

What's New in OSc 2025b?

A QUICK GUIDE TO THE LATEST OSC



The image shows a preview of the OmniScape 2025b software interface. At the top right is the Bruker logo. Below it is a horizontal banner with a sunset over a mountain range. On the left side of the banner is a blue square icon with the text "OSc" and a pink bar chart. Below the banner, the text "OmniScape™ 2025b" is displayed in a large, blue, sans-serif font. A small red dot is positioned to the left of the text. At the bottom center, the copyright notice "© 2024 Bruker Daltonics GmbH & Co. KG" is visible.

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Main features

Functional Improvements

- **Confirmation** workflow
 - Extended fragment walk
 - Introducing the SVP for better data interpretation
 - Clipping analysis
 - Addition of Metal ions for native MS (Zn, Fe, Cu)
 - Added filter options on fragment list
- **Result Combiner** workflow can now combine sequence maps from MS/MS and MS³ spectra
- **De novo sequencing** capabilities significantly improved in quality and speed
- **Sequence Editor** allows to add and delete sequence stretches or individual residues
- **Score Distribution Plot** has been added

Export Import Report

- **JPEAKS Data import** with meta information is supported – exported from DA 6.2 on as **JSON files**
- **Export** of graphics/plots as SVG files and of peaklists as CSV files (from Results as well as Confirmation tabs)
- **Report** generation

Extended fragment walk

The screenshot displays the Bruker software interface for an extended fragment walk. At the top, a sequence map shows two protein sequences: S H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V D I D T K A V V Q D and P A L K P L A L V Y G E A R R M V N N G H S F N V E Y D D S Q D K A V L K D. A blue callout box points to the 'GPEHW' region in the first sequence, with the text: "1. Select sequence stretch of interest: all fragments are listed in spectrum and fragments list".

Below the sequence map, a mass spectrum plot shows intensity versus m/z. Several peaks are highlighted in red and labeled: c16_4+, c17_4+, c18_4+, c16_3+, c17_3+, c18_3+, and c13_2+. A blue callout box points to the spectrum with the text: "2. Select option 'Keep list for fragment walk'. Click on a list entry (or arrow down) will cause the spectrum to zoom in, but the list doesn't disappear".

On the right side, a 'Proteoforms list' table is visible. Below it, a table of ion types is shown with checkboxes for 'a' [8.9%], 'b' [5.4%], 'c' [41.5%], 'x', 'y' [20.2%], 'z', 'd', 'w', and 'v'. A checkbox labeled 'Keep list for fragment walk' is checked. Below this is a table of selected peaks:

Selected	m/z	z	ppm	Score [a.u.]	ei Score	Max Int.
8/8	Filter by name			top 100.0%	top 100.0%	top 9.8%
<input checked="" type="checkbox"/>	c13_2+	773.8504	2	1.11	29.865	98.3
<input checked="" type="checkbox"/>	c16_3+	669.6350	3	1.45	49.963	99.1
<input checked="" type="checkbox"/>	c16_4+	502.4781	4	1.65	83.700	99.8
<input checked="" type="checkbox"/>	c17_3+	712.3334	3	1.91	30.470	100.0
<input checked="" type="checkbox"/>	c17_4+	534.5018	4	2.09	73.074	99.8
<input checked="" type="checkbox"/>	c18_2+	1125.5099	2	-1.09	4.753	
<input checked="" type="checkbox"/>	c18_3+	750.6757	3	1.88	49.102	
<input checked="" type="checkbox"/>	c18_4+	563.2586	4	2.20	79.472	

A blue callout box points to the '8/8' in the table with the text: "8 out of 8 peaks are listed, deselection of 1 fragment ion will cause a change to 7/8".

At the bottom right, a blue callout box points to the table with the text: "3. Control the length of the list by filter text and limiting to the top x% intense or scored peaks in that list".

Result Combiner workflow allows to combine MS/MS and MS³ spectra such as T³-Sequencing data from MALDI-ISD

Result Combination

Available Analyses

C:/Users/detlev.suckau/OmniScape/Analyses

- ▶ 240410_NISTmAb LC_z21_MS2 ECD 50ms#13-747_smooth_cal
- ▶ 240410_NISTmAb LC_z21_MS2 ECD 50ms#13-747_smooth_cal
- ▶ 240410_NISTmAb LC_z21_MS2 EID 50ms#12-763_smoothed_ca
- ▶ 240410_NISTmAb LC_z21_MS2 EID 50ms#12-763_smoothed_ca
- ▶ AMPK
- ▶ CA ETD denovo
- ▶ CA T3 MSMS Flex
- ▶ CA T3c18 MSMS Flex
- ▶ CA T3c9 MSMS Flex
- ▶ CA T3z13 MSMS Flex
- ▶ CA T3z9 MSMS Flex
- ▶ CAH FLEX confirmation
- ▶ CAH_ETD_confirmation
- ▶ CAH_ETD_de novo

+ Add to Selected (MSMS)

Equivalence Filter

Same Sequence Same Proteoform MSMS

Selected Analyses

Name	SC [%]

Remove Selected Save Combined Analysis

- ▶ 240410_NISTmAb LC_z21_MS2 ECD 50ms#13-747_smooth_cal
- ▶ 240410_NISTmAb LC_z21_MS2 ECD 50ms#13-747_smooth_cal
- ▶ 240410_NISTmAb LC_z21_MS2 EID 50ms#12-763_smoothed_ca
- ▶ 240410_NISTmAb LC_z21_MS2 EID 50ms#12-763_smoothed_ca
- ▶ AMPK
- ▶ CA ETD denovo
- ▶ CA T3 MSMS Flex
- ▼ CA T3c18 MSMS Flex

Sequence(s)	SC [%]	IC [%]	MS Score [a.u.]
CA2 c18 (Ace: N)	100.00	26.60	26.6049
CA2 c18 (Pho: S1)	82.35	11.60	9.5567
CA2 c18 (Pho: S1)	82.35	11.59	9.5469
CA2 c18 (unmod)	82.35	11.59	9.5469

- ▶ CA T3c9 MSMS Flex
- ▶ CA T3z13 MSMS Flex
- ▶ CA T3z9 MSMS Flex

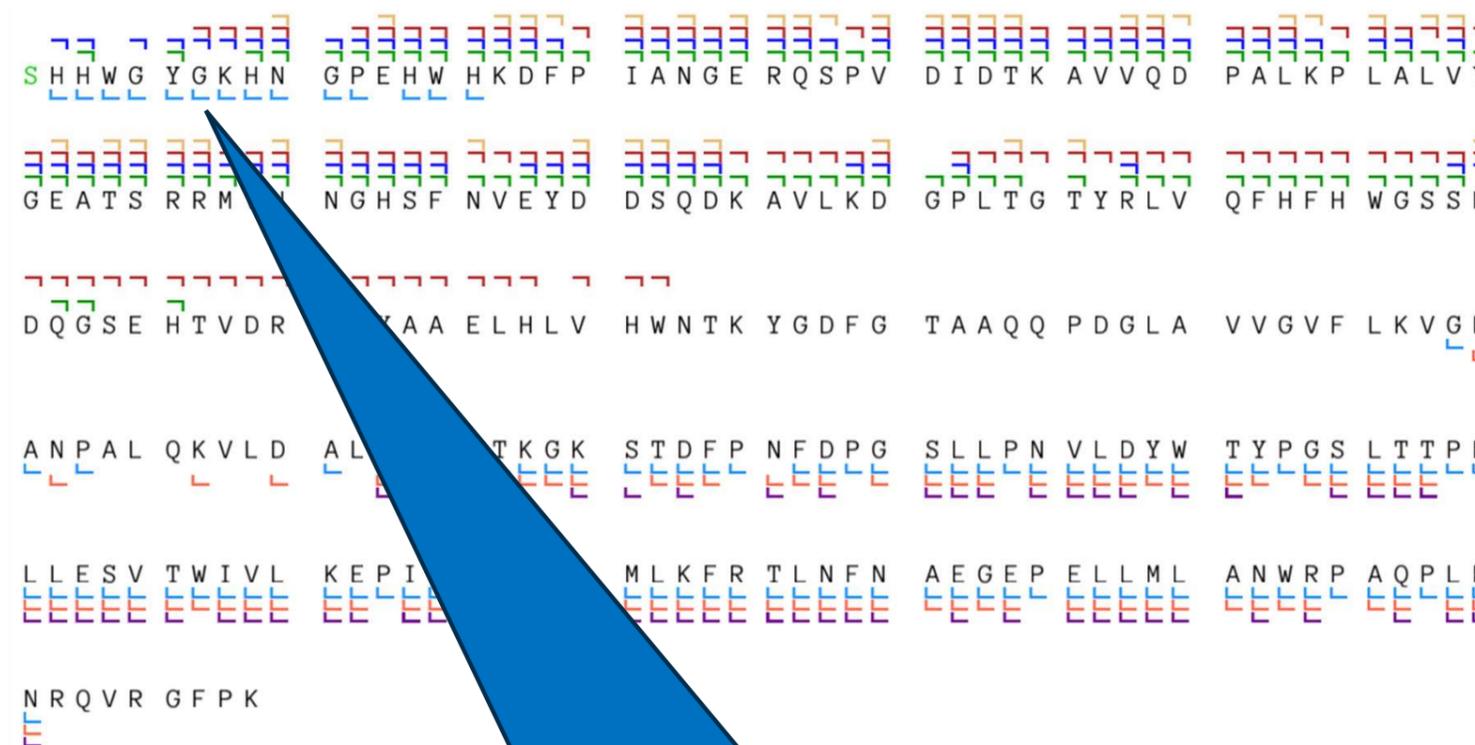
+ Add to Selected (MSn)

Equivalence Filter

Same Sequence Same Proteoform MSMS MSn

Selected Analyses

Name	SC [%]
▼ CA2_Nterm[Acetyl]	81.78
• CA T3 MSMS Flex	81.78



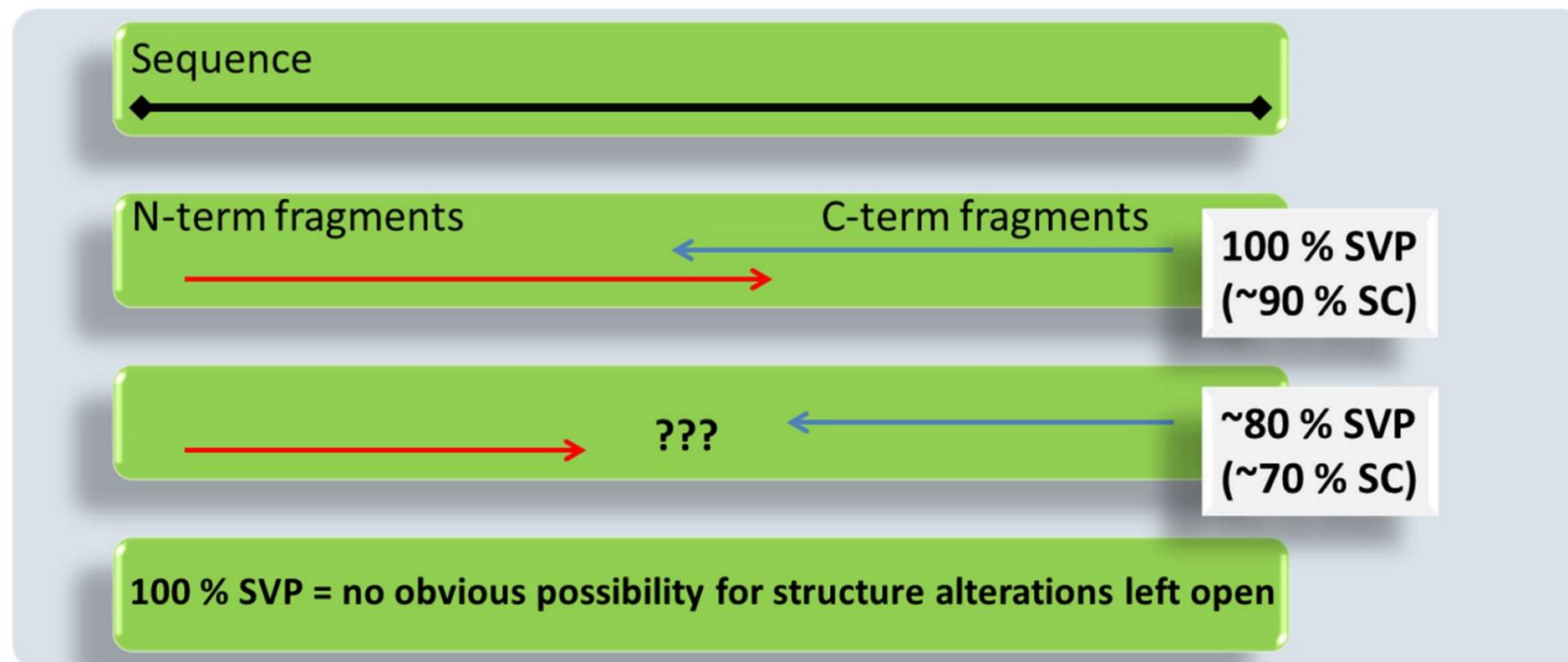
The MALDI-ISD sequence map is combined with the T³-Sequence map obtained from c₁₈ fragmentation

