Supplementary Material

Conformational and thermodynamic properties modulate the nucleotide excision repair of 2-aminofluorene and 2-acetylaminofluorene dG adducts in the *Nar*I sequence

Vipin Jain, Benjamin Hilton, Satyakam Patnaik, Yue Zou, M. Paul Chiarelli, and Bongsup P. Cho*

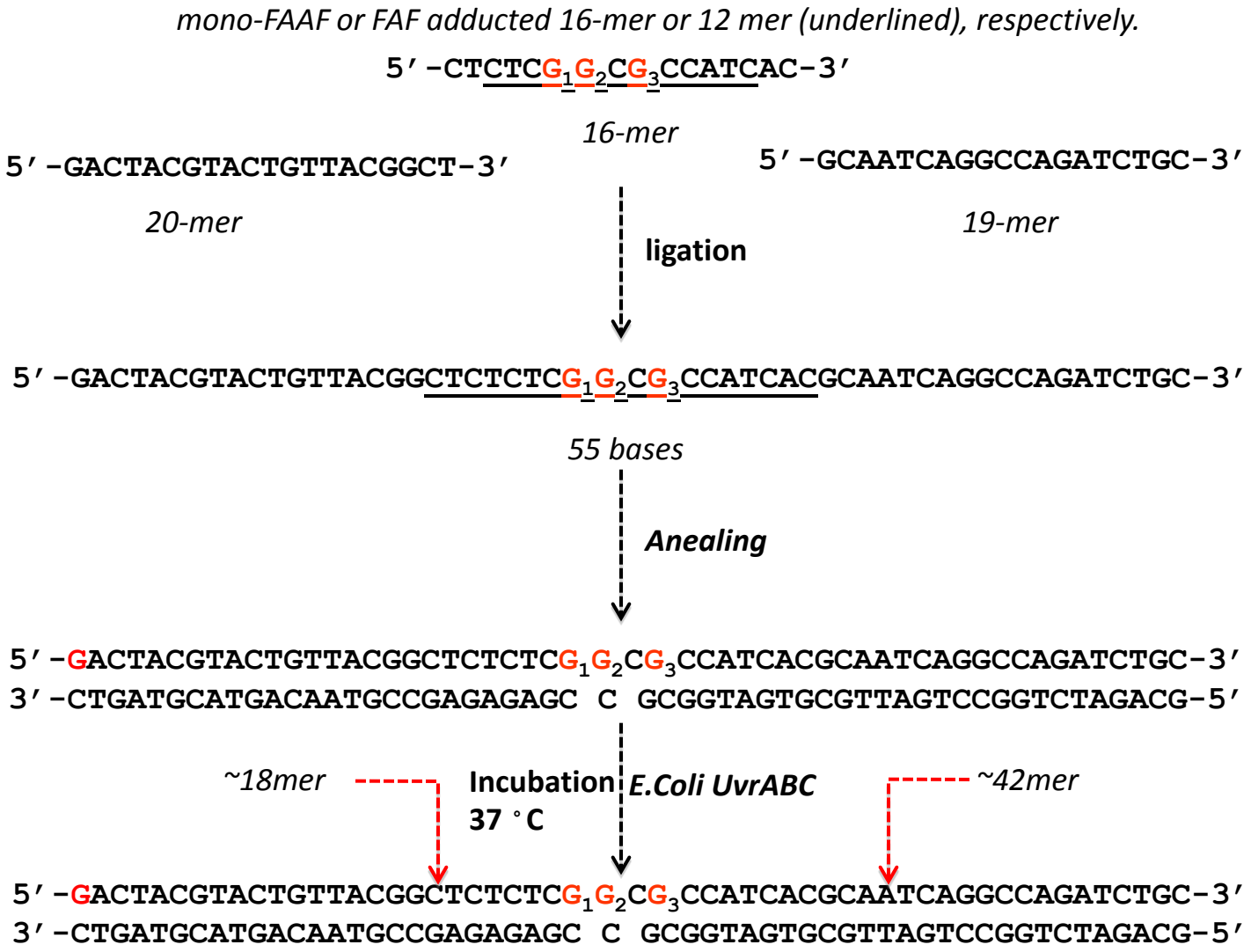

Figure S1: Schematic representation of strategy used for nucleotide excision repair by *E.coli* UvrABC for FAAF- and FAF-modified *Nar*I duplexes.

