

# What's new in MetaboScape® 2025

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One integrated solution for processing and interpreting of MS based non-targeted Metabolomics, Lipidomics, Phenomics and MALDI Imaging data



## Outline

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*in-silico* Derivatization

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dda-PASEF to prm-PASEF

03

iprm-PASEF SpatialOMx with rule-based lipid annotation via REST API

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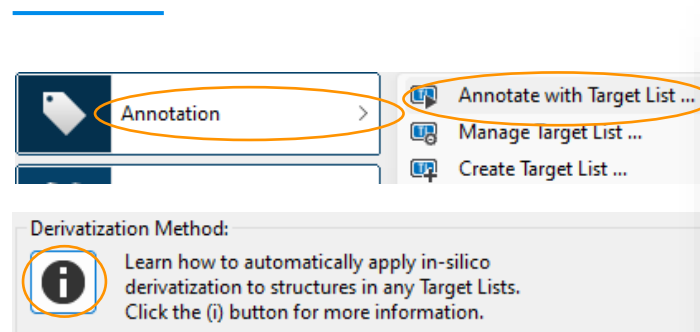
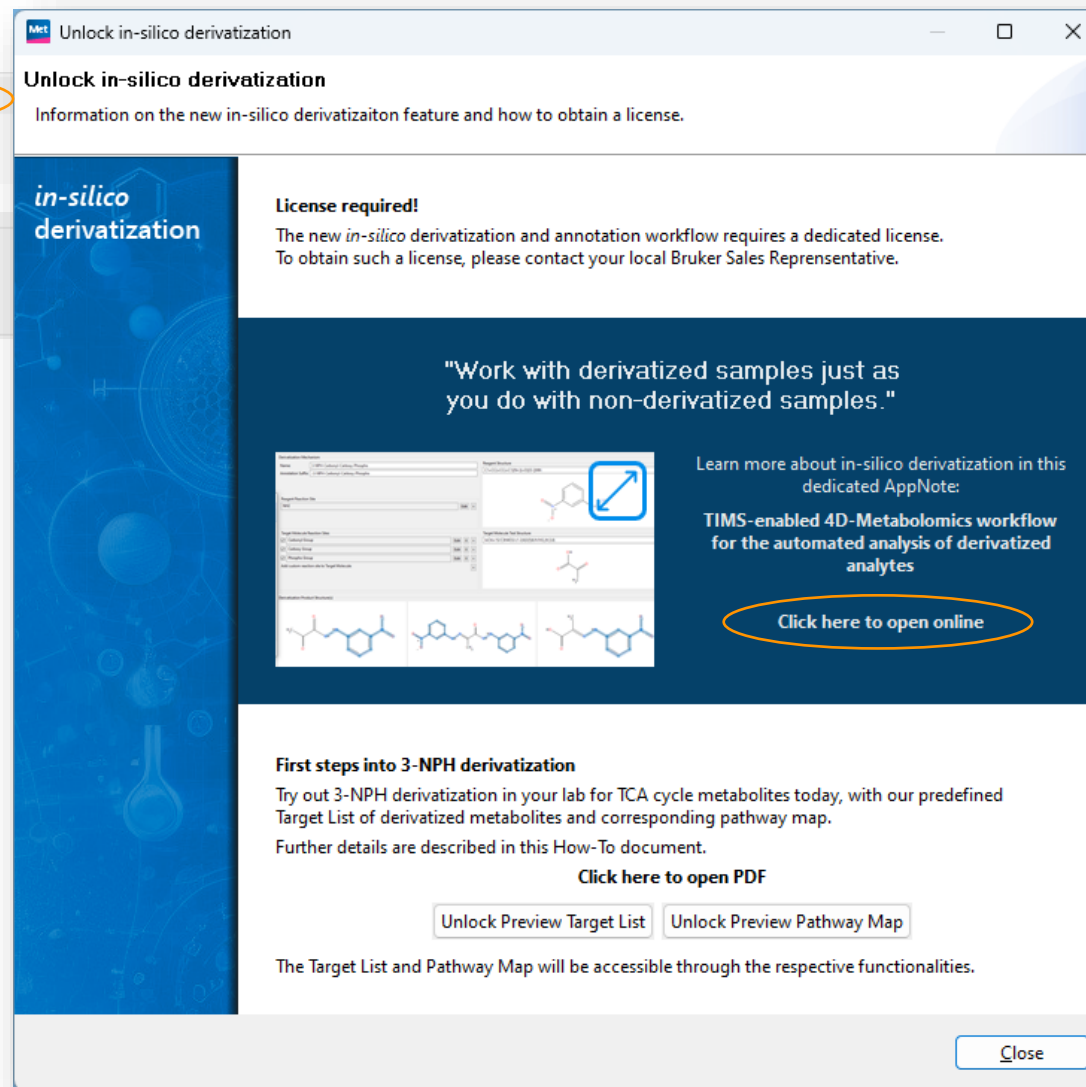
Export images from KMD (Survey) plot

07

Volcano plot colors according to statistical groups

# *in-silico* derivatization with MetaboScape

## Learn more about the new workflow in MetaboScape

The dialog box is titled 'Unlock in-silico derivatization' and contains the following text:

**Unlock in-silico derivatization**  
Information on the new in-silico derivatization feature and how to obtain a license.

**in-silico derivatization**

**License required!**  
The new *in-silico* derivatization and annotation workflow requires a dedicated license. To obtain such a license, please contact your local Bruker Sales Representative.

"Work with derivatized samples just as you do with non-derivatized samples."

Learn more about in-silico derivatization in this dedicated AppNote:  
**TIMS-enabled 4D-Metabolomics workflow for the automated analysis of derivatized analytes**

[Click here to open online](#)

**First steps into 3-NPH derivatization**  
Try out 3-NPH derivatization in your lab for TCA cycle metabolites today, with our predefined Target List of derivatized metabolites and corresponding pathway map. Further details are described in this How-To document.

**Click here to open PDF**

[Unlock Preview Target List](#) [Unlock Preview Pathway Map](#)

The Target List and Pathway Map will be accessible through the respective functionalities.

[Close](#)

The new *in-silico* derivatization feature is fully integrated with Target List annotation, but it requires a dedicated software license.

If the software license cannot be detected, the information shown on this slide will be presented instead.

To obtain such a license, please contact your local Bruker Sales Representative.

➔ [Open AppNote on bruker.com](#)

➔ The HowTo document, a prepared Target List with already derivatized TCA metabolites and the respective Pathway Map are installed with the MetaboScape 2025.

