
YOUNG
BeNeLux
METABOLOMICS centre
YoungBMC Symposium 2025
Amsterdam
Tuesday, 26th August 2025

Dear Colleague,

Thank you very much for registering for this event. It's a pleasure to have you here and during this exciting gathering. Events like these provide a fantastic opportunity for us to come together, share ideas, learn from one another, and foster connections that can lead to incredible collaborations and innovations.

The mission of the YoungBMC is to connect early-career metabolomics researchers from the BeNeLux.
We want to enable this by organizing several events, such as career symposia, lab tours, workshops and social events.

The goals of these events are to get to know each other in a low-key manner, learn from one another, build your own network within the Metabolomics Community, and find potential research partners for your own study.

We hope you have a wonderful time!

Kind regards,

The YoungBMC committee

Committee members

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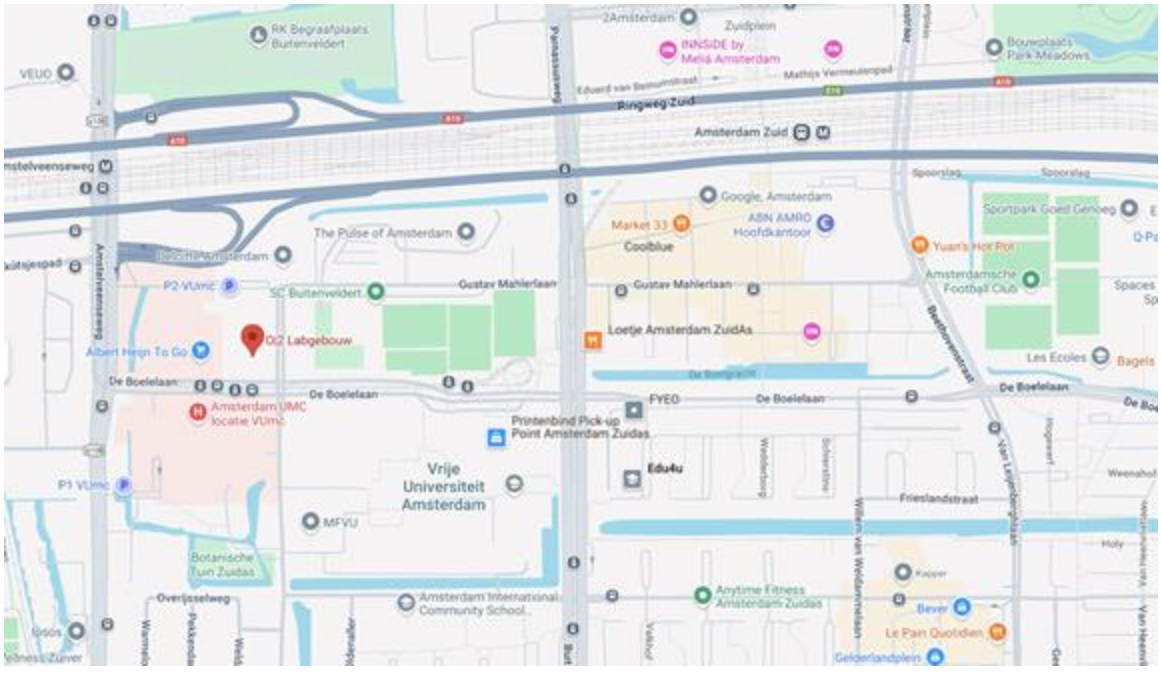
YOUNG BeNeLux METABOLOMICS centre

How to reach the event?

Directions and practicalities

We are located in the **O|2 Lab building**, of the **VU Amsterdam:**
O|2 Lab building
De Boelelaan 1108
1081 HZ Amsterdam
The symposium will take place on the auditorium of the O|2 building lobby on the ground floor – you can't miss it!



From Station Amsterdam Centraal to VU Amsterdam

-***RECOMMENDED*** **-** Metro 52 (Noord/Zuidlijn) towards Station Zuid (10 min) - get off at Station Amsterdam Zuid; see below how to reach O|2 building
-***RECOMMENDED*** **-** Metro 51 direction Isolatorweg (15 min) - stop Station Amsterdam Zuid; see below how to reach O|2 building
-Tram 24 direction VUmc (35 min) - De Boelelaan/VU exit stop; check VU campus map to reach O|2 building
-Tram 5 direction Amstelveen Stadshart - get off at A.J. Ernststraat; check VU campus map to reach O|2 building

