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A novel fluorescent biosensor for Adenosine Triphosphate detection based on the polydopamine nanospheres integrating with enzymatic recycling amplification

Xiaoting Ji, Bingqing Yi, Yujuan Xu, Yanan Zhao, Hua Zhong, Caifeng Ding*

Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology. Qingdao 266042, P.R. China.

*Corresponding author. Caifeng Ding, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology. Qingdao 266042, P.R. China, dingcaifeng2003@163.com

Abstract

Based on the protective performance of polydopamine nanospheres (PDANSs) for DNA against nuclease digestion and the specific recognition characteristic of aptamer, we have developed an enzymatic recycling signal amplification method for highly sensitive and selective detection of adenosine triphosphate (ATP). Fluorescence measurements were carried out to verify the DNA polymerase and exonuclease III (Exo III) assisted target recycling process and fluorescence signal amplification. In the absence of the ATP, initially, the signal DNA-PDANSs complex was in the "off" state due to the efficient fluorescence quenching of 6-carboxyfluorescein (FAM) adjacent to the surface of PDANSs. Due to the binding of the aptamer by ATP, it trigger DNA polymerase and Exo III assisted target recycling process by the product of release, the complex would change into the "on" state as a result of the dissociation of the FAM from the surface of PDANSs, thus providing greatly enhanced fluorescence emission intensity. The method allows quantitative detection of ATP in the range of 20

nM-600 nM with a detection limit of 8.32 nM. This biosensor requires no complex operations, and is a new high efficiency method for ATP detection.

Graphical Abstract

An enzymatic recycling signal amplification method for highly sensitive and selective detection of adenosine triphosphate (ATP), based on the protective performance of polydopamine nanospheres (PDANSs) for DNA against nuclease digestion and the specific recognition characteristic of aptamer.

Keywords: polydopamine nanospheres (PDANSs); fluorescence resonance energy transfer (FRET); exonuclease III (Exo III); polymerase, recycling amplification

1. Introduction

Owing to their small size, large surface-to-volume ratio, unique catalytic, optical and electronic properties, all manner of nanomaterials have been widely applied in the development of analytical detection method [1-4]. Especially, nanomaterials have been exploited as nanoquenchers for the design of novel biosensors [5,6]. Contrasted to the rigorous dependence of the quencher on the identity of the fluorophore in the conventional molecular beacon structure, the nanomaterials exhibit high quenching efficiencies towards a range of organic fluorophores. In the last few years, many nanomaterial-based, MBs-like fluorescent biosensors have been developed using nanomaterials as quenchers, such as gold nanoparticles (AuNPs) [7,8], carbon nanomaterials including 0D carbon nanoparticles (CNPs) [9,10], 1D carbon nanotubes (CNTs) [11,12] and 2D graphene oxide (GO) [13,14] and recently developed metal-organic frameworks (MOFs) [15] and MoS_2 nanosheets [16,17]. Moreover, for the admirable

biocompatibility and biodegradability of polydopamine nanospheres (PDANSs) [18,19], and the facile, lowcost preparation method, we may utilize PDANSs to protect fluorophore labeled DNA probe adsorbed on their surface from nuclease digestion, it has potentials to be developed into straightforward, fleet, and economical biosensors for molecular diagnostic.

To enhance the detection sensitivity, various target signal amplification techniques have been developed. Compared to the classic manner of polymerase chain reaction (PCR), enzyme-assisted target recycling (EATR) [20,21] do not an expensive thermal cycler. Furthermore, most of the EATR assays are isothermal process. Among the enzymes in EATR, DNA polymerase and Exonuclease III (Exo III) seems to be more commonly used [22,23]. In our previous work [21,24], we prove target recycling amplification can be most effectively achieved by DNA polymerase and Exo III. In the other hand, for the advantages of high affinity and specificity, low immunogenicity, as well as facile synthesis and modification [25], we can use aptamers to achieve detection of determinand.

ATP is the primary energy molecule in living cells, and it is generally called as the "molecular unit of currency" for intracellular energy transfer. Herein, as an exploration, we proposed an enzymatic target recycling signal amplification method for the analysis of adenosine triphosphate (ATP) based on PDANSs.

