Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Graphene Oxide-based Nano-beacon for DNA Phosphorylation Analysis

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Experimental Details

Materials and Instrumentation. DNA oligonucleotides were synthesized and purified by HPLC (Dalian Takara Bio Inc., Dalian, China). The sequences of the involved oligonucleotides are listed in Table 1. T4 DNA ligase and T4 polynucleotide kinase (PNK) were purchased from Fermentas International Inc. and New England Biolabs Inc., respectively. Graphite powder and other chemical reagent were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). All chemicals were of analytical grade and used as received. Milli Q Water was used for the whole procedure. GO was synthesized from graphite powder according to published protocols [1, 2]. Fluorescence spectroscopy was performed on Hitachi F-4500 Fluorescence spectrophotometer. The fluorescence intensity was performed with excitation at 494 nm and an emission of 524 nm was recorded. The emission spectra were obtained by exciting the samples at 494 nm and by scanning the emission from 504 to 610 nm. Spectrometer slits were set for 5 nm band-pass.

Table S1 Sequences of Oligonucleotides

Type	Sequence
HP[a]	5'-FAM-*CGCTC* TGG TGT GGT TGG GCT *GAGCG*-3'
MB[b]	5'-FAM-*CGCTC* TGG TGT GGT TGG GCT *GAGCG*-TAMRA-3'
C1[c]	5'-AGC CCA ACC ACA CCA-3'
C2[c]	5'-AGC CCA ACC ACA CCA *GAG* -3'
C3[c]	5'- AGC CCA ACC ACA CCA *GAG CG* -3'
C4[c]	5'-*CTC* AGC CCA ACC ACA CCA *GAG* -3'
M1[d]	5'-*CTC* AGC CCA ACG ACA CCA *GAG* -3'
M2[d]	5'-*CTC* AGC CCA ACA ACA CCA *GAG* -3'
M3[d]	5'-*CTC* AGC CCA ACT ACA CCA *GAG* -3'
Oligo A[c]	5'-*CTC* AGC CCA A -3'
Oligo B[c]	5'-CC ACA CCA *GAG* -3'
Oligo C[d]	5'-TC ACA CCA *GAG* -3'
Oligo D[d]	5'-AC ACA CCA *GAG* -3'
Oligo E[d]	5'-GC ACA CCA *GAG* -3'
Oligo F[c, d]	5'(PO_4)-CC ACA CCA *GAG* -3'

[a] Hairpin-structured probe. [b] Molecular beacon. [c] Perfectly complementary target.

[d] Single-base-mismatched target. [e] The PO_4 in Oligo F represents the phosphate at 5' end.

Improvement the fluorescence recovery in the presence of GO. 20 nM HP and 90 nM target (C1, C2, C3 and C4) were mixed in 995μl of hybridization buffer (10 mM PB, 100 mM NaCl, 10 mM MgCl2, pH 7.4) at RT. 5 μl of GO (0.47 mg/ml) was added into hybridization solutions and incubated 2 min at RT. The fluorescence measurements were performed with excitation at 494 nm and emission range from 504 to 610 nm. Spectrometer slits were set for 5-nm band-pass. In the SNP detection, 20 nM HP and 10 nM PC DNA or single base mismatch targets were mixed for 10min. The optimized experiments were performed at different temperature (temperature intervals of 5°C from 40°C to 60°C). And at every temperature dot, the temperature must be keep 5 minutes to equilibrium state. For allele frequency analysis, the test samples at various allele frequencies were used and which composed of C4 and M4 mixed at various ratios of 0, 2%, 5%, 10%, 20%, 50% and 100%. Total concentration of C4 and M4 is 10 nM.

