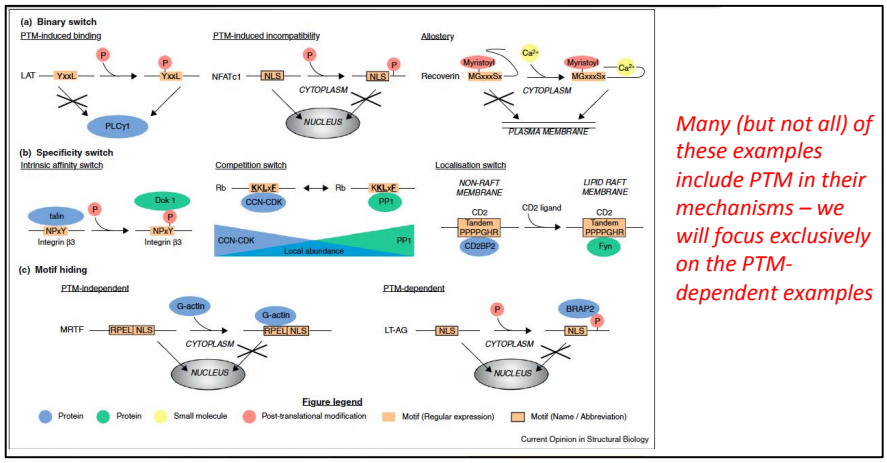
Molecular Switching Mechanisms (Simple)

- There are only 5 switch mechanisms here and they have PTMs involved within them (orange colour)
    - In many cases, it is going to be phosphorylation
- The top diagram is thought to be the core of signalling – includes idea about binary switching

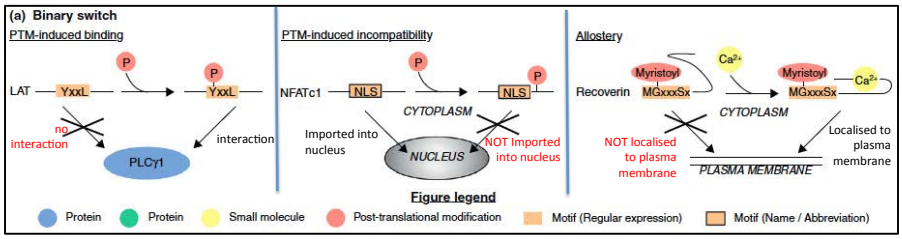


Many (but not all) of these examples include PTM in their mechanisms – we will focus exclusively on the PTM-dependent examples

PTMs – Simple Molecular Switching Mechanisms

Binary switching = on/off state (binary just means 2 states)

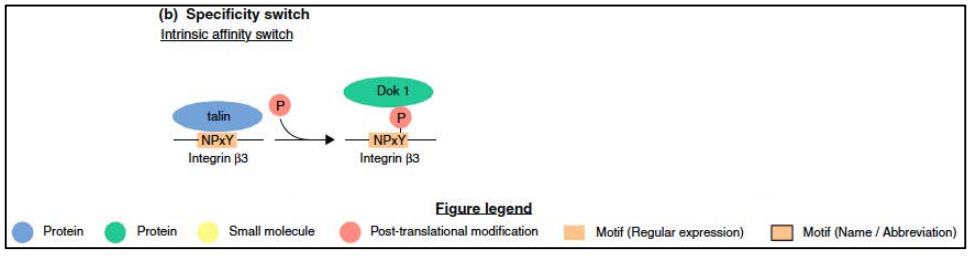
We can sub-classify binary switches into different types of mechanisms:



- *PTM-induced binding*
    - the modification is an essential part of the recognition of the protein, examples include the absolute requirement for p-Tyr in recognition by SH2 or PTB domains
    - We have an unmodified protein – here this is LAT protein – this is phosphorylated on Tyr following the engagement of a T-cell receptor. In its unphopshorylated state, it doesn't interact with protein-PLCγ1 but when phosphorylated it can interact with the protein. This is because PLCγ1 can recognise the phosphorylated LAT
    - This means there is an absolute requirement for this modification to occur
- *PTM-induced incompatibility*
    - the modification prevents the usual recognition; examples include that phosphorylation can act to prevent recognition of nearby Nuclear Localisation Sequences (NLS) – (and this prevent transport of this protein into the nucleus)
- *Allostery*
    - changes in protein conformations
    - this can depend on the presence of PTMs.
    - PTM-induced allosteric regulation of activity (not shown in the example above) is also possible!
    - This is an example of a constitutive modification – it is showing an event which is changes in Ca^{2+} that is starting to change the shape of the protein called recoverin. This protein is a neuronal Ca^{2+} binding protein in the photoreceptors of cells and it is highly modulated by the presence of Ca^{2+}
    - If we have a protein and there is a single event that will change the structure of the protein – this change of structure will have functional consequences
    - 2 states – off/on (localised or not localised to the plasma membrane)

