Bringing electron microscopy to life

Just last year, the Australia-based Clive and Vera Ramaciotto Centre of Structural Cryo Electron Microscopy, Monash University, installed its FEI Titan Krios cryo-electron microscope. Centre leader, Professor James Whisstock, tells *Microscopy and Analysis* about his plans to use electron microscopy in his structural biology research

HOW IMPORTANT DO YOU THINK ELECTRON MICROSCOPY IS GOING BE TO STRUCTURAL BIOLOGY IN THE FUTURE?

X-ray crystallography is an extremely powerful technique for determining molecular structures down to the level of individual atoms, but requires the molecule to be crystallized. For some molecules this is easy, but for larger and often flexible biologically-important molecules and molecular complexes, this is problematic. So, this is where we see tremendous promise for cryo electron microscopy. New developments, particularly in detector technology, allow us to resolve some larger or highly symmetrical structures at close to atomic resolution. And even at lower resolution, electron microscopy allows us to visualise elements such as sheets and helices, as well as the position of individual subunits in a molecular complex. What's more, at lower electron microscopy resolution, using crystallography to interpret EM data is particularly powerful.

FOR STRUCTURAL BIOLOGISTS, SYNCHROTRON TIME IS IMPORTANT BUT EXPENSIVE; CAN ELECTRON MICROSCOPY HELP?

Synchrotron radiation is also instrumental in any structural biology program, and combining this with the promise of electron microscopy provides a very powerful suite of tools to address the science that we are interested in. However, the Australian Synchrotron is a $250 million facility and researchers compete closely for experimental time. So, while electron microscopes cost a few million dollars apiece, this is inexpensive compared to a synchrotron. Given this, we anticipate applying electron microscopy to the problems that once were regarded as "crystallography only" will change this dynamic with respect to structural biology programs.

WAS INSTALLING THE TITAN KRIOS STRAIGHTFORWARD?

The Titan Krios TEM can produce more than two terabytes of data a day, so we have done a lot of work around the computational side of things and have built systems to harvest and distribute the data once it comes off the microscope. We've also been consulting extensively with FEI and other Krios users around the world to better understand issues relating to this. We are dealing with very advanced instrumentation that is operating quite literally at the edge of our knowledge of physics. I can't remember exactly how many pieces go together in a Krios, but it's more than 80,000 individual components, and so these are not trivial instruments to install.

WILL YOU USE THE INSTRUMENT IN COMMERCIAL APPLICATIONS?

We first want to use the Krios to understand basic mechanisms for biologically-important problems. For example, how immune complexes assemble on the surface cell and how those complexes function with respect to telling a cell that it has to die. Or alternatively, how these processes are perverted by autoimmune diseases where the immune system attacks the host inappropriately.

We then aim to use this information to better control the immune response in disease, particularly immune-driven diseases. If we understand mechanisms, we can then try to understand and develop therapeutics to target a particular condition. And this may lead to commercial outcomes.

We also want to combine crystallography with the Krios to develop reagents, in particular antibodies. As part of this aim, we have a high throughput monoclonal antibody production facility, run by Professor Mark Sleeman, who came to Monash through a monoclonal antibody company, Regeneron. One of our visions is to use the Krios as part of a screening process for developing therapeutic monoclonals. For example, we may want to target one particular part of a molecule over another and trigger one particular type of activity. Getting antibody/antigen complexes of big wobbly systems is extremely difficult - the determining the structure of the insulin receptor was a 25 year effort. So we see the Krios giving us a key advantage in dealing with these heterogeneous complexes.

NUCLEAR MAGNETIC RESONANCE (NMR) HAS BEEN INCLUDED ALONG WITH CRYSTALLOGRAPHY AND EM FOR INTEGRATED MOLECULAR STRUCTURAL ANALYSIS. IS NMR STILL IMPORTANT IN THIS KIND OF RESEARCH?

The problem with NMR is twofold. First, it is difficult to use NMR to solve larger structures such as large proteins. Secondly, unlike crystallography, once you get the data there is no well-defined quick and easy Molecular-Replacement-style approach to derive the structure. Thus, in most cases where you can use crystallography you do, as the robotics and automated approaches associated with this approach are so much easier to apply.

But ironically, I am now doing more NMR than I have ever done in my life simply because the method is absolutely essential in drug screening. In fragment-based drug design you take a library of small molecules that have attractive - compatible drug development - properties. By putting your protein in an NMR tube and looking at the spectrum of the small molecule you can tell quickly whether they are interacting or not. This is one of the things we are

doing with many of our targets – 100's of milligrams of protein that used to go into crystallography screens now go into NMR tubes for fragment-based discovery.

IS USING NMR FOR FRAGMENT–BASED DISCOVERY IMPORTANT?

Yes. A lot of the targets we are interested in hitting are not low hanging fruit, and aren't nice, "easy" enzymes with big deep crevices. High-throughput screening approaches can fail here, as the surfaces that you are trying to drug are hard to target. Indeed, you can easily spend up to $300,000 on a high-throughput screen and end up with no small molecule hits to take forward.

