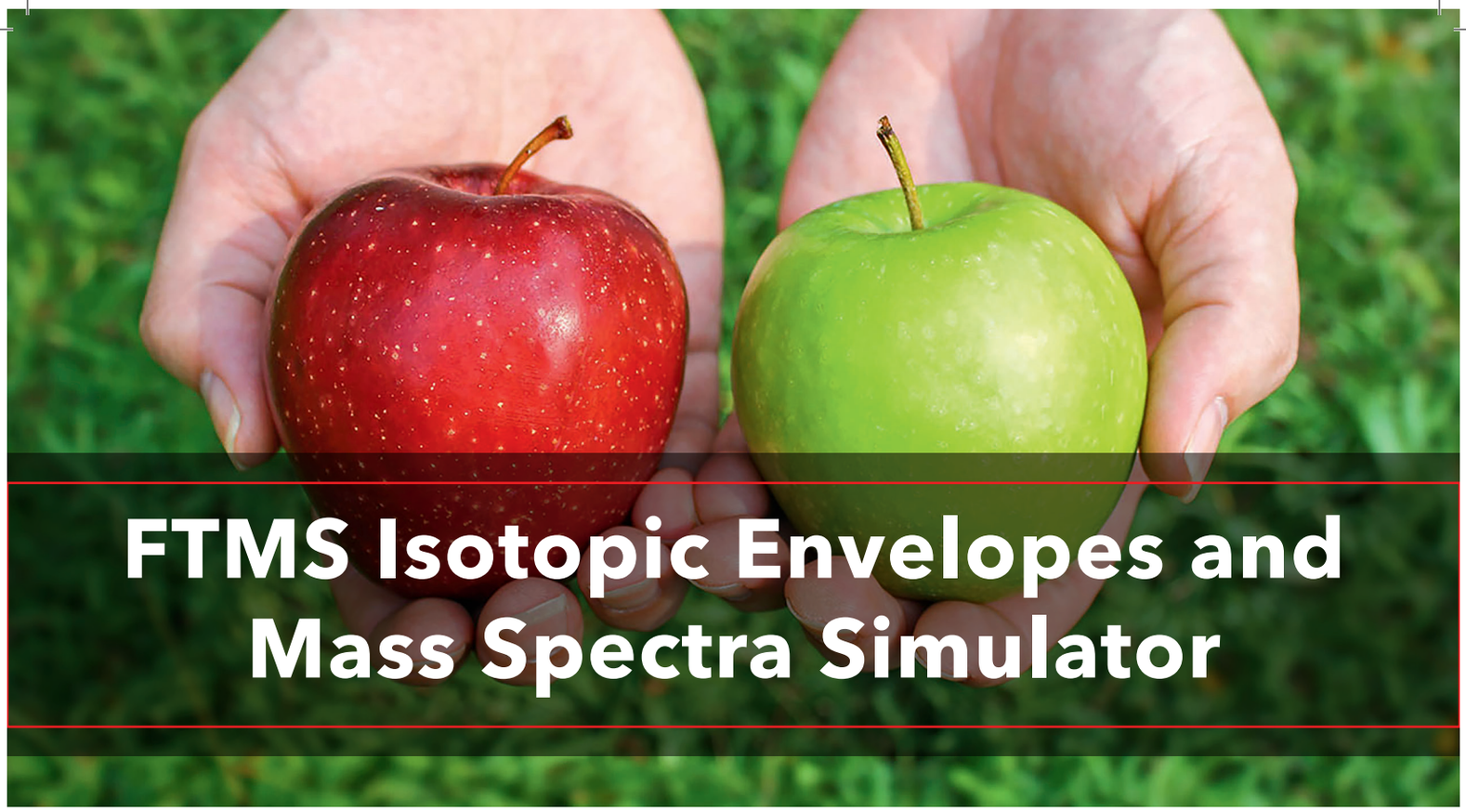


FTMS Isotopic Envelopes and Mass Spectra Simulator

FTMS DATA SIMULATOR

powered by

peakbypeak

FTMS DATA ANALYSIS

