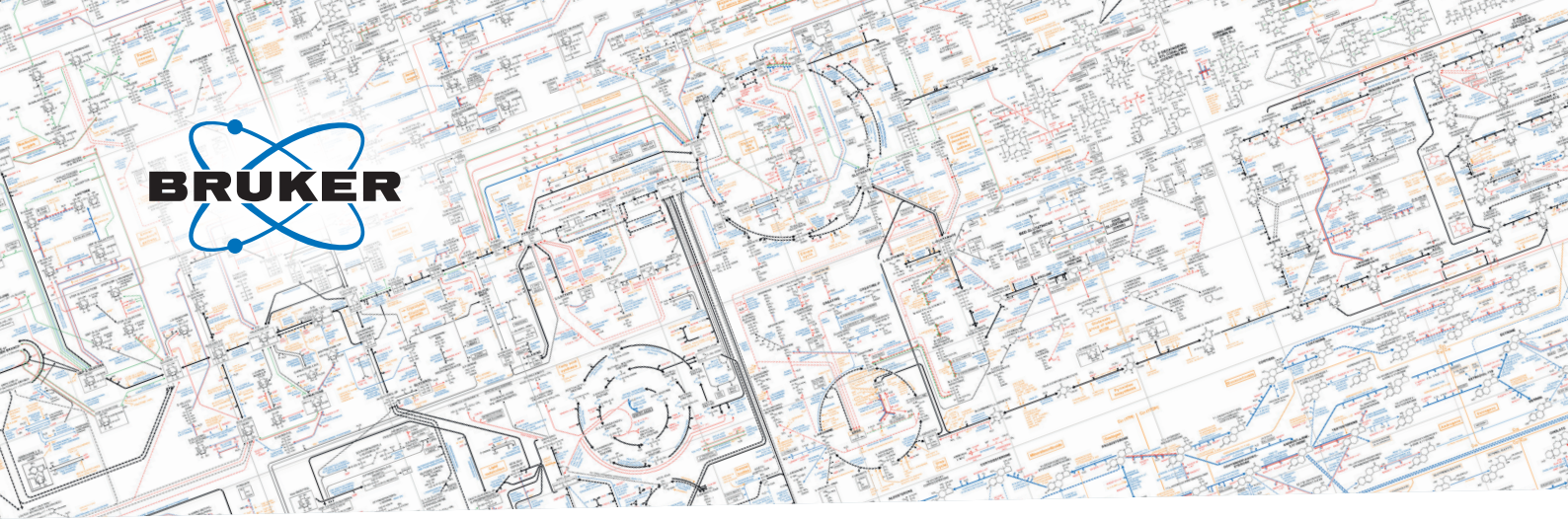


A novel data processing workflow for isotope tracing experiments in LC-MS and LC-TIMS-MS based metabolic pathway analysis and fluxomics

On the frontier of metabolic pathway analysis and fluxomics research, LC-MS and LC-TIMS-MS based isotope tracing experiments offer the opportunity to detect and quantify the incorporation of isotopically labelled atoms into an organism's metabolites.

Introduction

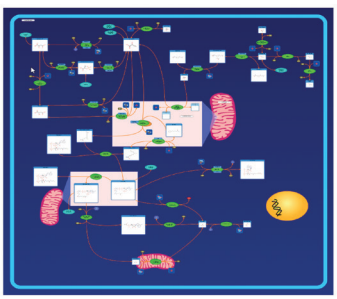
The analysis of isotope tracing data is not yet as mainstream and streamlined as other (e.g. biomarker discovery) workflows in metabolomics and lipidomics. To remedy this, we present here a completely integrated approach for the analysis of differential incorporation of ^{13}C or ^{15}N labelled isotopes in LC-MS data. The approach is fully capable of utilizing TIMS-based CCS-enabled measurements, providing a further dimension for both the separation and annotation of isomeric and co-eluting metabolites and lipids in LC-MS analyses of both labelled and unlabeled samples.

Keywords:
Fluxomics,
Metabolic Pathways,
Stable Isotope Labelling,
Ion Mobility Mass Spectrometry

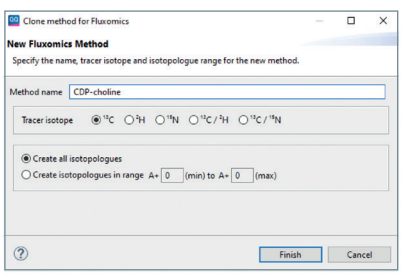
Target Lists

Name	Formula	Adduct	RT	CCS
Oxoglutaric acid	$C_5H_6O_5$	$[M+H]^+$	1.00	
Glutamine	$C_5H_{10}N_2O_3$	$[M+H]^+$	0.92	130.9
Glutamic acid	$C_5H_9NO_4$	$[M+H]^+$	1.10	
Guanosine	$C_{10}H_{13}N_5O_5$	$[M+H]^+$	2.44	163.5
...	...	...	...	...

