Electronic Supplementary Material (ESI) for Analytical Methods.


Supporting Information

# Isothermal Control of Probe-Target Interactions Using Graphene Oxide and Nuclease for Highly Sensitive and Specific Detection of DNA

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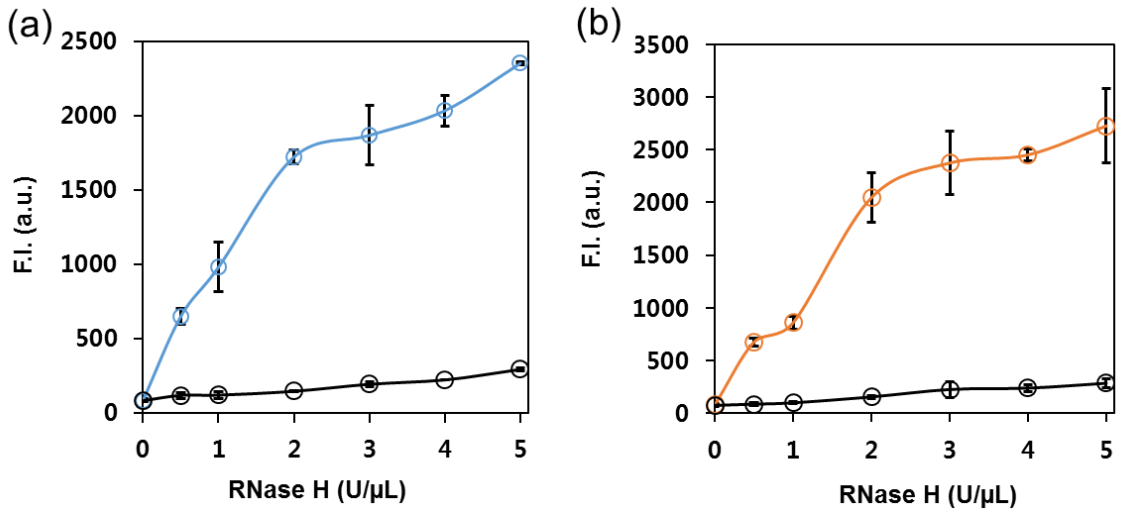


**Figure S1.** Optimization of RNase H. (A) and (B); Fluorescence intensity of the reacted sample at 520 nm with 100 nM of RNA probes, 100 nM of target DNA, and 10 μg/mL of GO depending on the concentration of RH, performed according to methods A and B, respectively.