Spectroswiss

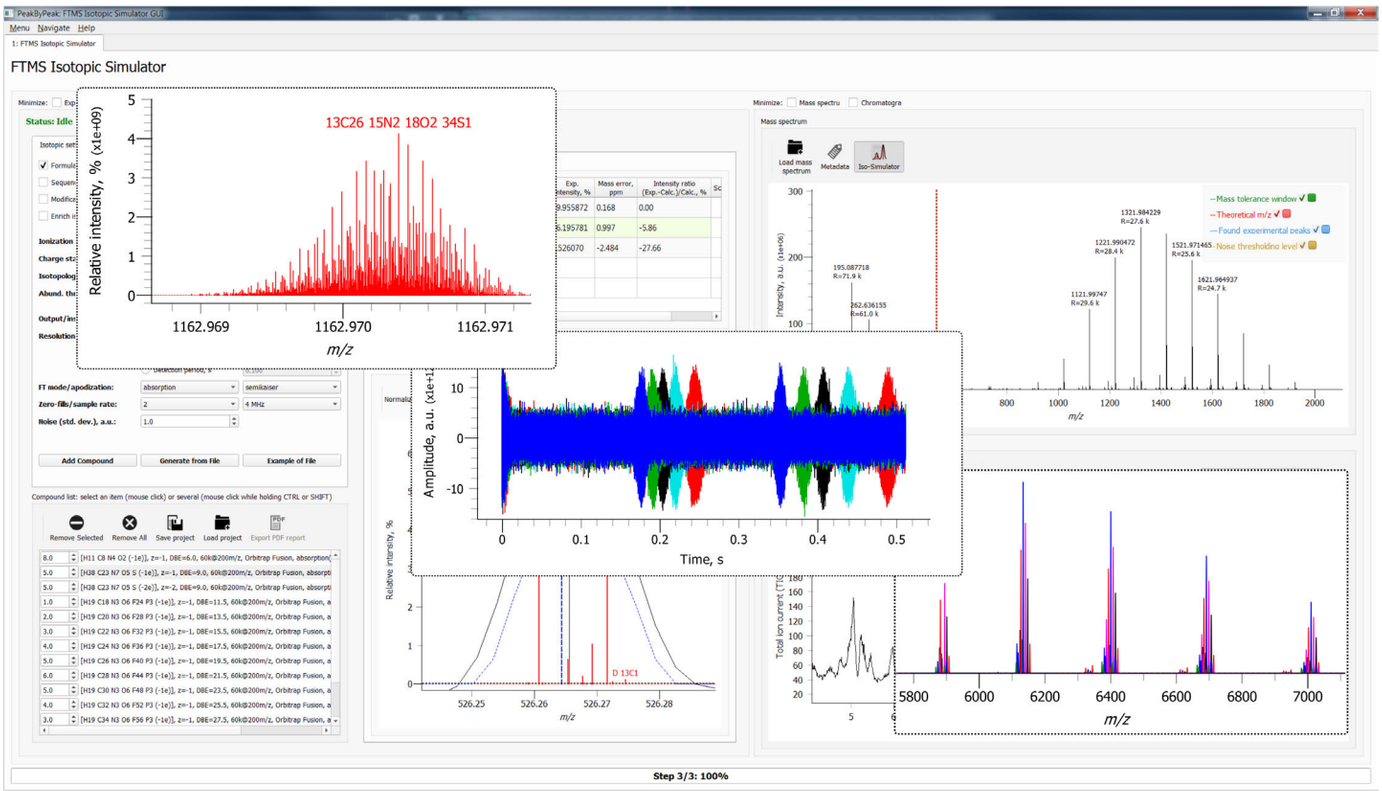
FTMS Data Simulator

The FTMS **Data Simulator** is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra to :

