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# A Review on Crystallography and Its Role on Drug Design

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**Abstract:** Crystallography is the study of crystals and their formation or determination of atom patterns in solid crystals. In the past, devices like goniometers were used for gaining information about crystal structures, but study of inner crystal structures became possible with Rontegin`s discovery of x-rays, which created x-ray crystallography. X-ray crystallography is the study of the determination of the atomic and molecular structure of crystals, whose crystal structure causes x-ray-emitted waves to diffract or divide. By measuring the angle and intensity of these diffracted waves, we can obtain the shape and size of a unit cell and a 3D photo of the electron density of crystals. This 3D photo of the electron density of crystals shows the location of electrons in crystal atoms. X-ray crystallography is a base for finding functions of biological molecules like vitamins, drugs, proteins, and nucleic acids.

Crystallography is used at quantative and qualitative analysis of crystallic phases and in the design and discovery of different drugs like anti-viral drugs (anti-HIV and anti-influenza drugs), hypertension drugs, antibiotics, etc. Structure-based drug design is used to find complete information about the molecular and spatial structure of lead compounds and analogs. In addition, crystallography has a direct role in drug design and discovery. (Ilari & Savino, 2018)[2].

Macromolecule crystallography, particularly protein crystallography, gives us information about proteins (enzymes, receptors, carriers, and nucleic acids), ligands, and protein-ligand complexes.

Brilliant developments in processing, measurement, and validation methods have removed x-ray crystallography`s limitations and increased usage of crystallography in related studies to drug design. Similarly, these improvements made crystallography a usual method to obtain structural information about macromolecules. (Verma et al., 2018)[4].

The purpose of this study is to explore the role of X-ray crystallography in drug design and discovery. It examines the structural analysis of molecules and atomic-level details using crystallographic techniques. The research demonstrates how X-ray crystallography, along with neutron diffraction and electron crystallography, has transformed drug design processes. It also highlights the importance of understanding the three-dimensional structure of biological targets, such as proteins, for creating effective and targeted therapies. Crystallography also helps in elucidating complex biological structures and interactions. (Bhatti & Hussain, 2000)[6].

***Keywords:*** Crystallography, crystal, drug design, x-ray crystallography, x-ray diffraction

## Introduction

Crystallography is the study of crystals, which are arranged in an orderly and structured microscopic state in three dimensions, extending in all directions. Crystallography determines the order of particles (atoms, molecules, and ions) within crystals. It investigates various materials, ranging from human cells to semimetals and from protein molecules to ceramics. Scientific achievements related to the use of crystallography have led to 29 Nobel Prizes. Crystallographic studies enable scientists to shed light on invisible molecules by directing X-ray beams, resulting in a clear depiction of an otherwise unknown or less distinct molecular structure. (Goldstein, 2003)[8].

Initially, crystallography aided scientists in understanding ionic, covalent, metallic, and hydrogen bonds, with the first crystalline structure identified through crystallography being table salt. Over the past decade, crystallography has astonishingly enhanced human knowledge regarding the structure and function of natural and synthetic molecules. Today, crystallography provides atomic order images of tens of thousands of new structures annually. (Noyan et al., 2007)[9].

X-ray crystallography emerged after the discovery of X-rays by Roentgen, and it was established a century ago through studies enabled by X-rays. Modern crystallography employs various methods, including electron crystallography, neutron scattering, and molecular modeling. X-ray crystallography assists in determining the spatial structure and ordering of atoms by measuring the shape and size of the unit cell, and it is used in the

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design of new drugs to combat various diseases Deschamps, 2005)[10]. Structural information obtained from crystallography is stored in databases such as PDB and CSD for drug design research. (Frank, 2006)[11].

With the advent of new X-ray sources such as neutrons, synchrotrons, and free-electron lasers, the processing and management of crystallographic data have improved. Protein crystallography, which has developed from X-ray crystallography, provides us with diffraction patterns from which electron density maps can be derived, allowing the design of atomic models(Suguna, 2014)[12]. Advancements in technology, the tools used, crystallographic equipment, and new perspectives in crystallography have created new insights that expand the field and enhance its application in drug design( Betts, 2014)[13].

**Crystalline Structure**

A crystalline structure is a state in which atoms, molecules, or ions are spatially organized in a crystal, typically with inter-atomic distances of 0.1 nanometers (100 picometers). The crystalline structure consists of the following components:

1. **Atoms or Base**
2. **Crystal Lattice**
3. **Unit Cell** (Caliandro et al., 2013)[14]

**Crystal Lattice**: A three-dimensional arrangement of points that are organized in specific geometric patterns (atoms, ions, and molecules).

**Unit Cell**: The smallest structural unit of crystals. A unit cell is defined in a three-dimensional lattice by specific lengths and angles between them. The lengths of the unit cell are defined as distances parallel to the three axes in three-dimensional space: parallel to the x-axis as a, parallel to the y-axis as b, and parallel to the z-axis as c. The angles between a and b, a and c, and c and a are denoted as γ, β, and α, respectively. The axes and angles of the unit cell are referred to as unit cell parameters.

