

# **Structures of 2-Acetylaminofluorene Modified DNA Revisited: Insight into Conformational Heterogeneity**

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## **Supplementary Information**

## EXPERIMENTALS

### Synthesis and Purification of FAAF-Modified Oligodeoxynucleotides

Crude oligodeoxynucleotides (ODN, 10  $\mu$ mol scale) used in this study were purchased in desalted form from Eurofins MWG Operon (Huntsville, AL) and purified by reverse phase high performance liquid chromatography (RP-HPLC). All solvents were purchased from Fisher Inc (Pittsburgh, PA). The HPLC system consisted of a Hitachi EZChrom Elite HPLC system with a L2450 diode array as a detector and a Luna column (10 mm $\times$ 150 mm; 5  $\mu$ m) (Phenomenex, Torrance, CA). The mobile phase consisted of a 40-min linear gradient of 5-15%(v/v) acetonitrile/ 0.1M ammonium acetate buffer (pH 7.0) with a flow rate of 2.0 ml/min. The activated model carcinogen *N*-acetoxy-*N*-(2-acetylamino)-7-fluorofluorene (AAFF) was prepared and characterized according to the methods published previously (1). 100 ODS of oligomer dissolved in a pH 6.0 sodium citrate buffer was made to react with 500 $\mu$ l of 20mg/ml solution of AAFF in ethanol at 37  $^{\circ}$ C. The mixture was allowed to stir for about 1 hr in dark following which the excess of ethanol was evaporated and the aqueous layer was extracted several times with water saturated diethyl ether. The aqueous layer containing the FAAF-modified ODN was concentrated and purified by semipreparative RP-HPLC. A typical yield for modification was 40-60% depending on sequences. The identity of the modified ODNs was confirmed by the electro-spray mass spectrometric procedures described previously (2) (calculated monoisotopic mass, 3745.67: found, 3745.8, for the FAAF-modified -CG\*C- sequence; calculated monoisotopic mass 3784.68: found, 3784.8 for the FAAF-modified TG\*A- sequence).

### $^{19}\text{F}/^1\text{H}$ NMR Experiments

Approximately 20-30 ods of pure modified ODNs were annealed with an equivalent amount of complementary sequences to produce duplex samples. The samples were filtered by ultracentrifugation using a Pall Microsep MF centrifugal device (Yellow,  $M_w$  cutoff = 1,000). The centrifuged samples were dissolved in 300  $\mu$ l of a neutral buffer (10%  $\text{D}_2\text{O}/90\%$   $\text{H}_2\text{O}$  containing 100 mM NaCl, 10 mM sodium phosphate, and 100  $\mu$ M tetrasodium EDTA (pH 7.0)), filtered through a 0.2  $\mu$ m membrane filter, and transferred to a Shigemi tube for NMR experiments.

All  $^1\text{H}$  and  $^1\text{H}$ -decoupled  $^{19}\text{F}$  NMR spectra were recorded using a dedicated 5 mm  $^{19}\text{F}/^1\text{H}$  dual probe on a Bruker DPX400 Avance spectrometer operating at 400.0 and 376.5 MHz, respectively. Imino proton spectra were obtained using phase sensitive jump-return sequences at 5  $^{\circ}$ C and referenced relative to DSS.  $^{19}\text{F}$  NMR spectra were referenced to  $\text{CFCl}_3$  by assigning external hexafluorobenzene in  $\text{C}_6\text{D}_6$  at -164.90 ppm. One-dimensional  $^{19}\text{F}$  NMR spectra at 5~60  $^{\circ}$ C were obtained by collecting 65,536 points using a 37,664 Hz sweep width and a recycle delay of 1.0 s between acquisitions. Additional temperatures were used as needed to clarify the signal exchange process. Temperatures were maintained by a Bruker VT unit by adding liquid nitrogen to the probe. A total of 1,200 scans were acquired for each spectrum. The spectra were processed by zero-filling, exponential multiplication using 20 Hz line broadening and Fourier transformation. The peak areas were base-line corrected and integrated using XWIN NMR software (Bruker, Billerica, MA). Two-dimensional NOESY/exchange  $^{19}\text{F}$  NMR spectra were carried out in the phase-sensitive mode using a NOESY pulse sequence: sweep width 4529 Hz, number of complex data points in  $t_2$  1024, number of complex free induction decays in  $t_1$  256, number of scans 96, dummy scans 16, recycle delays 1.0 s, and mixing time 400 ms. The data

were subjected to sine-bell apodization using 2 Hz line broadening in both dimensions and then zero-filled before Fourier transformation of the 1024x256 data matrix. The pH dependent NMR spectra were recorded with the initial pH of 6.8 with incremental adjustment towards acidic or basic pH's for the sample solution.

