Supporting Information

Binding and NMR Structural Studies on Indoloquinoline-Oligonucleotide Conjugates Targeting Duplex DNA

Andrea Eick, Fanny Riechert-Krause, and Klaus Weisz*

Institute of Biochemistry, Ernst-Moritz-Arndt University Greifswald, Felix-Hausdorff-Str. 4, D-17487 Greifswald, Germany

List of Contents

Table S1. Molar extinction coefficients, synthesis yields and mass spectral data for the TFO-PIQ conjugates

TFO	ε_{260nm} [M^{-1} cm^{-1}]	average yield [%]	MS (m/z)	
			found	calcd
PIQ-TTCTCCTTT	97618	70.8	3158.7	3156.6
PIQ-CCCTCCTTT	95118	62.8	3126.2	3126.6
PIQ-TCCTCCTTT	96718	59.8	3141.3	3141.6
PIQ-CTCTCCTTT	96018	62.7	3141.7	3141.6

Description of the fluorescence energy transfer experiments

To get additional information on binding interactions with the indoloquinoline conjugate, triplex **Tn4** was modified by replacing adenine in the purine-rich strand of the underlying duplex by a 2-aminopurine base (AP) at either position +1 (**AP1**) or -1 (**AP2**) with respect to the TFO recognition sequence. 2-Aminopurine has a more than one order of magnitude higher fluorescence quantum yield when compared to its adenine analog. Thus, with Ap incorporated at the triplex-duplex junction in **AP1-PIQ** and **AP2-PIQ**, an excitation energy transfer from the 2-aminopurine base to PIQ may be expected in case of intercalation of the ligand at the triplex-duplex junction. Because the absorbance maximum of Ap in DNA at 304 nm is separated from the π-π* absorbance of the major DNA bases at about 260 nm, it also allows for selective excitation of the fluorophore above 300 nm. It must be noted, however, that energy transfer efficiencies may be small due to the relatively low oscillator strength of PIQ with ε_{350} ~ 5300 $M^{-1}\cdot cm^{-1}$ in the wavelength region of spectral overlap between Ap emission and the absorption of bound PIQ (Figure S1A).

