DNA-Inspired Electrostatics

Not just the repository of our genetic information, DNA is also a fascinating, shape-shifting molecule whose behavior in solution counters our intuition and challenges our physical understanding.

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Under "physiological" conditions (a 0.1 molar solution of NaCl), a DNA molecule takes on the form of a disordered coil with a radius of gyration of several micrometers; if any lengths of the molecule come within 1 nm of one other, they strongly repel. But under different conditions—in a highly dilute aqueous solution that also contains a small concentration of polyvalent cations—the same DNA molecule condenses into a tightly packed, circumferentially wound torus. Figure 1a shows just such a DNA torus. Its average radius is about 50 nm, and the distance between the axes of neighboring, parallel portions of the molecule is only slightly larger than its diameter.

A torus with essentially the same structure and dimensions is formed with other condensing agents, and other genetic sequences, as well as with significantly smaller pieces of DNA.[1] A much larger torus is formed when DNA is ejected directly from bacterial viruses into aqueous solution containing polyvalent ions, as shown in figure 1b. Clearly, polyvalent counterions (oppositely charged ions) mediate an effective attraction between the negatively charged DNA molecules, in apparent and dramatic contradiction to the fundamental fact that like-charged objects repel each other.

The electrostatic phenomena behind the behavior of macro-ions (charged colloidal particles) in solution are only now being identified and understood. We can understand some of these phenomena within the context of mean-field theory, whereas other phenomena require the explicit inclusion of correlations. In this article, we focus on the physics of macro-ions and illustrate them with simplified models. By concentrating most directly on the fundamental electrostatic issues involved, we necessarily suppress discussion of the effects of solvent structure, describing instead the aqueous solution as a simple dielectric continuum.

In all our examples, DNA plays a key role. With an effective density of one fundamental (negative) charge every 0.17 nm of its length, DNA is about as highly charged as a linear polymer can be. Moreover, because of its uncontested status as the molecule of life, DNA has been subjected to overwhelmingly more structural, kinetic, and thermodynamic probes than any other molecule. However, we also discuss a number of generic features of macro-ion electrostatics that also apply to other charged biopolymers.

Long before DNA, proteins, and other charged macromolecules were discovered, charged colloidal particles had caught the attention of many outstanding physical scientists. Michael Faraday, in the mid-1800s, exploited the electrostatic repulsion between charged gold particles to prepare colloidal suspensions that remain stable to the present day. During the following decades, the names of Simeon-Denis Poisson and Ludwig Boltzmann and, later, Peter Debye and Ernst Hückel became indelibly associated with the mean-field theory of macro-ions.

Today, in the age of molecular dynamics and Monte Carlo studies of virtually all types of many-body systems, one mean-field theory after another has been subjected to stringent testing. Finite-temperature Coulomb systems and solutions containing macro-ions have been the last to succumb to this treatment because of the problematic nature of their long-range interactions and their spatial inhomogeneity. These same features render inapplicable many standard methods from the formal theory of many-body systems (notably, the virial expansion).

However, the recent resurgence of interest in macro-ions is not driven purely by issues of fundamental physics. Most of the important biomolecules (for example, nucleic acids and proteins) and large-scale biostructures (for example, cell membranes and extracellular protein networks) are highly-charged objects in aqueous solution. Indeed, they need to be charged to avoid precipitation and phase separation at the high concentrations that characterize them in vivo. Understanding how nature controls the electrostatic interaction between biomolecules and biostructures is a fascinating challenge.

DNA condensation

Because DNA has already been proclaimed as the hero of our story, a few words of introduction are necessary. DNA's double helical structure is well known, as is the fact that genetic information is carried in its sequence of base pairs. In this article, however, we ignore its internal chemical structure. For us, DNA's double helical form is significant only in that it makes the molecule quite stiff: DNA maintains essentially the same direction over its relatively long persistence length of about 50 nm.

Another of DNA's important structural properties is that the molecule is highly charged under standard, that is, physiological, pH conditions. This property gives rise to strong repulsions between neighboring molecules in a simple (for example, monovalent) salt solution.

DNA's extraordinarily large length-to-width ratio is the third key aspect of its structure. The diameter of the molecule is approximately 2 nm, whereas typical contour lengths range from micrometers in simple viruses to cen-

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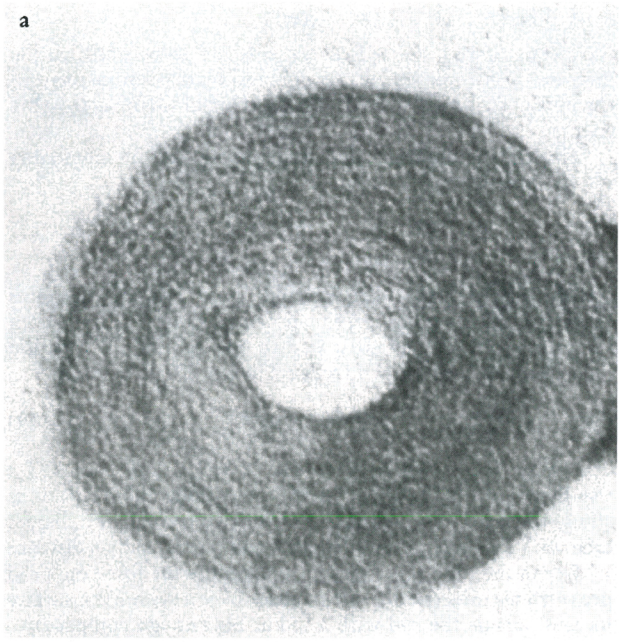

