Leveraging metabolomics to reveal how metabolic alterations drive colorectal cancer progression and enable patient stratification

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Colorectal cancer (CRC) is projected to have the highest global incidence and mortality by 2040, with rising rates of therapy resistance and an alarming increase among younger individuals. Despite advances in targeted and immune therapies, CRC's heterogeneity continues to limit universal treatment strategies. To address this, a deeper understanding of the mechanisms driving CRC progression is essential for improving patient stratification and enabling personalised care. Our recent work highlights metabolic rewiring as a key driver of disease evolution, with formate emerging as a metabolite of particular interest. Here, we present the implementation of a robust metabolomics strategy for large-scale cohort analysis applied to a well-characterised CRC cohort. This includes tumour and matched normal tissues, as well as longitudinal plasma samples from CRC patients and healthy controls. Our ongoing approach integrates clinical data, transcriptomic analysis and cytokine profiling with in-depth metabolomic and lipidomic analyses, with a particular focus on formate metabolism. This comprehensive multi-omics workflow is currently being developed to minimise technical variability, enhance analytical sensitivity, and uncover meaningful biological trends beyond statistical significance—particularly in longitudinal data. Additionally, our approach enables a multi-compartment analysis that expands biomarker discovery and provides mechanistic insight into metabolic reprogramming in cancer. With this strategy, we aim to demonstrate that the observable metabolic reprogramming in CRC, and the dysregulated formate metabolism, can serve as a powerful tool for patient stratification, predicting disease trajectories, and discovering novel therapeutic targets and strategies. We are also working toward developing predictive models based on combined molecular and metabolic features. The workflow we are developing in the context of CRC is broadly applicable to other disease cohorts, and we believe it holds real potential for advancing precision medicine.

Bioactive Natural Products: From Discovery to Chemo-diversification

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Over the past decade, we have witnessed a revolution in the methodologies applied to natural product research. Current approaches combine powerful metabolite profiling methods for compound annotation and prioritization. Targeted isolation is performed using high-resolution chromatographic methods that closely match those obtained for analytical profiling [1]. Thanks to these tools, minor bioactive compounds were identified. However, in plant extracts, the major compounds are generally common structures, apparently irrelevant for drug discovery. In this context, biotransformation could be an alternative to valorize them. For this proposal, we employ two biotech approaches: reactions using fungal secretomes (mixture of enzymes), and biotransformation using living organisms (whole-cell biotransformation). Saprophytic fungi, such as *Bothyis cinerea* and *Trametes versicolor*, were used as a source of enzymes [2]. Stilbenes, chalcones, phenylpropanoids, and terpenes from the Swiss flora were used as substrates. Biotransformations were first conducted at an analytical scale and monitored by UHPLC-PDA-ELSD-HRMS for the detection of unusual features. Promising reactions were scale-up at the gram scale, and high-resolution preparative chromatography combined with dryload was used for their purification [1]. Enantiomers were purified by chiral chromatography. HRMS, 2D NMR, and ECD were used for structural elucidation. Compounds were evaluated for their antibacterial and antiviral activity against relevant targets. A library of over 280 compounds was generated at the mg scale from common NPs. Some of the compounds obtained presented unique scaffolds and potent biological activities [3]. In most cases, it was possible to propose the enzymes and mechanisms involved in the synthesis of each compound [4]. The applications, possibilities, and limitations of these latest technologies will be illustrated with recent investigations carried out in our laboratory.

[1] Queiroz EF, Guillarme D, Wolfender JL. Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: from metabolite profiling to pure chemical entities. Phytochem Rev 2024; 23: 1415-1442; DOI; 10.1007/s11101-024-09928-w.

[2] Huber R, Marcourt L, Koval A, Schnee S, Righi D, Michellod E, Katanaev VL, Wolfender JL, Gindro K, Queiroz EF. Chemoenzymatic synthesis of complex phenylpropanoid derivatives by the *Botrytis cinerea* secretome and evaluation of their wnt inhibition activity. Frontiers in Plant Science 2021; 12: 805610; DOI; 10.3389/fpls.2021.805610.

