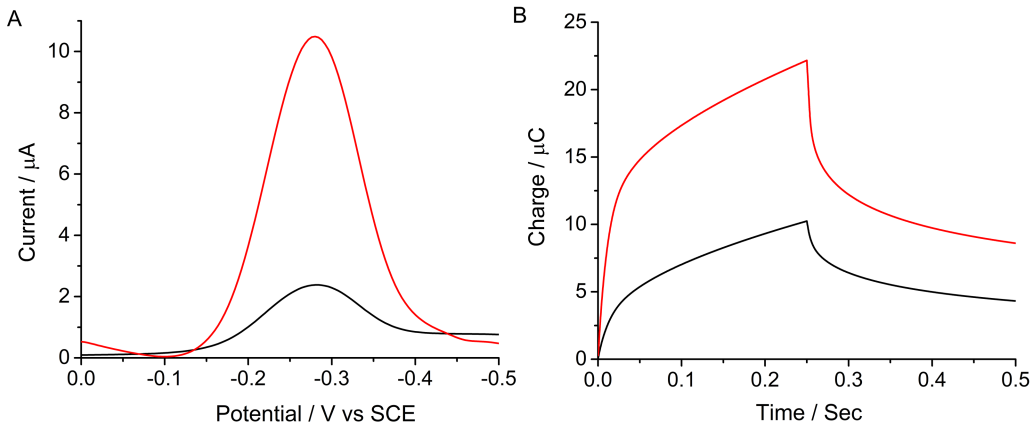
Table 1. Comparison between the developed biosensor with other reported sensors for LPS detection.

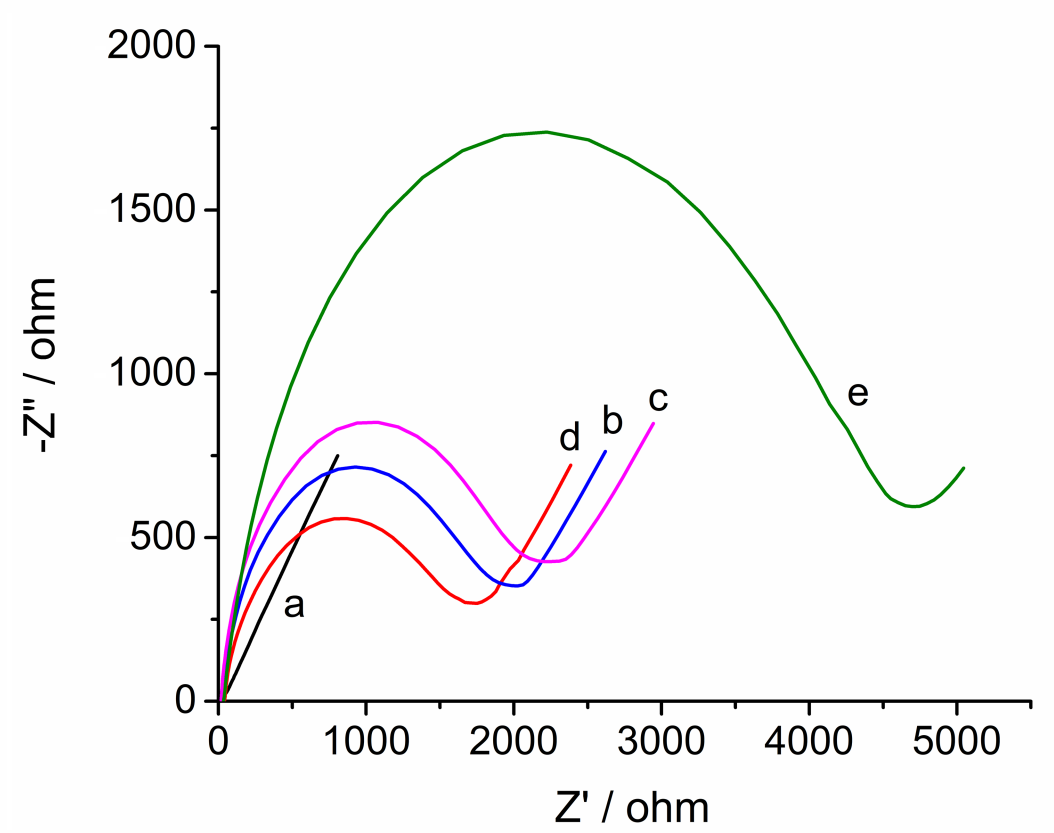
Sensor	Linear Range	LOD
Impedance biosensor [26]	0.001-1 ng mL^{-1}	1 pg mL^{-1}
Electrochemical biosensor [13]	0.01-1 ng mL^{-1}	10 pg mL^{-1}
Magnetic aptasensor [27]	0.01-100 ng mL^{-1}	3.6 pg mL^{-1}
Colorimetric aptasensor [28]	1-150 ng mL^{-1}	50 pg mL^{-1}
Fluorescent sensor [29]	0.10-1.50 µM	100 nM
The developed biosensor	2.5-1000 pg mL^{-1}	1 pg mL^{-1}

