Protein Metrics

Dedicated computational methods for feature detection and quantification in large molecule mass spectrometry 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

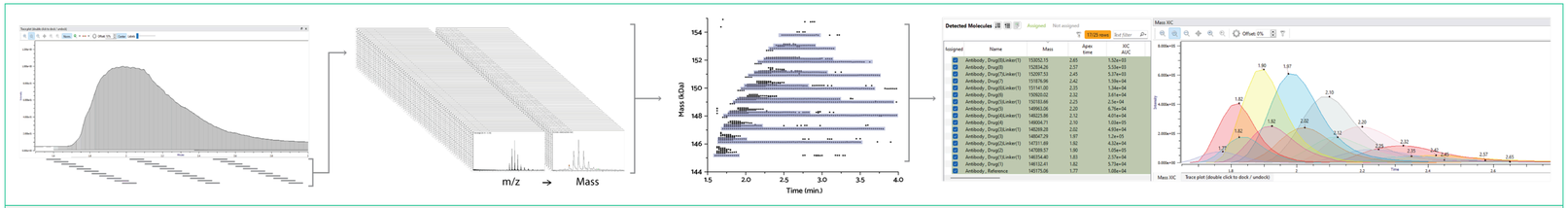
Introduction

As complex biomolecular constructs enter biopharmaceutical pipelines, advanced analytical methods are needed to assess their composition and quality. Intact mass spectrometry (MS) proteomics is a standard for such analyses.

When coupled with charge and isotope deconvolution, intact MS can provide knowledge of all unique masses in a sample. And when performed iteratively on sequential time ranges of a chromatogram, which we refer to as **progressive deconvolution**, co-eluting species can be discerned, and isomers can be distinguished based on their elution profile.

Here, we detail the incorporation of temporal resolution with charge deconvolution to profile the large mass composition of complex biologic samples.

Methods: Progressive Deconvolution for Large Mass Feature Finding

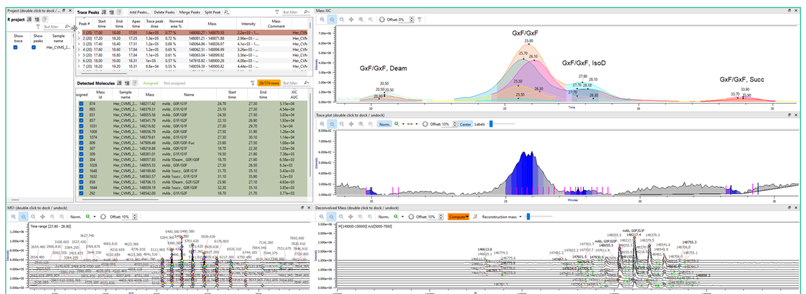


The workflow for large mass feature detection with progressive deconvolution begins with the sampling of short, fixed-width, overlapping segments of the chromatogram (a). The resulting deconvolved mass candidates from these trace segments (b) are transformed into a mass-time matrix and muti-dimensional 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).

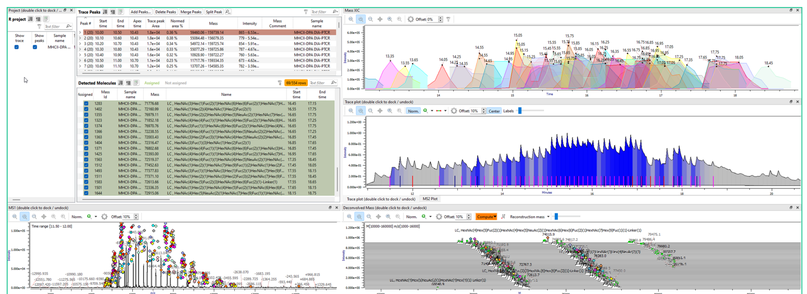
Results: Identification and quantification of biologics with high degrees of heterogeneity

Antibody drug conjugates (ADC) often elute as an offset mixture under one UV or TIC peak of a chromatogram. Here, we capture the Trastuzumab emtansine ADC (Kadcyla, Genentech) with varying amount of DM1* payload and MCC** linker, providing robust drug-to-antibody ratio calculation.

*DM1 = (N(20)-deacetyl-N(20)-(3-mercapto-1-oxopropyl)-maytansine)
**MCC = succinimidyl-4-(N-maleimidomethyl)cyclo-hexane-1-carboxylate



Deamidation, isomerization, succinimide formation, and glycosylation (including sialic acids) are common charge variant modifications on antibodies that lead to high sample heterogeneity which can be monitored at the intact level using weak cation exchange (WCX)-MS. The sample results above display the various forms of the unstressed Herceptin monoclonal antibody.



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