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# Quencher-free molecular beacon: Enhancement of the signal-to-background ratio with graphene oxide

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ABSTRACT

We report the highly improved version of quencher-free molecular beacon (**QF-MB**) system by using graphene oxide (GO) as an external quencher. This **QF-MB**/GO system provided a higher S/B ratio (31.0) relative to that (2.2) of the same system in the absence of GO, while retaining a high selectivity for fully matched over single-base-mismatched targets.



Since their invention by Tyagi and Kramer in 1996, molecular beacons (MBs) have been used widely for the detection of nucleic acids and single-nucleotide polymorphisms (SNPs).[1] A further development of MBs will require enhancements in their detection ability, namely their selectivity and signal-to-background (S/B) ratio. Previously, we reported[2] a 'quencher-free' (QF) MB that exhibits several advantageous features, including a high level discrimination between the target and its single-mismatched congeners and an economical device set-up due to the absence of the quencher. Nevertheless, because of its intrinsic fluorescence, the **QF-MB** exhibited a low S/B ratio of ca. 50%. To enhance the S/B ratios of fluorescent oligonucleotide probes, several techniques have been developed such as fluorescence resonance energy transfer, excimer signals, multivalent fluorophores and quenchers, fluorophore-labeled pyrrolo C–G base pairing, H-dimerization of phthalocyanine, and preparation of in-stem MBs containing threoninol nucleotides.[3] Although these approaches can be used to effectively quench the signal in the closed state, to improve both sensitivity and selectivity of MBs is the challenging task until now. Using graphene oxide (GO),[4] a highly oxidized form of graphene,[5] we here show that the **QF-MB** exhibits a very high S/B ratio with almost complete quenching. Because GO is readily prepared and cheaply purchased, this approach is quite practical and economical.

The oxidized functional groups of GO allow GO to be suspended in aqueous and organic solvents at high concentrations.[4] The interactions between GO and biomolecules have been investigated widely.[6] Recently, several groups reported a GO-based biosensor utilizing the quenching phenomena of single-stranded fluorescent oligonucleotides.[7] Here, we report that GO greatly enhances the S/B ratios of **QF-MB** by reducing the background signal (Fig. 1).

We prepared GO according to the modified Hummers method and investigated its structural properties using atomic force microscopy and infrared and Raman spectroscopy.[4] We then synthesized (Table 1) **QF-MB** and four of its matched and mismatched sequences [oligodeoxynucleotides (ODNs) **A–D**].

In the absence of GO, the S/B ratio of **QF-MB** was 2.2, suggesting that half of the emission intensity arose from incomplete quenching states, relative to that of the fully matched duplex. Because the incomplete quenching of **QF-MB** might result in false positive signals, its practical use requires an enhanced S/B ratio.

To determine the optimal concentration of GO to function as an external quencher, we prepared solutions of **QF-MB** at various concentrations of GO. The background signal of **QF-MB** was dependent on the concentration of GO (Fig. 2); at 0.1 mg/mL, the background signal of **QF-MB** exhibited almost complete quenching (ca. 97%).

Because we achieved a highly quenched state for **QF-MB** simply by adding the GO solution, this approach appears to be a practical means of reducing the background signal of **QF-MB**s. Thus, we prepared the sample of **QF-MB** in Tris–HCl buffer (pH 7.2) containing GO at a concentration of 0.1 mg/mL. The fluorescence emission of **QF-MB** in this solution exhibited almost complete quenching (Fig. 3). The addition of the fully matched sequence **ODN A** to the **QF-MB**/GO solution led to regeneration of the fluorescence emission intensity and an increase in the S/B ratio to 31.0. This high

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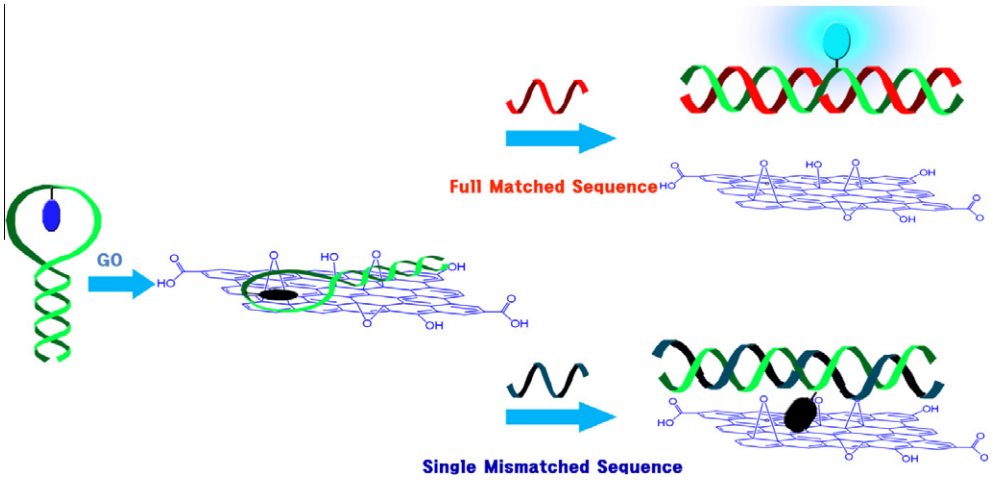


