Vienna BioCenter
CORE FACILITIES


# METABOLOMICS

## WHAT WE DO

Biological research in the post-genomic era has become largely dependent on technologies allowing large-scale analysis of biological systems and processes. One of these approaches, Metabolomics, aims for the comprehensive and quantitative picture of small biomolecules such as sugars, lipids, and nucleic acids in biological samples, which can then be mapped on biochemical pathways. The facility offers targeted and non-targeted quantitative analysis of metabolites and other chemicals.

### Quantification of (D)-2-hydroxyglutaric acid

IDH (isocitrate dehydrogenase) catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate; however, oncogenic mutations in the enzyme instead lead to the generation of (D)-2-hydroxyglutarate (2-HG), an oncometabolite. High levels of (D)-2-HG interfere with the activity of several enzymes, altering the methylation state of histones and DNA. Although 2-HG can be easily identified and quantified via liquid chromatography mass spectrometry (LC-MS/MS), (D)- and (L)-2HG cannot be separated with HILIC or reversed phase chromatography; therefore, only the total amount of 2-HG would be quantified. After derivatization with diacetyl-L-tartaric anhydride, the two enantiomers can be baseline-separated with reversed phase LC and can be now readily quantified. Using this set-up, we quantified the total amount of 2-HG and the relative amounts of both stereoisomers in metabolic extracts.

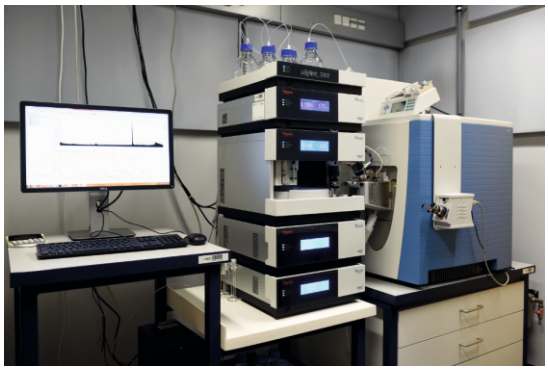
### Characterization of purine metabolism

Purine metabolism is a central element of general physiology in all living species, providing building blocks for DNA and RNA, main components of energy transfer and the involved coenzymes and a plethora of other essential elements of the biochemical activities of cells. Not surprisingly, the metabolic switch associated with oncogenic processes includes alterations of the purine metabolism, required for fast proliferation. Several users have been interested in quantifying components of the purine metabolism in their biological questions and the facility has developed a profound expertise in measuring the products and intermediates of the de novo synthesis and the salvage pathway of purines. Together with our customers, we also employed isotopic labelling techniques to measure the individual contributions and the time resolved dynamic of these pathways after perturbations.

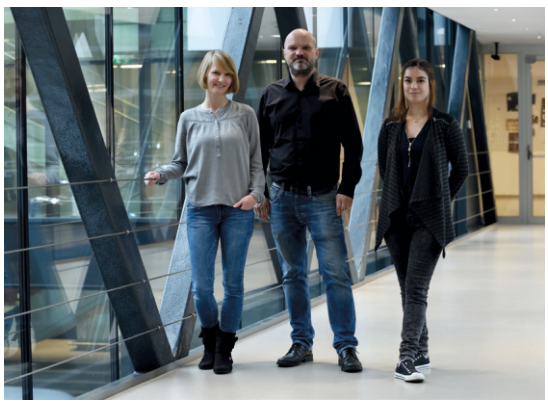
### Relative quantitation of methylated deoxycytidine

DNA methylation primarily occurs on cytosines and contributes, together with the modifications of histones, to the epigenetic regulation of gene transcription. In contrast to NGS-based methods, mass spectrometry provides a cheap way to determine the methylation state of DNA (however without providing sequence-specific information). We adapted a LC-MS/MS method allowing the measurement of both dC and methyl-dC to determine the percentage of global DNA methylation events.

TSQ Quantiva



Q-Exactive



Metabolomics team

## SERVICES AND METHODOLOGIES

- **Targeted LC-MS/MS** We offer the analysis of user-defined compounds in extracts from biological samples by targeted liquid chromatography–tandem mass spectrometry (LC-MS/MS), including the respective methods development. In addition to relative quantification, we also offer absolute quantification of metabolites (if the respective isotopically-labelled compounds are available) or semi-quantitative analysis via external calibration. The approach can also be used to assess the quantitative changes in important biochemical pathways in biological samples by using panels of pre-defined analytes (e.g. energy metabolism).

- **Non-targeted LC-MS/MS or metabolite profiling** Our facility offers non-targeted metabolite profiling, employing high-resolution mass spectrometry. This technique aims for a hypothesis-free description of metabolic differences between samples caused by different genotypes or any perturbations of the system. Using spectral libraries containing tandem mass spectrometry data from known substances, a subset of these compounds can also be identified.

## EQUIPMENT

- **TSQ Quantiva** (Thermo Fisher Scientific) is a state-of-the-art triple quadrupole mass spectrometer, optimal for targeted analysis of small molecules. The mass spectrometer is on-line coupled to an Ultimate 3000 HPLC (Dionex; Thermo Fisher Scientific). This is a bio-inert HPLC, to be operated at micro-flow conditions. A metal-free flow path ensures low binding of biomolecules to surfaces, allowing the analysis of phosphorylated compounds.

- **Q-Exactive** (Thermo Fisher Scientific) is a high-resolution instrument, mainly used for metabolite profiling and analysis of lipids. The mass spectrometer is on-line coupled to an inert Ultimate 3000 HPLC (Dionex; Thermo Fisher Scientific).

## CONTACT AND LOCATION

**Metabolomics**
**Vienna BioCenter Core Facilities (VBCF)**
Dr. Bohr-Gasse 3, 1030 Vienna, Austria
https://www.viennabiocenter.org/facilities/metabolomics/
thomas.koecher@vbcf.ac.at

Vienna BioCenter CORE FACILITIES