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# Supplementary Information

# Simultaneous detection of single-nucleotide polymorphisms in a DNA bulge structure using fluorine-modified bisbenzimide derivative

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## 1. Experimental Procedures

**ODNs and other reagents:** All oligodeoxyribonucleotides were purchased from Fasmac (Japan). 3,5-Bis(trifluoromethyl)benzene-modified bisbenzimide H33258 was prepared according to a literature method.[1] Other reagents were purchased from Sigma-Aldrich (CA), Wako Pure Chemical Industry (Osaka, Japan) or TCI (Tokyo, Japan).

**$^{19}$F NMR measurement:** $^{19}$F NMR spectra were recorded using AVANCE III 500 MHz NMR spectrometer (Bruker Biospin) equipped with a 5 mm probe head (PA BBO 500S2 BBF-H-D-05 Z, Bruker Biospin) at 470 MHz for $^{19}$F. The chemical shifts were corrected using TFA (–75.6 ppm) as an internal standard.

**Fluorescence measurement:** Fluorescence spectra were recorded using FP-6500 spectrofluorophotometer (JASCO, Tokyo, Japn) with excitation at 345 nm.

**Reversed-phase ultra-high performance liquid chromatographic analysis:** 2'-Deoxyribonucleosides (50 μM aq. soln.; 15 μL) were analyzed with a UPLC system (Aquity, Waters) equipped with BEH Shield RP18 column (1.7 μm, 2.1 × 50 mm, elution was with 0.05 M ammonium formate containing 0 to 0.5% $CH_3CN$, linear gradient (8 min) at a flow rate of 0.2 mL/min, 60 ºC). UV absorbance chromatograms were recorded at wavelength of 260 nm.

**Evaluation of partition coefficient ($P_{OW}$):** A solution of 2'-deoxyribonucleosides (200 μM in 50 mm Tris-HCl (pH 7.6) containing 100 mM NaCl; 2 mL) was mixed with 1-octanol (2 mL) that was presaturated with same buffer in a sample tube. After shaking 100 times at room temperature, the tube was centrifuged (1500 rpm, 5 min). UV absorbance at 260 nm of the aqueous layer was measured by spectrophotometer (V-630bio, JASCO) and $P_{OW}$ was calculated with the equation below;

$$P_{OW} = (A_0 - A_1) / A_0$$

where, $A_0$ and $A_1$ is the absorbance at 260 nm of the nucleoside solution before and after octanol treatment, respectively.

## 2. Supporting Table and Figures

**Table 1. Octanol-water partition coefficients ($P_{OW}$) of 2'-deoxyribonucleosides**

| | $P_{OW}$ |
|---|---|
| **dC** | **0.035** |
| **dG** | **0.044** |
| **dT** | **0.076** |
| **dA** | **0.236** |

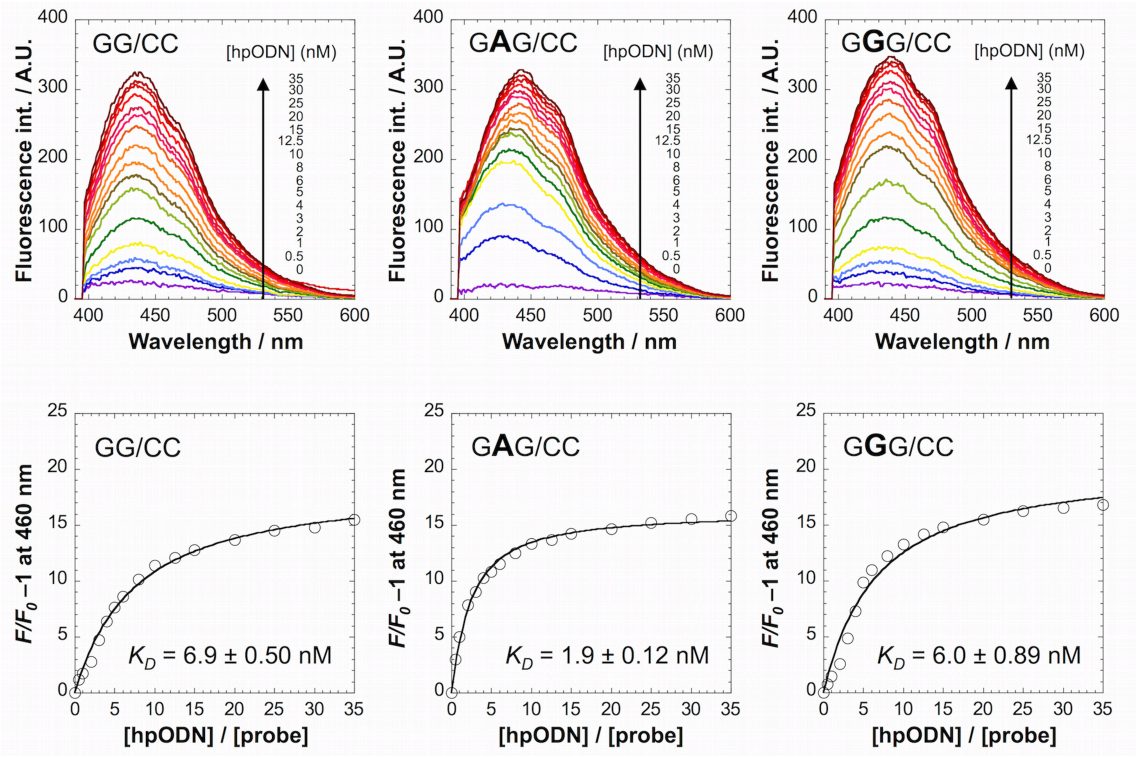


**Fig. S1** Fluorescence spectra and fluorescence titration curves of the probe versus hpODNs. [probe] = 1 nM in 50 mM Tris-HCl (pH 7.6) containing 100 mM NaCl. Measurements were performed at 25°C. Fluorescence intensity at 460 nm was used for preparing titration curves and $K_D$ values were calculated with non-linear least square fitting.

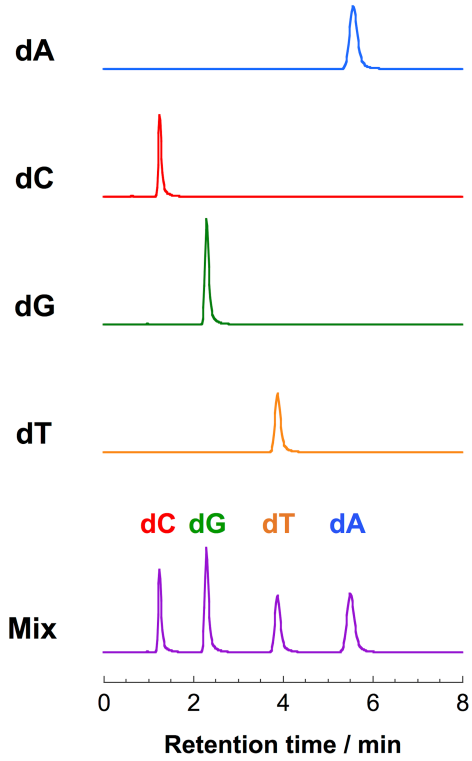


**Fig. S2** Reversed-phase ultra-high performance liquid chromatographic analysis of 2'-deoxyribonucleosides. Aqueous solutions of 2'-deoxyribonucleosides (50 μM each) were subjected to the analysis.

## References

1. T. Sakamoto, D. Hasegawa and K. Fujimoto, *Chem. Commun.*, 2015, **51**, 8749.