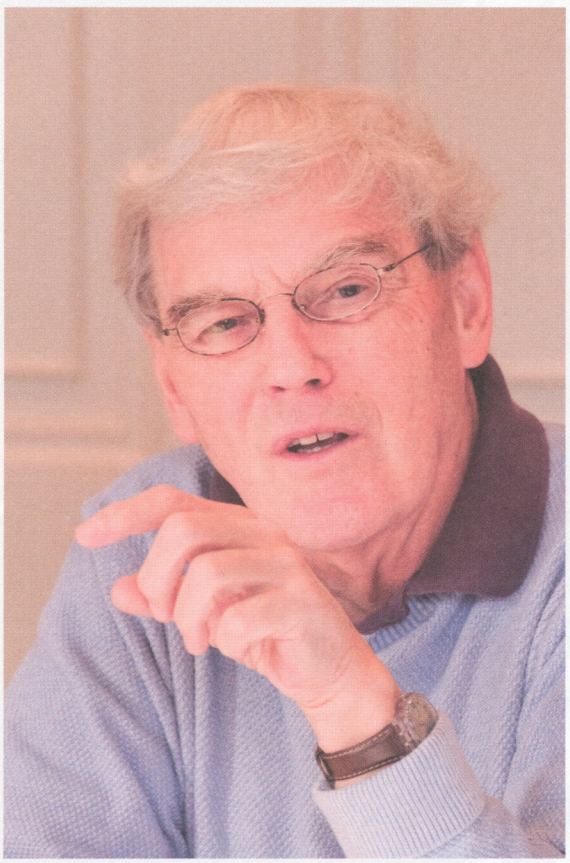
Emeritus Fellow, Richard Henderson: Chemistry Nobel Laureate 2017

An Interview by Harry Bhadeshia

Richard Henderson, Joachim Frank and Jacques Dubochet were jointly awarded the 2017 Nobel Prize for Chemistry. The Nobel citation reads, *"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution".*

This is in essence a technique that allows unfettered molecules to be imaged directly, leading to models of their atomic structure, and therefore, their functions in biochemical reactions.

Richard is based at the Medical Research Council Laboratory for Molecular Biology in Cambridge and has long been a Fellow of Darwin College in Cambridge. He kindly agreed to be interviewed by Harry Bhadeshia for this article.



Right:
Our new Nobel Laureate discusses his work. Photo Credit: Sir Cam

Harry: Many congratulations, we all are basking in your glory. There was uncontained delight in College about one of our own achieving the ultimate honour in science. How did you come to be in Darwin?

Richard: I came from Edinburgh for research in 1966. Keith Moffat (King's College) wrote to me with a paragraph about the pluses and minuses of each of the then around 25 Colleges in Cambridge. His number one recommendation was Darwin, followed by Corpus. However, I selected the latter because it had, at that time, more of a community at its Leckhampton campus. I enjoyed my time there but did not see eye-to-eye with the then Bursar, so after graduating, I did not have much contact with Corpus until I recently became an Honorary Fellow there.

In 1982, César Milstein invited me to dinner at Darwin and shortly afterwards, I learnt that I had been elected to a Fellowship of the College. I ran some discussion groups, was wine steward for three years, and assisted Torsten Krude in the organisation of the 2003 Darwin Lecture series on DNA. My role in the Lecture Series was that of a `fixer', in persuading busy and clever people to contribute to the series. At one point I felt my contribution to Darwin was insufficient but was persuaded otherwise by Geoffrey Lloyd.

Harry: Richard - we are delighted you stayed on! What happened on the day the prize was announced?

Richard: Well, I was in Leicester at a meeting listening to talks when *the* phone call came. I thought I could just go back into the meeting but some three hundred emails arrived in just one hour so I had to abandon the occasion. I spent three nights until the early hours responding to the messages. I imagined that there would be a spike of activity and then life would return to normal, but there have been some nice consequences. One of our former post-docs, Vinothkumar, who is now in India was having difficulties in getting adequate funds to do cryo-microscopy – that problem has now disappeared! I keep getting invitations to participate in meetings, but obviously, the demands on time are intense so I have to be selective.

Harry: Let me ask you some specific questions. During the attempts to determine the structure of DNA using X-rays, mistakes were made twice because of the difficulty of the problem. With this technique that you and your colleagues have developed, would it have been a lot easier to solve?

