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ABSTRACT
Over the last several decades, a range of experimental techniques from x-ray crystallography and atomic force microscopy to nuclear magnetic resonance and small angle x-ray scattering have probed nucleic acid structure and conformation with high resolution both in the condensed state and in solution. We present a computational study that examines the prospect of using electrostatic free energy measurements to detect 3D conformational properties of nucleic acid molecules in solution. As an example, we consider the conformational difference between A- and B-form double helices whose structures differ in the values of two key parameters—the helical radius and rise per basepair. Mapping the double helix onto a smooth charged cylinder reveals that electrostatic free energies for molecular helices can, indeed, be described by two parameters: the axial charge spacing and the radius of a corresponding equivalent cylinder. We show that electrostatic free energies are also sensitive to the local structure of the molecular interface with the surrounding electrolyte. A free energy measurement accuracy of 1%, achievable using the escape time electrometry (ETe) technique, could be expected to offer a measurement precision on the radius of the double helix of approximately 1 Å. Electrostatic free energy measurements may, therefore, not only provide information on the structure and conformation of biomolecules but could also shed light on the interfacial hydration layer and the size and arrangement of counterions at the molecular interface in solution.

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I. INTRODUCTION
Biomolecules, such as proteins and nucleic acids, in solution carry electrical charge, which plays a defining role in their thermodynamic properties and interactions. Modeling the electrostatics of proteins and nucleic acid molecules in solution has, therefore, attracted great interest in the field of computational biophysics. Indeed, the principles of classical electrostatics have been successfully applied to a great variety of biophysical problems, including determination of electrostatic potentials around macromolecules, solvation energies, pKₐ shifts in biomolecules, and acid–base equilibria. Electrostatic free energies, in particular, have been used to predict and model important molecular processes, such as folding, binding, and denaturation.

Recently, it has become experimentally possible to probe molecular electrostatic interactions in solution using the escape time electrometry (ETe) technique capable of delivering high-precision measurements (uncertainty ≈1%) of molecular electrostatic free energies at the single molecule level. In this study, we explore the relationship between electrostatic free energies and molecular structural properties of nucleic acids in solution. Specifically, we examine the possibility of gleaning conformational or structural detail on charged molecules in solution from experimentally measured interaction free energies.

Molecular simulations of biomolecules have made great advances in recent years. Explicit treatment of solvent molecules, as well as the atoms and ions in the system, arguably provides the most detailed description of the problem. However, in practice, as the system size, N, given by the number of water molecules and ions, increases, simulations based on explicit models become increasingly intractable. This is due to the high computational cost of the problem, which is of O(N³) for direct methods.
more efficient algorithms may reduce this cost to $O(N \log N)$, the explicit treatment of large systems still remains expensive. The continuum electrostatics approach, on the other hand, treats the solvent and ions implicitly, enabling greatly simplified computation of electrostatic interactions in solution.27

The Poisson–Boltzmann (PB) equation [Eqs. (3) and (4)] provides a continuum, mean-field electrostatic description of a charged object in an electrolyte. Solution of the PB equation, subject to the appropriate boundary conditions, yields the spatial distribution of electrostatic potential throughout the system, on which other derived quantities, such as free energies, depend.10,18 The PB equation plays a central role in implicit solvent models, and several numerical schemes and software packages have been developed, which accurately solve this equation around complex biomolecular structures. These packages are capable of solving different types of PB equation (linear and nonlinear) and rely on finite difference,19–21 finite element (FE)/volume,22,23 or boundary element methods.24–28 Recent molecular dynamics (MD) simulation studies have investigated the range of validity of PB models for nucleic acids, including RNA and DNA molecules.29–31 In general, these studies confirm that in the absence of multivalent species, ionic concentrations predicted by continuum models are in good agreement with all-atom calculations beyond some distance ($\approx 14–16$ Å) from the axis of the molecule.32,33 However, in the presence of mixtures of monovalent and divalent cations, both experimental observations and MD simulations have reported departures from the PB model.32,34 The discrepancies have been attributed to finite ion size, ion–ion correlations and competitive binding of divalent cations to DNA and RNA duplexes.

In this work, we focus on examining the relationship between electrostatic free energies and molecular structure for short stretches of double stranded nucleic acids in monovalent salt solution. We use FEniCS, an open-source finite element (FE) software package, to apply the finite element method to the molecular electrostatics problem. We solve the non-linear PB (NLPB) equation for both charged cylinders and molecular structural models of the double helix. The cylinder model, which serves as a convenient intermediate link between experimental measurements and molecular models, relates the molecular helix to a smooth charged cylinder of radius, $r$, and length, $l = nb$. Here, $n$ is the number of basepairs and $b$ is the rise per basepair (which corresponds to twice the linear charge spacing) of the molecular model.35 [Fig. 1(c)].

Importantly, cylinders provide a simple two-parameter system ($b$ and $r$) on the basis of which various double helical models may be classified or identified. Table I provides nominal values for the axial rise per basepair, $b_{\text{helix}}$, and the helical radius, $r_{\text{helix}}$, for A, B, and Z DNA, derived from data based on high resolution structural techniques, such as crystallography. Within the context of the Debye–Hückel equation, which is the linearized version of the PB equation, the rigid rod model of DNA has previously been applied to experiments focusing on the electrical mobility and interactions of DNA molecules in solution.

We present a computational study of the biomolecular electrostatic free energy problem in the context of the ET measurement.

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**FIG. 1.** (a) Two states of a charged cylinder or molecule in an electrostatic fluidic trap: the molecule is either outside the trap ("slit" state—1) or in the trap ("pocket" state—2) (top). Shaded regions denote the near field (NF—red), far field (FF—blue), and bulk (B—gray) of the system, and $\rho$ denotes the radial distance from the object axis (see Figs. 5 and 9). Electrical potential distributions for a charged cylinder of radius 10 Å and length 102 Å representing a 30 bp B-DNA molecule at $c = 1$ mM salt concentration in the two states (bottom). (b) Spatial distribution of the electrostatic free energy difference, $\Delta F_{\text{el}}$, for a representative potential well generated by an electrostatic fluidic trap. (c) Equivalent cylinder model for a DNA molecule depicting the various dimensions of interest.
ETe measures, with high accuracy, differences in electrostatic free energies of interaction, $\Delta F_{el}$, between two molecular spatial states in a nanostructured free energy landscape (Fig. 1). We parameterize both measured and calculated interaction free energy in terms of an effective charge, $q_{eff}$, that describes the properties of the molecule of interest (see Sec. I A). In the overall remit of the study, $q_{eff}$ serves a dual purpose. In the computational analysis, $q_{eff}$ serves as a connecting parameter that enables us to relate the electrostatic free energy calculated for the molecular double helix to that of a smooth charged cylinder model. Experiments, in turn, yield measured values for $q_{eff}$ that can be independently related to a molecular model, which may be either an atomic structural model or a simplified coarse-grained cylinder. Simple cylinders can provide a useful intermediate link connecting experimental measurements with particular molecular models. Mapping of the molecular helix to a cylinder fosters the extraction of helical structural parameters of interest from experimental measurements. An additional major advantage of this simplified view is that the computation of electrostatic potentials around a cylinder is substantially more efficient and rapid than when working directly with molecular structures. Thus, once an effective cylinder description is obtained from measurement, it can be independently tested for agreement against various molecular models.

