to narrow down the sites conferring the functional specificity of ACPM1 and ACPM2.

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1P.8 Electron transfer in *Eschericha coli* respiratory complex I

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The respiratory complex I couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. One flavin mononucleotide and seven iron–sulfur (Fe/S)-clusters build an electron transfer path from the NADH binding site to the ubiquinone binding site. The role of the protein in electron transfer was recently questioned. It is generally accepted that electron transfer takes place *via* tunnelling, that means through-space electron transfer with the Fe/S-clusters as the only centers of excess electrons. Recently it was proposed that electron transfer is performed *via* a hopping mechanism involving conserved aromatic amino acids between the Fe/S-clusters as stepping stones. The calculated transfer rates of the individual intramolecular electron transfer steps are in agreement with the experimental determined total transfer rate of $170 \pm 10\ s^{-1}$. To examine this hypothesis several aromatic amino acids in complex I from *E. coli* were mutated by λ-Red recombineering. The mutations led to a dramatic decrease in the NADH:oxidase activity of the mutant membranes, when the aromatic amino acid was replaced by a non-aromatic amino acid. Conservative substitution with another aromatic amino acid had a mild effect on the activity. The mutations showed the same effect on the k_{cat} of the NADH:ubiquinone oxidoreductase activity of the isolated complex I variants.

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1P.9 On complex I of *Neurospora crassa* — Role of the chaperone B17.2L

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Complex I or the NADH:ubiquinone oxidoreductase deficiency is a common cause of mitochondrial oxidative phosphorylation diseases, including Leigh syndrome. Mutations in nuclear genes encoding structural subunits or assembly factors of complex I have been increasingly identified as the cause of the diseases. One such assembly factor is a paralogue (B17.2L) of the B17.2 structural subunit of the enzyme, but the mechanism by which it exerts its function is still unclear. To better understand the requirement of B17.2L for complex I assembly we analyzed the mitochondrial respiratory chain of *Neurospora crassa* strains using one and two-dimensional blue-native PAGE. The results obtained indicate that disruption of both Neurospora genes, the one encoding the structural subunit (*nuo-13.4*) and its paralogue (*nuo-13.4L*), has no effect on the assembly or activity of complex I. Moreover, an anti-13.4L antibody does not recognize the holoenzyme but specifically associates with subassemblies present in several complex I mutant strains. The analyses performed revealed that the 13.4L protein associates with different subassemblies in different complex I mutants. Our results indicate that the 13.4L protein is a molecular chaperone involved in the assembly of complex I in *N. crassa*, probably stabilizing a subcomplex of assembly containing the membrane arm and the connecting part. Furthermore, dissociation of the 13.4L protein seems to occur only upon full complex I assembly, suggesting that the assembly factor functions as a sensor of integrity.

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1P.10 Crystallisation of the membrane domain of complex I from *Escherichia coli*

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Complex I is the first enzyme of the respiratory chain and plays a central role in cellular energy production, coupling electron transfer between NADH and quinone to proton translocation. The enzyme is L-shaped, consisting of peripheral and membrane arms. The structure of peripheral arm was previously determined, however the complete structure and the coupling mechanism of this large molecular machine are currently unknown. To determine the structure of the membrane domain of complex I form *E. coli*, we have developed an effective procedure for its separation from the hydrophilic domain. Isolated membrane domain was crystallised. Initially poor X-ray diffraction properties were improved after extensive optimisation of crystallisation conditions. After a broad screening of conditions for heavy atom derivatisation, MIRAS data were collected. The preliminary findings from crystallographic data will be discussed.

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1P.11 The Zn^{2+} inhibitive effects on the cytochrome bc_1 complex from *Rhodobacter capsulatus* as revealed by the FTIR difference spectroscopy

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The cytochrome bc_1 complex is the third enzyme in the respiratory chain and couples the redox reactions to the proton translocation across the membrane. It is widely accepted that the enzyme functions following the modified Q-cycle [1], first proposed by Mitchell [2]. Metal dications such as Zn^{2+} can bind to the proton translocating enzymes and blocks the proton transfer, preventing thus the generation of the proton gradient, ubiquitous for the ATP production [3, 4]. It is indeed crucial to understand the mechanism of inhibition of Zn^{2+}. The Zn^{2+} binding site was suggested to be located at the interface between the heme b_L and the Rieske protein. The binding site consists of two histidines, one aspartic acid, one asparagine, and one glutamic acid; all belong to the heme *b* subunit [5]. The effect of the Zn^{2+} binding to the bc_1 complex from *Rhodobacter capsulatus* is studied with the help of a redox induced FTIR difference spectroscopy combined with site directed mutagenesis. The experiment show that redox reaction rates of the protein becomes considerably slower in presence of Zn^{2+}. Furthermore, the FTIR difference spectroscopy shows that the mutation of the Glu295 to Val295 leads to a loss of the signature of the protonated acidic residue in

