and analyzed by SAXS. Comparison of the $SCa_{104\text{-}363}$ and $SCa_{106\text{-}324}$ shape showed that the additional loop region in $SCa_{104\text{-}363}$ consists of the C-terminal residues. Whereas $SCa_{104\text{-}363}$ is monomeric in solution, $SCa_{106\text{-}324}$ forms a dimer, indicating the importance of the very C-terminus in structure formation. Finally, the solution structure of $SCa_{104\text{-}363}$ and $SCa_{106\text{-}324}$ will be discussed in terms of the topological arrangement of subunit *a* and ARNO in V-ATPases [3].

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20P2

The mysterious cofactors of the cytochrome b_6f complex: contribution of *C. reinhardtii* mutants to structure/function relationships

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Cytochrome b_6f is a membrane complex essential to electron transfer in the oxygenic photosynthetic chain. This transfer is coupled to proton translocation across the membrane thank to a Q-cycle type catalysis which is achieved through an oxidation site of plastoquinol at the lumenal side (Qo) and a plastoquinone reduction site at the luminal side (Qi). 3D structure resolution of cytochrome b_6f in 2003 [1] relaunched the interest on the role of some of its cofactors. In particular, the structure has revealed the presence of an additional haem (c_i), with respect to the mitochondrial homolog, in the Qi site. This haem is strongly atypical, being covalently bound by a single thio-ether bond to the thiol side chain of Cys35 of cytochrome b_6, and having no strong protein axial ligand [2].

The other cofactor, whose role is still undetermined, is a chlorophyll *a* molecule. There is not a trivial explication for the presence of this molecule in the complex since the cytochrome b_6f is not light activated and moreover the complex has to deal with the problem of the chlorophyll triplet state deactivation.

In order to clear up the role of the c_i haem and of the chlorophyll in the cytochrome b_6f turn over, we constructed several mutants by site directed mutagenesis [3, 4]. Recently, we have obtained a c_i /Qi mutant that displays an important sensitivity to oxygen and light, those have dramatic consequences on the c_i haem integrity and on the function of the complex. Furthermore, we could obtain a chlorophyll mutant deprived of the chlorophyll molecule in the cytochrome b_6f after purification, but still assembled. Structural and functional characterization of these mutants will be presented in the optic to bring new elements to understand structure/function relationships in the cytochrome b_6f complex.

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20P3

Defining and manipulating proton transfer pathways in nitric oxide reductase

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Nitric oxide reductases (NORs) are part of the heme copper oxidase family (HCuO). Even though NORs are capable of reducing oxygen to water, their physiological roles are as NO reducers. NO is reduced via the following reaction:

$$2\ NO + 2\ e^- + 2\ H^+ \text{-->} N_2O + H_2O$$

NO reduction is performed by denitrifying bacteria, but also by pathogens that use it to break down the toxic NO produced by the immune system of the host. There are two types of NORs; one type can be reduced by a quinol (*q*NOR) the other is reduced via cytochrome *c* (*c*NOR). Crystal structures of both types of NORs have recently been published.

Unlike the oxidases of the HCuO family, *c*NOR does not build up a membrane potential. Since electrons are donated from the outside of the membrane, protons also have to be taken up from the outside. Using the structure of cNOR as a basis we have mutated charged residues lining the predicted proton entry pathways. Using the spectroscopic properties of the heme centers and a specific set-up called 'flow flash' (a stopped-flow machine combined with a laser set-up), we can follow the speed of a single turnover from fully reduced to fully oxidized *c*NOR. Mutants that interfere or block the proton entries pathways are expected to have a severe effect on the speed of the reaction and the pH dependence. In parallel we are trying to open up a pathway from the inside of the cell (analogous to the K-pathway in the oxydases of the HCuO family), to see if *c*NOR is capable of generating a membrane potential.

Proton pathways play an important role in many proteins but are often very difficult to study. They are composed of hydrogen-bonded chains of protonatable amino acids and/or water molecules, and can often not be identified in static crystal structures due to their transient nature. Because of the specific set-up for this class of proteins where proton transfer can be measured in a single turnover by following the oxidation state of the active centers, we can learn important lessons for proton transfer in general.

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20P4

Redox-Controlled Proton Gating in Bovine Cytochrome *c* Oxidase

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Cytochrome *c* oxidase (C*c*O) is the terminal enzyme in the electron transfer chain in both mitochondria and bacteria. C*c*O reduces oxygen to water and harnesses the energy to pump protons across the inner membrane of mitochondria in eukaryotic cells or the plasma membrane in aerobic bacteria. Despite extensive studies, the mechanism by which the redox reaction is coupled to proton translocation remains unresolved owing to the difficulty of visualizing proton movement within the massive protein matrix. Three pathways, H-, D-, or K-channels have been identified in the enzyme. There is strong evidence that in the bacterial isoforms of C*c*O, the proton translocation occurs via the D-channel, which terminates near the heme *a*3 binuclear center. In contrast, studies on mammalian C*c*O support a different proton pumping pathway, the H-channel, which passes by heme *a*, instead of the binuclear center [1].

