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# Signaling-Probe Displacement Electrochemical Aptamer-based Sensor (SD-EAB) for Detection of Nanomolar Kanamycin A

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ABSTRACT

The signal transduction of most target induced strand displacement-based assays relies on the conformational changes of aptamers, significantly limiting the general applications of these sensors. We report on a very simple and general sensor named signaling-probe displacement electrochemical aptamer-based sensor (SD-EAB), in which signal transduction is induced only by the affinity binding between an aptamer and its target and completely independent of the conformational state of the aptamer. A typical SD-EAB is comprised of a gold electrode immobilized with DNA duplexes formed between a thiolated capture probe (aptamer or its short complementary strand) and a redox tagged signaling probe (short complementary strand or aptamer). In the presence of target, the signaling probe is displaced and released from the electrode surface, leading to the decrease of current proportional to the logarithm of target concentrations. SD-EAB achieved the reagentless detection of kanamycin A with 7 orders of magnitude dynamic ranges (1 nM–10 mM). Amazingly, SD-EAB clearly differentiated kanamycin A from its structural analogues kanamycin B by showing opposite current change. In contrast, its counterpart, the label-free electrochemical impedance spectroscopy (EIS)-based sensor, was one thousand times less sensitive than SD-EAB and had a narrow dynamic range (1–100 μM) due to its limited tolerance of nonspecific adsorption of kanamycin A.



## 1. Introduction

Antibiotics revolutionized medicine in the 20th century due to their effectiveness and easy access. There are thousands of synthetic or natural antibiotics that have been synthesized or isolated as far. However, the abuse of them results in the serious environmental pollution due to the discharge of excrement and urine by human being and poultry, and the emergence of resistance of bacteria. In 2014, the World Health Organization classified the resistance of bacteria as "a serious threat that is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country" in "WHO's first global report on antibiotic resistance". The serious situation has prompted the issue of regulations in many countries and the development of methods for the detection of antibiotic residues in environment.

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Kanamycin A, one of the most widely used aminoglycoside antibiotics, is used to treat a wide variety of infections by inducing mistranslation and blocks translocation by interacting with proteins [1]. The chromatography-based methods [2–5] and immunoassays, typically, Enzyme-Linked Immunosorbent Assays (ELISAs) [6,7] and colloidal gold test strips [8], are the most widely used methods for the quantitative detection and screening of kanamycin A. However, the chromatography-based methods require sophisticated instruments, well-trained personnel, and long time, not suitable for on-site applications. The immunoassays are advantageous to the instrument-based methods because of its operational convenience, high sensitivity, specificity, and rapid turnaround time. Nanomolar kanamycin A was able to be detected with these

*Abbreviations:* ELISA, Enzyme-Linked Immunosorbent Assay; EAB, Electrochemical Aptamer-Based sensors; TREAS, Target-Responsive Electrochemical Aptamer Switch; EIS, Electrochemical Impedance Spectroscopy; EIS-AB, EIS-Aptamer-Based sensor; SD-EAB, Signaling-probe Displacement Electrochemical Aptamer-Based sensor.

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**Table 1**
Comparison of some recently reported aptamer-based sensors and immunoassays for detection of kanamycin A with SD-EAB.

| Sensor types | Limit of detection(M) | Dynamics range (M) | Reagent-less | Assay time (min) |
|---|---|---|---|---|
| electrochemical [19] | $9.4 \times 10^{-9}$ | $5 \times 10^{-8}$–$9 \times 10^{-6}$ | Yes | ~30 |
| electrochemical [21] | $5.8 \times 10^{-9}$ | $1 \times 10^{-8}$–$1.5 \times 10^{-7}$ | No | 60 |
| electrochemical [45] | $1.0 \times 10^{-8}$ | $1 \times 10^{-8}$–$1 \times 10^{-3}$ | Yes | ~30 |
| colorimetric [15] | $1.0 \times 10^{-8}$ | $1 \times 10^{-8}$–$1 \times 10^{-7}$ | No | 60 |
| luminescent [18] | $1.4 \times 10^{-7}$ | $2 \times 10^{-7}$–$1.5 \times 10^{-4}$ | No | 10 |
| FRET [17] | $9 \times 10^{-12}$ | $1 \times 10^{-11}$–$3 \times 10^{-9}$ | No | 210 |
| Rapid-ELISA [8] | $1.7 \times 10^{-9}$ ($IC_{50}$) | – | No | 40 |
| Colloidal gold immunoassay [8] | $1.0 \times 10^{-8}$ | – | Yes | 10 |
| SD-EAB | $1.0 \times 10^{-9}$ | $1 \times 10^{-9}$–$1 \times 10^{-2}$ | Yes | 30 |

$IC_{50}$: 50% inhibition value.

methods. However, the immunoassays need expensive antibodies and enzymes, which is not cost effective. Therefore, the development of fast, cheap, and easy detection systems is particularly important and urgently desired, especially in underdeveloped areas.

Aptamers are peptides or oligonucleotides that specifically recognize and bind to a wide range of targets ranging from small molecules to large proteins and even cells [9,10]. Aptamers have becoming attractive functional molecules for a variety of important applications including biosensors, imaging, drug delivery, and bioengineering [11,12]. Aptamers are particularly useful as biosensing elements as they are chemically stable, readily available, with high purity, and ease of modification in biosensor design [13,14].

