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Nature Protocols – 10 year Commentary - Advances in the field of cryo-electron microscopy over the last decade:

A Commentary

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I remember being approached at the reception of the meeting on *Structure and Function of Large Molecular Assemblies* in 2006, organized by the International School of Crystallography in Erice, Sicily. One of the editors of the newly created journal *Nature Protocols* invited me to contribute an article on single-particle cryo-EM. After years of mixed experiences with print journals, the idea of not having to worry about page limits and color charges, the idea of proving documents at the reader's fingertips via hyperlinks, and the promise of technical assistance in managing all these links all had an instant appeal to me. Eventually, in our follow-up correspondence, I agreed to write not one but three articles covering all aspects of the subject matter, from sample preparation¹ over data collection on the electron microscope² to image processing and 3D reconstruction using the SPIDER package³. The number of citations accumulated over the years referencing these three articles indicates that the time was ripe for dissemination of such meticulous step-by-step protocols, especially for the image processing part.

Since the appearance of our first contributions to the journal, a lot has happened in the field of cryo-EM, as has been noted in numerous recent commentaries, review articles, and cryo-EM-for-dummies introductions. Coinciding with our articles^{1–3} in *Nature Protocols* was the appearance of two method-oriented papers with general impact, one providing a way to interpret low-resolution density maps by fitting atomic structures⁴, the other addressing the need for a quantitative means to sort, or classify, single-particle projections from heterogeneous samples⁵. (A later version of this maximum-likelihood approach to resolving heterogeneity, developed by Sjors Scheres, is RELION⁶, now in widespread use.)

However, even though the structure of viruses could be solved at near-3Å resolution thanks to their icosahedral symmetry⁷, the resolution of single-particle cryo-EM reconstructions of asymmetric structures such as the ribosome showed little improvement for years, being limited in a fundamental way by the poor quality of traditional recording media. This is exemplified by our own progress with the ribosome from 6.7Å in 2008 (refs. 8,9) to 5.5Å in 2013 (ref 10). This discrepancy between resolution potentially achievable and resolution practically obtained was already strikingly apparent from Richard Henderson's study¹¹ in 1995 which predicted attainment of 3Å resolution for the *E. coli* ribosome from as few as 10,000 particles on theoretical grounds, while in practice 4,300 particles yielded a reconstruction of the ribosome with no better than 25Å resolution¹².

However, starting in 2012, as a consequence of the introduction of new direct electron detectors, the field as a whole has been marked by spectacular progress toward higher resolution, to a level that now frequently allows *de novo* atomic modeling. The era was

heralded by the appearance of an article¹³ entitled “Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles”, most notable here as it tended to close the gap in the aforementioned numerical discrepancy in particle counts. (For the record it should be mentioned, though, that the “near-atomic resolution” in the title referred to a value around 4.5Å, better by just 1Å than the film-based study of Hashem et al.⁹ which appeared at the same time, obtained from 160,000 particles, rather than millions). Since then numerous ribosome structures from both prokaryotes and eukaryotes have appeared in the 3Å range, the most recent one being the structure of the large subunit from *T. cruzi* solved in my lab¹⁴ at 2.5Å, a resolution sufficient to show water molecules as well as rRNA modifications, and to allow *de novo* atomic modeling. Most notable in this recent “resolution revolution” has also been the achievement of close-to-atomic resolution for small membrane-bound proteins, starting with the elucidation of the TRPV1 channel by the group of Yifan Chen at the University of California at San Francisco^{15,16}. In fact both kinds of structures, very large macromolecular assemblies, which are flexible and exist in multiple states, and small trans-membranous channels have long posed challenges to X-ray crystallography which are now overcome by cryo-EM.

What has been particularly evident in the past three years is the much accelerated pace in the development of techniques toward higher throughput, standardization and automation, inviting comparisons with the early years of X-ray crystallography by its practitioners old enough (yet not too old) to remember. While instrumentation has made a giant leap forward in automation, as reflected by the sophistication of new-generation cryo-electron microscopes with auto-loading capability, the craft of sample preparation has lagged far behind, and this area is ripe for inventions that are expected to be featured in many protocols to come. What comes to mind are robotic deposition methods (Spotiton¹⁷), whisker-assisted blotting¹⁸, exploration of graphene grids¹⁹, and time-resolved cryo-EM²⁰.

Especially explosive has been the development of software which now has acquired a much larger role encompassing frame processing, maximum-likelihood classification, validation, and model building that are augmenting, or entirely replacing, the stock of routines featured in the protocol of Shaikh et al.³. A good survey of these latest developments is found in the volume of *Methods in Enzymology* just edited by Tony Crowther²¹.

In fact, of the three areas covered by our original contributions, the development of software has exposed the problems of the old-fashioned, slow-moving publication process in scientific journals. Many would agree with the dictum that if something works, then the community should know right away, and not six months from now. Even though it is in the nature of all-electronic journals such as *Nature Protocol* that the tedious editorial and review process is somewhat shortened, many methods-oriented groups have now resorted to depositing their newest contributions in publicly accessible archives such as, *bioRxiv*²² following a practice long honored in the physics community, for rapid unedited dissemination. Thus the dissemination of “hot” material instantly useful for the community may be increasingly separated from its blessing by established authority through the vehicle of scientific journals. It is unlikely that publication of these “blessed” articles will be made redundant, but the trend nevertheless signifies a change in the role and scope of the scientific journals from past models.

In closing, I would like to recognize the role of *Nature Protocols* in facilitating the distribution of much more detailed methods and protocol information than has been traditionally provided. Looking back over the remarkable pace of development during the past decade in my own field makes it difficult to imagine what the next decade will bring, but I'm certain that our understanding of many molecular mechanisms, such as those propelling translation or activation and gating of channels, will be much more profound as a result of the spectacular process of cryo-EM.

Acknowledgments

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