Richard: Not DNA actually, because the way the method works is you take an image but it is a noisy image. When the molecular weight is in excess of approximately 10^5, it is possible in principle to determine the position and orientation of individual particles, by the application of averaging methods over a large number of images. The base pair of DNA has a much smaller molecular weight. So far, no one has done the cryo-reconstruction of bare DNA.

Harry: But what about the macroscopic features of DNA, the double helix?

Richard: Yes, you can see the double helix, but only when it is bound to something else. But in bare DNA you see it as a kind of a line with a random curvature, but you cannot at the moment get the structure, not even the 3.5 Å stacking of the bases. If you did, you would see the average of all the bases. It may become possible to get an averaged structure for DNA when our list of outstanding problems is solved. But the base pairing would require an added level of understanding.

X-ray crystallography has produced a massive databank of more than 130,000 protein structures and that will continue to grow. Cryo-microscopy however, has enabled a lot of difficult structures to be determined more quickly, even when the protein could not be crystallised or was difficult to purify.

Harry: You started off as an X-ray crystallographer trying to look at proteins in membranes but did not get very far for many years?

Richard: I started in 1972 to try very hard to make three-dimensional crystals for X-ray diffraction but never got crystals until 1980, but even then the crystals were very bad. Hartmut Michel who also tried and did not succeed at first but coming from a biochemistry background he tried many other membrane proteins and in 1982 succeeded in crystallising one that led to the first atomic structure of a membrane protein and his 1988 Nobel Prize for Chemistry. We adopted a biophysical approach and solved the second membrane protein structure in 1990 – had it been the other way round...

The Laboratory for Molecular Biology (LMB) recruited Nigel Unwin after he finished his PhD in metallurgy in Cambridge in 1969, to work on biological electron microscopy. I came back from Yale in 1973 and heard his talk about the observation of tobacco virus using stained samples, where one is not looking directly at the protein. But clearly, Nigel was thinking about directly observing proteins. So I said to him after his talk that I have a one-molecule thick crystallised specimen that does not need to be stained; we could take direct images. We worked together for a year and published the first low-resolution membrane protein structure using electron microscopy in 1975. But it was not good enough to see the amino acids. Between 1975 and 1990 we worked to improve the technique. At the same time the biochemists improved the crystallisation of membrane proteins so X-ray crystallography provided a huge boost to the protein structure databank. X-ray work has to date led to orders of magnitude more structure determinations than electron microscopy. Nevertheless, in the last 4 or 5 years, microscopy has resolved some of the most interesting protein structures, and has now become the method of choice for such investigations. All the X-ray people are now switching to electron microscopy.

So you are right, I started out in X-ray crystallography, we then moved to electron crystallography without staining the samples. We thought two-dimensional crystals would be easier to work with but it turned out they were not, so in 1996 we stopped that and our focus turned to single-particle imaging using Dubochet's method of rapidly freezing the sample in vitrified water. Joachim Frank had been doing single particle imaging but initially on stained particles. But the work needed a lot of problems to be solved, one of them being development of better detectors for the transmission microscope. We began detector developments in our laboratory at LMB with the expertise of Wasi Faruqi who did his PhD at Harwell, now the Rutherford Appleton Laboratory (RAL). Subsequent collaboration with the detector development group at RAL gave us a significant technological advantage because we knew what we were doing and in addition, the LMB had a lot of people working on structural biology who could take advantage of the equipment. Now these detectors are obtained commercially.

Harry: You made the data of the purple membrane protein freely available. What effect did that have on the subject?

Richard: The pharmaceutical companies were the first to request the data, and used them even though we did not think this would be useful. In 1990 when we had the structure, the internet was at an early stage, so instead of sending hundreds of magnetic tapes, we requested email addresses. None of the companies used emails at the time, but I remember one using an email address borrowed from a friend.

When the new and powerful synchrotrons came along in the mid-1990s, many switched from electron crystallography to X-ray crystallography, but with the new detectors, the pendulum has now swung back. Interpretation and computation have also become easier with the development of more powerful computer programs that use Bayesian statistics.

Harry: The 1990 paper is said to have rationalised many previously unexplained observations. Can you describe these?

Richard: In 1975, we said, this idea that a bundle of helices criss-crosses a membrane is likely to be the way all membrane proteins are made. It turns out that 90% of membranes have this structure and the remainder are weakly bonded sheets arranged into a closed barrel structure. But the high-resolution observations later revealed fine features that explain the chemical behaviour of the protein including details of interaction with light.

Harry: What was the reason for picking that particular purple membrane protein for your studies?