2. Experimental section

2.1. Materials and apparatus

2.1.1.Apparatus

Scanning electron microscopy (SEM) images were recorded using a JSM6700F scanning electron microsope (JEOL Ltd., Japan). Fluorescence measurements were performed by using a Hitachi F-4600 spectrofluorimeter (Tokyo, Japan) with a scan rate of 1200 nm/min. The excitation wavelength was set to 470 nm, and the 24 photomultiplier tube voltage was set to 700 V. The slits for excitation and emission were set at 10 nm/10 nm. The pH of all solutions was measured by a Model pHS-25 digital acidometer (Rex Electric Chemical Products Department, Shanghai Precision Scientific Instrument Co., Ltd.). Adjustable pipettes were from Thermoelectric instruments co., LTD., Shanghai. TG18KR

Centrifuge came from Dong Wang Instrument (Hunan, China). All glassware used in the experiment are immersed in a chromic acid solution, washed twice with deionized water and after drying use.

2.1.2. Materials

All oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Their base sequences are as below: template DNA, 5'-biotin-TTT TTT TTT TCT GAC ACC TGG GGG AGT ATT GCG GAG GAA GGT ATC ATA AAA TGA TAC CTT CCA AAA AA -3'; reporter DNA, 5'- CCC AGG TGT CAG CCC CCC CCC C -3'; primer DNA, 5'-GGA AGG TAT CAT -3'; signal DNA, 5'-FAM-GGG GGG CTG ACA CCT GGG -3'.

Streptavidin-coated magnetic beads (MBs) were ordered from Tianjin Baseline ChromTech Research Centre (Tianjin, China). Deoxynucleoside triphosphate (dNTP) and Klenow DNA polymerase were obtained from Dalian treasure biological engineering Co., Ltd. (Dalian, China). Dopamine hydrochloride, [Tris (hydroxymethyl) aminomethane] (Tris), Exonuclease III (Exo III), Adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) were obtained from Sangon Biotech Co.,Ltd. (Shanghai, China). ATP Assay Kit was obtained from Beyotime Biotech Co.,Ltd. (Jiangsu China). All the reagents were analytical grade and used without further purification. Phosphate buffer saline (PBS, pH 7.4) contained 0.15 M NaCl, 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4 were prepared by standard methods. Deionized and autoclaved water was employed to prepare all solutions. All aqueous solutions were of analytical grade.

2.2 Polydopamine nanospheres (PDANSs) synthesization.

Polydopamine nanospheres were synthesized according to previous report [19] with minor modification. Briefly, 100 mg dopamine hydrochloride (99%) was added to the mixture of 100 mL Tris-buffer (10 mM) and 40 mL isopropyl alcohol, after stirring for 72 h in dark, the final solution was centrifuged at 10000 rpm for 5 min and washed with distilled water for five times until the supernatant

become the same color as water. Then PDANDSs were re-suspended with suitable volume of distilled water for future experiment.

2.3 Preparation of Signal/PDANS Nanocomplex.

After the synthesis of PDANSs, enough PDANSs was introduced into the PBS buffer containing 500 nM signal, and the mixture was incubated at room temperature for 30 min to form the Signal/PDANSs nanocomplex. Then the obtained Signal/PDANSs nanocomplex was stored at 4 ℃ before further usage.

2.4 Immobilization of template DNA onto MBs.

In this assay, streptavidin-modified MBs (30 μL) were firstly washed three times. Then, template (100 nM) was added into the MBs, and the resultant mixture was incubated for 1 h at 37 ℃ with gentle shaking. The oligonucleotide-modified MBs were rinsed three times with PBS buffer (150 μL, 100 mM, pH 7.4 PBS containing 0.15 M NaCl), magnetically separated, and resuspended in PBS buffer containing BSA (1%) for 1 h to minimize nonspecific adsorption effects. The conjugates were resuspended in PBS buffer (100 μL) before use.

