# Supporting Information

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### SI Text

**SI Materials and Methods.** ***Chemicals.*** Salts used include potassium fluoride (>99.5%) from Fluka; dipotassium ethylenediaminetetraacetate ($K_2$EDTA, >98%), potassium chloride, sodium perchlorate ($NaClO_4$), guanidinium chloride (GuHCl), potassium bromide, and potassium phosphate dibasic (all >99%) from Sigma; ammonium sulfate, sodium chloride, potassium phosphate monobasic, disodium ethylenediaminetetraacetate (all >99%), ammonium chloride (>99.5%), and Tris (hydroxymethyl)-aminomethane (Tris base, >99.8%) from Fisher; sodium sulfate (>99%) from Baker; ultrapure GuHCl (>99.9%) from MP Biomedicals, and guanidinium sulfate (>99%) from Acros. All were used as provided, without further purification. Complementary HPLC-purified DNA oligonucleotides with sequences 5′-CGATTAGATAGC-3′ (S1) and 5′-GCTATCTAATCG-3′ (S2) were obtained from Integrated DNA Technologies. Purification and dialysis of the 62 residue helix-turn-helix *lac* repressor DNA-Binding Domain (*lac* DBD) were performed as described previously (1). All samples were dissolved in water purified with a Barnstead E-pure system (Thermo Fisher Scientific).

***UV spectrophotometry.*** The single-stranded oligomers were dissolved in separate buffer solutions containing 0.01 M Tris base, 0.001 M $Na_2$EDTA, and 0.1 M KCl, pH adjusted to 7.3 using HCl, and the concentrations were determined in a Beckman DU 640 spectrophotometer using the extinction coefficients (123,000 and 115,000 $L\,mol^{-1}\,cm^{-1}$, respectively). A PTC-200 Peltier Thermal Cycler was used to anneal a 1:1 mole ratio solution of the two strands by heating to 85 °C for 15 min and then slowly cooling to 25 °C (~24 h). Stock salt solutions ranging from 0.5 to 5.0 mol/kg, buffered with 0.1 mmol/kg $K_2$EDTA and 0.5 mmol/kg $KH_2PO_4/K_2HPO_4$ (run buffer; pH 7.0), were prepared gravimetrically. Experimental salt solutions (ranging from 0.05 to 3.7 mol/kg) were gravimetrically prepared in microcentrifuge tubes, using appropriate amounts of annealed duplex DNA, stock salt, and run buffer solutions. Thermal denaturation experiments were carried out on a Cary Model 1 Bio UV-visible spectrophotometer equipped with a Peltier heating/cooling accessory and a temperature probe. Absorbance was monitored at 260 nm in dual beam mode, with a slit width of 1.8-mm and a 1-cm path length. Scans typically were run in the range 6–95 °C, with temperature increasing at a rate of 0.5 °C/ min and data collected every 1.0 °C. Reversibility was demonstrated for 0.15 mol/kg solutions of $Na_2SO_4$, $NH_4Cl$, and GuHCl by heating solutions of the duplex from 12 to 65 °C, cooling the solution and then repeating the melt. Upon cooling, $\gtrsim$95% of initial double-stranded DNA signal was obtained, and equilibrium constants obtained from all transition curves were identical within 4%.

**CD Spectroscopy.** To reduce the buffer concentrations in studies of salt effects on stability of *lac* DBD, dialyzed protein in 25 mM $K_2HPO_4$ buffer (pH 7.3) was diluted with the appropriate stock salt solution and distilled deionized water for a final protein concentration in the range 15–30 μM and a final buffer concentration in the range of 2–8 mM. No dependence of stability on protein concentration in this range is observed. A molar extinction coefficient of 5400 $M^{-1}$ at 280 nm was used in determining the concentration (1). Protein solutions were monitored at 222 and 260 nm on an Aviv 62A DS CD spectrometer with a multicell attachment allowing simultaneous heating of five cuvettes. Heating curves typically spanned the range 2–98 °C, with temperature steps of 3°. After a temperature step, samples were equilibrated at the desired temperature (±0.6 °C instrumental deadband) for 7 min before readings were taken. Typically, 10–15 min elapsed between readings of the same cell at $n$ and $(n + 3)$ °C. Thermal unfolding of *lac* DBD in urea has been shown to be completely reversible, and a two-state transition is sufficient to model the process (1). In GuHCl, ~95% recovery of signal at 222 nm is attained upon cooling the samples to 4 °C after performing thermal melts to 80 °C. For stabilizing salts, >97% recovery of signal is attained.

1. Felitsky DJ, Record MT (2003) Thermal and urea-induced unfolding of the marginally stable lac repressor DNA-binding domain: A model system for analysis of solute effects on protein processes. *Biochemistry* 42(7):2202–2217.