Recently, we reported that the vibrational modes of each of the propionate groups of heme *a* and heme *a*3 can be resolved in the resonance Raman spectrum and are solvent isotope sensitive [2]. Thus, they serve as indicators of hydrogen/deuterium (H/D) exchange in the H-bonding network and offer a new method to follow proton migration through the enzyme. The application of this H/D exchange Raman spectroscopic method to studies of the proton pumping mechanism in bovine C*c*O revealed a redox-controlled proton gate near the heme *a* moiety in the middle of the H-channel, where protons can be stored prior to being pumped out to the p-side of the membrane. When heme *a* is reduced, the gate closes; upon oxidation of heme *a*, the gate opens facilitating proton uptake from the negative side of the membrane. Based on these novel findings, a new model for the molecular basis of proton translocation along the H-channel has been formulated by which the electron transfer from heme *a* to heme *a*3, associated with the oxygen reduction chemistry, is coupled to unidirectional proton translocation.

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20P5

The Paramagnetic and Redox Properties of the Hemes of *E. coli* Nitrate Reductase

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Escherichia coli nitrate reductase A (NarGHI) is an excellent model for studying electron transfer through membrane-bound *b*-type cytochromes. NarGHI serves to scaffold an electron transfer relay composed of a bis-molybdopterin cofactor, five Fe/S clusters and two *b*-type hemes, where quinol oxidation is coupled to nitrate reduction [1]. NarGH is anchored to the cytoplasmic face of the membrane by NarI, which coordinates two distinct EPR visible *b*-type hemes and is the site for quinol oxidation. Traditionally, multiple components and non-ideal electron stoichiometry have been used to fit EPR redox titration spectra of the NarGHI hemes and the NarI-proximal [3Fe-4S] cluster (FS4) of NarH. The alternative, and less arbitrary interpretation, is the existence of anti-cooperative interactions between the two hemes and FS4 of NarGHI.

Application of a three-center (FS4, heme b_d, heme b_p) redox model to this sub-system gives satisfactory fits for quinone-site mutants and quinone-like inhibitor bound states, but the three-center model does not fully account for redox titration line-shape of the wild-type enzyme. However, by extending the model to include the quinone redox transitions, the fit is improved considerably. This follows as NarI is a quinone binding protein with multiple redox centers. The inevitable consequence of multiple redox active centers being close in proximity and in midpoint potential is anti-cooperative redox interactions, which is what our analysis confirms. By considering multiple cofactors simultaneously and in the context of an interacting system, midpoint potentials different from previously reported values result, as is indeed the case for NarGHI. Moreover, the magnitude of the interaction potentials suggests a non-inverse squared distance relationship, which implies non-electrostatic effects (oxidation state-dependant conformational changes) may also be present in the system, as has been suggested for a number of proteins. Conformational coupling of the two hemes, for instance, is quite likely due to the fact that they are <6 Å from each other and coordinated axially by two common α-helices [1]. The research presented discusses the redox characteristics of the hemes and [3Fe-4S] cluster of NarGHI in the context of redox cooperativity, and the paramagnetic properties of the two hemes.

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20P6

Dehydration affects the stability of primary charge separation in bacterial reaction centers: Studies by optical and differential FTIR spectroscopy

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The photosynthetic reaction center (RC) of *Rb. sphaeroides* catalyzes light-induced electron transfer events which are connected to the conformational dynamics of the protein. The light-induced charge separation between the primary donor (P) and the quinone acceptor (Q_A) is stabilized by solvent/protein conformational rearrangements. After a laser pulse $P^+Q_A^-$ recombination, which occurs with a lifetime t~100 ms in room temperature solutions, is accelerated (t~20 ms) at cryogenic temperatures [1] and in dehydrated glassy matrices at room temperature [2]. After prolonged photoexcitation, a slow phase of recombination (t~250 s) is observed, attributed to additional conformational changes [3]. Differential FTIR bands of water associated with the Q_A/Q_A^- transition have been observed upon continuous illumination, leading to propose that weakly bound water molecules plays a role in $P^+Q_A^-$ stabilization [4]. By controlling the hydration level of RC-detergent films, through equilibration at given relative humidities (*r*), a strong inhibition of the $P^+Q_A^-$ conformational stabilization has been observed at low hydration [5]. We