
1 X-Ray Physics

1.1 Introduction

One of the main methods to decode the structure of condensed matter on atomic scale is x-ray diffraction. While it was originally used to analyze crystalline structures such as metals, it can also be used to gain insights on the structure of proteins. In that case, the protein must either be available in its crystallized form or contain crystalline components, as is the case for spider silk.

In the electromagnetic spectrum x-rays are to be found between ultraviolet light and high energy gamma rays. The wavelength range lies between 0.01 and 10 nm and is therefore of the order of distances between molecules and crystal lattice constants. X-rays with wavelengths longer than 1 nm are called *soft* x-rays, those of shorter wavelengths are considered *hard*. Hard x-rays may range into the regime of low energy γ rays, since the distinction between the two is derived from the source of radiation, not its wavelength. X-rays are generated by energetic electron processes, gamma rays by transitions in atomic nucleons.

1.2 Creation of x-rays

Today, there are two commonly used different methods to create x-rays. The first is based on decelerating very fast electrons by firing them at a heavy metal target. As explained by Maxwell's equations, the electrons will emit electromagnetic radiation upon deceleration, which is called the *Bremsstrahlung*. The true interest lies, however, in the electrons that have enough energy to knock metal electrons out of their shells. This will cause electrons from upper shells to drop down and fill the created gap, thereby emitting a photon with an energy corresponding to the energy difference between the levels (figure 1). The x-rays produced by transitions from the $n = 2$ to the $n = 1$ levels are called K_α x-rays, those for $n = 3 \rightarrow 1$ are called K_β x-rays. The energy efficiency of this process is only about 0.1%, most is lost in heat. Two of the most common elements used to create x-rays in this fashion are copper and molybdenum.

The alternative is to use x-ray radiation from synchrotrons, which are usually linear electron accelerators combined with storage rings. Here, the electrons are kept flying in a circle at a well-defined speed, giving off reasonably monochromatic x-rays as they go. The brilliance of the x-rays by far surpasses that of the ones created by characteristic radiation. Electron storage rings are huge and expansive, however, two of the most famous ones being the DAISY in Hamburg, the ESRF in Grenoble and the CERN in Genève.

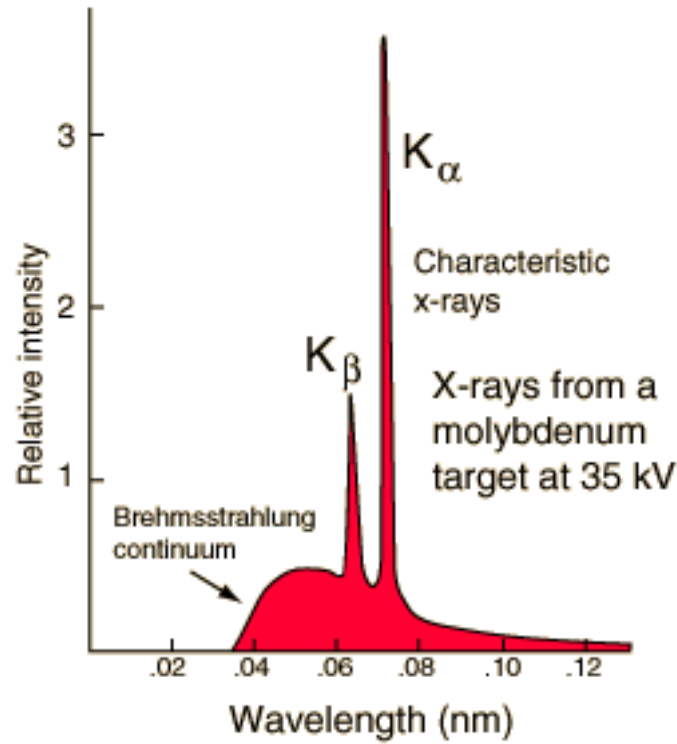


Fig. 1: Bremsstrahlung with characteristic radiation peaks. Taken from [1].

1.3 Basics of x-ray scattering

1.3.1 Thomson scattering

In the classical description of the scattering process, an electron is forced to vibrate by the electric field of an incoming x-ray beam. Because the mass of an electron is over a thousand times lighter than that of a nucleon, the resulting vibration and the corresponding emitted intensities are a lot higher. This is the reason why x-rays give a picture of the electron densities of a material and not of the nucleon positions themselves. To derive the scattering intensities of a material, it is therefore useful to start with the scattering by a single electron and move from there to atoms, molecules and crystals. Using Maxwell's equations, it can be derived that the ratio of magnitude between radiated and incident field of a single electron is given by:

$$\frac{E_{rad}(R, t)}{E_{in}} = - \left(\frac{e^2}{4\pi\epsilon_0 mc^2} \right) \frac{e^{ikR}}{R} \cos\psi. \quad (1)$$

The prefactor

$$r_0 = \frac{e^2}{4\pi\epsilon_0 mc^2} = 2.82 \cdot 10^{-6} \text{ nm}$$

1.3 Basics of x-ray scattering

is called the *Thomson scattering length*, the angle ψ is the angle between incident and scattered beam, R is the distance between the observer and the electron, $k = (2\pi)/\lambda$ is the absolute value of the wave vector, e is the elementary charge, c the speed of light, m the mass of the electron, λ the x-ray wavelength and ϵ_0 the electric constant.

1.3.2 Scattering by a single atom

Now the scattering from an atom with Z electrons can be described. For simplicity, a purely classical description is used, so that the electron distribution can be described as a number density $\rho(\vec{r})$. The resulting radiation field is the superposition of the contributions from all different volume elements of the charge distribution. Let \vec{k} and \vec{k}' be the wave vectors of the incident and the scattered wave, respectively. Then the phase difference between a wave that is scattered by a volume element at the origin and one scattered at point \vec{r} is:

$$\Delta\phi(\vec{r}) = (\vec{k} - \vec{k}') \cdot \vec{r} = \vec{Q} \cdot \vec{r}.$$

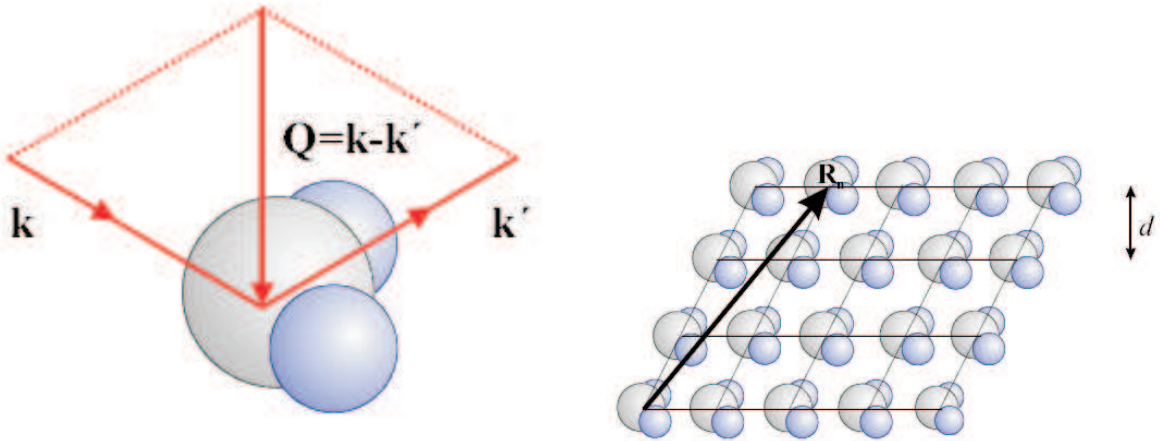


Fig. 2: Left: Wave vector transfer \vec{Q} during scattering from a molecule. Right: A two dimensional crystal lattice with intersheet spacing d . Taken from [2].

\vec{Q} is called the wave vector transfer. Assuming elastic scattering $|\vec{k}| = |\vec{k}'|$, the scattering triangle shown in figure 2 yields $|\vec{Q}| = 2|\vec{k}|\sin\theta$. So a volume element $d\vec{r}$ at \vec{r} contributes with an amount of $-r_0\rho(\vec{r})d\vec{r}$ to the scattered field with a phase factor of $e^{i\vec{Q}\cdot\vec{r}}$. The total scattering length of an atom is then given by

$$-r_0 f^0(\vec{Q}) = -r_0 \int \rho(\vec{r}) e^{i\vec{Q}\cdot\vec{r}} d\vec{r}. \quad (2)$$

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$f^0(\vec{Q})$ is the *atomic form factor*. In the limit of small \vec{Q} all volume elements scatter in phase, so that for $\vec{Q} = 0$ the atomic form factor can be taken to be Z , the number of electrons in the atom. The larger \vec{Q} becomes, the more the different volume elements scatter out of phase so that in the limit where \vec{Q} approaches infinity, the atomic form factor becomes zero. Dropping the classical approach from above, one has to consider the binding energy of the electron's shell, which will reduce its ability to respond to the driving field by $f'(\hbar\omega)$, and a phase lag with respect to the driving field related to absorption by $if''(\hbar\omega)$. So the full atomic form factor is:

$$f(\vec{Q}, \hbar\omega) = f^0(\vec{Q}) + f'(\hbar\omega) + if''(\hbar\omega). \quad (3)$$

Here $\hbar = h/(2\pi)$, where h is Planck's constant, and ω is the angular frequency.

