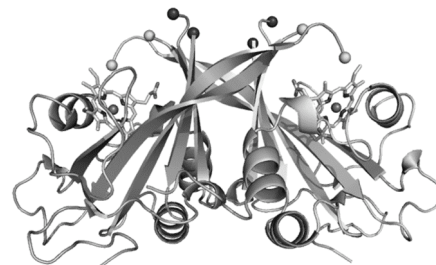


W0007

**The Crystal Structure of Cytochrome P460 of *Nitrosomonas europaea* Reveals a Novel Cytochrome Fold and Heme-protein Cross-link.** C.M. Wilmot\*, A.R. Pearson\*, B.O. Elmore\*, C. Yang#, J.D. Ferrara# & A.B. Hooper\*, Univ. of Minnesota, Minneapolis, MN 55455, Rigaku Americas Corp., The Woodlands, TX 77381.

We have determined the 1.8 Å X-ray crystal structure of a mono-heme *c*-type cytochrome, cytochrome P460, from *Nitrosomonas europaea*. The chromophore possesses unusual spectral properties analogous to those of the catalytic heme P460 of hydroxylamine oxidoreductase (HAO), the only known heme in biology to withdraw electrons from substrate coordinated to the iron. The structure was solved by S-SAD using 2.29 Å wavelength X-rays. The analysis reveals a homodimeric structure and elucidates a new *c*-type cytochrome fold that is predominantly  $\beta$ -sheet. In addition to the two cysteine thioether links to the porphyrin typical of *c*-type hemes, there is a third proteinaceous link involving a conserved lysine. The covalent bond is between the lysine side-chain nitrogen and the 13'-*meso* carbon of the heme, which following cross-link formation is  $sp^3$  hybridized demonstrating loss of conjugation at this position within the porphyrin. The protein underwent oxidation during crystallization, confirmed by mass spectrometry, leading to the presence of a hydroxyl group at the 5'-*meso* carbon of the heme. During X-ray data collection the hydroxyl group was lost in a dose dependent manner. Two structures were refined; the physiological structure using high dose data, and the structure with the additional hydroxyl using low dose data. The structure has implications for the analogous tyrosine-heme *meso* carbon cross-link observed in HAO.



This work was funded by NIH GM-66569 (CMW), NSF MCB 0093447 (ABH) and DOE DE-FG02-95ER20191A009 (ABH). Some data were collected at beam-line 4.2.2 at the Advanced Light Source, LBNL, Berkeley, CA.