Title : Analytical workflows development in omics toward merging untargeted and targeted methods in mass spectrometry

Key words : Multi-Omics, Analytical development, Mass Spectrometry, Retention modelling in chromatography

Context and state of the art : Metabolomics and lipidomics mass spectrometry (MS) strategies can be divided into untargeted and targeted approaches, each with their own advantages and limitations. The weaknesses encountered in untargeted or targeted approach are the respective strengths of the other. Untargeted metabolomics/lipidomics focuses on the analysis of all the detectable metabolites in a sample, including chemical unknowns. From an analytical perspective, both the measurement and identification of whole metabolomes presents a considerable challenge due to the vast structural heterogeneity of metabolites, their large number and their wide concentration ranges estimated to span 12 orders of magnitude. By contrast, targeted metabolomics/lipidomics is the measurement of defined and known of limited groups of metabolites. The targeted approach was more sensitive, accurate, and specific with the ability to cover a wide dynamic range of concentrations than the non-targeted metabolomics/lipidomics approach.

Metabolite identification remains the central bottleneck in untargeted metabolomics. Whereas in targeted analysis, information on metabolites of interest is established based on chemical reference standards, untargeted analysis uses MS2 data to establish putative identities of metabolites. The use of spectral libraries for identification represents the current practice, whereas the gold standard for MS-based identification is the comparison of tandem MS and retention time (RT) data for a chemical standard and biological sample under identical experimental conditions. But even identification by comparing MS/MS to reference data will result in numerous spurious identifications: Different metabolites can show similar or almost identical fragmentation patterns or RTs. To improve identification quality, combination of independent parameters such as mass, fragmentation pattern, and RT of a chemical reference standard have to be measured under identical analytical conditions and compared to those of the query molecule. The RT dimension can be used to reduce false positive identifications. However, experimental protocols employed in metabolomics are not standardized, and whereas the mass of a metabolite is a molecular property and is consistent across different experiments and laboratories, RTs arise from the combination of the metabolite and the employed chromatographic system. Different column chemistries and solvents lead to different RTs of the same metabolites; unfortunately, this remains true if the chromatographic setup is nominally identical but realized on different instruments. RT is often employed at a late stage of metabolite identification, typically when comparing with a chemical reference standard. However, it is not possible for a single laboratory to purchase and host standards of all possible standards for all putative annotations.

Recently, a new targeted Multiple Reaction Monitoring (MRM) acquisition mode, scout-triggered MRM was proposed by our research group and advantageously replace the conventional time-

scheduled acquisition mode when performing highly multiplexed assays by completely exempting the analyst from the use of absolute retention times. Basically, scout-triggered MRM relies on the monitoring of transition groups successively triggered by the detection of predefined compounds named Scout. Retention segmented windows do not have to be adjusted with all the standards even with different chromatographic set-up. Thus a database of several hundreds of molecules has been created with the different retention orders according to chromatographic conditions.

Thesis' objectives : The objective of phD project aims at investigating how merging untarged and targeted methods in liquid chromatography mass spectrometry. They will include : (1) Development of an analytical workflows that allow annotation of metabolites from scout-triggered MRM database after determining retention model parameters and Scout group attribution regardless of mode of chromatography (reversed phased and HILIC,..). (2) Development of an high-coverage targeted metabolomics workflow from untargeted data to perform targeted metabolomics at the untargeted Scale.
Then, we will applied our approach to discover potential metabolite biomarkers exposition of a fresh water crustacean, *Gammarus fossarum* in ecotoxicology and/or identification and semi-quantitation of metabolites and pathways modulated by adrenergic and cholinergic pharmacological signals in a variety of in vitro liver models.

Research profile : We are seeking a self-motivated and technically skilled person with a strong background in analytical chemistry especially in liquid chromatography, mass spectrometry and omics. Knowledge in python or R will be an asset. Organized person with
a good motivation to learn, autonomy, communication skills, curiosity, are also among important qualities.

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