[3] Huber R, Marcourt L, Heritier M, Luscher A, Guebey L, Schnee S, Michellod E, Guerrier S, Wolfender JL, Scapozza L, Kohler T, Gindro K, Queiroz EF. Generation of potent antibacterial compounds through enzymatic and chemical modifications of the *trans*-delta-viniferin scaffold. Scientific Reports 2023; 13,: DOI; 10.1038/s41598-023-43000-5.

[4] Huber R, Marcourt L, Félix F, Tardy S, Michellod E, Scapozza L, Wolfender JL, Gindro K, Queiroz EF. Study of phenoxy radical couplings using the enzymatic secretome of *Botrytis cinerea*. Front Chem 2024; 12: DOI; 10.3389/fchem.2024.1390066.

Computational Mass Spectrometry in mzmine - Hands-on Training

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This workshop will introduce non-target LC-MS^2 data processing workflows in mzmine. You will integrate feature detection, compound annotation, molecular networking, and statistical analysis. The new interactive molecular networking in mzmine clusters MS^2 fragmentation spectra by similarity reflecting the structural similarity of their underlying compounds. All the results from these workflows can be exported for downstream analysis in other popular tools like GNPS, SIRIUS, and statistical pipelines. The mzwizard aids in setting up workflows for various instruments and methods, e.g., for mass spectral reference library generation. We encourage you to bring your laptops for the best hands-on experience, but you can also take it as a live demo. Please download the provided dataset and install mzmine before the workshop.

Dataset

The dataset is constituted of HRMS/MS data acquired over *Pseudomonas* strains. It contains wild type or mutant strains together with medium blanks and QCs. More background on the dataset is available at https://doi.org/10.1111/1462-2920.15139.

Downloads

- Download the **dataset zip** file ~500 MB and unpack it.
  https://drive.google.com/drive/folders/12X1fGBcTpkcfSAijlpNXQxjU484xFL6Y?usp=sharing

- Open mzmine and register a **new user** for free or download the workshop user: https://drive.google.com/file/d/1H03wfwFCZEvlFSRiBggcvfFkUlYNTrSy/view?usp=sharing
- **Spectral library**:
  The Google Drive dataset **already** contains the MoNA (LC-MS^2 pos) library Alternative link if downloading from MassIVE

Installation

- **mzmine:** Download and install the latest version - Current **mzmine 4.2.0**. https://github.com/mzmine/mzmine/releases/latest
  There are platform-specific installers for Windows, Mac, and Linux. There is **NO** need to install any other tool or Java Virtual Machine (JVM): Refer to the documentation for installation instructions and post issues on GitHub: https://mzmine.org/documentation/

1. **mzmine paper**: https://www.nature.com/articles/s41587-023-01690-2
2. Development: https://github.com/mzmine/mzmine/
3. Documentation: https://mzmine.org/documentation/
4. Protocol: https://doi.org/10.26434/chemrxiv-2023-98n6q-v2
5. YouTube: https://www.youtube.com/@mzmineproject/playlists

Comprehensive Analysis of Small Molecule MS/MS Data with SIRIUS

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SIRIUS is a powerful tool for the automated analysis of tandem mass spectrometry (MS/MS) data, enabling the identification and annotation of small molecules with high confidence. In this workshop, we will guide participants through the entire SIRIUS data analysis pipeline, from loading LC-MS/MS data to feature detection, alignment, and molecular formula annotation. We will demonstrate how to search structure databases efficiently and validate results by integrating in-silico annotations, analog spectral library searches, and combinatorial fragmentation techniques. Additionally, we will explore strategies for processing large datasets, including classifying thousands of metabolites by chemical class and leveraging confidence scores to identify relevant compound annotations. This hands-on session will equip participants with practical skills for enhancing their MS/MS data analysis workflows.