Hybrid Boundary Element and Finite Difference Method for Solving the Nonlinear Poisson–Boltzmann Equation

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Abstract: A hybrid approach for solving the nonlinear Poisson–Boltzmann equation (PBE) is presented. Under this approach, the electrostatic potential is separated into (1) a linear component satisfying the linear PBE and solved using a fast boundary element method and (2) a correction term accounting for nonlinear effects and optionally, the presence of an ion-exclusion layer. Because the correction potential contains no singularities (in particular, it is smooth at charge sites) it can be accurately and efficiently solved using a finite difference method. The motivation for and formulation of such a decomposition are presented together with the numerical method for calculating the linear and correction potentials. For comparison, we also develop an integral equation representation of the solution to the nonlinear PBE. When implemented upon regular lattice grids, the hybrid scheme is found to outperform the integral equation method when treating nonlinear PBE problems. Results are presented for a spherical cavity containing a central charge, where the objective is to compare computed 1D nonlinear PBE solutions against ones obtained with alternate numerical solution methods. This is followed by examination of the electrostatic properties of nucleic acid structures.


Key words: Poisson–Boltzmann equation; boundary element method; finite difference; electrostatics

Introduction

Importance of Nonlinear Electrostatic Modeling

The ability to accurately model the electrostatic properties of highly charged biomolecular systems such as nucleic acids is critical because for such biopolyelectrolytes the structure–function relationships and molecular recognition events are strongly influenced by the electrostatic interactions. Due to the high charge density of its negatively charged sugar–phosphate backbone, the stability and interactions of nucleic acid structures with other charged molecules (e.g., drugs and proteins) are critically dependent on the ion type and concentration in the environment (see refs. 1–3). Because many biological systems contain both monovalent and multivalent ions it is important to model mixed salt solutions.4 In RNA and many other biomolecular systems both monovalent and divalent ions can trigger conformational transitions and play other important functional roles. For example, the helix-coil transition and folding of nucleic acids are dependent on the nonspecific effects of changes in the concentrations of both monovalent and divalent ions (e.g., refs. 3 and 5–8).

One formulation framework commonly adopted to treat nonspecific electrostatic interactions in highly charged systems is based on the classic continuum electrostatic model. In this model the solvent and dissolved ions are treated as a continuum environment, characterized by the solvent dielectric constant and bulk ionic strength, respectively, but the solute is treated explicitly. The 3D full (nonlinear) Poisson–Boltzmann equation (PBE) is one of the most popular continuum electrostatic approaches. In this method, the solute is treated in atomic detail with partial charges on all atoms and the dielectric interface between the solute and solvent regions is modeled by one of the molecular surfaces (e.g., solvent excluded surface9). For highly charged systems the linear PBE is no longer valid and the nonlinear PBE must be employed. For example, it has been shown that for highly charged enzymes such as superoxide dismutase (SOD) the electrostatic potential in the active site region obtained with the nonlinear PBE is weaker relative to the linear PBE case.10,11 Consequently, the diffusion-controlled reaction rate constants for the SOD–superoxide catalytic reaction obtained with the 3D nonlinear PBE are smaller than those obtained with the linear PBE.12 The Hecht et al.13 study shows that much better agreement with experimentally measured electrostatic potential at the surface of calf-thymus DNA is obtained using the nonlinear rather than linear PBE. Moreover, using the nonlinear PBE to predict various electrostatic properties of highly charged systems achieves good agreement with experimen-

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tal results or more accurate theoretical approaches (e.g., refs. 5, 14, and 15). All these studies highlight the importance of using the nonlinear PBE for highly charged biomolecular systems.

Previous Approaches

Even for the simplest systems, such as spherical molecular surface containing a central charge, analytic solutions to the nonlinear PBE are unavailable and numerical methods are necessary. The pioneering work of different groups (e.g., refs. 16–18) established that the mean-field nonlinear PBE for charged cylinder models adequately describes the electrostatic behavior of highly charged polyions (e.g., DNA) in monovalent salt solutions and thus stimulated the development of various numerical methods for solving the 3D nonlinear PBE. The computational tools available to solve the nonlinear PBE can be broadly categorized into (1) finite difference (FD) methods that here include finite element discretizations and (2) integral equation or boundary element methods (BEMs). Both methods are subject to well-known inherent advantages and limitations, which are closely tied to the underlying formulations. Below, a hybrid approach for the nonlinear PBE is introduced that combines the integral equation and FD approaches in a way that retains their relative strengths and eliminates their primary drawbacks. But, first a brief review of these methods is in order.

FD Method

In the FD approach the PBE solution is developed upon a mesh encompassing the molecule and its surrounding domain. Discrete approximations to the governing partial differential equation are obtained upon this grid using either FD procedures or variational principles leading to the finite element approximation. PBE codes developed from FD/finite element methods may be further distinguished according to the grid structure used. By far the most common arrangement is the uniformly spaced 3D lattice adopted in the widely used DelPhi, UHBD, and MEAD codes. Here, the molecule is placed inside this lattice and the mesh is allowed to “cut through” the molecular surface. Such meshes are said to be “nonconforming” because they do not align with the surface. One consequence to adopting a nonconforming mesh is that sizeable numerical errors in the computed potential and its spatial gradients are generated at the dielectric interface. The simple structure of lattice grids facilitates the implementation of efficient multigrid procedures to obtain the solution and, on a per-cell basis, minimizes storage costs compared to alternate mesh types. On the other hand, the regular grid structure can lead to severe resolution constraints. For example, halving the mesh spacing (to obtain more accurate results) or doubling the extent of the domain (to reduce errors at the outer boundaries) leads to an eightfold increase in the number of mesh points and thus storage. In linear PBE problems smaller grid domains can be employed by imposing higher-order conditions at the outer boundaries. However, for the nonlinear PBE problem no uniformly valid higher-order corrections seem available and the only reliable approach is to replace the outer boundaries sufficiently far away where the electrostatic potential is essentially negligible. It should be noted, however, that focusing and “parallel focusing” techniques have been employed in the FD solution of the PBE to alleviate the outer boundary errors and poor resolution of the dielectric interface associated with larger systems and/or highly charged systems.

Unstructured tetrahedral grids have been promoted as a way to address some of these limitations. In such grids, local solution errors can be controlled by adjusting the mesh spacing so that much higher accuracy can be achieved than with a regular 3D Cartesian grid method using the same number of grid points. For example, when solving the PBE finer mesh spacing can be used near the fixed charges and coarser spacing reverted to away from the molecule. On the other hand, per-node storage is high because pointer lists are needed to explicitly identify neighboring points and because the number of such neighbors per grid point is higher than in regular lattice methods. Also, geometric information such as grid point locations and the weights to evaluate the Laplacian are stored for individual edges and points. Multigrid schemes are more difficult and costly to develop upon unstructured grids. Finally, finite element discretizations of the Laplacian upon 3D unstructured grids in general fail to enforce a discrete maximum principle that is required to prove uniform convergence and stability. This leaves open the possibility of accuracy and convergence problems.

Generating unstructured grids is complex and CPU intensive, especially for large molecules. Grid generation is in particular difficult if the mesh is required to conform to the molecular surface. A boundary-conforming mesh would be desirable because it allows better predictions of the electrostatic field to be obtained at the surface. Specifically, a boundary-conforming grid would allow one to explicitly enforce continuity in the normal electrostatic displacement (which corresponds to a jump in the normal potential gradient) at the surface and reduce local solution errors. However, while unstructured grids offer a means of obtaining boundary conforming grids about complex geometries the associated mesh generation challenges have deterred wider use of such meshes for molecular electrostatic modeling.

Finally, near the charge site the electrostatic potential becomes singular and behaves as $\Phi(r) \sim A/r$, where $A$ is a constant and $r$ is the distance from the charge. Such variations are poorly resolved using a FD method and with some treatments, such as trilinear interpolation, the computed electrostatic energies do not converge with increased grid resolution. Often, one is interested solely in energy differences—for example, the change in electrostatic energy at zero and a specified salt concentration. Then, the errors associated with resolving singular fields essentially cancel when subtracting the total electrostatic energies. However, to obtain accurate estimates of total electrostatic energies or forces alternate numerical methods must be considered.

BEM

For the linear PBE, the potential solution inside and outside the molecule can be completely characterized in terms of surface integrals evaluated over the molecular boundary. These integrals involve products of the singular Green’s functions associated with the PBE and the surface potential or its normal gradient. The integral solution to the nonlinear PBE also involves additional volume integrals. In the BEM a surface mesh is generated upon the molecular surface and used to develop a discrete approximation to
the integral equation. Next, the unknown surface potential and normal gradient distributions are obtained by evaluating the governing integral equations at the surface. Once these distributions are known, the potential can be evaluated anywhere within or outside the molecule.

When used to solve the linear PBE, the BEM enjoys several intrinsic advantages over FD methods:

1. Accurate predictions of both the potential and its normal gradient (related to the induced charge density) are obtained at the molecular surface because the mesh coincides with this surface (i.e., it is boundary conforming). As a consequence, surface forces such as the dielectric and ionic boundary “pressure” components can be accurately determined.
2. The solution is characterized solely in terms of surface distributions so that there are fewer unknowns. Also, the same surface meshes can be reused when computing interaction energies for a pair of molecules in various relative positions and orientations.
3. The far-field boundary conditions are implicitly and exactly imposed. Thus, the challenges of specifying the solution at the outer boundary in a FD method are eliminated.
4. Adaptive techniques are easily introduced because boundary elements can be refined independently.
5. Graphical representation of surface properties is simplified because the primary properties—potential and surface polarization charge—are directly known at the molecular surface.
6. The total electrostatic potential and forces at charge sites are accurately determined using the exact analytic forms for the potential induced by a point charge or boundary element. Consequently, reliable estimates of total electrostatic energies can also be obtained.

A drawback of the BEM is the rapid (quadratic) growth in computational storage and CPU time with the number of elements. Efforts to address these costs have led to the development of so-called fast multipole methods. These methods combine a hierarchical decomposition of the domain and the active elements (i.e., fixed charges and boundary elements) and a multipole approximation to describe the long-distance influence of a collection of these elements. With careful organization and bookkeeping of these operations, computational costs are reduced to $O(N \log N)$, where $N$ is the number of elements; in practice, order of magnitude reductions in computational costs over conventional implementations are realized. The details of a fast multipole algorithm and its implementation within a BEM for solving the linear PBE are described elsewhere.$^{37,38}$ This work has successfully extended the fast BEM to problems involving finite salt concentrations (previously, only zero salt concentrations resulting in Coulombic interactions were amenable to fast treatments$^{39–41}$).

BEMs are also limited in their ability to treat nonlinear problems. It is formally possible to treat nonlinearities, ion-exclusion layers, and other inhomogeneous features within the framework of an integral equation solution representation and previous authors have pursued such an approach.$^{42–44}$ The nonlinear terms are then expressed as source distributions, which appear as volume integrals over the entire domain. However, our experience has been that the evaluation of these volume integrals is expensive even with the use of fast multipole methods. Moreover, the grid generation challenges are the same as those faced in a FD approach. For similar mesh spacings, one expects similar solution errors whether using an integral equation or a FD approach. However, the FD method is typically much faster because only immediately neighboring mesh nodes are directly coupled whereas in the integral equation method all cells mutually interact. Therefore, once volume integrals appear the computation times incurred by a conventional or multipole-accelerated integral equation method increase significantly and tend to be higher than a FD scheme of comparable accuracy.

