Characterizing the Invisible:
EPR and ENDOR Studies of Intermediates in N_2 Reduction by Nitrogenase

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Characterization of an enzyme mechanism can be viewed as the process of identifying and characterizing the structure and reactivity of intermediates that comprise its catalytic cycle. This talk will illustrate our approach to trapping such intermediates and using electron paramagnetic resonance (EPR) and electron-nuclear double resonance (ENDOR) spectroscopies to characterize them, through discussion of nitrogenase. Nitrogenase catalyzes the reduction of N_2 to NH_3 on the most complicated metalloenzyme active site: the molybdenum-iron cofactor (FeMo-cofactor), an $[Fe_7MoS_9X]$ cluster. Despite the availability of the beautiful and mysterious structure of this cofactor for more than a decade, it has yielded no clue as to the site(s) of substrate binding and reaction. We have now: *(i)* determined the site of alkyne and dinitrogen reduction, *(ii)* characterized an intermediate trapped during the reduction of propargyl alcohol ($HC\tilde{3}C2\text{-}C1H_2OH$; PA) through use of a novel set of *quantitative* ^{1,2}H ENDOR spectroscopic techniques, plus ^{13}C ENDOR measurements; *(iii)* used ^{1,2}H ENDOR to study an intermediate that is formed during proton reduction and has two $H^{+/-}$ bound to the cofactor; *(iii)* are in the process of characterizing *several* reduced forms of N_2 bound to the cofactor.