What’s New in OSc 2025b?

A QUICK GUIDE TO THE LATEST OSC

Main features

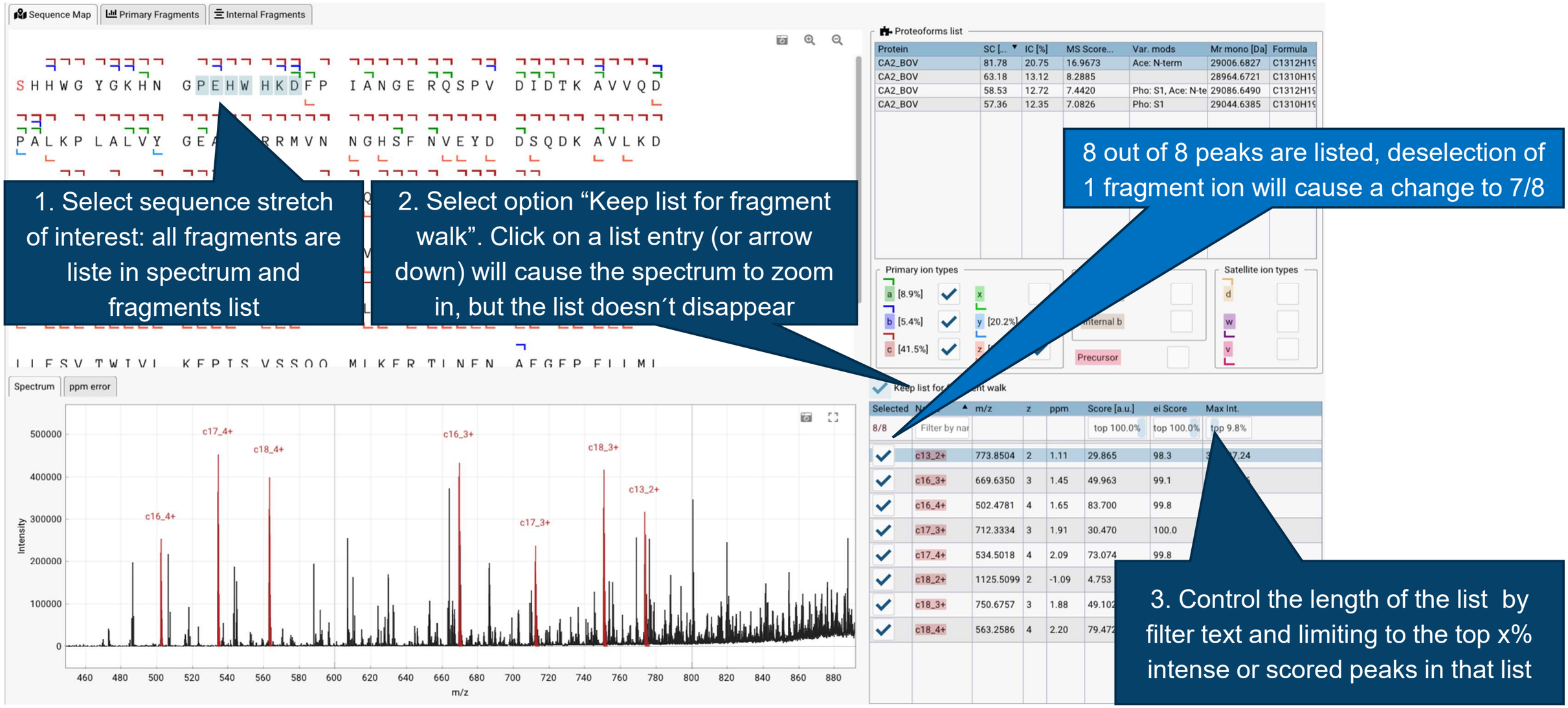
Functional Improvements

- 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 MS^3 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

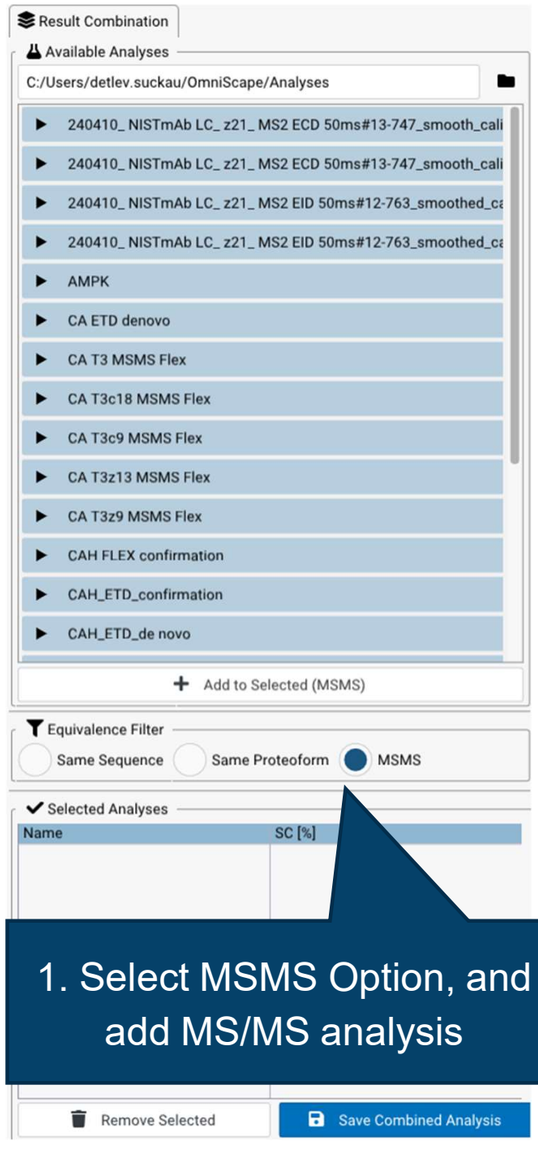
- JPEAKS Data import with meta information is supported – exported from DA 6.2 on as JSON files
- Export of graphics/plots as SVG files and of peaklists as CSV files (from Results as well as Confirmation tabs)
- Report generation

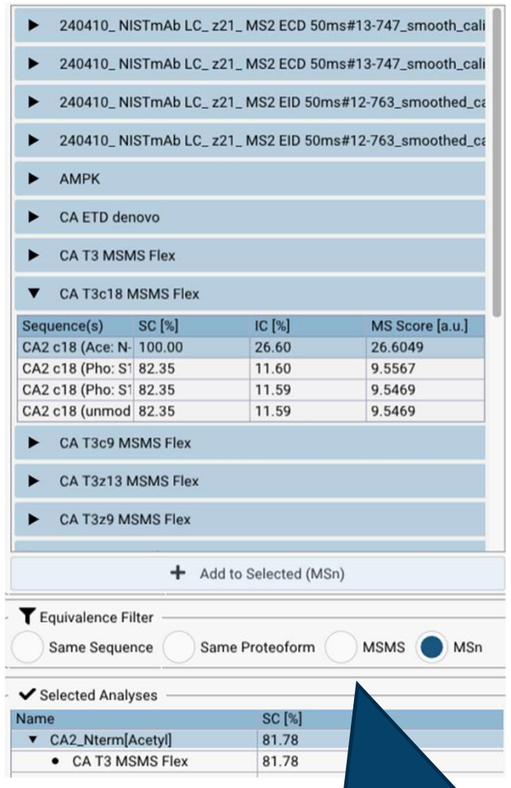
Extended fragment walk



Result Combiner workflow allows to combine MS/MS and MS³ spectra

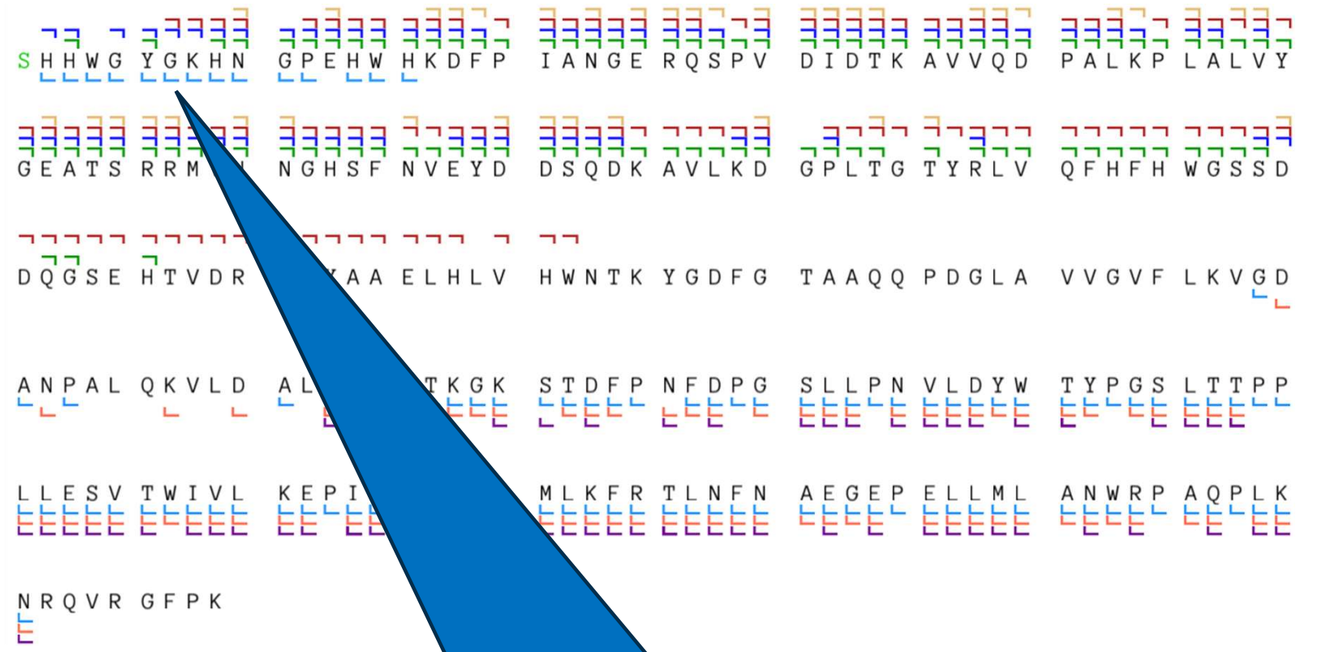
such as T³-Sequencing data from MALDI-ISD





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

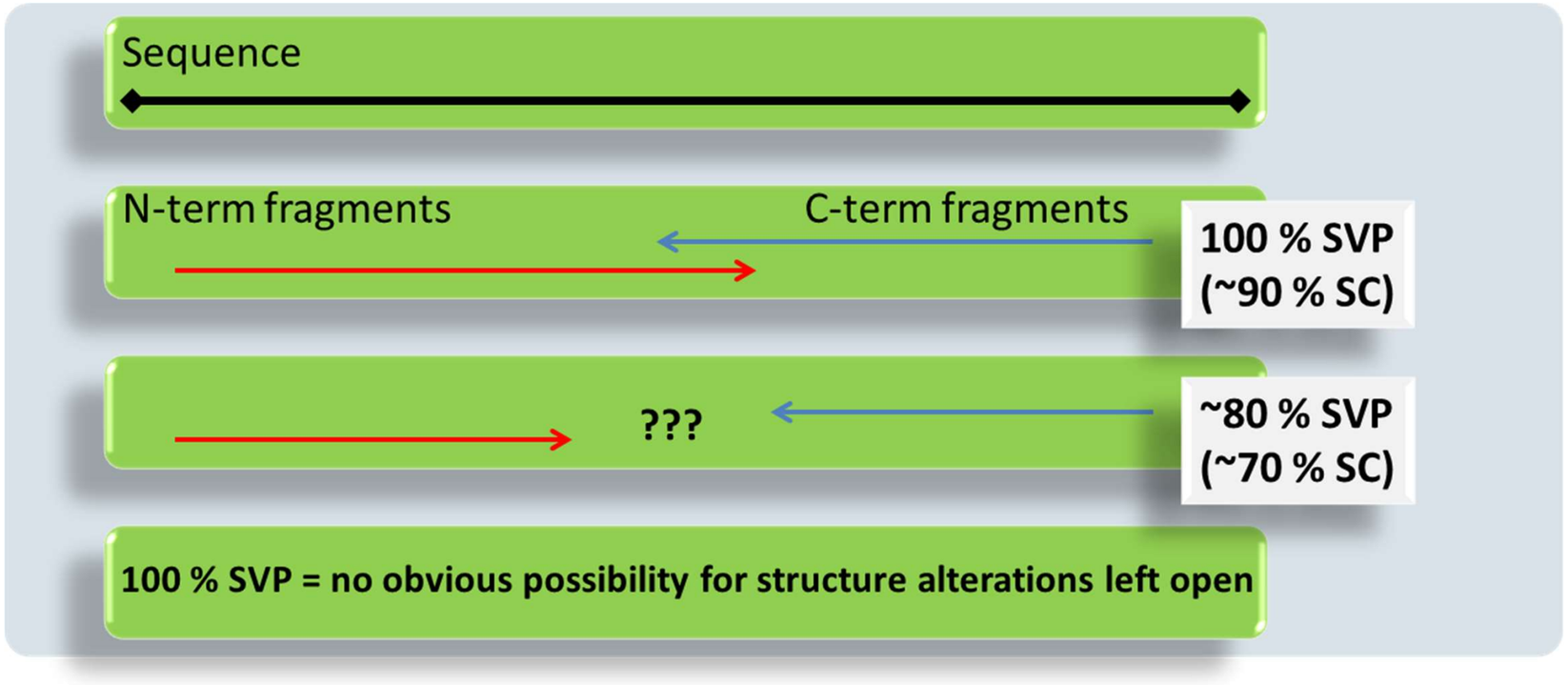
2. Add MS³ analysis



The MALDI-ISD sequence map is combined with the T³-Sequence map obtained from c_{18} fragmentation

Introducing SVP – the Sequence Validation Percentage

The Principle



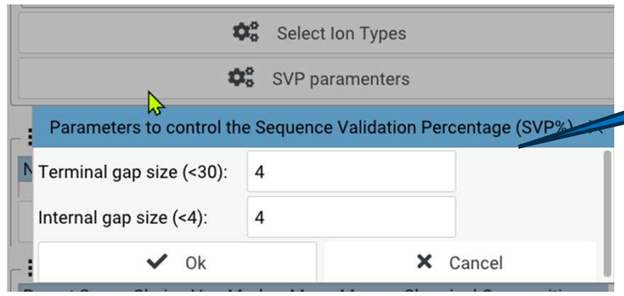
- For the calculation of the SVP, a certain tolerance for gaps in the fragment ion readout is introduced to obtain a continuous sequence readout
- The gaps that are left provide a good estimate of the remaining, unknown sequence

