

# What's new in MetaboScape 2023b

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



# Outline

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01 Home Perspective

02 Sample Characterization Table-based Annotation

03 Internal Standards and Normalization

04 REST API – Annotation Service

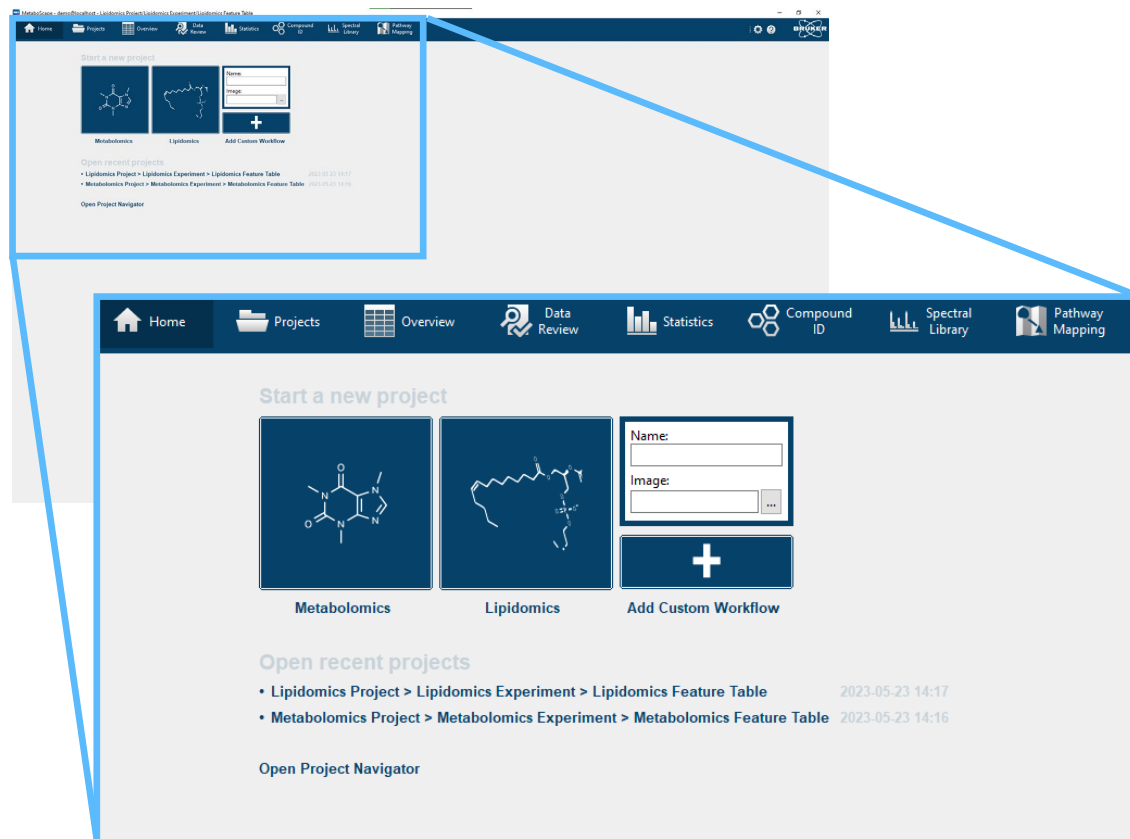
05 T-ReX Improvements

06 SMILES in Target Lists

07 Filter using MassQL Queries

08 Filter for MOMA Features

# Introducing the Home Perspective



## Define Research Types

- Initiate research projects effortlessly. Just select or create a research type and get started
- Benefit from two predefined but customizable Research Types, Metabolomics and Lipidomics, allowing you to kickstart your projects with a single click, optimized parameters included.

## Recent Projects Overview

- Stay on top of your work with the 'Recent Projects' list.
- This feature enables quick access to your most recently opened projects. Jump back into your work where you left off.

