DNA Condensation by Multivalent Cations

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Abstract

In the presence of multivalent cations, high molecular weight DNA undergoes a dramatic condensation to a compact, usually highly ordered toroidal structure. This review begins with an overview of DNA condensation:condensing agents, morphology, kinetics and reversibility, and the minimum size required to form orderly condensates. It then summarizes the statistical mechanics of the collapse of stiff polymers, which shows why DNA condensation is abrupt and why toroids are favored structures. Various ways to estimate or measure intermolecular forces in DNA condensation are discussed, all of them agreeing that the free energy change per base pair is very small, on the order of 1% of thermal energy. Experimental evidence is surveyed showing that DNA condensation occurs when about 90% of its charge is neutralized by counterions. The various intermolecular forces whose interplay gives rise to DNA condensation are then reviewed. The entropy loss upon collapse of the expanded wormlike coil costs free energy, and stiffness sets limits on tight curvature. However, the dominant contributions seem to come from ions and water. Electrostatic repulsions must be overcome by high salt concentrations or by the correlated fluctuations of territorially bound multivalent cations. Hydration must be adjusted to allow a cooperative accommodation of the water structure surrounding surface groups on the DNA helices as they approach. Undulations of the DNA in its confined surroundings extend the range of the electrostatic forces. The condensing ions may also subtly modify the local structure of the double helix.

1. Introduction

DNA condensation is the collapse of extended DNA chains into compact, orderly particles containing only one or a few molecules. The decrease in size of the DNA domain is striking, as is the characteristic toroidal morphology of the condensed particle, so the phenomenon of DNA condensation has drawn considerable attention.

Genomic DNA is a very long molecule, which must fit into a very small space inside a cell or virus particle. Fully extended, the 160,000 base pairs of T4 phage DNA span 54 μm. Yet the T4 DNA molecule has to fit in a virus capsid about 100 nm in diameter, a 540-fold linear compression. The 4.2 million base pairs of the *E. coli* chromosome extend 1.4 mm (half this as a fully-stretched circular chromosome), yet must fit into a nucleolar region about 1 μm across, a linear compression of about 1400. Thinking about the issue in a different way, the radius of gyration of the T4 wormlike coil is about 950 nm, so its volume compression ratio is about 6900. The molecular volume of the T4 DNA molecule, considered as a cylinder 2 nm in diameter phosphate-to-phosphate, is 1.7×10^5 nm^3; the internal volume of the capsid is about 5×10^5 nm^3, so the DNA occupies about 1/3 of the volume within the capsid. If an 0.3 nm shell of water is added to the DNA surface, the fractional volume occupancy rises to about 1/2.

Considering the obvious energetic barriers to such tight packaging - the loss of configurational entropy of the long DNA molecule, the tight bending of the stiff double helix, the electrostatic repulsion of the negatively charged DNA phosphates - it is no surprise that organisms expend considerable metabolic energy to accomplish the task. Recent estimates run about 1/2 ATP hydrolyzed per base pair packaged (Guo *et al*., 1987; Morita *et al*., 1993).

Thus it is a considerable surprise that DNA collapse, or condensation, can occur spontaneously in the test tube, upon adding a low concentration of multivalent cation to low ionic strength aqueous buffer (Gosule & Schellman, 1976). Even more surprising, the morphology of the condensed DNA particles is most commonly that of a compact, orderly toroid, strongly reminiscent of the structure of intraphage DNA gently lysed from virus capsids (Klimenko *et al*., 1967). X-ray scattering shows that the surface-to-surface spacing between DNA helices is only about 1-2 water diameters (Schellman & Parthasarathy, 1984), so that the packing density of DNA condensed *in vitro* is entirely comparable to that of intraphage DNA.

DNA condensation has become a lively area for research in diverse areas of science. In biochemistry, biophysics, and molecular biology it represents a process by which the genetic information is packaged and protected. In polymer physics and condensed matter physics, it presents intriguing problems of phase transitions, liquid crystal behavior, and polyelectrolytes. And in biotechnology and medicine, it provides a promising means whereby DNA containing genes of therapeutic interest can be prepared for transfer from solution to target cells for gene therapy applications.

In this review I concentrate on the energetics of DNA condensation provoked by multivalent cations, that is, on the intermolecular forces which provide the free energy which stabilizes the condensed state. There are essentially two contenders for the dominant attractive force in condensation:hydration forces and correlated counterion fluctuations. I shall discuss them both, but shall emphasize counterion fluctuations because it is most amenable to predictive theory and because it is the area on which my own work has focused.

2. Overview of Condensation Behavior

It is worthwhile to begin, however, with a brief overview of some broader aspects of DNA condensation (Bloomfield, 1991; Bloomfield *et al*., 1998).

Condensation is defined as a decrease in the volume occupied by a DNA molecule from the large domain dilutely occupied by a wormlike random coil, to a compact state in which the volume fractions of solvent and DNA are comparable. In the condensed state, DNA helices may be separated by just one or two layers of water. While condensation of single molecules has been observed, it is more common that several molecules are incorporated into the condensed structure. Thus condensation is difficult to distinguish rigorously from aggregation or precipitation. Use of the term condensation is generally confined to situations in which the aggregate is of finite size and orderly morphology.

Condensing agents

Condensing agents generally work either by decreasing repulsions between DNA segments (e.g. neutralizing of phosphate charge, and/or reorienting water dipoles near DNA surfaces, by multivalent cations) or by making DNA-solvent interactions less favorable (e.g. by adding ethanol, which is a poorer solvent than water for DNA, or by adding another polymer, such as polyethylene glycol (PEG), which excludes volume to the DNA). Multivalent cations may also cause localized bending or distortion of the DNA, which can also facilitate condensation.

In aqueous solutions, condensation normally requires cations of charge +3 or greater. Those most commonly used in condensation studies are the naturally occurring polyamines spermidine^{3+} and spermine^{4+} (Chattoraj *et al*., 1978; Gosule & Schellman, 1976) and the inorganic cation $Co(NH_3)_6^{3+}$ (Widom & Baldwin, 1980; Widom & Baldwin, 1983). Others include cationic polypeptides such as polylysine (Laemmli, 1975), and basic proteins such as histones H1 and H5 (Garcia-Ramírez & Subirana, 1994; Hsiang & Cole, 1977). Divalent metal cations do not provoke condensation in water at room temperatures except under special circumstances (Ma & Bloomfield, 1994), but they will do so in water-alcohol mixtures (Arscott *et al*., 1995; Votavová *et al*., 1986; Wilson & Bloomfield, 1979). A major goal of current research, and of this review, is to understand these counterion charge requirements.

Alcohols and neutral or anionic polymers can also provoke DNA condensation. High concentrations of ethanol are commonly used to precipitate DNA, but under carefully controlled conditions it can produce particles of well-defined morphology (Eickbush & Moudrianakis, 1976; Lang, 1973). Considerably less ethanol is required if $Co(NH_3)_6^{3+}$ is added at low ionic strength, since the two agents act synergistically (Arscott et al., 1995). Neutral polymers such as PEG, at high concentrations and in the presence of adequate concentrations of salt produce what has been termed Ψ-DNA, or psi-DNA, the acronym for Polymer-and-Salt-Induced which describes the condensation process (Lerman, 1971). Ψ-DNA, which has a distinctive circular dichroism spectrum due to the cholesteric liquid crystal structure of the aggregate, is also produced by anionic polymers, such as polyaspartate, polyglutamate, and the anionic peptides found in the capsid of bacteriophage T4 (Laemmli *et al*., 1974).

Much attention has been paid recently to the condensation of DNA with cationic liposomes, since the complex can be an efficient agent for transfection of eukaryotic cells. This is presumably because the condensed state of the DNA protects it from nucleases and allows it to pass more easily through small openings, while the lipid coating on the DNA increases its permeability through cell membranes. Interested readers are referred to recent reviews (Bloomfield, 1996; Duzgunes &

Felgner, 1993; Huang *et al*., 1993; Lasic, 1997).

Morphology

When condensation is induced by careful addition of polyamines or $Co(NH_3)_6^{3+}$ to very dilute aqueous DNA solutions, toroids and rods are the structures most commonly observed by electron microscopy. The toroids have similar size distributions regardless of DNA length, indicating that several small DNA molecules, or one large one, are incorporated in a single particle (Arscott *et al.*, 1990; Bloomfield, 1991; Chattoraj et al., 1978). Torus-shaped particles of spermidine-condensed DNA exist under the hydrated conditions of freeze fracture electron microscopy, and DNA double helical fibers can be seen circumferentially wrapped around the toroid (Marx & Ruben, 1983).