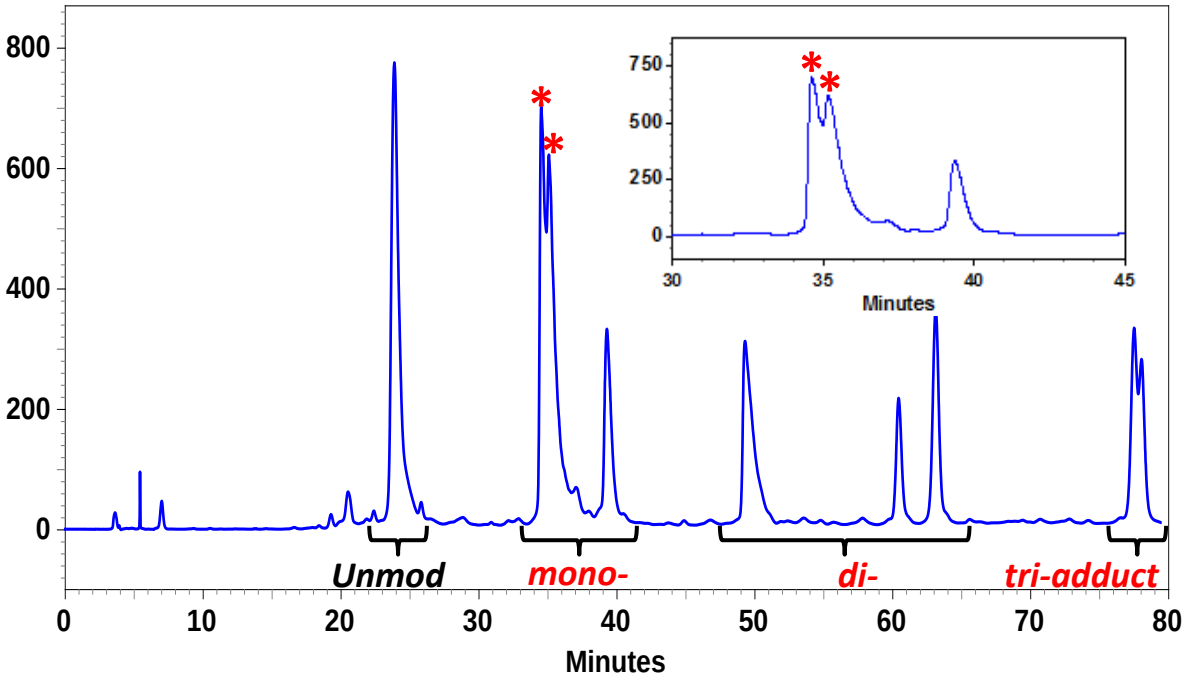

Figure S2: (a) HPLC chromatogram of a reaction mixture between 12-mer *Nar*I sequence (5'-CTCG$_1$G$_2$CG$_3$CCATC-3'**)** and N-acetoxy-N-2-(acetylamino)-7-fluorofluorene. The mono-, di-, and tri-FAAF adducts were eluted in the 33-41, 47-65, and 76-78 min ranges. The asterisked peaks could not be resolved. The gradient condition was 3-15% acetonitrile in ammonium acetate buffer (pH 7.0, 100 mM) for 40 min, followed by 15-35% for 60 min, and back to 3% acetonitrile in 10 min, flow rate 2 mL/min.

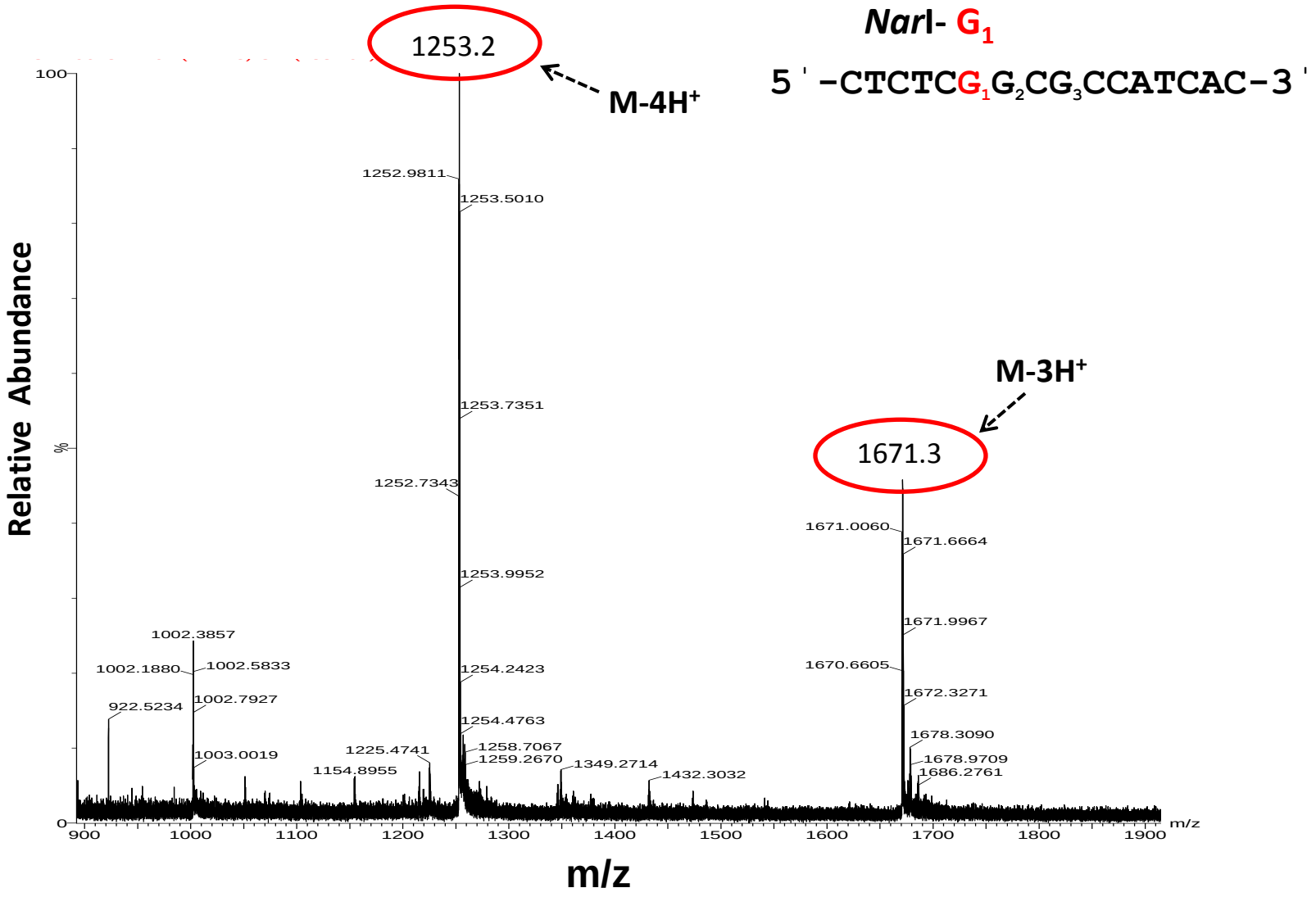

Figure S3: Molecular ion spectra of peak 1 obtained from FAAF-modification of *Nar*I 16-mer template conducted on Waters SYNAPT ESI-QTOF-mass spectrometer in negative ion and V-modes.