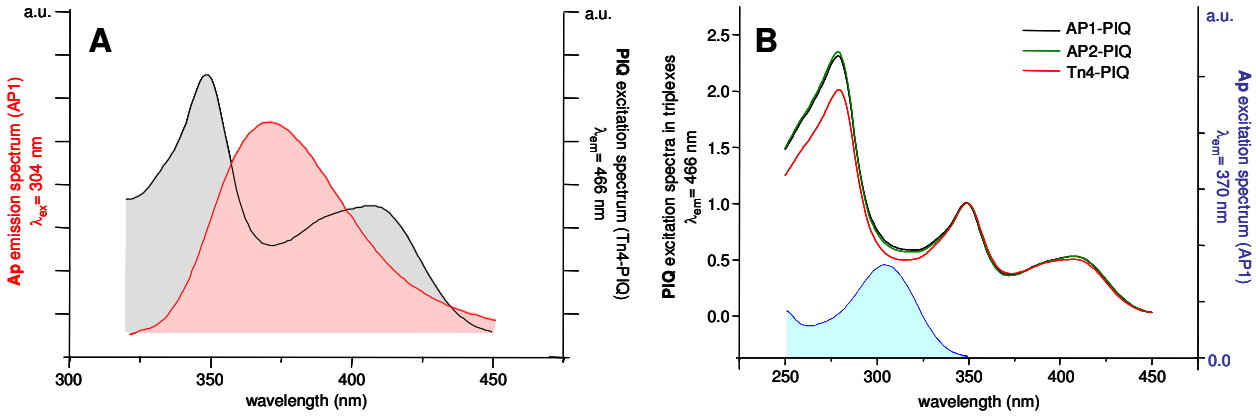

Figure S1. (A) Superposition of the fluorescence emission spectrum of 2-aminopurine (Ap) in triplex **AP1** with an excitation wavelength λ_{ex} = 304 nm and of the excitation spectrum of the PIQ ligand in triplex **Tn4-PIQ** recorded at its fluorescence emission maximum λ_{em} = 466 nm. The latter is identical in shape to the corresponding absorption spectrum but benefits from a better sensitivity. (B) Excitation spectra of PIQ in triplexes **Tn4-PIQ**, **AP1-PIQ** and **AP2-PIQ** with λ_{em} = 466 nm normalized to an excitation wavelength of 350 nm and excitation spectrum of Ap in **AP1** with λ_{em} = 370 nm. The latter is mostly identical in shape to the corresponding absorption spectrum but benefits from a better sensitivity and the elimination of high DNA absorbances at λ < 300 nm.

To assess potential energy transfer from Ap to PIQ in the triple-helical oligonucleotides, fluorescence excitation spectra were recorded for **Tn4-PIQ**, **AP1-PIQ** and **AP2-PIQ**. Initially, optical properties of PIQ in **Tn4-PIQ** and in the two Ap-modified triplexes were found to be equal within experimental error at excitation wavelengths > 340 nm beyond the AP absorption, indicative of a negligible impact on the absorbance and fluorescence properties from minor structural changes caused by the Ap substitutions. Assuming an unchanged ligand extinction coefficient upon Ap incorporation below ~340 nm as can be expected from the invariant PIQ absorbance at longer wavelengths, any change in the emission intensity measured for an excitation wavelength λ can thus be attributed to energy transfer processes from Ap or other DNA bases to the ligand. Because only PIQ shows absorption at wavelengths above 340 nm, fluorescence excitation spectra of **Tn4-PIQ**, **AP1-PIQ** and **AP2-PIQ**, acquired under the same experimental conditions at the emission maximum of PIQ at 466 nm, were normalized with respect to the intensity at an excitation wavelength of 350 nm and plotted in Figure S1B. Apparently, both Ap-modified triplexes exhibit an increased fluorescence intensity at lower wavelengths while being unaffected within experimental error above ~340 nm. With no DNA absorption from standard bases above 300 nm, the small but noticeable intensity increase in the range 300-330 nm coinciding with the Ap absorption must be attributed to some energy transfer from the 2-aminopurine. On the other hand, an increase in fluorescence intensity below 300 nm may also involve enhanced transfer efficiency from the natural DNA bases in the presence of Ap. It has to be noted, however, that DNA in the samples had a significant absorbance at 260 nm of up to 0.8, making the measured fluorescence intensity in this wavelength region less reliable. No attempt to correct for the absorption of excitation light in the 1-cm cells was made because in the wavelength range from $\lambda \geq 300$ nm maximum absorbances were < 0.05.

Experimental

Fluorescence measurements were carried out with 2-aminopurine modified triplexes (3 μM) in cacodylate buffer (0.1 M NaCl, 0.02 M cacodylate, 1 mM spermine, pH 5.0). Emission spectra were measured with an excitation wavelength λ_{ex} = 304 nm for Ap and λ_{ex} = 350 nm for PIQ. The excitation spectra were recorded with an emission wavelength λ_{em} = 370 nm for Ap and λ_{em} = 466 nm for the indoloquinoline. All experiments were performed at 20 °C and three spectra were accumulated each. All data were blank and volume corrected.