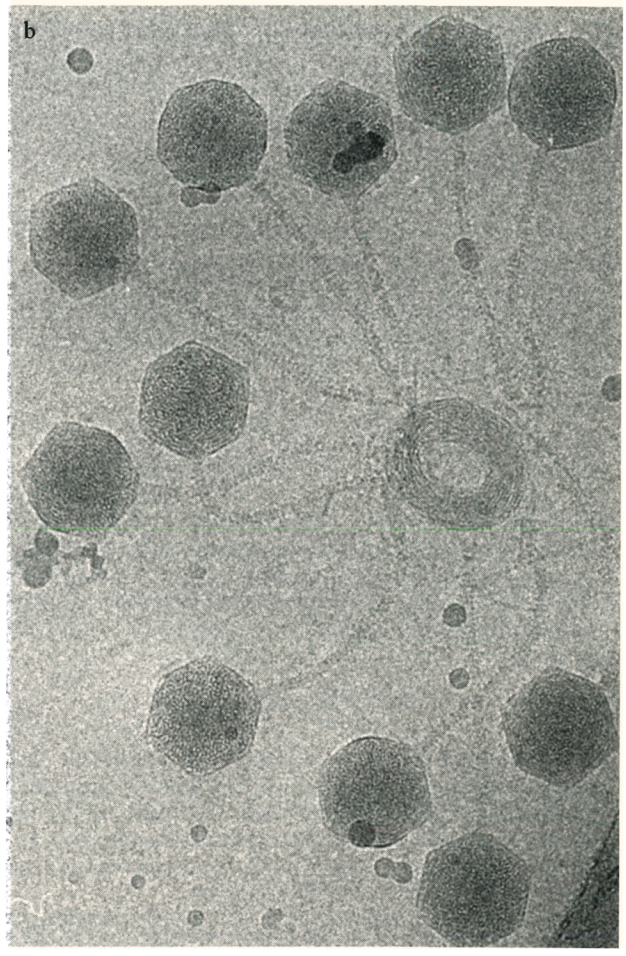

FIGURE 1. TOROIDAL DNA CONDENSATES. (a) The genome of the λ bacteriophage is wound circumferentially with local hexagonal packing and an interaxial spacing only 30% larger than the diameter. (Electron micrograph courtesy of J.-L. Sikorav.) (b) The much larger toroidal condensate in the process of being formed from the genomes of as many as 10 T5 bacteriophages. T5 closely resembles λ, but its genome is 2.5 times longer. (Adapted from O. Lambert *et al.*, *Proceedings of the National Academy of Sciences* (USA), volume 97, page 7248, 2000.)

timeters in humans.

Our article began by calling attention to the observation that DNA attracts itself under a wide range of solution conditions, many of which are commonly realized in vivo. This effect has been confirmed by well-controlled force measurement studies.[2] For example, consider a bundle of DNA molecules in osmotic equilibrium with a water-soluble polymer. The polymer exerts a lateral (osmotic) pressure on the bundle with a known magnitude that depends on the polymer concentration. For each pressure, the interaxial spacing between neighboring DNA molecules can be determined by x-ray scattering. In this way, one measures pressure–distance isotherms in the presence of different concentrations of polyvalent cations.[2] For concentrations above a certain threshold (about 10^{-2} mol l^{-1}), phase-coexistence plateaus become evident, as shown in figure 2. Such plateaus appear, for example, when simple liquid and gas phases coexist. Here, it provides direct evidence for the effective attraction between DNA strands.

Why does our intuitive understanding of electrostatics fail for highly charged macro-ions in the presence of polyvalent counterions? The mean-field treatment of the interaction between like-charged macro-ions leads inescapably to repulsive forces. The repulsive force between two charged rods falls off monotonically—inversely with spacing in salt-free solution, and exponentially otherwise. Nevertheless, to understand the origin of the attractions, we must start with the mean-field Poisson–Boltzmann (PB) theory, which is outlined in the box on page 40.

In PB theory, the effect of mobile counterions and co-ions turns out to be twofold. First, the counterions can reduce ("renormalize" is the technical term) the effective charge on the macro-ions. Second, the mobile ions of both signs act to screen the charge of the macro-ion. That is, they give rise to an exponentially decaying electrostatic potential at large distances. As a result, the interaction between two identical macro-ions always remains repulsive, albeit reduced in magnitude (relative, that is, to the "bare" value that occurs in the absence of the intervening mobile ions). How, then, can one understand the strong attractive interactions that act between highly-charged macro-ions such as the DNAs in figure 1?

Part of the answer follows from a closer examination of the counterion condensation concept, an idea developed independently by Fumio Oosawa and Jerry Manning in the late 1960s.[3] Counterion condensation essentially amounts to a battle fought between energy and entropy in minimizing the free energy of a solution of mobile charges near an isolated macro-ion.

