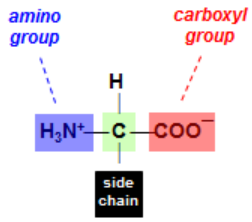
EXSS1029

- ***Understand the general concept of modular design. Explain in general terms the advantage of modular design in terms of up-scaling or down-scaling the top level assembly of the design***
- Modular design: smaller modules or subunits assembled into larger composite structures.
- One advantage is allowing up or down scaling of size of whole via addition or removal of base units.
- Applying this to muscle hypertrophy and atrophy: changes in whole muscle structure (i.e top level) due to training are caused by changes in rate of assembly of amino acids into contractile proteins (bottom level).
- The multi-level organisation allows coordination of individual units (for efficiency and functioning); distribution of responsibility (different base units perform different functions), recruitment/decrecruitment.

- ***Define the terms polymer and monomer***
- A polymer is a substance which has a molecular structure built up chiefly or completely from a large number of similar units bonded together. Monomers can be different, as long as they fit together.
- Thus, it's possible to have a polymer made from a variety of base modules.
- The same sequence can assume a number of different shapes (i.e different conformations) if one monomer can rotate relative to its neighbour.



- ***Describe the general structure of an amino acid***
- All proteins are polymers of amino acids (monomer units) bonded by a peptide bond. There are 20 different kinds of amino acid.
- All amino acids share an identical 'back bone' structure including an amino group and a carboxyl group linked by a single carbon atom (the alpha carbon).

- ***Explain in general terms, the primary structure of a protein and describe in general terms assembly of a protein in terms of the formation of peptide bonds between amino acids.***
- The carboxyl group of one amino acid bonds with the amino group of another to form a peptide bond (covalent), which continues to form a string or sequence of amino acids.
- This bond is formed by eliminating a water molecule. .

- ***Explain how proteins differ from each other in terms of their primary structure***
- They differ from each other in the structure of the side chain that is attached to the alpha carbon.
- Some have a charged side chain (either +/-), some have a polar side chain or a non-polar chain. This influences whether the protein has a specific shape or a different shape.
- The backbone of amino acids (the repeating sequence of atoms along core of the protein chain) that define the whole protein forms the shape. Projecting from the backbone are the amino acid side chains.

- ***Define the term protein conformation***
- A conformation is the 3D arrangement of atoms.

- ***Explain, using the example of ionic bonds, the causes of a protein folding into a specific conformation***
- The parts of the backbone don't twist, since the peptide bond is planar and doesn't permit rotation. However, rotation can occur about the N-C_α bond and the C_α-C bond.
- The protein backbone folds due to forces that arise from interactions between amino acid side chains:
  - ➔ Weak bonds which form between different parts of the backbone such as ionic and hydrogen bonds which are weak in comparison to strong covalent bonds).
  - ➔ Hydrophobic interactions
- For example, opposite charges attract each other, so attractive forces will bring the side groups together, bending the backbone into a specific shape.

- The weak bonds are either ionic bonds, hydrogen bonds or van der Waals attractions. Individual noncovalent bonds are 30-300 times weaker than the typical covalent bonds that create the molecules.
- But many weak bonds can act in parallel to hold two regions of a polypeptide chain tightly together. The stability of each folded shape is therefore determined by the combined strength of many noncovalent bonds.
- Hydrophobic molecules, like the nonpolar side chains of particular amino-acids, tend to be forced together in an aqueous environment in order to minimise their disruptive effect on the hydrogen-bonded network of water molecules. So the folding of proteins is influenced by the distribution of its polar and non-polar amino acids.
- Electrostatic attractive forces tend to bring side groups together, bending the backbone into a specific shape.
- As a result of all these interactions, each type of protein has a particular 3D structure determined by the order of amino acids in its chain.
- Thus, backbone rearranges its shape to minimise electrostatic repulsive forces and maximise attractive forces.

- ***Explain why adding a charged group to a protein may cause a change in it's conformation***
- If some event were to change the distribution of ionic bonds in a protein, then the shape (conformation) of the protein could be changed.
- In this way, some proteins can be made which have 'moving parts' and which behave like nano machines.
- For example, if an ATP molecule is introduced to the protein, the protein backbone folds to reposition charged side-chains.
- Although this example has only looked at ionic bonds, the binding of another chemical could also alter the pattern of non-covalent bonds (eg. Hydrogen bonds, hydrophobic interactins) which can effect protein conformation.

