

# Ion Atmosphere around Nucleic Acid<sup>†</sup>

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Received: March 29, 2005; In Final Form: August 9, 2005

The polyion–ion preferential interaction coefficient  $\Gamma$  describes the exclusion of coions and accumulations of counterions in the vicinity of a polyion in an aqueous solution. We give tight upper and lower bounds for  $\Gamma$  when the polyion can be modeled by a cylinder of infinite length but of arbitrary charge density. This case can be used as a model for long strands of DNA or RNA in an aqueous solution containing univalent cations. The salt dependence of  $\Gamma$  is predicted from low to intermediate and high salt concentrations. We also indicate how the bounds for the infinite polyion can be exploited to place bounds for polyions of length greater than a constant on the order of the inverse Debye screening length.

A wide range of theoretical<sup>1–36</sup> and experimental techniques<sup>37–56</sup> have been utilized in recent years to study the ion atmosphere of DNA and RNA in aqueous solution. The theoretical techniques include Monte Carlo,<sup>1–4</sup> molecular dynamics,<sup>5–15</sup> integral equation and density function approach to liquid state,<sup>16–20</sup> Poisson–Boltzmann equation,<sup>21–28</sup> and counterion condensation formalism.<sup>29–33</sup> These studies support the viewpoint that the stability of a nucleic acid in salt solution is governed by a delicate balance between electrostatic and nonelectrostatic interactions.<sup>1–36</sup>

In an aqueous solution, interactions between the salt ions and the polyion lead to accumulation of counterions and exclusion of coions in the vicinity of a polyelectrolyte.<sup>1–36</sup> A fundamental length scale for polyelectrolytes is the Bjerrum length  $l_B$  defined as the distance at which the coulomb energy between a pair of charges balances thermal fluctuation at temperature  $T$ .<sup>31,32</sup> Another length scale is the charge spacing  $b$ . For DNA and RNA,  $b$  is the axial spacing of the phosphate charges. Manning proposed that the relevant parameter that describes the electrostatic stability of nucleic acid is the linear charge density  $\xi$ ;  $\xi$  is defined as the ratio of the Bjerrum length to the axial spacing of the charges.<sup>31,32</sup> Since the linear charge density parameter  $\xi$  is larger than unity for DNA and RNA, the polyelectrolyte is electrostatically unstable.<sup>31,32</sup> To lower its energy, counterions from the bulk condense and form a delocalized cloud that screens the repulsive phosphate–phosphate interactions.<sup>30–34</sup> This is the phenomenon of diffuse binding.<sup>30–36</sup> A unique feature of counterion condensation is that the number of condensed counterions per polyion charge is insensitive to salt concentration up to about 0.1 M.<sup>30–32</sup>

In contrast to diffuse binding, ions that participate in site binding are viewed to be trapped<sup>36</sup> near the surface of the nucleic acid as a result of the electrostatic field.<sup>3–7</sup> Site bound ions can form an “outer-sphere” or an “inner-sphere” complex.<sup>36,57,58</sup> In the outer-sphere complex the ions share their solvation shells with nearby phosphates from the nucleic acid.<sup>36,58</sup> Ions in the

inner-sphere solvation complex interact with the nucleic acid by making direct contact with the phosphates in the absence of intervening water molecules.<sup>36,59</sup> Both nonelectrostatic and hydration effects contribute to site binding. The site-bound ions are in general more tightly bound than the delocalized counterions.<sup>31–34,36,60</sup>

To quantify the role that is played by the cations and the anions in determining the stability of a polyelectrolyte in aqueous solution, it is fruitful to introduce the Donnan coefficient or the polyion–ion preferential interaction coefficient  $\Gamma$ , defined as<sup>35,61–63</sup>

$$\Gamma = \lim_{m_u \rightarrow 0} \left( \frac{m - m'}{m_u} \right) \quad (1)$$

$\Gamma$  is measured in experiments that involve equilibrium dialysis. In eq 1,  $m'$  is the molar concentration of the salt in the compartment that does not contain the polyelectrolyte,  $m$  is the molar concentration of the salt in the compartment that contains the polyelectrolyte, and  $\mu_p$  is the concentration of the polyion monomers in units of molarity. The importance of  $\Gamma$  stems from the fact that it describes not only the nonideality due to short-range repulsive and long-range Coulombic interactions between a polyelectrolyte and an electrolyte solution<sup>27,28,35,36,41,42</sup> but also the exclusion of coions and accumulations of counterions around a polyion in an aqueous solution.<sup>27,28,35,62</sup>

