

STRUCTURE-BASED VACCINE DESIGN BY ELECTRON MICROSCOPY

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Modern vaccine design relies on multiscale, interdisciplinary efforts that take advantage of innovative technologies such as *in silico* identification of antigens, high throughput screening of antigen immunogenicity, and gene expression profiling to predict host immune responses. In recent years, structural analysis has played an increasingly important role in vaccine development as a means to improve antigen stability, immunogenicity and large scale production. Transmission electron microscopy (TEM), and in particular cryo-TEM, is an established and powerful imaging technique applicable to many specimens, including the three-dimensional (3D) reconstruction of macromolecules and their associated complexes to high resolution. The technique is parsimonious in its material requirements and captures the specimens in their fully hydrated state, close to their native environment. The resolution of cryo-TEM reconstructions was limited to the subnanometer range until the recent development of direct electron detectors and improvements in image processing software, which has led to a so-called “resolution revolution” in the cryo-TEM field. Several protein structures have now been solved at near atomic resolution, establishing the technique as a viable alternative to X-ray analysis for high resolution structure determination. We have determined several structures with and without bound compounds at 2.9 Å – 3.6 Å resolution, which are being integrated into drug discovery and development workflows by our clients. Here we present the 2.4Å resolution structure of apoferritin determined with our Titan Krios electron microscope as an example of the cryo-TEM services available at NIS. These services are significantly enhanced with unique access by NIS to a new instrument, Spotiton, a robotic device that dispenses picoliter-volumes of sample onto a self-blotting nanowire grid as it flies past en route to vitrification. This provides several advantages over standard vitrification methods, including more automated and reproducible preparation of specimens and reducing the deleterious effects of particles interacting with the air-water interface.

While high resolution 3D structure determination by cryo-TEM is at the forefront of structural biology, averages of 2D projection images at moderate resolution in negative stain or vitreous ice can also provide a wealth of information that may be difficult to obtain using other methods. This is illustrated in a number of case studies, including (1) mapping of neutralizing epitopes on the CMV pentameric glycoprotein complex; (2) mapping of neutralizing epitopes on the HIV-1 envelope glycoprotein trimer; (3) assessment of structure and conformational stability of pre- and post-fusion RSV-F protein; (4) characterization of novel adjuvants and protein delivery systems. In summary, both the moderate resolution TEM and high resolution cryo-TEM methods are well suited to extensively characterize antigen structure-function relationships, some of which may be refractory to other experimental methods.