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Advancing Top- and Middle-Down Antibody Analysis Using Simulated FTMS Datasets

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The structural complexity and heterogeneity of monoclonal antibodies (mAbs) continue to pose analytical challenges. Over the past three decades, top-down (TD) and middle-down (MD) MS approaches have become powerful tools for characterizing intact antibodies and their subunits [1]. These methods are routinely applied in our CRO operations to complement intact mass and bottom-up proteomics, particularly for resolving complex structural questions in real-world mAb samples. However, despite major advances in instrumentation and data analysis, the structural detail gained from current TD/MD MS workflows remains limited.

A key obstacle to improving TD/MD MS bioinformatics is the lack of standardized, annotated benchmark datasets. To address this, we launched the ProteoGold initiative. It is focused on generating high-quality, in silico FTMS datasets that mimic the complexity of real experimental spectra. Using the proprietary FTMS Simulator (Spectroswiss), we produce isotopically resolved datasets based on user-defined instrument settings and known protein sequences [2]. The simulator is available as a desktop application for full-spectrum simulations and as a web-based platform at www.peakbypeak.com for real-time isotopic modeling, profile-mode spectrum generation, and hybrid server-side processing.

As a proof of concept, we generated a simulated ETD TD MS dataset of carbonic anhydrase II, modeled after data from a 21 T FT-ICR MS at the MagLab [3]. Additional simulations include TD/MD MS datasets of mAbs and subunits acquired on various FTMS platforms, such as Orbitraps. For example, we simulated data from an antibody light chain analyzed on the Omnitrap-Orbitrap-Booster (OOB) platform at the Institute Pasteur, Paris [4].

These datasets form the foundation of the ProteoGold repository, supporting benchmarking, deconvolution evaluation, improved ion assignment, and driving innovation and education in TD/MD MS analysis.

1. Khristenko, et al., Mol. Cell Prot., in press
2. Nagornov, et al., JASMS (2022) 1113-1125
3. Weisbrod et al., JASMS (2017) 1787–1795
4. Garcia et al., submitted

User consent

yes

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