Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Graphene oxide as an efficient signal-to-background enhancer for DNA detection with a long range resonance energy transfer strategy

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Experimental Section

1. Apparatus

Fluorescence spectra were measured with an F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). A vortex mixer, QL-901 (Haimen, China), was employed to blend the solution, and a constant-temperature water-base boiler (Jiangsu, China) was employed to control the hybridization temperature to keep the temperature 37 ^{o}C. Circular dichroism (CD) measurements were performed with a model JASCO-810 spectropolarimeter (Hitachi, Tokyo, Japan).

2. Materials

A 21-mer synthetic oligonucleotide, corresponding to portions of the HIV-1 U5 LTR DNA segment, was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The linear probe DNA (P) has a sequence of 5′-ATG TGG AAA ATC TCT AGC AGT-3′, which was labeled with any dye molecule. The complementary target DNA (T) has a sequence of 5′-ACT GCT AGA GAT TTT CCA CAT-3′. Mismatch oligomers were employed to confirm the specificity of our protocol, including, one-base-mismatched oligomer (MT1), 5′-ACT GCT AGA ***T***AT TTT CCA CAT-3′; two-base-mismatched oligomer (MT2), 5’-ACT ***T***CT AGA ***T***AT TTT CCA CAT-3’; three-base-mismatched oligomer (MT3), 5′-ACT ***T***CT AGA ***T***AT TTT ***T***CA CAT-3′. Graphite powder was commercially purchased from Sinopharm chemical Reagent Co., Ltd. (Shanghai, China). SG (10000) was purchased from invitrogen inc, which was diluted to 250×with DMSO, then to 125×water to make a stock solution. The concentration of 125×SG solution is 2.45×10^{-4}M according to the research from Liu, et al in 2008.[1] Mili-Q purified water (18.2 MΩ) was used throughout. Tris-HCl buffer solution (pH 7.4) was used to control the acidity of the reaction solution.

3. Preparation of the Graphene Oxide

Graphene oxide was synthesized from natural graphite powder by modified Hummer’s method.[2] Generally, the graphite powder (3g) was oxidized in a solution of concentrated H_2SO_4 (24mL) containing P_2O_5 (2.5g), and $K_2S_2O_8$ (2.5g) for 4.5 hours, giving a dark blue mixture, which was diluted with water (500mL) and stood

overnight. The mixture then filtered with a membrane of 0.45μm to remove residual acid, and dried at 25 °C over a period of 24h. These pre-oxidized graphite powder were added to 120mL of cold H_2SO_4 (0 °C), to which $KMnO_4$ (15g) was slowly added under continuous stirring in ice-bath to control the temperature below 20 °C. After that, the mixture was further stirred for 2 hours at 35 °C and 250mL distilled water was added under 50 °C. The solution was rediluted, followed by addition 20mL H_2O_2 (30%).The product was washed with HCl (1:10), and then with water, and then suspended in water via sonication. The brown solution was dialyzed for two days to remove residual acids and metal.

4. Rate calculation of long range resonance energy transfer (LrRET)

If the acceptor is a carbon tube, the rate of the process can be calculated through following equation: [3]

$$K(\hbar\Omega) = \frac{3m^*}{8192\pi\hbar^3 E^2} \frac{\left(33\mu_z^{\pm^2} + 71\mu_\rho^{\pm^2}\right)}{R^5} \sum_{\{m_i\}} \frac{\mu_{eg}^2}{\sqrt{\frac{m^*\left(\hbar\Omega - \varepsilon_g\right)}{\hbar^2} - \frac{m_i^2}{a^2}}}$$

Where $\hbar\Omega$ is the transferred energy, m^* is the effective mass, m_i refers to the quantum number, ε is the permittivity of the medium separating the donor and the acceptor, ε_g is the band gap of the carbon nanotube, $\mu_z^{\pm}$ and $\mu_\rho^{\pm}$ are the transition dipolars of the lattice site of the carbon nanotube, $\pm$ in the $\mu_z^{\pm}$and $\mu_\rho^{\pm}$devote states above (+) and below (-) the band gap, and μ_{eg} is the transition dipolar of the donor, R is the distance between the dye and the carbon nanotube. Therefore, in the energy transfer from a dye

to a carbon nanotube, the rate has a (distance)$^{-5}$ dependence. Graphene is a two-dimensional sheet, which can be rolled to form 1D nanotube, even though, the rate of the energy transfer from a dye to graphene follows another expression:[3]

$$K(\hbar\Omega) = \frac{3\pi e^2}{256\,\Omega h^2 \varepsilon^2} \frac{\left(\mu_{eg}^2 (\sin\theta)^2 + 2\mu_{eg}^2 (\cos\theta)^2\right)}{z^4}$$

Where μ_{eg} is the transition dipolar of the donor, and θ is the angle that μ_{eg}^{D} ($|\mu_{eg}^{D}| = \mu_{eg}$) makes with the z-axis, Z is the distance between the dye and the graphene. So, the rate of energy transfer between the dye and GO has a (distance)$^{-4}$ dependence.

5. Detection of HIV-1 DNA

In a typical DNA assay, the probe DNA was hybridized with the target DNA in 20 mM Tris-HCl (pH 7.4) buffer solution containing 15 mM NaCl at 37 ^{o}C for 30 min. Then 50 μL of SG (2.45×10^{-6} M) was added and incubated at room temperature for 2 min. At last 15 μL GO (200 μg/mL) was added and incubated for further 12 min, and then the mixture was transferred for fluorescence measurements by an F-2500 fluorescence spectrophotometer with an excitation wavelength of 490 nm.

