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Deciphering Biotherapeutic Biotransformations with Top-Down Mass Spectrometry

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Today, within the R&D pipelines of pharmaceutical companies, monoclonal antibodies are gradually being replaced by new-generation biotherapeutics, including engineered hybrid and multispecific constructs. While these innovative formats hold great therapeutic promise, their structural complexity often leads to unexpected in vivo instabilities that may compromise efficacy or alter pharmacokinetics. Understanding their metabolic fate is therefore crucial to guide rational design and ensure functional performance.

However, current analytical workflows—originally designed for small molecules or peptide-level proteomics—struggle to capture the full heterogeneity of these large, hybrid biomolecules. Proteolytic digestion breaks apart structurally distinct proteoforms into overlapping peptide mixtures, leading to a loss of connectivity and incomplete characterization.

To overcome these limitations, we have developed and applied complementary middle-down and top-down mass spectrometry strategies capable of analyzing intact proteins and large subunits (25–100 kDa). These approaches were implemented on the timsOmni multimodal platform (Bruker) and the Orbitrap Eclipse Tribrid system (ThermoFisher), allowing high-resolution analysis with advanced dissociation methods (ECD, EID, CID, ETD, HCD).

We demonstrate that these techniques can detect and partially sequence low-abundance mAb proteoforms directly from in vivo plasma samples following administration in mice. A key part of this work involved the automated immunoenrichment of biotherapeutic metabolites from complex biological matrices, enabling their isolation and characterization despite low concentrations and high background.

Together, these innovative analytical workflows provide new insight into biotherapeutic metabolism, including the detection of truncated forms resulting from rapid in vivo cleavage—such as the loss of target-binding domains—which directly impacts the therapeutic mechanism of action. These results highlight the importance of proteoform-level analysis for next-generation biologics and open the path toward more robust, predictive tools in biotherapeutic development.

User consent

yes

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