Cryo-EM and single molecule biophysical studies of dsDNA packaging in *Bacillus subtilis* bacteriophage Phi 29

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Abstract

Genetic and physiological studies of dsDNA bacteriophage have established their assembly pathways in some detail, yet the precise mechanism of DNA packaging remains obscure. We combine two powerful techniques with the aim of further elucidating the mechanism of DNA packaging and ejection in bacteriophage: three-dimensional cryo-EM imaging to obtain precise structural measurements and optical tweezers to obtain direct measurements of the forces involved. We follow the process step-wise, from the initial state in which an empty capside engages a free, extended genome in solution, to the final state, in which 100% of the genome is tightly packed inside the capside. Current work has provided strikingly beautiful three-dimensional reconstructions of whole bacteriophage in which the ordered structure of the genome can be clearly appreciated. However, in these works, the DNA is averaged out of register from particle to particle, resulting in sets of quite perfect and independent rings. This could be a consequence of the effort to obtain a high resolution reconstruction of all protein components. The pitch and the threading of the dsDNA are lost, as is any idea of its actual trajectory throughout the packaging process. Alternatively, another possibility is that the DNA occupies the interior of the capside following a highly ordered overall architecture, but with a considerable degree of local disorder and defects. Our work aims at elucidating some of these DNA-specific details. Force measurements with optical tweezers provide the pressure generated inside the capside 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 on the interior of the viral capsid and the forces required to achieve this structure. The resulting density plots from simulations show a good qualitatively agreement with cryo-EM results; 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 across layers. We discuss the impact that such defects have on the overall order and the resistance to DNA packaging and ejection.