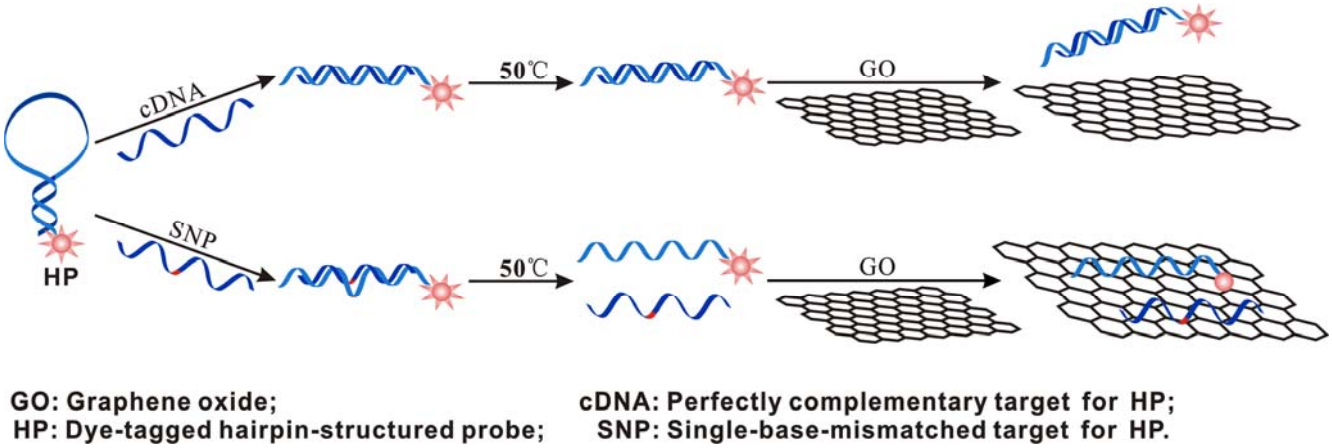
Assay of T4 PNK. The phosphorylation reactions were performed in 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 1.0 mM ATP using T4 DNA ligase, T4 PNK and various oligonucleotides. The oligonucleotides were altered depending on variant experiments. Each reaction mixture was incubated at 37 °C for 30 min and inactivated by heating at 70 °C for 10 min. Three samples were prepared to verify our postulated mechanism in scheme 1: sample I contained HP (10 nM) and Oligo A (20 nM) and Oligo F (20 nM); sample II and III both contained HP (10 nM), Oligo A (20 nM) and Oligo B (20 nM). Oligo A and Oligo B were designed to complement the 3' and 5' half part of the HP, respectively. And 10 Unit of T4 DNA ligase, 0.3 Unit of T4 PNK were introduced into each sample. But the T4 PNK in sample III was inactivated by heating at 70°C for 10 min before adding. After phosphorylation reaction, 6 μl reaction mixtures were mixed in 991.5 μl of assay buffer (10 mM PB, 100 mM NaCl, 10 mM MgCl2, pH 7.5) at 50 °C. After 5 min incubation, fluorescence measurements were performed with a quartz cuvette. 2.5 μl of GO (0.47 mg/ml) was added into assay solutions and fluorescence was recorded simultaneously. For T4 PNK activity quantification assay, different concentration T4 PNK (0, 0.001, 0.002, 0.01, 0.05, 0.1, 0.2, 0.3, 0.5, 0.75 or 1 Unit/ml) was used.

Specificity of DNA substrates based GO/HP complex system. A series of oligonucleotides (Oligo B to Oligo E) were carried out to explore the preference of the HP complex system for various substrates. The Oligo C, Oligo D and Oligo E are single-base-mismatch oligonucleotides with similar sequences to Oligo B. The samples were prepared with phosphorylation buffer mixed

with 0.3 U T4 PNK, 10 U T4 DNA ligase, HP (10 nM), Oligo A (20 nM) and Oligo B (20 nM) or single base mismatch target (20 nM). The phosphorylation process was incubated at 37 °C for 30 min and inactivated by heating at 70 °C for 10 min. Then, 6 μl reaction mixtures were mixed in 991.5 μl of assay buffer and pre-annealed at 50 °C for 10min. At this point, fluorescence measurement was performed with a quartz cuvette and 2.5 μl of GO (0.47 mg/ml) was added. Each sample was incubated at 50 °C for 10 min to obtain equilibrium.

Comparison between GO/HP and MB. Four samples were prepared to Comparison with the two systems: sample A and B both contained HP (10 nM), Oligo A (20 nM) and Oligo B (20 nM); sample C and D contained MB (10 nM), Oligo A (20 nM) and Oligo B (20 nM). 10 Unit of T4 DNA ligase was introduced into each sample and 0.3 Unit of T4 PNK was introduced into sample A and C. After phosphorylation reaction, 6 μl reaction mixtures were mixed in 991.5 μl of assay buffer at 50 °C. After 5 min incubation, fluorescence measurements were performed with a quartz cuvette. The fluorescence of MB systems was directly measured; 2.5 μl of GO (0.47 mg/ml) was added into assay solutions (GO/HP systems) and fluorescence was recorded simultaneously.

