analytical chemistry

Article

A novel ultrasensitive electrochemical biosensor of bacterial 16S rRNA gene based on polyA DNA probes

Qian Wang, Yanli Wen, Yan Li, Wen Liang, Wen Li, Yuan Li, Jiahuan Wu, Huichen Zhu, Keke Zhao, Jun Zhang, Nengqin Jia, Wangping Deng, and Gang Liu

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.9b02175 • Publication Date (Web): 14 Jun 2019

A novel ultrasensitive electrochemical biosensor of bacterial 16S rRNA gene based on polyA DNA probes

Qian Wang†§, Yanli Wen†, Yan Li†, Wen Liang†, Wen Li†, Yuan Li†, Jiahuan Wu†, Huichen Zhu†, Keke Zhao†, Jun Zhang†, Nengqin Jia§*, Wangping Deng‡*, Gang Liu†*

† Laboratory of Biometrology, Shanghai Institute of Measurement and Testing Technology, 1500 Zhang Heng Road, Shanghai 201203, People's Republic of China

§Department of Chemistry, College of Chemistry and Materials Science, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, China

‡ National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, 207 Rui Jin Er Road, Shanghai 200025, China.

ABSTRACT: Traditional microbiology analysis is usually hindered by the long time-cost and lack of portability in many urgent situations. In this work, we developed a novel electrochemical DNA biosensor (E-biosensor) for sensitive analysis of 16S rRNA gene of five bacteria, using a consecutive adenine (PolyA) probe. The polyA probe consists of a polyA tail and a recognition part. The polyA tail can combine onto the gold surface with improved controllability of the surface density, by conveniently changing the length of polyA. The recognition part of the capture probe, together with 2 biotin-labeled reporter probes, hybridize with the target DNA and form a stable DNA-tetramer sandwich structure, and then avidin-HRP enzyme was added to produce a redox current signal for the following electrochemical detection. Finally, we realized sensitive quantification of artificial target DNA with a LOD of 10 fM, and excellent selectivity, reusability was also demonstrated. Importantly, the detection capability was equally good when facing bacterial genomic DNA, due to the base-stacking force of our multi-reporter-probe system which can help to break the second structure and stabilized the probe-target complexes. Our biosensor was constructed on a 16-channel electrode chip without any PCR process needed, which took a significant step toward a portable bacteria biosensor.

Keywords: *poly-A probe, sandwich, bacterial, electrochemical sensor, identified, detection, 16S rRNA*

Introduction

Accurate, prompt and specific analysis of microbial pathogens is critical for a number of fields including food safety, clinical diagnosis, environmental analysis, and biological antiterrorism. Traditional microbiology analysis mainly relies on sample cultivation in artificial media, to achieve microbiology isolation and identification. However, the long time-cost hinders its application in many urgent situations, and the instrumental analysis is usually cumbersome. Phenotypic biochemical characteristics based on a process of trial and error and matrix-assisted laser desorption/ionization. Time-of-flight mass spectrometry (MALDI-TOF) further improved the accuracy of microbiology analysis based on a constantly updated database, but still relies on intricate instruments.

Along with the development of molecular biological techniques[1-3], DNA analysis[4] has attracted a large amount of research interest for bacteria analysis, with many advantages including fast analysis, high accuracy, and sensitivity. 16S rRNA gene is a component of the small subunit of prokaryotic ribosomes, which was first used as a target to detect and classify microorganisms in 1986[5]. Currently, analysis of 16S rRNA sequences has been extensively used for the identification of bacteria. 16S rRNA showed especially irreplaceable application at the species level, especially when facing closely related bacterial species. Thus, the 16S rRNA gene has been recognized as a specific target for molecular biological detection methods of bacteria.

Electrochemical DNA biosensors sensors (E-biosensor)[6-8] could sensitively and selectively quantify target nucleic acid[9-16], using the DNA probes on the electrode, based on DNA recognition and hybridization. Compared with traditional detection methods, E-biosensor[17] has unique advantages for quantitative analysis including high sensitivity, low cost, and good portability[17-28]. Furthermore, electrochemical provides methods to monitor bio-interaction and bio-recognition, while PCR can only detect the target DNA after an exponential amplification, thus, electrochemical biosensor is widely recognized as a preferred method for the biological quantification in many fields including point-of-care test, wearable device, anti-terrorism etc[29].

It is well known that the performance of E-biosensor is highly affected by the construction of self-assembled monolayer (SAM) of DNA probes on the electrode surface. A large amount of research energy has been paid onto the study of well-organized DNA SAMs[30, 31], and so many novel assembling methods have been reported, including organic polymer[32, 33] and nanoelectrode[34]. Unfortunately, current E-biosensor development is still hindered by the lack of a highly controllable DNA probe assembling strategy. The very intricate and costly regulation of the DNA interface state, including conformation and density, seriously limited the detection sensitivity and repeatability.

In this work, we successfully developed an electrochemical biosensor chip for sensitive analysis of 16S rRNA gene of 5 different bacteria, by applying a consecutive adenine (polyA) probe[35, 36]. The polyA probes consisted of two fragments: a polyA tail and a recognition part. The polyA tail could covalently combine onto the gold electrode surface, and interestingly, the surface density of the DNA probes could be conveniently regulated by adjusting the length of the polyA tail. The recognition part is used to capture the target sequence through DNA-DNA hybridization. Critical experiment conditions for polyA probe assembling were systematically studied to achieve an optimized biosensor interface.

Multi-reporter-probe system has been studied in reported work[37], which enhanced the signal gain by introducing more signal groups, and more importantly, the hybridization efficiency is improved due to the DNA base-stacking force. In this work, 3 different DNA reporter systems were compared toward the best signal-to-noise ratio. Finally, sandwich-like DNA hybridization strategies were successfully constructed for both specifically and universally detection of 5 kinds of bacteria, and most importantly, the biosensor chip showed excellent practicability when analyzing a complex mixture of bacterial genomic DNA without any PCR process needed.

EXPERIMENTAL SECTION

Materials

Ethylenediaminetetraacetic acid (EDTA) and MCH were purchased from Sigma-Aldrich (St. Louis, MO). The 3, 3′, 5, 5′-tetramethylbenzidine (TMB) substrate was purchased from Neogen as a ready-to-use reagent (H_2O_2 included). The avidin-horseradish peroxidase (avidin-HRP) was purchased from eBioscience (San Diego, CA). The diluent buffer was purchased from Fitzgerald Industries International (Acton, MA). Staphylococcus aureus (St, S.aureus, ATCC 25923) was from China General Microbiological Culture Collection Center (CGMCC). All solutions were prepared in Milli-Q water (18 MΩ·cm resistivity) without further purification. All oligonucleotides were synthesized and purified by Invitrogen Inc.