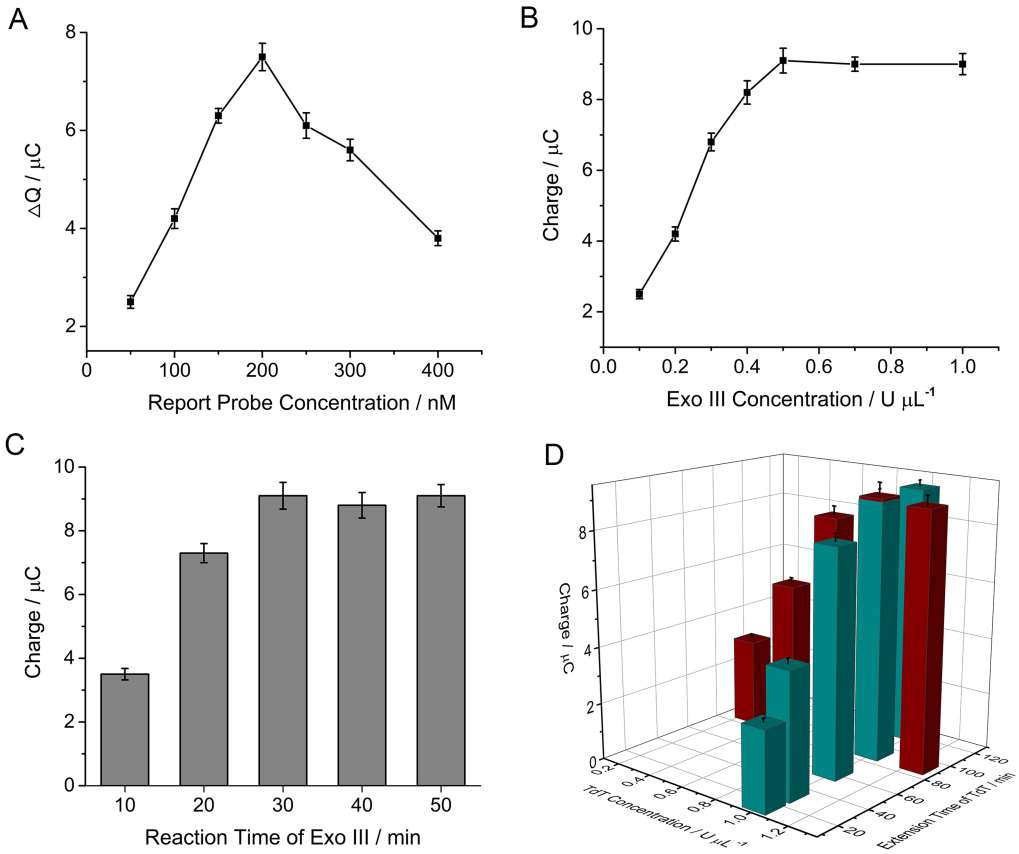
Table 2. Recoveries of the developed biosensor for LPS detection in real food samples.

