X-RAY CRYSTALLOGRAPHY: A SCIENCE OF TRUTH AND BEAUTY

Niloufar Ataie & Ho Leung Ng*†*

ABSTRACT

X-ray crystallography is a science in which mathematics and theory meet with experiment and art. Since its inception over a century ago, many crystallographers have contributed to this science where an appreciation for beauty and an eye for pattern are useful skills for extracting truth from data. X-ray crystallography has been and remains one of the most important tools for describing reality at a foundational level the organization of atoms.

1. INTRODUCTION

X-ray crystallography has been used for over one hundred years to determine the atomic positions of hundreds of thousands of molecules. Molecules and atoms can interact with incoming light whose wavelength is approximately the same size as they are. Since atomic bonds are on the order of 0.1-0.2 nanometers, X-rays, with wavelengths of approximately 0.1 nanometers, or 1 Å long, are used to produce diffraction patterns (Figure-1). The patterns produced by the interfering X-rays that hit the object can then be used to calculate the atomic coordinates of the electrons that give rise to the interference patterns through a mathematic operation called a 'Fourier transform'. The sensitivity limits on detectors require a crystal of the molecule, which can amplify the diffraction data. To calculate the electron density maps, the intensities of the reflections are used. The phase information of each reflection is also necessary to complete the Fourier transform, and that information is missing in a diffraction experiment and needs to be obtained by other methods. Since its development, X-ray crystallography has had a great impact on the understanding of physical and life processes. The thousands of maps of complex molecules that have been produced by crystallographers have provided deep insights into the understanding of disease, the design of medicines, the miracle of catalysis, the mysteries of molecular life processes, and the grandeur of complex systems.

2. THE PATTERNS OF CRYSTALLOGRAPHY

All things are made of atoms, and X-ray crystallography is the light that reveals atoms to scientists. A method that uncovers nature's foundational secrets, crystallography fills the hunger

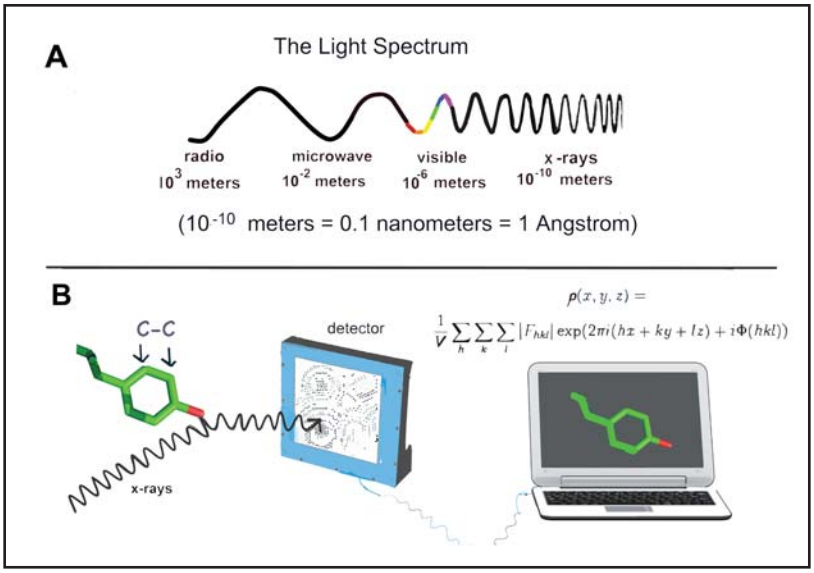

Figure-1: X-rays are Used to Collect a Diffraction Pattern and Solve the Structure

The wavelengths of X-rays are on the order of 10^{-10} meters which is equivalent to 0.1 nanometers or 1 Å
A) To use diffraction to resolve the details of a typical bond between atoms (which is 1-2 Å long) it is necessary to use light that is on the order of the object to be resolved; therefore, X-rays are used for objects on the molecular level. A single wavelength X-ray beam is directed toward a crystal of the molecule, and
B) the interference patterns are recorded on a detector. The reflections produced by the interacting waves are related to the electron density by a Fourier transform operation, where the intensities of the reflections are used to build a map of electron locations and calculate the structure of the molecule.

