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COMPLEMENTARITY OF NEUTRON AND ULTRA-HIGH RESOLUTION SYNCHROTRON X-RAY PROTEIN CRYSTALLOGRAPHY STUDIES

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Highly resolved crystal structures of protein molecules can be obtained now using X-ray crystallographic data measured at synchrotron radiation sources with high performance detectors and cryoprotected samples. Details of the mobility of the atoms is also available from their 'thermal parameters' in the refined molecular model. In this way we have now studied the sugar binding plant protein concanavalin A to 0.94A resolution [1]. The ease of exchange of particular protons for deuterium in the protein has then been studied using neutron Laue diffraction data measured at the ILL in Grenoble [2]. In addition we have determined the D_2O bound solvent structure of concanavalin A using a combined Cu K alpha X-ray refinement at 1.8 A and neutron at 2.4 A refinement approach, and then compared the results with the bound solvent in the 0.94A SR X-ray structure [3]. Many more bound water molecule deuteriums can be located via neutron protein crystallography ('X+n' approach) rather than the ultra-high resolution X-ray approach alone. However the latter does deliver a few very detailed water molecule hydrogen atoms. The two approaches are thus complementary.

In another aspect improvements in the diffraction resolution for room temperature protein X-ray crystallography are important because this is closer to physiological temperature. There are differences between cryo and room temperature protein structures as exemplified by an ensemble of experimental refined structures of concanavalin A [4]. Hence the extra mobility of bound waters at room temperature makes their study via X-rays yet more challenging, which further increases the utility and contribution of the neutron approach. The extension of neutron data collection methods in protein crystallography to encompass yet larger unit cells is important. We can currently record data on large crystals (approx 3mm in size) on the cubic concanavlin A with bound glucoside (space group I213, a=168A) to ~3.5 A on LADI at Grenoble [5]. In order to reach 3.0A or better resolution improved measuring techniques are needed and time-of-flight Laue diffraction is an attractive approach as the signal to noise can be improved, but is yet to be brought to fruition [6]. The proton synchrotron spallation 'short pulse' type neutron source (SNS) such as ISIS @ 160 kW, especially the proposed cold target station 2, and then the upcoming USA's SNS (@ 2 MW) and the Japanese spallation neutron project, will each allow the time-of-flight Laue approach to be utilised and at an increasing source power level. In the limit of the proposed ESS @ 5MW, with the short pulse target station, a factor of approx 30 gain in capability would be available over ISIS and 10 x ILL LADI [7]. Thus major strides can be made to address two fundamental limits of neutron protein crystallography at present. Firstly smaller protein crystals could be harnessed eg down to 0.5 mm on edge for a ‘typical’ 25, 000 molecular weight protein (in the crystal asymetric unit) [5]. Secondly larger molecular weight proteins (in the crystal asymmetric unit) could be studied where large crystals can be grown (eg 3mm on edge is a ‘typical’ maximum [5]). Microgravity provides a medium for convection free growth of large crystals [8]. Since the molecular weight histogram for eg the yeast genome peaks around 30,000 molecular weight, and many proteins make oligomers of single subunits, a larger molecular weight capability for neutron protein crystallography is vital. ESS can provide this. Overall ESS can allow then the detailed structural dissection of enzymes whose mechanisms need definition of key hydrogens (as deuteriums). Furthermore biological macromolecule ligand interactions involve water displacement and this is an even bigger set of targets for neutron structural study including proteins and nucleic acids.

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