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

Michela Castellani[a], Thomas Kleinschroth[a], Nina Morgner[b], Bernhard Brutschy[b], Francesco Malatesta[c], Bernd Ludwig[a]
[a]*Molecular Genetics, Institute for Biochemistry, J.W. Goethe University, Frankfurt am Main, Germany*
[b]*Institute for Physical and Theoretical Chemistry, J.W. Goethe University, Frankfurt am Main, Germany*
[c]*University of Rome, "La Sapienza" Rome, Italy*
E-mail: castellani@em.uni-frankfurt.de

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

Frauke Baymann[a], Anne-Lise Ducluzeau[a], Elodie Chenu[a], Agnès de Lacroix de Lavalette[b], Francesca Zito[b], Wolfgang Nitschke[a]
[a]*BIP/IBSM, UPR 9036, CNRS, 31, chemin Joseph Aiguier, 13402 Marseille Cedex 20, France*
[b]*IBPC, UMR7099, 13, rue Pierre et Marie Curie, 75005 Paris, France*
E-mail: baymann@ibsm.cnrs-mrs.fr

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

Ian M. Fearnley
Medical Research Council Dunn Human Nutrition Unit, Wellcome Trust/ MRC Building, Hills Road, Cambridge, CB2 0XY, UK
E-mail: imf@mrc-dunn.cam.ac.uk

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

Nick Morrice
MRC Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland
E-mail: n.a.morrice@dundee.ac.uk

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