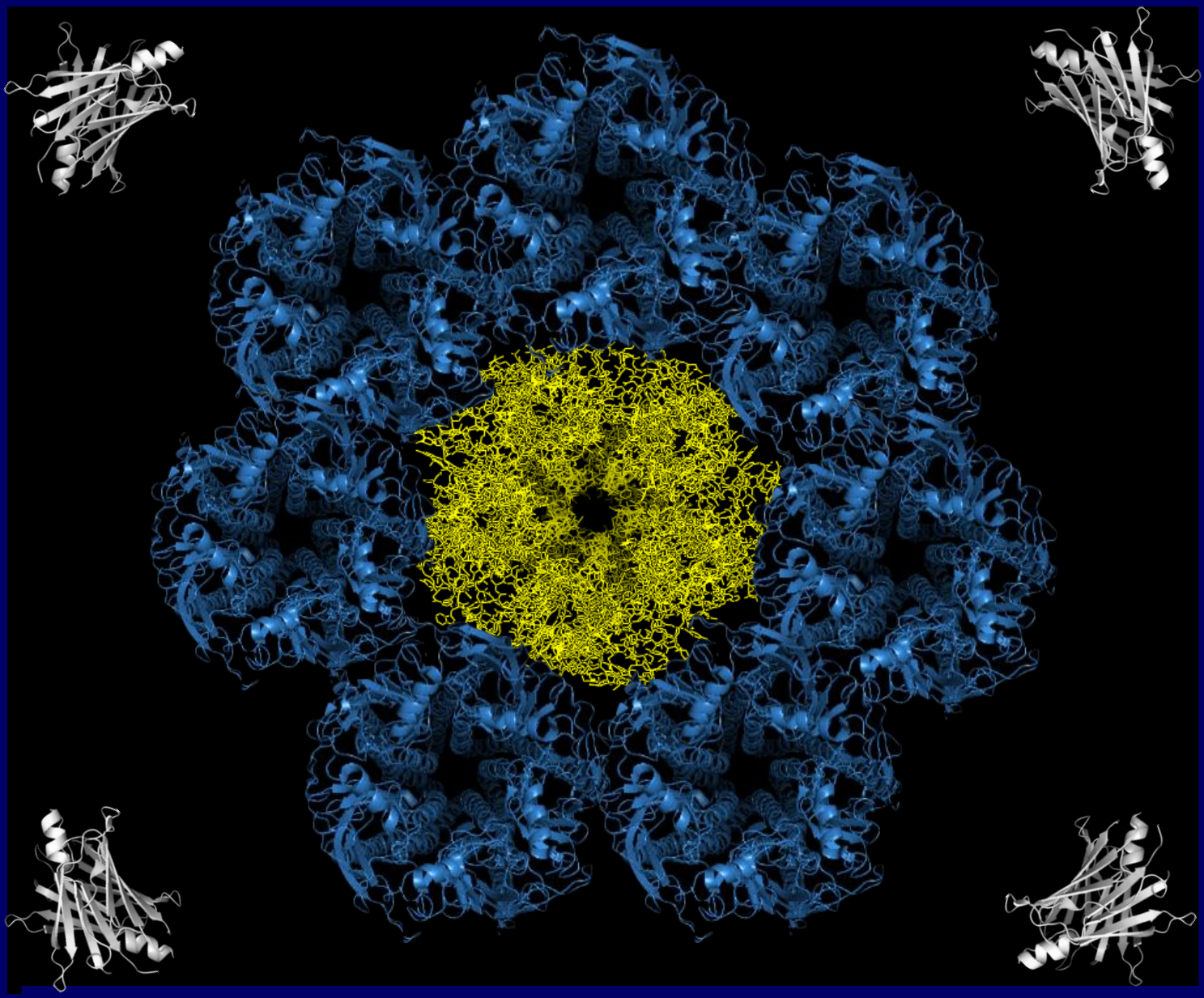


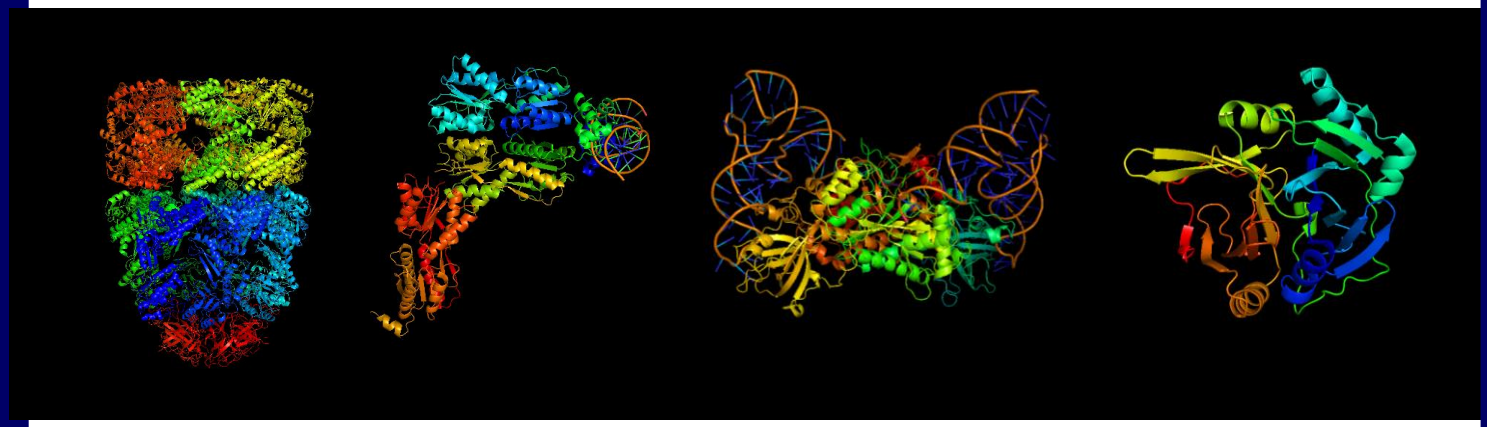
The Scholars Programme

Forget Me Not

[Term and year e.g. Autumn 2015]

Tutor Name Dr R. Porcari

Pupil Name _____



The Scholars Programme – [Spring- 2015/16] – Pupil Feedback Report

Grade	Marks	What this means
1st	70+	Performing to an excellent standard at first year undergraduate level
2:1	60-69	Performing to a good standard at first year undergraduate level
2:2	50-59	Performing to an excellent standard at GCSE level
3rd	40-49	Performing to a good standard at GCSE level
Workings towards a pass	0-39	Workings towards a pass
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Name of PhD Tutor	Dr R.Porcari		
Title of Assignment			
Name of Pupil			
Name of School			
ORIGINAL MARK / 100		**FINAL MARK / 100**	
DEDUCTED MARKS		**FINAL GRADE**	

If marks have been deducted (e.g. late submission, plagiarism) the PhD tutor should give an explanation in this section:

Learning Feedback Comment 1 – Key Learning Priority: Terminology	
What you did in relation to this Key Learning Priority	**How you could improve in the future**
Learning Feedback Comment 2 – *Key Learning Priority: Understanding of Biochemistry*	
What you did in relation to this Key Learning Priority	**How you could improve in the future**
Learning Feedback Comment 3 – *Key Learning Priority: Presentation*	
What you did in relation to this Key Learning Priority	**How you could improve in the future**
Resilience Comment	
How you showed learning resilience during the course	**How you could build learning resilience in the future**

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Course Rationale

Proteins play a key role in every single living organism on this planet and they are able to perform their specific tasks thanks to their peculiar and unique structures. This course will explain: how proteins are structured; how their structure enables them to carry out their role in our body, from the structural role of collagen to the carrier function of haemoglobin: how a protein reaches its structure. The student will also understand the importance of protein structure and function in degenerative or aging-related pathologies such as Alzheimer's, Parkinson's diseases and amyloidosis. Furthermore the course will have an insight on how scientists are working towards the cure of these pathologies.

Giving the pupils their first taste of applied biochemistry, this course will provide an overview of the importance of protein structure in our organisms and will deepen and test their biology and chemistry knowledge. Students will sample classical academic style teaching and learning and develop more awareness towards complex biology focusing on the molecular structure and interaction of proteins and other macromolecules.

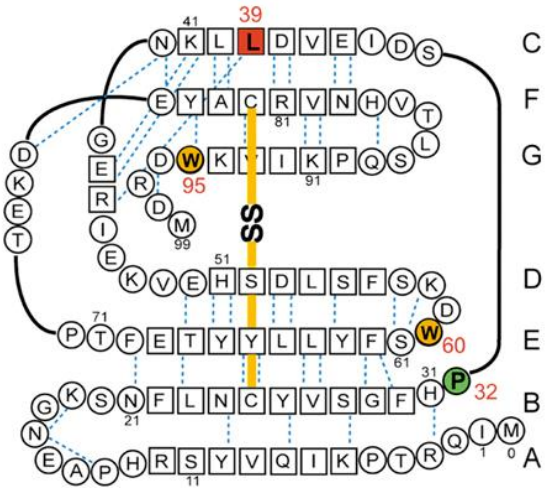
Mark Scheme

Key Skill	1st	2.1	2.2
Biochemistry understanding	☐ The pupil is able to analyse and express key biochemical concepts in an effective and synthetic way with contextual awareness. ☐ Good understanding of organic chemistry. ☐ The pupil is able to understand the importance of specific structures for peculiar biological functions. ☐ The pupil understands the dynamic of protein interaction with other proteins and its possible pathological outcome.	☐ The pupil shows good understanding of the subject ☐ Good understanding of organic chemistry. ☐ The pupil is able to analyse given information and learns from mistakes. ☐ The pupil is able to understand the importance of specific structures for peculiar biological functions.	☐ The pupil shows basic understanding of the subject. ☐ Basic understanding of organic chemistry. ☐ The pupil is able to analyse given data and learns from mistakes.
Application	☐ The pupil is able to apply the acquired knowledge to given problems in a realistic way. ☐ Links between structural features and biological aspects are made correctly. ☐ The pupil is able to properly compare the two diseases and make appropriate comments.	☐ The pupil is able to apply the acquired knowledge to given problems in a realistic way. ☐ Good linking between structural and biological features. ☐ Good comparison between the two diseases.	☐ The pupil is able to apply the acquired knowledge to given problems but not always in a realistic way. ☐ The student struggles in linking chemical features and biological features. Poor comparison between the two diseases.
Scientific Communication and Contribution	☐ The pupil independently researches and creatively illustrates the conclusions made. ☐ The pupil actively participates to the tutorials and to the scientific discussions ☐ The pupil comments during the course are relevant ☐ The pupil effectively uses the appropriate scientific terminology	☐ The pupil's effort towards independent research is good and attempts creative presentation of the conclusions made. ☐ The pupil effectively uses the appropriate scientific terminology ☐ The pupil participation to the tutorial is good ☐ The pupil comments during the course are relevant	☐ The pupil's effort towards independent research is modest and attempts creative presentation of the conclusions made. ☐ The pupil effectively uses the appropriate scientific terminology The pupil participation to the tutorial is modest

Glossary of Keywords

Keywords	Definitions	In your own words…
Protein Subunit	Protein subunit is a single protein molecule that assembles (or "*coassembles*") with other protein molecules to form a protein complex.	
Alpha Helix	The alpha helix (α-helix) is a common secondary structure of proteins and is a righthand-coiled or spiral conformation (helix)	
Beta- sheet	The β sheet (also β-pleated sheet) is the second form of regular secondary structure in proteins. Beta sheets consist of connected beta strands .	
Folding	Protein folding is the process by which a protein structure assumes its functional shape or conformation. It is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from random coil.[	
Neurodegeneration	Neurodegeneration is the umbrella term for the progressive loss of structure or function of neurons, including death of neurons.	
Amyloid fibre	Amyloid fibre insoluble fibrous protein aggregates sharing specific structural traits.	
Amyloidosis	Amyloidosis is a rare disease that results from the build up of misfolded proteins known as amyloids.	

Tutorial 1 – Structure of Proteins



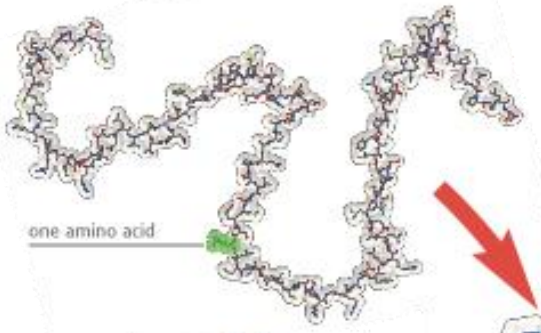
What is the Purpose of Tutorial 1?

- Introduce protein structure
- Differentiate between primary, secondary, tertiary and quaternary structures.

What is a Protein?

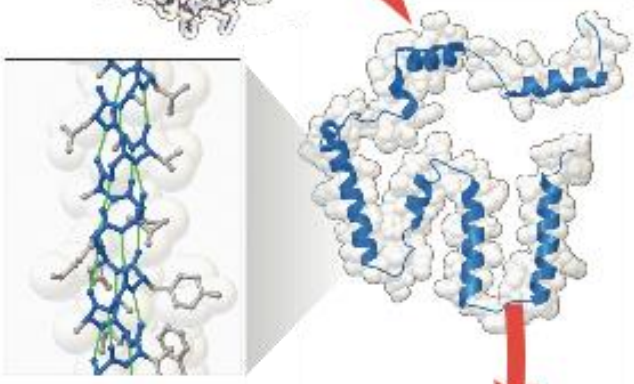
Proteins play countless roles throughout the biological world, from catalyzing chemical reactions to building the structures of all living things.

