Chemical Biological Methods for Mapping the Role of PTMs
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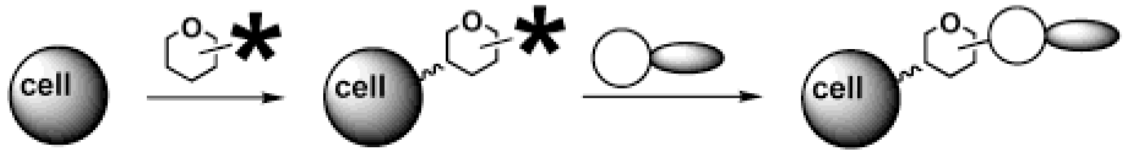
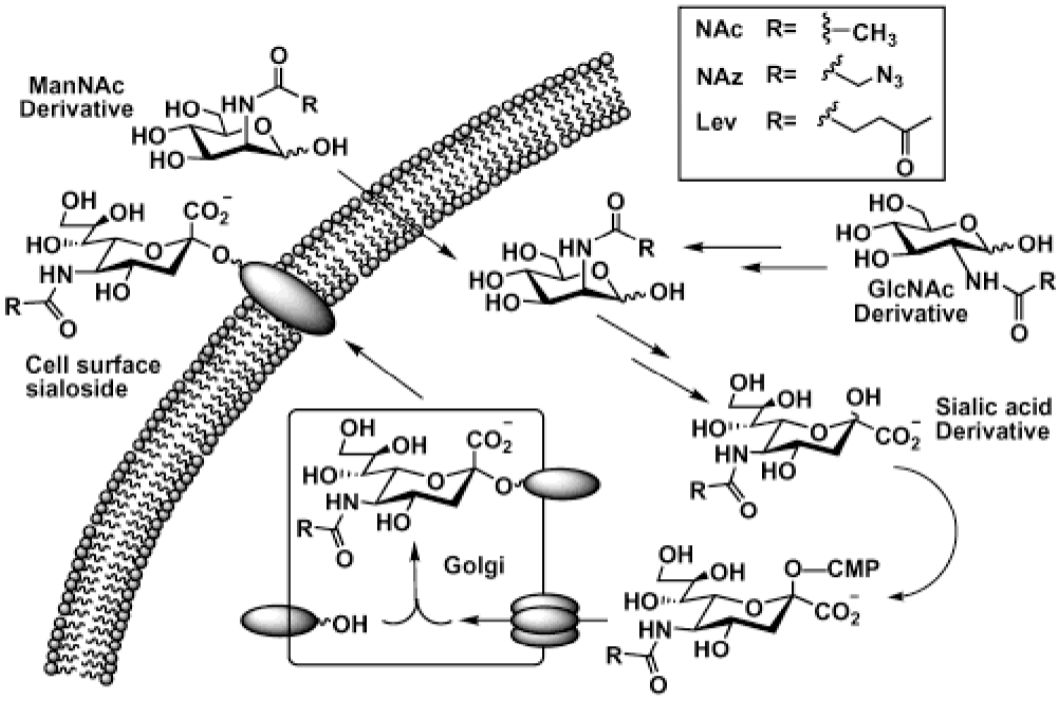
Introduction & Hypothesis
• PTMs expand the diversity of gene products enormously and may be the explanation for the differing biological complexity of organisms with similar levels of genes.
• Problem – when nature makes PTM modified proteins they are not synthesized under tight genetic control, they are synthesized by enzymes that create mixtures of multiply or partially modified PTM structures on the surface of proteins. These mean that it is difficult to dissect the function of PTM- modified proteins and then track their role.
• Can chemical methods allow us to create, quantify, track and/or delineate their roles by providing controllable ways of introducing modifiable PTMs? Two examples of PTMs are illustrated here: glycosylation and lipidation

Method Development
• Make precise mimics of proteins modified with PTMs and see what they do
OR
• Construct molecules that can be fed to organisms that will allow the presence of PTMs to be identified, perhaps through the display of the protein on a cell surface. These molecules might bear functional groups (or 'tags') for modification. A reaction is needed for these tags so that they can be visualized/identified/modified.