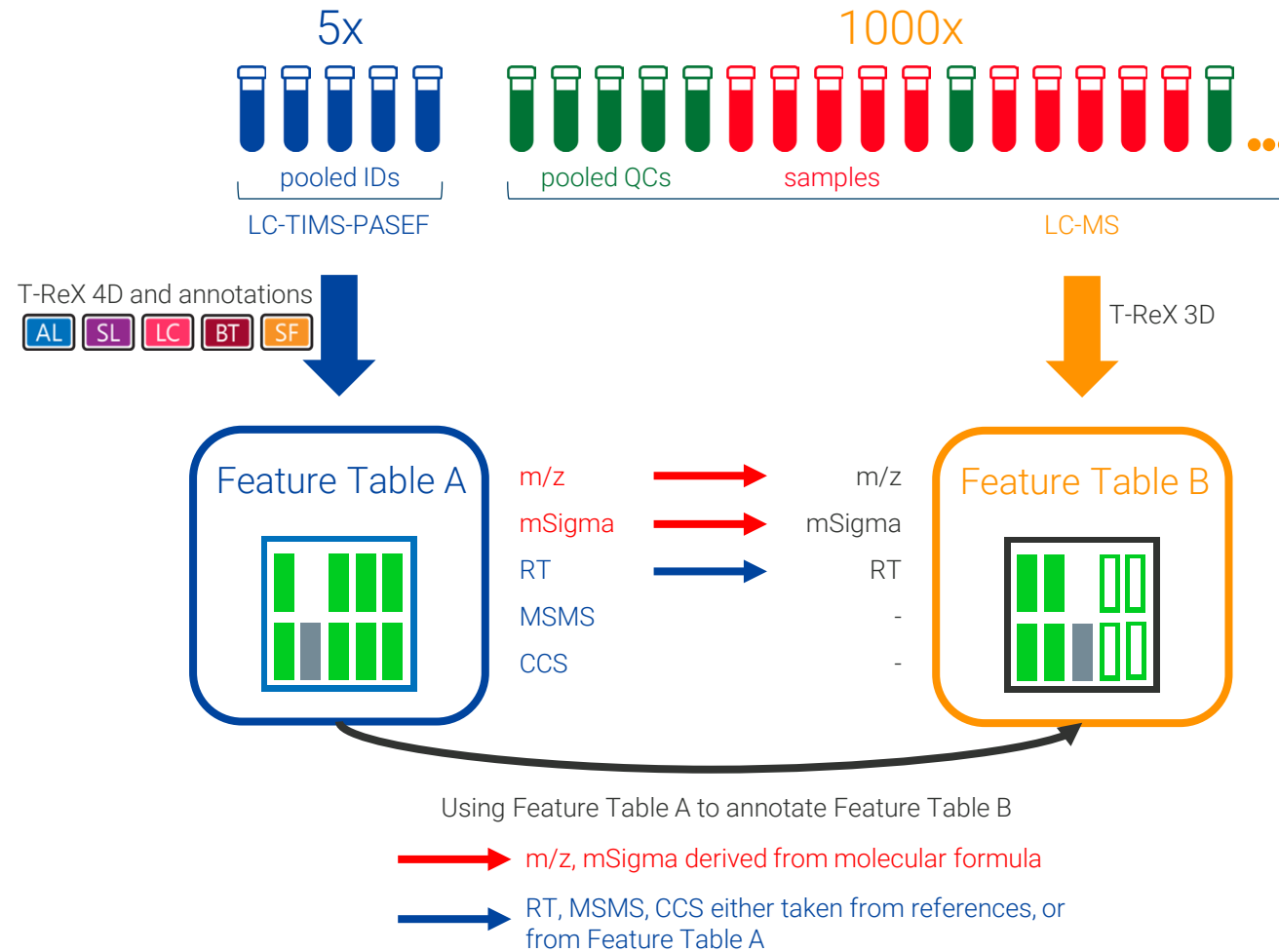
## Sample Characterization Table-based Annotation

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The Sample Characterization Table-based Annotation workflow allows separation of highly confident annotation and highly confident quantitation/high throughput.

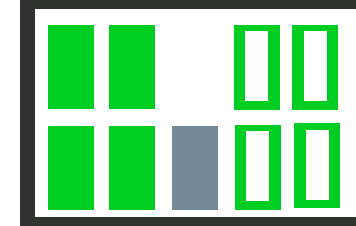
- Create a Feature Table A from pooled samples acquired with a high-dimensional mode like LC-TIMS-PASEF, for the sake of sample characterization. Use the CCS and MSMS information for highly confident annotation.
- Create a second Feature Table B from individual samples, acquired with e.g. LC-MS.
- Use the highly confident annotations from Feature Table A to annotate Feature Table B. This results in annotations in Feature Table B, referencing the CCS and MSMS annotation scores from Feature Table A.

