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REVIEW

Advances in methods for atomic resolution macromolecular structure determination

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Abstract

Recent technical advances have dramatically increased the power and scope of structural biology. New developments in high-resolution cryo-electron microscopy, serial X-ray crystallography, and electron diffraction have been especially transformative. Here we highlight some of the latest advances and current challenges at the frontiers of atomic resolution methods for elucidating the structures and dynamical properties of macromolecules and their complexes.

Keywords

Structural biology, x-ray crystallography, electron microscopy, electron diffraction, computational methods

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Introduction

Atomic-level structural information brings deep insight into macromolecular mechanism and function, but successful applications of structural biology methods are often challenging. Driven by the powerful insights they deliver, all of the major approaches—X-ray crystallography, multidimensional nuclear magnetic resonance (NMR), and electron microscopy—have evolved tremendously from their origins to their present forms through diverse technical innovations: improvements in instrumentation, analysis software, robotic automation, molecular engineering strategies, and so on. Major advances in structural biology have often come in waves, and structural biologists are now witnessing a sea change in the range and power of available methods for structure determination. These methodological advances are making it possible to illuminate molecular systems of growing complexity, and with sizes larger and smaller than have been possible before, at finer levels of spatial resolution. Likewise, new opportunities abound for dissecting the kinetic behavior and energetic landscapes of dynamic and polymorphic structures. Here we highlight some of the most recent developments and future prospects for applications of X-ray and electron-based crystallography and imaging methods.

Developments in single-particle cryo-electron microscopy

For some decades following proof-of-concept experiments in cryo-electron microscopy (cryo-EM), the technique occupied a somewhat limited niche in structural biology. Single-particle imaging in cryo-EM was primarily useful for the study of large complexes, typically reaching resolutions in the nanometer range, and particularly well-suited for the study of highly symmetrical structures such as viruses, for example. The prospects for single-particle cryo-EM changed with the introduction of new detectors, microscopes, and data analysis platforms[1,2]. These upgrades led to an explosion in the successful use of single-particle cryo-EM methods to illuminate the detailed structures of macromolecules[3,4]. Cryo-EM modalities now include single-particle methods, tomography, 2D crystallography, and microcrystal electron-diffraction (MicroED). The first two modalities rely on real space imaging of either many identical copies of a molecule (single particle) or a single sample from different angles (tomography). As a diffraction method, two-dimensional crystallography has traditionally achieved the highest resolutions from highly ordered single or multi-layer protein assemblies[5]. Based on diffraction from highly ordered three-dimensional biomolecular assemblies, MicroED has extended the attainable resolution in cryo-EM to the sub-ångstrom (Å) range using approaches borrowed from macromolecular crystallography.

The hardware and software improvements that drove the cryo-EM "resolution revolution" have enabled the technique, in favorable cases, to reach levels of detail that rival X-ray crystallography. Correspondingly, interest in cryo-EM has grown and with it a pressing need to expand accessibility to the technique. This has proven to be a major challenge owing to the high cost of purchasing and operating high-end electron microscopes. With current efforts to establish scientific centers and to develop tools that consolidate and streamline data collection, access is growing. Simultaneous efforts to improve the resolution obtainable using less-expensive microscopes are democratizing access[6,7]. As a result, two models are emerging for cryo-EM data collection. The first is driven by large national facilities that house high-end equipment and can be accessed by outside users through proposal-based systems. The second is an assortment of screening and data collection instruments operated independently by individual laboratories or research institutions.

Achievements in throughput and resolution

While computational methods had been under development since the 1970s for analyzing cryo-EM images with low signal-to-noise and performing three-dimensional image reconstructions[8], more recent hardware and software developments opened the door for high-resolution cryo-EM[1,2]. Specifically, the creation and application of cameras that could detect electrons directly allowed for the collection of microscopic data as movies rather than as individual frames[9]. This technological breakthrough led to the key data processing innovation known as "motion correction", whereby the radiation-induced drift of particles during electron exposure could be partially corrected[10]. With images less affected by blurring, microscopists could thereafter produce three-dimensional reconstructions of macromolecules at near-atomic resolution.

The dramatic improvements in image quality fueled a flurry of software developments directed toward automating data collection and processing and producing more accurate three-dimensional image reconstructions[11–13]. The current explosion of cryo-EM technology has also uniquely benefitted from coincident advances in computer science. Cryo-EM software developers have capitalized on high-performance GPU computing[14–16], cloud computing[17,18], and machine learning approaches for nearly user-free data processing[19–21]. The resulting increase in throughput is evident (Figure 1). To date, there are approximately 3,000 density map depositions and 2,000 atomic coordinate set depositions by cryo-EM at better than 4 Å resolution. If the exponential growth were to continue—we estimate the doubling time over the last 5 years to be about 1.1 years—then structure depositions by cryo-EM would be similar in number to those by X-ray diffraction by about 2023. The improvements in resolution are also notable. An example near the current resolution limit is a 1.65 Å structure of apoferritin[13]. Other recent reports in the literature, including structures of ribosomes bound to antibiotics[22] and a TRP channel bound to capsaicin and other vanilloid ligands[23], highlight the ability of cryo-EM to achieve the resolution needed for structural biology applications in areas such as drug discovery[24] (Figure 2).

Advances in imaging difficult macromolecules

Large macromolecular assemblies generally provide the easiest targets for cryo-EM, owing to the high signal-to-noise obtained in individual particle images. Recent successes elucidating the structures of large macromolecular complexes are

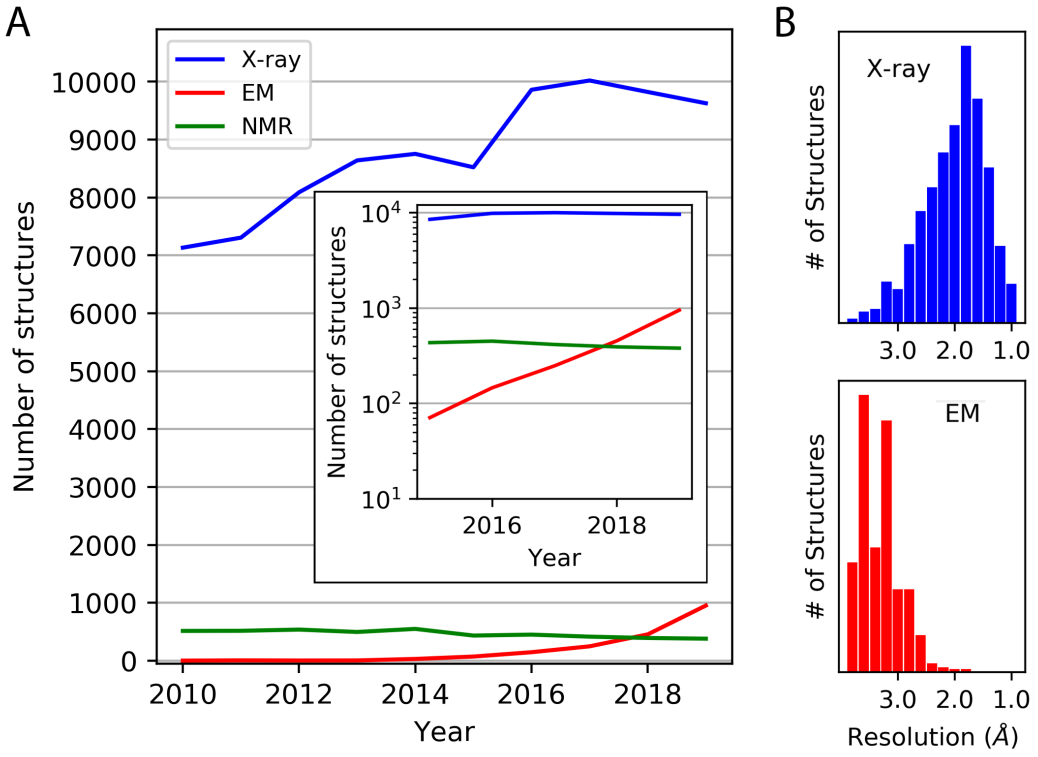


Figure 1. Deposition of atomic or near-atomic-resolution structures in the Protein Data Bank according to different experimental methods. (**A**) The number of structures deposited annually is shown between 2010 and the end of 2019, based on X-ray diffraction, cryo-electron microscopy (EM), and NMR methods. A sub-4 Å resolution criterion was imposed (for X-ray and EM). The inset shows the depositions since 2015 on a logarithmic scale. (**B**) The same set of structures are broken down by resolution. Those based on EM do not include those determined by electron diffraction methods (see Figure 4). This figure is an original image created by the authors for this publication.

too numerous to list here. Other categories of macromolecules are coming under increasing attention. Methods for analyzing filamentous assemblies, developed over the years in studies of filamentous viruses[25–27] and microtubules[28,29], are being applied to an increasing variety of systems[30–34]. Of particular interest in medicine, amyloid proteins and polypeptides, which assemble in ways that tend to be incommensurate with three-dimensional crystal formation, have been analyzed recently by cryo-EM, with numerous studies reaching near-atomic resolution[35–42].

Membrane proteins, which have been categorically difficult to crystallize and analyze by crystallography, have been challenging to study by cryo-EM as well. Nanodiscs—lipid bilayer disks bound by encircling protein molecules[43]—are providing new and fruitful routes to analyzing membrane proteins by cryo-EM[44–47]. To date, roughly 70 membrane protein complexes have been determined by cryo-EM in nanodiscs.

Proteins (and nucleic acid molecules) with molecular weights below about 100 kDa are especially challenging targets[48,49]. In a few favorable cases, near-atomic resolution has been possible for protein or enzyme assemblies in the 40–70 kDa range[50–53], but smaller proteins remain below practical (and perhaps theoretical) limits. Over the years, various ideas have been explored for using larger known structures—e.g. viral capsids, ribosomes, DNA arrays, and antibody fragments—as 'scaffolds' for attaching smaller 'cargo' proteins to make them amenable to cryo-EM imaging[54–58]. Problems, especially attachment flexibility between the scaffold and cargo, hindered much prior work. Recent advances have been made by adapting an alpha helical fusion approach, developed earlier in the area of protein design[59,60], to achieve more rigid connections between components[61–63]. The use of a modular adaptor system based on DARPins, as introduced by Liu *et al.*[62], has allowed the display and cryo-EM visualization of cargo proteins bound non-covalently to scaffolds built from symmetric protein assemblies[63,64]. Specific loop sequence mutations in the DARPin (or other adaptor protein) required to bind a given cargo protein can be identified experimentally based on separate laboratory evolution studies (see 65 for a recent review of DAPRin applications). Sub-4 Å resolution has been achieved by a scaffolding system of this type[57]. An important advance in visualizing RNA molecules in the 40 kDa range has also been demonstrated recently using a Volta phase plate to enhance image contrast, resulting in sub-4 Å resolution[66].

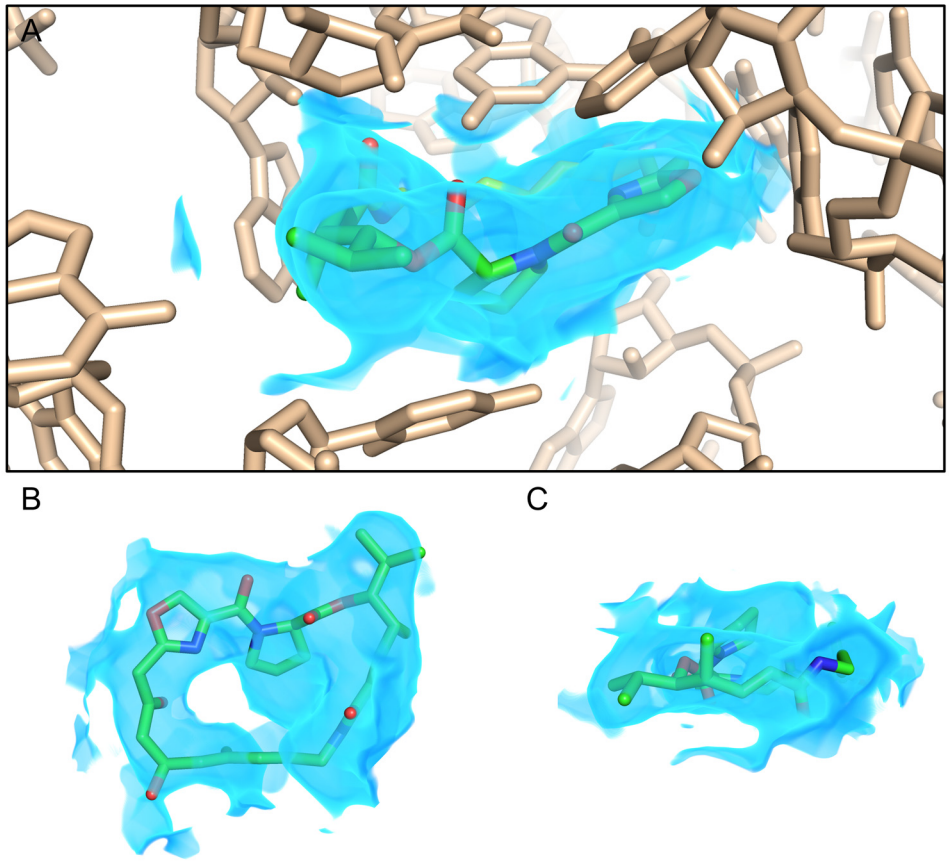


Figure 2. High-resolution cryo-electron microscopy (EM) as a tool for drug discovery. Cryo-EM density at a resolution of approximately 2.6 Å reveals a streptogramin antibiotic (green sticks) bound to the peptidyl transferase center (PTC) of a bacterial ribosome (wheat sticks), as described by Li, Pellegrino, *et al.* (https://doi.org/10.26434/chemrxiv.8346107.v2). The map is shown as a blue volume and is rendered only within 2 Å of the antibiotic for clarity (**A**). Views of the streptogramin molecule (ribosome deleted) normal to (**B**) and coplanar with (**C**) the macrocycle ring illustrate features such as the proline in the macrocycle backbone, isopropyl side chain, and carbonyl groups, allowing unambiguous placement of the drug in order to inform structure-based design. This figure is an original image created by the authors for this publication.

