

## Photosynthesis-triggered pH and NAD(P) redox signatures across plant cell compartments revealed by advanced illumination-imaging

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Plants undergo daily dark-light transitions, leading to dynamic changes of the metabolic and physiological status of their cells. Those changes, which include cofactors, ions and other small molecules such as ATP, NAD(P) redox status, ROS, Ca<sup>2+</sup> and pH, are a prerequisite for tuning protein functions through post-translational modifications (PTMs). However, it has been notoriously difficult to monitor those changes in vivo with sub-cellular precision.

To address this limitation, we have been investigating how photosynthetic activity affects key parameters within and beyond the chloroplast. We developed a new standard of monitoring subcellular energy physiology live by combining confocal imaging of genetically encoded fluorescent protein biosensors with advanced on-stage illumination technology to investigate pH, NADPH/NADP<sup>+</sup> and NADH/NAD<sup>+</sup> dynamics at dark-light transitions in living *Arabidopsis* leaf tissues, focusing on mesophyll cells. Our findings reveal a stromal alkalization signature induced by photosynthetic proton pumping, extending to the cytosol and mitochondria as a cellular 'alkalinization wave'. Moreover, we have been able to dissect the redox dynamics of the stromal and cytosolic NAD and NADP pools driven by photosynthesis-derived electron export.

I will discuss how both the technology as well as the novel insights into the subcellular physiology of photosynthesis will enhance our understanding of the intricate relationship between photosynthetic activity and the biochemical regulation of proteins in phototrophic cells.

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