Firstly, the first few lectures available at :
http://www.bioinfo.rpi.edu/~bystrc/courses/bcbp4870.html contain most of the slides we used in our class and a few useful extras.

The value α:
 δ, **α**, whatever, is a symbol. It is a phase difference. Doesn't have a special meaning. Just like x, y, and z we can use whatever we want. Called delta one place and somewhere else it is called **α** (he uses **α** for the phase difference of the resultant wave, and **δ** for the phase difference of the components we are adding up). It is just a symbol. There is no specific meaning, it just refers to the phase. Don't get scared just because there are several symbols denoting the same thing.

δ is the phase difference.

**Homework Problems on Wave Superposition/Interference:**

1)

$$\delta = 2\pi\Delta/\lambda$$

$\delta = 0$

A = 2.0, I = 4.0 in phase
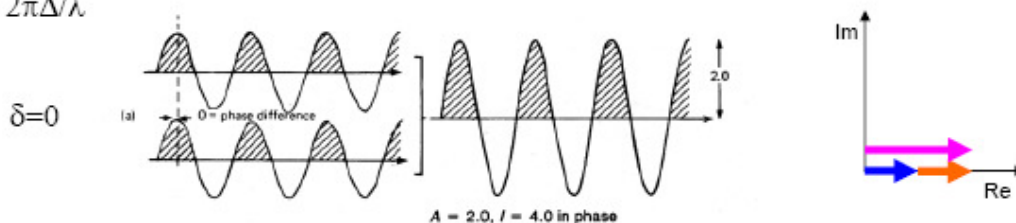
- You can use common sense to solve this problem, or the equation $E_t = E_0(1+e^{i\delta})$. Using euler's formula ($e^{i\delta} = \cos(\delta) + i\sin(\delta)$) this equation is $E_t = E_0(1+\cos(\delta) + i\sin(\delta))$. Plugging in **δ** =0 (phase difference relative to the origin), you get $E_t = E_0(1+1+0) = 2E_0$. Since the sign term in the equation is equal to zero, there is no imaginary component shown in the graph, only the 2E$_0$ on the real axis.

2)

$\delta = \pi/2$

A = 1.4, I = 2.0.
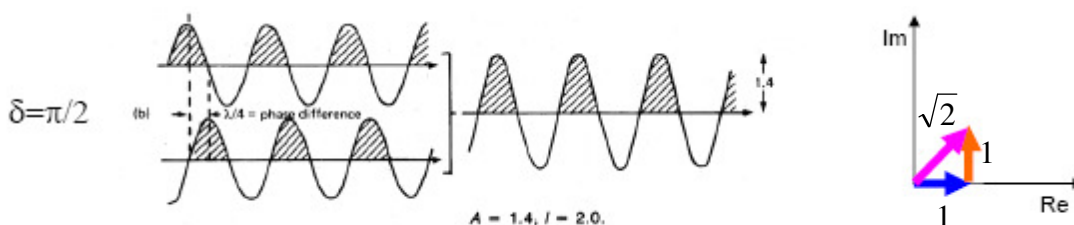
- This time $E_t = E_0(1+0+i)$ which means that there is an imaginary component of E$_0$ and a real component of E$_0$. Using vector addition, the magnitude of the resulting vector is $\sqrt{2} \cdot E_0$.
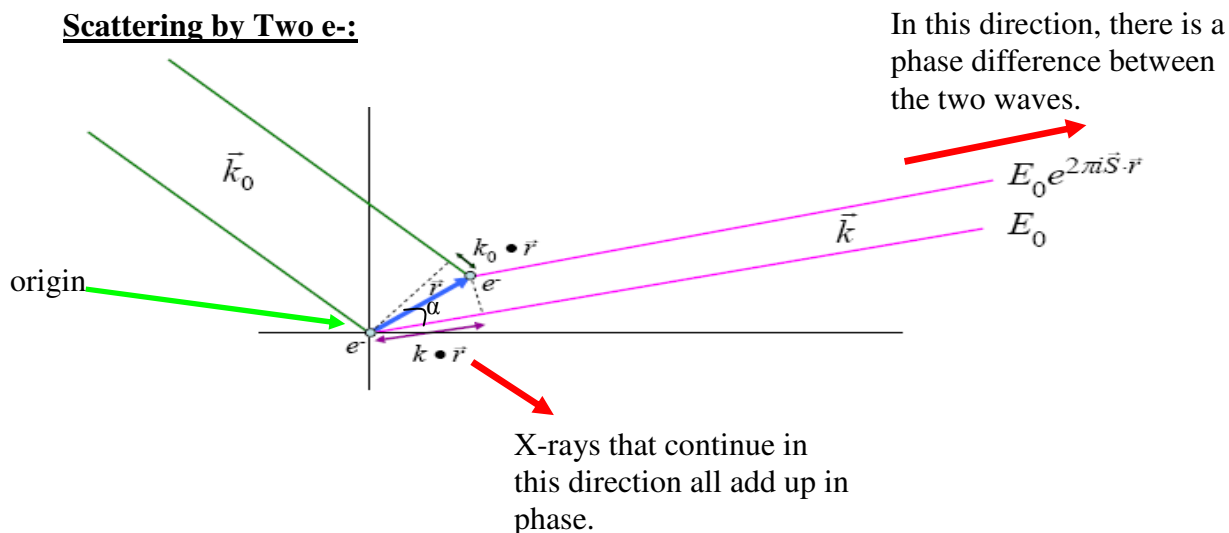
3)
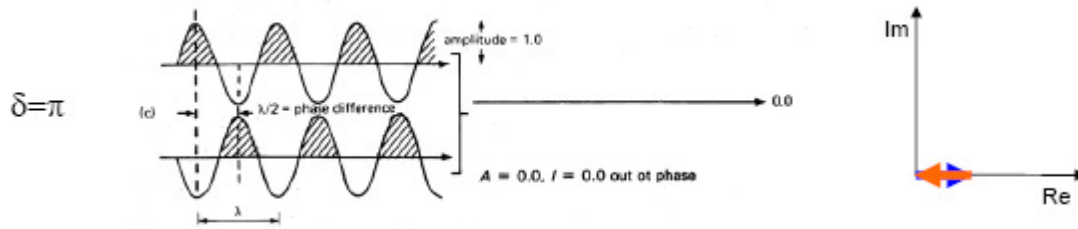
- In this case $E_t = E_0(1-1+0) = 0$. Thus no resulting amplitude is observed for the imaginary dimension or the real dimension.

