Electronic Supplementary Material (ESI) for Analytical Methods

Graphene oxide-based Fluorescent Detection of DNA and Enzymes Using Hoechst 33258 and their use for dual-output fluorescent Logic gate

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Materials and Methods

Reagents and instruments

All oligonucleotides (Tabel S1) used in the experiment were synthesized by the Invitrogen Company (Shanghai, China). The Graphene Oxide was obtained from the Nanoon Company (Hebei province, China). Hoechst 33258 dye was purchased from Beyotime Institute of Biotechnology. The Exonuclease III, Dam methyltransferase, Dpn I endonuclease, SAM and the corresponding reaction buffer solution were purchased from New England Biolabs Inc. The oligonucleotide stoke solution (100 μM) was prepared in Milli-Qultrapure water (Millipore, ⩾ 18 MΩ.cm). Double-stranded DNA (dsDNA) and hairpin DNA was annealed at high concentration in 10 mM Tris-EDTA, pH 8.0, 10 mM NaCl by heating to 95 ^{o}C for 5 minutes followed by slowly cooling to room temperature. All chemicals were of analytical grade and used without further purification. The fluorescence measurements were carried out on Perkin-Elmer LS-55 fluorescence spectrometer.

Table S1. oligonucleotides used in this study

Oligonucleotide	Sequence
P1	5'-AGTCAGTGTGGAAAATCTCTAGC-3'
T1	5'-GCTAGAGATTTTCCACACTGACT-3'
M1	5'-GCTAGAGATTGTCCACACTGACT-3'
M2	5'-GCTAGAGATGGTCCACACTGACT-3'
M3	5'-GCTAGAGATGGGCCACACTGACT-3'
H2	5'-AGTCAGTGTAAAATCTCTAGCTTTTTTGCTAGAGAT TTTACACTGACT-3'
P3	5'-CGAAAAGATCAAAAGG-3'
T3	3'-GCTTTTCTAGTTTTCC-3'

Fluorescent Detection of target DNA sequences

In a typical DNA assay, P1 was prepared as 100 nM in 20 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$) and mixed with solution containing 5 μM Hoechst 33258 and 25 ng/μL GO prior to the addition of different sequences (T1, M1, M2, M3). After allowing this mixture to hybridize for about 15 min at room temperature, then the flurescence of mixture was detected at 345 nm excitation.

In assay of fluorescence response of P1-GO towards T1 with different concentrations, P1 was prepared as 200 nM in 20 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$) and mixed with solution containing 5 μM Hoechst 33258 and 25 ng/μL GO prior to the addition of T1. The final T1 concentration in samples ranged from 5 nM to 150 nM. After allowing this mixture to bind for about 15 min at room temperature, the fluorescence of the mixture was detected at 345 nm excitation.

Exonuclease III activity assay by GO based platform

For Exo III activity assay, reaction mixture was prepared by mixing Exo III enzyme stock with 2 μM annealed DNA in 1X Exo III reaction buffer (10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0). After incubation at room temperature for 1 h, 20 μL of reaction mixture and the mixture containing 5 μM Hoechst 33258, 25 ng/μL GO, 10mM Tris-HCl buffer and 380 μL H_2O were added. Fluorescence spectra were measured at 345 nm excitation.

Dam methyltransferase activity assay by GO based platform

The methylation experiment was performed in 20 μL of methylase buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9) containing 1 μM DNA probe, 8 units DpnI, 160 μM SAM and different amounts of Dam MTase. The reaction mixture was incubated at 37 ^oC for 2 h. To protect the activity of enzymes, all of these standard solutions were thawed on ice and stored at -20 ^oC. After the methylation reaction, the mixture containing 5 μM Hoechst 33258, 25 ng/μL GO, 10 mM Tris-HCl buffer and 400 μL H_2O were added. Fluorescence spectra were obtained at 345 nm excitation.

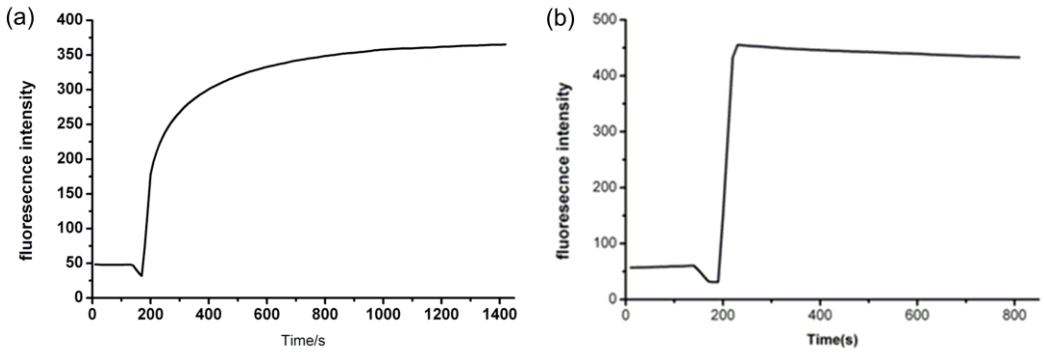


Figure S1. (a) The kinetic behavior of fluorescence restoration of P1-GO-Hoechst complex by target DNA T1 in Tris-HCl buffer. [P1]= [T1]= 100 nM. (b) Fluorescence restoration of GO-Hoechst complex in Tris-HCl buffer by dsDNA (P1 + T1) (50nM). [Hoechst 33258] = 5μM; [GO] = 25 ng/μL; Excitation: 345 nm, emission: 452 nm.

