CRYSTALLOGRAPHY IN A NUTSHELL

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Crystallography

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CHAPTER OVERVIEW

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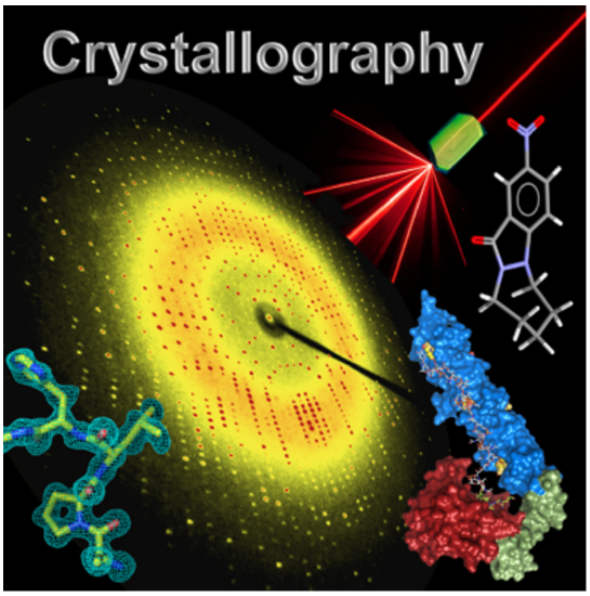
1.0: Introduction



Why water boils at 100ºC and methane at -161ºC; why blood is red and grass is green; why diamond is hard and wax is soft; why graphite writes on paper and silk is strong; why glaciers flow and iron gets hard when you hammer it; how muscles contract; how sunlight makes plants grow and how living organisms have been able to evolve into ever more complex forms…? The answers to all these problems have come from structural analysis.

***Max Perutz**, July 1996 (Churchill College, Cambridge)*

With the words pronounced by the Nobel laureate **Max Perutz** we open these pages **(*)**, a continuing work in progress, intended to guide the interested reader into the fascinating world of Crystallography, which forms part of the scientific knowledge developed by many scientists over many years. This allows us to explain what crystals are, what molecules, hormones, nucleic acids, enzymes, and proteins are, along with their properties and how can we understand their function in a chemical reaction, in a test tube, or inside a living being.

The discovery of X-rays in the late 19th century completely transformed the old field of Crystallography, which previously studied the morphology of minerals. The interaction of X-rays with crystals, discovered in the early 20th century, showed us that X-rays are electromagnetic waves with a wavelength of about 10^{-10} meters and that the internal structure of crystals was regular, arranged in three-dimensional networks, with separations of that order. Since then, Crystallography has become a basic discipline of many branches of Science and particularly of Physics, Chemistry of condensed matter, Biology and Biomedicine.

Structural knowledge obtained by Crystallography allows us to produce materials with predesigned properties, from catalyst for a chemical reaction of industrial interest, up to toothpaste, vitro ceramic plates, extremely hard materials for surgery use, or certain aircraft components, just to give some examples of small, or medium sized atomic or molecular materials.

Moreover, as biomolecules are the machines of life, like mechanical machines with moving parts, they modify their structure in the course of performing their respective tasks. It would also be extremely illuminating to follow these modifications and see the motion of the moving parts in a movie. To make a film of a moving object, it is necessary to take many snapshots. Faster movement requires a shorter exposure time and a greater number of snapshots to avoid blurring the pictures. This is where the ultrashort duration of the FEL (free electron laser) pulses will ensure sharp, non-blurred pictures of very fast processes (**European XFEL** or **CXFEL**).

We may suggest you to start **getting an overview about Crystallography**, or looking at **some interesting video clips collected by the International Union of Crystallography**. Some of them can directly be reached through the following links:

- **Presentation of the International Year of Crystallography**, 1.30 min video (**International Union of Crystallography**). In case of troubles, **use this link**.

- **Celebrating the centenary of the discovery of X-ray diffraction by crystals**, 3 min video (The Royal Institution, London). In case of troubles, **use this link**.
- "**The fascinating world of crystallography**", 2.30 min video, prepared by **Quidos** for the **International Year of Crystallography**. In case of troubles, **use this link**.
- **Georgina Ferry on X-ray crystallography**, a freelance science writer, editor and broadcaster, discussing the fascinating history and importance of crystallography. This video (7 min) was created as support of an exhibition of the **Wellcome Collection**. In case of troubles, **use this link**.
- **The humble Braggs and X-ray crystallography: Solving the patterns of matter**, 9 min video (The Royal Institution, London). In case of troubles, **use this link**.
- **A Century of Crystallography: the Braggs Legacy**, 44 min video (The Royal Institution, London). In case of problems, **use this link**.
- **Myoglobin: A brief history of structural biology**, 4 min video (The Royal Institution, London). In case of problems, **use this link**.
- **Elspeth Garman on the crystallization process**, 8 min video (The Royal Institution, London). In case of problems, **use this link**.
- **Stephen Curry explaining from the diffraction phenomena up to the structural resolution**, 8 min video (The Royal Institution, London).
- **Lecture of Prof. Stephen Curry**, 1 h video (The Imperial College, London).

In any case, we suggest you to get a previous **overview about the meaning of Crystallography**, and if you maintain your interest go deeper into the remaining pages that are shown in the menu on the left (if you don't see the left menu, **click here**). Enjoy it!

(*) We endeavor to assemble these pages and offer them to the interested reader, but obviously we are not immune to errors, inconsistencies or omissions. We are very grateful to several readers who have helped us to correct some previously undetected small errors or that have improved the wording of certain parts of the text. For anything that needs further attention, please, let us know through **Martín Martínez Ripoll**.

These pages were **announced by the International Union of Crystallography (IUCr)**, have been selected as **one of the educational web sites and resources of interest to learn crystallography**, offered as such in the **commemorative web for the International Year of Crystallography**, and suggested as **the educational website in the brochure prepared by UNESCO** for the **crystal growing competition for Associated Schools** (**even in subsequent calls of this competition**. The Cambridge Crystallographic Data Centre also offers this website through its Database of Educational Crystallographic Online Resources (**DECOR**).

Martín Martínez Ripoll (1946-) and **Félix Hernández Cano** (1941-2005+) were coauthors of a first version of these pages in the early 1990's. Later, in 2002 they produced a PowerPoint presentation dedicated to draw students' attention to the enigmatic beauty of the crystallographic world... This file, called XTAL RUNNER (totally virus free, although in Spanish) can be **obtained through this link**. If you understand Spanish we also offer you the possibility of reading a short general article of these authors published in 2003, entitled **Cristalografía: Transgrediendo los Límites**. Today we ask ourselves, **where are those glory days gone?**

Some relevant hints:

- This website is designed by combining three visible areas in the same window: a header and a menu on the left (which always remain visible), and a central area with the information obtained. It is what we can call **full-screen mode**, suitable for desktop computers and tablets.
- The full-screen mode may not be suitable for mobile, in which case we suggest using the **central-screen mode**, that is, the mode using the central area only, and starting the session with the Table of Contents.
- In both modes, a **small square logo** appears in the upper right corner of the central screen that links to the **Table of Contents**.
- All the links that appear in the menu on the left (in full screen mode), or in the Table of Contents (in any of the modes) lead to internal pages that are always displayed on the same window. The remaining links, which necessarily refer to external pages, will always be displayed in a "new window". Going back to a previous page can be achieved using the "**Back** " link in the top left of the header (full screen mode), or through the browser's own strategy.

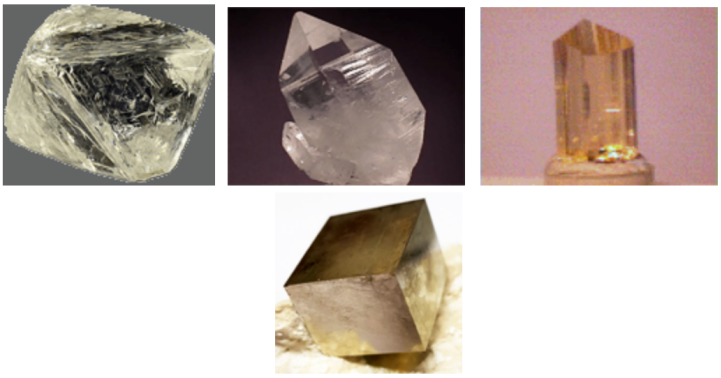
- From time to time we incorporate some novelties or small corrections into these pages, so occasionally we recommended to reload these pages in your browser, or to clear the navigator caché. This will avoid to see the previously existing pages stored on your computer's caché.
- Some companies offer documents directly extracted from these web pages and you have to pay for them. Please, do not participate in this fraud! All the material here presented is freely available to you, although for your personal use only, as it is shown below under the copyright condition.

1.1: The structure of crystals

In the context of this chapter, you will also be invited to visit these sections...

- **The crystalline state**
- **Interatomic forces in crystals**
- **Early historical notes about crystals and crystallography**

We all have heard about natural minerals and crystals. We find them daily without entering a museum. A rock and a mountain are made up of minerals, as crystalline as a lump of sugar, a bit of porcelain or a gold ring. However, only occasionally is the size of a crystal large enough to draw our attention, as is the case of these beautiful mineral examples of: *Diamond (pure carbon) - Quartz (silicon dioxide) - Scapolite (aluminum silicate) - Pyrite (iron sulfide)*



Several of these images are property of **Amethyst Galleries, Inc**.

Other excellent images of minerals **can be found through this link**.

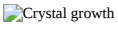
Although you can continue reading these pages without any special difficulty, probably you would like to know some aspects about the historical development of our understanding of the crystals. For these readers we offer **some further notes that can be found through this link**.

The ancient Greeks identified quartz with the word ***crystal*** (κρύσταλλος, *crustallos*, or phonetically *kroos'-tal-los* = cold + drop), ie, very cold icicles of extraordinary hardness. But the formation of crystals is not a unique property of minerals; they are also found (but not necessarily in a natural manner) in the so-called organic compounds, and even in nucleic acids, in proteins and in viruses...

A crystal is a material whose constituents, such as atoms, molecules or ions, are arranged in a highly ordered microscopic structure. These constituents are held together by **interatomic forces (chemical bonds) such as metallic bonds, ionic bonds, covalent bonds, van der Waals bonds, and others**.

The crystalline state of matter is the state with the highest order, ie, with very high internal correlations and at the greatest distance range. This is reflected in their properties: anisotropic and discontinuous. Crystals usually appear as unadulterated, homogenous and with well-defined geometric shapes (habits) when they are well-formed. However, as we say in Spanish, "the habit does not make the monk" (clothes do not make the man) and their external morphology is not sufficient to evaluate the crystallinity of a material.

The movie below shows the process of crystal growth of lysozyme (a very stable enzyme) from an aqueous medium. The duration of the real process, that takes a few seconds on your screen, corresponds approximately to 30 minutes.

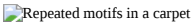


The original movie was found on an old website offered by **George M. Sheldrik**.

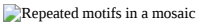
The figure on the left shows a representation of the faces of a given crystal. If your browser allows the **Java Runtime**, clicking on the image will open a new window and you will be able to turn this object. If you do not have this application, you can still observe the model rotation in continuous mode **from this link**.

Other Java pop-ups of faces and forms (habits) for ideal crystals can be obtained **through this link**.

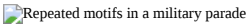
So, we ask ourselves, what is unique about crystals which distinguishes them from other types of materials? The so-called microscopic ***crystal structure*** is characterized by groups of ions, atoms or molecules arranged in terms of some ***periodic repetition*** model, and this concept (***periodicity***) is easy to understand if we look at the drawings in an carpet, in a mosaic, or a military parade...



Repeated motifs in a carpet



Repeated motifs in a mosaic



Repeated motifs in a military parade

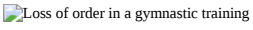
If we look carefully at these drawings, we will discover that there is always a fraction of them that is repeated. In crystals, the atoms, ions or molecules are packed in such a way that they give rise to "motifs" (a given set or unit) that are repeated every 5 Angstrom, up to the hundreds of Angstrom (1 Angstrom = 10^{-8} cm), and this repetition, in three dimensions, is known as the ***crystal lattice***. The motif or unit that is repeated, by orderly shifts in three dimensions, produces the network (the whole crystal) and we call it the ***elementary cell*** or ***unit cell***. The content of the unit being repeated (atoms, molecules, ions) can also be drawn as a point (the ***reticular point***) that represents every constituent of the motif. For example, each soldier in the figure above could be a reticular point.

But there are occasions where the repetition is broken, or it is not exact, and this feature is precisely what distinguishes a crystal from glass, or in general, from materials called amorphous (disordered or poorly ordered)...

Planar atomic model of an ordered material (crystal)

Planar atomic model of glass (an amorphous material)

However, matter is not entirely ordered or disordered (crystalline or non-crystalline) and so we can find a continuous degradation of the order (***crystallinity degree***) in materials, which goes from the perfectly ordered (***crystalline***) to the completely disordered (***amorphous***). This gradual loss of order which is present in materials is equivalent to what we see in the small details of the following photograph of gymnastic training, which is somewhat ordered, but there are some people wearing pants, other wearing skirts, some in different positions or slightly out of line...



In the crystal structure (ordered) of inorganic materials, the repetitive units (or motifs) are atoms or ions, which are linked together in such a way that we normally do not distinguish isolated units and hence their stability and hardness (ionic crystals, mainly)...

Crystal structure of an inorganic material: α-quartz

Where we clearly distinguish isolated units is in the case of the so-called organic materials, where the concept of the isolated entity (***molecule***) appears. Molecules are made up of atoms linked together. However, the links between the molecules within the crystal are very weak (***molecular crystals***). Thus, they are generally softer and more unstable materials than the inorganic ones.

Crystal structure of an organic material: Cinnamamide

Protein crystals also contain molecular units (molecules), as in the organic materials, but much larger. The type of forces that bind these molecules are also similar, but their packing in the crystals leaves many holes that are filled with water molecules (not necessarily ordered) and hence their extreme instability...

Crystal structure of a protein: AtHal3.

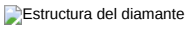
The molecular packing produces very large holes

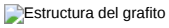
The different packing modes in crystals lead to the so-called ***polymorphic phases*** (allotropic phases of the elements) which confer different properties to these crystals (to these materials). For example, we all know the different appearances and properties of the chemical element carbon, which is present in Nature in two different crystalline forms, diamond and graphite:

Left: *Diamond (pure carbon)*

Right: *Graphite (pure carbon)*

Graphite is black, soft and an excellent lubricant, suggesting that its atoms must be distributed (packed) in such a way as to explain these properties. However, diamonds are transparent and very hard, so that we can expect their atoms very firmly linked. Indeed, their sub-microscopic structures (at atomic level) show us their differences ...





Left: *Diamond, with a very compact structure*

Right: *Graphite, showing its layered crystal structure*

In the diamond structure, each carbon atom is linked to four other ones in the form of a very compact three-dimensional network (covalent crystals), hence its extreme hardness and its property as an electric insulator. However, in the graphite structure, the carbon atoms are arranged in parallel layers much more separated than the atoms in a single layer. Due to these weak links between the atomic layers of graphite, the layers can slide, without much effort, and hence graphite's suitability as a lubricant, its use for pens and as an electrical conductor.

And speaking about conductors... The metal atoms in the metallic crystals are structured in such a way that some delocalized electrons give cohesion to the crystals and are responsible for their electrical properties.

Before ending this chapter let us introduce a few words about the so-called ***quasicrystals***...

A quasicrystal is an "ordered" structure, but not perfectly periodic as the crystals are. The repeating patterns (sets of atoms, etc.) of the quasicrystalline materials can fill all available space continuously, but they do not display an exact repetition by translation. And, as far as symmetry is concerned, while crystals (according to the laws of classical crystallography) can display axes of rotation of order 2, 3, 4 and 6 only, the quasicrystals show other rotational symmetry axes, as for example of order 10

In this website we will not pay attention to the case of quasicrystals. Therefore, if you are interested on it, please go to this **link, where Steffen Weber, in a relatively simple way, describes these types of materials from the theoretical point of view, and where some additional sources of information can also be found**..Advanced readers should also consult **the site offered by Paul J, Steinhardt at the University of Princeton**.

The Nobel Prize in Chemistry 2011 was awarded to Daniel Shechtman by the discovery of quasicrystals in 1984..

There are obviously many questions that the reader will ask, having come this far, and one of the most obvious ones is: ***how do we know the structure of crystals?*** This question, and others, will be answered in following chapters and therefore we encourage you to consult them...

1.2: X-rays

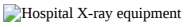
An unexpected result! Discovery of X-rays in 1895.

(Illustration by Alejandro Martínez de Andrés, CSIC 2014)

By the end of the 19th century, in 1895, **Wilhelm Conrad Röntgen (1845-1923)**, a German scientist from the University of Würzburg, discovered a form of radiation (of unknown nature at that time, and hence the name ***X-rays***) which had the property of penetrating opaque bodies. In the first paragraph of his **communication sent to the Society of Physics and Medicine of Wurzburg (1895)** he reports the discovery as follows:

*After producing an electrical discharge with a **Ruhmkorff's coil** through a **Hittorf**'s vacuum tube, or a sufficiently evacuated **Lenard**, **Crookes** or similar apparatus, covered with a fairly tight-fitting jacket made of thin, black paperboard, one sees that a cardboard sheet coated with a layer of platinum and barium cyanide, located in the vicinity of the apparatus, lights up brightly in the completely darkened room regardless of whether the coated side is pointing or not to the tube. This fluorescence occurs up to 2 meters away from the apparatus. One can easily be convinced that the cause of the fluorescence proceeds from the discharge apparatus and not from any other source of the line.*

To learn about some aspects of the discovery, as well as about personal aspects of Röntgen, see also the chapter dedicated to some **biographical outlines**. But if you can read Spanish, there is an extensive chapter dedicated to both the **historical details around Röntgen and his discovery.**



- Left: ***Wilhelm Conrad Röntgen (1845-1923)**, around 1895 with an X-ray photograph of his wife's hand showing her wedding ring . For his discovery Röntgen won the Nobel Prize in Physics in 1901.*
- Right: *Typical hospital radiology equipment*

X-rays are invisible to our eyes but they can produce visible images if we use photographic plates or special detectors...

Left: *Radiographic image of a hand*

Right: *Radiographic image of a monkey*

Left: *Radiographic image of a well-done weld*

Right: *Poorly-done weld (black line)*

A painting and its X-ray photograph showing two superimposed paintings on the same canvas

(Charles II of Spain, by Carreño de Miranda, Museo del Prado, Madrid)

We all know several applications of X-rays in the medical field: angiography (the study of blood vessels) or the so-called **CT scans**, but the use of X-rays has also been extended to detect failures in metals or for the analysis of paintings.

Many years passed from the discovery of X-rays in 1895 until that finding produced a revolution in the fields of Physics, Chemistry and Biology. The potential applications in these areas came in 1912 indirectly from the hand of **Max von Laue (1879-1960)**, professor at the Universities of Munich, Zurich, Frankfurt, Würzburg and finally Berlin.

Paul Peter Ewald (1888-1985) got his friend, Max Laue, interested in his own experiments on the interference between radiations with large wavelengths (practically visible light) on a "crystalline" model based on resonators (note that at that time the question on wave-particle duality was also under discussion). The idea then came to Laue that the much shorter electromagnetic rays, which X-rays were supposed to be, would cause some kind of diffraction or interference phenomena in a medium, and that a crystal could provide this medium.

Left: **Max von Laue (1879-1960)**

Right: **Paul. P. Ewald (1888-1985)**

Max von Laue demonstrated the nature of this new radiation by putting crystals of copper sulfate, and of the mineral zinc blende, in front of an X-ray source, obtaining confirmation of his hypothesis and demonstrating both, the undulatory nature of this radiation and the periodic nature of crystals. For these findings he received the Nobel Prize in Physics in 1914.

Left: **William H. Bragg (1862-1942)**

Right: **William L. Bragg (1890-1971)**

However, those who really benefited from the discovery of the Germans were the British Braggs (father and son), **William H. Bragg (1862-1942)** and **William L. Bragg (1890-1971)**, who together in 1915 received the Nobel Prize in Physics for demonstrating the usefulness of the phenomenon discovered by von Laue for obtaining the internal structure of crystals - but all this will be the subject of later chapters.

This chapter will deal exclusively with the nature and production of X-rays...

X-rays are electromagnetic radiations, of the same nature as visible light, ultraviolet or infrared radiations, and the only thing that distinguishes them from other electromagnetic radiations is their wavelength, which is about 10^{-10} m (equivalent to the unit of length known as one Angstrom).

*Graphic representation of an electromagnetic wave, showing its associated electric (**E**) and magnetic (**H**) fields, moving forwards at the speed of light.*

The continuous spectrum of visible light (wavelength decreases from red to violet)

Excellent information on the electromagnetic spectrum can be found in some pages offered by **NASA**. The reader can also learn about X-rays and their applications in **Medical Radiography** and in the pages of **The X-Ray Century**.

ν(Hz) λ(m) = 3 10^8 m Hz

E(J) = h(J/Hz) ν(Hz) = k(J/K molecule) T(K)

h = 6.6 10^{-34} (J/Hz); k = 1.4 10^{-23} (J/K molecule); 1 eV = 1.6 10^{-19} (J)

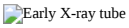
Figure taken from the **Berkeley Lab**

The most interesting X-rays for Crystallography are those having a wavelength close to 1 Angstrom (the ***hard X-rays*** in the diagram above), which is a distance very close to the interatomic distances occurring in molecules and crystals. These type of X-rays have a frequency of approximately 3 million THz (tera-hertz) and to an energy of 12.4 keV (kilo-electron-volts), which in turn would correspond to a temperature of about 144 million degrees Celsius. These wavelengths are produced in Crystallography laboratories and in large synchrotrons as **ESRF**, **ALBA**, **Diamond**, **DESY**, ...

X-ray generator in a Crystallography laboratory. The goniometric and detection systems are shown behind the X-ray tube.

*Aerial photograph of the synchrotron at the **ESRF** in Grenoble (France). Note its circular geometry*

The equipment used in crystallographic laboratories to produce X-rays is relatively simple. They have a high voltage generator (50,000 volts) that brings high voltage to the so-called X-ray tube, where the radiation is actually produced. You could also **take a look at the web page "The Cathode Ray Tube site"**.



*Early X-ray tube (image taken from **The Cathode Ray Tube** site)*

Conventional X-ray tubes used for crystallographic studies during the 20th century

Static sketch and animation of the X-ray production on a conventional X-ray tube

Those 50 kV are supplied as a potential difference (high voltage) between an incandescent filament (through which a low voltage electrical current of intensity ***i*** passes: around 5 A at 12 V) and a pure metal (usually copper or molybdenum). This produces an electrical current (of free electrons) between them of about 30 mA. From the incandescent filament (negatively charged) the free electrons jump to the anode (positively charged) causing (in the pure metal) a reorganization in its electronic energy levels.

This is a process that generates a lot of heat, so that X-ray tubes must be very well chilled. An alternative to conventional X-ray tubes are the ***rotating anode generators***, in which the anode in the form of a cylinder is maintained in a continuous rotation, so that the incidence of electrons is distributed over its cylindrical surface and thus a higher power can be obtained.

Left: *Rotating anode generator*

Right: *Rotating anode of polished copper (images taken from **Bruker-AXS**)*

The so-called "characteristic X-rays" are produced according to the following scheme:

a) Energy state of electrons in an atom of the anode that is going to be reached by an electron from the filament. **b)** Energy state of the same electrons after impact with the electron from the filament. The incident electron bounces and ejects an electron from the anode, producing the corresponding hole. **c)** An electron of a higher energy level falls and occupies the hole. This energy jump, perfectly defined, generates the so-called characteristic X-rays of the anodic material.

Left: *In an X-ray tube the electrons emitted from the cathode are accelerated towards the metal target anode by an accelerating voltage of typically 50 kV. The high energy electrons interact with the atoms in the metal target. Sometimes the electron comes very close to a nucleus in the target and is deviated by the electromagnetic interaction. In this process, which is called bremsstrahlung (braking radiation), the electron loses much energy and a photon (X-ray) is emitted. The energy of the emitted photon can take any value up to a maximum corresponding to the energy of the incident electron.*

Right: *The high energy electron can also cause an electron close to the nucleus in a metal atom to be displaced. This vacancy is filled by an electron further out from the nucleus. The well defined difference in binding energy, characteristic of the material, is emitted as a monoenergetic photon. When detected this X-ray photon gives rise to a characteristic X-ray line in the energy spectrum. Animations taken from **Nobelprize.org**.*

Apart from the developments made on the new synchrotron sources, there still exist several attempts to optimize efficiency and power of the "in-house" X-ray sources, as the ones based on the microfocus technology, that is, high brightness sources that additionally use very stable optics mounted to the tube housing, or those based on the use of a liquid metal as anode...

Left: *New microfocus X-ray tube. Image taken from **Incoatec***

Right: *New development for an of X-ray source based on liquid metal anodes.*

Taken from ***Excillum***. *There is an animation showing **this technology***

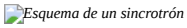
The energetic restoration of the excited anodic electron is carried out with an X-ray emission with a frequency that corresponds exactly to the specific energy gap (quantum) that the electron needs to return to its initial state. These X-rays therefore show a specific wavelength and are known as ***characteristic wavelengths*** of the anode. The most important characteristic wavelengths in X-ray Crystallography are the so-called ***K-alpha lines*** (***Kα***), produced by the electrons falling to the innermost layer of the atom (higher binding energy). However, in addition to these specific wavelengths, a continuous range of wavelengths, very close to each other, is also produced known as the ***continuous radiation*** which is due to the braking of the incident electrons when they hit the metal target.

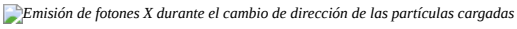
*Distribution of X-ray wavelengths produced in a conventional X-ray tube where the anode material is copper (**Cu**), molybdenum (**Mo**), chromium (**Cr**) or tungsten (**W**). Over the so-called continuous spectrum, the characteristic **K-alpha** (**Kα**) and **K-beta** (**Kβ**) lines are shown. The starting point of the continuous spectrum appears at a wavelength which is approximately 12.4 / V, (Angstrom) where V represents the amount of kV between anode and filament. For a given voltage between the anode and filament, only the characteristic wavelengths of molybdenum are obtained (figure on the left).*

In ***synchrotrons*****,** the generation of X-rays is quite different. A synchrotron facility contains a large ring (on the order of kilometers), where electrons move at a very high speed in straight channels that occasionally break to match the curvature of the ring. These electrons are made to change direction to go from one channel to another using magnetic fields of high energy. It is at this moment, when electrons change their direction, that the electrons emit a very high energy radiation known as ***synchrotron radiation***. This radiation is composed of a continuum of wavelengths ranging from ***microwaves*** to the so-called ***hard X-rays***.

Synchrotrons appearance is very similar to that shown in the following schemes:

A synchrotron scheme. The linear accelerator (Linac) and the circular accelerator (Booster) are seen in the center, surrounded by the outer storage ring. The emitted X-rays are directed to the beamlines.


Esquema de un sincrotrón


Emisión de fotones X durante el cambio de dirección de las partículas cargadas

Left: *General sketch of a synchrotron. The central circle is where the charged particles are accelerated (linac & booster). The outer circle is the storage ring, formed by crooked lines, at the end of which the experimental stations are installed.*

Right: *Outline of the junction of two crooked lines of the storage ring of a synchrotron. X-rays appear due to the change of direction of the charged particles.*

The interested reader can access a demonstration on the **operation of a synchrotron ring through this link**, or see the **same animation in a larger size through this other link**.

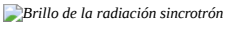
*Outline of the point between two straight segments in the storage ring of a synchrotron. Image taken from the **ESRF***

*Details of how X-rays are produced in a synchrotron in the curvature of the electrons' trajectory inside the storage ring. Image taken from the **ESRF***

The X-rays obtained in the synchrotrons have two clear advantages for crystallography:

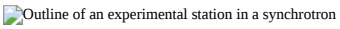
1. the wavelengths can be tuned at will, and
2. its brilliance is at least 10^{21} times higher that those obtained with a conventional X-ray tube (see the image below).

Here can you find a **list of synchrotrons and storage rings used as synchrotron radiation sources, and free electron lasers around the world**.


Brillo de la radiación sincrotrón

The brilliance of X-ray sources: conventional X-ray tubes, synchrotrons and the future ***XFEL***. *Image taken from the **ESRF**.*

The following image shows an outline of an experimental station of a synchrotron: a) the optics hutch, where X-rays are filtered and focused using curved mirrors and monochromators; b) the experimental hutch, where the goniometer, sample and detector are located and where the diffraction experiment is done and, c) the control cabin, where the experiment is monitored and, if required, also evaluated.


Outline of an experimental station in a synchrotron

Outline of an experimental station in a synchrotron

Lightsources.org contains news and science highlights from each light source facility, as well as photos and videos, education and outreach resources, a calendar of conferences and events, and information on funding opportunities.

The radiation used for crystallography is usually monochromatic (or nearly monochromatic), that is, a radiation with exclusively (or almost exclusively) a single wavelength. In order to achieve this, the so-called monochromators are used, which consist of a system of crystals that, based on **Bragg's Law (which will be presented in another chapter)**, are able to "filter" (through the interaction between the crystals and the X-rays) the polychromatic radiation, allowing only one wavelength (color), as shown below.

*Outline of a monochromator. Polychromatic radiation (white) coming from the left (below) is "reflected" , in accordance with **Bragg's Law**, (to be seen in subsequent chapter), in different orientations of the crystal to produce ("to filter") a monochromatic radiation that is reflected again ("filtered") in the secondary crystal. For the moment it is enough that the reader is aware that this law will allow us to understand how the crystals "reflect" the X-rays, behaving as special mirrors . Image taken from the **ESRF**.*

X-rays interact with the electrons of matter... A ***monochromatic beam*** (ie with a single wavelength) suffers an exceptional attenuation, proportional to the thickness being crossed. This attenuation may arise from several factors: **a)** the body heats up, **b)** a ***fluorescent radiation***, with different wavelength, is produced & accompanied by photoelectrons, both being characteristic of the material (this leads to the photo-electron spectroscopies, ***Auger*** and ***PES***); and **c)** scattered X-rays with the same wavelength (***coherent*** and ***Bragg***) or with slightly higher wavelengths (***Compton***), together with the scattered electrons.

