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Stable and Reusable Electrochemical Biosensor for Poly(ADP-ribose) Polymerase and Its Inhibitor Based on Enzyme-Initiated Auto-PARylation

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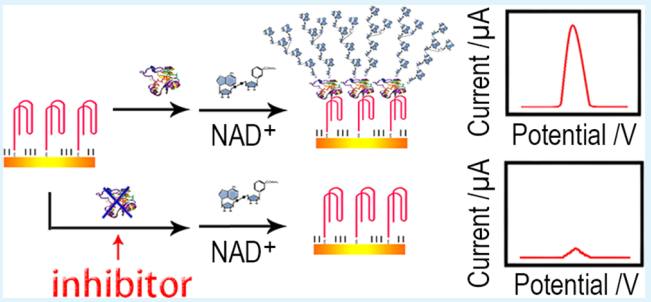
Supporting Information

ABSTRACT: A stable and reusable electrochemical biosensor for the label-free detection of poly(ADP-ribose) polymerase (PARP) is designed in this work. C-kit-1, a thiol-modified G-quadruplex oligonucleotide, is first self-assembled on a gold electrode surface. The G-quadruplex structure of c-kit-1 can specifically tether and activate PARP, resulting in the generation of negatively charged poly(ADP-ribose) polymer (PAR). On the basis of electrostatic attraction, PAR facilitates the surface accumulation of positively charged electrochemical signal molecules. Through the characterization of electrochemical signal molecules, the label-free quantification of PARP is simply implemented. On the basis of the proposed method, selective quantification of PARP can be achieved over the linear range from 0.01 to 1 U with a calculated detection limit of 0.003U. Further studies also demonstrate the applicability of the proposed method to biosamples revealing the broad potential in practical applications. Furthermore, inhibitor of PARP has also been detected with this biosensor. Meanwhile, benefited from self-assembly on solid surface, this biosensor possesses two important features, i.e., reusability and stability, which are desirable in related biosensors.



KEYWORDS: *electrochemical biosensor, stability and reusability, poly(ADP-ribose) polymerase, enzyme-initiated auto-PARylation, inhibitor*

INTRODUCTION

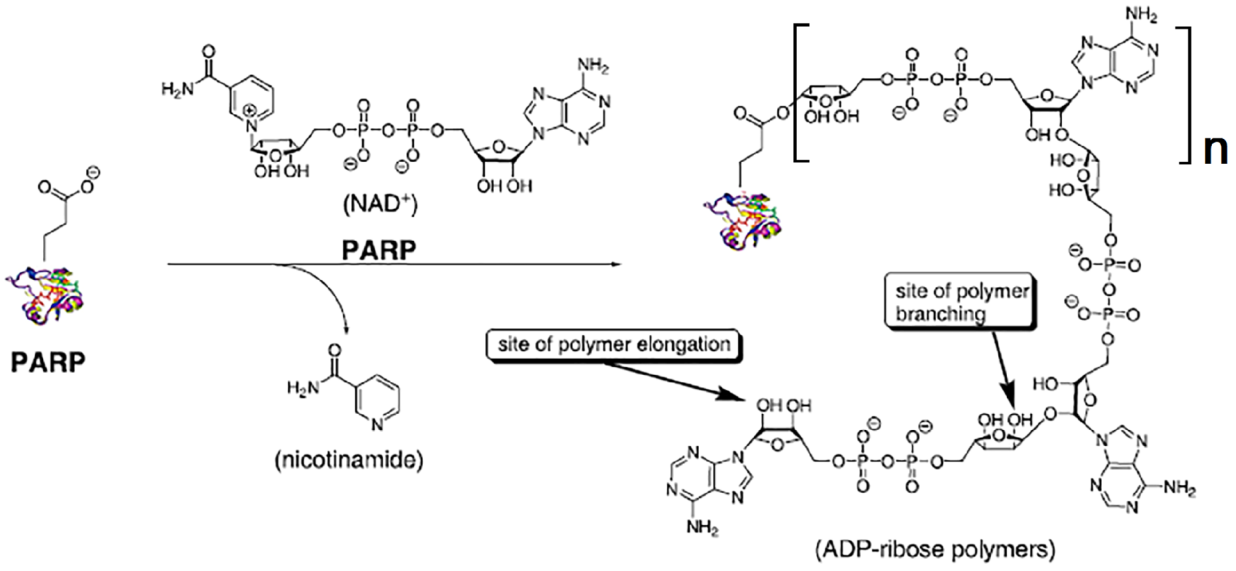
Poly(ADP-ribosyl)ation (PARylation) is an indispensable protein post-translational modification process catalyzed by the poly(ADP-ribose) polymerase family.[1,2] In this process, poly(ADP-ribose) polymerase is activated by specific DNA,[3,4] and the substrate, nicotinamide adenine dinucleotide (NAD^+), is subsequently cleaved into nicotinamide and an ADP-ribose unit that polymerizes onto multiple acceptor proteins to form a linear or branched poly(ADP-ribose) polymer (PAR) via a repeated reaction (Scheme 1).[5] The formed polymer consisting of a few to 200 ADP-ribose units is usually negatively charged.[6] Approximately 90% of the total PARylation in a living cell is assumed by poly(ADP-ribose) polymerase-1 (PARP), which plays important roles not only in DNA repair,[7−10] transcriptional control,[11] and DNA damage-induced cell death[12] but also in the restriction of aging rate.[13] Recent research demonstrates that the inhibitors of PARP are effective adjuvant therapeutics for various cancers.[14−16] Thus, the studies on PARylation, especially the fabrication of biosensors for PARP and its inhibitors, are of great importance.

