Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Biosensor for the Detection of Single Base Mismatches in microRNA

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Experimental Section

Materials. Graphite nanofibers was purchased from Catalytic Materials LLC (USA). Sulfuric acid (H_2SO_4) was purchased from Samchun chemical (Seoul, Korea). Hydrogen peroxide (30% in water) (H_2O_2) was purchased from Junsei (Japan). Potassium permanganate ($KMnO_4$), Potassium persulfate ($K_2S_2O_8$) and Phosphorus pentoxide (P_2O_5) were purchased from Sigma-Aldrich (MO, USA). RNA strands were purchased from Bioneer (Daejon, Korea). DNA strands were purchased from Genotech (Daejon, Korea). PNA probes were purchased from Panagene (Daejon, Korea). Temperature control was performed by using thermocycler (BioRad, USA). Fluorescence intensity and fluorescence image were obtained by fluorometer SynergyMx (Biotek, UK).

Preparation of graphene oxide. Graphene oxide (GO) was synthesized by using Hummers method with pre-oxidation treatment, using graphite nanofibers (Catalytic Materials LLC) as starting material. First, concentrated H_2SO_4 (5 ml) was heated to 80 °C in a round bottomed flask and then, $K_2S_2O_8$ (0.15 g) and P_2O_5 (0.15 g) were added to the sulfuric acid, keeping at 80 °C for 4.5 hours. After the mixture was cooled, the mixture was diluted with deionized (DI) water. This solution was filtered and rinsed with DI water (100 ml) for removing remained reactants. Obtained pre-oxidized graphite nanofiber was dried under ambient condition. The dried solid was transferred into a 50 ml round bottomed flask and concentrated H_2SO_4 (25 ml) was added and chilled to 0 °C using an ice bath. $KMnO_4$ (1 g) was slowly added to the mixture with stirring, keeping the temperature below 10 °C. After mixing, the flask was placed in 35 °C water bath for 12 hours. The reaction mixture was transferred into an Erlenmeyer flask (500 ml) in an ice bath. DI water (100 ml) was slowly added to the flask with stirring. During this step, the reaction temperature should be kept under 55 °C. Then, 5 ml of 30 % H_2O_2 solution was added to the mixture, of which the color turned into bright yellow. This mixture was collected, centrifuged and rinsed with 3.4 % HCl solution and acetone to get rid of residual salts and acid.

Obtained solid GO was dried under vacuum. To prepare GO stock solution, GO was dissolved in water and then, the solution was centrifuged at low speed 3000 rpm for 30 min to remove large chunks.

Temperature dependent mismatch discrimination using peptide nucleic acid and graphene oxide. Firstly, to prepare target/PNA probe duplex, 1 μL of 5 μM RNA target strand solution was mixed with 10 pmol of dye labeled PNA probe in 100 μL of pH 7.2 1XPBS buffer containing NaCl 137 mM, KCl 2.7 mM, followed by incubation at room temperature for 10 min. Then, 0.1 μg of GO was added to the mixture, followed by heating at high temperature (55 ℃ - 75 ℃) for 5 min to induce selective dissociation of relatively unstable duplex. After cooling down, fluorescence intensity of the mixture was measured.

Multiplex detection of miRNAs possessing only one base difference between them. For preparation of probe/target duplex, two RNA target strands (20 pmol) was incubated with FAM labeled let-7a probe and ROX labeled let-7c probe (each 10 pmol) in 100 μL of 1XPBS solution for 10 min. Then, 0.2 μg of GO was added to the mixture, followed by heating at 71 ℃ for 5 min to induce selective dissociation of two kinds of relatively unstable duplex. After cooling down, fluorescence intensity of the mixture was measured at ex492 nm/em518 nm for FAM-let7a probe and ex587 nm/ex608 nm for ROX-let7c probe.

