Electronic Supplementary Information

Experimental

All chemicals and oligonucleotides used within this study were purchased from Sigma-Aldrich.

UV spectroscopy was carried out on a Cary 300 spectrometer equipped with a 6x6 temperature controlled cell changer. UV spectra were recorded from 230-350 nm at 1°C intervals between 20-90 °C , with a temperature change rate of 1°C/min and a hold time of 1 min at each temperature. From these spectra UV melting curves were extracted at 260 and 295 nm.

Fig S1 – Representative UV spectra for HIF-1α S, 2uM DNA, 50mM sodium cacodylate pH 5.0

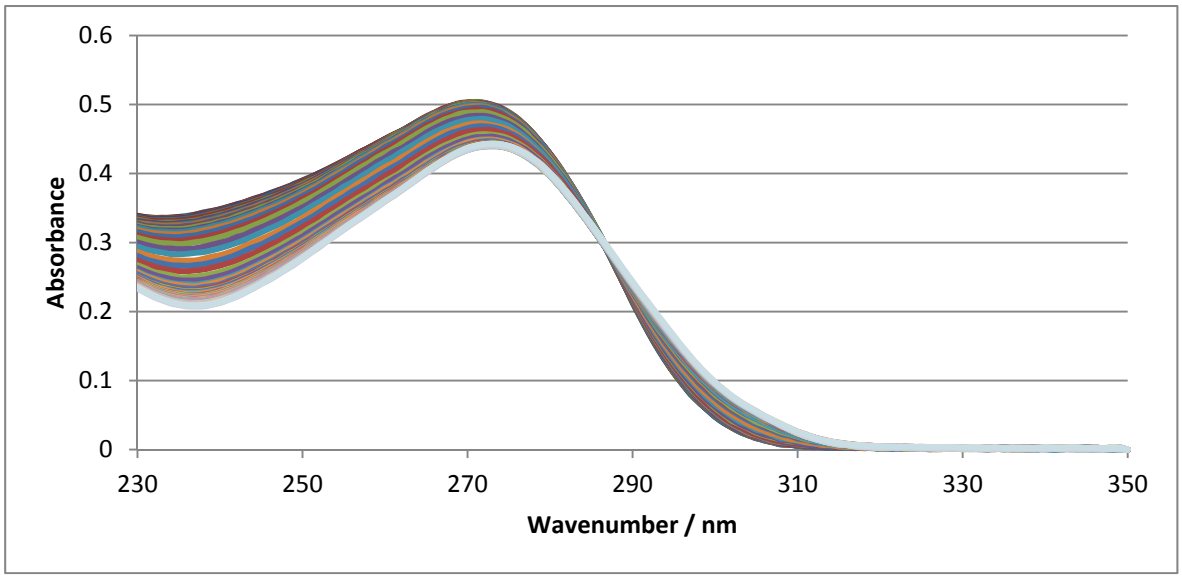


Fig S2 – UV melting profile at 260 nm for HIF-1α S, 2uM DNA, 50mM sodium cacodylate pH 5.0

