VMXm takes its First Users ‘where no synchrotron has gone before’

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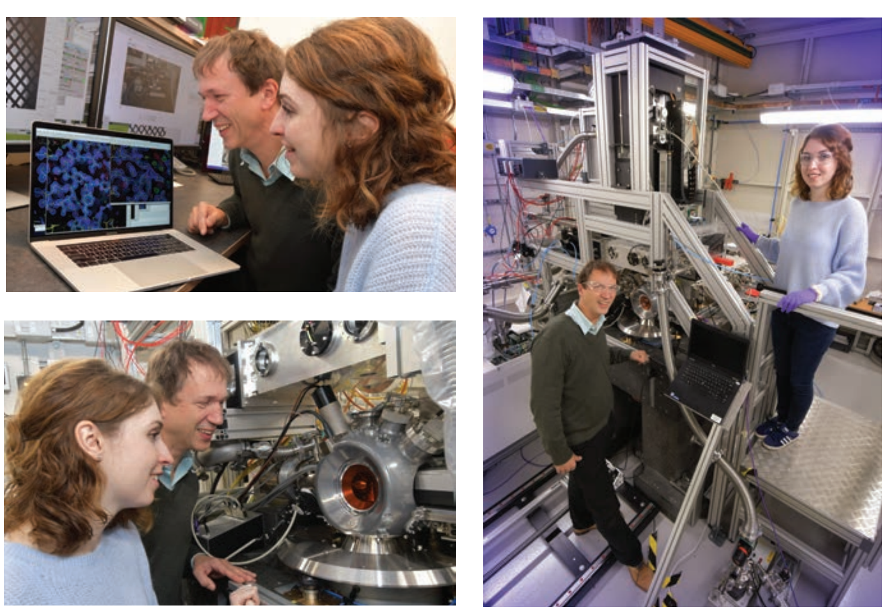
When the doors opened to Diamond Light Source’s newest beamline (the 32nd operational beamline at the UK’s national synchrotron), Dr Ivo Tews’ group from Biological Sciences at the University of Southampton were first in the queue to use it. They are researching photosynthetic or cyanobacteria, to find out more about how these phytoplankton thrive in nutrient-poor waters. Their project is embedded with work at the National Oceanography Centre at Southampton to understand the efficiency of the bacterial metabolism and of nutrient uptake. The team have taken *Prochlorococcus* samples from deep in the ocean, over 5,000 metres below the surface and acquired samples from particularly nutrient-poor regions of the world’s oceans in order to study microorganism adaptation.



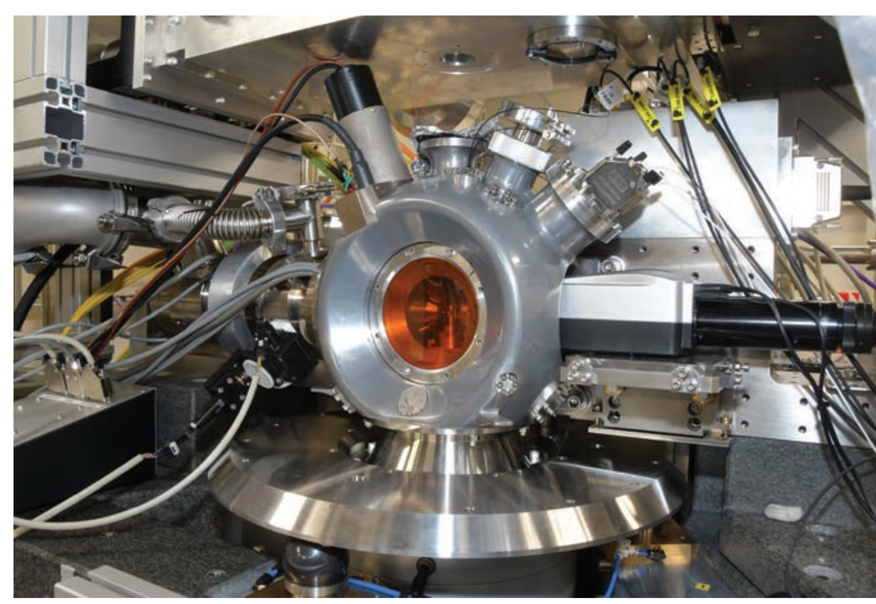
PhD student Rachel Bolton and Dr Ivor Tews, PI in Structural Biology at the University of Southampton using the new VMXm beamline

As PhD student Rachel Bolton explained, “The ocean bacteria *Prochlorococcus* dominates areas of the ocean in which nutrients are scarce and these cover 30% of Earth’s surface. *Prochlorococcus* is the most abundant bacteria in the ocean that performs photosynthesis and is responsible for the removal of approximately four gigatons of carbon a year. This figure is comparable to the amount of carbon produced by the world’s agriculture industry, highlighting the importance of these bacteria in global biogeochemical cycles. Understanding more about how these bacteria work could help with biotechnology and the production of biofuels.”

The group is investigating how *Prochlorococcus* takes up iron, an essential nutrient that is limited in waters where the bacteria thrive. The work focuses on determining the structure of the proteins involved in scavenging iron using X-ray crystallography, which requires protein crystals.

The new Versatile Macromolecular Crystallography micro/nanofocus (VMXm) beamline is unique and represents a significant landmark for Diamond. It is a specialist tuneable micro/nanofocus macromolecular crystallography (MX) beamline, with a variable X-ray beam size of between 0.5 and 5 microns, allowing even the tiniest of samples of microcrystals to be analysed. VMXm is managed and operated by a dedicated team of scientists at Diamond, including Principal Beamline Scientist Dr Gwyndaf Evans, senior beamline scientist Dr Jose Trincao, senior support scientist Dr Anna Warren and postdoctoral research associates Dr Emma Beale and Dr Adam Crawshaw.

