Mapping structure on the molecular highway

Image credit: "Dan Mao, Lunenfeld-Tanenbaum Research Institute
Lab website

"I'm a committed structural biologist," says Dr Elton Zeqiraj. "I like to identify structures and the mechanisms of how large molecular machines come together to operate in a cell. I'm interested in cell signalling and how molecules in a cell pass or transmit information from one part to another and so regulate events that are important for cellular growth, proliferation and eventually pretty much every aspect of the cell's life."

Dr Elton Zeqiraj is about to start his own lab as a Principal Investigator at the University of Leeds backed by a £1.1 million Sir Henry Dale Fellowship award from the Wellcome Trust.

"There are hundreds if not thousands of cellular pathways. Imagine them as highways or motorways that are interconnected in a large network communicating with each other. Even though it's complex and there are thousands of them, only about ten or fifteen of the highways are actually relevant in terms of disease. By studying them, you can learn a lot about how cellular life is regulated and you can get an idea about what you could do to reverse disease phenotypes in a patient. Add in detailed structural information and it can be a powerful tool to help design therapeutics and understand how disease arises."

Of the many processes involved in cell signalling, like most alumni of the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit at the University of Dundee (PPU), Dr. Zeqiraj's career has traced the action of proteins that add or remove phosphate or ubiquitin molecules to pass information on.

Deciphering the unexpected

As a PhD student co-supervised by Professors Dario Alessi and Daan van Aalten, he explored how protein kinases can be pushed into action in an unusual way. It's called allosteric activation. It means activating an enzyme through interactions only, so without passing it a phosphate.

"Many people are familiar with the idea that a kinase can phosphorylate and activate another kinase to pass a signal along. Rather unusually when I was in Dundee there was a kinase called LKB1 that didn't require this, it was somewhat different to the rest of the 500 or so kinases," says Dr. Zeqiraj.

"Professor Dario Alessi's team had already discovered that LKB1 needed another kinase called STRAD to activate it. But STRAD simply interacted with LKB1. It didn't pass it a phosphate like a kinase would normally do. There was no chemical transfer, it just stuck to it forming a complex, always together. Then we discovered a third protein called MO25 that kind of stitches the two together. "

When Dr. Zeqiraj started on the project the details of how STRAD activated LKB1 were missing, he says: "There was no precedent to follow in terms of mechanism from other

people or other examples. It was a new phenomenon, we knew it happened but not how it was done. So that was the aim of my PhD project, to figure out how you can activate a kinase without phosphorylating it."

Another PhD student, Dr Christine Milburn, had already made progress on understanding how MO25 recognises STRAD, so part of one of the components was becoming clear. Dr. Zeqiraj's challenge was to get the full crystal structure of the three molecules in the complex together. He did it.

"I crystallised all three of them together and that was essentially the work of my PhD," he explains. "A few key things came out of that. The first was that STRAD is what we call a pseudokinase – it has lost the ability to transfer a phosphate through evolution. It looks like it has the same shape as a kinase but it cannot transfer a phosphate. What was really surprising, and something that we never expected, was that STRAD can interact in a way that a kinase would usually interact with a substrate then stop there."

"In hindsight it actually makes sense because kinases will often carry out a reaction and then let go. The whole point of this was that STRAD has to stick to LKB1 to carry out its function and of course it can only stay there if no phosphorylation takes place. So it needs to be a dead enzyme, or pseudokinase, or the whole thing will fall apart."

Dr. Zeqiraj describes what happens if the genes that control STRAD are altered: "If you don't have functional STRAD you have problems. There are some mutations that truncate STRAD so that the bottom half is missing. Children are born with megalencephaly, a larger than usual head, they suffer severe epileptic seizures and sadly don't get to live a full life. There's a Mennonite community in Pennsylvania where this was identified and it turns out that these patients, are missing part of the STRAD gene and when that happens you basically get this severe malformation of the skull, so STRAD is really important."

On completion of his PhD Dr. Zeqiraj sought a new challenge. Together with his wife Dr Beatrice Maria Filippi, who is also PPU alumni and will feature later in the year, he moved to Toronto in 2009 to experience the North American research culture.

Scientist's suitcase

As he was preparing to leave colleague Professor Kei Sakamoto gave him a brilliant leaving gift – the opportunity to solve another structural puzzle. And so began his postdoctoral fellowship at the Lunenfeld–Tanenbaum Research Institute.

"As I was leaving, writing up and packing my bags Kei came to me and said we have this really interesting project on glycogen synthase. Would you like to take it with you? My supervisor in Toronto agreed to it. It turned out to be a really interesting study even though it's more or less a 50-year-old question. People are still trying to understand how glycogen is made. Glycogen is the main storage of glucose in liver and muscle cells so it's an extremely important molecule," says Dr. Zeqiraj.

