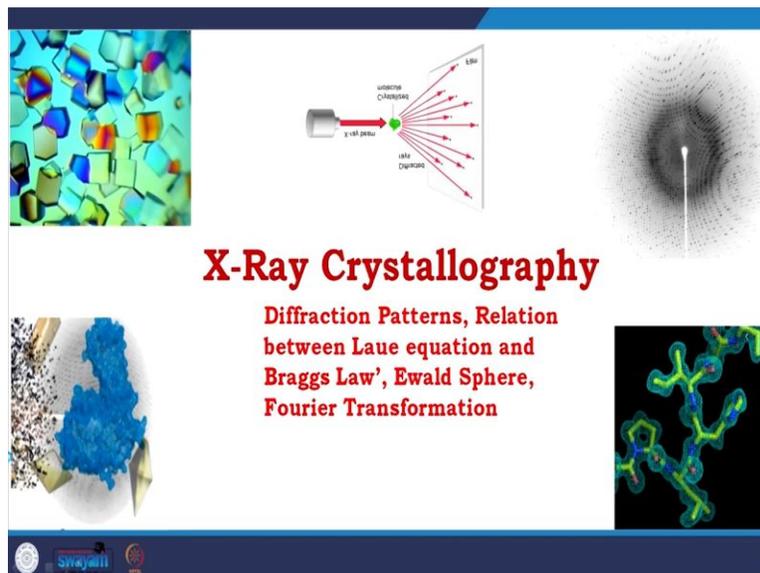


Structural Biology
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Lecture – 22
X-Ray Crystallography: Data Analysis, Part-II

Hi everyone, welcome to the structural biology course again. We are going through structural biology techniques to discuss crystallography techniques.

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X-Ray Crystallography
Diffraction Patterns, Relation between Laue equation and Bragg's Law', Ewald Sphere, Fourier Transformation

Today we are going to discuss few very critical things like the Ewald sphere, how the Ewald sphere is connected with Bragg's law and law equation which we discussed in the last class, how the structure factor is important how the structure factor is related to the development of electron density and what is phase problem and also what is the Fourier transformation.

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Laue Condition for Diffraction and Ewald Sphere:

From the concept of generation of reciprocal lattice, we can learn that each reciprocal lattice vector \mathbf{h} is an integer combination of the three reciprocal basis vectors \mathbf{a}^* , \mathbf{b}^* and \mathbf{c}^* :

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

where the triplet of integers (h, k, l) characterizes uniquely each reciprocal lattice vector

The reciprocal lattice vectors \mathbf{a}^* , \mathbf{b}^* and \mathbf{c}^* can be derived from the lattice vectors \mathbf{a} , \mathbf{b} and \mathbf{c} from the vector products

$$\mathbf{a}^* = \mathbf{b} \times \mathbf{c} / V \quad \mathbf{b}^* = \mathbf{c} \times \mathbf{a} / V \quad \mathbf{c}^* = \mathbf{a} \times \mathbf{b} / V$$

where V is the volume of the unit cell defined by the vectors \mathbf{a} , \mathbf{b} and \mathbf{c}

We will start with the Laue condition for diffraction connecting and Ewald sphere: From the concept of generation of the reciprocal lattice, which we will discuss in the last few classes, we learned that each reciprocal lattice vector \mathbf{h} is an integer combination of the three reciprocal basis vectors \mathbf{a}^* , \mathbf{b}^* and \mathbf{c}^* .

So, $\mathbf{h} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$ where the triplet of integers h, k, l , Miller indices uniquely characterizes each reciprocal lattice vector. The reciprocal lattice vectors \mathbf{a}^* , \mathbf{b}^* , and \mathbf{c}^* can be derived from the lattice vectors \mathbf{a} , \mathbf{b} , \mathbf{c} from the vector products of the real crystals.

$$\text{So, } \mathbf{a}^* = \mathbf{b} \times \mathbf{c} / V,$$

$$\mathbf{b}^* = \mathbf{c} \times \mathbf{a} / V \text{ and}$$

$$\mathbf{c}^* = \mathbf{a} \times \mathbf{b} / V$$

where V is the volume of the unit cell defined by the vectors \mathbf{a} , \mathbf{b} , \mathbf{c} in the real crystal.

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Laue Condition for Diffraction and Ewald Sphere:

It is convenient to introduce the vector \mathbf{s}_0 (\mathbf{k}_{in}) which is parallel to the direction of the incident beam

Its magnitude is set as the inverse of the wavelength λ of the incident beam:

$$\text{abs}(\mathbf{s}_0) = 1/\lambda$$

Similarly, the direction of the diffracted beam is described by the vector \mathbf{s} (\mathbf{k}_{out}) with its magnitude,

$$\text{abs}(\mathbf{s}) = 1/\lambda$$

The **Laue condition** states that the following equality must be fulfilled: $\mathbf{s} = \mathbf{s}_0 + \mathbf{h}$

Now it is convenient to introduce the vector \mathbf{s}_0 , which we define as \mathbf{k}_{in} in the last class, which is parallel to the direction of the incident beam. Its magnitude is set as the inverse of the wavelength λ of the incident beam.

So, $\text{abs}(\mathbf{s}_0)$ absolute value of $\mathbf{s}_0 = 1 / \lambda$.

Similarly, the direction of the diffracted beam is described by the vector \mathbf{s} , which we defined as the \mathbf{k}_{out} with its magnitude

So, $\text{abs}(\mathbf{s})$ the absolute value of $\mathbf{s} = 1 / \lambda$

The Laue condition states that the following equality must be fulfilled

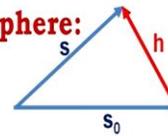
$$\mathbf{S} = \mathbf{s}_0 + \mathbf{h}$$

\mathbf{s} , which is the diffracted beam equal to \mathbf{s}_0 , which is the incident beam, and \mathbf{h} , which we already talked about in the last slide, which is you could see here it is an integer combination of the three reciprocal basis vector \mathbf{a}^* , \mathbf{b}^* and \mathbf{c}^* .

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Laue Condition for Diffraction and Ewald Sphere:

The Laue condition requires that the three vectors s_0 , s and h must form a triangle with two identical sides:



If a sphere is constructed with the center at the origin of s_0 and $|s_0|$ as radius, the diffracting conditions are satisfied only if the endpoint of a reciprocal lattice vector h is located on the surface of the Ewald sphere

The direction of the diffracted beam is then given by the direction of the vector s

By changing the orientation of the reciprocal lattice relative to the incident beam s_0 , each vector h which cuts the Ewald sphere gives rise to a diffracted beam which is parallel to the direction of the corresponding vector s .