When the polyion is in an aqueous solution containing univalent or divalent cations with univalent anions, then the electrostatic potential acting on the salt ions is well approximated by the solution to the nonlinear Poisson–Boltzmann (PB) equation:<sup>25</sup>

$$\Delta u = \sigma^2((1 - \alpha)e^u + \alpha e^{2u} - e^{-u}) \quad (2)$$

Here,  $u$  is the reduced potential, thus  $u = e\psi/k_B T$ , where  $e$  is the electron charge,  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature, and  $\Delta$  is the actual potential. Meanwhile,  $\Delta$  is the Laplacian. We will be modeling the polyion as a uniformly charged cylinder, so use the cylindrically symmetric version of the Laplacian,  $\Delta u = u_{\rho\rho} + 1/\rho u_\rho + u_{zz}$ , with the subscript denoting the derivative;  $\rho$  is the distance from the

<sup>†</sup> Part of the special issue “Irwin Oppenheim Festschrift”.

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polyion axis scaled by the radius  $a$  of the polyion, and  $z$  is the coordinate along the axis where  $\rho$  is zero. The details leading to eq 2 are discussed in Appendix A. In eq 2,  $\sigma^2 = \epsilon^2/(2 + \alpha)$ ,  $\epsilon = \kappa a$ ,  $\alpha = 1/(1 + n_m/2n_d)$ ,  $n_m$  is the concentration of monovalent ions,  $n_d$  is the concentration of divalent ions, and  $\kappa$  is the Debye screening parameter. We first consider the case of an infinitely long cylinder; this limit should be understood unless noted explicitly to the contrary. In this infinite cylinder case,  $u$  is taken to be independent of the coordinate  $z$ . The boundary conditions are (i)  $u \rightarrow 0$  as  $|\rho| \rightarrow \infty$ ; (ii) the  $\rho$ -derivative of  $u$  at  $\rho = 1$  obeys  $u_\rho = -2\xi$ , where  $\xi = e^2/\epsilon_0 k_B T b$ , with  $b$  the axial charge spacing on the cylindrical polyion and  $\epsilon_0$  is the dielectric constant of pure water. Thus,  $\xi$  is the ratio of the Bjerrum length to the axial charge spacing of the phosphates.

The concentration of polyelectrolytes is sufficiently dilute so that in the calculation of  $\Gamma$  the distribution of ions around a polyelectrolyte is unaffected by its interactions with other polyelectrolytes. The salt ions and the polyion are modeled as being excluded from each other for  $0 \leq r \leq a$ . In the infinite cylinder case, the local density of the ionic species of type  $i$  depends only on the distance from the cylinder.

In this paper, we will only treat the case of monovalent cations. This should be assumed henceforth. With this understood, we denote the ionic density at a distance  $r$  from the polyion axis by  $n(r)$ . Far from the polyion, the local density corresponds to uniform bulk density,  $n^{\text{bulk}}$ . The average concentration of the ions is given by

$$\begin{aligned}\bar{n} &\equiv \frac{2}{R_c^2} \int_a^{R_c} n(r) r \, dr \\ &= \frac{2n^{\text{bulk}}}{R_c^2} \int_a^{R_c} e^{-u(r)} r \, dr\end{aligned}\quad (3a)$$

where  $R_c$  is the radius of an annular region around the polyion within which the local density of the ionic species varies from its bulk value and  $u$  is the reduced electrostatic potential that satisfies the PB equation. It is fruitful to rewrite eq 3a as

$$\bar{n} = 2n^{\text{bulk}} \int_a^{R_c} (e^{-u(r)} - 1) r \, dr / R_c^2 + 2n^{\text{bulk}} \int_a^{R_c} r \, dr / R_c^2 \quad (3b)$$

Thus, the preferential interaction coefficient can be expressed as

$$\Gamma \equiv \frac{(\bar{n} - n^{\text{bulk}})}{c_u} = \frac{2n^{\text{bulk}}}{c_u R_c^2} \int_a^{R_c} (e^{-u(r)} - 1) r \, dr - \frac{n^{\text{bulk}} a^2}{c_u R_c^2} \quad (3c)$$

where  $c_u$  is the concentration of polyion monomers and can be expressed as  $c_u = N_p/(\pi R_c^2 b N_p)$ , where  $N_p$  is the number of polyion monomers.