From any other destination by train, check: ns.nl
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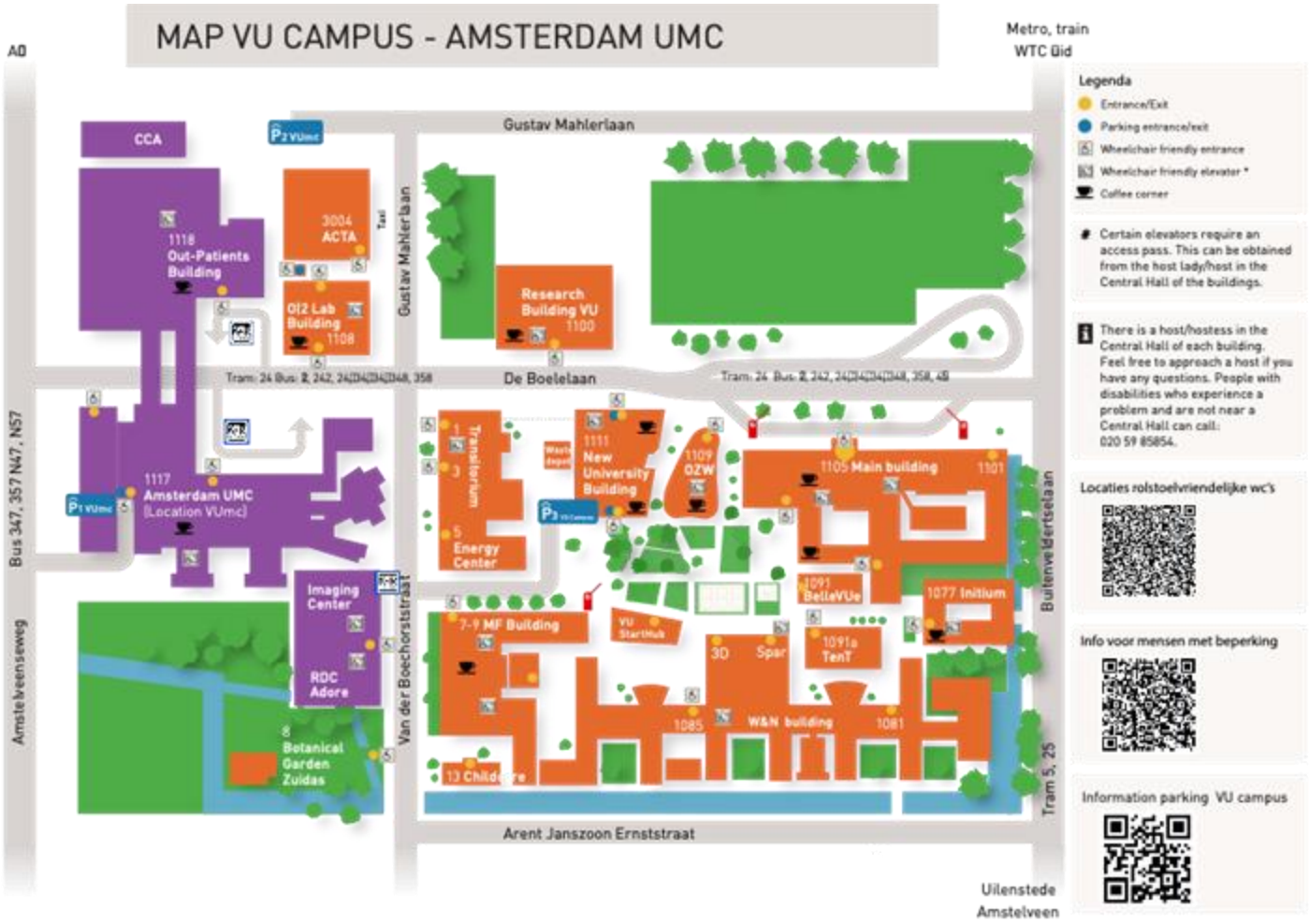
***RECOMMENDED* - It's a 15-minute walk to VU Amsterdam from Station Amsterdam Zuid.**

Alternatively you can take public transport, that takes approx. 2-4 minutes, get off at De Boelelaan / VU:
Tram 5 direction Amstelveen Stadshart; Bus 62 direction Haarlemmermeerstation; Bus 242 towards Mijdrecht Centrum; Bus 341 towards Hoofddorp Spaarne Gasthuis; Bus 346 direction Haarlem Station; Bus 348 direction Uithoorn; Bus 358 towards Kudelstaart

***to avoid* by car: from the A10 ring road to VU Amsterdam**
The A10 Amsterdam ring road can be reached from all directions. Follow the A10 to the Zuid/Amstelveen exit S 108. Turn left at the end of the slip road onto Amstelveenseweg: after about three hundred yards (at VU hospital building) turn left again onto De Boelelaan. VU Amsterdam can be reached via city routes S 108 and S 109.

Parking *is extremely expensive and limited!*
There is a limited amount of parking space around VU itself in De Boelelaan, which has parking bays, and also in Gustav Mahlerllaan. There is paid parking on VU Amsterdam parking lot to the right of the Hospital Outpatient Clinic. There is even more parking space on the east side of Buitenveldertselaan at the junction with Willem van Weldammelaan, within 5 minutes walking distance of VU Amsterdam. A number of parking places for people with a disability are reserved in front of Vrije Universiteit Amsterdam Main Building.

Map VU Campus



Program

Time	Presenter	Title of Presentation
9:30 - 10:00	all	Walk-in
10:00 - 10:15	Sofia Moco and Alessio Ciurli	Welcome and Introduction
SESSION 1: Bioinformatics and MultiOmics applications, CHAIR: Begoña Talavera Andújar		
10:15 - 10:45	**keynote 1: prof dr Bas Teusink**	**Understanding Metabolomics Data: in Need of a Systems Perspective**
10:45 - 11:00	Shauni Loopmans	Comprehensive Molecular Profiling Using the AstralTM Mass Spectrometer: a Single-Sample Workflow for Metabolomics, Lipidomics and Proteomics
11:00 - 11:15	Pablo Vangeenderhuysen	Development of a Fit-for-purpose Data Preprocessing Workflow for Untargeted DNA Adductomics in Exposomics Research
11:15 - 11:30	all	**Break**
SESSION 2: BioMedical applications, CHAIR: Pablo Vangeenderhuysen		
11:30 - 12:00	**keynote 2: dr Esther Zaal**	**Metabolic GPS: Live-Tracking Metabolic Routes in Cancer - Insights from Mass Spectrometry, Metabolomics and Isotope Tracing**
12:00 - 12:15	Victoria Pozo Garcia	Glycine: the Missing Link Between Carbohydrate and Xenobiotic Metabolism in iPSc-derived Hepatocyte Maturation
12:15 - 12:30	Tobias Ackermann	Mapping Anti-Ferroptotic Networks in CRC to Identify Novel Therapeutic Strategies
12:30	all	**group photo!!**
12:30 - 14:00	**Lunch + poster session**	
SESSION 3: Nutrition and veterinary applications, CHAIR: Victoria Pozo Garcia		
14:00 - 14:15	Pranas Grigaitis	Food to Brood: Metabolic Origins and Circulation of Nutrients in Ant Colonies
14:15 - 14:30	Manuel Alfaro-Gómez	Cryopreservation Effects on the Spermatic Metabolome and Lipidome of the Manchega Sheep
14:30 - 14:45	Fien Verdoodt	Medium Chain Triglycerides Induce Metabolic Shifts in Dogs with Idiopathic Epilepsy: a Multi-omics Approach
14:45 - 15:00	Ralph E. C. Monte	Using Machine Learning Models and Postmortem Metabolomics for Prediction of Ketoacidosis-related Deaths
15:30 - 17:30	all	**Lab tour + Meet the expert + poster session**
17:30 - 17:40	Sofia Moco and Alessio Ciurli	Wrap-up
17:40 - 19:00	all	Drinks