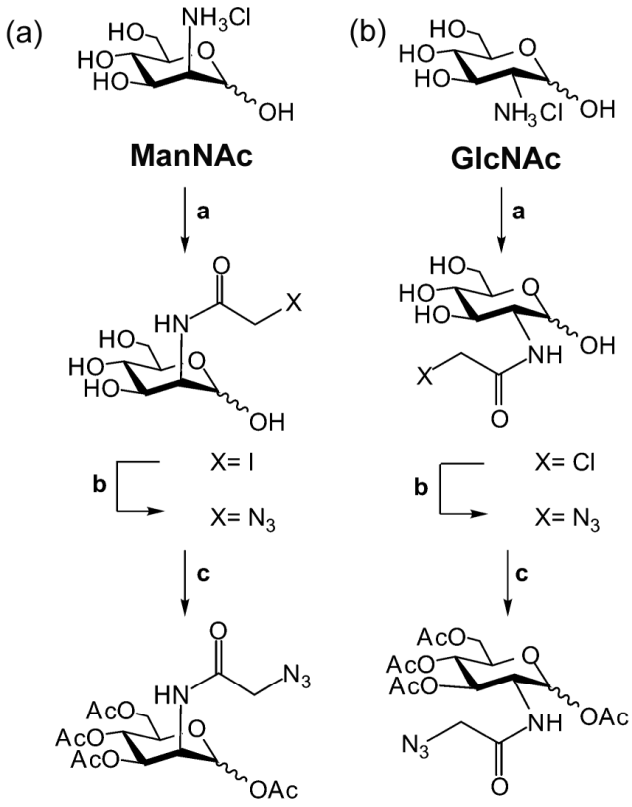
• One of the sugars that appears at the end of oligosaccharide chains that are added to proteins as PTMs is sialic acid (**SA**). SA is made enzymatically inside cells from the sugars *N*-acetyl glucosamine (GlcNAc) and *N*-acetyl mannosamine (ManNAc). A 'salvage' pathway means that as well as being internally synthesized by cells, ManNAc can be taken up by the cell from its external environment.



• Precursors bearing two types of tags (azidoacetamide NAz) and levuinoyl (Lev) were synthesized. The synthesis of the NAz compounds is shown (how would you make the ManLev?, explain the chemoselectivity).

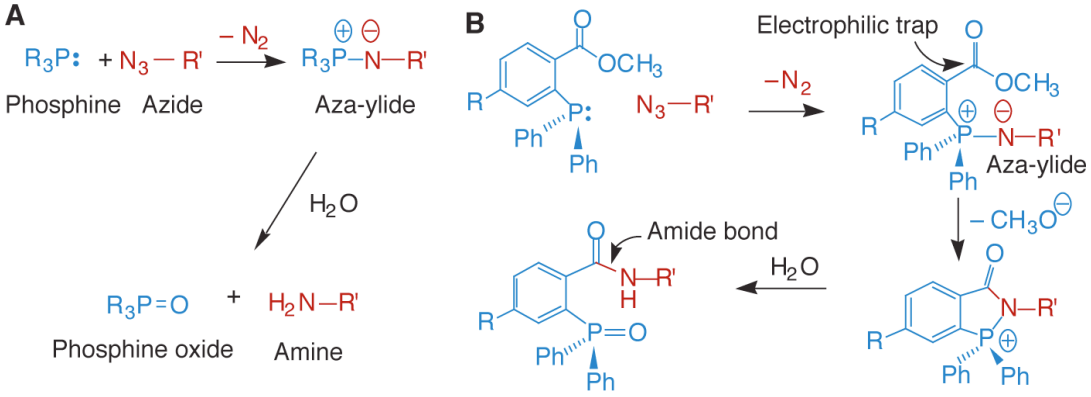
• Peracetylated compounds were made: acetylation increases the membrane permeability of the polar sugar; once the molecule is inside the cell, the acetyl groups are readily cleaved by cytosolic esterases.

• Reactions were needed for reacting with the tags present in ManNAz (azide) and ManLev (ketone). The reaction for reacting selectively with azide is shown below. From your knowledge of chemical tests used for ketones what would you use for reacting with the tag in ManLev?

