Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information:

Different thermodynamic signatures for DNA minor groove binding with changes in salt concentration and temperature

Shuo Wang[a], Arvind Kumar[a], Karl Aston[b], Binh Nguyen[a,c], James K. Bashkin[b], David W. Boykin[a], W. David Wilson[a*]

[a] Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA

[b] Department of Chemistry & Biochemistry, Center for Nanoscience, University of Missouri-St. Louis, St. Louis, MO 63121, USA

[c] Current address: Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

[*] To whom correspondence should be addressed:

Tel: +1.404.413.5503; Fax: +1.404.413.5505. Email: wdw@gsu.edu

Table of contents:

Figures

Table S1. Thermodynamic values for Net-AAAA, DB293-ATGA and KA1039-TGGCTT complexes.

Fig. S1 ITC titrations of 50 µM of Net into 10 µM of AAAA and ATAT hairpin duplexes at 25 ºC.

Fig. S2 ITC titrations of 50 µM of DB293 into 10 µM of AAAA and ATGA hairpin duplexes at 25 ºC.

Fig. S3 ITC titrations of 50 µM, 37.5 µM and 20 µM of KA1039 into 10 µM of TGGCTT hairpin duplexes at 25 ºC.

Fig. S4 Steady state analyses and global kinetic fits of SPR sensorgrams for KA1039 at 25 °C under various salt concentrations.

Fig. S5 Salt concentration dependence of kinetics for KA1039 binding.

Materials and methods

References

Table S1. Thermodynamic values for Net-AAAA, DB293-ATGA and KA1039-TGGCTT complexes [a].

Net - AAAA				**DB293 - ATGA**[b]				**KA1039 - TGGCTT**			
	ΔG_b	ΔH_b	$T\Delta S_b$		ΔG_b	ΔH_b	$T\Delta S_b$		ΔG_b	ΔH_b	$T\Delta S_b$
15 °C	-11.0 ±0.03	-6.2 ±0.1	4.8	**25 °C**	-8.9 ±0.03	11.0 ±0.2	-2.1	**25 °C**	11.0 ±0.02	10.8 ±0.2	0.2
25 °C	-11.0 ±0.03	-8.5 ±0.2	2.5	**30 °C**	-8.7 ±0.02	12.1 ±0.1	-3.4	**35 °C**	11.2 ±0.04	13.5 ±0.3	-2.3
35 °C	-10.9 ±0.05	-9.7 ±0.3	1.2	**35 °C**	-9.1 ±0.04	12.8 ±0.2	-3.7	**45 °C**	11.2 ±0.03	15.8 ±0.4	-4.6

[a] All values are in unit of kcal mol^{-1}. The errors of ΔG_b are calculated based on the SPR experimental K_a values obtained by global fit of 1:1 or 2:1 (DB293-ATGA) binding mode. The errors of ΔH_b are the standard errors for the fit of 1:1 or 2:1 (DB293-ATGA) binding determined by ITC. The $T\Delta S_b$ are calculated by $\Delta G_b = \Delta H_b - T\Delta S_b$. [b] Thermodynamic profile of DB293-ATGA are from reference 13 and are measured with MES buffer at pH 6.25 containing 10 mM MES, 200 mM NaCl and 1 mM EDTA.