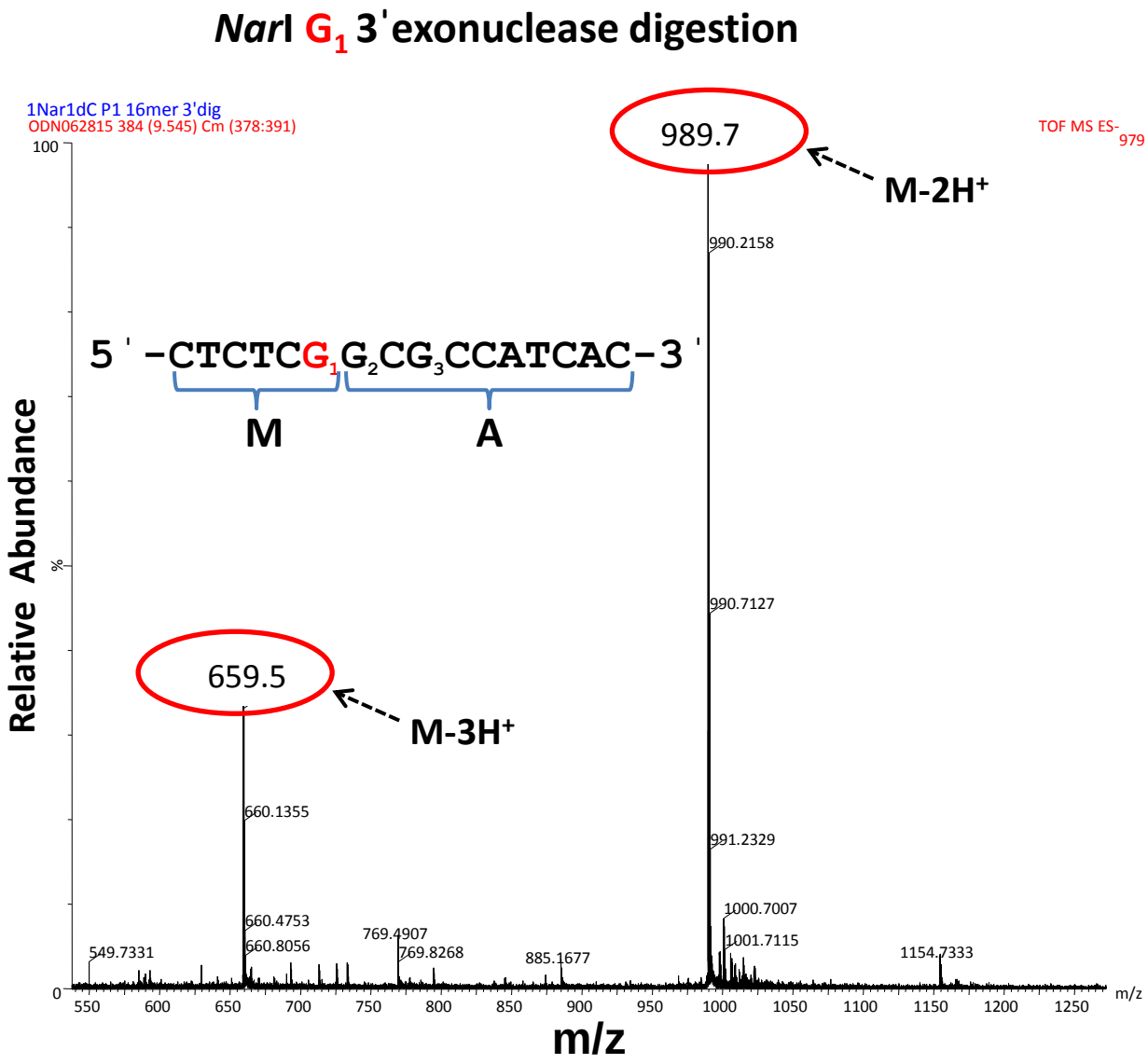

Figure S4: Molecular ion spectra of peak 1 after 3'-exonuclease digestion conducted on Waters SYNAPT ESI-QTOF-mass spectrometer in negative ion and V-modes.

