Electronic Supplementary Information

Probing structural changes of self assembled i-motif DNA†

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Contents

Experimental details

Calculations for binding constant

The binding constant value of ThT with the i-motifs has been determined from the emission intensity data following the modified Benesi–Hildebrand equation,[1, 2] graph plotting is done by originpro.

$1/ I-I_{min} = 1/ I_{max}-I_{min} + 1/ \{K[C] (I_{max}-I_{min})\}$

Where,

I_{min} = Emission intensities of ThT considered in the absence of i-motifs,

I = An intermediate i-motifs concentration, and

I_{max} = Concentration of complete saturation

K = Binding constant

C = i-motifs concentration respectively.

From the plot of $[1 / (I-I_{min})]$ against $[C]^{-1}$ for i-motifs, the value of K has been determined from the slope. The binding constant (K_a) as determined by fluorescence titration method for the motifs i1 (RET) with ThT is found to be 2.516×10^5 M^{-1} (error < 10%) and for the motifs i2 (Rb) with ThT is found to be 1.332×10^5 M^{-1} (error < 10%).

Calculations for sigmoidal transition midpoint value

The data fitting is done by sigmoidal fit in originpro. We calculated the transition midpoint values using boltzman equation.

$Y = A_2 + A_1 - A_2 / \{1 + e^{(x - x_0)/dx}\}$

$A_1 = I_{max}$; $A_2 = I_{min}$; dx = Slope or Width; X_0 = Center

Fluorescence spectroscopy Fluorescence experiments were done on a CARY Eclipse Fluorescence Spectrophotometer using either 480 nm excitation wavelength and emission spectra were recorded from 500 to 800 nm. Emission spectra, presented as an average of two successive scans.

CD spectroscopy All the CD (Circular Dichroism) experiments were done using a JASCO J-815 CD spectropolarimeter equipped with Peltier temperature controller. All the data were collected from 350 to 200 nm at a scan rate of 200 nm/min at 0.5 nm data intervals and are presented as an average of three successive scans unless specified.

CD melting curves All the CD (Circular Dichroism) experiments were done using a JASCO J-815 CD spectropolarimeter equipped with Peltier temperature controller. All the data of CD melting curves were collected from 20 ^{o}C to 95 ^{o}C at a scan rate of 2 ^{o}C /min and monitored at 290 nm.

The melting curves are measured by a RT (real time) monitoring of a heat-induced nucleic acid (i-motif) dissociation which can be checked by the change of the referenced mean/median CD intensity (MCI) at a defined temperature. By definition, the melting point (T_m) is the inflection point of the melting curve. On molecular level circa 50% of the nucleic acids are dissociated at T_m. The melting peak (Equation 1) can be determined from the first negative derivative (Equation 2) of the melting curve.

$$T_m = max(refMCI'(T)) \quad (1)$$

$$refMCI'(T) = -d(refMCI)/d(T) \quad (2)$$

At this temperature peak the rate of change is maximal. The T_m is highly reproducible, thus can be used as a "characteristic identity" to distinguish nucleic acid species. For this calculation, the Sigmoidal fit in the OriginPro was used.