* Department of Chemistry, University of Hawaii at Manoa, †Email: hng@hawaii.edu

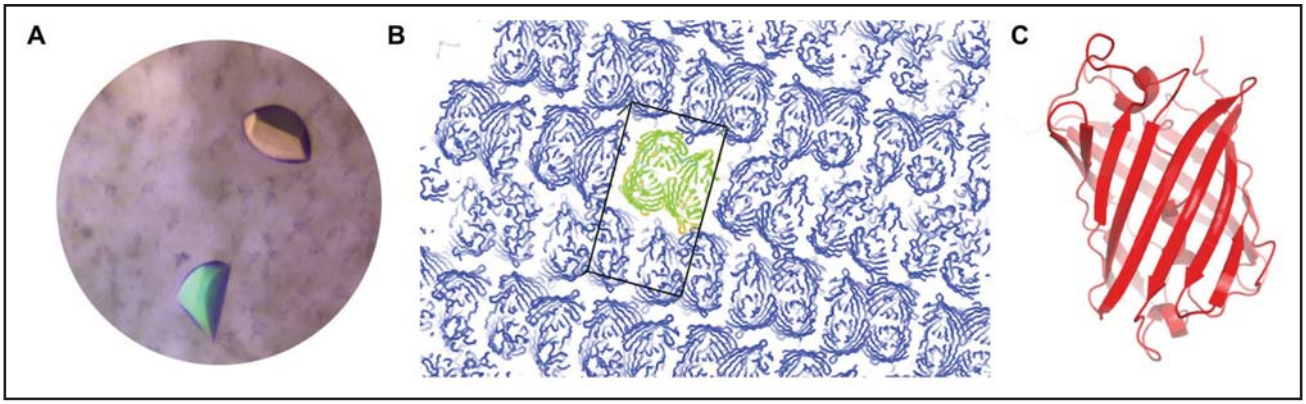

Figure-2: The Structure, Symmetry, and Beauty of Crystallography

Shown in **A** are high quality crystals of protein molecules. Crystals that are clear and free of visible imperfections tend to yield better data. Arrangement of protein molecules in a crystal lattice is shown in **B**, where part of the unit cell is outlined. The unit cell is the smallest geometric unit of the crystal that can be described by translations alone. The smallest part of the unit cell not subject to symmetry operators is the asymmetric unit, which is colored green in **B** and is composed of four molecules of the protein that is modeled in **C**. The protein in **C** is represented in a ribbon diagram rendering generated by the program PyMOL. The objective of a crystallography experiment is to obtain the coordinates for the atoms in the crystal. The atomic coordinates have many applications including molecular engineering and medicine design.

of those seeking to understand how the universe works and its structure. But crystallography is not just a tool for appeasing appetites of the curious; the symmetries, colors, shapes, and patterns that inundate a crystallographer's vision stimulate those with an appreciation for beauty too. Mastering crystallography demands years of training but offers huge scientific and aesthetic rewards. In crystallography, beauty and functionality go hand in hand. This is especially true of protein crystallography, which enables to understand the structure and function of the most complex molecules, the molecules of life. For example, only those protein crystals whose form and clarity rival the most beautiful gemstones are likely to give good data (Figure-2A). The better the ordering of the biological molecules in the crystal, the higher the probability of detecting waves diffracting off of the details of the crystal. This ordering is often propagated outward to the macro-morphology of the crystal so that crystals that appear beautiful and clear may very well represent a lattice that is well ordered and diffracts to a high resolution. Shown in Figure-2B is the arrangement of biological molecules in a crystal lattice. A homogeneous sample of the molecule is used to produce the crystal. The crystal lattice is composed of the molecule (Figure-2C) that is in an ordered array which can be described by various rotational and translational operations. The molecule in Figure-2C is represented by a ribbon diagram. Ribbon diagrams are the most common way of representing proteins and are the offspring of Dr. Jane S. Richardson, who drew the first ribbon diagrams by hand (Richardson, 2000).

Another example of where beauty and functionality go hand in hand in crystallography is with data extraction from a diffraction pattern. Extracting the data from the complex tapestries and hidden symmetries of a diffraction pattern (Figure-3) requires that the reflections are not smeared or overwhelmed by noise. An eye for pattern allows for more effective data processing, and ultimately, a more accurate picture of reality. The crystallographer strives for beauty but also latches on to beauty as a tool. The geometries, colors, symmetries, and patterns involved are an aesthetic treat and a scientific necessity.