In this study, we consider two canonical forms of the double helix, A-RNA and B-DNA, which are characterized by different values of axial rise per basepair, $b$, and are also thought to differ in their helical radii, $r$ (Table I). Although ETe measurements can yield values of both the radius and the rise per basepair of the double helix, in this work, we assume that $b_{cyl} = b_{doub}$, where the value of $b$ in the cylinder description is taken to be identical to that of the molecular helix. We, therefore, focus primarily on relating the radius of the cylinder model to various molecular models of the double helix. The main reason behind this choice is that the effective helical radius, and, therefore, the surface charge density of the molecular model, can be strongly affected by the parameters used to generate the molecular surface (described later), whereas the axial charge spacing, which is governed by the rise per basepair of the structure, remains effectively unperturbed across the various molecular models. Thus, we focus in this study on determining the values of the effective cylindrical radius, $r_{cyl}$, that yield the same value of interaction free energy as various models of the molecular helix. The analysis provides an estimate of the free energy measurement precision required to glean information on the radius of the double helix with $\approx 1$ Å precision. Because of the sensitivity of electrostatic free energies to the radius of the effective cylinder describing the DNA molecule, we expect that measured free energies will implicitly carry information on interfacial hydration and the size of counterners in solution.

### A. Probing electrostatic free energies in solution: The ETe measurement

ETe measures the reduction in system free energy associated with transferring a charged molecule in the solution phase from a gap between like-charged parallel plates (state 1) into a nanostructured “trap” region of very weak confinement (state 2) (Fig. 1). The electrostatic repulsion between the molecule and charged walls is strong in the parallel-plate gap (or “slit”) region and negligible, by design, in the trap (or “pocket”) region. In the experiment, an array of such electrostatic fluidic traps can be created by using periodic nanostructured indentations in one surface of a parallel-plate slit composed of silica surfaces separated by a gap of typical height, $2h = 75$ nm. We typically introduce nucleic acid molecules at a concentration of $\approx 50$ pM labeled with exactly two fluorescent dye molecules of ATTO 532, suspended in an electrolyte solution containing $c \approx 1$ mM monovalent salt at pH 9, into a system with multiple parallel lattices of traps (Fig. 1). Imaging the escape dynamics of trapped single molecules permits us to extract precise measurements of the molecular species’ average time to escape, $t_{esc}$. We convert measured $t_{esc}$ values to the potential-well depth of the trap, $W$, as described previously. The exponential dependence of the measured average escape time, $t_{esc} \propto \exp(W/\kT)$, on the potential-well depth, $W$, facilitates precise interaction energy measurements. Here, $\k$ is Boltzmann’s constant and $T$ is the temperature. Furthermore, observation of a large number of escape events, $N \approx 10^5$, reduces the fractional statistical uncertainty in the determination of $W$ to about 0.1% (Ref. 12). Importantly, the dominant contribution to the potential-well depth, $W = \Delta F_{el} + f$, is the electrostatic free energy of interaction, $\Delta F_{el}$, which has robust theoretical underpinnings in the PB framework for solution phase electrostatics. In the preceding equation, $f$ denotes an additional free energy contribution due to spatial fluctuations of the molecule. This contribution is very weakly-charge dependent and can be estimated by evaluating the local partition function as described in previous work. Thus, an experimental measurement of the average escape time, $t_{esc}$, of a molecular species yields a measure of the electrostatic interaction free energy cost of inserting a charged molecule into the center of a parallel-plate gap ($\Delta F_{el}$), as shown in Fig. 1.

We have previously shown that $\Delta F_{el}$ may be regarded in terms of the product of the effective charge of the molecule in solution, $q_{eff}$, and the electrical potential, $\phi_{m}$, at the midplane of the slit (Fig. 1) such that

$$\Delta F_{el} = q_{eff}\phi_{m}. \quad (1)$$

The above compact definition of the electrostatic free energy of interaction, $\Delta F_{el}$, indeed, includes both an electrostatic energy and an entropic contribution arising from mixing entropy of the ions, as shown in Eq. (6). We have shown in previous work that this free energy difference between two spatial locations of the molecule can be parameterized in terms of an effective charge, $q_{eff}$, and cast in a simple intuitive, electrostatic form, as shown in Eq. (1). Thus, if $\phi_{m}$ is accurately known, the measurable in our experiment is the effective charge, $q_{eff}$, of the molecular species under the experimental conditions.

### Table I. Nominal structural dimensions of different forms of the DNA double helix. Parameters $r_{doub}$ and $b_{doub}$ represent the helix radius and axial rise per basepair, respectively.

| Type | $r_{doub}$ (Å) | $b_{doub}$ (Å) |
|------|----------------|----------------|
| A    | 13.4, 12.8     | 2.6, 2.3       |
| B    | 10.9, 11.9     | 3.4           |
| Z    | 9.2            | 3.8           |

$^a$Values from Ref. 42.

$^b$Values from Ref. 43.
For a highly charged cylinder in solution, it has been shown that

\[ q_{\text{eff}} = q_{\text{net}} = f(n, b, r), \]

(2)

where \( \eta \) is a molecular geometry dependent charge renormalization factor depending on \( n, b \) and \( r \), which are the parameters of interest in this study (Refs. 46–50). \( q_{\text{net}} \) denotes the net electrical charge carried by the molecule and stems from the sum of charge carried by the ionized structural groups and ions that are bound or adsorbed to the molecular structure. A highly acidic molecule, such as DNA, \( n \) base-pairs in length and carrying a chemical modification at both 5′-end phosphates, has a structural charge, \( q_{\text{str}} = q_{\text{NA}} = -2ne \), at pH 7 and higher. Here, \( e \) is the elementary charge and \( q_{\text{NA}} \) is the total amount of charge due to backbone phosphate groups on the molecule, which are all fully ionized. However, if \( \delta \) number of positively charged counterions in solution associate with the molecule, e.g., via energetic interactions beyond the purely Coulombic that are already accounted for within the PB model, then \( q_{\text{str}} = q_{\text{NA}} - \delta e = f_{\text{ion}}q_{\text{NA}} \), where \( f_{\text{ion}} = (q_{\text{NA}} - \delta e)/q_{\text{NA}} \) represents an inverse ion affinity. Reference 46 places our definition of the renormalized or effective charge, \( q_{\text{eff}} \), given in Eq. (1), in the context of previous theoretical work on charge renormalization, including Manning’s renormalization factor. In general, we find good quantitative agreement between our interaction energy based definition of \( \eta \) and previous theoretical definitions. Importantly, our definition of \( q_{\text{eff}} \) in Eq. (1) directly relates to an experimental measurable. This is in contrast to previous theoretical definitions of the renormalized charge where the link to an experimental approach that would directly probe the calculated quantity is not always clear.47–49

For a given molecular geometry and structural charge, \( \eta \) is essentially independent of ion affinity for \( f_{\text{ion}} < 0.7 \). Importantly, although \( q_{\text{eff}} \) does exhibit some dependence on salt concentration, \( c \), this variation is negligible over the small range in experimental uncertainty in \( c \) in a given measurement.60–62 Furthermore, from the measurement point of view, we prefer to work with \( q_{\text{eff}} \) or \( \eta \) rather than \( \Delta F_{\text{el}} \) as the former two parameters describe properties of the molecule. The electrostatic interaction free energy, \( \Delta F_{\text{el}} \), on the other hand, depends on system size, \( kh \), and, therefore, strongly depends on small experimental variations, such as gap height, \( 2h \), and salt concentration, \( c \), via the Debye screening length \( \kappa^{-1} = (2\epsilon N_{A}e^{2}/\varepsilon_{0}k_{B}T)^{-1/2} \). Here, \( \varepsilon_{0} \) is the permittivity of the free space, \( \varepsilon_{r} \) represents the relative permittivity of the dielectric medium, and \( N_{A} \) is Avogadro’s number. In ET, we write the midplane electrical potential as \( \phi_{\text{mid}} = 2\phi_{i} \exp(-kh) \), which like the free energy, \( \Delta F_{\text{el}} \), strongly depends on the system size \( kh \). Here, \( \phi_{i} \) is a free surface electrical potential at the charged slit surfaces and is not the true surface electrical potential. The difference turns out to be unimportant as the molecule’s position is Boltzmann-weighted to the midplane of the slit so that the electrical potential close to the walls does not substantially influence interaction free energies for highly charged molecules. Division of \( \Delta F_{\text{el}} \) by \( \phi_{i} \) yields a quantity \( (q_{\text{eff}})_{\text{free}} \) that is robust to small experimental variations in \( k \) and \( h \) and is, therefore, amenable to further treatment, as reflected in Eq. (2).

