Swiss Metabolomics Society

Swiss Metabolomics Society Annual Meeting

September 15th 2023, ETH Zurich

Frontiers in Metabolomics

BOOK OF ABSTRACTS

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Program

September 15th, 2023

ETH Main Building, Rämistrasse 101, 8092 Zurich, **Room D1.1** (map on page 5)

Morning session

09:30	Welcome Coffee
10:00	Opening Remarks **Serge Rudaz (President), Nicola Zamboni (host)**
10:15	**Matej Orešič**, Örebro University, Sweden & University of Turku, Finland Gut Microbiome as Mediator of Chemical Exposome – Host Metabolism Crosstalk
11:00	**Mélina Richard**, University of Basel/UKBB, Switzerland *The Scent of Pain: Metabolic Fingerprints of Cold Pressor Test via Real-Time Breath Analysis in a Multisite Clinical Trial*
11:20	**Hector Gallart-Ayala**, University of Lausanne, Switzerland *Lipidome-wide association study across adipose tissue, liver and skeletal muscle: the effect of diet and bile acid metabolism*
11:40	**Raphael Morscher**, University Children's Hospital Zurich & Medical University Innsbruck, Austria *Elucidating in vivo Metabolic Dependencies of Translation by Stable Isotope Tracing*
12:00	Sponsors presentations: **Waters**, **Tommaso Miraval** *Metabolomics and Lipidomics at Bruker* **Shimadzu, Samantha Wörner** *New Mass Spectrometry Tools to Enhance Metabolomics Research* **SCIEX, Christian Baumann** *Deep Metabolomics and Lipidomics Profiling Quan/Qual using the Sciex ZenoTOF 7600* **Thermo Scientific, Florian Marty** *Simultaneous Quantitation and Discovery (SQUAD) of known and unknown metabolites using the Orbitrap Astral mass spectrometer*
12:20	Lunch break and poster session

Afternoon session

13:45	**Emma Schymanski, University of Luxembourg, Luxembourg** ***Navigating Millions of Chemicals in Metabolomics and Exposomics Workflows***
14:30	**Tingting Fu**, University of Geneva, Switzerland *Spatially resolved lipidome profiling of sentinel crustacean species by mass spectrometry imaging*
14:50	**Sergey Girel**, University of Geneva, Switzerland *Electron-activated dissociation for routine annotation and structure elucidation of endogenous steroids in bioanalysis*
15:10	Sponsors presentations: **Merck, Harry Wischnewski** *Metabolomics News from Merck* **Bruker, Pascal Looser** *Metabolomics and Lipidomics at Bruker* **Agilent, Moritz Wagner** *ChemVista and MassHunter Explorer, two new improved tools for data interpretation*
15:30	Coffee break and poster session
16:00	**Sofia Tsouka**, University Hospital Bern, Switzerland *Advantages and pitfalls of pathway analysis for the interpretation of metabolomics data*
16:20	**Louis-Felix Nothias, CNRS – Cote d'Azur University, France** *An Artificial Intelligence Agent for Navigating Knowledge Graph Experimental Metabolomics Data*
16:45	**Round Table** *Frontiers in Metabolomics*
17:25	Closing remarks
17:30	Apéro