Up to now, many efforts surrounding PARylation determination have been made, and most of them either use modified substrates or employ antibodies.[17−21] It is known that the labeling process is time-consuming, costly, and often leads to the denaturation of biomolecules. To address this problem, advanced methods to investigate PARylation have been developed.[20,22] For example, we reported a colorimetric method to detect protein PARylation based on NAD^+ protected gold nanoparticles, which avoids the usage of modified substrates.[23] Therefore, the procedure of the PARylation assay was greatly simplified. However, it is known that the stabilization of gold nanoparticles is vulnerable by multiple factors, which may restrict the application of the method in real sample detection.[24] Due to the advantages of the electrochemical method, such as ultrasensitivity, high selectivity, and limited interference, it is suitable to survey the protein post-translational modification processes with electrochemical methodologies, which have been demonstrated by the research reported before.[25−27] For example, protein biotinylation has been successfully detected by electrochemical

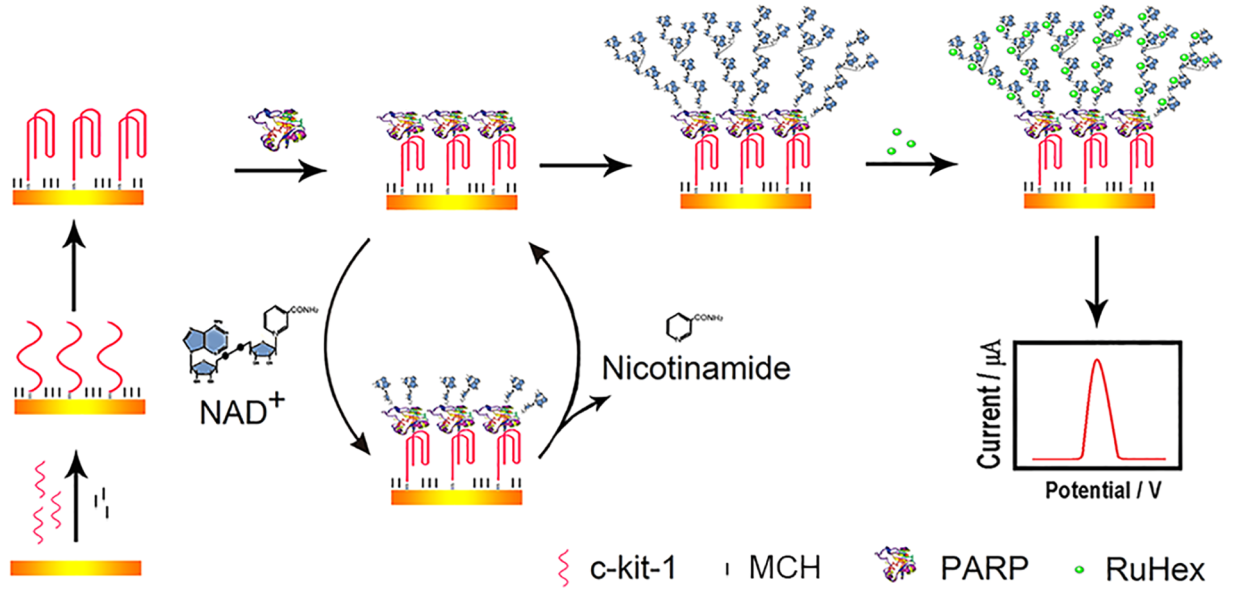
Received: February 17, 2016
Accepted: July 1, 2016

