**NSF-MCB – June 2016-June 2019 ($830,000) - Klaas J. van Wijk (PI)**

**CHLOROPLAST SOLUBLE PROTEASES AND THEIR PHYSIOLOGICAL SUBSTRATES:**

**An integrated genetic and targeted systems analysis of chloroplast proteolysis**

Proteolysis, the breakdown of proteins or peptides to amino acids, is critical for removal of unwanted or damaged proteins and regulation of cellular processes such as metabolism. Chloroplasts are essential organelles in plant productivity and agriculture, but determinants of chloroplast protein life-time and degradation are poorly understood. Cells and organelles contain hundreds of proteolytic systems; these must complement each other, by acting in sequence, in parallel and/or by sharing protein substrates. This research will provide insight into the network of chloroplast protease and serve as an example for protease network discovery in other subcellular compartments. The research findings can also be implemented in molecular farming and synthetic biology, since cellular compartments such as chloroplasts are favored for overexpression of products with nutritional or pharmaceutical value. The outcome of this research will allow more rational protein design for stable accumulation of chloroplast proteins, thus directly impacting these applications. This project will provide training in proteomics, mass spectrometry, molecular genetics, biochemistry and bioinformatics at the undergraduate, graduate and post-doctoral levels. Summer internships will be offered through our NSF-sponsored REU program and to high school students through the Cornell 4H extension program. To help to train the next generation scientists, a plant proteolysis workshop with invited experts on proteases and degradomics technology will be organized in year 2.



This research is built upon investments in Arabidopsis protease single and higher order mutants, previously identified protease substrates, specific biochemical tools and mass spectrometry-based workflows for detection of protein degradation events (e.g. TAILS). In AIM 1, investigators will study how key enzymes of major chloroplast metabolic pathways (e.g. tetrapyrroles and shikimate) are recognized and degraded by the Clp protease system in vivo. This essential Clp system is the most complex and abundant soluble chloroplast oligomeric protease consisting of 16 different gene products, including substrate selectors and chaperones. In AIM 2, additional Clp candidate substrates and perhaps adaptors will be identified, using affinity and trapping approaches, followed by in vivo physiological and in vitro interaction studies. AIM 3. Based on in vitro analysis, two additional chloroplast peptidases, PREP and OOP were proposed to degrade smaller proteins and protein fragments, including cleaved chloroplast transit peptides. Preliminary molecular genetics data indicate functional interactions between Clp, PREP and OOP. The in vivo physiological role of these peptidases will be determined by untargeted and targeted approaches and investigate if and how they form a functional protease network.