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Allosteric probe initiated triple signal recycles for sensitive analysis of adenosine triphosphate (ATP)

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Abstract

Adenosine triphosphate (ATP) is closely associated with a variety of pathophysiological process, providing energy for cell activities. However, it remains a huge challenge to develop a simple and sensitive ATP detection method. Herein, we describe an ultrasensitive approach for ATP detection by using an elegantly designed allosteric probe to bind with targets and to induce DNAzyme assisted triple signal recycles. To establish the approach, the allosteric probe is designed with three functional parts to bind with target ATP, to induce signal recycles and to assist chain extension. After recognition of ATP by allosteric probe, the triple signal recycles are initiated, including (i) released ATP-allosteric probe complex unfolds H1 probe, constituting the first signal recycle; (ii) DNAzyme based cleavage induces the second signal recycle; and (iii) chain extension induced release of ATP is the third signal recycle. Through a series of experiments, the approach exhibits a favorable ATP detection performance and shows a high anti-interference ability, indicating a potential application value in early diagnosis of diseases.

Keywords: ATP, DNAzyme, Allosteric probe, DNA polymerase, Cochlear striatal margin cells

Introduction

Adenosine triphosphate (ATP) plays a crucial role in regulating cellular metabolism and providing energy for different cell life activities such as regulating cochlear potential, maintaining cochlear internal environment stability, and controlling vascular tension (Bradley et al. 2010; Fontecilla-Camps 2021; Holzer and Granstein 2004). The variation of ATP concentration is directly associated with several pathological processes, such as the Parkinson's disease and malignant tumors (Boison and Yegutkin 2019; Di Virgilio et al. 2018; Kepp et al. 2021; Zhao et al. 2021). ATP can also be used for the treatment of nerve tinnitus or nerve deafness. For example, in the cochlea, ATP is a signal molecule that plays a key role in regulating the auditory acuity of sound transduction, actively amplifying the internal potential of the cochlea, stabilizing the internal environment of the cochlea and controlling vascular tension (Housley et al. 2013; Mammano 2013). Therefore, development of approaches that can sensitively analyze ATP concentration is pivotal for the diagnosis of various diseases and in-deep researches of pathophysiological mechanism.

In current years, a variety of ATP detection sensors have been reported based on different strategies, including electrochemistry, fluorescence, and electrochemiluminescence (Fang et al. 2018; Huo et al. 2016; Kashefi-Kheyrabadi and Mehrgardi 2013; Khojastehnezhad et al. 2021; Xu et al. 2021; Zhang and Wei 2020). For example, Xu et al. (2021) proposed an efficient electrochemiluminescence biosensor based on CRISPR-Cas12a and successfully applied for the analysis of ATP with a detection limit of 0.48 nM. Thereinto, fluorescence approach as a type of promising analytical technique has attracted abundant attention due to the advantages in selectivity, ease of operation and fast analysis. Thus, a variety of fluorescence approaches have been reported by

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integrating with various signal amplification strategies. Xue et al. (2020) developed a sensitive and label-free ATP detection method by using gold nanorods coupled with enzyme assisted target recycling amplification. Eventually, the method exhibited a low detection limit of 26 pM, providing robust tools for the ATP related researches. Despite that the reported fluorescence based strategies showed favorable detection performances, these methods are also faced the drawbacks of complicated procedures, and requirements of multiple enzymes, which limited their further applications. In addition, the sensitivity of the existed approaches needs to be improved to study the slight variation of ATP concentration. Therefore, it is urgent demand to develop a simple and sensitive ATP analysis approach.