# Sample Characterization Table-based Annotation

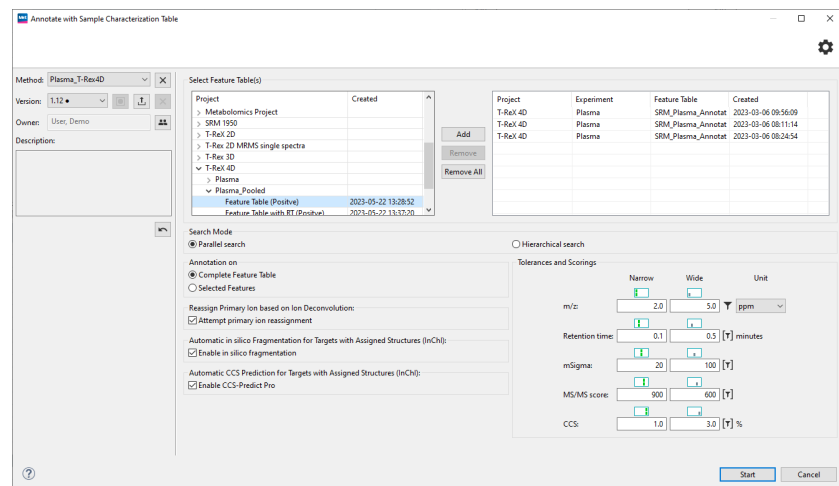



## Sample Characterization Table-based Annotation

































- Hollow AQ indicators mark scoring by Sample Characterization Table.
  - i.e., the set AQ Scoring was calculated against the information from the Sample Characterization Table
  
- New AQ symbols indicate referenced Retention Time, MS/MS and CCS matches from the Sample Characterization Table.



# Sample Characterization Table-based Annotation

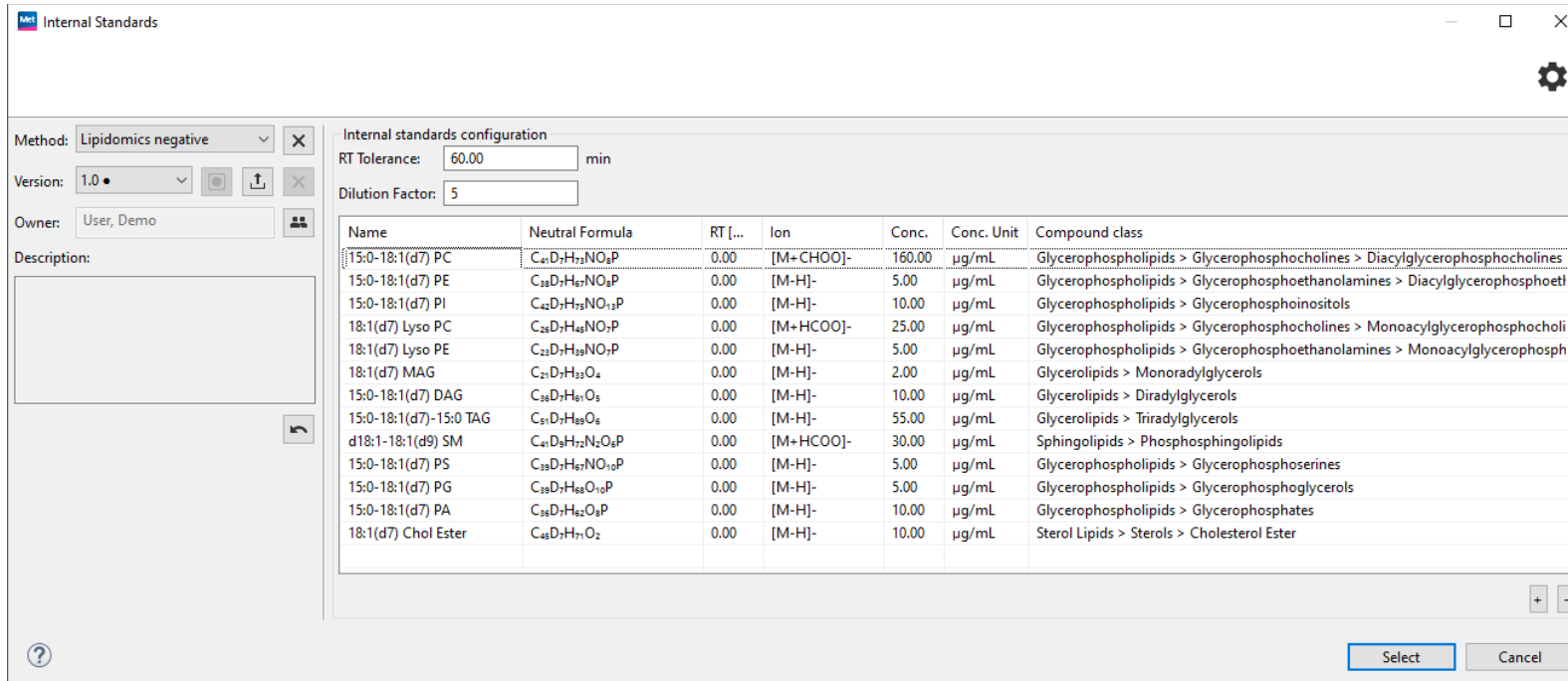


- Create an annotation method and select one or more already annotated feature tables for annotation.
- Use Search Mode, and optionally activate CCS Prediction, *in-silico* Fragmentation and set the tolerances and scorings.
- Annotated Features are marked with a “SC” symbol ()
- Annotations applied before version 2023b cannot be used for Sample Characterization Table Based Annotation

