Locating H atoms: active site protomer/tautomer state determination using routine, macromolecular X-ray diffraction and BUSTER/DivCon

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Active site protonation states play a critical role in enzyme mechanisms of action and understanding these states - in ligands, cofactors, and pocket residues - can be integral to successful structure based drug design (SBDD) campaigns. Neutron diffraction data are typically required for an accurate assessment of hydrogen positions in macromolecular crystallography. However, despite recent methodological advances, routing neutron diffraction experiments still remain very challenging. Recently we developed the XModeScore[1] method, which employs linear scaling, QM-based X-ray macromolecular refinement[2] coupled with rigorous experimental density statistical analysis in order to determine the correct protomer and tautomer states of protein residues and/or bound ligands even when challenged with data determined at routine resolutions.

We will discuss the influence of protonation states on reactivity, mechanisms of action, and ligand binding within the active through the combinatorial application of the XModeScore methodology as applied to an active site involving a mixture of multiple His, Glu and Asp residues. In particular, the impact of cooperative effects of protonation of key catalytic residues, as well as the influence of coordinated metals and bound water molecules, will be discussed.

References:

[1] Borbulevych, O., Martin, R.I., Tickle, I.J. & Westerhoff, L.M. (2016). Acta Cryst. D72, 586, http://dx.doi.org/10.1107/S2059798316002837

[2] Borbulevych, O., Martin, R.I., & Westerhoff, L.M. (2018). Acta Cryst. D74, 1063. https://doi.org/10.1107/S2059798318012913