While recent advances in working with difficult types of proteins have been notable, key challenges remain. Many studies are limited by protein denaturation at the air–water interface, combined with the tendency of proteins to adopt preferred orientations on EM grids[67,68]. Improved methods of sample preparation and freezing are being investigated to mitigate these challenges[69].

Cryo-electron tomography (cryo-ET) offers new promise, along with unique challenges of its own. Samples, often of intact cells or tissues, are tilted under low-dose exposure, allowing the reconstruction of three-dimensional images of subcellular structures and their constituent macromolecular assemblies (reviewed in 70,71). This has led to impressive views of macromolecular machines and the cellular milieu. Key challenges in cryo-ET concern resolution, which is limited by electron dose per volume. Important advances are facilitated by averaging; methods involving "sub-tomogram" averaging boost the signal-to-noise for particles present in large copy number or those exhibiting high internal symmetry, with concomitant improvements in resolution (reviewed in 72). Sample thickness is a further limitation, and focused ion beam (FIB) milling, which carves out a thin section of sample on the EM grid prior to electron beam exposure, is making a major impact[73,74]. Given resolutions typically insufficient to trace protein backbones and establish amino acid sequences, protein labeling and identification in a cellular context by cryo-ET is another area of vital investigation[75].

Improvements in interpretation

The resolution revolution led to a series of challenges and opportunities related to the interpretation of single-particle cryo-EM data. First, the ability of microscopists to reconstruct density maps with near-atomic resolution required the development of methods and software[76] that could be used to build and refine[77–79]

molecular models from cryo-EM data, in some cases utilizing complex automated modeling[80,81] or molecular dynamics calculations[82–85]. The new breakthroughs in cryo-EM resolution resulted in a new type of experimentally derived atomic model, which in turn necessitated the development of validation methods to assess data and model quality[86,87]. This was achieved through repurposing of existing tools for analysis of molecular geometries[88], which had already been used for years by X-ray crystallographers, as well as through the creation of new tools that analyze data quality[89–91] and the fit of atomic models to three-dimensional density map reconstructions[92,93].

An emerging frontier in cryo-EM data interpretation is the analysis of energetic landscapes and the dissection of structural heterogeneity[94]. In a cryo-EM data set with a large number of particles, the equilibrium ensemble of conformational states should be statistically represented, offering the ability to model different conformations of a molecule or complex from a single sample if one can correctly classify the particles into groups sharing the same conformational state. A number of different computational strategies for performing this task are under active development[58,58,95–98]. With sufficiently large data sets, it should be possible to structurally and energetically characterize the conformational landscape and functional motions for complex molecules[99–102]. Finally, for systems where computational sorting of alternative conformational states is challenging, the field of antibody engineering has increasingly come to the rescue. For example, engineered antibody fragments of various types developed through library selection methods have been used to trap specific conformational states of interest[103,104] (reviewed in 105,106) and/or to act as fiducial markers for molecules and complexes that have ambiguous orientations, sometimes due to pseudosymmetry[107,108].

Developments in X-ray crystallography

While rapid developments in cryo-EM have placed it in the spotlight over the past several years, X-ray crystallography remains the workhorse of structural biology, yielding the majority of structures deposited annually into the Protein Data Bank (PDB) by a wide margin (Figure 1a). Additionally, for structural studies of small soluble proteins that require Å-level precision, as is often the case in the fields of enzyme catalysis, drug discovery, and protein engineering, X-ray crystallography still reigns supreme[109] (Figure 1b). X-ray crystallography has benefited from decades of research on automation. Modern synchrotron beamlines currently measure hundreds of samples per day, with very little human intervention[80,109–111]. This rapid data collection has enabled experiments such as crystallographic fragment screening, which combines high-throughput compound screening with high-resolution structural measurements[112–115].

Two major synergistic advances in X-ray crystallographic data collection and interpretation have driven exciting technological developments that complement more traditional experimental measurements. These developments have broadened the scope of questions that can be addressed using the method. First, the view of X-ray crystallography as a method for determining a single, static structure of a molecule is evolving, as several recent developments are opening exciting opportunities for extracting information about the conformational landscape by visualizing multiple structural states for a crystallized macromolecule. Second, the dramatic increase in photon flux at synchrotron beamlines and the unprecedented X-ray pulse energies available at XFEL sources have opened the door to measuring diffraction from very small or highly radiation-sensitive macromolecular crystals, enabling a variety of interesting new experiments. While the majority of crystallographic experiments are still performed using traditional measurement strategies, recent examples from the literature, some of which are highlighted below, demonstrate the deep mechanistic insight that can be obtained using exciting new experimental methods.

Crystallography at near-physiological temperatures and modeling the conformational ensemble

A bane of traditional crystallography has been its mainly static nature, notwithstanding the important information contained in atomic displacement parameters and sparsely modeled alternative conformations. In the late 1970s, cryocrystallography—wherein a crystal is frozen in liquid nitrogen and maintained at cryogenic temperature (~100 K) throughout the course of data collection—took over the field of macromolecular crystallography[116,117]. Importantly, cryocooling increased the tolerance of crystals to radiation damage, a common limiting factor for successful data collection, by approximately two orders of magnitude[118–121]. While it was appreciated several decades ago that cryocooling would alter the intrinsic dynamics of a crystallized macromolecule[122–124], the effect of cryocooling on the interpretation of mechanism was not well documented until 2009, when a study of proline isomerase demonstrated that a crystal structure determined at 100 K showed only a single side chain conformation for most residues, whereas a structure determined at 277 K revealed a series of alternative side chain conformations that were shown by mutagenesis to be critical for catalysis[125]. In the decade since this key observation was made, a number of additional cases have demonstrated that structural information derived from data collected closer to physiological temperature (or across a range of temperatures) can provide a wealth of information about the conformational ensemble beyond a single static structure[115,126–128]. As noted above, similar ideas are emerging in electron microscopy[94].

Performing crystallography at non-cryogenic temperatures is now easier than ever because modern X-ray detectors and data processing software enable the measurement of X-ray diffraction using permissibly low X-ray doses[129–131]. Additionally, computational modelling software that can use experimental X-ray diffraction data to create models of alternative conformations or entire conformational ensembles is now available[132–136], allowing crystallographers to maximize the amount of structural and biophysical information that can be accessed from data collected at non-cryogenic temperatures. Frontiers in this area of research include the measurement of diffuse (non-Bragg) scattering for the analysis of protein dynamics[137–141] and the incorporation

of full molecular dynamics simulations (of crystallized molecules) into the modelling and refinement procedure[142–145].

Serial crystallography

Within the first decade of this millennium, first light was achieved at an X-ray free electron laser (XFEL). With it, macromolecular crystallographers gained access to a powerful tool that offered exciting new opportunities but which also required a reformulation of the traditional crystallographic experiment. XFELs produce ultrafast (tens of femtoseconds) X-ray pulses with gigawatts of peak power[146]. The benefit of this extreme X-ray fluence is the ability to measure useful X-ray diffraction signals from extremely small macromolecular crystals, as small as several hundred nanometers[147–149]. A concomitant challenge, however, is that the X-ray pulses are so intense that they strip atoms of their electrons and cause Coulombic explosion of the sample[150]. Diffraction is a nearly instantaneous process, and scattered X-rays can be recorded before destruction of the sample[151]. However, owing to the severe damage, a single crystal cannot be used for the repeated measurements required to fully sample three-dimensional reciprocal space. This required the development of a new experimental strategy, coined "serial crystallography"[152]. The technique was initially developed and refined prior to the operation of the first XFEL, at synchrotron facilities[153], and has now been successfully implemented at synchrotrons worldwide[154,155]. Instead of growing large, single crystals for data collection using the rotation method, serial crystallography relies on slurries of tiny microcrystals (tens of microns or less), which are serially replenished in the X-ray beam as they are destroyed by the intense X-ray pulses. Typically, a single X-ray pulse is used per individual microcrystal, destroying it in the process. Serial crystallography relies on rapid delivery of crystals to the X-ray beam, ideally in random orientations so that all reciprocal space can be sampled uniformly from thousands of microcrystals. These two needs can be met using either a microfluidic or a fixed-target approach (Figure 3). In the microfluidic setup, a freestanding stream or jet of microcrystals is generated perpendicular to the X-ray beam, and crystals are measured as they flow through the interaction region[156–160]. Alternatively, in a fixed-target experiment, many microcrystals are simultaneously mounted on some type of solid support chip, which is then rapidly translated through the X-ray beam using robotics to expose the crystals for measurement[161–166]. Additional experimental systems that deliver microcrystals via a drop-on-demand system have also been developed, but these are not yet as widely used[167,168].

The development of serial crystallography and other types of multi-crystal experiments has opened the door to interesting new opportunities for structural biology. While serial crystallography was invented to enable measurements at XFEL sources, similar experiments are now also routinely performed at very bright synchrotron beamlines[169–172], making them more easily accessible to the scientific community. Because these approaches use many crystals to construct a complete data set, traditional concerns about radiation damage become unimportant, either because of the "diffraction-before-destruction" phenomenon observed at XFELs or because the required X-ray dose per crystal is much lower than for traditional experiments when performed at a synchrotron source[173,174]. Consequently, serial crystallography is generally performed at room temperature[175], the benefits of which have already

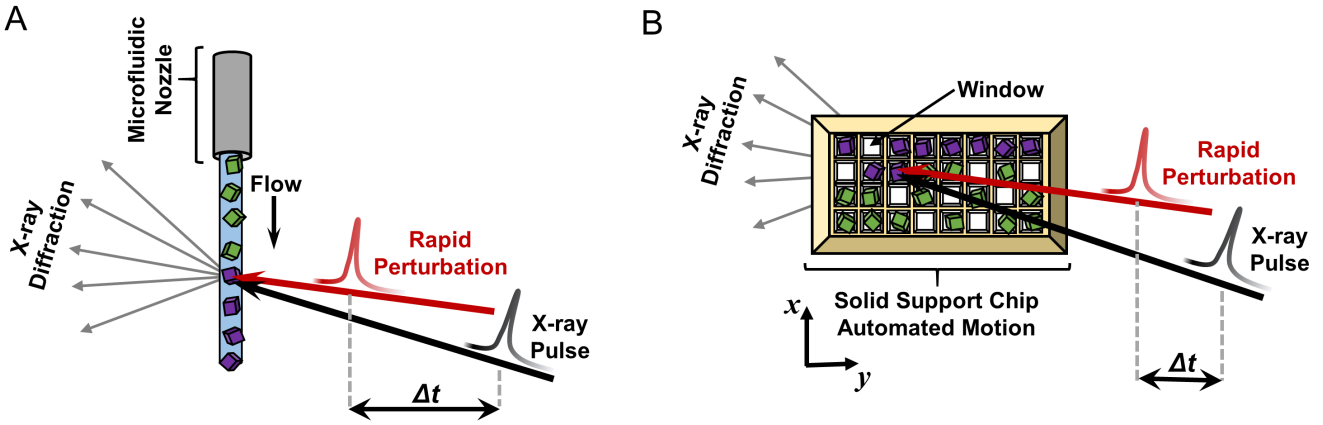
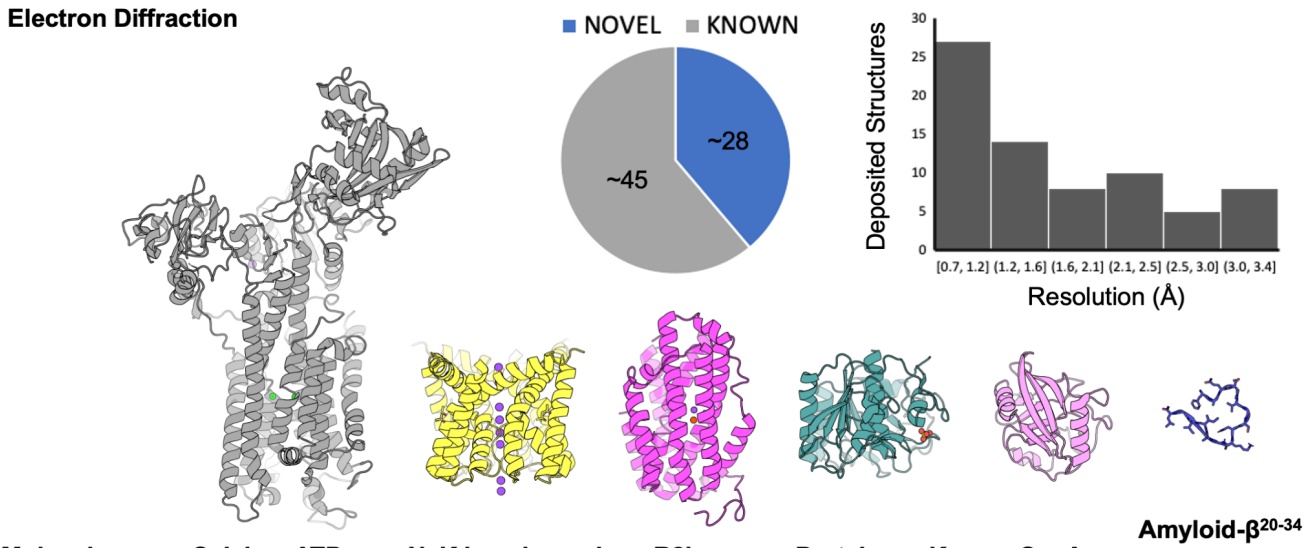


Figure 3. Sample delivery strategies for serial crystallography. In the microfluidic variety of the experiment (**A**), crystals are delivered to the X-ray beam using a microfluidic nozzle ranging from tens to hundreds of microns in diameter. A stream of randomly oriented microcrystals continuously flows perpendicular to the pulsing X-ray beam (black arrow). In the fixed-target version (**B**), microcrystals are mounted (by pipetting) in random orientations on a solid support chip that contains appropriately sized windows, and the chip is rapidly moved through the pulsing X-ray beam (black arrow) by automated translation in the x and y directions. In both diagrams, each crystal yields a single diffraction pattern capturing a random slice of reciprocal space. Crystals that have not been probed by the X-ray beam are colored green, and those that have been measured, and destroyed, are depicted in purple. In time-resolved serial crystallography, a perturbation (shown by the red arrow) is applied to the crystals with user-defined timing prior to the X-ray pulse (Δt). This figure is an original image created by the authors for this publication.



