Modelling and refinement of hydrogen atoms: new developments in REFMAC5

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Hydrogen (H) atoms represent a large fraction of the total atomic content of macromolecules. They often play critical roles in enzymatic reactions, as they are involved in the protonation of active site residues, in the organization and orientation of the solvent network, and in the establishment of hydrogen bonds.

However, the direct visualisation of H atoms in macromolecules is not straightforward. Since their contribution to the total scattering is minimal owing to their low electron content, only a small percentage of H atoms can be located using X-ray macromolecular crystallography (MX), even at sub-atomic resolution (<1.2 Å). In contrast with MX, neutron macromolecular crystallography (NMX) relies on the interaction between neutrons and atomic nuclei. Ratio of atomic scattering factors of deuterium (D) to that of typical heavy macromolecular atoms such as oxygen, carbon and nitrogen is not far from 1 (1.15, 1.08 and 0.71 respectively). Therefore, the observation of H atoms (typically in the form of D) is possible even at modest resolution (2.0–2.5 Å). Despite the major limitations of this technique, especially due to the low flux of neutron beams, NMX studies are particularly useful in answering research questions that require specific knowledge of H atom positions with the view of obtaining mechanistic insight. Whilst NMX maps show the nuclear positions of H atoms, MX maps indicate the positions of valence-electron density for H atoms shifted along their bond vector. Cryo-electron microscopy (cryo-EM) and electron diffraction experiments inform on both nuclear and electron localisation of H atoms.

This project aims at modelling and refining H atoms by using different experimental data (cryo-EM, NMX and electron diffraction) integrated in a common framework, to provide new insights in biological processes such as enzyme mechanisms. This contribution will describe the recent developments in the crystallographic refinement package REFMAC5 [1], for the refinement of structural models obtained by NMX data. The CCP4 Monomer Library [2], typically used as the source for prior chemical information in REFMAC5 and other programs such as Coot [3], has been recently extended to include accurate H atom nucleus to parent atom distances derived from neutron data analysis [4] and quantum mechanics (QM) calculations. New features for the refinement of mixture H/D parameters in NMX and the use of reference structure restraints will be presented.

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