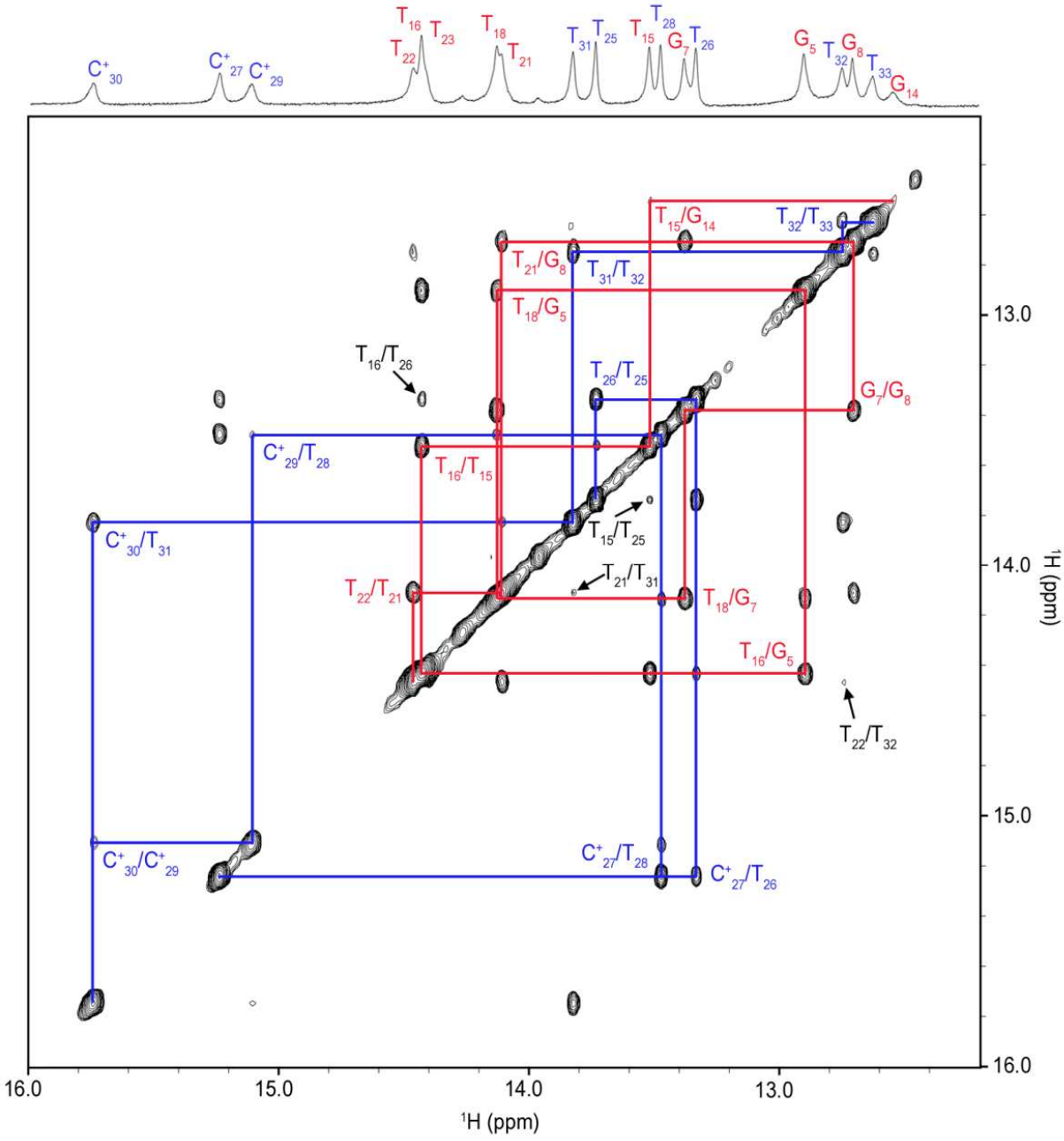

Figure S2. Portion of a NOESY spectrum (200 ms mixing time) of triplex **T1s** (0.65 mM) acquired at 10 °C in 90% H_2O/10% D_2O, 100 mM NaCl, 1 mM NaN_3, pH 5.0. Sequential imino-imino connectivities between Watson-Crick hydrogen-bonded iminos and Hoogsteen hydrogen-bonded iminos are indicated by red and blue lines, respectively. Intratriplet crosspeaks of the third strand iminos to the Watson-Crick iminos of the underlying duplex are marked by black arrows. A corresponding 1D spectrum with resonance assignments is shown on top.