Of all these effects, the most important one is ***fluorescence***, where the absorption increases by increasing incident wavelength. However, this behavior has discontinuities (***anomalous dispersion***) for those energies that correspond to electronic transitions between different energy levels of the material (this leads to the ***EXAFS*** spectroscopy).

*Spectrum emitted by a metallic anode showing its characteristic wavelengths (continuous line). In the same figure, but referred to a vertical axis of absorbance (not drawn) the increasing and discontinuous variation of the absorption (dashed line) of a given material is also shown. This gives an idea of the use of this property as a filter to obtain monochromatic radiation, at least separating the double **Kα1 - Kα2** from the rest of the spectrum. This approach, using concrete materials with specific absorption capacities, was used in Crystallography laboratories until the early 1970's to obtain monochromatic radiation.*

Special mention deserves the recent discovery introduced in the field of femtosecond X-ray protein nanocrystallography. Using this technique (**XFEL**: **X**-ray **F**ree **E**lectron **L**aser), based on the use of X-rays obtained from a free electron laser, "snapshots" of X-ray diffraction can be obtained in the femtoseconds scale. It has been proposed that femtosecond X-ray pulses can be used to outrun even the fastest damage processes by using single pulses so brief that they terminate before the manifestation of damage to the sample in less time than it needed to be damaged by the crystallites radiation.This will imply a giant step to remove virtually all the difficulties in the crystallization process, especially for proteins (see these articles: **Nature (2011) 470, 73-77**, **Nature (2013)** and **Nature(2014)**). In this sense, it is also worth quoting the article published in **Radiation Physics and Chemistry (2004) 71, 905-916**, which already warned on the future importance of the free electron laser on structural biology.


The X-ray free electron laser

The European XFEL generates ultrashort X-ray flashes, 27,000 times per second and with a brilliance that is a billion times higher than that of the best conventional X-ray radiation sources. Thanks to its outstanding characteristics, which are unique worldwide, the facility opens up completely new research opportunities for scientists and industrial users. It could be interesting to look at the video offered on the **web site of the international consortium**, or **directly through this link**.

Regarding the use of these powerful X-ray sources for determining the structure of biological macromolecules, the interested readers should consider the very promising results published in **Nature (2016) 530, 202-206**. This study provides the opportunity

to use not only the information contained in the diffraction spots generated by crystals, but also in the very weak intensity distribution found around and between the diffraction spots, the so called continuous diffraction.

With X-rays from free-electron lasers crystallographic applications are extended to nanocrystals, and even to single non-crystalline biological objects and **even movies of biomolecules in action can be produced**.

To generate the X-ray flashes, bunches of electrons will first be accelerated to high energies and then directed through special arrangements of magnets (undulators). In the process, the particles will emit radiation that is increasingly amplified until an extremely short and intense X-ray flash is finally created.

Recently, the modification that involves replacing the so-called material undulators (magnets) with a new optical device also based on laser technology, dramatically reduces the size of the XFEL by about 10,000 times and the size of the accelerator by 100 times, leading to an incredible reduction in size and price of the so called **CXFEL** (compact X-ray free-electron laser).

In any case, X-rays, like any light "illuminate" and "let to see", but in a different manner than we see with our eyes. We encourage you to go forward, to understand how X-rays allow us "to see" inside crystals, that is, to "see" the atoms and the molecules.

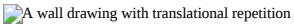
1.3: The symmetry of crystals

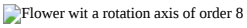
In the context of this chapter, you will also be invited to visit these sections...

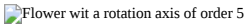
- **Symmetry elements**
- **The crystallographic restriction theorem**
- **Representation of crystal classes**
- **Representation of Laue groups**
- **Representation of Bravais lattices**

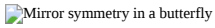
About symmetry in general

Often we don't realize it, but we continuously live with symmetry... Symmetry is the consistency, the repetition of something in space and/or in time, as is shown in the examples below: a wall drawing, the petals of flowers, the two sides of a butterfly, the succession of night and day, a piece of music, etc.









Symmetry by repetition of patterns in a wall drawing or in flowers. The wall drawing shows repetition by translation. The flowers show repetitions by rotations. The flower on the left shows repetition around an axis of rotation of order 8 (8 identical petals around the rotation axis). The flower in the middle shows an axis of rotation of order 5 (two different families of petals that are distributed around the rotation axis). In addition, each petal in both flowers shows a plane of symmetry which divides it into two identical parts (approximately), the same as it occurs with the butterfly shown on the right. If the reader is surprised by the fact that we say that the two parts separated by a plane of symmetry (mirror) are only "approximately" identical, is because they are really not identical; they cannot be superimposed, but this is an issue that will be explained in another section.

Symmetry by repeating events: Day - Night - Day

Symmetry in music. A fragment from "Six unisono melodies" by Bartók.

(The diagram at the bottom represents the symmetrization of the one shown above)

The word "Symmetry," carefully written with somewhat distorted letters, shows a two-fold axis (a rotation of 180 degrees) perpendicular to the screen.

The following sentence also serves to illustrate the concept of symmetry:

A MAN, A PLAN, A CANAL: PANAMA

where, if we forget the commas and the colon, it becomes:

AMANAPLANACANALPANAMA

which can be read from right to left with exactly the same meaning as above. It is a case similar to the "palindromic" numbers (**232** or **679976**).

There are many links in which the reader can find information on the concept of symmetry and we have selected some of them: **symmetry and shape of space**, some others in the **context of crystallographic concepts**, some **with decorative patterns**, or in the **context of minerals**. There is even an **international society for the study of symmetry**.

The essential knowledge on crystal morphology, symmetry elements and their combination to generate repetitive objects in space, were well established between the 17th and 19th Centuries, as **stated elsewhere in these pages**...

Specifically, in finite objects, there are a number of operations (***elements of symmetry***) describing repetitions. In the wall-drawing (shown above) we find ***translational operations*** (the motif is repeated by translation). The repetition of the petals in the flowers show us ***rotational operations*** (the motif is repeated by rotation) around a ***symmetry axis*** (or ***rotation axis***). And, although not exactly, the symmetry shown in the phrase or in the music fragment (shown above), lead us to consider other symmetry operations known as ***symmetry planes*** (***reflection planes***, or ***mirror planes***); the same operation that occurs when you look into a mirror. Similarly, for example, if we look at the relationship between the three-dimensional objects in some of the pictures shown below, we will discover a new element of symmetry called ***center of symmetry*** (or ***inversion center***), which is an imaginary point between objects (or inside the object) as shown in some drawings below.

Generally speaking, and taking into account that pure translational operations are not strictly considered as symmetry operations, we can say that finite objects can contain themselves, or may be repeated (excluding translation) by the following symmetry elements:

- The ***identity*** operation is the simplest symmetry element of all -- it does nothing! But it is important because all objects at the very least have the identity element, and there are many objects that have no other symmetry elements.
- The ***reflection*** is the symmetry operation that occurs when we put an object in front of a mirror. The image is found perpendicular to the reflection plane and equidistant from that plane, on the opposite side of the plane. The resulting object can be *distinguishable* or *indistinguishable* from the original, normally *distinguishable*, as they cannot be superimposed. If the resulting object is *indistinguishable* from the original, is because the reflection plane is passing through the object.
- The ***inversion*** operation occurs through a single point called the inversion center. Each part of the object is moved along a straight line through the inversion center to a point at an equal distance from the inversion center. The resulting object can be *distinguishable* or *indistinguishable* from the original, normally *distinguishable*, as they cannot be superimposed. If the resulting object is *indistinguishable* from the original, is because the inversion center is inside the object.
- The ***rotation*** operations (both *proper* and *improper*) occur with respect to a line called rotation axis. ***a)*** A *proper* rotation is performed by rotating the object 360°/*n*, where *n* is the order of the axis. The resulting rotated object is always indistinguishable from the original. ***b)*** An *improper* rotation is performed by rotating the object 360°/*n* followed by a reflection through a plane perpendicular to the rotation axis. The resulting object can be *distinguishable* or *indistinguishable* from the original, normally distinguishable, as they cannot be superimposed. If the resulting object is *indistinguishable* from the original, is because the improper rotation axis is passing through the object.

In addition to the name of the symmetry elements, we use **graphical and numerical symbols to represent them**. For example, a rotation axis of order 2 (a binary axis) is represented by the number **2**, and a reflection plane is represented by the letter **m**.

Left: *Polyhedron showing a two-fold rotation axis* ***(2)*** *passing through the centers of the top and bottom edges*

Right: *Polyhedron showing a reflection plane* ***(m)*** *that relates (as a mirror does) the top to the bottom*

Hands and molecular models related by a twofold axis ***(2)*** *perpendicular to the drawing plane*

Hands and molecular models related through a mirror plane ***(m)*** *perpendicular to the drawing plane*

Hands (left and right) related through a center of symmetry

Two objects related by a center of symmetry and a polyhedron showing a center of symmetry in its center

The association of elements of rotation with centers or planes of symmetry generates new elements of symmetry called ***improper rotations***.

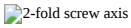
Left: *A four-fold improper axis implies 90º rotations followed by reflection through a mirror plane perpendicular to the axis. (Animation taken from **M. Kastner, T. Medlock & K. Brown, Univ. of Bucknell**)*

Right: *Axis of improper rotation, shown vertically, in a crystal of urea. The meaning of numerical triplets shown will be discussed in another chapter.*

Combining the rotation axes and the mirror planes with the characteristic translations of the crystals (which are shown below), new symmetry elements appear, with some "sliding" components: ***screw axes*** (or ***helicoidal axes***) and ***glide planes***.

Twofold screw axis. A screw axis consists of a rotation followed by a translation

Glide plane. A glide plane consists of a reflection followed by a translation



Twofold screw axis applied to a left hand. The hand rotates 180º and moves a half of the lattice translation in the direction of the screw axis, and so on. Note that the hand always remains as a left hand.

*(Animation taken from **M. Kastner, T. Medlock & K. Brown, Univ. of Bucknell**)*

Glide plane.applied to a left hand. The left hand reflects on the plane, generating a right hand that moves a half of the lattice translation in the direction of the glide operation.

*(Animation taken from **M. Kastner, T. Medlock & K. Brown, Univ. of Bucknell**)*

The symmetry elements of types ***center*** or ***mirror plane*** relate objects in a peculiar way; the same way that our two hands are related one to the other: they are not superimposable. Objects which in themselves do not contain any of these symmetry elements (center or plane) are called ***chiral*** and their repetition through these elements (center or plane) produce objects that are called ***enantiomers*** with respect to the original ones. The mirror image of one of our hands is the enantiomer of the one we put in front of the mirror. Regarding the chirality of the crystals and of their building units (molecular or not), advanced readers should also consult the **article by Howard D. Flack to be found through this link.**

*The mirror image of either of our hands is the enantiomer of the other hand. They are objects not superimposable and as they do not contain (in themselves) symmetry centers or symmetry planes, are called **chiral objects**.*

*Chiral molecules have different properties than their enantiomers and so it is important that we are able to differentiate them. The correct determination of the absolute configuration or absolute structure of a molecule (differentiation between enantiomers) can be done in a secure manner through X-ray diffraction only, but this will be explained in **another chapter***

Thus, any finite object (such as a quartz crystal, a chair or a flower) shows that certain parts of it are repeated by symmetry operations that go through a point of the object. This set of symmetry operations is known as a ***symmetry point group***. The advanced reader has also the opportunity to visit the nice work on ***point group symmetry elements*** offered through these links:

- **Dean H. Johnston from the Otterbein University**, and

- **Margaret Kastner, Timathy Medlock and Kristy Brown from the University of Bucknell.**

A good general web site about **symmetry in crystallography is offered by the Department of Chemistry and Biochemistry** of the Oklahoma University.

Additionally, the reader can download (totally virus free!!!) and run on his own computer **this Java application that, as an introduction to the symmetry of the polyhedra**, that was developed by Gervais Chapuis and Nicolas Schöni (École Polytechnique Fédérale de Lausanne, Switzerland).

Symmetry in crystals

In crystals, the symmetry axes (rotation axes) can only be ***two-fold*** (**2**), ***three-fold*** (**3**), ***four-fold*** (**4**) or ***six-fold*** (**6**), depending on the number of times (***order of rotation***) that a motif can be repeated by a rotation operation, being transformed into a new state indistinguishable from its starting state. Thus, a rotation axis of order **3** (3-fold) produces **3** repetitions (copies) of the motif, one every 120 degrees (= 360/**3**) of rotation. If the reader wonders why only symmetry axes of order **2**, 3, 4 and **6** can occur in crystals, and not **5**-, **7**-fold, etc., we recommend the **explanations given in another section**.

Improper rotations (rotations followed by reflection through a plane perpendicular to the rotational axis) are designated by the order of rotation, with a bar above that number.

The ***screw axes*** (or helicoidal axes, ie, symmetry axes involving rotation followed by a translation along the axis) are represented by the order of rotation, with an added subindex that quantifies the translation along the axis. Thus, a screw axis of type $\mathbf{6_2}$ means that in each of the six rotations an associated translation occurs of **2**/**6** of the axis of the elementary cell in that direction.

The ***mirror planes*** are represented by the letter **m**.

The ***glide planes*** (mirror planes involving reflexion and a translation parallel to the plane) are represented by the letters **a**, **b**, **c**, **n** or **d**, depending if the translation associated with the reflection is parallel to the reticular translations (***a***, ***b***, ***c***), parallel to the diagonal of a reticular plane (**n**), or parallel to a diagonal of the unit cell (**d**).

The letters and numbers that are used to represent the symmetry elements also have an equivalence with some **graphic symbols**.

But in order to keep talking about symmetry in crystals, it is necessary to introduce and remember the fundamental aspect that defines crystals, which is the periodic repetition by translation of motifs (atoms, molecules or ions). This repetition, which is illustrated in two dimensions with gray circles in the figure below, is derived from the mathematical concept of lattice that we will see more properly in **another chapter**.

In a periodic and repetitive set of motifs (gray circles in the two-dimensional figure above) one can find infinite basic units (***unit cells***) vastly different in appearance and specification, the repetition of which generates the same mathematical lattice. Note that all represented unit cells delimited by black lines contain in total a single circle inside them, since each vertex contains a certain fraction of a circle inside the cell. These are called ***primitive cells***. However, the cell delimited by red lines contains a total of two gray circles inside (one corresponding to the vertices and a complete one in the center). This type of unit cell is generically called ***non-primitive***.

Periodic repetition, which is a characteristic of the internal structure of crystals, is represented by a set of translations in the three directions of space, so that crystals can be seen as the stacking of the same block in three dimensions. Each block, of a certain shape and size (but all of them being identical), is called a ***unit cell*** or ***elementary cell***. Its size is determined by the length of its three edges (***a***, ***b***, ***c***) and the angles between them (alpha, beta, gamma: **α**, **β**, **γ**).

Stacking of unit cells forming an octahedral crystal and parameters which characterize the shape and size of an elementary cell (or unit cell)

As mentioned above, all symmetry elements passing through a point of a finite object, define the total symmetry of the object, which is known as the ***point group symmetry*** of the object. Obiously, the symmetry elements that imply any lattice translations (glide planes and screw axes), are not point group operations.

There are many ***symmetry point groups***, but in crystals they must be consistent with the crystalline periodicity (translational periodicity). Thus, in crystals, only rotations (symmetry axes) of order 2, 3, 4 and 6 are possible, that is, only rotations of 180º (= 360/2), 120º (= 360/3), 90º (= 360/4) and 60º (= 360/6) are allowed. See also the **crystallographic restriction theorem**. Therefore, only ***32 point groups*** are allowed in the crystalline state of matter. These ***32 point groups*** are also known in Crystallography as the ***32 crystal classes***.

point group . crystal translational periodicity = 32 crystal classes

Graphic representation of the 32 crystal classes

The motif, represented by a single brick, can also be represented by a lattice point. It shows the point symmetry **2mm**

The next three tables show animated drawings about the 32 crystal classes, grouped in terms of the so called ***crystal system*** (left column), a classification mode in terms of minimal symmetry, as **shown below**.

	Links below illustrate the 32 crystal classes using some crystal morphologies These interactive animated drawings need the **Java environment** and therefore will not run with all browsers						
Triclinic	1	1					
Monoclinic	2	m	2/m				
Orthorhombic	222	mm2	mmm				
Tetragonal	4	4	4/m	422	4mm	42m	4/mmm
Cubic	23	m3	432	43m	m3m		
Trigonal	3	3	32	3m	3m		
Hexagonal	6	6	6/m	622	6mm	6m2	6/mmm

	Links below illustrate the 32 crystal classes using some crystal morphologies These are non-interactive animated gifs obtained from the Java animations appearing in **http://webmineral.com**. **They will run with all browsers**						
Triclinic	1	1					
Monoclinic	2	m	2/m				
Orthorhombic	222	mm2	mmm				
Tetragonal	4	4	4/m	422	4mm	42m	4/mmm
Cubic	23	m3	432	43m	m3m		
Trigonal	3	3	32	3m	3m		
Hexagonal	6	6	6/m	622	6mm	6m2	6/mmm

	Links below show animated displays of the symmetry elements in each of the 32 crystal classes: (taken from **Marc De Graef**)						
Triclinic	1	1					
Monoclinic	2	m	2/m				
Orthorhombic	222	mm2	mmm				
Tetragonal	4	4	4/m	422	4mm	42m	4/mmm
Cubic	23	m3	432	43m	m3m		

Trigonal	3	3	32	3m	3m		
Hexagonal	6	6	6/m	622	6mm	6m2	6/mmm

Lluis Casas and Eugenia Estop, from the Department of Geology of the University of Barcelona, offer **32 pdf files** which, in an interactive way, allow very easily playing with the 32 point groups through the symmetry of crystalline solids.

Additionally, the reader can download and run on his own computer **this Java application that, as an introduction to the symmetry of the polyhedra**, was developed by Gervais Chapuis and Nicolas Schöni (École Polytechnique Fédérale de Lausanne, Switzerland).

Alternatively, the interested reader can interactively view some typical polyhedra of the 7 crystal systems, through the **Spanish Gemological Institute**.

Of the ***32 crystal classes***, only ***11 contain the operator center of symmetry***, and these 11 centro-symmetric crystal classes are known as ***Laue groups***.

crystal class . center of symmetry = 11 Laue groups

Graphic representation of the 11 Laue groups (centro-symmetric crystal classes)

In addition, the repetition modes by translation in crystals must be compatible with the possible point groups (the 32 crystal classes), and this is why we find only ***14 types of translational lattices which are compatible with the crystal classes***. These types of lattices (translational repetiton modes) are known as the Bravais lattices (**you can see them here**). The translational symmetry of an ordered distribution of 3-dimensional objects can be described by many types of lattices, but there is always one of them more suited to the object, ie: the one that best describes the symmetry of the object. As the lattices themselves have their own distribution of symmetry elements, we must fit them to the symmetry elements of the structure.

crystal translational periodicity . 32 crystal classes = 14 Bravais lattices

Graphic representation of the 14 Bravais lattices

A brick wall can be structured with many different types of lattices, with different origins, and defining reticular points representing the brick. But there is a lattice that is more appropriate to the symmetry of the brick and to the way the bricks build the wall.

The adequacy of a lattice to the structure is illustrated in the two-dimensional examples shown below. In all three cases two different lattices are shown, one oblique and primitive and one rectangular and centered. In the first two cases, the rectangular lattices are the most appropriate ones. However, the deformation of the structure in the third example leads to metric relationships that make that the most appropriate lattice, the oblique primitive, hexagonal in this case.

Adequacy of the lattice type to the structure. The blue lattice is the best one in each case.

Finally, combining the ***32 crystal classes*** (crystallographic point groups) with the ***14 Bravais lattices***, we find up to 230 different ways to replicate a finite object (motif) in 3-dimensional space. These ***230 ways to repeat patterns in space***, which are compatible with the 32 crystal classes and with the 14 Bravais lattices, ***are called space groups***, and represent the 230 different ways to fit the Bravais lattices to the symmetry of the objects. The interested reader should also consult the **excellent work on the symmetry elements present in the space groups**, offered by Margaret Kastner, Timathy Medlock and Kristy Brown through this link of the Bucknell University.

32 crystal classes + 14 Bravais lattices = 230 Space groups

*A wall of bricks showing the most appropriate lattice which best represents both the brick and its symmetry. Note that in this case the point symmetry of the brick and the point symmetry of the reticular point are coincident. The space group, considering the thickness of the brick, is **Cmm2**.*

The 32 crystal classes, the 14 Bravais lattices and the 230 space groups can be classified, according to their hosted minimum symmetry, into ***7 crystal systems***. The minimum symmetry produces some restrictions in the metric values (distances and angles) which describe the shape and size of the lattice.

32 classes, 14 lattices, 230 space groups / crystal symmetry = 7 crystal systems

All this is summarized in the following table:

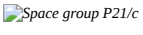
Crystal classes (* Laue)	Compatible crystal lattices and *their symmetry*	Number of space groups	Minimum symmetry	Metric restrictions	Crystal system
1 1 *	**P** **1**	**2**	**1** or **1**	none	**Triclinic**
2 m 2/m *	**P C (I)** **2/m**	**13**	One **2** or **2**	**α=γ=90**	**Monoclinic**
222 2mm mmm *	**P C (A,B) I F** **mmm**	**59**	Three **2** or **2**	**α=β=γ=90**	**Orthorhombic**
4 4 4/m * **422 4mm 42m 4/mmm ***	**P I** **4/mmm**	**68**	One **4** or **4**	**a=b** **α=β=γ=90**	**Tetragonal**
3 3 * **32 3m 3m ***	**P (R)** **3m 6/mmm**	**25**	One **3** or **3**	**a=b=c** **α=β=γ** (or Hexagonal)	**Trigonal**
6 6 6m * **622 6mm 6m2 6/mmm ***	**P** **6/mmm**	**27**	One **6** or **6**	**a=b** **α=β=90 γ=120**	**Hexagonal**
23 m3 * **432 43m m3m ***	**P I F** **m3m**	**36**	Four **3** or **3**	**a=b=c** **α=β=γ=90**	**Cubic**
Total: 32, 11 *	**14 independent**	**230**			**7**

The 230 crystallographic space groups are listed and described in the **International Tables for X-ray Crystallography**, where they are classified according to point groups and crystal systems. Chiral compounds that are prepared as a single enantiomer (for instance, biological molecules) can crystallize in only a subset of 65 space groups, those that do not have mirror and/or inversion symmetry operations.

A composition of part of the information contained in these tables is shown below, corresponding to the space group ***Cmm2***, where ***C*** means that the structure is described in terms of a lattice centered on the faces separated by the **c** axis. The first ***m*** represents a mirror plane perpendicular to the ***a*** axis. The second ***m*** means another mirror plane (in this case perpendicular to the second main crystallographic direction), the ***b*** axis. The number ***2*** refers to the two-fold axis parallel to the third crystallographic direction, the ***c*** axis.

*Summary of the information shown in the **International Tables for X-ray Crystallography** for the space group **Cmm2***

And this is another example for the space group ***P21/c***, centrosymmetric and based on a primitive monoclinic lattice, as it appears in the ***International Tables for X-ray Crystallography***



*Summary of the information shown in the **International Tables for X-ray Crystallography** for the space group **$P2_1/c$***

The advanced reader can also consult:

- the teaching and learning package offered by the University of Cambridge on **fundamental ideas and principles associated with the field of crystallography**
- the hypertext book "**Crystallographic Space Group Diagrams and Tables**"

- the article by Dauter & Jaskolski entitled **How to read and understand Volume A of International Tables for Crystallography**
- the **Bilbao crystallographic server**, which is an excellent tool for the management of symmetry in crystallography, and/or
- the so-called **space group decoder** offered by Bernhard Rupp.

Crystallographers never get bored! Try to enjoy the beauty, looking for the symmetry of the objects around you, and particularly in the objects shown below ...

Look for possible unit-cells and symmetry elements in these structures made with bricks

(the solution is obtained clicking on the image)

There is a question that surely the readers will have considered... In this chapter we have shown elements of symmetry that operate inside the crystals, but we have not yet said how we can find out the existence of such operations, when in fact, and in the best of cases, we could only visualize the external habit of the crystals if they are well formed! Although we will not answer this question here, we can anticipate that this response will be given by the behavior of the crystals when we illuminate them with that special light that we know as **X-rays**, but this will be the subject of another chapter.

In any case, it doesn't end here! There are many more things to talk about. Go on.

1.4: Direct and reciprocal lattices

Let's start with a summary of several concepts seen in previous chapters...

Any repetitive and periodic distribution of a ***set of objects*** (or ***motifs***) can be characterized, or described, by translations that repeat the set of objects periodically. The implied translations generate what we call a ***direct lattice*** (or ***real lattice***).

Left: *Fragment of a distribution of a set of objects that produce a direct lattice in 2 dimensions. As an example, one of the infinite sets of motifs (small tiles) that produce the repetitive and periodic distribution is shown inside the yellow squares. The dimensions of the yellow square represent the translations of the direct lattice*

Right: *Fragment of a mosaic in* ***La Alhambra*** *showing a 2-dimensional periodic pattern. These periodic translations can be discovered in the mosaic and produce a 2-dimensional direct lattice. The red square represents the translations of the smallest direct lattice produced by the periodic distributions of the small pieces of this mosaic.The yellow square represents another possible lattice, a bigger one, non primitive.*

Periodic stacking of balls, producing a 3-dimensional network (direct lattice). The motif being repeated in the three directions of space is the contents of the small box with blue edges, the so called "unit cell".

The translations that describe the periodicity in crystals can be expressed as a linear combination of three basic translations, not coplanar, ie independent, known as ***reticular or lattice axes*** (or unit cell axes). These axes define a parallelogram (in 2 dimensions), or a parallelepiped (in 3 dimensions) known as a ***unit cell*** (or elementary cell). This elementary area (in 2-dimensional cases), or elementary volume (in 3-dimensional cases), which holds the minimum set of the periodic distribution, generates (by translations) the full distribution which, in our atomic 3-dimensional case, we call ***crystal***.

In addition to the fact that the ***unit cell*** is the smallest repetitive unit as far as translations is concerned, the reader should note that the system of axes defining the unit cell actually defines the reference system to describe the positional coordinates of each atom within the cell.

Left: *Elementary cell (or unit cell) defined by the 3 non-coplanar reticular translations (cell axes or lattice axes)*

Right: *Crystal formation by stacking of many unit cells in 3 space directions*

In general, inside the unit cell there is a minimum set of atoms (ions or molecules) which are repeated inside the cell due to the **symmetry elements** of the crystal structure. This minimum set of atoms (ions or molecules) which generate the whole contents of the ***unit cell*** (after applying the **symmetry elements** to them) is known as the ***asymmetric unit***.

The structural motif shown in the left figure is repeated by a symmetry element (symmetry operation), in this case a screw axis

The repetition of the motif (asymmetric unit) generates the full content of the unit cell, and the repetition of unit cells generates the entire crystal

The lattice, which is a pure mathematical concept, can be selected in various ways in the same real periodic distribution. However, only one of these lattices "fits" best with the symmetry of the periodic distribution of the motifs...

Two-dimensional periodic distribution of one motif containing two objects (a triangle and a circle)

Left: *Unit cells corresponding to possible direct lattices (=real lattices) that can be drawn over the periodic distribution shown above. Only one of the unit cells (the red one) is more appropriate because it fits much better with the symmetry of the distribution*

Right: *The red cell on the left figure (a centered lattice) fits better with the symmetry of the distribution, and can be decomposed in two identical lattices, one for each object of the motif.*

As is shown in the figures above, although especially in the right one, any lattice that describes the repetition of the ***motif*** (triangle + circle) can be decomposed into two identical equivalent lattices (one for each object of the motif). Thus, the concept of lattice is independent of the complexity of the motif, so that we can use only one lattice, since it represents all the remaining equivalent ones. Once we have chosen a representative lattice, appropriate to the symmetry of the structure, any ***reticular point*** (or ***lattice node***) can be described by a vector that is a linear combination (with integer numbers) of the direct reticular axes: **R = m a + n b + p c**, where **m**, **n** and **p** are integers. ***Non-reticular points*** can be reached using the nearest **R** vector, and adding to it the corresponding fractions of the reticular axes to reach it:

$$\mathbf{r = R + r' = (m a + n b + p c) + (x a + y b + z c)}$$

Position vector for any non-reticular point of a direct lattice

where **x**, **y**, **z** represent the corresponding dimensionless fractions of axes **X/a**, **Y/b**, **Z/c**, and **X**, **Y**, **Z** the corresponding lengths.

Position vector for a non-reticular point (black circle)

The reader should also have a look into the chapters about **lattices** and **unit cells** offered by the University of Cambridge.

Alternatively, the reader can download and run on his own computer **this Java application that illustrates the lattice concept** (it is totally virus free and was developed by Gervais Chapuis and Nicolas Schöni, École Polytechnique Fédérale de Lausanne, Switzerland).

Let's now see some new concepts on direct lattices (= **real lattices) ...**

From a geometric point of view, on a lattice we can consider some ***reticular lines*** and ***reticular planes*** which are those passing through the ***reticular points*** (or reticular nodes). Just as we did with the lattices (choosing one of them from all the equivalent ones), we do the same with the reticular lines and planes. A reticular line or a reticular plane can be used as a representative of the entire family of parallel lines or parallel planes.

Following with the argument given above, each motif in a repetitive distribution generates its own lattice, although all these lattices are identical (**red** and **blue**). Of the two families of equivalent lattices shown (**red** and **blue**) we can choose only one of them, on the understanding that it also represents the remaining equivalent ones. Note that the distance between the planes drawn on each lattice (***interplanar spacing***) is the same for the **blue** or **red** families. However, the family of red planes is separated from the family of blue planes by a distance that depends on the separation between the objects which produced the lattice. This distance between the planes of different families can be called the ***geometric out-of-phase distance***.

