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Commentary

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X-ray Crystallography: One Century of Nobel Prizes

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ABSTRACT: In 2012, the United Nations General Assembly declared 2014 the International Year of Crystallography. Throughout the year 2014 and beyond, all the crystallographic associations and societies active all over the world are organizing events to attract the wider public toward crystallography and the numerous topics to which it is deeply interlinked. To increase awareness of modern crystallography and to diminish its halo of exoticism, the present commentary focuses on the fundamental contributions of X-ray crystallography during the last century in chemistry, physics, and medicine, as witnessed by the numerous Nobel Prizes that involved it.

2014 international year of crystallography

IUCr International Union of Crystallography

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In 2012, the United Nations declared 2014 the International Year of Crystallography. Among the purposes motivating the declaration is the need for increasing public knowledge of this scientific discipline, from its birth up to its current contributions to many relevant fields. Remarkable efforts in this respect have been already done in the past.[1]

Despite the fact that the World Directory of Crystallographers lists more than 22,000 practitioners, crystallography has not reached the wider public yet, and scarce awareness exists of the fact that it straddles numerous disciplines, including chemistry, physics, mathematics, materials science, biology, geology, and medicine. Mainly because of their shine and external symmetry, crystals have always appealed to humans: ancient Greeks and Romans, for example, left us a number of proofs in this regard.[2] Currently, crystals permeate our daily lives and are one of the pillars upon which modern academic institutions and industries rely to develop novel materials.

The birth of crystallography can be traced back to the 17th century with the work of Steno, Romé de L'Isle, and Haüy, among others.[3] *Modern* crystallography flourished only at the beginning of the 20th century, when X-rays appeared as a probe to "see" the structure of crystalline matter.[4] Since then, crystal structure determination has developed steadily;[5] starting from elements and binary compounds, it has become feasible to study even proteins. As such, it is not an exaggeration to consider modern crystallography as the most outstanding among the structural sciences.

The pervasiveness of modern crystallography is witnessed, inter alia, by the fact that it is the multidisciplinary field accounting for the highest number of Nobel Laureates: in one century, *at least* 25 Nobel Prizes have been awarded for work directly or indirectly involving crystallography (see Table 1 for a nonexhaustive list). Worthy of note is the exceptionally high fraction of women Nobelists in crystallography compared to other fields.

Table 1. Nonexhaustive List of the Nobel Prizes Involving X-ray Crystallography

Discipline, Year of Prize	Winners	Motivation Cited by the Nobel Prize Committee
Physics, 1901	W.C. Röntgen	Discovery of X-rays
Physics, 1914	M. von Laue	Discovery of X-ray diffraction by crystals
Physics, 1915	W.H. and W.L. Bragg	Use of X-rays to determine crystal structures
Chemistry, 1962	J.C. Kendrew, M. Perutz	Studies of the structures of globular proteins
Medicine or Physiology, 1962	F. Crick, J. Watson, M. Wilkins	Helical structure of DNA
Chemistry, 1964	D. Crowfoot Hodgkin	Structure of many biochemical substances, including vitamin B_{12}
Chemistry, 1976	W.N. Lipscomb	Structure of boranes
Chemistry, 1985	H. Hauptman, J. Karle	Development of direct methods for solving crystal structures
Chemistry, 1988	J. Deisenhofer, R. Huber, H. Michel	Determination of the 3D structure of a photosynthetic reaction center
Physics, 1992	G. Charpak	Discovery of the multiwire proportional chamber
Chemistry, 1996	R. Curl, H. Kroto, and R. Smalley	Discovery of the fullerene form of carbon
Chemistry, 2009	V. Ramakrishnan, T.A. Steitz, A.E. Yonath	Studies of the structure and function of the ribosome
Physics, 2010	A. Geim, K. Novoselov	Experiments regarding the 2D material graphene
Chemistry, 2011	D. Shechtman	Discovery of quasicrystals
Chemistry, 2012	R.J. Lefkowitz, B.K. Kobilka	Studies of G-protein-coupled receptors

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■ THE BEGINNINGS

Starting with a focus on X-ray crystallography, it is noteworthy that the first Nobel Prize in physics was conferred on Röntgen in 1901 for the discovery of X-rays. While working with a Lenard's tube on cathode rays, Röntgen was captivated by the light emitted by a sample of C_4BaN_4Pt far away from the tube, each time the latter was traversed by electric current. After this serendipitous observation, Röntgen tried to characterize as thoroughly as possible the unknown emission leaving the tube and provoking fluorescence of the C_4BaN_4Pt sample. The famous image of the hand bones and ring of Anna Berthe (Röntgen's wife), for example, demonstrated the penetrative power of X-rays. For almost 20 years, while X-ray tubes became more and more popular among medical doctors as diagnostic tool, numerous scientists—Röntgen, Haga, Wind, Walter, Pohl, Koch, among others—tried to unravel the (wave- or particle-like) nature of X-rays.

