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Investigation of viral DNA packaging using molecular mechanics models

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Abstract

A simple molecular mechanics model has been used to investigate optimal spool-like packing conformations of double-stranded DNA molecules in viral capsids with icosahedral symmetry. The model represents an elastic segmented chain by using one pseudoatom for each ten basepairs (roughly one turn of the DNA double helix). Force constants for the various terms in the energy function were chosen to approximate known physical properties, and a radial restraint was used to confine the DNA into a sphere with a volume corresponding to that of a typical bacteriophage capsid. When the DNA fills 90% of the spherical volume, optimal packaging is obtained for coaxially spooled models, but this result does not hold when the void volume is larger. When only 60% of the spherical volume is filled with DNA, the lowest energy structure has two layers, with a coiled core packed at an angle to an outer coaxially spooled shell. This relieves bending strain associated with tight curvature near the poles in a model with 100% coaxial spooling. Interestingly, the supercoiling density of these models is very similar to typical values observed in plasmids in bacterial cells. Potential applications of the methodology are also discussed.

Keywords: Viral packaging; Computer simulation; Molecular mechanics; Nucleic acid structure; DNA condensation; Bacteriophage P4

1. Introduction

Some bacteriophage have linear double-stranded DNA (ds-DNA) genomes that are tightly packaged into a roughly spherical volume inside the viral capsid [5]. The packaging of genomic DNA poses two problems for bacteriophage. First, a very high degree of condensation is required, with the final DNA concentration being on the order of 300 mg/ml [18]. This must be achieved without the benefit of higher order protein–DNA structures like those found in eukaryotic chromatin. Second, ejection of the DNA at the time of infection requires that the DNA not be knotted or entangled.

Diverse models have been proposed for the packaging of phage ds-DNA. The DNA might

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have local ordering but no long-range ordering, so that it is a liquid crystal [22]. Alternatively, there may be overall ordering into a fairly well-defined geometry. Among the latter possibilities are coaxial spooling [12,31], spiral folding [6], folded coaxial spooling [38], and a folded toroid [17]. Recent cryo-electron microscopy experiments appear to be most consistent with coaxial spooling, at least for bacteriophage T7 [8].

The issue of viral packaging has received renewed importance because of the quality of data from cryo-electron microscopy, and because of other experimental developments. Single molecule measurements are now capable of measuring the forces and pressures generated during packaging [19,40], and new 2D gel electrophoretic methods allow the quantitative determination of the relative frequencies of differently knotted conformers of closed circular DNAs like those found in bacteriophage P4 [47]. Furthermore, DNA packing in lipid complexes resembles packing in bacteriophage, with obvious implications for gene therapy [37]. Accurate modeling tools should assist in the interpretation of these and future experiments. Here we focus on the spooling model and its consequences, using P4 as a model.

Low resolution models for DNA can be used to examine issues of DNA packaging, supercoiling and knotting [26,30]. A number of approaches have been used to model DNA molecules in the size range 1–30 kilobasepairs (kb). The molecules have been described mathematically using finite elements [2,54], Fourier series [56], and B-splines [14,35,36]. DNA can also be treated as a segmented chain, using bead models (pseudoatom representations) suitable for hydrodynamic and molecular mechanics algorithms [20,23,27,28, 43,48], and that is the approach we use here.

Unlike traditional molecular mechanics models in which each atom is represented as a point mass [29], low resolution models use a reduced representation, with appropriate pseudoatoms representing pieces of the structure. Our original model [43] used three pseudoatoms to define the plane of each basepair. The potential energy function was parameterized so that the elastic moduli for stretching, bending and twisting deformations matched those for double helical DNA. The model is thus a discretized approximation to a continuum elastic model. It has been used to investigate a number of questions about the structure, topology and dynamics of small closed circular DNAs [41,45,46].

