Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602102327

Cryo-electron Microscopy of Large Complexes: A Cold Look at Fusion

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Cryo-electron microscopy (cEM) has become an important tool in the arsenal of the structural biologist. The introduction of a simple method for the vitrification of aqueous suspensions of particles [1] has led to an explosion of results [2]. The talk will illustrate a simple method of sample preparation for particles in suspension. The steps of a vitrification will be illustrated. Thephysics of vitrification [3-5] will be outlined in order to provide a rationale for optimizing the specimen preparation process. Some of the common artifacts that can arise from problems during vitrification (e.g. high salt concentrations and drying), specimen transfer (e.g. contamination, devitrification), microscopy (e.g. beam damage, devitrification), and imaging will be discussed. Images showing the effects of each of these will be presented.

Case studies of the image processing of an ordered structure, the icosahedral virus, Semliki Forest Virus (SFV) [6-12](panel A), and a poorly ordered structure, the non-icosahedral virus, Human Immunodeficiency Virus (HIV)[13-17] (panel B) will be presented. The effects of contrast transfer function correction [18-20] on the interpretation of the images and the reconstructions will be discussed. We will illustrate the complementarity of cEM and X-ray crystallography in the fitting of X-ray structures into cEM-derived densities and the deposition of the densities in the protein structure database [12,20-22].

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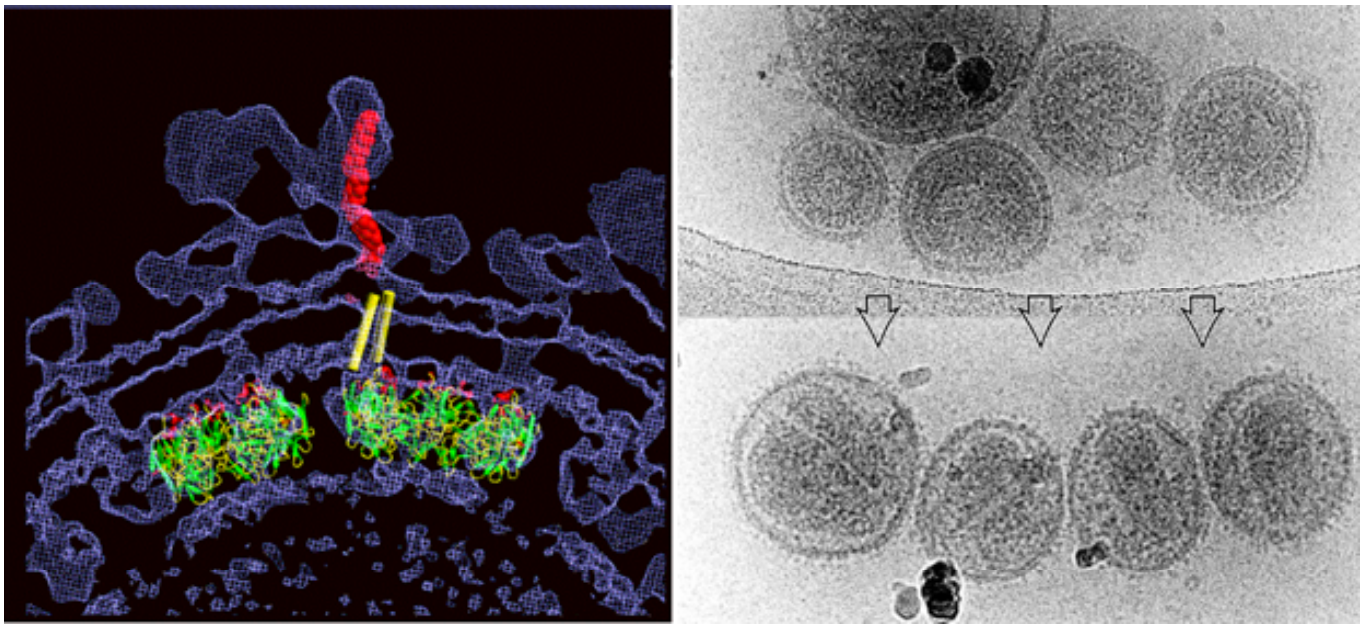

A -Semliki Forrest Virus (< 4nm>) B-Human Immunodeficiency Virus (<150nm>)

A- The placement of the several of the capsid, transmembrane helices and and elongated portion of protein E2 in Semliki Forest Virus are shown in this section from the 9A reconstruction.
B- The lifecycle of HIV is shown in cryo-electron micrographs of immature (top) and mature (bottom) virus. Note the variation in size of the virions and the condensation of the radially arranged Gag protein of the immature virion to form the cone-like capsid of the mature virion.