X-Ray Diffraction of Macromolecules

Determination of crystal morphology

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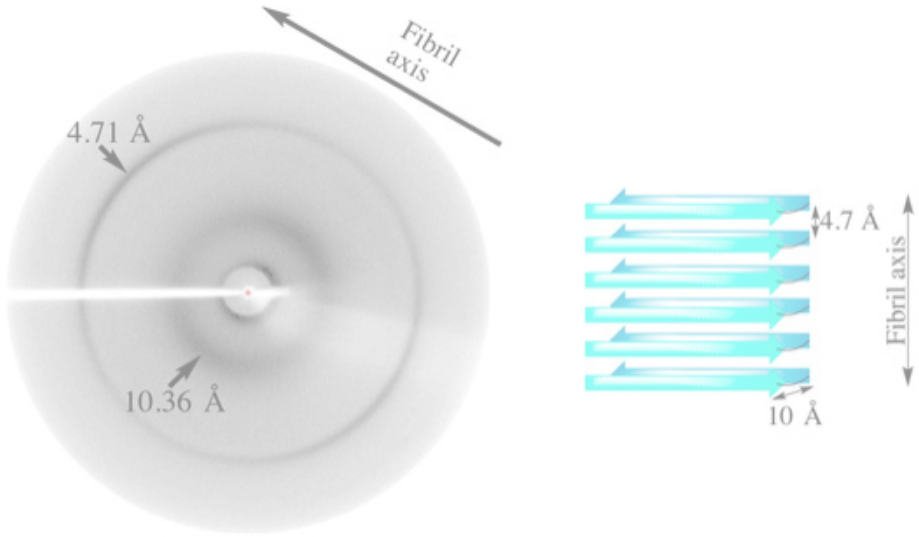
X-ray fiber diffraction

X-ray fiber diffraction for proteins



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X-ray Diffraction

X-ray diffraction is a technique used for determining the three-dimensional structure of molecules, such as proteins and nucleic acids, or any other biological macromolecule. It resolves the atomic arrangement from a crystal or a fiber for explaining the three-dimensional structure of the molecule. As a single crystal has a regular repeating of arranged atoms, therefore, the obtained diffracting X-ray data will be scattering off the periodic assembly of the atoms in the crystal or the molecule. The electronic map will then be used to construct the structure of the molecule. The X-ray diffraction is to provide information related to crystallization to build-up a model for the molecule, see Figure 1 for the x-ray diffraction components.

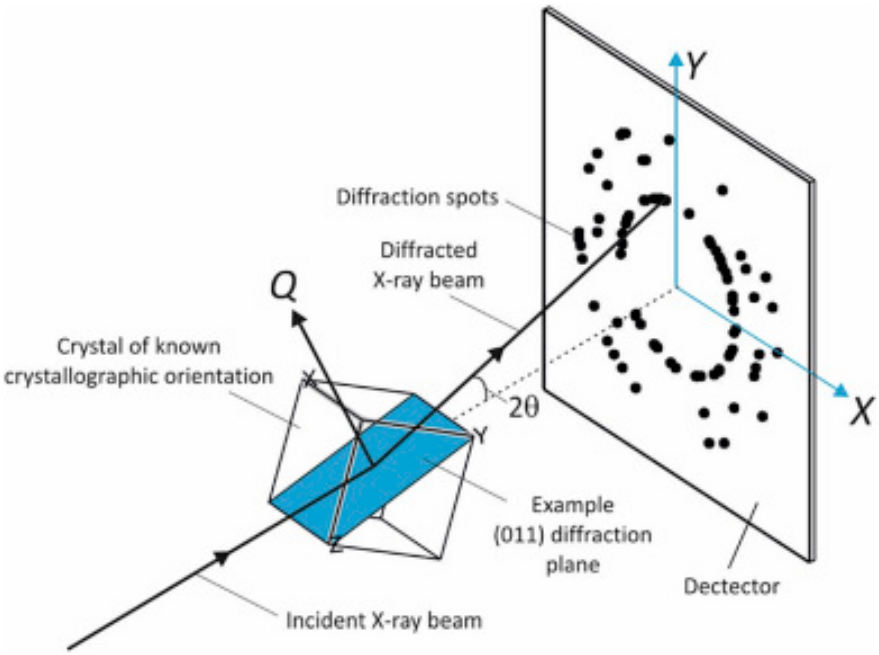

Figure 1. A diagram of x-ray diffraction technique.