Despite this wide range of functions all proteins are made out of the same twenty amino acids, but combined in different ways. The way these twenty amino acids are arranged dictates the folding of the protein into its unique final shape. Since protein function is based on the ability to recognize and bind to specific molecules, having the correct shape is critical for proteins to do their jobs correctly.



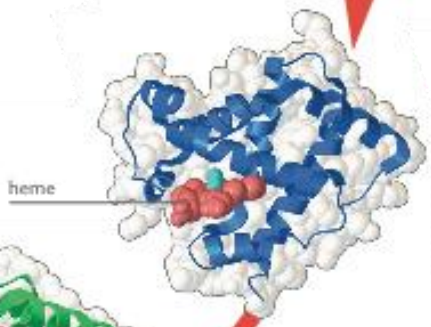
Primary Structure

Primary structure is the linear sequence of amino acids as encoded by the DNA. This sequence defines how the protein will fold and therefore also defines how it will function. A single change in the amino acid sequence of hemoglobin can cause the proteins to clump together, resulting in the disease sickle cell anemia.



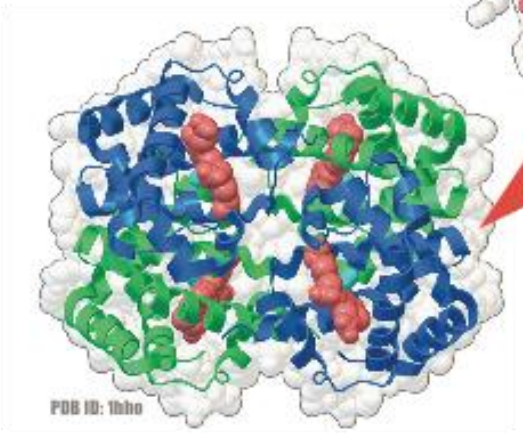
Secondary Structure

Hydrogen bonds between amino acids form two particularly stable structural elements in proteins: alpha helices and beta sheets. Alpha helices (shown in blue) are the basic structural elements found in hemoglobin, but many other proteins also include beta sheets. The inset highlights the pattern of hydrogen bonds (shown in green) that stabilizes alpha helices.



Tertiary Structure

Many functional proteins fold into a compact globular shape, with many carbon-rich amino acids sheltered inside away from the surrounding water. The folded structure of hemoglobin includes a pocket to hold heme, which is the molecule that carries oxygen as it is transported throughout the body.



Quaternary Structure

Two or more polypeptide chains can come together to form one functional molecule with several subunits. The four subunits of hemoglobin cooperate so that the complex picks up and delivers more oxygen than is possible with single subunits.

RCSB PDB
PROTEIN DATA BANK
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Tutorial 1 – Notes

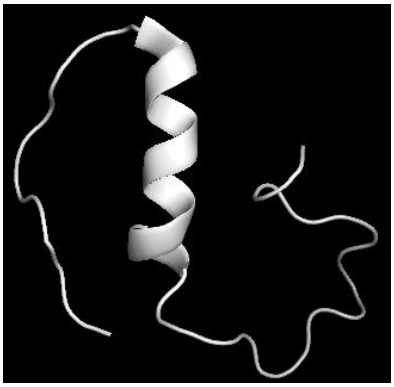
Tutorial 1 – Baseline Test

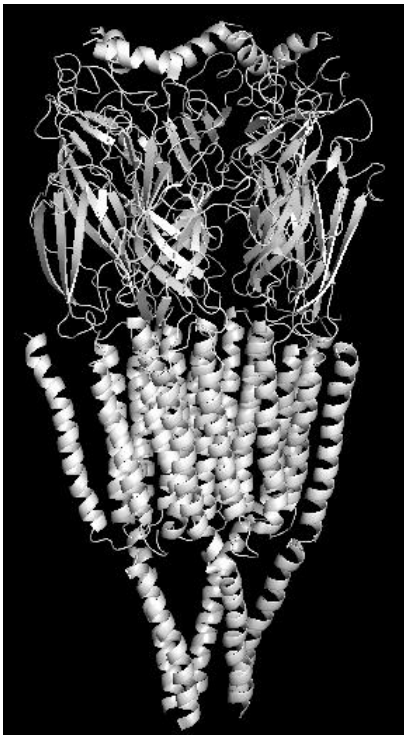
The homework assignment for the first tutorial is a baseline test to see your initial level of attainment in this subject area. The assignment will test for some or all of the subject specific skills that are required later in the final assignment. However, it is shorter than the final assignment and is will be an introduction to the subject as well as a challenge!

Do not worry too much about doing 'well' or 'badly' on the baseline test, it takes into account the fact that you may not be familiar with the subject area. It is designed to help you and your PhD tutor identify where you are at the start of the programme and to help you measure your progress along the way.

1) **Identify different secondary structures from these PDB images, colour them in using different colour for each secondary structure. Looking at protein A and protein B, do you think they have similar or different function?**

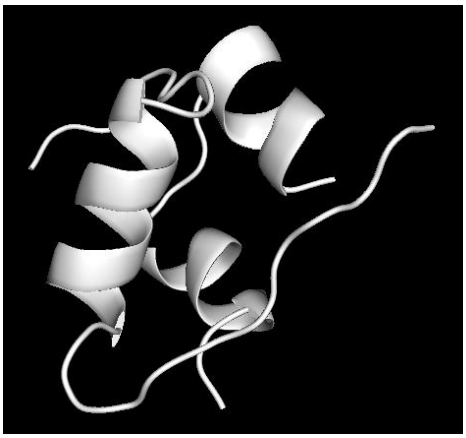
Abeta- protein

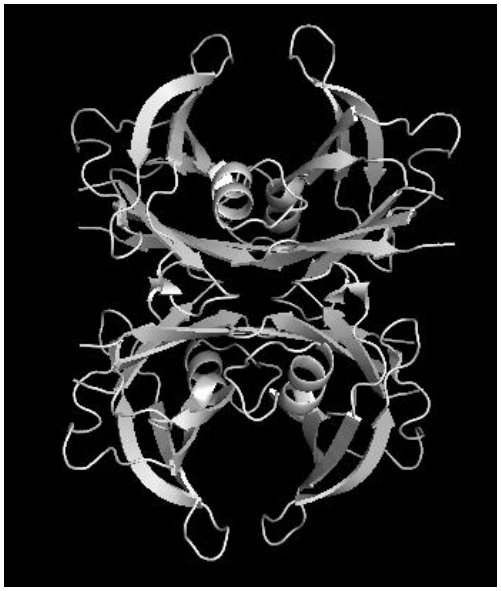




Nicotinic acetylcholine receptor

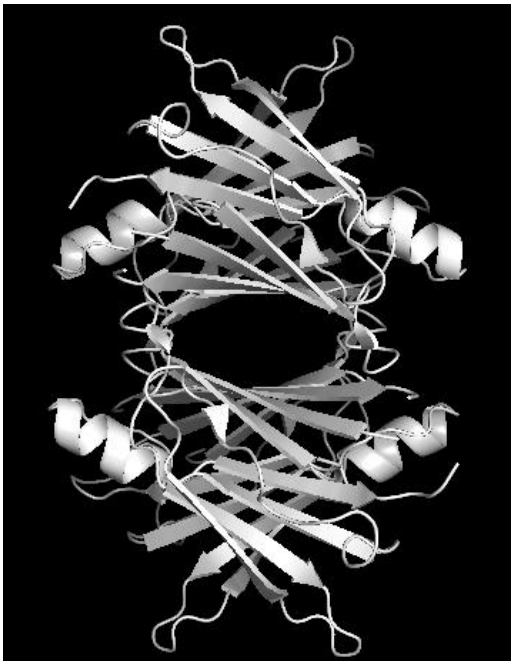
Insulin



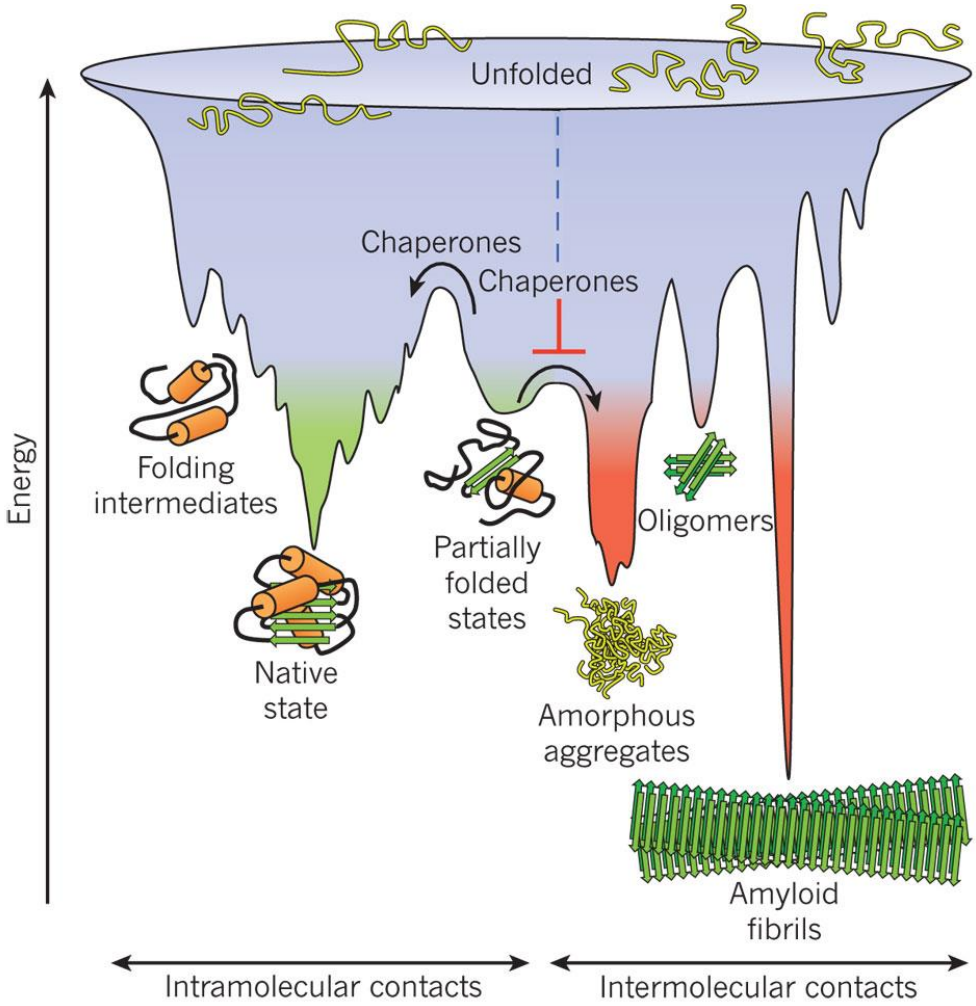


Protein A

Protein B



2) **Carry out a research about the role of one of the protein, where can be found in our body? Which is its function (max. 2 A4 paper, with pictures,) ? N.B. REFERENCE ALL YOUR FINDINGS. Can you also find out how the structure allows the function to be carried out?**



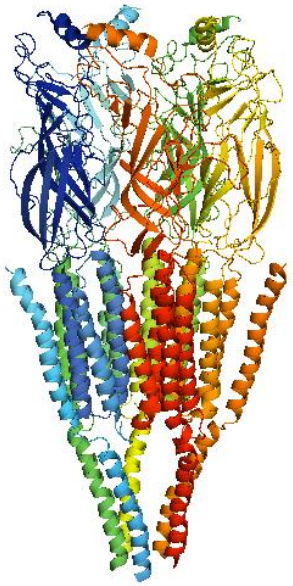
What is the Purpose of Tutorial 2?

