Photoelectrochemical and electrochemical ratiometric aptasensing: A case study of streptomycin

Xiuli Shen[a], Dong Liu[a,*], Chengxi Zhu[a], Yuye Li[a], Yang Liu[b], Tianyan You[a,*]

[a] Key Laboratory of Modern Agricultural Equipment and Technology, Ministry of Education, High-tech Key Laboratory of Agricultural Equipment & Intelligentization of Jiangsu Province, School of Agricultural Equipment Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China
[b] College of Science and Engineering, James Cook University, Townsville, Queensland 4811, Australia

ABSTRACT

There has been much interest in constructing ratiometric sensors using different sensing techniques because of their synergistic effect, although the simultaneous collection of the signals is challenging. Herein, we propose a ratiometric aptasensing strategy based on the dual-detection model with a photoelectrochemical (PEC) "signal-on" and an electrochemical (EC) "signal-off". As a proof-of-concept study, CdTe quantum dots (CdTe QDs) and a methylene blue-labeled aptamer (MB-Apt) were used to generate PEC and EC signals in the sensing system. The target-induced conformational change of MB-Apt pushed MB away from the electrode, thereby decreasing the EC signal; at the same time, the reduced steric hindrance favored the restoration of the PEC signal from the CdTe QDs. Thus, this PEC-EC strategy can achieve the PEC "signal-on" and EC "signal-off" states simultaneously, as well as allowing quantitative analysis of the target based on the ratio of the current intensities. As a model application, an aptasensor fabricated for streptomycin detection showed a wide linear range from 0.03 to 100 μM with a detection limit of 10 nM (S/N = 3). The proposed sensing platform displayed superior analytical properties compared with methods based on PEC or EC alone. Our work provides an efficient dual-detection model-based ratiometric strategy for advanced analysis, and paves the way to the simultaneous acquisition of signals.

1. Introduction

Ratiometric aptasensing has attracted extensive attention for advanced analysis owing to its superior sensitivity, selectivity and reliability [1]. A ratiometric sensor produces two separated signals and uses their ratio to eliminate interference factors via the built-in self-calibration strategy [2,3]. Among various state-of-the-art ratiometric aptasensors based on fluorescence (FL) [4], electrochemistry (EC) [5], surface-enhanced Raman scattering (SERS) [6], electrochemiluminescence (ECL) [7] and photoelectrochemistry (PEC) [8], the EC-based ratiometric aptasensors have exhibited better analytical performance, as measured by factors such as response time and sensitivity. In addition, the interface that plays a significant role in producing target-dependent ratiometric responses can be simply constructed using two EC-active probes. For example, in a pioneering work, self-designed ferrocene (Fc) and methylene blue (MB) labeled DNA were used to generate ratiometric signals through target-induced conformational change [9]. Since EC probes have different redox potentials, the dual signals of a ratiometric EC aptasensor can be collected simultaneously via many voltammetric techniques, including differential pulse

voltammetry. Recently, novel substrate materials and signal amplification strategies have been employed to enhance the analytical performance of ratiometric EC aptasensors, leading to rapid development of this field. Nevertheless, each technique, including EC, has its shortcomings, which significantly limit the application of these sensing devices. To overcome these limitations, the rational design of analytical strategies to produce synergistic effects between different sensing techniques opens a new avenue for ratiometric sensor development.

In this regard, a dual-channel sensing technique based on two signal transducers, such as ECL-FL [10], EC-FL [11] or ECL-EC [12], has emerged as an attractive strategy for the fabrication of high-performance ratiometric sensors. Recent investigations have revealed that the dual-channel sensing technique could empower the inherent characteristics of each transducer, as well as efficiently improving the reliability and accuracy of the analysis [13]. Additionally, more information about target recognition can be obtained. Feng et al. proposed an ultrasensitive ratiometric aptasensor for microRNAs based on an ECL-EC strategy, in which the target-triggered conformational change of the aptamer induced the EC "signal-off" while the ECL system displayed "signal-on" [14]. The study of dual-channel sensing

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techniques is currently still in its infancy, and thus novel strategies for extending ratiometric sensing applications are highly desirable.

