Advances in Structural Biology and the Application to Biological Filament Systems

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1. Introduction

Structural biology at atomic resolution is fundamental in understanding how the components of macromolecular machines generate biological processes. Through determining how the parts fit together and move relative to each other, and observing the architectures that define how chemistry is achieved, high resolution structure determinations can be used to comprehend the designs of biological machines, revealing how and why they work. Structural biology came to the world’s attention in the 1950s with the X-ray fiber diffraction-based DNA double helix hypothesis[1] and the first X-ray crystallography structures of proteins, myoglobin,[2] and hemoglobin.[3] This led to the 1962 Nobel prizes being awarded to Crick, Watson, and Watkins (Medicine) and to Perutz and Kendrew (Chemistry), but most importantly these studies demonstrated the importance of structure determination for understanding biology, through unlocking the genetic code and showing that protein architecture is key to biological function and chemistry. Celebrated discoveries have continued unabated, including the watershed publications of the structures of the ribosome[4] and the RNA polymerase,[5] both critical discoveries that propelled forward the understanding of the mechanics of these complex machines.

Since then, there have been many outstanding successes in structural biology, however, in the first decade of this century the general perception of the structural biology began to decline for two reasons. Firstly, protein crystallography became easier and saturating with the advances in molecular biology, robotic crystallization screening, advanced synchrotron sources, phasing techniques, and crystallographic software. This rapid accumulation of new structures lowered the impact of each individual structure on the wider audience. Secondly, biology sometimes took a backseat to engineering in the early structural genomics efforts, where there was a focus on numbers and structural variety rather than on protein interactions and chemistry.[6] Moderate biological motivation at the inception led to moderate biological significance revealed from the structures. The good news is that structural biology is firmly back in the limelight with exciting transformative technologies, which promise a new decade of startling discoveries. These technologies address issues that had previously rendered many areas underrepresented in atomic resolution structures. We assemble a model of a capped tropomyosin-actin minifilament to demonstrate the utility of combining structures determined by different techniques. Finally, we survey the methods that attempt to transform high resolution structural biology into more physiological environments, such as the cell. Together these techniques promise a compelling decade for structural biology and, more importantly, they will provide exciting discoveries in understanding the designs and purposes of biological machines.
important biological systems intractable for structure determination. Samples that have problems with heterogeneity, availability, or have behavioral issues are often unsuitable for protein X-ray crystallography, traditionally the most widespread high resolution structural technique. Here we discuss the advances in the technologies that have transformed these uncooperative samples into targets that are now highly amenable to structure determination.

2. Microcrystals – Small Becomes More Beautiful

Protein crystallographers often experience that their most exciting samples do not crystallize or form vanishingly small crystals. There have to be three notable recent advances in protein crystallography that have addressed structure determination from microcrystals, something that would have been impossible on traditional synchrotron beamlines. Diffraction from a microcrystal relies on the balance between the crystal size, intensity of the source, background scatter and radiation damage. The three methods have solved this equation in different ways. Together these techniques address the structure determination for rare or uncooperative proteins that are only capable of forming microcrystals.

2.1. Synchrotron X-Ray Microfocus Beamlines

The first method to address microcrystals was the introduction of the synchrotron microfocus beamline. Synchrotron X-ray studies produce optimal diffraction data when the X-ray beam size matches the crystal size, through maximizing the diffraction from the crystal while minimizing background scatter, leading to a maximum in signal-to-noise. Such microfocus beamlines are now widely available with typical minimum beam diameters of 1–10 μm. For reference, a 1 μm cube crystal will contain millions of copies of a typical protein. However, data collection from even smaller crystals is possible from well-ordered strongly diffracting samples. The experiment usually involves scanning the sample with X-rays to locate the crystal and determine its optimal diffraction position. Then partial diffraction data sets are collected from several crystals and later merged into a complete data set. This minimizes the radiation damage that would be incurred in measuring an entire data set from a single crystal. Alternatively, for needle-shaped crystals the beam can be translated along the length of the needle during data collection to continuously supply unexposed regions of the crystal. Given the high availability of microfocus beam time, this method can be routinely used structural biology laboratories with crystals that at are 1–10 μm × 1–10 μm in cross section.