Keynote talks: 25 min talk + 5 min (Q&A) | Short-talks: 12 min talk + 3 min (Q&A)

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BeNeLux METABOLOMICS centre

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VU VRIJE UNIVERSITEIT AMSTERDAM

Abstracts Keynote presentations

Keynote 1: Understanding Metabolomics Data: in Need of a Systems Perspective

Bas Teusink

Systems Biology Lab, Vrije Universiteit Amsterdam, the Netherlands

In this talk I will illustrate some concepts from a systems approach to metabolic networks and discuss how they can or cannot be integrated with a purely statistical approach to metabolomics data that is currently the mean stream. It is an invitation to combine fields and to more deeply integrate knowledge, theory and data to improve the understanding of the chemical fluxes that sustain life in health and disease.

Keynote 2: Metabolic GPS: Live-Tracking Metabolic Routes in Cancer *Insights from Mass Spectrometry, Metabolomics and Isotope Tracing*

Esther A. Zaal

Division of Cell Biology, Metabolism and Cancer, Department Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, CL, Utrecht, The Netherlands

Metabolism in cancer is a dynamic network of routes that are frequently rewired to sustain uncontrolled growth and survival. Isotope tracing, combined with high-resolution mass spectrometry and metabolomics, functions as a *Metabolic GPS*, revealing the real-time flow of atoms through cellular pathways. By following isotopically labeled nutrients, this approach uncovers pathway usage, detours, and bottlenecks that define the metabolic landscape of tumors.
In this talk, I will outline key principles of isotope tracing and share insights from our research into cancer metabolism, highlighting how flux analysis exposes hidden metabolic adaptations and identifies potential therapeutic vulnerabilities.

Abstracts short-talks presentations

Comprehensive Molecular Profiling Using the AstralTM Mass Spectrometer: a Single-Sample Workflow for Metabolomics, Lipidomics and Proteomics

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The central dogma of molecular biology traditionally outlines the unidirectional flow of genetic information from DNA to RNA to protein. While this framework captures the core logic of molecular biology, it overlooks the crucial roles of downstream molecular entities such as metabolites and lipids, that are essential for cellular function and regulation. By incorporating metabolomics and lipidomics alongside proteomics in a multiomics strategy, we can expand the central dogma into a more integrative model. Additionally, analyzing these molecular layers within the same sample enables direct mapping of the flow from genetic information to functional phenotype, offering a systems-level view of biology that bridges molecular structure and cellular function.

Here, we present an integrated multiomics workflow to extract and analyze metabolites, lipids and proteins from the same biological sample. As proof-of-priniciple, Jurkat cells were activated using anti-CD3 treatment and extracted using two-phase separation for efficient partitioning of polar metabolites, lipophilic compounds and proteins. Each molecular fraction was processed independently and prepared for liquid chromatography-mass spectrometry using the Thermo ScientificTM AstralTM, which combines high resolution and fast scanning to enable
sensitive and reproducible detection across the diverse molecular classes. Our integrated approach yielded high-quality data cross all three molecular layers with clear separation observed between control and activated Jurkat T cells in multivariate analyses. This single-sample strategy enabled direct cross-omic correlations, revealing coordinated changes in signaling proteins, metabolic pathways, and lipid composition upon activation. Compared to multiomics performed on separate samples, this methodology minimizes biological variability, preserves sample context, and enhances the interpretability of molecular interactions within a single cellular state.

Taken together, we developed a unified approach allowing high-throughput, quantitative profiling of metabolites, lipids and proteins from a single sample source, facilitating direct correlation across omics layers and minimizing inter-sample variability.

Development of a Fit-for-purpose Data Preprocessing Workflow for Untargeted DNA Adductomics in Exposomics Research

Pablo Vangeenderhuysen[1], Matthijs Vynck[1], Liesa Engelen[2], Adrian Covaci[3], Tim Nawrot[2,4], Lynn Vanhaecke[1,5], Lieselot Y. Hemeryck[1]

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DNA adductomics is used to study the whole of DNA adducts in a biological sample and demonstrates potential for application in exposomics studies since it can provide information on the (intermediate) effects and potential risks of environmental exposures. In latest years, LC-MS became the method of choice for characterization and quantitation of DNA adducts. Whereas other methods focus on the investigation of a smaller number of anticipated DNA adducts depending on the research's context (targeted analysis), LC-MS(/MS) based DNA adductomics allows to screen and analyze both known and unknown DNA adducts (non-targeted analysis). To date, a clear view on how to analyze bigger sample batches is however lacking, and preprocessing of untargeted DNA adductomics data is seldomly applied. This work aimed to optimize the DNA adductomics data (pre)processing workflow (in true non-target mode).

In this study, placental tissue samples of 376 mother-newborn pairs were selected from the Flemish ENVIRONAGE birth cohort. DNA from up to 100 mg of placental tissue was extracted at Biobank UZ Gent. DNA adduct extraction and analysis were performed as described by Hemeryck et al. (2015). LC-MS analysis was performed using a hybrid Quadrupole-Orbitrap High Resolution Accurate Mass Spectrometer (HRAM, Q-Exactive™, Thermo Fisher Scientific). The xcms R package was used for data preprocessing, including chromatographic peak detection, retention time correction and feature grouping. Xcms parameters were optimized based on two endogenous DNA adducts and three relevant internal standards. Next, to ensure reliable downstream data analysis, different sample-based normalization methods, i.e. total ion count (TIC), median intensity and different feature based signal correction methods based on pooled quality control runs such as quality control based robust LOESS (locally estimated scatterplot smoothing) signal correction (QC-RLSC) were tested and evaluated based on relative standard deviation (RSD), D-ratio and principal component analysis (PCA).