Results



Scheme S1 Schematic presentation of DNA and SNP detection using GO and HP

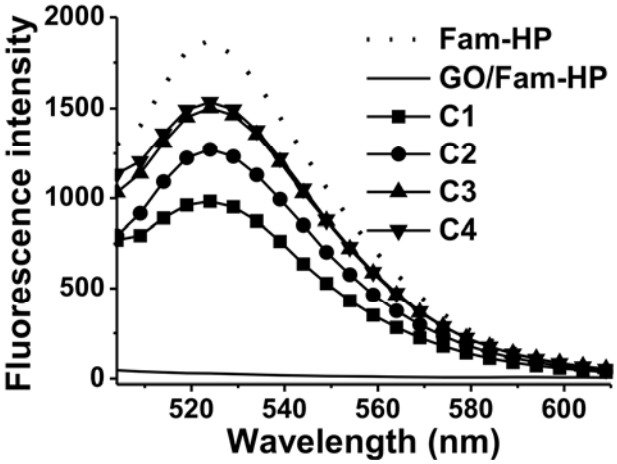


Fig. S1 Fluorescence spectra of HP (20 nM) in the absence (dotted line) and presence of GO (solid line), and HP hybridized with the C1 (90 nM, square), C2 (90 nM, circle), C3 (90 nM, uptriangle) and C4 (90 nM, downtriangle) in the presence of GO.

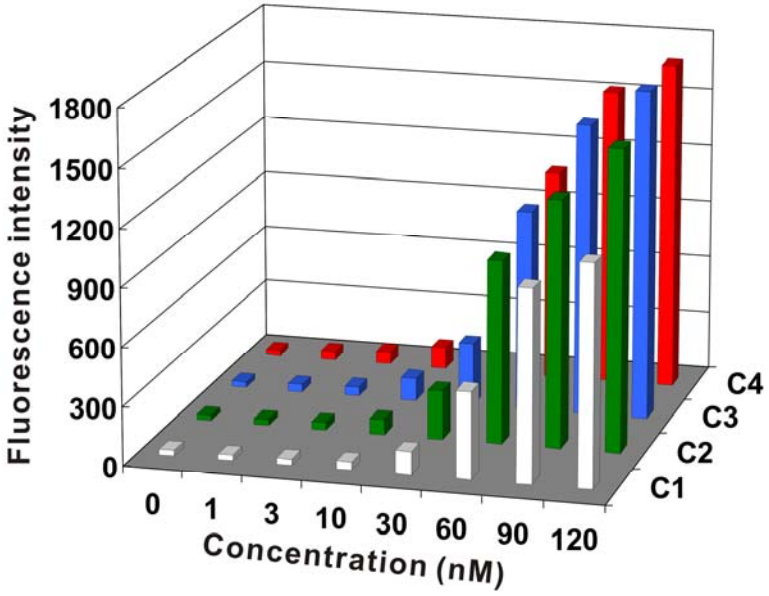


Fig. S2 Calibration curve for the fluorescence intensity vs. concentration of C1 (white), C2 (green), C3 (blue) and C4 (red).

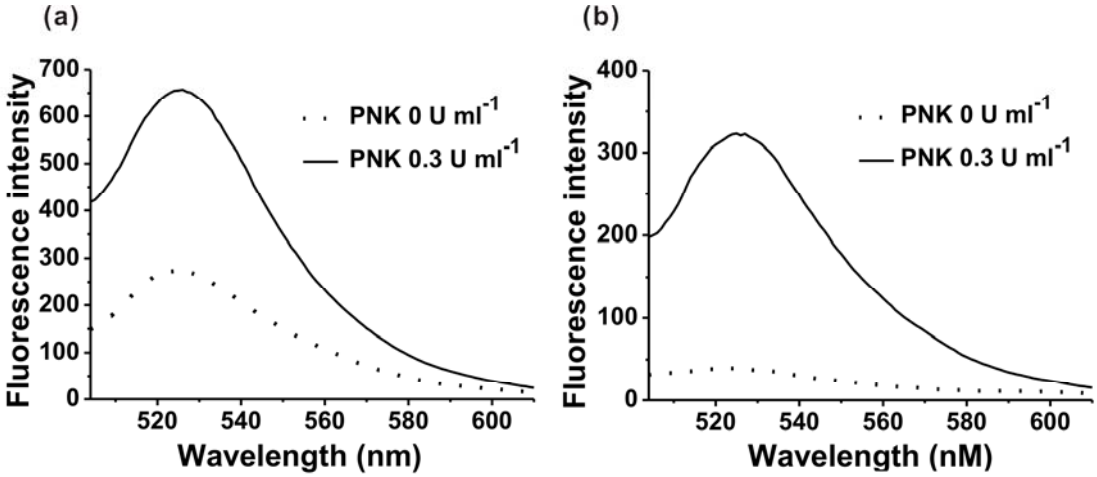


Fig. S3 (a) Fluorescence emission spectra for MB (10 nM) in the absence (…) and presence (–) of T4 PNK. (b) Fluorescence emission spectra for GO/HP (10 nM) complex in the absence (…) and presence (–) of T4 PNK.

References

1 S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang and C. Fan, *Adv. Funct. Mater.*, 2010, **20**, 453-459.

2 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339-1339.