The yeast *Saccharomyces cerevisiae* mitochondria are slightly peculiar since they do not exhibit a complex I but have a number of external and internal dehydrogenases in the inner mitochondrial membrane. These dehydrogenases give their electrons to the quinone pool. On the outer side of the inner membrane, two dehydrogenases activities are localized: the NADH dehydrogenase and the glycerol-3-phosphate dehydrogenase. On the matrix side of the inner membrane, one can find NADH dehydrogenase and succinodehydrogenase. We have previously shown that in mitochondria isolated from *S. cerevisiae*, electrons coming from certain dehydrogenases have the right of way on electrons coming from others. Typically, electrons coming from the external NADH dehydrogenases, have the right of way on other dehydrogenases. In order to understand the possible relationship between this process and the supramolecular organization of the respiratory chain, we undertook a functional and structural study of yeast mutants that do or do not exhibit a supramolecular organization. We have thus studied the electrons competition process in a cardiolipin delta mutant known to possess an altered respiratory chain supramolecular organization. We also studied the organisation of the respiratory chain by BN-PAGE method in a mutant that exhibit an altered electron competition process. Results pertaining to this study will be presented.

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S13.11 Towards crystallizing co-complexes of newly identified inhibitors with the QFR from *Wolinella succinogenes* and with other members of the superfamily of succinate:quinone oxidoreductases

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The members of the superfamily of succinate:quinone oxidoreductases exhibit a high degree of variability with respect to the sensitivity of their quinone sites to various inhibitors. The goal of the project is the identification of potent inhibitors of the diheme-containing membrane protein complex quinol:fumarate reductase (QFR) from *Wolinella succinogenes* and a comparison of these newly identified inhibitors to those of other members of the superfamily, e.g. 2-heptylquinoline-*N*-oxide (HQNO). We shall present first results which are a prerequisite for the co-crystallization of these inhibitors with the respective membrane protein complexes.

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S13.12 Hybrid protein-semiconductor photonic structures using bacteriorhodopsin and glucose oxidase

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The aim of this work is to characterize porous silicon (PSi) photonic structures impregnated with glucose oxidase (GOX) or solubilized bacteriorhodopsin (BR) as a first step in developing hybrid matrices for future biophotonic applications. PSi Bragg mirrors and microcavities are multilayered structures of periodic refractive index with layer thicknesses in the order of visible light wavelength. The pore size of PSi can be tuned to accommodate biomacromolecules, and the silicon surface can be functionalized for covalent protein attachment. Both proteins have previously been shown to possess nonlinear optical properties. We have used atomic force microscopy and multi-photon microscopy to characterise the surface and in depth, respectively, the GOX or BR impregnated PSi structures. Two photon fluorescence emission and second harmonic generation of the BR-PSi and the GOX-PSi systems were observed at some particular pores of PSi and subsequent enhancement of the signal arising from the proteins adsorbed within the pores was detected. The results constitute the first steps in an innovative biomimetic approach for the future design and development of protein based integrated optical devices.

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S13.13 Multiheme periplasmic cytochromes of *Geobacter sulfurreducens*: Optimized cellular devices to face extracellular electron acceptors?

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Multiheme cytochromes are key proteins in the reduction of extracellular metal ions as Fe(III) and U(VI) in *Geobacter sulfurreducens* (Gs). In contrast with cytoplasmic acceptors, theoretical studies showed that reduction of extracellular acceptors leaded to the dissipation of the membrane potential due to cytoplasm acidification. To counteract this, additional energy transduction steps are needed to generate energy. PpcA, a small periplasmic triheme cytochrome, was proposed to contribute to the energy transduction cycle that leads to ATP synthesis in Gs. Four homologs of PpcA were identified in Gs genome, being PpcB the most closely related, with 77% sequence identity. In this study the redox centers of PpcB were characterized using NMR and visible spectroscopy techniques. Despite being sequence and structurally homologous, the functional redox properties of PpcB and PpcA are quite distinct. This correlates with the results of phenotypic studies that showed that knock-out of PpcA gene disrupts electron transfer to extracellular Fe(III), while the effect of PpcB gene deletion is notorious on the U(VI) reduction activity. This suggests that each protein uniquely modulates the properties of their co-factors to assure effectiveness in the metabolic pathways they participate.

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S13.14 Spectroscopic and structural studies of the alternative oxidase

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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 (CcO) 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 ultrastructure of the inner membrane which possibly restricts the diffusion