METABOLOMIC WORKFLOWS ENABLED BY ISOTOPICALLY ENRICHED BIOMASS

Prof. Dr. Gunda Koellensperger

Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna

Vienna, Austria

Metabolomics is an umbrella term referring to the simultaneous analysis and understanding of the small -molecular complement (generally < 1200 Da) in the context of biological systems. Motivations for elucidating metabolites and their abundances range from bypassing complex biological regulatory networks (consisting of genes, transcripts, and proteins) which allows to directly observe the phenotype (an idea conceptualized as omics-cascade) to the construction of complex biochemical models summarized as systems biology.

Metabolomics is an interdisciplinary science. At its core, the analytical task of measuring the full scope of small molecules within biological systems remains challenging. Mass spectrometry is the unrivalled technology in the field. Successful approaches tackle multi-platform measurements defining and addressing sub-omes (such as metabolites and lipids) and analytical tasks (such as targeted and non-targeted analysis), individually. As a consequence, throughput and metabolome coverage are often conflicting goals.

Our group proposed different strategies overcoming this challenge. Our high resolution mass spectrometry methods are based on isotopically enriched biomass, multiplexed extractions and on-line combination of orthogonal chromatographic separations. By tailoring extractions and designing parallel chromatographic separations, we merge metabolomics and lipidomics within one analytical run, significantly increasing the analytical throughput.

In the lecture, a special emphasis will be given to the role of isotopically labelled biomass in metabolomics measurements. In the last decades, labeled organisms such bacteria, yeast or plants have been established deploying standard libraries of stable isotope labeled (^{13}C, ^{15}N, ^{34}S, ^{2}H) endogenous metabolites[1]. Simple organisms growing on non-complex controlled media such as *E. coli* or yeast (*Pichia pastoris)* were particularly successful offering unrivalled enrichment degrees higher than 99% and the production of hundreds of biological relevant labeled metabolites covering the highly conserved primary metabolome.

In metabolomics (including lipidomics), isotopically labeled biomass can be exploited in multiple ways, facilitating both analytical tasks, i.e. quantification and molecular identification. The major applications are (A) absolute quantification in wide targeted analysis of metabolites and lipids[2,3], (B) relative quantification[3,4], (C) credentialing, allowing to identify biologically relevant metabolites based on labeled and non-labeled metabolite pairs[5] and finally (D) offering a validation tool for isotopologue distribution measurement as accomplished in tracer/flux studies[6]. In the lecture, different example workflows will be showcased discussing their analytical figures of merit.

References

1. Rampler E, Abiead YE, Schoeny H, Rusz M, Hildebrand F, Fitz V, Koellensperger G. (2021) Recurrent topics in mass spectrometry-based metabolomics and lipidomics - Standardization, Coverage, and Throughput. *Analytical Chemistry*, *93*:519-545. DOI: 10.1021/acs.analchem.0c04698

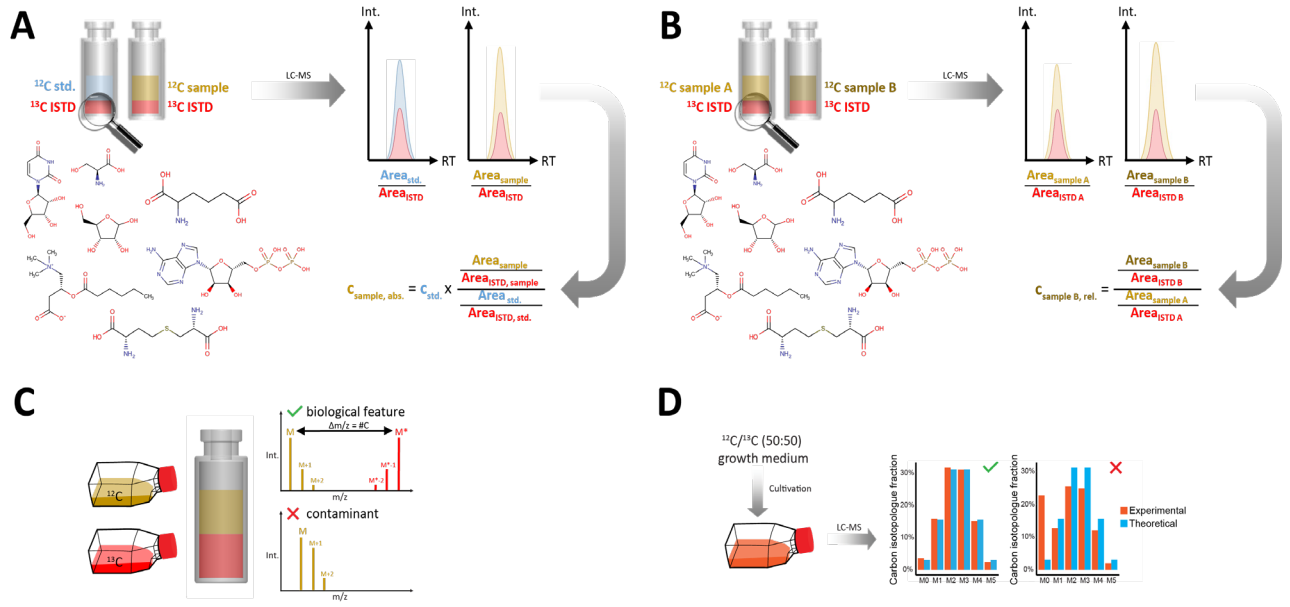

Figure 1: Applications of isotopically labelled biomass in metabolomics (A) absolute quantification (B) relative quantification, (C) credentialing and (D) validation tool for tracer studies/flux analysis

2. Schwaiger M, Schoeny H, El Abiead Y, Hermann G, Rampler E, Koellensperger G. (2019) Merging metabolomics and lipidomics into one analytical run. *Analyst*, *144*:220-229, DOI: 10.1039/C8AN01219A
3. Schoeny H, Rampler E, El Abiead Y, Hildebrand F, Zach O, Hermann G, Koellensperger G. (2021) A combined flow injection/reversed-phase chromatography-high-resolution mass spectrometry workflow for accurate absolute lipid quantification with 13C internal standards. *Analyst*, *146*:2591-2599. DOI: 10.1039/d0an02443k
4. E. Rampler, C. Coman, G. Hermann, A. Sickmann, R. Ahrends, G. Koellensperger, LILY-Lipidome Isotope Labeling of Yeast: In vivo synthesis of 13C labeled reference lipids for quantification by mass spectrometry, Analyst, 2017, 142, 1891-1899, doi: 10.1039/c7an00107j
5. Guijas C, Montenegro-Burke JR, Domingo-Almenara X, Palermo A, Warth B, Hermann G, Koellensperger G, Huan T, Uritboonthai W, Aisporna AE, Wolan DW, Spilker ME, Benton HP, Siuzdak G. (2018) METLIN: A Technology Platform for Identifying Knowns and Unknowns. *Analytical Chemistry*, 90 (5), pp. 3156-3164. DOI: 10.1021/acs.analchem.7b04424
6. Schwaiger-Haber, M., Hermann, G., El Abiead, Y., Rampler, E., Wernisch, S., Sas, K., Pennathur, S., Koellensperger, G.Proposing a validation scheme for 13C metabolite tracer studies in high-resolution mass spectrometry (2019) Analytical and Bioanalytical Chemistry, 411 (14), pp. 3103-3113. DOI: 10.1007/s00216-019-01773-7