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Understanding the Three-dimensional Architecture in Terms of the Spatial Arrangement of Organelle-bound Protein Complexes - Our Next Great Challenge.

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On a scale of a few nanometers the cell with very dynamic massive supramolecular architecture is largely uncharted terrain. We are working towards new technologies to provide 3D maps of cellular components at molecular resolution. Helium-cooled electron tomography of ultra-thin cryo-sections from unfixed or fixed cells is our method of choice for determining the three-dimensional structures of large macromolecular assemblies in cell organelles.

We focus on receptor-mediated signaling and endocytosis; which, when in a state of kinetic imbalance, may result in cancer. Our goal is to visualize supramolecular architecture in a native cellular state. By using the High Pressure Freezing techniques we were able to vitrify bacteria growing in sucrose and yeast growing in 20% dextran. So far in the mammalian system, only cartilage and skin could be vitrified but no mammalian cell culture or any other tissue without extensive pretreatment of cryo-protectant. We therefore explored two alternative solutions.

First we are working out conditions to cryo-section a monolayer of frozen hydrated cultured mammalian cells vitrified in the Vitrobot. Secondly, we vitrified thawed (immunolabeled) aldehyde fixed Tokuyasu cryo-section according to B. Geiger. Cryo-sections where picked up in a newly developed sandwich method allowing for better structural preservation. For both purposes however, we used the Vitrobot for blotting and vitrification by plunge freezing in ethane using a humidity controlled glove box attached to the Vitrobot.

The Vitrobot was in both instances used because just prior to vitrification, the 150nm thick layer (compare with a soap film) is vulnerable to heat and mass exchange. We found that vitrification of a monolayer of cells or a thawed cryo-section in a temperature and humidity-controlled environment is essential to prevent osmotic and temperature-induced alterations of structures.

Cryo-electron tomograms constructed from cryo-sections reveal remarkable membrane features at high resolution. By placing X-ray data into density maps derived from tomography, we are beginning to construct a three-dimensional molecular model for membrane protein complexes.

There is currently great enthusiasm among colleagues, since ongoing advances in cryo-sectioning of native material at ultra low temperature as well as cryo-EM instrumentation and computational methods may ultimately make it possible to obtain resolutions in the range of 4 nm, i.e. potentially high enough to locate individual proteins in a cell.