It proves convenient to rewrite  $\Gamma$  as follows. First, introduce the factor  $N_A/10^{-27}$ , where  $N_A$  is Avogadro's number to convert molar concentration to units of ions/Å<sup>3</sup>. Next, express the Debye screening parameter,  $\kappa$ , in terms of the bulk salt concentration and the Manning charge density parameter  $\xi$ . Third, rescale the radial distance  $r$  by the radius  $a$  of the polyion. Finally, note that since the concentration of polyion monomers is assumed to be dilute,  $R_c \gg a$ . Doing this, we obtain the desired expression for the polyion-ion preferential interaction coefficient per unit charge,  $\Gamma$ ,

$$\Gamma = -\frac{(\kappa a)^2}{4\xi} I - \frac{(\kappa a)^2}{8\xi} \quad (4)$$

Here,

$$I = \int_1^\infty (1 - e^{-u(\rho)}) \rho \, d\rho$$

and  $u(\rho)$  is the reduced electrostatic potential at a radial distance  $\rho$ .

In this paper we explain how to derive analytically tractable and reasonably tight upper and lower bounds for the polyion-ion preferential interaction coefficient, for molecules such as DNA or RNA. Our strategy for obtaining tight upper and lower bounds for  $\Gamma$  is to deduce upper and lower bounds for the electrostatic potential  $u$  by systematically exploiting the fact that no function has a local maximum where its Laplacian is positive nor a local minimum where its Laplacian is negative. This simple fact is called the "maximum principle". It is used with eq 2 to obtain bounds on  $u$ , which are then used to obtain upper and lower bounds for the integral  $I$  that defines the preferential interaction coefficient. We will implement this strategy for a polyion of linear charge density in an aqueous solution containing monovalent and divalent cations, such that  $n_d/2n_m \ll 1$ . Thus we are concerned with the  $\alpha = 0$  limit of eq 2. Even so, our techniques generalize to deal with  $\alpha > 0$  versions of eq 2, i.e., when both monovalent and divalent salts are present. With  $\alpha$  set equal to zero, two cases are discussed in detail: (A) an infinite length polyion with high and intermediate salt concentration, thus  $\sigma \geq 1$ , and (B) an infinite length polyion with very low salt concentration, thus  $\sigma < 1$ .

After discussing these cases, we indicate how they can be used to obtain lower and upper bounds for the analogue of  $\Gamma$  that arises when treating a linear polyion that is modeled by a cylinder of finite length  $L$ . In this case, we give tight bounds for  $\Gamma$  at intermediate and high salt concentrations. As before,  $\Gamma$  is expressible in terms of  $I$ , but the latter defined as

$$I = \int_\Omega (1 - e^{-u})$$

where the integration is over the part,  $\Omega$ , of three-dimensional space that is exterior to the cylinder. The function  $u$  still obeys the Poisson-Boltzmann equation that is depicted in eq 2, but it is now a function of the both coordinate  $\rho$  measuring distance from the cylinder axis and the linear coordinate  $z$  along the cylinder axis. In this finite length case, the boundary conditions for  $u$  are (i)  $u \rightarrow 0$  as distance from the cylinder goes to  $\infty$ ; (ii) the gradient of  $u$  is normal to the boundary of the cylinder; and (iii) the integral of the normal derivative of  $u$  over the boundary of the cylinder is  $-4\pi\xi(L + 1)$ ; thus  $-2\xi$  times the area of the boundary.

Let us now turn to the two cases where the cylinder length is assumed infinite. Case A:  $\sigma \geq 1$ . To obtain useful upper and lower bounds, we consider the solution  $w(\rho)$  to the equation

$$w_{\rho\rho} = \sigma^2(e^w - e^{-w}) \quad (5)$$

that satisfies the boundary conditions  $w_\rho|_{\rho=1} = -2\xi$ , and  $w \rightarrow 0$  as  $\rho \rightarrow \infty$ . The explicit solution to eq 5 is

$$e^{-w/2} = (1 - be^{-2^{1/2}\sigma(\rho-1)})/(1 + be^{-2^{1/2}\sigma(\rho-1)}) \quad (6)$$

where the constant  $b$ , obtained from the boundary conditions, is found to be  $b = (1 + 2(\sigma/\xi)^2)^{1/2} - 2^{1/2}\sigma/\xi$ . Since  $w_\rho < 0$  for all  $\rho$ , we have  $w_{\rho\rho} + w_\rho/\rho \leq \sigma^2(e^w - e^{-w})$ . By the maximum principle, this inequality implies that  $u \leq w$  everywhere. Making use of eq 6, we find that

$$\int (1 - e^{-w(\rho)}) \rho \, d\rho = 4 \left( b/(2^{1/2}(1+b)\sigma) + \frac{1}{2\sigma^2} \ln(1+b) \right) \quad (7)$$

Since  $u \leq w$ , the expression on the right-hand side of eq 7 is an upper bound for  $I$ . For a given value of linear charge density, salt concentration, and  $\sigma$ , we plug in the corresponding values of  $b$  so as to obtain upper bounds for preferential integral coefficient at high and intermediate salt concentrations (see Table 1).