1. Select MSMS Option, and add MS/MS analysis

2. Add MS³ analysis

Introducing SVP – the Sequence Validation Percentage

The Principle



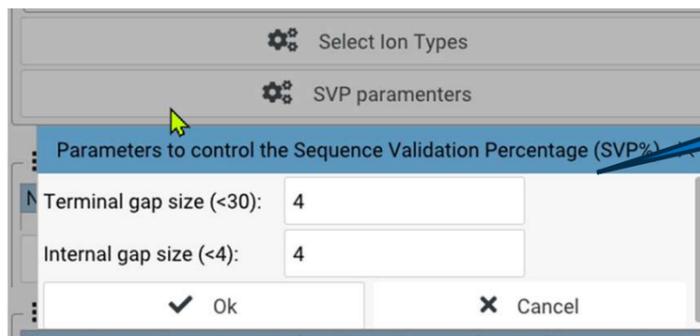
- For the calculation of the SVP, a certain tolerance for gaps in the fragment ion readout is introduced to obtain a continuous sequence readout
- The gaps that are left provide a good estimate of the remaining, unknown sequence

SVP was introduced in: A Resemann et al., [MAbs 2016;8\(2\):318-30](#). Full validation of therapeutic antibody sequences by middle-up mass measurements and middle-down protein sequencing.

Introducing SVP – the Sequence Validation Percentage

Control and Output

1. Click to open the SVP Parameters dialog



ESI: Terminal Gapsize 2-4, internal gapsize: 2-4

MALDI terminal Gapsize 7-14, internal gapsize: 1-2

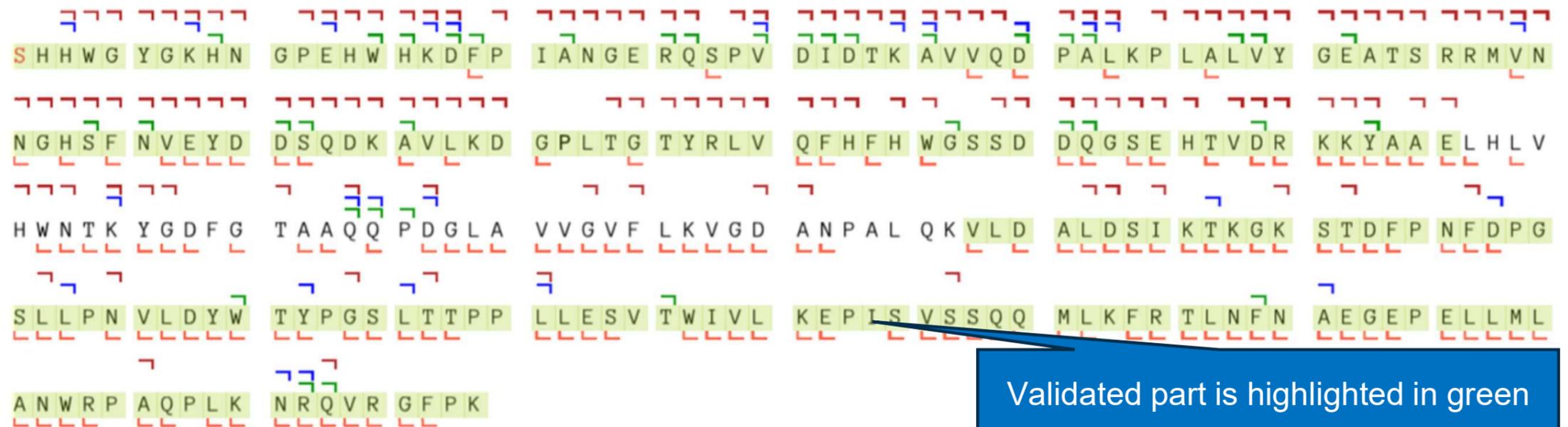
Protein	SC [%]	SVP [%]	IC [...]	MS Score [a.u.]	Var. mods	Mr m...
CA2_BOV	87.98	84.17	32.12	28.2607	Ace: N-term	29006.0
CA2_BOV	75.58	53.28	22.43	16.9504		28964.0
CA2_BOV	70.54	53.28	21.94	15.4736	Pho: S1, Ace: N-term	29086.0
CA2_BOV	69.77	53.28	20.68	14.4308	Pho: S1	29044.0

Primary ion types

- a [8.9%]
- b [5.4%]
- c [41.5%]
- x
- y [20.5%]
- z [50.8%]

Other

- SVP



Validated part is highlighted in green

SVP was introduced in: A Resemann et al., [MAbs 2016;8\(2\):318-30](#). Full validation of therapeutic antibody sequences by middle-up mass measurements and middle-down protein sequencing.

Clipping Analysis

here: CA2 with His tags added to N- and C-term

Name: CA2 His tagged

Sequence Length: 270 residues Monoisotopic Mass: 30472.3202 Da Chemical Formula: C1376H2071N391O394S3

10	20	30	40	50	60
H H H H H S H H W	G Y G K H N G P E H	W H K D F P I A N G	E R Q S P V D I D T	K A V V Q D P A L K	P L A L V Y G E A T
70	80	90	100	110	120
S R R M V N N G H S	F N V E Y D D S Q D	K A V L K D G P L T	G T Y R L V Q F H F	H W G S S D D Q G S	E H T V D R K K Y A
130	140	150	160	170	180
A E L H L V H W N T	K Y G D F G T A A Q	Q P D G L A V V G V	F L K V G D A N P A	L Q K V L D A L D S	I K T K G K S T D F
190	200	210	220	230	240
P N F D P G S L L P	N V L D Y W T Y P G	S L T T P P L L E S	V T W I V L K E P I	S V S S Q Q M L K F	R T L N F N A E G E
250	260	270	280	290	300
P E L L M L A N W R	P A Q P L K N R Q V	R G F P K	H H H H H		

Sequence(s) & Mass Filter Options

Name	Mass Type	Neutral Mass	Tolerance [mDa]	Clipping	Action
CA2 His tag	Monoiso	29006.6800	100.0000	<input checked="" type="checkbox"/>	

The visualization shows the sequence alignment with red brackets indicating the clipped regions at the N-terminus (residues 1-5) and C-terminus (residues 266-270). Blue brackets indicate the remaining sequence after clipping.

1. Enter measured **Neutral Mono Mass**
2. Select **Clipping** (if measured mono mass < mono mass calculated from sequence, clipping analysis can be performed)
3. **Start:** the N- and C-term His-tags are clipped off and CA2 is matched

Entirely revamped *De Novo* Sequencing

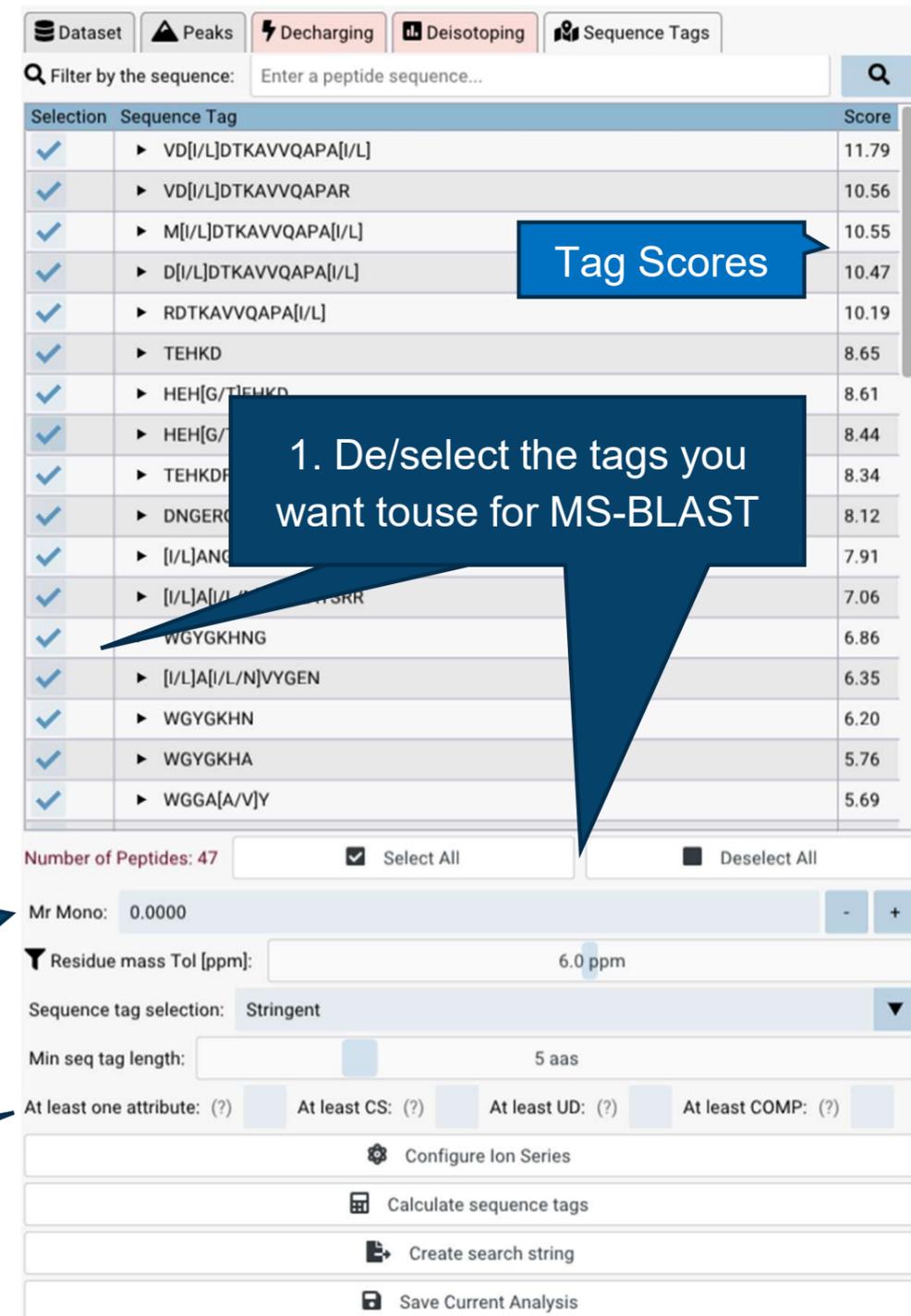
- The new Sequence Tags tab contains the **NEW *De Novo*** results, incl. Scores
- Dramatically increased speed (~10-fold)
- Higher quality and robust sequence tag generation

2. Define how many related tags you want to see in the Sequence Tag list

- Elitist 2
- Stringent 5
- Relaxed 20
- All

Start with Relaxed and reduce if too many hits are reported (~>100)

3. Leave empty and apply rules only if too many junk tags are proposed



Selection	Sequence Tag	Score
<input checked="" type="checkbox"/>	▶ VD[I/L]DTKAVVQAPA[I/L]	11.79
<input checked="" type="checkbox"/>	▶ VD[I/L]DTKAVVQAPAR	10.56
<input checked="" type="checkbox"/>	▶ M[I/L]DTKAVVQAPA[I/L]	10.55
<input checked="" type="checkbox"/>	▶ D[I/L]DTKAVVQAPA[I/L]	10.47
<input checked="" type="checkbox"/>	▶ RDTKAVVQAPA[I/L]	10.19
<input checked="" type="checkbox"/>	▶ TEHKD	8.65
<input checked="" type="checkbox"/>	▶ HEH[G/T]TEHKD	8.61
<input checked="" type="checkbox"/>	▶ HEH[G/T]TEHKD	8.44
<input checked="" type="checkbox"/>	▶ TEHKDF	8.34
<input checked="" type="checkbox"/>	▶ DNGERC	8.12
<input checked="" type="checkbox"/>	▶ [I/L]ANG	7.91
<input checked="" type="checkbox"/>	▶ [I/L]A[I/L]N	7.06
<input checked="" type="checkbox"/>	▶ WGYGKHNG	6.86
<input checked="" type="checkbox"/>	▶ [I/L]A[I/L/N]VYGEN	6.35
<input checked="" type="checkbox"/>	▶ WGYGKHN	6.20
<input checked="" type="checkbox"/>	▶ WGYGKHA	5.76
<input checked="" type="checkbox"/>	▶ WGGA[A/V]Y	5.69

Number of Peptides: 47 Select All Deselect All

Mr Mono: 0.0000

Residue mass Tol [ppm]: 6.0 ppm

Sequence tag selection: Stringent

Min seq tag length: 5 aas

At least one attribute: (?) At least CS: (?) At least UD: (?) At least COMP: (?)

