Improving super resolution cryo-CLEM

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Abstract

Cryogenic transmission electron microscopy (cryoEM) and super-resolution fluorescence microscopy (FM) are state-of-the-art methods that enable the observation of the function and structure of living matter at the molecular scale. The fundamental difference in contrast method between these two techniques can be used to gain novel insights.

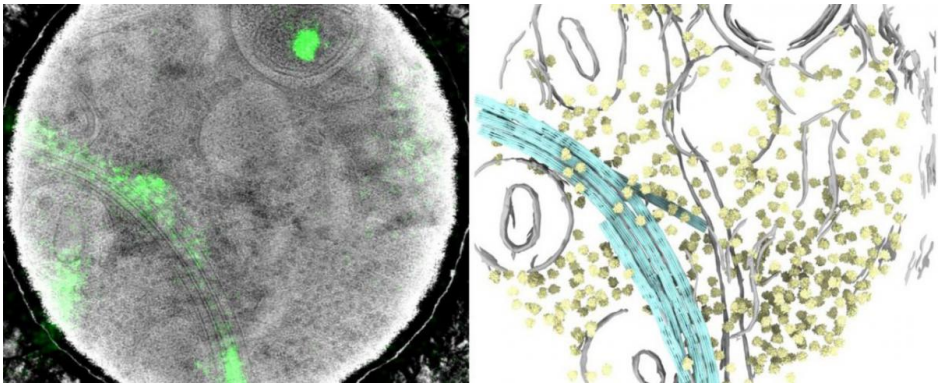
With cryo-TEM, electrons are used to probe the electron density of the entire sample. Though the technique can routinely deliver molecular scale images, this way of generating contrast also poses an issue: it is often difficult to identify structures of interest on the basis of electron density maps solely.

Using fluorescence microscopy, on the other hand, structures of interest can be made visible by use of target-specific, non-invasive probes such as fluorescent proteins or synthetic dyes. While the resolution of light microscopy is typically low in comparison to TEM, this ability to single out individual species of interest on top of an otherwise dark background is what makes fluorescence microscopy so exceptionally useful.

The combination of the two methods, called super-resolution cryogenic correlated light and electron microscopy (super resolution cryo-CLEM), leverages these different mechanisms of contrast generation in order to combine the high spatial resolution of cryo-TEM with the molecular specificity of FM.

In our lab, we work on the development of a super-resolution fluorescence microscope for imaging cryogenic samples. By developing this microscope as well as novel fluorescent probes, software, sample preparation methods, and other tools, we aim to increase the resolution, throughput, and correlation accuracy of super resolution cryo-CLEM experiments.

In parallel with method development, we apply super resolution cryo-CLEM to study various biological systems, such as the TAP antigen processing system and the nanopatterning of receptor proteins on the cell membrane. Within these projects, super resolution cryo-CLEM enables us to identify exactly where in the cell a protein of interest is located, and subsequently to image the environment of this protein with near molecular resolution.



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(left) Super-resolved reconstruction showing microtubule cytoskeleton. (right) Sub-tomogram average of microtubules extracted from cellular tomograms at regions located using super-resolution imaging fitted map back into the tomogram (cyan), together with identified ribosome locations (yellow) and membranes (grey).