1. X-ray crystal diffraction: Theory

A high concentration of a pure sample is grown to form crystals. The crystals are introduced to an *x*-ray beam, which is diffracting the atomic arrangement of the crystal as spots, appear in the detector. The resulting diffraction patterns (the spots) can then be served to obtain information about the symmetry of the crystal packing and the size (width, height, depth) of the repeating unit cells, which form the crystal molecule. The intensities and the distribution of the spots are useful to calculate a map of the electron density. A high quality electronic map permits the building of a molecular structure of the macromolecule or protein. Different methods and software are used to improve the quality of the electronic map, using the protein sequence, until it becomes clear enough to permit the building of the molecular structure.

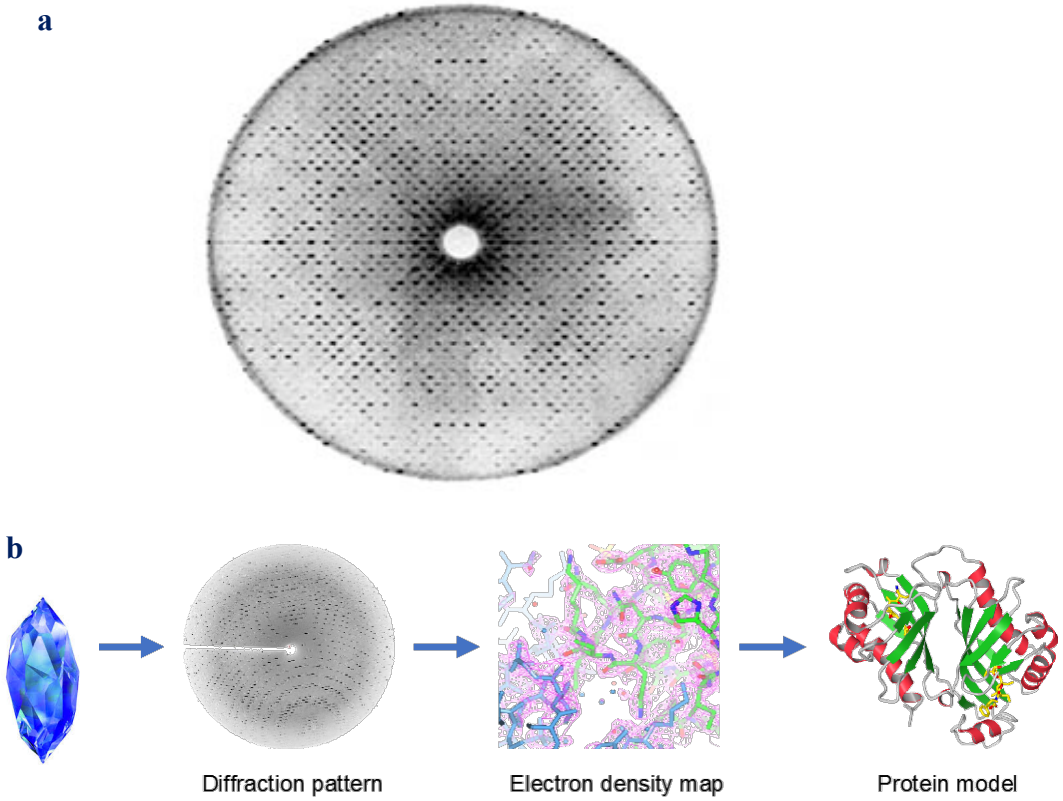

Figure 2. The x-ray diffraction output. a) x-ray crystal diffraction pattern appears as spots on the detector, b) the workflow of the x-ray crystal diffraction to model the structure of the molecule.

1.1. What is the unit cell?

Crystals have identical subunits that are repeated regularly and ordered in three-dimensional fashion. Repeating of these unit cells within the crystal molecule allows the x-ray signal to be improved into a diffraction pattern appears as spots in the detector. One unit cell has dimensions defined by vectors a, b, and c in the directions x, y, z with angles α, β and γ, see Figure 3.