Reporter (100 nM) was added to the solution of template-modified MBs, named MBs-T. After incubating for 1 h at 37 ℃ with gentle shaking, the MBs were washed three times with buffer (150 μL) and resuspended in buffer (100 μL) before use.

2.5 Detection of ATP.

Solutions of ATP at different concentrations (ranging from 0 to 1.0×10^{-6} M) were prepared before use. First, the MBs-T was mixed with different concentrations of ATP at 37 ℃ for 30 min to form the aptamer-ATP complex. Second, cyclic enzymatic signal amplification was performed by mixing the aptamer-ATP complex, DNA polymerase (10 U), dNTPs (0.2 mM) and primer solution at 37 ℃ for 1.5 h. Third, after separating magnetically, a solution of Signal/PDANS nanocomplex (40 μL) and Exo III (25 U) in buffer were added into the supernatant. The mixture was incubated with continuous shaking at 37 ℃ for 2 h. After incubation, the fluorescence of the resulting solution was measured. The

fluorescence emission spectra were recorded from 490 to 650 nm at an excitation wavelength of 470 nm, and the fluorescence intensity at 515 nm was used for quantitative analysis.

3. Results and discussion

We proposed an enzymatic target recycling signal amplification method for the analysis of adenosine triphosphate (ATP) based on PDANSs. Firstly, as illustrated in Scheme 1, an template DNA containing ATP aptamer hybridize with the reporter DNA was constructed. Meanwhile, due to PDANSs can strongly and effectively adsorb FAM-labelled signal DNA on its surface via π-π stacking interactions between unpaired DNA bases and PDANSs, the fluorescence of 6-carboxyfluorescein (FAM) labelled in the signal DNA would been quenched by PDANS through fluorescence resonance energy transfer (FRET). While, in the present of ATP, double-strand DNA begins to despiralize, and the reporter DNA strands are released. Furthermore, DNA polymerase-catalyzed primer strand extend along template DNA, as a consequence, magnetic beads (MB)-DNA compounds discharge to the freedom of DNA polymerase, which in turn catalyzes extension of numbers of primers, generating a powerful signal amplification effect. Simultaneously, the reporter DNA-signal DNA complexes desorb from the surface of PDANSs, and fluorescence of FAM is restored. The FAM-labelled signal DNA immediately becomes the substrate for Exo-III digestion, subsequently releasing the reporter DNA to bind to another signal DNA on PDANSs to initiate the next round of cleavage. This biosensor can provide a probability for detection of ATP.

3.1 Preparation and characterization of PDANSs.

The size of the obtained PDANSs was determined at 215.37±15.6 nm from the SEM image (Fig. 1A，Fig. 1B). Complementary IR spectrum, which was consistent with that in the previous report [26], provided evidence of successful PDANSs synthesis (Fig. 1C). As PDANSs shows broad band absorbance in the UV-vis spectrum (Fig. 1D), it would quench the fluorescence of the fluorophores with different emission wavelengths due to FRET [19].

3.2 The detection feasibility of strategy.

The feasibility of the developed strategy for target substance is validated from two sides. On the one hand, the conformational change of the template DNA caused by the elongation of primer in the presence of ATP was the essential principle in this work. On the other hand, the catalytic action of DNA polymerase and Exo III could produce target signal recycling amplification. To confirm the obtained fluorescence spectra in the present method were dependent on amount of ATP, control experiments were done. The result was shown in Fig. 2A. From the result we can see that the intensity of the fluorescence spectra of 4×10^{-7} M ATP (curve b) was more higher than the background level (curve a). As Fig. 2B shows, in the presence of DNA polymerase (10 U) and Exo III (20 U), fluorescence intensity was more higher than other level, it indicate target signal recycling amplification progress has been occurred by two kinds of enzyme.

3.3 Detection of ATP by the biosensing platform.

Under the optimize experimental conditions (more information see section 1.1-1.6 in supporting information), the fluorescence intensity emitted from the commercialization of ATP was shown in Fig. 3A. The results showed that the fluorescence intensities increased with the increasing of the concentration of the ATP. According to the fluorescence intensity, the working curve of ATP was obtained and shown in Fig. 3B. The ATP concentration from 2×10^{-8} M to 6×10^{-6} M has a good linear relationship with the fluorescence intensity in Fig. 3B. The detection limit was 8.32×10^{-9} M.