Short nucleic acid fragments of length, \( l \), smaller than the persistence length of the nucleic acid (150 bp for B-DNA and 430 bp for A-RNA) may be expected to display the conformational properties of rigid rods. Thus, accurate measurements of \( \eta \) on fragments shorter than about 60 bp can be expected to carry geometrical information on effective cylinders representing the double helix. According to Eq. (2), measurements on fragments of different lengths can serve to determine the values of both molecular geometrical unknowns, \( b \) and \( r \), within the cylinder model. In view of the grooved molecular surface of double stranded nucleic acids and the helicoid distribution of charge on the molecular backbone, we present a rigorous test of the quality of the smooth cylinder electrostatic model for the nucleic acid double helix, within the framework of experimental free energy measurements. Although the approximation of a fragment of double stranded DNA by a smooth charged cylinder can present a gross oversimplification, as discussed in Sec. IV B, we show that this rudimentary model, nonetheless, supports meaningful extraction of average molecular geometric parameters from measurements that sensitively probe interaction free energies.

II. METHODS

This section discusses the implementation of the NLBP equation in the experimental geometry shown in Fig. 1. To begin with, we describe the solution of the PB equation, the identification of the interfacial boundaries for the molecular structure, and the application of the boundary conditions. Finally, we present our approach to calculating free energies in a reduced computational geometry, which accurately captures free energy differences in the extended experimental system.

A. Governing equations

We solve the NLBP equation in the experimental geometry for two states of the molecule in the system: the “slit” state (1) and the “pocket” state (2), as shown in Fig. 1. The Poisson equation is given by

\[ -\nabla \cdot (\varepsilon_{r} \varepsilon_{0} \nabla \phi) = \rho_{e}, \]

(3)

and holds everywhere in the system. Here, \( \phi \) is the electric potential and \( \rho_{e} \) is the charge density. The charge density, \( \rho_{e} \), in the aqueous solution is described by the Boltzmann distribution for ionic species,

\[ \rho_{e} = \sum_{i} c_{i} N_{A} e_{i} z_{i} \exp\left(-\frac{z_{i} \phi_{i}}{k_{B} T}\right). \]

(4)

where \( c \) and \( z_{i} \) are bulk concentrations and the valences of \( i \)th ionic species, respectively, and \( z_{+} = -z_{-} = 1 \) holds for a binary, symmetric, and monovalent electrolyte. We further assume that the solution is electroneutral in the external reservoir to which the system is implicitly coupled, and therefore, \( c_{+} = c_{-} = c \).

While the charge density, \( \rho_{e} \), is given by the Boltzmann distribution within the electrolyte, in practice, ions and water molecules are largely excluded from the molecular interior. Thus, both the charge density, \( \rho_{e} \), and the dielectric function, \( \varepsilon_{r} \), are functions of spatial location in the system. The charge density, \( \rho_{e} \), must vanish in the charge-free interfacial region, which is inaccessible to ions. Furthermore, the dielectric function is expected to take values lower than the relative permittivity of water (\( \kappa=80 \)) in regions impenetrable to water.

A key task in computations involving molecular structural models is the definition of boundaries that distinguish these regions from one another. Section II B discusses several possibilities to define...
the required interfaces. Once the interfaces are defined, application of the proper boundary and jump conditions permits the NLPB equation to be solved using standard numerical schemes, such as finite element (FE) or finite difference methods, combined with Newton’s iterations to handle the nonlinearity of the problem.

B. Molecular surface and boundary conditions

1. Considerations in generating molecular surfaces from atomic structural models

The first step in electrostatic modeling of a molecular structure is the construction of a molecular surface. In general, there are three slightly different definitions of a molecular surface: (i) van der Waals (vdW) surface, (ii) Solvent Accessible Surface (SAS),1 and (iii) Solvent Excluded Surface (SES).55–57 The vdW surface of the molecular structure is simply the surface of the volume obtained by the union of all atoms in the molecule, which are treated as spheres of radii given by each atom’s respective vdW radius. The SAS, in turn, is a continuous surface generated by the center of a spherical probe of radius \( r_p \) rolled over the vdW surface. It can be readily demonstrated that the SAS is equivalent to a vdW surface where the vdW radii of all atoms are increased uniformly by an amount equal to \( r_p \) (Fig. 2). The SES, however, is defined by tracking the contact points of the rolling probe, as shown in Fig. 2. Accordingly, the SES can be regarded as a smooth manifold, which approximates the true vdW surface as \( r_p \to 0 \). We use a SES created by a small probe size of \( r_p = 1 \) Å in order to generate an effective vdW surface for the molecular structures that is as similar to the theoretical vdW surface as possible while still providing a surface with sufficient smoothness for the mesh generation and numerical solution procedure (Fig. 2). In this work, we consider the vdWS and SES as synonymous. In order to create the SAS, we simply generate a SES of a molecular structure where the values of default vdW radii of all atoms are increased by a constant value, \( \Delta r_p \). In the molecular simulation literature, the value of the probe radius \( r_p \) has substantial significance in lending physical meaning to the SAS. In general, the center of mass of a probe of radius \( r_p \approx 1.4 \) Å, representing the typical radius of a water molecule, can be used to distinguish the boundary of a region inaccessible to the center of mass of a solvent molecule. As a result, the dielectric function, \( \epsilon_p \), is expected to take small values (≈2–4) within this boundary (SAS) and to attain the bulk value (≈80) in the outer regions.58 The simplest form of a dielectric function satisfying the properties of a low dielectric “shell” region is a piecewise constant function with a sharp discontinuity across the interface.55,59 Where relevant, we use a discontinuous dielectric function in our study. We point out that a recent study proposes a distance dependent function based on MD simulations, which eliminates sharp discontinuities and captures the smooth transition to bulk values near the interface.60

The ion excluded region, in turn, may be represented by a SAS-like surface generated using a larger probe whose radius is determined by the size of the ions in the electrolyte; we term this surface the ion accessible surface (IAS). Clearly, the charge density, \( \rho_{\text{ion}} \), is zero inside the IAS and obeys the Boltzmann distribution outside this boundary. Figure 2 schematically depicts how these surfaces divide space into several subdomains with different dielectric properties and charge distributions. It is worth noting that the region within the IAS is often regarded as the Stern layer in the context of colloids and macroscopic surfaces in solution.59–61