Posters

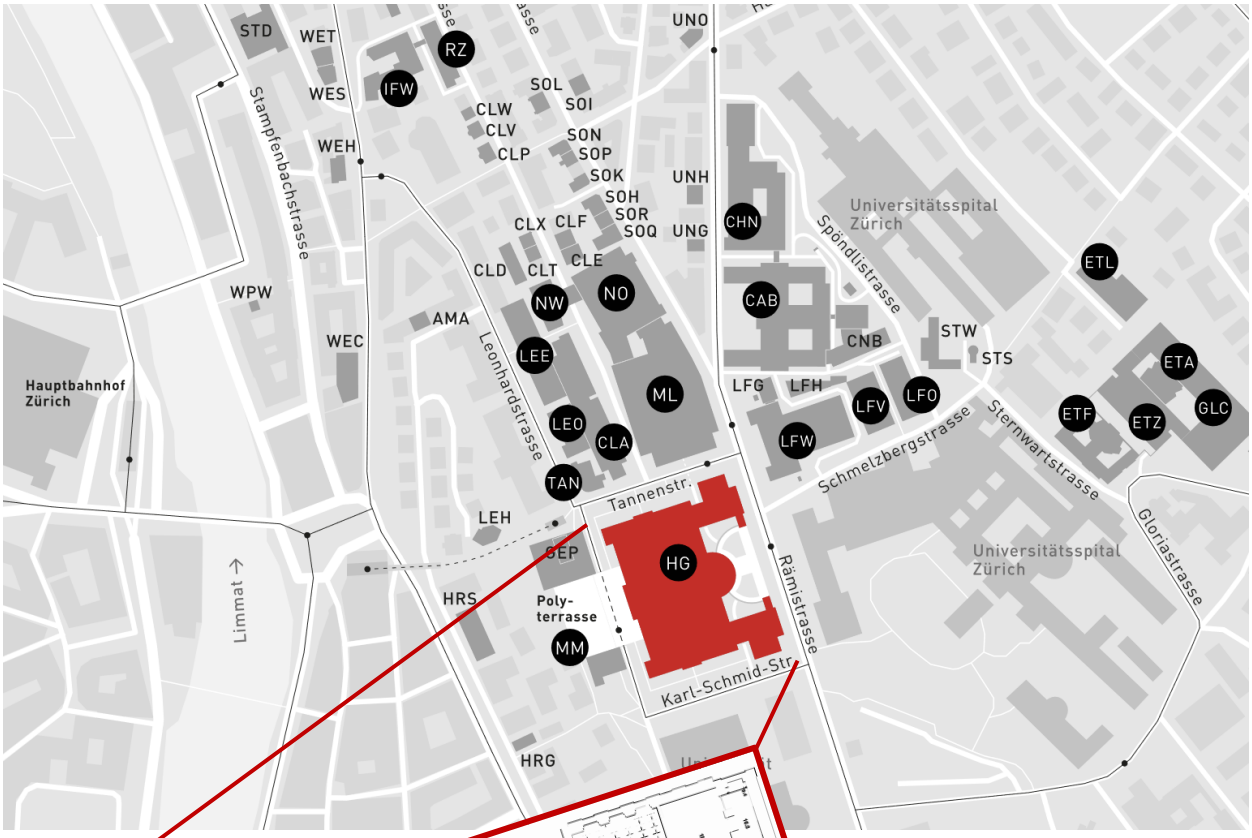
1	**Zhihong Yin,** *University of Basel, Switzerland* Breath pharmacometabolomics of stellate ganglion block intervention
2	**Andrew Quinn**, *University of Lausanne* A native gut symbiont alters tryptophan catabolism in the gut of the honey bee (*Apis mellifera*)
3	**A.M. Fouassier**, *Amazentis* Urolithin A improves mitochondrial and muscle health from cells to humans – evidence from biochemical and metabolomics-based assays
4	**Simona Rambousek**, *Nestlé Research* Impact of infant formulas compared to breast milk on metabolism of infants during the first 3 months of life studied by NMR metabolomics
5	**Viola Jeck**, *Bruker Daltonics* Novel Modes of Parallel Accumulation Serial Fragmentation (PASEF) for Mass Spectrometry-Based Lipidomics
6	**Simon Isfort**, *University Hospital Bern* Novel pre-treatment using chemical derivatization to allow spatial localization of Cholesterol using MSI in the liver
7	**Joachim Kloehn**, *University of Geneva* GNA1-mediated acetylation of glucosamine-6-phosphate is essential for *Toxoplasma gondii* and cannot be bypassed by GlcNAc salvage
8	**Mathieu Galmiche**, *University of Geneva* Towards extended steroid profiling in biological samples with the development of a multi-targeted UHPLC-MS/MS workflow
9	**Saurav Subedi**, *University of Bern* Exploring lipid metabolism as a translational tool to study Prostate cancer (PCa) progression
10	**Roshni M. Pasanna**, *St. John's Research Institute, Bangalore* Urinary metabolomic biomarkers of lysine supplementation in stunted children
11	**Tommaso Miraval**, *Waters AG* Enhancing the mobility resolution for co-eluting compound classes during plasma characterisation using multi-sequence IMS^n acquisitions
12	**Vincen Wu**, *ETH Zurich* Optimization of Electron Activated Dissociation for Phospholipids Annotation in Lipidomics Workflow
13	**Jiafa Zeng**, *University of Basel* Monitoring of propofol metabolism during surgical interventions via exhaled breath analysis
14	**A.F Babu**, *University of Eastern Finland, Kuopio* An integrated understanding of the metabolic benefits of a novel double-targeted intervention using genetically engineered probiotic expressing aldafermin with dietary changes on NAFLD
15	**Joëlle Houriet**, *Geneva* How Low Can You Go? Selecting Intensity Thresholds for Untargeted Metabolomics Data Preprocessing
16	**Isabelle Meister**, *University of Geneva* Metabolomic and lipidomic approaches in mindfulness-based intervention for health-care students
17	**Kapil Dev Singh**, *University Children's Hospital Basel* Recent developments in therapeutic drug management using exhaled breath
18	**Adriano Rutz**, *ETH Zurich* Prior Knowledge as a Compass Guiding Untargeted Metabolomics
19	**Luis-Manuel Quiros-Guerrero**, *University of Geneva* Integration of bioactivity and metabolomics structural novelty scores for efficiently targeted isolation of novel bioactive compounds from biodiverse natural extract collections

Location

The annual meeting takes place in the main building of ETH at Rämistrasse 101 (google maps), floor D, Room HG D1.1. If you enter from the main entrance at the East, in front of the University Hospital, you have to take the stairs down to you right (to the North).

The closest tram stop is ETH/Universitätsspital, right in front of the building. From the main station, it can be reached with tram (#10) or with a 10 minute walk. To avoid the steep climb, take the Polybahn from Central.

A public parking lot exists underneath the building and is accessible from the Karl-Schmid-Strasse.



The Scent of Pain:
Metabolic Fingerprints of Cold Pressor Test via Real-Time Breath Analysis in a Multisite Clinical Trial

Mélina Richard[1], Dilan Sezer[2], Sarah Bürgler[3], Luana Palermo[4], Yannick Schulz[5], Zhifeng Tang[6], Xin Luo[6], Xue Li[6], Jens Gaab[7], Pablo Sinues[8]

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Keywords: Breath Metabolomics, Pain, Cold Pressor Test, Validation, Breath Research

Abstract

The Cold Pressor Test (CPT) is a widely used experimental technique for human pain induction, involving immersion of the hand in cold water. Understanding the underlying molecular mechanisms associated with pain responses is crucial for the development of effective pain management strategies. In recent years, metabolomics has emerged as a powerful tool for investigating the comprehensive metabolic changes occurring in response to pain stimuli and metabolomics studies have revealed distinct metabolic profiles associated with pain response. This study is for the first time addressing the question of whether pain induces any measurable changes at the metabolic level in non-invasive real-time measurements of exhaled breath.

We conducted the study at two sites: Switzerland (n=19) and China (n=21). Exhaled breath metabolome was analyzed using Secondary Electrospray Ionization-High Resolution Mass Spectrometry (SESI-HRMS). Each participant performed two sets of exhalations before and after the CPT, with six exhalations in positive and negative modes respectively. A total of 1920 exhalations were examined using standard univariate and multivariate data analysis strategies.

Both for the participants in Switzerland and China, significant changes in peaks before and after the CPT were observed. We found an overlap of 149 significant features [FDR < 0.01, log2(fold change) > 2] between the two study sites with a consistent up-/down-regulation behavior.

Altered metabolite abundances suggest a metabolic response to CPT induced pain. The overlapping features in both study sites indicate consistent metabolic changes associated with pain across populations. We are currently conducting enrichment analysis to investigate the correlation networks of involved metabolic pathways.

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Lipidome-wide association study across adipose tissue, liver and skeletal muscle: the effect of diet and bile acid metabolism

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Abstract

The organismal lipidome is remarkably diverse and its composition and abundance are determined by tissue-specific *de novo* synthesis and metabolization of external lipid sources, mainly from food intake. Lipid profiles were shown to be organ-specific, however, the relative abundance and diversity of lipid species were not comprehensively investigated regarding the tissue function, effect of diet, and bile acid levels. Bile acids have been recognized as enterohepatic-derived hormones which control lipid metabolism and global metabolic health.