It turns out that the playing field for this contest is level only in the case of cylindrical macro-ions. For this special geometry, the Coulomb potential energy attracting the counterion to the rod depends logarithmically on the distance from the rod, with a magnitude scaling with the rod charge density. The counterion entropy also depends logarithmically on concentration (and, hence, logarithmically on distance from the rod). Whether the victor is Coulomb potential energy or entropy depends, therefore, on the charge density of the cylinder. The critical value of

Poisson–Boltzmann Mean-Field Theory of Macro-Ions

Let us approximate the free energy of a solution of macro-ions, counterions, and added salt by the following simple functional of the ion concentrations:

$$F_{PB}(\{n_i\}) = \int d\mathbf{r}\left\{\frac{1}{2}\rho\Psi + kT\sum_i n_i \ln(n_i/n_0)\right\}. \quad (1)$$

The second term is the mean-field entropic free energy of the ions, with n_i being the concentration of the ith ion species carrying charge $z_i e$ (n_0 sets the zero of the potential—see the following). The first term is the electrostatic energy, with the charge density $\rho(\mathbf{r})$ being the sum of charge densities of the macro-ions and the mobile ions:

$$\rho(\mathbf{r}) = \rho_{macro}(\mathbf{r}) + \sum_i z_i e n_i(\mathbf{r}). \quad (2)$$

The local electrostatic potential is $\Psi(\mathbf{r})$. The charge density and the potential are related by Poisson's equation, $-\nabla^2\Psi = (4\pi/\varepsilon)\rho(\mathbf{r})$, where ε is the dielectric constant of the continuum—the aqueous medium in which the ions are dissolved. Minimization of equation 1 with respect to the ion concentrations leads to the condition that they obey the Boltzmann distribution. More explicitly, using Poisson's equation, we obtain the relation

$$\nabla^2\Psi = -\frac{4\pi e n_0}{\varepsilon}\sum_i z_i \exp(-z_i e\Psi/kT) \quad (3)$$

for the potential outside the surface of the macro-ions. This nonlinear differential equation, which is known as the Poisson–Boltzmann (PB) equation, must be solved under the boundary condition (Gauss's law) that the electric field $\mathbf{E} = -\nabla\Psi$ at the surface of a macro-ion be consistent with its fixed charge density σ. That is, $-\nabla\Psi = (4\pi/\varepsilon)\sigma$.

The electrostatic self-energy of a macro-ion is computed by inserting the solution of the PB equation into equation 1 for an isolated macro-ion, and then subtracting the free energy with all charges set equal to zero. When this calculation is carried out for a charged rod, the self-energy is found to be positive; the increase in entropic free energy induced by the confinement of the ions near the rod exceeds the lowering of their electrostatic energy.

The force acting between macro-ions is found by integrating the stress tensor

$$\sigma_{ij} = -kT\left(\sum_i n_i\right)\delta_{ij} + (\varepsilon/4\pi)\{E_i E_j - (E^2/8)\delta_{ij}\}$$

across a surface surrounding each macro-ion. For large distances r, the dimensionless electrostatic potential $e\Psi(\mathbf{r})/kT$ in between the macro-ions is small compared to one and the PB equation reduces to the well-known Debye–Hückel (DH) equation:

$$\nabla^2\Psi = z^2\kappa^2\Psi. \quad (4)$$

The Debye screening length is κ^{-1} ($\kappa^2 \equiv 8\pi\lambda_B n_0$), $\lambda_B \equiv e^2/\varepsilon kT$ is the Bjerrum length, and z is the magnitude of the z_i. It is straightforward to solve equation 4 for two parallel line charges each with a charge per unit length λ and separated by a distance r. We can then use this solution to compute the force on a rod provided we integrate the stress tensor over a cylindrical surface located outside the rod with a radius big enough compared to κ^{-1} for the DH approximation to be valid. The effective interaction computed in this way is $V(r) \approx (2\lambda^*/\varepsilon)(\pi/2\kappa r)^{1/2}\exp(-\kappa r)$, $\kappa r \gg 1$. Here, λ^* is an effective or renormalized charge per unit length whose relation to the bare charge density λ must come from a complete solution of the PB equation. For $b < \lambda_B$, λ^*/λ is found to equal the Manning–Oosawa parameter $\xi \equiv \lambda_B/b$ and to equal one otherwise (b is the distance between fixed charges on the rod). A similar calculation for spherical macro-ions shows that the effective charge z^*e of an isolated sphere equals the bare charge ze, consistent with there being no counterion condensation in this case. For any nonzero concentration of spheres, however, z^*e is of order R/λ_B, with R being the sphere radius. For charged planar surfaces, on the other hand, the renormalized charge per unit area is effectively zero.

charge per unit length is e/λ_B, where λ_B is the Bjerrum length (see box), the distance at which the Coulomb interaction between two fundamental charges is equal to the thermal energy kT. Counterion condensation occurs when the distance between charges, b, is small enough for the dimensionless ratio $\lambda_B/b = \xi$ to exceed unity. Whenever $\xi > 1$, the renormalized rod charge is simply the bare value divided by ξ (which, for DNA in water at room temperature, is 4.2).