DOI: 10.1021/acsami.6b01883
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

Scheme 1. Schematic Illustration of Reaction Catalyzed by PARP



Scheme 2. Schematic Illustration of a Stable and Reusable Electrochemical Biosensor for PARP and Its Inhibitor Based on Enzyme-Initiated Auto-PARylation



methods,[28] and on the basis of the same experimental technology, protein glycosylation and phosphorylation have also been carried out.[25,27]

It is reported that a special DNA G-quadruplex, c-kit-1, could effectively combine with PARP and activate the enzyme.[29] This combination makes the quantification of PARP on an interface possible, because the modification of DNA onto an electrode surface has been widely applied in vast realms. More importantly, since the natural catalytic products of PARP are negatively charged, label-free electrochemical quantification of PARP could be easily implemented by employing positively charged molecules as electrochemical signal molecules. Therefore, the electrochemical technique is a preferential choice for PARP detection without the participation of modified substrates or antibodies. In this work, we fabricate a label-free electrochemical biosensor that can be used to detect PARP and its inhibitor selectively and sensitively (Scheme 2). The c-kit-1 with a thiol group is first immobilized on the electrode surface by forming an Au−S bond. Then, the c-kit-1 modified gold electrode is treated by mercaptohexanol (MCH), a thiolated 6 chain carbon, which is used to make the monolayer uniform and facilitate the formation of G-quadruplex.[28] Subsequently, c-kit-1 modified on the electrode surface constructs a parallel G-quadruplex structure.[30,31] It is known that PARP can bind to the G-quadruplex structure naturally and directly and subsequently form PARP homodimer to initiate PARylation on the electrode surface.[29] As a result, NAD^+ is cleaved into nicotinamide and an ADP-ribose unit that is covalently linked onto PARP itself to form a negatively charged polymer via repeated reaction. Due to an electrostatic interaction, positively charged signal molecules, hexaammineruthenium(III) chloride (RuHex), can be adsorbed onto the electrode surface, causing an intense electrochemical response. Accordingly, the label-free quantification of PARP can be actualized on the basis of the

DOI: 10.1021/acsami.6b01883
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

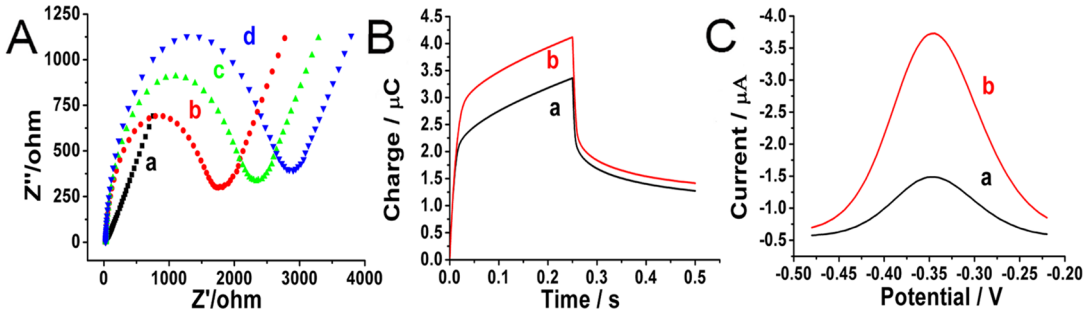


Figure 1. (A) Nyquist diagrams for electrochemical impedance measurements of the gold electrode at different modification stages (electrochemical impedance spectroscopy buffer: 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3; Init E: 0.224 V, 0.1−100 000 Hz): (a) the bare gold electrode, while (b−d) show the cases after (b) c-kit-1 immobilization, (c) PARP incubation, and (d) further treatment by NAD^+, respectively. (B) CC curves for the detection of PARylation obtained from the biosensor treated (a) without and (b) with 500 μM NAD^+ (Init E: 0 V; Final E: −0.5 V). (C) SWV curves for the detection of PARylation obtained from the biosensor treated (a) without and (b) with 500 μM NAD^+ (Init E: −0.2 V; Final E: −0.5 V; Frequency: 30 Hz).

characterization of electrochemical signals. To be noted, benefited from self-assembly on the electrode surface, strong stability of DNA-modified electrode is implemented, which makes the electrochemical biosensor readily useable for long-term detection. In addition, the convenient removal of the combination between c-kit-1 and PARP further endows the interfacial biosensor with the property of reusability, rendering it suitable for routine applications.