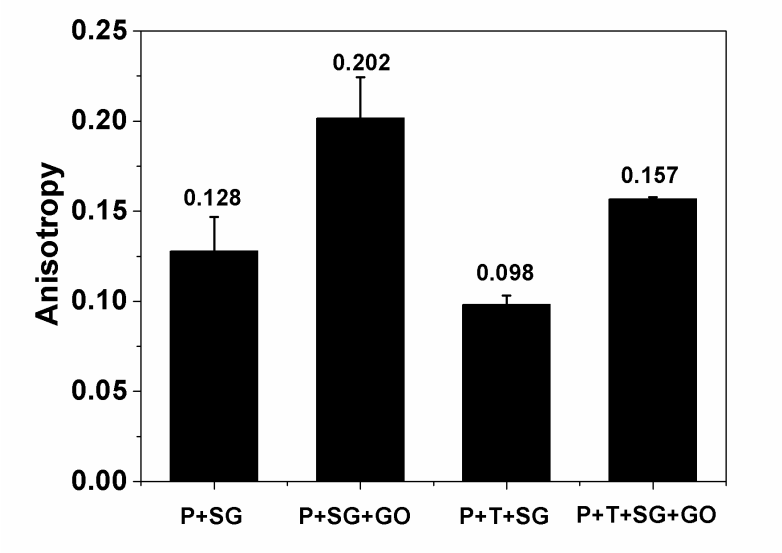

Figure S1. Fluorescence anisotropy changes of SG in the absence and presence of P, T and GO, respectively. Concentrations: P, 10 nM; T, 30 nM; SG, 2.45×10^{-7} M; GO, 3.0 μg/mL. The excitation was at 490 nm, and emission was recorded at 529 nm. All data were collected from three measurements, and the error bars indicate the standard deviation. The introduction of GO into P+SG and P+T+SG has an increase factor of 1.6.

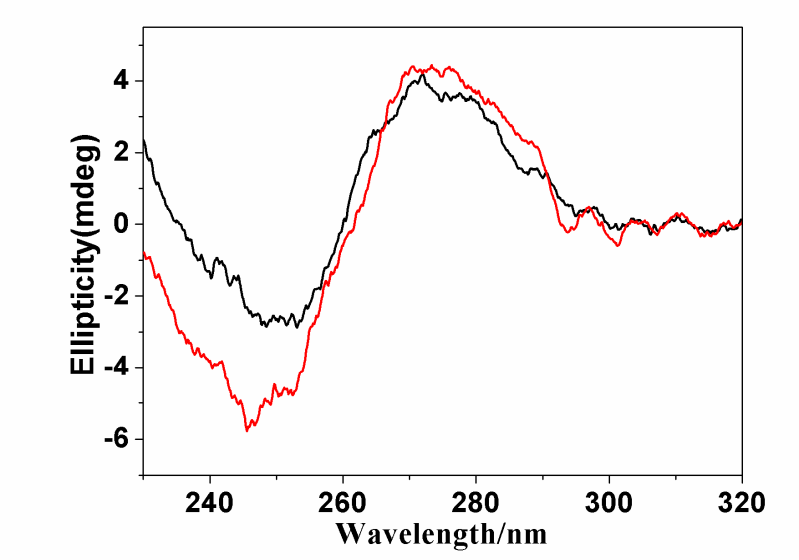

Figure S2. Circular dichroism of P in the absence (black) or presence (red) of T in pH 7.4 Tris-HCl buffer of SG/GO. Concentrations: P, 10 nM; T, 30 nM; SG, 2.45×10^{-7} M; GO, 3.0 μg/mL.

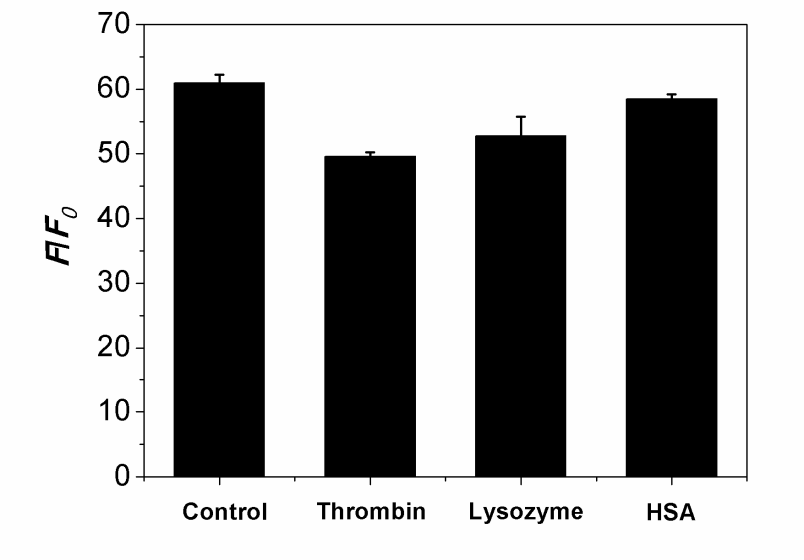

Figure S3. Signal-to-background ratio (F/F_0) of SG/P/GO incubated in the presence of 50 nM T with Tris-HCl buffer (control), Thrombin, Lysozyme and HSA. Concentrations: P, 10 nM; T, 50 nM; SG, 2.45×10^{-7} M; GO, 3.0 μg/mL; proteins, 1μM (solved in Tris-HCl buffer). The excitation was at 490 nm, and emission was recorded at 529 nm. All data were collected from three measurements, and the error bars indicate the standard deviation.

Reference

1. J. Wang and B. Liu, *Chem. Commun.*, 2008, 4759.
2. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.
3. R. S. Swathi and K. L. Sebastian, *J. Chem. Sci.*, 2009, **121**, 777.