Next we obtain lower bounds by taking the version of  $w$  from eq 6 where  $b$  is now determined by the boundary condition at  $\rho = 1$  given by  $w_\rho - w/2 = -2\xi$ . We then introduce the quantity  $z = w\rho^{1/2}$ . Observe that  $z$  obeys  $z_{\rho\rho} + z_\rho/\rho \geq \sigma^2(e^z - e^{-z})$  with  $z_{\rho}|_{\rho=1} = -2\xi$ . Consequently, by the maximum principle, we have  $u \geq z$  for all  $\rho \geq 1$ . The integral  $\int (1 - e^{-z}) \rho \, d\rho$  is now a lower bound for the integral,  $I$ , that appears in the preferential interaction coefficient. We estimate this integral numerically.

As the resulting lower and upper bounds are rather tight at high and intermediate salt concentrations, we have tabulated and summarized them in Table 1 for DNA for polyions with various linear charge densities.

Case B:  $\sigma \ll 1$ . To obtain upper and lower bounds for  $I$  in the low salt limit, we first introduce a nonnegative function,  $x(\tau)$ , of the rescaled variable  $\tau = 2^{1/2}\sigma\rho$ . We then set  $f \equiv -2\ln((1 - e^{-x})/(1 + e^{-x}))$  and consider the latter as a function of the variable  $\rho$ . The function  $f$  has the property that

$$f_{\rho\rho} + f_\rho/\rho \geq 2\sigma^2 \sinh(f) \quad (8)$$

if and only if

$$(1 + e^{-2x}/1 - e^{-2x})(x_\tau^2 - 1) \geq x_{\tau\tau} + \frac{1}{\tau}x_\tau \quad (9)$$

If it is also assumed that  $f$  obeys the same boundary conditions as  $u$ , then the maximum principle guarantees that  $f \leq u$  for all  $\rho \geq 1$ . Reversing the inequality in eq 9 reverses the inequality in eq 8 and so finds  $f \geq u$  for all  $\rho \geq 1$ . The requirement that  $f$  satisfies the boundary condition for  $u$ , is the following constraint at  $\tau = 2^{1/2}\sigma$

$$x_\tau e^{-x}/(1 - e^{-2x}) = \xi/(2^{3/2}\sigma) \quad (10)$$

To use all of this to obtain a lower bound for  $u$ , consider  $x(\tau) = \tau + \ln((1 + \beta\tau)^{1/2}/\eta)$ , where  $\beta > 0$  and  $\eta$  is determined from eq 10. For  $\sigma \ll 1$  and for both  $\xi = O(1)$  and  $\beta = O(1)$ , the constant  $\eta$  behaves as  $\eta \sim 1 + 2^{1/2}(1 + \beta/2)(1 - \xi/2)\sigma + O(\sigma^2)$ . With  $\eta$  determined in this way, we find, for example, that the desired inequalities are obeyed in the cases where  $0.1 \geq \sigma \geq 0.001$  and  $\xi = 4.2$  using  $\beta = 6$ .

To obtain an upper bound for  $u$ , we consider  $x(\tau) = \tau + \ln((1 + 16\tau^2)^\gamma/\eta)$ , where  $\gamma$  and  $\eta$  are constants that are specified below. The goal is to choose  $\eta$  and  $\gamma$  so that the equality in eq 10 holds and so that  $(1 + e^{-2x})(x_\tau^2 - 1)/(1 + e^{-2x}) \leq x_{\tau\tau} + x_\tau/\tau$ . In this regard, the boundary condition in eq 10 leads to

$$\eta = e\sqrt{2}\sigma(1 + 32\sigma^2)^{1/\gamma}[(1 + \Phi^2)^{1/2} - \Phi] \quad (11)$$

where  $\Phi = (2^{1/2}\sigma/\xi)(1 + 32/5)2^{1/2}\sigma/(1 + 32\sigma^2)$ . We find that the desired inequalities are obeyed if  $\gamma = 5.05$  for  $\sigma = 0.01$ . They are also obeyed if  $\gamma = 5$  for  $\sigma = 0.001$ .

Our upper and lower bounds for  $I$  when  $\xi = 4.2$  and  $\sigma = 0.1, 0.01$ , and  $0.001$  are displayed in Table 1.

