Density functional theory for encapsidated polyelectrolytes: A comparison with Monte Carlo simulation

Zhehui Jin and Jianzhong Wu^{a)}

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521, USA

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Genome packaging inside viral capsids is strongly influenced by the molecular size and the backbone structure of RNA/DNA chains and their electrostatic affinity with the capsid proteins. Coarse-grained models are able to capture the generic features of non-specific interactions and provide a useful testing ground for theoretical developments. In this work, we use the classical density functional theory (DFT) within the framework of an extended primitive model for electrolyte solutions to investigate the self-organization of flexible and semi-flexible linear polyelectrolytes in spherical capsids that are permeable to small ions but not polymer segments. We compare the DFT predictions with Monte Carlo (MC) simulation for the density distributions of polymer segments and small ions at different backbone flexibilities and several solution conditions. In general, the agreement between DFT and MC is near quantitative except when the simulation results are noticeably influenced by the boundary effects. The numerical efficiency of the DFT calculations makes it promising as a useful tool for quantification of the structural and thermodynamic properties of viral nucleocapsids *in vivo* and at conditions pertinent to experiments. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4737931>]

I. INTRODUCTION

In essence a viral particle is a self-assembled protein shell (i.e., capsid) containing RNA or DNA molecules. While the protein structure is often well characterized up to atomic details, much more difficult is to understand the biological function and self-organization of the generic material inside the capsid.¹ Experimental studies of the internal structure of a virus often rely on low-resolution methods such as cryo-electron microscopy that do not reflect the flexibility of confined RNA/DNA chains.² Despite the lack of chemical details, the “coarse-grained” structures are useful to study viral replication including genomic organization and assembly of viral capsids (or virus-like particles).³

In recent years, the rapid growth of computing power makes molecular modeling a valuable alternative to determine the internal structures of viral capsids.^{4–6} For example, molecular dynamics (MD) simulation was used to determine the structures of RNA viruses such as satellite tobacco mosaic virus ($T = 1$)⁷ and pariacoto virus ($T = 3$) at atomic details.⁸ Whereas in principle molecular simulations are able to describe the microscopic structure with few assumptions, simulation of the electrostatic interactions in a viral system is computationally demanding. The numerical difficulty arises not only from strong electrostatic interactions between nucleic acids and oppositely charged capsid proteins but from the influence of small ions in the thermodynamic background. Because of the boundary effects and the dynamic nature of RNA/DNA chains, atomistic simulation is impractical to study the viral behavior *in vivo* or at conditions pertinent

to experimental investigations. To establish the generic features underlying virus replication, most simulation and theoretical studies instead hinge on coarse-grained models that neglect the biochemical detail.^{9–18} With suitable parameters to describe non-specific interactions such as molecular excluded volume, van der Waals attractions, and electrostatic interactions, a coarse-grained model is able to predict low-resolution structure and thermodynamic properties of viral capsids such as the radial distribution of genomic materials and the osmotic pressure in good agreement with those from atomistic models or experimental data.^{19–21}

A major challenge in molecular modeling of most viral systems is associated with electrostatic interactions between the genomic materials and oppositely charged capsid proteins. The long-ranged electrostatic interactions are sensitive to local dielectric inhomogeneity and the boundary effects. Unlike bulk systems, charge neutrality may not hold within the space occupied by a viral particle. As a result, we must consider the thermodynamic properties of a viral system together with an ionic environment mimicking experimental or *in vivo* conditions. Because small ions are abundant, simulation of the confined biomolecules in an electrolyte environment is computationally prohibitive. To alleviate such difficulty, one may use analytical methods to account for the ion-mediated electrostatic interactions. The conventional approach to describe the salt effects relies on the Debye-Hückel screening parameter.^{3,12} Because the screened Coulomb potential is valid only when the ionic species are at infinite dilution and because it does not reflect the inhomogeneous distributions of small ions near biomolecules, one may question its applicability to highly charged polymeric species in a viral capsid. A more reliable description of ion screening requires analytical methods beyond the Poisson-Boltzmann theory.

^{a)} Author to whom correspondence should be addressed. Electronic mail: jwu@engr.ucr.edu.

The classical density functional theory (DFT) represents a powerful alternative to molecular simulation for describing microscopic structure and thermodynamic properties of molecular systems.^{22–24} Like simulation methods, DFT is formally exact, i.e., thermodynamic properties of an equilibrium system can be uniquely determined from the density profiles of constituent elements that minimize the grand potential. While “standard protocols” are available for molecular simulation, practical implementation of the DFT often relies on different approximations of the density functionals. The numerical performance of DFT depends heavily on its formulation. Nevertheless, DFT has several key advantages in comparison to molecular simulation methods: it provides explicit structure-property relationships, avoids artificial periodic boundary conditions and enumeration of microscopic events, and most importantly, it is innately applicable to systems with multiple length scales.

In previous work,^{19–21} we used a polymer DFT to study the osmotic pressure and packaging structure of double-stranded DNA in bacteriophages and electrostatic regulation of RNA encapsidation during replication of Hepatitis B virus. Whereas good agreement was found between the theoretical results and experiments, the DFT equations were suitable for systems containing flexible polyelectrolytes. Besides, no effort was made to compare the DFT predictions with simulation data, in particular under conditions that viral particles are surrounded by an external electrolyte solution. In the present work, we incorporate a bending energy into the DFT equations to account for the difference in the intrinsic flexibility of RNA and DNA chains. The numerical performance of the DFT is tested by comparison with extensive MC simulation data for a coarse-grained model of single-stranded RNA/DNA chains inside viral capsids. In addition, we study the responses of the polyelectrolyte structure and the net charge of the polymer-capsid complex to the surrounding solution conditions.

