Title

Three-Dimensional Architecture of the Bacteriophage phi29 Packaged Genome and Elucidation of its Packaging Process

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Genetic and physiological studies of dsDNA bacteriophages have established their assembly pathways in some detail, yet the precise mechanism of DNA packaging remains obscure. We follow the packaging process step-wise, from the initial state in which an empty capsid engages a free, extended genome in solution, to the final state, in which 100% of the genome is tightly packed inside the capsid. Our work is aimed at understanding details of the DNA packaging process. Force measurements with optical tweezers provide values of the pressure generated inside the capsid by the DNA, as a function of the percentage of the total genome already packaged. Cryo-EM provides a direct visualization of the architecture of the DNA inside the capsid and thus direct measurements of fundamental physical parameters, such as inter-strand distances, local curvatures, and local and long range degree of order. Using Monte Carlo simulations, we predict the conformation of the DNA in the interior of the viral capsid and the forces required to achieve this structure. The resulting density plots from simulations show a qualitatively good agreement with cryo-EM image analysis of bacteriophage with partially packaged genomes; the predicted forces are consistent with previous single-molecule measurements of the packaging forces, although small quantitative differences remain, and current work aims at obtaining a better agreement. Conformations from the Monte Carlo simulations tend to exhibit local layer ordering with frequent defects associated with chain segments jumping between layers. We discuss the impact that such defects have on the overall order and the resistance to DNA packaging and ejection.