In the present study, we first evaluated the relative distribution and diversity of lipid species across four organs playing a central role in lipid energy metabolism (including lipid synthesis, utilisation, and storage): subcutaneous white adipose tissue (scWAT), brown adipose tissue (BAT), skeletal muscle and liver. Furthermore, we aimed to determine the effect of diet on lipid composition of specified organs obtained from C57BL/6J mice fed with chow diet (CD) and high-fat diet (HFD). The mice (all males) were sacrificed at 29 weeks of age, following a period of 21 weeks on CD or HFD. The sacrifice was performed in the postprandial state (4h after physiological refeeding). Tissue lipidome was characterized using specific targeted SRM-based HILIC-MS/MS method screening for more than 2400 lipid species in the initial qualitative analysis followed by quantification of robustly detected species. Lipid quantification was achieved by a single point calibration with 75 isotopically labelled standards representative of different lipid classes, covering lipid species with diverse alkyl chain lengths and unsaturation degrees. Correction of isotopic overlap was performed using LICAR (https://slinghub.shinyapps.io/LICAR/). As a result, a wide panel of 24 lipid classes comprising 533 to 838 lipid species were quantified (CV<20%) depending on tissue type. Additionally, bile acids (15 species) were quantified in mice liver and plasma using stable isotope dilution approach.

The highest diversity of lipids was detected in quadriceps (n=838 species) while white adipose tissue contained the lowest (n=533 species) number of species. Main differences were found for triacylglycerols (TG) and glycerophospholipids such as glycerophosphocholines (PC) and glycerophosphoethanolamines (PE). In mice fed with HFD, we observed the accumulation of TG as a general trend for all tissues. Besides, a decreasing trend of some hexosylceramides (HexCer), and sphingomyelins (SM) was reported in a tissue-specific manner. The dataset is currently under investigation to identify the lipid signatures associated with measured physiological parameters including body and organ weight, blood glucose and bile acid levels.

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Spatially resolved lipidome profiling of sentinel crustacean species by mass spectrometry imaging

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Keywords: Lipidomics, Mass spectrometry imaging, MALDI MS, TOF-SIMS, *Gammarus fossarum*

Abstract

Lipids are important component in living organisms and play pivotal roles in various biological functions. In aquatic biomonitoring, lipidomic profiling of sentinel organisms is essential to understanding the molecular mechanism of the toxicity effect of endocrine disrupting chemicals. Currently, the most popular mass spectrometry (MS)-based lipidomic techniques include hyphenated chromatographic MS and direct infusion MS. Both methods require a destructive sample preparation step to isolate the analytes from the biosystems, during which the spatial information of the molecules is inevitably lost. Mass spectrometry imaging (MSI) is a well recognized technique for *in situ* mapping of a variety of molecular species. Compared to the above mentioned MS workflows, MSI has the unique advantage of preserving the spatial localization of the molecules, thus providing crucial information on biochemical processes within the biofunctional compartments. We herein propose a spatially resolved lipidomics workflow combining shotgun lipidomics and MSI to decipher the lipidome of a freshwater sentinel species *Gammarus fossarum*. Shotgun lipidomics was performed on whole body gammarids to generate a global reference lipidome of this species. Matrix assisted laser desorption ionization (MALDI) MS and high-resolution time of flight-secondary ion mass spectrometry (TOF-SIMS) imaging were employed to spatially map the lipids in whole-body gammarid tissue sections and specific organs, respectively. This spatial lipidomics workflow enabled a comprehensive examination of lipid composition and spatial distribution in *G. fossarum*. Particularly, MSI guided the discovery of unknown sulfate-based lipids in this organism and revealed a dynamic lipid composition in gammarids in the course of reproduction.

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Electron-activated dissociation for routine annotation and structure elucidation of endogenous steroids in bioanalysis

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Keywords: steroidomics, structure elucidation, electron-activated dissociation, prostate cancer

Abstract

Steroids represent a subclass of lipids with a common gonane-based framework and play a crucial role in regulation of physiological functions at cellular, tissue and systemic levels. In particular, they mediate reproductive functions, stress response and regulate inflammation. Their carbon skeleton possesses up to 15 possible chiral centres, resulting in immense structural diversity. Annotation and unambiguous identification of endogenous steroids in non-targeted fashion is thus complicated by a close proximity of their isomers in chromatographic dimension and extremely similar fragmentation patterns obtained from established collision-induced dissociation methods. Therefore, alternative fragmentation options are in demand to obtain unique discriminant fragments which are otherwise unavailable. In this work, we illustrate a problem of steroid annotation in a prostate cancer progression study. Next, we explore an electron activation dissociation (EAD) approach for steroid annotation in reversed-phase chromatographic conditions and fast scanning regime typical for routine non-targeted analysis. We demonstrate, how unique fragments can be derived for isomeric steroids in EAD mode and highlight prospective steroid annotation strategies for bioanalytical applications.

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Advantages and pitfalls of pathway analysis for the interpretation of metabolomics data

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Keywords: pathway analysis, computational, method, functional interpretation.

Abstract

Due to continuous advancements in the field of metabolomics, the importance of having robust computational tools that facilitate the functional interpretation of large-scale datasets has become essential. One of these tools is pathway analysis, a method that helps link changes in metabolic compounds to biological pathways. Although it is widely used, it is not well standardized and the impact of different considerations on the functional outcome is not well understood. The workflow consists of the selection of a suitable pathway collection, the conversion of these metabolic pathways to graphs, and the calculation of scores for each participating metabolite in the network. Subsequently, pathways are scored and ranked according to the metabolomics dataset. Multiple factors need to be taken into consideration when using pathway analysis in order to produce reliable results. We investigate two main aspects of pathway analysis, namely the consideration of non-human-native enzymatic reactions and the interconnectivity of individual pathways. The exclusion of non-human-native reactions leads to poorly represented reaction networks and to loss of information, particularly when it comes to microbiome related datasets. The connectivity between pathways illustrates better the importance of certain central metabolites in the network, such as aminoacids. However, it might result in hub compound effects, which are mitigated through a penalization scheme. Our findings aim to raise awareness on both the capabilities and limitations of current pathway analysis practices in metabolomics and provide insights on their standardization.

