Peptide Modifications

1. Amidation and Acetylation

This is a good idea if the peptide is from an internal protein sequence. In the protein the ends of the peptide fragment would be amides ($CONH_2$), and therefore amidation of the C-terminus and acetylation of the N-terminus replicates this. These modifications also protect the peptide from enzymatic degradation.

2. Special Amino Acids

Unnatural amino acids result in peptidomimetics that may increase *in-vivo* half life and potency. d-Amino acids are often used instead of natural l-amino acids to increase peptide stability against proteases. Replacing the N-H of the amide backbone with N-Methyl is useful for destabilising the formation of secondary structure and preventing peptide aggregation.

3. Phosphorylation

Peptides containing phospho-serine, -threonine and/or -tyrosine are useful in replicating phospho-protein downstream effects.

4. Biotinylation

Biotin (244 Da) can be added to your peptide at various positions. Due to the very strong non-covalent interaction between biotin and avidin / streptavidin, it is useful to have this small molecule as a tag to immobilise the peptide, or to pull-down the peptide from a mixture. Note that a spacer, such as a mini-PEG or Ahx (aminohexanoic acid), between biotin and the N-terminus give a better performance in biotechnological applications generally.

5. PEGylation

PEG stands for polyethylene glycol, which is an uncharged, hydrophilic and non-toxic polymer. A mini-PEG (11-amino-3,6,9-trioxaundecanoic acid) is useful as a spacer between modifications such as a biotin or fluorescent tag, as it decreases interference in peptide folding and the peptide-receptor binding interaction. Start your sequence with {mini-PEG} if you want this spacer before an N-terminal modification.

6. Fluorescent Labelling

Fluorescent labelling is the process of covalently attaching a fluorophore to your peptide, which can be done at a variety of positions and with a variety of fluorescent dyes. One of the most common dyes for labelling peptides is fluorescein and its derivatives, such as rhodamine. A common reagent used to label peptides with fluorescein is FITC,

which stands for fluorescein isothiocyanate. Other fluorescent dyes used frequently to label peptides are coumarins (MCA and AMC), p-nitroanilide and dansyl.

7. Cyclic Peptides

Cyclic peptides can be produced in several ways: (i) formation of a disulfide bond between two cysteine residues (up to three disulfide bonds can be made selectively); (ii) formation of an amide bond between the two ends of the peptide (head to tail); or by (iii) formation of an amide bond involving at least one side chain residue in your peptide.

8. KLH and BSA

Since a peptide alone is unlikely to be immunogenic enough for antibody generation, it is often conjugated to a carrier protein, particularly keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). KLH has the advantage in not interfering with western blots or ELISA assays. Note that KLH has limited solubility in water and may give a cloudy solution, but this does not affect immunogenicity.

9. MAPS

The multiple antigen peptide system (MAPS) uses a core peptide onto which your peptide is coupled. Either four or eight copies of the peptide (epitope) are displayed, depending on the core used. The core peptide is relatively small compared with carrier proteins (e.g. KLH). Also, the dense display of multiple copies of the antigenic epitope in a MAP produces a strong immunogenic response, without interference from a carrier protein.

10. Isotope Labelling

Peptides can be labelled with stable, nonradioactive isotopes, such as deuterium, carbon-13 and nitrogen-15. These isotopes are particularly useful for NMR spectroscopy. Please contact us with your peptide sequence and position of isotope label(s).