Enzymatic and mass spectrometric methodology for the selective investigation of gut microbiota-derived metabolites

Combining metabolomics with chemical biology methodologies

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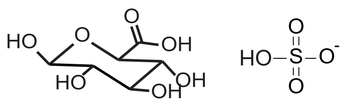
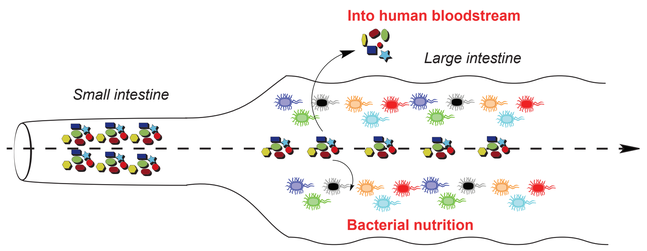
The discovery of biomarkers is the initial and crucial step for the development of new and sensitive diagnostic tools. The detailed investigation of small molecule metabolites in human samples including serum, plasma, urine, feces and tissues, carries a great potential for the identification of unknown biomarkers.[1] These endogenous biomolecules are altered in structure or quantity in a disease state compared to a normal "healthy" state. The field of small molecule biomarkers discovery has been termed "metabolomics", in which all small molecules are analyzed in parallel using diverse separation and detection methods including mass spectrometric analysis.[1] The parallel analysis of these molecules is challenging as these metabolites may differ radically in structure, polarity and other physical properties. Our interdisciplinary research group focuses on the development of new methodologies to overcome limitations in sample preparation and analysis. We are utilizing techniques at the interface of chemistry and biology to allow advanced quantitative and qualitative metabolite analysis for the discovery of disease-specific biomarkers. Further investigation of the biomarker's biosynthesis has tremendous potential for the identification of new drug targets and would lead to new opportunities for disease prevention, management and personalized medicine.

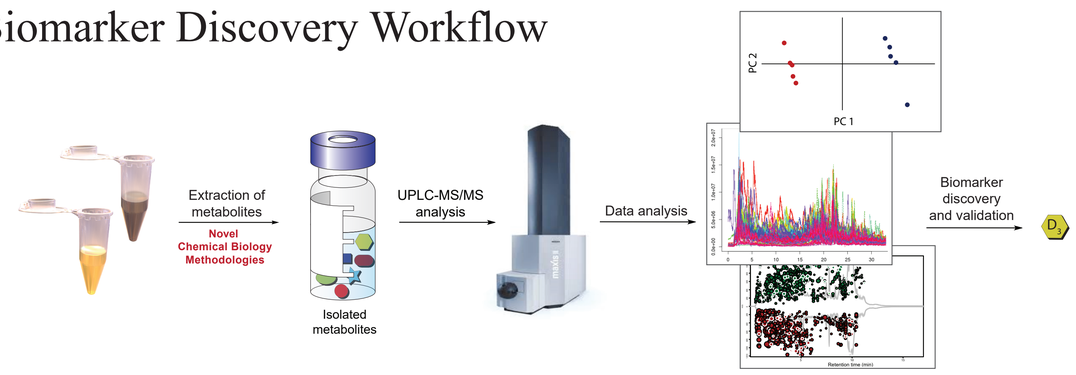
Phase II Modifications and Gut Microbiota

Glucuronidation and sulfation of metabolites are the two major phase II modifications in humans, which play a critical role in the xenobiotics clearance process and gut microbiota-host co-metabolism.

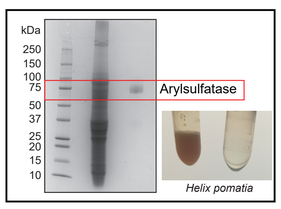




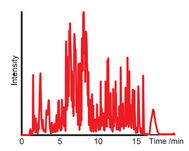
Biomarker Discovery Workflow



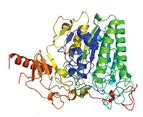
Host microbiota co-metabolism[2,3,4,5]



