# Analytical Methods

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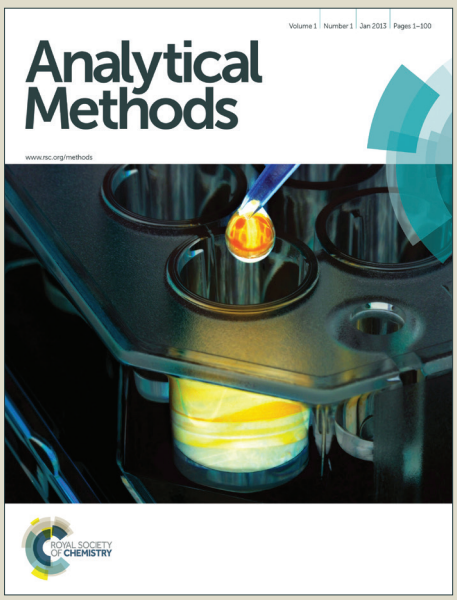





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Analytical Methods

ARTICLE

# A novel label-free biosensor based on self-assembled aptamer/GO architecture for sensitive detecting of biomolecules

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We developed a novel label-free biosensor for biomolecules detection based on the thioflavin T (ThT)-induced conformational change of guanine-rich oligonucleotide and self-assembled aptamer/GO nanosheets architecture. In the presence of target biomolecules, the aptamer sequence could specifically bind with target and release from surface of GO nanosheets to form G-quadruplex conformation with ThT as inducer, resulting in enhancement of fluorescence. The proposed biosensor exhibits a "turn-on" signal, which allow sensitive, selective and rapid detection of biomolecules.

## Introduction

Since the aptamer was first reported in 1990, design of aptamer-based sensors for a board range of analytes has attracted increasing attention in chemistry.[1] However, many aptamer-based sensors rely on fluorescence resonance energy transfer (FRET), which requires labeling of fluorophores or quenchers.[2] The labeling requires complex chemical modifications and purification, thereby resulting in a high cost for the sensor. The development of label-free biosensors still remains a major effort for analytical chemistry.[3]

Graphene oxide (GO) is a single carbon layer nanomaterial possessing strong adsorption properties for single-stranded DNA via non-covalent binding such as π-π stacking or Van Der Waals force.[4] Moreover, it only exhibits very weak affinity to double-stranded DNA or complex of aptamer with its target, which allows quick desorption of aptamer probe in response to target molecules.[5] Hence, GO is widely used as an attractive biosensing nanoplatform ascribed to its unique properties including high water solubility, good biocompatibility and low cost.[6]

Here, we report a novel label-free aptameric biosensor based on a self-assembled aptamer/GO architecture for sensitive detection of a wide range of targets. Mohanty *et al* reported that thioflavin T (ThT) could exclusively induce G-rich oligonucleotide sequences to fold into quadruplexes for sensing the quadruplexes motif through fluorescence light-up in visible region.[7] With this idea in mind, we design the aptamer sensor sequence to consist of two regions: one is the aptamer itself for recognition of the target biomolecules of interest; the other is a G-rich oligonucleotide sequence capable to fold into a G-quadruplex structure when induced by ThT. With the aptamer sequence absorbed on the GO surface, we obtain a self-assembled aptamer/GO architecture as the label-free sensor. The presence of the target would specifically bind to the target, which induces the release of the aptamer sequence away from the GO surface. Then, ThT can bind to the G-rich oligonucleotide region of the probe to form G-quadruplex conformation with a substantially enhanced fluorescence signal. To demonstrate the developed biosensor, we apply the aptamer/GO-based biosensor to detecting human α-thrombin and adenosine monophosphate (AMP). The result revealed that the proposed biosensor provides a label-free, sensitive, selective and rapid method for sensing different analytes.

## Experimental

### Chemicals and materials

The DNA oligonucleotides used in this work were synthesized by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Their sequences are listed in Table S1 in ESI.

