

Supporting Information for

Molecular Mechanism of Helicase on Graphene-Based Hybridization Reaction Platform for MicroRNA Detection

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1. Protein expression and purification of *E. coli* RecQ

The recombinant *E. coli* (RecQE) cells were grown in 50 ml LB medium containing 50 µg/ml kanamycin at 37°C. IPTG induction was proceeded when the culture was at an OD₆₀₀ of 0.6, with a final concentration of 0.5 mM. The growth of the culture was continued at 18°C with vigorous shaking (200 rpm) for 15 h. Cells were harvested by centrifugation at 8000 g for 10 min at 4°C, washed twice with sterile sodium phosphate buffer (100 mM, pH 7.0) and resuspended in the same buffer. To disrupt the cells, the cell suspension was sonicated for 5 min at 400 W, every 3 s with 7 s of interval. After centrifugation, the supernatant of cell lysate was loaded onto a nickel sepharose column (Sangon Biotech, Shanghai, China) and equilibrated with loading buffer (50 mM sodium phosphate and 300 mM NaCl, pH 7.0). After prewashing with 20 mM and 60 mM imidazole, the protein was eluted with 500 mM imidazole and dialyzed against sodium phosphate buffer (100 mM, pH 7.0). Finally, the sample was concentrated fourfold and stored at - 80°C for further use.

The protein was analyzed by 12% separating gel and 5% stacking gel. The gel was placed in 1 × tris-glycine buffer (25 mM Tris, 0.25 M Glycine and 0.1% SDS) at 150 V for 1 h and stained by coomassie brilliant blue R250 staining solution with microwave heating for 1 min. Gel imaging was performed after elution with coomassie brilliant blue destaining solution. Figure. S1 showed the SDS-PAGE image of the expressed RecQE with a molecular weight of about 69.0 kDa, which indicated that the protein was soluble and the high purity product could be used in the following experiments.

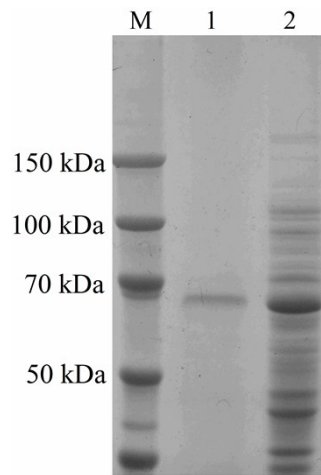


Figure S1. SDS-PAGE analysis for purification of recombinant RecQE helicase. M: protein molecular weight marker (kDa), 1: purified RecQE, 2: cell-free extract of RecQE.

2. Quenching effect of GO concentrations on the fluorescence of FDNA-BDNA

For the design of the FDNA-BDNA-GO system, it is crucial to know whether the fluorescence of FDNA-BDNA duplex can be effectively quenched by GO or not. As shown in Figure S2, with the increasing concentration of GO, the fluorescence intensity of FDNA-BDNA decreased obviously and tended to be completely quenched when the concentration of GO was above 6 $\mu\text{g/mL}$. Therefore, 6 $\mu\text{g/mL}$ GO was employed for analytical purpose in the whole work to ensure an exceeding 99.03% quenching efficiency. These results suggest that the partial double-stranded structure in the 3'-end does not affect the fluorescence quenching effect of FDNA labeled with a fluorescent group in its 5'-end.

