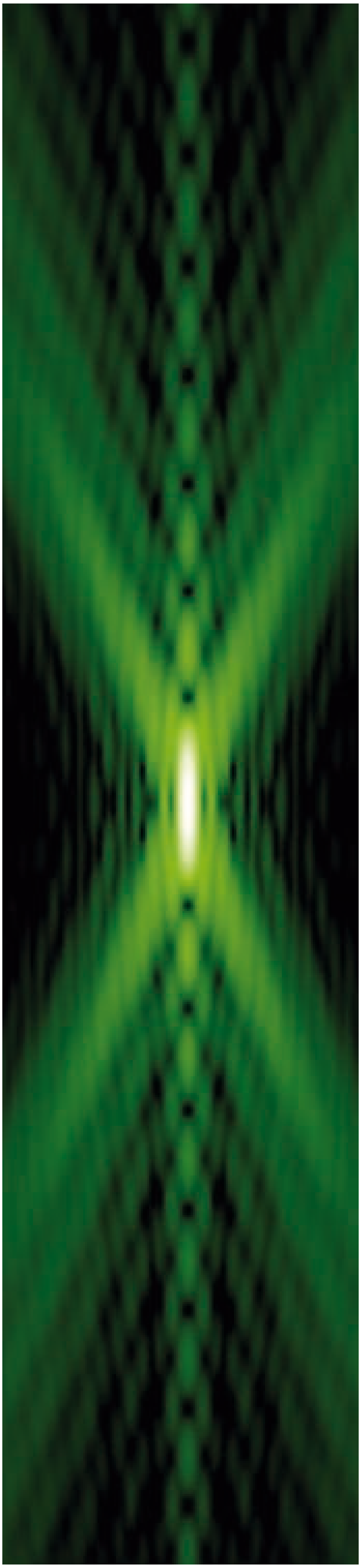


CENTRE OF EXCELLENCE FOR COHERENT XRAY SCIENCE TARGETS

New frontiers in imaging

La Trobe physicists and biologists and their partner-colleagues at the $17 million Australian Research Council Centre of Excellence for Coherent X-ray Science are collaborating with the world's leading beam-line scientists in a global race to develop novel, high-resolution X-ray techniques for imaging single protein molecules.

The Melbourne-based Centre is at the forefront of a close-knit, multi-national community of scientists fast-forwarding X-ray science towards new frontiers in the application of physics to biological, medical and environmental science.

They expect soon to cross thresholds as collaborators they might never otherwise have conquered, engaging a powerful combination of modern X-ray sources and algorithms to illuminate the sub-cellular architecture of the single protein molecule.

Centre of Excellence scientists from La Trobe, Monash, Melbourne and Swinburne universities and the CSIRO are working collaboratively with beam-line specialists in Japan, Taiwan, the United States and Europe to push third-generation X-ray sources to their limits in developing experimental new techniques to facilitate this.

Knowledge of some sub-cellular protein structures still elude science – and the pharmaceutical industry – despite all the advances of X-ray crystallography and other techniques. This is because it is impossible to make crystals for most proteins of interest involved in the human body, and X-ray crystallography requires the proteins to be in crystal form.

Intent on pushing the boundaries still further when X-ray Free Electron Lasers come on-stream – with X-ray light beams 10 orders of magnitude brighter than current Synchrotron sources – the Centre's scientists are developing imaging techniques they believe will take X-ray science beyond these limitations.

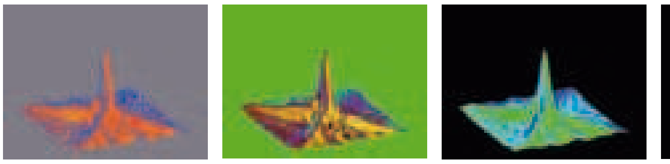
La Trobe physicists, in collaboration with US physicist Professor Chris Jacobsen from Stony Brook University, New York, have already brainstormed the conceptual approach for implementing this novel new imaging technique using existing light sources.

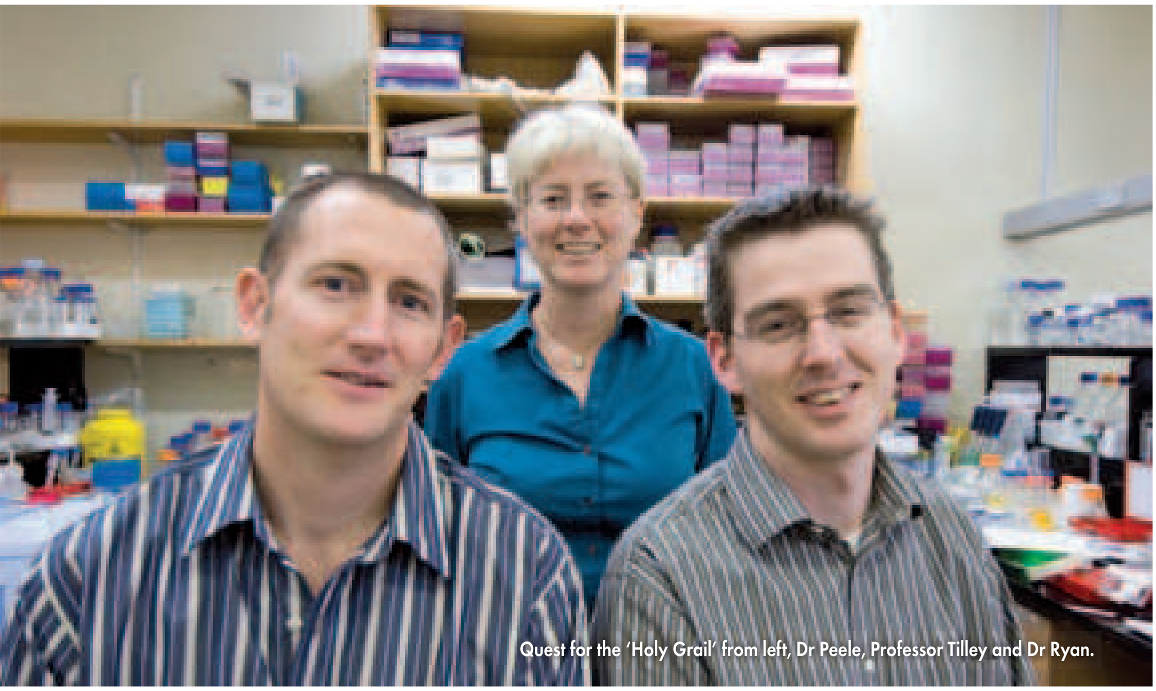
Head of the Centre's Experimental Group, La Trobe physicist and QE11 Research Fellow, Dr Andrew Peele, says: 'If it works we will have developed a mechanism for viewing the architecture and machinery of life, giving us a clearer understanding of cellular and sub-cellular function and the capability for improving rational drug design.'