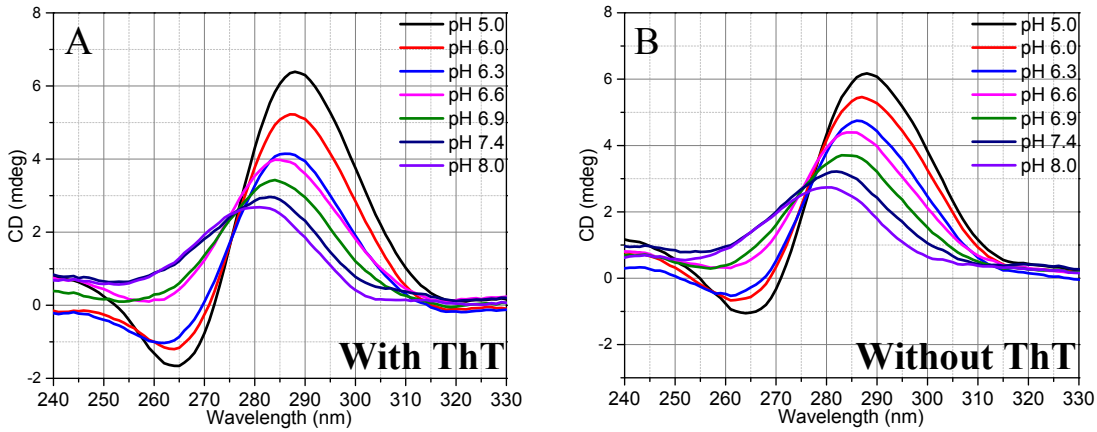

Fig. S1 CD spectra of **i1** (A) with and (B) without ThT at various pH values from pH 5.0 (black line, i-motif structure) to pH 8.0 (purple line, random coil). All samples were prepared by 1.0 μM DNA and 6.0 μM ThT in 50 mM Tris-HCl buffer at 25 °C.

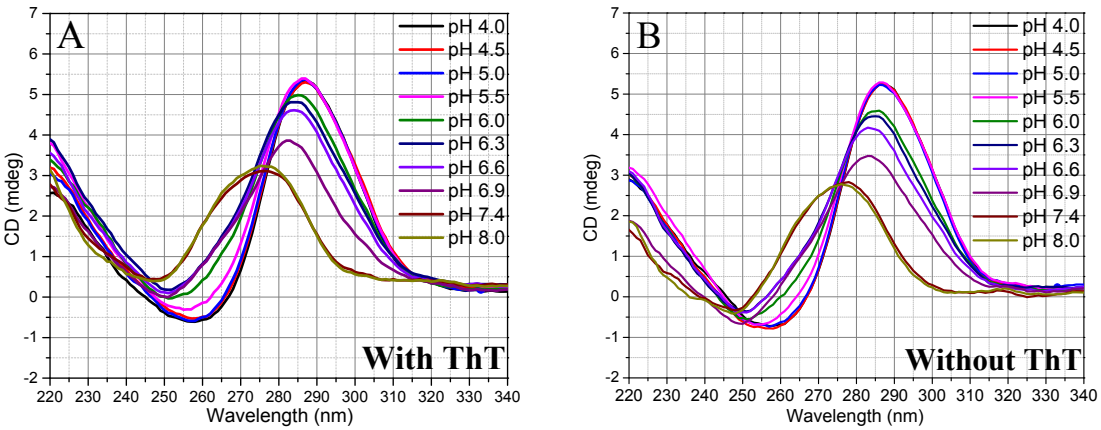

Fig. S2 CD spectra of **i2** (A) with ThT and (B) without ThT at various pH values from pH 4.0 (black line, i-motif structure) to pH 8.0 (dark yellow line, random coil). All samples were prepared by 1.0 μM DNA and 6.0 μM ThT in 50 mM Tris-HCl buffer at 25 °C.

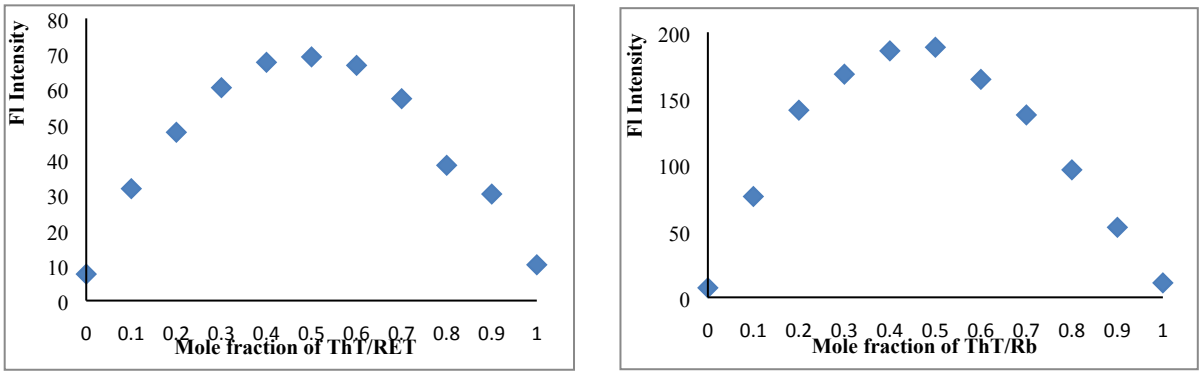

Fig. S3 Job's plot for the complex between i-motifs (**i1** and **i2**) and the ThT. All samples were prepared using **i1** (6.0 µM, pH 8.0), **i2** (6.0 µM, pH 5.0), and ThT (6.0 µM) in 50 mM Tris-HCl buffer at 25 °C. The fluorescence spectra were measured after excitation at 425 nm. Symmetric plots with maximum at 0.5 mole fraction indicate the 1:1 stoichiometry in the present system.

