# Structural Intermediates of Phenylalanine Hydroxylase Revealed by Disruption of a Key Intramolecular Interaction

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Phenylalanine hydroxylase (PAH, EC 1.14.16.1) is an enzyme that converts L-phenylalanine (Phe) to L-tyrosine, regulating normal Phe blood levels (~50-120 μM). In phenylketonuria (PKU), inherited defects in PAH result in hyperphenylalaninemia where Phe blood levels can become neurotoxic (up to ~2.5 mM). Recent atomic structures of mammalian PAH combined with solution scattering analyses have lent insight into the allosterically regulated conformational changes that occur between a resting state tetramer to a highly active form in rising Phe concentrations. This transition occurs via a conformational selection model, where the binding of allosteric Phe at a distal site within the regulatory (ACT) domain modulates this facile equilibrium.

Our recent study (Arturo et. al., Biochimie 2021) shows that disrupting a single cation-π interaction between the regulatory and catalytic domains (Phe80 variants) results in PAH variants with unique activity and biochemical profiles. In this study, we used a combination of analytical ultracentrifugation (AUC) and size-exclusion chromatography with synchrotron X-ray scattering (SEC-SAXS) to investigate the oligomerization and solution conformations of these variants. By modeling the SEC-SAXS data with atomistic models, we gained insights into the arrangement of these intermediates in different functional forms. Our work reveals the presence of various oligomeric assemblies and conformers in solution, and insights into the modulation of PAH activity by its regulatory domain.