the yeast Q_o pocket to mimic the *Plasmodium falciparum* and the human enzymes.

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S15.16 The *P. denitrificans* cytochrome bc_1 complex: A deletion in the acidic domain of cytochrome c_1

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The cytochrome bc_1 complex from *Paracoccus denitrificans* is a three-subunit transmembrane complex involved in the bacterial aerobic respiratory chain. Its cytochrome c_1 is made up of three domains: a C-terminal transmembrane domain, a core domain which covalently binds the redox cofactor, and an N-terminal acidic domain, a unique feature in *P. denitrificans*; it has a particular amino acidic composition, which gives the subunit an overall negative charge mostly due to the abundance of Glu residues. This feature has been exploited for the purification of the complex so far on ion exchange chromatography. To analyze the role of this extra sequence, we cloned, expressed, and purified a 10×His tagged mutant complex missing the acidic domain. This complex has been characterized via SDS- and BN-PAGE, Western Blots, kinetic tests, and LILBID MS. We obtained a fully assembled complex, forming dimers (unlike the wild type which forms tetramers), showing a standard activity of about 60% the wild type. Fast kinetic experiments and further BN-PAGE analysis are conducted presently to analyze the electron transfer between c_1 and c_{552}, and the potential role of this deletion complex in the *Paracoccus* respirasome.

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S15.17 Probing the environment of heme c_i of rieske/cytochrome *b* complexes by its phylogenetic diversity and by site directed mutagenesis

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One of the differences between cytochrome bc_1 and b_6f complexes is the presence of heme c_i in the Q_i binding site of the latter enzyme. The X-ray crystallographic structure of the b_6f complex showed that heme c_i is devoid of an axial protein ligand, leaving a vacant coordination site that might be involved in Q_i site turnover. In order to understand the function of this peculiar cofactor we investigated its presence and EPR characteristics in heliobacteria and in mutants of the b_6f complex from *Chlamydomanas reinhardtii*. EPR spectra and biochemical analyses confirmed the presence of heme c_i in the Rieske/cytochrome *b* complex from Heliobacteria. The spectra showed that a strong axial (5th) ligand to the heme iron is present and structural modelling places a Glu in a position suitable for heme c_i ligation. We compare the signature of heme c_i in heliobacteria to a mutant of the b_6f complex were a Phe in the vicinity of heme c_i iron was replaced by a Tyr and to the wild-type b_6f complex in the presence of NQNO. This inhibitor has been shown to be a ligand to heme c_i.

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(S16) Proteomics and mitochondria symposium lecture abstracts

S16/1 Advances in defining the mitochondrial membrane proteome

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The mitochondrial proteome is a precise description of the chemical composition and functions of mitochondrial proteins. The hydrophobicity of membrane proteins causes problems with their analysis and under-representation in proteomes. Improvements in membrane protein analyses have expanded our knowledge of the mitochondrial proteome. Organic solvent combinations have been employed for selective extraction of membrane proteins and also for purification with HILIC and reverse-phase chromatography on macroporous supports. Importantly, these methods are compatible with electrospray mass spectrometry and mitochondrial membrane proteins have been characterised by molecular mass measurements and identified directly by tandem mass spectrometry of intact proteins. Molecular masses, for all thirteen hydrophobic products of the bovine mitochondrial genome, demonstrate the absence of any modifications other than *N*-formyl. These measurements have resolved uncertainties concerning the interpretation of the mitochondrial genome. Also, the chemical composition of bovine mitochondrial complex I has been finalised by the definition of the natural, stable post-translational modifications on ND proteins. Sequence data from tandem MS experiments on protein ions, have identified many components of solvent extracts including some hydrophobic proteins undetected by proteomic analyses of peptides. Two small hydrophobic proteins of unknown function, DAPIT and a 6.8 kDa proteolipid associate with ATP synthase complex in the presence of phospholipids. The functions of others are under investigation.

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S16/2 Protein phosphorylation site analysis on different hybrid linear ion trap mass spectrometers

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Protein phosphorylation is most probably the most abundant reversible post translational modification (PTM) in the human proteome, regulated by 516 protein kinases and approx 100 protein phosphatases. Although it is an abundant PTM, it is not the easiest to study by conventional peptide mass spectrometry methods, as the combination of low stoichiometry and poor ionisation leads to a low representation of phosphopeptides in LC–MS based database searches. Utilising the selective scanning features of a 4000 Q-Trap mass spectrometer, such as precursor ion scanning and multiple reaction monitoring (MRM), protein phosphorylation site identification by LC–MS has become more routine in our laboratory. The rapid duty cycle of both precursor ion scanning and MRM permits phosphopeptide detection and sequencing by ms/ms in an LC–MS workflow. Neutral loss scanning, including multistage activation, on an LTQ-orbitrap mass spectrometer is an alternative to the precursor ion scanning used on triple quadrupole mass spectrometers. This high resolution and high mass accuracy mass spectrometer can be used successfully for the identification of protein phosphorylation sites and the identification sites in the protein UCP1 will be used as an example.

