Measured entropy and enthalpy of hydration as a function of distance between DNA double helices

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The temperature sensitivity of hydration forces between Mn^{2+}-DNA helices in ordered arrays has been used to measure the entropic part of the interaction free-energy versus helix separation. This entropy is positive, and like the hydration force itself, grows exponentially as helices move closer. Measured forces show an abrupt transition between regions of interactions with quite different characteristic decay lengths, with a discontinuous change in interhelical spacing. However, both the entropic and enthalpic components of the interaction free-energy maintain smooth single-exponential variation across this transition.

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I. INTRODUCTION

Both the popular "hydrophobic effect" of nonpolar molecules [1,2] and the ubiquitous powerful "hydration forces," which occur between interacting polar surfaces [3,4], recognize an ordering of water near a surface to create a structurally important difference in the entropy of this water compared to that of the bathing medium. In this paper, we report a measurement of the entropy associated with dehydration of an array of parallel DNA double helices as a function of distance between the molecules. To achieve this result we use the temperature sensitivity of the measured work needed to pack the helices as this work varies with interhelical distance.

Like the total hydration force, the entropic component of the work decays exponentially, with a decay distance of ~ 4 Å (similar to the 3–3.5-Å decay length for the net force between DNA double helices and between polysaccharides [5,6], and somewhat greater than the 1–3 Å measured for many kinds of lipids [7,8]). The strongest temperature dependence is observed [9] for hydration forces between DNA molecules with bound Mn^{2+} or Cd^{2+}. With increasing temperature, DNA assemblies lose water and become more ordered. The entropic drive is so strong in these systems that at high temperature it results in spontaneous assembly of DNA from dilute solution into a well-ordered precipitate. For the case of Mn^{2+}-DNA the entropy and enthalpy contribute almost equally to the free energy of boundary water but with opposite sign; the net free energy is about one-tenth the magnitude of each of the contending factors.

We use the osmotic stress technique [10] for the direct measurement of intermolecular forces under thermodynamically well-defined conditions. One can use the osmotic pressure, characterizing the activity of water, as a continuous thermodynamic variable. The sensitivity of osmotic stress versus interaxial spacing measurements to another experimental variable, such as temperature or salt activity, allows one to apply classical thermodynamic relations to link changes in different thermodynamic parameters. One can then join molecular assembly and structure with the energetics of dehydration in a rigorous manner.

We have been able to transform temperature-dependent force versus distance curves into entropy and enthalpy versus molecular separation by using Maxwell relations and a generalized Clausius-Clapeyron equation. Previous work [9] focused on the thermodynamics of the abrupt phase transition in helical packing observed with the Mn^{2+}-DNA system and on the influence of bulk water entropy on modulating that transition.

II. METHODS AND MATERIALS

The osmotic stress technique has been described in detail [5,7,10,11]. In the present application, DNA exposed to a polymer solution such as polyethylene glycol (PEG) forms an ordered phase. X-ray diffraction shows that DNA is packed into a two-dimensional (2D) hexagonal lattice with the long axes of the molecules perpendicular to the plane of the lattice. The osmotic stress method is based on the measurement of the interaxial distance in this phase as a function of the applied osmotic stress, varied by changing the concentration of the polymer.

High-molecular-weight chicken erythrocyte DNA is prepared as described previously [12] and exhaustively dialyzed against 10 m*M* tris-Cl (*p*H 7.5) and 1 m*M* ethylene diaminetetracetic acid (EDTA). DNA pellets (~ 250 μg) are prepared by either precipitating in ethanol or in 0.2*M* NaCl, 10 m*M* tris-Cl (*p*H 7.5), 1 m*M* EDTA, and 5% PEG (average molecular weight 20 000; Sigma Chemical Company) solution. These pellets are then equilibrated against PEG (average molecular weight 8000; Sigma Chemical Company) solutions of known osmotic pressure, salt concentration, and temperature generally for two weeks with several changes of bathing PEG solution.

All salts used are analytical grade.

Distances between helices are determined by x-ray diffraction using a fixed-anode Diffractis 601 (Enraf-Nonius) generator. The cameras are described elsewhere [13]. The sample cells hold the pellet and about 100 μl of PEG solution. The cell temperature is held to within ± 0.1 °C by a Peltier heat exchanger. Interhelical spacings are reproducible to within ± 0.2 Å for distances less than 30 Å. At larger spacings, reflections are more diffuse and reproducible to within only about ± 0.4 Å. The measurements are made under thermodynamic equilibrium conditions. DNA samples equilibrated against one set of osmotic pressure, $MnCl_2$ concentration, and temperature conditions can be reversibly equilibrated against any other. Interhelical spacings do not depend on the initial state of the pellet.

