

## Trapping Enzyme Reaction Intermediates by Freeze-Quench XAS

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The understanding of structure-function relationships in proteins has been significantly advanced with the advent of the biotechnology revolution. However, information on the solution-state of proteins and enzymes has also been of paramount importance, and structural spectroscopic techniques have been instrumental in defining protein structure-function relationships for a number of years. A goal yet to be realized for many metalloprotein systems is the nature of dynamic changes in structure that bridge the static endpoints provided by crystallography. We have developed data analysis strategy to analyze XAS data of enzyme-substrate intermediate complexes of alcohol dehydrogenase which were generated by freeze-quench methods. The relative contributions of each complex in the mixture were quantified by EXAFS analysis. An increase in the number of ligands in the active site of the enzyme was observed as a function of time. These results may be used as a fingerprint of the increasingly strong interaction between the enzyme and its substrate.