Actin and Myosin

- ***Describe or sketch the general shape of the myosin molecule and identify its components in terms of (i) myosin heavy chains and myosin light chains, (ii) myosin head and myosin tail, and (ii) the lever arm and motor domain. Be able to use the abbreviation: MHC. Define the term crossbridge***

- Myosin is a relatively large protein (sequence of about 2000 amino acids), that is the only source of active force production in muscle. While very tiny, in mass it has a large-scale effect on muscle contraction.
- The myosin molecule is an assembly of several proteins- 2 myosin heavy chains and 4 myosin light chains (6 proteins altogether).
- The shape of MHC is a long tail section and a globular head. The head is the crossbridge and is comprised of sub components of motor domain and the lever arm.
- The body of the molecule is formed by the tails of the 2 MHCs binding together, so shape is always like a double-headed golf club.
- The lever arm region of the MHC is subject to significant forces from both its attachments to the motor domain and the tail. If it were to bend significantly, it would fail to transmit force and movement (its job).

➔ Thus, 2 short protein chains- myosin light chains (MLCs) wrap around and bind to the lever arm to strengthen it.

- Most of the chain is coiled into a tight helix creating the extended tail segment of the protein. The helix then angles up (lever arm) to end in a globular region characterised by a complex folding- the motor domain.
- Collectively the motor domain and lever arm is known as the head region.
- Increasing myosin synthesis corresponds to muscle hypertrophy.

- ***Describe the general structure of ATP, ADP and Pi. Define the term ATP hydrolysis and write this reaction. Define the term: nucleotide***

- ATP refers to adenosine triphosphate. ADP refers to adenosine diphosphate, the product of ATP with the Pi removed. Pi is the inorganic phosphate molecule released from ATP during energy production.
- A nucleotide is a compound consisting of a nucleoside linked to a phosphate group.
- ATP hydrolysis is the reaction in which the 3rd phosphate group splits off the adenosine molecule.

- The splitting of ATP to ADP and Pi releases free energy, and so always proceeds ATP → Pi + ADP, NOT in the reverse direction (hence is irreversible).
- The feature that ATP hydrolysis always proceeds in 1 direction is used in the crossbridge cycle to 'drive' the cycle in the desired direction.
- The motor domain of the MHC has a specialised location, the nucleotide binding site, which can bind the fuel molecule ATP. Following binding ATP, the nucleotide biding site catalyses hydrolysis of ATP to form ADP and Pi. That is, myosin catalyses the reaction.
- Unlike many enzymes after which catalysing their particular reaction, immediately release their products, when ATP is hydrolysed, the resulting products of ADP and Pi are not released straight away.
- Instead the products are held trapped in the binding site until triggering events result in their release, which is in the specific order of Pi then ADP. After that, myosin can bind another ATP molecule and repeat the reaction.
- The binding of ATP, its hydrolysis and then the release of products from the myosin head causes a sequence of shape changes in the head:
  - ➔ Chemical steps of ADP release (from previous crossbridge cycle), then ATP binding to myosin head followed by hydrolysis of ATP with products ADP and Pi remaining bound until the myosin head returns to the pre-powerstroke conformation.
  - ➔ The chemical step of the release of Pi from the myosin head triggers the powerstroke, which ends with the myosin head in rigor conformation.
- So long as there is a supply of fresh ATP for the myosin head to repeatedly split to Pi and ADP, the myosin head can flip back and forth between conformations again and again.
- Without a ready supply of ATP, we encounter rigor mortis- muscle stiffness of death. Muscle cells are equipped with elaborate mechanisms to prevent ATP depletion during exercise, either by increasing TP supply sufficiently, or failing (limiting ATP use by myosin). This is fatigue, and though an undesirable circumstance, it is a protective mechanism preventing death.

- ***Describe or sketch the movements of the myosin molecule: Specifically i) describe the pre-powerstroke conformation and ii) rigor conformation in terms of the positions and orientation of the motor domain, lever arm relative to the tail.***

- A motor is an element that can changes its shape or position while exerting a force on an external object.
- A specialised feature of myosin is that the motor domain and the lever arm can change their position relative to each other, i.e the protein can change its conformation.
- This is possible due to 2 hinge regions: one between motor domain and lever arm, and between lever and tail.
- The extremes of the ROM of the myosin head region are called the pre-powerstroke conformation and the rigor conformation.
- Pre powerstroke conformation: lever arm of head is bent AWAY from the myosin tail (extended elbow)
- Rigor conformation: the lever arm is bent TOWARDS the tail region (bent elbow).
- The powerstroke is the basis of muscle force production and active shortening, however to be useful the force and movement developed by the powerstroke has to be transferred, which is done through attachment to the thin filaments.
- A protein's conformation can be altered by interactions. Since the ATP, ADP and Pi are all highly charged, they alter the pattern of ionic bonds in the area near the nucleotide binding site, causing conformation changes.
- Although the conformation change at the binding site is significant, its not far enough to match the displacement of the motor domain (from the prepowerstroke conformation to the rigor conformation).
- Part of the motor domain of the myosin acts to convert a displacement into a rotation of a lever thus amplifying the initial movement.
  1. While Pi is bound to the motor domain, its presence stabilises the shape of the relay in one conformation
  2. Release of Pi from the binding site causes conformation change that results in the relay changing to a different shape. This change causes a)rotation of the convertor which in turn causes b)a large scale rotation on the lever arm, called the powerstroke.