A. Thermodynamic methods

To obtain force-versus-distance curves at each concentration of $MnCl_2$, ordered arrays of DNA double helices are brought to thermodynamic equilibrium with a PEG-containing "reservoir" with well-defined salt and water activities. The DNA lattice spacing of the array or, equivalently, volume per base pair of the DNA containing phase V is measured as a function of the polymer osmotic pressure of the PEG bathing solution.

To analyze these data we assume that there is negligible preferential interaction of PEG with $MnCl_2$ and, therefore, no significant dependence of ion activity on PEG. To be sure of this assumption we experimentally verified that the osmotic pressure between the bathing solution with PEG and a similar solution without PEG, measured by a membrane osmometer, is independent of the concentration of ions. We also observed that the activity of water as measured by the vapor pressure osmometer is essentially an additive function of the $MnCl_2$ and polymer contributions measured separately.

The osmotic stress experiment is equivalent to pressing on the DNA phase with a semipermeable piston that passes water and salt but not DNA. (See the Appendix for a derivation showing the equivalence of this direct formulation with a traditional thermodynamic treatment.) The piston membrane separates the DNA-containing phase from a reservoir salt solution (without PEG) of fixed ionic strength and water activity. The mechanical pressure exerted is equivalent to the polymer osmotic pressure Π_{osm} corresponding to the solution used for the osmotic stress. To analyze these data we use a thermodynamic potential with independent intensive variables T and Π_{osm} for the temperature and pressure:

$$dG = -SdT + Vd\Pi_{osm} . \tag{1}$$

Here S and V are the corresponding extensive variables conjugate to T and Π_{osm}. A change in S defines the change in the system entropy as the DNA lattice is compressed by the semipermeable piston. V is the volume of species (water and ions) exchanging between compartments.

Since G is a state function and dG in Eq. (1) is a total differential, one can immediately write down a Maxwell relation

$$\left[\frac{\partial S}{\partial \Pi_{osm}}\right]_T = -\left[\frac{\partial V}{\partial T}\right]_{\Pi_{osm}} \tag{2}$$

relating the change in entropy as the lattice is compressed by the piston with the sensitivity of the volume to temperature.

Integration of Eq. (2) gives the entropy of condensing the DNA-containing phase:

$$\Delta S = -\int \left[\frac{\partial V(T,\Pi_{osm})}{\partial T}\right]_{\Pi} d\Pi_{osm} . \tag{3}$$

Equation (3) cannot, however, be applied to the region of abrupt change in the interaxial separation due to a phase transition in DNA packing. In the "classical" case, when the conjugate variables are hydrostatic pressure and system volume rather than Π_{osm} and V, the change of entropy across a phase transition is given by the Clausius-Clapeyron equation. Using an analogous approach [14], we can derive the "osmotic stress" variant of the Clausius-Clapeyron equation [15],

$$\Delta S = -\left[\frac{d\Pi_t}{dT_t}\right]\Delta V , \tag{4}$$

where Π_t and T_t are the observed transition point osmotic stresses and associated temperatures, and ΔS and ΔV are the entropy change and the volume transferred from the DNA phase into the bulk solution upon the transition.

Combining Eq. (3) with Eq. (4), we can calculate the entropy of the DNA phase over the full range of measured helical spacings or water volumes. Other thermodynamic potentials can be more directly calculated. The free energy or work W required for DNA dehydration is the integrated experimental osmotic stress Π_{osm} versus volume V at constant temperature,

$$W = \int dW = -\int \Pi_{osm} dV . \tag{5}$$

The enthalpy of DNA dehydration is then given by

$$\Delta H = W + T\Delta S . \tag{6}$$

Equations for the pressure-volume curves at different temperatures were obtained by least-square fitting to the experimental data. We also fitted the difference between these curves at each pressure to express the dependence of water volume in the DNA-containing phase on the temperature. The free energy, entropy, and enthalpy of the water release were then obtained by numerical integration of Eqs. (3) and (5). Fitting and numerical integration were done with MATHCAD software package (MathSoft, Inc., Cambridge, MA).