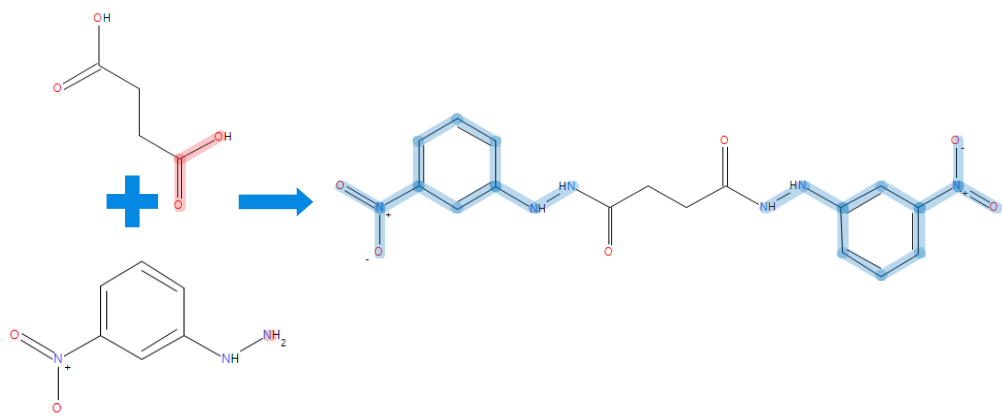
This Feature requires a dedicated software license.



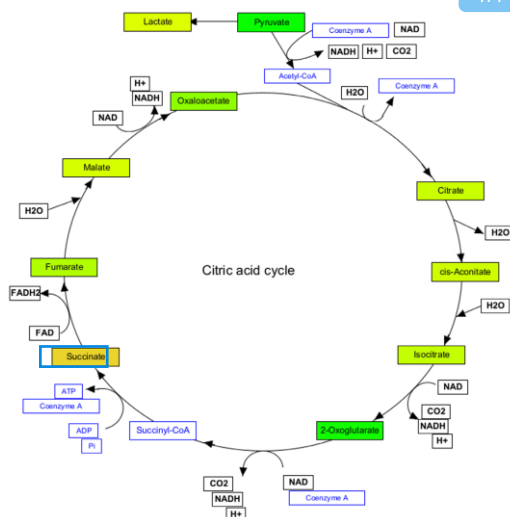
# *in-silico* derivatization with MetaboScape

## TIMS-enabled 4D-Metabolomics workflow for the automated analysis of derivatized analytes

### Derivatization



### *in-silico* Derivatization



CCS-Predict Pro

MetFrag  
*in-silico* fragmentation

True isotopic pattern

### Annotation Quality

m/z value fit  
Retention time fit  
Isotopic pattern fit  
MS/MS fit  
CCS value fit

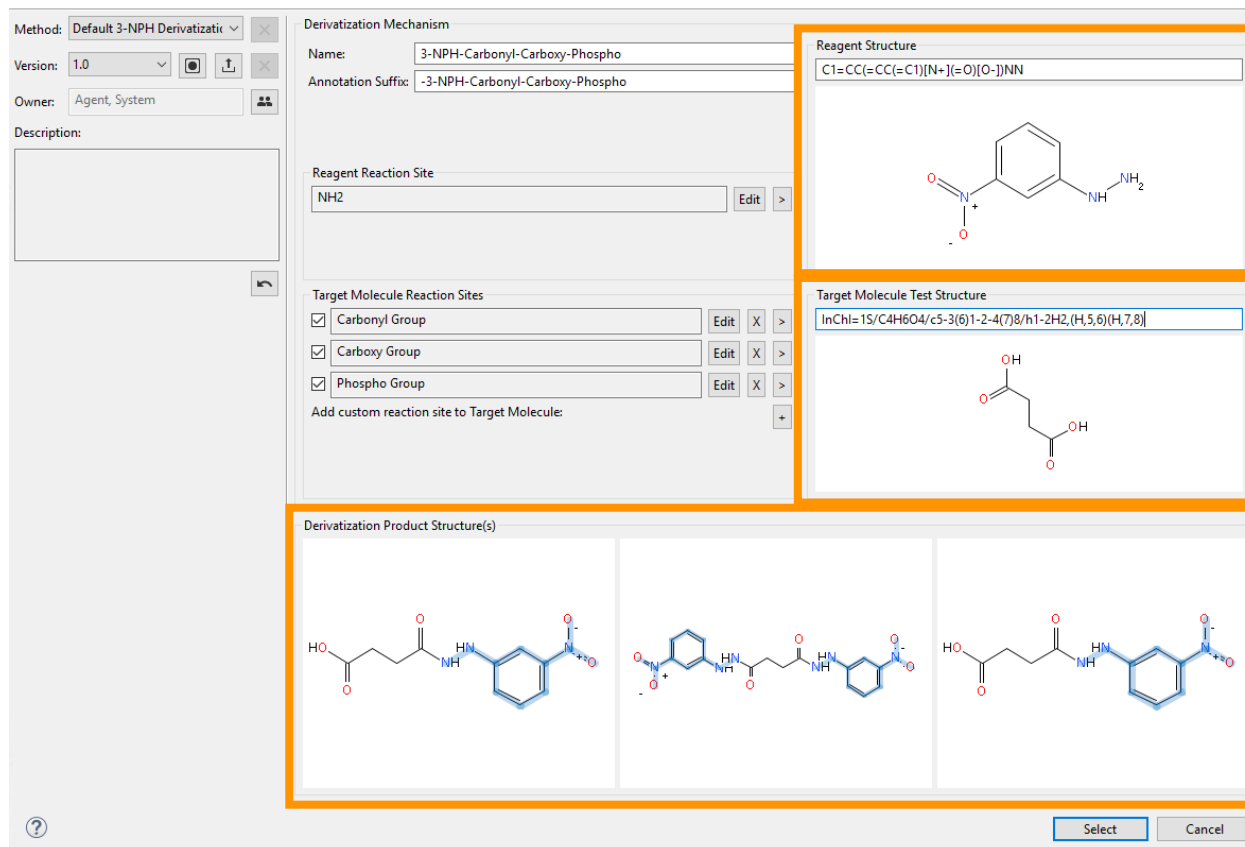


Starting from a known compound, e.g. from citric cycle, the derivatized structure, it's CCS value, and it's MSMS fragmentation are generated *in-silico* in MetaboScape.

## Work with derivatized samples just as you do with non-derivatized samples

# MetaboScape allows to easily tailor *in-silico* derivatization parameters for custom derivatization settings by....

This Feature requires a dedicated software license.



Method: Default 3-NPH Derivatizati...  
Version: 1.0  
Owner: Agent, System  
Description:

Derivatization Mechanism  
Name: 3-NPH-Carbonyl-Carboxy-Phospho  
Annotation Suffix: -3-NPH-Carbonyl-Carboxy-Phospho

Reagent Reaction Site  
NH2

Reagent Structure  
C1=CC(=CC(=C1)[N+](=O)[O-])NN

Target Molecule Reaction Sites  
 Carbonyl Group  
 Carboxy Group  
 Phospho Group  
Add custom reaction site to Target Molecule:

Target Molecule Test Structure  
InChI=1S/C4H6O4/c5-3(6)1-2-4(7)8/h1-2H2,(H,5,6)(H,7,8)

Derivatization Product Structure(s)

Select Cancel



## Your Choice!

Configure your derivatization reagent and how it reacts with functional groups.  
Default method provided!



