

Mechanistic Studies of Substrate Oxidation by Multiheme Enzyme Hydroxylamine Oxidoreductase from *Nitrosomonas europaea* using Rapid Freeze Quench EPR Spectroscopy

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Hydroxylamine oxidoreductase (HAO), with its 24 heme centers, is the most complex heme protein known. Its active site, a unique heme P460 has an additional covalent link to Tyr467 from an adjacent subunit of the enzyme. HAO catalyses the four electron oxidation of hydroxylamine to nitrite ($\text{NH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + 5\text{H}^+ + 4\text{e}^-$).

Intriguingly, the function of HAO is also unique: the electrons are *produced* during the catalytic turnover rather than being *taken up* from oxygen as is typical for most other redox heme enzymes. Hence we are interested in studying the mechanism of the reaction.

Stoichiometry, substrate binding mode and kinetics of oxidation of hydroxylamine and its analogs (*N*-methylhydroxylamine, hydrazine) using various electron acceptor systems were studied. The preliminary data from single turnover experiments using Rapid Freeze Quench (RFQ) EPR suggest

that we produced a new enzyme state, in which a substrate intermediate is bound (possibly $\{\text{FeNO}\}^6$), and one electron has been transferred to heme 2.