7518200 chromatographic peaks were detected and grouped into 15781 features. In all runs, 7.04% of feature values was missing and thus likely below the detection limit. All five targets for which the parameters were optimized could be retrieved in the feature list. After feature filtering, retaining only features with an RSD < 0.2 in technical replicates and a D-ratio < 0.4, it was noted that the number of retained features was smaller for the sample based normalization methods as compared to the feature based methods. The difference between the sample- and feature-based methods was also noted in the PCA. In a high quality dataset it is expected that QC and technical replicates cluster tightly in the center of the plot relative to the dispersion of sample runs. In this study, for the sample-based methods, QC and technical replicates clustered less and dispersion of sample runs was limited as compared to the feature based methods.

This work aimed to provide a fit-for-purpose framework for untargeted DNA adductomics analysis. Optimization of xcms parameters allowed for reliable, untargeted detection of known DNA adducts. Quantitative evaluation of normalization methods demonstrated the importance of method choice, as it determines downstream data quality. In this dataset, QC-RLSC came forward as the method of choice, retaining the largest number of high-quality features for further data analysis.

Glycine: the Missing Link Between Carbohydrate and Xenobiotic Metabolism in iPSc-derived Hepatocyte Maturation

Victoria Pozo Garcia, Tugce Su Cobanoglu, Konstantina Riga, Paul Jennings, J. Chris Vos, Sofia Moco

Department of Chemistry and Pharmaceutical Sciences, Amsterdam Institute of Molecular and Life Sciences (AIMMS), Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Modulation of cellular metabolism is a recognized driver in pluripotent stem cell (PSC) development and differentiation. Cellular differentiation is associated with decreasing glycolytic rates and enhancement of oxidative metabolism. Glycine has been identified as an essential nutrient driving the maturation of liver cell models, including hepatocyte-like cells (HLCs) differentiated from induced PSCs (iPSCs). HLCs acquire xenobiotic CYP3A4 activity under a glycine-supplemented nutritional regimen.

In this study, we unravel the link between carbohydrate and xenobiotic metabolism induced by glycine in the differentiation process of HLCs. Using semi-targeted LC-MS-based metabolomics and ^{13}C isotope-resolved metabolomics, we identified not only an expected increase in oxidative metabolism along differentiation but also enhancement of various other biosynthetic pathways. A glycine-rich environment boosted various metabolic pathways: collagen and bile acid biosynthesis, one-carbon metabolism, and heme biosynthesis, all promoting a hepatic phenotype. Enhancement of heme synthesis is of particular relevance during differentiation, as CYP3A4 is a heme-containing enzyme.

This study highlights the metabolic plasticity of *in vitro* iPSC models, undergoing metabolic reprogramming by altering nutrient composition. It also puts evidence of how modulation of central metabolic pathways may be crucial in the development of peripheric pathways, such as xenobiotic metabolism. This research unlocks the potential to maleate cell behavior for personalized therapeutical purposes.

This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 964537 (RISK-HUNT3R), which is part of the ASPIS cluster.

Mapping Anti-Ferroptotic Networks in CRC to Identify Novel Therapeutic Strategies

Tobias Ackermann[1,2], Lidia Atencia Taboada[1,2], Martin Huisman[1,2], Arezo Torang[1,2], Jan Paul Medema[1,2]

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[2] Oncode Institute

Colorectal cancer (CRC) is highly prevalent in the Western world. Despite recent therapy advances, mortality rates are still high. Specific subtypes respond poorly to standard-of-care therapy, underscoring the need for novel therapies. Ferroptosis, which is a form of programmed cell death caused by iron-dependent peroxidation of polyunsaturated fatty acids (PUFAs), could represent such a novel therapy. Ferroptosis is counteracted by the antioxidant activity of redundant, parallel working enzymes FSP1/AIFM2, GCH1, and selenoprotein GPX4. Our goal is to determine the dependence of different CRC subtypes on these anti-ferroptotic pathways.

We find that CMS4 subtype cell lines, the most aggressive CRC subtype, are more sensitive to GPX4 inhibition than cell lines from other subtypes. Moreover, GPX4 inhibitor sensitivity correlates with TSC-complex member TBC1D7, transcription factor MAFB, and phospholipase DDHD2 mRNA expression, identifying them as potential biomarkers. Additionally, inhibiting monounsaturated fatty acid biosynthesis or FSP1/AIFM2 sensitizes other CRC subtypes to GPX4 inhibitor-induced ferroptosis.
To advance this strategy, we are designing a drug repurposing screen to identify FDA-approved compounds that repress GPX4 protein expression. By mapping CRC anti-ferroptotic networks and identifying GPX4-repressing drugs, we aim to establish ferroptosis as a novel therapeutic target, potentially improving treatment outcomes—particularly for aggressive CRC subtypes.

Food to Brood: Metabolic Origins and Circulation of Nutrients in Ant Colonies

Pranas Grigaitis[1], Yuqi Wang[2,3], Andrew F. Brown[2], Helder Hugo[2,3], Brian L. Fisher[4], Bas Teusink[1], Adria C. LeBoeuf[2,3]

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Many insect orders, and most ant species, exhibit eusocial behavior, i.e., individuals live in groups, coordinated through cooperative action and reproductive division of labor. One of manifestations of eusociality is presence of socially exchanged nutritious fluids. In this way, the exchange of readily available molecular building blocks (biomass precursors) opens opportunities for distributing the metabolic labor among colony members: different individuals could specialize in production of compounds for the whole colony. However, both biosynthesis of biomass precursors in situ, and production of them to be shared, come with distinct investments and benefits. So, what profile of (re)distribution of biomass precursors through fluids are the most beneficial to the colony?

To answer this question, we combined multi-omics analyses with computational metabolic modeling. We sampled individuals and socially exchanged fluids from 5 ant species for proteomics and metabolomics analyses. We have found that despite very different lifestyles, the metabolic capacity encoded in the genomes of ants was largely overlapping. On the contrary, different small molecules were found in fluids originating from different colony tissues (also known as castes). By combining the computational models with multi-omics data, we identified metabolic objectives of colony tissues, reasoning that the division of labor among colony tissues fosters overall colony growth. We have correlated this with the observational data which suggests that species with more elaborate division-of-labor patterns perform better, i.e. can sustain larger colony sizes, than their more primitive counterparts (up to several orders of magnitude).