Left: *Family of reticular planes cutting the vertical axis of the cell in 2 parts and the horizontal axis in 1 part. These planes are parallel to the third reticular axis (not shown in the figure).*

Right: *Family of reticular planes cutting the vertical axis of the cell in 3 parts and the horizontal axis in 1 part. These planes are parallel to the third reticular axis (not shown in the figure).*

*The number of parts in which a family of planes cut the cell axes can be associated with a triplet of numbers that identify that family of planes. In the three previous figures, the number of cuts, and therefore the numerical triplets would be (**110**), (**210**) and (**310**), respectively, according to the vertical, horizontal and perpendicular-to-the-figure axes. In this figure, the numerical triplets for the planes drawn are (**022**), that is, the family of planes does not cut the **a** axis, but cuts the **b** and **c** axes in **2** identical parts, respectively.*

*The plane drawn on the left side of the figure above cuts the **a** axis in **2** equal parts, the **b** axis in **2** parts and the **c** axis in **1** part. Hence, the numerical triplet identifying the plane will be (**221**). The plane drawn on the right side of the figure cuts the **a** axis into **2** parts, is parallel to the **b** axis and cuts the **c** axis in **1** part. Therefore, the numerical triplet will be (**201**).*

A unique plane, as the one drawn in the top right figure, defined by the numerical triplet known as ***Miller indices***, represents and describes the whole family of parallel planes passing through every element of the motif. Thus, in a crystal structure, there will be as many plane families as possible numerical triplets exist with the condition that these numbers are ***primes***, one to each other (not having a common divisor).

The Miller indices are generically represented by the triplet of letters ***hkl***. If there are common divisors among the Miller indices, the numerical triplet would represent a single family of planes only. For example, the family with indices (***330***), which are not strictly reticular, can be regarded as the representative of **3** families of indices (***110***) with a *geometric out-of-phase distance* (among the families) of **1/3** of the original (see the figures below).

Left: *Three families of reticular planes, with indices (**110**) in three equivalent lattices, showing an out-of-phase distance between them of **1/3** of the interplanar spacing in each family.*

Right: *The same set of planes of the figure on the left drawn over one of the equivalent lattices. Therefore its Miller indices are (**330**) and its interplanar spacing is **1/3** of the interplanar spacing of the (**110**) family.*

Thus, the concept of Miller indices, previously restricted to ***numerical triplets*** (being prime numbers), can now be generalized to ***any triplet of integers***. In this way, every family of planes, will "cover" the whole crystal. And therefore, for every point of the crystal we can draw an infinite number of plane families with infinite orientations.

Through a point in the crystal (in the example in the center of the cell) we can draw an infinite number of plane families with an infinite number of orientations. In this case only 3 families and 3 orientations are shown.

Of course, interplanar spacings can be directly calculated from the Miller indices (***hkl***) and the values of the reticular parameters (unit cell axes). The table below shows that these relations can be simplified for the corresponding metric of the different lattices.

*Formula to calculate the interplanar spacings (d_{hkl}) for a family of planes with Miller indices **hkl** in a unit cell of parameters **a**, **b**, **c**, **α**, **β**, **γ**. Vertical bars (for the triclinic case) mean the function "determinant". In the trigonal case **a**=**b**=**c**=**A**; **α**=**β**=**γ**. In all cases, obviously, the calculated interplanar spacing also represents the distance between the cell origin and the nearest plane of the family.*

Interested readers should also have a look into the chapter on **lattice planes and Miller indices** offered by the University of Cambridge.

And now some more concepts on lattices: the so called **reciprocal lattice ...**

Any plane can also be characterized by a vector (σ_{hkl}) perpendicular to it. Therefore, the projection of the position vector of any point (belonging to the plane), over that perpendicular line is constant and independent of the point. It is the distance of the plane to the origin, ie, the ***spacing*** (d_{hkl}).

Any plane can be represented by a vector perpendicular to it.

Consider the family of planes ***hkl*** with the interplanar distance d_{hkl}. From the set of vectors normal to the planes' family, we take the one (σ_{hkl}) with length $1/d_{hkl}$. The scalar product between this vector and the position vector (d'_{hkl}) of a point belonging to a plane from the family is an integer (n), and this integer gives us the order of that plane in the ***hkl*** family. That is: (σ_{hkl}) . (d'_{hkl}) = ($1/d_{hkl}$) . ($n.d_{hkl}$) = n (see left figure below)

n will be 0 for the plane passing through the origin, 1 for the first plane, 2 for the second, etc.

Thus, σ_{hkl} represents the whole family of ***hkl*** planes having an interplanar spacing given by d_{hkl}. In particular, for the first plane we get: $|\sigma_{hkl}|\ d_{hkl} = 1$.

If we define 1/dhkl, as the length of the vector σ_{hkl}, the product of this vector, times the d_{hkl} spacing of the planes family is the unit.

If we take a vector 2 times longer than σ_{hkl}, the interplanar spacing of the corresponding new family of planes would be a half.

If from this normal vector σ_{hkl} of length $1/d_{hkl}$, we take another vector, n times (integer) longer ($n.\sigma_{hkl}$), the above mentioned product ($|\sigma_{hkl}|\ d_{hkl} = 1$) would imply that the new vector ($n.\sigma_{hkl}$) will correspond to a family of planes of indices ***nh***,***nk***,***nl*** having an interplanar spacing n times smaller. In other words, for instance, the lengths of the following interplanar spacings will bear the relation: $d_{100} = 2.(d_{200}) = 3.(d_{300})$..., so that $\sigma_{100} = (1/2).\sigma_{200} = (1/3).\sigma_{300}$ ... and similarly for other ***hkl*** planes.

Therefore, it appears that the moduli (lengths) of the perpendicular vectors (σ_{hkl}) are ***reciprocal to the interplanar spacings***. The end points of these vectors (blue arrows in figure below) also produce a periodic lattice that, due to this reciprocal property, is known as the ***reciprocal lattice*** of the original direct lattice. The ***reciprocal points*** obtained in this way (green points in figure below) are identified with the same numerical triplets ***hkl*** (***Miller indices***) which represent the corresponding plane family.

*Geometrical construction of some points of a reciprocal lattice (green points) from a direct lattice. To simplify, we assume that the third axis of the direct lattice (**c**) is perpendicular to the screen. The red lines represent the reticular planes (perpendicular to the screen) and whose Miller indices are shown in blue. As an example: the reciprocal point with indices **(3,1,0)** will be located on a vector perpendicular to the plane **(3,1,0)** and its distance to the origin **O** is inversely proportional to the spacing of that family of planes.*

Animated example showing how to obtain the reciprocal points from a direct lattice

It should now be clear that the direct lattice, and its reticular planes, are directly associated (linked) with the ***reciprocal lattice***. Moreover, in this reciprocal lattice we can also define a unit cell (***reciprocal unit cell***) whose periodic translations will be determined by three ***reciprocal axes*** that form ***reciprocal angles*** among them. If the unit cell axes and angles of the direct cell are known by the letters ***a***, ***b***, ***c***, ***α***, ***β***, ***γ***, the corresponding parameters for the reciprocal cell are written with the same symbols, adding an asterisk: ***a****, ***b****, ***c****, ***α****, ***β****, ***γ****. It should also be clear that these reciprocal axes (***a****, ***b****, ***c****) will correspond to the vectors σ_{100}, σ_{010} and σ_{001}, respectively, so that any reciprocal vector can be expressed as a linear combination of these three reciprocal vectors:

$$\sigma_{hkl} = h\ a^* + k\ b^* + l\ c^*$$

Position vector of any reciprocal point

Geometrical relation between direct and reciprocal unit cells

The figure below shows again the strong relationship between the two lattices (**direct with blue points**, **reciprocal in green**). In this case, the corresponding third reciprocal axes (**c** and **c***) are perpendicular to the screen.

And analytically the relationship between the direct (= real) and reciprocal cells can be written as:

Metrical relations among the parameters defining the direct and reciprocal cells. ***V*** *represents the volume of the direct cell and the symbol* ***x*** *means the cross product between two vectors. The same type of equations can be written by changing the asterisks to the right side of the equations. The volume of the direct cell can be calculated as:*

$V = (a \times b) \cdot c = a.\ b.\ c\ (1 - \cos^2\alpha - \cos^2\beta - \cos^2\gamma + 2\cos\alpha\cos\beta\cos\gamma)^{1/2}$

Note that, in accordance with the definitions given above, the length of a^* is the inverse of the interplanar spacing d_{100} ($|a^*| = 1/d_{100}$), and that $|b^*| = 1/d_{010}$, and that $|c^*| = 1/d_{001}$. Therefore, the following scalar products (dot products) can be written: $a.a^* = 1$, $a.b^* = 0$ and similarly with the other pairs of axes.

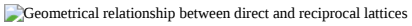
Summarizing:

- Direct space (= real space) is the space where we live..., where atoms are..., where crystals growth..., where we imagine the direct lattices (= real lattices).
- Reciprocal space is a mathematical space constructed on the direct space (= real space). It is the space where reciprocal lattices are, which will help us to understand the crystal diffraction phenomena.
- "*Big in direct space (that is, in real space)*", means "*small in reciprocal space*".
- "*Small in direct space (that is, in real space)*" means "*big in reciprocal space*".

In addition to this, we recommend to download and execute the Java applet by Nicolas Schoeni and Gervais Chapuis of the Ecole Polytechnique Fédéral de Lausanne (Switzerland) to **understand the relation between direct and reciprocal lattices and how to build the latter from a direct lattice**. (Free of any kind of virus).

See also the pages on ***reciprocal space*** offered by the **University of Cambridge through this link**.

And although we are revealing aspects corresponding to the next chapter (see the last paragraph of this page), the reader should also **look at the video** made by **www.PhysicsReimagined.com**, showing the geometric relationships between direct and reciprocal lattices, displayed below as an animated gif:



The reader is probably asking himself why we need this new concept (the ***reciprocal lattice***). Well, there are reasons which justify it. One of them is that a *family of planes can be represented by just one point*, which obviously simplifies things. And another important reason is that this new lattice offer us a very simple geometric model that can interpret the ***diffraction*** phenomena in crystals. But this will be described in another chapter. Go on!

1.5: Scattering and diffraction

In the context of this chapter, you will also be invited to visit these sections...

- **Group velocity**
- **Kinematic model**
- **Optical diffraction diagrams**
- **The Laue equations**
- **The Bragg's Law**
- **The Bragg's Law ("applet")**
- **The structure factor**
- **The Fourier transform**

Electromagnetic radiations (such as visible light) can interact among themselves and with matter, giving rise to a multitude of phenomena such as ***reflection***, ***refraction***, ***scattering***, ***polarization***...

Left: *Reflection and refraction of light in the interface between glass with a refractive index 1.5 and air with a refractive index 1.0. TIR = "Total Internal Reflection"*

Center: *Refraction of light after passing through a glass prism. Depending on the wavelength (color) of the incident beam (coming from the left), the angle of refraction varies, ie: it is scattered*

Right: *Polarization of light passing through a polarizer. Depending on the rotation of the polarizer, one of components of the incident beam (coming from the right) is filtered*

Animations originally taken from ***physics-animations.com***

X-ray diffraction is the physical phenomenon that expresses the ***fundamental interaction between X-rays and crystals*** (ordered matter). However, to describe the phenomenon, it is advisable to first introduce some physical models that (as all models) do not fully explain reality (as they are an idealization of it), but can be used to help understand the phenomenon.

On waves

A wave is an undulatory phenomenon (a disturbance) that propagates through space and time, and is regularly repeated.

Waves are usually represented graphically by a sinusoidal function (as shown at right), in which we can determine some general parameters that define it.

Transverse wave propagation of vibrating longitudinal and circular movements Animations originally taken from ***physics-animations.com***

Undulatory phenomena (waves) propagate at a certain ***speed*** (**v**) and can be modeled to meet the so-called wave equation, scalar or vectorial, depending on the nature of the disturbance. The solutions to this equation are usually combinations of trigonometric terms, each of them characterized by: 1) an ***amplitude*** (***A***), which measures the maximum (or minimum) of the disturbance with respect to an equilibrium value, and 2) a ***phase*** ϕ:

$$\phi = 2\pi\,(\mathbf{K}.\mathbf{r} - \boldsymbol{\nu}.\mathbf{t} + \alpha)$$

The ***intensity*** of an undulatory disturbance, at any point of the wave, is proportional to the square of the disturbance value at that point, and if it is expressed in terms of complex exponentials, this is equivalent to the product of the disturbance by its complex conjugate. The ***intensity*** is a measure of the energy flow per unit of time and per unit of area of the wavefront (spherical or flat, depending on the type of wave).

A wave is a regular phenomenon, ie it repeats exactly in time (with a period **T**) and space (with a period λ, the wavelength), so that $\lambda = \boldsymbol{\nu}.\mathbf{T}$, or $\lambda.\boldsymbol{\nu} = \mathbf{v}$.

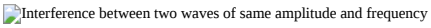
In the expression of the phase (ϕ), **K** is the so-called ***wave vector*** which gives the sense of progress of the wave (***the ray***), and is considered with an amplitude **1/λ**. Thus, **K** is the number of repetitions per unit of length.

V is the ***frequency*** (the inverse of the period), that is, the number of repetitions (or cycles) per unit of time. We give the name ***pulse*** to the magnitude given by: **2π.*V***, which measures the number of repetitions per radian (180/π degrees) of the cycle.

In the full **electromagnetic spectrum** (ie in the distribution of electromagnetic wavelengths) the ***hard X-rays*** (the high energy ones) are located around a wavelength of 1 Angstrom in vacuum (for ***Cu*** the average wavelength is 1.5418 Angstrom and for ***Mo*** it's 0.7107 Angstrom), while ***visible light*** has a wavelength in the range of 4000 to 7000 Angstrom.

t and **r** are, respectively, the time and the position vector with which we measure the disturbance, and **α** is the original ***phase difference*** relative to the other components of the wave.

We speak of waves being ***in phase*** if the difference between the phases of the components is an integer multiple of **2π**, and we say that the waves are in ***opposition of phase*** if that difference is an odd multiple of **π**. For an easy mathematical treatment to keep track of the relations between phases of the wave components, these terms are usually expressed in an exponential notation, where the exponential imaginary unit ***i*** means a phase difference of **+π/2**.



Possible states of interference of two waves shown at the top, having identical amplitude and frequency. The wave drawn at the bottom (bold line) shows the result of the interference, which has maximum amplitude when interfering waves overlap, i.e. they ***are in phase****. Complete destructive interference is obtained (resulting wave vanishes) when the maxima of one of the component waves coincide with the minima of the other, i.e., when the two waves* ***are in phase opposition****. Animation taken from* ***The Pennsylvania State University***

*Undulatory disturbance corresponding to the combination of two elementary waves (**blue** and **green**) of similar wavelengths (λ, **λ**), with the same amplitude (**A**, **A**) and relative difference of phase α. The disturbance is moving from left to right with a velocity **v**. The sum of these two elementary waves produces a wave (sum of the individual ones) depicted in red (λ).*

Interference usually refers to the interaction of waves which are ***correlated*** or ***coherent*** with each other, either because they come from the same source or because they have the same, or nearly the same, frequency.

The solutions to the **wave equation**, whose amplitude is not inversely dependent on the distance of origin, are called ***plane waves***, since at a given time all points belonging to the plane **K.r = constant** have the same phase, the plane is perpendicular to the propagation vector **K**, and propagates with speed **v**.

v is therefore the ***phase velocity***. For a wave resulting from the sum of several components, the pulse travels with the so-called ***group velocity*** and interested readers can consult the **simulation offered through this link**.

In the solutions to the equation in which the amplitude depends inversely on the distance, the planes become spheres and thus spherical waves are obtained. However if the distance of observation is very large, they can be considered similar to plane waves at that observation point.

Taking into account what it is shown in the figure above, the principle of superposition states that due to a number of ***coherent*** sources (which don't vary phase relationships between them), the wave measured at a given time and point, is the sum of the individual waves at that time and point, taking into account the individual phases (the process of ***interference***), as shown above.

If there is no coherence between waves, phase relationships vary over time, and to obtain the total intensity of the resultant wave, we just have to add intensities (see figure below):

The total disturbance of two non-coherent sources is just the sum of the individual intensities

To model the composition of simple trigonometric waves (of type sine or cosine, or in their imaginary exponential form) the Fresnel representation is normally used. In this representation it is assumed that each wave oscillates around the **X** axis, as the projection of the circular motion of a vector of length equal to its amplitude and with an angular speed equal to the wave pulse **ω**.

In this way, the resultant wave can be obtained by adding the individual vectors and projecting the resultant vector over the same X axis.

*Fresnel (or Argand) representation in which is shown the composition of several individual waves (*fj*).*

|F| *is the amplitude of the resultant wave and* Φ *its phase.*

Interaction of X-rays with matter

X-ray waves interact with matter through the electrons contained in atoms, which are moving at speeds much slower than light. When the electromagnetic radiation (the X-rays) reaches an electron (a charged particle) it becomes a secondary source of electromagnetic radiation that scatters the incident radiation.

According to the wavelength and phase relationships of the scattered radiation, we can refer to ***elastic processes*** (or ***inelastic processes: Compton scattering***), depending if the wavelength does not change (or changes), and to ***coherence*** (or ***incoherence***) if the phase relations are maintained (or not maintained) over time and space.

The exchanges of energy and momentum that are produced during these processes can even lead to the expulsion of an electron out of the atom, followed by the occupation of its energy level by electrons located in higher energy levels.

All these types of interactions lead to different processes in the materials such as: ***refraction***, ***absorption***, ***fluorescence***, ***Rayleigh scattering***, ***Compton scattering***, ***polarization***, ***diffraction***, ***reflection***, ...

The ***refractive index*** of all materials in relation to X-rays is close to 1, so that the phenomenon of refraction of X-rays is negligible. This explains why we are not able to produce lenses for X-rays and why the process of image formation, as in the case of visible light, cannot be carried out with X-rays. It does not explain why reflective optics (**catoptric system**) cannot be used. Only dioptric system is excluded.

Absorption means an attenuation of the transmitted beam, losing its energy through all types of interactions, mainly thermal, fluorescence, inelastic scattering, formation of free radicals and other chemical modifications that could lead to degradation of the material. This intensity decrease follows an exponential model dependent on the distance crossed and on a coefficient of the material (the linear absorption coefficient) which depends on the density and composition of the material.

The process of ***fluorescence***, in which an electron is pulled out of an atom's energy level, provides information on the chemical composition of the material. Due to the expulsion of electrons from the different energy levels, sharp discontinuities in the absorption of radiation are produced. These discontinuities allow local analysis around an atom (***EXAFS***).

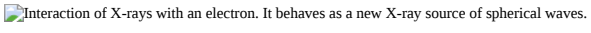
In the ***Compton effect***, the interaction is inelastic and the radiation loses energy. This phenomenon is always present in the interaction of X-rays with matter, but due to its low intensity, its incoherence and its propagation in all directions, its contribution is only found in the background radiation produced through the interaction.

By ***scattering*** we will refer here to the changes of direction suffered by the incident radiation, and NOT to ***dispersion*** (the phenomenon that causes the separation of a wave into components of varying frequency).

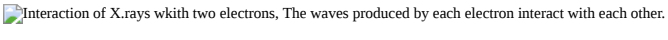
Left: *Variation in the absorption of a material according to the wavelength of the incident radiation*

Right: *Dispersion of visible light into its nearly monochromatic wavelengths*

Elastic scattering by an electron



Interaction of a X-ray front with an isolated electron, which becomes a new X-ray source, producing the X-rays waves in a spherical mode



The spherical waves produced by two electrons interact with each other, producing positive and negative interferences

Animations originally taken from ***physics-animations.com***

When a non-polarized X-ray beam (that is, when its electromagnetic field is vibrating at random in all directions perpendicular to the propagation), interacts with an electron, the interaction takes place primarily through its electric field. Thus, in a first approximation, we can neglect both the magnetic and nuclear interactions. According to the electromagnetic theory of Maxwell, the electron scatters electric waves which propagate perpendicular to the electric field, in such a way that the scattered energy (which crosses the unit of area perpendicular to the direction of propagation and per unit of time) is:

$$I_e(K_s) = I_0 \, [e^4 / R_0^2 \, m^2 \, c^4] \, [(1 + cos^2 2\theta) / 2]$$

Thomson scattering model

Ks is the scattering vector, R_0 is the distance to the observation point, 2θ is the angle between the incident direction and the direction where the scattering is observed; **e** and **m** are the charge and mass of the electron, respectively, and **c** is the speed of propagation of radiation in the vacuum.

The equation above describes the ***Thomson's model*** established in 1906 [**Joseph John Thomson (1856-1940)**] for the spherical wave elastically scattered by a free electron, which is similar to the ***Rayleigh scattering*** with visible light. The scattered wave is elastic, coherent and spherical. The mass factor (**m**) in the denominator justifies neglecting the nuclear scattering.

The binding forces between atom and electron are not considered in the model. It is assumed that the natural frequencies of vibration of the electron are much smaller than those of the incident radiation. In this "normal" scattering model (in contrast to the anomalous case in which those frequencies are comparable) the scattered wave is in opposition of phase with the incident radiation.

The second factor (in brackets equation above) which depends on the θ angle, is known as the ***polarization factor,*** because the scattered radiation becomes ***partially polarized***, which creates a certain anisotropy in the vibrational directions of the electron, as well as a reduction in the scattered intensity (depending of the direction). The scattered intensity shows symmetry around the incident direction. As the scattered wave is spherical, the inverse proportionality to the squared distance makes the energy per unit of ***solid angle*** a constant.

A solid angle is the angle in three-dimensional space that an object subtends at a point. It is a measure of how big that object appears to an observer looking from that point. Metrically it is the constant ratio between the intersecting areas of concentric spheres with a cone, and the corresponding squared radii of the spheres:

$$A1/R1^2 = A2/R2^2 = A3/R3^2 = ... = \text{solid angle in steradians}$$

The factor of the geometric "difference of phase"

With regard to the phenomenon of diffraction and interference, it is important to consider the phase relationship between two waves due to their different geometric paths. This affects the difference of phase α of the resultant wave:

$$\phi = 2\pi(K_0.r - \nu.t + \alpha)$$

in such a way that:

$$\alpha = 2\pi \, (K_s - K_0) \, r_{ij} + \alpha \, '$$

where K_0 is the wave vector of the incident wave, K_s is the wave vector in the direction of propagation and r_{ij} is the vector between the two propagation centers which produces the phase difference.

If we have several disturbance centers whose phase differences are measured from a common origin, and we consider the position vectors r_j of their phase differences, the phase difference of one of the centers can be written (using unit vectors in the directions of propagation with $\lambda K = s$) as:

$$\alpha_j = 2\pi\,[(s - s_0) / \lambda]\, r_j + \alpha\,'$$

This means that all r_j points in which the product $(s - s_0)\, r_j$ has a constant value (*cte*) , will have the same phase, given by:

$$\alpha = (cte.\, 2\pi / \lambda) + \alpha\,'$$

Scattering by an atom

An atom that can be considered as a set of **Z** electrons (its atomic number) can be expected to scatter **Z** times that which an electron does. But the distances between the electrons of an atom are of the order of the X-rays wavelength, and therefore we can also expect some type of ***partial destructive interferences*** among the scattered waves. In fact, an atom scatters **Z** times (what an electron does) only in the direction of the incident beam, decreasing with the increasing of the θ angle (the angle between the incident radiation and the direction where we measure the scattering). And the more diffuse the electronic distribution of electrons around the nucleus, the greater the reduction.

Phase relationships among the electrons in an atom

Diagram showing the variation of the amplitudes scattered by an electron, without considering the polarization (left figure), and an atom (right figure). The amplitude (intensity) scattered by an atom decreases with increasing scattering angle.

The intensity of the X-rays scattered by the electrons of an atom decreases with increasing scattering angle

Scheme taken from **School of Crystallography** (Birkbeck College, Univ. of London)

The ***atomic scattering factor*** is the ratio between the amplitude scattered by an atom and a single electron. As the speed of electrons in the atom is much greater than the variation of the electric vector of the wave, the incident radiation only "sees" an average electronic cloud, which is characterized by an electron density of charge $\rho(r)$. If this distribution is considered spherically symmetric, it will just depend on the distance to the nucleus, so that, with:

$H = 2\, sin\, \theta / \lambda$ (which is the length of the scattering vector $H = K_S - K_0 = (s - s_0) / \lambda$):

$$f(H) = 4\pi \int_{(0 \to \infty)} r^2\, \rho(r)\, (sin\, H\, r / H\, r)\, dr$$

Thus, the atomic scattering factor will represent a number of electrons (the effective number of electrons of a particular atom type) that scatter in phase in that direction, so that $\theta = 0$ and $f(0) = Z$. The hypothesis of isotropy, ie that this atomic factor does not depend on the direction of **H**, appears to be unsuitable for transition momentum in which **d** or **f** orbitals are involved, nor for the valence electrons.

By quantum-mechanics calculations we can obtain the values for the atomic scattering factors, and we can derive analytical estimates of the type:

$$f(H) = \sum_{(1 \to 4)} a_i\, exp\, [\, -b_i\, H^2\,] + c$$

Left: *Atomic scattering factors calculated for several ions with the same number of electrons as **Ne**. One can observe that the O^{--} has a more diffuse electronic cloud than Si^{4+} and thus it shows a faster decay*

Right: *Atomic scattering factors calculated for atoms and ions with different numbers of electrons. Note that the single electron of the hydrogen atom (H) scatters very little as compared with other elements, especially with increasing Θ. Hydrogen will therefore be "**difficult to see**" among other dispersion effects*

When the frequency of the incident radiation is close to the natural vibration of the electron linked to the atom, we have to make some corrections (Δ) due to the phase differences that occur between the individual waves scattered by electrons, whose vibration

(due to the incident wave) is affected by that linking. Thus:

$$\mathbf{f(H) = f_0 + \Delta' f + i \Delta'' f}$$

also written as:

$$\mathbf{f}(H) = \mathbf{f_0 + f' + i f''}$$

where $\mathbf{f_0}$ is the atomic scattering factor without ligation, as previously defined, and **i** is the imaginary unit that represents the phase differences between individual scattered waves. This situation occurs for atoms with large atomic numbers (heavy atoms), or with atomic numbers close (but smaller) to the metal atoms in the X-ray anode. **These corrections, that will be discussed in another chapter,** weakly depend on the θ angle, so that this anomalous effect is better seen at larger values of this angle, although this is where the scattered beams have lower intensity due to thermal effects (see below).

[These corrections allow us to distinguish the **chirality** (Bijvoet, 1951) of the crystals and provide us a **method for solving the structure of molecules** (*SAD*, *MAD*)].

Due to the movement of the atomic thermal vibrations within the material, the effective volume of the atom appears larger, leading to an exponential decrease of the scattering power, characterized by a coefficient **B** (initially isotropic) in the Debye-Waller (1913, 1923) exponential factor:

$$\mathbf{f}(H)\ exp\ [\ -\mathbf{B}_{iso}\ sin^2\theta\ /\ \lambda^2\ ]$$

B is $8\pi^2\langle u^2\rangle$, $\langle \mathbf{u^2} \rangle$ being the quadratic average amplitude of thermal vibration in the **H** direction. In the isotropic model of vibration, **B** is considered to be identical in all directions (with normal values between 3 and 6 Angstroms^2 in crystals of organic compounds). In the anisotropic model, **B** is considered to follow an ellipsoidal vibration model. Unfortunately, these thermal parameters may reflect not only thermal vibration, as they are affected by other factors such as atomic static disorder, absorption, wrong scattering factors, etc.

Decrease of the atomic scattering factor due to the thermal vibration

If the browser allows it, interested readers can also use **this applet made by Steffen Weber** which shows the ***decrease of the atomic scattering factor*** of an atom when the temperature increases its thermal vibration state. Just write in the left column of the applet the atomic number of an atom (eg 80 for mercury), and the same number in the box shown below. Then activate the box marked with the word "Execute" and note the decrease of the scattering factor as a function of the selected temperature. Now increase the temperature (eg 2), and re-activate the "Execute" box.

Scattering by a set of atoms

X-rays scattered by a set atoms produce X-ray radiation in all directions, leading to interferences due to the coherent phase differences between the interatomic vectors that describe the relative position of atoms. In a molecule or in an aggregate of atoms, this effect is known as the ***effect of internal interference***, while we refer to an ***external interference*** as the effect that occurs between molecules or aggregates. The scattering diagrams below show the relative intensity of each of these effects:

Scattering diagrams of a monoatomic material in different states. In the intensity axis we have neglected the background contribution. The figures mainly represent the effect of the external interference, while the internal interference (in this case due to a single atom only) is simply reflected by the relative intensity of the maxima. Note how the thermal movement in the liquid softens and reduces the scattering profile, and how the maxima produced by the glass also decrease. In the crystal, where the phase relations are fixed and repetitive, the scattering profile becomes sharp with well defined peaks, whereas in the other diagrams the peaks are broad and somewhat continuous. In the crystal case the scattering effect is known as diffraction. Note how the scattering phenomenon reflects the internal order of the sample -- the positional correlations between atoms.

In the case of monoatomic gases, the effects of interference between atoms **m** and **n** lead (in terms of the intensity scattered by an electron) to:

$$I(H) = I_e(H)\ \Sigma_m \Sigma_n\ f_m(H)\ f_n(H)\ exp\ [2\pi i\ (\mathbf{s} - s_0)\ r_{m,n}\ /\ \lambda]$$

which, when averaged over the duration of the experiment and in all **k** directions of space, gives rise to the ***Debye formula***:

$$<I(H)> = I_e(H) \Sigma_m \Sigma_n f_m(H) f_n(H) [sin\ 2\pi|H| |r_{m,n}| / 2\pi|H| |r_{m,n}|]$$

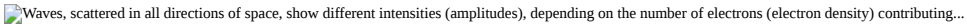
Geometry of the scattering produced by a set of identical atoms

In the case of monoatomic liquids some effects appear at short distances, due to correlations between atomic positions. If the density of atoms per unit of volume (at a distance **r** from any atom with spherical symmetry) is, on average, **$\rho(r)$**, then the expression **$4\pi\ r^2\rho(r)$** is known as the ***radial distribution***, and the ***Debye formula*** becomes:

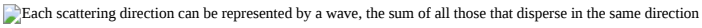
$$<I(H)> = I_e(H)\ N\ f^2(H) [1 + \int_{(0 \rightarrow \infty)} 4\pi r^2 \rho(r)\ sin\ (2\pi|H| |r|) / 2\pi|H| |r|\ dr]$$

All these relationships allow the analysis of the X-ray scattering in amorphous, glassy, liquid and gaseous samples.

