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Improved intact protein quantitation on an orbitrap fusion tribrid mass spectrometer using multiplexed SIM and PRM techniques

Steven Danielson[1], **Seema Sharma**[1], **Romain Huguet**[1], **Julian Whitelegge**[2] and **Tonya Second**[1]
[1]Thermo Fisher Scientific, USA
[2]University of California, USA

Quantitation of intact proteins provides information unavailable at the peptide level by preserving the molecular integrity of the protein state. Specific proteoforms can be monitored such as unmodified vs. modified forms (post-translational modification and truncation) these different forms may have functional consequences for protein activity. However there are several challenges to intact protein quantitation including loss of sensitivity and dynamic range due to distribution of signal among many charge states, complex highly charged isotope patterns that require high resolution mass spectrometry (MS) beyond the range of most MS instruments to resolve and a lack of MS scan speed to target multiple proteoforms on a chromatographic timescale. Here we describe a method for improved intact protein quantitation using recent advancements in chromatography and detection utilizing the Thermo Scientific™ Orbitrap Fusion Tribrid™ Mass Spectrometer. This very sensitive, fast, accurate mass instrument offers ultra high resolution (450,000 at 200 m/z), this in combination with its ability to optimize pressure of the HCD collision cell results in improved sensitivity and baseline resolved isotopic peaks for intact proteins <50kD. Additionally the Orbitrap Fusion mass spectrometer can multiplex (accumulation of multiple targets including the same species with different charge states in the HCD collision cell) either precursors through single ion monitoring (SIM) scans or by using parallel reaction monitoring (PRM) which targets specific fragment ions. The combination of these enhancements improved the sensitivity and reproducibility of intact proteins quantified including carbonic anhydrase II (2-172 pmol) and other plasma proteins at a flow rate of 0.5 mL/min.

Biography

Steven Danielson completed his PhD (Pharmacology) at the University of California, Davis in 2005 followed by Post-doctoral studies in the labs of Dr. Julie Andersen and Dr. Brad Gibson at the Buck Institute in Novato, CA where he published several papers on mass spectrometry techniques to study Parkinson's disease. He is currently a Demonstration Chemist focusing on LCMS proteomic applications for Thermo Fisher Scientific in San Jose CA.

steven.danielson@thermofisher.com

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