As the discovery of anti-kanamycin A aptamers, several aptamer-based kanamycin A biosensors including colorimetric [15,16], fluorescent [17], luminescent [18], and electrochemical sensors [19–21] have been recently reported (Table 1). However, these sensors have some of the following problems that limit their practical applications: narrow dynamic range [15,17–21], complicated sensor preparation process [20,21], or requirement of enzymatic reactions for signal amplification [16].

Target induced strand displacement is one of the most widely used signal transduction strategy in aptamer-based sensors for fluorescent [22,23], colorimetric [24,25], and electrochemical [26,27] detection of DNAs, proteins, ions, and small molecules. Typically, in those sensors, a short complementary DNA strand is hybridized with a DNA aptamer. A target can specifically bind with the aptamer and induce the displacement of the complementary strand, causing a corresponding signal change for quantitative detection of the target. Among these methods, electrochemical aptamer-based sensors (EAB) have attracted extensive attention because they possess many properties that are required for on-site applications: operational simplicity, high sensitivity, portability, and low cost [26–30]. For example, Xiao et al. reported the first target induced strand displacement-based EAB sensor for signal-on detection of thrombin, where a short methylene blue (MB)-tagged oligonucleotide hybridized partially with an anti-thrombin aptamer and partially with a DNA sequence linking the aptamer to the electrode. Thrombin binds with the aptamer and induces the release of one end of MB tagged oligonucleotide to approach the electrode surface, producing an increase of the Faradaic current [29]. Later on, based on the similar concept, they designed an ultrasensitive electrochemical sensor for DNA detection [30]. Fan et al. further simplified the sensor probe design and demonstrated a target-responsive electrochemical aptamer switch (TREAS) for detection of adenosine triphosphate (ATP) at nanomolar concentration, in which the anti-ATP aptamer dually labeled with SH and ferrocene (Fc) at the two terminals was self-assembled on gold electrodes and its complementary strand then hybridized with the aptamer to form the rigid duplex structure and limit the electron transfer of Fc with the electrode [28]. ATP binds with its aptamer and liberates the complementary DNA, leading to the structural switch from the duplex to the tertiary aptamer structure. An increase of current is generated as the Fc moiety approaches the electrode surface. Willner and Dong respectively reported an even simpler strategy by using label-free signaling probe to detection of adenosine using electrochemical impedance spectroscopy (EIS), here referred as EIS-aptamer-based sensor (EIS-AB) [26,27].

The purpose of this work is to develop a sensitive, cheap, and easy aptamer-based method that is able to detect the trace amount of antibiotics in the environmental sample. Taking the detection of kanamycin A as an example, here we report a simple and general signaling-probe displacement electrochemical aptamer-based sensor (SD-EAB, Fig. 1), capable of reagentless detection of kanamycin A with high sensitivity, selectivity, extremely broad dynamic ranges, and good tolerance of nonspecific adsorption.

Compared to the TREAS and EIS-AB, SD-EAB is also simple in design and generalizable while it has several advantages. First, the signal change of SD-EAB is induced only by the affinity binding between an aptamer and its target and completely independent of the conformational state of the aptamer before and after binding with target. The signal change of TREAS still highly relies on the conformational state of the aptamer binding with its target because it determines the distance between the redox moiety and the electrode and therefore has a strong impact on the sensitivity. The small increase of the distance can cause orders of magnitude decrease of the electron exchange rate and therefore seriously confound the sensitivity of the sensor. Similarly, the impedance change of EIS-AB is not only determined by the amount of aptamers or the short complementary probes that are displaced, but also strongly interfered by target itself or contaminants that

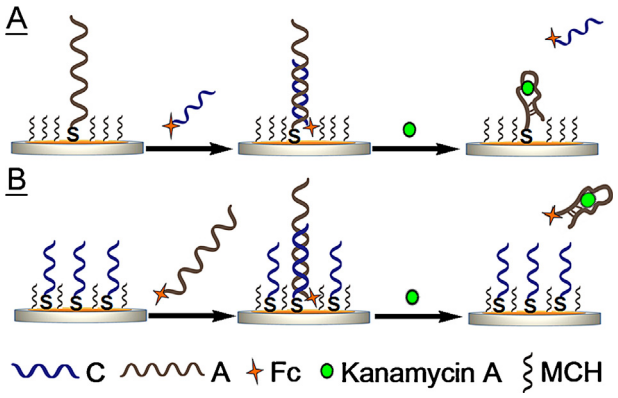


**Fig. 1.** Schematic illustrations of SD-EAB A (A) and B (B) for the detection of Kanamycin A. A SD-EAB is comprised of a gold electrode immobilized with DNA duplexes formed between (A) a thiolated aptamer capture probe and a Fc tagged short complementary signalling probe, or (B) a thiolated short complementary capture probe and a Fc tagged aptamer signalling probe. Kanamycin A binds with an aptamer and induced the displacement of a short complementary (A) or an aptamer signalling probe (B).