Cryopreservation Effects on the Spermatic Metabolome and Lipidome of the Manchega Sheep

Manuel Alfaro-Gómez [1,2], Begoña Talavera Andújar [3], Emma L. Schymanski [3], José Julián Garde [2], Virginia Rodríguez-Robledo [1,2], María del Rocío Fernández-Santos [1,2]

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Sperm cryopreservation is the gold standard technique for preserving the genetic material of the Manchega sheep (Ovis aries), since it enables long-term storage. However, it has deleterious effects on the sperm, associated with the extremely low temperatures needed. Several metabolites that play an important role in energy metabolism and maintaining the plasma membrane integrity may be involved in the regulation of cryotolerance (1). For this reason, the study of the metabolome and the lipidome of this breed, which has never been described before, could provide novel insights into cellular mechanisms affected by the cryopreservation. To investigate this, sperm samples were collected from 6 Manchega sheep, and the seminal quality parameters were evaluated. Subsequently, the seminal plasma was extracted, and then analyzed using metabolomics/lipidomics approaches following various steps of cryopreservation: fresh (37 oC), cooled (5 oC), and cryopreserved-thawed (–196 oC). Raw data obtained from the metabolomics/lipidomics analyses was preprocessed using MS-DIAL, while MetaboAnalyst 6.0 was employed to perform the statistical analysis. Principal Component Analysis (PCA) revealed that the fresh group clustered separately from the cooled and cryopreserved/thawed groups, for both metabolomics and lipidomics analyses. Moreover, potentially relevant metabolites were annotated, including those related to the TCA cycle (e.g. oxoglutaric acid), which were decreased in the fresh group, while some lysophosphatidylcholines, lysophosphatidylethanolamines, and aminoacides (e.g. L-methionine), were increased in the fresh group. Furthermore, some exogenous compounds that may be related to different kinds of pollution were tentatively identified (e.g. nicotine and 2-naphtalenesulfonic acid). This investigation opens new venues for in-depth studies about the altered metabolites in seminal plasma and how they affect the cellular function during the cryopreservation process. In addition, future studies could investigate the exposome of these animals to elucidate how exogenous compounds might impact the seminal quality.

Keywords: metabolomics, lipidomics, cryopreservation, seminal quality, ovine sperm

1. Ofosu J, Nartey MA, Mo X, Ye J, Zhang Y, Zeng C, et al. Ram sperm cryopreservation disrupts metabolism of unsaturated fatty acids. Theriogenology. 2023 Jul 1;204:8–17.

Medium Chain Triglycerides Induce Metabolic Shifts in Dogs with Idiopathic Epilepsy: a Multi-omics Approach

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Medium chain triglycerides (MCT) are a class of dietary lipids that are rapidly absorbed and transported directly to the liver (Watanabe and Tsujino, 2022), resulting in multiple nutritional benefits for animals and humans (Jadhav and Annapure, 2023). Their mechanism of action for specific diseases, such as epilepsy (Han et al., 2021), remain however unclear . Epilepsy is one of the most common neurological disorders both in dogs (Kearsly-Fleet et al., 2013) and humans (WHO, 2019). However, epileptic seizure management remains challenging, and as such nutritional interventions and manipulation of the gut-brain axis have sparked interest in recent years (Blanquet et al., 2025; Zhu et al., 2024). This study aimed to unravel the underlying mechanism of action of MCT by identifying alterations in the fecal and plasma metabolome and fecal microbiome of dogs with idiopathic epilepsy (IE) following an MCT-diet. To this purpose, a double-blinded placebo controlled dietary trial including 32 dogs with drug-resistant IE was established. Dogs were randomly allocated to an MCT (n = 16) or placebo diet (n = 16) for a period of 3 months. Feces and plasma were collected at the start (T0) and at the end of the dietary trial (T3). The metabolome of plasma and feces were analysed using state-of-the-art liquid chromatography coupled to high resolution mass spectrometry, and the fecal bacterial phylogeny was examined using 16S rRNA sequencing. Targeted metabolites were preprocessed using the TARDIS package (v0.1.3) (Vangeenderhuysen et al., 2025), followed by univariate comparison of the normalized peak areas of T3-T0 between placebo and MCT-diet. Untargeted components were preprocessed using Compound Discoverer 3.3, and analyzed using PCA and OPLS-DA. Subsequently, fecal metabolome, plasma metabolome and fecal microbiome data were integrated using the DIABLO framework (Singh et al., 2019).

MCT-induced alterations in histidine metabolism, e.g. lower fecal histamine in MCT vs. placebo, and energy metabolism, e.g. higher plasma hexanoyl carnitine, were observed using both the single and multi-analyses. Moreover, the MCT-diet resulted in a fecal microbiome with lower β-diversity compared to placebo (Bray-Curtis P = 0.037), and the relative abundancy of Escherichia-Shigella decreased following MCT, but not placebo diet. As such, a role for the microbiota-gut-brain axis in the mechanism of action of MCT was highlighted.

Using Machine Learning Models and Postmortem Metabolomics for Prediction of Ketoacidosis-related Deaths

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Introduction

Ketoacidosis is an imbalanced metabolic state in the body, where ketone bodies are overproduced and stay present in the body, leading to acidification of the blood. Ketone bodies are biomolecules which have ketone-groups and are formed from fatty acids. The three ketone bodies are acetoacetate, beta-hydroxybutyrate, and acetone. There are several reasons for ketoacidosis, including but not limited to, diabetes (diabetic ketoacidosis, DKA), alcoholism (alcoholic ketoacidosis, AKA), hypothermia and starvation. In forensic settings, ketoacidosis-related deaths are approximately 100 cases per year in Sweden, and often one or several ketone bodies are measured if it is deemed necessary. However, determining if someone has died from a ketoacidosis-related death cannot always be done accurately. Postmortem metabolomics have now been used in forensic settings to, e.g., determine cause of death or time of death. Then, only one blood sample and analysis are needed, which could aid in determining the cause of death. Here, we aim to find a machine learning model that can determine (1) if someone has died from ketoacidosis or not and (2) if that is the case, which ketoacidosis-related death it is.