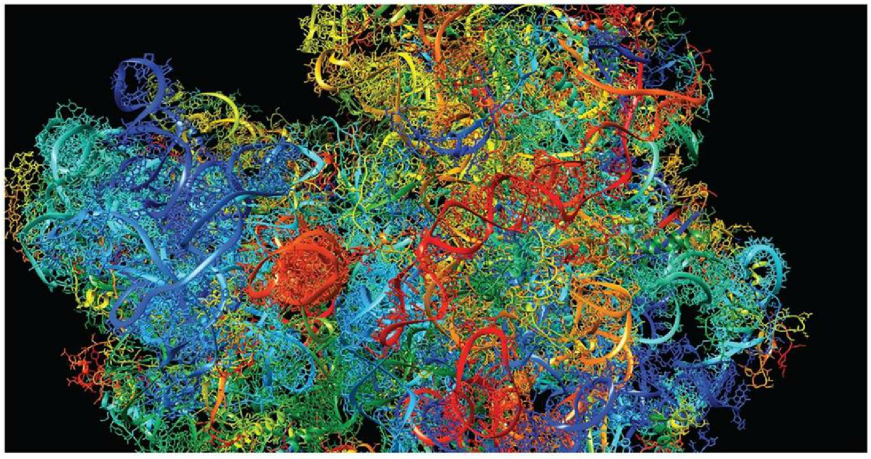
However, the beautiful thing about fragment-based discovery is that you should at least end up with a group of molecules that interact with your protein. You can then use surface plasmon resonance and crystallography to back up your results and elaborate your fragments into more drug-like molecules.

Given this, we have a 600 MHz NMR system here, with an auto changer, that is setup specifically for fragment-based discovery. NMR used to be about bigger and bigger instruments, but now I really feel that for most researchers, having a 1 GHz NMR is not especially useful, particularly given the running costs. NMR is still critical, but perhaps not in the way people once thought it was going to be.

WIIL THE ELECTRON MICROSCOPE BE USED FOR OTHER AREAS OF RESEARCH?

Yes. For example, we have a big structural immunology group led by Professor Jamie Rossjohn. He and colleagues are looking at how the handshake happens between an immune cell and a potential target.

We also have another large research group interested in DNA and RNA binding proteins, that is, transcriptional control, and this is where we see one of the big challenges for drug development. The nucleus is full of this incredibly complex machinery that makes sure the right gene is transcribed at the right time. However, understanding the control of such transcriptional events, and working out how to target specific events related to transcription is very difficult. I know my DNA and RNA colleagues are very excited about using electron microscopy to try and better target some of the machines that alter and transcribe DNA.



Ribosome structure determined by Mazdak Radjainia in the Monash Ramaciotti Centre for Structural Cryo-EM.

We also have several groups that are interested in transport, how bacteria transport [molecules] across membranes. Antibiotic resistant bacteria has become a major problem, and once again electron microscopy jumps out as the technique to use.

For example the ribosome, which is a target for a number of antibiotics, is very difficult from a crystallographic perspective. However, looking at a number of recently published papers, many researchers have used the Titan Krios to study the ribosome. So, here you can start to see the value of the high-throughput afforded by the Krios TEM.

SO WHY CHOOSE THE TITAN KRIOS?

One key reason was automation; the crystallography field has become kind of reliant on automation. We want an instrument that can capture data over long periods of time. Thus, it was clear when we were looking at the available instrumentation that traditional electron microscopy, though powerful, was in no way high throughput.

[In contrast] the Krios can collect data over one week running in fully automated mode - just capturing images - and has the computational approach to filter that data and process it. All this was very attractive to us because we could then see a reliable, predictable way to derive structures. As crystallographers, we are more comfortable using that kind of tool.

WHAT DO YOU THINK THE FUTURE HOLDS FOR ELECTRON MICROSCOPY IN YOUR CENTRE?

These are exciting times. I can't tell you how thrilled we are to be moving to use electron microscopy in our research. We have also seen what electron microscopy has done for the materials science community; we actually have an exceptional materials science centre that has a Titan and wonderful discoveries are coming out of there. We see electron microscopy as having similar impact in our field.

We are only at the beginning, anticipate growing our electron microscopy work here to a significant size, and expect additional investment in infrastructure and instrumentation so we continue to grow and thrive. We hope to add a cryo DualBeam - combined focused ion beam and scanning electron microscope - to our lab. This with our Titan Krios TEM will allow us to do even more interesting things.

Meet James Whisstock

James Whisstock is based at Monash University in Melbourne, Australia. He is a professor in the Department of Biochemistry and Molecular Biology at the university's School of Biomedical Sciences. He is also Senior Principal Research Fellow of the National Health and Medical Research Council.
His research interests include the structure, function and biology of medically-important proteins, particularly proteases, protease inhibitors and the perforin-like superfamily of pore forming toxins. From a disease perspective, his team focuses on infection, immunity, clotting diseases and cancer.