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Free NADH homeostasis in mitochondria

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Using fluorescence spectroscopy combined with spectral decomposition analysis we determine the NADH content of actively respiring mitochondria. The reduced coenzyme NADH has a central role in mitochondrial respiratory metabolism. However, reports on the amount of free NADH in mitochondria are sparse and contradictory. While the amount of bound NADH varies, the concentration of free NADH stays relatively unaffected by respiratory state. This suggests that there is a specific mechanism for free NADH homeostasis and that free NADH per se does not play a regulatory role in mitochondrial metabolism. These findings have far-reaching consequences for our interpretation of cellular metabolism.