Entropy, Energy, and Bending of DNA in Viral Capsids

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ABSTRACT Inspired by novel single-molecule and bulk solution measurements, the physics underlying the forces and pressures involved in DNA packaging into bacteriophage capsids became the focus of numerous recent theoretical models. These fall into two general categories: Continuum-elastic theories (CT), and simulation studies—mostly of the molecular dynamics (MD) genre. Both types of models account for the dependence of the force, and hence the packaging free energy (ΔF), on the loaded DNA length, but differ markedly in interpreting their origin. While DNA confinement entropy is a dominant contribution to ΔF in the MD simulations, in the CT theories this role is fulfilled by interstrand repulsion, and there is no explicit entropy term. The goal of this letter is to resolve this apparent contradiction, elucidate the origin of the entropic term in the MD simulations, and point out its tacit presence in the CT treatments.

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The genomic double-stranded (ds) DNA inside bacteriophage heads is highly stressed, leading to internal pressures of up to ~50 atmospheres, reflecting the tight packing and extreme bending of this highly charged and rigid molecule (1). The interaxial distance (d) between neighboring (nonbonded) dsDNA segments in the fully packaged virus is typically $\approx$2.5 nm (2,3), just slightly larger than the hard-core diameter of dsDNA ($b = 2.0$ nm) and well into the repulsive regime ($d \leq 2.8$ nm) of DNA-DNA interaction in ionic solutions (4–6). Moreover, free dsDNA in (physiological) solution is a fluctuating, semiflexible, wormlike chain (WLC), with persistence length $\xi \approx 50$ nm, larger than the radius of most viral capsids. Thus, on a molecular scale, packaging the long (e.g., the 330-ξ long λ-phage genome) viral DNA into its tiny capsid requires enormous mechanical work.

The force needed to package the DNA is provided by an ATP-driven motor protein situated at the capsid portal. Recent single molecule measurements reveal that this force, $f(L_{\text{int}})$, increases sharply with the loaded genome length, L_{int}, rising to ~30–100 pN, depending on the virus in question (7,8). These studies inspired the formulation of many theoretical models of DNA packaging in viral capsids, which fall roughly into two categories:

CONTINUUM-ELASTIC THEORIES

Similar to earlier theories of the problem (9–11), these models treat the dsDNA as a WLC whose packaging free energy involves two major contributions: $\Delta F = \Delta F_{\text{int}} + \Delta E_{\text{bend}}$, accounting for interstrand repulsion and DNA bending energy, respectively (12–14). Some models add DNA twist (15), attraction to the capsid wall (16), or surface energy terms (13). The encapsidated DNA is assumed to reel into an hexagonally ordered bundle, whose shape and interstrand distance, d, are determined as a function of L_{in} by variational minimization of the packaging free energy $\Delta F(L_{\text{in}})$. The bending energy, ΔE_{bend}, is evaluated as usual, by integrating the local curvature energy over the chain contour, (see Eq. S1 in the Supporting Material). The dependence of ΔF_{int} on d (and hence on L_{in}) is generally derived from osmotic stress measurements (4,6). Consistent with experiment, the continuum-elastic theory (CT) models predict that fully packaged genomes wind into a coaxial spool where $d \approx 2.5$ nm (3,17), and correctly reproduce the measured $f(L_{in})$ profiles. Remarkably, these models have correctly predicted (12,13) that by regulating the external osmotic pressure, one can control the extent (L_{out}) of genome ejection (18).

COMPUTER SIMULATIONS

DNA packaging into phage heads has been studied by several groups, using various simulation methods and WLC models (see, e.g., the literature (12,15,17,19–22)). Like the CT models, the simulations reproduce the observed $f(L_{\text{in}})$ behavior, and hence, following integration over L_{in} one obtains the work of loading which, assumed reversible, yields the packaging free energy ΔF. Harvey and coworkers (20–22), in a comprehensive series of molecular dynamics (MD) simulations, calculated ΔF or many viruses. Subtracting the sum of energetic contributions, ΔE, they found that the entropic contribution, $-T\Delta S = \Delta F - \Delta E$, provides a major, often the dominant, contribution to ΔF; for example, 88% of ΔF in the case of T7 and 74% for φ29 (20).

In contrast, as emphasized by Harvey and coworkers (20–22), there is no explicit entropy contribution to ΔF in

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the CT models. Curiously, however, the values of ΔF obtained by the MD and CT calculations are similar. The bending energies (ΔE_{bend}) are also similar, yet small, ~10% of ΔF (and not because of being unimportant but, rather, because the packaging stress is tolerated better by the softer interstrand repulsion mode, (13)). It thus follows that the role of interstrand repulsion, ΔF_{int}, in the CT models, is replaced by the entropic term, $-T\Delta S$, in the MD simulations, with each providing the major contribution to the respective ΔF.

The goal of this letter is to resolve this apparent contradiction, unravel the origin (and limited physical significance) of ΔS in the MD simulations, and reveal the (albeit tacit) presence of confinement entropy in the interstrand repulsion term (ΔF_{int}) of the CT models.

The qualitative clue to this puzzle is provided in Fig. 1, which shows two choices of $\varepsilon(d) = \Delta F_{\text{int}}(d)/NL$, the interaction free energy per unit length of a single dsDNA rod, in a bundle of N rods of length L, spaced by distance d from each other. (With six neighbors on average, the pairwise interstrand energy per unit length is $\varepsilon(d)/3$.)

In most CT models, $\varepsilon_{\text{CT}}(d)$ is derived by integrating the osmotic pressure versus d isotherms, $\Pi(d)$, of hexagonal DNA bundles in salt solution (4,6). In solutions containing monovalent and divalent counterions DNA-DNA repulsion is exponential, with a common decay length $\alpha \approx 3.3\ \text{nm}^{-1}$ but different preexponents for different salt solutions. (See Supporting Material for details). The red curve in Fig. 1 *A* represents ε_{CT} (d) for solutions containing Mg^{2+} and monovalent counterions, as derived by Purohit et al (14). using the results of Rau et al. (4).

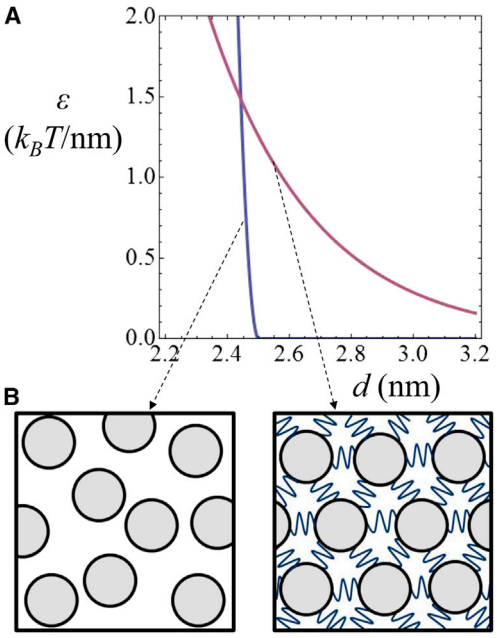

FIGURE 1 (*A*) Interaction energy per unit length of a single DNA rod in a bundle of parallel rods versus their average interaxial distance: CT (*red*) versus MD (*blue*). (*B*) Cross sections through the bundles.

The blue curve in Fig. 1 *A*, describing $\varepsilon_{\text{MD}}(d)$, is based on the WLC model of dsDNA by Locker et al. (20), whereby nearly spherical monomers (each representing six basepairs) are connected by (rather rigid) harmonic bonds of equilibrium length $b = 1.99$ nm. Interbond angle potentials, that allow only small fluctuations, $\Delta\theta = \sqrt{\langle\theta^2\rangle} \approx 16^0$, ensure $\xi = 51$ nm, (see Supporting Material). Intermonomer repulsion is modeled by a steep semiharmonic potential between nonbonded monomers that sets in at distances smaller than $d_0 = 2.5$ nm, the typical interstrand distance in fully packaged phage heads. This WLC model represents a semiflexible, slightly compressible, cylindrical molecule of diameter d_0. If packed in an hexagonal bundle with interstrand spacing d, the energy per unit length of this molecule is $\varepsilon_{\text{MD}}(d) = \kappa(d - d_0)^2$ for $d \le d_0$ and 0 for $d \ge d_0$. The blue curve in Fig. 1 *A* represents $\varepsilon_{\text{MD}}(d)$ for $\kappa = 445\ k_{\text{B}}T/\text{nm}^3$, based on the intermonomer potential of Locker et al. (21).

Fig. 1 *B* depicts cross sections through bundles of DNA rods governed by $\varepsilon_{\text{MD}}(d)$ versus $\varepsilon_{\text{CT}}(d)$, demonstrating their different implications with regard to ΔF. The analogy to the difference between a two-dimensional gas of hard disks versus a two-dimensional harmonic solid is apparent.

Compressing a perfectly hexagonal bundle obeying $\varepsilon_{\text{CT}}(d)$ appears as a purely energetic process involving no change in entropy. It should be noted, however, that the phenomenological (implicit solvent) interstrand potential, $\varepsilon_{\text{CT}}(d)$, is effectively a potential of mean force, i.e., an interaction free energy, and thus accounts for all the relevant entropic contributions due to hydration, electrostatic, and excluded volume interactions (all of which affect the orientational and translational entropy losses of the confined chain).

On the other hand, according to $\varepsilon_{\text{MD}}(d)$, nonbonded monomers do not repel each other unless they penetrate the strongly repulsive (and thus unlikely) d regime ($d \le 2.5$ nm), explaining the small interstrand repulsion energy ΔE_{int} in the MD simulations. The steep inter-monomer repulsion allows just a tiny inter-monomer penetration depth, $\Delta d = \sim 0.04$ nm (see Supporting Material for detail). Though small, this increase in the lateral range of monomer motion—from $d - d_0$ to $d - d_0 + 2\delta d \equiv \Delta d$—becomes significant when $d \to d_0$, thus affecting the value of the entropy loss, ΔS, inflicted upon on the confined chain by its neighbors.

Polymer confinement entropies have been studied by various authors, (23–25). However, for the MD model of interest here, a reasonable estimate can be obtained using the simple scheme in Fig. 2. Consider for instance the T7 phage, whose 39,937 basepairs genome was modeled as a WLC of $M = 6656$ monomers of diameter $b = 1.99$ nm and its capsid as a sphere of inner radius $R = 2.67$ nm (20). Assuming hexagonal packing of the fully packaged genome, one finds

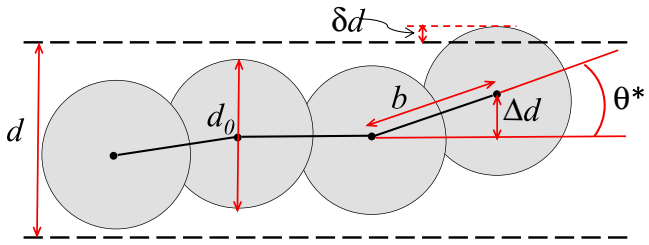

FIGURE 2 A section of the MD chain model confined within a cylindrical tube of diameter *d*.

$d = 2.64$ nm implying $\Delta d \equiv d - d_0 + 2\delta d = 0.22$ nm. (See Supporting Material). Suppose now that each monomer experiences (independently) this range of motion, and ignore local curvature effects. Then, the entropy change upon transferring the free WLC into the roughly cylindrical tube of diameter d prescribed by its neighbors is $T\Delta S/M = k_B T \ln(q^*/q^f) + \Delta\varepsilon_\vartheta$. Here, q^f and q^* are the bond rotation partition functions of the free and confined chains respectively (see Supporting Material), and $\Delta\varepsilon_\vartheta$ is the (negligible) change in the average bond rotation energy. With k_ϑ, d_0, and κ, as given above, this crude model yields $\theta^* \approx 3.3^\circ$ and hence $-T\Delta S \approx 11{,}000\ k_B T$, comparable to (though not surprisingly smaller than) the $14{,}000\ k_B T$ obtained in the MD simulations (20). The linear scaling with M is also consistent with the MD results regarding ΔS of T7 vs. φ29.

Two major conclusions emerge from the analysis above. The first is that—through the experimentally derived interstrand interaction free energy—the continuum theories do include, albeit indirectly, most of the important entropic contributions to the DNA packaging free energy. The second is that the value of ΔS obtained in the MD simulations depends sensitively on the choice of model parameters, primarily d_0. E.g., setting d_0 equal to the hardcore diameter of dsDNA (2.0 nm) would imply a much lower entropy loss and hence smaller ΔF. On the other hand, MD simulations relying upon DNA-DNA derived from experiment (or independent elaborate theory) can significantly substantiate their predictions of properties that coarse-grained continuum theories cannot provide, such as equilibrium bundle geometries and structural fluctuations.

SUPPORTING MATERIAL

Mathematical relations and numerical details complementing the main text are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(13)00430-X.

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