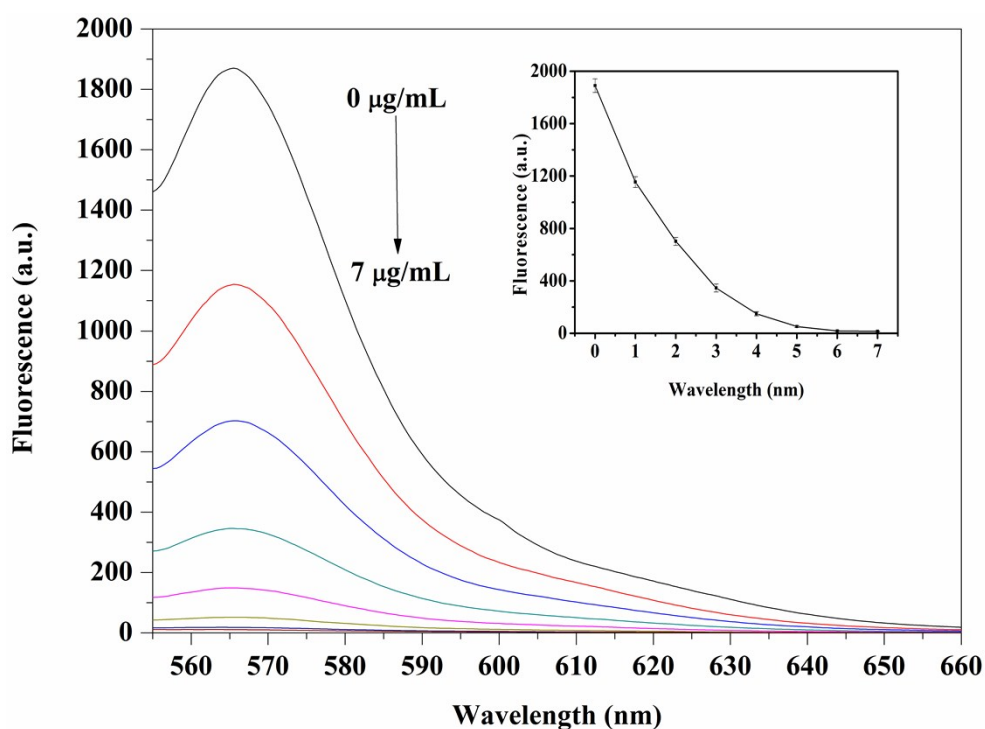


Figure S2. Quenching effect of GO concentrations on the fluorescence intensity of FDNA-BDNA. GO concentrations: 1, 2, 3, 4, 5, 6, 7 $\mu\text{g/mL}$ (from top to the bottom); the concentrations of FDNA and BDNA were both 50 nM. Inset: the plots of fluorescence intensity of FDNA-BDNA were recorded at 564 nm.

3. The function of BDNA on the system detection signal enhancement

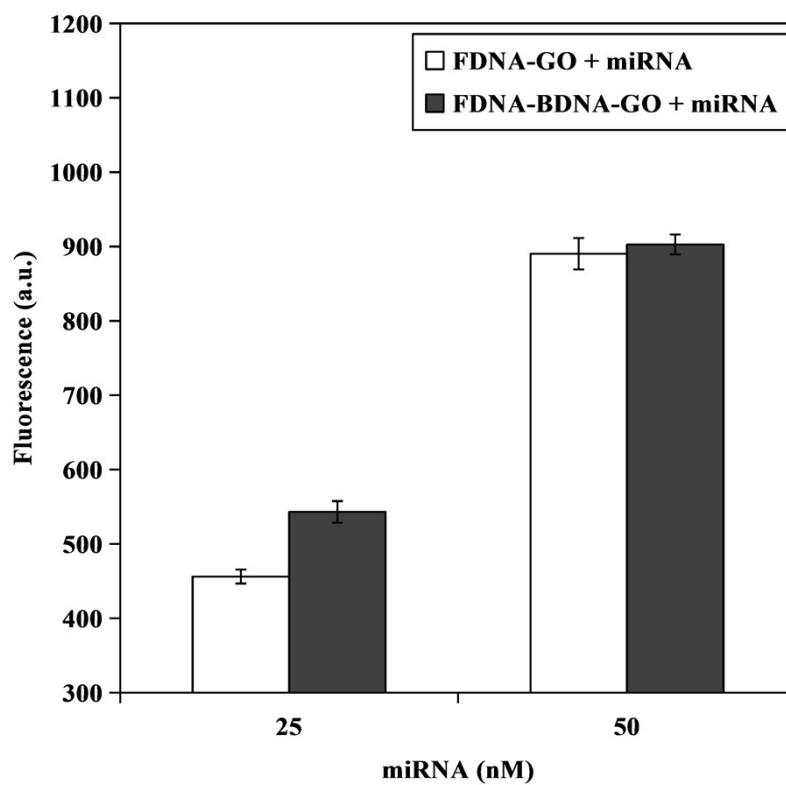


Figure. S3 Function of BDNA on signal enhancement. White: Fluorescent signal of target let-7a miRNA working in FDNA-GO system; Gray: Fluorescent signal of target let-7a miRNA working in FDNA-BDNA-GO system. MiRNA concentrations: 25, 50 nM.