RT [min]	m/z meas.	M meas.	Ions	MS/MS	Name	Molecular Formula	Annotations	AQ	Annotation Source	Boxplot	Flags	Include	AQ Details
1.70	204.12288	203.11560	± □		L-Acetylcarniti...	C <sub>9</sub> H <sub>17</sub> NO <sub>4</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
1.23	162.11244	161.10516	± □		L-Carnitine	C <sub>7</sub> H <sub>15</sub> NO <sub>3</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
1.28	148.06043	147.05315	± □		L-Glutamic acid	C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
1.22	147.07641	146.06913	± □		L-Glutamine	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
1.15	156.07681	155.06953	± □		L-Histidine	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
4.70	209.09224	208.08497	± □		L-Kynurenine	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
2.63	132.10177	131.09463	± □		L-Norleucine	C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	
4.80	166.08619	165.07887	± □		L-Phenylalanine	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>			SRM_Plasma_Annotatio...			<input checked="" type="checkbox"/>	

# Internal Standards with T-ReX 3D and 4D Processing

- Define Internal Standards as targets for T-ReX feature finding
- Stable Isotopic Labeled Standards allowed
- Internal Standards can be assigned to lipid classes for the sake of class-specific normalization



Internal Standards configuration

Method: Lipidomics negative

Version: 1.0

Owner: User, Demo

RT Tolerance: 60.00 min

Dilution Factor: 5

Name	Neutral Formula	RT [...]	Ion	Conc.	Conc. Unit	Compound class
15:0-18:1(d7) PC	C <sub>33</sub> D <sub>7</sub> H <sub>53</sub> NO <sub>8</sub> P	0.00	[M+CHOO]-	160.00	µg/mL	Glycerophospholipids > Glycerophosphocholines > Diacylglycerophosphocholines
15:0-18:1(d7) PE	C <sub>38</sub> D <sub>7</sub> H <sub>67</sub> NO <sub>8</sub> P	0.00	[M-H]-	5.00	µg/mL	Glycerophospholipids > Glycerophosphoethanolamines > Diacylglycerophosphoethanolamines
15:0-18:1(d7) PI	C <sub>42</sub> D <sub>7</sub> H <sub>71</sub> NO <sub>11</sub> P	0.00	[M-H]-	10.00	µg/mL	Glycerophospholipids > Glycerophosphoinositols
18:1(d7) Lyso PC	C <sub>28</sub> D <sub>7</sub> H <sub>49</sub> NO <sub>7</sub> P	0.00	[M+HCOO]-	25.00	µg/mL	Glycerophospholipids > Glycerophosphocholines > Monoacylglycerophosphocholines
18:1(d7) Lyso PE	C <sub>33</sub> D <sub>7</sub> H <sub>53</sub> NO <sub>7</sub> P	0.00	[M-H]-	5.00	µg/mL	Glycerophospholipids > Glycerophosphoethanolamines > Monoacylglycerophosphoethanolamines
18:1(d7) MAG	C <sub>27</sub> D <sub>7</sub> H <sub>53</sub> O <sub>4</sub>	0.00	[M-H]-	2.00	µg/mL	Glycerolipids > Monoradylglycerols
15:0-18:1(d7) DAG	C <sub>38</sub> D <sub>7</sub> H <sub>61</sub> O <sub>5</sub>	0.00	[M-H]-	10.00	µg/mL	Glycerolipids > Diacylglycerols
15:0-18:1(d7)-15:0 TAG	C <sub>51</sub> D <sub>7</sub> H <sub>89</sub> O <sub>6</sub>	0.00	[M-H]-	55.00	µg/mL	Glycerolipids > Triacylglycerols
d18:1-18:1(d9) SM	C <sub>41</sub> D <sub>9</sub> H <sub>71</sub> N <sub>2</sub> O <sub>8</sub> P	0.00	[M+HCOO]-	30.00	µg/mL	Sphingolipids > Phosphosphingolipids
15:0-18:1(d7) PS	C <sub>39</sub> D <sub>7</sub> H <sub>67</sub> NO <sub>10</sub> P	0.00	[M-H]-	5.00	µg/mL	Glycerophospholipids > Glycerophosphoserines
15:0-18:1(d7) PG	C <sub>38</sub> D <sub>7</sub> H <sub>65</sub> O <sub>10</sub> P	0.00	[M-H]-	5.00	µg/mL	Glycerophospholipids > Glycerophosphoglycerols
15:0-18:1(d7) PA	C <sub>38</sub> D <sub>7</sub> H <sub>65</sub> O <sub>9</sub> P	0.00	[M-H]-	10.00	µg/mL	Glycerophospholipids > Glycerophosphates
18:1(d7) Chol Ester	C <sub>48</sub> D <sub>7</sub> H <sub>71</sub> O <sub>2</sub>	0.00	[M-H]-	10.00	µg/mL	Sterol Lipids > Sterols > Cholesterol Ester

Select Cancel



# Internal Standards for Lipid Normalization

Internal Standards may be assigned to normalized specific lipid classes.