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Based on the *Thomson* scattering described above, we look at scattering from more complicated objects such as molecules and crystals. To allow for an easier description of such complex structures, the scattering is assumed to be weak. That is, the effect of multiple scattering will be completely neglected. This simplification is known as the *kinematical approximation*.

1.4.1 Two and more electrons

The simplest possible system to look at is that composed of two electrons. Assuming one is placed at the origin and the other at point \vec{r} , the scattering vector for elastically scattered x-rays is given by $|\vec{Q}| = \left(\frac{4\pi}{\lambda}\right) \sin \theta$, where θ is the scattering angle. For an incident beam polarized perpendicular to the plane spanned by \vec{k} and \vec{k}' to maximize the Thomson acceleration of the electrons, the scattering amplitude can be expressed as

$$A(\vec{Q}) = -r_0 \left(1 + e^{i\vec{Q}\vec{r}}\right), \quad (4)$$

which corresponds to an intensity of

$$I(\vec{Q}) = A(\vec{Q})A(\vec{Q})^* = 2r_0^2 \left(1 + \cos(\vec{Q} \cdot \vec{r})\right).$$

For an assembly of N electrons labelled by index j the amplitude can be generally written as:

$$A(\vec{Q}) = -r_0 \sum_{\vec{r}_j} e^{i\vec{Q}\vec{r}_j}. \quad (5)$$

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The problem with the above description is that the orientation of the vector \vec{r} has to be specified with respect to the wave vector transfer. For molecules or aggregates in solution this orientation varies with time and the measured intensity is a superposition of all possible orientations.

1.4.2 Orientational averaging

To calculate the scattering from two different particles with scattering amplitudes f_1 and f_2 , one stationed at the origin and the other at position \vec{r} , we start with the assumption, that the particles are stationary. Then we can write the scattering amplitude as

$$A(\vec{Q}) = f_1 + f_2 e^{i\vec{Q}\vec{r}}$$

and the intensity is given by

$$A(\vec{Q}) = f_1^2 + f_2^2 + f_1 f_2 e^{i\vec{Q}\vec{r}} + f_1 f_2 e^{-i\vec{Q}\vec{r}}.$$

Now we leave the distance between the molecules fixed, but allow its direction to vary randomly with time. The measured intensity can then be obtained by averaging over all possible orientations. Assuming spherical symmetry of the involved particles this gives:

$$\langle I(\vec{Q}) \rangle_{\text{orient. av.}} = f_1^2 + f_2^2 + 2f_1 f_2 \langle e^{i\vec{Q}\vec{r}} \rangle_{\text{orient. av.}}. \quad (6)$$

The orientational average of the phase factor is given by:

$$\langle e^{i\vec{Q}\vec{r}} \rangle = \frac{\int e^{iQr \cos \theta} \sin \theta \, d\theta d\phi}{\underbrace{\int \sin \theta \, d\theta d\phi}_{=4\pi}}$$

The integral in the enumerator may be evaluated as follows:

$$\int e^{iQr \cos \theta} \sin \theta \, d\theta d\phi = 2\pi \int_0^\pi e^{iQr \cos \theta} \sin \theta d\theta = 2\pi \left(\frac{-1}{iQr} \right) \int_{iQr}^{-iQr} e^x dx = 4\pi \frac{\sin(Qr)}{Qr}$$

So the orientational average of the phase factor may be written as:

$$\langle e^{i\vec{Q}\vec{r}} \rangle_{\text{orient. av.}} = \frac{\sin(Qr)}{Qr}. \quad (7)$$

Graphs resulting from such calculations are shown in figure 3.

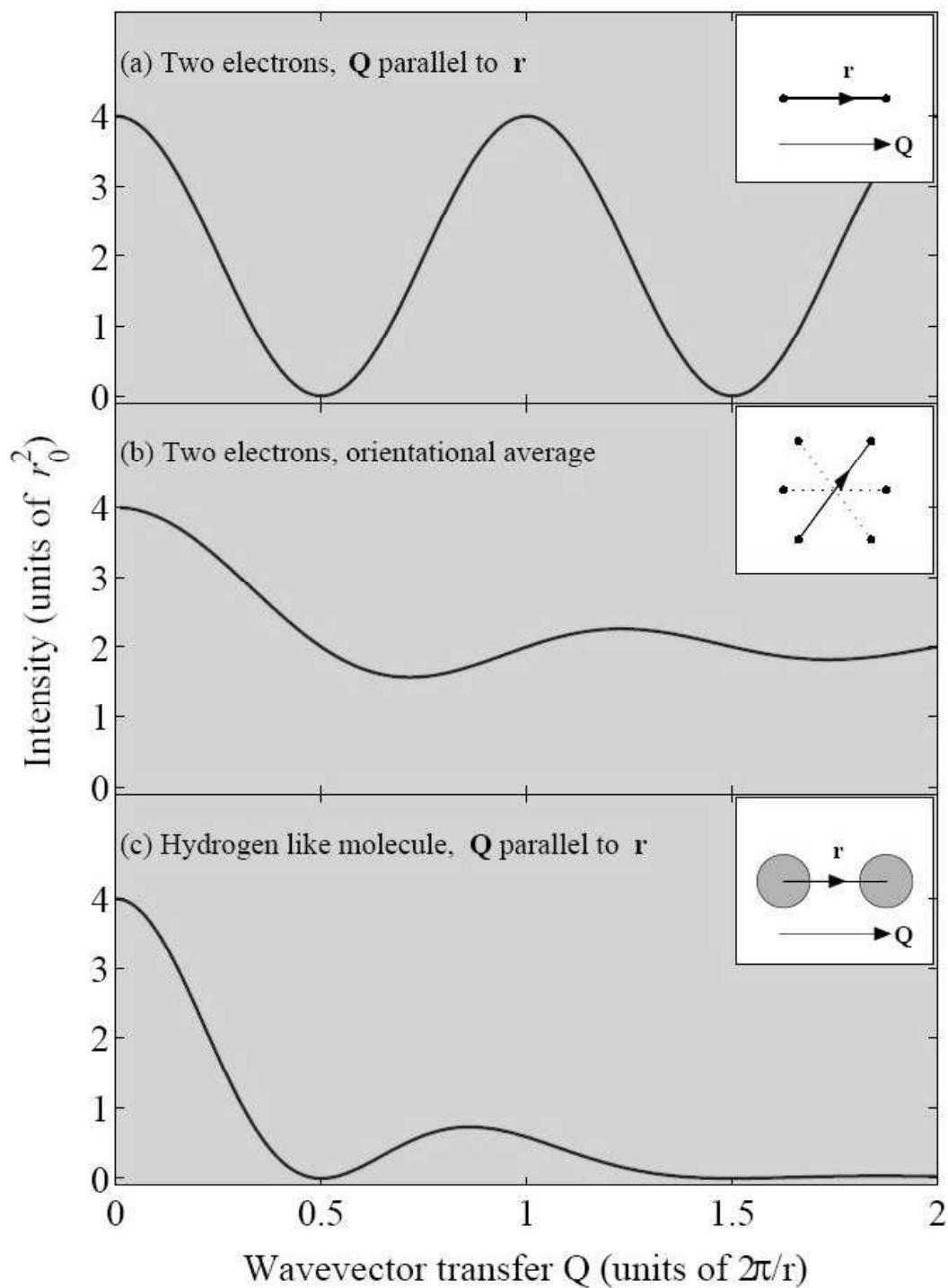


Fig. 3: Calculated intensity for two electrons (a) without and (b) with orientational averaging, (c) intensity of a hydrogen-like system.

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1.4.3 Scattering from a molecule

A molecule is a specific arrangements of atoms, so labelling the atoms by j , the scattering amplitude can be written as follows:

$$F^{mol}(\vec{Q}) = \sum_{\vec{r}_j} f_j(\vec{Q}) e^{i\vec{Q}\vec{r}_j}.$$

For molecules, the orientational averaging is more tedious to calculate. Nevertheless, Debye derived the following expression for a system of N particles with the scattering amplitudes $f_1, f_2 \dots f_N$ in 1915:

$$\begin{aligned} \left\langle \left| \sum_{j=1}^N f_j e^{i\vec{Q}\vec{r}_j} \right|^2 \right\rangle = & |f_1|^2 + |f_2|^2 + \dots + |f_N|^2 \\ & + 2f_1f_2 \frac{\sin(Qr_{12})}{Qr_{12}} + 2f_1f_3 \frac{\sin(Qr_{13})}{Qr_{13}} + \dots + 2f_1f_N \frac{\sin(Qr_{1N})}{Qr_{1N}} \\ & + 2f_2f_3 \frac{\sin(Qr_{23})}{Qr_{23}} + \dots + 2f_2f_N \frac{\sin(Qr_{2N})}{Qr_{2N}} + \\ & \dots + 2f_{N-1}f_N \frac{\sin(Qr_{N-1,N})}{Qr_{N-1,N}}, \end{aligned} \quad (8)$$

where $r_{ij} = |\vec{r}_i - \vec{r}_j|$.

