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ACS Appl. Mater. Interfaces, **Just Accepted Manuscript** • DOI: 10.1021/acsami.0c10542 • Publication Date (Web): 09 Jul 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Highly Sensitive Electrochemiluminescence Biosensor for Pyrophosphatase Detection Based on Click Chemistry Triggered Hybridization Chain Reaction in Homogeneous Solution

Xiaocui Huang[a,1], Jinpeng Jia[b,1], Yue Lin[a], Bin Qiu[a], Zhenyu Lin[a*] and Huixing Chen[c*]

[a]Ministry of Education Key Laboratory for Analytical Science of Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116, China.

[b]Department of Orthopaedics, General Hospital of Chinese People's Liberation Army, 28 Fuxing Road, Beijing 100853, China.

[c]Department of Hepatobiliary Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian, 350000, China.

Abstract

The abnormal expression of pyrophosphatase (PPase) is closely related to many diseases and malignant tumors, so the detection for PPase is of great significance in clinical diagnosis, disease monitoring and other biomedical aspects. In this study, a sensitive and specific electrochemiluminescence (ECL) biosensor combined highly specific Cu^{+} catalyzed azide-alkyne cycloaddition (CuAAC) with high efficiency of hybridization chain reaction (HCR) for the purpose of detecting pyrophosphatase has been designed. Highly efficient hybridization chain reaction amplification processed in homogeneous solution and the amplification products were connected to the electrode surface in one step, which solved the problem of low DNA amplification efficiency on the electrode surface because of the steric hindrance. $Ru(phen)_3^{2+}$ was embedded into the dsDNA and act as ECL probes, the enhanced ECL intensity of the system had a linear relationship with the logarithm of PPase concentration in the range of 0.025 - 50 mU with a detection limit of 8 μU. The method was proved to be of good specificity, repeatability and stability that could be used for screening and quantitatively determinating pyrophosphatase inhibitor sodium fluoride. The practicability of this method in clinical application has been proved through the detection of serum from the clinical arthritis patients. Moreover, the method can be used to monitor PPase activity of arthritis patients before and after administration, so as to provide reference for the effect of drug treatment.

KEYWORDS: electrochemiluminescence, pyrophosphatase, click chemistry, hybridization chain reaction, pyrophosphatase inhibitors

1. Introduction

Inorganic pyrophosphatase (PPase, EC 3.6.1.1), a widespread hydrolase, can specifically and irreversibly hydrolyze one molecule of inorganic pyrophosphate (PPi) into two molecules of orthophosphate (Pi). The hydrolysis reaction is a high-energy discharge reaction that provides thermodynamic power for biosynthetic reactions.[1-2] Early studies showed that PPase involved in various physiological processes such as calcium absorption, carbohydrate metabolism, lipid metabolism, and bone formation.[3-5] The abnormal expression of PPase has been confirmed to be associated with many clinical diseases such as colorectal cancer, lung cancer, hyperthyroidism, etc.[6-8] Therefore, the detection of PPase is of great importance in clinical diagnosis, disease monitoring and other biomedical aspects. To date, many analytical techniques like fluorescence, colorimetric, enzyme analysis, high-performance liquid chromatography and electrochemical, have been applied to develop sensitive methods for PPase detection.[9-12] However, all the current ways are either complicated or lack of sensitivity. Hence, much effort still needs to be paid on the development of simple, fast and sensitive methods for PPase detection.

Click chemistry was first proposed by Shapless et al. in 2001.[13] It was a series of chemical reactions that can efficiently and quickly join small units together with heteroatom links (C-X-C) to generate stable new substances. Cu^{+} catalyzed azide-alkyne cycloaddition was one of the most famous click chemistry reactions.[14] Due to the advantages of fast reaction, high selectivity, mild reaction conditions and high synthesis efficiency, the reaction had attracted much attention in wide range of