Specificity switch

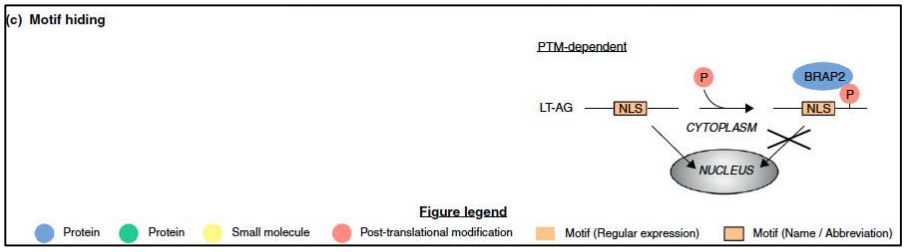
- *Intrinsic affinity switch* – a PTM can change the binding properties from recognition by 1 protein partner, to recognition by another distinct protein partner even though the sequences of amino acids themselves are NOT changed



    - In this example, the unmodified motif in integrin β3 is recognised by the protein called talin, but when the tyrosine in this motif is phosphorylated, this is recognised by a protein called Dok1

Motif hiding

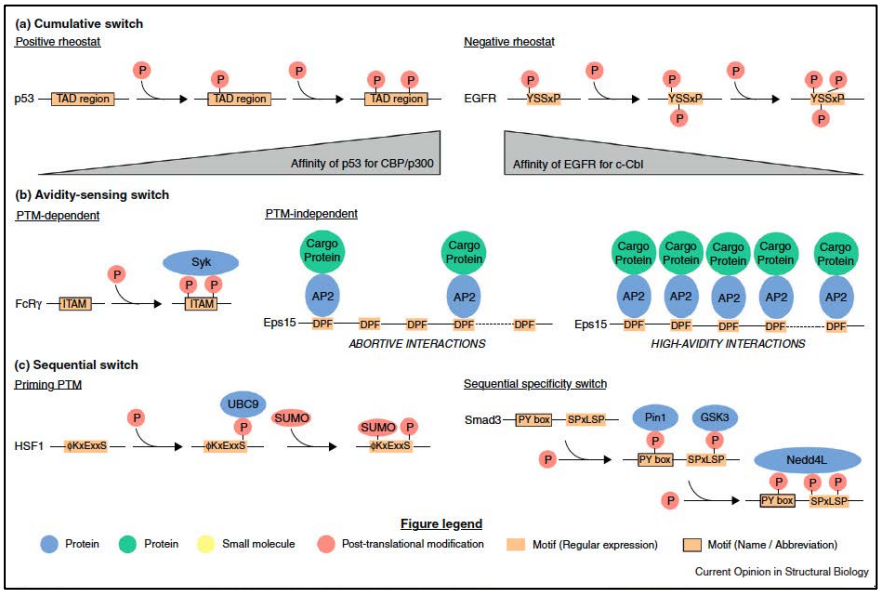
- PTM-dependent motif hiding – a PTM can increase to a specific protein partner, but this binding partner masks/blocks recognition of other motifs by their dedicated partners
    - In this example, the phosphorylation dependent binding to a protein called BRAP2 prevent the nuclear localisation sequence of LT-Ag being used for nuclear import



    - This is different to PTM-induced compatibility because in this we are bringing in another protein that will block the localisation (whereas in PTM-induced compatibility, the modification itself stops the localisation)

PTMs – Molecular Switching Mechanisms (Complex)

