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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