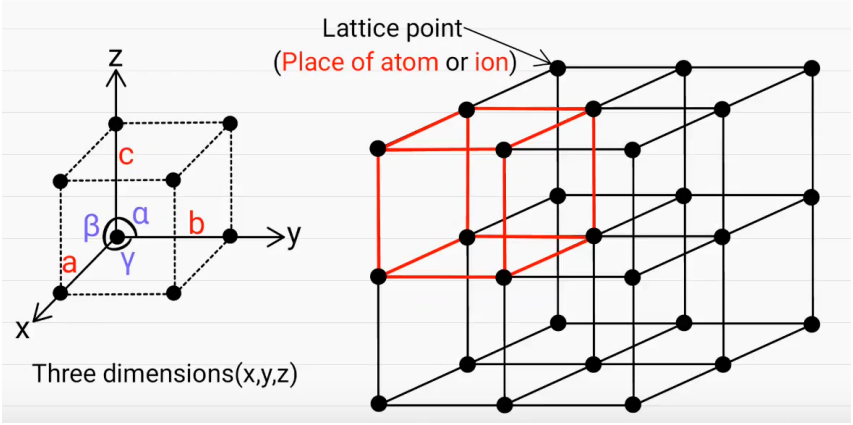

Figure 3. The crystal lattice illustrates the unit cell within the atomic plans.

In the crystal structure, each unit cell is identical and therefore, the position of atoms of the molecules are identical in each unit cell.
When x-ray penetrate the crystal, the light will interact with the electrons on each atom, therefore, some of the light will scatter. Because molecules in a crystal are in an order arrangement, the scattered lights will add up to be constructive interference and give a diffraction pattern. The diffraction pattern of a crystal appear on a detector is a series of black spots, which resemble the electron density (electron map), not only on the atoms, but in the molecule. This electronic map is essential to provide the structure of the crystal.
Only the in-phase waves are produced a signal because they are add-up to each other, therefore, the molecule will successfully diffract the light and the resulting pattern would appear as continuous distribution of electron density. However, most of the waves are out of phase will cancel each other, which is why we see black spots in the detector.

1.2. **The in-phase and out-of-phase waves**

What is the phase? The phase is the relationship between two waves in the same frequency represented as theta Θ. The wave has a frequency of number of cycles per second and an amplitude that strength the power of vibration. Waves are typically draw in terms of time in the x-axis and level of vibration in the y-axis. The period of length of time is one cycle; one cycle of wave is 360^{0} see Figure 4. The cycle starts at 0^{0} when the level of vibration is zero and reach to a maximum 90^{0} for a quarter cycle in a positive level. If two cycles began at the same time, they will have the same vibrational level so they are in-phase waves, but if they began at different times, they will be out of-phase waves.
Constructive and destructive interference occurs due to the superposition theory. The principle of superposition describes the way that the waves interfere to displace the resulting wave. If the several waves of the same type, they will interfere at a point and add to each other. Therefore, the resultant displacement at that point is the sum of the incident waves.
When two waves are oscillations (traveling) in the same stage, that means they are oscillating in phase. The phase difference between these two waves is a whole even-number multiple of pi (0, 2π, 4π,…), Figure 4.
If two waves are oscillating at opposite stages in the cycle, then they are oscillating **completely out-of-phase** or **in antiphase**. The phase difference between two waves that are in antiphase is a whole odd-number multiple of pi (π, 3π, 5π,…), see Figure 4