Compared with EC, the PEC technique has a relatively lower background and favorable sensitivity [15–17]. PEC aptasensing has become an important method for bioanalysis because it combines the advantages of PEC bioanalysis with the unique properties of aptamers, and considerable progress has been made in this field in past years [18]. Furthermore, integrating the PEC technique into a ratiometric strategy can eliminate interference factors, such as fluctuation of light intensity, leading to enhanced analytical reliability and sensitivity [19–21]. However, the routine approach to constructing a ratiometric EC sensor is not applicable to PEC-based sensors. More specifically, PEC sensors are incapable of collecting the signals of various different PEC active materials simultaneously [22]. A ratiometric PEC sensor producing wavelength-dependent photocurrents has been proposed; this employed a hybrid electrode with CdS quantum dots (QDs) and MB, while potentiometric-resolved ratiometric sensing was achieved using $BiPO_4$ NPs and $BiPO_4$-rGO NCs with different critical voltages [23,24]. However, special expertise and sophisticated equipment are usually required for device fabrication (e.g. dual working electrodes) or data acquisition (e.g. optical gratings) when using these techniques. For example, dual-working photoelectrodes fabricated using $NaYF_4$:Yb,Er UCNPs@CdTe nanocrystals are essential to produce two independent responses for spatially-resolved ratiometric sensors [2]. Thus, it is a challenge to construct a ratiometric PEC sensor in a similar way to an EC sensor. In particular, the target-dependent and built-in reference signals are usually derived from different sensing interfaces (PEC active species, etc.), whereas it would be preferable and more accurate to acquire signals induced by the same target recognition through one sensing interface.

Herein, we focused our research on exploring the PEC-EC technique to produce enhanced analytical properties. The EC-active MB was introduced into a PEC sensing interface consisting of CdTe QDs and an aptamer via the end-capping of the aptamer to eliminate the impact of steric hindrance of the aptamer at the electrode on the EC or PEC signals. As illustrated in Scheme 1, MB is expected to produce a redox EC signal at ca. -0.2 V, while the CdTe QDs continue to generate a PEC signal under illumination. In the proposed sensing platform, target-induced conformational change and subsequent stripping of the aptamer decreased the amount of MB at the electrode and reduced the EC response, while the lower steric hindrance contributed to the restoration of the PEC signal from the CdTe QDs. Taking streptomycin (STR) as a model target, a ratiometric aptasensor with a PEC (signal-on)-EC (signal-off) response model showed superior analytical properties compared with a sensor based on PEC or EC alone. The significance of this work lies in the novel and efficient strategy, which is promising for advanced analysis, and in understanding the response mechanism in the sensing interface.

2. Experimental

2.1. Fabrication of the aptasensor

ITO electrodes were treated with 1.0 M NaOH and sonicated in ultrapure water and alcohol. Then 10 μL CdTe QDs solution was cast onto the ITO electrode (diameter: 6 mm), and subsequently 5 μL chitosan solution was added to the electrode. The CdTe/ITO electrode was then incubated with 10 μL MB-Apt for 14 h in the dark at a temperature of 4 °C. Finally, the resulting MB-Apt/CdTe/ITO electrode was rinsed thoroughly with a phosphate-buffered solution (PBS, 0.1 M, pH 7.0) to remove the unbound aptamers.

For STR analysis, a 10 μL STR solution was cast onto the electrode and incubated for 60 min, then rinsed thoroughly with 0.1 M PBS. The EC and PEC signals of the electrodes were then used for STR detection.

2.2. Sample preparation

A stock solution of STR was prepared from ultrapure water and further diluted to obtain the different concentrations of STR solution required for analysis. The real samples used in the experiment were taken from Zhenjiang, China, and pretreated as described in an earlier report [25]. The samples were filtered through a 0.22 μm cellulose membrane to remove impurities. The treated samples were diluted to obtain the desired concentrations.

More experimental details and characterization data are available in the online Supplementary Information.

