Determination of crystalline structures using electron diffraction as a replacement for x-ray crystallography

Daniel Elston
University of Bristol - School of Physics

March 12, 2022

During the current global pandemic, efficiency of methods used is highly important in order to quickly produce vaccines and drugs used for treatment. X-ray crystallography has been an extremely effective tool in progressing many different areas of science, from pharmaceuticals and drug design, to vaccine development. However, there is currently immediate worldwide potential to significantly reduce the cost and time taken in developing these essential products.

X-ray crystallography is currently the accepted method of determining small molecular structures [1] while also developing insights into protein structures and new materials [2]. It can be further used to observe biological processes such as how drug interactions take place and observation of antibodies [3]. The main disadvantage of x-ray crystallography is that molecules must form large, periodic crystals in order to produce an x-ray interference pattern, requiring significant time investment. Furthermore, some molecules are unable to form the required crystal sizes at all [4]. This problem can be solved if electrons are used instead of x-rays to form an interference pattern. Most educational or research institutes world wide have access to a transmission electron microscopy setup, making this technique immediately available.

An electron diffraction pattern was first observed in 1927, paving the way for quantum mechanics [5]. Electron diffraction patterns can be produced with much smaller crystals than required for x-ray crystallography. This is because electrons have a smaller wavelength than x-rays. This effectively cuts out a large time consuming step from x-ray crystallography, the creation of large periodic crystals. A recent paper explores the benefits of using electron diffraction to determine micro crystalline molecular structures instead of x-ray crystallography.

A team led by Tim Gruene demonstrate the potential of electron diffraction in many different fields as appose to standard x-ray crystallography techniques. The team uses electron diffraction to determine the structure of two separate molecules and demonstrate the potential to determine structures of sub-micro sized crystals. Further benefits of using electron diffraction are stated, together with demonstrations of how electron diffraction could benefit sectors such as the pharmaceutical industry.

Experimental Method

The team of scientists use electron diffraction to determine the structure of active pharmaceutical ingredient of cold medicine, 'GrippostadU' which is a single-crystal structured drug, paracetamol [6]. Paracetamol crystals would need to be recrystallised to form a large enough crystal to produce interference patterns from x-rays [7]. Furthermore, the medicine contains inactive non-crystalline compounds, demonstrating a further ability of electron diffraction to distinguish between amorphous and crystal structures with ease.

A diffraction pattern of 0.9 was determined showing an atomic resolution using electron diffraction. Furthermore, due to electrons interacting with matter more strongly, hydrogen atoms were able to be observed which us highly rare in determination of crystal structures [4].

Using the same method on a methelyne blue derivative, the structure was determined [6]. This would have been difficult to achieve using x-rays due to the crystals produced requiring re-crystallisation into larger crystals to produce an x-ray interference pattern. Results of the electron diffraction were confirmed with x-ray crystallography methods later [4].

The team has demonstrated that electron diffraction can be used to determine crystalline structures that are unable to be determined using x-ray crystallography. Interference patterns of atomic resolution have been produced to high resolutions. Furthermore, they have shown that there is no need for re-crystallisation of small molecules to determine structure. This demonstrates the diversity, time efficiency and cost effectiveness of electron diffraction as appose to x-ray diffraction.

Crystallography

X-rays are normally used to determine crystalline structures, as their wavelengths are close to crystal atomic spacing's, approximately 1. The atoms in crystals are spaced just so that they can act as a diffraction grating for x-ray waves [8]. Waves diffract through atomic spacing and interfere either constructively or destructively creating a diffraction pattern [9]. Should two waves undergo constructive interference, there will be a maxima point [10]. This diffraction pattern is then solved mathematically, using Bragg's law,

$$2dSin\theta = n\lambda \quad (1)$$

where d is lattice, θ is x-ray incident angle, n is a positive integer and theta is wavelength of x-rays. Electron diffraction works similarly, however, electrons are used instead of x-rays. Electrons experience wave-particle duality, allowing them to behave similarly to x-rays [11]. An electrons wavelength is smaller than that of an x-ray by a factor of 10 on average. This results in electrons being able to diffract through smaller gratings, producing a higher resolution diffraction pattern than x-rays.

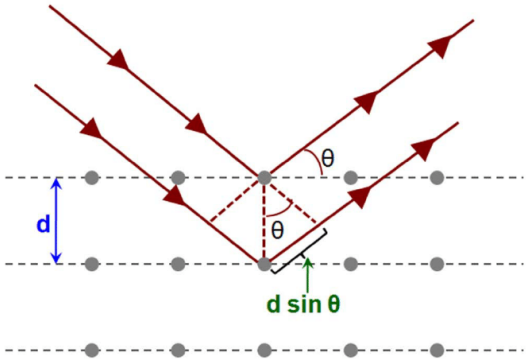

Figure 1: [12] Diagram showing incident x-ray waves being diffracted by crystalline lattice.

X-ray crystallography can be used to determine the structure of a 3D protein. To achieve this, scientists must first crystallise the protein sample, which is generally a trial and error process [13]. One crystallised protein is not sufficient to create a readable diffraction pattern. To overcome this, large numbers of identical crystals are created and arranged periodically in order to magnify the diffraction pattern produced [2]. This is a tedious process and could potentially be made redundant by electron diffraction.

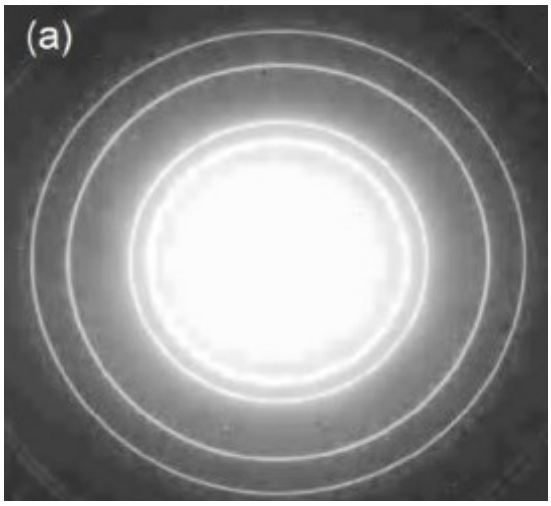

Figure 2: [14] Image showing an electron diffraction pattern.

Discussion

The ability to determine structures of sub atomic crystals without the need for further crystallisation techniques, gives electron diffraction a substantial edge over x-ray crystallography. The time and money spent arranging molecules into periodic crystalline structures in order to produce an x-ray interference pattern is tedious and inefficient. The ease of techniques used to determine structures compliments electron diffraction techniques further. Results using electron diffraction techniques are just as reliable as x-ray crystallography.

Electron diffraction could potentially distinguish between truly amorphous molecules and sub atomic crystals within a mixture. This has the potential to lower cost and timings of vaccine or drug development in a pharmaceutical setting. The fact that no further techniques are needed to separate molecules in a mixture in order to determine structure makes electron diffraction techniques incredibly useful in a range of sectors.

X-ray crystallography has limits in the lower size of crystals where as electron diffraction has virtually no lower sizing limits. The sub-atomic resolution provided by electron diffraction allows sub atomic crystals, previously unable to be seen using x-ray crystallography, to finally be determined. Furthermore, hydrogen atoms from paracetamol were observed, highlighting further potential of electron diffraction.

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