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Book of Abstracts

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Registration in Education Center: Sep 9, 18:30-19:30 and Sep 10, 8:00 - 8:45

Sep 9, Sunday

19:30 – 21:00	Welcome dinner reception in the Education Center
21.00 – 21.30	After dinner talk
	I0: Smilde A., Open issues in metabolomics data analysis
Afterwards	Get together at „Clubraum“ of the Education Center

Sep 10, Monday

08:50 - 9:00	Opening of MOVISS: Peter Filzmoser and Tomáš Adam

1) Morning Session: „Shiny design: What is important when designing experiments?“

Chairman: Reza Salek and David Friedecký

09:00 – 09:40	**I1: Neumann S., From profiling to biochemical insights**
09:40 – 10:00	Discussion
10:00 – 10:30	Short Oral Presentations (No. 1 – 5)
	1) Aksenov A., Molecular networking and data sharing as a gateway to new discoveries 2) Coman C., SIMPLEX: from extraction to underlying molecular mechanisms in cardiomyopathy 3) Mádrová L., Mass spectrometric analysis of purine de novo biosynthesis 4) Tengölics R., Method development for non-targeted metabolite profiling of yeast using FIA-HRMS 5) Richter C., Improving the resolution of metabolic flux analysis by tandem mass spectrometry
10:30 – 10:50	Discussion
10:50 – 11:20	Coffee break and poster viewing of oral presentations

11:20 – 11:50	Short Oral Presentations (No. 6 – 10)
	6) Kouřil Š., Removing false features in metabolomics data using correlations 7) Borgsmüller N., Optimization and automation of data processing in GC-MS metabolomics for large-scale processing 8) Buescher J., Fully automated processing of LC-MS and GC-MS data 9) El Abiead Y., Should the choice of your data preprocessing tool depend on your experimental conditions? 10) Najdekr L., Wide detection coverage of the human metabolome using multiple ultra performance liquid chromatography methods – the necessity of multiple separation conditions in untargeted metabolomics experiment
11:50 – 12:10	Discussion
12:10 – 13:00	Discussion (data clinic)
13:00 – 14:00	Lunch

2) Afternoon Session: „Misty mountains: How to find peaks hidden in mists? What is a real peak?"

Chairman: Karl Burgess and Jennifer Kirwan

14:00 – 14:40	**I2: Pluskal T., Reaching the summit: the importance of feature detection**
14:40 – 15:00	Discussion
15:00 – 15:30	Short Oral Presentations (No. 11 – 15)
	11) Reichl B., Multi-omic analysis facilitates a comprehensive characterization of lipid and protein patterns in cerebrospinal fluid from medulloblastoma patients 12) Bianchi F., A metabolomic approach to evaluate the attractant and phagostimulant mechanism of different yeast species to Drosophila suzukii 13) Blackburn G., A small scale study in trained Cyclists: Can trends be used to account for biological variability and push towards individualized metabolomics in personalised medicine? 14) Junghanns S., Stationary 13C MFA with HepG2 cells 15) Love K., Rapid diagnostic approaches for thoracic disease based on pleural effusions.
15:30 – 15:50	Discussion

15:50 – 16:20	Coffee break and poster viewing of oral presentations
16:20 – 16:50	Short Oral Presentations (No. 16 – 20)
	16) Narduzzi L., The CCC approach: a tool for improving confidence in natural compound identification 17) Trautwein C., Shifting, rising and shrinking peaks: Things to consider in 1H NMR-based metabolomics of blood and tissue 18) Piasecka A., Profiling of secondary metabolites related to pattern-triggered immunity in model plant Arabidopsis and evolutionary related species 19) Kokova D., NMR-based exploratory metabolomics study of opisthorchiasis 20) Shipley P., Nuclear Magnetic Resonance Spectroscopy Paired with Chemometric Analysis for the Discrimination, Characterization, and Classification of Highly Related Medicinal Plant Samples
16:50 – 17:10	Discussion
17:30 – 18:30	Short walk to the restaurant
18:30 – 22:00	Dinner

Sep 11, Tuesday

08:30 – 13:00	Hiking
13:00 – 14:00	Lunch

3) Afternoon Session: „Mountain safety: awareness of strengths and weakness of statistical tools"

Chairman: Peter Filzmoser and Age Smilde

15:00 – 15:40	**I3: Beata Walczak – Again about data pre-processing**
15:40 – 16:00	Discussion
16:00 – 16:30	Coffee break and poster viewing of oral presentations
16:30 – 17:10	**I4: Johan Westerhuis – Variable selection and classification in the presence of observation below the detection limit using error rate p-values**
17:10 – 17:30	Discussion
17:30 – 17:50	Short Oral Presentations (No. 21 – 23) 21) Kosmopoulou M., Metabolic Profiling of Human Melanoma during Metastasis 22) Panagiotakis A., Mapping the Metabolic Landscapes of Human Urothelial Bladder Carcinoma 23) Pecinova A., Tissue and plasma metabolomic analysis of DAPIT protein knockout rats – from mitochondria to glucose homeostasis
17:50 – 18:00	Discussion
18:15 – 19:15	Walk to the restaurant
19:30 – 22:00	Dinner

Sep 12, Wednesday

4) Morning Session: „Home run: Results Interpretation – Be creative, critical and think next?"

Chairman: Tomáš Adam and Jennifer Kirwan

09:00 – 09:40	**I5: Royston Goodacre: Mass Appeal: lessons learnt from large-scale mass spectrometry-based metabolomics**
09:40 – 10:20	Discussion
10:20 – 10:45	Short Oral Presentations (No. 24 – 28)
	24) Rusz M., Metabolomics in preclinical studies on metal based anticancer drugs 25) Saw N., Application of generalized additive models in metabolic changes of microbial community in enhanced biological phosphate removal 26) Široká J., Effect of cyclin-dependent kinase 4/6 inhibitors on cellular metabolome regarding selectivity 27) Tripathi A., Intermittent hypoxia and hypercapnia, a hallmark of obstructive sleep apnea, alters the gut microbiome and metabolome 28) Václavík J., Acylcarnitine profile of 3-hydroxy-3-methylglutaryl CoA lyase deficiency patients
10:45 – 11:00	Discussion
11:00 – 11:30	Coffee break and poster viewing of oral presentations
11:30 – 12:30	Discussion (data clinic)
12:30 – 13:00	Closing Moviss 2018
13:00 – 14:00	Lunch

Sunday, Sep. 9

The workshop will start with a welcome reception (warm buffett) at 19:30 in the rooms of the Education Center of the monastery of Vorau. Afterwards, at 21:00, Professor Age Smilde will give an "After Dinner Talk" with inspiring thoughts to the topic of the workshop. Discussions of the various open problems can then start at the "Clubraum" of the Education Center, which is open all day and night for the participants.

Monday, Sep. 10

At 17:30 we will start to walk to the restaurant Brennerwirt. We meet in front of the monastery, but people can also join the group from their hotels which are on our way. We walk around 3 km. Optionally, a bus service is available - please inform the organizers if you want to use this service. Also for going back, a bus service is available (and even recommended). After the dinner, some surprise will wait for you.

Tuesday, Sep. 11

We start at 8:30 with our hiking tour. Meeting point is in front of the monastery. The tour will depend on the whether conditions. In case of appropriate whether we will walk to a village called Pöllauberg, and the name already indicates that this will be on a mountain. It is recommended that you bring hiking shoes or at least some sports shoes. Also some warmer clothes could be advisable. The hiking tour will not be too demanding, and the plan is to be back at the monastery in Vorau at latest at 13:00, where lunch will wait for us. We will have one bigger and 3 smaller shuttle buses, which gives us the flexibility to pick up tired people on the way. After lunch there will be some time to relax until the scientific program starts again at 15:00.

In the evening we are ready for new adventures, and thus we meet at 18:15 in front of the monastery to walk to Mostschank Kuchlbauer. People can join the group from their hotels where we pass by. We walk around 3 km. Optionally, a bus service is available - please inform the organizers if you want to use this service. Also for going back, a bus service is available. Mostschank means that they have apple sider and products which they produce at the farm. Kuchlbauer's Most is excellent, and they always get awards for the good quality.

Invited Speakers

Age K. Smilde

Steffen Neumann

Tomáš Pluskal

Beata Walczak

Johan Westerhuis

Royston Goodacre

I0: Age K. Smilde

Open issues in metabolomics data analysis

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We have accomplished a lot in metabolomics data analysis as a community. We have many methods available to tackle metabolomics data and also data preprocessing tools are starting to become mature. Yet, it is also worthwhile to consider open issues since this gives avenues for future research and development thereby raising the quality of our work. I will discuss and illustrate some of these open issues such as study design, permissible statistics, measurement error correlation, cohort transfer and power.

I1: Steffen Neumann

From profiling to biochemical insights

Department of Stress and Developmental Biology
Leibniz Institute for Plant Biochemistry, Halle, Germany

Mass spectrometry is an important analytical technology in metabolite profiling. The first steps of the analysis pipeline for MS measurements are signal processing and peak picking tasks. We are actively developing and enhancing the open-source software XCMS and related bioconductor packages.