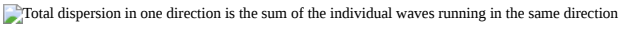
No matter the possible complexity with which the phenomenon of X-ray scattering is presented. The nonspecialist reader should only remember some simple ideas that are outlined below (drawings taken from the **lecture by Stephen Curry**)...



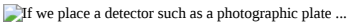
- X-rays are scattered by electrons contained in atoms. This dispersion effect (which is produced in the form of waves, scattered in all directions of space) contains different intensities (amplitudes), depending on the number of electrons (electron density) contributing to the scattered waves...



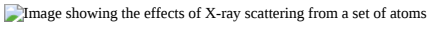
- Taking an origin in the atomic set and considering a given direction of dispersion, each of the waves scattered in that direction can be represented by a mathematical function (shown in the figure), whose amplitude depends on the electron density ***ρ(r)*** existing at the point where the wave arises. ***S*** is a magnitude which depends on the angle at which the scattering occurs.



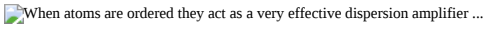
- The total scattered wave in each direction is the sum of all the individual waves which scatter in the same direction, ***f (S)***. Its intensity (amplitude) will be governed by the phase relationship between the contributing waves, which depends on ***r*** (the distance between the points where they originate). This will happen for all space directions...



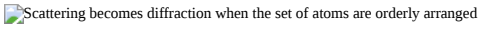
- If we place a detector (such as a photographic plate) to observe the scattered waves, ***f(S)***, we obtain a distribution of intensities as shown in the image below...



- This "map" of scattered waves (shape and intensities) contains information on the distribution of atoms that are producing the scattering. Mathematically this map is represented by the function ***f(S)***, which is the **Fourier transform** of the atomic distribution, that is, of the electron density function…



- We will see later that when the set of atoms are arranged in an orderly fashion, ie, in the form of a crystal, they behave as a very effective dispersion amplifier...



- In these circumstances, scattering effects concentrate in certain areas of the detector, very well defined and regularly distributed, known as diffraction... The diffraction allows us to obtain an information about the electronic distribution much richer than the one produced by the scattering of a set of disordered atoms...

Scattering by a monoatomic lattice: Diffraction

When the set of atoms is structured as a regular three-dimensional lattice (so that the atoms are nodes of the lattice), the precise geometric relationships between the atoms give rise to particular phase differences. In these cases, cooperative effects occur and the sample acts as a three-dimensional diffraction grid. Under these conditions, the effects of ***external interference*** produce a

scattering structured in terms of peaks with maximum intensity which can be described in terms of another lattice (**reciprocal of the atomic lattice**) which shows typical patterns, such as those you can see when you look at a streetlight through an umbrella or a curtain.

*Schematic diagram of diffraction patterns from several two-dimensional point distributions. The parameters of repetition in the diffraction patterns (reciprocal space) carry the * superscript and k means a constant scale factor which depends on the experiment. All points of the diffraction pattern have the same intensity, because it is assumed that the used wavelength is much larger than the points of the direct lattice (see above in the paragraph about scattering by an atom).*

*Relationship between two 2-dimensional lattices, direct lattice (on the left) and reciprocal lattice (on the right). The repetition parameters in reciprocal space carry the * superscript and **k** is a scale factor that depends on the experiment.*

***d_{10}** and **d_{01}** are the corresponding direct lattice spacings. Note that the figures show a direct unit cell and a reciprocal unit cell only, corresponding to the diffraction patterns shown on the left side of the page. See also **direct and reciprocal lattices**.*

Structured in a lattice, any atom can be defined by a vector, referred to a common origin:

$$\mathbf{R_{j,m1,m2,m3} = m1\ a + m2\ b + m3\ c}$$

where $\mathbf{R_j}$ represents the position of the **j** node in the lattice; **m1**, **m2**, **m3**, are integers and **a**, **b** and **c** are the vectors defining the lattice. According to this, the intensity scattered by a material would be:

$$\mathbf{I}(H) = \mathbf{I_e}(H) \Sigma_{m1}\Sigma_{m'1}\Sigma_{m2}\Sigma_{m'2}\Sigma_{m3}\Sigma_{m'3}\ \mathbf{f_j}(H)\ \mathbf{f_{j'}}(H)\ exp\ [2\pi \mathbf{i}\ (\mathbf{s} - \mathbf{s_0})\ \mathbf{r_{m,m'}} / \boldsymbol{\lambda}]$$

where:

$$\mathbf{r_{m,m'} = R_{m1,m2,m3} - R_{m'1,m'2,m'3} = (m1\text{-}m'1)\ a + (m2 - m'2)\ b + (m3 - m'3)\ c}$$

And calculating this sum we have:

$$\mathbf{I(H) = I_e(H)\ [\ [\ sin^2\ \pi(s - s_0)\ M1\ a / \lambda\ ] / [sin^2\ \pi(s - s_0)\ a / \lambda\ ]\ ]\ .}$$

$$\mathbf{[\ [\ sin^2\ \pi(s - s_0)\ M2\ b / \lambda\ ] / [sin^2\ \pi(s - s_0)\ b / \lambda\ ]\ ]\ .}$$

$$[\ [\ sin^2\ \pi(s - s_0)\ M3\ c / \lambda\ ] / [sin^2\ \pi(s - s_0)\ c / \lambda\ ]\ ]$$

$$\mathbf{= I_e(H)\ I_L(H)}$$

In this expression, **M1, M2, M3** represent the number of unit cells contained in the crystal along the a, b and c directions, respectively, so that in the total sample the number of unit cells would be **M = M1.M2.M3** (around 10^{15} in crystals of an average thickness of 0.5 mm).

$I_L(H)$ is the factor of external interference due to the monoatomic lattice. It consists of several products of type $(sin^2\ Cx) / sin^2\ x$, where **C** is a very large number. This function is almost zero for all x values, except in those points where x is an integer multiple of **π**, where it takes its maximum value of $\mathbf{C^2}$. The total value would be a maximum value only when all three products are other than zero, where it will take the value of $\mathbf{M^2}$. That is, the diffraction diagram of the direct lattice is another lattice that takes non-zero values in its nodes and that, due to the $\mathbf{I_e(H)}$ factor, varies from one place to another...

Due to the finite size of the samples, the small chromatic differences of the incident radiation, the ***mosaic*** of the sample, etc., the maxima show some type of spreading around them. Therefore, in order to set the experimental conditions for measurement, one needs a small sample oscillation around the maximum position (***rocking***) to integrate all these effects and to collect the total scattered energy.

Graphical representation of one of the products of the IL(H) function between two consecutive maxima.

*Note the transformation from scattering to diffraction, that is, from broad to very sharp peaks, as the number of cells **M1** increases.*

*The maxima are proportional to $\mathbf{M1^2}$ and the first minimum appears closer to the maximum with increasing **M1**.*

Diffraction by a crystal

When the material is not structured in terms of a monoatomic lattice, but is formed by a group of atoms of the same or of different types, the position of every atom with respect to a common origin is given by:

$$\mathbf{R}_{j,m1,m2,m3} = m1\,\mathbf{a} + m2\,\mathbf{b} + m3\,\mathbf{c} + \mathbf{r}_j$$

$$= \mathbf{T}_{m1,m2,m3} + \mathbf{r}_j$$

Reduction inside a unit cell of the absolute position of an atom through lattice translations

that is, that to go from the origin to the atom, at position **R**, we first go, through the **T** translation, to the unit cell origin, and from there with the vector **r** we reach the atom.

As the atom is always included within a unit cell, its coordinates referred to the cell are smaller than the axes, and often are expressed as fractions of them:

$$\mathbf{r} = X\,\mathbf{a} + Y\,\mathbf{b} + Z\,\mathbf{c} = X/a\,\mathbf{a} + Y/b\,\mathbf{b} + Z/c\,\mathbf{c} = x\,\mathbf{a} + y\,\mathbf{b} + z\,\mathbf{c}$$

where **x, y, z**, as fractions of axes, are now between -1 and +1.

Then, under the conditions initially raised, ie with a monochromatic and depolarised X-ray beam (as a plane wave, formed by parallel rays of a common front wave), perpendicular to the propagation unit vector $\mathbf{s}_0$ that completely covers the sample, the kinematic model of interaction indicates that the sample produces diffracted beams in the direction **s** with an intensity given by:

$$I(H) = I_e(H)\, I_F(H)\, I_L(H)$$

where I_e is the intensity scattered by an electron, I_L is the external interference effect due to the three-dimensional lattice structure, and I_F is the square of the so-called **structure factor**, a magnitude which takes into account the effect of all internal interferences due to the geometric phase relationships between all atoms contained in the unit cell. This internal structural effect is:

$$I_F(H) = |\, F^2(H)\,| = F(H)\, F^*(H)$$

As a consequence of the complex representation of waves, mentioned at the beginning, the square of a complex magnitude is obtained by multiplying the complex by its conjugate. Thus, specifically, we give the name **structure factor**, **F(H)**, to the resultant wave from all scattered waves produced by all atoms in a given direction :

$$F(H) = \Sigma_{(1 \to n)}\, f_j(H)\, exp\, [2\pi(\mathbf{s} - \mathbf{s}_0)\, \mathbf{r}_j / \lambda]$$

As already stated, the phase differences due to geometric distances **R** are proportional to $(\mathbf{s} - \mathbf{s}_0)\, \mathbf{R} / \lambda$. This means that if we change the origin, the phase differences will be produced according to the geometric changes, in such a way that as the exponential parts of the intensity functions are conjugate complexes, they will affect the intensities in terms of a proportionality constant only. Thus, a change of origin is not relevant to the phenomenon.

In the equation of the total intensity, **I(H)**, the conditions to get a maximum lead to the following consequences:

- The phenomenon of diffraction in crystal samples is discrete, spectral.
- The directions and the periodic repetitions in the reciprocal lattice do not depend on the structure factors. They only depend on the direct lattice. The knowledge of these directions give us the shape and size of the direct unit cell, which actually controls the positions of the diffraction maxima.
- The intensity of the diffraction maxima depends on the structure factor in this direction (at that reciprocal point), which only depend on the atomic distribution within the unit cell. In other words, the diffraction intensities are only controlled by the atomic distribution within the cell. Thus, through the intensities we can obtain information about the atomic structure within the unit cell.
- The total diffraction pattern is the consequence of the diffraction of the different atomic aggregates within the unit cell, sampled in the diffraction points produced by the crystal lattice (the reciprocal points).
- In summary, ***structural crystallography by X-ray diffraction*** consists of measuring the intensities of the largest possible amount of diffracted beams in the 3-dimensional diffraction pattern, to get from them the amplitudes of the structure factors, and from

these values (through some procedure to allocate the phases for each of these structure factors) to build the electronic distribution in the elementary cell (which can be described in terms of a function whose maxima will give us the atomic positions).

Diffraction patterns of: (a) a single molecule, (b) two molecules, (c) four molecules, (d) a periodically distributed linear array of molecules, (e) two linear arrays of molecules, and (f) a two-dimensional lattice of molecules. Note how the pattern of the latter is the pattern of the molecule sampled in the reciprocal points.

To clarify what has been said above, the reader can analyze **further objects and their corresponding diffraction patterns through this link**. Additionally we suggest you to watch the video prepared by the **Royal Institution** to demonstrate optically **the basis of diffraction using a wire coil (representing a molecule) and a laser (representing an X-ray beam)**.

Laue equations, Bragg's interpretation and Ewald's geometric diffraction model

We have seen that the diffraction diagram of a direct lattice defined by three translations, **a**, **b** and **c**, can be expressed in terms of another lattice (the reciprocal lattice) with its **reciprocal translations**: **a***, **b*** and **c***, and these translation vectors (direct and reciprocal) meet the conditions of reciprocity:

$$\mathbf{a\ a^* = b\ b^* = c\ c^* = 1}\ \textbf{and}\ \mathbf{a\ b^* = a\ c^* = b\ c^* = 0}$$

and they also meet that (for instance):

$$\mathbf{a^* = (b \times c) / V}$$ (x means vectorial or cross product)

where **V** is the volume of the direct unit cell defined by the 3 vectors of the direct cell, and therefore:

$$\mathbf{a^* = N_{100} / d_{100}}$$

where $\mathbf{N_{100}}$ is a unit vector perpendicular to the planes of indices **h=1, k=0, l=0**, and where $\mathbf{d_{100}}$ is the corresponding interplanar spacing. And similarly with **b*** and **c***.

In this way, any vector in the reciprocal lattice will be given by:

$$\mathbf{H^*_{hkl} = h\ a^* + k\ b^* + l\ c^* = N_{hkl} / d_{hkl}}$$

in such a way that:

$$\mathbf{|H^*_{hkl}|\ d_{hkl} = 1}$$

On the other hand, we have seen that the maxima in the diffraction diagram of a crystal correspond to the maximum function $\mathbf{I_L(H)}$, meaning that each of the products that define this function must be individually different from zero, as a sufficient condition to obtain a maximum for the diffracted intensity. If we remember that $\mathbf{H = (s - s_0) / \lambda}$, this also means that the three so-called ***Laue equations*** must be fulfilled [**Max von Laue (1879-1960)**]:

$$\mathbf{H\ a = \mathit{h},\ H\ b = \mathit{k},\ H\ c = \mathit{l}}$$

where $\mathbf{\mathit{h}}$, $\mathbf{\mathit{k}}$, $\mathbf{\mathit{l}}$ are integers

Laue equations

There is also a less formal way to derive and/or to understand the Laue equations, and therefore **we invite interested readers to visit this link** ...

These three Laue conditions are met if the vector **H** represents a vector of the reciprocal lattice, so that:

$$\mathbf{H} = h\,\mathbf{a}^* + k\,\mathbf{b}^* + l\,\mathbf{c}^*$$

since due to the properties of the reciprocal lattice, it can be stated that:

$$\mathbf{H}_{hkl}\cdot\mathbf{a} = h,\ \mathbf{H}_{hkl}\cdot\mathbf{b} = k,\ \mathbf{H}_{hkl}\cdot\mathbf{c} = l$$

Said in other words: the three conditions of Laue (Nobel Prize for Physics in 1914) are sufficient to establish that the vector **H** is a vector of the reciprocal lattice ($\mathbf{H} = \mathbf{H}^*_{hkl}$).

If these three conditions are fulfilled, and taking into account some relationships explained above, we can write:

$$|\mathbf{H}| = 2\sin\theta_{hkl} / \lambda = |(\mathbf{s} - \mathbf{s}_0)| / \lambda = |\mathbf{H}^*_{hkl}| = 1 / d_{hkl}$$

And this is ***Bragg's Law*** **[William L. Bragg (1890-1971)],** that can be rewritten in its usual form as:

$$\lambda = 2\,d_{hkl}\sin\theta_{hkl}$$

But taking into account that geometrically we can consider spacings of type $d_{hkl}/2$, $d_{hkl}/3$, and in general d_{hkl}/n (ie, $d_{nh,nk,nl}$, where **n** is an integer), the **Bragg's** equation (Nobel Prize in Physics in 1915) would be in the form:

$$\lambda = 2\,(d_{hkl}/n)\sin\theta_{nh,nk,nl}$$

that is: $n\lambda = 2\,d_{hkl}\sin\theta_{nh,nk,nl}$

where **n** is an integer number

Bragg's Law

There is also a less formal way to derive and/or to understand Bragg's Law, and therefore **we invite interested readers to visit this link**...

Moreover, if the Laue conditions are fulfilled (as explained in the following figure) all atoms located on the sequence of planes parallel to the one with indices ***hkl*** at a given distance (D_P) from the origin (D_P being an integer multiple of d_{hkl}) will diffract in phase, and their geometric difference-of-phase factor will be:

$$(\mathbf{s} - \mathbf{s}_0)\,\mathbf{r} = n\lambda$$

and consequently a diffraction maximum will be produced in the direction:

$$\mathbf{s} = \mathbf{s}_0 + \lambda\,\mathbf{H}^*_{hkl}$$

$$N_{hkl} = H^*_{hkl}\,d_{hkl}$$

The plane equation can, therefore, be written as:

$$H^*_{hkl}\,r = H^*_{hkl}\,r_i = |H^*_{hkl}|\,|r_i|\cos(H^*_{hkl}, r_i) = (1/d_{hkl})\,D_P = n$$

Bragg's equation has a very simple interpretation... When in the crystal-radiation interaction a maximum of diffraction occurs, ***it is equivalent to say that the incident beam reflects on the crystal planes*** of indices ***hkl*** and interplanar spacing d_{hkl}. That's why in talking about diffraction maxima, sometimes we use the phrase ***Bragg's reflection***.

Moreover, this equation holds all the traditional relations of reciprocity of diffraction, between ***spacing-direction*** or ***position-momentum***: the shorter spacing, the larger angle and vice versa; direct lattices with large unit cells produce very close diffracted beams, and vice versa.

The figure geometrically describes the direction of the diffraction beam due to the constructive interference between atoms located on the planes with interplanar spacing **d(hkl)**.

The figure depicts a description of Bragg's model when different types of atoms are located on their respective parallel planes with **Δd** *spacing. The separation between blue and green planes creates interferences and differences of phases (between the reflected beams) giving rise to changes in intensity (depending of the direction). These intensity changes allow us to get information on the structure of atoms that form the crystal).*

Readers with installed **Java Runtime** tools can play with **Bragg's model using this applet**.

On the other hand, we have seen that, in general:

$\mathbf{H} = (\mathbf{s} - \mathbf{s_0}) / \lambda = -\mathbf{s_0}/\lambda + \mathbf{s}/\lambda$

and this means that the vectors **H** can be considered as belonging to a sphere of radius $1/\lambda$ centered at a point defined by the vector $-\mathbf{s_0}/\lambda$ with respect to the origin where the crystal is. This is known as ***Ewald's sphere*** (Ewald, 1921), which provides a very easy geometric interpretation of the directions of the diffracted beams. When the **H** vectors belong to the reciprocal lattice and the end of the vector (a reciprocal point) lies on that spherical surface, diffracted beams are produced, and obviously the crystal planes are in Bragg's position.

It's amazing how quickly **Paul Peter Ewald (1888-1985)** developed this interpretation only some months after **Max von Laue** experiments. His original article, published in 1913 (in German), is **available through this link**. The advanced reader can also consult the article published by Ewald in **Acta Crystallographica (1969) A25, 103-108**.

This figure describes Ewald's geometric model. When a reciprocal point , **P*(hkl)**, touches the surface of Ewald's sphere, a diffracted beam is produced starting in the centre of the sphere and passing through the point P*(hkl). Actually the origin of the reciprocal lattice, **O***, coincides with the position of the crystal and the diffracted beam will start from this common origin, but being parallel to the one drawn in this figure, exactly as it is depicted in the figure below.

This figure shows the whole reciprocal volume that can give rise to diffracted beams when the sample rotates. Changing the orientation of the reciprocal lattice, one can collect all the beams corresponding to the reciprocal points contained in a sphere of radius $2/\lambda$ *known as the limit sphere. Reciprocal points are shown as small gray spheres .*

To obtain all possible diffracted beams that a sample can provide, using a radiation of wavelength λ, it is sufficient to conveniently orient the crystal and make it turn, so that its reciprocal points will have the opportunity to lay on the surface of Ewald's sphere. In these circumstances, diffracted beams will originate as described above. With larger wavelengths, the volume of the reciprocal space that can be explored will be smaller, but the diffracted beams will appear more separated.

Ewald's model showing how diffraction occurs. The incident X-ray beam, with wavelength **λ**, shown as a white line, "creates" an imaginary Ewald's sphere of diameter $2/\lambda$ (shown in **green**). The reciprocal lattice (**red points**) rotate as the crystal rotates, and every time that a reciprocal point cuts the sphere surface a diffracted beam is produced from the center of the sphere (yellow arrows).

This Java application can be downloaded from this link*. It is totally virus free, and based on the concept of the reciprocal lattice. It allows playing with the Ewald's model to understand the diffraction. Original by Nicolas Schoeni and Gervais Chapuis of the Ecole Polytechnique Fédéral de Lausanne (Switzerland).*

According to **Bragg's Law**, the maximum angle at which one can observe diffraction will correspond to the angle where the *sin* function is maximum (=1). This also means that the ***theoretical maximum resolution*** that can be achieved is $\lambda/2$. In practice, due to the decrease of the atomic scattering factors by increasing Bragg angles, appreciable intensities will appear only up to a maximum angular value of $\theta_{max} < 90^o$ and the ***real maximum resolution*** reached will be $d_{min} = \lambda/2 \sin \theta_{max}$.

Considering that the interplanar spacings d_{hkl} are a characteristic of the sample, by reducing the wavelength, Bragg's Law indicates that the diffraction angles (θ) will decrease; the spectrum shrinks, but on the other hand, more diffraction data will be obtained, and

therefore a better structural resolution will be achieved.

According to Ewald's model, the amount of reciprocal space to be measured can be increased by reducing the wavelength, that is, by increasing the radius of the Ewald's sphere

It is also very helpful to visit the pages that on ***reciprocal space*** are offered by the **University of Cambridge through this link**, as well as to **look at the video** made by **www.PhysicsReimagined.com**, showing the geometric relationships between direct and reciprocal lattices, displayed below as an animated gif:

Once the foundations of the theoretical model which describe the phenomenon of diffraction are set, we encourage the reader to visit the pages dedicated to the different experimental methods to measure the diffraction intensities.

1.6: Experimental diffraction

In the context of this chapter, you will also be invited to visit these sections...

- **Evaluating the diffraction pattern**
- **Understanding Weissenberg diagrams**

Regardless of the huge improvements that have occurred for **X-ray generation**, the techniques used to measure the intensities and angles of diffraction patterns have evolved over time. In the first diffraction experiment, Friedrich and Knipping (1912) used a film sensitive to X-rays, but even in the same year, Bragg used an ionization chamber mounted on a rotating arm that, in general, could more accurately determine angles and intensities. However, the film technique had the advantage of being able to collect many diffracted beams at the same time, and thus during the first years of structural Crystallography (from 1920 to 1970) an extensive use of photographic methods was made. Among them the following techniques should be highlighted: **Laue**, **Weissenberg**, **precession** and **oscillation**.

Since the mid-1970's, photographic methods have been gradually replaced by **goniometers** coupled with point detectors which subsequently have been replaced by **area detectors**.

The Laue method

For his first experiments, **Max von Laue (1879-1960** (Nobel Prize in Physics in 1914) used continuous radiation (with all possible wavelengths) to impact on a stationary crystal. With this procedure the crystal generates a set of diffracted beams that show the internal symmetry of the crystal. In these circumstances, and taking into account **Bragg's Law**, the experimental constants are the interplanar spacing ***d*** and the crystal position referred to the incident beam. The variables are the wavelength **λ** and the integer number ***n***:

$$n\,\lambda = 2\,d_{hkl}\,sin\,\theta nh,nk,nl$$

Thus, for the same interplanar spacing ***d***, the diffraction pattern will contain the diffracted beams corresponding to the first order of diffraction (***n*=1**) of a certain wavelength, the second order (***n*=2**) of half the wavelength (**λ/2**), the third order (***n*=3**) with wavelength **λ/3**, etc. Therefore, the Laue diagram is simply a stereographic projection of the crystal. See also the **Java simulation offered through this link**.

Laue diagram of a crystal

There are two different geometries in the Laue method, depending on the crystal position with regard to the photographic plate: transmission or reflection:

Left: *The Laue method in transmission mode*

Right: *The Laue method in reflection mode*

The Weissenberg method

The Weissenberg method is based on a camera with the same name, developed in 1924 by the Austrian scientist **Karl Weissenberg (1893-1976)**. In order to understand Weissenberg's contribution to X-ray crystallography one should read the two following articles that some years ago were offered to the British Society of Rheology: **"Weissenberg's Influence on Crystallography"** (by H. Lipson) (**use this link in case of problems**) and **"Karl Weissenberg and the development of X-ray crystallography**" (by M.J. Buerger).

The camera consists of a metallic cylinder that contains a film sensitive to X-rays. The crystal is mounted on a shaft (coaxial with the cylinder) that rotates. According to **Ewald's model**, the reciprocal points will intersect the surface of Ewald's sphere and

diffracted beams will be produced.

The diffracted beams generate black spots on the photographic film, which when removed from the metallic cylinder, appears as shown below.

Left: *Scheme and example of a Weissenberg camera. This camera type was used in crystallographic laboratories until about 1975.*

Right: *Camera developed by K. Weissenberg in 1924*

Two types of diffraction diagrams can be easily obtained with the Weissenberg cameras, depending on the amount of crystal rotation: ***oscillation diagrams*** (rotation of approx. +/-20 degrees) or ***full rotation diagrams*** (360 degrees) respectively. Oscillation diagrams are used to center the crystal, that is, to ensure that the rotation of axis coincides exactly with a direct axis, which is equivalent to saying that reciprocal planes (which by geometric construction are perpendicular to a direct axis) generate lines of spots on the photographic film. Once centering is achieved, the full rotation diagrams are used to evaluate the direct axis of the crystal, which coincides with the spacing between the dot lines on the diagram.

Scheme explaining the production of a Weissenberg diagram of the rotation or oscillation variety. When the reciprocal points, belonging to the same reciprocal plane, touch the surface of Ewald's sphere, they produce diffracted beams arranged in cones.

As shown in the diagram above, each horizontal line of points represents a reciprocal plane perpendicular to the axis of rotation as projected on the photographic plate. The figure on the left shows the real appearance of a Weissenberg diagram of this type, rotation-oscillation.

As explained below, the distance between the horizontal spot lines provides information on the crystal repetition period in the vertical direction of the film.

These diagrams were also used to align mounted crystals... This technique requires that the crystal rotation axis is coincident with an axis of its direct lattice, so that the reciprocal planes are collected as lines of spots as is shown on the left.

The crystal must be mounted in such a way that the rotation axis coincides with a direct axis of the unit cell. Thus, by definition of the reciprocal lattice, there will be reciprocal planes perpendicular to that axis. The reciprocal points (lying on these reciprocal planes) rotate when the crystal rotates and (after passing through the Ewald sphere) produce diffracted beams that arranged in cones, touch the cylindrical film and appear as aligned spots (photograph on the left).

It seems obvious that these diagrams immediately provide information about the repetition period of the direct lattice in the direction perpendicular to the horizontal lines (reciprocal planes). However, those reciprocal planes (two dimensional arrays of reciprocal points) are represented as projections (one dimension) on the film and therefore a strong spot overlapping is to be expected.

The problem with spot overlap was solved by Weissenberg by adding a translation mechanism to the camera, in such a way that the cylinder containing the film could be moved in a "back-and-forth" mode (in the direction parallel to the axis of rotation) coupled with the crystal rotation. At the same time, he introduced two internal cylinders (as is shown in the left figure, and also below). In

this way, only one of the diffracted cones (those from a reciprocal layer) is "filtered" and therefore allowed to reach the photographic film. Thus, a single reciprocal plane (a 2-dimensional array of reciprocal points) is distributed on the film surface (two dimensions) and therefore the overlap effect is avoided.

However, as a consequence of the back and forth translation of the camera during the rotation of the crystal, a deformation is originated in the distribution of the spots (diffraction intensities)

The appearance of such a diagram, **which produces a geometrical deformation of the collected reciprocal plane**, is shown below. Taking into account this deformation, one can easily identify every spot of the selected reciprocal plane and measure its intensity. To select the remaining reciprocal planes one just has to shift the internal cylinders and collect their corresponding diffracted beams (arranged in cones).

Left: *Details of the Weissenberg camera used to collect a cone of diffracted beams. Two internal cylinders showing a slit, through which a cone of diffracted beams is allowed to reach the photographic film. The outer cylinder, containing the film, moves back-and-forth while the crystal rotates, and so the spots that in the previous diagram type were in a line (see above) are now distributed on the film surface (see the figure on the right).*

Right: *Weissenberg diagram showing the reciprocal plane of indices hk2 of the copper metaborate.*

The precession method

The precession method was developed by **Martin J. Buerger (1903-1986)** at the beginning of the 1940's as a very clever alternative to collect diffracted intensities without distorting the geometry of the reciprocal planes.

As in the Weissenberg technique, precession methodology is also based on a moving crystal, but here the crystal moves (and so does the coupled reciprocal lattice) as the planets do, and hence its name. In this case the film is placed on a planar cassette that moves following the crystal movements.

In the precession method the crystal has to be oriented so that the reciprocal plane to be collected is perpendicular to the X-rays' direct beam, ie a direct axis coincides with the direction of the incident X-rays.

Two schematic views showing the principle on which the precession camera is based. μ *is the precession angle around which the reciprocal plane and the photographic film move. During this movement the reciprocal plane and the film are always kept parallel.*

The camera designed for this purpose and the appearance of a precession diagram showing the diffraction pattern of an inorganic crystal are shown in the figures below.

Left: *Scheme and appearance of a precession camera*

Right: *Precession diagram of a perovskite showing cubic symmetry*

Precession diagrams are much simpler to interpret than those of Weissenberg, as they show the reciprocal planes without any distortion. They show a single reciprocal plane on a photographic plate (picture above) when a circular slit is placed between the

crystal and the photographic film. As in the case of Weissenberg diagrams, we can readily measure distances and diffraction intensities. However, with these diagrams it is much easier to observe the symmetry of the reciprocal space.

The only disadvantage of the precession method is a consequence of the film, which is flat instead of cylindrical, and therefore the explored solid angle is smaller than in the Weissenberg case.

