Electronic Supplementary Information

Fluorescence-based detection of single-nucleotide changes in RNA using graphene oxide and DNAzyme

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Experimental details

Oligonucleotides and probe DNA. RNA oligonucleotides used in this study (wild type ABL RNA, T315I mutant ABL RNA, and ABL fragment RNA; sequences are shown in Table S1) were chemically synthesized and purified by HPLC and polyacrylamide gel electrophoresis (ST Pharm Co. Ltd. Seoul, Korea). DNA oligonucleotides (T315I DNAzyme and FITC-labeled probe DNA; sequences are shown in Table S1) were chemically synthesized and purchased (CosmoGenetech, Seoul, Korea).

RNA cleavage with DNAzyme and gel electrophoresis. The RNA cleavage reaction with DNAzyme was performed in reaction buffer (180 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.5). The cleavage reaction was initiated by adding DNAzyme (1 μM) to a reaction mixture containing substrate RNA (1 μM) that was preheated at 85 °C for 3 min and cooled to room temperature. The reaction mixture (15 μL) was incubated at 25 °C for 2 h, and then 1 μL of DNase I (5 U/μL, purchased from Takara, Tokyo, Japan) was added to degrade the DNAzyme and further incubated at 37 °C for 30 min. The reaction was quenched by adding 1.2 μL of Na_2EDTA (final concentration to 10 mM), and the oligonucleotides were fractionated by ethanol precipitation. The precipitated RNAs were dissolved with 10 μL of double-distilled water, and the sample was mixed with an equal volume of gel-loading dye containing 25 mM Na_2EDTA and 8 M urea. The cleavage products were resolved by 10% (w/v) denaturing polyacrylamide gel electrophoresis, and RNA product bands were visualized with SYBR Gold (Molecular Probes, Life technologies, Eugene, USA) (Fig. 1a).

Graphene oxide and atomic force microscopy (AFM). Graphene oxide (GO) that was prepared by Hummer's method was purchased from the vendor (cat#. SKU-HCGO-W-175,

Graphene Supermarket, Graphene Laboratories, Inc., Ronkonkoma, NY, USA). The AFM images were collected using an atomic force microscope with a NanoScope V controller (Model: Bruker Multimode 8, Bruker AXS Inc., Madison, WI, USA) at room temperature in tapping mode with spring constant of 40 N/m and tip radius of ≤8 nm. 10 μL of the graphene oxide solution (10 μg/mL) was used and then was placed on freshly cleaned silicon wafer washed by the piranha cleaning method. The sample was dried at room temperature. The scanning speed was at a line frequency of 1.0 Hz, and the original images were sampled at a resolution of 256 × 256 pixels.

Fluorescence measurement in the DNAzyme reaction with T315I mutant and wild-type ABL RNA. The same RNA-cleavage reaction (1 μM DNAzyme and 1 μM RNA) was conducted as described in the reaction mixture (216 μL). After the reaction was quenched by adding 24 μL of 100 mM Na_2EDTA, 30 μL of 10 μM FITC-labeled probe DNA (F-DNA) and 30 μL of annealing buffer (200 mM NaCl and 200 mM Tris-HCl, pH 7.5) were added to the reaction. RNA cleavage products and F-DNA were hybridized by heating at 85 °C for 3 min and slowly cooling to room temperature for 20 min. Then, 300 μL of RNA/F-DNA hybrid reaction mixture was added to a solution containing GO (50 μg/mL, final concentration) and distilled water to make up a total volume of 1 mL. After further incubation at 25 °C for 20 min, the emission spectra of the mixture (1 mL) were measured in the wavelength range of 500-600 nm upon excitation at 495 nm by using a spectrofluorophotometer (model RF-5301PC; Shimadzu Inc., Kyoto, Japan) (Fig. 1b).

GO-based fluorescence quenching assay in a 96-well plate. For the fluorescence quantification in the 96-well plate, the RNA cleavage reaction with DNAzyme was