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

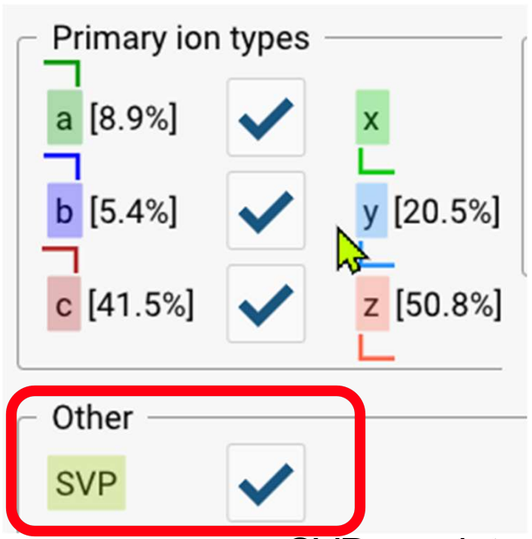


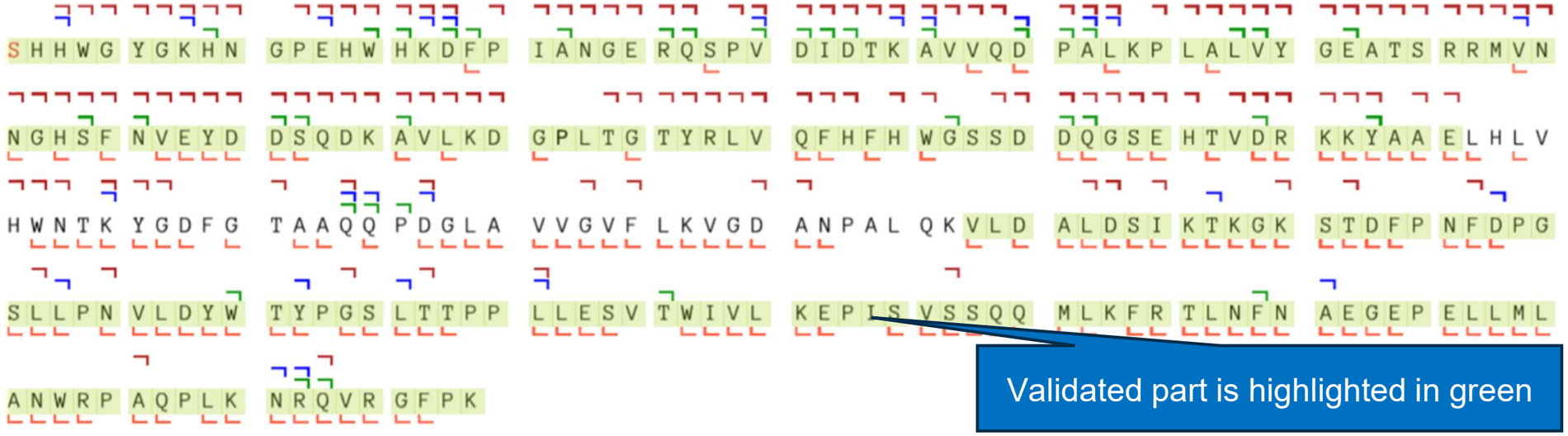
ESI: Terminal Gapsize 2-4, internal gapsize: 2-4

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

Proteoforms list

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.(
CA2_BOV	75.58	53.28	22.43	16.9504		28964.(
CA2_BOV	70.54	53.28	21.94	15.4736	Pho: S1, Ace: N-term	29086.(
CA2_BOV	69.77	53.28	20.68	14.4308	Pho: S1	29044.(



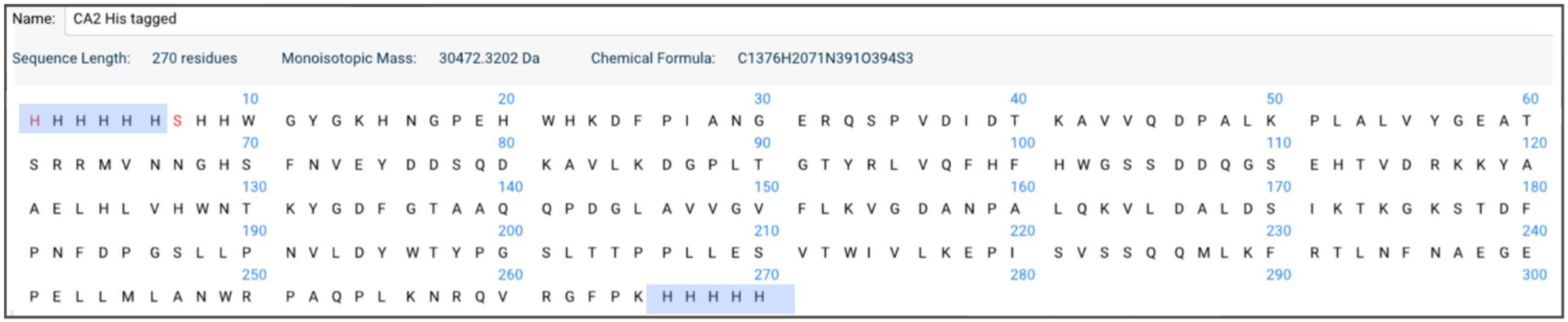


Validated part is highlighted in green

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



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

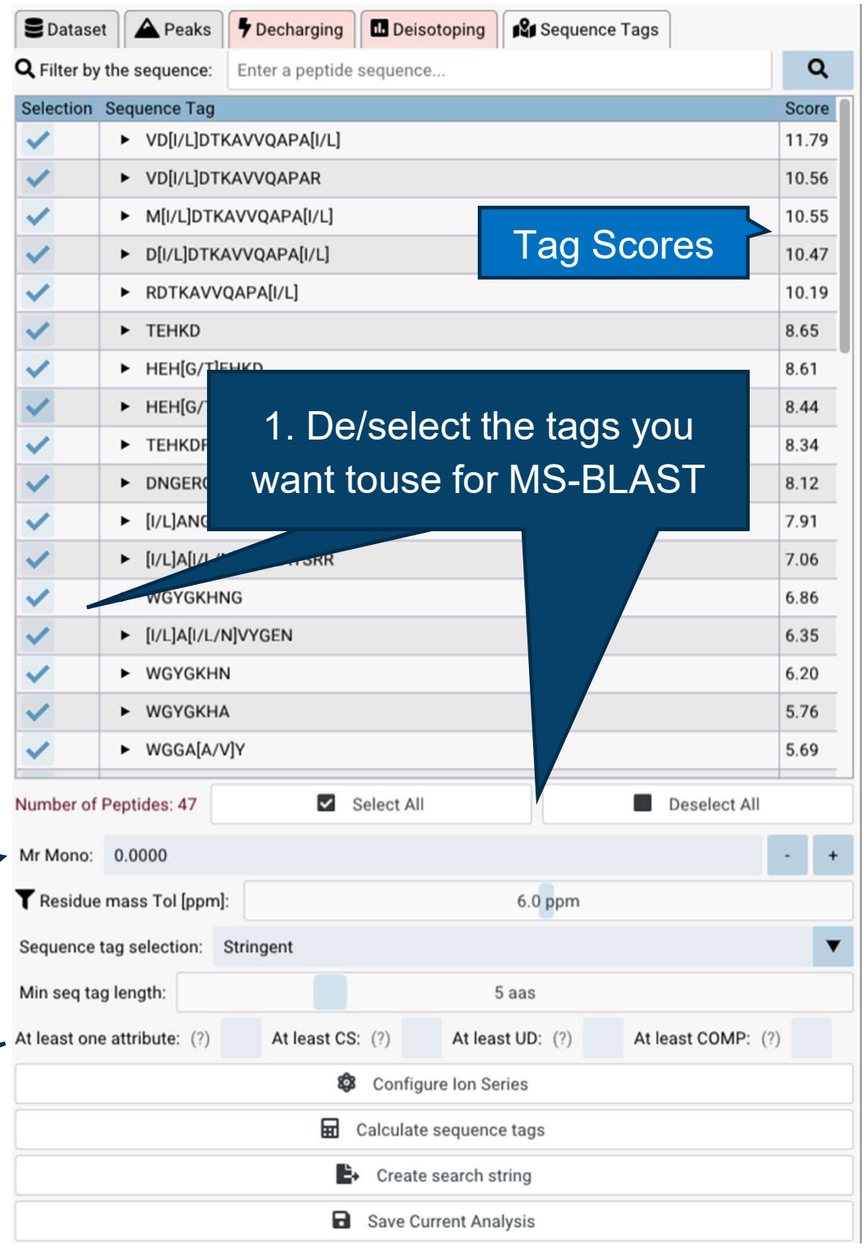
- The new Sequence Tags tab contains the **NEW *De Novo*** results, incl. Scores
- Dramatically increased speed (~10-fold)
- Higher quality and robust sequence tag generation

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

- Eliticist 2
- Stringent 5
- Relaxed 20
- All

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

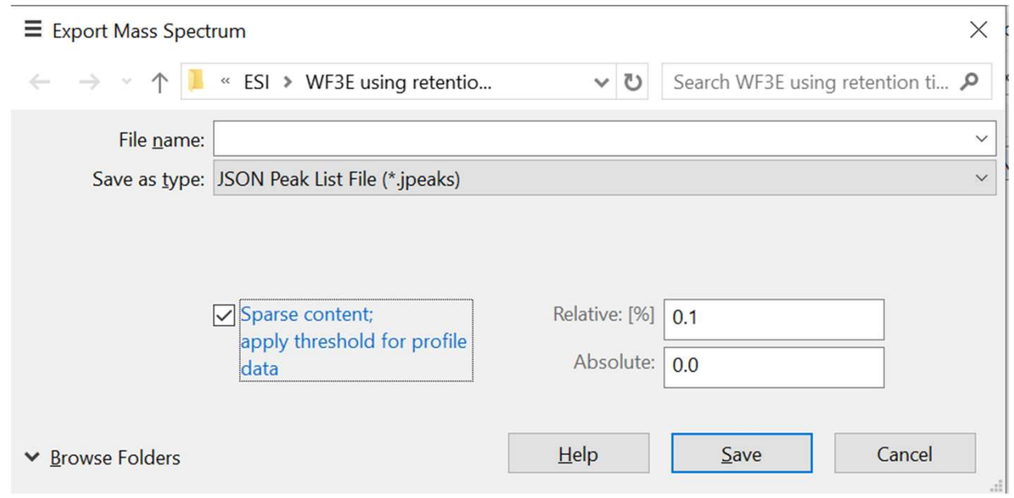
3. Leave empty and apply rules only if too many junk tags are proposed

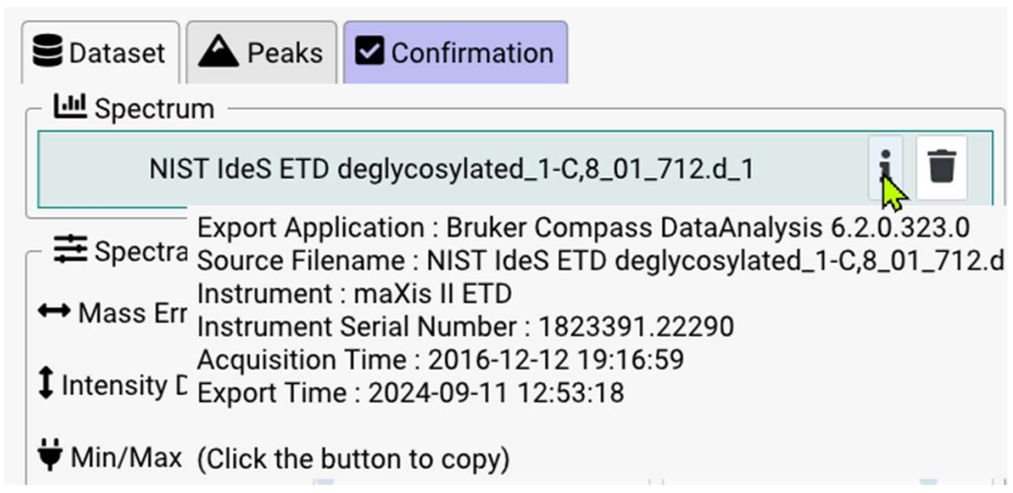


JPEAKS File from DA 6.2.323+ can be used in OSc

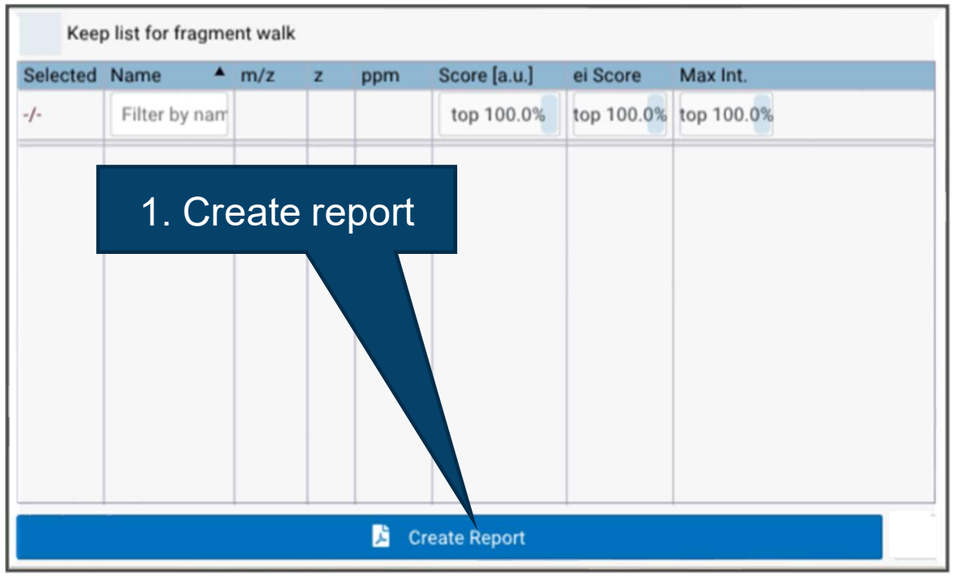
DA adds a new export format: JPEAKS

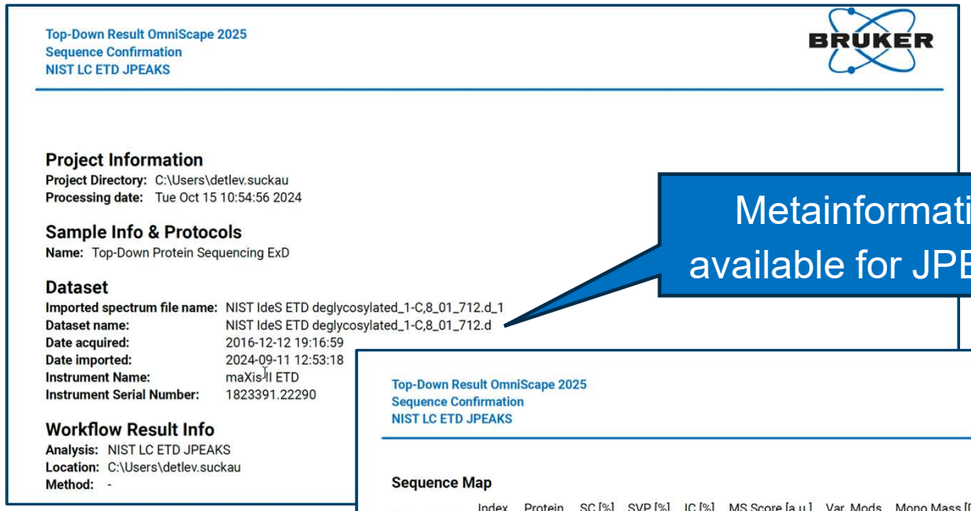
- Provides meta information of dataset
- Contains profile data, optionally peaklist, if generated
- Allows reimport to DA by drag-and-drop from the file manager
- JPEAKS spectra can be reprocessed in DA if needed
- JPEAKS files are large, can be reduced in size during export by low intensity filter (Abs/Rel)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