The precession method has been used successfully for many years, even for protein crystals:

Left: *Precession diagram of a lysozyme crystal. One can easily distinguish a four-fold symmetry axis perpendicular to the diagram. According to the relationships between direct and reciprocal lattices, if the axes of the unit cell are large (as in this case), the separation between reciprocal points is small.*

Right: *Precession diagram of a simple organic compound, showing mm symmetry (two mirror planes perpendicular to the diagram). Note that the distances between reciprocal points is much larger (smaller direct unit cell axes) than in the case of proteins (see the figure on the left).*

The oscillation method

Originally, the methods of rotating the crystal with a wide rotation angle were very successfully used. However, when it was applied to crystals with larger direct cells (ie small reciprocal cells), the collecting time increased. Therefore, these methods were replaced by methods using small oscillation angles, allowing multiple parts of different reciprocal planes to be collected at once. Collecting this type of diagrams at different starting positions of the crystal is sufficient to obtain enough data in a reasonable time. The geometry of collection is described in the figures shown below. Nowadays, with rotating anode generators, synchrotrons, and area detectors (***image plate*** or ***CCD***, see below), this is the method widely used, especially for proteins.

Outline of the geometrical conditions for diffraction in the oscillation method. The crystal, and therefore its reciprocal lattice, oscillate in a small angle around an axis (perpendicular to the plane of the figure) which passes through the center. In the figure on the right, the reciprocal area that passes through diffraction conditions, within Ewald's sphere (with radius $\mathbf{2.sin\ 90/\lambda}$*), is denoted in yellow. The maximum resolution which can be obtained in the experiment is given by* $\mathbf{2.sen\ \theta_{max}/\lambda}$*).*

When the reciprocal lattice is oscillated in a small angle around the rotation axis, small areas of different reciprocal planes will cross the surface of Ewald's sphere, reaching diffraction condition. Thus, the detector screen will show diffraction spots from the different reciprocal planes forming small "lunes" on the diagram (figure on the right). A "lune" is a plane figure bounded by two circular arcs of unequal radii, i.e., a crescent.

Four-circle goniometers

The introduction of digital computers in the late 1970s led to the design of the so-called ***automatic four-circle diffractometers***. These goniometers, with very precise mechanics and by means of three rotation axes, allow crystal samples to be brought to any orientation in space, fulfilling **Ewald's requirements** to produce diffraction. Once the crystal is oriented, a fourth axis of rotation, which supports the electronic detector, is placed in the right position to collect the diffracted beam. All these movements can be programmed in an automatic mode, with minimal operator intervention.

Two different goniometric geometries have been used very successfully for many years. In the ***Eulerian goniometer*** (see the figure below) the crystal is oriented through the three Euler angles (three circles): Φ represents the rotation axis around the goniometer head (where the crystal is mounted), χ allows the crystal to roll over the closed circle, and ω allows the full goniometer to rotate around a vertical axis. The fourth circle represents the rotation of the detector, 2θ, which is coaxial with ω. This geometry has the advantage of a high mechanical stability, but presents some restrictions for external devices (for instance, low or high temperature devices) to access the crystal.

Left: *Scheme and appearance of a four-circle goniometer with Eulerian geometry*

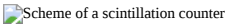
Right: *Rotations in a four-circle goniometer with Eulerian geometry*

An alternative to the Eulerian geometry is the so-called ***Kappa geometry***, which does not have an equivalent to the closed χ circle. The role of the Eulerian χ rotation is fulfilled by means of two new axes: κ (kappa) and ω_κ (see the figure below), in such a way that with a combination of both new angles one can obtain Eulerian χ angles in the range -90 to +90 degrees. The main advantage of this Kappa geometry is the wide accessibility to the crystal. The angles Φ and 2θ are identical to those in Eulerian geometry:

Scheme and appearance of a four-circle goniometer with Kappa geometry

The detection system widely used during many years for both geometries (***Euler*** and ***Kappa***) was based on small-area counters or point detectors. With these detectors the intensity of the diffracted beams must be measured individually, one after the other, and therefore all angles had to be changed automatically according to previously calculated values. Typical measurement times for such detector systems are around 1 minute per reflection.

One of the point detectors more widely used for many years is the **scintillation counter**, whose scheme is shown below:



Scheme of a scintillation counter

Area detectors

As an alternative to the point detectors, the development of electronic technology has led to the emergence of so-called ***area detectors*** which allow the detection of many diffraction beams simultaneously, thereby saving time in the experiment. This technology is particularly useful for proteins and generally for any material that can deteriorate over its exposure to X-rays, since the detection of every collected image (with several hundreds of reflections) is done in a minimum time, on the order of minutes (or seconds if the X-ray source is a synchrotron).

One of the area detectors most commonly used is based on the so-called ***CCD's*** (***Charge Coupled Device***) whose scheme is shown below:

*Schematic view of a CCD with its main components. The X-ray converter, in the figure shown as Phosphor, can also be made with other materials, such as GdOS, etc. The CCD converts X-ray photons at high speed, but its disadvantage is that it operates at very low temperatures (around -70 C). Image taken from **ADSC Products***

CCD-type detectors are usually mounted on Kappa goniometers and their use is widespread in the field of protein crystallography, with **rotating anode generators** or **synchrotron sources**.

Left: *Goniometer with Kappa geometry and CCD detector (Image taken from **Bruker-AXS**)*

Right: *Details of a Kappa goniometer (in this case with a fixed **κ** angle)*

Another type of detector widely used today, especially in protein crystallography, are the ***Image Plate Scanners*****,** which are usually mounted on a relatively rudimentary goniometer, whose only freedom is a rotation axis parallel to the crystal mounting axis. The sensor itself is a circular plate of material sensitive to X-rays. After exposure, a laser is used to scan the plate and read out the intensities.

Left: *Image Plate Scanner. (image taken from Marxperts)*

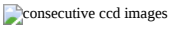
Right: *Components of an Image Plate Scanner*

The latest technology involves the use of area detectors based on ***CMOS*** (**c**omplementary **m**etal-**o**xide **s**emiconductor) technology that has very short readout time, allowing for increased frame rates during the data collection.

Area detectors

*XALOC, the beamline for macromolecular crystallography (left) at the **Spanish synchrotron ALBA** (right)*

In summary, a complete data collection with this type of detectors consists of multiple images such as the ones shown below. The collected images are subsequently analyzed in order to obtain the crystal unit cell data, symmetry (space group) and intensities of the diffraction pattern (reciprocal space). **This process is explained in more detail in another section**.



Left: *Diffraction image of a protein, obtained with the oscillation method in an Image Plate Scanner. During the exposure time (approx. 5 minutes with a rotating anode generator, or approx. 5 seconds at a synchrotron facility) the crystal rotates about 0.5 degrees around the mounting axis. The read-out of the image takes about 20 seconds (depending on the area of the image plate). This could also be the appearance of an image taken with a CCD detector. However, with a CCD the exposure time would be shorter.*

Right: *A set of consecutive diffraction images obtained with an Image Plate Scanner or a CCD detector. After several images two concentric dark circles appear, corresponding to an infinite number of reciprocal points. They correspond to two consecutive diffraction orders of randomly oriented ice microcrystals that appear due to some defect of the cryoprotector or to some humidity of the cold nitrogen used to cool down the sample. Images are taken from **Janet Smith Lab**. See also the **example published by Aritra Pal and Georg Sheldrick**.*

In all of these described experimental methodologies (except for the Laue method), the radiation used is usually ***monochromatic*** (or nearly monochromatic), which is to say, radiation with a single wavelength. Monochromatic radiations are usually obtained with the so-called ***monochromators***, a system composed by single crystals which, based on **Bragg's Law**, are able to "filter" the polychromatic input radiation and select only one of its wavelengths (color), as shown below:

*Scheme of a monochromator. A polychromatic radiation (white) coming from the left is "reflected", according to **Bragg's Law**, "filtering" the input radiation that is reflected again on a secondary crystal. Image taken from **ESRF**.*

At present, in crystallographic laboratories or even in the synchrotron lines, the traditional monochromators are being replaced by new optical components that have demonstrated superior efficacy. These components, usually known as "focusing mirrors", can be based on the following phenomena:

- total reflection (mirrors, capillaries and wave guides),
- refraction (refraction lenses) and
- diffraction (crystal systems based on monochromators, multilayer materials, etc.)

It can also be very instructive to look at this animated diagram showing the path of each X-ray photon in a given diffraction system:

- the photon leaves the source where X-rays are produced,
- goes through the various optical elements that channel it in the right direction (mirrors, slits and collimators)

- diffracts inside the single crystal, and
- finally generates the diffraction spots on a detector

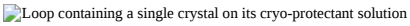
The original video can be seen in ***https://vimeo.com/52155723***

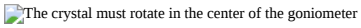
In order to get the largest and best collection of diffraction data, crystal samples are usually maintained at a very low temperature (about 100 K, that is, about -170 C) using a dry nitrogen stream. At low temperatures, crystals (and especially those of macromolecules) are more stable and resist the effects of X-ray radiation much better. At the same time, the low temperature further reduces the **atomic thermal vibration factors**, facilitating their subsequent location within the crystal structure.

Cooling system using dry liquid nitrogen. Image taken from ***Oxford Cryosystems***

To mount the crystals on the goniometer head, in front of the cold nitrogen stream, crystallographers use special loops (like the one depicted in the left figure) which fix the crystal in a matrix transparent to X-rays.

This is especially useful for protein crystals, where the matrix also acts as cryo-protectant (anti-freeze). The molecules of the cryo-protectant spread through the crystal channels replacing the water molecules with the cryo-protectant ones, thus avoiding crystal rupture due to frozen water.

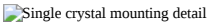




Left: *Detail of a mounted crystal using a loop filled with an antifreeze matrix*

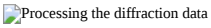
Right: *Checking the position of the crystal in the goniometric optical center. Video courtesy of* ***Ed Berry***

In any case, the crystal center must be coincident with the optical center of the goniometer, where the X-ray beam is also passing through. In this way, when the crystal rotates, it will always be centered on that point, and in any of its positions will be bathed by the X-ray beam.



Cryo-protection system mounted on a goniometer

The nitrogen flow at -170 º C (coming through the upper tube) cools the crystal mounted on the goniometer head. The collimator of the X-ray beam points toward the crystal from the left of the image. Note the slight steam generated by the cold nitrogen when mixed with air humidity.



Visually analyzing the quality of the diffraction pattern

In summary, all of these methodologies can be used to obtain a data collection, consisting of three Miller indices and an intensity for each diffracted beam, which is to say, ***the largest number of reciprocal points of the reciprocal lattice***.

This implies **evaluating both the geometry and the intensities of the whole diffraction pattern**.

All these data, crystal unit cell dimensions, crystal symmetry (space group) and intensities associated with the reciprocal points (diffraction pattern), will allow us to "see" the internal structure of the crystal, but this issue will be shown in another chapter...

1.7: Structural Resolution

In the context of this chapter, you will also be invited to visit these sections...

- **Crystallization methods for proteins**
- **Crystal symmetry and diffraction symmetry**
- **Conditions for systematic absences**
- **The Patterson function and the Patterson method**
- **The anomalous dispersion**

In previous chapters, we have seen how X-rays interact with periodically structured matter (crystals), and the implicit question that we have raised from these earlier chapters is:

Can we "see" the internal structure of crystals?, or in other words,

Can we "see" the atoms and molecules that build crystals?

The answer is definitely yes!

Left: *Molecular structure of a pneumococcal surface enzyme*

Center: *Molecular packing in the crystal of a simple organic compound, showing its crystallographic unit cell*

Right: *Geometric details showing several molecular interactions in a fragment of the molecular structure of a protein*

As the examples above demonstrate, crystallography can show us the structures of very large and complicated molecular structures (left figure) and how molecules pack together in a crystal structure (center figure). We can also see every geometric detail, as well as the different types of interactions, among molecules or parts of them (right figure).

However, for a better understanding of the fundamentals on which this response is based, it is necessary to introduce some new concepts or refresh some of the previously seen ones...

In previous chapters we have seen that crystals represent the organized and ordered matter, consisting of associations of atoms and/or molecules, corresponding to a natural state of it with a minimum of energy.

We also know that crystals can be described by repeating units in the three directions of space, and that this space is known as ***direct*** or ***real space***. These repeating units are know as ***unit cells*** (which also serve as a reference system to describe the atomic positions). This ***direct*** or ***real space***, the same in which we live, can be described by the electron density, ***ρ(xyz)***, a function defined in each point of the unit cell of coordinates ***(xyz)***, where, in addition, operate ***symmetry elements*** which repeat atoms and molecules within the cell.

Unit cell (left) whose three-dimensional stacking builds a crystal (right)

Motifs (atoms, ions or molecules) do repeat themselves by symmetry operators inside the unit cell.

Unit cells are stacked in three dimensions, following the rules of the lattice, building the crystal.

We have also learned that X-rays interact with the electrons of the atoms in the crystals, resulting in a ***diffraction pattern***, also know as ***reciprocal space***, with the properties of a lattice (***reciprocal lattice***) with a ***certain symmetry***, and where we also can define a repeating cell (***reciprocal cell***). The "points" of this reciprocal lattice contain the information on the diffraction intensity.

Left: *Interaction between two waves scattered by electrons. The resulting waves show areas of darkness (destructive interference), depending on the angle considered. Image originally taken from* ***physics-animations.com***.

Right: *One of the hundreds of diffraction images of a protein crystal. The black spots on the image are the result of the cooperative scattering (diffraction) from the electrons of all atoms contained in the crystal.*

Through this cooperative scattering (diffraction), scattered waves interact with each other, producing a single diffracted beam in each direction of space, so that, depending on the ***phase differences*** (advance or delay) among the individual scattered waves, they add or subtract, as shown in the two figures below:

Interference of two waves with the same amplitude and frequency (animation taken from ***The Pennsylvania State University****)*

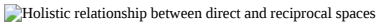
Composition of two scattered waves. ***A*** = resultant amplitude; I = *resultant intensity* ($\sim A^2$)

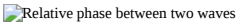
(a) totally in phase (the total effect is the sum of both waves)

(b) with a certain difference of phase (they add, but not totally)

(c) out of phase (the resultant amplitude is zero)

Between the two mentioned spaces (***direct*** and ***reciprocal***) there is a **holistic relationship** (every detail of one of the spaces affects the whole of the other, and vice versa). Mathematically speaking this relationship is a ***Fourier transform*** that cannot directly be solved, since the diffraction experiment does not allow us to know one of the fundamental magnitudes of the equation, the ***relative phases*** (Φ) of the diffraction beams.





Left: *Holistic relationship between direct space (left) and reciprocal space (right). Every detail of the direct space (left) depends on the total information contained in the reciprocal space (right), and vice versa... Every detail of the reciprocal space (right) depends on the total information contained in the direct space (left).*

Right: *Graphical representation of the out-of-phase between two waves. Relative phase between waves*

The diagram below, with the help of the following paragraph, summarizes what the resolution of a crystalline structure through X-ray diffraction implies ...

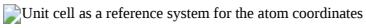
Atoms, ions, and molecules are packed into units (elemental cells) that are stacked in three dimensions to form a crystal in space that we call direct or real space. The diffraction effects of the crystal can be represented as points of a lattice mathematical space that we call the reciprocal lattice. The diffraction intensities, that is, the blackening of these points of the reciprocal lattice, represent the moduli of some fundamental vector quantities, which we call structure factors. If we get to know not only the moduli of these vectors (the intensities), but their relative orientations (that is, their relative phases), we will be able to obtain the value of the electron density function at each point of the elementary cell, providing thus the positions of the atoms that make up the crystal.

Outline on basic crystallographic concepts: direct and reciprocal spaces. The issue is to obtain information on the left side (direct space) from the diffraction experiment (reciprocal space).

ELECTRON DENSITY

In order to know (or to see) the internal structure of a crystal we have to solve a mathematical function known as the "electron density;" a function that is defined at every point in the **unit cell (a basic concept of the crystal structure introduced in another chapter)**.

The function of electron density, represented by the letter ρ, has to be solved at each point within the unit cell given by the coordinates (x, y, z), referred to the unit cell axes. At those points where this function takes maximum values (estimated in terms of electrons per cubic Angstrom) is where atoms are located. That means that if we are able to calculate this function, we will "see" the atomic structure of the crystal.



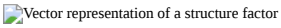
Formula 1. *Function defining the electron density in a point of the unit cell given by the coordinates* ***(x, y, z)***

- *F(hkl) represents the resultant diffracted beams of all atoms contained in the unit cell in a given direction. These magnitudes (actually waves), one for each diffracted beam, are known as structure factors. Their moduli are directly related to the diffracted intensities.*
- *h, k, l are the Miller indices of the diffracted beams (the reciprocal points) and Φ(hkl) represent the phases of the structure factors. V represents the volume of the unit cell. The function has limitations due to the extent to which the diffraction pattern is observed. The number of observed structure factors is finite, and therefore the synthesis will only be approximate and may show some truncation effects.*

Left: *Appearance of a zone of the electron density map of a protein crystal, before it is interpreted.*

Right: *The same electron density map after its interpretation in terms of a peptidic fragment.*

The equation above (***Formula 1***) represents the ***Fourier transform*** between the ***real or direct space*** (where the atoms are, represented by the function ***ρ***) and the ***reciprocal space*** (the X-ray pattern) represented by the ***structure factor amplitudes*** and their ***phases***. Formula 1 also shows the holistic character of diffraction, because in order to calculate the value of the electron density in a ***single point*** of coordinates ***(xyz)*** it is necessary to use ***the contributions of all structure factors*** produced by the crystal diffraction.



The structure factors ***F(hkl)*** *are waves and therefore can be represented as vectors by their amplitudes,* ***[F(hkl)]****, and phases* ***Φ(hkl)*** *measured on a common origin of phases.*

When the unit cell is centrosymmetric, for each atom at coordinates ***(xyz)*** there is an identical one located at ***(-x,-y,-z)***. This implies that **Friedel's law** holds ***F(h,k,l) = F(-h,-k,-l)*** and the expression of the electron density (***Formula 1***) is simplified, becoming ***Formula 1.1***. And the phases of the structure factors are also simplified, becoming 0° or 180°...

Formula 1.1. Electron density function in a point of coordinates (x, y, z) in a centrosymmetric unit cell.

It is important to realize that the quantity and quality of information provided by the electron density function, ***ρ***, is very dependent on the quantity and quality of the data used in the formula: the structure factors ***F(hkl)*** (amplitudes and phases!). We will see later on that the amplitudes of the structure factors are directly obtained from the **diffraction experiment**.

If your browser is Java enabled, as a practical exercise on Fourier transforms we recommend visiting he following links:

- **the applet by Steffen Weber**...
- or, even better, the **Java applet kindly provided by Nicholas Schöni y Gervais Chapui** (École Polytechnique Fédérale de Lausanne, Switzerland), that you can download (free of any virus) from the link shown and execute in your own computer. This applet calculates the Fourier transform of a two dimensional density function ρ(x) yielding the complex magnitude G(S), the reciprocal space. The applet is also able to calculate the inverse Fourier transform of G(S). The density function can be either periodic or non-periodic. Numerous tools including drawing tools can be applied in order to understand the role of amplitudes and phases which are of particular importance in diffraction phenomena. As an illustration, **the Patterson function** of a periodic structure can be simulated.

The analytic expression of the **structure factors**, ***F(hkl)***, is simple and involves a new magnitude (f_j) called atomic scattering factor (**defined in a previous chapter**) which takes into account the different scattering powers with which the electrons of the ***j*** atoms scatter the X-rays:

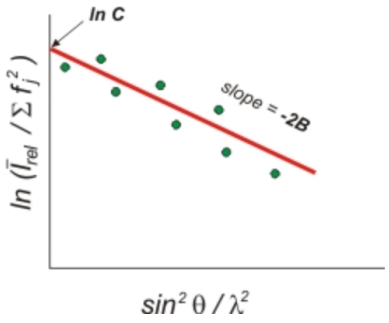
Formula 2. Structure factor for each diffracted beam. This equation is the Fourier transform of the electron density (**Formula 1**).

The expression takes into account the scattering factors ***f*** of all **j** atoms contained in the crystal unit cell.

From the experimental point of view, it is relatively simple to measure the amplitudes ***[F(hkl)]*** of all diffracted waves produced by a crystal. We just need an X-ray source, a single crystal of the material to be studied and an **appropriate detector**. With these conditions fulfilled we can then measure the intensities, ***I(hkl)***, of the diffracted beams in terms of:

Formula 3. *Relationship between the amplitude of the structure factors |F(hkl)| and their intensities I(hkl)*

K is a factor that puts the experimental structure factors, (F_{rel}) , measured on a relative scale (which depends on the power of the X-ray source, crystal size, etc.) into an absolute scale, which is to say, the scale of the calculated (theoretical) structure factors (if we could know them from the real structure, Formula 2 above). As the structure is unknown at this stage, this factor can be roughly evaluated using the experimental data by means of the so-called Wilson plot.



Wilson plot

I_{rel} represents the average intensity (in a relative scale) collected in a given interval of **θ** (the Bragg angle); f_j are the atomic scattering factors in that angular range, and **λ** is the X-ray wavelength.

By plotting the magnitudes shown in the left figure (green dots), a straight line is obtained from which the following information can be derived:

- The value of the y-axis intercept is the Naperian logarithm of ***C***, a magnitude related to the scale factor ***K*** (= *1 / √**C***), described above.
- The slope is equivalent to ***-2B***, where ***B*** is the isotropic overall atomic thermal vibration factor.

A is an absorption factor, which can be estimated from the dimensions and composition of the crystal.

L is known as the Lorentz factor, responsible for correcting the different angular velocities with which the reciprocal points cross the surface of Ewald's sphere. For four-circle goniometers this factor can be calculated as ***1/sin 2θ***, where ***θ*** is the Bragg angle of the reflections.

p is the polarization factor, which corrects the polarization effect of the of the incident beam, and is given by the expression $(1+\cos^2 2\theta)/2$, where ***θ*** also represents the Bragg angle of the reflections (the reciprocal points).

THE PHASE PROBLEM

However, in order to calculate the ***electron density*** (***ρ(xyz)*** in ***Formula 1***, above), and therefore to know the atomic positions inside the unit cell, we also need to know ***the phases*** of the different diffracted beams (***Φ(hkl)*** in Formula 1 above). But, unfortunately, ***this valuable information is lost during the diffraction experiment*** (there is no experimental technique available to measure the phases!) Thus, we must face the so-called ***phase problem*** if we want to solve Formula 1.

The phase problem can be very easily understood if we compare the diffraction experiment (as a procedure to see the internal structure of crystals) with a conventional optical microscope...

*Illustration on the **phase problem**. Comparison between an optical microscope and the "impossible" X-ray microscope. There are no optical lenses able to combine diffracted X-rays to produce a zoomed image of the crystal contents (atoms and molecules).*

In a ***conventional optical microscope*** the visible light illuminates the sample and the scattered beams can be recombined (with intensity and phase) using a system of lenses, leading to an enlarged image of the sample under observation.

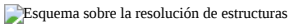
In what we might call the ***impossible X-ray microscope*** (the process of viewing inside the crystals to locate the atomic positions), the visible light is replaced by X-rays (with wavelengths close to 1 Angstrom) and the sample (the crystal) also scatters this "light" (the X-rays). However, we do not have any system of lenses that could play the role of the optical lenses, to recombine the diffracted waves providing us with a direct "picture" of the internal structure of the crystal. The X-ray diffraction experiment just gives us **a picture of the reciprocal lattice of the crystal on a photographic plate or detector**. The only thing we can do at this stage is to measure the positions and intensities of the spots collected on the detector. These intensities are proportional to the structure factor amplitudes, ***[F(hkl)]***.

But regarding the phases, ***Φ(hkl)***, nothing can be concluded for the moment, preventing us from obtaining a direct solution of the electron density function (Formula 1 above).

We therefore need some alternatives in order to retrieve the phase values, lost during the diffraction experiment...

STRUCTURAL RESolution

Once the ***phase problem*** is known and understood, let's now see the general steps (see the scheme below) that a crystallographer must face in order to ***solve the structure*** of a crystal and therefore locate the positions of atoms, ions or molecules contained in the unit cell...



General diagram illustrating the process of resolution of molecular and crystal structures by X-ray diffraction The process consists of different steps that have been treated previously or are described below:

- *Getting a crystal suitable for the experiment, with adequate quality and size. Something related will be seen in **another section**.*
- *Obtaining the diffraction pattern with the appropriate wavelength. This has been described in **another chapter**.*
- *Evaluating the diffraction pattern to get the **lattice parameters** (unit cell), **symmetry** (space group) and **diffraction intensities**.*
- *Solving the electron density function, obtaining any information about the phases of the diffracted beams. This is a key point for the structural resolution that will be discussed below.*
- *Building an initial structural model to explain the values of the electron density function and completing the model locating the remaining atomic positions. This will be seen below.*
- *Refining the model, adjusting all atomic positions to get the calculated diffraction pattern as similar as possible to the experimental diffraction pattern, and finally validate and show the total structural model obtained. This will be seen in **another chapter**.*

For the study to be successful, some important aspects must be taken into account, such as:

- The compound under study must be pure to be crystallized (if not already, as in the case of natural minerals).
- Crystals can be obtained using different techniques, from the most simple evaporation or slow cooling method up to the more complex: vapor (or solvent) diffusion, sublimation, convection, etc. There is enough literature available.. See, for example, the pages of the **LEC, Laboratory of Crystallographic Studies**, for additional information on specific crystallization techniques. For proteins, the procedure most extensively used is based on vapor diffusion experiments, usually with the "hanging drop" technique, **described elsewhere in these pages**. In this sense it is very relevant to note the recent advances introduced in the field of femtosecond X-ray protein nanocrystallography, which will mean a giant step to practically eliminate most difficulties in the crystallization process, and in particular for proteins (**see the small paragraph dedicated to the X-ray free electron laser**).
- If appropriate crystals are obtained, they are exposed to X-rays and their diffraction intensities measured using the methods and equipment **described in a previous chapter**. A careful data evaluation will provide us with the **dimensions of the unit cell**, the **symmetry** and, directly from the intensities, the amplitudes of the structure factors ***[F(hkl)]*** . Of all these subjects at this stage, the most difficult one concerns the determination of the **crystal symmetry**, a key question for the successful resolution of the structure. To obtain crystal symmetry, a visual study of the crystal would make no sense and therefore it must be deduced from the symmetry of the diffraction pattern, **as indicated in a specific section of these pages**.
- At this stage, the question about the unknown phases, ***Φ(hkl)***, arises, so that they must be somehow evaluated, as we will see below...

- If the evaluated phases are correct, the electron density function ***ρ(xyz)*** will show a distribution of maxima (atomic positions) consistent and meaningful from the stereochemical point of view. Once an initial structure is known, some additional steps (construction of the detailed model, mathematical refinement and validation) must be carried out. This will lead us to the so-called final model of the structure.

But let us come back to the most important issue: ***how do we solve the phase problem?***

THE PATTERSON FUNCTION

The very ***first solution to the phase problem*** was introduced by **Arthur Lindo Patterson (1902-1966)**.

Basing his work on the inability to directly solve the electron density function (Formula 1 above or below), and after his training (under the U.S. mathematician **Norbert Wiener**) on Fourier transforms convolution, Patterson introduced a new function ***P(uvw)*** (***Formula 4***, below) in 1934. This formula, which defines a new space (***the Patterson space***), can be considered as the most important single development in crystal-structure analysis since the discovery of **X-rays by Röntgen in 1895** or **X-ray diffraction by Laue in 1914**.

His elegant formula, known as the ***Patterson function*** (***Formula 4***, below), introduces a simplification of the information contained in the electron density function. The Patterson function removes the term containing the phases, and the amplitudes of the structure factors are replaced by their squares. It is thus a function that can be calculated immediately from the available experimental data (intensities, which are related to the amplitudes of the structure factors). Formally, from the mathematical point of view, the Patterson function is equivalent to the **convolution** of the electron density (***Formula 1***, below) with its inverse: $\rho(x,y,z) * \rho(-x,-y,-z)$.

Formula 1. *The electron density function calculated at the point of coordinates* (x,y,z).

Formula 4. *The Patterson function calculated at the point* (u, v, w). *This is a simplification of Formula 1, since the summation is done on* $F^2(hkl)$ *and all phases are assumed to be zero.*

It seems obvious that after omitting the crucial information contained in the phases [***Φ(hkl)*** in ***Formula 1***], the Patterson function will no longer show the direct positions of the atoms in the unit cell, as the electron density function would do.In fact, the Patterson function only provides a ***map of interatomic vectors*** (relative atomic positions), the height of its maxima being proportional to the number of electrons of the atoms implied. We will see that this feature means an advantage in detecting the positions of "heavy" atoms (with many electrons) in structures where the remaining atoms have lower atomic numbers. Once the Patterson map is calculated, it has to be correctly interpreted (at least partially) to get the absolute positions ***(x,y,z)*** of the heavy atoms within the unit cell. These atomic positions can now be used to obtain the phases ***Φ(hkl)*** of the diffracted beams by inverting Formula 1 and therefore this will allow the calculation of the electron density function ***ρ(xyz)***, but **this will be the object of another section of these pages**.

THE DIRECT METHODS

The phase problem for crystals formed by ***small and medium size molecules*** was solved satisfactorily by several authors throughout the twentieth century with special mention to **Jerome Karle** (1918-2013) and **Herbert A. Hauptmann** (1917-2011), who shared the Nobel Prize in Chemistry in 1985 (without forgetting the role of **Isabella Karle**, 1921-2017). The methodology introduced by these authors, known as ***the direct methods***, generally exploit constraints or statistical correlations between the phases of different Fourier components.

Left: ***Herbert A. Hauptman (1917-2011)***

Center: ***Jerome Karle (1918-2013)***

Right: ***Isabella Karle (1921-2017)***

The atomicity of molecules, and the fact that the electron density function should be zero or positive at any point of the unit cell, creates certain limitations in the distribution of phases associated with the structure factors. In this context, the ***direct methods*** establish systems of equations that use the intensities of diffracted beams to describe these limitations. The resolution of these systems of equations provides ***direct information on the distribution of phases***. However, since the validity of each of these equations is established in terms of probability, it is necessary to have a large number of equations to overdetermine the phase values of the unknowns (phases ***Φ(hkl)***).