## Works fully automated with your Target List annotation

All structures in the Target List are subjected to *in-silico* derivatization.



## All derivatization products generated *in-silico*

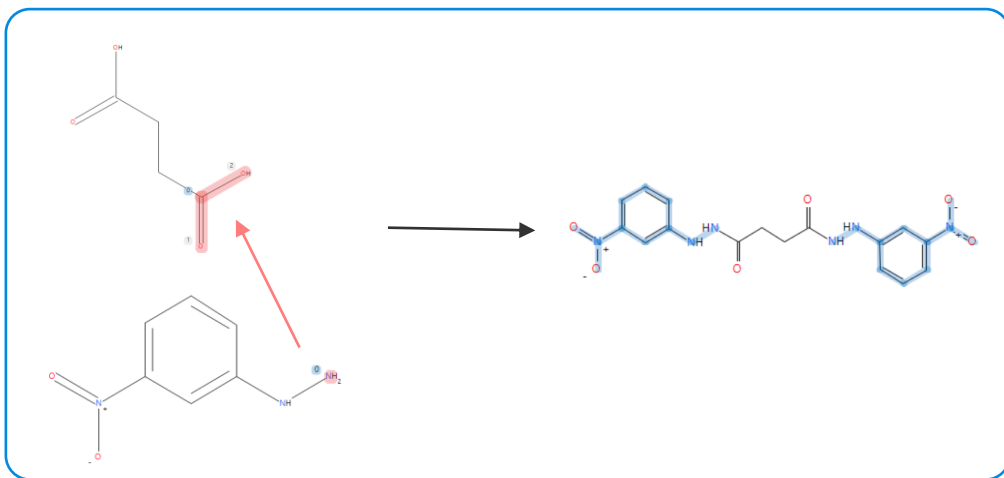
All combinations of derivatization sites are computed and matched against the Feature Table.

# Validate *in-silico* generated derivatization products with CCS-Predict Pro and MetFrag fragmentation

This Feature requires a dedicated software license.

4

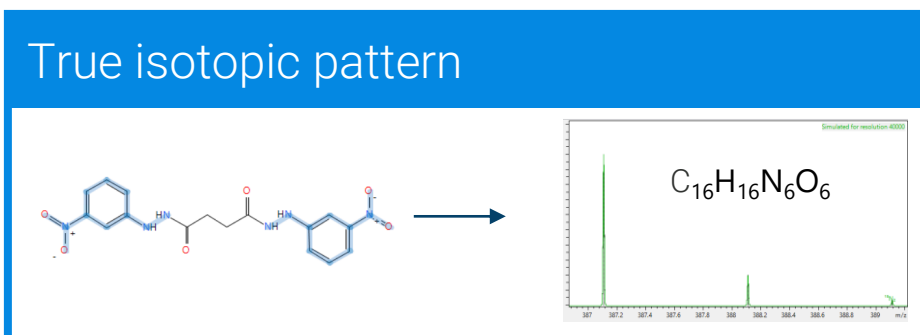
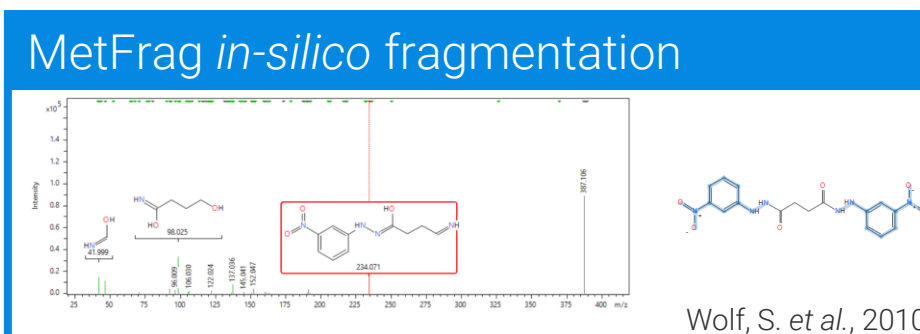
Detected Features in the Feature Table are matched against the compounds in the Target List and their derivatives.



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

### CCS-Predict Pro

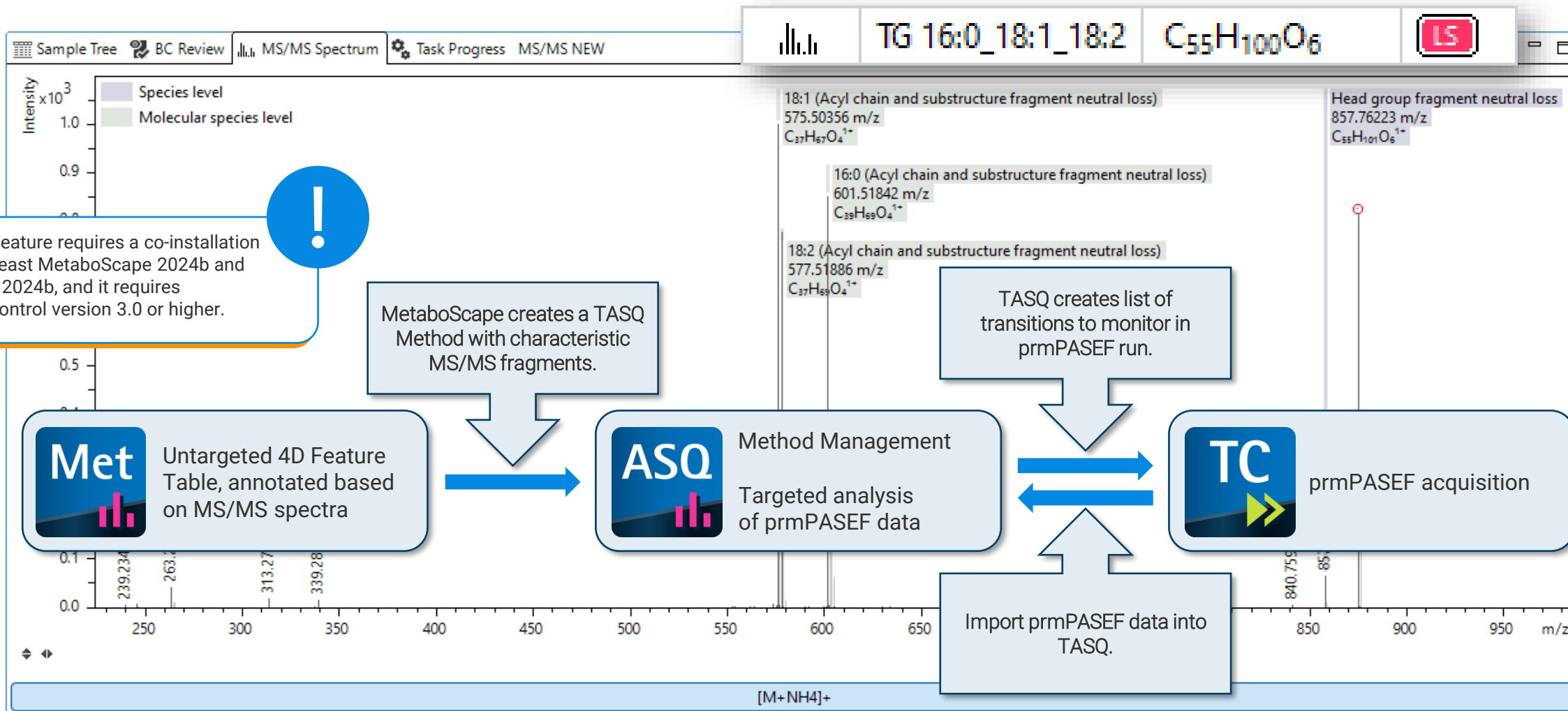
Mob. 1/K0	CCS (Å <sup>2</sup> )	ΔCCS [%]	Name
0.955	198.1	1.8	Succinic acid_2-3-NPH-Ca...



