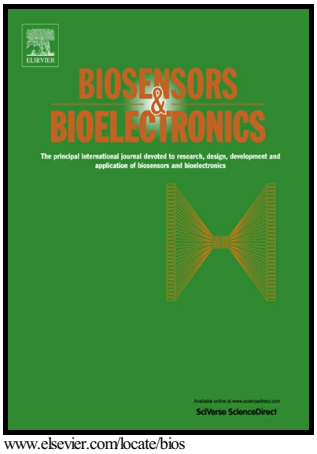

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Highly sensitive electrochemical assay for *Nosema bombycis* gene DNA PTP1 via conformational switch of DNA nanostructures regulated by H^+ from LAMP

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Abstract:

The portable and rapid detection of biomolecules via pH meters to monitor the concentration of hydrogen ions (H^+) from biological reactions (e.g. loop-mediated isothermal amplification, LAMP) has attracted research interest. However, this assay strategy suffered from inherent drawback of low sensitivity, resulting in great limitations in practical applications. Herein, a novel electrochemical biosensor was constructed for highly sensitive detection of *Nosema bombycis* gene DNA (PTP1) through transducing chemical stimuli H^+ from PTP1-based LAMP into electrochemical output signal of electroactive ferrocene (Fc). With use of target PTP1 as the template,

the H^+ from LAMP induced the conformational switch of pH-responsive DNA nanostructures (DNA NSs, Fc-Sp@Ts) that was assembled by the hybridization of Fc-labeled signal probe (Fc-Sp) with DNA-based receptor (Ts). Due to the folding of Ts into stable triplex structure at decreased pH, the configuration change of Fc-Sp@Ts led to the releasing of Fc-Sp, which was subsequently immobilized in the electrode interface through the hybridization with the capture probe modified with -SH (SH-Cp), generating amplified electrochemical signal from Fc. The developed biosensor for PTP1 exhibited a reliable linear range of 1 $fg \cdot \mu L^{-1}$ to 50 $ng \cdot \mu L^{-1}$ with the limit of detection of 0.31 $fg \cdot \mu L^{-1}$. Thus, by the regulation of H^+ from LAMP reaction on DNA NSs allostery, this novel and simple transduction scheme would be interesting and promising to open up a novel analytical route for sensitive monitoring of different target DNAs in related disease diagnosis.

Keywords: electrochemical DNA biosensor, loop-mediated isothermal amplification, pH-responsive DNA nanostructures, conformational switch, target transduction strategy

1. Introduction

Sensitive detection of biomolecules (e.g. sequence-specific DNA) has attracted increasing attentions due to its important roles in agricultural analysis, and infectious, pathogenic and genetic disease diagnosis (Sassolas et al., 2008; Ho et al., 2008; Liu et al., 2008). Polymerase chain reaction (PCR), as a regular standard method for DNA detection, requires complicated and expensive thermo cycler and is time-consuming due to multi-step thermal cycling process (Shu et al., 2017; Chen et al., 2017). This has

caused an active pursuit to develop simple and portable methods for the sensitive and rapid detection of DNAs. In recent years, several groups have attempted to use commercially available signal output devices such as personal glucose meters or pH meters to indirectly monitor DNAs in connection with enzymatic catalysis (Xiang et al., 2012a; Xiang et al., 2012b; Su et al., 2012; Xu et al., 2012), cross-priming isothermal amplification (CPA) (Zhang et al., 2014), and loop-mediated isothermal amplification (LAMP) (Kwon et al., 2013; Zhi et al., 2014). Among these sequence-specific DNAs, *Nosema bombycis*, a class of causative pathogen of pebrine disease, would cause serious decrease of the cocoon production by parasitizing in silkworm larva (Yan et al., 2014; Yang et al., 2014). So, to develop highly sensitive analytical methods for the biomarker gene PTP1 of *Nosema bombycis* is critical for the sericultural improvement and the agricultural development. Recently, our group proposed a novel detection strategy for genomic DNA PTP1 by using pH meter as signal reporter to monitor the activity change of H^+, which was dependently generated in PTP1-based LAMP reaction, successfully achieving indirect and portable detection of the target DNA PTP1 (Xie et al., 2014). These assay strategies via portable pH meters for signal readout, although handy and rapid, share an inherent drawback of low sensitivity (pH meter can only detect change of 0.001 pH value), which inevitably lead to great limitations in actual application, especially for those biomolecules at trace level. Therefore, to search for novel analytical methods with high sensitivity, portability and speediness is a big challenge. To face this challenge, an effort could be attempted to convert H^+ from target DNA-based LAMP reaction into amplified, detectable and more sensitive current signal of redox probes

through a simply and finely designed transduction strategy, realizing the highly sensitive and portable detection of the target DNA.

More recently, the novel DNA nanodevices and DNA nanomachines brought out promising application in nanoelectronic devices, smart materials, and biosensors due to highly steerable activity of DNA-based reactions (Wang et al., 2015; Zhang et al., 2017; Zhao et al., 2016). Particularly, some DNA nanostructures (DNA NSs) with pH-dependence can specifically respond to pH change, resulting in their conformational change at decreased or increased pHs (Porchetta et al., 2015; Nesterova, et al., 2014; Hoogsteen et al., 1963; Grosso et al., 2015). Thanks to the releasing or loading of sequence-specific ligand during the switch process, pH-responsive DNA NSs with versatility and modularity have attracted extremely research interest in pH sensors construction, diseases diagnosis, and drug delivery (Keum et al., 2012; Idili et al., 2014; Dvir et al., 2010). For example, Ricci et al. introduced a pH-induced allostery in DNA-based model receptors by designing a pH-sensitive stem and demonstrated the receptors can act as pH-responsive DNA nanomachines that load or release a specific target in a controlled fashion via pH changes (Porchetta et al., 2015). They also developed a kind of versatile pH-dependent DNA-based nanodevices by using H^+-producing or H^+-consuming enzymatic reaction to finely regulate their closing or opening, along with the releasing/loading of specific DNA ligand (Grosso et al., 2015). More importantly, the rational design of versatile DNA NSs with pH-response would provide significant accessibility and availability for the signal output transduction of sequence-specific target detection via pH change, which would be very desirable for

simple and sensitive assay of targeted DNAs.

Inspired by the great adjustability of pH-dependent DNA NSs, a novel electrochemical biosensor was developed for highly sensitive assay of *Nosema bombycis* gene DNA PTP1 by using LAMP-based H^+ as chemical stimuli that regulated the conformational switch of pH-dependent DNA NSs (Fc-Sp@Ts) and subsequently transducing the stimulated-response into the sensitive electrochemical signal of redox probe ferrocene (Fc). Firstly, the target PTP1 was used as the template DNA for LAMP reaction to produce H^+ that relied on the PTP1 concentration. Next, the generated H^+ as chemical transducers were introduced to drive the conformational switch of Fc-Sp@Ts, resulting in the liberation of specific signal probes labeled with electroactive Fc (Fc-Sp) due to the folding of Ts into stable triplex structures at decreased pH. Then, the released Fc-Sp was captured to the electrode surface through the hybridization with capture probes (SH-Cp), achieving amplified electrochemical signal readout of Fc and sensitively indirect detection of target PTP1. Thus, based on the regulation of DNA NSs conformation by LAMP-based H^+ as chemical stimuli, the proposed novel transduction route would open a new door for sensitive and portable assay of nucleic acids.

2. Experimental

2.1. Apparatus

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and square wave voltammetry (SWV) were performed on CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China) with a conventional three-electrode

system containing different modified bare glass carbon electrode (GCE, Φ = 4 mm) as working electrodes along with a saturated calomel electrode as reference and a platinum wire as supporting electrode. The morphology of the electrode surface after electrodeposited in $HAuCl_4$ was characterized with a scanning electron microscope (SEM, S-4800, Hitachi, Japan) at an acceleration voltage of 20-30 kV. Polyacrylamide gel electrophoresis (PAGE) was conducted via using a DYY-8C electrophoretic apparatus (Beijing, Wo De Life Sciences Instrument Co., Ltd., China). Gels images were obtained by a Bio-Rad imaging system (Hercules, CA, U. S. A). The morphology of prepared nanomaterials was tracked with transmission electron microscopy (TEM, H600, Hitachi, Japan).

2.2. Reagents and chemicals

Magnetic Fe_3O_4 microspheres (Fe_3O_4 MSs) were provided by BaseLine ChromTech Research Centre (Tianjing, China). Bst polymerase large fragments and $MgSO_4$ were supplied by New England Biolabs Ltd. (Beijing, China). Chlorauric acid ($HAuCl_4$), hexanethiol (96%, HT) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) and tris (2-carboxyethyl) phosphine (TCEP) were provided by Roche (Switzerland). Target *Nosema bombycis* gene DNA PTP1 and out/inner primers, as well as betaine and deoxynucleotide triphosphates (dNTPs) were synthesized and purified by high performance liquid chromatography by Sangon Biotech Co. Ltd (Shanghai, China). **Table 1** summarized the detailed base sequences of PTP1, primers and other oligonucleotides used in this work. The underlined letters of PTP1 (F1-F3 and B1c-B3c)

are six specific regions interacted with two inner-primers FIP (F1C-F2) and BIP (B1C-B2) and two out-primers (F3 and B3), respectively. F1 and B2c of PTP1 are complementary to F1c and B2, respectively. Moreover, the italicized letters and the bolded letters in triplex strand (Ts) refer to duplex portion and triplex-forming domain when Ts is folded. The signal probe labeled with electroactive ferrocene (Fc-Sp) is totally complementary to the capture probe modified with -SH in 5'-terminus (SH-Cp). The bolded letters in one-mismatch (M1), two-mismatch (M2) and three-mismatch (M3) refer to one, two and three base mismatches of B3, respectively.

Table 1 Base sequences of oligonucleotides used in this work.

Oligonucleotides	Base sequences
Nosema bombycis gene DNA PTP1	5'-CTACTGGATCAGGTGTTCCTTGTATTCCAGTAT CTTCAACCGGTGGC(**F3**) CATCCAAGTAATCTCGT TGGCGGTGGTTTC(**F2**) CCAAGTAATGCACAAGTC ACTCAAGCCCCATGTGTCCCATCACA(**F1**) AGCTC ATCATCCAGTAGCAAGTGTTCCAGTAACGAGTGT CCCAGTAAATGCTGTTCCAATGACCAGTGCCCC (**B1c**) TGTTACACCACTTGGCCCTTCTTATTA CGG ATCTTCAAGCCTTCCAC (**B2c**) CATCAGGCTCTCA TCCTACTGCTCCT(**B3c**) TGTTGT-3'
Inner-primer FIP (F1c-F2)	5'-TGTGATGGGACACATGGGGC(**F1c**)-TTTT-CTCG TTGGCGGTGGTTTC(**F2**)-3'
Inner-primer BIP (B1c-B2)	5'-CTGTTCCAATGACCAGTGCCCC(**B1c**)-TTTT-GTG GAAGGCTTGAAGATCCG(**B2**)-3'
Out-primer F3	5'-CAGTATCTTCAACCGGTGGC-3'
Out-primer B3	5'-AGGAGCAGTAGGATGAGAGC-3'
Triplex strand (Ts)	5'-SH-TTTTTTTTT-***TTCCTT***-TTTTT-*TTCCTT*-TTT GGCTAGAG-*AAGGAA*-3'
Signal probe (Fc-Sp)	5'-CTCTAGCCAAA-**Fc**-3'

Capture probe (SH-Cp)	5'-**SH**-$(CH_2)_6$-TTTGGCTAGAG-3'
One-mismatch (M1)	5'-AGGAGCAGTAGGATGAGAG**T**-3'
Two-mismatch (M2)	5'-AGGAGCAGTAGGATGAG**C**G**T**-3'
Three-mismatch (M3)	5'-AGGAGCAGTAGGATG**C**G**C**G**T**-3'

The stock solutions of different oligonucleotides for LAMP reaction and DNA NSs with a final concentration of 100 μM were prepared by suspending them in diethyl pyrocarbonate (DEPC) water, and were further diluted with DEPC when used. Normally, LAMP reaction proceeding would not exhibit a noticeable pH change due to its buffered conditions, which was preferred for DNA polymerase but would consume the released protons (Zhang et al., 2014; Idili et al., 2014). In order to minimize H^+ consumption and make pH change caused by LAMP noticeable, the compounds Tris-HCl were wiped off from reaction solution with low concentration of weak electrolyte NH_4^+ as much as possible. Low buffered conditions for LAMP were pH sensitive while retaining the amplification efficiency and specificity. Considering the DNA polymerase behaves well in alkaline condition, the initial pH of LAMP reaction mixture was adjusted to about pH 8.0. Meanwhile, low phosphate-buffered solution (PBS, pH 8.0) containing 0.01 mM Na_2HPO_4 and 10 mM $MgCl_2$ was used for the hybridization reaction of Ts and Fc-Sp to form pH-responsive DNA NSs (Fc-Sp@Ts) (Grosso et al., 2015). 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl, 1 mM TCEP and ethylene diamine tetraacetic acid (EDTA) was used to prepare SH-Cp solution. The electrochemical testing buffer was 0.1 M PBS (pH 7.0) containing 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. Unless noted otherwise, deionized water (DI water) was used in all the experiments.

2.3. Preparation of DNA NSs

Firstly, Fe_3O_4 MSs modified with -NH_2 was mixed with 5 mL of prepared gold nanoparticles (AuNPs) for sonicating 10 min and then shaking for 10 h at 4 ^oC. AuNPs were attached onto Fe_3O_4 MSs surface through strong Au-N bond. The obtained product Au@Fe_3O_4 nanocomposites (Au@Fe_3O_4) was collected via magnetic isolation and washing at least three times and re-dispersed in 1 mL of PBS (the TEM of Au@Fe_3O_4 was shown in **Fig. S1** in Supplementary Information). Then, in 50 μL PBS (pH 8.0) containing Au@Fe_3O_4 (1%, *w*/*w*), 5 μL of Ts (100 μM) modified with -SH was introduced at room temperature for keeping 12 h, forming the magnetic conjugates Au@Fe_3O_4-labeled Ts through Au-S bond. Followed that, 20 μL of HT (1 mM) was added for 0.5 h to block nonspecific binding sites of Au@Fe_3O_4. After magnetic separation, the obtained precipitates were incubated with 5 μL Fc-Sp (10 μM, pH 8.0) for 2 h, resulting in the assembly of pH-dependent DNA NSs (Fc-Sp@Ts) by loading of Fc-Sp at Ts labeled with Au@Fe_3O_4. Finally, the resultant products Fc-Sp@Ts were obtained by further magnetic separation and were allowed to use for the subsequent experiments.

2.4. LAMP reaction and conformational switch of DNA NSs

According to the reported reference (Xie et al., 2014), the sequences of target *Nosema bombycis* gene PTP1 as the template DNA, out-primers and inner-primers were designed for LAMP reaction. A solution with a total volume of 35 μL was prepared by mixing two inner-primers FIP and BIP (1.6 mM), two out-primers F3 and B3 (0.4 mM), 5 mM $MgSO_4$, 50 mM KCl, 5 mM NH_4Cl, 1 M betaine, 1 mg·mL^{-1} BSA, 0.1% Triton-X 100, 2.8 mM dNTPs, and 0.23 U·μL^{-1} Bst polymerase. In the resulting mixture,

a series of target PTP1 with different concentrations was added to reach to a final volume of 40 μL. After that, the obtained solution was kept at 65 ^{o}C for 1 h to carry out the LAMP reaction, followed by heating to 95 ^{o}C for 2 min to terminate the reaction. For comparison, we also performed two control LAMP reactions without PTP1 (Blank) or primers (No-primers), respectively. In this case, different LAMP reaction products containing released hydrogen ions (H^+) were introduced into the above obtained DNA NSs (Fc-Sp@Ts) for 1 h, allowing the conformational switch of DNA NSs due to pH change. Resultantly, the liberated Fc-Sp was obtained by magnetic separation and further introduced in the electrode surface for the fabrication of the electrochemical PTP1 biosensor.

2.5. Fabrication of designed biosensor for PTP1

Scheme 1 illustrated the preparation procedure of the proposed PTP1 biosensor, and the principle of the conformational switch of Fc-Sp@Ts stimulated by H^+ in LAMP. Prior to modification, bare GCE was pretreated by polishing with 0.3 μm and 0.05 μm Al_2O_3 powder and sonicating in DI water, followed by the electrodeposition in $HAuCl_4$ solution under -0.2 V potential for 30 s (depAu/GCE). After that, 20 μL of thiol-modified capture probes (SH-Cp, 2.5 μM) dissolved in Tris-HCl (pH 7.4) was incubated in the resulting electrode surface for 16 h (SH-Cp/depAu/GCE), followed by the introduction of blocking agent HT (1 mM) for 0.5 h (HT/SH-Cp/depAu/GCE). Finally, 25 μL of the specific strands Fc-Sp, which was liberated from DNA NSs Fc-Sp@Ts due to the conformation switch stimulated by H^+ from LAMP, were modified in the modified electrode surface for 3 h (Fc-Sp/HT/SH-Cp/depAu/GCE).

After being rinsed with DI water, the fabricated biosensor was used for the next electrochemical characterization and measurement.

2.6. Electrochemical measurements

CV and EIS response of the proposed biosensor were measured in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{4-/3-}$ at 100 mV/s scan rate in the potential range from -0.2 to 0.6 V. SWV signal recorded for the condition optimization and analytical performance in the total experiments was conducted in 0.1 M PBS (pH 7.0) in the potential range from 0.25 V to 0.65 V, with a step of 4 mV, a frequency of 6 Hz, and an amplitude of 25 mV.

2.7. Polyacrylamide gel electrophoresis (PAGE)

PAGE was used to demonstrate LAMP reaction. 12 μL of tested sample and DNA loading buffer with a volume ratio of 5:1 was placed into 16% non-denaturing polyacrylamide gel, and carried out in 1×TBE (pH 8.3) at a constant voltage of 100 V for 2.5 h. After that, the resultant solution was separated and stained with ethidium bromide, and gels images were taken under Gels Imagers.

3. Results and discussion

3.1. Proposed transduction strategy for PTP1 detection

In response to the effective improvement of low sensitivity via pH meter to monitor H^+ from LAMP reaction, herein we proposed a novel transduction strategy by converting the chemical stimuli (H^+) into sensitive electrochemical current signal of electron mediator Fc. In the LAMP reaction with *Nosema bombycis* gene PTP1 as the

template DNA (see **Fig. S2** for the PAGE analysis of LAMP reaction in the absence of and in the presence of DNA PTP1 with different concentrations, and **Fig. S3** for the working principle of PTP1-based LAMP), the produced H^+ as chemical stimuli drove the structure change of pH-responsive DNA NSs (Fc-Sp@Ts) and liberated signal probes labeled with electroactive Fc (Fc-Sp).

Additionally, the specially designed stem (TTCCTT) as pH-responsive domain was introduced in DNA-based receptors (Ts) previously modified with magnetic Au@Fe_3O_4, which could load or release signal probes (Fc-Sp) in a controlled fashion via pH changes. Herein, the stable pH-responsive DNA NSs (Fc-Sp@Ts) were assembled by the loading of Fc-Sp onto Ts through the hybridization reaction at pH 8.0 (Porchetta et al., 2015). When H^+ dependent on LAMP reaction was introduced, the bases T and C in pH-dependent stem of Ts (TTCCTT) were protonated at decreased pH (Grosso et al., 2015), forming stable triplex structure T-A*T and C-G*C through parallel Hoogsteen paring (Hoogsteen et al., 1965). In this case, the conformational switch of Fc-Sp@Ts followed the releasing of specific Fc-Sp. Next, the liberated Fc-Sp was further hybridized onto the electrode surface that was previously electrodeposited in $HAuCl_4$ solution and incubated with capture probes (SH-Cp). Specifically, the electrochemical response signal generated by electron mediator Fc was relied on the concentration of the target gene PTP1. Based on the proposed transduction strategy by using H^+ from LAMP as chemical stimuli, the output signal of the developed biosensor was successfully converted and efficiently promoted. Thus, the highly sensitive and accurate detection of target DNA could be effectively achieved, which would be very

promising to avoid the lower sensitivity of pH meters.

3.2. Electrochemical characterization of the proposed biosensor

To verify the modification procedure of the electrochemical biosensor, the CV response of each fabrication step was measured and displayed in **Fig. 1A**. Obviously, a pair of well-defined and reversible redox peak for GCE (curve a) and increased CV signal for the GCE after electrodeposited in $HAuCl_4$ (depAu/GCE) (see **Fig. S4** for the SEM images of depAu) were observed (curve b), suggesting the acceleration of depAu with excellent conductivity to the electron transfer in electrode interface. Then the sequential introduction of SH-Cp as capture probes (SH-Cp/depAu/GCE) and HT as blocking reagent (HT/SH-Cp/depAu/GCE) led to an obvious decrease in CV peak current (curve c and d). This was ascribed to the electrostatic repulsion between negatively charged $[Fe(CN)_6]^{4-/3-}$ and phosphate backbones of DNA (Yang et al., 2017), and the hindrance of non-conducting HT to electron transfer in electrode interface. Furthermore, the EIS response of the developed biosensor in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{4-/3-}$ was also investigated and shown in **Fig. S5** (see Supplementary Information).

To demonstrate and confirm the successful immobilization of the specific ligand (Fc-Sp) onto the electrode surface, we measured the SWV response of the modified electrode HT/SH-Cp/depAu/GCE before and after incubated with released Fc-Sp after the conformational regulation of pH-dependent DNA NSs (Fc-Sp@Ts) with H^+ from LAMP. As shown in **Fig. 1B**, compared with the hardly detectable SWV signal of the electrode HT/SH-Cp/depAu/GCE (curve a), significantly rising SWV current was

observed after the strong hybridization of specific Fc-Sp in the electrode substrate (electrode Fc-Sp/HT/SH-Cp/depAu/GCE, curve b). This demonstrated that Fc-Sp was liberated from the pH-responsive Fc-Sp@Ts due to the fact that Ts was folded into more stable triplex structure driven by H^+ from LAMP. In addition, the conformation switch of Fc-Sp@Ts was also demonstrated via PAGE analysis. The insert figure of **Fig. 1B** displayed the PAGE bands of the before and after regulated by decreased pH. Compared with the band of single strand Ts containing pH-dependent stem (lane I), a slightly lower electrophoretic mobility (lane II) was obtained after the thermal annealing reaction of Ts (34 nt) with Fc-Rs (11 nt) in low-buffered PBS (pH 8.0), indicating that pH-responsive DNA NSs (Fc-Rs@Ts) were successfully formed by the hybridization reaction of Ts and Fc-Rs. When Ts and Fc-Rs were mixed in low-buffered PBS (pH 6.0), the corresponding PAGE band exhibited further decreased mobility (lane III). This was attributed to that DNA Nss Fc-Rs@Ts could not be formed due to the protonation of pH-dependent stem in Ts at decreased pH (Grosso et al., 2015). And the stem-loop configuration of the folded triplex structure of Ts presented greater steric hindrance (Zhang et al., 2017).

3.3. *Optimization of experimental conditions*

In order to achieve the optimal amplified efficiency and capability of this system, different experimental parameters were optimized by measuring CV or SWV signal, which included each concentration of SH-Cp incubated in the electrode surface, dNTPs and Bst polymerase for LAMP reaction, and pH of tested buffer. The experimental results are shown in **Fig. 2.** From **Fig. 2A**, the oxidation peak current in CV curves

decreased with the rising concentration of SH-Cp from 0.5 μM to 2.5 μM and reached to the largest at 2.5 μM level, attributing to growing electrostatic repulsion between negatively charged $[Fe(CN)_6]^{4-/3-}$ and phosphate backbones of DNA strand (Shi et al., 2015). When the concentration of SH-Cp was larger than 2.5 μM, slowly dropped CV signal was observed, suggesting that the presence of 2.5 μM SH-Cp in the electrode interface was the optimal to obtain the most significant electrochemical response.

It has been reported that non-essential specific hydrolysis of dNTPs would influence the LAMP amplification efficiency (Shiddiky et al., 2006). We firstly introduced six different concentrations of dNTPs in the range of 1.2 mM to 3.2 mM to carry out LAMP reaction with 0.5 $ng \cdot \mu L^{-1}$ PTP1. The corresponding LAMP products containing H^+ were allowed to interact with Fc-Sp@Ts, and the released Fc-Sp was introduced in the biosensor surface. The SWV signal of modified electrodes was measured and shown in **Fig. 2B**. The SWV peak currents gradually increased with the rising of the dNTPs concentration and levelled off at 2.8 mM. The amount of Bst polymerase in LAMP reaction is also an important factor to improve the amplification efficiency. Similarly, we used different concentrations of Bst polymerase in the range of 0.05 to 0.29 $U \cdot \mu L^{-1}$ to perform the LAMP reaction. From **Fig. 2C**, one can see that the increased SWV responses with the prolonged amount of Bst polymerase reached to a plateau after 0.23 $U \cdot \mu L^{-1}$. Thus, in the proposed electrochemical system, 2.8 mM dNTPs and 0.23 $U \cdot \mu L^{-1}$ Bst polymerase were the optimal for the LAMP reaction with 0.5 $ng \cdot \mu L^{-1}$ PTP1 as the template DNA.

Moreover, as shown in **Fig. 2D**, the most significant SWV response was obtained at

pH 8.0 for the hybridization reaction of Ts and Fc-Sp to form pH-responsive DNA NSs (Fc-Sp@Ts). So, pH 8.0 was the most suitable for the stable assembly of DNA NSs and used in all experiments unless otherwise noted.

3.4. Analytical performance of the proposed biosensor

To evaluate the sensitivity and potential quantitative range of the developed biosensor, the SWV response to different concentrations of PTP1 was measured under the optimal experimental conditions. From **Fig. 3A**, we can see that the SWV signal significantly elevated with the increasing of PTP1 concentrations in the range from 1 $fg \cdot \mu L^{-1}$ to 50 $ng \cdot \mu L^{-1}$. This was attributed to the fact that more target PTP1 for LAMP dependently released more H^+, and more H^+ as chemical transducers resulted in the releasing of more Fc-Sp, and more liberated Fc-Sp was subsequently captured in the electrode surface, generating significantly amplified electrochemical signal readout. In this case, as shown in **Fig. 3B**, the corresponding SWV peak currents exhibited an excellent linear relationship relied on the logarithm of PTP1 concentrations from 1 $fg \cdot \mu L^{-1}$ to 50 $ng \cdot \mu L^{-1}$. The regression equation was I (μA) = $0.2422 \lg c_{PTP1}$ ($ng \cdot \mu L^{-1}$) + 1.576 with a correlation coefficient of 0.9977. According to the $3s_B/m$ rule (where s_B is the standard deviation of the blank and m is the slope of the calibration curve) (Radi et al., 2006), the limit of detection (LOD) of the developed biosensor was calculated to be 0.31 $fg \cdot \mu L^{-1}$. Moreover, as summarized in **Table 2**, the analytical sensitivity of the developed biosensor was greatly improved, which was comparable with or superior to those of different detection methodologies for the detection of DNA. This may be originated from two main concerns in the proposed scheme. (i) Amplified hydrogen

ions (H^+) from LAMP reaction, dependent on target PTP1, were used as chemical stimuli to achieve signal readout transduction. (ii) The generated H^+ specifically regulated the conformation of pH-responsive DNA NSs (Fc-Sp@Ts). Due to the folding of Ts into stable triplex structure at decreased pH, electroactive signal probes Fc-Sp were released from Fc-Sp@Ts and further captured to the modified electrode surface, giving significantly amplified electrochemical output signal dependent on the PTP1 concentration. As a result, the proposed biosensor here exhibited high sensitivity while retaining portability and rapidness. Thus, this vigorous and robust transduction strategy via the conformational switch of DNA NSs regulated by target-LAMP-based H^+ would be promising and potential to extend the highly sensitive monitoring of different target DNAs.

Table 2 Comparison of analytical performance of different detection methodologies for DNA.

Methods	Target	Detection range	LOD	Ref.
PAGE	Salmonella DNA	1 ng·μL^{-1} to 3.5 μg·μL^{-1}	0.32 ng·μL^{-1}	Bai et al., 2015
Microfluidic	Streptococcus pneumoniae DNA	0.17 pg·μL^{-1} to 0.17 μg·μL^{-1}	56.1 fg·μL^{-1}	Doua et al., 2017
Turbidimetry	H1N1 virus DNA	100 fg·μL^{-1} to 1 ng·μL^{-1}	33.2 fg·μL^{-1}	Fang et al., 2011
pH meter	PTP1	0.5 pg·μL^{-1} - 50 ng·μL^{-1}	0.17 pg·μL^{-1}	Xie et al., 2014
CV	PTP1	50 fg·μL^{-1} - 50 ng·μL^{-1}	17 fg·μL^{-1}	Xie et al.,

				2015
SWV	PTP1	1 fg·μL^{-1} - 50 ng·μL^{-1}	0.31 fg·μL^{-1}	The work

3.5. Specificity, stability and reproducibility of the proposed biosensor

To evaluate the specificity and affinity of the developed electrochemical biosensor based on H^+ from LAMP as signal transduction stimuli, it was challenged with five different LAMP operation systems including three base mismatches of the out-primer B3 (one-mismatch, M1; two-mismatch, M2; and three-mismatch, M3), without the involvement of target PTP1 (Blank) or primers (including out- and inner-, No-primers), respectively. The corresponding SWV signals of the same batch biosensors were measured under the optimal conditions, and illustrated in the inset of **Fig. 4A**. Obviously, almost negligible response current was observed for the five control systems M1, M2, M3, Blank and No-primers. However, the proposed platform with 0.5 ng·μL^{-1} PTP1 yielded much higher response signal, indicating satisfactory specificity of the developed biosensor for PTP1. This was owing to the successful operation of LAMP reaction dependent on the corporate participation of target PTP1 and all the primers with complementary base sequences. In this case, the conformation switch of DNA NSs regulated by H^+ from LAMP enabled the proposed electrochemical transduction route more reliable, and higher efficient in amplified detection of DNA by the interpretation of LAMP reaction.

The long-term stability of the developed biosensor was investigated by testing SWV signal when stored at 4 ^{o}C for 5, 10, 15, 20 days. As shown in **Fig. 4B**, the SWV current still maintained 97.3%, 90.5%, 89.8%, and 85.0% of its original level,

respectively, indicating acceptable stability possibly due to the long-term stable electroactivity of redox probes presented in the electrode surface. In addition, five electrodes independently prepared in the same batch showed almost equal SWV response with a relative standard deviation (RSD) of 5.71% (**Fig. 4C**), suggesting a reliable reproducibility.

3.6. Evaluation of real sample

To survey the practical applicability of the novel electrochemical transduction system, the silkworm healthy eggs, which were kindly provided by the State Key Laboratory of Silkworm Genome Biology of China (Southwest University), were employed to prepare the tested sample solutions. By using the standard addition method, different concentrations (0.5 $pg \cdot \mu L^{-1}$ to 5 $ng \cdot \mu L^{-1}$) of PTP1 standard solutions were introduced into the samples for LAMP reaction, and the SWV signal of the fabricated biosensor was measured under the optimal conditions. As listed in **Table S1**, the obtained recoveries for five additions varied in the range from 92.0% to 106% with RSD in the range of 2.2% to 5.6%, demonstrating acceptable reliability of the proposed biosensor for the sensitive detection of DNA PTP1.

Conclusions

In this work, by converting target-LAMP-based hydrogen ions (H^+) into electrochemical output signal of redox mediator Fc in a controlled fashion via simple pH change, we developed a novel transduction strategy to construct a highly sensitive biosensor for the detection of *Nosema bombycis* gene DNA PTP1. During PTP1-based

LAMP reaction, the generated H^+ as chemical stimuli regulated the conformational switch of pH-dependent DNA NSs (Fc-Sp@Ts), leading to the consequent releasing of specific signal probes labeled with electroactive Fc (Fc-Sp) due to the folding of Ts into stable triplex structure at decreased pH. The liberated Fc-Sp was subsequently captured in the modified electrode surface, dependently generated sensitive and amplified current signal of Fc that was relied on the concentration of PTP1 in the range of 1 $fg \cdot \mu L^{-1}$ to 50 $ng \cdot \mu L^{-1}$ with a limit of detection as low as 0.31 $fg \cdot \mu L^{-1}$. The designed biosensor also displayed convenience, rapidness, easy operation, and low background response. More importantly, in comparison to the conventional potentiometry with pH meter as signal reporter, the developed electrochemical system exhibited higher sensitivity through the simple conformational switch of DNA NSs driven by PTP1-LAMP-based products H^+, which was interesting and promising to extend the analytical application of LAMP technique for the sensitive and portable assay of different biomolecules, such as DNA and RNA. In the future, we would attempt to further improve the long-term stability and assay sensitivity of the target-LAMP-based electrochemical transduction system by engineering different pH-responsive DNA NSs and integrating with other amplification technologies. Possibly, a potentially universal method could be developed for the highly sensitive detection of various targets by switching the corresponding template DNA or primers of LAMP reaction.

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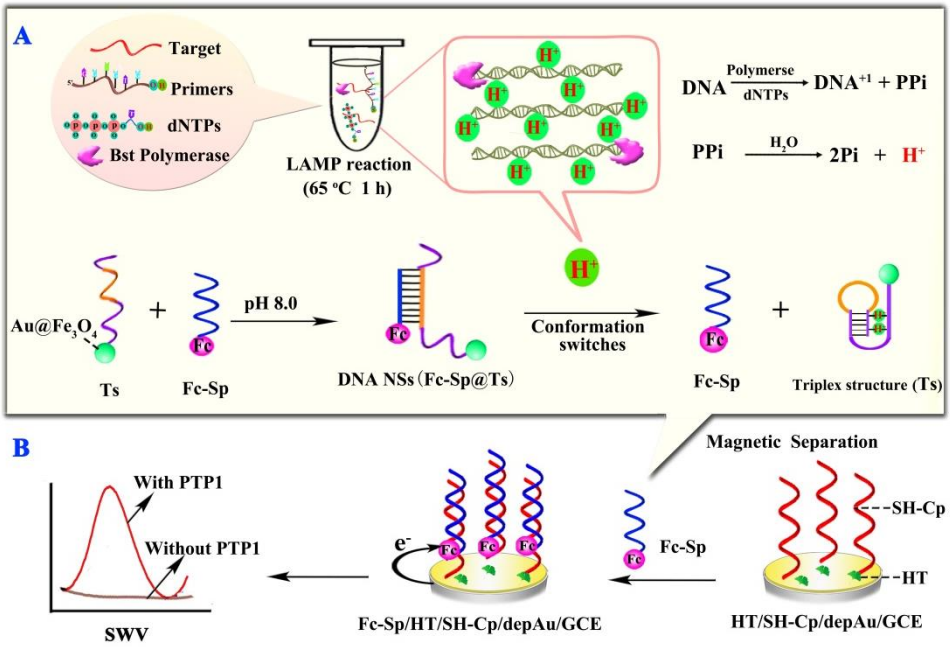

Scheme 1. The schematic diagram (A) of the conformational switch of pH-responsive DNA nanostructures (DNA NSs, Fc-Sp@Ts) induced by hydrogen ion (H^+) released from PTP1-based LAMP reaction initiated by the primers with the help of dNTPs and Bst polymerase, liberating the signal probe labelled with electroactive ferrocene (Fc-Sp), and (B) of the fabrication process of the proposed electrochemical biosensor.

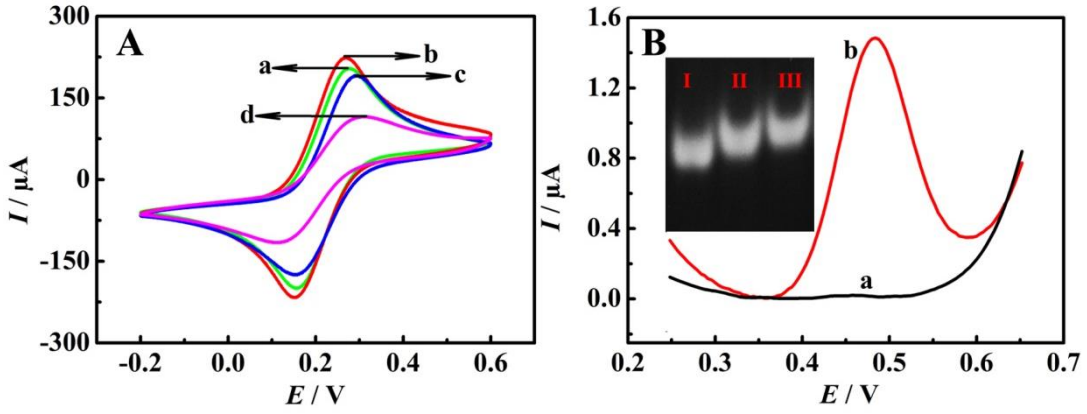

Fig. 1. (A) CV response of stepwise modification process of the developed biosensor: (a) bare GCE; (b) depAu/GCE; (c) SH-Cp/depAu/GCE; (d) HT/SH-Cp/depAu/GCE. (B) SWV response of the electrode HT/SH-Cp/depAu/GCE before (a) and after (b) incubated with the specific ligand Fc-Sp (Fc-Sp/HT/SH-Cp/depAu/GCE), which was released after the interaction of Fc-Sp@Ts with H^+ from LAMP by using 0.5 ng·μL^{-1} PTP1 as template DNA. The inset of (B) is the PAGE images of different samples (2.0 μM) including Ts in pH 8.0 PBS (lane I), Ts and Fc-Rs in pH 8.0 PBS (lane II) and pH 6.0 PBS (lane III), respectively.

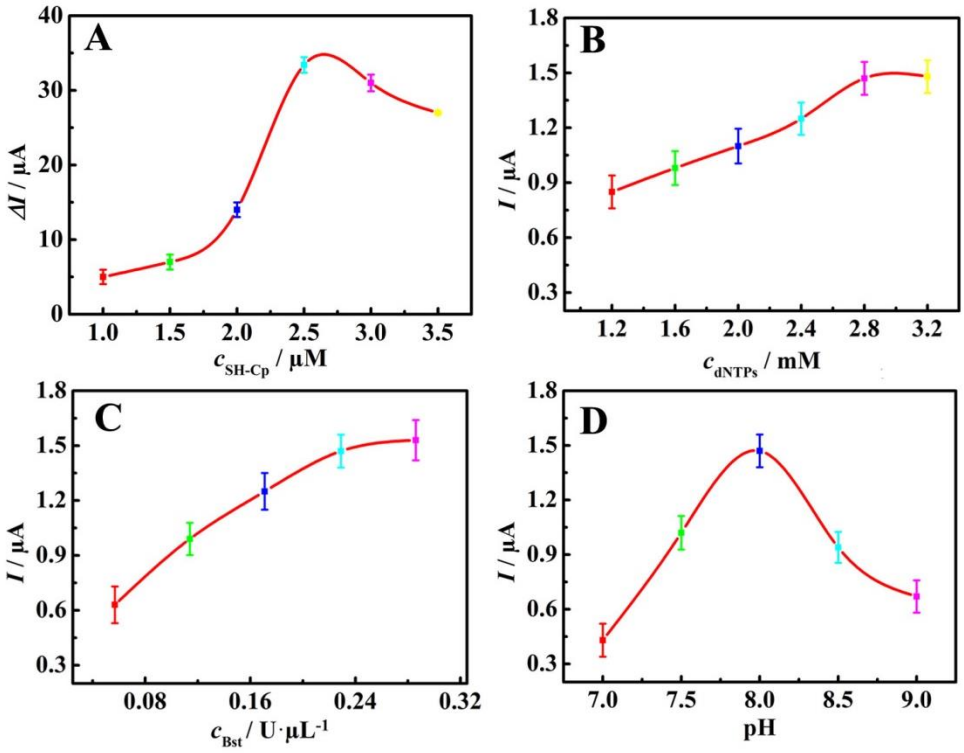

Fig. 2. The effect of different experimental conditions on the electrochemical signal of the proposed biosensor for PTP1: the concentrations of (A) SH-Cp incubated in the electrode interface, (B) dNTPs and (C) Bst polymerase for LAMP reaction with 0.5 ng·μL^{-1} PTP1. The corresponding CV (A) and SWV (B, C and D) signals were measured in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{4-/3-}$ and 0.1 M PBS (pH 7.0), respectively. Error bars: *SD*, *n*=3.

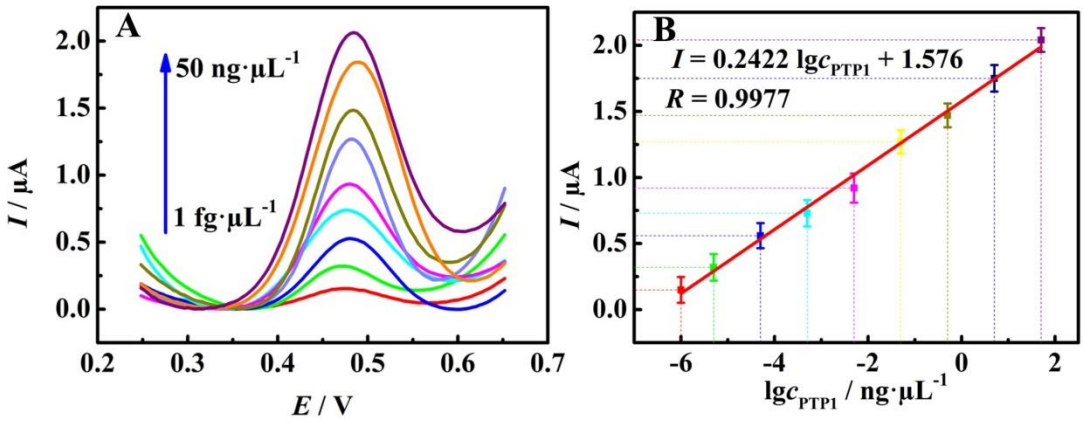

Fig. 3. (A) SWV response of the prepared biosensor to target PTP1 with different concentrations; (B) Corresponding calibration curve plotted by SWV peak current vs the logarithm of PTP1 concentrations (1 fg·μL^{-1} to 50 ng·μL^{-1}). SWV was measured in 0.1 M PBS (pH 7.0) under the optimal conditions. Error bars: *SD*, *n*=3.

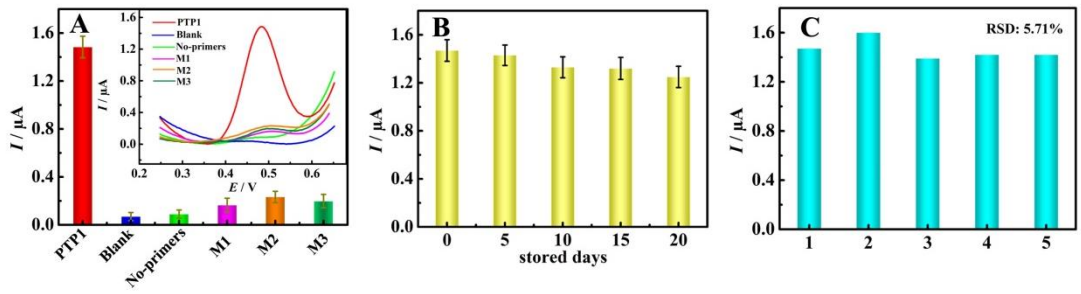

Fig. 4. SWV responses of the developed biosensor under the optimal conditions in 0.1 M PBS (pH 7.0). (A) Specificity toward PTP1 *vs* five control systems for LAMP reactions including different base mismatches of the out-primer B3 (M1, M2 and M3), Blank without PTP1 and No-primers; (B) Stability after storing 0-20 days; (C) Reproducibility by investigating five biosensors prepared in the same batch. The PTP1 concentration was 0.5 ng·μL^{-1}. *SD*, *n*=3.

Highlights

- A novel electrochemical transduction strategy was developed to construct sensitive DNA biosensor.
- *Nosema bombycis* DNA PTP1 was used as the template of LAMP to produce hydrogen ions (H^+).
- The H^+-induced conformation switch of pH-responsive DNA nanostructures released signal probe.
- Amplified electrochemical signal was dependent on the concentration of the target PTP1.