energy metabolism and mitochondrial function. Whereas insect stage parasites maintain a well developed single mitochondrion that produces ATP via oxidative and substrate-level phosphorylation, the bloodstream stage generates ATP exclusively via glycolysis. Although the cytochrome-containing respiratory complexes are absent from the inner membrane of the bloodstream stage mitochondrion, other activities, including respiratory complex V (ATP synthase) and an alternative oxidase, are expressed and in fact essential. While the presence or absence of respiratory complexes II–V in the bloodstream stage mitochondrion is firmly established, the existence of mitochondrial complex I in trypanosomes is the subject of a long-standing controversy in the field. Genes encoding putative subunits of complex I can be identified in the *T. brucei* genome [1] and several of the corresponding proteins have been identified in a putative oxidoreductase complex isolated from insect stage parasites [2]. In order to definitively establish whether complex I exists and is functional in bloodstream stage trypanosomes we use a combination of affinity purification strategies and gene knockout studies. We have expressed tagged versions of four putative subunits — NUBM (51 k, Nqo1), NUKM (NdhK, Nqo6), acyl-CoA ligase-like protein (ACSL) and LYR motif protein 4 (LYRM4) — and have demonstrated that at least three of these localize to the mitochondrion and that ACSL and LYRM4 comigrate in a possible complex on glycerol gradients. Tag-mediated pulldown of NdhK also pulled down ACSL, corroborating an association between the two molecules. Thus, our current data suggest that at least a partial complex I is assembled in bloodstream form *T. brucei*. Interestingly, we were able to generate null mutants for NUBM and NUKM, indicating that this complex is non-functional as an NADH:ubiquinone oxidoreductase or redundant.

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1P.39 Inhibition of the NADH:ubiquinone oxidoreductase (complex I) by Zn^{2+}

Marius T. Schulte[1], Dinah Mattay[2], Katerina Dörner[1], Petra Hellwig[3], Thorsten Friedrich[1]
[1]*Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität Freiburg, Germany*
[2]*BAG Health Care GmbH, Germany*
[3]*Instituté de Chimie, Laboratoire d'electrochimie Strasbourg, France*
E-mail: Marius-in-Freiburg@web.de

The energy-converting NADH:ubiquinone oxidoreductase (complex I) couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. It was shown that Zn^{2+} inhibits proton translocation of many proton-translocating membrane proteins. We studied the effect of Zn^{2+} on electron transfer and proton translocation by the *E. coli* complex I and the NADH-dehydrogenase fragment of the complex. It turned out that Zn^{2+} inhibited both activities of complex I in a pH-dependent manner. The electrontransfer of the NADH dehydrogenase fragment was also inhibited but at a lower IC_{50}. This indicates that complex I has at least two Zn^{2+} binding sites. Complex I was not inhibited by other mono- or bivalent cations except Ag^{+} [1], which is expected to react with the flavin mononucleotide [2]. The most distal iron–sulfur cluster N2 [3], expected to be involved in quinone binding, was only partially reduced in the presence of Zn^{2+}. As Zn^{2+} is expected to block proton translocation this finding is the first experimental evidence for a conformational change of the surrounding of cluster N2 due to proton translocation.

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1P.40 Assembly of the *Escherichia coli* NADH:ubiquinone oxidoreductase (complex I)

Stefan Steimle, Thomas Pohl, Thorsten Friedrich
Albert-Ludwigs-Universität Freiburg, Institut für Organische Chemie und Biochemie, Germany
E-mail: stefansteimle@web.de

The energy-converting NADH:ubiquinone oxidoreductase, the respiratory complex I, of *Escherichia coli* consists of 13 subunits named NuoA – NuoN [1]. We used *E. coli* strains in which the *nuo*-genes, coding for the complex I subunits, are individually disrupted by insertion of a resistance cartridge to study the assembly of the complex in the mutants [2]. No complex I specific activity was detected in the membranes of the mutants. However, the cytoplasmic fraction of some of the mutants contained the fully assembled NADH dehydrogenase fragment of the complex. In addition, a partially assembled complex I was detected in the membranes of the *nuoL* mutant. For characterization of this fragment all *nuo*-genes but *nuoL* were overexpressed using the system established in our lab [3]. The overproduced complex I variant was isolated from the mutant. Two populations were obtained. In both populations the subunit NuoL was missing. One population showed no activity and was lacking Fe/S cluster N2. This preparation was associated with a *bona fide* chaperone. The other population contained all Fe/S clusters of complex I. It showed about two thirds of the electron transfer activity of the wild type complex I. After reconstitution in proteoliposomes this preparation showed a proton translocation activity which was approximately half of that of the wild type complex I.

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1P.41 Statistical analysis of experimental data on titration of metal centers in respiratory complex I

Emile S. Medvedev[1], Vernon A. Couch[2], Alexei A. Stuchebrukhov[2]
[1]*Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Russia*
[2]*Department of Chemistry, University of California, Davis, CA 95616, USA*
E-mail: stuchebr@chem.ucdavis.edu