In our work, we applied an electrochemical array chip (26 mm*77 mm) contains 16 groups of sensors (Fasteur Inc. China), and each group has a gold working electrode (about 2.3 mm in diameter), a gold reference electrode (small square) and a gold auxiliary electrode (circle).[33]

Design of oligonucleotide probes

Analysis probes consisted of capture probes (CP) and reporter probes (RP). RP was complementary to the target sequences with a biotin label at the 5′ end. CP had 2 parts: a capturing part that was complementary to the target and an assembling part that was 30 consecutive adenines (PolyA). The design of CP was mainly according to our former work[38]. The hybridization between the target DNA and the probes was shown in figure 1. All the gene sequence information was obtained from the Genebank database, and the sequence information of the target DNA and the probes were listed in table 1.

Table 1, Sequences of the targets and DNA probes applied in our experiment:

Oligonucleotides	Sequence (5′-3′)
St	GGGAAGAACATATGTTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTA ACTAC
St-CP	ACTTACACATATGTTCTTCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAA
St-RP-2.1	TGATTAGGTACCGTCAAGATGTGCACAGTT
St-RP-2.2	GTAGTTAGCCGTGGCTTTC
St-RP-1	TTGACACGTGTAGAACTGCCATGGATTAGTCTTTCGGTGCCGATTGATG
St-RP-3-1	TGATTAGGTACCGTCAA
St-RP-3-2	GATGTGCACAGTTGTAG
St-RP-3-3	TTAGCCGTGGCTTTC
En	GAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCA CGG CTA ACT AC
En-CP	ACTAACGTCCTTGTTCTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
En-RP-2.1	GGTTAGATACCGTCAGGGGACGTTCAGTT
En-RP-2.2	GTAGTTAGCCGTGGCTTTCT
Es	GGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAA GCACCGGCTAACTCC
Es-CP	TAACTTTACTCCCTTCCTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Es-RP-2.1	TCTGCGGGTAACGTCAATGAGCAAAGGTAT
Es-RP-2.2	GGAG TTA GCC GGT GCT TCT
Ps	GGA GGA AGG GCA GTA AGC TAA TAT CCT TGC GGT TTT GAC GTT ACC AAC AGA ATA AGC ACC GGC TAA CTT CGT
Ps-CP	AGC TTA CTG CCC TTC CTC C AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
Ps-RP-2.1	ATT CTG TTG GTA ACG TCA AAA CCG CAA GGA TAT T
Ps-RP-2.2	ACG AAG TTA GCC GGT GCT T
Ci	GGA GGA AGG TGT TGT GGT TAA TAA CCG CAG CAA TTG ACG TTA CTC GCA GAA GAA GCA CCG GCT AAC TCC GT
Ci-CP	TAACCACAACACCTTCCTCC AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
Ci-RP-2.1	CTTCTGCGAGTAACGTCAATTGCTGCGGTTAT
Ci-RP-2.2	ACGGAGTTAGCCGGTGCTT
Un	CCA CAC TGG AAC TGA GAC ACG GTC CAG ACT CCT ACG GGA GGC AGC AGT AGG GAA TCT TC
Un-CP	TGTCTCAGTTCCAGTGTGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Un-RP-2.1	TCCCGTAGGAGTCTGGACCG
Un-RP-2.2	GAAGATTCCCTACTGCTGCC

Artificial target DNA: St: Staphylococcus aureus, En: Enterococcus faecalis, Es: Escherichia coli, Ps: Pseudomonas aeruginosa, Ci: Citrobacter freundii, Un: Universal.

Preparation of the electrochemical Sensor

Multi-channel chip electrodes were rinsed with deionized water and dried with nitrogen, and then, the cleaned electrodes were incubated in immobilization buffer (pH 7.0, 10 mM Tris·HCl, 1 mM EDTA, 1 M NaCl) containing 0.1μM CP overnight under room temperature. Then, 5 μL of 0.1 mM MCH was added onto the electrode surface for 30 min under room temperature to construct the CP/MCH

SAM[38]. The prepared electrode was stored in TE buffer at 4 °C before DNA analysis.

Electrochemical Measurement

Step 1, The target DNA was mixed with the biotinylated RP (100 nM) in hybridization buffer (pH 7.4, 10 mM PB containing 1 mM NaCl and 20 mM $MgCl_2$), and the solution was heated to 80 °C for 5 min and then cooled down to room temperature for 20 min.

Step 2, The pre-hybridization solution (3 μL) was added onto the CP-covered working electrode and incubated under 40 °C for 4 h.

Step 3, The electrode was rinsed with washing buffer (pH 7.4, 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl), dried lightly with N_2, and then incubated with 3 μL of avidin-HRP (0.5 U/mL) at 37 °C for 15 min. Finally, the electrode was rinsed again with washing buffer and dried lightly with N_2.

Step 4, 35 μL of the TMB substrate solution (H_2O_2 included) was added onto the electrode. The electrochemical analysis was performed on a 16-channel electrochemical work station (LP-1501, Fasteur Inc. China) at room temperature. Cyclic voltammetry (CV) was performed between -200 to +370 mV. For I-t assay, the voltage (V) was -200 mV (vs gold reference), and the steady-state amperometric current signal (I) was measured at 60 s.

Bacterial genomic DNA extraction

Single S. aureus colony was picked and inoculated in the nutrient broth at 37 °C for 18 h~24 h. The inoculum was inoculated onto Baird-Parker medium at 37 °C to cultivate colonies. Then, ten colonies were randomly picked for Gram staining and microscopic identification as following: the bacteria are arranged in a spherical shape, without spores, without capsules, and the diameter was between 0.5 μm to 1 μm. Then, total DNA was extracted from 1.5 mL of bacterial broth culture and purified by using alkali wash and heat lysis methods.

Results and Discussion

Construction of the SAM on the 16-channel electrode array

In our work, the electrochemical analysis was performed on an electrode array that consisted of 16 groups of electrodes, and each group contained a working electrode, a reference electrode, and an auxiliary electrode. The electrode was fabricated by plating a thin gold layer on a hydrophobic plastic chip using vacuum evaporation. During the electrode preparation and analysis process, the chip was covered with a hydrophobic plastic sealing strip, and the strip had 16 pores at the position of the electrodes. With the assistance of the strip, we insulated the analysis system on each electrode between each other. Thus, this electrode array allows us to analysis multiple targets simultaneously.