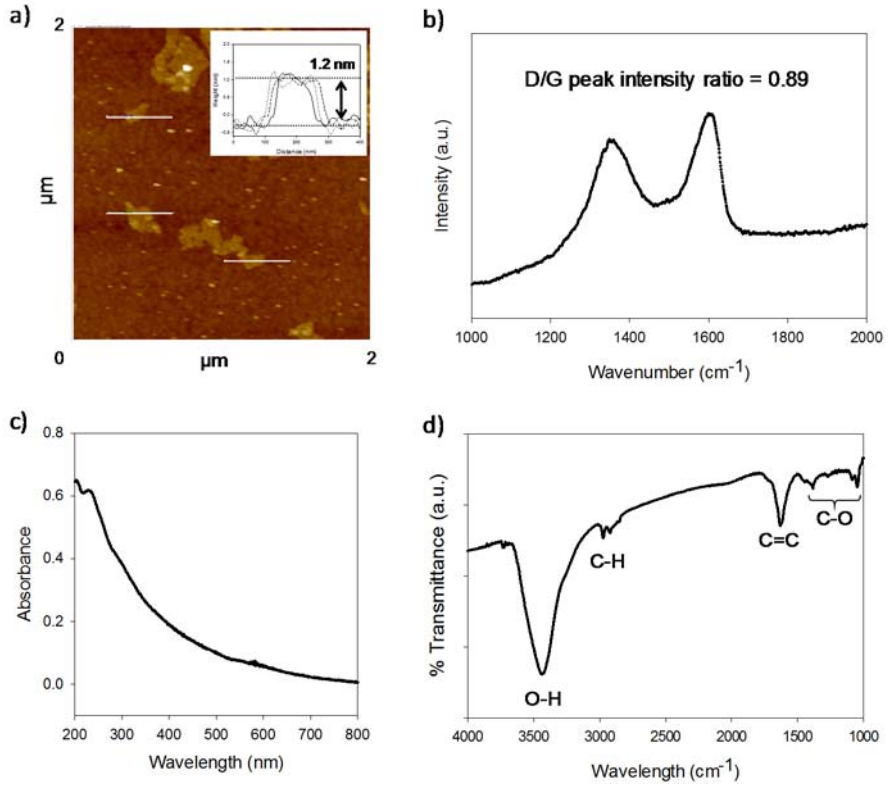

Figure S1. Characterization of GO. (a) AFM image and height profile of GO (inset) showed the dimensions of the prepared GO as 0.2–1 µm in width and ca. 1.2 nm in height. (b) Raman spectrum showed peaks at 1359 and 1609 cm^{-1}, which correspond respectively to the D band related to structural disorder and G band associated with ordered sp^2 carbon domain. (c) The UV-Vis absorption spectrum showed intense absorption at 231 nm corresponding to the π-π* transition of aromatic C=C bonds. (d) The peaks in Fourier transform infrared (FT-IR) spectrum were assigned as various oxygen containing functional groups of GO.

