

Supplementary materials for “Sequence dependence of an intramolecular interaction that inhibits DNA binding by p53”

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Supplementary Table S1: Kds (nM) of p53 constructs binding consensus and scrambled DNA at various ionic strengths.

IS	DBD		ND		ND DE		ND NP		ND QS	
	Con	Scram	Con	Scram	Con	Scram	Con	Scram	Con	Scram
15mM	0.018 ± 0.001	0.034 ± 0.004	0.71 ± 0.08	2.3 ± 0.1	NA	NA	NA	NA	NA	NA
55mM	0.61 ± 0.01	5.51 ± 0.03	0.97 ± 0.03	69 ± 3	NA	NA	NA	NA	NA	NA
85mM	2.08 ± 0.01	22 ± 2	7 ± 0.6	170 ± 10	2.1 ± 0.2	25.8 ± 0.2	1.4 ± 0.1	55 ± 6	3.08 ± 0.04	150 ± 6
125mM	0.90 ± 0.07	89 ± 5	43 ± 3	193 ± 8	4.6 ± 0.3	108 ± 2	3.2 ± 0.2	82 ± 2	7 ± 1	213 ± 9
145mM	1.39 ± 0.01	113 ± 7	230 ± 7	578 ± 40	7.6 ± 0.5	245 ± 7	9.4 ± 0.7	333 ± 7	17 ± 3	329 ± 25
165mM	7.1 ± 0.4	285 ± 25	473 ± 29	1500 ± 46.4	17.9 ± 0.7	593 ± 23	19 ± 3	830 ± 27	31.3 ± 0.7	855 ± 7
185mM	19 ± 2	590 ± 26	883 ± 49	1743 ± 105	96 ± 16	761 ± 69	52 ± 7	2006 ± 119	160 ± 9	1042 ± 58
205mM	58 ± 15	717 ± 62	1574 ± 163	2727 ± 66	290 ± 43	1083 ± 50	84 ± 6	2777 ± 85	347 ± 8	2402 ± 81
225mM	105 ± 5	1388 ± 44	3861 ± 40	3705 ± 230	384 ± 16	1676 ± 38	218.1 ± 15	3386 ± 31	889 ± 340	2760 ± 38

All values were obtained by fluorescence anisotropy. Each data point represents three titrations of protein into a stable concentration of DNA. ND mutants were not evaluated at ionic strengths below 85 mM, represented here as 'NA.'

Supplementary Table S2: Standard error of estimate.

	DBD		ND		ND DE		ND NP		ND QS	
	Con	Scram	Con	Scram	Con	Scram	Con	Scram	Con	Scram
15mM	0.0450	0.0744	0.0852	0.0003	NA	NA	NA	NA	NA	NA
55mM	0.0543	0.0180	0.0193	0.0198	NA	NA	NA	NA	NA	NA
85mM	0.0368	0.0161	0.0518	0.0134	0.0411	0.021	0.0186	0.0224	0.0351	0.0235
125mM	0.0310	0.0180	0.0254	0.0332	0.0276	0.0306	0.0402	0.032	0.0514	0.0274
145mM	0.0317	0.0173	0.059	0.0189	0.0332	0.0185	0.0345	0.0138	0.0377	0.0161
165mM	0.0318	0.0104	0.037	0.0323	0.0186	0.0168	0.0272	0.0121	0.0344	0.0438
185mM	0.0263	0.0243	0.0395	0.0201	0.0331	0.0379	0.0148	0.0173	0.0291	0.0178
205mM	0.0776	0.0249	0.0164	0.0172	0.0901	0.0248	0.0206	0.0432	0.052	0.0087
225mM	0.0315	0.0250	0.0399	0.0212	0.082	0.0344	0.0433	0.0117	0.0368	0.0232

Triplicate runs of fluorescence anisotropy were evaluated using standard error of estimate as a measure of data quality using the following:

$$\frac{(Actual - Expected)^2}{dof}$$

Where *Actual* is the observed anisotropy values at a given concentration of protein and DNA, *Expected* is the predicted anisotropy value at these concentrations, and *dof* is degrees of freedom. ND mutants were not evaluated at ionic strengths below 85 mM, represented here as 'NA.'

