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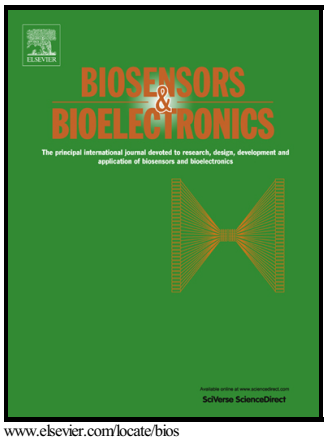

www.elsevier.com/locate/bios

PII: S0956-5663(14)00992-0
DOI: http://dx.doi.org/10.1016/j.bios.2014.12.040
Reference: BIOS7356

To appear in: *Biosensors and Bioelectronic*

Received date: 1 October 2014
Revised date: 9 December 2014
Accepted date: 17 December 2014

Cite this article as: Abdolkarim Abbaspour, Fatemeh Norouz-Sarvestani, Abolhassan Noori and Noushin Soltani, Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of *staphylococcus aureus*, *Biosensors and Bioelectronic*, http://dx.doi.org/10.1016/j.bios.2014.12.040

Aptamer-conjugated Silver nanoparticles for Electrochemical Dual-Aptamer-Based Sandwich Detection of *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is one of the most important human pathogens and causes numerous illnesses. In this study, we report a sensitive and highly selective dual-aptamer-based sandwich immunosensor for the detection of *S. aureus*. In this bioassay system, a biotinylated primary anti-*S.aureus* aptamer was immobilized on streptavidin coated magnetic beads (MB), which serves as a capture probe. A secondary anti-*S.aureus* aptamer was conjugated to silver nanoparticles (Apt-AgNP) that sensitively reports the detection of the target. In the presence of target bacterium, an Apt/*S.aureus*/apt-AgNP sandwich complex is formed on the MB surface and the electrochemical signal of AgNPs followed through anodic stripping voltammetry. The proposed sandwich assay benefits from advantageous of a sandwich assay for increased specificity, MB as carriers of affinity ligands for solution-phase recognition and fast magnetic separation, AgNPs for signal amplification, and an electrochemical stripping voltammetry read-out as a simple and sensitive detection. The electrochemical immunosensor shows an extended dynamic range from 10 to 1×10^6cfu/mL with a low detection limit of 1.0 cfu/mL (S/N=3). Furthermore, the possible interference of other analogue bacteria was studied. To assess the general applicability of this sensor, we investigated the quantification of *S. aureus* in real water samples. The results were compared to the experimental results obtained from a plate counting method, which demonstrated an acceptable consistency.

Keywords: Aptamer, *Staphylococcus aureus,* Sandwich assay, Silver nanoparticle, Electrochemical Biosensor

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the most important human pathogens that can cause different kinds of illnesses from minor skin infections to life threatening diseases (Chang et al. 2013). Since, *S. aureus* infections are contagious, it emerged as one of the common infectious worldwide (Ferreira et al. 2014; Török and Day 2014). *S. aureus* has also the ability to make seven different toxins that are frequently responsible for food poisoning (Nelles et al. 1985). *S. aureus* is one of the five most common causes of infections after injury or surgery. For the subsequent treatment of infectious diseases caused by *S. aureus*, its rapid and accurate identification is critical.

S. aureus has specifically been identified by traditional microbiological laboratory procedures, which involve culturing the bacteria followed by either specific genotype or phenotype diagnostic assays (Štěpán et al. 2004) .Even though these methods are specific and sensitive, they require skilled staff, expensive laboratory facilities, pretreatment procedures and one to two days for the identification of pathogenic bacteria. This delay is unacceptable in emergency and critically ill situations. For this reason, several ultrasensitive detection methods such as polymerase chain reaction (Cheng et al. 2006), ligase chain reaction (Moore and Curry 1998), strand displacement amplification (Edman et al. 2000), enzyme-linked immunosorbent assay (Chang and Huang 1994) have been introduced. High costs and complex procedures limit the wide spread use of these technologies for clinical diagnosis.

Biosensor technology is a rapid and reproducible approach for the detection of pathogens or toxins and other biomolecules (Abbaspour and Noori 2012a; Mello and Kubota 2002). Several biosensing strategies such as surface plasmon resonance (Balasubramanian et al. 2007; Subramanian et al. 2006), fluorescence (Su et al. 2007), chromatographic (Kaláb and Skládal 1997) and electrochemical (Berrettoni et al. 2004) methods have been developed for fast detection of *S. aureus* pathogen. Antibody (Ab) based immunoassays for bacterial identification are well established and have been used for many years (Swaminathan and Feng 1994). However, the use of Ab in the analysis of samples could encounter some limitations (Tombelli et al. 2007). Ab-based biosensors are suffering from high cost, low screening efficiency, low stability, and also difficulties associated with their isolations and modifications. Aptamers are a class of high-affinity molecules with the potential to play a role in point of care assays. This group of synthetic nucleic-acid molecules can be generated using a repetitive in vitro selection and partitioning technology, called SELEX, against virtually any target molecule. The affinities of aptamers for their targets are comparable to, or even higher than most monoclonal antibodies. Typical dissociation constants for aptamer-target complexes are found in the picomolar to low micromolar ranges (Kim and Gu 2014).

Several bacterial aptamers had been isolated and recently used in identification of bacteria, including Escherichia coli (Lee et al. 2009), Mycobacterium tuberculosis (Chen et al. 2007), Salmonella enteric (Joshi et al. 2009), and Bacillus anthracis (Zhen et al. 2002). However, none of these studies reported the capability of identifying extremely low numbers of target bacteria without a PCR reaction. Recently, DNA aptamers that selectively bind *S. aureus* have been selected with binding constants of a few tens of nanomolar (Chang et al. 2013; Huang et al. 2014), and *S. aureus* aptasensors have also been constructed (Chang et al. 2013; Wu et al. 2014). There is a continuous need for point-of-care assays that are simple, sensitive, and economical. Compared with optical-based biosensors,

electrochemical methods, in general, show the potential for construction of fast, simple, low-cost, sensitive and high throughput biosensors that can be miniaturized (Duan et al. 2013; Jiang et al. 2014; Lian et al. 2015; Moradi et al. 2013; Wu et al. 2014). In addition, recent advances in nanobiotechnology and conjugation of nanomaterials to biomolecules provided a major expansion in the field of biosensing, diagnostics and therapeutic developments (Abbaspour and Noori 2012b; Kashefi-Kheyrabadi and Mehrgardi 2012; Sapsford et al. 2013).

