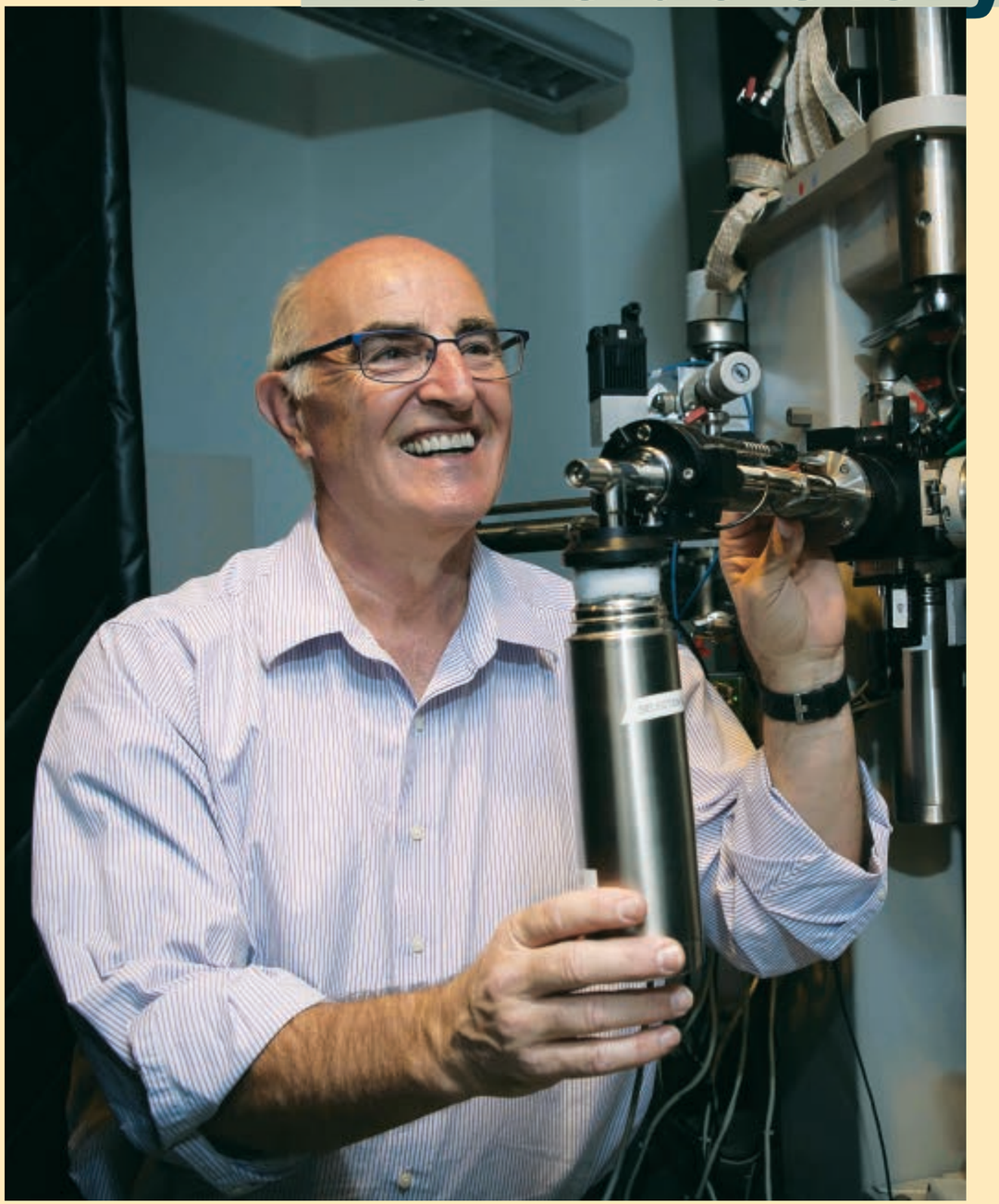
# The Inside Story



## A Q&A with Alasdair McDowall, cryo-electron microscopist

Alasdair McDowall has been working in the field of electron microscopy for 45 years, starting at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, and in 2008 joining the Caltech laboratory of Grant Jensen, professor of biophysics and biology and a Howard Hughes Medical Institute Investigator. He was on the scientific front lines when Jacques Dubochet—one of the three scientists awarded the 2017 Nobel Prize in Chemistry "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"—was establishing how best to keep cells in an electron microscope hydrated, given that the scope's vacuum evaporates liquid. The solution? Vitrification, the cooling of water so rapidly that it doesn't create cell-and-organelle-destroying crystals.

McDowall played a key role in optimizing the process of vitrification, which made cryo-electron microscopy (cryo-EM) possible. McDowall was first author on several of the early papers from the Dubochet lab that the Nobel Prize recognized and was considered so integral to this work that Dubochet invited McDowall and his wife, Leta, to attend last year's Nobel ceremonies: Dubochet also gave McDowall one of the three replica medals offered to each Nobelist.