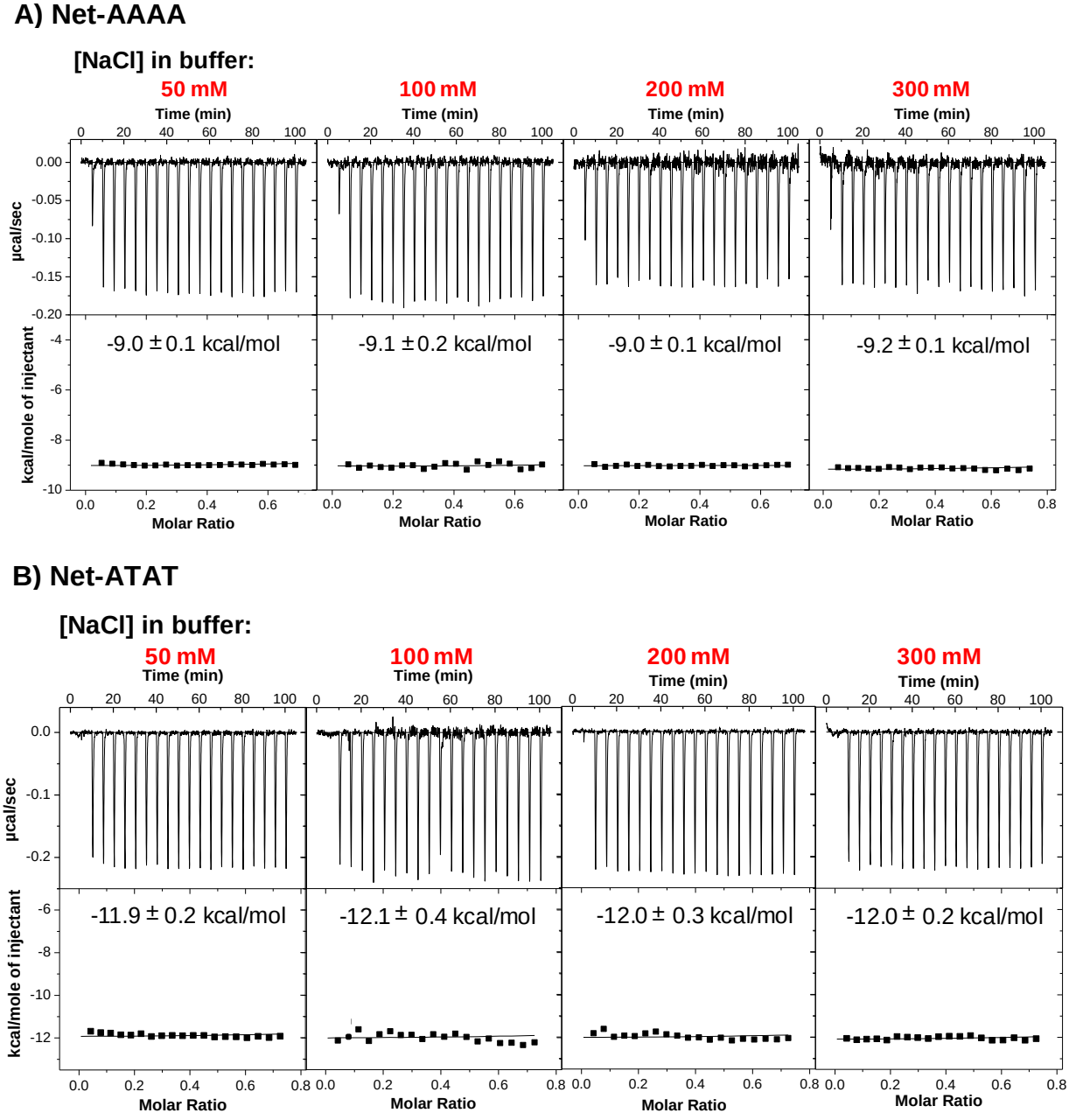
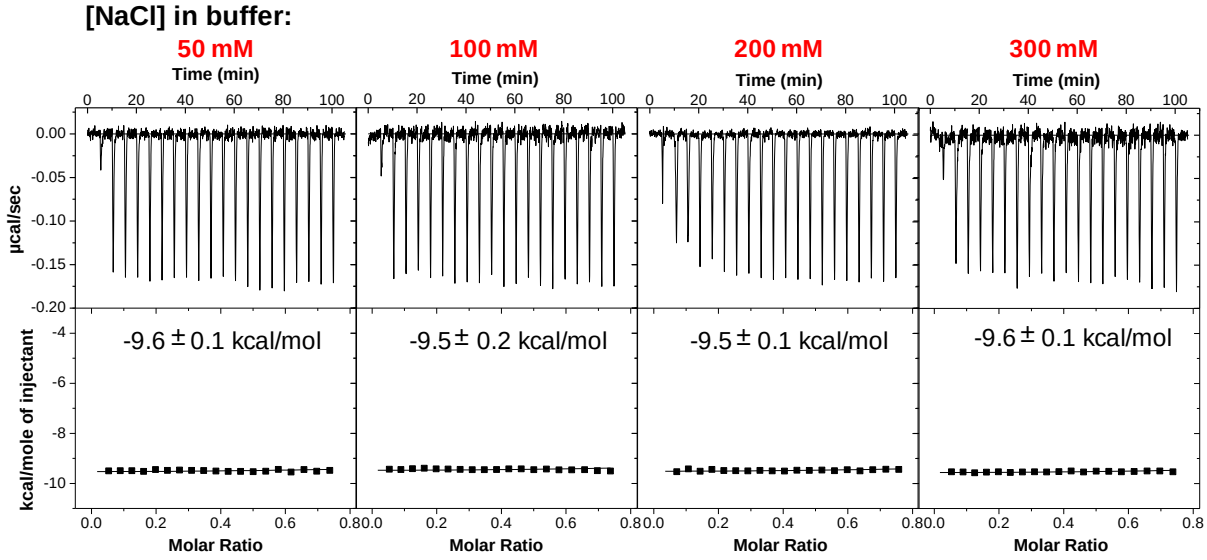