The Laue condition requires three vectors s_0 , s , and h must form a triangle with two identical sides. Suppose a sphere is constructed with the center at the origin of s_0 and the absolute value of s_0 as radius. In that case, the diffracting conditions are satisfied only if the endpoint of a reciprocal lattice vector h is located on the surface of the Ewald sphere. The direction of the vector s then gives the direction of the diffracted beam. Changing the orientation of the reciprocal lattice relative to the incident beam s_0 , each vector h that cuts the Ewald sphere gives rise to a diffracted beam that is parallel to the direction of the corresponding vector s .

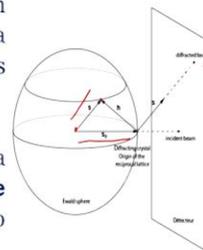
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The Ewald Condition of Diffraction:

Based on the Ewald sphere, it is clear that each reciprocal lattice vector can only give rise to a diffracted beam if the reciprocal lattice takes successively different orientations

This means that with a **monochromatic beam**, i.e. a unique wavelength, the **crystal has to be moved** relative to the incident beam in order to generate diffraction peaks.

For a complete measurement of all the accessible diffraction intensities, the crystal must be displaced so that each reciprocal lattice vector satisfies the Ewald conditions.



Based on the Ewald sphere, it is clear that each reciprocal lattice vector can only give rise to a diffracted beam if the reciprocal lattice takes a successively different orientation. So, look at the

picture you see here in the center; it is s , s_0 , and this is h , now where it would cut, then you will get the diffracted beam for complete measurement of all the accessible diffraction intensities the crystal must be displaced. So that each reciprocal lattice vector satisfied the Ewald condition, and that is why I was talking about that Ewald is like Ewald sphere so critical.

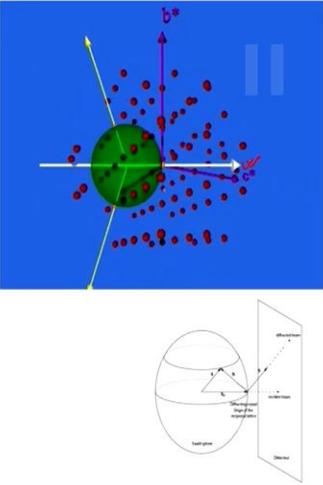
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Ewald Sphere: Animation

The white beam in this animation is the incident beam and the yellow beams are the diffracted beams

Here the origin of the diffracted beam has been placed at the center of the Ewald sphere

In practice, the diffracted beam originates from the diffracting crystal, i.e. from the origin of the reciprocal lattice



If you look at the animation, you will understand it much better. So, if you see here, you will see that the white beam you are looking at is the incident beam, and then you see the yellow beams are generated; they are diffracted beams. Here the origin of the diffracted beam has been placed at the center of the Ewald sphere. But in practice, the diffracted beam originated from the diffracting crystal from the origin of the reciprocal lattice. And you will see that only when it is touching can only get the diffraction.

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The Ewald Sphere: Summary

The **Ewald sphere** is a **geometric construction** used in electron, neutron, and X-ray crystallography which demonstrates the relationship between:

- the **wavevector** of the incident and diffracted x-ray beams,
- the **diffraction** angle for a given reflection,
- the **reciprocal** lattice of the crystal

It was conceived by **Paul Peter Ewald**, a German physicist and crystallographer.

So, in summary, the Ewald sphere is a geometric construction used in electron, neutron, and X-ray crystallography which demonstrates the relationship between the wave vector of the incident and diffracted X-ray beams s and s_0 , the diffraction angle for a given reflection, and the reciprocal lattice of the crystal and it was conceived by Paul Peter Ewald a German physicist and crystallographer.

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The Ewald Sphere:

A tool to visualize the conditions under which Bragg's law is satisfied and therefore a reflection (diffraction spot) will be observable.

This occurs when the surface of a sphere centered about the crystal with radius = $1/\lambda$ intersects with a point on the reciprocal lattice.

Also, the Ewald sphere is used to visualize the condition under which Bragg's law is satisfied. Therefore, a reflection will be observable, or you could better say diffractions spot in the collector. This occurs when the sphere's surface centered about the crystal with a radius equal to

$1/\lambda$ intersects with a point on the reciprocal lattice. So, now we will come to the structure solution to calculate structure factor amplitudes from the merged intensities.

So, we have already talked about the diffraction pattern where we see that there are spots that are either very high intensity or relatively lower intensity or even lower than those that would influence the calculation of the structure factor.

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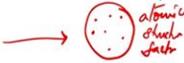
Structure Factors and Diffraction Pattern:

The structure factors, $F(hkl)$, are the fundamental quantities on which the function of electron density depends

These are very important magnitudes, since the maxima of the electron density function, $\rho(xyz)$ show the location of the atoms, that is, the internal structure of the crystals

The structure factors represent the diffracted waves, which when colliding with a photographic plate, or a detector, leave their mark in the form of well-defined spots that form the diffraction pattern

Therefore, from an experimental point of view, a crystal structure is defined by as many structure factors as spots contained in the diffraction pattern found in the detector or plate which is used for data collection

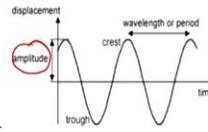


The structure factor $F(hkl)$ are the fundamental quantities on which electron density function depends if you see the structure factor and the diffraction pattern. These are important magnitudes since the maxima of the electron density function $\rho(xyz)$. So, the atoms' location is the crystal's internal structure. So, if you get the electron density function, you could have gotten the crystal's entire information. The structure factor represents that diffracted waves that, when colliding with a photographic plate usually used in old days or a detector that are now used, leave their mark in the form of well-defined spots that form the diffraction pattern. Therefore, from an experimental point of view, a crystal structure is defined by many structure factors spots content in the diffraction pattern found in the detector or plate used for the data collection. So, if you have many spots, all of them represent their atomic structure factor. And together, they are presenting the structure factor.

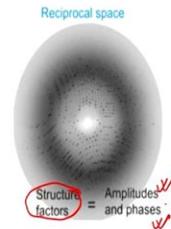
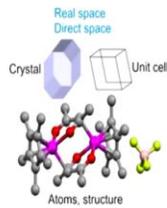
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Structure Factors and Diffraction Pattern:

Analytically speaking, each structure factor can be considered as a vector, with its amplitude and phase (referred to an arbitrary origin of phases)



Also it represents the total wave resulting from the cooperative dispersion (diffraction), caused by all the atoms of the cell, in a given direction of space.



So, if you talk analytically, each structure factor can be considered a vector with its amplitude and phase (referred to as an arbitrary origin of phases). So, if you see it is a wave where you get the wave's amplitude, which is the value that will give you the intensity. Also, it represents that total wave resulting from the cooperative dispersion, which is diffraction, caused by all the atoms of the cell in a given direction of space.