- Understand the concept of folding
- Analyse the process of protein folding
- Learn about the different folding theories
- Learn about the role of chaperones
- Understand how chaperones work

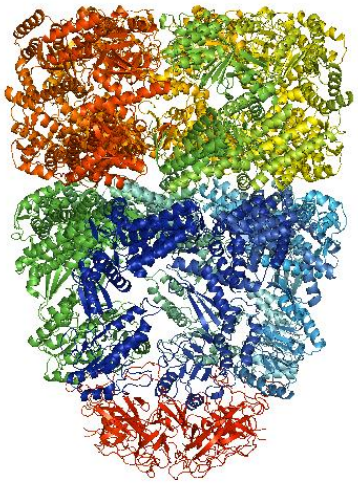
Tutorial 2 – Notes

Tutorial 2 – Homework

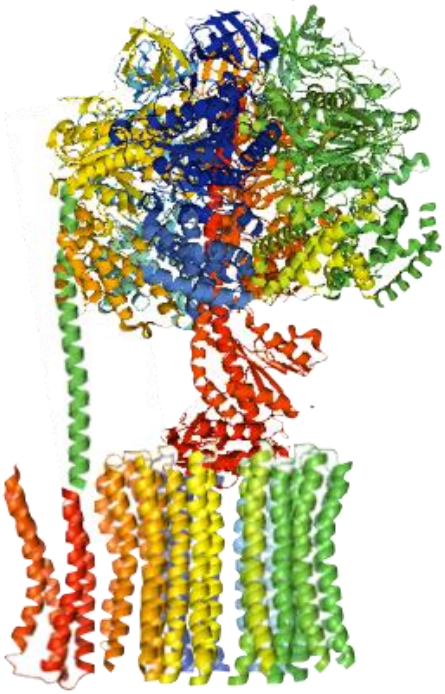
1) **Is this a soluble or trans membrane protein (images)? Explain your choices**



PROTEIN A



PROTEIN B



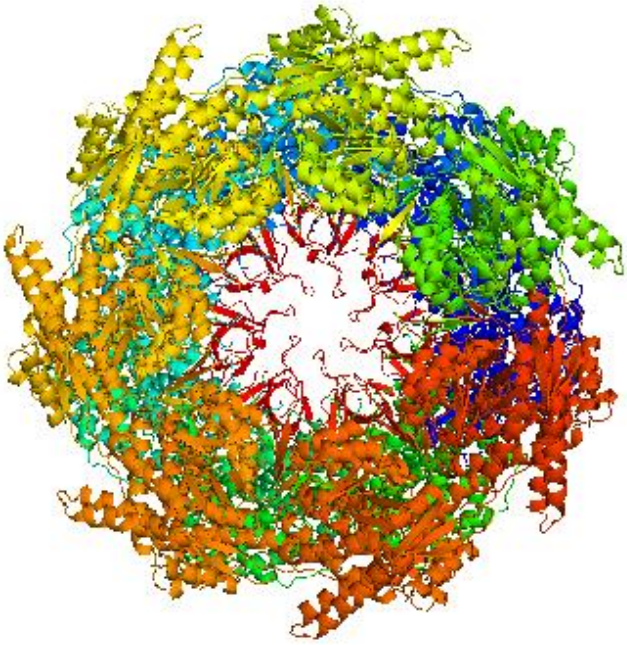
PROTEIN C

PROTEIN A….

PROTEIN B….

PROTEIN C…..

Tutorial 3 – The Misfolding Process



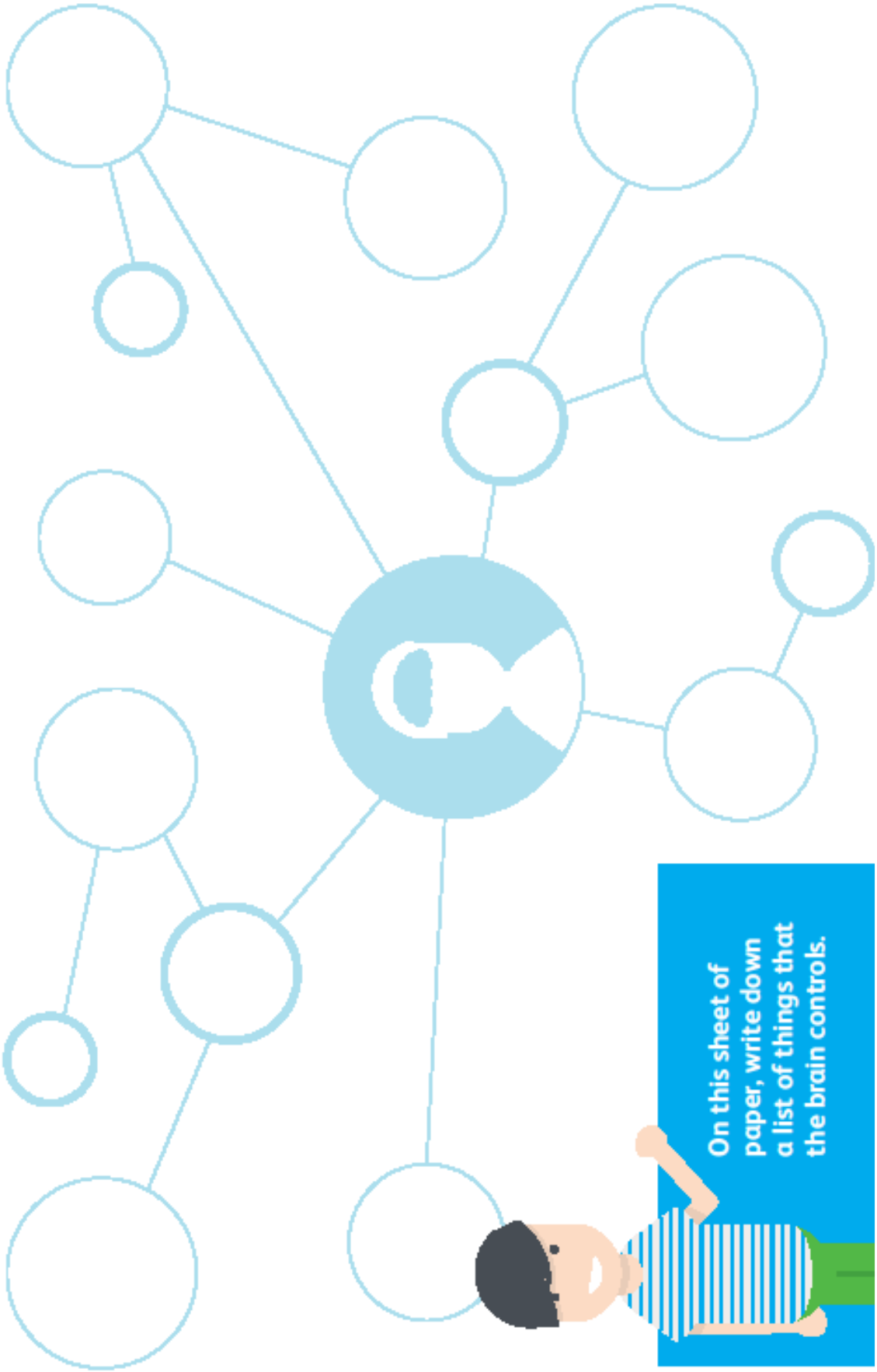
What is the Purpose of Tutorial 3?

- Learn about misfolding
- Understand the importance of post translational modifications

Tutorial 3 – Notes

Tutorial 3 – Homework

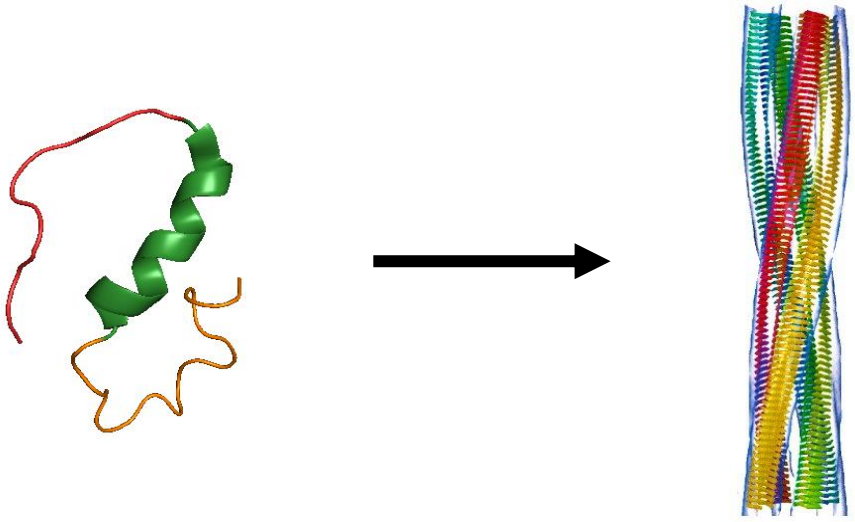
Research about Alzheimer's disease, fill in the mind map and answer the questions on the next page.



Alzheimer's Society | Leading the fight against dementia

Can you find the answers to the following questions?

1. What is dementia?	
2. What causes dementia?	
3. How many people in the UK have dementia?	
4. Is dementia a natural part of ageing?	
5. What help and support is available for people living with dementia?	
6. How can people learn more about dementia?	



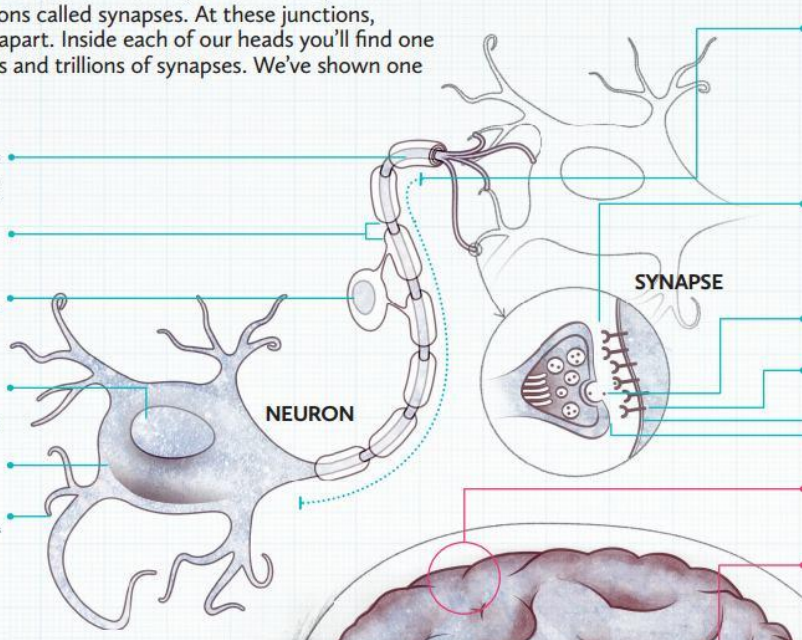
What is the Purpose of Tutorial 4?

- Learn about Alzheimer's disease
- Understand the molecular mechanism of Alzheimer's disease

Inside the brain

BigPicture

Our brains contain some 86 billion neurons, or nerve cells, which communicate via junctions called synapses. At these junctions, neurons are just 20 nm apart. Inside each of our heads you'll find one brain, billions of neurons and trillions of synapses. We've shown one of each in detail below.



Axon: The long projection that carries signals away from the cell body towards other neurons. The membrane voltage change from an incoming signal here triggers the opening of channels that allow ions (charged atoms) to flow into the cell from outside. This causes more channels further along the axon to open, creating an electrical signal (action potential) that propagates along it.

Myelin sheath: Many neurons are insulated by myelin: multiple layers of fatty cell membrane that wrap around the axon. The sheath is interrupted at regular intervals ('nodes of Ranvier'), where the channels that generate the electrical signal are located.

Nodes of Ranvier: Areas of the axon without myelin. The action potential (electrical signal) jumps rapidly from one node of Ranvier to the next, speeding up conduction.

Glial cell: Non-neuronal cells found in the brain and other parts of the nervous system. Their roles include supporting neurons and forming the myelin sheath.

Cell body (soma): Contains many components typically found in other types of cell. This includes DNA, in the nucleus, which holds instructions for producing the proteins that determine the shape and function of the cell.

Cell membrane: A film of fatty molecules (phospholipids) that controls substances entering and leaving the cell.

Dendrites: Protrusions from the cell body that form branches connecting to other cells. These connections are input synapses (see right), which receive signals from the axons of neighbouring neurons.

Synapse: The junction between neurons. When a nerve signal travelling along an axon reaches a synapse, it triggers the release of a neurotransmitter that diffuses across the synaptic gap and binds to receptor proteins on the surface of the receiving neuron. This binding causes an influx of ions, changing the membrane voltage and initiating an electrical signal in the second neuron.

Neurotransmitter: A chemical that neurons use to communicate with one another. There are many kinds, including serotonin, dopamine and noradrenaline.

Receptor: A protein molecule embedded in the cell membrane, which binds neurotransmitter molecules released by other neurons.

Postsynaptic membrane

Presynaptic membrane

Cortex: The thin, folded structure on the outside surface of the brain.

Cerebral hemispheres: The two halves of the brain, each of which controls and receives information from the opposite side of the body.

Limbic system: A set of structures (pink) sitting above the brain stem, which are involved in emotion, motivation and memory. It includes:

Hypothalamus: The interface between the brain and pituitary gland, which controls the production and release of hormones.

Amygdala: A small, almond-shaped structure involved in emotions, particularly fear.

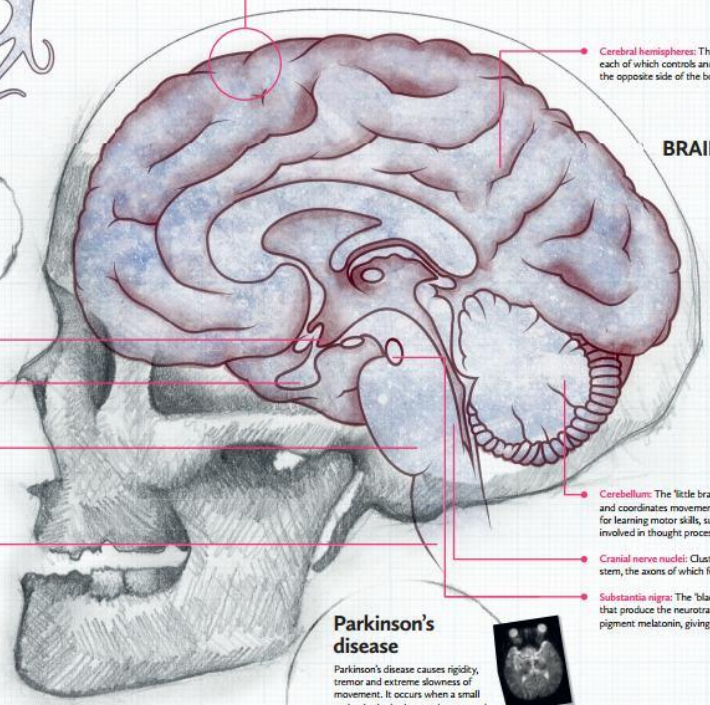
Hippocampus: Essential for making new memories and finding your way around.