*Caltech* magazine caught up with McDowall just before his trip to Stockholm, Sweden, to talk about the evolution of cryo-EM, the next challenges researchers in that field will face, and how he, Jensen, and their colleagues are working to bring cryo-EM to the next level.

**Caltech Magazine [CM]:** Tell us about how cryo-EM evolved.

**Alasdair McDowall [AM]:** The history of cryo-EM more or less started for me back in the early '80s. I grew up in Scotland, and did my undergrad work in Edinburgh. I was introduced to electron microscopy there at a young age. I became interested in it, and then after my master's, I accepted a position at the EMBL.

They were just setting up, and their focus was driven by the director at the time, John Kendrew, who had won the Nobel Prize for the structure of myoglobin. His goal was to bring in groups that could look at structures in their more realistic native state.

They invested a lot in getting specific lenses and microscopes that would better protect a sample. The vacuum of an electron microscope is very alien for biological samples. We don't live in a vacuum, so putting biological samples into a vacuum is not natural. That's the way we have to look at them with electrons, but they don't like going in that environment, so we have to protect them. That's what one of these new instruments that Kendrew was building was hopefully going to help us do.

I was there in the background, trying to prepare better samples from biological materials that would work in this new superconducting helium-cooled lens electron microscope. Up to that point, from the '30s to the '80s, everything was dry when it went into the electron microscope. It was dead. It was pickled. It was cut. It was cooked. We wanted to see more subtle things in the microscope, and so we had to think of better ways of saving this liquid that we all live in.

That's what the goal was, and that's where the big quantum leap came. We managed to take cells or parts of cells and immobilize them by freezing them very fast. It was

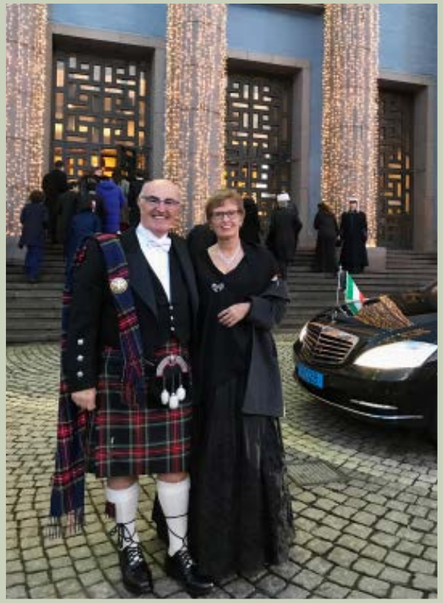
believed by the physicists and the theorists that vitrification was impossible at ambient pressures. Generally, when you cool water, it will form crystals, and that will just damage everything.

**CM:** So, by freezing the water quickly, you preserve its structure in solid amorphous water.

**AM:** That's right. The water molecule doesn't have the chance to arrange itself into a crystal. The minute you freeze something successfully in that state, it's stable, and life in that state looks very real. It looks as if it was still in the biological system outside.

This was a big jump, being successful in getting that to work. Many others were trying other things with metal salts or sucrose to support structures, and getting close, but nobody had done the vitrification of cells for electron microscopy until we did it at Heidelberg.

Then the research took off for the next 10 years, and the Dubochet team worked on that, and we had lots of success and trained a lot of people. Many of the group leaders who are working in the field now came to that lab to learn how to do the work, including Richard Henderson, one of the three 2017 Nobel laureates in chemistry.



Center: Alasdair and Leta McDowall attending the Nobel Prize ceremonies at the Stockholm Concert Hall in December 2017.

**CM:** When did you work with Jacques Dubochet?

**AM:** I joined the Dubochet group in 1978, and I worked with him in Heidelberg for 10 years. I became an assistant professor at UTSW [University of Texas Southwest] Dallas, and then I went to a director professorship in Brisbane, Australia. Jacques came out and did a sabbatical with me there. We collaborated, and we published a bit more throughout the years.

**CM:** Why was achieving vitrification so important?

**AM:** Because the cells go into the microscope vacuum dry, you had to somehow support the sample. You just can't remove the liquid and expect to see the structure as it is. It's like seeing a sun-dried tomato and a vine tomato. They are totally different.

We struggled with making vitrification work. We were using all sorts of different cryogens, cold liquids. Nitrogen is one obvious one. When it's liquid, it's very cold, but it doesn't remove the heat fast enough. This is where the success came in the early '80s, when I tried different cryogens and eventually found one or two that could cool the sample in a freezing action much faster than liquid nitrogen. We now use liquid ethane, propane. These hydrocarbons are much more effective in removing the heat before the water can crystallize.