Supplementary Table S3: Percentage of Gibbs free energy originating from salt-dependent and salt-independent components estimated using Record's interpretation of the counterion condensation model.

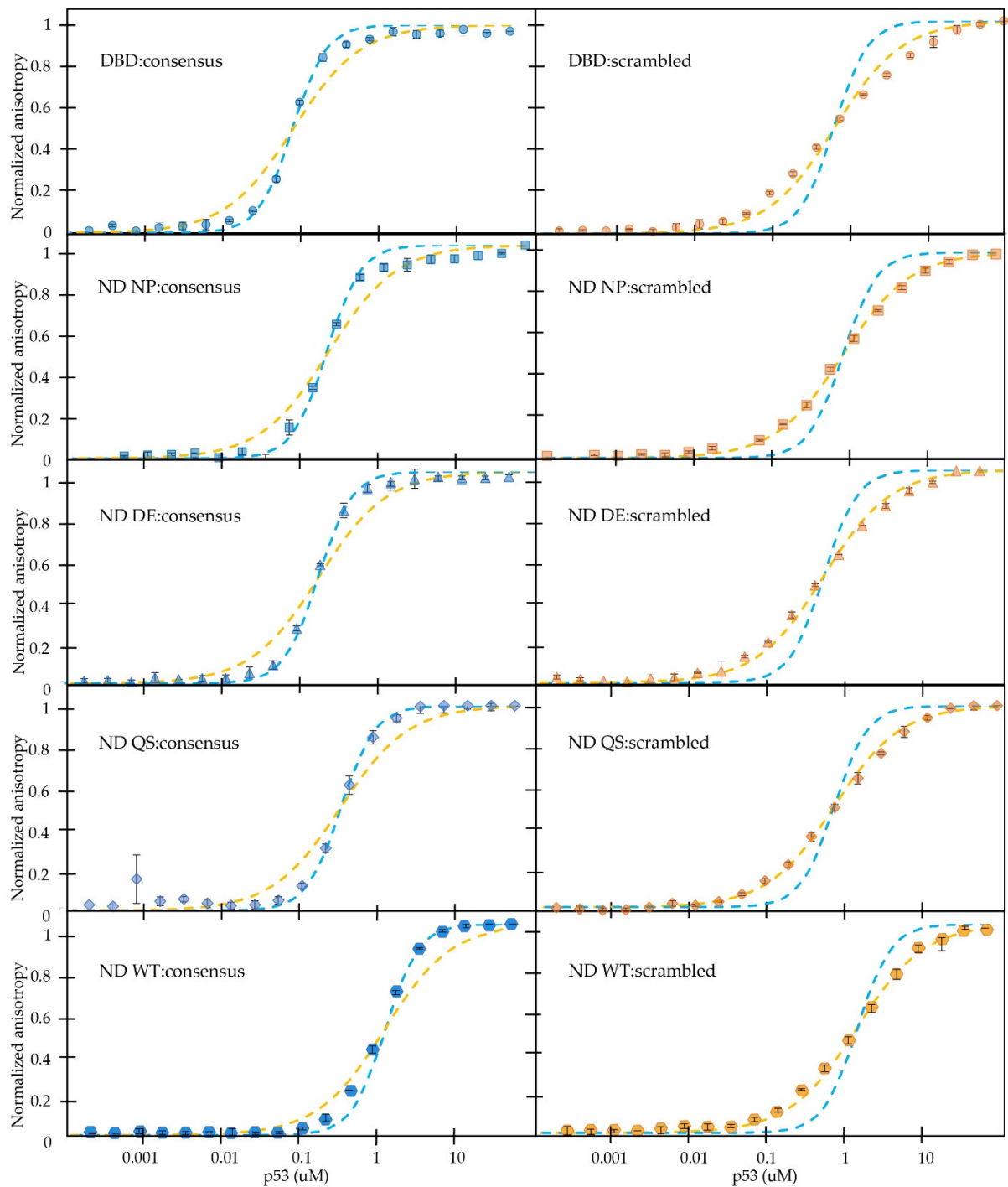
Consensus DNA		
	% Electrostatic	% Nonelectrostatic
DBD	79.67	20.33
ND DE	82.34	17.66
ND NP	67.98	32.02
ND QS	85.05	14.95
ND WT	81.02	18.98
Scrambled DNA		
	% Electrostatic	% Nonelectrostatic
DBD	55.95	44.05
ND DE	53.86	46.14
ND NP	97.68	2.32
ND QS	57.12	42.88
ND WT	60.56	39.44