**Drug Design**The term drug design is synonymous with rational drug design, simply referring to the creative process of discovering new drugs based on information and knowledge about a biological target or a specific binding site. Essentially, drug design involves creating molecules that complement the biological target in form and size, which bind to or interact with this target.

Drug design is often, but not always, dependent on computational techniques, known as Computer-Aided Drug Design (CADD).

**Ligand-Based Drug Design**: This type of drug design relies on knowledge of molecules that bind to the desired biological targets. In this method, a model of the biological target is constructed based on which molecules bind to this model, and then QSAR (Quantitative Structure-Activity Relationship) is considered.

**Structure-Based Drug Design**: This type of drug design is based on knowledge of the three-dimensional structure of the biological target, obtained from methods such as X-ray crystallography and nuclear magnetic resonance spectroscopy(Thagadurai et al., 2005)[15].

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**X-ray Crystallography**

The study of the internal structure of crystals using X-rays gives rise to the field of X-ray crystallography. The investigation of the internal structure of crystals became possible after the discovery of X-rays by the German scientist Roentgen in 1895. X-ray crystallography is an empirical science for determining the atomic and molecular structure of crystals, where the crystalline structure causes incoming X-ray waves to diffract in specific directions. By measuring the angles and intensities of these diffracted waves, we can produce a three-dimensional image of the electron density within the crystals. The positions of the atoms within the crystals can be determined from this electron density Zheng et al., 2014)[16].

Generally, X-ray crystallography involves obtaining the spatial structure and determining their order by measuring the shape and size of the unit cell. Since X-rays have wavelengths similar to the size of atoms, this similarity leads to the diffraction of X-rays and facilitates research into crystals. Additionally, it provides information regarding chemical relationships. Since materials such as metals, salts, minerals, semimetals, and biological, organic, and inorganic molecules can form crystals, X-ray crystallography has been foundational for advancements in various fields of science(Borchardt Ott & Gould, 2011)[17].

In the early decades of X-ray crystallography, this method was used to determine atomic sizes, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals and

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alloys. This method also helped determine the structures and functions of many biological molecules, including vitamins, drugs, proteins, and nucleic acids such as DNA, hemoglobin, vitamin B12, egg white proteins, and more (Che et al., 2001)[18].

Additionally, this method is used in qualitative and quantitative analysis of crystalline phases. X-ray crystallography is recognized as the gold standard for determining the structures of proteins, where the structures of biological molecules directly relate to their functions, making this topic particularly significant in biological molecules compared to other areas. X-ray crystallography remains the primary method for identifying the atomic structures of new materials and distinguishing substances that appear similar by other methods and experiments. Furthermore, this method is also utilized in the design of new drugs to combat various diseases.

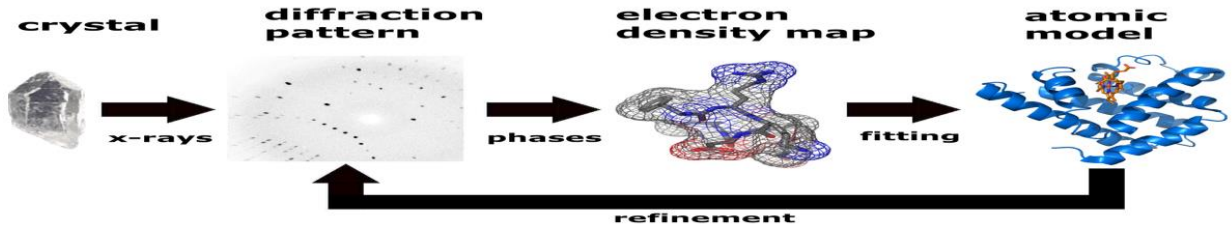


*Figure (3) Obtaining the crystalline structure and constructing atomic models with X-ray crystallography. Y. Che, J. Zheng, J. Hao, and L. Chu, (2001).*

**X-ray Diffraction**

Diffraction is a wave phenomenon where waves encounter an obstacle and spread out. In crystals, atoms act like a natural lattice. Because atoms are relatively large, the internal distances between them are comparable to the wavelength of X-rays. For this reason, the atoms in a crystal are capable of diffracting X-rays. The pattern of this diffraction varies for different crystals due to differences in atomic spacing and the distances between various planes. Consequently, the diffracted X-rays from a specific sample produce patterns on radiographic films that function like fingerprints. The technique of X-ray diffraction is a powerful qualitative tool for studying crystals.

There is a specific relationship between the angle of diffraction, the wavelength of the radiation, and the spacing between the lines in the lattice. The wavelength can be compared with the distance between atoms in crystals, leading to the proposal by von Laue that crystals can act as three-dimensional diffraction lattices for X-rays. This was experimentally confirmed by Friedrich and Knipping.