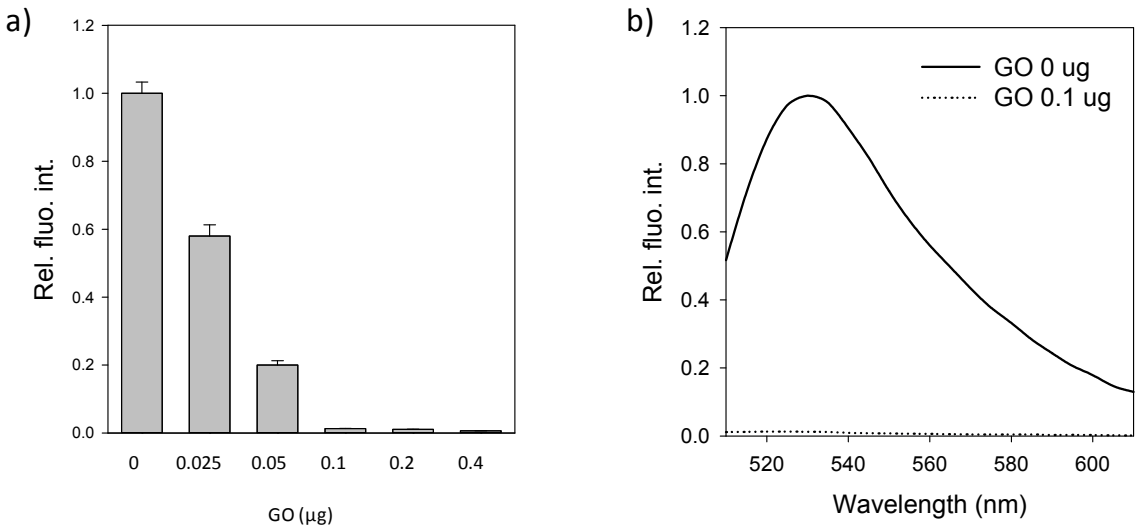

Figure S2. (a) Relative fluorescence intensity and (b) fluorescence emission spectra of FAM conjugated PNA in the presence of GO. More than 97% of the fluorescence intensity of 10 pmol of FAM coujugated PNA was quenched by 0.1 ug of GO.

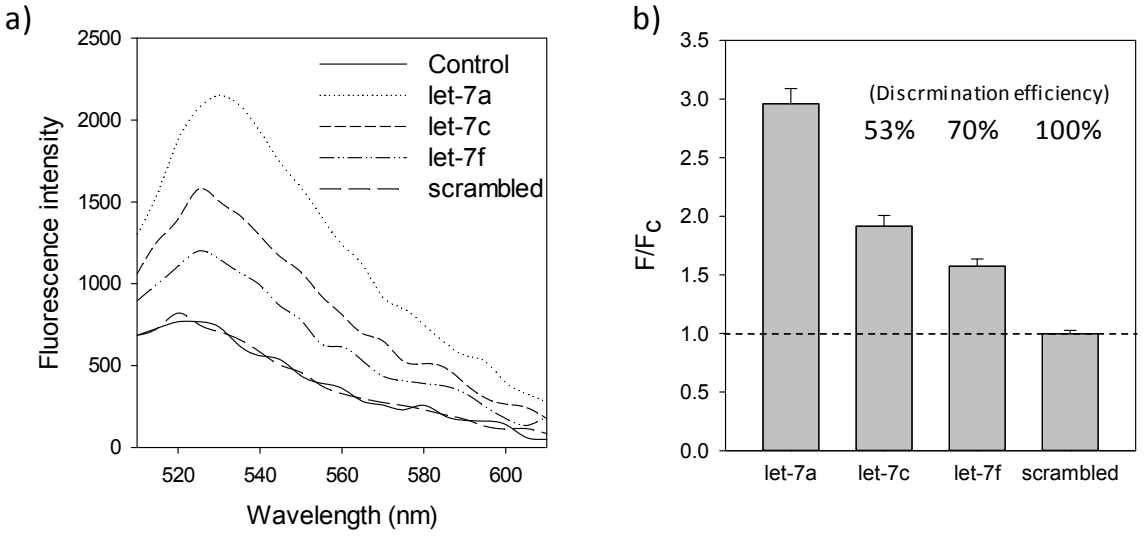

Figure S3. a) Fluorescence spectra and b) relative fluorescence intensity (F/Fc) of FAM labeled let-7a probe/GO complex in the presence of let-7 miRNA family. (F : fluorescence intensity of the complex in the presence of each target. Fc : fluorescence intensity of the complex without any target.)