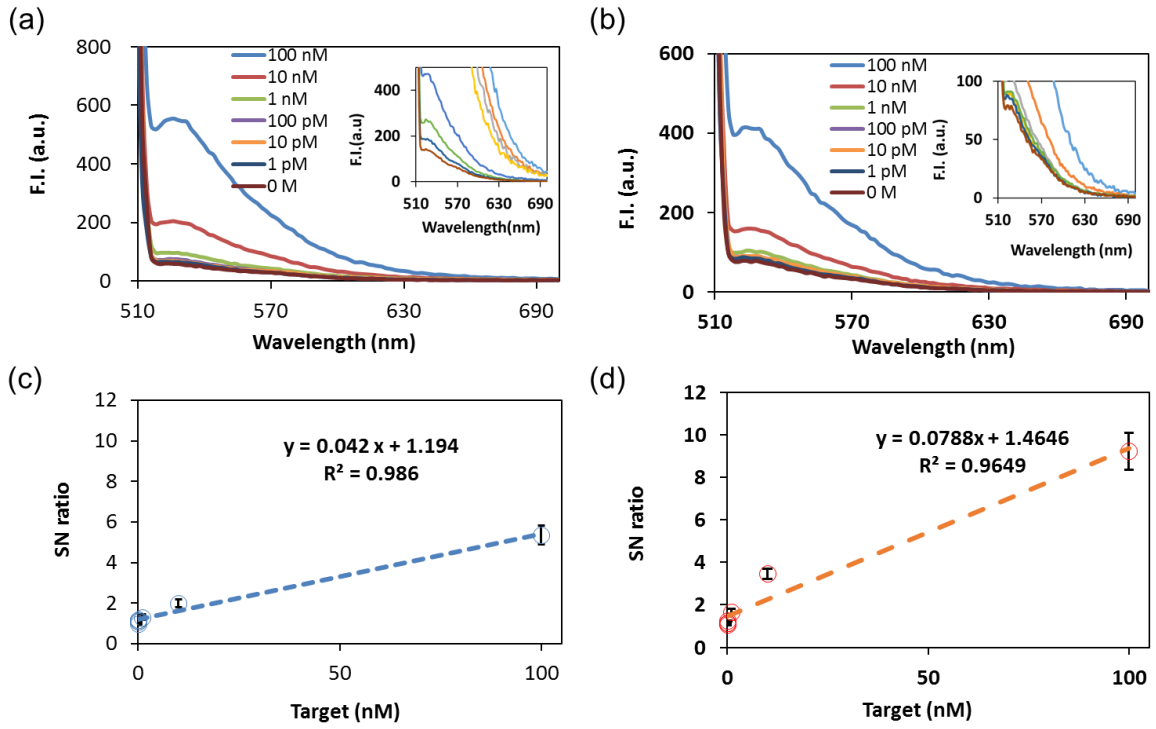


**Figure S2.** DNA target detecting sensitivity of the proposed assays in the absence of RH. (a)/(c) and (b)/(d): Fluorescence spectra/SN ratio of the reacted samples with different amounts of target DNA (0 to 100 nM) and optimized amounts of GO in the absence of RH according to methods A and B, respectively. Fluorescence intensity of each sample at 520 nm was used for determining the SN ratio. Inset in each figure is enlarged image of the fluorescence spectra.

