Electronic Supplementary Information (ESI)

A New Strategy for Designing Graphene Oxide-based DNA Hairpin Probe: Fluorescence upon Switching the Orientation of Sticky End

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1. DNA sequences

All of the DNA sequences used in this work were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The hairpin probes used for DNA detection were adopted from Pierce et al.'s design, [1] and the target DNA were adopted from our previous work [2]. All the sequences were listed as below (from 5' to 3', the sticky ends are underlined and loops are italicized):

H0: FAM-CACGCCGAATCCTAGACT***CAAAGT***AGTCTAGGATTCGGCGTG

H1: FAM-<u>TTAACC</u>CACGCCGAATCCTAGACT***CAAAGT***AGTCTAGGATTCGGCGTG

H2: AGTCTAGGATTCGGCGTG***GGTTAA***CACGCCGAATCCTAGACT<u>ACTTTG</u>-FAM

Matched target: AGTCTAGGATTCGGCGTGGGTTAA

Mismatched target: AGTCTAGGATTCAGCGTGGGTTAA

Deleted target: AGTCTAGGATTC_GCGTGGGTTAA

Inserted target: AGTCTAGGATTCTGGCGTGGGTTAA

2. AFM Image of Graphene oxide (GO)

GO was obtained from Boson Technology Co. Ltd. (Shanghai, China). Morphologies of GO were determined on an atomic force microscope (AFM) (SPI3800/SPA400, Seiko Instrument, Japan) using tapping mode to simultaneously collect height and phase data. Figure S1 showed the typical imaging of the GO, which could be seen that the thickness of single-layer and bilayer GO was about 1.2 nm and 2.3 nm, respectively. According to the cross-section analysis, the average thickness of GO sheet was about 1.1 nm.

3. Fluorescence quenching efficiency of GO

The fluorescence quenching efficiency of GO in the SPSC buffer (0.75 M NaCl, 50 mM Na_2HPO_4, pH 7.4) was estimated by measuring the fluorescence intensity of 400 nM H1 with the additional of GO. Different concentrations of GO, ranging from 5 to 40 μg/mL, was tested with the fixing concentration of H1 to get an optimal GO concentration. Figure S2 showed that, with the increase of GO concentration, the fluorescence intensity dramatically decreased. Here, 30 μg/mL was chosen as optimized GO concentration.

4. DNA detection procedures

According to our previous work [2], the hairpin probes were heated to 95 ºC for 2 min and then allowed to cool to room temperature for 1 h before use. For the GO-quenched single hairpin probe assay, different concentrations of target DNA were incubated with 400 nM H1 in SPSC buffer for 30 min. Subsequently, 30μg/mL GO was added into the reaction solution and the final mixture was further incubated for 30 min. For the GO-quenched duple hairpin probe assay, different concentrations of target DNA were incubated with 200 nM H1 and 200 nM H2 in SPSC buffer for 2 h. Subsequently, 30μg/mL GO was added into the reaction solution and the final mixture was further incubated for 30 min. Finally, fluorescence signals were recorded on a F-7000 spectrofluorometer (Hitachi, Japan) by setting 480 nm as the excitation wavelength, 500 to 650 nm as the emission wavelength range.

5. Gel electrophoresis procedures

H1 and H2 were heated to 95 ºC for 2 min and then allowed to cool to room temperature for 1 h before use. Then 4 μM H1 and H2 were incubated with 0 and 400 nM target DNA, respectively. The 1% agarose gels were prepared using TAE buffer. The gel was run at 55 V for 2 h in TAE buffer and finally visualized under UV light.