Small numbers of rods are sometimes seen in electron micrographs of mainly toroidal condensates. The diameters and lengths of the rods are similar to the thicknesses and circumferences, respectively, of the toroids; but one does not seem to be the precursor of the other. In water-alcohol mixtures, however (Arscott et al., 1995; Eickbush & Moudrianakis, 1976; Lang, 1973; Lang *et al*., 1976), or with permethylated spermidine as condensing agent (Plum *et al.*, 1990), a greater proportion of rods are seen. This choice of rodlike over toroidal morphology may rest on the nonpolarity of the solvent or condensing agent. Theories for determination of morphology are discussed in more detail below.

As the alcohol concentration becomes higher, discrete toroids and rods are replaced by more extensively aggregated structures. With sufficient alcohol to lower the dielectric constant below 65, and in the presence of $Co(NH_3)_6^{3+}$, DNA collapses into a network of multistranded fibers (Arscott et al., 1995). Circular dichroism spectroscopy indicates that the DNA has undergone a B-A transition, albeit at concentrations at which neither ethanol nor $Co(NH_3)_6^{3+}$ could induce the transition alone. The A-DNA then apparently strongly self-adheres and rapidly aggregates into fibrous networks, not allowing time for more compact and orderly condensates to form.

Kinetics and Reversibility

Light scattering kinetic studies of DNA condensation have been carried out in dilute DNA solutions (ca. 1–5 μg/ml) where extensive intermolecular aggregation is minimized though not totally eliminated (He, 1992; Widom & Baldwin, 1980). These experiments typically show an initial rapid rise in scattering intensity over the first few minutes, followed by a slower rise to a plateau after 30-120 minutes. The increase in intensity is attributable to a collapse of individual DNA molecules (resulting in a larger particle structure factor); and, in the case of plasmid condensation, to the association of several DNA molecules in the condensing particle. Further moderate rise in intensity after several hours may be due to secondary aggregation of toroids or rods. Experiments on the millisecond time scale (Porschke, 1984) indicate an induction period during which the condensing agent (spermine in this case) must rearrange on the DNA to reach a critical binding fraction before intramolecular condensation can occur.

While condensation may be relatively slow, reflecting nucleation lags and slow bimolecular association in these very dilute DNA solutions (typically on the order of 10^{-10} M DNA molecules), decondensation provoked by diluting the condensing agent is very rapid (Widom & Baldwin, 1980), being complete within the measurement time of a few seconds. Thus DNA condensation is a readily reversible process, which depends sensitively on the association of sufficient condensing ligand with the DNA.

Minimum DNA Size

A striking feature of DNA condensation is that the dimensions and morphology of condensed DNA particles are largely independent of the size of the DNA. This generalization holds only above a minimum length, however. Widom and Baldwin (Widom & Baldwin, 1980) found that DNA fragments shorter than about 400 base pairs will not condense into orderly, discrete particles. This indicates that the net attractive interactions per base pair are very small:at least several hundred base pairs must interact, either intramolecularly or intermolecularly, to form a stably condensed particle (Bloomfield, 1991).

3. Statistical Mechanics of Polymer Collapse

DNA condensation is an example of a polymer coil-globule transition, whose general nature is discussed in several articles by Lifshitz, Grosberg and co-workers (Grosberg & Khokhlov, 1994; Grosberg & Kuznetsov, 1992a; Grosberg & Kuznetsov, 1992b; Grosberg & Kuznetsov, 1992c; Grosberg & Kuznetsov, 1992d; Grosberg & Kuznetsov, 1993; Lifshitz *et al.*, 1978). An important insight arising from theory is that the coil-globule transition is abrupt for stiff polymers such as DNA, but broad for flexible polymers. A lucid exposition of this problem has been presented by Post and Zimm, who first considered condensation of a single DNA molecule (Post & Zimm, 1979) and then treated the competition between intramolecular collapse and intermolecular aggregation (Post & Zimm, 1982b). Their approach is based on the familiar Flory-Huggins lattice theory of polymer solutions, but including third virial coefficient effects to take into account the high local concentration of polymer segments in the condensed phase. They obtain an equation for the total mixing free energy ΔG of polymer and solvent

$$\frac{\Delta G}{k_B T} = \frac{\Delta G_{ext}}{k_B T} + n_2 \frac{\Delta G_{int}}{k_B T} \tag{1}$$

where k_B is the Boltzmann constant, T is the temperature, ΔG_{ext} is the external free energy of mixing polymer and solvent molecules

$$\frac{\Delta G_{ext}}{k_B T} = n_1 \ln v_1 + n_2 \ln v_2 + \chi n_1 v_2 \tag{2}$$

and ΔG_{int} is the internal free energy of dissolution of a single polymer chain

$$\frac{\Delta G_{int}}{k_B T} = N\left[(\chi - 1) + \frac{B_2 \omega}{2^{3/2} \alpha^3} + \frac{B_3 \omega^2}{2\ 3^{5/2} \alpha^6} + \frac{3}{2}\left(\alpha^2 - 1\right) - \ln \alpha^3\right]. \tag{3}$$

In these equations, n_1 and n_2 are the numbers of solvent and polymer molecules, respectively, v_1 and v_2 are the volume fractions of solvent and polymer, and N is the ratio of molecular volume of polymer to that of solvent. The expansion parameter α is the ratio of the polymer radius of gyration to that in the uncondensed state with no intramolecular excluded volume (α in the condensed state « 1). B_2 and B_3 are the second and third virial coefficients, defined in terms of χ, the difference in interaction free energies of like and unlike species:

$$B_2 = \frac{1}{2} - \chi, \tag{4}$$

$$B_3 = 1 + 12\frac{\chi^2}{q} - 16\frac{\chi^3}{q^2} \tag{5}$$

where q is the lattice coordination number. The parameter ω is defined as

$$\omega = \left(\frac{9}{\pi\langle h_0^2\rangle}\right)^{3/2} V_p \tag{6}$$

where $\langle h_0^2 \rangle$ is the mean-square end-to-end distance in the unperturbed state, and V_p is the molecular volume of the polymer. Thus ω/α^3 is the volume fraction of polymer in the solution.

The key parameter is χ - the difference in free energy between solvent-solvent, polymer segment-segment, and solvent-segment interactions. Although the theories of intermolecular forces we shall explore are not explicitly phrased in terms of χ, it is implicitly this parameter which we shall try to understand.

The equations for ΔG can be differentiated with respect to n_1 and n_1 to obtain the chemical potentials μ_1 and μ_1 of solvent and polymer. When χ is > 1/2, the chemical potentials are no longer monotonic functions of polymer concentration, indicating coexisting phases. The boundaries of the phase diagram depend on DNA molecular weight, but three regions occur:"extended random coils in solution, collapsed DNA in dilute solution, and concentrated precipitate ("aggregated")." The aggregated phase is predicted to be stable, relative to the collapsed phase, except in very dilute solutions, in qualitative agreement with experiment (Post & Zimm, 1982a). The extended-collapsed coil transition is predicted to be discontinuous because of the stiffness of DNA, but the transition should appear macroscopically diffuse because individual molecules will collapse independently. These predictions are nicely borne out experimentally in fluorescence microscopic studies of single T4 phage DNA molecules (Yoshikawa *et al*., 1996a; Yoshikawa *et al*., 1996b).

4. Why Toroids?

The toroidal conformation of condensed DNA, with a well-defined hole in the middle surrounded by circumferentially wound double helical strands, is one of the most striking aspects of the condensation phenomenon. Given that solvent conditions are sufficient to cause collapse of the extended DNA chain, why are toroids formed, rather than spherical globules, or rods, or lamellae? Or indeed, why ordered particles at all, rather than random aggregates? Most of the answer lies in the stiffness of DNA, the rather weak attractive force between DNA segments, and the very low DNA concentrations at which condensation experiments are generally done.

A tenable theory has been developed by Grosberg and Zhestkov (Grosberg & Zhestkov, 1986), who consider that the total free energy of DNA is the sum of compressive, repulsive, and elastic contributions. The compressive free energy is due either to external osmotic pressure (e.g., from high molecular weight PEG in Ψ-condensation) or from poor solvent quality (χ > 1/2 in Eq. (4)). The repulsive term comes from the excluded volume of the DNA, accentuated by its high asymmetry (persistence length/diameter). The elastic contribution comes from bending and other

conformational entropy terms. The relative importance of these free energy terms depends also on polymer chain length. The result is a complicated phase diagram, from which it is deduced that relatively short DNA molecules will form toroids, while very long molecules will form compact spherical globules. (Of course, very short DNAs cannot form toroids either, since they cannot bend sufficiently without kinking.) Greater compressive forces will favor spherical globules without an inner hole, while greater stiffness and excluded volume will favor toroids. These predictions have been verified experimentally for condensation by $Co(NH_3)_6^{3+}$ of DNA from bacteriophages λ (48 kbp) and T4 (166 kbp) (Vasilevskaya *et al*., 1997). Toroids are seen with both molecules at low $Co(NH_3)_6^{3+}$ concentration, but spherical globules become predominant as the concentration of condensing agent increases.

