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Regulative peroxidase activity of DNA-linked hemin by graphene oxide for fluorescence DNA sensing†

Quanbo Wang, Nan Xu, Jianping Lei and Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P.R. China

Experimental

Materials and reagents. Graphene oxide (GO, purity 99%, single layer ratio 99%) was obtained from Nanjing XFNano Materials Tech Co. Ltd. (Nanjing, China). Tyramine, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) and tris(hydroxymethyl) aminomethane were purchased from Sigma–Aldrich Inc (USA). Tris−HCl buffer (50 mM, containing 300 mM NaCl, pH 7.4) was used for homogeneous fluorescence measurements. All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays.

All oligonucleotides were synthesized and purified by TAKARA Biotechnology (Dalian, China), and their sequences are listed in Table S1. The probe (oligo 1) contained a 5′-hemin label, which was characterized with mass spectrum and high-performance liquid chromatography (HPLC). The sequence of target DNA (oligo 2) was perfectly matched to the sequence of the probe. The oligo 3 contained one-base mismatch, while the oligo 4 was a three-base mismatch strand.

Table S1. Oligonucleotides employed in this work

Oligonucleotides	**Oligonucleotides Sequence**
oligo 1 (probe)	5′-hemin-CTGTCTTGAACATGAGTT-3′
oligo 2 (target)	5′-AACTCATGTTCAAGACAG-3′
oligo 3 (sm-target)	5′-AACTCATGTTCAA<u>C</u>ACAG-3′
oligo 4 (tm-target)	5′-AACTCAT<u>C</u>TT<u>G</u>AA<u>C</u>ACAG-3′

Apparatus and gel electrophoresis. Fluorescence (FL) spectra were recorded on a RF-5301PC spectrofluorometer (Shimadzu, Japan) equipped with a xenon lamp. To measure catalytic fluorescent signal using tyramine and H_2DCFDA as substrates, the excitation wavelengths were set to 320 nm and 500 nm, respectively, and the excitation and emission slits were both set to 10 nm. Mass spectrum was recorded on an Autoflex III Smartbeam mass spectrometer (Bruker, Germany). HPLC was performed by a CTO-10AVP system (Shimadzu, Japan).

The 12% native polyacrylamide gel electrophoresis (PAGE) was performed with 5×Tris-Borate-EDTA (TBE) buffer. The loading sample was prepared by mixing 7 μL of DNA sample, 1.5 μL 6×loading buffer with 1.5 μL UltraPower dye, and kept for 3 min so that the dye could integrate with DNA completely. The gel was run at 90 V for 90 min in 1×TBE buffer, and then scanned with Molecular Imager Gel Doc XR (BIO-RAD, USA).

Inhibition effect of GO toward the probe. The probe in 50 mM Tris-HCl buffer (pH 7.4, containing 300 mM NaCl) was mixed with the GO for 2 min. Then the non-fluorescent substrat (tyramine or H_2DCFDA) and H_2O_2 were added into the mixture to react for 10 min at room temperature, and the fluorescence spectrum was measured. The inhibition efficiency was

calculated with $[(F_0 - F) / F_0] \times 100\%$, where F_0 and F is the fluorescence intensity in absence and presence of GO, respectively.

Homogenous fluorescence detection of DNA. After 197 μL mixture of probe (10.15 nM) and target was incubated in 50 mM Tris-HCl buffer (pH 7.4, containing 300 mM NaCl) at 37 °C for 10 min, 1.0 μL 150 μg mL^{-1} GO was added in the mixture to react for 2 min, and then 1.0 μL 140 mM non-fluorescent substrat tyramine and 1.0 μL 400 mM H_2O_2 were added to initiate enzymatic catalsysis for 10 min. Finally, the fluorescence spectrum was recorded at an excitation wavelength of 320 nm.