3. Results and discussion

3.1. The feasibility of the proposed aptasensor

The proposed PEC-EC based ratiometric aptasensor was fabricated through layer-by-layer assembly (Scheme 1). To monitor the assembly process and confirm its feasibility, EC, PEC and electrochemical impedance spectroscopy (EIS) methods were used (Fig. 1). No characteristic current signal was observed (Fig. 1A, curves a and a′) at the bare ITO with a low electronic transfer resistance (R_{et}) of 44 Ω (Fig. 1B, curve a). After modification with CdTe QDs, a significant PEC current (I_{PEC}) of 125 nA (Fig. 1A, curve b') was recorded while the corresponding value of R_{et} (73 Ω) showed an obvious increase, due to its poor electron transfer ability (Fig. 1B, curve b) [26]. The subsequent addition of MB-Apt to the CdTe/ITO led to not only the further enlargement of R_{et} (138 Ω) (Fig. 1B, curve c) but also a significant reduction in I_{PEC} (57 nA, Fig. 1A, curve c′). Meanwhile, an EC current (I_{EC}) of 89 nA with a peak potential of -0.22 V (Fig. 1A, curve c) was observed for the MB-Apt/CdTe/ITO, which was assigned to the redox characteristics of MB [27]. Thus, the observed PEC and EC current signals as well as the gradually increasing R_{et} demonstrate the feasibility of our proposed dual-signal output strategy.

As a model application, the proposed aptasensing platform was used for the specific recognition of STR (1 μM). The value of I_{EC} showed a sharp decrease to 61 nA while the value of I_{PEC} increased to 99 nA (Fig. 1A, curve d and d′). These changes in the signals resulted from the reduced amount of MB and steric hindrance due to the stripping of MB-Apt, which was demonstrated by the lower value of R_{et} (Fig. 1B, curve d) [28]. Thus, our proposed aptasensor displayed the "signal-off" EC response and the "signal-on" PEC response in the presence of STR, and the ratio of the intensities of I_{PEC} and I_{EC} could be used for quantitative analysis with high selectivity.

3.2. Optimization of experimental conditions

The experimental conditions, including the incubation time of MB-Apt and the reaction time for STR with MB-Apt, were optimized to obtain the best results (Fig. 2). To evaluate the optimal incubation time, I_{EC} and I_{PEC} were recorded at the CdTe/ITO after incubation with MB-Apt. There was no significant increase in I_{EC} (decrease in I_{PEC}) after 14 h of incubation time due to saturation of the active sites on MB-Apt [29]. Therefore, 14 h was chosen as the optimal time for MB-Apt incubation (Fig. 2A).

To investigate the effect of STR reaction time on the aptasensor response, I_{EC} and I_{PEC} were recorded at the MB-Apt/CdTe/ITO after incubation with STR. Clearly, the change in I_{PEC} increased (I_{EC} decreased) when the interaction time was between 20 and 50 min and then reached a plateau after 60 min, indicating that 60 min was the optimal reaction time within the operating range (Fig. 2B).

