Supplementary Information

Development of 19F-NMR chemical shift detection of DNA B-Z equilibrium using 19F-NMR

Shigetaka Nakamura, Hui Yang, Chihiro Hirata, Florian Kersaudy, and Kenzo Fujimoto*

School of Materials Science, Japan Advanced Institute of Science and Technology, Asahi-dai 1-1, Nomi, Ishikawa, 923-1292, Japan

General

^{1}H-NMR and 19F-NMR spectrum were recorded on a Bruker AVANCE III 400 system and Bruker AVANCE III 500 system, respectively. Mass spectra were recorded on a Voyager PRO-SF, Applied Biosystems. HPLC was performed on a Chemcosorb 5-ODS-H column with JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA sysnthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite , and CPG support were purchased form Glen research.

Synthesis ODN containing ^{TF}C and ^{TF}T

The phosphoramidite of ^{TF}C and ^{TF}T was prepared according to a method reported in literature. The modified oligonucleotides containing ^{TF}C and ^{TF}T were prepared, according to standard phosphoramidite chemistry, on a DNA synthesizer using the phosphoramidite of ^{TF}C and ^{TF}T as shown in Figure 2a. Synthesized ODN were detached from the support and deprotected in 50 mM K_2CO_3 in MeOH for 8 h at room temperature. Then it was neutralize by trichloroacetic acid and removing solvent by speedvac, and the crude oligomer was purified by reverse phase HPLC and lyophilized.

19F-NMR measurements

19F-NMR spectra were measured on a Bruker AVANCE III 500 instrument with a 5 mm probe head (PA BBO 500S2 BBF-H-D-05 Z) at 470 MHz for fluorine. The solvent for 19F-NMR measurement was 10 mM Tris-HCl buffer (pH7.0) containing 10 μM trifluoroacetic acid (for internal standard, -75.6 ppm) and 10% D_2O. When calculation of ratio of B-, Z-, and ss-DNA based on 19F NMR data, each peak was normalized by the peak of TFA as the internal standard .

UV melting analysis

UV melting curves of a solution of the 10 μM duplex DNA in 10 mM Tris-HCl buffer(pH7.0) containing 10 mM TFA and 10% D_2O were measured (260 nm, 1°C/min) by a spectrophotometer equipped with a temperature controller.

CD spectra

CD spectra of a solution of the 20 μM duplex DNA in 10 mM Tris-HCl buffer(pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C on a JASCO J-720 spectropolarimeter.

Table S1. MALDI-TOF-MS data of synthesized ODN

Entry	Sequence(5'-3')	Calcd. for $[M+H]^+$	Found
ODN 1	$CG^{TF}CGCG$	1861.32	1861.35
ODN 2	$CG^{TF}CGTGCGCG$	3112.52	3112.06
ODN3	$CGCG^{TF}TGCGCG$	3098.45	3099.01

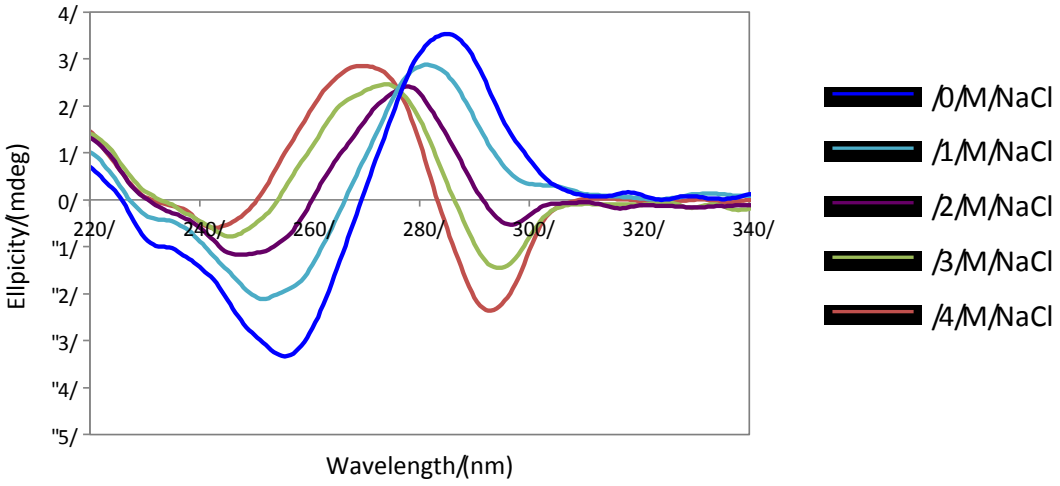

Figure S1. CD spectrum of ds DNA consisting of ODN 1 with the addition of NaCl. 20 uM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C.

