

The role of protein misfolding in human disease

Current research suggests that errors in the way protein molecules fold into three-dimensional structures contribute to the development of literally hundreds of diseases including the majority of diseases not caused by an infectious agent. These diseases include cystic fibrosis, Parkinson's and Gaucher's diseases, as well as Alzheimer's disease - the neurological disorder that continues to elude treatment.

A better understanding of the processes involved in protein folding could lead to new treatments for diseases or may even be a key to their prevention. However, given the variety of human proteins the challenge for scientists is enormous: the total number of different proteins in the human proteome is not known exactly, but on the basis that "one gene = one protein" there should be at least 20,000 nonmodified human proteins. When one takes account of mechanisms such as alternative splicing, single amino acid polymorphisms and posttranslational modifications, the actual number could well be several times higher¹.

Proteins are large, complex molecules that carry out many structural and physiological functions in the human body, including the coordination of biological processes between cells, tissue and organs. In the form of antibodies, for instance, they help the body fight disease. To perform these many and varied tasks, proteins need to fold correctly into three-dimensional shapes.

In this article we discuss how this folding process can go awry and the implications for human disease. We also report on what some pharmaceutical companies are doing to address this problem.

Four levels of protein structure

In order to appreciate the nature of protein folding and what can go wrong with it, it is necessary to understand the structure of protein molecules. A detailed description is beyond the scope of this article, but in essence there are four levels of protein structure. The primary structure of a protein is simply the sequence of amino acid residues in its molecule, each bound to the next via a peptide bond. This sequence is determined by the order of codons in the relevant gene.

Protein molecules typically contain many amino acid residues and rarely exist as a simple linear chain. Rather, they tend to bend and fold upon themselves as a result of attraction (or repulsion) between individual amino acid residues. For example, an amino acid with a net negative electrostatic charge may be attracted to another with a positive charge further along the molecule. Unless the molecule folds correctly, the protein cannot function correctly.

This folding is referred to as the secondary structure of the protein. For a given protein, there may be more than one way in which it can fold upon itself, but protein folding obeys the laws of thermodynamics such that the preferred, native configuration is usually the one with the lowest energy state.

The tertiary structure of a protein refers to a higher order of folding and the formation of disulfide bonds, hydrogen bonds and/or hydrophobic/hydrophilic interactions between various parts of the secondary structure. Such interactions

are determined by the position of individual amino acid residues within the protein's secondary structure.

The quaternary structure of a protein is the result of two or more polypeptide chains binding to each other via intermolecular interactions.

It can thus be seen that protein folding is a complicated process and consequently one which is susceptible to errors. It is very difficult for scientists to investigate: researchers have traditionally relied on time-consuming and difficult techniques such as NMR spectroscopy, X-ray crystallography, cryoelectron microscopy and others to determine the three-dimensional structure of a protein. The Holy Grail of protein research is to be able to predict a protein's secondary and/or tertiary structure simply from the sequence of amino acid residues in the molecule. If that were achieved, it would make it much simpler to design strategies to modify the folding process. In this context, CASP (Critical Assessment of protein Structure Prediction) is a global competition to assess the state of the art in modelling protein structure from amino acid sequence data which has taken place every two years since 1994. It is not merely an academic exercise but also attracts interest from industry. In the most recent CASP, in 2018, the most successful entrant was DeepMind, a European artificial intelligence (AI) company owned since 2014 by Google.

Misfolding and disease

There are a number of ways in which incorrect folding can give rise to disease. Perhaps the best understood is the process of amyloid accumulation, which is implicated in some neurodegenerative diseases. The most prevalent type of secondary structure in protein molecules is known as an α -helix, in effect a right-handed spiral. When a protein becomes toxic, it assumes a conformation known as a β -sheet (the β -sheet also exists in many functional native proteins). The transition from α -helix to β -sheet exposes hydrophobic amino acid residues that promote aggregation of the protein. Such insoluble protein aggregations are referred to as amyloid deposits.

A simple example of amyloid accumulation leading to disease occurs in the eye, where proteins known as crystallins are a major component of the lens. In their native form they are transparent, but they are prone to forming amyloid fibres that scatter light rather than transmitting it, a process that manifests itself as cataracts. A more complex example is Alzheimer's disease, where there is an accumulation of an abnormally folded protein, amyloid beta ($A\beta$) in the brain. $A\beta$ monomers are soluble and are largely α -helical in structure (although they do contain a small proportion of β -sheets). However, as they accumulate in the brains of Alzheimer's patients they undergo a conformational change to a β -sheet-rich tertiary structure that aggregates to form amyloid fibrils. These fibrils are deposited in dense plaques around the neurons.

