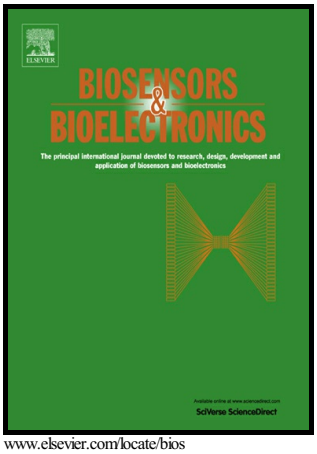


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# An ultrasensitive electrochemical biosensor for the detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus*

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## Abstract:

Electrochemical DNA biosensor has unique advantages for on-site pathogenic microorganism detection, yet the detection of long DNA towards genome DNA (gDNA) analysis remains challenge. In this work, we report a novel electrochemical biosensor for the ultrasensitive analysis of *mecA* DNA on methicillin-resistant *Staphylococcus aureus* (MRSA) genome, using a multi-signal probes (MSP) system. The MSP consists of 7 biotin-labelled signal probes that will combine to the target DNA in a prehybridization step, and then the complex will be captured by a DNA tetrahedron structure probe (TSP) on the electrode surface. Then, after the introduction of the strepavidin-labelled HRP enzyme, a catalysis current signal is detected that is found to be corresponding to the concentration of the target DNA. MSP in this work plays a critical role not only for the signal amplification through bringing 7 biotins, but also dramatically improves the accessibility of the target sequence embedded in the double-strand DNA molecules and complex second structures. The 3-D DNA TSP here provides steady support and optimized surface density for the very "large" complex of MSP system and gDNA, as a base of the capture probe. Finally, as low as 10 fM synthetic target DNA was successfully detected, which is at least 3 magnitudes lower than that using single signal probe. Most importantly, we demonstrated the practicability of our analysis method by analyzing a 57 fM MRSA gDNA sample showing excellent selectivity, and the reliability of the analysis was also demonstrated by digital PCR.

**Keywords:** *mecA*, methicillin-resistant *Staphylococcus aureus* (MRSA), electrochemical DNA biosensor, multi-signal probe (MSP), DNA tetrahedron structure probe (TSP), digital PCR

## 1. Introduction:

Along with many variations of resistance patterns spread with remarkable speed in worldwide, antimicrobial resistance is becoming a great threat for human health, leading to treatment difficulty, increased mortality and higher cost. Methicillin-resistant *Staphylococcus aureus* (MRSA)(Brumfitt and Hamiltonmiller 1989) is one of the most famous pathogens in public healthcare, which has resistance not only to methicillin but also to all other antibiotics, including the β-lactams. MRSA is widely reported to be related with many serious health problems, including food poisoning, skin infection and lung inflammation(Rossi 2014; Stefani et al. 2012).

Traditional culture methods(Bessede et al. 2011; Conza et al. 2013) based on the growth of microorganism on plates and colony counting by instruments or naked eyes, have advantages in sensitivity but require long period for adequate growth. In order to perform fast and convenient MRSA detection, various analysis strategies were developed. Molecular biological methods such as PCR, microarray(Zhou et al. 2011) and next generation sequencing (NGS)(Kuroda et al. 2001) have advanced sensitivity and specificity for microbiology detection, however, the requirement of long operation time, laborious sample treatment and complex instruments, is still hindering their application for on-site test. To monitor MRSA contamination and prevent MRSA outbreak, there is a pressing demand for rapid and convenient detection methods,

towards on-site microbiological analysis, with adequate sensitivity and specificity.

Electrochemical biosensor(Liu et al. 2008; Wen et al. 2013) has shown great promise for DNA hybridization analysis(Fan et al. 2003), with many excellent features such as time and economy saving, multiple-target detection(Rossi 2014) and operational convenience etc., however, their detection capability is usually limited when facing longer DNA or untreated genome DNA (gDNA) samples before enzyme digestion or PCR amplification. Several challenges need to be overcome before direct analysis of gDNA with larger molecular length(Ganta et al. 2008) and complex second structures: Firstly, it is difficult for the DNA probes to approach and hybridize with the target sequence in the double-stranded gDNA, especially when the reaction kinetics is quite limited on the confined interface of the electrochemical biosensor. Secondly, the extracted genome samples usually contain unspecific DNA fragments, which will bring higher blank signal due to the unspecific surface adsorption.

Aiming at both higher hybridization efficiency and signal amplification for MRSA gDNA detection, in this work, we developed a multi-signal probes (MSP) strategy, using 7 signal probes to hybridize onto the target DNA, along the target DNA strands one next to another. Firstly, multiple probes would hybridize to the target sequence and help to open the second structures and greatly improve the accessibility of the target DNA sequence, which is very important for the target recognition and combination by the DNA capture probe on the electrode surface. Secondly, the application of multiple signal probes would bring us effective signal amplification, through introducing more biotin tags. On the other side, 7 signal probes

can bring more signal labels, and thus, produce higher current signal.