1.4.4 Example: CF₄

Now let us consider tetrafluoromethane (figure 4), i.e. CF₄, as an example. The four fluor atoms are placed on the vertices A, B, C, D of a tetrahedron with the carbon atom in the center O . Labelling the four CF bonds by R , the length of each of the six FF -bonds is given by $\sqrt{8/3}R$. For simplicity let us assume that the scattering vector \vec{Q} be parallel to one of the CF -bonds. Then the molecular scattering factor may be expressed as:

$$F^{mol}(\vec{Q}) = f^C(Q) + f^F(Q) (3e^{-iQR/3} + e^{-iQR})$$

Calculating the orientational average according to equation 6 gives (figure 5):

$$|F^{mol}|^2 = |f^C|^2 + 4|f^F|^2 + 8f^C f^F \frac{\sin(QR)}{QR} + 12|f^F|^2 \frac{\sin(Q\sqrt{8/3}R)}{Q\sqrt{8/3}R}$$

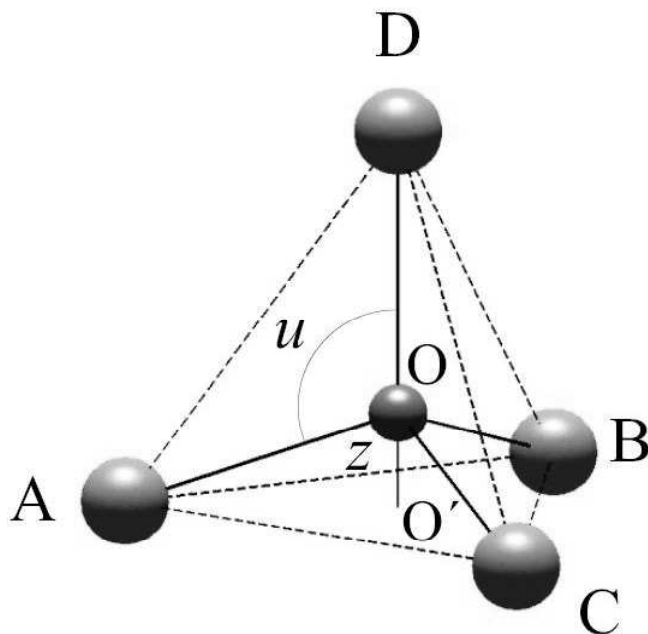
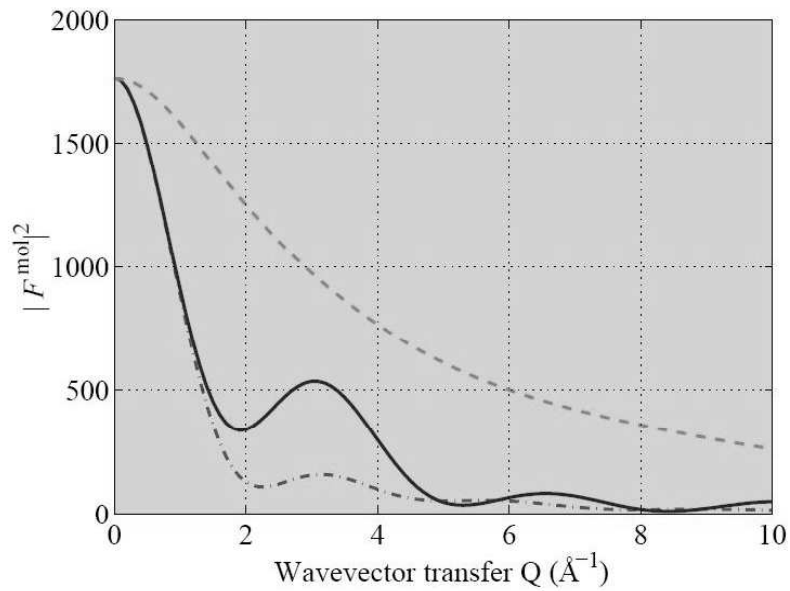
Fig. 4: The CF_4 molecule

Fig. 5: Amplitude of the structure factor squared. Solid line: without, dash-dotted line: with orientational average, dotted: molybdenum, which possesses the same number of electrons

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1.4.5 Side note: Primitive and conventional unit cell

Prior to discussing the scattering from crystals, it seems appropriate to repeat the two main definitions of unit cells as they are commonly used in condensed matter physics. Any three dimensional lattice may be represented by a set of lattice vectors as follows:

$$\vec{R}_n = n_1\vec{a}_1 + n_2\vec{a}_2 + n_3\vec{a}_3$$

Every such (*Bravais*-) lattice possesses characteristic translational and rotational symmetries. Furthermore we can choose a set of vectors for each lattice, such that the volume of the corresponding unit cell is minimized. The unit cell of minimum volume is called the *primitive* unit cell, the vectors spanning it are the *primitive* lattice vectors. In case the unit cell ought to better represent the structure of the crystal, a non-minimal choice for the unit cell might be desirable. Such unit cells are called *conventional* (figure 6).

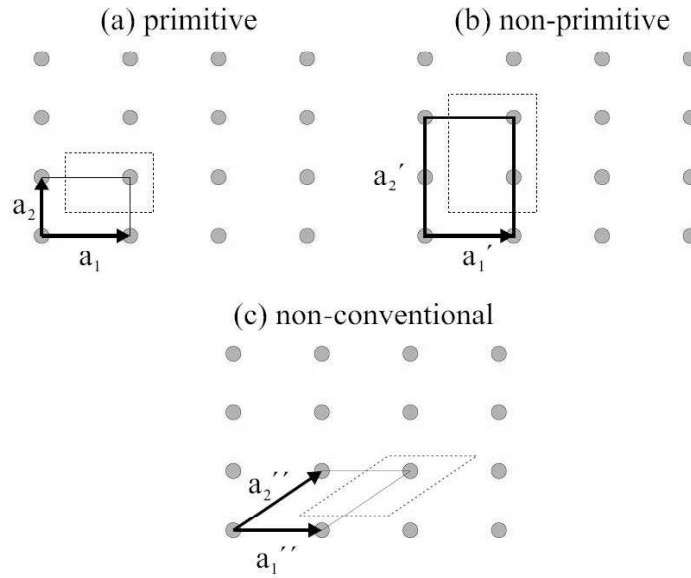


Fig. 6: primitive (a), non-primitive (b) and non-conventional (c) unit cells

To represent a complete crystal structure, one additionally requires a basis of atoms or molecules to be assigned to each lattice point (figure 7).

Considering all imaginable symmetries of basis and lattice results in a variety of 32 possible point groups and 230 symmetry groups. Refer to section 4.1 for some information on the Miller indices used to describe different sets of planes. The lattice in real space is called *direct* lattice to allow a distinction from lattices defined on different spaces, such as the *reciprocal lattice* (figure 8).

(d) Crystal=lattice+basis

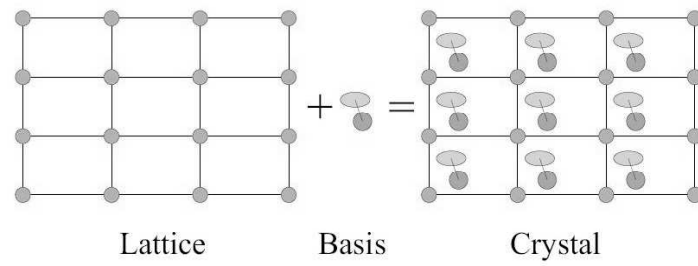


Fig. 7: Creation of a crystal structure using its lattice and basis

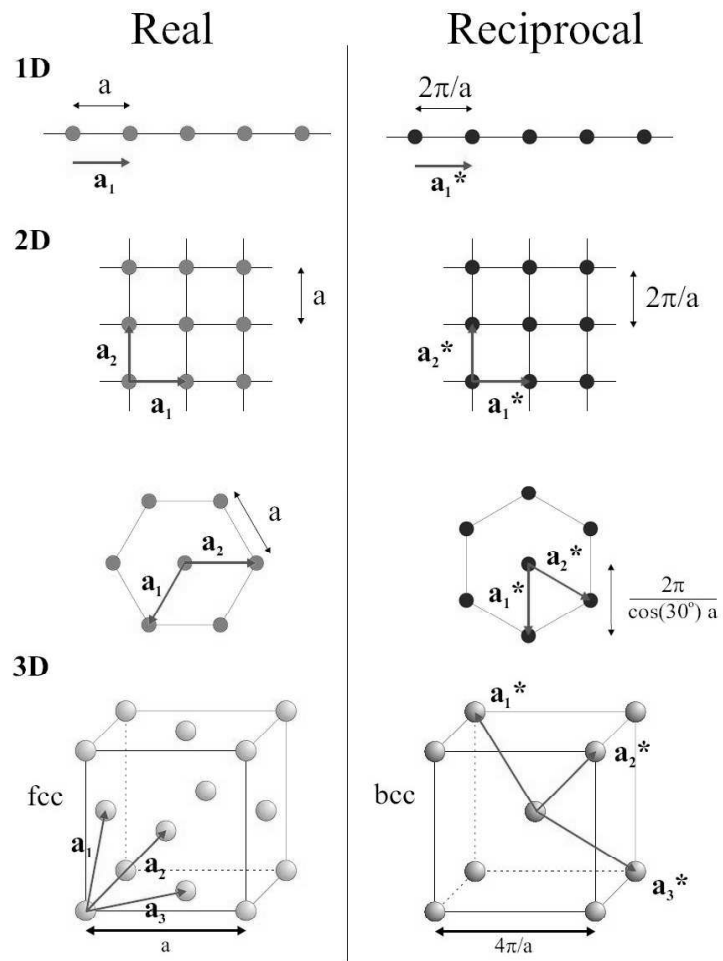


Fig. 8: Examples for direct and reciprocal lattice in one, two and three dimensions.