3. During the powerstroke, the motor domain displaces and the lever arm ends relatively bent towards the tail- the rigor conformation.

- Thus, release of Pi is the key event triggering the powerstroke. The lever arm can be returned to pre-powerstroke conformation if Pi is returned to nucleotide binding site to re-stabilise the relay.
- However, for this to occur, the myosin head must first eject the ADP molecule, bind a fresh ATP molecule and split this to Pi and ADP. The lever arm will then rotate back from rigor to pre-powerstroke conformation.

- ***Describe the approximate location and purpose of the nucleotide binding site on myosin.***

- A nucleotide binding site is an enzyme catalytic site that can bind ATP or ADP + Pi. Since ATP is a stable fuel, it won't spontaneously combine with water to hydrolyse, it must first bind to a specialised catalytic site which reduces the activation energy.
- The myosin head contains the catalytic site, called the nucleotide binding site that binds ATP and catalyses its breakdown to ADP and Pi.
- The converter region converts small movements of the relay to large movements of the lever arm (thus amplifying the effect).

- ***Describe i) the general shape of the protein actin, ii) the structure of the thin filament and ii) the general process of thin filament assembly.***

- Actin is a medium-sized (377 amino acids) globular protein. Each molecule has a binding site for a myosin head.
- The structure of the thin filament of actin is a double spiral (like a twisted chain of pearls).
- Every thin filament is assembled to have the exact same length, which is 1300um. It's called the thin filament because its width (8nm) is much less compared to the myosin filament (thick).

- ***Identify the approximate locations i) the myosin binding sites on the thin filament ii) the actin binding site on myosin***

- The protein actin possesses a specific region that fits a complementary region on the myosin motor domain. Myosin can reversibly attach, exert a force on it, then detach to this binding site on actin.

- ***Describe the events of the crossbridge cycle: specifically in terms of attachment, the power stroke, detachment and return to the pre-powerstroke conformation. Include events at the nucleotide binding site in terms of binding, reaction, and product release. Explain the overall purpose of the crossbridge cycle and it component stages including the powerstroke.***

1. **Attachment**: starting with lever arm extended relative to tail (prepowerstroke) and with ADP and Pi bound to the motor domain, the crossbridge attaches to an actin binding site
   - The motor domain continually undergoes slight movements relative to its tail as its bombarded by water molecules; a process called random thermal motion.
   - If there is an exposed binding site near the myosin head, then due to these random motions, occasionally the head will contact the region of the actin molecule (complementary to myosin head).
   - When this occurs, the motor domain is stabilised in his region of the actin molecule- this first step of binding is called the 'weak bonding' state.
2. **Powerstroke**: attachment triggers release of Pi, which in turn triggers the powerstroke- the lever arm rotates towards the tail, pulling on the thin filament. The crossbridge finishes in rigor conformation.
   - The interaction between charges in myosin and actin cause a slight change in the shape of the motor domain as it nestles into the binding site. This re-arrangement breaks a weak bond holding the Pi in the nucleotide binding site, allowing its release from the head.
   - In turn, the release of Pi allows the myosin head to form further non-covalent bonds with actin- this is called the 'strong bonding' state (i.e myosin head is now locked onto the thin filament).
   - The release of Pi and formation of the strong bond with actin triggers the myosin powerstroke.

3. **Detachment:** at end of powerstroke, ADP is released and ATP is bound. ATP binding triggers detachment from thin filament.
    - At the end of the powerstroke, rotation of the lever arm has resulted in an opening of the nucleotide binding site so that ADP can be released. At this stage, the binding site is vacant.
    - ATP, present in high concentrations in solution around the myosin, quickly occupies the site. This binding brings the negative changes on the terminal phosphate of the molecule into a position on the head that causes a slight shape change, breaking a number of non-covalent bonds that had stabilised the myosin-thin filament connection.
    - In effect, the ATP binding 'unlocks' the head from the thin filament, which then immediately detaches from the thin filament.
4. **Return to pre-powerstroke conformation**: motor domain splits ATP to ADP and Pi, both of which remain bound to the motor domain at this stage. The appearance of Pi in the nucleotide binding site causes the lever arm to rotate to the pre-powerstroke conformation.

- If another actin binding site is near the myosin head, the cycle can repeat again.