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FTMS DATA SIMULATIONS IN PROTEIN ANALYSIS

Spectroswiss

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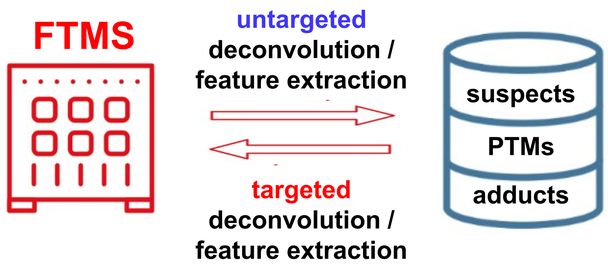
Targeted deconvolution and feature extraction workflow



Data analysis in FTMS typically begins with mass spectra deconvolution and deisotoping (an **untargeted** approach). The obtained monoisotopic or average mass lists are then searched against the suspect database by mass accuracy, retention time, fragments, and isotopic ratios.

An alternative approach is to do the inverse (a **targeted** approach), namely to start with the suspect database, targeted or large-scale, simulate the isotopic envelopes in diverse charge states, and identify compounds directly from the experimental data by their signals correlation with the simulated data. **What is the value of the targeted approach?**

Define molecule(s) → **Define PTMs / Ions / Charges** → **Shuffle PTMs etc.** → **Simulate profiles** → **SIC extraction, feature detection**

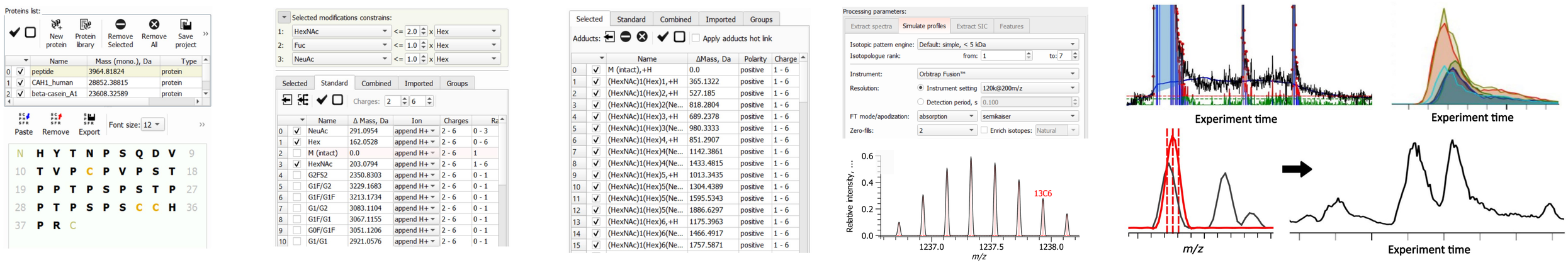
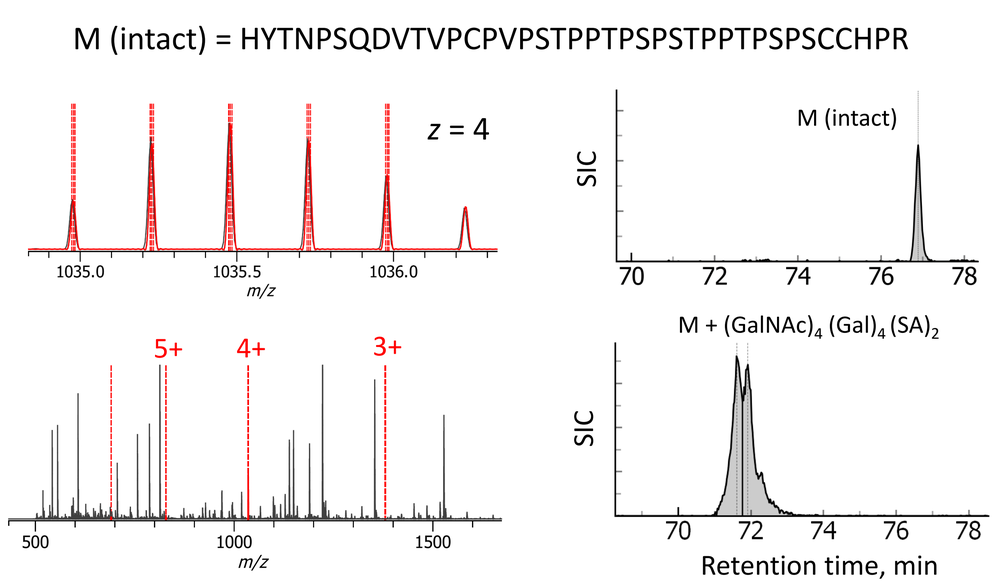