Supplementary Table S4: Percentage of Gibbs free energy originating from salt-dependent and salt-independent components estimated using Manning's interpretation of the counterion condensation model.

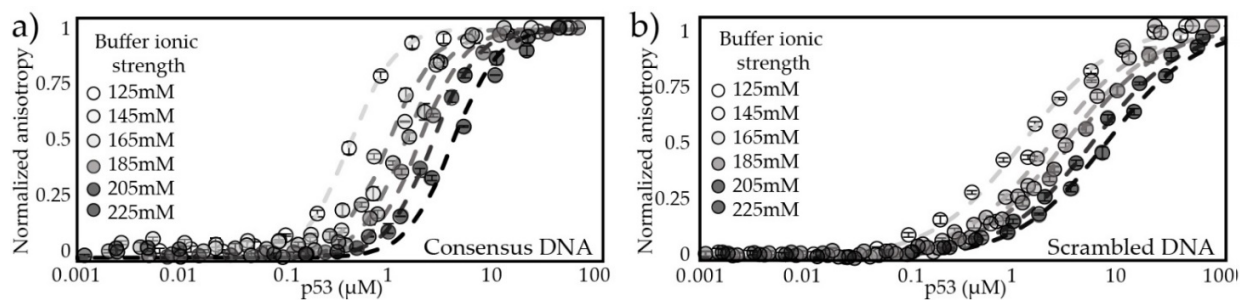
Consensus DNA		
	%Electrostatic	%Nonelectrostatic
DBD	68.97	31.03
ND DE	70.81	29.19
ND NP	46.56	53.44
ND QS	74.81	25.19
ND WT	63.00	37.00
Scrambled DNA		
	%Electrostatic	%Nonelectrostatic
DBD	20.31	79.69
ND DE	19.39	80.61
ND NP	84.76	15.24
ND QS	18.00	82.00
ND WT	24.46	75.54

Supplementary Table S5: van't Hoff plot-derived thermodynamics. All values in kcal/mol.

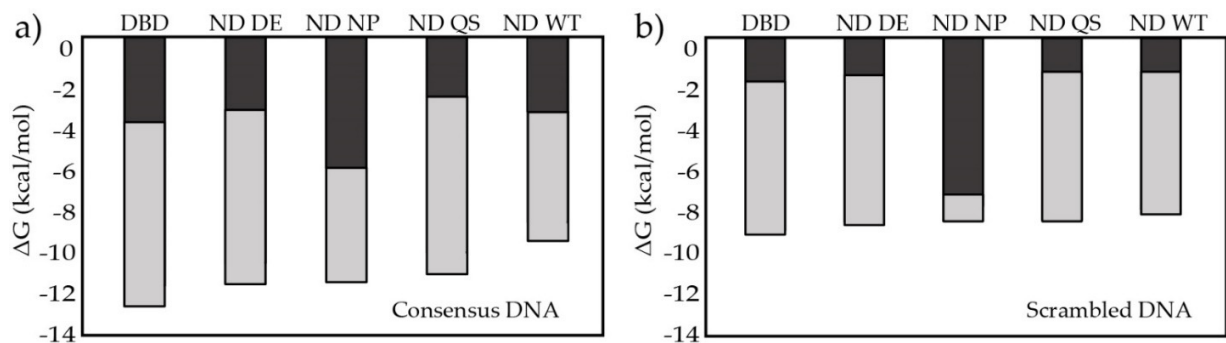
Consensus DNA						
	ΔH	ΔS	$T\Delta S$	$\Delta H - T\Delta S$	Actual ΔG	Entropic %
DBD	-5.252	0.022	6.601	-11.853	-11.838	56
ND	-2.843	0.021	6.198	-9.041	-9.047	69
Scrambled DNA						
	ΔH	ΔS	$T\Delta S$	$\Delta H - T\Delta S$	Actual ΔG	Entropic %
DBD	-6.401	0.008	2.456	-8.856	-8.870	30
ND	-4.371	0.012	3.454	-7.825	-7.824	49