“In the 1990’s, two great biochemists, Professor Bill Whelan at the University of Miami and Professor Philip Cohen in Dundee, described how the enzymes, glycogen and glycogen synthase start the process. But what wasn’t known is how they interact and come together. With Kei, I managed to get a crystal structure of the two proteins in action and see how that process begins. It is still a very interesting project.”

It was always Dr. Zeqiraj and Dr. Filippi’s intention to return to Europe, to the UK. People living in the UK voted to leave the European Union the weekend they were packing to return after nearly seven years of post-doctoral research in Toronto.

Brexit blow

“It has been a bit of a shock,” Dr. Zeqiraj says, “I can only hope that when the dust settles and everyone starts thinking with a cooler head that things will not be as bad as we think they might be in science. We will lose a lot of our European funding. British science was doing rather well with European funding. I seriously do not think any future government is going to bridge the funding gap.”

“Money aside, I think the bigger damage is that which the vote has done to our community. It has made our European colleagues and collaborators from around the world feel unwelcome in Britain and that is not good. It has an impact, one that cannot be measured or easily quantified, but one that is devastating non the less,” he says.

“It is such a shame because the vote has created barriers. Britain is so open minded and such a great country and to have these kind of conversations going on in the community, not just the scientific community but around the country, it’s something I never thought I would see.”

Picture perfect structures

Despite the uncertain funding future Dr. Zeqiraj remains optimistic. He is excited by the research opportunities he will be able to create at the University of Leeds thanks to the University’s early investment in advanced microscopy techniques.

“At Leeds, they recognised three or four years ago that electron microscopy would become the method of choice to visualise protein structures,” says Dr. Zeqiraj. “So far X-ray crystallography has been the best available tool, and it still works well, but in the past five years, electron microscopy has become the method of choice. It’s amazing how fast things have improved - both instruments and the software for analysing data. This has helped create exponential progress in identifying the molecular details of protein – protein interactions and protein structures.”

When Dr. Zeqiraj starts his new lab in August he will have access to two of the latest electron microscopy instruments. This will enable him to explore how molecules relate to one another when they form linked structures called protein assemblies. Dr. Zeqiraj plans to use both X-ray crystallography and cryo-electron microscopy, a technique that freezes

samples and visualises individual molecules in unprecedented detail, to learn how components in assemblies relate to one another.

“Leeds looks like it is going to be an excellent place for the research I plan to do,” he says. “Right now I’ve moved a little further away from phosphorylation and have taken up ubiquitin signalling.” Though he is sticking with the theme of pseudokinases and how they activate real kinases.

Hidden abilities

“Pretty much every enzyme family has a few rogue ones that look like they do nothing or like they won’t carry out a catalytic action because they are missing certain residues so they have been ignored,” Dr. Zeqiraj says. “But the pseudokinase field has shown that they are really important and when they are mutated in disease can cause huge problems.”

During his postdoc it became clear that there are many layers of interaction involved in allosteric activation of ubiquitin processing enzymes that just couldn’t be imagined without having revealed the structures of molecules as they interact.

This has significance in the search for future drug targets, Dr. Zeqiraj says: “Both the pharmaceutical industry and academia have been mainly targeting active sites of enzymes with drugs. That has advantages but creates its own challenges as it can risk cross reactivity. Trying to inhibit a large enzyme complex is not easy but it creates opportunities, for example if you can target an allosteric pocket unique to that complex, you could make a drug more specific. This is where our expertise in structural biology comes in. Protein complexes can be so similar but do different things. If you have detailed structural information it can be a very powerful tool.”

“There are so many components of molecular complexes that are still unknown. What I hope to do in Leeds is define more of the structures I have an interest in. You only need to intervene in a few different cell signalling pathways to create a therapeutic benefit for a patient. I think the ubiquitin signalling field is going to reveal new drug targets in the future. It’s a huge part of what I plan to do in Leeds,” he says.

In his new post, Dr. Zeqiraj will lead a lab for the first time. He plans to draw on his experiences of the research culture in Dundee and Toronto and try to put the best of both into practice as a supervisor.

Build your network

Dr. Zeqiraj’s advice for PhD students and postdocs today is to remember that both are endeavours in research training. “The majority of the time you have to be doing or thinking about experiments and that’s really important, but the other major part of being a PhD student or postdoc is being open minded and getting input from other people. You really have to go to as many poster sessions or presentations as you can and talk to people when you are there.”

One of the things he most enjoyed about Dundee was the collaborative atmosphere in the unit. “I knew pretty much what everyone in the unit was doing and they knew what I was doing. People in Dundee go out of their way to encourage this kind of cross talk and networking and I think this is a great thing. I found it happens less so in Toronto. At PPU senior scientists would make a point of going to events where research information was shared and this encouraged everyone else to go. I found it very useful,” Dr. Zeqiraj says.

He has also learnt that it is crucial to pause and take time to think about research problems. “Downtime is important if you want to be effective,” he says. “If things are working really well be happy about it and keep working hard. If not and you are getting frustrated, pause a little bit more. If you don’t then you risk repeating the same mistakes over and over again.”

