Leading Edge
Review

Cell

A Glimpse of Structural Biology through X-Ray Crystallography

Yigong Shi[1,*]
[1]Center for Structural Biology, Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences and School of Medicine, Tsinghua University, Beijing 100084, China
*Correspondence: shi-lab@tsinghua.edu.cn
http://dx.doi.org/10.1016/j.cell.2014.10.051

Since determination of the myoglobin structure in 1957, X-ray crystallography, as the anchoring tool of structural biology, has played an instrumental role in deciphering the secrets of life. Knowledge gained through X-ray crystallography has fundamentally advanced our views on cellular processes and greatly facilitated development of modern medicine. In this brief narrative, I describe my personal understanding of the evolution of structural biology through X-ray crystallography—using as examples mechanistic understanding of protein kinases and integral membrane proteins—and comment on the impact of technological development and outlook of X-ray crystallography.

Brief History

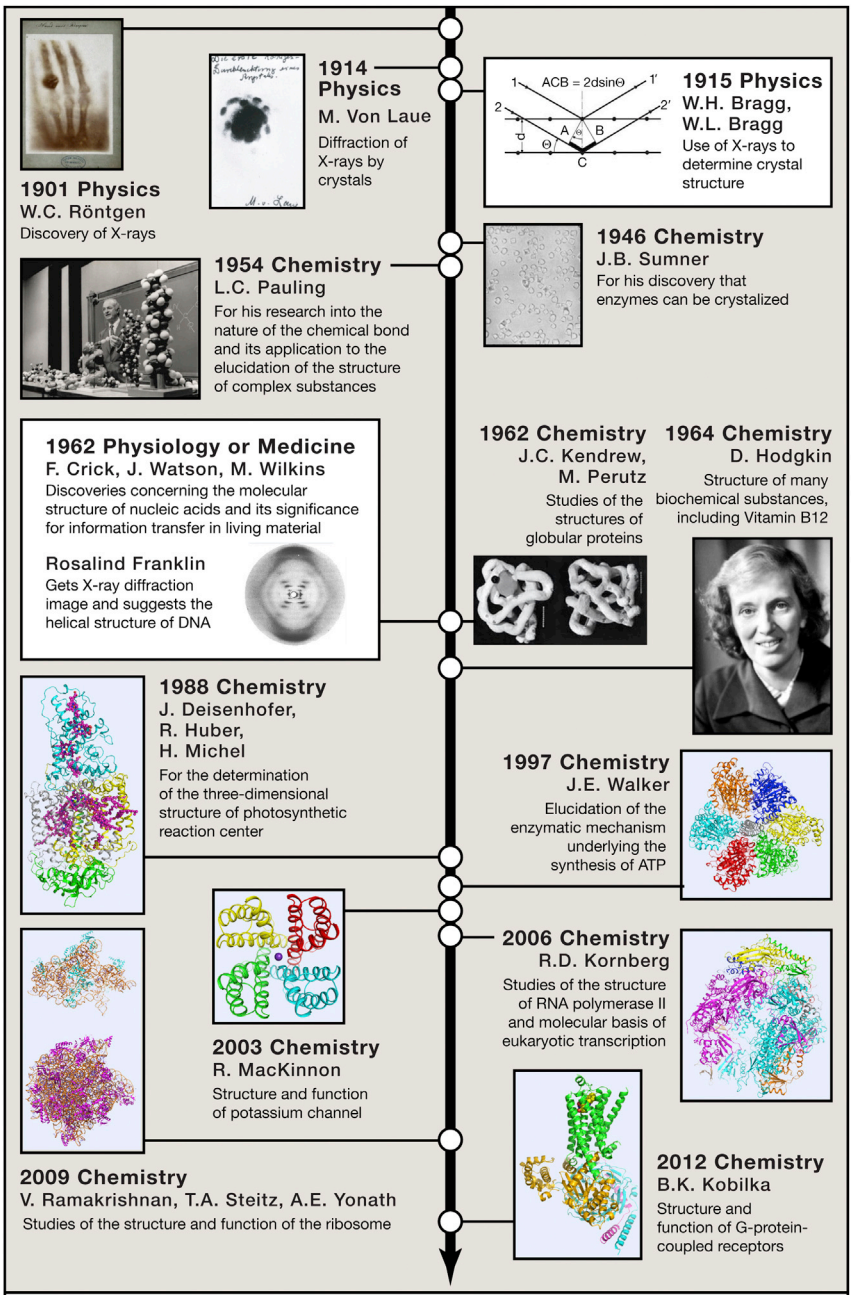
When Wilhelm Roentgen discovered X-ray in 1895, he could not have imagined the powerful applications of X-ray diffraction on crystals of biological samples. Max von Laue showed X-ray diffraction pattern of crystals in 1912, and William Lawrence Bragg derived a general equation, known as the Bragg's Law, to describe the founding principle of image formation by X-ray diffraction (Bragg, 1913) (Figure 1). James Sumner obtained the first crystal of jack bean urease in 1926 and showed the enzyme to be a protein (Figure 1). Max Perutz and John Kendrew decided to pursue crystal structures of proteins—hemoglobin and myoglobin—beginning in the 1940s at the Cavendish Laboratory, University of Cambridge. Their pioneering effort was encouraged by William Lawrence Bragg, who served as the Director of the Cavendish Laboratory between 1938 and 1954. In 1953, James Watson and Francis Crick, both employed at the Cavendish Laboratory, deduced a DNA double-helix model on the basis of X-ray fiber diffraction images of DNA generated by Rosalind Franklin (Watson and Crick, 1953).

