compared FTIR light-minus-dark ($P^+Q_A^-/PQ_A$) differential spectra in hydrated ($r = 76\%$) and dehydrated ($r = 11\%$) RC films over the 4000-1000 cm^{-1}. The spectra differ significantly in the 3750-3550 cm^{-1} range, the band attributed to weakly hydrogen bonded water molecules [5] being strongly reduced in the dried film. Dehydration also affects the 1800-1200 cm^{-1} range, which includes contributions from P, the quinones and the peptide. Optical absorption measurements performed under the same photoexcitation regime reveal a slow (t~5 s) kinetic component of $P^+Q_A^-$ recombination which disappears in the dehydrated sample, indicating at low *r* a destabilization of the charge separated state. As a whole the data suggest a correlation between the hydration shell dynamics and the conformational RC dynamics which stabilize the charge separated state.

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20P7

Molecular basis for semiquinone stabilization in respiratory enzymes: a pulsed EPR study of the menasemiquinone binding mode in *E. coli* nitrate reductase A

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Quinone binding sites (or Q sites) in respiratory complexes are the primary places for the production of reactive oxygen species that occurs as side reactions during the catalytic cycle and leads to cellular oxidative stress. This is mainly due to the transient formation of the highly reactive semiquinone species at these sites during electron transfer processes. Indeed, semiquinone stabilization is an obligate step during these processes since quinols/quinones are two-electron redox components while the proximal metal centers within respiratory complexes are one-electron transfer systems. The stabilization degree of a protein-bound semiquinone can differ by several orders of magnitude depending on the enzyme, and its importance for the function of the enzyme remains to be established. Moreover, the molecular determinants that drive this stability remain to be elucidated.

Due to the very high stability of the menasemiquinone bound to its quinol oxidation site (QD), *E. coli* nitrate reductase A (NarGHI) is a prime model for investigating the relationship between semiquinone binding mode and stabilization. Indeed, we have previously shown that this radical exhibits the highest stability measured so far for a quinone-utilizing respiratory enzyme [1]. Taking advantage from this peculiar property, the radical was used as a magnetic probe of its immediate environment. The detection of weak magnetic couplings between the unpaired electron and neighboring nuclei provided unprecedented information on the menasemiquinone binding mode [2, 3]. Combining multifrequency high-resolution pulsed EPR methods and H_2O/D_2O exchange experiments, several hydrogen atoms were unambiguously detected in the vicinity of the radical. They were assigned to specific chemical groups from either the quinone itself or from a single H-bond having unusual characteristics. Taken together, these results indicate a peculiar binding mode of the menasemiquinone at the NarGHI QD site which we consider to strongly contribute to its unusual redox properties.

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20P8

Computational investigation of the electronic structure of the Cu_A site in bovine cytochrome *c* oxidases: the functional role of the axial methionine residue

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Cytochrome *c* oxidase (C*c*O), the terminal enzyme of the electron transport system, reduces an oxygen molecule, and thereby generates the gradient of the proton concentration between the matrix and the intermembrane space of mitochondria or the periplasmic space of bacteria. In the Cu_A site of C*c*O, two Cu ions form a covalent bond, and receive electrons from cytochrome *c*, thereby providing the electrons with heme *a*. The previous experiments revealed that the substitutions of the axial Met ligand, which coordinates to a Cu ion, with leucine largely induced the changes of the redox potentials in various species. For example, for *R. sphaeroides* C*c*O, the redox potential of the M207L mutant increases by 118 mV (large) from that of wild type. In contrast, for *T. thermophilus* ba_3 oxidase, the redox potential of the mutant increases by 53 mV (middle), and for the engineered azurin, which is a reconstructed azurin possessing a similar Cu-coordination in the Cu_A site, the redox potential of the mutant increases by 16 mV (small). Thus, since the effects of the Met residue on the mutants are likely to be different among the species, the functional roles of the Met residue are still ambiguous.