Configure Ion Series

Calculate sequence tags

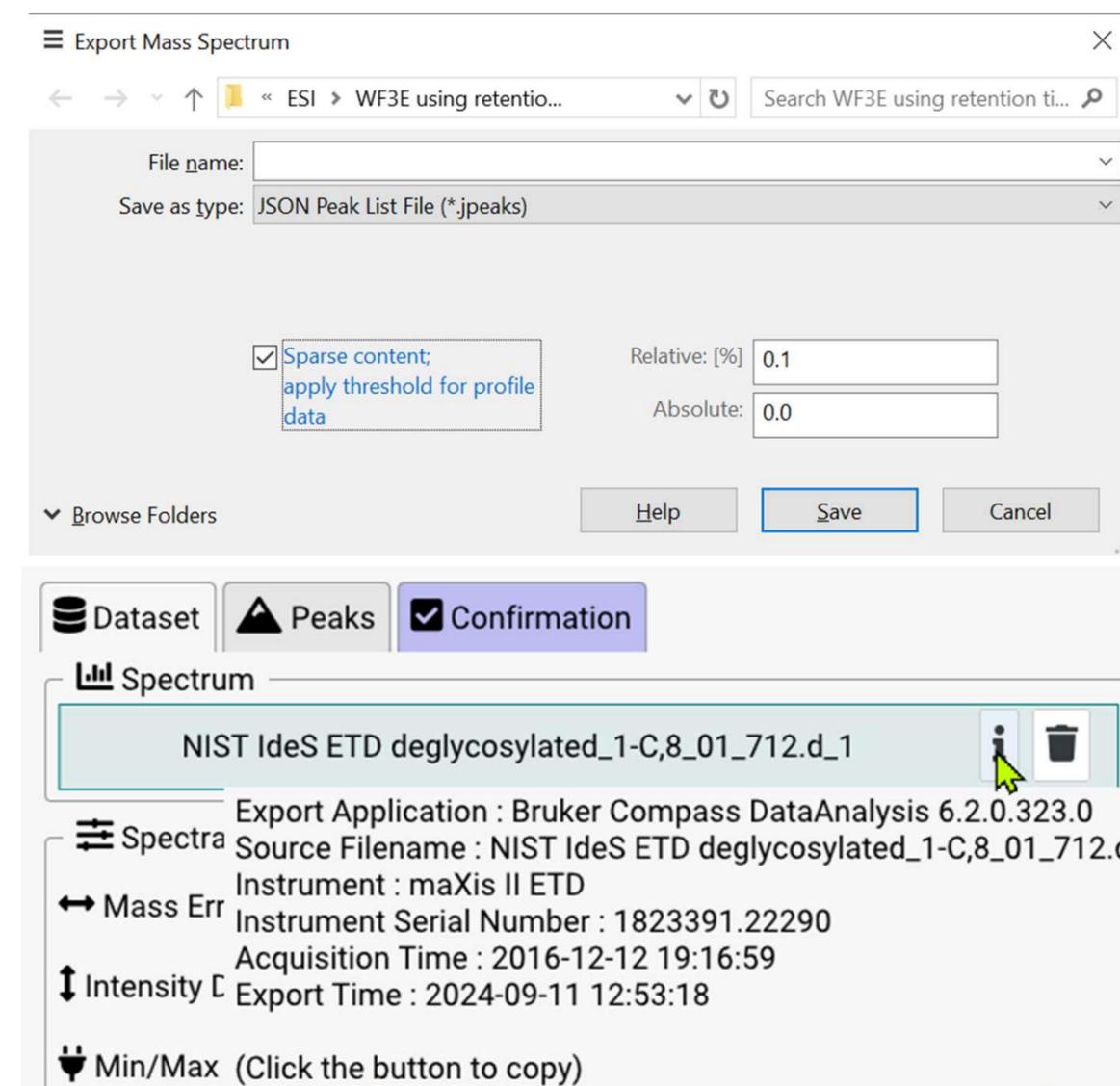
Create search string

Save Current Analysis

JPEAKS File from DA 6.2.323+ can be used in OSc

DA adds a new export format: JPEAKS

- Provides **meta information** of dataset
- Contains **profile data**, optionally peaklist, if generated
- Allows **reimport to DA** by drag-and-drop from the file manager
- JPEAKS spectra can be **reprocessed in DA** if needed
- JPEAKS files are large, can be reduced in size during export by **low intensity filter (Abs/Rel)**
- JPEAKS is also used for **export to BPC** for HR MALDI spectra (fleX, MRMS)





Confirmation Report (pdf)

Keep list for fragment walk

Selected	Name	m/z	z	ppm	Score [a.u.]	ei Score	Max Int.
-/-	Filter by name				top 100.0%	top 100.0%	top 100.0%

1. Create report

Create Report

Top-Down Result OmniScape 2025
Sequence Confirmation
NIST LC ETD JPEAKS

Project Information
Project Directory: C:\Users\detlev.suckau
Processing date: Tue Oct 15 10:54:56 2024

Sample Info & Protocols
Name: Top-Down Protein Sequencing ExD

Dataset
Imported spectrum file name: NIST IdeS ETD deglycosylated_1-C,8_01_712.d_1
Dataset name: NIST IdeS ETD deglycosylated_1-C,8_01_712.d
Date acquired: 2016-12-12 19:16:59
Date imported: 2024-09-11 12:53:18
Instrument Name: maXis4i ETD
Instrument Serial Number: 1823391.22290

Workflow Result Info
Analysis: NIST LC ETD JPEAKS
Location: C:\Users\detlev.suckau
Method: -

Metainformation only available for JPEAKS files

Top-Down Result OmniScape 2025
Sequence Confirmation
NIST LC ETD JPEAKS

Sequence Map

Proteoform	Index	Protein	SC [%]	SVP [%]	IC [%]	MS Score [a.u.]	Var. Mods	Mono Mass [Da]	Chemical Formula
1		NIST LC	75.47	36.62	65.02	49.07		23113.3042	C1020H1578N2700330S7

DIQMT QSPST LSASV GDRVT ITCSA SSRVG YMHVY QKQPG KAPKL LIYDT SKLAS GVPSR FSGSG SGTEF TLTIS SLOPD
DFATY YCFQG SGYFP TFGGG TKVEI KRIVA APSVF IFFPS DEQLR SGTAS VVCLL NNFYP REARK QKQVD NALQS GNSQE
SVTEQ DSKDS TYSLT STLT SKADY EKHKV YACEV THOGL SSPVT KSFNR GEC

Report Configuration

Include:
 Top proteoforms 1
 Select proteoforms

Meta Information
 Method
 Sequence

Selected	Protein	SC [%]	SVP [%]	IC [%]	MS Score [a.u.]	Var. mods	Mr mono [Da]	Formula
<input checked="" type="checkbox"/>	NIST LC	80.66	37.09	60.25	48.60		23113.3042	C1020H1578N2700330S7

For each proteoform report:
 Sequence Map
 Primary Fragment's Plots
 Error Plot
 Internal Fragment's Plots

Export Cancel

3. Click export and define target directory

2. Select report options allow to in/exclude certain elements from reporting

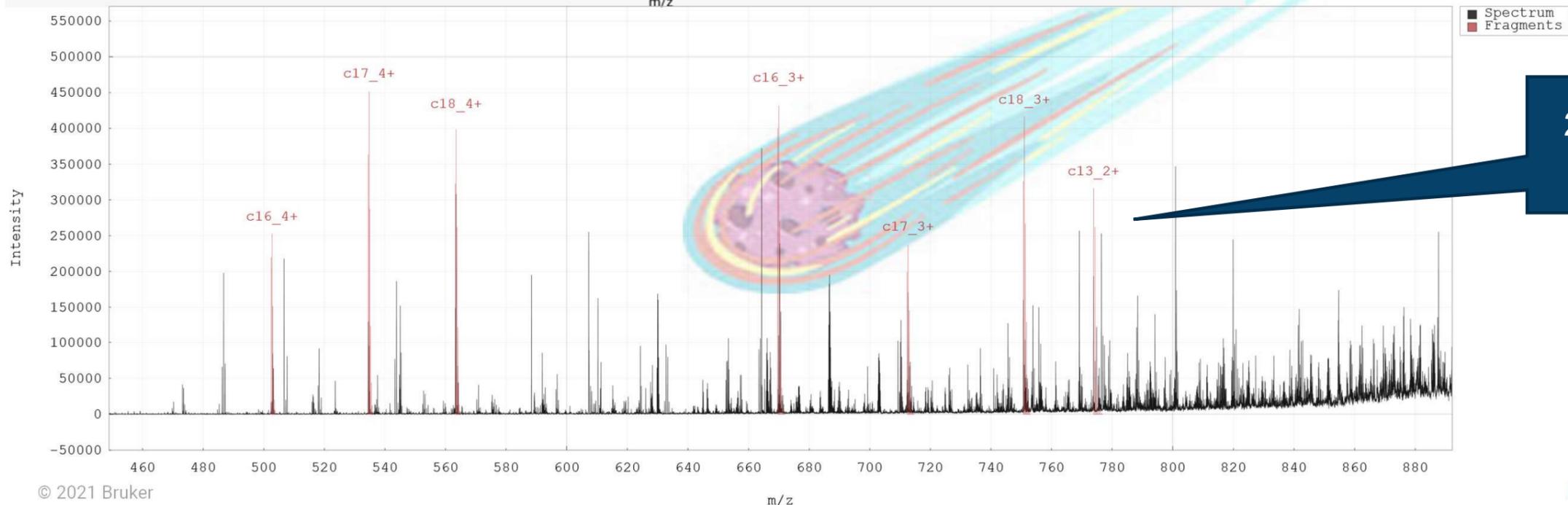
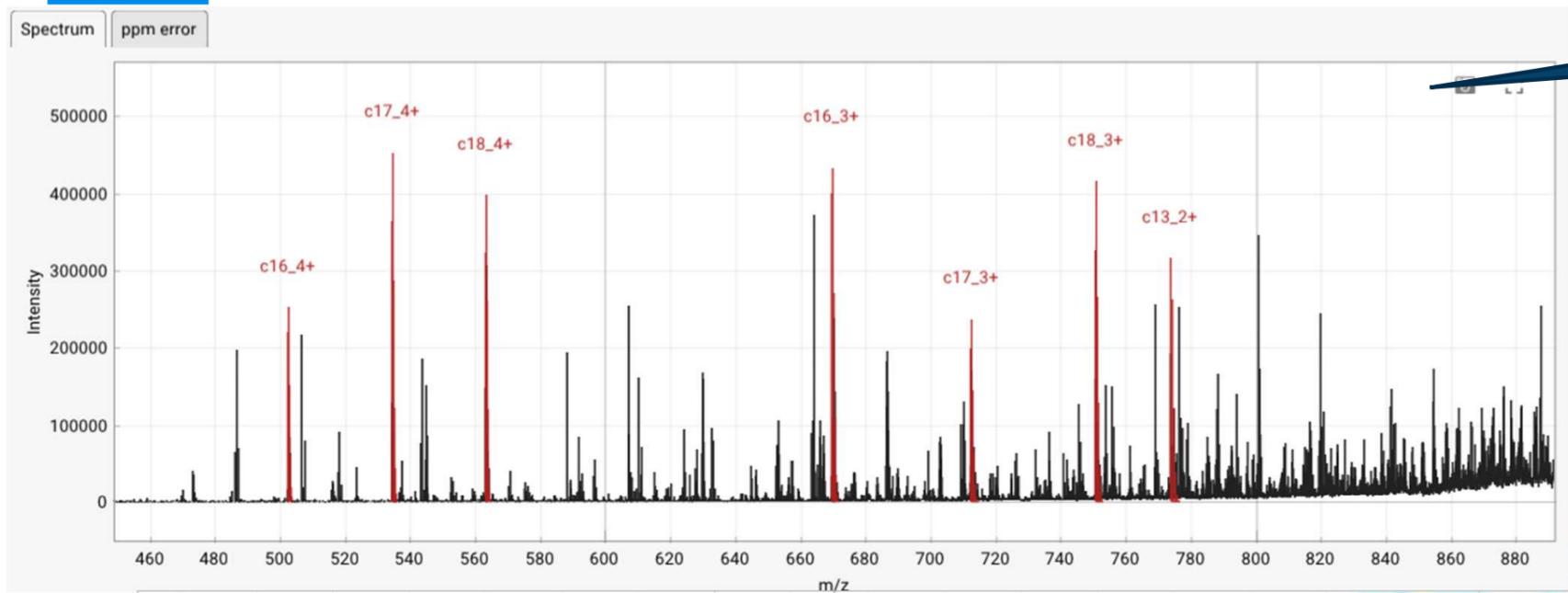
Top-Down Result OmniScape 2025
Sequence Confirmation
NIST LC ETD JPEAKS

