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) $= -265 \pm 10$ and /E_m /(N2) $= -205 \pm 10$ (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

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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

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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

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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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1P.45 Purification and characterisation of complex I from *Paracoccus denitrificans*

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Complex I is a multisubunit integral membrane protein which catalyses the transfer of 2 electrons from NADH to ubiquinone coupled to the translocation of about 4 protons across the membrane. It is the first enzyme in the electron transport chain and along with complex III and IV, it provides the proton motive force required for ATP synthesis. There is a lack of knowledge regarding the structure and mechanism of complex I and research is hindered by the difficulty in isolating stable protein. Here we show the purification of complex I from *Paracoccus denitrificans* where 13 out of the 14 "core" subunits have been identified by mass spectrometry. Initial single particle analysis studies show the protein to be L-shaped. This L-shape is consistent with EM studies of complex I from other studies. The protein has ubiquinone reductase activity and is inhibited by piericidin A indicating the protein is intact and active. Further studies should enable the other bands in the SDS-gel to be identified and more work will be done on increasing the yield and purity for structural and functional studies.

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