### Annotation Quality

m/z value fit  
Retention time fit  
Isotopic pattern fit  
MS/MS fit  
CCS value fit

# Towards targeted and quantitative 4D Lipidomics or 4D Metabolomics From PASEF<sup>®</sup> to prm-PASEF<sup>®</sup>





# Generate TASQ methods from within MetaboScape

Characteristic and/or abundant fragments from annotated Features

**LS** | **TL** | **SL** | **MF** ...

**Met**

**Create TASQ Method**

MS/MS peak selection strategy (is not applied for Lipid Species annotations)

Select most intense peaks  Select characteristic peaks

Number of most intense peaks: 5

Ion selection strategy

Only Main Ion peaks

Instrument Profile

TOF  TQ  timsTOF

[timsTOF series negative]

Select one or more Features of interest from the Feature Table to incorporate them as precursors (Ion m/z, RT, and CCS) into a TASQ method. Specifically for Lipidomics, i.e., for features annotated with the rule-based lipid species tool, characteristic MS/MS fragments will be included. For all other types of annotations, the most abundant MS/MS fragments and/or characteristic MS/MS fragments (unique in their retention time range) are included.

Upon submission from MetaboScape, the new method is instantly displayed in TASQ. This method can then be further refined and optimized using TASQ's dedicated method editor, providing a seamless and efficient workflow for method creation and curation.

**ASQ**

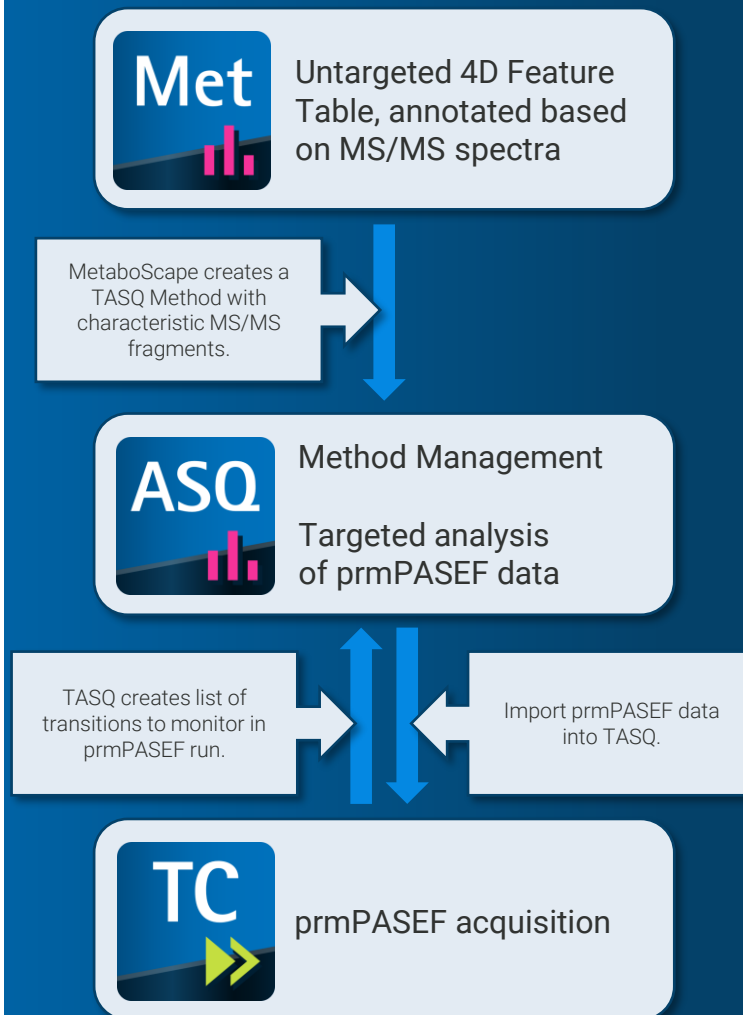
Method Navigator

Applied filter: None

	Ion	Ion formula	m/z	Precursor m/z	Mandatory	Charge	Quant. ion
1	[M+Na] <sup>+</sup>	C <sub>55</sub> H <sub>98</sub> NaO <sub>6</sub> <sup>1+</sup>	877.7256		<input type="checkbox"/>	1	<input type="checkbox"/>
2	[M+NH <sub>4</sub> ] <sup>+</sup>	C <sub>55</sub> H <sub>102</sub> NO <sub>6</sub> <sup>1+</sup>	872.7702		<input checked="" type="checkbox"/>	1	<input checked="" type="checkbox"/>
3	[M+K] <sup>+</sup>	C <sub>55</sub> H <sub>98</sub> KO <sub>6</sub> <sup>1+</sup>	893.6995		<input type="checkbox"/>	1	<input type="checkbox"/>
4	C <sub>55</sub> H <sub>99</sub> O <sub>6</sub> <sup>1+</sup> Head group fragment neutral loss	C <sub>55</sub> H <sub>99</sub> O <sub>6</sub> <sup>1+</sup>	855.7436	872.7702	<input type="checkbox"/>	1	<input type="checkbox"/>
5	C <sub>55</sub> H <sub>97</sub> O <sub>5</sub> <sup>1+</sup> Head group fragment neutral loss	C <sub>55</sub> H <sub>97</sub> O <sub>5</sub> <sup>1+</sup>	837.7331	872.7702	<input type="checkbox"/>	1	<input type="checkbox"/>
6	C <sub>39</sub> H <sub>67</sub> O <sub>4</sub> <sup>1+</sup> Acyl chain and substructure fragment neutral loss	C <sub>39</sub> H <sub>67</sub> O <sub>4</sub> <sup>1+</sup>	599.5034		<input type="checkbox"/>	1	<input type="checkbox"/>
7	C <sub>37</sub> H <sub>67</sub> O <sub>4</sub> <sup>1+</sup> Acyl chain and substructure fragment neutral loss	C <sub>37</sub> H <sub>67</sub> O <sub>4</sub> <sup>1+</sup>	575.5034		<input type="checkbox"/>	1	<input type="checkbox"/>

Both MS ions and MS/MS fragments are reflected in the TASQ method. The method can still be edited.

