membrane. Here EPR spectroscopy is applied to address several issues relating to the structure and function of haem copper oxidases;

(i) Multi-frequency EPR together with site-directed mutagenesis and isotope labelling is used to identify obligate paramagnetic intermediates within the catalytic cycle of cytochrome *c* oxidase (C*c*O), providing impetus for a discussion of the natural catalytic cycle.
(ii) 2D pulsed EPR and quantum chemical (DFT) calculations are used to identify and characterise the putative site of switching from a 2 electron donor to sequential electron transfer, as yet not observed in crystallographic models.
(iii) PELDOR spectroscopy is used to probe the recent suggestion of coupling of electron input to proton pumping that may involve conformational changes within the protein.

Here we demonstrate the use of various EPR techniques and especially the correlation with modern DFT calculations to address several of these issues. The findings are discussed in relation to the function of this important class of enzymes.

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(S11) Terminal oxidase symposium abstracts (poster and raised abstracts)

S11.8 The coupling of electron and proton transfer in haem copper oxidases as studied by peldor spectroscopy

Christian Teutloff[a], Gaye White[a], Robert B. Gennis[b], Andrew J. Thomson[a], Fraser MacMillan[a]
[a]*Henry Wellcome Laboratory for Biological EPR, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich UK*
[b]*University of Illinois, Champaign-Urbana, Illinois, USA*
E-mail: C.Teutloff@uea.ac.uk

Haem copper oxidases constitute the terminal complex of the respiratory chain and catalyse the reduction of oxygen to water. This exergonic redox reaction is coupled to proton pumping across the inner mitochondrial or bacterial membrane. O_2 reduction occurs at the binuclear haem-Cu_B centre. Despite high resolution X-ray crystallographic structures, the properties of the catalytic redox states of the metal centres and their relation to protonation states within this class of enzyme remain still poorly understood. Using a cysteine-free strain of quinol oxidase from *E. coli* (*bo*$_3$), cysteines were introduced at positions R134 and R309 and labelled with a spin label probe (MTSL). Using EPR these positions were probed as a function of different catalytic intermediate states. Pulsed ELDOR spectroscopy was used to resolve potentially subtle distance changes on the doubly labelled system (R134/309). Conditions for trapping intermediate states of the enzyme while maintaining the spin labels in their paramagnetic states were successfully developed permitting the study of local conformational changes in great detail. This allows us to probe the recent suggestions for electron/proton-transfer coupling, which may involve a conformational change within the proton uptake channels.

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S11.9 Characterisation of ubiquinol oxidase activity in a native-like membrane using voltammetry

Sophie A. Weiss[a,b], Stephen D. Evans[a], Richard Bushby[b], Peter J.F. Henderson[c], Lars J.C. Jeuken[a,b]
[a]*School of Physics and Astronomy, University of Leeds, UK*
[b]*SOMS Centre, University of Leeds, UK*
[c]*Institute for Membrane and Systems Biology, University of Leeds, UK*
E-mail: s.a.weiss@leeds.ac.uk

The aim of this study was to characterise the activity of the *E. coli* terminal oxidase, cytochrome *bo*$_3$ (*cbo*$_3$) with its lipophilic substrate ubiquinol in a native-like lipid membrane. Inner membrane vesicles extracted from an *E. coli* strain which overexpresses *cbo*$_3$, were mixed with *E. coli* phospholipid extract containing ubiquinol-10 (UQ-10). These mixed vesicles were added to electrodes functionalised with cholesterol tether molecules to form planar membranes on the surface. C*bo*$_3$ activity was monitored using cyclic voltammetry with electron transfer to *cbo*$_3$ mediated by UQ-10. The apparent K_M for oxygen was determined as 1.06(±0.38) μM which is in line with apparent K_M values determined using other assay methods and experimental conditions. The UQ-10 concentration in the membrane was varied to investigate the activity of *cbo*$_3$ with its lipophilic co-substrate. Increasing the concentration of UQ-10 in the membrane above physiological levels leads to a further increase in *cbo*$_3$ activity. A K_M cannot be determined due to the limit to how much UQ-10 can be incorporated into the bilayer and this limit appears to be lower than the concentration required for maximum *cbo*$_3$ activity. This native-like membrane model provides new insights into the interaction of transmembrane enzymes with hydrophobic substrates which contrasts with studies using hydrophilic UQ analogues.

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S11. 10 Steady state redox interactions of cytochrome *c* oxidase

Maria G. Mason, Peter Nichols, Chris E. Cooper
Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK.
E-mail: mgmaso@essex.ac.uk

The aim of this study was to characterise the behaviour of cytochrome *c* oxidase during steady state, at different levels of reductive pressure. Firstly, we addressed whether the partially oxidised intermediates 'P' and 'F' were significantly populated at low reductive pressure as previously proposed. Secondly we determined whether the two initial electron-accepting centres of cytochrome *c* oxidase, CuA and heme *a*, are in redox equilibrium with their electron-donating partner cytochrome *c*. Steady state visible and NIR spectra of pure cytochrome *c* oxidase and cytochrome *c* were acquired at different levels of reduction by adjusting the concentrations of ascorbate and TMPD; samples were oxygenated by addition of H_2O_2 plus catalase. Although our system was able to detect low levels (5–10%) of the intermediates 'P' and 'F' none could be detected above these limits, at any level of reduction. We found that cytochrome *c*, CuA and heme *a* are in rapid redox equilibrium, with CuA at 234 mV, 16 mV more negative than that of cytochrome *c*. Heme *a* equilibrates in a biphasic manner with redox potentials of 262 mV and 322 mV. This effect is probably due to modulation of heme *a* by the redox state of other centres within the enzyme.

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S11.11 FTIR detection of carboxyl groups in bovine heart cytochrome *c* oxidase

Maréchal Amandine, Peter R. Rich
Glynn Laboratory of Bioenergetics, University College London, UK
E-mail: a.marechal@ucl.ac.uk

FTIR spectroscopic studies of bovine heart cytochrome *c* oxidase (C*c*O) have revealed carboxyl group changes linked both to CO dissociation from heme *a*$_3$ and to redox changes of heme *a*. In both