The entire biological research community was both excited and shocked to see the very first crystal structure of a macromolecule in 1957—that of sperm whale myoglobin by John Kendrew (Kendrew et al., 1958). The structure of myoglobin, initially determined at 6 Å resolution but quickly improved to 2 Å (Kendrew et al., 1960), confirmed the α-helical conformation as proposed by Linus Pauling and Robert Corey (Pauling and Corey, 1951a, 1951b, 1951c; Pauling et al., 1951). Kendrew's success in structure determination of myoglobin was indispensably assisted by Perutz' solution to the phase problem—multiple isomorphous replacement through heavy atom soaks. Max Perutz presented his own X-ray structure on the larger protein hemoglobin at 5.5 Å (Perutz et al., 1960) and took a few years to improve the resolution to 2.8 Å (Perutz et al., 1968a, 1968b). Kendrew founded the *Journal of Molecular Biology* and served as Editor-in-Chief for a number of years. Kendrew also helped establish the European Molecular Biology Laboratory in Heidelberg and became its founding director. Perutz, on the other hand, founded and directed the MRC Laboratory of Molecular Biology (Figure 1). Notably, the double-helix structure of DNA was finally visualized in 1980 by the X-ray structure of a 12-base-pair palindromic DNA, known as the Dickerson dodecamer (Wing et al., 1980).

DNA is the genetic material of almost all living matters, and proteins are the engines of life. Structural elucidation of DNA and protein is arguably the most important scientific discovery in the 20th century. Proposal of the double-helix structure of DNA has fundamentally changed our perception of life and has ushered in a new era of modern biology. Crystal structures of myoglobin and hemoglobin allowed us to link protein function to its chemical details. In many respects, the atomic details offered by X-ray crystallography allowed mechanistic understanding of protein function, which marks the beginning of molecular biology. Kendrew and Perutz have been fondly named fathers of molecular biology.

Early crystallographic studies focused on abundant proteins, most often enzymes, from animal organs and tissues. Following the successes on myoglobin and hemoglobin, structural information was obtained for at least seven additional proteins in the 1960s, including the first enzyme hen egg white lysozyme (Blake et al., 1965), ribonucleases A and S (Kartha et al., 1967; Wyckoff et al., 1967), chymotrypsin (Matthews et al., 1967), papain (Drenth et al., 1968), carboxypeptidase A (Lipscomb et al., 1969), and subtilisin (Wright et al., 1969). These structures, together with those of many other enzymes in the 1970s and beyond, reveal the active site conformations and catalytic mechanisms, which form the physical basis of molecular enzymology.

The Protein Data Bank (PDB), a central repository for three-dimensional structural data of macromolecules, was established in 1971 at the Brookhaven National Laboratory with seven initial entries. As of August 26, 2014, there were 102,863 total entries in the PDB, of which 88.7% were determined by X-ray crystallography, 10.3% by nuclear magnetic resonance (NMR), and 0.8% by electron microscopy (EM) (Figure 2A). Following structure determination of the lysozyme from bacteriophage T4 (T4 lysozyme) (Matthews and Remington, 1974), it became a paradigm



1917	Physics	C.G. Barkla	Discovery of the characteristic Röntgen radiation of the elements
1929	Physics	L.-V. de Broglie	The wave nature of the electron
1936	Chemistry	P.J.W. Debye	For his contributions to our knowledge of molecular structure through his investigations on dipole moments and on the diffraction of X-rays and electrons by crystals
1937	Physics	C.J. Davisson G. Thompson	Diffraction of electrons by crystals
1972	Chemistry	C.B. Anfinsen	Folding of protein chains
1982	Chemistry	A. Klug	Development of crystallographic electron microscopy and discovery of the structure of biologically important nucleic acid-protein complexes
1985	Chemistry	H. Hauptman J. Karle	Development of direct methods for the determination of crystal structures
1994	Physics	C. Shull, N. Brockhouse	Neutron diffraction
2013	Chemistry	M. Karplus, M. Levitt, A. Warshel	Development of multiscale models for complex chemical systems

Figure 1. The History of X-Ray Crystallography in the Eyes of Nobel Prizes

Major achievements in the development and application of X-ray crystallography have been recognized by at least 14 Nobel Prizes. The first Nobel Prize in physics was awarded to Roentgen in 1901 for his discovery of X-rays. The next two Nobel Prizes in 1914 and 1915 were given to Laue for his discovery of X-ray diffraction by crystals and to the Bragg father and son for the use of X-rays to determine crystal structure. Sumner was awarded a Chemistry Prize in 1946 for crystallization of the enzyme urease. Pauling won a Chemistry Prize in 1954 for his research into the nature of chemical bond and its application in structure determination. The year 1962 was quite special, with the Chemistry Prize awarded to Kendrew and Perutz for their pioneering work in protein structure determination, and the Physiology or Medicine Prize bestowed on Crick, Watson, and Wilkins for their contribution in the discovery of DNA structure. Hodgkin was awarded a Chemistry Prize in 1964 for structural elucidation of many biochemical substances, including vitamin B12. The next six Nobel Prizes were awarded to macromolecular crystallographers: Deisenhofer, Huber, and Michel in 1988 for the structure of bacterial photosynthetic reaction center; Walker in 1997 for the structure of F1-ATPase; MacKinnon in 2003 for potassium channels; Kornberg in 2006 for the structure of RNA polymerases; Ramakrishnan, Steitz, and Yonath in 2009 for the structure of ribosome; and Kobilka in 2012 for the structure of GPCR. Listed in the lower left corner are nine Nobel Prizes that are closely related to X-ray crystallography.