To obtain upper and lower bounds in the case  $\xi = 4.2$  that hold uniformly for all sufficiently small values of  $\sigma$ , we note

**TABLE 1: Lower and Upper Bounds for  $I(u)$  at Various Values of  $\sigma$**

$\xi$	$\sigma$	lower bound	upper bound
2	5	0.07	0.079
	1	0.12	0.177
	4.2	0.22	0.268
	1.2	1.18	1.665
	0.1	63.4	85
4.2	0.01	$0.48\sigma^{-2}$	$0.795\sigma^{-2}$
	0.001	$0.45\sigma^{-2}$	$0.79\sigma^{-2}$
	6	0.155	0.177
	1	1.70	2.41

<sup>a</sup> By virtue of eq 4, the preferential interaction coefficient can be written directly in terms of  $I$  and  $\sigma$  as follows:  $\Gamma = -(\sigma^2/2\xi)I - (\sigma^2/4\xi)$ .

first that the  $\beta = 4$  version of  $x(\tau) = \tau + \ln((1 + \beta\tau)^{1/2}/\eta)$  makes the corresponding function  $f$  an upper bound for  $u$  if  $\sigma$  is sufficiently small when  $\xi = 4.2$ . Thus  $I(f) \geq I(u)$ . To obtain a lower bound that works for all sufficiently small values of  $\sigma$ , we use the function  $x(\tau) = \tau(2 + 1/3(\ln\tau/3)^2)$ . Because the resulting version of  $f$  obeys  $f_\rho \geq -2\xi$  at  $\rho = 1$  when  $\xi = 4.2$ , we can still employ the maximum principle to deduce that  $f \geq u$  at all  $\rho \geq 1$ . Thus,  $I(f) \leq I(u)$  for the latter version of  $f$ . Granted the preceding, crude numerical evaluations of the integrals for the two versions of  $I(f)$  find that the following low salt bounds must hold as  $\sigma \rightarrow 0$ :

$$0.24\frac{1}{\sigma^2} \leq I(u) \leq 0.60\frac{1}{\sigma^2} \quad (12)$$

**Finite Length Polyion.** The techniques introduced above can be generalized to the more realistic case of finite length ionic oligomers. In particular, the bounds for the infinite cylinder case can be used to obtain bounds for a cylinder of length  $L$ . For example, a detailed analysis indicates that

$$\int_\Omega (1 - e^{-u}) \leq 4\pi L \left(1 + \frac{2(1 + 2\sigma)}{\sigma L}\right) \hat{I}(\sigma, \xi) \quad (13)$$

where  $\hat{I}(\sigma, \xi)$  denotes the upper bound obtained in Case A. This upper bound will be tight in the case that  $\sigma L \gg 1$ . The details of the steps that lead to eq 13 are given in Appendix B. Somewhat different upper and lower bounds are given by

$$\frac{\xi}{(e^c + 1)\sigma^2} \leq \int_\Omega (1 - e^{-u}) \leq \frac{\xi}{2\sigma^2} \quad (14)$$

In eq 14,  $c$  is the value of  $u$  on the boundary of the cylinder. In this regard, we can show that  $c$  is no greater than the  $s = 1$  value of the function  $q(s)$  that satisfies the equation  $q_{ss} = \sigma^2(e^q - e^{-q})$  with the condition  $q_s = -2\xi$  at  $s = 1$ . In any case,  $c$  is no greater than the minimum of  $2^{1/2}/\pi\xi(1 + 1/L)$  and  $2\xi/\pi\sigma$ . Note that the bounds in eq 14 are thus tight in the case that  $\sigma/\xi \geq 1$ .

In his seminal work on counterion condensation, Manning predicted that the low salt limiting value of the preferential interaction coefficient for univalent ions is  $\Gamma = -1/4\xi$  for  $\xi \geq 1$ .<sup>31,35</sup> By comparison, our bounds on the preferential interaction coefficient in the limit of very low salt ( $\sigma \rightarrow 0$ ) and  $\xi = 4.2$  are  $-1.20 \leq \Gamma \leq -0.48$ . We can also compare our bounds with the low salt calculations of  $\Gamma$  that were obtained from the formal solution of the nonlinear PB equation as an asymptotic series in a small parameter,  $1/\ln\tilde{\epsilon}$  where  $\tilde{\epsilon} = 1/(2^{1/2}\sigma) = 1/(ka)$ .<sup>27</sup>

$$\Gamma = -\frac{1}{4\xi} - \frac{1}{4\xi} \left( \frac{\pi}{\ln \tilde{\epsilon} + 1.897 + (\xi - 1)^{-1}} \right)^2 + \dots \quad (15)$$

The overall accuracy of eq 15 assessed by Shkel et al. is  $(\ln \tilde{\epsilon})^{-5}$ , which is the order of the largest term that was neglected in eq 15.<sup>27</sup> For the 19.05  $\mu\text{M}$  case,  $\tilde{\epsilon} = 68.7$  and eq 15 leads to  $\Gamma \approx -0.074$ . In contrast, our bounds lead to  $-0.057 > \Gamma > -0.095$ .