If you have three waves, you can solve it by using the formula just the same with some geometry.

<u>Scattering by Two e-:</u>

In this direction, there is a phase difference between the two waves.

origin

X-rays that continue in this direction all add up in phase.

- If we want to convert the phase difference into length difference we have to multiply by 2π

and divide by wavelength $\left(\delta = \dfrac{2\pi\Delta}{\lambda}\right) = \dfrac{2\pi(\vec{k}\bullet\vec{r} - \vec{k}_0\bullet\vec{r})}{\lambda} = 2\pi\,\vec{S}\bullet\vec{r}$

- In the situation where two waves are being scattered by two electrons, we can use the equation

$E_t = E_0 + E_0 e^{2\pi i\,\vec{S}\bullet\vec{r}} = \sum_{n=1}^{2} E_0 e^{2\pi i\,\vec{S}\bullet\vec{r}_n}$ which is essentially the same as the equation used in the homework problems. In this case we have to find the phase difference between the two waves. If we assume one of the waves if passing through the origin, this wave has 0 phase difference and the other has a phase difference. If you do the calculation with both of the electrons away from the origin, the phase difference between the two waves is the same.

- The scattering vector, $\vec{S}$, is the difference between the directions we

observe the scattered radiation ($\vec{k}$) and the incoming radiation ($\vec{k}_0$).

- $\vec{k}$ (the unit length vector in the direction of scattering) indicates only the direction of radiation.

- $\vec{s}$ and $\vec{s}_0$ indicate the wavelength of the radiation and the direction

denoted by $\vec{k}$ ($\vec{s} = \dfrac{\vec{k}}{\lambda}$). So $\vec{s}$ gives us more information about the wave.
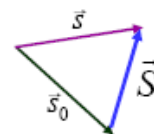
$$\vec{S} = \vec{s} - \vec{s}_0$$

Scattering vector

- The phase difference depends only on the scattering vector and the position of the second wave relative to the wave that passes through the origin.

