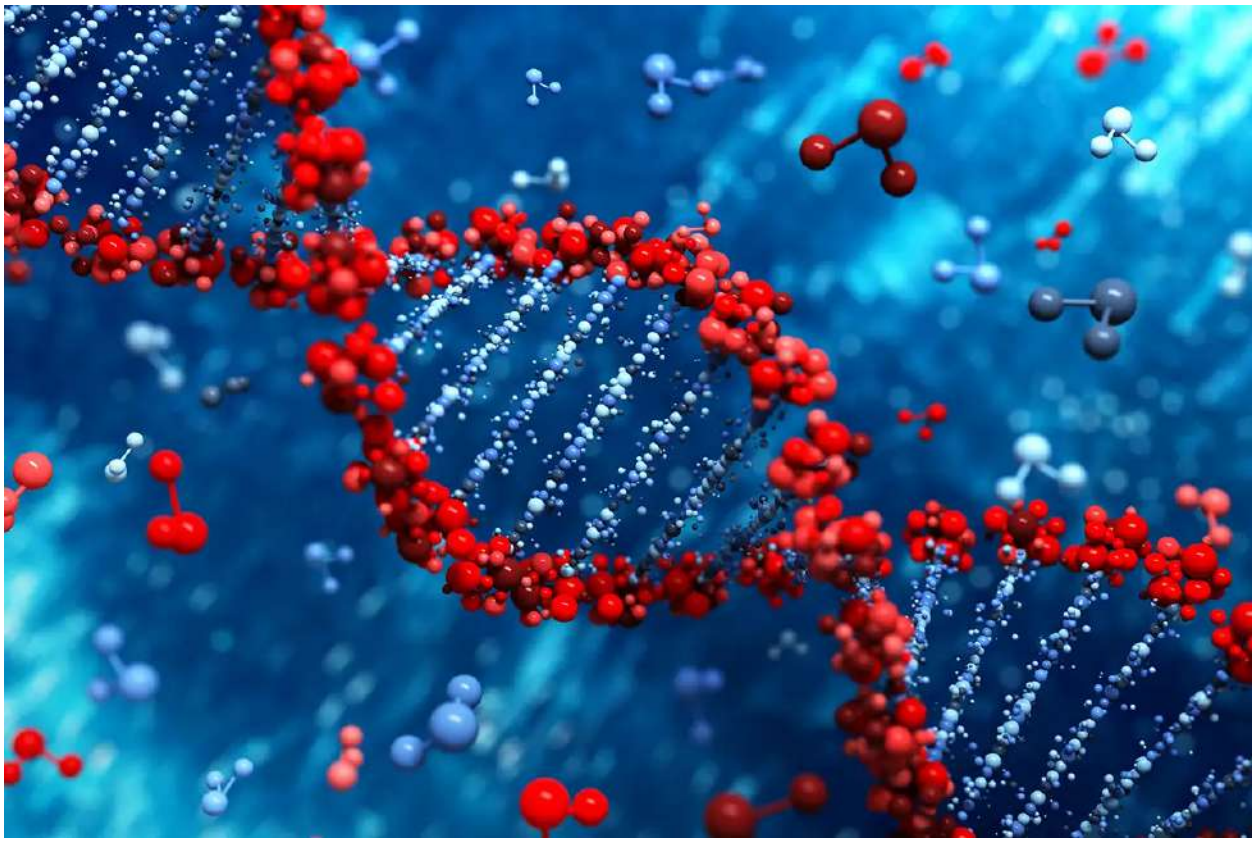
DECEMBER 2022

BIOLOGY NEWSLETTER



As an early christmas gift, here is the last Biology newsletter of 2022! This one is focused all around biochemistry, specifically proteins and their complications. If you ever feel bored over the holidays, here are a few interesting (well, hopefully!) articles to keep to entertained!

Wishing you all a Merry Christmas and Happy Holidays,

Aahana

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Chaperone Proteins

When the body makes proteins, the polypeptide chain is folded from its primary structure all the way to the tertiary or even quaternary structure. This is very handy for us, but have you ever wondered how the body regulates this protein folding? With so many possibilities in the way amino acids could interact with one another, the body relies on (yes, another protein!) molecular chaperones.