Thrombin was purchased from Sigma-Aldrich (U.S.A.). AlwI, Endonuclease IV, HOGG1 and HhaI were acquired from New England Biolabs (U.S.A.). Adenosine monophosphate (AMP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), and thymidine triphosphate (TTP) were all bought from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). ThT (3, 6-dimethyl-2-(4-dimethylaminophenyl) benzo-thiazolium cation) was also obtained from Sigma-Aldrich (U.S.A.). GO was synthesized according to the modified Hummers' method.[8] Atomic force microscopy (AFM) was used to characterize the GO nanosheets (Fig. S1 in ESI). All other reagents were of analytical grade and were used without further purification.

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Ultrapure water obtained from a Millipore Milli-Q water purification system (resistance>18.2 MΩ $cm^{-1}$) was used throughout the experiments.

The fluorescence measurements were performed on a F-7000 fluorescence spectrometer (Hitachi, Japan). The slit width for both excitation and emission was set at 5 nm. The emission spectra were collected by exciting the samples at 425 nm and scanning the emission from 450 to 540 nm at room temperature.

### Procedures for Thrombin and AMP Detection

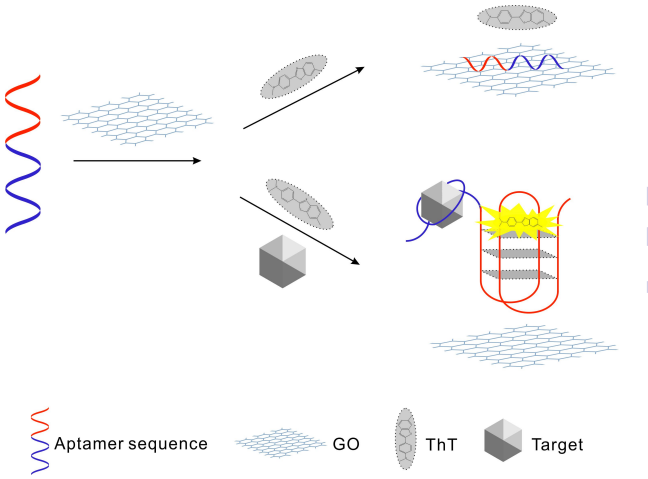
Thrombin aptamer sequence (100 nM) was incubated with GO (10 μg $mL^{-1}$) for 5 min at 37 °C to form the aptamer/GO complex in 10mM Tris-HCl buffer (pH 7.3, containing 50 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$). Then, the targets (different concentrations of thrombin solution) and 2 μM ThT were added into the aptamer/GO solution, following by incubating at 37 °C for 10 min. After incubation, the fluorescence emission spectrum of the mixture was recorded. For AMP detection, the same procedure was used with AMP aptamer sequence to replace the thrombin counterpart.

## Results and discussion

### Working Mechanism

The detection mechanism of the proposed biosensor is shown in Scheme. 1. ThT is a fluoregenic dye with weak fluorescent by itself, but exhibits a dramatic fluorescence enhancement upon binding to DNA molecular G-quadruplexes formation. Taking advantages of the unique absorption for single-stranded DNA probes and good biological compatibility of GO nanosheets, the aptamer sequence is self-assembled on the surface of GO. In the absence of target and upon the addition of ThT, the aptamer sequence, being captured by GO nanosheets, is not able to fold into a G-quadruplex structure. As a result, we can only detect a weak fluorescence from free ThT itself. In contrast, in the presence of the target, the sequence probe can be released from GO nanosheets due to the specific binding between the aptamer and its target. Then, the G-rich oligonucleotide region of the probe is further induced by ThT to form ThT-incalated-G-quadruplex which shows dramatic fluorescence enhancement. The unique nature of ThT and the high specific interaction between aptamer and its target make the aptamer/GO-based biosensor a promising label-free and sensitive assay for detecting biomolecules.