A theory (Ubbink & Odijk, 1995; Ubbink & Odijk, 1996) of toroids with hexagonally aligned DNA which also takes into account surface free energy (molecules on the outside have only four nearest neighbors, while those on the inside have six) enables prediction of inner and outer toroid dimensions. Unfortunately, no data are available which can clearly test this theory.

If the DNA is a covalently closed circle, its linking number is a topological invariant. Condensation of such a molecule increases its writhe, and the increase in its torsional elastic free energy must be taken into consideration. This leads to the prediction (Grosberg & Zhestkov, 1985) that the equilibrium radius of the toroid formed by supercoiled DNA will be less than that for linear or relaxed circular molecules. This prediction has been verified experimentally (Arscott et al., 1990), though the effect is not large.

Rodlike particles are occasionally seen in electron micrographs of condensed DNA, but they are rarely in high proportion unless the solvent or the condensing agent is somewhat nonpolar (Arscott et al., 1995; Eickbush & Moudrianakis, 1976; Lang, 1973; Lang et al., 1976; Plum et al., 1990). It seems likely that a nonpolar environment may lower the free energy of exposed heterocyclic bases, favoring sharp local kinking over gradual bending.

Theory (Post & Zimm, 1982b) predicts, and most experiments confirm, that DNA condensation will be multimolecular except at extraordinarily low concentration. However, the aggregated material still generally consists of toroidal condensates, connected by strands of DNA. Thus the constraints of stiffness and excluded volume still dominate morphology, except when condensing conditions are very strong (Arscott et al., 1995). If attractive interactions are too firm, DNA segments will stick where they first touch, and will be kinetically constrained from annealing into the thermodynamically favored hexagonally packed, tightly curved toroidal conformation.

5. Experimental Estimates of Intermolecular Free Energy

When DNA condenses spontaneously in the presence of multivalent cations, what is the magnitude of the attractive free energy holding the molecule in the condensed state? Several estimates may be made, with varying degrees of precision.

The crudest back-of-the-envelope estimate can be made by recalling that DNA molecules smaller than 400 bp do not form ordered, compact particles (Widom & Baldwin, 1980). Assuming that the total attractive free energy must be at least an order of magnitude greater than thermal energy, to maintain at least a side-by-side bimolecular complex, one may estimate that the attractive free energy per base pair is on the order of $10k_BT$/400 bp = 1/40 k_BT/bp = 0.06 kJ/mole bp = 0.015 kcal/mole bp.

A similar value comes from measurements on single λ DNA molecules using optical tweezers

(Baumann *et al*., 1997b). The force *vs*. extension curve is measured in low concentrations of salt as spermidine or $Co(NH_3)_6^{3+}$ is added, with the DNA molecule held at less than 80% full extension so that intramolecular looping can occur. As the multivalent cation concentration reaches a value which would normally cause DNA condensation, the force F rises abruptly from near zero to about 4 pN with $Co(NH_3)_6^{3+}$ or 0.5 pN with spermidine. Since the change in free energy upon extending the molecule a distance Δx is $\Delta G = F\Delta x$, the free energy change in moving one base pair, 3.4 Å, is about 13.6×10^{-22} J, or 1/3 k_BT. ΔG with spermidine is about 1/8 of this.

An argument which treats plasmid condensation as a monomer–n-mer association equilibrium (Bloomfield, 1991) gives somewhat smaller values. If the initial mole fraction of plasmid monomer is X_1^0, the mole fraction of n-mer is X_n, and the fraction of uncondensed plasmid is f_u, then the equilibrium constant is

$$K = \frac{X_n}{X_1^n} = \frac{(1 - f_u)\left(X_1^0\right)^{1-n}}{n f_u^{\,n}}. \qquad (7)$$

With typical conditions of a 3000 bp plasmid at 5 µg/ml (so $X_1^0 = 5 \times 10^{-11}$), n = 5, and f_u between 0.1 and 0.9, we find $K \approx 5 \times 10^{-41}$, and since $\Delta G° = -RT\ln K$, $\Delta G \approx -0.007\, k_BT$ / bp.

Of course, the most satisfactory way to determine the free energy of DNA condensation is to measure it directly. This can be done using the osmotic stress technique with a thermodynamic analysis (Leikin *et al*., 1991; Rau & Parsegian, 1992a; Rau & Parsegian, 1992b). DNA in an ordered fiber is equilibrated with a reservoir containing water and ions that can exchange, and a large inert polymer (e.g. polyethylene glycol, PEG) that cannot exchange. The distance between DNA molecules is measured from the Bragg spacing in x-ray diffraction, assuming hexagonal packing. The osmotic pressure is controlled by the polymer concentration. Since stress is force per unit area, a measured osmotic stress is readily converted to a force between surfaces, giving osmotic stress vs. distance curves.The force per unit length on a DNA molecule a distance R from its neighbors is $f = R\Pi/\sqrt{3}$, where Π is the osmotic stress or pressure, and the $\sqrt{3}$ comes from the hexagonal packing of the double helices. Techniques for osmotic stress measurements are described in detail by Parsegian et al (Parsegian *et al*., 1986).

Thermodynamic analysis begins with the standard relation between free energy, temperature, and pressure changes, but with osmotic pressure $\prod$ replacing external pressure P:

$$dG = -SdT + Vd\Pi \qquad (8)$$

Differentiating both sides with respect to T and Π gives the Maxwell relation

$$\left(\frac{\partial S}{\partial \Pi}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_\Pi \qquad (9)$$

This can be integrated to give the entropy change accompanying a change of osmotic stress

$$\Delta S = -\int \left(\frac{\partial V(T,\Pi)}{\partial T}\right)_\Pi d\Pi \qquad (10)$$

In the region of abrupt change due to a packing transition, one uses a variant of the Clausius-Clapeyron equation,

$$\Delta S = -\left(\frac{d\Pi_{trans}}{dT_{trans}}\right)\Delta V \tag{11}$$

and the total entropy change as a function of helix spacing (which is in turn related to water volume) is a sum of the continuous and transition contributions.

The total free energy change equals the work done on the system by changing the array spacing at constant osmotic pressure

$$W = \Delta G = -\int \Pi dV \tag{12}$$

and the enthalpy change accompanying the approach of the DNA helices to their equilibrium distance is

$$\Delta H = W + T\Delta S. \tag{13}$$

Such an analysis shows that the free energy minimum at the equilibrium center-to-center separation of 28 Å between DNA helices in 20 mM $Co(NH_3)_6^{3+}$ and 0.25 M NaCl is -0.17 k_BT /bp, and that a change of 0.20 ions bound per bp accompanies the spontaneous (Π = 0) condensation transition. (Rau & Parsegian, 1992b).

Thus we see that a variety of lines of reasoning all lead to an estimate of the free energy of DNA condensation in the range of 10^{-2} to 10^{-1} k_BT per base pair. At this stage the point is not to argue what is the “correct” value (since this will, in any case, vary with condensing ligand and solution conditions), but rather to recognize that $\Delta G/k_BT$ per bp is a very small number, so that DNA condensation must be a delicately poised, highly cooperative process.

6. The Importance of Ionic Interactions

Electrostatic forces must be important in condensation. The laws of physics require that the strong repulsive interactions must be substantially neutralized as negatively charged DNA segments approach closely. Ionic effects on condensation were investigated systematically by Wilson and Bloomfield (Wilson & Bloomfield, 1979), who studied condensation of T7 bacteriophage DNA with spermidine, spermine, and other multivalent cations by light scattering, which enabled convenient detection of condensation under a wide range of ionic conditions. They observed that the critical concentration of multivalent cation required to produce DNA condensation increases with increasing salt. When the amount of binding of multivalent cation and simple salt to the DNA was calculated using Manning's counterion condensation theory (Manning, 1978), a striking regularity emerged which has since been confirmed under a wide variety of circumstances:approximately 90% of the DNA charge must be neutralized for condensation to occur.

The equations for competitive counterion condensation of two cations with different valences are (Manning, 1978; Wilson & Bloomfield, 1979)

$$1 + \ln\frac{1000\theta_1}{c_1 V_{P1}} = -2z_1\xi\left(1 - z_1\theta_1 - z_2\theta_2\right)\ln\left(1 - e^{-\kappa b}\right), \tag{14}$$

$$\ln\frac{\theta_2}{c_2} = \ln\frac{V_{P2}}{1000e} + \frac{z_2}{z_1}\ln\frac{1000\theta_1 e}{c_1 V_{P1}}, \tag{15}$$

and

$$V_P(z) = 4\pi e N_A (1+z)\left(\xi - \frac{1}{z}\right)b^3 \tag{16}$$

where Z_1, c_1, V_{P1}, and θ_1 are the valence, molar concentration, molar territorial binding volume, and fraction of DNA phosphate binding sites occupied, for cations of type 1 (the lower valent ion). Z_2, c_2, V_{P2}, and θ_2 are the corresponding quantities for the higher valent ion. ξ is the counterion condensation parameter, the ratio of charge spacing b to Bjerrum length $q^2/\varepsilon k_B T$, and κ is the inverse Debye-Hückel screening length. Iterative numerical solution of these equations for θ_1 and θ_2 allows calculation of the total fraction of DNA phosphate charges neutralized,

$$r = z_1\theta_1 + z_2\theta_2. \tag{17}$$

The charge neutralization predicted by these equations has been directly verified by gel electrophoresis of plasmids in buffers containing various amounts of multivalent cations (Ma & Bloomfield, 1995).