I. search the experimental data with a like-for-like theoretical data using similarity score for ranking the results ;

II. design FTMS experiments by selecting appropriate instrumentation settings and FT data processing parameters ;

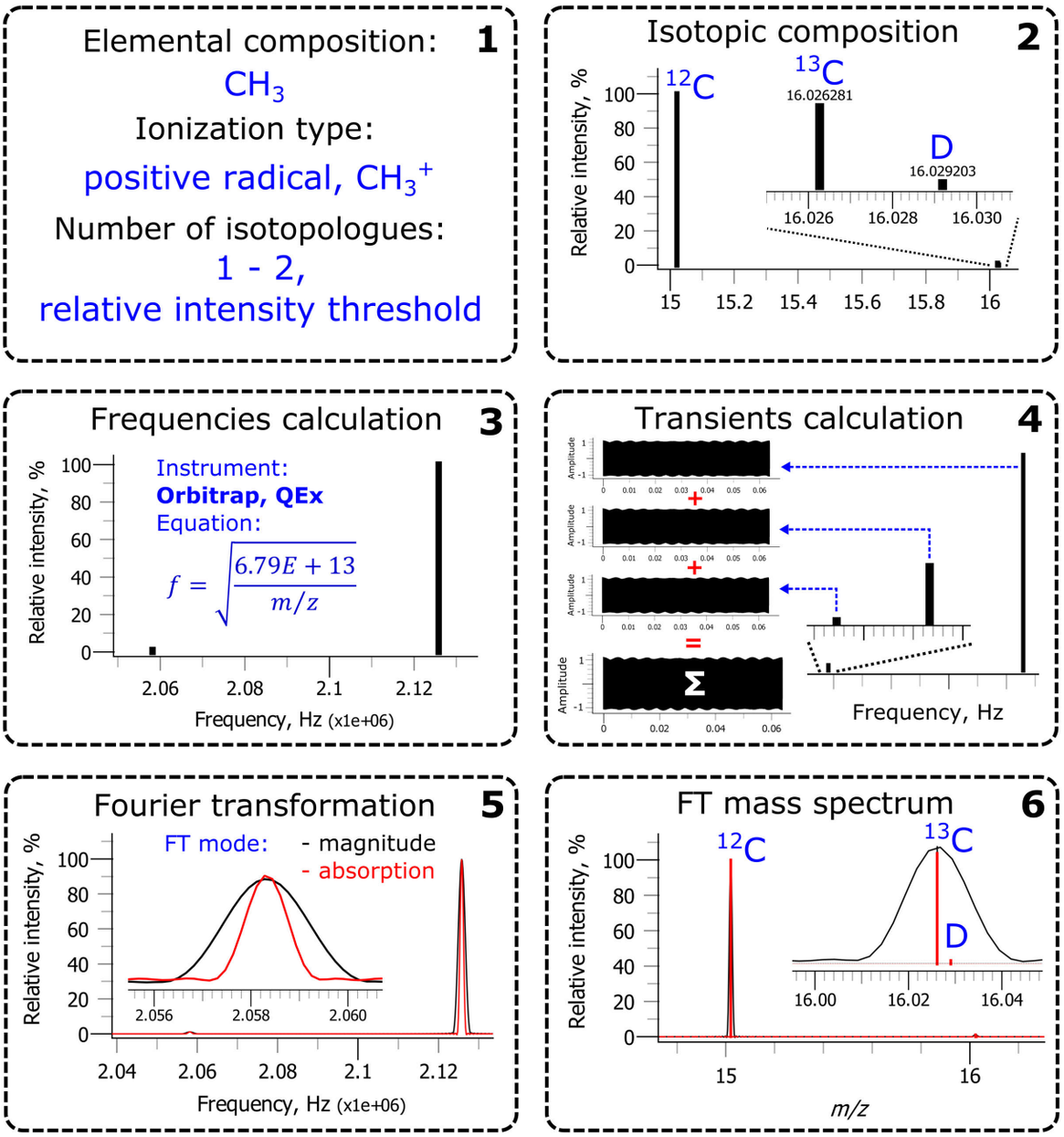
III. teach the FTMS subject by demonstrating both transients and corresponding mass spectra, containing typical FT artefacts, *etc*.