DNAzyme, as a kind of intrinsically catalytic nucleic acid sequences selected by SELEX, shows high catalytic hydrolytic cleavage activities toward a specific substrate (Jouha and Xiong 2021; Khan et al. 2021). Inspired by the robust enzyme catalysis capability of DNAzyme, we propose here a novel simple and sensitive ATP detection method through integrating allosteric probe based recognition of ATP and DNAzyme assisted triple signal amplifications (Fig. 1). In the proposed method, an hairpin structure allosteric probe was designed containing three functional sections, including ATP aptamer for specifically identifying ATP, Initiator section to initiate the following signal amplifications, and P' sequence to induce the DNA polymerase based third signal amplification. Moreover, the ATP aptamer sequence is partially complementary with Initiator sequence to form the stem of allosteric probe, blocking Initiator sequence to induce subsequent signal amplification. When ATP existed in the sensing system, ATP aptamer could specifically bind with ATP, leading to the allosterism of allosteric probe and releasing P' section and Initiator sequence. The released Initiator sequence could bind with the stem (green) in H1 probe to gradually unfold H1 probe, forming Initiator-H1 probe complex. Afterward, the H2 probe could bind with H1 probe to release ATP-allosteric probe complex. The released ATP-allosteric probe complex unfolded a next H1 probe, constituting the first signal recycle. The two toehold sequences in the terminal of formed H1–H2 probe complex could form DNAzyme conformation under the assistance of added substrate probe. With the addition of Mg^{2+}, the DNAzyme was activated to cut the upper sequences in the substrate probe, releasing the ***p*** sequence to bind with P' section in the ATP-allosteric probe complex and generating fluorescence signals. The resulted H1–H2 probe complex could bind with a next substrate probe to form the second signal recycle. Under the assistance of DNA polymerase, a chain that is complementary with ATP aptamer was formed with the ***p*** sequences as primer, leading to the liberation of ATP to attend a bind with allosteric probe, constituting the second signal recycle.

