Chemical Metabolomics - Advanced chemoselective probe-based mass spectrometric investigation of the human carbonyl-metabolome

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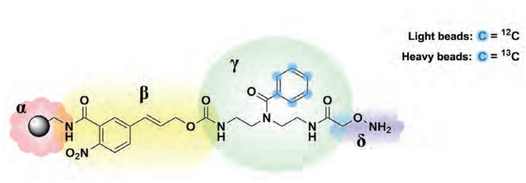
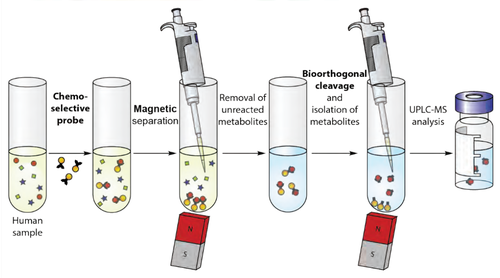
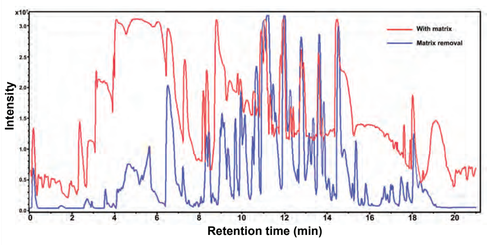
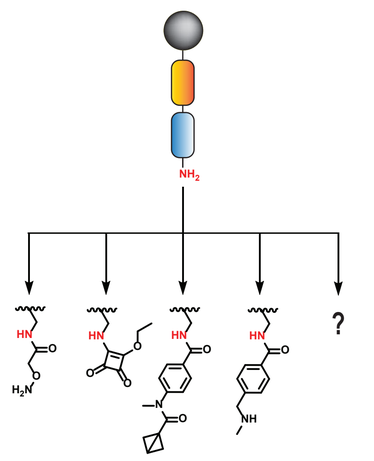
New approaches are required for the discovery of unknown biomarkers. Carbonyl-containing metabolites are a highly reactive compound class that can form conjugates with DNA and proteins. They are also involved in a wide range of biochemical pathways and their dysregulation has been linked to pathological conditions as well as linked to microbiome-metabolism. Six carbonyl-containing metabolites have been classified as carcinogens by the International Agency for Research of Cancer. We have recently developed a new methodology based on chemoselective probes to investigate this compound class after derivatization at enhanced mass spectrometric sensitivity due to removal of the captured metabolites from the matrix.[1-4] To improve the current bottleneck of metabolomics analyses, we have enhanced our analysis with the timsTOF technology for structure elucidation of metabolites through in-silico derivatization, CCS prediction and annotation. We have utilized our Quantitative Sensitive CHEmoselective MetAbolomics (quant-SCHEMA) methodology for the investigation of biological samples.[1-3] This method allows for the simultaneous and semi-quantitative analysis at the femtomole level as well as qualitative analysis at attomole quantities that allows for detection of more than 200 metabolites in diverse human samples. To improve our analysis and metabolite structure elucidation workflow, we treated NIST® SRM® 1950 plasma samples with the quant-SCHEMA method and analyzed the derivatized carbonyl metabolites with a UHPLC coupled to DDA-TIMS-MS/MS. The data was acquired on a timsTOF instrument (Bruker) in positive ionization mode. Data was processed with a development version of MetaboScape 2026, using its in-silico derivatization workflow to automatically annotate and identify the captured compounds.

Chemoselective Probe Design, Workflow and Results [1-7]