For classic electrochemical biosensors, the hybridization efficiency and interfacial support was usually a critical challenge, especially when facing long target DNA and more signal probes. However, like many other signal amplification strategies such as rolling circle amplification (RCA)(Yan et al. 2014; Zhao et al. 2012), nanomaterials(Song et al. 2010; Xu et al. 2014) and polymerized enzyme(Bessede et al. 2011), our MSP system, together with the target gDNA and the signal enzyme, has a very large molecular dimension, which require a better improved interfacial engineer on the electrode surface. Thus, the construction of the self-assembly monolayer (SAM) of the capture probes plays a key role of our biosensor, to provide steady support and optimized surface density for the following hybridization system, which consists of 7 signal probes and a long target DNA or even a whole gDNA.

Recently, the development of three-dimensional DNA architectures(Li et al. 2015; Li et al. 2014; Lin et al. 2014) has attracted tremendous research interest because of their unique advantages including mechanical rigidity and excellent capability of modulating the surface density of the DNA capture probes(Pei et al. 2010). In this work, a 3-D TSP with 3 thiol groups and 1 capture probe was designed through the self-assembly of 4 single-strand DNA (ssDNA). As reported in our previous work(Wen et al. 2013), the DNA nanostructure could effectively modulate the surface density, and thus controlled the distance between the probes, avoiding surface crowding and improving the hybridization efficiency. On the other side, TSP provided rigid support for the target gDNA and the pre-hybridized signal probes. Finally, we

developed a signal amplification system using 7 signal probes, and an ultra-high sensitivity of 10 fM DNA was achieved. The practicability of our biosensor was demonstrated by analyzing a MRSA gDNA sample, which is accurately quantified by digital PCR(Baker 2012; Liang et al. 2016; Vogelstein and Kinzler 1999) as a validation.

## 2. **Experimental**

### *2.1 Materials.*

A 130 nt ssDNA target was synthesized by Integrated Device Technology, Inc. (USA), and all other oligonucleotides were purchased from Invitrogen Inc. (Shanghai, China). The DNA sequences are listed in Table S1. Polymerized streptavidin-HRP conjugate (poly-HRP80) and its buffer were obtained from Fitzgerald Industries International (Acton, MA). A bacterium genomic DNA (gDNA) extraction kit was purchased from Tiangen Biotech (Beijing, China) Co., LTD. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-Mercaptohexanol (MCH) was from Sigma. The TMB substrate (TMB= 3, 3', 5, 5'-tetramethylbenzidine) was purchased from Neogen (USA) in the form of a ready-to-use reagent (Enhanced K-Blue TMB Substrate, $H_2O_2$ included). The MRSA strain (methicillin resistant *Staphylococcus aureus*) was provided by Shanghai center for clinical laboratory. *E. coli* Dh5α and the QuantStudio™ 3D digital PCR master mix were purchased from ThermoFisher. All other chemicals were of at least analytical grade. All solutions were prepared with Milli-Q water (18 MΩ·cm resistivity) from a Millipore system.

### *2.2 Construction of the DNA tetrahedral nanostructure-based biosensing surface*

Gold electrodes were cleaned following a reported protocol(Zhang et al. 2007). Briefly, gold electrodes (2 mm in diameter, CH Instruments Inc., Austin, TX) were firstly polished with micropolish alumina suspensions then sonicated in ethanol and Milli-Q water for 2 min respectively. Then the electrodes were electrochemically treated in a freshly prepared 0.5 M $H_2SO_4$ solution. Finally, the electrodes were rinsed with Milli-Q water and then blow-dried with nitrogen.

Tetrahedral nanostructure-based capture probes (TSP) were constructed mainly following our previously reported work(Wen et al. 2013): Firstly, 1 μL of 50 μM of each strand was mixed with 1 μL of TCEP (500 mM) and 45 μL of TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), and the resulted mixture was then heated to 95 °C for 2 min, and then cooled down to 4 °C for 30 s using a thermal cycler (S1000, BIO-RAD., USA). Then TSP was self-assembled onto the electrode surface: 3 μL of the prepared TSP probes (1 μM) were dispensed on the cleaned electrode surface and incubated overnight at room temperature (RT).