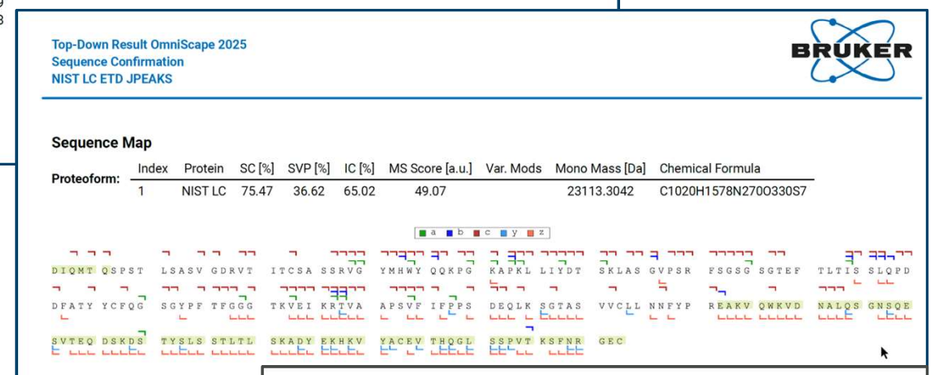


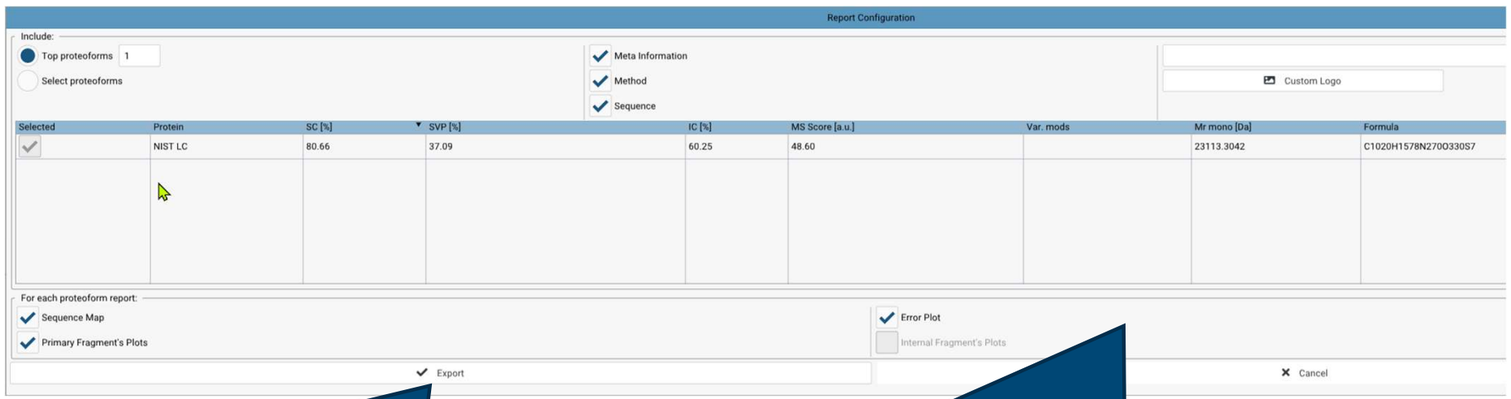
Confirmation Report (pdf)

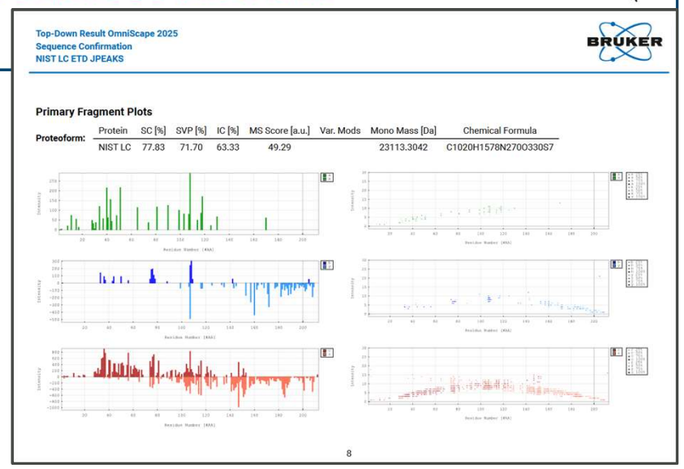




Metainformation only available for JPEAKS files





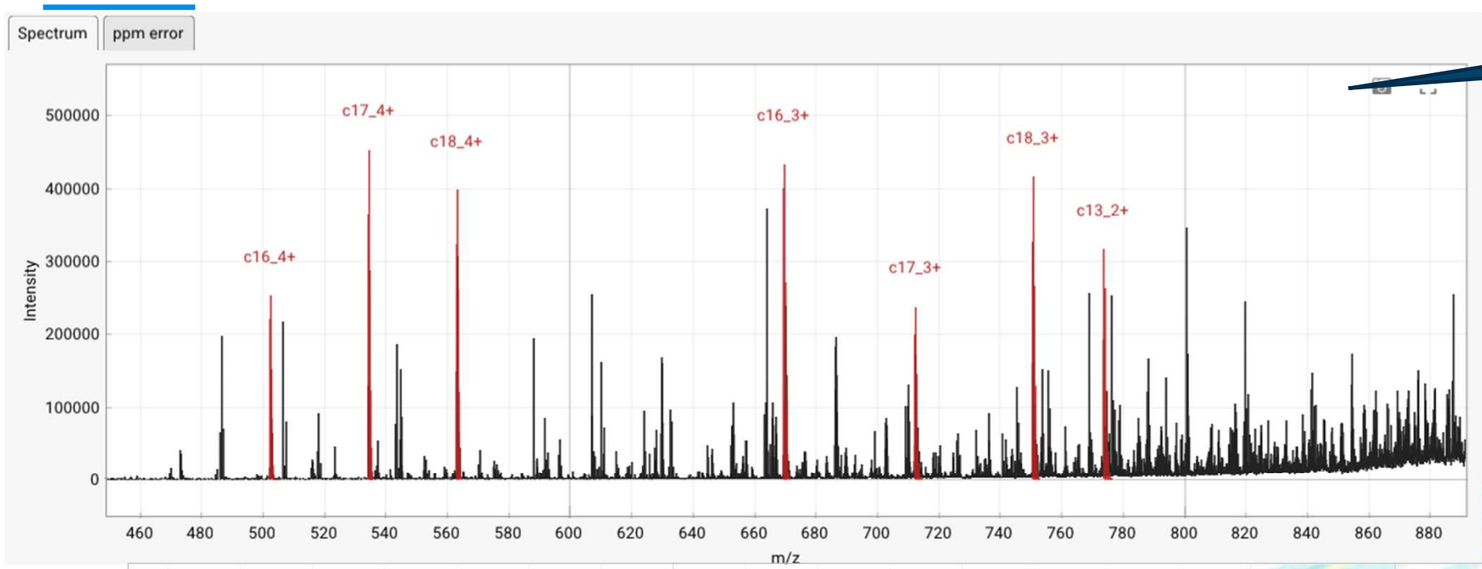


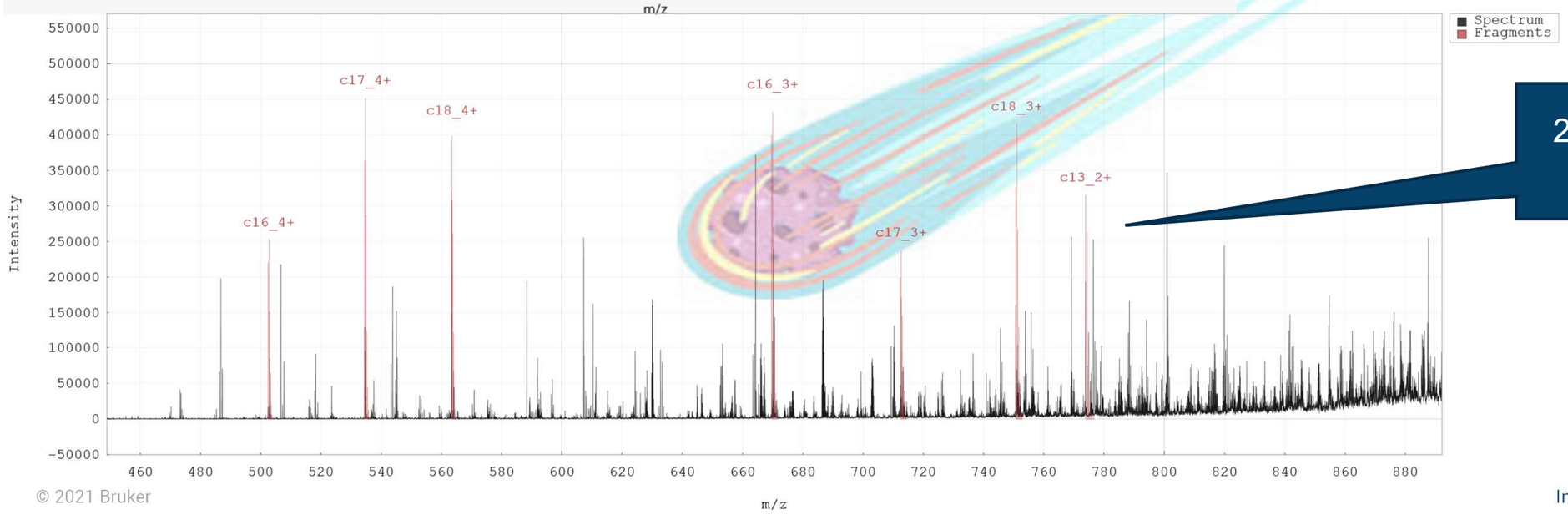
3. Click export and define target directory

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

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

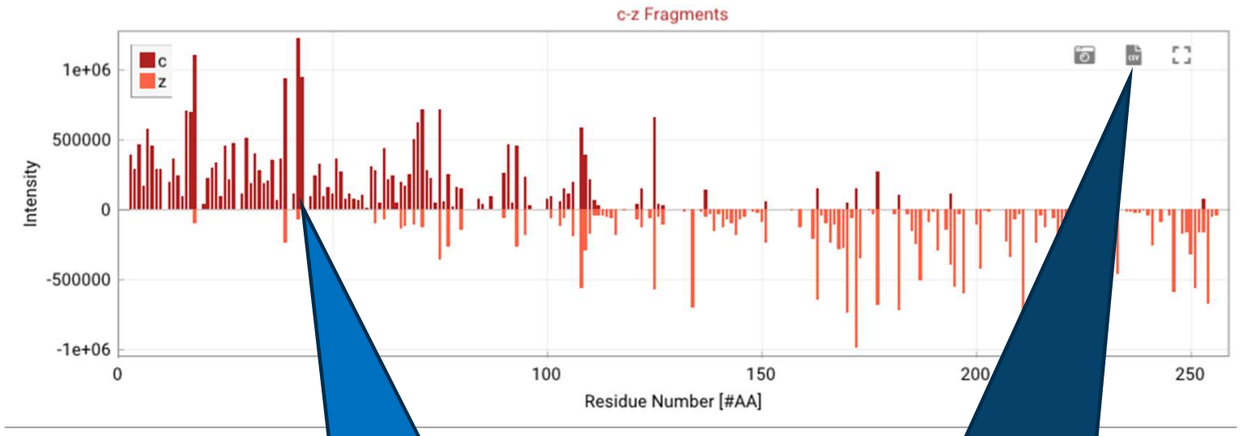
Keep list for fragment walk

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%
✓	c13_2+	773.8504	2	1.11	29.865	98.31	316437.24
✓	c16_3+	669.6350	3	1.45	49.963	99.08	447391.26
✓	c16_4+	502.4781	4	1.65	83.700	99.83	253361.06
✓	c17_3+	712.3334	3	1.91	30.470	99	239588.11
✓	c17_4+	534.5018	4	2.09	73.074		453403.73
✓	c18_2+	1125.5099	2	-1.09	4.7		
✓	c18_3+	750.6757	3	1.88	49.		
✓	c18_4+	563.2586	4	2.20	79.		

