Modern Methods in Peptide Conjugation

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Background

The search for viable therapeutics requires the consideration of a number of properties: specificity for a particular biological receptor; bioavailability to ensure that the therapeutic is present in sufficiently high concentrations to ensure efficacy; be non-toxic and non-immunogenic.

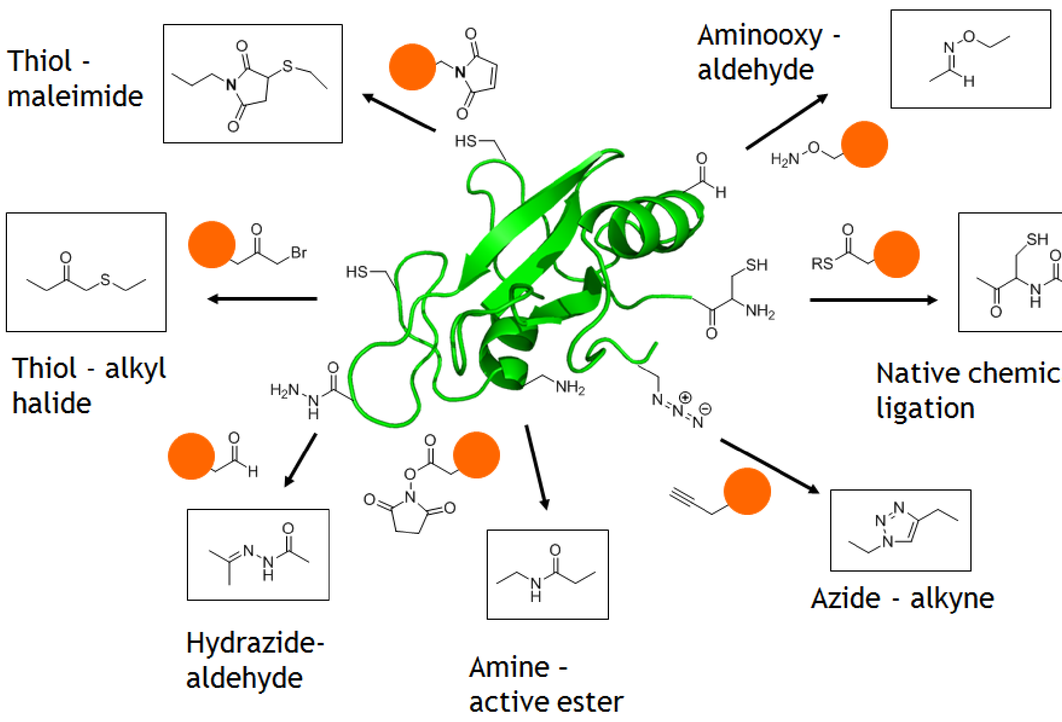
Peptides are a favourable class of molecule as therapeutics because they tend to have very specific interactions with receptors. This also results in lower toxicological effects than, for example, small molecule therapeutics. However, the inherent bioavailability of peptides tends to be low due to natural breakdown through proteolysis. It is therefore useful to be able to enhance the stability of the peptide through conjugation to other moieties. Alternatively, the peptide moiety may not be the therapeutic, but when conjugated to, for example, a small molecule therapeutic, may impart properties which result in more specific receptor targeting of the therapeutic.

In this poster we describe the variety of chemistries available when considering conjugation approaches, and use examples of conjugating folded proteins and peptide ubiquitylation to demonstrate how even complex structures can be used in conjugation.

Conjugation Chemistries

A wide variety of chemistries are available in order to conjugate peptides with other moieties. Examples of such chemistries are shown in *Figure 1*. When choosing the type of chemistry for the conjugation, a number of factors should be taken into account:

i. Is the relevant functionality available on the peptide and conjugate partner, or will it have to be incorporated separately?
ii. Selectivity of the site of conjugation – for example peptides will tend to have more than one free amine, and are hence likely to react at more than one site if amine/NHS conjugation is used.
iii. Will the site of conjugation impact on the biological function of the peptide or conjugate partner?



Conjugating disulfide-bridged peptides

Many peptides rely on intramolecular disulfide bridges to create a stable tertiary structure which is responsible for the peptide's function. The correct disulfide bridge configuration is created by a controlled oxidation step, whereby the most thermodynamically favoured configuration is allowed to form. External factors, such as modification of the sequence, can negatively impact the effectiveness of the oxidation step, and so performing a conjugation step prior to oxidation is likely to lead to severe disruption of the disulfide bridge forming step.

Thiol-maleimide chemistry offers a high degree of selectivity in conjugation reactions. However, an extra thiol in a peptide about to undergo oxidation will lead to disulfide scrambling, and unlikely to result in the required tertiary structure. In order to capitalise on the selectivity of the thiol-maleimide chemistry, whilst retaining the ability to reliably form tertiary structure through disulfide bridges, one approach is to use masked thiol functionality through the thiazolidine group.

Therefore, after oxidation to form the disulfide bridges, the thiazolidine is ring-opened to unmask the thiol functionality and this is then reacted with a maleimide-functionalised moiety. Figure 2 illustrates how this technology has been applied to the site-specific labelling of endothelin 1, a 21 amino acid peptide containing 2 disulfides, with a bis-maleimide linker, for conjugation with an antibody.