By interlocking new techniques in X-ray physics, computational mathematics and structural biology, the scientists have developed a process they believe will help them to 'see' the physical and chemical structure of a single protein molecule by calculating backwards from a projected, finely-resolved three-dimensional 'image' of a protein cell sample.

The 'image' is actually a diffracted pattern of light projected onto a detector by shining a powerful X-ray beam through a cryogenically-frozen cell sample – a technique commonly used in crystallography to 'see' backwards to the internal structure of the original crystalline object.

The techniques of conventional crystallography use the knowledge that the diffraction pattern resulting from light scattered from a periodic (i.e. crystalline) structure makes it possible to deduce one of the critical features of the wavefield





Quest for the 'Holy Grail' from left, Dr Peele, Professor Tilley and Dr Ryan.

generated, that is, its 'phase.'

It is the knowledge of 'phase' in combination with another critical feature, 'intensity', that allows scientists to calculate back from the diffraction pattern to obtain information about the internal structure of the original object.

(If you think of 'intensity' as you might perceive the height of a water wave, and 'phase' as the position of the 'peaks and troughs', you won't be far wrong.)

When the diffraction pattern is produced by an object that is not 'periodic', the crystallographic method does not work, and scientists must find another way to determine the phase.

Physicists call this difficulty in obtaining the phase in the diffraction pattern 'the phase problem', describing 'solving for the phase' as 'the Holy Grail' of computational physics.

The Melbourne-based group says the crux of the matter is finding another piece of information about the sample, for instance that it is small and isolated, which allows them to design an algorithm to facilitate a 'guess the phase' technique, thereby enabling the same process for 'seeing backwards' that works in conventional crystallography.

They have developed a variant of the standard algorithm for doing this which involves illuminating the sample object with a pin-point of light instead of a parallel beam, resulting in the projected beam spreading out in a spherical diffraction pattern like a balloon, instead of a straight wave. Knowing the characteristics of the illuminating beam is another piece of information that helps determine the phase.

Initial experiments have proved successful and they are now constructing the hardware which will improve on the concept, including the phase-defining algorithm. Tests will be conducted initially using a Melbourne-based source of laser X-rays, and subsequently the highly coherent beamlines at overseas synchrotrons.

Dr Peele explains: 'This technique of taking the 'intensity' and hazarding a guess at the 'phase' and then doing this circular iteration is a standard method known as the Gerchberg Saxton algorithm.

'What we've been developing at the Centre is a variant on that idea, which says that instead of shining a parallel beam onto the sample and getting a characteristic type of diffraction pattern, we can take a beam that is structured – that is, focused down to a point and spreading out in a spherical way – in what is known as curved beam illumination.

'What that does is to change the nature of the diffraction pattern, and it gives us a better handle on the inversion technique. It makes the iterative process for determining the phase faster, it makes solution more robust to certain problems in the beam, and it allows you to reconstruct certain types of samples that you can't reconstruct using other methods.

'The down side is that it's a lot harder to do experimentally. With the traditional method you can put a sample in the beam and it's very forgiving of the sample moving slightly. With our method the sample has to be kept stable, possibly to the level of nanometers, and that's a very difficult engineering problem.

'It is a novel idea, and we're developing ways to improve it, but it's a difficult thing to design. That's where Chris Jacobsen comes in.' (See next page.)

PARTNERS IN THE CENTRE

ARC Centre of Excellence for Coherent X-ray Science groups involved in the experiment include the Theory and Modelling Group (led by Dr Harry Quiney from the University of Melbourne); the Experimental X-ray Science Group (led by Dr Peele, La Trobe); the Biochemistry and Structural Biology Group (led by La Trobe biochemists Professor Leann Tilley and Dr Mike Ryan); the Laser Physics and Technology Group (led by Professor Lap Van Dao, Swinburne University of Technology); and the Detectors & Instrumentation Group (led by Professor Rob Lewis, Monash University.)

Synchrotrons involved include the Advanced Photon Source Synchrotron in Chicago, the Spring 8 Synchrotron in Japan, the National Synchrotron Radiation Research Centre in Taiwan, and the European Synchrotron Research Facility in France. ●