Figure 1. Targeted deconvolution and feature extraction workflow as implemented in Peak-by-Peak Multiomics, empowered by FTMS Simulator software (Spectroswiss).

Peptides, High-resolution

Glycosylated peptide profiling, quantification



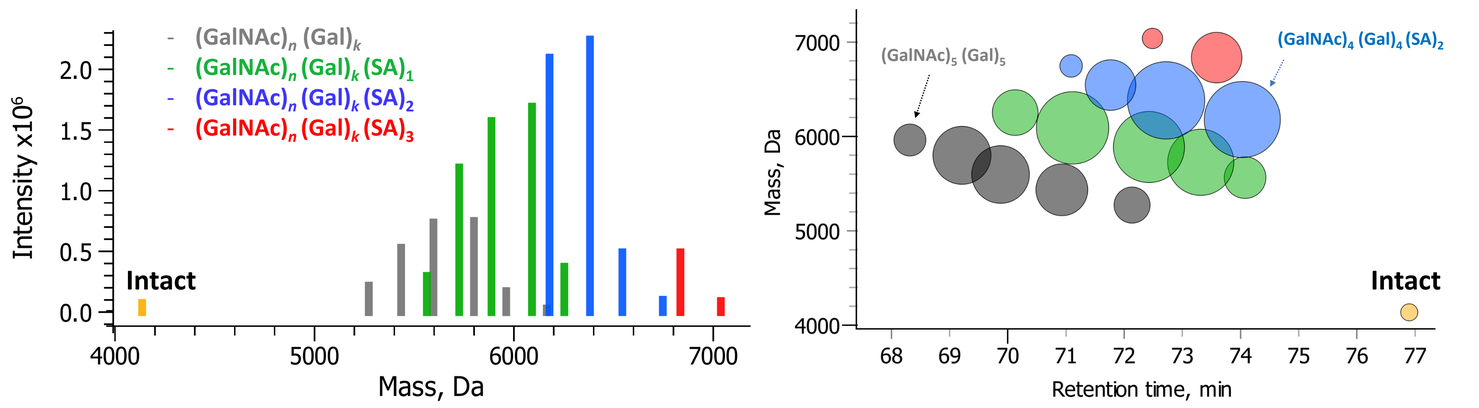


Figure 2. Bottom-up (DDA, HCD) MS analysis of a human IgA1 (Sigma Aldrich). The data of IgA1 tryptic digest are acquired using Orbitrap™ Fusion™ (Thermo Fisher Scientific) and analyzed using the targeted deconvolution workflow. The identified PTMs were quantified via area under the curves of SICs. The MS-only SICs were extracted using 4 highest isotopologues in several charge states.

