

## Measuring Inter-DNA Potentials in Solution

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Interactions between short strands of DNA can be tuned from repulsive to attractive by varying solution conditions and have been quantified using small angle x-ray scattering techniques. The effective DNA interaction charge was extracted by fitting the scattering profiles with the generalized one-component method and inter-DNA Yukawa pair potentials. A significant charge is measured at low to moderate monovalent counterion concentrations, resulting in strong inter-DNA repulsion. The charge and repulsion diminish rapidly upon the addition of divalent counterions. An intriguing short range attraction is observed at surprisingly low divalent cation concentrations,  $\sim 16$  mM  $\text{Mg}^{2+}$ . Quantitative measurements of inter-DNA potentials are essential for improving models of fundamental interactions in biological systems.

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The large charge density of DNA,  $\sim 2e/3.4 \text{ \AA}$ , is fundamentally important to the biological functions of this molecule of life. Interactions between highly charged DNA strands are modulated by oppositely charged macromolecules, molecules, or ubiquitous counterions and range from strongly repulsive to strongly attractive [1,2]. In spite of their biological importance, measurements of fundamental inter-DNA interaction parameters, such as charge, remain sparse. This Letter reports on measurements of the modulation of DNA-DNA interactions by nonspecifically bound counterions, and on the application of models to extract essential physical parameters.

The primary role of nonspecifically bound counterions is electrostatic screening of DNA's large negative charge. Spatial distributions of counterions around DNA have been successfully described with the mean field nonlinear Poisson-Boltzman (NLPB) theory [3,4]. The theoretical description of screened interactions between DNAs follows the pioneering work of Derjaguin, Landau, Verwey, and Overbeek (DLVO) [5], which gives a repulsive Yukawa pair potential. However, the DLVO theory is based on the linearized PB approximation, which breaks down near the DNA due to strong electrostatic potentials. As a compromise, Alexander *et al.* justified the use of the convenient Yukawa potential form provided that the DNA interaction charge is renormalized to match the linearized PB solution to the exact NLPB solution at the boundary of a Wigner-Seitz (WS) cell, i.e., midway between DNAs [6]. This renormalized charge,  $Z_{\text{ren}}$ , is always smaller than the bare DNA charge due to "condensed" counterions. Although these mean field approaches provide much insight into the screening by counterions, they fail to predict the biologically important attraction of like-charged polyelectrolytes. With more realistic models accounting for discrete ion charges and their spatial correlations, extensive theoretical studies and numerical simulations reveal short range attraction between DNAs for counterions of valence  $Z \geq 2$  [7]. Attractive interactions of different ori-

gins have also been suggested [8,9], and are under active investigation [10,11].

Experimental studies of interactions between isolated DNAs in solution are scarce in comparison to theoretical efforts [11]. The often cited measurements of Rau and Parsegian [12] of inter-DNA forces resulting from osmotic stress were made on condensed, not isolated, DNAs. Small angle scattering (SAS) experiments on DNAs in solution revealed strong electrostatic repulsion at low monovalent ion concentrations, reflected by the presence of interference peaks as the charged molecules self-organize into a rough lattice [2,13]. As the counterion valence is increased, this repulsion vanishes. Recent experiments by Bai *et al.* [14] provided upper limits on inter-DNA attractive and repulsive potentials, and showed no evidence for strong attractive forces at divalent counterion concentrations as large as  $0.6 \text{ M Mg}^{2+}$ . Intriguingly, Borsali *et al.* reported an "upturn" at low scattering angle with neutron SAS studies of moderate length ( $\sim 400$  base pairs) DNAs in monovalent and divalent salts [15]. This upturn was interpreted as loose cluster formation due to short range attraction, though at the high concentrations used ( $42 \text{ mg/mL}$ ) entanglement of the nonrigid DNAs may be a complication. These studies suggest that any divalent ion induced attractive force must be weak, consistent with the observation that divalent ion induced condensation of DNA occurs only in reduced dimensions [16]. In the presence of more highly charged counterions ( $Z \geq 3$ ), the existence of strong inter-DNA attraction is supported by DNA condensation in bulk solution [1]. In spite of these experimental findings, quantification of the inter-DNA potentials is still lacking, precluding direct comparison with theoretical studies [11].