Key features

- Analyte parameters : elemental composition, amino acid sequence, charge carrier, modifications, isotopic enrichment/depletion, number of charge states and isotopologues
- FTMS experiment parameters : FTMS instrument selection (models of Orbitrap™ & ICR), resolution setting (@ *m/z*), transient sampling rate, add noise, set thresholds
- Data processing parameters : absorption/magnitude FT, apodization function, number of zero-fills, full or reduced profile mass spectra, centroids, visualize transients
- Use mass spectra : set ratios of intensities, visualize isotopic envelopes, plot multiple envelopes, extract high quality figures, extract data points for analysis
- Results output : search experimental data, rank search results by the similarity score (*m/z*, intensity), save project for future use, create report as a PDF file

FTMS Data Simulator : Workflow



Main workflow steps :

1. Define elemental composition, experiment and signal processing parameters ;
2. Calculate all possible isotopes within a specified relative intensity threshold ;
3. Convert isotopic masses to frequencies according to the specified instrument ;
4. Calculate time domain sinusoidal signals for each isotopic peak and sum them together ;
5. Perform Fourier transformation, including zero-filling and apodization ;
6. Convert frequencies to masses and visualize data : transients, mass spectra, *etc.*

FTMS Data Simulator : Architecture

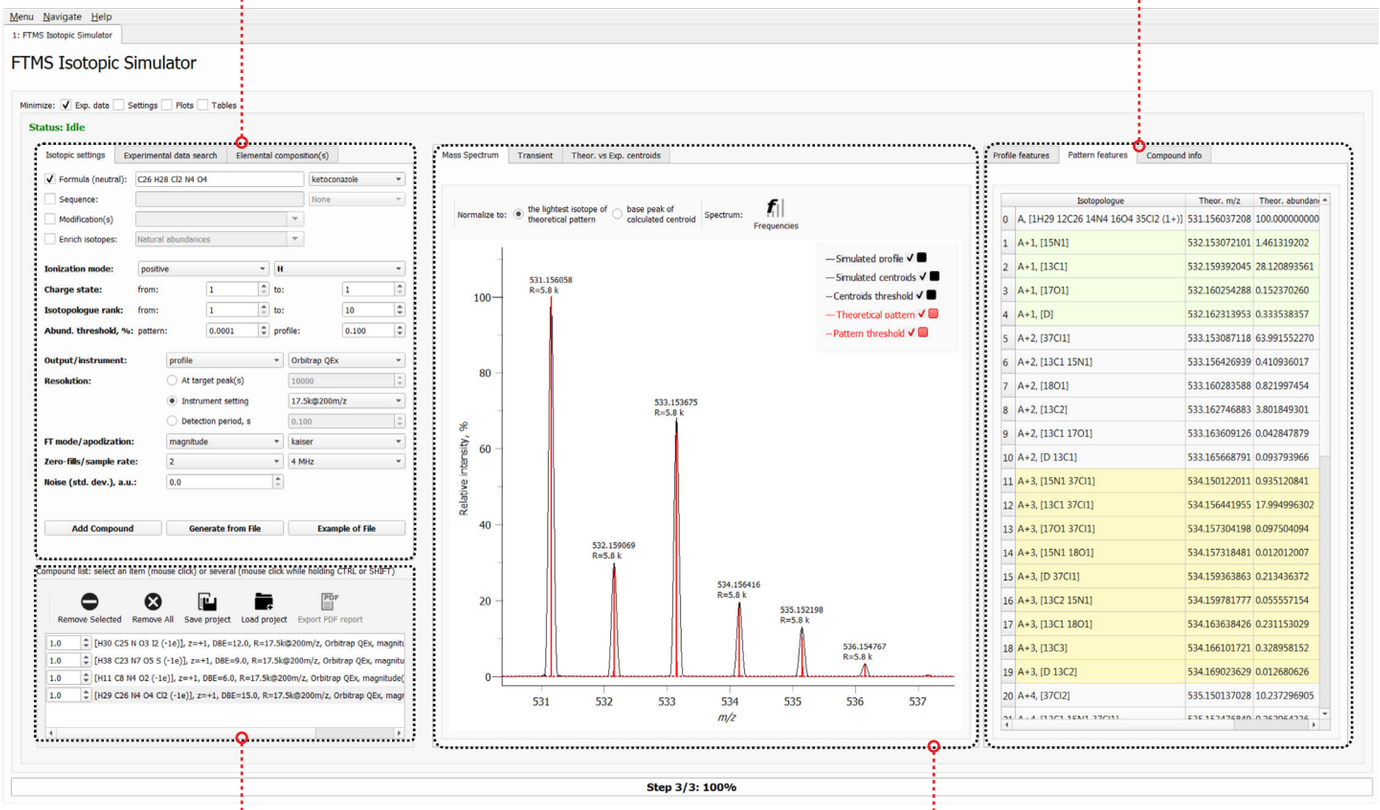
Simulation/Search settings :

