Neutron Diffraction Studies of Pyridoxal-5'-Phosphate Dependent Enzymes.

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The vitamin $B_6$ derived cofactor pyridoxal-5'-phosphate (PLP) is the predominate coenzyme in metabolism and the wide variety of PLP catalyzed reactions has intrigued researchers for many decades. PLP is found in at least five protein fold types and is involved in a multitude of chemical transformations including decarboxylations, $\beta$ and $\gamma$ elimination, racemization, transamination, and dephosphorylation. A search of the RCSB Protein Data Bank for the PLP ligand indicate over 900 X-ray diffraction derived structures have been submitted. With the crystal structures providing positions of the heavier atoms, UV-Vis and NMR spectroscopy have provided evidence for the positions of hydrogens. Recently, we solved the complete structure of aspartate aminotransferase (AAT), in both the internal and the external aldimine forms, using joint refinement of X-ray and neutron diffraction data on perdeuterated enzyme (Dajnowicz et al, Nat. Comm., 2017). Hydrogen mainly contributes to an incoherent isotropic background in the neutron diffraction experiment. In contrast, deuterium has a coherent signal with a comparable scattering length to carbon, oxygen, and nitrogen. The diffraction from crystals of perdeuterated protein in $D_2O$ provide direct evidence for the position of hydrogens in the molecule. The ability to visualize the entire molecule, including the presence of unpredicted low-barrier hydrogen bonds, offers more accurate models as inputs for computational analysis. In the transamination reaction, the $C\alpha$ – H bond in the external aldimine is in a "stereochemical alignment" perpendicular to the cofactor conjugated ring, an alignment, deemed necessary in the "Dunathan Hypothesis". Using the new joint refined model in computational studies, we have been able to establish that hyperconjugation enhances catalysis several orders of magnitude.