Hypothalamus: See above.

Pituitary gland: The 'master gland' of the body, which releases hormones that control growth, blood pressure, the stress response and the function of the sex organs.

Medulla oblongata: Controls vital involuntary functions such as breathing and heart rate.

Spinal cord: A large bundle of millions of nerve fibres, which carries information back and forth between the brain and the body. It extends down the back and is protected by the spine.



Cerebellum: The 'little brain' that controls balance and coordinates movements. It's normally required for learning motor skills, such as riding a bike, and is involved in thought processes.

Cranial nerve nuclei: Clusters of neurons in the brain stem, the axons of which form the cranial nerves.

Substantia nigra: The 'black substance' contains cells that produce the neurotransmitter dopamine and the pigment melatonin, giving it a black appearance.

Alzheimer's disease

Alzheimer's disease can cause severe problems with mood, memory, speech and more. It occurs when abnormally folded proteins form plaques and tangles in the brain. These disrupt the normal working of neurons, which eventually die.

Right: MRI scan showing brain shrinkage and expansion of the ventricular system (central black region). Dementia Research Centre, UCL.

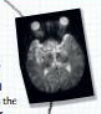
Stroke

Strokes can cause death or serious disability, often affecting speech and movement. Stroke occurs when the blood supply to the brain is reduced or blocked, by a blood clot or haemorrhage (bleed).

Left: MRI scan from a woman who had a stroke at 18, showing damaged brain (dark area, left).

Parkinson's disease

Parkinson's disease causes rigidity, tremor and extreme slowness of movement. It occurs when a small region in the brain stem known as the substantia nigra degenerates. Cells within it produce the neurotransmitter dopamine. When inadequate amounts of dopamine are produced, Parkinson's disease can occur.



Above: MRI scan of a 63-year-old's brain, showing plaques (dark areas) in the [illegible] muscle in the basal ganglia. Zuckerkorn Photo Library

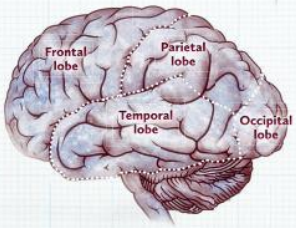
KNOW YOUR LOBES

The frontal lobe performs complex mental tasks such as making abstract judgements and decisions, thinking about the future, paying attention and inhibiting behaviour (so-called 'executive functions'). It is also involved in short-term memory, language and movement.

The parietal lobe processes information from the body and senses, and integrates it to help orient the body and carry out movement in space.

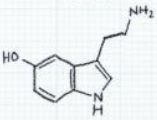
The occipital lobe is the part of the brain that manages vision, containing dozens of areas that are specialised for processing inputs from the eyes.

The temporal lobe contains areas specialised for processing sound, language and memory. It is involved in mood, appetite, sleep and learning.



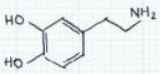
KNOW YOUR NEUROTRANSMITTERS

Serotonin



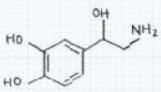
Made in the central nervous system, but mostly found in the gut, serotonin is involved in things like body temperature, mood, appetite and sleep. Drugs to increase serotonin levels are used to treat depression.

Dopamine



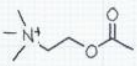
Involved in the control of movement, posture and mood. L-dopa (the precursor to dopamine and noradrenaline) is used to treat Parkinson's disease.

Noradrenaline



As a neurotransmitter, noradrenaline influences emotions, sleeping and learning. It is also a hormone, which affects blood vessel contraction and heart rate.

Acetylcholine



A neurotransmitter in the central and peripheral nervous system. Drugs to increase acetylcholine levels are sometimes used to treat Alzheimer's disease.

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www.wellcome.ac.uk/bigpicture/brain/poster

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Tutorial 4 – Notes

Tutorial 4 – Homework

Read the following article and underline the key concepts. Identify the differences and analogies with the Alzheimer's diseases.

The term amyloidosis describes a group of disorders caused by abnormal folding, aggregation and accumulation of certain proteins in the tissues, in an abnormal form known as amyloid deposits. These deposits are composed of abnormal protein fibres, the so-called amyloid fibrils that accumulate more quickly than they are cleared away, and which progressively interfere with the structure and function of affected organs throughout the body. Normal healthy proteins are cleared away at about the same rate that they are produced, but proteins that have formed amyloid are broken down only very slowly.

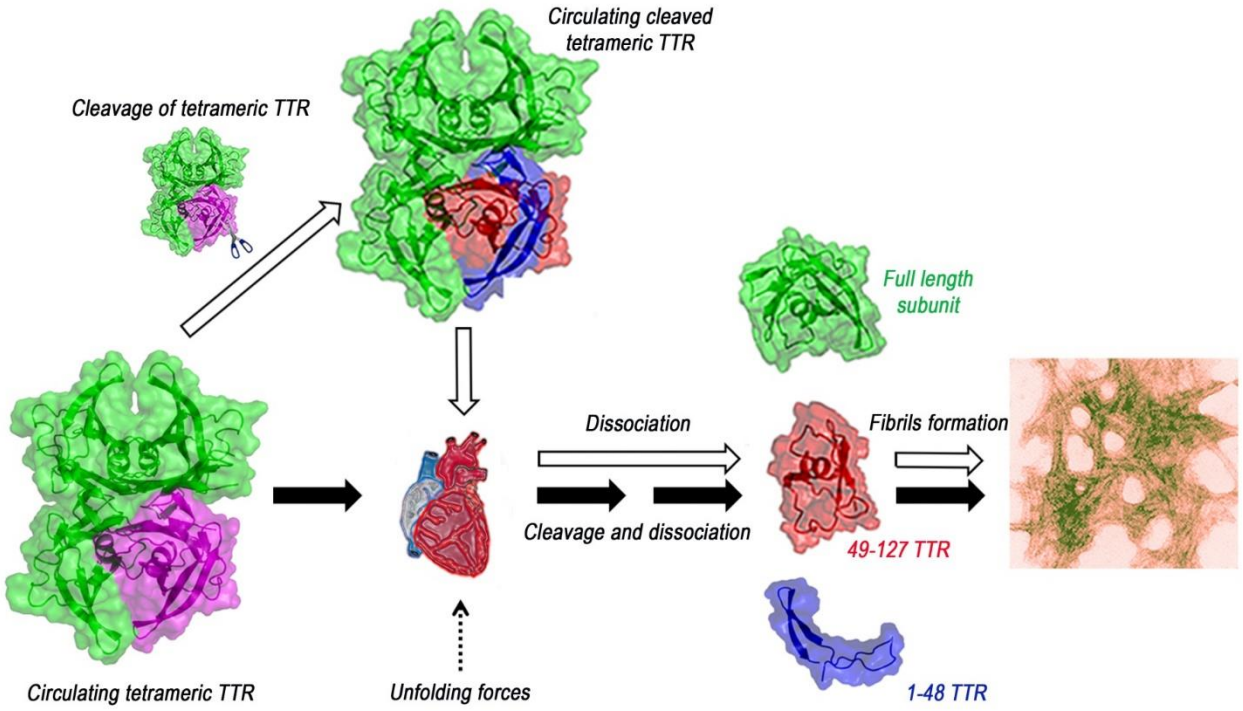
About 30 different proteins have been found to form amyloid in man, but only a few are associated with clinically significant disease. Amyloidosis is classified according to the protein that forms the amyloid fibrils and the clinical picture and symptoms can differ greatly between one amyloid type and another. The proteins that cause two of the common types of systemic amyloidosis (i.e. the types that can affect many parts of the body) are produced in the presence of other disorders. Patients with systemic AL amyloidosis (formerly known as primary amyloidosis) have an underlying bone marrow disorder, and patients with systemic AA amyloidosis (formerly known as secondary amyloidosis) have some form of long-standing inflammatory disorder. Some types of systemic amyloidosis have a genetic basis, most of which can now be identified by DNA tests. One relatively common type of amyloidosis, known as senile systemic amyloidosis, is not related to an underlying disease, and does not have a genetic basis.

Amyloid deposits can accumulate virtually anywhere in the body or can remain localized to one particular organ or tissue. Symptoms occur as a result of progressive damage to affected organs and tissues, for example the kidneys or heart, and may vary greatly from patient to patient.

Although various specific anti-amyloid drugs are under development, none as yet has been introduced into routine clinical practice. However, available treatments for the various conditions that underlie amyloidosis can stabilize or improve organ function, and may greatly improve the outlook. Contrary to previous beliefs, we have shown that amyloid deposits often gradually diminish in patients whose underlying conditions respond to treatment.

Sometimes truly localized forms of amyloidosis can cause significant disease, for example in the airways, skin, bladder, genitals or eye. Until recently, almost all patients with end-stage kidney failure who were treated with dialysis for over 5 years developed so-called dialysis related amyloidosis. In this condition, amyloid deposits in the bones and joints are formed from a protein called β2-microglobulin. This type of amyloidosis is becoming less common due to modern dialysis procedures which have improved the clearance of β2-microglobulin. Localized amyloid deposits composed of a protein called Aβ occur in the brains of patients with Alzheimer's disease, but it is not known whether they are the cause of the disease. Fortunately, the brain is almost never directly involved in systemic amyloidosis.

Tutorial 5 – Amyloidosis



What is the Purpose of Tutorial 5?

- Learn about Amyloidosis
- Understand the molecular mechanism of Amyloidosis

Tutorial 5 – Notes

Tutorial 5 – Homework: Draft Assignment – Review Analysis

Read the review and resume its content in a 3-4 slide presentation. Each slide cannot contain more than 10 words. Write a speech for your short presentation.

REVIEW ARTICLE

MECHANISMS OF DISEASE

Molecular Mechanisms of Amyloidosis

Giampaolo Merlini, M.D., and Vittorio Bellotti, M.D., Ph.D.

THE AMYLOIDOSES CONSTITUTE A LARGE GROUP OF DISEASES IN WHICH misfolding of extracellular protein has a prominent role. This dynamic process, which occurs in parallel with or as an alternative to physiologic folding, generates insoluble, toxic protein aggregates that are deposited in tissues in bundles of β-sheet fibrillar protein (Fig. 1). (A β-sheet consists of strands of polypeptides in zigzag formation, as shown in Fig. 2.) These amyloid deposits are identified on the basis of their apple-green birefringence under a polarized light microscope after staining with Congo red and the presence of rigid, nonbranching fibrils 7.5 to 10 nm in diameter, on electron microscopy (Fig. 2).[1] Amyloid deposits are the basis of several conditions that have an enormous social and medical impact as well as the cause of rare conditions that challenge the physician's diagnostic capability (Table 1).

The deposition of amyloid in brain tissue underlies Alzheimer's disease,[2,3] which affects more than 12 million people worldwide. The central nervous system is also the target of prion proteins, the cause of a group of rare hereditary or acquired neurodegenerative conditions.[4] The approximately 1 million patients who are receiving dialysis worldwide are at risk for symptomatic amyloidosis.[5] The two most common forms of systemic amyloidosis are light-chain (AL) amyloidosis, with an incidence of approximately 1 case per 100,000 person-years in Western countries,[6] and reactive amyloidosis due to chronic inflammatory diseases (e.g., rheumatoid arthritis and chronic infections). Hereditary amyloidosis is an ever-expanding group of disorders that pose difficult diagnostic problems.[7] The clinical features of systemic amyloidosis were reviewed in the *Journal* in 1997.[8]

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MOLECULAR MECHANISMS

BIOCHEMICAL CHARACTERISTICS OF AMYLOIDOGENIC PROTEINS

Isolation of the protein components of natural amyloid and the chemical characterization of these components are indispensable investigative tools.[9] To date, at least 21 different proteins have been recognized as causative agents of amyloid diseases.[1] Despite having heterogeneous structures and functions, all these proteins can generate morphologically indistinguishable amyloid fibrils (Fig. 2).[10] The generic fibrillar form of proteins can be regarded as a primordial structure dominated by hydrogen bonding between the amide and the carbonyl groups of the main chain, rather than by specific interactions of the side chains, which dictate the structure of functional globular proteins.[11] The essence of amyloidosis lies in the capacity of these proteins to acquire more than one conformation, a feature that has earned them the sobriquet of chameleon proteins.[12]

A DYNAMIC VIEW OF THE PATHOGENIC PROCESS

The conversion of the structure of the native protein into a predominantly antiparallel β-sheet secondary structure (in which the N- and C-terminals are oriented in opposite directions) is a pathologic process closely related to physiologic protein folding. The folding of a newly synthesized polypeptide occurs in a rapid sequence of conformation-

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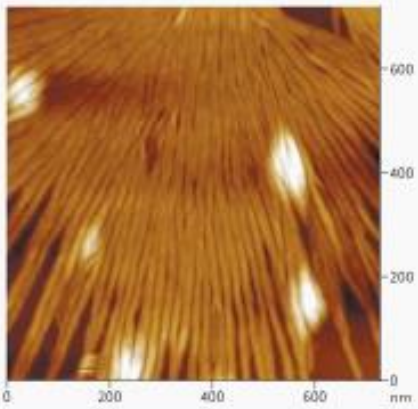

Figure 1. Atomic-Force Microscopy of Natural Amyloid Fibrils.

