Josef Komenda`s group

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Title of the project: A Proteolytic Trap: Identifying FtsH4's Role in Stress Conditions

For how many student/s: 1

Description of the project: Maintaining the delicate balance of life requires a sophisticated internal cleaning crew capable of identifying and removing damaged proteins before they compromise the cell. Membrane-bound FtsH proteases are elite agents of this process. They are universally present in prokaryotes and in the mitochondria and chloroplasts of eukaryotic cells, acting as critical metalloproteases that maintain cellular homeostasis through their dual roles as proteases and chaperones. In contrast to most bacteria that possess a single *ftsH* gene, cyanobacterium *Synechocystis* PCC 6803 typically possess four FtsH proteases (FtsH1–4) which assemble into heteromeric (FtsH1/3 and FtsH2/3) and homomeric (FtsH4) complexes. Our previous work has proven that heteromeric complex FtsH1/3 is involved in iron stress acclimation by degrading Fur protein while FtsH2/3 is well-characterized as the primary machinery for degrading the damaged D1 protein during the Photosystem II (PSII) repair cycle. The specific role of the homomeric FtsH4 complex remained largely unknown until our study utilized a proteolytically inactivated "trap" version of the enzyme to capture its targets. This approach identified Flavodiiron proteins (Flv) as exclusive potential substrates, which is particularly significant as the Flv2/4 heterodimer acts as a specialized photoprotective shield that prevents the photosynthetic machinery from becoming over-reduced during high-light or low-CO_2 stress. These findings indicate that FtsH4 acts as a regulator, fine-tuning the availability of the Flv2/4 shield and thereby modulating the flow of electrons through the photosynthetic apparatus. When this balance is disrupted, the PSII reaction center becomes susceptible to damage. This damage subsequently triggers the second phase of the response—the repair cycle—in which the FtsH2/3 complex mediates the degradation of the damaged D1 protein. However, further experimental evidence is required to determine whether FtsH4 directly degrades Flv proteins *in vivo* and to validate this proposed mechanism.

Student's role in the lab:

In this project, student will take ownership of the experimental process from beginning to end. This includes:

- **Cultivating** FtsH4 mutants and preparing them for study.
- **Extracting proteins** by harvesting the biomass and performing cell disruption.
- **Analyzing samples** using 1D/2D electrophoresis and Western Blotting to find our target proteins.
- **Visualizing data** through specialized staining protocols.

Requirements:.

I'm looking for a curious student who is eager to dive into "wet-lab". You don't need a perfect CV—just a drive to learn, a grasp of English, and passion for new findings.