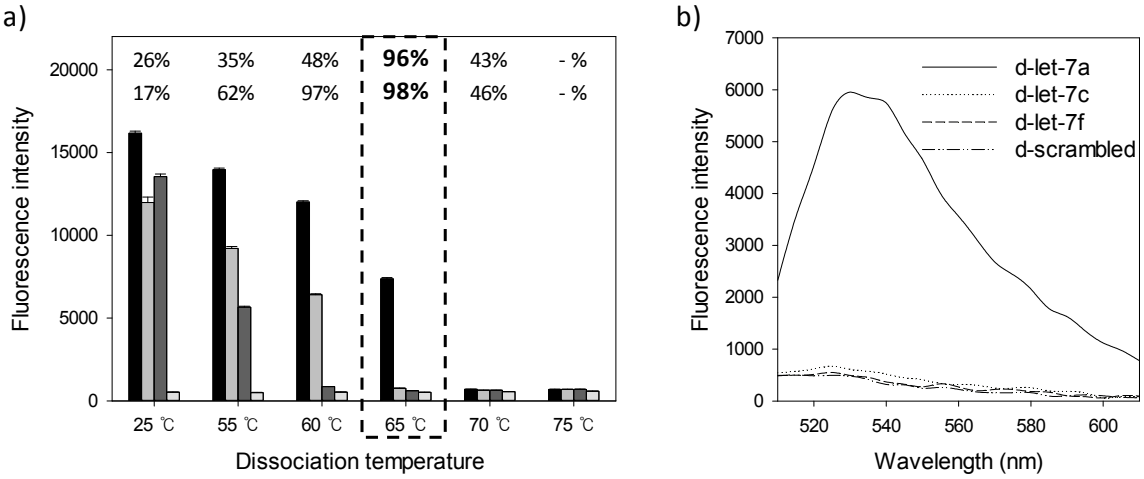

Figure S4. Fluorescence intensity of FAM labeled let-7a PNA probe in the presence of each DNA target of which sequence is corresponding to let-7 miRNA family (d-let-7) after thermal dissociation step in a broad range of temperature in the presence of 0.1 μg of GO. The discrimination efficiencies of let-7a PNA probe against d-let-7c (upper) and d-let-7f (bottom) were presented above each bar graphs. (b) Fluorescence spectra of FAM conjugated PNA with target DNA after termal dissociation at 70 ℃ in the presence of GO.

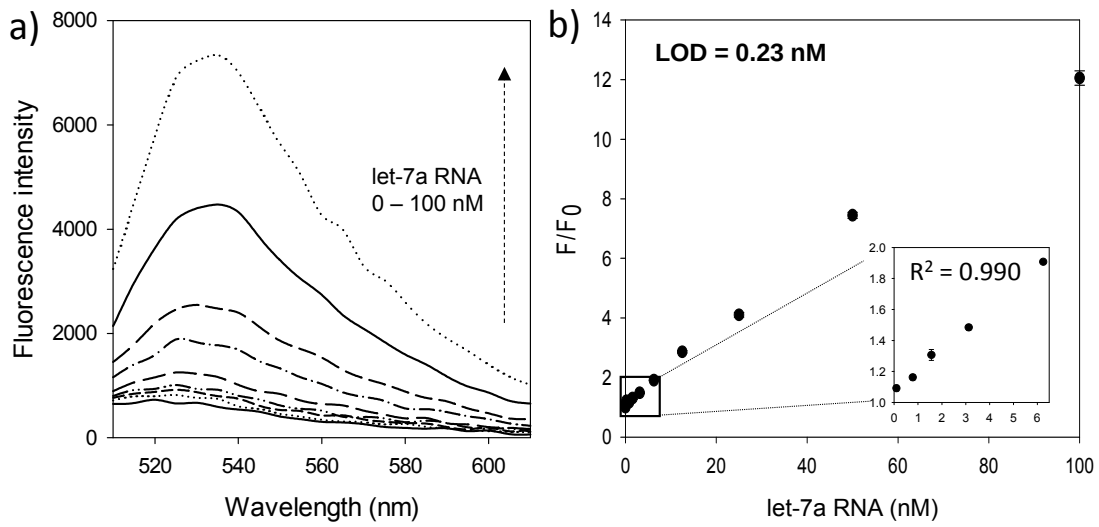

Figure S5. (a) Fluorescence spectra of PNA probe in the presence of various concentrations of let-7 miRNA in buffered solutions. (b) Fluorescence intensity enhancement (F/F0) with wide concentration range of target concentrations (F: fluorescence intensity of PNA probe with target, F0: fluorescence intensity of PNA probe without target).