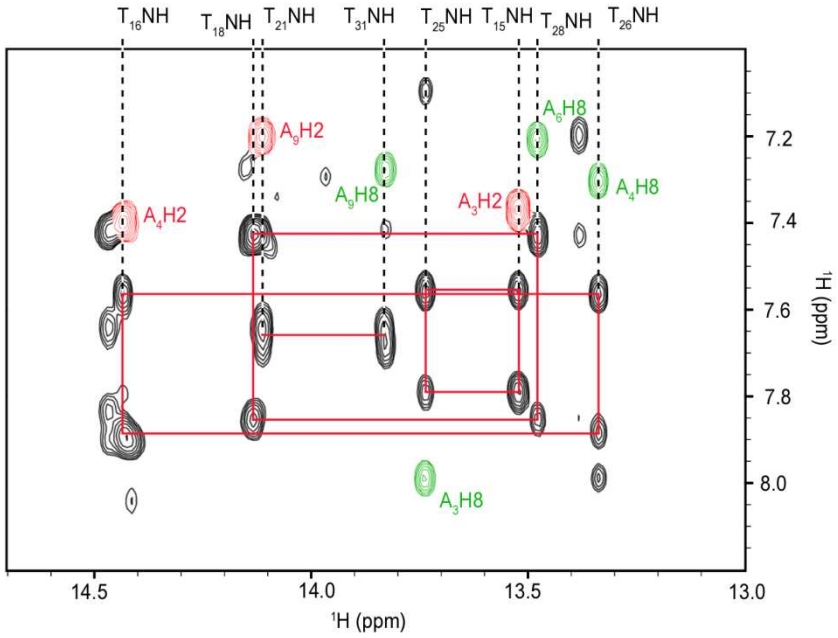

Figure S3. Region of a NOESY spectrum of **T1s** (0.65 mM) showing intratriplet crosspeaks of Watson-Crick and Hoogsteen hydrogen-bonded thymine iminos to adenine H2 (red) and H8 protons (green) as well as to the shared adenine aminos (connected by red lines). The spectrum was acquired at 10 °C in 90% H_2O/10% D_2O, 100 mM NaCl, 1 mM NaN_3, pH 5.0 with a mixing time of 200 ms.