**Hybrid Approach**

The method presented here combines the intrinsic advantages of a BEM and FD scheme to solve the nonlinear PBE for arbitrary complex-shaped biomolecules. The fast BEM is used to solve the linear PBE problem at finite salt concentrations.$^{38}$ Because only surface but no volume integrals appear in the solution, the computational efficiencies of a fast BEM are preserved. Also, the inherent advantages of a BEM listed above (high accuracy, precise representation of the surface, robust treatment of point charge singularities, etc.) are retained. To progress from the linear to nonlinear PBE solution a correction potential and its governing equation are developed. Because this correction potential is non-singular (including at charge sites) and everywhere continuous (although slope discontinuity at the surface is present) it can be accurately resolved by a FD method. The problem of computed self-energies is also eliminated. The coupling between the linear and correction potentials is one way so that the linear solution effectively “drives” the correction terms. This property facilitates algorithm development and overall flow of the program.

This article describes the hybrid algorithm for solving the nonlinear PBE and contrasts it with a pure integral equation description. The article concludes with demonstration calculations involving both idealized geometries and highly charged biopolymers such as nucleic acids.

**Methodology**

The problem geometry is represented by three distinct regions: (1) the molecular interior, $\Omega_1$, which contains the atomic point charges and has dielectric constant, $\varepsilon_1$; (2) the exterior region, $\Omega_2$, which has dielectric constant, $\varepsilon_2$, and contains the dissolved ions; and (3) a charge-free layer (or ion exclusion region), $\Omega_3$, of specified thickness that separates the interior and exterior regions and whose dielectric constant is also $\varepsilon_3 = \varepsilon_2$. The ion-exclusion layer accounts for the ion size and its thickness corresponds roughly to the hydrated radius of the ion. These regions are delimited by surfaces whose precise location remains open to debate.$^{45}$ The most common choices are the van der Waals, solvent-accessible, and solvent-excluding surfaces.$^9$

The PBE is based on the mean-field treatment of the mobile ions, which neglects ion–ion correlations and ion size effects. Some recent theoretical studies (e.g., refs. 46–48) of highly charged biomolecules (i.e., DNA) immersed in pure 1:1 salt solutions (up to $\sim 1 \text{ M}$) show that thermodynamic properties obtained
with the nonlinear PBE approach are in good agreement with more accurate theoretical accounts that account for ion–ion correlations and ion size. This agreement worsens in the presence of multivalent ions and asymmetrical salts.\(^{57,49-51}\) However, some studies (e.g., refs. 5 and 52–54) have shown that under certain mixed monovalent and divalent salt concentrations the nonlinear PBE predictions are in good agreement with experimental results. On the other hand, the nonlinear PBE cannot predict attractive electrostatic interactions between like-charged polyions (e.g., DNA condensation by multivalent ions) and overcharging effects, which have been observed both theoretically and experimentally, due to the mean-field approximation (e.g., refs. 55–61). For a more detailed discussion on the limitations of the nonlinear PBE due to the mean-field approximation the reader is referred elsewhere (e.g., refs. 32 and 62–64).

With these assumptions the reduced (or dimensionless) electrostatic potential, \(\Phi\), of biopolyelectrolytes immersed in a mixture of 1:1 and 2:1 salts is governed by the PBE\(^{55}\):

\[
\nabla \cdot [\varepsilon(\mathbf{r}) \nabla \Phi(\mathbf{r})] + \frac{4\pi e}{k_B T} \rho(\mathbf{r}) = 0 \quad (1)
\]

where \(\varepsilon\) is the dielectric constant, \(e\) represents the protonic charge, \(k_B\) denotes the Boltzmann constant, and \(T\) is the absolute temperature of the solution. The charge density in the different regions is given by

\[
\rho(\mathbf{r}) = \rho^0(\mathbf{r}) = \sum_k Q_k \delta(\mathbf{r} - \mathbf{r}_k), \quad \mathbf{r} \in \Omega_1 \quad (2a)
\]

\[
\rho(\mathbf{r}) = \rho^\infty(\mathbf{r}) = -2eI_{1,2} \sinh(\Phi) - \frac{2}{3} eI_{2,3} [\exp(\Phi) - \exp(-2\Phi)], \quad \mathbf{r} \in \Omega_2 \quad (2b)
\]

\[
\rho(\mathbf{r}) = 0, \quad \mathbf{r} \in \Omega_3 \quad (2c)
\]

where \(\rho^0\) represents the contribution to the total charge density from the fixed charges in the solute and \(\rho^\infty\) denotes the corresponding contribution from the mobile ions, which is approximated by the Boltzmann distribution. \(Q_k\) is the value of the 4th charge positioned at \(\mathbf{r}_k\), \(\delta(\mathbf{r})\) is the 3D Dirac delta function, and \(I_{1,2}\) and \(I_{2,3}\) denote the ionic strengths of the 1:1 and 2:1 salts, respectively. In terms of the bulk salt concentrations, \(I_{1,2} = c_{1,2}^{\text{bulk}}\) and \(I_{2,3} = 3c_{2,3}^{\text{bulk}}\). It is convenient to introduce the Debye–Hückel screening parameters:

\[
\kappa_{1,2}^2 = \frac{8\pi e^2 I_{1,2}}{\varepsilon k_B T}, \quad \kappa_{2,3}^2 = \frac{8\pi e^2 I_{2,3}}{\varepsilon k_B T} \quad (3)
\]

which allows one to express the equation for the electrostatic potential in the exterior domain, \(\Omega_2\), as

\[
\nabla \cdot [\varepsilon \nabla \Phi] = e\kappa_{1,2}^2 \sinh(\Phi) + \frac{e\kappa_{2,3}^2}{3} [\exp(\Phi) - \exp(-2\Phi)] = f(\Phi) \quad (4)
\]

For small electrostatic potentials,

\[
f(\Phi) = f_1(\Phi) = e\kappa_{1,2}^2 + \kappa_{2,3}^2 \Phi \quad (5)
\]

so that the effective Debye–Hückel screening parameter to use in a linearized PBE is

\[
\kappa^2 = \kappa_{1,2}^2 + \kappa_{2,3}^2 \quad (6)
\]

By solving (1) and obtaining the electrostatic potential all other electrostatic properties such as the electrostatic free energy, electrostatic solvation free energy, and counterion concentrations can be derived. Both the linear and nonlinear forms of (1) can be solved using either a FD or an integral equation-based approach. Here, we combine both methods into a hybrid scheme to exploit the unique strengths of each method. First, however, we describe how the nonlinear PBE can be solved using an integral equation approach. We adopted this approach initially, but it proved extremely costly in terms of CPU time and was subsequently replaced by the hybrid technique presented here. Nevertheless, to compare the integral and hybrid approaches, and motivate the latter, a brief description of the pure integral equation method is presented here.

**Integral Equation Approach**

If one defines the nonlinear correction term

\[
\chi(\Phi) = \frac{1}{\varepsilon} \{ f(\Phi) - f_1(\Phi) \} = \kappa_{1,2}^2 \sinh(\Phi) + \frac{2}{3} \kappa_{2,3}^2 \{ \exp(\Phi) - \exp(-2\Phi) \} - \kappa^2 \Phi \quad (7)
\]

and considers the case of a vanishing ion-free layer, then (1) can be expressed as

\[
\nabla^2 \Phi(\mathbf{r}) = -\sum_k q_k \delta(\mathbf{r} - \mathbf{r}_k), \quad \mathbf{r} \in \Omega_1 \quad (8a)
\]

\[
(\nabla^2 - \kappa^2) \Phi(\mathbf{r}) = \chi(\Phi), \quad \mathbf{r} \in \Omega_2 \quad (8b)
\]

where \(q_k = (4\pi e^2/\varepsilon k_B T)Q_k\). Note that the dielectric constant has been taken outside the \(\nabla\) operator. This is valid because within each region \(\Omega_i\), \(\varepsilon\) is constant. However, to solve (8) additional conditions must now be provided at the molecular surface, \(\Omega_1 \cap \Omega_2\). Elementary considerations require that the electrostatic potential, \(\Phi\), and the normal electric displacement, \(\varepsilon\Phi/\varepsilon n\), be continuous at the surface, where \(\mathbf{n}\) is the surface normal. Note that the only difference between the linear and nonlinear PBE is the appearance of the nonlinear correction term, \(\chi(\Phi)\), on the right side of (8b).

The general solution for the electrostatic potential can be developed by standard application of Green’s second theorem using the fundamental solution (Green’s function) for the linear PBE:
\[ G_s(R, \rho) = \frac{e^{-\kappa R}}{4\pi |R - \rho|} \]  

(9)

Note that \( G_0 \) is the fundamental solution for the Laplace and Poisson equations. The general solution is (8) expressed in terms of surface integrals taken over the molecular surface is then

\[ \Phi(R) = \int_S \left[ G_0(R, \rho) h(\rho) - \frac{\partial G_0}{\partial n}(R, \rho) \Phi(\rho) \right] dS(\rho) + \Phi^r(R), \]

\( R \in \Omega_1 \)  

(10a)

\[ \Phi(R) = \int_S \left[ -G_s(R, \rho) \frac{1}{\varepsilon} h(\rho) + \frac{\partial G_s}{\partial n}(R, \rho) \Phi(\rho) \right] dS(\rho) + \Phi^{NL}(R), \]

\( R \in \Omega_2 \)  

(10b)

\[ \Phi^r(R) = \sum_k q_k G_0(R, \rho_k), \]

\[ \Phi^{NL}(R) = -\int_{\Omega_1} \chi(\Phi) G_s(R, \rho) dV(\rho) \]  

(10c,d)

where the surface conditions have been imposed. Here, the ratio of dielectric constants is \( \varepsilon = (\varepsilon_0/\varepsilon_\infty) \), \( \varepsilon_\infty \) is the surface normal pointing outward from \( \Omega_1 \) to \( \Omega_2 \), \( S \) is the molecular surface, \( V \) is the electrolyte volume, and \( h(\rho) \) is the normal gradient, \( \partial \Phi/\partial n \), as one approaches the surface from the interior domain, \( \Omega_2 \).

Before the electrostatic potential can be evaluated at selected points, the unknown surface distributions, \( \Phi(\rho) \) and \( h(\rho) \), must be determined. This is accomplished by considering the limiting forms of (10) as one approaches the surface. Except for rare cases, the resulting integral equations can only be solved numerically using a BEM. In such a method, the surface is represented by an assembly of elements and the surface distributions, \( \Phi(\rho) \) and \( h(\rho) \), approximated by interpolants of specified order. The solution process first determines these surface distributions. Once they are known, the electrostatic potential can be evaluated everywhere using the discrete approximation of (10).