2.2. X-Ray Free Electron Laser

Secondly, the advent of the X-ray free electron laser (XFEL) provided an incredibly intense X-ray source in extremely short pulses (femtoseconds). In the serial femtosecond crystallography (SFX) approach, a single diffraction image is recorded before the energy of the pulse destroys the crystal. The pulsed operation mode of XFEL allows for continuous sampling of a moving stream of crystals. Bad images are then discarded, while thousands of good diffraction images, each arising from a different crystal, are scaled into a complete data set. In this experiment the diffraction image is recorded before radiation damage occurs, maximizing the diffraction and signal-to-noise despite the high background scatter. The technique has been typically applied to solving high resolution structures from ~1 μm crystals (meaning that the crystals dimensions are at least 1 μm in each axis), and to time-resolved structural studies. A recent report has demonstrated that 2 Å diffraction data is possible from 0.25 μm crystals, and 0.05 μm crystals are theoretically amenable for structure determination. Thus, crystals in the 0.25–1.0 μm range are optimal for data collection at XFEL beamlines and the technique is uniquely capable of fast time resolved studies, particularly for light-activated processes such as oxygen bond formation in photosystem II.

2.3. MicroED

X-rays and electrons are scattered by atoms in different ways. X-rays interact with the electron cloud around an atom, however, the probability of a diffraction event is low. In X-ray diffraction, it is estimated that only one photon in a million interacting with the protein is scattered leading to low signal-to-noise and high radiation damage. Electrons, by contrast, are scattered by their charge interaction with the charges on the protons and electrons of an atom and their probability of scatter is high. Thus, the attractiveness of electrons over X-rays is that there is less sample damage (per diffraction event), by a factor of more than two orders of magnitude, which allows for diffraction from smaller crystals than is possible from non-pulsed X-rays. Electron diffraction has been used as a method for protein structure determination for more than 40 years, including the 3.5 Å structure of bacteriorhodopsin in 1990, which was recognized in award of the 2017 Nobel Prize. However, the routine implementation of electron crystallography suffered from two hurdles, which were solved in 2013 by the introduction of micro electron diffraction (MicroED). Firstly, the crystal size, electron dose and radiation damage were optimized. Large crystals (greater than 3 μm) cannot be penetrated by the beam of a standard cryo-electron microscope, yet small crystals experience too much radiation damage. Thus, the solution was to drastically reduce the electron dose and use crystals of a thickness of 0.5–1.0 μm. Secondly, data indexing was previously challenging from single images due to the large numbers of reflections (the large Ewald sphere) at the wavelengths used in electron microscopy. At 200 kV the wavelength is 0.025 Å in contrast to the 1 Å typically used in X-ray diffraction experiments. This hurdle was removed by the use of low dose exposures that allow continuous crystal rotation exposures (>400 oscillations), which can then be indexed in standard X-ray crystallographic software. The benefits of MicroED over SFX are that only a handful of crystals are required and that cryo-electron microscopy (cryoEM) instruments, which are available at many research centers, require a minor modification to tilt the samples and consequently are likely to become widely available, as opposed to the XFEL for which it is notoriously difficult to obtain beamtime.
extended the minimal size of crystals suitable for structure
determination, solving some of the problems posed by scarce
and uncooperative proteins, however, they do not address
samples that cannot be crystallized, such as the majority of
biological filament systems.

3. Non-Crystalline Samples – A Continuing
Revolution in Resolution

The numbers of techniques available to study these non-crystalline
samples are few. In the special cases of filament systems, fiber
diffraction studies and solid-state NMR spectroscopy have
produced a limited number of high resolution structures,[22,23]
however, these reports are rare.[24] Protein structure determination
without the need for crystals was one of the initial goals of XFEL.
The intensity of the XFEL pulses is theoretically sufficient to collect
a diffraction pattern from a single molecule before it is destroyed by
radiation damage.[10] However, signal-to-noise levels in the
diffraction patterns measured thus far have not proven sufficient
for high resolution structure determination.[24] Significant
advances, particularly in sample introduction methods, will be
needed before the averaging of XFEL single molecule diffraction
patterns will lead to high resolution electron density maps. By
contrast, innovations in cryoEM have propelled it to be the
dominant technique available for studying the structures of non-
crystalline large protein assemblies.