Characterization of hemin labelled probe

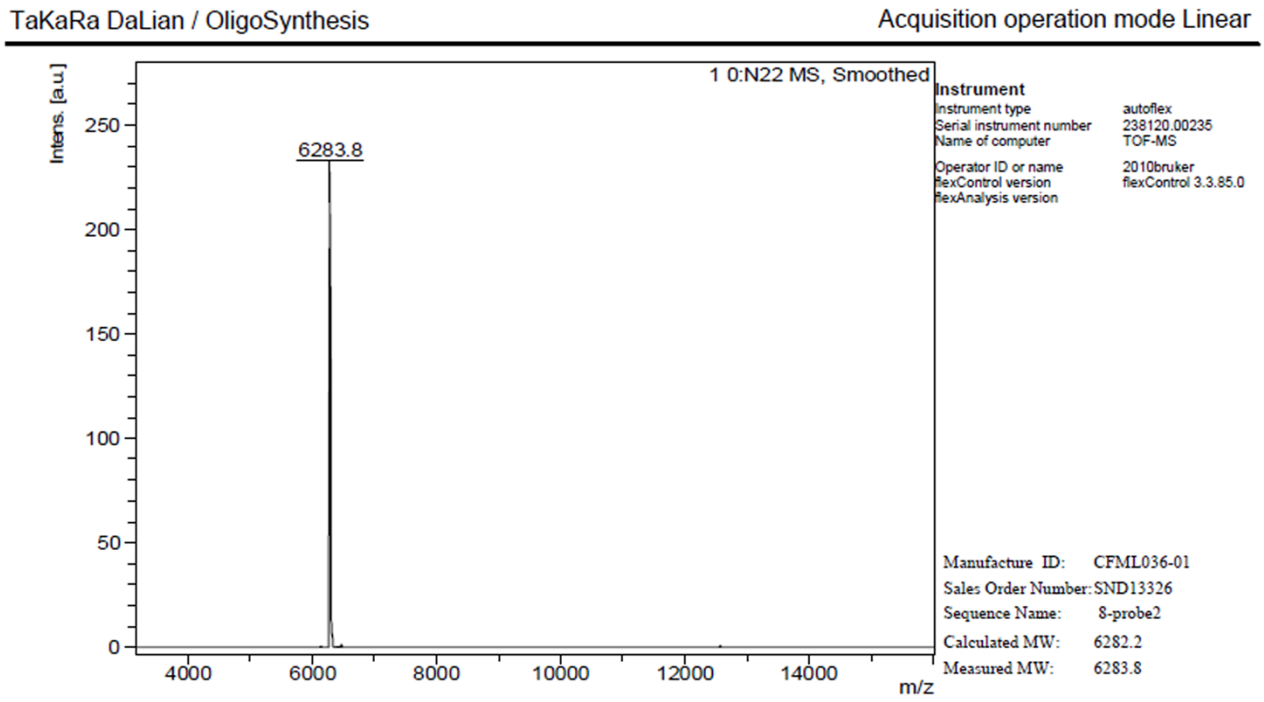
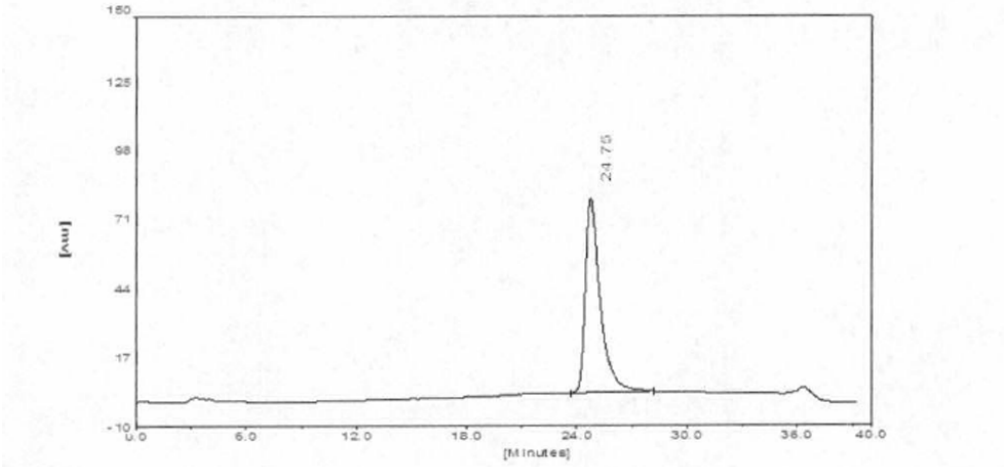


Fig. S1 Mass spectrum of probe. Calculated MW: 6282.2 $(M\text{-}Cl)^+$, found MW: 6283.8.