Fecal or urine sample

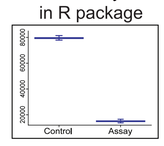


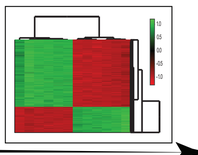
UPLC-MS chromatogram



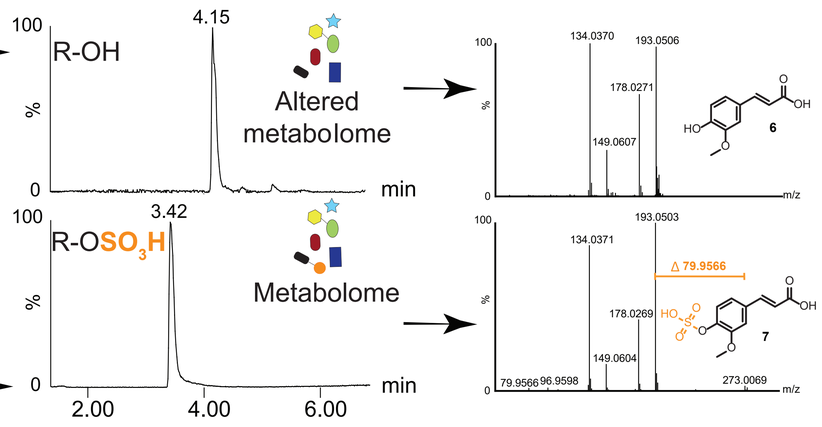
ArS treatment

XCMS analysis in R package



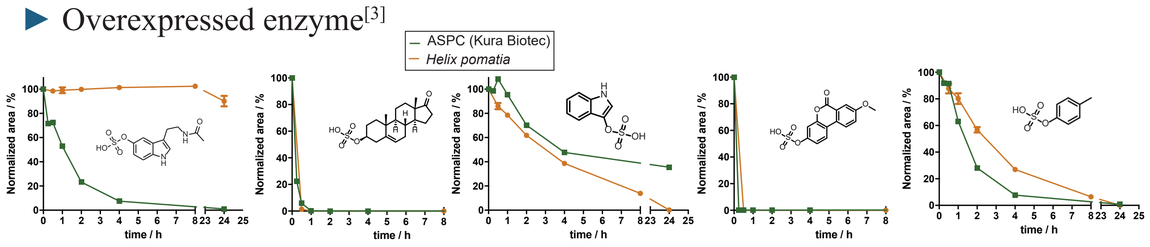


No treatment

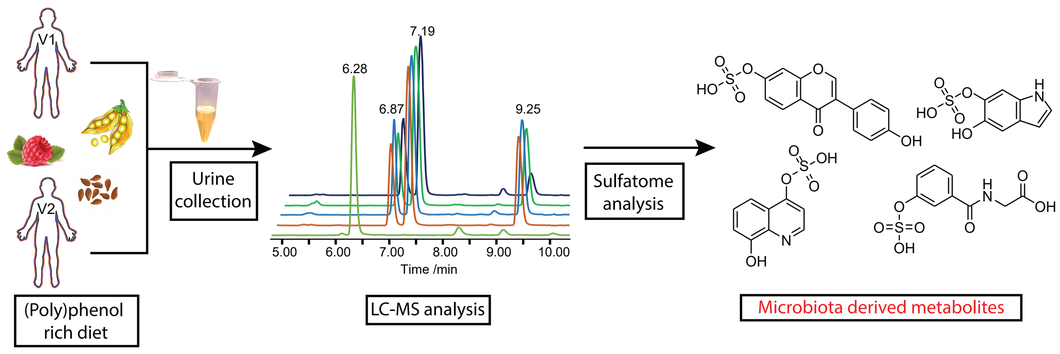


Urine sample analysis	Feature number
Identified features	3620
Potential sulfated metabolites	261
MS^2-validation of sulfate ester	206
Identified by MS^2 fragmentation	36
Identified by synthetic internal standard	9