It is convenient to enforce the ion-accessibility condition by multiplying Eq. (4) by an ion-accessibility function, which depends on the radius of \( i \)th ionic species. The ion-accessibility function, \( \lambda_i(x) \), is accordingly a step function in space, which is 0 inside the IAS and 1 elsewhere.61 However, the radii of hydrated ions in solution are not precisely known,62–64 and this necessitates the introduction of parameters associated with some degree of uncertainty into the model. This is true, in general, for models that attempt to account for finite ion size, including size-modified PB (SMPB) models. Values for ion size may be taken from all-atom MD simulations,65 from experimental ionic conductivity measurements,66 or treated as fit parameters.33,67 Note that in this work, we only use a SAS region in our molecular modeling and do not explicitly invoke an IAS. We, in fact, show that the IAS may be indirectly captured within the point-ion PB model by adjusting the radius of the equivalent cylinder. Thus, the present study which is based on the point-ion PB model (1) maps the electrostatic free energy of the double helix into the model. This is true, in general, for models that attempt to account for finite ion size, including size-modified PB (SMPB) models. Values for ion size may be taken from all-atom MD simulations,65 from experimental ionic conductivity measurements,66 or treated as fit parameters.33,67

29,59–61

![Schematic representation of molecular surfaces. The gray region denotes the interior of the molecule, while the van der Waals surface (vdWS) (red lines) and ion accessible surface (IAS) (blue lines) demarcate low dielectric regions inaccessible to water molecules and ions, respectively. The solvent accessible surface (SAS) (green line) denotes the surface of the closest approach of water molecules to the vdw. The charge distribution outside the IAS is governed by the Boltzmann distribution.](image)
2. Construction of molecular models for electrostatic calculations

The starting point for generating the molecular surface is an atomic level structure of the double helix. In this study, we focus on 30 bp double stranded B-DNA and A-RNA molecules, which carry the same amount of structural charge \( q_{str} = -60e \) but differ significantly from the structural point of view. We consider sequences 5′-GGA TGG GAC GGA CCC GGA CAC AGA CAG TGC-3′ and 5′-GGA UGG GAC GGA CCC GGA CAC AGA CAG UGC-3′ for B-DNA and A-RNA, respectively. We generated atomistic models of the above molecules using 3DNA, a software package, which utilizes experimental crystallographic data to build model structures of DNA and RNA molecules. Note that some molecular modeling platforms, such as Nucleic Acid Builder (NAB), provide double stranded DNA structures free of terminal phosphate groups. In this case, the total charge on a 30 bp DNA fragment would be \( q_{str} = -58e \).

Next, we proceeded to construct the molecular surface. MSMS is a molecular surface rendering software package, which generates the SES by rolling a spherical probe of radius \( r_p \) over the surfaces of spheres representing atoms in the molecular structure. The radii of these spheres are taken to be equal to the corresponding vdW radius of each atom. MSMS is embedded as a third-party package into UCSF Chimera, a molecular visualization software. We point out that Chimera has different algorithms to determine the vdW radii of atoms based on whether the structure contains explicit H-atoms or not. In our case, since the atomic structures generated by 3DNA do not contain explicit hydrogen atoms, “united atom radii” based on the protein organic set (ProtOr) are used. For more detail, we refer the reader to the Chimera User’s Guide.

As illustrated in Fig. 2, the SES does not considerably increase the overall size of the molecule obtained by the union of vdW spheres but rather tends to smooth out the sharp edges and cusps of the vdW surface. This is particularly useful in ensuring a better quality for the FE mesh. In order to generate the SAS, we use a slightly different approach. We first increase the default vdW radii of all atoms by a constant value, \( w \), and then roll a sphere of radius \( r_p = 1 \) Å over the surface of the volume resulting from the union of the modified vdW spheres.

The next stage in the process is the treatment of the structural charge of the molecule, which is localized at the negatively charged O-atoms of the backbone phosphate groups. Only atoms that are responsible for the net electrical charge on the molecule in solution are endowed with charge in our model. We do not consider any atomic partial charges on the remaining atoms in the structure. Some software packages, such as APBS and DelPhi, treat structural charge as Dirac-distributed source terms. These packages benefit from automated algorithms to process atomic structures, which greatly reduce the overhead associated with mesh generation. APBS, for example, has implemented algorithms to distribute the atomic partial charges to the nearest grid points in a volumetric fashion. It is known, however, that such an approach can lead to inaccuracies near discrete charges, rendering the potential field in the vicinity of these points mesh density dependent. In contrast, in our work, we distribute the charge over “surface patches” representing the vdW atomic spheres. This approach is not only physically intuitive in view of the non-point-like nature of electron density in atoms but also renders our results robust to mesh refinement.

Furthermore, under typical experimental conditions (pH \( \approx 7-9 \)), the constant charge assumption for DNA is an excellent approximation due to the highly acidic nature of the phosphate groups (pK\(_a\) \( \approx 2 \)). The molecules in our study, therefore, carry a total structural charge of \( q_{str} = -2ne = -60e \) localized at the negatively charged O-atoms in the backbone phosphate groups. We distribute this total charge over the spheres corresponding to the “O1P” and “O2P” atoms (Fig. 3) using the following procedure. Having generated the required molecular surfaces, we identify all surface patches that are made up of points lying at a minimum distance from the coordinates of the nearest “O1P” and “O2P” atoms. This essentially distinguishes the surfaces of the charge carrying O backbone atoms from all other atoms in the structure (Fig. 3). Subsequently, the surface charge density at the dielectric interface is weakly imposed by applying the following jump condition:

\[
\sigma_p = -\left(\epsilon_o \nabla \phi_o - \epsilon_i \nabla \phi_i\right) \cdot \mathbf{n},
\]  

where \( \sigma_p = \frac{q_{str}}{A_p} \) is the surface charge density of the charged atomic regions and \( \epsilon = \epsilon_o \epsilon_r \). The subscripts (o) and (i) distinguish between the functions or quantities evaluated on the inside and on the outside of the dielectric boundary. Here, \( A_p \) represents the total surface area of all the “O1P” and “O2P” patches in the molecule, and \( \mathbf{n} \) is the unit normal vector pointing from (i) to (o).

![FIG. 3. (a) Atomic structure of a B-DNA molecule generated using 3DNA. (b) Molecular surface produced by the MSMS package using a rolling probe of \( r_p = 1 \) Å and default vdW radii \((\epsilon = 0)\). Red patches depict surfaces attributed to “O1P” and “O2P” atoms.](image-url)
In Sec. III A, we will demonstrate that from the perspective of the electrostatic interaction free energy, $\Delta F_{el}$, our molecular models can be further simplified by neglecting the region within the dielectric boundary, which comprises not only the dielectric shell region but also the entire molecular interior. As a result, the charge carrying molecular surface turns into an exterior boundary of the computational domain and the surface charge density is applied by using a Neumann boundary condition at the surface, i.e., $\sigma = -\epsilon \nabla \phi \cdot \mathbf{n}$. Note that here $\epsilon$ corresponds to the bulk dielectric constant. We have further verified that the silica walls of the nanostructure geometry can also be treated with constant charge boundary conditions as the molecular surfaces in the system, we enforce $\sigma = -0.1\text{e}\text{n}^{-2}$ is a nominal surface charge density of the wall and $\mathbf{n}$ is the unit normal vector pointing into the electrolyte.

Having constructed the molecular surface, we used GMSH\textsuperscript{15} for the subsequent mesh generation and solved the governing equations using FEniCS,\textsuperscript{13} which is an open-source FE software package.