Approach

In this collaborative effort with RMV (Rättsmedicinalverket, English: Swedish National Board of Forensic Medicine), we obtained postmortem metabolomic data from 109 AKA cases, 29 alcoholic controls, 220 DKA cases, 40 diabetic controls, 140 hypothermia cases, 29 starvation cases, and 1,229 hanging cases (controls). For both binary classification (ketoacidosis or not) and multinomial classification (AKA, DKA, hypothermia, or hanging), we used the following supervised machine learning methods: a random forest model, a logistic regression model with a LASSO penalty, and a support vector machines model.

Results and discussion

Overall, different supervised machine learning models can accurately predict cases based on the postmortem metabolome. Thus, this indicates that the postmortem metabolome could be a very useful aid in forensic settings. Further research is needed to validate these results.

Abstracts poster presentations

An LC-MS/MS Workflow to Study the NAD$^+$ Metabolome across Biological Samples

Valentina Ferro[1], Nathalie Reuss[1], Brian van der Kieft[1], Victoria Pozo Garcia[1], Johan van Heerden[2] and Sofia Moco[1]

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Nicotinamide adenine dinucleotide (NAD$^+$) and its reduced form (NADH) are essential coenzymes involved in cellular energy metabolism and redox balance. They function as hydride carriers in redox reactions, supporting cellular energy production and catabolic pathways. Due to its crucial biological role, the study of NAD$^+$ metabolism has become a key focus in understanding cellular metabolic states and their links to disease. In fact, altered NAD$^+$ levels are correlated with ageing, obesity, neurodegenerative diseases and cancer.

The goal of this study is to develop a comprehensive metabolomics workflow for the analysis of the NAD$^+$ metabolome across biological matrices using liquid chromatography coupled with mass spectrometry (LC-MS). The high polarity of these metabolites, their pH sensitivity and the rapid interconversion between the oxidized and reduced forms still represent a challenge in sample preparation and chromatographic separation.

Here, we present an approach based on hydrophilic interaction chromatography (HILIC) coupled with high resolution multiple reaction monitoring (MRM^{HR}) MS. Different chromatographic gradients and mobile phase conditions (e.g. pH) were used to optimize peak shape and intensity, as well as chromatographic resolution. Additionally, different extraction protocols were tested to assess metabolite recovery and matrix composition. Our optimized method includes NAD$^+$ metabolic intermediates, precursors and catabolites. Using this approach, we measured the NAD$^+$ metabolome of human hepatoma cell lines, which are widely used in drug metabolism and hepatotoxicity testing. To ensure a wider applicability, the method was extended to other sample types, including urine, serum, plasma, whole blood, and cerebrospinal fluid (CSF).

By leveraging the sensitivity and specificity of LC-MS, this workflow provides a detailed and comprehensive assessment of NAD$^+$ metabolic pathways across different biological matrices. Such approaches will assist the development of therapeutic strategies around NAD$^+$, by enabling systems-wide investigations of its role in health and disease.

Optimisation of Sample Preparation Methods for Studying Xenobiotic Metabolism using Liver Microsomes

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The human body is constantly exposed to xenobiotics, substances foreign to the body, including pollutants, pesticides, food additives, heavy metals, plasticizers, fertilizers, household chemicals, and pharmaceutical drugs. Exposure may occur through different ways, with the oral route being the most common. Although some xenobiotics, such as pharmaceutical drugs, have therapeutic value, the body automatically recognises them as foreign and may biotransform them into water-soluble metabolites, of easier excretion. Xenobiotic metabolism takes place mainly in the liver, and influences the bioefficacy of drugs, depending on how and how quickly they are metabolised. Xenobiotic metabolism consists of two phases. In phase I, xenobiotics are modified via oxidation or reduction, typically mediated by cytochrome P450 (CYP450) enzymes.

The gold standard for studying xenobiotic metabolism involves mimicking the metabolism in the liver by using liver microsomes (LM). LMs are rich in CYP450 enzymes, making them a suitable in vitro model. Incubating xenobiotics with LMs results in the formation of metabolites, which can be analysed using liquid chromatography coupled to mass spectrometry (LC-MS).

This study aims to optimise the microsomal incubation protocol to a 96-well plate format, allowing high-throughput screening, increased efficiency, and more cost-effectiveness. Key steps include the isolation of microsomes from rat liver tissue, assessment of enzyme activity, optimisation of extraction volumes, and the use of midazolam as a model compound due to its well-characterised CYP450 metabolism.

This research will allow testing more drugs in parallel, in a more efficient format, facilitating the study of xenobiotic metabolism, essential in the drug development programs.

Polymer-Driven Innovation in Ambient Metabotyping: Toward Scalable Pediatric Biofluid Analysis

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Childhood overweight and obesity's rising prevalence and lasting health impacts demand early intervention via precision medicine. Although the metabolome offers valuable insights to guide prevention and treatment, traditional analytical workflows using sample extraction, chromatography and mass spectrometry (MS) are labor-intensive, costly, and challenging to scale. Moreover, biofluid sampling and transport compromise metabolome stability unless stringent cold chain measures are maintained.

To address these limitations, we developed a high-throughput chromatography-free laser-assisted rapid evaporative ionization MS (LA-REIMS) method assisted by biofluid-specific MetaSAMP® electrospun sampling membranes[1]. The latter increase metabolome stability during transport and storage while supporting surface-assisted ambient ionization, reducing matrix effects and increasing metabolome coverage without extensive sample pre-treatment[1]. However, the use of toxic solvents in MetaSAMP®'s production, its limited scalability, and long-term reproducibility still present challenges.