Here we investigate the packaging of molecules in the size range of genomic phage DNA inside viral capsids, approximating the icosahedron by a sphere. Since we are using the model to investigate questions of global organization rather than local structural details, we do not need atomic detail, or even to represent each basepair. ds-DNA in bacteriophage does not differ significantly from normal B-DNA [1] and does not interact strongly with the capsid [39], so we can use a very simple model with a single pseudoatom to represent one turn of the double helix. This reduces the computational requirements and permits rapid calculations on molecules in the size range 10–30 kb. A variety of traditional molecular mechanics algorithms (energy minimization; Monte Carlo; simulated annealing; molecular dynamics (MD); Brownian dynamics) can be used to refine static models, to sample conformational space, to estimate average properties of thermodynamic ensembles, and to investigate conformational transitions.

2. Methods

All modeling and data analysis were carried out with YAMMP [44], a molecular modeling package developed specifically for reduced representation models. See http://uracil.cmc.uab.edu/YammpWeb for more information on the YAMMP package.

The model uses one pseudoatom to represent ten basepairs (approximately one turn of the double helix). Successive pairs of pseudoatoms are held together by harmonic bonds with an ideal length of 34 Å, the pitch of the double helix. The bond stretching force constant can be parameterized using the relationship between this stiffness and the root mean square (rms) fluctuations in the bond. The energy of a bond of length b is

$$E = \frac{k}{2}(b - b_0)^2$$

where b_0 is the ideal bond length and k is the stretching force constant. The distribution of bond energies is specified by the Boltzmann distribution,

$$P(b) = e^{-E(b)/RT} = e^{-k(b-b_0)^2/2RT}$$

where R is the universal gas constant and T is the temperature. This function is a Gaussian, with mean b_0 and variance

$$\sigma^2 = RT/k$$

A force constant of 50 kcal/(mol Å^2) was chosen for this study. It allows rms fluctuations of approximately 0.1 Å at $T=300$ K. The properties of the model are insensitive to this parameter, since the principal deformation of the double helix on packaging into a virus is bending, not longitudinal compression.

To represent DNA bending deformations, successive triplets of pseudoatoms describe harmonic bond angles with an ideal value of π (180°); the force constant for angular deformations is 4.35 kcal/(mol rad^2), which gives an rms fluctuation of 21.4° per 10 bp segment, or 6.6° per basepair. It can be shown [33] that this corresponds to the experimental persistence length of 150 bp [13]. As will be seen below, over 99% of the deformation energy of well packed models arises from this term in the energy function. This is as it should be, since the principal deformation of the DNA on packaging into the viral head is bending.

A nonbonded repulsion term is required between all pairs of pseudoatoms to prevent overlap between different regions of the double helix and to prevent strand crossing. The interhelical center-to-center distance is in the range 25–30 Å in DNA condensed by polyvalent cations [34], and the average distance in T4 is 27 Å [12]. Cryo-EM shows that the packing distance in T7 depends on the amount of DNA that is in the capsid, ranging from 25 Å when the capsid is packed with the full genome to 28 Å when DNA corresponding to 84% of the full genome is packaged [8]. Two extreme cases were examined in the present pilot study, one with an exclusion diameter of 25 Å and the other with an exclusion diameter of 30 Å. Volume exclusion effects are enforced by a semiharmonic repulsion: the nonbonded energy is zero for interparticle distances greater than the exclusion diameter and rises as the square of the overlap distance for distances less than that, with a force constant of 50 kcal/(mol Å^2). The spacing of successive pseudoatoms is sufficient to prevent chain crossings during optimization. This was verified by monitoring the degree of knotting of closed circular models, using the *knot* program [15]. The optimal conformations are not very sensitive to the value of the force constant, but, as will be seen, variations in the exclusion diameter over the range 25–30 Å have significant effects on the final global conformations, because the DNA volume is proportional to the square of the exclusion diameter, and the optimal conformation depends on what fraction of the capsid volume is filled.

