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Preliminary Studies of Bacteriophage Sf6 Virions: Icosahedral, Asymmetric, and Tomographic Reconstructions

Kristin N. Parent[*], Robert S. Sinkovits[*], Sherwood R. Casjens[**], and Timothy S. Baker[*,***]

[*]University of California, San Diego, Department of Chemistry & Biochemistry, La Jolla, CA
[**]University of Utah School of Medicine, Division of Microbiology & Immunology, Pathology Department, Salt Lake City, UT
[***]University of California, San Diego, Division of Biological Sciences, La Jolla, CA

Shigella is the causative agent of bacillary dysentery and is responsible for 165 million cases worldwide, with 1.1 million deaths reported annually in developing countries [1]. Bacteriophage Sf6 infects *S. flexneri*, the most commonly isolated *Shigella* strain from infected patients with acute dysentery. Sf6 infection has the potential to alter the pathogenicity of *S. flexneri* by horizontal gene transfer [2], and an understanding of the morphogenic pathway of Sf6 is needed. Genomic sequencing has characterized Sf6 as a member of the "P22-like" phages, yet bioinformatics indicates that P22 and Sf6 have low homology between structural proteins (for example, ~14 % sequence identity of the respective coat proteins) [3]. Highest divergence between P22 and Sf6 occurs in portions of those proteins that control assembly, indicating that Sf6 morphogenesis is expected to differ from P22 [3]. Here, we report preliminary analysis of the Sf6 virion architecture as visualized by cryo-electron microscopy and 3D image reconstruction. Details of the phage head were reconstructed at 8.2-Å resolution by means of icosahedral averaging procedures [4, 5]. Unexpectedly, the coat protein interacts with the genome in several places, a phenomenon not observed in other "P22-like" phages. Also, a comparison of Sf6 and P22 coat proteins reveals the presence of a conserved, surface-exposed, telokin domain that, in Sf6, is rotated and extended relative to the domain in P22.

An asymmetric cryo-reconstruction was also computed from the same data set of Sf6 images and yielded structural details of the entire Sf6 virion, including the tail machinery (tailspike proteins and tail needle proteins) at an overall resolution of 22-Å. Virions exhibit symmetry mismatches between the tail (6-fold symmetry), the portal (12-fold symmetry), and the capsid lattice (5-fold). Cryo-electron tomography was also performed on Sf6 virions captured in the process of "infecting" lipid vesicles *in vitro*. Two types of phage were observed in cryo-tomograms: 1) phage that appear "full" and contain most if not all of their genomes, and 2) phage that appear "empty" and have injected their genomes into the lipid vesicles. Our goal is to compare the structure of the native, isolated virion derived from our asymmetric reconstruction with Sf6 docked to the lipid vesicles, which will help identify changes that occur in the tail machinery during host attachment and subsequent genome injection.