There are three types of chaperones: foldases, holdases and disaggregases[1]. Foldases are chaperones that prevent the formation of aggregated proteins by using ATP to change misfolded proteins back into their native (or normal) conformation. Holdases, in contrast, hold unfolded proteins until there is a foldase available, preventing protein damage by providing favourable conditions.

Chaperones are very costly to a cell because of their large size, so our body tries to strike a balance between the number of foldases and holdases in order to maintain low levels of unfolded proteins[2]. Their expensive nature also explains why they are usually only translated during times of stressful conditions in the cell. This quality led to the name of one family of chaperones being called heat-shock proteins (HSPs) - can you work out why? This is because at higher temperatures, the body produces more of these proteins as heat destabilises proteins and makes misfolding more common. Two important HSPs in eukaryotes are HSP-60 and HSP-70[3].

Acting as a sort of 'protein control system', disaggregate chaperones prevent potential misfolding (most severely, **aggregation**) of the polypeptide. Most chaperones do this through binding to hydrophobic surfaces of non-native proteins in order to stabilise them[4]. However, if this chaperone catalysis fails, there is another way the body combats this misfolded protein; the ubiquitin proteasome pathway (UPP).

The UPP eliminates distorted proteins through protein degradation. The 'U' in 'UPP' names proteasome's **cofactor** (the non-protein chemical compound that is required for this enzyme's role as a catalyst). The ubiquitin (or Ub) is used as a sort of tag to mark out the aggregated proteins and is attached to the proteins by 3 enzymes and ATP[5]. The first enzyme activates Ub, the second enzyme carries the Ub, but the third and most important enzyme - the Ub-protein ligase - transfers the prepared Ub onto the recognised misfolded protein. So many enzymes!

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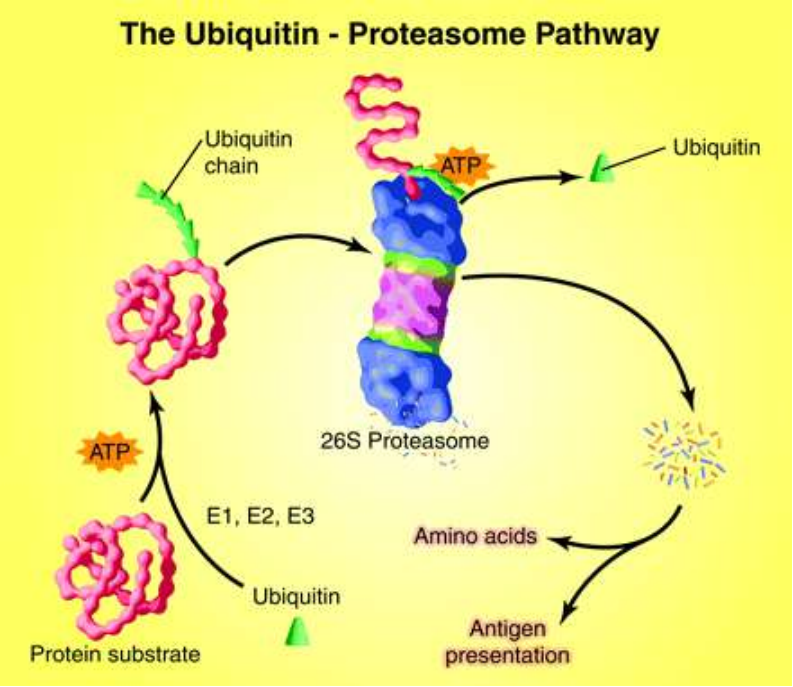
[1] Chaperones | Functions & Types
[2] Proteostasis is adaptive: Balancing chaperone holdases against foldases | PLOS Computational Biology
[3] PDB-101: Molecule of the Month: Chaperones
[4] Prevention and reversion of protein aggregation by molecular chaperones in the E. coli cytosol: implications for their applicability in biotechnology - ScienceDirect.
[5] Protein Degradation by the Ubiquitin–Proteasome Pathway in Normal and Disease States | American Society of Nephrology

But we're not done yet! The second part of 'UPP' names the kind of enzyme needed to disaggregate the misfolded protein: a protease. After the tagging process, ubiquitinated proteins (which need about 5 Ub attached) can be recognised by a very large multicatalytic protease complex. This 26S proteasome irreversibly digests the protein into peptides and removes the ubiquitin from the polypeptide. The resulting peptides are then further degraded into amino acids by other cytoplasmic peptidases.