The equations to obtain the surface distributions, \( \Phi(\rho) \) and \( h(\rho) \), can be stated in different ways, which, although mathematically equivalent, give rise to algebraic systems with different numerical conditioning properties. The development by Jufer et al.\(^{36}\) has been shown to promote excellent numerical conditioning\(^{67}\) and is therefore adopted in the present work. Following their approach, the equations for the surface distributions are

\[ \frac{1}{2} \left( 1 + \frac{1}{\varepsilon} \right) \Phi(R) = \int_S \left[ (G_0 - G_s) h(\rho) - \left( \frac{\partial G_0}{\partial n} - \frac{\partial G_s}{\partial n} \right) \Phi(\rho) \right] dS + \Phi^r(R) + \varepsilon \Phi^{NL}(R) \]  

(11a)

\[ \frac{1}{2} \left( 1 + \frac{1}{\varepsilon} \right) h(R) = \int_S \left[ \left( \frac{\partial G_0}{\partial n} - \frac{1}{\varepsilon} \frac{\partial G_s}{\partial n} \right) h(\rho) - \frac{\partial^2}{\partial n^2} (G_0 - G_s) \Phi(\rho) \right] dS + \frac{\partial}{\partial n} \left\{ \Phi^r(R) + \Phi^{NL}(R) \right\} \]  

(11b)

The first result is obtained by linearly combining (10a) and (10b). The second follows upon forming the normal gradients of (10) (i.e., forming \( \partial \Phi/\partial n_0 = n_1 \cdot \nabla \Phi \)) and linearly combining the results to eliminate the most singular terms. Except for the appearance of the volume integrals, \( \Phi^{NL}(R) \), containing the nonlinear terms, eqs. (11) are completely identical to the ones used in the fast BEM solver for the linear PBE.\(^{38}\) In that work, planar surface elements were used and a piecewise constant approximation adopted for the surface distributions. The discrete representations of (11) using \( N \) boundary elements, \( j \), are\(^{38}\)

\[ \frac{1}{2} \left( 1 + \frac{1}{\varepsilon} \right) \Phi_j = \sum_{j=1}^N A_{1,j} \Phi_j + \sum_{j=1}^N A_{2,j} h_j + \Phi^r(\rho_j) + \varepsilon \Phi^{NL}(\rho_j) \]  

(12a)

\[ \frac{1}{2} \left( 1 + \frac{1}{\varepsilon} \right) h_j = \sum_{j=1}^N A_{3,j} \Phi_j + \sum_{j=1}^N A_{4,j} h_j + \frac{\partial}{\partial n_0} \left\{ \Phi^r(\rho_j) + \Phi^{NL}(\rho_j) \right\} \]  

(12b)

where \( \Phi_j = \Phi(\rho_j) \) and \( h_j = h(\rho_j) \) are at the element centroids. The coefficients, \( A_{1,j} \) to \( A_{4,j} \), are obtained by integrating the terms in (11). For the linear PBE, the 2N surface “strengths” \( \Phi_j \) and \( h_j \) are obtained by inverting the 2N equations in (12) with \( \Phi^{NL}(\rho_j) = 0 \). Fast multipole methods are used to expedite evaluation of the sums appearing in (12) and efficient generalized minimum residual (GMRES)-based iteration procedures applied to perform the inversion. Full details of these steps and methods for numerically evaluating the coefficients are given elsewhere.\(^{38}\)

Appearance of the nonlinear terms in (12) poses two important challenges. The first concerns the efficient and accurate evaluation of the associated singular volume integrals (10d). Because a volume mesh is now required, related issues such as mesh generation and the development of good numerical quadratures must be addressed. The other challenge results from the inherent coupling between the nonlinear terms, \( \Phi^{NL} \), and the surface distributions. Hence, an iterative method is needed that reliably converges both the surface solutions and nonlinear volume source distributions.

The simplest mesh structure is a regular 3D lattice laid over the molecule. Assuming that the nonlinear term, \( \chi(\Phi) \), defined in (7) is constant within each mesh cell, then the volume integral (10d) reduces to the discrete sum:
A mesh containing \( N_v \) grid cells each having volume, \( V_k = \Delta s^3 \) (\( \Delta s \) is the grid spacing). Here, \( \chi_k = \chi(\Phi(\rho_k)) \), where \( \rho_k \) is the center of cell, \( k \). Away from charge sites and the molecular surface, solutions to both the linear and nonlinear PBE vary smoothly. Therefore, rather than investing in the development of complicated quadrature schemes to evaluate the volume integrals in (13), one can adopt a simple regularization technique and approximate (13) as

\[
\Phi_{NL}(\mathbf{r}) = -\sum_{i=1}^{N_v} \chi_i V_i G_c(\mathbf{r}, \rho_i) \]

where for smoothing parameter, \( \sigma \), the “desingularized” Green’s function\(^{68} \) is

\[
G_c(\mathbf{r}, \rho) = \frac{e^{-\kappa r}}{r_\sigma} \left( \frac{r^2 + \frac{3}{2} \sigma^2}{r^2 + \sigma^2} \right), \quad r_\sigma = \sqrt{R - \rho^2 + \sigma^2}
\]

(15a,b)

Formal convergence proofs require that \( \sigma \) be larger than the grid spacing, \( \Delta s \); however, it should not be made much larger than this because \( \sigma \) controls the effective resolution of the solution. From empirical studies, \( \sigma = 1.5 \Delta s \) represents a good choice.

To update the potential solution, (14) must be evaluated at the centers of all \( N_v \) cells and the \( N \) surface boundary elements. Direct evaluation therefore requires \( O(N_v (N_{vol} + N)) \); for a moderate mesh containing \( N_{vol} = 128^3 \) cells the operation count is at least \( 4.4 \times 10^{12} \) operations, which is unacceptable for routine use. To reduce this cost, fast multipole methods are used.\(^{37,38} \) Essentially, to evaluate the nonlinear potential at a point, \( \mathbf{R} \), the mesh cells are first assembled into hierarchical groups with the aid of an octree.\(^{70} \) Next, the octree is searched to distinguish between near- and far-field groups. The contributions from cells in the near-field groups to the nonlinear potential are evaluated directly as indicated in (14). However, the contributions from far-field groups are approximated using multipole expansions. The introduction of a fast multipole algorithm reduces the asymptotic cost of evaluating \( \Phi_{NL} \) at all field points from \( O(N_{vol} (N_{vol} + N)) \) to \( O((N_{vol} + N) \log N_{vol}) \). For \( N_{vol} = 128^3 \), this translates into an \( O(10^5) \) reduction with negligible degradation in accuracy.

It was already indicated that the surface and field potentials are coupled and must be converged simultaneously using an iterative method. Various iteration strategies can be considered, but no attempt was made to exhaustively explore and compare these options. In our work, the following iteration sequence, which minimizes the number of nonlinear field evaluations, was used:

1. Solve the linear problem by setting \( \Phi_{NL} = 0 \) in (12) and obtaining the surface distributions, \( \Phi_i \) and \( h_i \).
2. Starting with the linear potential field, relax to the nonlinear solution by repeating the following steps until neither the surface distributions (\( \Phi_i \) and \( h_i \)) nor field potentials (\( \chi_k \)) exhibit significant change:
   a. From the current estimate of \( \Phi_i \), \( h_i \), and \( \chi_k \), compute the potential at the grid cell centers, \( \rho_k \), over the entire domain using (10b).
   b. Update the nonlinear corrections, \( \chi_k \), at the cell centers, \( \rho_k \).
   c. Using the updated values, calculate the potential, \( \Phi_{NL} \), and its normal gradient, \( \partial \Phi_{NL}/\partial n_{hi} \), at the boundary element points, \( \rho_i \).
3. Conduct postprocessing.

Calculations using this approach were successfully carried out (see Results), but CPU times using the fast integral equation method were typically \( O(100) \) times longer than with the hybrid scheme. This is not surprising because evaluating the potential at a single domain point requires at least \( O(C + \log N + \log N_{vol}) \) evaluations, where \( C \) represents the cost of evaluating the near-field contributions. There are at least 27 near-field grid points so that for \( N_{vol} = 128^3 \) the number of interactions per potential evaluation exceeds 41. Moreover, each of these interactions requires an expensive square root and exponential function evaluation as well as several floating point operations. By comparison, in a FD method implemented upon a regular lattice updating the potential at a point requires only six additions and one multiply. Thus, even with fast multipole acceleration the integral method is at least two orders of magnitude slower than finite differencing. Upon the same grid, one expects each method to offer comparable accuracy because the truncation and boundary errors are similar. Therefore, it appears that fast BEM only remains competitive with FD methods as long as the solution can be completely characterized in terms of surface integrals. Once volume integrals appear, the CPU cost incurred by the fast BEM increases considerably. In all cases involving the nonlinear PBE that we have considered, the computational cost of fast BEM has been considerably higher than for the FD or hybrid approach.

**Hybrid Approach**

The long CPU times incurred when solving the nonlinear PBE by the integral method motivates consideration of alternate approaches. One option is the FD method, which is well suited for addressing problems involving nonlinearities and varying material properties (e.g., dielectric constant). However, FD methods are poor at resolving rapidly varying potentials near point charges. Therefore, a hybrid approach is considered where the fast BEM is used to solve the singular and linear components of the problem.
The linear PBE is given by (8) with $\chi(\Phi) = 0$ and $\kappa$ defined by (6) and is efficiently solved using a fast BEM.\textsuperscript{38} The ion-free layer is also omitted in this form of the linear PBE. Both finite thickness ion-exclusion layers and/or nonlinear behavior are captured by the FD method described below. Denoting the solution to the linear problem by $\Phi^L(R)$ and expressing the full nonlinear solution to (1) as the sum, $\Phi = \Phi^L + \phi$, then it is not hard to see [e.g., by subtracting (8) from (1)] that the correction potential, $\phi(R)$, is governed by

$$\nabla \cdot (\kappa \nabla \Phi) - \nabla \cdot (\kappa \nabla \phi) = \nabla \cdot (\kappa \nabla \phi) = g(\Phi^L, \phi) \quad (16)$$

where the source terms in each domain, $\Omega_i$, are

$$g(\Phi^L, \phi) = 0, \quad R \in \Omega_1 \quad (17a)$$

$$g(\Phi^L, \phi) = f(\Phi^L + \phi) - \kappa \nabla^2 \Phi^L, \quad R \in \Omega_2 \quad (17b)$$

$$g(\Phi^L, \phi) = -\kappa \nabla^2 \Phi^L, \quad R \in \Omega_3 \quad (17c)$$

and the function, $f(\Phi)$, is defined in (4).

Note that the point charges and associated singularities have been removed and the correction potential, $\phi$, over the interior domain, $\Omega_1$, is now governed by a Laplace equation. Hence, one expects that the solution is well behaved and readily reproduced upon a FD grid. Also, the linear problem makes no reference to the nonlinear solution and so can be solved independently. The linear solution, $\Phi^L$, then “drives” the correction potential as indicated in (17b) and (17c). This one-way coupling facilitates the solution strategy because no iteration between the linear and correction solution procedures is necessary. Instead, a two-step solution strategy is adopted where $\Phi^L$ is obtained first using the fast BEM and then $\phi$ is procured by solving (16) upon a FD mesh. Moreover, data structures used in the procurement of the linear solution are available for reuse in the nonlinear calculation.

**FD Approximation**

To solve (16) a cubic lattice of grid points, $\{ R_{ijk} : i, j, k \in [0, n] \}$ is laid over the molecule. The number of mesh points in a direction, $n$, and grid spacing, $\Delta s$, are specified by the user. Next, each grid point is examined to establish whether it lies inside or outside the molecule. For general surfaces and large numbers of grid points, this determination is computationally nontrivial. One option is to march outward from a grid point and count the number of surface crossings (if the number is odd, the point lies inside the molecule). This approach can fail if the surface contains small gaps or if roundoff errors at a crossing location (e.g., if the radiating line lands exactly on an edge) are not handled consistently. Therefore, the inside/outside status is determined on the basis of the solid angle subtended by the surface$^{71}$:

$$\Lambda(R) = -\oint_S \frac{\partial G_0}{\partial n}(R, \phi) dS = \begin{cases} +1, & R \in \Omega_1 \\ 0, & R \in \Omega_2 \cup \Omega_3 \end{cases} \quad (18)$$

This expression can be obtained from (10a) upon setting $h(\rho) = \Phi^L(R) = 0$ and $\Phi(\rho) = 1$. Because the fast multipole method is employed to rapidly evaluate (10a) it is available for the solid angle calculation also. Therefore, the inside/outside status of all mesh points is efficiently determined using the multipole-accelerated implementation of (18).

