anchor peptides and heme *b* composition. Delineation of mechanism of action of these enzymes is highly desirable due to the medical disorders caused by their dysfunctions in eukaryotic organisms. Although several 3D structures of complex II exist, the exact mechanism of action remains unclear. Here we present identification, isolation, purification and characterisation of SQR from an extreme thermophile *Thermus thermophilus,* analysis of which is additionally interesting from the evolutionary perspective and the aspect of protein thermostability. The *Thermus* SQR is constituted by flavoprotein (SdhA), iron–sulfur protein (SdhB) and two membrane anchor proteins (SdhC&D). It has been purified to homogeneity following series of chromatographic steps yielding 8 mg per 100 g of *Thermus* biomass. The EPR analysis revealed unusual influence of succinate on the properties of the [2Fe2S] center and presence of two heme *b* molecules in the protein. The latter places the *Thermus* SQR in the Type A SQOR, crystal structure and detailed characterisation of which are not available to date. The enzyme exists in trimeric form and unexpectedly, it is not stable at temperatures above 70 °C in the purified state as judged by Circular Dichroism analysis. Steady-state kinetic measurements show that the *Thermus* SQR displays standard Michaelis–Menten kinetics at low temperature (30 °C) but exhibits positive cooperativity for succinate oxidation at the optimum temperature of the enzyme (70 °C). To our knowledge, this is the first example of allosterically regulated complex II. To facilitate purification, increase the production yield and enable mutagenesis studies of the *Thermus* complex II, a homologous expression system has been developed. Recombinant form of the enzyme has been produced, purified and its biochemical and biophysical characterisation has also been performed.

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1P.24 Structure of a soluble subcomplex of NADH:ubiquinone oxidoreductase from *Aquifex aeolicus*

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The proton-pumping NADH:ubiquinone oxidoreductase (complex I) catalyzes the transfer of two electrons from NADH to ubiquinone via one FMN and a series of iron–sulfur (Fe–S) clusters. This process is coupled with the translocation of four protons across the membrane. Electron microscopy revealed that complex I is made up of a peripheral and a membrane arm. The peripheral arm contains all known cofactors and the binding site for NADH. We have obtained a subcomplex consisting of subunits NuoE and NuoF by heterologous expression of the corresponding genes from *A. aeolicus* in *E. coli*. This subcomplex comprises the NADH- and FMN-binding sites and harbours two Fe–S clusters. The NuoEF subcomplex was purified to homogeneity by chromatographic methods. The cofactors of the preparation were characterized by EPR- and UV/vis-spectroscopy [1]. The structure of the subcomplex was determined at 1.9 Å resolution. To define the substrate–protein interactions the structure of the subcomplex with nucleotides was examined.

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1P.25 The study of cbb_3-type oxidase

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Heme-copper superfamily are terminal respiratory oxidases in mitochondria and in many bacteria, coupling the oxygen reduction to water in order to generate proton-motive force across the membrane. The most established heme-copper oxidases are aa_3-type oxidases followed by the second abundant cbb_3-type oxidases. However, the sequence homology between aa_3- and cbb_3-type oxidases is low and they are highly different within the heme-copper superfamily. aa_3-type oxidases utilize two proton uptake pathways known as K-pathway and D-pathways for both catalytic and pumped protons. Whereas, the pattern of conserved polar residues supports the one proton uptake pathway in cbb_3-type oxidases. cbb_3-type oxidases express under low oxygen tension and have a lower K_M for oxygen than aa_3-type oxidases. Moreover, cbb_3-type oxidases are the closest to the bacterial nitric oxide reductases (NOR), which reduce NO to N_2O. This reaction is not electrogenic and do not pump protons or generate proton-motive force. In this study, we investigate the molecular details of the reasons for the differences between the subfamilies of heme-copper oxidases by focusing on cbb_3-type oxidases.

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1P.26 Kinetic and structural analysis of succinate:ubiquinone oxidoreductase (complex II) inhibition by thiapronil

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Mitochondrial succinate:ubiquinone oxidoreductase (complex II, SQR, EC 1.3.5.1) catalyzes the oxidation of succinate to fumarate as part of the tricarboxylic acid cycle. With ubiquinone (Q) as its terminal electron acceptor the SQR is also an entry point for electrons into the respiratory chain. The catalytic core of complex II consists of a flavoprotein (FP) with covalently bound FAD and an iron–sulfur protein (IP) carrying three Fe–S clusters. These two subunits are anchored to the inner membrane by two small transmembrane subunits [1,2]. The precise structural requirements for ubiquinone binding and the mechanistic details of its reduction remain to be elucidated. X-ray crystallographic studies of Q-site inhibitors bound to SQR have indicated that amino acid residues of the IP and of the transmembrane subunits constitute the putative Q-site [3,4]. Under these circumstances, chemically diverse specific inhibitors may be valuable tools for functional and structural studies of complex II. Evidence is presented here that the cyanoketone insecticide thiapronil [3-(2-chlorophenyl)-3-oxo-2-(4-phenyl-1,3-thiazol-2-yl)propanenitrile] blocks quinone reduction by purified housefly (*Musca domestica*) SQR at low nanomolar concentrations. Non-competitive inhibition is observed with respect to the electron acceptor decylubiquinone. However, Yonetani–Theorell analysis indicates that the established Q site inhibitor atpenin A5 and thiapronil bind in a mutually exclusive fashion. The structure of thiapronil bound to the chicken complex II has been solved at 2.2 Å resolution. A number of interactions contribute to the high binding affinity of thiapronil. Two H-bonds are formed between the carbonyl oxygen and the residues TyrD58 (transmembrane subunit D) and TrpB173 (iron–sulfur protein). The 3.9 Å distance between TrpB173 and the thiazole sulfur

implicates a bifurcated H-bond to both the inhibitor's carbonyl oxygen and the thiazole sulfur. The nitrile nitrogen is H-bonding Ser39 of the anchor subunit C. Additional van der Waals contacts between the aromatic rings of thiapronil and putative Q site amino acid residues are discussed.