- define molecules ;
- define FTMS experiment ;
- define data processing ;
- define search parameters.

peakbypeak
FTMS DATA ANALYSIS
by spectroswiss

Isotopic pattern/profile features :

- compound info ;
- mass/intensity error ;
- similarity score



Project manipulation section :

- single or multiple compound(s) selection ;
- generate from file ;
- load/save project ;
- export PDF report.

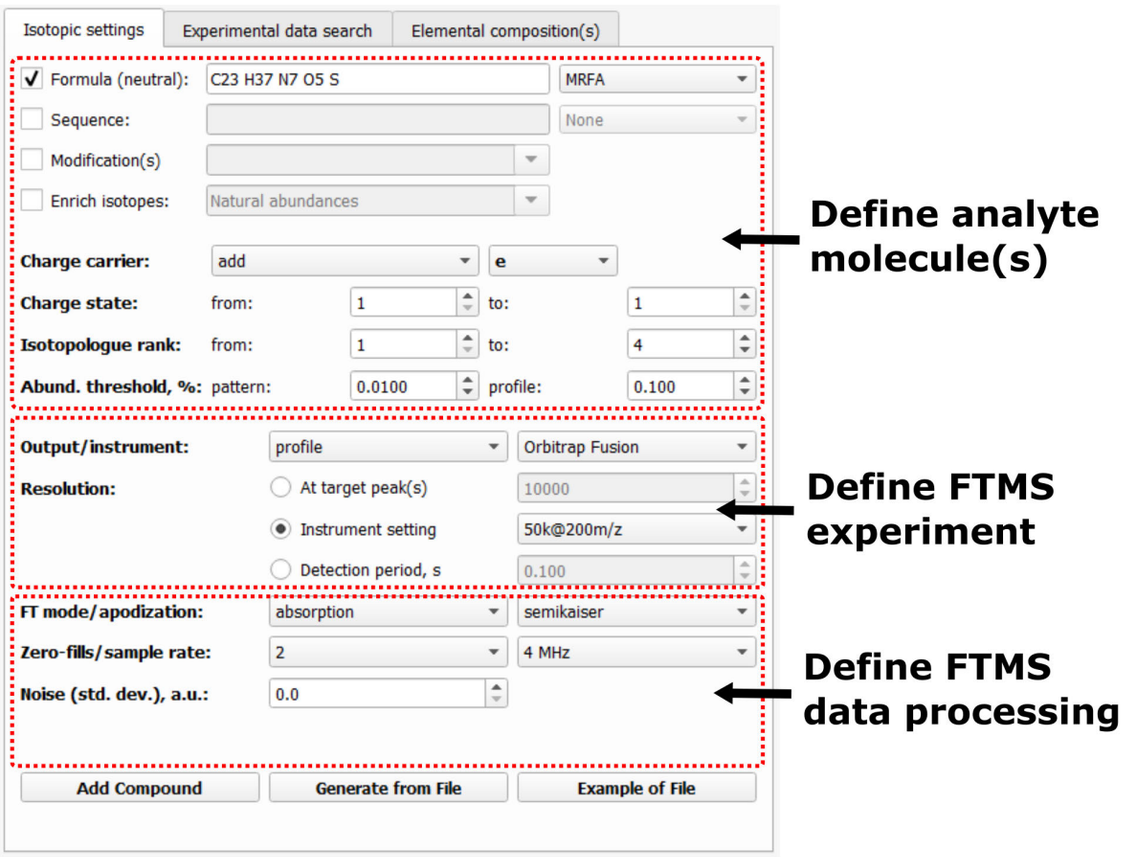
Data visualization section :