But, as with most of the body's functions, there is something that can go wrong - some aggregated proteins resist the UPP. This lack of protein degradation leads to the formation of prions. Whilst still not completely understood, prions may also trigger misfolding of proteins and are most abundantly found in the brain. Some prion diseases include Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD)[6].



[6] Prion Diseases | CDC.

Prions and their diseases

After writing the article above, I started ‘Genome’ by Matt Ridley (definitely recommend it if you're interested in genetics or just generally DNA and disease). On the chromosome 20 chapter, Ridley describes prion diseases, such as Bovine Spongiform Encephalopathy (BSE), a.k.a. Mad Cow Disease, as well as the research behind establishing Creutzfeldt-Jakob Disease’s origin and its mechanism of spread. I thought it was a great explanation, and wanted to take you with me as I explore the current knowledge on prion diseases further.

I’ll start with what research has discovered so far.

Prions are abnormally folded infectious agents able to self-propagate (reproduce by themselves) in their hosts. They are proteinaceous (consists of/contains protein) and are associated with transmissible spongiform encephalopathies in mammals. This group of neurodegenerative diseases affects a wide variety of species, including humans, cows, mink and cats.[7] The main or sole component of the infectious agent is a misfolded protein (PrP^{Sc})2.

There are 3 kinds of prion diseases: sporadic, genetic and acquired[8]. PrP^{Sc} (scrapie isoform of the prion protein) prions are the infectious agent behind diseases such as Creutzfeldt–Jakob disease in humans, bovine spongiform encephalopathy in cattle, and sheep scrapie[9]. PrP^{Sc} is an alternatively folded variant of the cellular prion protein, PrPC, which is a regular protein that is present on the cell surface of neurons and other cell types. PRNP is the human gene on chromosome 20[10] encoding for this major **Pr**ion **P**rotein. As you probably guessed from its location, the expression of the protein is most predominant in the nervous system but also occurs in many other tissues throughout the body[11]. Whilst the structure of PrPC is well studied, the structure of PrP^{Sc} resisted high-resolution determination due to its general insolubility and propensity to aggregate.

Now what is being researched about these fiddly things at the moment?

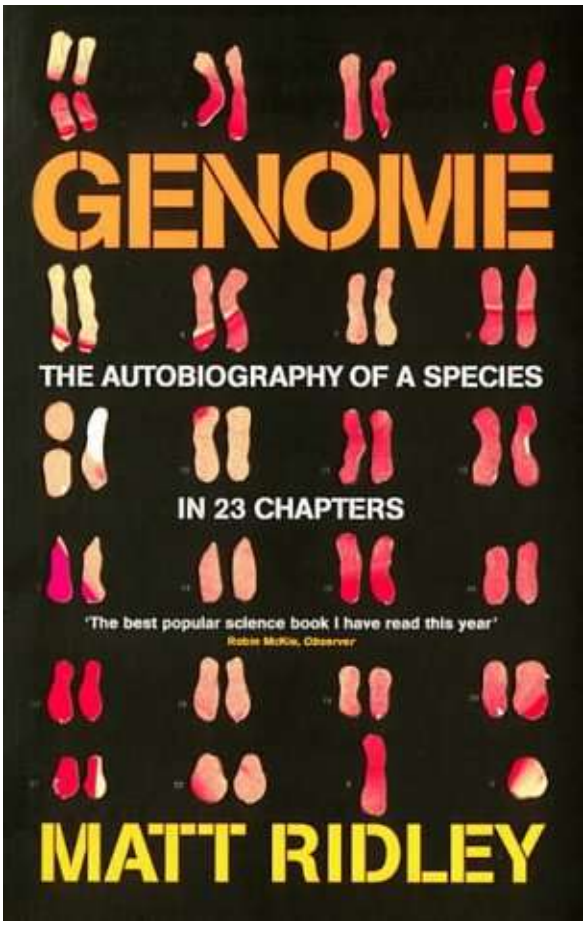
Researchers are still unsure of the mechanism in which these prions cause disease. Whilst microbes are usually able to be killed by various methods, such as exposure to UV light; very fine filtering; boiling; and drenching in a solution of formalin (formaldehyde - IUPAC name methanal), prions have shown to be resistant to all of these methods. And by the misfortune of a