III. RESULTS

Plots of the osmotic stress Π_{osm} versus interaxial separation for DNA assemblies in $MnCl_2$ solution are shown in Fig. 1. Since the DNA helix diameter is approximately 20 Å, the 23–40-Å range in interaxial distance corre-

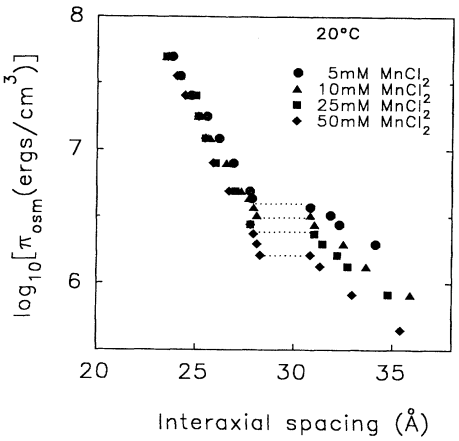

FIG. 1. Molecular force curves. Applied osmotic pressure vs separation between DNA helices in different $MnCl_2$ solutions at 20 °C.

sponds to a 3–20-Å range in surface separation.

Three regions of force behavior are observed. At close separation (3–7 Å), the repulsive force grows exponentially upon dehydration with a decay length $\lambda \simeq 1.5$ Å. At larger separations (10–20 Å), the observed repulsion is also exponential, but with a decay length $\lambda \simeq 3.5$ Å. An abrupt change in surface separation between 7 and 10 Å at a well-defined transition stress π_t connects the two exponential forces. Both the force or stress at constant spacing and the transition stress magnitudes are sensitive to bulk $MnCl_2$ concentration.

The temperature sensitivity of the osmotic stress-distance curves is illustrated in Figs. 2 and 3. The magnitudes both of the exponential forces at constant spacing and of the transition stress decrease significantly with increasing temperature. At temperatures above some critical value dependent on $MnCl_2$ concentration (about 40 °C for 50 m*M*, for example), DNA will spontaneously assemble (precipitate) from aqueous solution (no PEG) [9]. The surface separation between helices in the precipitate is approximately 7 Å.

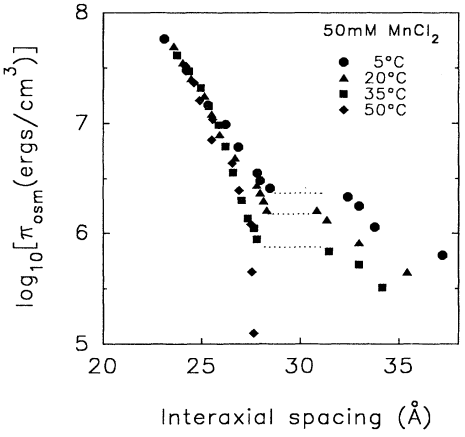

FIG. 2. Applied osmotic pressure vs DNA separation in 25-m*M* $MnCl_2$ at different temperatures.

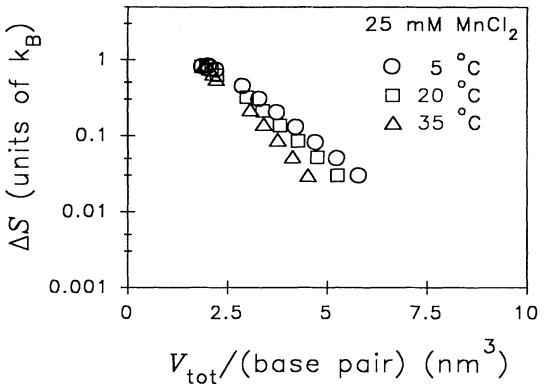

FIG. 3. Entropy of DNA assemblies vs total volume V_{tot} per base pair in the assembly. Entropy is given in units of the Boltzmann constant k_B per base pair. (The total volume V_{tot} per DNA base pair in the assembly differs from exchangeable volume V by the fixed volume of the base pair itself. We show the points rather than continuous lines to emphasize that results are obtained by direct thermodynamic transforms of experimental data. However, these transforms were performed on smooth curves obtained by least-squares fitting of the data, and the points represent smooth functions with gaps in the region of the phase transition in the packing of DNA.)

