From interactomes to in situ structures: what proteomics adds to structural biology

By Prof. Martin Beck, Max Planck Institute of Biophysics, Frankfurt, Germany

Understanding how proteins fold, organize, and function within cells requires approaches that preserve their native architecture. We combine cryo-electron tomography (cryo-ET), crosslinking mass spectrometry, affinity purification MS, and AI-assisted integrative modeling to determine structures and interaction networks of macromolecular machines directly inside human cells.

Using this approach, we dissected the duty cycle of the essential chaperonin TRiC in situ. We resolved open, closed, substrate-bound, and prefoldin-associated states in the cytoplasm, demonstrating that TRiC operates at near-full occupancy in actively translating cells. Mass spectrometry data guided identification of PDCD5 as a cofactor specific to the open conformation. Extending this to the nucleus, we detect TRiC duty cycle intermediates and candidate nuclear substrates, pointing to a role in nuclear proteostasis.

Applying the same strategy to the nuclear pore complex, we substantially extend its known architecture and find that GANP, Centrin-2, and ENY2 — core subunits of the TREX-2 mRNA export complex — are integral components of the nuclear ring, establishing a structural basis for coupling mRNP remodeling to nucleocytoplasmic transport.

Together, these studies illustrate the power of combining cryo-ET with mass spectrometry-based proteomics to reveal how molecular machines are built, regulated, and spatially organized within the cell.