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1.4.6 Scattering from a crystal lattice

A crystal lattice in three dimension can be characterized by a set of vectors

$$\vec{R}_n = n_1 \vec{a}_1 + n_2 \vec{a}_2 + n_3 \vec{a}_3.$$

Here n_1 , n_2 and n_3 are integers, while the vectors \vec{a}_1 , \vec{a}_2 and \vec{a}_3 define the three dimensional unit cell of the crystal. To gain a complete description of a crystal, we need to construct a basis of molecules or atoms which should be the same for each and every lattice site. The simplest possible approach to x-ray diffraction from such a crystal is to treat it as an interference of rays scattered by a multitude of equidistant lattice planes. Maximum constructive interference occurs, when the path difference between scattered waves is an integer multiple of the wavelength λ . For an incident ray making an angle θ with the scattering lattice planes, set a distance d apart from each other, the path difference between the incoming and the scattered ray is given by $2d \sin \theta$. The formula to find the maxima of the corresponding interference pattern known as *Bragg's law* is

$$m\lambda = 2d \sin \theta, \quad (9)$$

where m is an integer value (see figure 9). For a more advanced treatment of x-ray diffraction, that gives insight on the precise intensities between the maxima, it is important to have a good way of describing the families of lattice planes responsible for the observed interference patterns. The convention is to use the Miller indices (h, k, l) defined as follows: For a given family of planes, the plane closest to the origin has intercepts $(a_1/h, a_2/k, a_3/l)$ with respect to the axes $\vec{a}_1, \vec{a}_2, \vec{a}_3$ and h, k, l all have integer values. Negative intercepts are represented with a bar over the number. It may be shown that for a cubic lattice with lattice parameter a , the spacing between a certain family of planes is given by

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}.$$

Any atom can now be found by the sum of a vector \vec{R}_n , specifying its unit cell, and a vector \vec{r}_j , specifying its position within the unit cell. This means that the scattering amplitude for a crystal factorizes into two terms and may be written as

$$F^{crystal}(\vec{Q}) = \underbrace{\sum_{\vec{r}_j} F_j^{mol}(\vec{Q}) e^{i\vec{Q}\vec{r}_j}}_{\text{structure factor}} \underbrace{\sum_{\vec{R}_n} e^{i\vec{Q}\vec{R}_n}}_{\text{lattice sum}}. \quad (10)$$

The first term is the so-called *structure factor* from the basis of the molecules, the second is the *lattice sum*. The latter term is of enormous importance, because the summation is over a huge number of terms ($\geq 10^{12}$). Each of the terms is a complex number

1.4 Kinematical diffraction

located somewhere on the unit circle. Because the orientation is random, the summation is of order unity. But in case all phases are 2π or a multiple thereof, the sum will suddenly be equal to the huge number of terms. We are therefore interested in solving.

$$\vec{Q}\vec{R}_n = 2\pi \cdot \text{integer}. \quad (11)$$

The ideal solution is to construct a lattice in wave vector or *reciprocal* space spanned by the basis vectors $(\vec{a}_1^*, \vec{a}_2^*, \vec{a}_3^*)$ which fulfill

$$\vec{a}_i \cdot \vec{a}_j^* = 2\pi\delta_{ij}.$$

Here δ_{ij} is the Kronecker delta, i.e. $\delta_{ij} = 1$ for $i = j$ and zero otherwise. Using this definition all points on the reciprocal lattice are described by

$$\vec{G} = h\vec{a}_1^* + k\vec{a}_2^* + l\vec{a}_3^*,$$

where h, k, l are the Miller indices. It is now evident, that all vectors on the reciprocal lattice satisfy equation 11 because the scalar product of \vec{G} and \vec{R}_n is given by

$$\vec{G} \cdot \vec{R}_n = 2\pi(hn_1 + kn_2 + ln_3),$$

where there are only integer values within the parenthesis so the sum is also an integer. This implies a non-vanishing scattering amplitude will be measured, if \vec{Q} equals a reciprocal lattice vector. This condition is called the *Laue* condition for the observation of x-ray diffraction.

$$\vec{Q} = \vec{G} \quad (12)$$

To find the reciprocal vectors corresponding to a given lattice, the following algorithm may be used

$$\vec{a}_1^* = \frac{2\pi}{V_{uc}} \vec{a}_2 \times \vec{a}_3, \quad \vec{a}_2^* = \frac{2\pi}{V_{uc}} \vec{a}_3 \times \vec{a}_1, \quad \vec{a}_3^* = \frac{2\pi}{V_{uc}} \vec{a}_1 \times \vec{a}_2,$$

where $V_{uc} = \vec{a}_1 \cdot (\vec{a}_2 \times \vec{a}_3)$ is the volume of the unit cell in real space and a, b, c are its side lengths. It is worthwhile to note that this condition is fully equivalent to the *Bragg* condition for reflection from atomic planes with the spacing $d = 2\pi / |\vec{G}|$.

$$\lambda = 2d \sin \theta \quad (13)$$

The proof can be found in [2] and is indicated in figure 9.

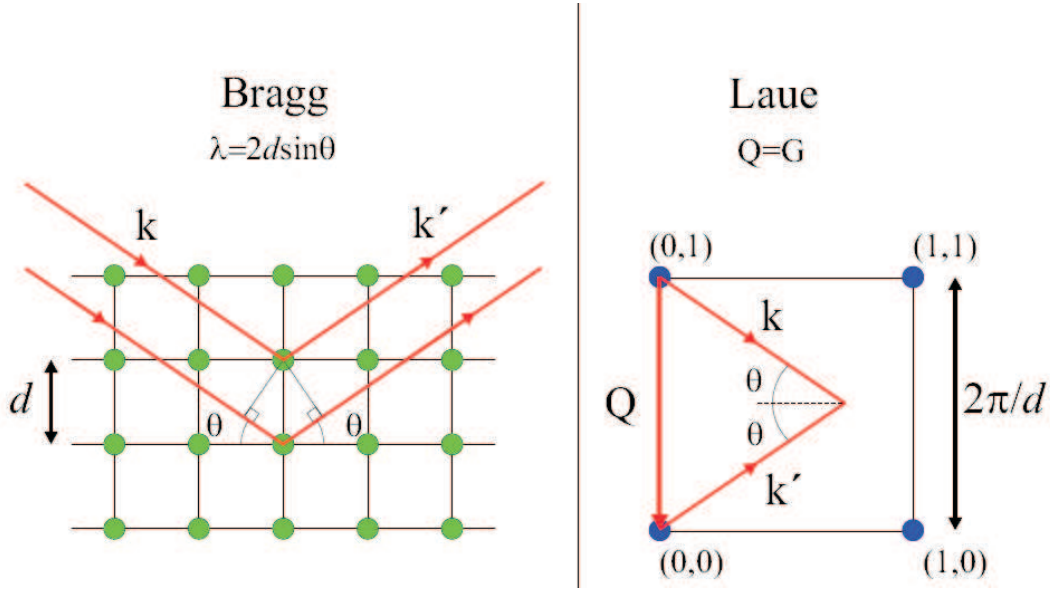


Fig. 9: Equivalence of Bragg (left) and Laue (right) [2].

1.4.7 Reconstruction of crystal structures

The seemingly simple issue of going from a measured x-ray structure back to the corresponding real space lattice is in reality quite complicated, since not the F , but the square of the scattering amplitude $|F|^2$ is measured. This means that any phase information is lost in the process. This is referred to as the *phase problem* and is one of the main topics of crystallography.

To get a decent idea of what the diffraction pattern of a certain crystal looks like despite this problem, it suffices to take equation 10 and assume that $F_j^{mol}(\vec{Q}) = F_j^{mol} = Z$. The real problem is of course to figure out what kind of crystal structure is responsible for the scattering pattern observed in an experiment. In most cases this is an under-determined inverse problem and it is usually solved by making assumptions about the structure beforehand. This is necessary, because only the intensity, i.e. the square of the structure factor, can be measured in an experiment and the phase information is lost in the process. Therefore, a simple fourier back transform will not yield the desired result. Let us start with the simplest possible assumption, that the scattering pattern is derived from a cubic lattice. In this case the spacing of the system using Miller indices is given by $\frac{1}{d^2} = \frac{(h^2+k^2+l^2)}{a^2}$, where a is the side length of the unit cell. If this assumption is correct according to Bragg's law the following equation must hold

$$\sin^2 \theta = \frac{\lambda^2}{4a^2} (h^2 + k^2 + l^2) = \frac{\lambda^2}{4a^2} s = \text{const.}$$

Depending on whether the cubic lattice is body-centered, face-centered or diamond cubic the values of s vary accordingly and allow a decision between the three types. For

1.5 The Debye-Scherrer formula

non-cubic crystal systems the expressions for the lattice spacings turn out to be more complicated. A few examples are given below

$$\begin{aligned}\text{tetragonal} : 1/d^2 &= a(h^2 + k^2) + cl^2 \\ \text{hexagonal} : 1/d^2 &= a(h^2 + hk + k^2) + cl^2 \\ \text{orthorombic} : 1/d^2 &= ah^2 + bk^2 + cl^2\end{aligned}$$

For more complex structures, such as folded proteins, a simple crystal system will not suffice as approximation to give a decent result. So in the case of crystallized proteins, the diffraction patterns are taken at a variety of different angles and from those patterns an electron density profile is calculated. Combining this with the initial knowledge about the structure of proteins even this kind of problem becomes solvable.