EXPERIMENTAL SECTION

Materials. MCH, ethylenediamine tetraacetic acid (EDTA), RuHex, tris(2-carboxyethyl) phosphine hydrochloride (TCEP), diethyl pyrocarbonate (DEPC), NAD^+, and bovine serum albumin (BSA) from human plasma were obtained from Sigma-Aldrich (Shanghai, China). 3-Aminbenzamide (3-AB, an accepted PARP inhibitor) was purchased from Aladdin (Shanghai, China).[23] Human PARP (113kD) was obtained from Trevigen (Gaithersburg, MD, USA). Other chemicals were of analytical grade and were used without further purification. The oligonucleotides (c-kit-1) were synthesized and purified by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). The sequence of c-kit-1 was as follows, and the specific base pairs used to form a G-quadruplex structure is highlighted in bold:[29,30] c-kit-1:5′-SH-CCC**GGGCGGG**CGCGA**GGGGAGGG**-GAGG-3′.

The buffers employed in this work were prepared with DEPC treated ddH_2O, which was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA). The immobilization buffer (I-buffer) contained 10 mM Tris-HCl, 10 mM TCEP, and 0.1 M NaCl (pH 7.4). The ingredients of the quadruplex formation buffer (Q-buffer) were 50 mM Tris-HCl and 100 mM KCl (pH 7.4). The reaction buffer (R-buffer) consisted of 50 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, and 50 μM $Zn(OAc)_2$ (pH 7.4). Electrochemical measurement was carried out in the solution containing 10 mM Tris-HCl and 5 μM RuHex (pH 7.4).

Preparation of c-kit-1 Quadruplex Modified Gold Electrode. Before use, the disk gold electrode was first polished with alumina (1 and 0.3 μm) slurry on silk to obtain a mirror surface. After being thoroughly rinsed with ddH_2O, the electrode was ultrasonicated in ethanol and ddH_2O for 5 min each. Then, the electrode was immersed in a freshly prepared piranha solution for 5 min. *(CAUTION: "Piranha" solution reacts violently with organic materials; it must be handled with extreme care.)* Afterward, the electrode was electrochemically swept in 0.5 M H_2SO_4 by cyclic voltammetry to remove any remaining impurities. After drying with nitrogen, the gold electrode was immersed into 100 μL of I-buffer containing 0.2 μM c-kit-1 for 12 h. After the modified electrode was immersed into 100 μL of 1 mM MCH for 1 h, a well-aligned DNA monolayer was formed on the electrode surface. The DNA quadruplexes were then prepared by immersing the electrode into 100 μL of Q-buffer and heating to 95 °C for 5 min. After slowly cooling down to room temperature, the c-kit-1 modified electrode was obtained.

Electrochemical Detection for Protein PARylation. The PARP catalyzed assay was carried out as follows. The above c-kit-1 modified gold electrode was immersed into 100 μL of R-buffer containing different amounts of PARP (0, 0.005, 0.01, 0.1, 0.25, 0.5, 0.75, 1, 2, and 5 U). After incubation for 1 h at 25 °C, the modified c-kit-1 was fully combined with PARP. Then, the electrode was subsequently incubated with 100 μL of 500 μM NAD^+ diluted by R-buffer for 1 h at 25 °C. After that, the electrode was thoroughly rinsed and prepared for electrochemical measurements. Square wave voltammetry (SWV) was performed on a CHI 660E (CH Instruments) electrochemical analyzer. Control experiments were conducted using R-buffer, BSA, heat deactivated-PARP, and 3-AB treated-PARP instead of PARP. Then, a series of experiments for PARP in fetal bovine serum was carried out. Before determination, the serum was diluted 10-fold with R-buffer. To testify reusability of the biosensor, the electrode was simply treated with 5% SDS solution and then immersed into 100 μL of Q-buffer after one detection of protein PARylation. Then, the electrode was heated to 95 °C for 5 min to reform the G-quadruplex structure. Afterward, the electrode was ready to repeatedly detect PARylation. The stability of the biosensor was evaluated by storing the c-kit-1 modified electrode at 4 °C in I-buffer. The modified electrode was used for the electrochemical detection of protein PARylation every 5 days.