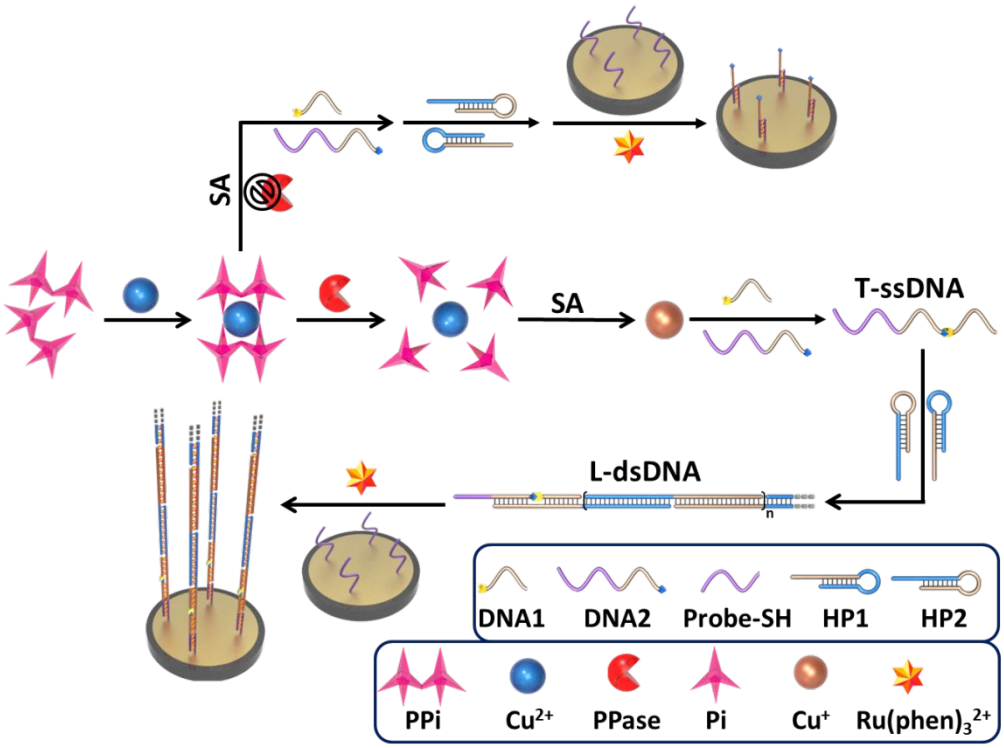
fields, especially in the modification and functionalization of biomacromolecules. This reaction had been combined with different detection techniques and applied to develop many sensitive biosensors for different targets.[15-19] For example, Ge et al. established a colorimetric Cu^{2+} biosensor through click chemistry and hemin/G-quadruplex HRP-mimicking DNAzyme.[20] Li and his colleagues constructed a dual amplification biosensor for CFP-10 detection using the DNA click ligation induced target cycling and gold nanoparticles loaded with G-quadruplex DNA motifs.[21] Our group also committed to the application of click chemistry in biological detection, and has developed a variety of biosensors. For example, by introducing DNA-modified CuS particle to a DNA sandwich assay, a highly sensitive and selective fluorescence sensor for DNA detection had been proposed.[22] And by combining personal glucose meters (PGM) with click chemistry, a portable chemical sensor for sensitive and selective determination of histidine had been developed.[23] In addition, PPi can form complexes with Cu^{2+}, and can also be hydrolyzed by PPase. This principle had been applied to develop many biosensors for PPi or PPase.[12, 24-26] In early studies, this principle had been coupled with the click chemistry to develop sensitive fluorescence biosensors for PPase detection.[17,27]

Electrochemiluminescence (ECL) is the process that luminophores are oxidized or reduced on the electrode surface undergo high-energy electron-transfer reactions to form excited states that emit light.[28-29] As a powerful analytical technique, ECL has been widely used in immunoassay, food safety, environmental monitoring and other fields.[30] Integrating electrochemical controllability and high sensitivity of

chemiluminescence, ECL is particularly suitable for developing detection strategies for low abundance biomarkers.[31-33] However, in most reported ECL biosensors, the nucleic acid signal amplification is restricted to only occur on the electrode surface, and the steric hindrance on the electrode surface limits the efficiency of DNA hybridization, which affects the effect of signal amplification. Meanwhile, the DNA hybridization reaction in homogeneous solution is convenient and efficient. Therefore, connecting the amplification product to the electrode surface probe in one step can shorten the time required for DNA growth on the electrode surface and reduce the effect of steric hindrance on the amplification effect.

$Ru(phen)_3^{2+}$, an efficient and stable ECL molecular beacon, has been widely used to construct various highly sensitive ECL nucleic acid sensors because it can be embedded in the groove of double strand DNA (dsDNA).[34-37] As an enzyme-free isothermal nucleic acid amplification technology, hybridization chain reaction (HCR) is simple and rapid and does not require repeated temperature changing steps, which can form dsDNA in homogeneous solution easily.[38-39] In an early report, HCR had been coupled with click chemistry to develop sensitive biosensor for tumor exosomes.[19] But the HCR occurred on the interface of the solid electrode surface and the solution, the efficiency is relative low. In this study, by combining the high selectivity of click chemistry, the high amplification efficiency of HCR in homogeneous solution and the high sensitivity of ECL, an ECL biosensor for PPase detection was developed. The principle of the proposed system was shown in **Scheme 1**. Cu^{2+} was first complexed with PPi to form a coordination complex (PPi/Cu^{2+}). The

