Larger Crystals a Step Forward for Bioscience and ESS User Program

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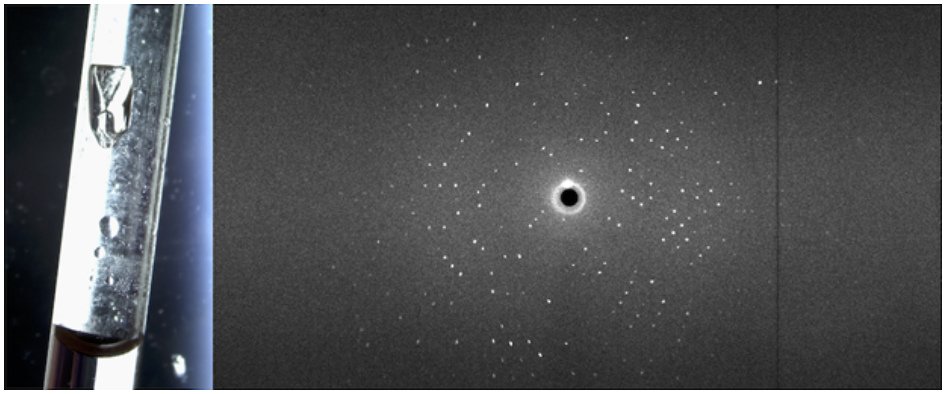
Support Labs. The first successful user experiment at ESS demonstrates the importance of neutrons to the life sciences, and shows the way forward for ESS as a user facility.

LUND and GARCHING — The heavy machinery and rapid development of the European Spallation Source (ESS) construction site make it easy to see that the project is a reality on the ground. What is less visible is that ESS is already taking shape as a user facility. The first successful ESS user experiment was begun last fall in collaboration with researchers from the Lund University (LU) Department of Immunotechnology. The organization and management of that partnership is a preview of the large-scale user program that ESS will become over the next decade. It is also helping to spread the word in life sciences circles that ESS is already working to expand research options in this area.



Photograph (left) of a large, single crystal of X-2 L110F in complex with XXXG mounted in a quartz capillary undergoing H/D exchange (dimensions are ~1.3 x 1.2 x 1.0 mm; 1.6 mm3); and a representative monochromatic neutron diffraction image collected using BIODIFF at FRM-II. IMAGES: ESS

'Neutrons have the power to discern the exact locations of protons in large molecular complexes, information that is key to understanding molecular functional mechanisms in biology," explains Prof. Sindra Petersson Årsköld, senior science advisor at ESS. 'However, in order to obtain this information, samples often have to be deuterated and crystallized, which poses some challenges."

A team of researchers led by Prof. Mats Ohlin of the LU Department of Immunotechnology is studying protein-carbohydrate binding as part of a larger effort to expand knowledge of the role carbohydrates play in our immune systems. The work also has implications for a

variety of plant bioscience and biomedical applications.

To unlock the secrets of carbohydrates, a researcher needs proteins to show the way. The LU Department of Immunotechnology is compiling libraries of millions of proteins, and incrementally resolving their atomic structures. The library will aid researchers in the design or selection of proteins exhibiting the attributes needed to probe particular carbohydrates. To date, those protein structures have been determined by X-ray crystallography at MAX-lab, but now the group is looking at what neutrons can provide.

'Often when you hear people talk about neutron scattering, they don't really know what to use it for, there is some confusion as to what kind of questions it may actually answer," says Ohlin. In April 2014, the immunotechnology department hosted a seminar on science at ESS, and invited Petersson Årsköld and crystallographer Dr. Zoë Fisher, a scientist for deuteration and crystallization at ESS. Their presentation had immediate results.


Prof. Mats Ohlin (left) of Lund University's Department of Immunotechnology; and ESS scientist Dr. Zoë Fisher. PHOTOS: Courtesy of Mats Ohlin; Courtesy of Zoë Fisher

'At that time," explains Ohlin, 'we had a collaboration with MaxLab and SARomics Biostructures to solve the structure using X-ray crystallography, and we became aware of how important hydrogen atoms were in these interactions. Even if we had high-resolution structures, it was not feasible to see all the hydrogens in them. So when [Petersson Årsköld and Fisher] described what neutron scattering could do, what kind of information you would get, it immediately caught my attention."

X-ray structural studies usually do not give explicit information on hydrogen atom positions and, as a result, many of the details of Ohlin's protein-carbohydrate interactions have to be inferred. The ability to assign the precise locations of the hydrogen atoms will help predict which modifications in a protein might produce the desired probe. The high neutron flux of ESS, and the ability of some of its instruments to make use of smaller sample sizes, will allow researchers to put hydrogen atoms in their proper positions. By supplying this missing link in a more routine way than existing neutron sources, ESS is expected to unlock some long-held secrets of macromolecular biological materials.

'SARomics was very successful at growing the crystals," continues Ohlin, 'but not to the size required for neutron diffraction. It had not really been on the table to discuss actually using neutron diffraction. That's why I approached Zoë immediately after her presentation."

Many user experiments at ESS will rely on spectroscopy and diffraction instruments that require samples to be converted to crystalline form. Synchrotron X-ray sources—like ESRF in Grenoble and MAX IV in Lund (located a few hundred meters southwest of ESS and expected to be commissioned next year)—can make use of small crystals, but neutron sources require the crystals to be larger. A crystal is not a naturally occurring form for most biological samples, and these large crystals can be difficult, and sometimes impossible, to produce.