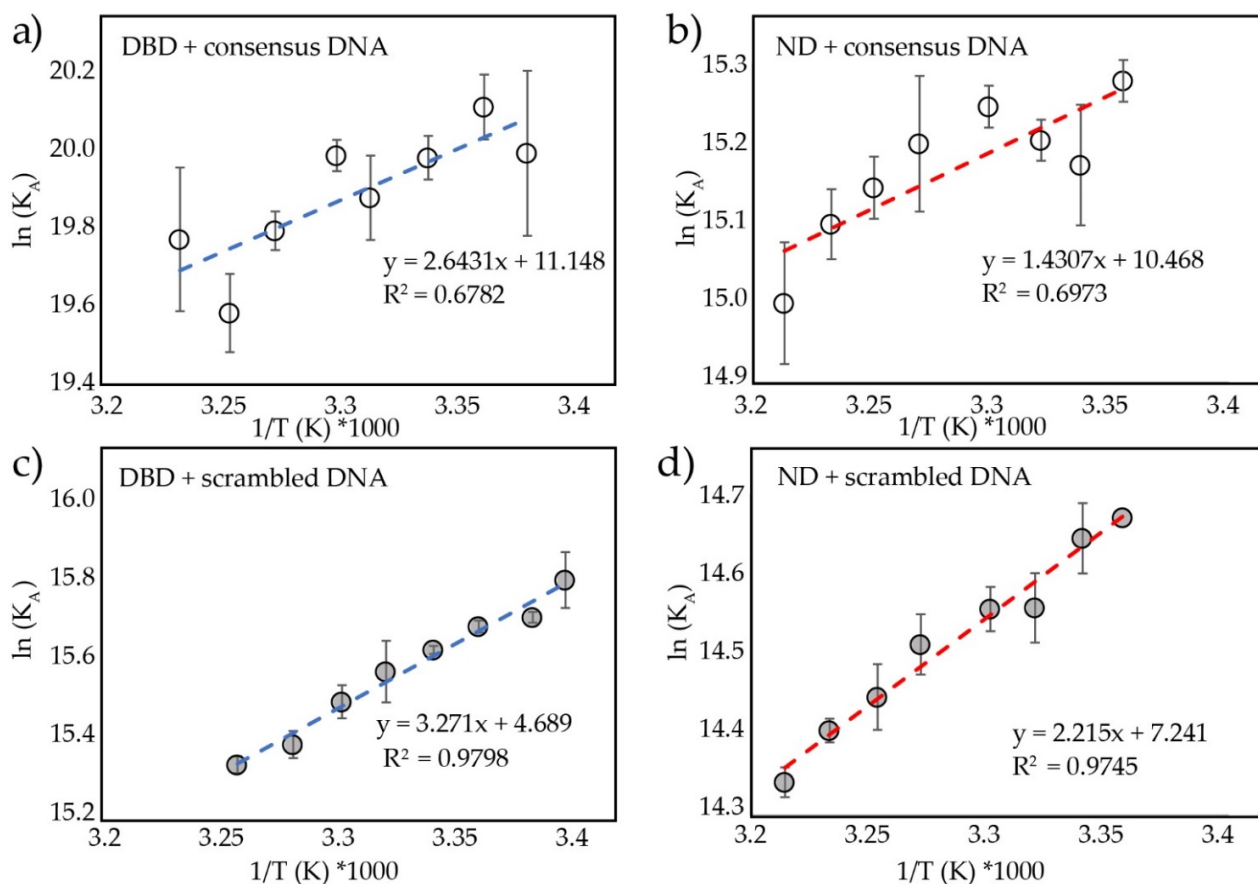
Supplementary Figure S1. Hill Coefficients of p53 constructs. Data points of p53 binding to consensus or scrambled DNA at 145mM ionic strength, filled shapes, are fit using an equation in which the Hill coefficient is set to 1 (orange dashed lines) or 2 (blue dashed lines). Data for consensus DNA binding is best fit with a Hill coefficient of 2; data for scrambled DNA binding is best fit with a Hill coefficient of 1.