Proteins, High-resolution

Protein mixture analysis, middle-up analysis

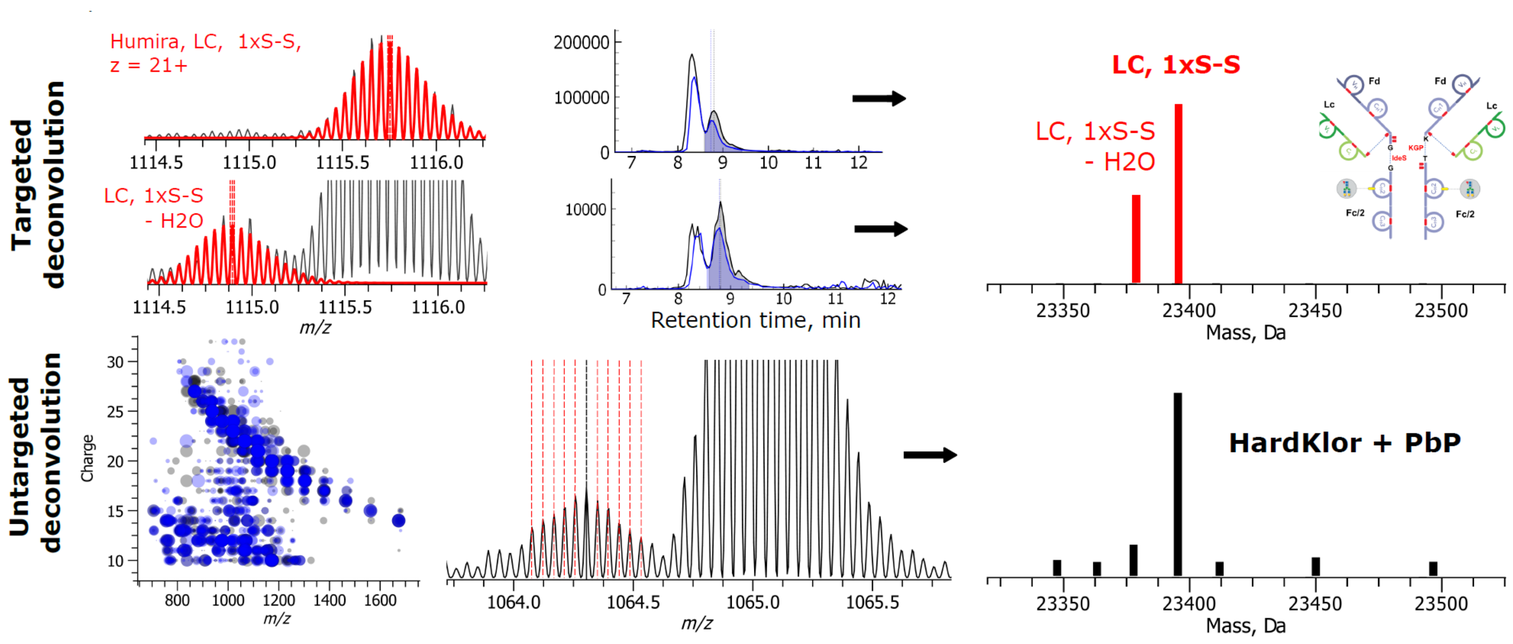


Figure 3. A combined integrated targeted & untargeted high-resolution deconvolution method: targeted - developed in this work and untargeted deconvolution method (e.g., HardKlor).

The isotopically resolved patterns (profile, multiple charge states) were simulated for several subunits of IdeS-digested adalimumab (Humira) and a Q Exactive HF Orbitrap (red).

Targeted SICs were extracted for the simulated reference patterns and features were detected according to the algorithm in Figure 1. Specifically, SIC is extracted using 10 highest isotopologues for several (> 5, consequent) charge states passed via isotopic similarity filtering (simulated vs experimental). Targeted approach distinguishes 1-2 Da difference features (e.g., S-S bond) with a high confidence.

