

Almac Voice

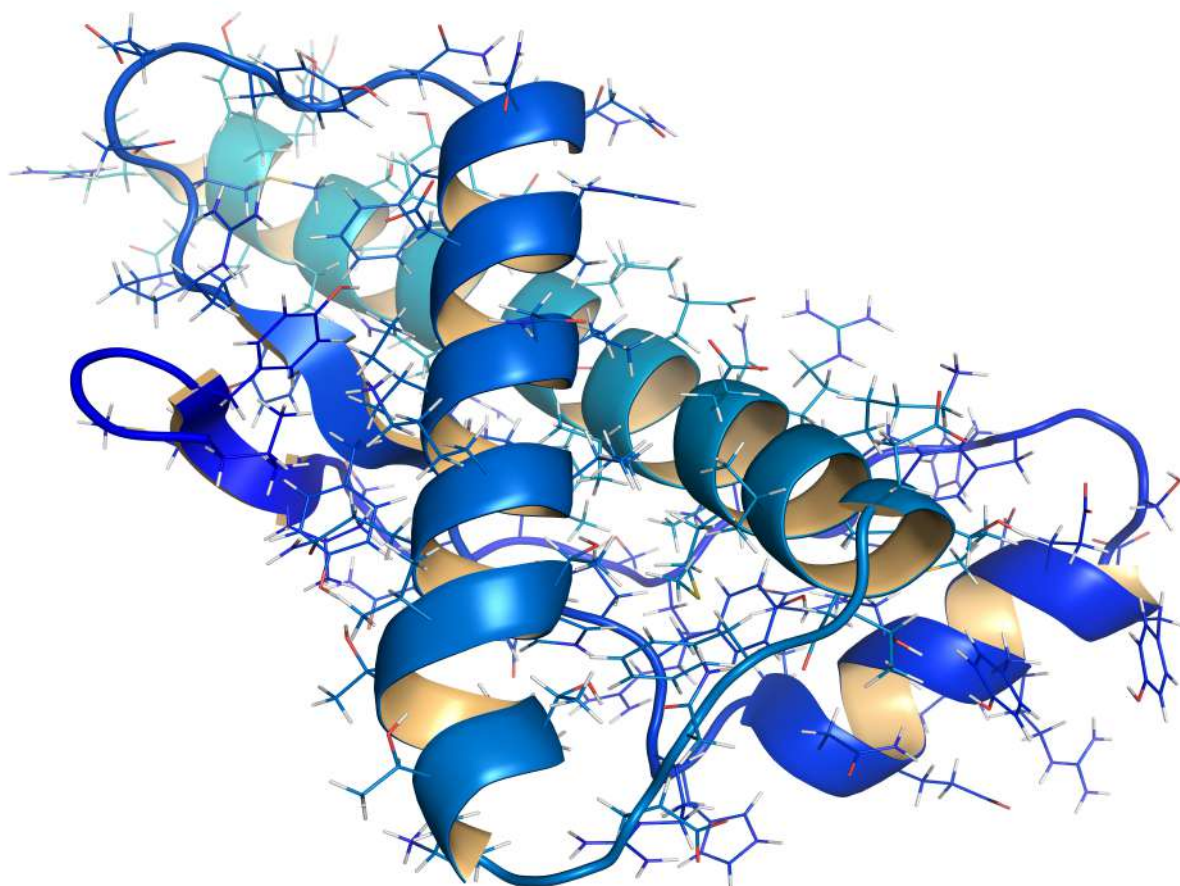
Peptide mapping – navigating the way through structural characterisation



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Peptide mapping is a widely used structural characterisation test for biopharmaceuticals and accepted as one of the principle tests for identity confirmation in support of regulatory submissions (and for lot release purposes).



Peptide mapping is a comparative procedure which confirms the structure of proteins, while also enabling the detection of modifications to primary structure. Peptide mapping provides a unique 'fingerprint' that is characteristic for each protein, which can be used for confirmation of desired product structure for lot release purposes. It's a very powerful technique that can identify single amino acid changes that can result from point mutations. As such, validated peptide mapping methods can also be applied to the evaluation of genetic stability of product-producing cell lines.

Peptide mapping is not a general method but involves development of specific strategies for each unique protein. Strategies developed at Almac are based upon compendial methods (USP Chapter <1055> and Ph.Eur. 2.2.55), involving the use of a range of sequencing-grade proteolytic enzymes to selectively cleave the protein of interest into distinct peptides. The resulting peptide mixture is subsequently separated by

reverse phase chromatography, and peptide fragments analysed using suitable detection methods, such as UV and mass spectrometry.

Combining multiple fragment strategies during peptide mapping work provides greater assurance of confirmation of protein structure. Peptide mapping using a single enzyme typically provides sequence coverage of 10-90%. By combining two or more enzymes, this coverage can be increased to 95-100%. Based on the structural information supplied for the test sample, our experts can propose an appropriate analytical strategy for characterisation of your biopharmaceutical products.

Sulfhydryl group and Disulfide bond analysis

The number and location of cysteine residues and disulfide bonds in a protein play a crucial role in correct folding. Intra-chain disulfide bonds can stabilise tertiary structure, and



inter-chain linkages join multi-subunit proteins together to stabilise quaternary structure. The correct structural conformation of a protein is critical for correct function. Where cysteine residues are known to be present in a protein (as determined from the encoding gene sequence or available amino acid sequence information), the number and positions of any free sulfhydryl groups must be determined – this is a key characteristic that should be determined according to ICH Q6B.

At Almac, structural characterisation strategies provide confirmation that disulfide bonds are not mismatched. This is typically accomplished using peptide mapping techniques employing mass spectrometry. Analysis is conducted under both reducing and non-reducing conditions to reveal the location of disulfide bonds.

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