Next, eq. (10b) (with $\Phi^{NL} = 0$) is employed to evaluate the linear potential, $\Phi^L$, at every exterior grid point using the fast BEM algorithm. The linear potential at interior points is not needed because it does not enter into (16). The correction potential is then obtained by solving the second-order accurate discrete approximation to eq. (16):

$$[\nabla \cdot (\kappa \nabla \phi) - g]_{R = R_a} = \Delta_s \phi_{ijk} - g(\Phi^L_{ijk}, \phi_{ijk}) \quad (19a)$$

$$\Delta_s = e_{i,j-1,k}(\phi_{i,j-1,k} - \phi_{ijk}) + e_{i,j+1,k}(\phi_{i,j+1,k} - \phi_{ijk}) + e_{i-1,j,k}(\phi_{i-1,j,k} - \phi_{ijk}) + e_{i+1,j,k}(\phi_{i+1,j,k} - \phi_{ijk}) + e_{i,j+1,k}(\phi_{i,j+1,k} - \phi_{ijk}) + e_{i,j-1,k}(\phi_{i,j-1,k} - \phi_{ijk}) \quad (19b)$$

This FD approximation is the same as the one originally introduced by Warwicker and Watson\textsuperscript{72} and is both compact (only refers to mesh points that are immediate neighbors of $R_{ijk}$) and second order accurate.

**Outer Boundary Conditions**

By necessity, the mesh used to solve (19) is of finite extent so that the problem of imposing appropriate conditions at the outer boundary must be addressed. Unlike the linear problem, where the Debye–Hückel approximation can legitimately be used, setting outer boundary conditions for $\phi$ (or $\Phi$) is difficult because of the lack of a corresponding simple, uniformly valid formula for the nonlinear potential at a distant point. Moreover, computer results show that $\phi$ and $\Phi$ are negligible only when $\Phi^L$ is negligible (i.e., $\Phi^L \ll 1$). In the absence of a higher-order outer boundary treatment, the only reliable approach is to make the grid sufficiently large and set $\phi = 0$ at the outer boundaries. Fortunately, by evaluating $\Phi^L$ at the outer boundaries one can determine a priori whether the mesh extends sufficiently far to produce accurate results. However, for problems involving highly charged systems at low salt concentrations, where $\Phi^L$ in general extends farthest from the molecule, the grid must be correspondingly large, thus raising computational storage costs.

**Iterative Solution Strategy for $\phi$**

The discrete approximation, (19), represents a nonlinear and sparsely coupled set of algebraic equations to be solved for $\phi_{ijk}$. An iterative approach is necessary and an effective strategy is one that promotes rapid and robust convergence. Developing a reliably convergent iteration sequence can be a challenge because of the physical behavior associated with the ion-exclusion layer and nonlinear terms. The general effect of the ion-free layer is to increase the magnitude of the local potential because there is no screening within this region. Conversely, the nonlinear terms increase the effective local screening as can be readily demonstrated
charged systems. In Gauss–Seidel iteration the mesh points are
removing every other grid point from the previous finer one.
here closely adheres to the standard full approximation scheme
of increasingly coarse meshes. The multigrid strategy adopted
errors at all wavelengths by projecting the solution onto a hierar-
are effectively eliminated by applying Gauss–Seidel at the coarser
errors appear as short wavelength ones upon the coarser grid, they
wavelength errors. On the other hand, these long wavelength errors
number of grid points grows. This is because Gauss–Seidel itera-
tion is much less than its linear counterpart because of the increased

by considering a monovalent salt. In that case linearizing about the
local solution, \( \Phi \)
\[
\kappa^2 \sin h(\Phi + \delta \Phi) - \kappa^2 \cos h(\Phi + \delta \Phi) = 0
\]
shows that the effective local screening parameter, \( \kappa \sqrt{\cos h(\Phi)} \geq \kappa \).
Suppose now that the linear solution has been obtained. For
highly charged biological systems it is not unusual to encounter
regions where [\( \Phi \)'] exceeds \( \Theta(100) \), resulting in numerical over-
flow when evaluating the exponential terms. Ironically, in these
regions the final converged full nonlinear potential is typically
much less than its linear counterpart because of the increased
screening.

The solution procedure utilized here is based upon limited
Gauss–Seidel iteration. Linearizing (19) about the current solution:
\[
[\Delta e \phi \bar{g}]_{\delta \phi} - \left\{ \bar{e}_{ijk} + \frac{\partial g}{\partial \phi} \right\}_{\delta \phi} \Delta \phi_{ijk} = 0
\]
\[
\bar{e}_{ijk} = e_{i-1,j-1,k} + e_{i-1,j,k-1} + e_{i,j,k-1} + e_{i+1,j,k} + e_{i-1,j+1,k} + e_{i,j+1,k} + e_{i,j,k+1}
\]
readily furnishes the local update, \( \Delta \phi_{ijk} \). It is found useful to limit
updates to \( |\Delta \phi_{ijk}| < 1 \), which helps prevent divergence for highly
charged systems. In Gauss–Seidel iteration the mesh points are
visited in succession. For each point, \( i, j, k \), the solution is updated
according to
\[
\phi_{ijk} \leftarrow \phi_{ijk} + \min\{+1, \max\{-1, \Delta \phi_{ijk}\}\}
\]
before proceeding to the next point. This iteration sequence re-
quires little additional storage and smooths solution errors. To
prevent overflows in the exponential evaluations the initial solu-
tion is set to \( \phi = -\Phi' \) in the exterior regions so that \( \Phi = 0 \) and
hence \( f(\Phi) = 0 \) initially.

Gauss–Seidel iteration becomes increasingly inefficient as the
number of grid points grows. This is because Gauss–Seidel itera-
tion is good at damping short wavelength errors (i.e., ones that
vary significantly from point to point) but poor at eliminating long
wavelength errors. On the other hand, these long wavelength errors
can be accurately resolved upon a coarser grid and, because these
errors appear as short wavelength ones upon the coarser grid, they
are effectively eliminated by applying Gauss–Seidel at the coarser
level. This insight motivates multigrid, which attempts to eliminate
errors at all wavelengths by projecting the solution onto a hierar-
chy of increasingly coarse meshes. The multigrid strategy adopted
here closely adheres to the standard full approximation scheme
described in ref. 73. The coarser grids are developed recursively by
removing every other grid point from the previous finer one.
Linear interpolation is used to transfer coarse-level corrections
from coarser to finer levels and a full-weighted approximation
used to transfer the solution and errors from the finer to coarser
grids. Definitions of these operators and additional details of the
multigrid method are given in ref. 73.

Postprocessing
For biopolyelectrolytes immersed in a mixed NaCl and MgCl₂
solution, the total electrostatic energy expression is taken from eq.
(8) of ref. 65. After integration by parts of the last term and
substituting using the governing eq. (4) one obtains the total
electrostatic free energy, \( G^I \), in \( k_B T \) units:
\[
G^I = \left( \frac{(k_B T)^2}{8 \pi e^2} \right) G^I = \left( \frac{(k_B T)^2}{8 \pi e^2} \right) \times \left( G^I_m + G^I_{Na-1} + G^I_{Na-2} + G^I_{Mg-1} + G^I_{Mg-2} \right)
\]
\[
G^I_m = \int_{\Omega_2} \frac{4\pi e}{k_B T} \Phi dV
\]
\[
G^I_{Na-1} = \int_{\Omega_2} \Phi \sinh(\Phi) dV \equiv \tilde{G}^I_{Na} \sum_{\ell=0} \left[ \Psi(\Phi) \sinh(\Phi) \right]_{\ell}
\]
\[
G^I_{Na-2} = -\tilde{G}^I_{Na} \int_{\Omega_2} (2 \cos h \Phi - 2) dV
\]
\[
G^I_{Mg-1} = \frac{\tilde{G}^I_{Mg}}{3} \int_{\Omega_2} \Phi(e^{\phi} - e^{-2\beta}) dV
\]
\[
G^I_{Mg-2} = -\frac{\tilde{G}^I_{Mg}}{3} \int_{\Omega_2} (2e^\phi + e^{-2\beta} - 3) dV
\]
where \( \Phi_{ijk} = \Phi(R_{ijk}) = \Phi_k(R_{ijk}) + \phi(R_{ijk}) \). The electrostatic
energy contribution from the interior region, \( G^I_{int} \) is computed by
summing the product of charge times electrostatic potential at the
fixed charge sites. The electrostatic energy contribution from the
exterior region is approximated by summing over exterior nodes in
\( \Omega_2 \). Equations (24c) and (24e) are the osmotic pressure terms
arising from the NaCl and MgCl₂ salts, respectively. In the linear
solution to the PBE, (24a) is the sole contributor to the total
electrostatic free energy because, by assumption, \( \Phi \) is sufficiently
small in the exterior region that the net contributions from the
other terms, (24b)–(24e), can be neglected (in general, the summed
contribution of these energies is proportional to \( \Phi^3 \); for pure NaCl
this nonlinear energy sum is proportional to \( \Phi^4 \)).
Results

We begin by examining an idealized problem involving a sphere with a central charge to assess overall accuracy and its dependence upon grid spacing, CPU performance, and robust convergence at high charges and/or low ion screening values. The hybrid method is then applied to some highly charged nucleic acid molecules. The objective here is to help identify under what circumstances the retention of nonlinear effects is important for accurately predicting the electrostatic behavior of these highly charged systems.

Sphere Containing a Central Charge

In the first set of test cases the electrostatic properties of a spherical cavity containing a single central charge and immersed in an aqueous salt solution are examined. For all results presented in this article, the ion-exclusion layer is omitted. To obtain the linear PBE solution using the fast BEM, the spherical surface is discretized into a collection of 1280 curvilinear triangular elements and the surface solution (electrostatic potential and normal component of the electric field) is developed by solving (12) with nonlinear contributions omitted. The nonlinear correction is obtained by the FD method using a 65³ regular 3D mesh. The total extent of the 3D uniform Cartesian grid was at least four times the sphere diameter. In most calculations, five multigrid levels were considered to solve the FD equations (19). The nonlinear PBE for a central charge inside a spherical cavity reduces to a second-order ordinary differential equation (ODE) that can be solved by alternative means.

The first study examines solutions to the linear and nonlinear PBE for a centrally located 50e charge inside a 20-Å radius sphere. Such a large net charge is chosen to see the difference between the electrostatic properties obtained with the linear and nonlinear PBE.

Table 1. Reduced Surface Electrostatic Potentials and Electrostatic Free Energies (in Units of k_BT) for a Single Centrally Located Charge of 50e Inside a 20-Å Radius Sphere Obtained With the Nonlinear and Linear PBE Under Different Ionic Strengths (I).