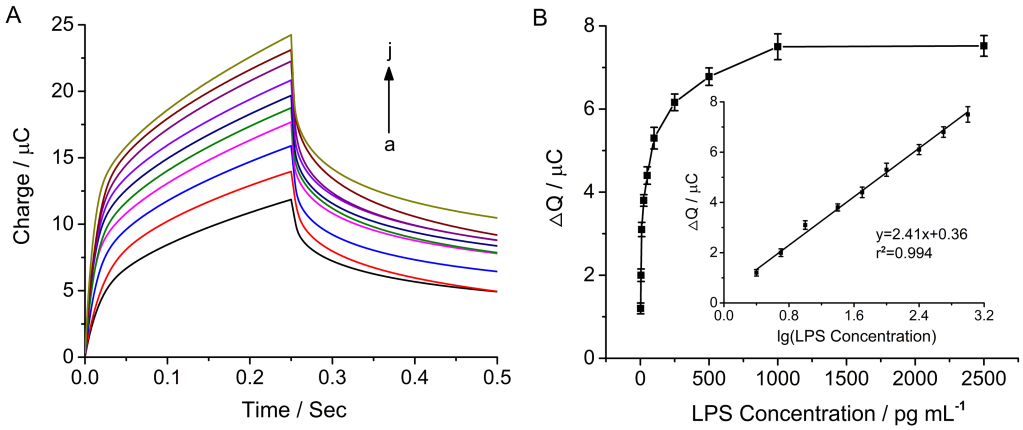
Real Sample	Added (pg mL^{-1})	Detected (pg mL^{-1})	Recovery (%)	Relative Error (%)
Drinking Water	5	5.6	112	5.2
	20	19.5	97.5	3.5
	200	186	93	5.5
	600	620	103	4.2
	900	826	91.8	4.9
Milk	5	4.7	94	6.3
	20	18.4	92	4.1
	200	181	90.5	4.7
	600	575	95.8	2.4
	900	981	109	3.3

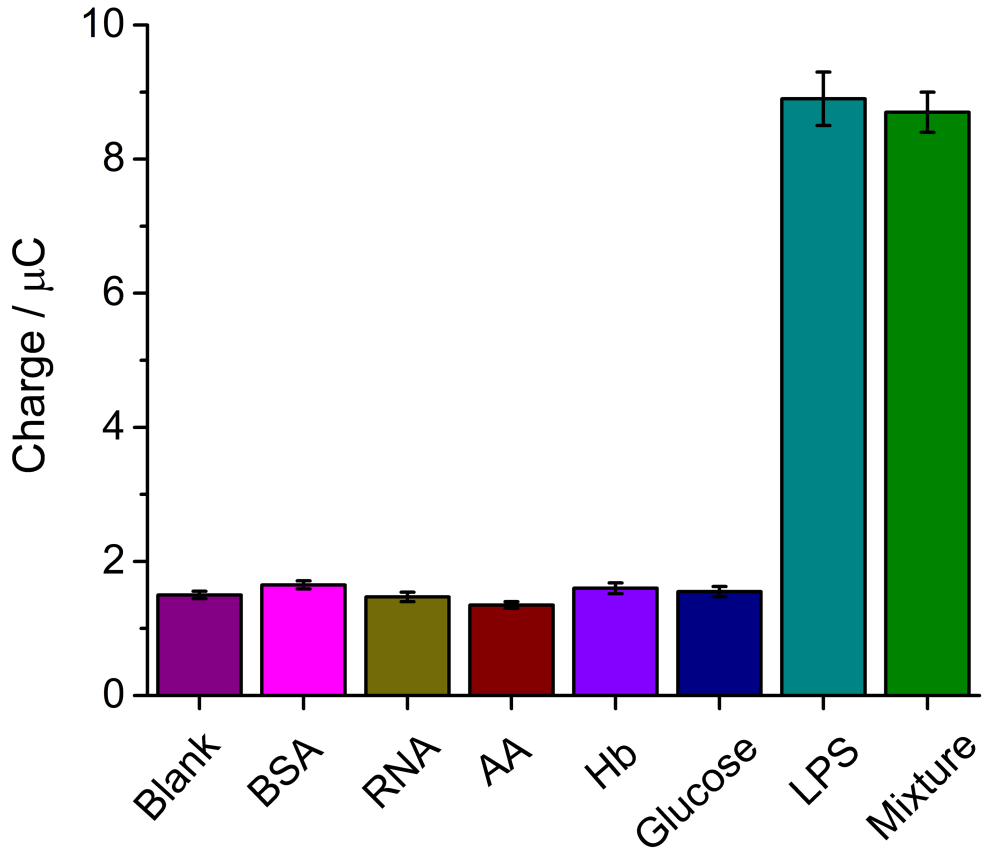
Independent experiments for the measurement of each concentration were performed in triplicate.