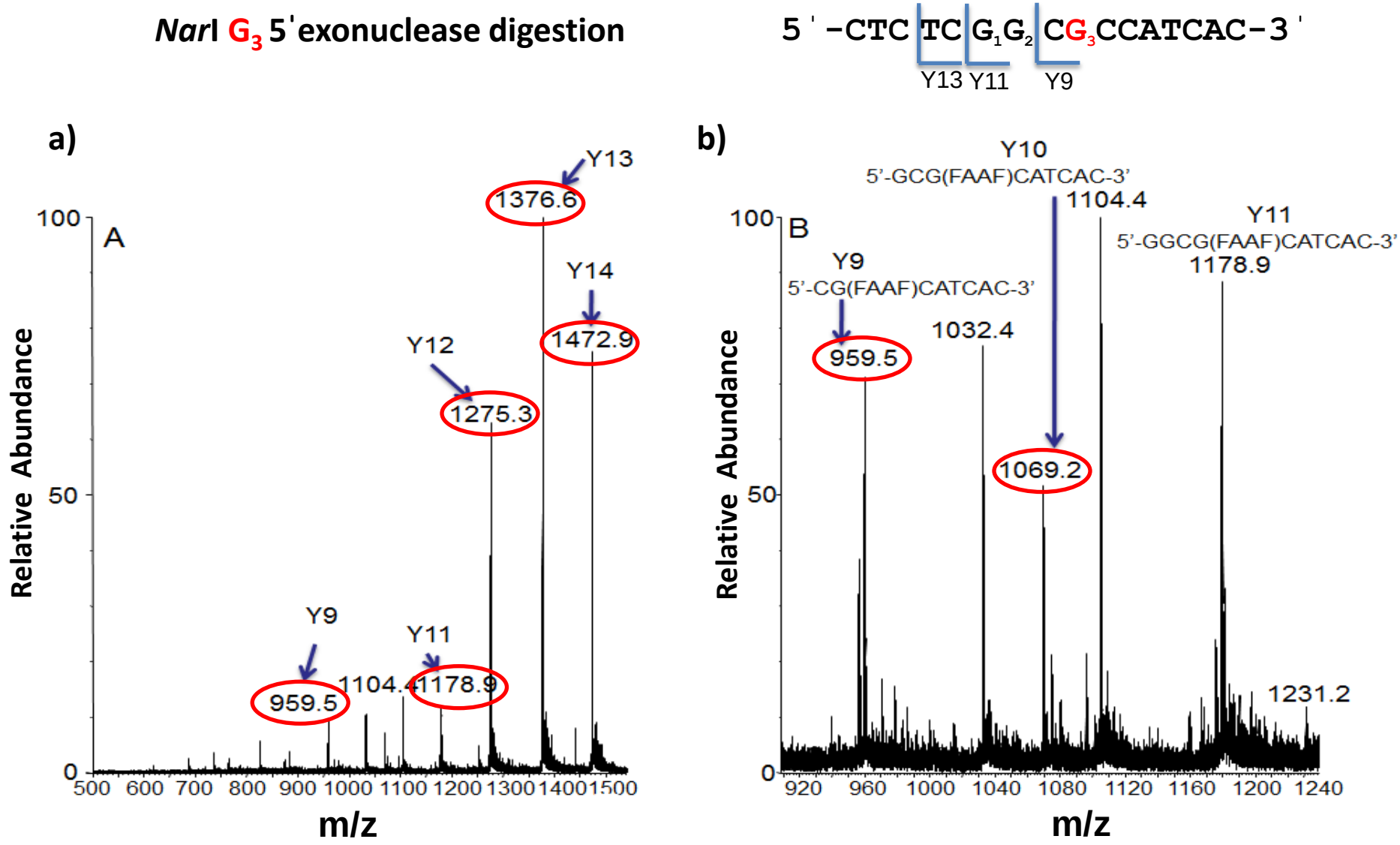

Figure S5: Molecular ion spectra of peak 2 after 5'-exonuclease digestion conducted on Waters SYNAPT ESI-QTOF-mass spectrometer in negative ion and V-modes (a) complete m/z range and (b) m/z range from 900-1240. The 5'-exonuclease digest products are labeled Y consistent with the accepted nomenclature first proposed by McLuckey and Habibigoudarzi (*J. Am. Chem. Soc.* **1993,** *115*, 12085–12095).