Fig. S1 ITC titrations of 50 µM of Net into 10 µM **(A)** AAAA and **(B)** ATAT hairpin duplexes at 25 ºC are shown at the top of each panel. The integrated heats after subtraction of the heat of dilution are plotted versus Net/DNA molar ratio in the bottom of each panel. Errors listed are the standard errors for the fit to the 1:1 binding model.

A) DB293-ATGA



B) DB293-AAAA

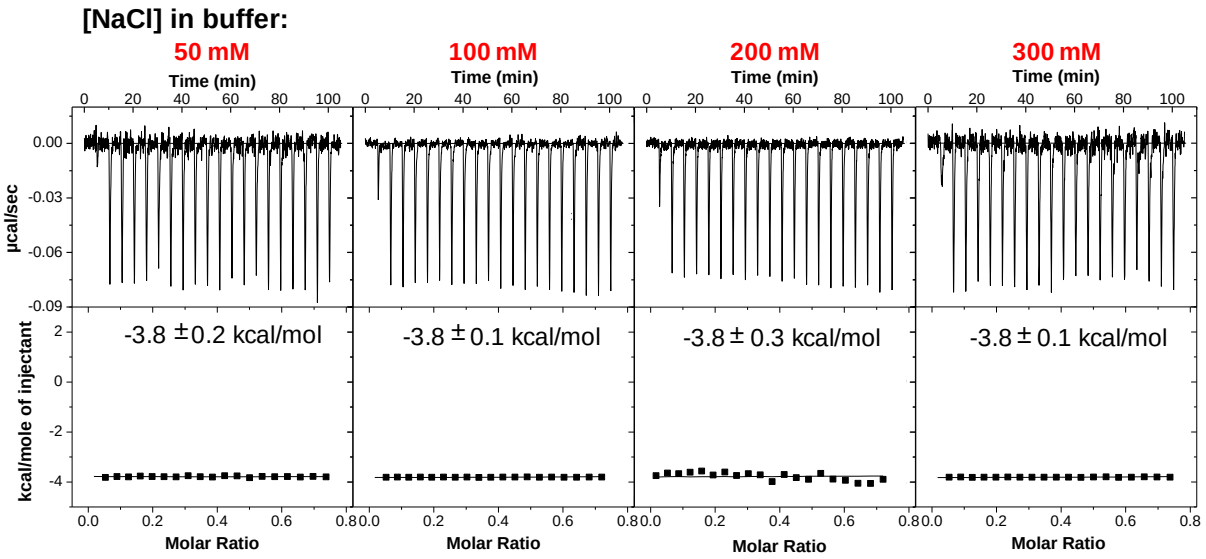


Fig. S2 ITC titrations of 50 µM of DB293 into 10 µM **(A)** ATGA and **(B)** AAAA hairpin duplexes at 25 ºC are shown at the top of each panel. The integrated heats after subtraction of the heat of dilution are plotted versus DB293/DNA molar ratio in the bottom of each panel. Errors listed are the standard errors for the fit to the 1:1 binding model.