Richard: It was readily available and easily formed two-dimensional crystals. In fact, when I went to Yale, I was intending to work on enzymes, but my sponsor, Ray Wang, suggested there are thousands of enzymes and you should instead pick a project that may come to fruition in 20 years, so I chose to work on membrane proteins. But after working on voltage-gated ion channels in membranes for two years, it became clear that the tools to investigate detail were simply not good enough, so I switched to a more tractable membrane protein – it actually took from 1972-1990 to solve even this one.

There is a rule that if you do a careful calculation of the time to complete work, you multiply by two and take the next time scale to convert that calculation into reality. So if you think it will take 2 months, it will actually take 4 years.

Harry: So I presume that the sponsors of your work are very tolerant?

Richard: It took us a year beginning 1973, to get a low-resolution structure. But then we were stuck for about 15 years. At one stage we said we would not publish anything until we had the structure. But the MRC then brought in 5-yearly reviews, and we had several referees stating "this will not work". We laughed this off, but as the years went by, we thought we ought to be pragmatic, and published incremental papers to show some semblance of progress.

Harry: Young academics these days would not survive waiting to publish until the problem is solved. Is the LMB special in supporting dedication to science rather than indicators?

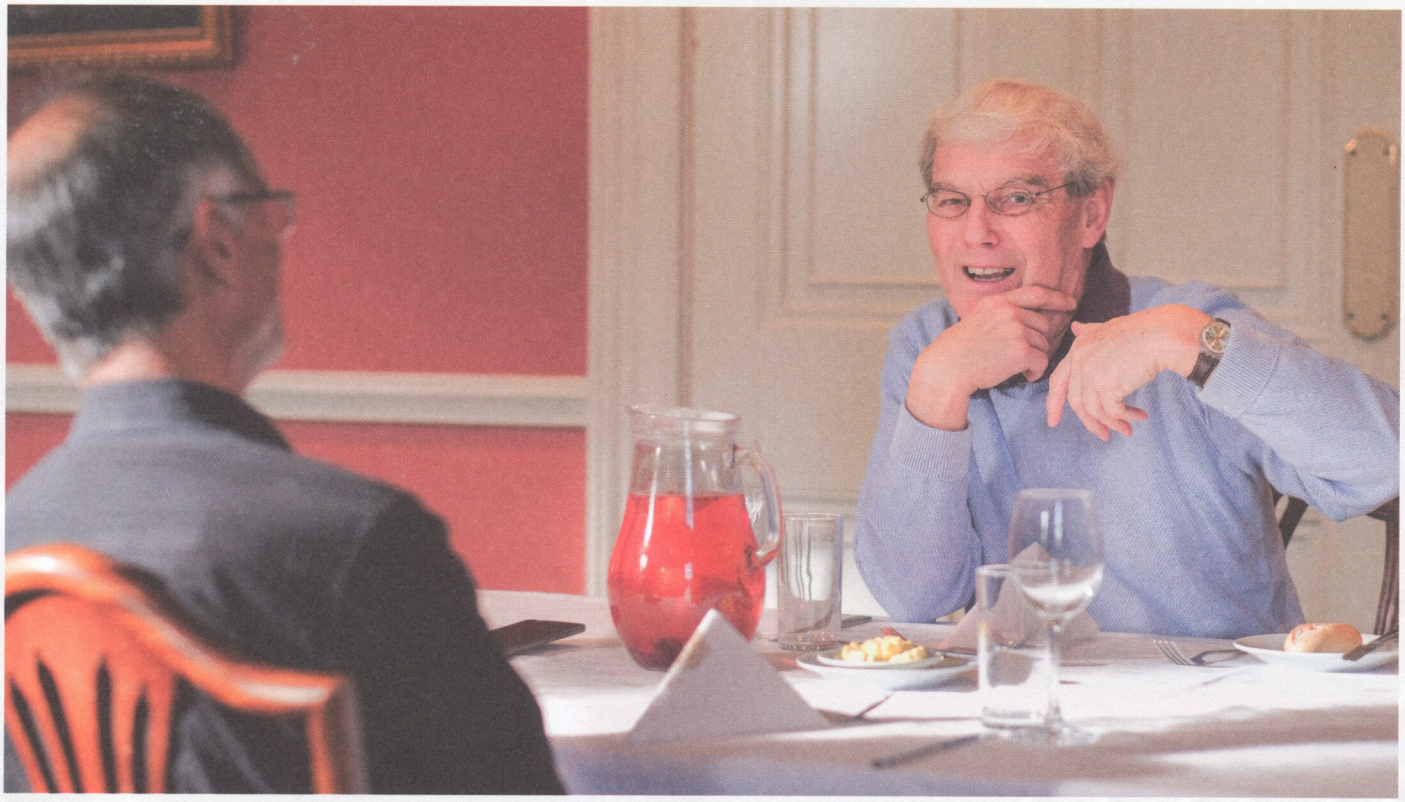
Richard: The one difference is that the LMB recruits people who like doing the work themselves. A lot of the universities however, have Professors who are good at lecturing, writing proposals and getting the work done by students and post-docs. Something is lost in this process. I myself never wanted any students, and my first post-doc was thrust on me. I came here in 1973 and worked by myself or with Nigel Unwin who then went on to Stanford around 1978. The one student who I shared with Linda Amos, attended a course with Dubochet at my behest – the student came back and decided `this is the future' and left to work with others. I later had three or four students who worked on other aspects somewhat differentiated from my work.