Fibrils were extracted from the heart of a patient with a familial form of apolipoprotein A-I amyloidosis. The image was obtained by tapping mode atomic-force microscopy; the fibrils were dried on mica. The scan size was 720 nm, with a Z range of 14 nm. The image was kindly provided by Dr. Annalisa Relini, Department of Physics, University of Genoa, Genoa, Italy.

al modifications in the cytoplasm. According to the "folding energy landscape theory," the process follows a funnel-like pathway (Fig. 3) in which the conformational intermediates progressively merge into a final species.[13] In addition, at a minimum of energy similar to that reached by the native protein, the polypeptide can acquire an alternative and relatively stable "misfolded state,"[14] which is prone to aggregation. Once the folding process has been completed and the native protein secreted (Fig. 4), many proteins are in dynamic equilibrium with a partially folded conformation, and in this state, they retrace the final part of the folding pathway, ultimately forming either a native or misfolded protein.

In amyloid disease, potentially pathogenic misfolded proteins can form in different ways. The protein may have an intrinsic propensity to assume a pathologic conformation, which becomes evident with aging (e.g., normal transthyretin in patients with senile systemic amyloidosis)[15] or at persistently high concentrations in serum (e.g., beta$_2$-microglobulin in patients undergoing long-term hemodialysis).[16] Another mechanism is the replacement of a single amino acid in the protein, as occurs in hereditary amyloidosis.[17] A third mechanism is proteolytic remodeling of the protein precursor, as in the case of β-amyloid precursor protein (APP) in Alzheimer's disease.[2] These mechanisms can act independently or in association with one another. In addition to the intrinsic amyloidogenic potential of the pathogenic protein, other factors may act synergistically in amyloid deposition. For example, the protein precursor must reach a critical local concentration to trigger fibril formation, a process enhanced by local environmental factors and by interactions with extracellular matrixes.[18]

MUTATIONS AND THE MOLECULAR MECHANISM OF AMYLOID FORMATION

Immunoglobulin Light Chains

Only a small proportion of immunoglobulin light chains are amyloidogenic; for example, AL amyloidosis occurs in only 12 to 15 percent of patients with myeloma. Certain structural features are related to amyloidogenicity: the λ isotype and the $V_{\lambda VI}$ variability subgroup (a homologous family of light-chain variable regions).[19] Two V_λ gene segments — $6a$ and $3r$ — contribute equally to the encoding of 42 percent of amyloidogenic λ chains.[20] The variable domains of light chains V(L), including the amyloidogenic chains,[21] mutate during the immune response. Some of these physiologic mutations can affect critical structural sites, destabilizing the domain and favoring the generation of an aggregation-prone state.[22,23]

Familial Amyloidosis

In the familial amyloidoses, the substitution of a single amino acid transforms a normal protein into an amyloidogenic one; prototypical proteins are transthyretin[24] and lysozyme.[25] Transthyretin is a homotetrameric protein with a prominent β-sheet secondary structure, whereas lysozyme consists of a single polypeptide with a predominantly helical structure (Fig. 2). Approximately 80 different mutations in transthyretin have been reported[26]; a few mutations are not associated with amyloidosis, and a couple are thought to protect against the deposition of amyloid. Four pathogenic variants of lysozyme have been reported[27,28]; a fifth variant, Thr70Asn,[29] is apparently not pathogenic.

The Role of Instability

The property shared by these amyloidogenic variants and confirmed in studies of cystatin C,[30] immunoglobulin light chains,[31] and gelsolin[32] is a native conformation that is thermodynamically less

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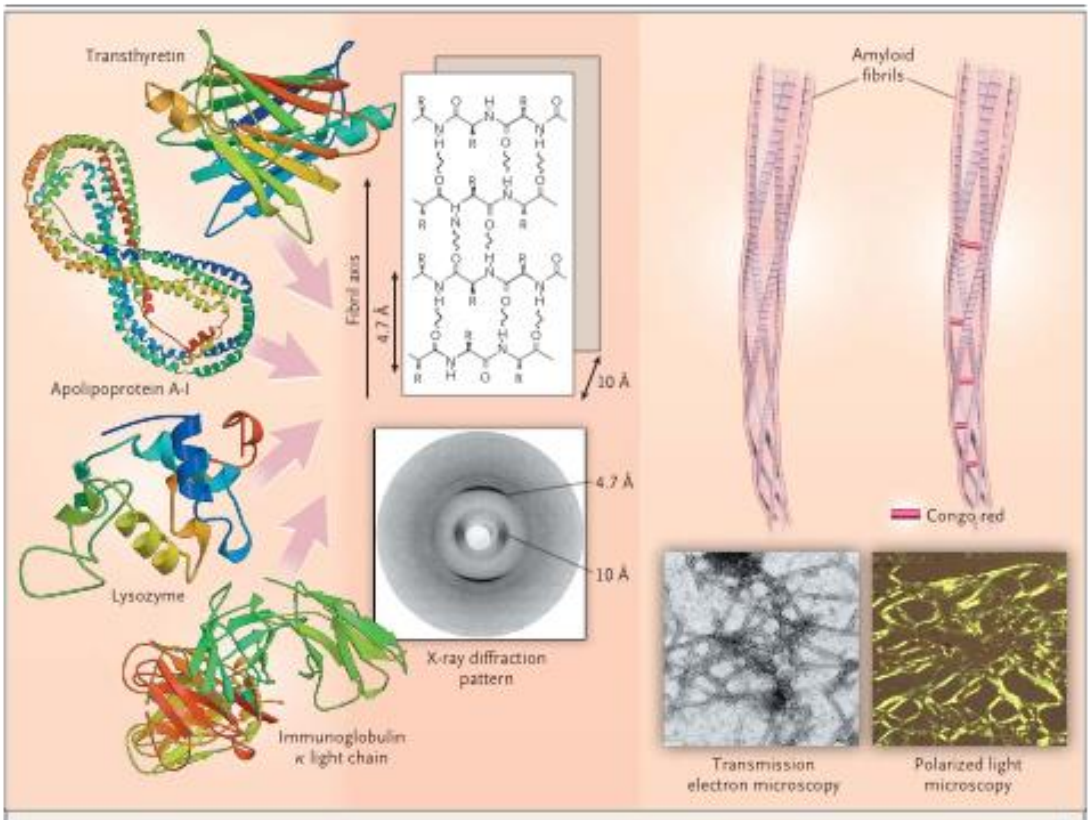

Figure 2. Structural Features of Amyloid.

The three-dimensional structures of lysozyme (Protein Data Bank code 1LYY), transthyretin (Protein Data Bank code 1TTA), apolipoprotein A-I (Protein Data Bank code 1AV1), and immunoglobulin κ light chain (Protein Data Bank code 1BRE) are shown on the left. As shown in the middle panel, all these polypeptide chains converge into a cross-beta super-secondary structure that has been well characterized by x-ray diffraction, with prototypical interstrand and intersheet distances of 4.7 and 10 to 13 Å, respectively. The original conformation of the precursor protein can no longer be distinguished at this stage. Contiguous β-sheet polypeptide chains constitute a protofilament. As shown on the right, several (four to six) protofilaments are wound around one another to form an amyloid fibril, with a distinct diameter of 7.5 to 10 nm visible on transmission electron microscopy (×100,000). This ultrastructure of the fibril allows the regular intercalation of Congo red dye, conferring a diagnostic optical property to amyloid such as apple-green birefringence under polarized light microscopy. The Protein Data Bank is accessible at http://www.rcsb.org/pdb/.

stable than that of the normal counterpart. A reduction in the stability of transthyretin should make it easier for the tetramer to dissociate into monomers,[15] whereas lysozyme mutations destabilize the tertiary structure and thus give rise to partially folded conformers[33] (alternative spatial arrangements of the same polypeptide). Monomers of transthyretin and partially folded conformers of lysozyme have a strong propensity to self-aggregate and assemble into fibrils.

The role of protein stability in the formation of fibrils in vivo has been clarified by studies of natural, nonamyloidogenic variants of both transthyretin and lysozyme. The Thr119Met variant of transthyretin has a thermodynamic stabilizing effect on transthyretin tetramers in association with both the wild-type polypeptide (wild type/Met119 genotype) and the amyloidogenic variant Val30Met.[34] Persons with transthyretin tetramers reflecting the Met30/Met119 genotype are protected from the disease that

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Table 1. Amyloid Proteins and Their Precursors.*

Amyloid Protein	Precursor	Distribution	Type	Syndrome or Involved Tissues
Aβ	Aβ protein precursor	Localized Localized	Acquired Hereditary	Sporadic Alzheimer's disease, aging Prototypical hereditary cerebral amyloid angiopathy, Dutch type
APrP	Prion protein	Localized Localized	Acquired Hereditary	Sporadic (iatrogenic) CJD, new variant CJD (alimentary?) Familial CJD, GSSD, FFI
ABri	ABri protein precursor	Localized or systemic?	Hereditary	British familial dementia
ACys	Cystatin C	Systemic	Hereditary	Icelandic hereditary cerebral amyloid angiopathy
Aβ2M	Beta$_2$-microglobulin	Systemic	Acquired	Chronic hemodialysis
AL	Immunoglobulin light chain	Systemic or localized	Acquired	Primary amyloidosis, myeloma-associated
AA	Serum amyloid A	Systemic	Acquired	Secondary amyloidosis, reactive to chronic infection or inflammation including hereditary periodic fever (FMF, TRAPS, HIDS, FCU, and MWS)
ATTR	Transthyretin	Systemic Systemic	Hereditary Acquired	Prototypical FAP Senile heart, vessels
AApoAI	Apolipoprotein A-I	Systemic	Hereditary	Liver, kidney, heart
AApoAII	Apolipoprotein A-II	Systemic	Hereditary	Kidney, heart
AGel	Gelsolin	Systemic	Hereditary	Finnish hereditary amyloidosis
ALys	Lysozyme	Systemic	Hereditary	Kidney, liver, spleen
AFib	Fibrinogen Aα chain	Systemic	Hereditary	Kidney

* Data were adapted from Westermark et al.[1] The following proteins may also cause amyloidosis: immunoglobulin heavy chain, calcitonin, islet-amyloid polypeptide, atrial natriuretic factor, prolactin, insulin, lactadherin, keratoepithelin, and Danish amyloid protein (which comes from the same gene as ABri and has an identical N-terminal sequence). CJD denotes Creutzfeldt–Jakob disease, GSSD Gerstmann–Sträussler–Scheinker disease, FFI fatal familial insomnia, FMF familial Mediterranean fever, TRAPS tumor necrosis factor receptor–associated periodic syndrome, HIDS hyper-IgD syndrome, FCU familial cold urticaria, MWS Muckle–Wells syndrome, and FAP familial amyloidotic polyneuropathy.

occurs in those with the Met30/wild-type genotype. The folding stability of the newly identified Thr70Asn variant of lysozyme is between that of normal and amyloidogenic species.[35] However, although destabilized, this molecule is not amyloidogenic, which suggests that certain proteins have a marginal degree of protection against amyloidogenesis, even when their thermodynamic stability is less than that of wild-type protein.

Destabilization is necessary but probably not sufficient to confer an amyloidogenic propensity on a protein; other structural features are required for the formation of fibrils. Recently, the role of charged residues in modulating the aggregation process, acting as structural gatekeepers by means of repulsive forces, has been highlighted.[36] In certain proteins, such as gelsolin,[37] the partial unfolding caused by the mutation renders the protein susceptible to the attack of proteases, thus provoking the release of highly amyloidogenic polypeptides.