As the first step of the biosensor fabrication, the working electrode was coated with a novel SAM consisted of polyA CP and MCH. The polyA CP has a capturing part and an assembling part: the capturing part was expected to hybridize with a DNA target and the assembling part (a polyA tail) was capable of combining onto the gold surface. Compared with traditional thiol-labeled DNA probe, the first advantage of polyA CP was "label-free", thus obviously decreased the economic cost for probe production, secondly, the surface density of the capture probe can be easily adjusted by changing the length of the polyA tail, due to space occupying effect. MCH was added to further decrease the surface density of the DNA probe on the electrode surface, and occupy the exposed electrode surface to prevent unspecific absorption.

The construction of the polyA-based SAM was characterized by using CV method in ferricyanide solution (0.5 mM $K_3Fe(CN)_6$ in 0.1 M KCl). A pair of clear redox peaks (at -110 mV VS gold reference electrode) of $[Fe(CN)_6]^{3-}$ was observed, and the consistency among the electrode array was very good. When the SAM was assembled, the current signal was distinctively suppressed (blue lines in Fig S1), suggesting the SAM was successfully constructed on the electrode surface with excellent repeatability. Obviously, with the densely packing SAM on the electrode surface, we achieved a very low background, which is critical for the detection performance.

Analysis strategy

In this work, we analyzed 6 DNA targets on the 16S rRNA genes: 5 species-specific targets were fragments from the variable regions of 5 bacteria (St: Staphylococcus aureus, En: Enterococcus faecalis, Es: Escherichia coli, Ps: Pseudomonas aeruginosa, Ci: Citrobacter freundii) and 1 universal target (Un) was from the conserved regions of the bacterial 16S rRNA gene.

We designed a polyA-tailed capture probe and two biotin-labeled reporter probes according to the target sequences (Fig. 1, top left), including the specific targets from the variable regions and the universal target from the conserved regions. The target and two reporter probes were firstly mixed together for a pre-hybridization step, and then, the hybridization production was added onto the polyA probe covered electrode. Thus, A "sandwich-like" structure was formed through subsequent hybridization. Then, the avidin-HRP was combined onto the reporter probes through the biotin-avidin reaction and produced a catalysis current signal in TMB-H_2O_2 substrate. The I-t analysis (Fig 1, bottom right) provided quantitative results, showing that the current signal at 100 s was 1051 nA for 1 nM target, 64 nA for the blank and 73 nA for 1 nM nontarget control.

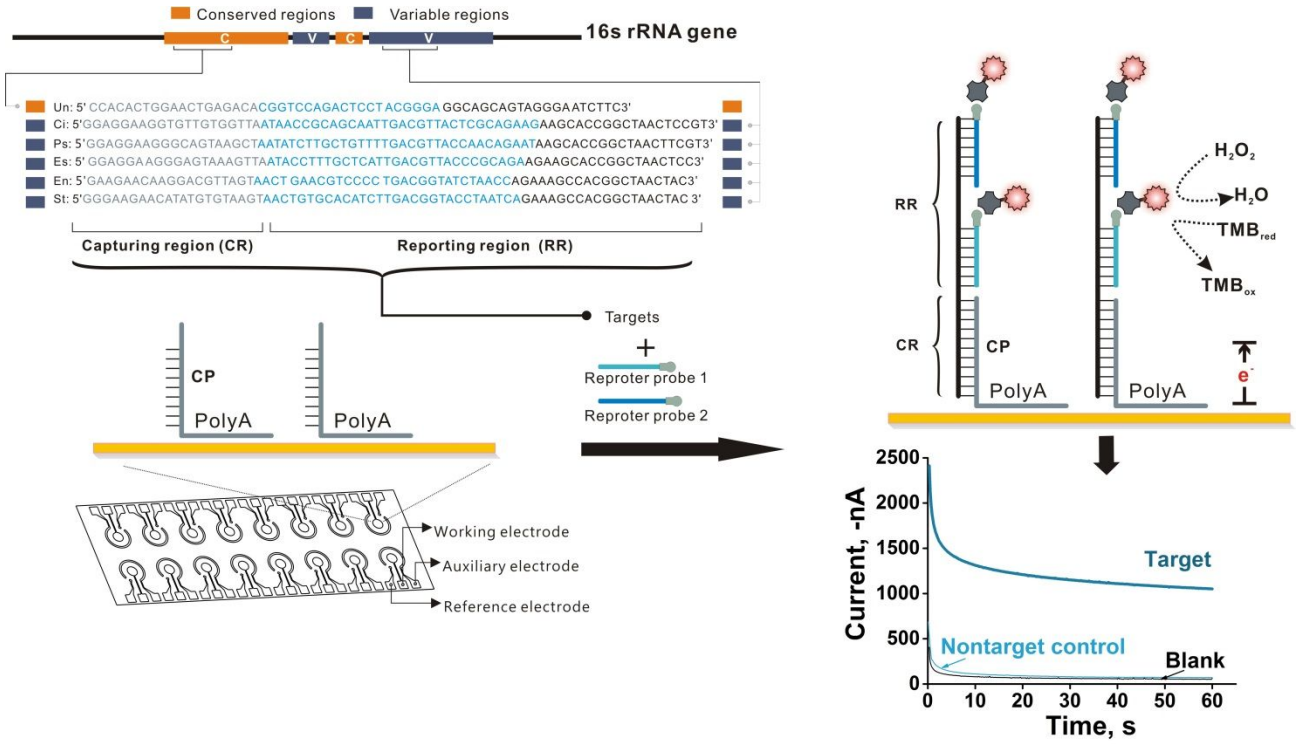

Figure 1. Schematic illustration of our 16S rRNA gene biosensor using a polyA capture probe. The reporter probes hybridized with different reporting regions (RR1 or RR2) of the targets, then, the polyA capture probe on electrode surface hybridized with the capturing region (CR) of the target. Up left: Target sequences from the 16S rRNA gene. The universal target was selected from conserved regions and the specific targets were from variable regions on 16S rRNA genes of 5 different bacteria. Bottom left: 16-channel electrode array. Bottom right: I-t curves of the analysis using St capture probe: target (10 nM St target DNA), nontarget control (10 nM En target DNA), Blank (Hybridization buffer)

Optimization and validation of the signal reporting system

Currently, traditional sandwich-like DNA biosensors are widely applied, but their sensitivity and specificity are usually limited, especially when analyzing long DNA targets or facing samples in a biological matrix. The application of a multi-probe system can help to open the secondary structure of long DNA targets and increase the stability of the hybridization complex due to the increased DNA base-stacking forces.

