remains a subject of intense debate. We have performed structure-based calculations of the activation barriers for the initial coupled ET/PT steps in CcO [1]. The calculations have, for the first time, reproduced the barriers that account for the directionality and sequence of events in the primary PT in CcO. We have also addressed the effect of the conformational change of Glu286 and the role of bridging water molecules. (2) Nitric oxide reductase (NOR) of denitrifying bacteria belongs to the superfamily of heme-copper oxidases and, due to high structural similarities to CcO, is generally believed to be the evolutionary ancestor of cytochrome oxidases. However, in contrast to CcO, there were no crystal structures of NOR available until recently, and previous analyses of PT in NOR were based on a homology-built model [5]. We will report preliminary results of our simulations of PT in NOR, which are based on the recently solved crystal structure [6]. This includes both the large-scale MD simulations that were performed to identify specific PT pathways leading to the active site and explicit EVB calculations of the barriers for individual PT steps.

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11P.20 Cytochrome *c* oxidase activity in mitochondria is regulated by the ATP/ADP ratio and by the phosphorylation pattern of the enzyme

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The conditions for measuring the allosteric ATP-inhibition of cytochrome *c* oxidase (CcO) in isolated mitochondria were investigated. The oxygen consumption of mitochondria in the presence of 1% Tween-20 was recorded polarographically with ascorbate as substrate at increasing concentrations of cytochrome *c* in the presence of ADP and of ATP. Only by increasing the ATP/ADP ratio with the ATP-regenerating system phosphoenolpyruvate and pyruvate kinase to high values full ATP-inhibition of CcO could be seen. The extent of allosteric ATP-inhibition was found to vary between different preparations of mitochondria from heart, liver and kidney of rat and bovine. The phosphorylation pattern of CcO was determined by isolating the enzyme complex by Blue Native PAGE and subsequent Western blots with antibodies against phosphoserine, phosphothreonine and phosphotyrosine. The correlations between kinetics of allosteric ATP-inhibition and the phosphorylation patterns are discussed.

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11P.21 NO reduction by heme-copper oxidases

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The mechanism by which certain heme-copper oxidases (HCuO) reduce nitric oxide to nitrous oxide is far from being understood despite increasing efforts. It is not the chemistry alone that is intriguing but the accompanying proton transfer that deserves attention. The nitric oxide reductases (NORs) are predicted to be structurally similar to traditional members of the superfamily of heme-copper oxidases but their proton chemistry is fundamentally different. Early work on NOR from *Paracoccus denitrificans* revealed its non-electrogenic character for both substrates O_2 and NO. Later, it was shown that this is due to the uptake of electrons and protons from the same side of the membrane and the lack of proton pumping. We are trying to understand why the reduction of NO, a reaction as exergonic as the reduction of O_2, is not coupled to endergonic vectorial proton transfer in a system that seems to possess a proton pumping machinery, using proton-pumping members of the HCuO family capable of NO reduction. Our recent studies showed that in the cbb_3-type oxidase from *Rhodobacter sphaeroides*, typical proton pumping was only observed for the reaction with O_2, whereas the reaction with NO resulted in a small membrane potential not big enough to account for proton pumping. Studying the ba_3 oxidase from *Thermus thermophilus* extends our investigation into the B-family with well-described chemical intermediates and a high-resolution protein structure. We are currently characterizing the reaction between the fully reduced ba_3 oxidase and NO, results from such optical flow-flash experiments will be presented.

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11P.22 Potential generation during CO photodissociation from the fully reduced cytochrome *c* oxidase from *Paracoccus denitrificans*

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Cytochrome *c* oxidase (CcO) is the terminal oxidase of the electron transfer chain. CcO uses electrons donated by cytochrome *c* from the P-side and protons from the N-side of the inner mitochondrial membrane to reduce molecular oxygen to water and associates the released energy to pumping of four protons per one O_2 reduced. The aim of this study was to elucidate the nature of the 1.5 μs phase of potential generation upon the photodissociation of CO from the fully reduced CcO from *Paracoccus denitrificans*. The 1.5 μs phase is absent in the two electron reduced CO-bound enzyme and emerges only upon additional reduction of two other redox centers: heme *a* and Cu_A. It was found that the amplitude of this phase depends only on enzyme concentration and can be used as an internal ruler for the calibration of the electrogenic events in the enzyme. The obtained data shows that the fast phase is followed with an additional phase which is growing and slowing down with increase of pH. This additional phase emerges when the fully reduced CO bound enzyme is first oxidized by an oxygen pulse and then immediately re-reduced with sodium dithionite. The amplitude of the slow phase is not stable and it fades away with a time constant of about 15–20 min. Comparison of results from mutant enzymes suggests that these events are linked to the proton conducting channel K of CcO.

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11P.23 Interaction of acidic cytochrome *c* with wild and mutant B-type cytochrome oxidases from thermophilic Bacillus

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