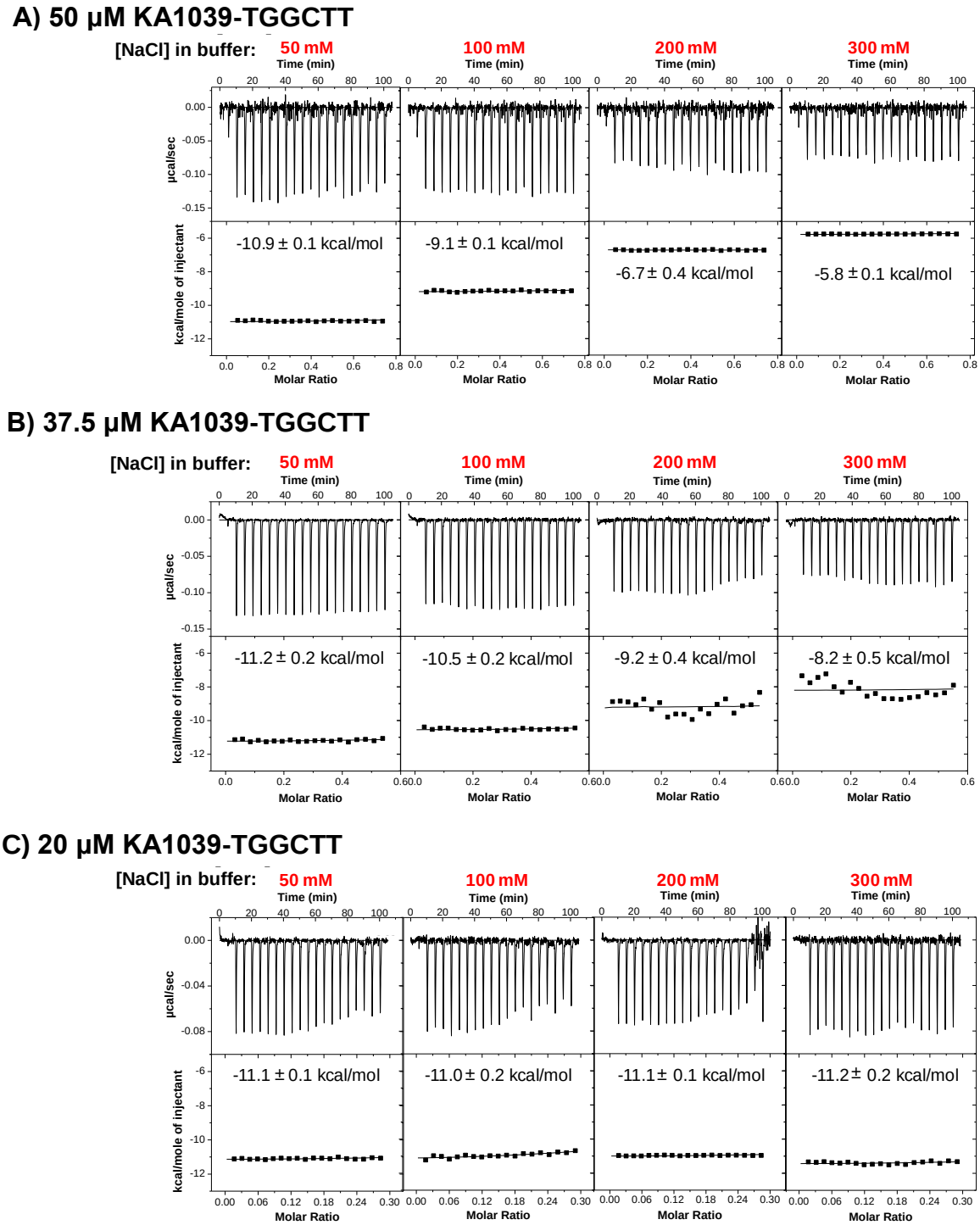


Fig. S3 ITC titrations of 50 µM **(A),** 37.5 µM **(B)** and 20 µM of KA1039 **(C)** into 10 µM TGGCTT hairpin duplexes at 25 ºC are shown at the top of each panel. The integrated heats after subtraction of the heat of dilution are plotted versus KA1039/DNA molar ratio in the bottom of each panel. Errors listed are the standard errors for the fit to the 1:1 binding model.