<table>
<thead>
<tr>
<th>I (M)</th>
<th>κ (1/Å)</th>
<th>(\Phi^e(\alpha))</th>
<th>(\Phi(\alpha))</th>
<th>(G_{el,lin}^{\alpha})</th>
<th>(\Delta G_{el}^{\alpha})</th>
<th>(G_{el,Na^{-1}}^{\alpha})</th>
<th>(G_{el,Na^{-2}}^{\alpha})</th>
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<tbody>
<tr>
<td>0.001</td>
<td>0.01040</td>
<td>14.8</td>
<td>8.18</td>
<td>−8394.32</td>
<td>−162.9</td>
<td>108.27</td>
<td>−38.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.8)</td>
<td>(8.28)</td>
<td>(−8392.03)</td>
<td>(−162.7)</td>
<td>(92.25)</td>
<td>(−31.45)</td>
</tr>
<tr>
<td>0.01</td>
<td>0.03287</td>
<td>10.8</td>
<td>5.94</td>
<td>−8494.39</td>
<td>−118.5</td>
<td>75.47</td>
<td>−35.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10.8)</td>
<td>(6.09)</td>
<td>(−8492.29)</td>
<td>(−117.1)</td>
<td>(73.28)</td>
<td>(−33.87)</td>
</tr>
<tr>
<td>0.04</td>
<td>0.06575</td>
<td>7.71</td>
<td>4.67</td>
<td>−8571.11</td>
<td>−73.80</td>
<td>62.56</td>
<td>−35.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.71)</td>
<td>(4.79)</td>
<td>(−8568.79)</td>
<td>(−73.12)</td>
<td>(58.28)</td>
<td>(−32.90)</td>
</tr>
<tr>
<td>0.08</td>
<td>0.09298</td>
<td>6.23</td>
<td>4.02</td>
<td>−8608.19</td>
<td>−53.28</td>
<td>51.72</td>
<td>−32.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.24)</td>
<td>(4.15)</td>
<td>(−8605.52)</td>
<td>(−52.30)</td>
<td>(50.46)</td>
<td>(−31.53)</td>
</tr>
<tr>
<td>0.12</td>
<td>0.1139</td>
<td>5.44</td>
<td>3.66</td>
<td>−8628.38</td>
<td>−42.51</td>
<td>46.54</td>
<td>−31.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.45)</td>
<td>(3.79)</td>
<td>(−8625.43)</td>
<td>(−41.52)</td>
<td>(45.91)</td>
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<td>0.16</td>
<td>0.1315</td>
<td>4.91</td>
<td>3.41</td>
<td>−8641.79</td>
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<td>43.19</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(4.92)</td>
<td>(3.53)</td>
<td>(−8638.65)</td>
<td>(−34.67)</td>
<td>(42.73)</td>
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<tr>
<td>0.20</td>
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<td>4.52</td>
<td>3.21</td>
<td>−8651.61</td>
<td>−30.74</td>
<td>40.74</td>
<td>−29.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.53)</td>
<td>(3.34)</td>
<td>(−8648.34)</td>
<td>(−29.85)</td>
<td>(40.30)</td>
<td>(−28.69)</td>
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<tr>
<td>0.30</td>
<td>0.1801</td>
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<td>2.87</td>
<td>−8668.06</td>
<td>−23.01</td>
<td>36.50</td>
<td>−27.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.88)</td>
<td>(2.99)</td>
<td>(−8664.61)</td>
<td>(−22.22)</td>
<td>(36.01)</td>
<td>(−27.06)</td>
</tr>
<tr>
<td>0.40</td>
<td>0.2079</td>
<td>3.45</td>
<td>2.64</td>
<td>−8678.62</td>
<td>−18.40</td>
<td>33.64</td>
<td>−26.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.46)</td>
<td>(2.69)</td>
<td>(−8675.09)</td>
<td>(−17.70)</td>
<td>(33.07)</td>
<td>(−25.76)</td>
</tr>
<tr>
<td>0.8</td>
<td>0.2940</td>
<td>2.58</td>
<td>2.12</td>
<td>−8700.23</td>
<td>−10.06</td>
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<tr>
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<td></td>
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<td>(2.21)</td>
<td>(−8696.75)</td>
<td>(−9.583)</td>
<td>(26.46)</td>
<td>(−22.27)</td>
</tr>
</tbody>
</table>

The dielectric constant inside and outside the sphere is set to 4 and 78.5, respectively. The temperature of the aqueous salt solution is set to 298 K. The numbers in parentheses represent the 1D solutions obtained with the finite element PBE analysis described in the appendix. One-thousand two-hundred and eighty curvilinear boundary elements were considered and the FD mesh spacing is 2.5 Å.
and nonlinear cylindrical PBE solutions due to the known error in the linearized PBE solution under such conditions. Also, this behavior should hold true even for biomolecules of arbitrary shape and charge distribution.

The net nonlinear electrostatic free energy contribution is reduced at higher salt concentrations. For instance, at 1 mM NaCl the nonlinear contribution to the total electrostatic free energy is 0.7% whereas it is 0.1% at 0.20 M NaCl. This behavior concurs with observations that the nonlinear contribution to the total electrostatic free energies becomes larger with decreasing salt concentration. A third observation is that the nonlinear energy contributions are small compared to the total electrostatic free energy. Thus, for this idealized case anyway, if one is interested in electrostatic free energies only the linear PBE results seem adequate. On the other hand, the surface electrostatic potentials differ significantly, suggesting that nonlinear behavior may be important in docking or molecular recognition applications.

Next, the integral equation and hybrid PBE methods are compared with the 1D PBE results. The configuration is the same as before and the NaCl concentration is fixed at 0.16 M. The nonlinear integral equation method was applied using two different volume grid resolutions: a fine 64³ mesh with \( \Delta x = 1.5 \) Å and a coarser 32³ mesh with \( \Delta x = 3.3 \) Å. The coarse and fine mesh integral equation nonlinear PBE calculations required CPU times of 2600 and 8100 s, respectively. A hybrid mesh calculation was also performed with mesh spacing, \( \Delta x = 3.3 \) Å. This computation required only 43 s to compute both the linear solution and nonlinear corrections using FD. The predicted nonlinear potential in the exterior region is shown in Figure 1. The analytic linear PBE result is also shown for comparison. All three approaches are seen to produce results that are in close agreement. Also, significant deviation from the linear PBE case is noted. Both the integral equation and hybrid schemes produce smoothly varying results that are devoid of the numerical “noise” evident in some previous publications (e.g., Fig. 4 in ref. 44). Thus, the integral equation and hybrid schemes both deliver accurate nonlinear predictions, but the hybrid method is much faster.

The next study examines the behavior of the nonlinear PBE solution and its departure from the linear PBE result as the net charge is increased. The spherical cavity now has a radius of 15 Å and interior dielectric constant of 2. The spherical cavity is represented using 1280 curvilinear boundary elements and embedded in an aqueous salt solution having dielectric constant 78 and temperature of 298 K. The FD mesh used to obtain the nonlinear corrections contains 65³ nodes and has spacing \( \Delta x = 1.875 \) Å (the total extent is 120 Å, which is four times the sphere diameter). Results are shown for NaCl concentration of 30 and 150 mM. The net charge is increased from 1e, where the PBE solution is essentially linear, to 5000e, where nonlinear behavior dominates and the ability of the FD solver to converge the correction potential in a robust manner is put to the test. Figure 2 shows how the ratio, \( \Phi/\Phi^\text{lin} \), at the surface varies with net charge. At low charge values (\( Q < 5e \)) the ratio is close to unity implying that nonlinear effects are weak. This same prediction was made by Holst and coworkers based on the exterior electrostatic potential. At higher net charges, the nonlinear surface potentials are considerably lower than their linear counterparts. Agreement between the results obtained from the 3D hybrid scheme and the 1D solution is sustained up to \( Q = 100e \). At higher charge values, the associated curves begin to deviate. This is due to a combination of outer boundary errors and inadequate grid spacing. As the charge increases, the potential extends further into the exterior domain so that eventually outer boundary potentials become significant unless the boundary is extended further from the molecule. Simultaneously, higher potentials imply higher local screenings and thus exponential decay rates that demand higher grid density for property resolution. Nevertheless, the FD solver converges reliably even at the highest charge values, where converged results are obtained using 10 single-level Gauss–Seidel iterations followed by 70 multigrid cycles. As expected, the ratio \( \Phi/\Phi^\text{lin} \) is closer to 1 in the range of low charge values and at the higher salt concentration.

It is also of interest to examine the variations in various electrostatic free energies as the charge is increased. Equation (24) lists three contributions to the total electrostatic free energy for the monovalent salt considered here (i.e., NaCl). These electrostatic free energy contributions, normalized by \( G^\text{el} \) for the linear problem, \( G^\text{el(lin)}_m \), are recorded in Figure 3. Note that \( G^\text{el(lin)}_m \) can be expressed analytically and the resulting values are in close agreement with the numerical predictions. The reason for normalizing the electrostatic free energy contributions this way is to highlight the relative importance of the various nonlinear contributions. Good agreement between the 1D and hybrid PBE code results is established, attesting to the good predictive capabilities of the hybrid PBE method. The change in normalized reaction field energy, \( \Delta G_m = (G^\text{el(lin)}_m/G^\text{el(lin)}_m) - 1 \), is seen to be negligible at small charge values but to dominate the nonlinear contributions at higher charge values. Also, \( \Delta G_m \) seems to asymptote to a constant value at very high net charges. The opposite trend holds for the other two normalized electrostatic free energy contributions, \( G_{\text{Na}^+} = G^\text{el} / G^\text{el(lin)}_m \) and \( G_{\text{Na}^+} = G^\text{el}/G^\text{el(lin)}_m \), which individually contribute a fixed fraction of total electrostatic energy.
at the low charge range. As net charge increases, however, their relative contributions diminish to zero. Also, because these two electrostatic free energy terms have opposite signs their combined contribution is small over the entire charge range. None of the nonlinear electrostatic free energy contributions seem significant compared to the linear term. For example, the change in reaction field energy due to nonlinear effects is less than 1.4% of the linear reaction field energy. The other two nonlinear electrostatic free energy terms are even smaller and remain less than 0.4% of the linear electrostatic free energy (24a). Finally, nonlinear effects are seen to be more significant in the low-salt region as evidenced by the somewhat higher normalized electrostatic free energy contributions.

The last example considered with the idealized model examines the nonlinear electrostatic behavior when the spherical cavity containing a central charge is embedded in a mixed salt solution containing NaCl and MgCl₂. In particular, the variation in computed surface potential and free energies with salt composition is examined. Curves are developed by varying \( I_{\text{mix}} \) while holding the combination, \( I_{\text{mix}} = I_{1:1} + I_{2:1} = c_{\text{bulk}}^{1:1} + 3c_{\text{bulk}}^{2:1} \) (and hence also the Debye–Hückel screening parameter, \( \kappa \)), constant. Two ionic strengths are considered: \( I_{\text{mix}} = 0.03 \) M and \( I_{\text{mix}} = 0.15 \) M. The interior charge, \( Q = -400e \), sphere radius, \( a = 20 \AA \), and the net charge range is examined.

Figure 2. Charge dependence of the nonlinear/linear surface electrostatic potential ratio for (a) \( I = 0.03 \) M and (b) 0.15 M. Results are obtained using the hybrid scheme and the 1D finite element solution for the case of a spherical cavity with centrally located charge.