3.3. Analytical performance of the proposed aptasensor

Under the optimal conditions, the analytical properties of the proposed PEC-EC ratiometric aptasensor were evaluated by detecting
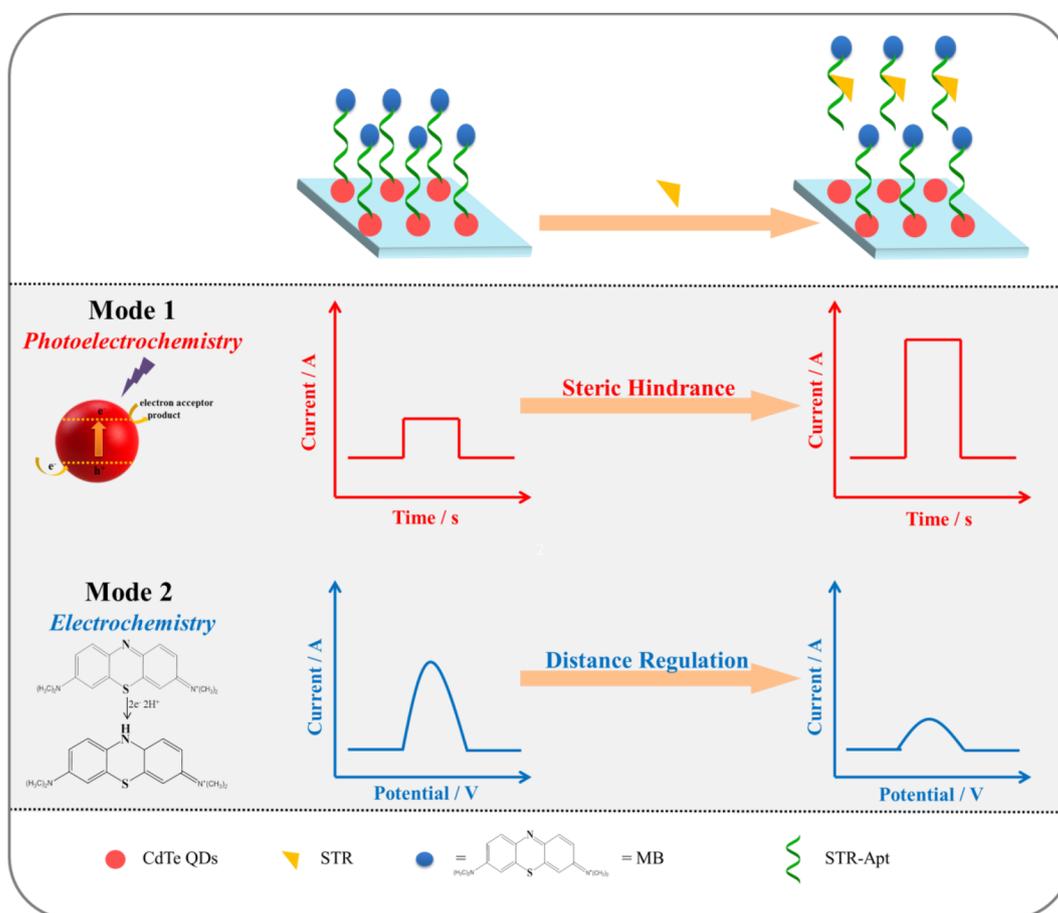
Scheme 1. Schematic illustration of the fabrication and working principle of the proposed aptasensor.
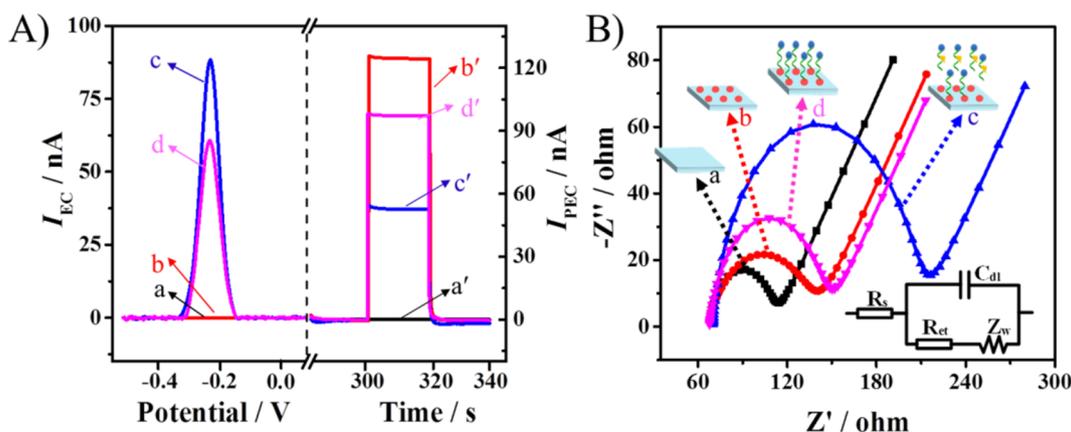
Fig. 1. (A) EC and PEC responses of (a, a′) bare ITO, (b, b′) CdTe/ITO, (c, c′) MB-Apt/CdTe/ITO and (d, d′) upon the addition of 1 μM STR in 0.1 M PBS. (B) Nyquist plots from EIS: (a) bare ITO, (b) CdTe/ITO, (c) MB-Apt/CdTe/ITO and (d) upon the addition of 1 μM STR (the inset shows the equivalent circuit of the Nyquist plots).

standard samples of STR at different concentrations. As the concentration of STR (C_{STR}) increased, the I_{EC} gradually decreased and the I_{PEC} increased (Fig. 3A). Their intensities showed a well-behaved linear relationship with the logarithmic values of C_{STR} in the range 0.10 Mm–1.0 μM for PEC and 0.03 Mm–10 μM for EC (Fig. 3B). Furthermore, the limit of detection (LOD, S/N = 3) for PEC and EC were calculated to be 0.03 μM and 0.01 μM, respectively. The PEC and EC responses toward each C_{STR} are shown in Fig. 3C, which indicates that the trend in the dual-signal changes clearly with the increase in C_{STR}. Fig. 3D reveals the linear relationship between the value of I_{PEC}/I_{EC} and the logarithm of C_{STR} from 0.03 μM to 100 μM (I_{PEC}/I_{EC} = 6.24 + 0.76logC_{STR}, correlation coefficient (R^2): 0.9991); the obtained LOD was 10 nM (S/

N = 3). Obviously, the use of a ratiometric strategy led to a significantly expanded linear range compared with PEC or EC tests alone.

