PhD offer for October 2024

Research institute: CEISAM (Interdisciplinary Chemitry: Synthesis, Analysis, Modelling), MIMM team https://ceisam.univ-nantes.fr/equipe-mimm/

Title of the subject:
Development of new signal identification approaches of 1D, fast 2D NMR and HRMS data for metabolomics

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Funding: Ministerial allowance (MESR) – doctoral contract

Context

Metabolomics, the study of small molecules contained in biological mixtures, is mainly based on the use of two analytical tools, mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. Although NMR is a well-established technique in metabolomics, thanks to its robustness and repeatability and because it provides quantitative and structural information. However, it suffers from strong signal overlaps that prevent the elucidation and quantification of biomarkers.

In this context, since 2015 the CEISAM laboratory has been one of the pioneers in developing methods to improve the separation of signals from complex mixtures by spreading them through a second dimension while providing additional information for their identification. In particular, fast 2D methods using strategies to reduce the acquisition times of conventional 2D methods, such as ultrafast spectroscopy, non-uniform sampling (NUS) and aliasing, present a growing interest in metabolomics. These new techniques could form the basis of more robust and reliable analytical strategies, but their complementarity, both within each other and with MS methods, remains unexplored.

In this context, we have acquired several datasets using these fast 2D methods on a variety of metabolomics issues. We now need to develop a strategy for identifying this type of data as comprehensively as possible, taking advantage of their respective benefits. Two specific approaches will need to be developed, one for metabolic extracts and a second for lipid extracts. In addition, high-resolution MS (HRMS) data, coupled with gas chromatography (GC-HRMS) and liquid chromatography (LC-HRMS) in the reverse chromatography phase and hydrophilic interaction phase (HILIC) and in positive and negative ionisation mode, will also have to be exploited to aid NMR identification and explore NMR-HRMS complementarity. This combination represents a range of analytical methods not previously deployed in metabolomics. The first aim of the project is to explore the ability to discriminate metabolic profiles by applying the widest possible range of analytical methods and placing them in competition through multi-block integration. The second perspective, which is the aim of this thesis work, is to explore the complementarity of these methods in terms of metabolic coverage.

Objective of the thesis

The main objective of this thesis is to achieve the most complete annotation possible of the signals observed on various fast 1D and 2D NMR data sets, as well as that of numerous HRMS data sets. This will make it possible to assess the complementarity of these analytical methods, as well as any redundancies between them. To achieve this objective, it will be necessary to develop new data annotation strategies, particularly for fast 2D NMR techniques, which have been little used in metabolomics to date. The identification of signals at confidence level 1, according to the Metabolomics Standards Initiative, will be achieved by acquiring spectra of pure compounds and spike-in experiments (addition of the suspected compound to a representative sample). The identification of these signals will highlight the metabolites and lipids commonly detected by several of the methods and those detected only by one analytical method. This study will be supplemented by an in-depth analysis of the literature, in order to assess the extent of the metabolic coverage achieved by deploying this approach, and to identify the metabolic pathways not covered. The methods developed will be applied to a variety of problems, such as characterising the exposure of mini-pigs exposed to the endocrine disruptor Bisphenol A (BPA), in collaboration with the LABERCA, and pre-clinical studies in collaboration with Nantes University Hospital.

More specifically, the doctoral student will be responsible for:

- Developing a strategy for annotating signals from rapid 1D and 2D NMR data sets and HRMS data sets, by appropriating and adapting the relevant software tools for identifying metabolites.
- Evaluate the contribution of fast 2D NMR methods in terms of signal identification in comparison with conventional ^{1}H NMR and HRMS methods.
- Confirm the identification annotations by analysing pure compounds by NMR and HRMS.
- Evaluate the complementarity and metabolic redundancy of the different methods and explore any shortcomings by comparing with the literature.

Environment and partnerships

The doctoral student recruited will interact with his/her supervisors, who are specialists in the conventional and fast 2D NMR methods used to acquire the data for this project, and experts in metabolomics. They will also interact with the LABERCA and Nantes University Hospital for the processing and annotation of the HRMS datasets. The thesis work will be carried out in the stimulating collaborative environment of the MIMM team, involving numerous PhD students, post-docs, engineers and researchers in NMR methodology and metabolomics. CEISAM's collaborative environment will be an advantage (particularly in synthesis, to confirm the identification of certain metabolites).

CEISAM is the molecular chemistry laboratory of Nantes University and brings together 5 renowned research teams in theoretical, physical and analytical chemistry, and organic synthesis. The CEISAM laboratory's NMR platform is the largest NMR platform in western France. It has a wealth of equipment, including 6 high-field spectrometers (400 - 700 MHz) and 3 low-field spectrometers. It is also part of the MetaboHub national metabolomics and fluxomics research infrastructure, which will enable the person recruited to work in a rich and stimulating national network. CEISAM is located in the dynamic environment of the city of Nantes, close to the Atlantic coast and southern Brittany.

Profile

The candidate will have a background in analytical chemistry, and should have a strong interest in structural elucidation using NMR and mass spectrometry in a metabolomics context. A strong interest in these analytical methods is expected to ensure a good understanding of the spectra to be exploited. A taste for programming will be an advantage. Given the collaborative nature of the project, good

writing and communication skills in French and English are required. The PhD candidate will be required to pass on their knowledge to other students (Masters, PhD) and to present their work at national and international conferences. As this thesis will be funded by a ministerial grant, candidates must be in the top third of their Master 2 class.

References

- Dona et al., *CSBJ* 2016, **14**, 135-153
- Marchand et al., *Metabolomics* 2018, **14**(5), 60
- Joesten et al., *Metabolomics* 2019, **15**(1), 5
- Letertre et al., *Anal. Chem.* 2021, **93**(1), 500–518