Electrochemical Detection for 3-AB. A series of 3-AB standard solutions at different concentrations was mixed with 1 U PARP and was diluted by R-buffer to the final concentrations of 3-AB to 1, 5, 10, 30, and 50 nM, respectively. After incubation for several hours at 4 °C, c-kit-1 modified gold electrode was immersed into 100 μL of the above mixture solution. Subsequently, the electrode was incubated with 100 μL of 500 μM NAD^+ for 1 h at 25 °C. Then, the electrode was ready for electrochemical measurements.

RESULTS AND DISCUSSION

Feasibility of the Proposed Biosensor. First, the stepwise modification process on the electrode surface has been characterized by electrochemical impedance spectroscopy (EIS). As shown in Figure 1A, there is low impedance for the bare gold electrode (curve a). After modification of the c-kit-1 quadruplex, the resistance increases because of the electrostatic repulsion between oligonucleotides and negatively charged molecules, $Fe(CN)_6^{3-/4-}$ (curve b). After further incubation with PARP and NAD^+, an increase in charge transfer resistance is observed, owing to the repulsion of $Fe(CN)_6^{3-/4-}$ by the negatively charged PAR (curves c and d). The experimental results indicate that PARylation can occur on the surface of an electrode. RuHex that can be concentrated onto the electrode

DOI: 10.1021/acsami.6b01883
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

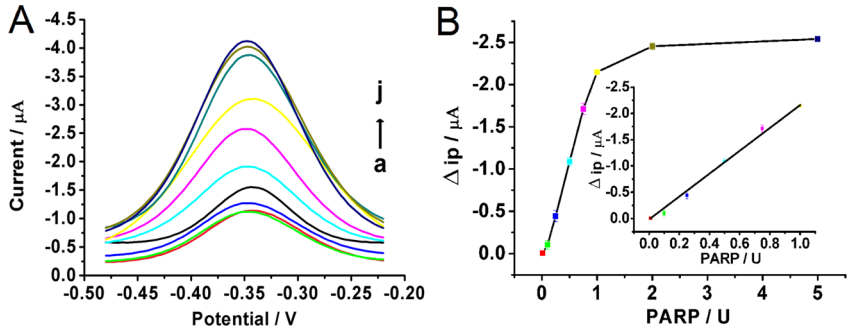


Figure 2. (A) SWV curves for the range of C_{PARP} from 0 to 5 U (from bottom up: 0, 0.005, 0.01, 0.1, 0.25, 0.5, 0.75, 1, 2, and 5 U). (B) Calibration curve for Δip versus C_{PARP}. Inset: a linear range of C_{PARP} from 0.01 to 1 U (Error bars indicate the standard deviation of average values measured in three reduplicated parallel experiments, n = 3).

surface via electrostatic attraction is further chosen to confirm the process of protein PARylation by chronocoulometry (CC).[32] As shown in Figure 1B, the signal increases after PARylation because RuHex can adsorb around the polymer immobilized on the electrode surface, greatly improving the electron transfer efficiency. Meanwhile, to detect the process of PARylation more sensitively, square wave voltammetry (SWV), an accurate electrochemical technique with fine back-ground correction capability, is employed to demonstrate the catalytic process.[33] It can be observed that the peak drastically increases after the occurrence of protein PARylation (Figure 1C), indicating the same conclusion.

Optimization of the Proposed Biosensor. After demonstrating the feasibility of the biosensor, the experimental condition for sensitive detection is optimized. Because both the concentrations of signal molecules and c-kit-1 have important effects on analytic performance, the concentrations of RuHex and c-kit-1 are investigated, respectively. According to the results in Figure S1, the electrochemical signal increases with the addition of RuHex. When the concentration of RuHex reaches 5 μM, a plateau is obtained. Similarly, as shown in Figure S2, the electrochemical signal of c-kit-1 at 0.2 μM is obviously larger than other concentrations. Thus, 5 μM RuHex and 0.2 μM c-kit-1 are selected for the following experiments. The experiments for the incubated and catalytic time of PARP are also carried out. It can be observed that, with the extension of the incubation time for c-kit-1 and PARP, the signal of RuHex decreases (Figure S3), which can be explained by the fact that the combination of c-kit-1 and PARP partly shields the negativity of c-kit-1. As expected, the electrochemical signal increases with the prolongation of the catalytic time for PARP (Figure S4). According to the results in Figures S3 and S4, 1 h is the optimal time for both PARP incubation and catalysis.