The selectivity of the proposed aptasensor was investigated by detecting STR in the presence of some other antibiotics, including CIP, KAN, and GEN. Although the aptasensor showed a slight signal change for each interferent (10 μM), remarkable responses were observed for STR (1 μM) or mixtures of STR and different interferents, revealing the specificity of the aptasensor toward STR (Fig. 4A). Additionally, the reproducibility of the electrode used in the ratiometric aptasensor was studied by testing with six different electrodes. The relative standard deviation (RSD) was calculated to be 1.40%, which is satisfactory for practical applications (Fig. 4B).
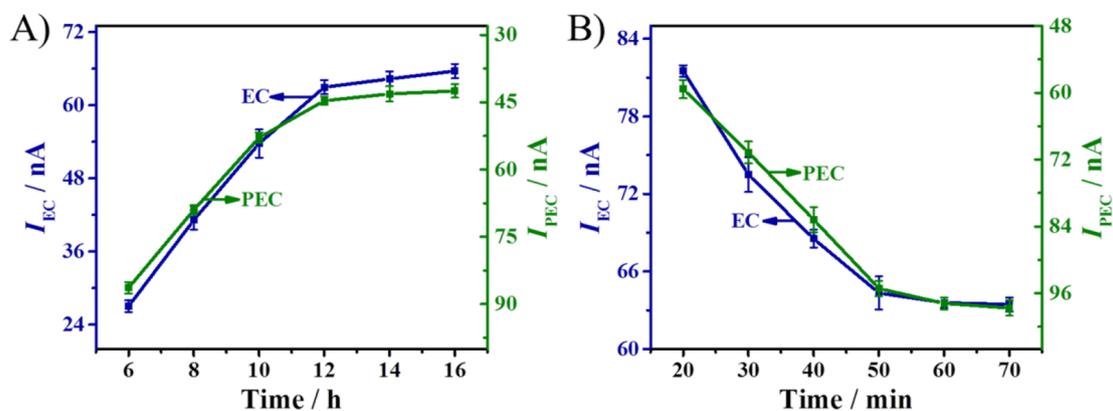
Fig. 2. Effects of (A) incubation time of MB-Apt and (B) reaction time for STR and MB-Apt on the EC and PEC response.
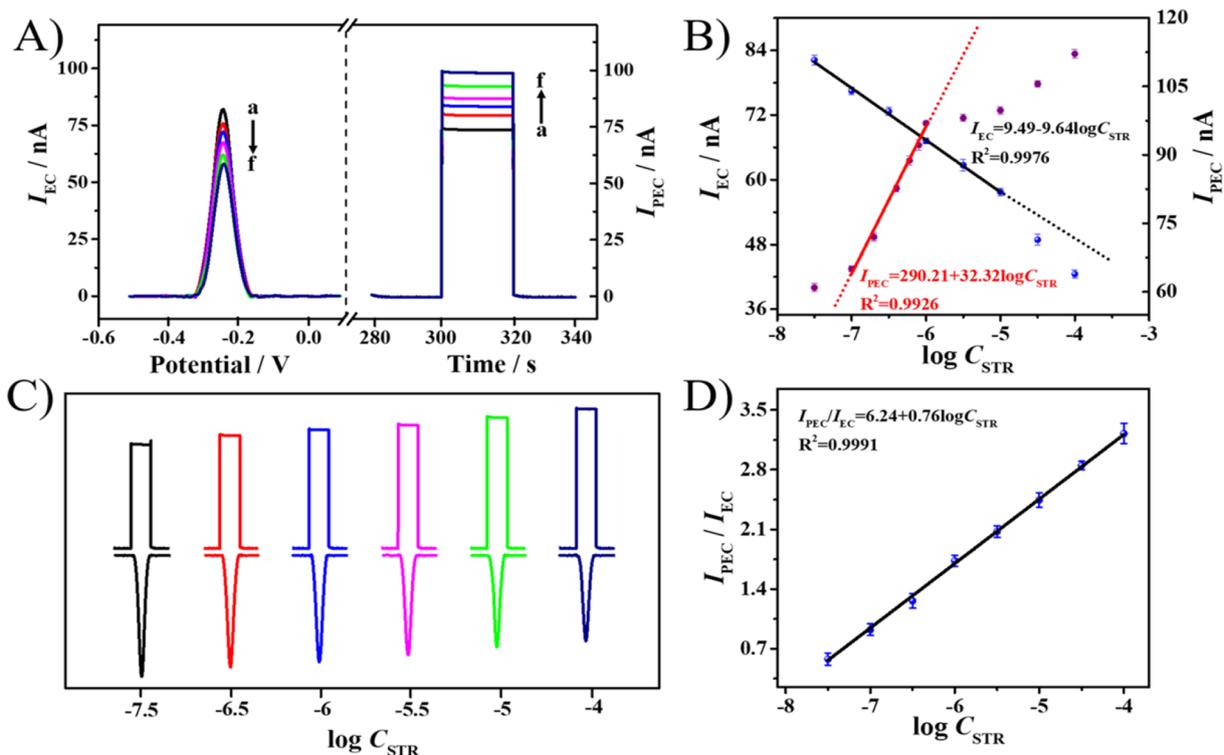

