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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