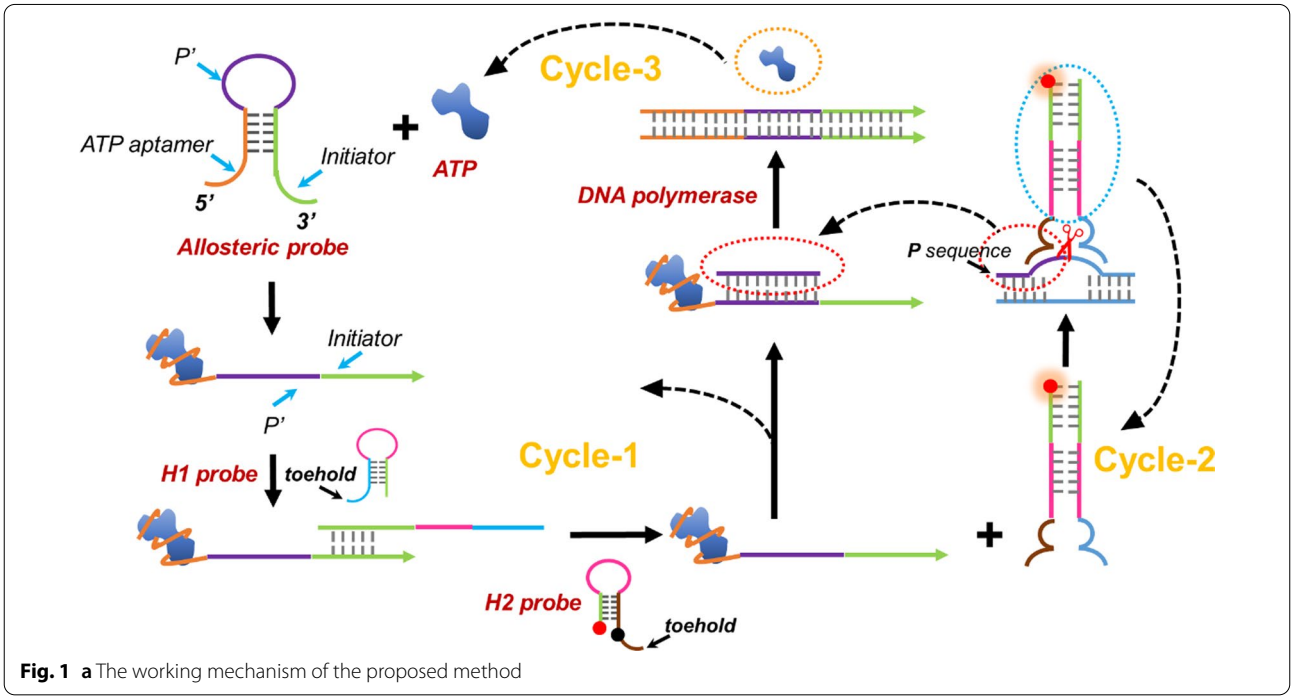

Fig. 1 **a** The working mechanism of the proposed method

Materials and methods

Materials and chemicals

The HPLC-purified oligonucleotide sequences used in this research were brought from Sangon Biotech Co., Ltd. (Shanghai, China) and details of the sequences are listed in Additional file 1: Table S1. All other chemicals were obtained from Shanghai Chemical Reagents (Shanghai, China) and used without further purification. DNA polymerase was purchased from New England Biolabs Ltd. (Ipswich, MA, USA). Adenosine triphosphate (ATP) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Fluorescence signals were detected by Hitachi fluorospectro photometer F-4600 (Tokyo, Japan).

ATP detection through the established approach

Assembly of allosteric probe

10 μL the obtained allosteric probe was firstly heated to 90 °C and incubated for 10 min. Afterward, the solution was gradually cooled to room temperature. The assembly of H1, H2 and dsDNA substrate follows the former steps.

Detection procedures

10 μL the assembled allosteric probe was mixed with the obtained ATP in the solution containing 10 mM Bis Tris Propane-HCl, 10 mM MgCl2, 1 mM DTT, pH 7.0, and incubated for 15 min. Then, 10 μL H1 probe and 10 μL H2 probe were added in the mixture and incubated for 30 min. Afterward, the substrate and DNA polymerase were added in the mixture and the fluorescence signal was detected.

Results and discussion

Feasibility of the allosteric probe-mediated identification of ATP and initiation of signal recycles

We firstly investigated whether the designed allosteric probe could specifically bind with ATP and release the Initiator sequences through a fluorescence assay as illustrated in Fig. 2a. In details, the two terminal of allosteric probe is labeled with fluorescence moiety (FAM) and corresponding quenching moiety (BHQ), respectively. In the hairpin structure, FAM signal is quenched by BHQ, which was demonstrated by the decreased signal of assembled allosteric probe compared with linear one in Fig. 2b. In the present of ATP, it could specifically bind with aptamer section, leading to the re-appear of FAM signal. We then studied the detection stability of allosteric in different experimental conditions, such as DMEM, PBS buffer and fetal bovine serum. The result in Fig. 2c showed no significant fluorescent change between the three groups, indicating a high detecting stability of allosteric probe even in complicated environments. We then studied the feasibility of the approach when ATP existed or not. As shown in Fig. 2d, the obtained fluorescence signal greatly increased when only ATP existed

