Most of the information that we have on protein structure comes from x-ray crystallography. The basic steps in finding a protein structure using this method are:

- a high quality crystal is formed from a sample of protein
- the crystal is placed in an x-ray beam and the intensities of the diffraction spots are measured
- after finding the phases, an electron density map is computed from the diffraction intensities and phases using Fourier analysis.
- The coordinates of the atoms are found from the electron density.
- The structure is refined by checking that, for example, the atoms do not get too close to each other.

We will briefly discuss some of the mathematics involved in finding the electron density from the diffraction intensities and phases. This requires studying Fourier series for functions periodic on lattices.

6.1. **Lattices.** The basic structure of a crystal is that of a lattice. A crystal is formed by many copies of the same protein in a pattern is formed which fills a unit cell. The unit cell is a parallelepiped which is used as a tile whose translations fill up space. For mathematical simplicity, we can suppose that there are an infinite number of copies of the same protein.

A lattice is easy to describe mathematically. A lattice $L$ in three dimensions is generated by three linearly independent vectors $\mathbf{a}, \mathbf{b}, \mathbf{c}$; it is the set of all vectors $h\mathbf{a}+k\mathbf{b}+l\mathbf{c}$ where $h, k$ and $l$ are integers. A unit cell is the set of points $x\mathbf{a}+y\mathbf{b}+z\mathbf{c}$ for $0 \leq x \leq 1, 0 \leq y \leq 1, 0 \leq z \leq 1$. Similarly we can define a lattice in two dimensions as the set of integer combinations of two linearly independent vectors $\mathbf{a}$ and $\mathbf{b}$ and the unit cell is the parallelogram formed by the points $x\mathbf{a}+y\mathbf{b}$ for $0 \leq x \leq 1, 0 \leq y \leq 1$. In the pdb file for a crystal structure, you can find the lengths and angles between the vectors $\mathbf{a}, \mathbf{b}$ and $\mathbf{c}$ which generate the lattice for the crystal.

The origin of the coordinate system can be put at any point crystallized protein. If the origin is placed at an atom in the protein, then every lattice point will be on exactly the same atom in a translation of the protein.

6.1.1. **Examples of lattices.** First consider two dimensions. The vectors $\mathbf{a} = (1,0)$ and $\mathbf{b} = (0,1)$ generate the *square lattice*. The vectors $\mathbf{a} = (1,0)$ and $\mathbf{b} = (1/2, \sqrt{3}/2)$ generate the *hexagonal lattice*. 
In three dimension the vectors \( \mathbf{a} = (1, 0, 0) \), \( \mathbf{b} = (0, 1, 0) \), and \( \mathbf{c} = (0, 0, 1) \) generate the cubic lattice. The vectors \( \mathbf{a} = (1, 1, 0) \), \( \mathbf{b} = (1, 0, 1) \), \( \mathbf{c} = (0, 1, 1) \) generate the face centered cubic lattice. The face centered cubic lattice can also be described as the set of points \( (x, y, z) \) with integer coordinates such that \( x + y + z \) is even.

For an illustration of lattices see the Maple demo.

The lattices described above are examples of lattices with symmetries. A symmetry of a lattice is a rotation such that the rotation and its inverse maps every point in the lattice onto another point of the lattice. We say the rotation leave the lattice unchanged. The square lattice, for example, is left unchanged by 90 degree rotation, an order 4 symmetry. The hexagonal lattice is left unchanged by 60 degree rotation, an order 6 symmetry. The cubic lattice is left fixed by 90 degree rotation about any axis. These are order 4 symmetries. The face centered cubic lattice is left fixed by 120 degree rotation about the axis in the direction \( (1, 1, 1) \). This is an order 3 symmetry.

6.2. The crystallographic restriction. A symmetry of the lattice for a crystal shows up in a symmetry of the diffraction pattern. It can be proved mathematically that the symmetry of a lattice has the crystallographic restriction: it has no rotational symmetries of order 5 or greater than 6. The crystallographic restriction can be stated as follows.

