Supporting Information

A Simple Fluorometric Assay for DNA Exonuclease Activity Based on Graphene Oxide

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Materials

Natural graphite (FP 99.95%) was purchased from Graphit Kropfmühl AG (Hauzenberg, Germany). Sulfuric acid (H_2SO_4) was purchased from Samchun chemical (Seoul, Korea). Sodium nitrate ($NaNO_3$) and hydrogen peroxide (30% in water) (H_2O_2) were purchased from Junsei (Japan). Potassium permanganate ($KMnO_4$) and aurintricarboxylic acid (ATA) were purchased from Sigma-Aldrich (MO, USA). Fluorescent dye labelled hairpin DNAs for Exo III substrate (5' FAM— GGCCGAGC TTTTTT GCTCGGCC – 3') was purchased from Genotech (Daejon, Korea). Exonuclease III (Exo III) was purchased from New England BioLabs (MA, UK). Ethylenediamine tetraacetic acid (EDTA) was purchased from BIO-RAD (CA, USA). Fluorescence intensity was measured by fluorometer SynergyMx (Biotek, UK)

Preparation of graphene oxide

Graphene oxide (GO) was prepared according to modified Hummers method (Cote et al., 2009). 0.5 g of natural graphite, 0.5 g of $NaNO_3$, and 23 mL of H_2SO_4 were mixed under vigorous stirring in an ice bath. Then, 3 g of $KMnO_4$ was slowly added. After the addition, the mixed solution was transferred to 35 ^oC water bath and incubated for one hour with stirring. Next, 40 mL of distilled water was added and the bath temperature was increased up to 90 ^oC for 30 min. Another 100 mL of distilled water was then added. Next, dropwise addition of 3 mL of 30% H_2O_2 changed the color of the solution from dark brown to yellow. Synthesized GO solution was filtered using a Buchner funnel and washed with copious amount of distilled water. The filter cake was dried in a desiccator and redispersed in distilled water.

Exo III activity assay by GO based platform

To prepare hairpin structured DNA substrate of Exo III, 2.5 μL of 100 μM fluorescent dye labeled DNA in pH 8.0 buffer containing 50 mM Tris-HCl and 50 mM NaCl was self-annealed by heating to 90 ^oC for 5 min and followed by slow cooling at room temperature for 1 hr. For Exo III activity assay, reaction mixture was prepared by mixing Exo III enzyme stock with 200 nM annealed DNA in 1X Exo III reaction buffer (pH 7.0, 10 mM Bis-Tris Propane, 10 mM $MgCl_2$, and 1 mM dithiothreitol). After incubation at room temperature for designated time, 30 μL of reaction mixture and 30 μL of 11 μg/ml GO solution were mixed in a 96-well plate. Fluorescence intensity was measured at 520 nm.

Exo III inhibition assay by GO based plarform

For EDTA-Exo III inhibition assay, EDTA solutions with various concentrations were mixed with 200 nM DNA in 1X Exo III reaction buffer followed by addition of 1 Unit of Exo III. After incubation at room temperature for 30min, each 30 μL of reaction mixtures were mixed with 30 μL of 11 μg/ml GO solutions in a 96-well plate. In case of ATA-Exo III, ATA solutions with various concentrations were mixed with 200 nM DNA in 1X Exo III reaction buffer and followed by addition of 1 Unit of Exo III. After incubation at room temperature for 1hr, each 30 μL of reaction mi xtures were mixed with 30 μL of 11 μg/ml GO solutions in a 96-well plate. Fluorescence intensity was measured at 520 nm.

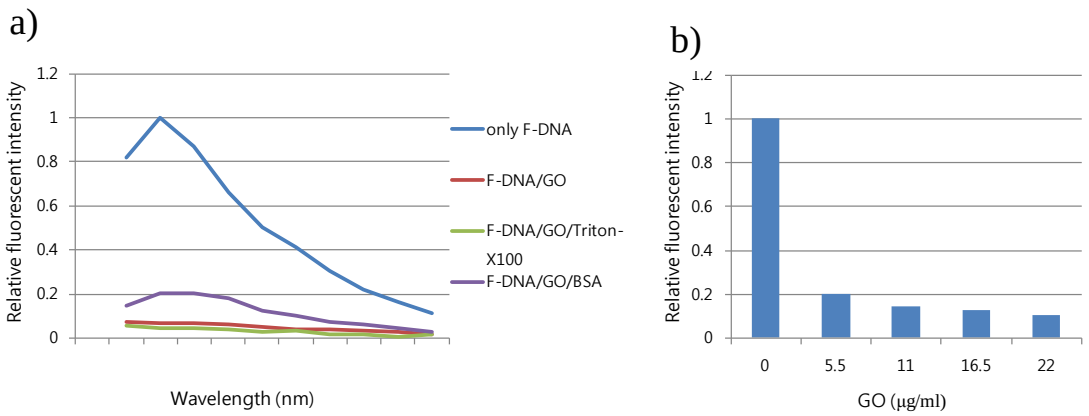

Figure S1. To show the effect of proteins and detergents on the assay, 0.1% BSA and 0.1% Triton X were added in the mixture of FAM-ssDNA and GO. a) Fluorescence spectra of FAM-ssDNA (13mer, FAM – AGT ATG ATA TCC A) with or without GO, 0.1% BSA and 0.1% Triton X. b) Relative fluorescence intensity of FAM-ssDNA/GO complex in the enzyme reaction buffer containing 0.1% BSA can be tuned by changing the concentration of GO .