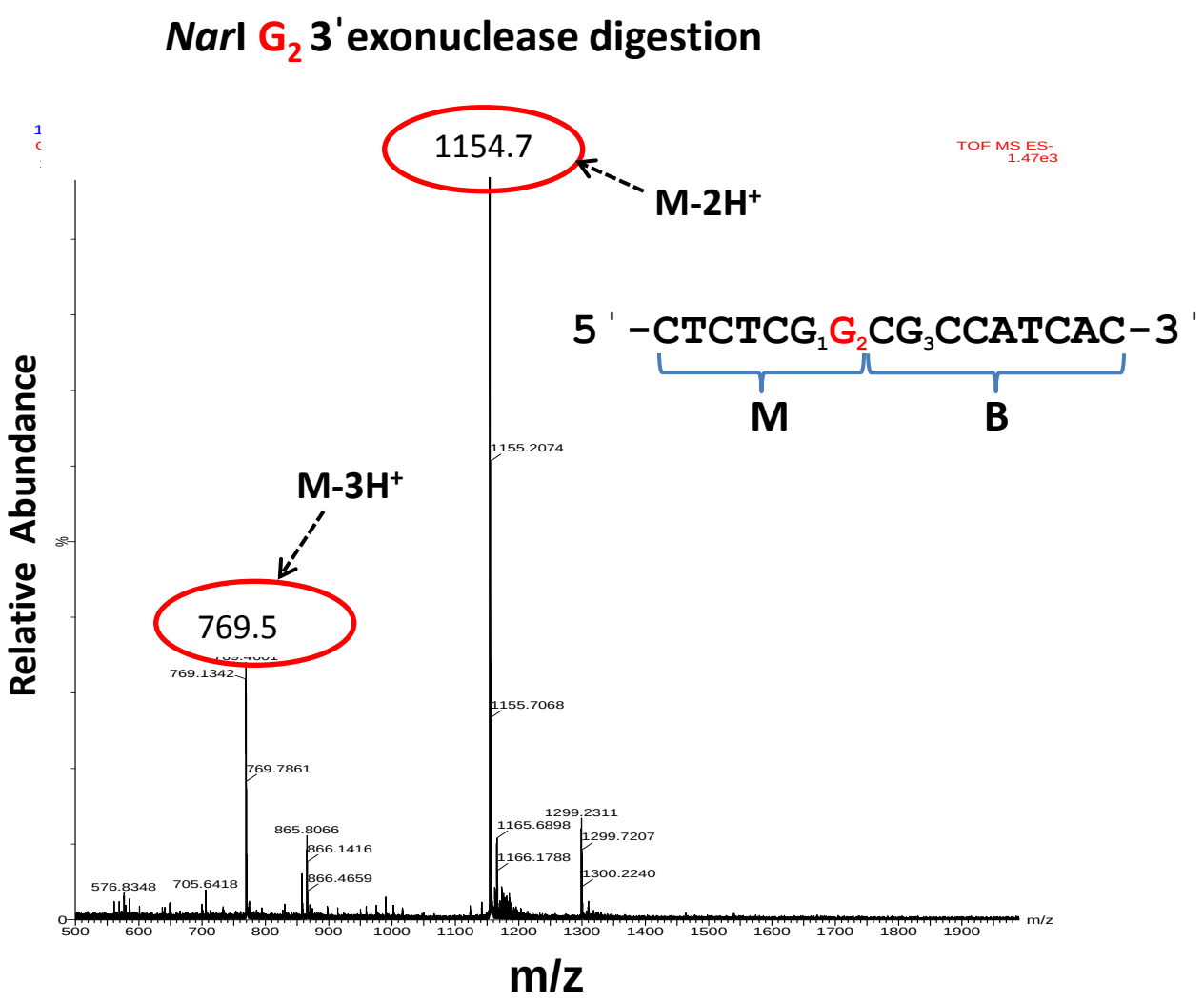

Figure S6: Molecular ion spectra of peak 3 after 3'-exonuclease digestion conducted on Waters SYNAPT ESI-QTOF-mass spectrometer in negative ion and V-modes.

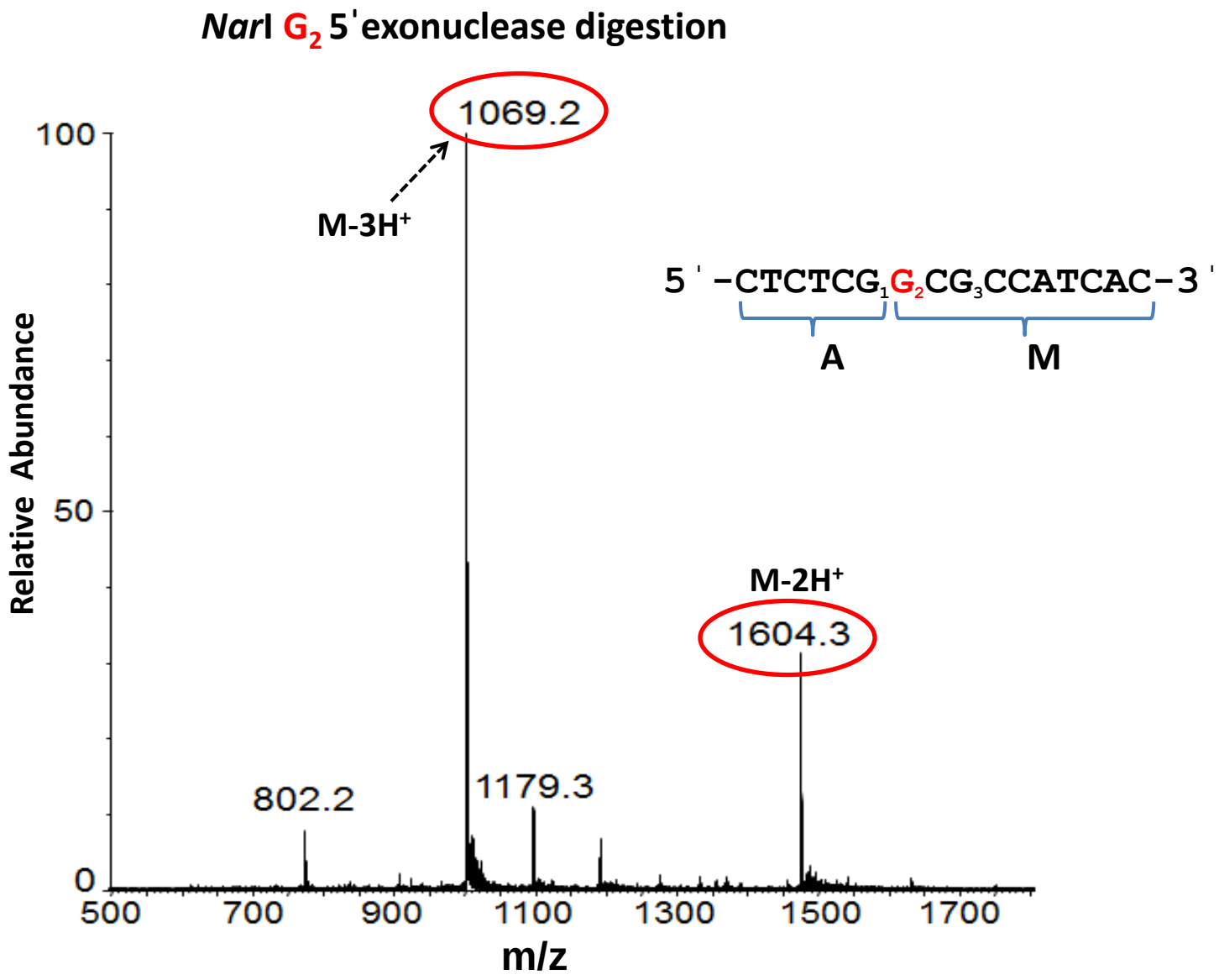

Figure S7: Molecular ion spectra of peak 3 after 5'-exonuclease digestion conducted on Waters SYNAPT ESI-QTOF-mass spectrometer in negative ion and V-modes.