Figure 3. Charge dependence of electrostatic free energy ratios for (a) \( I = 0.03 \) M and (b) 0.15 M. Results are obtained using the hybrid scheme and the 1D finite element solution for the case of a spherical cavity with centrally located charge. The plotted electrostatic free energies are normalized by the reaction field energy obtained from the linear PBE (for this model problem there is no Coulombic energy), \( G_{\text{in}}^{\text{el(lin.)}} \), specifically, the change in (normalized) reaction field energy, \( \Delta G_{\text{in}} = \{ G_{\text{in}}^{\text{el}} - G_{\text{in}}^{\text{el(lin.)}} \} / G_{\text{in}}^{\text{el(lin.)}} \); the normalized nonlinear electrostatic free energy contribution \( G_{\text{Na}^{-1}} = G_{\text{Na}^{-1}}^{\text{el}} / G_{\text{in}}^{\text{el(lin.)}} \); and the normalized osmotic pressure contribution, \( G_{\text{Na}^{+2}} = G_{\text{Na}^{+2}}^{\text{el}} / G_{\text{in}}^{\text{el(lin.)}} \).
The interior and exterior dielectric constants are $\varepsilon_1 = 2$ and $\varepsilon_2 = 78$. The electrostatic free energies for the linear problem are similar: $1.108 \times 10^6 k_B T$ (for $I_{\text{mix}} = 0.03$ M) and $1.1138 \times 10^6 k_B T$ (for $I_{\text{mix}} = 0.15$ M). Figure 4 depicts the surface potential and nonlinear contribution to the reaction field energy as the salt composition is varied. The salt composition ranges from pure NaCl ($\varepsilon_{2:1}^{\text{bulk}} = 0$ M) to pure MgCl$_2$ ($\varepsilon_{2:1}^{\text{bulk}} = I_{\text{mix}}/3$). Again, both the 1D results and those obtained with the hybrid scheme are shown. These results are seen to be in good agreement with maximum relative errors occurring at the lower salt concentration and pure MgCl$_2$. These differences are attributable to errors at the outer boundary and finite resolution. The nonlinear surface potentials shown in Figure 4(a) differ considerably from their linear counterparts: $\Phi_{\text{lin}}(a) = -67.1 k_B T/e$ (for $I_{\text{mix}} = 0.03$ M) and $\Phi_{\text{lin}}(a) = -40.4 k_B T/e$ (for $I_{\text{mix}} = 0.15$ M). Thus, the nonlinear surface potentials are approximately one-tenth of the linear values. Despite the large reduction in surface potentials when nonlinear effects are included, the energy changes are small. The nonlinear contributions to the reaction field energy recorded in Figure 4(b) are less than 1% of the linear contribution. This energy increases by about $800 k_B T$ as the salt composition is changed from pure NaCl to pure MgCl$_2$, which again is small compared to the total energy. The same observations apply to the other energy measures recorded in Figure 5 except that the energy values are in general even smaller but the variations with salt concentration more pronounced (although net energies obtained by summing the four energy contributions show weaker variations due to cancellation). Although the nonlinear energy contributions are small compared to the linear component, they are wholly responsible for any variations in energy as the salt composition is varied. This is because for fixed $I_{\text{mix}}$ (and hence $\kappa$) the linear reaction field energy is constant. Thus, changes in salt composition are only reflected in the nonlinear free energy contributions. Summing all nonlinear contributions to the total electrostatic free energy, one obtains that the increase in $|G^{\text{el}} - G^{\text{el}}|$. 

Figure 4. Surface electrostatic potential and nonlinear contribution to reaction field energy for sphere, with $-400e$ central net charge, embedded in NaCl/MgCl$_2$ salt mixture.

Figure 5. Nonlinear electrostatic free energy contributions for sphere with central $-400e$ net charge embedded in NaCl/MgCl$_2$ salt mixture.
gies are not very sensitive to the grid resolution. Rather coarse 3D mesh grid resolutions can be used in the FD calculation because the electrostatic free energy of an ideal d(CGCGAATTCGCG)₂ B-DNA dodecamer. The electrostatic potential near the surface of calf-thymus DNA has been measured experimentally. To assess the accuracy of the PBE for nucleic acids Hecht and coworkers compared the electrostatic potentials of different models of DNA obtained with the linear and nonlinear PBE against the measured electrostatic potentials obtained using electron–electron double-resonance spectroscopy. This study showed that the electrostatic potentials obtained using the nonlinear PBE are in much better agreement with the experimentally measured potentials. As depicted in Figure 6 the linear PBE results are significantly different—electrostatic potentials are more negative and respond more strongly to changes in ionic strength—than the nonlinear PBE results. These results are in good qualitative agreement with the Hecht et al. study.

Next, we examine the salt dependence of the ion contribution of the electrostatic solvation free energy of an idealized d(A)₁₂–d(T)₁₂ B-DNA fragment using the linear and nonlinear PBE. The salt contribution to the electrostatic solvation free energy is defined as the difference between the electrostatic solvation free energy at finite and zero salt concentrations, \( \Delta G_{\text{solv}} = \Delta G_{\text{solv}}(c_2, \kappa) - \Delta G_{\text{solv}}(c_2, 0) \). As shown in Figure 7, the salt dependencies of \( \Delta G_{\text{solv}} \) predicted by the linear and nonlinear PBE are different, with the linear PBE predicting a stronger variation with the ionic strength of the solution. These differences are most pronounced in the low-salt regime but remain significant at the higher salt concentrations.

We now consider the effect of mixed 1:1 and 2:1 salts on the salt dependence of the ion contribution to the electrostatic free energy of the dA₁₂–dT₁₂ B-DNA dodecamer. In Figure 8(a) the effect of added 2:1 salt concentration \( c_{2,1} \) on the \( G_{\text{el}} \) vs. \( c_{1,1} \) bulk behavior is shown. At \( c_{2,1} = 0 \), a linear dependence of \( G_{\text{el}} \) on log(\( c_{1,1} \) bulk) is observed whereas as \( c_{2,1} \) is increased this behavior becomes increasingly nonlinear. With increasing amounts of magnesium ions \( G_{\text{el}} \) decreases, especially at the low NaCl concentration end. At the highest MgCl₂ concentration, \( c_{2,1} = 0.1 \) M, \( G_{\text{el}} \) is essentially independent of the NaCl concentration because the electrostatic free energy is now dominated by the fixed concentration of the divalent ions. As \( c_{2,1} \) increases the \( G_{\text{el}} \) curves converge to the pure 1:1 salt \( G_{\text{el}} \) curve. The observed curvature in the plots is a manifestation of the competing effects of the monovalent and divalent ions. Figure 8(b) shows the \( G_{\text{el}} \) vs. \( c_{2,1} \) bulk behaviors at various 1:1 salt concentrations for the dA₁₂–dT₁₂ B-DNA dodecamer. Here, increasing amounts of monovalent salt

![Image](https://example.com/image.png)

**Figure 6.** Electrostatic potential 14 Å from the helical axis of the d(CGCGAATTCGCG)₂ B-DNA based on the linear and nonlinear solutions of the PBE. The solute and solvent dielectric constants are fixed at 1 and 80, respectively. The electrostatic potentials were evaluated in the plane passing through the position of the middle phosphorus atom (residue 6) of the B-DNA.

**Figure 7.** Salt contribution to the electrostatic solvation free energy of d(A)₁₂–d(T)₁₂ B-DNA as a function of the ionic strength, \( I_{1,1} \), of the NaCl solution. Both the linear and nonlinear PBE were employed. The solute and solvent dielectric constant are fixed at 2 and 80, respectively.

**Electrostatic Properties of B-DNA**

Due to the highly charged nature of nucleic acids the electrostatic properties of nucleic acids are strongly dependent on the ionic strength of the solution. Here, we examine the salt dependence of different electrostatic properties of ideal 12 base pair (bp) B-DNA fragments. The atomic coordinates of the ideal d(CGCGAATTCGCG)₂; d(A)₁₂–d(T)₁₂ B-DNA oligonucleotides are based on the fiber diffraction model and were generated with the 3DNA program. The hydrogen atoms were added to the DNA structure and satisfy stereochemistry requirements. The OPLS charges and radii were employed and the solute and solvent dielectric constant were fixed at 1 (or 2) and 80, respectively. The temperature of the solution was 298 K. The MSMS program was employed to triangulate the solvent-excluded molecular surface with a probe radius of 1.4 Å and thus develop the BEs (flat triangles) used in the linear PBE solver. Rather coarse 3D mesh grid resolutions can be used in the FD calculation because the electrostatic free energies are not very sensitive to the grid resolution.

We first examine the ionic strength dependence of the exterior electrostatic potential of an ideal d(CGCGAATTCGCG)₂ B-DNA dodecamer. The electrostatic potential near the surface of calf-thymus DNA has been measured experimentally. To assess the accuracy of the PBE for nucleic acids Hecht and coworkers compared the electrostatic potentials of different models of DNA obtained with the linear and nonlinear PBE against the measured electrostatic potentials obtained using electron–electron double-resonance spectroscopy. This study showed that the electrostatic potentials obtained using the nonlinear PBE are in much better agreement with the experimentally measured potentials. As depicted in Figure 6 the linear PBE results are significantly different—electrostatic potentials are more negative and respond more strongly to changes in ionic strength—than the nonlinear PBE results. These results are in good qualitative agreement with the Hecht et al. study.

Next, we examine the salt dependence of the ion contribution of the electrostatic solvation free energy of an idealized d(A)₁₂–d(T)₁₂ B-DNA fragment using the linear and nonlinear PBE. The salt contribution to the electrostatic solvation free energy is defined as the difference between the electrostatic solvation free energy at finite and zero salt concentrations, \( \Delta G_{\text{solv}} = \Delta G_{\text{solv}}(c_2, \kappa) - \Delta G_{\text{solv}}(c_2, 0) \). As shown in Figure 7, the salt dependencies of \( \Delta G_{\text{solv}} \) predicted by the linear and nonlinear PBE are different, with the linear PBE predicting a stronger variation with the ionic strength of the solution. These differences are most pronounced in the low-salt regime but remain significant at the higher salt concentrations.

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MgCl$_2$. The solute and solvent dielectric constants are fixed at 2 and 80, respectively, and the temperature of the solution is 298 K. (b) Salt dependence of the ion contribution to the electrostatic free energy with varying concentration of NaCl. The solvent-excluded surface is used to represent the dielectric interface that separates the solute and solvent regions. This surface was triangulated with the MSMS algorithm, resulting in 120,000 elements for the larger 30 bp DNA molecules.

The atomic coordinates of the Dickerson–Drew dodecamer B-DNA structure [d(CGCGAATTCGCG)]$_2$ were taken from the RCSB Protein Data Bank (PDB entry code: 1BNA). The Arnott X-ray fiber diffraction RNA triplex 2r(U)$_{15}$–r(A)$_{15}$ and RNA duplex r(U)$_{15}$–r(A)$_{15}$ structures were obtained using the 3DNA program. Hydrogen atoms were added and the AMBER (or CHARMM) atomic charges were used. For the Dickerson–Drew B-DNA dodecamer the AMBER charges were considered whereas for the RNA duplex and triplex structures the CHARMM charges were adopted. PARSE-like atomic radii (in Å) were employed: H = 1.0, C = 1.7, N = 1.5, O = 1.4, and P = 2.0.

In Figure 9 the surface electrostatic potential of the Dickerson–Drew B-DNA dodecamer obtained by solving the linear and nonlinear PBE is displayed. In both cases, the region of most negative potential is in the minor groove. However, the electrostatic potential is significantly reduced when the nonlinear PBE is invoked. The larger negative electrostatic potential predicted by the linear PBE results in an overestimation of the counterion concentration in the grooves. The minimum electrostatic potential obtained with the linear PBE is a factor of 1.2 smaller than the minimum nonlinear electrostatic potential.