Molecule	Calcium ATPase	NaK ion channel	R2lox	Proteinase K	CypA	Amyloid-β$^{20-34}$ (L-isoAsp23)
Weight (kDa)	110	40	38	29	18	1.5
Resolution (Å)	3.4	2.5	3.0	1.5	2.5	1.1
Date	2014	2018	2019	2019	2019	2019
PDB Code	3J7T	6CPV	6QRZ	6LAW	6U5G	6NB9

Figure 4. A survey of biomolecular structures determined by electron diffraction from three-dimensional crystals. An aggregate analysis of Protein Data Bank (PDB) depositions shows the breakdown of novel versus known structures and the distribution of structures according to their reported resolution (in Å). Cartoon diagrams of several representative structures are shown; below each structure are properties (molecular weight and resolution) as well as identifiers (date associated with deposition release and PDB code). This figure is an original image created by the authors for this publication.

been described, and the approach has proven useful for studies of radiation-sensitive samples, such as metalloproteins[176–179]. Furthermore, because serial crystallography enables efficient measurements from small crystals, the technique has produced crystal structures of challenging targets for which large crystals could not be obtained, including proteins such as GPCR–arrestin complexes[180] and RNA polymerase[181]. Some proteins naturally form small crystals within cells, as exemplified by early structures of virus polyhedra[182,183]; serial X-ray methods have proven useful in structure determination for multiple types of such *in vivo* protein crystals[184–186]. Measurement of very small crystals with highly coherent X-rays can also produce signal between Bragg peaks. Solving the phase problem from an oversampled molecular Fourier transform represents an additional frontier in the field[187–191]. Finally, because serial crystallography allows the rapid measurement of many crystalline specimens, it is possible to exploit clustering methods that allow grouping of similar measurements, potentially leading to additional structural insight through the comparison of crystal polymorphs[192,193].

The advent of serial crystallography using femtosecond XFEL pulses has led to a renaissance in time-resolved structural studies of macromolecules[194]. Traditional high-resolution structural techniques are ensemble measurements, which yield information only about conformational states of molecules that are significantly populated at equilibrium. This limitation makes it challenging to study macromolecular dynamics, because protein motions can involve the formation of transient, high-energy configurations, on timescales shorter than traditional X-ray measurement. Although challenging to study, dynamics are vital for function. Dynamics play fundamental roles in enzyme catalysis[125,195–198], protein–protein interactions[199], allosteric signaling[115], and protein evolution[127,200]. Dynamics also have important practical implications, as they can result in the formation of cryptic binding sites that are actionable for drug discovery[201–203].

In the late 1980s and early 1990s, several groups demonstrated the first successful time-resolved macromolecular X-ray crystallography (and solution scattering) experiments, essentially bringing together elements of ultrafast pump-probe spectroscopy and X-ray structural analysis to observe molecular motions[204]. In these experiments, a rapid perturbation was applied to the crystallized proteins to synchronize conformational changes[205], and then one of two methods was used to observe the resulting dynamics in a time-dependent manner. In one form of the experiment, the initial perturbation was followed by freeze-trapping

of the excited states by rapid cryocooling at defined time delays, followed by X-ray structure determination using traditional methods[206–209,209]. Because protein dynamics are dependent on external perturbations, but also highly temperature-dependent, the freeze-trapping method was determined to be of limited utility owing to challenges in data interpretation. It was soon replaced by methods that could utilize the pulsed nature of the synchrotron beam (approximately 100 ps pulse duration) to act as a high-speed camera, capable of capturing structural snapshots of the motions in real time[210]. By varying the time delay between the perturbation and X-ray pulses, researchers could capture molecules in various stages of conformational transitions[211–214]. Time-resolved crystallography (and X-ray solution scattering) proved to be an extremely powerful tool for studying macromolecular dynamics[215–217], but the need to synchronize conformational changes in a significant fraction of the crystallized molecules still posed a substantial technical challenge, and time-resolved measurements were relegated to a handful of systems with well-characterized photoactivity, such as myoglobin and photoactive yellow protein, where it was straightforward to initiate conformational changes using a pulsed laser.

The use of serial crystallography for time-resolved studies has facilitated a series of recent technological developments and experimental achievements[194]. Studies have revealed the sub-picosecond chromophore gymnastics that result in photoactivation of photoreceptors and fluorescent proteins[218–222]. The long-sought mechanism of photosynthetic water splitting by photosystem II has been revealed using ultrafast time-resolved crystallography paired with simultaneous X-ray spectroscopy[223–225]. Covalently bound enzyme–substrate reaction intermediates, which had been theorized but never observed, have been identified[226,227]. Additionally, a substantial effort has been made to expand time-resolved measurements to systems that do not have any photoactivity, making these experiments amenable to essentially any protein of interest. New perturbation methods that have been successfully utilized include temperature-jump[228], rapid mixing[226,227], ligand or substrate photocaging[229], and rapid application of electric fields[230]. Finally, it is worth noting that the success of time-resolved crystallography has inspired the more recent development of time-resolved cryo-EM[231]. In such an experiment, molecules are mixed as they are sprayed onto a cryo-EM sample grid, and a time delay is introduced between the mixing and vitrification processes[232–234]. The time-resolution of these experiments is limited to tens of milliseconds by the time required to vitrify the sample, but they have been successfully applied to study relatively slow motions of large complexes, such as ribosomes during protein translation[235]. Although limited in their temporal resolution, these experiments offer the opportunity to study large-amplitude motions that are incompatible with a crystal lattice.

The resurgence of electron diffraction

For the past half century, the use of electron diffraction remained limited, often applied to unique samples or used as a complement to X-ray studies for macromolecular analysis. Common approaches to electron diffraction relied on the electron microscope, often configured to sample from the back focal plane of its objective lens. Use of the electron microscope as a tool for three-dimensional characterization of macromolecules dates to the mid-twentieth century when Klug and colleagues pioneered molecular electron microscopy at the MRC Laboratory of Molecular Biology in Cambridge[236,237]. In contrast to single molecules or small molecular assemblies, extended two-dimensional molecular crystals are ideally suited for diffraction. Translational invariance in the transforms of two-dimensional crystals facilitates the measurement of high-resolution diffraction. This was exploited by Henderson and Unwin to characterize crystals of bacteriorhodopsin (bR)[238]. In fact, their use of electron diffraction necessitated thin crystals of bR to avoid extensive multiple scattering and absorption artifacts[239]. The potent interaction of the electron beam with the single layer of molecules in two-dimensional bR crystals was sufficient to yield an initial molecular resolution structure of the protein and later several atomic models[240,241]. Two-dimensional electron crystallography has since produced a number of important high-resolution structures, including the plant light-harvesting complex[242] and aquaporin[243–245]. At 1.9 Å, the structure of the water pore protein, aquaporin-0, was the highest resolution structure determined by two-dimensional electron crystallography[246]. The small quantity of high-resolution structures determined by electron crystallography may be because of its unique sample demands. For two-dimensional crystals, missing wedge effects impact resolution in the direction normal to the molecular layer, while crystal bending and in-plane defects may limit resolution along the layer[247]. Despite these limitations, electron microscopes offer an immediate benefit for structure determination, since phases can be obtained by direct imaging of two-dimensional crystals[248].

In the early 1970s, electron microscopy of protein nanocrystals in liquid-filled cells demonstrated the power of using electron beams for three-dimensional crystallography[249]. By mitigating radiation damage, cryogenic cooling of samples further facilitated the extraction of high-resolution information from three-dimensional crystals by electron diffraction[250]. Beam attenuation measurements in these early studies demonstrated that crystals of the protein catalase—a few hundred nanometers thick—could be investigated under the electron microscope[251]. Despite these early efforts, electron diffraction of three-dimensional macromolecular crystals was impacted by many of the same challenges that affected two-dimensional electron crystallography. Multiple scattering and absorption effects presented added concerns[252,253]. These limitations would ultimately delay the widespread application of electron diffraction to three-dimensional macromolecular crystals. In contrast, three-dimensional electron crystallography of small molecules and materials continued to progress, ultimately yielding structures of various inorganic and organic structures before those of macromolecules could be determined[254–256].

Electron diffraction is now widely used for the study of three-dimensional inorganic and organic crystals[257]. Its application to three-dimensional protein crystals re-emerged recently in

work by Gonen[258], Abrahams[259], Yonekura[260], and many others. These efforts benefitted from several compelling aspects of electron diffraction, including the large electron scattering cross section, the shorter electron wavelength (and flatter Ewald sphere) in conventional electron microscopes, and the ability of the method to adopt many of the computational developments and software tools honed for X-ray crystallography over the last several decades[261,262]. This lowered the barriers for application of the method. However, the early studies in three-dimensional electron diffraction of protein crystals brought to light key distinctions between the use of X-rays and electrons for crystallography. Because of the strong interaction of electrons with matter, electron crystallography of three-dimensional protein crystals resuscitated concerns over multiple scattering, the resulting fidelity of diffraction measurements, and ultimately the accuracy of structures determined by these methods[253]. While some of these concerns have been dispelled by the determination of novel and *ab initio* macromolecular structures[263–266] (Figure 4), there is considerable room for improvements in our understanding and practice of electron diffraction from protein nanocrystals.

The ability to measure diffraction from sub-micron-sized areas of three-dimensional protein nanocrystals, on the scale of the conventional domain block, has further facilitated the interrogation of individual crystallites derived from bundles or larger clusters[267]. Crystals as thick as half a micrometer might be tolerated, but thinner specimens avoid heavy absorption and minimize multiple scattering[268]. The term 'MicroED' was coined to emphasize the requirement for small crystals and is complemented by terms like three-dimensional electron diffraction (3DED) and continuous rotation electron diffraction (CRED)[257]. Some self-associating proteins—amyloidogenic peptides, for example—tend to grow small or needle-like crystals, making them natural targets for study. For general applications, methods are being investigated for reliably producing microcrystals, or for nano-machining thin sections from larger protein crystals by FIB milling[268–270]. To date, less than a hundred structures determined by electron diffraction from three-dimensional crystals can be found in the PDB. Among the largest is a 110 kDa calcium ATPase; most are small proteins and peptides (Figure 4).

One notable challenge still faced by electron diffraction is *de novo* phasing. The majority of MicroED structures deposited to date have not been novel (Figure 4); many were determined by molecular replacement based on highly similar known structures. Some structures have been determined by direct methods, thus far only when macromolecules were small (e.g. oligopeptides) and diffracted to very high resolution. The weaker scattering for heavier atoms by electrons compared to X-rays, and the absence of anomalous scattering effects, may call for remedies to the phasing problem that could be unique to electron diffraction. For example, owing to the strength of electron scattering, information useful for structure determination might be extractable from secondary or 'dynamic' scattering phenomena[252,271]. Scattering factors for electrons are distinct from those used in X-ray experiments and may need to be further refined. Theoretical and computational efforts to more accurately analyze electron diffraction data will no doubt benefit from ongoing improvements to detectors and from the collection of energy-filtered patterns[272].

As electron diffraction develops beyond initial demonstrations, new opportunities and challenges arise. Recent studies have demonstrated increasingly facile structure determination of organic molecules, and the scope of substrates continues to grow. Electron nanobeams (5–100 nm in diameter) have enabled some early studies on the fine structure of macromolecular crystals and offer growth in new areas of serial or multi-structure crystallography[273–275]. However, the advantageous features of automation, speed, and process integration offered by most modern X-ray beamlines are not yet available for electron diffraction. Wider adoption of the method will necessarily be linked to its accessibility and its capacity to rapidly determine informative and novel structures.