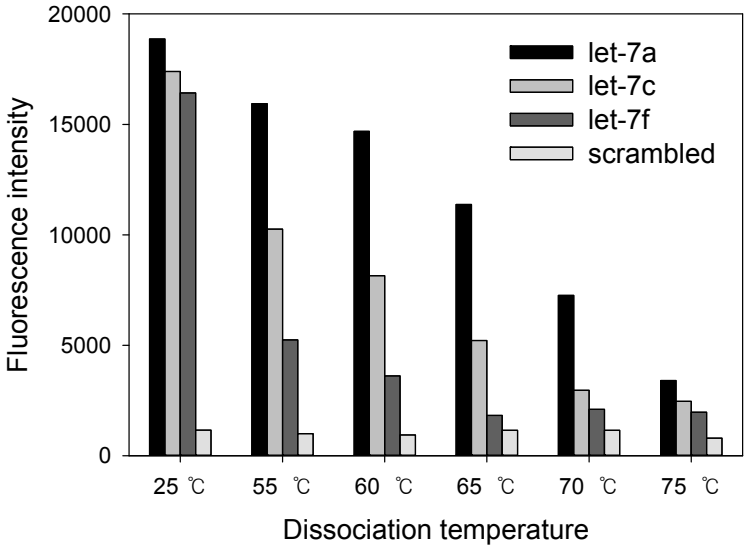

Figure S6. Fluorescence intensity of FAM labeled PNA probe in the presence of each spiked let-7 miRNA in HeLa cell lysate (10000 cells per well) after thermal dissociation step with broad range of temperature in the presence of 0.1 μg of GO. Although the signal of scrambled RNA was almost quenched, perfect matched target could not be discriminated from mismatched target efficiently, even at high temperature under the experimental condition. To successfully discriminate single base mismatches under the condition, the amount of GO was raised to 0.5 μg, as described in the main manuscript.

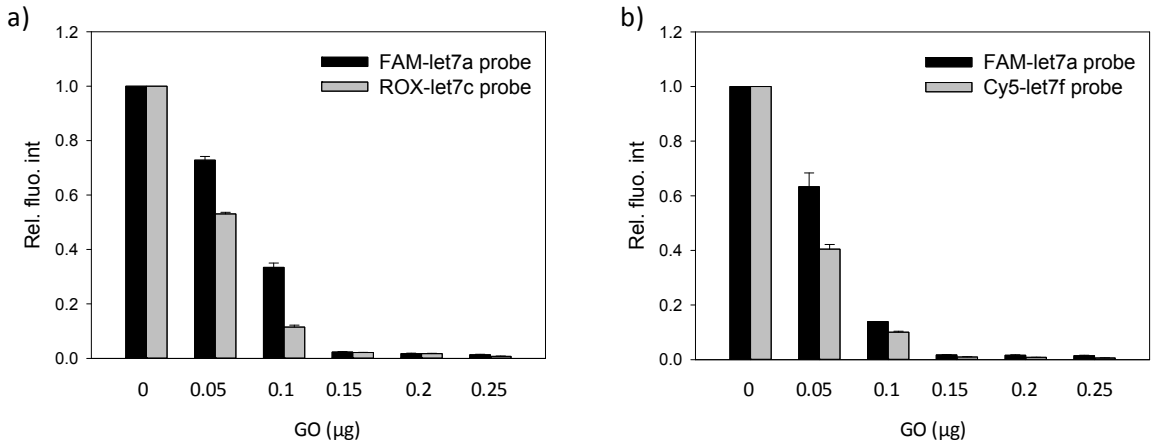

Figure S7. Relative fluorescence intensity of a) FAM-let-7a PNA probe and ROX-let-7c PNA probe, b) FAM-let-7a PNA probe and Cy5-let-7f PNA probe in the presence of various amounts of GO in a mixed solution.

