the less rigid models. Thus, the large frequency shift ascribable to the presence of copper (I) suggests that the copper ion resides in close proximity to the bound CO. The existence of two conformers is discussed. Additional IR studies of oxygen reactivity with these model compounds were also investigated.

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11P.17 Cyanide inhibition and pyruvate-induced recovery of cytochrome *c* oxidase

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The mechanism of cyanide inhibition of the mitochondrial cytochrome *c* oxidase (COX) as well as the conditions for its reversal is not yet fully explained. With regard to the inhibition by KCN and its reversal by pyruvate, we investigated three parameters of COX function, namely the transport of electrons in the terms of oxygen consumption, the proton pumping evaluated as mitochondrial membrane potential ($\Delta\psi_m$) and the enzyme affinity to oxygen by means of p_{50} value calculation. We analyzed the function of COX in intact rat liver mitochondria, either within the respiratory chain or as a sole enzyme, using succinate or ascorbate + TMPD to fuel respiration. We found that 250 μM KCN completely inhibited both electron and proton transport function of COX, and this inhibition was reversible after washing of mitochondria. The addition of 60 mM pyruvate induced the maximal recovery of both parameters to 60–80% of original values. Using KCN in the low concentration range up to 5 μM, we observed a profound (30-fold) decrease of COX affinity to oxygen. Again, this decrease was completely reversed by washing of the mitochondria while pyruvate induced only a partial yet still significant recovery of oxygen affinity. Our results demonstrate the reversible nature of inhibition of COX by cyanide and reveal the limited potential of pyruvate to act as a cyanide poisoning antidote. Importantly, we also show that the COX affinity to oxygen is the most sensitive indicator for the detection of toxic effect of cyanide.

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11P.18 Evaluation of the mitochondrial metabolism of two invertebrates' species using permeabilized fibres in high-resolution respirometry

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The use of permeabilized fibres instead of mitochondrial isolations allows the estimation of mitochondrial metabolism in an in situ approach. This approach has several advantages compared to the *in vitro* approach, notably being closer to the real physiological environment (review in Kuznetsov et al., 2008) and has never been used to assess mitochondrial functions in invertebrates. Measurement of O_2 consumption using permeabilized fibers from high energetic muscles flight of *Drosophila* simulans were used for classic assessment of mitochondrial performances at several steps of the ETS. In another example on the whole body musculature of the polychaete *Nereis virens*, we evaluated the normal and the alternative oxidative pathways in order to understand the conditions of maximum efficiency of ETS and the intervention of alternative oxidase as terminal electron acceptor in some invertebrate species. In flies, results showed very good RCR for complex I with high state 3 respiration. The assessment of complex II showed significant contribution of succinate on the electron transport system. It is the first time that respiration from supplying complex II has been quantify in *Drosophila*. When ubiquinol pool was supplied through complex I, complex II and glycerol-3-phosphate dehydrogenase, the activity of the electron transport system reached a maximum state 3 and further uncoupling showed that the OXPHOS capacity was not overwhelmed suggesting that ATP synthase can support the maximum electron flux measured in the electron transport system. In *Nereis*, RCR for complexes I and II showed low values but consistent with previous studies on mitochondrial isolations from *Nereis pelagica* (Tschischka et al., 2000). When inhibiting complex III, O_2 consumptions measurements showed that 28.97% of state 3 respiration are dedicated to supply alternative oxidase in electrons as well as to the backflux of electrons to complex I and/or complex II. SHAM was used to further inhibit alternative oxidase and allowed us to corroborate the significant contribution of the alternative pathway. We demonstrated here that high-resolution respirometry with permeabilized fibres in invertebrates can be use as an accurate tool to evaluate the mitochondrial metabolism at each steps of the ETS and may insure better understanding of the regulation of several processes not detected in vertebrates like the alternative oxidase.

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11P.19 Computer simulations of proton transfer in cytochrome *c* oxidase and nitric oxide reductase

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Simulation of proton transfer (PT) in proteins through bridging water molecules and ionizable amino acid residues is a challenging task: classical MD simulations cannot, in principle, describe individual PT steps; on the other hand, ab initio QM/MM simulations of biosystems are still limited by many factors (e.g., sampling, simulation time, convergence). One of the most efficient approaches is the empirical valence bond (EVB) method. Recently, we have adopted an EVB-based multi-level modeling strategy for simulations of the coupled ET/PT events in proteins [1, 2]. (1) We will present the results of our recent computational study [1] of cytochrome *c* oxidase (CcO), a system that has long presented a conceptual challenge in bioenergetics [3]. After its structure has been solved more than a decade ago, CcO was the focus of numerous works, including a number of computational studies with different methods [2, 4]. Although these studies have shed light on many aspects of CcO functioning, the detailed molecular mechanism of proton pumping

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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