1.5 The Debye-Scherrer formula

At this point we drop the assumption of an infinitely large crystal and instead focus on the influence of the crystal size on the scattering intensity profile, which will then no longer be composed of delta peaks as equation 11 implies. If the path difference between rays scattering in the first and second layer of a crystal is only a quarter wavelength, these two rays do not cancel each other completely. It is the wave from the third layer that is necessary to gain a path difference of half a wavelength and completely cancel the ray reflected from the first layer. Analogously, the second and fourth layer along with the third and fifth and so on would cancel each other. If the path difference between the first two planes is just slightly less than an integral number of wavelengths, as is the case near Bragg peaks, the plane scattering a ray completely out of phase with the one scattered in the first layer lies deep within the crystal. So if the crystal is too small for this plane to exist, the intensity cannot be cancelled out completely. Let us now examine a crystal of thickness t in direction perpendicular to the reflecting planes. The Bragg angle θ now serves as variable with θ_B being the angle that exactly satisfies the Bragg condition.

$$\lambda = 2d \sin \theta_B$$

In figure 10 the rays A , D and M make precisely this angle with the reflecting planes. Ray D' , scattered by the first plane below the surface, is one wavelength out of phase with A' , ray M' is m wavelengths out of phase with it. At the diffraction angle $2\theta_B$ all these rays are in phase and unite to form a beam of maximum amplitude. Ray B makes a slightly larger angle θ_1 with the reflecting plane, such that ray L' from the m th plane is $(m + 1)$ wavelengths out of phase with B' . This means that in the middle of the crystal there is a plane scattering a ray that is exactly an integer plus one-half wavelength out of phase with B' . So the rays scattered by the upper half of the crystal cancel exactly with those scattered by the lower half of the crystal and θ_1 is the smallest angle where complete destructive interference occurs. This is also the case for an angle θ_2 which is

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a bit smaller than θ_B so that the path difference between the ray scattered by the first and the last plane is $(m - 1)$ wavelengths. These are the two limiting angles where the intensity of the diffracted beam drops to zero. Note that this implies that the intensity is greater than zero all the way from θ_2 to θ_1 as depicted in figure 11.

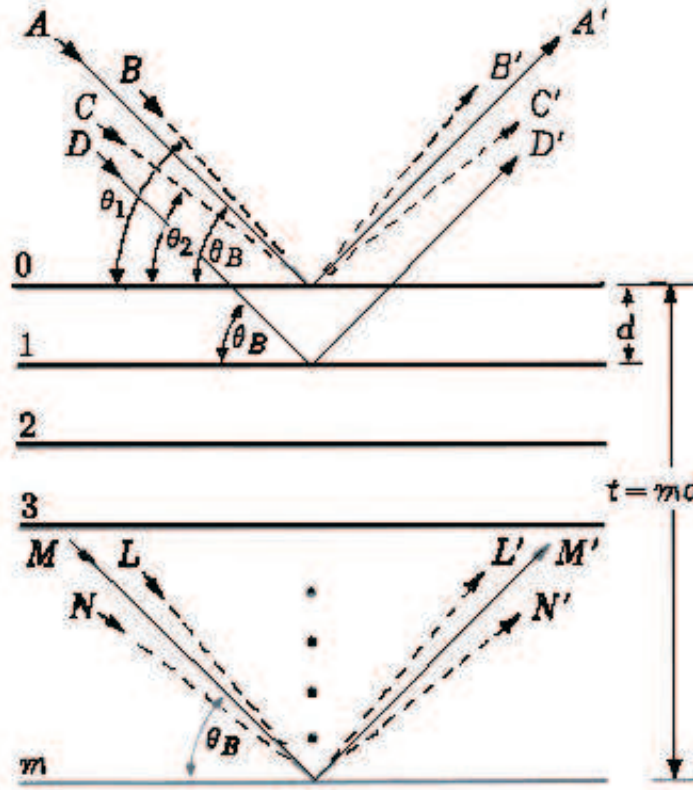


Fig. 10: Scattering from a finite number of equidistant planes [3].

The width of diffraction curves increases as the thickness of the crystal decreases, because the angular range $(2\theta_1 - 2\theta_2)$ increases as m decreases. As a measure of the peak width, the full width at half maximum *FWHM*, denoted by B , is used. As an approximation $B = \frac{1}{2}(2\theta_1 - 2\theta_2) = \theta_1 - \theta_2$ is chosen, since this yields the exact FWHM for a Gaussian. The path difference equations for these two angles related to the entire thickness of the crystal are given by:

$$2t \sin \theta_1 = (m + 1)\lambda$$

$$2t \sin \theta_2 = (m - 1)\lambda$$

Subtracting the above equations yields:

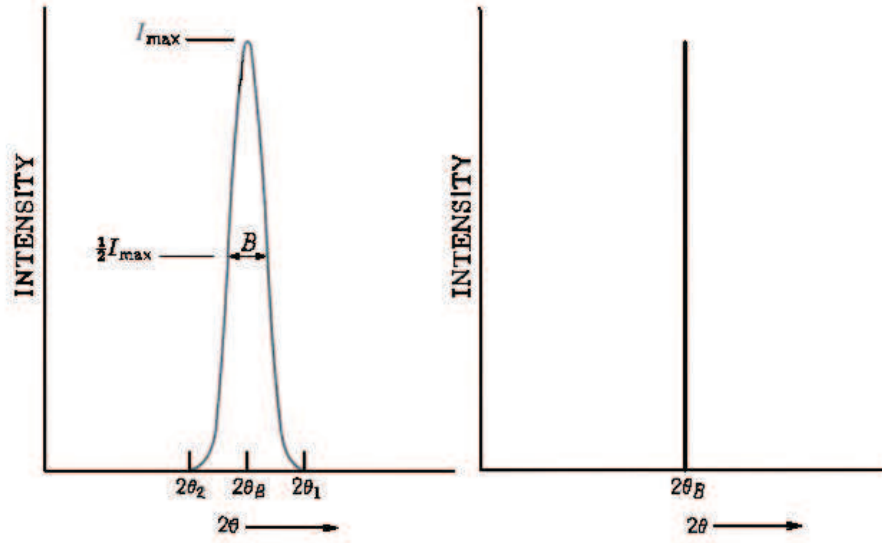


Fig. 11: FWHM for a crystal of finite (left) and infinite (right) size [3].

$$t(\sin \theta_1 - \sin \theta_2) = \lambda$$

$$\Leftrightarrow 2t \cos \left(\frac{\theta_1 + \theta_2}{2} \right) \sin \left(\frac{\theta_1 - \theta_2}{2} \right) = \lambda$$

Since θ_1 and θ_2 are very close to θ_B it is reasonable to make the following approximations:

$$\sin \theta_1 + \sin \theta_2 = 2 \sin \theta_B$$

$$\sin \left(\frac{\theta_1 - \theta_2}{2} \right) = \left(\frac{\theta_1 - \theta_2}{2} \right)$$

With these approximations we obtain

$$2t \left(\frac{\theta_1 - \theta_2}{2} \right) \cos \theta_B = \lambda$$

and using the definition of the FWHM introduced above gives a crystal depth $t = m \cdot d$ of

$$t = \frac{\lambda}{B \cos \theta_B}.$$

1.5 The Debye-Scherrer formula

A more rigorous mathematical treatment of the problem results in the *Debye-Scherrer* formula:

$$t = \frac{K\lambda}{B \cos \theta_B} \quad (14)$$

Here K is a dimensionless constant that may range from 0.89 to 1.39 depending on the specific geometry of the scattering objects. For a perfect two-dimensional lattice, where every point on the lattice emits a spherical wave, numerical calculations yield the lower bound of 0.89 for K [4]. A cubic three-dimensional crystal is best described by $K = 0.94$, while analytical calculations for a perfectly spherical object yield $K = 1.33$ [5]. For a crystal of 50 nm thickness, a wavelength of 0.15 nm and a plane spacing of 0.15 nm the diffraction curve would broaden to $4 \cdot 10^{-3}$ radians or 0.2° which is easily observable.

2 Diffraction from proteins and organic materials

2.1 Differences to diffraction from crystals

Differences between diffraction from crystals and diffraction from polymers, proteins or other organic substances are multiple (see figure 12), we decided to name the most important here:

- very large molecules with large molecular weights
- may be crystalline, semi-crystalline or amorphous
- unit cell very large, so reciprocal unit cell very small
- anisotropy of ordering in polymer samples
- frequently encountered in form of fibers
- way more diffraction reflexes, amorphous halos,...