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An Artificial Intelligence Agent for Navigating Knowledge Graph Experimental Metabolomics Data

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Keywords: Knowledge graph, mass spectrometry, artificial intelligence, natural products.

Abstract (250 words)

An experimental knowledge graph (KG) driven framework (10.26434/chemrxiv-2023-sljbt) was recently introduced to facilitate the integration of heterogenous data types, encompassing both experimental data (mass spectrometry annotation, results from biological screening and fractionation) as well as web-wide meta-information (such as taxonomy and metabolite databases). Although this KG efficiently encapsulates the different data structures and semantic relationships, retrieving specific information visually or programmatically is not trivial.

To unlock the full potential of KGs for scientists of all fields, we have engineered a KG Artificial Intelligence (AI) agent that can convert natural language questions into programmatic data-mining tasks and generate adapted visualization. By leveraging the potential of emerging Large Language Models (LLMs) to understand semantic relationships encapsulated in KGs, the KGAI-agent autonomously iterates to construct a SPARQL request – the widely-used query language – of inputted natural question. After retrieving the necessary information from the KG, the KGAI-agent provides a preliminary interpretation of the results in natural language, along with relevant visualizations and statistics. In addition, follow-up interactions between the LLM and the user the AI-agent can be expert-guided to refine the initial results and interpretation. Released as an open-source tool, the KGAI-agent acts as a powerful assistant capable of exploring any KG, democratizes access to such semantic databases without requiring bioinformatics expertise, and is accessible in over 50 human languages.

Here we will describe the core principles and components of the KGAI-agent and illustrate how we can interrogate metabolomics information from a KG to discover bioactive metabolites from a collection of 16,000 plant extracts.

Breath pharmacometabolomics of stellate ganglion block intervention

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Keywords: stellate ganglion block, real-time and non-invasive breath analysis, secondary electrospray ionization high resolution mass spectrometry.

Abstract

Stellate ganglion block (SGB) has been widely used in clinic as a therapeutic approach to reduce the symptoms of persistent sympathetic pain related diseases in the head, neck and upper limbs, such as insomnia, facial paralysis and postoperative pain. There have been many reports about the excellent clinical performance of SGB, but little is known about its mechanism of action at the metabolic level. We hypothesize that breath-based metabolomics may the help for understanding the mechanism of SGB from the perspective of expiratory metabolomics.

In this study, we collected 52 sets of real-time online exhalation data before and after SGB intervention from n= 28 subjects, 17 healthy volunteers and 11 patients with persistent sympathetic pain, using secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS). All subjects received 5-6 mL 1% lidocaine in the stellate ganglion on one side of the neck and showed Horner's syndrome and increase of facial temperature on the same side.

SGB induced significant changes (FDR < 0.01 & mean (Log2FC) > 2) in 200+ expiratory volatile organic compounds (VOCs) could be rapidly captured by real-time high resolution mass spectrometry within 5 minutes after the Horner effect appears. It provides effective expiratory metabolomics information on the mechanism of SGB. Furthermore, this present study provided a rapid, reliable and sensitive approach for the monitoring of the lidocaine metabolite 2,6-DMA.

# These authors contributed equally to the work; * Correspondent author.

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A native gut symbiont alters tryptophan catabolism in the gut of the honey bee (*Apis mellifera*)

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Keywords: Microbiome, Tryptophan, Gut-brain axis, Social insects

Abstract

Animal physiology and behavior is tightly regulated by neuroactive compounds. Many of these compounds, including serotonin, tryptamine, and indole derivatives, are products of tryptophan catabolism. It is well established, in humans and other species, that the gut microbiota participates in tryptophan metabolism, and therefore directly affect their host. Further investigating diverse animal species will enable us to better understand the bidirectional impacts of tryptophan metabolism in the gut. The western honey bee (*Apis mellifera*) is an ideal model for answering such questions, as bees engage in complex, eusocial behaviors while possessing a well-defined, stable, and manipulatable gut microbiome. Recent research has further found that the native microbiota enhances bee learning, memory, and social interactions compared to microbiota-free controls. Working with a betaproteobacterium (*Snodgrassella alvi*) that first co-diversified with social bees >85 mya, we recently found that it performs a key step of tryptophan metabolism, transforming host synthesized L-kynurenine into anthranilic acid, without a clear benefit for itself. Intriguingly, this biochemical transformation is absent in the honey bee host. Feeding tryptophan to bees colonized with the symbiont further elevates levels of tryptamine, indole-3-acetic acid and kynurenic acid in the gut. Only some of these effects are recapitulated *in vitro* with kynurenine supplemented media. Our ongoing work seeks to identify whether other members of the native microbiota further modulate tryptophan metabolism, whether those compounds are exported outside of the gut, particularly to the brain, and whether they induce physiological effects on the gut or on the host behavior.

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Urolithin A improves mitochondrial and muscle health from cells to humans – evidence from biochemical and metabolomics-based assays

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Keywords: Urolithin A, muscle function, aging, mitophagy, acylcarnitines

Abstract

Urolithin A (UA) is a natural compound produced by the gut microbiome from dietary precursors found in foods such as pomegranate, berries, and nuts. Our studies showed that UA positively impacts on mitophagy and mitochondrial metabolism. The decline of these processes is considered a hallmark of aging and health. The positive effects by UA was observed across various cell lines, preclinical models, and clinical studies. We first showed that UA induces mitophagy markers and enhances mitochondrial metabolic function in C2C12 muscle cells. Consistent results were confirmed in vivo, where UA increased protein markers of mitophagy and mitochondrial respiration in gastrocnemius muscles of both old mice and dystrophic animals, compared to controls. We also showed improved mitophagy and mitochondrial function by UA in human chondrocytes from both healthy donors and osteoarthritis (OA) patients. Human muscle proteomics data from healthy middle-aged subjects administered with UA for four months described elevated levels of proteins involved in mitophagy and oxidative phosphorylation compared to placebo. Plasma metabolomics analysis of the same subjects, as well as in additional clinical studies in healthy elderly, revealed a significant reduction of several acylcarnitines following UA administration. Such decrease is indicating higher systemic mitochondrial efficiency by UA. Finally, the enhanced mitochondrial health induced by UA was linked to a significant improvement in muscle strength and endurance in both preclinical models and multiple clinical studies. These findings provide evidence for the use of UA as a nutritional intervention targeting mitochondria to support the health of multiple tissues and promote healthy aging.