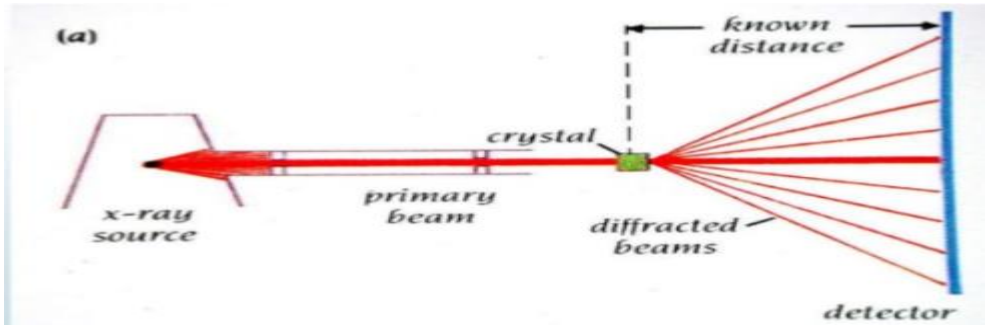


*Figure (4) Working principle of X-ray scattering devices*

**X-ray Scattering Uses**

X-ray scattering is a non-destructive technique that some of its uses include:

1. Separation of crystalline materials from amorphous
2. Determination of the structure of crystalline materials
3. Determine the distribution of electrons in atoms and throughout the unit cell
4. Determine the orientation of single crystals

**The Use of Crystallography in Drug Design and Discovery:**

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Crystallographic studies play a vital role in drug design. Crystallography has been used for more than a decade in drug design based on structure, or Structure-Based Drug Design (SBDD). Protein crystallography is used to obtain 3D images of the structures of biologically active substances and their targets, forming the foundation of drug discovery. The growth of protein crystals can be challenging and highly sensitive during their formation. Accurate analysis of the crystal structures of macromolecular targets and ligand-macromolecule complexes is crucial at every stage, with X-ray crystallography playing a key role in this process.

Among the many drugs discovered through this method are the AIDS drugs Veracept and Criksowan, the flu medication Tamiflo, leukemia treatments, and anti-cancer drugs like Tarceva. X-ray crystallography is often used alongside other techniques to enhance the efficacy and utility of drug design. For example, scientists combined it with SAR analysis to develop new hydroxamate inhibitors as potent inhibitors of the enzyme TNF-alpha converting enzyme, which increases in autoimmune disorders like Rheumatoid arthritis.
At the pharmaceutical company Merck, researchers used molecular modeling and X-ray crystallography to develop highly selective inhibitors of the β-site amyloid precursor protein cleaving enzyme (BACE), which is involved in the treatment of Alzheimer's disease. Another team developed a strong and selective Feminase inhibitor using molecular modeling and X-ray crystallography, which has entered clinical trials for asthma and dermatitis.
Recently, researchers have utilized neutron crystallography, a technique that has been used in recent decades, to determine the structure of human enzymes. The first team to use this technique successfully determined the structure of carbonic anhydrase in its bound state with acetazolamide. Scientists noted that neutron crystallography allows the detection of hydrogen molecules, hydrogen bonds, charge states, and molecular properties that are not visible through regular X-ray crystallography. This is highly valuable for drug design. While significant advances have been made in structural biology, particularly in X-ray crystallography, further progress in computational methods is on the horizon. Crystal structures can be reinterpreted, and future experiments can build on these models. The macromolecular structures derived from X-ray diffraction data have limitations and require careful evaluation and understanding to be effectively used in structure-based drug discovery.

**Conclusion**

Most solids form crystal structures, and about 80% of drugs are formulated in their final form as crystals. Thus, using various crystallographic methods, we can determine the structures of drugs and use this structural information in the design and discovery of analogs of those drugs. Additionally, using protein crystallography, we can determine the structures of unknown proteins such as receptors, carriers, enzymes, and nucleic acids. Since most drugs exert their effects through interactions with the active sites of proteins, studies that obtain information about proteins, ligands, and protein-ligand complexes are essential in creating protein models and designing new drugs. These structural models significantly aid in drug discovery, with tens of thousands of new structures being obtained through crystallography each year.

Recent advances in crystallography, with the introduction of new X-ray sources such as neutrons, synchrotrons, and free-electron lasers, as well as techniques like neutron diffraction and electron crystallography, have made it one of the standard methods used to determine the structures of unknown molecules.
With the development of modern technology and the use of advanced equipment, crystallography tools and new techniques have completely transformed scientists' understanding of crystallography, broadening its scope far beyond its initial uses and highlighting its role in drug design. Combining X-ray crystallography with electron microscopy studies and nuclear magnetic resonance spectroscopy (NMR) provides detailed information about atoms. This method is highly effective for studying the structures of complex biological molecules, such as ribosome complexes, t-RNA, and protein factors

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