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S16/3 Mammalian liver cytochrome *c* is tyrosine-48 phosphorylated *in vivo*, inhibiting mitochondrial respiration

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Cytochrome *c* (Cyt *c*) is part of the mitochondrial electron transport chain (ETC), accepting electrons from bc_1 complex and transferring them to cytochrome *c* oxidase (CcO). Considering its central role in life (respiration) and death (apoptosis) decisions one would expect tight regulation of Cyt *c* function. We have recently shown that Cyt *c* isolated from cow heart tissue is phosphorylated on tyrosine 97 *in vivo*, which leads to inhibition of respiration in the reaction with CcO. In this study we isolated Cyt *c* from a different organ, cow liver, under conditions preserving the physiological phosphorylation state. Western analysis with a phospho-tyrosine specific antibody suggested that liver Cyt *c* is phosphorylated. Surprisingly, the phosphorylation site was unambiguously assigned to Tyr-48 by immobilized metal affinity chromatography/nano-liquid chromatography/electrospray ionization mass spectrometry, and not to the previously identified phospho-Tyr-97 in cow heart. As is true of Tyr-97, Tyr-48 is conserved in eukaryotes. As one possible consequence of Tyr-48 phosphorylation we analyzed the *in vitro* reaction kinetics with isolated cow liver CcO revealing striking differences. Maximal turnover of Tyr-48 phosphorylated Cyt *c* was 3.7 s^{-1} whereas dephosphorylation resulted in a 2.2 fold increase in activity to 8.2 s^{-1}. Effects of Tyr-48 phosphorylation based on the Cyt *c* crystal structure are discussed.

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S16/4 Mitochondrial comparative proteomics: Strength and pitfalls

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In this review, we describe the various techniques available to carry out valid comparative proteomics, their advantages and their disadvantages according to the goal of research. The two-dimensional gel electrophoresis and the 2D-DIGE (Differential in-gel electrophoresis) are compared with the shotgun proteomics and SILE (Stable isotopic labeling experiments). We deliver our opinion on their best fields of application in the domain of the comparative proteomics. We underline the utility of these new tools, providing mass data to study physiology and mitochondrial plasticity in front of a specific mitochondrial insufficiency or exogenous stresses. We illustrate our matter with results obtained in our laboratory specifying the importance of an approach of comparative proteomics combined on mitochondria and the cell which makes it possible to obtain important information on the statute of the mitochondrial function at the cellular level. Finally, we draw attention to the dangers of the extrapolation of the data of proteomics to metabolic flows which require the greatest care.

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(S16) Proteomics and mitochondria symposium abstracts (poster and raised abstracts)

S16.5 High sensitivity identification of membrane proteins by MALDI TOF-mass spectrometry using polystyrene beads

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Uncoupling proteins (UCPs) are transporters present in the inner membrane of mitochondria. They are found in all mammals and in plants, and they belong to the family of anionic mitochondrial carriers. Their roles are quite well established for UCP1 in nonshivering thermogenesis process, but are still in debate for the others UCPs. We study theses proteins by biophysical approaches such as mass spectroscopy (MS). Peptide mass fingerprinting methods using techniques such as MALDI-TOF MS, for example, have become an important analytical tool in the identification of proteins. However, PMF of membrane proteins is a real challenge for at least three reasons. First, membrane proteins are naturally present at low levels; second, most of the detergents strongly inhibit proteases and have deleterious effects on MALDI spectra; and third, despite the presence of detergent, membrane proteins are unstable and often aggregate. We improved and showed that differential acetonitrile extraction of tryptic peptides combined with the use of polystyrene Bio-Beads triggered high resolution of the MALDI-TOF identification of mitochondrial membrane proteins solubilized either with Triton-X100 or CHAPS detergents. The sequence coverage of UCP1 obtained by this approach is close to 90% and allows further investigations for characterizing regulators bound to UCP1.

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S16.6 Analysis of synaptic and non-synaptic mitochondria using colorless- and blue-native PAGE

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Mitochondrial dysfunction contributes to a wide range of human diseases, including neurodegenerative diseases. Proteomic technology can give novel insights into the structure and composition of the brain mitochondrial electron transport chain (ETC) complexes. Using beef heart mitochondria as a control and reference point, this study reports a comparison of rat synaptic and non-synaptic mitochondrial protein profiles using blue-native and colorless-native (CN) gel electrophoresis combined with Tricine-SDS PAGE and MALDI-TOF mass spectrometry. BN-PAGE was found to be a straightforward tool for proteomic analysis of ETC complexes (I, III, IV and V) and especially for the identification of very hydrophobic membrane protein constituents that are not accessible by common isoelectric focusing/sodium dodecyl sulphate gel electrophoresis. The introduction of CN-PAGE into a three dimensional electrophoresis greatly improved the isolation and