BMC Systems Biology

Poster presentation

Towards *quantitative* metabolome analysis

Nadine Stein*, Tina Zeller and Marco Oldiges

Address: Forschungszentrum Juelich GmbH, Juelich Germany

Email: Nadine Stein* - n.stein@fz-juelich.de

* Corresponding author

from BioSysBio 2007: Systems Biology, Bioinformatics and Synthetic Biology
Manchester, UK. 11–13 January 2007

Published: 8 May 2007

BMC Systems Biology 2007, **1**(Suppl 1):P14 doi:10.1186/1752-0509-1-S1-P14

This abstract is available from: http://www.biomedcentral.com/1752-0509/1?issue=S1

Background

Meanwhile mass spectrometry serves as essential methodology for metabolome analysis in bacterial, mammalian and plant systems. Nevertheless, quantitative approaches are complicated to realise, because unknown amounts of metabolites are often lost during sample preparation. Recovery is difficult to acquire due to the lack of "blank" cell extracts. Therefore, methods like standard addition or isotope dilution – where stable isotopes of an analyte are used as internal standards – were introduced to bacterial metabolome analysis. Thus, there is an increasing demand for the quantification of different labelling states of metabolic intermediates. Methods to meet these demands have been developed in our group. They have been applied successfully to the analysis of cell extracts from various biological sources such as bacterial and mammalian cells.

Results

Strong distinctions in metabolite concentrations were found with the quantification strategies applied. For dilutions ranging from 1:2 to 1:20 internal calibration and isotope dilution mass spectrometry were found to be most suitable for the compensation of matrix effects. Further more, the results showed that U13C4-aspartate as internal standard could be used for various amino acids in the chromatographic run. Using isotope dilution, where each metabolite has its own labelled analogue, the linear range of the calibration curve and the coefficient of correlation (up to 0.9999) were improved. The procedure of standard addition was only necessary for very low concentrated metabolites. To avoid this time consuming procedure, samples can as well be concentrated and measured via internal calibration instead. External calibration was sufficient for biological samples in dilutions of 1:20 or higher, because the measurements were hardly influenced by matrix effects.

Materials and methods

In the present study we investigated the impact of the matrix effect based on a comparative analysis applying different quantification methods. Therefore, *E. coli* MG 1655 was cultivated in a bioreactor and analysed in the growth phase. Cells were sampled directly into -50°C cold methanol, disrupted with Chloroform and intracellular metabolites were measured by LC-MS/MS (Figure 1). Investigated quantification methods were standard addition, internal calibration, isotope dilution and external calibration. Internal standards such as U13C4-aspartate and 13C-labelled biomass were introduced either previous to or after cell disruption (Figure 1) to compensate for matrix effects and losses during sample preparation.

Conclusion

Measuring accurate intracellular concentrations is essential for the reflection of metabolic changes after physiological stimuli or genetic modifications of an organism. Especially for anabolic reaction sequences the identification of rate limiting steps is very important to identify possible metabolic engineering targets in production

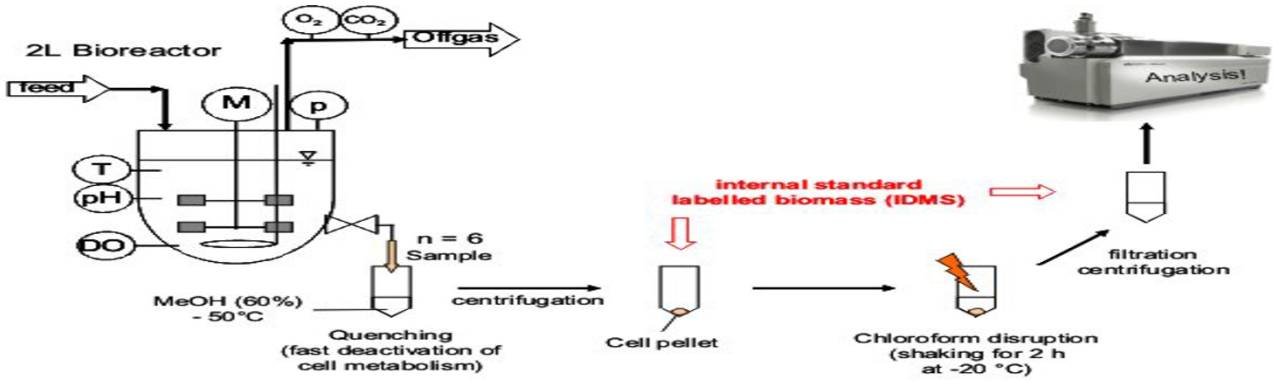

Figure 1
Mechanism for measuring the impact of the matrix effect

processes. Thus, the here presented approach builds the basis for modeling of in vivo enzyme kinetics, metabolic fluxes or thermodynamic states.