Electronic supplementary information

Interaction of single-stranded DNA with graphene oxide: fluorescence study and its application for S1 nuclease detection

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1. Supporting Experimental Section

1.1. Optimization of the concentration of GO

The purchased GO was sonicated in Milli-Q purified water for 5 h to give a homogeneous black solution and stored at 4 ºC for use.

To optimize the concentration of GO, 2 μL of the ssDNA stock solution (10 μM), was diluted with CH_3COONa-CH_3COOH buffer (pH 4.5) to 20 μL. Then 0, 5, 10, 15, 20, 25, 30, 35 and 40 μL GO solution (100 μg/mL) as prepared was added into the above solution and diluted with Tris-HCl buffer to 500 μL and incubated for 10 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

1.2. Optimization of the reaction time between 20F and GO, as well as 20F and S1 nuclease

To optimize the reaction time between 20F and GO, 2 μL of the ssDNA stock solution (10 μM), was diluted with CH_3COONa-CH_3COOH buffer (pH 4.5) to 20 μL. Then 30 μL GO solution (100 μg/mL) as prepared was added into the above solution and diluted with Tris-HCl buffer to 500 μL and incubated for 0, 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

To optimize the reaction time between 20F and S1 nuclease, 2 μL of the ssDNA stock solution (10 μM), and 0.02 units/mL of S1 nuclease solution were mixed, the mixed solution was diluted with CH_3COONa-CH_3COOH buffer (pH 4.5) to 20 μL. The above prepared solution was incubated for 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50,

55 and 60 min at 37 ºC. Then 30 μL GO solution (100 μg/mL) as prepared was added to the solution, the mixed solution was diluted with Tris-HCl (pH 7.4) buffer to 500 μL. The above prepared solution was incubated for 10 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

2. Supporting Discussion

2.1. Effect of the concentration of GO

To achieve the best sensing performance, the concentration of GO was optimized. As shown in Figure S3, fluorescence intensity of 20F decreases sharply as the concentration of GO increases from 0 to 8 μg/mL. When GO concentration is up to 6 μg/mL or larger, the fluorescence intensity of FAM is quenched down to 4% of original fluorescence signal. As a result, 6 μg/mL was taken as the optimized concentration for GO.

2.2. Effect of reaction time

After optimizing the concentration of GO, we are in the position to unravel the optimal reaction time between 20F and GO, and the optimal reaction time between 20F and S1 nuclease. Figure S4A shows the fluorescence quenching of 20F in the presence of GO as a function of incubation time. 20F adsorption on the surface of GO is very fast at room temperature. The process of adsorption reaches equilibrium in 5 min. Figure S4B shows fluorescence intensity versus different reaction time of 20F (40 nM) with S1 nuclease (0.02 units/mL). It indicates that the reaction of 20F with S1 nuclease becomes slower and is completed in about 30 min at 37 ºC. So we introduced GO into the sensing solution after the system had reacted for 30 min at 37 ºC.

2.3. Effect of adding order between S1 nuclease and GO

In order to achieve the best sensing performance, we then investigate the influence on the final results caused by the adding order of S1 nuclease and GO. Figure S5 shows the result of influence on the final results caused by the adding order

of S1 nuclease and GO. Figure S5, curve a shows that considerably high fluorescence signal of the system appears when we first mixed the 20F with 0.02 units/mL S1 nuclease, the mixed solution was incubated for 30 min at 37 ºC. Then 6 μg/mL GO was added and the solution was incubated for 10 min at room temperature. In contrast, in Figure S5, curve b, which shows the result of the reversed adding order of S1 nuclease and GO of Figure S5, curve a, only exhibits very low fluorescence signal. Therefore the adding order of S1 nuclease and GO has significant influence on the final results. This phenomenon can be explained by the properties of GO which can protect DNA against enzymatic cleavage and intracellular stability of DNA compared with free DNA probes[1,2]. As a result, to achieve the sensing performance of S1 nuclease detection, S1 nuclease should be added before the addition of GO.

