SUPPLEMENTAL DATA

Electrostatic control of intersegmental translocation by the ETS transcription factor ETV6

June 4, 2017

Tam Vo[1], Shuo Wang[1], Gregory M. K. Poon[1,2,*], and W. David Wilson[1,2,*]

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

[2] Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30303

* To whom correspondence should be addressed at: P.O. Box 3965, Atlanta, GA 30302-3965. Email: gpoon@gsu.edu and wdw@gsu.edu. Tel. (404) 413-5491 and (404) 413-5498.

SUPPLEMENT INDEX **PAGE**

Table S1

SPR analysis of site-specific DNA binding by the ETS domain of ETV6

Kinetic and equilibrium parameters for ETV6 binding to DNA^{sp} in the absence and presence of salmon sperm DNA at the indicated total concentrations were estimated (± S.E.) according to a 1:1 binding model as previous reported and summarized in *Materials and Methods*. *, Inferred from the equilibrium and dissociation rate constants.

No salmon sperm DNA

$[Na^+]$, x10^{-3} M	k_a, x10^6 $M^{-1}s^{-1}$	k_d, x10^{-3} s^{-1}	K_D, x10^{-9} M	
			Kinetic fit	Steady-state
150	15.1 ± 0.02	7.34 ± 0.07	0.5 ± 0.006	1.70 ± 0.16
200	5.78 ± 0.06	20.4 ± 0.2	3.53 ± 0.05	5.92 ± 0.40
250	3.21 ± 0.02	47.7 ± 0.2	14.9 ± 0.10	16.87 ± 0.38
300	1.67 ± 0.02	178.9 ± 1.8	107.1 ± 1.53	99.94 ± 1.78
350	1.44 ± 0.04	483 ± 11	335 ± 11.3	334.9 ± 1.38
400	-	-	-	1048.6 ± 8.5

10 μM salmon sperm DNA

$[Na^+]$, x10^{-3} M	k_a, x10^5 $M^{-1}s^{-1}$	k_d, x10^{-3} s^{-1}	K_D, x10^{-9} M	
			Kinetic fit	Steady-state
125	3.45 ± 0.7	1.0 ± 0.5	2.9 ± 1.5	-
150	1.8 ± 0.3	1.4 ± 0.4	8.2 ± 2.5	9.8 ± 1.2
200	6.8 ± 0.9*	10.8 ± 0.8	-	15.7 ± 1.0
250	5.3 ± 0.8*	26.0 ± 0.4	-	48.6 ± 0.8
300	12.4 ± 0.5	157.6 ± 0.7	126.6 ± 2.1	132.9 ± 2.8
400	-	-	-	1642.8 ± 5.4

Table S1 (continued)

50 μM salmon sperm DNA

[Na^+], x10^{-3} M	k_a, x10^5 $M^{-1}s^{-1}$	k_d, x10^{-3} s^{-1}	K_D, x10^{-9} M	
			Kinetic fit	Steady-state
125	2.1 ± 0.2	1.9 ± 0.1	8.9 ± 0.4	-
150	1.1 ± 0.4	2.1 ± 0.1	20.3 ± 0.7	-
200	6.8 ± 0.5*	10.8 ± 0.2	-	15.2 ± 0.4
250	8.9 ± 0.6	31.2 ± 0.4	35.1 ± 0.8	33.3 ± 0.5
300	9.9 ± 0.8	117.6 ± 0.7	118.7 ± 0.6	125.1 ± 7.1
350	15.3 ± 0.4	493.2 ± 0.8	320.4 ± 0.5	342.0 ± 20.8
400	-	-	-	1291.6 ± 166.0
450	-	-	-	3674.5 ± 74.7

100 μM salmon sperm DNA

[Na^+], x10^{-3} M	k_a, x10^5 $M^{-1}s^{-1}$	k_d, x10^{-3} s^{-1}	K_D, x10^{-9} M	
			Kinetic fit	Steady-state
100	-	-	-	18.03 ± 1.7
150	1.1 ± 0.5	2.4 ± 0.2	21.3 ± 0.6	25.0 ± 2.2
200	4.5 ± 0.3	21.8 ± 0.1	49.1 ± 0.4	55.3 ± 1.8
250	9.2 ± 0.6	69.4 ± 0.4	75.6 ± 0.7	77.2 ± 1.4
300	20.4 ± 0.7	231.4 ± 0.1	113.4 ± 0.6	113.0 ± 1.9
350	9.8 ± 0.4	458 ± 12	467 ± 23	471 ± 64
400	-	-	-	862 ± 30
450	-	-	-	2890 ± 52

Table S2

Nonspecific DNA binding by the ETS domain of ETV6 to oligomeric DNA

Equilibrium dissociation constants for ETV6 binding to the 23-bp nonspecific sites SD1 and SD2 (c.f. Table 1) were estimated (± S.E.) according to the McGhee-von Hippel equation modified for oligomeric DNA, Eq. **Error! Reference source not found.** in the main text. The binding site size was fixed at 5.2 according to the value determined by McIntosh and coworkers.[1] To constrain the fit for the binding data at high salt, each data set shared a common capacity (RU_{max}), whose fitted value corresponds to ~9 equivalents of ETV6 per duplex hairpin, a reasonable value given for 23 bp if the protein was assumed to occupy only one face of the double helix as in its site-specific co-crystal structure (PDB: 4MHG).