For planar geometry of fixed charge, the counterions are always condensed, independent of surface charge density. This situation arises because the electrostatic energy (in effect, the one-dimensional Coulomb energy) varies linearly with distance, and, therefore, always overwhelms the entropic contribution. Conversely, for an isolated spherical macro-ion the counterions always remain free because the three-dimensional Coulomb potential falls off as an inverse first-power law.

The connection between the counterion-induced compensation of macro-ion charge and the appearance of attractive forces has been recognized for some time. Two mechanisms have been identified that lead to counterion-mediated attractions.

The first mechanism involves a Gaussian fluctuation correction to the PB mean-field theory. This attractive term may be considered as a Casimir force, analogous to the familiar dispersional or van der Waals interactions between molecules. For molecules, the crucial fluctuations in position are quantum mechanical in nature, whereas for dissolved macro-ions, they are statistical mechanical.

Oosawa was the first to study correlated long-wavelength thermal fluctuations of the condensed counterion density along a pair of rod-like macro-ions (ref. 3, Oosawa). He treated the counterion cloud of each rod as a one-dimensional ideal gas. By including correlations between the fluctuations of the two rods, he obtained a nonspecific long-range attractive contribution to the force between rods that varies inversely with the square of the separation distance and decreases linearly with temperature. But because this fluctuation term was computed as a lowest-order perturbation correction to the mean-field repulsive force, it could not be concluded whether the overall interaction was indeed attractive. Furthermore, because of the Coulomb potential's long range, this inverse-square force of counterion fluctuation cannot be pairwise-additive. In fact, the interactions among molecules in a DNA array do not occur in this simple form.[4]

The second mechanism, which has been investigated more recently, focuses on the short-range electrostatic correlations between the counterions of the two clouds. By using a combination of computer simulation approaches and analyses of simplified models, a strong short-range interaction is obtained whose strength of attraction increases on lowering the temperature.[5] This form of

attraction is related to forces explored in earlier work on charged planar surfaces, which suggested that, at low enough temperature, counterions should form a self-ordered two-dimensional Wigner crystal, and that the two mobile surface lattices should attract each other.[6] The threshold for low enough temperature depends strongly on the valence of the counterions. Direct comparisons with experiment are problematic because measurements necessarily include contributions from many nonelectrostatic interactions.

The above two attraction mechanisms are not necessarily in conflict with each other. The long-wavelength fluctuation effect appears for high temperatures, whereas the short-range correlations predominate at low temperatures. Evidence for the two regimes comes from applying molecular dynamics to a model system of two charged rods in the presence of neutralizing polyvalent counterions, with no added salt (ref. 5, Gronbech-Jensen *et al.*). The computed force is very weakly repulsive at high temperatures, with a magnitude consistent with PB theory. As the temperature is lowered, a significant short-range attraction appears and the condensed counterions develop structure resembling that of a highly correlated one-dimensional fluid.

In actual measurements on DNA, the temperature cannot be varied over a large range. Even so, information concerning the nature of counterion structure can be obtained from synchrotron x-ray measurements of the density–density correlation function along the chain direction. Cyrus Safinya's group at the University of California, Santa Barbara, recently carried out such a study of aligned DNA strands confined to a flat substrate of positively charged lipids. Unlike the case of DNA in bulk, they found that divalent ions such as Ca^{2+} can make surface-confined DNA condense. They also observed that the counterion structure is indeed consistent with that of a highly correlated, one-dimensional liquid.

These results suggest that shorter-range correlations may provide the key to the attractive interaction of DNA, but there is no evidence yet of a true Wigner crystal phase in this context. In an alternative approach, Alexei Kornyshev and Sergei Leikin have proposed an electrostatic zipper motif that posits a helical path for the specific (chemisorbed) binding sites of polyvalent counterions on DNA.[7] The resulting axial separation of these positive charges from the (displaced) helices of negative phosphates is shown to give rise to an attractive interaction between strands.

Independent of the precise physical mechanism, we can exploit the curious self-attraction of DNA to manipulate viral activity in an interesting way, with possible therapeutic uses. All biological cells are surrounded by closed bilayers comprised of many different (neutral and charged) phospholipid molecules. (Each phospholipid consists of two hydrocarbon chains topped with a phosphate containing complex.) These membranes also contain many proteins that are essentially insoluble in water and that act as ion channels or receptors, thereby controlling the transport and chemical life of the cell. For a virus to infect a cell, it must inject its DNA across this membrane into the cell interior.

Figure 1b shows an electron micrograph of several T5 bacteriophages, which are all in the process of ejecting their DNA molecules into a shared toroidal condensate, a process made possible because of the presence of a tetravalent cation (spermine) in aqueous solution. Note that each viral DNA is originally confined within a rigid protein coat (the capsid) that is icosahedral in shape, hence appearing hexagonal in cross section. If the spermine concentration is lowered (below about 50 mM), then the self and mutual repulsion of the DNA predominates and viral ejection leads in that case to independent, disordered DNA coils.

