In cryoelectron tomography (cryo-ET), cells or suspensions of biological macromolecules are flash-frozen to preserve them in a native-like state, so that they can be imaged in a transmission electron microscope. In subtomogram averaging (STA), multiple copies of macromolecular complexes from a three-dimensional tomographic reconstruction are combined to yield, under ideal conditions, near-atomic-resolution structures.

One research area of our group is the molecular architecture of neurons, both in their healthy state and in the context of neurodegenerative diseases. Another focus of our group is the study of membrane contact sites (MCS), structures where two cellular membranes come into proximity in order to exchange metabolites. We wish to use the computational resources offered by the HLRN cluster to analyze data for these projects using STA.

Structural basis of proteasome inhibition by poly-GA aggregates.

Mutations in the C9orf72 gene are the most common cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Such mutations lead to loss of native function of the C9orf72 protein and to aggregation of proteins such as poly-GA. We investigated the structural basis of this phenomenon imaging poly-GA aggregates within neurons using cryo-ET [1]. We found that poly-GA aggregates recruit large amounts of neuronal proteasomes (Fig. 1), which are protein complexes which degrade proteins in the cell. STA suggested a functional impairment of the proteasome, suggesting that proteasome impairment may be a relevant phenomenon in these diseases. We seek to understand the structural basis of proteasome inhibition by poly-GA at high resolution. To that end, we aim to reconstitute the poly-GA/proteasome interaction in vitro, and image these complexes cryo-ET.

Structural templating in α-synuclein aggregate spreading.

Deposits of misfolded α-synuclein (α-syn) amyloids, along with the death of dopaminergic neurons within the brain, are characteristic of Parkinson's Disease. A picture emerges in which different neurodegenerative diseases are characterized by different amyloid folds, but the underlying mechanisms are not understood. Additionally, aggregates spread throughout the brain following anatomic connections, imprinting their misfolded conformation on the soluble proteins of neighboring cells. How this seeding process takes place also remains mysterious. We are tackling these questions using cryo-ET. We have recently analyzed the architecture of neuronal α-syn inclusions within cells by cryo-ET [2]. Currently, we are developing image processing workflows to determine amyloid structures within cells by STA. To investigate the mechanisms of templating during spreading, in cellulo structures will be compared with in vitro structures of aggregate seeds obtained by cryo-ET.

Molecular organization of synaptic vesicles.

Synaptic vesicles (SVs) are membrane-bound organelles containing neurotransmitters which are released during impulse propagation at the synapse. We have previously used cryo-ET to investigate factors controlling synaptic vesicle distribution, clustering and fusion at the presynaptic terminal [3],[4]. We aim to identify the major protein complexes residing on SV membranes. We hypothesize that

protein clustering is important within SVs, allowing the formation of pre-assembled functional units. We are incubating SVs with recombinant proteins shown by our collaborator Dragomir Milovanovic (Charité/ DZNE Berlin) to induce SV clustering in vivo and in vitro. We are currently testing the hypothesis that those proteins may form the connections that we have observed interlinking SVs [3].

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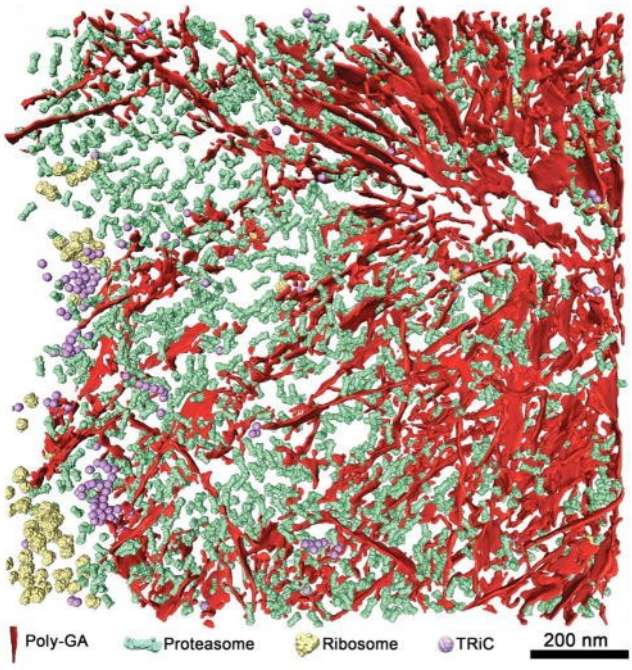

Figure 1. Segmented 3D rendering of a poly-GA aggregate within a neuron showing different macromolecules found either within or at the periphery of the aggregate. Red, poly-GA ribbons; green, 26S proteasomes; yellow, ribosomes; purple, TRiC/CCT chaperonins. Orientations were computationally determined by template matching and STA. (From [1])