denoted P. During A→P transition, heme b_{595} is oxidized whereas heme b_{558} remains reduced. The P formation is not coupled to membrane potential generation. Reduction of O_2 by two electrons is sufficient to produce (hydro)peroxide bound to ferric heme *d*. Hence, if O–O bond is left intact in the P state, P is a true peroxy complex of heme *d* (Fe_d^{3+}–O–O–(H)) corresponding to compound 0 in peroxidases. If O–O bond is broken, P is heme *d* oxoferryl species ($Fe_d^{4+}=O^{2-}$) with a nearby radical (most likely amino acid residue), analogous to compound I of cytochrome *c* peroxidase or P_M species of cytochrome *c* oxidase. Decay of P to oxoferryl species is accompanied by heme b_{558} oxidation and this process is electrogenic.

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S11.36 Crystallisation and preliminary X-ray diffraction analysis of *CAA$_3$*-cytochrome *c* oxidase from *Thermus thermophilus*

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The last step in the respiratory chain uses a proton-pumping cytochrome *c* oxidase to reduce molecular oxygen to water. The extreme thermophilic, gram negative bacterium *Thermus thermophilus* expresses two distantly related cytochorme *c* oxidases, *ba$_3$*- and *caa$_3$*-oxidase. The latter is unique among the heme-copper oxidase superfamily because it exists as a complex of the oxidase enzyme and its substrate, cytochrome *c*. The crystal structures of the *ba$_3$*-oxidase and it substrate cytochrome c_{552} have been reported to high resolution. Our current aim is to solve the structure of its *caa$_3$* counterpart. The *caa$_3$*-oxidase has been solubilised from *Thermus* membranes and purified according to an established protocol by ion exchange and gel-filtration chromatography. Purification takes approximately two weeks and yields about 10 mg purified enzyme from 100 g biomass. The purified enzyme has been characterised by UV-visible spectroscopy and SDS-PAGE. Crystals of the *caa$_3$*-oxidase have been obtained by vapour diffusion sitting drop (*in surfo*) and cubic phase (*in meso*) methods. *In meso*-grown crystals diffracted to 2.8 resolution at ID-23-2, ESRF (Grenoble) but were found to be radiation sensitive. Optimisation of crystallisation conditions and stabilisation of the crystals for data-collection are in progress. Presented here are details of the purification, characterisation, *in surfo* and *in meso* crystallisation and initial diffraction results.

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S11.37 Ultrafast ligand binding dynamics in the active site of native bacterial nitric oxide reductase

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The catalytic subunit of nitric oxide reductase (NOR) from *Paracoccus denitrificans* is evolutionarily related to that of heme-copper oxidases. With the aim of exploring the interactions of external ligands with NOR, using ultrafast transient absorption spectroscopy we investigated the dynamics of the physiological substrate NO, and of CO, with the active site, which contains heme (heme b_3) and non-heme iron (Fe_B). We find that, upon photodissociation from heme b_3, 20% of the CO rebinds in 170 ps, suggesting that not all the CO transiently binds to the non-heme iron. The remaining 80% do not rebind within 4 ns and likely migrate out of the active site without transient binding to the non-heme iron. Rebinding of NO to ferrous and ferric heme takes place in ~13 ps. Our results reveal that heme-ligand recombination in this enzyme is considerably faster than in heme-copper oxidases and point at a constrained active site and (at least for CO) a low probability of transient binding to the close lying Fe_B site.

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S11.38 The reversibility of P → F state transition in cytochrome *c* oxidase from *Paracoccus denitrificans*

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Here we demonstrate a bound peroxide ($O–O^-$) intermediate within the catalytic cycle of cytochrome *c* oxidase (C*c*O). The reaction of C*c*O from *P. denitrificans* using differing H_2O_2 concentrations provides further insight into the overall mechanism. Terminal oxidases require four electrons for cleavage of dioxygen (O=O). The **P** intermediate is an oxoferryl state implying the lack of an electron for the **R** → **P** transition. Using electron paramagnetic spectroscopy (EPR) it was shown that Y167 hosts a radical species in the H_2O_2-induced P_H state which suggests that Y167 could provide this "missing electron". While X-ray structural models of C*c*O suggest bound peroxide in the O state, optical and EPR studies indicate that other intermediates may also contain such peroxide species. Stoichiometric and excess amounts of H_2O_2 induce the $\mathbf{P}_H/\mathbf{F}_{.H}$ and F_H states, respectively and catalase-treatment of the $\mathbf{F}_H$ state leads to the apparent transition $\mathbf{F}_H$ → **P**/**F**. which is accompanied by the reappearance of an EPR signal from Y167. radical EPR signal. Here we present these *novel* $\mathbf{P}_{FH}/\mathbf{F}_{.FH}$ states and postulate that the $\mathbf{F}_H$ state hosts a superoxide (or peroxide) adduct at Cu_B (in the active site). A new model for the natural catalytic cycle is proposed incorporating the concept of a complexed peroxide bound in the **O** state.

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S11.39 Identification of a putative quinone-binding site of the alternative oxidase

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Through accumulated data and our bioinformatics searches we have identified a region of the alternative oxidase (residues 236 to 266 of the *Sauromatum guttatum* protein) that we suggest constitutes a putative quinone-binding pocket located between α-helices II and III. Within this region we have identified six residues (Q242, N247, Y253, S256, H261 and R262) that are either totally or very highly conserved amongst all alternative oxidase sequences available to date (including plants, fungi and protists). We are using site-directed mutagenesis together with a yeast expression system