2.4. Specificity

The specificity of this method for S1 nuclease was investigated by testing the response of the assay to other nucleases (Exo I, MNase, DNase I and Exo Ⅲ). Under the same conditions as S1 nuclease, the fluorescence intensity of this GO-based biosensor changed less for Exo I, MNase, DNase I and Exo Ⅲ, while a significant fluorescence increase was observed for S1 nuclease (shown in SI. Fig. S6). The observation indicates the specificity of the GO-based biosensor for S1 nuclease testing.

2.5. Inhibitor detection

The cleavage reaction of DNA with nuclease can be prohibited when the nuclease inhibitor is present. ATP is known to be an inhibitor of S1 nuclease.[3] In the

presence of ATP, the fluorescence intensity of the biosensor dramaticly increases with the increasing concentration of S1 nuclease (shown in SI. Fig. S7). The control experiments indicated that ATP itself could not result in the fluorescence intensity change (shown in SI. Fig. S8). This result is fully in accordance with the fact that ATP is known as an inhibitor of S1 nuclease.

3. Supporting Figures 1-8 with Legends

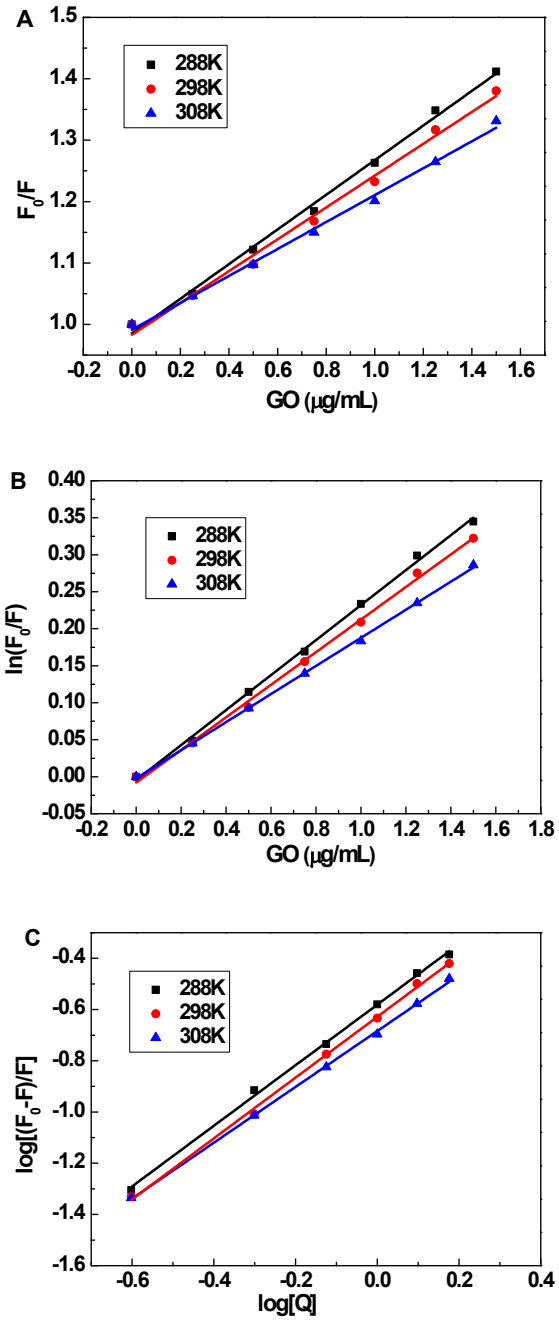

Fig. S1 (A) Stern-Volmer plot for the binding of 10F with GO at 288K, 298K and 308K; (B) Perrin plot for the binding of 10F with GO at 288K, 298K and 308K; (C) Plots of log[(F_0 −F)/F] vs. log([Q] for the binding of 10F with GO at 288K, 298K and 308K.