The narrow minor groove of this B-DNA molecule contains an extended region of negative potential (see Fig. 10) due to the close approach of the phosphate groups across the groove. This result is in good agreement with the nonlinear PBE calculations performed by Young and coworkers using the DelPhi program. As shown in Figure 10, there are also patches of negative potential in the major groove and in the sugar–phosphate backbone. However, our results (see Fig. 9) show that the region of most negative potential is located in the grooves as opposed to the phosphodiester backbone (this effect is not captured by the formal charge model, where only the phosphate groups have a net $-1e$ charge; in this case the region of most negative potential is along the sugar–phosphate backbone; results not shown). This same qualitative prediction was made earlier by two different groups using Coulombic potentials. Many experimental studies (e.g., NMR, X-ray crystallography and magnetic relaxation dispersion) show that the minor groove in A-tracts (or AT-tracts), the Dickerson–Drew B-DNA structure decreases $\Delta G^{	ext{el}}$. Also, the convergence of the $\Delta G^{	ext{el}}$ curves with increasing MgCl$_2$ is observed. However, in contrast to the behavior shown in Figure 8(a), the curvature effects due to the competition of the monovalent and divalent ions are significantly smaller. Only at the highest NaCl concentration does one observe a slight curvature in the $\Delta G^{	ext{el}}$ curve with decreasing MgCl$_2$ concentration.

Thus, here the competition effects between monovalent and divalent counterions are much less pronounced due to the smaller charge of the counterion. Our results are in good agreement with the study of Chen and Honig of mixed salt effects on the ligand–DNA electrostatic binding free energies as well by the Sharp study on a charged cylinder in mixed salt conditions.

**Surface Electrostatic Potential of Nucleic Acids**

The accurate and fast calculation of surface electrostatic potential maps for nucleic acids is crucial to understand molecular recognition events. Also, surface electrostatic potential maps are widely used to determine potential metal ion binding sites in nucleic acids. In contrast to proteins, nucleic acids contain solely negatively charged residues. However, the resulting electrostatic potentials of nucleic acids, based on either formal (charges on phosphate groups only) or all-atom partial charges, are far from homogeneous and are sensitive to base sequence effects (for all-atom partial charge models) and different structural motifs.

In all the calculations here reported the solute and solvent dielectric constants are 2 and 80, respectively. The temperature of the solution is 298 K and the ionic strength of the solution is 0.1 M NaCl. The solvent-excluded surface is used to represent the dielectric interface that separates the solute and solvent regions. This surface was triangulated with the MSMS algorithm, resulting in 120,000 elements for the larger 30 bp DNA molecules.

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In Figure 9 the surface electrostatic potential of the Dickerson–Drew B-DNA dodecamer obtained by solving the linear and nonlinear PBE is displayed. In both cases, the region of most negative potential is in the minor groove. However, the electrostatic potential is significantly reduced when the nonlinear PBE is invoked. The larger negative electrostatic potential predicted by the linear PBE results in an overestimation of the counterion concentration in the grooves. The minimum electrostatic potential obtained with the linear PBE is a factor of 1.2 smaller than the minimum nonlinear electrostatic potential.

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Many experimental studies (e.g., NMR, X-ray crystallography and magnetic relaxation dispersion) show that the minor groove in A-tracts (or AT-tracts), the Dickerson–Drew B-DNA structure decreases $\Delta G^{	ext{el}}$. Also, the convergence of the $\Delta G^{	ext{el}}$ curves with increasing MgCl$_2$ is observed. However, in contrast to the behavior shown in Figure 8(a), the curvature effects due to the competition of the monovalent and divalent ions are significantly smaller. Only at the highest NaCl concentration does one observe a slight curvature in the $\Delta G^{	ext{el}}$ curve with decreasing MgCl$_2$ concentration.

Thus, here the competition effects between monovalent and divalent counterions are much less pronounced due to the smaller charge of the counterion. Our results are in good agreement with the study of Chen and Honig of mixed salt effects on the ligand–DNA electrostatic binding free energies as well by the Sharp study on a charged cylinder in mixed salt conditions.

Thus, as pointed out by Sharp for the nonlinear PBE the electrostatic free energy does not depend only on the ionic strength but also on the ion composition. Our results and those of others agree with the experimental observation that divalent ions, such as magnesium, reduce the effects of monovalent salt concentration on thermodynamic properties of nucleic acids (ref. 46 and references therein).
Figure 9. Surface electrostatic potential of the Dickerson–Drew B-DNA dodecamer according to the (a) linear PBE and (b) nonlinear PBE, respectively. The electrostatic potential ranges from yellow → red → white → blue → green where yellow and red are negative potential, white is neutral, and blue and green are positive potential. The electrostatic potential range is: $-11.8 \text{kT/e}$ (yellow) to $+1.7 \text{kT/e}$ (green).

Figure 10. Two different views of electrostatic potential of the classical Dickerson–Drew B-DNA dodecamer mapped on the solvent-excluded surface. (a) Colors for the electrostatic potential follow the GRASP convention (red, white, and blue) with the electrostatic potential ranging from $-8.4 \text{kT/e}$ (red) to $1.7 \text{kT/e}$ (blue). (b) Red represents the electrostatic potential in the range $(-15.7, -3.4) \text{kT/e}$ and white represents the range, $(-3.4, +4.4) \text{kT/e}$. 
being one example, is a counterion-binding region. Moreover, all-atom molecular dynamics simulations of the classical Dickerson–Drew B-DNA dodecamer also show a continuous well-defined counterion density around the minor groove and density patches in the major groove.\textsuperscript{86} Our results and those of others all agree that the minor groove of the classical Dickerson–Drew B-DNA dodecamer is a region of high electrostatic potential and thus a region of high counterion concentration. An analysis of other NMR and X-ray crystallographic A-tract nucleic acid structures (work in progress) will reveal if the minor groove of all these structures is indeed the region of most negative potential and thus a unique potential binding site for ions or other positively charged molecules.

As shown in Figure 11 the association of the third strand in the major groove of triplex RNA creates a region of high negative potential due to the close approach of the phosphate groups. Thus, the well-defined region of high negative potential, shown Figure 11(b), is a potential ion binding region. Also, the electrostatic potential of the triplex is more negative compared to the duplex RNA structure, thus suggesting that more counterions will be associated with the triplex as opposed to the duplex structure of the same length. This is due to the larger charge density of the triplex relative to the duplex RNA structure of the same length. For the A-RNA duplex structure the electrostatic potential is more negative in the deep major groove, especially near the phosphodiester backbone and in the outer mouth of the major groove. However, there are regions of negative potential in the minor groove as well [see Fig. 11(c)]. These latter results are in good qualitative agreement with other published results for RNA duplexes that adopt the A-form.\textsuperscript{81,92}

Last, we examine the surface electrostatic potential of the different canonical forms of DNA: A-, B-, and Z-DNA. For these calculations 30 bp oligomeric structures were considered. For A- and B-DNA, the d(A)\textsubscript{30}–d(T)\textsubscript{30} sequence was considered whereas for Z-DNA, the d(CG)\textsubscript{15}–d(CG)\textsubscript{15} fragment was considered. These represent ideal conformations of DNA obtained with the 3DNA program. The atomic charges were taken from the AMBER force field and PARSE atomic radii were employed. Figure 12 compares the surface electrostatic potential of the different canonical forms of DNA. As observed by others the electrostatic potential of B-DNA is more negative in the minor groove, whereas the region of most negative potential in A-DNA occurs in the deep major groove. On the other hand, in Z-DNA the region of most negative potential is in the deep minor groove and the convex major groove contains patches of positive potential. Similar qualitative observations have been made by other groups using different theoretical approaches (e.g., refs. 81, 93, and 94). Due to the higher charge density of A- and Z-DNA relative to B-DNA the electrostatic potential is more negative for these DNA structures.

\textit{Comparison of Hybrid PBE Results with Those Obtained with FD PBE Solvers}

We first compare the electrostatic free energy for the sphere model at different salt concentrations obtained by the hybrid PBE scheme and the FD-based DelPhi code with the 1D nonlinear PBE solution results. The interior of the sphere was set to a dielectric constant of 2 and the solvent to 80. The sphere radius is 20 Å and the net central charge is +50e. The solution temperature is 298 K. The same hybrid PBE code parameters described above are assumed except that the spherical surface is panelized into 320 curvilinear triangular elements. Moreover, the nonlinear correction was obtained by the FD approach based on 49\textsuperscript{3}, 65\textsuperscript{3}, or 129\textsuperscript{3} cubical lattices.

In the DelPhi\textsuperscript{22} (version 4.0, release 1.0) calculations the “per-nil” parameter, which describes the percentage of the solute’s longest linear dimension to the lattice linear dimension, was set to either 20 or 40% and the grid sizes are 129\textsuperscript{3} or 257\textsuperscript{3}. For the
DelPhi computations the potentials at the lattice points on the boundary are approximated analytically using the Debye–Hückel equation (“dipolar” option). The relaxation parameter was set to 1.0 for the +500e sphere model and had to be reduced to values as small as 0.005 to obtain converged results for the +500e sphere case. Such small relaxation parameters slow the convergence process significantly. To improve the accuracy of the DelPhi results a four-step focusing procedure was employed for a 129^3 grid. In the first and second focusing steps the linear PBE was used to improve the convergence behavior of the solution and the nonlinear PBE was adopted in the last two steps. In the initial calculation, the potentials at the boundary points of the grid are approximated analytically using the Debye–Hückel equation (“Coulombic” option) and the solute encompasses only 0.5% of the 3D lattice. The boundary potentials in the three steps in which the grid is made four times finer (perfil = 2.5, 10, and 40%) are interpolated from the previous step.

As observed in Table 2, the hybrid 3D PBE results are closer to the 1D results than the DelPhi results. This is true even when relatively coarse grids are used for the hybrid analysis. It should be pointed out that even at such moderately charged systems at low salt the outer boundary errors can be significant and can lead to inaccurate electrostatic properties.

For the more highly charged model, +500e sphere, our results shown in Table 3 remain accurate even at rather low 3D grid resolution. Again our results are closer to the 1D solution than DelPhi. The hybrid PBE calculation takes 24 and 14 s, at low and high salt concentration, respectively. Thus, there is little CPU overhead to obtain converged and reliable hybrid PBE results for the more highly charged systems under low salt conditions. Low relaxation parameters are needed in DelPhi to successfully converge solutions; consequently thousands of Gauss–Seidel iterations are required to complete the calculation. The DelPhi calculation at 0.001 M NaCl takes 3162 s (a calculation at zero salt concentration is required to obtain the salt contribution to the reaction field energy) when focusing is applied. Also, considerable user intervention was necessary to pick optimal DelPhi code parameters for successful convergence.