In this work, we systematically studied the multi-probe system. By dividing the reporting region of a target DNA into several parts. In this way, more reporter probes were combined, so that more avidin-HRP can be introduced to produce a higher current signal. We compared the performance of our biosensor when using one, two and three reporting probes on the same reporting region of St 16S rRNA gene. The signal-to-noise ratio (S/N) of our biosensor was shown in figure 2. When we utilized two reporter probes, the current signal increased while the blank signal decreased. Finally, the S/N was 4.4 which was 2.8 times higher than that of one reporter probe. On the contrary, when 3 shorter reporter probes were designed for the same reporting region, the S/N decreased to 0.9. Probably, when the reporting region was divided into 3 parts, the length of each reporting probe was too short for stable hybridization, thus the signal gain decreased to even lower than the 1-reporter-probe situation. On the other side, more signal probes increased the opportunity of unspecific adsorption, causing higher background.

In summary, using 2 reporter probes, we achieved the highest signal and the lowest blank signal. Thus, 2-reporter-probe (RP2) system was applied in the following experiments as an optimized condition.

Analysis performance

Critical conditions were optimized, including the concentration of the reporter probes (Fig S2), the temperature of hybridization (Fig S3), and temperature of enzyme reaction (Fig S4), and then, the analysis performance of our biosensor was verified including sensitivity, selectivity, reusability, and practicability.

5 artificial DNA targets corresponding to 5 bacteria were analyzed using our biosensor, and the results were shown in figure 3. As the I-t results showed (Fig3 A), when the concentration of the target DNA of St 16s rRNA gene increased from 10 fM to 1 nM (six orders of magnitude), the current signal increased gradually. The working curve (Fig3 B) for St analysis was constructed between the DNA concentration and the current signal, and the analysis below 1 pM was displayed as inset histograms. The nonlinear fitting result was: y = 1146 - 1070exp(-lgx/338) - 46exp(-lgx/0.07), where y is the current signal and the x is the concentration of the St 16S rRNA gene target. The LOD was calculated to be 5 fM, when $x = S_0 + 3\delta$ (S_0 = 28.75 nA is the background signal and δ = 2 nA is the SD of S_0).

Analysis of 4 other artificial DNA targets was also performed, corresponding to the 4 bacterial 16S rRNA genes: En (Fig3 C), Es (Fig3 D), Ps (Fig3 E) and Ci (Fig3 F). The similar analysis capability of different bacteria strongly demonstrated the universality of our biosensor, and there is a high possibility that the analysis targets can be further extended through simply modified the sequence of our CP and RPs.

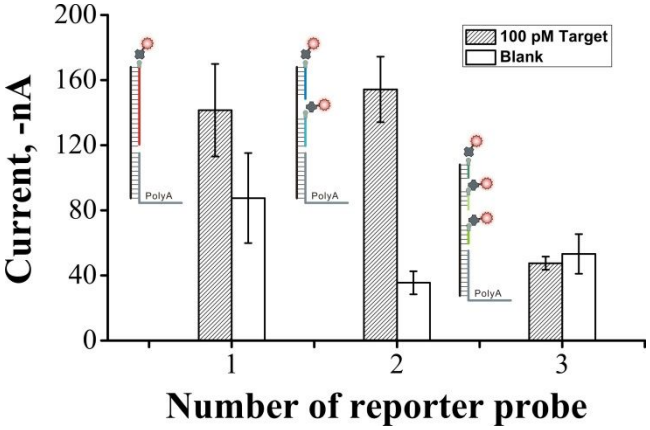

Figure 2. the analysis results of St using 1, 2 and 3 reporting probes, targeting the same reporting region of St 16S rRNA gene. The concentration of target DNA was 100 pM.

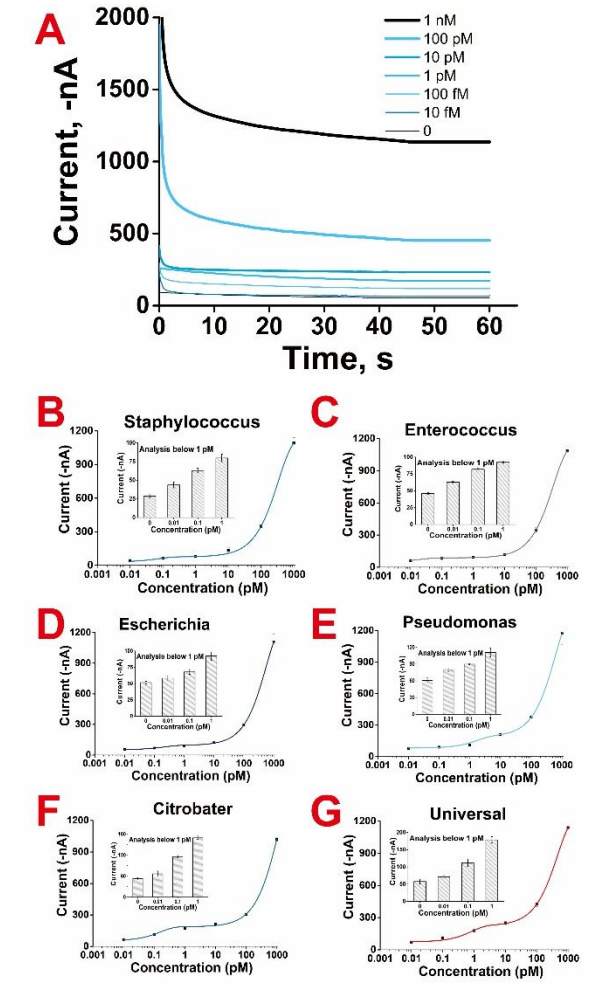

Figure 3. A) the I-t curves of the analysis of St DNA target, and the working curves of the analysis of 5 different bacteria DNA targets: B) Staphylococcus, C) Enterococcus, D) Escherichia, E) Pseudomonas, F) Citrobacter. G) Universal Inset: the detection results of low-concentration DNA. The error bars showed the standard deviations of at least three experiments. Hybridization buffer was analyzed as the blank signal.