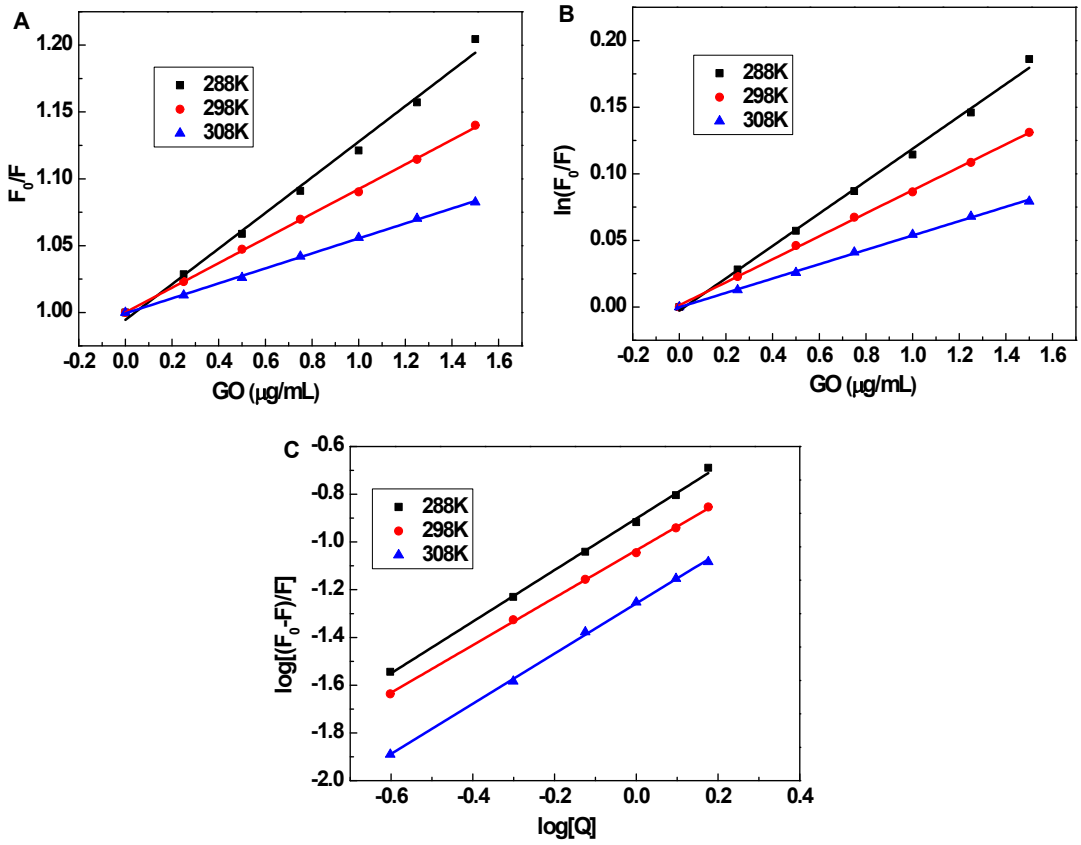

Fig. S2 (A) Stern-Volmer plot for the binding of 5F with GO at 288K, 298K and 308K; (B) Perrin plot for the binding of 5F with GO at 288K, 298K and 308K; (C) Plots of $\log[(F_0 - F)/F]$ vs. log([Q] for the binding of 5F with GO at 288K, 298K and 308K.

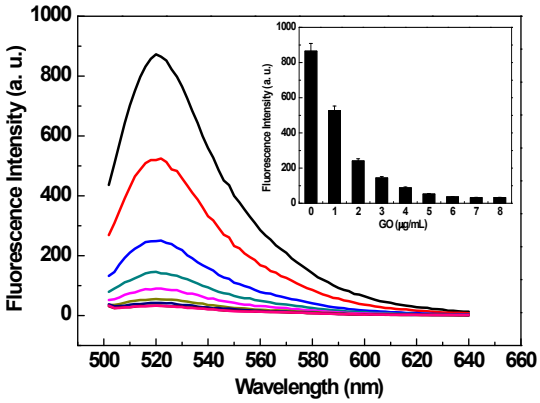

Fig. S3 Fluorescence emission spectra of 20F upon the addition of different concentrations of GO. Inset: fluorescence intensity versus concentration of GO. Concentration: 20F, 40 nM; GO, 0, 1, 2, 3, 4, 5, 6, 7, 8 μg/mL. Excitation: 480 nm.