3.1. CryoEM

Recent technological advancements in cryoEM have led to higher
resolutions, and as a consequence, higher quality structures.
This has taken cryoEM structural biology into the realms of near
atomic resolution and, with it, recognition through the award of
the 2017 Nobel Prize in Chemistry for developing cryoEM to
Jacques Dubochet, Joachim Frank, and Richard Henderson. A
recent significant technological advance was the advent of direct
electron detectors (DED), which have superior signal-to-noise
resolution over charge-couple device (CCD) cameras, and have a
data collection frame rate that allows for frames to be captured as
a “movie.” Realignment of individual movie frames is used to
account for beam-induced sample movement, damage, and grid
drift,[25] factors that blurred earlier electron micrographs.
Currently, high resolution cryoEM structural determinations
are still subject to a few limitations. These include protein size
(larger structures are easier to identify and align), preference for
samples with inherent symmetry, and high sample rigidity
(characteristics that allow averaging between identical units).
However, the size and symmetry criteria are becoming relaxed,
as evidenced by the 100 kDa protein size “barrier” being broken
with the determination of the structure of isoctate dehydroge-
nase, solved as a dimer with twofold symmetry at 3.8 Å
resolution.[26] In cryoEM individual images of molecules are
selected and averaged into a composite electron density map.
This allows relatively heterogeneous samples to be analyzed,
such from a range of ribosome assembly intermediates.[27] Thus,
the strength of cryoEM is the ability to elucidate structures from
non-crystalline, non-homogeneous samples.

While there is an encouraging growth in the number of
cryoEM structures solved for samples without any inherent
symmetry, symmetry in the sample improves the resolution of
the final electron density maps by increasing the number of
asymmetric units available for averaging.[28] Using data available
in Electron Microscopy Data Bank (EMDB) from 2010 to 2017,
the average resolution of the top 10% of all maps deposited has
risen in the later half of that time period to a level that is typically
acceptable in X-ray crystallography structures (3 Å, Figure 1).
Furthermore, the highest resolution structures published each
year continue to push the resolution boundaries (Figure 1). The
numbers of cryoEM electron density maps deposited each year is
increasing, as are the numbers of maps to 4 Å or better deposited
over the last 4 years, a resolution that allows for chain tracing
(Figure 2A). Within the “4 Å or better” set, approximately half of
the maps are generated using symmetrical averaging
(Figure 2B). Prior to 2017, the numbers of ribosomes or
ribosomal complexes dominated the set of “4 Å or better” maps
produced without symmetrical averaging (60%, Figure 2C).
This was due, in part, to the large size and rigidity of ribosomes
making them excellent samples for cryoEM. A closer look at the
high resolution maps generated using symmetrical averaging,
reveals that prior to 2012 viruses accounted for most structures,
due to their high symmetry, however, over the later years lower
symmetry structures have become more prevalent, as the
reliance on averaging from high symmetry became less essential
due to improved data (Figure 2D). Thus advances in cryoEM
allow for near atomic resolution structures to be determined for
proteins and protein complexes in solution.

4. F-Actin: Charting the Evolution of
Resolution

To understand how the recent resolution advances in cryoEM have
transformed structural biology for filament systems, we chart the
history of the determination of the actin filament structure. It is
important to note that helical filament systems, such as actin,
cannot generally pack together to adopt the symmetry needed to

