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DIA-HUNTER: Increased plant N-terminome coverage by library-free data independent mass spectrometry

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The N-termini of chloroplast proteins are a common site of co- and post-translational protein modifications, including N-terminal acetylation, transit peptide cleavage and subsequent proteolytic processing, that result in distinct proteoforms that may differ in activity, interactions and location. However, protein N-terminal peptides are often missed in standard shotgun proteomics experiments.
Over the last two decades, multiple enrichment methods have been developed that overcome this problem. Terminal Amine Isotope Labeling of Substrates (TAILS), for example, enabled us to characterize N-terminal determinats of transit peptide cleavage and subequent N-terminal modifications in chloroplasts of both Arabidopsis thaliana and Physcomitrium patens. However, dertermination of sub-stoichiometric changes have often been hampered by poor reproducibility and insufficient sensitivity.
In recent years, data independent acquisition (DIA) methods, combined with advances in mass spectrometry instrumentation and new computational tools, have massively improved the reproducibility and sensitivity of mass spectrometry-based proteomics. We have now established analysis of protein N-terminal peptides enriched by our improved Hypersensitive Undecanal-mediated N-TERmini enrichment (HUNTER) protocol in library-free DIA mode using FragPipe. We evaluated the performance of DIA-HUNTER by rigorous benchmarking to traditional DDA-analysis across multiple instruments. We consistently observe substantial increases in reproducibly quantified N-terminal peptides in DIA-mode compared to DDA-mode, independent of the mass spectrometry system used. This will improve future analysis of dynamic, regulatory N-terminal modifications in plastids.

Primary author: HUESGEN, Pitter (University of Freiburg)

Co-authors: BAETA, Henrique (University of Freiburg); NIEDERMAIER, Stefan (University of Heidelberg); COSENZA-CONTRERAS, Miguel (University of Freiburg); STOCKERT, Fabian (University of Freiburg); HOERNSTEIN, Sebastian (University of Freiburg)

Presenter: HUESGEN, Pitter (University of Freiburg)

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