So, if crystals give rise to real space or direct space, there will be unit cells and information about the atoms. On the other hand, you will have the reciprocal lattice, which will come in the spots, you will have structure factors we are talking about, you will get amplitude and phase.

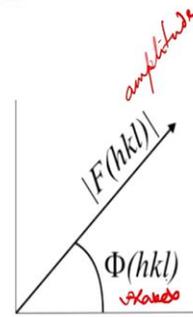
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Structure Factors and Diffraction Pattern:

The graphical representation of diffraction waves as vectors is equivalent to considering that the waves can also be represented as **complex numbers**

The real and imaginary parts of it correspond to the projections of the wave amplitude on the axes of the representation

The phase is the angle that forms the vector with the horizontal axis, which acts as the origin to which phases are referred.



The graphical representation of diffraction waves as the vector is equivalent to considering that the waves can also be represented as a complex number. If you see here, it is the amplitude, and this is the angle. The real and imaginary part of it corresponds to the projection and the wave amplitude on the axes of the representation. The phase is the angle that forms the vector with the horizontal axes, which act as the origin to which phases are referred. So, you see here amplitude and here phases.

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Structure Factor - $F(hkl)$:

Structure factor, $F(hkl)$, is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms contained in the unit cell:

$$F(hkl) = f_1 e^{i\phi} + f_2 e^{i\phi} + f_3 e^{i\phi} + \dots \\ + f_N e^{i\phi} + f_1 e^{i\phi} + f_2 e^{i\phi} + f_3 e^{i\phi} + \dots$$

or, $F(hkl) = \sum f_j e^{i\phi}$

The term f_j describing the diffractive contributions of each atom is called the atomic scattering factor of atom j .

The scattering factor essentially describes the amplitude for the scattering contributed by a particular species of atom.

The structure factor $F(hkl)$ is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms contained in the unit cell. So, As I was talking about the $F(hkl)$ the original structure factor combines all the small contribution from each atom.

So,

$$F(hkl) = \sum f_j e^{i\phi}$$

where ϕ is the phase, the term f_j describing the diffractive contribution of each atom is called the atomic scattering factor. So, instead of the atomic structure factor, it is called the atomic scattering factor because it comes in a proportional way how an atom would be scattered when the incoming ray is coming. The scattering factor essentially describes the amplitude for the scattering contributed by a particular atom species.

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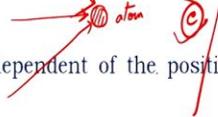
Atomic Scattering Factor:

Structure factor, $F(hkl)$, is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms contained in the unit cell: $F(hkl) = \sum f_i e^{i\phi}$

Each of these waves shows an amplitude proportional to the **atomic scattering factor**, f_j , that measures the X-ray scattering power of each atom

The atomic scattering factor is independent of the position of the atom in the unit cell

It depends only on the type of atom and the direction of scattering, so that it reaches a maximum in the same direction of the incident X-rays, and decreases as a function of the angle of departure



The structure factor $F(hkl)$ is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms content in the unit cell.

$$F(hkl) = \sum f_i e^{i\phi}$$

Each wave shows an amplitude proportional to the atomic scattering factor f_j that measures each atom's X-ray scattering power. The atomic scattering factor is independent of the atom's position in the unit cell. It depends only on that type of atom and that scattering direction. It reaches a maximum in the same direction of the incident X rays and decreases as a function of the departure angle. So, if you think about suppose this is an n atom. So, it could be coming from a different angle, and depending on that, the scattering happens. So, when you see the spots you have remembered, or you go back and see that there are very bright spots, there are very light spots that are because of this. So, the atomic scattering factor is independent of the atom's position in that unit cell. It stays in one position, but it changes when you rotate them. It changes their position like it is in the unit cell, but how the rays are coming and where it is hitting them changes. So, it depends only on the type of atom we will discuss and the direction of the scattering, as I told you.

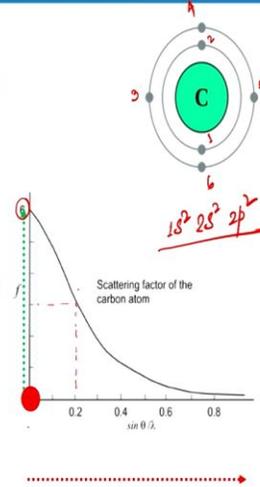
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Atomic Scattering Factor:

To understand the variation of the scattering factor let us take the example of carbon atom

At the angular value corresponding to $(\sin \theta) / \lambda = 0$ the magnitude of the atomic scattering factor is always equal to the total number of electrons in the atom

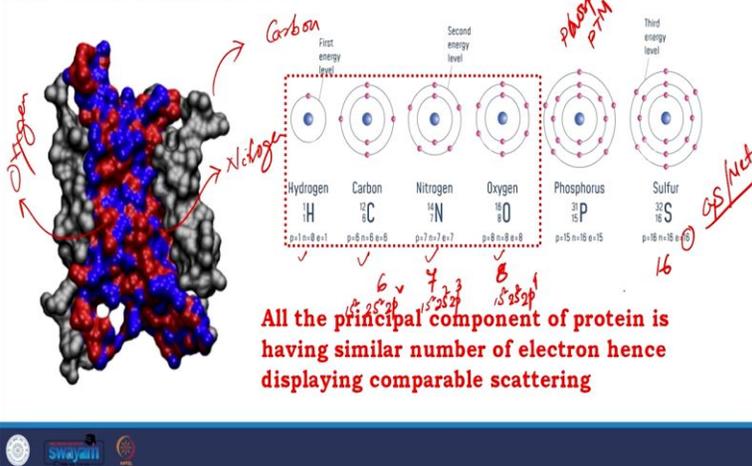
But it decreases strongly as the angular value increases



To understand the variation of the scattering factor, let us take the example of the carbon atom. So, at the angular value corresponding to $\sin \theta / \lambda = 0$, the magnitude of the atomic scattering factor is always equal to the total number of electrons. So, here the number is 6, and you see carbon has 6 electrons, you know that $1s^2, 2s^2, 2p^2$. But then the angle changes and see how rapidly it is reduced, and it decreases strongly. That is why it is written. It decreases strongly as the angular value increases from 0 to 0.2. It nearly reduces up, and at 0.8 it is almost 0.