II. MOLECULAR MODEL AND THEORY

A. Molecular model

The purpose of this work is not to study the polymer structure or ion conformation of a specific viral capsid but to calibrate the numerical performance of the DFT by comparison with simulation data. Toward that end, we inherited a coarse-grained model proposed by Linse and co-workers^{25,26} and their MC simulation results provide a benchmark for calibration of the DFT equations. As shown schematically in Fig. 1, the thermodynamic system consists of a single polyelectrolyte chain confined in a capsid represented by a rigid but semi-permeable spherical shell with inner radius designated as R_c and wall thickness as D_c . The capsid bears a total positive charge of Q_c uniformly distributed within the capsid wall at radial distance R_q from the center. The capsid wall is impermeable to the polyelectrolyte segments but permeable to small ions. In MC simulations, the model parameters were selected to approximately represent a $T = 3$ phage virus containing a single-stranded RNA chain.^{25–27}

FIG. 1. A schematic representation of the nucleocapsid system studied in this work. The dark blue spheres stand for polyelectrolyte segments, the green spheres for anions, the red spheres for cations, and the dark red shell with shaded background for a semi-permeable protein capsid. In MC simulations, the capsid is embedded in a spherical cell such that the boundary (solid line) has negligible effects.

The primitive model is used to describe the confined polyelectrolyte and small ions in the thermodynamic background.^{28,29} The polymer is modeled as a linear chain of tangentially connected charged spheres of diameter $\sigma = 4\text{Å}$ and valence $Z_p = -1$. Approximately, each segment represents a nucleotide of a single-stranded RNA molecule. In addition to the bond length defined by touching spheres in a tangent chain, the neighboring segments along the polyelectrolyte backbone are subject to a bending potential³⁰

$$E_b = \phi_0 (1 + \cos \gamma), \quad (1)$$

where ϕ_0 characterizes the bending energy, and γ is the angle of two immediate neighboring bonds for $\gamma \geq \pi/3$, otherwise $E_b = \infty$. Small cations and anions in the thermodynamic background are depicted as charged hard spheres with valence Z_+ and Z_- , respectively. The diameters of these small ions are set to be identical to that of a polyelectrolyte segment. As in the simulation,^{25,26} the solvent (water) and capsid wall are represented by continuous media of the same relative dielectric permittivity. In other words, we assume that the dielectric constant of the capsid wall ϵ_c is the same as that of the solvent, i.e., $\epsilon_c = \epsilon$. While apparently the dielectric constant for the interior of the capsid protein is different from that of an aqueous solution, the dielectric homogeneity greatly simplifies MC simulation and the DFT calculations.

For the convenience of DFT calculations, the bonding potential described above is different from that used in the Monte Carlo (MC) simulation. In Monte Carlo simulations reported by Angelescu *et al.*,^{25,26} the chain connectivity is described within the framework of the bead-spring model where harmonic potentials are used to describe both bond stretching and bending energies. The equilibrium bond length of the polymer chain is $r_0 = 5\text{Å}$ instead of fixed at 4Å in the tangent sphere chain. Besides, MC simulation used discrete charges for the capsid and the viral system was embedded in a spherical cell of radius R_{cell} to mimic the thermodynamic background. It was assumed that the spherical cell

was sufficiently far from the capsid surface such that the ionic properties near the cell boundary are the same as those corresponding to a bulk solution. To facilitate comparison, we select bonding parameters ϕ_0 and γ in our DFT calculations such that the equilibrium bond angle and the intrinsic persistence length of a single polymer chain can be reproduced. The artificial cell is not necessary in DFT calculation; the boundary condition simply is defined by the bulk concentrations of the ionic species.

The coarse-grained model proposed by Linse *et al.* is not accurate for any realistic viral capsid because it does not include molecular-level details such as the nucleotide sequence or the secondary structure of the RNA chain, nor does it reflect the charge distribution of proteins and the icosahedral symmetry of the capsid.¹⁶ But to our knowledge, Linse *et al.* were the first to consider the electrolyte environment outside the viral capsid explicitly. Most previous theoretical studies of virus packaging ignore the ionic distribution outside the viral capsid and assume charge neutrality within the space occupied by the viral particle. Although the model by Linse *et al.* oversimplifies a realistic virus, it does capture electrostatic interactions and excluded volume effects important for viral packaging. In our previous work, the DFT method was used to describe the electrostatic regulation of genome packaging in hepatitis B virus wherein the fenestrated capsid structure, including its flexible C-terminal domain and charge distribution, was considered explicitly.^{21,31}

B. Interaction potential

In dimensionless form, the interaction potential between any pair of charged spheres, denoted by i and j , is given by

$$\beta u_{ij}(r) = \begin{cases} \infty, & r < \sigma \\ Z_i Z_j l_B / r, & r \geq \sigma \end{cases}, \quad (2)$$

where $\beta = 1/(k_B T)$, k_B is the Boltzmann constant, T stands for the absolute temperature, r is the center-to-center distance between spherical particles, and Z_i is the valence of sphere i . The Bjerrum length, $l_B \equiv e^2/(4\pi\epsilon k_B T)$, characterizes the distance between two unit charges (e) in a solvent of dielectric constant ϵ such that the electrostatic potential is equal to thermal energy $k_B T$. For an aqueous solution at temperature $T = 298$ K, $\epsilon = 78$ and the Bjerrum length is $l_B = 0.715$ Å.