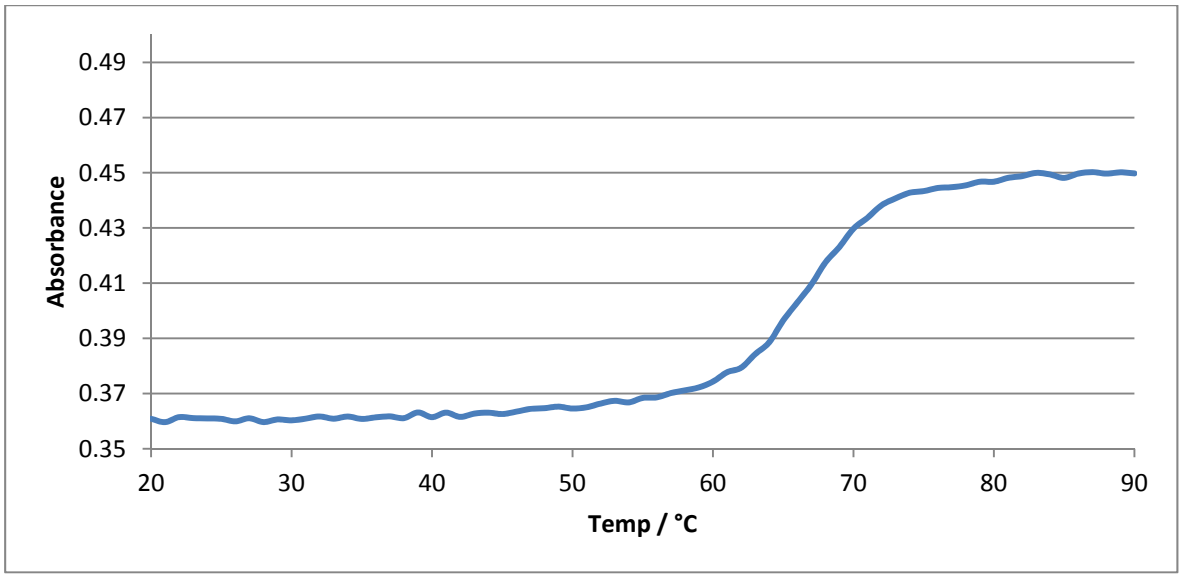


Fig S3 – UV melting profile at 295 nm for HIF-1α S, 2uM DNA, 50mM sodium cacodylate pH 5.0

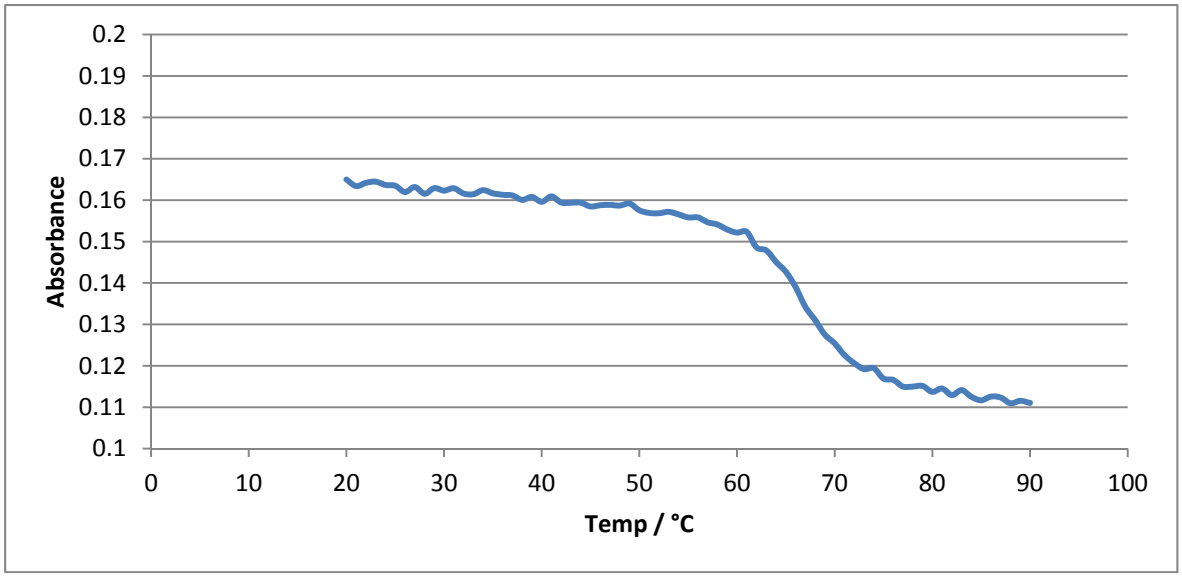


Table 1 – T_m values calculated at pH 5 and pH 7 for cooling (90-20) and heating (20-90) experiments. n.f. – no i-motif formation evident. n.d. I-motif formation evident, T_m not calculated.