A lattice in the plane or in three-dimensional space cannot have rotational symmetries of order 5 or of order greater than 6.

Proof Here is a proof in dimension 2. The proof in dimension 3 is similar.

Let \( L \) be a two dimensional lattice generated by vectors \( \mathbf{a} \) and \( \mathbf{b} \),

\[
L = \{ h \mathbf{a} + k \mathbf{b} \mid h \text{ and } k \text{ are integers} \}.
\]

Suppose that the rotation

\[
R = \begin{pmatrix} \cos \theta & -\sin \theta \\ \sin \theta & \cos \theta \end{pmatrix}
\]

is a symmetry of \( L \), that is, \( R \) moves \( L \) onto itself. Since \( Ra \) and \( Rb \) are in \( L \),

\[
Ra = h_1 a + k_1 b \\
Rb = h_2 a + k_2 b
\]

for integers \( h_1, h_2, k_1, k_2 \).

Using the frame \((\mathbf{a}, \mathbf{b})\) write \( R(a, b) \) as

\[
R(a, b) = (a, b) M
\]
or
\[(a, b)^{-1} R (a, b) = M\]
where
\[M = \begin{pmatrix} h_1 & h_2 \\ k_1 & k_2 \end{pmatrix}.\]

The trace of a matrix \( A \) (\( \text{tr} \ A \)) is the sum of its diagonal entries. A basic fact in linear algebra is that
\[\text{tr} A = \text{tr}(B^{-1}AB)\]
for all invertible matrices \( B \). Applying this to (3),
\[(4) \quad \text{tr} R = 2 \cos \theta = \text{tr} M = h_1 + k_2.\]

Now from (4) it follows that \( 2 \cos \theta \) is an integer, and so must be equal to \(-2, -1, 0, 1, \) or \(2\). Thus the only possibilities for \( \theta \) are \(0^\circ, \pm60^\circ, \pm90^\circ, \pm120^\circ, \) and \(180^\circ\) and this proves the crystallographic restriction in dimension 2. \( \square \)

6.3. **Diffraction.** Molecules arranged in a lattice are arranged in many sets of parallel planes. You can see this by plotting a lattice, for example the face centered cubic lattice, in Maple and rotating it [Maple demo].

An X-ray beam focused on a crystal creates a pattern of diffraction spots. The position of an X-ray diffraction spot depends on the distance \( d \) between the parallel planes creating it, the normal vector for the planes, and the wave length of the x-rays.

The simplest and oldest rule of diffraction is Bragg’s law derived by the English physicists Sir W.H. Bragg and his son Sir W.L. Bragg in 1913,
\[(5) \quad 2d \sin \theta = n \lambda\]
where \( \theta \) is the angle of incidence of the x-ray, \( \lambda \) is the wave length of the x-ray, and \( n \) is some integer. The formula holds when refraction yields a bright spot at angle \( \theta \).

The formula is explained by looking at figure [Java applet]. If \( d \) is the distance between the planes, the ray \( R_2 \) travels a distance of \( 2d \sin \theta \) farther than \( R_1 \). If this is an integer multiple of the wavelength, the two rays are in phase after reflection and create a bright spot. Otherwise there is cancellation and no spot is seen.

Here is a link to a [Java applet] illustrating Bragg’s Law.
Figure 1. Illustration of Bragg’s Law. \( R_1 \) and \( R_2 \) are diffracted X-rays. The angle \( \theta \) is the angle of incidence with planes containing the indicated atoms. Bragg’s law can be derived by looking at the triangle ABC. The extra distance travelled by \( R_2 \) is \( 2BC \).

6.3.1. Mathematical statement of Bragg’s law. Bragg’s law is an equation for the phase difference between two sine waves. We say the functions \( \sin(\frac{2\pi}{\lambda}x) \) and \( \sin(\frac{2\pi}{\lambda}x + p) \) have phase difference \( p/2\pi \) cycles. The wave length is \( \lambda \).

