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The Development of Bubblegrams is Dose Rate-Dependent.

Naiqian Cheng[1], Weimin Wu[1], Norman R. Watts[2] and Alasdair C. Steven[1]

[1.] Laboratory of Structural Biology Research and [2.] Protein Expression Laboratory, National Institute for Arthritis, Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

The state of preservation of vitrified biological specimens is known to depend on the electron dose. Under standard imaging conditions for specimens maintained at ~ 83°K, exposures of 0.5 - 1.0 sec with 10 - 15 electrons/Å^2, structural information is retained on protein complexes to resolutions beyond 4 Å. At higher doses, radiation damage is manifested by progressive blurring ("delocalization") of finer details (1), leading eventually to the appearance of bubbles of hydrogen gas at high pressure (2). Recently we found that bubbling starts unusually early in proteins that are embedded in densely packed DNA (3). We call these images "bubblegrams". They offer a way to discriminate between nucleic acid (bubble-negative) and protein in nucleoprotein complexes and we used it to define the cylindrical "inner body" of bacteriophage ϕKZ (4). We hypothesized that the early onset of bubbling is due to the DNA impeding the diffusion of protein-derived radiation products away from their site of origin. Here, we tested this hypothesis by recording and analyzing dose series micrographs of bacteriophage T7 whose genomic DNA is wrapped tightly around a protein "core" (inner body) of known structure (5).

T7 was grown and purified as follows: 100 ml of *E. coli* BL21 cells growing in LB broth at 37°C in a shaker flask were infected with wild-type phage, a kind gift of Dr F. W. Studier. Following lysis, clarification, DNase and RNase treatment, pelleting, and resuspension in buffered CsCl the phage were banded by ultracentrifugation, collected by piston displacement, and dialyzed extensively against 50 mM Tris, 10 mM $MgCl_2$, 50 mM NaCl (pH 7.5). Drops of purified virions were blotted and vitrified on a Leica KF80 cryo-station and observed in a Philips CM200-FEG operating at 120 keV and a magnification of 38,000x. In recording the dose series, each exposure was for 1.0 sec and imparted a dose of 17 electrons/Å^2. In a given series, the interval between exposures was fixed in the range 10 sec to 10 min. In the series shown (Figures 1 & 2), the defocus was fixed at - 1.15 μm.

Figure 1 shows two dose series of purified T7 virions. The virion consists of an icosahedral capsid of 55 nm in diameter with a triangulation number of T = 7 *levo*. At one of the twelve vertices is the portal protein, a 12-membered ring. Attached to the portal are two protein complexes. Extending outwards is the conical tail, which observes 6-fold rotational symmetry. Extending inwards and surrounded by densely packed DNA is the core, a 25 nm-long cylindrical structure comprising stacked rings of three proteins in multiples of 4 subunits. It is not distinguishable in the first-exposure images. With short times (up to 5 min) between exposures, bubbles first appeared in the 3rd exposure, corresponding to a cumulative dose of 51 electrons/Å^2 (Figs. 1A & 2). One or two bubbles per virion nucleated in the core region and grew rapidly. Of note, no bubbling was observed in the surrounding capsid. However, increasing the interval between exposures from 5 min to 10 min resulted in a dramatic retardation in the onset of bubbling (Figs. 1B & 2), i.e. an approximate 3-fold increase in the threshold for bubbling. These observations support the inferred mechanism that bubblegrams arise from the DNA retarding the outwards diffusion of radiation products so that their concentration builds up more rapidly to bubble-generating levels. They also suggest that in cryo-electron tomography in which cumulative electron doses are necessarily high, structural preservation may be enhanced by allowing at least 10 minutes between successive frames of a tilt series (6).