In this study, we explored alternative in-house synthesized polymers as substrates for biofluid collection, storage and ambient ionization, using green solvents and scalable fabrication methods. Two novel polymeric electrospun membrane types were produced as single fiber networks with crosslinking to ensure water stability (confirmed by ATR-FTIR or UV-VIS depending on the polymer). Membrane morphology and fiber distribution were studied via SEM before and after water exposure. Additionally, contact angle measurements were taken to confirm water absorption. Upon impregnation with pooled biological samples (saliva and feces, each pooled from 6 children, n=10 replicates), for both matrices the number of features detected with novel samplers was comparable to or higher than MetaSAMP® and respective raw biofluids (e.g., 2100 features in novel samplers for saliva vs. 1873 features in MetaSAMP® and 1938 features in raw counterpart). Reproducibility improved over MetaSAMP® , though raw biofluids still showed the highest number of features with CV<30%. A similar trend was observed for feces. Our results demonstrate that alternative polymer substrates may address the current limitations, supporting their potential for sustainable, scalable point-of-care biofluid analysis.

[1]De Spiegeleer et al. Sci Adv 9 (23), 2023

Distinct Metabolic Alterations associated with NLRP3 Inflammasome activation Induced by Neurodegenerative Disease-Linked Protein Aggregates

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The NLRP3 inflammasome plays a key role in the microglial innate immune response, and its activation leads to the secretion of inflammatory cytokines. Dysregulation of the NLRP3 inflammasome has been implicated in various neurodegenerative diseases including Alzheimer's disease (AD), Parkison's disease (PD) and Amyotrophic lateral sclerosis (ALS). In both, AD human brain and animal models NLRP3 inflammasome activation is significantly increased, while genetic ablation of NLRP3 mitigates hallmark disease features such as neuroinflammation, amyloid-beta (Aβ) accumulation, and cognitive decline.

The aim of this study was to investigate the metabolic alterations associated with NLRP3 inflammasome activation induced by pathological protein aggregates implicated in neurodegenerative diseases, including TDP-43, Tau, Aβ1−42, and α-synuclein (α-syn). While previous studies have investigated metabolic changes following NLRP3 activation, comparisons of the effects of different disease-associated proteins remain limited. This study seeks to fill this critical knowledge gap. To achieve this, non-target high resolution mass spectrometry (HRMS) coupled to liquid-chromatography (LC) was employed to analyze the media of THP-1 wild-type (WT) and NLRP3 knockout (KO) cells treated with different pathological proteins (n=5 per condition and group). An open cheminformatics workflow using MS-DIAL, ms2query MetaboAnalyst, and R-based software was employed to analyze the LC-HRMS data. To validate and correlate the metabolic changes with inflammasome activation, lactate dehydrogenase (LDH) release, interleukin-1β (IL-1β) secretion, and caspase-1 activity were assessed. Preliminary results indicate that exposure to Aβ, Tau, and α-syn induces distinct metabolic signatures associated with alterations in key intermediates of the tricarboxylic acid (TCA) cycle and closely associated metabolic pathways including amino acids (e.g., glutamine and glutamate) and lipid metabolism (e.g., carnitine and acetylcarnitine). Ongoing analyses aim to define specific metabolite pathways linked to inflammasome activation and determine their potential as biomarkers or therapeutic targets in neurodegenerative diseases.

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Studying the metabolic interplay of subcellular NAD^+ pools

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NAD(H) plays a central role in health and disease making the study of NAD^+ metabolism essential for understanding physiological perturbations, and age-related pathological changes.

The importance of NAD+ in cellular metabolism has generated widespread interest, leading to numerous investigations of its metabolic roles. NAD+ is involved in key pathways such as glycolysis, the tricarboxylic acid (TCA) cycle, and β-oxidation. It also serves as a substrate for several enzyme families, including poly(ADP-ribose) polymerases (PARPs), sterile alpha and toll/interleukin receptor motif-containing protein (SARM), sirtuins (SIRTs), and cluster of differentiation proteins 38 and 157 (CD38 and CD157).

Within cells, NAD(H) is closely associated with mitochondrial function, with mitochondria containing the largest NAD+ pool. This highlights the importance of understanding NAD+ compartmentalization, particularly between mitochondrial and cytosolic fractions.

This study aims to develop a protocol for isolating mitochondria from mammalian cells and tissues to enable NAD(H)-focused metabolomic analysis. The goal is to assess mitochondrial NAD(H) levels and compare them to total cellular levels. For this purpose, a hepatic cell line (HepG2), and murine liver tissue were used as starting material. The isolation procedure is being optimized to obtain pure mitochondria. Purity is assessed by western blot using markers specific to the cytosol, nucleus, endoplasmic reticulum, and mitochondria. The successive and final isolation fractions will be analyzed by liquid chromatography (LC)-mass spectrometry (MS) metabolomics. The NAD^+ metabolome, as well as specific cytosolic and mitochondrial metabolites will be monitored.

With the development of this protocol, we hope to characterize organelle-specific metabolic differences, in distinct cellular NAD^+ pools. Since the largest NAD(H) pool is found inside the mitochondria, significant changes in whole-cell NAD(H) levels may reflect shids within these organelles. For this reason, more in-depth studies focused on mitochondrial NAD(H) levels, could shed light on the interplay between mitochondria and NAD(H) dependent metabolic processes.

ADME Processes of Thiabendazole in Human and Rat *In Vitro* Models

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INTRODUCTION. Thiabendazole (TBZ) is a food preservative, anti-fungal agent and anthelmintic drug which prevents the polymerization of beta-tubulins. Its major metabolite 5-hydroxy-thiabendazole (5-OH-TBZ) has been found in the urine of over 90% of the Swedish population between 2000-2017. Traditionally human health risk assessment is conducted *in vivo*, but there are indications that TBZ is only hepatotoxic in rats while being hepato- and nephrotoxic in mice and humans. In human liver microsomes it has been shown that the major metabolite 5-OH-TBZ can undergo metabolic activation to form nephrotoxic molecules. Organic cation transporter (OCT) is believed to be involved in the uptake in the proximal tubule cells. TBZ is first metabolized by CYP1A2 in the liver and then further metabolized by UGT, SULT and GST.

