Biophysical Perspective

A Reminiscence about Early Times of Vitreous Water in Electron Cryomicroscopy

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A white blackbird is not an issue, until you see one. I saw it in an article by Taylor and Glaeser (1) presenting frozen delicate biological specimens with high contrast and exquisite details. I am still unsure of what really happened with this specimen but, what is certain, is that frozen water was present and that the unstained biological material was more beautiful than anything I had seen before. For me, the path was marked and chance helped.

The problem lies with water. Arguably, it is the best part of us but it evaporates in the vacuum of the electron microscope, leaving the frustrated observer with the tortuous task to guess what the native object was when it was hydrated. Attempts were made to keep vapor pressure—and some water—in a small volume around the specimen (2). It was technically difficult and met with limited success. Fernández Morán (3) explored another avenue: preventing the loss of water by cooling the whole specimen chamber to a temperature where evaporation is suppressed. Too cold, too expensive, too ambitious, the program never achieved practical use.

Filled with enthusiasm—and with Glaeser's results!—I was hired by John Kendrew at the newly formed European Molecular Biology Laboratory with the task of mastering water in electron cryomicroscopy. We learned a lot from the literature plus a little bit from our own observations. We wrote as if we were experts (4). One of the greatest advances was that Alasdair McDowall (Fig. 1) found that water can be vitrified (5). We were not the first, as Mayer and Brüggeler (6) did it shortly before. This group, together with many other water specialists, embraced the difficult task of understanding why vitreous water does exist when the theory at the time said it could not. The work is still in progress. For us, it was easier: with our simple vitrification method and the relatively simple electron cryomicroscope, observation of vitrified specimens became routine. We had in our hand a method to optimize and plenty of specimens to look at. Interested water specialists came to look with us and, with their help, we started to seriously understand that water is beautiful and that vitrification is useful. Then came Marc Adrian (Fig. 2). He was unimpressed with my idea that the controlled formation of an unsupported thin film of pure water or of a dilute solution should be impossible because of the high surface tension of water. So he found no difficulty in spanning a 100-nm-thick layer of virus solution over the 16-μm empty holes of a 400-mesh grid. For more control, the thin-film vitrification method was further developed with the optimal use of the calibrated holes of a supporting film. Since then, the method has not changed much. Furthermore, Marc had no ethical problem—as I had—to record terribly underfocused micrographs. They turned out to be much better. In the early 1980s (long after Scherzer), I should have known this, but the push by Marc's observations was decisive to put us on the right track. This is how, with the help of experienced specialists in three-dimensional reconstruction, we could obtain, for the first time with electron cryomicroscopy, a reconstruction of a virus at 35 Å resolution (7). More about this early time of vitreous water in electron cryomicroscopy can be read in Dubochet (8).

Thirty-five Ångstroms! Not bad at the time, but critical people today call it "blobology" (9). They are right because, in most cases, the function of a biological structure can only be understood at the much smaller atomic scale. It is thus understandable that the interest for the new method was modest at first. Ten years after our initial findings, electron cryomicroscopists of vitreous specimens could be counted on one's fingers (or perhaps on one's hands and feet). Ten years later there were perhaps 10 times as many. Nowadays, their number has perhaps increased by another factor of 10. What is sure, however, is that the resolution is also 10 times better now.

Thirty years! The thin-film vitrification method has hardly changed but near-atomic resolution is now (nearly) routine. At 10 times better resolution. That means one-thousand times smaller structural elements that can be resolved. How is this possible? Giants and many "normal" scientists are behind this achievement. They have not been ignored. Aaron Klug won the Noble Prize for "his development of crystallographic electron microscopy". For the young electron microscopist that I was in the

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FIGURE 1 Alasdair McDowall, circa 1985. To see this figure in color, go online.

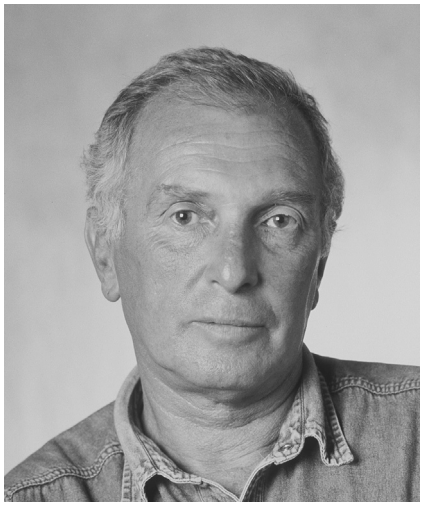

FIGURE 2 Marc Adrian (1945–2013), circa 2005.

1970s, my hero was Nigel Unwin. Except for vitrification, most of the ingredients were already in his hands. Most, but not all. The present-day success of electron cryomicroscopy required the extraordinary development of image processing, in particular single-particle three-dimensional reconstruction and tomography. The new cameras gave the surprising and unanticipated final push. There are simply too many names of people who have contributed to cite them all, but I have a special admiration for the work of the Cambridge group and its numerous ramifications, and also for the sustained effort of the Albany group's work with ribosomes. The avalanche of published results is the testament of today's electron cryomicroscopy revolution.

Learning from the observations of nature has been a necessity for me since childhood. When it came the time to be serious about it, electron microscopy was an easy choice; it was the time when DNA was made beautifully visible by Kleinschmidt's method (10) and when a Nobel Prize rewarded the elucidation of the DNA's structure. The road was set: solve the structure and you will understand how it works! As we now know, the road turns out to be rocky and the rather simple DNA structure-function paradigm was not easily reproduced. Now, retired, I am filled with the same emotion as 50 years ago, when I look, for example, at the RNA strands embracing each other in CRISPR (11). Yes, seeing is understanding. The dream has become true and the white blackbird a familiar specimen. This issue of the *Biophysical Journal* is all about this.

The story is not finished, however: understanding also brings the capability for controlling and acting. At a time when modifications of human embryos and ecosystems become a reality, the responsibility of acting is a vital challenge (12,13). Are we going to face it?

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