![Figure 1](https://www.ebi.ac.uk/pdbe/emdb/index.html). Data accessed 03/01/2018. Orange: average resolution of the top 10% of all maps deposited in the Electron Microscopy Data Bank (EMDB) from 2010 to 2017. The average resolution of the top 10% of all maps deposited has risen in the later half of that time period to a level that is typically acceptable in X-ray crystallography structures (3 Å, Figure 1). Furthermore, the highest resolution structures published each year continue to push the resolution boundaries (Figure 1). The numbers of cryoEM electron density maps deposited each year is increasing, as are the numbers of maps to 4 Å or better deposited over the last 4 years, a resolution that allows for chain tracing (Figure 2A). Within the “4 Å or better” set, approximately half of the maps are generated using symmetrical averaging (Figure 2B). Prior to 2017, the numbers of ribosomes or ribosomal complexes dominated the set of “4 Å or better” maps produced without symmetrical averaging (60%, Figure 2C). This was due, in part, to the large size and rigidity of ribosomes making them excellent samples for cryoEM. A closer look at the high resolution maps generated using symmetrical averaging, reveals that prior to 2012 viruses accounted for most structures, due to their high symmetry, however, over the later years lower symmetry structures have become more prevalent, as the reliance on averaging from high symmetry became less essential due to improved data (Figure 2D). Thus advances in cryoEM allow for near atomic resolution structures to be determined for proteins and protein complexes in solution.
build a crystal, ruling out the possibility of using X-ray crystallography to solve their structures. The first detailed model of the actin filament (1990) was generated by fitting of the newly solved X-ray crystal structure of the actin monomer into X-ray fiber diffraction data arising from aligned actin filaments collected on a CuKα rotating anode (Figure 3A).[29,30] The positioning of the monomer in the filament was confirmed later that year by the determination of a 25–30 Å resolution cryoEM map.[31] Manual positioning of the hydrophobic plug, in the original model (Figure 3A),[29] proved to be an artifact introduced by an erroneous estimation of the filament diameter from an assumed radius of gyration of 25 Å. The later higher resolution fiber diffraction model[23] and 13.8 Å cryoEM[32] models reduced the radius of gyration to 23.7 Å, abrogating the need to extend the hydrophobic plug. In 2009, higher resolution fiber diffraction data (3.3–5.6 Å) were reported that were collected from superconducting-magnet aligned gelsolin-capped filaments using the SPring-8 third generation synchrotron X-ray source. This allowed for refinement of the protomer structure within the filament,[23] and determined that there is a 20° rotation of the two domains of actin, flattening the G-actin structure on polymerization, and an ordering of the DNase I binding loop,[30] which is disordered in the native G-actin structure.[33] These changes were revealed two decades later in the fiber diffraction (2009) and cryoEM (2010) models (Figure 3C and D).[29,34] Calculation of the fiber diffraction pattern from the latest cryoEM F-actin model with the original experimental data shows excellent correlation (Figure 3E), demonstrating that fiber diffraction is an informative technique in filament structure determination. However, sample preparation of suitably aligned samples for fiber diffraction studies is non-trivial,[24] leaving cryoEM as the most practical method for determining high resolution filament structures.

5. Filament Structures: Recent Successes

The implementation of direct electron detectors in cryoEM has led to some stunning new structures over the last few years. These structures fall into three broad classes. The first class of filament system structure determination belongs to the normal single particle reconstruction. The dynactin complex is an outstanding
example (Figure 4A and B) of how a minifilament system can be reconstructed from single particles.\[17\] The dynactin minifilament is constructed from ten actin-like protomers – eight protomers of the actin-related protein 1 (Arp1), one protomer of actin-related protein 11 (Arp11), and one protomer of actin. This minifilament is capped at each end. At the barbed end, two Arp1 protomers interact with capping protein (CapZ), which is usually found capping actin filaments. By contrast, at the pointed end, there is a capping complex (p25, p27, p62) that is unique to the dynactin filament and interacts with one copy each of Arp1 and Arp11. The shoulder complex (p150Glued, p50, p24) binds to the side of the minifilament and extends out four shoulder peptides that interact with the each of the Arp1 subunits. Together the “shoulder” and capping complexes specify the composition and length of the minifilament, through interacting with each of the filament protomers. The shoulder also integrates p150\textsuperscript{Ghard}, firmly attaching it to the side of minifilament. Together, this allows p150\textsuperscript{Ghard} to link the cargo-binding dynactin filament to the microtubule motor, dynein to produce transport of large cargos, such as organelles and vesicles.