In this study, we have proposed a dual-aptamer-based sandwich assay for electrochemical detection of *S. aureus*. First, a biotinylated anti-*S.aureus* aptamer is immobilized on streptavidin-coated magnetic beads (MB) via biotin-streptavidin affinity reaction. When a sample containing *S. aureus* is added to an aptamer-immobilized MB suspension, bacterium is captured via the specific interaction between the aptamer and the bacterium. Then, secondary anti-*S.aureus* aptamer-conjugated AgNPs (Apt-AgNP) is added to the solution, thus completing the sandwich. In the next step, MBs that are carrying the affinity complex are separated with an external magnet. After dissolution of AgNPs in an acidic medium, differential pulse stripping voltammetric signal of AgNPs is followed as an analytical signal for the detection and quantification of *S. aureus* (Scheme 1). Finally, the applicability of the immunosensor was evaluated for the detection of *S. aureus* in some real samples. To the best of our knowledge, this is the first report on an aptamer-based sandwich immunosensing of *S. aureus*.

2. Experimental

2.1. Chemicals and reagents

Streptavidin-coated magnetic beads (1.05 μm), silver nitrate, sodium borohydride, sodium citrate trihydrate, Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and Tris–HCl were purchased from commercial sources (Merck or Sigma) in analytical grade. *S. aureus* ATCC 25923, E. coli ATCC: 43896 and S. epidermidis ATCC: 155 were provided from the American Type Culture Collection (ATCC).

Biotinylated anti-*S. aureus* primary aptamer and thiolated anti-*S. aureus* secondary aptamer were obtained from Anaspec Co.(Canada) with the following sequences:
Primary aptamer: 5'-Biotin-$(CH_2)_6$-TCC CTA CGG CGC TAA CCC CCC CAG TCC GTC CTC CCA GCC TCA CAC CGC CAC CGT GCT ACA AC-3'
Secondary aptamer: 5'-SH-$(CH_2)_6$-TCC CTA CGG CGC TAA CCT CCC AAC CGC TCC ACC CTG CCT CCG CCT CGC CAC CGT GCT ACA AC-3'

2.2. Apparatus

Electrochemical measurements were conducted using a μAutolab type III (The Netherland) controlled via GPES 4.9 software. Disposable screen-printed electrodes (DS 110) consisting of a carbon working electrode, a carbon counter electrode, and a silver pseudo-reference electrode printed on a substrate were purchased from DropSens (Spain) for electrochemical measurements. A cable connector (DRP-BICAC connector, DropSens, Spain) operates as an interface between a disposable screen-printed electrode and μAutolab potentiostat/galvanostat. A homemade magnetic separation stand was used for capturing the magnetic beads on the side wall of micro tubes. A homemade sample mixer with 10-tube mixing wheels was used for all the mixing and washing steps. A Metrohm 780 pH meter was used for pH measurements. A model CM10 transmission electron microscope (TEM, Philips) was used to characterize the products. The UV–Vis spectra of the samples were recorded by PERKIN–ELMER Lambda 2 spectrophotometer in a 1-cm quartz cell.

2.3. Bacterial culture

The bacterial strains used in the study were S. aureus (ATCC:25923), E. coli (ATCC: 43896) ,S. epidermidis (ATCC: 155). All of them were provided as culture grown on nutrient agar plates (Himedia, India). The bacteria were inoculated in LB broth (Himedia, India) and incubated in a shake flask at 37°C for

24 h. Bacteria were gently washed out from the agar using sterile distilled water. To remove liquid remains of the medium, bacteria were centrifuged at 4000 rpm for 5 min. The numbers of bacteria were determined via a plate count method. The plate count method is the standard method used in microbiology. The purpose of plate counting is to estimate the number of cells present based on their ability to give rise to colonies under specific conditions of nutrient medium, temperature and time. This method consists of growing the bacteria in a nutrient culture petri dish and counting colonies, which develop. To calculate the exact number of bacteria in the suspension, the suspension was serially diluted. One milliliter of each diluted solution was transferred to plate count agar. After 24 h incubation at 37°C under aerobic conditions, colonies on plates were counted to determine the number of colony-forming units per milliliter (cfu/ mL). Colonies counting was performed manually using a pencil and a click-counter.

2.4. Buffers and solutions

The compositions of the buffers used for the experiments were as follows: buffer for bead washing: 10 mM Tris-HCl, 0.5 mM EDTA, 1.0 M NaCl and 0.1 % Tween 20 pH 7.5 (buffer A); buffer for the immobilization of the primary aptamer onto the magnetic beads: 10 mM Tris-HCl, 0.5mM EDTA, and 1.0 M NaCl pH 7.5 (buffer B); buffer for *S. aureus*-captured MB washing, 10 mM Tris-HCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 150 mM NaCl pH 7.5 (buffer C). All experiments were performed at room temperature. The stock solutions of the aptamers (50 μM) were prepared using 10 mM Tris-HCl buffer solution (pH=7.4) and kept at -20 °C until use.