Primary Fragment Plots

Protein	SC [%]	SVP [%]	IC [%]	MS Score [a.u.]	Var. Mods	Mono Mass [Da]	Chemical Formula
NIST LC	77.83	71.70	63.33	49.29		23113.3042	C1020H1578N2700330S7

Export of SVG graphics from all kinds of plots

1. Click on camera to export an SVG file



2. Import as transparent vector graphics into MS Office etc.

Export of lists into CSV

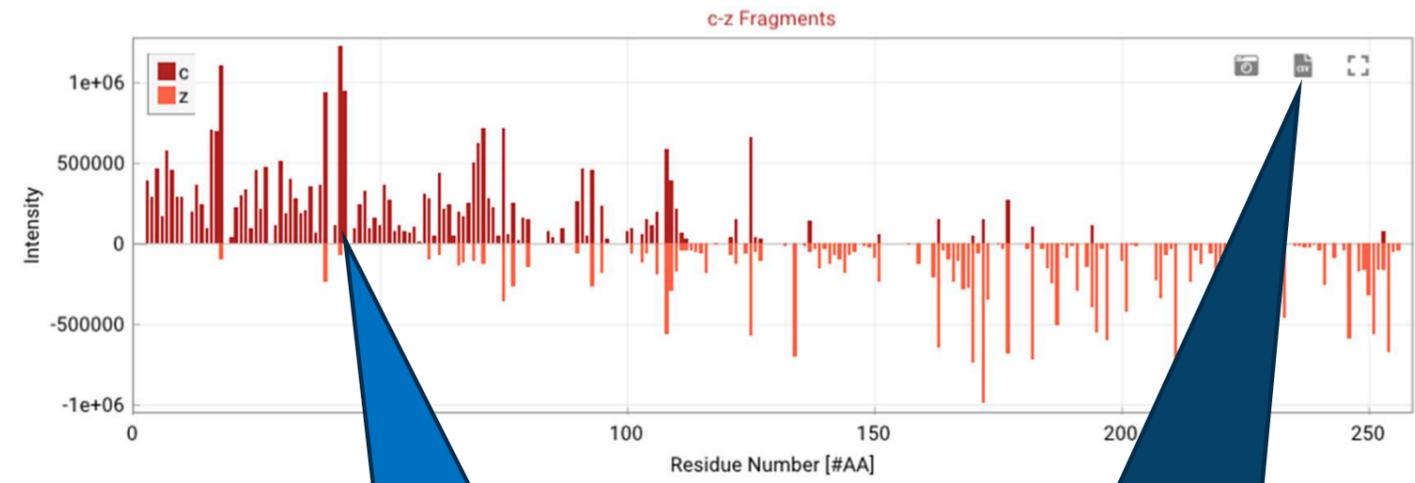
Keep list for fragment walk

Selected	Name	m/z	z	ppm	Score [a.u.]	ei Score	Max Int.
8/8	Filter by				top 100.0%	top 100.0%	top 7.8%
<input checked="" type="checkbox"/>	c13_2+	773.8504	2	1.11	29.865	98.31	316437.24
<input checked="" type="checkbox"/>	c16_3+	669.6350	3	1.45	49.963	99.08	447391.26
<input checked="" type="checkbox"/>	c16_4+	502.4781	4	1.65	83.700	99.83	253361.06
<input checked="" type="checkbox"/>	c17_3+	712.3334	3	1.91	30.470	99.99	239588.11
<input checked="" type="checkbox"/>	c17_4+	534.5018	4	2.09	73.074	99.77	453403.73
<input checked="" type="checkbox"/>	c18_2+	1125.5099	2	-1.09	4.753	94.73	271028
<input checked="" type="checkbox"/>	c18_3+	750.6757	3	1.88	49.102	99.71	416953.8
<input checked="" type="checkbox"/>	c18_4+	563.2586	4	2.20	79.074	99.07	411176.4

1. Right mouse-click provides handle to export .csv file from peaklist

A	B	C	D	E	F	G	H	I	J
#	Selected	Name	m/z	z	ppm	Score [a.u.]	ei Score	Max Int.	Tag
1	TRUE	c13_2+	773.8504	2	1.11	29.865	98.31	316437.2	
2	TRUE	c16_3+	669.635	3	1.45	49.963	99.08	447391.3	
3	TRUE	c16_4+	502.4781	4	1.65	83.7	99.83	253361.1	
4	TRUE	c17_3+	712.3334	3	1.91	30.47	99.99	239588.1	
5	TRUE	c17_4+	534.5018	4	2.09	73.074	99.77	453403.7	
6	TRUE	c18_2+	1125.51	2	-1.09	4.753	94.73	271028	
7	TRUE	c18_3+	750.6757	3	1.88	49.102	99.71	416953.8	
8	TRUE	c18_4+	563.2586	4	2.20	79.074	99.07	411176.4	

2. Open in EXCEL etc.

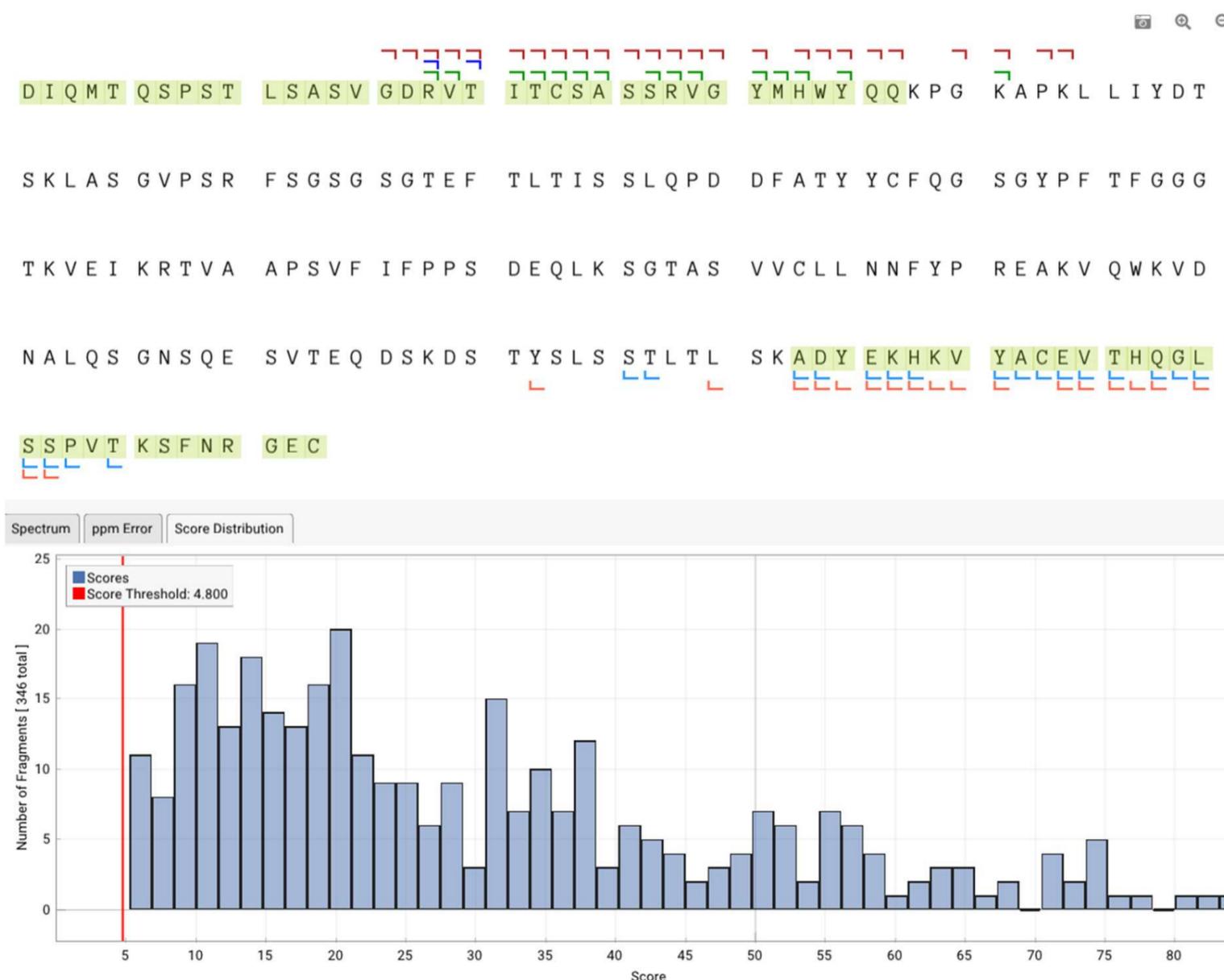


1. Click on sheet icon to export as .csv file

Each bar represents the total intensity of a fragment ion across multiple charge states

Score Distribution plot

- **Score Threshold [a.u.]** = 3 per default (defined in the Confirmation tab)
- Here, it was set to 4.8
- The plot allows to rationally define reasonable threshold values



What's New in OSc 2025?

