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1P.35 The structure of complex I from the hyperthermophilic eubacterium *Aquifex aeolicus*

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Complex I from *Aquifex aeolicus* is highly stable and active. Image analysis and 2D and 3D reconstruction by electron micrographs revealed a complete complex I particle of typical L-shape, and a pronounced invariant angle (90°) between the cytoplasmic arm [1–2] and the membrane arm. It showed many details in its external arm. The isoforms of the complex have been detected by mass spectrometry. So far, the subunits in the hydrophilic domain could be clearly assigned to two isoforms. The partial structure of one isoform of *Aquifex* complex I containing all subunits of hydrophilic domain has been determined by X-ray at a 2.9 Å resolution. Interestingly, *Aquifex* complex I contains one extra iron sulfur cluster, which is not found in that of *E. coli* and *T. thermophilus*. These data allow us to describe and discuss the mechanistic hypotheses and models of bacterium complex I [3–5].

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1P.36 A systematic approach to membrane-protein reconstitution in liposomes, applied to the M2 protein of Influenza virus A

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We present an improved methodology for production of large unilamellar vesicles and reconstitution of membrane-proteins, using gradual detergent removal. We also present two novel membrane-impermeable pH sensors, the porphyrin-based Glu3 and TCHP (Leiding et al., 2009, Anal. Biochem. 388: 296–305). The solubilization behavior of vesicles in different detergents is reported, and the effect of protein-to-lipid concentration on passive ion permeability of the liposomes. The effects of cholesterol and lipid composition on vesicle integrity are also explored — all for the purpose of understanding and optimizing the protein reconstitution process. As a proof of concept, successful unidirectional reconstitution of the Influenza protein A/M2 is reported. The integrity of the proteoliposomes allowed detailed, quantitative data collection over tens of minutes, providing a wealth of new information on ion flux through the protein (cf. Thom Leiding's poster). This reliable reconstitution method, together with pH sensors that stay within vesicles and a semi-automated titration and data-analysis system, provides a strong platform for investigating proton-translocating bioenergetic complexes.

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1P.37 A novel *c*-type cytochrome transfers electrons between sulfite oxidase and cytochrome c_{552} in the respiratory chain of *Thermus thermophilus*

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We here describe a novel *c*-type cytochrome from the extreme thermophile *Thermus thermophilus*. N-terminal sequencing of the purified protein led to the identification of the corresponding gene TTHA1326. The 23 kDa cytochrome possesses two heme *c* binding sites and demonstrates a high sequence identity to cytochrome c_{552}, the substrate of the ba_3-type cytochrome *c* oxidase. Because of the low yield, we have succeeded in its recombinant production in *E. coli* with the simultaneous expression of the *ccm* genes involved in the maturation of cytochrome *c* in the same organism. We have generated milligram quantities of the holo-protein allowing the investigation of its properties and physiological function. There is no evidence that cytochrome c_{550} acts as an electron shuttle between the *bc* complex and Thermus cytochrome *c* oxidases. We have shown that, surprisingly, cytochrome c_{550} clearly mediates electrons to cytochrome c_{552}. Further analysis of the putative operon encoding the protein led to the identification of a potential electron donor namely sulfite oxidase. In order to assess the subsequent electron transfer, sulfite oxidase (SO) TTHA1325 was produced recombinantly in *E. coli* and was shown to utilize the cytochrome c_{550} as the electron acceptor following oxidation of sulfite. To the best of our knowledge, this is the first characterization of the sulfite respiration system from a thermophilic bacterium.

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1P.38 Functional analysis of respiratory complex I (NADH:ubiquinone oxidoreductase) in the early-branching eukaryote *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* alternates between a mammalian host and an insect vector, and these environmental changes have resulted in dramatic regulation of the organism's

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+}

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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)

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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

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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