2.5. Procedure

2.5.1. Synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were prepared by the method reported previously (Mulfinger et al. 2007). Briefly, an aliquot of 5 mL silver nitrate (1.0 mM) was added drop-by-drop (about 1 drop/s) to15.0 mL of 2 mM sodium borohydride

solution at 0 °C. The reaction mixture was stirred vigorously on a magnetic stirrer. The solution turned to light yellow after the addition of 2 mL of silver nitrate. It turned to brighter yellow when the rest of the silver nitrate solution was added. In order to minimize the aggregation of AgNPs, 30 mg sodium citrate trihydrate was then added to the resulting solution. The AgNPs could be stored in a dark bottle (4 °C) for several weeks. The average size of the AgNPs, measured through TEM analysis (**Fig. 1a**), was about 20 nm. The UV–Vis spectrum of the AgNPs is presented in **Fig. 1b**. Based on what has been reported by Solomon research group (Mulfinger et al. 2007), the wavelength of the plasmon absorption maximum can be used to calculate the size of the nanoparticles. Absorption maximum at 415 nm is consistent with 20 nm diameter nanoparticles, which confirms the results of the TEM analysis.

2.5.2. Preparation of aptamer-conjugated AgNPs

AgNPs-conjugated secondary aptamers (Apt-AgNPs) were prepared based on a procedure reported in literature (Kashefi-Kheyrabadi and Mehrgardi 2012). First, the disulfide bond of the secondary aptamer was reduced by TCEP (200 µL of 5 µM secondary aptamer was incubated with 20 µL of 5 mM TCEP in dark for 1 h). Then, 1000 µL of AgNPs solution was added to the de-protected secondary aptamer and kept for 2 h for the assembling of AgNPs to thiol group of aptamer segment. After that, Apt-AgNPs were centrifuged at 10000 rpm for 10 min and washed twice. The sediment was re suspended in 200 µL of the10 mM Tris-HCl buffer solution.

2.5.3. Assay Protocol

(a) Immobilization of primary aptamer onto magnetic beads. The streptavidin-coated magnetic beads (MB) were first washed with 400 µL of buffer A before use, as advised by the manufacturer in order to remove the NaN_3 preservative. In this study, every washing step consists of: 2 min incubation on a sample mixer, 1

min rest on a magnetic separation stand, and after that aspiration of the supernatant (Noori et al. 2010). Then, 100 μL of the MB suspension was introduced in a microtube containing 100 μL of primary aptamer (1.0 μM). After 30 min of incubation, the microtube was positioned on a magnetic separation stand that attracts the MBs from solution to the sidewall of the test tube; the supernatant was then aspirated and the beads were washed twice with 400 μL of buffer B. To eliminate the effect of the nonspecific adsorptions on MB's surface, the remaining active streptavidin sites on MB should be blocked or inactivated. Thus, the beads were resuspended in 100 μL of 1% BSA solution for a definite time to saturate the sites of streptavidin that have not bound to the biotinylated primary aptamer. The beads were then washed with buffer B and resuspended in 100 μL of that buffer. Aptamer-immobilized MBs were prepared in advance and kept at +4 °C for several weeks.

(b) **Addition of the Analyte**. 20 μL of aptamer-immobilized MBs suspension was mixed with 100 μL of a solution containing *S. aureus* with definite concentration. After 5 min of incubation, the beads were magnetically separated to remove the supernatant, and then washed twice with buffer C.

(c) **Addition of the Apt-AgNPs**. After capturing the target *S. aureus* bacterium onto MB's surface, the beads were resuspended in 100 μL of Apt-AgNPs solution (1.0 μM), incubated for 5 min, and then separated and washed twice using buffer C.

(d) Electrochemical Detection.

The beads carrying the affinity complex (Apt/*S.aureus*/Apt-AgNPs) were resuspended in 100 μL of 0.1 M HNO_3 for about 10 min to fully dissolve AgNPs. After magnetic separation, a portion of the supernatant (which now contains Ag^+ ions) was transferred onto the surface of an electrode for differential pulse anodic stripping voltammetric measurement. Concentration of Ag ions in solution is directly proportional to *S. aureus* concentration in the sample. For quantification of Ag ions, the potential was held at -1.0 V for 120 s followed by sweeping the

potential from -0.1 to 0.5 V with modulation amplitude of 25 mV. The control experiments were performed in a similar fashion but without adding the *S. aureus*.

3. Results and discussion

3.1. Characterization of Apt-AgNP conjugation

Metallic nanoparticles of gold and silver are conventionally used for functionalization of thiol-terminated DNA sequences. To characterize the conjugation of the AgNPs to secondary aptamer, UV-Vis spectra of AgNPs, aptamer and Apt-AgNPs were recorded (Supplementary information, **Fig. S1**). AgNPs show absorption maximum at 408 nm (**Fig. S1,** a). Aptamer also exhibits a characteristic absorption band at about 260 nm (**Fig. S1**, b). After conjugation of the AgNPs to aptamers, the absorption peak of aptamer and AgNPs are observed, which indicates a successful formation of Apt-AgNPs bioconjugates (**Fig. S1**, c). The absorption peak of AgNPs, are weakened and red-shifted (to 430 nm) after conjugation to aptamers that is due to aggregation of AgNPs and changes in plasmon on the NPs surface (Dougan et al. 2007). For immobilization of primary aptamer on magnetic beads surface, we used the high affinity of streptavidin (on MB surface) to biotin (tagged to aptamer), which has provided the basis of many important biotechnological processes.

3.2. Parameters optimization

A highly sensitive method for quantification of *S. aureus* pathogen is proposed using a dual-aptamer-based sandwich assay, applicable for analysis in complex real samples. In a sandwich assay several parameters may influence the overall performance of the immunosensor, thus those parameters should be optimized. In this study, we tried to optimize the concentration of primary aptamer as a capture probe, and secondary aptamer as a signaling probe to achieve the highest signal-to-noise ratio of the electrochemical read-out signal.