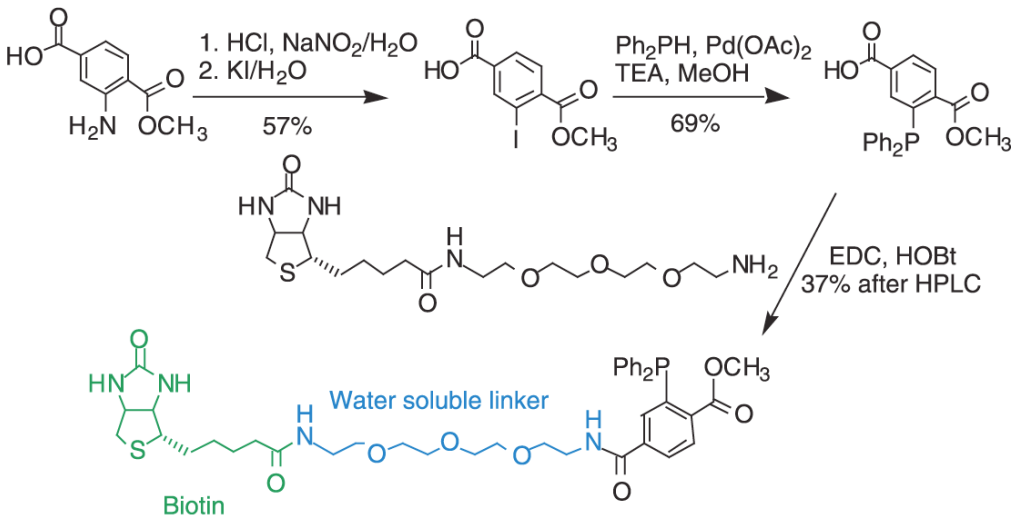


[a] (a) NaOMe, iodoacetic anhydride, MeOH. (b) NaN_3, MeOH. (c) Ac_2O, pyridine. [b](a) NaOMe, chloroacetic anhydride, MeOH. (b) LiN_3, DMF. (c) Ac_2O, pyridine.

• The azide reaction is based on the reaction of phosphines with azide to form aza-ylid iminophosphoranes. Here it has been cleverly adapted to allow reaction with a neighbouring ester leading to amide formation. This is often referred to as the 'Staudinger ligation' reaction.

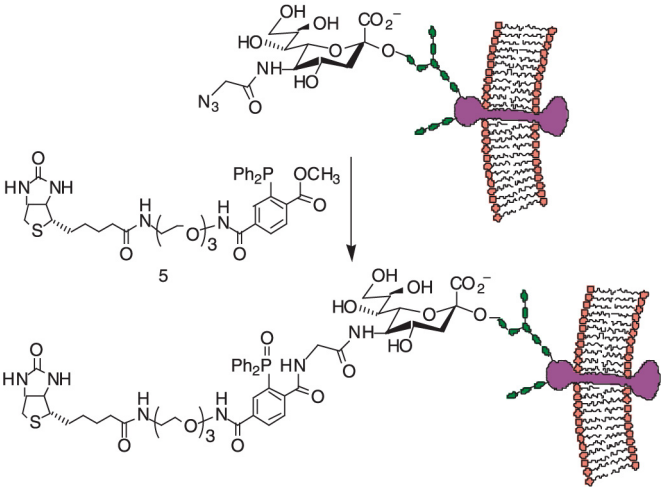


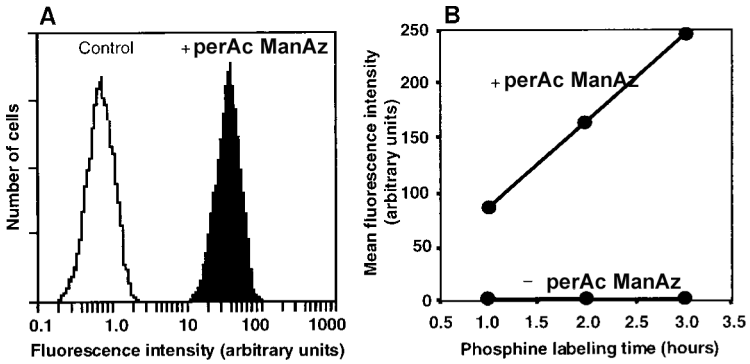
• A reagent was created that contains a well known ligand biotin that has a strong affinity for proteins known as avidins.