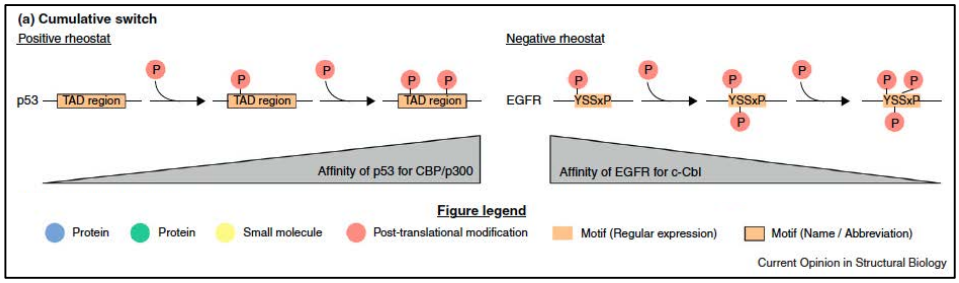
- PTMs are changing some sort of action of the protein and in this case they are complex mechanisms because they are many modification events (as opposed to a single event)



PTMs – Complex Molecular Switching Mechanisms

PTM-dependent Cumulative switched

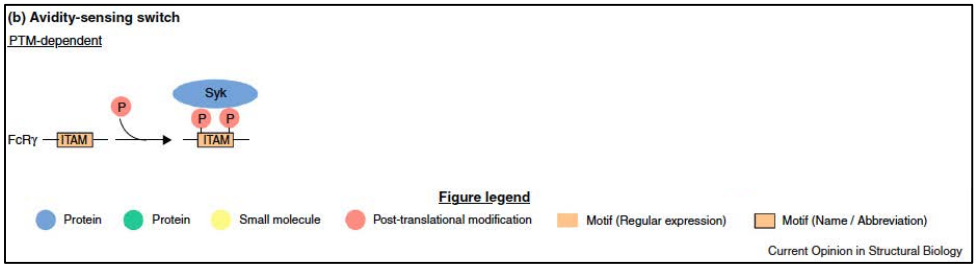
- POSITIVE or NEGATIVE Rheostat
    - A rheostat in electronics is something that gives variable electrical resistance – so it is not something that goes on/off (lights we can dim)



- Positive – as phosphorylation events increase the affinity increases
- The PTM regulatory potential can be dramatically increased by multi-site modifications
- Progressive/sequential additions will modulate the affinities of interactions with binding partners – in these examples, we see that this can either INCREASE or DECREASE the affinity for interaction with a specific partner by the progressive addition of phosphate groups to the protein

PTM dependent avidity sensor

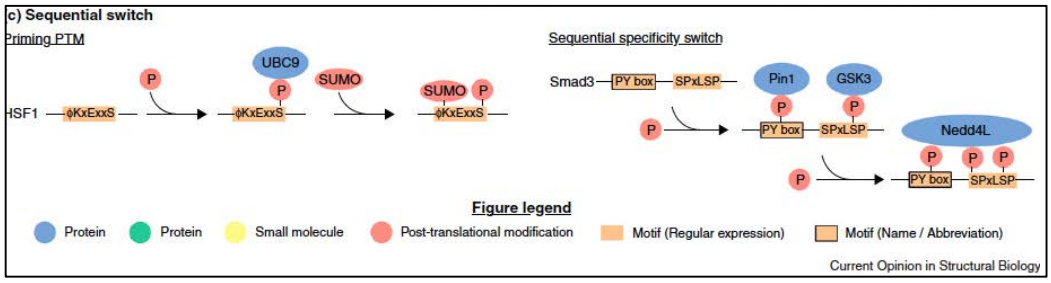
- May appear similar to the cumulative switch, but in this example there is a cooperative use of multiple low-affinity interaction sites that will drive a high avidity interaction (Appears to be more on-off in regulation rather that the continuum of a rheostat)



- Multiple modifications are essential, but it appears that the order of their addition is not a critical factor in determining partner binding

Sequential switches

- Require the correct sequence/timing of events
- i.e. an ORDERED transition from 1 form of a functional protein to another

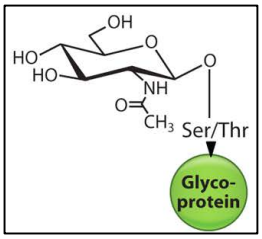


- Priming PTM – one PTM is essential for the second PTM to occur – in this example, the phosphorylation within a specific sequence context directly recruits the SUMO conjugating enzyme UBC9
- PTM-dependent sequential specificity switch – a change in the protein partner depending on the number/sum of PTMs – in this example we see a di-phosphorylated protein recruiting specific partners, but the further addition of a 3rd phosphate group will allow for binding by a different protein