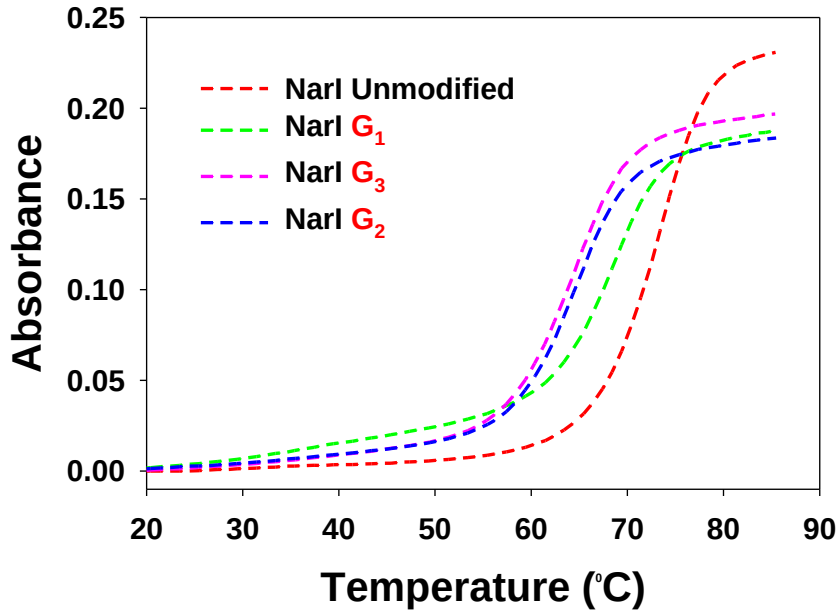

Figure S8: UV-melting curves of the three FAAF-*Nar*I duplexes and an unmodified control duplex, all at 6.4 μM in 0.2 M NaCl, 10 mM sodium phosphate, and 0.2 mM EDTA at pH 7 .

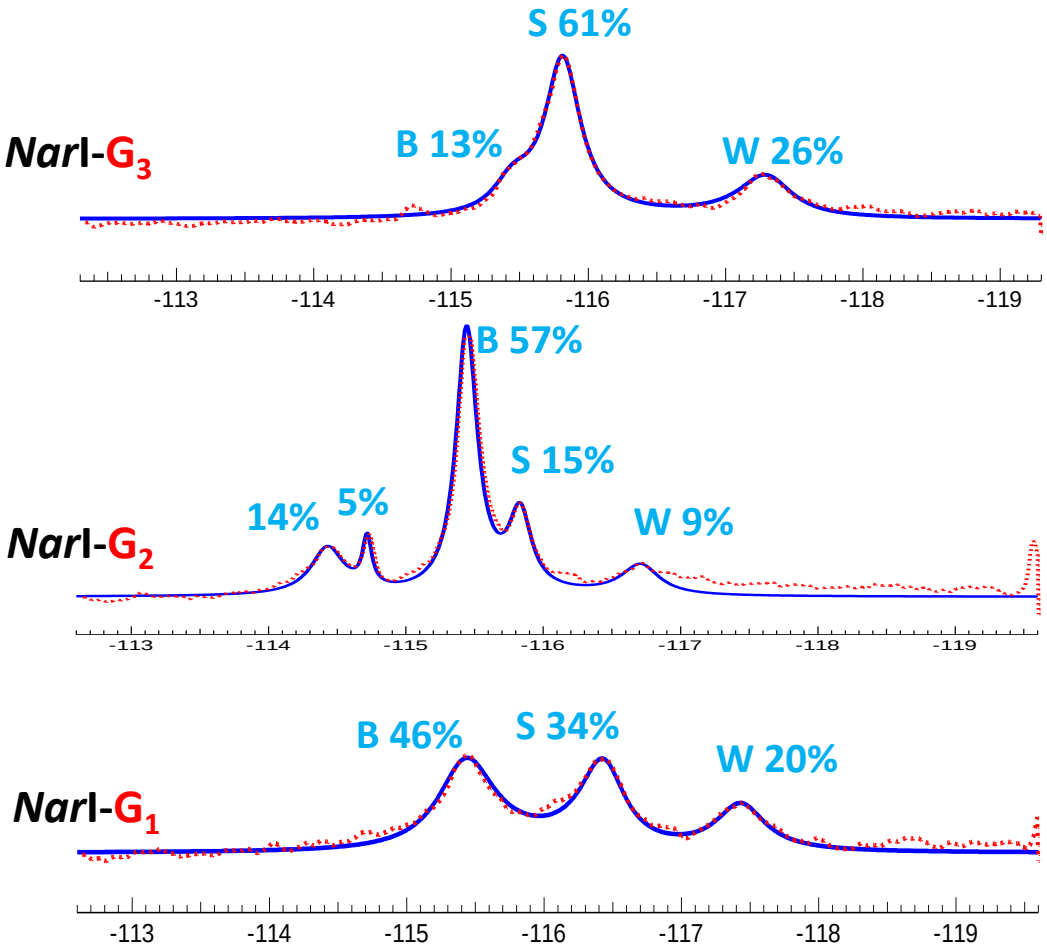

Figure S9: Line simulation of fully paired FAAF-modified *Nar*I 16-mer (5'-CTCTCG$_1$G$_2$CG$_3$CCATCAC-3') duplexes at 5 ˚C.