Additional complications arise in making crystals for life science research as the attempts must often be made with a limited supply of deuterated materials. Deuteration, another service the support labs will provide, is the process of replacing a molecule's more common hydrogen-1 atoms, protium, with hydrogen-2 atoms, deuterium. Because neutrons interact differently with the two hydrogen isotopes, this process allows the hydrogen atoms in a sample to be identified in neutron scattering experiments.

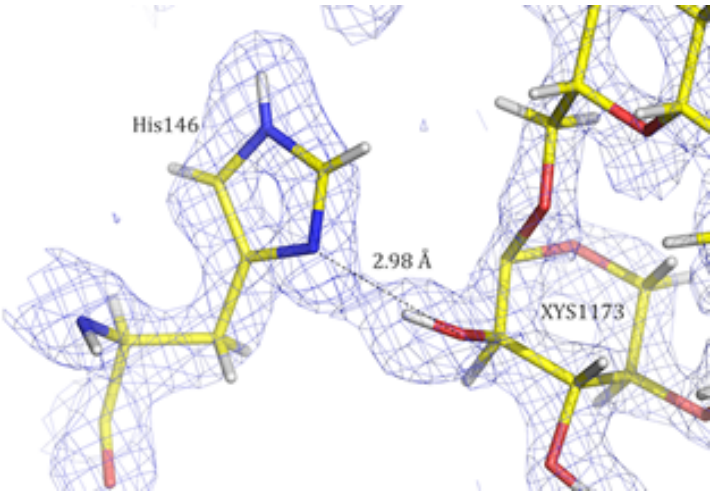
'A major bottleneck in neutron protein crystallography is crystal volume," explains Fisher. 'Large crystals are often difficult to attain due to the amount of deuterated protein needed to grow them."

The development of some crystallization processes has taken years, whereas others may benefit from the prior knowledge of a scientist who specializes in growing materials into crystals. Fisher is one such master. Recruited to ESS from the Los Alamos National Laboratory in the US, she has developed the crystals and assisted in resolving the 3D atomic structures of dozens of unique biological samples.

Once Ohlin handed over his carbohydrate-binding sample—incorporating protein variant derived from a thermophilic microbe found in Icelandic hot springs—Fisher was able to produce large crystals (around 2 mm^3 in volume) of the structure known only as 'X-2 L110F". These crystals were carefully sealed in capillary tubes and have now been zapped with X-rays at MAX-lab and bombarded with neutrons on the BIODIFF instrument at the FRM II facility in Munich. The combination of these two high-resolution diffraction experiments will result in a very precise 3D model of the protein structure.

'We have been chasing certain hydrogens in the binding cleft for years, and with this breakthrough in large crystal growth we have a good chance at unravelling the key binding interactions," says Ohlin. 'The next step is to move on and study additional interactions, much as we did with X-ray crystallography, but to complement those results with neutron diffraction." These and other related studies in protein-carbohydrate interactions are important for development of new tools that would facilitate development of improved

biofuels, biomedical diagnostic tools, drug administration, new vaccines, more profitable crops, and more.



An example of how neutrons are useful for visualization of protein residues and how they interact with substrates or ligands. Shown are representative nuclear density maps of key residues after several rounds of PHENIX joint refinement and manual checking in *Coot*. The H/D atoms are shown in white; *2Fo — Fc* nuclear density is shown as a blue mesh and is contoured at 1.5σ. IMAGE: Ohlin et al.

The ESS Biocrystallization Lab is temporarily housed in Medicon Village, and already serves to provide services for users in need of assistance with biological samples to be analysed at existing neutron and X-ray sources. The ESS lab has established close ties to Lund University and collaborates with their Lund Protein Production Platform (LP3), which provides a range of complimentary services to produce, purify, and analyse protein samples—like Ohlin's—that will be used in spectroscopy and diffraction experiments.

Working together, these facilities are establishing an efficient administrative and scientific workflow that will be essential to making the best use of precious ESS beam time once the facility is commissioned. ESS will be used by the neutron science community to conduct experiments on materials samples across the whole range of organic and inorganic matter. Each sample must be managed either by the researcher or the facility where the research is done.

'It is an added value to have scientific support labs here in Lund, to offer an expert workflow for future ESS users," says Prof. Arno Hiess, head of the ESS Scientific Activities Division. ESS plans to provide access to a number of on- and off-site labs that will serve to facilitate users throughout the life cycle of their sample—its preparation, testing environment, handling, and disposal. 'We will be able to follow up or provide expert support, we can keep bench and beam time allocation efficient," explains Hiess. 'We are here to enable the user through interwoven access modes, bearing in mind also that MAX-lab is there. Co-hosting allows us to achieve critical mass in competence and resources, that also gives a scale advantage."

'Knowing the difficulty of even getting crystals suitable for X-ray crystallography, and the added difficulty of neutron studies, it's going to be a larger hurdle to cross," adds Ohlin. 'Support labs will be very helpful. There will be a lot of hands-on experience that is required to get these large crystals to materialize. I think core facilities that can aid people in such aspects with specialized knowledge are going to be very helpful."

Further Reading:

- Ohlin, M., von Schantz, L., Schrader, T. E., Ostermann, A., Logan, D. T. & Fisher, S. Z. (2015). Acta Cryst. F71, 1072-1077.
  http://journals.iucr.org/f/issues/2015/08/00/nj5231/index.html

- More information on Dr. Ohlin's research at LU can be found here.
  http://www.immun.lth.se/research/principle-investigators/mats-ohlin/research/molecular-design-carbohydrate-binding-scaffold/

- ESS Science Focus Team page for Soft Condensed Matter & Life Science.
  https://europeanspallationsource.se/page/soft-condensed-matter-life-science

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