Here, we present experimental studies that quantify the interaction potentials of isolated DNAs in solution by measuring the small angle x-ray scattering (SAXS) structure factor  $S(Q)$  ( $Q = \frac{4\pi}{\lambda} \sin\theta$ ,  $\lambda$  is the x-ray wavelength, and  $2\theta$  is the scattering angle). The measured interparticle interference function  $S(Q)$  can be modeled to obtain the

inter-DNA Yukawa pair potentials following the generalized one-component method (GOCM) with mean spherical approximation closure pioneered by Hayter *et al.* and Chen *et al.* [17,18]. This quantitative analysis has not been previously applied to DNA-DNA structure factors [2,13,15]. The GOCM computes the DNA-DNA correlations, starting with the inter-DNA Yukawa potential including a hard sphere core,  $\phi(r)|_{r>\sigma} = Z_{\text{eff}}^2 \exp[-\kappa(r - \sigma)] / \epsilon(1 + \kappa\sigma/2)^2 r$ , where  $\epsilon$  is the solvent dielectric constant. In this study, the inverse Debye screening length  $\kappa$  and the equivalent diameter  $\sigma$  of the cylindrical DNA are calculated from solution conditions. The only remaining parameter is the effective interaction charge  $Z_{\text{eff}}$ , which is to be obtained by fitting the data.

We selected a short DNA (25 base pairs, length  $L \approx 80$  Å, purchased from Integrated DNA Technologies), which is monodisperse and rigid rodlike. The DNA sequence, sample preparation, and beam line setup at the C1 station of the Cornell High Energy Synchrotron Source (CHESS) were described in Ref. [3]. Each sample was dialyzed against the corresponding monovalent (NaCl) and/or divalent ( $\text{MgCl}_2$ ) salt solution made up with  $\text{pH} = 7$  1 mM NaMOPS buffer. Neither  $\text{Na}^+$  nor  $\text{Mg}^{2+}$  displays site-specific binding to DNA [19]. Standard SAXS data corrections and error propagations were applied [4].

SAXS experiments measure the total scattering profile  $I(Q) = NP(Q)(1 + \frac{\langle F^2 \rangle}{\langle F^2 \rangle} [S(Q) - 1])$ , where  $N$  is a scale factor,  $P(Q) \equiv \langle F^2 \rangle$  is the form factor, and  $\langle \rangle$  indicates an average over all DNA orientations. The decoupling approximation [20] is applied to account for the cylindrical DNA shape (height/diameter  $\sim 4$ ) in the absence of inter-DNA orientational correlations. This approximation has been successfully used by Nossal *et al.* [20] to study a cylindrical protein (height/diameter  $\sim 3.5$ ) at higher concentrations. The ratio  $\frac{\langle F^2 \rangle}{\langle F^2 \rangle}$  is calculated using an 80 Å (height) by 20 Å (diameter) cylinder. The  $P(Q)$  can be computed from the known DNA atomic coordinates [21]. To accurately reproduce the solvent effects, the computation also accounted for the excluded volume, the ion atmosphere, and the hydration shell, as follows. The scattering factors of ions are first corrected for electrorestriction effects [3]. The solution background scattering density is then computed taking the ions into account. The program CRY SOL [22] is used to assign the weight and excluded volume of each atom (group). The electrostatic potential around the DNA is then determined using APBS [23]. Counterions are randomly distributed consistent with calculated Boltzmann factors with this potential. With the hydration shell (HS) volume ( $9700 \text{ \AA}^3$ ) predicted by CRY SOL and excess scattering density of  $0.07e/\text{\AA}^3$  [24], the total contrast of the HS amounts to  $680e$ . Dummy HS atoms were randomly distributed (number: 240,  $Z$ :  $2.83e$ ) along the boundary of the solvent accessible volume obtained from APBS. Finally, the Debye formula was used to sum over the DNA atoms, counterions, and HS atoms to

compute the form factor  $P(Q)$ . This procedure was repeated 100 times and the results were averaged. Figure 1 shows the excellent agreement of the calculated and measured  $I(Q)$  for a solution of dilute DNA.

