

## Where In the World Are the Hydrogens?

Dagmar Ringe  
Brandeis University  
Waltham, MA

Abstract:

Perhaps the most important atom in a protein is the smallest one, the one that is almost impossible to see - hydrogen. It plays a central role in the fields of mechanistic enzymology, protein folding, protein engineering, and computational (or rational) drug design. Enzyme mechanisms can often be determined through careful kinetic analyses; however, assignment of mechanistic roles to individual side chains within the active site is almost never straightforward. This is often due to difficulties in assigning protonation states in enzyme active sites, as it is well established that the pKa value of an amino acid in the environment of an enzyme active site can differ significantly from that in solution. In addition to yielding mechanistic information about enzymes, knowledge of the presence or absence of hydrogen atoms on ionizable side chains plays an important role in computational experiments. It has been shown that significant improvements in the scoring functions in computational ligand docking are possible when the correct protonation states are used.

In each of the aforementioned research areas, three-dimensional protein structures are critically important for success; yet, protein structures are almost exclusively presented without hydrogen atoms. This is not surprising, as even in ultra-high resolution structures ( $<1.2 \text{ \AA}$ ), many hydrogen atoms remain invisible, especially those attached to heteroatoms, which are generally the hydrogens of greatest interest. This can, however, be accomplished via neutron diffraction, as deuterium atoms are readily visible at moderate resolution (2 - 2.5  $\text{\AA}$ ). By comparison of ultra-high resolution X-ray crystallography and neutron diffraction analysis, we seek correlate inter-heavy-atom distances (and possibly hydrogen atom positions) with protonation states determined from neutron diffraction analyses.