Lecture 11 – O-GLcNAc and its crosstalk with phosphorylation

[Don't need to know the structure of O-GlcNAc/ But we need to be able to explain important features]

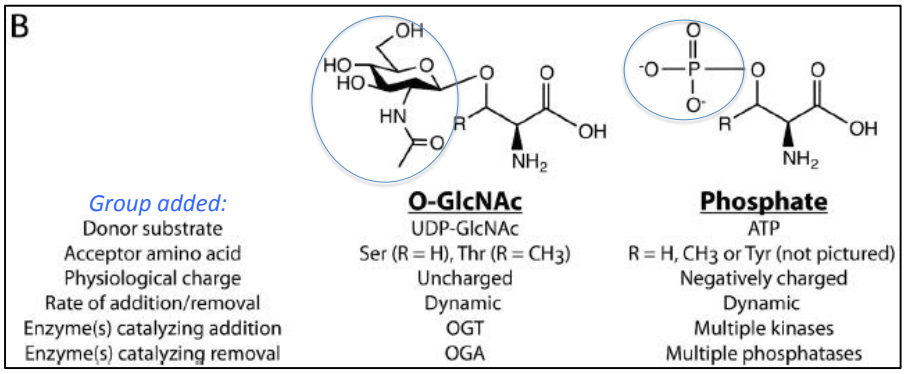
- O-GlcNAcylation – Definition and Scope
  - =The addition of the monosaccharide β-D-N-acetylglucosamine to hydroxyl (OH) containing residues (Ser or Thr) of proteins
  - =O-linked β-N-glucosamine (O-GlcNAc)
  - a single sugar modification – so there is only a single transfer of a single monosaccharide (no other further modifications)
  - The monosaccharide is linked through its O/OH group to the hydroxyl group of the Ser or Thr residue



- This is different to normal glycosylation which involves the glycosylation of proteins in the ER predominantly and sent out through the golgi – so this result in extracellular proteins
- O-GlcNAcylation results in the formation of intracellular proteins which forms part of their regulation. As this is related to glucose metabolism, there is an interest in the events which might be related to different disease types especially metabolic diseases i.e. diabetes, cancer cell biology

O-GlcNAcylation

- α-Carbon (carbon with amine and –COOH groups)
- The side chain of OGlcNAc should be either Ser, Thr or Tyr. And in this diagram, the R group can be H or CH_3 – so either Ser or Thr (this is because this reaction doesn't happen for Tyr residues (as far as we know) because the active site of the enzyme will not accommodate the big bulky ring of the Tyr group)

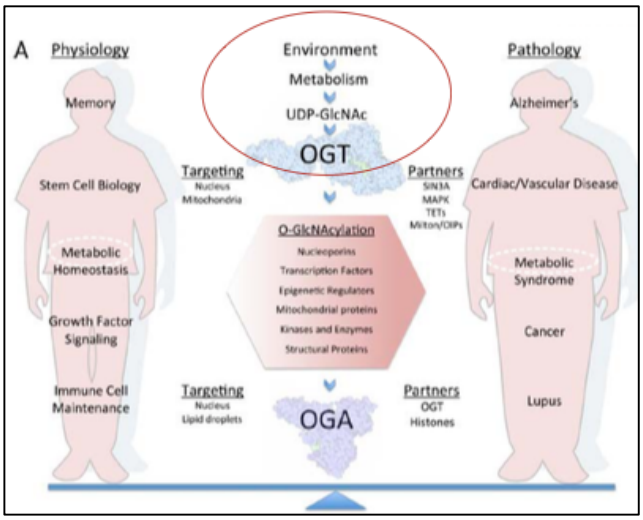


- In comparison, in the phosphate, Ser and Thr can be modified as well as Tyr
- Similarities and differences
  - The group (that modified of the O-GlcNAc) is much larger than that of the phosphate due to the ring structure
  - O-GlcNAc will not change its charge/physiological pH because the atoms of the group are not ionisable in the normal physiological charge range. But the pKa of the phosphate hydroxyls is such that the physiological pH becomes completely ionised – i.e. phosphate carries a -2 charge
  - Both are dynamic – the are going to have proteins that are going to catalyse the addition of these and the removal of specific substrates
  - There is a single transferase and a single removing enzyme (OGA enzyme) for O-GlcNAc. But phosphate has multiple phosphatases and kinases which are regulators of the phosphorylation reaction
- Is considered by many to be "similar to phosphorylation" in terms of amino acids (at least in terms of Ser and Thr) targeted stoichiometry, and cycling (i.e. it is reversible)

