MS10-04 Crystal Structure of Human Lysosome-associated Membrane Protein 3 (LAMP-3). Sonja Wilke, Joern Krausze, Konrad Büssow, [a] *Helmholtz Centre for Infection Research, Braunschweig, Germany*
E-mail: konrad@buessow.com

The family of lysosome-associated membrane proteins (LAMP) comprises the multifunctional, ubiquitous LAMP-1 and LAMP-2, and the cell type-specific proteins LAMP-3 (DC-LAMP), BAD-LAMP (UNC-46, C20orf103) and macrosialin (CD68). LAMPs have been implicated in a multitude of cellular processes including phagocytosis, autophagy, lipid transport and aging. LAMP-2 acts as a receptor in chaperone-mediated autophagy. Deficiency causes the fatal Danon disease. The abundant proteins LAMP-1 and LAMP-2 are major constituents of the glycoconjugate coat present on the inside of the lysosomal membrane, the 'lysosomal glycocalyx'. The LAMP family is characterized by a conserved domain of 150-200 amino acids with two disulfide bonds. We have solved the crystal structure of the conserved domain of human LAMP-3. It is the first high-resolution structure of a heavily glycosylated lysosomal membrane protein. The structure represents a novel β-prism fold formed by two β-sheets bent by β-bulges and connected by a disulfide bond. Flexible loops and a hydrophobic pocket represent possible sites of molecular interaction. Computational models of the glycosyated luminal regions of LAMP-1 and LAMP-2 indicate that the proteins adopt a compact conformation in close proximity to the lysosomal membrane. The models correspond to the thickness of the lysosomal glycoprotein coat of only 5-12 nm, measured by electron microscopy.

Keywords: lysosome; LAMP family; CD107a

MS10-05 Phenylalanine Hydroxylase from *Legionella pneumophila*: a Thermostable Dimer Marte I. Flydal [a], Hanna-Kirsti S. Leiros [b], Nicholas Cianciotto[c], Aurora Martinez [a] [a]*Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway.* [b]*Norstruct, Department of Chemistry, University of Tromsř, 9037 Tromsř, Norway.* [c]*Department of Microbiology-Immunology, Northwestern University Medical school, 320 East Superior Ave, Chicago, IL 60611, USA*
E-mail: marte.flydal@biomed.uib.no

We have recently solved the structure of phenylalanine hydroxylase (PAH) from the pathogenic bacterium *Legionella pneumophila* (lpPAH). This is the third structure of a bacterial PAH to be solved. In contrast to those from *Chromobacterium violaceum* and *Colwellia psychrerythraea* that are both monomers, lpPAH is organized into a dimer. In humans, PAH (hPAH) is a tetramer with each subunit consisting of three domains; regulatory, catalytic and oligomerization domain. It is present in liver where it catalyzes the first and rate-limiting step in degradation of excess L-Phenylalanine (L-Phe) to avoid neurotoxic accumulation. To ensure that the L-Phe level is still retained in sufficient amounts for protein synthesis, the human enzyme is closely regulated by mechanisms such as substrate-activation and also positive cooperative behaviour toward increasing concentration of substrate. The sophisticated regulatory behaviour is possible due to its complex structure and we can therefore learn about evolutionary adaptation by studying bacterial forms of PAH. In addition to being much simpler in both size and structural organization we expect the enzyme to play a less important role in organisms without a nervous system that needs protection. The role and importance of PAH in bacteria in general have not been established, but our recent results show that in *L. pneumophila* this enzyme is important for synthesis of pyomelanin, a tyrosine-derived pigment implicated in resistance to light [1] and acquisition of iron [2]. We have also characterized the recombinant enzyme and found that it has several unusual traits compared to other PAHs. In addition to its particular dimeric structure it exhibits an unexpectedly high thermal stability. Its activity is dependent upon the concentration of iron, the essential metal cofactor in the active sites of all PAHs characterized so far that is also important for the virulence of *L. pneumophila*. These special properties can now be rationalized based on the crystal structure of lpPAH.

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Keywords: phenylalanine hydroxylase; dimeric crystal structure; high thermostability.