The system entropy versus separation, extracted using Eqs. (3) and (4), is shown in Fig. 3 for DNA in 25-m*M*-$MnCl_2$ solutions at three different temperatures and in Fig. 4 for DNA at 35 °C in four different $MnCl_2$ solutions. This total entropy is a general function of the system. It contains contributions not only from the differences in structure and translational freedom of ions and water between the DNA phase and the bulk solution, but also from the change in configurational entropy of DNA helices. We will argue in the Discussion section, however, that the dominating contribution comes from the release of the water itself from the DNA-containing phase to the bathing solution.

The dependence of free energy (or work) W [Eq. (5)] and enthalpy H [Eq. (6)] of solution transfer from the DNA phase to the bulk on DNA associated water volume is shown in Figs. 5 and 6 for 25-m*M*-$MnCl_2$ solu-

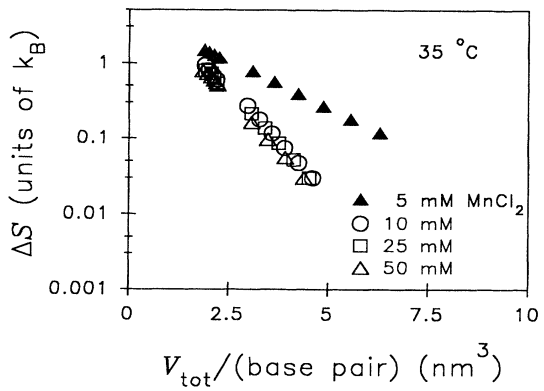

FIG. 4. Entropy vs volume per DNA base pair for different $MnCl_2$ concentrations.

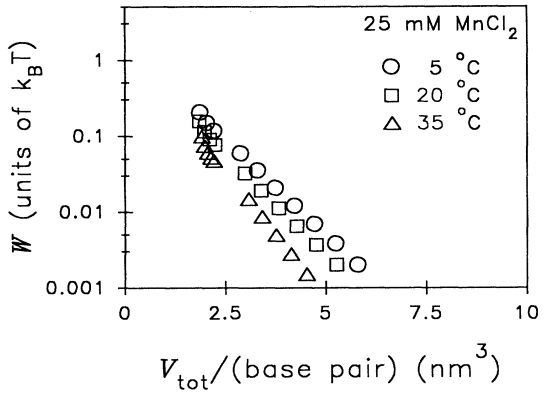

FIG. 5. Work or free energy of DNA dehydration (in units of k_BT per base pair) as dependent on the volume per DNA base pair.

tions, at $T=5$, 20, and 35 °C. These are, again, exponential functions.

IV. DISCUSSION

The entropy of the DNA array increases as DNA helices pack more closely. The sign of the entropy alone supports the idea that hydration forces come from the structuring of water by polar surfaces. Why the water? The other two components that can contribute to the entropy are the DNA molecules themselves and the added ions, but their contributions have the wrong sign. The entropies both of DNA and of ions decrease with decreasing interhelical spacing due to concomitant ordering of DNA molecules and additional adsorption (i.e., immobilization) of Mn^{2+} ions.

The DNA and ion entropy contributions are not only of the wrong sign, but also of a magnitude negligible in comparison with the observed entropy. Figure 4 shows that the entropy is independent of the ionic strength for $MnCl_2$ concentrations above 10 mM. This implies that DNA helices are nearly saturated with bound ions at these concentrations and that the additional entropy cost of ion adsorption with dehydration must be negligible compared with the total entropy change. Thermal undulations of helices in DNA assemblies have been measured previously [11,16]. Not only does the DNA configurational entropy decrease as helices pack more tightly, but also we estimate the configurational entropy to be at least an order of magnitude smaller than the entropy measured here.

One might then imagine two extreme possibilities to explain the entropy of water release. First, there is a spatially varying entropy of boundary water around DNA helices with progressively greater entropy of water release as helices move together. Second, there is a continuously changing distribution of Mn^{2+} ions among different classes of DNA binding sites with release of water directly associated with the ion.

It is instructive to compute an entropy $\tilde{s}_w$ of transferring a single water molecule from the DNA-containing phase into the bulk $MnCl_2$ solution (free of PEG) from the measured forces. This entropy can be calculated from $\partial S/\partial V$ and the volume v_w of a water molecule by

$$\tilde{s}_w = -\left[\frac{\partial S}{\partial V}\right]_T v_w \ . \tag{7}$$

The result, shown in Fig. 7, demonstrates that the entropy of water associated with DNA is negative relative to the reference bulk phase. The entropy of water release decays exponentially with the increase in interhelical spacing. The characteristic length ranges from 3.5 to 5 Å. The decay length for the structural perturbation is about the same as seen for the exponential force between helices, demonstrating a link between water restructuring and helix interaction.