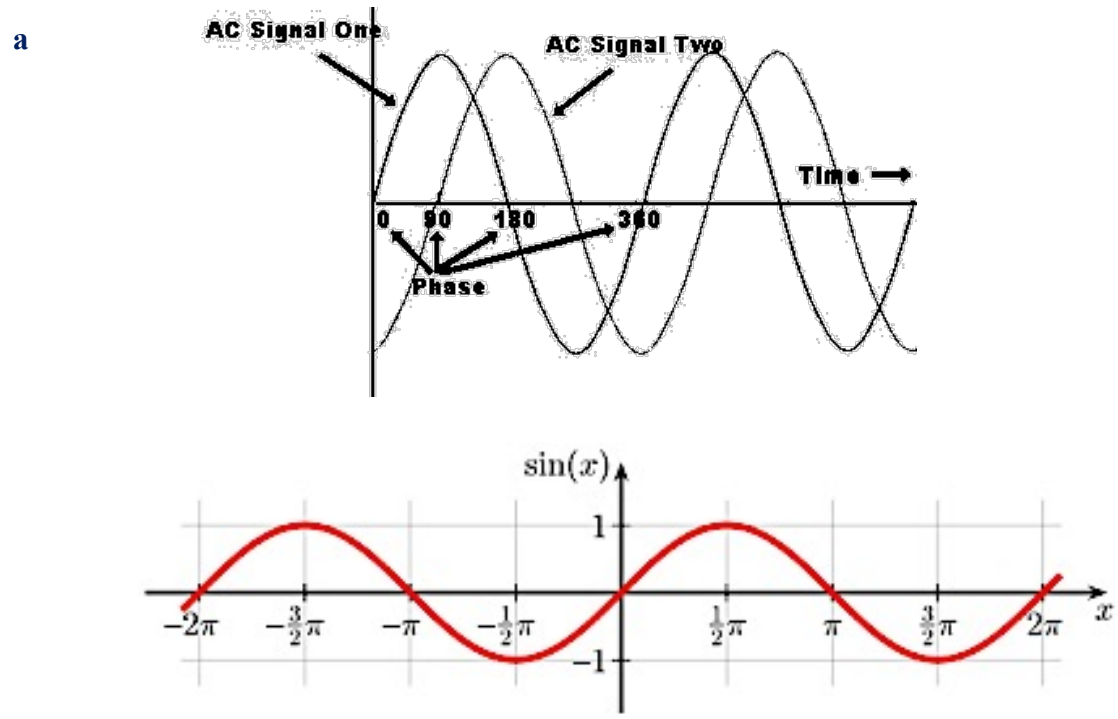

Figure 4. The in and out of phase waves. a) the degree of the in-phase wave, b) two in-phase waves that are add to each other, c) partially and completely out of phase waves.

1.3. The constructive and destructive interference of waves

When the waves are in-phase, they will meet each other and display in the same direction, which means they are reinforce each other. The resulting wave will be in a higher amplitude than the individual amplitude of any single wave and define as a constructive interference, see Figure 5.

The destructive interference occurs when two waves have their out of phase interference (partially or completely display in opposite directions). The produced wave would show a little amplitude if the two opposite waves have different amplitude. If the opposite waves have similar amplitude, they will cancel each other and the resultant wave will have zero amplitude, see Figure??