The brilliant Dr. Dorothy Crowfoot Hodgkins (born 1910 in Cairo, Egypt) was a pioneer crystallographer and a Nobel laureate who solved the structures of the largest and most complex molecules of her day, including penicillin, vitamin B-12, and insulin. She also pushed and developed importance practices such as refining the fit of models to diffraction data. It was said that early in her career, she would measure the intensity of the spots from a diffraction experiment under a lamp by eye (Eleanor Dodson, 2014). The intensities of the reflections and the patterns formed are used to calculate electron density maps. The reflections are made by X-ray waves that scatter off a crystal lattice onto a detector (Figure-3). The crystal amplifies the diffraction signal enough to be detected. Since the early years of crystallography, great improvements have been made in measuring

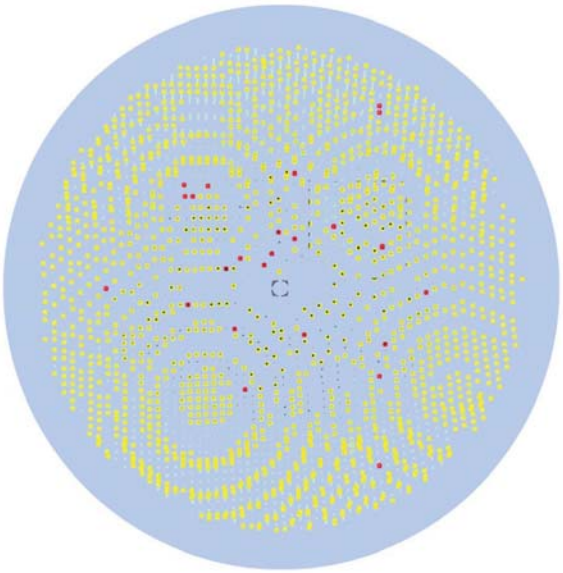

Figure-3: Diffraction Pattern from a Protein Crystal

Monochromatic X-rays diffracting from a protein crystal produces patterns that are used to calculate the coordinates of the atoms in the crystal. Yellow circles correspond to the diffraction pattern calculated for the crystal. The intensities of the reflections and the geometric relationship between the positions are used to solve structures. The crystallographer needs to discount noise and ice crystal reflections, which manifest in spots and features that do not fit the calculated protein diffraction pattern.

diffraction data. But even today, distinguishing protein diffraction patterns from artifacts caused by noise and salt and ice crystals require the discerning eye of the crystallographer.

3. COMPUTATION AND CRYSTALLOGRAPHY

Crystallographers use and write computer programmes to help solve, improve, and interpret structural data. Professor Eleanor Dodson, of York University in the UK, was a student of Dorothy Hodgkin, and excels at developing programmes used for structure determination and refinement. Her pioneering techniques in molecular replacement, phase determination (Dodson, and Woolfson, 2009), data distribution (Dodson, Winn, and Ralph, 1997; Winn, et al., 2011), and model refinement (Murshudov, et al., 1999; Murshudov, Vagin, and Dodson, 1997) have contributed greatly to the rapid increase in high quality macromolecular structures. The measured intensities of X-rays scattering diffracting from a crystal are used to calculate the distribution of electron density corresponding to the atoms of the molecules in the crystal. But a key ingredient to solving the structure, the phases of the diffracting X-ray waves, cannot be measured directly. Eleanor Dodson and others have written, developed and shared software that can estimate and refine phases from the relationships within the complex tapestry of diffraction spots. By understanding how the patterns, intensities, and symmetries of an image of spots relate to three-dimensional atomic structures, deep thinkers have made processes, once believed impossible, accessible to researchers and students. Today, through software included in the CCP4 suite (a collaboration that Professor Dodson helped lead), phases can be assigned and structures can be solved, sometimes, in minutes.

Also included in the CCP4 software suite are programmes that can improve the model through the process of refinement. After the phases are assigned and the structure is solved, the model can be made to better fit the data by using known experimental parameters like bond lengths and angles to maximize the agreement between the model and data. During this process of changing the model to better fit the data, a measure called the 'R-factor' is used to measure the agreement between the model and the observed amplitudes. This process can greatly improve the description of the electron density to better describe the positions of the atoms in a macromolecule.

4. BIOLOGICAL CRYSTAL GROWTH

Accurate measurement of X-ray diffraction requires uniform, high quality crystal lattices, but crystallizing large biomacromolecules is not easy. Often, many solution conditions, sometimes thousands, must be screened for crystallization. Initial crystals are often small and of poor quality. The quality and size of those crystals must be optimized through further solution screening. As with all scientific research, human persistence is essential for success, but chemical knowledge is also vital. Solution compositions,