The next step in bioinformatics is the statistical analysis, checking e.g. for significant differences between samples even in the presence of high biological variation. This can reveal „interesting" features, and subsequent tandem MS experiments provide powerful structural hints for the elucidation of these unknown mass spectral features.

Using data from modern mass spectrometers it is possible to combine both steps with our MetFamily tool, and seamlessly navigate between statistical analysis and structural characterisation of metabolites.

I2: Tomáš Pluskal

Reaching the summit: the importance of feature detection

Whitehead Institute for Biomedical Research, Cambridge, USA

Feature detection is typically the first step of data processing in liquid chromatography mass spectrometry (LC-MS)-based metabolomic studies. Although downstream statistical analyses rely on precise identification and integration of peaks (features), the importance of this step is often underappreciated in practice. I will present an overview of various existing approaches to feature detection in high-resolution LC-MS data based on extracted ion chromatograms, wavelet transform algorithms, Kalman filters, and others. All methods have their potential strengths and pitfalls, but none of them can produce optimal results without parameter optimization, which should ideally be done systematically using a design-of-experiment approach. Since benchmarking feature detection algorithm is a non-trivial problem by itself, I will outline potential approaches to this problem. Finally, I will discuss possible future developments towards more intelligent methods for feature detection.

I3: Beata Walczak

Again about data pre-processing

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Each experimental research involves many steps and each step essentially influences final result. Such steps as, e.g., experimental design, data analysis and its validation are supported by the established knowledge and the well defined rules. There are also the rules concerning sample collection and preparation, as well as a 'good practice' concerning measurements. Unfortunately, situation is quite different, when data pre-processing is considered. This step is data- and goal-dependent and as such, it requires a unique treatment, well suited for the data at hand. Nowadays, pre-processing is the most time consuming and challenging step. It aims at elimination of an undesired data variance associated with data noise, background and shifts, as well as at elimination of size effect (present due to an unknown overall sample concentration) and differences of contributions of the studied variables to the total data variance. There are many available methods of data pre-processing, but they cannot be treated as alternative approaches. Their assumptions and principles should be taken into the account and their choice should be based on data characteristics.

This presentation aims at outlining important problems of data pre-processing and their illustration upon the real and simulated data sets. Some rules for data pre-processing will be discussed and few diagnostics tools for data characterization will be demonstrated.

Special attention will be given to the data normalization step, particularly important in the metabolomics studies. Recently, a new approach to normalization of metabolomics data, based on Compositional Data Analysis (CODA) is popularized (1). It consists of an attractive idea of working with the log ratios, thus eliminating data normalization step. CODA was tested in our previous simulation study and its performance was compared with Total Sum Normalization, Probabilistic Quotient Normalization and Pair-wise Log Ratios, which showed that the CODA transformations should not be applied to identify biomarkers (2). These conclusions do not coincide with that presented in (1). As stated in (1), the discrepancy was probably caused by a limited number of variables considered in our study. Thus we undertook a new simulation study, working with the data sets of much higher numbers of variables. An additional motivation appeared, when we realized that there was another question to be answered. Namely, it was observed that the Pair-wise Log Ratio (plr) method performed well (2), whereas CODA, based on a similar concept, did not. The presented results allow an explanation of this apparent contradiction in terms of local and global characteristics of the features transformed by plr and clr, respectively.

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References: 1. A. Gardlo, A. K. Smilde, K. Hron, M. Hrda, R. Karlıkova, D. Friedecky, T. Adam, Normalization techniques for PARAFAC modeling of urine metabolomic data, Metabolomics, 12, 117; (2016); 2. P. Filzmoser, B. Walczak, What can go wrong at the data normalization step for identification of biomarkers?, Journal of Chromatography A 1362, 194-205 (2014)

I4: Johan Westerhuis

Variable selection and classification in the presence of observation below the detection limit using error rate p-values

Mari van Reenen[3], Johan A Westerhuis[1,3], Carolus J Reinecke[3], J Hendrik Venter[2]

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[2]Centre for Business Mathematics and Informatics, Faculty of Natural Sciences, North-West University (Potchefstroom Campus), Private Bag X6001, Potchefstroom, South Africa.
[3]Centre for Human Metabolomics, Faculty of Natural Sciences, North-West University (Potchefstroom Campus), Private Bag X6001, Potchefstroom, South Africa

We introduce an approach using minimum classification error rates as test statistics to find discriminatory variables. The thresholds resulting in the minimum error rates can be used to classify new subjects. This approach transforms error rates into p-values and is referred to as ERp. ERp can handle unequal and small group sizes, as well as account for the cost of misclassification. In metabolomics studies, often many values below the detection limit (indicated by zero's in the data table) are observed. We extended ERp (to XERp) to address two sources of zero-valued observations: (i) zeros reflecting the complete absence of a metabolite from a sample (true zeros); and (ii) zeros reflecting a measurement below the detection limit. XERp is able to identify variables that discriminate between two groups by extracting information from the difference in the proportion of zeros and shifts in the distributions of the non-zero observations simultaneously. To demonstrate the utility of XERp, it is applied to GC-MS data from a metabolomics study on tuberculosis meningitis in infants and children. We find that XERp is able to provide an informative shortlist of discriminatory variables, while attaining satisfactory classification accuracy for new subjects in a leave-one-out cross-validation context.

I5: Royston Goodacre

Mass Appeal: lessons learnt from large-scale mass spectrometry-based metabolomics

School of Chemistry, Manchester Institute of Biotechnology
University of Manchester, Manchester, UK

Metabolomics is a growing discipline that allows the analysis of the thousands of structural different small molecules found within a biological system. These metabolites can be measured using a variety of different analytical approaches and at Manchester we have developed GC-MS and LC-MS (Dunn et al., 2011). I shall provide an overview of metabolomics and lessons learnt from of our large-scale human serum metabolome project (www.husermet.org) where we profiled 1200 healthy individuals (Dunn et al., 2015). Using these protocols we then went on to profile another ~1200 ageing individuals and identified key Metabolic dysregulation which were drivers behind human frailty (Rattray N.J. et al., under review).

Key aspects for discussion will be based around a SWOT analysis of the field.

Dunn, W.B., et al. (2011) Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols 6, 1060-1083.

Dunn, W.B., et al. (2015) Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics 11, 9-26.

Short oral presentations

O1: Molecular networking and data sharing as a gateway to new discoveries

K Alexander A. Aksenov[1], Robert Quinn[1], Mingxun Wang[1], Louis-Félix Nothias[1], Alexey V. Melnik[1], Pieter C. Dorrestein[1]

[1]*Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093, United States*

Mass spectrometry (MS)-based metabolomics is going through a renaissance. A flurry of new developments, especially creation of new tools for data analysis and interpretation has spurred new discoveries that were not possible in preceding better part of the century the MS has been around. Also, there is an increasing understanding within the MS user community that the openness in science in general and metabolomics in particular, including sharing of data, libraries and depositories, is a key ingredient necessary for further progress1. Public sharing enables better use of the data as some researcher may try their analysis tools that were not available to those who originally collected the data, or re-use of previously analyzed and published datasets can provide new insight, or, simply, an increasing sample size can be possible by combining multiple datasets. The Global Natural Product Social Molecular Networking (GNPS)2 platform at UCSD is one of the mass spectrometry "social networking" platforms which enables users to deposit and analyze their data. GNPS promotes the open access model by allowing the storage of full raw mass spectrometry data and upgrading the collaborative model to the crowdsourcing model, where community-contributed data are constantly evaluated by peer scientists, creating a virtuous cycle of improvement. GNPS provides a variety of tools beyond simple compound annotation, in particular molecular networking – an ability to represent the similarities in tandem mass spectra as networks. This enables much deeper understanding of the underlying chemical relationships. Here we demonstrate few recent examples of applications of molecular networking and use of public data that enabled discoveries of novel chemistries: N-acyl amides, the new bacterial compounds that are used by microbes to manipulate metabolism of the host; bacterially-made bile acids, only discovered recently despite more than a century of active research in this area; and novel mechanism of tobramycin antibiotic inactivation via propionylation by Pseudomonas aeruginosa in cystic fibrosis patient's lungs. Currently we installing a tagging functionality that enables users to indicate origin and importance of discovered molecules, among other things. Feedback from user community that now counts over 25,000 would be essential in guiding how to maximize the utility of this functionality to maximize the amount of knowledge that can be gained from the data.

References

1. Aksenov, A. A., da Silva, R., Knight, R., Lopes, N. P. & Dorrestein, P. C. Global chemical analysis of biology by mass spectrometry. 1, 54 (2017).