***2.3 Electrochemical Measurement***

Firstly, the TSP-modified gold electrode was incubated in 100 μL MCH solution (0.2 mM) for 1 h at RT. After the MCH passivation, the gold electrode was rinsed thoroughly with the washing buffer (10 mM phosphate buffer (PB) containing 0.01 M NaCl) and blow-dried with nitrogen. Meanwhile, pre-hybridization was performed by mixing the target with the biotinylated reporter probes (50 nM) in 10 mM phosphate buffer (PB) containing 1 M NaCl and 20 mM $MgCl_2$ (pH 7.4), then heated to 95 °C for 5 min, and then cooled down to RT for 20 min.

Secondly, the TSP-modified and MCH-blocked gold electrode was immersed into 100 μL of the pre-hybridization mixture at 37 °C for 4 h, to perform the hybridization between the TSP capture probe and the target DNA, and then, the gold electrode was rinsed with washing buffer and dried lightly with nitrogen, and then incubated with 3 μL of HRP (SA-HRP80) (10 ng/μL) for 20 min at RT. Finally, the sensors were rinsed with washing buffer and subjected to electrochemical measurements.

Cyclic voltammetry (CV) and amperometric detection were performed with a CHI 630 electrochemical workstation (CH Instruments Inc., Austin), employing a conventional three-electrode system, consisting of a gold working electrode, a platinum wire auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode. CV was carried out at a scan rate of 100 mV/s. Amperometric detection was performed at 100 mV (versus Ag/AgCl reference) and the electrocatalysis reduction current was measured at 100 s after the HRP redox reaction reached the steady state.

***2.4 Digital PCR Quantification***

Digital PCR was performed for the quantification of synthetic target ssDNA and the MRSA gDNA with a microchip digital PCR system (QuantStudio® 3D Digital PCR System, ThermoFisher, USA). The DNA samples were firstly evaluated by UV and then serially diluted to an optimized concentration level (about 350 copies/μL) for dPCR measurement (The optimization of dPCR is not shown in this paper). 20 μL PCR reaction mix was prepared with 1×digital PCR master mix (with dNTPs and *Taq* polymerase), 0.9 μM primer forward, 0.9μM primer reverse, 0.25μM probe, DNA template. Then 5 μL reaction mixture was immediately loaded on a microchip with

20,000 reaction wells. The chip was then placed on a thermal cycler for PCR amplification under the experiment procedure as follows: 96 °C，10 min；60 °C，2 min，98 °C，30 sec，40 cycles；60 °C，2 min；10 °C，hold. Finally, imaging and analyzing of the chip was performed using the QuantStudio™ 3D Instrument.

2.5 Tm value test

Tm value test was performed on a QuantStudio® 12K PCR platform. The final concentration of each signal probe was 2.5 μM, and the concentration of the target was 0.5 μM. The fluorescent dye was purchased from ThermoFisher as a 10,000× concentrate in DMSO (SYBR® Green I, ThermoFisher), and diluted to 1× in the Tm test. Before the PCR melting curve analysis, an annealing procedure was performed as following: 95 °C 10 min, 20 °C 20 min, 95 °C 15 s, 20 °C 1 min.

2.6 Electrochemical impedance spectroscopy (EIS) measurement

Electrochemical impedance spectroscopy (EIS) measurements were performed using a μAUTOLAB III electrochemical workstation (Metrohm Autolab, Switzerland) and a three-electrode system with an Ag/AgCl reference electrode, a Pt counter electrode, and a gold working electrode. The EIS analysis solution was 10 mM PBS (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ and 0.1 M KCl.



## 3. Results and discussion

### *3.1 Strategy of the MSP biosensor*

A MSP electrochemical biosensor was constructed aiming at sensitive and specific analysis of the MRSA gene, and the strategy of our biosensor was schematically

described in figure 1. In general, a classic "sandwich-like" strategy was applied in our biosensor. A 130 nt fragment of the *mecA* gene of MRSA was taken as the target. In the pre-hybridization step, 7 signal probes hybridized to the target for signal amplification and to improve the accessibility of the target sequence. The pre-hybridized product was then added to the electrode surface that was covered by TSP with 3 thiol-labeled vertices and a capture probe on the top vertex. The TSP provided steady support for the target and the signal probes, and optimized surface density between the probes. After the duplex of the target and signal probes was captured by the TSP on the electrode, polymerized streptavidin-HRP conjugate (poly-HRP80) was combined the biotin tags to produce a detectable electrochemical catalysis signal in a commercially purchased substrate solution containing TMB and $H_2O_2$.

The construction of the biosensor was characterized by electrochemical impedance spectroscopy (EIS). As the results shown (figure S2), when TSP was assembled on the electrode and the following hybridization of the target and the signal probes happened step by step, the impedance gradually increased accordingly.