This Feature requires a co-installation of at least MetaboScape 2024b and TASQ 2024b, and it requires timsControl version 3.0 or higher.







This Feature requires  
SCiLS Lab 2025a.



# *iprm-PASEF* – identify your images with confidence!

## *iprm-PASEF* – identify your images with confidence



Accurate MS/MS-based molecular identification



25 precursors in a single *iprm-PASEF* acquisition



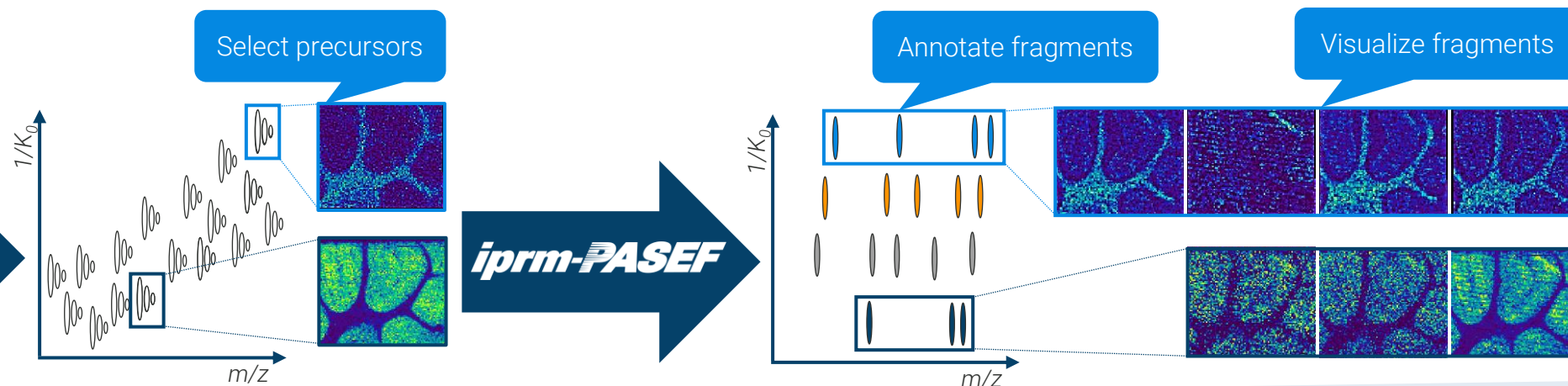
Integrated workflow: Acquire, Analyze & Annotate  
powered by *timsTOF fleX*, *timsControl*, *SCiLS Lab* & *MetaboScape*

## *iprm-PASEF*

Powered by 4D-Multiomics



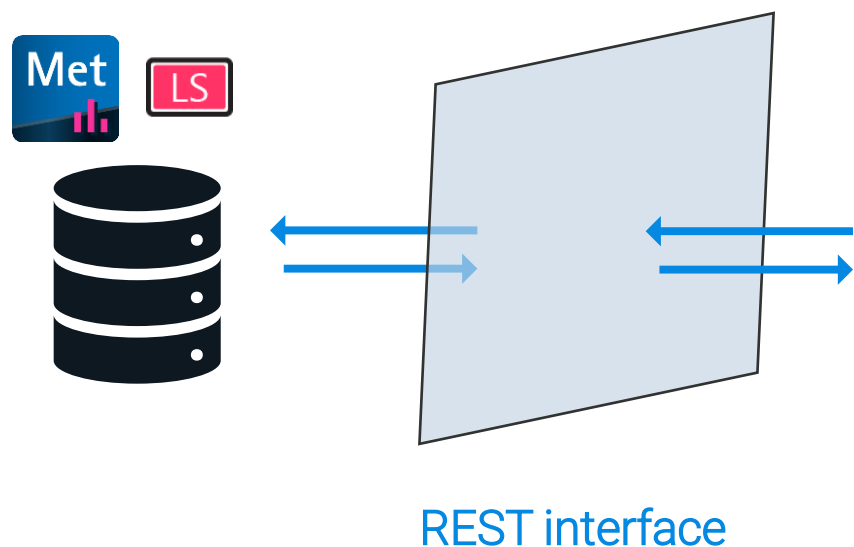
CCS-enabled  
MALDI Imaging



# REST API

## Annotate your Features with Rule-Based Lipid Annotation

### MetaboScape server



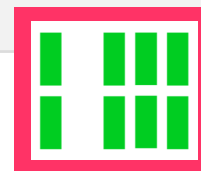
### Your code:

```
annotations = metaboscape_api.create_lipid_annotations_with_method
```