2. Wang, M. et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat. Biotechnol. 34, 828–837 (2016).

O2: SIMPLEX: from extraction to underlying molecular mechanisms in cardiomyopathy

Cristina Coman[1], Andreas Roos[1], Evelyn Rampler[2], Gunda Koellensperger[2], Robert Ahrends[1]

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Despite significant therapeutic advances, there is no unified, complete mechanism explaining how exactly various cardiomyopathies biochemically manifest and progress nor explaining why this cardiac conditions result in heart failure. "Caveolinopathies" are caused by mutations in the sarcolemmal protein Caveolin-3 (CAV3) and can manifest as myopathic diseases often associated with cardiomyopathy. To gain deeper insights into the underlying molecular mechanisms, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, the previously established SIMPLEX procedure (SImultaneous Metabolite, Protein, Lipid EXtraction) (1) was applied to examine the molecular basis of an inherited dilative cardiomyopathy from a transgenic mouse model carrying a dominant mutation (P104L) in CAV3 (2).

In brief, the cardiac tissue was incubated with cold MeOH, MTBE was added and water was utilized to induce phase separation. The individual fractions containing lipids (top phase), metabolites (lower phase) and proteins (pellet) were then subjected to individual mass spectrometry based workflows. The application of the SIMPLEX workflow pinpointed multiple molecular mechanisms involved in the pathogenesis of functional CAV3-deficiency. In order to maintain the protein homeostasis, the mutant protein is excessively degraded while the integrity of the cellular membranes and cytoskeleton are affected. The increased oxidative stress burden upon P104L mutant CAV3 expression was directly reflected by the altered cardiolipin and plasmalogen levels. Similar trends were observed for TAG species along with a downregulation of glycolytic and TCA cycle intermediates and an accumulation of acylcarnitines. Protein analysis corroborated these findings and indicates a change in substrate utilization as an attempt of the heart to maintain a normal cardiac function. The interconnection provided by the lipidomics, metabolomics and proteomics data revealed the cardiomyopathic phenotype as a severe metabolic disease, not only as a structural one.

References

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2. Ohsawa, *et al*. Overexpression of P104L mutant caveolin-3 in mice develops hypertrophic cardiomyopathy with enhanced contractility in association with increased endothelial nitric oxide synthase activity. *Hum. Mol. Genet*. 13, 151-7 (2004).

O3: Mass spectrometric analysis of purine *de novo* biosynthesis

Lucie Mádrová[1,2], Matyáš Krijt[3], Veronika Barešová[3], Jan Václavík[1,2], David Friedecký[1,2], Dana Dobešová[1], Olga Součková[3], Václava Škopová[3], Marie Zikánová[3], Tomáš Adam[1,2]

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Purine nucleotides have vital functions in nucleic acid synthesis, energetic homeostasis, cell signalling and others in both prokaryotes and eukaryotes. Supply of purines is provided by two pathways – salvage pathway and *de novo* synthesis (PDNS). Although PDNS activity varies during cell cycle, it becomes an important source of purines especially for rapidly dividing cells. Method for detail studying of PDNS is missing due to analytical reasons (sensitivity) and commercial unavailability of the compounds.

The aim was to fully describe mass spectrometric fragmentation behaviour of newly synthesized PDNS related metabolites and build up analytical method. With the exception of four initial ribotide PDNS intermediates that preferred losing water, phosphate or cleave forming base of purine ring, all the other metabolites studied cleaved the glycosidic bond in the first fragmentation stage. Fragmentation was possible to 3^{th}-6^{th} stages. Liquid-chromatography-high resolution mass spectrometric method was developed and applied in the analysis of CRISPR-Cas9 genome-edited HeLa cells deficient in individual steps of PDNS and salvage pathway. Identity of newly synthesized intermediates forming under pathological conditions of known and theoretically possible defects of PDNS was confirmed by comparing fragmentation patterns of synthesized metabolites of PDNS with those produced by cells. Use of stable isotope incorporation allowed confirmation of fragmentation mechanisms and provide data for future fluxomic experiments. The method may find its use in diagnosing of PDNS disorders, investigation of purinosome formation, cancer research, enzyme inhibition studies and other applications.

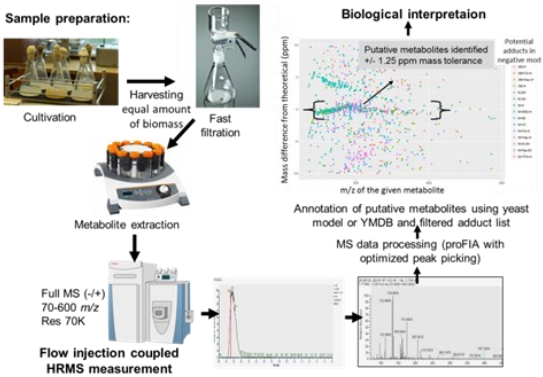
Supported by the MEYSCR LO1304, the MHCR (AZV 15-28979A), programmes PRIMUS/17/MED/6 and by IGA_LF_2018_010.

O4: Method development for non-targeted metabolite profiling of yeast using FIA-HRMS

Roland Tengölics[1], João B. Mokochinski [1,2], Balázs Szappanos[1], Dorottya Kalapis[1], Ivett Török[1], Stefániai Erdei[1], Szilvia Z. Tóth[2], Balázs Papp[1]

[1] *Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged Hungary*
[2] *Biological Research Centre of the Hungarian Academy of Sciences, Institute of Plant Biology, Szeged Hungary*

Metabolite levels are especially important links between genotype and complex phenotypes. Budding yeast (*S. cerevisiae*) is arguably a key eukaryotic model organism for studying metabolism both *in silico* and *in vivo* as well. Hence, there's a high demand to develop a fast and easy-to-operate global metabololite profiling method.



LC-MS based methods give high metabolome coverage and sensitivity, although in case of large sample batches system and sample instability could strongly bias the results.large sample sizes and sample instability could strongly bias the results. Therefore we aimed to optimize a comprehensive flow-injection fingerprinting workflow to provide a global overview of all the ionized intracellular metabolites of yeasts in a short time.

S. cerevisiae genotypes were cultivated in liquid minimal media. Equal amount of biomass is harvested by fast filtration and immediately placed to extraction solution (40:40:20 ACN:MetOH:H2O) and frozen in liquid nitrogen. Yeast material was extracted by using 10 minutes vortexing at 4C°. For data acquisition, an UHPLC was coupled with a Thermo Q-Exactive Focus HRMS. The mobile phase for positive and negative mode consists of H2O 0.1% Acetic acid:ACN (20:80 v/v)and H2O 0.2mM NH_4F:MetOH (20:80 v/v), respectively. In both ionisation modes, the system was operated in isocratic mode at a constant flow rate (0.2 mL/min). Mass spectra were acquired in full scan mode from 70 - 600 m/z mass range at 70K resolution. Data were processed using the Pro-FIA R toolbox and compounds were annotated using Yeast metabolome database and a strict (+/- 1.25 ppm) mass tolerance. This method enabled us to identify metabolic differences between closely related yeast strains and to study the metabolic signatures of yeast domestication.

The work was supported by GINOP-2.3.2-15-2016-00014; GINOP-2.3.2-15-2016-00026 and Welcomme Trust 098016/Z/11/Z

O5: Improving the resolution of metabolic flux analysis by tandem mass spectrometry

Chris Richter, Eva Grafahrend-Belau, Björn Junker

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GC-MS is usually the source for isotope labeling data used for ^{13}C Metabolic Flux Analysis (MFA). Unlike NMR, MS data only partially resolves positional labeling by analyte fragmentation, which does not result in full isotopomer distributions. Tandem-MS has been applied to overcome this problem, however, due to technical restrictions and poor availability of labeled standards the improvement of the data situation so far was limited.

By a combination of a fully labeled amino acid (AA) standards, high-resolution tandem mass spectrometry and chemical considerations, we identified 30 new MS/MS fragments that allow to obtain the full isotopomer distribution of all AA with up to 4 carbons.

For all other non-cyclic AA, the data situation was significantly improved over the single-MS situation.

We are currently testing how this new data situation improves the accuracy of ^{13}C-MFA.

We suggest that in the MFA field, the major workhorse GC-MS should be replaced by GC-MS/MS in the near future.

O6: Removing false features in metabolomics data using correlations

Štěpán Kouřil[1,2], Julie Rendlová[1,3], David Friedecký[1,2], Tomáš Adam[1,2]

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Untargeted LC-HRMS analysis produces a large number of features (measured ions with unique *m/z* and retention time) which corresponds to the potential compounds in the analyzed sample. During the data processing, it is necessary to merge associated features belonging to one metabolite to prevent multiplicities and possible misidentification. There are many LC-MS processing tools available (open source – XCMS and CAMERA [1], MZmine2 [2], vendor software Compound Discoverer and many others). All of them use complex algorithms to merge features belonging to one compound like isotopic peaks, adducts and multiply charged features but none of them can do it without mistakes and none can deal with fragments formed in ion source during the analysis. There are some independent post-processing tools, which can improve the merging of associated features like Ion Fusion [3] or MS-FLO [4] – however, they cannot detect fragments yet. Our approach is more comprehensive, revealing all correlated features in the data. It combines graphical tool called correlation network with Pearson's pairwise correlations and retention time range to group all required features to one compound and then uses the largest sample mean of the peak area as a reference. We assume that some biological correlated compounds will be also accidentally merged during the process – but it will not affect further statistical analysis considerably since it is rather rare. The new approach was tested on the metabolomics data from patients with Phenylketonuria and healthy controls. Twenty percent of features, which could be falsely considered as potential biomarkers, were removed using this tool. A slightly modified version of the tool can be also used to reveal biological correlations in the sample with the aim to find out relationships between metabolites as well as to help to reveal new biomarkers correlated with the known ones.