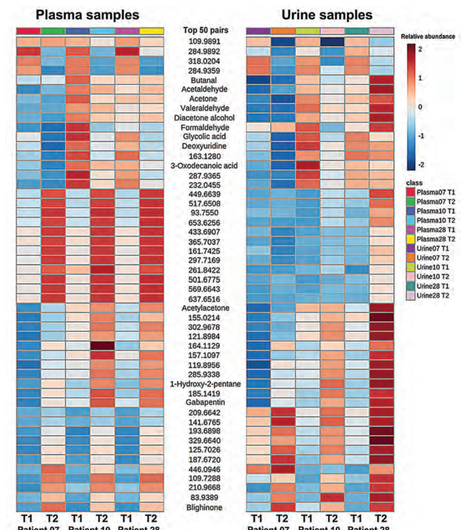


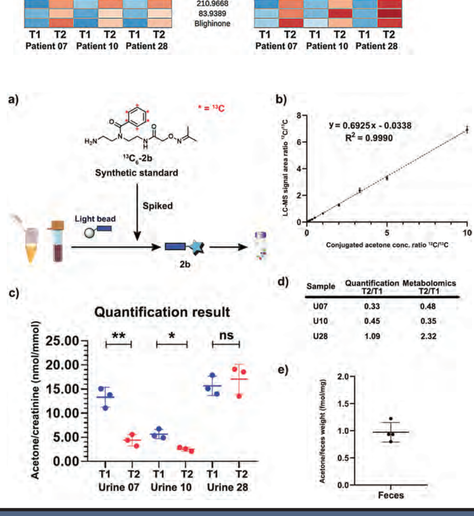


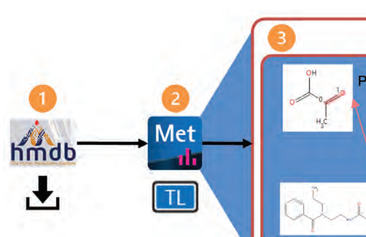




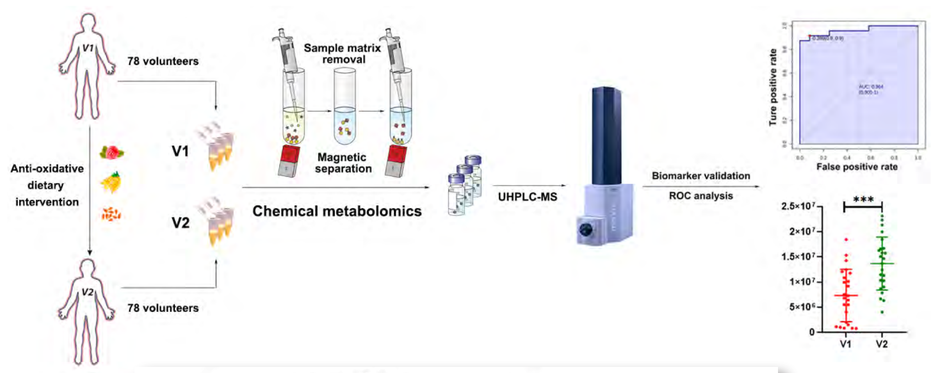
Level of confidence	Metabolite identification methods	Human sample type		
		Urine	Plasma	Feces
1	Validation with authentic synthetic standards	43	42	60
2	Pairs detection by XCMS analysis with specific 6.0201 Dalton difference	203	180	239
3	Annotation by molecular weight from HMDB	669	989	796
4	LC-MS features after initial data processing	5280	4018	5930

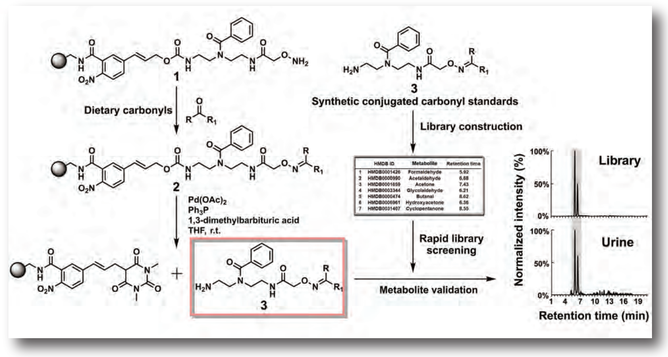


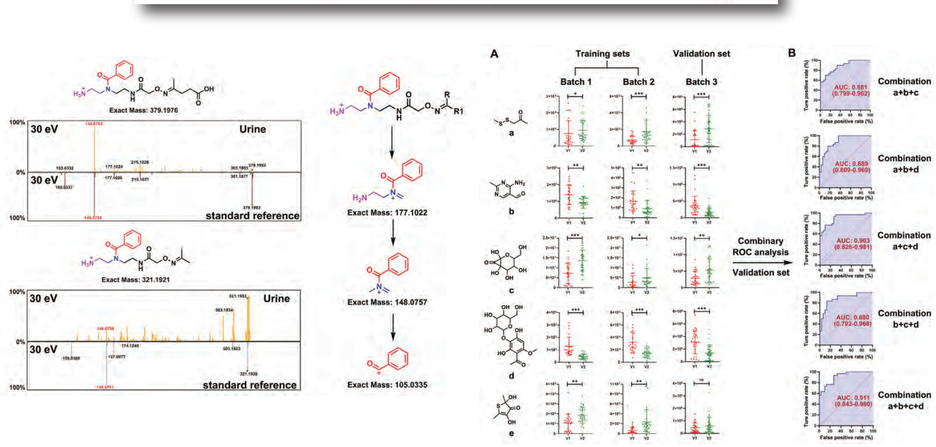




Dietary intervention study [3]