**Figure 1.** Schematic of the **QF-MB** operation in the presence of GO.

**Table 1**
ODN sequences employed in this study

| Name | Sequence |
|---|---|
| **QF-MB** | 5′d-TTC TGA CTC **F** CTT TCA GAA |
| **ODN A** | 5′d-TTC TGA AAG A GAG TCA GAA |
| **ODN B** | 5′d-TTC TGA AAG T GAG TCA GAA |
| **ODN C** | 5′d-TTC TGA AAG C GAG TCA GAA |
| **ODN D** | 5′d-TTC TGA AAG G GAG TCA GAA |

F =

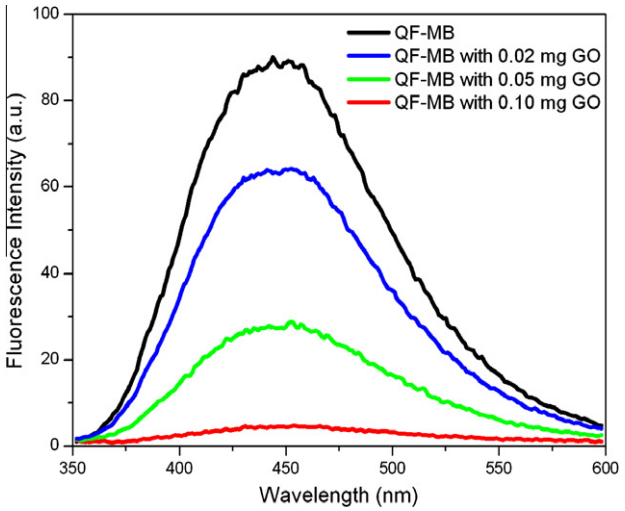


**Figure 2.** Fluorescence emission spectra of **QF-MB** (1.5 μM) in the presence of GO at various concentrations in Tris–HCl buffer (pH 7.2) containing 100 mM NaCl and 20 mM $MgCl_2$ at 20 °C, $\lambda_{ex}$ = 335 nm.

S/B ratio meant that the closed and open states of **QF-MB** could be clearly discriminated by the naked eye (Fig. 3).

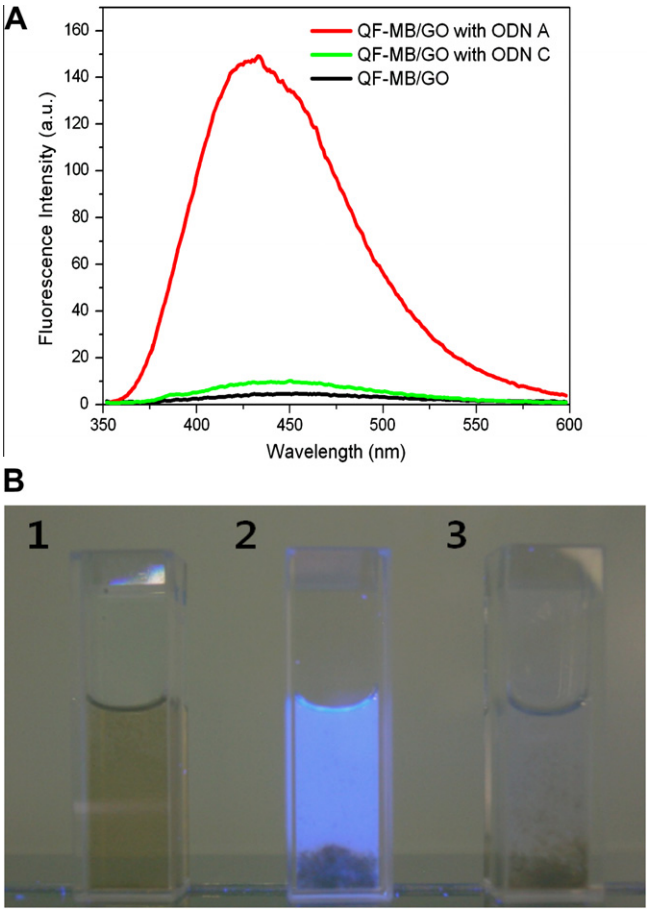


**Figure 3.** Fluorescence emission spectra of **QF-MB** in the absence (black) and presence of the fully matched strand **ODN A** (red) and single-base-mismatched strand **ODN C** (green) and corresponding photographs of the solutions in under UV irradiation: (1) **QF-MB**, (2) **QF-MB**/**ODN A**; and (3) **QF-MB**/**ODN C**. Buffer: 100 mM Tris–HCl, 10 mM $MgCl_2$, and 100 mM NaCl (pH 7.2); temperature: 20 °C; concentration of each ODN: 1.5 μM; additive: GO, 0.1 mg/mL, $\lambda_{ex}$ = 335 nm.