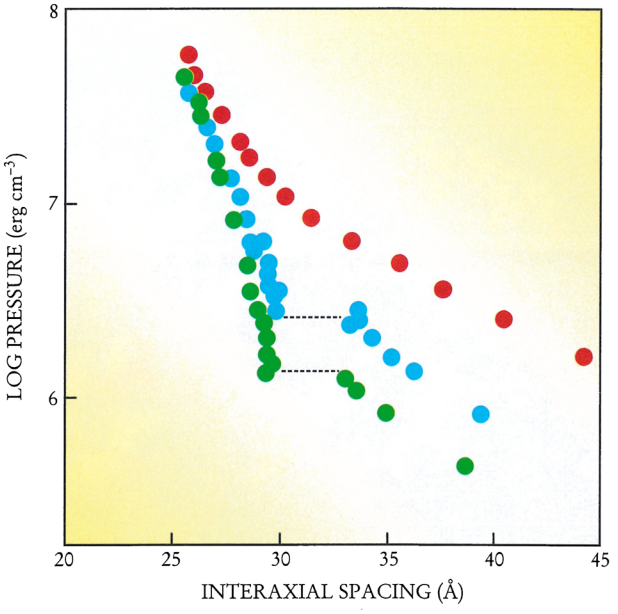

FIGURE 2. MEASURED INTERMOLECULAR FORCE as a function of interaxial separation for DNA in simple salt solutions (0.25 molar NaCl) that contain increasing concentrations (2 mM, red; 8 mM, blue; 12 mM; green) of the trivalent cation $Co(NH_4)_6^{+3}$. (Adapted from ref. 2, Rau *et al.*)

This physics can be used to trick the T5 virus into giving up its DNA in a controlled way. The key is FhuA, a unique membrane protein molecule that binds to the tip of the tail of the T5 virus. In doing so, the protein triggers the opening of the viral capsid and hence the expulsion of its DNA. A (bacterial) cell whose membrane includes even one molecule of the protein FhuA is susceptible to attack by that virus.

Suppose we incorporate FhuA proteins in the membrane of a liposome (a spherical bilayer of phospholipid molecules) whose interior contains spermine in an aqueous salt (NaCl) solution. Each T5 virus recognizes a target cell only through its interaction with FhuA in the outer cell membrane, so it can be fooled into attacking a liposome reconstituted with FhuA. As shown on the magazine cover, this leads to injection of its DNA into the liposome. The spermine assists the ejection and condenses the DNA into a torus, which contains the DNA of several viruses (three, in this case). The viral DNA has now reached a "dead end" in the form of a highly ordered, densely packed torus trapped within the liposome.

Counterion release and charge reversal

The dense cloud of condensed counterions surrounding DNA represents a large number of hidden degrees of freedom. When two different macro-ions of opposite charge approach each other, they interact at large separations via the Debye–Hückel interaction (see box), with their effective charge renormalized. At smaller separations, however, the counterion clouds can undergo dramatic rearrangements that have important, counterintuitive consequences.

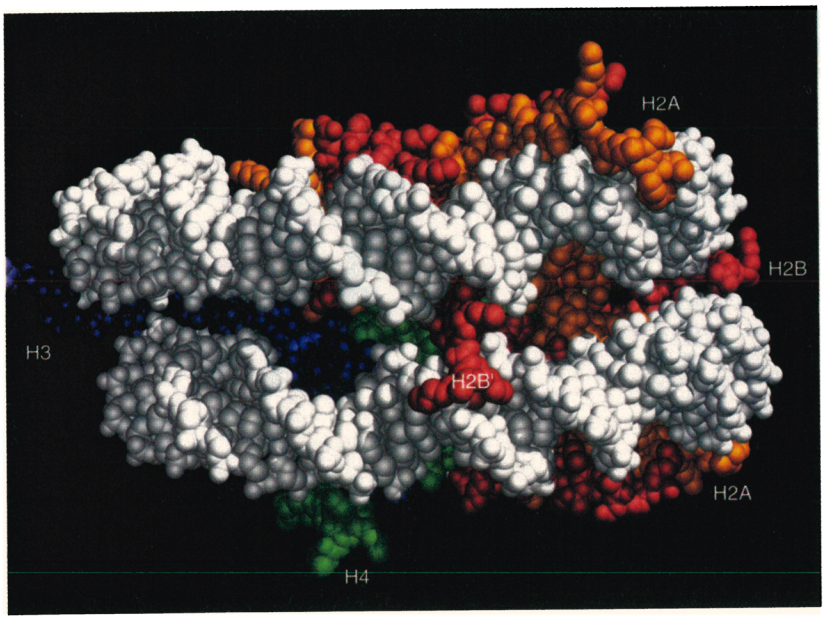

FIGURE 3. THE STRUCTURE of nucleosome core particle, as determined by x-ray diffraction. Such particles form the building blocks of chromosomal DNA, in which a length of DNA containing 146 base pairs wraps 1.75 times around a cylinder-shaped octamer of the four histone proteins H2A, H2B, H3, and H4. (Adapted from K. Luger *et al.*, *Nature*, volume 389, page 251, 1997.)