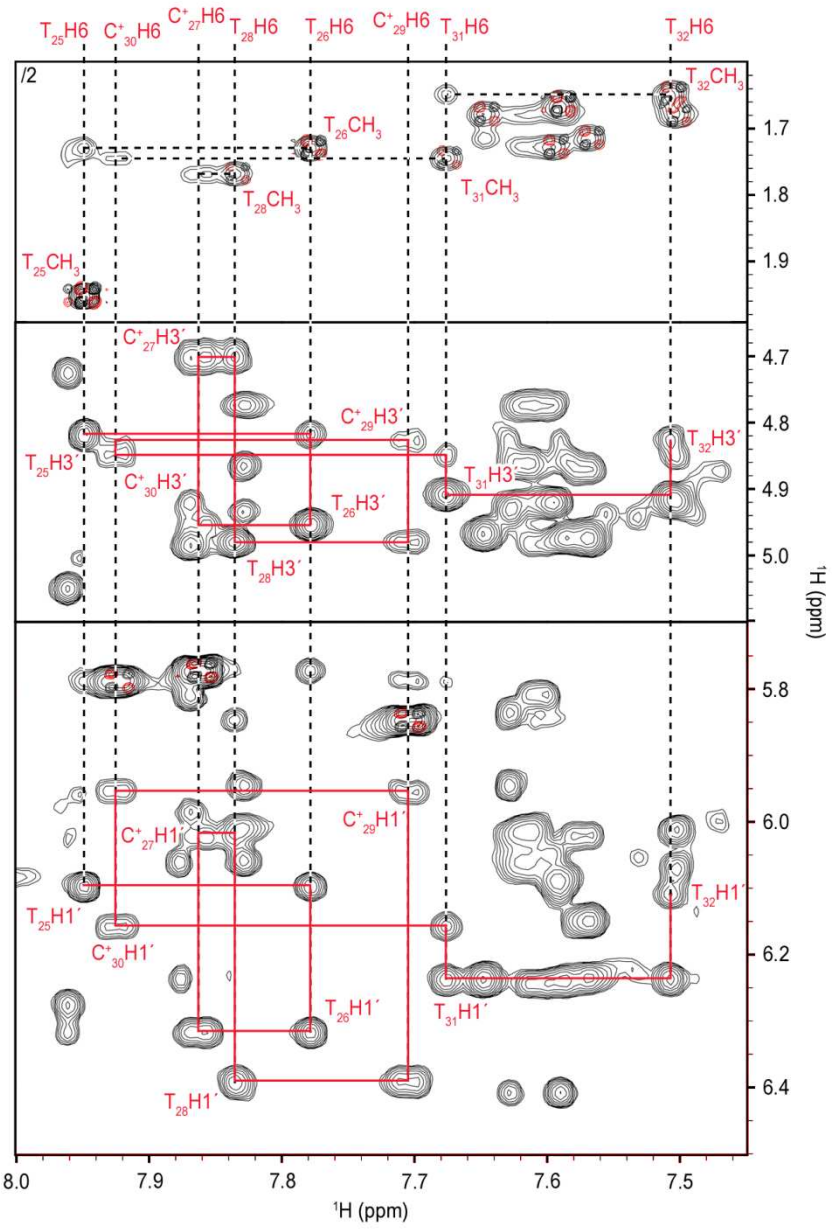

Figure S4. NOESY spectral regions with H6-H1', H6-H3' and H6-CH_3 NOE crosspeaks. Sequential walks within the TFO of the triplex **T1s** (0.65 mM) are indicated by red lines. The spectrum was acquired at 20 °C in 100% D_2O, 100 mM NaCl, 1 mM NaN_3, pH 5.0 with a mixing time of 200 ms. A superimposed DQF-COSY spectrum identifies the scalar coupled cytosine H5 and H6 as well as thymine CH_3 and H6 protons.

Table S2. Chemical shifts δ (in ppm) of exchangeable[a] and non-exchangeable[b] protons of the triplex T1s[c]

nucleotide	imino	amino		H1‘	H3‘	H5/H2/CH_3	H6/H8
		NH_2(1)	NH_2(2)				
G_1	----	----	----	6.00	4.91		7.86
C_2		8.17	6.57	5.54	4.72	5.14	7.09
A_3		7.79	7.54	6.27	5.04	7.36	7.95
A_4		7.87	7.55	----	4.86	7.39	7.27
G_5	12.89	n.d	----	----	----		7.40
A_6		7.84	7.42	----	----	----	7.17
G_7	13.37	----	----	----	----		7.33
G_8	12.70	----	----	----	----		7.39
A_9		7.66	7.63	----	----	7.19	7.25
A_{10}		----	----	----	----	----	7.62
A_{11}		----	----	----	----	----	7.82
C_{12}		8.02	6.85	----	----	5.22	7.35
C_{13}		8.14	6.83	----	----	5.59	7.60
G_{14}	12.54	----	----	5.80	4.97		7.86
T_{15}	13.52			5.98	4.92	1.67	7.30
T_{16}	14.42			6.08	----	1.71	7.56
C_{17}		8.27	7.25	----	----	5.53	7.61
T_{18}	14.12			----	----	1.68	7.50
C_{19}		8.52	7.18	----	----	5.53	7.61
C_{20}		8.25	6.68	----	----	5.63	7.57
T_{21}	14.10			----	----	----	----
T_{22}	14.46			----	----	----	----
T_{23}	14.40			----	----	----	----
G_{24}	----	----	----	----	----		----
T_{25}	13.73			6.09	4.81	1.94	7.94
T_{26}	13.33			6.31	4.95	1.72	7.77

C^{+}_{27}	15.23	9.99	9.26	6.01	4.69	5.77	7.86
T_{28}	13.47			6.38	4.97	1.76	7.83
C^{+}_{29}	15.10	9.99	8.82	5.95	4.82	5.84	7.70
C^{+}_{30}	15.74	10.10	9.46	6.15	4.84	5.79	7.92
T_{31}	13.82			6.23	4.90	1.74	7.67
T_{32}	12.74			6.10	----	1.64	7.50
T_{33}	12.62			----	----	----	----

[a] At 283 K in H_2O.
[b] At 293 K in D_2O.
[c] 0.65 mM triplex in 100 mM NaCl, 1 mM NaN_3, pH 5.0.