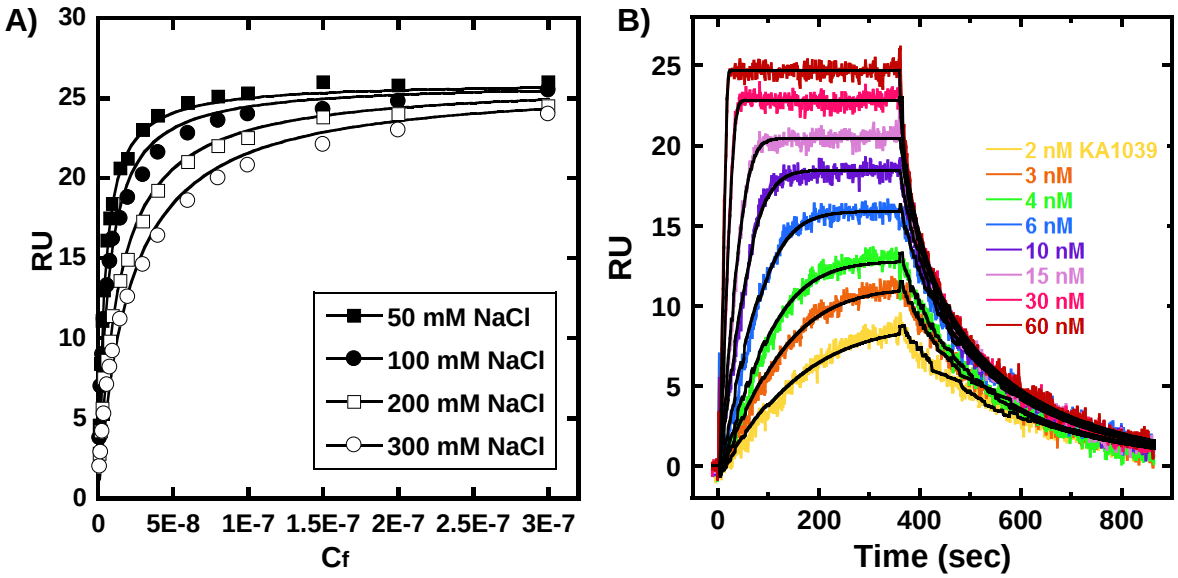


Figure S4. (A) Steady state analyses of SPR sensorgrams for KA1039 binding with TGGCTT at 25 °C under various salt concentrations. **(B)** SPR sensorgrams (color) and global kinetic fits (black overlays) for KA1039 binding at 25 °C with 50 mM NaCl buffer.

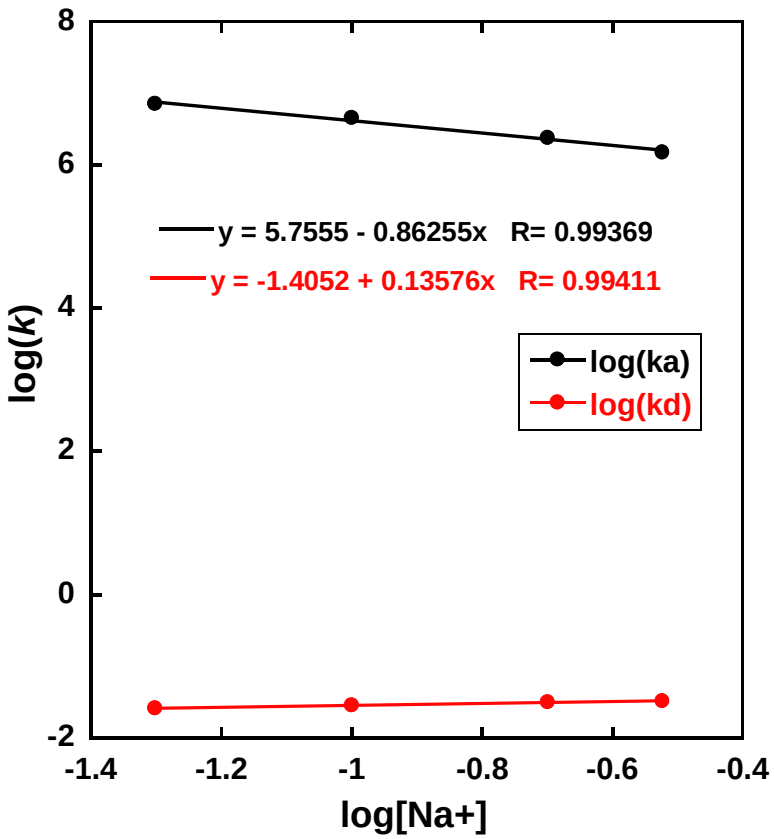


Figure S5. Salt concentration dependence of kinetic constants for KA1039 binding. All values are calculated based on data in Table 1.