Fig. 3. (A) EC and PEC responses of the proposed aptasensor for the detection of different concentrations of STR. (B) Logarithmic dependence of EC (black line) and PEC (red line) current on target STR concentration. (C) EC and PEC responses of the proposed aptasensor for the detection of STR. (D) Linear relationship between I_{PEC}/I_{EC} and the logarithm of target STR concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

In summary, a novel ratiometric sensing strategy was developed by combining PEC and EC techniques. A proof-to-concept sensing platform was fabricated using CdTe QDs and MB-Apt of STR to assemble a dual-signal response interface. For STR analysis, the proposed PEC-EC-based ratiometric aptasensor showed significantly enhanced properties compared with those based on the PEC or EC method alone, revealing its potential for advanced analysis. Additionally, the aptasensor has been successfully used to detect STR in real samples, including tap water and Yangtze River water (see Section 1.7 and Table S2 in the Supplementary Information). Using this new strategy, the dual-detection responses, which are PEC "signal-on" and EC "signal-off", operated simultaneously for target recognition, however, the PEC and EC signals were collected separately. Further work is therefore underway to achieve the simultaneous acquisition of multiple signals, which will provide new opportunities for the accurate and sensitive detection of target molecules.

CRediT authorship contribution statement

Xiuli Shen: Investigation, Data curation, Writing - original draft. **Dong Liu:** Methodology, Conceptualization, Formal analysis, Writing - review & editing. **Chengxi Zhu:** Resources, Software. **Yuye Li:** Resources, Software. **Yang Liu:** Writing - review & editing. **Tianyan You:** Supervision, Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
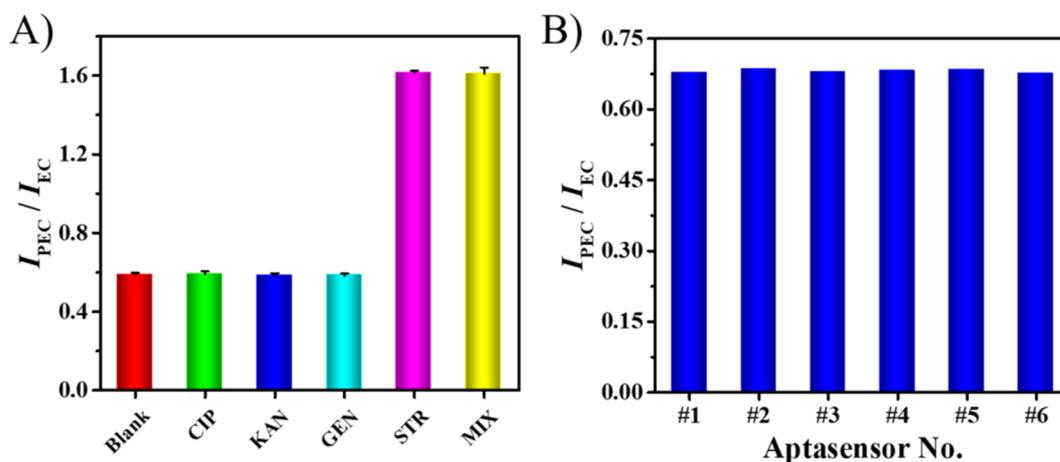
Fig. 4. (A) The selectivity of the developed aptasensor toward STR ($n = 3$). (B) Reproducibility obtained using six parallel measurements.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.elecom.2019.106637.