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Scattering Factor and its dependency of number of electrons in an atom:



So, here is a very interesting fact to discuss this protein structure. If you look at it, you will see that it has carbon, nitrogen, and oxygen, they are the main components, hydrogen is there. Still, we are not considering hydrogen as a candidate when considering the scattering. So, I mentioned

before that the protein molecule is mostly composed of hydrogen, carbon, nitrogen, and oxygen. And then, sulfur and phosphorus come in the form of phosphate as a post-translational modification. Interestingly, if you compare carbon, nitrogen, and oxygen, the major scatter is here 6, 7, and 8 electrons $1s^2 2s^2 2p^2$, $1s^2 2s^2 2p^3$, $1s^2 2s^2 2p^4$. So, you could easily understand now, as you have seen, that the scattering is mostly dependent on the number of electrons present. So, carbon, nitrogen, or oxygen all behave as a similar scatter. It isn't easy to differentiate between carbon, nitrogen, and oxygen. Sulfur having 16 is better, but not a huge difference in its scattering ability. So, I am trying to say that if you think in the protein the major population, you would not get any different significant difference in terms of scattering, so that is a problem, especially when you want to find a reference. And later, when we will go to the phase problem, I will show you why that is a big problem and how we solve it. So, these are the major molecules and atoms, and I say all the principal components of protein have a similar number of electrons hence displaying comparable scattering.

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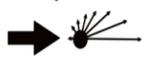
Anomalous Dispersion Affect Scattering Factor:

In a first approach the scattering power of the different atoms does not depend on the wavelength of the X-ray radiation

However, there are side effects that make them different. How?

If the incident X-ray radiation has a frequency close to the natural oscillation frequency of the electrons of a given atom, there occurs the so-called **anomalous dispersion**, which modifies the atomic dispersion factor, $f_i (= f_0)$.

In this situation, the expression of scattering factor is modified with two terms, f' and f'' , which represent the real and imaginary components, respectively, of the anomalous fraction of the atomic scattering factor



$$f = f_0 + f' + i f''$$

Coming to anomalous dispersion effect on the scattering factor. In a first approach, the scattering power of the different atoms does not depend on the wavelength of the X-ray radiation. However, there are side effects that make them different. Suppose the incident X-ray radiation has a frequency close to the natural oscillation frequency of the electrons of a given atom. In that case, the so-called anomalous dispersion occurs, which modifies the atomic dispersing factor f_i . In this situation, the expression of the scattering factor is modified with two terms f' and f''

double prime, which represent the real and imaginary components, respectively, of the anomalous fraction of the atomic scattering factor

$$f = f_0 + f' + i f''$$

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Analytical expression of the phase:

Suppose a crystal formed by the repetition of the atomic model constituted by the pair of atoms (red and blue) shown in the figure

Of course, any crystalline model can be decomposed into as many simple lattices as atoms

Diffraction Patterns Correlated to number of atoms:

Suppose there is a crystal formed by the repetition of the atomic model constituted by the pair of atoms which we are showing you is a crystal with a pair of atoms red and blue. Of course, any crystalline model can be decomposed into as many simple lattices as atoms. Similarly, here you see the lattice. But now, before going further, I want to talk about another very interesting thing: diffraction patterns that I discussed in detail in the last class correlated to the number of atoms this is very interesting. So, here there is a single atom, and you get the type of spots, two atoms you see how significantly the spots change from these two that this is, and you already start getting spots which we have seen in our actual protein diffraction pattern. These are couples where you have many molecules in one array, and you get a spot. So, this is kind of you could say like a 1D array in you get two there it is not 2D it is still 1D. Still, here you have the molecules, which are into two groups and then multiple groups. If you see here, you have already started getting the diffraction pattern to look very similar to what we get in the diffraction pattern of a protein. So, this is the diffraction pattern of protein, so that is how that diffraction patterns are correlated to the number of atoms present in the molecule. So, now we will continue with the crystal composed by repeating the atomic model constituted by the red and blue atoms.

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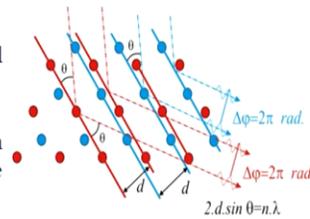
Analytical expression of the phase:

Considering the condition of **Bragg's Law**, the phase difference between the two reflected red beams will be 0° ($= 360^\circ = 2\pi$ radians).

Also the same will happen for the two reflected blue beams.

However, since there is a separation between the two lattices, there will be a phase shift between red and blue waves

Therefore the total diffracted intensity will be less than the arithmetic sum of both, red + blue intensities



So, now, if we see we consider Bragg's law, the phase difference between the two reflected red beams would be 0 because they are the same atom or 360 degrees which is equal to 2π radians. This should be the same thing between the blue beams, but as there is separation between the two lattices, red and blue lattices, there will be a phase shift between the red and blue waves, which you can see here. Therefore the total diffracted intensity will be less than the arithmetic sum of both red + blue intensities.

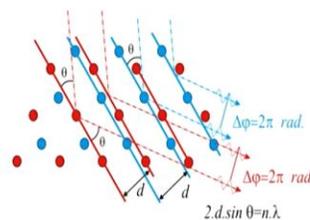
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Analytical expression of the phase:

The resulting amplitude (ie the diffraction intensity) is controlled by the separation between the two lattices (ie by the shape of the motif being repeated)

But the resulting diffraction geometry is the same as the one produced by of each single lattice

Because the diffraction geometry depends only on the lattice geometry



What is the effect? The resulting amplitude, that is, the separation between the two lattices, controls the diffraction intensity, that is, by the repeated shape of the motif. Still, the resulting

diffraction geometry is the same as the one produced of every lattice because the deflection geometry depends only on the same lattice geometry.

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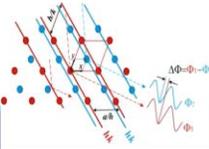
Analytical expression of the phase:

The red X-ray beams, which are being reflected on the red planes of indices hk , fulfill the Bragg's Law, and same is applied for blue beams on the blue planes hk

Now if, Bragg's Law is fulfilled, the phase shift between waves reflected on planes of the same color, shifted (a/h) in the direction of the a -axis, must be $2\pi = 360^\circ$

For the same reason, the phase difference due to the plane separation (b/k) in the direction of the b -axis must also be $2\pi = 360^\circ$

But the beams reflected on the blue planes show a phase shift respect to the red ones by a quantity $(\Delta\Phi)$ radians that depends on the separation between the two lattices



So, the red X-ray beams reflected on the red planes of indices hk fulfill Bragg's law, and the same is applied for the blue beams on the blue planes hk . If Bragg's law is fulfilled, a phase shift between waves reflected on planes of the same color, shifted a / h in the direction of the a -axis, must be $2\pi = 360^\circ$. For the same reason, the phase difference due to the plane separation b / k in the direction of the b -axis must be $2\pi = 360^\circ$. But the beams reflected on the blue planes show a phase shift respect to the red ones by a quantity called $\Delta\phi$ that depends on the separation between the two lattices. So, if it is the same lattice, it is the same, and if it is another lattice, there is a change. The change is expressed by a difference which is $\Delta\phi$.