Materials and Methods

Hairpin DNA oligomers were purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA), with HPLC purification and mass spectrometry characterization. Netropsin was purchased from Sigma-Aldrich (St Louis, MO), and the syntheses of DB293 and KA1039 were reported previously.[1] The full DNA sequences and compounds structure are shown in Figure 1. HEPES buffer containing 10 mM HEPES, 1 mM EDTA and a certain amount (50 mM, 100 mM, 200 mM or 300 mM) of NaCl at pH 7.4 was used in ITC experiments. The SPR experiments were performed in the buffer with 0.05% (v/v) surfactant P20. All the ligands in this work are dissolved in water.

ITC experiments were performed using a MicroCal VP-ITC (GE Healthcare, Inc., Piscataway, NJ) with VP-2000 software for instrument control and Origin 7.0 for data analysis. The sample cell was filled with 10 μM target DNA in HEPES buffer, and 20 injections of 10 μL of compound solution (netropsin and DB293 are 50 μM, KA1039 is 37.5 μM or 20 μM) were performed incrementally. A delay of 300 sec was used between each injection to ensure the equilibration of baseline. The heat for each injection was obtained by integration of the peak area as a function of time. The heats of dilution, determined by injecting compound into the sample cell containing only buffer, were subtracted from those in compound/DNA titrations to obtain the corrected binding-induced enthalpy changes. Because all the ligands bind quite strongly to the sequences in this work, the heat/mole of added compound is essentially constant in the initial titration region where all added compound is bound to DNA. The ΔH_b can be determined by a linear fit as shown in Figs. S1-3.

SPR measurements were performed with a Biacore 2000 optical biosensor system (GE Healthcare, Inc., Piscataway, NJ). The target DNA of KA1039 (Figure 1) with 5'-labeled biotin was immobilized onto a streptavidin-coated sensor chip as previously described.[2] In a typical experiment, 150 μL of KA1039 at different concentrations (from 1 nM to 300 nM) were injected onto the chip surface with a flow rate of 25 μL/ min and a 500 sec dissociation period. The surface was regenerated with a pH 2.5 glycine solution followed by multiple buffer injections. Kinetic analyses were performed by globally fitting the binding results for the entire concentration series using a standard 1:1 kinetic model with integrated mass transport-limited binding parameters as described previously.[2-3]

Steady-state analyses were also conducted and data were fit with a one site model: $r = (K_{eq} \times C_f) / (1 + K_{eq} \times C_f)$, where r represents the moles of bound compound per mole of DNA hairpin duplex, K_{eq} is equilibrium binding constants, and C_f is the free compound concentration in equilibrium with the complex. The observed steady-state response unit, RU_{obs}, at saturation of binding site divided by the calculated response per bound compound, RU_{cal}, gives the binding stoichiometry (n) of KA1039, $n = RU_{obs}/RU_{cal}$. For the thermodynamic profiles, binding entropy $T\Delta S_b$ was calculated by the equation: $\Delta G_b = \Delta H_b - T\Delta S_b$, where ΔG_b was measured by SPR and ΔH_b was determined by ITC.

References

1 (a) K. T. Hopkins, W. D. Wilson, B. C. Bender, D. R. McCurdy, J. E. Hall, R. R. Tidwell, A. Kumar, M. Bajic, D. W. Boykin, *J. Med. Chem.* 1998, **41**, 3872; (b) C. M. Dupureur, J. K. Bashkin, K. Aston, K. J. Koeller, K. R. Gaston, G. He, *Anal. Biochem.* 2012, **423**, 178.

2 R. Nanjunda, M. Munde, Y. Liu, W. D. Wilson in *Methods for Studying Nucleic Acid/Drug Interactions,* eds: M. Wanunu and Y. Tor, CRC Press, Boca Raton, 2011, pp. 91-122.

3 Y. Liu, W. D. Wilson, *Methods Mol. Biol.* 2010, **613**, 1.