- O-GlcNAcylation differs from other forms of protein glycosylation
    - The sugar moiety is not further modified or elongated into complex structures – so a single sugar is added or removed
    - Addition/removal is highly dynamic and inducible in response to cellular changes
    - It is a modification on a range of nucleocytoplasmic proteins (rather than cell surface or in luminal compartments of the cell)
- >1000 proteins are known to be modified in this way, and the number continues to grow rapidly with improvements in the technology that allows its detection
- GlcNAcylated proteins are involved in TRANSCRIPTION, UBIQUITINATION, CELL CYCLE AND STRESS RESPONSES. So these proteins don't seem to fit within a single subset of functions like processes in the nucleus, regulating processes (protein degradation), regulating cell cycle, modulating stress responses
- Abnormal levels of O-GlcNAcylation appear to correlate with the development of pathologies such as neurogenerative diseases or insulin resistance and glucose toxicity
- O-GlcNAcylation (like other forms of PTM)
    - Influences protein-protein interactions (influence partners of O-GlcNAcylation)
    - Modulates protein localisation

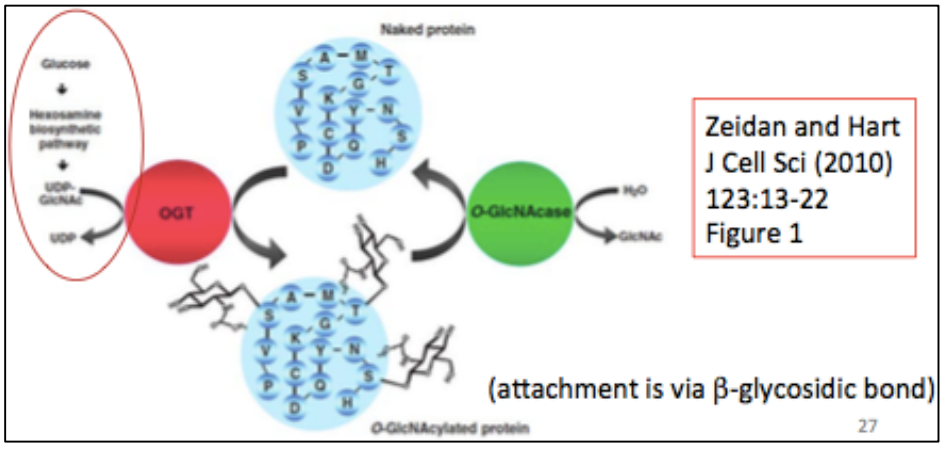
O-GlcNAc dynamically impacts biological homeostasis and disease pathologies

- Physiology: There is a range of physiological processes of the body that we have recognised that are being critically regulated by this type of PTM
- Pathology: There seems to be correlations between abnormal levels of O-GlcNAcylated proteins and the development of some types of diseases
- O-GlcNAcylation provides an immediate link towards the environment and metabolism because the precursor, UDP-GlcNAc which is going to be used in this reaction, is the direct product of metabolism



The O-GlcNAcylation "cycle"

- Glucose metabolism via the hexosamine biosynthetic pathway yields the high energy intermediate UDP-GlcNAc that serves as the sugar donor in the OGT-catalysed reaction
- OGT catalyses the transfer of GlcNAc from UDP-GlcNAc to Ser/Thr residues of the substrates proteins
- O-GlcNAcase catalyses the removal of GlcNAc from the protein



- The circled pathway in the diagram – this pathway is critically important because if we don't have this particular substrate (UDP-GlcNAc) OGT will not work. Also if there is too much flux through the pathway, too much of its substrate will be given to OGT and hence we will be able to catalyse this reaction more efficiently and more frequently. This is another instance where we see the links to glucose are important because it dictates the levels of one of the substrates that this enzyme will use.
- UDP-GlcNAc is the sugar donor and is used by the transferase enzyme called OGT. The other product of this is UDP. The most important product of this reaction [left side of diagram] is the modified protein (the O-GlcNAcylated protein)
- The O-GlcNAcylated protein can be subjected to enzymatic removal of the O-GlcNAc residue in a typical hydrolysis reaction (releasing water) and this can convert it back to the naked protein