[7] The Extent of Protease Resistance of Misfolded Prion Protein Is Highly Dependent on the Salt Concentration - PMC
[8] Genetic PrP Prion Diseases - PMC
[9] The Structure of PrP Sc Prions
[10] Genomic structure of the human prion protein gene. - PMC
[11] PRNP - Wikipedia

couple of epileptics, prions were discovered to even withstand surgical sterilisation, as these two sufferers developed CJD after electrodes used on a CJD patient were also used for the two exploratory brain surgery volunteers.

Since in prion diseases, the protein PrPc is affected, identification of the physiological functions of PrPc in the different cell types appears crucial for understanding the progression of prion diseases[12], so research is being undertaken to see how and where the PrPC protein works in the body.

I know this is a lot of information, so feel free to ask me any questions! Or alternatively, read Genome's chapter on chromosome 20, which gives a more basic overview, but perhaps an easier to understand story about prions and the diseases!

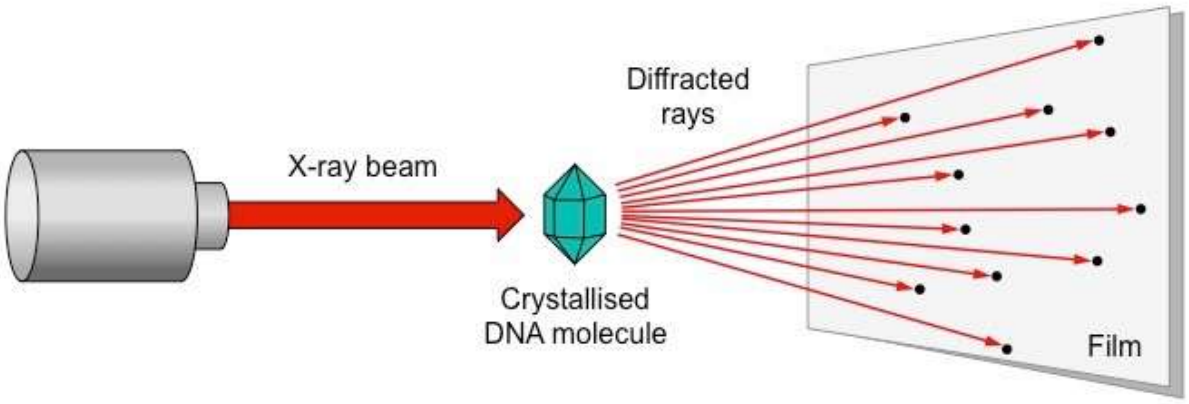


[12] [Functions of prion protein PrPc]

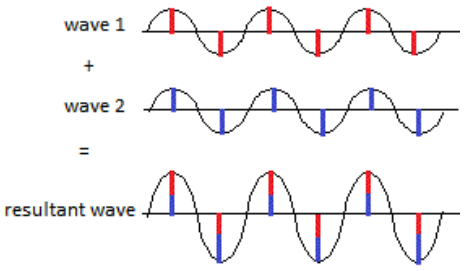
Techniques

Crystallography in used in so many fields in science that is has, directly or indirectly, produced the largest number of Nobel Laureates throughout history. 29 of the prizes, these awarded to 48 Laureates[13], have been awarded due to work using this kind of technique. This technique links lots of multidisciplinary sciences and their frontier areas of research together. But how does it work?

