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Phage-like Packing Structures with Mean Field Sequence Dependence

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Summary

Packing of double-stranded DNA in phages must overcome both electrostatic repulsions and the problem of persistence length. We consider coarse-grained models with the ability to kink and with randomly generated disorder. We show that the introduction of kinking into configurations of the DNA polymer packaged within spherical confinement results in significant reductions of the overall energies and pressures. We employ a kink model which has the ability to deform every 24 bp, close to the average length predicted from phage sequence. The introduction of such persistence length defects even with highly random packing models increases the local nematic ordering of the packed DNA polymer segments. Such local ordering allowed by kinking not only reduces the total bending energy of confined DNA due to nonlinear elasticity, but also reduces the electrostatic component of the energy and pressure. We show that a broad ensemble of polymer configurations is consistent with the structural data.

Graphical abstract

DNA sequence determines the response to packing stress in some phages.

Left is a purely elastic model and right is a mean field sequence model with structural defects determined by the nonlinear elastic response to sequence thermodynamics.

A. Introduction

The physical confinement of double-stranded DNA within viral capsids is a theoretically and experimentally long-studied problem [1]-[3]. Single-molecule experiments [4]-[8] and

osmotic pressure ejection inhibition experiments [9]-[11] determine the dynamics of packaging and the thermodynamics of the various states of packaged phages. Structural distributions of the nucleic acid within packaged phages are generated via cryo-electron microscopy (cryo-EM) by averaging over many virus particles [12]-[15]. Here, we propose a model which explains both the thermodynamics and existing structural information.

Models of phage packaging have most often assumed highly-ordered spool-like configurations, known as “inverse spool” models in which the DNA bends smoothly and isotropically while maintaining a 50-nm persistence length [16]-[19]. Experiments indicate the forces measured for phage-packaging motors to be as much as six times greater than predicted by these coaxially-spoiled models which approximate the DNA inside phages as highly-ordered, smoothly bent rings with hexagonal packing [8]. More recent models have moved beyond such essentially analytic models by implementing coarse-grained simulations of electrostatically-interacting DNA beads-on-a-string [20]-[23]. Most of these models, with the exception of our own [24], assume the entire genome remains in canonical B-form well within the phage capsid, thus maintaining a harmonic-bending potential with the stiff persistence length of ~50 nm. While these elastic rod models do not demand the perfect inverse spool, a high degree of spool-like ordering is still found in the final structures by using relatively long equilibration times (microseconds) allowing the rod-like model to approach equilibrium at each stage of a gradual packaging process [21], [22]. Experiments, however, indicate heterogeneity in packaging rates due to glassy dynamics and that final packaged states are nonequilibrium, glassy states [25]. There is evidence that such structures may not accurately represent actual phage packaging structures because the subsequent simulated ejection times [26] are much less (on the order of microseconds) than the *in vitro* and *in vivo* experimental ejection times, which are on the order of seconds to minutes [27], [28]. It would be expected that knotted configurations amongst final packaging states would frustrate the ejection process and thereby slow it down. It has been also observed that 97% of knots found in tailless phage mutants have greater than 10 crossings [29], further suggesting that packaged conformations are highly knotted and frustrated states. Some models, applied to the study of knotting distributions of lower complexity phage knots (rather than the thermodynamics of the packaged DNA structures), employ cholesteric nematic-ordering interactions which help to favor certain aligned structures [20]. Highly-ordered elastic models have been popular based on interpretations of experimental structural data, initially from small-angle x-ray diffraction indicating spacings on the order of close-packed DNA helices [1], and, more recently, from 3-D symmetric and asymmetric reconstructions using cryo-electron microscopy (cryo-EM) [12]-[15]. Such reconstructions are dependent upon alignments of the capsid and portal proteins, and typically give lower (non-atomic level) resolution density maps of the DNA packaged inside the mature phages. These density maps contain shells of DNA density that appear as rings within 2-D cross sections, which have often been interpreted as implying highly-ordered spooled structures [18], [19]. However, we previously demonstrated that the cryo-EM structural data do not necessarily imply highly-ordered spooled structures of the DNA packaged within viral capsids [23]. That work showed that density distributions consistent with those seen in cryo-EM density maps could be obtained from time-averaging across simulation trajectories of a random liquid of beads with the diameter of dsDNA. We found the density shells and rings