### C. Molecular free energy calculations in an electrostatic fluidic trap

Figure 1 schematically represents a charged cylinder or a DNA molecule suspended in solution within a nanostructured landscape consisting of a parallel-plate slit region and a nanostructured "pocket" region that serves as an electrostatic trap. Thermodynamically, the direction of the trapping process is determined by the difference in the free energy of the system between two different states given by the molecule occupying the slit region (state 1) and residing in the pocket region (state 2). Neglecting the angular (orientational) and spatial degrees of the freedom of the molecule, the problem can be simplified to a calculation of the free energy for two configurations given by the molecule positioned at the local axial depth for a charged molecule (green sphere). A free energy calculation for the full region C in both configurations (gray regions in Fig. 4) remains unaffected for sufficiently large A and B, which implies $F_2^C - F_1^C = 0$.

The dimensions of the nanostructured system used for electrostatic trapping are typically of the order of 100 nm.\textsuperscript{15,44} Since a DNA molecule involves considerably smaller dimensions ($r \approx 1$ nm), resolving such a small object within the domain of interest (Fig. 1) is computationally expensive. On the other hand, in light of the properties of the PB equation, it is known that the perturbation in electrical potential due to the molecule decays exponentially with distance from its surface. In practice, the perturbation of the molecular vanishes after several Debye screening lengths, $\kappa^{-1}$, leaving the rest of the domain essentially unperturbed. Since we are ultimately interested in the difference between free energies of two states discussed above, the overall problem can be reduced to solving three smaller ones. Figure 4 represents a domain decomposition, which we use for this purpose. The domain, $\Omega$, is decomposed to three subdomains (A–C). The molecule is contained within subdomains B and A for configurations 1 and 2, respectively. We define $F_{el}/k_B T = \int_{\Omega_i} f(\psi)dx$ as the contribution of the subdomain $j$ to the free energy of state $i$. Accordingly, the difference in free energy, $\Delta F_{el}/k_B T$, can be described by

$$\Delta F_{el}/k_B T = \int_{\Omega_2} f(\psi)dx - \int_{\Omega_1} f(\psi)dx = (F_2^A + F_2^B + F_2^C) - (F_1^A + F_1^B + F_1^C).$$  

Region C in both configurations (gray regions in Fig. 4) remains unaffected for sufficiently large A and B, which implies  $F_2^C - F_1^C = 0$.

Here, the function $\lambda(x)$ is a step function as defined previously, which takes the value 1 in the regions accessible to ions and is 0 elsewhere. This function restricts the integral of the second term (entropic term) to the regions in which charge density, $\rho_i$, is described by the Boltzmann distribution. Clearly, the calculation of the free energy, $F_i$, only requires the electrical potential, $\psi$, which is determined by solving Eq. (3) in the domain of interest $\Omega$.

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Concurrently, region A in configuration 1 is far from any charged surfaces, and therefore, the potential distribution in this region is nearly uniform and equal to zero (bulk electrical potential), i.e., $F^a_1 = 0$ [Fig. 4(a)]. Consequently, the difference in free energy only depends on the solution of the PB equation in three subdomains: $\Omega^a_1$, $\Omega^b_1$, and $\Omega^2_1$. The first case, $\Omega^a_1$, represents a DNA molecule in a 3D rectangular domain of width and depth $L \gg \kappa^{-1}$, which is bounded by the charged slit walls on the top and bottom. The second domain, $\Omega^b_1$, is an electrolyte filled rectangular region of the same dimensions, but devoid of the molecule, and the third case, $\Omega^2_1$, corresponds to that of a DNA molecule at the center of a spherical domain of radius $R \gg \kappa^{-1}$ that is large enough to fully capture the perturbation to the electrical potential due to the molecule.

The virtual boundaries that delimit the subdomains (defined by parameters $L$ and $R$) are chosen such that the electric potential does not change normal to those boundaries, and we apply a homogeneous Neumann boundary condition, $\nabla \phi \cdot \mathbf{n} = 0$, on these surfaces.

The salt concentration in the present study is $c = 1\, \text{mM}$, which corresponds to a Debye screening length of $\kappa^{-1} \approx 9.6$ nm. For all simulations, we consider $L = R = 90$ nm to ensure that the subdomains A and B are significantly larger than both the Debye length, $\kappa^{-1}$, and the lengths, $L$, of a 30 bp nucleic acid molecule. Using this approach, the slit height, $2h$, is the only physical dimension of the device, which enters the calculations. For the purpose of this study, we have chosen $2h = 75$ nm, which represents a typical value in experimental measurements.

Calculating the free energy, $\Delta F_{el} = F_1 - F_2$, we further define the effective charge of the molecule via the relation $q_{eff} = \Delta F_{el}/\phi_{midd}$ (Ref. 45), where the midplane potential, $\phi_{midd}$, is determined from the numerical solution in domain $\Omega^b_1$ (Fig. 1). We then compare the effective charge values calculated for the molecular models with simple cylindrical geometries. We deduce the equivalent radius, $r_{cyl}$, of a cylindrical object, which leads to the same value of effective charge, $q_{eff}$, as the molecular helix. A detailed description of this comparison follows.

### III. RESULTS

#### A. Role of the interfacial region in free energy differences

In general, we solve Eq. (3) for a molecular model or a cylindrical object and calculate the difference in free energy, $\Delta F_{el}$, for the two states of interest (Fig. 1). These values are then converted to $q_{eff}$ and $\eta = \frac{q_{eff}}{q_{str}}$, which represent the effective charge and charge renormalization factor, respectively.

In order to explore the contribution of the interfacial region to the free energy difference, $\Delta F_{el}$, we begin with a general case of a B-DNA molecule. Our structure consists of the dielectric interior of the molecule, defined by the SES ($r_p = 1, w = 0$ Å), surrounded by another dielectric shell whose exterior is defined by another molecular surface ($r_p = 1, w = 1.4$ Å; purple surface in Fig. 5) representing the surface accessible to water molecules (SAS) (Fig. 5). The dielectric constant within both the molecular interior and the shell is fixed at $\epsilon_i = 2$, and we assume that relative permittivity in the region exterior to the SAS is equal to that of water, i.e., $\epsilon_r = 80$. As discussed above, the structural charge is distributed on “O1P–O2P” patches of the inner surface, which are located at the interface of the molecular interior and the dielectric shell around it (green dashed line in Fig. 2). Furthermore, the volumetric charge density outside the SAS is given by Eq. (4). Solving the NLBP equation for this B-DNA structural model, as previously described, we obtain the potential distribution at the outer surface of the dielectric shell or SAS [Fig. 5(c)].

Considering Eq. (6) and the fact that $\lambda(x) = 0$ in all regions inaccessible to ions, the contribution of the inner regions to free energy is restricted to the first term of Eq. (6), which corresponds to the electrical energy of the dielectric media (i.e., both the molecular interior and the dielectric shell regions). We compared the computed $q_{eff}$ (or $\Delta F_{el}$) values for models in Fig. 5 that either included or excluded the dielectric regions interior to the SAS. This entailed a comparison of the model in Fig. 5(c) and its simplified counterpart model IIIB shown in Fig. 7, where the charge is carried on the outer SAS. We found that the contribution of the dielectric shell and the molecular interior to the free energy difference $\Delta F_{el}$ is negligible (≈1%) in comparison to that of the solvent-filled electrolyte region. Therefore, given the complexity of the mesh generation process and the computational cost of resolving the interior regions, for the rest of the study, we ignore all interior dielectric regions and distribute the charge directly on the “O1P–O2P” patches of the outermost surface (SAS), which is in contact with the electrolyte (purple surface in Fig. 5; model IIIB in Fig. 7).