Summary

Methods for elucidating the three-dimensional structures of macromolecules continue to expand and diversify. The technical advances surveyed here push the envelope in terms of the biological systems that can be elucidated in atomic detail and the kinds of mechanistic insights that might be extracted. On one hand, improvements in robustness and automation are lowering the barriers to entry and making applications accessible to a growing body of scientists working across expansive areas of molecular biology. On the other hand, forward progress is bringing new challenges into view. Interestingly, some of the emerging challenges have historical roots, having arisen in somewhat different contexts in other areas of structural biology. The phase problem, re-emerging now in MicroED, was surmounted in the field of X-ray diffraction by heavy atom and anomalous phasing methods. The challenges of interpreting polymorphic and dynamic systems, a key goal of many of the methods discussed here, invoke connections to NMR and molecular dynamics simulation methods, which championed the study of dynamics for several decades. And efforts to expand the reach of various X-ray and electron microscopy and diffraction methods to new kinds of macromolecular systems are drawing increasingly on advances in the area of protein design. Thus, exciting developments in structural biology methods, aided by advanced computing and other approaches (including many not discussed here), are steadily expanding our ability to dissect the structure and dynamics of biological molecules in atomic detail. Combinations of different methods, new and old, should bring us closer to this ultimate goal.

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References

1. Callaway E: **The revolution will not be crystallized: A new method sweeps through structural biology.** *Nature.* 2015; **525**(7568): 172–4.
PubMed Abstract | Publisher Full Text

2. Subramaniam S: **The cryo-EM revolution: Fueling the next phase.** *IUCrJ.* 2019; **6**(Pt 1): 1–2.
PubMed Abstract | Publisher Full Text | Free Full Text

3. F Lyumkis D: **Challenges and opportunities in cryo-EM single-particle analysis.** *J Biol Chem.* 2019; **294**(13): 5181–97.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

4. Bai XC, McMullan G, Scheres SHW: **How cryo-EM is revolutionizing structural biology.** *Trends Biochem Sci.* 2015; **40**(1): 49–57.
PubMed Abstract | Publisher Full Text

5. Glaeser RM ed: **Electron crystallography of biological macromolecules.** Oxford; New York: Oxford University Press; 2007.
Reference Source

6. F Herzik MA, Wu M, Lander GC: **Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV.** *Nat Methods.* 2017; **14**(11): 1075–8.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

7. F Naydenova K, McMullan G, Peet MJ, *et al.*: **CryoEM at 100 keV: A demonstration and prospects.** *IUCrJ.* 2019; **6**(Pt 6): 1086–98.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

8. Frank J, Goldfarb W, Eisenberg D, *et al.*: **Reconstruction of glutamine synthetase using computer averaging.** *Ultramicroscopy.* 1978; **3**(3): 283–90.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Faruqi AR, Henderson R, Pryddetch M, *et al.*: **Direct single electron detection with a CMOS detector for electron microscopy.** *Nucl Instrum Meth A.* 2005; **546**(1–2): 170–5.
Publisher Full Text

10. F Li X, Mooney P, Zheng S, *et al.*: **Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM.** *Nat Methods.* 2013; **10**(6): 584–90.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

11. F Punjani A, Rubinstein JL, Fleet DJ, *et al.*: **cryoSPARC: Algorithms for rapid unsupervised cryo-EM structure determination.** *Nat Meth.* 2017; **14**(3): 290–6.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

12. Grant T, Rohou A, Grigorieff N: ***cis*TEM, user-friendly software for single-particle image processing.** *eLife.* 2018; **7**: e35383.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Zivanov J, Nakane T, Forsberg BO, *et al.*: **New tools for automated high-resolution cryo-EM structure determination in RELION-3.** *eLife.* 2018; **7**: e42166.
PubMed Abstract | Publisher Full Text | Free Full Text

14. Li X, Grigorieff N, Cheng Y: **GPU-enabled FREALIGN: Accelerating single particle 3D reconstruction and refinement in Fourier space on graphics processors.** *J Struct Biol.* 2010; **172**(3): 407–12.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Zhang X, Zhang X, Hong Zhou Z: **Low cost, high performance GPU computing solution for atomic resolution cryoEM single-particle reconstruction.** *J Struct Biol.* 2010; **172**(3): 400–6.
PubMed Abstract | Publisher Full Text | Free Full Text

16. F Kimanius D, Forsberg BO, Scheres SH, *et al.*: **Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2.** *eLife.* 2016; **5**: e18722.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

17. Baldwin PR, Tan YZ, Eng ET, *et al.*: **Big data in cryoEM: Automated collection, processing and accessibility of EM data.** *Curr Opin Microbiol.* 2018; **43**: 1–8.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Cianfrocco MA, Lahiri I, DiMaio F, *et al.*: **cryoem-cloud-tools: A software platform to deploy and manage cryo-EM jobs in the cloud.** *J Struct Biol.* 2018; **203**(3): 230–5.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Langlois R, Pallesen J, Frank J: **Reference-free particle selection enhanced with semi-supervised machine learning for cryo-electron microscopy.** *J Struct Biol.* 2011; **175**(3): 353–61.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Wang F, Gong H, Liu G, *et al.*: **DeepPicker: A deep learning approach for fully automated particle picking in cryo-EM.** *J Struct Biol.* 2016; **195**(3): 325–36.
PubMed Abstract | Publisher Full Text

21. F Li Y, Cash JN, Tesmer JJG, *et al.*: **High-Throughput Cryo-EM Enabled by User-Free Preprocessing Routines.** *Structure.* 2020; S0969-2126(20)30080-0.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

22. Li Q, Pellegrino J, Lee DJ, *et al.*: **Synthesis and Mechanism of Action of Group A Streptogramin Antibiotics That Overcome Resistance.** 2019.
Reference Source

23. F Cao E, Liao M, Cheng Y, *et al.*: **TRPV1 structures in distinct conformations reveal activation mechanisms.** *Nature.* 2013; **504**(7478): 113–8.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

24. Merino F, Raunser S: **Electron Cryo-microscopy as a Tool for Structure-Based Drug Development.** *Angew Chem Int Ed Engl.* 2017; **56**(11): 2846–60.
PubMed Abstract | Publisher Full Text

25. Finch JT, Klug A: **Three-dimensional reconstruction of the stacked-disk aggregate of tobacco mosaic virus protein from electron micrographs.** *Philos Trans R Soc Lond B Biol Sci.* 1971; **261**(837): 211–9.
PubMed Abstract | Publisher Full Text

26. Ge P, Zhou ZH: **Hydrogen-bonding networks and RNA bases revealed by cryo electron microscopy suggest a triggering mechanism for calcium switches.** *Proc Natl Acad Sci U S A.* 2011; **108**(23): 9637–42.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Wang YA, Yu X, Overman S, *et al.*: **The structure of a filamentous bacteriophage.** *J Mol Biol.* 2006; **361**(2): 209–15.
PubMed Abstract | Publisher Full Text

28. F Zhang R, Alushin GM, Brown A, *et al.*: **Mechanistic Origin of Microtubule Dynamic Instability and Its Modulation by EB Proteins.** *Cell.* 2015; **162**(4): 849–59.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

29. Tan D, Rice WJ, Sosa H: **Structure of the kinesin13-microtubule ring complex.** *Structure.* 2008; **16**(11): 1732–9.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Fujii T, Kato T, Namba K: **Specific arrangement of alpha-helical coiled coils in the core domain of the bacterial flagellar hook for the universal joint function.** *Structure.* 2009; **17**(11): 1485–93.
PubMed Abstract | Publisher Full Text

31. Braun T, Vos MR, Kalisman N, *et al.*: **Archaeal flagellin combines a bacterial type IV pilin domain with an Ig-like domain.** *Proc Natl Acad Sci U S A.* 2016; **113**(37): 10352–10357.
Publisher Full Text

32. F Yamaguchi T, Toma S, Terahara N, *et al.*: **Structural and Functional Comparison of *Salmonella* Flagellar Filaments Composed of FljB and FliC.** *Biomolecules.* 2020; **10**(2): 246.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

33. F Poweleit N, Ge P, Nguyen HH, *et al.*: **CryoEM structure of the *Methanospirillum hungatei* archaellum reveals structural features distinct from the bacterial flagellum and type IV pilus.** *Nat Microbiol.* 2016; **2**: 16222.
Publisher Full Text | Faculty Opinions Recommendation

34. Zheng W, Andersson M, Mortezaei N, *et al.*: **Cryo-EM structure of the CFA/I pilus rod.** *IUCrJ.* 2019; **6**(Pt 5): 815–821.
PubMed Abstract | Publisher Full Text | Free Full Text

35. F Gremer L, Schölzel D, Schenk C, *et al.*: **Fibril structure of amyloid-β(1–42) by cryo–electron microscopy.** *Science.* 2017; **358**(6359): 116–119.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

36. Schmidt M, Wiese S, Adak V, *et al.*: **Cryo-EM structure of a transthyretin-derived amyloid fibril from a patient with hereditary ATTR amyloidosis.** *Nat Commun.* 2019; **10**(1): 5008.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Li B, Ge P, Murray KA, *et al.*: **Cryo-EM of full-length α-synuclein reveals fibril polymorphs with a common structural kernel.** *Nat Commun.* 2018; **9**(1): 3609.
PubMed Abstract | Publisher Full Text | Free Full Text

38. F Fitzpatrick AWP, Falcon B, He S, *et al.*: **Cryo-EM structures of tau filaments from Alzheimer's disease.** *Nature.* 2017; **547**(7662): 185–190.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

39. Cao Q, Boyer DR, Sawaya MR, *et al.*: **Cryo-EM structures of four polymorphic TDP-43 amyloid cores.** *Nat Struct Mol Biol.* 2019; **26**(7): 619–627.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Boyer DR, Li B, Sun C, *et al.*: **Structures of fibrils formed by α-synuclein hereditary disease mutant H50Q reveal new polymorphs.** *Nat Struct Mol Biol.* 2019; **26**(11): 1044–1052.
PubMed Abstract | Publisher Full Text | Free Full Text

41. F Hervas R, Rau MJ, Park Y, *et al.*: **Cryo-EM structure of a neuronal functional amyloid implicated in memory persistence in *Drosophila*.** *Science.* 2020; **367**(6483): 1230–4.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

42. Schweighauser M, Shi Y, Tarutani A, *et al.*: **Structures of α-synuclein filaments from multiple system atrophy.** *Neuroscience.* 2020.
Publisher Full Text

Faculty Opinions Recommended

43. Bayburt TH, Sligar SG: **Membrane protein assembly into Nanodiscs.** *FEBS Lett.* 2010; **584**(9): 1721–7.
PubMed Abstract | Publisher Full Text | Free Full Text

44. F Gao Y, Cao E, Julius D, *et al.*: **TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action.** *Nature.* 2016; **534**(7607): 347–51.
Publisher Full Text | Faculty Opinions Recommendation

45. F Liu S, Chang S, Han B, *et al.*: **Cryo-EM structures of the human cation-chloride cotransporter KCC1.** *Science.* 2019; **366**(6464): 505–8.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

46. Thonghin N, Kargas V, Clews J, *et al.*: **Cryo-electron microscopy of membrane proteins.** *Methods.* 2018; **147**: 176–86.
PubMed Abstract | Publisher Full Text

47. Nasr ML, Wagner G: **Covalently circularized nanodiscs; challenges and applications.** *Curr Opin Struct Biol.* 2018; **51**: 129–34.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Henderson R: **The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules.** *Q Rev Biophys.* 1995; **28**(2): 171–93.
PubMed Abstract | Publisher Full Text

49. Glaeser RM, Hall RJ: **Reaching the information limit in cryo-EM of biological macromolecules: Experimental aspects.** *Biophys J.* 2011; **100**(10): 2331–7.
PubMed Abstract | Publisher Full Text | Free Full Text

50. F Merk A, Bartesaghi A, Banerjee S, *et al.*: **Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery.** *Cell.* 2016; **165**(7): 1698–707.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

51. F Khoshouei M, Radjainia M, Baumeister W, *et al.*: **Cryo-EM structure of haemoglobin at 3.2 Å determined with the Volta phase plate.** *Nat Commun.* 2017; **8**: 16099.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

52. F Herzik MA Jr, Wu M, Lander GC: **High-resolution structure determination of sub-100 kDa complexes using conventional cryo-EM.** *Nat Commun.* 2019; **10**(1): 1032.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

53. F Fan X, Wang J, Zhang X, *et al.*: **Single particle cryo-EM reconstruction of 52 kDa streptavidin at 3.2 Angstrom resolution.** *Nat Commun.* 2019; **10**(1): 2386.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

54. Kratz PA, Böttcher B, Nassal M: **Native display of complete foreign protein domains on the surface of hepatitis B virus capsids.** *Proc Natl Acad Sci U S A.* 1999; **96**(5): 1915–20.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Martin TG, Bharat TAM, Joerger AC, *et al.*: **Design of a molecular support for cryo-EM structure determination.** *Proc Natl Acad Sci U S A.* 2016; **113**(47): E7456–E7463.
PubMed Abstract | Publisher Full Text | Free Full Text

56. Wu S, Avila-Sakar A, Kim JM, *et al.*: **Fabs enable single particle cryoEM studies of small proteins.** *Structure.* 2012; **20**(4): 582–92.
PubMed Abstract | Publisher Full Text | Free Full Text

57. Aksel T, Yu Z, Cheng Y, *et al.*: **Molecular goniometers for single-particle cryo-EM of DNA-binding proteins.** *Biophysics.* 2020.
Publisher Full Text

58. F Zhang C, Cantara W, Jeon Y, *et al.*: **Analysis of discrete local variability and structural covariance in macromolecular assemblies using Cryo-EM and focused classification.** *Ultramicroscopy.* 2019; **203**: 170–80.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

