

Tips for designing a good peptide immunogen

What to aim for and what to avoid.

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- As many features and their combinations affect immunogen design, machine-learning approaches and protein 3D structure/informatics-based programs are used to design peptide immunogens.
- UniprotKB provides essential information on protein function, structure and properties.
- Establish domain regions. Domains will be present in other proteins and should be avoided in the sequence of the immunogen as they might increase cross reactivity.
- Avoid complex and inaccessible regions such as alpha helices and beta sheets. Instead aim for accessible regions of the native protein which are surface-exposed, hydrophilic and flexible.
- Epitopes (antigenic determinants) are preferably located at β turns or loops with amphipathic helices. The C- and N-termini of proteins are often surface-exposed with a high degree of flexibility.
- Peptide length – in general 10-20 amino acids. A long peptide increases immunogenicity, but also increases the chance for cross-reactivity while a short peptide improves the specificity, but may not be immunogenic.
- Avoid long chains of hydrophobic residues. Peptide solubility can be improved by changing the peptide length and/or adding 1-2 hydrophilic amino acid residues to the N- or C-terminus.
- Avoid N-terminal glutamine or asparagine and C-terminal proline or glycine in a peptide.
- Avoid internal cysteine, which can be replaced with serine.
- Avoid multiple (more than three) serine or proline residues in a sequence. Also avoid multiple glutamine residues as this can result in hydrogen bonding between peptides.
- Conservative residues can be used to replace any undesirable residues to improve peptide properties.
- Avoid tripeptide arginine-glycine (RGD motif), small molecule binding sites, biologically active regions and post-translational modification site(s) as they may mask the antibody recognition site.
- A terminal cysteine may be added to the peptide sequence to allow peptide conjugation to carrier proteins. It is added away from the epitope location if known. If the peptide is derived from the N-terminus of the protein the cysteine should be added to the C-terminus of the peptide and vice-versa.