These findings provide support for the mechanism shown in Figure 4. Amyloidogenic and normal counterparts are synthesized and secreted as native proteins, but the system of intracellular quality control appears to be incapable of recognizing and removing dangerous mutants.[38] Outside the cell, the amyloidogenic variants ultimately reach a state of equilibrium between fully folded and partially folded forms, but there is a much greater fluctuation in the concentrations of the two forms than would be expected. All factors that perturb the three-dimensional structure — such as a low pH, oxidation, in-

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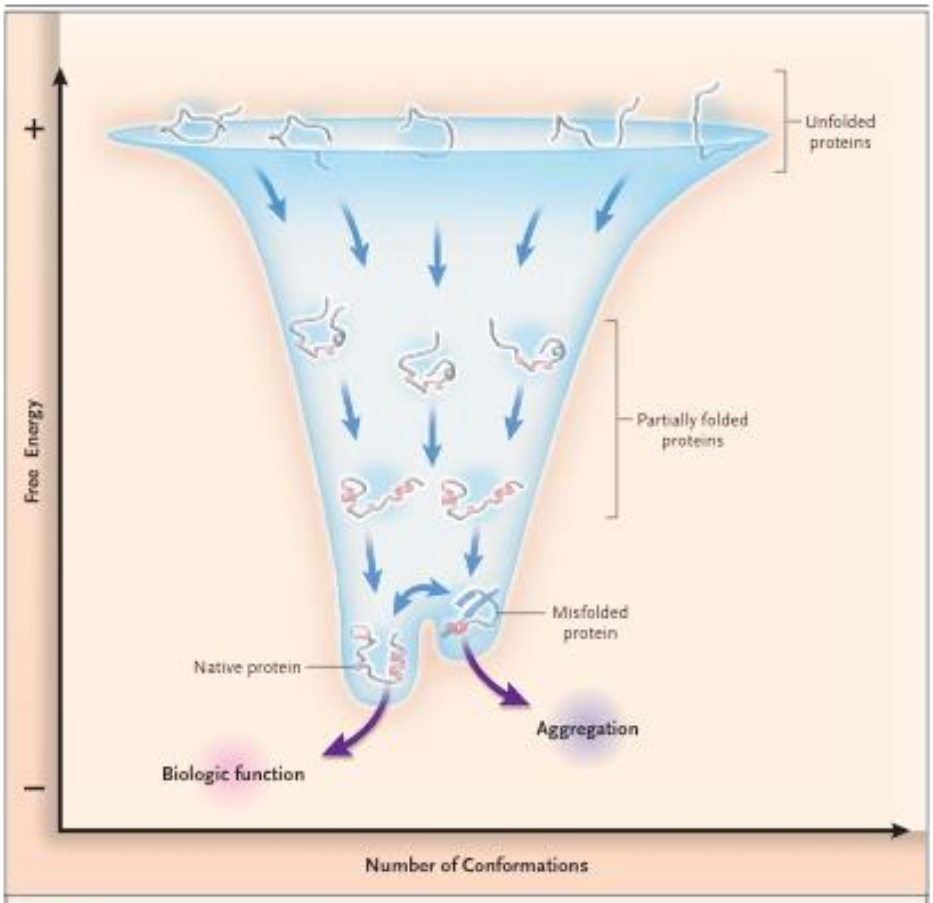

Figure 3. The Process of Protein Folding.
From a random coil conformation, the unfolded polypeptide enters a funnel-like pathway in which the conformational intermediates become progressively more organized as they merge, resulting in the most stable native state. In this state, there is a minimum of free energy, which results from the balance between the level of enthalpy, the internal energy that in folded protein is mainly determined by the kind and number of intramolecular bonds, and the level of conformational entropy, the level of randomness of the polypeptide in solution.

creased temperature, limited proteolysis, metal ions, and osmolytes — can shift the equilibrium toward the partially folded amyloidogenic state. For example, urea at the concentrations present in the inner renal medulla enhances the formation of fibrils by reducing the time required for a nucleus to form, which in turn initiates rapid growth of the fibril.[39] In addition, local microenvironmental conditions affect the ultrastructural organization of protein deposits. For example, pH influences the processing of immunoglobulin light chains, causing them to form either fibrillar amyloid aggregates or amorphous aggregates characteristic of light-chain–deposition disease.[40] Common components of amyloid deposits, such as glycosaminoglycans and serum amyloid P (SAP) component, may exert identical effects by hastening the integration of a soluble polypeptide into a more stable fibril.

Proteolysis

In certain amyloidoses, only a limited portion of the amyloid protein precursor forms the fibril; the pro-

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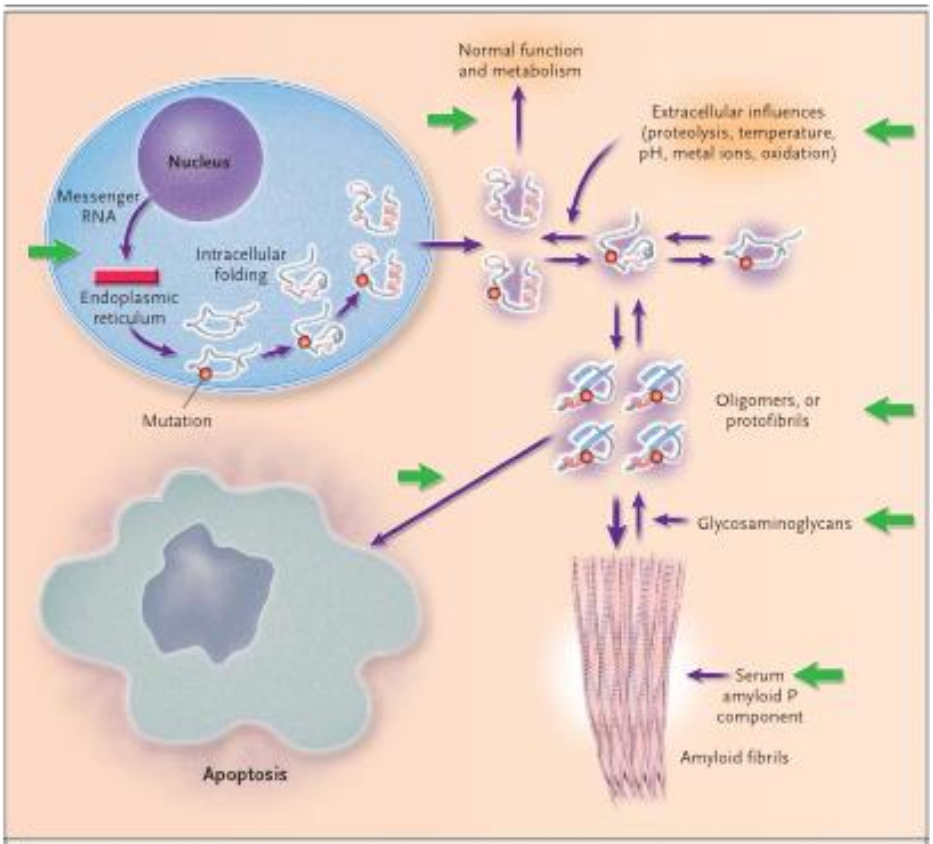

Figure 4. Pathway Followed by a Newly Synthesized Polypeptide Chain in a Patient Who Is Heterozygous for an Amyloidogenic Mutation.

The chains undergo synthesis in the endoplasmic reticulum, fold, pass through the cellular quality-control mechanism, and are secreted. In the extracellular environment, the mutants may change from a fully folded to a partially folded state and then retrace the final part of the folding pathway. Normal proteins are functionally active and are normally metabolized. The partially folded polypeptides can generate misfolded molecules, which have a high propensity to self-aggregate. Environmental conditions, chemical modifications, and common constituents favor the pathologic pathway. Oligomers, or protofibrils, may mediate cellular toxicity through a mechanism that activates apoptosis in cells of the target tissues. The green arrows indicate therapeutic targets.

totypical example is Alzheimer's disease, in which the fibrils consist of proteolytic fragments of 39 to 43 residues derived from the 753-residue APP. In lysozyme amyloidosis,[25] the full-length protein is detectable in natural fibrils. There is a wide gradation of proteolytic remodeling of the protein precursor in all types of amyloidosis, but the remodeling of light chains in AL amyloidosis can be considered the archetype of the heterogeneity of this process.[41] Proteolysis is generally ascribed to extracellular, or pericellular, enzymes, such as those that cleave serum amyloid A.[42] However, in the cases involving gelsolin[43] or the amyloid ABri protein,[44] the proteases act in the Golgi apparatus. The structural flexibility of the target protein allows limited proteolysis and therefore the release of polypeptides that cannot display conformational plasticity in the constrained structure of the original protein.[31]

AMYLOIDOSIS AS A CONFORMATIONAL DISEASE

Amyloidosis properly belongs to the category of conformational diseases because pathologic protein

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aggregation is largely due to reduced folding stability and a strong propensity to acquire more than one conformation.[45] The risk of protein aggregation, which poses toxic threats to the cell, is minimized by protein sequences that confer the properties of high stability and fast folding kinetics, both of which minimize the concentration of easily aggregating, partially folded proteins. Certain proteins, however, seem to require a high degree of structural disorder in their native states to fulfill their function. For instance, the structural plasticity of the emerging class of "natively unfolded proteins"[46] favors their interaction with ligands. These proteins represent an intriguing gamble of molecular evolution in which the subtle border between risky self-aggregation and sophisticated function is easily crossed. Amyloidogenic lipoproteins are examples. On the basis of its amino acid composition, lipid-free apolipoprotein A-I should behave as a natively unfolded protein; this state guarantees the plasticity of the protein, which partially unfolds when lipids are released and refolds when lipids are taken up. These properties are particularly evident in the N-terminal domain of apolipoprotein A-I, the major constituent of apolipoprotein A-I amyloid fibrils.[47] Other lipoproteins, such as apolipoprotein A-II,[48] apolipoprotein E,[49] and serum amyloid A,[50] also form amyloid or are implicated in amyloidogenesis and thus constitute a unique group of proteins. They have structural similarities that confer the conformational plasticity necessary for their function and at the same time favor the formation of amyloid.

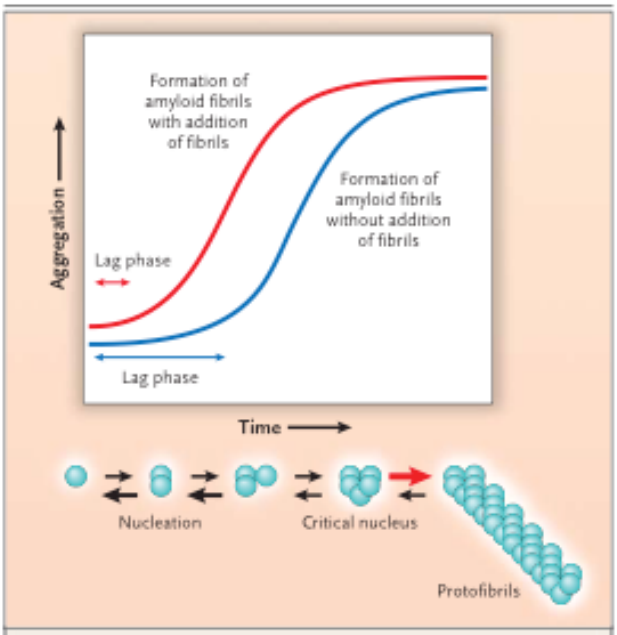

Figure 5. Kinetics of Fibril Formation.
The blue line indicates the formation of amyloid fibrils, beginning from a solution of monomeric proteins, which may temporarily assume an amyloidogenic conformation. Initially, conditions do not favor aggregation, and this period corresponds to the lag phase that precedes the formation of fibrils. Once a critical nucleus has been generated, the conditions change to favor aggregation with very fast kinetics. Any available monomers in the amyloidogenic conformation quickly become entrapped in the fibril. The red line represents a similar condition in which preformed fibrils are added, thus making the lag phase much shorter.

IS AMYLOIDOSIS A PROTEIN-MEDIATED TRANSMISSIBLE DISEASE?

Certain aspects of animal models of the amyloidosis caused by serum amyloid A[51] and apolipoprotein A-II[52] have introduced the possibility that amyloidosis is transmissible. In mice, amyloid protein A amyloidosis (AA) is caused by an inflammatory reaction that results in overproduction of the acute-phase protein serum amyloid A. Injection or oral administration of amyloid-enhancing factor, a crude homogenate of natural amyloid fibrils, accelerates the deposition of amyloid during the inflammatory process.[51,53] These findings are consistent with the capacity of fibrillar seeds to catalyze conformational changes in the soluble protein. The capacity of preformed fibrils to trigger fibrillogenesis has been demonstrated in vitro for amyloid β (Aβ) peptides,[54] lysozyme,[55] and beta$_2$-microglobulin[56] (Fig. 5).

With the exception of prion diseases, there is no evidence that amyloidosis is transmissible in humans. However, the formation of amyloid can be accelerated by the presence of fibril nuclei in tissues. A pertinent example is the patient with transthyretin variants who has cardiac involvement and receives a liver transplant. The transplanted liver minimizes the production of amyloidogenic transthyretin but does not halt the progression of amyloid deposition in the heart.[57] Wild-type instead of mutant transthyretin continues to accumulate in the heart.[58] This finding is reminiscent of the capacity of the pathologic prion protein (PrP^{sc}) to convert its normal counterpart (PrP^{c}) into a pathologic conformation.[4] The main difference is that the dominant negative effect, in the case of transthyretin, is due entirely to fibrils within the patient and is not transmitted from one person to another, as in prion disease.

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THE COMMON CONSTITUENTS OF AMYLOID

All amyloid deposits contain SAP, a glycoprotein that belongs to the pentraxin family and binds amyloid independently of the protein of origin. It has a specific binding motif for the common conformation of amyloid fibrils.[59] This property makes radiolabeled SAP a diagnostic tool for the imaging of amyloid deposits.[60] SAP is highly protected against proteolysis and thus makes amyloid fibrils resistant to degradation.[61] Proteoglycans are also common in amyloid deposits and contribute extensively to the carbohydrate composition of natural amyloid.[62] Heparan sulfate proteoglycans, in particular, have kinetics of deposition in tissue similar to that of fibrillar proteins[63] and localize with constitutive elements of the extracellular matrix, such as perlecan, laminin, entactin, and collagen IV. These molecules can constitute a scaffold, facilitating the initial phases of fibril nucleation,[18] and could have a targeting role in the localization of amyloid deposits in tissue. For example, apolipoprotein E is reportedly a common constituent of amyloid deposits,[64] and epidemiologic studies have shown an increased risk of Alzheimer's disease among white persons carrying the $\epsilon 4$ allele of apolipoprotein E.[65] However, the role of apolipoprotein E in systemic amyloidoses is less clear.

Figure 6 (facing page). Distribution of Organ Involvement in 445 Patients with Light-Chain Amyloidosis.