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		1.88	49.102	99.71	416953.8	
8	TRUE	c18_4+	563.2586						

2. Open in EXCEL etc.

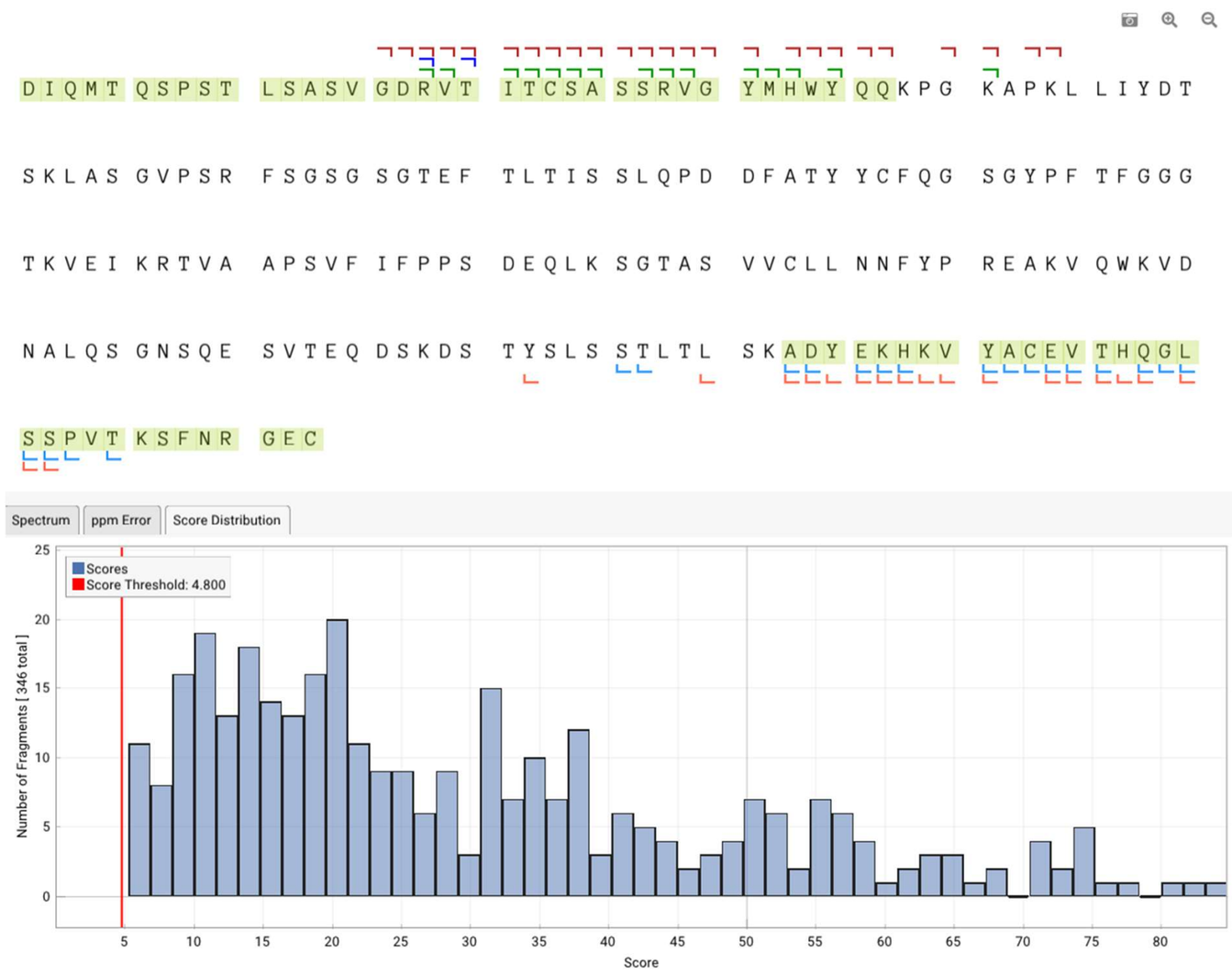


1. Click on sheet icon to export as .csv file

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

Main features

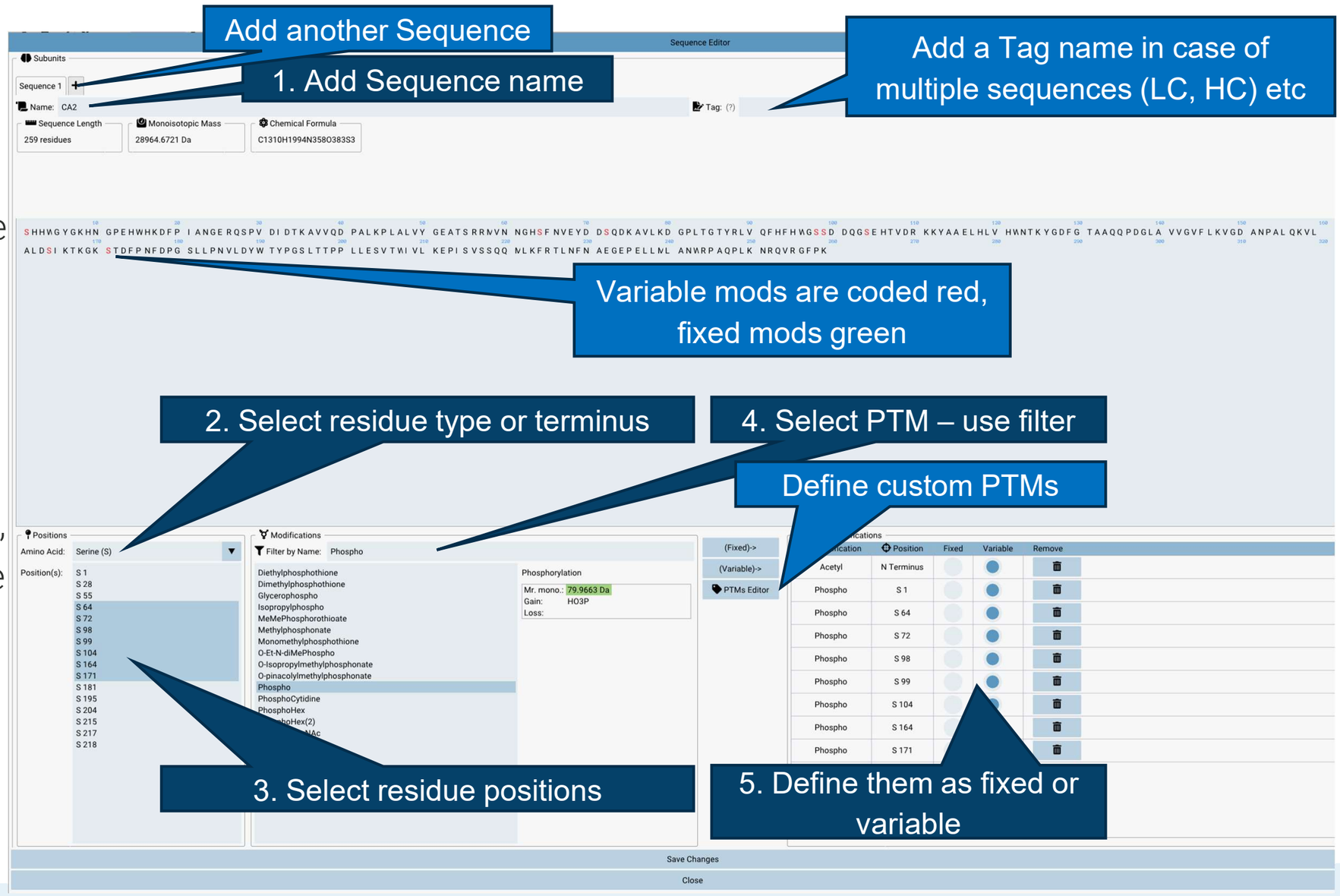
- Software with predefined Top-Down protein sequencing workflows to for best ease-of-use and reproducibility:
  - Sequence Editor to define sequences and PTMs
  - Confirmation workflow with scored proteoform assessment
  - Extended Confirmation for many variable modification sites
  - Result combiner 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

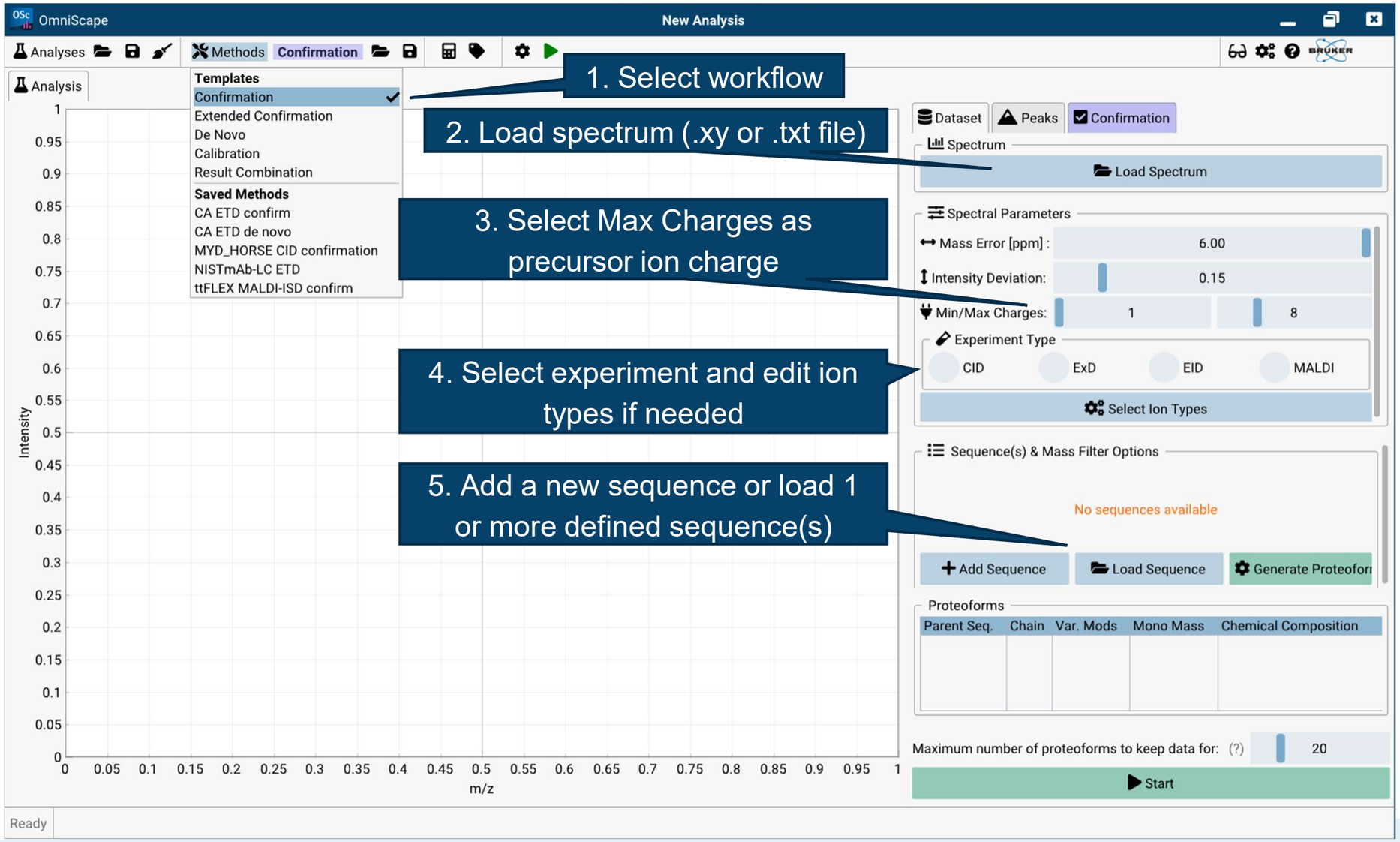
SEQUENCE EDITOR

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

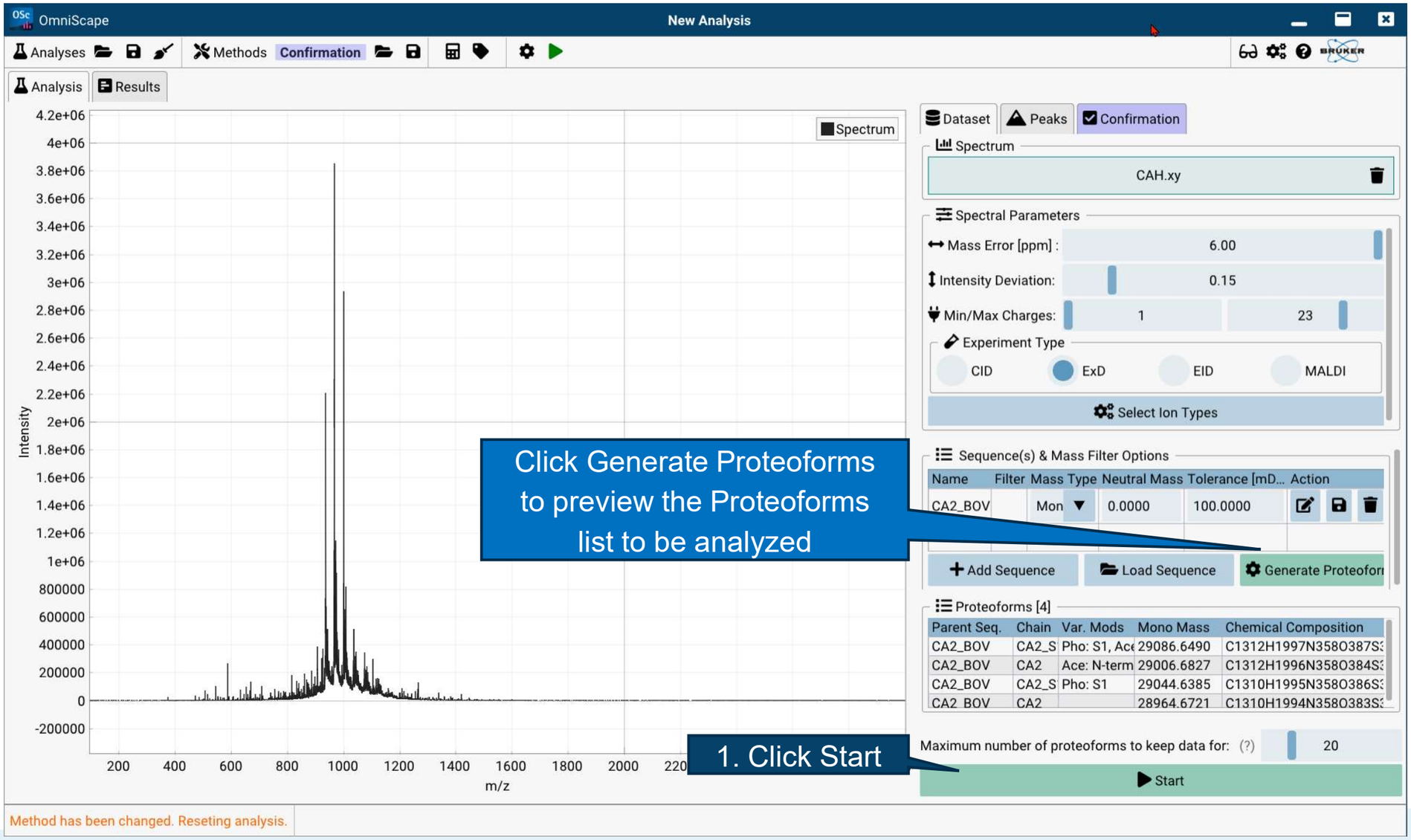
- Opens empty from the Add Sequence button (sequence must be pasted – it cannot be typed!)
- Opens with Sequence and PTMs from the Load Sequence button
- Save Changes activates them, but does not save them to the file system yet