### **Circular Dichroism (CD) Experiments**

CD measurements were conducted on a Jasco J-810 spectropolarimeter equipped with a variable Peltier temperature controller. All the dichroism experiments were performed at 20 °C unless until stated otherwise. Typically, 2 ODS of a FAAF-modified template strand were annealed with an equivalent of primer strands. The samples were dissolved in 400 µl of a neutral buffer (0.2 M NaCl, 10 mM sodium phosphate, 0.2 mM EDTA, pH 7.0) and placed in a 1.0 mm path-length cell. The pH dependent CD spectra were recorded with the initial pH of 7.0 with incremental adjustment towards acidic or basic pH's for the sample solution. The sample was heated at 85 °C for 5 min and then cooled to 15 °C over a 10 min period to ensure duplex formation. Spectra were measured from 200 nm to 400 nm at a rate of 50 nm/min; the final data were averaged from ten accumulations. Data points were acquired every 0.2 nm with a 2 s response time and smoothed using the 17 point adoptive algorithms provided by Jasco.

### **UV-Thermomelting Experiments**

Melting experiments were conducted on a Cary 100 Bio, UV/Vis spectrophotometer equipped with a 6 x 6 chamber, 1.0-cm path-length  $T_m$  cell. Sample cell temperatures were controlled by a built-in Peltier temperature controller. All modified ODN sequences were hybridized to their complementary strands in solutions with a total concentration in the range of 0.8 µM-6.4 µM containing 0.2 M NaCl, 10 mM sodium phosphate, and 0.2 mM EDTA (pH 7.0). The concentration of each oligo sample was estimated based on UV absorbance at a wavelength of 260 nm. Samples were equilibrated at 10 °C for 5-10 min prior to data collection Thermal melting curves were constructed by varying the temperature of the sample cell (1 °C/min) and monitoring the absorbance of the sample at 260 nm. A typical melting experiment consisted of forward/reverse scans and was repeated three times.

Coordinates for the FAAF-modified B, S, W-conformers illustrated in Figure 1c are courtesy of Dr. Lihua Wang, Biology Department, New York University.