3.4 Specificity.

In order to verify the selectivity of this design, the influence of interference of a variety of biorelevant analytes on monitoring ATP were also tested for control experiments. The results in Fig. 4 indicate that various bioanalytes produced no or negligible interference with ATP detection. The applications of the proposed method were performed using the CTP, GTP and UTP samples. The suitability of the developed method was carried out with respect to ICH guidelines [27].

3.5 Validity.

Comparing to commercial detection method, we further evaluated the veracity of this PDANSs-functionalized enzymatic recycling amplification system in sample detection. The ATP standards and their detection results with RSD% by our designed method and ATP Assay Kit have been listed in Table 1. It can be seen that this method has provided accuracy detection results with good repeatability, indicating its feasibility in sample detection.

4. Conclusions

All in all, a fluorescence biosensing platform based on PDANSs intergrating with DNA polymerase and Exo III was constructed. The developed strategy presents several advantages. Firstly, due to the DNA polymerase and Exo III assisted target recycling, the signal was amplified for the detection of ATP, the detection limit for ATP was 8.32 nM, which was 4 orders of magnitude and 6 orders of magnitude lower than that without DNA polymerase (more information see section 2 in supporting information) and heat-treated enzyme (more information see section 3 in supporting information). And the assay time was largely shortened due to the faster signal recovery kinetics. Futhermore, the DNA polymerase and Exo III assisted target recycling strategy was also applied to conduct an aptamer-based biosensing platform. The fluorescence intensity was also enhanced for the assay of ATP. Besides the advantage of the reported PDANSs-based biosensors such as simple, lowcost and easy to automate, this design provides a new method for ATP detection and get more lower detection limit compared with other literature results [28,29]. Last but not least, this works has a shorter time of ATP detection than our former work [30]. In conclusion, with its sensitivity, specificity, simplicity and celerity, it is reasonable to believe that this platform holds great promise for molecular diagnostics.

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Scheme 1. Schematic illustration of the enzymatic target recycling signal amplification method for the analysis of ATP based on PDANSs.

Fig. 1 Structural characterization of PDANSs. (A) The SEM image of PDANSs （2.0 mg/mL）, (B) the SEM image of PDANSs （0.1 mg/mL）, (C) the IR spectrum of the PDANSs, (D) the UV-visible spectrum of the PDANSs.

Fig. 2 The intensity of fluorescence to strategy feasibility in PBS. (A) Fluorescence intensities of ATP (curve b: 4×10^{-7} M), blank (curve a). (B) Fluorescence intensities of no ATP (curve a), heat-treated enzyme (curve b), only Exo III (curve c: 20 U) , DNA polymerase and Exo III (curve d: 10 U and 20 U). Spectra recorded in the presence of ATP (4×10^{-7} M), signal DNA (2×10^{-7} M), PDANSs (0.24 mg/mL).

Fig. 3 The ATP detection of strategy. (A) Fluorescence intensities of sensing system to different concentrations of commercial ATP (curve from a to i: 0 M, 2×10^{-8} M, 4×10^{-8} M, 6×10^{-8} M, 8 ×

10^{-8} M, 1×10^{-7} M, 2×10^{-7} M, 4×10^{-7} M, 6×10^{-7} M). (B) Standard curve for detection of ATP. ΔI_F=

Sample	ATP standards (nM)	ATP concentration using this method (nM)	RSD (%, n=5)	ATP concentration using ATP Assay Kit (nM)	RSD (%, n=5)
1	100	99.3	3.6	98.9	4.7
2	200	198.9	3.1	203.5	3.7
3	400	401.3	2.9	406.7	3.6