The measured perturbation in entropy per water molecule caused by the DNA surface ($\tilde{s}_w \simeq 0.001-0.1$ cal/mol deg in our measurements) is very small compared with the heat capacity of water (18 cal/mol deg). Its corresponding free energy ($T\tilde{s}_w \simeq 0.3-3$ cal/mol) is tiny compared to the magnitude of water-water interactions,

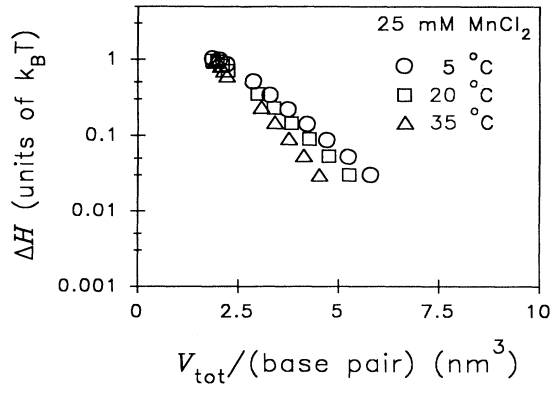

FIG. 6. Enthalpy of DNA assemblies (in units of k_BT per base pair) vs volume per DNA base pair.

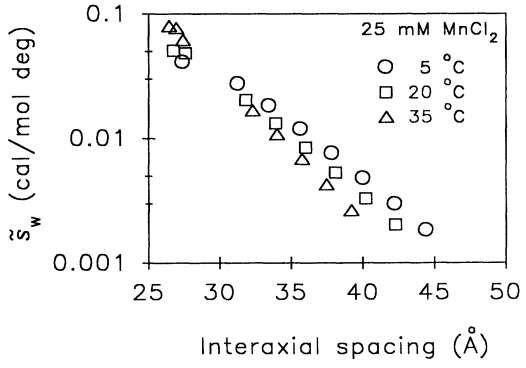

FIG. 7. Entropy of transfer of a single water molecule from the DNA-containing phase into 25-mM bulk solution of $MnCl_2$ as a function of interhelical separation. Positive entropy of the transfer means that water entropy in the DNA phase is negative relative to the bulk. Decay length of the entropy varies from 3.7 Å at 35 °C to 5.1 Å at 5 °C.

e.g., the energy of a hydrogen bond ($\sim$5 kcal/mol). Summed over all water molecules surrounding DNA, however, it becomes energetically significant and can result in such entropically favored effects as the thermal precipitation of DNA in the presence of Mn^{2+}. The dominance of hydration forces at close separation of helices is due to the huge number of water molecules per unit length of DNA.

The free energy change (or net work) W for DNA dehydration in $MnCl_2$ solutions is about an order of magnitude smaller than its enthalpic and entropic components. The contributions to the Gibbs free energy of hydration from entropy and enthalpy almost completely compensate each other. Similar compensation has been observed for many reactions involving changes in water binding [17].

A surprising feature of the results shown in Figs. 3–5 is that entropy of water release versus volume exhibits no obviously discontinuous or phase-transition behavior in the region of the sudden change in water volume. Since there is an abrupt change in equilibrium spacing at the critical stress, entropy-versus-water-volume curves, of course, show gaps in this region. Unlike the force-volume or work-volume curves, however, there is no apparent change in slope or discontinuity in magnitude between the small separation, high stress and the large separation, low stress parts of the curves.

One possible, but only formal, explanation can be qualitatively developed entirely within the framework of existing theories of hydration forces. As originally formulated [18], water structure at polar surfaces can be described by an unspecified order parameter. Hydration forces arise from perturbations in the order parameter caused by the close approach of two surfaces with their own characteristic surface structured water. A connection between a water order parameter and water entropy has been described previously [19]. For surfaces, such as DNA, with fixed backbone negative charge and bound, but laterally mobile, positive Mn^{2+} ions, both of which strongly structure water, hydration interactions depend sensitively on the mutual arrangement of these on two apposing helices. Some features of such mutual arrangement have been described elsewhere [20,21].