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Analytical expression of the phase:

In fact, this offset can be easily calculated using the following "rule of three" proportions, applied to the three independent space directions:

$$\begin{array}{ccc} a/h \dots 2\pi & b/k \dots 2\pi & c/l \dots 2\pi \\ x \dots \Delta\Phi_a & y \dots \Delta\Phi_b & z \dots \Delta\Phi_c \end{array}$$

$$\Delta\Phi_a = 2\pi h x/a \quad \Delta\Phi_b = 2\pi k y/b \quad \Delta\Phi_c = 2\pi l z/c$$

Combining the three phase shifts, and generalizing to the three dimensions:

$$\Delta\Phi = 2\pi (h x/a + k y/b + l z/c)$$

Finally, taking fractional coordinates (assuming $x=x/a$, $y=y/b$, $z=z/c$) and replacing $\Delta\Phi$ by Φ :

$$\Phi = 2\pi (h x + k y + l z) \text{ radians} \quad \text{Formula 1}$$

This offset can be easily calculated using the following rule of three proportions, applied to the three independent space directions. So, there are three differences $\Delta\phi_a$, $\Delta\phi_b$ and $\Delta\phi_c$. $2\pi h x / a$, $2\pi k y / b$ and $2\pi l z / c$. Now, if you combine those 3 phase shifts in 3 different axes and generalize to the three dimensions, you will get

$$\Delta\phi = 2\pi (h x / a + k y / b + l z / c)$$

Now finally when you take the fractional coordinates assuming $x = x / a$, $y = y / b$, $z = z / c$ and replacing $\Delta\phi$ by ϕ :

$$\phi = 2\pi (h x + k y + l z) \text{ radians}$$

We consider this formula 1 for our mathematical analysis for the next level.

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Analytical expression of the structure factor:

Once the mathematical expression of the phase is established in terms of the shape of the crystallographic model, let's see how to arrive at the analytical expression of the structure factor

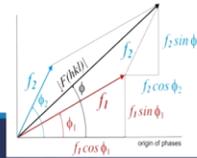
Suppose that f_1 represents the dispersion of the red atoms, and f_2 for the blue atoms (figure on the left). The total resultant dispersion of both atom types will be $F(hkl)$

Writing it using vector notation

$$F(hkl) = f_1 + f_2$$

According to the scheme, the module of this vector sum will be:

$$|F(hkl)| = \sqrt{(f_1 \cos \phi_1 + f_2 \cos \phi_2)^2 + (f_1 \sin \phi_1 + f_2 \sin \phi_2)^2}$$



Now where we get the mathematical expression of the phase. So, this is the mathematical expression of the phase. So, once we get the mathematical expression of the phase in terms of the crystallographic model's shape, let us now see how to arrive at the analytical expression of the structure factor. We know the expression, but we want to suppose that f_1 represents the red atom's dispersion and f_2 for the blue atoms. This is the figure where you could see the red dots, the red one represented by f_1 and the blue one represented by f_2 . So, write it using vector notation $F(hkl) = f_1 + f_2$.

According to the scheme, the module of this vector sum will be

$$F(hkl) = \sqrt{(f_1 \cos \phi_1 + f_2 \cos \phi_2)^2 + (f_1 \sin \phi_1 + f_2 \sin \phi_2)^2}$$

(Refer Slide Time: 32:10)

Analytical expression of the structure factor:

It's corresponding phase, referred to an arbitrary phase origin:

$$\phi = \tan^{-1} \frac{(f_1 \sin \phi_1 + f_2 \sin \phi_2)}{(f_1 \cos \phi_1 + f_2 \cos \phi_2)}$$

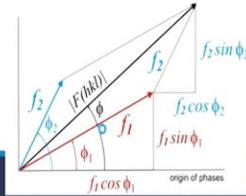
Generalizing now for all atoms, and taking into account the general expression for the phase (**Formula 1**, above), the module of the structure factor will be:

$$|F(hkl)| = \sqrt{\left[\sum_j f_j \cos 2\pi(hx_j + ky_j + lz_j) \right]^2 + \left[\sum_j f_j \sin 2\pi(hx_j + ky_j + lz_j) \right]^2}$$

Formula 2

And its relative phase:

$$\phi = \tan^{-1} \frac{\sum_j f_j \sin \phi_j}{\sum_j f_j \cos \phi_j}$$



It is corresponding phase, referred to an arbitrary phase or origin:

$$\phi = \tan^{-1} (f_1 \sin \phi_1 + f_2 \sin \phi_2) / (f_1 \cos \phi_1 + f_2 \cos \phi_2)$$

Now generalizing for all attempts and taking into account the general expression for the phase (formula 1) the module of the structure factor will be if

$$F(hkl) = \sqrt{\left[\sum f_j \cos 2\pi(hx_j + ky_j + lz_j) \right]^2 + \left[\sum f_j \sin 2\pi(hx_j + ky_j + lz_j) \right]^2}$$

So, this is now Formula 2, and the relative phase would be

$$\phi = \tan^{-1} \sum_j f_j \sin \phi_j / \sum_j f_j \cos \phi_j$$

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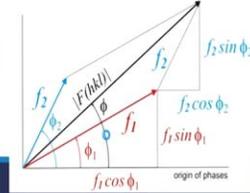
Analytical expression of the structure factor:

We have used the vector graphic representation to deal with diffraction waves, and this is equivalent to considering that waves can be represented as complex numbers

In this type of representation, the real and imaginary parts correspond to the projections of the wave amplitude on the Cartesian axes, and the phase is the angle that forms the vector with the horizontal axis, which acts as an origin to which phases are referred.

Therefore, and taking into account **Formula 2**, the complex expression for the structure factor will be:

$$F(hkl) = \sum_{j=1}^n f_j \cos [2\pi(hx_j + ky_j + lz_j)] + i \sum_{j=1}^n f_j \sin [2\pi(hx_j + ky_j + lz_j)]$$



We have used the vector graphic representation to deal with diffraction waves, and this is equivalent to considering that waves can be represented as a complex number. In this type of representation, the real and imaginary parts correspond to the projection of the wave amplitude on the Cartesian axes. The phase is the angle that forms the vector with the horizontal axis you could see here, which acts as an origin to which phases are referred.

