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BOOK OF ABSTRACTS OF THE 8TH MEETING ON MEDICINAL BIOTECHNOLOGY

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Insights into the structure and activity of LONP2, a peroxisomal AAA+ ATPase

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Peroxisomes are single membrane-bound organelles that participate in a wide range of metabolic pathways, often involving multiple H_2O_2-producing oxidases. The large amounts of H_2O_2 generated inside these organelles, combined with the absence of protein repair systems renders peroxisomal proteins particularly susceptible to oxidative damage. Notably, distinct peroxisomal matrix proteins were shown to display heterogeneous half-lives, pointing to the existence of Protein Quality Control mechanisms (PQC) that degrade damaged proteins. In mammalian peroxisomes, two proteases were identified: TYSND1 (Peroxisomal leader peptide processing protease) and LONP2 (Lon protease homolog 2). TYSND1 cleaves flexible loops within some peroxisomal proteins and the type 2 peroxisomal targeting signals; however its restricted substrate specificity makes a role in PQC system unlikely. In contrast, LONP2 belongs to the AAA+ family of ATP- dependent proteases, a family typically involved in PQC. Structurally, LONP2 comprises an N-terminal domain (NTD) of ~200 amino acid residues, an ATPase AAA+ domain, a Lon proteolytic domain, and a peroxisomal targeting signal type 1. Mammalian LONP2 is most related to mitochondrial LONP1 (38% identity), the second LONP member expressed in mammals. LONP1 degrades misfolded, oxidized, or unassembled proteins using its NTD to both recruit substrates and regulate allosterically the proteolytic domain. Unfortunately, the NTDs of LONP1 and LONP2 are poorly conserved, and so data on LONP1 cannot be easily extrapolated to LONP2. Thus, the nature of the substrates targeted by LONP2 and the mechanisms regulating its activity remain largely unknown. In this work, we aim to comprehensively investigate the role of LONP2 in peroxisomes. To this end, LONP2 was heterologously expressed in *E. coli* and purified using affinity and size-exclusion chromatography. The hexameric, catalytically active fraction of LONP2 was subsequently used in proteolytic assays with β-casein as a substrate. Structural analysis of LONP2 reveals distinct features compared to mitochondrial LONP1. Further studies, including the characterization of mutant variants, are currently underway.

KEYWORDS: *lonp2, peroxisomes, aaa+ atpases*