To test the feasibility of this strategy, the aptamer/GO platform for detecting the thrombin was studied. Fig. 1A shows the fluorescence emission spectra of the thrombin aptamer sequence P1 under different conditions. In the absence of thrombin, only a quite weak fluorescence signal from ThT is detected (curve c). On addition of thrombin, however, the P1 is subject to be released from GO surface and is induced by ThT to form G-quadruplexs, resulting in the sharply enhancement of fluorescence (curve d). Compare with P1, the control DNA sequence P3 was also self-assembled on the surface of GO nanosheets, leading to a poor fluorescence signal (curve a). After thrombin was added into the solution (curve b), the response signal remained almost unchanged. The fact indicated that the desorption was caused only by specific interaction between thrombin and its aptamer. Hence, the proposed detection principle in Scheme. 1 worked well. For investigating the kinetic behaviour of the whole aptamer/GO biosensor, we recorded the fluorescence intensity as a function of time after adding thrombin and ThT, and found that the fluorescence signal reached up to equilibrium within 10 min (Fig. 1B), revealing that the biosensor have rapid response to thrombin.




**Scheme. 1** Schematic of the label-free aptamer/GO-based platform for biomolecules analysis.

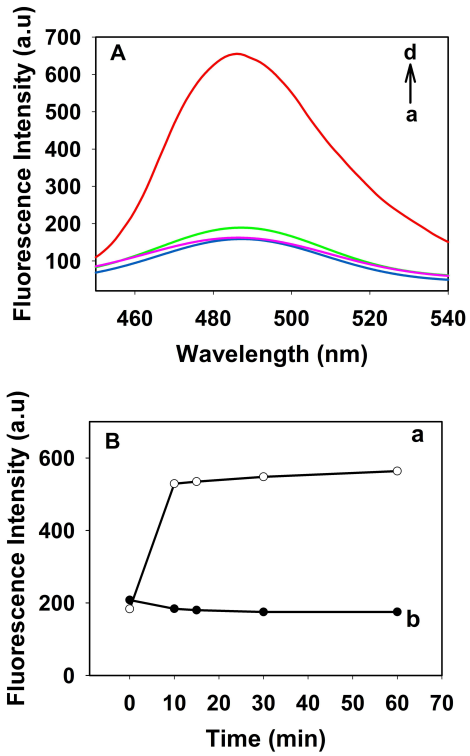



**Fig. 1** (A) Fluorescence emission spectra of P1 and P3 under different conditions: (a) P3 + GO + ThT; (b) P3 + GO + ThT + thrombin; (c) P1 + GO + ThT and (d) P1 + GO + ThT + thrombin (GO 10 μg $mL^{-1}$, ThT 2 μM, P1 100 nM, P3 100 nM, thrombin 100 nM). (B) (a) Fluorescence enhancement of P1 in GO nanosheets solution by thrombin and ThT as a function of time , and (b) Fluorescence signal of P1 in Tris-HCl buffer by GO nanosheets as a function of time (GO 10 μg $mL^{-1}$, P1 100 nM, thrombin 100 nM, ThT 2 μM).

### Optimization of Experimental Conditions

In order to obtain the highest sensitive response, the concentration of ThT and the amount of GO nanosheets were optimized. Fig. 2A displays the changes of fluorescence intensity with different concentration of ThT. It was observed that the fluorescence intensity increased significantly with increasing the concentration of ThT, and the ratio of signal to background also increased gradually until the concentration reached to 2 μM. As a result, 2 μM was used as the optimized cencentration for ThT in this assay. In addition, the effect of GO nanosheets on the assay performance was also investigated. Fig. 2B depicts the effect of GO nanosheets on the fluorescence intensity. It showed the maximum fluorescence enhancement was obtained in the presence of 10 μg $mL^{-1}$ GO nanosheets. Therefore, 2 μM of ThT and 10 μg $mL^{-1}$ of GO nanosheets were used in the assay to ensure a good signal-to-background ratio.