To restrain the DNA within a spherical volume representing the capsid, an additional pseudoatom is placed at the origin of coordinates and constrained to that position using the YAMMP *lock* function. (*Lock* is a true constraint, not a force-dependent restraint, so the central pseudoatom cannot move at all.) This pseudoatom is not attached to any other atom through bond or angle interactions, and it is not included in the nonbonded interactions, so it has no exclusion volume. All other atoms are restrained within the sphere by a semiharmonic energy function with $E=0$ when the distance r from the atom of interest to the central atom is less than a specified bounding radius, R, and $E=k(r-R)^2$ when $r>R$. This is mathematically equivalent to the NOE restraint commonly used for refining NMR structures, but $R=190$ Å in the present case. The bounding radius is equal to the inner radius of the spherical shell (205 Å) minus the nonbonded radius of each DNA pseudoatom (15 Å). A large force constant, $k=50$ kcal/(mol Å^2), was used to make the capsid very stiff, but the results are not sensitive to this parameter, since changes in this parameter simply alter the effective radius of the sphere a little, and DNA packing is not altered by changes in the radius on the order of 1% or so.

The energy function in YAMMP is expressed in a descriptor file. The YAMMP utility *mkchaindes* was used to generate appropriate descriptors for the models used in this investigation.

The P4 ds-DNA is a linear molecule, but the ends are believed to be in close proximity [7,25].

This prevents knotting or entangling and is a consequence of the attachment of one end of the DNA to an anchoring protein in the connector region [9]. In mutant P4 lacking the anchoring protein, the cohesive ends of the linear ds-DNA can find each other and anneal together in the virus, producing a variety of knotted closed circular molecules [25,47]. To guarantee that the molecules not be entangled, we confined this initial study to unknotted closed circles. (Suitable linear models with two ends in proximity could be easily generated by cutting the circle near the surface of the capsid.)

Starting conformations were generated using various in-house utility programs, and a variety of procedures were tested for optimizing the structures. Conjugate gradient energy minimization is sufficient to package any initial structure, but the energy and quality of the resulting model depend strongly on the initial conformation. Models were further optimized using simulated annealing with MD, followed by minimization. MD can produce substantial collective motions that are evidently useful in refining such structures, and the 'flying icecube' artefact of velocity rescaling [16] can be used to good advantage in this respect. A timestep of 25 fs was used in all the MD simulations reported here. Temperature was maintained by either rescaling or reassigning velocities at intervals of 100 timesteps. Monte Carlo simulated annealing did not prove effective in these studies, because it does not represent inertial effects and cannot easily generate large collective motions.

Writhe was calculated with an in-house program using two independent algorithms, the Gauss integral [53] and the projection method [4]. Agreement between these methods guarantees accuracy of the calculations.

3. Results

3.1. Random packing of a closed circular DNA molecule

The power of the method is illustrated by the compression of a relaxed closed circular DNA molecule into the volume corresponding to that of a small viral capsid (Fig. 1). With an exclusion diameter of 30 Å for the DNA, this model occupies over 90% of the available volume. A variety of starting conformations were generated, each corresponding to a typical structure for a relaxed closed circular 10 kb ds-DNA. The starting structures are quite extended, with radii of gyration on the order of 100 nm. The relaxed closed circle has a very low energy (14 kcal/mol), all of which is due DNA bending, arising from the slight deformation of bond angles connecting successive triplets of atoms along the chain. Wild-type P4 anchors one end of its chromosome to prevent knotting, but randomly generated closed circular ds-DNA chains of this size have a 3% probability of being knotted [32], so we verified that all conformers in this study were unknotted, using the *knot* program [15].

When the spherical constraint force on all the pseudoatoms is applied, the energy is greater than 10^9 kcal/mol, and the corresponding forces are immense. Packing is achieved by simply crumpling the DNA into a compact ball (Fig. 1). Conjugate gradient energy minimization is sufficient to do this in a few minutes of CPU time, but the resulting energy is quite high (2720 kcal/mol). Again, all of this is due to the bending deformations: the only component of the total energy greater than 1 kcal/mol is the bond angle bending term.