performed in a small volume (9.8 μL). The reaction mixtures containing 1 μM DNAzyme and 1 μM RNA in the reaction buffer were incubated at 25 ˚C for 2 h. For control experiments, DNAzyme and/or the RNA substrate was excluded from the reaction mixture (reactions #3-#7 in Fig. 2a). RNA fragments were added to the reaction mixture rather than the RNA substrate (reaction #4 and #7). After 2 h of incubation, 1 μL of DNase I (5 U/μL) was added to degrade the DNAzyme and further incubated at 37 ˚C for 30 min. After the reaction was quenched by addition of 1.2 μL of 100 mM Na_2EDTA, 1.5 μL of 10 μM FITC-labeled probe DNA (F-DNA) and 1.5 μL of annealing buffer (200 mM NaCl and 200 mM Tris-HCl, pH 7.5) were added to the reaction. In the control experiments, 1.5 μL of DNAzyme (final 1 μM) was added to the F-DNA hybridization mixture (reactions #4 and #5). RNA cleavage products and F-DNA were hybridized by heating at 85 ˚C for 3 min and slowly cooling to room temperature for 20 min. Then, 15 μL of the RNA/F-DNA hybrid reaction mixture was transferred to each well containing 35 μL of GO solution (GO; 50 μg/mL, final concentration) and distilled water in the 96-well plate. After incubation at 25 ˚C for 20 min, the FITC fluorescence was measured using a multilabel plate reader (VICTOR X3; PerkinElmer, Waltham, MA, USA) with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Fluorescence images of the reaction in a black 96-well plate (SPL Life Sciences, Gyeonggi-do, Korea) were obtained using a fluorescent imaging system (IVIS-Lumina II; Caliper Life Sciences, Hopkinton, MA, USA) (Fig. 2b)

Optimization of GO concentration for maximal difference in fluorescence. The RNA cleavage reaction with the DNAzyme and each RNA substrate (wild-type ABL RNA and T315I mutant ABL RNA) was performed as described in the multi-well plate setup. The reaction was subsequently quenched and subjected to F-DNA hybridization as described

above. Then, 15 μL of the RNA/F-DNA hybrid reaction mixture was supplemented with a solution containing GO (0, 1, 3, 5, 10, 20, 30, 50, and 100 μg/mL, final concentration) and distilled water in a total volume of 50 μL. After further incubation at 25 ˚C for 20 min, the fluorescence was measured by using a multilabel plate reader (VICTOR X3) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm (Fig. 1c).

Selectivity and sensitivity of GO-based fluorescence quenching assay. To assess the selectivity of the GO-based mutant RNA detection, the RNA cleavage reaction with the DNAzyme (1 μM) and RNA substrate mixture (1 μM) with different mixing ratios of wild-type ABL RNA and mutant T315I ABL RNA (Fig. 3a) was performed as described in the multi-well plate setup. The sensitivity of the GO-based mutant RNA detection method was also examined by performing the same RNA cleavage reaction (1 ml) with the DNAzyme (1 μM) and increasing concentrations of RNA substrates (1.5 to 15 nM wild-type ABL RNA and mutant T315I ABL RNA; Fig. 3b), as described in the multi-well plate setup. The aliquot of RNA cleavage reaction (12 μL) was subsequently quenched and subjected to F-DNA hybridization as described above, and the RNA/F-DNA hybrid reaction mixture (15 μL) was supplemented with a solution containing GO (50 μg/mL, final concentration) and distilled water in a total volume of 50 μL. After incubation at 25 ˚C for 20 min, fluorescence images of the reaction in a black 96-well plate were obtained using a fluorescent imaging system (IVIS-Lumina II), and the fluorescence was measured by using a multilabel plate reader (VICTOR X3) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm (Fig. 3). The RNA cleavage reaction was also prepared to contain the DNAzyme (1 μM) and various concentrations of mutant RNA (1.5 to 15 nM) in the presence of a defined amount (15 nM) of wild-type ABL RNA (Fig. S3).

Table S1. Oligonucleotides used in this study.

Oligonucleotide (nts)	Sequence (from 5′ to 3′)
Wild-type ABL RNA (45)	GGA GCC CCC GUU CUA UAU CAU CA**C** UGA GUU CAU GAC CUA CGG GUU
T315I mutant ABL RNA(45)	GGA GCC CCC GUU CUA UAU CAU CA**U** UGA GUU CAU GAC CUA CGG GUU
ABL fragment RNA (23)	GGA GCC CCC GUU CUA UAU CAU CA
FITC-DNA (23)	FITC – TGA TGA TAT AGA ACG GGG GCT CC
T315I DNAzyme (36)	CAT GAA CTC AAG GCT AGC TAC AAC GAG ATG ATA TAG

*Underlined nucleotide represents a single base difference in ABL RNA.