3.2.1. Optimization of the primary aptamer concentration

Density of aptamers onto MBs is of prime importance since strongly influences the detection sensitivity of the immunosensor. A low density of immobilized aptamer onto MBs results in a narrow linear range with a diminished sensitivity. On the other hand, aptamers always adopt a secondary structure with a high level of steric hindrance. Thus, a highly dense aptamer layer onto MBs can cause a limited accessibility of the aptamers to the target molecule. For optimization of the primary aptamer concentration, a set of aptamer solutions with different concentrations in the range of 0.1 to 2.0 μM, were used for MBs modification. The electrochemical signal of a 1×10^6cfu/mL *S. aureus* solution was followed, the results of which are presented in **Fig. 2** and the corresponding DPVs are presented in supplementary information (**Fig. S2**). As shown in this figure, the intensity of the electrochemical signal increased with increasing the aptamer concentration and reached a maximum at 1.5 μM of aptamer. Additional increase in the aptamer concentration causes the electrochemical signal to decrease. It's probably due to the steric hindrances that impose to the primary aptamer at its higher concentrations, which lower the overall signal of the sensor. Moreover, electrochemical signal of a solution that is 1.0 μM in aptamer is 46.2 μA, which is about 90% of the maximum signal, i.e.51.7 μA obtained in a solution that is 1.5 μM in aptamer. To save the aptamer consumption of the immunoassay, we used a solution that is 1.0 μM in aptamer for preparation of aptamer-immobilized MBs. Although part of the sensitivity was sacrificed for saving the aptamer consumption, the biosensor was still sensitive enough for quantification of the bacterium at a very low level.

3.2.2 Optimization of the Apt-AgNPs concentration

In the next step, we attempted to optimize the concentration of Apt-AgNPs as a detection probe. Different concentrations of Apt-AgNPs bioconjugate, in the range of 0.1 to 2.0 μM, were used to establish a sandwich structure. The net

electrochemical signal increased with increasing the Apt-AgNPs concentration and reached a maximum at 1.0 μM Apt-AgNPs bioconjugate concentration. With subsequent increase in concentration of Apt-AgNPs bioconjugate, the electrochemical read-out signal starts to decrease (**Fig. 2**). Thus we used a solution that is 1.0 μM in Apt-AgNPs as an optimal concentration for making a MB-Apt/bacterium/Apt-AgNPs sandwich.

Different kinds of NPs (including metals, semiconductor quantum dots, upconverting, and magnetic NPs) have been synthesized and used thus far. Based on the intended detection method, each kind of NPs has special advantages. For example, while upconversion NPs are the best choice for *in-vivo* bio-imaging with fluorometric detection (Muhr et al. 2014), AuNPs and QDs are always the first choice in colorimetric detections (Hutter and Maysinger ; Shi et al. 2014). Here, the reason behind the selection of the AgNPs as a signaling probe stems comes from its superior electrochemical behavior over other NPs. Although the total number of biosensing strategies reported with AuNPs is much higher than those based on AgNPs, the use of AgNPs in electrochemical biosensors has some advantages. For example, the electrochemical redox reactions of silver occur at lower potentials and it gives a well-defined and sharp voltammetric peak. In addition, the oxidative peak of silver is about 100-fold more intense than that of colloidal AuNPs with the same diameter and concentration (Cai et al. 2002). Moreover, silver metal can be easily oxidized without poisonous reagents. The high sensitivity of an electrochemical immunoassay with a metal nanoparticle tag stems in the release of huge number of metal ions from each single nanoparticle (i.e.10^6 ions from a 40-nm particle) and the subsequent detection of metal ions using highly sensitive voltammetric techniques.

3.3. Analytical Application

Under optimal conditions, the proposed biosensor shows a linear response to *S. aureus* concentration over an extended dynamic range of 10 to 10^6 cfu/mL (**Fig.**

3a). The linear regression equation is I_p (μA) = 10.6 Log [C (cfu/mL)] – 8.2 with a correlation coefficient of 0.9935 (**Fig. 3b**). Limit of detection of the biosensor is down to 1.0 cfu/mL (S/N=3). To the best of our knowledge, this is the best detection limit reported for electrochemical detection of *S. aureus* thus far.

In a common sandwich assay, the choice of antibodies (Ab) is of prime importance. A monoclonal Ab binds to the antigen via one epitope thus provide a high level of specificity. By contrast, a polyclonal Ab provides a higher level of sensitivity, since multiple Ab could be bind to a single antigen. However, due to less precisely defined epitope for a polyclonal Ab on an antigen, cross-reactivity is a potential problem. Thus, in a common dual-Ab-based immunoassay a monoclonal Ab is chosen as a capture probe and a polyclonal Ab is chosen as the detection probe to fulfill both the high level of specificity and sensitivity. Here we have presented a dual-aptamer based sandwich assay with outstanding superiorities over traditional Ab-based immunosensing. The number of aptamer molecules bind to a single *S. aureus* is 900 to 1200 molecules of primary aptamer, and 1500 to 2300 molecules of secondary aptamer per a single *S. aureus* bacterium. Thus, the high binding affinities of anti-*S.aureus* aptamers to their target bacterium (35 and 129 nM for primary and secondary aptamer respectively), and the large number of aptamer per a single bacterium ensures recognition of *S. aureus* at such a low concentration.

Table 1 shows a comparison study of the analytical characteristics of the present immunosensor with relevant immunosensors for bacterial analysis. As can be seen from the table, the obtained results are better than the results presented by other research groups. The limit of detection achieved in this work being considerably lower than those reported before for *S.aureus.*