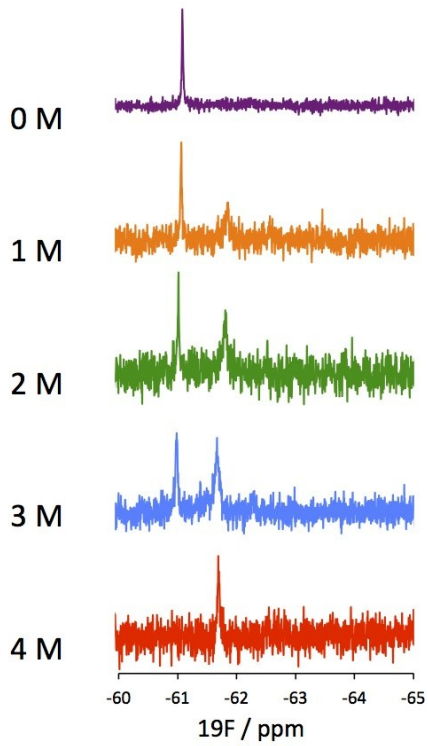

Figure S2. 19F-NMR spectra of duplex consisting of ODN 1 at each NaCl concentration. 20 μM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C.

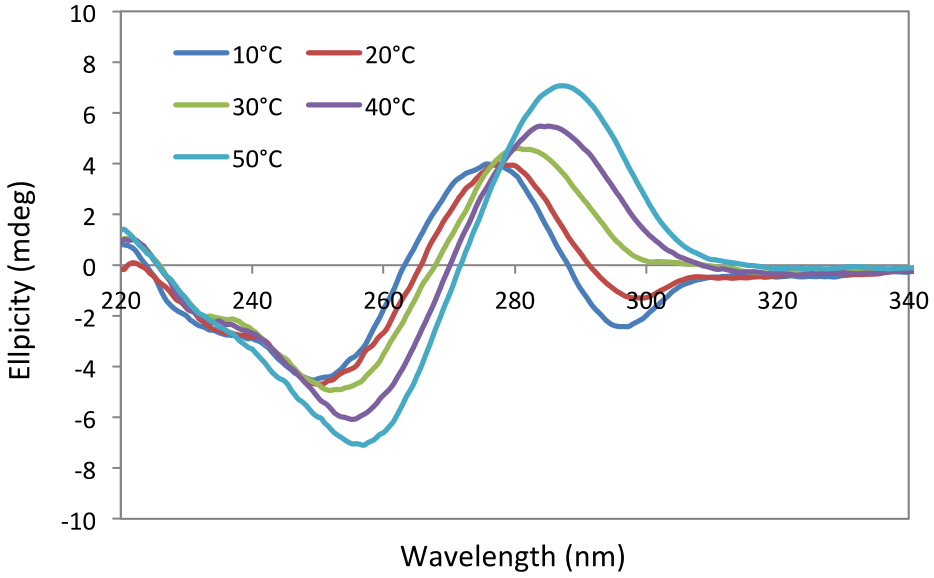

Figure S3. CD spectrum of ds DNA consisting of ODN 2 and ODN 4 at each temperature. 20 μM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O containing 4 M NaCl were measured at 10°C.

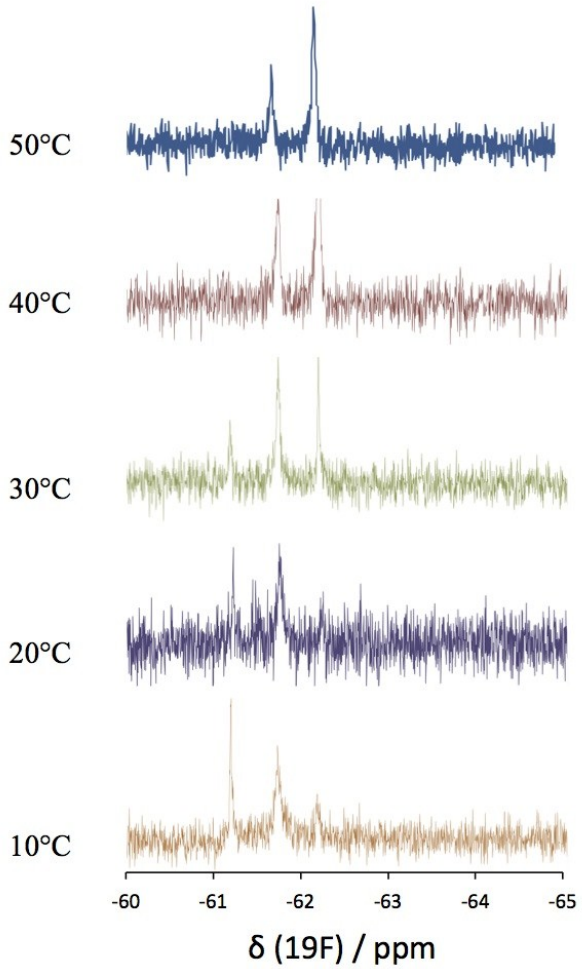

Figure S4. 19F-NMR spectra of duplex consisting of ODN 2 and ODN 4 at each temperature. 20 μM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O containing 4 M NaCl were measured at 10°C.