Supplementary Figure S2. ND WT binds DNA across ionic strengths. (a) fluorescence anisotropy plots of ND bound to consensus DNA at 125-225mM ionic strength; (b) fluorescence anisotropy plots of ND bound to scrambled DNA at 125 – 225mM ionic strength.

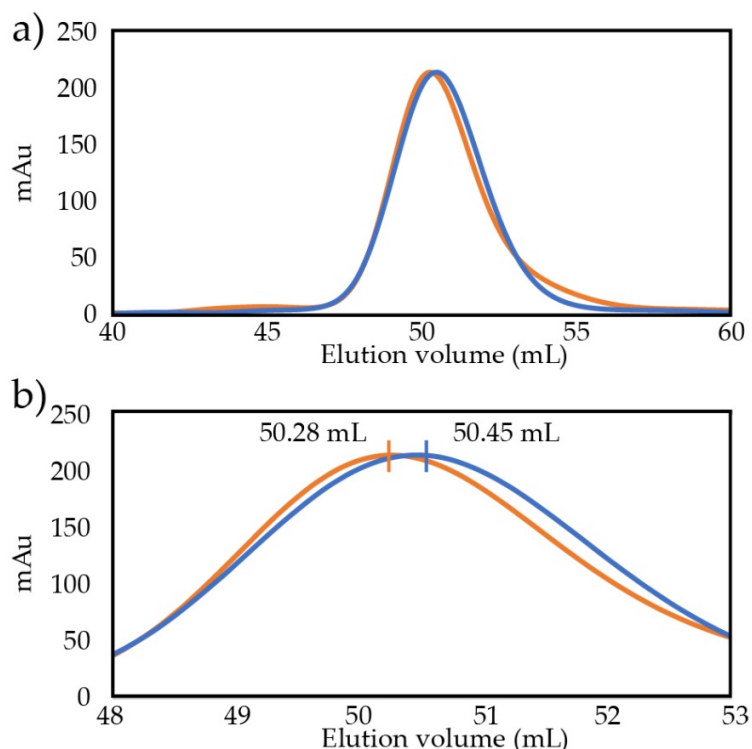


Supplementary Figure S3. Salt-dependent and salt-independent components of Gibbs free energy at physiological ionic strength using Manning's model where ■ is the salt-dependent component and □ is the salt-independent component for (a) consensus DNA, or (b) scrambled DNA.



Supplementary Figure S4. Van't Hoff plots of DBD and ND with consensus and scrambled DNA derived from fluorescence anisotropy gives an estimate of enthalpic and entropic components of binding.

Thermodynamic values derived from a van't Hoff plot are inferred rather than directly measured. Enthalpic components such as buffer protonation and folding that occurs during binding is not measured here with the result that enthalpic values are always underestimated [1, 2]. The nearly linear plots of DBD and ND bound to scrambled DNA suggests minor heat capacity change [3], which supports predictions of DBD's structure when bound to nontarget DNA [4-6] and the general observation that nonspecific protein-DNA binding exhibits low heat capacity change [7]. The nonlinear plots of DBD and ND bound to consensus DNA suggest a non-negligible heat capacity change upon binding.



Supplementary Figure S5. Separation of ND WT in size exclusion column in low and high ionic strength buffer. a) ND WT in 150mM ionic strength buffer (orange line) and 410mM ionic strength buffer (blue line), b) inset of these with the maxima labeled. Note, elution volumes differ from that in main text Figure 7 because a different size exclusion column was used here; however, the Stokes radius of ND WT is the same as reported in the main text.

References

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