Statistical Pattern Recognition for Biotechnological Process Characterization Based on High Resolution Mass Spectrometry

Authors : S. Fröhlich, M. Herold, M. Allmer

Abstract : Early stage quantitative analysis of host cell protein (HCP) variations is challenging yet necessary for comprehensive bioprocess development. High resolution mass spectrometry (HRMS) provides a high-end technology for accurate identification alongside with quantitative information. Hereby we describe a flexible HRMS assay platform to quantify HCPs relevant in microbial expression systems such as E. Coli in both up and downstream development by means of MVDA tools. Cell pellets were lysed and proteins extracted, purified samples not further treated before applying the SMART tryptic digest kit. Peptides separation was optimized using an RP-UHPLC separation platform. HRMS-MSMS analysis was conducted on an Orbitrap Velos Elite applying CID. Quantification was performed label-free taking into account ionization properties and physicochemical peptide similarities. Results were analyzed using SIEVE 2.0 (Thermo Fisher Scientific) and SIMCA (Umetrics AG). The developed HRMS platform was applied to an E. Coli expression set with varying productivity and the corresponding downstream process. Selected HCPs were successfully quantified within the fmol range. Analysing HCP networks based on pattern analysis facilitated low level quantification and enhanced validity. This approach is of high relevance for high-throughput screening experiments during upstream development, e.g. for titer determination, dynamic HCP network analysis or product characterization. Considering the downstream purification process, physicochemical clustering of identified HCPs is of relevance to adjust buffer conditions accordingly. However, the technology provides an innovative approach for label-free MS based quantification relying on statistical pattern analysis and comparison. Absolute quantification based on physicochemical properties and peptide similarity score provides a technological approach without the need of sophisticated sample preparation strategies and is therefore proven to be straightforward, sensitive and highly reproducible in terms of product characterization.