Table S3. Chemical shifts δ (in ppm) of exchangeable and non-exchangeable DNA protons in the selectively ^{15}N-,^{13}C-labeled triplex conjugate T1s-PIQ at 283 K.[a,b] Labeled nucleotides are written in bold

nucleotide	imino	amino		H5/H2/CH_3	H6/H8
		NH_2(1)	NH_2(2)		
G_1	----	----	----		----
C_2		8.05	6.67	4.99	7.02
A_3		7.03	6.66	----	----
A_4		7.62	7.35	----	----
G_5	12.71	----	----		----
A_6		----	----	----	----
G_7	13.32	----	----		----
G_8	12.69	----	----		----
A_9		----	----	----	----
A_{10}		----	----	----	----
A_{11}		----	----	----	----
C_{12}		7.96	6.74	5.10	7.27
C_{13}		7.87	6.64	5.35	7.43
G_{14}	11.70	----	----		----
G_{14}*	11.76	----	----		----
T_{15}	12.50			1.41	----
T_{15}*	12.00			1.17	----
T_{16}	14.27			1.71	----
T_{16}*	14.28			1.63	
C_{17}		8.10	7.05	5.45	7.59
T_{18}	14.11			----	----
C_{19}		8.49	7.17	5.47	7.60
C_{20}		8.22	6.68	5.61	7.55
T_{21}	14.05			----	----
T_{22}	14.44			----	----

T_{23}	14.38			----	----
G_{24}	----	----	----		----
T_{25}	13.66			1.60	----
T_{25}*	13.45			----	----
T_{26}	13.03			----	----
T_{26}*	13.17			----	----
C^{+}_{27}	15.17	9.89	9.23	5.77	7.86
C^{+}_{27}*	14.87	----	----	----	----
T_{28}	13.45			----	----
C^{+}_{29}	14.93	9.96	8.80	5.84	7.70
C^{+}_{30}	15.76	10.07	9.43	5.78	7.92
T_{31}	13.80			----	----
T_{32}	12.71			----	----
T_{33}	12.57			----	----

[a] 0.42 mM triplex in 90% H_2O/10% D_2O, 84 mM NaCl, 1 mM NaN_3, pH 5.0.
[b] Nucleotides of minor complex are marked by asterisks.

