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REVIEW

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The Nobel Prize Factory

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Nobel Prize Laureate Richard Henderson introduces structural biology and electron cryo-microscopy, and talks about the successful journey of the MRC Laboratory of Molecular Biology. See video at https://youtu.be/3D7m6qXRpzA

Mejd Alsari (MA). Richard, you have shared the 2017 Chemistry Nobel Prize with Jacques Dubochet and Joachim Frank *for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution*,[1] can you briefly explain what is structural biology?

Richard Henderson (RH). Structural biology started in 1926-1927 here in Cambridge in the physics department. William Astbury[2] and John Desmond Bernal[3] were young scientists who decided to begin by shining X-ray beams at biological structures, either crystals of biological molecules or fibrous structures.[4] They sort of kicked the field off and that's how structural biology started.

It was defined then as - it has grown of course - the study of the structure of all the molecules in biology using various methods, initially X-ray crystallography but then other methods like electron microscopy and nuclear magnetic resonance (NMR) spectroscopy have come in. These technical methods are used to dissect and analyse the structures so that you can then understand what it is that makes the whole of biology tick because of the molecules that are underlying all the processes. That's the core of structural biology.

The first time structural biology was used as a term was about 1960, when Donald Caspar[5] created a department and put 'Structural Biology' on the door.[6] Now we all say we are structural biologists because it's been a very successful method. There's now hundreds of thousands of structures and thousands of people doing structural biology.

MA. What is cryo-EM in a nutshell?

RH. Of the three principal methods in structural biology, electron microscopy itself started in about 1930 when it was realized you could focus electrons and make images just like you do with light: with a lens.[7,8] The first lenses were developed in the 1930s. The difficulty with electron microscopy is that electrons only pass through a vacuum.[9] If you have them in air they scatter and then you can't image them. They get scattered and then the image would be very blurry.

In the early stages of electron microscopy people could only look at structures that had heavy metal stains on them, such as uranium, platinum, etc.[10] The early progress of electron microscopy was all in material science. It wasn't until later that people started looking at biological structures. The problem with biological structures is that they are made up of organic molecules, carbonaceous material. If you shine a beam of electrons on them they get damaged, bonds break, they get ionized. You get radiation damage and they fall apart. All the early work in structural biology was done with either metal shadowing[11] or negative stain[12].

It was realized that if you could freeze the specimens down to a very low temperature so that the atoms wouldn't move, the structures would be preserved. You can freeze structures and then you can thaw them out and the enzymes are still alive, cells are still alive. The development of electron cryo(genic) microscopy (cryo-EM) didn't start until about 1980, when methods were developed by Jacques Dubochet, one of those who shared the 2017 Nobel Prize in Chemistry.[13] He developed ways of freezing them. What you do then is you make a thin film of your biological molecules embedded in amorphous ice and then you put the electron beam through it, collect it with a lens, and make images.[14] You're just magnifying images of the biological molecules without any use of metal stains. You're looking at the molecules, at the atoms (carbon, nitrogen, oxygen, hydrogen, etc.) that make up the biological molecules and then you get an image just like if you take a photograph of us with light. You take a photograph of the molecules with electrons and then you can see them. They've just been magnified a million times.

Figure 1 | Richard Henderson, group leader at the MRC Laboratory of Molecular Biology, Cambridge..

MA. An outsider to the field would normally ask are you doing scanning electron microscopy (SEM) or transmission electron microscopy (TEM)? Why do you call it cryo-EM instead of cryo-TEM?

RH. Originally the best way of using the electrons to make the images to see what you're interested in wasn't clear. The difference between what they call transmission electron microscopy (TEM) and scanning electron microscopy (SEM) is that in TEM the electrons go all the way through the sample, they get scattered, and then you image. The molecules also don't absorb electrons but scatter them. They alter the phases of the electron waves. You're imaging essentially by looking through a transparent object. The biological molecules are transparent, but you get the modulation of the electron beam, the phase contrast.[9]

Whereas with SEM you put the electrons on the specimen and you look at the ones that are back-scattered.[15] You can look at a solid object.