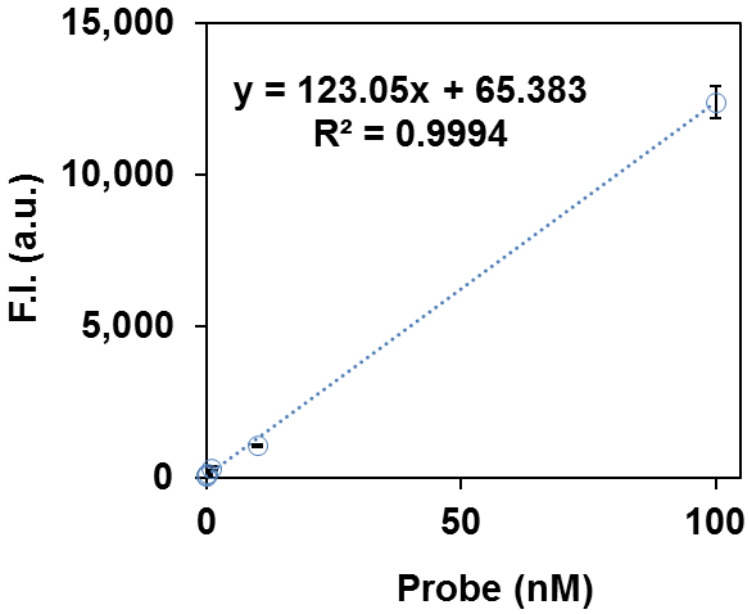


**Figure S3.** Standard curve of fluorescence intensity for RNA probes

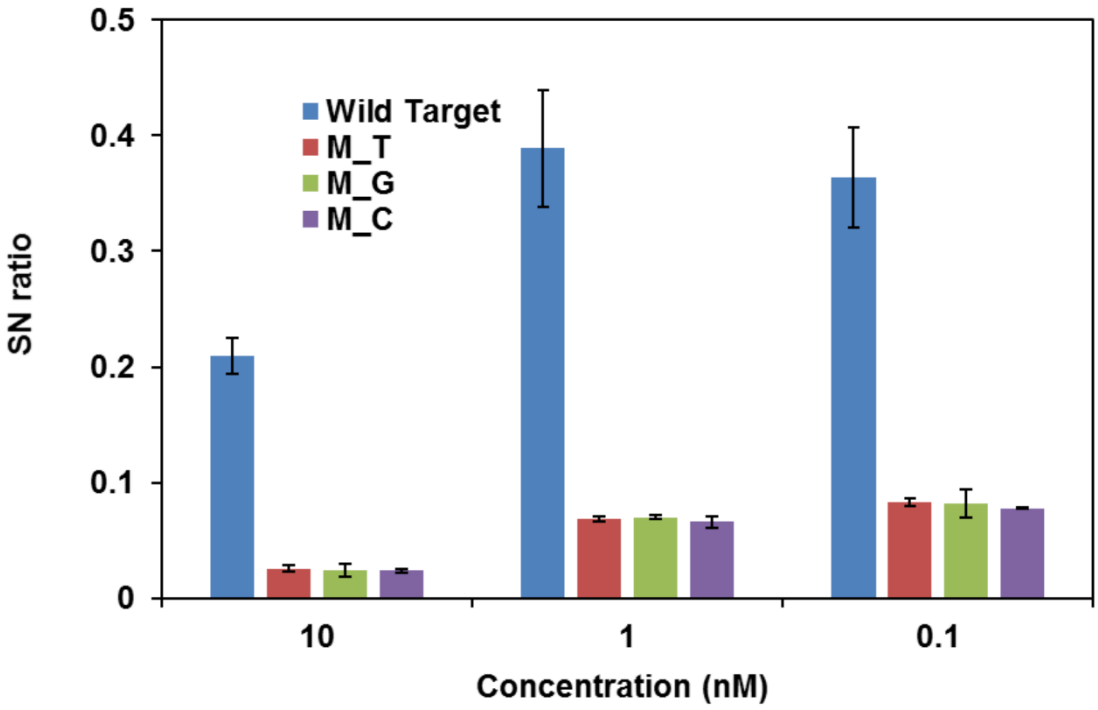


**Figure S4.** Relative SN ratio of the RNA probes in the presence of the fully matched DNA (wild target) and 3 single-base mismatched DNAs, T (M_T), G(M_G) and C(M_C). Relative SN ratio was obtained by dividing the SN ratio of the reacted samples according to method A by SN ratio of the reacted samples according to method B.

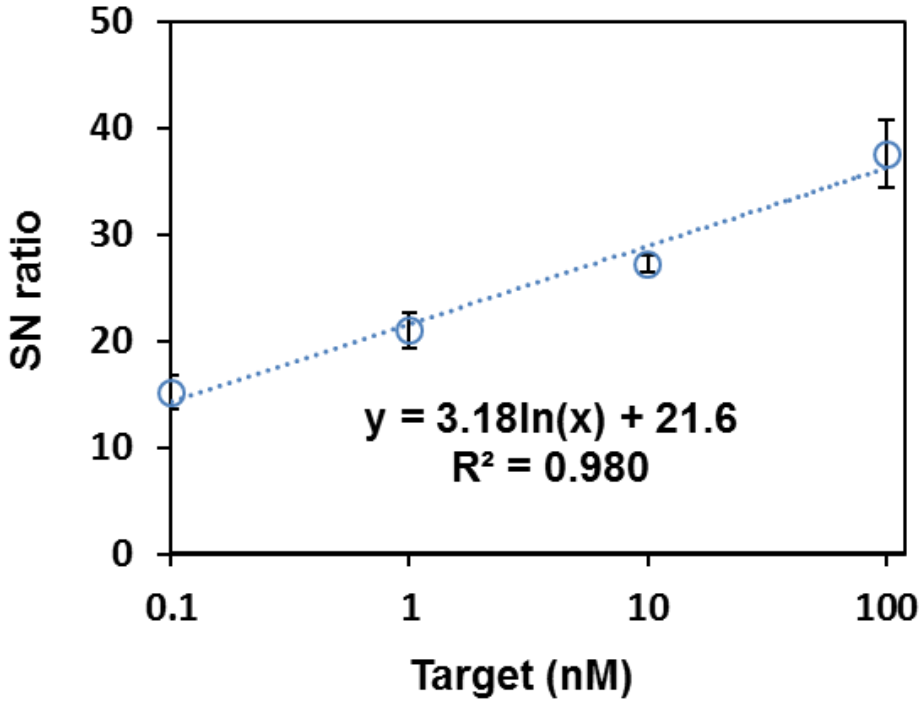


**Figure S5.** SN ratio of the reacted samples with different amounts of target DNA in presence of RH according to method A for target DNA between 0.1 to 100 nM