More Complex Things…
Atomic scattering factor:

For One Atom:
- Electrons are in certain orbitals. The electrons are moving pretty fast, and we don't really see them. We describe the e- in an atom by the e-density distribution as a function of position or distance.
- In a certain small volume there is a certain e- density corresponds to that volume multiplied by the probability of finding an e- in that volume. (for example mass/volume=mass density and likewise e- density x volume=the charge in that volume or how many e- are in that volume on average).

origin

$\rho(r)$

+r

ΔV_n

nucleus

-r

$\rho(-r)$
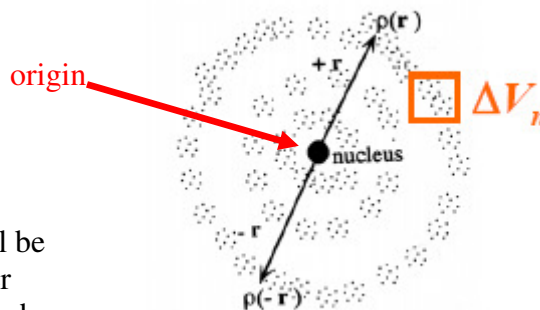
- With hydrogen, the e- density in this small volume will be less than 1, because there is only one electron spread over the whole atom. The e-density is a fraction of an e- for each tiny volume ΔV. For the entire volume surrounding the nucleus the e- density is 1e-.

- For the scattering in a direction with $\vec{s}_0$ and $\vec{s}$ ($\vec{S} = \vec{s} - \vec{s}_0$): we need to add the scattering from all these little volumes. If you divide the atom into very small volumes, the sum will have many terms. (many volumes making up the atom). If you make the volume very very tiny then it will be equivalent to an integral.
- Since the e-density changes as a function of the distance from the nucleus, then e-density might be slightly different at a different point. So summation is not the best way, an integral is better. ρ (e-density) at a specific point multiplied by volume around that tiny point multiplied by Phase of the wave scattered from that one point = scattering from an atom.

$$f_{atom}(\vec{S}) = \sum_n \rho(\vec{r}_n) \Delta V_n e^{2\pi i \vec{S} \cdot \vec{r}_n}$$

e-density at
a distance
r_n from the
nucleus

volume

phase
difference

3

- In fact: Because these values depend on e-density. We can calculate e- density quantum mechanically. we can actually calculate these values for an individual atom. Can calcualte these values as a function of S for each atom.

- For individual atoms if we put the origin in the center of the atom, then the atom has spherical symmetry to the first approximation. So this function is no longer a function of $\vec{S}$ as a vector, but of S as an value. Which means for every vector $\vec{S}$ that has the same modulus, no matter in which direction, the scattering from an atom is the same because the atom has spherical symmetry when we put the center of the atom at the origin (only in that case)

- Fourier transform: it turns out that this is a very well known mathmatical function (integral). It is called the fourier transform.

$$f_{atom}(\vec{S}) = \int_V \rho(\vec{r}) e^{2\pi i \vec{S} \cdot \vec{r}} dV \equiv f(S)$$

atomic scattering factor

Fourier transform

- The function depends on direction of S and direction of r. However, if it has spherical symmetry (as in the case of an atom at the origin), then all the directions are sort of the same. All that matters is the angle between $\vec{s}_0$ and $\vec{s}$. If you do the math, then it turns out if the e-density is spherically symmetric about the origin, the scattering only depends on the modulus of $\vec{S}$ and not on its direciton.

- The modulus of $\vec{S}$, what does it really mean? $\vec{S}$ will have the same length for values of $\vec{s}$ that rotate around $\vec{s}_0$ making the same angle. $\vec{s}_0$ is given and they have constant lengths, the only way you can keep the same S is to rotate it around $\vec{s}_0$. So the surface of the cone contains all the values of S that have the same length.
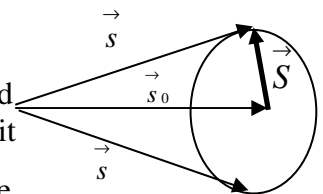
- If you change the angle, then you no longer have the same modulus (value, length) of S.

All the same values of S on a cone have a given angle of $\vec{s}$ relative to incident radiation.

- We call what we calculated an **atomic scattering factor**. This gives the value of scattering for an certain atom relative (as compared to) to a single electron.

- So if we look in the direction where $\vec{S} = 0$ ($\vec{s} = \vec{s}_0$ so we're looking in the same direction as the incoming radiation) then there is no path difference in between any of these ΔV (volumes).

- In the zero direction ($\vec{S} = 0$), all the vectors add up in phase. If we look in the zero direction, then we get the integral over the e- density of the whole atom.

▪ So the integral over the e-density of a carbon atom for example, will give us 6 electons (scattering 6x as big as a single e-).