The amplitude at a point \( x \) in space of parallel sine waves with wavelength \( \lambda \) moving in the direction of the unit vector \( u_1 \) can be written as

\[
\sin \left[ \frac{2\pi}{\lambda} x \cdot u_1 \right].
\]

This represents an x-ray beam moving in the direction \( u_1 \). The reflection off a point \( x_0 \) along a line through \( x_0 \) in the direction \( u_2 \), the wave has the equation

\[
\sin \left[ \frac{2\pi}{\lambda} \left( x \cdot u_2 + x_0 \cdot (u_1 - u_2) \right) \right].
\]

This can be seen by checking that [6] and [7] agree at \( x = x_0 \). Similarly the reflection off the point \( x_1 \) along a line through \( x_1 \) in the same direction \( u_2 \), the wave has the equation

\[
\sin \left[ \frac{2\pi}{\lambda} \left( x \cdot u_2 + x_1 \cdot (u_1 - u_2) \right) \right].
\]
See figure 2. The difference in phase between (7) and (8) is

\( \lambda (x_1 - x_0) \cdot (u_1 - u_2) \)

and when the difference is a integer \( n \),

\( (x_1 - x_0) \cdot (u_1 - u_2) = n\lambda, \)

the outgoing waves are in phase at the point at infinity in the direction \( u_2 \).

Suppose \( x_0 \) and \( x_1 \) are points in a lattice, so they differ by a lattice vector \( x_1 - x_0 = \mathbf{v} \). Equation (10) shows that all such reflections are in phase if

\( \mathbf{v} \cdot (u_1 - u_2) = n\lambda \)

for all lattice vectors \( \mathbf{v} \). For fixed \( n \), (11) says that \( x_0 \) and \( x_1 \) lie in parallel planes perpendicular to \( u_1 - u_2 \).

Equation (11) is the mathematical expression of Bragg’s law (5). It says, for example, that on the integer lattice we get reflections in the direction \( u_2 \) from the planes normal to \( u_1 - u_2 \) only if \( (u_1 - u_2)/\lambda = (h,k,l) \) for integers \( h, k, \) and \( l \). The reflection spot in the direction \( u_2 \) is labelled \( (h,k,l) \).

6.4. **Electron density.** In crystallography, it is not the atoms of a molecule that are seen, but the electrons surrounding the atoms. To describe an electron cloud, a non-negative number giving the density of electrons is assigned to each point in the crystal. This number can be thought of as the amount of charge per unit volume. Although the information about protein structure is given in the form of a file containing the coordinates of each atom, in reality what the crystallographer sees is the electron density. The points representing the centers of the atoms are extrapolated from the electron density function using a computer.
Suppose that a structure is crystallized into a lattice consisting of all integer combinations of vectors $\mathbf{a}$, $\mathbf{b}$ and $\mathbf{c}$. Let $r(x, y, z)$ be the electron density at the point $x\mathbf{a} + y\mathbf{b} + z\mathbf{c}$, where $x$, $y$ and $z$ are real numbers, not necessarily integers. The function $r$ is called the electron density function. If a level surface of the electron density function is plotted in one unit cell, it will look as in figure 3 for 6 Angstrom resolution and figure 4 for 1A resolution.

![Figure 3. An electron density map of a molecule at 6 Angstrom resolution. The atoms are not clearly seen.](image)

The level surface shows more structural detail at higher (1 Angstrom) resolution.

The electron density map is the level surface of a function computed from a Fourier series. Here is how Maple deals with level surfaces of functions.