to be a reflection of the correlation among DNA segments and with the confining capsid. That work implied that much larger ensembles of polymer configurations could be consistent with cryo-EM maps and that neither spooled conformations nor correlations induced by less-ordered polymeric configurations are necessary for structural agreement with experimental data [23]. Some cryo-EM studies have suggested more disordered final states (with few individual DNA segments resolved) which indicate highly-disordered intermediate states undergoing disorder-to-order transitions in the later stages of packaging near 70-100% genome-filling [30], calling into question the validity of more spool-like structures, which would require ordering earlier in the packaging, in favor of late transitions of glassy, knotted, nonequilibrium final states. This did not imply much about the thermodynamics of any specific less-ordered hypothetical polymeric configuration; however, it did suggest a re-examination of the thermodynamic principles of packaged phage DNA that control the allowed conformations of fully-packaged genomes.

Motor proteins of various phage portals have been studied in some detail. Although the precise mechanisms that phage motors utilize to drive DNA packaging into the capsid continue to be debated, single-molecule experiments have established a rotation of the DNA, presumably inducing negative-twist into the molecule [5]. In addition, multiple models of packaging have been hypothesized with mechanisms of motor packaging involving torsional compression with transitions to and from A-form DNA [31], [32]. Cryo-EM experiments of phage portal DNA show toroidal shapes indicating torsional strain on the DNA molecule [33], [34]. The effects of torsional strain on the DNA molecule may be important to the thermodynamics of phage packing.

The effects of torsional strain on the elasticity of DNA have been demonstrated via both experiment [35] and simulation [36]. All-atom simulations of DNA sequences have shown mild negative and large positive twist to be capable of inducing localized structural variations [36] with the potential for marked changes in persistence length. Cryo-EM density maps of DNA inside phage portals contain densities which seem to indicate deviations from helical structures and may imply structures with strand- opening holes [12] reminiscent of holes induced by negative-twist torsional strain within the all-atom simulations mentioned above. Using sequence-specific models of portal phage dsDNA, simulations have shown that sequence-dependent twist-induced persistence length defects can explain the cryo-EM density data [24]. Our combining of those simulations with the phage portal sequence information indicates that such defects occur within an AT-rich tract (e.g., ATATTA) containing multiple TpA steps, which are known to be the most thermodynamically-unstable dinucleotide steps [37].

The known torsional strain placed on DNA during the packaging process and the sequence-specific effects such as inducing kinking defects imply that sequence-dependent effects may be important in the overall thermodynamics of phage genome packaging. In addition to the helical strand separation believed to occur in the portal channel, many more similar local structural variations may occur within the rest of the packaged genome. Previous models of phage packaging have not incorporated sequence data. Here, by modeling the average sequence-dependent effects of torsional strain combined with model random packings, we compare with the experimental data of phage-packaging structure and thermodynamics.

B. Methods

1. Coarse-Grained Modeling

To reduce the computational demand required to simulate tens of kilobase-pair-long genomes, we model the phage genome using coarse-grained (CG) models rather than all the atoms. We model mechanically-relaxed DNA as 6-bp beads-on-a-string with screened repulsive electrostatic interactions and angle-dependent bending and stretching contributions between adjacent 6-bp segments with parameters corresponding to a 50-nm persistence length and the dimensions of B-DNA.

$$V_{total}(R) = V_{bend}(\theta) + V_{stretch}(R) + V_{el}(R) \\ = k_b(\theta - \theta_0)^2 + k_s(R - R_0)^2 + V_{el}(R)$$

where V_{bend} is the bending potential between three adjacent beads, $V_{stretch}$ is the bond potential between two adjacent beads, and V_{el} is the screened electrostatic potential. Here

