Visual Proteomics or From Words to Literature in Protein Science  
(Wolfgang Baumeister)

At a glance

Humans are a highly visual species and in the wake of new imaging technologies the life sciences enter another visual phase. Superresolution light microscopes have surpassed the ‘diffraction barrier’ and electron microscopy has left behind the artefact-stricken methods of the past. Cryoelectron tomography fills a critical gap between techniques with atomic resolution and light microscopy bridging the divide between molecular and cellular structural studies.

The challenges of post-reductionist protein science

Cellular processes rely on the concerted action of individual macromolecules or functional modules. At one end of the scale, there are highly stable interactions that are robust enough to withstand the rigors of isolation and purification. Structures of a large proportion of those stable complexes will be solved by the established methods of structural biology, x-ray crystallography or EM single particle analysis or by a combination of these methods (‘hybrid methods’). At the other end of the scale, there are interactions that occur more fleetingly, in response to specific signals, for example. The chances for determining the structures of such transient assemblies or networks by using any type of crystallography are relatively poor.

Proteomics has greatly advanced our knowledge of the molecular inventories (‘parts lists’) of cells and organelles but conjecture remains regarding the spatio-temporal organization of proteomic networks. On a systems level, our current understanding of protein interaction networks is still rather limited, mainly because experimental methods for the analysis of supramolecular structures subject to stochastic variability are underdeveloped. But how much easier would it be to comprehend the workings of a cell if we could obtain 3-D images of cells with molecular resolution, ideally in a non-invasive manner. We have to tackle the methodological challenges of studying proteins in situ without disrupting their physiological context.
Cryoelectron tomography: A key player in visual proteomics

Electron tomography enables us to obtain 3-D images of large and pleomorphic objects, such as cells and organelles with unprecedented resolution. When applied to samples embedded in vitreous ice, i.e. samples frozen so rapidly that the water molecules do not form damaging ice crystals, then the notorious artefacts associated with conventional specimen preparation for EM are avoided. At a resolution of a few nanometers, cryoelectron tomograms are essentially 3-D snapshots of the entire proteome of a cell or organelle, and they contain precise information about the spatial relationships of macromolecules in cellular networks. However, the price to pay for a close-to-life preservation of the cellular samples embedded in vitreous ice is low contrast and the need to minimize exposure to the ionizing electron beam results in a low signal-to-noise ratio of the tomograms. Therefore, it is not a trivial task to retrieve the imposing amount of information contained in such tomograms. Sophisticated computational tools are needed for the segmentation and visualization of cellular tomograms and for their molecular interpretation.

Where we stand and the challenges ahead of us

At present, the resolution of cellular tomograms is limited to 3 to 5 nm, depending on specimen thickness. While this is sufficient for studying large molecular machines such as nuclear pore complexes or ribosomes in situ, higher resolutions are needed for charting the molecular landscapes of cells in a comprehensive manner.

To achieve this goal a number of technological and methodological challenges must be addressed. To name a few: The ‘field of view’ of tomograms is small compared with the landscape of an entire cell. Therefore, correlative light microscopy-electron microscopy approaches are needed to guide the tomographic studies. Fluorescence microscopy is a powerful tool to identify a particular molecular or cellular structure and can be used for the navigation of cellular landscapes prior to zooming in on a feature of interest with the electron microscope.

New micromachining tools such as focused ion beam technology will enable us to dissect frozen-hydrated cells and tissues in a controlled manner and make them
accessible to electron tomography. There is huge potential in marrying the power of identification (mass spectrometry) with the power of visualization (electron tomography) and the spatially controlled dissection of complex samples will supersede the traditional ‘slice and dice’ approaches. Advances in electron optics promise to improve resolution and enhance contrast. The fully automated segmentation of noisy tomograms will be a prerequisite for the improvement of throughput and it will make the interpretation of tomograms more objective and reproducible. And last but not least, advanced pattern recognition techniques will allow us to fully exploit the wealth information contained in tomograms.

**Outlook**

The need for experimental methods enabling us to study proteins in their natural habitat without perturbing their functional context is self-evident. Cryoelectron tomography has unique potential to meet this challenge. It is reasonable to expect that advances in instrumentation and in computational image analysis will allow us to attain resolutions in the range of 1.5 to 3 nm in a not too distant future. *A priori*, it is clear that even a complete molecular atlas of a single cell – which, of itself, would be prodigious technical accomplishment – would yield only limited functional insight. For a deeper understanding of the trends of co-association and compartimentation – i. e. the molecular sociology of the cell – interaction patterns have to be deduced from many tomograms on statistical grounds. This propositions represents just one of many arenas where computational analysis moves into center-stage.
References:


Figures

Fig. 1
Cryoelectron tomography of polyribosomes.
Left: Tomogram of a polyribosome; interpretation is based on pattern recognition (‘template matching’).
Right: Snapshot of a Brownian Dynamics simulation of this polyribosome with nascent polypeptide chains.
Fig. 2
Cryoelectron tomography of a presynapse. Highlighted is the network of connectors (red) and tethers (blue) interconnecting the synaptic vesicles.