During their time at Diamond, the Southampton team prepared their samples by placing the tiny crystals onto standard cryo electron microscopy grids using an automatic plunge freezer system. Crystals are pipetted onto a grid inside a humidity chamber and blotted from behind to remove any excess liquid. Minimal liquid on the grids is required to maximise the signal to noise and to ensure crystals on the grid aren’t visually obscured. Grids are then plunge-cooled in liquid ethane and can be screened using an offline scanning electron microscope (SEM) in the sample preparation area.



The Versatile Macromolecular Chrystallographymicro/nanofocus (VMXm) beamline is the 32nd operational beamline at Diamond Light Source.

The beamline is able to hold five cryogenically cooled grids in the evacuated sample chamber and as each grid can carry hundreds of crystals, enough material can be provided for several hours of measurements. The ‘in vacuum’ sample environment gives VMXm a huge advantage as it reduces unwanted X-ray background arising from the interaction of X-rays with air, particularly important as the measured diffraction signal from microcrystals is exceedingly small. The end-station also incorporates its own SEM, which allows users to precisely locate each microcrystal, characterise its size and shape and then align it into the X-ray beam ready for data collection, a task that would be very difficult for the usual visual camera.

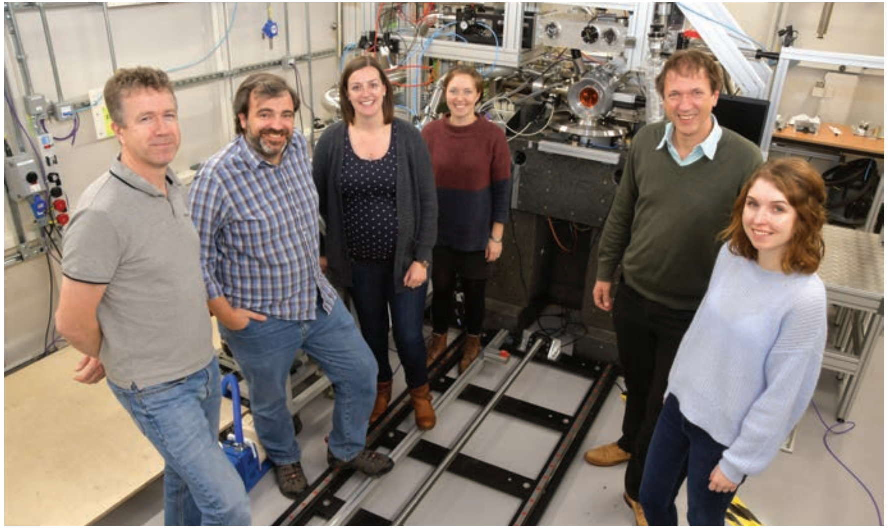
Rachel said: “What drew us to VMXm was the ability to record data from microcrystals, from a small volume of sample and to work in vacuum to get a better signal-to-noise ratio. The crystals of the iron-containing protein are extremely sensitive to X-ray; VMXm is designed to push the limits in terms of size of crystals but also to reduce the number of X-ray exposures needed. We wanted to test the limits of the beamline and find out whether we could get a full dataset from microcrystals. It was such a great feeling when we got our first diffraction patterns from the first samples; it’s really, really exciting to be involved in something so new.”

The team is still processing their data but the initial results looked good and their next line of investigation involves pushing the capabilities of the beamline by trying to further minimise X-ray radiation damage to the crystal samples. “One of the biggest problems in X-ray crystallography is sample radiation damage as it can cause structural artefacts, leading to a misinterpretation of protein structure,” explained Rachel. It has been hypothesised that as protein crystals get smaller the majority of photoelectrons (responsible for causing much of radiation damage in protein crystals) actually leave the crystal before they do any harm, an effect that will hopefully be harnessed at VMXm. This will be particularly useful for proteins sensitive to radiation, for example metal-binding proteins like the iron uptake proteins in *Prochlorococcus* bacteria.

Dr Ivo Tews is PI in Structural Biology at Southampton and a member of the VMXm proposal team, which is made up of fourteen UK academic and industrial structural biologists representing the wider community. Dr Tews understands the significance of the new instrument to research projects like this one: "The protein crystals investigated contain iron and are very sensitive to X-ray radiation induced damage. I was very excited to see that complete data with minimal damage could be collected from very few micro-crystals, requiring very little material!"

Rachel has a unique perspective on the VMXm beamline as her PhD is a collaboration between Diamond and the University of Southampton, with her time split between the two institutions. This means that Rachel has been involved with the beamline for over a year and has had the opportunity to learn much more about beamline design and processes which has benefitted her research. "It's so great to be involved in something so cutting edge and to be part of the beamline improvements and optimisation as we moved forward," she said. Rachel will now be involved in welcoming more users to the VMXm beamline and in pushing its capabilities even further. There are plans, for example, to investigate ways in which the inevitable radiation damage to the sample can be reduced.

Dr Gwyndaf Evans summed up: "VMXm will allow researchers to push synchrotron data measurement beyond the previously explored limits of crystal size. It's also a significant milestone for the Diamond design and construction team: making such a complex instrument work so well at the first attempt is a remarkable achievement, and they should be immensely proud of themselves. We are now looking forward to really exploring the limits of what VMXm is capable of and using it to answer important biological questions."



First Users on the VMXm beamline: From left, Dr Gwyndaf Evans, Principle Beamline Scientist at Diamond Light Source, Dr Jose Trincao, senior support scientist, Dr Anna Warren and postdoctoral research associate Dr Emma Beale, with on the right Dr Ivor Tews and Rachel Bolton, Biological Sciences at the University of Southampton.