The second class of filament reconstruction is that of isolated extended filaments. In this case, short segments within the filament, which contain multiple protomers (usually more than 10), are treated initially as single particles. After 3D reconstruction, the averaging is extended to treat each protomer as a single particle allowing for extensive averaging within and between the filaments. The aforementioned tropomyosin:actin copolymer exemplifies this method of reconstruction (Figure 4C). The final class of filament reconstruction is where the filament system is saturated with a binding partner, such as myosin (Figure 4D). Here, the innate symmetry of the filament is exploited for averaging to produce a high resolution image of the interaction between the two proteins, and to reveal the structure of the binding partner.

Comparison of the core filament structures from the actin: tropomyosin and dynactin complexes gives the impression that filament systems constructed from the actin fold are highly conserved (Figure 5A and B). This is certainly true within eukaryotes, however, far greater variety can be found in the prokaryotic world.\[18\] For example, the ParM filament that segregates the R1 plasmid forms a parallel left-handed helical filament,\[19\] rather than right-handed architecture observed for actin (Figure 5B and C). Furthermore, MreB forms antiparallel untwisted filaments, which when analyzed by protein

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**Figure 3.** Evolution of resolution for the actin filament. A) Comparison of the 10 Å 1990 fiber diffraction (FD) filament\[29\] with the 3.7 Å 2015 cryoEM filament.\[35\] The two strands of each filament are shown in light blue and fawn, and were aligned based on the central protomer (red) with bound ADP (yellow). Circled regions show enlargements of the hydrophobic plug. The pointed and barbed ends are indicated. B–D) Superimposition of the 2015 cryoEM filament protomer structure\[35\] (cyan) with: (B) the 1990 FD filament protomer structure\[29,30\] (red); (C) the 3.3–5.6 Å 2009 FD filament protomer structure\[23\] (green); and (D) the 6.6 Å 2010 protomer cryoEM structure\[34\] (blue). The subdomains are labeled 1–4 in (B). E) Comparison of the experimental FD pattern (red boundary, top right and bottom left quadrants) with the calculated FD pattern from the 2015 cryoEM structure\[35\] (cyan boundary, bottom right and top left quadrants).
Figure 4. Examples of near atomic resolution cryoEM filament structures. A and B) Two views of the 4.0 Å dynactin complex (2015). The central filament consists of eight Arp1 (shades of green/brown), one Arp11 (yellow) and one actin (purple) protomer. This filament is capped at the barbed end by the heterodimeric capping protein (alpha subunit in red, beta subunit in blue, schematic) and at the pointed end by p25/p27/p62 (red/blue surface). The shoulder (p150Glued, p50, p24) and the p150Glued projection are shown in cyan. The shoulder peptides (black) specify the organization and length of the filament and are thought to correspond to N-terminal extensions of the tetrameric p50 emanating from the shoulder. The p150Glued projection was determined at 8.6 Å. C) The 3.7 Å tropomyosin/actin complex (2015). The two strands of the actin filament are colored in shades of brown or shades of green. The tropomyosin dimer (Tm) is shown in gray and black. D) The 3.9 Å myosin/tropomyosin/actin complex (2016). Colors as in C) with four myosin heads (M1, M2, M3, M4) depicted as schematic in purple.
crystallography, were seen to form protofilaments within the crystal packing. These designs are likely to represent just a fraction of the total filament architectures formed from the actin fold. Negative stain EM data point to other designs that are comprised of four filaments arranged as closed or open tubules, thus we expect the wider application of high resolution cryoEM to uncover many more architectures.