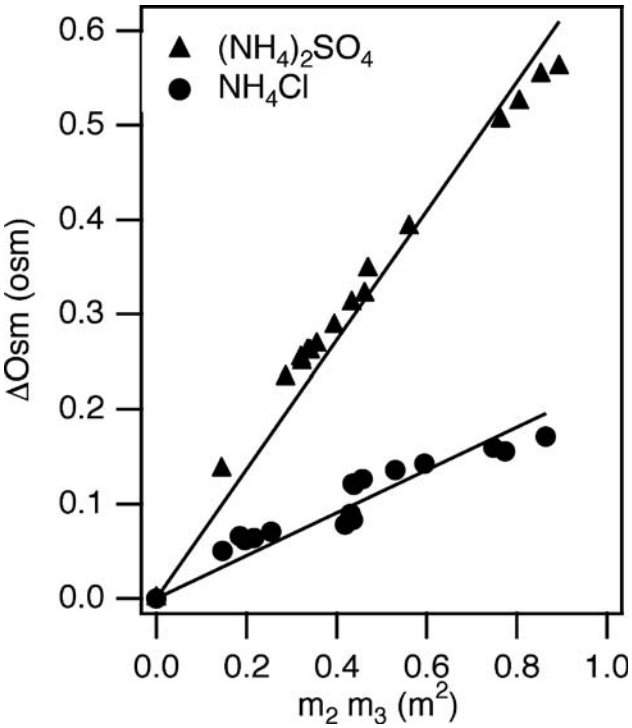


**Fig. S1.** Vapor pressure osmometry data for the three-component aqueous solutions of $NH_4Cl$ and $(NH_4)_2SO_4$ with Acetyl-Ala-amide. The quantity $\Delta Osm$ is defined as the difference between the osmolality of the three component solution and the two component solutions of interest (i.e. $\Delta Osm = Osm(m_2, m_3) - Osm(m_2, 0) - Osm(0, m_3)$), and $\mu_{23}/RT$ is the slope of a plot of $\Delta Osm$ vs. $m_2 m_3$ (1).

1. Capp MW, et al. (2009) Interactions of the osmolyte glycine betaine with molecular surfaces in water: Thermodynamics, structural interpretation, and prediction of *m*-values. *Biochemistry* 48:10372–10379.

**Table S1. Contribution of different surface types to Δ*ASA* of protein unfolding and DNA duplex dissociation**

| Biopolymer or process | ASA contribution, Å$^2$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Aliphatic. C | Hydroxyl O | Amide O * | Amide N * | Carbox. O | Cat. N | Arom. C | Amino N | Other ring N |
| *lac* DBD unfolding[†] | 1658 | 66 | 369 | 90 | 6 | 109 | 148 | 0 | 0 |
| DNA 12mer melting[‡] | 242 | 0 | 455 | 358 | 0 | 0 | 662 | 428 | 547 |
| (a) $ASA_{total}$ for S1 bases[§] | 243 | 0 | 495 | 377 | 0 | 0 | 813 | 550 | 793 |
| (b) $ASA_{total}$ for S2 bases[§] | 324 | 0 | 584 | 468 | 0 | 0 | 787 | 493 | 571 |

*For DNA melting, "amide N" and "amide O" refer to amide-like groups on the bases, e.g., N1 and O6 on G.

[†]The 20 conformers of PDB (1) ID 1OSL (2) were used as the model of the folded state; averages for total *ASA* and composition were calculated using the first 51 residues of each headpiece monomer (40 conformers). The web application ProtSA (3, 4) was used to generate an unfolded ensemble for these 51 residues, and water-accessible surface areas were calculated for 1,919 conformations. The resulting average Δ*ASA* composition is shown in the table.

[‡]The Δ*ASA* for DNA 12-mer melting is calculated as $33\%(a) + 50\%(b)$, based on the extent of unstacking determined for each strand as a function of temperature (5).

[§]The Δ*ASA* values for each strand are determined by adding the total amount of surface area of each base, weighted by its occurrence in the strand.

1. Berman HM, et al. (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235–242.
2. Kalodimos CG, et al. (2004) Structure and flexibility adaptation in nonspecific and specific protein-DNA complexes. *Science* 305(5682):386–389.
3. Bernado P, Blackledge M, Sancho J (2006) Sequence-specific solvent accessibilities of protein residues in unfolded protein ensembles. *Biophys J* 91(12):4536–4543.
4. Estrada J, Bernado P, Blackledge M, Sancho J (2009) ProtSA: A web application for calculating sequence specific protein solvent accessibilities in the unfolded ensemble. *BMC Bioinformatics* 10:104–111.
5. Anderson MW (2008) Thermodynamics of the unfolding of a 12 base pair DNA duplex and the interaction of the lacI-DNA binding domain with weak and strong operator DNA. PhD thesis (University of Wisconsin, Madison).

**Table S2. Fit values of *x* (Eq. 7) and corresponding NLPB values**

| | | NLPB (cylindrical model) | | |
|---|---|---|---|---|
| salt | *x* | 12bp in polymeric DNA | oligomeric 12bp DNA | NLPB (all-atom) |
| KF | 0.15 ± 0.03 | 0.09 (1:1 salts) | 0.23 (1:1 salts) | 0.37 (1:1 salts) |
| KCl | 0.15 ± 0.03 | — | — | — |
| NaCl | 0.15 ± 0.03 | — | — | — |
| KBr | 0.15 ± 0.03 | — | — | — |
| GuHCl | 0 | — | — | — |
| $NaClO_4$ | 0.20 ± 0.04 | — | — | — |
| $Na_2SO_4$ | 0.20 ± 0.05 | — | — | 0.37 (1:2 salts) |
| $(GuH)_2SO_4$ | 0 | — | — | — |