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Impact of infant formulas compared to breast milk on metabolism of infants during the first 3 months of life studied by NMR metabolomics

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*Drs. Godin and Picaud are co-senior authors

Disclosures: SR, NPH, SKD and JPG are employees of Société des Produits Nestlé S.A., the sponsor of the study.

Keywords: NMR metabolomics, infant nutrition, metabolic maturation

Abstract

Adaptation of protein content in infant formulas to be closer to the dynamic composition of human milk may reduce possible metabolic differences between breastfed and formula-fed infants. We assessed the urinary metabolome in healthy term infants fed with an age-adapted formula regimen or standard starter formula in comparison with breastfed infants.

Urinary metabolomics was performed using nuclear magnetic resonance (NMR) spectroscopy in 0, 1 and 3 months old infants randomized to receive either Control (CTRL; standard starter formula with 1.85g protein/100kcal from enrollment [age ≤7d] until 3 months) or Experimental (EXPL; new formula with 2.5g protein/100kcal from enrollment until age 1 month followed by CTRL formula until 3 months) interventions. The formula regimens were compared to a reference group of breastfed infants using univariate or multivariate analysis and based on their metabolic maturation age derived from changes in urinary metabolites related to infant age.

Metabolomics of infants at age 1 month was influenced by differences in formula protein concentration, inducing changes of microbiota-related metabolites (phenylacetylglutamine, 4-hydroxyphenylacetate, indoxyl sulfate, and hippurate) and essential amino acids (isoleucine, leucine, threonine and lysine) as detected in urine, predominantly in EXPL group. The metabolic maturation age trajectories for both infant formula regimens were similar but significantly different from the breastfed trajectory.

In conclusion, urinary metabolomics doesn't indicate superiority of the higher protein content formula (2.5 g protein/100 kcal) from birth to 1 month of age over the standard starter formula (1.85 g protein/100 kcal). The effect of the metabolite differences on health requires further evaluation.

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Novel Modes of Parallel Accumulation Serial Fragmentation (PASEF) for Mass Spectrometry-Based Lipidomics

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Keywords: Lipidomics, Trapped Ion Mobility Spectrometry (TIMS), Mass Spectrometry (MS), Hydrophilic Interaction Liquid Chromatography (HILIC).

Abstract

Addressing the complexity of the lipidome with sufficient depth requires multi-dimensional analytical techniques. Conventional liquid chromatography-mass spectrometry (LC-MS) approaches can be enhanced by trapped ion mobility spectrometry (TIMS) to increase selectivity and lipidome coverage.

Our contribution outlines the benefits of TIMS-enabled LC-IMS-MS/MS plasma lipidomics workflows based on Parallel Accumulation Serial Fragmentation (PASEF). In PASEF experiments, electrospray generated ions are subjected to cycles of trapping/elution in the TIMS device followed by sequential fragmentation of ions, before entering a time-of-flight (TOF) analyzer. Variation of the TIMS conditions and quadrupole filtering windows enables unique MS/MS experiments with distinct benefits for lipidomics studies.

Data dependent acquisition (dda-)PASEF, suitable for non-targeted approaches, produces high-quality CCS and MS/MS data for the identification of phospholipid species based on accurate precursor mass and fragment ion spectra. Compared to dda-PASEF, data independent acquisition (dia-)PASEF and parallel reaction monitoring (prm-)PASEF do not require an MS1 scan for precursor picking. In dia-PASEF, broad-band fragmentation occurs via predefined mass/mobility windows. It leads to a particularly high coverage in the generation of MS/MS spectra. prm-PASEF is based on a target list with user defined retention time and mobility sections, leading to better quality of MS/MS spectra for low abundant lipids. Therefore, prm-PASEF is the method of choice for targeted lipidomics in unknown samples.

Experiments were carried out on a timsTOF flex (Bruker, Germany) coupled to an Ultimate 3000 LC system (Thermo Scientific, Germany) using negative-mode electrospray ionization. Experimental details can be found in Rudt et al., Analytical Chemistry 2023, 95, 9488-9496.

Novel pre-treatment using chemical derivatization to allow spatial localization of Cholesterol using MSI in the liver

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Cholesterol plays a crucial role in diverse physiological processes such as regulating cell membrane fluidity and facilitating cellular transport [1]. Its perturbation is associated with MASLD [2] although its zone-specific distribution is not known. Precise detection and localization are therefore essential to understand its involvement in MASLD.

Desorption electrospray ionization (DESI) mass spectrometry has been employed to detect and identify multiple molecular species and simultaneously map their distribution in tissue sections [3],[4]. Cholesterol among other sterols, tends to be poorly ionized by conventional DESI and is often disregarded [5]. To solve this problem, on-tissue derivatization has been developed by adding reagents to the electrospray solvents. Betaine aldehyde, which contains a pre-charged group, reacts selectively and rapidly with alcohols, including cholesterol, and significantly decreases the limits of detection [6]. This however, imposes limitations to the range of suitable solvents for DESI analysis.

This poster explores the detection of cholesterol throughout the liver, integrating a novel and rapid pre-treatment technique to allow high-resolution spatial mapping of cholesterol in conjunction with other lipids classes. Meanwhile, the integrity of cellular structures remains intact, allowing subsequent pathophysiological assessment of the same tissue slide using conventional staining methods. Given the limited tissue amounts required, this method allows the detection of cholesterol with other lipid classes simultaneously.

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GNA1-mediated acetylation of glucosamine-6-phosphate is essential for *Toxoplasma gondii* and cannot be bypassed by GlcNAc salvage

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Keywords: *Toxoplasma gondii*, Apicomplexa, N-acetylglucosamine, GPI-anchors, parasite, metabolism, nutrient salvage, glycosylation, stable isotope labelling.