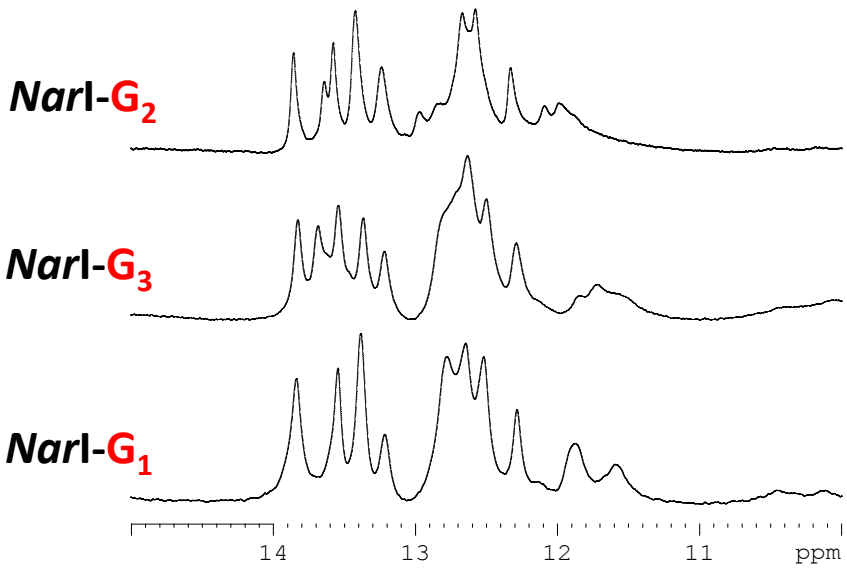

Figure S10: Imino proton region (10-15 ppm) of proton NMR of fully paired FAAF-modified *Nar*I 16-mer duplexes at 5 ˚C.

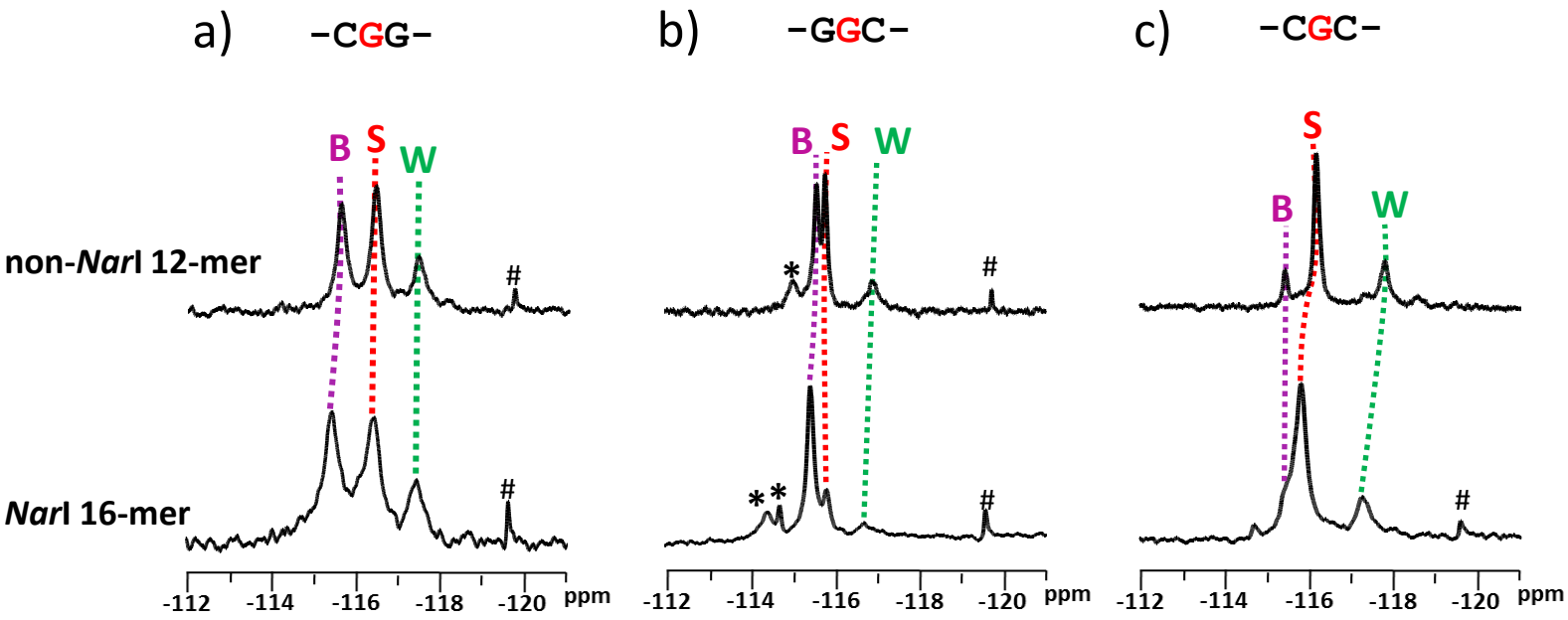

Figure S11: ^{19}F NMR chemical shifts comparison of FAAF-modified *Nar*I 16-mer and FAAF-modified non-*Nar*I 12-mer duplexes. a) -CGG-, b) -GGC-, and c) -CGG- sequence context at 5 ˚C. * unknown conformers; # impurity.