Other mechanisms have been identified by which misfolded protein may cause disease. One is that the misfolded protein is incorrectly transported within the cell, so that it is unable

to fulfil its normal function. Alternatively, the misfolded protein simply loses functionality, or acquires some form of undesirable function. A good example of the latter is the lipid transport protein apolipoprotein E (APOE): as many as 80% of Alzheimer's disease patients carry a gene for the variant APOE4, which prevents the correct formation of the α -helix. This means that the protein is unable to fold properly. The resultant change in the structure of the protein is associated with impaired lipid binding, mitochondrial dysfunction, and increased accumulation of $A\beta^2$.

In other situations, the misfolded protein may retain some functionality, but be recognised by the body as 'foreign' and therefore eliminated. This leads to a loss of the regular function of the protein. An example of this is CFTR (cystic fibrosis transmembrane conductance regulator), a membrane chloride channel. In patients with cystic fibrosis, a mutation causes CFTR to fold incorrectly and, although it retains some of its normal function, the misfolded CFTR is targeted for degradation by the endoplasmic reticulum. This disrupts chloride transport in cells in the lungs, contributing to the symptoms of cystic fibrosis.

One other important situation where protein misfolding is related to human disease should be mentioned. This is the phenomenon known as infectious proteins, whereby some misfolded proteins have the ability to catalyse the transition of native proteins to the toxic state. The best-known example of this is the prion proteins, which are responsible for the transmissible spongiform encephalopathies including Creutzfeldt-Jakob disease.

Defence mechanisms

Given the relative ease with which proteins can become misfolded, cells have evolved a number of mechanisms to mitigate its effects or even prevent misfolding from happening at all. One way in which cells defend themselves is to degrade misfolded proteins via autophagy or some other mechanism. Dysfunction of these mechanisms may lead to disease. However, in terms of the development of novel medicines probably the most significant defensive mechanism relates to the so-called molecular chaperones. These are small protein molecules that assist in the correct folding and assembly of macromolecular structures; they may also have other related functions, such as optimising the structure of a protein for transport across membranes or for degradation.

Any disturbance in the function of a chaperone may lead to a pathological state, just like disturbances of protein folding itself. On the other hand, chaperones may be a target for pharmacological intervention in protein folding disorders. For example, heat shock protein 90 (HSP90) is a molecular chaperone that assists in the folding and function of a variety of proteins, some of which are essential for cellular survival. Pharmacological inhibition of HSP90 destabilises these proteins and leads to their degradation through the proteasome: tanespimycin is an example of an HSP90 inhibitor that has been evaluated clinically, in this case by Bristol-Myers Squibb and others, for use in the treatment of various types of cancer³. Alternatively, upregulating the expression of the relevant chaperone molecule may be an effective way of encouraging a particular protein to fold correctly.

A number of other strategies for treating disorders relating to protein misfolding have been postulated. These include reducing the production of disease-associated precursor proteins liable to misfold; inhibiting modifications to the structure of post-translational proteins into forms more likely to misfold; inhibiting the aggregation of misfolded proteins; restricting the spread of aggregated misfolded proteins, or removing them from the body; and modifying other cellular mechanisms in order to offset the toxic effects of incorrectly folded protein.

Unsurprisingly, Big Pharma has been interested in protein folding for some time. In 2010, Pfizer acquired the privately-held company FoldRx Pharmaceuticals, which had developed a drug called tafamidis for the treatment of familial amyloid polyneuropathy. In these patients, a protein called transthyretin, which is normally made up of four strands, dissociates and forms aggregates which can damage nerve cells. Tafamidis functions as a chaperone to stabilise the correctly folded tetrameric form of the protein.

Under the trade name Vyndaqel, tafamidis was granted marketing authorization in the EU in 2011 for the treatment of transthyretin amyloidosis in adult patients with stage 1 symptomatic polyneuropathy. It was also approved in Japan in 2013, but in the US the FDA rejected an NDA for the product in 2012, requesting further information and a second efficacy study. In January 2019, however, the FDA accepted for filing two NDAs for tafamidis for the treatment of transthyretin amyloid cardiomyopathy.