**Table S1.** Sequences of oligonucleotides used in the study

| *Name* | *Sequence* |
|---|---|
| Probe(RNA) | 5'- rArArG rArArG rArUrG rArGrG rCrArU rArGrC rArGrC rArGrG rArU -Fluorescein-3' |
| Wilde Target | 5'ATC CTG CTG CTA TGC CTC ATC TTC TT-3' |
| NCD | 5'- TCC GTC GCT ACC GTG CCA AAG CC -3' |
| M_A | 5'- ATC CTG CTG CTA AGC CTC ATC TTC TT-3' |
| M_G | 5'- ATC CTG CTG CTA GGC CTC ATC TTC TT -3' |
| M_C | 5'- ATC CTG CTG CTA CGC CTC ATC TTC TT -3' |

r of the sequences in Probe indicates ribonucleotide. Mismatched sequence was underlined

**Table S2.** Fluorescence quenching efficiency of GO and partial recovery of the fluorescence upon addition of the target DNA

| *Sample* | *Fluorescence intensity at 520nm (a.u.)* | *Recovered fluorescence (%)* | *Quenching efficiency (%)* |
|---|---|---|---|
| w/o GO | 21773 | - | - |
| w/o target w/ GO | 131.67 | - | 99.39 |
| w/ target w/ GO | 3278 | 15.1 | - |

Quenching efficiency by GO and recovered fluorescence was calculated using following equations, $Q = (F_0 - F)/F_0 \times 100$ and $R = F/F_0 \times 100$, respectively, where, Q is quenching efficiency, R is recovered efficiency, F is Fluorescence intensity of RNA probe in the presence of GO, $F_0$ is: Fluorescence intensity of RNA probe in the absence of GO, and $F_t$ is Fluorescence intensity of RNA probe with target in the presence of GO

**Table S3.** The number of degraded RNA probes per single target depending on the concentration of RH for methods A and B, respectively

| Target (nM) | 10 | 1 | 0.1 | 0.01 | 0.001 | 0.0001 |
|---|---|---|---|---|---|---|
| Method A | 7 | 27 | 208 | 151 | 1013 | 8420 |
| Method B | 3 | 23 | 165 | 317 | 1546 | 9725 |

The number of degraded RNA probes was estimated using the standard curve of Fig. S3 and measuring fluorescent intensity of supernatant collected after centrifugation of the reacted sample

**Table S4.** Recovery test for target DNA in human serum

| ***Added DNA*** | ***Type*** | ***Detected DNA*** [a] | ***Recovery (%)*** |
|---|---|---|---|
| 10 nM | Wild | 9. 2 ± 1.01 nM | 92 |
| | M_T | 0 | 0 |
| | M_G | 0 | 0 |
| | M_C | 0 | 0 |
| 1 nM | Wild | 1.1 ± 0.1 nM | 110 |
| | M_T | 0 | 0 |
| | M_G | 0 | 0 |
| | M_C | 0 | 0 |
| 100 pM | Wild | 83 ± 10 pM | 83 |
| | M_T | 0 | 0 |
| | M_G | 0 | 0 |
| | M_C | 0 | 0 |
| 10 pM | Wild | 10. 6 ±1.1 pM | 106 |
| | M_T | 0 | 0 |
| | M_G | 0 | 0 |
| | M_C | 0 | 0 |
| 1 pM | Wild | 1.1±0.1pM | 110 |
| | M_T | 0 | 0 |
| | M_G | 0 | 0 |
| | M_C | 0 | 0 |

[a] Concentration of DNA which has SN ratio exceeding 15 and lower than 15 was calculated using the equation on Figure S5 and the equation on inset of Figure 5 (c) in respectively. In case of a real sample, of which concentration of DNA is unknown, concentration of DNA which has SN ratio around 15 can be calculated by diluting the sample and measuring SNR again. Since SNRs of the mutated DNAs isolated from the DNA spiked serum were around 1, the concentration of the 4 kinds of the spiked mutant DNA was estimated to be 0 regardless of the amount of DNA added to the serum