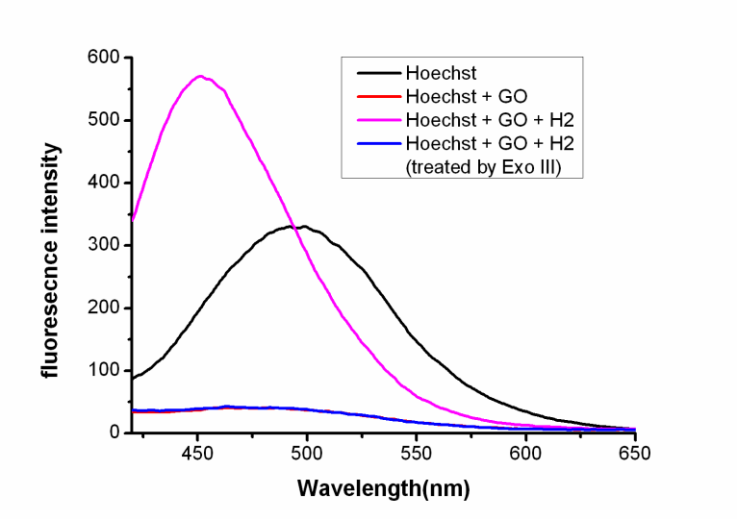
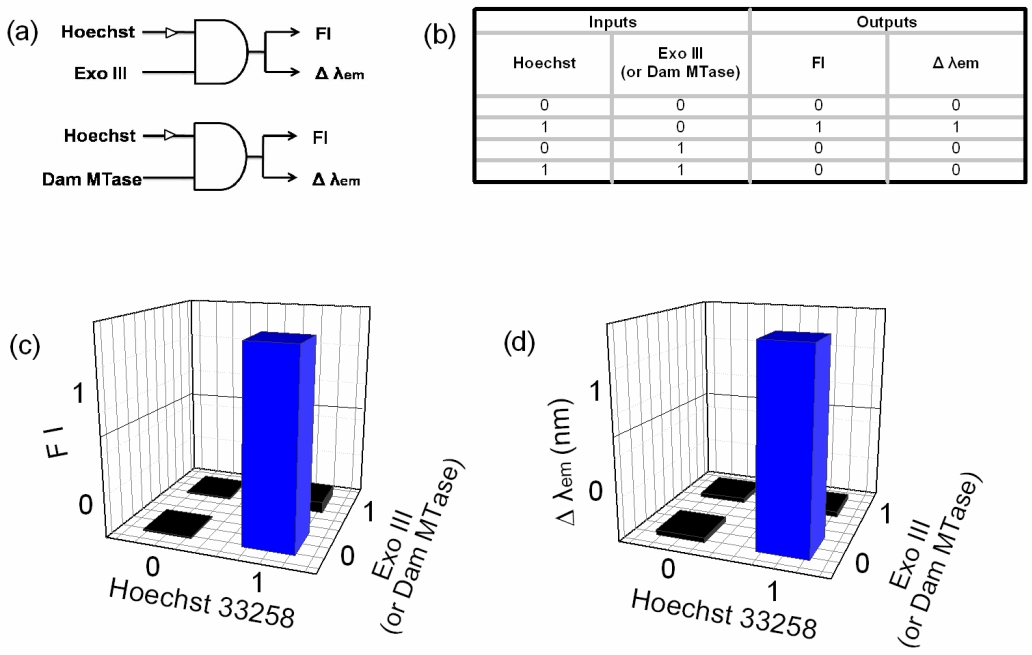


Figure S2. Fluorescence spectra of the Exo III detection system by hairpin DNA (H2) substrate. The fluorescence emission spectra with (blue) and without (rose-red) Exo III digestion to the GO-Hoechst complex. [H2]= 100 nM; [Hoechst 33258]= 5μM; [GO]= 25 ng/μL.



Inputs		Outputs	
Hoechst	Exo III (or Dam MTase)	FI	Δ λem
0	0	0	0
1	0	1	1
0	1	0	0
1	1	0	0

Figure S3. (a) The symbol of the logic gate. (b) Truth table for the two-input and two-output INHIBIT logic gate. (c) Fluorescence intensity at 452 nm in the form of a bar representation. (d) Fluorescence wavelength in the form of a bar representation.