At 51.0 mM,  $\tilde{\epsilon} = 2.89$  and eq 15 yields  $\Gamma = -0.154$ . Meanwhile, our bounds for  $\Gamma$  are  $-0.153 > \Gamma > -0.226$ . In this case, a Monte Carlo simulation and a numerical solution to the PB equation give  $\Gamma = -0.164$  and  $\Gamma = -0.175$ , respectively.<sup>35</sup> Note that these values for  $\Gamma$  from the Monte Carlo simulation, the numerical solution to the PB equation, and the asymptotic expansion in terms of a small parameter ignore the right-most term in eq 4.<sup>35,27,28</sup> Since the coions are excluded from the region occupied by the cylindrical polyelectrolyte, the last term reflects the volume excluded per mole of charges of the polyanion.

There is also a formal asymptotic solution of the nonlinear Poisson–Boltzmann equation in the high salt limit, where  $\sigma \rightarrow \infty$ . This formal solution is presented as an expansion now in powers of the small parameter  $\tilde{\epsilon} = 1/(2^{1/2}\sigma)$ , and it results in the following expression for the preferential interaction coefficient:<sup>28</sup>

$$\Gamma = -\frac{1}{2} + \frac{1}{4}\xi\tilde{\epsilon} - \frac{1}{4}\xi\tilde{\epsilon}^3 + \dots \quad (16)$$

This high salt asymptotic expansion for  $\Gamma$  has been used at salt concentration where  $\tilde{\epsilon}$  is slightly larger than unity.<sup>28</sup>

It should be remarked that the limit of  $1/2$  in eq 16 is also our high salt limit for the first term on the right-hand side of eq 4. Obviously, the right-most term in eq 4 diverges in the infinite salt concentration limit. At 2.21 M and 0.255 M concentrations,  $\tilde{\epsilon} = 0.202$  and  $\tilde{\epsilon} = 0.594$ , respectively. With  $\xi = 4.2$  and with these values for  $\tilde{\epsilon}$ , the respective values of the preferential interaction coefficient from the first few terms of eq 16 are  $\Gamma = -0.342$  and  $\Gamma = -0.228$ . Meanwhile, our bounds using only the first term on the right-hand side of eq 4 are  $-0.322 > \Gamma > -0.392$  and  $-0.199 > \Gamma > -0.281$  at 2.21 M and 0.255 M, respectively. At 210 mM corresponding to  $\tilde{\epsilon} = 0.655$ , Monte Carlo and numerical solutions to the PB equation yield  $\Gamma = -0.246$  and  $\Gamma = -0.273$ , respectively,<sup>35</sup> in the case where  $\xi = 4.2$ . For this same value of  $\tilde{\epsilon}$ , the first few terms of the asymptotic solution to the PB equation in eq 16 give  $\Gamma = -0.22$  when  $\xi = 4.2$ . Our bounds for  $\Gamma$  in this case, again without the right-most term in eq 4, are  $-0.192 > \Gamma > -0.276$ .

The validity and the mean-field nature of the cylindrical nonlinear PB equation in describing the ionic characteristics of a charged polyanion such as DNA and RNA in monovalent or divalent salts has been extensively discussed in the literature.<sup>1,5,17–22</sup> These studies suggest that the Monte Carlo simulations are reasonably accurate in describing  $\Gamma$  for univalent counterions for both high and low salt concentrations. However, one such study indicates that there is less accuracy for the case of divalent cations.<sup>35</sup> This paper contributes to the discussion by providing new theoretical techniques that give tight, mathematically rigorous bounds for the preferential interaction coefficient that are valid from low to intermediate and high salt concentrations. By way of contrast, a formal solution of the nonlinear PB equation that is given as an asymptotic expansion in a small parameter has two principle drawbacks: First, it can be used only if higher order terms in the expansion are neglected. Second, the range of validity of such an expansion or any truncation at finite order can be assessed only if the exact

solution is known. In contrast, our upper and lower bounds are mathematically rigorous and so the true solution must lie between them.