A QUICK GUIDE TO OSC



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Main features

- Software with predefined **Top-Down protein sequencing workflows** to for best ease-of-use and reproducibility:
 - **Sequence Editor** to define sequences and PTMs
 - **Confirmation** workflow with scored proteoform assessment
 - **Extended Confirmation** for many variable modification sites
 - **Result combiner** workflow which can combine multiple sequence maps into 1 better one
 - **De novo sequencing** workflow with MS-BLAST homology searches for protein ID
 - **Calibration** workflow to recalibrate spectra internally with matching fragments
- **Entire analyses can be saved** and reloaded – they can be shared between OSc users
- **Datasets need to be calibrated, smoothed and background subtracted (optionally) in DA** and exported as Simple ASCII (.xy) datasets; (.txt files from 3rd party programs can be used as well) prior to import into OSc

Define sequence and proteoforms in the Sequence Editor for the Confirmation workflow

- Opens empty from the **Add Sequence** button (sequence must be pasted – it cannot be typed!)
- Opens with Sequence and PTMs from the **Load Sequence** button
- **Save Changes** activates them, but does not save them to the file system yet

1. Add Sequence name

2. Select residue type or terminus

3. Select residue positions

4. Select PTM – use filter

5. Define them as fixed or variable

Variable mods are coded red, fixed mods green

Define custom PTMs

Add another Sequence

Add a Tag name in case of multiple sequences (LC, HC) etc

Sequence Editor

Subunits

Sequence 1

Name: CA2

Sequence Length: 259 residues

Monoisotopic Mass: 28964.6721 Da

Chemical Formula: C1310H1994N3580383S3

Tag: (?)

SHHWG YGKHN GPEHWHKDFP I ANGERQSPV DI DTKAVVQD PALKPLALVY GEATSRMVN NGH S FNV EYD DS QDKAVLKD GPLTGT YRLV QFHFHVGSSD DQGS EHTVDR KKYAAELHLV HWNTK YGDFG TAAQQPDGLA VVGVF LKVG D ANPAL QKVL ALDSI KTKGK STDFPNFDPG SLLPNVLDYW TYPGSLTTPP LLESVTW I VL KEPI SVSSQQ MLKFR TLNFN AEGEP ELLML ANWRPAQPLK NRQVRGFPK

Positions

Amino Acid: Serine (S)

Position(s): S 1, S 28, S 55, S 64, S 72, S 98, S 99, S 104, S 164, S 171, S 181, S 195, S 204, S 215, S 217, S 218

Modifications

Filter by Name: Phospho

Phosphorylation

Mr. mono.: 79.9663 Da

Gain: H03P

Loss:

Modification	Position	Fixed	Variable	Remove
Acetyl	N Terminus	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 1	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 64	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 72	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 98	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 99	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 104	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 164	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>
Phospho	S 171	<input type="radio"/>	<input checked="" type="radio"/>	<input type="button" value="Remove"/>

Save Changes

Close

Confirmation workflow

1. Select workflow

2. Load spectrum (.xy or .txt file)

3. Select Max Charges as precursor ion charge

4. Select experiment and edit ion types if needed

5. Add a new sequence or load 1 or more defined sequence(s)

Ready

After import of spectrum and sequence

The screenshot shows the Bruker OmniScope software interface. The main window displays a mass spectrum plot with Intensity on the y-axis (ranging from -200,000 to 4.2e+06) and m/z on the x-axis (ranging from 200 to 2200). A prominent peak is visible at approximately m/z 900. The interface includes a top menu bar with 'Analyses', 'Methods', and 'Confirmation' options. On the right side, there are several panels: 'Spectrum' (showing 'CAH.xy'), 'Spectral Parameters' (with sliders for Mass Error [ppm] at 6.00, Intensity Deviation at 0.15, and Min/Max Charges at 1 and 23), 'Experiment Type' (with radio buttons for CID, ExD, EID, and MALDI), and 'Sequence(s) & Mass Filter Options' (containing a table of sequences and a 'Generate Proteoforms' button). Below this is a 'Proteoforms [4]' table and a 'Start' button. A blue callout box points to the 'Generate Proteoforms' button with the text: 'Click Generate Proteoforms to preview the Proteoforms list to be analyzed'. Another blue callout box points to the 'Start' button with the text: '1. Click Start'. A status bar at the bottom left reads 'Method has been changed. Resetting analysis.'

Click Generate Proteoforms to preview the Proteoforms list to be analyzed

1. Click Start

Name	Filter	Mass Type	Neutral Mass	Tolerance [mD...	Action
CA2_BOV	Mon	▼	0.0000	100.0000	[Edit] [Lock] [Delete]

Parent Seq.	Chain	Var. Mods	Mono Mass	Chemical Composition
CA2_BOV	CA2_S	Pho: S1, Ac	29086.6490	C1312H1997N358O387S...
CA2_BOV	CA2	Ace: N-term	29006.6827	C1312H1996N358O384S...
CA2_BOV	CA2_S	Pho: S1	29044.6385	C1310H1995N358O386S...
CA2_BOV	CA2		28964.6721	C1310H1994N358O383S...

Results are directly displayed after START

Result tab automatically selected

Sequence map of selected proteoform

Click Separator to expand Sequence Map

Display parameters

Score-sorted proteoform list with Sequence Coverage, Intensity Coverage and MS Score

Protein	S...	IC...	MS Sc...	Var. mods	Mr mon...	Form...
CA2_BOV	66.67	23.55	15.7025	Ace: N-term	29006.68	C1312F
CA2_BOV	46.12	14.17	6.5354		28964.67	C1310F
CA2_BOV	43.41	13.59	5.8995	Pho: S1, Ace:	29086.64	C1312F
CA2_BOV	41.86	13.52	5.6585	Pho: S1	29044.63	C1310F

Type	Percentage	Checked
a	8.5%	<input checked="" type="checkbox"/>
b	2.3%	<input checked="" type="checkbox"/>
c	34.9%	<input checked="" type="checkbox"/>
x		<input type="checkbox"/>
y	10.5%	<input type="checkbox"/>
z	35.3%	<input type="checkbox"/>

Ion Type	Checked
Internal a	<input type="checkbox"/>
Internal b	<input type="checkbox"/>
Precursor	<input type="checkbox"/>

Ion Type	Checked
d	<input type="checkbox"/>
w	<input type="checkbox"/>
v	<input type="checkbox"/>

Selected	Name	m/z	z	ppm	Score [a.u.]	ei Score	Max...

Save Current Analysis

Validation of matching fragments

The screenshot displays the Bruker Proteome Discoverer interface. At the top, there are tabs for 'Analysis' and 'Results', and sub-tabs for 'Sequence Map', 'Primary Fragments', and 'Internal Fragments'. The 'Sequence Map' shows a protein sequence with various residues highlighted in different colors (red, green, blue, orange) and brackets indicating fragmentation sites. A blue callout box points to a selected range of residues, stating: 'Sequence range selected cause the spectrum to show matching fragments'. Another blue callout box lists keyboard shortcuts: 'CNTRL-A: select full sequence', 'Click-LMB: select 1 residue', 'SHIFT-Click LMB: extend the selection to another residue', and 'ESC: deselect all'. Below the sequence map is a mass spectrum plot with 'Intensity' on the y-axis (0 to 50,000) and 'm/z' on the x-axis (1282 to 1320). Several peaks are highlighted in red and labeled with their charge state and mass: (z+1)90_8+, (z+2)91_8+, (z+2)92_8+, (z+1)93_8+, (z+2)90_8+, and (z+2)91_8+. A blue callout box points to the spectrum with the text: '1. Curate fragment list by de/selection'. On the right side, there is a 'Proteoforms list' table and a 'Filter by Name' section. The 'Proteoforms list' table has columns for Protein, SC, IC, Var. mods, Mr mono, and Formula. The 'Filter by Name' section has a dropdown for 'Fragment Name' and a table of selected fragments. A blue callout box points to the 'Save Current Analysis' button at the bottom right of the interface, with the text: '2. Save analysis to file system if satisfied with result (needed for result combination)'.

Sequence range selected cause the spectrum to show matching fragments

CNTRL-A: select full sequence
 Click-LMB: select 1 residue
 SHIFT-Click LMB: extend the selection to another residue
 ESC: deselect all

1. Curate fragment list by de/selection

2. Save analysis to file system if satisfied with result (needed for result combination)

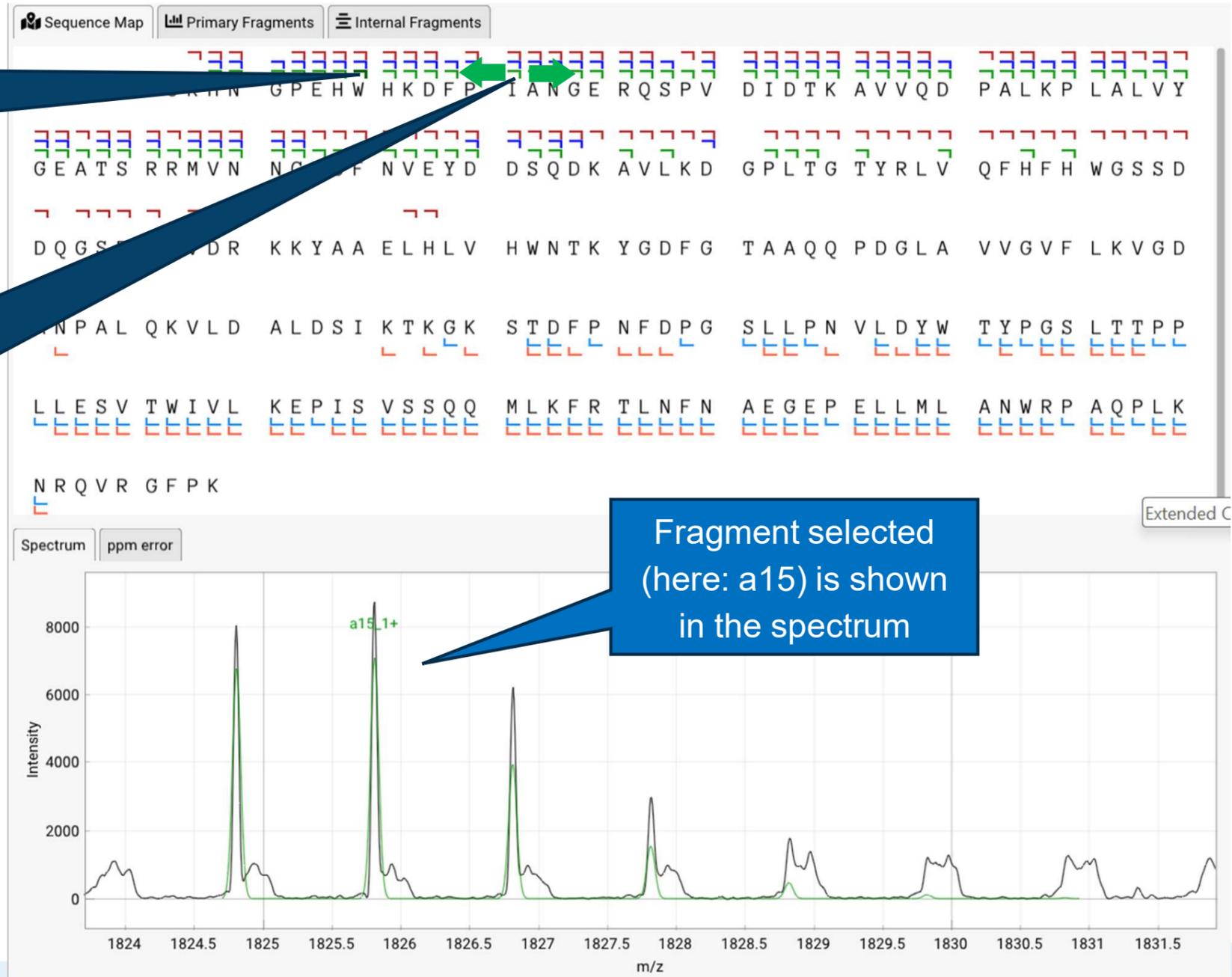
Protein	SC	IC	Var. mods	Mr mono	Formula
CA2_BOV	51.16	11.99	Ace: N-term	29006.6827	C1312H1996N358O38
CA2_BOV	32.56	6.52		28964.6721	C1310H1994N358O38
CA2_BOV	30.23	6.24	Pho: S1, Ace: N-	29086.6490	C1312H1997N358O38
CA2_BOV	28.68	6.12	Pho: S1	29044.6385	C1310H1995N358O38