Therefore with those understanding and taking the formula 2 to have structure factor the complex expression from the structure factor will be

$$F(hkl) = \sum f_j \cos 2\pi (hx_j + ky_j + lz_j) + i \sum f_j \sin 2\pi (hx_j + ky_j + lz_j)$$

$\sum_{j=1}^n$ for the n atom present

(Refer Slide Time: 34:36)

Analytical expression of the structure factor:

$$F(hkl) = \sum_{j=1}^n f_j \cos [2\pi(hx_j + ky_j + lz_j)] + i \sum_{j=1}^n f_j \sin [2\pi(hx_j + ky_j + lz_j)]$$

which, according to **Euler's formula**, can also be written as:

$$F(hkl) = \sum_{j=1}^n f_j e^{2\pi i(hx_j + ky_j + lz_j)} \quad \text{Formula 3}$$

Euler's formula, named after Leonhard Euler, is a mathematical formula in complex analysis that establishes the fundamental relationship between the trigonometric functions and the complex exponential function

Euler's formula states that for any real number x :

$$e^{ix} = \cos x + i \sin x$$

So, when we get this expression according to Euler's formula this expression we could write as

$$F(hkl) = \sum_{j=1}^n f_j \cos [2\pi(hx_j + ky_j + lz_j)] + i \sum_{j=1}^n f_j \sin [2\pi(hx_j + ky_j + lz_j)]$$

$\sum_{j=1}^n$ for the n atom present

Which, according to Euler's formula can be written as

$$F(hkl) = \sum_{j=1}^n f_j e^{2\pi i(hx_j + ky_j + lz_j)}$$

$\sum_{j=1}^n$ for the n atom present

this is called formula 3 and Euler formula which we have done the conversion this is named after Leonhard Euler is a mathematician who developed this mathematical formula in complex analysis that establishes the fundamental relationship between the trigonometric functions and the complex exponential functions which states that for any real number, which is x

$$e^{ix} = \cos x + i \sin x$$

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Alternative form of Structure Factor - F(hkl):

F(hkl), as a complex number, can be expressed in terms of its real and imaginary components:

$$F(hkl) = A(hkl) + i B(hkl)$$

where, $A = \sum f_i \cos \alpha_i = f_{\text{resultant}} \cos \alpha_{\text{resultant}}$

and, $B = \sum f_i \sin \alpha_i = f_{\text{resultant}} \sin \alpha_{\text{resultant}}$

f_j are the atomic scattering factor and α_j are the phase angles of the waves scattered from individual atoms

This is just an alternate, mathematically equivalent representation for the structure factor

So, F (hkl) as a complex number can be expressed in its real and imaginary components.

$$So, F (hkl) = A (hkl) + i B (hkl)$$

where $A = \sum f_i \cos \alpha_i = f_{\text{resultant}} \cos \alpha_{\text{resultant}}$ and

$$B = \sum f_i \sin \alpha_i = f_{\text{resultant}} \sin \alpha_{\text{resultant}}$$

f_j are the atomic scattering factor, and α_j are the phase angles of the waves scattered from individual atoms. So, this is an alternate mathematically equivalent representation for the structure factor.

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Evaluation of the structure factor:

If we know.

A) The internal structure of any crystal, ie the types of atoms (f_j) that constitute it,

B) The positions (x, y, z) of all atoms (n) contained in the unit cell,

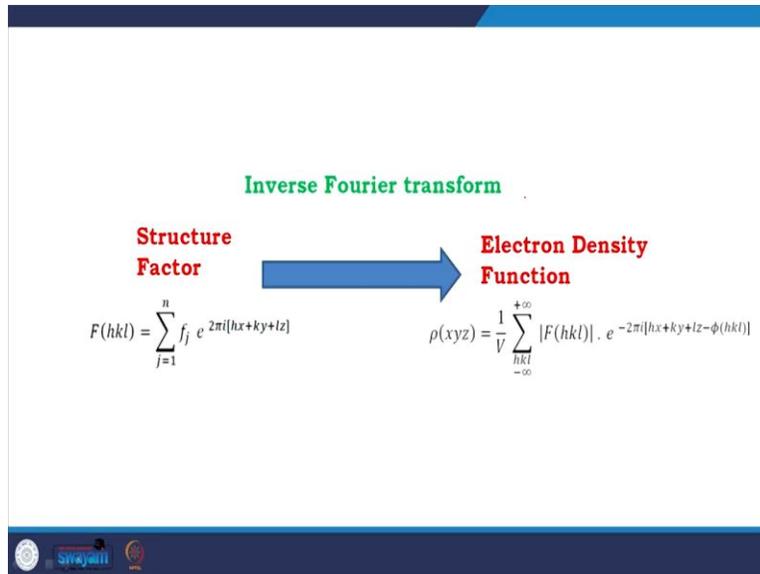
We can immediately calculate the structure factors, $F(hkl)$, that define the crystal.

To do this, it is enough to apply **Formula 3**, which actually involves calculating the inverse **Fourier transform** of the **electron density function**

$$F(hkl) = \sum_{j=1}^n f_j e^{2\pi i(hx+ky+lz)} \quad \text{Formula 3}$$

Evaluation of the structure factor. So, suppose we know that the internal structure of any crystal is the type of atoms f_j that constitute it and the positions (x, y, z) of all atoms (n) content in the unit cell. In that case, we can now immediately calculate the structure factors $F(hkl)$ that define the crystal. To do this, it is enough to apply formula 3, which involves calculating the inverse Fourier transform of the electron density function. We know Formula 3, but we do not know what Fourier transform and electron density function?.

(Refer Slide Time: 37:23)



So, we talked about the structure factor,

which is

$$F(hkl) = \sum f_j e^{2\pi [hx + ky + lz]}$$

$\sum j = 1$ to n for the n atom present

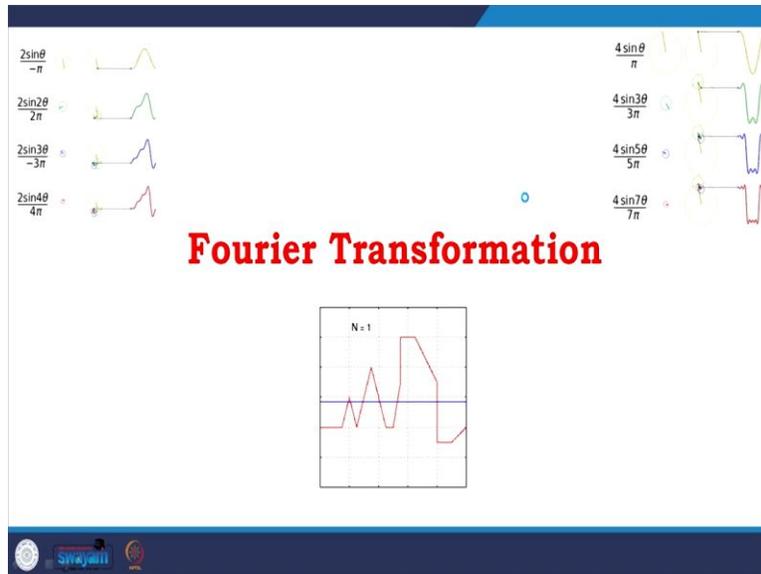
and electron density function which is

$$\rho(xyz) = 1 / V \sum |F(hkl)| * e^{2\pi [hx + ky + lz - \phi(hkl)]}$$

summation hkl - infinity to + infinity

So, to convert the structure factor to an electron density function, we have to perform an inverse Fourier transform. Now we know about these, but we do not know what inverse Fourier transform is.