The skills of a rigorously trained scientist are in demand in many disciplines and Dr. Zeqiraj acknowledges that not everyone who studies for a PhD will make it as a Principal Investigator, he says: “As I progressed through my postdoc it became more obvious to me that it’s hard for everyone who graduates from a PhD to end up having a career in academia. I think PhD students need to have an open mind about moving to, or being trained for, industry, or even moving outside of science. I know people that have done both with huge success. I applied to both industry and academia until I secured the post in Leeds. In science, one of the biggest challenges can be getting other people to see and understand your point of view. It’s a skill that if you learn to do well will be useful wherever you go.”

Dr Zeqiraj is keen to get start building his new lab. His research is driven by curiosity but the challenges of human health problems tug at the edges. “There are big issues facing science,” he says. “How to find solutions to disease, how to tackle antibiotic resistance but also how to keep basic research alive. It is difficult for one team to do both. It is a huge challenge for academics as we move forward.”

Collaborating with industry in ways that see both partners get a good deal is another area he sees as a both a challenge and an opportunity. He cites the Division of Signal Transduction Therapy, PPU’s collaboration with industry as a a model he hopes other institutions will adopt in the future.

Make the most of your time

“We sometimes forget that science is a hard endeavour, one that gives few rewards and sometimes the rewards aren’t that obvious in the short term, so it’s important that you enjoy yourself. You have to celebrate when things are going well and know that things will somehow work out in the end.”

“I spoke at a meeting in Miami last year, it was the 90th birthday of Professor Bill Whelan the biochemist who discovered glycogenin and how it builds glycogen. Professor Philip Cohen and Professor Edmond Fischer gave talks, some of my collaborators from Spain were there, people who had worked on these problems for 30 or 40 years. It was excellent. And I realised how much dedication these people have put into their work – there was a story about Bill Whelan selling his pension bonds to fund his lab. It just shows you how dedicated you have to be to get something done.”

Final Word

"In science it's really difficult to predict what's going to happen and dangerous to even try. It's one of the great things about it because you never know where a research question will take you. Ten years from now, I hope I still have interesting problems to solve and that I still have friends I can talk to who are excited about what I do, and that I am equally fascinated by what they do," says Dr. Elton Zeqiraj.

Career Highlights

2016 Sir Henry Dale Fellowship, University of Leeds, Leeds, UK

2014 TD Bank Postdoctoral fellowship, *Mount Sinai Hospital Foundation, Toronto, Canada*

2010 Sir Henry Wellcome Postdoctoral Fellowship

2009 Howard Elder Prize for Cancer Research, University of Dundee, Scotland
Awarded annually to a PhD student or postdoctoral researcher in the College of Life Sciences, Dundee, deemed to have published the most significant paper in an area related to cancer research.

2005 Raymond Whittle prize, University of Westminster, London, UK
Awarded annually to an undergraduate student in the Science division who achieves the highest level of performance in experimental work.

2005 Leica-Bitplane Prize, University of Westminster, London, UK
Awarded to an undergraduate/postgraduate student that produces the best research project using confocal imaging techniques.

Top publications

Dr Elton Zeqiraj picks his top three papers and tells us why he has chosen each one.

Higher order assembly of BRCC36-KIAA0157 is required for DUB activity and biological function.
Molecular Cell, 59: 970-983, Sept 2015
E. Zeqiraj, T. Lei, C. Pigott, M. Pillon, N. M. Duffy, D. F. Ceccarelli, A. F. Keszei, K. Lorenzen, I. Kurinov, S. Orlicky, G. D. Gish, A. J. R. Heck, Alba A. Guarné, R. Greenberg and F. Sicheri.

"This paper describes the structure of BRCC36 deubiquitinating (DUB) enzyme and provides the first example of how a pseudo-DUB allosterically activates a real DUB. Pseudo-DUBs, like pseudokinases are likely to be the underappreciated, yet fascinating members of the DUB family."

Structural Basis for the Recruitment of Glycogen Synthase by Glycogenin.
PNAS, 15;111(28):E2831-40, Jun 2014

E. Zeqiraj, J. Tang, R. W. Hunter, M. Garcia-Rocha, A. Judd, M. Deak, A. von Wilamowitz-Moellendorff, I. Kurinov, J. J. Guinovart, M. Tyers, K. Sakamoto and F. Sicheri.

"Glycogenin and glycogen synthase interact together to kickstart the process of making the glucose polymer glycogen. This paper describes the structural features of that interaction and we are beginning to picture the molecular details of how glycogen synthesis begins."

Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation.
Science, 326(5960):1707–1711, Dec 2009.
E. Zeqiraj, B. M. Filippi, M. Deak, D. R. Alessi, and D. M. van Aalten.

"There are around 50 presumed pseudokinases and with each elucidated structure there is a new and surprising story. This paper describes the crystal structure of the LKB1-STRAD-MO25 kinase complex and provides the first example of how a pseudokinase activates a real kinase."

Lab website

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