Intra-plastid proteolysis is essential in plastid biogenesis, differentiation and protein homeostasis (proteostasis). Furthermore, protein N-termini are a major determinant of protein stability in bacteria and eukaryotes, resulting in the concept of the ‘N-end rule’. In bacterial systems proteins with N-terminal degradation signals are degraded by the Clp protease system. Determinants of chloroplast protein life-time and stability are poorly understood, even if this is of critical importance for chloroplast proteostasis. In my talk, I will discuss the organization and function of Clp protease system present in chloroplasts (and other non-photosynthetic plastids) and demonstrate its essential function in plant and chloroplast development and function. The plastid Clp system consists of a tetradecameric proteolytic core with catalytically active ClpP and inactive ClpR subunits, hexameric ATP-dependent chaperones (ClpC,D), and adaptor protein(s) (ClpS1) enhancing delivery of subsets of substrates. ClpT proteins are found associated with the Clp core and are unique to higher plants plastids. I will illustrate structural and functional features of the plastid Clp system in Arabidopsis thaliana though extensive reverse genetics analysis combined with biochemical analysis, X-ray crystallography, as well as large scale quantitative proteomics for loss-of-function mutants of Clp core, chaperone, ClpT and ClpS1 subunits. Multiple substrates are identified based on their direct interaction with the ClpS1 adaptor or screening of different loss-of-function protease mutants. Finally, I will present our latest efforts to determine degradation signals (degrons), as well as functional interactions of Clp with other plastid proteases. This includes the use of terminal amine isotopic labeling of substrates (TAILS) and mass spectrometry to characterize the N-termini of chloroplast proteins (chloroplast the N-terminome).

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in 3440 Molecular Sciences

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