A triplex-forming linear probe for sensitive and sequence-specific detection of duplex DNA

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Most of the strategies for detection of target DNA have relied on the denaturation of double-strand DNA (dsDNA) into single strands, which prevents a simple detection. In this study, we report a triplex-forming linear probe tethering multiple fluorophores at intervals of native oligonucleotides with D-threoninol as scaffold, which allows simple detection of the dsDNA without denaturation. The principle of the linear probe is schematically illustrated in Figure 1 on the left: At single-stranded state, it self-quenches due to hydrophobic interaction among fluorophores, while it sequence-specifically forms triplex with dsDNA via Hoogsteen base-pairing.[1] As a result, fluorescence remarkably recovers because weakly stacked fluorophores are separated by their intercalation between the base-pairs.

By the incorporation of perylene derivative L and anthraquinone derivative gQ, we have successfully designed an optimal probe 3L1gQ, targeting human Androgen Receptor (AR) gene. Signal/background ratio of 3L1gQ was as high as 278 (Figure on the right). Moreover, the melting temperature of the triplex with this linear probe (61.9 ^{o}C) was much higher than that of native DNA (40.0 ^{o}C), suggesting remarkable improvement on triplex stability. As possible application, detection of PCR products was performed: 3L1gQ showed clear light-up in the presence of product-dsDNA containing target site after PCR reaction, only by the addition of the probe to PCR solution (data not shown). These results demonstrate remarkable ability as a fluorescent probe for detecting dsDNA, and its possibility for applying to dsDNA-targeted diagnosis in cell.

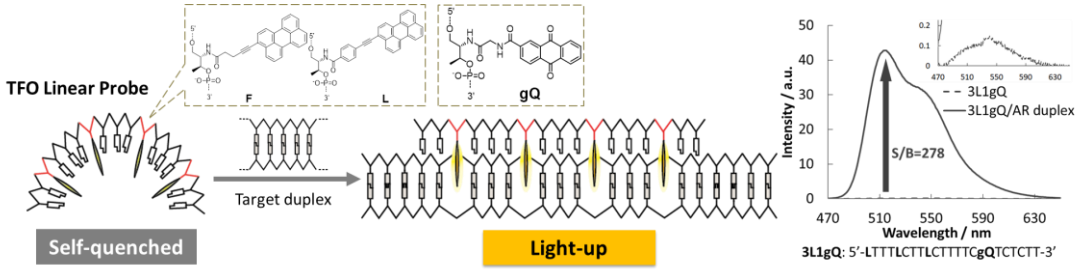

Figure. Schematic illustration of the linear probe (left panel) and its light-up in the presence of target dsDNA (right panel).

1) Y. Chen *et al.*, *Chem. Commun.*, **2020**, *56*, 5358-5361.