To validate the selectivity of our system, we challenged our biosensor by analyzing different bacteria target DNA using different probes. Theoretically, only the specific DNA target can produce a high current signal. The results (figure 4) ideally matched the anticipation. For example, when we analyzed 5 different bacteria using the specific probe for Ci, the current signal of Ci target DNA was 1023, which was about 10 times higher than the average of other 4 bacteria and 19 times higher than the blank signal. The analysis using all other probes also successfully achieved excellent selectivity. These results demonstrated the capability of bacteria identification even when mixed with more than one interferent bacteria.

In this work, the stability of the polyA-MCH SAM was significantly improved. As a demonstration experiment, an electrode array was applied for the analysis for 10 nM St target and then treated by urea solution (8 M) for 30 min before applied into the I-t analysis in TMB solution. Interestingly, the current signal dramatically decreased to blank signal level, suggesting that the reporting probes were washed away clearly. More importantly, when the electrode array was applied again for 1 nM St target detection, the signal gain was recovered to the level close to a new electrode array, which demonstrated the analysis capability of the regenerated electrode. Our biosensor can be repeatedly used for at least 5 detection-washing cycles (Fig 5A). These results proved the ideal reusability of our polyA-based SAM, based on its excellent stability.

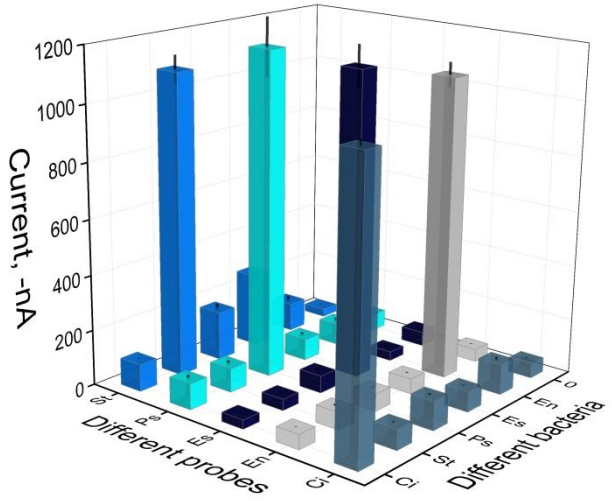

Figure 4. Analysis of different bacteria using different probes. The concentration of the bacteria DNA target was 1 nM.

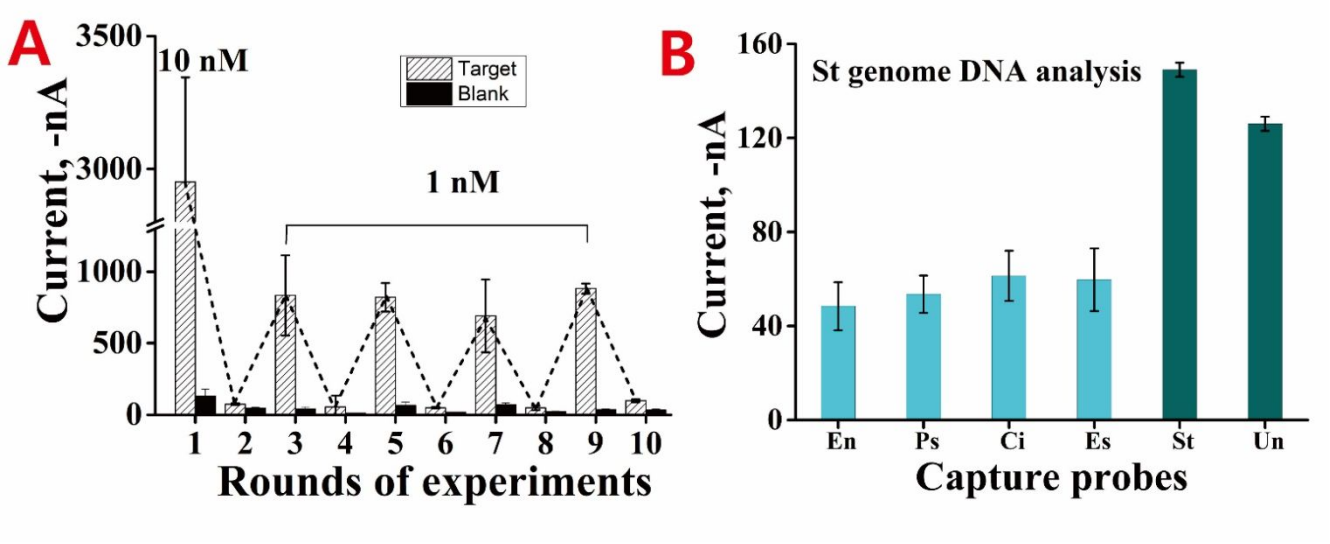

Figure 5. A) Results of reusability validation when analyzing St target. The concentration of the initial analysis was 10 nM, and the concentration for the following cycles was all 1 nM. Rounds 1, 3, 5, 7, 9 were the analysis of target, and Rounds 2, 4, 6, 8, 10 were results right after a urea washing step. B) Analysis of St genome DNA using different probes. The concentration of the genome DNA was evaluated to be 10 pM.

To challenge the practicability when facing real samples, we applied our biosensor to the analysis of genome DNA sample. In this section, Staphylococcus aureus was taken as a classic example. The concentration of St genome DNA was 10 pM, which was accurately quantified by digital PCR (Fig S5). Six different capture probes were applied to the analysis of the same St genome DNA. As anticipated, the biosensor with Un CP achieved a positive signal of -126 nA, and St CP generated a current signal of -149 nA that was more than 2 times higher than other CPs. More importantly, the signal gain was equally good to that of artificial DNA target of the same concentration (-135 nA for 10 pM St DNA) (Fig 5B), which strongly demonstrated the insusceptible detection capability of our biosensor in real samples.

Conclusions

Robustness, portability, and sensitivity are critical problems of electrochemical biosensor toward excellent practical application. Fortunately, our work made encouraging progress in two aspects: firstly, we developed a polyA-based SAM and dramatically improved the reusability of our electrochemical biosensor. The bacteria biosensor can be repeatedly used for at least 5 times with nearly constant detection performance, which is very outstanding among recent similar works (Table 2). Secondly, we developed a multi-probe system, and we compared the S/N of our biosensor when using one, two and three reporting probes on the same reporting region. Finally, 2 reporter probes were applied for enhanced signal reporting and higher hybridization efficiency due to DNA base-stacking forces. As a result, we achieved ultra-high sensitivity at about 10 fM, which is quite competitive among bacteria biosensors (Table 2). What is worth mentioning is that the detection capability was strongly demonstrated with equally good S/N and selectivity when facing bacterial genome DNA.

