

## **INVESTIGATION OF IRON-LIMITATION INDUCIBLE PROTEINS OF *NEISSERIA MENINGITIDIS* USING THE ISOBARIC LABELING APPROACH TANDEN MASS TAGS (TMT®)**

<sup>1</sup>PRINZ T., <sup>1</sup>KUHN K., <sup>1</sup>LEGLER H., <sup>1</sup>SCHMID P., <sup>1</sup>BAUMANN C., <sup>2</sup>VAN ULSEN P., <sup>2</sup>TOMMASSEN J.

<sup>1</sup>*Proteome Sciences R&D GmbH & Co. Kg, Frankfurt/M/. Germany.* <sup>2</sup>*Department of Molecular Microbiology, Utrecht University, Utrecht, The Netherlands*

Isobaric mass tags have recently been developed for MS-based protein quantitation and identification in complex mixtures. This approach was applied for the investigation of the pathogenic bacterium *Neisseria meningitidis*. This bacterium is known to express additional proteins under iron-limitation. Several key-player proteins, eg. the receptor complexes in the outer membrane for iron-uptake from host proteins, are well-known. For many other proteins the influence of iron availability on their abundance has not been described. Consequently there is potential to find new anti-microbial targets by performing such analysis.

In this study, we analysed the total lysate of cells either grown under iron-restriction or iron-excess. Starting from the cell lysates, the TMTduplex approach was applied in triplicate. After cell lysis, the proteins were subjected to reduction/alkylation of the cystein residues and to proteolytic digestion with trypsin. The peptide mixtures of both cell samples were then labelled with the TMTduplex reagents TMT<sup>2</sup>-126 or TMT<sup>2</sup>-127, combined, fractionated by SCX chromatography and analysed by LC-MS/MS on a Q-TOF instrument. The obtained MS/MS data were subjected to an automated data analysis by SEQUEST, PeptideProphet and ProteinProphet. Quantitative data analysis was performed based on the reporter intensities of the identified peptides. For an independent confirmation of the TMT<sup>®</sup>-reported protein regulation, quantitative Western blots were carried out for several known iron-responsive proteins.

609 proteins in total could be identified and quantified in this investigation, whereby 418 proteins were found in each of the biological replicates. 36 proteins were found with a regulation of .1.25fold (up or down) in at least two of the three experiments.

For six known iron-regulated proteins the TMTduplex data were confirmed by quantitative Western blots.

This study enhances former reported DNA microarray data adding protein abundance results and identifying new iron-regulated proteins in meningococci.