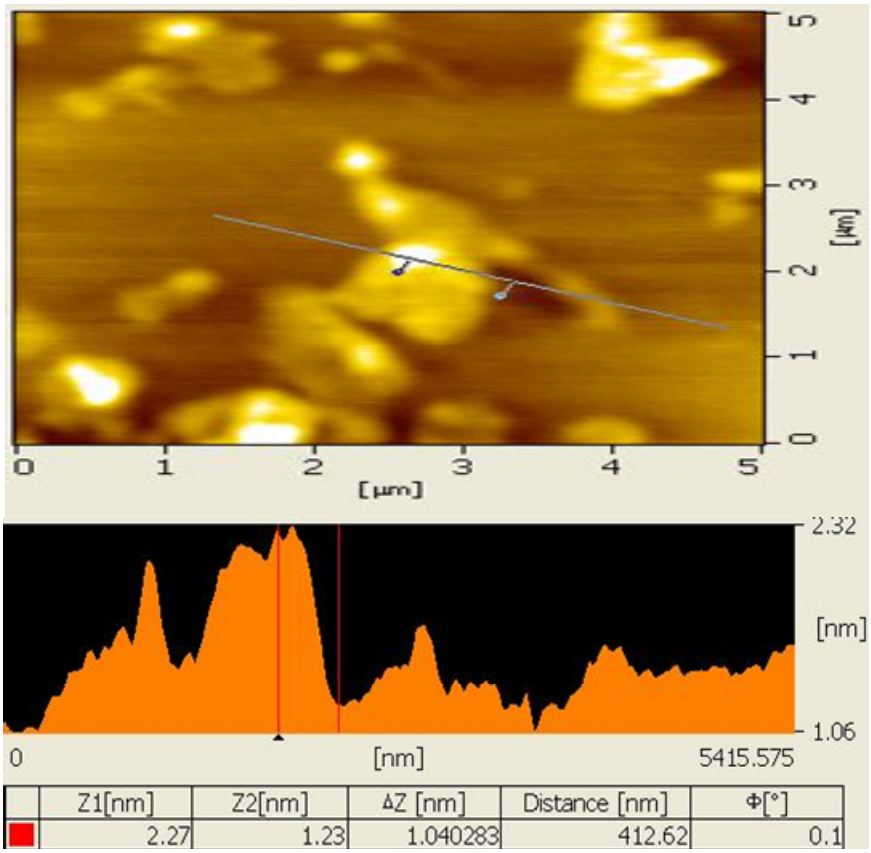

Figure S1. Tapping mode AFM image (top) and height profile (bottom) of GO sheets deposited on mica substrates.

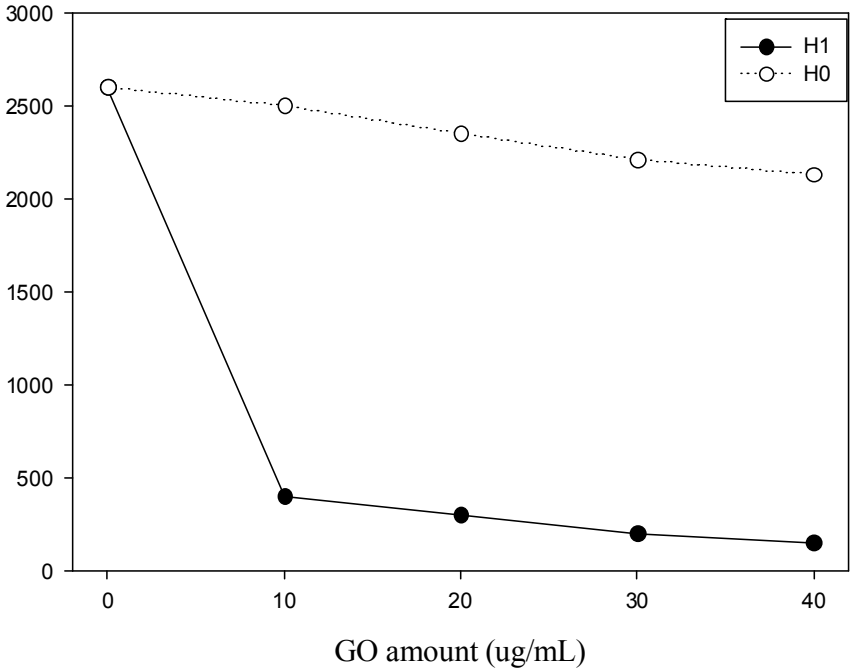

Figure S2. The fluorescence intensity of 400 nM H1 or H0 versus the concentration of GO.