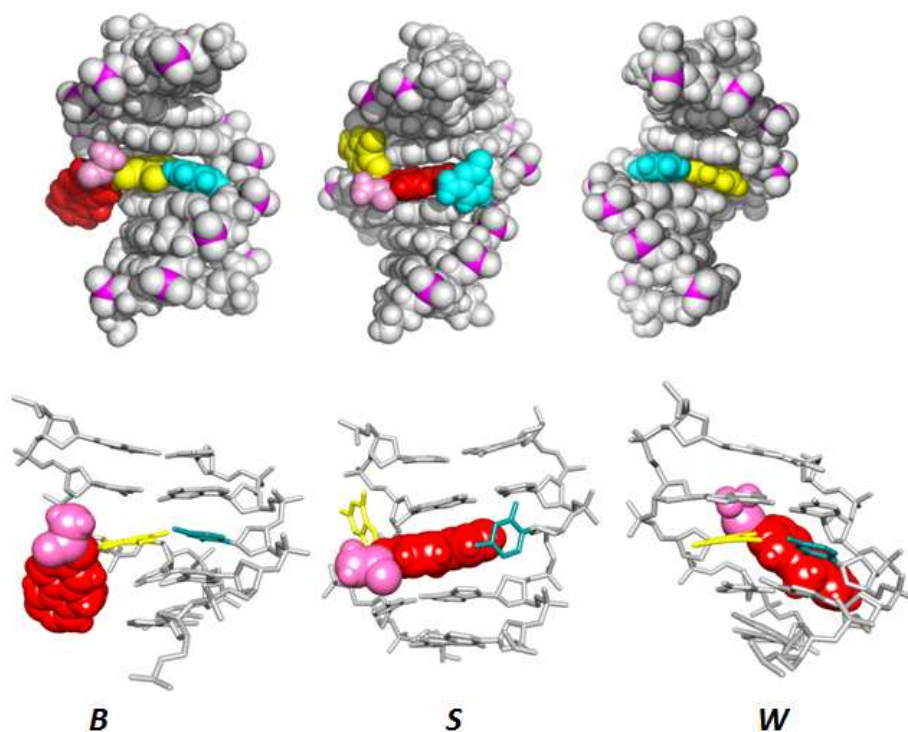
### **References**

1. Cho, B. P., and Zhou, L. (1999) Probing the conformational heterogeneity of the acetylaminofluorene-modified 2'-deoxyguanosine and DNA by  $^{19}\text{F}$  NMR spectroscopy, *Biochemistry* 38, 7572-7583.
2. Meneni, S. R., D'Mello, R., Norigian, G., Baker, G., Gao, L., Chiarelli, M. P., and Cho, B. P. (2006) Sequence effects of aminofluorene-modified DNA duplexes: thermodynamic and circular dichroism properties, *Nucleic Acids Res.* 34, 755-763.

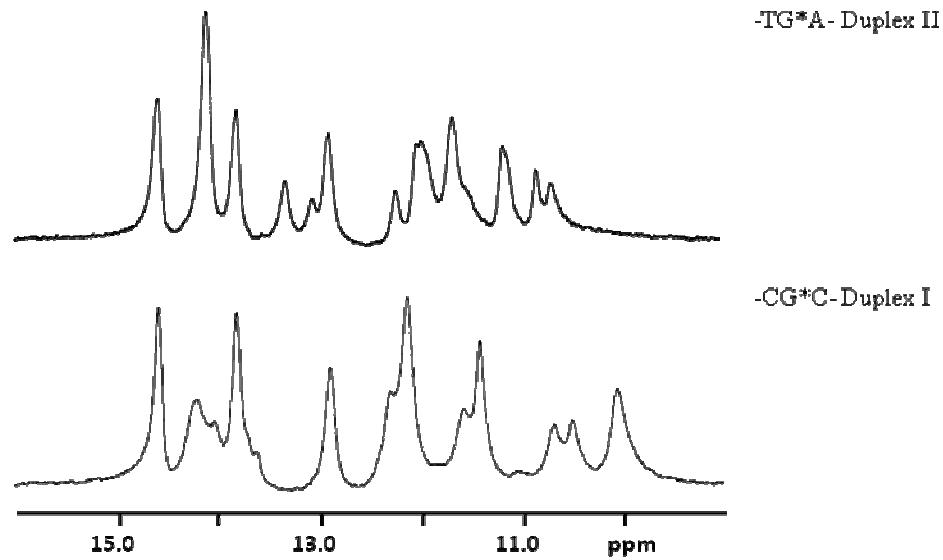
Torsion angles	B	S	W
$\gamma'$ (C8-N-C-Cm)	32.0 (~trans)	13.0 (~trans)	48.8 (~trans)
$\beta'$ (C8-N-C2-C1)	36.5	-46.9	-1.0
$\alpha'$ (N9-C8-N-C2)	48.6	95.3	-134.5
$\chi$ (O1'-C1'-N9-C4)	-158.9 (anti)	58.2 (syn)	59.4 (syn)

$\gamma'$  0 (trans) 180 (cis)  
 $\chi$  170-300 (anti) 20-100 (syn)