**<Figure 1 inserted here>**



### *3.2 Electrochemical behavior of the MSP DNA biosensor*

Cyclic voltammetry (CV) and amperometry (I-t) were applied to investigate the electrochemical behavior of the MP-based electrochemical DNA biosensor. In this part, a synthetic DNA sequence was analyzed as a model target, which is a 130 nt

fragment of the *mecA* DNA from MRSA genome, following the strategy described above. As shown in figure 2A, when CV was performed, 2 pairs of well-defined clear redox peaks were obtained, representing the good electron communication between TMB and the underlying gold electrode, which were assigned to the two-electron reduction and oxidation reactions of TMB. Interestingly, after hybridization with the target DNA, a typical HRP-based electrocatalysis process occurred at about 250 mV (Fig. 2A). Then, we employed the amperometry method to achieve a more visualized characterization of the HRP-catalyzed electrochemical process, by analyzing the steady-state current signal achieved at 100 second. As shown in figure 2B, the current signal increased with the increase of target DNA concentration (Fig. 2B).

**<Figure 2 inserted here>**

### *3.3 Study of the MSP system*

In our work, 7 different signal probes were designed to combine with different hybridization site on the long DNA target, with 2 nt spacer between each other, along the target DNA strand (As shown in figure 3A). The sequences of the signal probes were listed in table S1 and the length of the signal probes were between 12-15 nt. Firstly, the melting temperature (Tm) was studied when the target DNA hybridized with different single signal probe or a certain group of them, and the results was shown in figure 3B, Tm value of the sample contains only target sequence is 55.57 °C, which we attributed to the second structures of the target sequences and the unspecific hybridization between each other. When 7 probes were tested one at a time, the probe

"a" (P-a) showed the highest Tm value which is 65.04, demonstrating its highest hybridization efficiency with the target, while the Tm value of P-c with the target DNA was only 52.88, which was even lower than the target DNA itself.

Due to the Tm analysis method principle, when a group of the signal probes was added to the target DNA, the Tm value would be mainly determined by the highest Tm in the group under the same probe amount, for example, when P-a and P-d (P-ad in figure 3B) was tested together, the Tm value was 65.22, which was very close to the result of P-a, Instead, when P-c and P-e (P-ce in figure 3B) was tested, the Tm was only 54.23. The situation was exactly the same when P-abcdef group and P-bcdefg group was compared, that the Tm of P-abcde was much higher than the latter, mostly because the P-abcde group contained the "best" signal probe: P-a. The original melting curves were provided in a supplementary file (Fig. S1).

Then, we investigated the multi-probe strategy on our electrochemical platform, by analyzing a 100 pM target DNA sample using different single or MSP, and the results were showed in figure 3C. Interestingly, the electrochemical signal was highly related to the amount and the Tm value of the probes. Firstly, signal probes with higher Tm value would help to increase the current signal, due to their better hybridization effect with the target sequence. For example, the current signal of P-ad is much higher than that of P-ce, even with the same amount of signal probes, and similarly, the current signal of P-ad is much higher than that of P-ce, which is highly consistent with Tm value analysis results. Secondly, it is easy to understand that when the amount of the signal probes was increased, current signal would be increased

accordingly because more biotin labelled HRP enzyme was captured. As shown in figure 3C, when we increased the signal probes gradually, the electrochemical signal increased accordingly as following: P-a < P-ad < P-abcd < P-abcdef < P-abcdefg. In our work, 7 signal probes were finally applied as an optimized research model with the highest signal-to-noise ratio (S/N).

**<Figure3 inserted here>**

### *3.4 Investigation of the hybridization time*

An important parameter for DNA biosensors is the hybridization time on the electrode interface, especially for the detection of a relatively long DNA target. Due to the low reaction dynamics and complex second structures, the hybridization between the target DNA strands and the capture probes on electrode surface is relatively slow than normal DNA biosensor. In order to perform sufficient hybridization, the hybridization time was investigated. As the result showed in figure S3, the hybridization time was studied in the analysis of a synthetic DNA target: When the hybridization time increased from 0.5 h to 5 h, the current signal increased accordingly, but when the hybridization time was longer than 5 h, the current signal decrease obviously, maybe because of degradation of the DNA strands, including the TSP, the signal probes or the target sequences. Finally, 4 h was chosen as the optimized hybridization time for the following study of our biosensor.

### *3.5 Performance of the MRSA biosensor*

Based on the optimized conditions above, we achieved an ultra-high sensitivity

of DNA analysis. As shown in Figure 4, when the concentration of the target sequence increased, starting from 10 fM, the signal gain increased accordingly. The highest signal growing rate was between 31.6 pM and 1 nM, showing the best responding concentration range of our biosensor. When the target concentration was below 31.6 pM, the signal distinction between each concentration was getting relatively lower, however, a very low limit of detection (LOD) was achieve as 10 fM, based on the very steady and low blank signal, which was only 58.1 nA. When the concentration exceeded 1 nM, the current signal reached a platform. Finally, a very good exponential fitting was achieved, on the whole analysis range between 10 fM and 10 nM, covering 6 orders of magnitude, which is very competitive among reported electrochemical biosensors.