References

[1] T. Yang, R.Z. Yu, Y.H. Yan, H. Zeng, S.Z. Luo, N.Z. Liu, A. Morrin, X.L. Luo, W.H. Li, A review of ratiometric electrochemical sensors: from design schemes to future prospects, Sens. Actuators B 274 (2018) 501–516.

[2] Z.L. Qiu, J. Shu, J.F. Liu, D.P. Tang, Dual-channel photoelectrochemical ratiometric aptasensor with up-converting nanocrystals using spatial-resolved technique on homemade 3D printed device, Anal. Chem. 91 (2019) 1260–1268.

[3] C.X. Zhu, D. Liu, Y.Y. Li, X.L. Shen, L.B. Li, Y. Liu, T.Y. You, Ratiometric electrochemical, electrochemiluminescent, and photoelectrochemical strategies for environmental contaminant detection, Curr. Opin. Electrochem. 17 (2019) 47–55.

[4] X. Yan, H.X. Li, X.S. Han, X.G. Su, A ratiometric fluorescent quantum dots based biosensor for organophosphorus pesticides detection by inner-filter effect, Biosens. Bioelectron. 74 (2015) 277–283.

[5] L. Cui, M.F. Lu, Y. Li, B. Tang, C.Y. Zhang, A reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence for sensitive detection of adenosine, Biosens. Bioelectron. 102 (2018) 87–93.

[6] H.Y. Shi, N. Chen, Y.Y. Su, H.Y. Wang, Y. He, Reusable silicon-based surface-enhanced Raman scattering ratiometric aptasensor with high sensitivity, specificity, and reproducibility, Anal. Chem. 89 (2017) 10279–10285.

[7] M. Wei, C.L. Wang, E.S. Xu, J. Chen, X.L. Xu, W. Wei, S.Q. Liu, A simple and sensitive electrochemiluminescence aptasensor for determination of ochratoxin A based on a nicking endonuclease-powered DNA walking machine, Food Chem. 282 (2019) 141–146.

[8] R. Hua, N. Hao, J.W. Lu, J. Qian, Q. Liu, H.N. Li, K. Wang, A sensitive potentiometric resolved ratiometric photoelectrochemical aptasensor for *Escherichia coli* detection fabricated with non-metallic nanomaterials, Biosens. Bioelectron. 106 (2018) 57–63.

[9] Y. Du, B.J. Lim, B.L. Li, Y.S. Jiang, J.L. Sessler, A.D. Ellington, Reagentless, ratiometric electrochemical DNA sensors with improved robustness and reproducibility, Anal. Chem. 86 (2014) 8010–8016.

[10] H. Wang, Q. Ma, Y.F. Wang, C.H. Wang, D.D. Qin, D.L. Shan, J. Chen, X.Q. Lu, Resonance energy transfer based electrochemiluminescence and fluorescence sensing of riboflavin using graphitic carbon nitride quantum dots, Anal. Chim. Acta 973 (2017) 34–42.

[11] A. Mars, M. Hamami, L. Bechnak, D. Patra, N. Raouafi, Curcumin-graphene quantum dots for dual mode sensing platform: electrochemical and fluorescence

[12] G.F. Jie, L. Tan, Y. Zhao, X.C. Wang, A novel silver nanocluster in situ synthesized as versatile probe for electrochemiluminescence and electrochemical detection of thrombin by multiple signal amplification strategy, Biosens. Bioelectron. 94 (2017) 243–249.

[13] D.G. Rackus, M.H. Shamsi, A.R. Wheeler, Electrochemistry, biosensors and microfluidics: a convergence of fields, Chem. Soc. Rev. 44 (2015) 5320–5340.