$k_b = 3.5 \frac{\text{kcal}}{\text{mol radian}}$, $k_s = 22.4 \frac{\text{kcal}}{\text{mol \AA}}$, $R_0 = 20 \text{ \AA}$ and $\theta_0 = 180^\circ$. The bending force constant is taken as zero every 4 beads to model the average effects of sequence dependent twist induced double strand pairing defects. (see below)

Even in the presence of condensed counterions, the electrostatics comprise the largest contribution to the overall potential energies. The form of our CG model employs ion-penetrable spheres to represent six-base pair segments of double-stranded DNA which interact via an electrostatically- repulsive potential derived from classic DLVO theory of charged colloids [38], given by

$$V_{el}(R) = \frac{q^2 L_B}{(1 + \kappa \alpha)^2} \frac{\exp(-\kappa(R - 2\alpha))}{R}$$

where $q = 12e^-$ is the electrostatic charge from backbone phosphate groups $L_B = 7.135 \text{ \AA}$ is the Bjerrum length, $\kappa = 0.31 \text{ \AA}^{-1}$ is the inverse Debye screening length, $\alpha = 19.9 \text{ \AA}$ is the radius of the DNA segments, and R is the separation between such segments. Such coarse-grained models are similar to other models which also treat DNA as polymeric chains of ion-penetrable spheres or ellipsoids with electrical double layer interactions and have been successfully applied to surface-bound DNA microarray experiments [39]-[41], as well as models of phage packaging [22]. Our choice of potential includes the full negative charges of the phosphate backbone with simple Gouy-Chapman double layer ionic-screening and depends only on the ionic-screening conditions as input variable parameters. This allows us to study some effects which changes in ionic distributions and in counterion condensation can have on the thermodynamics.

2. Average Sequence Twist-Induced Kinking Effects

As mentioned, the possible effects of torsional strain on the local elasticity of DNA have been previously demonstrated. As TpA steps are the most thermodynamically-unstable dinucleotide steps [37], we expect regions containing them to be the most likely regions for localized structural defects to occur due to torsional strain. By plotting the frequencies of various dinucleotide steps across the λ -phage genome within 100 kbp sliding windows, we found non-trivial distributions for certain steps including TpA and CpG (Fig. 1). The prevalence of TpA steps in the vicinity of the *ori* sequence, for example, is consistent with our hypothesis of decreased thermodynamic stability favoring strand opening and denaturation since this region serves as the origin of replication for the phage genome once it has circularized within its host cell.

We expect the thermodynamic instability under the torsional strain of cyclization to aid the necessary strand separation for replication to occur within the infected host bacterium analogous to our prediction of denaturation due to torsional strain within the phage. To simplify the computational modeling, we average over the details of the non-trivial distribution and determine that *on average*, across the entire λ genome, TpA dinucleotide steps occur every ~22 basepairs. We model this mean probability of kinking as a uniform distribution every 1 in 24 basepairs, occurring every 4 beads in our CG model in which each bead represents a 6-bp segment of dsDNA. This represents an average or mean- field approximation for the likelihood of sequence-informed twist-induced kinking and subsequent reduction in the local bending elasticity. As mentioned, mechanical twist comes from the phage motor during packing. As a coarse-grained model of the twist induced change in persistence length we assume freely-jointed chain connections every 24 bp. Since every dinucleotide step has a melting probability depending on context and other correlations this model is probably an upper bound on the number of allowed defects.

3. Polymer Configuration Sampling

We utilize path sampling techniques to generate initial polymer configurations of fully-connected dsDNA polymers per phage for MD simulation by starting from initially disconnected bead segments. In order to generate near-equilibrium globules—each containing one fully-connected, continuous chain of DNA per phage—we employ Monte Carlo path sampling techniques which grow the polymer chain by selecting nearest-neighbor monomers in the vicinity of the growing end of the polymer chain, accepting or rejecting each move according to a Boltzmann-weighted probability for the newly-formed bending energy.

To avoid inescapable traps in conjunction with our sampling of polymer paths, we employ techniques from the polymer literature known as “backbite” moves (also known as an “end-bridging” moves). Specifically, we employ an off-lattice type II internal rebridging move (backbite) which was shown to be the most efficient such algorithm in a Monte-Carlo study of conformational sampling of stiff polymer melts for long-chain polymers of greater than 400 monomers [42]. Such a move involves growing the polymer chain by allowing the addition of not only free monomers but also those monomers previously connected in the growing polymer chain (hence the “backbite”), thereby altering the connectivity of the

polymer chain. Such a move is accepted or rejected per the Boltzmann-weighted probability of the energy difference between the proposed and current bonds. If accepted, the current bond is broken, a new bond is formed, and a new end of the growing polymer chain is formed. Similar methods have previously been used in the studies of coil-globule transitions of homopolymers [43] and to generate configurations of polymers for MD simulation [44]. This algorithm was recently applied to smaller test systems of DNA phage packaging used to probe the effects of chain stiffness and nematic ordering in DNA knotting [45].

By varying the ionic strength of the potential, we also consider the effects of salt on the pressures and energies of packaged polymeric DNA. While a bulk ionic concentration is known for the experimental solutions, the ion concentrations within the capsid is not measured by experiment. Some experiments indicate possible *local* Mg^{2+} concentrations as high as 2-4 times the bulk concentration within the capsid [46].

We previously utilized a Debye screening length ($1/\kappa$) of 3.3 Å, appropriate to the experimental bulk conditions with ionic concentrations of 100 mM Na^+ and 5 mM Mg^{2+} . This corresponds to an average bulk concentration and does not necessarily equal the concentration inside the phage since ions may be pulled into the capsid, consistent with a simple view of ion-condensation around DNA [47]. We also determine the hydrostatic pressure as a function of the effective ionic screening in the form of varying the Debye screening length ($1/\kappa$) within the above formula. We note that the hydrostatic pressure we calculate is not strictly the osmotic pressure.

4. Molecular Dynamics Simulations

As in our previous work we modeled the enclosed volume of the expanded λ -phage capsid by a spherical surface of radius 290 Å. All simulations were performed using modified versions of the Extended System Program (ESP) [48] Molecular Dynamics (MD) package developed in our lab. The interactions of the capsid with the DNA inside were modeled as the repulsive part of a Lennard-Jones interaction via a WCA decomposition [49]. Simulations were performed, after minimization and equilibration, for 100 ns in the microcanonical ensemble near 300 K with a 100 fs time-step. Coordinates were sampled every 2 ps. Thermodynamic quantities were averaged over 21 independent simulations of independently-generated initial configurations.

We generated configurations with and without the potential terms for kinking. The model consists of 6-bp beads bound into tetramers by elastic bending and stretching terms corresponding to the known 50 nm persistence length (at 300 K) of B-form DNA. Our mean-field approximation of sequence-specific twist-induced kinking effects based on the lambda phage genome predicts that such kinking should occur on average every 22 bps, approximated here as once every 24 bps, or every 4 beads in our 6-bp CG representation of DNA segments.

The DNA-packed lambda phages and similar systems are more glass-like than fluid, and thus, in order to sample an ensemble of possible starting configurations of packaged DNA, we use a procedure analogous to simulated annealing whereby we initially sample random conformations of the 24 bp segment tetramers at very high temperatures (near 1000K). We

select 21 independent initial configurations for polymer sampling followed by minimization into the correct spherical volume confinement of 290Å radius approximately representing the λ -phage capsid near physiological temperatures. We then perform simulations for 100 ns for each of the 21 independent initial configurations taken from snapshots of the initial simulation at regular time intervals.

We can turn on the sequence dependent kinking and thus generate both an ensemble of both fully- elastic and mean-field sequence-specific twist-induced kinked elastic configurations. Examples of configurations generated using conformational sampling with type-II internal rebridging (“backbite”) moves [42] and following energy minimization and MD simulation are shown in Figure 2 with full persistence length and Figure 3 with sequence-allowed kinking.

This produces a model which is less random than the fluid of CG beads used previously [23] but much more disordered than spooled configurations [18], [19], [22], [26]. We note that our random packings are not derived from any sort of a portal filling procedure. This and the mean field nonlinear elasticity mean that this model has the possibility of a very high level of disorder that a phage may not achieve. Our purpose is to consider how these various disorderings of the DNA chain contribute to packing in an ideal setting. We leave the problem of how these effects contribute in the context of a model with portal based filling for later.

C. Results

1) Interior order

In Figure 3 we see the spontaneous generation of order near the capsid surface. An alignment of our stiff segments happens with high probability. We find that, although the introduction of sequence- specific twist-inducible kinking creates global disorder which deviates from spooled or elastic-toroidal model structures, such kinking actually induces regions of local cholesteric order in our configurations. That is, segments of the DNA spontaneously adopt local nematic-phase-like alignments with the helical axis, thereby lowering the energetic penalty of the system’s repulsions. Utilizing models with more detailed sequence-dependence (see methods), we would expect to find both longer and shorter segments similarly aligned.

2) Density Maps

As mentioned, the cryo-EM experimental resolution for this system is currently not high enough to discern atomic detail of the packaged DNA. This may not be as much a result of the physical limitations of the technique as it is a limitation of the methods of sampling and refinement. Advances in cryo-EM have led to the development of “asymmetric reconstructions” of viral capsids which do not require the imposition of symmetry. However, phages are aligned with respect to the outer capsid portal or tail proteins, and typically 15,000 to 19,000 particles are averaged to produce a single density map [13]-[15]. For certain features to be visible within the final reconstructions, such features must be somewhat uniform not only across multiple particles but also relative to the point of

alignment, such as the tail or portal. While this allows precise determination of the structure of well-ordered icosahedral capsid shells, this assumption may not be valid for the DNA packaged within some phages. Because the density maps are obtained by averaging thousands of particle images, if the DNA structure differs significantly between particles at a position measured relative to the point of orientation, then structural elements of the packaged DNA will be an average over position and conformation within virus particles. This is the case expected for an ensemble of liquid like or glassy objects.

Figure 4 shows the effects of the sequence specific persistence length on the density distribution. The correlations induced by the capsid wall are enhanced with the kinked configurations. This model explains previous observations of DNA spacing in phages without need to invoke highly-ordered spooled conformations. Other authors have previously noted [50] that a possible over-interpretation [1] of the x-ray scattering data may have led to the assumption of spooled structures.

We see that correlations induced by surface-confinement, combined with negative twist and the possibility of sequence related local structural defects, induces conformations that on average have higher probabilities of finding portions of the DNA helix near the capsid wall where experiment finds regions of higher “average” density. We interpret that a conformation/configuration similar to that seen in Figure 3 is reminiscent of the local ordering seen in projections of single virus particles [51]. Local ordering combined with capsid confinement contribute to the appearance of ring-like densities. Such conformations, when averaged over many particles and combined with the “smearing” effect due to the independence of the DNA orientation from portal alignment, results in shell like density distributions seen here (Figure 4B) and in the asymmetrically refined cryo-EM density maps (Figure 4C).

3) Pressure from Simulation

Given the significant contribution of the elastic bending potential to the interior pressure, it is not surprising that we find that adding sequence-dependent kinking into elastic models of phage packing significantly reduces the energies and pressures of the packaged conformations within λ -phage-like confinement. More interestingly, however, because the kinking induces local ordering, we find the introduction of sequence-induced kinking also decreases the electrostatic component of the energies and pressures. As shown in Table 1, we find, at the bulk ionic strength of physiological (and the experimental) conditions, hydrostatic pressures of ~23 atm for the mean-field sequence-dependent twist-induced kinked-elastic model under the bulk salt conditions. This is, in fact, quite consistent with the interpretations of osmotic pressure ejection-inhibition experiments of the lambda phage which have estimated the equilibrium osmotic pressure differences of the fully packaged phage to be 20-25 atmospheres [9], [10].

We also find, as expected, that decreasing the ionic strength ($1/\kappa = 10 \text{ \AA}$) of the solution increases the overall hydrostatic pressure to nearly 190 atm, and that increasing the ionic strength ($1/\kappa = 1 \text{ \AA}$) decreases this pressure to around 8 atm (see Table 1). This is consistent within a well-tested qualitative continuum ion atmosphere approximation and explicit ion/solvent correlations would be expected to change the results quantitatively.

D. Discussion

We have shown that introducing sequence-dependent changes in persistence length (kinking) into configurations of DNA packaged in phage-like confinement results in significant reductions of energies and pressures as well as reproducing the known average density rings within the capsid. We previously showed that more disordered conformations *could* be consistent with cryo-EM density maps and implied that a highly-ordered spool model or even the ordering due to the polymeric path of less ordered models is not required [23] to reproduce the cryoEM density rings. That study implied little information toward the favoring of any specific conformations, and we instead hypothesized that a broad ensemble of polymer configurations could be consistent with the data.

We find here, perhaps surprisingly, that the introduction of twist-induced local structural departures from B-form DNA (defects) increases the local nematic ordering of the phage packaging without a need to invoke any cholesteric interaction potentials. This increase in the local ordering reduces not only the bending energy due to reduced elasticity but also reduces the electrostatic contribution to the energy and pressure. While many improvements still need to be made, this model of phage packaging incorporates a level of sequence-dependence which affects the packing pressures and energies.

As we mentioned, our random packings are not derived from the filling via a portal. The disorder we generated here is thus not strictly in the same class as would be found with a filling proceeding from a single orifice. It is interesting however that even given a random initial chain construction and a potential energy model with the ability to model persistence length breaks the simulations give rise to spontaneously ordered alignments of the DNA segments.

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Figure 1. Dinucleotide Frequencies Across λ Phage Genome

Figure 2.

Fully-Elastic Configurations, as shown above, were generated from path sampling employing backbite moves[42] followed by energy minimization and Molecular Dynamics simulations at 300 K employing fully elastic B-form DNA bending potentials

Figure 3.
Mean-Field Sequence-Specific Twist-Induced Kinked Elastic Configurations, as shown above, were generated from path sampling employing backbite moves followed by energy minimization and Molecular Dynamics simulations at 300 K employing mean-field kinked elastic bending potentials

Figure 4.
Average Density Distributions over 21 DNA Configurations Each Simulated for 100 ns of Molecular Dynamics after minimization using (A) Fully Elastic and (B) Kinked Configurations, for comparison to (C) Cryo-EM density map from asymmetric phage reconstructions of P22 with capsid density graphically removed [15]

Table 1

Pressures from Molecular Dynamics Simulations, in atmospheres, averaged over 21 different fully-packaged configurations simulated for 100 ns near 300 K with a fully elastic model ($P_L=50$ nm) and a sequence-allowed kinked elastic model. The simulations were performed at three ionic strengths.

	Salt Model 1	Salt Model 2	Salt Model 3
Debye length	$1/\kappa = 10 \text{ \AA}$	$1/\kappa = 3.3 \text{ \AA}$	$1/\kappa = 1 \text{ \AA}$
Fully Elastic Model ($P_L=50$ nm)	198.	32.7	15.7
Sequence-Specific Twist-Induced Kinked Elastic Model with kinks Every 24 bps	186.	23.0	7.92