Analysis with these equations of the ionic conditions at which DNA collapse occurs shows that an essentially constant fraction, $r \approx 0.89 - 0.90$, of the DNA charge is neutralized by counterion condensation. This constancy holds with Na^+ or Mg^{2+} as the lower valent ion, with spermidine^{3+} or spermine^{4+} as the higher valent ion, and in a wide range of mixtures of water with alcohol or osmolytes as cosolvents (Arscott et al., 1995; Bloomfield *et al.*, 1994; Flock *et al.*, 1995; Flock *et al.*, 1996; Wilson & Bloomfield, 1979). It also holds with phage φW14 DNA (Benbasat, 1984), which has variable and lower charge density owing to the incorporation of the positively charged base α-putrescinylthymine^{2+}. Widom and Baldwin (Widom & Baldwin, 1980; Widom & Baldwin, 1983) found that $Co(NH_3)_6^{3+}$ is a 5-fold more efficient condensing agent than spermidine^{3+}, despite equal binding of these isovalent compounds (Plum & Bloomfield, 1988), with collapse occuring at about 85% charge neutralization. However, the dependence of the critical $Co(NH_3)_6^{3+}$ concentration on added salt confirms the ion exchange behavior underlying Eqs. (14)–(17). Electrophoretic light scattering shows directly that the condensation of λ DNA by spermidine^{3+} or spermine^{4+} is accompanied by a decrease in charge density to approximately the 1- r = 0.1 level (Yen & Ware, 1982).

All of these results indicate that DNA condensation in the presence of multivalent cations is determined by the total charge neutralization of the DNA, rather than by the binding of the multivalent cation per se, which can be as low as θ_2 = 0.1 for Mg^{2+} - spermine^{4+} solutions (Wilson & Bloomfield, 1979).

7. Forces in DNA Condensation

Bending

If a length L of DNA with persistence length a is bent in a path with radius of curvature R_C, the bending free energy is

$$\Delta G_{bend} = \frac{RTaL}{2R_c^{\,2}}. \tag{18}$$

Inside a condensed particle, R_C will vary from point to point, being less at the inside of a toroid than at the periphery, so a mean value should be used (Riemer & Bloomfield, 1978). Probably more important are the possibility of sequence-directed bending (Reich *et al*., 1992), or bending due to localized binding of multivalent cations (see below), in which case one may even have $\Delta G_{bend} < 0$ (Marquet & Houssier, 1991). At the very least, Eq. (14) provides an order of magnitude estimate of the energetic cost of bending. Typically, the radius of curvature of the DNA in a toroid, or in a bacteriophage capsid, is on the order of the persistence length:$R_C \approx a$. If we consider a length of DNA equal to one persistence length, $L = a$, then $\Delta G_{bend} \approx RT/2$. Since a persistence length of DNA contains about 150 bp, this corresponds to 1/300 k_BT per base pair.

Mixing entropy

When a polymer such as DNA undergoes a coil-globule transition, entropy is lost due to the demixing of polymer and solvent. This is essentially given by the first two terms on the right hand side of Eq. (2). Neglecting the complications of stiffness and χ value considered by Post and Zimm, a simple approximation is (Riemer & Bloomfield, 1978)

$$\Delta G_{mix} = -T\Delta S_{mix} = RT\frac{L}{a}. \tag{19}$$

This corresponds to about 1/150 k_BT per base pair.

Coulombic

Bending and mixing free energies resist condensation. In the quest for energetic contributions that favor condensation, at least under some conditions, there are two prime contenders:fluctuating counterion electrostatics and hydration force. We shall take them in order.

Although the calculated charge of the DNA is reduced to about 10% of its original value under the ionic conditions required for condensation, it is not reduced to zero. In fact, the amount of remaining charge is impressively large. If a condensed DNA particle contains 40,000 bp, it has 80,000 negative phosphates, or about 8,000 elementary charges after 90% neutralization. This much charge concentrated in a particle a few hundred Å in radius will generate a powerful repulsive force. The free energy change ΔG_{elec} due to electrostatic repulsions in going from uncondensed to condensed DNA has been estimated (Marquet & Houssier, 1991; Riemer & Bloomfield, 1978) from the results of Oosawa (Oosawa, 1968; Oosawa, 1971) to be

$$\Delta G_{elec} = \frac{n_{tot}k_BT}{2\xi z_2^{\,2}} \ln\frac{V_{uncond}}{V_{cond}} \tag{20}$$

where n_{tot} is the total number of DNA phosphate charges before neutralization, and z_2 is the valence of the condensing cation. The uncondensed molecule is assumed to be a sphere of radius equal to its radius of gyration R_G:$V_{uncond} = (4\pi/3)R_G^3 = (4\pi/3)(aL/3)^{3/2}$. The volume of the condensed particle is $V_{cond} = 2\pi R_c \times \pi[(R_o - R_i)/2]^2$, where R_o and R_i are the outer and inner radii of the toroid. Assuming a ratio $V_{uncond}/V_{cond} = 10^4$, this gives $\Delta G_{elec} \approx 0.24\ k_BT$ per base pair with $z_2 = 3$, and 0.14 k_BT /bp with $z_2 = 4$.

Substantial reduction of the repulsive electrostatic interaction between DNA helices does not in itself account for the attractive interaction stabilizing the condensed form. One possible source of attraction, suggested by Oosawa (Oosawa, 1968; Oosawa, 1971), is induced dipole interactions between the fluctuating ion atmospheres surrounding rodlike macroions such as DNA. This is conceptually similar to the London dispersion force arising from the mutual polarization of electron clouds. (However, analysis of the balance between polyelectrolyte repulsive forces and attractive forces due to London dispersion interactions leads to the conclusion that dispersion forces would have to be 2-5 times larger than normal, in order to cause DNA collapse when the repulsions have been reduced by the presence of multivalent counterions (Bloomfield *et al*., 1980).)

Marquet and Houssier (Marquet & Houssier, 1991) have used the Oosawa theory to obtain a simple equation for the attractive fluctuation free energy

$$\Delta G_{fluct} = -\frac{3Lk_BT}{X_{cond}} \frac{\left(\theta_2 z_2^2 \xi\right)^2}{\left(1+\theta_2 z_2^2 \xi\right)^2} \tag{21}$$

where X_{cond} is the center-to-center interhelix distance in the condensed DNA. Typical values for the parameters in this equation lead to $\Delta G_{fluct} \approx -0.3\ k_BT$ per base pair in aqueous solution.

Addition of Eqs. (18)–(21) gives the total free energy ΔG_{tot} (Marquet & Houssier, 1991). In water, the result is a net attraction of -0.11 to -0.24 kJ/mole bp (-0.044 to -0.097 k_BT/bp) with cations of charge +3 or greater, in accord with experiment. ΔG_{tot} with divalent cations in 50% methanol-water is very slightly negative, indicating stable condensates consistent with experiment (Wilson & Bloomfield, 1979). While this treatment is at least semiquantitatively successful and captures much of the important underlying physics, it is not perfect. In particular, Eq. (21) neglects the effects of excess monovalent counterions and of Debye-Hückel screening, which will tend to reduce the attractive free energy.

A somewhat different attraction theory which utilizes the concept of correlated ionic fluctuations has been developed by Rouzina and Bloomfield (1996). This theory emphasizes the pseudo-two-dimensional character of the counterion distribution very close to the highly charged DNA surface. Coulombic repulsion between these surface-adsorbed but mobile ions leads to a two-dimensional ionic lattice. When two DNA molecules with ionic surface lattices approach each closely, the lattices adjust in complementary fashion, positive charge opposite negative, leading to a net attraction. The magnitude of this attraction is determined by the surface charge density σ and the solution dielectric constant ε. The average coulomb energy between neighboring mobile counterions at the surface is

$$W_c = \frac{z^2 q^2}{\varepsilon a_z} \tag{22}$$

where a_z is the average distance between z-valent ions on the surface

$$a_z = (zq/\sigma)^{1/2}. \quad (23)$$

The critical parameter is Γ_2, the 2D coupling strength or ratio of W_c to thermal energy

$$\Gamma_2 = W_c / k_B T. \quad (24)$$

Association of macroions will occur when $\Gamma_2 \approx 2$. For B-DNA in water, with $\sigma = 0.150$ C/m^2 ($q/\sigma = 1.07$ nm^2), $a_z = 1.03$, 1.46, and 1.79 nm for $z = 1$,2, and 3 respectively. This corresponds to values of $\Gamma_2 = 0.68$, 1.92, and 3.53 respectively. Thus the attraction is calculated (in agreement with experiment) to be stable with respect to disruptive thermal motions of the ions so long as the counterions have charge +3 or greater. The condensed state in the presence of divalent ions is predicted to be marginally unstable, also in agreement with experiment. Unfortunately, this theory has not yet been sufficiently worked out to predict reliably the magnitude of the net attractive free energy, after thermal disorder and the competition of lower valent cations are taken into account. However, preliminary calculations indicate that the net free energy is of order -0.1 k_BT /bp.