Within the tangent chain model described above, the bonding potential of the polyelectrolyte chain $V_b(\mathbf{R})$ satisfies^{21,32}

$$\begin{aligned} \exp[-\beta V_b(\mathbf{R})] &= \prod_{i=1}^{M-1} \frac{\delta(|\mathbf{r}_{i+1} - \mathbf{r}_i| - \sigma)}{4\pi\sigma^2} \\ &\times \prod_{i=2}^{M-1} \exp\left[-\phi_0 \left(1 + \frac{|\mathbf{r}_i - \mathbf{r}_{i-1}| \cdot |\mathbf{r}_{i+1} - \mathbf{r}_i|}{\sigma^2} \cos \gamma\right)\right], \end{aligned} \quad (3)$$

where M is the degree of polymerization, $\mathbf{R} = (\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_M)$ is a set of coordinates representing the polymer configuration, and δ denotes the Dirac-delta function. The first term on the right side of Eq. (3) ensures that the bond length is equal to the segment diameter, and the second defines the chain flexibility.

The latter reduces to Eq. (1) by replacement of $(|\mathbf{r}_i - \mathbf{r}_{i-1}| \cdot |\mathbf{r}_{i+1} - \mathbf{r}_i|)/\sigma^2 = 1$.

The capsid exerts different effective potentials on polyion segments $\Psi_p(r)$ and monomeric ions $\Psi_{+,-}(r)$ because it is permeable to small ions but not to polymer segments. These potentials are given by, respectively,

$$\begin{aligned} \beta \Psi_p(r) &= \begin{cases} \frac{Z_p Q_c l_B}{R_c + \sigma/2}, & r \leq R_c - \sigma/2 \\ \infty, & r > R_c - \sigma/2, \end{cases} \\ \beta \Psi_{+,-}(r) &= \begin{cases} \frac{Z_{+,-} Q_c l_B}{R_c + \sigma/2}, & r \leq R_c - \sigma/2 \\ \infty, & R_c - \sigma/2 < r < R_c + D_c + \sigma/2 \\ \frac{Z_{+,-} Q_c l_B}{r}, & r \geq R_c + D_c + \sigma/2 \end{cases}. \end{aligned} \quad (4)$$

As discussed later, the electrostatic interaction due to the capsid charge is included in the overall mean electrostatic potential.¹⁹

C. Polymeric density functional theory

Most DFT equations used in this work can be retrieved from our previous publications.^{28,29,33} Here we recapitulate the key equations for polyelectrolyte systems. For a system containing polyions and monomeric ions, the grand potential is given by

$$\begin{aligned} \Omega[\rho_M(\mathbf{R}), \{\rho_\alpha(\mathbf{r})\}] &= F[\rho_M(\mathbf{R}), \{\rho_\alpha(\mathbf{r})\}] \\ &+ \int [\Psi_M(\mathbf{R}) - \mu_M] \rho_M(\mathbf{R}) d\mathbf{R} \\ &+ \sum_{\alpha=+,-} \int [\Psi_\alpha(\mathbf{r}) - \mu_\alpha] \rho_\alpha(\mathbf{r}) d\mathbf{r}, \end{aligned} \quad (5)$$

where $d\mathbf{R} = d\mathbf{r}_1 d\mathbf{r}_2 \dots d\mathbf{r}_M$ represents a set of differential volumes, $\rho_M(\mathbf{R})$ is the polyelectrolyte density as a function of the polymer configuration $\mathbf{R}$, $\rho_\alpha(\mathbf{r})$ is the density profile of small ion α , and μ_M and μ_α are the chemical potentials of the polyelectrolyte chain and small ions, respectively. In Eq. (5), F stands for the intrinsic Helmholtz energy; $\Psi_\alpha(\mathbf{r})$ is the external potential for small ions, and $\Psi_M(\mathbf{R})$ is the external potential for a polyelectrolyte chain given by

$$\Psi_M(\mathbf{R}) = \sum_{i=1}^M \Psi_M(\mathbf{r}_i). \quad (6)$$

The intrinsic Helmholtz energy consists of an ideal-gas term (F^{id}) that is determined by the bond potentials and the molecular configuration, and an excess (F^{ex}) that accounts for the thermodynamic non-ideality due to different contributions of non-bonded inter-segment interactions. For the polyelectrolyte system, the intrinsic Helmholtz energy includes five inter-related contributions:

$$F = F^{id} + F_{hs}^{ex} + F_{ch}^{ex} + F_C^{ex} + F_{el}^{ex}, \quad (7)$$

where F^{id} stands for the ideal-gas term, F_{hs}^{ex} is the excess Helmholtz energy due to hard-sphere repulsion, F_{ch}^{ex} is the excess Helmholtz energy due to chain connectivity, F_C^{ex} is the excess Helmholtz energy due to direct Coulomb interaction,

and F_{el}^{ex} is the excess Helmholtz energy due to electrostatic correlations.

The ideal-gas term F^{id} depends on the chain configuration, the bonding potential, and the segmental distribution of small ions,

$$\beta F^{id} = \int d\mathbf{R} \rho_M(\mathbf{R}) [\ln \rho_M(\mathbf{R}) - 1] + \beta \int d\mathbf{R} \rho_M(\mathbf{R}) V_b(\mathbf{R}) + \sum_{\alpha=+,-} \int d\mathbf{r} \rho_\alpha(\mathbf{r}) [\ln \rho_\alpha(\mathbf{r}) - 1]. \quad (8)$$

where bond potential $V_b(\mathbf{R})$ includes both bond length and bond angle contributions as described by Eq. (3).

