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# An enzyme-free strategy for ultrasensitive detection of adenosine using a multipurpose aptamer probe and malachite green

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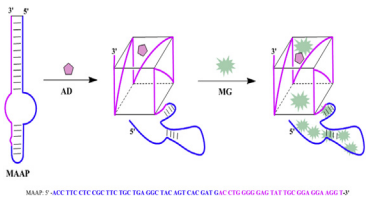
## HIGHLIGHTS

- A novel multipurpose aptamer probe for adenosine (MAAP) was assembled.
- The use of such a MAAP can detect adenosine at concentration as low as 23 pM.
- The MAAP can also be used for fluorescence or spectrophotometric assay of adenosine.
- The conformational switching mechanism of MAAP by adenosine was verified.

## GRAPHICAL ABSTRACT

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## ABSTRACT

We report on an enzyme-free and label-free strategy for the ultrasensitive determination of adenosine. A novel multipurpose adenosine aptamer (MAAP) is designed, which serves as an effective target recognition probe and a capture probe for malachite green. In the presence of adenosine, the conformation of the MAAP is converted from a hairpin structure to a G-quadruplex. Upon addition of malachite green into this solution, a noticeable enhancement of resonance light scattering was observed. The signal response is directly proportional to the concentration of adenosine ranging from 75 pM to 2.2 nM with a detection limit of 23 pM, which was 100–10,000 folds lower than those obtained by previous reported methods. Moreover, this strategy has been applied successfully for detecting adenosine in human urine and blood samples, further proving its reliability. The mechanism of adenosine inducing MAAP to form a G-quadruplex was demonstrated by a series of control experiments. Such a MAAP probe can also be used to other strategies such as fluorescence or spectrophotometric ones. We suppose that this strategy can be expanded to develop a universal analytical platform for various target molecules in the biomedical field and clinical diagnosis.



## 1. Introduction

Adenosine (AD) has received much attention owing to its multiple important biological functions [1–4]. The accumulation of adenosine of high concentration has been found in solid tumors [2,5]. Thereby, adenosine has been considered as a possible biomarker for cancer [1–4]. Traditional methods, such as high-

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performance liquid chromatography and gas/liquid chromatography-mass spectrometry [6,7], allow sensitive and selective analysis of adenosine. However, most of them are highly specialized and expensive, which limit their wide application. Over the past decades, the discovery of aptamers has shown tremendous potential and versatility in the analytical and bioanalytical fields [1,8–12]. With the successful selection of the adenosine aptamer [13], several aptamer-based methods for detecting adenosine were also reported in recent years, including fluorescence [1,14], electrochemical biosensor [10], Raman scattering [8], chemiluminescence [12,15,16], flow cytometry [17], surface plasmon resonance [18], aptamer-gold nanoparticle aggregation [19], and enzyme-free and label-free fluorescence aptasensor [20]. These advances in developing aptasensors are impressive. Nevertheless, these methods exhibit only moderate sensitivity with detection limits for adenosine in the nanomolar range, and also involve the chemical modification of the probes [1,10,15–18]. This would make experiments relatively more complex and expensive. Various new approaches have been explored to boost detection sensitivity of sensing processes via the enhancement/amplification of sensing responses, including rolling cycle amplification (RCA) [21], polymerase chain reaction (PCR) [22], nicking enzyme signal amplification (NESA) [23], electrochemical impedance spectroscopy aptasensor [24], and exonuclease III assisted signal amplification [25]. Although enhanced sensitivities were demonstrated by these amplification strategies, they suffer some drawbacks of increased complexity and cost of the assay. For example, RCA needs the circular single strand DNA templates, and PCR requires precise control of the temperature cycling to achieve amplification. These limit their practical applications in many cases. Therefore, the exploration of new enzyme-free amplification detection methods can substantially facilitate adenosine detection.