**CM:** What are some of the other challenges?

**AM:** One other challenge is that the beam is like a nuclear reaction on the biological samples. Really, it's like a nuclear bomb going off inside a cell when a beam goes in there. It's burning and cooking the cell. Yes, we got a prep that was well frozen, but now we have to find machines in which we can control the beam dose, the dose of the number of electrons that are bombarding the sample. We have to record enough electrons just to sensitize the camera but not to boil the sample.

Stability is another challenge. You cannot afford to have anything drifting around or moving during a few-microsecond exposure or something like that.

**CM:** What kind of things do you think researchers will be able to look at using cryo-EM?

**AM:** There is a huge explosion of information coming out. Structures are being solved so rapidly now as opposed to even just five years ago. And medicine will be helped along the way by understanding what's happening inside the cell or nucleus when it's dividing and replicating and doing all sorts of its operations.

The Jensen Lab has been working for the last 10 years on bacteria and viruses, and some of the bacteria that cause problems in the Third World in terms of health and disease. Cryo-EM is opening up this window inside the cells that we never knew existed.

We're trying to also correlate the information we obtain from cryo-EM with what we see with the other microscopies—fluorescence and light microscopy, that is—with which you can look at a live cell. This is called correlative light electron microscopy. That's one very strong area.

**CM:** What about the microscopes themselves? How are they improving?

**AM:** The environment that the microscope is in is being controlled better. Now, if you go over and look at the new microscopes in Caltech's Beckman Institute, they put them in their own cabinet, so you don't see the microscope anymore. You operate outside the room, and the human body heat, the human noise, the human airflow is taken out of the equation. Those advances, together with better stability, cameras, and computers have made the new microscope much better. It is more expensive, of course.

**CM:** When did Caltech get its first cryo-EM?

**AM:** I think the Jensen group was formed around 2003.

**CM:** Pretty recently then.

**AM:** Yes. They've been here 15 years. The microscope arrived soon after that in the form of this 300 kV Polara, still an excellent workhorse cryo-electron microscope. They bought two under the Moore Foundation and Agouron Institute grants. Then the new microscopes just arrived a couple of months ago.

**CM:** It must be exciting seeing the cryo-EM field getting so much recognition now after the Nobel Prize.

**AM:** There is a lot of gratification, because the field struggled for quite a while to get going. Now there's this tsunami of information coming out from the data from so many labs. I was just on the phone this morning with the salespeople who sell the microscopes, who said they just can't keep up. The instruments are not cheap. They're $10-million-plus, each one. Labs are desperately trying to get hold of these machines.

Grant Jensen's group here actually realized that there aren't enough people to train those who want to use his microscope, so Grant is putting a lot of his effort into teaching now and very successfully making YouTube videos of how to actually prepare samples, to drive the microscopes. He's redoing them and creating more advanced ones for the new microscopes this year.

We spent many years training people in our little institutes, wherever we were, in groups of 10 or 15 or 20, and that's just never going to work for the hundreds of people who need to know how to get into this field now. The YouTube videos are hands-on, very specific, detailed. A beginner can learn how to do it.

**CM:** That is great.

**AM:** That's what the goal is now, to get everybody educated. It's exciting times.

# In Memoriam

Read more about their lives at **magazine.caltech.edu/post/in-memoriam**

## Jerry Pine 1928–2017

Jerome "Jerry" Pine, a Caltech professor of physics, emeritus, passed away on November 8. He was 89 years old. Pine served as a professor at Caltech for more than 50 years. In his early career, he undertook research in particle physics at several particle colliders, improving our understanding of the structure of elementary particles. Later, he transitioned into biophysics, developing new ways to study and visualize living neural cells. Pine was also passionate about science education.

## J. N. Franklin 1930–2017

Joel (J. N.) Franklin, who taught mathematics at Caltech for nearly a half century, passed away on November 18 at the age of 87. Franklin joined Caltech in 1957 and worked closely with Gilbert McCann, professor of applied science, who was one of the early champions of computing at Caltech. Franklin was the author of textbooks on methods of mathematical economics and matrix theory, and was the recipient of Associated Students of the California Institute of Technology (ASCIT) Teaching Awards for the 1977–78 and 1979–80 academic years.

## Kevin Austin 1953–2017

Kevin Austin, longtime director of Caltech's health and counseling services, died on November 4. He was 64. Austin, who retired from Caltech in December 2015 after more than 25 years of service as a therapist and administrator, worked with countless students, faculty members, and student-affairs professionals during his tenure at Caltech.

## Joseph Polchinski 1954–2018

Joseph Polchinski (BS '75), the Pat and Joe Yzurdiaga Professor of Theoretical Physics, Emeritus, at UC Santa Barbara, passed away on February 2. Polchinski was perhaps best known for his discovery of D-branes in string theory. In 2017, with two other physicists, he won the prestigious Breakthrough Prize in Fundamental Physics.