Selected	Name	m/z	z	ppm	Score [a.u.]	ei Score	Max Int.
<input checked="" type="checkbox"/>	(z+1)90_8+	1282.4242	8	1.69	30.337	0.87	21901.83
<input checked="" type="checkbox"/>	(z+1)92_8+	1305.5637	8	2.43	15.005	0.83	16694.69
<input checked="" type="checkbox"/>	(z+1)93_8+	1318.1947	8	1.91	8.272	0.87	13804.64
<input checked="" type="checkbox"/>	(z+2)90_8+	1282.5502	8	1.58	3.770	0.88	7852.69
<input checked="" type="checkbox"/>	(z+2)91_8+	1289.6779	8	0.53	30.240	0.70	19874.68

Validation of matching fragments (2): the fragment walk

1. Click on a fragment mark causes the spectrum view to zoom close to that fragment

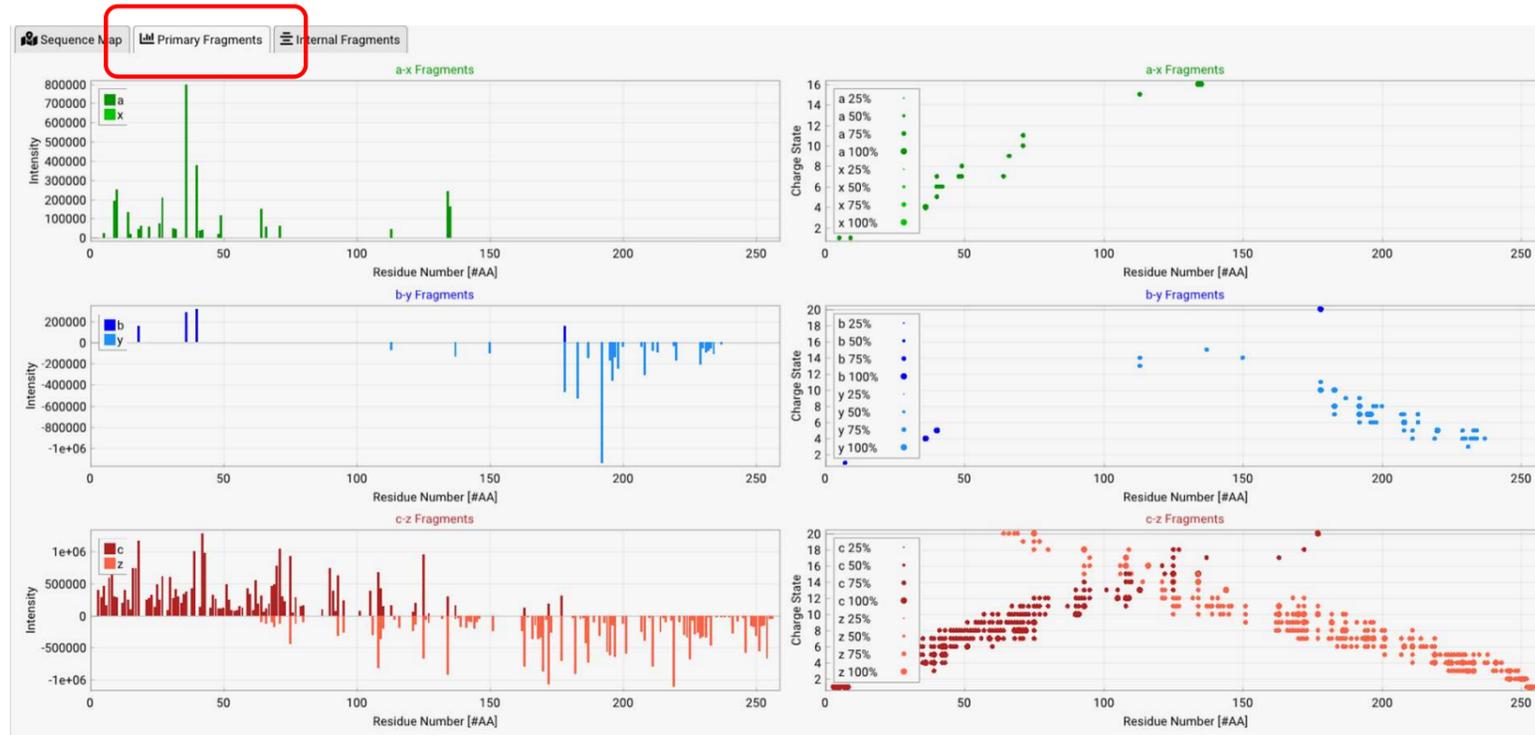
2. Keyboard arrow keys (←/→) allow to walk through the sequence and verify the fragments of the selected ion type.



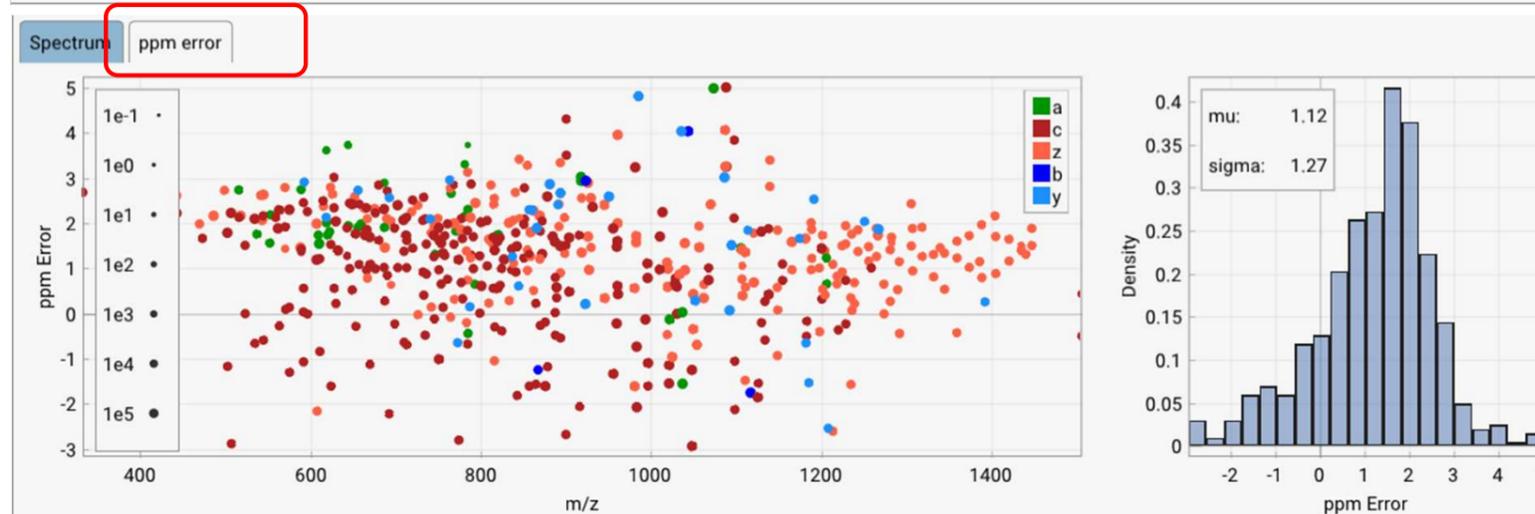
Fragment selected (here: a15) is shown in the spectrum

Additional Results tabs provide tools to inspect the data quality

- Primary fragments statistics can be inspected



- ppm Errors can be visualized



Extended Confirmation Workflow

- What is different from the Confirmation Workflow?
 - Averagine based deconvolution is performed.
 - **Speed**: Greatly reduced calculation time, at least 10x faster than the standard confirmation
 - **Data Volume**: disk space is saved and tasks can be performed that otherwise would need much larger SSD sizes
 - **Lower Interactivity** in the results
 - The **Sequence Coverage might be slightly lower** because weak isotope patterns might not match
- When is it needed?
 - If a high number ($\sim > 7-10$) of variable modification sites are defined in the sequence and many proteoforms need to be calculated

Define the De Novo Sequencing workflow

1. Select workflow

2. Load spectrum (.xy or .txt file)

3. Select Max Charges as precursor ion charge

4. Specify precursor neutral monoisotopic mass

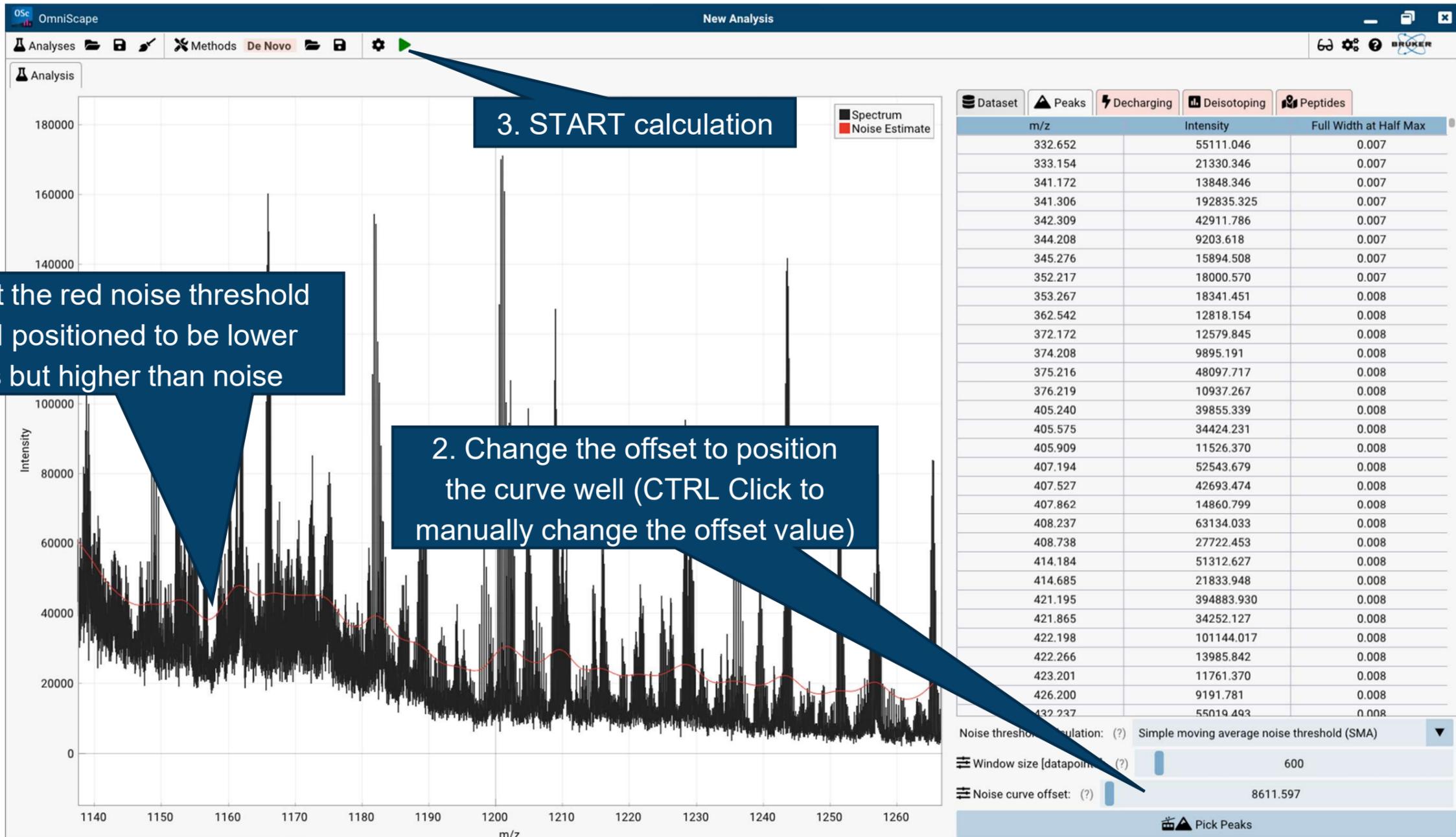
5. Select experiment and edit ion types if needed

6. Properly set the threshold curve under the Peaks tab

The screenshot shows the 'New Analysis' window in OmniScope. The 'Methods' menu is open, showing 'De Novo' selected. The 'Spectrum' tab is active, displaying a mass spectrum with intensity on the y-axis (ranging from -200,000 to 4.2e+06) and m/z on the x-axis (ranging from 200 to 3000). The configuration panel on the right includes:

- Dataset:** CAH.xy
- Spectral Parameters:** Mass Error [ppm]: 6.00, Intensity Deviation: 0.15, Min/Max Charges: 1 to 20, Mr Mono: 28964.6721
- Peptide Generator Options:** Experiment Type (CID, ExD, EID, MALDI), Complementary (a-y, a-x, b-y, c-z), and buttons for 'Check All Options', 'Uncheck All Options', and 'Start'.

