MS05-139 LATE | Structural Characterization of the Linear Ubiquitin Chain Assembly Complex

Rodriguez, Alan (Institute of Molecular Biotechnology, Vienna, AUT); Vogel, Antonia (IMP - Research Institute of Molecular Pathology, Vienna, AUT); Orban-Nemeth, Zsuzsanna (Universitäts-Sportinstitut, AUT); Mechtler, Karl (AUT); Clausen, Tim (Department für Mikrobiologie, Immunbiologie und Genetik, AUT); Haselbach, David (AUT); Ikeda, Fumiyo (AUT)

The Linear Ubiquitin Chain Assembly Complex (LUBAC) is the only known mammalian ubiquitin ligase complex capable of assembling linear ubiquitin chains, which are essential in immune response, autophagy, and development. LUBAC is comprised of two ubiquitin ligases, HOIP and HOIL-1L, as well as the accessory protein Sharpin. HOIP is the ligase necessary in generation of linear ubiquitin chains, which are assembled by formation of a peptide bond between the C-terminal Gly of one ubiquitin and the N-terminal Met of another. HOIL-1L and Sharpin are needed for activation and stabilization of LUBAC although their precise roles remain unclear. The biological functions of LUBAC have been extensively studied, however structural work on the holoenzyme or its constituent components has been difficult given that recombinant expressions yield little protein of poor quality and stability. Furthermore gel filtration analysis indicates that the complex cannot be readily reconstituted *in vitro.* We have recently established a method to isolate recombinant LUBAC with high yield, stability, and purity. Using this protein we have begun to determine the structure of LUBAC by single particle negative stain- and cryo-electron microscopy. Sorting negatively stained particles into 2D class averages reveals a distinct elongated structure with a long axis of 12-15nm. We are aiming to obtain the first 3D map of the LUBAC holoenzyme from these 2D class averages and to collect micrographs from cryo-preparations for high-resolution structural determination. Additionally we are carrying out cross-linking mass spectrometry analysis to determine the relative domain positioning of the proteins in the complex.