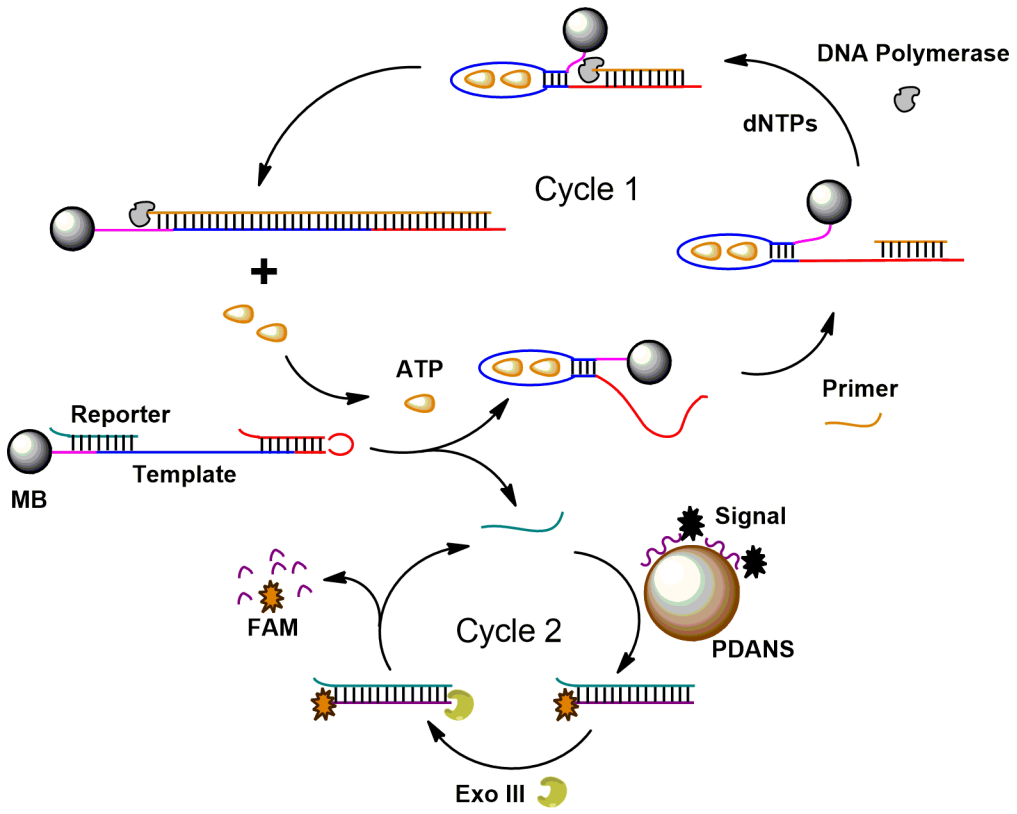
$64.49C_{ATP}+16.86$ (R^2=0.99648).

Fig. 4 The intensity of fluorescence to diverse bioanalytes in PBS. (the concentration of ATP, CTP, GTP, UTP were 2×10^{-7} M, 1×10^{-4} M, 1×10^{-4} M, 1×10^{-4} M)

Table 1 ATP concentration was validated using this method and ATP Assay Kit (Beyotime).

Highlights

- A new strategy was designed for the detection of ATP by the polydopamine nanospheres integrating with enzymatic recycling amplification.
- A new fluorescent biosenor that exhibits high sensitivity, specificity.
- A promising approach to molecular diagnostics that has extensive potential.