Electrochemical Detection of PARP. Under optimal experimental conditions, the sensitivity of the biosensor is investigated. As shown in Figure 2A, SWV peaks increase with the increase of PARP from 0.01 to 5 U. This increase indicates that the electrochemical signal is highly dependent on the content of PARP. After repeated trials (n = 3), the average increase of the peak values shows a good linear correlation with PARP ranging from 0.01 to 1 U (~0.45 to 45 ng, Figure 2B, inset). The linear equation is calibrated as $y = 0.0165 - 2.18 \times [\mathrm{PARP}]$ (R^2 = 0.998), and a calculated detection limit is estimated to be 0.003 U (~0.135 ng, which is 3 times signal-to-noise ratio, n = 5).[32] Compared with the gold nanoparticle-based colorimetric method previously reported,[23] it can be observed that the linear detection range is expanded more than an order of magnitude, and the calculated detection limit is decreased about 30 times. To study the selectivity of the biosensor, a series of control experiments is carried out. The experimental results in Figure S5 indicate that there is nearly no change of electrochemical signal by replacing PARP (Column a) with BSA (Column b), heat deactivated-PARP (Column c), and control assay (Column d), suggesting the satisfactory selectivity of the biosensor. Furthermore, results of real sample assays show acceptable recovery rate and relative standard deviation (Table 1). According to the reference, about 2 mg of PARP could be extracted from about 500 g of calf thymus.[34] On the basis of the calculation, PARP in 0.1125 to 11.25 mg of tissue can be detected by the proposed method. We found that the content of PARP in three pieces of calf thymus (5.0 g) was 0.38, 0.45, and 0.52 U, respectively.

Table 1. Results of PARP Detection in Serum Samples

samples	added (U)	detected (U)	recovery rate (%)	relative standard deviation (%, n = 3)
1	0.25	0.24122	96.45	4.611
2	0.50	0.50598	101.96	4.015
3	0.75	0.77795	103.73	4.744

Reusability and Stability of the Proposed Biosensor. Reusability means a biosensor can accomplish detection for several times, while stability means a biosensor can accomplish detection in a long time. Both properties are important features for a biosensor but are rare in other PARP detection methods.[20,23] In comparison with detection systems that occur in solution, electrochemical biosensors possess the advantages to achieve reusability and stability,[35,36] because assembly and dissociation on the interface are easier to be manipulated. Therefore, we attempt to use this DNA assembled electrode to repeatedly detect PARP. After one instance of PARylation detection, the electrode is simply treated with 5% SDS solution and then immersed into 100 μL Q-buffer. After heating to 95 °C for 5 min, the electrode surface is recovered and could be used to detect PARylation once again. As shown in Figure 3A, the peak value increased after PARylation, while decreasing by interfacial treatment. Besides, both the procedures of PARylation and recovery can be obtained repeatedly, suggesting that the biosensor can detect PARP at least four times. Meanwhile, due to the stability of the DNA-modified electrode, we further try to test the stability of the biosensor. The long-term stability of the biosensor is evaluated by storing a c-kit-1 modified electrode at 4 °C and measuring the electrochemical signals every 5 days under the same

DOI: 10.1021/acsami.6b01883
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

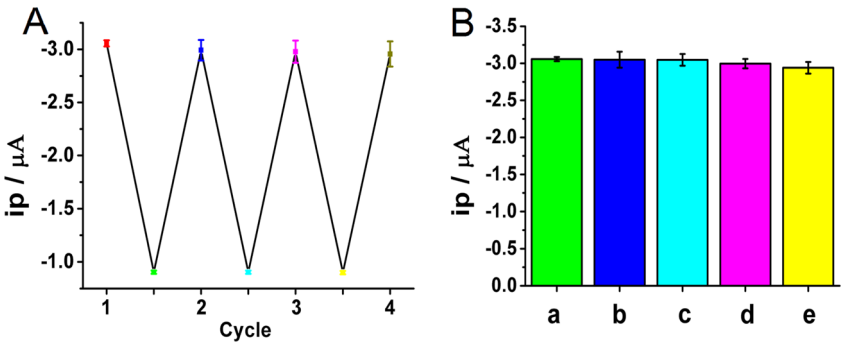


Figure 3. (A) The reusability of the biosensor by the comparison of ip after four times of PARylation and three times of recovery. In this assay, PARP is fixed at 1 U (B). The stability of the biosensor by comparison of ip at different times (a) 1 day, (b) 6 days, (c) 11 days, (d) 16 days, and (e) 21 days (Error bars indicate the standard deviation of the average values measured in three reduplicated parallel experiments, n = 3).