Recently, Euro et al. (Biochem., 2008, **47**: 3185) have reported titration data for seven of nine FeS redox centers of complex I from E. coli. There is a significant uncertainty in the assignment of the data. Four of the titration curves were assigned to N1a, N1b, N6b, and N2; one curve either to N3 or N7; one more either to N4 or N5; and the last one denoted Nx could not be assigned at all. In addition, the assignment of the N6b signal is also uncertain, and the signal might belong to N6a. In this paper, using our calculated interaction energies (Couch et al., 2009, Biochim. Biophys. Acta **1787**: 1266), we perform statistical analysis of these data and determine the intrinsic redox potentials of the centers; out of 24 possible assignments of the data we find the best fit, and a few less

likely but still possible ones, thus greatly reducing the uncertainty of the assignments. The intrinsic potentials can be determined with uncertainty of less than ±10 mV at 95% confidence level for best fit assignments. We also find that the best agreement between theoretical and experimental titration curves is obtained with the N6b–N2 interaction equal to 71 ± 14 or 96 ± 26 mV depending on the N6b/N6a signal assignment to N6b or N6a, respectively, which is stronger than was expected, and may indicate a close distance of N2 center to the membrane surface. The potentials of N1b and N2 were determined to be /E_m /(N1b) = −265 ± 10 and /E_m /(N2) = −205 ± 10 (N6b/N6a is assigned to N6b) or −217 ± 10 mV (N6b/N6a is assigned to N6a). Implications of the present results for the titration data assignments are discussed.

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1P.42 Turning the mitochondrion into a chloroplast: the light-activation of the respiratory function in *Saccharomyces cerevisiae* allows its time-resolved analysis

Martin Trouillard[1], Brigitte Meunier[2], Fabrice Rappaport[1]
[1]*Institut de Biologie Physico-Chimique, UMR 7141 CNRS-Univ. P. et M. Curie, Paris, France*
[2]*Centre de Génétique Moléculaire, CNRS-Univ P. et M. Curie, Gif sur Yvette, France*
E-mail: martin.trouillard@ibpc.fr

Studies on the photosynthetic functions have greatly benefited from the possibility to trigger electron transfer with high accuracy through the control of the delivery of the photosynthetic substrate, i.e. photons. The respiratory chain lacks this handiness, since its substrates are continuously consumed, making real-time analysis difficult to achieve. We therefore adapted to intact living cells of *Saccharomyces cerevisiae* the "flow-flash" method, first designed in 1963 by Gibson & Greenwood [1], and successfully used since then for studying the reaction sequence of cytochrome c oxydase (CcOx) (reviewed in [2–5]). This technique relies on the lability of the CcOx inhibitor Carbon Monoxide (CO) under strong light conditions, allowing the binding of O_2 and consecutive electron transfer reactions once flashed off. Thus, conditioning the availability of the respiratory substrate (O_2) to a light input allowed us to probe the mitochondrial function almost as if it were a chloroplast. Time-resolved changes of the redox states of the CcOx cofactors (hemes *a* and a_3), cytochromes *c* and *b* could be monitored by absorption spectroscopy. Following the photodissociation of CO, hemes a_3 and *a* transfer their electrons to oxygen, while cytochrome *c* oxidation significantly lags in time. This appears hardly compatible with the predominant actual model, supported by numerous biochemical and functional reports of isolated III–IV supercomplexes, in which cytochrome *c* would be expected to be prebound to the CcOx and thus oxidized within a few dozens of microseconds. Further insight in the understanding of the *in vivo* respiratory electron transfers could also be obtained by varying the relative stoichiometries of active complexes III and IV.

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1P.43 Crystallization of mitochondrial complex I

Volker Zickermann[1], Blanka Wrzesniewska[1], Carola Hunte[2], Ulrich Brandt[1]
[1]*Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt "Macromolecular Complexes", Center for Membrane Proteomics, Goethe University, Frankfurt am Main, Germany*
[2]*Centre for Biological Signalling Studies (bioss), Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany*
E-mail: Zickermann@zbc.kgu.de

Mitochondrial complex I (proton pumping NADH:ubiquinone oxidoreductase) has remained the least understood enzyme complex of the respiratory chain and progress in understanding complex I function is severely impeded by the lack of sufficient structural information [1, 2]. The holo-enzyme complex has been thoroughly characterized by electron microscopy [3] and the structure of a hydrophilic fragment from a bacterial complex I has been determined by X-ray crystallography [4, 5]. However, an X-ray structure of the complete enzyme complex is still missing. We have crystallized complex I (approximately 940 kDa, 40 subunits) from the aerobic yeast *Yarrowia lipolytica*. Key steps for obtaining diffracting crystals of this large membrane protein complex are discussed.

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1P.44 Overproduction of *Aquifex aeolicus* complex I in *Escherichia coli nuo*-deletion strains

Marta Vranas, Markus Kohlstädt, Thorsten Friedrich
Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität Albertstr. 21, D-79104 Freiburg, Germany
E-mail: marta.vranas@sgbm.uni-freiburg.de

The NADH:ubiquinone oxidoreductase, also called complex I, is the main entrance point of electrons into the respiratory chains. Within the complex electrons are transferred from NADH to Ubiquinone (Q) coupled with a proton translocation across the membrane according to:

$$NADH + Q + 5H^+ \rightarrow NAD^+ + QH_2 + 4H^+$$

The enzyme comprises a noncovalently bound flavin mononucleotide and several iron–sulfur clusters as co-factors. In *Aquifex aeolicus,* a hyperthermophilic bacterium with an optimal growth temperature of 95 °C, the 13 *nuo*-genes, coding for NuoA-N subunits of complex I, are organized in three different loci. Heterologous production of *A. aeolicus* complex I is attempted in different *Escherichia coli* strains where the chromosomal *nuo*-operon was deleted. The deletion of the 16 kb *nuo*-operon was performed by Lambda-Red Recombineering technique, a PCR mediated gene replacement method. Plasmids were constructed containing various groups of the *A. aeolicus nuo*-genes, including the entire *nuo*-operon from the three loci mentioned above. The *nuo*-deletion strains were transformed with the expression plasmids and the heterologous overproduced proteins were isolated.

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