Schematic 1

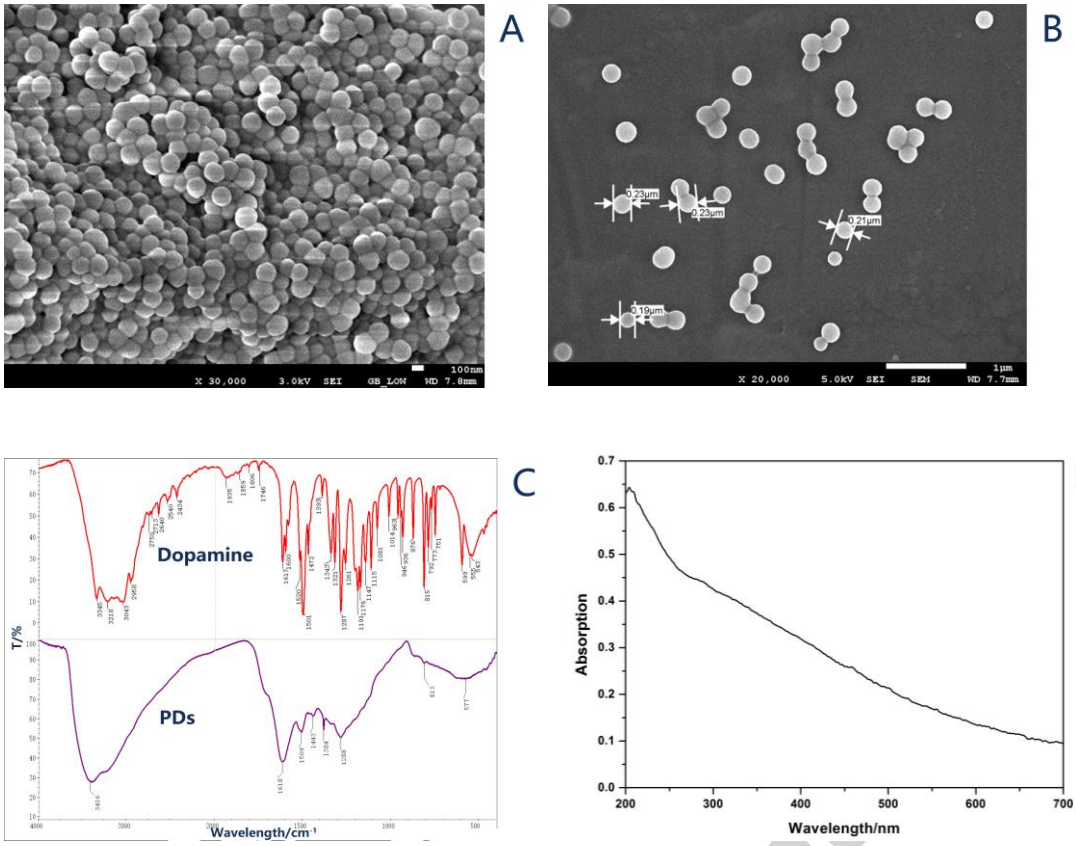
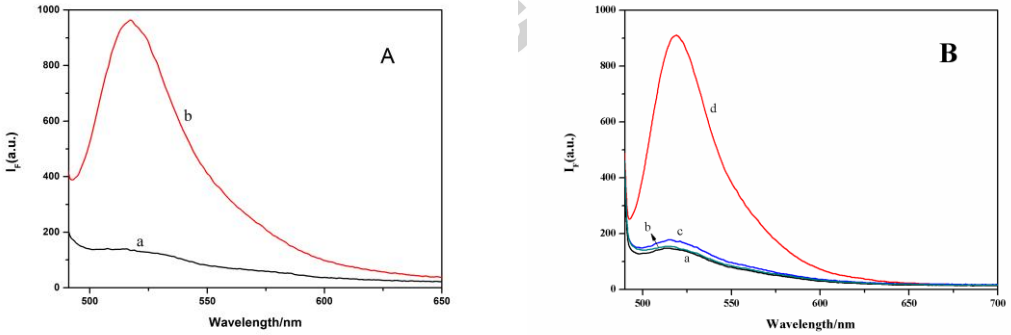