		260 nm				295 nm			
		pH 5		pH 7		pH 5		pH 7	
		90-20	20-90	90-20	20-90	90-20	20-90	90-20	20-90
c-kit	2uM	50	52	n.f.	n.f.	53	53	n.f.	n.f.
	10uM	50	52			53.5	53.5		
c-myb	2uM	58	59.5	n.f.	n.f.	59	60	n.f.	n.f.
	10uM	57.5	59			58.5	59		
HIF-1α S	2uM	64.5	66	25	27	65.5	66	25	27
	10uM	65.5	66			66	66		
HIF-1α S -CG	2uM	66	66	23	24	66.5	66.5	23	24.5
	10uM	66	67			66.5	67		
HIF-1α S2	2uM	51	53	n.f.	n.f.	53.5	54.5	n.f.	n.f.
	10uM	51	54			53.5	54		
HIF-1α LG	2uM	61.5	64	<25	~27	62.5	63.5	n.d.	n.d.
	10uM	63.5	64.5			62.5	64		
h-tert	1uM	66	69	n.f.	n.d.	67	69.5	n.d.	n.d.
	5uM	66.5	69			67	69		
h-tert 1	2uM	56	58	n.f.	n.f.	55.5	55.5	n.f.	n.f.
	10uM	55	57			55	56		
h-tert 2	2uM	65	66	<25	<25	65	66	<25	<25
	10uM	66	67.5			67	67		
PDGF	2uM	78	78.5	27	32	78	78.5	27	32
	10uM	78.5	78.5			78.5	79		

NMR Experiments

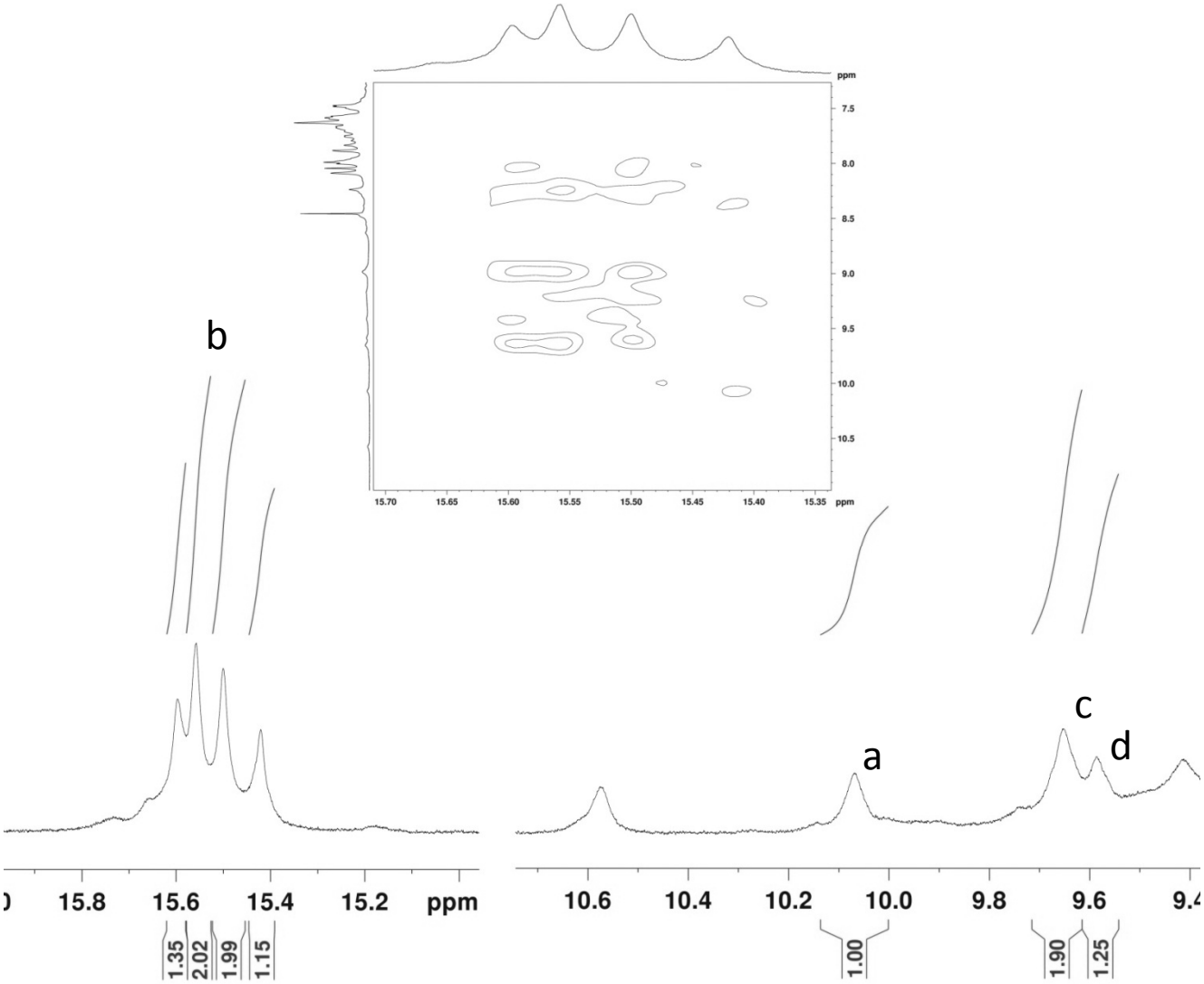
"The sample (*ca.* 7 mg) was prepared for NMR by dissolution in 90%H_2O/10%D_2O (*ca.* 0.5 ml; the solvent also contained d_4-TSP as a reference). All NMR experiments were performed using a Bruker Avance III 700 MHz instrument, equipped with a TCI cryoprobe.