(Refer Slide Time: 38:23)



So, let us try to understand what Fourier transform is, and it is not with all of you, but when I teach in the class I call Fourier transformation, and people become afraid. So, just to tell you we are not going into the mathematical complexity of the Fourier transformation. Still, because you know you do not have to do it when you are actually solving a crystal structure, the software could do this. Still, the more you understand what is happening better you can control the process.

(Refer Slide Time: 39:03)

Fourier Transformation:

We have different waves of X-rays superimposed on one another during diffraction

Now it is difficult to isolate the contribution of each diffraction event to determine the lattice structure

To solve this problem, we use a mathematical tool known as the Fourier transform

A Fourier transform is a representation of some function in terms of a set of sine-waves

The set of sine-waves of different frequencies has orthogonal properties

In general, any function can be represented in terms of a Fourier series, which is just the sum of enough sine-waves of the appropriate frequency, amplitude and phase

So, as we have seen, Fourier transforms, we have different waves of X rays superimposed on one another during the diffraction. Now it is difficult to isolate the contribution of each diffraction event to determine the lattice structure. So, what to do, to solve this problem. We use a mathematical tool which is known as the Fourier transform. A Fourier transform represents some function in terms of a set of sine waves. The set of sine waves of different frequencies has orthogonal properties. In general, any function can be represented in a Fourier series, which is the sum of enough sine waves of the appropriate frequency amplitude and phase.

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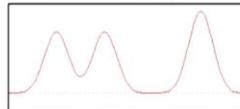
Fourier Transformation:

If we have a function that contains superposition of many different waves with different amplitudes, frequency and phase, such as in our case of the diffracted X-ray signal we pick up on our detector: what we should do?

Here, we can perform an inverse Fourier transform to determine the individual frequencies of the diffracted X-ray waves as well as their relative strengths

To illustrate the notion of stacking sine-waves, consider an imaginary one-dimensional crystal

The unit cell contains three atoms: two carbons and an oxygen. From our X-ray diffraction experiment, we determined that the electron density in the unit cell looks like the following:



Now, if we have a function that content superposition of many different webs with different amplitudes frequency and phase such as in our case the diffracted X-ray signal we pick up on the

detector in an experiment, what should we do here, we can perform an inverse Fourier transform which we talked about to determine the individual frequencies of the diffracted X-ray waves as well as their relative strengths.

To illustrate the notion of tracking sine waves, consider an imaginary one-dimensional crystal to make this an easy case. And by looking at that, we could have developed our idea on the complex crystal that unit cell content three atoms is a simple 1-dimensional crystal where the unit cell contains three atoms to carbon and oxygen. From our X-ray diffraction experiment, we determined that the electron density in the unit cell looks like the following. So, this is the thing we get initially.

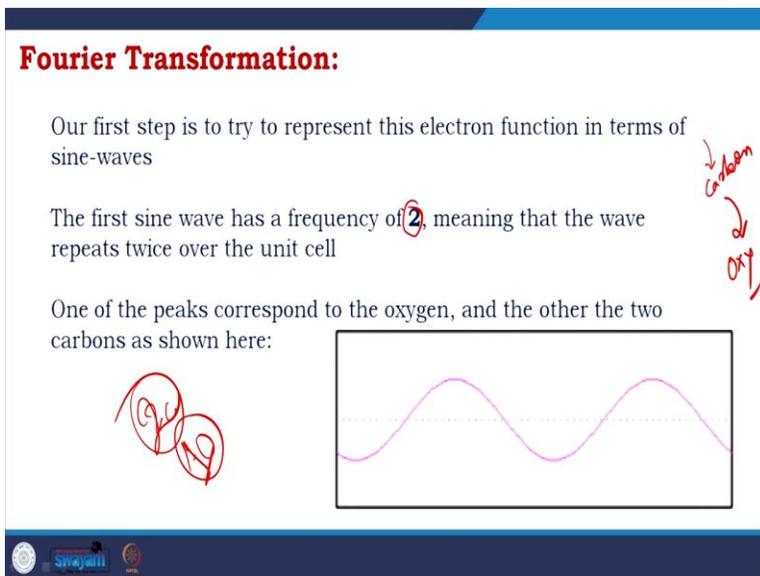
(Refer Slide Time: 41:21)

Fourier Transformation:

Our first step is to try to represent this electron function in terms of sine-waves

The first sine wave has a frequency of 2, meaning that the wave repeats twice over the unit cell

One of the peaks correspond to the oxygen, and the other the two carbons as shown here:



The slide contains a diagram of a sine wave with two peaks and a handwritten diagram of three atoms labeled 'C', 'O', and 'C'. There are also handwritten red annotations: 'Carbon' with an arrow pointing to the first peak, and 'oxy' with an arrow pointing to the second peak.

Our first step is to represent this electron function in sine waves. The first sine wave has a frequency of 2, which means that the wave repeats twice over the unit cell. So, you have three atoms, two carbon, and oxygen. One would be representing the carbon, and one would be the present oxygen. So, one of the peaks corresponds to the oxygen and the other the two carbons, as we can see here.

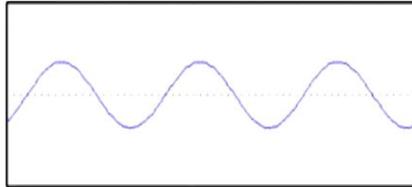
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Fourier Transformation:

The second sine wave has a frequency of **3**

Which means that there are three repeats of the wave across the unit cell

It has a different phase: That mean we start at a different place on the wave. The amplitude is also different as shown here:



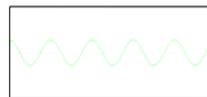
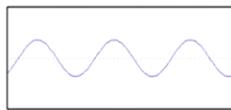
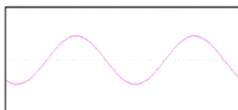
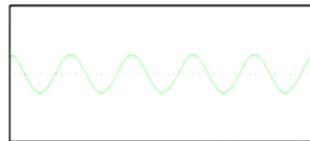
Then we take a second sine wave with a frequency of 3, which means that there are three wave repeats across the unit cell. It has a different phase that means we start at a different place on the wave; the amplitude is also different, you can see here.

