APPLICATION NOTE

Cryo-electron tomography for neuroscience

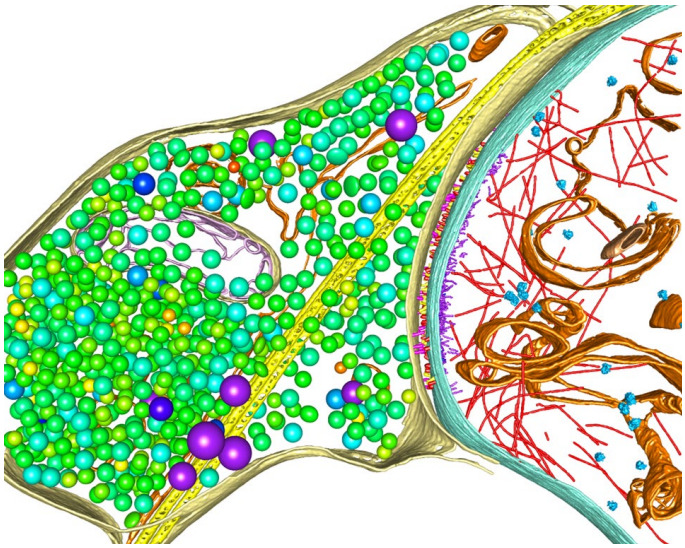
How do the building blocks of a synapse work together?

In order to understand synaptic mechanisms and their components it is necessary to visualize them. Understanding subcellular mechanisms and components also is essential to understanding larger life processes.

Most cellular structures, proteins and organelles are too small to be resolved by light microscopes, but they can be resolved by a cryo-electron microscope. However, single particle cryo-electron microscopy (cryo-EM) is limited to highly purified and isolated proteins lacking a connection to the cellular context. Cryo-electron tomography (cryo-ET) expands cryo-EM by visualizing proteins that contribute to a mechanism within their functional cellular environments from a flash-frozen cell—all within a single cryo-ET 3D dataset. Our integrated cryo-electron tomography workflow provides label-free, fixation-free, nanometer-resolution imaging. Seeing the entire picture on multiple levels from molecules to organelles, complements existing dynamic techniques for highly accurate data in order to facilitate breakthrough discoveries.

Why use cryo-ET for sub-cellular imaging?

High-resolution cryo-ET avoids the alterations caused by conventional preparation techniques such as chemical fixation to allow imaging of cellular morphology in fully hydrated conditions. For researchers to understand complex biological mechanisms, protein structures and complexes are imaged in 3D at nanoscale resolution within a cell while maintaining their context.



3D view of an excitatory synapse between cultured hippocampal neurons revealed by cryo-electron tomography. *Image courtesy of Guoqiang Bi of USTC and Hong Zhou of UCLA.*

Research highlights

In situ analysis of ultrastructural organization underlying distinct synaptic functions.

Tao C.-L., Liu Y.-T., Sun R., Zhang B., Qi L., Shivakoti S., Tian C.-L., Zhang P., Lau P.M., Zhou Z.H., et al. 2018 Differentiation and characterization of excitatory and inhibitory synapses by cryo-electron tomography and correlative microscopy. JNeurosci 1548-17. doi: 10.1523/JNEUROSCI.1548-17.2017

The image above shows a 3D view of an excitatory synapse between cultured hippocampal neurons revealed by cryo-ET. Segmented structures are rendered as colored surfaces to facilitate visualization of synaptic structures, including:

- Presynaptic (light yellow) and postsynaptic (cyan) membranes
- ER and endosomes (orange)
- Mitochondrion (outer: gold and light pink) microtubules (yellow)
- Ribosomes (cyan)

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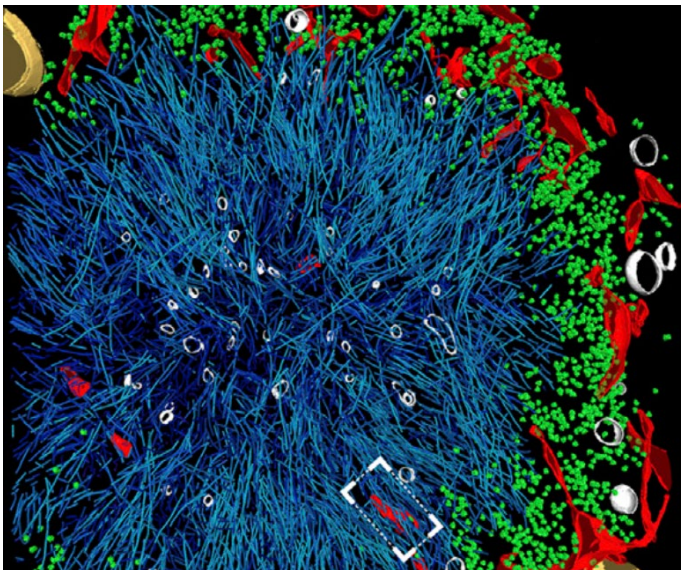
- Actin filaments (red)
- Putative presynaptic (magenta) and postsynaptic (yellow) adhesion molecules
- Putative glutamate receptors (red)
- Postsynaptic density filaments either attached to (blue) or away from (purple) the postsynaptic membrane
- Dense core vesicles (purple) and synaptic vesicles of various sizes