Internal Standards are detected and annotated according to the defined method.

Name	Molecular For...	Annotations	AQ	Internal Standard					
d18:1-18:1(d9) SM	C <sub>41</sub> D <sub>9</sub> H <sub>72</sub> N <sub>2</sub> O <sub>6</sub> P			⊖ Phosphosphingolipids	6,000	6,000	6,000	6,000	6,000
18:1(d7) Lyso PE	C <sub>23</sub> D <sub>7</sub> H <sub>39</sub> NO <sub>7</sub> P			⊖ Monoacylglycerophosphoethanolamines	1,000	1,000	1,000	1,000	1,000
18:1(d7) Lyso PC	C <sub>26</sub> D <sub>7</sub> H <sub>45</sub> NO <sub>7</sub> P			⊖ Monoacylglycerophosphocholines	5,000	5,000	5,000	5,000	5,000
15:0-18:1(d7) PS	C <sub>39</sub> D <sub>7</sub> H <sub>67</sub> NO <sub>10</sub> P			⊖ Glycerophosphoserines	1,000	1,000	1,000	1,000	1,000
15:0-18:1(d7) PG	C <sub>39</sub> D <sub>7</sub> H <sub>68</sub> O <sub>10</sub> P			⊖ Glycerophosphoglycerols	1,000	1,000	1,000	1,000	1,000
15:0-18:1(d7) PA	C <sub>38</sub> D <sub>7</sub> H <sub>62</sub> O <sub>8</sub> P			⊖ Glycerophosphates	2,000	2,000	2,000	2,000	2,000
15:0-18:1(d7) PE	C <sub>38</sub> D <sub>7</sub> H <sub>67</sub> NO <sub>8</sub> P			⊖ Diacylglycerophosphoethanolamines	1,000	1,000	1,000	1,000	1,000
15:0-18:1(d7) PC	C <sub>41</sub> D <sub>7</sub> H <sub>73</sub> NO <sub>9</sub> P			⊖ Diacylglycerophosphocholines	32,000	32,000	32,000	32,000	32,000
SM 34:1;O2	C <sub>39</sub> H <sub>79</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	76.366	74.224	73.740	78.413	81.218
SM 42:2;O2	C <sub>47</sub> H <sub>93</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	21.682	19.962	20.736	21.683	21.961
SM 34:2;O2	C <sub>39</sub> H <sub>77</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	12.445	12.075	11.716	12.546	12.848
SM 42:1;O2	C <sub>47</sub> H <sub>95</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	11.307	10.694	10.273	10.958	11.623
SM 40:1;O2	C <sub>45</sub> H <sub>91</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	10.911	10.624	10.475	11.303	12.538
SM 36:1;O2	C <sub>41</sub> H <sub>83</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	10.822	10.337	10.455	10.876	11.335
SM 42:3;O2	C <sub>47</sub> H <sub>91</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	10.388	10.273	10.201	11.276	11.485
SM 32:1;O2	C <sub>37</sub> H <sub>75</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	8.886	8.104	8.099	8.594	9.185
SM 40:2;O2	C <sub>45</sub> H <sub>89</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	8.092	8.044	7.479	8.221	8.319
SM 36:2;O2	C <sub>41</sub> H <sub>81</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	6.303	6.183	5.944	6.801	7.013
SM 38:1;O2	C <sub>43</sub> H <sub>87</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	5.413	5.316	4.903	5.541	5.472
SM 38:2;O2	C <sub>43</sub> H <sub>85</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	2.541	2.495	2.276	2.554	2.534
SM 42:4;O2	C <sub>47</sub> H <sub>89</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	1.762	1.647	1.494	1.745	1.871
SM 40:3;O2	C <sub>45</sub> H <sub>87</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	1.159	1.104	1.158	1.183	1.203
SM 36:3;O2	C <sub>41</sub> H <sub>79</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	0.800	0.819	0.836	0.911	0.871
SM 32:2;O2	C <sub>37</sub> H <sub>73</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	0.635	0.852	0.850	0.823	0.965
SM 42:5;O2	C <sub>47</sub> H <sub>87</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	0.310	0.319	0.272	0.302	0.276
SM 30:1;O2	C <sub>35</sub> H <sub>71</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	0.299	0.438	0.465	0.414	0.446
SM 44:2;O2	C <sub>49</sub> H <sub>97</sub> N <sub>2</sub> O <sub>6</sub> P			Phosphosphingolipids	0.243	0.219	0.200	0.206	0.248