X-ray crystallography is a key method for the determination and characterisation of three-dimensional structures at the atomic level of small molecules from many different sources. This method was discovered in 1912 and is based on the diffraction of incident X-ray beams by crystalline samples. The angles and intensities of the diffracted reflections are used for the calculation of atom positions and building of the three-dimensional model of the molecule.

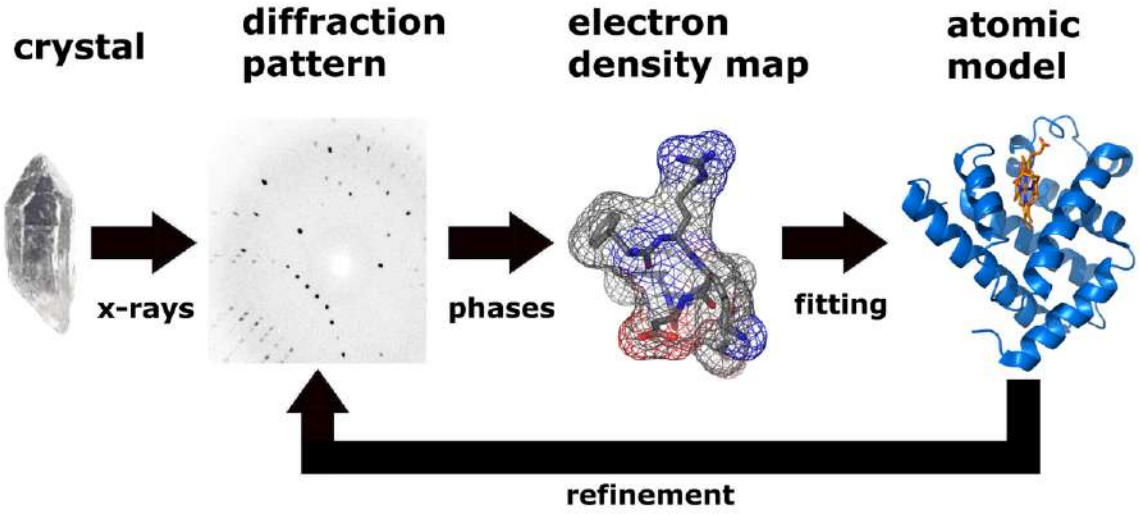


This diffraction pattern can be used to determine the electron density of the molecule hence it's 3D arrangement through the Laue pattern[14]. The Laue pattern is the location of the spots on the photographic film and it depends upon the relative locations of the atoms, or ions, in the crystal lattice. These spots come from the fact that the X-ray waves have hit the electrons of the crystal's atoms, are then absorbed then reemitted. As the physicists of you know, the emitted waves can undergo something called **constructive interference** where two waves meet 'in phase' so that the resultant wave, with an unchanged wavelength, has a larger peak and trough than the original two waves. This increase in amplitude leads to bright spots on the film (whilst the opposite causes the lack of a spot):



[13] Nobel Laureates through Crystallography
[14] Crystallography: Introduction to X-Ray Diffraction Chemistry Tutorial

The more electrons there are, the more interactions there are, however, the maths of discerning electron density from diffraction patternIf you imagine the electrons around atoms and ions, they form 'charge clouds'. These clouds cannot overlap, so the arrangement of the atoms/ions can be mapped out, and this in turn can be related to their proteins.