The poor quality of the final model in Fig. 1 is a consequence of having squashed a large, extended molecule into the capsid. No amount of optimization—even extended high temperature MD—is capable of generating the extensive structural reorganization that would be required to generate a well-relaxed final structure. A better starting model is needed.

3.2. Refinement of a densely packed coaxially spooled model

A much better structure can be generated by placing each pseudoatom along a path that approximates a coaxial spool, and then refining this model. The initial conformer is generated using a set of parametric equations in spherical coordinates. The first atom is placed on the surface of the sphere ($r=R$) at a longitude of 0 ($\theta=0$) and a colatitude specified by the user ($\phi=\phi_0$, typically

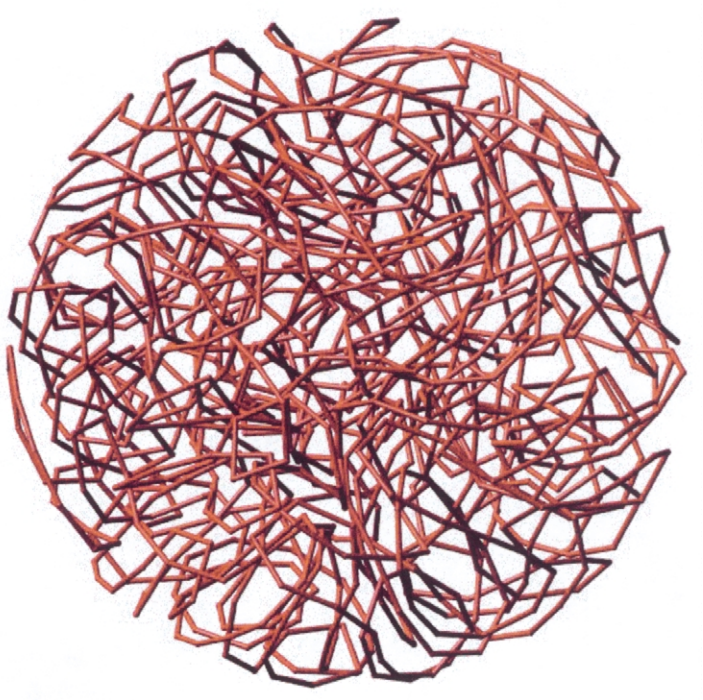

Fig. 1. Forced packing of a relaxed closed circular DNA containing 10 kb into a spherical volume with radius 190 Å. The DNA occupies over 90% of the available volume. The initial structure was very extended, with a radius of gyration about an order of magnitude larger than that of the packed structure. The initial model was not set up in a way designed to produce smooth packaging, so the final structure is badly distorted and has a large energy associated with the many sharp bends. In this and subsequent figures, the cylindrical representation shows the path of the center of the DNA double helix. The graphical diameter is only a fraction of the true diameter of the helix, in order to permit a clear view of the interior.

approximately 20°, i.e. a latitude of 70°). The spherical coordinates of successive atoms (r', θ', ϕ') are obtained by incrementing those of the previous atom according to the following rules:

$$r' = r - d^3/4\pi R^2$$

$$\phi' = \phi + sbd/[2\pi r^2 \sin(\phi)]$$

$$\theta' = \theta - b/[r\sin(\theta)]$$

where d is the diameter of the DNA double helix (again 30 Å), b is the interatom bond length, R is the radius of the sphere, and s is a sign function that controls the direction of spiral packing, downward (from north to south) when $s=-1$ and upward (from south to north) when $s=+1$. With s initially set to -1, ϕ is decremented until packing reaches the latitude limit near the south pole, at which point s is set to $+1$ and the direction of winding is reversed. (With $\phi_0 = 20°$, reversal occurs at a latitude of $-70°$.) Successive reversals of the sign occur whenever ϕ passes the latitude limit approaching either pole. The molecule is closed into a circle by adding a bond from atom 1000 back to atom 1.

The rough approximation to an ideally packed coaxial spool from the above equations produces some bad nonbonded overlaps in the unrefined structure, giving a nonbonded energy in excess of 3×10^6 kcal/mol. The very long bond between atoms 1000 and 1 is another serious structural problem, with an initial bond energy of more than 10^6 kcal/mol. Both of these problems are quickly repaired by a few 1000 steps of conjugate gradient energy minimization, yielding a structure (Fig. 2) that does not differ substantially from the starting spool. The deformation energy of the final structure (456 kcal/mol) is contained entirely in the

Fig. 2. Initial packing of a relaxed closed circular DNA containing 10 kb into a small spherical volume. A set of parametric equations (see text) was used to give a coaxially spooled initial model. Refinement with energy minimization gives a structure with a residual bending energy of 456 kcal/mol. The roughly parallel strands are spaced at the interstrand contact distance of 30 Å.

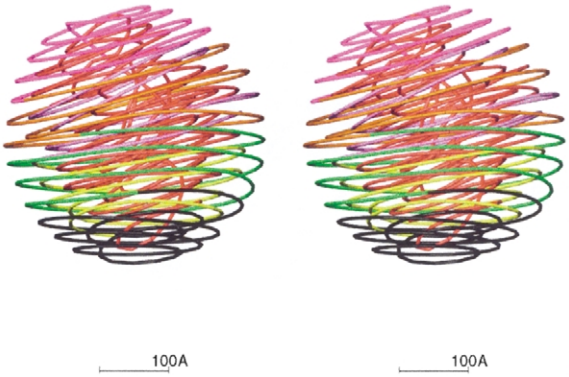

Fig. 3. Optimal packing of a relaxed closed circular DNA containing 10 kb into a small spherical volume. Simulated annealing of the structure in Fig. 2, with velocity rescaling at intervals of 100 steps to drive the temperature linearly from 300 to 10 K over a period of 2.5 ns (100 000 steps), was followed by energy minimization, giving a model with a residual bending energy of 446 kcal/mol. Longer annealing schedules failed to achieve a lower energy structure, as did simulated annealing using MD with velocity reassignments, or using Monte Carlo.

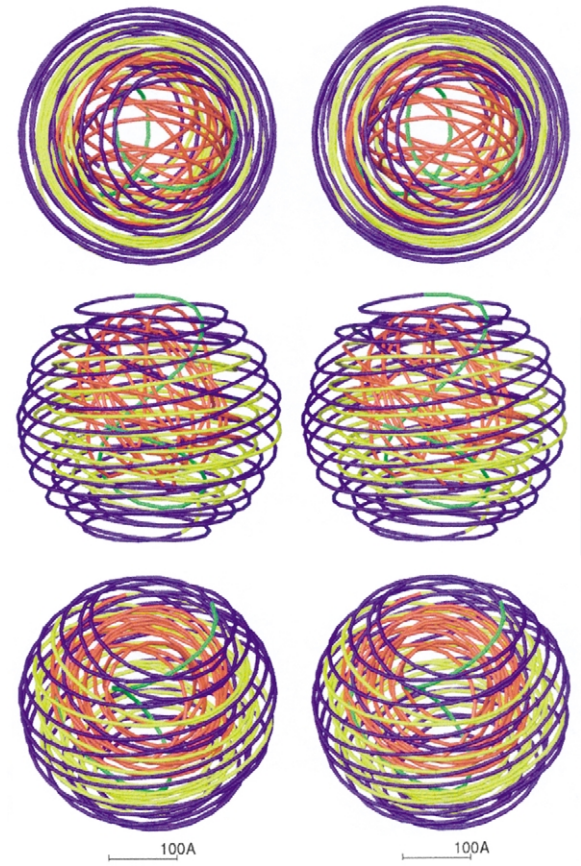

Fig. 4. Optimal packing of a relaxed closed circular DNA containing 10 kb into a sphere with substantial free volume (stereo views). All parameters for this model are the same as those for the models in Figs. 2 and 3, except the nonbonded exclusion diameter has been reduced from 30 to 25 Å. The volume occupied by the DNA is thus $(25/30)^2 = 69\%$ of that in the other models, or approximately 60% of the total volume inside the capsid. The initial structure was axially spooled along the full length of the molecule, except for a short connector running down the center of the spool, so it was organized in a fashion similar to that shown in Fig. 2. In the refined model, which has been extensively optimized, there are two separately coiled regions. The outer region consists of two coaxially spooled layers, containing approximately 7.5 kb (blue and yellow). The cavity inside these layers is occupied by the second coil (red), containing approximately 2.2 kb and packed at a sharp angle to the first. Approximately 0.3 kb (green) are required to connect the innermost and outermost layers. The top and middle images are from approximately orthogonal views, while the bottom image shows a view that is roughly along the principle axis of the innermost coil. The structure is not knotted.

bond angle bending term, with all other components being less than 0.1 kcal/mol.

The structure was further refined by simulated annealing followed by energy minimization to generate two additional models. The lowest energy model was derived from a simulated annealing run using MD with velocity rescaling, followed by energy minimization (Fig. 3); velocity reassignments were also tried. A substantial reorganization of the entire DNA molecule takes place (compare Fig. 2 and Fig. 3). Some loops that were originally in interior layers expand by pushing through the outer layers on one side of the capsid. Two or three of the innermost loops turn almost 90°, producing strands that run vertically through the central cylindrical hole, then doubling back. This extensive reorganization generates an energy drop of only 10 kcal/mol, to 446 kcal/mol. When velocities are reassigned, rather than rescaled, there is some reorganization of the model, but the structure does not change as dramatically as in rescaling. With reassignments, the final energy after a long MD run and energy minimization (461 kcal/mol) is slightly higher than that obtained by minimization alone (456 kcal/mol). The success of the simulated annealing run with velocity rescaling provides additional evidence for our earlier observation that rescaling leads to a violation of the principle of equipartion of kinetic energy, with most or all of the kinetic energy going into low frequency collective modes [16]. This artefact is obviously useful in the present case, where large collective reorganization is needed.

3.3. Refinement of a less densely packed model

To examine volume exclusion effects, we next built and refined a series of spooled models for the case where the interhelix contact distance is 25 Å, which is at the lower limit of published values for viral DNA packing. All other parameters, including length of the DNA, were kept constant. The reduction in DNA diameter produces a dramatic reduction in packing density, with the DNA occupying slightly less than 70% of the available volume inside the capsid.

Before optimization with simulated annealing, the spooled DNA is coaxially spooled along 98% of its length, but simulated annealing with velocity reassignments causes a drastic reorganization of the molecule (Fig. 4). This reorganization relieves the bending deformation of those parts of the DNA that are packed in the innermost layers and at relatively high latitudes. Initially, these regions are more sharply curved than those parts of the DNA in the outermost layer and packed around the equator. By forming a second spirally packed spool structure inside the first and at an angle to it, the curvature can be reduced. The packing of the outer two layers (blue and yellow in Fig. 4) is not perfectly regular. One loop of the yellow region has pushed through to the outside of the ball (right side of center view). The outer coil is thicker near the south pole (bottom of center view) than near the north pole, so the cavity occupied by the inner coil (red) lies largely in the northern hemisphere. This asymmetry is almost certainly a consequence of the fact that the connection between the innermost and outermost layers (green) is through the north polar region.

4. Discussion

Cryo-electron microscopy on oriented tailless T7 heads reveals a quasi-crystalline packing of the DNA into six coaxial shells, consistent with the coaxial spooling model [8], and it is likely that this motif occurs in some other viruses with ds-DNA genomes. Here we have described methods for modeling packaging in such systems, using energy minimization to generate idealized models for coaxially spooled DNA. When the packing density is high, we find that little reduction in energy is obtained by extensive optimization, consisting of simulated annealing followed by minimization. This suggests that a coaxially spooled structure is indeed an optimal form in viruses with little free volume. When the packing density is reduced, however, the lowest energy structure contains a tightly coiled internal region packed in the central cavity of an outer spooled region, with the axes of these two regions at a substantial angle to one another. These deviations from perfect coaxial spooling allow the DNA to reduce the bending energy associated with the tight curvature near the two poles of the capsid. It remains to be seen

whether such structures occur in real viruses, but we note here that strands in the inner layer are less regularly packed than those in the outer region, so they will be more difficult to see by diffraction methods or by image reconstruction methods.

Packaging of a closed circular DNA molecule into a spherical capsid can be considered from the standpoint of DNA supercoiling. The average superhelical density in a plasmid in a bacterial cell is approximately -0.05 [55]. Since the DNA double helix is much more resistant to twisting deformations that bending deformations, almost all of the supercoiling pressure is normally relieved by writhing. The calculated writhe for the conformer in Fig. 3 is -45.2; since the model corresponds to a DNA containing approximately 1000 turns of the double helix, this corresponds to a supercoiling density of approximately -0.45. The initial spooling trajectory was generated with a set of empirical equations, without any consideration of the consequences for supercoiling, but it is interesting to note the remarkable coincidence of the resulting supercoiling density with typical biological values.

Structures similar to that shown in Fig. 4 were recently reported in a series of Brownian dynamics simulations in which the DNA is injected into the capsid in a step-by-step fashion [19]. Interestingly, those authors observed structures like that shown in Fig. 4 only when their force field contained an attractive term mimicking the forces responsible for DNA condensation; they stated that 'in the absence of attractions between nearby chain segments, a much more disordered structure is formed during loading.' The difference between our result and their attraction-free result is probably due to some combination of three factors. First, free volume must play a role, since we find a substantial inner coil when there is greater free volume (compare Fig. 3 and Fig. 4). Second, ours is a closed circular molecule, while theirs is a linear molecule with unconstrained ends; it is not clear if this plays a role or not. Third, kinetic barriers may prevent conversion of our final structure to one resembling theirs, or vice versa; the energy difference between the two structures is unknown, and it is unclear which structure is at the global energy minimum and which is at a local minimum.

The pressure inside viral capsids has recently been calculated from single molecule measurements of the forces generated by the portal motor that drives DNA into bacteriophage $\phi 29$ [40]. It will be an interesting challenge to understand the relative contributions of DNA bending (which we can measure with these models) and the attractive and repulsive contributions arising from DNA–DNA self interactions (which we have not yet included). The first steps toward this goal have been taken in a recent set of elegant calculations on a continuum model, parameterized to match experimental data [19].

5. Conclusions

Reduced representations like the one reported in the present work are potentially powerful tools for teasing out the interplay of the various energetic factors on the structural, kinetic and thermodynamic aspects of DNA packaging. It is already clear that the optimal structure will depend on the free volume within the capsid, and on whether or not attractive interactions are present. Little is presently known about the proper functional form for treating attractive interactions for DNA condensation within the molecular mechanics formalism, although the Lennard-Jones approximation [19] is not an unreasonable first approach.

The modeling tool developed here will also have utility for examining a number of other problems in large-scale DNA structure. One potential application is the investigation of knots and catenanes in closed circular DNA molecules in the 10–100 kb size range. DNA topology has important biological ramifications [10,11,42,49,50,52] and can be investigated experimentally by a variety of methods, including cryo-electron microscopy [3,24] and sedimentation and gel electrophoresis [21,47,51]. Our methods can be used to generate ensembles of linear molecules and to evaluate the distribution of knotted and catenated isomers that result after those are closed into circular forms, either free in solution or within the confines of viral capsids.

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