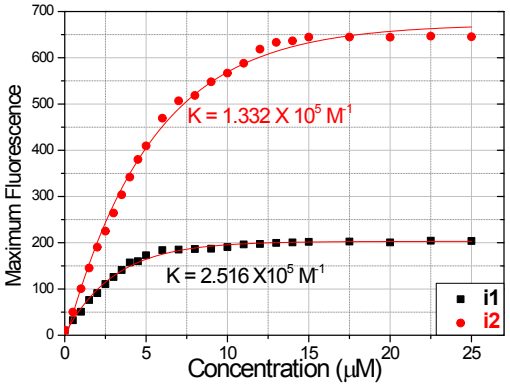

Fig. S4 Binding curves at 485 nm and evaluated binding constant values of ThT (6 µM) with different concentrations of i-motifs (0.5 µM – 25 µM). All samples were prepared at pH 8.0 for **i1** (RET) and at pH 5.0 for **i2** (Rb), titration in 50 mM Tris-HCl buffer at 25 °C and the fluorescence spectra were measured after excitation at 425 nm.

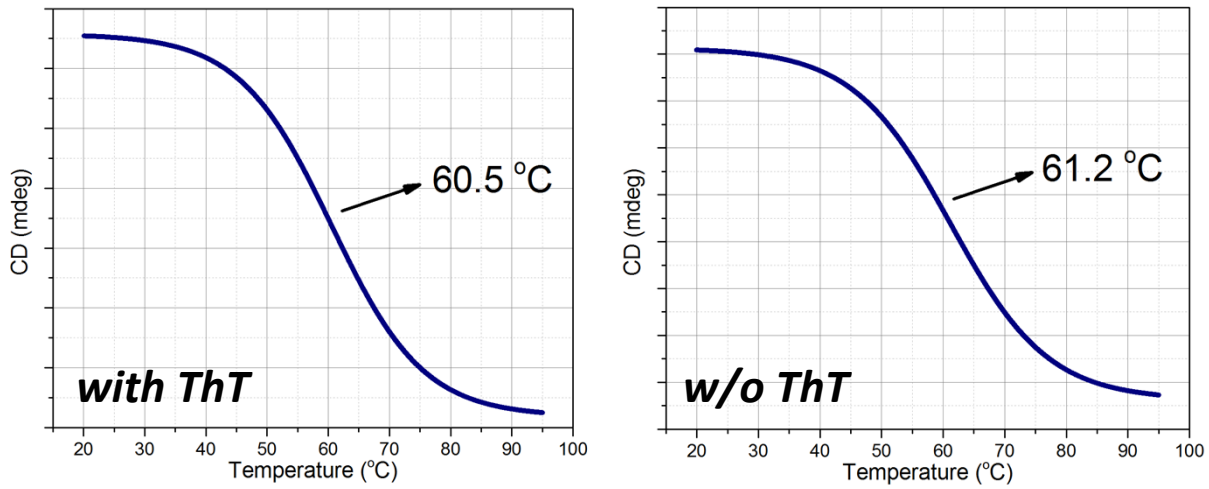

Fig. S5 Melting curves measured by CD for **i1** at pH 5.0 with and without ThT. All samples were prepared by 1.0 μM DNA and 6.0 μM ThT in 50 mM Tris-HCl buffer. Melting of i-motif structure was monitored at 290 nm.