Recent developments

Now a growing number of companies are moving into the protein folding space. In January 2019, Wren Therapeutics, a spin-off company from the University of Cambridge in the UK and Lund University in Sweden, announced the completion of a Series A financing round that raised £18 million from an international syndicate. Wren focuses on the discovery and development of drugs to treat protein misfolding diseases, and says it will use the proceeds to expand its pipeline of small molecule and antibody therapeutics and diagnostics. Wren's CEO Dr Samuel Cohen says the company's approach focuses on the chemical kinetics of protein misfolding, "thereby creating a predictive and quantitatively driven platform with the potential radically to advance drug discovery in this class of diseases"⁴.

Also in the news recently was the Swiss company Gain Therapeutics, which is developing "next generation brain-penetrant non-competitive pharmacological chaperones" that are able to stabilise misfolded proteins and restore their enzymatic activity. Established in Lugano in 2017, Gain was initially funded by the Swiss seed and early stage venture capital fund, TiVenture, but has recently announced a €1 million investment by Helsinn Investment Fund. Gain has an exclusive licence to an advanced computational technology referred to as Site-directed Enzyme Enhancement Therapy (SEE-Tx), which was invented by the company's Chief Scientific Officer, Dr Xavier Barril. The technology permits the identification of pharmacological chaperones which are non-competitive with the natural substrate and which have improved drug-like properties. This is achieved by exploiting previously uncharacterised binding sites which do not bind the enzyme substrate: the resulting chaperones

Company	Focus
Orphazyme (Copenhagen, Denmark)	Technology is based around the drug arimoclomol, which stimulates an increased production of heat-shock proteins, protecting cells from an accumulation of misfolded proteins. Product is in clinical trials for amyotrophic lateral sclerosis, sporadic inclusion body myositis, Niemann-Pick disease Type C and Gaucher's disease.
Proclara Biosciences (Cambridge, US)	Proprietary GAIM (General Amyloid Interaction Motif) technology simultaneously targets multiple misfolded proteins implicated in both neurodegenerative and progressive peripheral diseases, potentially creating a more robust response that could be suitable for patients at all stages of disease. Has two candidates in clinical trials: NPT088 is in Phase 1 for Alzheimer's disease and may also be considered for Parkinson's disease; and NPT189 is in Phase 1 for transthyretin amyloidosis and antibody light chain amyloidosis.
Proteostasis Therapeutics (Boston, US)	Disease-Relevant Translation (DRT) platform integrates genomics, proteomics and functional assays to identify small molecule therapeutics to treat cystic fibrosis and other diseases caused by dysfunctional protein processing. Pipeline includes three products for cystic fibrosis, one of which is in Phase 1 trials in the US. Also researching the effect of modulating the unfolded protein response pathway, in collaboration with Astellas Pharma.
Prothena (Dublin, Ireland)	Spun out of Elan in 2012. Specialises in developing immune system-based drugs to treat neurodegenerative diseases. Pipeline includes: prasinezumab, a monoclonal antibody that targets α -synuclein, a protein believed to misfold and form aggregates in Parkinson's disease; and PRX004, a monoclonal antibody designed to target and clear the misfolded forms of amyloid transthyretin protein in transthyretin amyloidosis cardiomyopathy and neuropathy
Yumanity Therapeutics (Cambridge, US)	Discovery and development of drugs to correct the cellular pathologies of neurodegenerative diseases driven by misfolded proteins. The company has three types of proprietary drug discovery platform: ultra-high-throughput, phenotypic screening, in which different protein misfolding pathologies are modelled in yeast; a human neuronal platform, in which neurons produced from the cells of patients with disease-causing genetic mutations are used to validate the molecules discovered in yeast; and a yeast-based drug-target identification platform.

are drug-like and non-inhibitors, hence offering a wider therapeutic window.

Gain says its technology is applicable to all diseases with a known genetic mutation in target proteins. Its current focus is lysosomal storage disorders, specifically Gaucher's disease and GM1 gangliosidosis. The company is also developing programmes in three additional inborn errors of metabolism caused by enzyme deficiencies, two lysosomal storage diseases and one organic aciduria⁵.

A selection of other companies actively involved in developing treatments for protein misfolding-related diseases is shown in the table.

Wren's Samuel Cohen describes protein misfolding diseases as "one of the most critical healthcare challenges of the 21st century", while the Centre for Misfolding Diseases at the University of Cambridge in the UK says that protein misfolding disorders are rapidly becoming the greatest threat to modern healthcare. When it comes to research, protein misfolding diseases have traditionally been the poor cousin to diseases such as cancer, but as we approach the third decade of the 21st century, it seems at last that these conditions are starting to get the attention they deserve.

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