The alternative oxidase is a terminal respiratory chain protein found in plants, fungi and some parasites that still remains physically uncharacterised. Current model of the AOX, predicts that the enzyme is a monotopic integral membrane protein associating with one leaflet of the lipid bilayer. Although it is generally accepted that AOX is a non-haem diiron carboxylate protein in which the metal atoms are ligated by amino acid residues that all reside within a 4-helix bundle there is little biophysical experimental evidence in favour of this notion. We present EPR evidence from parallel mode experiments which reveal signals at approximately $g=16$ in both purified plant alternative oxidase protein ($g=16.9$), isolated plant mitochondrial membranes ($g=16.1$), and in the trypanosomal AOX expressed in *Escherichia coli* membranes ($g=16.4$). Of particular importance is the finding that such signals disappear in the presence of inhibitors of the AOX. Such signals are indicative of a dicarboxylate diiron centre at the active site of the enzyme. To our knowledge these data represent the first EPR signals from AOX present in its native environment.

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S13.15 Far infrared spectroscopic studies on hydrogen bonding features in proteins from the respiratory chain

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In order to understand the molecular basis of energy transduction, we have extensive interest in experiments which reveal at the molecular level how protons are drawn through proteins. It is crucial to determine the structural, dynamic and energetic requirements for the proton transferring groups in the proton pumping enzymes and the cofactor sites that rule them. A significant part of the proton conduction is made by channels that orient specifically bound water molecules. These water molecules can be monitored by X ray crystallography. However, high resolution structures of membrane proteins are difficult to obtain. Interestingly, water molecules and their hydrogen bonding interactions are expected to contribute in the far infrared spectral range (<400 cm^{-1}). Experiments at synchrotron far infrared beamlines have been made with large membrane proteins from the respiratory chain and their models, clearly demonstrating the presence of this hydrogen bonding signature signal and, importantly, that it can be manipulated.

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S13.16 The role of the cross-linked Tyr in the catalytic cycle of cytochrome *c* oxidase

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Tyr-280 in cytochrome *c* oxidase (C*c*O) from *Paracoccus denitrificans* undergoes posttranslational modification that results in a covalent cross-link to histidine residue 276 that is a ligand of Cu$_B$. The Tyr-280 has been predicted to be a proton (or a proton and electron) donor for oxygen activation. The aim of our studies was to test the role of the cross-linked Tyr-280 as a proton donor for oxygen reduction and to resolve the stage in catalysis when it becomes reprotonated. The combination of three time-resolved techniques: visible spectroscopy, electrometry and FTIR spectroscopy were used. Electrometry showed positive charge transfer across a distance consistent with that from Tyr-280 to oxygen in the reaction site, and FTIR spectroscopy revealed a band at 1308 cm^{-1} that was assigned to deprotonated Tyr-280. The obtained results strongly indicate that Tyr-280 provides a proton for oxygen activation in fully reduced enzyme. According to the FTIR spectra it is fully deprotonated at the next step after O–O bond splitting (ferryl) and partly reprotonated in the fully-oxidized state, depending on pH. We showed that full reprotonation of Tyr-280 takes place in the one-electron reduced state when Cu$_B$ becomes reduced.

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S13.17 Reverse redox loop enzymes for driving endergonic reactions in bacterial electron transport

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The study deals with enzymes that drive endergonic reactions of electron transport by a reverse redox loop mechanism and the proton potential. Succinate dehydrogenase (succinate: menaquinone (MK) reductase) from MK-containing bacteria catalyzes an endergonic reaction ($\Delta E_0' = +110$ mV). The soluble subunits (SdhAB) of the enzyme are similar in composition and function to succinate: ubiquinone reductase from mitochondria and ubiquinone containing bacteria. The quinone reactive subunit SdhC is a membrane integral diheme protein which allows electron transfer from the cytoplasmic to the extracellular side of the membrane. Function of succinate: MK reduction depends on the membrane potential and is inhibited by protonophores and ionophores. Bioenergetic studies and membrane topology of SdhC suggest that the active site for MK reduction is close the extracellular side of the membrane, resulting in the uptake of H$^+$ from the outside, whereas succinate oxidation and the accompanying proton release take place in the cytoplasm. In this way the enzyme consumes a proton potential and uses a reverse redox loop (2 H$^+$/2e) for driving the redox reaction. The same type of enzyme is found in anaerobic electron transport of sulphate reducing bacteria. Database screening demonstrated that a reverse redox loop mechanism is predicted for other bacterial respiratory enzymes catalyzing endergonic reactions. Reverse redox loop enzymes therefore are of general significance for driving endergonic redox reactions in bacteria.

