Cryo-Transmission Electron Microscopy: From Sample Preparation To Reconstruction Of 3D Models

Andrea Villanueva, Reika Watanabe, and Elizabeth Villa

The Department of Chemistry & Biochemistry, University of California San Diego, 9500 Gilman Drive, La Jolla, California

ABSTRACT

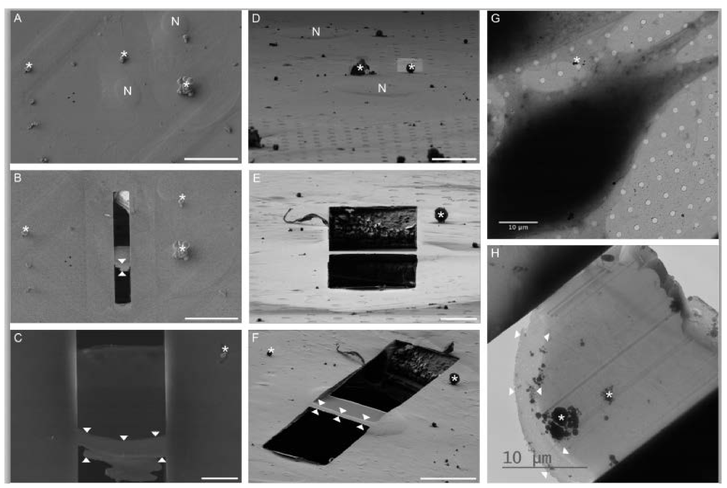
The use of cryo transmission electron microscopy (Cryo-TEM) has revolutionized the way in which we can observe biological specimens in their native state, without the need for staining. Cryo-TEM looks at samples in a subatomic resolution at cryogenic temperatures. This technique works well with biological samples and we are able to look inside of cells at a near atomic level. The frozen sample must be very thin (100-300 nm) to get the best results. Still, this way is technically challenging and the sample is more vulnerable due to radiation. Cryo-EM is most desirable technique because the data can be collected in a near-native state.

To prepare the samples for observation under cryo-TEM, the sample is placed on a grid and rapidly frozen in liquid nitrogen. The sample is now vitrified to preserve the hydrogen bonds and does not let ice crystals form to damage the sample. From here, we place the sample into the Dual beam microscope equipped with a scanning electron microscope (SEM) and Focused Ion Beam (FIB) system, where we are able to create a thin slice called lamella. The advantages of using the FIB is that it is fast and precise. After milling, the frozen hydrated state of the lamella allows the sample to be inserted into the high vacuum of the TEM. Electrons are then shot at the sample to create an image. Because these biological samples are sensitive to radiation, only a small amount of electrons can be used without damaging the sample.

Using the TEM, we can take a series of two dimensional projections at different angles (called tomography or a tilt series) and use a computer program such as IMOD to combine them and create a 3D image called a tomogram. Through this process, we are able to see structures such as nanoparticles, ribosomes, mitochondria, nucleus, etc. at near atomic resolution. This process can be used in a wide range of areas such as structural biology or cancer research.

The Villa lab is currently working in collaboration with Bryan Spring at Harvard Medical School to look at how a new photoactivatable drug in the form of liposomes can be used for local tumor treatment. In the following data, we obtained the data using the TEM and applied reconstruction methods to visualize such encapsulated nanoparticles.

Lamella Under Various Microscopes



SEM View **FIB View** **TEM View**

Figure 1: In this figure, we see FIB milling and transmission electron micrographs of mouse NIH3T3 fibroblast cells at different angles. The top row are pictures before milling, while the bottom two rows are after milling. It is clear that without milling, there is limited access to visibility of the cells. (Wagner, F 2015 submitted)

How Tomography Works

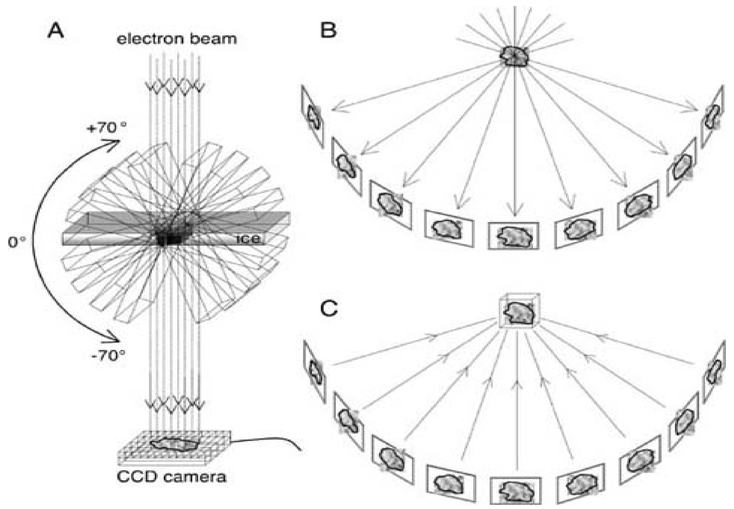


Figure 2: Reproduced from Steven and Belnap, Current Protocols in Protein Science, 2005