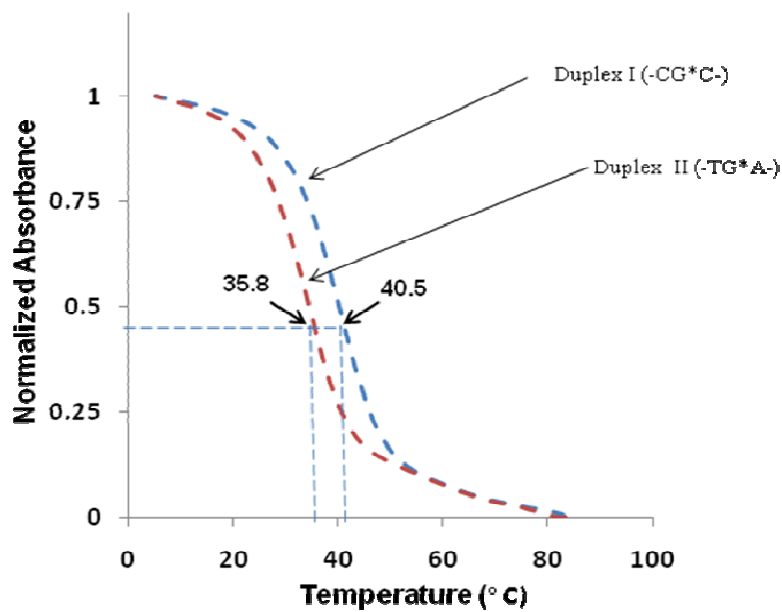
Supplementary Table 1: Various dihedral torsion angles for the B, S, and W-conformer FAAF-duplex II



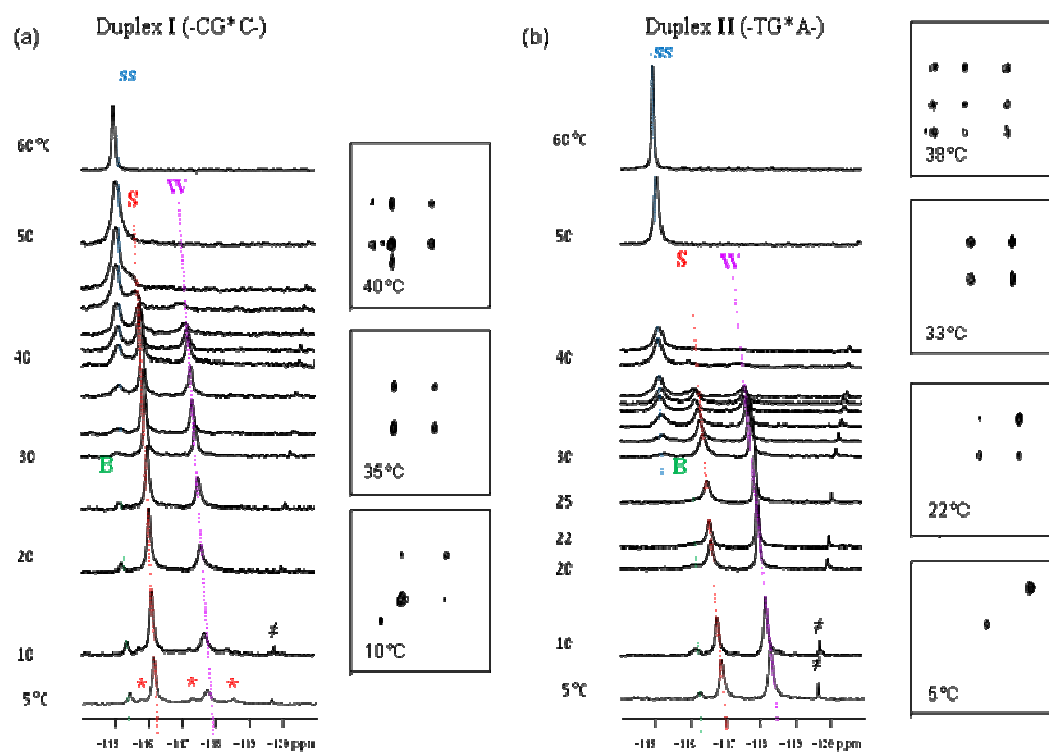
**Supplementary Fig. S1:** Views from the major-grooves of a duplex for three FAAF-induced conformational motifs: B, S, and W-conformers in (a) CPK and (b) stick models. Color code: modified G (yellow) and complementary C (cyan) at the lesion site; fluorene (red) and N-acetyl (pink).



