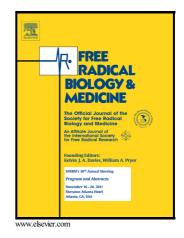
### Author's Accepted Manuscript

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#### **ACCEPTED MANUSCRIPT**

# Real-time and high-throughput analysis of mitochondrial metabolic states in living cells using genetically encoded NAD<sup>+</sup>/NADH sensors

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### Abstract

Mitochondria are central organelles that regulate cellular bioenergetics, biosynthesis, and signaling processes. NADH, a key player in cell metabolism, is often considered as a marker of mitochondrial function. However, traditional methods for NADH measurements are either destructive or unable to distinguish between NADH and NADPH. In contrast to traditional methods, genetically encoded NADH sensors can be used for the real-time tracking and quantitative measurement of subcellular NADH levels in living cells. Therefore, these sensors provide innovative tools and address the limitations of current techniques. We herein summarize the properties of different types of recently developed NADH biosensors, discuss their advantages and disadvantages, and focus on the high-throughput analysis of mitochondrial function by using highly responsive NAD<sup>+</sup>/NADH sensors.

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