Supporting Information

- for -

"A High-Throughput, High-Resolution Pathway to the Study of Site-Selective DNA Binding Agents: Analysis of a 'Highly Twisted' Benzimidazole-Diamidine"

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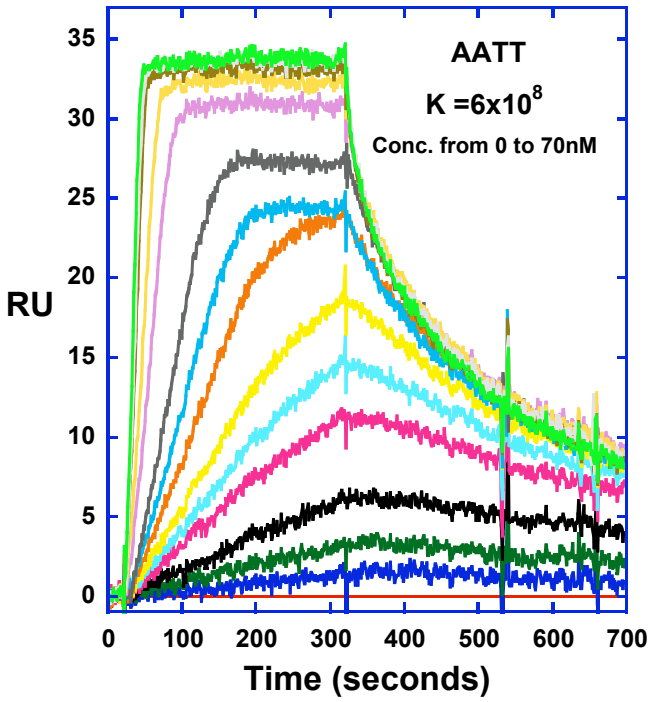
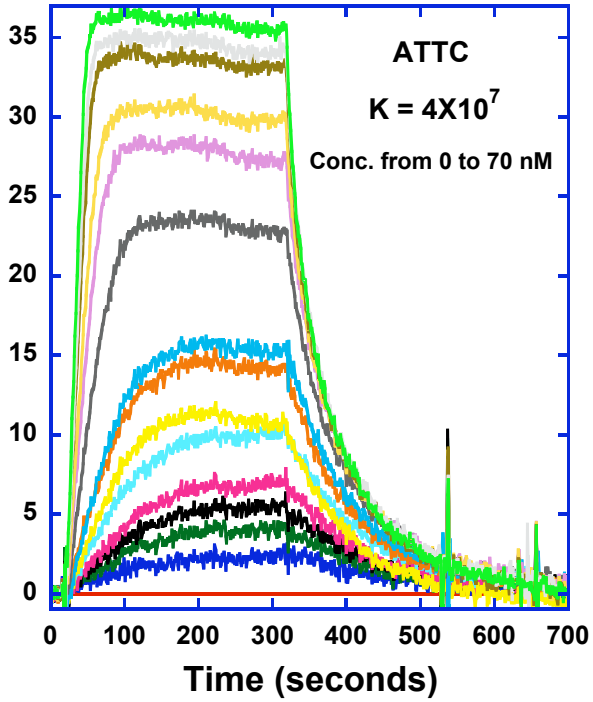
Figure S1. **Biosensor-SPR Results for RT29 Binding**. Experiments were conducted in 0.01 M MES, 0.001M EDTA, 0.2M NaCl, pH 6.2, using biotin labeled DNA hairpins of the following compositions:

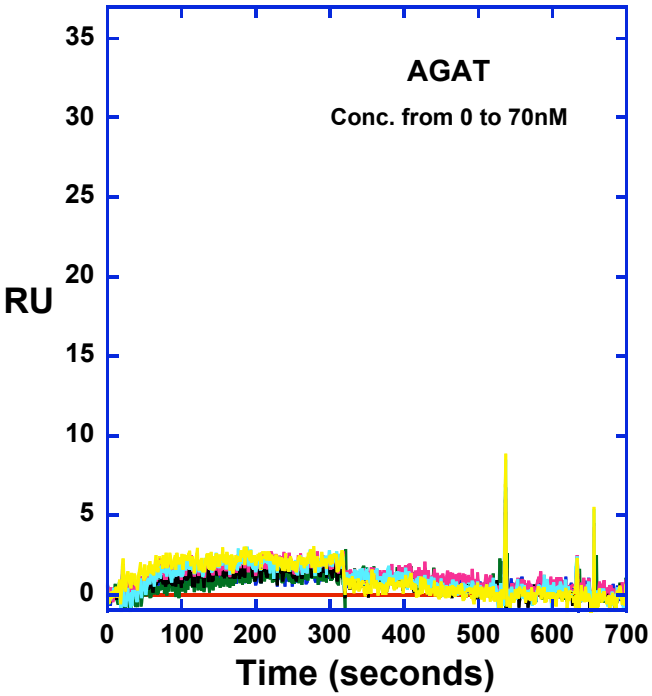
5´-CG-**XXXX**-C-A_4-G-**YYYY**-CG-3´

where:	-**XXXX**-	-**YYYY**-
	-AATT-	-AATT-
	-ATTC-	-GAAT-
	-AGAT-	-ATCT-

Biosensor-SPR experiments to determine **RT29**-DNA binding constants (K) were conducted with a BIAcore 3000 (Biacore, Uppsala, Sweden) instrument as described previously (Davis, T. M., and Wilson, *Methods Enzymol.* **2001**, *340*, 22-51). Briefly, the 5´-biotin labeled DNA hairpins shown above were immobilized on four channel BIAcore SA sensor chips via non-covalent streptavidin capture. Three flow cells contained the DNA samples and one flow cell was left blank as a reference. For the DNA samples with rapid **RT29** binding kinetics, steady-state binding studies were carried out by averaging the resonance unit values (RU) over a selected time region at different compound concentrations. The K values were obtained from the best fit of RU versus free compound concentration with a one-site binding model. Fits with more than one site did not improve the correlation coefficient or residuals significantly. Kinetics analyses to obtain K were performed by global fitting of the binding data with mass transport limited 1:1 kinetic binding models (Karlsson, R., *J. Mol. Recognit.* **1999**, *12*, 285-292).


AATT
K =6x10^8
Conc. from 0 to 70nM
RU
Time (seconds)


ATTC
K = 4X10^7
Conc. from 0 to 70 nM
Time (seconds)


AGAT
Conc. from 0 to 70nM
RU
Time (seconds)

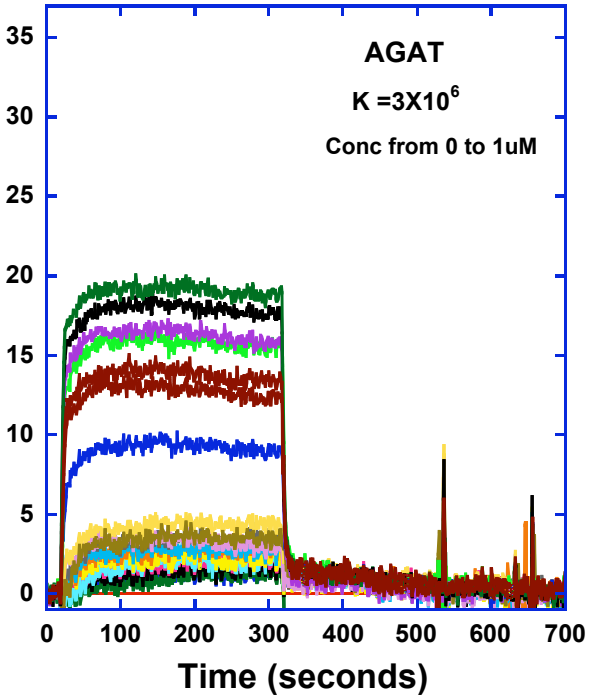

AGAT
K =3X10^6
Conc from 0 to 1uM
Time (seconds)

Table S1. Hydrogen bonds between RT29 and AATT DNA.

RT29 atom	DNA atom	Chain	Distance (Å)
N1	O4' of A5	B	2.8
N3	O4' of T6	B	3.3
N3	O2 of T13	G	3.0
N5	H_2O..N3 of A10	G	2.6/2.6
N5	H_2O..O4' of A11	G	2.6/2.9

Table S2. Hydrogen bonds between RT29 and ATTC DNA.

RT29 atom	DNA atom	Chain	Distance (Å)
N1	O4' of A5	B	2.9
N2	H_2O..N3 of A14	G	3.0/2.7
N2	H_2O..O4' of A15	G	3.0/3.3
N3	O4' of A6	B	3.3
N5	O4' of T11	G	3.2

Table S3. Hydrogen bonds between RT and AATT DNA in the presence and absence of RT29.

Residue	Atom	Atom	Nucleotide	Distance (Å)	RT29
Tyr 64	OH	O2	C1	3.2	+
		O4'	T2	3.4	--
Asp 114	Oδ2	N2	G16	3.0/3.3	--/+
Leu 115	N	O3'	G16	3.0/2.9	--/+
Arg 116	Nη1	O2	T2	3.4	+
		O4'	T3	2.8/3.0	--/+
		O2	T3	3.3	+
	Nη2	N2	G16	3.2/3.3	--/+
		O2	T2	2.7/2.8	--/+
		O4'	T3	3.3/3.4	--/+
Gly 191	O	O3'	G16	2.9/3.1	--/+

Table S4. Hydrogen bonds between RT and ATTC DNA in the presence and absence of RT29.

Residue	Atom	Atom	Nucleotide	Distance (Å)	RT29
Tyr 64a*	OH	O2	C1	3.1/3.3	-/+
Tyr 64a,b*	OH	H_2O..N2	G16	2.5,2.6..3.4	+
		H_2O..O4'	T2	2.5,2.6..3.3	+
Asp 114	Oδ2	N2	G16	3.0/3.0	-/+
	OH	H_2O..N2	G16	2.7..3.4	+
		H_2O..O4'	T2	2.7..3.3	+
Leu 115	N	O3'	G16	3.0/3.0	-/+
Arg 116	Nη1	O4'	T3	2.7/2.9	-/+
		O2	T3	3.4/3.2	-/+
	Nη2	N2	G16	3.2/3.2	-/+
		O2	T2	2.7/2.6	-/+
		O4'	T3	3.3/3.3	-/+
Gly 191	O	O3'	G16	2.9/2.9	-/+