Detector A (254 nm)

Pk#	RetentionTime	Area	Area%	Height	Height%
1	24.75	4299.63	100.000	76.05	100.000
Totals		4299.63	100.000	76.05	100.000

Fig. S2 HPLC analysis of probe.

Inhibition effect of GO using H_2DCFDA as substrate

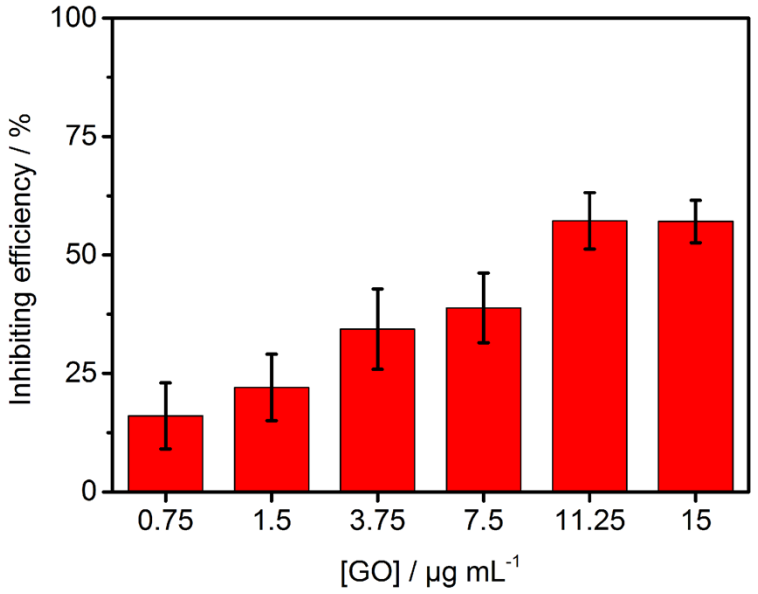


Fig. S3 The inhibiting efficiency of GO at different concentrations.

pH dependence of fluorescent signal

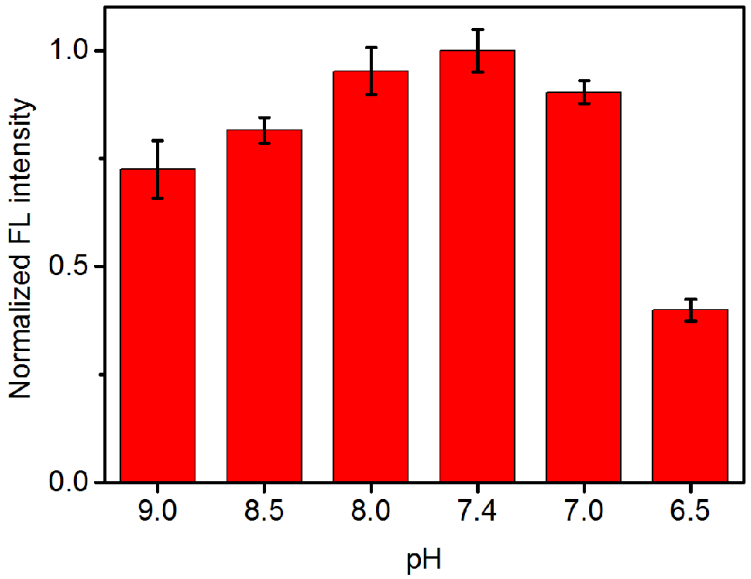


Fig. S4 Effect of pH on the normalized fluorescent signal. The catalytic reaction is carried out in 50 mM Tris-HCl with 0.7 mM tyramine and 2 mM H_2O_2.

Gel electrophoresis

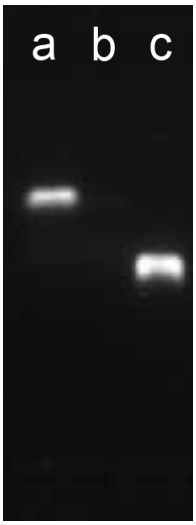


Fig. S5 PAGE analysis of 1.0 μM probe (lane a), 1.0 μM probe treated with 75 μg mL^{-1} GO for 2 min (lane b) and 1.0 μM probe firstly hybridized with 1.0 μM target at 37 ^{o}C for 10 min and subsequently treated with 75 μg mL^{-1} GO for 2 min (lane c).