Metabolic Pathways



http://www.smpdb.ca



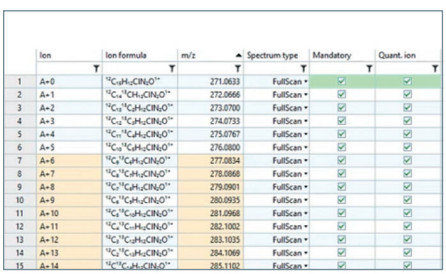


Figure 1
Targeted labeling methods are derived from existing TASQ methods or by utilizing SMPDB pathway maps. Each isotopologue ion trace can be defined with molecular formula, adduct, retention time and optionally CCS values from reference measurements or online repositories [3].

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Methods

The basis for the described workflow is the creation of a target compound list. This can be obtained from SMPDB pathways or from simple, text-based formats. The software constructs all relevant extracted ion chromatograms from the molecular formula and the labeling experiment. Quantitative information for all possible isotopologue traces of the target molecules is generated in the initial data processing step. Successive correction for natural occurring isotopes utilizing the AccuCor [2] algorithm, together with the integrated computation of fractional enrichment, allows for a comprehensive interpretation of the data. The TASQ® batch processing functionality simplifies the elucidation of large-scale tracer experiments. Furthermore, in-depth manual review options and the possibility to correct chromatographic and mobility peak boundaries can generate highest confidence in results from tracer experiments.

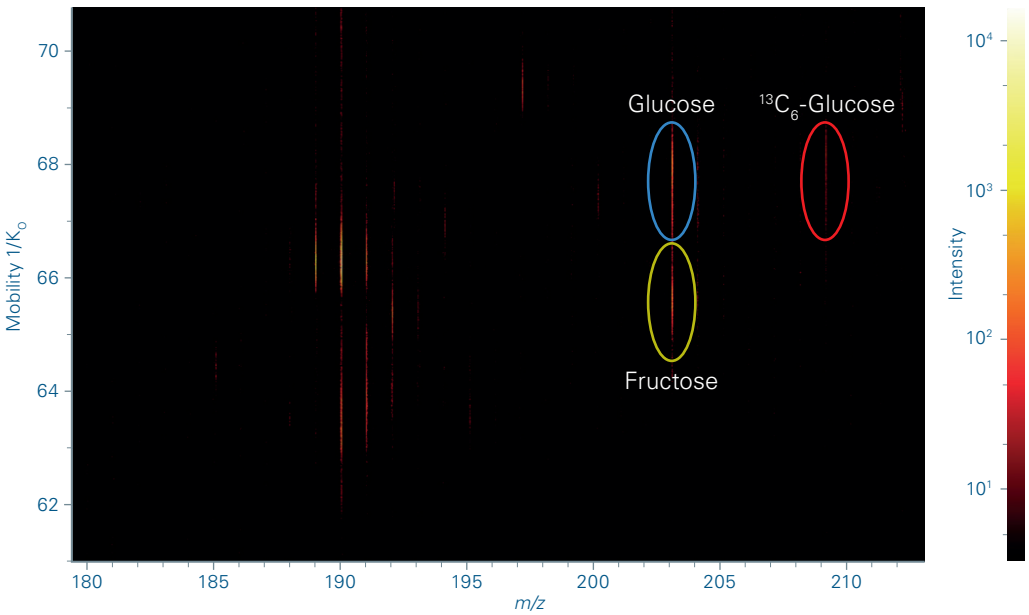

Figure 2
Heatmap display of isobaric compounds separated by ion mobility. The x-axis depicts *m/z* and the y-axis represents the mobility dimension. The ion intensity of measured signals is color coded. The separation of two isobaric compounds as well as the conserved mobility for isotopologues are highlighted.

Results

- **CCS-enabled** - CCS values are stable across isotopologues adding orthogonal separation capability
- Both **single** and **dual tracer** support for **^{13}C**, **^{15}N** and **^{2}H** labeling
- **Accurate mass** and true isotopic pattern support for all generated molecular formulas and adducts
- **Correction of natural abundances** for single and dual tracer experiments for high resolution accurate mass data
- Computation and reporting of **fractional enrichment per compound**

A visual overlay of all isotopologue traces enables rapid data quality assessment both for individual samples and across complete batches. The interactive visualizations also allow for direct manual integration of ion mobility resolved chromatographic peaks.

All respective quantitative information is recomputed directly after manual integration. The seamless, detailed export in ready to use tabular formats simplifies any downstream fluxomics analysis.

