more single-subunit, non-proton-motive NADH dehydrogenases encoded by nuclear DNA. The rotenone-insensitive NADH dehydrogenase from yeast (Ndi1) was successfully used for alleviation of complex I defects in multiple models including nematodes, fruit flies, rats, and human cells. It was also used as a remedy in Parkinson disease models and as an anti-cancer treatment. Many other physiological effects were also discovered in organisms expressing Ndi1, such as extended lifespan and resistance to particular stresses. Recently, an alternative respiratory chain was found in several animal taxa, including tunicates. However, the properties of alternative NADH dehydrogenases from tunicates, which are supposed to be evolutionary closer to humans than fungi, remain uninvestigated. Previously, the alternative oxidase (AOX) from ascidian *Ciona intestinalis* was successfully exploited to by-pass defects in complexes III and IV in fruit fly, mouse and human cell models. The goal of the present work was to express the *C. intestinalis* alternative NADH dehydrogenase (NDX) in a suitable model organism and investigate its properties. We have found that the gene coding NDX in *C. intestinalis* contains about 4% polymorphisms which may lead to 1.7–2.1% amino acid changes, thus the cloned gene might code non-functional protein. However, NDX was localized to mitochondria when expressed in *Drosophila* S2 cells, and conferred about 20% rotenone-insensitive respiration for mitochondria of adult fruit flies *Drosophila melanogaster*. The exhibiting of rotenone-insensitive respiration, provided by NDX, required high levels of the expression. NDX conferred also resistance of the fruit flies to 20 mM menadione, heat and cold stresses. However, NDX-expressing flies were more sensitive to the salts of chromium and molybdenum. The resistance of NDX-expressing *D melanogaster* to multiple stresses indicates a possible role of NDX in stress responses of tunicates.

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S7.P7

The relationship between cytochrome redox state and oxygen consumption in isolated mouse and beef heart mitochondria during hypoxia

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This study describes a low-noise, rapid spectrophotometric system using visible light (440–605 nm) for the measurement of cytochrome redox state combined with a high-resolution respirometry. The system was tested in an investigation using beef and mouse heart isolated mitochondria (BHImt, MHImt) in order to determine the relationship between respiratory rate and cytochrome redox state at steady-state levels of hypoxia. Monophasic hyperbolic relations were observed between respiratory rate, j (with glutamate + malate and saturating ADP concentrations), and oxygen partial pressure, pO2, in the range <1.1 kPa for both BHImt and MHImt with $p50, j$ (pO2 at $j = 0.5\,j$max) of 0.015 and 0.021 kPa respectively. The oxidation fractions of cytochromes *aa*3 and *c* were biphasic hyperbolic functions of pO2. The relationships between cytochrome oxidation states and j were more complex with an initial steep decrease in the oxidation fraction of cytochrome *c* to a value of j of approximately 0.7 followed by a plateaux and a further steep decrease at $j < 0.2$. This relationship was less apparent with cytochrome *b* redox state. Using these functions, it was possible to create a model that successfully described the measured relationship between cytochrome oxidation state and oxygen consumption.

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S7.P8

Combined high-resolution respirometry and fluorometry. Validation of safranin for determination of mitochondrial membrane potential

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Mitochondrial membrane potential (mtMP) is closely intertwined with oxidative phosphorylation (OXPHOS). The exact nature of the interactions of respiration (flux) and mtMP (force) under various physiological and pathological conditions remains unclear, partially due to methodological limitations. We introduce the combination of high-resolution respirometry and fluorometry with the OROBOROS Oxygraph-2 k, using the widely applied mtMP indicator safranin. OXPHOS analysis with mouse brain homogenate revealed that safranin inhibits Complex I linked OXPHOS capacity at commonly applied concentrations and targets primarily the phosphorylation system, without effect on LEAK respiration. Complex II linked OXPHOS capacity was inhibited by <20% at 2 μM safranin sufficient for mtMP monitoring. mtMP was higher in the LEAK state without adenylates (*L*N) than at identical LEAK respiration after ADP stimulation and inhibition by oligomycin (*L*Omy). Maximum ETS capacity was reached in uncoupler titrations before mtMP was fully collapsed, whereas respiration was inhibited at increasing uncoupler concentrations and further reduction of mtMP. Examining a pharmacologically induced state of Complex II dysfunction, mtMP was rather insensitive to 50% inhibition of OXPHOS, but responded strongly to addition of inhibitors when respiration was minimized by substrate depletion. The optimum uncoupler concentration supporting maximum ETS capacity varied as a function of pharmacological intervention. Taken together, combined measurement of respiration and mtMP greatly enhances the diagnostic potential of OXPHOS analysis. Respirometric validation of inhibitory and uncoupling effects is mandatory for any fluorophore applied for probing mtMP, in any respiratory state, type of tissue and pathophysiological condition.

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S7.P9

Succinate dehydrogenase regulation in normoxic and anoxic conditions in mammal heart and brain

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Succinate dehydrogenase (SDH; complex II) is a pivotal mitochondrial component connecting the TCA cycle and respiratory chain. The enzyme is a subject of feedback control by oxaloacetate (OAA) which is a product of the malate dehydrogenase. It is a competitive inhibitor of SDH with high affinity (Kd ~ 17 nM) and an extremely low dissociation rate (~0.02 min^{-1}) [1,2]. OAA binding depends on the redox state of the enzyme which is fully reduced in the absence of oxygen [2]. The