Summary

This thesis describes different parts of the analytical workflow during metabolomics analysis and shows two examples of metabolomics applications. The introduction section explains general terms and provides an overview on why metabolomics studies are performed, how those studies can be subdivided, what analytical platforms can be used and what the general advantages and disadvantages of those platforms are. In addition, this section addresses different steps in sample-processing, analysis and subsequent data interpretation.

The following three chapters provide examples of assay development, sample preprocessing steps and automated data processing, including correction for time related bias. The last two chapters provide an example of the application of metabolomics in order to answer different biological questions.

In Chapter 1 we have investigated quenching, a sample-processing step that provides a fast and complete stop of the cellular metabolism during a metabolomics workflow, necessary when intracellular metabolites are to be measured. We provided in the introduction an overview of quenching approaches and their advantages and disadvantages. In this chapter we tested a set of different organic solvent mixtures as quenching solutions for metabolism analysis of Lactococcus lactis. Our goal was to stop L. lactis metabolism by cooling it down to -40°C while preserving membrane integrity. From a long list of tested organic solvents, we ended up with two final quenching solutions candidates. To prove that quenching was accomplished without leaking of intracellular metabolites to extracellular fractions, mass balance analysis was used. Our data showed that a 2-methyl-1-propanol based quenching solution is the best choice for an organic-solvent-based quenching solution, that enables quantitative analysis of 28 intracellular and extracellular metabolites. The key to a successful quenching method turned out to be the solubility of compounds in the water and organic phase at low temperatures, not so much the prevention of leakage itself.

Chapter 2 describes the implementation and validation of three analytical platforms for amino acid analysis. In the introduction section of this chapter we provide a long review of analytical platforms used for amino acid analysis describing their advantages and disadvantages. In the experimental section we provide validation data and a comparison of three analytical platforms: gas chromatography in combination with mass spectrometry, reversed
phase chromatography in combination with mass spectrometry and hydrophilic interaction liquid chromatography (HILIC) coupled to mass spectrometry. During the comparison we discuss issues such as a sample pretreatment, platform performance, the use of $^{13}$C-labeled internal standards or the application of methods to a complex biological sample, and describe the overall advantages and disadvantages of all three platforms. We concluded that all three platforms showed comparable results but some of them showed specific advantages.

The effect of instrumental instability, the so-called instrumental drift, on the quality of metabolomics data, was investigated in Chapter 3. To monitor and remove time dependent bias, we developed an R script that enables automated data detrending and correction based on the quality control sample (QC) values observed over time. We showed that when the instrumental drift takes a place it leads to an overall increase in bias in all analytical data including QC, calibration, and biological data. If such biological data were to be used for metabolite flux calculations, they would lead to unnecessary errors or masking of biological effects. We showed that the R-script that was developed could remove such a bias.

We used metabolomics in Chapter 4 to investigate physiological functions of ATP Binding Cassette Subfamily C Member 2 (ABCC2), one member of the ATP-binding cassette (ABC) transporters family. We developed a new approach to discover new substrates of ABC transporters in which urine extracts were incubated with membrane vesicles containing an ABC transporter, after which the compounds transported into the vesicles were analyzed by LC/MS-based metabolomics. Using this transportomics approach we identified many new substrates of ABCC2 such as glucuronides and sulfo-glucuronides of plant-derived xenobiotics. Moreover, we confirmed using vesicular transport experiments that the excretion of these compounds in vivo depends on ABCC2 activity.

An untargeted metabolomics approach was used to investigate the nitrogen metabolism of Bordetella pertussis, the causative agent of Whooping Cough, in Chapter 5. During the metabolomics workflow, we accelerated the time-consuming identification step by combining LC-MS data after scanning the exometabolome of B. pertussis with comprehensive, genome-scale metabolic reconstructions. We reasoned that genome-scale reconstructions provide a context for interpretation and allow for prioritization of candidate metabolites. The idea behind this was that only those metabolites predicted by the genome-scale model needed to be considered. Using this model-
guided filtering lead to 50% success rate in metabolite identification and enabled us to identify eleven new metabolites involved in nitrogen metabolism of *B. pertussis*.

The **general discussion section** of this thesis contains my personal evaluation per chapter and provides suggestions for future developments. Altogether, the methodologies and topics described in this thesis will hopefully help the metabolomics field move forward.