the region of 1750–1700 cm^{-1}. The Glu295 is a crucial acidic residue to the proton translocation. In addition, the intensity of the Glu295 is decreased by the addition of Zn^{2+} meaning that the dication leads to the deprotonation of the Glu295. The bc_1 complex lacking the Rieske protein, shows a slower reaction rates in presence of Zn^{2+} without showing a loss of the signature arising from the redox active protonated acidic residue.

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1P.12 Localization and dynamics of the OXPHOS complexes in *Escherichia coli* by *in-vivo* fluorescence microscopy

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Biological membranes are known to be highly organized. Lipids and membrane proteins are not regularly distributed within biological membranes but are organized in distinct and dynamic clusters. It has been proposed that protein complexes connected by a common substrate chain form supercomplexes which are located in specific areas within the membrane. Besides their structural role for stabilization of the individual complexes, the functional role of supercomplexes is catalytic enhancement. We focused on the OXPHOS system of aerobically grown *E. coli* to determine whether the OXPHOS complexes are localized in distinct lipid areas and if so, whether there is a dynamic exchange of the complexes between the areas. To address these questions, we labeled the membrane protein complexes with different fluorescent proteins and visualized their distribution in the membrane by fluorescence microscopy. FP-decorated variants of the NADH:ubiquinone oxidoreductase, the succinate dehydrogenase, the cytochrome-bd complex and the F_OF_1-ATP-Synthase, were created by means of λ-RED mediated mutagenesis. Biochemical analysis revealed that the modified enzymes were catalytically active and assembled to stable complexes in the membrane. Fluorescence microscopy of living *E. coli* cells showed an uneven distribution of the respiratory complexes in the cytoplasmatic membrane. These data suggest that all complexes reside in distinct membrane domains, which might be of functional importance.

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1P.13 A new method of preparation of cytochrome *c* oxidase vesicles from bovine heart mitochondria

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Cytochrome c oxidase is an integral membrane protein that catalyzes the oxidation of ferrocytochrome c by molecular oxygen. This electron transport reaction is coupled to the generation of transmembrane gradient of protons. Investigations of the coupling mechanism between the electron and proton transfer processes by spectroscopic methods rely on the availability of small vesicles with a high concentration of incorporated enzyme. Typically proteoliposomes are prepared by reconstitution of the purified protein into phospholipid membranes in the presence of detergent. However, we have found that the process of preparation of oxidase vesicles that exhibit respiratory control can be simplified by the fusion of isolated mitochondrial membranes with small preformed asolectin vesicles. These proteoliposomes, formed without detergent, can be purified further by chromatographic methods and used in studies of the proton translocation processes.

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1P.14 Mitochondrial respiratory supercomplexes: Structural organization and functional role

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The model of the respiratory chain depicting the enzyme complexes as independent units embedded in the lipid bilayer of the inner mitochondrial membrane and connected by randomly diffusing coenzyme Q and cytochrome c is mostly favored [1], although the presence of stable supramolecular aggregates (respiratory supercomplexes) has been also demonstrated in mitochondria [2]. Besides the structural evidence reported in the literature, the functional analysis of the supercomplexes is still poor. In the present study, we have applied the flux control analysis method [3] to intact liver mitochondria from aged rats in order to measure the extent of metabolic control that each respiratory complex exerts over respiration under phosphorylating condition (state 3). Our results indicate that both complex I and complex III are rate limiting, thus supporting the idea that they physiologically behave as a supercomplex [4, 5]. The presence of the I+III assembly was also confirmed by 2D BN/SDS PAGE in mitochondria after digitonin solubilization. Further experiments are available to investigate the role of complex IV in frozen–thawed rat liver mitochondria. In this condition, we can observe that complex IV kinetically behaves as an independent enzyme, not comprised in a functional supramolecular assembly, although I+III+IV supercomplexes can be detected by 2D BN/SDS PAGE. We suggest that the role of cytochrome c can be crucial for the functionality of such supercomplexes.

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