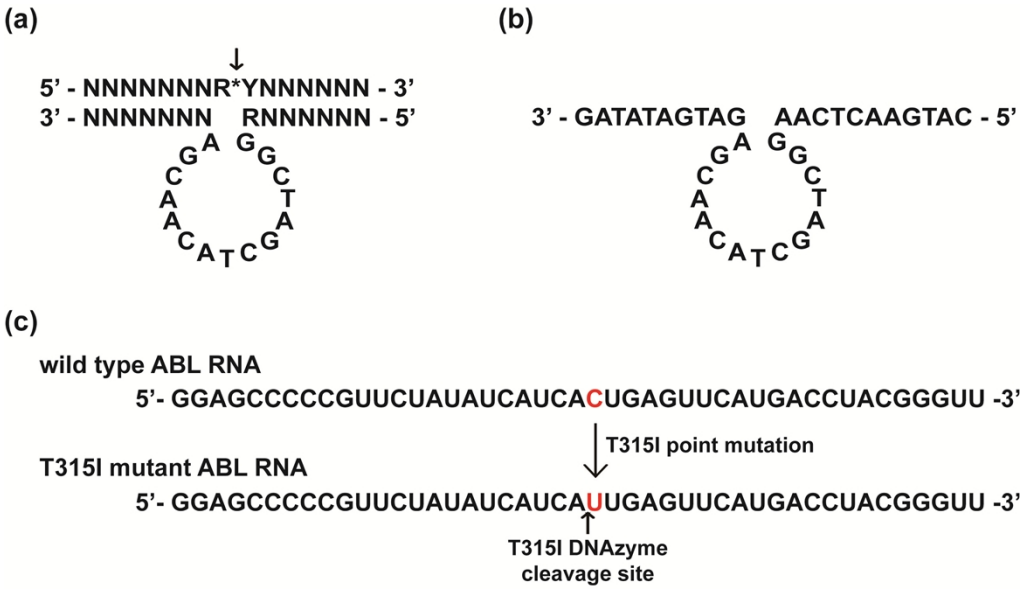

Fig. S1. Sequences of DNAzyme and substrate RNAs. (a) General sequence of the RNA-cleaving deoxyribozyme (DNAzyme). DNAzyme recognizes RNA (upper strand) through Watson-crick base pairing and cleaves its target at a phosphodiester bond located between an unpaired purine (R) and pyrimidine (Y). N represents a random base. (b) Sequence of T315I DNAzyme for cleavage of T315I mutant ABL RNA. (c) Sequences of wild-type ABL RNA and T315I mutant ABL RNA. T315I mutant ABL RNA was generated by replacing threonine with isoleucine through a single base change (C to U). The arrow indicates the T315I

DNAzyme cleavage site in RNA.

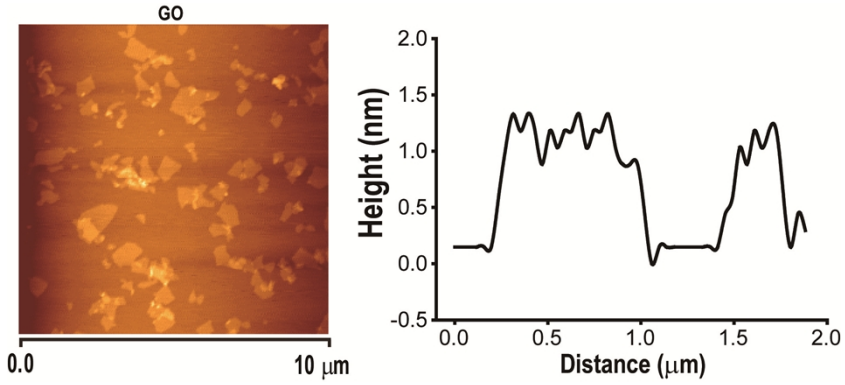

Fig. S2. Atomic force microscopic (AFM) analysis of GO. The average thickness was estimated to be 0.684 nm for the GO sheet, which corresponds to one atomic layer.

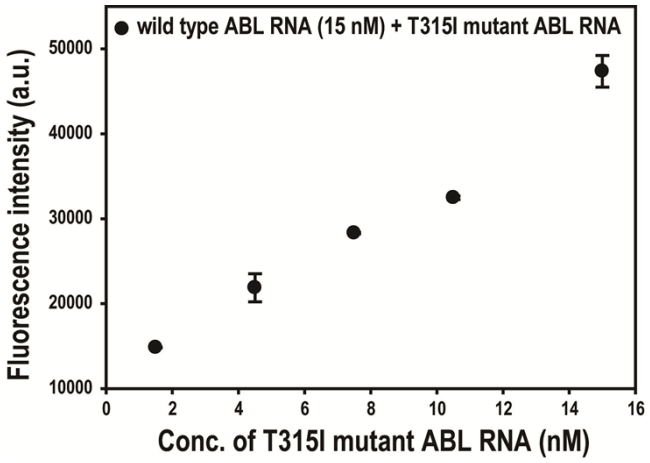

Fig. S3. Fluorescence analysis of the RNA cleavage reaction mixture containing 15 nM of wild-type ABL RNA and various concentrations of T315I mutant ABL RNA. The fluorescence of the mixture after addition of GO in the 96-well plate was measured using a multilabel plate reader.