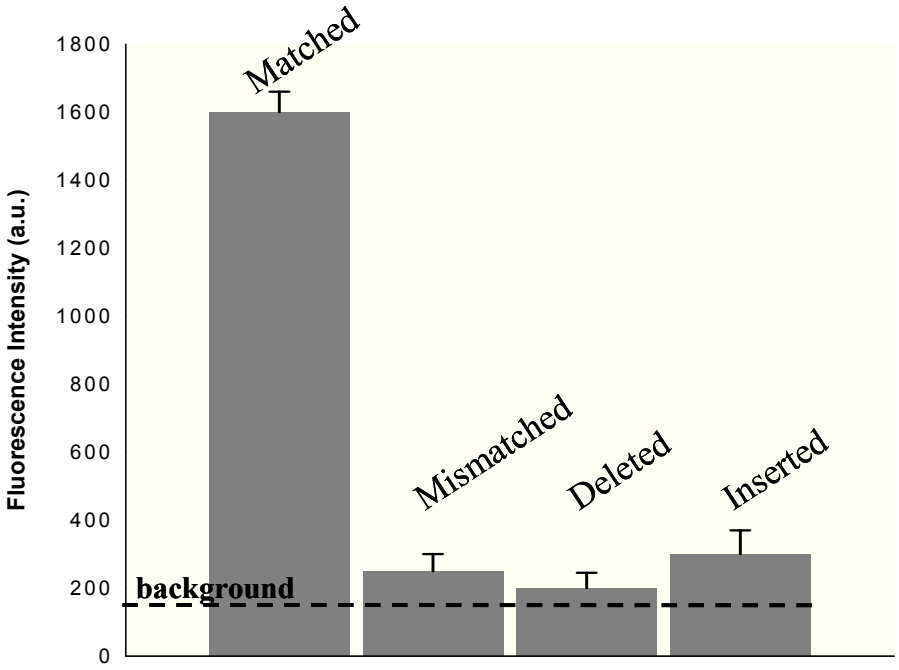

Figure S3. Responses of the GO-quenched single hairpin probe to matched, mismatched, deleted and inserted target DNA .

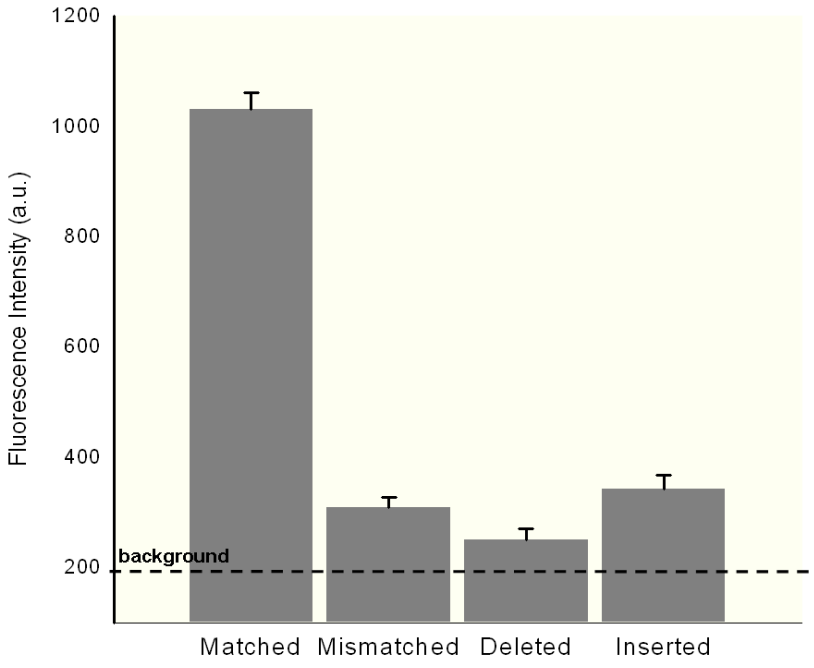

Figure S4. Responses of the GO-quenched duple hairpin probe to matched, mismatched, deleted and inserted target DNA .

[1] R. Dirks and N. Pierce, Proc. Natl. Acad. Sci. U.S.A., 2004, 101, 15275-15278;

[2] J. Huang, Y. Wu, Y. Chen, Z. Zhu, X. Yang, C. Yang, K. Wang and W. Tan, Angew. Chem. Int. Ed., 2001, 50, 401-404.