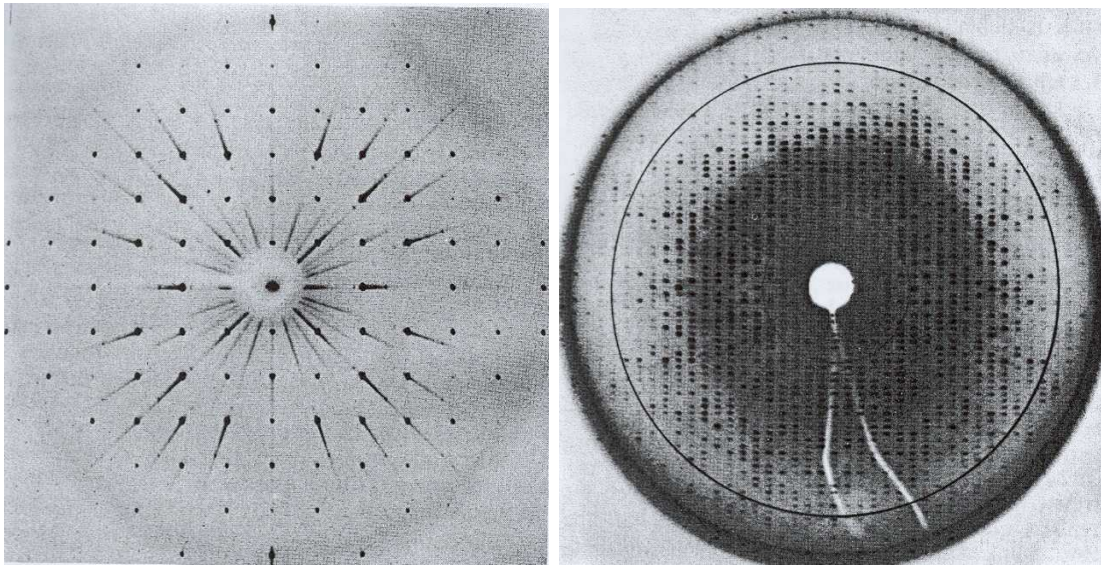


Fig. 12: Examples of diffraction patterns for a crystalline material (left) and the protein myoglobin (right).

2.2 Scattering from a single helix

As an example of more complex scattering, we will now derive the scattering pattern for a single helix. For sake of simplicity, assume the helix to be infinitely long and have the material be uniformly distributed (figure 13). Let the radius be labelled R and the period

2.2 Scattering from a single helix

P . As we know $F(\vec{Q}) \propto \int e^{i\vec{Q}\vec{r}} dz$. Letting the axis of the helix point in the direction of the z-axis, we may rewrite its position vector as:

$$\vec{r} = \left(R \cos\left(\frac{2\pi z}{P}\right), R \sin\left(\frac{2\pi z}{P}\right), z \right)$$

The integral above now decomposes into a sum over all periods multiplied by a structure factor of a single period. To calculate the phase, we express \vec{Q} in cylindrical coordinates:

$$\vec{Q} = (Q_{\perp} \cos(\psi), Q_{\perp} \sin(\psi), Q_z)$$

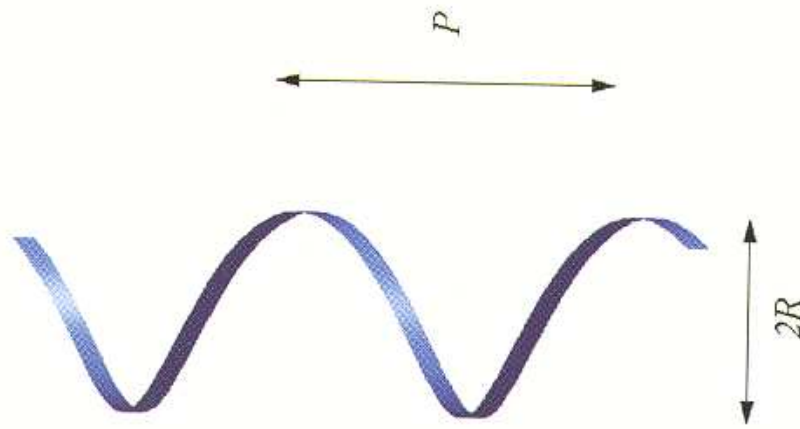


Fig. 13: Sketch of a helix.

After a little calculation, we obtain the scattering amplitude:

$$F(\vec{Q}) \propto e^{in\psi} \int e^{iQ_{\perp} R \cos \psi + in\phi} dz$$

where $\phi = \frac{2\pi z}{P} - \psi$. Recalling the definition of Bessel functions

$$J_n(\xi) = \frac{1}{2\pi i^n} \int_0^{2\pi} e^{i\xi \cos \phi + in\phi} d\phi,$$

we may rewrite our solution as follows:

$$F(Q_{\perp}, \psi, Q_z) \propto e^{in\psi} J_n(Q_{\perp} R) \quad (15)$$

The intensity resulting from this equation is shown in figure 14. The blue dots are the principle maxima arising from peaks in the Bessel functions. The gray circles are secondary maxima of the zeroth order Bessel function. The scattering vector \vec{Q} has been decomposed into its components parallel and perpendicular to the axis of the helix.

2.3 Scattering from a double helix

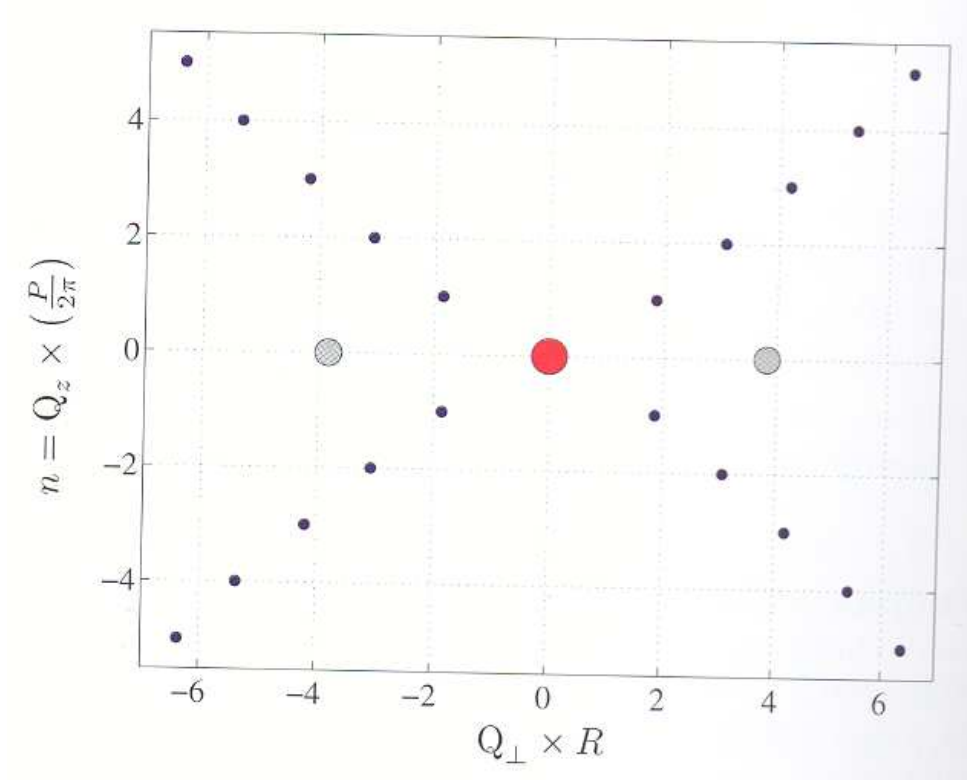


Fig. 14: The squared structure factor obtained from the analytical solution (\approx intensity).

2.3 Scattering from a double helix

To bridge the gap to double-stranded DNA, assume that two helices are displaced along a common z -axis by Δ . This corresponds to an azimuthal angle of $\psi = 2\pi(\Delta/P)$, which leads to a scattering amplitude of:

$$F(Q_{\perp}, \psi, Q_z) \propto (1 + e^{in(2\pi/P)\Delta}) J_n(Q_{\perp}R) \quad (16)$$

A comparison of this calculated intensity with the experimental results obtained for double-stranded DNA is shown in figure 15. Based on the diffraction results, the following helix properties may be calculated:

- period of the helix ≈ 2.4 nm from position of the peaks on vertical axis
- radius ≈ 1 nm from angle of cross
- displacement of helices $\approx 3/8 \cdot P$