59. Padilla JE, Colovos C, Yeates TO: **Nanohedra: Using symmetry to design self assembling protein cages, layers, crystals, and filaments.** *Proc Natl Acad Sci U S A.* 2001; **98**(5): 2217–21.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Kwon NY, Kim Y, Lee JO: **The application of helix fusion methods in structural biology.** *Curr Opin Struct Biol.* 2020; **60**: 110–6.
PubMed Abstract | Publisher Full Text

61. F Coscia F, Estrozi LF, Hans F, *et al.*: **Fusion to a homo-oligomeric scaffold allows cryo-EM analysis of a small protein.** *Sci Rep.* 2016; **6**: 30909.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

62. Liu Y, Gonen S, Gonen T, *et al.*: **Near-atomic cryo-EM imaging of a small protein displayed on a designed scaffolding system.** *Proc Natl Acad Sci U S A.* 2018; **115**(13): 3362–7.
PubMed Abstract | Publisher Full Text | Free Full Text

63. Yao Q, Weaver SJ, Mock JY, *et al.*: **Fusion of DARPin to Aldolase Enables Visualization of Small Protein by Cryo-EM.** *Structure.* 2019; **27**(7): 1148–1155.e3.
PubMed Abstract | Publisher Full Text | Free Full Text

64. F Liu Y, Huynh DT, Yeates TO: **A 3.8 Å Resolution cryo-EM Structure of a Small Protein Bound to an Imaging Scaffold.** *Nat Commun.* 2019; **10**(1): 1864.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

65. Mittl PR, Ernst P, Plückthun A: **Chaperone-assisted structure elucidation with DARPins.** *Curr Opin Struct Biol.* 2020; **60**: 93–100.
PubMed Abstract | Publisher Full Text

66. F Zhang K, Li S, Kappel K, *et al.*: **Cryo-EM Structure of a 40 kDa SAM-IV Riboswitch RNA at 3.7 Å resolution.** *Nat Commun.* 2019; **10**(1): 5511.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

67. Noble AJ, Dandey VP, Wei H, *et al.*: **Routine Single Particle CryoEM Sample and Grid Characterization by Tomography.** *eLife.* 2018; **7**: e34257.
PubMed Abstract | Publisher Full Text | Free Full Text

68. Glaeser RM, Han BG: **Opinion: Hazards Faced by Macromolecules When Confined to Thin Aqueous Films.** *Biophys Rep.* 2017; **3**(1): 1–7.
PubMed Abstract | Publisher Full Text | Free Full Text

69. F Noble AJ, Wei H, Dandey VP, *et al.*: **Reducing effects of particle adsorption to the air-water interface in cryo-EM.** *Nat Methods.* 2018; **15**(10): 793–795.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

70. Wagner J, Schaffer M, Fernández-Busnadiego R: **Cryo-electron tomography-the cell biology that came in from the cold.** *FEBS Lett.* 2017; **591**(17): 2520–33.
PubMed Abstract | Publisher Full Text

71. Henderson LD, Beeby M: **High-Throughput Electron Cryo-tomography of Protein Complexes and Their Assembly.** *Methods Mol Biol.* 2018; **1764**: 29–44.
PubMed Abstract | Publisher Full Text

72. Leigh KE, Navarro PP, Scaramuzza S, *et al.*: **Subtomogram averaging from cryo-electron tomograms.** *Methods Cell Biol.* 2019; **152**: 217–59.
PubMed Abstract | Publisher Full Text

73. Marko M, Hsieh C, Schalek R, *et al.*: **Focused-ion-beam thinning of frozen-hydrated biological specimens for cryo-electron microscopy.** *Nat Methods.* 2007; **4**(3): 215–7.
PubMed Abstract | Publisher Full Text

74. Schaffer M, Mahamid J, Engel BD, *et al.*: **Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins.** *J Struct Biol.* 2017; **197**(2): 73–82.
PubMed Abstract | Publisher Full Text

75. Oda T: **Three-dimensional structural labeling microscopy of cilia and flagella.** *Microscopy (Oxf).* 2017; **66**(4): 234–44.
PubMed Abstract | Publisher Full Text

76. DiMaio F, Chiu W: **Tools for Model Building and Optimization into Near-Atomic Resolution Electron Cryo-Microscopy Density Maps.** *Meth Enzymol.* 2016; **579**: 255–76.
PubMed Abstract | Publisher Full Text | Free Full Text

77. Afonine PV, Poon BK, Read RJ, *et al.*: **Real-space refinement in PHENIX for cryo-EM and crystallography.** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 6): 531–44.
PubMed Abstract | Publisher Full Text | Free Full Text

78. Brown A, Long F, Nicholls RA, *et al.*: **Tools for macromolecular model building and refinement into electron cryo-microscopy reconstructions.** *Acta Crystallogr D Biol Crystallogr.* 2015; **71**(Pt 1): 136–53.
PubMed Abstract | Publisher Full Text | Free Full Text

79. Nicholls RA, Tykac M, Kovalevskiy O, *et al.*: **Current approaches for the fitting and refinement of atomic models into cryo-EM maps using CCP-EM.** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 6): 492–505.
PubMed Abstract | Publisher Full Text | Free Full Text

80. Terwilliger TC, Adams PD, Afonine PV, *et al.*: **A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps.** *Nat Methods.* 2018; **15**(11): 905–8.
PubMed Abstract | Publisher Full Text | Free Full Text

81. Wang RYR, Song Y, Barad BA, *et al.*: **Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta.** *eLife.* 2016; **5**: e17219.
PubMed Abstract | Publisher Full Text | Free Full Text

82. F Singharoy A, Teo I, McGreevy R, *et al.*: **Molecular dynamics-based refinement and validation for sub-5 Å cryo-electron microscopy maps.** *eLife.* 2016; **5**: e16105.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

83. F Kim DN, Moriarty NW, Kirmizialtin S, *et al.*: **Cryo_fit: Democratization of flexible fitting for cryo-EM.** *J Struct Biol.* 2019; **208**(1): 1–6.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

84. Igaev M, Kutzner C, Bock LV, *et al.*: **Automated cryo-EM structure refinement using correlation-driven molecular dynamics.** *eLife.* 2019; **8**: e43542.
PubMed Abstract | Publisher Full Text | Free Full Text

85. Tama F, Miyashita O, Brooks CL: **Normal mode based flexible fitting of high-resolution structure into low-resolution experimental data from cryo-EM.** *J Struct Biol.* 2004; **147**(3): 315–26.
PubMed Abstract | Publisher Full Text

86. F Afonine PV, Klaholz BP, Moriarty NW, *et al.*: **New tools for the analysis and validation of cryo-EM maps and atomic models.** *Acta Crystallogr D Struct Biol.* 2018; **74**: 814–40.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

87. F Neumann P, Dickmanns A, Ficner R: **Validating Resolution Revolution.** *Structure.* 2018; **26**(12): 785–795.e4.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

88. Williams CJ, Headd JJ, Moriarty NW, *et al.*: **MolProbity: More and better reference data for improved all-atom structure validation.** *Protein Sci.* 2018; **27**(1): 293–315.
PubMed Abstract | Publisher Full Text | Free Full Text

89. Heymann JB, Marabini R, Kazemi M, *et al.*: **The first single particle analysis Map Challenge: A summary of the assessments.** *J Struct Biol.* 2018; **204**(2): 291–300.
PubMed Abstract | Publisher Full Text | Free Full Text

90. F Avramov T, Vyenielo D, Gomez-Blanco J, *et al.*: **Deep Learning for Validating and Estimating Resolution of Cryo-Electron Microscopy Density Maps †.** *Molecules.* 2019; **24**(6): 1181.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

91. Rohou A: **Fourier shell correlation criteria for local resolution estimation.** *Biophysics.* 2020.
Publisher Full Text

92. F Herzik MA, Fraser JS, Lander GC: **A Multi-model Approach to Assessing Local and Global Cryo-EM Map Quality.** *Structure.* 2019; **27**(2): 344–358.e3.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

93. Barad BA, Echols N, Wang RYR, *et al.*: **EMRinger: Side chain–directed model and map validation for 3D cryo-electron microscopy.** *Nat Methods.* 2015; **12**(10): 943–6.
PubMed Abstract | Publisher Full Text | Free Full Text

94. F Chen CY, Chang YC, Lin BL, *et al.*: **Temperature-Resolved Cryo-EM Uncovers Structural Bases of Temperature-Dependent Enzyme Functions.** *J Am Chem Soc.* 2019; **141**(51): 19983–7.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

95. Liao HY, Hashem Y, Frank J: **Efficient Estimation of Three-Dimensional Covariance and its Application in the Analysis of Heterogeneous Samples in Cryo-Electron Microscopy.** *Structure.* 2015; **23**(6): 1129–37.
PubMed Abstract | Publisher Full Text | Free Full Text

96. Scheres SHW: **Processing of Structurally Heterogeneous Cryo-EM Data in RELION.** *Methods Enzymol.* 2016; **579**: 125–57.
PubMed Abstract | Publisher Full Text

97. F Nakane T, Kimanius D, Lindahl E, *et al.*: **Characterisation of molecular motions in cryo-EM single-particle data by multi-body refinement in RELION.** *eLife.* 2018; **7**: e36861.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

98. Zhang W, Kimmel M, Spahn CMT, *et al.*: **Heterogeneity of large macromolecular complexes revealed by 3D cryo-EM variance analysis.** *Structure.* 2008; **16**(12): 1770–6.
PubMed Abstract | Publisher Full Text | Free Full Text

99. Tagare HD, Kucukelbir A, Sigworth FJ, *et al.*: **Directly reconstructing principal components of heterogeneous particles from cryo-EM images.** *J Struct Biol.* 2015; **191**(2): 245–62.
PubMed Abstract | Publisher Full Text | Free Full Text

100. Dashti A, Schwander P, Langlois R, *et al.*: **Trajectories of the ribosome as a Brownian nanomachine.** *Proc Natl Acad Sci U S A.* 2014; **111**(49): 17492–7.
PubMed Abstract | Publisher Full Text | Free Full Text

101. Frank J, Ourmazd A: **Continuous changes in structure mapped by manifold embedding of single-particle data in cryo-EM.** *Methods.* 2016; **100**: 61–7.
PubMed Abstract | Publisher Full Text | Free Full Text

102. Chen B, Frank J: **Two promising future developments of cryo-EM: Capturing short-lived states and mapping a continuum of states of a macromolecule.** *Microscopy (Oxf).* 2016; **65**(1): 69–79.
PubMed Abstract | Publisher Full Text | Free Full Text

103. Maeda S, Koehl A, Matile H, *et al.*: **Development of an antibody fragment that stabilizes GPCR/G-protein complexes.** *Nat Commun.* 2018; **9**(1): 3712.
PubMed Abstract | Publisher Full Text | Free Full Text

104. Zimmermann I, Egloff P, Hutter CA, *et al.*: **Synthetic single domain antibodies for the conformational trapping of membrane proteins.** *eLife.* 2018; **7**: e34317.
PubMed Abstract | Publisher Full Text | Free Full Text

105. Uchański T, Pardon E, Steyaert J: **Nanobodies to study protein conformational states.** *Curr Opin Struct Biol.* 2020; **60**: 117–123.
PubMed Abstract | Publisher Full Text

106. Basu K, Green EM, Cheng Y, *et al.*: **Why recombinant antibodies — benefits and applications.** *Curr Opin Biotechnol.* 2019; **60**: 153–158.
PubMed Abstract | Publisher Full Text | Free Full Text

107. F Dutka P, Mukherjee S, Gao X, *et al.*: **Development of "Plug and Play" Fiducial Marks for Structural Studies of GPCR Signaling Complexes by Single-Particle Cryo-EM.** *Structure.* 2019; **27**(12): 1862–1874.e7.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

108. Kim J, Wu S, Tomasiak TM, *et al.*: **Subnanometre-resolution electron cryomicroscopy structure of a heterodimeric ABC exporter.** *Nature.* 2015; **517**(7534): 396–400.
PubMed Abstract | Publisher Full Text | Free Full Text

109. Grimes JM, Hall DR, Ashton AW, *et al.*: **Where is crystallography going?** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 2): 152–166.
PubMed Abstract | Publisher Full Text | Free Full Text

110. Cymborowski M, Klimecka M, Chruszcz M, *et al.*: **To automate or not to automate: This is the question.** *J Struct Funct Genomics.* 2010; **11**(3): 211–21.
PubMed Abstract | Publisher Full Text | Free Full Text

111. F Sanchez-Weatherby J, Sandy J, Mikolajek H, *et al.*: **VMXi: A fully automated, fully remote, high-flux in situ macromolecular crystallography beamline.** *J Synchrotron Rad.* 2019; **26**(Pt 1): 291–301.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

112. Krojer T, Talon R, Pearce N, *et al.*: **The XChemExplorer graphical workflow tool for routine or large-scale protein-ligand structure determination.** *Acta Crystallogr D Struct Biol.* 2017; **73**(Pt 3): 267–78.
PubMed Abstract | Publisher Full Text | Free Full Text

113. Pearce NM, Bradley AR, Krojer T, *et al.*: **Partial-occupancy binders identified by the Pan-Dataset Density Analysis method offer new chemical opportunities and reveal cryptic binding sites.** *Struct Dyn.* 2017; **4**(3): 32104.
PubMed Abstract | Publisher Full Text | Free Full Text

114. F Pearce NM, Krojer T, Bradley AR, *et al.*: **A multi-crystal method for extracting obscured crystallographic states from conventionally uninterpretable electron density.** *Nat Commun.* 2017; **8**: 15123.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