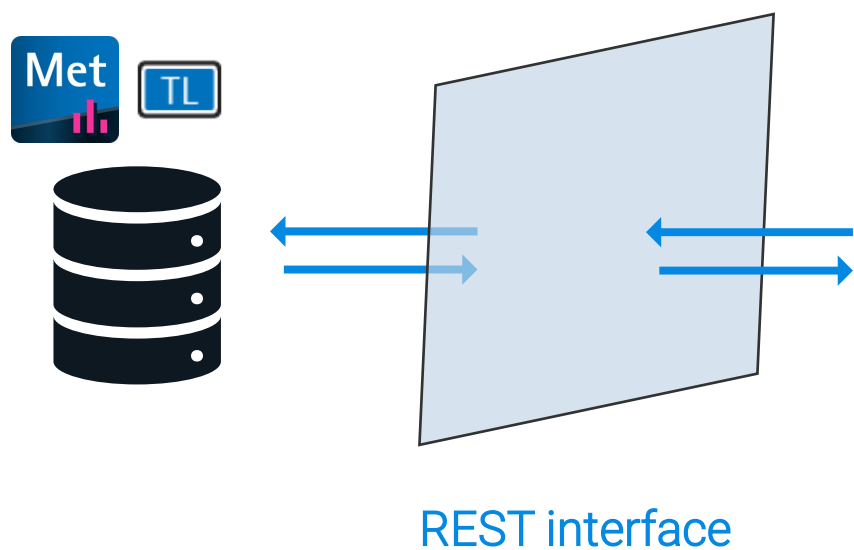
Normalized quantities are calculated, considering the amounts of the standards and the dilution factor.

Lipids annotated by the Lipid Species annotation tool are normalized by the Internal Standard assigned to their classes.

# REST API

## Annotate your Features with Target List Annotation

### MetaboScape server



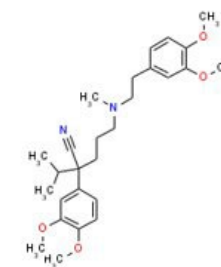
### Your code:

```
annotations = metaboscape_api.create_target_list_annotations_with_method
```

Scored annotations



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



Features

Use the full potential of the Target List annotation algorithm including Annotation Quality scoring of up to 5 qualifiers (retention times, m/z, isotope patterns, MS/MS spectra, CCS values) to annotate your features in your own code.

## T-ReX 4D for timsTOF data: peak picking improved

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- Algorithmic improvement for calculation of feature intervals
- Optimized parameterization for peak shape analysis

- Improved consistency of feature intensities

It may be necessary to increase the intensity threshold to achieve an equivalent number of features, compared to older versions of T-ReX.

- Targeted detection of Features and Isotope Patterns from SIL standards




See “Internal Standards with T-ReX 3D and 4D Processing” above.

# Support for SMILES in Target List annotation

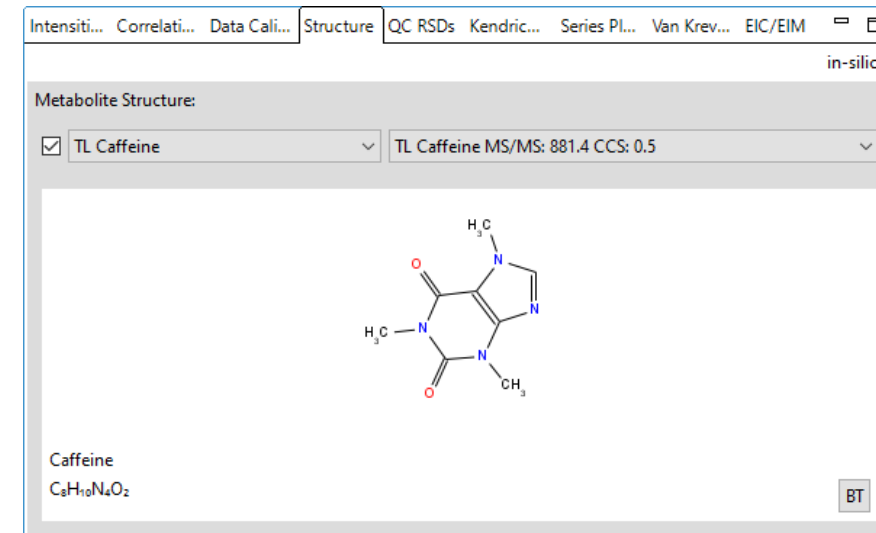
Import references with SMILES structures

TL	Name	Formula	Smiles
	Caffeine	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	<chem>CN1C=NC2=C1C(=O)N(C(=O)N2C)C</chem>



