Protein Metrics

Automated LC–MS Characterization of Disulfide Bonds in Biotherapeutics Using Positive Controls and MS1 Isotope Envelope Confidence

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Summary

Disulfide bond characterization in complex biologics by LC–MS is challenged by scrambling and low signal intensity.

Neutral-pH digestion without alkylation increases shuffled species from ~8% to 69%, with tenfold more unique species.

Automated decision-tree filtering cuts manual review candidates from 415 to 60, supported by MS1 isotope envelope scoring.

A novel cysteine-based grouping strategy enables quantitative disulfide analysis in highly complex modalities.

Introduction

- **Biologics & disulfide bonds:** ~30% of FDA approvals over the past decade are biologics (esp. mono-, bi-, trispecific antibodies), all relying on correct disulfide bonds for structural integrity and efficacy.
- **Analytical challenge:** Non-reducing LC–MS peptide mapping is the standard for disulfide characterization, yet disulfide scrambling during digestion and low signal intensity of native disulfide-linked peptides hamper reliable identification.
- **Our approach:** We combine a scrambling-induced positive control with MS1 isotope envelope scoring, automated decision-tree filtering, and a novel quantitation strategy to improve disulfide characterization in a trispecific antibody.

Results

- **Shuffling induction:** Neutral-pH digestion without alkylation dramatically increased disulfide scrambling — shuffled species rose from ~8% to 69% relative abundance, with unique shuffled species increasing tenfold (22 → 225) and mean Byonic scores improving from 416 to 725
- **Automated filtering:** A decision-tree-based software tool reduced candidates requiring manual review from 415 to 60, restoring throughput without sacrificing confidence.
- **Quantitation strategy:** Cysteine-based peptide grouping links expected disulfide species with their shuffled variants, enabling group-wise normalization for quantitative assessment of disulfide scrambling.

Conclusion

- **Improved Shuffling detection:** Neutral-pH digestion without alkylation, combined with MS1 isotope envelope confidence scoring, substantially enhances the identification of shuffled disulfide species in complex biologics
- **Efficient data evaluation:** Automated decision-tree-based filtering reduces manual review burden by ~85%, enabling high-throughput disulfide characterization without compromising reliability.
- **Quantitative framework:** The proposed cysteine-based grouping and normalization strategy provides a novel, robust approach for quantitative disulfide analysis in highly complex modalities such as trispecific antibodies.