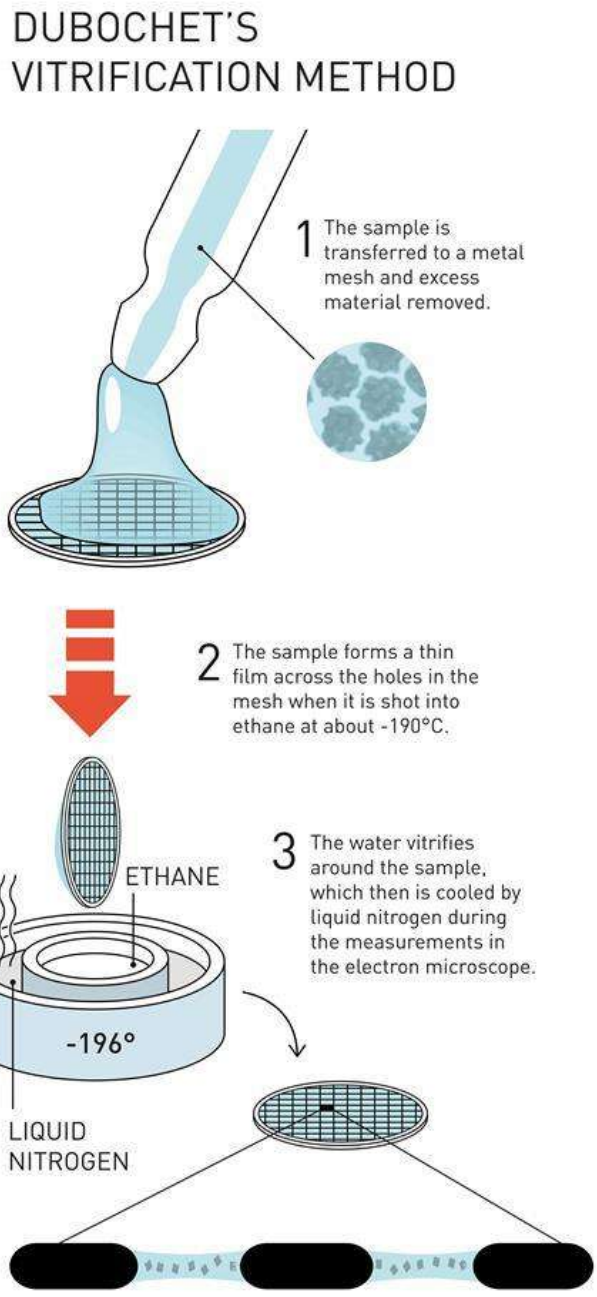
Why is this important? Well, as the Alphafold website states, being able to known a protein's structure allows us to use that knowledge towards understanding it's function e.g. determining the structure for hameoglobin can allow us to treat sickle cell anaemia or even combating diseases e.g. Zika virus. Alphafold can be used to add to the Protein Data Bank (showing how creative scientists are) as a model in order to document the electron density maps of proteins, allowing a 'prediction' of their structure. Really intense computing can be involved in this, adding to the biological, chemical and physical aspects of this topic, showing the multifaceted nature of scientific research.

But some molecular biologists predict a relatively new technique called cryogenic electron microscopy, co-developed by 2017 Nobel Prize in Chemistry winner Richard Henderson, could replace x-ray crystallography as the dominant method of visualising larger and complex molecules.[15] In another data bank - the Electron Microscopy Data Bank (EMDB) - cryo-EM is a major tool used.

As mentioned before, X-ray crystallography involves crystallizing proteins, pummelling them with X-rays and reconstructing their shape from the resulting tell-tale patterns of diffracted light. X-ray crystallography produces high-quality structures, but some proteins can take months to crystallize, and others never crystallize at all.[16] On the other hand, cryo-EM doesn't require protein crystals, but the technique called 'blobology'. Whilst it tends to produce low resolution structures, it's use of frozen samples, gentler electron beams and sophisticated image processing to overcome common TEM problems - Vacuum conditions, intense electron beams and high energy electron burn.

[15] X-ray crystallography: Revealing our molecular world | Science Museum
[16] Revolutionary cryo-EM is taking over structural biology

Overall, the method of cryo-electron microscopy involves freezing water-based TEM samples so rapidly that the water forms a disordered glass, rather than crystalline ice, so that ice isn't present to strongly diffract the microscope's electron beam. Catapulting (yes, you read that right, being shot into) the sample into a liquid nitrogen-cooled ethane bath freezes the thin film of water on the sample so quickly that the water molecules don't have time to arrange into a crystalline lattice. In this 'vitrified' sample, the water is disordered but the 3D structure of the biomolecules in the sample is retained. Here's a link if you would like to read about it in more detail, or look at a diagram[17]:



[17] https://www.chemistryworld.com/news/explainer-what-is-cryo-electron-microscopy/3008091.article