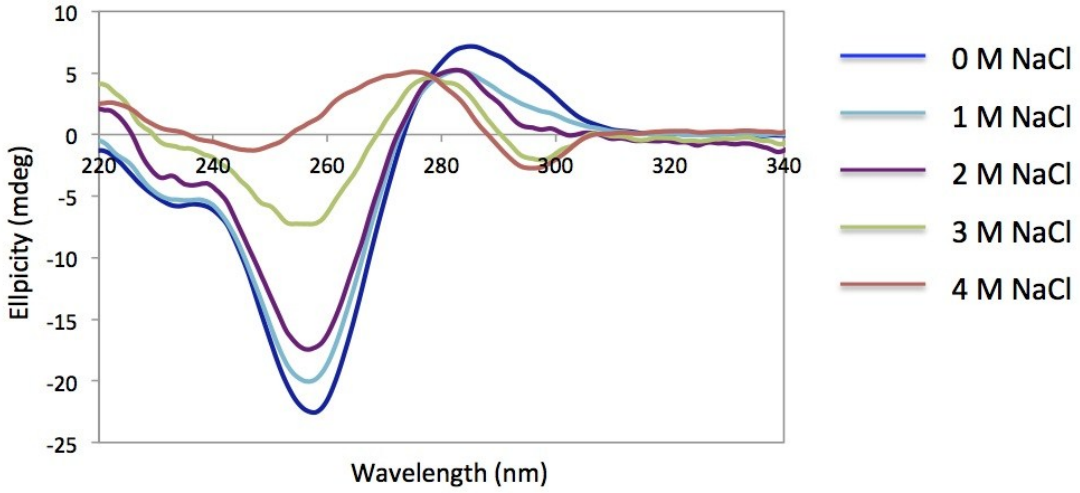

Figure S5. CD spectrum of ds DNA consisting of ODN 3 and ODN 4 with the addition of NaCl. 20 uM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C.

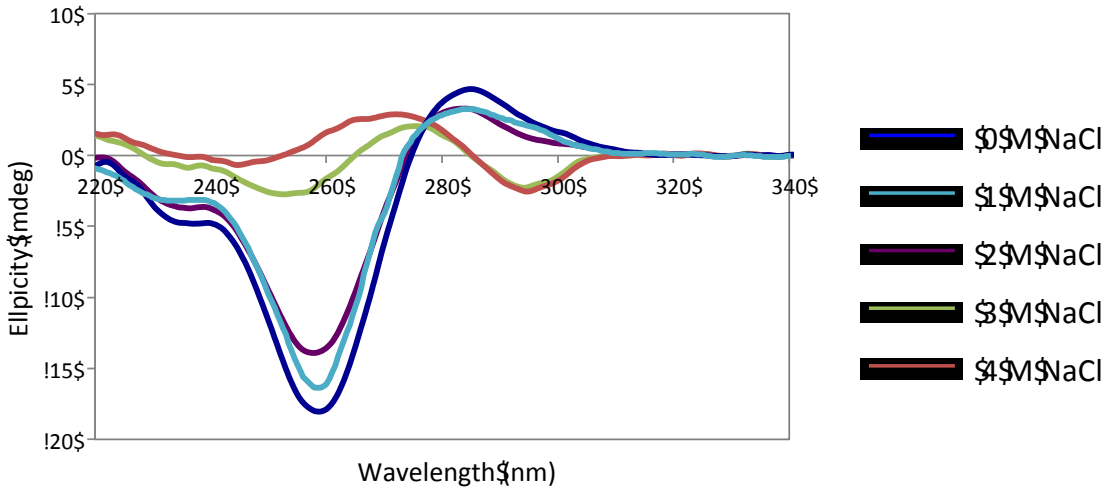

Figure S6. CD spectrum of ds DNA consisting of ODN (5'-CGCGTGCGCG) and ODN 4 with the addition of NaCl. 20 uM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C.

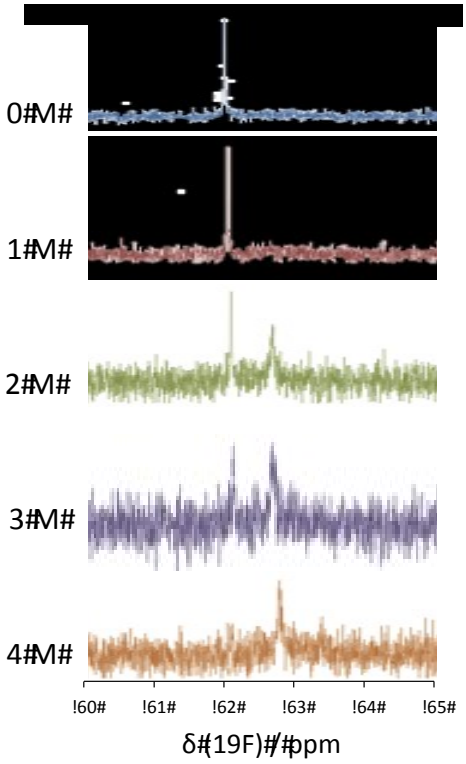

Figure S6. 19F-NMR spectra of duplex consisting of ODN 3 and ODN 4 at each NaCl concentration. 20 μM DNA in 10 mM Tris-HCl (pH7.0) containing 10 μM TFA and 10% D_2O were measured at 10°C.