Since the crystal is unchanged if it translated by $\mathbf{a}$, $\mathbf{b}$ or $\mathbf{c}$, the electron density is the same at 

$$(x + 1)\mathbf{a} + y\mathbf{b} + z\mathbf{c}$$

as at 

$$x\mathbf{a} + y\mathbf{b} + z\mathbf{c}.$$ 

Thus $r$ has period 1 in the variable $x$,

$$r(x, y, z) = r(x + 1, y, z).$$ 

A similar equation holds for the variables $y$ and $z$. The electron density function is a periodic function, of period 1 in each of the three variables. Periodic functions are studied using Fourier series. We begin by looking at the theory for one variable. This will generalize to the three variable case which we are interested in.
6.5. **Fourier series.** The electron density function is constructed using Fourier Analysis. Fourier analysis is the approximation of periodic functions by sines and cosines. The basic idea of Fourier analysis is that any real valued function \( f(x) \) of period 1 can be approximated by sums of the type

\[
a_0 + \sum_{j=1}^{n} [a_j \cos(2\pi j x) + b_j \sin(2\pi j x)]
\]

for suitable choice of the coefficients \( a_j \) and \( b_j \) and for \( n \) large enough. A sum of type (12) is called a Fourier series.

The surprising part about Fourier’s discovery is that even a function such as a square wave can be approximated by Fourier series. For example consider the period 1 square wave defined on the interval \([0, 1]\) by the function

\[
f(x) = \begin{cases} 
0, & \text{if } \frac{1}{4} < x < \frac{3}{4} \\
2, & \text{otherwise.}
\end{cases}
\]

This is a function of period 1, and \( \int_{0}^{1} f(x) \, dx = 1 \). The Fourier cosine series

\[
1 + \sum_{j=0}^{n} a_j \cos 2\pi (2j + 1)x
\]

with coefficients

\[
a_j = \frac{4(-1)^j}{\pi(2j + 1)}
\]

approximates \( f \) closely for large \( n \). Here is a Maple demo.
6.5.1. **Complex form of Fourier series.** We will discuss how the coefficients of a Fourier series are computed. The simplest formula uses the complex form of the Fourier series. Complex numbers give a convenient way of writing Fourier series, even for real functions.

The complex form of Fourier series is based on Euler’s formula

\[ e^{ix} = \cos x + i \sin x. \]

from which it follows that

\[ \cos 2\pi jx = \frac{e^{2\pi ijx} + e^{-2\pi ijx}}{2} \]

and

\[ \sin 2\pi jx = \frac{e^{2\pi ijx} - e^{-2\pi ijx}}{2i}. \]

Substituting these expressions in the general form for the Fourier sum (12) get

(16) \[ \sum_{j=-n}^{n} c_j e^{2\pi ijx} \]

where

\[ c_j = \frac{1}{2} (a_j - ib_j), \quad j = 1, \ldots, n \]

\[ c_0 = a_0 \]

and

\[ c_{-j} = \overline{c_j}, \quad j = 1, \ldots, n. \]

So now we have a sum (16) of complex functions equal to the real sum (12). The sum (16) adds up to a real function since every term \( c_j e^{2\pi ijx} \) is added to its conjugate \( \overline{c_j} e^{-2\pi ijx} \).

If

(17) \[ f(x) = \sum_{j=-n}^{n} c_j e^{2\pi ijx} \]

then the Fourier coefficients \( c_j \) can be recovered from the function \( f \) by the formula

(18) \[ c_j = \int_{0}^{1} f(x) e^{-2\pi ijx} dx. \]

Here is a [demo] in Maple of complex Fourier series. It is left as an exercise to show that formula (18) gives coefficients (15) for the square wave.