References

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O7: Optimization and automation of data processing in GC-MS metabolomics for large-scale processing

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High false positive rates in GC-MS metabolomics peak detection is a common issue that impedes automated analysis of large-scale data. There is a growing need for improving the reliability and scalability of data analysis workflows. Many algorithms are available for peak detection, a crucial step for the data analysis, but performance and outcome can differ widely depending on both algorithmic approach and data acquisition method. This makes it difficult to compare and contrast between algorithms without extensive manual intervention. We present a parameter optimized, multi-algorithm peak detection workflow for GC-MS metabolomics, which automatically evaluates the quality of detected peaks using machine learning-based classification. First, the classifier is trained to distinguish between real compound related peaks and false positive peaks. Then the algorithm parameters are scored based on the quality of detected peaks and optimized accordingly. This procedure is repeated for two peak detection algorithms and subsequently both algorithms are run in parallel on the entire data set with the optimized parameters. The qualitative information returned by the classifier for every peak is then used to merge individual algorithm results into one final high confidence peak set. Using this approach we show that automated detection and evaluation of peak quality is possible with an average sensitivity of 90% on a standard compound mixture. The additional quantitative and qualitative information generated by the classifier allows:

i) optimization of individual peak detection algorithms parameters to increase sensitivity and specificity, ii) an objective way to assess peak detection algorithm performance, iii) optional exclusion of peaks which are less robustly defined as true peaks based on class membership probability. We demonstrate that this workflow automatically recovers over 90% of the compounds identified in biological data by manual curation.

The modular design allows extension, adjustment and improvement of the workflow using different or additional peak detection algorithms and classifiers. Importantly, due to the fully automated implementation, the workflow is suitable for large-scale studies.

O8: Fully automated processing of LC-MS and GC-MS data

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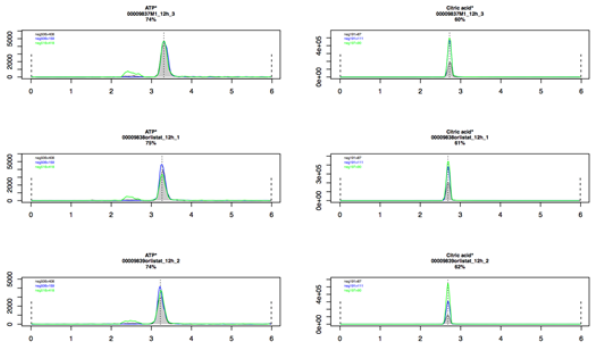
The capacity to generate metabolomics data exceeds the capacity to process this very data in many laboratories. This is particularly true for core facilities that operate multiple analytical pipelines. In order to best utilize the capacity of our machines and to enable fast delivery of results despite very limited manpower we implemented a fully automated data processing pipeline that reads raw data and metadata from our in-house sample database and generates outputs in excel and pdf format that are readable by life scientists.

We store both raw data and user-provided metadata in an SQL database. Users can select samples and start their processing online. In a first step, we use ProteoWizard(1) to convert data in vendor's format to .mzML. Next an R script is triggered that reads the raw data, identifies, integrates, and scores chromatographic peaks. This step is highly specific to the employed chromatographic method. For example, for data that was generated by HILIC chromatography, large deviations from the expected retention time must be allowed, whereas data generated by gas chromatography yields highly reproducible retention times. Only peaks that pass a strict multi-step scoring process are then reported in Excel format and used for statistical and graphical outputs. In our experience this automatically generated output suffices for many experiments. In addition, expert-supervised "manual" peak integration can still be used to report data on peaks that were filtered out in the automatic pipeline.



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O9: Should the choice of your data preprocessing tool depend on your experimental conditions?

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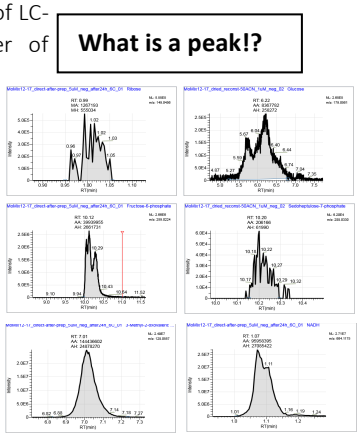
With the increasing number of people publishing in the field of LC-MS facilitated untargeted metabolomics also the number of software tools for data preprocessing rose within the last decade. Comparisons between those tools are often only looking at the number of peaks being detected neglecting detected peak boundaries[1] or focus on a specific chromatographic conditions[2]. However, employed chromatographic conditions as well as well as employed mass spectrometer scan rates can heavily impact on observed peak shapes. Moreover, the threshold of what needs to be detected as a peak might depend on the research question. This will naturally lead to different demands for used data preprocessing tools – especially at the step of peak detection.



We will evaluate the performance of different peak detection algorithms by measuring a multi component standard including more than 80 compounds utilizing different experimental setups. All acquired data will then be inspected for expected analyte peaks in a targeted manner. Moreover peak integration will be manually curated. The comparison between the untargeted and the targeted results will then be carried out automatically by in-house developed Microsoft Excel macros.

Targeted and untargeted results will be compared with regard to peak picking/detection and peak area. Finally we will show how the choice of software tools would impact on the output of an untargeted metabolomics study displaying different scenarios regarding peak picking.

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3. Chromatograms in the picture were generated using Tracefinder 4.1 (Thermo Fisher Scientific)

O10: Wide detection coverage of the human metabolome using multiple ultra performance liquid chromatography methods – the necessity of multiple separation conditions in untargeted metabolomics experiment

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Analysis of complex human biological matrices is still a challenge in the field of metabolomics. Biofluids including plasma and urine as well as tissue extracts are highly complex and to build the whole biological picture, multiple analytical methods are typically necessary. Ideally, the best option is to apply multiple and several different analytical techniques for individual classes/groups of metabolites. Unfortunately, we do not always have the luxury of having enough sample material or finances available to apply several different analytical techniques. In this presentation, I will introduce the concept and importance of complementary high throughput UPLC-MS methods for untargeted metabolomics experiments. Special focus will be provided on the chromatographic separation of water-soluble metabolites using HILIC separation techniques including the importance of mobile phases, modifiers and sample reconstitution.

O11: Multi-omic analysis facilitates a comprehensive characterization of lipid and protein patterns in cerebrospinal fluid from medulloblastoma patients

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Lipids are a highly diverse class of biomolecules, which play many essential roles in cellular functions. These compounds received growing attention in recent years due to their correlation with several diseases, such as cardiovascular disease, inflammatory diseases, neurological disorders, cancer and many more [1]. Phosphatidylcholines are the most abundant phospholipids in mammalian cell membranes. Hence, diseases that affect membrane integrity or lead to a high demand of membrane constituents (for example, during tumor cell proliferation) are supposed to be reflected by the pattern of phosphatidylcholines in body fluids. Eicosanoids, derived from polyunsaturated fatty acids, are key signaling molecules, playing important roles for example in immune response.

In the present study, an integrative characterization of lipid and protein patterns in cerebrospinal fluid from pediatric patients suffering from medulloblastoma, the most common malignant pediatric brain tumor, was accomplished. The presented poster will give an overview on the analytical approaches and exemplary results from this study.

Reference

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This work was conducted under the frame of „Programm Interreg ATCZ52 Österreich – Tschechische Republik: Infrastruktur für Metabolomik-Forschung und Klinische Chemie“.

O12: A metabolomic approach to evaluate the attractant and phagostimulant mechanism of different yeast species to *Drosophila suzukii*

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Since 2011 the invasive species *Drosophila suzukii* has affected the cultivation of soft and stone fruits in South Tyrol causing important harvest losses. Up until today the chemical control available is not enough efficient in controlling the pest. Yeasts have been shown to influence the fitness and be a primary food source in the nutrition of many Drosophila species [1,2]. The project Dromytal has the aim of developing an "attract&kill" formulation as a control strategy against *Drosophila suzukii* based on the association of an attractant yeast and a last generation insecticide. The yeasts applicated on the plant surface should not only attract the insect, but also favor phagostimulant activity in the flies to promote the insecticide ingestion on the targeted treated surfaces enhancing insect mortality. For the experimental design, nutritionally relevant yeast species have been chosen based on previous studies [1]. Yeast cultures were inoculated in liquid growth media for 30 hours. After quenching, intracellular metabolites were extracted. To yield a wide-ranging view of metabolic differences between the various yeast species, an untargeted metabolomic analysis was performed. Hydrophilic interaction liquid chromatography–quadrupole/time-of-flight mass spectrometry (HILIC-QTOFMS) was used to detect diverse chemical compound classes. Overall, more than a hundred distinct metabolites have been annotated and/or identified. PLSDA analysis showed a discrimination between the four different yeast species on the first two components. ChemRICH [3] analysis allowed to show the significantly impacted metabolite clusters between the species by chemical similarity and ontology mapping.In summary, we found significant global metabolic alterations in the different yeast species and were able to detect significantly altered metabolite classes.