The excess Helmholtz free energy due to hard-sphere repulsion F_{hs}^{ex} can be accurately represented by the modified fundamental measure theory (MFMT)^{34,35}

$$\beta F_{hs}^{ex} = \int \Phi^{hs} [n_w(\mathbf{r})] d\mathbf{r}, \quad (9)$$

where the reduced excess free energy density Φ^{hs} is represent by six weighted densities $n_w(\mathbf{r})$ ³⁶

$$\Phi^{hs} = -n_0 \ln(1 - n_3) + \frac{n_1 n_2 - \mathbf{n}_{V1} \mathbf{n}_{V2}}{1 - n_3} + \frac{1}{36\pi} \left[n_3 \ln(1 - n_3) + \frac{n_3^2}{(1 - n_3)^2} \right] \frac{n_2^3 - 3n_2 \mathbf{n}_{V2} \mathbf{n}_{V2}}{n_3^3}. \quad (10)$$

The detailed expressions for weighted densities $\{n_w, w = 0, 1, 2, 3, V1, V2\}$ can be found in Rosenfeld's original work.³⁶ The hard-sphere repulsion depicts the molecular excluded volume effects; it plays a key role in short-range interactions between oppositely charged species and thus is important for describing packaging of a polyelectrolyte chain in an oppositely charged capsid.

In addition to the direct bonding energy, the Helmholtz energy functional should include a contribution due to intra-chain correlations. According to an extension of the first-order thermodynamic perturbation theory (TPT1),^{29,37} the excess Helmholtz free energy due to the polymer chain connectivity F_{ch}^{ex} is given by

$$\beta F_{ch}^{ex} = \frac{1 - M}{M} \int n_{0p} \zeta_p \ln y(\sigma, n_w) d\mathbf{r}, \quad (11)$$

where $\zeta_p = 1 - \mathbf{n}_{V2p} \mathbf{n}_{V2p} / n_{2p}^2$ accounts for the effect of local density variation, and $y(\sigma, n_w)$ is the contact value of the cavity correlation function (CCF) of the polymeric segments. A key assumption of TPT1 is that CCF can be represented by that corresponding to a monomeric reference system^{31,38}

$$y(\sigma, n_w) = \left[\frac{1}{1 - n_3} + \frac{n_2 \sigma (1 - \mathbf{n}_{V2} \mathbf{n}_{V2} / n_2^2)}{4(1 - n_3)^2} \right] \times \exp \left(-\frac{\Gamma^2 a_p^2}{4\pi^2 l_B \sigma} \right) \exp \left(\frac{l_B Z_p^2}{\sigma} \right), \quad (12)$$

where parameters Γ and a_p are calculated from

$$\Gamma = \sqrt{\pi l_B \sum_{k=p,+, -} n_{0k} \left(\frac{1}{1 + \Gamma \sigma} \right)^2 \left(Z_k - \frac{\pi P_n \sigma^2}{2(1 - n_3)} \right)^2}, \quad (13)$$

$$a_p = \frac{2\pi l_B \left(Z_p - \frac{\pi P_n \sigma^2}{2(1 - n_3)} \right)}{\Gamma (1 + \Gamma \sigma)}, \quad (14)$$

with

$$P_n = \sum_{k=p,+, -} \frac{2n_{1k} Z_k}{1 + \Gamma \sigma} \left/ \left(1 + \frac{3}{(1 - n_3)} \sum_{k=p,+, -} \frac{n_{3k}}{1 + \Gamma \sigma} \right) \right. \quad (15)$$

The excess Helmholtz free energy due to the electrostatic interactions includes two parts: one is due to the direct Coulomb interaction as that in the Poisson-Boltzmann (PB) equation,³⁹ and the other part accounts for electrostatic correlations.²⁹ Given the distributions of charged species, the energy due to the direct Coulomb interactions is

$$\beta F_C^{ex} = \frac{l_B}{2} \sum_{i,j=p,+, -} \iint d\mathbf{r} d\mathbf{r}' \frac{Z_i Z_j \rho_i(\mathbf{r}) \rho_j(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|}, \quad (16)$$

where $p, +, -$ stand for polyion segments, monomeric cations, and anions, respectively. Because of the long-range nature of the electrostatic potential, the integrals on the right side of Eq. (16) diverge for any pair interaction. To avoid the numerical problem, we need to calculate the electrostatic energy from the local mean electrostatic potential

$$\psi(\mathbf{r}) = \int d\mathbf{r}' \sum_{j=p,+, -} \frac{Z_j e \rho_j(\mathbf{r}')}{4\pi \epsilon |\mathbf{r} - \mathbf{r}'|}, \quad (17)$$

where e stands for the unit charge. Equation (17) is the integrated form of the Poisson equation

$$\nabla^2 \psi(\mathbf{r}) = -\frac{\rho_c(\mathbf{r})}{\epsilon}, \quad (18)$$

where $\rho_c(\mathbf{r})$ denotes the local net charge density. Neglecting contributions due to hard sphere repulsion and intra-chain correlations, we would have a Helmholtz energy functional [Eqs. (8) plus (16)] equivalent to that from the conventional Boltzmann distribution for charged species. Therefore, we may consider the Poisson-Boltzmann equation as a simplified version of the classical DFT.

Besides the direct Coulomb energy, the excess Helmholtz free energy includes a contribution due to electrostatic correlations F_{el}^{ex} . The electrostatic correlation effect can be obtained by a quadratic functional expansion of excess Helmholtz free energy with respect to that for a bulk fluid

of uniform densities $\{\rho_i^b\}$,^{40,41}

$$\begin{aligned} \beta F_{el}^{ex} = & \beta F_{el}^{ex} [\{\rho_i^b\}] - \int d\mathbf{r} \sum_{i=+,-} \Delta C_i^{(1)el} [\rho_i(\mathbf{r}) - \rho_i^b] \\ & - \frac{1}{2} \iint d\mathbf{r} d\mathbf{r}' \sum_{i,j=+,-} \Delta C_{ij}^{(2)el} (|\mathbf{r} - \mathbf{r}'|) \\ & \times [\rho_i(\mathbf{r}) - \rho_i^b] [\rho_j(\mathbf{r}') - \rho_j^b], \end{aligned} \quad (19)$$

where $\Delta C_i^{(1)el}$ and $\Delta C_{ij}^{(2)el}$ are the first-order and the second-order direct correlation functions (DCF) due to the electrostatic correlations in the reference monomeric system

$$\Delta C_i^{(1)el} = -\beta \mu_i^{el} = -\delta \beta F_{el}^{ex} / \delta \rho_i(\mathbf{r}) \Big|_{\rho_i(\mathbf{r})=\rho_i^b}, \quad (20)$$

$$\Delta C_{ij}^{(2)el} (|\mathbf{r} - \mathbf{r}'|) = -\delta^2 \beta F_{el}^{ex} / \delta \rho_i(\mathbf{r}) \delta \rho_j(\mathbf{r}') \Big|_{\rho_i(\mathbf{r})=\rho_i^b, \rho_j(\mathbf{r}')=\rho_j^b}. \quad (21)$$

