

PW12 Detailed Annotation of Qualitative Differences in Recombinant Protein Samples by Top-Down LC-MALDI-reISD – A QC Exercise.

Matthias Pelzing¹, Leith J. Fremlin¹, Anja Resemann², Detlev Suckau²

1 Bruker Biosciences Pty. Ltd, Parkville, Victoria, Australia

2 Bruker Daltonik, Bremen, Germany

Keywords: Top-Down, LC-MALDI, ABRF

Top down LC-MALDI analysis of heterogeneous recombinant protein batches providing high sequence coverage plus definitive assignment of all termini

Introduction: Detailed characterisation of recombinant proteins including the differentiation of isoforms or structural aberrations is a lot more difficult than the protein ID problem and its routine solution in proteomics workflows. Typically, a method mix is required that almost certainly involves protein separations, top-down (TD) plus bottom-up (BU) sequence characterisation tools.

Methods: The samples were separated by LC, and fractions were collected on AnchorChip MALDI plates. Linear mode protein spectra were obtained using a MALDI-TOF/TOF. Reflector In-Source Decay (reISD) spectra were acquired manually from the fractions containing the main protein components of samples A and B. In addition, LC-MALDI-MS/MS spectra were obtained from Glu-C digested samples to increase sequence coverage.

Preliminary Results: Two batches of an undigested recombinant protein preparation were analysed by LC-MALDI and three different proteins were detected based on retention time and molecular weight. Three different forms of “advanced glycosylation end product-specific receptor isoform” were identified by TD-sequencing using reISD-MALDI. Terminal truncation variants were assigned and fully annotated to sequences from the NCBI-MR95clean protein sequence database + the N-terminal sequence tag as defined in BioTools. LC-MALDI analysis of the Glu-C digest provided 100 % sequence coverage, including the N-terminal peptide that was not amenable to database searching due to the His-tag it contained. TD and BU sequencing together with the TD-LC-MALDI analysis provided 100 % sequence coverage of all three detected protein forms.