115. Keedy DA, Hill ZB, Biel JT, *et al.*: **An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering.** *eLife.* 2018; **7**: e36307.
PubMed Abstract | Publisher Full Text | Free Full Text

116. Haas DJ: **The early history of cryo-cooling for macromolecular crystallography.** *IUCrJ.* 2020; **7**(2): 148–57.
Publisher Full Text

117. Pflugrath JW: **Practical macromolecular cryocrystallography.** *Acta Crystallogr F Struct Biol Commun.* 2015; **71**(Pt 6): 622–42.
PubMed Abstract | Publisher Full Text | Free Full Text

118. F Nave C, Garman EF: **Towards an understanding of radiation damage in cryocooled macromolecular crystals.** *J Synchrotron Rad.* 2005; **12**(Pt 3): 257–60.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

119. Garman E: **'Cool' crystals: Macromolecular cryocrystallography and radiation damage.** *Curr Opin Struct Biol.* 2003; **13**(5): 545–51.
PubMed Abstract | Publisher Full Text

120. Holton JM: **A beginner's guide to radiation damage.** *J Synchrotron Rad.* 2009; **16**(Pt 2): 133–42.
PubMed Abstract | Publisher Full Text | Free Full Text

121. Garman EF, Weik M: **Radiation Damage in Macromolecular Crystallography.** *Methods Mol Biol.* 2017; **1607**: 467–89.
PubMed Abstract | Publisher Full Text

122. Tilton RF Jr, Dewan JC, Petsko GA: **Effects of temperature on protein structure and dynamics: X-ray crystallographic studies of the protein ribonuclease-A at nine different temperatures from 98 to 320 K.** *Biochemistry.* 1992; **31**(9): 2469–81.
PubMed Abstract | Publisher Full Text

123. Halle B: **Biomolecular cryocrystallography: Structural changes during flash-cooling.** *Proc Natl Acad Sci U S A.* 2004; **101**(14): 4793–8.
PubMed Abstract | Publisher Full Text | Free Full Text

124. Frauenfelder H, Chen G, Berendzen J, *et al.*: **A unified model of protein dynamics.** *Proc Natl Acad Sci U S A.* 2009; **106**(13): 5129–34.
PubMed Abstract | Publisher Full Text | Free Full Text

125. F Fraser JS, Clarkson MW, Degnan SC, *et al.*: **Hidden alternative structures of proline isomerase essential for catalysis.** *Nature.* 2009; **462**(7273): 669–73.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

126. Keedy DA, Kenner LR, Warkentin M, *et al.*: **Mapping the conformational landscape of a dynamic enzyme by multitemperature and XFEL crystallography.** *eLife.* 2015; **4**: e07574.
PubMed Abstract | Publisher Full Text | Free Full Text

127. Biel JT, Thompson MC, Cunningham CN, *et al.*: **Flexibility and Design: Conformational Heterogeneity Along the Evolutionary Trajectory of a Redesigned Ubiquitin.** *Structure.* 2017; **25**(5): 739–749.e3.
PubMed Abstract | Publisher Full Text | Free Full Text

128. Acker TM, Gable JE, Bohn M-F, *et al.*: **Allosteric Inhibitors, Crystallography, and Comparative Analysis Reveal Network of Coordinated Movement Across Human Herpesvirus Proteases.** *J Am Chem Soc.* 2017; **139**(34): 11650–3.
PubMed Abstract | Publisher Full Text | Free Full Text

129. Winter G, Gildea RJ, Paterson NG, *et al.*: **How best to use photons.** *Acta Crystallogr D Struct Biol.* 2019; **75**(Pt 3): 242–61.
PubMed Abstract | Publisher Full Text | Free Full Text

130. Winter G, Waterman DG, Parkhurst JM, *et al.*: **DIALS: Implementation and evaluation of a new integration package.** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 2): 85–97.
PubMed Abstract | Publisher Full Text | Free Full Text

131. F Ueno G, Shimada A, Yamashita E, *et al.*: **Low-dose X-ray structure analysis**

of cytochrome c oxidase utilizing high-energy X-rays. *J Synchrotron Rad.* 2019; **26**(Pt 4): 912–21.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

132. van den Bedem H, Dhanik A, Latombe JC, *et al.*: **Modeling discrete heterogeneity in X-ray diffraction data by fitting multi-conformers.** *Acta Crystallogr D Biol Crystallogr.* 2009; **65**(Pt 10): 1107–17.
PubMed Abstract | Publisher Full Text | Free Full Text

133. Keedy DA, Fraser JS, van den Bedem H, *et al.*: **Exposing Hidden Alternative Backbone Conformations in X-ray Crystallography Using qFit.** *PLoS Comput Biol.* 2015; **11**(10): e1004507.
PubMed Abstract | Publisher Full Text | Free Full Text

134. van Zundert GCP, Hudson BM, de Oliveira SHP, *et al.*: **qFit-ligand Reveals Widespread Conformational Heterogeneity of Drug-Like Molecules in X-Ray Electron Density Maps.** *J Med Chem.* 2018; **61**(24): 11183–98.
PubMed Abstract | Publisher Full Text | Free Full Text

135. F Lang PT, Ng HL, Fraser JS, *et al.*: **Automated electron-density sampling reveals widespread conformational polymorphism in proteins.** *Protein Sci.* 2010; **19**(7): 1420–31.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

136. Burnley BT, Afonine PV, Adams PD, *et al.*: **Modelling dynamics in protein crystal structures by ensemble refinement.** *eLife.* 2012; **1**: e00311.
PubMed Abstract | Publisher Full Text | Free Full Text

137. van Benschoten AH, Liu L, Gonzalez A, *et al.*: **Measuring and modeling diffuse scattering in protein X-ray crystallography.** *Proc Natl Acad Sci U S A.* 2016; **113**(15): 4069–74.
PubMed Abstract | Publisher Full Text | Free Full Text

138. Wall ME, van Benschoten AH, Sauter NK, *et al.*: **Conformational dynamics of a crystalline protein from microsecond-scale molecular dynamics simulations and diffuse X-ray scattering.** *Proc Natl Acad Sci U S A.* 2014; **111**(50): 17887–92.
PubMed Abstract | Publisher Full Text | Free Full Text

139. F de Klijn T, Schreurs AMM, Kroon-Batenburg LMJ: **Rigid-body motion is the main source of diffuse scattering in protein crystallography.** *IUCrJ.* 2019; **6**(Pt 2): 277–89.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

140. Wall ME: **Internal protein motions in molecular-dynamics simulations of Bragg and diffuse X-ray scattering.** *IUCrJ.* 2018; **5**(Pt 2): 172–81.
PubMed Abstract | Publisher Full Text | Free Full Text

141. F Meisburger SP, Case DA, Ando N: **Diffuse X-ray scattering from correlated motions in a protein crystal.** *Nat Commun.* 2020; **11**(1): 1271.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

142. Janowski PA, Liu C, Deckman J, *et al.*: **Molecular dynamics simulation of triclinic lysozyme in a crystal lattice.** *Protein Sci.* 2016; **25**(1): 87–102.
PubMed Abstract | Publisher Full Text | Free Full Text

143. Cerutti DS, Freddolino PL, Duke RE, *et al.*: **Simulations of a Protein Crystal with a High Resolution X-ray Structure: Evaluation of Force Fields and Water Models.** *J Phys Chem B.* 2010; **114**(40): 12811–24.
PubMed Abstract | Publisher Full Text | Free Full Text

144. Cerutti DS, Case DA: **Molecular dynamics simulations of macromolecular crystals.** *Wiley Interdiscip Rev Comput Mol Sci.* 2019; **9**(4): e1402.
PubMed Abstract | Publisher Full Text | Free Full Text

145. F Moriarty NW, Janowski PA, Swails JM, *et al.*: **Improved chemistry restraints for crystallographic refinement by integrating the Amber force field into Phenix.** *Acta Crystallogr D Struct Biol.* 2020; **76**(Pt 1): 51–62.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

146. Emma P, Akre R, Arthur J, *et al.*: **First lasing and operation of an ångstrom-wavelength free-electron laser.** *Nat Photonics.* 2010; **4**: 641–7.
Publisher Full Text

147. Schlichting I, Miao J: **Emerging opportunities in structural biology with X-ray free-electron lasers.** *Curr Opin Struct Biol.* 2012; **22**(5): 613–26.
PubMed Abstract | Publisher Full Text | Free Full Text

148. Schlichting I: **Serial femtosecond crystallography: The first five years.** *IUCrJ.* 2015; **2**(Pt 2): 246–55.
PubMed Abstract | Publisher Full Text | Free Full Text

149. Martin-Garcia JM, Conrad CE, Coe J, *et al.*: **Serial femtosecond crystallography: A revolution in structural biology.** *Arch Biochem Biophys.* 2016; **602**: 32–47.
PubMed Abstract | Publisher Full Text | Free Full Text

150. Neutze R, Huldt G, Hajdu J, *et al.*: **Potential impact of an X-ray free electron laser on structural biology.** *Radiat Phys Chem.* 2004; **71**: 905–16.
Publisher Full Text

151. Chapman HN, Caleman C, Timneanu N: **Diffraction before destruction.** *Philos Trans R Soc Lond B Biol Sci.* 2014; **369**(1647): 20130313.
PubMed Abstract | Publisher Full Text | Free Full Text

152. F Chapman HN, Fromme P, Barty A, *et al.*: **Femtosecond X-ray protein nanocrystallography.** *Nature.* 2011; **470**(7332): 73–7.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

153. Shapiro DA, Chapman HN, Deponte D, *et al.*: **Powder diffraction from a continuous microjet of submicrometer protein crystals.** *J Synchrotron Radiat.* 2008; **15**(Pt 6): 593–9.
PubMed Abstract | Publisher Full Text

154. Rossmann MG: **Serial crystallography using synchrotron radiation.** *IUCrJ.* 2014; **1**(Pt 2): 84–6.
PubMed Abstract | Publisher Full Text | Free Full Text

155. Spence JCH: **Serial Crystallography: Preface.** *Crystals.* 2020; **10**(2): 135.
Publisher Full Text

156. DePonte DP, Weierstall U, Schmidt K, *et al.*: **Gas dynamic virtual nozzle for generation of microscopic droplet streams.** *J Phys D Appl Phys.* 2008; **41**(19): 195505.
Publisher Full Text

157. Sierra RG, Laksmono H, Kern J, *et al.*: **Nanoflow electrospinning serial femtosecond crystallography.** *Acta Crystallogr D Biol Crystallogr.* 2012; **68**(Pt 11): 1584–7.
PubMed Abstract | Publisher Full Text | Free Full Text

158. F Weierstall U, James D, Wang C, *et al.*: **Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography.** *Nat Commun.* 2014; **5**: 1119.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

159. Tono K: **Fluid sample injectors for x-ray free electron laser at SACLA.** *High Pow Laser Sci Eng.* 2017; **5**.
Publisher Full Text

160. Wolff AM, Young ID, Sierra RG, *et al.*: **Comparing serial X-ray crystallography and microcrystal electron diffraction (MicroED) as methods for routine structure determination from small macromolecular crystals.** *IUCrJ.* 2020; **7**(Pt 2): 306–23.
PubMed Abstract | Publisher Full Text | Free Full Text

161. Cohen AE, Soltis SM, González A, *et al.*: **Goniometer-based femtosecond crystallography with X-ray free electron lasers.** *Proc Natl Acad Sci U S A.* 2014; **111**(48): 17122–7.
PubMed Abstract | Publisher Full Text | Free Full Text

162. Roedig P, Ginn HM, Pakendorf T, *et al.*: **High-speed fixed-target serial virus crystallography.** *Nat Methods.* 2017; **14**(8): 805–10.
PubMed Abstract | Publisher Full Text | Free Full Text

163. Baxter EL, Aguila L, Alonso-Mori R, *et al.*: **High-density grids for efficient data collection from multiple crystals.** *Acta Crystallogr D Struct Biol.* 2016; **72**(Pt 1): 2–11.
PubMed Abstract | Publisher Full Text | Free Full Text

164. Mueller C, Marx A, Epp SW, *et al.*: **Fixed target matrix for femtosecond time-resolved and *in situ* serial micro-crystallography.** *Struct Dyn.* 2015; **2**(5): 054302.
PubMed Abstract | Publisher Full Text | Free Full Text

165. Mathews II, Allison K, Robbins T, *et al.*: **The Conformational Flexibility of the Acyltransferase from the Disorazole Polyketide Synthase Is Revealed by an X-ray Free-Electron Laser Using a Room-Temperature Sample Delivery Method for Serial Crystallography.** *Biochemistry.* 2017; **56**(36): 4751–6.
PubMed Abstract | Publisher Full Text | Free Full Text

166. Lyubimov AY, Murray TD, Koehl A, *et al.*: **Capture and X-ray diffraction studies of protein microcrystals in a microfluidic trap array.** *Acta Crystallogr D Biol Crystallogr.* 2015; **71**(Pt 4): 928–40.
PubMed Abstract | Publisher Full Text | Free Full Text

167. Roessler CG, Agarwal R, Allaire M, *et al.*: **Acoustic Injectors for Drop-On-Demand Serial Femtosecond Crystallography.** *Structure.* 2016; **24**(4): 631–40.
PubMed Abstract | Publisher Full Text | Free Full Text

