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Opinion

The SIV Surface Spike Imaged by Electron Tomography: One Leg or Three?

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Strategies to inhibit the cellular entry of human immunodeficiency virus type 1 (HIV-1) constitute an important element of current approaches to develop an effective vaccine against AIDS [1]. The entry of viruses such as HIV-1, and its closely related simian counterpart simian immunodeficiency virus (SIV), is mediated by the interaction of glycoprotein spikes on the viral membrane surface with receptors on the target cell membrane. The viral spike itself is a trimeric complex of gp41, a membrane protein which anchors the spike to the membrane, and gp120 which is non-covalently associated with gp41. Cartoon representations of the spike as a trimeric entity abound, but what does it really look like? So far, structural information on the spike has come largely from X-ray crystallographic analysis of the core of the gp120 monomer in the CD4-liganded [2] or unliganded forms [3], and of trimers of the helical regions of gp41 in a post-fusion conformation [4]. The structure of an intact trimeric spike itself has, nevertheless, remained elusive.

In this issue of *PLoS Pathogens,* Zanetti and coworkers [5] present electron tomographic studies of purified virions aimed at obtaining an average 3-D structure of the SIV surface spike. They report that the overall shape of the spike resembles that of a mushroom with a single stalk forming the junction with the viral membrane. By manually positioning the X-ray structure of an unliganded gp120 monomer [3] into this map, they suggest two atomic models for the probable structure of a trimeric spike displayed on the surface of their SIV specimens. This paper follows closely at the heels of a recent report by Zhu and coworkers [6], who have carried out almost exactly the same type of electron tomographic analysis of SIV, but conclude that the spike has a more globular "tripod-like" shape, with three separated legs near the surface of the viral membrane, and who propose a very different putative atomic model for the gp120 trimer. Nevertheless, both Zhu et al. and Zanetti et al. are confident that their models account for, and are consistent with, what is known about the structure of the spike from numerous biochemical experiments.

How is it possible that two different groups carrying out essentially the same experiment can arrive at completely different density maps for the viral spike, and contradictory models for the Env trimer? The simplest possibility is that there are genuine differences in the viruses imaged by the two groups. This, however, appears to be unlikely, given that both viruses are closely related and express very similar Env molecules that carry truncations in the C-terminal tail. There are subtle differences in the electron microscope hardware used by the two groups, but these differences are minor, and, indeed, the raw images obtained in both cases appear to be closely comparable. There are however, many aspects of data collection and image analysis that could have a profound impact on the final result. Here I attempt to provide a perspective on the possible origins of the discrepancy between the conclusions arrived at in these two papers, and focus primarily on experimental and computational aspects of using cryo-electron tomography that need careful attention to avoid artifacts and inaccuracies in structural determination.

Images recorded in a transmission electron microscope are "projection" images, meaning that they contain information from all regions of the specimen through which the beam was transmitted. Thus, each image contains information from all heights of the specimen collapsed into a single plane. From these projection images, one can derive 3-D information using one of two computational methods. In one method, often referred to as "single particle" electron microscopy [7], the poor signal-to-noise ratios inherent to biological electron microscopy are overcome by averaging images recorded from thousands of identical copies of specimens randomly oriented relative to the electron beam. The key to averaging multiple images is the requirement that the variations from one image to another are solely due to differences in its orientation relative to the electron beam. This type of averaging is even more powerful when the objects display intrinsic icosahedral, helical, or crystalline order. The second method, referred to as electron tomography [8,9], involves the imaging of "one-of-a-kind" objects such as nonsymmetric viruses and subcellular organelles. By recording a series of images in which the orientation of the specimen is varied relative to the incident beam, it is possible to obtain a series of projection views of the object (Figure 1), which can be converted into a 3-D volume using methods that generally rely on the use of weighted back-projection algorithms. Since the first use of these methods nearly four decades ago [10], they have been applied to a variety of biological objects to describe 3-D structures at varying resolutions. The two methods intersect in special cases as demonstrated in the work of Zhu et al. and Zanetti et al.: tomographic imaging of

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Abbreviations: SIV, simian immunodeficiency virus

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