An interesting series of papers (Guldbrand *et al.*, 1986; Lyubartsev & Nordenskiöld, 1995; Nilsson *et al.*, 1991) has calculated ion-mediated forces between DNA molecules using the Monte Carlo method. The most recent paper in the series (Lyubartsev & Nordenskiöld, 1995) makes some important methodological advances, notably giving the ions a finite radius rather than treating them as point charges, and considering added salt. Previous calculations had resulted in fairly strong attractions between hexagonal arrays of DNA rods in the presence of point divalent cations. When a more realistic ionic radius of 4 Å is assigned, a very weak attraction is found. This adds weight to the conclusion, based on both experimental and theoretical evidence, that divalent cations are borderline in provoking DNA condensation in water.

Ray and Manning (Ray & Manning, 1994) have used the framework of counterion condensation theory to calculate the potential of mean force of a pair of identical rodlike polyions, oriented in parallel, in a solution of monovalent cations. The polyions are modeled as infinite linear arrays of uniformly spaced monovalent charge sites, not as continuous line charges. According to the results we have surveyed up to this point, we would expect this model to give repulsive interactions at all distances, since it involves only monovalent cations. That is not what happens, however. The mean force between the polyions is indeed repulsive for distances much less than the Debye length κ^{-1} and for distances on the order of κ^{-1}. There is an intermediate range of distances, however, where the polyions attract each other. The physical interpretation of this attraction is expansion of the counterion condensation region into the space between the polyions, thus allowing counterions to remain condensed but be shared, thus raising their translational entropy. Since this theory does not predict attraction at the close surface-to-surface distances characteristic of DNA condensation, it does not appear to be relevant to understanding the forces stabilizing the condensed state. However, its possible relevance to the multimolecular aggregation of concentrated solutions of mononucleosomal DNA fragments in NaCl (Wissenburg *et al.*, 1994; Wissenburg *et al.*, 1995) should be noted.

Hydration Force

The hydration force is due to reconfiguration of water between macromolecular surfaces. It appears to be a general force in biomolecular systems (Leikin *et al.*, 1993), being observed with charged, zwitterionic, and neutral phospholipid bilayers, neutral and charged polysaccharides, protein assemblies such as collagen fibers and hemoglobin, and voltage-gated transmembrane ion

channels as well as with DNA. It can be either attractive or repulsive. The repulsive component is short-ranged, exponentially decaying, independent of ionic strength, and similar in behavior for all types of molecular systems examined. This suggests a common underlying force, probably involving polarization of water by polar groups on the surface of a charged or polar macroion.

The concept of hydration forces developed largely from the direct measurement of osmotic stress as a function of separation between macromolecules. Force-distance measurements of parallel DNA helices as a function of salt concentration and valence show a surprising result:all ionic influences nearly disappear when the separation between DNA surfaces is 5-15 Å (Rau *et al.*, 1984). This holds true under very different ionic conditions. The repulsive pressure decays exponentially, with a characteristic length of 2.5 - 3.5 Å. Similar behavior is seen even within intact bacteria (Reich *et al*., 1995). It should be noted, however, that this lack of ionic dependence at close approach does not prove that electrostatics is not important, since Monte Carlo simulation of osmotic pressure in hexagonally packed DNA, taking ionic size and correlation effects into account, reproduces the essential features of the osmotic stress experiments while using a continuum solvent model (Lyubartsev & Nordenskiöld, 1995).

At interaxial distances of 26-30 Å typical of DNA packing in bacteriophage, the packing energy per base is 0.1 - 0.4 kcal/mol, corresponding to an osmotic pressure of 1.2 - 5.5 x 10^7 dyne/cm^2. Parameters for repulsive forces between DNA molecules in solutions of monovalent cations have been tabulated for practical use by Podgornik *et al*. (Podgornik *et al*., 1994).

Hydration forces can sometimes be attractive as well as repulsive, when the osmotic pressure vs. distance curve exhibits a discontinuity or drops abruptly to zero. These curves have several properties that are not easily explicable by more traditional forces (Rau & Parsegian, 1992a; Rau & Parsegian, 1992b). They can extend 8-10 Å surface-surface, do not depend on ionic strength or composition, are two orders of magnitude greater than those predicted by London dispersion force theory, and decay exponentially over a characteristic distance 1.4-1.5 Å independent of the condensing ligand.

This type of behavior has been explained by a theory that postulates the rearrangement of surface-bound water by condensing ligands, to create regions of hydration attraction, or water bridging, between helices. The behavior of the condensing ligands, in turn, is influenced by the surface lattice of DNA phosphates and other polar groups. Leikin et al (Leikin et al., 1991) state "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."

Hydration forces are very difficult to predict from first principles, since they depend on summation of hundreds or thousands of very weak intermolecular interactions. However, some general statements can be made using an order parameter formalism (Leikin et al., 1991). This predicts a repulsive hydration pressure between two similar, homogeneous planar surfaces separated by distance *h*:

$$P_{rep}^{homo} = \frac{R}{\sinh^2\left(h/2\lambda_w\right)} \approx 4\mathrm{Re}^{-h/\lambda_w} \tag{25}$$

and an attractive pressure between two complementary surfaces

$$P_{attr}^{homo} = -\frac{A}{\cosh^2\left(h/2\lambda_w\right)} \approx 4Ae^{-h/\lambda_w} \tag{26}$$

where R and A are coefficients and λ_w is the water correlation length (about 4-5 Å). This is the value observed for Na^+ or tetramethyl ammonium$^+$ as counterions.

If the surfaces themselves have a lattice structure, of periodicity a, then the homogeneous pressure is replaced by an inhomogenous pressure

$$P_{rep}^{inhomo} = -\frac{\alpha\left\langle\sigma^2\right\rangle}{\sinh^2\left\{h\left[\frac{1}{\lambda_w^2}+\left(\frac{2\pi}{\alpha}\right)^2\right]^{1/2}\right\}} \tag{27}$$

proportional to the mean-square value of the surface "charge" density σ. For B-DNA, with a = 34 Å and λ_w = 4-5 Å, the effective correlation length

$$\lambda = \frac{1}{2}\left[\frac{1}{\lambda_w^2}+\left(\frac{2\pi}{\alpha}\right)^2\right]^{-1/2} \tag{28}$$

is in the range 1.6 to 1.8 Å, compared with the measured 1.3 Å for DNA condensed with $Co(NH_3)_6^{3+}$ or Mn^{2+}.

Further insight into hydration forces can be achieved by thermodynamic analysis (Leikin et al., 1991; Rau & Parsegian, 1992a; Rau & Parsegian, 1992b). Such analysis shows that attraction is driven by the entropy increase upon rearrangement or release of water, which in turn depends on counterion binding. Attraction is enhanced by increasing bulk water entropy with chaotropic anions such as perchlorate. When this analysis is applied to DNA in aqueous $MnCl_2$ solutions (Leikin et al., 1991; Rau & Parsegian, 1992a), ΔS and ΔH are found to be positive and increase exponentially as the helices approach, with a characteristic length ≈ 4 Å. The thermodynamic parameters show no discontinuity in slope or magnitude associated with the collapse transition that occurs under applied osmotic stress (though it does not occur spontaneously in water except at elevated temperatures). $T\Delta S$ and ΔH are of nearly equal magnitude; ΔG is about 10% of either, in the range 0.1 to 0.001 k_BT depending on interhelix distance. The exponential decay length decreases with increasing T, but is independent of $MnCl_2$ concentration at 10 mM and above. $T\Delta S$ for transfer of a single water molecule from the DNA-containing phase into the bulk $MnCl_2$ solution is 0.3-3 cal/mol. Although this is a very small number, it becomes significant when summed over all the waters associated with DNA. The small values of the thermodynamic quantities for MnDNA arise from an almost equal mix of attractive and repulsive contributions. A transition from repulsion to attraction may require only small rearrangements of bound Mn^{2+} ions. On the other hand, application of the same sort of analysis to DNA condensation by $Co(NH_3)_6^{3+}$ (Rau & Parsegian, 1992b) shows that a change of 0.20 ions bound per bp accompanies the spontaneous (Π = 0) condensation transition.