An important example of this form of macro-ion association is the interaction between DNA and DNA-associating proteins. It is the task of certain proteins, known as repressors, to search along the DNA strands in chromosomes to find a particular target sequence of base pairs. When it finds the sequence, the repressor must bind and block the expression of some gene (or set of genes). The recognition of the target site involves hydrogen bonding between the protein's amino acids and the DNA's nucleotides. However, the nonspecific interaction between repressor proteins and DNA—that is, the attractive interaction between the protein and DNA away from the target site—is essentially electrostatic in nature. Positively charged amino acids of the repressor face the negatively charged (phosphate) groups of the DNA's nucleotides.

In a naive electrostatics picture in which binding is due to the association of fixed positive and negative charges, one would expect the binding of proteins to DNA to be dominated by an enthalpy decrease. In fact, thermodynamic studies of the enthalpic and entropic contributions to the binding free energy indicate that protein–DNA binding is dominated by an entropy increase that depends logarithmically on the ambient salt concentration.[8]

The key to understanding why the naive model fails is again provided by the counterions. Even when two macro-ions are far apart, a large part of their charge is already compensated by their counterions. Consequently, the enthalpy change that accompanies macro-ion association is modest. However, when the macro-ions do associate, a number of opposite charges on the two macro-ions are brought in close proximity and a proportional number of counterions are no longer needed and can be freed. It is the entropy gain of these released counterions that is largely responsible for the binding process. The release of water molecules, associated with changes in solvent structure on protein–DNA association, is also important, but is beyond the scope of simple dielectric continuum theory.

Figure 3 shows the high-resolution (0.28 nm) x-ray structure solved recently for the nucleosome, the basic building block of chromosomes. Each unit (the core particle) consists of a length of DNA containing 146 base pairs and wrapped around an octamer of proteins called histones. Short lengths of so-called linker DNA attach the nucleosomes to each other. It is interesting that the radius of the histone octamer is only about 5 nm—that is, 10 times smaller than the persistence length of DNA. Also, the negative charge brought to the nucleosome core particle by wrapped DNA is significantly larger (by about 20%) than the total positive charge carried by the histones, implying that the core is overneutralized (overcharged).

The effective negative overcharge of the histones can be dramatically illustrated in the lab. When long DNA strands are mixed in vitro with histone octamers, chains of DNA-linked nucleosomes form. Such chains constitute the basic structure of chromosomal DNA, the so-called 10 nm fiber, which is organized on successively larger length scales, all the way up to 1400 nm-thick chromosomes. Electron microscopy reveals that the next largest stage of structural organization, the so-called 30 nm fiber, forms when counterions are added,[9] indicating the vital role played by electrostatics in the first steps of the folding of chromosomal DNA.

But why are nucleosomes negatively charged? Consider a simple model system of a long, flexible, negatively charged chain that wraps around a positively charged sphere in aqueous solution. One can easily imagine why the chain would continue to adsorb until the ball charge was neutralized: to saturate the electrostatic interaction. Less naively, the entropic free energy gain associated with the chain's counterion release would appear to be maximized when the negative charge of the wrapped part of the chain just compensates the sphere charge. But why would it continue to adsorb beyond that point? If we also consider the elastic bending energy of the chain, then we might expect that the wrapping would not proceed even this far and that the ball–chain complex should have a net positive charge.

To see why these arguments are incorrect, suppose that we measure the voltage difference $\Delta\Psi$ between the surfaces of a neutralized complex and of the chain far away. Let c(chain) and c(complex) be the concentrations of condensed (positive) counterions along the chain and on the surface of the complex, respectively. Because the complex has a zero net charge, we expect that $c(\text{chain}) \gg c(\text{complex})$. In that case, the Boltzmann distribution requires that $c(\text{chain})/c(\text{complex}) = \exp(e\Delta\Psi/kT)$, implying a large voltage difference $\Delta\Psi$ between the complex and the chain. This difference pulls in more (negatively charged) chain material onto the complex, so the complex acquires a net negative charge. As the process continues, the counterion density on the complex starts to rise and the voltage difference drops.

Spontaneous overcharging appears to be counterintuitive, but it is actually encountered in many other areas of macro-ion electrostatics. The phenomenon is seen most