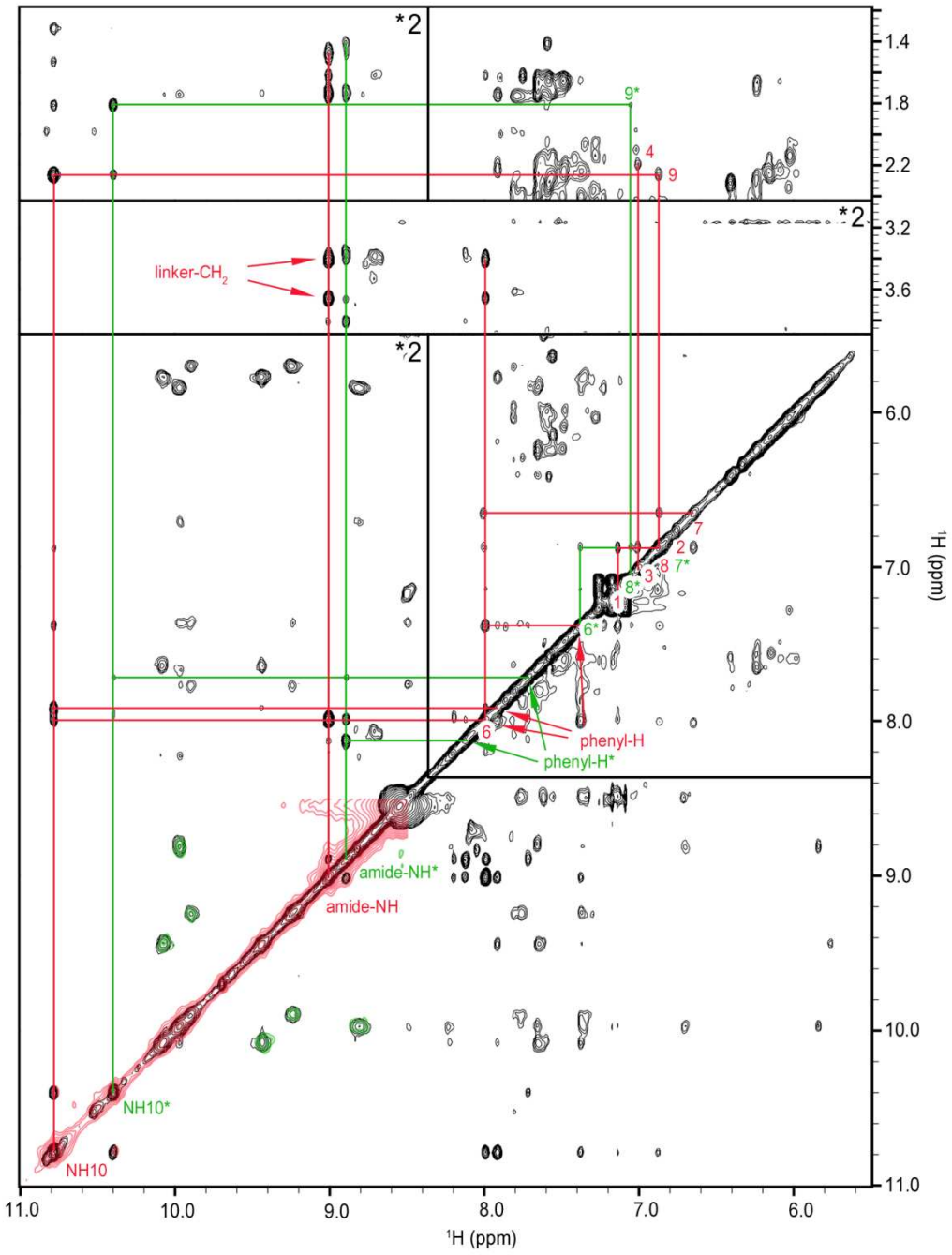

Figure S5. Portions of a 2D NOE spectrum of triplex **T1s-PIQ** (0.42 mM) acquired at 10 °C with a mixing time of 200 ms in 90% H_2O/10% D_2O, 84 mM NaCl, 1 mM NaN_3, pH 5.0. To better identify crosspeaks of the PIQ protons in case of extensive spectral overlap with intra-DNA contacts of lower intensity, corresponding spectral regions are displayed with a higher threshold level as indicated in the figure. In the spectral region from 8.5-11.0 ppm a superimposed ROESY spectrum confirms chemical exchange crosspeaks between PIQ protons of the two complexes. The intramolecular NOE connectivities of the PIQ moiety in the minor and the major complex are indicated by green and red lines, respectively.

Table S4. ^{1}H chemical shift δ (ppm) of PIQ protons in the free TFO-PIQ conjugate[a] and in the triplex conjugate T1s-PIQ[b]

PIQ protons	TFO-PIQ conjugate	**T1s-PIQ**	
		major complex	minor complex
H1	7.64	7.13	----
H2	7.47	6.86	----
H3	7.67	7.01	----
4Me	2.92	2.18	2.41
NH^+5	----	11.21	----
H6	8.47	8.00	7.38
H7	7.19	6.65	6.86
H8	7.43	6.88	7.05
9Me	2.41	2.25	1.80
NH10	----	10.75	10.36
phenyl-H	7.77 (2H), 8.08 (2H)	7.91, 7.99, 7.36, 7.99	7.97, 8.12, 7.71, 8.20
amide-NH	----	9.00	8.88
linker CH_2-	----	3.65, 3.39, 3.80	3.79, 3.34, 3.64

[a] 0.62 mM TFO-PIQ conjugate in100% D_2O at 293K.
[b] 0.42 mM **T1s-PIQ** in 90% H_2O/10% D_2O, 84 mM NaCl, 1mM NaN_3, pH 5.0 at 283K.