The direct methods use equations that relate the phase of a reflection (*hkl*) with the phases of other neighbor reflections (*h',k',l'* y *h-h',k-k',l-l'*), assuming that these relationships are "***probably true***" (***P***) ...

where E_{hkl}, $E_{h'k'l'}$ and $E_{h-h',k-k',l-l'}$ are the so called "normalized structure factors", that is, structure factors corrected for thermal motion, brought to an absolute scale and assuming that structures are made of point atoms. In other words, structure factor normalization converts measured $|F|$ values into "point atoms at rest" coefficients known as $|E|$ values.

At present, direct methods are the preferred ones for phasing structure factors produced by small or medium sized molecules having up to 100 atoms in the asymmetric unit. However, they are generally not feasible by themselves for larger molecules such as proteins. The interested reader should look into an **excellent introduction to direct methods through this link** offered by the International Union of Crystallography.

METHODS OF STRUCTURAL RESolution FOR MACROMOLECULES

For crystals composed of ***large molecules***, such as proteins and enzymes, the ***phase problem*** can be solved successfully with three main methods, depending of the case:

(i) introducing atoms in the structure with high scattering power. This methodology, known as ***MIR*** (Multiple Isomorphous Replacement) is therefore based on the Patterson method.

(ii) introducing atoms that scatter X-rays anomalously, also known as ***MAD*** (Multi-wavelength Anomalous Diffraction), and

(iii) by means of the method known as ***MR*** (Molecular Replacement), which uses the previously known structure of a similar protein.

MIR (**M**ultiple **I**somorphous **R**eplacement)

This technique, based on the **Patterson method**, was introduced by **David Harker**, but was successfully applied for the first time by **Max F. Perutz** and **John C. Kendrew** who received the Nobel Prize in Chemistry in 1962, for solving the very first structure of a protein, hemoglobin.

Left: ***David Harker (1906-1991)***

Center: ***Max Ferdinand Perutz (1914-2002)***

Right: ***John Cowdery Kendrew (1917-1997)***

The *MIR* method is applied after introducing "heavy" atoms (large scatterers) in the crystal structure. However, the difficulty of this methodology lies in the fact that the heavy atoms should not affect the crystal formation or unit cell dimensions in comparison to its native form, hence, they should be isomorphic

This method is conducted by soaking the crystal of the sample to be analyzed with a heavy atom solution or by co-crystallization with the heavy atom, in the hope that the heavy atoms go through the channels of the crystal structure and remain linked to amino acid side chains with the ability to coordinate metal atoms (eg SH groups of cysteine). In the case of metalloproteins, one can replace their endogenous metals by heavier ones (for instance Zn by Hg, Ca by Sm, etc.).

Heavy atoms (with a large number of electrons) show a higher scattering power than the normal atoms of a protein (C, H, N, O and S), and therefore they appreciably change the intensities of the diffraction pattern when compared with the native protein. These differences in intensity between the two spectra (heavy and native structures) are used to calculate a ***map of interatomic vectors***

between the heavy atom positions (**Patterson map**), from which it is relatively easy to determine their coordinates within the unit cell.

*Scheme of a **Patterson function** derived from a crystal containing three atoms in the unit cell. To obtain this function graphically from a known crystal structure (left figure) all possible interatomic vectors are plotted (center figure). These vectors are then moved parallel to themselves to the origin of the Patterson unit cell (right figure). The calculated function will show maximum values at the end of these vectors, whose heights are proportional to the product of the atomic numbers of the involved atoms. The positions at these maxima (with coordinates **u**, **v**, **w**) represent the differences between the coordinates of each pair of atoms in the crystal, ie* $u=x_1-x_2$, $v=y_1-y_2$, $w=z_1-z_2$.

With the known positions of the heavy atoms, the structure factors are now calculated using Formula 2 (see also the diagram below), that is their amplitudes $|F_c(hkl)|$ and phases $\Phi_c(hkl)$, where the **c** subscript means "calculated". By using Formula 1, an electron density map, $\rho(xyz)$, is now calculated using the amplitudes of the structure factors observed in the experiment, $|F_o(hkl)|$ (containing the contribution of the whole structure) combined with the calculated phases $\Phi_c(hkl)$. If these phases are good enough, the calculated electron density map will show not only the known heavy atoms, but will also yield additional information on further atomic positions (see diagram below).

In summary, the *MIR* methodology steps are:

- Prepare one or several heavy atom derivatives that must be isomorphic with the native protein. A first test of isomorphism is done in terms of the unit cell parameters.
- Collect diffraction data from both native and heavy atom derivative(s).
- Apply the Patterson method to get the heavy atom positions.
- Refine these atomic positions and calculate the phases for all diffracted beams.
- Obtain an electron density map with those calculated phases.

MAD (**M**ulti-wavelength **A**nomalous **D**iffraction)

The changes in the intensity of the diffraction data produced by introducing heavy atoms in the protein crystals can be regarded as a chemical modification of the diffraction experiment. Similarly, we can cause changes in the intensity of diffraction by modifying the physical properties of atoms. Thus, if the incident X-ray radiation has a frequency close to the natural vibration frequency of the electrons in a given atom, the atom behaves as an "anomalous scatterer". This produces some changes in the atomic scattering factor, f_j (see Formula 2), so that its expression is modified by two terms, f' and f'' which account for its real and imaginary components, respectively. For atoms which behave anomalously, its scattering factor is given by the expression shown below (Formula 5).

Formula 5. *In the presence of anomalous scattering, the atomic scattering factor, f_0, has to be modified adding two new terms, a real and an imaginary part.*

The advanced reader should also **read the section about the phenomenon of anomalous dispersion**.

The f' and f'' corrections *vs.* X-ray energy (see below for the case of Cu Kα) can be calculated taking into account some theoretical considerations...

*Real and imaginary components of the **Selenium** scattering factor vs. the energy of the incident X-rays. The vertical line indicates the wavelength for **CuKα**.*

For X-ray energy values where resonance exists, f' increases dramatically, while the value of f'' decreases. This has practical importance considering that many heavy atoms used in crystallography show absorption peaks at energies (wavelengths) which can be easily obtained with synchrotron radiation. Diffraction data collected in these conditions will show a normal component, mainly due to the light atoms (nitrogen, carbon and hydrogen), and an anomalous part produced by the heavy atoms, which will produce a global change in the phase of each reflection. All this leads to an intensity change between those reflections known as Friedel pairs

(pairs of reflections which under normal conditions should have the same amplitudes and identical phases, but with opposite signs). The detectable change in intensity between these reflection pairs (Friedel pairs) is what we call anomalous diffraction.

The *MAD* method, developed by Hendrickson and Kahn, involves diffraction data measurement of the protein crystal (containing a strong anomalous scatterer) using X-ray radiations with different energies (wavelengths): one that maximizes f'', another which minimizes f' and a third measurement at an energy value distinct from these two. Combining these diffractions data sets, and specifically analyzing the differences between them, it is possible to calculate the distribution of amplitudes and phases generated by the anomalous scatterers. The subsequent use of the phases generated by these anomalous scatterers, as a first approximation, can be used to calculate an electron density map for the whole protein.

In general, there is no current need to introduce individual atoms as anomalous scatterers in protein crystals. It is relatively easy to obtain recombinant proteins in which methionine residues are replaced by selenium-methionine. Selenium (and even sulfur) atoms of methionine (or cysteine), behave as suitable anomalous scatterers for carrying out a *MAD* experiment.

The *MAD* method presents some advantages *vs*. the *MIR* technique:

- As the *MAD* technique uses data collected from a single crystal, the problems derived from lack of isomorphism, common in the *MIR* method, do not apply.
- While in the absence of anomalous dispersion, the atomic scattering factor (f_0) decreases dramatically with the angle of dispersion, its anomalous component ($f' + if''$) is independent of that angle, so that this relative signal increases at a higher resolution of the spectrum, which is to say, at high Bragg angles. Thus, the estimates of phases by *MAD* are generally better at high resolution. On the contrary, with the *MIR* method, the lack of isomorphism is larger at high resolution angles and therefore the high resolution intensities (> 3.5 Angstrom) are not suitable for phasing.

*Argand diagram showing the scattering contribution from an anomalous scatterer in a matrix of normal scatterers. This effect implies that Friedel's law fails. Image taken from "**Crystallography 101**".*

- Fp represents the contribution from the normal scatterers to the **structure factor** (of indices *hkl*).
- Fa and Fa"represent the real ($f_0 + f'$) and imaginary (f'') parts, respectively, of the scattering factor from the anomalous scatterers.
- -Fp, -Fa and -Fa" represent the same as Fp, Fa and Fa", but for the reflection with indices -h, -k, -l.

The anomalous behavior of the atomic scattering factor only produces small differences between the intensities (and therefore among the amplitudes of the structure factors) of the reflections that are related by a centre of symmetry or a mirror plane (such as for instance, ***I(h,k,l)*** *vs*. ***I(-h,-k,-l)***, or ***I(h,k,l)*** *vs*. ***I(h,-k,l)***. Therefore, to estimate these small differences between the experimental intensities, additional precautions must be taken into account. Thus, it is recommended that reflections expected to show these differences are collected on the same diffraction image, or alternatively, after each collected image, rotate the crystal 180 degrees and collect a new image. Moreover, since changes in f' and f'' occur by minimum X-ray energy variations, it is necessary to have good control of the energy values (wavelengths). Therefore, it is essential to use a synchrotron radiation facility, where wavelengths can be tuned easily.

The advanced reader should also **have a look into the web pages on anomalous scattering**, prepared by Bernhard Rupp, as well as the **practical summary** prepared by Georg M. Sheldrick.

MR (**M**olecular **R**eplacement)

If we know the structural model of a protein with a homologous amino acid sequence, the phase problem can be solved by using the methodology known as molecular replacement (MR). The known structure of the homologous protein is regarded as the protein to be determined and serves as a first model to be subsequently refined. This procedure is obviously based on the observation that proteins with similar peptide sequences show a very similar folding. The problem in this case is transferring the molecular structure of the known protein from its own crystal structure to a new crystal packing of the protein with an unknown structure. The positioning of the known molecule into the unit cell of the unknown protein requires determining its correct orientation and position within the unit cell. Both operations, rotation and translation, are calculated using the so-called rotation and translation functions (see below).

Scheme of the molecular replacement (MR) method.

*The molecule with known structure (**A**) is rotated through the **[R]** operation and shifted through **T** to bring it over the position of the unknown molecule (**A'**).*

The rotation function. If we consider the case of two identical molecules, oriented in a different way, then the **Patterson function** will contain three sets of vectors. The first one will contain the Patterson vectors of one of the molecules, ie all interatomic vectors within molecule one (also called eigenvectors). The second set will contain the same vectors but for the second molecule, identical to the first one, but rotated due to their different orientation. The third set of vectors will be the interatomic cross vectors between the two molecules. While the eigenvectors are confined to the volume occupied by the molecule, the cross vectors will extend beyond this limit. If both molecules (known and unknown) are very similar in structure, the rotation function $R(\alpha,\beta,\gamma)$ would try to bring the Patterson vectors of one of the molecules to be coincident with those of the other, until they are in good agreement. This methodology was first described by **Rossman and Blow**.

$$R(\alpha,\beta,\gamma) = \int_u P_1(u) \times P_2(u_r)\, du$$

Formula 6. *Rotation function*

P_1 is the Patterson function and P_2 is the rotated Patterson function, where **u** is the volume of the Patterson map, where interatomic vectors are calculated.

The quality of the solutions of these functions is expressed by the correlation coefficient between both Patterson functions: the experimental one and the calculated one (with the known protein). A high correlation coefficient between these functions is equivalent to a good agreement between the experimental diffraction pattern and the diffraction pattern calculated with the known protein structure. Once the known protein structure is properly oriented and translated (within the unit cell of the unknown protein), an electron density map is calculated using these atomic positions and the experimental structure factors. It is worth consulting the **article published on this methodology by Eleanor Dodson**.

Probably it is valuable for the advanced reader to **consult a nice article** that, despite having been published in 2010, has not lost its validity in relation to the description of the different methodologies for the determination of the relative phases of the diffraction beams.

COMPLETING THE STRUCTURE

All these methods (Patterson, direct methods, *MIR*, *MAD*, *MR*) provide (directly or indirectly) knowledge about approximate phases which must be upgraded. As indicated above, the calculated initial phases, $\Phi_c(hkl)$, together with the observed experimental amplitudes, $|F_o(hkl)|$, allow us to calculate an electron density map, also approximate, over which we can build the structural model. The overall process is summarized in the cyclic diagram shown below.

The initial phases, $\Phi_c(hkl)$, are combined with the amplitudes of the experimental (observed) structure factors, $|F_o(hkl)|$, and an electron density map is calculated (shown at the bottom of the scheme). Alternatively, if the initial known data are the coordinates (xyz) of some atoms, they will provide the initial phases (shown at the top of the scheme), and so on in a cyclic way until the process does not produce any new information.

*Scheme showing a cyclic process to calculate electron density maps **ρ(xyz)** which produce further structural information.*

From several known atomic positions we can always calculate the structure factors: their amplitudes, |Fc(hkl)|, and their phases, Φc(hkl),as shown at the top of the scheme. Obviously, the calculated amplitudes can be rejected, because they are calculated from a partial structure and the experimental ones represent the whole and real structure. Therefore, the electron density map (shown at the bottom of the scheme) is calculated with the experimental (or observed) amplitudes, |Fo(hkl)|, and the calculated phases, Φc(hkl). This function is now evaluated in terms of possible new atomic positions that are added to the previously known ones, and the cycle repeated. Historically this process was known as "successive Fourier syntheses", because the electron density is calculated in terms of a Fourier sum.

In any case, from atomic positions or directly from phases, if the information is correct, the function of electron density will be interpretable and will contain additional information (new atomic coordinates) that can be injected into the cyclic procedure shown above until structure completion, which is to say until the calculated function $\rho(xyz)$ shows no changes from the last calculation.

The lighter atoms of the structure (those with lower atomic number, ie, usually hydrogen atoms) are the most difficult ones to find on an electron density map. Their scattering power is almost obscured by the scattering of the remaining atoms . For this reason, the location of H atoms is normally done via a somewhat modified electron density function (the ***difference electron density***), whose coefficients are the differences between the observed and calculated structure factors of the model known so far:

Formula 7. *Function of "difference" electron density*

In practice, if the structural model obtained is good enough, if the experiment provided precise structure factors, and there are no specific errors such as X-ray absorption, the difference map $\Delta\rho$ will contain enough signal (maxima) where H atoms can be located. Additionally, to get an enhanced signal from the light atoms scattering, this function is usually calculated with the structure factors appearing at lower diffraction angles only, usually with those appearing at $\sin\theta / \lambda < 0.4$, that is, using the region where the scattering factors for hydrogens are still "visible".

1.8: The Structural Model

The analysis and interpretation of the electron density function, ie the resolution of a crystal structure (molecular or non-molecular) leads to an initial distribution of atomic positions within the unit cell which can be represented by points or small spheres:

Left: Initial model of the 3-dimensional structure of a molecule. Atoms, also labelled, are represented by small spheres. Center: Initial model of the 3-dimensional structure of a molecule. Despite the increased complexity or beauty of the model, the level of information is basically the same as in the model on the left. Right: Initial model of the 3-dimensional structure of a molecule, including its crystal structure, that is, the molecular packing within the unit cell.

Once the structural model is completed, having stereochemical sense and including its crystal packing, it is necessary to make use of all the information we can extract from the experimental data, since the diffraction pattern generally contains much more data (intensities) than needed to locate the atoms at their 3-dimensional coordinates. For instance, for a medium sized structure, with 50 independent atoms in the asymmetric unit (in the structural unit which is repeated by the symmetry operations), the diffraction pattern usually contains around 2500 **structure factors**, which implies approximately 50 observations per atom (each atom needs 3 coordinates). However, for more complex structures, as in the case of macromolecules, the amount of experimental data available normally does not reach these limits.

REFINING THE FINAL MODEL

The basic parameters associated with a three-dimensional structure are, obviously, the three positional coordinates (***x, y, z***) for each atom, given in terms of unit cell fractions. But, in general, given the experimental overdetermination mentioned above, the atomic model can become more complex. For instance, associating each atom with an additional parameter reflecting its thermal vibrational state, in a first approach as an isotropic (spherical) thermal vibration around its position of equilibrium. This new parameter is normally shown in terms of different radius of the sphere representing the atom. Thus an isotropic structural model would be represented by 4 variables per atom: 3 positional + 1 thermal.

However, for small and medium-sized structures (up to several hundred of atoms), the diffraction experiment usually contains enough data to complete the thermal vibration model, associating a tensor (6 variables) to each atom which expresses the state of vibration in an anisotropic manner, ie distinguishing between different directions of vibration in the form of an ellipsoid (which resembles the shape of a baseball). Therefore, a crystallographic anisotropic model will require 9 variables per atom (3 positional + 6 vibrational).

Left: *Three bonded atoms represented with the isotropic thermal vibration model* Right: *The same three atoms shown on the left, but represented using the anisotropic thermal vibration model*

Left: *Anisotropic model of the 3-dimensional structure of a molecule, showing some atoms from neighboring molecules.*

Right: *Anisotropic model of the 3-dimensional structure of a molecule showing its crystal packing.*

Regardless of the model type, isotropic or anisotropic, the above-mentioned overabundance of experimental data allows a description of the structural model in terms of very precise atomic parameters (positional and vibrational) which lead to very precise geometrical parameters of the whole structure (interatomic distances, bond angles, etc.).

This refined model is obtained by the analytical method of least-squares. Using this technique, atoms are allowed to "move" slightly from their previous positions and thermal factors are applied to each atom so that the diffraction pattern calculated with this model is essentially the same as the experimental one (observed), ie minimizing the differences between the calculated and observed structure factors. This process is carried out by minimizing the function:

$$\sum w||F_o| - |F_c||^2 \rightarrow 0 \qquad (1.8.1)$$

Least-squares function used to refine the final model of a crystal structure

where w represents a "weight" factor assigned to each observation (intensity), weighting the effects of the less-precise observations vs. the more accurate ones and avoiding possible systematic errors in the experimental observations which could bias the model. **Fo** and **Fc** are de observed and calculated structure factors, respectively.

Although usually the mentioned experimental overdetermination ensures the success of this analytical process of refinement, it must always be controlled through the stereochemical aspects, ie, ensuring that the positional movements of the atoms are reasonable and which therefore generate distances within the expected values. Similarly, the thermal vibration factors (isotropic or anisotropic) associated with the atoms must always show reasonable values.

In addition to the aforementioned control of the model changes during the refinement process, it seems obvious that (if everything goes well), additionally the diffraction pattern calculated (**F_c**) with the refined model (coordinates + thermal vibration factors) will show increasing similarity to the observed pattern **(F_o)**. The comparison between both patterns (observed vs. calculated) is done via the so-called R parameter, which defines the "disagreement" factor between the two patterns:

$$R = \frac{\sum[||F_o| - |F_c||]}{|F_o|} \tag{1.8.2}$$

Disagreement factor of a structural model, calculated in terms of differences between observed and calculated structure factors with the final model

The value of the disagreement factor (**R**) is estimated as a percentage (%), ie, multiplied by 100, so that "well" solved structures, with an appropriate degree of precision, will show an **R** factor below 0.10 (10%), which implies that the calculated pattern differs from the observed one (experimental) less than 10%.

The diffraction patterns of macromolecules (enzymes, proteins, etc.) usually do not show such large overdetermination of experimental data and therefore it is difficult to reach an anisotropic final model. Moreover, in these cases the values of the **R** factor are greater than those for small and medium-sized molecules, so that values around or below 20% are usually acceptable. In addition, as a result of this relative scarcity of experimental data, the analytical procedure of refinement (least-squares) must be combined with an interactive stereochemical modeling process and by imposing certain "soft restraints" to the molecular geometry.

MODEL VALIDATION

The reliability of a structural model has to be assessed in terms of several tests, a procedure known as ***model validation***. Thus, the structural model should be continuously checked and validated using consistent stereochemical criteria (for example, bond lengths and bond angles must be acceptable). For instance a C---O distance of 0.8 Angstrom would not be acceptable for a carbonyl group (C = O). Similarly, the bond angles must also be consistent with an acceptable geometry. These criteria are very restrictive for small or medium-sized structures, but even in the structures of macromolecules they must meet some minimum criteria.

Maximum dispersion values generally accepted for interatomic distances and bond angles in the structural model of a macromolecule

In the case of proteins, the peptide bond (the bond between two consecutive amino acids) must also satisfy some geometrical restrictions. The torsional angles of this bond should not deviate much from the acceptable values of the usual conformations shown by the amino acid chains, as is shown in the so-called Ramachandran plot:

Left: *Schematic representation of the peptide bond, showing the two torsional angles (Ψ and Φ) defining it.*

Right: *Ramachandran plot showing the different allowed (acceptable) areas for the torsional angles of the peptide bonds in a macromolecule. The different areas depend on the different structural arrangements (α-helices, β-sheets, etc.)*

Similarly, the values of the thermal factors associated with each atom should show physically acceptable values. These parameters account for the thermal vibrational mobility of the different structural parts. Thus, in the structure of a macromolecule, these values should be consistent with the internal or external location of the chain, being generally lower for the internal parts, and higher for external parts near the solvent.

DEGREE OF RELIABILITY OF THE MODEL

A model that has been "validated" according to the criteria described above, that is, which demonstrates:

- a reasonable agreement between observed and calculated structure factors,
- bond distances, bond angles and torsional angles that meet stereochemical criteria, and
- physically reasonable thermal vibration factors,

is a reliable model. However, the concept of reliability is not a quantitative parameter which can be written in terms of a single number. Therefore, to interpret a structural model up to its logical consequences one has to bear in mind that it is just a simplified representation, extracted from an electron density function:

$$\rho(xyz) = \frac{1}{V}\sum_{hkl}^{+\infty} |F(hkl)| \cdot e^{-2\pi i[hx+ky+lz-\phi(hkl)]} \qquad (1.8.3)$$

on which the atoms have been positioned and which is being affected by some conditions **described in another section, which we invite you to read**.

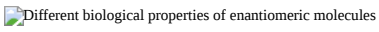
But, in any case, well-done crystallographic work always provides atomic parameters (positional and vibrational) along with their associated precision estimates. This means that any direct crystallographic parameter (atomic coordinates and vibration factors) or derived (distances, angles, etc.) is usually expressed by a number followed by its standard deviation (in parentheses) affecting the last figure. For example, an interatomic distance expressed as 1.541 (2) Angstroms means a distance of 1.541 and a standard deviation of 0.002.

THE ABSOLUTE CONFIGURATION (OR ABSOLUTE STEREOCHEMISTRY)

As **stated in a previous chapter**, all molecules or structures in which neither mirror planes nor centres of symmetry are present, have an **absolute configuration**, that is, that they are different from their mirror images (they cannot be superimposed).

Structural models showing two enantiomers of a compound (the two molecules are mirror images)

These particular structural differences, very important as far as the molecular properties are concerned, can be unambiguously determined through the diffraction experiment (without using any external standard). This can be carried out using the so-called anomalous scattering effect which atoms show when appropriate X-ray wavelengths are used. This feature is also very succesfully used as a **method to solve the phase problem for macromolecular crystals**. It doesn't seem difficult to understand that the molecular enantiomers have different properties, as in the end they are different molecules, but regarding their biological activity (if any) the situation is particularly striking.


Different biological properties of enantiomeric molecules

Enantiomeric molecules that are represented in the left figure were introduced in the market by a pharmaceutical company and, obviously, they showed different properties.

The properties of DARVON (Dextropropoxyphene Napsylate) **are available through this link**, while production of NOVRAD (Levopropoxyphene Napsylate) was discontinued.

The experimental diffraction signal that allows this structural differentiation is a consequence of the fact that the **atomic scattering factor** does not behave as a real number when the **frequency of X-rays is similar to the natural frequency of the atomic absorption**. See also the **chapter dedicated to anomalous dispersion**.

Under these conditions, **Friedel's Law** is no longer fulfilled and therefore structure factors such as $|\mathbf{F}_{h,k,l}|$ and $|\mathbf{F}_{-h,-k,-l}|$ will be slightly different. These differences are evaluated in terms of the so-called **Bijvoet** estimators, which compare the ratios for observed structure factors for such reflection pairs with the corresponding ratios for the calculated structure factors using the two possible absolute models. Only one of these two comparisons will maintain the same type of bias:

$$\frac{|F(hkl)|_o}{|F(\bar{h}\bar{k}\bar{l})|_o} \text{ vs. } \frac{|F(hkl)|_c}{|F(\bar{h}\bar{k}\bar{l})|_c} \qquad (1.8.4)$$


Johannes Martin Bijvoet

Comparison of Bijvoet ratios - **Johannes Martin Bijvoet (1892-1980)**

Thus, if the quotient between the observed structure factors is <1, the same quotient for the calculated structure factors should also be <1. Or, on the contrary, both quotients should be >1. If this is true for a large number of reflection pairs it will indicate that the absolute model is the right one. If it is not so, the structural model has to be inverted.

The interested reader should also **have a look into the web pages on anomalous scattering**, prepared by Ethan A. Merritt.

THE FINAL RESULT

The information describing a final crystallographic model is composed of:

- Data from the diffraction experiment: wavelength and diffraction pattern (the intensity of thousands or even hundreds of thousands of diffracted waves with their ***hkl*** indices),
- Unit cell dimensions as derived from the diffraction pattern (from the reciprocal cell),
- The symmetry present in the crystal, derived from the reciprocal lattice (from the diffraction pattern), and
- Atomic positions (coordinates and thermal vibration factors) and, if needed, the so-called population factor, as indicated in the table below.

The atomic positions are usually given as fractional coordinates (fractions of the unit cell axes), but sometimes, especially for macromolecules where the information usually refers to the isolated molecule, they are given as absolute coordinates, ie, expressed in Angstrom and referred to a system of orthogonal axes independent of the crystallographic ones (see below).

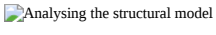
Information about several atoms of a protein structure using the so-called PDB format (Protein Data Bank), ie atomic coordinates in Angstrom on a system of orthogonal axes, different from the crystallographic ones. For clarity, the estimated standard deviations have been omitted.

The population factor is the fraction of atom located in a specific position, although this factor is usually 1. The meaning of this parameter requires an explanation for the beginner, since it could be understood that atoms could be divided in parts, which obviously has no physical meaning. Due to atomic vibrations, and to the fact that the diffraction experiment has a duration in time, it is possible that in some of the unit cells atoms are missing. Thus, instead of a complete occupancy (population factor = 1), the corresponding site, in an average unit cell, will contain only a fraction of the atom. In these cases it is said that the crystal lattice has defects and population factors smaller than 1 reflect a fraction of unit cells where a specific atomic position is occupied. Obviously, a fraction of unit cells where the same position is empty complements the population factor to unity. Therefore, the crystallographic model reflects the average structure of all unit cells during the experiment time.

The atomic coordinates and in general all information collected from a crystallographic study, is stored in accessible databases. There are different databases, depending of the type of compound or molecule, but this will be **discussed in another chapter of these pages**.

GRAPHICAL REPRESENTATIONS OF THE MODEL

The final structural model (atomic coordinates, thermal factors and, possibly, population factors) directly provide additional information which leads to a detailed knowledge of the structure itself, including bond lengths, bond angles, torsional angles, molecular planes, dipole momentum, etc., and any other structural detail that might be useful for understanding the functionality and/or properties of the material under study.


Analysing the structural model

In the case of complex biological molecules, the use of high-quality graphic processors and relatively simple models, greatly facilitates the understanding of the relationship between structure and function, as shown in the figure on the left.

At present the available computational and graphic techniques allow us to obtain beautiful and very descriptive models which help to visualize and understand structures, as is shown in the examples below:

Left: *Model of balls and sticks to represent the structure of a simple inorganic compound.* Right: *Representation of an inorganic compound, in which a partial polyhedral representation has been added*

Left: *Animated model of sitcks to represent the packing and molecular structure of a simple organic compound.* Right: *Given the complexity of biological molecules, the models which represent them are usually simple, showing the overall folding and the different structural motifs (α-helices, β-strands, loops, etc.) shown with the ribbon model. The example also shows a stick representation of a cofactor linked to the enzyme.*

Left: *Combined model of ribbons and sticks to represent the dimmeric structure of a protein which also shows a sulfate ion in the middle--represented with balls* Right: *Representation of the surface of a biological molecule where the colours represent different properties of hydrophobia. The arrow represents the dipolar momentum of the molecule.*

Finally, using additional information from other techniques (such as cryo-electron microscopy), or combining two different crystal conformations of a molecule, other models are available as shown below. Moreover, using the ultrashort exposure times of X-rays produced by free electron lasers (**European XFEL**), crystallographers are able to collect diffraction data of macromolecules in different conformations, that is, during the course of performing their respective tasks. In this manner, using a huge number of X-ray snapshots we can produce like a film where we are able to follow the molecular modifications and therefore to understand their function.

Left: *Combined model of the molecular structure of a protein and an envelope (as obtained by high-resolution electron microscopy) showing a pore formed by the association of four protein molecules* Right: *Simplified animated model showing the backbone folding of an enzyme and the structural changes between two molecular states: active (open) and inactive (closed). The structures of both states were determined by crystallography*

1.9: Crystallographic computing

Readers who have arrived at this chapter in a sequential manner will notice that, apart from the **phase problem**, the relationship between the diffraction pattern (reciprocal space) and the crystal structure (direct space) is mediated by a **Fourier transform** represented by the electron density function: $\rho(xyz)$ (see the drawing on the left).

Readers will also know that the relationship between these two spaces is "holistic", meaning that the value of this function, at each point in the unit cell of coordinates (xyz), is the result of "adding" the contribution of "all" structure factors [ie diffracted waves in terms of their amplitudes $|F(hkl)|$ and phases $\Phi(hkl)$] contained in the diffraction pattern. They will also remember that the diffraction pattern contains many structural factors (several thousand for a simple structure, and hundreds of thousands for a protein structure).

The "jump" between direct and reciprocal spaces, mediated by a Fourier transform represented by the electron density function

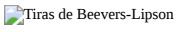
Moreover, the number of points in the unit cell, where the ***ρ*** function has to be calculated, is very high. In a cell of about 100 x 100 x 100 Angstrom^3, it would be necessary to calculate at least 1000 points in every unit cell direction to obtain a resolution of 100/1000, which equals 0.1 Angstrom in each direction. This means calculating at least 1000 x 1000 x 1000 = 1,000,000,000 points (one billion points) and at each point to "add" several thousand (or hundreds of thousands) structure factors ***F(hkl)***.

