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AiDA Accelerates Top-Down and Middle-Down MS Data Analysis Across Multiple Antibody Variants

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Mass spectrometry (MS) is essential for characterizing biotherapeutics, with Top-Down (TD) and Middle-Down (MD) approaches offering faster alternatives to peptide mapping. Despite achieving high sequence coverage using advanced fragmentation techniques like HCD, ETD, and UVPD, traditional data analysis presents challenges. These include time-consuming fragmentation map creation, difficulty localizing post-translational modifications (PTMs) in non-fragmented regions, and missed diagnostic ions due to monoisotopic peak detection errors in complex spectra.

To overcome these issues, the All ion Differential Analysis (AiDA) method was developed for online antibody variant characterization. (1) AiDA enables rapid identification of diagnostic spectral differences across multiple MS spectra before fragment assignment, significantly accelerating data analysis. It introduces a quantitative layer to TD and MD workflows by analyzing preferential fragmentation patterns, particularly near aspartic and iso-aspartic acid residues, to localize PTMs with statistical confidence.(1, 2) AiDA also helps detect interactions between neighboring residues and has proven effective in characterizing deamidation, sequence variant and sequence positional isomers of oxidation and iso-aspartic acids in antibodies. Finally, AiDA supports multi-level validation of internal fragments N-terminal to Proline which can be used to increase MD sequence coverage by up to 34% and access otherwise non-fragmented regions.(1) Both 1D and 2D AiDA applications will be discussed.

(1) Griaud F, Denefeld B, Kao-Scharf CY, Dayer J, Lang M, Chen JY, Berg M. Anal Chem. 2019 Jul 16;91(14):8845-8852.

(2) Denefeld B, Hajduk J, Cerar J, Rondeau JM, Dayer J, Lang M, Kern W, Griaud F. J Am Soc Mass Spectrom. 2025 May 7;36(5):969-979.

User consent

yes

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