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S13.18 Light-activating the respiratory chain: Toward the time-resolved studies of the electron transfer chain in vivo

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Although the structure and function of most of the individual complexes involved in the respiratory chain are known with great details, the accurate understanding of the function of the chain as a whole is still missing. Yet, an increasing number of evidence suggests it may significantly differ from the simple combination of the functions of the individual complexes. Indeed, the supramecular association of complexes within identified super-complexes or the ultra-structure of the inner membrane which possibly restricts the diffusion of soluble electron carriers may have significant functional consequences since they would introduce macroscopic heterogeneities in the chain. The expected signature of such heterogeneities is essentially kinetic calling for new methods allowing the time-resolved analysis of the electron transfer sequence associated with the mitochondrial respiration *in vivo*. We are currently developing such a method based on the flash-induced photolysis of CO in the presence of O_2, as fruitfully conducted for the mechanistic dissection of complex IV. The advantages and pitfalls of the approach will be described and preliminary results will be presented and discussed.

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S13.19 A role for sodium ions in the respiratory chain of *Rhodothermus marinus*

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Rhodothermus marinus is a strictly aerobic and thermohalophilic organism isolated from submarine hot springs in Iceland and Açores. Its respiratory complexes have been studied and include a complex I (NADH:menaquinone oxidoreductase), a complex II (succinate: menaquinone oxidoreductase), a novel complex III and at least three different dioxygen reductases. Since it is a halophilic organism, and because a proton/sodium antiporter gene was found among its complex I genes, a possible role of sodium ions in *R. marinus* bioenergetics was investigated. We prepared inside-out vesicles from *R. marinus* and demonstrated that the vesicles maintained an electrochemical K^+ potential imposed by K^+/valinomycin. The membrane potential driven by the addition of substrates NADH and succinate to *R. marinus* membrane vesicles was followed using the sensitive dye oxonol V. It was observed that the NADH-driven membrane potential was sodium ion dependent, while the build-up of a membrane potential during succinate oxidation seems not to be influenced by Na^+. To investigate the mode of Na^+ transport during NADH respiration, ^{23}Na in membrane vesicles was followed by NMR spectroscopy.

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S13.20 Structural characterization of respiratory complexes in potato tuber

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The aim of this study was to determine the structures of potato respiratory supercomplexes. Therefore, mitochondrial inner membranes from potato tuber cells were isolated, mildly solubilized with digitonin and the respiratory supercomplexes were separated by sucrose gradient ultra centrifugation. Finally, content of sucrose gradient fractions was inspected with Blue Native electrophoresis and electron microscopy. Single particle analysis of our data revealed several projection maps of complex I, monomeric and dimeric ATP synthase, supercomplex III_2+IV_1, supercomplex I+III_2 and larger unassigned supercomplexes. In some side-view projection maps of complex I the structure of carbonic anhydrase shows its trimeric features. Furthermore, one projection map revealed an extra unknown density at the intermembrane side of complex I. Top-view projection maps of I+III_2 supercomplex showed similar features found in other plant species including the presence of carbonic anhydrase. Besides the top-views, two different side-views and several angular views of the I+III_2 supercomplex were revealed which allowed a better assignment of interaction between complex I and III_2 within the supercomplex. The side-views of the largest supercomplex most likely do not represent the structure of the I+III_2+IV_1 supercomplex, also known as the respirasome. The largest particles represent probably a supercomplex composed of two copies of complex I and one copy of complex III_2.

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S13.21 Production, characterization, and determination of the real catalytic properties of the 'succinate dehydrogenase' from *Wolinella succinogenes*

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The genomes from both of the ε-proteobacteria *Wolinella succinogenes* and *Campylobacter jejuni* contain operons (*sdhABE* operons) that encode for hitherto uncharacterized enzyme complexes annotated as 'non-classical' succinate dehydrogenases. In the framework of a functional genomics project, a genetic system has been established for the homologous (over-)production and manipulation of the SdhABE complex from *W. succinogenes*. The catalytic properties of the purified enzyme were examined using various possible electron donor and acceptor substrates. Strikingly, for the SdhABE complex annotated as a 'succinate dehydrogenase', no succinate oxidation activity could be detected, neither with DCPIP, nor with methylene blue, nor with the high-potential quinone EQ-0 as electron donor. Although the complex catalyzes fumarate reduction with the menaquinol-6 analog 2,3-dimethyl-1,4-naphthoquinol ($DMNH_2$) the activities are very low. In addition to menaquinol-6, membranes of *C. jejuni* and of *W. succinogenes* contain a second quinol, 8-methylmenaquinol-6 (8-$MMKH_2$-6). Supplying an 8-$MMKH_2$-6 analog as a substrate increased specific quinol:fumarate reductase activity by about one order of magnitude. Furthermore, studies on variant enzymes demonstrated that the hydrophilic subunits of the complex are, in contrast to all other members of the superfamily, exported into the periplasm via the tat-pathway. Our studies reveal that the putative succinate dehydrogenase is in fact a novel periplasmic 8-methylmenaquinol:fumarate reductase with no detectable succinate dehydrogenase activity. These results provide an explanation for apparently puzzling previously published observations on the regulation of the *C. jejuni sdhABE* operon.

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