Features

Scored annotations including fragment explanations

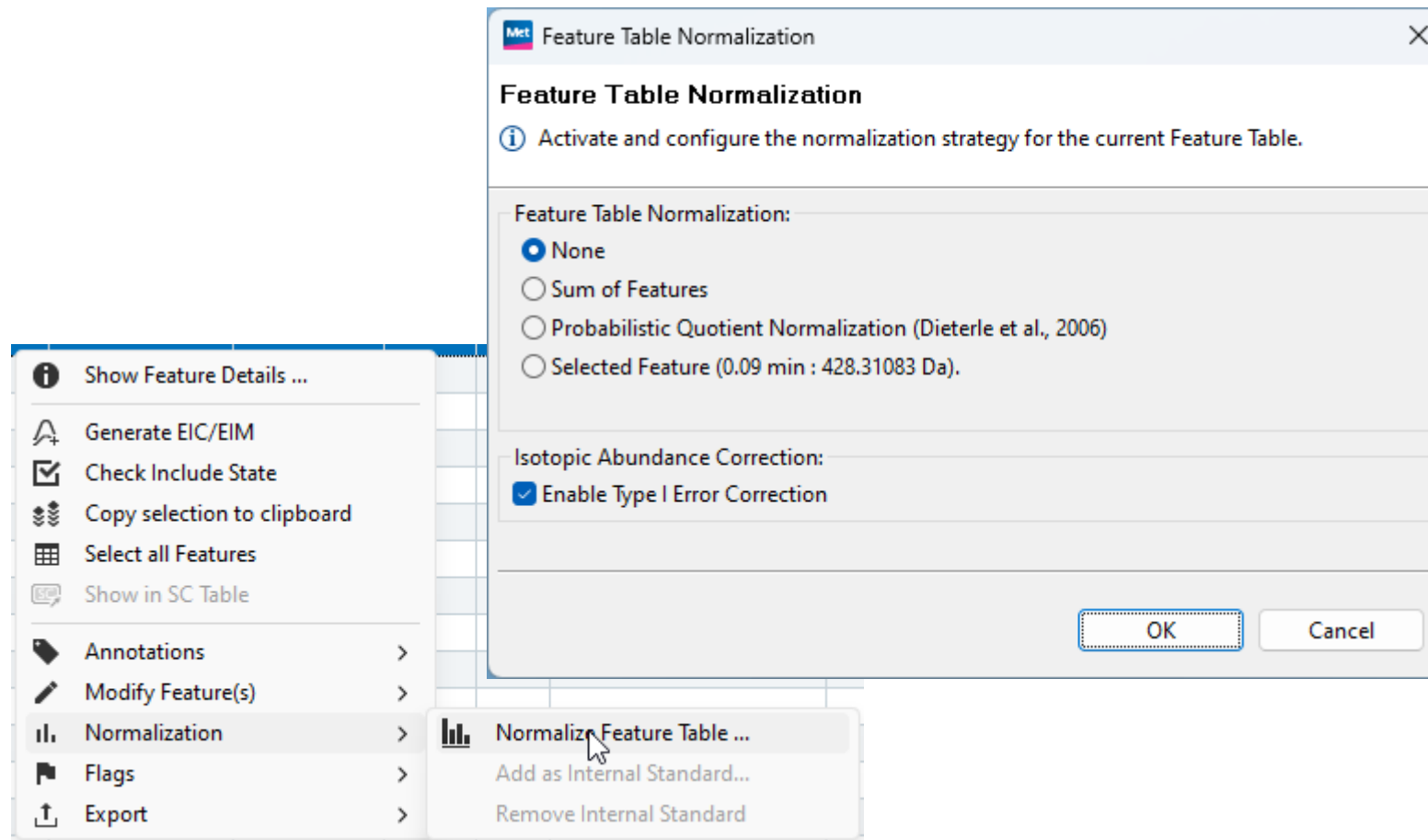
18:1 (Acyl chain and substructure fragment neutral loss) 575.50356 m/z C <sub>37</sub> H <sub>67</sub> O <sub>4</sub> <sup>+</sup>	Head group fragment neutral loss 857.76223 m/z C <sub>38</sub> H <sub>69</sub> O <sub>4</sub> <sup>+</sup>
16:0 (Acyl chain and substructure fragment neutral loss) 601.51842 m/z C <sub>36</sub> H <sub>69</sub> O <sub>4</sub> <sup>+</sup>	
18:2 (Acyl chain and substructure fragment neutral loss) 577.51886 m/z C <sub>37</sub> H <sub>65</sub> O <sub>4</sub> <sup>+</sup>	



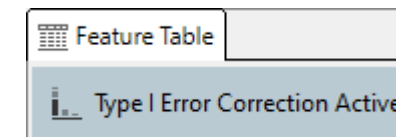
m/z value fit  
Retention time fit  
Isotopic pattern fit  
MS/MS fit  
CCS value fit

Use the full potential of the rule-based Lipid Species annotation algorithm including Annotation Quality scoring of up to four qualifiers (m/z, isotope patterns, MS/MS spectra, CCS values) to annotate lipid features in your own code.

# Type-1 abundance error correction



The screenshot shows the 'Feature Table Normalization' dialog box in the MetaboScape software. The dialog has a title bar with the Bruker logo and the text 'Met Feature Table Normalization'. Below the title bar, the main heading is 'Feature Table Normalization' followed by an information icon and the text 'Activate and configure the normalization strategy for the current Feature Table.' The dialog is divided into two sections: 'Feature Table Normalization:' and 'Isotopic Abundance Correction:'. In the first section, there are four radio button options: 'None' (selected), 'Sum of Features', 'Probabilistic Quotient Normalization (Dieterle et al., 2006)', and 'Selected Feature (0.09 min : 428.31083 Da)'. In the second section, there is a checked checkbox for 'Enable Type I Error Correction'. At the bottom right of the dialog are 'OK' and 'Cancel' buttons. To the left of the dialog, a context menu is open over a table, with the 'Normalization' option selected, which has opened a sub-menu containing 'Normalize Feature Table ...', 'Add as Internal Standard...', and 'Remove Internal Standard'.



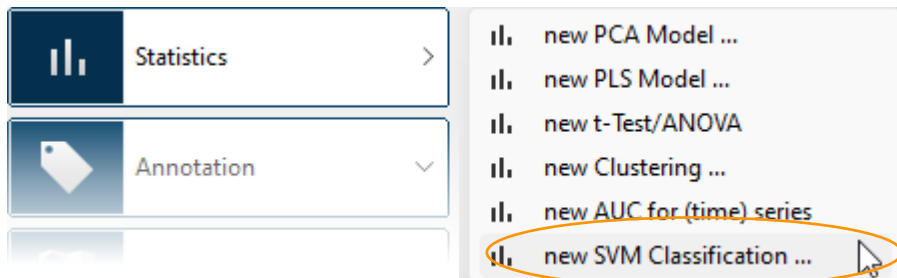
The relative amount of the total intensity of an ion, that is distributed across its naturally occurring isotopologues, depends on its elemental composition. For instance, with each additional carbon atom in a molecule, due to naturally occurring  $^{13}\text{C}$  atoms, the relative abundance of isotopologues increases while the relative abundance of the monoisotope decreases.

Especially in Lipidomics this „type 1 abundance error“ hampers internal standard normalization, as lipid class specific standards have to cover a range of different side chain lengths and thus different type 1 abundance errors.

The Lipidomics Standards Initiative (LSI) recommends correcting for type 1 abundance errors to ensure accurate quantitation.

➔ <https://lipidomicstandards.org/lipid-species-quantification/> [23.07.2024]

# Support Vector Machine (SVM) Classification as Statistical View



SVM Classification now is accessible through the Statistics menu and adds a new view to the bottom of the Overview perspective.

The classification will react to changes in the Include States of Analyses and Features.

Selecting analyses from the Classification Results, will select them throughout the User Interface – for example in the Sample Tree and in other statistical views.