3.4. Selectivity of the assay

In this study, we have used two aptamers that recognize the *S. aureus* with high selectivity or even specificity. The employed process for aptamer identification

has been cell-SELEX in which the oligonucleotides are mixed with *S. aureus* and the bounded sequences separated (Chang et al. 2013). Then the bound aptamers are incubated with Staphylococcus epidermidis (which is a bacterium with the same genus as *S. aureus*) as a counter-selection. In the second round of the cell-SELEX process, just the sequences that survive the counter-selection process have been used. Thus, the aptamers that finally have been identified are specific/highly selective to *S. aureus*. The two aptamers that have been used in this study display dissociation constants (K_d) of 35 and 129 nM to *S. aureus*. The aptamer with K_d of 35 nM shows no clross-reactivity with other bacteria, and the aptamer with K_d of 129 nM just shows very week cross-reactivity with two other bacteria (S. epidermidis and P. aeruginosaout of 15 bacteria that their cross-reactivity has been tested). Thus, in this study, we used the aptamer with K_d of 35 nM as a primary aptamer. Because, first, the higher affinity of that aptamer to *S. aureus* (35 nM vs. 129 nM) ensures a better and faster recognition of the *S. aureus* from the media, and second, its specificity to *S. aureus* (complete survival from cross-reactivity test), ensures the specific capturing of *S. aureus* to the MB surface among many possible interfering species. The aptamer with K_d of 129 nM was conjugated to AgNPs and served as a signaling probe. The number of secondary aptamer molecules bind to a single *S. aureus* bacterium is higher than that of primary aptamer (1500 to 2300 apt-2/bacterium vs. 900-1200 apt-1/bacterium)), thus that would be a better choice as a signaling probe. We expect a large number of reporter molecules on every single *S. aureus* bacterium surface. Consequently we anticipated that the proposed sandwich assay would show outstanding results for the detection and quantification of the *S. aureus*.

To assess how specific the proposed dual-aptamer-based immunosensor is, we measured the electrochemical signal of other pathogenic bacteria. The intensity of the read-out signals originated from *Staphylococcus epidermidis* (*S. epidermidis*) and *Escherichia coli* (*E. coli*), in their equimolar concentrations (i.e. 10^6 cfu/mL), were measured (**Fig. S3)**. Response of the immunosensor to *S. epidermidis* and *E.*

coli was 17.6 ±0.93 % and 5.63± 0.80 % that of *S. aureus*, respectively. This is, while, *S. epidermidis* is the most potential interfering bacterium to detection of *S. aureus*. These results show that the present assay has a high selectivity for discrimination of *S. aureus* from other pathogenic bacteria. The specificity of the proposed biosensor arises from the high affinity of the aptamers that are used for sandwiching the target.

3.5. Real sample analysis

Finally, the applicability of the aptasensor was evaluated by detecting *S. aureus* in real samples. In this step, the concentration of *S. aureus* was measured in tap and river water samples without any pretreatment. The obtained results were compared to those of the plate counting methods, which is a standard method for cell and bacteria counting in microbiology (Jeffrey 2007; Winn and Koneman 2006). The results of each analysis are presented in **Table 2**, which indicate that there is no significant difference between the results obtained via the proposed aptasensor and those obtained through the standard plate counting method.

4. Conclusion

In this study, an electrochemical sandwich aptasensor was developed for the detection of *S. aureus.* Primary aptamer immobilized on magnetic beads surface captures the *S. aureus* and the secondary aptamer conjugated to silver nanoparticles provides a well-defined electrochemical characteristic that considerably improves sensitivity of the assay. Excellent discriminatory power of the biosensor originates from the use of two specific aptamer sequences against the target bacterium, and the use of magnetic beads for capturing the *S. aureus* in a solution phase. The electrochemical detection method showed many advantages in term of simplicity, analysis time, low cost and detection limit compared to state-of-the-art detection protocols. The high sensitivity of proposed method opens up

the possibility of using aptamers for detecting trace levels of bacteria that cannot be measured by conventional methods.

Acknowledgment

The authors express their gratitude to Shiraz University Research Council for the support of this work.

Table 1

A comparison of the analytical characteristics of the immunosensor developed in this work with relevant immunosensors for pathogenic bacteria

Microorganism Target	**Detection Method**	**Dynamic range (cfu/mL)**	**Detection limit (cfu/mL)**	**Ref.**
S.Tiphimurium	Absorbance	10-10^6	7	(Yuan et al. 2014)
S.Typhimurium	Fluorescence	10-10^5	5	(Duan et al. 2012)
S.aureus	Fluorescence	10-10^5	8	(Duan et al. 2012)
L.monocytogenes	Fluorescence	10^2 -10^7	75	(Duan et al. 2013)
S.aureus	Amperometry	$1.3x10^3$ –$7.6x10^4$	$3.7x10^2$	(Escamilla-Gómez et al. 2008a)
S.Paratyphi A	Fluorescence	10^3-10^7	10^3	(Yang et al. 2013)
E.coli	Differential Pulse Voltammetry	10^2-2 $x10^3$	50	(Paniel et al. 2013)
E.coli	Amperometry	10^3-$5x10^5$	5	(Paniel et al.

				2013)
S.aureus	Amperometry	10-10^8	10	(Majumdar et al. 2013)
S.aureus	Surface Plasmon resonance	-	10^4	(Balasubramanian et al. 2007)
S.aureus	Potentiometric	10^3-10^8	8 x10^2	(Zelada-Guillén et al. 2012)
S.aureus	Amperometry	4.4×10^5 - 1.8×10^7	1.7 x10^5	(Escamilla-Gómez et al. 2008b)
S.aureus	Differential pulse voltammetry	10^1-10^7	1	This work

Table 2

Comparison of the results obtained by the proposed method with Plate counting method as a standard method in real water sample analyses of *S. aureus*.