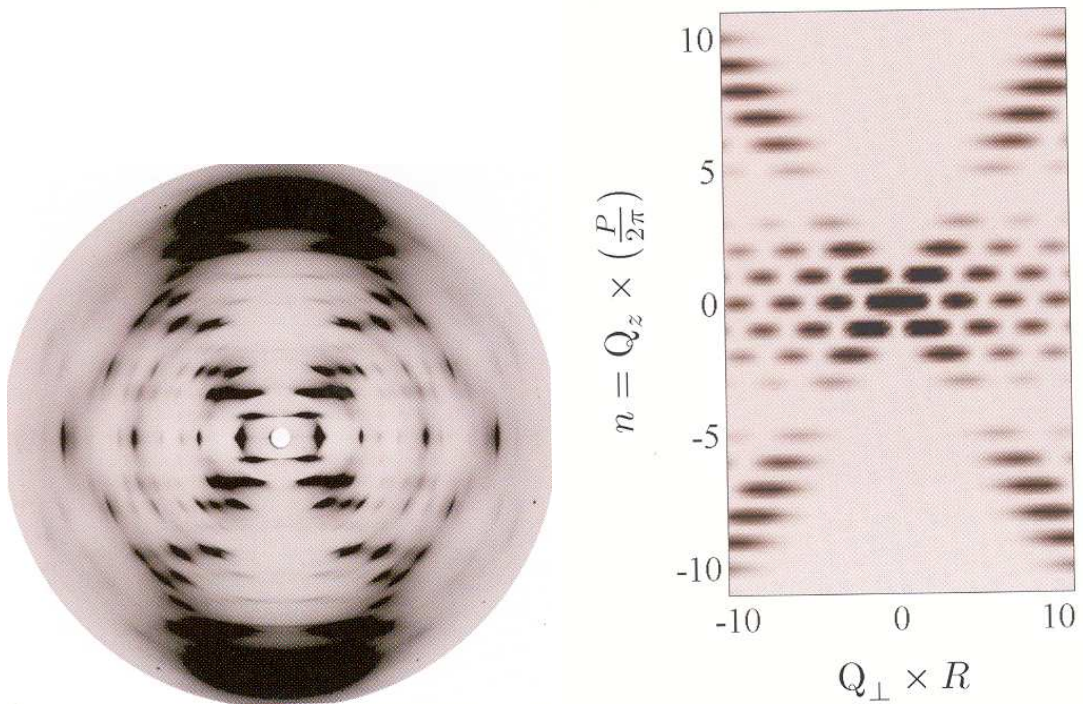


Fig. 15: Scattering intensity for double helix: experimental for DNA (left) and calculated (right).

3 Notes on other x-ray topics

3.1 Powder and fiber diffraction

For a huge perfect crystal, one would expect to see only dots on a 2D x-ray detector. For a perfect powder sample, one would expect to see rings, since the Bragg condition specifies the distances between the resulting maxima, but there is no distinguished direction. For fiber diffraction, crystalline regions inside are often aligned along the fiber axis, but never perfectly, so the diffraction pattern shows peaks smeared out into circles, which allow computation of the alignment. An x-ray scattering result for spider silk obtained at the ESRF in Grenoble from a single fiber is shown below in figure 16.

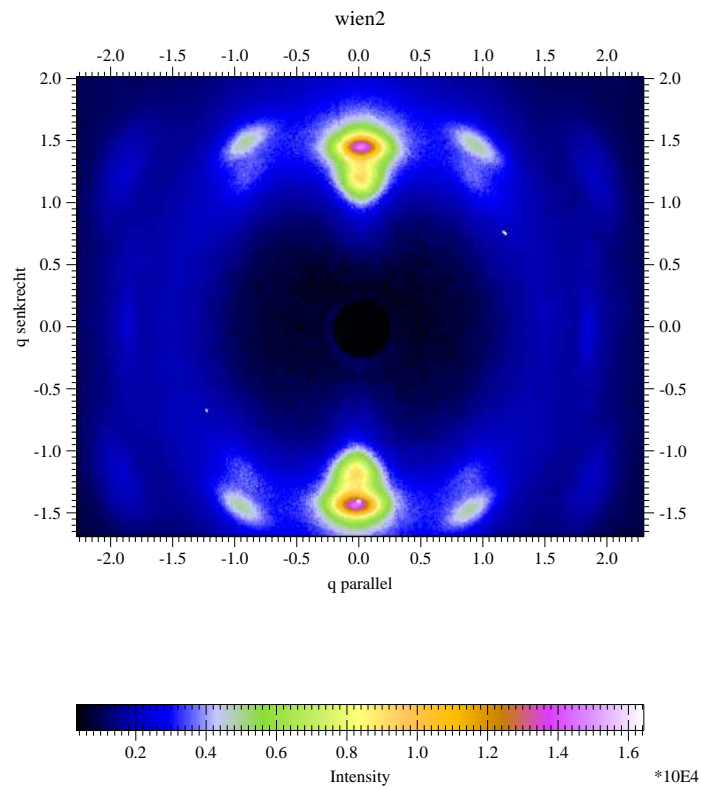


Fig. 16: WAXS results for single spider silk fiber obtained at the ESRF.

3.2 WAXS and SAXS

The abbreviations mean wide angle and small angle x-ray scattering. Note that WAXS is the standard method, since large scattering distances mean small real space dimensions, which one is usually interested in. When doing SAXS one is interested not in the unit cell, but possibly in an ordering between crystallites in an amorphous matrix or so. To this end, the detector has to be placed a couple meters away from the sample with enough intensity must still arriving there to make out the peaks. As an example, one can see the distance between crystallites in spider silk using SAXS. However, the silk must be wet for those peaks to appear. The reason is that there must be a density difference between the structures to make them visible in x-ray scattering. When dry, this is not the case, but the water only penetrates the amorphous regions leading to a density contrast between them and the crystallites.

3.3 Debye-Waller factor

The Debye-Waller factor (DWF), named after Peter Debye and Ivar Waller, is used in condensed matter physics to describe the attenuation of x-ray scattering or neutron scattering caused by thermal motion or quenched disorder. It has also been called the B factor or the temperature factor.

The factor depends on q , the absolute value of the scattering vector \vec{q} . For a given q , it gives the fraction of elastic scattering; $(1-DWF(q))$ correspondingly the fraction of inelastic scattering. In diffraction studies, only the elastic scattering is useful; in crystals, it gives rise to distinct Bragg peaks. Inelastic scattering events are undesirable as they cause a diffuse background - unless the energies of scattered particles are analysed, in which case they carry valuable information (inelastic neutron scattering).

Assuming harmonicity of thermal motion in the material under study, the DWF takes the form:

$$DWF = e^{-\langle [\vec{q}\vec{u}]^2 \rangle} = e^{-q^2 \langle [\vec{u}(0)]^2 \rangle / 3}$$

where $\langle \dots \rangle$ denotes thermal averaging and $\vec{u}(t)$ is the displacement of a scattering center as a function of time t [6].

4 Connected topics and details

4.1 Miller indices

Since one is interested in different sets of planes when dealing with x-ray physics, it is important to be able to find and describe equivalent planes efficiently. For this purpose the Miller indices were developed. The Miller indices corresponding to a certain set of planes are calculated as follows (figure 17):

- i. Initially compute the intersect of the planes with the crystallographic axes. The result in 3D will be a set of three fractions, which indicate the intersection point between the normalized basis vectors of the unit cell and the planes. In case there is no such point, ∞ is used.
- ii. Then one calculates the reciprocal value of the above fractions.
- iii. The resulting fractions are expanded to give natural numbers. Obviously, for $1/\infty$ this yields zero.
- iv. Negative numbers are represented by a bar above.
- v. The triplet hkl will be placed in parentheses, when it refers to a specific plane, in accolades, when it refers to the entirety of all crystallographic planes described by the same indices.

Now the distance between two sets of planes may also be calculated based upon the Miller indices. Of course, this depends on the type of unit cell. In the simplest case of a cubic lattice, one obtains:

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

where a refers to the side length of a cubic unit cell.

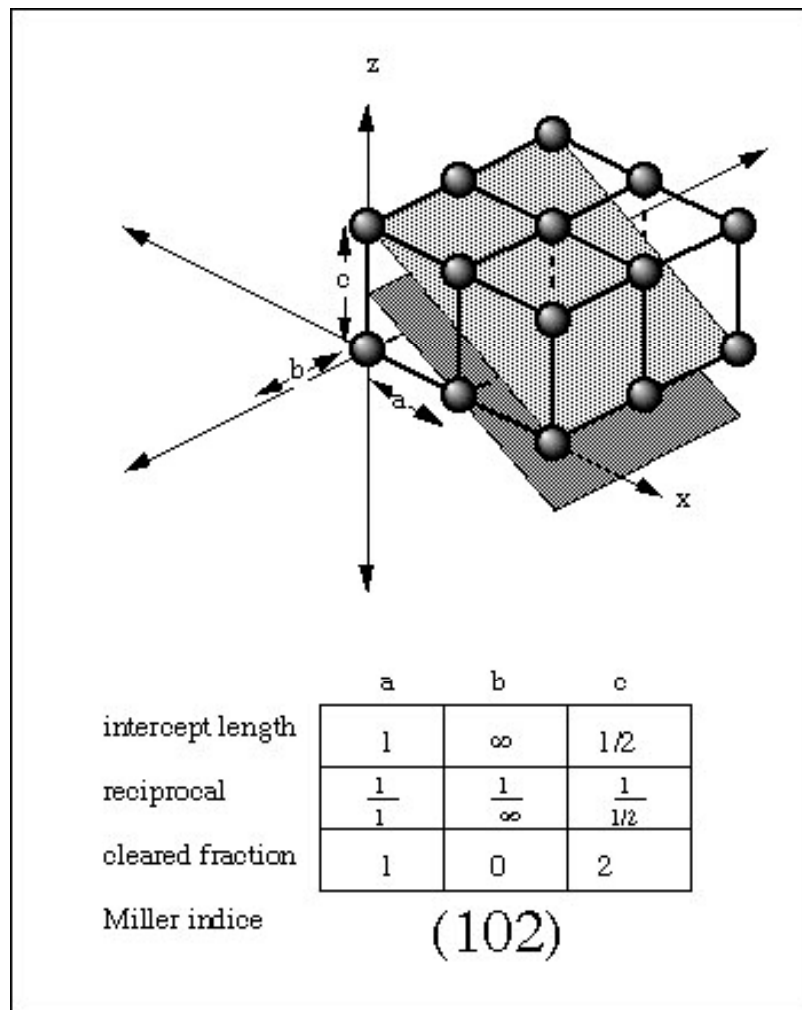


Fig. 17: Determination of the Miller indices for the (102) plane.