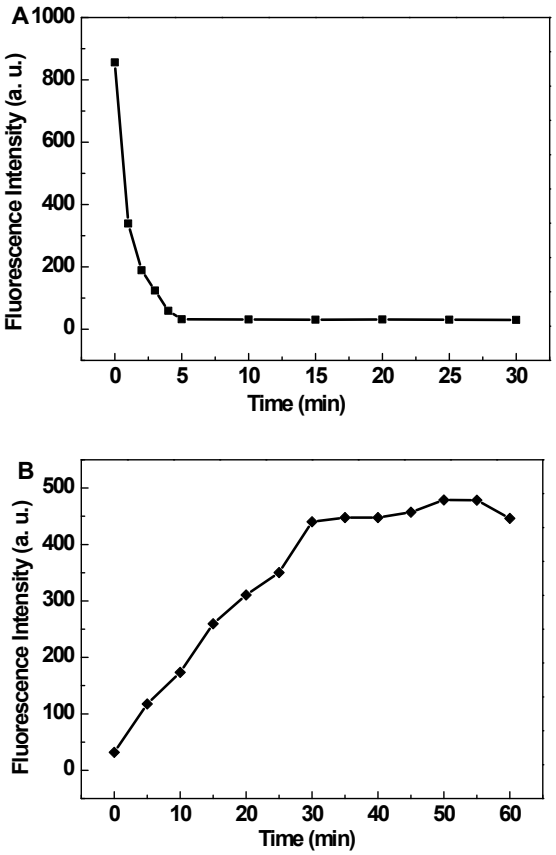

Fig. S4 (A) Fluorescence quenching of 20F (40 nM) in Tris-HCl buffer by GO as a function of time. (B) Fluorescence intensity versus different reaction time of 20F (40 nM) with S1 nuclease (0.02 units/mL).

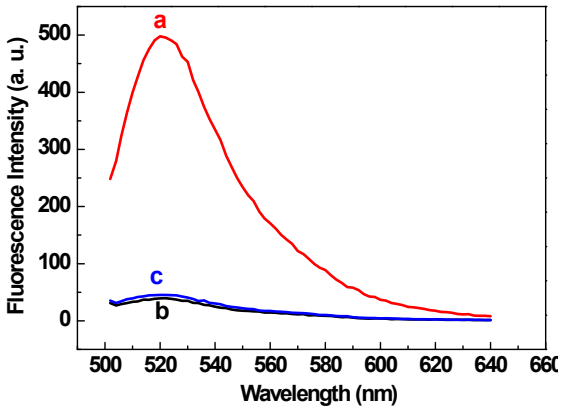

Fig. S5 Comparison the fluorescence intensity caused by the adding order of S1 nuclease and GO: (a) (20F + S1 nuclease) + GO; (b) (20F + GO) + S1 nuclease; (c) 20F + GO. Concentration: 20F, 40 nM; S1 nuclease, 0.02 units/mL; GO, 6 μg/mL. Excitation: 480 nm.

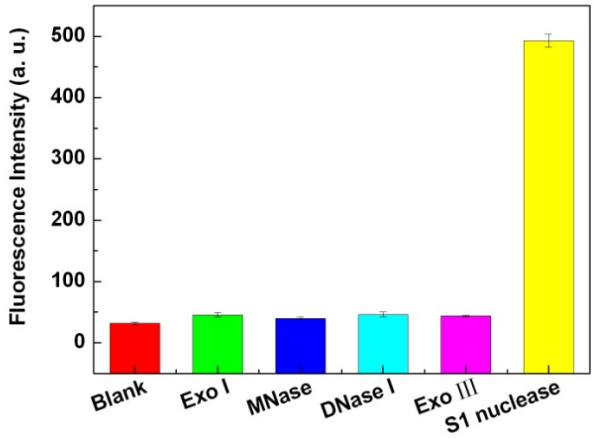

Fig. S6 Fluorescence intensity of GO-based biosensor in the presence of different nucleases: blank control (without S1 nuclease); Exo I (0.02 units/mL); MNase (0.02 units/mL); DNase I (0.02 units/mL) Exo Ⅲ (0.02 units/mL) and S1 nuclease ((0.02 units/mL)). Excitation: 480 nm.