The potentials calculated with FD methods depend on the boundary potential. Because the boundary potentials are computed with the linear approximation of the Debye–Hückel, it is important to keep the boundary away from the source of the potential. As shown above this is especially important in the case of highly charged systems at low salt concentrations. In such cases the potential does not attenuate to zero at the boundary of the grid and one needs to apply focusing techniques starting from very low grid filling. A discussion of convergence for highly charged systems using DelPhi is presented elsewhere.22

The osmotic free energy term is directly related to the variation of the electrostatic free energy with salt concentration. Thus, to accurately predict salt dependencies of the electrostatic binding free energies, melting temperatures, and other electrostatic free energy-derived properties requires either precise total electrostatic free energies or osmotic pressure terms. For the 50e sphere at 0.001 M NaCl, relative errors in the computation of the osmotic pressure and electrostatic stress terms are around 2% for the hybrid PBE scheme with a 129^3 grid. With DelPhi the calculations of such free energy terms have larger relative errors compared to the hybrid scheme, but focusing reduces the relative errors substantially. For this same model problem, the DelPhi relative errors for the osmotic pressure and electrostatic stress terms are 36 and 90%, respectively. When focusing is applied the relative errors reduce to 10 and 52% for the osmotic pressure and electrostatic stress terms, respectively. For the 500e sphere at 0.001 M NaCl, the relative errors in the computed osmotic pressure and electrostatic stress terms are 1 and 15%, respectively, when using the hybrid PBE code (129^3 grid). On the other hand, even with focusing the corresponding relative errors for this highly charged case exceed 100% in the FD PBE solution. We suspect that these large discrepancies in the computations of the individual nonlinear free energy terms using DelPhi are due to outer boundary errors.

In summary, as shown in previous sections, the hybrid PBE scheme accurately predicts the correct salt dependence of the electrostatic free energies and the individual nonlinear free energy terms (i.e., osmotic pressure term) even at coarse grid resolutions, which is important when studying salt effects on conformational stability, folding, and binding energetics of nucleic acids and other highly charged systems. It also converges reliably and rapidly even at very high net charges and/or low salt concentration, thus increasing its utility in the study of salt effects in biomolecular systems.

Next, we compare the electrostatic solvation free energy of idealized A- and B-RNA (CCAAGUGGGG) at 0.1 M NaCl.

**Figure 12.** Surface electrostatic potential of 30 base pairs (a) B-DNA, (b) A-DNA, and (c) Z-DNA, respectively. The colors follow the GRASP convention (red, white, and blue) with the electrostatic potential ranging from $-5.1 \ kT/e$ (red) to $+0.8 \ kT/e$ (blue). The solute and solvent dielectric constants are 2 and 80, respectively. The ionic strength of the NaCl solution is 0.1 M. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
obtained with the hybrid nonlinear PBE scheme and the UHBD code. Partial charges and radii on each atom were taken from the AMBER force field. The solute and solvent regions are assigned a dielectric constant of 1 and 78.5, respectively. In the first hybrid 3D PBE run the mesh spacing of the regular lattice used in the finite difference nonlinear correction calculation ranges between 2.3 and 2.8 Å, whereas in the second run it is reduced by a factor of 1.75, with a final grid spacing ranging from 1.3 to 1.6 Å. The temperature of the solution is fixed at 298 K. To assure that adequate surface mesh resolution is achieved mesh adaptation is invoked and the surface resolution is around 40,000 boundary elements for the nucleic acid molecules here studied. For the UHBD calculations a two-step focusing protocol is employed. In the UHBD1 run the grid size and grid spacing is 553 and 1.6 Å and 1103 and 0.4 Å in the first and second runs, respectively. On the other hand, in the UHBD2 run the grid size and grid spacing is 553 and 1.6 Å and 1403 and 0.32 Å in the first and second runs, respectively. An ion-exclusion radius of 2 Å was used in all the UHBD runs because convergence of the nonlinear PBE solution was not obtained in the absence of this region. The boundary potential is set according to a Debye–Hückel approach and dielectric boundary smoothing was not invoked.

As shown in Table 4, neither the absolute nor relative electrostatic solvation free energies appear sensitive to the FD grid resolution in the hybrid scheme. Moreover, coarse FD grids lead to accurate electrostatic solvation free energies. The relative electrostatic solvation free energies obtained at the different grid resolutions differ by less than 0.1%. UHBD results show higher sensitivity to very small changes in grid resolution and finer FD grids are probably required to eliminate these fluctuations. However, the relative electrostatic solvation free energies for these small RNA fragments obtained with the UHBD program at the two different

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<tr>
<td>G0(0.05)</td>
<td>−17381.61</td>
<td>−17390.03</td>
</tr>
<tr>
<td>G0(0.1)</td>
<td>−17403.28</td>
<td>−17411.29</td>
</tr>
<tr>
<td>G0(0.005) − G0(0.001)</td>
<td>−51.36</td>
<td>−51.94</td>
</tr>
<tr>
<td>G0(0.1) − G0(0.05)</td>
<td>−26.17</td>
<td>−21.26</td>
</tr>
</tbody>
</table>

The dielectric constant inside and outside the sphere is set to 2 and 80, respectively. The temperature of the aqueous salt solution is set to 298 K. The 1D solutions were obtained with the finite element nonlinear PBE analysis described in the appendix. Three-hundred and twenty curvilinear boundary elements were considered in the hybrid 3D PBE scheme.

The nonlinear PBE is considered in these studies.

| Table 2. Comparison of the Electrostatic Free Energies, G0, (in Units of k_BT) For a Single Centrally Located Charge of +50e Inside a 20-Å Radius Sphere Obtained With the Hybrid 3D Nonlinear PBE Scheme and the FD-based DelPhi Code at Different NaCl Concentrations. |
|---|---|---|---|---|
| Hybrid PBE | DelPhi | DelPhi |
| G0(0.001) | −174054.1 | −174504.68 | −1615110.4 | −1715435.1 | −1746724.1 |
| G0(0.005) | −1741013.0 | −1745780.9 | −1708572.6 | — | −1747974.2 |
| G0(0.01) | −1741468.0 | −1746113.4 | −1721237.5 | — | −1747830.6 |
| G0(0.05) | −1742836.5 | −1746909.9 | −1732096.5 | — | −1748603.7 |
| G0(0.1) | −1743616.5 | −1747281.1 | −1735574.0 | — | −1748934.6 |
| G0(0.005) − G0(0.001) | −858.9 | −734.1 | −93462.2 | −773.1 |
| G0(0.1) − G0(0.05) | −780.0 | −371.2 | −3477.5 | −330.9 |

The dielectric constant inside and outside the sphere is set to 2 and 80, respectively. The temperature of the aqueous salt solution is set to 298 K. The 1D solutions were obtained with the finite element nonlinear PBE analysis described in the appendix. Three-hundred and twenty curvilinear boundary elements were considered in the hybrid 3D PBE scheme. The nonlinear PBE is considered in these studies.
and fine grid resolutions differ by less than 1%, which is adequate for most applications. As observed for the model sphere problem the hybrid 3D nonlinear PBE scheme provides accurate results with rather coarse FD grids. This will be an advantage when treating larger biomolecular systems. Thus, the hybrid PBE scheme should be useful when analyzing the molecular dynamics trajectories of large and highly charged biomolecular systems using the molecular mechanics/Poisson–Boltzmann solvent accessible (MM/PBSA) model.95

Conclusions

This article describes a hybrid approach that combines the intrinsic advantages of a BEM and FD scheme for solving the nonlinear 3D PBE for arbitrary complex-shaped biopolyelectrolytes immersed in either single or mixed salt solutions. An integral equation-based nonlinear PBE algorithm is also presented. The accuracy of both 3D nonlinear PBE codes was verified using a fast finite element-nonlinear PBE algorithm is also presented. The accuracy of both in either single or mixed salt solutions. An integral equation-based PBE for arbitrary complex-shaped biopolyelectrolytes immersed over a large range of salt concentration and for highly charged systems will allow reliable and faster predictions of salt effects on binding, folding, and conformational/thermal stability of highly charged biomolecular systems. The computed electrostatic properties of nucleic acids are in good agreement with similar published results.

Acknowledgments

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Appendix: Solution to the 1D Nonlinear PBE Equation with Spherical Symmetry

The solution to the nonlinear PBE for a spherical cavity containing a centrally located charge is useful for validation of the full 3D numerical method. A closed-form analytic result for the 1D problem does not seem available. Zhou44 developed a numerical solution to the 1D problem based upon the integral form of the solution. Here, an alternate finite element approximation is presented that is as accurate but considerably faster.

In the exterior region, the 1D form of (16) becomes

$$\frac{d}{dr} \left( r^2 \frac{d\Phi}{dr} \right) = \frac{r^2}{\varepsilon_2} g(\Phi^L, \Phi)$$  \hspace{1cm} (A1)

where $\Phi$ is again the correction potential, $r$ is the radial distance, and $g(\Phi^L, \Phi)$ is the nonlinear source term. Formally, (A1) applies everywhere except at the charge site and across the dielectric boundary. The full potential is obtained by adding the correction to the linear potential:

$$\Phi^L(r) = \left( \frac{q}{4\pi} \right) \frac{1}{a\varepsilon_2(1 + \kappa a)} + \frac{1}{\varepsilon_1} \left( \frac{1}{r} - \frac{1}{a} \right), \quad r < a$$  \hspace{1cm} (A2a)

$$\Phi^L(r) = \frac{q e^{-r/a}}{4\pi r \varepsilon_2 (1 + \kappa a)}, \quad r > a$$  \hspace{1cm} (A2b)

where $a$ is the spherical cavity radius. At the surface, the potential gradient

$$\left. \frac{\partial \Phi}{\partial n} \right|_{r=a} = -\frac{q}{4\pi \varepsilon_2 a^2}$$  \hspace{1cm} (A3)

Because this relation holds for both the linear and full nonlinear potentials it follows that for the correction potential

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Table 4. Comparison of the Electrostatic Solvation Free Energies, $\Delta G_{\text{solv}}^{\text{el}}$, in kcal/mol, of A and B Forms of RNA r(CCAACGUUGG) 2 at 0.1 M NaCl.

<table>
<thead>
<tr>
<th>PBE program</th>
<th>A-DNA</th>
<th>B-DNA</th>
<th>$\Delta G_{\text{solv}}^{\text{el}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid scheme run 1</td>
<td>-4978.60</td>
<td>-4748.85</td>
<td>-229.75</td>
</tr>
<tr>
<td>Hybrid scheme run 2</td>
<td>-4978.59</td>
<td>-4749.02</td>
<td>-229.57</td>
</tr>
<tr>
<td>UHBD1</td>
<td>-4963.65</td>
<td>-4740.93</td>
<td>-222.72</td>
</tr>
<tr>
<td>UHBD2</td>
<td>-4949.84</td>
<td>-4727.86</td>
<td>-221.98</td>
</tr>
</tbody>
</table>

The solute and solvent dielectric constants are 1 and 78.5, respectively. For an explanation of the protocol used in the different PBE runs, see the main text.
The discrete approximation to (A1) is obtained by substituting for the potential solution, \( \Phi \), over each element is approximated by a collection of interpolation functions. Here, a linear variation (A4). At the outer boundary, \( \Phi \) vanishes everywhere. Adopting a Galerkin formulation, the inter-element results in the following matrix equation for element, \( i \):

\[
- [K_i] \left[ \phi_{i-1} \right] = \left[ \frac{s_{i,1}}{s_{i,2}} \right]
\]  

The right side is obtained by evaluating the source terms, \( g(\Phi^L, \Phi) \), at the element nodes and assuming a linear variation in \( g(\Phi^L, \Phi) \) over the element. Note that in general the source terms depend upon \( \Phi^L \) [evaluated from (A2)] and the nodal values of the potential correction, \( \phi \). The final step is to assemble the element equations. This results in a global equation set having the form

\[
[K_G] \{ \phi \} = \{ s(\Phi^L, \Phi) \}
\]  

The references provide additional context and support the theoretical framework discussed in the text. The authors acknowledge the contributions from various sources, including journals and books, which are listed at the end of the document. These references are essential for further exploration into the topic of computational chemistry and the methods discussed in the paper.