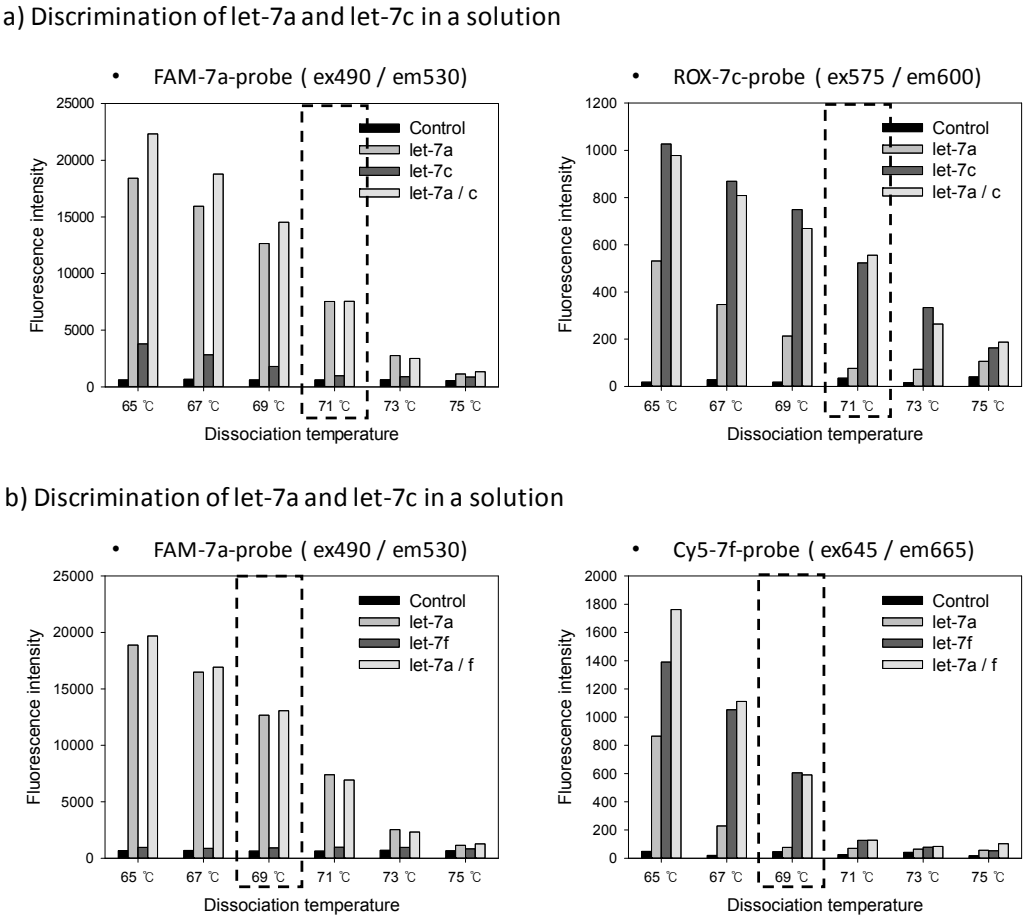

Figure S8. Temperature dependent multiplex detection of a) let-7a and let-7c , b) let-7a and let-7f with each corresponding PNA probe in a solution after thermal dissociation step from 65 ℃ to 75 ℃ in the presence of GO.

Notes on the usage of PNA in SNP detection

PNA is an artificial nucleic acid, possessing higher sequence specificity compared to natural DNA or RNA probes. However, it has hardly replaced DNA probe in general applications of molecular beacon (MB) due to the extremely stable PNA-PNA interaction. Instead, PNA MB is usually designed as stemless form using arrangement of oppositely charged residues at each ends (J. Am. Chem. Soc. 2002, 124, 1097; Appl. Environ. Microbiol. 2003, 69, 5673). In several previous reports using PNA MB, controlling the temperature was also required for highly efficient single mismatch discrimination with low noise (J. Am. Chem. Soc. 2002, 124, 1097; Angew. Chem. Int. Ed. 2008, 47, 9555). Moreover, the combination of hydrophobic PNA strand and charged residues could restrict the length and sequence of probe (J. Am. Chem. Soc. 2002, 124, 1097). In the present work, the combination of minimally modified ssPNA probe and GO enabled discrimination of the base substitution in target possessing any length or sequence without any dis-benefit originated from high stability of PNA. The relatively high temperature applied for dissociation of unstable duplex was caused by the usage of fully complementary PNA and it can be controlled by adjusting the length of PNA probe. Also, fine-tuning of dissociation temperature, as described in the manuscript, was just an additional step to represent the similar sensing sensitivity between two targets possessing only one base difference in multiplex detection.