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

structure as well as some protonation states of specific residues of the protein [1] and the far-IR domain (500–50 cm^{-1}) on the metal–ligand vibrations of the cofactors and the hydrogen bonding network [2–4]. When Infrared spectroscopy is coupled to electrochemistry, changes in secondary structure, protonation states of residues and metal ligand vibrations induced by the redox reaction can be monitored. The immobilization of proteins on rough metal surfaces leads to an enhancement of the intensity of the IR vibrational peaks (SEIRA effect) [5–8]. The magnitude of the enhancement depends on the topography of the metal surface as well as on the nature of the interaction between the protein and the surface. Covalent immobilization is usually more efficient than physisorption. We will describe the immobilization of complex I and QFR from *E. coli* on thin layers of gold sputtered on a silicon crystal and the reconstitution of the lipid bilayer. We have studied the effect of different types of lipids. All the immobilization procedure has been followed by SEIRAS. The redox behavior of these immobilized enzymes will also be reported.

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1P.30 Red complex I — Using cytochrome c_{550} from *Bacillus subtilis* as a fusion domain to study NADH:quinone oxidoreductase

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Complex I (NADH:quinone oxidoreductase) contains four very large membrane spanning protein subunits that hitherto have been difficult to express individually in any appreciable amounts in *Escherichia coli*. The polypeptides contain no prosthetic groups or visual redox pigments and are poorly antigenic. In this work we have constructed fusion proteins where the C-terminal end of complex I protein subunits NuoH, NuoL, NuoM and NuoN from *E. coli* were genetically fused to the cytochrome c domain of *Bacillus subtilis* cytochrome c_{550}. A naturally occurring transmembrane helix anchor was removed from the cytochrome c_{550} and was substituted by the membrane spanning polypeptide to be tagged. To facilitate purification of the expressed proteins, a C-terminal his-tag was added to the protruding cytochrome domain. The fusion proteins were expressed from plasmids in a wild type *E. coli* strain, together with a plasmid containing the operon encoding the *E. coli* cytochrome c maturation (*ccm*) proteins [1], enabling holo-cytochrome c synthesis under aerobic conditions. The rationale behind cytochrome tagging was to be able to monitor the proteins. The heme in cytochrome c is covalently bound to the polypeptide, renders the proteins visible by optical spectroscopy, and can be used to monitor and quantify the proteins, and to determine the orientation of the polypeptides when reconstituted in liposomes. Particularly the three large antiporter-like subunits NuoL, NuoM and NuoN, that previously had been particularly cumbersome to produce in *E. coli*, could be made in unprecedented amounts when expressed with a fused cytochrome c domain. Finally, a gene fragment encoding the NuoN-cytochrome c fusion protein was reintroduced into the *nuo* operon on the *E. coli* chromosome using the recombination plasmid pKOV [2] allowing the production and characterization of cytochrome-tagged whole complex I.

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1P.31 The evolution of respiratory chain complex I from an 11-subunit last common ancestor

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Respiratory chain complex I is a large complex enzyme that has evolved from the combination of smaller functional building blocks. The NuoE and F are part of a family of flavin-containing NADH dehydrogenases, NuoG resembles a Fe-only hydrogenase/formate dehydrogenase, NuoB and D are homologous to NiFe-hydrogenase [1] and NuoKLMN make up an antiporter module homologous to the Mrp antiporter complex [2]. Small membrane-bound hydrogenases consisting of homologues of NuoB, D, H, I and one antiporter-like subunit are also found in nature. The ancestor of complex I did however not resemble these present day small membrane-bound hydrogenases, instead the membrane bound hydrogenases and complex I have a common ancestor. We postulate that this last common ancestor was composed of 11 subunits, homologous to NuoA, B, C, D, H, I, J, K, L, M and N. It was noticed early on that chloroplasts and cyanobacteria contain such a complex I-like protein complex with 11 subunits [3]. We have conducted a detailed survey of the distribution of 11-subunit complex I in the tree of life, using the 656 whole genome sequences currently available in CMR. Notably, 11-subunit complex I are found both in the archeal and the eubacterial kingdoms, whereas the 14-subunit classical complex I is only found in some eubacterial phyla. A complex I-homologous enzyme, F_{420} dehydrogenase, that contains a 12th subunit, FpoF, is found in Euryarchaeota but not in Crenarcheyota that only contain 11-subunit complex I. A convergent evolutionary event has occurred in eubacteria, evident today in the ε-proteo-bacteria such as *Helicobacter* and *Campylobacter* where a NuoG-like 12th subunit has been added to the 11-subunit ancestor. The evolution of classical complex I in the eubacterial lineage has occurred in two steps, first NuoG is recruited to the 11-subunit complex I, followed by a second event, where the NuoE and F subunits are added, forming classical complex I.

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1P.32 Affinity of *Escherichia coli* complex I variants to NADH and NADPH

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