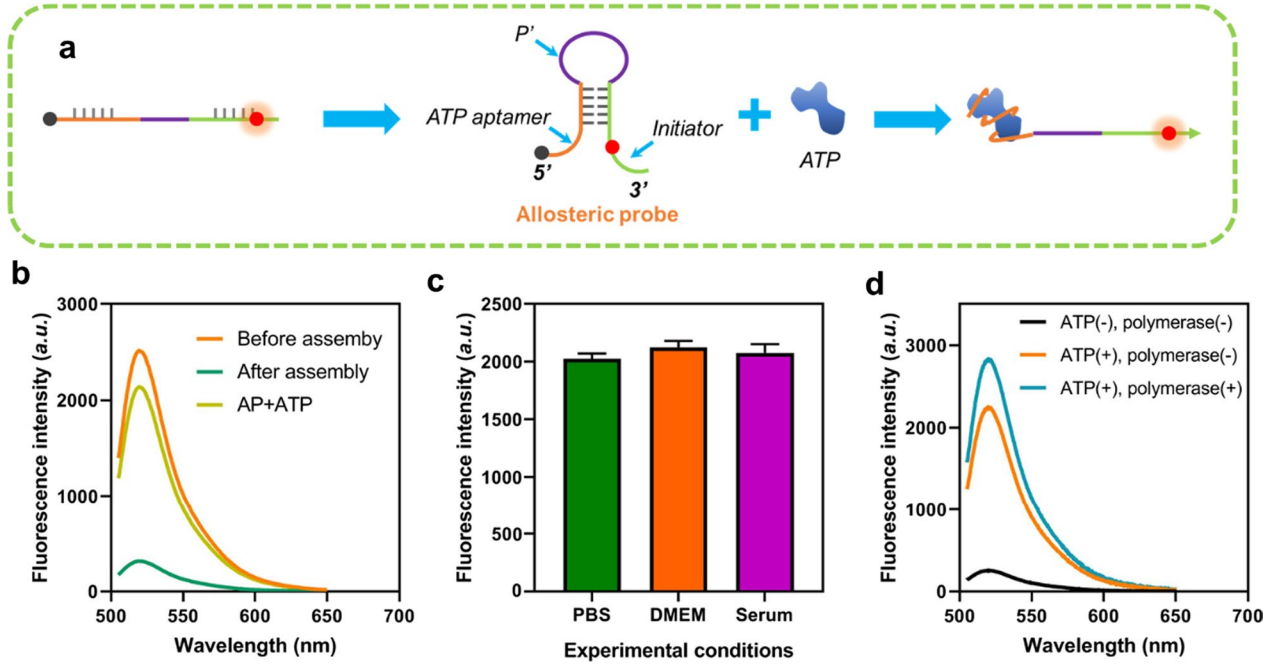

Fig. 2 Feasibility allosteric probe initiated triple signal recycles. **a** Illustration of assembly of allosteric probe; **b** fluorescence spectrum of allosteric probe during the assembly process; **c** fluorescence intensity of allosteric probe when incubated in different buffer solutions; **d** fluorescence spectrum of the approach when ATP and DNA polymerase existed or not

which was 8.8 times higher than control group. Upon the addition of DNA polymerase, the FAM signal exhibited a 1.26 times increasements compared with only ATP, indicating that DNA polymerase could induce an amplified signal.

Optimization of experimental conditions

We then optimized the experimental conditions for a better detection performance, including the length of stem section in allosteric probe, concentration of DNA polymerase, and incubating time. The stem section in allosteric probe is formed by complementary sequences of ATP aptamer and Initiator, which determines the hairpin structure and ATP recognition efficiency. Thus, we firstly studied the complementary length of stem section. From the result in Fig. 3a, the F/F_0 value (refers to the ratio of obtained fluorescence signal of allosteric probe when ATP exited (F) or not (F_0)) of the approach increased when complementary length ranged from 3 to 6 bp, and gradually decreased when the stem section was designed with more than 6 bp. Thus, the stem section in allosteric probe was designed with 6 bp in length for the following experiments. The former experimental result in Fig. 2d showed an enhanced fluorescence signal when DNA polymerase existed. Thus, we explored the detection performance of the approach when incubated with different concentrations of DNA polymerase. The result in Fig. 3b showed a gradually increased fluorescence signal of the method when concentration of DNA polymerase increased from 0.01 to 0.5 U/L. There were no significant enhancements when incubated with more than 0.5 U/L DNA polymerase. Lastly, we optimized incubating time. The obtained signal enhanced when the incubating time ranged from 0 to 45 min and exhibited no more increasements when incubated with more than 45 min. Thus, 45 min was selected in the following experiments. Fluctuations in experimental temperature greatly affect the activity of enzymes and thus influencing the detection performance of the approach. Therefore, we optimized the experimental temperatures. From the result in Additional file 1: Fig. S1, the recorded fluorescence intensity increased with the temperature increased from 4 to 37 °C, indicating an improved detection performance of the approach. When the approach was performed with more than 40 °C, a sharp decrease in fluorescence signal was observed, indicating the detection capability of the approach was diminished. The variation of fluorescence signal indicated a 37 °C was the optimal experimental temperature.