A
B
C
D
Dopamine
PDs
T/%
Wavelength/cm-1
Absorption
Wavelength/nm

Fig. 1


A
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Wavelength/nm

Fig. 2

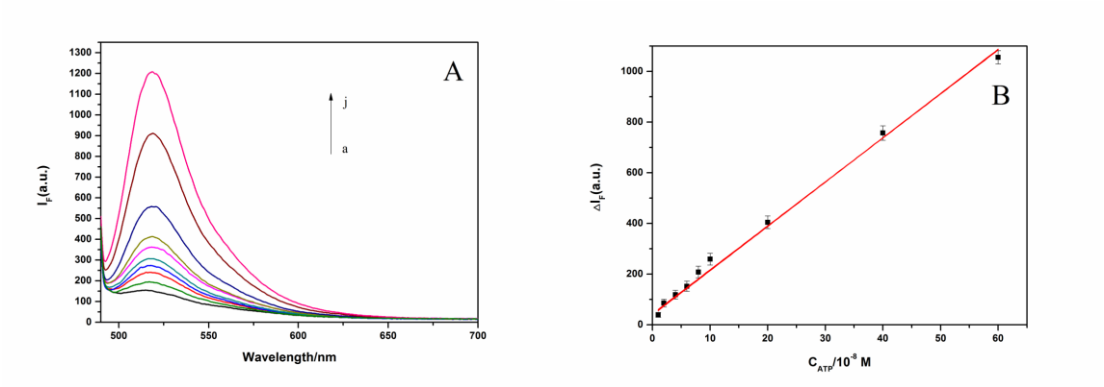


Fig. 3

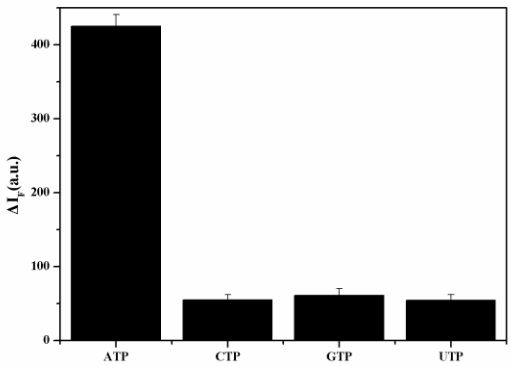
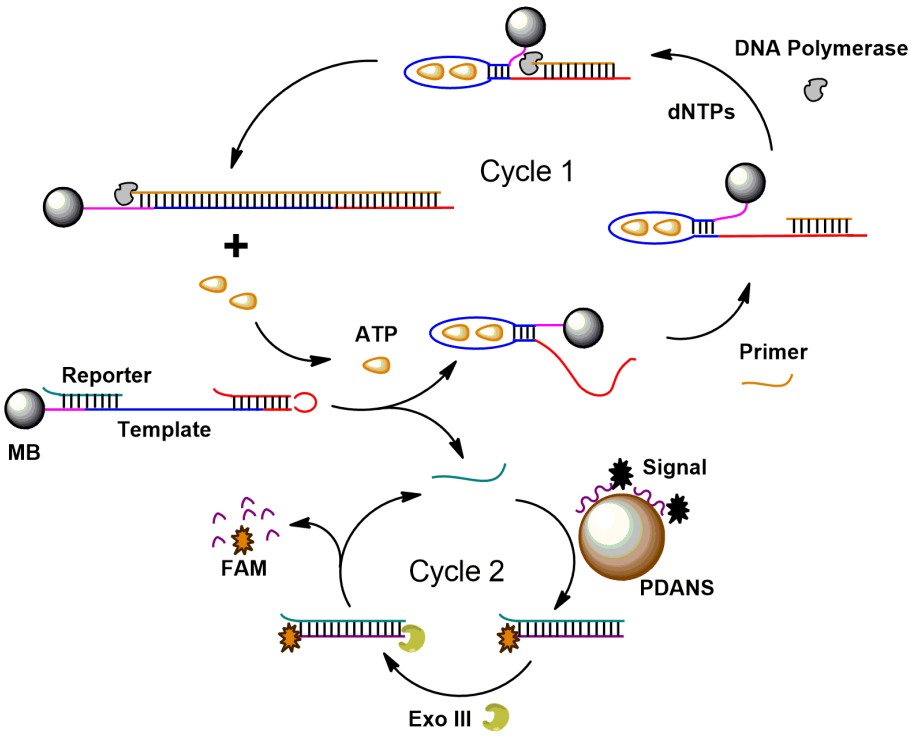


Fig. 4


DNA Polymerase
dNTPs
Cycle 1
+
ATP
Primer
Reporter
Template
MB
Signal
FAM
Cycle 2
PDANS
Exo III