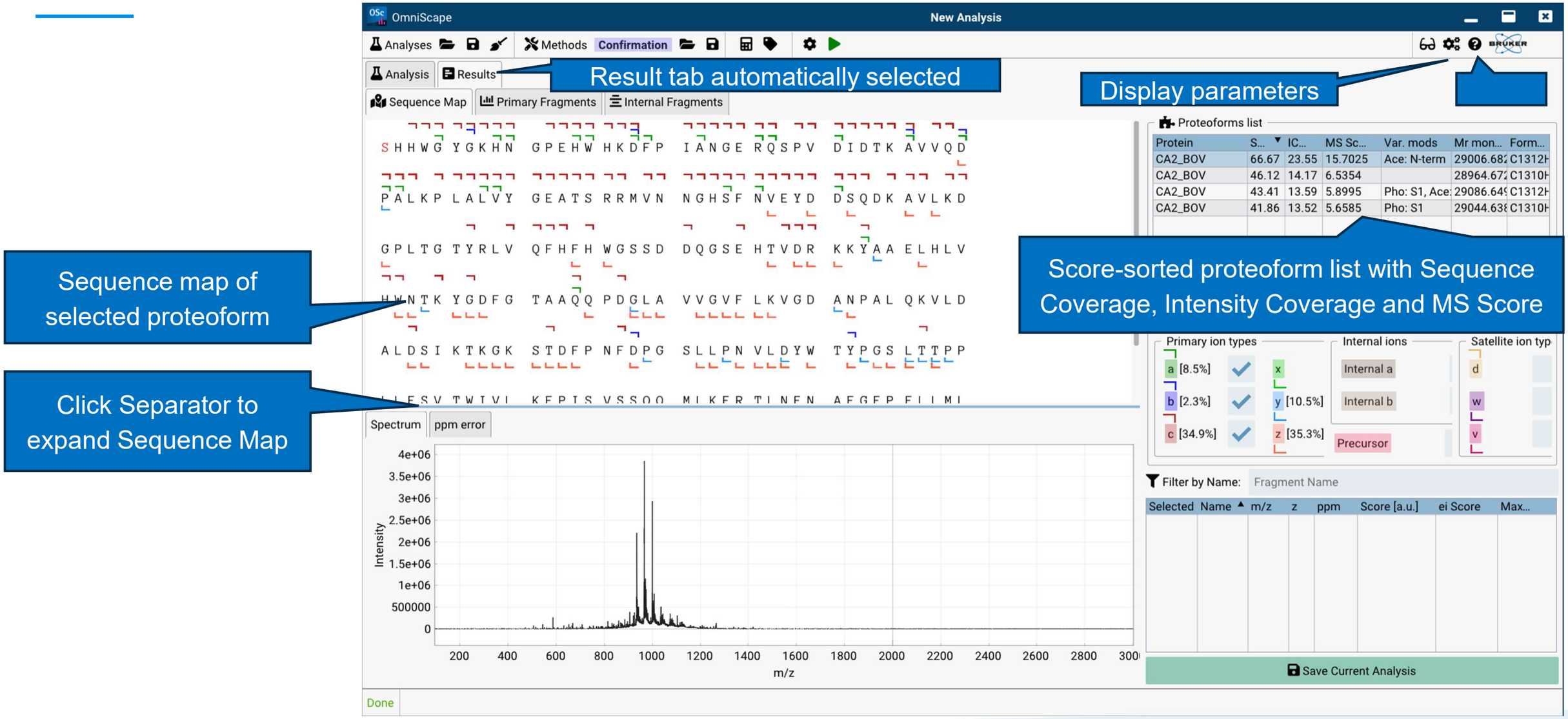
Confirmation workflow



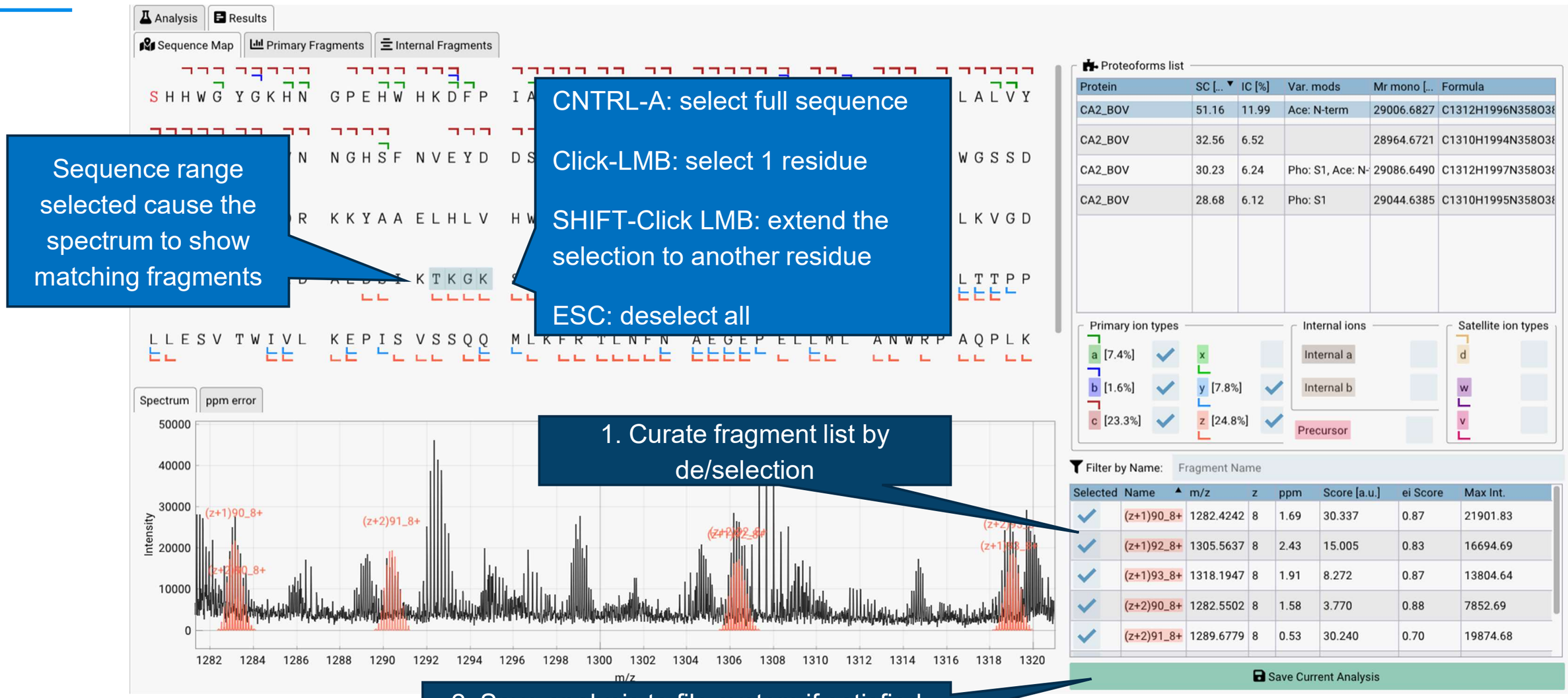
After import of spectrum and sequence



Results are directly displayed after START



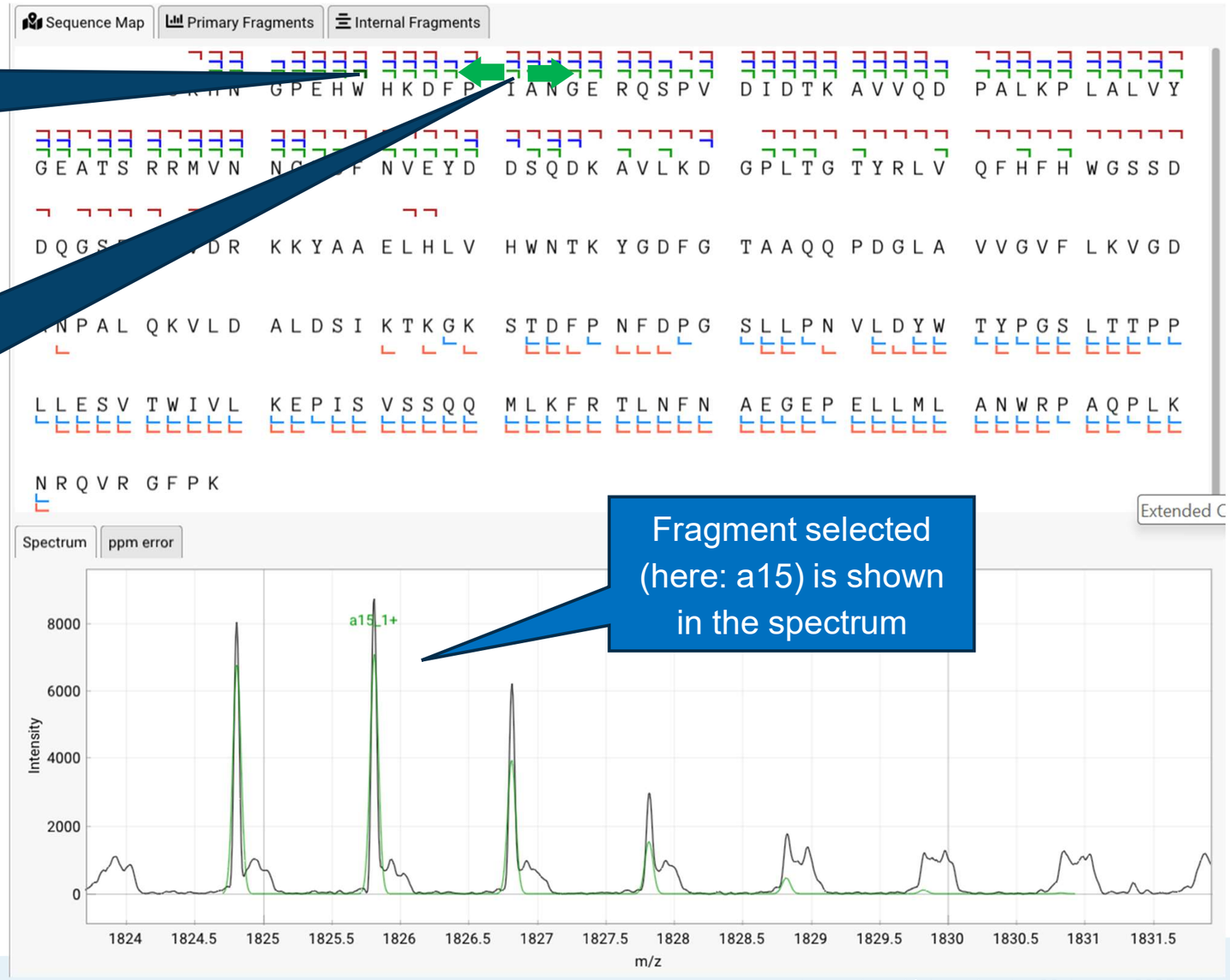
Validation of matching fragments



Validation of matching fragments (2): the fragment walk

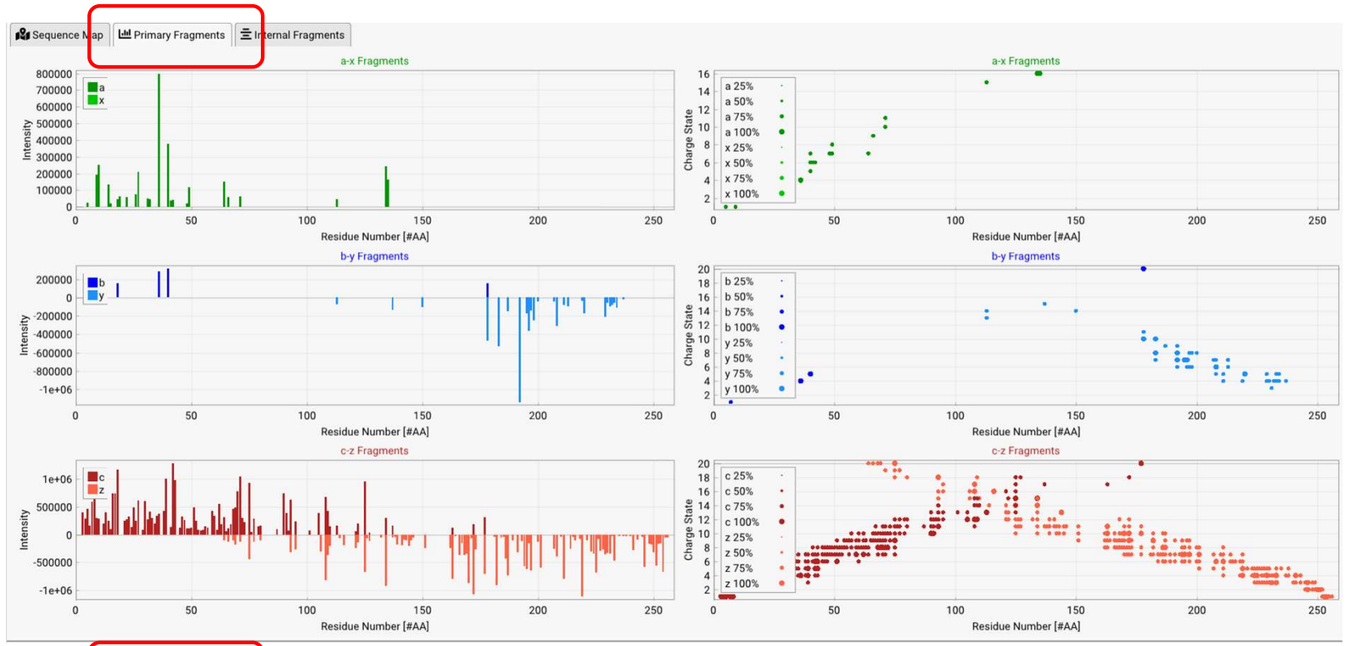
1. Click on a fragment mark causes the spectrum view to zoom close to that fragment