Harry: So what is the function of a group leader in LMB?

Richard: Some of the leaders are managers, who delegate, others do things themselves and therefore, can, I think be bolder. Both Nigel and I are in this second category. There is a third category where the leader has broken the back of the problem but then a great deal of backup work needs to be done by others who are given considerable freedom to explore. The LMB has all these structures with the focus on the science getting priority rather than on personalities.

Harry: To an ordinary person, an image means what you see is what you get. But there is in fact a huge amount of analysis in your 1990 paper in order to get to the atomic model. Can you also explain the validation of any model?

Richard: The initial validation comes from examining the density features in the image. You know you are right, when those density features show you the amino acid sequence that someone else has determined using biochemistry. Later on, the knowledge of the structure can explain a wide range of other biochemical data. But you are right, there were a lot of problems to solve to interpret the images. I wrote a program to help, a very difficult one to write, so we never knew whether it was correct or not. Joyce Baldwin created some test data that revealed an important error; the corrected software stands to this day, albeit in a friendlier format.



Above:
Richard Henderson and Harry Bhadeshia
Photo Credit: Sir Cam

Harry: How long was it between obtaining the images and creating the model?

Richard: The big change between 1975 and 1990 was from indirect methods to direct high resolution imaging using cryo-microscopy. My first effort was here in Cambridge. John Meurig Thomas in Physical Chemistry had a liquid helium electron microscope, but the instrument was not good. Dubochet's team at EMBL then built their own cryo-microscope, which we used to get the first high-resolution image. But that instrument still was cumbersome. I then went to Berlin to work with Fritz Zemlin where we got many more images in the Fritz-Haber-Institut that Ernst Ruska had set up. More good images were obtained in collaboration with Ken Downing by using the Berkeley field emission gun microscope, which had a higher coherence. It took from 1984 to 1990 to get enough good images to analyse together using our programs. So there were many years of visiting and accumulation of images. We were completely focused.

Harry: In 2004 you wrote an article saying that more validation methods are required.

Richard: This is because some people would take images and produce a self-consistent structure, but this does not prove the structure is right. There was one structure that five groups had worked on independently, but they were all different in important detail. We felt that such work might cause a scandal in the subject, and hence the article. But the problem has now disappeared because the resolution is sufficient to avoid significant misinterpretation.

Harry: It is refreshing to see your focus on science rather than indicators that even in this University are used in appointments committees. Your most important work seems to be published in ordinary journals. Would you care to comment?

Richard: Sydney Brenner was asked recently what he thinks about the trend that people only want to publish in high-impact journals. He replied that often papers in these journals have large numbers of authors whereas the seminal work may have been done by a few key people. He also noted that papers in these journals have a higher probability of being wrong!

Harry: You were at work when you received the Nobel Prize and I hope you were as elated as we were.

Richard: I am delighted. My 14-year-old granddaughter told her chemistry teacher that her grandfather has won the Nobel Prize. The teacher asked her to see if I could visit the school - I shall be going to the chemistry class at her local school early in 2018. But bear in mind that in the LMB there have been 16 Nobel Prizes so we do not get overly excited. For example, we have a tree named after Hugh Huxley, who although he discovered the mechanism of muscle contraction unfortunately was never awarded a Nobel Prize.

Harry: Thank you very much indeed for sparing the time, it has been wonderful to talk to you.

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Darwin College and the Nobel Institute

Richard Henderson is interviewed by Harry Bhadeshia

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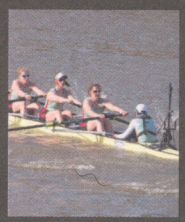

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