Undulations

Direct measurements of the electrostatic repulsive force as a function of distance between aligned DNA molecules show that it cannot be explained just by a simple application of screened Debye-Hückel interactions (Podgornik *et al*., 1989). Instead, undulations of the somewhat flexible DNA helices roughly double the range of the repulsive interactions, and broaden the x-ray diffraction peaks in osmotic stress experiments. Odijk (Odijk, 1993b) has developed a mean-field theory that explains these data fairly well, using the persistence length a to estimate the elastic bending properties of DNA that govern the undulations. The characteristic length scale of the undulations (the deflection length) is $d^{2/3}a^{1/3}$, where d is the effective diameter of the the cylindrical tube in which each DNA molecule is confined by its neighbors in a hexagonal array. For the theory to be self-consistent, the lattice spacing R should be larger than $D + 2d + 2\kappa^{-1}$ where D is the DNA diameter. The effect can be substantial, especially at high salt. For example, in 0.8 M NaCl at R = 4.5 nm, which corresponds to $\kappa d = 1.80$, the force per unit length is calculated to be 0.19 dyne/cm, compared with 0.042 dyne/cm if the polyion were infinitely stiff. The experimental value is 0.35 dyne/cm. For DNA confined in a tightly curved environment such as a toroid or phage head, with radius of curvature R_c, the deflection length is predicted to be proportional to R_c^2/a (Odijk, 1993a).

Podgornik *et al.* (Podgornik et al., 1994) have used directly measured forces between DNA helices in uni-univalent salt solutions to assemble a "toolbox" of force coefficients and mathematical expressions for the forces between pairs of molecules, both parallel and skewed. The parameters reflect short-range exponential interactions, presumed to be hydration forces, and longer-range, undulation-enhanced electrostatic forces. They are sensitive to the univalent cation species, but less so to the anion or to temperature. While the tabulated values are only for univalent cations, and therefore do not bear directly on the forces involved in condensation of DNA by multivalent cations, this approach will be most valuable in developing a quantitative understanding of interactions leading to condensation.

8. Helix Distortion by Multivalent Cations

Up to this point we have assumed that multivalent cations bind electrostatically near the DNA surface, and cause DNA condensation by their correlated fluctuations and effects on water polarization, but that they have no other impact on DNA structure. This assumption is inherent in most continuum electrostatic treatments, which treat the polyion simply as a charged surface. It seems intrinsic to the concept of "territorial binding" in counterion condensation theory (Manning, 1978), which postulates that the condensed counterions can move freely along the polyion backbone, a postulate supported by NMR studies of spermine^{4+} mobility in DNA solutions (Wemmer *et al*., 1985). The enthalpy of binding of spermine to DNA is 0 cal/mol P, compared with -300 cal/mol P for poly(L-lysine) and +350 cal/mol P for Mg^{2+} (Ross & Shapiro, 1974). Thus polyamine binding is entropy-driven, a characteristic of purely electrostatic interactions without significant dehydration or structural changes. The circular dichroism of DNA condensed with spermidine or spermine indicates that it remains in the B-conformation (Gosule & Schellman, 1978).

However, there is increasing evidence that multivalent cations can distort the local helical structure of the DNA. Early models of DNA-polyamine binding (Liquori *et al*., 1967; Suwalsky *et al*., 1969), based on crystal structures of spermidine·HCl and spermine·HCl, postulated that the

polyamines bind in the minor groove, where they can form H-bonds to the backbone phosphates without distorting the double helix structure. In fact, a high resolution crystal structures of DNA (Drew & Dickerson, 1981) with spermine in the crystallization buffer showed that the spermine is generally in the *major* groove, associated with a bend or distortion of the double helix, a conclusion supported by molecular mechanics computer studies (Feuerstein *et al.*, 1986; Feuerstein *et al.*, 1990). While $Co(NH_3)_6^{3+}$ bound to DNA generally has high diffusive mobility when assayed by NMR, a small fraction appears to interact with G-G sequences in a more localized fashion, in some cases inducing a B-A transition (Arscott et al., 1995; Braunlin & Xu, 1992; Xu *et al.*, 1993a; Xu *et al.*, 1993b).

Transient electric dichroism measurements by Pörschke (Porschke, 1986) indicate that DNA becomes more bent and/or more flexible in the presence of Mg^{2+}, spermine^{4+}, and $Co(NH_3)_6^{3+}$. Similar work by Marquet *et al.* (Marquet & Houssier, 1988; Marquet *et al.*, 1985; Marquet *et al.*, 1987) suggests that spermine both bends DNA (in AT-rich regions) and stiffens it (in GC-rich regions). Recent experiments in which single DNA molecules are stretched by optical tweezers yield a decrease in persistence length in the presence of these ions (Baumann *et al.*, 1997a; Wang *et al.*, 1997). Theoretically, increased bending and increased flexibility both decrease the persistence length (Schellman & Harvey, 1995; Trifonov *et al.*, 1987).

Coming from quite a different starting point, Mirzabekov and Rich speculated that asymmetric charge neutralization of DNA phosphate groups produces part of the driving force for nucleosome folding:the DNA tends to bend toward the neutralized face. This proposal was examined quantitatively by Manning *et al.* (Manning *et al.*, 1989) and shown to be feasible. It has been experimentally verified by Maher and coworkers who characterized DNA bending by gel electrophoretic mobility when one face of the DNA was neutralized by substitution of neutral methylphosphonate linkages for negatively charged phosphates (Strauss & Maher, 1994), and when ammonium ions were tethered by hexamethylene chains near specific phosphates (Strauss *et al.*, 1996).

An interesting question is how, if the ions are freely diffusable along the DNA backbone with a sub-nanosecond residence time at any given base pair, they are able to induce local bending of the relatively massive and slowly responding double helix. The answer appears to be that the time-average cation charge cloud favors greater occupancy of the major groove, which in turn is consistent with a DNA conformation bent into the major groove (Rouzina & Bloomfield, 1997).

Thus it is becoming increasingly clear that multivalent cations profoundly affect the structure of DNA, both by condensing it and by modifying its local structure. The two processes are likely to be interrelated, and may together control higher-order aspects of DNA structure and function (Levin-Zaidman *et al.*, 1996).

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References

Arscott, P. G., A.-Z. Li & V. A. Bloomfield. (1990). Condensation of DNA by trivalent cations. 1. Effects of DNA length and topology on the size and shape of condensed particles. *Biopolymers.* 30: 619-630.

Arscott, P. G., C. Ma, J. Wenner & V. A. Bloomfield. (1995). DNA condensation by cobalt hexaammine(III) in alcohol-water mixtures: Dielectric constant and other solvent effects. *Biopolymers.* 36: 345-365.

Baumann, C. G., S. B. Smith, V. A. Bloomfield & C. Bustamante. (1997a). Ionic Effects On the Elasticity Of Single DNA Molecules. *Proc. Natl. Acad. Sci. USA.* 94: 6185-6190.

Baumann, C. G., S. B. Smith, C. Bustamante & V. A. Bloomfield. (1997b). Intramolecular Collapse in Stretched Single DNA Molecules. *in preparation.*

Benbasat, J. A. (1984). Condensation of bacteriophage øW14 DNA of varying charge densities by trivalent counterions. *Biochemistry.* 23: 3609-3619.

Bloomfield, V. A. (1991). Condensation of DNA by multivalent cations: Considerations on mechanism. *Biopolymers.* 31: 1471-1481.

Bloomfield, V. A. (1996). DNA condensation. *Curr. Opinion Struct. Biol.* 6: 334-341.

Bloomfield, V. A., D. M. Crothers & I. Tinoco, Jr. (1998). *Nucleic Acids: Structures, Properties and Functions*, University Science Press, Mill Valley, CA.

Bloomfield, V. A., C. Ma & P. G. Arscott. (1994). Role of multivalent cations in condensation of DNA. In *Macro-Ion Characterization: From Dilute Solutions to Complex Fluids* (Schmitz, K. S., ed.), pp. 195-209. American Chemical Society, Washington, D.C.

Bloomfield, V. A., R. W. Wilson & D. C. Rau. (1980). Polyelectrolyte effects in DNA condensation by polyamines. *Biophys. Chem.* 11: 339-343.

Braunlin, W. H. & Q. Xu. (1992). Hexaamminecobalt(III) binding environments on double-helical DNA. *Biopolymers.* 32: 1703-1711.

Chattoraj, D. K., L. C. Gosule & J. A. Schellman. (1978). DNA condensation with polyamines. II. Electron microscopic studies. *J. Mol. Biol.* 121: 327-337.

Drew, H. R. & R. E. Dickerson. (1981). Structure of a B-DNA dodecamer. III. Geometry of hydration. *J. Mol. Biol.* 151: 535-556.

