Neutron Cryocrystallography of Proteins

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Neutron diffraction can directly determine the hydrogen isotope positions of a protein and its bound solvent. By collecting the data at cryo-temperatures the quality of the resulting maps can be improved. It has proved possible to cryo-cool very large concanavalin A protein crystals (> 1.5 mm^3) suitable for high resolution neutron and X-ray structure analysis. We can thereby report the neutron crystal structure of the saccharide-free form of concanavalin A to 2.5 Å resolution [1]. This is the first cryo- neutron protein crystal structure ever to be reported and the first 15K to 293K neutron protein crystal structure comparison. Comparison with the 293K neutron structure [2] shows that the bound water molecules are better ordered and have lower average B-factors than those at room temperature. Overall, twice as many bound waters (as D_2O) are identified at 15K than at 293K.

Methodologically, this successful neutron cryo protein structure refinement opens up new categories of neutron protein crystallography, including freeze trapped structures. Other large crystals of proteins have also proved amenable to cryo-cooling and examples of these will be presented too (Blakeley, Meilleur, Myles, Bau *et al* to be published).

[1] Blakeley M.P., Kalb (Gilboa) A.J., Helliwell J.R., Myles D.A.A., *PNAS*, 2004, **101(47)**, 16405. [2] Habash J, Raftery J, Nuttall R, Price H.J., Wilkinson C, Kalb (Gilboa) A.J., Helliwell J.R., *Acta Cryst.,* 2000, *D***56,** 541.

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