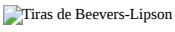
It should therefore be clear that, regardless of the difficulties of the phase problem, solving a crystal structure implies the use of computers.

Finally, the analysis of a crystal or molecular structure also implies calculating many geometric parameters that define interatomic distances, bond angles, torsional angles, molecular surfaces, etc., using the atomic coordinates ***(xyz)***.

The "hardware"

For the reasons described above, since the beginning of the use of Crystallography as a discipline to determine molecular and crystal structures, crystallographers have devoted special attention to the development of calculation tools to facilitate crystallographic work. With this aim, and even before the early computers appeared, the crystallographers introduced the so-called "Beevers-Lipson strips," which were widely used in all Crystallography laboratories.





The Beevers-Lipson strips

The Beevers-Lipson strips (which were strips of paper containing the values for some trigonometric functions) were used in laboratories to speed up the calculations (by hand) of the Fourier transforms (see above: the electron density function, for example).

These strips were introduced in 1936 by A.H. Beevers and H. Lipson. In the 1960s, more than 300 boxes were distributed to nearly all the laboratories in the world. You can also have a look into the **description made by the International Union of Crystallography**. The nightmare was maintaining upright this box, which had a very narrow base, otherwise it was impossible to maintain the strips correctly stored!

As expected, the introduction of early computers (or electro-mechanic calculators) inspired great hope in crystallographers...

ENIAC (Electronic Numerical Integrator and Computer, 1945) -- the very first electronic computer. Some pictures of the rooms where it was installed.

ENIAC, short for Electronic Numerical Integrator And Computer, was the first general-purpose electronic computer, whose design and construction were financed by the United States Army during the Second World War. It was the first digital computer capable of being reprogrammed to solve a full range of computing problems, especially calculating artillery firing tables for the U.S. Army's Ballistic Research Laboratory.

The ENIAC had immediate importance. When it was announced in 1946, it was heralded in the press as a "Giant Brain". It boasted speeds one thousand times faster than electro-mechanical machines, a leap in computing power that no single machine has matched. This mathematical power, coupled with general-purpose programmability, excited scientists and industrialists.

Besides its speed, the most remarkable thing about ENIAC was its size and complexity. ENIAC had 17,468 vacuum tubes, 7,200 crystal diodes, 1,500 relays, 70,000 resistors, 10,000 capacitors and around 5 million hand-soldered joints. It weighed 27 tons, was roughly 2.6 m by 0.9 m by 26 m, took up 63 m^2, and consumed 150 kW of power.

Later, with the development of Electronics and Microelectronics, which introduced integrated circuits, computers became accessible to crystallographers, who flocked to these facilities with large boxes of "punched cards" (the only means for data storage at that time), containing the diffraction intensities and their own computer programs.

A punch card or punched card (or punchcard or Hollerith card or IBM card), is a piece of stiff paper which contains digital information represented by the presence or absence of holes in predefined positions. It was used by crystallographers until the end of the 1970s.

Punched paper tape (shown in yellow) and different magnetic tapes (as well as some small disks) used for data storage during the 1970s and 1980s.

Around the early 1970s, and for over a decade, crystallographers became a nightmare for the managers and operators of the so-called "computing centers," running in some universities and research centers.

In the 1980s the laboratories of Crystallography became "flooded" with computers, which for the first time gave crystallographers independence from the large computing centers. The VAX series of computers (sold by the company Digital Equipment Corporation) marked a splendid era for crystallographic calculations. They allowed the use of magnetic tapes and the first hard disk drives, with limited capacity (only a few hundred MB) -- very big and heavy, but they eliminated the need for the tedious punched cards. **Nostalgics should have a look into this link**.!!!

A typical computer (of the VAX series) used in many Crystallography laboratories during the 1980s.

Over the years, crystallographic computing has become easy and affordable thanks to personal computers (PC), which meet nearly all the needs of most conventional crystallographic calculations, at least concerning crystals of low and medium complexity (up to hundreds of atoms). Their relative low price and their ability to be assembled into "farms" (for distributed calculation) provide crystallographers the best solution for almost any type of calculation.

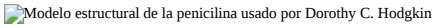
Left: *A typical personal computer (PC) used in the 2000s*

Right: *A typical PC-farm used in the 2000s*

However, the crystallography applied to macromolecules not only needs what we could call "hard" computing. The management of large electron density maps, which are used to build the molecular structure of proteins, as well as the subsequent structural analysis, requires more sophisticated computers with powerful graphic processors and, if possible, with the capability of displaying 3-dimensional images using specialized glasses...

A Silicon Graphics computer used to visualize 3-dimensional electron density maps and structures. The processor and the screen are complemented by an infrared transmitter (black box on the screen) and the glasses used by the crystallographer.

The current computing facilities represent a big jump respect to the capabilities available during the mid-twentieth century, as it is shown in the representation of the structural model used for the structural description of penicillin, based on three 2-dimensional electron density maps... And even 3d maps where also used!...



Left: *Three-dimensional model of the structure of penicillin, based on the use of three 2-dimensional electron density maps, as used by **Dorothy C. Hodgkin**, Nobel laureate in 1964*

Right: *Representation of 3d electron density maps used until the middle of the 1970's. The contours are lines of electron density and show the positions of individual atoms in the structure*

A typical personal computer commonly used since 2010 for crystallographic calculations and also for their graphic capabilities

The software

At present there are enough personal, institutional or commercial computer program developments, or even computing facilities through remote servers, to fulfill nearly all of the needs for crystallographic computing, as well as many sources from which one can download most of those programs. In this context, it could be useful to check the following links:

Crystallographic computer programs

- Macromolecules: **The Web-Book of the Department of Crystallography & Structural Biology (CSIC)**
- Of general interest: **The crystallographic software list maintained by the International Union of Crystallography** - (**IUCr**)

Specifically for compounds of small and medium size (molecular or not) we recommend using the **Wingx package which can be freely downloaded** by courtesy of Louis J. Farrugia, (University of Glasgow, UK). It is easy to install on a PC and contains an interface which includes the most important programs for small and medium size crystallographic problems. Also, for these types of compounds there is a very useful computer program (Mercury), user-friendly and free, which includes powerful graphics and some other analytical tools to analyze crystal structures. It can be **downloaded from the Cambridge Crystallographic Data Centre, UK**.

Protein crystallographers need more specific programs, and in this context we recommended using the link offered by **CCP4, Collaborative Computational Project No. 4, Software for Macromolecular X-Ray Crystallography**.

On the other hand, crystallographic work is currently unimaginable without having access to crystallographic databases, which contain all the structural information that is being published and which have a clear added value for the researcher. The type of structure is what determines its inclusion in any of the existing databases. Thus, metals and intermetallic compounds are made available in the database **CRYSTMET**; inorganic compounds are centralized in the **ICSD** database (Inorganic Crystal Structure Database); organic and organometallic in **CSD** (*Cambridge Crystallographic Database*); and proteins in PDB (*Protein Data Bank*), which is a databank (not a database). Other databases, databanks, etc., do not necessarily contain structural information in the most precise sense, but they can also be very helpful for crystallographers. And this is the case of **WebCite** published by the ***Cambridge Crystallographic Data Centre*** (*CCDC*), containing over 2000 articles with very important information for structural chemistry research in its broadest sense, and in particular to pharmaceutical drug discovery, materials design or drug development, among others.

Structural databases and databanks

- **CRYSTMET**: Metals and intermetallic compounds (license required)
- **ICSD**: Inorganic compounds (license required)
- **CSD**: Organic and organometallic compounds (license required)
- **glycoSCIENCES.de**: Carbohydrates
- **LipidBank**: Lipids
- **PDB**: Proteins, Nucleic acids and large complexes

- **NDB**: Nucleic acids

As indicated, some of these databases (or databanks) are public (**glycoSCIENCES.de**, **LipidBank**, **PDB** and **NDB**), and therefore can be searched online. However, others (**CRYSTMET**, **ICSD** and **CSD**) require a license or even a local installation.

During the period 1990-2012, **CRYSTMET**, **ICSD** and **CSD** have been licensed free of charge to all **CSIC** research institutes (**CRYSTMET** and **ICSD**) and to all academic institutions in Spain and Latin American countries (**CSD**). However, due to economic constraints, the **CSIC**'s authorities decided to reduce drastically this program that was managed through the **Department of Crystallography and Structural Biology** (at the **Institute of Physical Chemistry "Rocasolano"**). Nowadays this program is maintained in a reduced manner, only for Spanish institutions, **as it can be seen through this link**.

1.10: Biographical outlines

In the context of this chapter, you will also be invited to visit these sections...

- **Nobel Laureates through Crystallography**
- **Ewald Prize Laureates by the IUCr**

As mentioned in the **introduction**, Crystallography is one of the scientific disciplines that has most clearly influenced the development of Chemistry, Biology, Biochemistry and Biomedicine. Although in other pages we made some reference to the scientists directly involved at the early stages, this chapter is aimed at presenting short biographical outlines.

As a supplement of the biographical notes presented in this chapter, the reader can also consult the **early historical notes about crystals and Crystallography offered in another section**.

The biographical outlines object of the present chapter (shown below) have been distributed in groups, in chronological order, using the terminology of some musical sections and tempos, trying to describe their relevance, at least from a historical perspective.

- **Prelude (1901)**
- **Overture (1914)**
- **Allegro ma non tropo (1915)**
- **Allegro molto (1934-1935)**
- **Andante (1940-1960)**
- **Finale (1970-1980...)**

1901"Prelude", by Wilhelm Conrad Röntgen

Wilhelm Conrad Röntgen (1845-1923). None of this would have been possible without the contribution of Wilhelm Conrad Röntge***n,*** who won the first Nobel Prize in Physics (1901) for his discovery of X-rays.

Although many other biographical personal references to Röntgen **can be found on the internet**, we **recommend visiting the site prepared by Jose L. Fresquet** (in Spanish). In the following paragraphs we summarize the most relevant details and add a few others.

Wilhelm Conrad Röntgen was born on March 27, 1845, at Lennep in the Lower Rhine Province of Germany, as the only child of a manufacturer and merchant of cloth. His mother was Charlotte Constanze Frowein of Amsterdam, a member of an old Lennep family which had settled in Amsterdam. When he was 3 years old his family moved to Holland. From 16 to 20 years old he studied at the *Technical School in Utrecht*, and he then moved to Zurich where he got the corresponding academic degree in mechanical engineering.

After some years in Zurich, as assistant professor of physics under August Kundt, in 1872 (27 years old), he moved to the University of Würzburg. However, as he couldn't find any job (he previously couldn't pass his exams in Latin and Greek) he moved to Strasbourg where he finally got a position as professor in 1874. Five years later he accepted a teaching position at the *University of Giessen* and finally at 45 years old, he obtained a professorship in physics at Würzburg, where he became Rector.

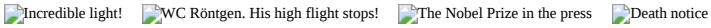
His work on cathode rays led him to the discovery of a new and different kind of rays. On the evening of November 8, 1895, working with an enclosed and sealed discharge tube (to exclude all light), he found that a paper plate (covered on one side with barium platinocyanide and placed accidentally in the path of the rays) became unexpectedly fluorescent, even when it was as far as two metres from the discharge tube.

It took a month until Röntgen understood the importance of this new radiation and he immediately sent a scientific communication to the *Society for Physics and Medicine* in Würzburg...Specifically, the first sentences of his official statement (written in a nice German language) read:

Lässt man durch eine Hittorf'sche Vacuumröhre, oder einen genügend evacuirten Lenard'schen, Crookes'schen oder ähnlichen Apparat die Entladungen eines grösseren Ruhmkorff's gehen und bedeckt die Röhre mit einem ziemlich eng anliegenden Mantel aus dünnem, schwarzem Carton, so sieht man in dem vollständig verdunkelten Zimmer einen in die Nähe des Apparates gebrachten, mit Bariumplatincyanür angestrichenen Papierschirm bei jeder Entladung hell aufleuchten, fluoresciren, gleichgültig

ob die angestrichene oder die andere Seite des Schirmes dem Entladungsapparat zugewendet ist. Die Fluorescenz ist noch in 2 m Entfernung vom Apparat bemerkbar. Man überzeugt sich leicht, dass die Ursache der Fluorescenz vom Entladungsapparat und von keiner anderen Stelle der Leitung ausgeht.

*After producing an electrical discharge with a **Ruhmkorff's coil** through a **Hittorf**'s vacuum tube, or a sufficiently evacuated **Lenard**, **Crookes** or similar apparatus, covered with a fairly tight-fitting jacket made of thin, black paperboard, one sees that a cardboard sheet coated with a layer of platinum and barium cyanide, located in the vicinity of the apparatus, lights up brightly in the completely darkened room regardless of whether the coated side is pointing or not to the tube. This fluorescence occurs up to 2 meters away from the apparatus. One can easily be convinced that the cause of the fluorescence proceeds from the discharge apparatus and not from any other source of the line.*



Röntgen's discovery quickly produced a social commotion... "Incredible light!". However, almost at the same speed, his public celebrity dropped to a minimum... "his high-flying stopped...". It was during the first months of 1896, after sending to the *British Medical Journal* an X-ray photograph of a broken arm, that Röntgen began to regain the public's confidence, demonstrating the diagnostic capacity of his discovery. However, it took still many years until his "incredible light" was recognized as of medical interest. It was awarded the first **Nobel Prize for Physics in 1901**. Wilhelm Conrad Röntgen died in Munich on 10 February 1923 from carcinoma of the intestine. It is not believed his carcinoma was a result of his work with ionizing radiation because of the brief time spent on those investigations and because he was one of the few pioneers in the field who used protective lead shields routinely.

If you can read Spanish, there is also an extensive chapter dedicated to both the **historical details around Röntgen and his discovery.**

1914 ***"Overture", by Max von Laue, with accompaniment by Paul P. Ewald***

Max von Laue (1879-1960). If Röntgen's discovery was important for the development of Crystallography, the second qualitative step forward was due to another German, Max von Laue, Nobel Prize for Physics in 1914, who trying to demonstrate the undulatory nature of X-rays, discovered the phenomenon of X-ray diffraction by crystals. A complete **biographical description can also be found through this link**.

Max von Laue was born on October 9, 1879 at Pfaffendorf, in a little town near Koblenz. He was the son of Julius von Laue, an official in the German military administration, who was raised to hereditary nobility in 1913 and who was often sent to various towns, so that von Laue spent his youth in Brandenburg, Altona, Posen, Berlin and Strassburg, going to school in the three last-named cities. At the Protestant school at Strassburg he came under the influence of Professor Goering, who introduced him to the exact sciences, where he studied Mathematics, Physics and Chemistry. However, he soon moved to the *University of Göttingen* and in 1902 to the *University of Berlin*, where he began working with Max Planck. A year later, after obtaining his doctorate degree, he returned to Gottingen, and in 1905 he went back to Berlin as assistant to **Max Planck, who also won the Nobel Prize for Physics in 1918**, ie four years after von Laue. Between 1909 and 1919 he went through the Universities of Munich, Zurich, Frankfurt and Würzburg, and he finally returned to Berlin where he earned a position as a professor.

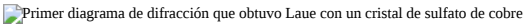
Paul Peter Ewald (1888-1985). It was during this last period, namely in 1912, when he met Paul Peter Ewald in Munich. Ewald was then finishing his doctoral thesis under **Arnold Sommerfeld (1868-1951)**, and he got Laue interested in his experiments on the interference between radiations with large wavelengths (practically visible light) on a "crystalline" model based on resonators. Note that at that time the question on wave-particle duality was also under discussion.

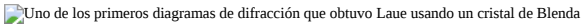
The idea then came to Laue that the much shorter electromagnetic rays, which X-rays were supposed to be, would cause some kind of diffraction or interference phenomena in a medium and that a crystal could provide this medium. An excellent historical description of these facts and the corresponding experiments, conducted by Walter Friedrich and Paul Knipping under the direction of Max von Laue, can be found in an **article by Michael Eckert**. The original article of that experiment, signed by Friedrich, W.,

Knipping, P. and Laue, M., was published with the reference: **Sitzungsberichte der Kgl. Bayer. Akad. der Wiss. (1912) 303–322**, although it was later collected by **Annalen der Physik (1913) 346, 971-988**.

It's amazing how quickly Ewald developed the interpretation of Max von Laue experiments, as it can be seen in **his original article, published in 1913 (in German), available through this link**. Recognizing the role played by Ewald for the development of Crystallography, **the International Union of Crystallography grants the Prize and Medal that carry the name of Paul Peter Ewald**.

And so was it that using a crystal of copper sulfate and some others from zinc blende, in front of an X-ray beam, how Laue got the confirmation on the undulatory nature of the rays discovered by Röntgen (see images below). For this discovery, and **its interpretation**, Max von Laue received the **Nobel Prize for Physics in 1914**. But at the same time, his experiment created many questions on the nature of crystals...





Left: *First X-ray diffraction pattern obtained by Laue and his collaborators using a crystal of copper sulphate*

Right: *One of the first X-ray diffraction patterns obtained by Laue and his collaborators using some crystals of the mineral Blende*

Laue was always opposed to National Socialism, and after the Second World War he was brought to England for a short time with several other German scientists contributing to the **International Union of Crystallography**. He returned to Germany in 1946 as director of the *Max Planck Institute* and professor at the *University of Göttingen*. He retired in 1958 as director of the *Institute of Physical Chemistry Fritz Haber* in Berlin, a position to which he had been elected in 1951. On 8 April, 1960, while driving to his laboratory, Laue's car was struck by a motorcyclist in Berlin, The cyclist, who had received his license only two days earlier, was killed and Laue's car flipped. Max von Laue (80 years old) died from his injuries sixteen days later on April 24.

1915"Allegro, ma non troppo", by Bragg *(father & son)*

Izquierda: **William Henry Bragg (1862-1942)**

Derecha: **William Lawrence Bragg (1890-1971)**

This time it did not happen as with Röntgen. Max von Laue's discovery became immediately known, at least by the British **William Henry Bragg (1862-1942)** and his son **William Lawrence Bragg (1890-1971)**, who in 1915 shared the Nobel Prize for Physics for demonstrating the usefulness of the phenomenon discovered by von Laue (X-ray diffraction) in studying the internal structure of crystals. They showed that X-rays diffraction can be described as specular reflection by a set of parallel planes through all lattice elements in such a way that a diffracted beam is obtained if:

$$2.d.sin\,\theta = n.\lambda$$

where d is the distance between the planes, θ is the angle of incidence, n is an integer and λ is the wavelength. Through this simple approach the determination of crystal structures was made possible.

William Henry Bragg studied Mathematics at the *Trinity College* in Cambridge and subsequently Physics at the Cavendish Laboratory. At the end of 1885, he was appointed professor at the *University of Adelaide* (Australia), where his son (William Lawrence Bragg) was born. W. Henry Bragg became successively Cavendish Professor of Physics at Leeds (1909-1915), Quain Professor of Physics at the University College London (1915-1925), and Fullerian Professor of Chemistry in the Royal Institution.

His son, William Lawrence, studied Mathematics at the University of Adelaide. In 1909, the family returned to England and W. Lawrence Bragg entered as a fellow at *Trinity College* in Cambridge. In the autumn of 1912, during the same year that **Max von Laue** made public his experiment, the young W. Lawrence Bragg started examining the phenomenon that occurs when putting a crystal in front of the X-rays, presenting its first results (**The diffraction of short electromagnetic waves by a crystal**) at the headquarters of the Cambridge Philosophical Society during its meeting in November 11th, 1912.

In 1914, W. Lawrence Bragg was appointed Professor of Natural Sciences at *Trinity College*, and that same year he was awarded the Barnard Medal. The two years (1912-1914) he worked with his father on the experiments of refraction and diffraction by crystals led to a lecture of W.H. Bragg (**Bakerian Lecture: X-Rays and Crystal Structure**) and to the famous article **X-rays and Crystal Structure**, also published in 1915. That same year, he (25 years old!) and his father, shared the Nobel Prize in Physics.

Father and son were able to explain the phenomenon of X-ray diffraction in crystals through crystallographic planes acting as special mirrors for X-rays (**Bragg's Law**), and showed that the crystals of substances such as sodium chloride (NaCl or common salt) do not contain molecules of NaCl, but simply ions of Na^+ and Cl^-, both regularly ordered. These ideas revolutionized Theoretical Chemistry and caused the birth of a new science: X-ray Crystallography.

Unfortunately, after the First World War, some difficulties arose between William Lawrence and his father when the general public did not directly credit W. Lawrence with his contributions to their discoveries. Lawrence Bragg desperately wanted to make his own name in research, but he sensed the triumph of their discoveries passing to his father, as the senior man. W. Henry Bragg tried his best to remedy the situation, always pointing out which aspects of their work were his son's ideas; however, much of their work was in the form of joint papers, which made the situation more difficult. Sadly, they never discussed the problem, and the trouble lingered for many years. The close collaboration between father and son ended, but it was natural that their work would continue to overlap. They decided to divide up the available work, and agreed to focus on separate areas of X-ray crystallography. W. Lawrence was to focus on inorganic compounds, metals and silicates, whereas William H. Bragg was to focus on organic compounds.

In 1919, William Lawrence was made Langworthy Professor of Physics at *Victoria University*, Manchester, where he married and remained until 1937. There, in 1929, he published an excellent article on the use of the Fourier series to determine crystal structures, **The Determination of Parameters in Crystal Structures by means of Fourier Series**.

In 1941 father and son were knighted (Sir) and a year later (1942) William Henry died. In subsequent years, William Lawrence was interested in the structure of silicates, metals, and especially in the chemistry of proteins. He was appointed Director of the *National Physical Laboratory* in Teddington and professor of Experimental Physics at the *Cavendish Laboratory* (Cambridge). In 1954, he was appointed Director of the *Royal Institution* in London, establishing his own research group aimed at studying the structure of proteins using X-rays. William Lawrence Bragg died in 1971, aged 81. The **IUCr** published an **obituary that you can reach through this link**.

The year 2012 represents the centennial of the first single crystal X-ray experiments, performed at the Ludwig Maximilian Universität, Munich (Germany), by Paul Knipping and Walter Friedrich under the supervision of Max von Laue, and especiallythe the experiments done by the Braggs. **The interested reader can enjoy reading the chapters published as a reminder by the International Union of Crystallography, to be found through the links shown below.**

34-1935"Allegro molto", *by Arthur Lindo Patterson, and David Harker as soloist*

Arthur Lindo Patterson (1902-1966). It is unexplainable how the name of Arthur LIndo Patterson is slowly fading and entering history almost as a stranger, at least since the last decade of the Twentieth Century. Probably his name remains associated only with some crystallographic calculation subroutine. However, as **mentioned in another chapter**, the contribution of Patterson to Crystallography can be seen as the single most important development after the discovery of **X rays by Röntgen** in 1895.

Arthur Lindo Patherson was born in the early years of the Twentieth Century in New Zealand, but his family soon emigrated to Canada, where he spent his youth. For some unknown reason, he went to school in England before returning to Montreal (Canada) to study Physics at *McGuill University*, where he obtained his master's degree with a thesis on the production of hard X-rays (with small wavelengths) using the interaction of Radio β radiation with solids. He performed his first experiments on X-ray diffraction during a period of two years at the laboratory of **W.H. Bragg** at the *Royal Institution* in London. At that time he was aware that, although in small crystal structures the location of atoms in the unit cell was a relatively simple problem, the situation was virtually unfeasible in the case of molecular compounds, or in general with more complex compounds.

After a stay in the lab of **W.H. Bragg**, Lindo Patterson spent a very productive year in the *Kaiser-Wilhelm Institute* in Berlin, with a grant from the **National Research Council** of Canada to work under **Hermann Mark**. With his work, he contributed decisively to the determination of particle size using X-ray diffraction, and started to become interested in the theory of the Fourier transform, an idea that some years later would become his obsession in connection with the resolution of crystal structures.

In 1927, he returned to Canada and a year later completed his PhD at *McGuill University*. After two years with R.W.G. Wyckoff in the *Rockefeller Institute* in New York, he accepted a position at the *Johnson Foundation for Medical Physics*in Philadelphia which gave him the chance to learn X-ray diffraction applied to biological materials. In 1931 he published two articles on Fourier series as a tool to interpret X-ray diffraction data: **Methods in Crystal Analysis: I. Fourier Series and the Interpretation of X-ray Data** and **Methods in Crystal Analysis: II. The Enhancement Principle and the Fourier Series of Certain Types of Function**.

In 1933, he moved to the *MIT* (*Massachusetts Institute of Technology*) where, through his friendship with the mathematician **Norbert Wiener**, he started learning Fourier theory, and especially the properties of the Fourier transform and convolution. That was how, in 1934, his equation (the **Patterson Function**) was formulated in an article entitled **A Fourier Series Method for the Determination of the Components of Interatomic Distances in Crystals**, opening enormous expectations for the resolution of crystal structures. However, due to the technological precariousness of those days in addressing the large amount of sums involved in his function, it took some years until his discovery became effective in indirectly resolving **the phase problem**.

Patterson's death, in November 1966, resulted from a massive cerebral hemorrhage.

In addition to the technical difficulties existing at that time in solving complex mathematical equations, the function introduced by **Arthur L. Patterson**, clearly presented significant difficulties in the case of complex structures. At least it was so until, in 1935, **David Harker (1906-1991)**, a "trainee", realized the existence of special circumstances that significantly facilitated the interpretation of the **Patterson Function**, and of which **Arthur L. Patterson** had not been aware.

David Harker was born in California, and graduated in 1928 as a chemist at Berkeley. In 1930, he accepted a job as a technician in the laboratory of the Atmospheric Nitrogen Corp. in New York, where, through the reading of articles related to crystal structures, his interest in crystallography increased. Due to the great economic depression in 1933, he lost the job and returned to California. Using some savings, he was able to enter the California Institute of Technology. There, supervised by **Linus Pauling**, he began to experiment with the resolution of some simple crystal structures.

During one of the weekly talks in **Pauling's** lab, the **function recently introduced by Arthur L. Patterson** was described and Harker was immediately aware of the difficulties implied in the many calculations in attaining the Patterson map, but especially the difficulty in interpreting it in structures with many atoms. However, a few nights after the speech, he woke up suddenly and said it has to work!. Indeed, it became clear to Harker that the Patterson map contains regions where the interatomic vectors (between atoms related by symmetry elements) are concentrated. Therefore, in order to look for interatomic vectors, one has only to explore certain areas of the map, and not the entire Patterson unit cell, which simplifies the interpretation qualitatively.

From 1936 until 1941, Harker had a professor position to teach Physical Chemistry at Johns Hopkins University, where he learned classical Crystallography and Mineralogy. During the remaining years of the 1940's, he obtained a research position at the General Electric Company and from there, together with his colleague, John S. Kasper, made another important contribution to Crystallography: **the Harker-Kasper inequalities**, the first contribution to the so-called **direct methods** for solving the **phase problem**.

During the 1950's, Harker accepted the offer of joining the Irwin Langmuir Brooklyn Polytechnic Institute to solve the structure of ribonuclease. This opportunity helped him to **establish the methodology** that, years later (1962), was used by **Max Perutz** and **John Kendrew** to solve the structure of hemoglobin. In 1959, Harker moved his team and project to the Roswell Park Cancer Institute and completed the ribonuclease structure in 1967. He retired officially in 1976, but remained somewhat active at the Medical Foundation of Buffalo (today the Hauptman-Woodward Institute), until his death in 1991 from pneumonia. There is a nice **Harker's obituary** written by William Duax.

1940-1960"Andante", score by John D. Bernal

John Desmond Bernal (1901-1971). Following the findings and developments by Arthur Lindo Patterson and David Harker, interest was directed to the structure of molecules, especially those related to life: proteins. And in this movement an Irishman settled in England, John Desmond Bernal, played a crucial role to the further development of crystallography.

John Desmond Bernal was born in Nenagh, Co. Tipperar, in 1901. The Bernals were originally Sephardic Jews who came to Ireland in 1840 from Spain via Amsterdam and London. They converted to Catholicism and John was Jesuit-educated. John enthusiastically supported the Easter Rising, and, as a boy, organized a Society for Perpetual Adoration. He moved away from religion as an adult, becoming an atheist. Bernal was strongly influenced by the Russian Revolution of 1917 and became a very active member of the Communist Party of Britain.

John graduated in 1919 in Mineralogy and Mathematics (applied to symmetry) at the *University of Cambridge*. In 1923, he obtained a position as assistant in the laboratory of **W.H. Bragg** at the *Royal Institution* in London, and in 1927, he returned as a professor to Cambridge. His fellow students in Cambridge nicknamed him ‘Sage’ because of his great knowledge. From there, he

attracted many young researchers from *Birkbeck College* and *King's College* to the field of macromolecular crystallography. In 1937, he obtained a professor position in London at *Birkbeck College*, from where he trained many crystallographers (Rosalind Franklin, Dorothy Hodgkin, Aaron Klug and Max Perutz, among others). Undoubtedly, John D. Bernal has earned a prominent position in the Science of the Twentieth Century. He showed that, under appropriate conditions, a protein crystal can maintain its crystallinity under exposure to X-rays. Some of his students were able to solve complex structures such as hemoglobin and other biological materials of importance, such that crystallographic analysis started to revolutionize Biology. John Bernal, who died at the age of 70, was also the engine of crystallographic studies on viruses, together with his collaborator, Isadore Fankuchen.

The developments of the **Bragg's**, based on the previous discovery of **Laue** and the work by **Patterson and Harker**, raised the expectations of structural biology. Due to the Second World War, England became an attractive center, especially around **John D. Bernal**.


Max Ferdinand Perutz

Max Ferdinand Perutz (1914-2002) was born in Vienna, on May 19th, 1914, into a family of textile manufacturers. They had made their fortune in the 19th Century by the introduction of mechanical spinning and weaving to the Austrian monarchy. Max was sent to school at the Theresianum, a grammar school derived from an officers' academy at the time of the empress Maria Theresia. His parents suggested that he should study law in preparation for entering the family business. However, a good schoolmaster awakened his interest in chemistry and he entered the *University of Vienna* where he, in his own words, "wasted five semesters in an exacting course of inorganic analysis". His curiosity was aroused, however, by organic chemistry, and especially by a course of organic biochemistry, given by F. von Wessely, in which Sir F.G. Hopkins' work at Cambridge was mentioned. It was here that Perutz decided that Cambridge was the place he wanted to work on his Ph.D. thesis.