Duzgunes, N. & P. L. Felgner. (1993). Intracellular delivery of nucleic acids and transcription factors by cationic liposomes. *Meth. Enzymol.* 221: 303-306.

Eickbush, T. H. & E. N. Moudrianakis. (1976). The compaction of DNA helices into either continuous supercoils or folded-fiber rods and toroids. *Cell.* 13: 295-306.

Feuerstein, B. G., N. Pattabiraman & L. J. Marton. (1986). Spermine-DNA interactions: A theoretical study. *Proc. Natl. Acad. Sci. USA.* 83: 5948-5952.

Feuerstein, B. G., N. Pattabiraman & L. J. Marton. (1990). Molecular mechanics of the interactions of spermine with DNA: DNA bending as a result of ligand binding. *Nucleic Acids Res.* 18: 1271-1282.

Flock, S., R. Labarbe & C. Houssier. (1995). Osmotic effectors and DNA structure: Effect of glycine on precipitation of DNA by multivalent cations. *J Biomol Struct Dynam.* 13: 87-102.

Flock, S., R. Labarbe & C. Houssier. (1996). Dielectric constant and ionic strength effects on DNA precipitation. *Biophys J.* 70: 1456-1465.

Garcia-Ramírez, M. & J. Subirana. (1994). Condensation of DNA by basic proteins does not depend on protein composition. *Biopolymers.* 34: 285-292.

Gosule, L. C. & J. A. Schellman. (1976). Compact form of DNA induced by spermidine. *Nature*. 259: 333-335.

Gosule, L. C. & J. A. Schellman. (1978). DNA condensation with polyamines I. Spectroscopic studies. *J. Mol. Biol.* 121: 311-326.

Grosberg, A. Y. & A. R. Khokhlov. (1994). *Statistical Physics of Macromolecules*, American Institute of Physics, New York.

Grosberg, A. Y. & D. V. Kuznetsov. (1992a). Quantitative theory of the globule-to-coil transition. 1. Link density distribution in a globule and its radius of gyration. *Macromolecules.* 25: 1970-1979.

Grosberg, A. Y. & D. V. Kuznetsov. (1992b). Quantitative theory of the globule-to-coil transition. 2. Density-density correlation in a globule and the hydrodynamic radius of a macromolecule. *Macromolecules.* 25: 1980-1990.

Grosberg, A. Y. & D. V. Kuznetsov. (1992c). Quantitative theory of the globule-to-coil transition. 3. Globule-globule interaction and polymer solution binodal and spinodal curves in the globular range. *Macromolecules.* 25: 1991-1995.

Grosberg, A. Y. & D. V. Kuznetsov. (1992d). Quantitative theory of the globule-to-coil transition. 4. Comparison of theoretical results with experimental data. *Macromolecules.* 25: 1996-2003.

Grosberg, A. Y. & D. V. Kuznetsov. (1993). Single-chain collapse or precipitation? Kinetic diagram of the states of a polymer solution. *Macromolecules.* 26: 4249-4251.

Grosberg, A. Y. & A. V. Zhestkov. (1985). On the toroidal condensed state of closed circular DNA. *J. Biomolec. Struct. Dynam.* 3: 515-520.

Grosberg, A. Y. & A. V. Zhestkov. (1986). On the compact form of linear duplex DNA: Globular states of the uniform elastic (persistent) macromolecule. *J. Biomolec. Struct. Dynam.* 3: 859-872.

Guldbrand, L., L. G. Nilsson & L. Nordenskiöld. (1986). A Monte Carlo simulation study of electrostatic forces between hexagonally packed DNA double helices. *J. Chem. Phys.* 85: 6686-6698.

Guo, P., C. Peterson & D. Anderson. (1987). Prohead and DNA-gp3-dependent ATPase activity of the DNA packaging protein gp16 of bacteriophage ø29. *J. Mol. Biol.* 197: 229-236.

He, S. (1992). Kinetics of DNA Condensation by Hexaammine Cobalt(III), University of Minnesota.

Hsiang, M. W. & R. D. Cole. (1977). Structure of histone H1-DNA complex: Effect of histone H1 on DNA condensation. *Proc. Natl. Acad. Sci. USA.* 74: 4852-4856.

Huang, L., A. L. Klibanov, A. Mori & X. Gao. (1993). Targeted delivery of drugs and DNA with liposomes. *J. Liposome Res.* 3: 505-515.

Klimenko, S. M., T. I. Tikkchonenko & V. M. Andreev. (1967). Packing of DNA in the head of bacteriophage T2. *J. Mol. Biol.* 23: 523-533.

Laemmli, U. K. (1975). Characterization of DNA condensates induced by poly(ethylene oxide) and polylysine. *Proc. Natl. Acad. Sci. USA.* 72: 4288-4292.

Laemmli, U. K., J. R. Paulson & V. Hitchins. (1974). Maturation of the head of bacteriophage T4. V. A possible DNA packaging mechanism: *in vitro* cleavage of the head proteins and the structure of the core of the polyhead. *J. Supramol. Struc.* 2: 276-301.

Lang, D. (1973). Regular superstructures of purified DNA in ethanolic solutions. *J. Mol. Biol.* 78: 247-254.

Lang, D., T. N. Taylor, D. C. Dobyan & D. M. Gray. (1976). Dehydrated circular DNA: Electron microscopy of ethanol-condensed molecules. *J. Mol. Biol.* 106: 97-107.

Lasic, D. D. (1997). *Liposomes in Gene Delivery*, CRC Press, Boca Raton, FL.

Leikin, S., V. A. Parsegian, D. C. Rau & R. P. Rand. (1993). Hydration forces. *Annu. Rev. Phys. Chem.* 44: 369-395.

Leikin, S., D. C. Rau & V. A. Parsegian. (1991). Measured entropy and enthalpy of hydration as a function of distance between DNA double helices. *Phys. Rev. A.* 44: 5272-5278.

Lerman, L. S. (1971). A transition to a compact form of DNA in polymer solutions. *Proc. Natl. Acad. Sci. USA.* 68: 1886-1890.

Levin-Zaidman, S., Z. Reich, E. J. Wachtel & A. Minsky. (1996). Flow of structural information between four DNA conformational levels. *Biochemistry.* 35: 2985-2991.

Lifshitz, I. M., A. Y. Grosberg & A. R. Khokhlov. (1978). Some problems of the statistical physics of polymer chains with volume interaction. *Rev. Mod. Phys.* 50: 683-713.

Liquori, A. M., L. Constantino, V. Crescenzi, V. Elia, E. Giglio, R. Puliti, M. D. Savino & V. Vitagliano. (1967). Complexes between DNA and polyamines: A molecular model. *J. Mol. Biol.* 24: 113-122.

Lyubartsev, A. P. & L. Nordenskiöld. (1995). Monte Carlo simulation study of ion distribution and osmotic pressure in hexagonally oriented DNA. *J Phys Chem.* 99: 10373-10382.

Ma, C. & V. A. Bloomfield. (1994). Condensation of Supercoiled DNA Induced by $MnCl_2$. *Biophys. J.* 67: 1678-1681.

Ma, C. & V. A. Bloomfield. (1995). Gel Electrophoresis Measurement of Counterion Condensation on DNA. *Biopolymers.* 35: 211-216.

Manning, G. S. (1978). The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11: 179-246.

Manning, G. S., K. K. Ebralidse, A. D. Mirzabekov & A. Rich. (1989). An estimate of the extent of folding of nucleosomal DNA by laterally asymmetric neutralization of phosphate groups. *J. Biomolec. Struct. Dynam.* 6: 877-889.

Marquet, R. & C. Houssier. (1988). Different binding modes of spermine to A-T and G-C base pairs modulate the bending and stiffening of the DNA double helix. *J. Biomol. Struct. Dynam.* 6: 235-246.

Marquet, R. & C. Houssier. (1991). Thermodynamics of cation-induced DNA condensation. *J. Biomol. Struct. Dynam.* 9: 159-167.

Marquet, R., C. Houssier & E. Fredericq. (1985). An electro-optical study of the mechanisms of DNA condensation induced by spermine. *Biochim. Biophys. Acta.* 825: 365-374.

Marquet, R., A. Wyart & C. Houssier. (1987). Influence of DNA length on spermine-induced condensation. Importance of the bending and stiffening of DNA. *Biochim. Biophys. Acta.* 909: 165-172.

Marx, K. A. & G. C. Ruben. (1983). Evidence for hydrated spermidine-calf thymus DNA toruses organized by circumferential DNA wrapping. *Nucleic Acids Res.* 11: 1839-1854.

Morita, M., M. Tasaka & H. Fujisawa. (1993). DNA packaging ATPase of bacteriophage T3. *Virology.* 193: 748-752.

Nilsson, L. G., L. Guldbrand & L. Nordenskjöld. (1991). Evaluation of the electrostatic osmotic pressure in an infinite system of hexagonally oriented DNA molecules. A Monte Carlo simulation. *Mol. Phys.* 72: 177-192.