**<Figure 4 inserted here>**

For the construction of the optimized interfacial support for the MSP system, the TSP played an important role in our biosensor, providing both improved surface density between each probe and strong support for the upper conjugate of long DNA target and signal probes. As evidence, we compared TSP with a normal single-strand DNA probe (SS probe) by analyzing the same concentration of target DNA. The results were showed in figure 4. The analysis ability was quite limited when normal SS probe was applied even the same signal probes were applied. Generating obviously lower signal gain at every concentration level, and the LOD of the biosensor using TSP was at least 4 orders of magnitudes lower than that of normal ssDNA probe. The stability of our biosensor under 4 °C storage was investigated, by

analyzing 100 pM target, as the result shown in figure S3, the signal gain and the blank was maintaining good stability during 2 days, and slightly decreased to a plateau after 4 days.

### *3.6 Analysis of MRSA gDNA*

Finally, our MSP biosensor was applied for the analysis of an extracted MRSA gDNA. In order to provide a reliable validation for our ultra-sensitive MRSA biosensor, we firstly quantified the target sample with digital PCR (dPCR). Digital PCR is a recently developed method for DNA quantification, showing optimized specificity and accuracy than traditional PCR. The dPCR firstly distributed the DNA sample onto a microarray chip containing tens of thousands of reaction chambers, and then perform a PCR reaction. Finally, by analyzing the number of positive signals, the gDNA molecules containing the target sequence were quantified without any calibration curve needed, and more importantly, any unspecific DNA fragments were eliminated as negative signal, thus, the dPCR results showed much higher specificity and accuracy than UV evaluation. In our work, the extracted gDNA sample was quantified by dPCR to be 5.7 pM, and then diluted and analyzed by our electrochemical biosensor. Meanwhile, a gDNA sample extracted from *Escherichia coli* (*E. coli*) was prepared and analyzed as a negative control. The quantification results were showed in figure 5. Interestingly, our sensor performed equally well despite the fact that gDNA is much longer than the 130 nt synthetic oligo and the purity of the gDNA sample is limited than model system described above. We found that the current signal of the 57 fM MRSA gDNA was -117 nA, which was highly

consistent with the analysis result of synthetic DNA (Figure 4), and significantly higher than the blank, demonstrating the accuracy and sensitivity of our sensor. On the contrary, the current signal of a length-matched, non-complementary *E. coli* gDNA was statistically insignificant from blank sample, which demonstrated the excellent specificity of our sensor.

**<Figure 5 inserted here>**

**Table 1,** Comparison of electrochemical DNA biosensors for the detection of *mecA* gene on MRSA gDNA:

| **Sensors type** | **Target** | **Sensitivity** |
|---|---|---|
| electrochemical biosensor using AuNPs(Liu et al. 2014) | 50 nt synthetic ssDNA | 23 pM |
| FRET biosensor based on GQDs and AuNPs pairs(Shi et al. 2015) | 20 nt synthetic ssDNA | 1 nM |
| isothermal strand-displacement polymerization reaction (ISDPR)(Wang et al. 2015) | 27 nt synthetic ssDNA | 63 fM |
| SPR-DNA in combination with loop-mediated isothermal amplification (LAMP)(Nawattanapaiboon et al. 2015) | 203 bp LAMP amplicons | 10 copies/mL |
| Interference-based detection of nucleicacid targets on optically coated silicon(Jenison et al. 2001) | 38 nt synthetic ssDNA | 10 fM |
| electrochemical biosensor based on | 130 nt synthetic ssDNA and | 10 fM synthetic |

| | | |
|---|---|---|
| MSP and TSP (this work) | whole genomic DNA | ssDNA and 57 fM genomic DNA |

## 4. Conclusions

Our MSP electrochemical sensor presented a novel application of signal probes in the analysis of long DNA target and even whole gDNA of MRSA. In our work, the signal probes not only bring more biotin labels, but also help to expose the target sequence by breaking the double strands and the second structures of the long DNA molecules through an annealing step.

We studied the hybridization between signal probes and target DNA, and demonstrated the advantages of the MSP system in electrochemical biosensing. We found that the current signal was highly related to both the signal probe amount and the Tm value of the hybridization between the target and the signal probes. Our study of the relationship between Tm and electrochemical signal demonstrated the reliability of our results and provided a valuable strategy for the optimization of signal amplification systems.

To steadily support the MSP system on the electrode interface, we utilized a DNA tetrahedron nanostructure probe, which also provided optimized density of the capture probes. The comparison with normal signal capture probes strongly suggested the advantage of TSP.