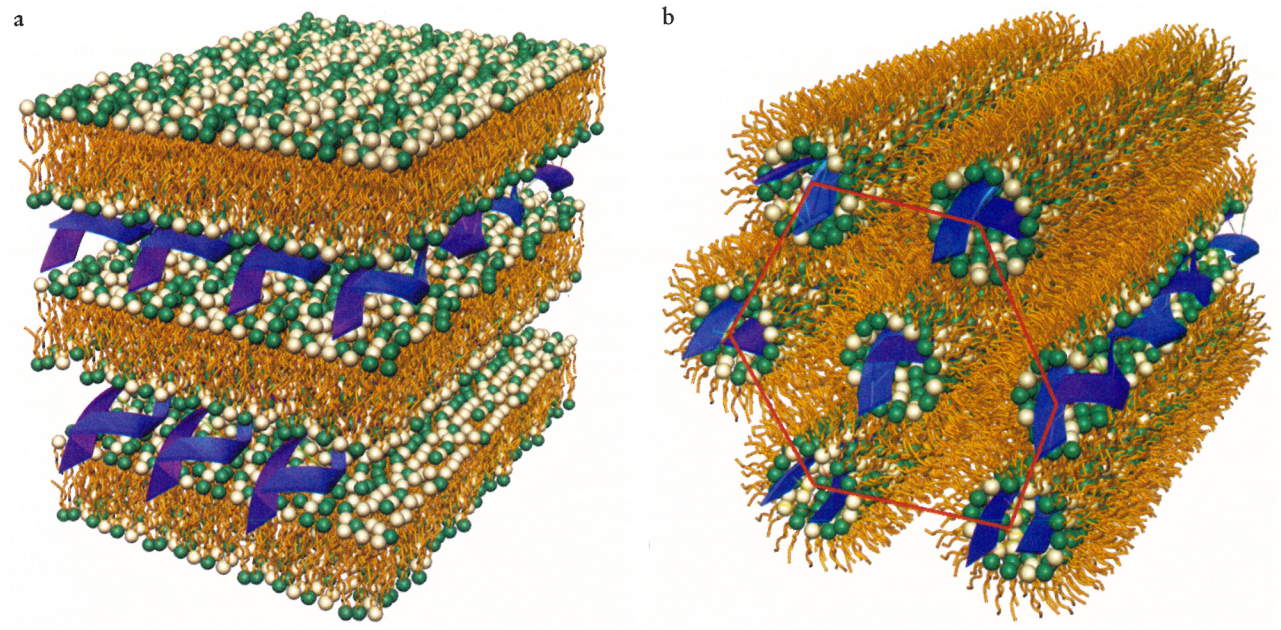

FIGURE 4. CATIONIC–LIPID COMPLEXES. (**a**) A model of the local arrangement of complexes in the lamellar phase showing the alternating lipid bilayer and DNA monolayer. (Adapted from J. O. Radler *et al.*, *Science*, volume 275, page 810, 1997.) (**b**) Model of the local arrangement of cationic lipid–DNA complexes in the inverted hexagonal phase. The cylinders consist of DNA coated with a lipid monolayer and arranged on a hexagonal lattice. (Adapted from I. Koltover *et al.*, *Science*, volume 281, page 78, 1998.)

straightforwardly in the case of a macroscopic planar surface immersed in a solution of counterions and added salt. The PB equation of a charged planar surface in salt solution can be solved exactly. Although the free ions screen the plate charge, the effective surface charge never undergoes sign reversal no matter how large the surface charge density or how large the concentration and valence of salt. On the other hand, Monte Carlo simulations of a concentrated solution of monovalent and divalent salt in the region of a highly charged planar surface show[10] that—as a function of distance from the surface—the sign of the local charge density oscillates (in qualitatitive contrast to the monotonic PB charge profile). This means that ions with the same sign as the plate charge (co-ions) are attracted to the plate because counterions have overcompensated for the plate charge.

This form of spontaneous overcharging is not due to counterion release, but rather to the effect of short-range correlations between the ions at high ion densities, and it is in fact closely related to the correlation-induced attraction between charged rods and plates discussed previously. These short-range correlations, and specifically their effect on overcharging, have recently been incorporated into an analytical theory by Boris Shklovskii.[11] In his theory, the condensed polyvalent counterions are treated as a two-dimensional strongly correlated liquid whose cohesive energy sucks in additional counterions, with the result that the surface charge is more than compensated at sufficiently high valence and concentration.

Indeed, whenever spontaneous overcharging is encountered, it always arises from some form of correlation between the mobile charges. In the case of a flexible charged chain complexing with a ball, overcharging can arise entirely within PB theory because the charges on the chain are correlated through their connectivity. This effect can be illustrated most simply by removing all the counterions from the sphere–chain complex and directly minimizing the electrostatic energy at zero temperature. For a sufficiently flexible chain, the ground state of the chain–ball complex is still overcharged, due to connectivity-induced correlation between the chain charges.[12] Clearly, the mechanism that drives spontaneous overcharging in general can be both entropic and enthalpic.

Experimentally, there are quite dramatic examples of sign reversal due to overcharging. One is provided by electrophoretic mobility measurements on colloidal particles. Under most circumstances, applying an electric field to a colloidal suspension of charged balls in polyvalent salt solution causes the balls to move in the direction of the field. One can then extract a mobility from the balls' steady-state velocity, which, by definition, is equal to force times mobility. But for sufficiently high surface charge densities and large enough salt concentration and valence, the mobility is seen to go negative—that is, the colloidal particle appears to move in the wrong direction under the influence of the electric field![13] The effective charge of the colloids has had its sign reversed because of the strong overneutralizing effect of the counterion condensation. Again, this is possible only because of correlations in mobile ion positions that arise from their high concentration and valence in the presence of strong surface fields.

Dramatic correlation effects due to chain connectivity, on the other hand, are also observed experimentally. When a highly charged colloidal sphere (say, positive) is dipped into a solution of anionic polymer, the charge of the colloid is reversed.[14] Successive dippings into polymer solutions of alternating sign confirm as many as 10 reversals of sign, pointing out unambiguously the extent to which charged surfaces can be overneutralized by polyelectrolyte adsorption.