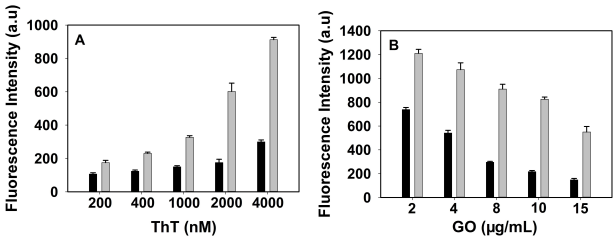



**Fig. 2** (A) Fluorescence intensity histogram of P1 + GO + ThT (black) and P1 + GO + ThT+ thrombin (gray) in the presence of 0.2, 0.4, 1, 2 and 4 μM ThT (P1 100 nM, GO 10 μg $mL^{-1}$, thrombin 100 nM). (B) Fluorescence intensity histogram of P1 + GO + ThT (black) and P1 + GO + ThT+ thrombin (gray) in the presence of 2, 4, 8, 10 and 15 μg $mL^{-1}$ GO (P1 100 nM, ThT 2 μM, thrombin 100 nM).

### Sensitivity and Specificity

Fluorescence signals for different concentration of thrombin were measured under optimal conditions to demonstrate the feasibility of our approach. As shown in Fig. 3A, the fluorescence responses of the system gradually increased with increasing concentrations of thrombin in the range from 0 to 100 nM. The calibration equation was F = 330.80 + 122.27 lg C, where F is the fluorescence intensity and C is the concentration of thrombin. There was a good linear correlation with correlation coefficient of 0.987 between the fluorescence intensity and the logarithm of thrombin concentration in the range from 1 to 100 nM, as shown in the inset of Fig. 3B. The detection limit of thrombin was 180 pM (according to the 3σ rule), which was better than that obtained using dye-labeled aptamer/GO-based sensor.[9] In addition, the detection limit was comparable to or better than many reported label-free aptamer-based thrombin detections.[10]

To further evaluate the selectively of this biosensor for thrombin, other proteins including HOGG1, AlWI, HhaI and Endonuclease IV, each at a concentration of 100 nM, were detected under the same conditions (see Fig. S2 in ESI). In the control experiment using other kinds of protein, the fluorescence signals did not show significant change. However, when the target molecule thrombin was present, the ratio of the $F/F_0$ was significantly higher compared than the control protein. This result demonstrated that the proposed biosensor had excellent selectively toward thrombin, which was contributed by the extraordinary capability of high selectively of aptamer.

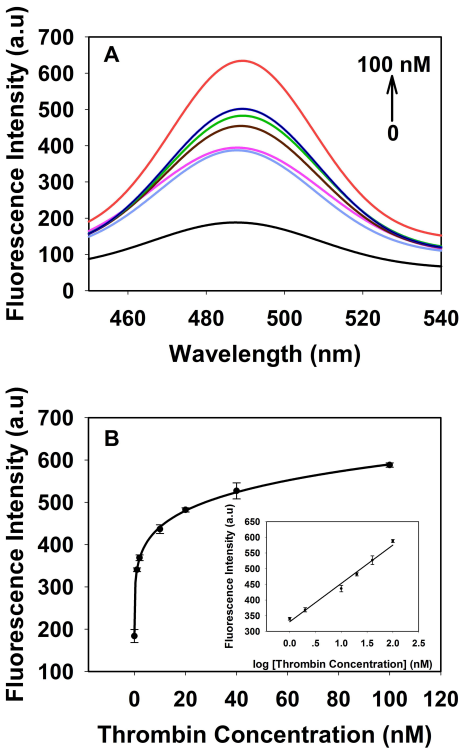



**Fig. 3** (A) Fluorescence emission spectra of aptamer/GO-based platform in the presence of increasing amounts of thrombin, the arrow indicating the signal changes with increases in thrombin (0, 1, 2, 10, 20, 40 and 100 nM). (B) Fluorescence spectral response of the proposed aptamer/GO-based platform at 495 nm versus thrombin concentration. Inset: dependence of fluorescence intensity on the thrombin concentration.