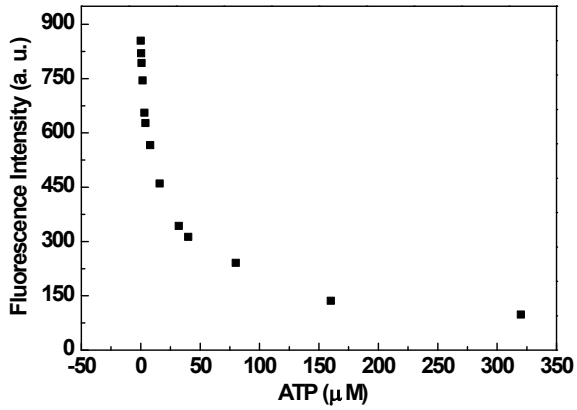

Fig. S7 Inhibition efficiency of S1 nuclease activity by ATP. Concentration: 20F, 40 nM; S1 nuclease, 0.032 units/mL; GO, 6 μg/mL. Excitation: 480 nm.

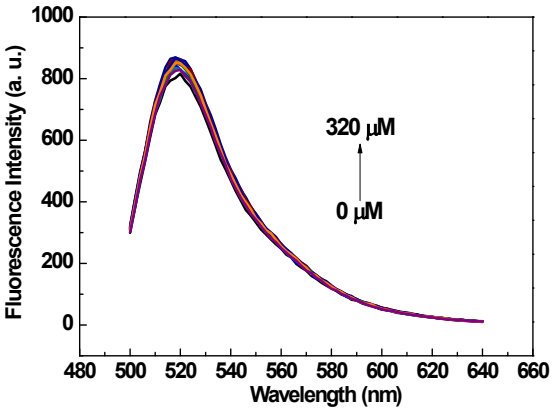

Fig. S8 Fluorescence emission spectra of 20F (40 nM) upon the addition of ATP at different concentrations. Excitation: 480 nm.

4. Supporting Tables

Table S1 Original data for element analyses for GO and computation of atom ratio for GO.

Element	**C**	**H**	**O**
wt%	51.69	2.14	42.80
Atom ratio	2.10	1.00	1.25

Table S2 Results for the determination of S1 nuclease.

Concentration (μM)	Mean	SD (n=3)	RSD (%)
0	28.90	4.83	16.71
0.0008	46.95	3.88	8.23
0.0016	62.40	3.49	5.60
0.0024	85.96	5.83	6.78
0.0032	107.7	7.31	6.79
0.004	126.3	5.05	4.00
0.008	196.8	6.87	3.49
0.012	326.4	15.06	4.61
0.016	383.5	28.41	7.41
0.02	500.3	18.41	3.68
0.024	633.9	25.36	3.99
0.028	713.6	39.95	5.60
0.032	825.4	23.01	2.79
		The average RSD is 6.13%	

Table S3 Comparison of detection limit between the proposed method with other reported methods for S1 nuclease.

Method	Linear range	LOD	References
The proposed method	0.0008-0.032 units/mL	5.8×10^{-4} units/mL	
G-quadruplex based fluorescent method	0.04-0.4 units/mL	Not given	4
Perylene derivative based fluorescent method	0-80 units/mL	9.2×10^{-2} units/mL	5
Conjugated polymer and DNA/intercalating dye complex based fluorescent method	0-0.45 units/mL	2.6×10^{-3} units/mL	6
Anionic water-soluble conjugated polymer based fluorescent method	0-12 units/mL	Not given	7
Gold nanoparticles and graphene oxide based colorimetric method	1-12 units/mL	Not given	8
Positively-charged gold nanoparticles based colorimetric method	0-30 units/mL	Not given	9

5. Supporting References

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