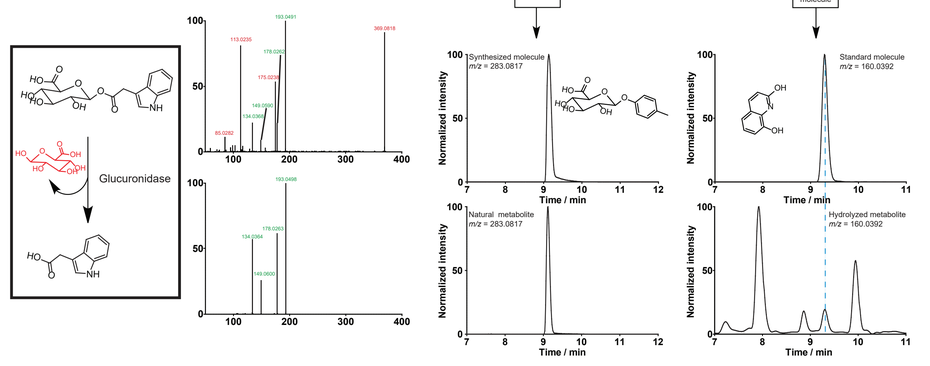
Overexpressed enzyme[3]



Dietary sulfated metabolome analysis[4]

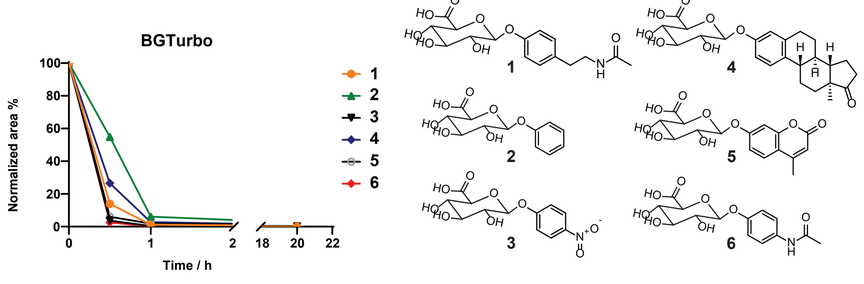


Glucuronidated metabolites[5]

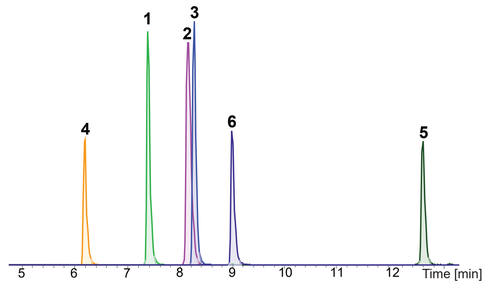


New recombinant enzymes[6]

Full hydrolysis within 1 hour



Excellent chromatographic separation



BGTurbo and ASPC urine application

Name	m/z	Retention time
p-Cresol glucuronide	283.0826	9.66
Indoxyl glucuronide	308.0780	8.61
Dihyroxy-1H-indole glucuronide I	324.0729	5.75
Acetaminophen glucuronide	326.0884	6.07
Urolithin A-3-*O*-glucuronide	403.0678	9.80
11-beta-Hydroxyandrosterone-3-glucuronide	481.2450	13.79

Name	m/z	Retention time
p-Cresol sulfate	187.0075	9.22
Indoxyl sulfate	212.0023	7.71
3-Methoxyphenol sulfate	203.0022	8.73
Thymol Sulfate	229.0544	13.09
Hippuric acid sulfate	258.0082	6.75
Ferulic acid 4-*O*-sulfate	273.0079	8.60
Sinapic acid sulfate	303.0186	8.45

Column: Acquity UPLC HSS T3 1.8 µM

References

[1] J. A. Gilbert, M. J. Blaser, ... R. Knight, *Nat. Med.* **2018**, *24*, 392-400; M. S. Donia, M. A Fischbach, *Science*, **2015**, 349-395.
[2] C. Ballet, M. S. P. Correia, L. P. Conway, T. L. Locher, L. C. Lehmann, N. Garg, M. Vujasinovic, S. Deindl, J. M. Löhr, D. Globisch, *Chem. Sci.,* **2018**, *9*, 6233–6239.
[3] A. Jain, M.S.P.Correia, H. Meistermann, M. Vujasinovic, J.M. Löhr, D. Globisch, *J Pharm Biomed Anal.*, **2021**, *195*, 113818.
[4] M.S.P Correia, A. Jain, W. Alotaibi, P. Young Tie Yang, A. Rodriguez-Mateos, D. Globisch, *Free Radic. Biol. Med.*, **2020**, *160*, 745-754.
[5] M.S.P Correia, M. Rao, C. Ballet, D. Globisch, *Chembiochem*, **2019**, *20 (13)*, 1678-1683.
[6] Collaboration with Kura Biotec - https://www.kurabiotec.com

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