▪ At different $\vec{S}$, we start having phase difference, as a result the scattering factor of an atom goes down. In the direction of $\vec{s}_0$ (zero direction), the scattering amplitude will be equal to the # of e- that the atoms have. As we go away from the 0 direction, phase difference cuases the amplitude to decrease.

For carbon, when $\left|\vec{S}\right| = 0$, get scattering amplitude 6 times larger than for a single e-

▪ $\sin(\theta/\lambda) = \sin\left(\dfrac{\theta}{\lambda}\right) = \left|\vec{S}\right|$
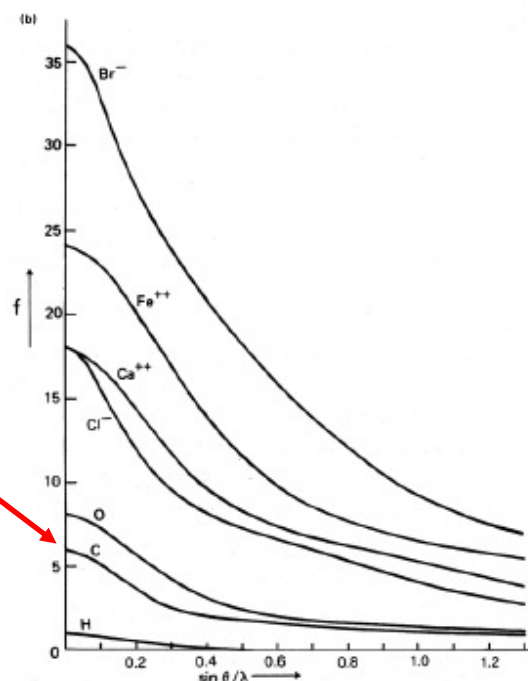
▪ Why are we assuming the atoms are symmetrical?
The assymetry in the atom is in the outer shell. The departure from symmetry is not high. We can assume the e- density is approximately symmetrical. If we talk about the e-density of a bond, this is not symmetric. This can be studied for small molecules by the model. But what we are discussing such as carbon, nitrogen, and hydrogen, we can think of as spherical.

▪ Fourier transform: describes the relationship between e- density and scattering amplitude. The relationship is the Fourier transform.

▪ The mathematics of this show that if we have two functions ($\rho(\vec{r})$ (ρ as a function of $\vec{r}$) and f($\vec{S}$) (scattering amplitude as a function of scattering vector) variables) related by such a relationship, we can describe e-density of an integral of scattering amplitude with slightly different…equations.

▪ The forward transform allows us to derive the reverse transform. If we know one of them, we can calculate the other. If we know e-density we can calculate the scattering.

▪ Also if we know the scattering effects, we can calculate e-density (!!!)

Forward transform

Inverse transform

$$F(\vec{S}) = \int_V \rho(\vec{r}) e^{2\pi i \vec{S} \cdot \vec{r}} dV \qquad \rho(\vec{r}) = \int_{V^*} F(\vec{S}) e^{-2\pi i \vec{S} \cdot \vec{r}} dS$$

note the minus

▪ All the wave combination stuff is important in order to be able to see these relationships or the forward and reverse fourier transform. If we can measure structure factor, we can calculate e-density that causes that scattering that we have measured. We will measure scattering effects and calculate the e-density indirectly (so not measuring the e-density directly).

▪ Measure the scattering effects, and back-calculate the e-density. Measure the influence of e-density on x-rays passing through this medium.

- Note that the two (or V and V*) r's in the equation are not the same. The $\rho(\vec{r})$ is in real space, the distance from the nucleus, and the r_n that is in S space

Temperature factor B:
- When we calcualte the scattering from an atom, we assume the atom is staying still. The scattering corresponds to a fixed atom. In a solid, it will not be completely still, except at 0° K.
- The effect of this is that the e-density averages because we don't know where the individual e- of the atom are or where the atom is at every moment. The nucleus also moves, so we have a smearing, which affects the scattering amplitude.
- If the atom is imobile, then the atomic scattering amplitude is the one that we showed previously, calculated from classic quantum mechanics.

