Pyrene-PNA Twin Probe Detects Sequence-specific DNA by the Excimer Fluorescence

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Detection of sequence-specific nucleic acids can be applied to the diagnosis for gene diseases. Fluorescent probe methods are expected to be useful for such detection of nucleic acids. In this work, we have developed a novel fluorescent probe, pyrene-PNA twin probe (**P1** and **P2**). **P1** and **P2** were consisted of PNA (Peptide Nucleic Acid) as the main chain backbone and contained a pyrenyl group at the terminal to detect the target nucleic acids from fluorescence. We synthesized the probes using Fmoc-based solid-phase peptide method. As these probes **P1**/**P2**, form hybrids with complementary DNA simultaneously, the pyrenyl groups in the probes are expected that arranged to face each other, resulting in fluorescence because of excimer will be formed (Fig. 1, upper).

To assess it, we measured fluorescence spectra of a mixture of **P1**/**P2** with a complementary DNA and the pyrenyl group-derived excimer emission was observed. In contrast, no excimer emission was observed from the mixture of **P1**/**P2** with or without a non-complementary DNA (a scrambled sequence). These results clarified that the probes could detect the target DNA as we expected.[1]

Furthermore, we attempt to enhance the fluorescence intensity of the excimer emission of this probe to improve the contrast of nucleic acid detection. For this purpose, we are currently synthesizing a cross-linker was added to conjugate these two probes together for promoting of an overlap of the two pyrenes (Fig. 1, lower).

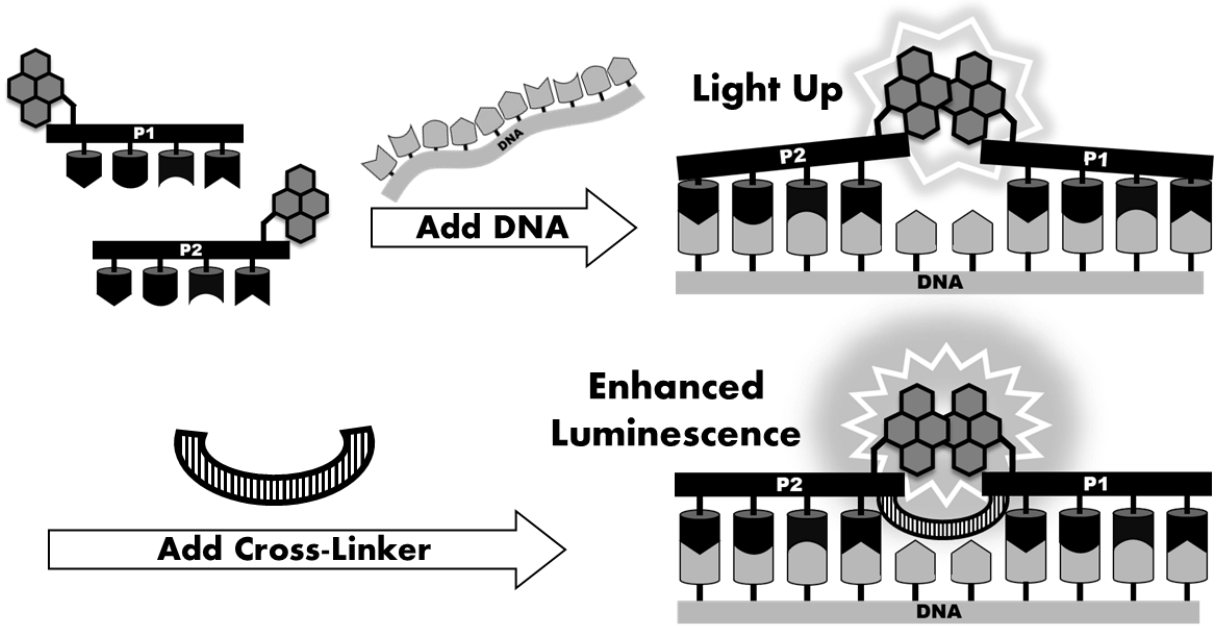

Fig.1 Schematic illustration of pyrene-PNA twin probes (**P1** and **P2**) for detecting a target DNA.

References

[1] Ishii K., Tsuchitani S., Kitamatsu M, et al. Fluorescence Ratiometric DNA Detection by Peptide Nucleic Acid-Pyrene Binary Probes, *Bioorg. Med. Chem. Lett.*, 71, 128838-128841 (2022).