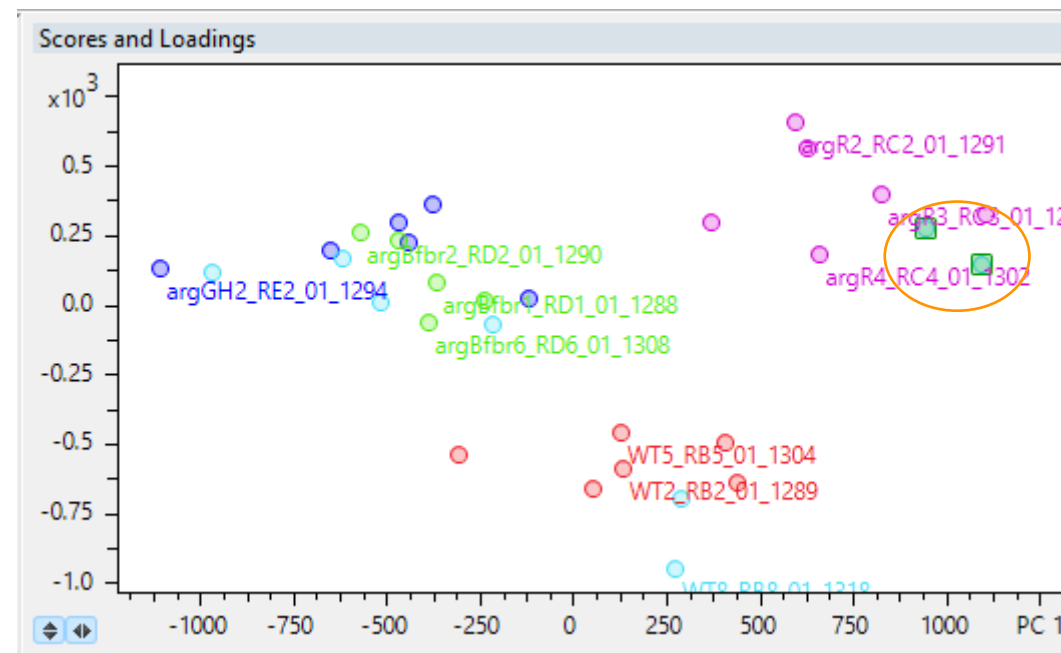
**SVM Classification**

Training Results  
In 4-fold cross validation:  
91.30 percent accuracy

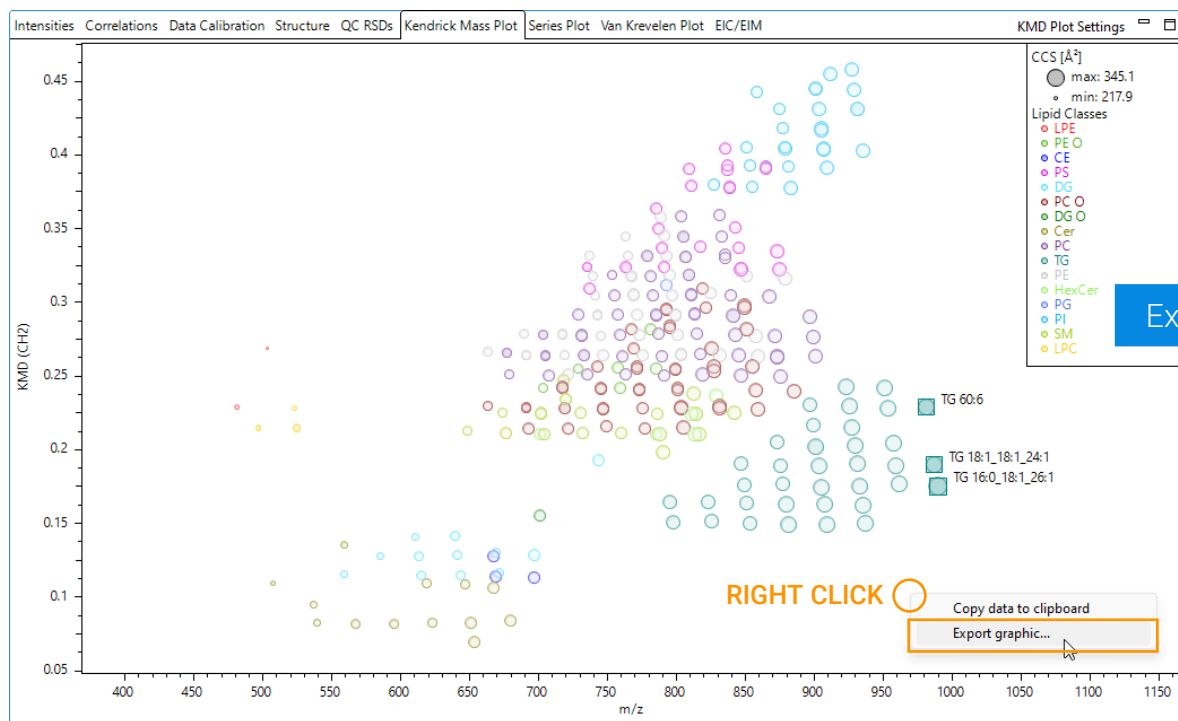
Classification Results

Analysis	Prediction
WT7_RB7_01_1314	WT
WT8_RB8_01_1318	WT
argBfbr7_RD7_01_1313	argBfbr
argBfbr8_RD8_01_1321	argBfbr
argGH7_RE7_01_1316	argGH
argGH8_RE8_01_1317	argGH
argR7_RC7_01_1315	argR
argR8_RC8_01_1320	argR

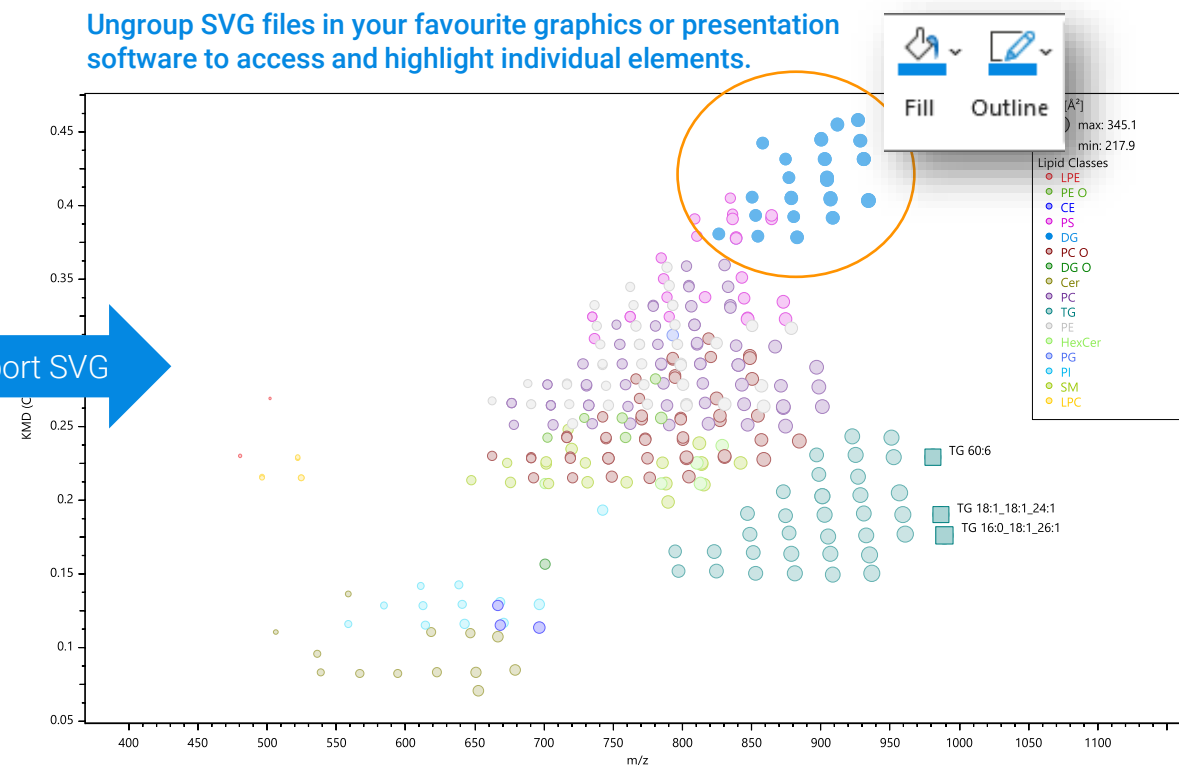
Sample Name	T
28	unknown
29	argR7_RC7_01_1315
30	argGH7_RE7_01_1316
31	argGH8_RE8_01_1317
32	WT8_RB8_01_1318
33	argR8_RC8_01_1320
34	argBfbr8_RD8_01_1321
35	argBfbr7_RD7_01_1313
36	WT7_RB7_01_1314