The project Dromytal is supported by the European Regional Development Fund 1021 CUP: H32F16000420009

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O13: A small scale study in trained Cyclists: Can trends be used to account for biological variability and push towards individualized metabolomics in personalised medicine?

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Inherent variation in metabolites across a population can render small scale studies useless when attempting to look for reproducible changes in single data-points. To combat this most researchers will increase the power of their experiment by including more samples across the different groups under investigation[1]. For large studies this approach works well, but it can be prohibitive for small scale studies, such as pilot data generation or student projects, where access to even relatively small numbers of subjects may be difficult and only small amounts of funding may be available.

When changes are expected to occur over time, looking for trends in metabolites across individual subjects provides a way to eliminate the need for a greater power within the experiment. While individual metabolites may vary widely between individuals, the change in these metabolites over time may be expected to be similar for all subjects. This reduces the variability, lowering the number of subjects required to produce meaningful data.

A small scale study involving 7 fasted, trained cyclists was carried out to generate both metabolomics and physiological data sets, including lab based measurements of lactate and respiratory exchange ratio (RER). Briefly, the cyclists were fasted for 12 hours, inserted with a cannula and exercised on a stationary bike at ~70% maximum exertion for 1 hour, ~50% maximum exertion for 1 hour and were kept at rest for 2 hours. Breath and blood samples were taken every 15 minutes to produce the physiological data and a subset of these samples were processed for metabolic analysis.

On analysis of the data it was found that, while variation between subjects was high for individual metabolites at single time-points, trends over time were observed that closely matched the expected changes in metabolism for the protocol. The data were then further investigated using linear models to determine if other metabolites exhibited time-trends across different subjects.

O14: Stationary 13C MFA with HepG2 cells

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In drug development 30% failure are due to toxicity, majorly hepatotoxicity[1]. Hepatotoxicity has one of the poorest correlations to regulatory animal studies[2]. HepG2 cells however have 80% sensitivity for detection of hepatotoxicity and 90% specificity[3]. ^{13}C Metabolic Flux Analysis is a method to quantify intra- and extracellular fluxes and map the metabolism of organisms[4]. Therefore, it might be a useful method to detect hepatotoxicity.

We performed metabolic and isotopic stationary ^{13}C-MFA with HepG2 cells. To get the basic computational model, some experimental data needed to be integrated, which included biomass composition, uptake and secretion rates of amino acids and lactate. For the label experiment the cells were fed with differently labeled glucose, like U^{13}C-Glucose and 1^{13}C-Glucose. Amino acids, intermediates, RNA and Glycogen were extracted after seven days of cultivation and measured via GC/MS. The evaluated label data was compared to the data of the label predictions of the computational model. Based on this data the model was corrected until the best fit with the experimental data was achieved. The resulting flux maps visualize quantitative carbon fluxes in liver cells.

The next step is to test different substances, predict the changes in the metabolic flux of the liver and subsequently liver toxicity. With this hepatotoxicity cases in clinical trials could be reduced to a minimun.

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O15: Rapid diagnostic approaches for thoracic disease based on pleural effusions

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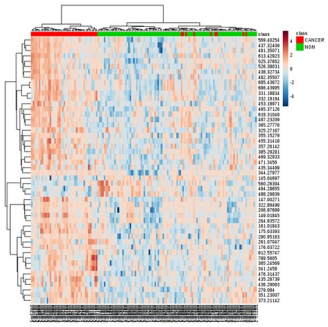
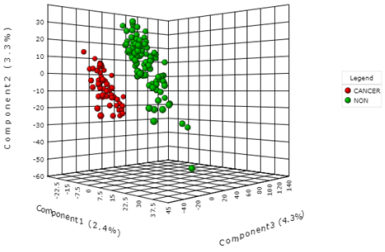


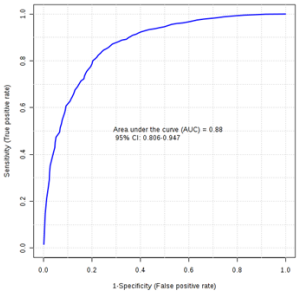
Around 71% of the world's population will die prematurely this year due to non-communicable diseases (NCDs), which are health conditions not caused by infection. Cardiovascular disease (CVD) causes 17.9 million of these deaths a year, followed by cancer with 9.0 million.

Thoracic diseases such as CVD, lung cancer and mesothelioma, a massively aggressive and invasive cancer, with a 5yr survival rate of only 5-10% represent major threats to public heath globally; particularly with aging populations, increasing obesity, wide-spread smoking and pollution in some areas. To address the challenges posed by such diseases, robust and cost-effective tests, which allow their rapid and early diagnosis, are required.



My project aims to develop such tests based on biomarkers derived following metabolomic assessments of differences between cancers and non-cancers, such as CVD. They all share initial symptoms; coughing, chest pain, breathlessness and present 'pleural effusions', which is fluid accumulation on the lungs.

Using untargeted Flow-infusion mass-spectrometry (FI-MS) on pleural fluid footprints, and cellular debris from 100 patients from a biobank, statistical analysis shows clustering, along with distinct differences between clinically relevant disease states. These results suggest the validity of the metabolomic approach to the diagnosis of thoracic disease. This data can now be both validated, using a larger sample set, including double blind trails and to further confirm the identity of the significant masses using targeted MS.



O16: The CCC approach: a tool for improving confidence in natural compound identification

Luca Narduzzi[1], Jan Stanstrup[1], Fulvio Mattivi[1] & Pietro Franceschi[1]

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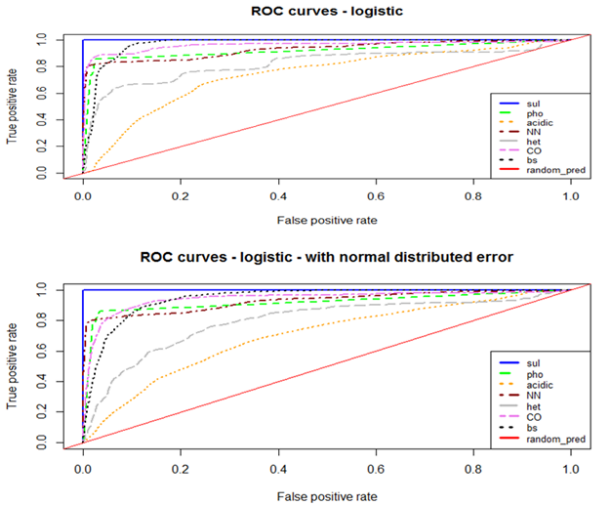
Compound identification is the main hurdle in LC-HRMS based metabolomics, given the high number of 'unknown' metabolites. In recent years, numerous in-silico fragmentation simulators have been developed to simplify and improve MS spectra interpretation and compound annotation. Nevertheless, expert mass spectrometry users and chemists are still needed to select the correct entry from the numerous candidates proposed by automatic tools, especially in the plant kingdom due to the huge structural diversity of natural compounds occurring in plants.

In this work, we propose to use a supervised machine learning approach to predict molecular substructures from isotopic patterns, training the model on a large database of grape metabolites. This approach, called 'compound characteristics comparison' (CCC) emulates the experience of a plant chemist who 'gains experience' from a (proof-of-principles) dataset of grape compounds. The results show that the CCC approach is able to predict with good accuracy most of the substructures proposed. In addition, after querying MS/MS spectra in Metfrag 2.2 and applying CCC predictions as scoring terms with real data, the CCC approach helped to give a better ranking to the correct candidates, improving users' confidence in candidate selection. Our results demonstrated that the proposed approach can complement current identification strategies based on fragmentation simulators and formula calculators, assisting compound identification.

The CCC algorithm is freely available as R package (https://github.com/lucanard/CCC) which includes a seamless integration with Metfrag. The CCC package also permits uploading additional training data which can be used extend the proposed approach to other systems biological matrices.



O17: Shifting, rising and shrinking peaks: Things to consider in 1H NMR-based metabolomics of blood and tissue

Christoph Trautwein[1], Marcel A. Krueger[1], Andreas M. Schmid[1], Bernd J. Pichler[1]

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Objective: Metabolite peak and spectrum characteristics in NMR-based *ex-vivo* metabolomics are in several points fundamentally different to GC- or LC-MS data. First of all, metabolites of the same concentration may have once a strong singlet peak, but eventually also several multiplet peaks of low intensity. Secondly, a huge portion of ^{1}H NMR extract spectra consists of metabolites with fast turnover. This is especially the case for the aromatic region (5 - 10 ppm) of the spectrum, where many signals from coenzymes like NAD+/NADH and energy-carrying molecules like AMP/ADP/ATP can arise. The turnover of these metabolites also directly changes concentrations of metabolites having their main signals in the aliphatic region of the spectrum (0 - 5 ppm) like GSH or GSSG. Immediate quenching and redox stabilization is therefore crucial to keep the metabolome as close to *in-vivo* conditions as possible. Finally, ongoing enzymatic reactions or physical destruction of the sample during data acquisition (e.g. by rotation at high frequency as necessary in high-resolution magic-angle spinning spectroscopy) can totally change the spectrum within a short period of time. The purpose of this study was to evaluate how blood and different tissue metabolomes change under different sample preparation and NMR analysis techniques.

