## Neutron Diffraction Studies of PLP-Dependent Enzymes

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Vitamin $B_6$ derivative, pyridoxal 5-phosphate (PLP), is the cofactor to many different chemical reactions. PLP-dependent enzymes catalyze a broad range of chemistry including transamination, racemization, phosphorylation, $\alpha$-decarboxylation, aldol cleavage, $\beta$ and $\gamma$-elimination and replacement reactions. PLP-dependent enzymes have important roles in the metabolism of amino acids and glycogen and are found in numerous pathways, including the interconversion of $\alpha$-amino acids and the biosynthesis of antibiotic compounds (Schneider *et al.*, 2000). As ubiquitous proteins with significant metabolic functions, PLP-dependent enzymes are attractive targets for specific inhibitor design. As a cofactor, PLP forms a covalent intermediate with the substrate, generating an external aldimine. The external aldimine stabilizes the transition state carbanion formed by the deprotonation of the C$\alpha$. While considered a rather well-defined enzymatic mechanism, recent work on PLP-dependent catalysis has suggested that some features of the mechanism, like the thermodynamics of decarboxylation and the role of molecular strain in the protonated and deprotonated PLP-enzyme complex, need reevaluated (Bach *et al.*, 1999; Hayashi *et al.*, 2003). To better understand and characterize the role of protonation in PLP-dependent catalysis, we have begun neutron diffraction studies on several PLP-dependent enzymes, including Aspartate aminotransferase, transamination model, and Tryptophan synthase, $\beta$-elimination model. We have recently published the neutron diffraction structure of Aspartate aminotransferase, in both the internal and external aldimine forms (Dajnowicz *et al*, 2017). Additionally, neutron data sets of Aspartate aminotransferase with the pyridoxamine and $\beta$-hydroxyaspartate (Figure 1) external aldimines have been collected. Large crystals of Tryptophan synthase have been grown and beam time at Oak Ridge National Laboratory has been approved. Furthermore, microgravity crystallization efforts are being made to improve crystal quality and size for neutron diffraction. AAT and TS flew on SpaceX CRS-15 in a preliminary study, showing that microgravity improved the size and mosaicity of the crystals. Both AAT and TS will fly on SpaceX CRS-18 in June 2019 with optimized conditions and perdeuterated materials.

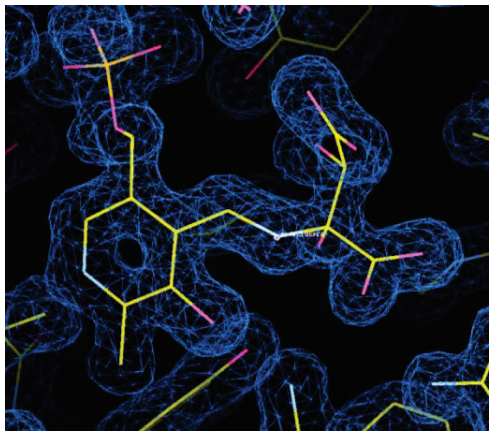

*Figure 1:$\beta$ -hydroxyaspartate external aldimine in active site AAT*

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