

s14.m39.o5 **Neutron protein structures at 12K.** Matthew Blakeley, EMBL outstation, 6, rue Jules Horowitz, Grenoble, FRANCE, E-mail: [blakely@embl-grenoble.fr](mailto:blakely@embl-grenoble.fr)

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Using neutron crystallography, one can assign the positions of hydrogen atoms of a protein and its bound solvent. Knowing the positions of the hydrogen atoms can be crucial in understanding the mechanisms behind a protein's function. Furthermore, by collecting neutron data at cryo-temperatures, the dynamic disorder within the protein crystals is reduced which can lead to improved definition of the nuclear density. Using flash-cooling protocols that enable large protein crystals (1-3mm<sup>3</sup>) to be cooled, transferred and mounted for data collection at cryogenic temperatures we have determined the low temperature neutron structures of two proteins, concanavalin A and a rubredoxin mutant from *Pyrococcus furiosus* (Pf).

The iron-sulfur redox protein rubredoxin (Rd) from *Pyrococcus furiosus* (Pf), found in deep undersea superheated vents, is stable for days in boiling water, whereas most other bacterial rubredoxins are readily denatured within minutes at 100°C. We are using high-resolution neutron diffraction to probe this temperature stability by analysing the extent of H/D exchange at main chain N-H bonds in a series of rubredoxins that have been subjected to varying degrees of H<sub>2</sub>O/D<sub>2</sub>O exchange (by boiling for different times). This should help to identify flexible regions exposed to the environment that readily exchange (N-D) from those more rigid and/or protected regions that do not (N-H). Analysis of neutron data collected to 1.7 Å resolution and at 12K from the first of these structures shows, remarkably, that 5 specific internal H-bonded positions do not undergo H/D exchange, even under these harsh conditions. Interpretation is continuing.

Concanavalin A from the Jack bean (*Canavalia ensiformis*), is a sugar-binding protein capable of specific recognition and reversible binding to complex carbohydrates that provides a good model system for the study of ligand binding interactions. We have shown that neutron analysis at room temperature to 2.4 Å resolution enables ~7 times more water molecules to be fully modelled as complete D<sub>2</sub>O molecules than in a 0.94Å X-ray analysis at 110K. In order to improve and extend this analysis, we have re-determined the neutron structure of the protein at 12K on LADI to 2.5 Å resolution. Comparison with the 293K neutron structure shows that at 12K the bound water molecules are better ordered and have lower average B factors. Overall, twice as many bound waters (as D<sub>2</sub>O) are identified at 12K than at 293K. In particular, the definition of D<sub>2</sub>O water molecules at the saccharide binding site is greatly improved. Whereas at 293K, where the waters were identified as a cluster, at 12K the waters are resolved, allowing the D atom positions to be placed with confidence. This has allowed a hydrogen bonding network to be proposed between key amino acid residues in the saccharide binding site and the conserved water molecules. Furthermore, knowledge of the orientation of these waters to the approach of the ligand is important for more complete thermodynamics and modelling studies.

s14.m40.o1 **Teaching crystallography in a changing world.** Henk Schenk, Laboratory for Crystallography, HIMS, FNWI, Universiteit van Amsterdam, Nieuwe Achtergracht 166, 1018WV Amsterdam, The Netherlands. Email: [schenk@science.uva.nl](mailto:schenk@science.uva.nl)

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In present years the character of science is changing and along science teaching changes as well. Students in many countries turn away from it in favour of other fields. Where Science Faculties used to have thousands of students now numbers are halved or smaller. Also the way students study and the content of curricula changed in the last two decades and are more focussed on broader issues. Students have to use their time more efficiently in order to adopt the other challenges of modern life. Universities are permanently reorganising their personnel in order to cope with the changed interests of students and to fulfil the request of society for increased accountability. On top of this they are generally faced with shrinking budgets and therefore try to work market-oriented to compensate decreasing finances, e.g. by playing a role in permanent education, by making courses for industry and society, and also by changing subjects much quicker than before.

In the past industry undertook research only when the financial results enabled it. At present, however, even additional profit and stockholders confidence has more priority. So industry has less interest than before in research in basic and applied science, and consequently in skilled scientists. Multinationals also outsource their research to any country with science for a better price. There is also a more positive side of the medal: small firms are coming up as young scientists are setting up research-based small enterprises, in many cases with venture capital. In our own area we see for instance companies in high-throughput protein crystallography and high throughput polymorph screening coming up.

All of this makes it more difficult for basic science and its teaching to survive. For crystallography there is one more handicap: it is a small subject compared with e.g. chemistry and physics. When faculties grow, small fields may grow at the same pace. However, when faculties shrink, it could well be that they decide to stop small subjects completely rather than to work out other ways without reducing knowledge and skills.

In this environment the teaching crystallography in an undergraduate science curriculum should be attractive and related to the other fields of science and applied science. For master education the level should be such that it enables working with crystallographic methods in the relevant fields. And in PhD studies a level should be reached that enables qualified teaching to undergraduate studies and promoting crystallography. Moreover, it would be advisable also to work on skills enabling PhD's to start small enterprises when finished.

Another challenge for our field is to teach some basics and fun of crystals, their properties and use to children of primary and secondary schools. This hopefully will develop and bring back interest and enthusiasm for science in general and in particular for crystallography.