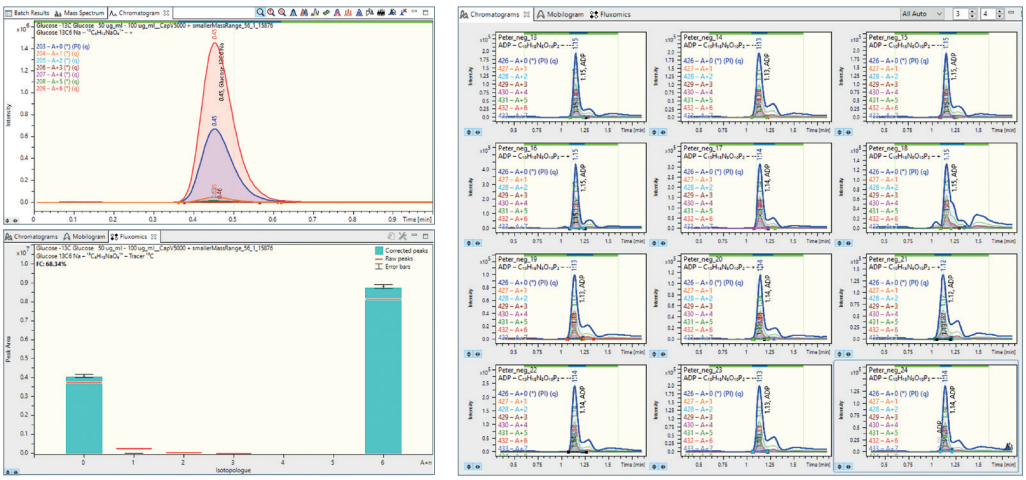

Figure 3
Isotopologue details and batch overview. Comprehensive and interactive visualization of ^{13}C labeling data in the TASQ user interface. Fractional contribution and natural abundance correction are presented. Left: a 1:2 mixture of glucose vs. fully labeled ^{13}C glucose with the respective corrections is shown. Right: batch overview for isotopologues of labeled ADP. ADP data was kindly provided by Dr. Jesper Havelund and Professor Nils J. Faergeman of University of Southern Denmark.

Detailed Ion Results

	Ion Formula	Ion Type	Mandatory	Area	Δm/z [mDa]	Δm/z [ppm]	ΔRT [min]	mSi
1	$^{12}C_{10}H_{13}N_5O_7P^{1-}$	A+0	☑	40007	0.10	0.28	0.05	
2	$^{12}C_9{}^{13}CH_{13}N_5O_7$...	A+1	☑	10667	0.56	1.61	0.05	
3	$^{12}C_8{}^{13}C_2H_{13}N_5O$...	A+2	☑	18482	-0.11	-0.31	0.05	
4	$^{12}C_7{}^{13}C_3H_{13}N_5O$...	A+3	☑	25226	-0.32	-0.92	0.05	
5	$^{12}C_6{}^{13}C_4H_{13}N_5O$...	A+4	☑	37957	-0.20	-0.57	0.05	
6	$^{12}C_5{}^{13}C_5H_{13}N_5O$...	A+5	☑	248455	-0.11	-0.30	0.05	
7	$^{12}C_4{}^{13}C_6H_{13}N_5O$...	A+6	☑	253809	-0.11	-0.30	0.05	
8	$^{12}C_3{}^{13}C_7H_{13}N_5O$...	A+7	☑	188893	0.17	0.47	0.05	
9	$^{12}C_2{}^{13}C_8H_{13}N_5O$...	A+8	☑	128709	-0.25	-0.69	0.05	
10	$^{12}C^{13}C_9H_{13}N_5O_7$...	A+9	☑	46384	0.17	0.48	0.05	
11	$^{13}C_{10}H_{13}N_5O_7P^{1-}$	A+10	☑	1248	-2.35	-6.61	0.05	

Figure 4
Detailed quantitative result visualization. Each isotopologue trace is generated and can be scored with respect to expected mass, retention time and CCS (ion mobility). Comprehensive quantitative information in the form of area, height and intensity is generated for each isotopologue and ready for export in downstream, tabular formats.

Summary

- A novel, integrated and streamlined workflow for high confidence analysis of stable isotope labeling data obtained by either LC-MS and LC-TIMS-MS is presented.
- Ion mobility can contribute to the generation of accurate quantitative information for complex isotopologue labeling patterns by removing background signals and allowing for the separating of co-eluting compounds in the mobility dimension.
- Batch processing of dozens to hundreds of samples is simplified through TASQ automation and workflow wizards. Interpretation of the data in a batch context simplifies quality control and the identification of outlier samples.
- Interactive correction of peak integration boundaries and batch-wise application of manual corrections allows for rapid and consistent generation of high-quality data.
- Quantitative information for downstream analysis with already incorporated correction for natural abundances of ^{13}C, ^{15}N and ^{2}H isotopes.
- Fractional enrichment is computed and reported on a per-compound basis

Conclusion

- ^{13}C / ^{15}N labeling experiments supported in the TASQ 2022b targeted screening and quantitation software
- Automated batch processing in TASQ streamlines the processing of labeling data, scoring with regards to RT, CCS and mass supported
- Interactive review and manual correction capabilities ensure confidence and accuracy in quantitative labeling data
- CCS-enabled workflows allow to exploit the benefits of additional ion mobility separation in 4D fluxomics studies

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