Calculation of the structure factors requires determination of the Yukawa potential parameters  $\kappa$  and  $\sigma$ , given the known DNA bare charge ( $48e$ ) and geometry. The charge renormalization prescription [6,25] defines the effective inverse Debye screening length  $\kappa$  as calculated from the ionic concentrations at the WS cell boundary in the NLPB solution. This  $\kappa$  can differ significantly from that of the dialyzing buffer when the counterions dissociated from DNAs are not negligible, e.g., under low salt conditions. These model calculations were carried out for two geometries: an infinite rod with the same charge density as DNA and a sphere with the same second virial coefficient and bare charge. The values of  $\kappa$  were only weakly dependent on the geometry, and were used as fixed parameters in  $S(Q)$  fits. In addition, the calculations give the theoretical renormalized charge  $Z_{\text{ren}}$ . Consistently smaller  $Z_{\text{ren}}$  values were obtained from the rod model and will be used for comparisons with experimental charge  $Z_{\text{eff}}$ . The effective DNA diameter,  $\sigma$ , is determined by the DNA interaction volume representing the “hard core” in the GOCM. Notably, this volume includes the high potential region around the DNA containing “localized” counterions. The increase in size, relative to the bare DNA diameter, is accounted for using an empirical potential threshold to locate the effective “boundaries” of the DNA. Because of the cylindrical geometry, the effective DNA  $\sigma$  was set equal to the diameter of a sphere with the same second virial coefficient [20]. To test this approach, we fit the data in a regime where the interference is strong [e.g., Figs. 2(a) and 2(b)], and both the effective diameter  $\sigma$  and the charge

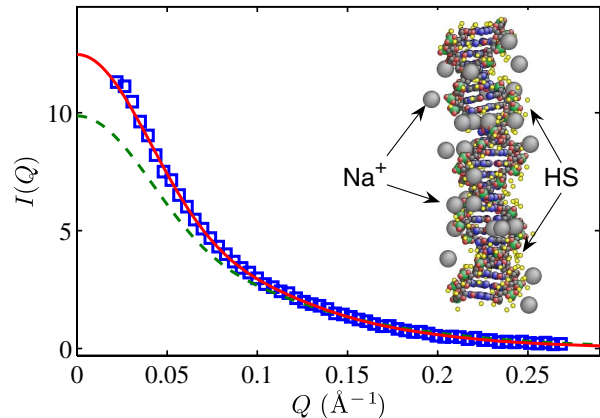


FIG. 1 (color online). The experimental  $I(Q)$  of 0.05 mM DNA ( $\square$ ) is compared with the calculated  $I(Q)$  from DNA atoms only (dashed line) and the  $I(Q)$  including solvent effects (see text) (solid line). Note that  $I(Q) = NP(Q)$  in the absence of inter-DNA interference [ $S(Q) = 1$ ] under such dilute conditions. The curves are matched between  $Q$  values of 0.1 and 0.25  $\text{\AA}^{-1}$ . The inset shows a model consisting of DNA, distributed  $\text{Na}^+$  counterions, and dummy HS atoms.

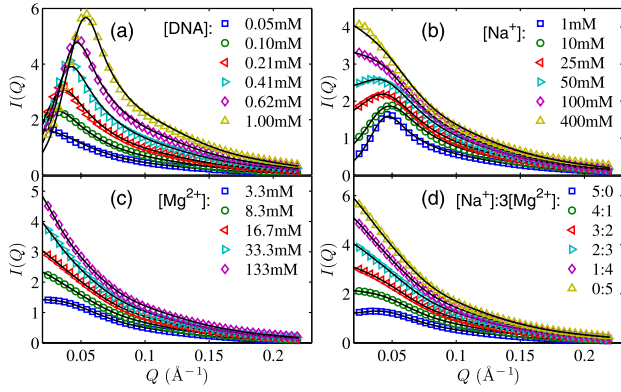


FIG. 2 (color online). Experimental  $I(Q)$ s are indicated with symbols; fits are shown as lines. The data are offset for clarity. (a) data with no excess salt. (b),(c), and (d) data at fixed DNA concentrations (around 0.60 mM) as counterion concentration and valence are varied as indicated.

$Z_{\text{eff}}$  can be free parameters [20]. The simultaneous fitting of both parameters consistently yields a potential threshold around 3.8 kT (previous studies suggested 4 kT [25]). Thus, we used 3.8 kT to determine the effective DNA sizes under all conditions for consistency. Home written MATLAB codes were used for all analysis unless otherwise noted.

