

ELECTRONIC SUPPLEMENT

“Annexin I and Annexin II N-terminal Peptides Binding to S100 Protein Family Members: Specificity and Thermodynamic Characterization” by Werner W. Streicher, Maria M. Lopez and George I. Makhatadze

Supplementary Table S1: Summary of AnI and AnII interactions with S100 protein family members using intrinsic Trp to screen for binding for AnI and ITC for AnII.

Protein	Trp Fluor.		ITC	
	AnI + Ca ²⁺	AnI - Ca ²⁺	AnII + Ca ²⁺	AnII - Ca ²⁺
S100A1	-	-	-	-
S100A2	**	-	-	-
S100A3	-	-	-	-
S100A4	-	-	-	-
S100A5	-	-	-	-
S100A6	Binding*	-	-	-
S100A7	-	-	-	-
S100A8	-	-	-	-
S100A9	-	-	-	-
S100A10	-	-	Binding	Binding
S100A11	Binding*	-	Binding	-
S100A12	-	-	-	-
S100A13	-	-	-	-
S100B	-	-	-	-
S100P	-	-	-	-
S100Z	-	-	-	-
Calmodulin	**	-	-	-

- represents no detectable binding;

* confirmed using ITC;

** small changes in fluorescence emission spectra have been observed by the interaction could not be confirmed by ITC.

Supplementary Table S2: Thermodynamic parameters determined using ITC for AnI and AnII binding their respective S100 protein binding partners.

Peptide	Protein	Temperature (°C)	K_{d1} (μ M)	ΔH_1 (kJ/mol)	K_{d2} (μ M)	ΔH_2 (kJ/mol)
AnI	S100A11	15	2.7	-4		
		20	4.6	-11		
		25	5.4	-15		
		30	6.5	-22		
		35	7.2	-30		
	S100A6	10	16.0	14		
		15	15.7	11		
		20	10.1	6		
		25	ND	0		
		30	9.3	-8		
AnII	S100A10	10	0.6	-53	0.8	1
		10 ^a	0.7	-54	0.8	1
		15	0.1	-63	0.2	-14
		20	1.1	-93	0.7	-18
		25	0.8	-100	0.6	-36
		30	0.1	-97	0.2	-49
		30 ^a	0.1	-101	0.3	-51
		S100A11	6 ^b	3.9	31	9.7
	6	1.9	31	11.5	20	
	10 ^b	1.8	29	11.3	18	
	10	0.9	28	7.6	21	
	15	0.9	21	6.8	17	
	20 ^b	1.0	9	8.4	11	
	25	ND				

^a experiments performed in the absence of calcium;

^b experiments performed in the presence 100 mM NaCl

Errors in enthalpies are ~10%. Average values for dissociation constants are: S100A6 AnI: 13±4 μ M; S100A11 AnI: 5±2 μ M; S100A10 AnII: K_{d1} : 0.5±0.4 μ M; K_{d2} : 0.5±0.3 μ M; S100A11 AnII: K_{d1} : 2±1 μ M; K_{d2} : 9±2 μ M.