DNA–lipid complexes

Charge-reversal of colloids also plays an important role in our final example of DNA-inspired electrostatics, which is

drawn from the field of gene therapy.[15] DNA–lipid complexes are important biomedical materials because they have been shown to be effective carriers of DNA inside living cells. When we prepare a solution that contains both DNA strands and positively charged lipids at various mixing ratios, the negatively charged DNA molecules associate spontaneously with the lipid molecules. Usually, lipids are either neutral or negatively charged, so the cell membrane tends to repel negatively charged DNA molecules that one wants to inject into the cell for purposes of gene therapy. By complexation of DNA with positively charged lipids, the electrostatic barrier for DNA injection can be lowered and gene delivery facilitated.

DNA–cationic lipid complexation produces colloidal particles with sizes about 0.1 μm. The effective charge of these colloids depends on the DNA-to-cationic lipid mixing ratio. From electrophoresis experiments we learn that if the total DNA charge exceeds the total cationic lipid charge, then the colloids are negatively charged and coexist with excess DNA in solution, whereas in the opposite case they are positively charged with excess lipid material in solution. Cationic lipids can thus overcompensate the DNA charge.

The special mixing ratio for which the DNA and lipid charges cancel each other is known as the isoelectric point. What type of correlation effect is involved in the DNA–lipid complexes? Why do we not always see neutral complexes forming, with the DNA charge exactly compensating the lipid charge?

An important—and surprising—clue comes from recent experiments probing the interior structure of DNA–cationic lipid complexes using high-resolution (synchrotron) x-ray measurement (see figures 4a, b). Instead of finding cationic lipid vesicles adsorbed on the DNA chains, we see various liquid crystal-type structures, reminiscent of the lamellar and hexagonal states formed in pure lipid or pure DNA solutions at high concentration.

In the case shown in figure 4a, the lipids are arranged in a lamellar stack of nearly flat bilayers, with the DNA intercalated between each pair of bilayers. The width of the aqueous layers is only slightly bigger than the diameter of the DNA molecules, leaving just enough room for water molecules to complete a hydration shell around the DNA. The DNA strands are parallel to one another in each layer.

The observed intralayer DNA–DNA spacing, d_{DNA}, depends on the cationic lipid to DNA weight ratio ρ. As we cross the isoelectric point where $d_{DNA} = d_{iso}$, d_{DNA} increases rapidly. For the linear DNA charge density ($-e$ per 0.17 nm), and the lipid bilayer surface density ($+e$ per 1.4 nm^2), corresponding to a 50/50 mixture of neutral and cationic lipids), d_{iso} is about 4 nm. For $d_{DNA} < d_{iso}$, the complexes are undercharged, whereas for $d_{DNA} > d_{iso}$, they are overcharged.

Consider a model system of a row of charged rods (the DNA strands) separated by a spacing d_{DNA} and sandwiched between two plates, with the plates containing mobile positive charges (the cationic lipids) that have a prescribed mean surface charge density (fixed by the lipid composition). Free ions are present to neutralize the whole system. The equilibrium spacing d_{DNA} is then determined by requiring that this system be in chemical equilibrium either with excess DNA or with excess lipid.

Within PB theory, the formation energy of this structure is again dominated by counterion release. By bringing the rods and the plate together we can free counterions into solution. Part of the mobile plane charges (cationic lipids) collect near the fixed charges (DNA phosphates) to enhance local surface charge neutrality, with the remaining plane charges compensated by solution counterions. The dependence of d_{DNA} on the mixing ratio, computed from PB theory,[16] agrees well with experiment. Recall from our discussion of nucleosomes that extra DNA adsorbs on the histone aggregate beyond the neutralization point because condensed counterions are released from the DNA. In the case of the DNA–lipid complexes, extra DNA is incorporated into the cationic bilayer complex for the same reason.

It is interesting to compare the physics of the lamellar structure with that of a second type of liquid crystal structure encountered for the colloidal complexes: the inverted hexagonal phase shown in figure 4b. Complexes with this structure perform better than the lamellar case for gene therapy purposes. The hexagonal geometry arises in solutions of DNA and cationic (plus neutral) lipid to which has been added a lipid species that prefers negative curvature at the water interface. The radius of the inverted bilayer is essentially imposed by the requirement of surface charge density matching with the hexagonally packed DNA. In this case, the geometry imposes local electrical neutrality; there is no self-adjusting structural degree-of-freedom, such as the DNA–DNA spacing, that allows the system to deviate from its isoelectric point.

We have seen that electrostatic phenomena in the context of colloidal and biophysical systems play a central role in determining how macro-ions interact. When dealing with macro-ion association in solution, it is quite misleading to assume that we can assign an effective charge to the macro-ion and apply our intuition of electrostatics in vacuum. Rather, the entropy and local structure of the counterion hidden variables produce some wonderful and surprising effects. Many conceptual questions still remain, but we are making progress with the analytical and computational techniques—and the novel and powerful experimental methods—required to answer them.

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