We first discuss the “no excess salt” series with DNA concentration  $c_{\text{DNA}}$  ranging from 0.1 to 1.0 mM (strictly speaking, the buffering 1 mM NaMOPS is the excess salt). The scattering profiles  $I(Q)$  [Fig. 2(a)] show pronounced interference peaks, indicating significant structural ordering due to strong inter-DNA repulsion. The “Bragg distance” ( $2\pi/Q_{\text{max}}$ ,  $Q_{\text{max}}$  is the peak position) corresponds to the mean inter-DNA distance ( $\propto c_{\text{DNA}}^{-1/3}$ ) within a few percent [13]. The satisfactory fits obtained support the validity of the decoupling approximation under these experimental conditions, which are well below the “entanglement” concentration ( $1/L^3 \sim 2.6$  mM). Figure 3(a) shows the fitted  $Z_{\text{eff}}$  along with the predicted  $Z_{\text{ren}}$ . Although both parameters increase with increasing DNA concentration, deviations are apparent. The calculated effective diameter is 86 Å, considerably larger than 54 Å for hydrated DNA. This is not surprising considering the Debye screening length is about 100 Å.

To study the ionic strength dependence of the potential, the DNA concentration is fixed at 0.6 mM and monovalent salts (NaCl) are added. The data of Fig. 2(b) reveal significant inter-DNA interference even up to  $[\text{Na}^+]$  of 100 mM, though the suppression of the interference peak is apparent. Thus, electrostatic repulsion between DNAs persists, up to monovalent ion concentrations of order 0.1 M. The effective charge  $Z_{\text{eff}}$  [Fig. 3(b)] drops slightly with increasing monovalent ion concentration, in contrast to the predicted renormalized charge  $Z_{\text{ren}}$  which is significantly larger (26.9e versus 9.1e at 100 mM  $\text{Na}^+$ ) and displays a steady increase. The calculated effective diameter decreases slowly with increasing salt concentration, dropping from 61 Å (10 mM  $\text{Na}^+$ ) to 54 Å (400 mM  $\text{Na}^+$ ).

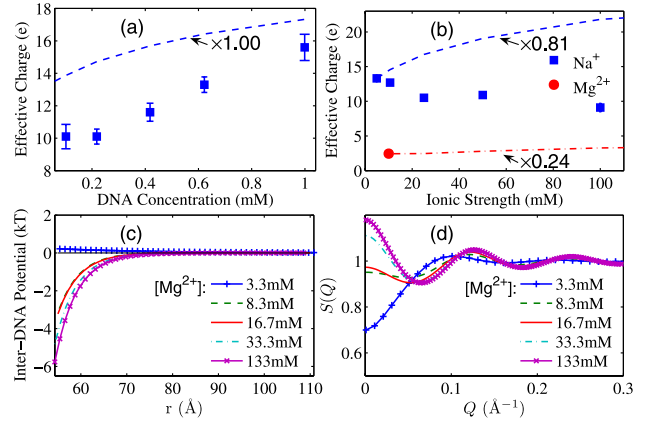


FIG. 3 (color online). (a) and (b) show the experimental effective charge  $Z_{\text{eff}}$  (symbols) and the theoretical renormalized charge  $Z_{\text{ren}}$  (lines) with scaling factors denoted below. Only the results from the one Yukawa repulsive potential refinements are shown. (c) The fitted inter-DNA potentials are shown as functions of inter-DNA distance. (d) The model  $S(Q)$ s corresponding to the inter-DNA potentials in (c).

Qualitatively different behavior is observed in the presence of divalent counterions [Fig. 2(c)] at the same ionic strengths ( $I$ ) as the monovalent ion series. At  $[\text{Mg}^{2+}] \sim 3.3$  mM ( $I = 10$  mM),  $Z_{\text{eff}}$  is  $2.5e$ , drastically smaller than  $Z_{\text{eff}}$  of  $12.7e$  at  $[\text{Na}^+] = 10$  mM. Clearly, divalent cations screen more efficiently than monovalent cations. Deviations from the predicted  $Z_{\text{ren}}$  value ( $10.0e$  at  $[\text{Mg}^{2+}] \sim 3.3$  mM) become more pronounced [Fig. 3(b)] as the counterion valence increases. At 8.3 mM  $\text{Mg}^{2+}$  the interparticle interference almost completely vanishes. Above  $[\text{Mg}^{2+}]$  of 16.7 mM, a low  $Q$  upturn (relative to the form factor) emerges and increases with increasing  $\text{Mg}^{2+}$  concentration. Low  $Q$  upturns in SAXS profiles generally signify a local “clustering” of macromolecules [15,26,27]. Thus, an intriguing short range inter-DNA attraction appears at rather low divalent counterion concentrations.