Detection performance of the method

Under the obtained experimental parameters, the allosteric probe initiated triple recycles system was utilized to detect target ATP. As shown in Fig. 4a, constant increasements of FAM signal were observed with the ATP concentration ranged from 100 pM to 100 nM. The correlation between fluorescence intensity and concentration of ATP is displayed in Fig. 4b, exhibiting a good linear relations and a linear fitting equation of $F/F0 = -640.0 * \lg C + 182.2$ ($R^2 = 0.9969$). LOD was determined to be as low as 32 pM (S/N ¼ 3), which is much lower compared to other systems reported previously (Additional file 1: Table S2). We then studied the specificity of the established approach thorough comparing obtained fluorescence signal for 1 nM ATP and interfering substances. Blank was utilized as control. According to former references, we added 0.5 mM interfering substances to test the anti-interference of

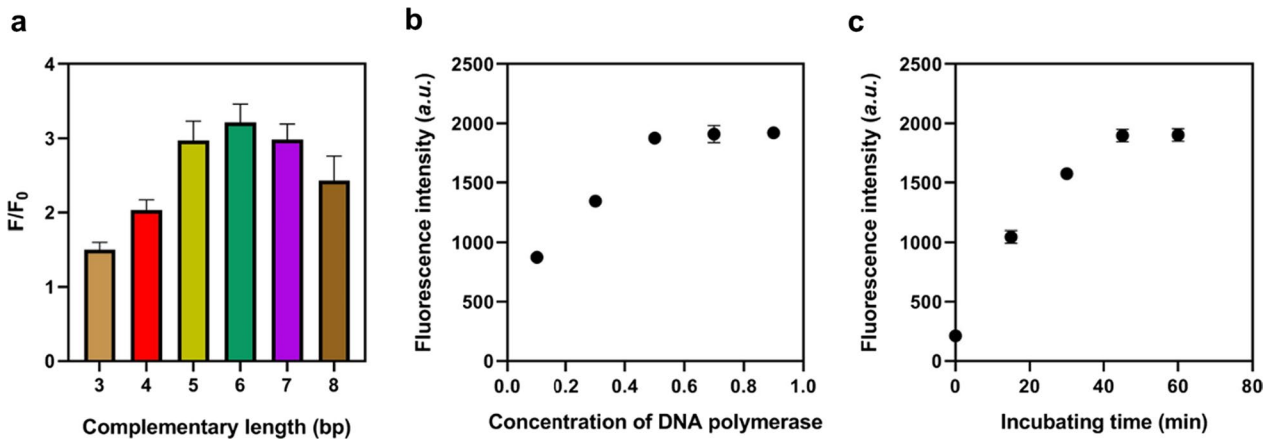

Fig. 3 Optimization of experimental conditions. **a** F/F_0 result of the allosteric probe with different complementary lengths when ATP existed or not. **b** Fluorescence intensity of the approach for ATP detection when incubated with different DNA polymerase. **c** Fluorescence intensity of the approach for ATP detection with different incubation times

