an alanine residue. It was found that this substitution significantly altered the charge transfer band seen in wild-type QFR and SQR with oxaloacetate. The spectral and kinetic data are consistent with a loss of catalysis showing the importance of the H-bond to substrate in the mechanism of fumarate reduction and succinate oxidation by both SQR and QFR. The X-ray crystallography of the FrdA Thr234Ala enzyme also shows a dramatic domain rearrangement between the capping domain and flavin domain in FrdA. This movement opens a substrate channel to the active site of the enzyme by altering the capping domain position. A comparison of the location of the capping domain in the open and closed states in the mutant protein suggests that in complex II enzymes, movement of the domain may be coupled to stabilization of the transition state by the threonine side chain.

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S13/3 Activated Q-cycle as a common mechanism for the cytochrome bc_1 and cytochrome b_6f complexes

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The aim of our studies is to understand the electron/proton coupling in the cytochrome bc_1 complex (bc_1). The functioning of bc_1 can be described by a dimeric Q-cycle scheme. The main traits of such a mechanistic scheme, which we have been developing during the last decade, are (i) the possibility of electron exchange between the monomers, (ii) the alternating ubiquinol binding in two ubiquinol-oxidizing centers *P*, (iii) the electrostatic compensation of electron transfer up to the ultimate step of the center *N*-catalyzed ubiquinol formation that is coupled with major voltage generation. Based upon own data on the kinetic correlation between the flash-induced redox changes of cytochrome *b*, voltage generation, and proton transfer in membrane vesicles of *Rhodobacter capsulatus*, we have put forward a scheme of a dimeric, activated Q-cycle. This scheme implies that under physiological conditions the bc_1 is maintained in an "activated" state, with a bound semiquinone in center *N* of one monomer and a reduced high-potential heme *b* in the other monomer, owing to continual priming by oxidation of membrane ubiquinol via center *N*. If bc_1 is pre-activated, then, in accordance with experimental observations, oxidation of each ubiquinol molecule in center *P* leads to ubiquinol formation in the one of enzyme's centers *N* and to the voltage generation. The applicability of this scheme to the plant cytochrome b_6f-complexes will be discussed.

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S13/4 Structural and biochemical characterisation of the alternative oxidases

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In addition to the conventional cytochrome *c* oxidase, plant mitochondria contain a nonprotonmotive alternative oxidase (AOX) that couples the oxidation of ubiquinol directly to the reduction of molecular oxygen. In thermogenic plants, AOX is responsible for heat generation, whilst in non-thermogenic species, the oxidase is thought to play a more fundamental role in the regulation of energy metabolism. AOX may be involved in facilitating TCA cycle turnover, protection against oxidative stress, and preservation of plant growth homeostasis. AOX proteins are not restricted to plants, but also occur in pathogenic organisms including the blood parasite *Trypanosoma brucei* and the intestinal parasite *Cryptosporidium parvum*. Because of their absence in the mammalian host, AOX proteins are potential therapeutic targets in these systems. Although no high-resolution AOX structure is available to date the accepted structural model predicts that AOX is an integral (~32 kDa) interfacial membrane protein that interacts with a single leaflet of the lipid bilayer, and contains a non-haem diiron carboxylate active site. This model is supported by extensive site-directed mutagenesis studies and EPR spectroscopic experiments have confirmed the presence of a binuclear iron centre. This talk will focus on the recent identification of other residues and regions important for enzyme catalysis, access of oxygen to the active-site and ubiquinol-binding.

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(S13) Electron transport chain and proton pumps symposium abstracts (poster and raised abstracts)

S13.5 An alternative site for proton entry from the cytoplasm to the quinone binding site in the *Escherichia coli* succinate dehydrogenase

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Escherichia coli succinate dehydrogenase (Sdh) belongs to the highly conserved Complex II family of enzymes which does not generate a proton motive force during catalysis. Because of its electroneutrality, the quinone reduction reaction must consume cytoplasmic protons which are released stoichiometrically from succinate oxidation. The X-ray crystal structure of *E. coli* Sdh shows that residues $SdhB^{G227}$ and $SdhC^{D95}$, as well as $SdhC^{E101}$, are located at or near the entrance of an observed water channel that has been proposed to function as a proton wire connecting the cytoplasm to the quinone binding site. However, the pig and chicken Sdh enzymes show an alternative entrance to the water channel that is greeted by the conserved $SdhD^{Q78}$ residue. In this study, these four residues were studied by site-directed mutagenesis. We show that the observed water channel in the *E. coli* structure is the functional proton wire *in vivo*, while *in vitro* results indicate alternative entrances for protons, possibly located at $SdhD^{Q78}$. *In silico* examination of the *E. coli* Sdh also reveals a possible H-bonding network leading from the cytoplasm to the quinone binding site, also via $SdhD^{Q78}$. Based on these results we propose an alternative proton pathway in *E. coli* Sdh that is functional only *in vitro*.

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S13.6 Heme-free variants of *Escherichia coli* succinate dehydrogenase

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