SEEING IS BELIEVING: STRUCTURES AND FUNCTIONS OF BIOLOGICAL MODULES

The ability to elucidate the structure and function of biological molecules holds great importance in a variety of domains. Nawsheen Boodhun explores techniques used to understand macromolecules

Structural biology is a field focused on understanding biological macromolecules such as proteins and RNAs. Deciphering the effects of changes to the structure of these biological macromolecules helps elucidate their function and the part they play in diseases. This ultimately assists in various fields including biotechnology, drug discovery and design of therapeutics.

RNA is one of the three major biological macromolecules, along with DNA and proteins, which are essential for all life forms. DNA is required for the creation of RNA that in turn makes proteins. This is known as the 'Central Dogma' of molecular biology. The key working molecules and building blocks in all cells are proteins and they are produced in a similar two-step process in all living things; transcription of DNA into RNA and translation of RNA into proteins.

Proteins are polymers. They are large, complex molecules that are made from long chains of different amino acids with a particular sequence that gives the protein a specific three-dimensional shape. Proteins play a crucial role in the body and the specificity of their shape is uniquely suited to their function. For instance, antibodies protect the body from foreign invaders such as viruses whilst collagen, the most abundant protein in the body accounting for about a third of the total [1], plays a structural role and holds our skin, tendons, muscles and bones together.

The field of structural biology is an expanding area and it has experienced a number of transformative advances [2]. The 19th and early 20th centuries saw the introduction and improvements of the compound light microscope and Max von Laue's discovery of the diffraction of x-rays by crystals for which he was awarded a Nobel Prize in 1914. In 1912–13,

Lawrence Braggs, who was shown Laue's data by William Braggs, his father, found that treating the crystalline diffraction as the reflection of x-rays from the planes of atoms within the crystal lattice allowed the atomic positions to be deduced [3]. This was a key finding by the Braggs' and they were awarded a Nobel Prize in 1915 'for their services in the analysis of crystal structure by means of x-rays'. By the end of the 1950s, x-ray crystallography had made a leap from physics to chemistry to biology and the structure of DNA as well as several proteins had been revealed. These discoveries contributed to an explosive era revealing a world that was once invisible.

Today, there are a number of techniques researchers utilize in order to view proteins and determine their structure. These include cryo-electron microscopy, x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. The structure of more than 122,000 proteins has been unravelled using these techniques [4] and x-ray crystallography has been responsible for determining more than 85% of known protein structures [5].

x-ray crystallography

x-ray crystallography is a widespread method employed to determine the structure of proteins and biological macromolecules. The process involves utilizing a purified sample at high concentration and crystallization of the specimen. The crystalline atoms cause a beam of x-rays to diffract into many specific directions. Measurement of the angles and intensities of the diffracted beams allows for the production of a three-dimensional image of the density of electrons within the crystal. From this electron density map, a model of the protein can be built to fit the map. A number of information can be obtained

Structural image obtained by x-ray crystallography shows the broadly neutralizing antibody B12 (green) in contact with a critical target (yellow) for vaccine developers on HIV-1 gp120 (red).

Credit: NIAID.

via this method such as the mean position of the atoms in the crystal and their chemical bonds [6].

x-ray crystallography has been utilized to reveal the structure and function of many biological molecules and was involved in the discovery of the double helix structure of DNA. It has been reported that over 80% of all the macromolecular structure, in the Protein Data Bank were determined by x-ray crystallography [7]. Although this process is considered a gold standard for the determination of macromolecular structure and protein substrate interactions, there are a few crucial aspects of the technique that cause limitations. This includes the unpredictability of producing protein crystals. Protein crystallography requires a reliable source of protein along with a purification or concentration protocol that will yield high-quality, homogeneous, soluble material [8]. In the absence of quality crystals, protein structures cannot be determined as this limiting factor prevents an image of sufficient resolution from being achieved. Crystallization also changes the environment of the specimen to that which is non-physiological, thus in certain cases, due to the alteration of structure, the information obtained is not representative of the molecule [6]. It is also important to note that not all specimen can be crystallized, for instance there are great challenges in crystallizing proteins that are embedded in cell membranes. Membrane proteins represent between 20–30% of the proteomes of most organisms and more than 40% of drug targets [9]. However, very few structures of these types of molecules have been elucidated by x-ray crystallog-

raphy. It can take researchers many years to find a way around these challenges in order to crystallize certain specimen but there has been progress made in the field. It has been found that the addition of additives such as small molecules, detergents or metal ions facilitate or enhance crystal formation or growth [10]. In 2006, Tomcova *et al.* crystallized the newly discovered di-haem cytochrome c_4 from the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina* by adding metal-ion salts to the standard vapour-diffusion method [11]. This was the first cytochrome c_4 to be crystallized from an anaerobic organism.