Optimization for homogenous fluorescence DNA sensing

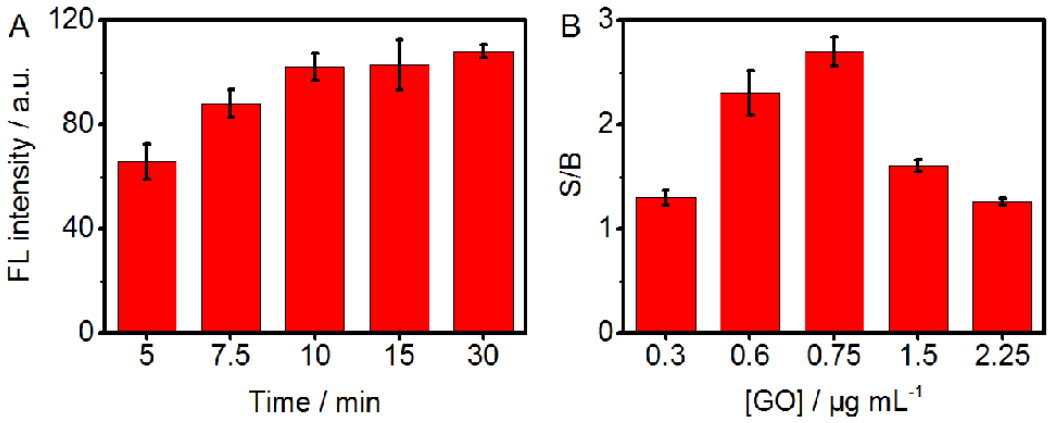


Fig. S6 Effects of (A) incubation time between 10 nM probe and target at 37 °C on fluorescent signal, and (B) GO concentration on the ratio of catalytic fluorescent signal to background (S/B) in presence of 10 nM probe and target. The catalytic reaction is carried out in 50 mM Tris-HCl (pH 7.4, containing 300 mM NaCl) with 0.7 mM tyramine and 2 mM H_2O_2.

Specificity of DNA sensing protocol

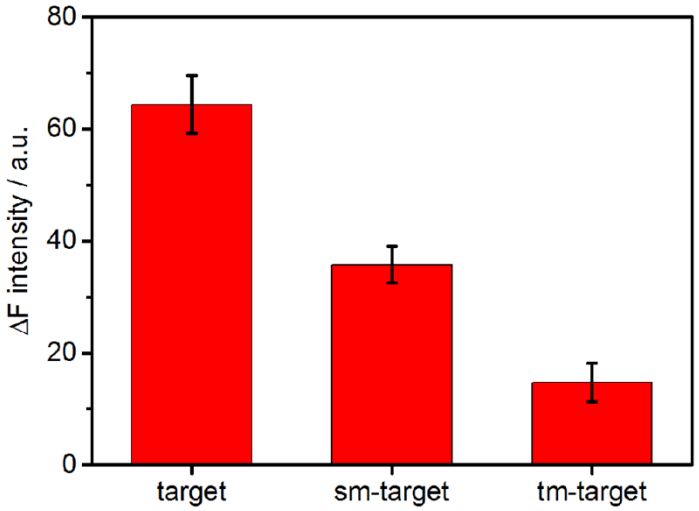
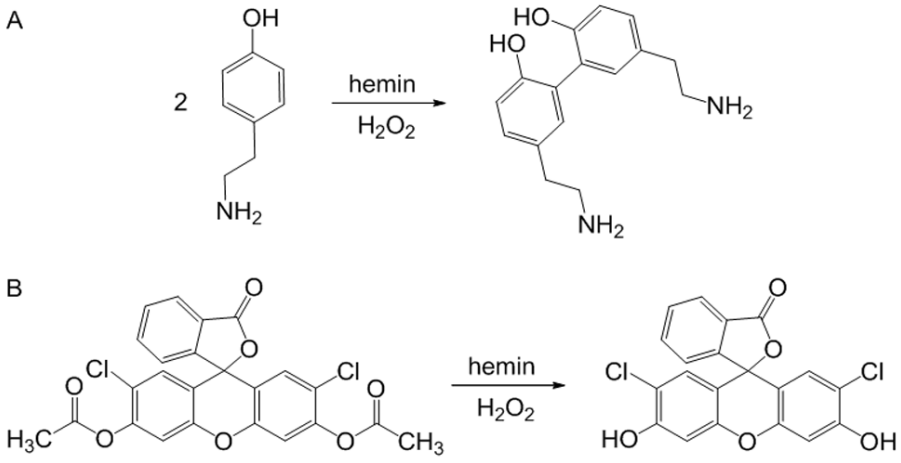