Resonance light scattering (RLS) method, one of the most widely used optical sensing strategy, has many inherent advantages such as high sensitivity, simplified operation, and homogenous single-phase detection [26,27]. Malachite green (MG) is an inexpensive, commonly available triphenylmethane dye [28]. It can effectively bind to G-quadruplexes by a π stack interaction on the faces of the G-quadruplex [28,29]. Inspired by these findings, we rationally designed a novel multipurpose aptamer probe for adenosine (MAAP) (Scheme 1). This MAAP contains an adenosine aptamer sequence (the red portion) and a 37-mer fragment (the blue portion), which were elegantly integrated into one oligonucleotide to be used as both a recognition probe for target and a capture probe for malachite green. We presumed that MAAP could fold into a hairpin structure by additional 37 nucleotide sequences which are partly complementary to aptamer sequence (Scheme 1). The combination of adenosine with the aptamer part of MAAP possibly could result in the conformational change of MAAP to form a G-quadruplex and release a 37-mer fragment. The resulting G-quadruplex with a 37-mer fragment could interact with several MG to lead to a noticeable increase of RLS intensity of the sensing system. Thereby, a novel enzyme-free and label-free strategy for the ultrasensitive assay of adenosine could be developed. This strategy can sensitively and selectively detect adenosine at concentration as low as 23 pM, which is comparable with those amplified methods based on enzymes such as RCA and PCR, and also much lower than those of related literature reports. This method does not require any other reagents or enzymes, without tedious procedure or the requirement of sophisticated equipment. It is particularly suitable for aptamer-based biosensing for wide applications in clinical diagnoses and biomedical fields.

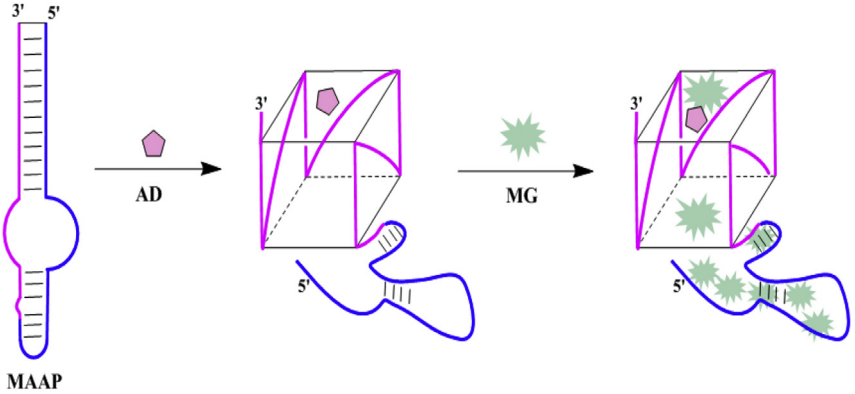
## 2. Materials and methods

### 2.1. Reagents

All chemicals used were analytical grade, and sterilization doubly distilled water (18.2 MΩ cm) was used throughout this work. Adenosine, guanosine, cytidine, thymidine and uridine were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Their stock solutions of 1.0 mM were prepared by dissolving an appropriate amount of them in water, then diluting to 100 mL with water. Oligonucleotides (5′-ACC TTC CTC CGC TTC TGC TGA GGC TAC AGT CAC GAT GAC CTG GGG GAG TAT TGC GGA GGA AGG T-3′) designed according to the literature [13] were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China), and its concentration of working solution was 1.0 μM, stored at 4 °C before use. Malachite green was obtained from Tianjing Chemical Reagent Research Institute (Tianjing, China), and its concentration of stock solution was 0.50 mM. Tris-HCl buffer was bought from Aladdin Chemistry Co., Ltd. (Shanghai, China), and its concentration of working solution was 50 mM with 50 mM NaCl and 5 mM $MgCl_2$ (pH 7.4).

### 2.2. Apparatus

All RLS spectra were completed on a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). The absorption spectra were obtained with a Shimadzu UV-2550 spectrophotometer (Kyoto, Japan). The circular dichroism spectra were recorded with a Jasco J-815 circular dichroism spectrometer (Tokyo, Japan).




**Scheme 1.** Schematic diagram for analytical principle of MAAP-AD-MG system.