^{1}H NMR spectra were collected with presaturation of water at the transmitter frequency (O1 = 4.697 ppm) which was applied during both the mixing time and relaxation delay of the pulse sequence "noesygppr1d". 10240 repetitions of the pulse sequence were made, with a relaxation delay of 4 s between scans (total experiment time of 15 hours 18 mins - including 4 dummy scans at the start of the experiment). For each increment, 65536 data points were sampled in a total acquisition time of 1.34 s (FID resolution 0.747 Hz). A negative exponential function (line broadening = 0.3 Hz) was applied to the resulting co-added time data prior to Fourier transformation to a spectrum which consisted of 65536 points, covering a spectral width of 35 ppm.

2D-NOESY spectra were collected in phase sensitive mode with water suppression by excitation sculpting with gradients at the transmitter frequency (O1 = 4.697 ppm; pulse sequence "noesyesgpphpp"). 2048 data points were sampled in a total acquisition time of 59 ms (FID resolution 17 Hz) in the directly acquired dimension (F2); with 256 increments used to create the second dimension (F1). 264 repetitions of the pulse sequence were made for each increment, with a relaxation delay of 1 s between repetitions (total experiment time of 22 hours 54 mins - including 32 dummy scans at the start of the experiment). QSINE window functions (SSB = 2) were applied in both direct and indirect dimensions to the resulting co-added time-data, prior to two-dimensional Fourier transformation to a spectrum which consisted of 4096 points in both F2 and F1 (linear prediction was applied in F1). The spectral width was 25 ppm in both dimensions, and the mixing time for build up of the nuclear Overhauser enhancement was 100 ms."

The 2D NOESY spectra allowed the identification of a single cytosine amino proton (labelled **a** in figure S4). The imino protons (Fig S4, peaks **b**) were subsequently integrated with respect to proton **a**. For verification amino proton peaks labelled c and d in Fig S4 were also integrated, with an expected integral of 2 and 1 respectively)

Fig S4 – 1D ^{1}H NMR of **ODN5a**, inset – 2D NOESY showing imino and amino proton regions.



Potential loop size

Based on the NMR observation of 6 protonated cytosine-cytosine base pairs, and taking into account the sequence composition the following loop sizes are possible:

3:4:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
2:5:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
1:6:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
3:3:3 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
2:4:3 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
1:5:3 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
3:4:1 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
2:5:1 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
1:6:1 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
3:3:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
2:4:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC
1:5:2 CGCGCTCCCGCCCCCTCTCCCCTCCCCGCGC

All of these potential loop arrangement result in either one long loop and two shorter loops, or three short loops. Although there is an ability to form a long central loop, this the tempered by the shortness of the other two loops, this is clearly not a Class II arrangement consisting of three long loops. The arrangement in which the loops are all short in length results in a structure that would be classified as Class I.