Reconstructing A Tomogram

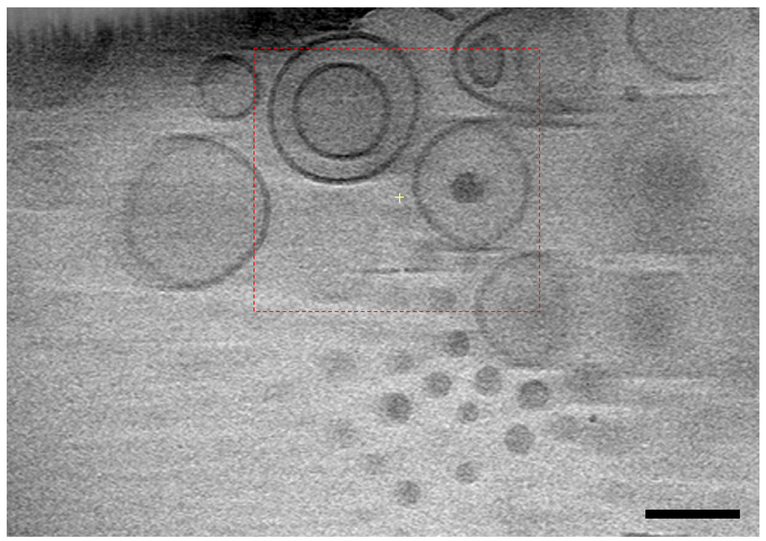


Figure 3: After taking many projections, we can put them together to reconstruct the 3D volume of the sample. This is a tomogram of the liposomes with encapsulated and floating nanoparticles. Here we see one slice of the tomogram. Because the sample is relatively thick, as we view more stacks, we can get a fuller image of the rest of the sample.

3D Model Representation

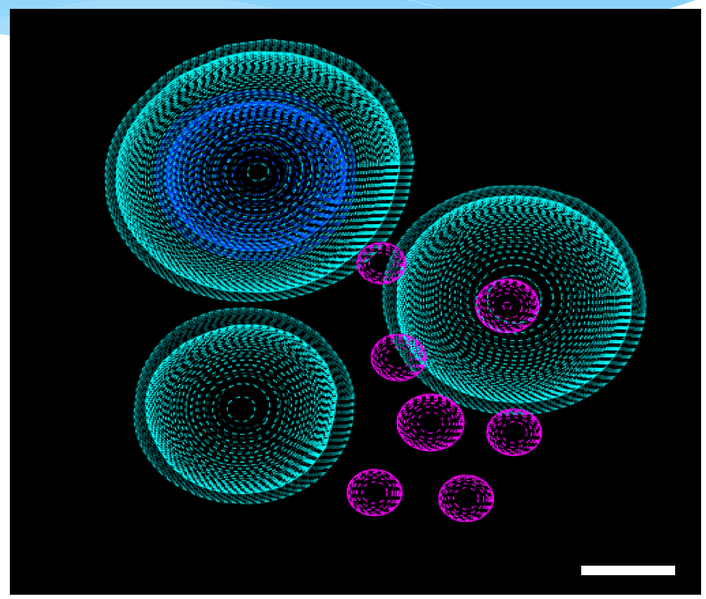


Figure 4: By using IMOD, we can create models with 3D features. 3D modeling can help us visualize difficult complexes at various angles. In this figure, we see the boxed area from Figure 3. This is a representation of 3 liposomes with a bilayer membrane (In Cyan) with one liposome having an encapsulated nanoparticle (In Magenta).

CONCLUSIONS

In this data, we found two types of nanoparticles: free floating nanoparticles surrounding the liposomes and nanoparticles encapsulated by the liposomes. The collaborators have found that both of these nanoparticle forms are photoactivatable. The next steps would be to determine which form of these nanoparticles functions the best in terms of local control and a reduction in toxicity for cancer treatment. By using mice models, we can attempt to differentiate the efficiency of the nanoparticles by its form (Free floating nanoparticles vs. encapsulated nanoparticles). This can help us determine the best treatment as well as optimize the way liposomes are made.

FUTURE RESEARCH

Cryo-EM has a bright future ahead in the way it images cells in a near-native state. The microscopes themselves are still evolving. The FIB itself is still a prototype, but is looking promising for the future of imaging. Cryo-EM is technically demanding, so as more people become familiarized with this technique, the better growth there can be. As for the drug, despite there being many nanoparticles outside of the liposomes, there is still room for refinement.