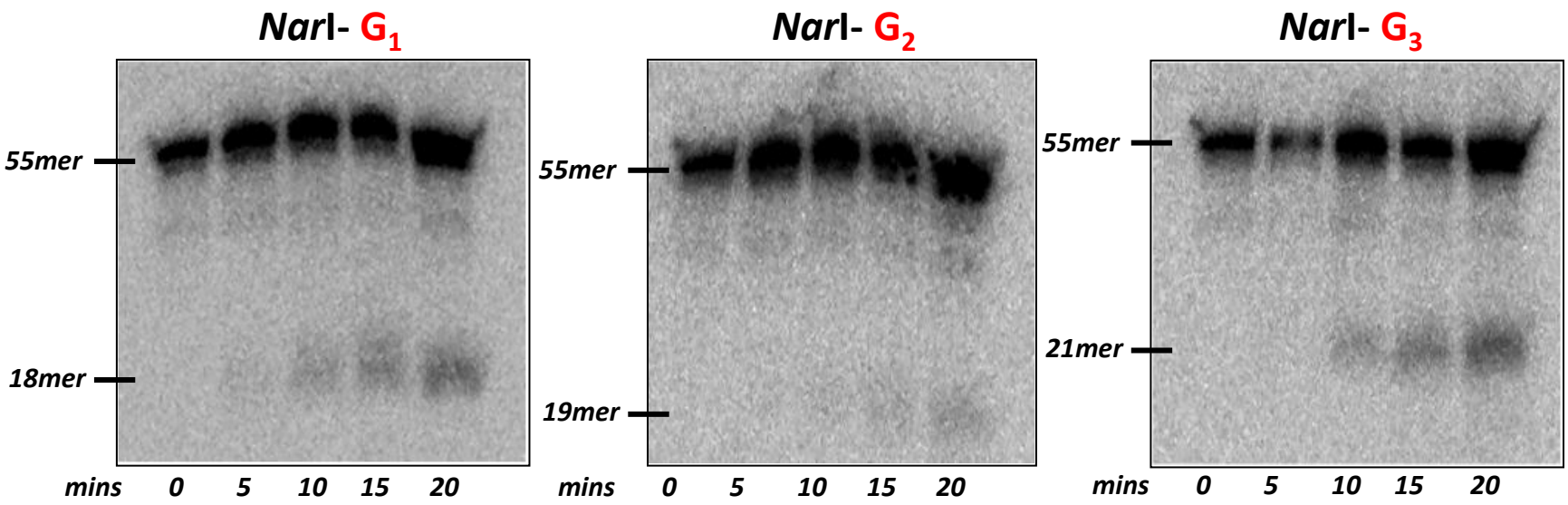

Figure S12: The 5'-terminally labeled DNA substrates containing the FAAF modified *Nar*I sequence (2 nM) were incubated with UvrABC (UvrA, 10 nM, UvrB, 250 nM, and UvrC, 100 nM) in UvrABC reaction buffer at 37°C for the time period mentioned above. The incision products were then analyzed on a 12% polyacrylamide sequencing gel under denaturing condition. The 55-mer represents the intact DNA substrates, and the 18mer, 19mer and 21mer represent the 5'-incised DNA fragments for *Nar*I G_1, *Nar*I G_2 and *Nar*I G_3, respectively.

Supplementary Table S1. Thermal and thermodynamic parameters of FAAF modified *Nar*I duplexes obtained from UV-melting curves

5'-CTCTCG$_1$G$_2$CG$_3$CCATCAC-3'
3'-GAGAGC C GCGGTAGAG-5'

	$-\Delta H$ *kcal/mol*	$-\Delta S$ *eu*	$-\Delta G_{37^\circ C}$ *kcal/mol*	T_m[b] ^oC	$\Delta\Delta H$[c] *kcal/mol*	$\Delta\Delta S$[d] *eu*	$\Delta\Delta G_{37^\circ C}$[e] *kcal/mol*	ΔT_m[f] ^oC
Control[a]	121.9	324.7	21.2	70.6	-	-	-	-
*Nar*I-G$_1$-FAAF[a]	117.2	315.8	19.2	66.0	4.7	8.9	2.0	-4.6
*Nar*I-G$_2$-FAAF[a]	116.2	317.1	17.9	61.9	5.7	7.6	3.3	-8.7
*Nar*I-G$_3$-FAAF[a]	111.9	304.4	17.5	61.8	10.0	20.3	3.7	-8.8

a) The results of curve fit and T_m-lnC_t dependence were within 15% of each other, and these numbers are averages of the two methods.The average standard deviations for -Δ*G*, -Δ*H*, and T_m are ±0.2, ±3.2, and ±0.4, respectively.
b) T_m values at 0.1mM extrapolated from these two methods.
c) $\Delta\Delta H$ = ΔH (modified duplex) - ΔH (control duplex).
d) $\Delta\Delta S$= ΔS (modified duplex) - ΔS (control duplex).
e) $\Delta\Delta G$ = ΔG (modified duplex) - ΔG (control duplex).
f) ΔTm = Tm (modified duplex) - Tm (control duplex).

Supplementary Table S2. Chemical shift information of different conformers exhibited by FAAF-modified *Nar*I and non-*Nar*I duplexes

FAAF modified duplexes	^{19}F Chemical Shifts at 5˚C (ppm)			
	Minor conformers	***B***	***S***	***W***
*Nar*I-C$G_1$$G_2$		-115.4	-116.4	-117.4
*Nar*I-$G_1$$G_2$C	-114.4, -114.7	-115.4	-115.8	-116.7
*Nar*I-CG_3C	-114.7	-115.5	-115.8	-117.3
non-*Nar*I-CGG		-115.6	-116.4	-117.4
non-*Nar*I-GGC	-115.0	-115.6	-115.8	-116.9
non-*Nar*I-CGC		-115.4	-116.2	-117.8