6. Structure Versus Chemistry: Resolution Is Critical

An oft-posed question is whether resolution is directly comparable between CryoEM and X-ray crystallography. There is an issue in that standardization in the methods used to calculate resolution has yet to be fully implemented in the EM field, with different research groups using different metrics, something that has been normalized in the X-ray field for many years. The truest test of resolution is to directly compare electron density maps. The electron density maps at various resolutions can be seen to be essentially equivalent across a range of X-ray and cryoEM structures (Figure 6). At 4.3 Å the protein backbone is usually well defined but sidechains often have poor density (Figure 6F and I). At 3.5–3.6 Å the sidechains become more visible (Figure 6C, G, and H). At resolutions beyond 2.5 Å, sidechains orientations become reliable and water molecules can be identified (Figure 6A, B, D, E, and J). Thus at resolutions around 4 Å the general fold of a
protein can be determined, but it is only when the resolution exceeds 2.5 Å that the structures can be determined with sufficient accuracy to understand the chemistry. Genuine atomic resolution occurs at resolutions better than \( \frac{1}{\sqrt{2}} \) Å. Clearly, the highest resolution cryoEM structures are now able to reveal chemistry (Figure 1A), however, the publication of cryoEM structures has no rules with respect to minimum resolution, placing the onus on the reader to decipher the quality of the structure. Currently, X-ray crystallography structures are not normally published unless the resolution at least reaches 3 Å, a level that is rather stringent.

7. Dynamic Structures

Exposure of proteins to intense X-rays or electrons leads to reaction with free radicals generated from the surrounding solvent and breakage or rearrangement of bonds. These effects are minimized by freezing the samples in liquid nitrogen or helium in many of the techniques discussed above, and as such damage is not applicable in the femtosecond exposures of the XFEL SFX approach. However, one consequence of immobilizing samples, by freezing or trapping within crystals, is that the structural range of dynamic cellular processes, such as polymerization, DNA segregation, protein recycling by chaperons or motility of motor proteins, can only be inferred from defined transient states. Currently, X-ray crystallography structures are not normally published unless the resolution at least reaches 3 Å, a level that is rather stringent.

Figure 6. Comparison of electron density maps from cryoEM and X-ray crystallography. Purple box – X-ray crystallography electron density maps at the indicated resolutions: (A–C) surrounding the metal ion in ADP/G-actin\(^{[67]}\); (D–F) surrounding the metal ion in ATP/G-actin\(^{[68]}\); (G) surrounding the \( \beta \)-phosphate from ADP in Arp1 in the dynactin complex\(^{[37]}\); (H) surrounding the metal ion in ADP/F-actin\(^{[36]}\); (I) surrounding the \( \gamma \)-phosphate from AMPPNP in the ParM filament\(^{[39]}\); (J) surrounding the metal ion in beta-galactosidase.\(^{[49]}\) Water molecules can be reliably placed into the maps at resolutions below 2.5 Å. The lower resolution electron density maps in B, C, E, and F were obtained by placing resolution cut-offs on the data shown in A and D.

7.1. Atomic Force Microscopy (AFM)

AFM has found many applications since its introduction,\(^{[45]}\) with recent developments including a high-speed AFM (HS-AFM), which has been used to visualize movements and structural changes in chaperons in real time.\(^{[46]}\) A recently developed tip-scan AFM was able to visualize tropomyosin (diameter 20 Å) with a temporal resolution of 10 s per frame.\(^{[47]}\) Thus, the AFM-based techniques promise to answer some of the outstanding questions concerning dynamic biological systems in real time at a comparatively high resolution (20–30 Å). EM has also been implemented in the liquid phase, with acrosomal actin bundle being imaged at 27 Å.\(^{[48]}\) However, the applications of wet EM may be limited due to the effects on dynamics from the energy of the electron beam.

8. Physiological Structures – Imaging Within Cells

The ultimate aim of structural biology is to determine the structural components within cells and to follow their changes in various physiological processes.