In order to further examine the role played by the dielectric shell region in the interaction free energy problem, we undertook a similar study on a simpler system consisting of a charged cylinder surrounded by a dielectric shell. We compared the results of $q_{eff}$ for such cylinders with their dielectric-free counterparts, which are hollow objects carrying the same amount of total charge on their surface. Figure 6 represents the ratio of effective charges, $\frac{q_{eff}}{q_{str}}$, for the two cases of interest where the subscript “a” represents the cylinder with a dielectric shell and “b” denotes the hollow cylinder case. We examine the ratio $\frac{q_{eff}}{q_{str}}$ for a range of values of the shell dielectric...
constant, $\epsilon_s$, and different shell thicknesses, $t$. As shown in Fig. 6, neglecting the dielectric region and distributing the same amount of charge on the outer surface of the cylinder has a very small impact on the calculated effective charge (0.7% difference between the models for $\epsilon_s = 2, t = 3$ Å), and this trend, indeed, holds for a range of values of shell thickness and dielectric constants relevant to the problem. Thus, based on the above result, we neglect the dielectric molecular interior and the dielectric shell for all subsequent calculations involving molecular structures and cylinders. Note that the relative insensitivity of the problem to shell thickness, $t$, and shell dielectric constant, $\epsilon_s$, provides an early indication of the impact of ion size on the effective charge problem. Essentially, these results suggest that finite cation size in real experiments is likely to be captured by a point-ion PB model where the molecular charge is simply distributed over the surface of a hollow cylinder whose radius, $r_o$, reflects a contribution from the radius of the counterion species. To the first approximation, the analysis suggests $r_c = r_i + a_{II}$, where $a_{II}$ is the radius of the counterion species in solution.

B. Relating the B-DNA molecular model to an equivalent cylinder

As discussed previously, we are interested in mapping the molecular electrostatic problem onto a simpler case given by a cylindrical object of radius, $r_{cyl}$, and length, $l$. In this model, the total structural charge is uniformly distributed over the lateral surface of the cylinder, i.e., $\sigma_{cyl} = q_{str}/2\pi r_{cyl} l$. According to our definition, $r_{cyl}$ is the radius of an equivalent cylinder whose calculated effective charge is equal to that of the molecular entity. In other words, the equivalent cylinder is expected to create the exact same electrostatic free energy difference as a molecule in our system, and such a cylinder would, therefore, be effectively indistinguishable from the molecule in an ETe experiment.

Figure 7 depicts three different molecular surfaces corresponding to the B-DNA molecule. We first focus on models IB and IIB, generated using two different values of probe radius, $r_p = 1$ and 5 Å. Here, default vdW radii were used to generate the molecular surface and $\omega = 0$. We find that the larger probe radius smooths out the details of the molecular surface but preserves its global features. Interestingly, this increase in $r_p$ leads to a small impact on effective charge (increase in magnitude of =1%), which enlarges $r_{cyl}$ from 8.8 to 9.4 Å, corresponding to an increase of =7% (Fig. 7). It is worth noting that the effective cylinder radii in these cases are slightly smaller than the nominal radius of about $r_c = 10$ Å obtained from high resolution structural techniques (Table I, Fig. 7). This may be reasoned by the fact that the molecular surface is corrugated, and point ions are, in fact, able to penetrate the cylindrical helical envelope of radius $r_c$, effectively creating a potential field resembling that of a slightly narrower cylinder.

Our third model for B-DNA, model IIIIB, displays the influence of a “virtual” dielectric layer, demarcated by the SAS, which arises
from the finite size of water molecules \(w = 1.4 \text{ Å}\). Note that, as previously described, neither the molecular interior nor the SAS shell domain is explicitly resolved, and the SAS surface is the sole charge carrying surface in the molecular model. We note that incorporation of the SAS layer into the model leads to a larger equivalent cylinder radius of \(\approx 10.8 \text{ Å}\). Overall, the equivalent cylinder radii calculated for the various models lie in the range of 9–11 Å, depending on the structural parameters used \((r_p\text{ and } w)\). This range is in remarkable agreement with the radius of B-DNA obtained using structural techniques, such as x-ray crystallography and NMR \((r_{\text{helix}} = r_c = 10 \text{ Å}, \text{Table I})\). Assuming that \(b_{\text{helix}}\) is known, a high enough precision in the effective charge measurement (uncertainty \(\approx 1\%\)) would be sufficient to distinguish between at least two scenarios—IB and IIIB, which differ in \(q_{\text{eff}}\) by \(\approx 6\%\). This suggests that the dimensions of a B-DNA molecule can be inferred from the free energy associated with the electrostatic trapping process, which is an experimentally measurable quantity. The analysis also suggests that such measurements could reveal details of interfacial hydration and the size of cations in solution.

1. Comparing electrical potential distributions for the double helix and a smooth charged cylinder

We now compare the spatial distribution of electrical potential for model IB and its corresponding equivalent cylinder (Fig. 8). We first consider both rod and molecule in the “pocket state,” i.e., in domain \(\Omega^2\), which essentially mimics the situation in free solution (Fig. 4). On first glance, we find that the potential distributions are remarkably similar [Fig. 8(b)]. However, there are, indeed, small differences that stem from the disparities in overall spatial charge distribution caused by differences due to the topography and the charge non-uniformity of the actual molecular surface [Fig. 8(a)].

In order to make a quantitative comparison between the two models, we considered the plane perpendicular to the slit wall containing the axis of the molecule or cylinder (Fig. 1). We extracted the potential distribution and the magnitude of the electrical potential difference, \(\Delta\psi\), between the two models on this plane. Figure 8(b) displays the potential values as a function of radial distance from the object axis, \(\rho\), for both objects. We then determined the electrical potential averaged over the lengths of the two objects [demarcated by dotted lines in Fig. 8(a)] as a function of \(\rho\). We note a larger discrepancy (\(\Delta\psi \approx 0.5\)) in the potential fields in the vicinity of the molecular surface (\(\rho < 20 \text{ Å}\)). We empirically define this region as the electrostatic near field (NF) of the molecule. The difference in length-averaged electrical potential decreases rapidly at larger distances from the object axis reaching (\(\Delta\psi \approx 10^{-2}\)) at about \(\rho = 30 \text{ Å}\), regardless of whether the object is in the “slit” or “pocket” states [gray curve in Fig. 8(b)]. In summary, we find that, as expected, due to differences in the exact spatial distribution of charge between the molecular model and the cylinder, there is, indeed, a disparity in the average potentials in “near fields” (NFs) of the two objects (\(\rho < 2 \text{ nm}\)). This difference in average potential, however, decays monotonically with distance and practically vanishes in the “bulk” (B) and “far field” (FF) regions (\(\rho \geq 2 \text{ nm}\)) (Fig. 1).