The Fourier coefficients, like all complex numbers, have an absolute value and an argument, that is, we can write them in polar form as

(19) \[ c_j = |c_j| e^{i\delta_j}. \]

The number \( \delta_j \) is called the phase and \( |c_j| \) the norm.
6.5.2. Delta function. The function (13) is an example of an approximation to a periodic 1 Dirac delta function. The periodic delta function \( \delta_{Z} \), or the delta function of the one dimensional integer lattice \( \mathbb{Z} \), is thought of as a “function” of period 1 such that

\[
\int_{-1/2}^{1/2} \delta_{Z}(x) f(x) \, dx = f(0)
\]

for any smooth periodic function \( f(x) \). In particular,

\[
\int_{-1/2}^{1/2} \delta_{Z}(x) \, dx = 1.
\]

There is no function that has these properties, but the delta function can be thought of as the limit as \( M \to \infty \) of functions such as

\[
f(x) = \begin{cases} \frac{M}{2} & \text{if } n - \frac{1}{M} < x < n + \frac{1}{M}, n \text{ an integer;} \\ 0 & \text{otherwise,} \end{cases}
\]

of which (13) is the special case \( M = 4 \). Similarly by replacing 0 by \( a \) in (20), get the definition of the periodic delta function \( \delta_{Z+a} \).

A way to think about electron density function of a crystal and the phase problem is to use a delta function. Think of the periodic delta function \( \delta_{Z+a} \) in one dimension as describing the electron density of a crystal formed by a single atom of zero radius at each point \( a + n \) where \( n \) is an integer. By (18), the Fourier coefficients of \( r_{Z+a} \) are given by

\[
c_j = e^{-2\pi ja}.
\]

So \( \delta_{Z+a} \) can be approximated by

\[
\delta_{Z+a}(x) \approx \sum_{k=-N}^{N} e^{2\pi ik(x-a)}
\]

for large \( N \). Look at the Maple worksheet to see what this function looks like. In fact, simplifying (23) gives

\[
\delta_{Z+a}(x) \approx \frac{\sin \left[ 2\pi \left( N + \frac{1}{2} \right) (x-a) \right]}{\sin[\pi(x-a)]}.
\]

The phase of \( c_j \) is \(-2\pi ja\) and the norm is 1. If we did not know the phase but put 0 for the phase instead, then the coefficients \( c_j \) would all be 1 and we would get an atom at \( x = 0 \) instead of \( x = a \). This illustrates the importance of phase in computing the electron density function.

6.6. Three variable Fourier series. Fourier series can be used to analyze periodic functions in any number of variables, for example the electron density function \( \rho \) for a 3D crystal.
Given a real function $\rho(x, y, z)$ of period 1 in each variable, it can be approximated by a triple sum of the form

$$
\sum_{h=-n}^{n} \sum_{k=-n}^{n} \sum_{l=-n}^{n} c_{hkl} e^{2\pi i (hx + ky + lz)}
$$

written more compactly as

$$
(25) \quad f(x, y, z) = \sum_{hkl} c_{hkl} e^{2\pi i (hx + ky + lz)}
$$

where

$$
c_{-h,-k,-l} = \bar{c}_{hkl}
$$

since the function is real, and where $|h|$, $|j|$, and $|k|$ are $\leq n$ where $n$ is a large enough integer.

Write the complex Fourier coefficient as

$$
c_{hkl} = |c_{hkl}| e^{i\alpha_{hkl}}
$$

The number $\alpha_{hkl}$ is called the phase.

Similar to (18) the Fourier coefficients of (25) are

$$
(26) \quad c_{hkl} = \int_{0}^{1} \int_{0}^{1} \int_{0}^{1} f(x, y, z) e^{-2\pi i (hx + ky + lz)} dx dy dz.
$$

In x-ray crystallography, the values $|c_{hkl}|$ are measured from the intensities of the spots in the diffraction pattern. The phases $\alpha_{hkl}$ must be supplied by other means to find the electron density function. This is called the phase problem. There are methods for finding the phases but also some guess work is involved.

6.6.1. Delta function. Similar to the delta function for the one dimensional integer lattice, the delta function $\delta_{Z^3}$ for the three dimensional integer lattice, or cubic lattice, can be approximated by a sum of the form (25) with $c_{hkl} = 1$ for all integer triples $hkl$. Also $\delta_{Z^3+a}$ is approximated by setting the Fourier coefficients $c_{\ell} = e^{-2\pi i a \cdot \ell}$ where $\ell = (h, k, l)$.