vibrations

$\langle u \rangle =$ average displacement from the equilibrium position
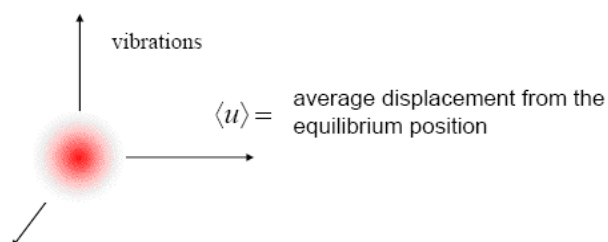
- If the atom is moving, then we introduce the **temperature factor B**, which is related to the average displacment of the atom from its equilibrium position.
- So, remember high B values corresponds to large movement, low B values corresponds to small movement.
- May not see something in a polypeptide chain b/c it has high B-value. This tells you that the part is higher mobility relative to the rest of the peptide. nmr gives dynamic info. Crystallography gives you an average picture over time and space. It is not a dynamic picture info but dynamics can be indirectly inferred from the B-value.
- High B-value, very dynamic situation.

$$f(S) \Rightarrow f(S) \cdot e^{-1/4BS^2}, \quad where \quad B = 8\pi^2(\langle u \rangle^2/3)$$

Typical B for protein atoms at ~2Å resolution is ~20 Å². That corresponds to $<u> \approx 0.8$Å and $<u_x> \approx 0.5$Å

- Graph: B-factor affects the scattering. In the 0-direction there is no difference. Temperature factor affects the scattering. B value of 10 decreases a lot the scattering effects. Value of 20 even more. Value between 20 and 50 and up to 100 for a protein. So proteins vibrate a lot. Small molecules will have a b-value around 3-5, so they are much more solid.

scattering by a carbon atom expressed in electrons

B=0

B=10
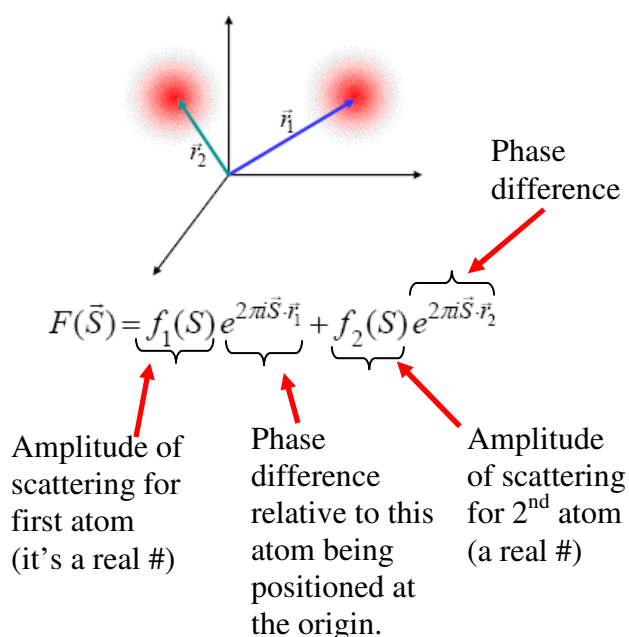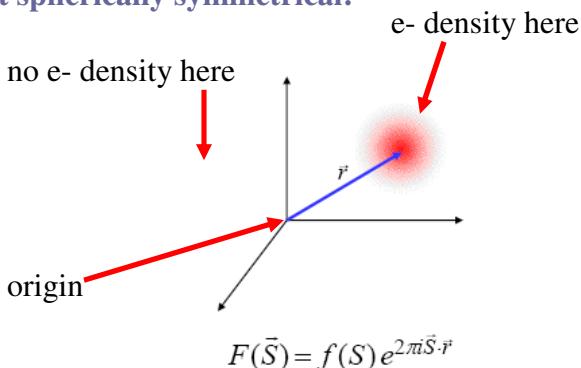
B=20

$\frac{2\sin\theta}{\lambda}$
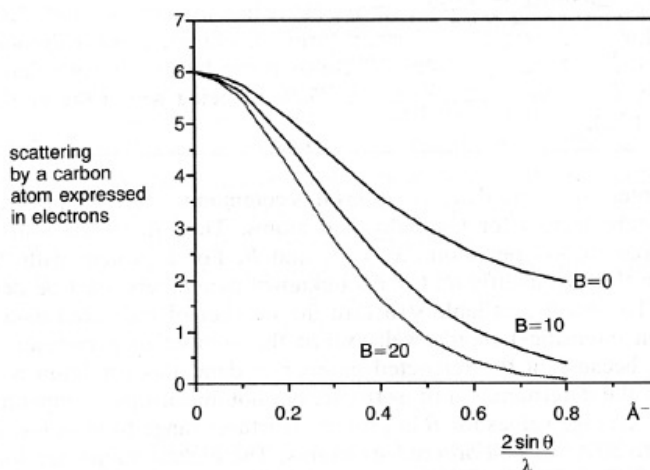
Scattering for more than one atom:

- We can not repeat the same thing for more than one atom. The wave that passes through the origin will have phase 0, and everything else will have a phase depending on the distance (from the origin0. There is nothing different than the scattering for an electron, now this is just scattering for an atom.
- The only thing that changes when we move the atom away from the origin is the phase term. We aren't scattering from the origin anymore. The atom at the origin is spherically symmetric relative to the coordinate system. If the single atom is not at the origin, then it is **no** longer spherically symmetric relative to our origin.
- Since it is not spherically symmetrical, the phase is dependent not only on the modulus of $\vec{S}$, but also on the direction.