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Fourier Transformation:

Lastly, we add the last sin wave with a frequency of **5**

You see that two of the peaks of this wave are lined up with the carbon atoms:



Lastly, we added the last sine wave with a frequency of 5. Now, you can see there at 2 of the peaks. These waves are lined up with the carbon atom. And now, if we take all the three and combine them, we will get the original one.

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Fourier Transformation:

In this fashion we have just shown that we can decompose our original density function into three different sine-waves

Since different lattice spacing's will diffract X-rays differently, each decomposed sine-wave will give us a characteristic of the crystal structure

Therefore by reconstructing the frequency spectrum from the inverse Fourier transform, we can determine the relative strength of different waves that are contributing to form our signal

Therefore, performing an inverse Fourier transforms on the signal will give us valuable information on our crystal structure.

In these ways, we have just shown that we can decompose so that we can decompose our original density function into three different sine waves from the main. Since different lattice spacings will diffract X-rays differently, each decomposed sine wave will give us a crystal structure characteristic. Therefore, by reconstructing the frequency spectrum from the inverse Fourier transform, we can determine the relative strength of different waves contributing to our signal. Therefore, performing an inverse Fourier transform on the signal will give us valuable information on our crystal structure.

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Fourier Transformation:

In our example, you can see that the sum of the three sine-waves is a good approximation of the original density function

This indicates that we can describe the unit cell quite well using only three sine-waves, given the correct choice of frequency, amplitude and phase

In general however, we would need to use many different sine-waves to build an accurate reconstruction of our original function

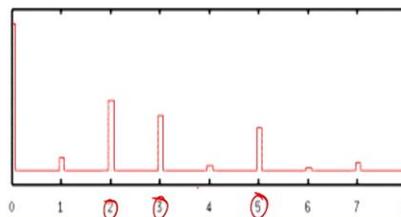
In our example, you can see that the sum of the three sine waves is a good approximation of the original density function. This indicates that we can describe the unit cell quite well using only three sine waves given the correct choice of frequency amplitude and phase. But in general, we would need to use many different sine waves to build accurate reconstructions of our original function.

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Fourier Transformation:

Now if we look at the inverse Fourier transform of the unit cell, we find that the result consists of a series of peaks, the largest of which are at **2**, **3** and **5** on the frequency axis

These peaks correspond exactly to the sine-wave frequencies that we used to reconstruct the unit cell. The height of the peaks also correspond to the amplitude of the three waves:



Now, if we look at the inverse Fourier transform the unit cell, we find that the result consists of a series of peaks, the largest of which are 2, 3, and 5 on the frequency axis. These peaks correspond exactly to the sine wave frequencies we used to reconstruct the unit cell. The height of the peaks also corresponds to the amplitude of the three waves.

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Fourier Methods in Diffraction Theory:

In the case of protein crystallography, the aperture function is regularly periodic (due to the repetition of the unit cell in the lattice) electron density distribution within our crystals.

The electron density is the inverse Fourier transform of the diffraction pattern expressed as follows:

$$\rho(x, y, z) = 1/V_{\text{unit cell}} \sum_h \sum_k \sum_l F(hkl) e^{-2\pi i(hx+ky+lz)}$$

where $V_{\text{unit cell}}$ = volume of one unit cell and $F(hkl)$ is called the structure factor for a particular set of Miller indices h , k and l . We can do a summation here, instead of integrating, because we know we will only have reflections at integer values for h , k and l .

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$V_{\text{unit cell}}$ is the volume of one unit cell, and $F(hkl)$ is the structural factor for a particular set of Miller indices h , k , and l . We can do a summation here instead of integrating because we know we will only have reflection at integer values for h , k and l .

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Fourier Methods in Diffraction Theory:

For each point in a diffraction, there is a corresponding spatial frequency.

Therefore, the distribution of a far-field diffraction pattern is the Fourier transform of the aperture function.

(aperture - an opening, often adjustable, that controls the amount of light reaching the lens on a camera or other optical instrument.)

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For each point in diffraction, there is a corresponding spatial frequency. So, the far field diffraction pattern distribution is the Fourier transform of the aperture function. Aperture is an opening often adjustable that controls the amount of light reaching the lens on a camera or other optical instrument which controls the array.

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Phase Problem:

The structure factors calculated with **Formula 3**, ie from the known atomic structure, are represented by vectors (modules and phases)

$$F(hkl) = \sum_{j=1}^n f_j e^{2\pi i[hx+ky+lz]} \quad \text{Formula 3}$$

Their numerical values, corresponding to the so-called absolute scale, since they are calculated with the dispersion factors (f_j) that depend on the atom numbers

However, the conventional situation is the opposite one

That is, we normally pretend to solve **Formula 4**, to determine the structure of the crystal by solving the function of electronic density at each point of the unit cell



The structure factor, calculated with formula three from the known atomic structure, is represented by vectors of the modules and phases.

$$F(hkl) = \sum f_j e^{2\pi i[hx + ky + lz]}$$

Their numerical values correspond to the so-called absolute scale, since they are calculated with the dispersion factor (f_j) that depends on the atom numbers.

However the conventional situation is the opposite one.

We normally pretend to solve formula 4, where I am coming to determine the crystal structure by solving the function of electronic density at each point of the unit cell.

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$$\rho(xyz) = \frac{1}{V} \sum_{hkl} |F(hkl)| \cdot e^{-2\pi i[hx+ky+lz-\phi(hkl)]} \quad \text{Formula 4}$$

For this purpose we have to measure experimentally the structure factors using the X-ray diffraction

However, we must remember that experimentally we can only measure their modules, and therefore we have to face the so-called **phase problem**

The modules of the experimental structure factors are related to the **intensities of the diffracted beams**, but these are in a relative scale

Because they depend on multiple experimental aspects, such as the crystal dimensions and the brightness of the primary X-ray beam.

So, as I talked about formula 4, this is the formula electron density

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

summation hkl - infinity to + infinity

For this purpose, we have to measure the structure factor experimentally using X-ray diffraction, which is possible. However, we must remember that experimentally we can only measure their modules amplitude, and therefore we have to face the phase problem.

The module of the experimental structure factors is related to the diffracted beams' intensities, but these are in relative scale. Because they depend on multiple experimental aspects such as the crystal dimension and the brightness of the primary X-ray beams.