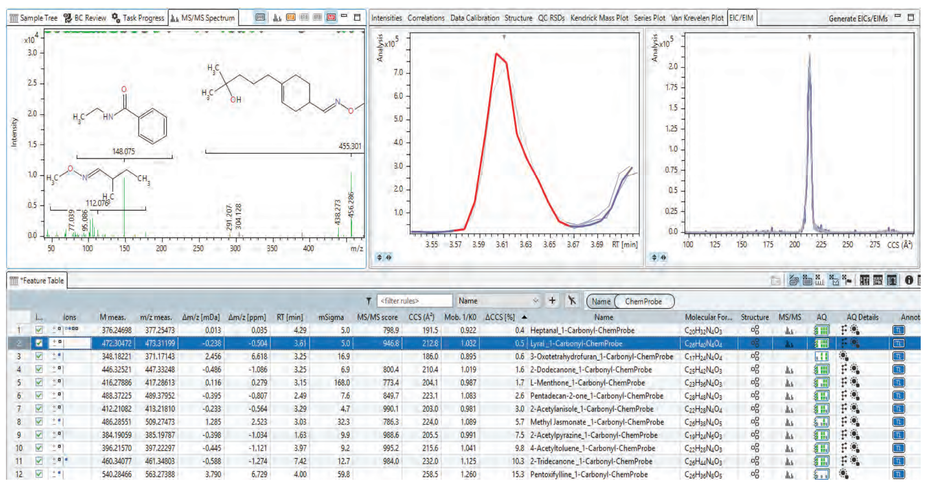
In-silico derivatization with CCS-Predict Pro

1 Applicable to any list of compound structures, for example the **Human Metabolome Database (HMDB)**.

2 Import as Target List into MetaboScape. **Select this Target List and a derivatization mechanism** to annotate a Feature Table.

3 On the fly, structures in the Target List are subjected to ***in-silico* derivatization according to the selected derivatization mechanism.**

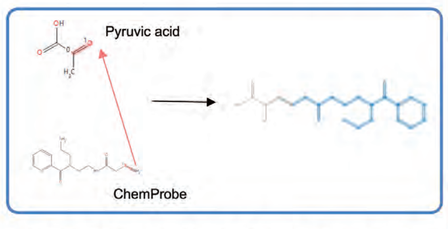
4 Detected Features in the Feature Table are **matched against the compounds in the Target List and their derivatives.**



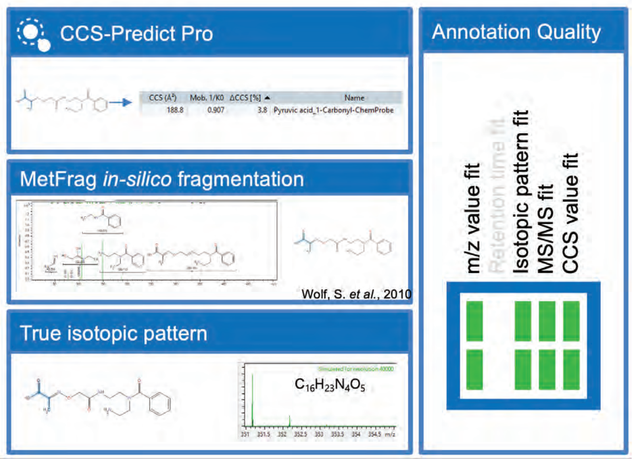
Name	CCS	Neutral Formula	CAS	HMDB
2-Ethylbutyraldehyde	205.1	C19H30N4O3	97-96-1	HMDB0031220
Jasmonic acid	217.3	C25H36N4O5	6894-38-8	HMDB0032797
trans -Cinnamaldehyde	217.0	C22H26N4O3	104-55-2	HMDB0003441
Levulinic acid	187.4	C18H26N4O5	123-76-2	HMDB0000720
Naringenin	221.2	C28H30N4O7	480-41-1	HMDB0002670
2-Octanone	214.4	C21H34N4O3	111-13-7	HMDB0031294
3-Methyl-2-oxovaleric acid	206.1	C19H28N4O5	24809-08-3	HMDB0000491
Safranal	218.5	C23H32N4O3	116-26-7	HMDB0036061
Pyruvic acid	188.7	C16H22N4O5	127-17-3	HMDB0000243
trans -2-Hexenal	208.4	C19H28N4O3	6728-26-3	HMDB0031496
4-Hydroxybenzaldehyde	209.1	C20H24N4O4	123-08-0	HMDB0011718
Dihydroxyacetone	193.6	C16H24N4O5	96-26-4	HMDB0001882
2',4'-Dihydroxyacetophenon	205.5	C21H26N4O5	89-84-9	HMDB0029659
2-Deoxy-D-ribose	205.5	C18H28N4O6	533-67-5	HMDB0245099
Benzaldehyde	204.3	C20H24N4O3	100-52-7	HMDB0006115
Raspberry ketone	209.1	C23H30N4O4	5471-51-2	HMDB0033723
o -Tolualdehyde	208.6	C21H26N4O3	529-20-4	HMDB0029636
Acetone	188.8	C16H24N4O3	67-64-1	HMDB0001659
p -Tolualdehyde	209.9	C21H26N4O3	104-87-0	HMDB0029638