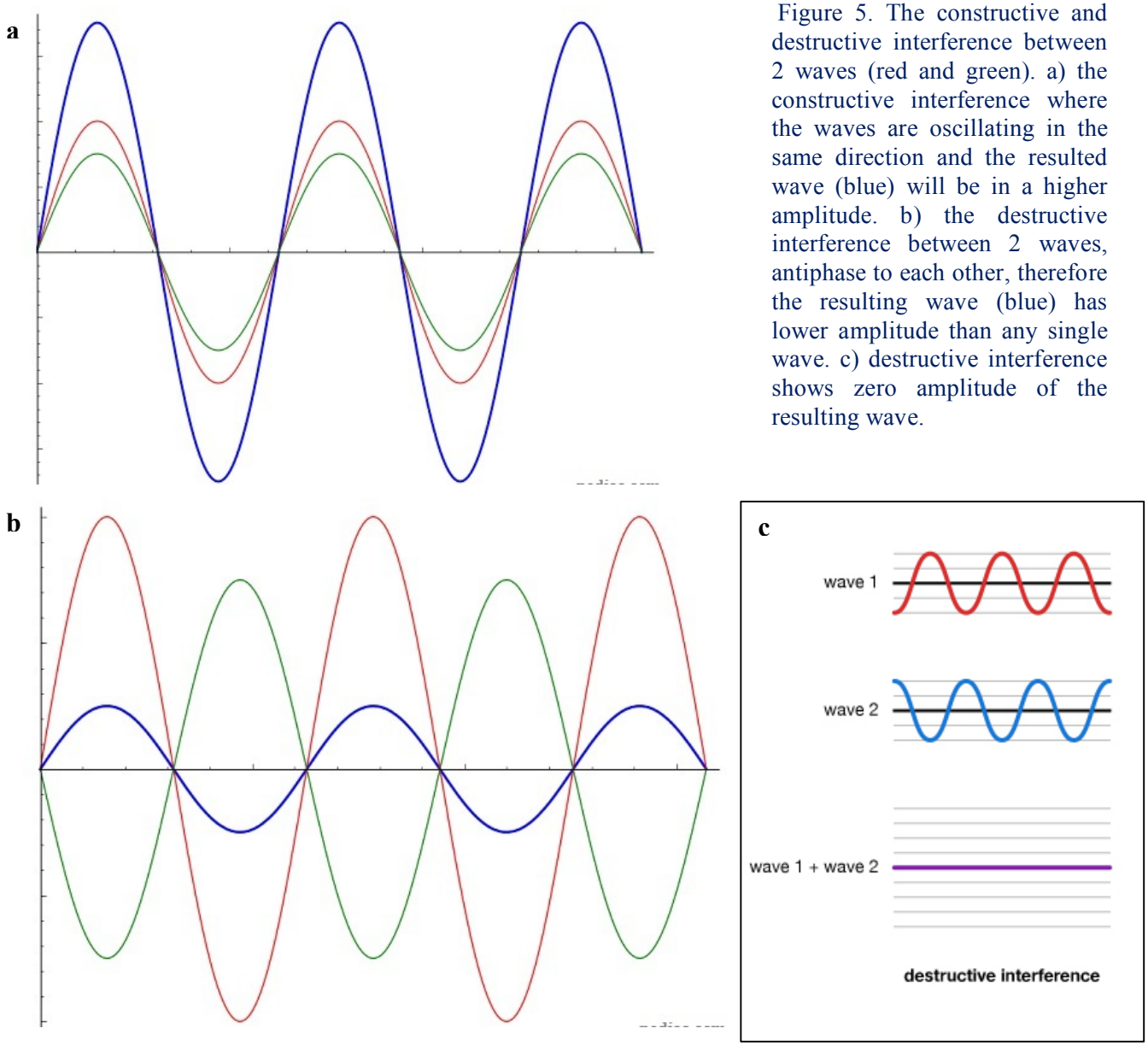

Figure 5. The constructive and destructive interference between 2 waves (red and green). a) the constructive interference where the waves are oscillating in the same direction and the resulted wave (blue) will be in a higher amplitude. b) the destructive interference between 2 waves, antiphase to each other, therefore the resulting wave (blue) has lower amplitude than any single wave. c) destructive interference shows zero amplitude of the resulting wave.

1.4. X-Ray crystal diffraction according to Bragg`s low

Bragg`s law describes the relationship between the x-ray wave that hit the crystal with the spots that appear on the detector, see Figure 6 . The figure shows the diffracted waves off the atomic plans within the crystal molecule. Two incident (in-phase) light with a wavelength λ hit two atomic plans that separated a distance d and diffract off the atomic plan at a specific angle θ. Mathematically, **Bragg`s law is: nλ= 2d sinθ** where n=integer, λ= the wavelength of the x-ray, θ= the incident and the diffract angle**.** The Bragg`s law is satisfied only with in-phase waves, when n= integer, which successfully diffract the light and produce the black spots on the detector.

Waves that in -phase will constructively interfere by an integer number of wavelength (2 π, 4π,…). Waves that are partially out of-phase are interfere by half-integer multiplies (1/2 π, 1 ½ π….) and waves that are out of phase will destructively interfere by odd integer numbers (3 π, 5π…). These half and odd integer numbers will cancel out and therefore, no spots will appear on the detector with out of phase waves.

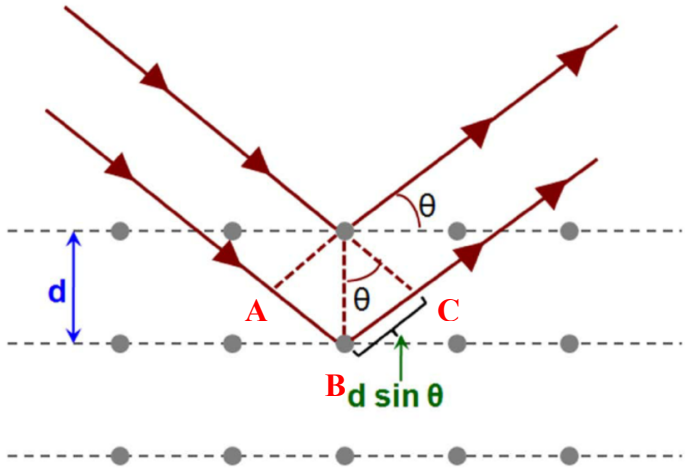

Figure 6. Interaction of x-ray with the atomic plans according to Bragg`s law.
Bragg`s law = nλ= 2d sin θ :
nλ= AB+BC
AB=BC
Then nλ= 2AB
AB= d sin θ
nλ= 2 d sin θ
n= integer

The distance between atomic plans (d) is related inversely with (θ), which means when the space is low, the angle of constructive interference will be high. The diffraction pattern on the detector represents a crystal structure in a real place, i.e. the relationship of the spots to the crystal is in reciprocal space: 1/a, 1/b and 1/c. Therefore, the smaller the unit cells makes the distance between the spots be larger. The reciprocal lattice vector 1/a, 1/b and 1/c are related to Miller indices h, k, and I.