With financial help from his father, in September 1936, Perutz became a research student at the *Cavendish Laboratory* in Cambridge under **John D. Bernal**. His relationship with **Lawrence Bragg** was also critical, and in 1937 he conducted the first diffraction experiments with hemoglobin crystals which had been crystallized in *Keilin's Molteno Institute*. Thus, from 1938 until the early fifties, the protein chemistry was done at *Keilin's Molteno Institute* and the X-ray work at the *Cavendish*, with Perutz busily bridging the gap between biology and physics on his bicycle.

After the invasion of Austria by Hitler, the family business was expropriated, his parents became refugees, and his own funds were soon exhausted. Max Perutz was saved by being appointed research assistant to **Lawrence Bragg**, under a grant from the *Rockefeller Foundation*, on January 1st, 1939. The grant continued, with various interruptions due to the war, until 1945, when Perutz was given an *Imperial Chemical Industries Research* Fellowship. In October 1947, he was made head of the newly constituted *Medical Research Council Unit for Molecular Biology*. His collaboration with Sir Lawrence Bragg continued through many years. As a memorial to Perutz you probably may consult this **obituary published in *Nature* on the occasion of his death in 2002** (otherwise you always may **download this obituary written in Spanish**).


John Cowdery Kendrew

John Cowdery Kendrew (1917-1997) was born on 24th March, 1917, in Oxford. He graduated in Chemistry in 1939 from *Trinity College*. He spent the first few months of the war doing research on reaction kinetics in the *Department of Physical Chemistry* at Cambridge under the supervision of E.A. Moelwyn-Hughes. The personal influence of **John D. Bernal** led him to work on the structure of proteins and in 1946 he joined the *Cavendish Laboratory*, working with **Max Perutz** under the direction of **Lawrence Bragg**, where he received his Ph.D. in 1949. Kendrew and Perutz formed the entire staff of the Molecular Biology Unit of the recently established (1947) *Medical Research Council*.

Although the work of Kendrew focused on myoglobin, **Max Ferdinand Perutz** and **John Cowdery Kendrew** received the Nobel Prize in Chemistry in 1962 for their work on the structure of hemoglobin and both were the first to successfully implement the **MIR methodology** introduced by **David Harker**.


Rosalond Frabklin

Rosalind Elsie Franklin (1920-1958). One of the great scientists of those years who also emerged under the direct influence of **John D. Bernal**, was the controversial and unfortunate Rosalind Franklin. There are many texts concerning Rosalind, but perhaps it is worthwhile to read the detailed pages (in Spanish) prepared by Miguel Vicente: **La dama ausente: Rosalind Franklin y la doble hélice** and **Jaque a la dama: Rosalind Franklin en King's College**, both of which do justice to her personality and to her short but fruitful work in the science of the mid-twentieth century.

In the summer of 1938, Rosalind Franklin went to *Newnham College*, Cambridge. She passed her finals in 1941, but was only awarded a titular degree, as women were not entitled to degrees from Cambridge at the time. In 1945, Franklin received her PhD from *Cambridge University*. After the war Franklin accepted an offer to work in Paris at the *Laboratoire de Services Chimiques de L'Etat* with Jacques Mering, where she learned X-ray diffraction techniques on coal and related inorganic materials. In January 1951, Franklin started working as a research associate at *King's College*, London, in the *Medical Research Council*, in the Biophysics Unit, directed by **John Randall**. Although originally she was to have worked on X-ray diffraction of proteins and lipids in solution, Randall redirected her work to DNA fibers before she started working at King's, as Franklin was to be the only experienced experimental diffraction researcher at King's in 1951.

In Randall's laboratory, Rosalind's trajectory crossed with that of **Maurice Wilkins (1916-2004)**, as both were dedicated to DNA research. Unfortunately, unfair competition led to a conflict with Wilkins which finally "took its toll". In Rosalind's absence, Wilkins showed the diffraction diagrams, which Rosalind had taken from DNA fibers, to two young scientists lacking excessive scruples... **James Watson** and **Francis Crick**.

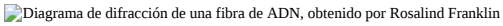
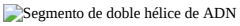


Diagrama de difracción de una fibra de ADN, obtenido por Rosalind Franklin


Segmento de doble hélice de ADN

John Bernal called her DNA X-ray photographs "the most beautiful X-ray photographs of any substance ever taken." Rosalind's DNA diagrams provided the establishment of the double helical structure of DNA. It might be interesting for the reader to **see this short video prepared by "My Favourite Scientist"** (**also available through this link**). Using a laser pen and some bent wire Andrew Marmery from the Royal Institution in London demonstrates the principles of diffraction and **reproduces the characteristic diffraction pattern of the helical structure of DNA** (**use this other link in case of problems**). The interested reader can also access **the original manuscripts prepared by Rosalind Franklin on the structure of DNA**. Rosalind Franklin died very young, at age 37, from ovarian cancer.


Maurice Wilkins

Maurice Wilkins (1916-2004) was born in New Zealand. He graduated as a physicist in 1938 from *St. John's College*, Cambridge, and joined **John Randall** at the *University of Birmingham*. After obtaining his PhD in 1940, he joined the Manhattan Project in California. After World War II, in 1945, he returned to Europe when **John Randall** was organizing the study of biophysics at the *University of St. Andrew* in Scotland. A year later, he obtained a position at *King's College*, London, in the newly created *Medical Research Council*, where he became deputy director in 1950.


James Watson

James Dewey Watson (1928-), born in Chicago, obtained a PhD in Zoology in 1950 at the *University of Indiana*. He spent a year in Copenhagen as a Merck Fellow and during a symposium held in 1951 in Naples, met **Maurice Wilkins**, who awoke his interest in the structure of proteins and nucleic acids. Thanks to the intervention of his director (**Salvador E. Luria**), Watson in the same year got a position to work with **John Kendrew** at the *Cavendish Laboratory*, where he also met **Francis Crick**. After two years at the *California Institute of Technology*, Watson returned to England in 1955 to work one more year in the *Cavendish Laboratory* with Crick. In 1956 he joined the *Department of Biology* at Harvard.


Francis Crick

Francis Crick (1916-2004) was born in England and studied Physics at *London University College*. During the war, he worked for the *British Admiralty* and later went to the laboratory of **W. Cochran** to study biology and the principles of crystallography. In 1949, through a grant from the *Medical Research Council*, he joined the laboratory of **Max Perutz**, where, in 1954, he completed his doctoral thesis. There he met **James Watson**, who later would determine his career. He spent his last years at the *Salk Institute for Biological Studies* in California.

In connection with the unfortunate story of Rosalind Franklin, **Maurice Wilkins**, **James Watson** and **Francis Crick** received the Nobel Prize in Physiology or Medicine in 1962 for the discovery of the right handed double helix structure of DNA. The decisive role of **Rosalind Franklin** was forgotten. it is very instructive to observe the video that **hhmi biointeractive** offers about this discovery.


Dorothy C. Hodgkin

Dorothy C. Hodgkin (1910-1994), was born in Cairo, but she also spent part of her youth in Sudan and Israel, where her father became director of the *British School of Archeology* in Jerusalem. From 1928 to 1932 she settled in Oxford thanks to a grant from *Sommerville College*, where she learned the methods of crystallography and diffraction, and soon was attracted by the character and work of **John D. Bernal**. In 1933, she moved to Cambridge where she spent two happy years, making many friends and exploring a variety of problems with Bernal.

In 1934, she returned to Oxford, from where she never left, except for short periods. In 1946, she obtained a position as Associate Professor for Crystallography and although she was initially linked to Mineralogy, her work soon pointed towards the area which had always interested her and which she had learned under **John D. Bernal**: sterols and other interesting biological molecules. Dorothy Hodgkin took part in the meetings in 1946 which led to the foundation of the **International Union of Crystallography** and she visited many countries for scientific purposes, including China, the USA and the USSR. She was elected a Fellow of the *Royal Society* in 1947, a foreign member of the *Royal Netherlands Academy of Sciences* in 1956, and of the *American Academy of Arts and Sciences* (Boston) in 1958. In 1964 she was awarded the Nobel Prize in Chemistry.

1970-1980..."Finale", with an unfinished melody...

Although what happened in the first 60 years of the Twentieth Century is astonishing and somewhat unique, the "crystallographic melody" continued, and in this sense it is still worthwhile to mention other scientists who made Crystallography go further.

William N. Lipscomb

William Nunn Lipscomb (1919-2011) was born in Cleveland, Ohio, USA, but moved to Kentucky in 1920, and lived in Lexington throughout his university years. After his bachelors degree at the *University of Kentucky*, he entered graduate school at the *California Institute of Technology* in 1941, first in physics. Under the influence of Linus Pauling, he returned to chemistry in early 1942. From then until the end of 1945 he was involved in research and development related to the war. After completing his Ph.D., he joined the *University of Minnesota* in 1946, and moved to *Harvard University*in 1959. Harvard recognitions include the Abbott and James Lawrence Professorship in 1971, and the George Ledlie Prize, also in 1971. In 1976 Lipscomp was awarded the Nobel Prize in Chemistry for his contributions to the structural chemistry of boranes.

This chapter cannot be concluded without mentioning the efforts made by other crystallographers, who during many years tried to solve the **phase problem** with approaches different from those provided by the **Patterson method**, ie, trying to solve the problem directly from the intensities of the diffraction pattern and based on probability equations: **direct methods**.

Herbert A. Hauptman

Herbert A. Hauptman (1917-2011), born in New York, graduated in 1939 as a mathematician from *Columbia University*. His collaboration with **Jerome Karle** began in 1947 at the *Naval Research Laboratory* in Washington DC. He earned his PhD in 1954 from the *University of Maryland*. In 1970, he joined the crystallographers group at the *Medical Foundation* in Buffalo, where he became research director in 1972. Hauptman was the second non-chemist to win a Chemistry Nobel Prize (the first one was the physicist Ernest Rutherford).

Jerome Karle

Jerome Karle (1918-2013), also from New York, studied mathematics, physics, chemistry and biology, obtaining his master's degree in Biology from *Harvard University* in 1938. In 1940, he moved to the *University of Michigan*, where he met and married Isabella Lugosky. He worked on the *Manhattan Project* at the *University of Chicago* and earned a doctoral degree in 1944. Finally, in 1946, he moved to the *Naval Research Laboratory* in Washington DC, where he met **Herbert Hauptman**.

Isabella Karle

The monograph published in 1953 by Hauptman and Karle, *Solution of the Phase Problem I. The Centrosymmetric Crystal*, already contained the most important ideas on probabilistic methods which, applied to the **phase problem**, made them worthy of the Nobel Prize in Chemistry in 1985. However, it would be unfair not to mention the role of Jerome's wife, **Isabella Karle (1921-2017)**, who played an important role, putting the theory into practice.

Karle & Hauptman during the XIII Iberoamerican Meeting of Crystallography, Montevideo, 1994

In memory of these important persons, we show this photograph taken in 1994, during the *XIII Iberomerican Congress of Crystallography* (Montevideo, Uruguay).

Left (front to back): *Jerome Karle, Isabella Karle and Martin Martinez-Ripoll (author of these pages).*

Right (front to back): *Herbert A. Hauptman and* **Ray A. Young** *(neutron expert and one of the pioneers of the Rietveld method)*

Crystallography is (and has been) one of the most inter- and multidisciplinary sciences. It links together frontier areas of research and has, directly or indirectly, produced **the largest number of Nobel Laureates throughout history**.

Additionally, the **International Union of Crystallography (IUCr)** established, since 1986, the existence of the **Ewald Prize awarded every three years for outstanding contributions to the science of Crystallography**.

This chapter is dedicated to the many scientists who have made Crystallography one of the most powerful and competitive branches of Science for looking into the "tiny" world of atoms and molecules. It could definitely have been more extensive and detailed, because we cannot forget the participation and effort of many other scientists, past and present, but the important issue is that, after our "finale", "crystallographic music" plays on ...

The United Nations in its General Assembly A/66/L.51 (issued on 15 June 2012), after considering the relevant role of Crystallography in Science decided to proclaim **2014 International Year of Crystallography**. Click also on the left image!

We send congratulations to Gautam R. Desiraju, President of the **IUCr**, and Sine Larsen, former President of the **IUCr**, when this initiative was launched!

In this context, 11 November 2012 marked the centenary of the presentation of the paper by a young **William Lawrence Bragg (1890-1971)**, where the foundations of X-ray crystallography where outlined. For this reason, the International Union of Crystallography (**IUCr**) published a fascinating set of articles that the reader can find via the following links:

- **Celebrating 100 years of X-ray crystallography (S.W. Wilkins)**
- **A tribute to W.L. Bragg by his younger daughter (Patience Thomson)**
- **Sir Lawrence Bragg (M.F. Perutz)**
- **Background to the Nobel Prize to the Braggs (Anders Liljas)**
- **Lawrence Bragg's interest in the deformation of metals and 1950–1953 in the Cavendish – a worm's-eye view (Anthony Kelly)**
- **Lawrence Bragg, microdiffraction and X-ray lasers (J.C.H. Spence)**
- **The Bragg legacy: early days in macromolecular crystallography (Brian W. Matthews)**
- **The early development of neutron diffraction: science in the wings of the Manhattan Project (T.E. Mason, T.J. Gawne, S.E. Nagler, M.B. Nestor and J.M. Carpenter)**
- **The significance of Bragg's law in electron diffraction and microscopy, and Bragg's second law (C.J. Humphreys)**
- **Evolution of diffraction methods for solving crystal structures (Wayne A. Hendrickson)**
- **Early days in drug discovery by crystallography – personal recollections (Peter M. Colman)**

The first 50 years of X-ray diffraction were commemorated in 1962 by the International Union of Crystallography (**IUCr**) with the publication of an interesting book entitled **Fifty Years of X-Ray Diffraction, edited by Paul Peter Ewald**.

Bart Kahr and Alexander G. Shtukenberg wrote an interesting chapter, **Histories of Crystallography by Shafranovskii and Schuh**, (included in **Recent Advances in Crystallography**, where they offer a short summary of the two volumes on the *History of Crystallography* written by **Ilarion Ilarionovich Shafranovskii** (1907-1994), a Russian crystallographer who assumed the **E.S. Fedorov** (1853-1919) Chair of Crystallography at the Leningrad Mining Institute. The chapter of Kahr and Shtukenberg also include many other references, especially those taken from Curtis P. Schuh, author of at least a remarkable book entitled **Mineralogy & crystallography: an annotated bio-bibliography of books published 1469 through 1919**.

M.A. Cuevas-Diarte and S. Alvarez Reverter.are the authors of an **extensive and commented chronology on crystallography and structural chemistry**, starting in the IV Century BC.

It is noteworthy **the exhibition offered by the University of Illinois** (Vera V. Mainz and Gregory S. Girolami, *Crystallography - Defining the Shape of Our Modern World*, University of Illinois at Urbana-Champaign), commemorating the 100th Anniversary of the Discovery of X-ray Diffraction, as well as a lecture of Prof. Seymour Mauskopf from the Duke University, to be found also directly through these links: **PowerPoint format** or **pdf format**.

It is also very interesting to read the articles collected in the **special issue of Nature (2014)**, dedicated to Crystallography, especially:

- **Crystallography: Atomic secrets**

- **X-ray science: The big guns**
- **Policy: Crystallography needs a governing body**
- **History: Women in crystallography**

among other from the archive **included in the same special issue**. Nearly in the same context, Nature has also released this interesting article, entitled **Structural biology: More than a crystallographer**, about the training currently expected from crystallographers working in the field of structural biology.

Science, the journal, also joined the celebration of the **International Year of Crystallography**, devoting **a special issue that you can find via this link**.

- **Going from Strength to Strength**
- **Cutting-Edge Techniques Used for the Structural Investigation of Single Crystals**
- **Developments in X-ray Crystallographic Structure Determination of Biological Macromolecules**
- **Femtosecond Crystallography with Ultrabright Electrons and X-rays: Capturing Chemistry in Action**
- **Crystallography and Geopolitics**

1.11: Crystallographic Associations

The ollowing table shows links to some scientific associations of crystallographic interest, distributed around the world and alphabetically ordered ...

Look also at this link from the IUCr

- American Association for Crystal Growth **American Association for Crystal Growth**
- American Crystallographic Association **American Crystallographic Association**
- Asian Crystallographic Association **Asian Crystallographic Association**
- Asociación Argentina de Cristalografía **Asociación Argentina de Cristalografía**
- Associacao Brasileira de Cristalografia **Associaçao Brasileira de Cristalografia**
- Association Française de Cristallographie **Association Française de Cristallographie**
- Association Marocaine de Cristallographie **Association Marocaine de Cristallographie**
- Associazione Italiana di Cristallografia **Associazione Italiana di Cristallografia**
- Belgian National Committee for Crystallography **Belgian National Committee for Crystallography**
- British Crystallographic Association **British Crystallographic Association**
- Canadian National Committee for Crystallography **Canadian National Committee for Crystallography**
- Clay Minerals Society **Clay Minerals Society**
- Comité Español de Cristalografía **Comité Español de Cristalografía**
- Croatian Crystallographic Association **Croatian Crystallographic Association**
- Crystallographic Society of Japan **Crystallographic Society of Japan**
- Czech and Slovak Crystallographic Association **Czech and Slovak Crystallographic Association**
- Czechoslovak Association for Crystal Growth **Czechoslovak Association for Crystal Growth**
- Deutsche Gesellschaft für Kristallographie **Deutsche Gesellschaft für Kristallographie**
- Deutsche Gesellschaft für Kristallwachstum und Kristallzüchtung e.V. **Deutsche Gesellschaft für Kristallwachstum und Kristallzüchtung e.V.**
- Deutsche Mineralogische Gesellschaft **Deutsche Mineralogische Gesellschaft**
- Egyptian National Committee of Crystallography **Egyptian National Committee of Crystallography**
- European Crystallographic Association **European Crystallographic Association**
- European High Pressure Research Group **European High Pressure Research Group**
- European Mineralogical Union **European Mineralogical Union**
- European Neutron Scattering Association **European Neutron Scattering Association**
- Finnish National Committee for Crystallography **Finnish National Committee for Crystallography**
- Grupo Especializado de Cristalografía y Crecimiento Cristalino **Grupo Especializado de Cristalografía y Crecimiento Cristalino (España)**
- Hellenic Crystallographic Association **Hellenic Crystallographic Association**
- Indian Crystallographic Association **Indian Crystallographic Association**
- International Council for Applied Mineralogy **International Mineralogical Association**
- International Organization for Biological Crystallization **International Organization for Biological Crystallization**
- International Union of Crystallography **International Union of Crystallography**
- Korean Crystallographic Association **Korean Crystallographic Association**
- Latin American Crystallographic Association **Latin American Crystallographic Association**
- Mineralogical Society **Mineralogical Society of Great Britain and Ireland**
- Mineralogical Society of America **Mineralogical Society of America**
- Nederlandse Vereniging voor Kristallografie **Nederlandse Vereniging voor Kristallografie**
- Pittsburgh Diffraction Society **Pittsburgh Diffraction Society**

- Polish Crystallographic Association **Polish Crystallographic Association**
- Polish Synchrotron Radiation Society **Polish Synchrotron Radiation Society**
- Real Sociedad Española de Física **Real Sociedad Española de Física**
- Real Sociedad Española de Química **Real Sociedad Española de Química**
- Russian National Committee for Crystallography **Russian National Committee for Crystallography**
- Sociedad Mexicana de Cristalografia **Sociedad Mexicana de Cristalografía**
- Italian Society for Synchrotron Light **Società Italiana Luce di Sincrotrone**
- Société Française de la Neutronique **Société Française de la Neutronique**
- Société Française de Minéralogie et Cristallographie **Société Française de Minéralogie et Cristallographie**
- Society of Crystallographers in Australia and New Zealand **Society of Crystallographers in Australia and New Zealand**
- Swiss Society for Crystallography **Swiss Society for Crystallography**
- Turkish Crystallographic Association **Turkish Crystallographic Association**
- US National Committee for Crystallography **US National Committee for Crystallography**

1.12: Crystallography in Spain

In the context of this chapter, you will also be invited to visit these sections...

- **Crystallography in Spain. Brief historical outlines**
- **Participants in the Meeting on Anomalous Dispersion (Madrid, 1974)**
- **Some pictures taken during IUCr2011 (Madrid, 2011)**

Crystallography is one of the branches of Science whose importance has been critical to the development of Chemistry around the world. Its influence, which was spectacular in Spain during the last third of the twentieth century, led (through many efforts) to the establishment of several groups of crystallographers whose relevance is nowadays beyond any doubt. However, contrary to what happened in other developed countries, Crystallography in Spain, and especially in academic institutions, seems in general to remain an unresolved matter. This is probably due to the fact that it has erroneously been considered as a minor technical issue, whose application and interpretation is trivial.

No scientific discipline has so profoundly influenced the field of structural Biochemistry as that of X-ray diffraction analysis when applied to the crystals of macromolecules. It is among the most prolific techniques available for providing significant new data. In theory, there is no limit in molecular size, and thus it includes, in addition to proteins, the viruses, ribo- and deoxyribonucleic acids and protein complexes. Its influence has also affected the development of Biology and Biomedicine, leading to the so-called structural genomics. The detailed knowledge of the structure of biological macromolecules enables us not only to understand the relationship between structure and function, but also to make rational functional improvement proposals. In contrast with the importance of these issues, the rather large number of research groups in Spain (very competitive in Cellular and Molecular Biology), and the lack of resources dedicated to the few Spanish laboratories active in macromolecular Crystallography becomes very apparent.

In an separate part of this chapter the reader will find a **brief historical outline on the initial development of Crystallography in Spain**.

Most crystallographers working in Spain are associated with the Specialized Group for Crystallography and Crystal Growth (**Grupo Especializado de Cristalografía y Crecimiento Cristalino, GE3C**), a group associated with the **Spanish Royal Society of Chemistry**. Similarly, European crystallographers are associated with the **European Crystallographic Association, ECA**.

Further, the **Spanish Committee of Crystallography** is the Spanish association responsible for coordinating the official Spanish representation to the **International Union of Crystallography, IUCr**.

There are some other Spanish associations related to Crystallography, organized according to various radiation sources of interest in the field:

- **Spanish Association of Synchrotron Users (AUSE)**
- **Spanish Association for Neutron Techniques (SETN)**

XXII Congress and General Assembly, Madrid 2011

Crystallographers working in Spain had the responsibility to organize the ***XXII Congress and General Assembly*** of the **International Union of Crystallography, IUCr**, that was held in Madrid (August 22-30, 2011). This type of congress, held every three years in a different country, had in Madrid over 2,800 worldwide participants and implied an explicit recognition by the **IUCr** of the "Spanish crystallography". The event was officially supported by the **IUCr**, the **Spanish Ministry of Science and Innovation**, the **Spanish National Research Council (CSIC)**, several Spanish universities (especially **Alcalá**, **Complutense of Madrid**, **Autonomous of Madrid**, **UIMP**, **Oviedo**, **Cantabria** and **Barcelona**). Special mention has to be done to the support received by the **BBVA Foundation** (which specifically supported the participation of the **three 2009 Chemistry Nobel Laureates**). **AECID** and **Metro-Madrid** helped to the participation of a large number of young researchers from less developed countries. The "**Madrid Convention Bureau**" supported, in 2005, the preparation of the candidature of Spain to organize this unique event. Crystallographers working in Spain and specially those implied in the Local Organizing Commitee, appreciate the suport obtained from all these organizations.

See also the contents of this link.

The most relevant research groups located in Spain and using Crystallography as a main research tool can be found below. **See also the following link**.

Visite La Factoría de Cristalización

Nine of the groups listed below made an association in the context of a joint project called **The Factory of Crystallization**, a collaborative project to create an integrated platform for research and services in crystallization and crystallography. The project was thought as a setting to combine advanced research on crystallization and crystallography and service delivery in these fields to companies and research groups in biomedical, pharmacological, biotechnological, nanotechnological, natural or material sciences. The aim was that any group extracting, synthesizing or, in general, developing a new molecule or potentially interesting material could have access, with an adequate level of confidentiality, to the knowledge and technology needed for crystallization, diffraction data collection and structure solution. The project was funded with € 5.0 M by the former Spanish Ministry of Education and Science (now **Ministry of Science and Innovation**), as part of the Consolider-Ingenio/2010 program.

The following information corresponds to a relatively splendid stage of Crystallography in Spain (during the first decade of the 21st century). Unfortunately, with the passage of time the situation has worsened, so many of the links shown below may no longer be operational..

The list of groups shown below may contain involuntary errors or omissions. Groups who would like to be included here should **get in contact through this link**.

Select a region on the map

Andalusia

Division for X-ray Diffraction, University of Cádiz

Campus Universitario del Río San Pedro, E-11510 Puerto Real

General Service Unit, Institute for Materials Science of Seville (CSIC-University of Seville)

Americo Vespuccio 49, Isla de la Cartuja, E-41092 Sevilla

Group of Coordination Chemistry and Structural Analysis, University of Granada

Avda. Fuentenueva s/n, E-18071 Granada

Group of Organometallic Chemistry and Homogeneous Catalysis, Institute of Chemical Reseach (CSIC-Universidad de Sevilla)

Américo Vespucio 49, E-41092 Sevilla

Group of Protein Structure, Department of Physical-Chemistry, Biochemistry and Inorganic Chemistry, University of Almería

Edificio Científico Técnico de Química, Ctra. Sacramento, La Cañada de San Urbano, E-04120 Almería

Laboratory of Crystallographic Studies (LEC), CSIC-University of Granada

Edifício Inst. López Neyra, Avenida del Conocimiento s/n, PT Ciencias de la Salud, E-18100 Armilla

X-ray Diffraction Service, University of Málaga

Bulevar Louis Pasteur 33 , Campus de Teatinos, Edificio SCAI, Planta 1, B1-04, E-29071 Málaga

Ir al mapa **Go to the map**

Aragon

Institute of Chemical Synthesis and Homogeneous Catalysis (iSQCH), CSIC-University of Zaragoza

Pedro Cerbuna 12, E-50009 Zaragoza

Department of Physics of Condensed Matter, CSIC-University of Zaragoza

Plaza de San Francisco s/n, E-50009 Zaragoza

Ir al mapa **Go to the map**

Asturias

Department of Physics, University of Oviedo

Julián Clavería 8, E-33006 Oviedo

Crystallography and Mineralogy, Department of Geology, University of Oviedo

Jesús Arias de Velasco, E-33005 Oviedo

Synthesis, Structure and Technological Application of Materials, Department of Physical and Analytical Chemstry, Faculty of Chemistry, University of Oviedo

Julián Clavería 8, E-33006 Oviedo

Go to the map

Balearic Islands

No data available

Go to the map

Cantabria

Group of High Pressure and Spectroscopy, Faculty of Sciences, University of Cantabria

Avda. de los Castros s/n, E-39005 Santander

Group of Matter Magnetism, University of Cantabria

Avda. de los Castros s/n, E-39005 Santander

Go to the map

Canary Islands

Laboratory of X-Rays and Molecular Materials, Department of Fundamental Physics II, University of La Laguna

Avda Astrofísico Francisco Sánchez s/n, E-38204 La Laguna

Some other groups (no web link) from the Dept. of Fundamental Physics II, making use of the **Integrated Service for X-Ray Diffraction, University of La Laguna**

Avda. Astrofísico Francisco Sánchez s/n, E-38206 La Laguna

Go to the map

Castile and León

Department of Physics of the Condensed Matter, Crystallography and Mineralogy, University of Valladolid

E-47002 Valladolid

Structural Biology Group, Cancer Research Center, CSIC-University of Salamanca

Campus Miguel de Unamumo, E-37007 Salamanca

X-ray Diffraction Service, University of Burgos

Plaza de Misael Bañuelos s/n, E-09001 Burgos

X-ray Diffraction Service, University of Salamanca

Plaza de la Merced s/n, E-37008 Salamanca

Go to the map

Castile - La Mancha

Grupo de Química Organometálica y Catálisis, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha

Avenida de Camilo José Cela 10, E-13071 Ciudad Real

Ir al mapa **Go to the map**

Catalonia

Department of Crystallography, Institute of Material Sciences of Barcelona, CSIC

Campus de la Universidad Autónoma de Barcelona, E-08193 Bellaterra

Department of Chemical Engineering, Polytechnic University of Catalonia

Escola d'Enginyeria Barcelona Est, Campus Diagonal Besos, Building (EEBE) I 2.21, c/ Eduard Maristany 10-14, E-08019 Barcelona

Department of Crystallography, Mineralogy and Mineral Deposits, Faculty of Geology, University of Barcelona

c/ Martí i Franqués s/n, E-08028-Barcelona

Department of Structural Biology, Institute of Molecular Biology of Barcelona, CSIC

Parque Científico de Barcelona, Baldiri i Reixach 15-21, E-08028 Barcelona

Group for Physics and Crystallography of Nanomaterials, Faculty of Chemistry, University Rovira i Virgili

Marcel.li Domingo s/n, Campus Sescelades, E-43007 Tarragona

Unit for X-ray Diffraction, Autonomous University of Barcelona

Campus de la Universidad Autónoma de Barcelona, E-08193 Bellaterra

X-ray Diffraction Unit, Institute of Chemical Research of Catalonia

Avgda. Països Catalans 16, E-43007-Tarragona

Ir al mapa **Go to the map**

Extremadura

No data available

Ir al mapa **Go to the map**

Galicia

Metallosupramolecular Chemistry Group (QI5)

Universidad de Vigo, Facultad de Química, E-36310 Vigo

Research Group on Molecular and Structural Chemistry (GIQIMO), University of Santiago de Compostela

Campus Universitario Sur, E-15782 Santiago de Compostela

Structural Analysis Unit, Service for Research Support, University of A Coruña

E-15001 A Coruña

Support Center for Science and Technology, University of Vigo

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