Imaging enzyme active site chemistry using multiple fields up to 35.2T: NMR crystallography of tryptophan synthase

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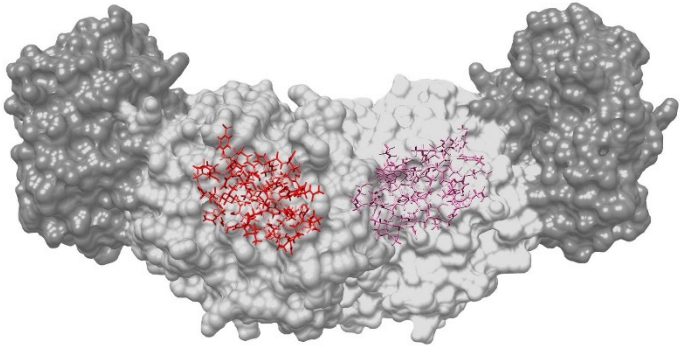
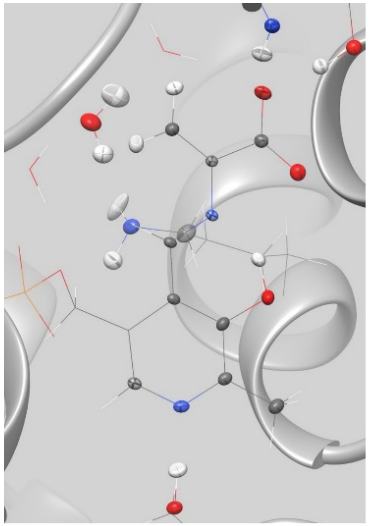
Funding : G.S. Boebinger (NSF DMR-1644779); L.J. Mueller (NSF CHE-1710671; NIH GM097569); J.R. Long (NSF CHE-1229170; NIH GM122698)

The determination of active site protonation states is critical for a full mechanistic understanding of enzymatic transformations; however, hydrogen atom positions are challenging to extract using the standard tools of structural biology.

Here we make use of an integrated approach using high-magnetic-field solid-state NMR, X-ray crystallography, and first-principles computation that enables the investigation of enzyme catalysis at a fine level of chemical detail. The X-ray crystal structure provides a coarse framework upon which models of the active site are built using first-principles computational chemistry and various active site chemistries are explored. *These competing computed models are differentiated based on their agreement with experimental chemical shift restraints measured at multiple magnetic fields of 9.4T, 14.1T, 21.1T, and 35.2T – the latter being uniquely available at the MagLab*.

A detailed three-dimensional picture of structure and reactivity emerges, highlighting the fate of the substrate L-serine hydroxyl leaving group and the reaction pathway back to the preceding transition state. Subsequent characterization of the complex with the inhibitor benzimidazole shows it bound in the active site and poised for, but unable to initiate, the subsequent bond formation step. The chemically-rich structure from this NMR-assisted crystallography is key to understanding why this inhibitor does not react, while the natural substrate indole does.





Top: The protein tryptophan synthase showing the active site (red) with hydrogen atoms.

Left: The chemically-detailed view of the tryptophan synthase active site showing the position of hydrogen atoms (colored white), including anisotropic displacement parameters .

Facilities and instrumentation used: NMR Facility: 14.1T/600MHz (DNP) and 21.1T/900MHz; DC Facility: 36T Series Connected Hybrid

Citation: Holmes, J.; Liu, V.; Caulkins, B.; Hilario, E.; Ghosh, R.; Drago, V.; Young, R.; Romero, J.A.; Gill, A.; Bogie, P.; Paulino, J.; Wang, X.; Riviere, G.; Bosken, Y.; Struppe, J.; Hassan, A.; Guidoulianov, J.; Perrone, B.; Mentink-Vigier, F.; Chang, C.; Long, J.R.; Hooley, R.; Mueser, T.; Dunn, M.; Mueller, L., *Imaging active site chemistry and protonation states: NMR crystallography of the tryptophan synthase α-aminoacrylate intermediate,* **Proceedings of the National Academy of Sciences of the USA (PNAS)**, **119** (2), e2109235119 (2022) doi.org/10.1073/pnas.2109235119