Application to Cells

• Several cell types (Jurkat, HeLa) were incubated with peracetylated ManNaz and then treated with the biotin-containing phosphine reagent. Analysis of the cell surface using fluorescently-labelled avidin protein (which binds to biotin) showed high levels of fluorescence on cells.



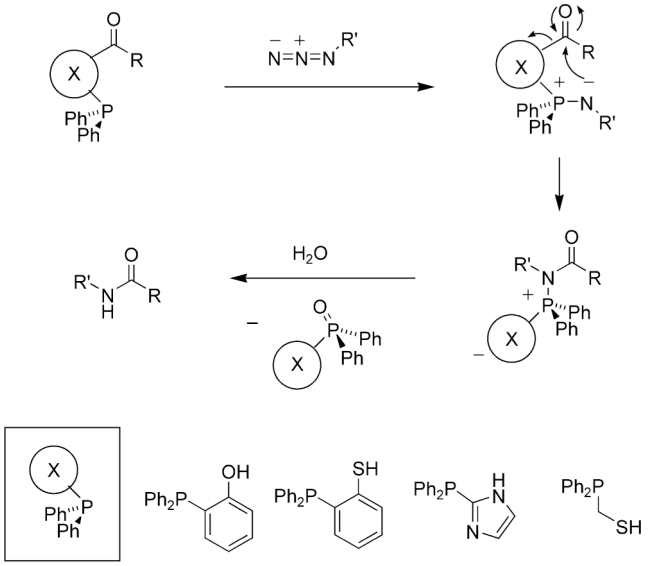


• The incubation with peracetylated ManNAz is not toxic as cells grow at a normal rate despite the fact that they are now incorporating a non-natural group.

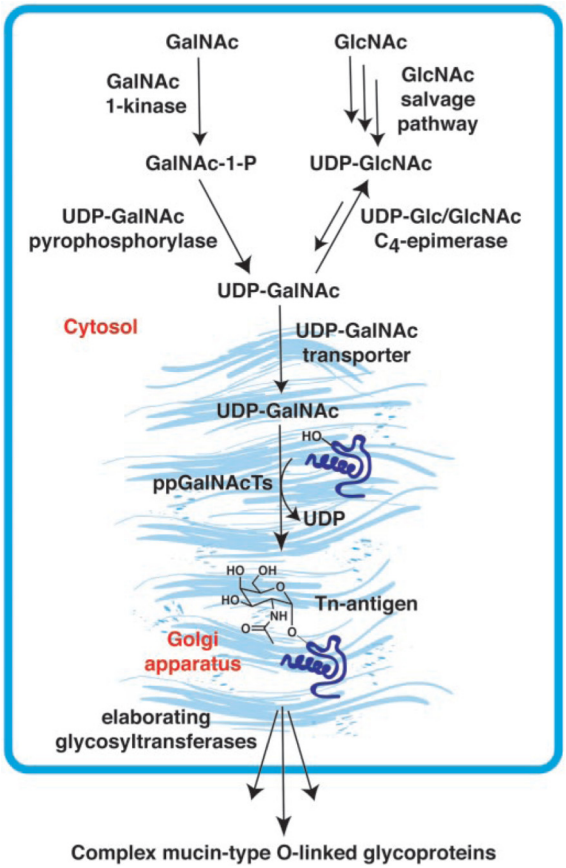
Problems?

• Can you think of groups that other PTMs in proteins that might be disrupted by the phosphine?
• In aqueous environments containing oxygen the phosphine is prone to other side reactions – can you see what these might be?

Future Applications and Developments



• ‘Traceless’ variants of the ligation reaction involve loss of the phosphine following hydrolysis and allow the formation of a product that contains amide but not phosphine oxide. This can also be used as tagging method.



• SAs appear on the tips of both *N* and *O*-linked glycans on glycoproteins. A method that uses the galactose analogue GalNAz has been applied to investigating the presence of GalNAc.