The light chains are predominantly λ (ratio of λ to κ light chains, 4 to 1). Usually, a bone marrow plasma-cell clone, identified here by means of an immunofluorescence assay with rhodamine-labeled anti-lambda antibody (anti-λ), synthesizes monoclonal light chains, which are best identified by immunofixation (IF) of serum and urine. The light chains can produce amyloid deposits in virtually every organ, with the exception of parenchymal brain tissue. Percentages indicate the frequency of dominant organ involvement. Only one quarter of the patients had involvement of a single organ at presentation; the remaining patients had involvement of two organs (36 percent) or three or more organs (39 percent). The heart mass can be markedly increased, and the interventricular septum is thickened. Involvement of the gastrointestinal tract may cause life-threatening bleeding. Shown is one example of peripheral nervous system involvement: infiltration of the carpal tunnel by amyloid, which may damage the median nerve, with consequent marked hypotrophy of the hand muscles. Soft tissues that may be infiltrated by amyloid include the tongue and the submandibular regions; bruising is typical and is caused by vascular fragility resulting from the infiltration of the vessel walls by amyloid (as indicated by the apple-green birefringence on polarized light microscopy).

CLINICAL IMPLICATIONS

TISSUE SPECIFICITY OF AMYLOID DEPOSITION

The remarkable diversity in the organ distribution of amyloid deposits remains one of the most important unsolved problems in amyloid research. Specific proteins aggregate predominantly in defined target organs: beta$_2$-microglobulin in joints, the fibrinogen Aα chain in the kidney, and the transthyretin Met30 variant in peripheral nerves. In light-chain amyloidosis, the deposits can involve virtually any organ (Fig. 6). One quarter of patients present with clinical involvement of a single organ, and the organ affected establishes the prognosis. Localized deposition of proteins that are normally deposited systemically can also occur, such as in localized AL amyloidosis, an intriguing condition characterized by localized growth of monoclonal plasma cells and the restriction of amyloid deposits to sites adjacent to the synthesis of the precursor.

The site of deposition may depend on the concurrence of several factors favoring the formation of fibrils, such as a high local protein concentration, a low pH, the occurrence of proteolytic processing, and the presence of fibril seeds. Specific interactions with tissue glycosaminoglycans[66] or cell-surface receptors such as the receptor for advanced glycation end-products (RAGE) may be important.[67] In AL amyloidosis, recognition of particular tissue constituents (i.e., collagen)[68] by amyloidogenic light chains may determine the specificity of tissue deposition. A specific kidney tropism of the λ light chains derived from the 6a germ-line gene has been demonstrated[20,69]; the tropism may occur because of the interaction of these proteins with mesangial cells.[69]

MECHANISM OF TISSUE DAMAGE

There is lively debate about the mechanism by which aggregation causes tissue damage and organ dysfunction. The deposition of large amounts of fibrillar material can subvert the tissue architecture and consequently cause organ dysfunction. Amyloid fibrils may also cause organ dysfunction by interacting with local receptors, such as RAGE.[67,70] In Alzheimer's disease, an inflammatory response in the cerebral cortex elicited by the progressive accumulation of Aβ contributes to the pathogenesis of the disease.[71] In Aβ and transthyretin amyloidosis, soluble oligomeric intermediates of fibril assembly are cytotoxic in vitro[72-75] and in vivo.[76] Soluble fibril

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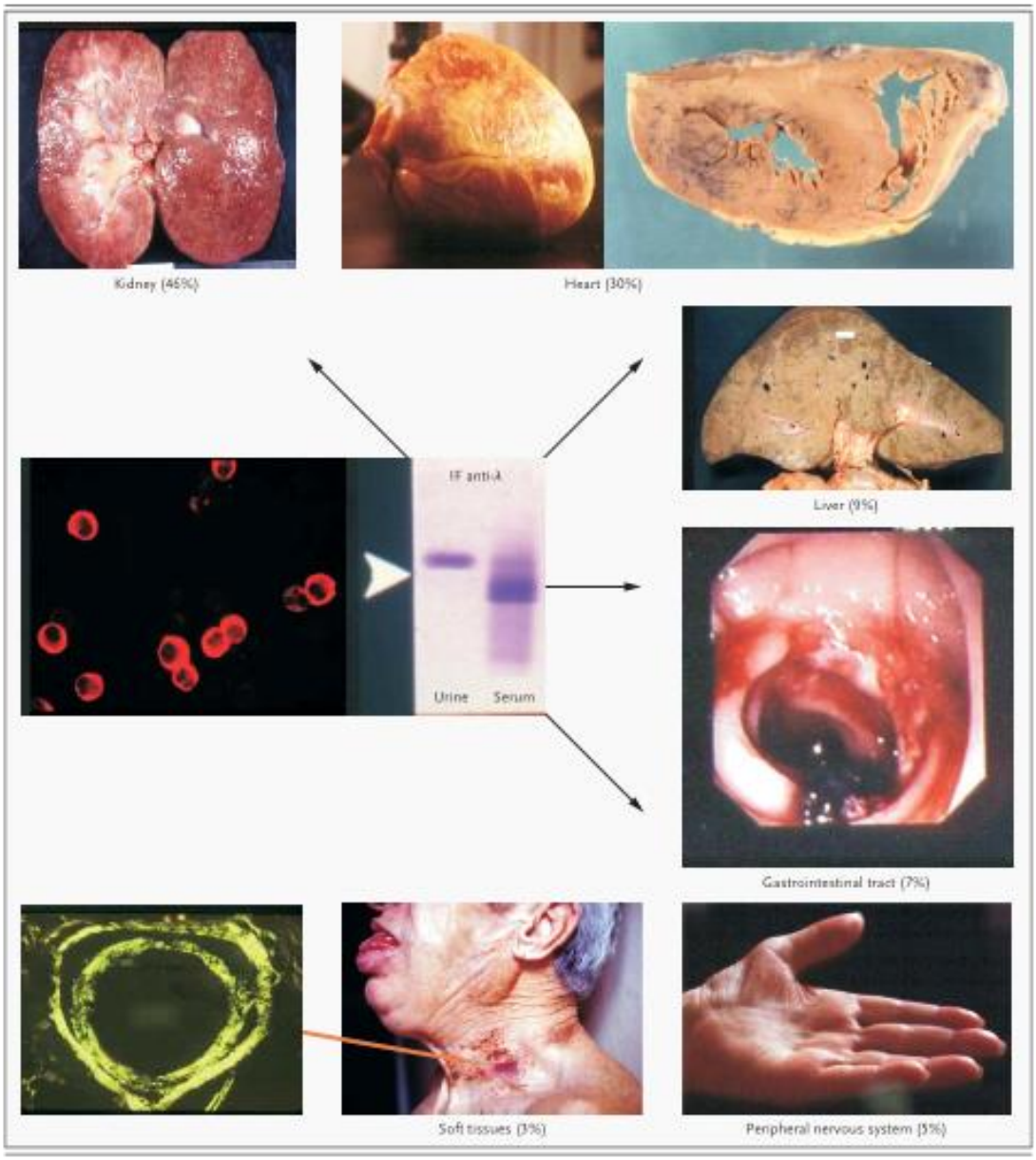

precursors are likely to be the quaternary structures that mediate cellular toxicity through a mechanism that causes oxidative stress and activates the apoptotic pathway. According to this hypothesis, mature amyloid fibrillary deposits are inactive proteinaceous reservoirs that are in equilibrium with smaller, putatively toxic assemblies[2] (ordered aggregates). Several clinical clues suggest that in AL amyloidosis as well, soluble oligomers are cytotoxic and contribute to organ dysfunction. For example, peripheral neuropathy and renal and cardiac function improve dramatically after chemotherapy has

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halted the production of amyloidogenic light chains but before the expected resolution of amyloid deposits.[77,78] The ongoing elucidation of the mechanism of tissue damage by other amyloid proteins may ultimately redirect therapeutic efforts.

DIAGNOSTIC PROBLEMS AND PITFALLS

The number of recognized amyloidogenic proteins is ever expanding, posing increasing difficulties in formulating a correct diagnosis. Unequivocal identification of the deposited amyloidogenic protein is essential in order to avoid misdiagnosis and inappropriate treatment, to assess the prognosis, and to offer genetic counseling when appropriate.[26] The diagnostic approach is multidisciplinary and demands careful clinical evaluation combined with refined histochemical studies,[79] biochemical tests,[80] genetic analyses,[7,26] and possibly functional imaging studies (for cerebral amyloidosis).[81] The presence of a family history is important in the diagnosis of hereditary systemic amyloidoses. However, the clinical onset of these autosomal dominant diseases can be modulated by genetic or environmental factors or both. Furthermore, a low penetrance in carriers of the mutation, the involvement of organs usually affected in acquired (AL and AA) amyloidoses (the heart, liver, kidneys, and peripheral nervous system), and failure of immunohistochemical typing to identify the mutant protein can lead to the misdiagnosis of hereditary systemic amyloidosis as an acquired syndrome.[7,26] These factors are important because early diagnosis is the key to effective treatment.[82]

MOLECULAR TARGETS AND THERAPEUTIC STRATEGIES

Amyloid deposits can be reabsorbed and organ dysfunction reversed if the synthesis of the amyloidogenic protein is shut down. There seems to be a fine balance between the rate at which amyloid is formed and its clearance. It may therefore be possible to promote the resorption of amyloid by reducing the concentration of the amyloidogenic protein to a level below a critical threshold and not necessarily eliminating the precursor. This principle is supported by the excellent outcome of liver transplantation in patients with hereditary apolipoprotein A-I amyloidosis, which can decrease the supply of the amyloid fibril precursor by 50 percent.[83] The same principle also applies to the new formation of amyloid: to halt the pathogenic process, it may be sufficient to reduce the concentration of the precursor to a level below the threshold at which oligomers form. Further studies are necessary to define these thresholds in order to exploit them in clinical settings.

The mechanism of resorption is still unknown, although recent data obtained in studies of immunotherapy for Aβ amyloidosis indicate that phagocytosis has a substantial role.[84] Knowledge of the amyloidogenic pathway (Fig. 4) is the basis of some recently proposed therapeutic strategies.

EFFECTIVE THERAPIES

At present, the most effective approach to the treatment of the systemic amyloidoses involves shutting down or substantially reducing the synthesis of the amyloid precursor. In AL amyloidosis, reduction or elimination of the amyloidogenic clone by chemotherapy almost invariably improves the function of the affected organs.[77,78,85] In reactive amyloidosis (AA), control of the underlying inflammatory disorders can result in regression of the disease. In familial Mediterranean fever, which belongs to the expanding group of hereditary periodic fever syndromes, colchicine controls the febrile attacks and the synthesis of the acute-phase protein SAA, preventing amyloid from forming and reversing amyloid-induced organ dysfunction.[86] Similarly, the recent identification of the genetic defects in other syndromes of periodic fever has led to several breakthroughs in the understanding of the molecular basis of the inflammatory response[87,88] and has opened the way to tailored therapies for these syndromes.[89] In transthyretin amyloidosis, elimination of the circulating pathogenic protein by means of liver transplantation arrests the progression of the neurologic symptoms,[90] although wild-type protein may continue to be added to the existing deposits in the myocardium.[58] This finding suggests that liver transplantation should be performed early, before amyloid nuclei have been deposited in the heart.

FUTURE PERSPECTIVES

The concentration of amyloid protein could be reduced by interfering with the expression of the corresponding gene by means of antisense oligonucleotides and small interfering RNA. These strategies have proved successful experimentally in reducing the synthesis of amyloidogenic light chains[91] and of a neurotoxic polyglutamine disease protein that forms nonamyloid intracellular aggregates.[92] However, technical difficulties related to targeting specific messenger RNA and to modulating the intracel-

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lular concentration of small interfering RNA need to be overcome before clinical applications become feasible.

The conversion of native, fully folded protein into a highly amyloidogenic, partially folded conformer can be blocked by stabilizing native proteins with a specific ligand. In vitro studies have shown that stabilization of the transthyretin tetramer with the natural ligand thyroxin inhibits the formation of fibrils.[93] These results have led to structure-based designs, which in turn have yielded several structurally distinct families of small ligands that stabilize the transthyretin tetramer and inhibit the formation of amyloid fibrils.[94] Problems persist in vivo with regard to the specificity with which these small molecules bind to transthyretin and to the high dose required to saturate circulating transthyretin. In principle, this approach is applicable to the numerous other amyloidogenic proteins for which a stabilizing ligand can be identified.

The inhibition of proteases that generate amyloidogenic fragments is another approach. In Alzheimer's disease, the inhibition of β- and γ-secretases that generate the amyloidogenic peptide is a leading therapeutic strategy,[95] although the toxicity of γ-secretase and the selectivity of β-secretase inhibitors are major unresolved issues. Epidemiologic studies indicate that statins (cholesterol-lowering drugs) might prevent Alzheimer's disease,[96] probably by modulating the ability of secretases to cleave the amyloid precursor. Also, certain antiinflammatory drugs used in the treatment of Alzheimer's disease may have direct effects on secretases.[97] As other proteases involved in amyloidogenesis are identified (e.g., furin for gelsolin[43] and the amyloid ABri protein[44]), they will become targets for specific inhibitors.