Abstract

Like in other eukaryotic cells, many proteins of the apicomplexan parasite *Toxoplasma gondii* entering the secretory pathway are *N*- or *O*-glycosylated or linked to membranes via glycosylphosphatidylinositol (GPI) anchors. Formation of these post-translational modifications relies on activated sugar nucleotides, including uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc). A critical enzyme in UDP-GlcNAc synthesis is the glucosamine-phosphate-*N*-acetyltransferase (GNA1). To decipher the importance of GNA1 for the parasite, conditional depletion of the gene was achieved using the auxin-degron system. While GNA1 was assigned a high positive fitness score in a genome-wide fitness screen, indicating dispensability, we demonstrate here that GNA1 is essential for *T. gondii*. This discrepancy is likely due to a functional shorter splice variant which is significantly shorter than the annotated protein. Functional characterization of parasites downregulated in GNA1 revealed that GNA1 depletion impairs GPI synthesis via two distinct modes: Depletion of its crucial substrate UDP-GlcNAc as well as a toxic accumulation of glucosamine-6-phosphate (GlcN6P). GNA1-depleted parasites show no or only a modest recovery in fitness following GlcNAc supplementation, despite stable isotope labelling experiments demonstrating *T. gondii*'s ability to take up and phosphorylate GlcNAc. Metabolomic analyses reveal that in glucose-replete conditions, GlcNAc appears not to be efficiently utilized and fails to restore UDP-GlcNAc levels. In glucose-deplete conditions, on the other hand, GlcNAc is efficiently used and fully restores UDP-GlcNAc levels. However, the absence of glucose does not alleviate GlcN6P accumulation, which continues to inhibit GPI synthesis. This vulnerability highlights the evolutionary divergent apicomplexan GNA1 as a promising drug target in *T. gondii*.

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Towards extended steroid profiling in biological samples with the development of a multi-targeted UHPLC-MS/MS workflow

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Keywords: Steroids, Sample Preparation, Liquid Chromatography, Mass Spectrometry, Targeted analysis.

Abstract

Dysregulation of steroidogenesis and steroid metabolism can be responsible for a wide range of diseases such as diabetes, prostate cancer or male infertility.

For extended steroid profiling, untargeted analytical workflows can be implemented. However, they lack absolute quantification and require high-end mass spectrometers for meaningful annotation.

The sensitivity and wide availability of low-resolution mass spectrometers makes the emergence of targeted analysis a promising alternative for extended steroid profiling at very low concentrations.

The main challenges associated with the analysis of steroids in biological samples are the complexity of the matrix causing high background noise and interferences, the wide dynamic range of analyte concentrations (from < 5 ppt to > 5 ppm), the weak ionization efficiencies of some steroids, and the coexistence of multiple isobars with similar MS/MS spectra that can be difficult to separate.

In this work we explore this multi-targeted approach and address these challenges. Sample clean-up was optimized, including phospholipid removal and solid phase extraction of steroids, with comparison of different sorbents. The separation of isobaric pairs was fine-tuned using a biphenyl stationary phase. Attention was also paid to the optimization of steroid ionization conditions with post-column addition of ammonium fluoride combined with different formic acid concentrations, and to the optimization of MS/MS transitions for improved selectivity and sensitivity.

The method performance was evaluated on pooled plasma samples in which 41% of the target steroids were successfully detected.

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Exploring lipid metabolism as a translational tool to study Prostate cancer (PCa) progression

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Keywords: Metabolomics, Prostate cancer progression, Machine learning, Metabolic Signature.

Abstract

Lipid metabolism has a proven role in the pathogenesis and progression of many cancers, and growing evidence support a role for dysregulated lipid metabolism and prostate cancer (PCa). Several studies have shown that PCa metabolome could be used as non-invasive diagnostic and prognostic tool. In this study, we analyzed the plasma of 73 prostate cancer patients to identify metabolites with diagnostic or prognostic value. Patient plasma was collected during fasting shortly before radical prostatectomy. Targeted metabolomics was run on the pre-operative plasma. Using machine learning algorithms, we identified a signature of 41 metabolites either positively or negatively associated with the development of bone metastatic PCa. Furthermore, we studied the effect of high- and low-fat diet on the progression and on the morbidity of prostate cancer using inducible genetically engineered mouse models (GEMMs) of prostate cancer of different grades. Mice were switched to either low- or high-fat diet since week 6 and the Cre recombinase was activated during week 8 to induce the phenotype in the GEMMs. Mice were kept on low- or high-fat diet until the time of harvest (1, 3 and 5 months). Faeces were collected longitudinally to perform a metagenomics study on the effect of diet on microbiota, while prostate, plasma and liver tissues were collected at harvest point. In conclusion, our studies associated a metabolic signature to a risk of metastasis development and will provide more insights on the role of diet on prostate cancer metabolism.

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Urinary metabolomic biomarkers of lysine supplementation in stunted children

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Keywords: Lysine, stunting, urine, metabolomics

Abstract

The risk of essential amino acid deficiency (EAAD), like lysine in cereal-based diets, can impact growth in young children. Urinary metabolomic biomarkers can be used for rapid non-invasive screening for EAAD in stunted children, and to design targeted nutritional therapies.

A parallel group interventional trial of lysine supplementation (80 mg/kg/day in an orange flavored drink) was conducted in stunted (height-for-age Z-score <-2SD, n=24) 6-11 years, South Indian children to evaluate urinary biomarkers for EAAD, in comparison with control non-stunted children (n=27) who received an orange flavored placebo drink for 3-months.

At baseline and monthly intervals, clinical examinations, height, weight, circumferences (cranial, forearm, upper-arm, waist), skin folds, muscle strength, food intake-recalls were measured, along with urine and blood sampling at baseline and end-line. The urine metabolome was analyzed by Q Exactive orbitrap-based mass spectrometer using Compound Discoverer software (Thermo Scientific). Differences in anthropometry were analyzed by t-test and repeated measures models.

Anthropometric measurements were significantly different ($p<0.038$) at baseline and after 3-months ($p<0.0077$). Preliminary urinary metabolomic profiles showed a difference between groups in lysine-related metabolites at baseline and alterations with lysine intervention. Metabolites of tryptophan degradation and utilization, threonine, methionine, cysteine, and branched chain amino acid biosynthesis pathways were altered at baseline between groups and with lysine intervention.

The urine metabolomic profile with EAAD is different between stunted and non-stunted children at baseline and in response to lysine supplementation. A validation of urine metabolomic profiles using the blood metabolomic profiles could provide more insights towards designing targeted nutritional therapies.

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Enhancing the mobility resolution for co-eluting compound classes during plasma characterisation using multi-sequence IMS^n acquisitions.