Finally, the sensitivity for synthetic ssDNA detection is proved to be 10 fM, which is quite comparable to the previously reported work (Table 1). More

importantly, we successfully detected MRSA gDNA samples without PCR, and the concentration was demonstrated to be as low as 57 fM by a commercial dPCR platform. It is worth pointing out that the direct analysis of gDNA without any treatment or PCR amplification is an important progress for the development of on-site analysis for pathogenic microorganism (Table 1).

In summary, we report a novel MSP-based electrochemical DNA biosensor for the sensitively and specifically detection of *mecA* gene, and the electrochemical analysis platform gives us great potential for portable on-site detection of MRSA, based on this proof-of-concept sensor.

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## Figure Captions:

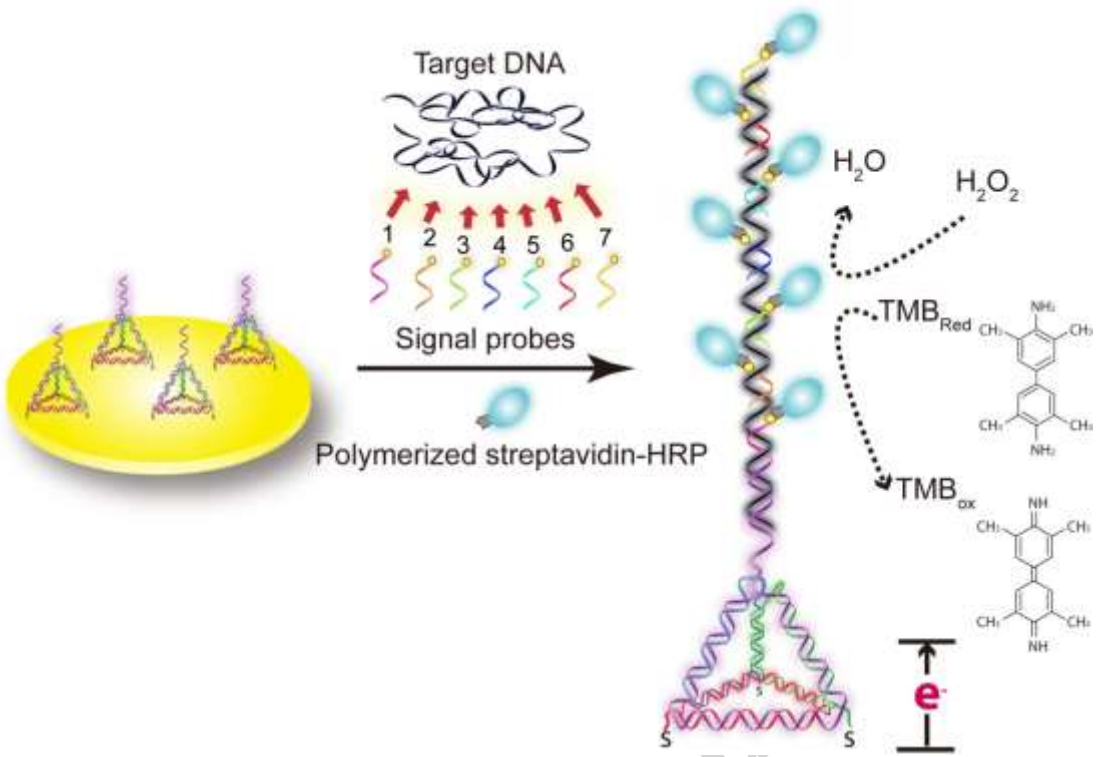


**Figure 1,** Schematic instruction for the electrochemical DNA biosensor using a MSP system containing 7 signal probes and a tetrahedral nanostructure-based capture probe for MRSA DNA analysis.

