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Comments to the Editor

Reply to the Comment by S. Harvey on "Entropy, Energy, and Bending of DNA in Viral Capsids"

ABSTRACT The comment by Stephen Harvey in this issue of the *Biophysical Journal* concludes with two statements regarding my recent letter about DNA packaging into viral capsids. Harvey agrees with my interpretation of the origin of the large confinement entropy predicted by the molecular-dynamics simulations of his group, and its sensitive dependence on the molecular parameters of their wormlike chain model of double-stranded DNA. On the other hand, he doubts my assertion that the confinement entropy is already included in the interstrand repulsion free energy derived from osmotic stress measurements, which constitutes the major contribution to the packaging free energy used in recent continuum theories of this process. Harvey suggests instead that the confinement entropy should be added to this free energy as a separate term (using, for instance, the method described in my letter). I will argue that this addition is redundant, and, in a brief discussion of continuum theories, will also discuss his comments as relates to the work of other researchers.

INTRODUCTION

As correctly noted in Stephen Harvey's comment in this issue of the *Biophysical Journal* (1), the goal of my recent letter (2) was to reconcile differences between his group's studies of DNA packaging into bacteriophage heads (3–6) and several continuum theory analyses of this process (7–10). A central issue to resolve has been the origin of the substantial contribution of DNA confinement entropy predicted by their molecular-dynamics (MD) simulations. Notably, the dominant free energy component in the continuum theories is the short-ranged repulsion between neighboring strands, and there is no explicit contribution due to chain confinement entropy. Nevertheless, both approaches have correctly predicted or reproduced, qualitatively at least, the measured DNA loading force and the internal pressure in the capsid as a function of the loaded genome length (11,12). Applying a simple, very approximate, statistical thermodynamic analysis to the beads-on-a-string model describing the double-stranded (ds)DNA chain in the MD simulations, I showed that the large confinement entropies obtained in these studies reflect the suppression of local chain undulations owing to the tight packing of the DNA within the viral capsid. I also emphasized that the magnitude of the calculated entropy depends sensitively on the model parameters, in particular the dimensions of the beads comprising the wormlike chain (WLC) model of the dsDNA and the interaction potential between nonbonded beads. Harvey appears to agree with these conclusions, but rejects my assertion that the DNA undulation entropy is naturally embodied in the interstrand repulsive potential derived from osmotic stress measurements (13,14). I will address this issue in the following section, after rectifying a few statements made in Harvey's comment regarding the reference to the work of Riemer and Bloomfield (15) in our own work (7,8) and that of the Caltech group of Purohit et al. (9,10).

CONTINUUM THEORY OF DNA PACKAGING

In a pioneering article published 35 years ago, Riemer and Bloomfield (15) presented a theoretical model of DNA packaging into viruses. Their free energy expression included a confinement entropy term, originally referred to as "condensation entropy". Assuming that the encapsidated genome is perfectly ordered (and thus of zero entropy), their confinement entropy is the entropy of the free chain in solution. Applying Flory-Huggins theory to the free chain in solution (not to the packaged chain as implied by Harvey's comment), they obtained $(L/\xi)k_B$ for the entropy loss of the chain upon confinement, with L and ξ denoting, respectively, the contour length and the persistence length of the viral DNA, and k_B is Boltzmann's constant. For the T7 phage, for instance, this yields a confinement entropy of ~300 k_B, far smaller than the ~10^4 k_B obtained in the MD simulations of Harvey and co-workers (Locker et al. (3), Petrov et al. (4,5), and Petrov and Harvey (6)). One could argue that the entropy estimate of Riemer and Bloomfield (15) is indeed too low and even suggest possible improvements of their model, yet this is irrelevant for the continuum theories of interest here, as explained below. First, however, to rectify one of Harvey's comments, it should be mentioned that while we have naturally cited the theory of Riemer and Bloomfield (15) in our work (7,8), we did not use nor did we even quote their confinement entropy estimate. The Caltech team indeed quoted

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*Correspondence: abs@fh.huji.ac.il

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the result from Riemer and Bloomfield (15), but did not make use of it in their theory (9,10).

The packaging free energies used in the above-mentioned continuum models (7–10) comprise two major contributions, each based on independent experimental measurements:

1. The known bending elastic energy of dsDNA in solution associated with its 50-nm persistence length; and
2. The repulsive interstrand free energy (i.e., a potential of mean force) obtained from integrating the osmotic pressure isotherms measured by Rau et al. (13) and Rau and Parsegian (14).

The relevant range of interaxial distances in these experiments is generally $d \approx 2.5$–4 nm. For solutions containing polyvalent counterions, an additional term (of marginal importance) is added to the packaging free energy, to account for the (relatively long-ranged) attraction between DNA strands. The strength of this attraction has been evaluated (8) based on the dimensions of DNA toroids in solution (16,17). No fitting parameters were used in our work, which mainly intended to unravel the origin, and estimate the magnitudes, of the forces and pressures involved in DNA packaging and release from viral capsids. One prediction of our theory was that DNA injection can be inhibited by regulating the osmotic pressure in the external solution (8), as has been amply confirmed experimentally, first by Evilevitch et al. (18).

The dsDNA helices in the osmotic stress measurements are arranged in bundles of, on average, hexagonally packed parallel strands. Within these bundles, the dsDNAs undergo positional fluctuations and angular undulations whose amplitudes decrease as the packing density increases. The suppression of these motions is the origin of the confinement entropy loss. As explained by Podgornik et al. (19), Podgornik and Parsegian (20), and Strey et al. (21), this entropic penalty constitutes a major contribution to the measured interstrand repulsive force, coupled to hydration and electrostatic effects. The reference state of dsDNA in these measurements is that of vanishing repulsion, as obtained by extrapolating the repulsive force to infinite interaxial spacing. This reference state is not identical to the initial state of free DNA in single molecule experiments. Yet the difference is small if not negligible, because the repulsive undulation forces are predominantly short-ranged, implying that the entropic penalty in DNA packaging is mainly paid at the short interaxial distances, as is also evident from the single molecule force measurements (11,12). The entropic undulation forces in the bent DNA bundles inside the viral capsid are not identical to those in the straight bundles in the osmotic stress measurements. Yet again, the difference is expected to be small, because the wavelength of the relevant (short-range) fluctuations is considerably smaller than the radii of curvature along most of the packaged DNA contour. Note also that the reference state in the MD simulations, where there is no free chain outside the capsid, is also not identical to that in the single molecule experiments.

Because positional and short-range orientational fluctuations are accounted for by the interstrand repulsion measurements on parallel DNA bundles, there is no need to supplement the packaging free energy by a separate repulsive contribution due to chain confinement—it is already included there.

Not included in the osmotic stress measurements is the elastic DNA bending energy, which is therefore accounted for by a separate term in the continuum free energy models. Adding the interstrand repulsion and the elastic bending energy, the pressure inside the virus head and the loading force can then be evaluated based on variational minimization of the packaging free energy with respect to the geometry of the packaged genome (8).

A NOTE ON THE SIMULATIONS

Using a beads-on-a-string model of dsDNA, Locker et al. (3), Petrov et al. (4,5), and Petrov and Harvey (6) carried out a comprehensive series of MD simulations for various viral systems and solution conditions. In their early studies (3), repulsive interactions between nonbonded beads were modeled using a steeply rising semiharmonic (almost hard-core repulsion) potential that sets in when the interhelical distance falls below $d_0 = 2.5$ nm. The bond length ($b = 1.99$ nm) and the force constant of the (harmonic) interbond angle potential were adjusted to reproduce the DNA persistence length. This model is quite similar to the WLC model used in our own (Brownian dynamics) simulations of DNA packaging (7), where a $d_0 = 2.5$ nm cutoff was used because this is typically the minimal interstrand distance in fully packaged capsids. (However, we did not calculate the confinement entropy.) The simulations of Locker et al. (3), Petrov et al. (4,5), and Petrov and Harvey (6) revealed confinement entropies of ~10^4 k_BT, constituting the dominant contribution to the genome packaging free energy.

In my recent letter (2) published in the *Biophysical Journal*, using the chain model of Locker et al. (3), Petrov et al. (4,5), and Petrov and Harvey (6), I presented an approximate analytical scheme that explains the origin of the large confinement entropy predicted by their simulations, as well as its linear dependence on the (fully packaged) genome length. In this scheme, the chain is regarded as being confined to an effective (approximately) cylindrical tube prescribed by its neighbors, and its confinement entropy reflects the suppression of the angular fluctuations of its constituent links. This approach resembles the more sophisticated models of Odijk (22), Podgornik et al. (19), Podgornik and Parsegian (20), and Strey et al. (21), and accounts for the nonzero diameter of the WLC. Because the effective chain diameter ($d_0 = 2.5$ nm) is barely smaller than the tube diameter ($d \approx 2.7$ nm), the calculated entropy loss is substantial, and its numerical value strongly

dependent on the model parameters, in particular d_0. For instance, $d_0 = 2.2$ nm would imply a (roughly) twofold smaller entropy penalty.

In later applications of their model, Locker et al. (3), Petrov et al. (4,5), and Petrov and Harvey (6) superimposed a long-ranged potential representing electrostatic repulsion on their (essentially) hardcore ($d_0 \approx 2.5$ nm) interbead potential. The effective chain diameter is now even larger and the confinement entropy loss is accordingly ~15% higher. In his comment, Harvey argues that the modified interaction potential is very similar to the relatively moderate potential of the continuum models (the *red curve* in Fig. 1 of my letter (2)). While similar in shape, the two potential curves span different ranges of interaxial distance. In particular, the modified potential still does not allow for interhelical distances smaller than 2.5 nm, which are not entirely forbidden in the continuum treatment (8). Indeed, cases are known where longer-than-wild-type genomes were packaged into capsids, resulting in an interhelical distance of ~2.3 nm (23).

It would be interesting and instructive to know the magnitudes of the confinement entropies predicted by the MD simulations using the same chain model, but with a smaller effective hardcore diameter (say $d_0 \approx 2.0$ nm), with (or without) a soft repulsive potential (of longer range than in the soft sphere MD model) superposed on it. Since the smaller hard-core diameter allows for more conformational freedom of the packaged DNA, one should expect a smaller confinement entropy. In parallel, the longer range of the soft inter-helix potential is expected to result in a larger contribution to the packaging free energy due to DNA-DNA repulsion. It would also be of interest to examine the dependence of the calculated confinement entropy on other parameters of the WLC model, e.g., the intersegment bond length.

SUMMARY

Continuum theories and molecular simulation models are frequently used to study the same system, often complementing each other. In the present case, the continuum models and the MD simulations yield comparable (DNA packaging) free energies, but differ in identifying their ingredients. As a simple example that may shed some light on this issue, consider two systems, both consisting of N pointlike particles of mass m, whose equilibrium positions are the centers of the (square) sites of a two-dimensional square lattice, each site of area $a = A/N$. In one system, every particle can translate freely within its own cell, but a hard wall potential prevents it from crossing to a neighboring cell. The configurational partition function per particle in this system is $q_{tr} = a$. The interaction potential between the particles comprising the second system is softer and of longer range, enabling them to partially penetrate each other's territory. The motion of each particle is governed by an effective (mean field) two-dimensional harmonic potential with a restoring force constant κ, allowing lateral fluctuations of amplitudes

$$\langle x^2 \rangle^{1/2} = \langle y^2 \rangle^{1/2} = a^{1/2}.$$

The configurational partition function in this case is

$$q_{\mathrm{vib}} = k_{\mathrm{B}}T/\kappa = \sqrt{\langle x^2 \rangle \langle y^2 \rangle} = a,$$

where we have used the equipartition result, $\kappa \langle x^2 \rangle = k_{\mathrm{B}}T$. The configurational free energies per particle ($f = -k_{\mathrm{B}}T \ln q$) in the two systems are thus identical. The momentum factors in both systems are also equal, ($2\pi m k_{\mathrm{B}}T/h^2$). However, the average potential energies are different: 0 and 1 $k_{\mathrm{B}}T$, respectively, and hence also the entropies. The first model, because of the hard wall potential, yields the higher entropy, as expected. For a macroscopic system of N particles, the difference between the entropic terms is substantial: $T\Delta S = Nk_{\mathrm{B}}T$. The analogy to the difference between the predictions of the MD versus the continuum models of DNA packaging is quite obvious.

In my letter, I suggested a qualitative explanation along these lines to account for the differences between the continuum and MD treatments of DNA packaging. The continuum theories are based on experimental evidence but ignore molecular details. Nevertheless, they explained the basic thermodynamic aspects of genome packaging, and provided interesting predictions, such as the controllability of genome injection by external osmotic pressure. The MD simulations provided interesting information on the microstructure and the configurational variance of the packaged genome, but some of their conclusions depend on the molecular parameters of the model. When modeling a system as complex as that of a virus, approximations are inevitable for both analytical and simulation studies. Together, they complement one another, and it is hoped they will provide additional insights into the experimental findings.

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Avinoam Ben-Shaul*
Institute of Chemistry and the Fritz Haber Research Center, The Hebrew University, Jerusalem, Israel

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