Define the threshold curve before Start



From de novo sequencing to protein identification

View and load previously saved analyses.

Analysis

Intensity

180000

160000

140000

120000

100000

80000

60000

40000

20000

0

114

m/z

340 1360 1380 1400 1420

Sequence search string

ANGERQ-QREGNA-ANWRQ-QRWNA-FNAW-DL...-YGEATSRR-YWATSRR-EG

Copy Open MS-BLAST Close

Calculated sequence tags

1. Click Create search string

2. Click Copy and then Open MS-BLAST

Dataset Peaks Decharging Deisotoping Peptides

Filter by the sequence: Enter a peptide sequence...

Selected	Sequence	Rules	Ion Type	a	b	c	x	y	z
<input type="checkbox"/>	ANGERQ	Mz		0	0	0	0	0	0
<input type="checkbox"/>	ANWRQ	Mz		0	0	0	0	0	0
<input type="checkbox"/>	D[I/L]DT[K/Q]	Mz		0	0	0	0	0	0
<input type="checkbox"/>	EG[K/Q]T	Mz Ud	a	1	0	0	0	0	0
<input type="checkbox"/>	WANF	Mz Ud	z	0	1	0	0	0	3
<input type="checkbox"/>	YGEATSRR	Mz Ud	a	1	0	0	0	0	0
<input type="checkbox"/>	YWATSRR	Mz Ud	a	1	0	0	0	0	0

Peptides: 7

Mr Mono: 28964.6721

Ruleset: At least CS

Min seq tag length: 4 aas

Residue mass Tol [ppm]: 6.00 ppm

Min count of ion types: 1 aas

Allow ion type transitions in bidirectional tags:

Configure Ion Series

Calculate sequence tags

Create search string

Save Current Analysis

Browser with internet connection allows for the MS-BLAST Search

1. Select suitable protein sequence database

2. Paste the search string into the sequence field

3. Submit Query

MS-BLAST Search 

[Tips/Help](#) | [Disclaimer](#) | [Citation \(PubMed\)](#)

Choose a database for your search and set the number of unique peptides and score table:
Database unique peptides score table

Apply LC-MS/MS Presets
[What is it and when to use presets?](#)

Enter below your sequence in [FASTA](#) or raw format:

ANGERO-OREGNA-ANWRO-ORWNA-FNAW-DLDTZ-ZTDLD-YGEATSRR-YWATSRR-EGZT

Enter your query description:

Options for the BLAST server:
Matrix **Filter** **Echofilter** **Expect**
Cutoff **Descriptions** **Alignments**
Histogram **Other advanced options:** **Parsed HTML**

[Browser cookies must be enabled!](#)



The search result provides an overview of the found matches and provides access to the identified sequence(s)

Echofilter:

>Unfiltered+0
 ANGERQ-QREGNA-ANWRQ-QRWNA-FNAW-DLDTZ-ZTDLD-YGEATSRR-YWATSRR-EGZT

Database: uniprot_sprot-2011_03_msblast.fasta
 525,997 sequences; 185,874,894 total letters.
 Searching...10....20....30....40....50....60....70....80....90....100% done

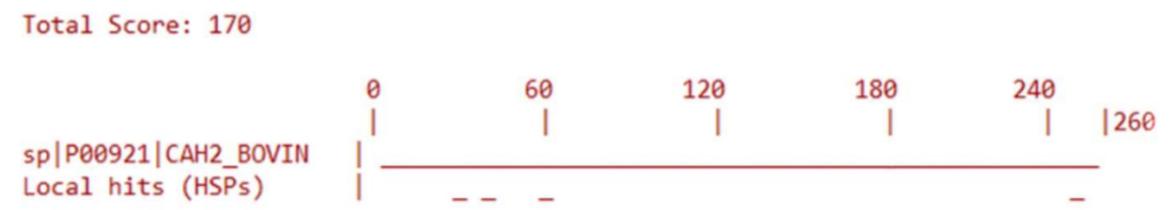
Color Key: red = positive hit; green = borderline hit; black = negative result

Summary:

Sequences producing High-scoring Segment Pairs:	High Score	Total Score	
/:sp P00921 CAH2_BOVIN Carbonic anhydrase 2 OS=Bos taurus...	59	170	<input checked="" type="checkbox"/>
/:sp P00922 CAH2_SHEEP Carbonic anhydrase 2 OS=Ovis aries...	38	139	<input checked="" type="checkbox"/>

The sequence found here can be the starting point for a Confirmation workflow in OSc

[^ = sp|P00921|CAH2_BOVIN](#) Carbonic anhydrase 2 OS=Bos taurus GN=CA2 PE=1 SV=3/
 Length = 260



Score = 59 (31.0 bits)
 Identities = 8/8 (100%), Positives = 8/8 (100%)

Query: 44 YGEATSRR 51
 YGEATSRR
 Sbjct: 51 YGEATSRR 58

Score = 44 (23.6 bits)
 Identities = 6/6 (100%), Positives = 6/6 (100%)

Query: 1 ANGERQ 6
 ANGERQ
 Sbjct: 23 ANGERQ 28

Score = 35 (19.1 bits)
 Identities = 4/4 (100%), Positives = 4/4 (100%)

Query: 15 ANWR 18
 ANWR
 Sbjct: 242 ANWR 245

Score = 32 (17.7 bits)
 Identities = 3/5 (60%), Positives = 5/5 (100%)

Query: 32 DLDTZ 36
 D+DT+
 Sbjct: 32 DIDTK 36

Result combiner allows to select multiple datasets to be combined on the level of the Sequence Map

1. Select Result Combination method

2. From the saved analyses select those (from a single directory) that are to be combined

3. Click Add to Selected

The combined map is visualized

Sequence...	SC [%]	IC [%]	MS Score...
CA2_BOV (/ 72.87	30.17	21.9822	
CA2_BOV (L 59.30	16.91	18.9272	
CA2_BOV (F 34.88	14.80	5.1623	
CA2_BOV (F 33.72	14.79	4.9867	

Sequence...	SC [%]	IC [%]	MS Score...
CA2_BOV (/ 75.58	31.54	23.8360	
CA2_BOV (L 60.85	24.10	14.6628	
CA2_BOV (F 52.33	22.72	11.8888	

Name	SC [%]
CA2	95.35
• CAH_ETD_confirm	83.33
• Neoflex CA	75.58
• CA FLEX	72.87

The combined map is visualized

OmniScope New Combined Analysis

Combined Analyses Methods Result Combination a[9.69%] b[5.43%] c[44.19%] x[0.00%] y[21.32%] z[53.88%] d[0.00%] w[0.00%] v[0.00%] M[0.00%]

Result Combination

Available Analyses

C:/Users/detlev.suckau/OmniScope/Analyses

CA FLEX_2

CAH FLEX confirmation

Sequence(s)	SC [%]	IC [%]	MS Score [a.u.]
CA2_BOV (Ace: N)	72.87	30.17	21.9822
CA2_BOV (unmo)	59.30	16.91	10.0272
CA2_BOV (Pho: S)	34.88	14.80	5.1623
CA2_BOV (Pho: S)	33.72	14.79	4.9867

CAH Neoflex confirmation

Sequence(s)	SC [%]	IC [%]	MS Score [a.u.]
CA2_BOV (Ace: N)	75.58	31.54	23.8360
CA2_BOV (unmo)	60.85	24.10	14.6628
CA2_BOV (Pho: S)	52.33	22.72	11.8888
CA2_BOV (Pho: S)	50.78	22.66	11.5058

CAH_confirmation

CAH_ETD_confirmation

CAH_ETD_de novo

CA_ETD

MLC-1v

MVD_HORSE

Add to Selected

Equivalence Filter

Same Sequence Same Proteoform

Selected Analyses

Name	SC [%]
CA2	95.35
• CAH_ETD_confirmation	83.33
• Neoflex CA	75.58
• CA FLEX	72.87

Remove Selected Save Combined Analysis

Added analysis: CA FLEX

Combined View Paired View

S H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V D I D T K A V V Q D P A L K P L A L V Y

G E A T S R R M V N N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V Q F H F H W G S S D

D Q G S E H T V D R K K Y A A E L H L V H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D

A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G S L L P N V L D Y W T Y P G S L T T P P

L L E S V T W I V L K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L A N W R P A Q P L K

N R Q V R G F P K

1. Selected Sequence Map is displayed

2. Click through the individual maps for comparison

3. Save combined analysis

The individual map can be visualized against the combined map

OmniScope File View Preferences

Combined Analyses Methods Result Combination a[7.36%] b[2.71%] c[37.60%] x[0.00%] y[14.34%] z[46.12%] d[0.00%] w[0.00%] v[0.00%] M[0.00%]

Result Combination

Available Analyses
C:/Users/detlev.suckau/OmniScope/Analyses

- ▶ AMPK
- ▶ CA ETD denovo
- ▼ CA FLEX
- ▶ CA FLEX_2
- ▶ CAH_confirmation
- ▶ CA_ETD
- ▶ MLC-1v
- ▶ MYO_HORSE
- ▼ Neoflex CA
- ▶ neofleX Ubi
- ▶ NIST_LC_MALDI-MSD val
- ▶ T3 UBI 2234

Add to Selected

Equivalence Filter
 Same Sequence Same Proteoform

Selected Analyses

Name	SC [%]
▼ CA2	93.80
• CA FLEX	72.87
• Neoflex CA	75.58
• CAH_confirmation	74.42

Remove Selected Save Combined Analysis

Combined View Paired View

S H H W G Y G K H N O H W H K D F P I A N G E R Q S P V D I D T K A V V Q D P A L K P L A L V Y

G E A T S R R M V N N G H S F N V E Y D D S Q P L T G T Y R L V Q F H F H W G S S D

D Q G S E H T V D R K K Y A A E L H L V H W N T K

A N P A L Q K V L D A L D S I K T K G K S T D F P

L L E S V T W I V K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L A N W R P A Q P L K

