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On the state of crystallography at the dawn of the electron microscopy revolution

Matthew K .Higgins[1] and Susan M Lea[2]

While protein crystallography has, for many years, been the most used method for structural analysis of macromolecular complexes, remarkable recent advances in high-resolution electron cryo-microscopy led to suggestions that 'the revolution will not be crystallised'. Here we highlight the current success rate, speed and ease of modern crystallographic structure determination and some recent triumphs of both 'classical' crystallography and the use of X-ray free electron lasers. We also outline fundamental differences between structure determination using X-ray crystallography and electron microscopy. We suggest that crystallography will continue to co-exist with electron microscopy as part of an integrated array of methods, allowing structural biologists to focus on fundamental biological questions rather than being constrained by the methods available.

Addresses
[1] Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
[2] Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

Corresponding authors: .Higgins, Matthew K (matthew.higgins@bioch.ox.ac.uk) and Lea, Susan M (susan.lea@path.ox.ac.uk)

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Introduction

Since the 1950s, the method of choice for the determination of protein structures has been X-ray crystallography, and innovations in sample handling, X-ray sources, detectors and software have since dramatically reduced the time taken to determine a structure [1]. Data collection at a 'standard' synchrotron source generally takes only a few seconds [2], while automated pipelines facilitate data collection [3], and allow many structures to be solved without intervention by the user [3,4]. The high level of automation and speed of the experiment have revolutionized how crystallography is performed, making it standard to collect data from several tens to hundreds of crystals and allowing determination of structures from crystal systems that would previously have been considered intractable. Recent advances include quick data collection, free at the point of access synchrotron facilities and simple to use or highly automated beamlines [5] and software [3]. These have contributed to an ever growing number of coordinate sets deposited in the Protein Data Bank. Indeed crystallography is still by far the most used method for structure determination (Table 1).

Advances in both synchrotron hardware and in software suites have made the determination of novel structures more streamlined, with a massive case history helping the community to employ the best strategies to collect data [6]. While experimental phasing previously relied on introduction of non-native heavy atoms into the macromolecule under study, long wavelength beams are allowing phasing using weak anomalous signal from naturally occurring atoms, such as sulphur, making resolution of 'the phase problem' increasingly routine [7]. Coupling these weak signals with molecular replacement, using search models derived from the latest protein modeling tools, is providing increased power for *de novo* structure determination [8]. Advances in automatic data collection are also improving the throughput of crystallography as a tool for drug design. For systems that generate well diffracting crystals, screening platforms, including semi-automated crystal mounting, together with high-throughput automatic data collection and processing, allow rapid screening of small molecules and molecular fragments, to identify those with promise as part of molecules of medicinal value (for example http://www.diamond.ac.uk/Beamlines/Mx/Fragment-Screening.html) [9]. It is therefore easier to both determine a novel structure and to exploit this structure for therapeutic use.

While the ability to grow a crystal remains limiting for standard crystallography, what defines a useful crystal is in constant flux, with the absolute size of crystals, and their required degree of order, continuously decreasing. Improvements to synchrotron facilities include the availability of microfocus sources, such as beamline I24 at Diamond Light Source [10], providing small and intense beams to coax diffraction from crystals a few micrometers across. These crystals can even be imaged in crystallization plates or collected onto mesh supports, with small numbers of images collected from individual crystals, and complete diffraction patterns obtained by piecing these

Table 1

Structures deposited in the protein data bank (www.rcsb.org) determined by the major structural methods

Method	2015	2016
Solution NMR	346	450
Solid state NMR	11	7
X-ray	7662	9964
XFEL	15	57
EM	216	410

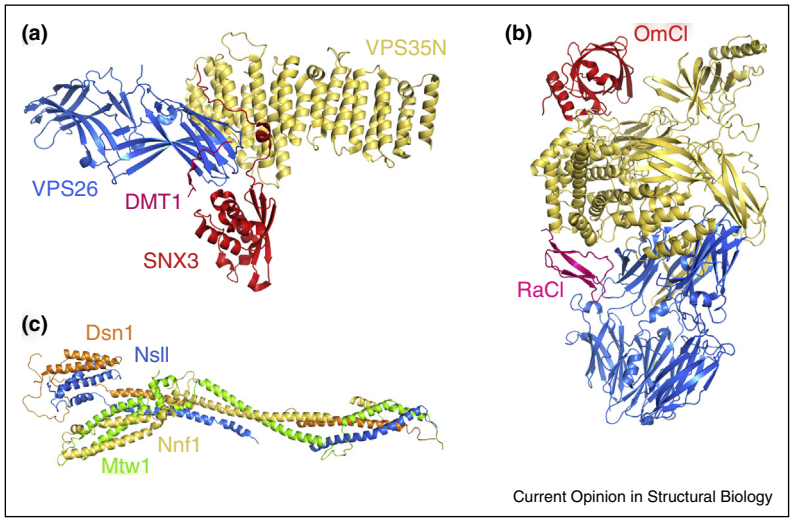
together [11–13]. Serial crystallography, with single diffraction patterns collected from microcrystals or nanocrystals, and data collection using X-ray free-electron lasers, are turning the size restrictions on crystals on their head, making small crystals desirable, and allowing collection of diffraction data from crystals at room temperature, untainted by beam-induced radiation damage. These methods have great power to determine structural changes induced in a macromolecule by light or by ligand.

In this review we will briefly highlight how these developments place X-ray techniques at the heart of integrated structural biology and will describe how fundamental differences in the basis of structure determination by different methods mean that all the structural techniques will continue to have roles to play for the foreseeable future. While there is no doubt that advances in electron microscopy are opening exciting new possibilities for the structural biologist, claims of the demise of crystallography seem premature, if not unfounded.

Crystallography at the heart of integrative structural biology: some recent triumphs

Many exciting studies over recent years illustrate the continuing power of classical crystallography to underpin integrative structural and cellular science. Examples that have caught the eyes of the authors include structural analysis of cellular trafficking [14], complement regulation [15], kinetochore assembly [16] and nuclear pore formation [17] (Figure 1). Crystal structures of a large complex from the retromer system involved in membrane protein recycling, supported by small angle X-ray scattering, and biophysical and cellular analysis, have revealed new insight into the process by which signal recognition leads to membrane recruitment in this trafficking system [14]. Novel crystal structures, combined with NMR, electron microscopy and functional and biophysical analysis have shown how antibodies and proteins from tick salivary glands can inhibit critical complement pathways [15]. Structures of the MIND complex, determined using powerful crystallographic tools to overcome the challenges associated with anisotropic data and small crystals, have given important insights into kinetochore assembly [16]. Finally, a study has generated a molecular model for the mRNA export platform of the nuclear pore complex using a combination of mass spectrometry, cross-linking, electron microscopy and molecular modeling, allowing the assembly of previously determined crystal structures into a larger assembly [17]. Each of these studies highlights how modern synchrotrons, advanced detectors and the latest generation of processing software are allowing determination of increasingly complex structures and show how crystallography can be integrated with other

Figure 1



Crystallography addressing major problems in cell biology. **(a)** The structure of the retromer complex gives insight into cargo recruitment (PDB code: 5F0P) [14]. **(b)** Crystal structure of human Complement C5 with two inhibitors derived from tick saliva, *Ornithodoros moubata* OmCI and *Dermacentor andersoni* RaCI3 (PDB code: 5HCC). Adapted from [15]. **(c)** The structure of the MIND complex and the assembly of yeast kinetochores (PDB code: 5T58) [16].