Odijk, T. (1993a). Physics of tightly curved semiflexible polymer chains. *Macromolecules.* 26: 6897-6902.

Odijk, T. (1993b). Undulation-enhanced electrostatic forces in hexagonal polyelectrolyte gels. *Biophys. Chem.* 46: 69-75.

Oosawa, F. (1968). Interaction between parallel rodlike macroions. *Biopolymers.* 6: 1633-1647.

Oosawa, F. (1971). *Polyelectrolytes*, Marcel Dekker, New York.

Parsegian, A. V., R. P. Rand, N. L. Fuller & D. C. Rau. (1986). Osmotic stress for the direct measurement of intermolecular forces. *Methods Enzymol.* 127: 400-416.

Plum, G. E., P. G. Arscott & V. A. Bloomfield. (1990). Condensation of DNA by trivalent cations. 2. Effect of cation structure. *Biopolymers.* 30: 631-643.

Plum, G. E. & V. A. Bloomfield. (1988). Equilibrium dialysis study of binding of hexammine cobalt(III) to DNA. *Biopolymers.* 27: 1045-1051.

Podgornik, R., D. C. Rau & V. A. Parsegian. (1989). The action of interhelical forces on the organization of DNA double helices: Fluctuation-enhanced decay of electrostatic double-layer and hydration forces. *Macromolecules.* 22: 1780-1786.

Podgornik, R., D. C. Rau & V. A. Parsegian. (1994). Parametrization of direct and soft steric-undulatory forces between DNA double helical polyelectrolytes in solutions of several different anions and cations. *Biophys. J.* 66: 962-971.

Porschke, D. (1984). Dynamics of DNA condensation. *Biochemistry.* 23: 4821-4828.

Porschke, D. (1986). Structure and dynamics of double helices in solution: Modes of DNA bending. *J. Biomolec. Struct. Dynam.* 4: 373-389.

Post, C. B. & B. H. Zimm. (1979). Internal condensation of a single DNA molecule. *Biopolymers.* 18: 1487-1501.

Post, C. B. & B. H. Zimm. (1982a). Light scattering study of DNA condensation: Competition between collapse and aggregation. *Biopolymers.* 21: 2139-2160.

Post, C. B. & B. H. Zimm. (1982b). Theory of DNA condensation: Collapse vs. aggregation. *Biopolymers.* 21: 2123-2137.

Rau, D. C., B. K. Lee & V. A. Parsegian. (1984). Measurement of the repulsive force between polyelectrolyte molecules in ionic solution: Hydration forces between parallel DNA double helices. *Proc. Natl. Acad. Sci. USA.* 81: 2621-2625.

Rau, D. C. & V. A. Parsegian. (1992a). Direct measurement of temperature-dependent solvation forces between DNA double helices. *Biophys. J.* 61: 260-271.

Rau, D. C. & V. A. Parsegian. (1992b). Direct measurement of the intermolecular forces between counterion-condensed DNA double helices. Evidence for long range attractive hydration forces. *Biophys. J.* 61: 246-259.

Ray, J. & G. S. Manning. (1994). Attractive force between two rodlike polyions mediated by the sharing of condensed counterions. *Langmuir.* 10: 2450-2461.

Reich, Z., R. Ghirlando & A. Minsky. (1992). Nucleic acids packaging processes: effects of adenine tracts and sequence-dependent curvature. *J. Biomol. Struct. Dynam.* 9: 1097-1109.

Reich, Z., E. J. Wachtel & A. Minsky. (1995). In Vivo Quantitative Characterization Of Intermolecular Interactions. *J. Biol. Chem.* 270: 7045-7046.

Riemer, S. C. & V. A. Bloomfield. (1978). Packaging of DNA in bacteriophage heads: Some considerations on energetics. *Biopolymers.* 17: 785-794.

Ross, P. D. & J. T. Shapiro. (1974). Heat of interaction of DNA with polylysine, spermine, and Mg^{++}. *Biopolymers.* 13: 415-416.

Rouzina, I. & V. A. Bloomfield. (1997). DNA bending by small multivalent cations. *Biophys. J.* submitted:

Schellman, J. A. & S. C. Harvey. (1995). Static contributions to the persistence length of DNA and dynamic contributions to DNA curvature. *Biophys Chem.* 55: 95-114.

Schellman, J. A. & N. Parthasarathy. (1984). X-ray diffraction studies on cation-collapsed DNA. *J. Mol. Biol.* 175: 313-329.

Strauss, J. K. & L. J. Maher, III. (1994). DNA bending by asymmetric phosphate neutralization. *Science.* 266: 1829-1834.

Strauss, J. K., C. Roberts, M. G. Nelson, C. Switzer & L. J. Maher, III. (1996). DNA bending by hexamethylene-tethered ammonium ions. *Proc Natl Acad Sci USA.* 93: 9515-9520.

Suwalsky, M., W. Traub, U. Shmueli & J. A. Subirana. (1969). An X-ray study of the interaction of DNA with spermine. *J. Mol. Biol.* 42: 363-373.

Trifonov, E. N., R. K.-Z. Tan & S. C. Harvey. (1987). Static persistence length of DNA. In *Structure & Expression* (Olson, W. K., Sarma, M. H., Sarma, R. H. & Sundaralingam, M., eds.), Vol. 3: DNA Bending and Curvature, pp. 243-253. Adenine Press, Schenectady, NY.

Ubbink, J. & T. Odijk. (1995). Polymer- and salt-induced toroids of hexagonal DNA. *Biophys J.* 68: 54-61.

Ubbink, J. & T. Odijk. (1996). Deformation of toroidal DNA condensates under surface stress. *Europhysics Lett.* 33: 353-358.

Vasilevskaya, V. V., A. R. Khokhlov, S. Kidoaki & K. Yoshikawa. (1997). Structure of collapsed persistent macromolecule: Toroid vs spherical globule. *Biopolymers.* 41: 51-60.

Votavová, H., D. Kucerová, J. Felsberg & J. Sponar. (1986). Changes in conformation, stability and condensation of DNA by univalent and divalent cations in methanol-water mixtures. *J. Biomolec. Struct. Dynam.* 4: 477-489.

Wang, M. D., H. Yin, R. Landick, J. Gelles & S. M. Block. (1997). Stretching DNA With Optical Tweezers. *Biophysical Journal.* 72: 1335-1346.

Wemmer, D. E., K. S. Srivenugopal, B. R. Reid & D. R. Morris. (1985). Nuclear magnetic resonance studies of polyamine binding to a defined DNA sequence. *J. Mol. Biol.* 185: 457-459.

Widom, J. & R. L. Baldwin. (1980). Cation-induced toroidal condensation of DNA: Studies with $Co^{3+}(NH_3)_6$. *J. Mol. Biol.* 144: 431-453.

Widom, J. & R. L. Baldwin. (1983). Monomolecular condensation of λ−DNA induced by cobalt hexammine. *Biopolymers.* 22: 1595-1620.

Wilson, R. W. & V. A. Bloomfield. (1979). Counterion-induced condensation of deoxyribonucleic acid. A light-scattering study. *Biochemistry.* 18: 2192-2196.

Wissenburg, P., T. Odijk, P. Cirkel & M. Mandel. (1994). Multimolecular aggregation in concentrated isotropic solutions of mononucleosomal DNA in 1 M sodium chloride. *Macromolecules.* 27: 306-308.

Wissenburg, P., T. Odijk, P. Cirkel & M. Mandel. (1995). Multimolecular aggregation of mononucleosomal DNA in concentrated isotropic solutions. *Macromolecules.* 28: 2315-2328.

Xu, Q., S. R. B. Jampani & W. H. Braunlin. (1993a). Rotational dynamics of hexaamminecobalt(III) bound to oligomeric DNA: Correlation with cation-induced structural transitions. *Biochemistry.* 32: 11754-11760.

Xu, Q., R. K. Shoemaker & W. H. Braunlin. (1993b). Induction of B-A transitions of deoxyoligonucleotides by multivalent cations in dilute aqueous solution. *Biophys. J.* 65: 1039-1049.

Yen, W. S. & B. R. Ware. (1982). Counterion condensation of DNA studied by electrophoretic light scattering. *Biophys. J.* 37: 291a.

Yoshikawa, K., S. Kidoaki, M. Takahashi, V. V. Vasilevskaya & A. R. Khokhlov. (1996a). Marked Discreteness On the Coil-Globule Transition Of Single Duplex DNA. *Berichte der Bunsen Gesellschaft fur Physikalische Chemie.* 100: 876-880.

Yoshikawa, K., M. Takahashi, V. V. Vasilevskaya & A. R. Khokhlov. (1996b). Large discrete transition in a single DNA molecule appears continuous in the ensemble. *Phys. Rev. Lett.* 76: 3029-3031.