N R Q V R G F P K

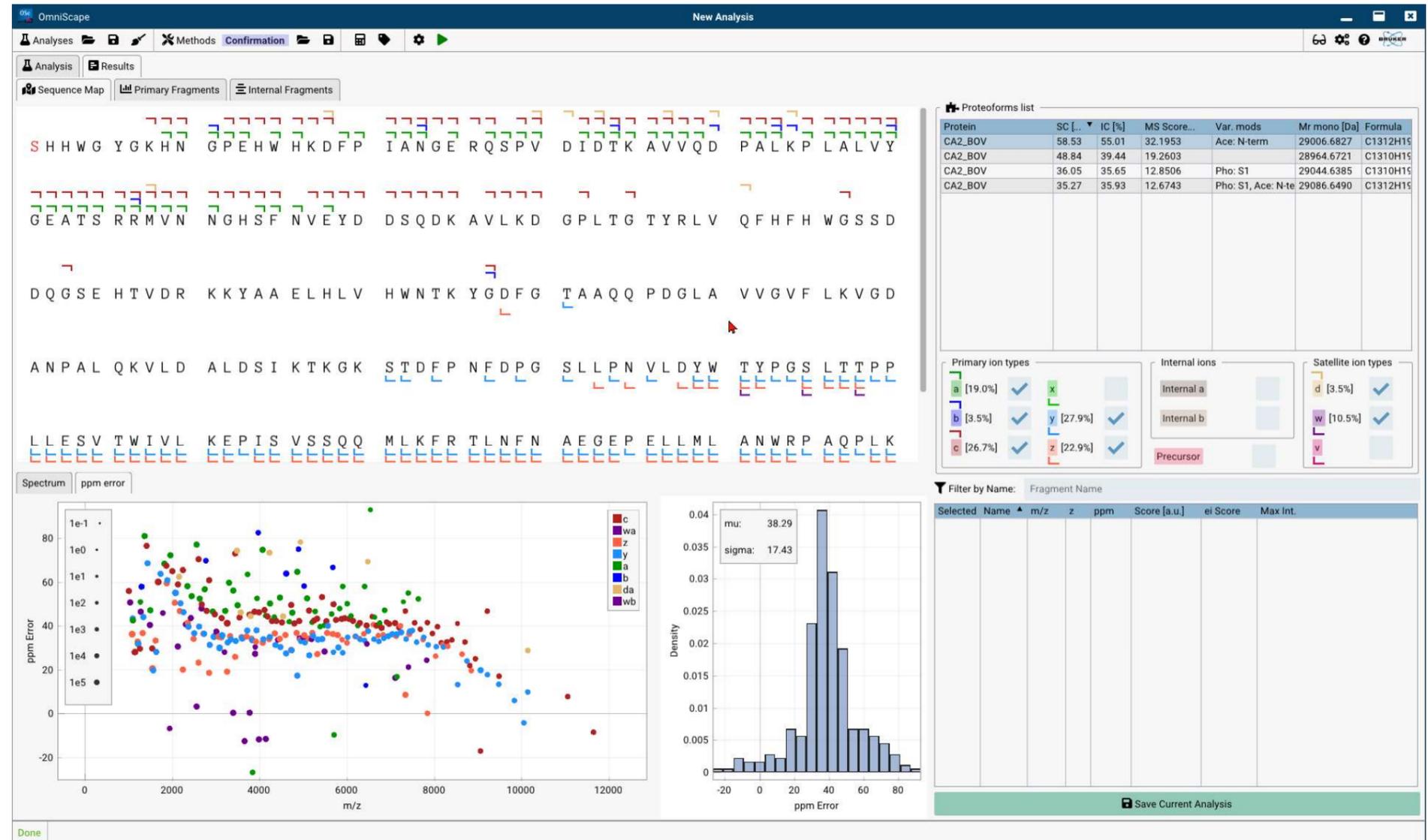
Combined view and Paired view can be selected

Greyed fragments are not contained in the selected single results

1. Save combined analysis

After Confirmation workflow completion and large ppm errors are observed...

- The ppm error plot allows to identify useful calibration points for a quadratic or cubic calibration function
- Also, the m/z tolerance for calibration can be estimated (here: 100 ppm)
- Consider a list of calibrants (e.g., c12, c25, c50, c70) that you expect to be good ones



Setup for calibration: select method type Calibration

1. Select Calibration tab

2. Select Proteoform

3. Enter ppm Tolerance

4. Click Generate fragments...

5. In the spectrum zoom to the first calibrant mass

Protein	SC [%]	IC [%]	MS Score...	Var. mods	Mr mono [Da]	Formula
CA2_BOV	58.53	55.01	32.1953	Ace: N-term	29006.6827	C1312H19
CA2_BOV	48.84	39.44	19.2603		28964.6721	C1310H19
CA2_BOV	36.05	35.65	12.8506	Pho: S1	29044.6385	C1310H19
CA2_BOV	35.27	35.93	12.6743	Pho: S1, Ace: N-ter	29086.6490	C1312H19

Name	m/z	z	ppm	Score [a.u]
c8_1+	1012.4748	1	55.8599	11.405
wa9_1+	1041.5952	1	50.6477	23.463
(z+2)9_1+	1086.6167	1	36.1602	61.171
y9_1+	1101.6276	1	43.3378	32.681
a9_1+	1104.5122	1	42.4657	13.196
c9_1+	1149.5337	1	27.9960	46.142
da10_1+	1175.5493	1	43.4746	0.151
(z+2)10_1+	1214.7117	1	32.8084	48.730

Fragment	Measured m/z	Theoretical m/z	ppm Error Before	ppm Error After

Calibration Parameters

Calibration Method: Quadratic

ppm Tolerance: 100.0 ppm

Generate fragments for selected proteoform

Calibrate | Export Calibrated Spectrum

Fragmentation of sequence CA2_BOV_1 finished.

Zoom in to first calibrant and do:

5. Click Add to Calibration Points

4. Click on top of matching measured peak

3. Click on top of calculated peak

2. Select peak in corresponding peaklist

1. Specify calibrant mass tolerance

Calibrant is added to the list of calibrants

Dataset: Peaks Calibration

Protein	SC [%]	IC [%]	MS Score...	Var. mods	Mr mono [Da]	Formula
CA2_BOV	58.53	55.01	32.1953	Ace: N-term	29006.6827	C1312H19
CA2_BOV	48.84	39.44	19.2603		28964.6721	C1310H19
CA2_BOV	36.05	35.65	12.8506	Pho: S1	29044.6385	C1310H19
CA2_BOV	35.27	35.93	12.6743	Pho: S1, Ace: N-ter	29086.6490	C1312H19

Name	m/z	z	ppm	Score [a.u]
y12_1+	1439.8594	1	68.5456	33.053

Fragment	Measured m/z	Theoretical m/z	ppm Error Before	ppm Error After
y12_1+	1439.9599	1439.8594	69.78	-

Calibration Parameters

Calibration Method: Quadratic

ppm Tolerance: 100.0 ppm

Generate fragments for selected proteoform

Calibrate Export Calibrated Spectrum

Not enough calibration points to calibrate the spectrum. Needed: 3, available: 1

Repeat until the calibration curve can be calculated

Expected calibrated spectrum

Current spectrum

Theoretical isotope pattern

Expected calibration effect: reduce errors from ~50 to 5 ppm

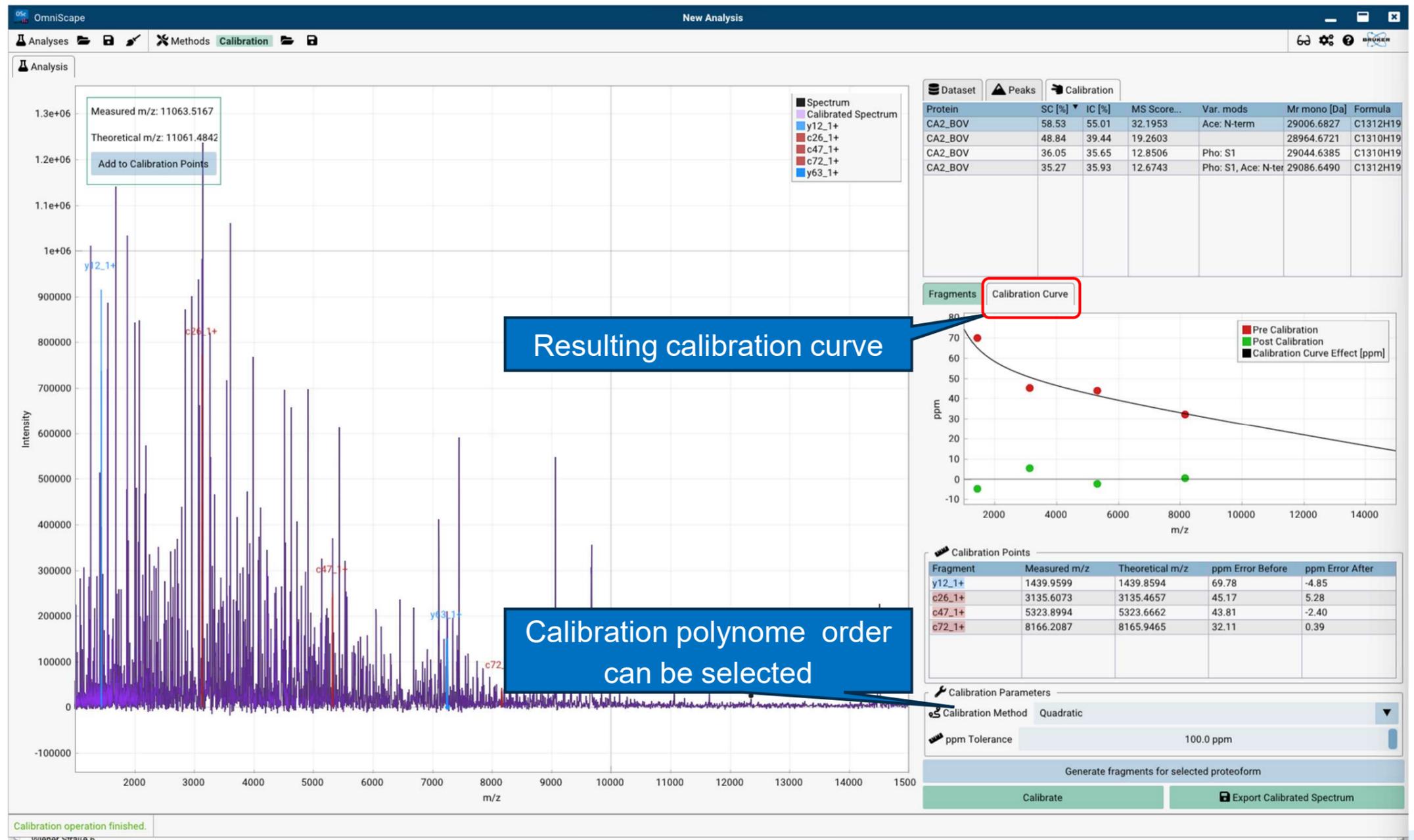
1. Click Calibrate

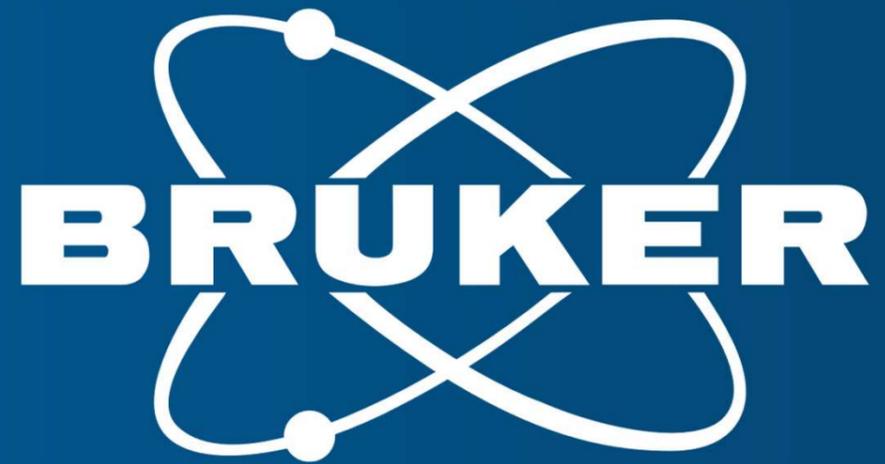
2. Export calibrated spectrum (must then be reloaded)

Protein	SC [%]	IC [%]	MS Score...	Var. mods	Mr mono [Da]	Formula
CA2_BOV	58.53	55.01	32.1953	Ace: N-term	29006.6827	C1312H19
CA2_BOV	48.84	39.44	19.2603		28964.6721	C1310H19
CA2_BOV	36.05	35.65	12.8506	Pho: S1	29044.6385	C1310H19
CA2_BOV	35.27	35.93	12.6743	Pho: S1, Ace: N-ter	29086.6490	C1312H19

Fragment	Measured m/z	Theoretical m/z	ppm Error Before	ppm Error After
y12_1+	1439.9599	1439.8594	69.78	-4.85
c26_1+	3135.6073	3135.4657	45.17	5.28
c47_1+	5323.8994	5323.6662	43.81	-2.40
c72_1+	8166.2087	8165.9465	32.11	0.39

The calibration curve can be visualized





Innovation with Integrity