8.1. Cryo-Electron Tomography (cryoET)

CryoET is a technique that obtains 3D information from individual projection images, taken through a range of tilt angles, of vitrified biological specimens by EM. The acquired projection stack is then processed and merged into a tomogram
Figure 7. A hybrid model of a capped actin filament by superimposition of X-ray and cryoEM structures. A) The capped actin filament model. B–D) Enlargement of the pointed end capping model, which was obtained by superimposing the X-ray crystal structures of fragments of tropomodulin/actin onto the terminal actin protomers from the cryoEM tropomyosin/actin structure, keeping only the fragments of tropomodulin (red/cyan). Tropomodulin/tropomyosin interactions and linker regions (yellow/purple) were taken from the model proposed by Rao et al. E–G) Enlargement of barbed end capping model that was obtained by superimposing the cryoEM structures of the dynactin and tropomyosin/actin filaments, keeping only capping protein (alpha subunit in red, beta subunit in blue) from the dynactin complex. F) represents an end-on view at the barbed end.
(the 3D volume of the specimen). Individual components within the tomogram (e.g., actin filaments, microtubules, or ribosomes) can be extracted and averaged to obtain medium-to-high resolution structures (sub-tomogram averaging). Newly developed phase plates further increase image contrast, making it easier to identify cellular components within cells. In vitro cryoET structures have been determined at medium resolution (8–9 Å) for structures with high symmetry like GroEL and the HIV capsid in the intact virus. Thus, using sub-tomogram averaging (which is similar to single particle analysis in cryoEM) cryoET has become a reliable method to study the macromolecular assemblies inside cells at 10–20 Å resolution, and has been used to investigate the cytoskeleton in cellular processes like cell adhesion or mitosis, and its morphology during interactions with viruses and parasites.

CryoET is limited by how far electrons can penetrate through a biological sample, ≈1 μm, which was shown to be feasible for Ostreococcus tauri, the smallest eukaryotic cell. In order to examine bulkier specimens the sample has to be thinned. This can be achieved by cryo-sectioning, however, even the most advanced oscillatory knife causes mechanical stress and deformation, resulting in loss of resolution. Recently, focused ion beam scanning electron tomography (FIB-cryoET) has emerged as the new and superior method of sample thinning in cryoET. FIB-cryoET allows for the visualization of cellular structures like the endoplasmic reticulum, mitochondria, microtubules, and ribosomes in cells and the intracellular organization of cells from embryos to adults in developing Caenorhabditis elegans. FIB-cryoET combined with a phase plate has allowed the Golgi apparatus to be visualized within cells at unprecedented resolution and for the identification of new components. CryoET has recently been applied as a diagnostic tool to identify structural differences in microtubules between healthy and diseased states of clinical samples of patients with primary ciliary dyskinesia or with invasive ovarian cancer. Thus, cryoET is becoming an invaluable tool to study the internal structure of cells, in order to build realistic models of cellular machineries by docking higher resolution structure determined in vitro.

The future of EM imaging of biological molecules may follow the techniques that are being developed to image robust non-biological molecules, where one current trend is to decrease the electron energy to levels where the radiation damage is acceptable for imaging stable single molecules like graphene at atomic resolution, without the need for averaging. Low energy electrons when combined with holographic electron microscopy (HEM), which retains the phase information without the use of lenses and thus minimizes distortion, has been used to image graphene at 2 Å resolution. Significant advances in sample introduction will be necessary to adapt these techniques to biological molecules.

10. Conclusions

Recent advances in structural biology techniques are transforming the landscape of what can be considered as reasonable targets for structure determination. Microcrystals are now routinely used to reveal high resolution structures of rare or moderately well-behaved samples. However, it is the development of cryoEM that has most dramatically changed the perspective, with whole new classes of molecules, such as filament systems, yielding their structures to this technique. Furthermore, cryoET techniques are quickly improving the resolution of structures that can be observed within cells. The costs of these new structural biology tools are significant. Nonetheless, state-of-the-art cryoEM instruments are relatively inexpensive, at less than 1% of XFELs. Research centers are currently scrambling to install high resolution cryoEM instruments, and often multiple instruments, with the current demand outstripping supply. Given this rapid expansion, it is foreseeable that we will have a thorough structural understanding of all cellular machineries in the coming decade.

Abbreviations

(HS-)AFM, (high-speed) atomic force microscopy; CCD, charge-couple device; cryoEM, cryo-electron microscopy; cryoET, cryo-electron tomography; DED, direct electron detector; FD, fiber diffraction; FIB, focused ion beam; HEM, holographic electron microscopy; MicroED, micro electron diffraction; SFX, serial femtosecond crystallography; XFEL, X-ray free electron laser.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

actin, capping protein, cryo-electron microscopy, filaments, ParM, tropomodulin, tropomyosin

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