Trends in Biochemical Sciences | Technology of the Month

Monitoring subcellular NADP redox state with NAPstar biosensors

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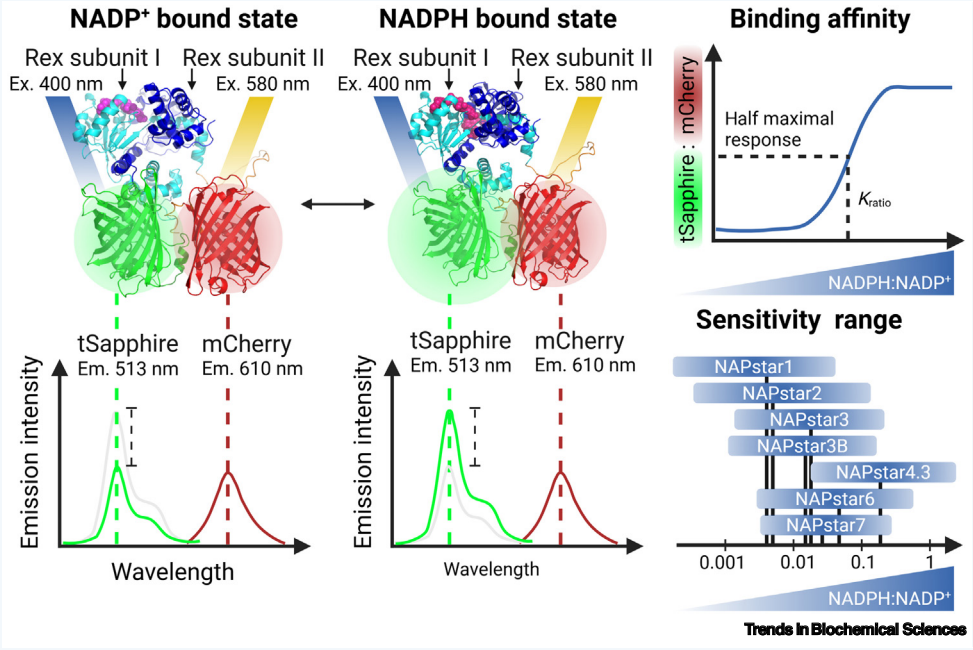
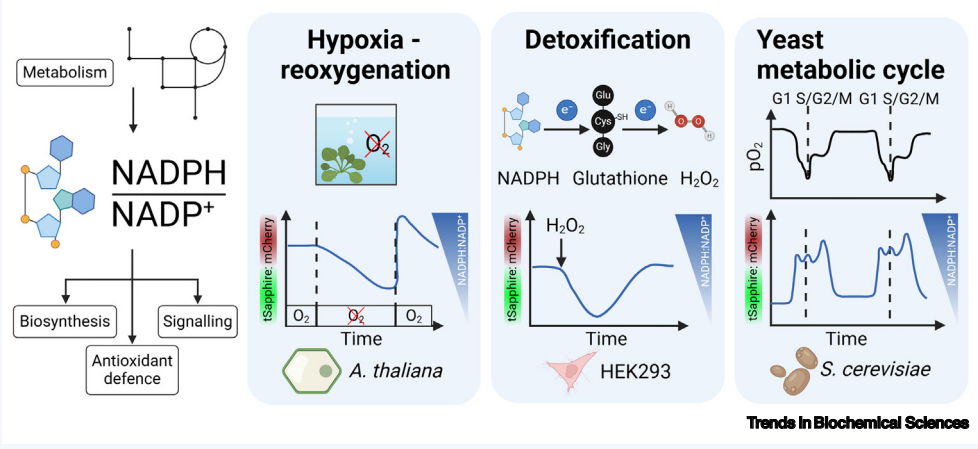


NADP is a crucial coenzyme in all living organisms, fulfilling important and distinct functions in different subcellular compartments. The NADP redox state is intimately coupled to anabolic metabolism, antioxidative defence, and signalling. However, reliable and specific monitoring of subcellular NADP redox state dynamics has proven remarkably challenging. The NAPstar family of genetically encoded fluorescent NADP redox state probes overcomes key limitations of previous approaches.



NAPstar measurements indicate an unexpectedly oxidized cytosolic NADP redox state in different eukaryotic species with important implications for our understanding of NADP function *in vivo*. For example, NAPstars reveal robust maintenance of cytosolic NADP redox state under acute oxidative challenge, facilitated by primary metabolism. Additionally, they uncover dynamic fluctuations in NADP redox state associated with physiological situations including hypoxia-reoxygenation and cell division.

ADVANTAGES:

NAPstars allow specific monitoring of dynamic changes in the cytosolic NADP redox state (i.e., the NADPH:NADP⁺ ratio) in live cells.

Eight current NAPstar variants allow measurements across a broad range of NADPH:NADP⁺ ratios, from 0.001 to 5.

High suitability to fluorescence lifetime imaging microscopy (FLIM) due to a large response range in fluorescence lifetime.

A very limited pH-sensitivity in comparison to many other sensors.

Self-contained design, functioning independently of expression levels, unlike dimerization-dependent sensors.

NAPstars work in different species; validated in yeast, plants, and mammalian cells.

Reveal NADP redox changes in physiologically relevant contexts including light–dark cycle in plants, cell division, and hypoxia-reoxygenation.

CHALLENGES:

NAPstars can in principle be used for absolute quantification of the NADP redox state, though appropriate calibration regimes remain to be developed.

NAPstars monitor dynamic changes in steady state and are not suitable for measuring electron flux through the NADP pool *in vivo*.

Measurements are limited to soluble NADPH/NADP⁺; protein-bound NADP cannot be detected, which may be biologically important.

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Declaration of interests

No interests are declared

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The *in vivo* model system to be studied needs to be amenable to genetic modification to permit NAPstar measurements.

Targeting NAPstars to other organelles such as mitochondria and plastids has yet to be established.

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