Name	Annotations	AQ	$\Delta m/z$ [mDa]	mSigma	MS/MS score	$\Delta CCS$ [%]	AQ Details
Caffeine	TL		0.382	9.8	881.4	0.5	 

CCS-Predict Pro  
Metfrag



Metabolite Structure:

TL Caffeine TL Caffeine MS/MS: 881.4 CCS: 0.5

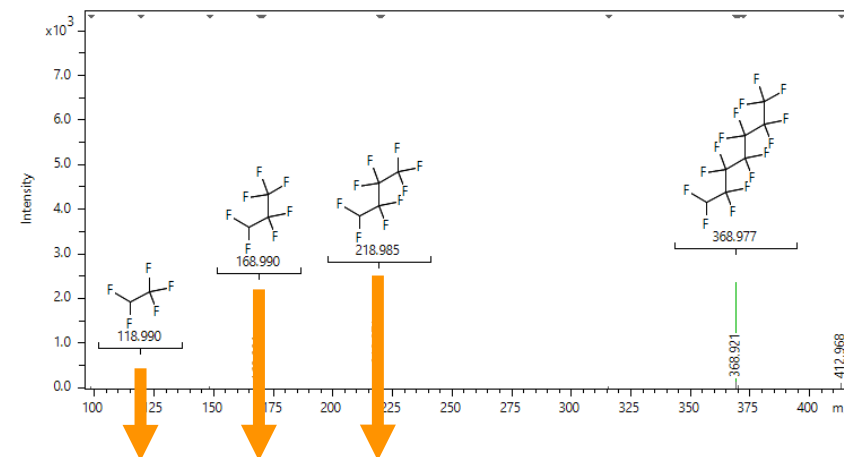
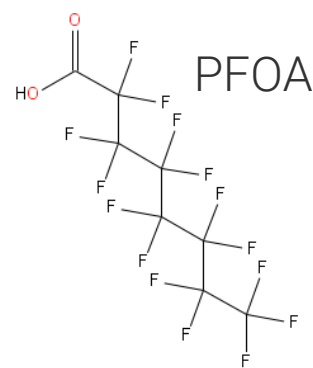
Caffeine  
C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>

BT

Use Target List to annotate SMILES and benefit from structure-based Metfrag in-silico fragmentation and CCS prediction with CCS-Predict Pro.

# Filter Features using MassQL Queries

*“The Mass Spec Query Language (MassQL) is a domain specific language meant to be a succinct way to express a query in a mass spectrometry centric fashion. It is inspired by SQL, but it attempts to bake in assumptions of mass spectrometry to make querying much more natural for mass spectrometry users.”*



```
QUERY scaninfo(MS2DATA) WHERE
MS2PROD=118.987:TOLERANCEMZ=0.005 AND
MS2PROD=168.987:TOLERANCEMZ=0.005 AND
MS2PROD=218.986:TOLERANCEMZ=0.005 AND
MOBILITY = range(min=120,max=210)
```

Design and validate MassQL Queries using the interactive User Interface of MetaboScape.

