Electronic supplementary information

A turn-on fluorescent aptasensor for adenosine detection based on split aptamer and graphene oxide

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Experimental Section

Materials and reagents

Graphite oxide dispersion (2 mg/mL) was purchased from XF Nano (Nanjing, China). All oligonucleotides were synthesized and HPLC purified by Shanghai Sangon Biotechnology Co. Ltd (Shanghai, China). The sequences of oligonucleotides are given as follows.

ABA1-FAM: 5'-FAM-ACC TGG GGG AGT AT-3';

ABA2: ABA2: 5'-TGC GGA GGA AGG T-3'

Tris(hydroxymethyl)aminomethane (Tris), Adenosine, thymidine, cytidine and uridine were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification. All work solutions were prepared with 20 mM Tris-HCl buffer solution (pH 7.4, 100 mM NaCl, 5 mM KCl , 5 mM $MgCl_2$). Deionized water was used for the preparation of aqueous solution.

Fluorescence spectroscopic analysis

Fluorescence measurements were performed on F-2500 fluorescence spectrometer (Hitachi, Japan). The optical path length of a quartz fluorescence cell was 1.0 cm. Under the excitation wavelength of 480 nm, the fluorescence spectra were recorded from 500 to 650 nm. Both the excitation and emission slits were set at 10 nm and the PMT detector voltage was 400 V. All fluorescence detections were carried out under room temperature unless otherwise indicated.

Fluorescence anisotropy measurement

Fluorescence anisotropy was measured by a LS 55 Fluorescence Spectrometer with an excitation wavelength at 480 nm and an emission wavelength at 519 nm (PerkinElmer, American). Anisotropy value (r) is a ratio, defined as the difference between the linearly polarized components of emission divided by the total light intensity, which is sensitive to changes in the rotational motion of fluorescently labeled molecules.[1] The observed r can then be calculated by eq 1.

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \qquad \text{eq 1}$$

where the subscripts V and H refer to the orientation (vertical or horizontal) of the polarizer for the intensity measurements, with the first subscript indicating the position of the excitation polarizer and the second for the emission polarizer. The G factor is defined and calculated according to the following eq 2.

$$G = \frac{I_{HV}}{I_{HH}} \qquad \text{eq 2}$$

Six anisotropy measurements were taken each time using an integration time of 1 s for each sample, and the resulting anisotropy values were averaged. The relative standard deviation is <5% for all measurements.

Adenosine assay

All experiments were performed in 20 mM Tris-HCl buffer (pH 7.4, 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$). A solution containing 50 nM of ABA1-FAM, 50 nM of ABA2 and different concentrations of adenosine was incubated for 30 min at room temperature, then GO was added into the solution to make the final volume 1 ml. The final concentration of GO was 18 μg/ml. After the mixture was incubated for 10 min at room temperature, the fluorescence emission spectra were recorded with an excitation wavelength of 480 nm. The same procedures were repeated in the presence of other adenosine analogues instead of adenosine to assess the selectivity.

Supplementary Figures

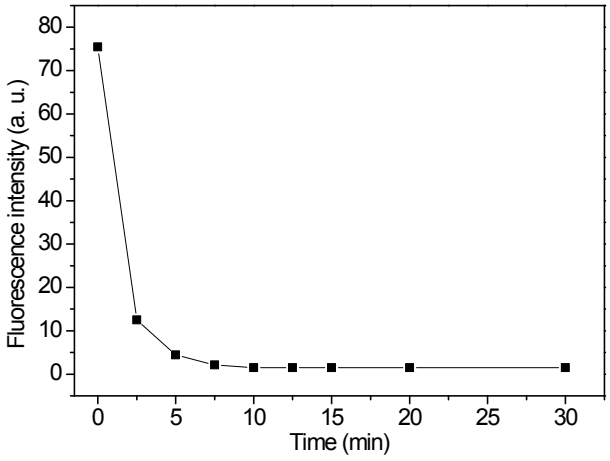

Fig. s1 Fluorescence quenching of ABA1-FAM (50 nM) by GO (18 μg/ml) as a function of time.

Table s1 Performance comparison of this work with other aptamer-based methods for small

molecule detection.

Analytical method	Sensor strategy	Operation	Linear range	Detection limit	Real sample	Ref.
Fluorescence	Split aptamer/ GO	Simple	0-1400 μM	6 μM	Serum samples	This work
Fluorescence	Nicking endonuclease/ AgNCs	Simple	2–50000 nM	Cocaine 2 nM	Serum	2
Time-resolved fluorescence	Layer-by-layer self-assembly/ Eu	Complex	0–100 nM	0.5 nM	Human serum	3
Fluorescence	KF polymerase/ Split aptamer/ SYBR Green dye	Simple	0–1.5 mM	12 μM	Not reported	4
Fluorescence	Target-catalyzed hairpin self-assembly/ G-quadruplex/ NMM	Simple	30–680 μM	6 μM	Human serum	5
Fluorescence	DNA/RNA chimeric aptamer/ MG	Simple	0–1 mM	20 μM	Not reported	6
Fluorescence	Silica coated photon up converting nanoparticles / Split aptamer	Complex	2–16 μM	1.7 μM	Not reported	7
Visual	Split aptamer/ AuNPs	Simple	Not reported	Cocaine 2 μM	Not reported	8
Colorimetric	Split aptamer labeled AuNPs	Simple	Not reported	0.25 mM	Not reported	9
Colorimetric	Double-functionalized AuNPs with split aptamer	Complex	0–400 μM	24 μM	Fetal calf serum	10
Electrometry	Split aptamer/ Electrode	Complex	Not reported	1 μM	Cell lysates	11

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