As remarked previously, we have generalized the technique to deal with values of  $\alpha$  greater than zero, thus for DNA and RNA in divalent and mixed salts. A detailed comparison of our predictions for mixed salts, values of  $\alpha$  greater than zero, with asymptotic expansion<sup>27,28</sup> and simulation data<sup>35</sup> will be described elsewhere. The maximum principle techniques described in this paper can also be generalized to the important case of secondary structures in nucleic acid, such as three-way or four-way helix RNA junctions or hairpin ribozymes, in aqueous solution containing various concentrations of divalent cations. In fact, conformation changes in these systems have been recently measured using single-molecule fluorescence resonance energy transfer spectroscopy techniques.<sup>55,56</sup>

**Acknowledgment.** This work was supported by the National Science Foundation (U.M.; S.C. and C.H.T.).

## Appendix A

Our starting point is the following nonlinear PB equation in terms of the reduced potential  $u = e\psi/k_B T$ , where  $\psi$  is the actual potential:

$$\nabla^2 u = -4\pi l_B \sum_i z_i n_i e^{-z_i u} \quad (A.1)$$

Here,  $l_B$  is the Bjerrum length, and  $z_i$  and  $n_i$  are respectively the valence and the density of the ion labeled  $i$ . To proceed further it is fruitful to rewrite eq A.1 in the form

$$\nabla^2 u = -4\pi l_B \left( \sum_i z_i^2 n_i \right) \left( \frac{\sum_i z_i n_i e^{-z_i u}}{\sum_i z_i^2 n_i} \right) \quad (A.2)$$

In the presence of univalent and divalent cations and univalent anions, the charge neutrality condition is

$$n_+ - n_- + 2n_{2+} - 2n_{2-} = 0 \quad (A.3)$$

Consequently, we can write the term  $\sum_i z_i^2 n_i$  that appears in eq A.2 as

$$\begin{aligned} \sum_i z_i^2 n_i &= 2n_+ + 6n_{2+} \\ &\equiv 2n_m + 6n_d \end{aligned} \quad (A.4)$$

where  $n_m$  and  $n_d$  are the density of the univalent and the divalent cations. Similarly on simplifying the term  $\sum_i z_i n_i e^{-z_i u}$  that appears in eq A.2, we find

$$\sum_i z_i n_i e^{-z_i u} = n_+ e^{-u} - 3n_- e^u + 2n_{2+} e^{-2u} \quad (A.5)$$

Next we make use of eqs A.5 and A.6 to further simplify the PB equation as<sup>25</sup>

$$\nabla^2 u = -8\pi l_B \frac{(n_m + 2n_d)}{(2n_m + 6n_d)} \left\{ \frac{n_m}{(n_m + 2n_d)} e^{-u} + e^u + \frac{2n_d}{(n_m + 2n_d)} e^{-2u} \right\} \quad (A.6)$$



where  $I$  is the ionic strength of the aqueous solution. Scaling all distances by the radius of the cylinder and introducing the Debye screening parameter  $\kappa$ , eq A.6 reduces to

$$\nabla^2 u = -\frac{(\kappa a)^2}{2 + \alpha} \{ (1 - \alpha)e^{-u} - e^u + \alpha e^{-2u} \} \quad (\text{A.7})$$

where  $\alpha = 2n_d/(n_m + 2n_d)$ . Finally, on letting  $u \rightarrow -u$  and introducing the quantity  $\sigma^2 = \epsilon^2/(2 + \alpha)$ , where  $\epsilon = \kappa a$ , we obtain the expression for the PB equation used in the text

$$\nabla^2 u = \sigma^2 \{ (1 - \alpha)e^u - e^{-u} + \alpha e^{2u} \} \quad (\text{A.8})$$

## Appendix B

In this appendix we explain in detail how the bounds for the infinite cylinder can be exploited to obtain finite cylinder bounds for the case  $\alpha = 0$ . Let us suppose that  $u$  is the solution to the equation

$$\Delta u = \sigma^2 (e^u - e^{-u}) \quad (\text{B.1})$$

for a cylinder of length  $L \geq \sigma^{-1}$ . Thus,  $u$  is constant where  $\rho = 1$  and  $|z| \leq L/2$ , and where  $|z| = L/2$  and  $\rho \leq 1$ . Moreover,  $u$  satisfies the boundary condition  $f_{\partial\Omega} \hat{n} \cdot \nabla u = -4\pi\xi(L + 1)$ , where  $\hat{n}$  is the unit, outward pointing normal vector to the cylinder and the integration is over the boundary  $\partial\Omega$  of the cylinder.