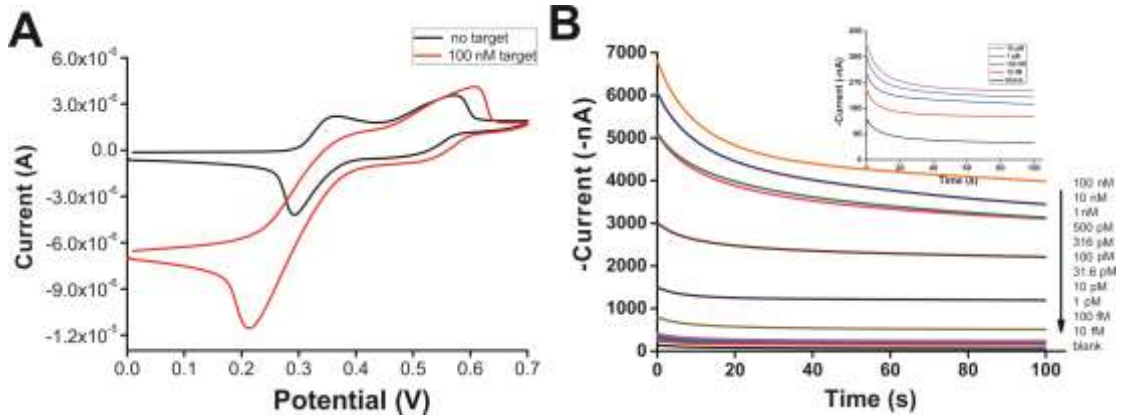


**Figure 2,** A) CV results of our biosensor in the absence (black line) and presence (red line) of 100 nM synthetic target DNA. Scan rate: 100 mV/s. B) Aerometric curves (I-t curves) of a series of synthetic target DNA concentrations. From top to bottom: 100 nM, 10 nM, 1 nM, 500 pM, 316 pM, 100 pM, 31.6 pM, 10 pM, 1 pM, 100 fM, 10 fM and Blank. Insert shows the I-t curves of low-concentration range target DNA between 10 pM to Blank. The potential for I-t analysis was 100 mV.

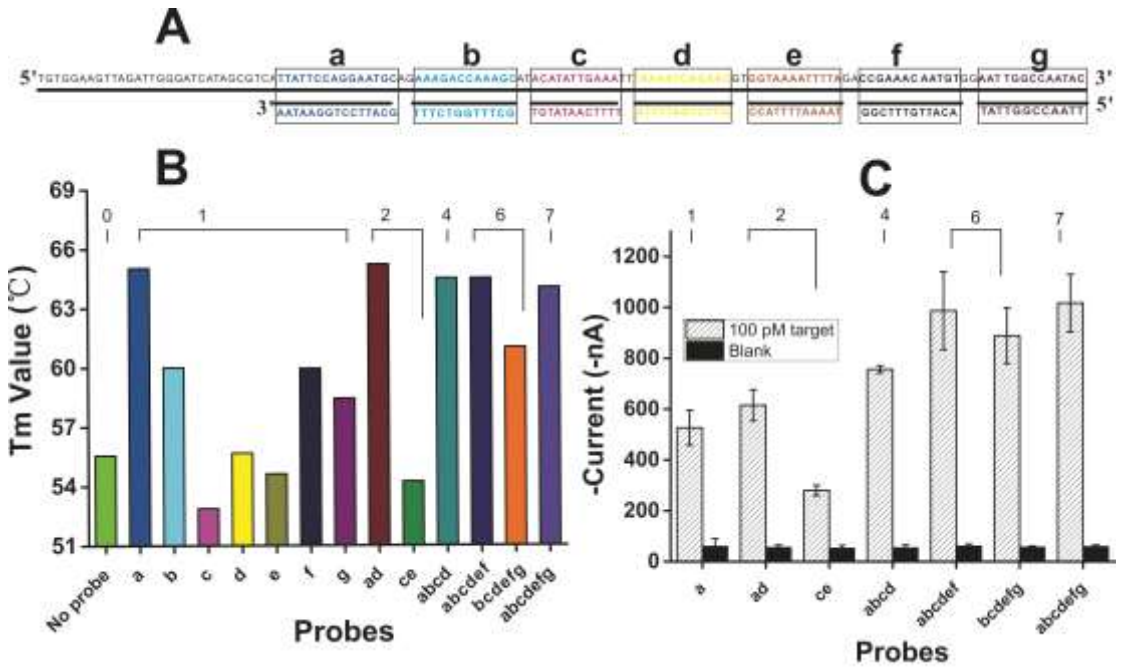


**Figure 3,** Investigation results of the signal probe number: A) the schematic illustration of the hybridization position of the 7 signal probes, B) Tm value analysis results using different signal probes, C) Electrochemical analysis results using different signal probes.