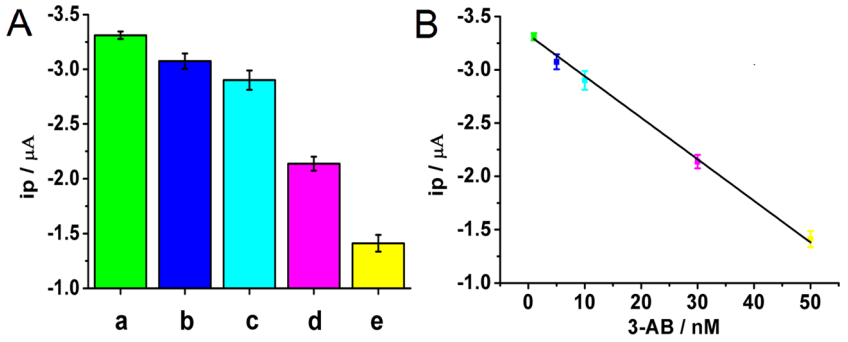


Figure 4. (A) Histograms of peak values corresponding to (a) 1 nM; (b) 5 nM; (c) 10 nM; (d) 30 nM; (e) 50 nM 3-AB. (B) Calibration curve corresponding to the SWV peak values for variable concentrations of 3-AB (Error bars indicate the standard deviation of the average values measured in three reduplicated parallel experiments, n = 3).

conditions. At the 21st day, the SWV current maintains 96.17% of its initial current (Figure 3B), which suggests the proposed biosensor has a satisfying stability for PARylation measurement.

Electrochemical Detection for 3-AB. Finally, the application of this biosensor is extended to screen the inhibitor of PARP. 3-AB, a well-known PARP inhibitor, is used as a model.[23] After full incubation with 3-AB at different concentrations, activity of PARP is significantly depressed, causing the decrease of PARylation product (negatively charged polymer). Accordingly, absorption of positively charged electrochemical signal molecules is reduced. As shown in Figure 4A, it can be observed that the electrochemical signal is gradually reduced with the increase of 3-AB, indicating the obvious effect of inhibitor on protein PARylation. After three parallel tests, the average of the peak values shows a strong linear correlation to the concentration of 3-AB ranging from 1 to 50 nM (Figure 4B). The linear equation is calibrated as $y = -3.32915 + 0.03898 \times [3\text{-AB}]$ (R^2 = 0.997).

CONCLUSIONS

In summary, the negatively charged product of PARylation is employed to actualize the label-free electrochemical detection of PARP. On the basis of the proposed method, quantification of PARP can be achieved in a linear range from 0.01 to 1 U with a calculated detection limit of 0.003 U, which is better than the PARP biosensor reported before. Therefore, this biosensor is suitable to detect PARP in ultralow concentrations, and fewer samples are needed in the detection assay. Besides, the inhibitor of PARP can also be quantified by the proposed biosensor with a simplified procedure. In addition, it is desirable that this biosensor possesses the properties of reusability and stability. Because many biological processes are modulated by PARylation, the proposed biosensor is of help to PARylation-related studies. More importantly, the proposed biosensor is versatile to survey other DNA−protein interactions by simply replacing the corresponding DNA.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b01883.

The optimization of the concentrations for RuHex and c-kit-1 (Figures S1 and S2). The optimization of the incubation time and catalyzed time (Figures S3 and S4). The demonstration of the selectivity of the proposed biosensor (Figure S5). The analytical performance of PARylation by using different methods (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is supported by the National Natural Science Foundation of China (Grant Nos. 31502033, 21505077, 21475062), the China Postdoctoral Science Foundation Funded Project (Grant No. 2015M581823), the Fundamental

DOI: 10.1021/acsami.6b01883
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

Research Funds for the Central Universities (Grant No. KJQN201615), the Natural Science Foundation of Jiangsu Province (Grant No. BK20130688), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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DOI: 10.1021/acsami.6b01883
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