Weeds in the brain

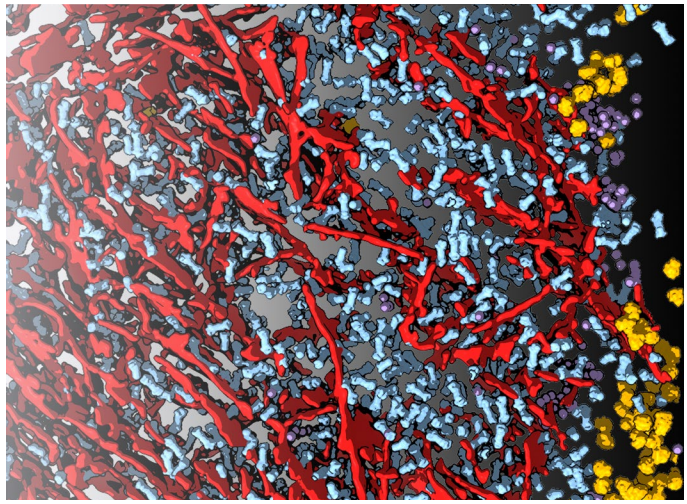
Protein aggregates involved in motor neuron disease (or ALS) have been captured stalling the molecular machines needed for normal protein degradation. Protein aggregates are a hallmark of neurodegeneration. High-resolution snapshots of the structure of one such aggregate offer an unprecedented view of how these proteins disrupt crucial cellular functions. Details of contrasting mechanisms of Poly(GA) and Poly(Q) aggregates are detailed in the publications of Guo et al. (Cell, 2018) and Bauerlein (Cell, 2017), respectively, and highlighted in the next section.

Bäuerlein F.J.B., Saha I., Mishra A., et al. 2017 *In Situ* Architecture and Cellular Interactions of PolyQ Inclusions. Cell 171, 179-187.e10. doi: 10.1016/j.cell.2017.08.009

A common feature of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's is the accumulation of toxic protein deposits in the nerve cells of patients. Once these aggregates appear, they begin to proliferate like weeds. If and how these deposits damage nerve cells and lead to their demise remains largely unexplained. A detailed insight into the three-dimensional structure of the protein aggregates should help researchers to solve this puzzle. Source: MPI of Biochemistry, 07 Sept 2017



PolyQ fibrils (cyan) interact with endoplasmic reticulum membranes (red) while ribosomes (green) barely penetrate the inclusion. Image © 2017 Elsevier Inc.



3D rendering of a an aggregate within a neuron: poly-GA ribbons (red), 26S proteasomes (blue), ribosomes (yellow), TRiC/CCT chaperonins (purple).

Are ALS dipeptide repeat ribbons entangling proteasomes?

Guo Q., Lehmer, C., Martínez-Sánchez A., et al. 2018 In Situ Structure of Neuronal C9orf72 Poly-GA Aggregates Reveals Proteasome Recruitment. Cell 172, 696-705.e12. doi: 10.1016/j.cell.2017.12.030

In neurons, ALS/FTD poly-Gly-Ala peptides aggregate into a dense network of twisted ribbons. The ribbons sequester a large fraction of the cells' proteasomes. Many trapped proteasomes are frozen in a catalytic transition state. Source: Daisuke Ito, ALZ Forum, 02 Feb 2018

Further reading

Editorial. Vaites L.P., Harper, J.W. 2018 Protein aggregates caught stalling. Nature 555, 449-451. doi: 10.1038/d41586-018-03000-2

Gruber A., Hornburg D., Antonin M., et al. 2018 Molecular and structural architecture of polyQ aggregates in yeast. PNAS 115, E3446-E3453. doi: 10.1073/pnas.1717978115

Shahmoradian S.H., Genoud C., Graff-Meyer A., Hench J., Moors T., Schweighauser G., Wang J., Goldie K.N., Suetterlin R., Castano-Diez D., et al. 2017 "Lewy pathology in Parkinson's disease consists of a crowded organellar membranous medley" BioRxiv. doi: 10.1101/137976

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