# Export Kendrick Mass (Survey) plot into high quality graphics formats

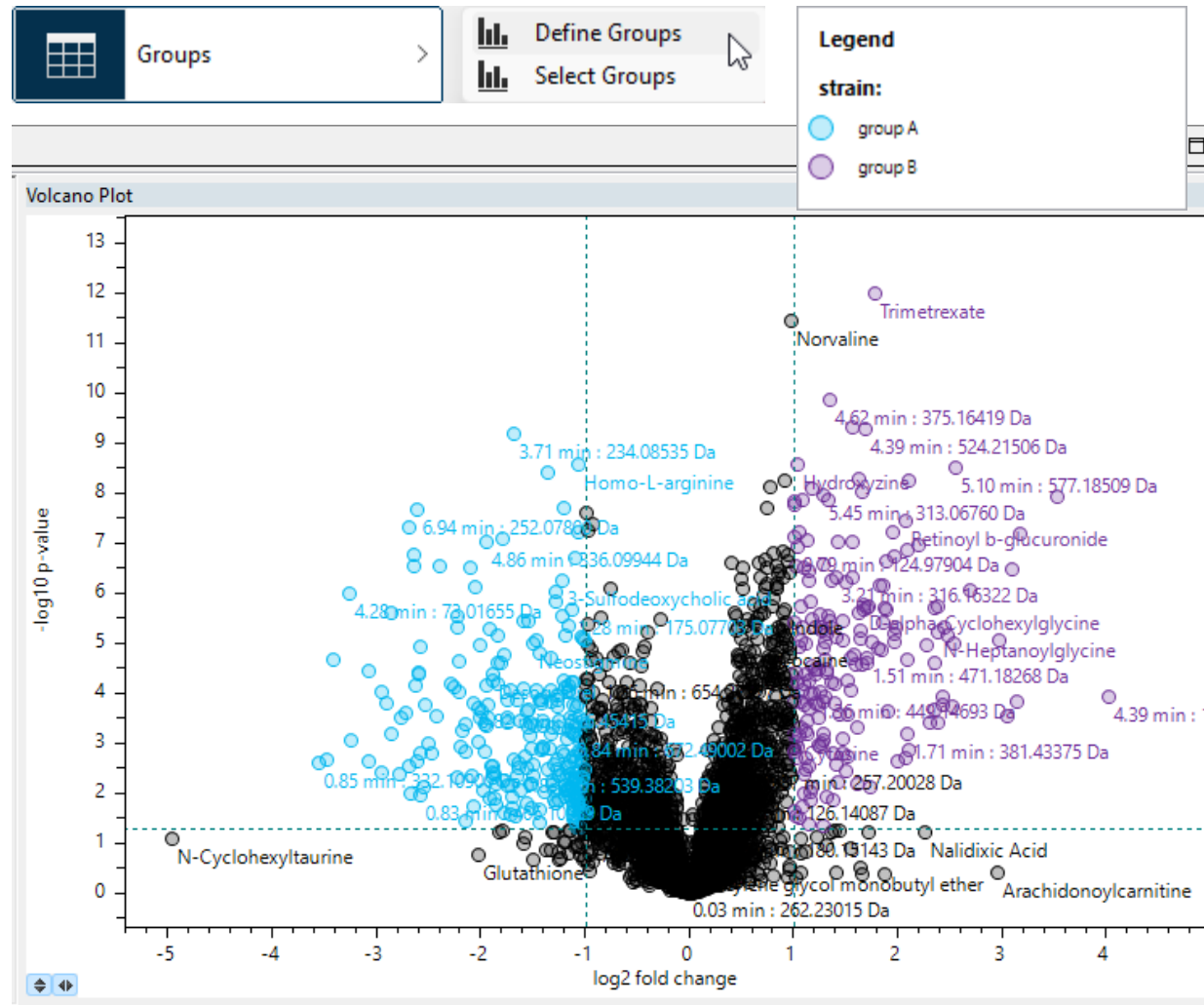


Ungroup SVG files in your favourite graphics or presentation software to access and highlight individual elements.



Export your Kendrick Mass (Survey) plot as you see it. The file formats SVG, PNG, EMF and GIF are available. Especially SVG files are highly accessible in many graphics and presentation softwares and provide high quality for use in presentations and publications.

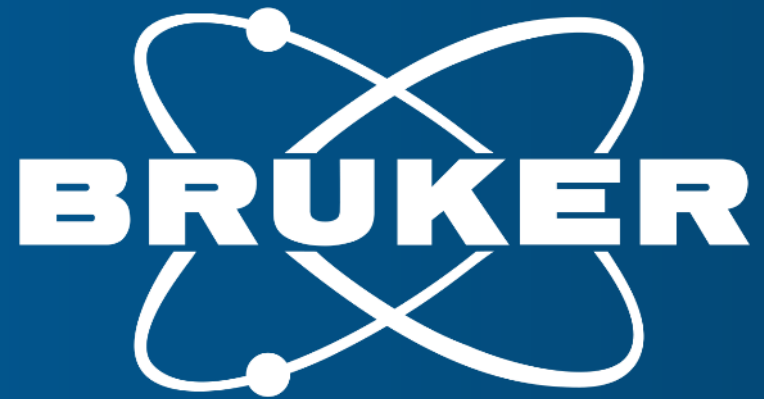
# Volcano plot compound coloring according to statistical groups



The visualization of volcano plots now considers the colors assigned to the respective statistical groups.

In this example, the light blue compounds are more abundant in group A, while the purple compounds are more abundant in group B.

The volcano plot visualization can also be exported as SVG, PNG, EMF or GIF files.



Innovation with Integrity