Two e- scattering:

Would you know how to do it graphically? Which is the amplitude, which is the modulus, which term is the phase? The length would be which one??

Not spherically symmetrical:

e- density here

no e- density here

origin

$$F(\bar{S}) = f(S)\, e^{2\pi i \vec{S} \cdot \vec{r}}$$

Phase difference

$$F(\bar{S}) = \underbrace{f_1(S)}\, e^{2\pi i \vec{S} \cdot \vec{r}_1} + \underbrace{f_2(S)}\, e^{2\pi i \vec{S} \cdot \vec{r}_2}$$

Amplitude of scattering for first atom (it's a real #)

Phase difference relative to this atom being positioned at the origin.

Amplitude of scattering for 2nd atom (a real #)

We can add the contributions of the different atoms together using this formula:

$$F(\bar{S}) = \sum_{n=1}^{N} f_n(S)\, e^{2\pi i \vec{S} \cdot \vec{r}_n}$$

the atomic scattering factors are different for different atoms.

Diffraction from one-dimensional array of same atoms:
- Let's imagine a case where we have a one-dimensional array of atoms.
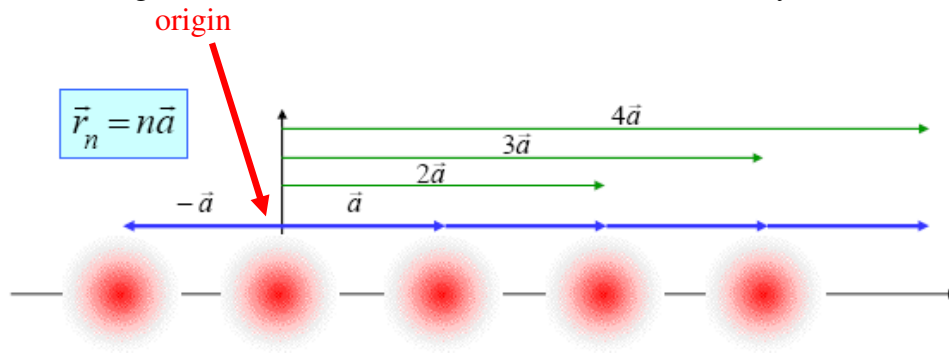
- All the atoms are in one line and they are equidistant from eachother (separated by the vector $\vec{a}$). We have a regular arrangement of atoms in a line of atoms.
- Imagine that it is a very long line of atoms. This line has translational symmetry. If we take the line and shift it by the distance from neighboring atoms ($\vec{a}$), then the whole line will fall on itself. Where we had an atom b/f translation, we will have an atom after translation. By shifting the line by a vector $\vec{a}$, the whole line would fall on itself.
- Shifting by less than ($\vec{a}$) creates a new line. If we shift by 2a then the translational symmetry still holds (and for $3\vec{a}$, $-\vec{a}$, $-2\vec{a}$ etc)
- The equation below describes the situation above. Sum up the phase difference terms for each atom corresponding to the different positions relative to the origin ($n\vec{a} = -1\vec{a}$, $n\vec{a} = 1\vec{a}$, $n\vec{a} = 2\vec{a}$ etc)

$$F(\vec{S}) = \sum_{n=-\infty}^{\infty} f(S)\, e^{2\pi i \vec{S}\cdot n\vec{a}} = f(S) \cdot \sum_{n} e^{2n\pi i \vec{S}\cdot\vec{a}} = f(S) \cdot G(\vec{S})$$

- With incoming radiation of $\vec{s}_0$, observing in direction $\vec{s}$ which corresponds to scattering vector $\vec{S}$, we have to add the contributions from all the atoms. (Note: we would not have translational symmetry if the atoms were not all the same).
- There is scattering for each atom. You can take the scattering for each atom and just add them together. If we assume it is a very long line, we sum from $-\infty$ to $+\infty$. f($\vec{S}$) is the same in every term, so we can take it out of the summation.
- Scattering from a long line is the same as f($\vec{S}$) multiplied by G($\vec{S}$) (the shape function) that describes the distribution of the atoms along the line.
- G($\vec{S}$) is a very special function (trust cygler). If it goes from $-\infty$ to $+\infty$, then the shape function has only two values. Values of 0 (most of the time), and very very large values (a number of terms in here) when $\vec{S} \bullet \vec{a}$ is an integer.

