**Supplementary material** for "The natural DNA bending angle in the *lac* repressor headpiece - O1 operator complex is determined by protein-DNA contacts and water release", by Daniel Barr and Arjan van der Vaart



**Supplementary Figure S1**. Convergence of the variance-covariance matrix for the unrestrained and restrained protein-DNA simulations (see text).



**Supplementary Figure S2**. Hotspot analysis for the system at (*A*) the unrestrained angle, as well as for the restrained simulations at angles of (*B*)  $27^{\circ}$ , (*C*)  $29^{\circ}$ , (*D*)  $30^{\circ}$ , (*E*)  $34^{\circ}$ , (*F*)  $39^{\circ}$ , (*G*)  $42^{\circ}$ , (*H*)  $47^{\circ}$ , (*I*)  $51^{\circ}$ , and (*J*)  $55^{\circ}$ . Secondary structure elements are labeled as in Fig. 4 and 5. The intensity of color for any pairwise interaction represents the intensity of energetic coupling between those residues.



**Supplementary Figure S3**. Distortion energy of the DNA throughout the restrained angle simulations for (*A*) 27°, (*B*) 29°, (*C*) 30°, (*D*) 34°, (*E*) 39°, (*F*) 42°, (*G*) 47°, (*H*) 51°, and (*I*) 55°.



**Supplementary Figure S4**. (*A*) total distortion energy of DNA, and (*B*) the total contact occupancy of all base-specific protein-DNA contacts.



**Supplementary Figure S5**. Number of interstitial waters in the protein-DNA interface for (A) the left (black) and right (gray) sites, and (B) the polar (black) and nonpolar (gray) surfaces of the complex.