[14] X.B. Feng, N. Gan, H.R. Zhang, T.H. Li, Y.T. Ca, F.T. Hu, Q.L. Jiang, Ratiometric biosensor array for multiplexed detection of microRNAs based on electrochemiluminescence coupled with cyclic voltammetry, Biosens. Bioelectron. 75 (2016) 308–314.

[15] Z. Chen, D. Liu, C.X. Zhu, L.B. Li, T.Y. You, Facile preparation of hemin-functionalized electrochemically reduced graphene oxide nanocomposite for H_2O_2 biosensing, Sensor. Mater. 31 (2019) 1167–1179.

[16] L. Ge, Y.H. Xu, L.J. Ding, F.H. You, Q. Li, K. Wang, Perovskite-type $BiFeO_3$/ultrathin graphite-like carbon nitride nanosheets p-n heterojunction: Boosted visible-light-driven photoelectrochemical activity for fabricating ampicillin aptasensor, Biosens. Bioelectron. 124–125 (2019) 33–39.

[17] W.W. Zhao, J.J. Xu, H.Y. Chen, Photoelectrochemical DNA biosensors, Chem. Rev. 114 (2014) 7421–7441.

[18] W.W. Zhao, J.J. Xu, H.Y. Chen, Photoelectrochemical aptasensing, Trend. Anal. Chem. 82 (2016) 307–315.

[19] P.C. Yan, D.S. Jiang, H.N. Li, J. Bao, L. Xu, J.C. Qian, C. Chen, J.X. Xia, $BiPO_4$ nanocrystal/BiOCl nanosheet heterojunction as the basis for a photoelectrochemical 4-chlorophenol sensor, Sens. Actuators B 279 (2019) 466–475.

[20] Y. Zang, J.P. Lei, H.X. Ju, Principles and applications of photoelectrochemical sensing strategies based on biofunctionalized nanostructures, Biosens. Bioelectron. 96 (2019) 8–16.

[21] W.W. Zhao, J.J. Xu, H.Y. Chen, Photoelectrochemical enzymatic biosensors, Biosens. Bioelectron. 92 (2017) 294–304.

[22] N. Hao, Y. Zhang, H. Zhong, Z. Zhou, R. Hua, J. Qian, Q. Liu, H.N. Li, K. Wang, Design of a dual channel self-reference photoelectrochemical biosensor, Anal. Chem. 89 (2017) 10133–10136.

[23] Q. Hao, X.N. Shan, J.P. Lei, Y. Zang, Q.H. Yang, H.X. Ju, A wavelength-resolved ratiometric photoelectrochemical technique: design and sensing applications, Chem. Sci. 7 (2016) 774–780.

[24] Y. Zhang, N. Hao, Z. Zhou, R. Hua, J. Qian, Q. Liu, H.N. Li, K. Wang, Potentiometric resolved ratiometric photoelectrochemical aptasensor, Chem. Commun. 53 (2017) 5810–5813.

[25] X.H. Liu, L.J. Luo, L.B. Li, Z.X. Di, J.Y. Zhang, T.Y. You, An electrochemiluminescence aptasensor for analysis of bisphenol A based on carbon nanodots composite as co-reaction of $Ru(bpy)_3{}^{2+}$ nanosheets, Electrochim. Acta 319 (2019) 849–858.

[26] Y.X. Lin, Q. Zhou, D.P. Tang, R. Niessner, H.H. Yang, D. Knopp, Silver nanolabels-assisted ion-exchange reaction with CdTe quantum dots mediated exciton trapping for signal-on photoelectrochemical immunoassay of mycotoxins, Anal. Chem. 88 (2016) 7858–7866.

[27] T. Hou, W. Li, X.J. Liu, F. Li, Label-free and enzyme-free homogeneous electrochemical biosensing strategy based on hybridization chain reaction: a facile, sensitive and highly specific microRNA assay, Anal. Chem. 87 (2015) 11368–11374.

[28] X.X. Xu, D. Liu, L.J. Luo, L.B. Li, K. Wang, T.Y. You, Photoelectrochemical aptasensor based on CdTe quantum dots-single walled carbon nanohorns for the sensitive detection of streptomycin, Sens. Actuators B 251 (2017) 564–571.

[29] F.L. Li, Y.M. Guo, X.Y. Wang, X. Sun, Multiplexed aptasensor based on metal ions labels for simultaneous detection of multiple antibiotic residues in milk, Biosens. Bioelectron. 115 (2018) 7–13.