1.5. How to determine the morphology of the crystal

This section will discuss the general features of the crystal diffraction pattern and its relation to the morphology that predicts from the unit cells.

According to Bragg`s law:

$$2 \sin \theta = \frac{n\pi}{d}$$

the spacing of the reflected lights (2 sin θ) are inversely proportional to lengths of atomic plane (the unit cell of the crystal). The spacing are not affected by the number of atoms, or the types, positions of the atoms for each unit cell. Therefore, these spacing are used to determine the length of the unit cell in a crystal. Moreover, the orientation of the reflections is usually mirror the angles between the edges of the unit cells. As a result, the symmetry of the diffraction pattern explained by the symmetry within the unit cell. The lengths, angles, as well as the symmetry in the unit cell will collectively define the final morphology of the crystal.

Experimentally, to determine the length of the unit cell, at least 2 perpendicular photographs of the diffracted crystal (at 0 and 90 ^{0}) should be collected on the detector, to understand the 3D structure of the crystal.

The pattern in Figure 7 can be useful to define the unit cell of the crystal. The photograph must first be indexed in terms of the 3 Miller Indices *h, k* and *l*, then it will be ready to define the lengths and angles (α, β and γ) of the unit cells between each principle axis, which means the unit cell`s geometry of the crystal. The reciprocal vectors 1/a, 1/b and 1/c are related to the Miller Indices.

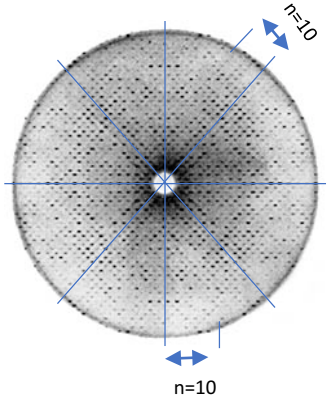

Figure 7. A photograph of the tetragonal crystal of lysozyme. The primary axes are the vertical and the horizontal axes which are used for indexing. Other set of axes used for indexing are indicated along the diagonals. In this photograph, the unit cell is defined to be larger than the set chosen. The distance of diagonal layer lines is smaller than that appears in the vertical layer line, which indicates a larger unit cell along the diagonal.

2. In Figure 8, the black boxes are showing the unit cells and the atomic planes for each unit cell is shown in yellow, green red and blue. The distance between the yellow planes is ***a*** (one unit cell edge), which is related to the *h* indexing and described as [1 0 0]. The red planes are separated by 1/3 of ***a*** and therefore indexed as [3 0 0]. In the ***b*** direction, the plane is related to *k* indexing and describe as [0 1 0] and in the ***c*** direction, the plane is related to *l* indexing and described as [0 0 1].

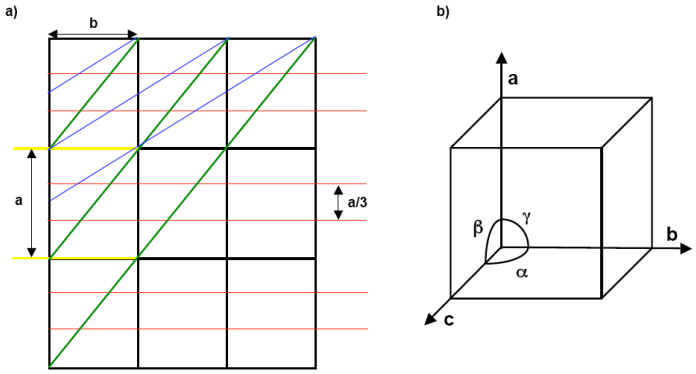

Figure 8. a) the atomic planes of Bragg law, b) three-dimensional representation of the unit cell.