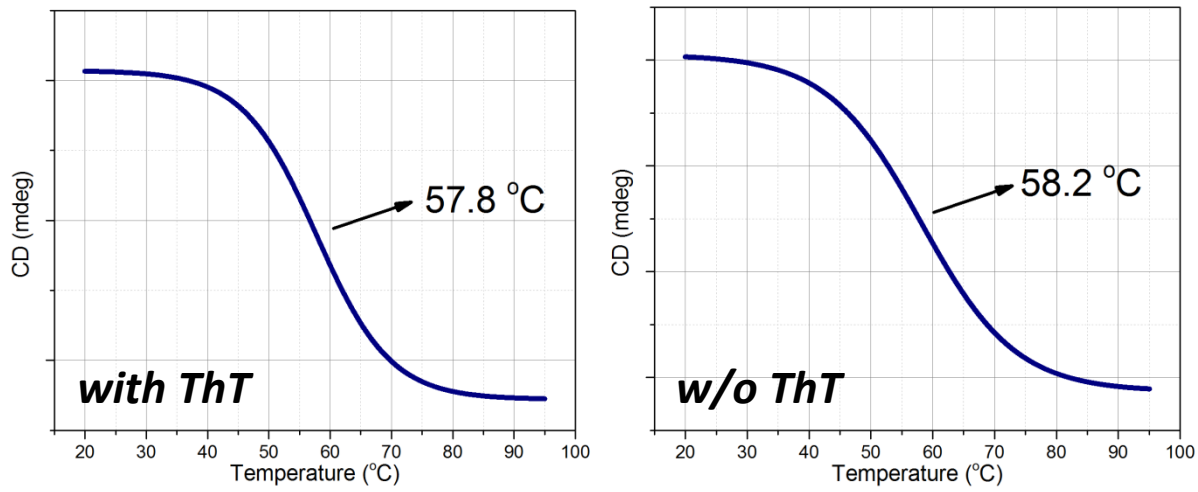

Fig. S6 Melting curves measured by CD for **i2** at pH 6.3 with and without ThT. All samples were prepared by 1.0 μM DNA and 6.0 μM ThT in 50 mM Tris-HCl buffer. Melting of i-motif structure was monitored at 290 nm.

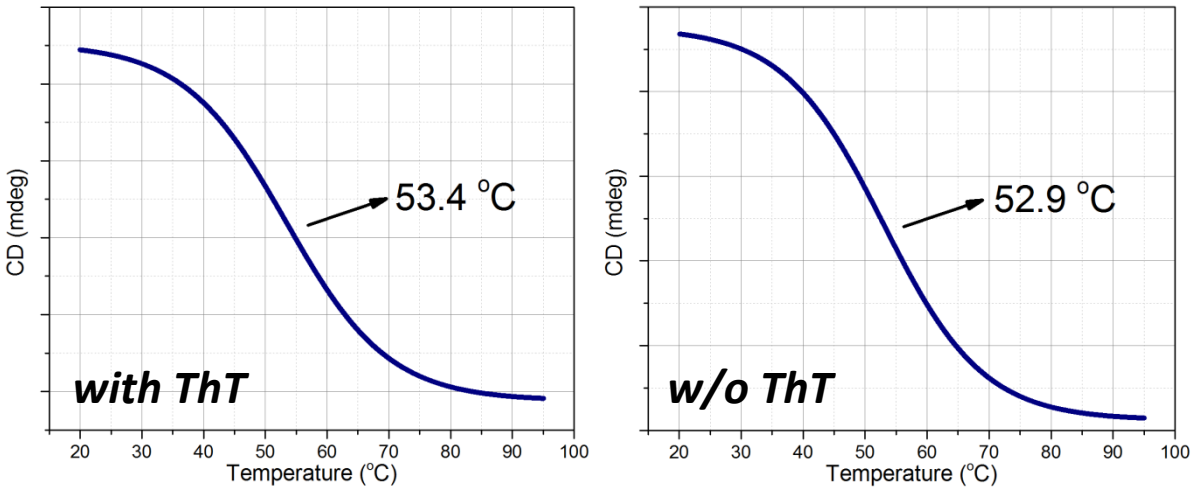

Fig. S7 Melting curves measured by CD for **i2** at pH 4.0 with and without ThT. All samples were prepared by 1.0 μM DNA and 6.0 μM ThT in 50 mM Tris-HCl buffer. Melting of i-motif structure was monitored at 290 nm.

1. H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*,1949,**71**, 2703.
2. S. Goswami, K. Aich, S. Das, A. K. Das, A. Mannaa and S. Halder, *Analyst*, 2013, **138**, 1903.