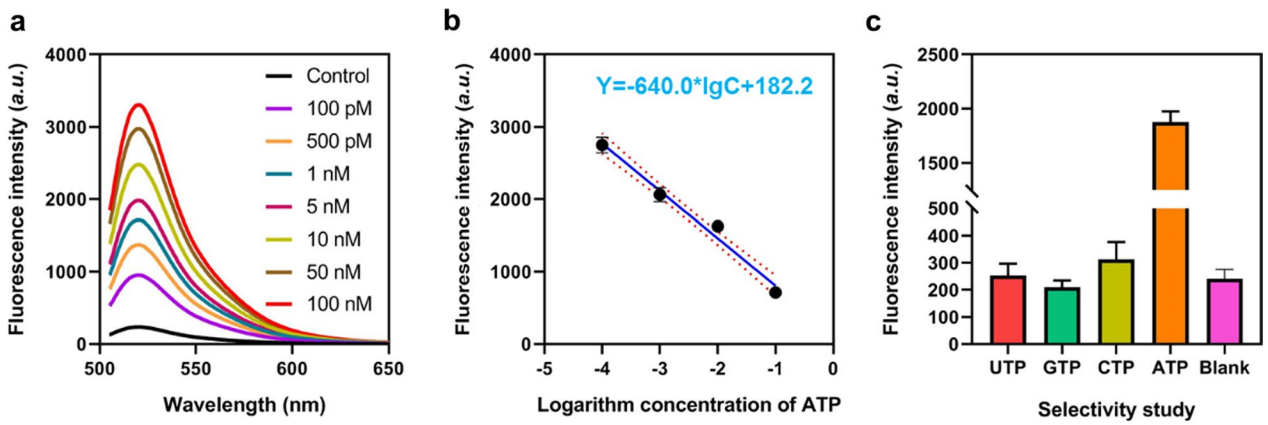

Fig. 4 Detection performance of the method. **a** Fluorescence spectrum of the approach when detecting different concentrations of ATP; **b** correlation equation of the obtained signal and ATP concentration; **c** fluorescence intensity of the approach when detecting UTP, CTP, GTP and ATP

Table 1 Detection result of ATP in artificial clinical samples

Samples	Added (pM)	Detected (pM)	Recovery (%)	RSD, $n=3$
1	50	49.7	99.4	4.3
2	100	102.9	102.9	3.5
3	500	505.6	101.1	4.8
4	800	789.3	98.6	4.7
5	1000	1010.3	101.0	5.8

RSD relative standard deviation

the approach. From the result in Fig. 4c, the obtained intensities of UTP, CTP, GTP and blank showed little difference. Only when detecting ATP, the sensor exhibited a greatly enhanced fluorescence intensity, indicating a great selectivity of the established allosteric probe initiated triples recycles biosensing system. The stability of the developed approach was subsequently studied. From the result in Additional file 1: Fig. S2, CV (coefficient of variation) of the recorded fluorescence signals of 10 sample duplicates was 5.36%, implying a high stability of the approach.

Clinical application of the approach

To study the applicable potential of the established approach in clinical practice, artificial serum samples were prepared through diluting ATP to DMEM solution. The ATP in the constructed samples were detected by the established approach and the concentration of ATP were calculated through fitting the obtained fluorescence intensities to the correlation equation in Fig. 4b. From the result in Table 1, a good recovery rate between 98.6 and 102.9% was observed, indicating that the established approach could potentially be applied in clinical practice.

Conclusion

In summary, the allosteric probe integrating the function of binding with target ATP, initiating subsequent signal recycle and inducing DNA polymerase based chain extension was fully developed for the construction of triple signal recycle sensing system for the detection of ATP. The triple signal recycles were formed by: (i) releasing ATP-allosteric probe complex during the formation of H1–H2 duplex to unfold H1 probe, constituting the first signal recycle; (ii) DNAzyme based cleavage induced the second signal recycle; and (iii) chain extension induced release of ATP is the third signal recycle. Based on the triple signal recycle, the method exhibited a LOD of 32 pM and a wider detection range. As a simple and sensitive ATP detection platform, it would find a broad spectrum of applications in bioanalysis, disease diagnostics, and clinical biomedicine. In addition to ATP detection, the proposed approach could be potentially extended to other biomarker detection through replacing the aptamer, showing a wide applicable scene in practice.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00341-6.

Additional file 1. Table S1. Sequence of synthesized oligonucleotide probes. **Table S2**. A brief comparison of the established approach with former ones. **Figure S1**. Fluorescence intensity of the approach with different experimental temperatures. **Figure S2**. Fluorescence intensity of the approach when detecting 10 sample duplicates.

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Author contributions

LY is the supervisor of the team in all research steps including designing, data analysis and manuscript writing. LC, as the first author, has the main role for

experimental data collection, data gathering, preparation of results, and data analysis. All authors read and approved the final manuscript.

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Availability of data and materials
Almost all details of experimental data are presented in the article or additional file.

Declarations

Ethics approval and consent to participate
The manuscript does not contain clinical or trial studies on patients, humans, or animals.

Competing interests
The authors declare that they have no competing interests.

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