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Catching Catalysis in the Act: Using Single Crystal Microspectrophotometry to Trap Reaction Intermediates. A.R. Pearson, T. De la Mora Rey, K. Watts, E. Hoeffner, C.M. Wilmot. Univ. of Minnesota, Dept. of Biochem., Mol. Biol. & Biophys., Minneapolis, MN 55455, U.S.A.

Methylamine dehydrogenase (MADH) contains a novel quinone cofactor, TTQ, derived from two modified tryptophan residues. MADH catalyses the oxidation of methylamine, with concomitant reduction of TTQ. To complete the catalytic cycle, TTQ is reoxidized by two electron transfer (ET) events.

In the *P. denitrificans* enzyme, the first ET partner is amicyanin, a blue-copper protein. A stable MADH-amicyanin catalytically competent complex can be crystallized, and the structure has been solved¹.

TTQ and copper have spectral features that change during turnover to reflect electron distribution in the complex. Using single crystal visible microspectrophotometry (SCVM) and freeze trapping, catalytic intermediates of MADH in complex with amicyanin have been trapped in the crystalline state. However, these intermediates are extremely sensitive to X-radiation and decay during data collection. We have used in-line SCVM to monitor the redox state of these crystals during data collection at BioCARS (14BM-C). This information enables us to generate composite datasets of each intermediate before radiation induced decay occurs.

¹Chen, L. *et al.* (1992) *Biochemistry* 31 4959-64.