In writing Eq. (19), we assume that the electrostatic correlation due to the chain connectivity can be ignored.²⁹ The excess chemical potential and the DCF can be obtained from mean-spherical approximation (MSA) for the monomeric ions.^{31,42}

At equilibrium, the grand potential is minimized with respect to the density profiles of polyion segments and small ions, leading to the Euler-Lagrange equations:

$$\rho_M(\mathbf{R}) = \exp \left\{ \beta \mu_M - \beta V_b(\mathbf{R}) - \sum_{i=1}^M \left[\beta \Psi_p(\mathbf{r}_i) + \frac{\delta \beta F^{ex}}{\delta \rho_p(\mathbf{r}_i)} \right] \right\}, \quad (22)$$

$$\rho_\alpha(\mathbf{r}) = \exp \left[\beta \mu_\alpha - \beta \Psi_\alpha(\mathbf{r}) - \frac{\delta \beta F^{ex}}{\delta \rho_\alpha(\mathbf{r})} \right]. \quad (23)$$

For the polymer systems considered in this work, the density profiles of polyion segments and monomeric ions vary only in the r direction, i.e., the radial direction of a spherical capsid. As a result, the Euler-Lagrange equation for small ions can be simplified as

$$\rho_\alpha(r) = \exp [\beta \mu_\alpha - \beta \lambda_\alpha(r)]. \quad (24)$$

The effective one-body potential for a small ion (or a polyion segment), $\lambda_\alpha(r)$, depends on the external potential $\Psi(r)$, the mean electrostatic potential $\psi(r)$, and local excess chemical potentials due to intermolecular interactions and correlation effects:

$$\lambda_k(r) = \Psi'_k(r) + Z_k e \psi(r) + \delta (F_{hs}^{ex} + F_{ch}^{ex} + F_{el}^{ex}) / \delta \rho_k(r). \quad (25)$$

In Eq. (25), the mean electrostatic potential is obtained from integration of the Poisson equation

$$\begin{aligned} \psi^*(r) \equiv \beta e \psi(r) = & \psi^*(r=0) - 4\pi l_B \\ & \times \int_0^r dr' \left(r' - \frac{r'^2}{r} \right) \sum_{k=p,+, -} Z_k \rho_k(r'). \end{aligned} \quad (26)$$

Equation (26) satisfies boundary condition $d\psi(r)/dr|_{r=0} = 0$. at the capsid center. By applying another boundary condition $\psi(r=\infty) = 0$, we can obtain mean electrostatic potential at capsid center, e.g. $\psi^*(r=0)$. Because the electrostatic potential accounts for contributions from all charges, the external potential $\Psi'_k(r)$ in Eq. (25) excludes the direct Coulomb energy.

The local density of polyion segments $\rho_p(\mathbf{r})$ is determined from

$$\rho_p(\mathbf{r}) = \sum_{i=1}^M \rho_{si}(\mathbf{r}) = \sum_{i=1}^M \int d\mathbf{R} \delta(\mathbf{r} - \mathbf{r}_i) \rho_M(\mathbf{R}). \quad (27)$$

If the polyelectrolyte chain is intrinsically flexible (i.e., no bending potential), the density profile of the polyion segments is calculated from

$$\rho_{p,flex}(r) = \frac{\sum_{i=1}^M \exp[-\beta \lambda_p(r)] G_{flex}^i(r) G_{flex}^{M+1-i}(r)}{\int 4\pi r^2 dr \exp[-\beta \lambda_p(r)] G_{flex}^i(r) G_{flex}^{M+1-i}(r)}, \quad (28)$$

where $\lambda_p(r)$ represents the effective potential for a segment, $G_{flex}^i(r)$ stands for the propagator function (i.e., Green function), which is determined from the recursive relation

$$G_{flex}^i(r) = \int dr' G_{flex}^{i-1}(r') \exp[-\beta \lambda_p(r')] \frac{\theta(\sigma - |r - r'|)}{2\sigma}, \quad (29)$$

for $i = 1, 2, \dots, M$ with $G_{flex}^1(r) = 1$.

For a sufficiently long polyelectrolyte ($M \gg 1$), the end effect is negligible.¹⁰ In that case, the Green function becomes indistinguishable for all segments (i.e., $G_{flex}^i(r) \approx G_{flex}(r)$) and Eq. (28) is simplified to

$$\rho_{p,flex}(r) = \frac{M \exp[-\beta \lambda_p(r)] G_{flex}^2(r)}{\int 4\pi r^2 dr \exp[-\beta \lambda_p(r)] G_{flex}^2(r)}, \quad (30)$$

with

$$G_{flex}(r) = \int dr' G_{flex}(r') \exp[-\beta \lambda_p(r')] \frac{\theta(\sigma - |r - r'|)}{2\sigma}. \quad (31)$$

For a semi-flexible polyelectrolyte chain, we must consider the bending potential. With the assumption of no-end-effect,^{21,43} the segmental distribution is given by

$$\rho_{p,semi}(r) = \frac{\exp[-\beta \lambda_{p,semi}(r)] S(r)}{\int 4\pi r^2 dr \exp[-\beta \lambda_{p,semi}(r)] S(r)}, \quad (32)$$

where

$$\begin{aligned} S(r) = & \int_{|\sigma_p - r'|}^{\sigma_p + r'} dr' r' G_{semi}(r, r') \\ & \times \exp[-\beta \lambda_{p,semi}(r')] G_{semi}(r', r). \end{aligned} \quad (33)$$

The propagator for a semi-flexible polyelectrolyte chain is

$$G_{semi}(r, r') = \frac{1}{2\sigma r} \int_{|r-\sigma|}^{r+\sigma} dr'' r'' G_{semi}(r'', r) P(\alpha, \theta, r''), \quad (34)$$

where

$$P(\alpha, \theta, r'') = \exp[-\beta \lambda_{p,semi}(r'') - \phi_0(1 + \cos \theta \cos \alpha)] I_0(\sin \theta \sin \alpha). \quad (35)$$

In Eqs. (34) and (35), α and θ are the inside angles formed by the i th bond and $i - 1$ th bond with vector $\mathbf{r}_i$ and $\mathbf{r}_{i-1}$, respectively. $I_0(x) = \frac{1}{2\pi} \int_0^{2\pi} \exp(-x \cos \Phi) d\Phi$ is a modified Bessel function. More details about the segmental distribution function of semi-flexible polyelectrolyte are referred to the supplementary materials of Ref. 21.

We solve the density distributions of polyion segments and small ions with the Picard iteration method. The numerical procedure starts with an initial guess for the density profiles of polyions and monomeric ions based on their bulk densities. The reduced electrostatic potential $\psi^*(r)$, the effective fields for polyion segments and monomeric ions, $\lambda_k(r)$, and the Green function $G^i(r)$ for polyions are then calculated by iterations. Subsequently, we update the new density profiles using Eqs. (24) and (28). The new density profiles are mixed with the previous results as the new inputs. The entire iteration procedure repeats until the change in the input and output density profiles of polyions and monomeric ions is smaller than 0.01% at all positions.

III. RESULTS AND DISCUSSIONS

The main objective of this work is to validate the numerical performance of the polyelectrolyte density functional theory (DFT) with molecular simulations for polyelectrolytes in spherical capsids. To explore a broad parameter space, we consider polyelectrolytes with different bending energies and electrolyte solutions with various salt concentrations and ionic valences. We compare the density profiles of polyions and small ions to those obtained from Monte Carlo simulations.

Because of numerical difficulties in calculation of electrostatic interactions in an extended environment, simulation results are rare for confined polyelectrolytes. Fortunately, Angelescu *et al.*^{25,26} reported extensive MC studies on the structure and thermodynamic properties of polyions confined in charged capsids. For the sake of comparison, the parameters in our DFT calculations are chosen to be the same as those used in their studies, i.e., $\sigma = 0.4$ nm for ionic and segment diameters, $R_c = 5.0$ nm for the inner radius of the spherical capsid, and $D_c = 1.2$ nm for the capsid thickness. For numerical convenience, our DFT calculations are based on the tangent-chain model for the polymer backbone but the simulation used the bead spring model. With a reasonable choice of the parameters for the bond connectivity, we expect that the difference between these two models is insignificant. The cut-off distance in the DFT calculations (or the size of calculation cell) is chosen to be the same as the simulation cell. Because the simulations were performed in the NVT ensemble, we use

the chemical potentials as the Lagrange multipliers to impose the conservation of mass for each species inside the computation cell.

A. Flexible polyelectrolytes

We first compare the density profiles of polyelectrolyte segments and small ions with the MC results. For a flexible polyelectrolyte chain, the parameter specifying the intrinsic bending stiffness satisfies $\beta\phi_0 = 0$.

Figure 2 shows the distributions of polyion segments obtained from the DFT calculations and from MC simulations for flexible polyelectrolytes of different chain lengths in a spherical capsid. The total capsid charge is $Q_c = +250$, and the valences of cations and anions are $Z_+ = +1$ and $Z_- = -1$, respectively. As in MC simulation, we consider three different polyion chains with length $M = 100$, $M = 250$ and $M = 400$; the corresponding radii of the computation cells are $R_{cell} = 50$ nm, $R_{cell} = 56.3$ nm and $R_{cell} = 61.4$ nm, respectively. As indicated by Angelescu *et al.*,^{25,26} the total charges of capsid and polyelectrolytes in a real virus are typically much larger than those used in the MC simulation. Even for a very small virus the capsid carries more than 2000 positive charges and the number of monomers in the RNA/DNA chain is about the same order of magnitude.²⁵ Because simulation of the viral particle in an electrolyte environment is

FIG. 2. (a) Density distribution of polymer segments $\rho_p(r)$ is plotted as a function of radial distance r for a flexible polyelectrolyte confined in a capsid with total charge $Q_c = +250$. Here the polymer chain length is $M = 100$ and each segment carries unit negative charge, the valences of cations and anions are $Z_+ = +1$ and $Z_- = -1$, respectively. In MC simulation,²⁵ the cell radius is $R_{cell} = 50$ nm, the numbers of cations and anions simulated are $N_+ = 100$ and $N_- = 250$, respectively. (b) The same as Fig. 2(a) except that the polymer chain length is $M = 250$, cell radius $R_{cell} = 56.3$ nm, and the number of cations simulated is $N_+ = 250$. (c) The same as Fig. 2(a) except that the chain length is $M = 400$, the cell radius is $R_{cell} = 61.4$ nm, and the number of cations simulated is $N_+ = 400$.

extremely time-consuming, meaningful MC results were obtained only for capsids with genome size and surface charges much smaller than those in real capsids. In all cases, the small ions reach bulk properties near the cell boundary. In MC simulations, the number of anions is fixed at $N_- = 250$, the same as the number of charges of the spherical capsid, and the number of cations are $N_+ = 100$, $N_+ = 250$, and $N_+ = 400$ for three different polyion chain lengths. The total cation charge is the same as the charge of the confined polyelectrolyte. The model system mimics a capsid dispersed in a dilute aqueous electrolyte solution.

Despite different models for the polymer backbone, the DFT is able to reproduce the MC results near quantitatively. However, the distinctive features of soft and hard potentials are noticeable near the capsid surface within a distance comparable to the bond length. While the density profile generated by MC is quite smooth due to the use of a soft potential for the chain connectivity, the tangent hard-sphere chain model exhibits sharp edges near the hard wall and such effects become more visible as the polymer concentration/chain length increases. The polyelectrolyte segments accumulate near the inner surface of capsid due to strong electrostatic attraction from the positively charged surface. The density of polymer segments is near zero at the capsid center until the total charge of polyelectrolyte is significantly larger than the capsid charge (e.g., $M = 400$). Despite that the polyelectrolyte and the capsid surface have opposite charges, the DFT predicts that the polymer segments are slightly repelled from inner surface. Such depletion, also noticeable in the simulation, arises from the intra-chain correlations.

Figures 3 and 4 show, respectively, the density profiles of monomeric anions and cations for the systems discussed above. The agreement between DFT and MC simulation is excellent for both cases. Because the cations are coupled with

FIG. 3. The same as Fig. 2 but for the density profiles of anions.

FIG. 4. The same as Fig. 2 but for the density profiles of cations.

polyelectrolyte segments, the difference between DFT and MC simulation shown in Figure 4 is probably due to discrepancies on polymer segment distributions near the inner capsid surface. When the net charge of the confined polyelectrolyte is less than that of the capsid wall, the capsid contains more anions than cations. However, when the polyelectrolyte has equal or more net charge than the capsid, strong accumulation of polyion segments near the inner capsid surface makes the integrated charge distribution function $P(r) = \int_0^r dr' \sum_{k=p,+,-} Z_k \rho_k(r')$ negative at $r \approx R_c - \sigma/2$. In this case, the electric field produced by such charge distribution (according to the Gauss law) points inward to the capsid center and therefore repels the anions from accumulating at the inner capsid surface but attracts cations. On the other hand, the positive electric field from the capsid out surface has opposite effect and a competition between the electric field and excluded-volume effect results in a maximum in the distribution of cations at $r = 4$ nm.

When the polyion charge is sufficiently less than the capsid charge (e.g., $M = 100$), the polyelectrolyte-capsid complex is positively charged and thus attracts anions to and repels cations from the outer capsid surface. The preferential distributions of anions and cations are shown in Figures 3(a) and 4(a), respectively. When the polyion charge is significantly larger than the capsid charge (e.g., $M = 400$), on the other hand, the complex is negatively charged and thus attracts cations to and repels anions from the outer capsid surface. The overcharged capsid attracts extensive number of cations into the capsid, as shown in Figure 4(c). Although electrostatic interactions facilitate the polymer segments to reside at the inner surface of the capsid, the polymer segments extend into the capsid center due to strong excluded-volume effect and intra-chain correlations. When the polyelectrolyte and capsid have the same electric charge ($M = 250$), some an-

ions are released from the capsid and some cations reside inside the capsid, making the net charge of the complex slightly positive. Overall, the small ion distributions inside the capsid do not compensate the net charge of polyelectrolyte and capsid complex and the net charges inside the capsid are $108e$, $6e$, and $-23e$, respectively, in good agreement with the simulation data.²⁵ Our DFT calculation affirms that the overall charge of a capsid complex is not neutral. In general, the charge neutrality is valid only for the entire system that includes both polymer-containing capsid and small ions in the bulk solution.

B. The intrinsic rigidity of polyion

Now we validate the DFT results for confined semi-flexible polyelectrolytes with MC simulation data,²⁵ again by comparing the density profiles for the polymer segments as well as for small ions. The model parameters are similar to those used in the previous subsection except that the potential for chain connectivity takes in to account the bending stiffness ($\beta\phi_0 = 10$). The value of the bending stiffness is calculated from the relation between the persistence length l_p and bond length r_0 approximated by Woodward and Forsman^{21,43}

$$l_p \approx \phi_0 r_0. \quad (36)$$

In MC simulation by Angelescu *et al.*,²⁵ the persistence length of polyelectrolyte is $50 \text{ \AA}$ and the equilibrium bond length is $r_0 = 5 \text{ \AA}$.

Figure 5 shows the DFT and MC simulation results for the density distributions of semi-flexible polyelectrolyte segments in a spherical capsid. Here the model parameters are the same as those used in Figure 2 but with the bending stiffness for the polymer backbone. A close comparison of Figures 2 and 4 shows noticeable influences of the chain stiffness on

FIG. 5. The same as Fig. 2 but for a semi-flexible polyelectrolyte with bending stiffness parameter $\beta\phi_0 = 10$.

FIG. 6. The same as Fig. 5 but for the density profiles of anions.

the density distributions of polymer segments. In contrast to flexible polyelectrolytes, the density profiles for semi-flexible polyions do not exhibit sharp edges near the capsid surface and show stronger affinity. Evidently, the bending rigidity suppresses the penalty of chain configurational entropy. Furthermore, the contact density of semi-flexible polymer at inner capsid surface is slightly higher than that of flexible polyelectrolyte. Although we take into account the bending energy on the mean-field basis, the DFT predictions corroborate well with the simulation data.²⁵

Figures 6 and 7 present the density profiles of anions and cations for the confined semi-flexible polyelectrolytes, respectively. Similar to encapsidation of intrinsically flexible polyions discussed above, the agreement between DFT and MC simulation is very good. Unlike polymer segment distributions, the effect of chain stiffness on the distribution of small ions is insignificant. Because a semi-flexible polyelectrolyte chain is closer to the inner surface of capsid, the capsid contains slightly fewer cations in comparison to the case of flexible polymer. At the same solution condition, the net charge of semi-flexible polyion-capsid complex remains similar to that of flexible polyion-capsid complex. In other words, it appears that the stiffness of the polymer backbone has little effect on the net charge of the polyelectrolyte-capsid complex.

C. Effects of salt valences

We also compare the DFT calculations with MC simulation for the segmental distributions of polyelectrolytes in electrolyte solutions with various ionic valences. In this case, the valences of polyion segment and anion remain the same (-1), but cations have three different valences ($Z_+ = 1, 2, 3$). Because the effect of chain stiffness was considered in the

FIG. 7. The same as Fig. 5 but for the density profiles of cations.

previous subsection, the comparison here is focused on systems containing a flexible polyelectrolyte.

Figure 8 shows the DFT and MC simulation results for the density distributions of flexible polyelectrolyte segments

FIG. 8. (a) Density distribution of polymer segments $\rho_p(r)$ versus radial distance r for a flexible polyelectrolyte in a spherical shell. Here the polyelectrolyte chain length is $M = 252$ and each segment carries unit negative charge. The total charge of the spherical shell is $Q_c = +252$. In MC simulation, the cell radius is $R_{cell} = 10$ nm, the numbers of cations and anions simulated are $N_+ = 252$ and $N_- = 252$, respectively, and the valences of cations and anions are $Z_+ = +1$ and $Z_- = -1$, respectively. The solid lines represent results from the DFT calculation and symbols are from MC simulation.²⁶ (b) The same as (a) except for $Z_+ = +2$ and that the number of cations used in the simulation is $N_+ = 126$. (c) The same as (a) except for $Z_+ = +3$ and that the number of cations simulated is $N_+ = 84$.

FIG. 9. The same as Fig. 8 but for the density distributions of monomeric anions.

in a spherical capsid surrounded by electrolytes of different cation valences.²⁶ In all cases, the cell radius is fixed at $R_{cell} = 10$ nm, and the polyion chain length is $M = 252$. As the cation valence increases, the polyion segments are more depleted from the inner surface because of stronger screening effects. The polymer packing density near the capsid inner surface falls as the cation valence increases; more polymer segments are distributed near the capsid center. While the DFT captures the simulation results qualitatively, the quantitative discrepancy increases with the cation valence. Such difference can be attributed in part to approximations of the DFT and in part due to the small simulation range,²⁶ which makes the properties of small ions near the boundary different from those in the bulk. Because of the overall small ionic concentration, such difference has a drastic effect on the density profiles. Furthermore, MC simulation introduced an interaction between the small ions and the cell wall and the hard-wall potential is absent in the DFT calculations. Because electrostatic interactions are sensitive to the ionic valence, the cell boundary might influence the distributions of small ions and polymer segments.²⁶ While the size effect is significant in MC simulation, the DFT calculation fully incorporates the long-ranged electrostatic interactions and the properties of small ions at the boundary are exactly the same as those in the bulk.

Figures 9 and 10 present the density distributions of anions and cations, respectively, for the system discussed above. As the cation valence increases, the capsid contains more anions to neutralize the positively charged capsid surface. The accumulation of cations drives polyion segments away from the capsid surface toward the center (Fig. 8). For the reasons discussed above, for multivalent ions the DFT results corroborate MC simulation only qualitatively.

FIG. 10. The same as Fig. 8 but for the density distributions of cations.

IV. CONCLUSIONS

We have used a polymer density functional theory (DFT) to study the structural and electrostatic properties of flexible and semi-flexible polyelectrolytes confined within spherical capsids. The DFT equations are similar to those used in our previous works for inhomogeneous polyelectrolyte solutions.^{19,29} Specifically, we adopted the modified fundamental measure theory to account for the excess Helmholtz energy functional due to the hard-sphere repulsion. The extended first-order thermodynamic perturbation theory accounts for the excess Helmholtz free energy functional due to the polyion chain connectivity. The mean-spherical approximation accounts for the excess Helmholtz energy functional due to the electrostatic correlation. The mean electrostatic potential from Poisson equation takes into account the excess Helmholtz energy functional due to direct Coulomb interactions. Unlike most previous models for viral packaging,^{18,19} this work considers the polyelectrolyte containing capsids in equilibrium with a bulk salt solution.

Extensive comparison of the DFT and simulation results indicates that the DFT provides an efficient and reliable way to describe the density distributions of polyelectrolyte segments and small ions as well as net charges of capsid complex for different chain lengths and bending stiffness. Similar to our previous work for inhomogeneous polyelectrolytes, the agreement between DFT and MC simulation²⁵ is near quantitative over a broad range of the parameter space pertinent to polyelectrolyte encapsidation. The numerical performance is less satisfactory for multivalent systems when there is certain discrepancy in the thermodynamic conditions defining the DFT and MC calculations. Similar to MC simulation,²⁵ the DFT calculations reveal that the small ions do not compensate the net charge of the polyelectrolyte and capsid complex and the assumption of local charge neutrality is in general not valid.

Whereas computer simulation of viral packaging is often hindered by the expensive computational cost due to the explicit consideration of small ions and the surrounding environment, DFT is very efficient to account for the electrostatic interaction and packaging effect explicitly. The good agreement between DFT and MC simulation ensures applicability of DFT to the study of virus packaging in realistic systems that often have a few thousands of opposite charges for the capsid protein and nucleic acids.

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