- when $\vec{S} \cdot \vec{a}$ is an integer, the e^ 's exponent is always a multiple of 2π, which referring back to euler's formula means that we have cosine of a multiple of 2π (which is always =1) and sine of that, which is always =0. So we get the summation of many ones (cos(2π multiple) when $\vec{S} \cdot \vec{a}$ is an integer. If this is not the case, there is a lot of cancellation, and we end up with 0 (in the infinity case)

- So for each specific value of h and a specific value of $\vec{a}$, $\vec{S}$ describes a cone with a certain angle around $\vec{a}$. $\vec{a}$ is a constant value, $\vec{S}$ is a constant value and length is constant value.if cosine alpha (angle between the two) stays the same, so $\vec{S} \cdot \vec{a}$ stays the same.

$$G(\bar{S}) = \begin{cases} \gg 1, & when \ \bar{S} \cdot \bar{a} = h \ (integer) \\ 0, & elsewhere \end{cases}$$

Defines conical surface for **S**
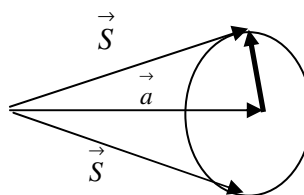
The Shape Function G(S):

- For one atom at the origin, this is the atomic scattering amplitude. Max at s0 and falling off as we go to different scattering lengths as before. This function is the phase angle for one atom.

- The maxima are at integer values of $\vec{S} \cdot \vec{a}$.

- The more terms you have, the sharper the maxima are (at integer values of $\vec{S} \cdot \vec{a}$), and they are higher and higher and higher values.

- When we have many terms (ex: ~20 atoms):

Basically the function is high at integer values of $\vec{S} \cdot \vec{a}$ and 0 elsewhere. the scattering occurs in only in certain directions where $\vec{S} \cdot \vec{a}$ is an integer value.

Several atoms: looks like this and now we have two terms (f(S) and G(S))

$G(\vec{S})$

$f(S) \cdot G(\bar{S})$ Max at $\vec{s}_0$
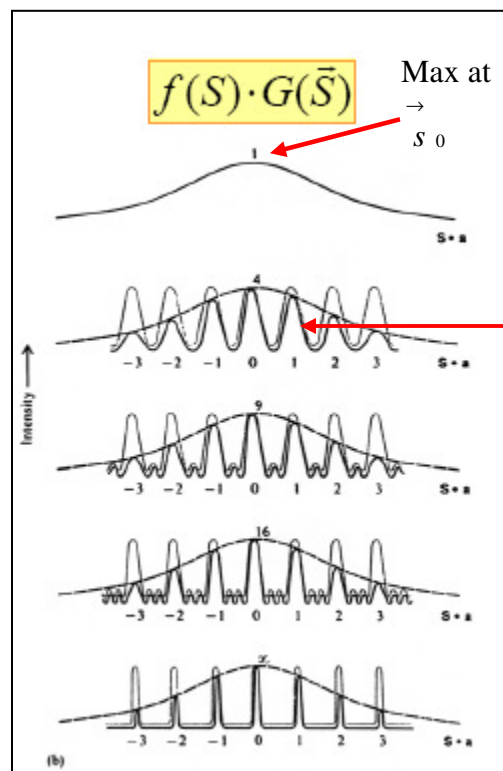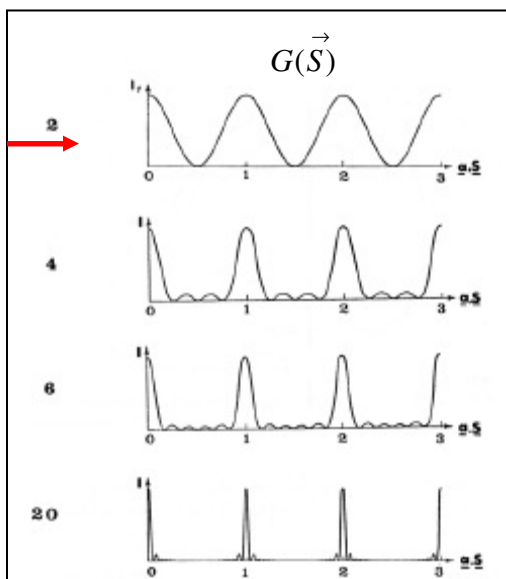
The darker line is the product of the two functions.

