Progressive deconvolution provides robust feature detection and quantitation for large mass intact MS analyses

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Summary

The size and heterogeneity of modern biotherapeutic molecules present unique challenges to analytical pipelines

Novel analytical and computational approaches are required to accurately characterize and quantify complex biologics

Progressive deconvolution of intact mass spectrometry analyses coupled with multi-dimensional feature detection robustly addresses such needs

Introduction

Older MS-based deconvolution techniques are insufficient for modern multispecifics and complex biologics. This poster describes how Progressive Deconvolution from Protein Metrics can reveal low-abundance forms, faithfully render all proteoforms, and provide clear visualizations to analytical scientists and biologists alike. And when performed iteratively on sequential time ranges of a chromatogram, referred here as 'progressive deconvolution', can distinguish isomers based on differential elution patterns.

Here we present automated large mass feature detection for the identification and quantification of intact analytes in complex biological mixtures.

Methods: Progressive Deconvolution for Large Mass Feature Finding

The workflow for large mass feature detection with progressive deconvolution begins with the sampling of short, fixed-width, overlapping segments of the chromatogram **(a)**. The resulting deconvolved mass candidates from these trace segments **(b)** are transformed into a mass-time matrix and multi-dimensional segmentation provides elution times of unique masses **(c)**. Subsequent peak segmentation of an extracted mass chromatogram identifies peak bounds of the eluting species and can distinguish isomeric forms if they exist. Results are organized and viewable in a user interface **(d)**.

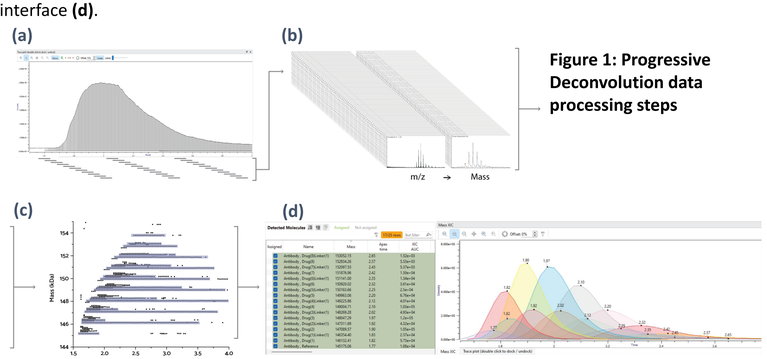
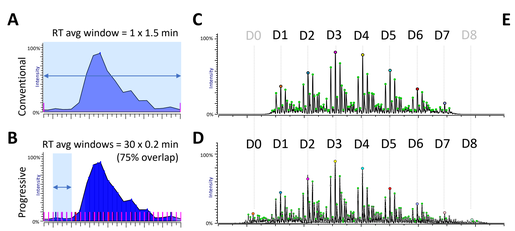


Figure 1: Progressive Deconvolution data processing steps

Results - ADC Analysis

Antibody drug conjugates (ADC) often elute together under one peak of a chromatogram. Here, we capture the **Trastuzumab emtansine ADC (Kadcyla, Genentech)** with varying amount of drug payload and PEG linker, providing robust drug-to-antibody ratio calculation.



E

	Conventional (1 x 1.5 min)	Progressive (30 x 0.2 min)
Total # Validated IDs (90 possible)	63	81
Highest Intensity Validated ID	2.8e5	2.2e5
Lowest Intensity Validated ID	1.9e4	5.3e3
Validated Dynamic Range	14.7	42.5
Average DAR (G0F/G1F)	**3.62**	**3.40**

Figure 2: Quantitative comparison of conventional vs. progressive deconvolution analysis for Kadcyla
A raw data file from a native SEC-Orbitrap MS analysis of Kadcyla was processed using a **(A)** conventional deconvolution approach, based on a single average RT window (1.5 min width), versus **(B)** a progressive deconvolution approach based on numerous narrower average windows (0.2 min widths, 75% overlap). Deconvolved intact mass results from **(C)** conventional versus **(D)** progressive methods were searched using a database of 90 possible species: 5 glycoforms x 9 linker-drug states (0-8 MCC-DM1) x 2 linker-only states (0-1 MCC). **(E)** A summarized comparison of conventional vs. progressive deconvolution results show that progressive deconvolution yields many additional high confidence IDs at lower abundance.

Results - Biologics with high degrees of heterogeneity - Herceptin

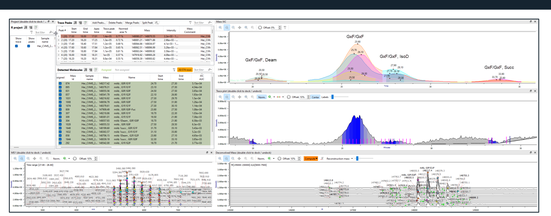


Figure 3: Analysis of Herceptin
Deamidation, isomerization, succinimide formation, and glycosylation (including sialic acids) are common charge variant modifications on antibodies which reflect sample degradation. Such degradation can be characterized at the intact level using weak cation exchange (WCX)-MS. The results above display a high dynamic range of modified proteoforms of the unstressed Herceptin monoclonal antibody.

Results - Biologics with high degrees of heterogeneity

Figure 4: Analysis of Glycosylated Biotherapeutics
Biologics with even small numbers of glycosylation sites can produce significant heterogeneity if there is microheterogeneity at each glycol-site. The analysis of a class II major histocompatibility complex with 4 glycol-sites and considering 9 possibility glycans identifies hundreds of individual glycoforms and their elution profile (https://doi.org/10.1038/s41467-024-47693-8)

Conclusion

- Protein Metrics' parsimonious deconvolution algorithm is a best-in-class option for deconvolution of intact mass spectra.
- The method presented here proved to be a robust and sensitive feature detection tool to assure lower abundance intact species are detected with high confidence
- Relative Quantitation of proteoforms present in the sample can be performed by utilizing the resulting massXICs – which are providing a more comprehensive assessment of relative levels across the time range of the progressive deconvolution experiment. Results are comparable to conventional methods.
- Progressive Deconvolution yields additional high confidence IDs compared to conventional methods of peak based summed spectra deconvolution which improvement can be contributed to the higher validated dynamic range.

Conflict of Interest Statement

The authors are employees of Abcellera and Protein Metrics LLC.

References

Schachner, L.F., Mullen, C., Phung, W. *et al.* Exposing the molecular heterogeneity of glycosylated biotherapeutics. *Nat Commun* **15**, 3259 (2024). https://doi.org/10.1038/s41467-024-47693-8