To validate the observed upturn, we carefully reexamined the calculation of the form factor  $P(Q)$ . Agreement between the calculated form factor and the low  $Q$   $I(Q)$  data can be improved, but only by including unphysical solvent effects and at the expense of significant deterioration in agreement at medium-high  $Q$ . We then recalculated the structure factor by adding a short range Yukawa-form attractive potential with a fixed empirical decay length of 5 Å ( $\sim$ hydrated diameter of  $\text{Mg}^{2+}$ ).  $S(Q)$  calculations with two Yukawa potentials used a method recently developed by Liu *et al.* [26,27]. The data were fit by fixing the repulsive potential to have  $Z_{\text{eff}} = 2.5e$  (as at  $[\text{Mg}^{2+}] \sim 3.3$  mM) and varying only the second, attractive, potential. Figs. 3(c) and 3(d) show the fitting results of the total inter-DNA potentials and the corresponding structure factors  $S(Q)$ s, respectively. The excellent agreement between theory and experiment [Fig. 2(c)] further supports the existence of a weakly attractive potential, though we do not know its origin. A similar upturn in  $S(Q)$  has been pre-

dicted from Monte Carlo (MC) simulations [28] in the presence of divalent salts due to overcharging. Interestingly, Liu *et al.* recently showed that a  $Q = 0$  or very low  $Q$  peak results from a long or short range attraction [26,27], respectively. In the future, lower  $Q$  data will be acquired to reliably distinguish these two cases.

We also examined the competition of monovalent and divalent counterions for charge neutralization of the DNA, by varying the ratio of  $[\text{Na}^+]$  to  $[\text{Mg}^{2+}]$  while maintaining a fixed ionic strength of 50 mM. Systematic changes in  $I(Q)$ s [Fig. 2(d)] corroborate the higher efficiency of divalent counterions in electrostatic screening. The low  $Q$  upturn is again observed for  $[\text{Mg}^{2+}] > 10$  mM.

The excellent agreement of the  $S(Q)$  refinements over a wide range of solution conditions (Fig. 2) supports the application of this approach. The well-defined DNA system under study provides a stringent test of our current understanding of polyelectrolyte interactions. The Yukawa pair potentials appear to provide reasonably accurate theoretical descriptions of these systems *with properly determined parameters*. Our experimental quantification of the inter-DNA interaction parameters should provide valuable guides for theoretical studies (e.g., MC or molecular dynamics simulations) and contribute to the understanding of biologically important nucleic acid dynamics, such as DNA condensation or RNA folding. Finally, we suggest two possible explanations for the large discrepancies between the measured effective charge  $Z_{\text{eff}}$  and the theoretical  $Z_{\text{ren}}$  obtained from the charge renormalization prescription. (i) The DLVO theory considered two macroions approaching from infinity, and showed a distance dependent, but neglected, prefactor between 0.6 and 1.0 [5], which may not be warranted for precise measurements. (ii) The  $Z_{\text{ren}}$  prescription [6,25] addresses DNA-ion interactions in a WS cell, while the DNA-DNA interactions are only explicitly accounted for by charge neutrality and cell size. Further theoretical work is required to resolve these discrepancies.

In summary, we have experimentally quantified inter-DNA interactions by modeling the effect of inter-DNA interference on SAXS profiles. We find that electrostatic repulsion dominates in the presence of monovalent ions, and is reduced by increasing ion concentration. Divalent ions are much more effective at reducing this repulsion than monovalent ions. The extracted effective DNA interaction charge appears to be smaller than predicted from Alexander's prescription within a WS cell. Notably, we observe the onset of a short range attractive force in the presence of even small amounts of divalent ions.

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