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

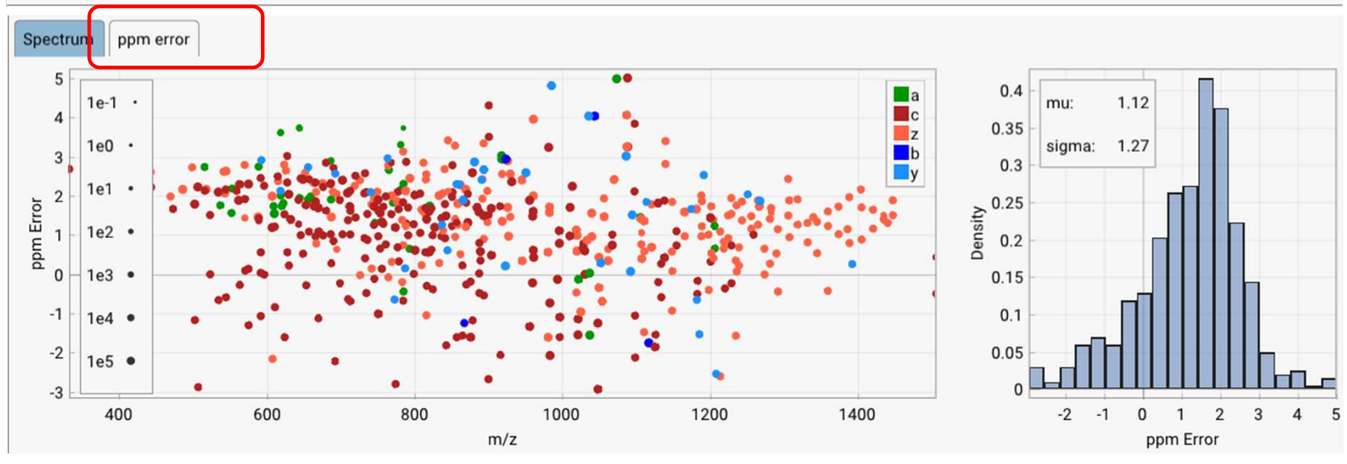


Additional Results tabs provide tools to inspect the data quality

- Primary fragments statistics can be inspected



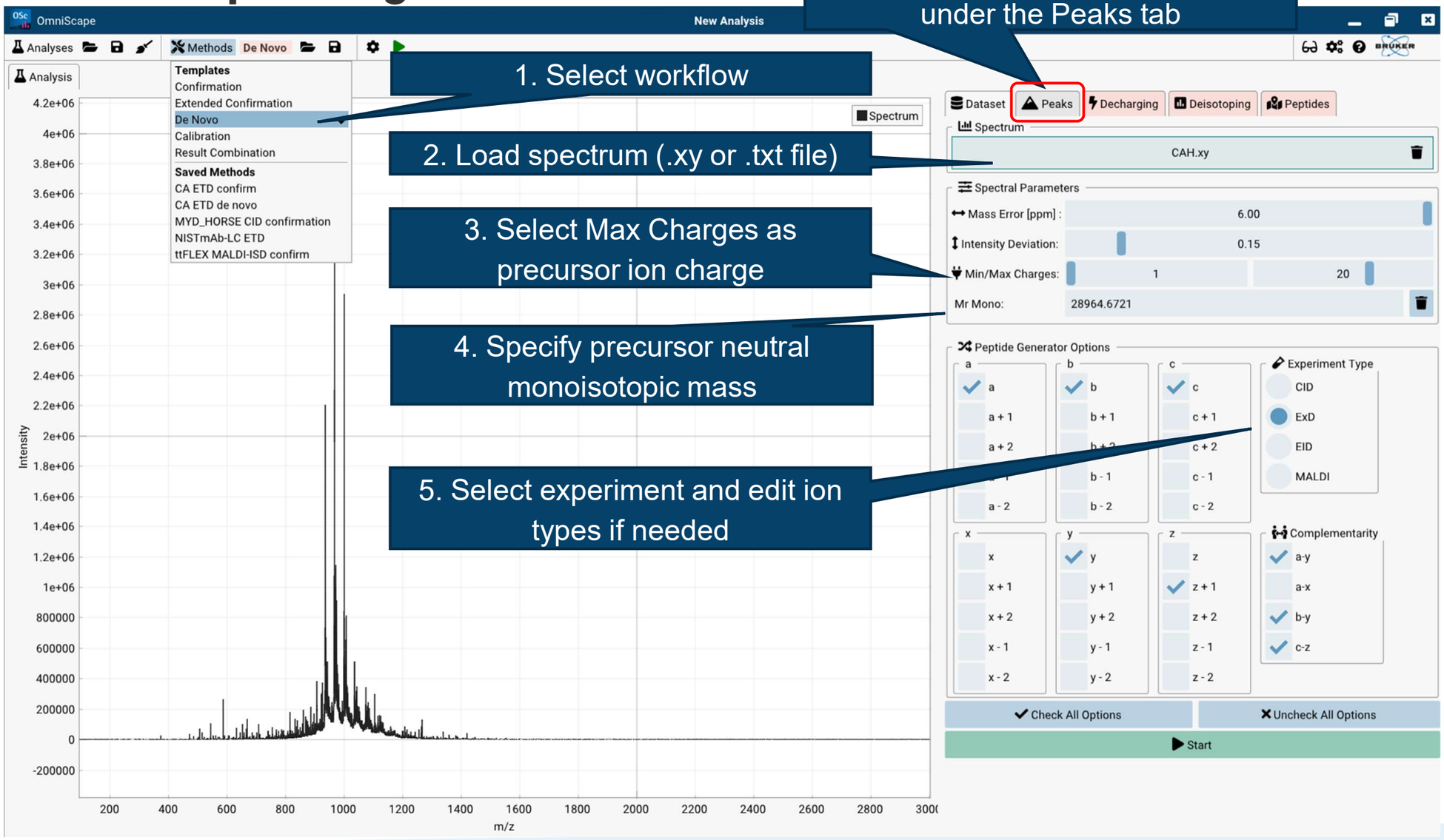
- ppm Errors can be visualized



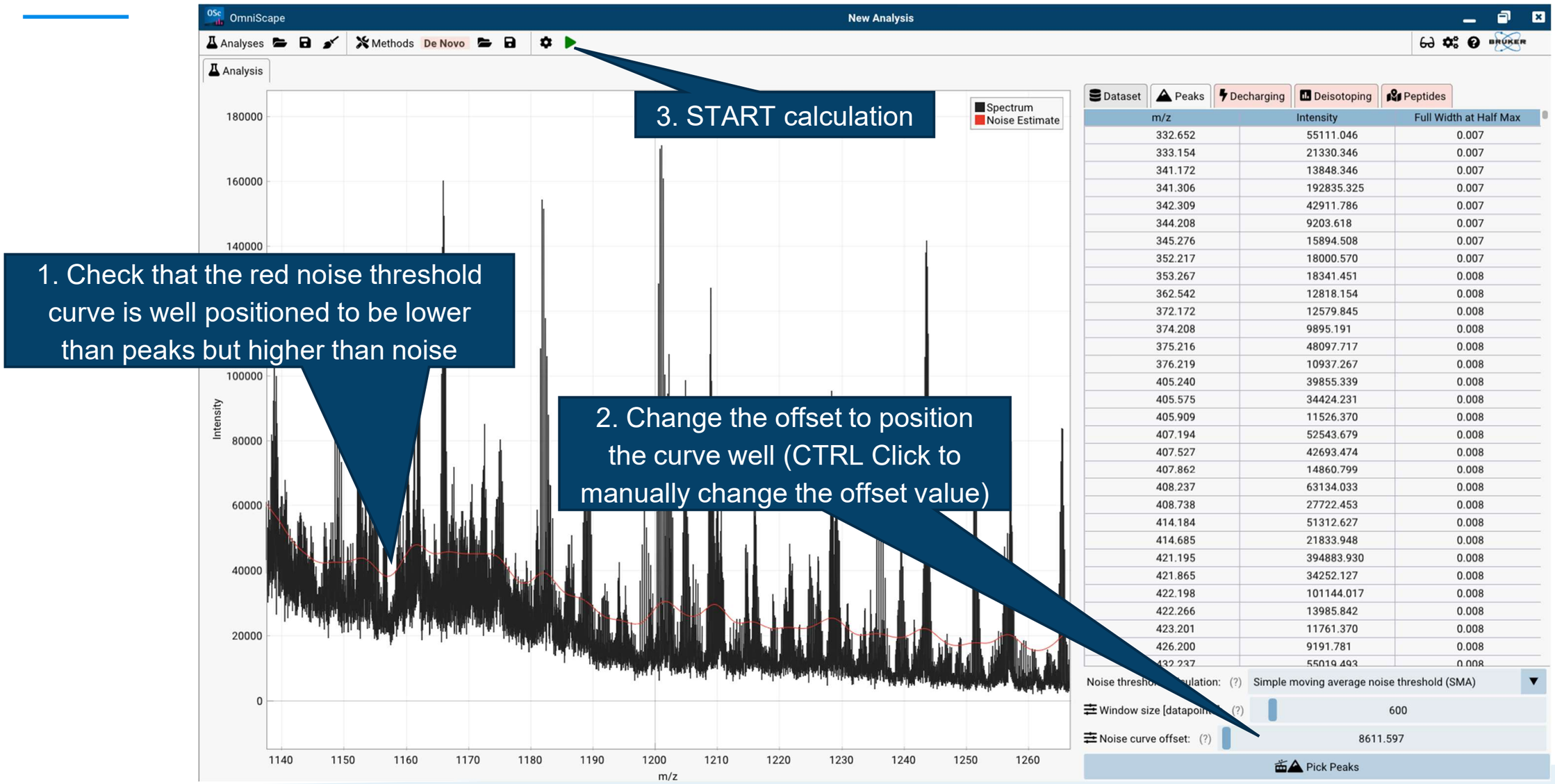
Extended Confirmation Workflow

- 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 (~>7-10) of variable modification sites are defined in the sequence and many proteoforms need to be calculated

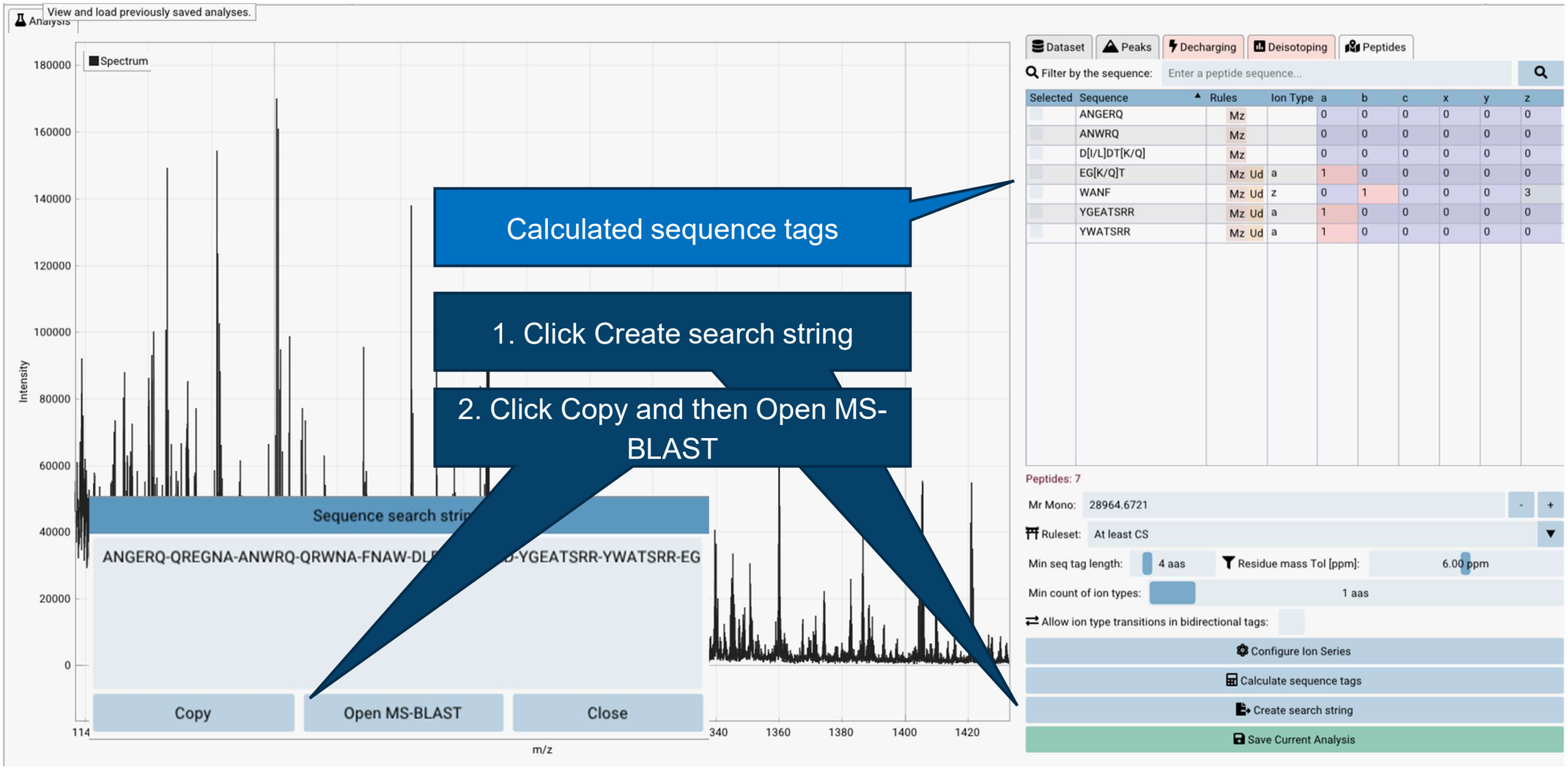
Define the De Novo Sequencing workflow



Define the threshold curve before Start



From de novo sequencing to protein identification

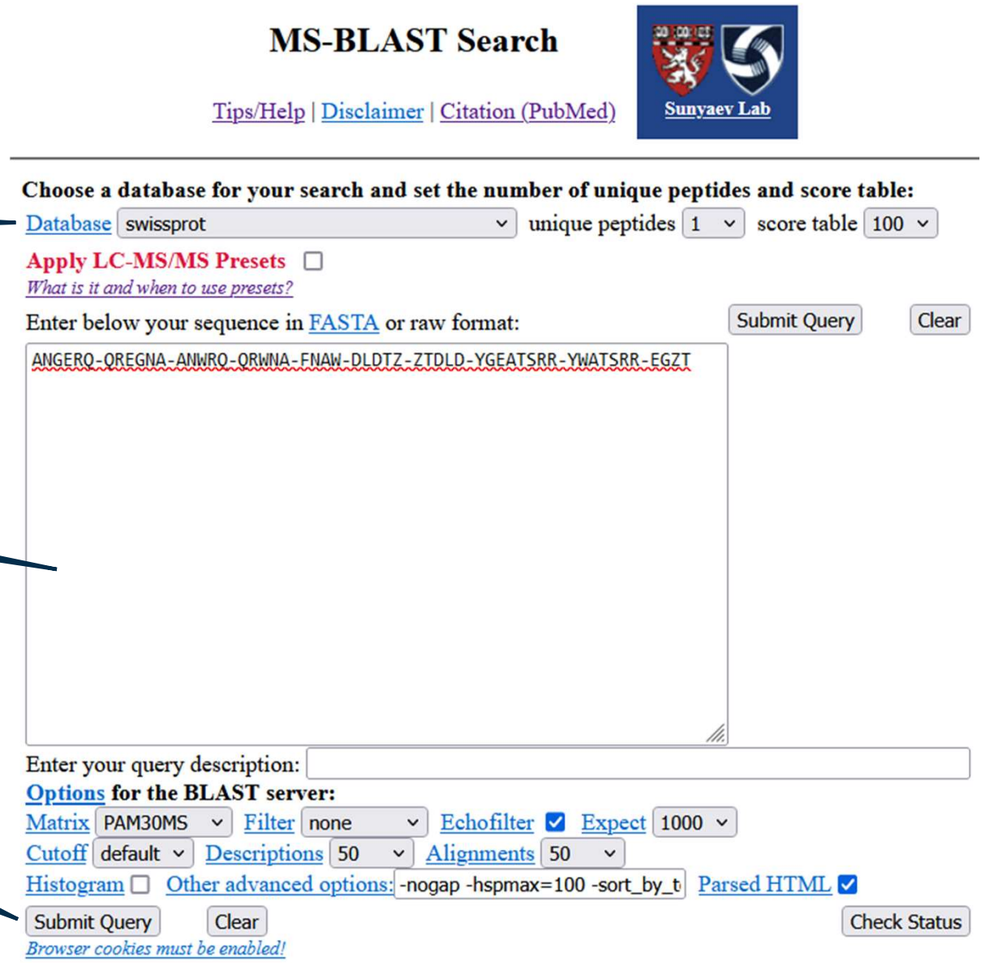


Browser with internet connection allows for the MS-BLAST Search

1. Select suitable protein sequence database

2. Paste the search string into the sequence field

3. Submit Query



DE NOVO SEQUENCING

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: Get the selected sequences Reset