Methods: For whole blood metabolomics we kept to a recent protocol [1], however aside from vortexing and normal sonication of human heparinized blood (500 µL), we additionally used focused ultrasonication for metabolite extraction. For tissue metabolomics, we collected murine brain, liver and tumor samples (10-20 mg) either with a customized tissue-milling robot [2] or by direct punching and freezing in liquid nitrogen after manual organ excision. All biopsies were cryogenically pulverized and directly transferred into 1 mL ceramic bead-containing extraction glass tubes. Both, blood and tissue specimen were then filled up with either a Methanol/MTBE or Methanol/Chloroform solvent mixture and then extracted with focused ultrasound for 10 minutes at 4° C. The solutions were spun down for 30 min at 14,000 g and the supernatants transferred into fresh 2 mL polypropylene cups. Phase separation was induced by adding 500 µL of ultrapure water. The aqueous phase was evaporated to dryness and reconstituted in a NaN_3 containing deuterated phosphate buffer with 100 µM TSP and transferred to 5 mm NMR tubes. ^{1}H NMR spectra under different temperatures were recorded on a 600 MHz Bruker Avance III spectrometer equipped with a 5 mm TXI RT probe. Spectral processing and metabolite assignment was done with ChenomX NMR Suite 8.2.

Results and Discussion: Generally, the ^{1}H chemical shifts of most fast-turnover metabolites showed a high dependence on temperature, pH and salt concentration. This could be prevented by sufficient buffering (e.g. 200 mM K_2HPO_4) and pH adjustment (pH 7.4) of the samples. For longer experiments sample stability is crucial for any reliable statistical analysis. Therefore we added sufficient NaN_3 (200 µM) to every extract. In whole blood analysis we found 1.5 - 2 fold higher and more reproducible metabolite concentrations using focused ultrasonication instead of only vortexing and normal sonication, so this could be an add-on to the current protocol [1]. For tissue extracts we generally found higher concentrations of reduced coenzymes and antioxidant species when using Methanol/Chloroform extraction. Interestingly, ratios of ATP/ADP/AMP strongly differed between brain, liver, tumor and excision technique, rising the questions if the often used adenylate energy charge determination is still the appropriate measure for quenching efficiency and what metabolites under which technique should be eventually excluded from further statistical analysis.

Acknowledgements: The authors gratefully acknowledge funding through the ImageLink project of the European Research Council (Grant agreement no. 323196).

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O18: Profiling of secondary metabolites related to pattern-triggered immunity in model plant Arabidopsis and evolutionary related species

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Plants – pathogen interactions start with recognition of microbes by sensing microbe-associated molecular patterns (MAMPs) and leads to activation of plant immunity mechanisms such as transcriptional reprogramming, phytohormone and secondary metabolite production. Well characterized MAMP, bacteria-derived oligopeptides flg22, was sprayed on plants from Brassicaceae family including model plant *A. thaliana* and related species *C. rubella, C. hirsuta,* and *E. salsugineum* in order to study changes in secondary metabolome during pattern-triggered immunity responses.

High resolution UPLC - microQTOF system was applied for metabolites profiling and then MZmine software was used for pre-processing of metabolomic data prior to ion detection and chromatograms building, followed by chromatograms deconvolution into individual peaks by Wavelets (XCMS) and then isotopes and adducts removal. Finally, alignment of all data files was performed using Join Aligner algorithm and corrected by Peak Finder. Exported data table was subjected to further statistical analysis in order to select differentially accumulated metabolites (DAMs).

Most of changes were related to increase accumulation of metabolites The highest metabolomics response was observed in *C. hirsuta* and the lowest in *E. salsugineum*. Interestingly, flg22 - induced changes in secondary metabolome were species-specific and no metabolite was commonly affected by flg22 in all species. This strong diversification of metabolomics responses to flg22 can be related with evolutionary divergence in immune systems within Brassicaceae family. In addition, *A. thaliana* and *E. salsugineum* which are the most distantly related tested species showed the highest similarities in PCA based on DAMs indicating low correlation between flg22 - induced metabolomics response and the Brassicaceae phylogeny.

O19: NMR-based exploratory metabolomics study of opisthorchiasis

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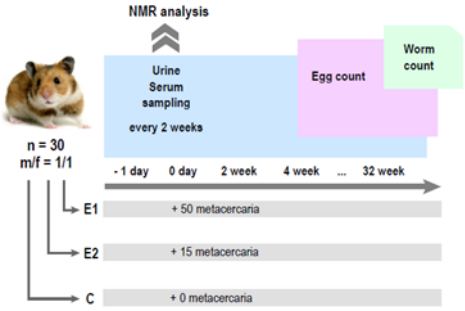
Helminthic infections may appear as a minor threat to the global health in Europe. However, in the endemic regions e.g. Africa, South-East Asia the burden of helminthiases remains heavy. The main problem is the chronic forms which are leading to a development of a variety of the pathological conditions. The metabolic profiling of helminth infections has a special position as it describes not only metabolism of the host but also the "parasite-host" system, where both of organisms are dynamically adapting to the mutual existence. Here we use experimental infection of the laboratory animals (golden hamster) with a liver fluke *Opisthorchis felineus* for an experimental assessment of the metabolic response to the helminthiasis.

Methods and findings. We conducted a NMR-based longitudinal metabolomics study of urine and serum samples on a cohort of 30 animals with two degrees of infection and a control group. Exploratory analysis performed on each body fluid separately shows a difference in the variance influencing the data. For instance, a PCA model built of the urinary data (PCs(1+2) – 34 %) shows a noticeable gender related trend. On contrary, in the serum dataset (PCs(1+2) – 60 %) a clustering according to the experimental groups is apparent.



Using supervised modeling methods such as PLS-class analysis and ANOVA-simultaneous component analysis (ANOVA-ASCA) were selected list of metabolites associated with the infection. The bulk of selected metabolites is associated with the lipid metabolism and metabolic stress.

Conclusions. Our data show that the main metabolic response is detectable at acute stage of infection. And physiological mechanisms of the response are best described as a state of metabolic stress and changes in lipid metabolism.

The work was supported by the Russian Science Foundation [grant number 14-15-00247].

Reference: 1. Kokova, D.A. *et al.* Exploratory metabolomics study of the experimental opisthorchiasis in a laboratory animal model (golden hamster, Mesocricetus auratus). *PLoS Negl Trop Dis*. 11(10): e0006044, 1-14 (2017).

O20: Nuclear Magnetic Resonance Spectroscopy Paired with Chemometric Analysis for the Discrimination, Characterization, and Classification of Highly Related Medicinal Plant Samples

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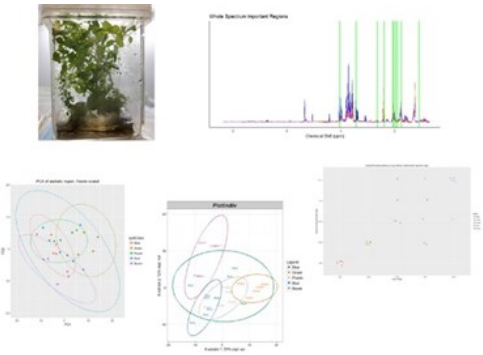

The use of metabolomics techniques to classify unrelated species, in particular for the detection of adulteration, has proven to be robust and relatively method-independent. In contrast, metabolomics analysis of more closely related samples, such as highly related species or different growing conditions, both classification and identification of significant metabolites can approach the false discovery rate.

Crataegus spp., or hawthorn, are flowering, fruit bearing shrubs that are found throughout the northern temperate zones. Hawthorn is well described in traditional medicine systems in Europe, Asia, and North America for its use in the treatment and prevention of cardiovascular ailments. Representative samples of European and North American hawthorn species have been collected. NMR data for methanolic extracts of both leaf material and fruits have been acquired and investigated by multi- and univariate statistical methods to see if the known cardioprotective compounds vary significantly between species. These models were used to identify significant metabolites that distinguish the species.

Hypericum perforatum, or St. John's wort, is a flowering plant native to parts of Asia and Europe, and invasive to several other regions including North America. St. John's wort has a long history of use in traditional medicine for the treatment of depression. In this study, clonally propagated St. John's wort were grown in culture with different light conditions. NMR data for the lyophilized and extracted plants were acquired and investigated using uni- and multivariate statistical methods. Regions of the spectra found to drive variance between different growth conditions were identified and their significance determined.

The work was supported in part by an NSERC Strategic Research Project grant (STPGP 381703).