$[Na^+]$, M	K_D, mM	
	SD1	SD2
0.15	0.0069 ± 0.0004	0.0051 ± 0.0003
0.20	0.030 ± 0.012	0.021 ± 0.001
0.25	0.24 ± 0.02	0.18 ± 0.01
0.30	0.76 ± 0.08	0.57 ± 0.05
0.40	5.4 ± 3.2	3.3 ± 1.3
RU_{max}	700 ± 14	702 ± 13
Δm_{NS} (z)	-6.8 ± 0.4 (7.7 ± 0.5)	

Table S3

Nonspecific DNA binding by the ETS domain of ETV6 to polymeric salmon sperm DNA

Equilibrium dissociation constants for ETV6 binding to polymeric salmon sperm DNA in the presence of 0.05 M to 0.45 M NaCl were estimated (± S.E.) according to the McGhee-von Hippel equation, Eq. **Error! Reference source not found.** in the main text. In each fit, the binding site size was constrained at m = 5.2 and $[ETV6]_t$ = 200 nM (known). *, ω fixed at unity, since no statistically significant improvement on the fit by letting ω float (Fisher's F-test on sums of square, p = 0.05).

[NaCl], M	K_D^{ns} **, μM**	**ω**
0.125	0.10 ± 0.01	1*
0.15	0.29 ± 0.16	31 ± 1
0.20	0.39 ± 0.03	34 ± 8
0.25	0.41 ± 0.04	31 ± 6
0.30	2.1 ± 0.3	(6.7 ± 4.0) × 10^2
0.45	60 ± 10	(10 ± 2) × 10^3
Δm_{NS} (z)	-4.5 ± 0.9 (5.1 ± 1.0)	

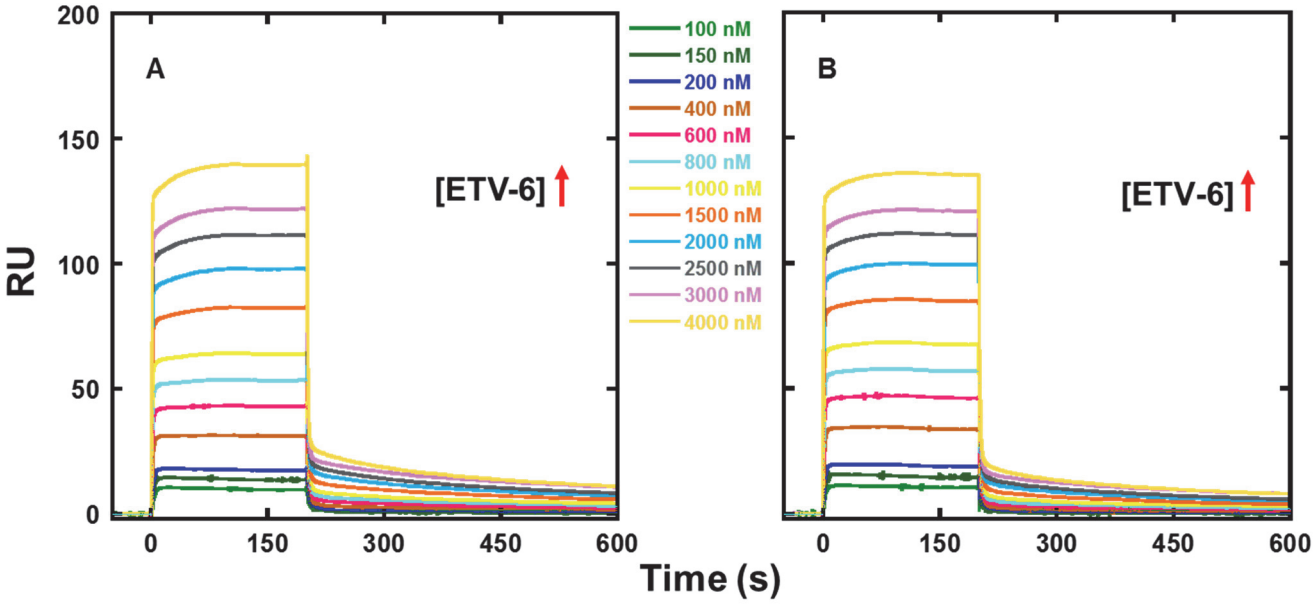

Figure S1. Representative sensorgrams of DNA^{ns} binding by the ETS domain of ETV6 to oligomeric nonspecific DNA. A) SD1 and B) SD2 at 0.2 M Na^{+}. ETV6 concentrations from 100 nM to 4 μM are shown in the figure legend. In each case, the association and dissociation rate were both rapid beyond the limit of the instrumental detection. At over 10^{-6} M concentrations, ETV6 was sometimes observed to randomly adsorb the biosensor surface as shown in the sensorgrams, but was readily removed by washing after each cycle of injection.

Supplemental References

1. De, S.; Chan, A. C.; Coyne, H. J., 3rd; Bhachech, N.; Hermsdorf, U.; Okon, M.; Murphy, M. E.; Graves, B. J.; McIntosh, L. P., Steric mechanism of auto-inhibitory regulation of specific and non-specific DNA binding by the ETS transcriptional repressor ETV6. *J Mol Biol* **2014,** *426* (7), 1390-406.