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Merging disciplines: Chemical bases of life processes are revealed by X-ray crystallography

YONATH Ada*

Weizmann Institute of Science, Rehovot 76100, Israel

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This article has been written for concluding the International Year of Chemistry. Chemistry dominates almost all branches of modern science, since once reaching the molecular level, chemical events are being investigated. In life sciences the borders between the traditional scientific categories are practically vanishing. Thus, almost all biological questions are answered in chemical terms.

Biological X-ray crystallography is the research tool that shaped our notion on biological processes at the molecular level. By mapping the position of each and every atom that makes up the studied object, being a biological macromolecule or a molecular assembly, X-ray crystallography provides the information required for creating accurate 3D models, thus generating the information vital to understand life processes. This method utilizes physical properties, namely diffraction caused by the interaction between waves and matter alongside mathematical principles such as Fourier transform and group theory, and provides results in terms of bond lengths, chemical affinities, molecular mobility etc.

The scattering of X-rays from a single molecule is a continuous function. Crystals, namely periodically arranged entities along three dimensions, scatter waves in discrete spots according to their periodicity (with reciprocal relation). Consequently the scattered waves are convoluted with the lattice contribution. In this way the diffraction of the X-rays becomes discrete, hence measurable. In addition, in crystals a huge number of entities with the same orientation are diffracting simultaneously so that the diffracted signals are significantly increased. This explains the essentiality of crystalline samples.

The use of X-ray crystallography begun in the twenties of the last century, as a result of Max von Laue discovery of the discreet diffraction of X-rays by crystals, followed by the successful application, performed by Sir William Henry Bragg and his son, William Lawrence Bragg, of X-ray diffraction for the determination of the electronic distribution within simple inorganic molecules. Initially, this method was used for the determination of the structures of small inorganic molecules. Then, the structures of rather simple organic compounds could be determined. The extension of X-ray crystallography towards biological molecules met originally with considerable skepticism. For over two decades many respectable scientists doubted whether it could even be done.

Despite severe bottlenecks, since its first successful application, the superiority of X-ray crystallography over all other approaches for shedding light on functional aspects at the molecular level became evident. Consequently, the use of this method in life sciences gained more popularity and led to a constant growth of the number of structures, some of which were considered formidable even a few years ago. Thus, throughout the span of biological crystallography, its horizons exceeded what was considered to be realistic expectations. Consequently it can be described as ongoing intensive attempts at pursuing problems that require means with ever growing sophistication. Thus, the obtained structures inspired crystallographic studies of increasingly complexity, which led to spectacular technical innovations, which vastly accelerated its incredible expansion. The growing number of structures of bio-molecules yielded new insights into intricate functional aspects, such as membrane transport, signaling pathways, protein/nucleic acids recognition, biologically active RNA molecules.

*Corresponding author (email: ada.yonath@weizmann.ac.il)

One of the major bottlenecks is sample preparation. Thus, throughout the existence of biological crystallography and even today, when automated systems for crystal growth became available, growing useful crystals from biological macromolecules has been a major bottleneck in the determination of their three dimensional structures. This is because of their inherent internal flexibility and the acute requirement for being immersed in supporting solvent in order to maintain their functional meaningful conformation. In the case of membrane proteins, the latter is particularly limiting. The membranes that encapsulate cells or internal cellular specialized compartments, are water-impermeable barriers in which proteins, controlling the transport through the membranes, are embedded. As by their nature these proteins are hardly soluble in aqueous media, their crystallization is significantly more complex than the crystallization of soluble proteins. Therefore among the total number (~80000) of structures of proteins that are currently known, only about 280 structures of membrane proteins have been deposited in the protein data bank (PDB) during the last 26 years, since the first membrane protein structure has been determined. Remarkably, about a half of the structures were determined during the last few years. Also, an impressive increase in the resolution limits is constantly being achieved.

Currently biological crystallography is being employed for investigating a large variety of key life processes with incredible progress. Selected highlights of the recent impressive progress in understanding the chemical aspects of fundamental life process by deciphering structures are listed below. These include transcriptional regulation of genes controlling the ratio of unsaturated/saturated fatty acids; oxidoreductase complexes; complexes of multimeric membrane and outer membrane proteins; membrane activators; polypeptide transport-associated domains; G-protein-coupled receptors with transmembrane helices used for transducing signals into cells; human lipases that play key roles in endocannabinoid signaling; secreted lysophospholipases that generate lipid interacting mediators; actin regulatory complex that controls cytoskeletal dynamics by promoting filament nucleation; outer membrane palimitoyl transferease; plant self activating G-protein; the catalytic domain of human calmodulin; chaperons from plant pathogens; tumor suppressor proteins; estrogen receptors and more.

X-ray crystallography of RNA molecules and their complexes has also expanded dramatically recently. Examples are riboswitches and ribosomes. The latter are the universal cellular assemblies that translate the genetic code into proteins. The first molecular structures of bacterial ribosomal particles became available about a decade ago. These structures and those determined later illuminated key issues concerning in the process of protein production and also paved the way for the design on improved antibiotics. Interestingly the first three decades of ribosomal crystallography only prokaryotic ribosome structures were investigated crystallographially. During the last year the first two structures of eukaryotic ribosomal particles have been determined: of the small ribosomal subunit from protozoa, in complex with a translation initiation protein and of a functional complex of a eukaryotic ribosome from the yeast. Considering the much higher complexity of eukaryotic ribosomes, which are 40% larger than their bacterial counterparts, the recent structures demonstrate a remarkable progress.

