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A Comprehensive Approach for Top-Down Biopharmaceutical Characterization, PTM Assessment, and Unknown Identification

This study discusses the advanced software-supported Top-Down characterization of biopharmaceutical proteins, addressing the limitations of traditional bottom-up analyses. Despite historical challenges in Top-Down sequence analysis due to inadequate software and instrumentation, this research presents a comprehensive method using modern software tools and fragmentation techniques for high sequence coverage. We examined proteins such as monoclonal reference antibody NISTmAb fragments, including LC, Fd, and Fc/2, utilizing a script to identify chromatographic peaks and generate average MS1 and MS2 spectra. These spectra were matched to expected sequences, assessing putative PTMs.

A novel workflow allowed method optimization and analysis via direct-infusion setups with various acquisition methods, including charge state and fragmentation chemistries (such as CID, EAD, ECD), and fragmentation strengths (reaction time, collision energy). The OmniScape software facilitated direct spectrum comparisons and combined sequence maps for optimal SC and PTM/proteoform assignments.

Additionally, workflows for identifying unknown proteins and side products through de novo sequence tag generation and homology-based database searches using MS-BLAST were developed.

The challenge of simultaneous identification and localization of multiple PTMs was addressed using heuristic algorithms in OmniScape, screening billions of proteoforms to retrieve top-scoring candidates for confirmation. Using this PTM Screening approach, we analyzed the poly-phosphorylated protein AMPK, identifying a proteoform phosphorylated at multiple specific residues. This comprehensive software-supported method enhances the accuracy and efficiency of biopharmaceutical characterization, facilitating improved PTM assessment and unknown identification.

User consent

yes

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