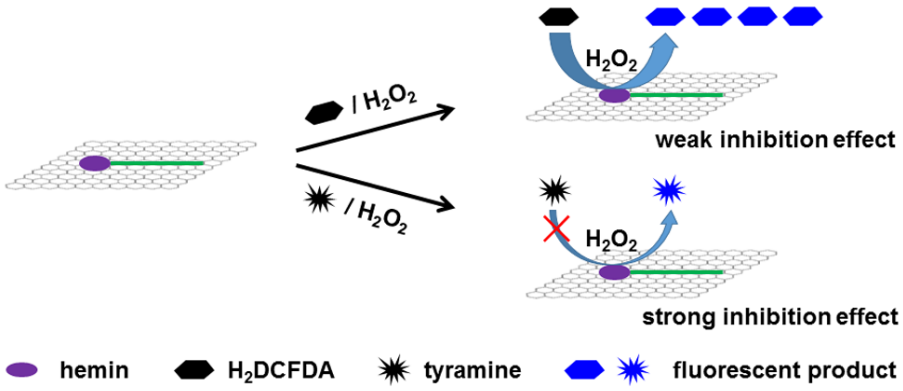
Fig. S7 Catalytic fluorescence intensity increment (ΔF) of 10 nM probe in the presence of 10 nM complementary target, single-base and three-base mismatched DNA.

Catalytic reaction of hemin



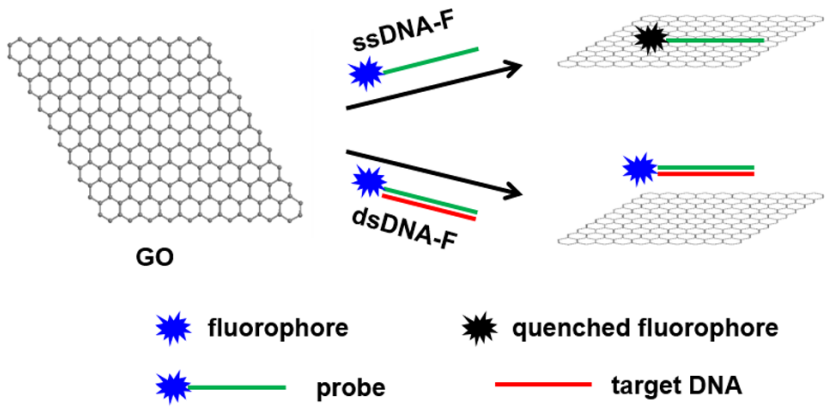
Scheme S1 The catalytic reactions of non-fluorescent substrates (A) tyramine and (B) H_2DCFDA for formation of fluorescent products in the presence of hemin and H_2O_2.

Proposed inhibition mechanism



Scheme S2 The proposed inhibition mechanism of GO toward the peroxidase activity of DNA-linked hemin.

Conventional fluorescence DNA sensing strategy



Scheme S3 Conventional fluorescence DNA sensing strategy using GO as fluorescent quencher and fluorophore labelled on the probe as signal reporter, in which one target DNA could only recover the fluorescence of one fluorophore labelled on the probe.

Table S2. Analytical performances of the proposed and previously reported methods using nanomaterials as quenchers for fluorescence sensing of nucleic acid

Nanomaterial	Linear Range	Detection Limit	Assay Time	Reference
	0.5-40 nM	**0.2 nM**	**22 min**	**this work**
Graphene oxide	50-1500 nM	12 nM	not reported	S1
	not repoeted	5 nM	20 min	S2
Pd nanowire	10-100 nM	6.0 nM	> 1 h 20 min	S3
MoS_2 nanosheet	0-15 nM	0.5 nM	15 min	S4
Metal-organic framework	10-100 nM	3.0 nM	> 4 h	S5
Carbon nitride nanosheet	3.0-30 nM	2.1 nM	12 min	S6

Supporting references

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