Let  $q(s)$  denote the solution to the equation

$$q_{ss} = \sigma^2 (e^q - e^{-q}) \quad (\text{B.2})$$

where  $s \geq 1$  with the condition  $q_s = -2\xi$  where  $s = 1$ . Extend  $q$  as a constant function with the value  $q(1)$  where  $s \leq 1$ . Now let us set  $w(\rho, z) \equiv \min(q(\rho), q(|z| - L/2 + 1))$ . This function obeys the equation

$$\Delta w \leq \sigma^2 (e^w - e^{-w}) \quad (\text{B.3})$$

Note that  $w$  is a constant on  $\partial\Omega$  and  $f_{\partial\Omega} \hat{n} \cdot \nabla w = -4\pi\xi(L + 1)$ .

Since  $\Delta(u - w) \geq \sigma^2(e^u - e^{-u}) - \sigma^2(e^w - e^{-w})$  and  $f_{\partial\Omega} \hat{n} \cdot \nabla(u - w) = 0$ , we have the inequality  $u \leq w$  and

$$\int_{\Omega} (1 - e^{-u}) \leq \int_{\Omega} (1 - e^{-w}) \quad (\text{B.4})$$

We have thus shown that the upper bounds developed for the preferential interaction coefficient for the infinite cylinder case can be used to derive an upper bound for the preferential interaction coefficient for the finite cylinder.

To say more about this last conclusion, we rewrite the right-hand side of the integral that appears in eq B.4 as

$$\begin{aligned} \int_{\Omega} (1 - e^{-w}) &= 2\pi L \int_{s>1} (1 - e^{-q(s)}) s \, ds + \\ &2\pi \int_{s>1} (1 - e^{-q(s)}) \, ds + 4\pi \int_{s>1} (1 - e^{-q(s)}) (s - 1) \, ds + \\ &2\pi \int_{s>1} (1 - e^{-q(s)}) (s^2 - 1) \, ds \end{aligned} \quad (\text{B.5})$$

Combining the various terms in eq B.5, we get

$$\begin{aligned} \int_{\Omega} (1 - e^{-w}) &= 2\pi(L - 2) \int_{s>1} (1 - e^{-q(s)}) s \, ds + \\ &6\pi \int_{s1} (1 - e^{-q(s)}) s^2 \, ds \end{aligned} \quad (\text{B.6})$$

The first integral on the right-hand side of (B.6) is given by eq 7 in the text under Case A. The second integral can be computed

by a similar strategy:

$$\begin{aligned} \int_{s>1} (1 - e^{-q}) s^2 \, ds &= 2\sqrt{2} \frac{b}{\sigma(1 + b)} - 4 \frac{\ln(1 + b)}{\sigma^2} + \\ &\frac{4}{\sigma^2} \int_{s>1} \ln(1 + b e^{-\sqrt{2}\sigma(s-1)}) \, ds \end{aligned} \quad (\text{B.7})$$

Noting that the last integral in eq B.7 can be expressed as an infinite series, we have the upper bound for  $f_{\Omega} (1 - e^{-w})$ :

$$\begin{aligned} \int_{\Omega} (1 - e^{-w}) &\leq 4\pi(L - 2) \left( \sqrt{2} \frac{b}{\sigma(1 + b)} + \frac{1}{\sigma^2} \ln(1 + b) \right) + \\ &12\sqrt{2}\pi \left( \frac{b}{\sigma(1 + b)} + \sqrt{2} \frac{\ln(1 + b)}{\sigma^2} + \frac{1}{b^3} \sum_{n \geq 1} (-1)^{n+1} \frac{b^n}{n^2} \right) \end{aligned} \quad (\text{B.8})$$

Since the sum in the final term in eq B.8 is an infinite alternating series, we obtain the following upper bound on  $f_{\Omega} (1 - e^{-w})$ :

$$\begin{aligned} \int_{\Omega} (1 - e^{-w}) &\leq 4\pi(L - 2) \times \\ &\left( 1 + \frac{2(1 + 2\sigma)}{\sigma L} \right) \left( \sqrt{2} \frac{b}{\sigma(1 + b)} + \frac{1}{\sigma^2} \ln(1 + b) \right) \\ &= 4\pi L \left( 1 + \frac{2(1 + 2\sigma)}{\sigma L} \right) \hat{i}(\sigma, \xi) \end{aligned} \quad (\text{B.9})$$

where  $\hat{i}(\sigma, \xi)$  denotes the upper bound obtained in Case A. Equation B.9 is precisely what is stated as eq 13 in the text.

## References and Notes

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