168. Fuller FD, Gul S, Chatterjee R, *et al.*: **Drop-on-demand sample delivery for studying biocatalysts in action at X-ray free-electron lasers.** *Nat Methods.* 2017; **14**(4): 443–9.
PubMed Abstract | Publisher Full Text | Free Full Text

169. Botha S, Nass K, Barends TRM, *et al.*: **Room-temperature serial crystallography at synchrotron X-ray sources using slowly flowing free-standing high-viscosity microstreams.** *Acta Crystallogr D Biol Crystallogr.* 2015; **71**(Pt 2): 387–97.
PubMed Abstract | Publisher Full Text

170. Jaeger K, Dworkowski F, Nogly P, *et al.*: **Serial Millisecond Crystallography of Membrane Proteins.** *Adv Exp Med Biol.* 2016; **922**: 137–49.
PubMed Abstract | Publisher Full Text

171. Martin-Garcia JM, Conrad CE, Nelson G, *et al.*: **Serial millisecond crystallography of membrane and soluble protein microcrystals using synchrotron radiation.** *IUCrJ.* 2017; **4**(Pt 4): 439–54.
PubMed Abstract | Publisher Full Text | Free Full Text

172. Stellato F, Oberthür D, Liang M, *et al.*: **Room-temperature macromolecular serial crystallography using synchrotron radiation.** *IUCrJ.* 2014; **1**(Pt 4): **204–12.**
PubMed Abstract | Publisher Full Text | Free Full Text

173. F de La Mora E, Coquelle N, Bury CS, *et al.*: **Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures.** *Proc Natl Acad Sci U S A.* 2020; **117**(8): 4142–51.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

174. Nass K: **Radiation damage in protein crystallography at X-ray free-electron lasers.** *Acta Crystallogr D Struct Biol.* 2019; **75**(Pt 2): 211–8.
PubMed Abstract | Publisher Full Text | Free Full Text

175. Johansson LC, Stauch B, Ishchenko A, *et al.*: **A Bright Future for Serial Femtosecond Crystallography with XFELs.** *Trends Biochem Sci.* 2017; **42**(9): 749–62.
PubMed Abstract | Publisher Full Text | Free Full Text

176. Kern J, Yachandra VK, Yano J: **Metalloprotein structures at ambient conditions and in real-time: Biological crystallography and spectroscopy using X-ray free electron lasers.** *Curr Opin Struct Biol.* 2015; **34**: 87–98.
PubMed Abstract | Publisher Full Text | Free Full Text

177. Ebrahim A, Moreno-Chicano T, Appleby MV, *et al.*: **Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins.** *IUCrJ.* 2019; **6**(Pt 4): 543–51.
PubMed Abstract | Publisher Full Text | Free Full Text

178. F Sauter NK, Kern J, Yano J, *et al.*: **Towards the spatial resolution of metalloprotein charge states by detailed modeling of XFEL crystallographic diffraction.** *Acta Crystallogr D Struct Biol.* 2020; **76**(Pt 2): 176–92.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

179. Suga M, Shimada A, Akita F, *et al.*: **Time-resolved studies of metalloproteins using X-ray free electron laser radiation at SACLA.** *Biochim Biophys Acta Gen Subj.* 2020; **1864**(2): 129466.
PubMed Abstract | Publisher Full Text

180. Kang Y, Zhou XE, Gao X, *et al.*: **Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser.** *Nature.* 2015; **523**(7562): 561–7.
PubMed Abstract | Publisher Full Text | Free Full Text

181. F Lin G, Weiss SC, Vergara S, *et al.*: **Transcription with a laser: Radiation-damage-free diffraction of RNA Polymerase II crystals.** *Methods.* 2019; **159–160**: 23–8.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

182. F Coulibaly F, Chiu E, Ikeda K, *et al.*: **The molecular organization of cypovirus polyhedra.** *Nature.* 2007; **446**(7131): 97–101.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

183. Coulibaly F, Chiu E, Gutmann S, *et al.*: **The atomic structure of baculovirus polyhedra reveals the independent emergence of infectious crystals in DNA and RNA viruses.** *Proc Natl Acad Sci U S A.* 2009; **106**(52): 22205–10.
PubMed Abstract | Publisher Full Text | Free Full Text

184. Sawaya MR, Cascio D, Gingery M, *et al.*: **Protein crystal structure obtained at 2.9 Å resolution from injecting bacterial cells into an X-ray free-electron laser beam.** *Proc Natl Acad Sci U S A.* 2014; **111**(35): 12769–74.
PubMed Abstract | Publisher Full Text | Free Full Text

185. Colletier JP, Sawaya MR, Gingery M, *et al.*: ***De novo* phasing with X-ray laser reveals mosquito larvicide BinAB structure.** *Nature.* 2016; **539**(7627): 43–7.
PubMed Abstract | Publisher Full Text | Free Full Text

186. F Tetreau G, Banneville AS, Andreeva EA, *et al.*: **Serial femtosecond crystallography on in vivo-grown crystals drives elucidation of mosquitocidal Cyt1Aa bioactivation cascade.** *Nat Commun.* 2020; **11**(1): 1153.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

187. Chen JPJ, Donatelli JJ, Schmidt KE, *et al.*: **Shape transform phasing of edgy nanocrystals.** *Acta Crystallogr A Found Adv.* 2019; **75**(Pt 2): 239–59.
PubMed Abstract | Publisher Full Text

188. Millane RP, Chen JPJ: **Aspects of direct phasing in femtosecond nanocrystallography.** *Philos Trans R Soc Lond, B Biol Sci.* 2014; **369**(1647): 20130498.
PubMed Abstract | Publisher Full Text

189. Kirian RA, Bean RJ, Beyerlein KR, *et al.*: **Phasing coherently illuminated nanocrystals bounded by partial unit cells.** *Phil Trans R Soc B.* 2014; **369**(1647): 20130331.
PubMed Abstract | Publisher Full Text | Free Full Text

190. Chen JPJ, Spence JCH, Millane RP: **Direct phasing in femtosecond nanocrystallography. I. Diffraction characteristics.** *Acta Crystallogr A Found Adv.* 2014; **70**(Pt 2): 143–53.
PubMed Abstract | Publisher Full Text | Free Full Text

191. Chen JPJ, Spence JCH, Millane RP: **Direct phasing in femtosecond nanocrystallography. II. Phase retrieval.** *Acta Crystallogr A Found Adv.* 2014; **70**(Pt 2): 154–61.
PubMed Abstract | Publisher Full Text

192. F Ebrahim A, Appleby MV, Axford D, *et al.*: **Resolving polymorphs and radiation-driven effects in microcrystals using fixed-target serial synchrotron crystallography.** *Acta Crystallogr D Struct Biol.* 2019; **75**(Pt 2): 151–159.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

193. Thompson MC, Cascio D, Yeates TO: **Microfocus diffraction from different regions of a protein crystal: Structural variations and unit-cell polymorphism.** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 5): 411–421.
PubMed Abstract | Publisher Full Text | Free Full Text

194. Neutze R: **Opportunities and challenges for time-resolved studies of protein structural dynamics at X-ray free-electron lasers.** *Philos Trans R Soc Lond B Biol Sci.* 2014; **369**(1647): 20130318.
PubMed Abstract | Publisher Full Text | Free Full Text

195. F Eisenmesser EZ, Millet O, Labeikovsky W, *et al.*: **Intrinsic dynamics of an enzyme underlies catalysis.** *Nature.* 2005; **438**(7064): 117–21.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

196. Henzler-Wildman KA, Lei M, Thai V, *et al.*: **A hierarchy of timescales in protein dynamics is linked to enzyme catalysis.** *Nature.* 2007; **450**(7171): 913–6.
PubMed Abstract | Publisher Full Text

197. Henzler-Wildman KA, Thai V, Lei M, *et al.*: **Intrinsic motions along an enzymatic reaction trajectory.** *Nature.* 2007; **450**(7171): 838–44.
PubMed Abstract | Publisher Full Text

198. Sawaya MR, Kraut J: **Loop and subdomain movements in the mechanism of Escherichia coli dihydrofolate reductase: Crystallographic evidence.** *Biochemistry.* 1997; **36**(3): 586–603.
PubMed Abstract | Publisher Full Text

199. Chakrabarti KS, Agafonov RV, Pontiggia F, *et al.*: **Conformational Selection in a Protein-Protein Interaction Revealed by Dynamic Pathway Analysis.** *Cell Rep.* 2016; **14**(1): 32–42.
PubMed Abstract | Publisher Full Text | Free Full Text

200. F Dellus-Gur E, Elias M, Caselli E, *et al.*: **Negative Epistasis and Evolvability in TEM-1 β-Lactamase--The Thin Line between an Enzyme's Conformational Freedom and Disorder.** *J Mol Biol.* 2015; **427**(14): 2396–409.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

201. Cimermancic P, Weinkam P, Rettenmaier TJ, *et al.*: **CryptoSite: Expanding the Druggable Proteome by Characterization and Prediction of Cryptic Binding Sites.** *J Mol Biol.* 2016; **428**(4): 709–19.
PubMed Abstract | Publisher Full Text | Free Full Text

202. Bowman GR, Geissler PL: **Equilibrium fluctuations of a single folded protein reveal a multitude of potential cryptic allosteric sites.** *Proc Natl Acad Sci U S A.* 2012; **109**(29): 11681–6.
PubMed Abstract | Publisher Full Text | Free Full Text

203. Hart KM, Moeder KE, Ho CMW, *et al.*: **Designing small molecules to target cryptic pockets yields both positive and negative allosteric modulators.** *PLoS One.* 2017; **12**(6): e0178678.
PubMed Abstract | Publisher Full Text | Free Full Text

204. Moffat K: **Time-resolved crystallography.** *Acta Crystallogr A.* 1998; **54**(Pt 6 Pt 1): 833–41.
PubMed Abstract | Publisher Full Text

205. Schlichting I, Goody RS: **Triggering methods in crystallographic enzyme kinetics.** *Meth Enzymol.* 1997; **277**: 467–90.
PubMed Abstract | Publisher Full Text

206. Chen CC, Herzberg O: **Inhibition of β-Lactamase by Clavulanate. Trapped Intermediates in Cryocrystallographic Studies.** *J Mol Biol.* 1992; **224**(4): 1103–13.
PubMed Abstract | Publisher Full Text

207. Moffat K, Henderson R: **Freeze trapping of reaction intermediates.** *Curr Opin Struct Biol.* 1995; **5**(5): 656–63.
PubMed Abstract | Publisher Full Text

208. Ding X, Rasmussen BF, Petsko GA, *et al.*: **Direct Structural Observation of an Acyl-Enzyme Intermediate in the Hydrolysis of an Ester Substrate by Elastase.** *Biochemistry.* 1994; **33**(31): 9285–93.
PubMed Abstract | Publisher Full Text

209. Teng TY, Srajer V, Moffat K: **Photolysis-induced structural changes in single crystals of carbonmonoxy myoglobin at 40 K.** *Nat Struct Biol.* 1994; **1**(10): 701–5.
PubMed Abstract | Publisher Full Text

210. Bourgeois D, Ursby T, Wulff M, *et al.*: **Feasibility and Realization of Single-Pulse Laue Diffraction on Macromolecular Crystals at ESRF.** *J Synchrotron Radiat.* 1996; **3**(Pt 2): 65–74.
PubMed Abstract | Publisher Full Text

211. Hajdu J, Acharya KR, Stuart DI, *et al.*: **Catalysis in the crystal: Synchrotron radiation studies with glycogen phosphorylase b.** *EMBO J.* 1987; **6**(2): 539–46.
PubMed Abstract | Publisher Full Text | Free Full Text

212. Schlichting I, Almo SC, Rapp G, *et al.*: **Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis.** *Nature.* 1990; **345**(6273): 309–15.
PubMed Abstract | Publisher Full Text

213. Ng K, Getzoff ED, Moffat K: **Optical Studies of a Bacterial Photoreceptor Protein, Photoactive Yellow Protein, in Single Crystals.** *Biochemistry.* 2002; **34**(3): 879–90.
PubMed Abstract | Publisher Full Text

214. Genick UK, Borgstahl GE, Ng K, *et al.*: **Structure of a protein photocycle intermediate by millisecond time-resolved crystallography.** *Science.* 1997; **275**(5305): 1471–5.
PubMed Abstract | Publisher Full Text

215. F Schotte F, Lim M, Jackson TA, *et al.*: **Watching a Protein as it Functions with 150-ps Time-Resolved X-ray Crystallography.** *Science.* 2003; **300**(5627): 1944–7.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

216. Schotte F, Soman J, Olson JS, *et al.*: **Picosecond time-resolved X-ray crystallography: Probing protein function in real time.** *J Struct Biol.* 2004; **147**(3): 235–46.
PubMed Abstract | Publisher Full Text

217. F Ihee H, Rajagopal S, Srajer V, *et al.*: **Visualizing reaction pathways in photoactive yellow protein from nanoseconds to seconds.** *Proc Natl Acad Sci U*

S A. 2005; **102**(20): 7145–50.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

218. F Nogly P, Weinert T, James D, *et al.*: **Retinal isomerization in bacteriorhodopsin captured by a femtosecond x-ray laser.** *Science.* 2018; **361**(6398): eaat0094.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

219. F Nango E, Royant A, Kubo M, *et al.*: **A three-dimensional movie of structural changes in bacteriorhodopsin.** *Science.* 2016; **354**(6319): 1552–7.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

220. F Pande K, Hutchison CDM, Groenhof G, *et al.*: **Femtosecond structural dynamics drives the trans/cis isomerization in photoactive yellow protein.** *Science.* 2016; **352**(6286): 725–9.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