Qualitatively, hydration force magnitudes depend on the strength of surface water ordering, while the decay length and sign, attraction or repulsion, depend on the mutual structuring of water on the two surfaces. Attraction results from a complementary ordering, while repulsion is due to symmetrical structuring. For DNA helices with bound Mn^{2+} ions the force is an almost equal mix of the attractive and repulsive contributions. A transition from net repulsion to net attraction may only require minor rearrangement of bound ions. The residual 1.5-Å decay length exponential is a "second-order" force seen when the repulsion and attraction almost completely compensate each other. Physically, this "second-order" effect is due to the steric exclusion of the water required for full solvation of one helix by the water-inaccessible cores of neighboring helices [20,21]. This interaction, reminiscent of an electrostatic image-charge force, is repulsive for both complementary and symmetric opposing surface patches. It has a decay length about half that of the main repulsive and attractive forces [20,21], and therefore manifests itself only at small distances.

The water entropy, unlike the net hydration force, is related to a sum of order parameter magnitudes on repulsive and attractive patches, not a difference. A shift in the fine balance between repulsion and attraction, responsible for the phase transition in packing of helices, might only slightly perturb total water ordering. It is just another manifestation of the same entropy-enthalpy compensation seen with the free-energy change. In this way, it is possible to have continuously varying entropy versus volume (or molecular separation) across the phase transition (Figs. 3, 4, 6, and 7).

Quantitative examination of such ideas requires significantly higher-resolution data than reported here. More detailed force measurements, in progress, should allow observation of small second-order perturbations in water entropy, as well as comparison with theoretical predictions. Higher-resolution data should also enable us to quantitate heat-capacity changes as ordered water is released from the DNA phase to the bulk (implicit in the temperature dependence of enthalpies and entropies shown in Fig. 5). Spectroscopy of ion binding at different sites might allow one to distinguish ionic contributions.

A striking number of macromolecular assembly reactions in biochemistry and biology are entropically favored. Among the more important are the assembly of collagen into fibers, of myosin into myofibrils, of actin into the thin filaments, of tubulin into the cytoskeleton, and the gelation of sickle hemoglobin [22]. A favorable entropy for the interaction with DNA specifically is seen in the binding of several sequence-specific gene-regulatory proteins, for example, the *lac* repressor [23]. Typically, these reversible interactions are under sensitive cellular control and regulation. Many of the energetic characteristics of these assembly reactions have been associated with the interaction of nonpolar surfaces, although, for the most part, interacting monomers are very water soluble. The emerging picture of hydration forces shows that the same characteristics are observed in the interaction of polar or hydrophilic surfaces. The release of structured water drives surface attraction. Unlike hydrophobic surfaces, however, the arrangements of charged and polar groups on two hydrophilic surfaces must be complementary, must "fit" each other, for assembly to occur. This gives the interaction of polar molecules greater possibilities for control and regulation through chemical modification, e.g., phosphorylation, ligand binding, and conformational change than does traditional hydrophobic bonding.

V. CONCLUSIONS

There is an entropically favored release of *structured* water from *polar* surfaces sufficient to create ordered arrays of macromolecules. The enthalpies and entropies of water release are very small per water molecule. However, the summation of these small quantities over the many solvent molecules per DNA double helix creates significant, even dominant, forces of molecular organiza-

tion that can act at distances of several water layers.

The negative entropy of structured water associated with Mn^{2+}-DNA in an ordered phase is an exponentially decaying function of interhelical separation with a $\sim$4-Å decay length. This is about the same decay length as seen for hydration forces; it establishes another link between these forces and the structure of water at polar surfaces.

For the Mn^{2+}-DNA system, the entropic and enthalpic free energies of water release from the vicinity of the DNA molecule are of comparable magnitude but of opposite sign. The net free energy of interaction of helices is about an order of magnitude less than the size of the entropy or enthalpy alone.

The delicate balance between large entropic and enthalpic free energies creates DNA packing transitions. This strength and delicacy of hydration forces, so dependent on details of the polar surface, provide a new and largely neglected logic for thinking about the control and regulation of molecular contact and conformation.