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1P.27 Photosynthesis with simplified cytochrome b_6f complexes: Are all hemes required?

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Cytochrome bc_1 and b_6f complexes are key players in bioenergetic electron transfer chains. Their quinol oxydoreductase activity participates to the formation of the proton motive force through the Q-cycle. Structural data (Stroebel *et al.,* 2003, Nature **426**: 413–418) showed that b_6f differs from bc_1 by three additional cofactors: one β-carotene, one chlorophyll *a* and a singular heme, named c_i, located in the quinone reduction site. The CCB maturation pathway specifically responsible for the delivery of c_i and its covalent binding has been described recently (Kuras *et al.,* 2007, Proc. Natl. Acad. Sci. USA **104**: 9906–9910). A *ccb* mutant shows low accumulation level of functional b_6f complex and, hence, cannot grow photosynthetically (Saint-Marcoux *et al.,* 2009, J. Cell Biol. **185**: 1195–1207). This inability to grow under phototrophic conditions grounded a screen for suppressor mutations allowing accumulation level of functionally active b_6f complex compatible with photosynthetic growth, yet still lacking the c_i heme. The genetic analysis of the thereby rescued mutants showed that the suppressor mutation is nuclear, monogenic and affects a chloroplast protease. Although phototrophic, this mutant is highly photosensitive in the presence of oxygen. Spectroscopic study of the purified b_6f complex confirmed the absence of c_i. *In vivo* functional analysis showed that the turnover of the variant b_6f complex is not electrogenic showing that the quinone reduction site is inactive. Yet, we could observe the usual oxidant induced reduction of a *b* heme and this reduction phase was similar to the WT one, thus showing that the quinone oxidation site is not impaired. Altogether these findings show that b_i, the *b* heme of the quinone reduction site, does not participate to the turnover of the complex. Consistent with this, redox titration evidenced a strong down-shift of the midpoint potential of one of the two *b* hemes and we assigned the more negative midpoint potential to the b_i heme, excluding it, on thermodynamic ground, from the functional field. The combination of the suppressor mutation to a mutant bearing a substitution of the His202 axial ligand of the b_i heme, allowed us to construct a mutant lacking the b_i heme but still accumulating a high level of b_6f complex. This variant grows under photosynthetic conditions providing the final demonstration that the turnover of this minimal b_6f complex sustains an electron transfer flux compatible with photosynthetic growth despite its inactive Q-cycle.

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1P.28 Synthesis of cardiolipin analogues bearing a biophysical probe at any position of the four acyl chains

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Cardiolipin (CL), a negatively charged phospholipid bearing four fatty acid chains, is a major phospholipid found in mammalian mitochondria (up to 20–25%) with a multitude of biological functions. For instance, CL is responsible for regulation of the activity of several mitochondrial enzymes involved in ATP biosynthesis, though the precise molecular mechanism of regulation remains to be elucidated. This is primarily because CL analogues used in the previous biochemical studies are limited to natural and/or a few commercially available CL analogues; in the former, the chain moiety is a mixture of various fatty acids, and in the latter, the chemical variation of fatty acid chains is very poor. Therefore, to explore in detail the molecular mechanisms of both the formation of the cyt *c*-CL complex and the induction of peroxidase activity of cyt *c*, biochemical studies using structurally variable CL analogues are needed. Several different procedures for the synthesis of CL have been reported. Previous studies however were not necessarily concerned with generating structurally diverse CL analogues. For example, some procedures for the synthesis of CL bearing only saturated fatty acid chains are not suitable for the synthesis of CL containing linoleic acid(s) (C18:2), which is a major fatty acid of natural CL in mammalian mitochondria, because the *cis*-1,4-diene structure in linoleic acid is remarkably degradable under the conditions. In addition, some methods are not feasible for the routine preparation of large quantities owing to use of highly unstable intermediates or expensive reagents. The phosphoramidite approach, widely exploited in oligonucleotide chemistry, described by Ahmad et al. is an excellent way to obtain large quantities of CL analogues in high yields [1]. Unfortunately, they did not use linoleic acid as the acyl chain(s), and their procedure do not give asymmetrically substituted CL analogues. We now developed a concise procedure for the synthesis of CL using phosphoramidite chemistry, which produces diverse CL analogues bearing linoleic acid(s) at any position of the four acyl chains on a gram scale. This approach also allows for the production of CL containing a biophysical probe (nitroxide spin-label, fluorescent label, etc.) in one of the four chains.

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1P.29 Surface enhanced infrared absorption spectroscopy (SEIRAS) of complex I and QFR from *Escherichia coli*

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Infrared spectroscopy was established as a very valuable method for the study of the structure and dynamics of enzymes. The mid-IR domain (4000–500 cm^{-1}) gives information on the secondary