In summary, we constructed a 16-channel electrochemical biosensor, realizing sensitive quantification of five bacteria 16S rRNA gene. This work took an encouraging step toward a convenient and portable bacteria biosensor, and we believe the sensitivity can be further improved by using signal amplification strategies such as rolling circle amplification (RCA) and hybridization chain reaction (HCR).

Table 2. Comparison of similar electrochemical biosensors for pathogenic bacteria detection.

SAM	Renewability	Linearity range	Detection limit	Ref
SH-DNA probe on HRP functionalized Fe_3O_4 nanoparticles	Not reported	50 pM - 500 nM	7.1 pM	39
SH-DNA probe on gold electrode	Not reported	1 pM - 100 nM	1 pM	40
SH-DNA probe on gold nanoparticle-modified graphene oxide	Not reported	0.37 - 10 nM	160 pM	41
Amino-modified probe on silver-deposited gold nanoparticles	Not reported	1.0 - 70 pM	0.3 pM	42
SH-DNA probe on gold electrode	Not reported	1 pM - 10 nM	1 pM	43
Molecular beacon probe	Not reported	1- 40 nM	0.17 nM	44
Amino-modified probe	Not reported	2 - 16 nM	0.6 nM	45
Gold modified with LPA	Not reported	2-50 μM	5 μM	46
Gold modified with LPA	Not reported	0.1-2 μM	0.1 μM	47
11-mercaptoundecanol-modified gold electrode	Not reported	1-6 μM	44 pM	48
SH-DNA probe on gold electrode	Not reported	0.5-80 pM	0.12 pM	49
GCE modified with multiwall carbon nanotubes-chitosan-bismuth	Not reported	/	31.7 fM	50
SH-DNA probe on gold electrode	Not reported	10 pM-100 nM	10 pM	7
Label-free DNA probe on gold electrode	5 times	10 fM - 1 nM	10 fM	Our work

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Characterization of the polyA-based SAM on electrode array (Figure S1)

Optimization of reporter probe concentration. (Figure S2)

Optimization of the hybridization temperature (Figure S3)

The study of the reaction temperature between avidin-HRP and the reporter probes (Figure S4)

Digital PCR results of the St genome DNA quantification (Figure S5)

ACKNOWLEDGMENTS

This work was financially supported by the National Quality Infrastructure Program of China (2018YFF0212800), National Natural Science Foundation of China (No. 21775104, 21373138, 21605026, 81672720).

AUTHOR INFORMATION

Corresponding Authors:

*E-mail: liug@simt.com.cn

*E-mail: dengwp@nipd.chinacdc.cn

*E-mail: nqjia@shnu.edu.cn

Notes

The authors declare no competing financial interest.

REFERENCES:

1. Sun, C. P.; Liao, J. C.; Zhang, Y. H.; Gau, V.; Mastali, M.; Babbitt, J. T.; Grundfest, W. S.; Churchill, B. M.; McCabe, E. R.; Haake, D. A., Rapid, species-specific detection of uropathogen 16S rDNA and rRNA at ambient temperature by dot-blot hybridization and an electrochemical sensor array. *Mol. Genet. Metab.* **2005,** *84* (1), 90-99.
2. Zhu, D.; Pei, H.; Yao, G.; Wang, L.; Su, S.; Chao, J.; Wang, L.; Aldalbahi, A.; Song, S.; Shi, J.; Hu, J.; Fan, C.; Zuo, X., A Surface-Confined Proton-Driven DNA Pump Using a Dynamic 3D DNA Scaffold. *Adv. Mater.* **2016,** *28* (32), 6860-6865.
3. Chao, J.; Zhu, D.; Zhang, Y.; Wang, L.; Fan, C., DNA nanotechnology-enabled biosensors. *Biosens. bioelectron.* **2016,** *76*, 68-79.
4. Wang, X.; Hou, T.; Lu, T.; Li, F., Autonomous exonuclease III-assisted isothermal cycling signal amplification: a facile and highly sensitive fluorescence DNA glycosylase activity assay. *Anal. Chem.* **2014,** *86* (19), 9626-9631.
5. Kohne, D.; Hogan, J.; Jonas, V.; Dean, E.; Adams, T., Novel approach for rapid and sensitive detection of microorganisms: DNA probes to rRNA. In *Microbiology*, American Society of Microbiology, Washington, DC: 1986; pp 110-112.
6. Zeng, D.; Wang, Z.; Meng, Z.; Wang, P.; San, L.; Wang, W.; Aldalbahi, A.; Li, L.; Shen, J.; Mi, X., DNA Tetrahedral Nanostructure-Based Electrochemical miRNA Biosensor for Simultaneous Detection of Multiple miRNAs in Pancreatic Carcinoma. *ACS Appl. Mater. Interfaces* **2017,** *9* (28), 24118-24125.
7. Yang, C.; Shi, K.; Dou, B.; Xiang, Y.; Chai, Y.; Yuan, R., In situ DNA-templated synthesis of silver nanoclusters for ultrasensitive and label-free electrochemical detection of microRNA. *ACS Appl. Mater. Interfaces* **2015,** *7* (2), 1188-1193.
8. Dong, S.; Zhao, R.; Zhu, J.; Lu, X.; Li, Y.; Qiu, S.; Jia, L.; Jiao, X.; Song, S.; Fan, C.; Hao, R.; Song, H., Electrochemical DNA Biosensor Based on a Tetrahedral Nanostructure Probe for the Detection of Avian Influenza A (H7N9) Virus. *ACS Appl. Mater. Interfaces* **2015,** *7* (16), 8834-8842.
9. Pei, H.; Li, F.; Wan, Y.; Wei, M.; Liu, H.; Su, Y.; Chen, N.; Huang, Q.; Fan, C., Designed diblock oligonucleotide for the synthesis of spatially isolated and highly hybridizable functionalization of DNA-gold nanoparticle nanoconjugates. *J. Am. Chem. Soc.* **2012,** *134* (29), 11876-11879.
10. Lapierre, M. A.; O'Keefe, M.; Taft, B. J.; Kelley, S. O., Electrocatalytic detection of pathogenic DNA sequences and antibiotic resistance markers. *Anal. Chem.* **2003,** *75* (22), 6327-6333.
11. Yang, F.; Zuo, X.; Li, Z.; Deng, W.; Shi, J.; Zhang, G.; Huang, Q.; Song, S.; Fan, C., A bubble-mediated intelligent microscale electrochemical device for single-step quantitative bioassays. *Adv. Mater.* **2014,** *26* (27), 4671-4676.
12. Ge, Z.; Pei, H.; Wang, L.; Song, S.; Fan, C., Electrochemical single nucleotide polymorphisms genotyping on surface immobilized three-dimensional branched DNA nanostructure. *Sci. China: Chem.* **2011,** *54* (8), 1273-1276.
13. Wan, Y.; Wang, P.; Su, Y.; Wang, L.; Pan, D.; Aldalbahi, A.; Yang, S.; Zuo, X., Nanoprobe-Initiated Enzymatic Polymerization for Highly Sensitive Electrochemical DNA Detection. *ACS Appl. Mater. Interfaces* **2015,** *7* (46), 25618-25623.
14. Zhang, J.; Wang, L. H.; Pan, D.; Song, S. P.; Fan, C. H., DNA hybridization "turns on" electrocatalysis at gold electrodes. *Chem. Commun.* **2007,** (11), 1154-1156.
15. Choi, J. H.; Kim, H.; Choi, J. H.; Choi, J. W.; Oh, B. K., Signal enhancement of silicon nanowire-based biosensor for detection of matrix metalloproteinase-2 using DNA-Au nanoparticle complexes. *ACS Appl. Mater. Interfaces* **2013,** *5* (22), 12023-12028.
16. Su, S.; Zuo, X.; Pan, D.; Pei, H.; Wang, L.; Fan, C.; Huang, W., Design and applications of gold nanoparticle conjugates by exploiting biomolecule-gold nanoparticle interactions. *Nanoscale* **2013,** *5* (7), 2589-2599.
17. Wen, Y. L.; Pei, H.; Wan, Y.; Su, Y.; Huang, Q.; Song, S. P.; Fan, C. H., DNA Nanostructure-Decorated Surfaces for Enhanced Aptamer-Target Binding and Electrochemical Cocaine Sensors. *Anal. Chem.* **2011,** *83* (19), 7418-7423.
18. Fan, C. H.; Plaxco, K. W.; Heeger, A. J., Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** *100* (16), 9134-9137.
19. Lin, M. H.; Wen, Y. L.; Li, L. Y.; Pei, H.; Liu, G.; Song, H. Y.; Zuo, X. L.; Fan, C. H.; Huang, Q., Target-Responsive, DNA Nanostructure-Based E-DNA Sensor for microRNA Analysis. *Anal. Chem.* **2014,** *86* (5), 2285-2288.
20. Liu, C.; Zeng, G. M.; Tang, L.; Zhang, Y.; Li, Y. P.; Liu, Y. Y.; Li, Z.; Wu, M. S.; Luo, J., Electrochemical detection of Pseudomonas aeruginosa 16S rRNA using a biosensor based on immobilized stem-loop structured probe. *Enzyme Microb. Technol.* **2011,** *49* (3), 266-271.
21. Liu, G.; Lao, R. J.; Xu, L.; Xu, Q.; Li, L. Y.; Zhang, M.; Song, S. P.; Fan, C. H., Single-nucleotide polymorphism genotyping using a novel multiplexed electrochemical biosensor with nonfouling surface. *Biosens. bioelectron.* **2013,** *42*, 516-521.
22. Ricci, F.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Sumner, J. J., Effect of molecular crowding on the response of an electrochemical DNA sensor. *Langmuir* **2007,** *23* (12), 6827-6834.
23. Su, S.; Cao, W. F.; Liu, W.; Lu, Z. W.; Zhu, D.; Chao, J.; Weng, L. X.; Wang, L. H.; Fan, C. H.; Wang, L. H., Dual-mode electrochemical analysis of microRNA-21 using gold nanoparticle-decorated MoS2 nanosheet. *Biosens. bioelectron.* **2017,** *94*, 552-559.
24. Su, S.; Sun, H. F.; Cao, W. F.; Chao, J.; Peng, H. Z.; Zuo, X. L.; Yuwen, L. H.; Fan, C. H.; Wang, L. H., Dual-Target Electrochemical Biosensing Based on DNA Structural Switching on Gold Nanoparticle-Decorated MoS2 Nanosheets. *ACS Appl. Mater. Interfaces* **2016,** *8* (11), 6826-6833.
25. Wen, Y. L.; Liu, G.; Pei, H.; Li, L. Y.; Xu, Q.; Liang, W.; Li, Y.; Xu, L.; Ren, S. Z.; Fan, C. H., DNA nanostructure-based ultrasensitive electrochemical microRNA biosensor. *Methods* **2013,** *64* (3), 276-282.
26. Wen, Y. L.; Wang, L. L.; Xu, L.; Li, L. Y.; Ren, S. Z.; Cao, C. M.; Jia, N. Q.; Aldalbahi, A.; Song, S. P.; Shi, J. Y.; Xia, J. Y.; Liu, G.; Zuo, X. L., Electrochemical detection of PCR amplicons of Escherichia coli genome based on DNA nanostructural probes and polyHRP enzyme. *Analyst* **2016,** *141* (18), 5304-5310.
27. Shi, X. M.; Fan, G. C.; Shen, Q.; Zhu, J. J., Photoelectrochemical DNA Biosensor Based on Dual-Signal Amplification Strategy Integrating Inorganic-Organic Nanocomposites Sensitization with lambda-Exonuclease-Assisted Target Recycling. *ACS Appl. Mater. Interfaces* **2016,** *8* (51), 35091-35098.
28. Kou, B. B.; Zhang, L.; Xie, H.; Wang, D.; Yuan, Y. L.; Chai, Y. Q.; Yuan, R., DNA Enzyme-Decorated DNA Nanoladders as Enhancer for Peptide Cleavage-Based Electrochemical Biosensor. *ACS Appl. Mater. Interfaces* **2016,** *8* (35), 22869-22874.
29. Drummond, T. G.; Hill, M. G.; Barton, J. K., Electrochemical DNA sensors. *Nat. Biotechnol.* **2003,** *21* (10), 1192-1199.
30. Chen, P.; Pan, D.; Fan, C.; Chen, J.; Huang, K.; Wang, D.; Zhang, H.; Li, Y.; Feng, G.; Liang, P.; He, L.; Shi, Y., Gold nanoparticles for high-throughput genotyping of long-range haplotypes. *Nat. Nanotechnol.* **2011,** *6* (10), 639-644.
31. Opdahl, A.; Petrovykh, D. Y.; Kimura-Suda, H.; Tarlov, M. J.; Whitman, L. J., Independent control of grafting density and conformation of single-stranded DNA brushes. *Proc. Natl. Acad. Sci. U. S. A.* **2007,** *104* (1), 9-14.
32. Gui, A. L.; Luais, E.; Peterson, J. R.; Gooding, J. J., Zwitterionic phenyl layers: finally, stable, anti-biofouling coatings that do not passivate electrodes. *ACS Appl. Mater. Interfaces* **2013,** *5* (11), 4827-4835.
33. Liu, G.; Wan, Y.; Gau, V.; Zhang, J.; Wang, L. H.; Song, S. P.; Fan, C. H., An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets. *J. Am. Chem. Soc.* **2008,** *130* (21), 6820-6825.
34. Das, J.; Ivanov, I.; Sargent, E. H.; Kelley, S. O., DNA Clutch Probes for Circulating Tumor DNA Analysis. *J. Am. Chem. Soc.* **2016,** *138* (34), 11009-11016.
35. Zhu, D.; Zhao, D.; Huang, J.; Zhu, Y.; Chao, J.; Su, S.; Li, J.; Wang, L.; Shi, J.; Zuo, X.; Weng, L.; Li, Q.; Wang, L., Poly-adenine-mediated fluorescent spherical nucleic acid probes for live-cell imaging of endogenous tumor-related mRNA. *Nanomedicine:* **2018,** *14* (6), 1797-1807.
36. Zhu, D.; Song, P.; Shen, J.; Su, S.; Chao, J.; Aldalbahi, A.; Zhou, Z.; Song, S.; Fan, C.; Zuo, X.; Tian, Y.; Wang, L.; Pei, H., PolyA-Mediated DNA Assembly on Gold Nanoparticles for