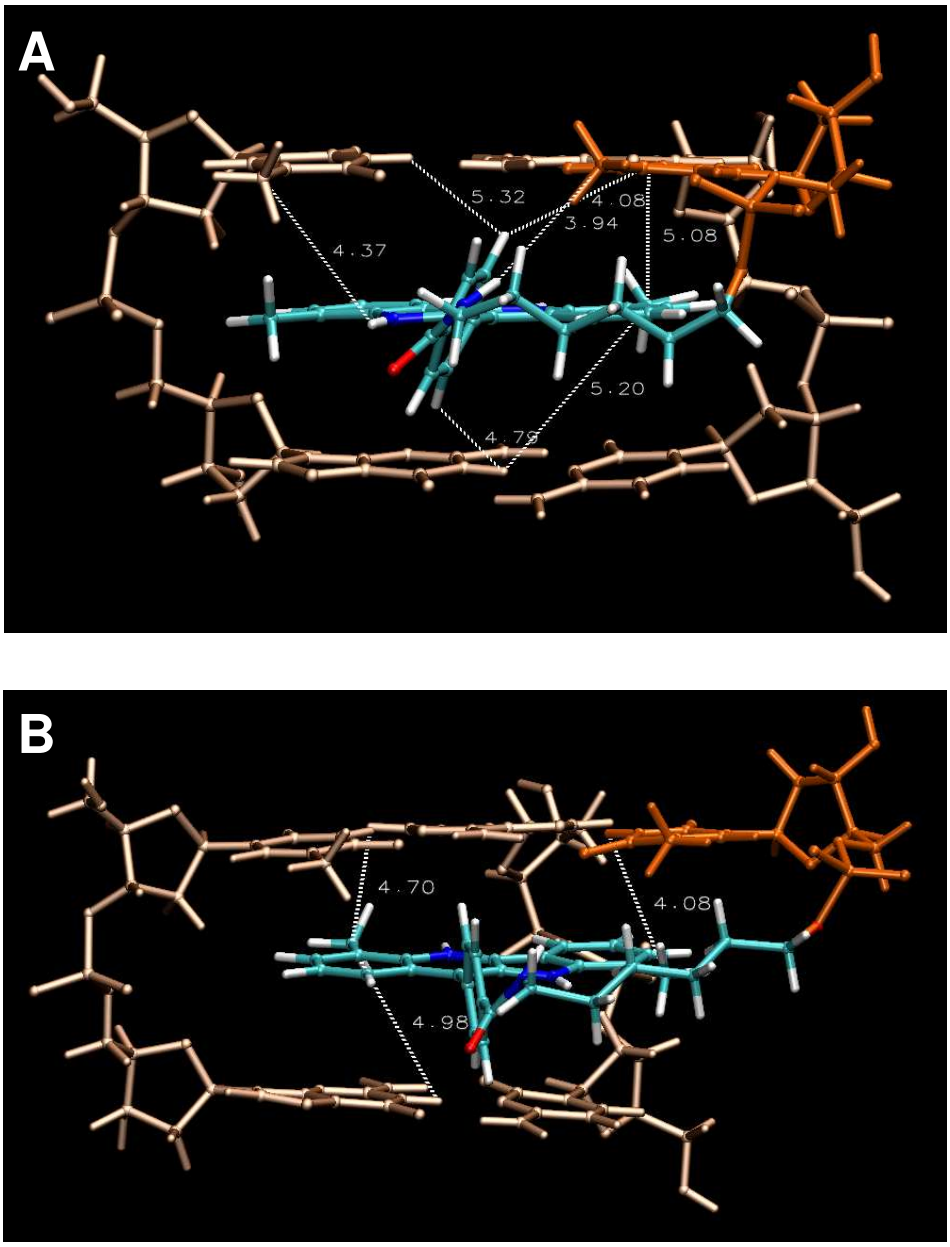

Figure S6. Molecular models of the intercalation site for the major (A) and minor complex (B) of triplex **T1s-PIQ**. NOE-derived PIQ-DNA distance restraints used for the model building are indicated together with the corresponding distances of the final complex geometries.