Proteins, Low-resolution

Proteoform mixture analysis, intact mass analysis

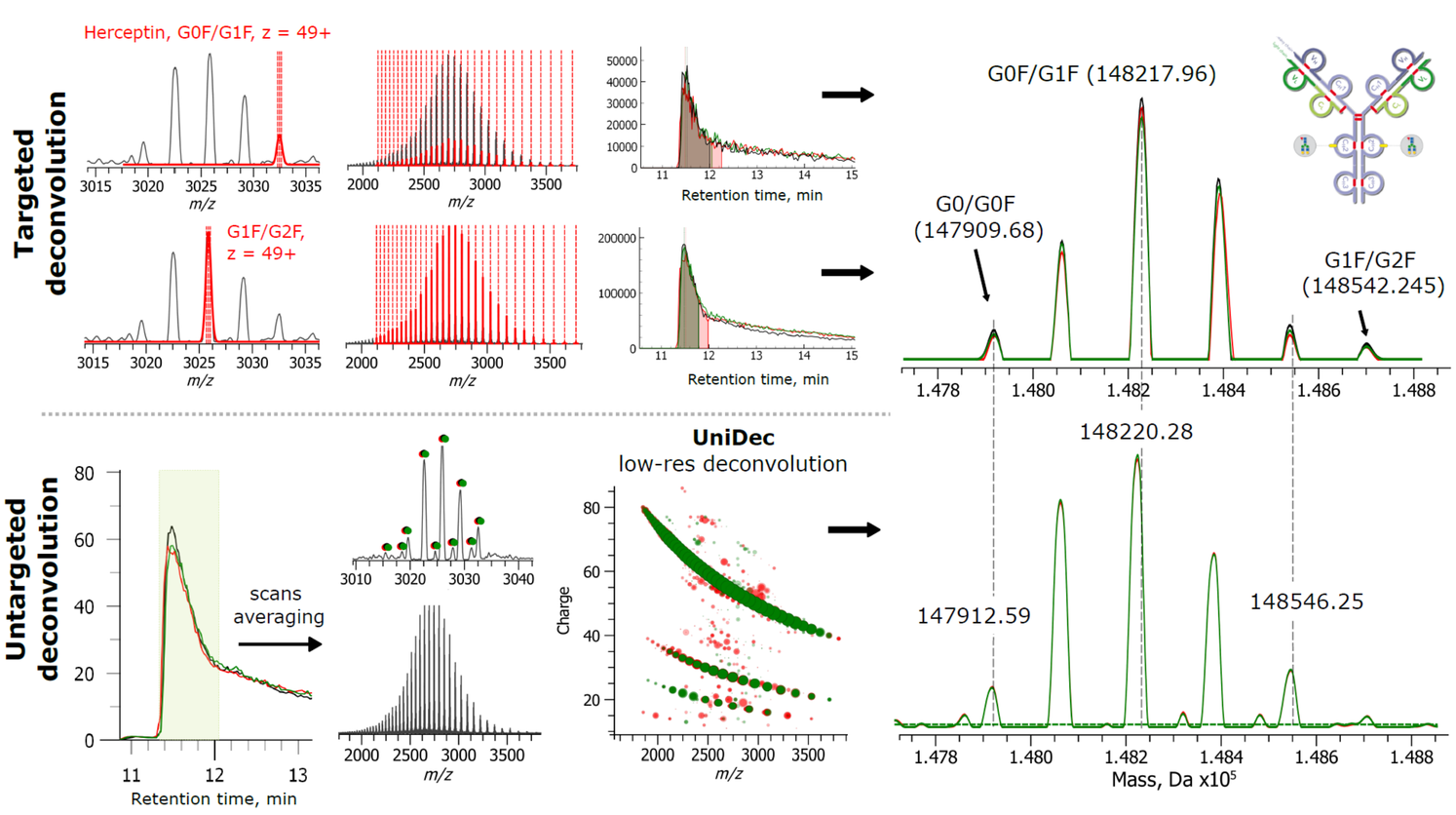


Figure 4. A combined integrated targeted & untargeted low-resolution deconvolution method: targeted - developed in this work and untargeted deconvolution method (e.g., UniDec, DOI: 10.1021/acs.analchem.5b00140). Low-resolution intact mass analysis of trastuzumab (Herceptin) IgG (~150 kDa) performed on a Q Exactive HF Orbitrap reveals the typical glycosylation pattern. Targeted SICs were extracted using several (>10) charge states (in the 30 – 80 range). Combination of both targeted and untargeted deconvolution methods allows direct annotation of the deconvolved features.

Conclusions

Targeted approach applied in protein and peptide modifications analysis demonstrates high analytical specificity, sensitivity, and quantitative precision. Analysis of N- and O-glycosylated tryptic peptides from IgG/IgA demonstrated the identification and quantitation of modified species in a wide dynamic range using the MS-only data. The MS/MS data act as an additional results validation filter. The approach intrinsically supports automation. Implemented in **Peak-by-Peak Multiomics** software package.