

## **The missing atom in function: reliability of the determination of hydrogen positions in protein structures**

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Arguably the most important atom in a protein is the smallest one, hydrogen. However, it is also the most difficult to visualize. It plays a central role in enzyme catalysis, protein stability and folding, protein engineering, and computational drug design. Assignment of mechanistic roles to individual residues often depends on the ability to assign protonation states. Presence of a proton and its movements during a reaction are inferred from what is known about the chemistry of molecules. However, that inference is not always accurate, especially in the active site of an enzyme. Because the environment of an active site can influence the pKa of a residue, it is often difficult to identify ionizable residues from kinetic analyses. Protein structures are generally presented without hydrogens, since X-ray structural methods do not identify them. Consequently, other methods are required to assign hydrogens. Ultra-high resolution crystallography (<1.0Å resolution) can provide information about some hydrogens, although those of most interest are often still invisible. Computational methods are cumbersome and not useful for routine analyses. Neutron diffraction analysis, however, identifies protons, and their positions can be correlated with the results of ultra-high resolution analyses and computational analyses, thereby filling this important mechanistic gap.