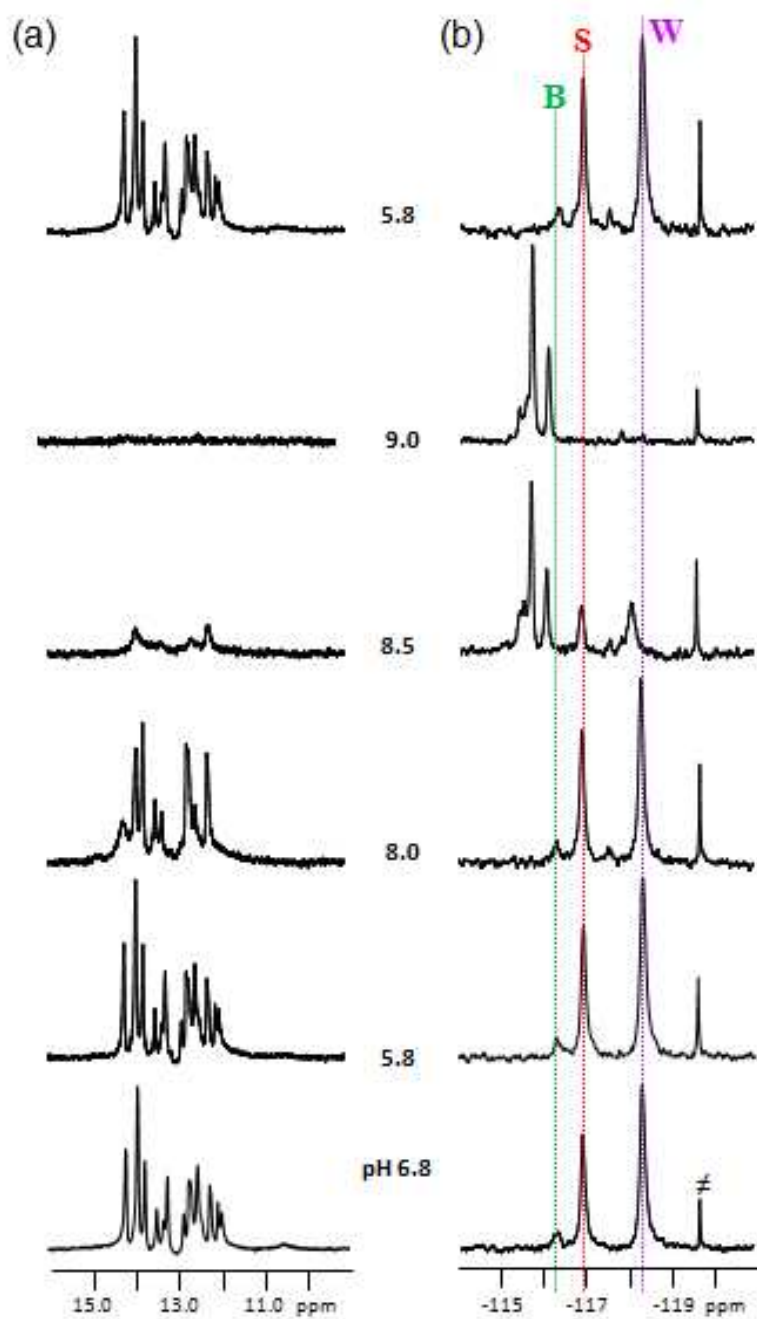
**Supplementary Fig. S2:** (a) Imino proton region (9 - 16 ppm) of <sup>1</sup>H NMR spectra of FAAF-modified 12-mer duplexes II (top) and I (bottom) recorded at pH 6.8 at 5 °C.



**Supplementary Fig. S3:** Normalized UV melting curves for the FAAF-modified Duplex I and II.



**Supplementary Fig. S4:** Temperature dependence of 1D  $^{19}\text{F}$  NMR (-114 ~ -121 ppm) and NOESY (-116 ~ -121 ppm) spectra of the FAAF-modified duplex (a) I and (b) II recorded in 10% $\text{H}_2\text{O}$  buffer at pH 6.8. ss (single strand, blue), B (green), S (red), W (purple) conformers. # impurity.



**Supplementary Fig. S5:** pH dependence of (a) imino proton (9~16 ppm) and (b) fluorine (-114 ~ -121 ppm) NMR spectra of the FAAF-modified duplex II recorded in 10% H<sub>2</sub>O buffer at 5°C. # impurity