All the compounds that inhibit the formation of fibril nuclei could prove important, both to prevent the deposition of amyloid and to avoid the cytotoxicity of the soluble oligomers. Synthetic peptides that bind natural amyloidogenic peptides and prevent further polymerization have already been designed[98,99] and used successfully in vitro[99] and in transgenic mice with amyloidosis.[100] The effect of chelation of metal ions (copper and zinc) in modulating the aggregation of Aβ is also being studied.[101] Amyloid-induced cytotoxicity can be mitigated by compounds that inhibit the toxic mediators, such as free radical scavengers and antioxidants,[2] and by inhibitors and antagonists of cell-surface receptors.[67]

Another approach works directly on amyloid deposits targeting the common fibrillar architecture and common protective elements. Molecules capable of clearing SAP from amyloid deposits[102] or of inhibiting their interaction with glycosaminoglycans[103] have been designed and tested in animal models and phase 2 clinical trials. Several small ligands avidly bind the common ultrastructure of amyloid fibrils; iodinated anthracycline (4'-iodo-4'-deoxydoxorubicin) is a prototype of these molecules. It binds specifically and with high affinity to all the natural amyloid fibrils and promotes the disaggregation of fibrils both in vitro[104,105] and in vivo.[106] However, its clinical efficacy remains to be determined.[107,108]

An innovative approach is immunization against fibrillar proteins. The immune response to these proteins increases the clearance of amyloid deposits. In transgenic mouse models of Alzheimer's disease, immunization with the Aβ peptide attenuates existing abnormalities and prevents neurodegeneration.[109,110] However, phase 2 clinical trials with an Aβ vaccine were stopped when a central nervous system inflammatory reaction occurred in some patients.[111] Manipulation of the immunization protocols might circumvent this problem.[112] This approach also seems viable as an intervention in prion disease[113] and in light-chain amyloidosis.[114]

The increase in our knowledge of the structure and abnormal metabolism of the proteins involved in amyloidosis is closely paralleled by the search for remedies for this disease. On both fronts, the rate of progress is rapidly accelerating, hence legitimizing the hope that effective therapy, exploiting integrated strategies, will soon become a reality.

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Tutorial 6 – Drug Design

What is the Purpose of Tutorial 6?

- How drugs are designed
- The complexity of drug testing
- How Amyloidosis are tackled pharmacology
- Feedback on the draft assignment
- Prepare for the final assignment and the mini conference

How Do DRUGS Work?

Examples from the PDB archive

PROTEINS are tiny molecular machines that perform most of the tasks needed to keep cells alive. These machines are far too small to see, so you might imagine that it is impossible to affect their action. However, drugs can be used to turn proteins on or off. **DRUGS** are small molecules that bind to one specific protein and modify its action. Some very powerful drugs, such as antibiotics or anticancer drugs, are used to completely disable a critical molecular machine. These drugs can kill a bacterial or cancer cell. Other molecules, such as aspirin, gently block less-critical proteins for a few hours. With the use of these drugs, we can make changes inside our own cells, such as the blocking of pain signals. Many structures of drugs that bind to proteins have been determined by scientists. These atomic structures allow us to see how drugs work, and perhaps how to modify them to improve their action. A few examples are shown here. Some of these drugs, like penicillin, were discovered in nature. Other drugs, such as HIV protease inhibitors, were created by using the target protein structure to design new drug molecules. These structures of proteins and drugs, along with many others, can be explored at the RCSB Protein Data Bank (PDB).

1 2 Antibiotics & Antivirals

Antibiotics and antiviral drugs are specific poisons. They need to kill pathogenic organisms like bacteria and viruses without poisoning the patient at the same time. Often, these drugs attack proteins that are only found in the targeted bacterium or virus and which are crucial for their survival or multiplication. For instance, **penicillin** attacks the enzyme that builds bacterial cell walls, and HIV protease inhibitors like **saquinavir** attack an enzyme that is needed for HIV maturation.

1. D-alanyl-D-alanine carboxypeptidase with penicillin (**1pwc**)
2. HIV protease with saquinavir (**1hxb**)

3 4 Anticancer Chemotherapy

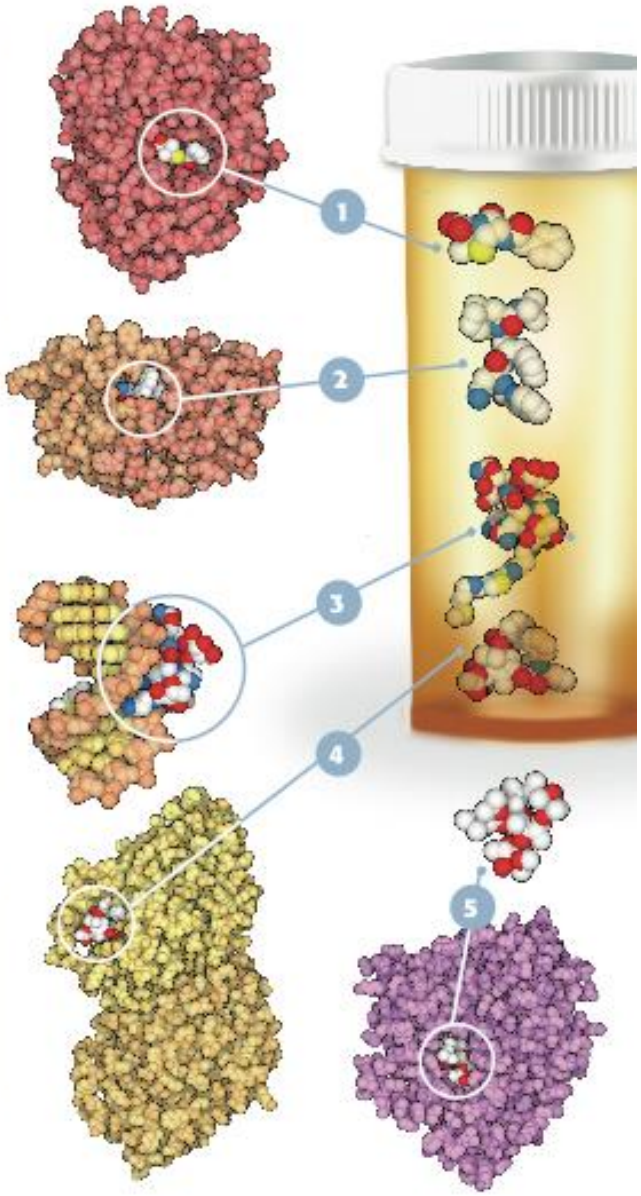
Cancer cells grow and multiply without control. Since these cells are still similar to normal cells, it is difficult to kill them selectively with drugs that can't distinguish between the two. Many drugs currently used for cancer chemotherapy attack all growing cells, including cancer cells and normal cells. This causes the severe side effects of cancer chemotherapy because the drugs attack rapidly-growing cells in hair follicles and the stomach. Two examples are shown here. **Bleomycin** attacks DNA in actively growing cells, often cleaving the DNA chain and killing the cell. **Paclitaxel** (**Taxol**) binds to tubulin, preventing the action of microtubules during cell division.

3. DNA with bleomycin (**1mxk**)
4. Tubulin with taxol (**1jff**)

5 Drug Metabolism

You have probably noticed that when you take drugs, the effects gradually wear off in a few hours. Enzymes like cytochrome P450 continually search for drugs and destroy them. This is important because it protects us from poisonous molecules in our diet and in the environment, but it means that we have to take multiple doses of drugs when being treated for a disease.

5. Cytochrome P450 3A4 with erythromycin (**2j0d**)



Tutorial 6 – Notes

Tutorial 6– Homework: Final Assignment

For your final assignment you will have to read the given material and carry out research about a specific type of Amyloidosis and prepare a report (max 2 A4 sides with no pictures, otherwise 3 with pictures) and a presentation [10 minutes (8-10 slides max)] where you need to:

- List the characteristic of the disease;
- Compare the disease with Alzheimer's highlighting the differences;
- Explain the current therapeutic strategies;
- Hypothesize a possible target for the treatment of the disease

The best presentations will be chosen and presented to the classroom.
Suggested Websites: www.proteopedia.org

Tutorial 7– Mini conference - Feedback

The best and more interesting pieces of work will be presented to the classroom in a scientific conference.

What is the Purpose of Tutorial 7?

- Reflect on skills learned on the programme with a view to encouraging resilience
- Receive feedback on final assignment

What are the key questions?

- What strengths have I demonstrated in my work and what areas of development are there left for me to address?
- What steps do I need to take to improve my academic output?

Activity 2 – Marking your own

Using the mark scheme, identify three things you did well and three things you could improve.

What went well?

1.

2.

3.

What could be improved?

1.

2.

3.

Activity 3 – Responding to feedback

Read through your feedback sheet and highlight on your assignment where you received positive feedback (one colour) and areas for improvement (different colour). You might find it helpful to annotate this with a brief comment or two.

(Acknowledgement: The structure and the activities in this feedback tutorial were first designed by Hannah Manktelow in January 2015. We have repeated them here as they demonstrate thoughtful and excellent planning, and can be used by all tutors to ensure a successful and productive feedback tutorial.)

University Applications Guidance

For course choices and careers information: www.brightknowledge.org.uk

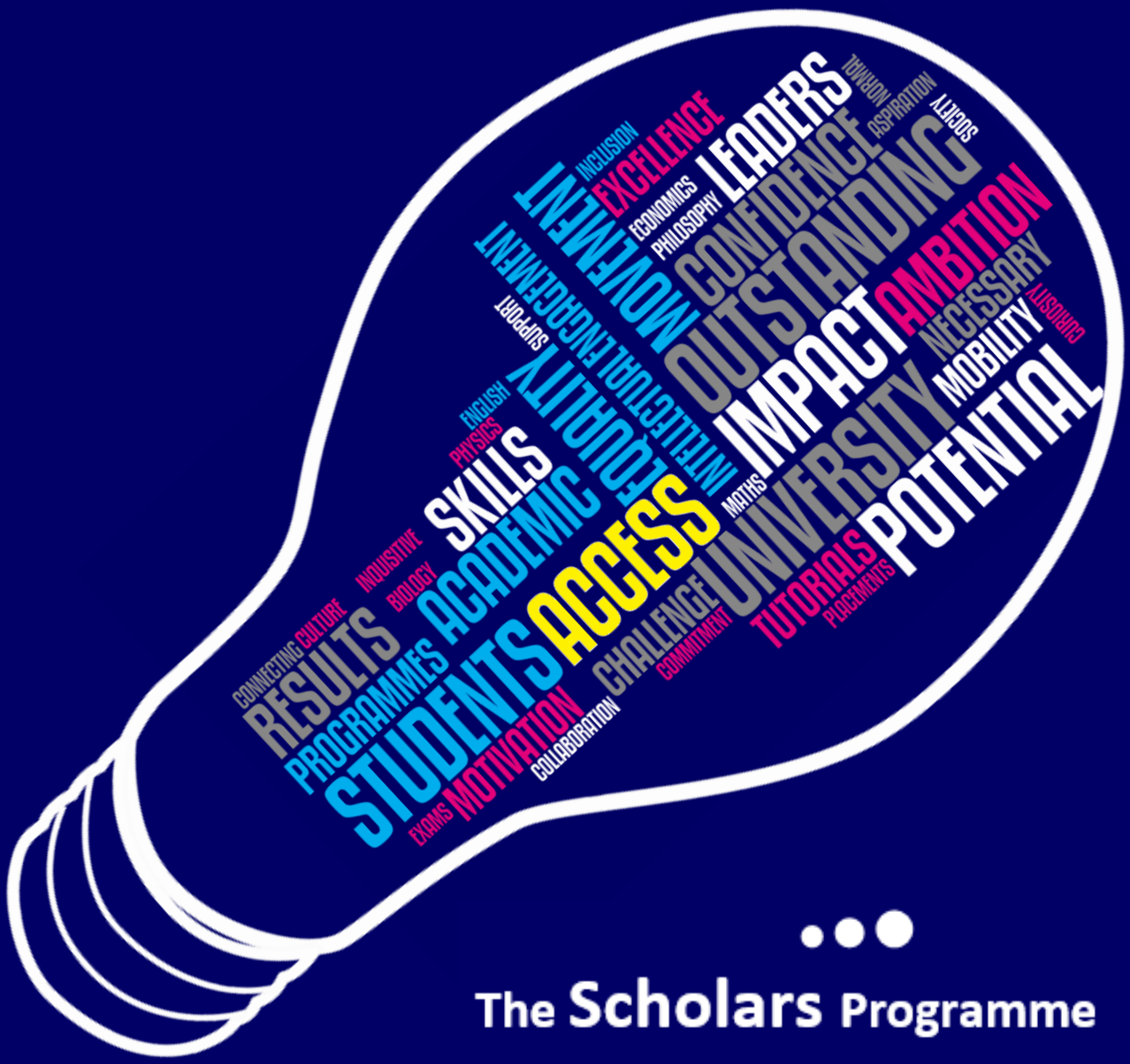
For information and statistics on universities and course: www.unistats.direct.gov.uk

For comprehensive information on universities, including rankings:
www.thecompleteuniversityguide.co.uk

PLEASE NOTE – The Scholars Programme is designed to support the university applications process at your school. The work you complete for the programme should add to your schoolwork rather than detract

Subject Specific Top Tips for Strengthening your application

What could you read around the subject?
Suggestions of podcasts/videos that can be found online? Are there any age-appropriate, good films on this topic?
What cheap or free events and talks would it be good to attend?



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