Correlative Cryo-Fluorescence Light Microscopy and Cryo-Electron Tomography of Streptomyces

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Light microscopy (LM) and electron microscopy (EM) are imaging techniques that are commonly employed to study cellular structure and function. Fluorescence microscopy (FM) can provide dynamical and positional information of multiple chemically or genetically labeled proteins within tissues and whole cells at a resolution in the order of several hundreds of nanometers. Transmission electron microscopy (TEM) can obtain structural and morphological information of macromolecules and cellular compartments of thin fixed specimens at the subcellular level with a resolution of several nanometers. Combining the plethora of labelling possibilities for FM with the structural imaging capabilities of TEM in a correlative light and electron microscopy approach, in which a single specimen object is imaged by both techniques, makes it possible to identify and target sparse or transient structures for three-dimensional imaging *in situ* at nanometer resolution.

We developed instrumentation and employed workflows for cryo-correlative light and electron microscopy (cryo-CLEM), combining cryo-fluorescence light microscopy and cryo-electron tomography (cryo-ET).We used both a separate approach, combining FM using a Linkam cryo-LM stage and cryo-ET on a FEI Titan Krios, as well as an integrated approach, using an FEI iCorr system on a Tecnai F20 equipped with an energy filter (Gatan) for investigation of *Streptomyces* bacteria samples.

Streptomycetes are mycelial bacteria that grow as long hyphae and reproduce via sporulation. They are well known for the production of antibiotic, anti-parasitic and anti-fungal drugs. On solid media, Streptomyces subsequently form distinct aerial mycelia where cell division leads to the formation of unigenomic spores which separate and disperse to form new colonies. In liquid media, only vegetative hyphae are present divided by non-cell separating crosswalls. Their multicellular life style makes them exciting model systems for the study of bacterial development and cell division. Their thickness and branched structure make them a suitable model system for cryo-CLEM. Cryo-CLEM using chemical fluorescent labels for DNA, lipids and cell wall was used to target sparse and transient structures in *Streptomyces* bacteria.

Available instrumentation and possible workflows for cryo-CLEM and the and the resulting complex intracellular structures of *Streptomyces* will be presented.