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Keywords: Lipids, Ion Mobility, HILIC

Abstract

Human plasma comprises of many small molecules including amino acids, organic acids, and larger lipid classes. To accurately characterize this biological fluid requires multiple extraction and complimentary LC-MS acquisition methodologies to deconvolve certain compounds not efficiently resolved across methods. When analyzing small polar analytes by HILIC based methods, many moderately polar and non-polar compounds e.g. lipids are also present but co-elute in class specific regions or at the solvent front, meaning specific analytical methods must be employed to separate out these features. Coupling ion mobility separation with mass spectrometry has improved the separation of many of these co-eluting compounds, increase the peak capacity and improving spectral quality. But different compounds require different levels of separation to achieve the desired resolution.

The new multi-sequence IMS^n analysis assisted in improving the depth of characterization for plasma metabolomics. Areas of chromatographically resolved small molecules had sufficient separation using a single pass of the cyclic device, whilst larger lipid classes required 5 additional passes to enhance the isolation of co-eluting lipid phosphatidylcholine features. The additional passed for these lipid precursor ions has enabled improved confidence in annotation using drift separated elevated collision energy spectra, reducing the impact of chimeric spectra that result from fragmentation of co-eluting molecules.

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Optimization of Electron Activated Dissociation for Phospholipids Annotation in Lipidomics Workflow

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Keywords: Lipidomics, EAD, Annotation.

Abstract

The aim of lipidomics is to analyse lipid species across all biological samples, which may reveal a comprehensive metabolic network and interactions of lipids. Current paradigms for identification of detectable lipids are rooted on the concept of diagnostic fragments, that usually provide up to molecular species level. These are derived primarily from the spectra measured for pure standards. Albeit this approach is optimised for the analysis of fragments obtained by collisional activation, it fails to capture the richness of MS2 spectra obtained by electron-based dissociation, i.e. electron-activated dissociation (EAD). The latter allows to break virtually all C-C bonds in acyl chains and, thereby, obtain fine-grained spectra that are only partly decoded with canonical diagnostic fragments. Here, we optimise the necessary parameters for the optimal fragmentation of phospholipids for accurate, automated analysis within a standard high-throughput lipidomics workflow.

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Monitoring of propofol metabolism during surgical interventions via exhaled breath analysis

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Keywords: Propofol, breath metabolic profiling, serum concentration.

Abstract

Propofol is one of most frequently used anesthetics in clinical practice to induce and maintain general anesthesia and procedural sedation. It is administered intravenously. Propofol acts quickly, with its maximum effect taking place about two minutes after administration and typically lasts five to ten minutes. Propofol is a relatively safe and efficient anesthetic, however, it has been reported about 1 in 300 patients suffered adverse effects. Therefore, monitoring propofol and its metabolites systemic concentrations during surgical interventions may provide an opportunity to flag such events.

Exhaled breath from patients (n = 7) undergoing surgery was collected in Nalophan bags at baseline (5 min prior propofol administration) and every 30 minutes post-propofol. Collected breath samples were immediately transferred within the hospital for mass spectrometric analysis using secondary electrospray ionization combined with high-resolution mass spectrometry. Paired blood samples were collected during surgery to subsequently determine correlations between exhaled metabolites and systemic propofol levels.

A total of 34 breath samples and 27 blood samples from n = 7 patients were collected. Propofol, along with propofol metabolites 4-Hydroxypropofol, 2,6-Diisopropyl-1,4-quinone and 2-propanol-6-isopropyl-1,4-quinol were consistently detected in exhaled breath of all patients. Large inter-patient variabilities were observed for propofol metabolization. A correlation with r = 0.54 of breath propofol and serum propofol concentration was found.

In conclusion, we showed that breath analysis can be deployed in a real clinical setting to monitor propofol and its metabolites during surgical interventions. Integration of this experimental procedure in clinical practice may provide a more personalized approach towards propofol dosage.

An integrated understanding of the metabolic benefits of a novel double-targeted intervention using genetically engineered probiotic expressing aldafermin with dietary changes on NAFLD

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Keywords: Non-alcoholic fatty liver disease (NAFLD), *Escherichia coli* Nissle 1917, aldafermin, transcriptomics, non-targeted metabolomics

Abstract

Lifestyle changes toward a healthy diet and increased physical activity are the cornerstone interventions in the treatment of non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide. However, due to its increased prevalence, new therapeutic approaches targeting the gut-liver-axis such as the use of microbial therapeutics and gut-hormonal interventions have been suggested. The present study introduces a seven-week double-targeted intervention using genetically engineered probiotic *Escherichia coli* Nissle 1917 to continuously express aldafermin (a non-tumorigenic analog of a human intestinal peptide hormone, fibroblast growth factor 19) along with dietary change (EcNA). The safety, efficacy, and mechanisms of action of the EcNA intervention were demonstrated using a high-fat-diet-induced NAFLD mouse model. The beneficial effects of the EcNA intervention were evidenced by the decrease in body weight, liver steatosis, and plasma concentrations of aspartate aminotransferase and cholesterol. Comprehensive integrated transcriptomics and non-targeted metabolomic analyses further revealed alterations in NAFLD-related genes and metabolites from the host and gut-microbial origin; along with a switch in amino acid, lipid, and their associated receptor signaling pathways. These results suggest the potential efficacy of EcNA in ameliorating NAFLD by decreasing insulin resistance, steatosis, oxidative stress, and maintaining gut-liver axis homeostasis; and highlight the potential of exploring multi-targeted interventions combining microbial therapeutics with the diet for NAFLD.

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How Low Can You Go? Selecting Intensity Thresholds for Untargeted Metabolomics Data Preprocessing

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Keywords: Mass spectrometry metabolomics, pre-processing, feature height intensity cut-offs, natural product.