- theoretical pattern(s);
- isotope label(s) ;
- profile and centroided mass/frequency spectra
- single/summed transient(s)

FTMS Data Simulator: Settings

Define analyte molecules:

- Elemental composition(s)
- Amino acid sequence(s)
- Add adduct(s)/modification(s)
- Charge carrier, modifications
- Isotopic enrichment/depletion
- # charge states, isotopologues



Define FTMS data processing:

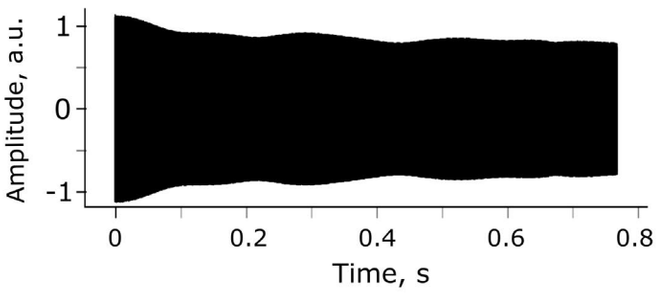
- Absorption/magnitude FT
- Apodization function
- # zero-fills
- Full/reduced profile, centroids

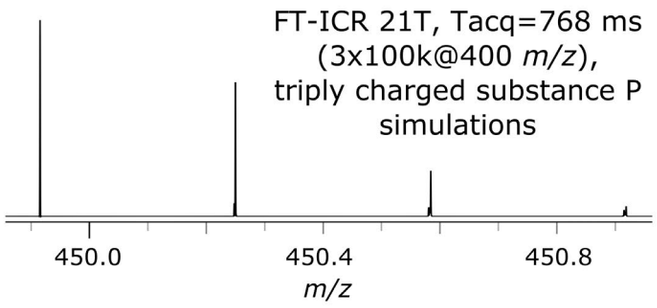
Define FTMS experiment:

- FTMS instrument selection: Orbitrap™ & FT-ICR MS
- Resolution target (@ *m/z*)
- Transient sampling rate
- Add noise, set thresholds

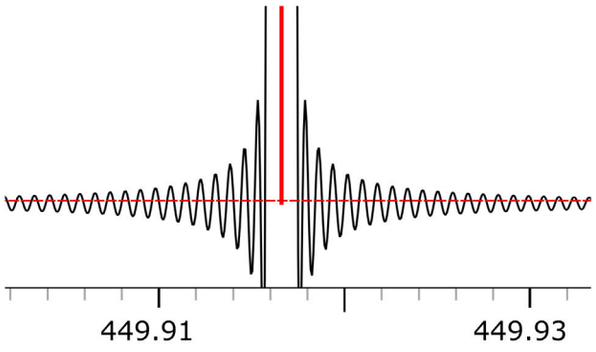
FTMS Data Simulator: Isotopic Distributions

FTMS Data Simulator enables accurate representation of isotopic fine structure profiles for small molecules, peptides, and proteins

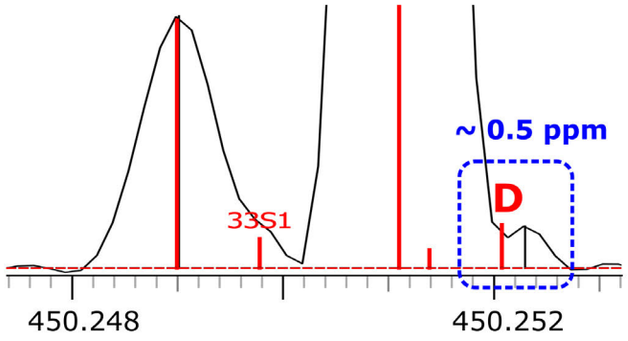




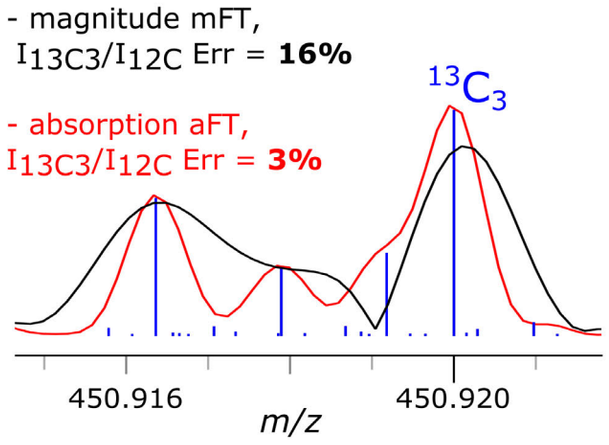
FTMS features: side-lobes, negative values in aFT mode



