GLOBAL RELATIVE AND ABSOLUTE PROTEIN QUANTITATION IN GRAM POSITIVE BACTERIA

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Introduction. For a comprehensive understanding of physiological processes in a cell, the integration of tailored approaches is needed. Therefore *Bacillus subtilis*, a Gram-positive prokaryote serving as a model organism, was subject to a wide range of transcriptomic, proteomic and metabolomic studies. Unlike transcriptome analysis, proteomic studies do not necessarily cover all possible gene products of the cell. For a long time, the entirety of all proteins represented a far too complex mixture to be analysed at once, due to the large ranges of protein abundance, isoelctric point, molecular weight, solubility or localization. Therefore new methods have to be developed to analyze the proteome of bacteria in a relative and/or absolute quantification manner at a global scale.
The objective of our work is the development and application of methods enabling a comprehensive quantitative proteome analysis of Gram-positive bacteria to gain new insights in regulatory processes and cellular stress response and as a base for systems biology studies.

Methods. For relative protein quantification at a global scale we applied a metabolic labelling approach and investigated cytosolic proteins, two different membrane fractions consisting of transmembrane and membrane-attached proteins (membrane-shaving fraction (MSF) and enriched membrane fraction (EMF)), proteins attached to the cell surface (biotinylation-enrichment fraction (BEF)) and secreted extracellular proteins (extracellular fraction) of *B. subtilis*. (Fig.1)

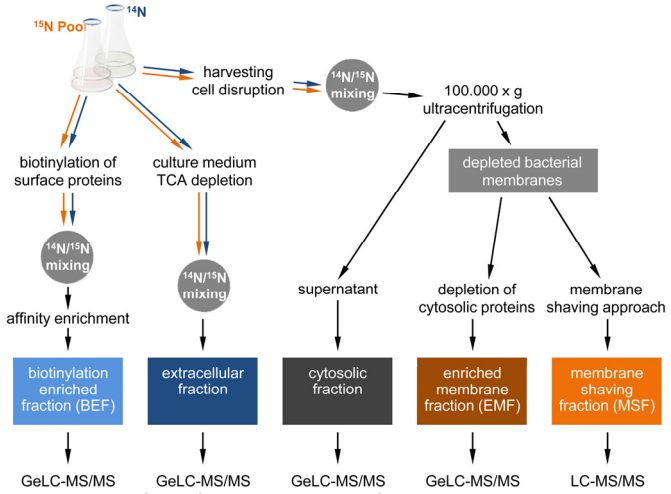

Fig.1. Workflow of a proteome quantification experiment based on metabolic labelling and fractionation.

Absolute protein quantification as a prerequisite for systems biology studies was achieved by integrating targeted mass spectrometric analyses of selected anchor proteins with quantification of all proteins accessible on calibrated two-dimensional gels. The number of accessible proteins was increased by application of label free and gel free quantification approaches.

Results. By application of the described workflow it is possible to include more the 50 % of the predicted proteome of an organism in relative proteome quantification studies. Membrane proteins as well as membrane and surface associated proteins are well represented in such studies (Fig.2).

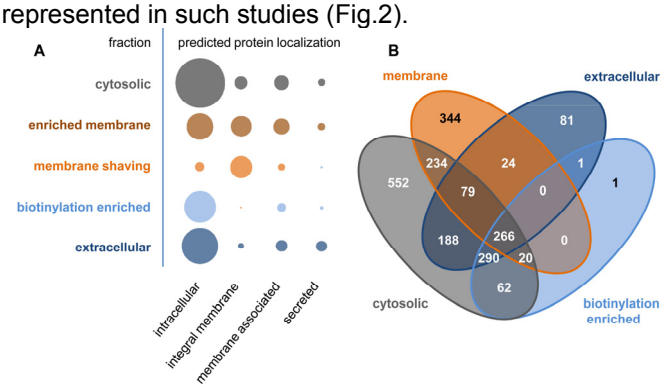

Fig.2 (A) Distribution of proteins identified. The area of the circles is proportional to number of identified proteins. (B) VENN plot of protein identifications in the different designated subcellular fractions.

Combining gel-free and 2D gel based methods we were able to determine the absolute copy number per cell for more than 400 proteins in *B. subtilis*. This number could be significantly increased by application of mass spectrometry based, label free approaches. The pros and cons of these methods will be presented.

Conclusions. With newly developed proteomics approaches comprehensive insights into cellular processes at a global scale are possible. The current state of method development enables the delivery of absolute copy numbers per cell for more than 50% of the cytosolic proteins.

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