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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