Samples	Plate counting method (log *S. aureus*(cfu/ml))	Proposed method (log *S. aureus*(cfu/ml))
Tap water	4.9	4.98 ±0.04
River water	4.9	5.01±0.07

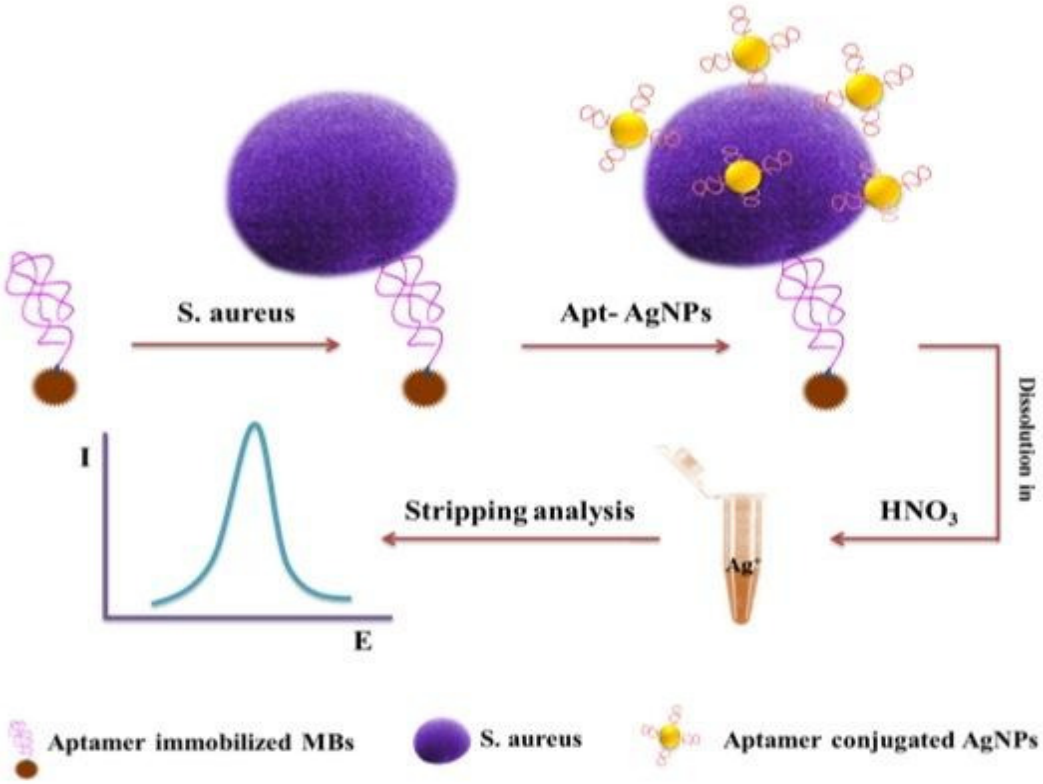
Figure captions

Scheme 1. Schematic representation of the aptamer-based electrochemical determination of *S. aureus*

Fig 1. TEM image (a) and UV–Vis spectrum (b) of synthesized silver nanoparticles

Fig. 2. Optimization of the primary and secondary aptamer concentration. The data points represent the average and standard deviation of measurements obtained from three independent tests. The readout signal was obtained in a 10^6cfu/mL solution of *S.aureus*.

Fig. 3. (a)Differential pulse anodic stripping voltammograms of AgNPs in the presence of different concentrations of *S.aureus.* (b)The linear relationship between the peak current signal of AgNPs and concentration of *S.aureus*



Scheme 1

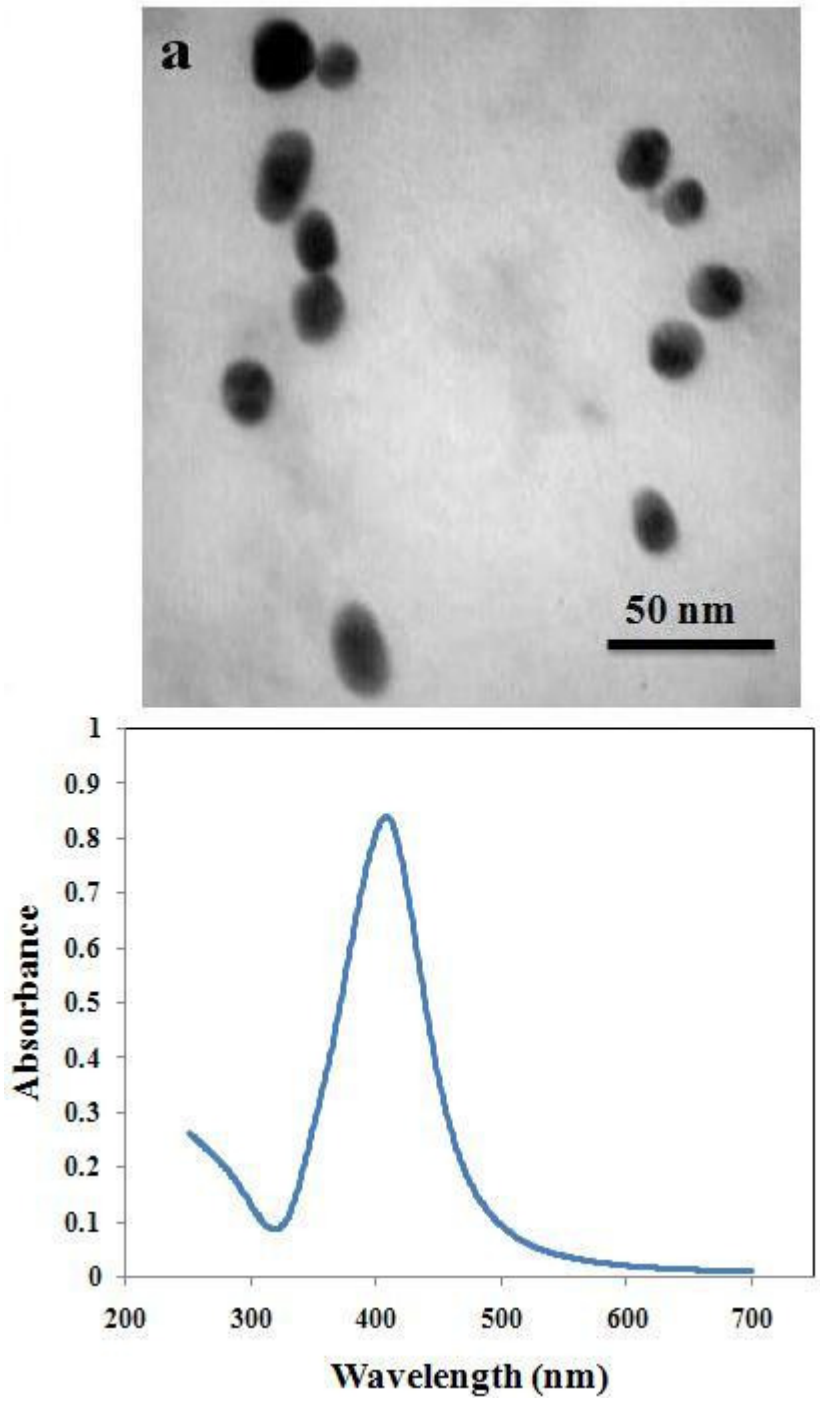

a
50 nm
Absorbance
Wavelength (nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
200
300
400
500
600
700