Since the free energy functional, given by the integral of Eq. (6), depends on \(\psi\), the quantitative impact of small differences in spatial potential distribution on the calculated interaction free energies is not immediately obvious. In order to address this question, we perform a domain decomposition of the free energy in the system, where once again we split the system into three virtual domains. The first domain represents the near field (NF) region. The molecular NF is demarcated by a cylindrical capsule of radius 20 Å enclosing the molecule or cylinder. This capsule is not only composed of a cylindrical region of the same length as the object of interest but also has spherical caps at each end in order to account for end effects [Fig. 8(a)]. By symmetry, we consider a corresponding near field region at the slit wall. Due to the distance of the wall from the molecule (\(h \approx 38 \text{ nm} \approx 4\kappa^{-1}\)), the near field of the wall is regarded as the electrostatic far field (FF) from the perspective of the molecule. The FF region is thus represented by a layer of thickness 40 Å at the slit walls (Fig. 1).
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C. Electrostatic modeling of A-RNA

For B-DNA, we found that an increase in the probe radius (from 1 to 5 Å) resulted in a small increase in the magnitude of the effective charge by ~1%. This is because an increase in \( r_p \) only slightly modifies the B-DNA surface by smoothing out the surface detail of the molecular geometry and, therefore, as noted above, has a relatively minor impact on the calculated effective charge. Interestingly, the impact of \( r_p \) on the calculated \( q_{el} \) of A-RNA can be much larger (≈12%). Figure 10 displays the influence of probe radius on the surface potentials for various models of A-RNA. A comparison of models IA and IIA (entailing \( r_p = 1 \) and 5 Å, respectively, and \( w = 0 \)) reveals that contrary to B-DNA, the A-RNA structure undergoes a significant change in topography, losing its narrow and deep major groove, which reflects the fact that the grooves become progressively impenetrable to rolling probes of larger radii, as shown in Fig. 10 (top view).

Importantly, the \( q_{el} \) values calculated for A-RNA with \( r_p < 5 \) Å suggest equivalent values of cylinder radii, \( r_{cyl} < r_{helix} \), that are much smaller than the values of nominal structural radii quoted in Table I. While there is no requirement per se for the effective electrostatic cylinder radius to closely reflect the radius of the helical envelope as detected using high resolution structural techniques, we point out that this indication for model IA may, in fact, reflect an unphysical result for the following reasons. One consideration is the continuum character of the PB equation, which also treats ions as point-like particles. It is known that at high magnitudes of electrical potential, the finite size of ions causes the point-ion assumption to break down. This is because the close packing condition—which specifies an upper bound on ionic concentrations—is violated. The maximum magnitude of surface potentials for A-RNA structural models under experimental conditions (\( \psi_{max} \approx 11.5 \)) suggests local counterion concentrations of around 100M \( \gg 9M \), where the latter value represents the close packing concentration for Na\(^+\) ions whose hydrated ion radius, \( q_{el} \approx 2.8 \) Å (Ref. 62). Another aspect of the electrostatics...
influenced by the deep major groove of A-RNA concerns the “hollow spine” along the molecular axis, visible when the molecular structure is viewed from the top (Fig. 10, model IA). In general, electrical potentials in the major groove and hollow spine tend to be high and the point-ion PB model predicts high counterion concentrations in these regions, yet again, it is not clear that these values point to physically plausible concentrations under experimental conditions where ionic species have finite size.

We, therefore, also consider a third model of A-RNA (model IIIA), which involves a hydration sheath (or SAS region) of thickness \( w = 3 \) Å (model IIIA). Similar to model IIA, the major groove and the hollow spine regions disappear, effectively occluding ions from these regions of the molecular structure. The average surface electrical potential in model IIIA drops in relation to models IA and IIA, and we note a corresponding increase to \( r_{\text{cyl}} \approx 11.7 \) Å in the radius of the effective cylinder.

We note that the above three models for A-RNA yield very different values of \( q_{\text{eff}} \) that vary by about 25\% (−19e for model IÀ to about −24e for model IIIA). These \( q_{\text{eff}} \) values, in turn, imply equivalent cylinder radii, \( r_{\text{cyl}} \), that vary by more than a factor 2 (5 Å for model IA to about 12 Å for model IIIA). Thus, if \( b_{\text{helix}} \) is known, a measurement of \( q_{\text{eff}} \) (or \( \eta \)) with an uncertainty of ±2\%–3\% would be able to distinguish between models of the molecular interface whose \( r_{\text{cyl}} \) values differ by about 1 Å (Fig. 11).

![Figure 11](https://example.com/figure11.png)

**FIG. 11.** Equivalent cylinder radii, \( r_{\text{cyl}} \), deduced from mapping the renormalization factor, \( \eta \), for the 30 bp double helix to that of charged cylinders corresponding to B-DNA (\( b = 3.4 \) Å, red line) and A-RNA (\( b = 2.6 \) Å, gray line). Also shown is the dependence of \( \eta \) on the value of the rise per basepair, \( b \), where \( r_{\text{cyl}} \approx 10 \) Å is held constant (blue dashed line). The gradients of the two relationships yield estimates of the expected accuracies on \( b \) and \( r \) in an electrometry measurement. Here, we have \( q_{\text{eff}} \Delta \psi / \Delta b = 0.3 \) Å/\( e \) and \( q_{\text{eff}} \Delta \psi / \Delta r = 1.3 \) Å/\( e \). Assuming a 2\% measurement uncertainty in \( q_{\text{eff}} \) (corresponding to ±0.5e for 30 bp DNA), the aforementioned values imply uncertainties of ±0.2 and ±1 Å in the determination of \( b \) and \( r \), respectively. Electrostatic free energy measurements are, therefore, expected to display greater sensitivity to the rise per basepair of the double helix compared to its radius.

**IV. DISCUSSION**

**A. The role of finite ion size**

Clearly, as alluded to previously, real ions have finite size and we expect the point-ion description to break down or require appropriate adjustment under specific experimental conditions. Such conditions typically arise when ion concentrations exceed the close packing limit. However, importantly, they can also occur at the molecular interface where the electrolyte continuum acquires a “granular” structure whose properties influence, e.g., the location of the boundary (IAS) beyond which the continuum point-ion description could be invoked (blue dashed boundary, Fig. 2). This shortcoming of the PB framework has long been recognized. Using a lattice gas approach, Borukhov et al. proposed a modified PB (MPB) model, which uses the close packing limit to define an upper bound for local ionic concentrations. 17 Importantly, use of this MPB model and the related free energy functionals in our calculations gives free energies that are effectively identical to the point-ion model for B-DNA, as expected. For A-RNA, however, depending on the ion size assumed, the MPB model can yield larger values of \( \Delta F_{\text{el}} \) compared to the point-ion PB model. Larger free energy differences correspond to larger \( q_{\text{eff}} \) and \( \eta \) values, which, in turn, imply larger values of \( r_{\text{cyl}} \) within the point-ion model. This trend is expected on the grounds that the average surface electrical potential for the A-RNA models tends to be higher than that for B-DNA (\( \psi_{\text{max}} \approx 11.5 \) for model IIIA; \( \psi_{\text{max}} \approx 8.7 \) for model IIIB). The trends are also in line with the general expectation from Fig. 6, where finite ion size is accommodated within the point-ion model by a mapping to larger values of \( r_{\text{cyl}} \). However, we point out that the close packing limit is a purely geometric condition, which prohibits unphysical overlap of ions in a lattice model. This particular MPB model does not take into account short-range interactions between ions and surfaces due to which steric effects can manifest at ion concentrations much lower than the close packing limit, as demonstrated in more recently described MPB models. 27 A more refined MPB model may therefore be able to account for ion size effects even in the dilute limit, self-consistently capturing ion size effects for B-DNA. We emphasize, nonetheless, that the point-ion PB model is amenable to a simple adjustment that facilitates modeling of real experiments as summarized below.

Despite the fact that the simple point-ion PB equation does not explicitly incorporate finite ion size, we have found that the model does, indeed, lend itself well to accounting for finite ion size at the molecule/electrolyte interface in our work. The basis of the underlying argument is illustrated in Fig. 2. Essentially, the existence of a hydration layer around the molecule and the finite size of counterions implies the presence of a low dielectric “shell” region in the vicinity of the molecular structure, whose thickness, \( t \), may be expected to be well approximated by the radius of a hydrated counterion. The study presented in Fig. 6 shows that, to a good approximation, a dielectric shell region of thickness at least up to \( t = 5 \) Å surrounding a charged cylinder simply has the effect of inflating the effective radius of the corresponding bare charged cylinder. Note that 5 Å corresponds to the radius of some of the largest (organic) ions in solution. 63 The exterior surface of the dielectric shell region may be regarded as equivalent to an “ion accessible surface” (IAS) beyond which the point-ion description should hold, provided that the local electrical potential \( \psi \lesssim 9 \) for an ion concentration in the
bulk of $c = 1 \text{mM}$. This condition is largely met for B-DNA under the stated experimental conditions. Thus, within the framework of the point-ion PB model, we expect the effective cylindrical radius parameter, $r_{\text{cyl}}$, to successfully capture the effect of finite ion size in the interaction free energy problem.

B. Ion-specific and sequence dependent effects on double helix structure and interactions

We have shown that the simple cylinder model, in conjunction with PB theory, should provide satisfactory ability to discriminate between significantly different helical forms. However, we point out that the uniformly charged cylinder does not adequately describe intricate structural detail arising, e.g., from sequence-specific and ion-specific effects on local helical structure. For instance, recent MD simulations have revealed distinct condensation behaviors for double stranded (ds) DNA and dsRNA molecules in the presence of multivalent ions. Multivalent ions can lead to strong attractions between DNA double helices and facilitate condensation of molecules, while dsRNA resists condensation under similar conditions. This highly distinct behavior has been attributed to different modes of ion binding in DNA and RNA structures, which potentially has an impact on the flexibility of the duplexes. Several studies have also pointed to sequence dependent variations in local helical structure. These effects include deviations from ideal rigid helices, fluctuations in helical coherence length, sequence dependent hydration of duplexes, and ion-dependent structural properties. All these findings suggest that a more realistic model for nucleic acid structure and interactions must go beyond the simple cylinder model by taking into account additional microscopic geometrical and mechanical details. We emphasize, however, that our study has sought to capture coarse-grained average properties of significantly different helical forms that appear to be nicely reflected in the simple cylinder model. The smooth charged cylinder view will have to be relinquished in favor of more detailed, albeit computationally more expensive, molecular structures in order to glean detailed structural information from experimental measurements of free energy.

Moving beyond considerations of molecular structure, multivalent ions have been implicated in other complex phenomena, such as ion correlations, which can have significant impact on interaction free energies and ionic distributions around macromolecules. Although ETc measurements deal with dilute solutions of monovalent ions, multivalent ions are important in the study of dynamic behavior and interactions between helical duplexes and finite ion size in solution. We find that the presence of both a hydration layer and an ion excluded region may be well captured within the simple NLPB model through the use of a larger effective cylindrical radius describing an actual experimental measurement should carry a quantitative signature of interaction free energies (or $q_{\text{eff}}$ values) by about 5%. The corresponding models IA and IIIA for A-RNA, in turn, display a much larger discrepancy of about 25%. A major implication of these findings is that precise experimental measurements (uncertainty <1%) of molecular interaction free energies would be able to distinguish between similar molecular models, thereby shedding light on important aspects of experimental measurements made possible by the application of an $\varepsilon$-MPB model to B-DNA molecules and cylinders. The application of an $\varepsilon$-MPB model to B-DNA molecules and cylinders has shown that these effects are weak for monovalent ions but can lead to considerable departure from the standard PB model in the presence of multivalent ions. In general, however, the PB equation is found to provide a satisfactory description of long range electrostatic interactions of DNA molecules in monovalent electrolytes, which is also in agreement with Monte Carlo (MC) simulations and hypernetted chain approximations. It is worth mentioning that although, in numerous circumstances, PB models can be adapted to provide results, which are in agreement with MD/MC simulations, the results are not necessarily universally applicable due to ion-specific binding properties of different ionic species.

V. CONCLUSION

This study has focused on the use of the PB electrostatics framework to compute molecular electrostatic free energies relevant to high-precision experimental measurements made possible by the single molecule ETc technique. As exemplified in Figs. 7 and 10 and the related results, the calculated free energies can be sensitive to the choice of particular values for parameters, such as effective atomic radii, reflected in the value of the parameter $a$, and the rolling probe radius, $r_p$, used to construct molecular structural models. Nonetheless, our study demonstrates that for a fixed set of input parameters, electrostatic free energies may themselves be computed with the accuracy required to make free energy measurement-based structure/conformation studies meaningful and feasible. Furthermore, development of accurate parameter models for ion interactions with nucleic acids has proved to be a difficult problem, with multiple competing models providing different predictions. Future studies comparing calculated interaction energies with high-precision experimental measurements may, indeed, shed light on elusive parameter values in computational modeling and aid in the testing of atomistic force-fields.

In demonstrating that the electrostatic interactions of the nucleic acid double helix can be successfully mapped onto that of a smooth charged cylinder in solution, we have shown that the radius of the effective cylinder can carry a quantitative signature of interfacial structural detail describing the hydration shell of the molecule and finite ion size in solution. We find that the presence of both a hydration layer and an ion excluded region may be well captured within the simple NLPB model through the use of a larger effective cylindrical radius. Our calculations further suggest that subtle differences in various structural models due to, e.g., the thickness of the hydration shell, as reflected in models IB and IIIB for B-DNA, would differ in their interaction free energies (or $q_{\text{eff}}$ values) by about 5%. The corresponding models IA and IIIA for A-RNA, in turn, display a much larger discrepancy of about 25%. A major implication of these findings is that precise experimental measurements (uncertainty <1%) of molecular interaction free energies would be able to distinguish between similar molecular models, thereby shedding light on important aspects of experimental measurements made possible by the application of an $\varepsilon$-MPB model to B-DNA molecules and cylinders. The dielectric shell model analysis in Fig. 6 shows that the effect of size of real ions in solution can be captured simply by an inflated effective cylindrical radius.
radius. In particular, we expect that \( r'_{cyl} \approx d_{H1} + r_{cyl} \), where \( r'_{cyl} \) represents the effective cylinder in a real electrolyte, \( d_{H1} \) is the radius of the hydrated cation, and \( r_{cyl} \) is the radius of the cylinder in an idealized point-ion scenario. This implies that if the radius of the hydrated counterion in solution is known, it can be subtracted from the measured effective cylindrical radius to obtain an estimate for \( r_{cyl} \) that can be directly compared to the point-ion model results in Fig. 11. Agreement between measured \( r_{cyl} \) values and those calculated for particular molecular models would then define additional molecular parameters, such as the appropriate value for the thickness of the SAS, given by \( t \).

In conclusion, our results suggest that conformational changes in nucleic acids, such as those arising from the formation of secondary and tertiary structures in nucleic acids, e.g., due to internal sequence complementarity or the binding of small molecules, can be detected at the single molecule level by free energy (or effective charge) measurements. Such measurements could prove particularly useful in studies on RNA conformation, where structural investigations lag far behind those for proteins. Molecular modeling of these measurements using the framework presented here could then, permit access to the “conformation spectrum” of a heterogeneous mixture of molecular conformational states in solution.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request and openly available in Zenodo at http://dx.doi.org/10.5281/zenodo.22558 (Ref. 100).

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