Abstract

Untargeted mass spectrometry metabolomics is an increasingly popular approach for characterizing complex mixtures. Recent studies have highlighted the impact of data preprocessing for determining the quality of metabolomics data analysis. The first step in data processing with untargeted metabolomics requires that signal thresholds be selected for which features (detected ions) are included in the dataset. Analysts face the challenge of knowing where to set these thresholds; setting them too high could mean missing relevant features, but setting them too low could result in a complex and unwieldy dataset. Our study compared data interpretation for an example metabolomics dataset when intensity thresholds were set at a range of feature heights. The main observations were that low signal thresholds (1) improved the limit of detection, (2) increased the number of features detected with an associated isotope pattern and/or an MS−MS fragmentation spectrum, and (3) increased the number of in-source clusters and fragments detected for known analytes of interest. When the settings of parameters differing in intensities were applied on a set of 39 samples to discriminate the samples through principal component analyses (PCA), similar results were obtained with both low- and high-intensity thresholds. We conclude that the most information-rich datasets can be obtained by setting low-intensity thresholds. However, in the cases where only a qualitative comparison of samples with PCA is to be performed, it may be sufficient to set high thresholds and thereby reduce the complexity of the data processing and amount of computational time required.

Metabolomic and lipidomic approaches in mindfulness-based intervention for health-care students

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Keywords: Metabolomics, lipidomics, stress, mindfulness, untargeted, health-care students, longitudinal.

Abstract

Anxiety, stress, and manifestations of depressive cases are the most prevalent psychiatric disorders and affect an increasingly large proportion of the population worldwide. In the case of health-care professionals, high levels of stress are not only impacting their personal life but can also lead to a decrease in empathy and compassion, which translates to worse patient outcomes and increased rates of professional errors. In this context, strategies such as mindfulness-based intervention (MBIs) are a promising approach to stress management. Benefits related to MBIs have already been reported in several studies, however, the biological and physiological effects of these interventions on stress reactivity are not well understood. We therefore aimed to characterize the biochemical responses to MBI in a cohort of 28 health care students in Geneva, Switzerland. The intervention was organized over 8 weekly sessions and students were sampled longitudinally before and after the program as well as 6 months later, with the control group was undergoing the MBI after the third sampling. Blood serum was collected and analyzed using untargeted metabolomics and lipidomics platforms and stress was evaluated using several well-established protocols, such as the perceived stress scale and the prosocialness scale in adults. A total of 124 metabolites and 774 lipids were annotated. Metabolic profiles were varied with MBI impacting amino acid and nucleoside in females, while males displayed increased acetylneuraminic acid after the intervention. In terms of lipids, lower triglycerides and phosphatidylethanolamines (PE) and higher etherPE were associated to MBI in male volunteers.

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Recent developments in therapeutic drug management using exhaled breath

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Keywords: Breath metabolomics, therapeutic drug monitoring, offline sampling, SESI-HRMS

Abstract

In medicine, serum concentrations of antiseizure medication (ASM) are measured to assure the most efficacious and safe treatment. However, this does not always lead to the intended outcome. As such pharmacotherapy of epileptic patients remains a challenge and would profit from a more comprehensive phenotyping approach. Consequently, recently, we reported the successful implementation of real-time breath analysis using secondary electrospray ionization-high resolution mass spectrometry (SESI-HRMS) to provide reliable estimations of systemic drug concentrations along with risk estimates for drug response and side effects in epileptic patients. In this presentation, we intend to show our latest and ongoing efforts to further improve/supplement the abovementioned method. Firstly, we developed a new method (tested on 40 patients) that involves transitory collection of exhaled breath in Nalophan bags prior to SESI-HRMS analysis. We conclude that the clinically relevant free fraction of valproic acid (VPA, an ASM) can be predicted reasonably well by this combination of off-line breath collection with rapid SESI-HRMS analysis (Lin's CCC between actual and predicted free VPA was 0.6). Furthermore, we would also like to show the results from our still ongoing follow-up of previously mentioned study. Finally, we will present another study, where we successfully used SESI-HRMS to characterize the volatolome (and by extension metabolome) of patients undergoing treatment with Methotrexate (MTX, a chemotherapy agent). In conclusion, various studies presented here make an important contribution to the implementation of breath analysis by SESI-HRMS in precision medicine.

Prior Knowledge as a Compass Guiding Untargeted Metabolomics

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Keywords: Metabolite annotation, prior knowledge, data accessibility.

Abstract

While chemical analysis of complex biological matrices has been limited for long by technology, current analytical instrumentation allows for high-throughput, high-quality data. Thus, the main challenge of metabolomics today is no longer data acquisition, but data interpretation. Over the past decade, computational tools to guide the analysis of metabolomics data have improved remarkably. However, these improvements are often siloed and highly domain-specific, preventing interpretation, translation, and re-use of the data within a broader context. Access to high quality linked open data therefore appears essential to allow cross-fertilization of the different Life sciences. Through different examples, we will show how the inclusion of prior knowledge can be used to improve mass spectrometry-based metabolite annotation.

Integration of bioactivity and metabolomics structural novelty scores for efficiently targeted isolation of novel bioactive compounds from biodiverse natural extract collections

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Keywords: Natural Products, Inventa, Structural Novelty, Wnt pathway, Bioactive compounds.

In natural products (NP) research, efficiently prioritizing samples in natural plant extract (NEs) libraries is crucial for discovering novel bioactive molecules. Here we sought to investigate the UHPLC-HRMS^2 metabolite profiling, dereplication outcomes, and Wnt-pathway inhibition biological screening results for a set of 1,600 NEs. The results of the biological screening initially drove the selection of NEs. Only non-toxic NEs with an inhibitory $IC_{50} \leq 5$ μg/mL were considered, resulting in a subset of only 30 NEs. To increase the chances of selecting NEs containing novel NPs, *Inventa* was used to calculate priority scores for structural novelty. *Inventa* is a computational tool we have developed to automate the prioritization of extracts to assess the potential of NEs to contain novel NPs. It automatically integrates spectral fingerprint specificity, metabolite profiling dereplication results, and previous literature on the profiled organism. The integration of these scores in the selection revealed 9 active NEs with a high potential of containing structurally novel NPs. The most promising candidate was *Hymenocardia punctata*. Further phytochemical investigations of the *H*. *punctata*'s leaves ethyl acetate extract resulted in the isolation of 10 novel bicyclo[3.3.1]non-3-ene-2,9-diones and 3 prenylated flavones. Assessment of the Wnt inhibitory activity of these compounds revealed that three novel bicyclic compounds and two prenylated flavones showed interesting activity without apparent cytotoxicity. This study highlights the potential of combining *Inventa*'s structural novelty scores with biological screening to discover potentially novel bioactive compounds in large natural extract collections.