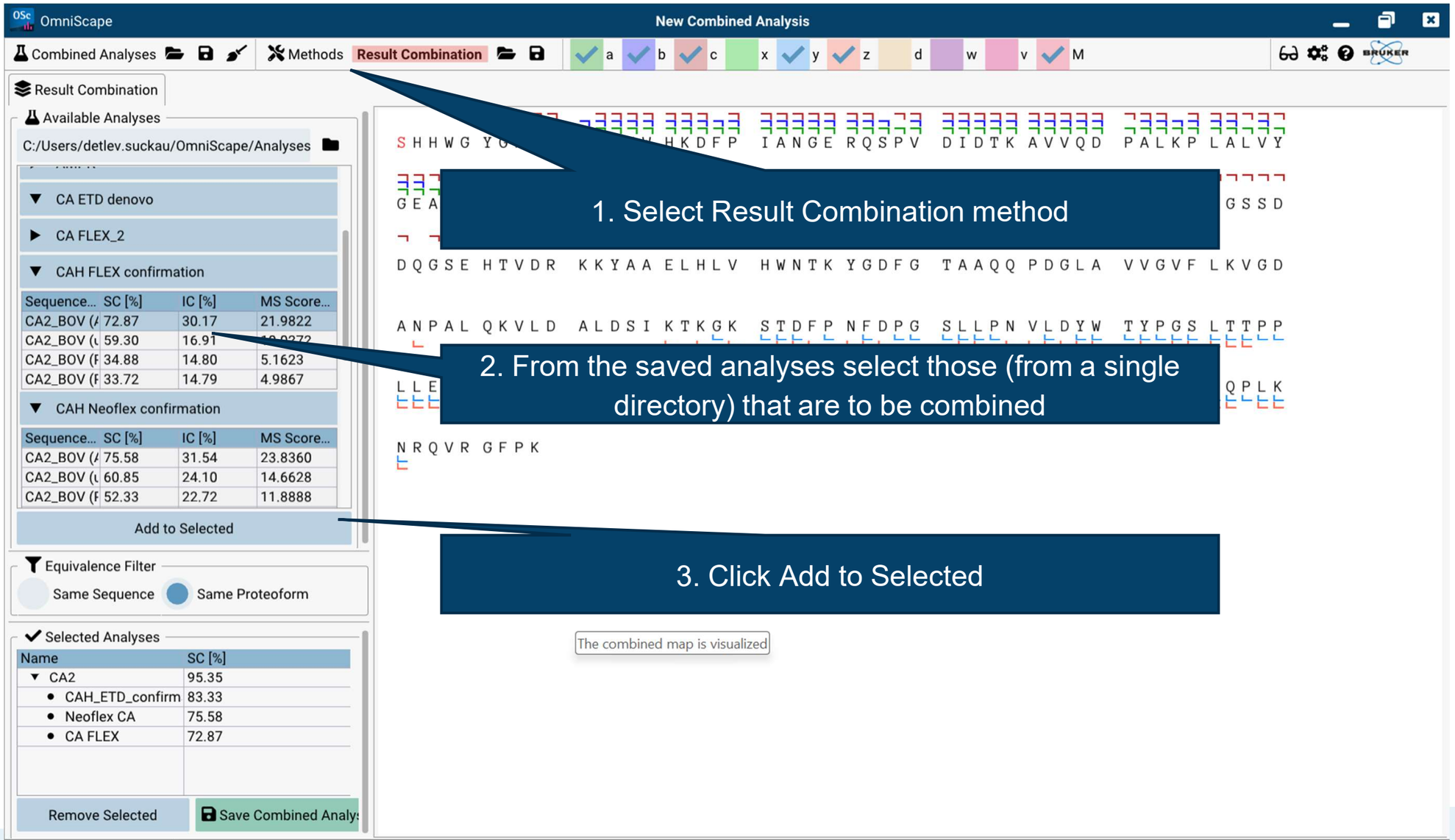
Sequences producing High-scoring Segment Pairs:	High Score	Total Score	
/:sp|P00921|CAH2_BOVIN Carbonic anhydrase 2 OS=Bos taurus...	59	170	☑
/:sp|P00922|CAH2_SHEEP Carbonic anhydrase 2 OS=Ovis aries...	38	139	☑