In order to evaluate whether the label-free aptamer/GO-based biosensor can be extended to detecting other biomolecules, we further challenged the performance of the biosensor on adenosine monophosphate (AMP). As expected, in the absence of the AMP, only a weak fluorescence intensity by ThT could be detected; in the presence of the AMP, the fluorescence intensity dramatically increased (Fig. S3 in ESI). Under optimal conditions (Fig. S4 and Fig. S5 in ESI), the sensitively of the developed biosensor for AMP detection was investigated. The fluorescence intensity increased with increasing concentrations of AMP was also observed, as shown in Fig. 4A. There is a good linear correlation between the peak fluorescence intensity and the logarithm of AMP concentration in range from 10 μM to 5 mM (Fig. 4B). The linear calibration equation was F = 96.563 + 21.691 lg C (F is the fluorescence intensity and C is the concentration of AMP) and the correlation coefficient $R^2$ = 0.962. The limit of detection was found to be 30 μM (according to the 3σ rule). The linear dependence of fluorescence intensity on the logarithm of AMP concentration indicates that the biosensor is suitable for accurate quantitation of AMP. The selectivity of the developed sensor was also investigated by employing nonspecific target biomolecules (see Fig. S6 in ESI).

The dramatic change of fluorescence intensity in the presence of AMP was observed, while the sensor showed no obvious response to CTP, GTP or TTP. These results indicate that the proposed assay was highly selective to its target.

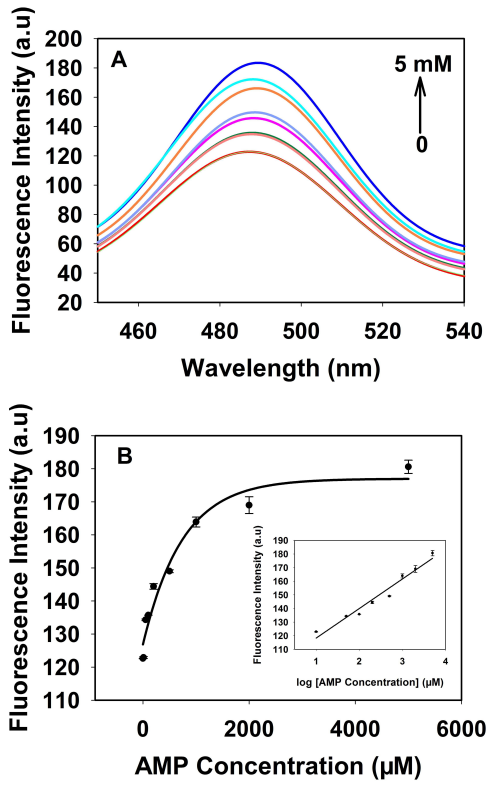



**Fig. 4** (A) Fluorescence emission spectra of aptamer/GO-based platform in the presence of increasing amounts of AMP, the arrow indicating the signal changes with increases in AMP (0, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mM). (B) Fluorescence spectral response of the proposed aptamer/GO-based platform at 495 nm versus AMP concentration. Inset: dependence of fluorescence intensity on the AMP concentration.

## Conclusions

In summary, we have reported a novel label-free fluorescence biosensor utilizing aptamer probes self-assembled on GO nanosheets for detection protein and small molecule targets. The highly specific interaction between aptamer and its targets, the unique property of GO nanosheets and ThT induced G-quadruplex system all contribute to the sensitive and selective assay of biomolecules. Moreover, this biosensor avoids the complex processes of labeling aptamers with fluorophore or quencher units, which significantly lowered the detection cost. In addition, the whole detection process takes only approximately 10 minutes, implying the ability of the sensor for rapid assays. Furthermore, by using different aptamers, this sensor may provide a promising platform for detecting a wide range of analytes.

## Acknowledgements

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