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Establishing fluorescent protein-based biosensing of NADPH:NADP+ dynamics in living plants

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The pyrimidine nucleotide cofactors NAD and NADP operate at the critical intersection between the cellular metabolic network, Cys-based systems and redox regulation. In plants, the redox states of the NAD and NADP pools of the cytosol and the chloroplast stroma are physically separated, but connected by metabolic shuttles, such as the malate/oxaloacetate and the triose-phosphate shuttle. Changes in photosynthetic activity trigger a major transition in subcellular redox dynamics going far beyond the chloroplast. While the biochemical importance of NAD(P) has been well established, its subcellular regulation and its dynamics in living cells have been difficult to explore. I will present my recent efforts of rationally engineering a fluorescent protein-based biosensor for NAD redox status to generate and characterize a novel biosensor family that specifically responds to NADPH:NADP+. The design of the new sensor family enables the monitoring of the biologically important NADP redox status and overcomes critical downsides of previous NADPH sensors. Plant lines expressing the sensor enable the dynamic mapping of NADP redox dynamics and the resolution of NADP responses down to the single cell. I will discuss the properties of the new sensor family for exploration of plant cell physiology and will highlight our attempts to dissect NADP redox dynamics at the interface of metabolism and signalling.

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