

## E0024

**Preliminary Neutron Studies of Protocatechuate 3,4-Dioxygenase.** Douglas H. Ohlendorf, C. Kent Brown, Paul Langan, Benno P. Schoenborn,.

The metalloenzyme protocatechuate 3,4-dioxygenase (3,4-PCD) forms large well ordered crystals that diffract to  $\sim 2.0$  Å resolution using  $\text{CuK}\alpha$  x-ray produced by in-house rotating anodes. A key question concerning 3,4-PCD is the protonation state of the iron ligands during the reaction cycle. Since neutrons possess greater scattering sensitivity to H/D atoms, protonation states of the enzyme and enzyme-substrate complexes can be experimentally determined.

Single-crystal neutron analysis of 3,4 PCD from *Pseudomonas putida* has begun with collecting native data of the unliganded enzyme to  $\sim 2.4$  Å at the Protein Crystallography Station of the spallation source at the Los Alamos Neutron Science Center. 3,4-PCD is the largest biological molecule ever studied at high resolution using single crystal neutron techniques. The 3,4-PCD aggregate is a dodecamer of 587,000 Da, half of which is in the crystallographic asymmetric unit of a C2 unit cell ( $a = 223$  Å,  $b = 127$  Å,  $c = 134$  Å,  $\beta = 61^\circ$ ). A progress in collecting the initial native data set will be reported and the quality of the data will be evaluated. We expect this work to further demonstrate the power of neutron studies and provide a technical basis for future neutron studies of other biological molecules.