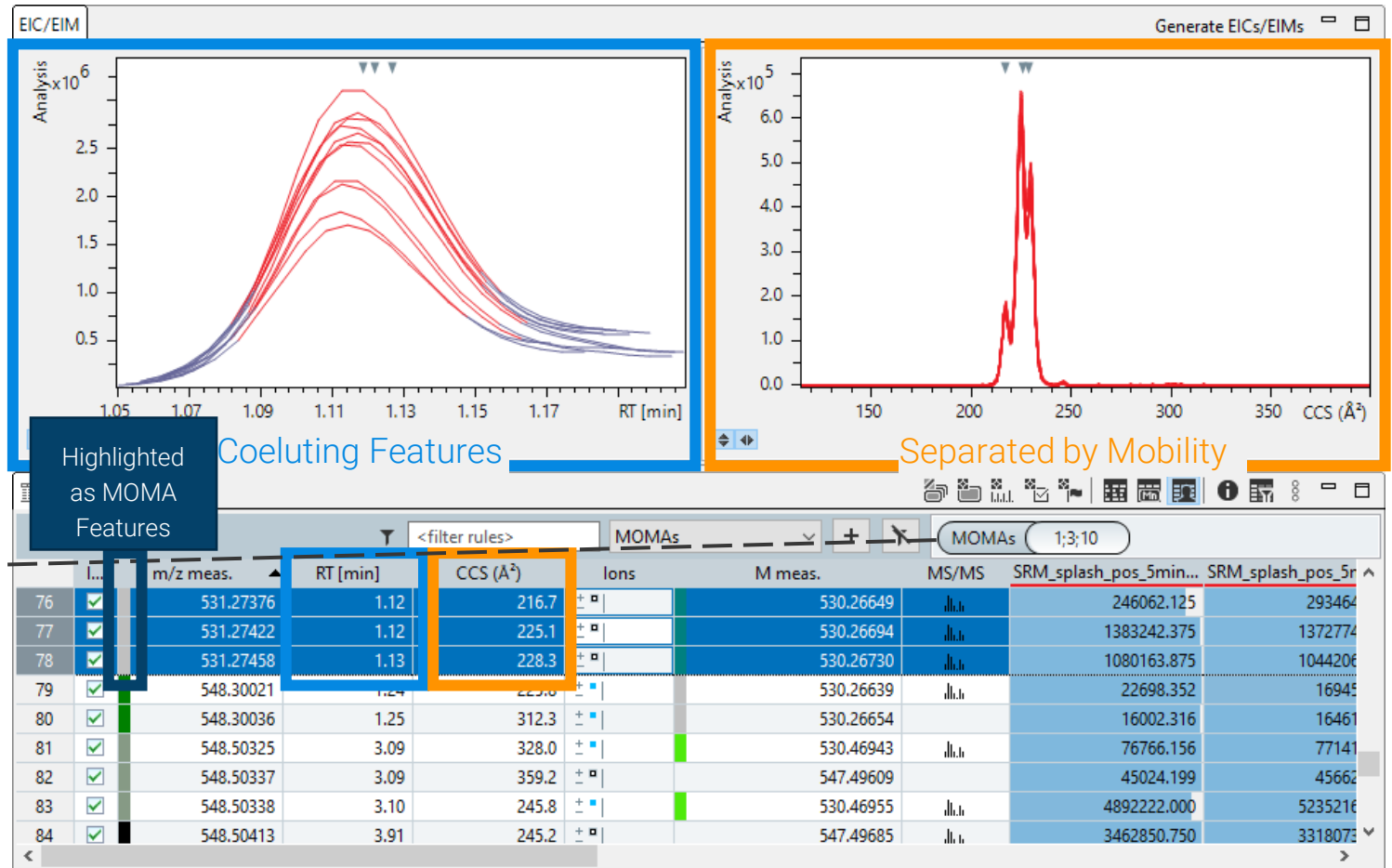
MassQL (beta)   MassQL (beta) QUERY scaninfo(MS2DATA) WHERE MS2PROD=118.987 (...)

CCS (Å <sup>2</sup> )	MS/MS	Name	Molecular For...	Annotations
152.8		PFOA	C <sub>8</sub> HF <sub>15</sub> O <sub>2</sub>	<input type="button" value="TL"/> <input type="button" value="SF"/>
172.3		PFDA	C <sub>10</sub> HF <sub>19</sub> O <sub>2</sub>	<input type="button" value="TL"/> <input type="button" value="SF"/>
180.7		PFUdA	C <sub>11</sub> HF <sub>21</sub> O <sub>2</sub>	<input type="button" value="TL"/> <input type="button" value="SF"/>
136.7		1-hydro-penta...	C <sub>7</sub> HF <sub>15</sub>	<input type="button" value="CC"/> <input type="button" value="SF"/>

Jarmusch, A. K., Aron, A. T., Petras, D., Phelan, V. V., Bittremieux, W., Acharya, D. D., ... & Wang, M. (2022). A universal language for finding mass spectrometry data patterns. <https://massql.gnps2.org/>

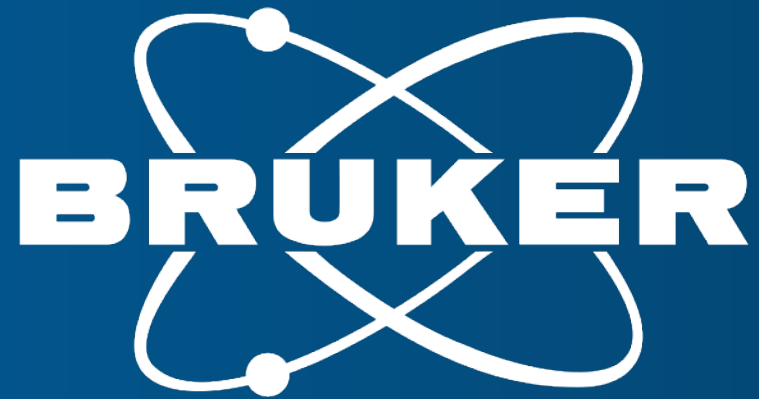
# Filter for MOMA Features

Use the MOMA (=Mobility Offset Mass Aligned) filter to detect potential isomers.



MOMAs 1;3;10

$\Delta m/z$  [mDa];  $\Delta RT$  [s]; Min.  $\Delta CCS$  [Å²]; Max.  $\Delta CCS$  [Å²]



Innovation with Integrity