In this study, to explain this diversity, we theoretically investigated the electronic structures of the Cu_A site by employing hybrid *ab initio* quantum mechanics / molecular mechanics calculation. As a result of the analysis, we revealed that the Met residue creates the characteristic feature in the electronic structure of the Cu_A site, *without the significant rearrangements*. Furthermore, we calculated the inner-sphere reorganization energy of the Cu_A site with respect to the wild type and the mutants, and found that the effects of the Met residue are not significant. Accordingly, we concluded that the Met residue may act as the "fine-modulator" of the properties relevant to the various reactions occurring in C*c*O. Moreover, we investigated the

primary and tertiary structures around the Cu_A site employing amino acid sequence analysis and structural modeling, and found that the presence/absence of an *inserted structural element* close to the Cu_A site may be related to the diversity of the redox potential.

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20P9

Interfacial water provides the pathway for proton transport along membranes

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Proton diffusion along membrane surfaces is thought to be crucial for many cellular processes such as energy transduction. It is commonly regarded as a succession of jumps between membrane-anchored proton binding sites. Our experiments provide evidence for an alternative model. We released protons at the interface, and monitored their arrival at distant sites by fluorescence measurements. The kinetics of the arrival was probed as a function of distance (i) for membranes of various compositions [1] and (ii) for the decane/water interface [2]. We found that long-range proton diffusion along the interface required neither the presence of ionizable groups [1] nor of lipids [2]. Salt removal altered the diffusion constant but did not inhibit long range lateral proton migration. Surface to bulk transfer was delayed by an energy barrier, which according to measurements at various temperatures amounted to at least 8.7 kT [2]. The observation of a large isotope effect supported the conclusion that interfacial water provided the pathway for rapid lateral proton migration.

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20P10

Microanalysis of the quinone reducing site Qi from the cytochrome *b6f* complex

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Most electron transfer chains share a complex from the Rieske/cytochrome *b* family. A common mechanism based on the Q-cycle is usually widely accepted. Yet, the quinone reducing site Q_i of the complex harbors an additional heme, called ci, in cyanobacteria and chloroplast, heliobacteria and low GC gram positive bacteria. The configuration of that haem suggests a possible coordination with a substrate, an unusual feature for supposed electron transfer site. Despite the accumulation of data, the mode of action of the action of the Q_i site remains to be elucidated. We propose here that the use of anomalous scattering can be used to overcome the limiting resolution of the available structures in order to gain further insights on the heme states and to correlate spectroscopic and crystallographic data.

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20P11

Heterologously expressed Aio: A system to study biogenesis and structure/function relationships of the Rieske superfamily

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Structural similarities between the small AioB subunit of arsenite oxidase Aio, harboring a [2Fe-2S] cluster, and PetA, the Rieske-subunit of Rieske/cyt *b* complex, show that AioB is a member of the Rieske protein superfamily. AioB and PetA indeed are so closely related that the conspicuous absence of the canonical disulfide bridge in several AioB proteins, presenting yet unchanged spectral and redox properties, was surprising [1]. This disulfide bridge is considered to be essential for Rieske cluster redox and spectral properties [2,3]. AioB furthermore distinguishes itself from PetA through the apparent fate of its leader sequence. Despite a similar predicted N-terminal Twin-arginine translocation (Tat) signal sequence, PetA invariably is membrane-anchored *via* its uncleaved Tat signal peptide, whereas Aio was either found in the periplasm or associated with the cytoplasmic membrane, depending on the species [4,5]. Heterologous expression of Aio from *Ralstonia* sp. S22 and *Rhizobium* sp. NT-26 in *Escherichia coli* allowed us to address both a) the nature of Aio's membrane-association by biochemistry and b) the influence of the disulfide bridge in this enzyme by EPR. The results with the *Ralstonia* sp. S22 enzyme suggest that the Tat signal sequence is sufficient to attach the enzyme to the membrane. The study of a Cys106Ala mutant, devoid of the first Cys involved in the disulfide bridge formation, confirmed that this bridge has no significant influence on properties of the Rieske protein from Aio. Our study furthermore revealed an oxidation-induced EPR spectral conversion of AioB centre. We propose an interaction between the [3Fe-4S]- and the [2Fe-2S]-center to be responsible of this conversion.

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