Reference: "Differentiation of Crataegus spp. guided by nuclear magnetic resonance spectrometry with chemometric analyses" Jensen A. Lund, Paula N. Brown, Paul R. Shipley Phytochemistry (2017) **141**, 11-19

O21: Metabolic Profiling of Human Melanoma during Metastasis

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The recent advances in mapping metabolomic landscapes have unearthed the important roles of plasticity and reprogramming of cellular metabolism in tumorigenesis, thus paving the way to novel drug-treatment strategies and therapies. Metabolic activities, which are tightly correlated with malignant hallmarks, such as cell survival under stress conditions and the ability to successfully encounter high-energy demands, may significantly differ between primary tumor- and metastatic-cell populations of the same type of cancer, leading to distinct metabolic networks and metabolomes. In this study, we present a comparative NMR- and LC-MS/MS-mediated untargeted metabolic profiling of melanoma to landscape the metabolic alterations tumor cells are subjected during metastasis, while we, also, discuss methodological issues on the metabolomic analysis.

Two different cell lines, the WM115 and WM266-4 ones (10 individual samples from each cell line), were examined. Both cell lines have derived from the same patient, with WM115 cells having originated from a primary, pre-metastatic, tumor and WM266-4 cells clonally expanded from individual lymph-node metastases. Application of orthogonal partial least-squares discriminant analysis (OPLS-DA), following receiver operating characteristic (ROC) curve of NMR data, revealed significantly differentiated metabolite profiles in each cell-malignancy grade, with WM115 cells being mainly characterized by upregulated levels of choline, guanosine and inosine. Interestingly, WM266-4 cells showed notably increased contents of hypoxanthine, myo-inositol, glutamate and organic acids, as well as AMP, ADP, ATP and UDP(s), strongly indicating the critical roles of purine, pyrimidine and amino acid metabolism, during metastasis. Further analysis of the supernatants ("fluxomics") obtained from cells cultured with 1x PBS for 24 h (starvation conditions), allowed the identification of extracellular metabolites, where guanosine and inosine were clearly enriched in WM115, while myo-inositol and hypoxanthine were significantly upregulated in WM266-4 cells, thus corroborating the importance of these metabolites in melanoma progression, invasion and metastasis, both at intra- and extra-cellular settings.

Funding Resource: the present work was supported by Stavros Niarchos Foundation (SNF).

O22: Mapping the Metabolic Landscapes of Human Urothelial Bladder Carcinoma

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Plasticity and reprogramming of cellular metabolism have proved to act as strong forces in tumorigenesis. Malignant hallmarks, such as cell survival under stress conditions, as well as the ability to utilize nutrients and successfully encounter high-energy demands, are tightly correlated with metabolic alterations, thus indicating the important roles of metabolic landscapes in Cancer Biology and therapy. Metabolic activities may significantly differ between different malignancy stages (grades) of the same type of cancer, leading to distinct metabolic networks and metabolomes. In this study, we present a comparative NMR- and LC-MS/MS-mediated untargeted metabolic profiling of human urothelial bladder cancer (hUBC) to landscape the metabolic alterations tumor cells are subjected during cancer progression, while we also discuss methodological issues on the metabolomic analysis herein performed.

The metabolic landscapes of grade 1 (Gr1) to grade 4 (Gr4) human urinary bladder cancer cell lines were extensively investigated, using four different cell lines; the RT4 (Gr1), RT112 (Gr2), T24 (Gr3) and TCCSUP (Gr4) ones (10 individual samples from each cell line were examined). The obtained results unveiled diverse and malignancy-grade-specific metabolite collections, critically implicated in amino acid metabolism, tricarboxylic acid (TCA) cycle and energy metabolism, as well as purine and pyrmidine metabolism. A small, but significant, increase was observed in Gr2 (RT112) compared to Gr1 (RT4) cells, while Gr3 (T24) cells proved to carry surprisingly upregulated levels of most metabolites, with the amino acid metabolic network being emerged as a powerful and promising novel platform for bladder cancer targeted therapy. However, in TCCSUP (Gr4) cells, the levels of most identified metabolites, including those involved in amino acid metabolism, were notably reduced compared to RT112 (Gr2) and T24 (Gr3) cell-line respective ones, likely indicating a “metabolic inversion” of the cellular machinery during late metastasis. In accordance, amino acid levels were also increased in the supernatants (“fluxomics”) derived from T24 (Gr3) cells, as compared to RT4 (Gr1) ones, and, again, restored to the initial levels or less in the TCCSUP (Gr4) cells, thus further supporting the “metabolic inversion” phenomenon.

Funding Resource: the present work was supported by Stavros Niarchos Foundation (SNF).

O23: Tissue and plasma metabolomic analysis of DAPIT protein knockout rats – from mitochondria to glucose homeostasis

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F_oF_1-ATP synthase is a key enzyme of mitochondrial energy provision, responsible for production of most of cellular ATP. Recently, small 7 kDa proteolipid DAPIT, originally recognised as "diabetes associated protein in insulin sensitive tissues" (also termed Usmg5), has been recognize as a loosely attached subunit of the enzyme, but its biological role is largely enigmatic.

To elucidate the importance of this novel protein we produced zinc-finger rat knockout model of DAPIT deficiency on unique SHR background. DAPIT$^{-/-}$ animals were fully viable and contrary to previous data on cell lines, we observed normal levels of fully assembled ATP synthase, which, however, was predominantly present in its monomeric form. Contrary to proposed role of ATP synthase dimers in mitochondrial cristae formation, we observed only minor changes in cristae morphology in heart of DAPIT$^{-/-}$ animals. We observed analogous phenotype of ATP synthase dimers absence and almost normal cristae morphology in HEK293 DAPIT knockdown model, further verifying our animal observations. From the biochemical standpoint, we detected mild isolated ATP synthase deficiency in DAPIT$^{-/-}$ animals. Both ADP phosphorylating and ATP hydrolyzing activities were reduced by approx. 10% in liver and heart.

DAPIT$^{-/-}$ animals had 20-30% lower body weight and pronounced decrease in total adiposity (by 40%). Based on indirect calorimetry, DAPIT$^{-/-}$ animals preferred utilization of glucose to other substrates. This was also replicated at the tissue level, with higher glucose oxidation in DAPIT$^{-/-}$ skeletal muscle. Serum levels of glucose were unchanged in both fed and fasted state, but DAPIT$^{-/-}$ animals were significantly more insulin sensitive with decreased levels of serum insulin as well as area under curve in OGTT test. This is due to the improved peripheral insulin sensitivity, as glucose -stimulated insulin secretion from pancreatic islets was normal in DAPIT$^{-/-}$ animals.

Using targeted metabolomics panel, we evaluated rat plasma, liver and heart by LC-MS/MS (QTRAP 5500) focusing on TCA cycle and glycolytic intermediates as well as on acylcarnitines and amino acids. We observed lower levels of branched-chain amino acids, 3 – hydroxy butyrate and overall decrease in acyl carnitines, suggesting role of DAPIT in regulation of mitochondrial glucose oxidation, ketogenesis and fatty acid oxidation.

In conclusion, absence of DAPIT protein leads towards preferential oxidation of glucose, increased insulin sensitivity and decreased total adiposity in rat. In addition, it implicates for the first time that mitochondrial ATP synthase can be directly involved in regulation of glucose homeostasis.

Supported by Czech Science Foundation grant 14-36804G.

O24: Metabolomics in preclinical studies on metal based anticancer drugs

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In this work we aimed at the optimisation of metabolomic approaches for the investigation of 3D multicellular tumour spheroids, which emerged as models for testing of anticancer compounds as they better mimic certain features of *in vivo* tumour tissue. Since their cell number is usually lower than that of two-dimensional cell cultures, it is challenging to set up a reliable tool kit for profiling their metabolome. Therefore, we addressed fundamental questions regarding all steps of analysis. Different sample preparation and normalization procedures were employed for non-targeted and targeted metabolomics by high resolution mass spectrometry.

The application of metabolomics in research of acquired drug resistance based on *in vitro* models is of paramount importance as the mechanisms underlying the development of resistance on the metabolome level remain unclear. Furthermore, hypoxia - a key feature of poorly vascularized solid tumours and tumour spheroids - also triggers cellular adaptations and metabolic shifts. Hence, we addressed metabolomic rearrangements and lead metabolomic alterations in hypoxic and non-hypoxic 3D models in comparison with 2D (monolayer) cultures and upon incubation with oxaliplatin and KP1339, a clinically investigated Ru-based drug. The monolayer and spheroid cultures grown from oxaliplatin-sensitive vs –resistant colorectal cancer cell lines reveal metabolomics patterns correlating with sensitivity and resistance.