The resolution limit of the determined structures is one of the major issues in crystallographic structure determination. Aiming at illuminating the mechanisms of biological processes at the molecular level, high resolution structures are desired. Diffraction that can yield electron density map showing the exact position for each atom is preferably obtained from samples in which all atoms are rather fixed in their positions and all crystallized molecules are in identical conformations. Because proteins are fairly flexible, and because crystals of biological macromolecules contain significant amount of solvent, the lattice is somewhat disordered and therefore the diffraction pattern cancels out at lower resolution limits. The impressive result that was documented in 2011, namely the structure of the very small protein crambin that was determined at a resolution limit of 0.48 Å, is clearly an exception in this field.

Throughout, biological crystallography has been suffering from numerous bottlenecks and obstacles. Challenging these hurdles inspired not only major innovations and outstanding technological advances, but also led to conceptual revolutions in the philosophy of the experimental design. Consequently, installation of advanced technologies and innovative computational tools was required. An example is synchrotron radiation (SR) that became useful about four decades ago. Furthermore, the exploitation of its favorable properties, namely beam brightness, beam collimation and wavelength tunability, have been constantly optimized and led to striking progress in accurate structure determination. Originally, using synchrotron radiation met with severe skepticism. Once its usefulness became evident, many technical problems hampered its efficient exploitation. For example, the damage of most biological crystals caused almost instantaneously by the synchrotron X-ray beam brightness (required for obtaining measurable data from crystals of biological macromolecules), could be reduced to tolerable level by the introduction of a novel procedure for conducting data collection at cryo temperature.

In addition, despite the shortcomings of synchrotron radiation, it was soon realized that it provides unprecedented experimental tools for the determination structures. Noteworthy is the exploitation of its tunability for the determination of the crystallographic phases, which are required for constructing electron density maps, but cannot be directly measured. For filling in this missing link, resonant diffraction methods capable of direct phase determination were pioneered, based on discovering the critical properties of

discrete wavelengths within the synchrotron radiation continuous spectrum. This phasing method utilizes selected wavelengths that span the absorption edge of specific atoms serving as anomalous scatterers. The genuine and overwhelming payoff of this method, called multi/single-wavelength anomalous dispersion (MAD/SAD), is the benign introduction of selenium instead of sulfur into proteins, as a vehicle for direct protein structure determination. Benefiting from the incredible development in genetic manipulations that exploits selenomethionine instead of native methionine, as selenium is one of the atoms possessing a large anomalous dispersion, around 90% of the currently available atomic-level de novo structures were determined.

A major bottleneck in determining three dimensional structures is growing useful crystals. The essentiality of suitable crystals hampered many exciting structural studies. Therefore, alongside the impressive latest progress in growing crystals from difficult to crystallize molecules and assemblies, massive attempts are being made to rid this hurdle. Consequently, an exciting conception for offering new opportunities in experimental structure determination by using the free electron laser (FEL), was recently developed. As this technology exploits extremely bright and coherent X-ray pulses (a billion times brighter than conventional SR sources), it is capable of extracting accurate diffraction data at very high resolution from nano-crystals. This beam can also be utilized for many other applications, such as constructing rapid single-shot three-dimensional images of complex macromolecules, observing short-lived intermediates, following role of radiation damage of sensitive macromolecules and deciphering the interior structure of partially organized systems, such as intact huge viruses.

Future prospects: The future structural biology as a tool for understanding the chemical bases of life seems now to be several orders of magnitude brighter than expected. The ingenious introduction of advances in synchrotron radiation alongside crystallographic algorithms have yielded a huge number of structures of biologically relevant macromolecules and their complexes, and the pioneering of cutting edge approaches in X-ray sources opened new routes in structural studies. Furthermore, the realization that a huge number of photons allows structure determination form nano crystals alongside visualization of the internal organization of non crystallized molecules in vacuum before they explode, paves the way to better understanding of extremely complex systems with minimal or no periodic organization (e.g. chromosomes, membranes, cellular organelles, etc.). This also provided means for following snapshots of dynamic biochemical processes, such as catalysis, membrane transport, signal transduction, etc. Furthermore, the examination of the recently determined structures of the huge macromolecular assembly, the ribosome, and its complexes with substrate analogs yielded truly unexpected gains. An example is the identification of a ribosomal universal pocket-like semi symmetrical internal feature, accounting for 3%-4% of the ribosomal RNA, which appears to be a remnant of an RNA prebiotic bonding apparatus (called proto-ribosome). Remarkably, this presumed prebiotic RNA machine seems to be still functioning in all contemporary ribosomes.

A note concerning citations: Because of the nature of this minireview, citations are not provided. For those, the readers are directed to the more detailed account in A. Yonath, *Curr Opin Struct Biol*, 2011, 21: 1–5

YONATH Ada E. is a Nobel Laureate and the Martin S. and Helen Kimmel Professor of Structural Biology at the Weizmann Institute of Science, Israel. She grew up in Jerusalem, and received her undergraduate and M.Sc. studies in chemistry, biochemistry, and biophysics at the Hebrew University of Jerusalem. Yonath conducted Ph.D. studies at the Weizmann Institute of Science, and postdoctoral work at the Mellon Institute in Pittsburg, Pennsylvania, and the Massachusetts Institute of Technology, USA. She shared the 2009 Nobel Prize in Chemistry with Venkatraman Ramakrishnan and Thomas A. Steitz for "*for studies of the structure and function of the ribosome*" [1], among many honors and awards she has received.

[1] Announcement of the 2009 Nobel Prize in Chemistry.
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2009/