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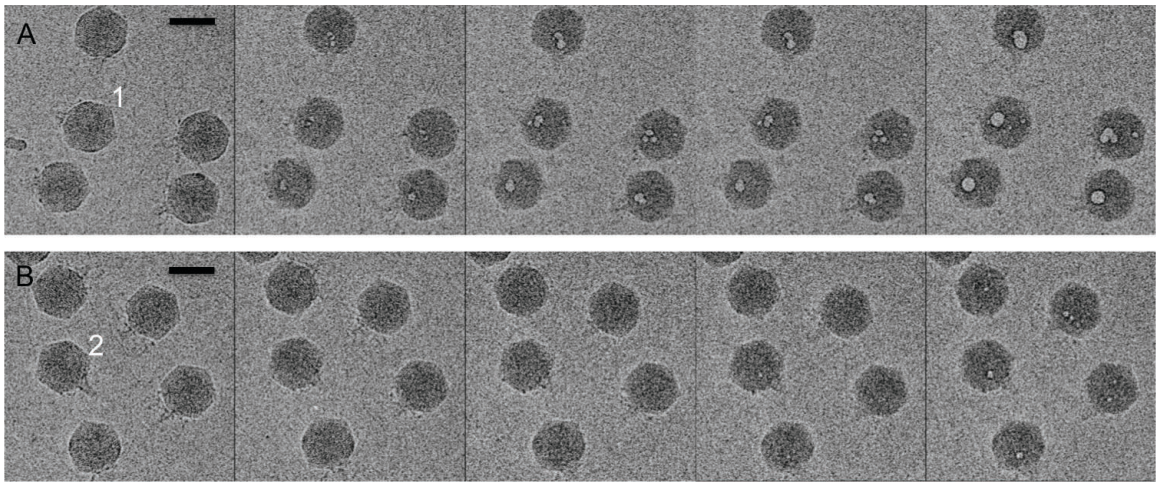

Figure 1. Two dose series (A and B) of images recorded on film as the 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th} exposures (from left to right). The cumulative doses were 17, 51, 85, 119, 153 electrons/Å^2 respectively. Wait times of 10 sec (A) and 10 min (B) were applied between successive exposures. Scale bar, 50 nm.

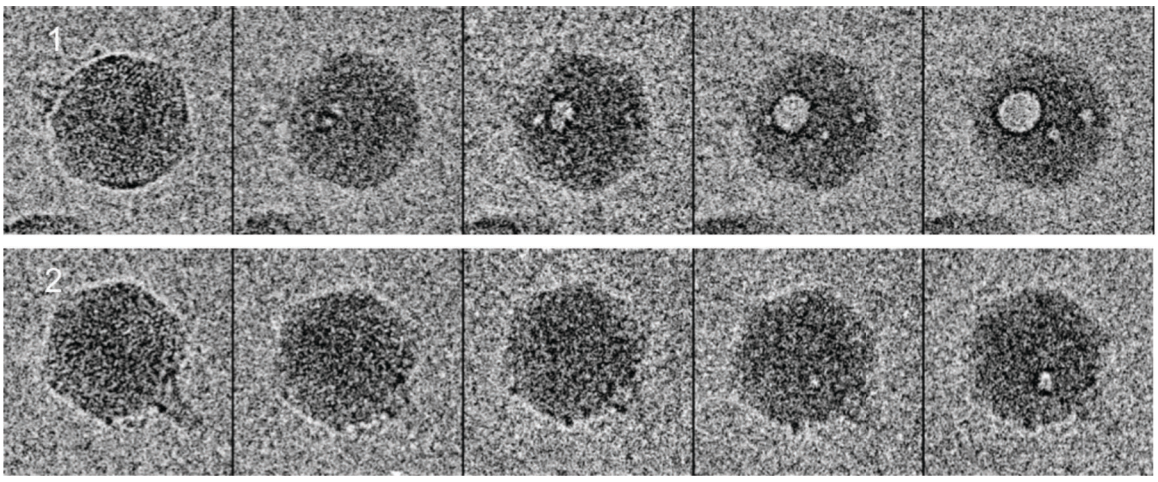

Figure 2. Higher magnification images of two particles labeled 1 and 2 selected from the two montages in Figure 1 to highlight the DNA fingerprints and other fine features.

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