Probing the Reactivity of New Metalloproteins With High-Field Pulsed Electron Magnetic Resonance

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Metalloproteins catalyze the most challenging chemical reactions seen in nature. R2lox metalloproteins have an unusual "mixed-metal" manganese-iron (Mn/Fe) active site and are recognized as a virulence factor in pathogens like *Mycobacterium tuberculosis,* the causative agent of tuberculosis. *While diiron (Fe/Fe) proteins are well-studied, Mn/Fe proteins represent a new class of metalloprotein for which the chemistry is largely unexplored*. In this work, the reactivity of R2lox towards O_2 and the structure of a key, short-lived oxidized state have been characterized, demonstrating unusually weak electronic interactions between the Fe and Mn centers and a critical hydrogen bond within the active site that likely play an important role in determining the reactions that R2lox can perform.

Experiments at the MagLab EMR program capitalized on the sensitivity and resolution of the HiPER spectrometer to study transient biochemical species that could only be obtained at low concentrations. *High-field experiments enable resolution of the spin Hamiltonian parameters of the Mn center, suggesting the presence of an open coordination site that a target molecule could subsequently use to bind to the metal(s) and undergo reaction(s)*. Researchers also took advantage of the high-power pulsed EPR capabilities to directly examine protons near the active site using the Electron-Detected NMR technique, which indicated a strongly coupled proton in this short-lived state that disappears as the reaction proceeds, consistent with the proton-coupled reactivity hypothesized for these proteins.

This research revealed what R2lox looks like before it reacts with a target, which will aid in identifying the role that R2lox plays in enhancing tuberculosis virulence. Moreover, this study provides a roadmap for understanding electronic structure-function relationships in novel Mn/Fe metalloproteins.

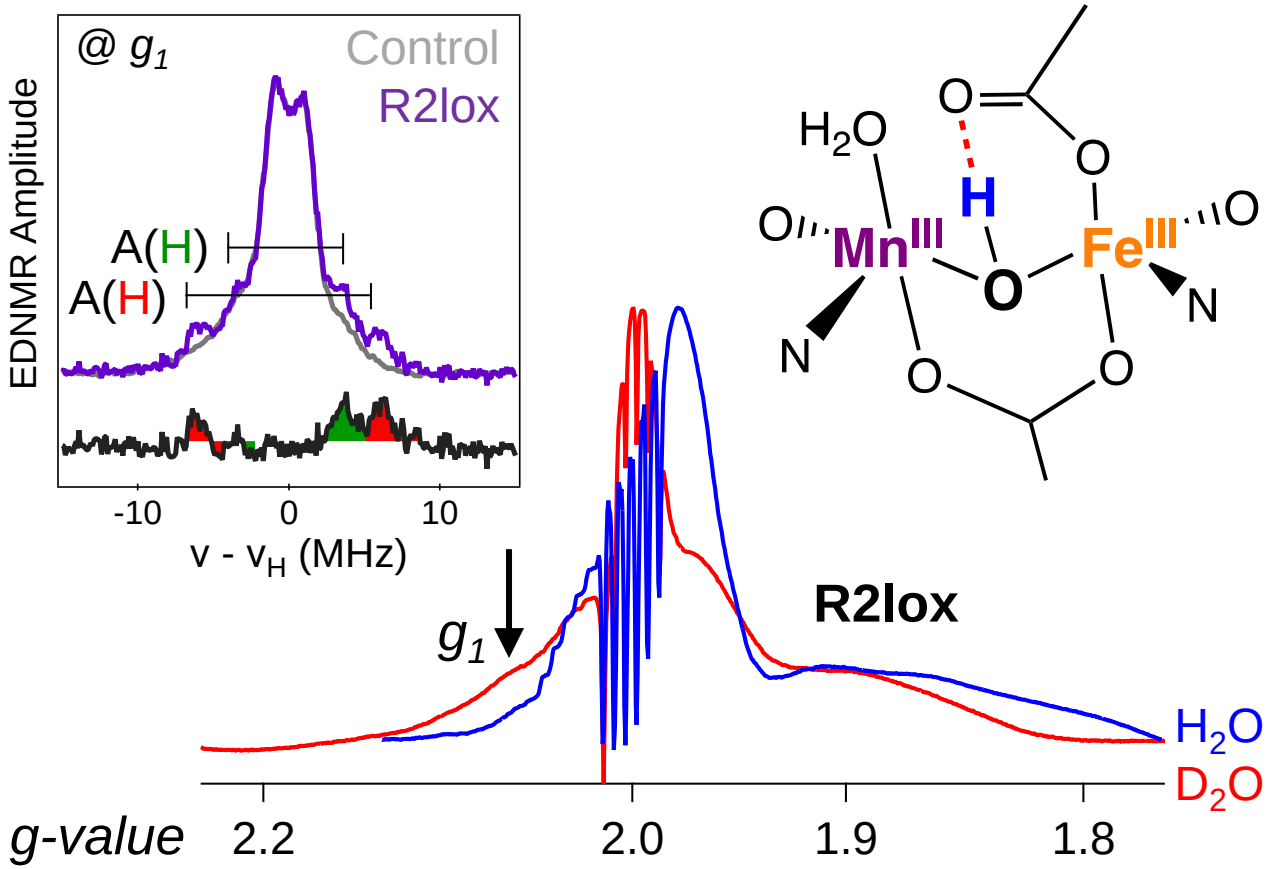

Figure. (*Center*) Magnetic field-swept pulsed electron echo-detected (ED) spectrum of a reactive, short-lived state of Mn/Fe R2lox after binding oxygen. (*Top left*) High-field ED-NMR spectrum of the same Mn/Fe R2lox state. The weak signals due to the observed protons [A(H)] are highlighted. (*Top right*) Structure of the trapped species proposed from EPR spectroscopy.

Facilities and instrumentation used: EMR (W-Band HiPER Pulsed EPR Spectrometer).

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