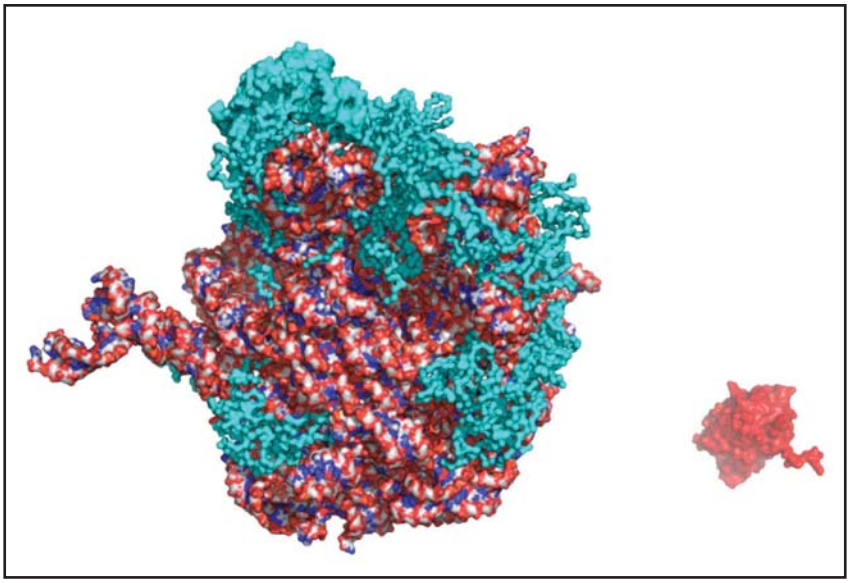

Figure-4: The Ribosome Crystal Structure

For comparison, shown on the left is a model of the 50S large ribosomal subunit (Harms, Schluenzen, Zarivach, et al., 2001) (pdb ID 1NKW) and on the right is a model of a more typically sized protein, with 270 residues. The ribosome has over ten-fold more atoms, is irregularly shaped, unstable, and composed of dozens of molecular chains. These features discourage ribosome crystallization. The form of the ribosome is conserved across species, and extremophilic species have ribosomes that are extra sturdy. Professor Ada Yonath and colleagues used this knowledge to crystallize ribosomes and produce atomic maps of one of the most important macromolecules of life. The ribosome portrayed here comes from the remarkably radiation resistant bacterium, *Deinococcus radiodurans.*

instruments, temperature, and other experimental variables must be considered. Even after thousands of attempts, a protein may not form usable crystals. Sometimes clever scientists use alternative ways to crystallize macromolecules, like using molecular engineering to add on groups that may contribute to enhanced crystal contact surface area. Alternatively, crystallographers may remove floppy or charged residues that lower the probability of molecules coming together to form a crystal contact surface. Often, biological homology and diversity are taken advantage of to crystallize an evolutionary related molecule that is very similar but has enhanced crystal forming properties. Nevertheless, failure is the constant friend of the crystallographer.

Most biomolecules are large, but some of the most important are gigantic. For example, the ubiquitous and age-old ribosome is easily ten times larger than most biomolecules that have been crystallized (Figure-4). The ribosome's size (over 60,000 atoms), irregular shape, instability, and composition (it consists of dozens of chains) make crystallization very improbable. Against the advice of most, Dr. Ada Yonath was determined to solve its structure. Many felt the task impossible, but Dr. Yonath kept searching for a way. Through her keen understanding of biology, she hypothesized that the fundamental structure and function of the ribosome would be preserved even in species that live under very extreme conditions. For extremophiles to live, their macromolecules and ribosomes must be chemically sturdy. She reasoned that those sturdy ribosomes would be more likely to crystallize. She solved the ribosome structure from a radiation sensitive bacterium, earning her the 2009 Nobel Prize in Chemistry.

5. CONCLUSION

Crystallography is a tool developed by the most creative and curious people to answer some of the most important questions in chemistry, biology, and medicine. Love for beauty and truth meet in this field that has been aglow with discovery since its inception. Crystallography has not only uncovered deep foundational secrets but has also led to discoveries with great benefits in the development of pharmaceuticals and the understanding of disease. For example, many drugs are targeted to biomolecules and the crystal structures have allowed for the design of drugs with enhanced specificity. Such a design is rational and has increased drug efficacy and lowered the probability of debilitating side-effects.

The three-dimensional structures of proteins have also helped uncover the secrets of catalysis, and this is giving way to the rational design of catalysts, which will revolutionize the way we live. Applications of crystallography continue to bear fruit in ways that surpass the imagination too, as the coming of age of computational chemistry is showing. The three-dimensional structures have opened the door to computer simulations of macromolecules, which are getting more and more powerful every year at predicting the behavior of complex molecules and systems of molecules.

The fruits of crystallography are many, and many scientists have contributed to the field. Many women scientists have made great contributions to the field, and perhaps, this is a reflection of the rewards and challenges that crystallography bestows.

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