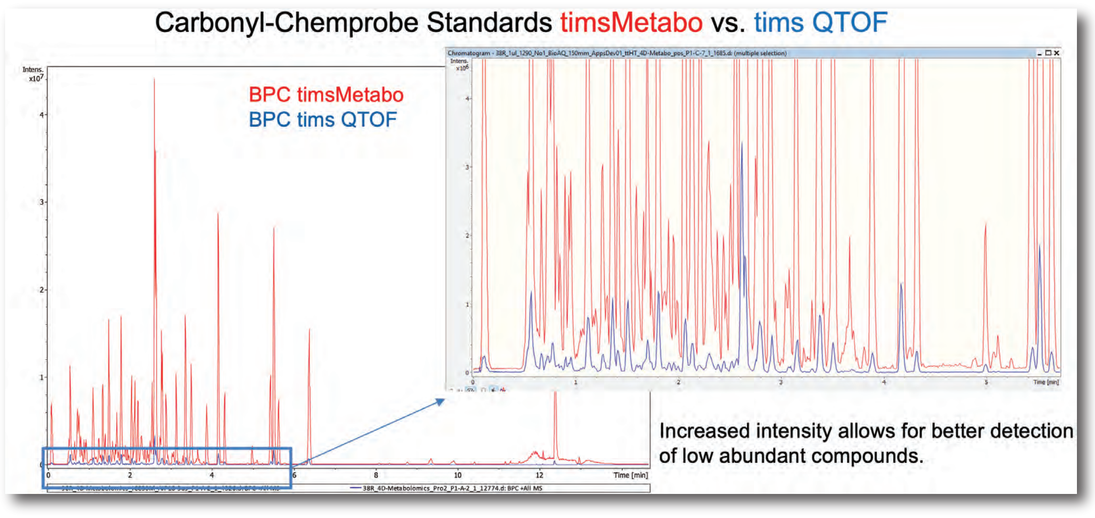
CCS reference library for **>90 metabolites** created for **targeted annotation** at a high confidence level

4 Detected features in the feature table are **matched against the compounds in the Target List with their derivatives.**



Starting from a known compound, e.g. from the citric cycle, the **derivatized structure, its CCS value, and its MS/MS fragmentation are generated *in-silico* in MetaboScape.**





References

[1] W. Lin, L.P. Conway, M. Vujasinovic, J.-M. Löhr, D. Globisch, ***Angew. Chem. Int. Ed.* 2021**, *60*, 23232-23240.
[2] Lin, Gerullat, Braadland, Fournier, Hov, Globisch ***Angew. Chem. Int. Ed.* 2024**, *63*, e202318579.
[3] Lin, Mellinghaus, Rodriguez-Mateos, Globisch ***Food Chem.* 2023**, *425*, 136481.
[4] Lin, Conway, Block, Sommi, Vujasinovic, Löhr, Globisch ***Analyst* 2020**, 145, 3822.
[5] N. Garg, L.P. Conway, C. Ballet, M.S.P. Correia, F. Olsson, M. Vujasinovic, J.-M. Löhr, D. Globisch, ***Angew. Chem. Int. Ed.* 2018**, *57*, 13805–13809.
[6] A. Kaur, W. Lin, V. Dovhalyuk, L. Driutti, M.L. Di Martino, M. Vujasinovic, J.-M. Löhr, M. Sellin, D. Globisch, ***Chem. Sci.* 2023**, *14*, 5291-5301.
[7] W. Lin, F. Romero García, E.L. Norin, D. Kart, L. Engstrand, J. Du, D. Globisch, ***Chem. Comm.* 2023**, *59*, 5843-5846.

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