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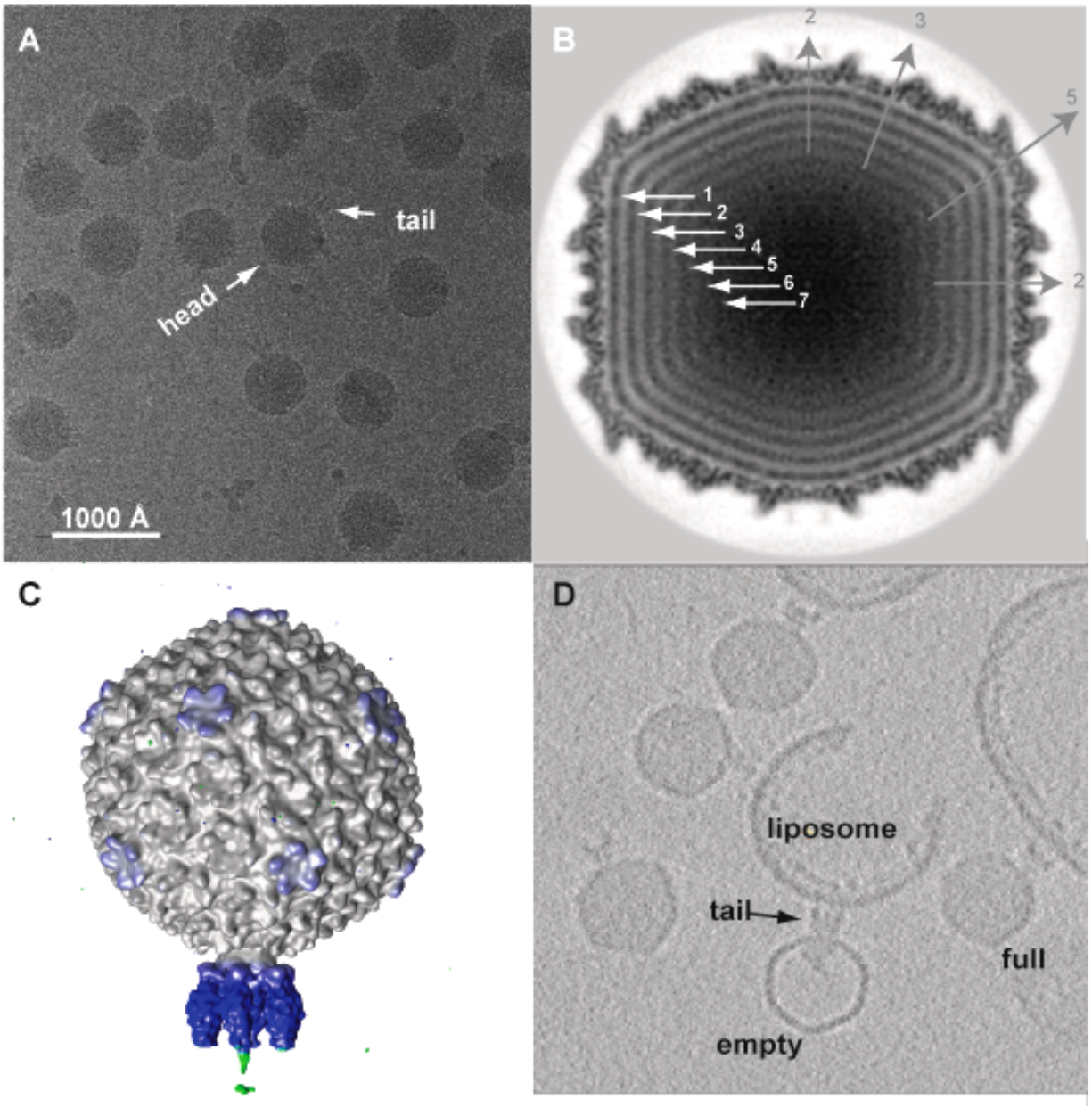

FIG 1. A) Micrograph of a vitrified, purified sample of Sf6 virions imaged on Kodak SO-163 film at 59,000X nominal magnification in an FEI Technai G^2 Polara microscope operated at 200 keV. B) Planar, 1-Å-thick equatorial section through an icosahedrally-averaged reconstruction at 8.2-Å resolution (darkest regions correspond to densest regions in the density map). The dsDNA genome is organized into a series of concentric shells (arrows point to seven discreet layers). C) Surface-shaded representation of an asymmetric reconstruction (from the same data set as B) at 22-Å resolution. The phage head is comprised of coat protein organized as oligomers in arrangements of six ("hexons", colored grey) or five ("pentons", colored blue) copies, the tail appendages (six trimers of the tailspike protein) are shown in blue, and the tail needle is rendered in green. D). Slab through a tomographic reconstruction of Sf6 docked to lipid vesicles. One particle ("empty") has released its genome into the labeled vesicle. Phage that still contain genome ("full") are also observed free in solution and docked to a liposome.

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