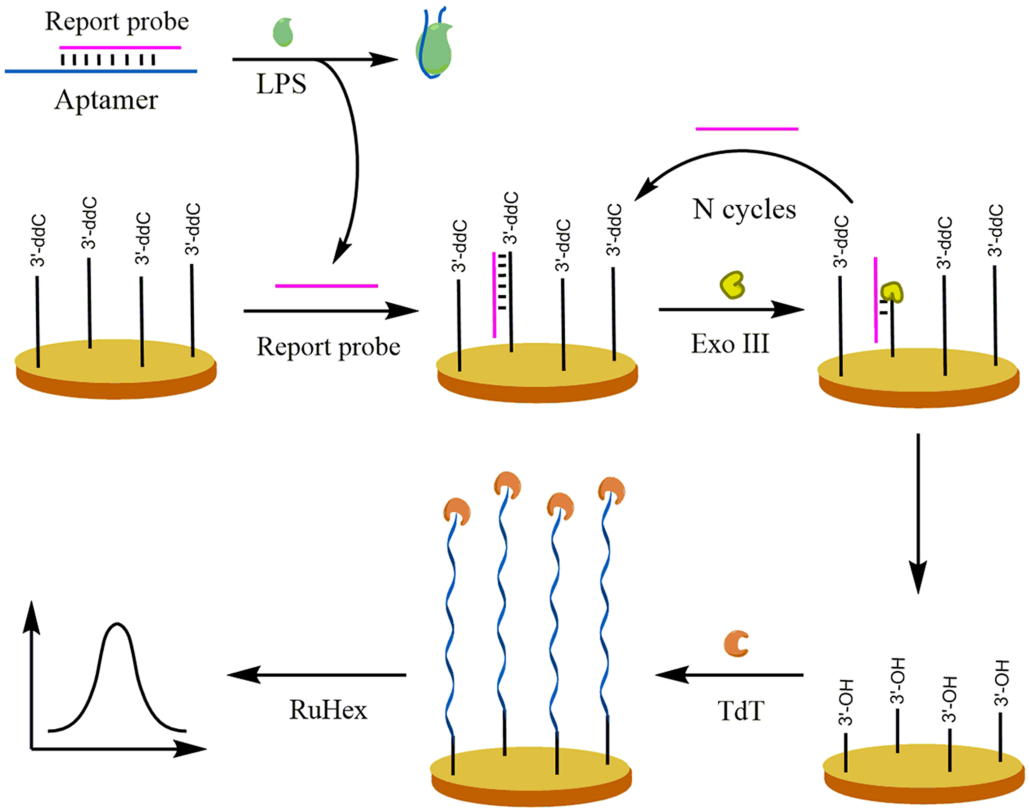



2000
1500
1000
500
0
-Z'' / ohm
0
1000
2000
3000
4000
5000
Z' / ohm
a
d
b
c
e


A
△Q / μC
Report Probe Concentration / nM
B
Charge / μC
Exo III Concentration / U μL-1
C
Charge / μC
Reaction Time of Exo III / min
D
Charge / μC
Tgt Concentration / U μL-1
Extension Time of Tgt / min







Highlights:

- A novel electrochemical biosensor is developed for LPS detection.
- The biosensor is based on collaborative amplification of dual enzymes.
- The biosensor shows high sensitivity, specificity and acceptable reproducibility.
- LPS is detected effectively in real food samples by the biosensor.

CRediT authorship contribution statement

Yue Huang: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Lei Wang:** Investigation, Validation. **Lingjun Sha:** Formal analysis, Validation. **Yaosong Wang:** Validation, Writing - review & editing. **Xuguo Duan:** Writing - review & editing. **Genxi Li:** Resources, Writing - review & editing, Supervision.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: