# The Role of DNA Twist in the Packaging of Viral Genomes

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ABSTRACT We performed molecular dynamics simulations of the genome packaging of bacteriophage P4 using two coarse-grained models of DNA. The first model, 1DNA6 (one pseudo-atom per six DNA basepairs), represents DNA as a string of beads, for which DNA torsions are undefined. The second model, 3DNA6 (three pseudo-atoms per six DNA basepairs), represents DNA as a series of base planes with torsions defined by the angles between successive planes. Bacteriophage P4 was packaged with 1DNA6, 3DNA6 in a torsionally relaxed state, and 3DNA6 in a torsionally strained state. We observed good agreement between the packed conformation of 1DNA6 and the packed conformations of 3DNA6. The free energies of packaging were in agreement, as well. Our results suggest that DNA torsions can be omitted from coarse-grained bacteriophage packaging simulations without significantly altering the DNA conformations or free energies of packaging that the simulations predict.

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As part of its life cycle, a double-stranded DNA bacteriophage packages its genome into a preassembled capsid using an ATP-driven motor. The motor must package its genome against a significant force due to the stiff, self-repulsive nature of double-stranded DNA and due to the significant entropic penalty associated with confining the genome to a small volume (1–4). One of the central problems in bacteriophage research is the structure of DNA inside individual capsids. Cryo-electron microscopy has been applied with great success to imaging bacteriophages, but it can only provide average structures based on thousands of individual bacteriophages (5–8).

In recent years, bacteriophage genome packaging has been simulated, in silico, by a number of investigators who have attempted to predict the structure of DNA inside a single capsid (2,3,8–16). Some of these studies have calculated the force of packaging (2,4,15), as well as the Helmholtz free energy of packaging (2,4). The free energy can be decomposed into the internal energy and entropic contributions (2,4).

Previous studies have assumed that DNA is torsionally relaxed on the timescale of packaging, but no simulations have been carried out to determine whether or not this assumption is valid. Spakowitz and Wang have presented simulations in which DNA torsional stiffness is present but in which the end of DNA inside the capsid is fixed to the capsid wall (14). Such a constraint is necessary if one wishes to decompose the linking number of DNA into its twist and writhe components, but it prevents torsional relaxation in the same way that closed circular DNA is incapable of changing its linking number. In addition, it affects the conformation attained by the DNA in much the same way that the addition of a protein core would: it provides a focal point around which the DNA is organized.

We simulated the packaging of bacteriophage P4 using an established molecular dynamics protocol (2–4,16,17) to determine the effect of twist on the conformation of the genome inside the capsid. The 11,600 basepair genome of P4 was represented using two coarse-grained models of DNA (Fig. S1 in Supplementary Material), one with torsions undefined (1DNA6) and the other with torsions parameterized based on the torsional modulus of DNA (3DNA6). The 1DNA6 representation resembles a string of beads, in which each bead corresponds to six basepairs of DNA. The 3DNA6 representation is a series of base planes. Each base plane is defined by three pseudo-atoms, and each base plane corresponds to six DNA basepairs. Packaging was performed using 1DNA6, a torsionally relaxed chain of 3DNA6, as well as a torsionally strained chain of 3DNA6. In the torsionally strained case, each twist angle was twisted away from its equilibrium geometry by 156° before entering the capsid. This deformation corresponds to overtwisting DNA by 26° per basepair ($\approx$13 kcal/mol per basepair). Upon entering the capsid, each segment of the chain was free to relax its torsional strain.

The capsid was modeled as a regular icosahedron, 45 nm in diameter, in accord with cryoEM structures of P4 (18). Only the DNA inside the capsid was subjected to dynamics. We did not fix or constrain the end of DNA inside the capsid in any way. Liu et al. have shown that knotting occurs in 20% of wild-type P4 and 100% of tailless P4 mutants (19), which suggests that the sticky ends of the genome are free to interact. Thus, we did not impose any constraints on the free end of DNA inside the capsid (see Supplementary Material for more detail on the models and the packaging protocol).

The free energies of packaging for each DNA representation are within 10% of each other at all stages of packaging

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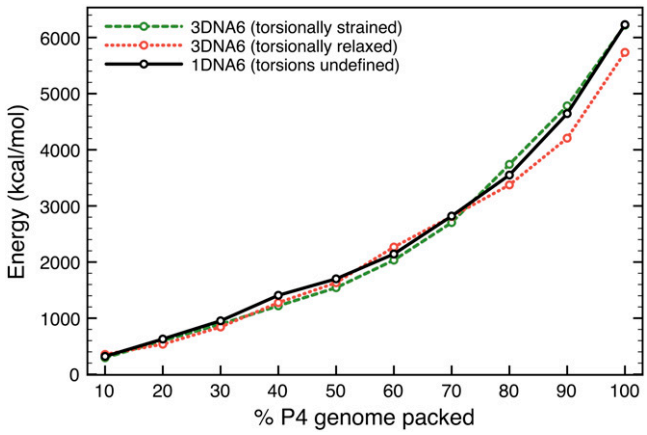


**FIGURE 1 Helmholtz free energy of packaging.**

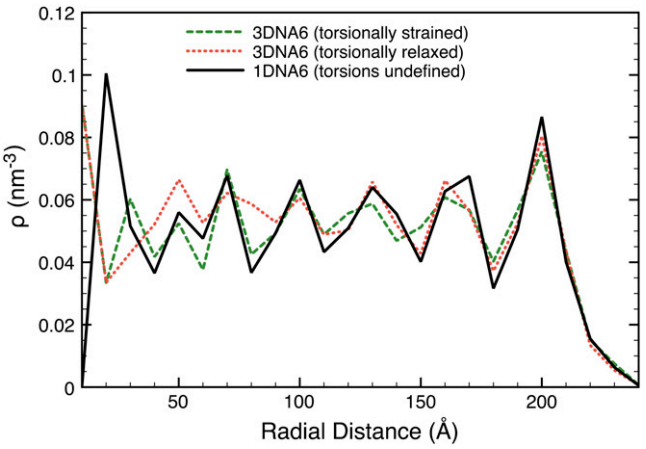


**FIGURE 3 Radial density distribution for DNA inside P4.**

(Fig. 1). Thus, the additional twist energy added to the system during packaging in the overtwisted simulation is quickly relaxed and does not accumulate or increase the free energy required to pack the genome. This is not surprising since the electrostatic repulsion and loss of entropy due to confinement are far more significant than the elastic energy terms (2,3). The elastic energy is focused into the softest degrees of freedom, the bending modes.

In all three cases, regardless of how DNA was represented, the genome was spooled at an angle of ~45° to the major axis of the capsid. The addition of twist deformation did not hinder the formation of this spooled conformation. The spooling is not perfect; the conformations appear disordered with strands meandering back and forth on the outside of the capsid. This disorder is a feature of single bacteriophage conformations, so it is not observed in cryoEM structures, which are average structures.

The final conformations shown in Fig. 2 provide a qualitative sense of the organization of DNA inside the P4 capsid. For a quantitative representation, we plot the density of DNA as a function of radial distance from the center of the capsid (Fig. 3). The ordering is equally well defined in all three cases, which supports the qualitative similarity of the conformations shown in Fig. 2. The 1DNA6 conformation has seven sharp density peaks. The 3DNA6 density plots are almost identical to the 1DNA6 plot. The ordering inside of 5 nm is slightly different for each plot, but the inner shells are the smallest in volume, so they are more sensitive to the exact number of DNA pseudo-atoms they contain.

Spakowitz and Wang have suggested that twist deformation acts as a driving force for the bacteriophage genome to adopt a spoollike conformation (14). They found that a different conformation is formed depending on whether the DNA is twisted as it enters the capsid or not, but the DNA in their model was topologically constrained.

Our results show that twist deformation plays little, if any, role in DNA organization in bacteriophage P4. Twist deformation did not alter the radial density of packed P4, nor did it change the observed conformation of DNA. Further, the free

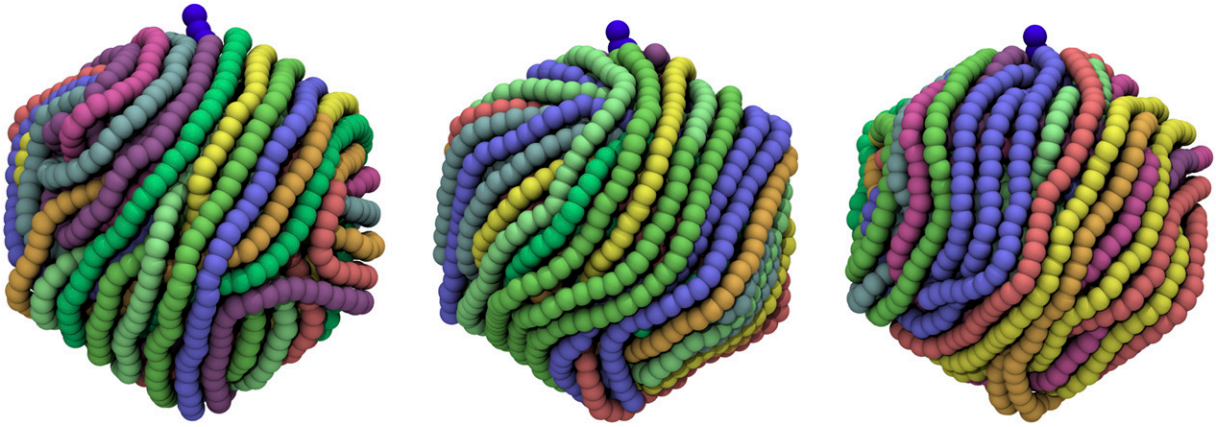

**FIGURE 2 P4 genome fully packed conformations: 1DNA6 (*left*), 3DNA6 relaxed (*middle*), and 3DNA6 strained (*right*). We have used a rainbow coloring scheme for the DNA: red corresponds to the first fraction of the genome to enter the capsid while violet corresponds to the last fraction to enter.**

energy required to pack the genome of P4 into its capsid was not significantly changed by the addition of DNA torsional stiffness.

When DNA is packaged without topological constraints, the conformation of DNA is determined primarily by the size and shape of the capsid, and whether or not a protein core is present (17). This result validates the assumption made by a number of previous investigators that DNA is torsionally relaxed on the timescale of packaging. The packaging energetics and the global conformation of the genome inside the capsid are not substantially altered by the use of a torsionless DNA model in place of a coarse-grained model with defined torsions.

## SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

## ACKNOWLEDGMENTS

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