present PPase can hydrolyzed PPi to release Cu^{2+} from the PPi/Cu^{2+}, and the free Cu^{2+} was then reduced to Cu^{+} by sodium ascorbate (SA), thereby catalyzing the click chemistry of the two single strand DNA (ssDNA) modified with alkynyl groups and azide groups at the end to form new long ssDNA (T-ssDNA), which can be used as a trigger to initiate HCR amplification for the formation of long dsDNAs amplification products with 5'-protruding ends (L-dsDNA). The capture probe modified on the gold electrode surface with Au-S bonds could hybridize with the 5'-protruding end of L-dsDNA and then capture the amplification product onto the electrode surface. At the same time, the ECL molecular beacon $Ru(phen)_3^{2+}$ would embed in the dsDNA. The intensity of the signal was dependent on the amount of $Ru(phen)_3^{2+}$ embedded in the dsDNA as the HCR product hybridizes to the capture probe. Therefore, the more $Ru(phen)_3^{2+}$ accumulates on the electrode surface, the stronger the ECL signal can be detected in this biosensor. Conversely, if PPase was absent and HCR amplification did not occur in the system, the capture probe can only capture free DNA2 in the solution, and only a small amount of $Ru(phen)_3^{2+}$ accumulates on the electrode surface, hence just weak ECL signals could be detected. The designed biosensor showed good selectivity and high sensitivity to PPase, and could be used for screening and quantitatively determinating PPase inhibitor sodium fluoride (NaF). Furthermore, the analysis results of the serum samples were also satisfactory.



Scheme 1. Schematic illustration of the ECL biosensor for PPase based on click chemistry-induced hybridization chain reaction.

2. EXPERIMENTAL SECTION

2.1. Reagents and instrumentation. Baker's yeast inorganic PPase (EC3.6.1.1), 6-mercapto-1-hexanol (MCH), dichlorotris (1, 10-phenanthroline) ruthenium (II) hydrate ($Ru(phen)_3^{2+}$), tripropylamine (TPA) and 1,4-dithiothreitol (DTT) were purchased from Sigma-Aldrich (Shanghai, China). Sodium pyrophosphate was provided by Beilian Fine Chemicals Co. Ltd. (Tianjin, China). Sodium ascorbate (SA) and copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) were purchased from Alfa Aesar China Co. Ltd. (Tianjin, China). Tris-borate-EDTA (5× TBE) and all oligonucleotides used in this work were purchased from Sangon Inc. (Shanghai, China). The oligonucleotides sequences were listed in Table S1. Clinical serum samples were obtained from the volunteers of Fujian Medical University Union Hospital (Fujian,

China).

The ECL signals were measured with a CHI660D electrochemical workstation (Chenhua Instruments, Shanghai, China) and a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China). Gold electrode (3 mm in diameter), platinum electrode, and Ag/AgCl electrode were used as working, counter and reference electrodes, respectively.

2.2. Preparation of HCR amplification solution. 20 μM Cu^{2+} and 50 μM PPi were mixed in 1× NEBuffer 2 containing DNA1 and DNA2, and reacted at 37 ℃ for 20 min to form a stable PPi/Cu^{2+} complex ($Cu(P_2O_7)_2^{6-}$). PPi was hydrolyzed to Pi by adding PPase of a certain concentration for 1 h to release Cu^{2+} from the PPi/Cu^{2+} complex. Subsequently, 7.5 μM SA was added to the solution and reacted in the dark for 30 min at room temperature. This process reduced the free Cu^{2+} to Cu^{+}, while the DNA1 and DNA2 in the solution underwent a click chemistry reaction under Cu^{+} catalysis. The terminal alkynyl group of DNA1 and the terminal azide group of DNA2 were linked by click chemistry to form T-DNA. Then the mixture of HP1 and HP2 of 10 μM was added and the resulted product was incubated at 37 ℃ for 1.5 h. T-DNA can open the material hairpin probe HP1 of HCR and thereby triggering HCR amplification, which produced a great number of L-dsDNA until the HCR material hairpin HP1 or HP2 was depleted.

2.3. Pretreatment of gold electrode and ECL detection. The gold electrode with a diameter of 3 mm was first polished with alumina powder of 0.3 μm and 0.05 μm, and then ultrasonically cleaned with ethanol and ultrapure water in that order.

Afterwards, the electrode treated as described above was immersed in a 0.5 M H_2SO_4 solution and subjected to cyclic voltammetry scanning in the range of 0 to 1 V until a stable cyclic voltammogram of three oxidation peaks and one reduction peak was obtained, indicating that the electrode has been activated. Followingly, 0.5 μM thiol-modified capture probe (Probe-SH) was dropped onto the activated gold electrode and placed at 37 ℃ for 2 h to allow Probe-SH to bind to the gold electrode by the Au-S bond. The unmodified sites of the modified gold electrode surface were blocked with 1 mM MCH. In the next step, the treated gold electrode was then immersed in the HCR amplification solution while 0.1 μM $Ru(phen)_3^{2+}$ was added and incubated at 37 ℃ for 90 min. In this process, L-dsDNA was captured by the Probe-SH onto the electrode while a sufficient amount of $Ru(phen)_3^{2+}$ embedded into the groove of the dsDNA. The gold electrode was washed with ultrapure water after each step above to remove non-specific adsorption on the electrode surface.

At last, the gold electrode after the above reaction was immersed in 2 mL PBS buffer (containing 20 mM TPA) for ECL analysis. The photomultiplier tube voltage was set to -500 V, and the measurement was performed at a scan rate of 100 mV/s in the voltage range of 0.4 V to 1.6 V.

2.4. Polyacrylamide gel electrophoresis analysis. Electrophoresis was performed using a 12% polyacrylamide gel to verify the occurrence of click chemistry and explore the feasibility of click chemistry-induced HCR amplification. The working voltage was 80 V, the running buffer was 0.5× TBE, and electrophoresis was carried out for 120 min at room temperature.

2.5. Electrochemical impedance spectroscopy. Electrochemical impedance spectroscopy characterization of the proposed strategy was performed using the CHI 660D software. The detection buffer was 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (containing 100 mM KCl), the operating voltage was 0.235 V, and the impedance frequency range was $10^{-1} \sim 10^4$ Hz.

3. RESULTS AND DISCUSSION

3.1. Feasibility study. The feasibility of the proposed strategy was characterized by polyacrylamide gel electrophoresis, electrochemical impedance spectroscopy and ECL, respectively. The result of polyacrylamide gel electrophoresis (**Figure 1A**) confirmed that click chemistry reaction had occurred in the system, and the HCR amplification triggered by the click chemistry was successfully achieved. In the absence of PPase, Cu^{2+} was confined in Cu^{2+}/ppi complex, and DNA1 (lane a) and DNA2 (lane b) cannot be linked (lane c). When PPase was added, click chemistry occurred in the system, and T-DNA (lane d) was formed by connecting DNA1 and DNA2 (lane g) showed that T-DNA could open up HP1 (lane e), one of the material hairpin of HCR, and triggered HCR amplification (lane j). In the absence of PPase, no HCR amplification occurred in the system (lane i), and the band position of lane i was comparable to that of the mixture of HCR material hairpin (lane h).

The electrochemical impedance spectroscopy was applied to characterize different electrode states. **Figure 1B** showed that the capture probe was successfully modified to the electrode surface (the impedance of curve b was larger than that of curve a). Moreover, the impedance of the system (curve e) was significantly larger

when the target was present than that when the target was absent (curve d), which also verified that the addition of the target triggered the occurrence of click chemistry and further induced HCR amplification. The product of HCR was captured by the capture probe onto the electrode surface, and the surface resistance of the electrode was greatly enhanced. The ECL analysis also confirmed the feasibility of the proposed strategy (**Figure 1C**). Without the target, the capture probe modified on the electrode surface could only hybridize with the free DNA2 in the solution, and just a small amount of $Ru(phen)_3^{2+}$ would be captured to the electrode surface and therefore only weak ECL signal could be detected (curve a), while with the addition of the target, HCR amplification occurred in the system. The HCR product containing large quantities of dsDNA could hybridized with the probe modified on the electrode surface to capture plenty of dsDNA onto the electrode surface. In the meantime, lots of $Ru(phen)_3^{2+}$ embedded in the groove of the dsDNA and a strong ECL signal was detected (curve b).

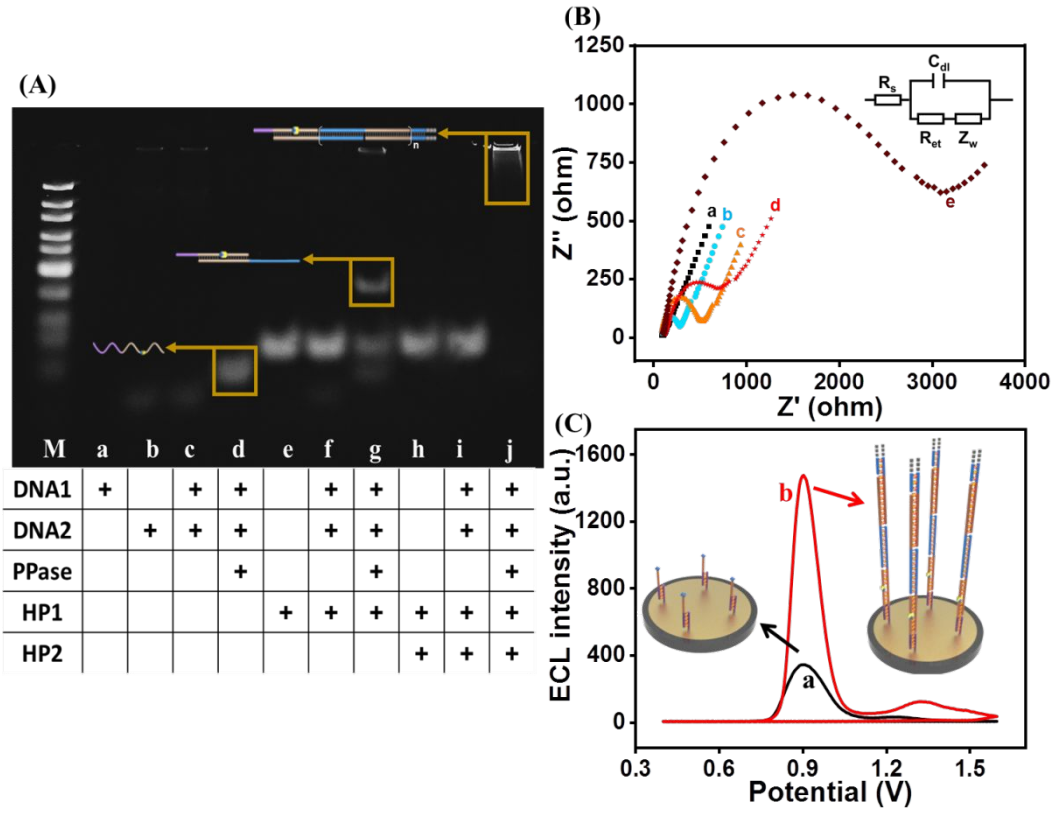


Figure 1. Feasibility of the ECL biosensor. (A) Polyacrylamide gel electrophoresis analysis of different conditions; (B) Electrochemical impedance spectroscopy of various probes modified at electrode (a: bare Au electrode; b: electrode 'a' + Probe-SH; c: electrode 'b' + MCH; d: electrode 'c' + (Cu(PPi) + SA + DNA1 + DNA2 + HP1 + HP2); e: electrode 'd' with 10 mU PPase) in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ including 100 mM KCl; (C) ECL intensity of the proposed sensing system in the (a) absence and (b) presence of 0.25 mU PPase.

3.2. Optimization of the experimental conditions. The immobilization of the amplified product on the gold electrode surface is performed through the hybridization of the DNA2 in the amplified product and the capture probe on the electrode surface. In order to fully capture the amplified products, gold electrodes modified with 0.5 μM Probe-SH were used to capture DNA2 at different

concentrations in the experiment, so as to optimize the concentration of DNA2. As shown in **Figure S1**, when the concentration of DNA2 was 0.4 μM, the measured ECL signal tended to be stable, indicating that the DNA2-Probe-SH complex formed on the electrode surface reached saturation at this time. Therefore, 0.4 μM DNA2 was selected for subsequent experiments. It is worth noting that in the experiment, the binding ratio of DNA1 and DNA2 was 1: 1, so the concentration of DNA1 was also determined to be 0.4 μM. In addition, the high sensitivity of this method mainly depends on the efficiency of HCR amplification, so the amount of amplification materials HP1 and HP2 is also optimized. **Figure S2** shows that when the concentration of HP1 (also the concentration of HP2) was 10 μM, the measured ECL signal reached the platform. Even if the amplification material continued to increase afterwards, there was no significant improvement in HCR efficiency, indicating that within 90 min of amplification time, 10 μM HP1 and HP2 (25 times the concentration of 0.4 μM DNA2) was sufficient to obtain the highest amplification efficiency.

In order to obtain the best sensing performance, several experimental parameters were optimized in this experiment. Coordination of Cu^{2+} with PPi was a prerequisite for this strategy. Incomplete complexation of Cu^{2+} by PPi caused free Cu^{2+} in the solution and resulted in a high background signal, while excessive PPi directly affected the sensitivity of the sensor. Therefore, the ratio of PPi and Cu^{2+} and their reaction time were first optimized. As shown in **Figure 2A** and **Figure 2B**, with the increase of the ratio of PPi and Cu^{2+} and their reaction time, the free Cu^{2+} in the system decreased, hence, the finally detected ECL response signal also dropped.

Besides, Cu^{2+} were fully complexed by PPi and the ECL signal was minimized and reached the platform when the ratio of PPi and Cu^{2+} was 2.5 : 1 and the reaction time was 20 min, which was consequently chosen as the complexation condition for Cu^{2+} and PPi in subsequent experiments.

The reaction time of PPase also had an influence on the efficiency of the experiment. As depicted in **Figure 2C**, the measured *Δ*ECL intensity ($\Delta ECL = ECL - ECL_0$, *ECL* was the measured ECL intensity, and ECL_0 was the ECL intensity when no target was present) increased with the extension of the PPase reaction time in 0-60 min, and reached the plateau when reacted 60 min. Therefore, 60 min was chosen as the optimal hydrolysis time for PPase.

Finally, the effect of the time of $Ru(phen)_3^{2+}$ embedded in dsDNA (also the hybridization time between the capture probe and the HCR reaction product) for the experiment was investigated. As shown in **Figure 2D**, *Δ*ECL intensity increased with the increasing of hybridization time and stabilized over 90 min, indicating that 90 min was sufficient for the hybridization of the capture probe and the HCR reaction product, and that $Ru(phen)_3^{2+}$ was fully embedded into dsDNA.

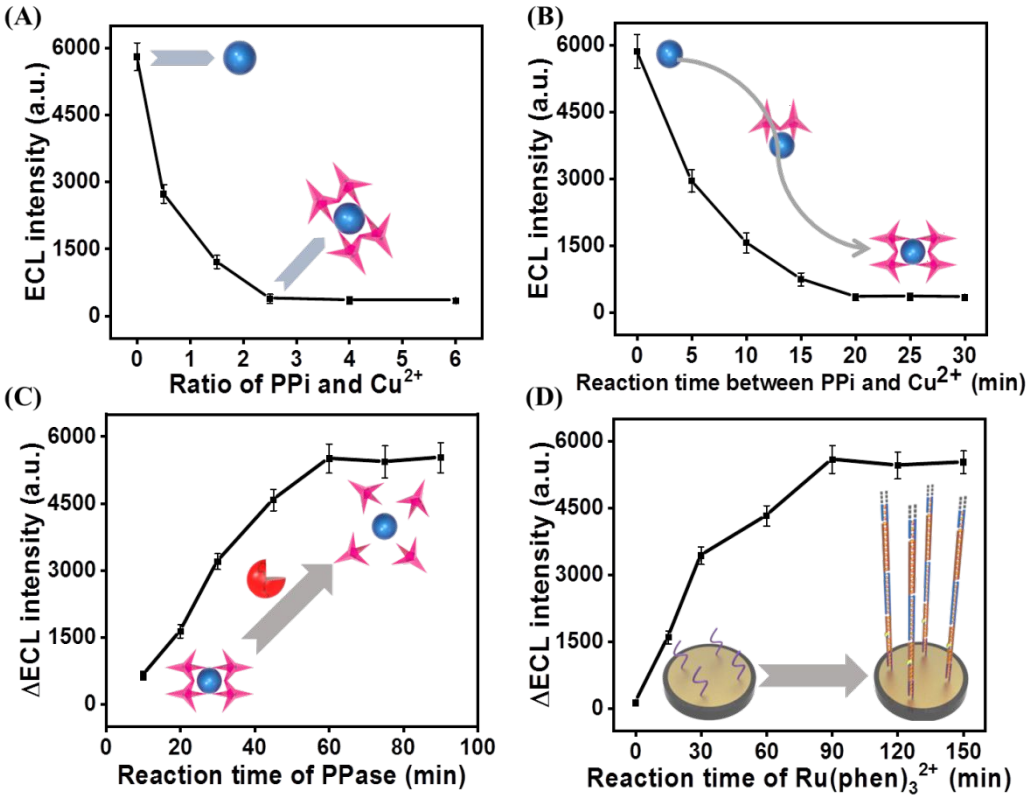


Figure 2. Optimization of the detection conditions: (A) Ratio of PPi and Cu^{2+}; (B) Reaction time between PPi and Cu^{2+}; (C) Reaction time of PPase; (D) Reaction time of $Ru(phen)_3^{2+}$. The PPase concentration was 50 mU in the above experiments. The illustrated error bars represented the standard deviation of three repeated measurements.

3.3. Performance of the developed biosensor. Under the optimal experimental conditions, the relationship between the ECL response signal and the concentration of PPase was explored. As depicted in **Figure 3A**, the ECL intensity grew as the PPase concentration increased in the range of 0 - 50 mU. In addition, *ΔECL* intensity and the logarithm of PPase concentration had a good linear relationship in the range of 0.025 - 50mU (illustration in **Figure 3B**). The linear fitting equation was as follows:

$$\Delta ECL = 1351.6 \text{ Lg } C_{PPase} + 2608.6 \qquad R^2 = 0.9908$$

where C_{PPase} was the concentration of PPase (mU) and R was the correlation linear coefficient. The limit of detection (LOD) was 8 μU (S / N = 3). Compared with some methods for detecting PPase in recent years, the strategy proposed in this study possessed a lower limit for the detection of PPase (see **Table 1**), higher sensitivity and wider dynamic range of the biosensor, and the latter two were attributed to: (1) the conversion from the detection of the target PPase into a response of Cu^+ catalyzed click chemistry reaction, which effectively increased the sensitivity of the target response; (2) the signal amplification of ECL further achieved by the integration of HCR and the signal conversion of $Ru(phen)_3^{2+}$, which was beneficial to the detection of low abundance targets.

Table 1. Comparison of the present method with other reported strategies.

Detection Method	Dynamic Range (mU)	Limit of Detection (mU)	References
Label-free colorimetric analysis	6.0 to 80	5.4	[10]
Fluorescent method	4.2 to 70	1.4	[9]
Electrochemical assay	2.0 to 100	1.2	[11]
Photocurrent analysis	0.8 to 5000	0.41	[12]
ECL method	0.025 to 50	0.008	This work

3.4. Selectivity, stability and reproducibility of the system. Limited by the complementary base pairs in the stem of the hairpin structure, the amplified material DNA cannot stretch freely, while the amplification reaction can only be triggered by the initiator generated by the CuAAC reaction, which lays the foundation for the

biosensor to specifically respond to the target. To evaluate the selectivity of the prepared biosensor for PPase, five typical interfering proteins (Thrombin, HSA, Urease, CEA, and MUC 1) were tested for ECL under the same experimental conditions. As shown in **Figure 3C**, in the absence of the specific target, the Cu^{2+}/PPi complex cannot hydrolyze and release Cu^{2+} to initiate the subsequent reaction, so the ECL signal generated is weak. The ECL responses of the selected five interfering proteins were almost identical to the experimental background, and only PPase could cause a significant increase in the signal, indicating that the biosensor had good selectivity for PPase detection.

The stability of the ECL biosensor was assessed by 5 cycles of consecutive potential scans between 0.4 V and 1.6 V. **Figure S3** shows that the ECL response is stable and the relative standard deviation (RSD) was calculated to be 1.45%, indicating that the ECL biosensor exhibited good stability.

As shown in **Figure 3D**, with no target added as a control, the reproducibility of the ECL biosensor was studied by repeatedly performing PPase (0.06 mU, 0.2 mU, 2 mU and 6 mU) detection for three times. The P value calculated using ANOVE is much less than 0.01, indicating that the inter-group difference is significant, the concentration of PPase has a very significant effect on the measurement results. And the RSD of each group at the same concentration was calculated to be 2.69% - 5.18%, the deviation within each group is small. In addition, the reproducibility was also investigated with six electrodes toward 10 mU PPase, RSD of intra-assay was 3.41%. All these results demonstrated that the ECL biosensor had a high reproducibility.

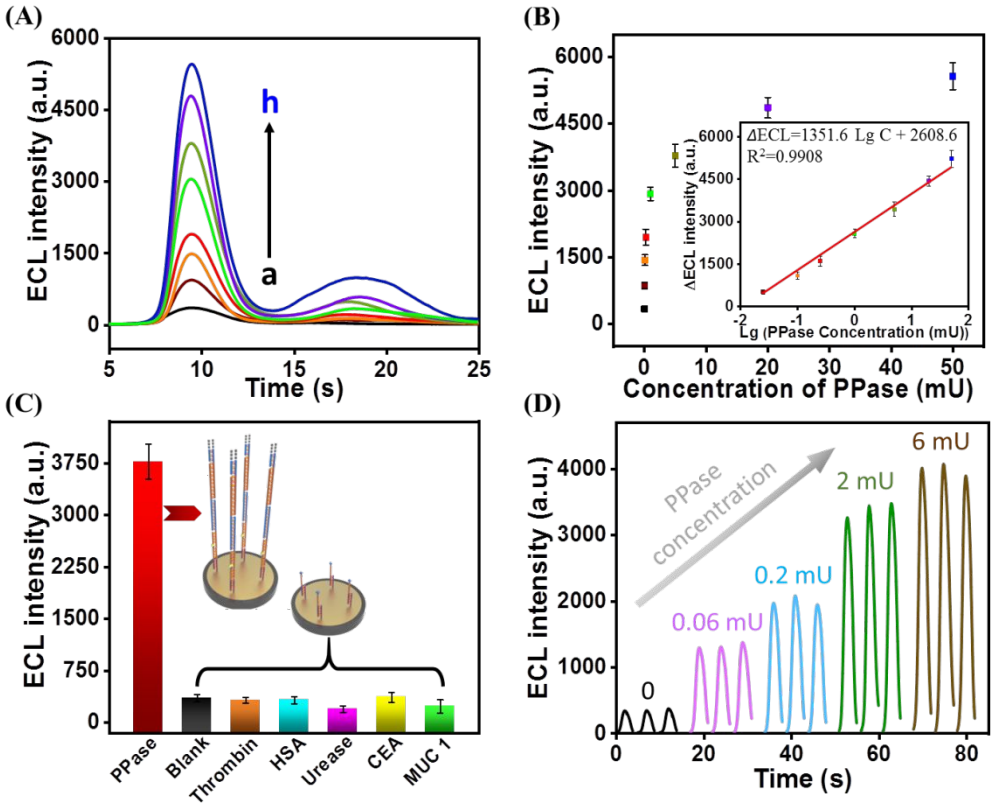


Figure 3. (A) ECL responses for different PPase concentrations (a to h: 0, 25 μU, 100 μU, 250 μU, 1 mU, 5 mU, 20 mU, and 50 mU). (B) The linear relationship between ⊿ECL intensity and the logarithm of the PPase concentration. (C) ECL response in the presence of 5 mU PPase and other analytes (50 μg/mL thrombin, 50 μg/mL HSA, 50 μg/mL urease, 50 μg/mL CEA and 50 μg/mL MUC 1). (D) The reproducibility of the ECL biosensor. The illustrated error bars represented the standard deviation of three repeated measurements.

3.5. Real sample analysis. In the treatment of arthritis patients, the regulation of calcium deposition by PPase plays a vital role in the metabolic activity of cartilage and the formation of new bone. So PPase can act as a potential evaluation index during the treatment of arthritic and the proposed biosensor has been applied to measure the activity of PPase in the serum from four clinical arthritic patients. Take

10 μL of serum samples diluted 50-fold for recovery experiment by a standard addition method. The results in **Table 2** indicate that this method can be used for the detection of PPase in clinical samples, and the activity levels of PPase in the serum of different arthritis patients show large differences.

Table 2. Detection results of PPase in diluted arthritic patient serum.

Sample No.	PPase added (mU)	Total found (mU)	Recovery (%)	RSD (%, n=3)
	-	0.0413	-	3.78
#1	0.100	0.144	103	4.73
	1.00	1.03	98.9	4.26
	-	0.358	-	5.09
#2	0.100	0.454	96.0	4.35
	1.00	1.37	101	3.77
	-	0.194	-	3.34
#3	0.100	0.298	104	2.72
	1.00	1.17	97.6	5.13
	-	0.0771	-	4.62
#4	0.100	0.182	105	3.97
	1.00	1.09	101	4.98

3.6. Inhibitor assay. Sodium fluoride (NaF) is commonly known as PPase inhibitor that can form an intermediate product [F^--PPase] with PPase harbor the function of reducing the activity of PPase. In this study, NaF with different concentrations were added to the system to assess the ability of the system to screen for inhibitors. As shown in **Figure 4A**, the ECL signal decreased significantly with the addition of NaF, indicating that NaF can be used as a model inhibitor to explore the inhibitory effect on PPase activity. Moreover, the reduced ECL signal intensity showed a linear correlation with the logarithm of NaF concentration in the range of 0.1 μM to 10 μM (**Figure 4B**). The above results proved that the sensing system can be used for quantitative analysis of the PPase inhibitor NaF.

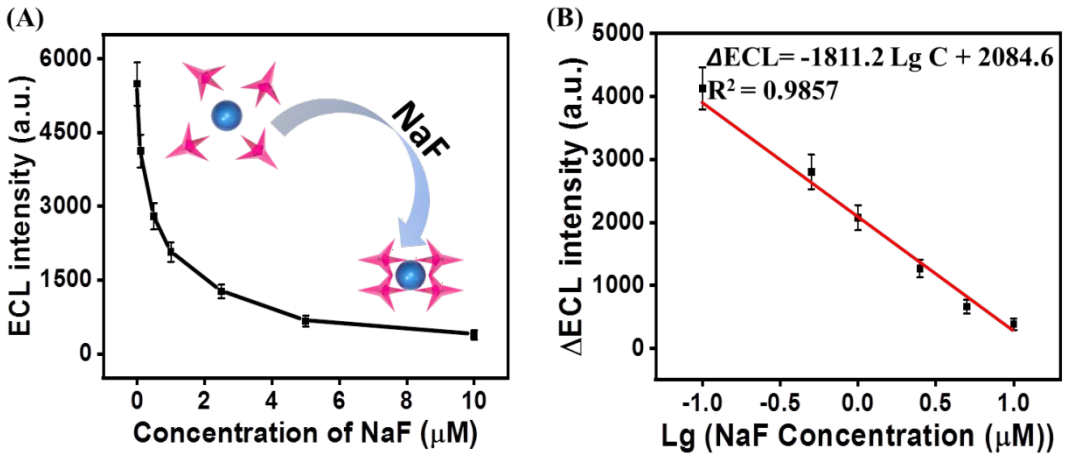


Figure 4. Inhibition efficiency of PPase by NaF. (A) ECL response with different concentrations of NaF. (B) The relationship between the reduced value of ECL signal and the logarithm of NaF concentrations. The PPase concentration was 50 mU. The illustrated error bars represented the standard deviation of three repeated measurements.

4. CONCLUSIONS

By combining highly specific click chemistry with HCR signal amplification technology, an rapid ECL biosensor with mild condition was successfully constructed for sensitive and specific detection of PPase. DNA hybridization reaction in homogeneous solution was convenient and efficient, which could shorten the time required for DNA growth on the electrode surface and reduce the influence of steric hindrance from the solid electrode on the amplification effect. Due to the highly efficient HCR amplification processed in homogeneous solution and the highly sensitive characteristic of ECL, the sensing strategy owned high sensitivity with a detection limit of 8 μU. The proposed method had been applied to detect PPase in the serum from clinical arthritic patients and quantitatively determine PPase inhibitor with satisfactory results. Since PPase can act as a potential evaluation index during the treatment of arthritic, the proposed method can be applied to evaluate the effectiveness of treatment and screen potential PPase inhibitor.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Figures for nucleic acid probe concentration optimization (Figure S1 and Figure S2) and biosensor stability (Figure S3), and table for oligonucleotides sequences used in this study (Table S1).

AUTHOR INFORMATION

Corresponding Author

* Email: zylin@fzu.edu.cn (Zhenyu Lin)

* Email: (chenhuixing@163.com) (Huixing Chen)

Author Contributions

The manuscript was written through contributions of all authors.

X.H., H.C. and Z.L. conceived the projects, and X.H., J.J., Y.L. and Z.L. designed and performed the experiments and collected the data. X.H., J.J., Y.L., B.Q., Z.L. and H.C. analyzed and discussed the data. All authors discussed the results and contributed to the writing of this manuscript. All authors have given approval to the final version of the manuscript

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This project was financially supported by National Sciences Foundation of China (21775026，21974020), the cooperative project of production and study in University of Fujian Province (2018Y4007), the Sciences Foundation of Fujian Province (2018J01685, 2018J01682), STS Key Project of Fujian Province (2017T3007).

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