O25: Application of generalized additive models in metabolic changes of microbial community in enhanced biological phosphate removal

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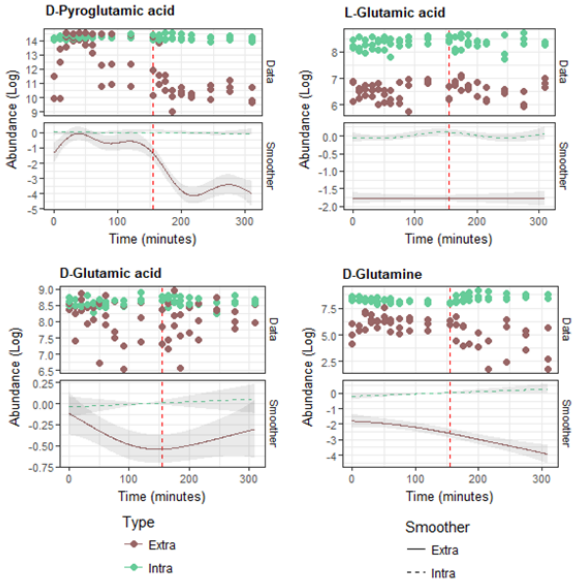
Phosphorus accumulating organisms (PAOs) play a key role in the process of enhanced biological phosphorus removal (EBPR) for the removal of phosphorus (P) from wastewater. The biochemistry and physiology of PAO is still incompletely understood, in part due to unculturability of these microbes, which require enrichment bioreactor protocols for their investigation. No metabolome level data on PAO enrichment communities has been collected to date. In this study, we obtained time series from laboratory scale activated sludge reactors enriched for PAO bacteria *Tetrasphaera* spp. for 19 time points during the P-release/uptake cycle. Extra- and intra-cellular metabolites were extracted by methanol/water (1:1 v/v) solvent mixture and untargeted metabolomics were analysed by ultraperformance liquid chromatography mass spectrometry (UPLC-MS). Generalized additive models (GAM) are the powerful statistical analysis for time resolved experiments to model and compare the "trends" of different metabolites [1]. In this study, we applied GAMs to infer the time dependents profile of extra- and intracellular metabolites of PAOs enriched communities across the EBPR cycle.

The models demonstrated a defined reproducible trajectory of extracellular metabolites over the EPBR cycle, which is distinct from the intracellular metabolite production.



An example of using GAMs in comparison of the time-course profile of selected metabolites observed both extra- and intracellular compartments are presented in the figure. The points in the upper pane show the time dependent production of metabolites over the experiment. The lower panels instead, show the results of GAM analysis on the overall time course. These plots indicate clearly visible changes of extracellular metabolites from anaerobic to aerobic phase while the intracellular metabolites tend to remain stable. These results suggest that the application of generalized additive models is a powerful approach to explore the direct surveys of metabolic state of PAO-enriched EBPR communities and dependent changes in metabolism of microbial community in EBPR.

Reference

Saw NMMT, Moser C, Martens S, Franceschi P (2017). .*Hortic Res*. **4**: 17038.

O26: Effect of cyclin-dependent kinase 4/6 inhibitors on cellular metabolome regarding selectivity

Jitka Široká[1], Lucie Mádrová[2], Radana Karlíková[2], Štěpán Kouřil[2], Radek Jorda[1], Vladimír Kryštof[1], David Friedecký[2], Tomáš Adam[2]

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Inhibitors of cyclin-dependent kinases CDK4/6 are effective anticancer drugs. Some of them has been approved in breast cancer treatment (e.g. palbociclib) and some are undergoing pre/clinical trials (e.g. ON-123300). Their effect dwell on inhibition of enzymes essential for cell cycle progression preventing cell proliferation.

The aim of this project is to study the connection between changes in metabolic/lipid profile induced by treatment with selected CDK4/6 inhibitors in mouse embryonic fibroblast (MEFs) cells with homozygous double knockout cdk4/cdk6 (k.o.). Wild type MEFs (WT) were used for comparison. The cells (1x106) were treated by 0.25 µmol/L of palbociclib (WT_PD, n=6; ko_PD, n=6), 2.5 µmol/L of ON-123300 (WT_ON, n=6; ko_ON, n=6) and 0.1% DMSO as control (WT_0, n=6; ko_0, n=6), and incubated for 16 hours. The cellular metabolites/lipids were extracted and subjected to targeted metabolic or untargeted lipidomic liquid chromatography mass spectrometry analysis.

The results imply that the effect of palbociclib on cellular metabolome is rather in concordance with CDK4/6 inhibition for both basic metabolic and lipid profile. Different are the results of ON-123300, which also suggest the function of CDK4/6 inhibition in basic metabolism, but also through a target other than CDK4/6 influencing the lipid profile.

Our results demonstrate changes of metabolic/lipid profiles in terms of selectivity of studied compounds towards preferential targets.

O27: Intermittent hypoxia and hypercapnia, a hallmark of obstructive sleep apnea, alters the gut microbiome and metabolome

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Obstructive sleep apnea (OSA) is a common disorder characterized by episodic obstruction to breathing due to upper airway collapse during sleep. OSA has been associated with adverse cardiovascular and metabolic outcomes, although data regarding potential causal pathways are still evolving. Because O2 and CO2 affect the ecology of the gut microbiota and the microbiota has been shown to contribute to various cardio-metabolic disorders, we hypothesized that the downstream physiological consequences of OSA are linked to functional alterations in the gut ecosystem. Here, we model human OSA and its cardiovascular consequence using atherosclerosis-prone (Ldlr -/-) adult mice fed high-fat diet (resembling western dietary practices). As episodic hypoxia and hypercapnia mimic the changes in blood gases that occur in OSA, these mice were longitudinally exposed to intermittent hypoxia and hypercapnia (IHH; analogous to chronic OSA) in a computer-controlled atmosphere chamber system (treatment group; n=8) or housed in room air (control group; n=8), and examined for 6 weeks. We had previously shown that IHH exacerbates atherosclerosis plaque formation in this model system. Fecal samples, a representative of the gut ecosystem, were collected at baseline and twice each week thereafter, and microbiome and metabolome were profiled using 16S rRNA amplicon sequencing and LC-MS/MS-based untargeted mass-spectrometry, respectively. We estimated relative abundances of microbial features (using QIIME2) and molecular features (using GNPS and MZmine2) per sample and compared OSA-mimicking and control mice using multivariate statistical models.

Starting from a highly congruent gut composition at baseline, both microbiome and metabolome of IHH-exposed mice cumulatively diverged from controls and co-varied with increasing duration of IHH-exposure. We noted significant compositional changes in both microbial (>10%, mostly increases in Clostridia) and molecular (>22%) species in the gut.

Furthermore, top molecules altered in abundance included microbe-derived secondary bile acids, enterolignans and fatty acids (identified to the highest level of annotation per metabolomics standards), highlighting the impact of IHH on host-commensal co-metabolism in the gut. Thus, we present the first evidence that IHH functionally perturbs the gut ecosystem, setting the stage for understanding its involvement in associated cardio-metabolic disorders.

Importance

Intestinal dysbiosis mediates various cardiovascular diseases comorbid with OSA. To understand the role of dysbiosis in cardiovascular and metabolic disease caused by OSA, we systematically study the effect of intermittent hypoxic/hypercapnic stress (IHH, mimicking OSA) on gut microbes in an animal model. We take advantage of a longitudinal study design and paired '-omics to investigate the microbial and molecular dynamics in the gut to ascertain the contribution of microbes on intestinal metabolism in IHH. We observe microbe-dependent changes in the gut metabolome that will guide future research on unrecognized mechanistic links between gut microbes and comorbidities of OSA. Additionally, we highlight novel and non-invasive biomarkers for OSA-linked cardiovascular and metabolic disorders.

O28: Acylcarnitine profile of 3-hydroxy-3-methylglutaryl CoA lyase deficiency patients

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3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency (HMGCLD, OMIM 246450) is a rare autosomal recessively inherited metabolic disorder caused by mutations in *HMGCL* gene. The mitochondrial enzyme is responsible for catalysing the cleavage of HMG-CoA to acetyl-CoA and acetoacetic acid. This conversion is a common last step in leucine catabolism and ketogenesis from fatty acids. Diagnosis is established by tandem mass spectrometry based newborn screening that usually contains SRM transition for non-butylated C5-OH carnitine (262 à 85) which represents four different compounds (methylmalonylcarnitine, 3-hydroxyisovalerylcarnitine, succinylcarnitine and 2-methyl-3-hydroxybutyrylcarnitine), pointing to different inborn errors of metabolism.

We analysed plasma samples of two HMGCLD patients with a liquid chromatography coupled with high-resolution mass spectrometry.

Apart from 3-hydroxyisovalerylcarnitine and 3-methylglutarylcarnitine, other acylcarnitine species derived from intermediates in the leucine degradation pathway were observed. These acylcarnitines were annotated based on accurate mass of precursor ions and predicted fragmentation behavior.

All newly found elevated acylcarnitines could hypothetically be also expected in one other condition, 3-methylglutaconyl-CoA hydratase deficiency (MGCA) of which defective enzyme is located one step upstream of the leucine degradation pathway. HMGCLD could be distinguished from MGCA by measuring of 3-hydroxy-3-methylglutarylcarnitine. The result suggests that 3-hydroxy-3-methylglutarylcarnitine could be specific marker in dried blood spots by FIA-MS screening (based on SRM transition 306 à 85) and thus speed up differential diagnoses among all the diseases characterized/screened by C5-OH transition (262 à 85). That would allow for earlier introducing dietary intervention directly from first screening sample without necessity of re-sampling.

This work was supported by NPU I (LO1304) and IGA_LF_2018_010.