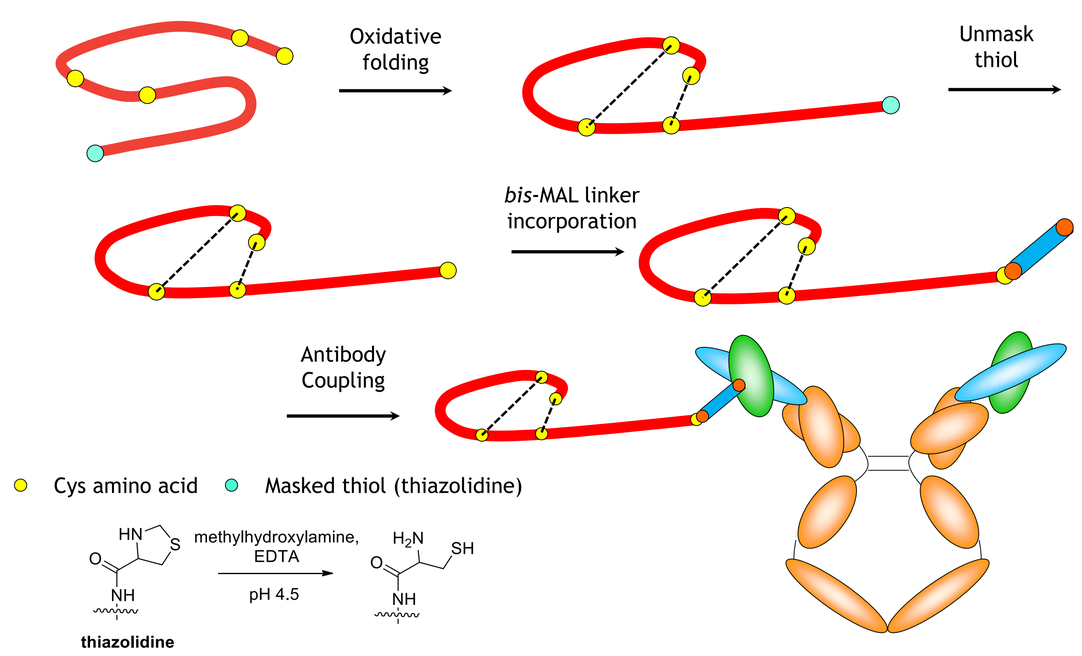


Figure 2: *Schematic of endothelin-1 functionalization for conjugation*

Peptide ubiquitylation

Ubiquitylation is a reversible post-translational protein modification that occurs at the epsilon-amino group of peptide or protein substrate. The function of ubiquitin (Ub) is chiefly to regulate peptide / protein degradation in the cells. The ability to ubiquitylate peptide substrates enables researchers to study the enzyme-mediated ubiquitylation and deubiquitylation processes.

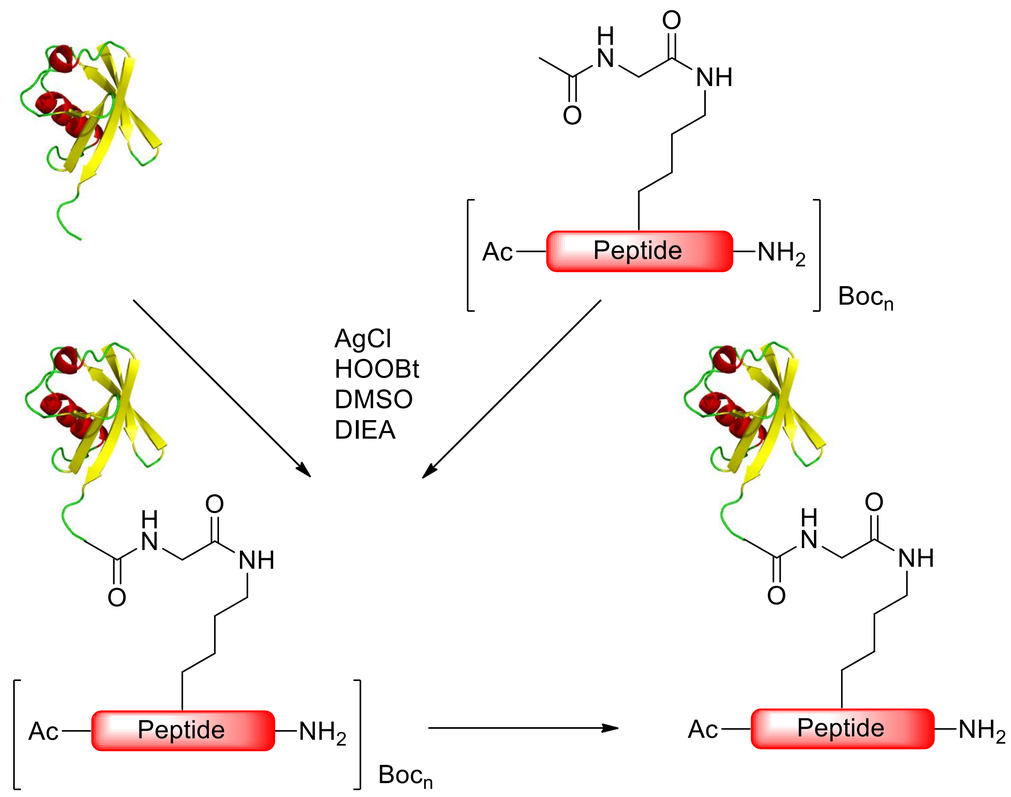


Figure 3: *Peptide ubiquitylation*

Using the approach above, Almac has produced several reagents that incorporate full length ubiquitin site-specifically onto a peptide. Products are entirely synthetic, ensuring precision over the labeling position and product purity. Linkage is via the native isopeptide-bond, using the above high yielding methodology. These reagents are physiologically relevant Ub substrates for DUB assays, and fluorescent analogues are available for drug screening and profiling applications.

Conclusion

Almac has a broad and deep experience of performing conjugation reactions with peptides. Using the challenging examples of conjugation to disulfide-bridged peptides, and the unique product class of ubiquitylated peptides we have illustrated the enhancement of product classes available to the researcher.