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Towards fluorescent protein-based biosensing of thylakoid lumen pH

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The pH within the thylakoids is essential for photosynthesis. As part of the photosynthetic light reactions, proton translocation across the thylakoid membrane generates a proton motive force that consists of a pH gradient (ΔpH) and an electrical gradient (ΔΨ) to drive ATP synthase. While genetically encoded pH biosensors have been instrumental in dissecting pH dynamics of different plant cell compartments, in vivo pH biosensing in the subcompartments of the chloroplast had remained challenging. We recently established pH biosensing in response to photosynthetic activity and revealed a major impact on pH dynamics not only in the stroma but also in the cytosol and the mitochondria. While the observed pH dynamics clearly mirror photosynthetic proton pumping, measuring the bona fide pH gradient across the thylakoid membrane as the major determinant of the proton motive force, requires monitoring pH in the thylakoid lumen. Yet, establishing luminal pH monitoring by genetically-encoded biosensors presents unique challenges, such as import across three membrane systems, low pH values that may be adopted in the light, silencing of sensor expression and direct vicinity of photosynthetic pigments. To address those challenges and to optimize luminal pH biosensing in tobacco and Arabidopsis leaves, we have generated a collection of constructs that include different pH biosensors, signal peptides and promoters. Initial experiments show that illumination induces an inverse response between luminal and stromal targeted pH sensors, which provides evidence for correct subcellular targeting. I will present my recent progress in developing luminal pH sensors while highlighting remaining challenges.

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