

P015 Location of cluster N4 and N5 in Respiratory Complex I determined by Pulsed EPR

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Depending on the organism, up to 9 iron-sulphur (FeS) clusters exist in the respiratory complex I, making it the most elaborate FeS protein assembly known. They are involved in electron transfer reactions and have been studied extensively by EPR spectroscopy. The individual characterization of the FeS centres by EPR spectroscopy is hampered by the strong spectral overlap of their signals. We have shown that at 17 K, where only N1 and N2 are visible in the EPR spectra, both spectra can be separated by their different T_1 relaxation and individually hyperfine characterized (REFINE: Relaxation Filtered Hyperfine Spectroscopy). Recently, we have shown that this method can be extended to more than two paramagnetic species in a two-dimensional version (2D-REFINE). Here we applied this method to characterize all 5 FeS clusters (N1-N5) of Complex I from *Y. lipolytica* at 5 K. We could spectrally resolve all clusters and therefore determine the individual transversal and longitudinal relaxation times. Based on this data and assuming a similar structure of our complex as in *T. thermophilus*, we calculated the expected dipolar relaxation between the FeS clusters, which allowed an assignment of the positions of N4 and N5.