6.7. Diffraction pattern and Fourier series. Here we show how the diffraction intensities give the absolute values of the Fourier coefficients of the electron density function. Consider the electron density of a single atom of mass $m$ modeled approximately as $m$ times a delta function. For simplicity, assume the lattice of the crystal is the integer lattice, $Z^3$. In order to get a finite Fourier sum consider just a part of the lattice,

$$
L = \{(x, y, z) | \ x, y, z \ \text{integers}, \ -N \leq x \leq N, -N \leq y \leq N, -N \leq z \leq N\}
$$
for $N$ large. Now write the electron density function for atoms at points $x_0 + \ell$, $\ell \in L$ as

$$
\rho(x) = m \sum_{\ell \in L} e^{-2\pi i x_0 \cdot \ell} e^{2\pi i x \cdot \ell}.
$$

The Fourier coefficients of the electron density function are $c_\ell = m e^{-2\pi i x_0 \cdot \ell}$ for $\ell \in L$. Note that $\rho(x)$ is an approximation of the delta function $\delta_{x_0 + Z^3}$.

Now look at the diffraction pattern. As before, consider the reflection of an x-ray beam in the direction $u_1$, reflected in the direction $u_2$ from all of these atoms. The equation for the beam reflected from the atom at $x_0$ is given as (7). The computation simplifies if we replace the sine function by the complex exponential. Also assume that the intensity of the reflected beam is proportional to the electron density $m$ at the point. Then the outgoing beam has equation

$$
m \exp \left[ \frac{2\pi i}{\lambda} (x \cdot u_2 + x_0 \cdot (u_1 - u_2)) \right]
$$

for the amplitude of the beam at a point $x$ in space. The beam from the entire lattice of points is the sum of the intensities of each point in the lattice,

$$
\begin{align*}
w(x) &= \sum_{\ell \in L} m \exp \left[ \frac{2\pi i}{\lambda} (x \cdot u_2 + (x_0 + \ell) \cdot (u_1 - u_2)) \right] \\
&= m \exp \left[ \frac{2\pi i}{\lambda} x \cdot u_2 \right] \exp \left[ \frac{2\pi i}{\lambda} x_0 \cdot (u_1 - u_2) \right] \sum_{\ell \in L} \exp \left[ \frac{2\pi i}{\lambda} \ell \cdot (u_1 - u_2) \right].
\end{align*}
$$

Writing $\ell^* = (u_1 - u_2)/\lambda$ equation (29) becomes

$$
w(x) \approx m \exp \left[ \frac{2\pi i}{\lambda} x \cdot u_2 \right] \exp [2\pi i x_0 \cdot \ell^*] \delta_{Z^3}(\ell^*)
$$

since

$$
\delta_{Z^3}(\ell^*) \approx \sum_{\ell \in L} \exp [2\pi i \ell \cdot \ell^*].
$$

Integrating it with respect to $\ell^*$ near a lattice point gives the value of 1. It follows that the total reflected wave for $(u_1 - u_2)/\lambda$ near $\ell^*$ is

$$
m \exp \left[ \frac{2\pi i}{\lambda} x \cdot u_2 \right] \exp [2\pi i x_0 \cdot \ell^*]
$$

where $x$ is a point along the reflected beam. (Since all the beams are parallel in the direction $u_2$, we merge them all into one beam at infinity where they are collected.) The expression (31) is $(c_{\ell^*}) \exp \left[ \frac{2\pi i}{\lambda} x \cdot u_2 \right]$ where $c_{\ell^*}$ is the $\ell^*$ Fourier coefficient of the electron density function (27). Since only the amplitude of the beam can be measured from the intensity of the diffraction spot, the data gives only $|c_{\ell^*}| = m$, the absolute value of the Fourier coefficient, and not the phase.

The same result holds when we add electron densities of atoms to get the electron density of a molecule. Only the absolute value of the Fourier coefficient can be determined from the diffraction pattern.