# NMR-Assisted Crystallography: Imaging Active Site Chemistry with Protons

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The determination of active site protonation states is critical to gaining a full mechanistic understanding of enzymatic transformations; yet proton positions are challenging to extract using the standard tools of structural biology. Here we make use of a joint solid-state NMR, X-ray crystallography, and first-principles computational approach that unlocks the investigation of enzyme catalytic mechanism at this fine level of chemical detail. Through this process, we are developing a high-resolution probe for structural biology that is keenly sensitive to proton positions – rivaling that of neutron diffraction, yet able to be applied under conditions of active catalysis to microcrystalline and non-crystalline materials. For tryptophan synthase, this allows us to peer along the reaction coordinates into and out of the α-aminoacrylate intermediate. By uniquely identifying the protonation states of ionizable sites on the cofactor, substrates, and catalytic side chains, as well as the location and orientation of structural waters in the active site, a remarkably clear picture of structure and reactivity emerges. Most incredibly, this intermediate appears to be mere tenths of angstroms away from the preceding transition state in which the β-hydroxyl of the serine substrate is lost. The position and orientation of the structural water immediately adjacent to the substrate β-carbon suggests not only the fate of the hydroxyl group, but also the pathway back to the transition state and the identity of the active site acid-base catalytic residue. Reaction of this intermediate with benzimidazole (BZI), an isostere of the natural substrate, indole, shows BZI bound in the active site and poised for, but unable to initiate, the subsequent bond formation step. When modeled into the BZI position, indole is positioned with C-3 in contact with the α-aminoacrylate Cβ and aligned for nucleophilic attack.

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