It is without a doubt that the well-established method of x-ray crystallography has enabled researchers to make great advances in the field of structural biology. The technique has contributed to the allocation of more than a dozen Nobel prizes, which includes that of 1962 for the revelation of DNA's double helix. It is clear that from its birth in 1912, when only the most simple of structures could be determined, x-ray crystallography has progressed tremendously, paving the way for macromolecular structures containing thousands of independent non-hydrogen atoms to be solved [12]. Advancement of the technique over the years has been driven by great progress in technology ranging from development of synchrotron x-ray sources to powerful computers and systems that handle the data processing and programmes employing complex algorithms for the elucidation of structures.

However, a new dawn is upon us. Recent years have seen a rise in the use of cryo-electron microscopy for deducing the structure of molecules. This technique is advantageous in studying molecules that have resisted x-ray crystallography as well as other approaches. In comparison to x-ray crystallography, cryo-electron microscopy allows for the study of a protein structure that has been frozen in many different conformations, enabling the mechanism by which it works to be deduced. The great increase in the number of cryo-electron microscopy publications and the use of the technique to map more than a 100 molecules in the past few years suggests that this technique is quickly surpassing x-ray crystallography as the method of choice.

Cryo-electron microscopy

In October 2017, Jacques Dubochet, Joachim Frank and Richard Henderson were awarded a Nobel Prize in Chemistry for their work in developing cryo-electron microscopy, a technique used to image and determine the structure of biomolecules. In cryo-electron microscopy, biological molecules are rapidly frozen so that the water in, and surrounding the specimen is fixed in a vitreous state to prevent the formation of crystals [13]. An electron microscope is used to fire a beam of electrons at the frozen sample. The emerging scattered electrons pass through a lens creating a two-dimensional and amplified image, which is recorded on the detector. This device can be photographic film, charged coupled device cameras or direct electron detectors. Combining a number of images taken from various angles and taking the average of these angles, a 3D model of the protein structure can be generated. However, if freezing does not occur swiftly or the specimen is warmed, crystalline ice formation will occur and

From left to right, advances in cryo-electron microscopy technology can be seen that result in improved resolutions in the atomic detail of protein and drug binding sites. Credit: National Cancer Institute.

ice contamination will compromise the structural integrity of the specimen. The crystalline ice also diffracts electrons which in turn degrades the image quality.

Historically, the use of traditional transmission electron microscopy presented a number of challenges that ranged from the high-energy electron beams burning and destroying the molecule to shrinkage of samples by dehydration in vacuum. These cause a hindrance as they distort the morphology and chemical characteristics of the specimen [14]. To overcome the challenges seen with traditional transmission electron microscopes, cryo-electron microscopy uses frozen samples, gentler electron beams and improved image processing. Cryo-electron microscopy has seen recent advances that allow for the production of highly detailed images of proteins and other biological structures, including larger structures such as RNA–protein complexes. There have been major improvements with regards to instrumentation and image processing making structure determination with cryo-electron microscopy possible for complexes with or without icosahedral symmetry [15].

The cryo-electron microscopy technique has been found to play a significant role in understanding cellular processes and pathogenesis, and is leading the way for drug development. The recent Zika outbreak raised concerns globally and the World Health Organization declared ‘a public health emergency of international concern’. The virus, which is transmitted primarily by *Aedes* mosquitoes, has been associated with birth defects and autoimmune-neurological syndromes including microcephaly and Guillain–Barré syndrome. In 2016, Sirohi *et al.*, published findings from their study in *Science* where they presented the 3.8Å resolution structure of mature Zika virus, determined by cryo-electron microscopy [16]. They compared it with the structure of other flaviviruses to provide a foundation for detailed analyses and understanding of the virology, antigenicity, and pathogenesis of the virus and threat to public health. Through use of the cryo-electron microscopy technique, the team of researchers found the structure of the Zika virus to be similar to that of other flaviviruses except

for a particular segment that surrounds the Asn154 glycosylation site found in each of the 180 envelope glycoproteins that make up the icosahedral shell. This highlights the potential of this moiety to act as an attachment site of the virus to the host cells and its likelihood of playing a role in disease severity.

Cryo-electron microscopy provides the advantage of visualizing macromolecules in a hydrated state that is as close as possible to its natural form. The ability to capture images of biological molecules, their structure and interactions at atomic resolution is a critical step that greatly aids researchers in understanding crucial biological processes and making the link between structure and function. Ultimately, this holds promises for identification of new drug targets and therapeutic agents to assist in addressing public health concerns and tackling debilitating conditions. The technique enables

heterogeneous samples to be studied and provides structural details about large biomolecules and dynamic multimolecular complexes that would otherwise be difficult to examine with other structural biology techniques.

Determining the structure of a biological molecule is one piece of the puzzle. At the heart of structural biology is the challenge of making the link between structure and function. Cryo-electron microscopy has emerged as a rival to x-ray crystallography and time will tell whether they will be seen as complementary or alternative techniques. With the level of advancements made already, there is great prospect for the future. Research is being conducted to improve the resolution for revealing the structure and function of a range of specimen including even smaller protein complexes, and utilization of the technique to better understand cellular processes, pathogenesis and development of treatments.

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