Thermodynamically Favorable and Rapid Hybridization Analysis. *Anal. Chem.* **2016,** *88* (9), 4949-4954.
37. Xu, L.; Liang, W.; Wen, Y.; Wang, L.; Yang, X.; Ren, S.; Jia, N.; Zuo, X.; Liu, G., An ultrasensitive electrochemical biosensor for the detection of mecA gene in methicillin-resistant Staphylococcus aureus. *Biosens. Bioelectron.* **2018,** *99*, 424-430.
38. Li, L. Y.; Wang, L. L.; Xu, Q.; Xu, L.; Liang, W.; Li, Y.; Ding, M.; Aldalbahi, A.; Ge, Z. L.; Wang, L. H.; Yan, J.; Lu, N.; Li, J.; Wen, Y. L.; Liu, G., Bacterial Analysis Using an Electrochemical DNA Biosensor with Poly-Adenine-Mediated DNA Self-Assembly. *ACS Appl. Mater. Interfaces* **2018,** *10* (8), 6895-6903.
39. Dong, X. Y.; Mi, X. N.; Wang, B.; Xu, J. J.; Chen, H. Y., Signal amplification for DNA detection based on the HRP-functionalized Fe3O4 nanoparticles. *Talanta* **2011,** *84* (2), 531-537.
40. Liu, J.; Tian, M.; Liang, Z., DNA analysis based on the electrocatalytic amplification of gold nanoparticles. *Electrochim. Acta* **2013,** *113*, 186-193.
41. Saeed, A. A.; Sanchez, J. L. A.; O'Sullivan, C. K.; Abbas, M. N., DNA biosensors based on gold nanoparticles-modified graphene oxide for the detection of breast cancer biomarkers for early diagnosis. *Bioelectrochemistry* **2017,** *118*, 91-99.
42. Zhang, Y.; Geng, X.; Ai, J.; Gao, Q.; Qi, H.; Zhang, C., Signal amplification detection of DNA using a sensor fabricated by one-step covalent immobilization of amino-terminated probe DNA onto the polydopamine-modified screen-printed carbon electrode. *Sens. Actuators, B* **2015,** *221*, 1535-1541.
43. Zhang, J.; Lao, R.; Song, S.; Yan, Z.; Fan, C., Design of an Oligonucleotide-Incorporated Nonfouling Surface and Its Application in Electrochemical DNA Sensors for Highly Sensitive and Sequence-Specific Detection of Target DNA. *Anal. Chem.* **2008,** *80* (23), 9029-9033.
44. Gerasimova, Y. V.; Kolpashchikov, D. M., Detection of bacterial 16S rRNA using a molecular beacon-based X sensor. *Biosens. bioelectron.* **2013,** *41*, 386-390.
45. Spehar-Deleze, A. M.; Gransee, R.; Martinez-Montequin, S.; Bejarano-Nosas, D.; Dulay, S.; Julich, S.; Tomaso, H.; O'Sullivan, C. K., Electrochemiluminescence DNA sensor array for multiplex detection of biowarfare agents. *Anal. Bioanal. Chem.* **2015,** *407* (22), 6657-6667.
46. She, Z.; Topping, K.; Ma, T.; Zhao, T.; Zhou, W.; Kamal, A.; Ahmadi, S.; Kraatz, H.-B., Detection of the Lipopeptide Pam3CSK4 Using a Hybridized Toll-like Receptor Electrochemical Sensor. *Anal. Chem.* **2017,** *89* (9), 4882-4888.
47. She, Z.; Topping, K.; Shamsi, M. H.; Wang, N.; Chan, N. W. C.; Kraatz, H.-B., Investigation of the Utility of Complementary Electrochemical Detection Techniques to Examine the in Vitro Affinity of Bacterial Flagellins for a Toll-Like Receptor 5 Biosensor. *Anal. Chem.* **2015,** *87* (8), 4218-4224.
48. Kalimuthu, P.; Tkac, J.; Kappler, U.; Davis, J. J.; Bernhardt, P. V., Highly Sensitive and Stable Electrochemical Sulfite Biosensor Incorporating a Bacterial Sulfite Dehydrogenase. *Anal. Chem.* **2010,** *82* (17), 7374-7379.
49. Xiong, E.; Li, Z.; Zhang, X.; Zhou, J.; Yan, X.; Liu, Y.; Chen, J., Triple-Helix Molecular Switch Electrochemical Ratiometric Biosensor for Ultrasensitive Detection of Nucleic Acids. *Anal. Chem.* **2017,** *89* (17), 8830-8835.
50. Abdalhai, M. H.; Maximiano Fernandes, A. n.; Bashari, M.; Ji, J.; He, Q.; Sun, X., Rapid and sensitive detection of foodborne pathogenic bacteria (staphylococcus aureus) using an electrochemical DNA genomic biosensor and its application in fresh beef. *J. Agric. Food Chem.* **2014,** *62* (52), 12659-12667.

For Table of Contents Only