The decisive proof as to the nature of X-rays came with the experiment planned by von Laue and realized by Friedrich and Knipping. In early 1912, when Ewald paid a visit to von Laue to illustrate to him the problems he was facing while studying the behavior of visible light passing through a 3D lattice, von Laue was struck by a crucial question: What would happen if the wavelength of the incident electromagnetic wave were of similar size to the spacing of the lattice? He thought right away of X-rays and crystals: as a matter of fact, it was known from the work by Wien and Sommerfeld that if X-rays were waves, their wavelength would be of the order of 10^{-11} m. On the other hand, Fedorov, Sohnke, and others had proposed that atoms, in crystals, were periodically arranged with a spacing of the order of 10^{-10} m. When Friedrich and Knipping irradiated a crystal of copper sulfate with X-rays, the photographic plate behind it showed spots undoubtedly due to the diffraction of the incident X-rays by the crystal.[6] The implications of this observation were tremendous: it proved, at the same time, the wavelike nature of X-rays and the 3D periodicity of atoms in crystals. von Laue went even further: he elaborated a mathematical theory modeling the diffraction of X-rays by a 3D lattice, thus allowing the structure of crystalline matter to be unraveled. Exactly one century ago, in 1914, von Laue was awarded the Nobel Prize in Physics for his impressive discovery.[7]

von Laue's work raised two research lines, namely crystal structure determination and X-ray spectroscopy. Along the first path went W. L. Bragg: after working on the diffraction pictures of zincblende[8] acquired by Friedrich and Knipping, using his father's spectrometer[9] he studied, among others, sodium and potassium chlorides,[10] diamond,[11] calcite,[8] fluorite,[8] iron pyrites,[8] naphthalene, and anthracene.[12] Remarkably, the determination of these first crystal structures threw new light on the nature of chemical bonds: the structure of sodium chloride, for example, allowed chemists to dismiss (reluctantly!) the existence of NaCl molecules in favor of the formation of ions reciprocally bound by electrostatic forces.[13] W. H. Bragg, among others, focused on X-ray spectroscopy and, inter alia, proved that X-ray tubes emitted the characteristic K- and L-lines first proposed by Barkla.[14] For their pioneering work, the Braggs were awarded the Nobel Prize in Physics in 1915.

■ THE FIRST CRYSTAL STRUCTURES OF BIOMOLECULES

Given the available tools, the first crystal structures were necessarily simple. In the 1930s, two major advancements, Patterson synthesis[15] and Beevers–Lipson strips,[16] allowed more challenging problems to be tackled. As Hodgkin recognized in her 1964 Nobel Prize lecture, she relied deeply on both developments to determine the structure of manifold biochemical substances: making extensive use of Patterson maps and Fourier syntheses, the latter computed with the strips, she determined the crystal structures of a range of compounds as varied as cholesteryl halides,[17] penicillin,[18] cephalosporin C,[19] vitamin B_{12},[20] cobyric acid–Factor V Ia,[21] and coenzyme B_{12}.[22]

Needless to say, in the 1930s no crystal structure of proteins had been determined yet, and many scientists were skeptical about this possibility. The first bottleneck to be faced was the growth of suitable crystals. Northrop, Stanley, and Sumner shared the 1946 Nobel Prize in Chemistry for their pioneering studies in the crystallization of enzymes in the 1920s.[23] In 1937, when protein crystallization methods were rather established, Perutz decided to study hemoglobin, a 10,000-atom protein, in an attempt to understand the molecular basis of its biological function. He worked for six years on human oxygen-free and horse oxygenated hemoglobin. Adopting the isomorphous replacement method, he eventually determined the first crystal structures[24] at a resolution of about 5.5 Å, adequate to show the fold of the polypeptide chains and the positions of the heme groups, and to reveal that the structures of the oxygen-free and oxygenated forms differ. Concomitantly, Kendrew concentrated on myoglobin, four times smaller yet still containing ~2,600 atoms. Its complete diffraction pattern featured about 25,000 reflections, so that the Fourier syntheses required managing about 5×10^9 numbers. When Kendrew started his work, no computer existed to do this. As such, Kendrew's first efforts focused only on 400 and 10,000 reflections, mostly acquired by photographic methods, and resulted in 3D models of myoglobin at 6 and 2 Å resolution, respectively.[25,26] Because of rapidly increasing computer power, an incomplete model at 1.4 Å was derived later using all the data possible to acquire by means of the diffractometer engineered by Arndt and Phillips.[27] Perutz and Kendrew were awarded the Nobel Prize in Chemistry in 1962 for having determined the crystal structures of these two key proteins.

The year 1962 witnessed another Nobel Prize involving X-ray crystallography, that awarded in Medicine to Crick, Watson, and Wilkins for the molecular structure of nucleic acids. While examining DNA-oriented films for UV dichroism, Wilkins noted extremely uniform fibers, serendipitously produced while manipulating DNA gel. He immediately brought the fibers to Raymond Gosling, who was in charge of the X-ray equipment and, with the valuable contributions of Rosalind Franklin, the first, encouraging diffraction patterns were acquired. X-ray diffraction indicated regular and helical DNA molecules. Regularity was explained by Watson and Crick through the formation of hydrogen-bonded nucleotide pairs. Based on this hypothesis, the two scientists built a molecular model of DNA featuring two polynucleotide chains arranged in an antiparallel fashion to form a double helix.[28] Importantly, the axial repeat of the model matched that suggested by X-ray diffraction. The two scientists distinguished three different configurations of DNA and proved that the double-helix was not an artifact, but could be found also in vivo.[29]

This remarkable work was vital for other Nobelists; for example, Ochoa and A. Kornberg received the Nobel Prize in Medicine in 1959 for elucidating the mechanisms in the biological synthesis of RNAs and DNAs.