So one of them is transmitted and the other one is reflected. The different characteristics of SEM and TEM mean that SEM is good for sort of big objects. You don't get very high resolution and you have to cover the sample usually with metal.

In structural biology, SEM is used as a sort of diagnostic tool but it's not very powerful. With transmission electron microscopy you can go in principle to very high resolution, get very detailed pictures

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of all the atoms. So in our field basically SEM is not a strong candidate, it doesn't really exist.

We just say we are electron microscopists or, better, we like to call ourselves structural biologists because then that doesn't mean that you have to say "I am doing X-ray crystallography". Now most structural biologists can go back and forward between all the different methods.

One big debate is whether we should, when we have the specimen cold at liquid nitrogen temperature, call it *electron cryo-microscopy* or, some people say, *cryo-electron microscopy*. You're just saying "it's cold", but it's the specimen that's cold, the electrons are actually hot, they come out at ~2000 °C. So we prefer electron cryo-microscopy.[16]

MA. You have been working at the (MRC) LMB[17] since the 1970s, can you tell us what makes this place unique?

RH. When I was an undergraduate in Edinburgh doing physics I looked into where physics was going. From all of the different types of physics, particle physics, solid-state physics, fusion research to produce unlimited power and so on, I thought biophysics might be something that you wouldn't need a team of a thousand people. You know, for the gravitational wave discoveries recently they needed a thousand people all over the world.[18] In biophysics you could do work with yourself or with one or two people. So I thought that was a good idea.

I looked around in the UK, I didn't want to go abroad and hadn't quite decided. I went to talk to the physics professor in Edinburgh, Bill Cochran. He said "I don't think you should go to Norwich or London or Oxford's and you should write to Max Perutz". Max Perutz was the first director of the MRC Laboratory of Molecular Biology.[19]

When I came here in 1966, it was on a Saturday and everyone was working. I thought "This is marvellous!".

Then I went away for a postdoc and came back in 1973.

I keep looking around for good labs, but I still haven't found a better one.

The LMB had been founded in 1960 by the merger of the biophysics group from the Cavendish, which was Francis Crick, John Kendrew, Max Perutz and so on. [19] The more biochemically oriented group from the biochemistry partnership Fred Sanger, Brian Hartley, Ieuan Harris and so on.[20] So there was a merger of two groups from two different Cambridge University departments, who didn't like the molecular biologists. They wanted them to go. So they said "please leave". They got money from the MRC, built a new building, and then recruited people also from London such as Aaron Klug.

It was a new lab created from people who had a clear research plan, a clear vision. It was a very good lab in the 60s. Many people predicted it would just die out with the second generation. Obviously the first generation was great. Six months after the lab opened they had four Nobel Prize winners in 1962.[21] The critics said "The second generation will be less talented scientists than the founders, and then the third generation will be even worse, eventually it will just disappear".

Actually the opposite has happened. What has happened is that the founders attracted good people and it's continued. It went from sort of 70 or 80 people up to about 500-600 people. It's really quite productive. The lab really gets very good students, postdocs, and young group leaders now and it's not necessarily because they're recruited. It's because they have noticed the lab, they've identified it, and they think this would be a good place for them to work, just like it happened for me, that's 53 years ago for me.

I'm kind of trying to retire now. The younger ones are obviously working harder and they're full of good ideas. There's still a long way to go. It's a medical research council lab. We're supposed to have our eye on medical research, often really important revolutionary treatments for, you know, say human welfare or in agriculture and so on. You often come not because you're working on liver cancer, you're working on some basic biology and you don't realize it will have an impact on a much wider area. I think a lot of the work in the lab is basic biology that develops completely unknown ideas that do revolutionary improvements in health and wealth rather than evolutionary improvements. That still seems to be happening.

Figure 2 | MRC Laboratory of Molecular Biology, Cambridge.

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Conflicts of Interest
There are no conflicts to declare.

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