

Neutron Crystallography of Insulin using a radically small volume crystal

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Neutron crystallography is an important complementary technique to X-ray crystallography because it provides details of the H-atom and proton (H⁺) positions in biological macromolecules and, given the absence of radiation damage with neutrons, the resulting structures are 'damage-free' even at room temperature. Knowledge of the positions of the H-atoms and protons is important since details of protonation and hydration are often necessary for understanding macromolecular function at an atomic level, such as enzyme mechanisms [1,] and in drug-binding [2,]. Although, historically, the study of biological macromolecules using neutron crystallography had been limited-due to the requirement for extremely large crystals of several cubic millimetres-recent improvements to the quasi-Laue diffractometer LADI-III at the Institut Laue-Langevin (ILL) are allowing us to extend the limits for neutron macromolecular crystallography using crystals of smaller dimensions and studying larger unit-cell systems [3]. This has resulted in a typical lower limit for useful crystal volumes of ~0.1 mm³, however, from a very recent study of human recombinant insulin it has been shown that in fact much smaller crystals can be used for high-resolution neutron diffraction studies. Neutron quasi-Laue diffraction data have been collected to 2.2 Å resolution from a crystal of human insulin with a radically small volume of 0.02-0.03 mm³ (~250-300 µm on edge) using the LADI-III instrument at the ILL. Given that the insulin crystal used for data collection was H/D-exchanged this implies that the limit for perdeuterated crystals (in which all H are replaced by its isotope deuterium) should be even smaller, possibly less than 0.01 mm³. Given that crystal volumes of this order are much more feasible to grow, a large number of potential neutron crystallography studies are now within range. Herein we will describe, in detail, the neutron structure of human recombinant insulin, which has not been determined previously using neutron diffraction, and which clearly shows details of protonation and hydration that are not attainable even with ultra-high resolution X-ray crystallography.

[1] Casadei et al., (2014) Science, 345, 193-197.

[2] Gerlits et al., (2016) Angew. Chem. Int. Ed., 55, 4924-4927.

[3] Blakeley et al., (2015) IUCr, 2, 464-474.

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