Moreover, in the presence of single-base-mismatched sequences, the fluorescence emissions of the **QF-MB**/GO systems remained highly quenched. As a result, the **QF-MB**/GO system exhibited a 14-fold enhancement in its S/B ratio and a discrimination factor of 15.9 for the fully matched and single-base-mismatched target

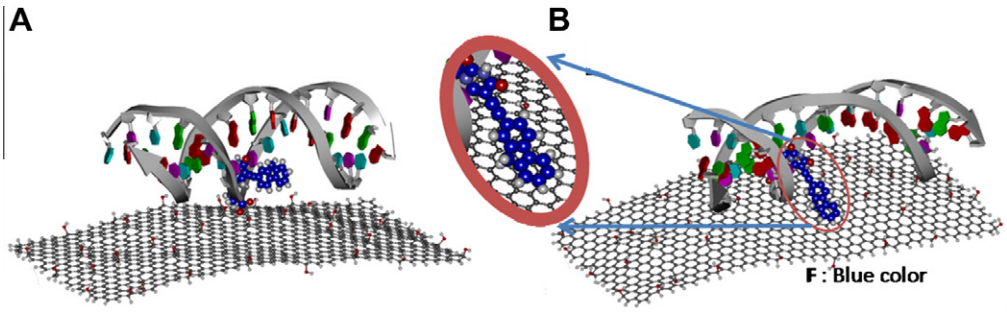


**Figure 4.** Representative snapshot from the MD simulations of (a) **QF-MB**/**ODN A**/GO and (b) **QF-MB**/**ODN B**/GO complex systems.

**Table 2**
Effect of GO on the S/B ratio

| | Without GO | With GO |
|---|---|---|
| S/B ratio[a] | 2.2 | 31.0 |
| Discrimination factor[b] | 14.7 | 15.9 |

[a] The ratio of fluorescence emission intensity between **QF-MB**/GO and **QF-MB**/**ODN A**/GO complex in Tris–HCl buffer (pH 7.2) containing 100 mM NaCl and 20 mM $MgCl_2$ at 20 °C.

[b] Ratio in fluorescence intensity of the fully matched to single-base-mismatched duplexes (**QF-MB** with **ODN A** or **ODN C**).

sequences (**ODN A** and **ODN C**)—that is, high sensitivity and selectivity (Table 2). Other discrimination factors in the **QF-MB**/GO system are as follows: 15.3 (**ODN A** and **ODN B**), 14.8 (**ODN A** and **ODN D**).

In order to understand the origin of fluorescence quenching or enhancing in the **QF-MB**, **QF-MB**/**ODN A**, and **QF-MB**/**ODN B** upon complexing with GO, we have performed molecular dynamics simulations of **QF-MB**/GO, **QF-MB**/**ODN A**/GO, and **QF-MB**/**ODN B**/GO complex systems. In **QF-MB**/**ODN A**/GO, due to the existence of two H-bonds between **A** and **F**(3-N–H and 4-C=O), the normal duplex geometrical features are retained and fluorophore has no interaction with aromatic surface of the GO. Therefore, the unique and strong florescent intensity of the fluorophore is observable. In the **QF-MB**/**ODN B**/GO, the base of **F**(2-C=O and 3-N-H) makes two H-bonds with **T** and hence duplex geometry is distorted significantly during the simulation. As a result, fluorophore moiety of **F** makes strong π–π interactions with the aromatic rings in GO. We also notice that fluorophore moiety of **F** in **QF-MB**/GO complex forms the π-π interactions with the aromatic rings in GO. Thus, due to the π-π interaction in **QF-MB**/GO and **QF-MB**/**ODN B**/GO complex systems, the unique fluorescent properties of the fluorophore in **F** is almost fully quenched (Fig. 4).

In summary, the addition of GO as an external quencher enhances the S/B ratio of a **QF-MB** system. Our **QF-MB**/GO system exhibited a higher S/B ratio (31.0) relative to that (2.2) of the same system in the absence of GO, while retaining a high selectivity for fully matched over single-base-mismatched targets. Because GO is a cheap, readily prepared, and useful material in the aqueous medium, this approach will enable the application of **QF-MB**s to biological systems and the development of additional biosensing systems.

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## Supplementary data

Supplementary data (characterization data for the ODNs and GO) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.004.

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