

SPIN STATE CHARACTERIZATION OF METALLOENZYMES VIA X-RAY EMISSION SPECTROSCOPY

SAHAND EMAMIAN, *Department of Physics, Emory University, Atlanta, Georgia, USA*; KENDRA A IRELAND, VATSAL PUROHIT, KIRKLIN L McWHORTER, *Department of Chemistry, Emory University, Atlanta, GA, USA*; OLGA MAXIMOVA, YULIA N PUSHKAR, *Department of Physics, Purdue University, West Lafayette, IN, USA*; KATHERINE M DAVIS, *Department of Chemistry, Emory University, Atlanta, GA, USA*.

Enzyme reactivity is often enhanced by changes in oxidation state, spin state, and metal-ligand covalency of associated metallocofactors. The development of spectroscopic methods for studying these processes coincidentally with structural rearrangements is essential for elucidating metalloenzyme mechanisms. Herein, we demonstrate the feasibility of collecting X-ray emission spectra of metalloenzyme crystals at a third-generation synchrotron source.

In particular, we report the development of a von Hamos spectrometer for the collection of Fe $K\beta$ emission optimized for analysis of dilute biological samples. We further showcase the application of this high-resolution, "DIY" mini-spectrometer in crystals of the immunosuppressive heme-dependent enzyme indoleamine 2,3-dioxygenase (IDO).

Spectra obtained from reference compounds were compared to catalytically-relevant states of IDO via integrated absolute difference analysis to determine their spin numbers. Complementary density functional calculations assessing covalency support our spectroscopic analysis and identify active site conformations that correlate to high- and low-spin systems. These experiments validate the suitability of an X-ray emission approach for determining spin states of previously uncharacterized metalloenzyme reaction intermediates.