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

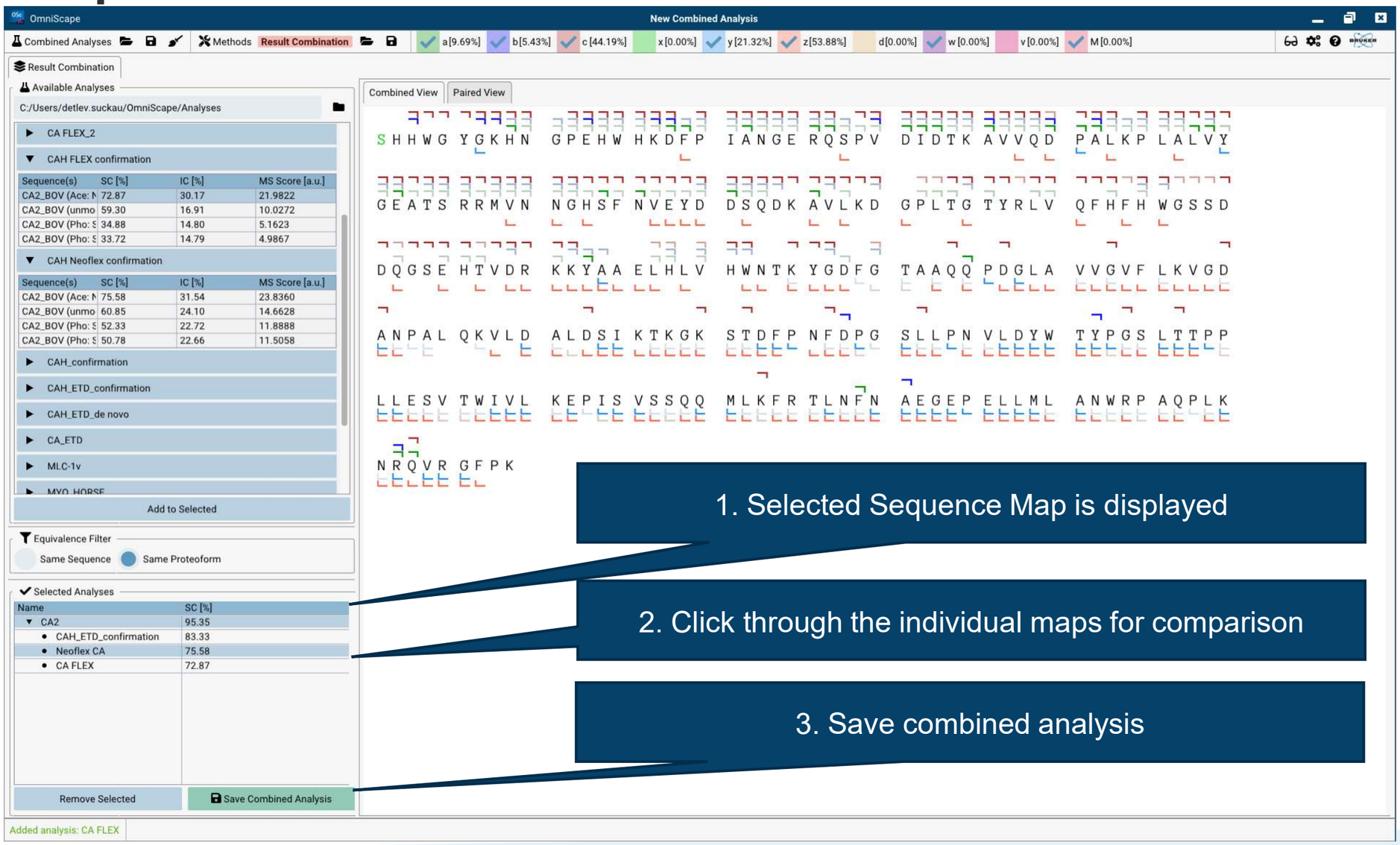
```
^ = sp|P00921|CAH2_BOVIN Carbonic anhydrase 2 OS=Bos taurus GN=CA2 PE=1 SV=3/
      Length = 260

Total Score: 170

                          0         60        120       180       240
                          |         |         |         |         |   |260
sp|P00921|CAH2_BOVIN    | ____________________________________________
Local hits (HSPs)        |      _ _   _                            _

 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

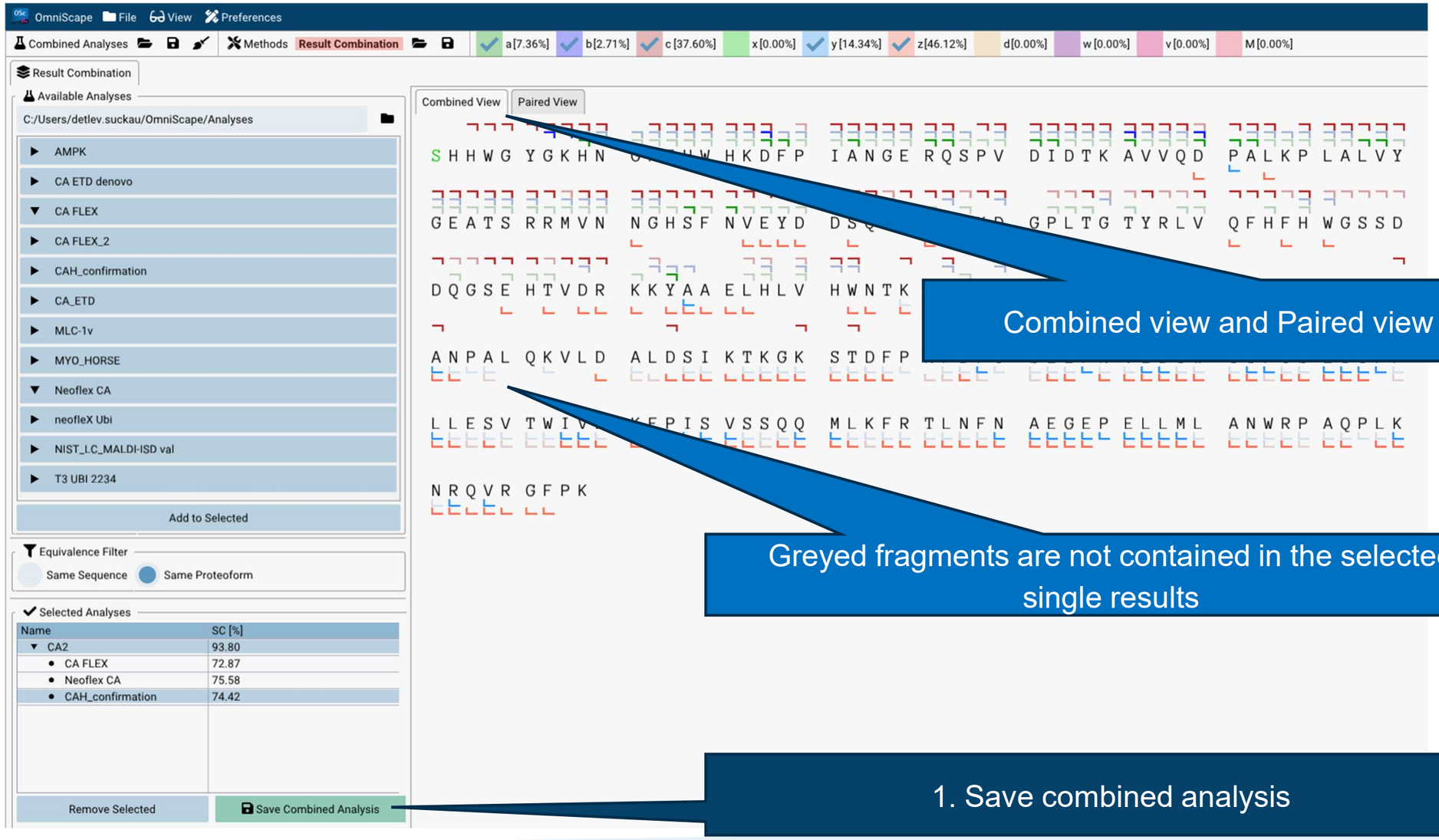
Result combiner allows to select multiple datasets to be combined on the level of the Sequence Map



The combined map is visualized



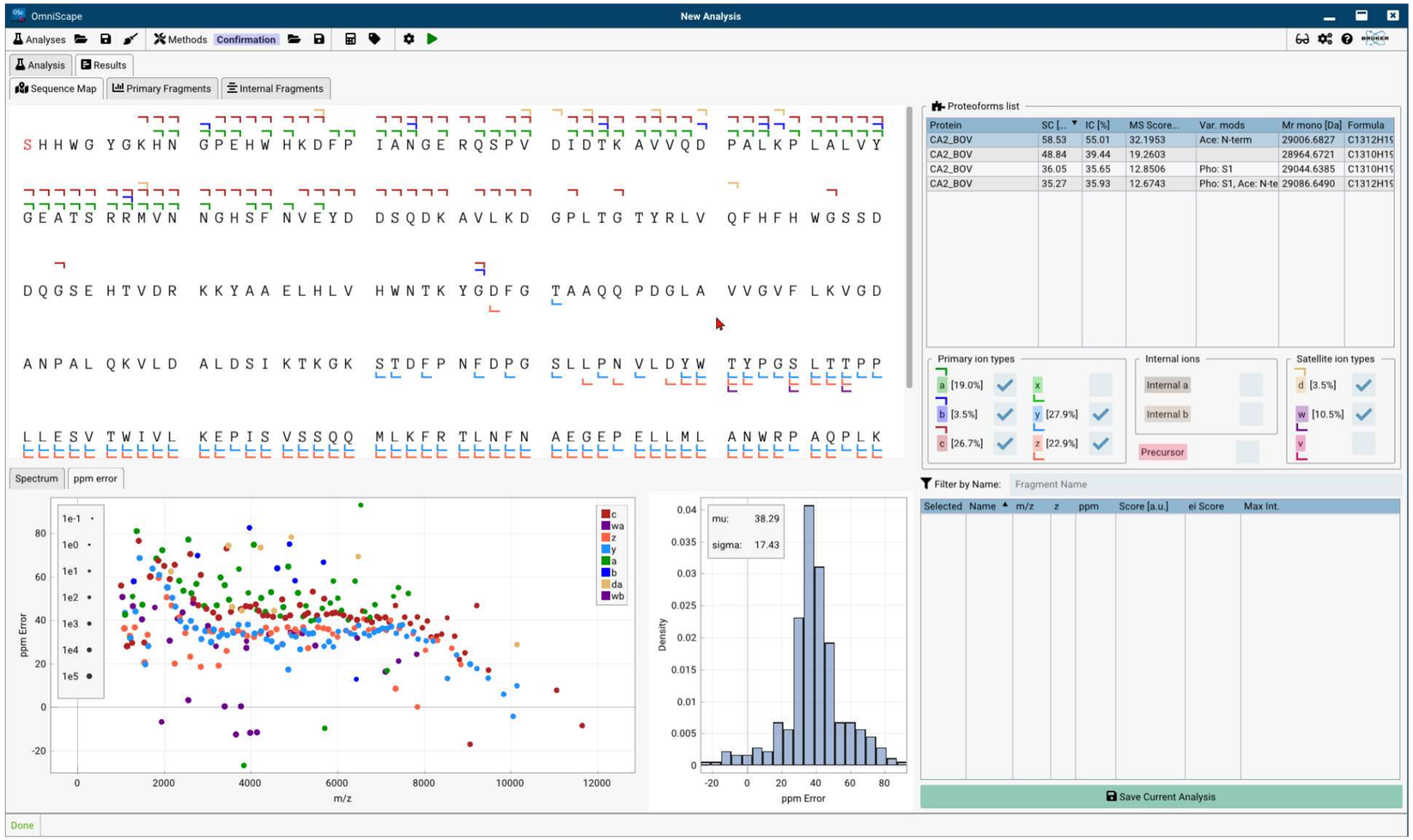
The individual map can be visualized against the combined map



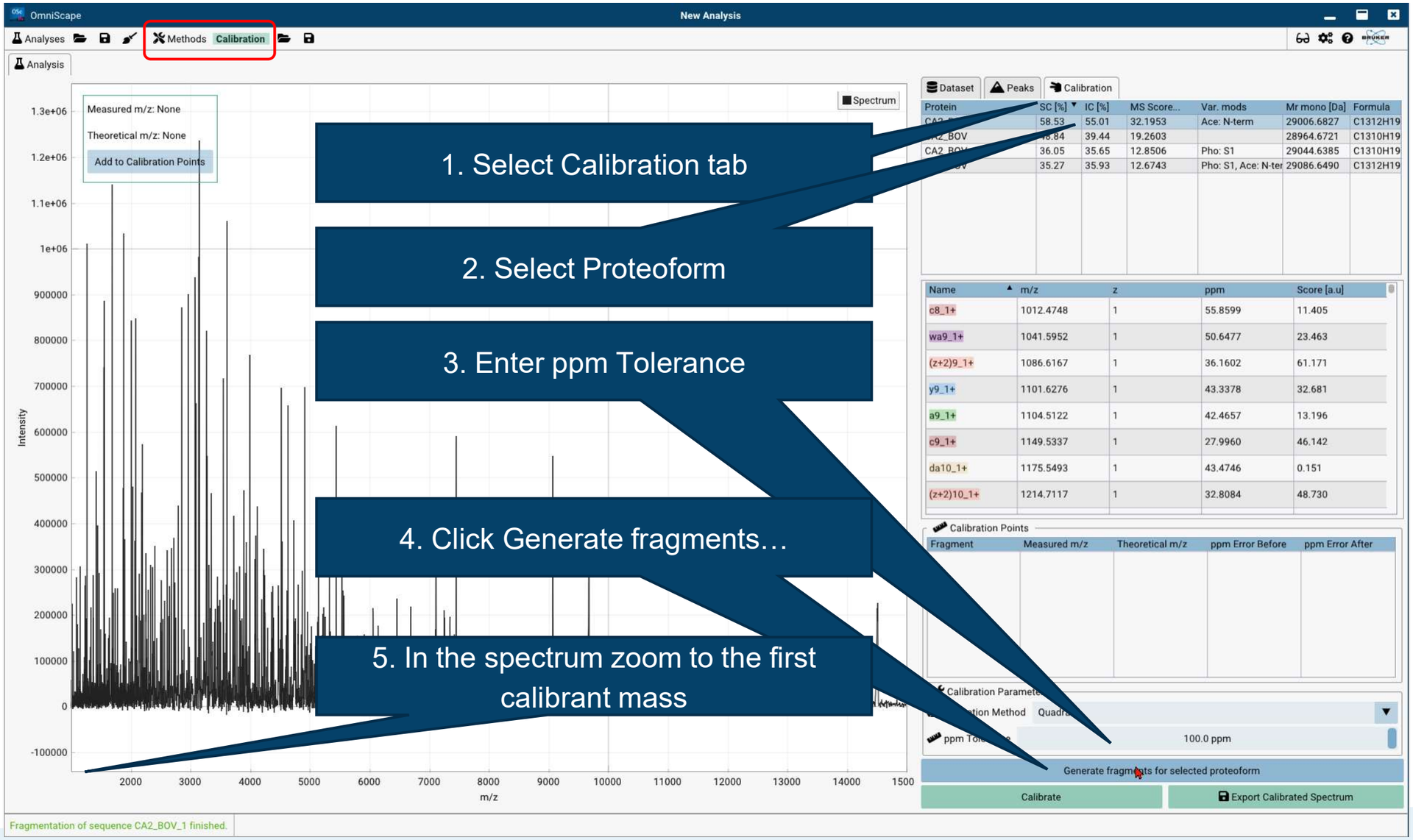
CALIBRATION

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

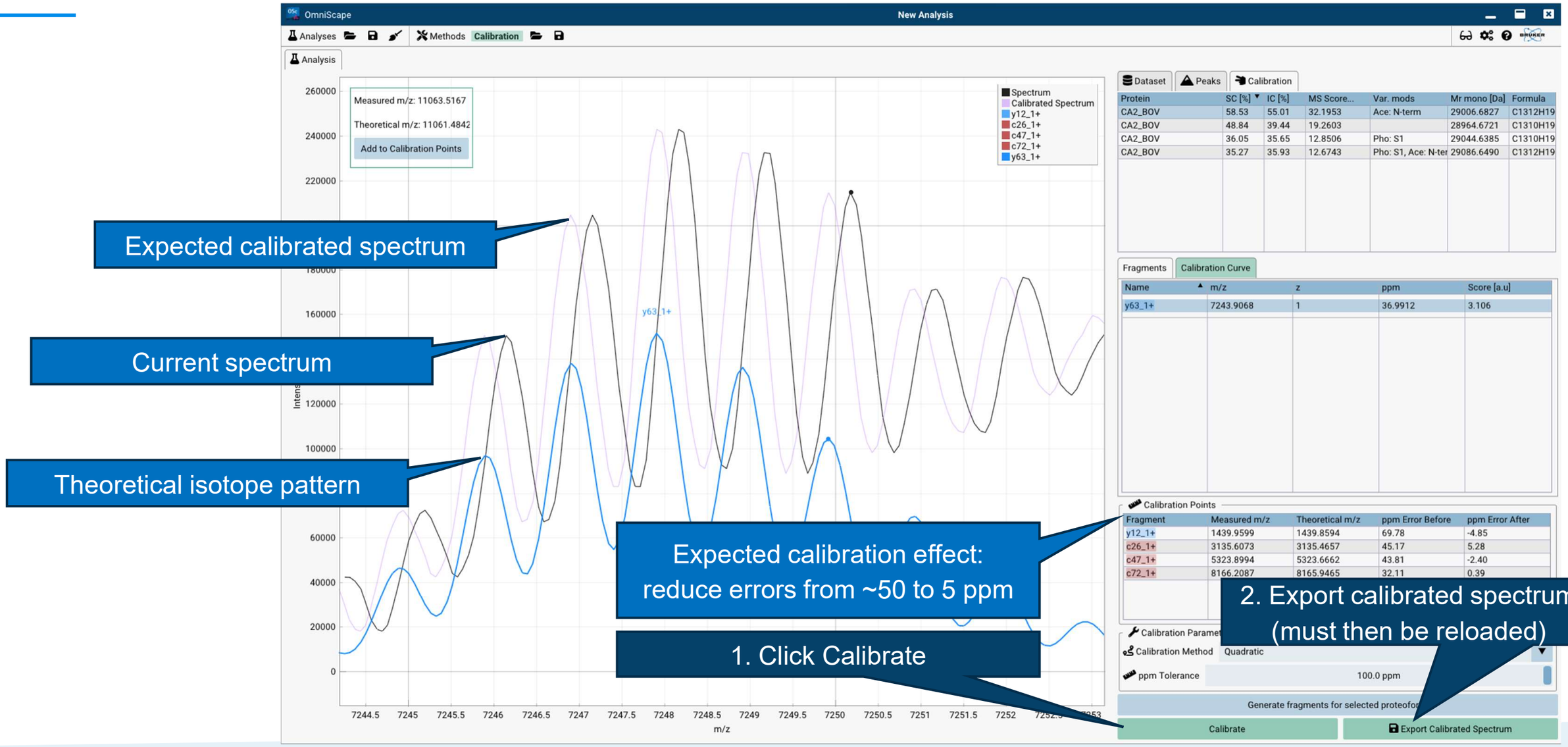


Setup for calibration: select method type Calibration

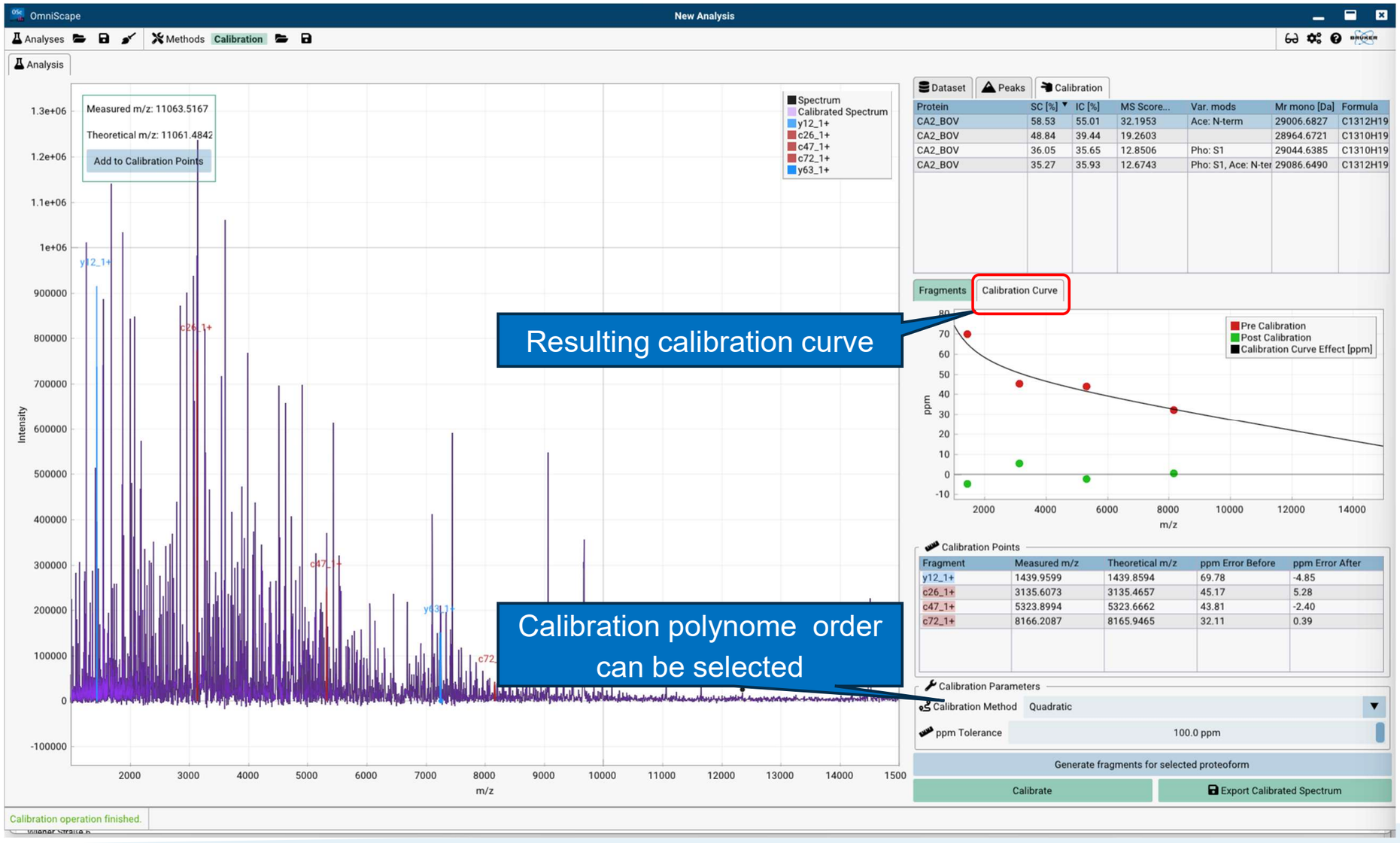


Zoom in to first calibrant and do:

Repeat until the calibration curve can be calculated



The calibration curve can be visualized



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