

P002 Transient oxo-ferryl tryptophan cation radicals in cytochrome aa_3 and bo_3 oxidase from *P.denitrificans* and *E.coli*
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Cytochrome oxidases are the final electron acceptor enzymes in Archaea, Bacteria and Eucarya catalyzing the following reaction: $4 \text{ cyt } c^{2+} + \text{O}_2 + 8 \text{ H}^+_{\text{in}} \rightarrow 4 \text{ cyt } c^{3+} + 2 \text{ H}_2\text{O} + 4 \text{ H}^+_{\text{out}}$. The catalytic mechanism of this reaction has been studied the past 50 years with a great number of different techniques. One of the remaining questions is whether a radical is formed during O-O bond cleavage and if so, what is its nature? A Microsecond freeze-HyperQuench (MHQ) setup with a rotating cold plate at 77 K to quench the reaction has been built, capable of freeze quenching within 60 μs and has been used in experiments with cytochrome oxidase from *E.coli* and *P.denitrificans*. Samples were reacted with O_2 and quenched in the 60 to 200 μs range. UV-Vis spectra showed that the samples were in the P_m state. In both cytochrome bo_3 (*E.coli*) and cytochrome aa_3 oxidase (*P.denitrificans*) an oxo-ferryl tryptophan cation radical was detected by EPR. This is the first time a transient radical is identified in cytochrome oxidase in the direct reaction with O_2 .