Disulfide Bond Mapping Services

Introduction

A protein's three-dimensional structure is based initially upon the amino acids of the protein's sequence. Additionally for therapeutic proteins, the correct configuration of disulfide bonds is required for determining structural integrity and biological activity. This structural integrity is achieved and stabilized by the formation of intra- and inter-chain disulfide bonds.

Mispaired disulfide bonds may result in structural changes, aggregation and ultimately loss of function. Thus, monitoring for correct disulfide bond formation is necessary for the structural integrity and biological activity of most therapeutic proteins. Moreover, characterizing a protein's native disulfide linkage pattern is critical in understanding the structure–function relationship of the protein. Mass spectrometry has become the predominant method to ensure efficacy and safety both in biological drug development and in academic research.

Why Conduct Disulfide Analysis?

Developability of protein

Understanding disulfide bonding is critical for protein characterization and in helping solve a proteins structure. Incorrect disulfide bond formation, or exchange, can be side-effects of long-term mAb storage that can cause antibody aggregation. Providing early insight into the integrity of a biotherapeutic can allow identification of potential quality issues.

Misfolding due to scrambling

Scrambling can result in a misfolded protein, leading to inactivity, immunogenicity or susceptibility to aggregation. Disulfide bonds can contribute to aggregation and must be monitored in regard to patient safety. In biopharmaceutical production, elucidating the cysteine connections is necessary to prevent disulfide scrambling and incorrect folding. CovalX services are typically used to identify the number and/or position of disulfide bridges present or to detect mismatched or scrambled disulfide bridge formation.

Submission guidelines

IND submission guidelines for biotherapeutic proteins state that it is necessary to characterize sulfhydryl group(s) and disulfide bridges. If cysteine residues are expected, the number and positions of any free sulfhydryl groups and/or disulfide bridges should be determined, to the extent possible. Peptide mapping (under reducing and non-reducing conditions), such as mass spectrometry is useful for this evaluation.

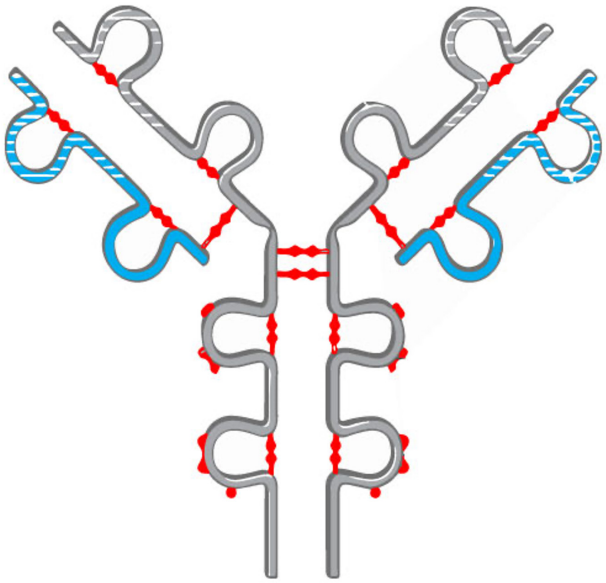
Why does CovalX conduct Disulfide Analysis?

After recombinant or purified proteins are provided, we will require the sequence information to be provided by the client or it can be measured by De Novo sequencing. Mass spectra from multiple enzyme proteolysis are then compared before and after reduction to determine the location and type of disulfide bond present.

Multiple enzyme digestion coupled with the latest nano-LC coupled with high-resolution Orbitrap MS/MS instrumentation and software provide the highest sequence coverage and the most accurate detection. A full report and thorough client discussion are included upon completion.

Why choose CovalX's Disulfide Mapping Services?

- Over 15 years characterizing Biologics by MS
- Experienced mass spec scientists overseeing all analysis
- Latest MS Instrumentation and Software
- Reliable delivery time
- Proven professional results, trusted industry partner



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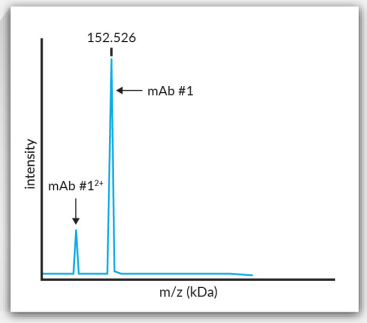
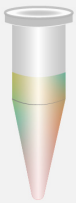
Disulfide Bond Mapping by High-Resolution Mass Spectrometry: 3 weeks

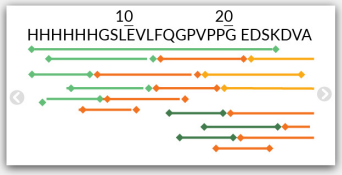
Step 1. Intact high-mass MALDI MS measurements are conducted as an initial QC step. This is a rapid step that can confirm stability after transport directly before disulfide bond mapping analysis.



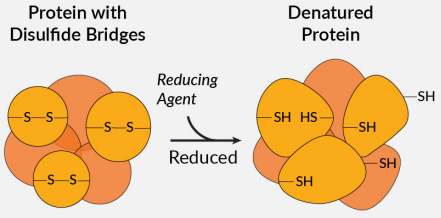


Step 2. Using multiple enzyme digestion, nano-LC and high-resolution Orbitrap MS/MS analysis, our analysis accurately detects a large number of peptides covering the sequence of the protein. This data is included in a table format and showing sequence detected coverage and main post translational modifications.

Sequence	Modifications	Position Peptide
REDACTED		1-19
REDACTED	1xCarbamidomethyl [C9]	88-98
REDACTED		99-123
REDACTED	1xCarbamidomethyl [C11]	135-148
REDACTED	2xCarbamidomethyl [C6;C9]	219-245
REDACTED	2xCarbamidomethyl [C5;C8]	220-245



Step 3. Dithiothreitol (DTT) is used to reduce disulfide bonds before repeating the multiple enzyme digestion, nano-LC and high-resolution Orbitrap MS/MS analysis. This analysis accurately detects disulfide bonds within proteins and identifies the position of cysteins involved in the bridges.



Sequence	Identification	Site	Protein	Enzyme
REDACTED	2:E1-Y27/2:F95-F106=...	2:C22/2:C96	HC/HC	Chymotrypsin
REDACTED	1:S12-Y32/1:C88-Y91=...	1:C23/1:C88	LC/LC	Chymotrypsin
REDACTED	1:S12-Y32/1:C88-F97=...	1:C23/1:C88	LC/LC	Chymotrypsin
REDACTED	1:S12-Y32/1:F87-Y91=...	1:C23/1:C88	LC/LC	Chymotrypsin
REDACTED	2:V115-L146/1:N208-C...	2.~C132,~C145...	HC/LC/HC	Chymotrypsin
REDACTED	2:K219-K245/2:S220-K...	2.~C224,~C227...	HC/HC	Trypsin

Table 13: Detection of the peptide linked by disulfide bonds for the protein mAb#1

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