Figure 1

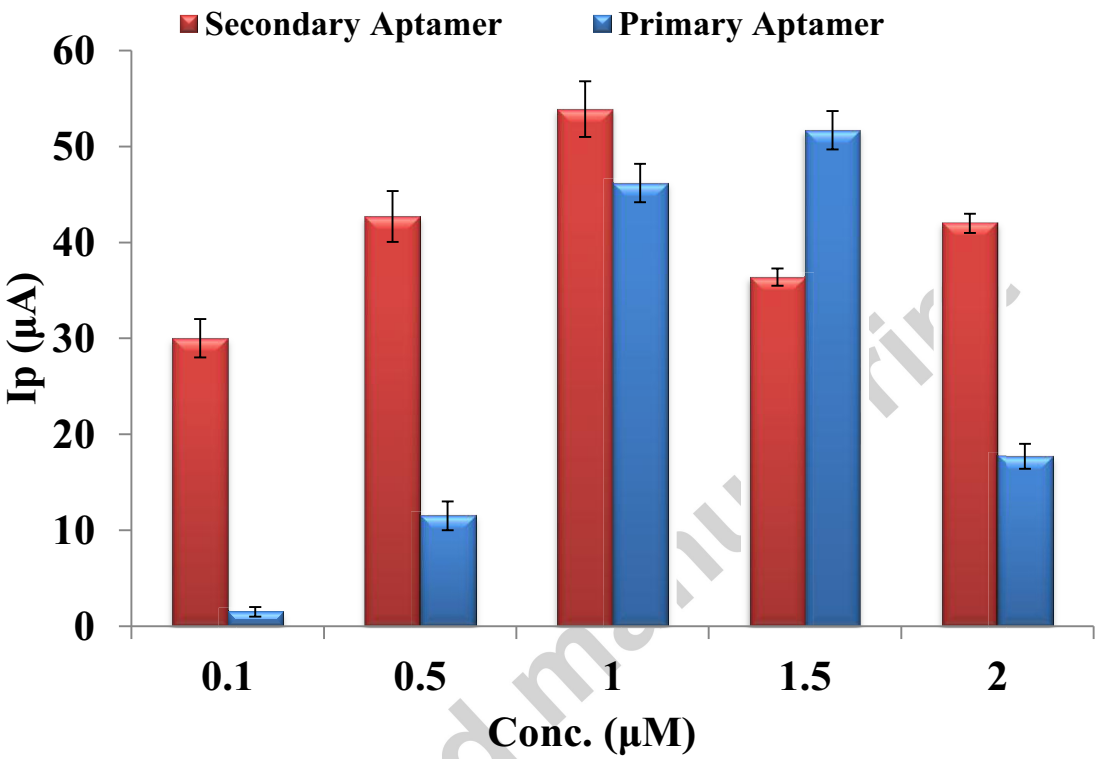


Figure 2

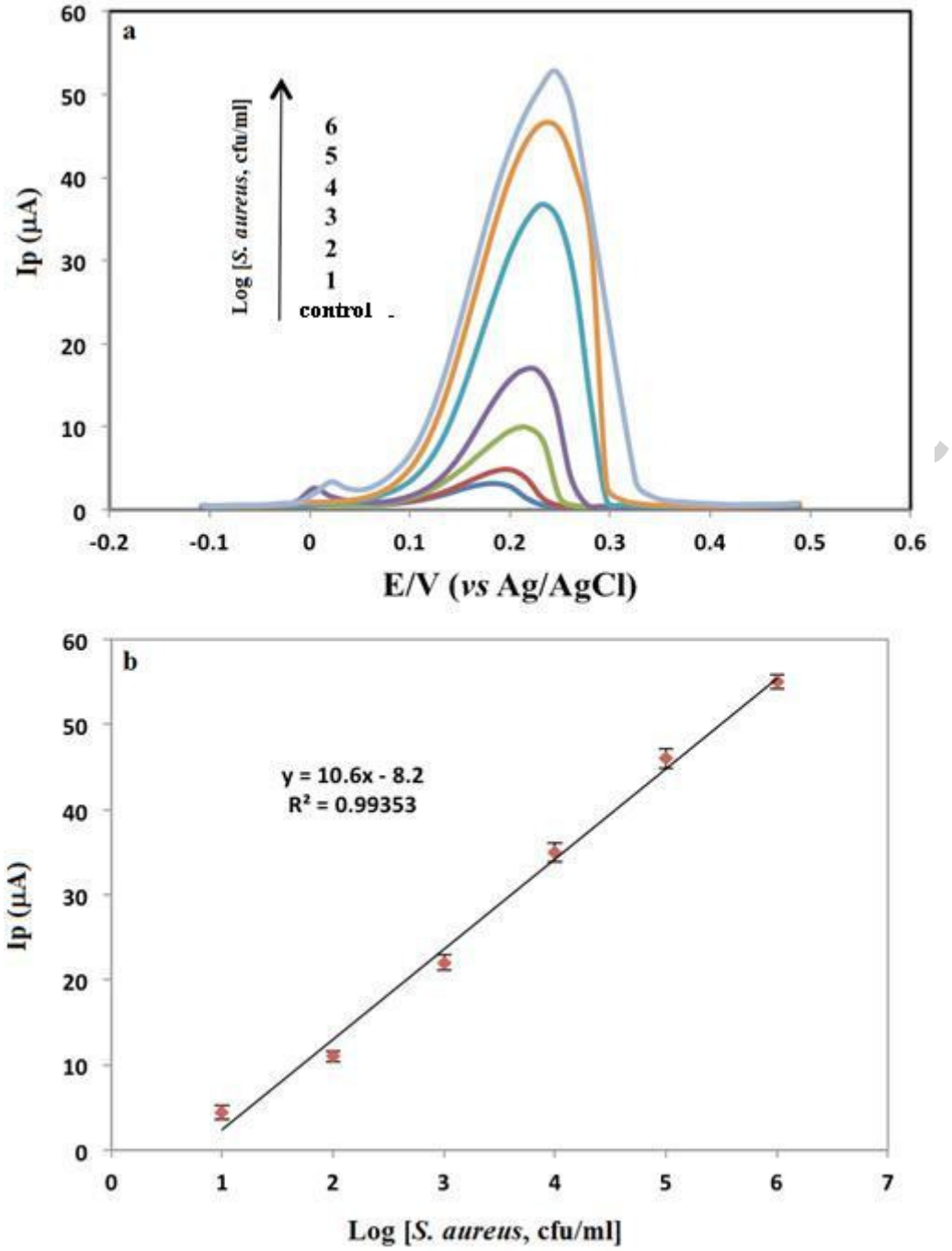

a
Log [S. aureus, cfu/ml]
6
5
4
3
2
1
control
Ip (µA)
E/V (vs Ag/AgCl)
b
y = 10.6x - 8.2
R² = 0.99353
Ip (µA)
Log [S. aureus, cfu/ml]

Figure 3

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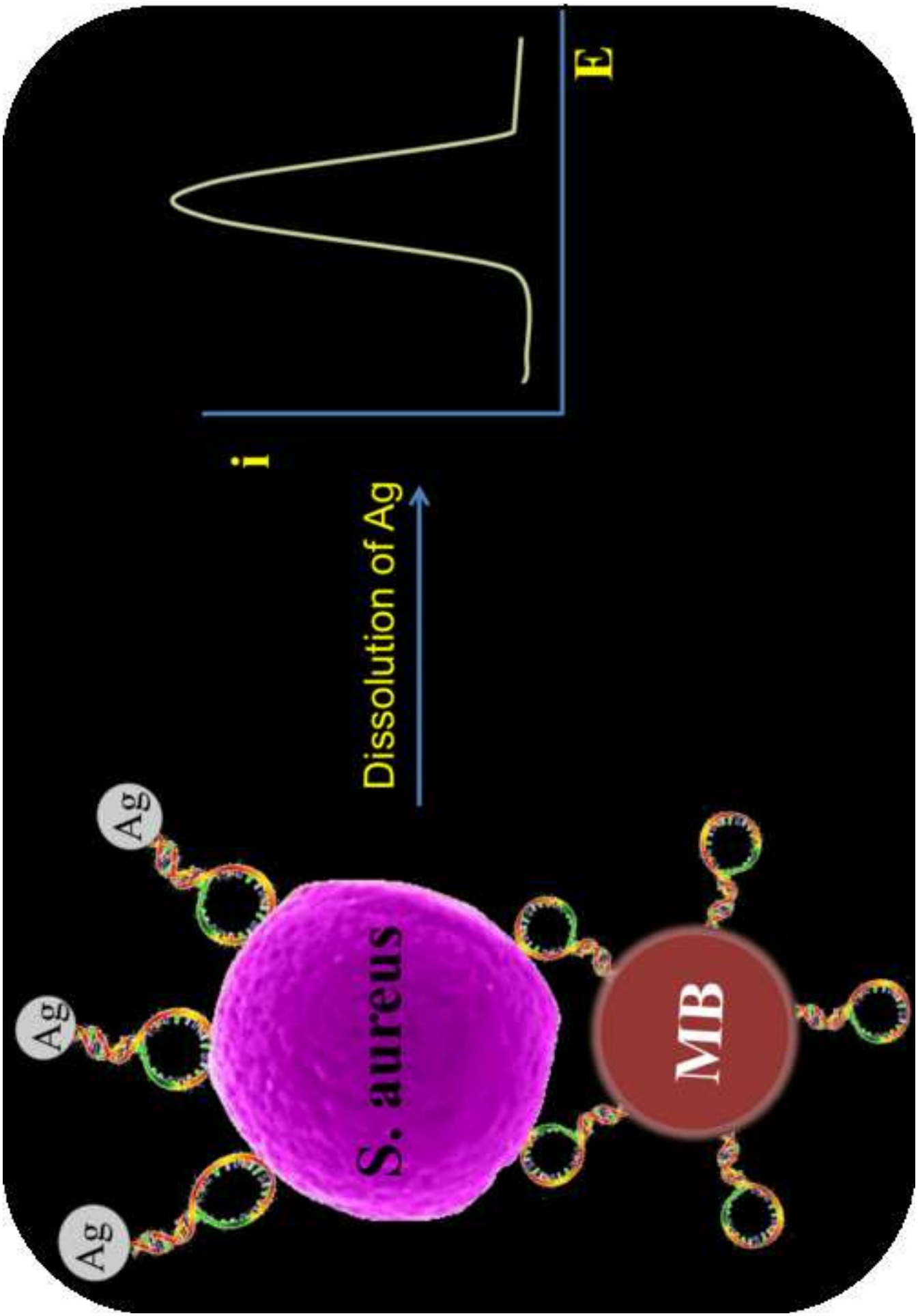
Highlights

- Dual-aptamer-based sandwich immunosensing of *S. aureus*
- primary anti-*S. aureus* aptamer immobilized on magnetic beads as a capture probe
- Secondary anti-*S. aureus* aptamer conjugated to AgNPs as a signaling probe
- Dissolution of AgNPs in acid and its stripping analysis as a readout signal
- Quantification of S. aureus in an extended dynamic range free from interferences of other analogue bacteria



Graphical Abstract