THE INCESSANT GROWTH OF MACROMOLECULAR CRYSTALLOGRAPHY

The early work on globins and DNA opened the vast and ever-growing field of macromolecular crystallography, the development of which is highlighted by cornerstone results. As representative examples, Anfinsen, sharing the Nobel Prize in Chemistry in 1972 with Moore and Stein, collaborated with the group of crystallographers at MIT determining the structure of ribonuclease.[30] Deisenhofer, Huber, and Michel received the 1988 Nobel Prize in Chemistry for the determination of the crystal structure of a photosynthetic reaction center,[31] crucial for understanding the mechanisms regulating photosynthesis. Boyer and Walker, sharing the 1997 Nobel Prize in Chemistry with Skou, relied on the crystal structure of F1-ATPase determined by Walker to elucidate the enzymatic mechanism behind the synthesis of ATP.[32] MacKinnon, sharing the 2003 Nobel Prize in Chemistry with Agre, intensely investigated the mechanistic and structural aspects of ion channels.[33] R. D. Kornberg received the 2006 Nobel Prize in Chemistry for his studies on the molecular basis of eukaryotic transcription, including the determination of the crystal structure of RNA polymerase II.[34] Ramakrishnan, Steitz, and Yonath shared the 2009 Nobel Prize in Chemistry for their studies on the structure and function of prokaryotic ribosomes, including the introduction, by Yonath, of cryo biocrystallography, which became routine in structural biology.[35] Finally, Kobilka shared the 2012 Nobel Prize in Chemistry with Lefkowitz for the studies on G protein-coupled receptors, exemplified by the molecular structure of the β_2-adrenergic receptor.[36]

THE INCESSANT GROWTH OF INORGANIC CRYSTALLOGRAPHY

Inorganic systems have also been the subject of unprecedented studies deserving of a Nobel Prize. Lipscomb, for example, was awarded the Nobel Prize in Chemistry in 1976 for his contributions within the vast field of boranes. In the 1880s, it was known that boranes exist in the gaseous mixture that forms when alloys between boron and certain metals are attacked by acids. In 1912, Stock first produced boranes in pure form. Given the ratios between boron and hydrogen, the existence of boranes was a sort of puzzle; to solve this, the so-called "banana bond" was theorized in 1949. Due to his work begun in 1954, which coupled NMR, theoretical calculations, and low-temperature diffraction experiments, Lipscomb proved the existence of banana bonds[37] and shed light on the molecular structure and reactivity of boranes.

In the realm of inorganics, we must also cite the 1996 Nobel Prize in Chemistry awarded to Curl, Kroto, and Smalley for the discovery of fullerenes: their observations by ^{13}C NMR, mass spectrometry, IR, and UV spectroscopy and their subsequent rationalizations deeply benefitted from the X-ray crystal structure determination by Krätschmer et al.[38]

DATA TREATMENT AND INSTRUMENTATION

On the side of data treatment, we have to recall the Nobel Prize in Chemistry awarded in 1985 to Hauptman and Karle for their achievements in the development of direct methods. Their complementary skills—Hauptman possessed a background in mathematics and Karle was an expert in physical chemistry—enabled them to successfully tackle the so-called "phase problem". Their first monograph, introducing probabilistic methods for phasing centrosymmetric crystals, appeared in 1953.[39] Extension of direct methods to noncentrosymmetric crystals was accomplished a few years later. By the end of the 1960s, many scientists recognized the potentiality of direct methods; to the present day, this is still the most widely used phasing approach for small molecule structure determination.

In the field of instrumentation, we must recall the work of Charpak, awarded the 1992 Nobel Prize in Physics for inventing and developing particle detectors, in particular the multiwire proportional counters,[40] a main application of which are the 2D detectors ubiquitous in current X-ray crystallography.

At this stage, it should definitely appear clear that X-ray crystallography has allowed us to obtain numerous unforeseen results in different fields of crucial importance. For example, who would have ever believed that crystals possessing forbidden symmetry exist? Yet, this is indeed the case: in spite of claims as harsh as that by Pauling ("There is no such thing as quasicrystals, only quasiscientists"), the Swedish Academy of Sciences assigned the 2011 Nobel Prize in Chemistry to Shechtman for the discovery of quasiperiodic crystals.[41]

CONCLUSIONS

This brief treatment of Nobel Prizes related to X-ray crystallography may pique readers' interest and curiosity about the burgeoning development of X-ray crystallography during the last century. Far from being a mature and stagnant discipline, X-ray crystallography possesses the potential to keep contributing to unexpected outcomes in fields as varied as instrumentation, data treatment, and complex systems, allowing for decisive chemicophysical properties or biological mechanisms to be rationalized.

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Notes

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