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APPENDIX: THERMODYNAMIC DERIVATION

We consider the DNA array as a thermodynamic subsystem comprised of water, ions, and DNA itself. The Gibbs free energy G of this subsystem at temperature T and pressure p with N_i moles or molecules of each component has the form

$$dG=-S_{\rm tot}dT+V_{\rm tot}dp+\mu_{\rm DNA}dN_{\rm DNA}+\sum\mu_{\rm ions}dN_{\rm ions}+\mu_w dN_w\ , \tag{A1}$$

where $S_{\rm tot}$ and $V_{\rm tot}$ are the total volume and entropy of the DNA-containing phase, $\mu_{\rm ions}$ and μ_w are the chemical potentials of ions and water. Since we work here at constant atmospheric pressure, $V_{\rm tot}dp=0$ throughout.

The nature of the experiment is to change temperature and the concentration of a polymer added to the salt solution of known molarity where the polymer-plus-salt solution forms a reservoir whose volume is in essentially infinite excess to that of the DNA phase being studied. Thus our experimental variables are the temperature and the chemical potentials of the ions and water. The thermodynamic potential $G_{\rm DNA}$ of the DNA-containing phase appropriate for this experiment has the form

$$G_{\rm DNA}=G-\sum N_{\rm ions}\mu_{\rm ions}-N_w\mu_w\ . \tag{A2}$$

Since the number of DNA molecules in the array is fixed, i.e., $\mu_{\rm DNA}dN_{\rm DNA}=0$, we find

$$dG_{\rm DNA}=-S_{\rm tot}dT-\sum N_{\rm ions}d\mu_{\rm ions}-N_w d\mu_w\ . \tag{A3}$$

The interface between the DNA-containing phase and the polymer-containing reservoir is thermodynamically equivalent to a semipermeable membrane that excludes only the large species and passes salts and water. There is no osmotic pressure gradient between the phases; water and salt activities are equal in the two compartments. Equilibrium is achieved through the adjustment in water and salt activity in the DNA phase as the helices come closer to each other. However, osmotic pressure $\Pi_{\rm osm}$, measured in a separate experiment with a membrane osmometer between the PEG solution and a reference aqueous solution of the same ionic composition without PEG, is a convenient auxiliary parameter to characterize water activity changes induced by PEG.

We use the assumption (see Sec. II) that there is no preferential interaction of ions with PEG and neglect the dependence of ion activity on PEG. Since the chemical potentials of ions and water are the same in the DNA-containing phase and in the PEG solution, they now can be written via the osmotic pressure $\Pi_{\rm osm}$ and chemical potentials $\mu_w^{\rm ref}$ and $\mu_{\rm ions}^{\rm ref}$ in the reference phase. Using that $d\mu_{\rm ions}\simeq d\mu_{\rm ions}^{\rm ref}$ and $d\mu_w=d\mu_w^{\rm ref}-v_w d\Pi_{\rm osm}$, where v_w is the molecular volume of water, we find

$$\sum N_{\rm ions}d\mu_{\rm ions}+N_w d\mu_w=\sum N_{\rm ions}d\mu_{\rm ions}^{\rm ref}+N_w d\mu_w^{\rm ref}-Vd\Pi_{\rm osm}\ . \tag{A4}$$

Here V is the exchangeable volume between the DNA-containing phase and the PEG solution. We neglect the volume of ions in comparison with water volume and recognize water to be effectively incompressible at the pressures involved here.

The important changes in the chemical potentials with temperature can be derived from a Gibbs-Duhem relation for the reference solution

$$\sum_w N_{\rm ions}d\mu_{\rm ions}^{\rm ref}+N_w d\mu_w^{\rm ref}=-S_{\rm ref}dT\ . \tag{A5}$$

Substituting Eqs. (A4) and (A5) into Eq. (A3), we find

$$dG_{\rm DNA}=-SdT+Vd\Pi_{\rm osm}\ , \tag{A6}$$

where

$$S=S_{\rm tot}-S_{\rm ref}\ . \tag{A7}$$

Note that S now automatically subtracts the entropies of water and ions in the (uninteresting) reference phase from the entropy of the (interesting) DNA-containing phase. This function, therefore, defines the entropy associated with condensing the DNA phase by transfer of water and ions into the bulk solution not containing PEG (as if this condensing is done by mechanical pressure applied to a piston permeable for water and ions but impermeable for DNA). This brings us back to Eq. (1) introduced in Sec. II and shows the equivalence of the osmotic stress and the mechanical stress exerted by a semipermeable piston.

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