221. Coquelle N, Sliwa M, Woodhouse J, *et al.*: **Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography.** *Nat Chem.* 2018; **10**(1): 31–7.
PubMed Abstract | Publisher Full Text

222. F Woodhouse J, Nass Kovacs G, Coquelle N, *et al.*: **Photoswitching mechanism of a fluorescent protein revealed by time-resolved crystallography and transient absorption spectroscopy.** *Nat Commun.* 2020; **11**(1): 741.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

223. F Kern J, Chatterjee R, Young ID, *et al.*: **Structures of the intermediates of Kok's photosynthetic water oxidation clock.** *Nature.* 2018; **563**(7731): 421–5.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

224. F Young ID, Ibrahim M, Chatterjee R, *et al.*: **Structure of photosystem II and substrate binding at room temperature.** *Nature.* 2016; **540**(7633): 453–7.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

225. Kern J, Tran R, Alonso-Mori R, *et al.*: **Taking snapshots of photosynthetic water oxidation using femtosecond X-ray diffraction and spectroscopy.** *Nat Commun.* 2014; **5**: 4371.
PubMed Abstract | Publisher Full Text | Free Full Text

226. Olmos JL, Pandey S, Martin-Garcia JM, *et al.*: **Enzyme intermediates captured "on the fly" by mix-and-inject serial crystallography.** *BMC Biol.* 2018; **16**(1): 59.
PubMed Abstract | Publisher Full Text | Free Full Text

227. Dasgupta M, Budday D, de Oliveira SHP, *et al.*: **Mix-and-inject XFEL crystallography reveals gated conformational dynamics during enzyme catalysis.** *Proc Natl Acad Sci U S A.* 2019; **116**(51): 25634–40.
PubMed Abstract | Publisher Full Text | Free Full Text

228. Thompson MC, Barad BA, Wolff AM, *et al.*: **Temperature-jump solution X-ray scattering reveals distinct motions in a dynamic enzyme.** *Nat Chem.* 2019; **11**(11): 1058–66.
PubMed Abstract | Publisher Full Text | Free Full Text

229. F Tosha T, Nomura T, Nishida T, *et al.*: **Capturing an initial intermediate during the P450nor enzymatic reaction using time-resolved XFEL crystallography and caged-substrate.** *Nat Commun.* 2017; **8**(1): 246.
Publisher Full Text | Faculty Opinions Recommendation

230. Hekstra DR, White KI, Socolich MA, *et al.*: **Electric-field-stimulated protein mechanics.** *Nature.* 2016; **540**(7633): 400–5.
PubMed Abstract | Publisher Full Text | Free Full Text

231. Frank J: **Time-resolved cryo-electron microscopy: Recent progress.** *J Struct Biol.* 2017; **200**(3): 303–6.
PubMed Abstract | Publisher Full Text | Free Full Text

232. Kaledhonkar S, Fu Z, White H, *et al.*: **Time-Resolved Cryo-electron Microscopy Using a Microfluidic Chip.** *Methods Mol Biol.* 2018; **1764**: 59–71.
PubMed Abstract | Publisher Full Text

233. F Kontziampasis D, Klebl DP, Iadanza MG, *et al.*: **A cryo-EM grid preparation device for time-resolved structural studies.** *IUCrJ.* 2019; **6**(Pt 6): 1024–31.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

234. Feng X, Fu Z, Kaledhonkar S, *et al.*: **A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM.** *Structure.* 2017; **25**(4): 663–670.e3.
PubMed Abstract | Publisher Full Text | Free Full Text

235. F Kaledhonkar S, Fu Z, Caban K, *et al.*: **Late steps in bacterial translation initiation visualized using time-resolved cryo-EM.** *Nature.* 2019; **570**(7761): 400–4.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

236. de Rosier DJ, Klug A: **Reconstruction of three dimensional structures from electron micrographs.** *Nature.* 1968; **217**(5124): 130–4.
PubMed Abstract | Publisher Full Text

237. Crowther RA, Amos LA, Finch JT, *et al.*: **Three Dimensional Reconstructions of Spherical Viruses by Fourier Synthesis from Electron Micrographs.** *Nature.* 1970; **226**(5244): 421–5.
PubMed Abstract | Publisher Full Text

238. Henderson R, Unwin PN: **Three-dimensional model of purple membrane obtained by electron microscopy.** *Nature.* 1975; **257**(5521): 28–32.
PubMed Abstract | Publisher Full Text

239. Grigorieff N, Ceska TA, Downing KH, *et al.*: **Electron-crystallographic Refinement of the Structure of Bacteriorhodopsin.** *J Mol Biol.* 1996; **259**(3): 393–421.
PubMed Abstract | Publisher Full Text

240. F Henderson R, Baldwin JM, Ceska TA, *et al.*: **Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy.** *J Mol Biol.* 1990; **213**(4): 899–929.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

241. Kimura Y, Vassylyev DG, Miyazawa A, *et al.*: **Surface of bacteriorhodopsin revealed by high-resolution electron crystallography.** *Nature.* 1997; **389**(6647): 206–11.
PubMed Abstract | Publisher Full Text

242. Kühlbrandt W, Wang DN, Fujiyoshi Y: **Atomic model of plant light-harvesting complex by electron crystallography.** *Nature.* 1994; **367**(6464): 614–21.
PubMed Abstract | Publisher Full Text

243. F Gonen T, Sliz P, Kistler J, *et al.*: **Aquaporin-0 membrane junctions reveal the structure of a closed water pore.** *Nature.* 2004; **429**(6988): 193–7.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

244. F Hiroaki Y, Tani K, Kamegawa A, *et al.*: **Implications of the aquaporin-4 structure on array formation and cell adhesion.** *J Mol Biol.* 2006; **355**(4): 628–39.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

245. Walz T, Hirai T, Murata K, *et al.*: **The three-dimensional structure of aquaporin-1.** *Nature.* 1997; **387**(6633): 624–7.
PubMed Abstract | Publisher Full Text

246. F Gonen T, Cheng Y, Sliz P, *et al.*: **Lipid-protein interactions in double-layered two-dimensional AQP0 crystals.** *Nature.* 2005; **438**(7068): 633–8.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

247. Glaeser RM: **Specimen flatness of thin crystalline arrays: Influence of the substrate.** *Ultramicroscopy.* 1992; **46**(1–4): 33–43.
PubMed Abstract | Publisher Full Text

248. F Righetto RD, Biyani N, Kowal J, *et al.*: **Retrieving high-resolution information from disordered 2D crystals by single-particle cryo-EM.** *Nat Commun.* 2019; **10**(1): 1722.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

249. Matricardi VR, Moretz RC, Parsons DF: **Electron Diffraction of Wet Proteins: Catalase.** *Science.* 1972; **177**(4045): 268–70.
PubMed Abstract | Publisher Full Text

250. Taylor KA, Glaeser RM: **Electron diffraction of frozen, hydrated protein crystals.** *Science.* 1974; **186**(4168): 1036–7.
PubMed Abstract | Publisher Full Text

251. Dorset DL, Parsons DF: **Electron diffraction from single, fully-hydrated, ox-liver catalase microcrystals.** *Acta Cryst A.* 1975; **31**: 210–5.
Publisher Full Text

252. Grigorieff N, Henderson R: **Comparison of calculated and observed dynamical diffraction from purple membrane: Implications.** *Ultramicroscopy.* 1996; **65**(1–2): 101–7.
Publisher Full Text

253. Subramanian G, Basu S, Liu H, *et al.*: **Solving protein nanocrystals by cryo-EM diffraction: Multiple scattering artifacts.** *Ultramicroscopy.* 2015; **148**: 87–93.
PubMed Abstract | Publisher Full Text

254. Mugnaioli E, Gorelik T, Kolb U: **"*Ab initio*" structure solution from electron diffraction data obtained by a combination of automated diffraction tomography and precession technique.** *Ultramicroscopy.* 2009; **109**(6): 758–65.
PubMed Abstract | Publisher Full Text

255. Sun J, Zou X: **Structure determination of zeolites and ordered mesoporous materials by electron crystallography.** *Dalton Trans.* 2010; **39**(36): 8355–62.
PubMed Abstract | Publisher Full Text

256. Jiang L, Georgieva D, Nederlof I, *et al.*: **Image Processing and Lattice Determination for Three-Dimensional Nanocrystals.** *Microsc Microanal.* 2011; **17**(6): 879–85.
PubMed Abstract | Publisher Full Text

257. Gemmi M, Mugnaioli E, Gorelik TE, *et al.*: **3D Electron Diffraction: The Nanocrystallography Revolution.** *ACS Cent Sci.* 2019; **5**(8): 1315–29.
PubMed Abstract | Publisher Full Text | Free Full Text

258. F Shi D, Nannenga BL, Iadanza MG, *et al.*: **Three-dimensional electron crystallography of protein microcrystals.** *eLife.* 2013; **2**: e01345.
PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

259. Nederlof I, van Genderen E, Li YW, *et al.*: **A Medipix quantum area detector allows rotation electron diffraction data collection from submicrometre three-dimensional protein crystals.** *Acta Crystallogr D Biol Crystallogr.* 2013; **69**(Pt 7): 1223–30.
PubMed Abstract | Publisher Full Text | Free Full Text

260. Yonekura K, Kato K, Ogasawara M, *et al.*: **Electron crystallography of ultrathin 3D protein crystals: Atomic model with charges.** *Proc Natl Acad Sci U S A.*

2015; **112**(11): 3368–73.
PubMed Abstract | Publisher Full Text | Free Full Text

261. F Shi D, Nannenga BL, de La Cruz MJ, *et al.*: **The collection of MicroED data for macromolecular crystallography.** *Nat Protoc.* 2016; **11**(5): 895–904.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

262. Clabbers MTB, Gruene T, Parkhurst JM, *et al.*: **Electron diffraction data processing with *DIALS*.** *Acta Crystallogr D Struct Biol.* 2018; **74**(Pt 6): 506–18.
PubMed Abstract | Publisher Full Text | Free Full Text

263. F Rodriguez JA, Ivanova MI, Sawaya MR, *et al.*: **Structure of the toxic core of α-synuclein from invisible crystals.** *Nature.* 2015; **525**(7570): 486–90.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

264. Sawaya MR, Rodriguez J, Cascio D, *et al.*: ***Ab initio* structure determination from prion nanocrystals at atomic resolution by MicroED.** *Proc Natl Acad Sci U S A.* 2016; **113**(40): 11232–6.
PubMed Abstract | Publisher Full Text | Free Full Text

265. F Xu H, Lebrette H, Clabbers MTB, *et al.*: **Solving a new R2lox protein structure by microcrystal electron diffraction.** *Sci Adv.* 2019; **5**(8): eaax4621.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

266. Warmack RA, Boyer DR, Zee CT, *et al.*: **Structure of amyloid-β (20-34) with Alzheimer's-associated isomerization at Asp23 reveals a distinct protofilament interface.** *Nat Commun.* 2019; **10**(1): 3357.
PubMed Abstract | Publisher Full Text | Free Full Text

267. de la Cruz MJ, Hattne J, Shi D, *et al.*: **Atomic-resolution structures from fragmented protein crystals with the cryoEM method MicroED.** *Nat Methods.* 2017; **14**(4): 399–402.
PubMed Abstract | Publisher Full Text | Free Full Text

268. F Martynowycz MW, Zhao W, Hattne J, *et al.*: **Collection of Continuous Rotation MicroED Data from Ion Beam-Milled Crystals of Any Size.** *Structure.* 2019; **27**(3): 545–548.e2.
PubMed Abstract | Publisher Full Text | Free Full Text |
Faculty Opinions Recommendation

269. Zhou H, Luo Z, Li X: **Using focus ion beam to prepare crystal lamella for electron diffraction.** *J Struct Biol.* 2019; **205**(3): 59–64.
PubMed Abstract | Publisher Full Text

270. Duyvesteyn HME, Kotecha A, Ginn HM, *et al.*: **Machining protein microcrystals for structure determination by electron diffraction.** *Proc Natl Acad Sci U S A.* 2018; **115**(38): 9569–73.
PubMed Abstract | Publisher Full Text | Free Full Text

271. Brázda P, Palatinus L, Babor M: **Electron diffraction determines molecular absolute configuration in a pharmaceutical nanocrystal.** *Science.* 2019; **364**(6441): 667–9.
PubMed Abstract | Publisher Full Text

272. F Yonekura K, Ishikawa T, Maki-Yonekura S: **A new cryo-EM system for electron 3D crystallography by eEFD.** *J Struct Biol.* 2019; **206**(2): 243–53.
PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

273. Wang B, Zou X, Smeets S: **Automated serial rotation electron diffraction combined with cluster analysis: An efficient multi-crystal workflow for structure determination.** *IUCrJ.* 2019; **6**(Pt 5): 854–67.
PubMed Abstract | Publisher Full Text | Free Full Text

274. Gallagher-Jones M, Ophus C, Bustillo KC, *et al.*: **Nanoscale mosaicity revealed in peptide microcrystals by scanning electron nanodiffraction.** *Commun Biol.* 2019; **2**: 26.
PubMed Abstract | Publisher Full Text | Free Full Text

275. F Bücker R, Hogan-Lamarre P, Mehrabi P, *et al.*: **Serial protein crystallography in an electron microscope.** *Nat Commun.* 2020; **11**(1): 996.
PubMed Abstract | Publisher Full Text | Free Full Text |
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