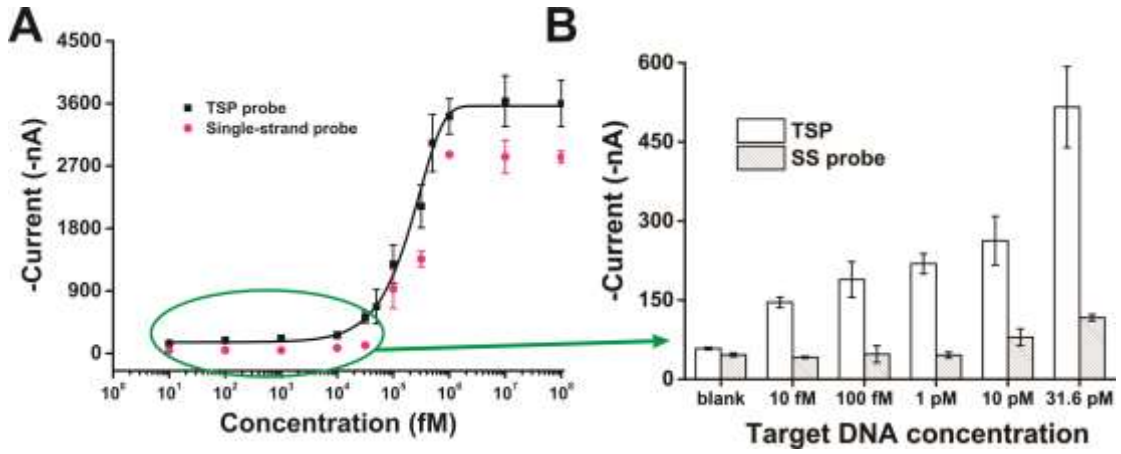


**Figure 4,** Analysis results of a synthetic DNA: A) The working curve of a series of synthetic target DNA concentrations. From right to left: 100 nM, 10 nM, 1 nM, 500 pM, 316 pM, 100 pM, 50 pM, 34.6 pM, 10 pM, 1 pM, 100 fM, 10 fM. The black squares were the results using TSP, and the red dots were normal ssDNA probes. B) The histogram of the low concentration range between 10 fM to 31.6 pM. The potential for I-t analysis was 100 mV.

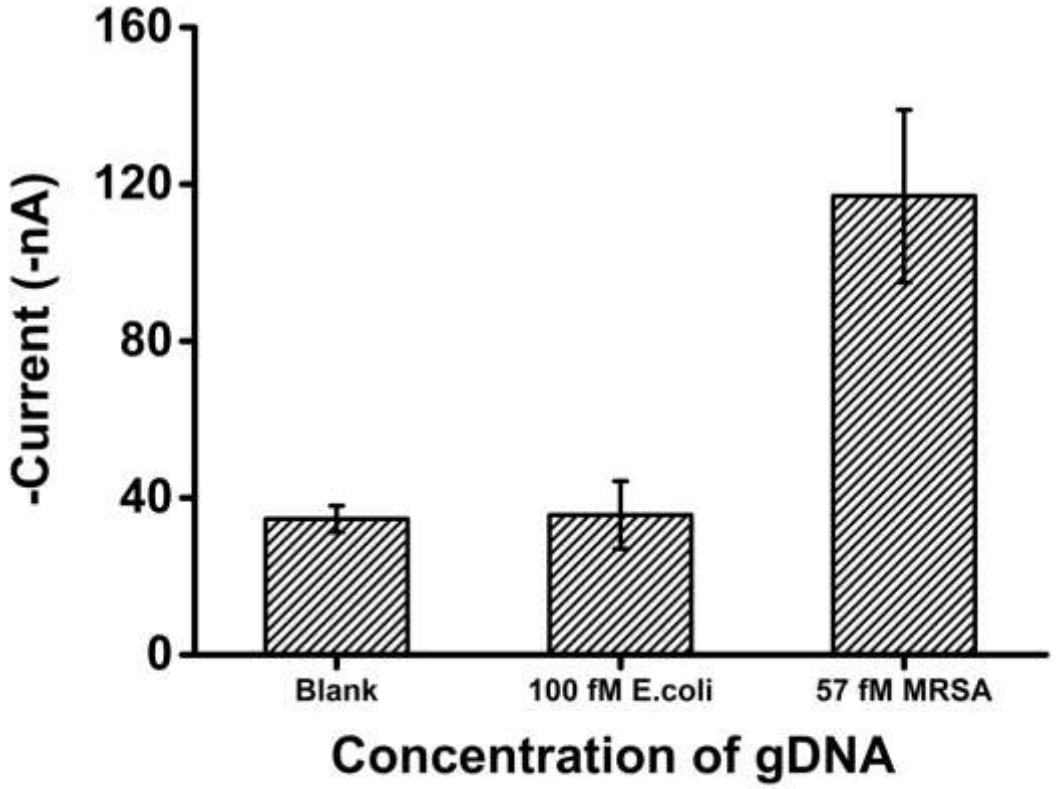


**Figure 5,** Electrophoretic analysis of MRSA gDNA and *E.coli* gDNA. The concentration of the target gDNA was determined by dPCR.

## Highlights:

1, A multi-signal probe system was developed for the improvement of the hybridization and signal amplification for long DNA detection.

2, A 3-D DNA tetrahedron structure probe was used as a base of the capture probe.

3, 10 fM synthetic target DNA and 57 fM MRSA gDNA was successfully detected.

4, The reliability of MRSA gDNA analysis was demonstrated by digital PCR.