4.2 Scattering of a plane wave

A plane wave is given by $Ae^{i(\vec{k}_0\vec{r}-\omega t)}$, where A is the amplitude, \vec{k}_0 the wave vector and ω the angular frequency. Planes of equal phase for an infinitely large plane wave are planes perpendicular to the direction of the wave's propagation and therefore also perpendicular to the wave vector. After a time Δt these planes will have travelled a certain distance into the direction of the wave vector (figure 18).

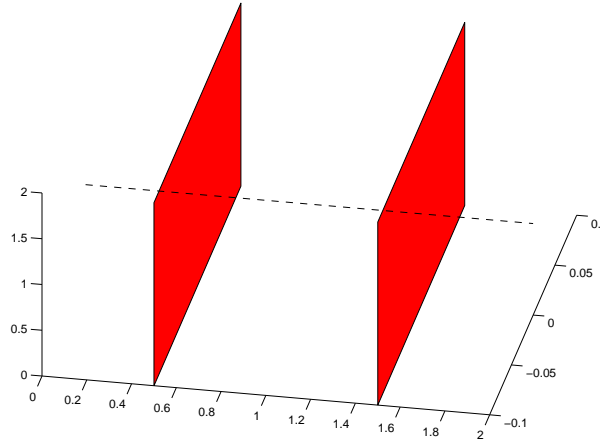


Fig. 18: The dotted line indicates the direction of the wave vector, the planes are areas of equal phase at the times t and t_0 .

When a plane wave encounters a point particle, it will emit spherical (elementary) waves in response:

$$e^{i\vec{k}_0\vec{r}} \rightarrow e^{i\vec{k}_0\vec{r}} + f(r, \Omega)e^{i\vec{k}_1\vec{r}}$$

The factor $f(r, \Omega)$ incorporates anisotropic and distance dependent scattering amplitudes. This shall be neglected and set to one for the following computations. In the far field the elementary waves may be treated as plane waves again. We shall look at the phase difference of waves scattered by two neighboring particles, which depends on the scattering vector $\vec{q} = (\vec{k}_1 - \vec{k}_0)$. We can see on the left side of (figure 19) that the optical retardation δ between particle one and two is given by:

$$\delta = \frac{1}{k_1}\vec{k}_1\vec{r}_e - \frac{1}{k_0}\vec{k}_0\vec{r}_e$$

Since we intend to treat elastic scattering, the amplitude of the wave vector is identical before and after scattering. Letting $k_1 = k_0 = k$, we obtain:

$$\delta = \frac{1}{k}(\vec{k}_1 - \vec{k}_0)\vec{r}_e$$

4.2 Scattering of a plane wave

The phase difference $\Delta\phi$ is now nothing other than $\frac{2\pi}{\lambda}\delta$ - or $k\delta$, so we have:

$$\Delta\phi = (\vec{k}_1 - \vec{k}_0) \cdot \vec{r}_e = \vec{q} \cdot \vec{r}_e$$

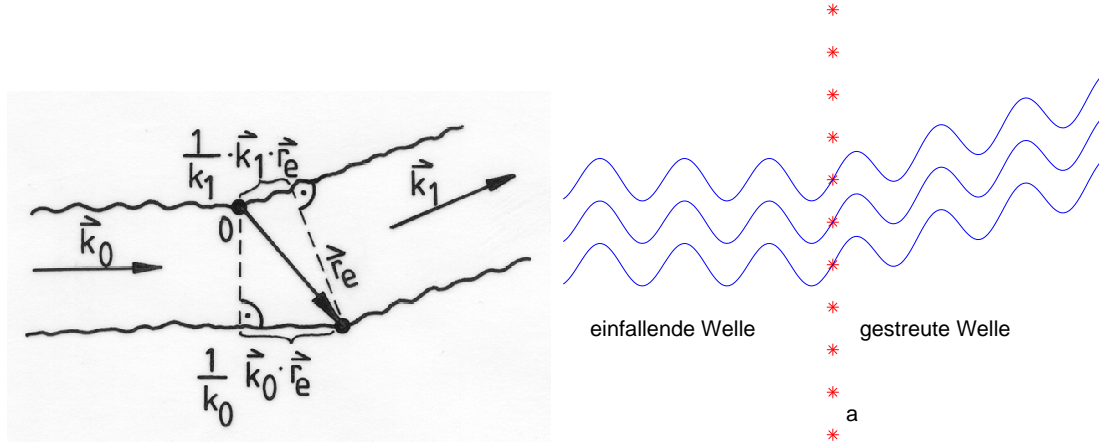


Fig. 19: Scattering from two particles (left) and from N particles arranged periodically along the z -axis (right).

Of greater interest is the intensity resulting from scattering from N particles as illustrated on the right of figure 19. The wave function after scattering from N particles is simply the superposition of the waves emitted by each of the particles. We label the particle from zero to $(N-1)$ and place the first particle at the origin for prettier mathematical results.

$$\begin{aligned} \psi_{res} &= \psi_0 + \psi_2 + \dots + \psi_{N-1} = e^{i\vec{k}_0 \cdot \vec{r}} e^{i\phi_0} + e^{i\vec{k}_0 \cdot \vec{r}} e^{i\phi_1} + \dots + e^{i\vec{k}_0 \cdot \vec{r}} e^{i\phi_{N-1}} \\ &= e^{i\vec{k}_0 \cdot \vec{r}} (e^{i\vec{q} \cdot \vec{r}_{00}} + e^{i\vec{q} \cdot \vec{r}_{01}} + \dots + e^{i\vec{q} \cdot \vec{r}_{0(N-1)}}) \end{aligned}$$

Thanks to the simple geometry we chose $\vec{r}_{0n} = na\vec{e}_z$, which leads to a phase difference of $\vec{q} \cdot \vec{r}_{0n} = naq_z$. So the resulting wave function is:

$$\begin{aligned} \psi_{res} &= e^{i\vec{k}_0 \cdot \vec{r}} (1 + e^{iaq_z} + e^{i2aq_z} + \dots + e^{iNaq_z}) \\ &= e^{i\vec{k}_0 \cdot \vec{r}} \left(\sum_{n=0}^{N-1} e^{inaq_z} \right) \end{aligned}$$

Since the intensity is the absolute value squared of the wave function, i.e. $I = |\psi|^2$, we may write:

4.2 Scattering of a plane wave

$$\begin{aligned}
 I &= \left| e^{i\vec{k}_0 \vec{r}} \sum_{n=0}^{N-1} e^{inaq_z} \right|^2 = \left| e^{i\vec{k}_0 \vec{r}} \right|^2 \left| \sum_{n=0}^{N-1} e^{inaq_z} \right|^2 \\
 &= \left| \sum_{n=0}^{N-1} e^{inaq_z} \right|^2
 \end{aligned}$$

For the last step, we used $|e^{ix}| = 1$. Remembering the geometric series:

$$\sum_{n=0}^N x^n = \frac{1 - x^{N+1}}{1 - x} \Leftrightarrow \sum_{n=0}^{N-1} x^n = \frac{1 - x^N}{1 - x}$$

we may simplify the expression further. In our case $x = e^{iaq_z}$. Notice that the above formula holds true for all complex values, not just for those where $|x| < 1$. This condition is only required in the limit $N \rightarrow \infty$. Our simplification yields:

$$I = \left| \sum_{n=0}^{N-1} (e^{iaq_z})^n \right|^2 = \left| \frac{1 - e^{iNaq_z}}{1 - e^{iaq_z}} \right|^2$$

For any complex number $|z|^2 = z\bar{z}$ holds, where \bar{z} is the complex conjugate of z . Additionally using $e^{ix} = e^{-ix} = \cos(x) - i\sin(x)$ allows the following transformations:

$$\begin{aligned}
 I &= \left(\frac{1 - e^{iNaq_z}}{1 - e^{iaq_z}} \right) \left(\frac{1 - e^{-iNaq_z}}{1 - e^{-iaq_z}} \right) = \frac{2 - e^{iNaq_z} - e^{-iNaq_z}}{2 - e^{iaq_z} - e^{-iaq_z}} \\
 &= \frac{2 - 2\cos(Naq_z)}{2 - 2\cos(aq_z)} = \left[\frac{\sqrt{\frac{1 - \cos(Naq_z)}{2}}}{\sqrt{\frac{1 - \cos(aq_z)}{2}}} \right]^2 \\
 &= \left| \frac{\sin(\frac{Naq_z}{2})}{\sin(\frac{aq_z}{2})} \right|^2
 \end{aligned}$$

For the last step the trigonometric identity $\sin(\frac{\alpha}{2}) = \sqrt{\frac{1 - \cos\alpha}{2}}$ was used, leading to the desired simplified result without complex exponentials.

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