AIMS. We aim to investigate interspecies differences in the absorption, distribution, metabolism and excretion (ADME) of TBZ in liver, kidney and gut microbiome *in vitro* systems using a tiered approach.

MATERIALS AND METHODS. *In vitro* models including human and rat renal subcellular fractions, RPTEC/TERT1 cells, human and rat primary hepatocytes and human gut microbiome samples were exposed to TBZ or 5-OH-TBZ at different timepoints. The formation of different metabolites over time was explored using LC-MS. Additionally the renal transporter involved in the uptake of TBZ will be studied using suitable OCT inhibitors and quantified by transported TBZ.

RESULTS AND DISCUSSION. Exposure of 99 µM TBZ induced thioredoxin reductase 1 (TXNRD1) in RPTEC/TERT1 after 24 h exposure. Kinetic experiments in RPTEC/TERT1 and primary hepatocytes were performed with non-toxic maximum concentrations of 10 µM TBZ. Phase I metabolite 5-OH-TBZ was detected in rat liver microsomes. Phase II 5-OH-glucuronide formation was detected in human and rat renal microsomes. There was no 5-OH-sulfate measured in human and rat renal cytosol. Additionally, TBZ does not seem to be metabolized by the human gut microbiome.

CONCLUSION. Phase I and II metabolites of TBZ were detected in human and rat liver and kidney *in vitro* models. The experimental data will be integrated into physiologically based kinetic (PBK) models. This will help to improve these *in silico* models and lead to more accurate xenobiotic human health risk assessment.

Keywords: Thiabendazole, ADME, human and rat *in vitro* models

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Discovery of Microbial Metabolite Biomarkers for Early Prediction of Immunotherapy Response in Non Small Cell Lung Cancer

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Lung cancer is the leading cause of cancer incidence and mortality worldwide, with non-small cell lung cancer (NSCLC) comprising approximately 85% of cases. Immunotherapy is considered a breakthrough for NSCLC treatment. However, not all patients respond properly to it, and the underlying mechanism remains unclear. Gut microbial metabolites, particularly short-chain fatty acids (SCFAs), are believed to play a key role in immune regulation and may enhance the immunotherapy response in NSCLC. Our study aims to identify microbial metabolite biomarkers for early immunotherapy response prediction by exploring
the fecal metabolome of 200 NSCLC patients from the DEDICATION-1 study (NCT04909684).

A targeted RPLC-MS metabolomics method was developed for absolute quantification of key gut microbial metabolites, including SCFAs, bile acids, and indole derivatives. To ensure reliable analysis of SCFAs in the RP column, a pre-analytical derivatization with 3-nitrophenylhydrazine was employed to convert the targets to their 3-nitrophenylhydrazones. Derivatization conditions, such as reaction time and reagent concentration, were optimized for method reliability. For fecal sample preparation, freeze-drying is commonly recommended before extraction to control water content variation among fecal samples. However, being volatile organic acids, SCFAs are likely to evaporate during the freeze-drying process. Therefore, we investigated the impact of freeze-drying on the targets by comparing their signals in 150 mg wet samples and those freeze-dried for 24h, 48h, and 72h. Samples were extracted with 50% acetonitrile in water (mg:µL, 1:10) before LC-MS analysis.

Our results show that with 3-nitrophenylhydrazine derivatization, the developed RPLC-MS method achieved reliable and sufficient separation of the targeted metabolites. SCFAs show no significant change after freeze-drying for 24 ,48, and 72 hours compared to wet samples. This optimized method will be fully validated and applied to final fecal sample analysis. Additionally, we will develop an untargeted method to explore unknown microbial metabolites in the future.

Microbial Indoles as Modulators of Hepatic CYP2E1 Activity

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Indole derivatives are a class of molecules produced by gut bacteria from the essential amino acid tryptophan. They are increasingly recognized for their protective and detrimental effects on host health. In this study, we aim to unravel the effect of indoles on xenobiotic metabolism, the process by which the body prevents the accumulation and potential toxicity of foreign substances.

A link between indole-3-carboxylic acid (ICA) and protection against acetaminophen induced liver damage was recently established [1]. A main superfamily of phase I metabolism enzymes, Cytochrome P450 (CYP), were implicated, specifically 2E1 isoform (CYP2E1). To further investigate the impact of microbial indoles on CYP2E1 activity, a structure-activity analysis using a fluorescence-based enzymatic assay with recombinant CYP2E1 was performed. A series of indoles were tested individually and in combination to identify the most potent modulators of CYP2E1 activity. The observed effects were validated using the gold standard of xenobiotic metabolism studies: liver microsomal incubations in combination of LC-MS-based metabolomics, to asses metabolite production and enzymatic activity.

This study will deepen our understanding of the gut-liver axis by linking microbial metabolites to hepatic xenobiotic metabolism.

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Studying Dynamics of Cellular Metabolism by Metabolomics

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Cellular metabolism is dynamic and time dependent. Bioactive compounds, such as pharmaceutical drugs, vitamins or phytochemicals, induce cellular metabolic changes. These metabolic alterations inform about the bioactive's mechanism-of-action or their metabolic fate. The dynamics of metabolite concentrations and pathway turnover are then a proxy of cellular metabolic status.

In our lab, we use a combination of mass spectrometry (MS) and nuclear magnetic resonance (NMR) metabolomics approaches to study metabolism, including central, redox, and xenobiotic metabolism in human mammalian cells. On one hand, NMR gathers several advantages in studying cellular metabolism: it allows screening many cellular metabolites in parallel, in a quantitative fashion, and it even allows monitoring kinetic changes over time. And on the other hand, MS allow us to focus on selected panel of metabolites and study them at high sensitivity.

These strategies – snapshot metabolomics and stable isotope ratio metabolomics - are then applied to study cellular biochemistry, such as stem cell differentiation, oxidative stress and drug metabolic fate, at the mechanistic level. These biochemical readouts, integrated with other functional readouts, contribute to understanding and developing pharmaceutical and therapeutic programs.