- So we if we have a row of atoms, we get a selective amplification of the signal at certain values of $\vec{S} \cdot \vec{a}$ because the value of the shape function is very large.
- The shape function is proportional to the number of atoms in the row. If we have 1000 atoms, then the value is 1000, if we have 3 atoms, then the value is only three.
- The energy scattered by one atom is very small.
- If there are many atoms on a line (as in our 1D same atom ex), then in most directions they won't scatter at all, but in some directions they will scatter and the scattering will be multiplied by the number of atoms in that row.
- So we have a selective amplifier. The scattering is amplified for certain values of scattering vector ($\vec{S}$).
- Scattering from a single atom is very weak. So weak that we are just not able (yet) to measure the scattering from a single atom.
- However, if we can amplify the signal, we can measure it. If we can amplify by adding the signal together then maybe we can measure the signal. So if the signal from two atoms is twice as much, then we can assemble these atoms together, then maybe measure their scattering. If they are randomly distributed, then the scattering will not add together in phase anymore for all directions. If we make the array, we have shown that we can amplify the signal, but not for all directions. We can amplify significantly, but only in specific directions.

One-dimensional array of atoms:

molecular scattering
amplitude
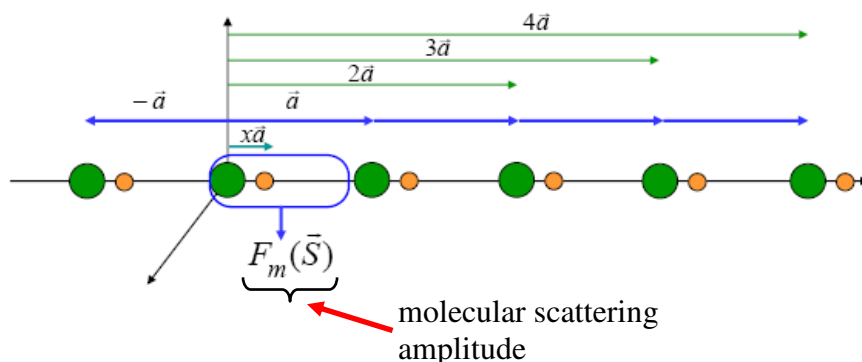
- Now we have a molecule made of several atoms. This molecule is translated along a single line at equidistant spaces. Now we calculate the molecular scatter amplitude (the scattering from a molecule).
- The scattering from a molecule that is shifted by a vector ($\vec{a}$) can be calculated just as for an atom. Now in the equation for the scattering of the row of molecules, we add all the terms using molecular scattering factor ($F_m(S)$) rather than atomic scattering factor ($f(S)$).
- The scattering factor of the row is still directed by the same shape function. So the G function is non-zero only when S dot a is an integer. So it is non-zero as a function of h.

$$F(\vec{S}) = F_m(\vec{S}) \cdot G(\vec{S})$$

$$F(\vec{S}) \neq 0 \quad only \ when \ \vec{S} \cdot \vec{a} = h$$

-
$$F_m(\vec{S}) = \sum_{n=1}^{N} f_n(S) e^{2\pi i \vec{S} \cdot x_n \vec{a}} = \sum_{n=1}^{N} f_n(S) e^{2\pi i (\vec{S} \cdot \vec{a}) x_n} = \sum_{n=1}^{N} f_n(S) e^{2\pi i h x_n} = F_m^h$$

Position of sector relative to the origin.

- In terms of obtaining the molecular scattering factor for just one molecule ($F_m(S)$), we calculate it as before, for the case when we have more than one atom. We assume that one of the atoms is at the orgin, and the other is some distance away from the origin (this is shown as x_n in the diagram which relates the distance between the atoms (a) to the distance between the molecules)
- We can go from a single line of atoms to a plane. We create another line of atoms by shifting the line in different directions. We now have a regular plane of atoms. This is a 1D array of atoms ordered in the 2nd dimension. We can take the plane and make it into a 3D array of planes. Every atom is at a position as a multiple of $\vec{a}$, multiple of $\vec{b}$ and multiple of $\vec{c}$.