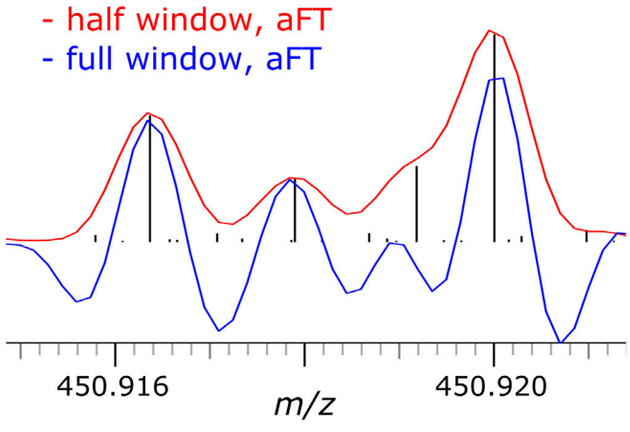
Side-lobes influence



Fourier transformation mode influence



Apodization function influence

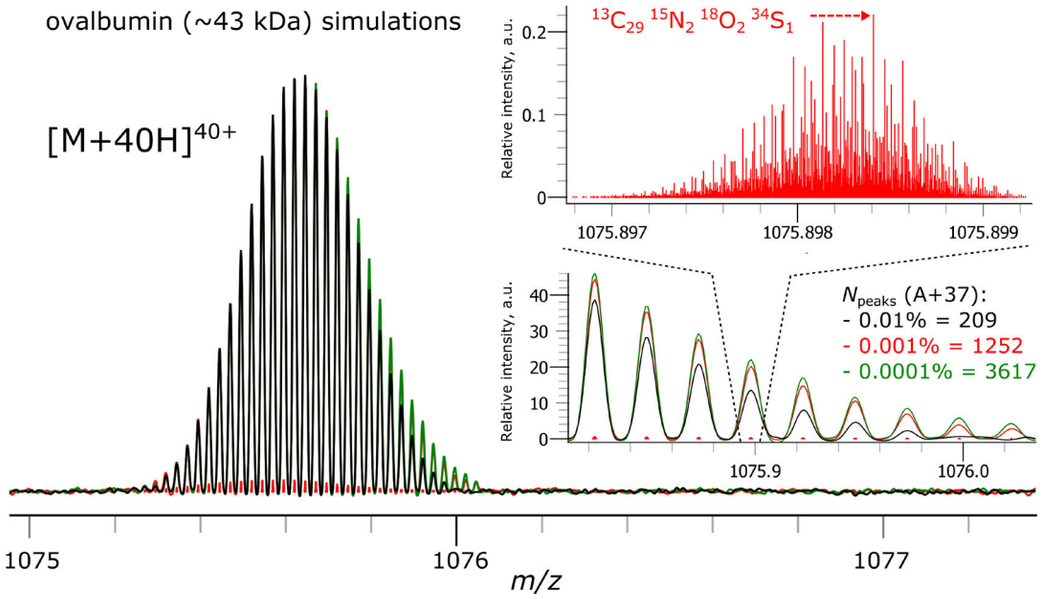


Possible artefacts in isotopic fine structure FTMS analysis of peptides: in-silico analysis of a triply charged substance P peptide using a 21 T FT-ICR MS (768 ms transients). The simulated transient converted into a mass spectrum using user-defined parameters: Fourier transformation mode (magnitude, mFT, and absorption, aFT); apodization windows (half and full, Kaiser-type), 2 zero-fills.

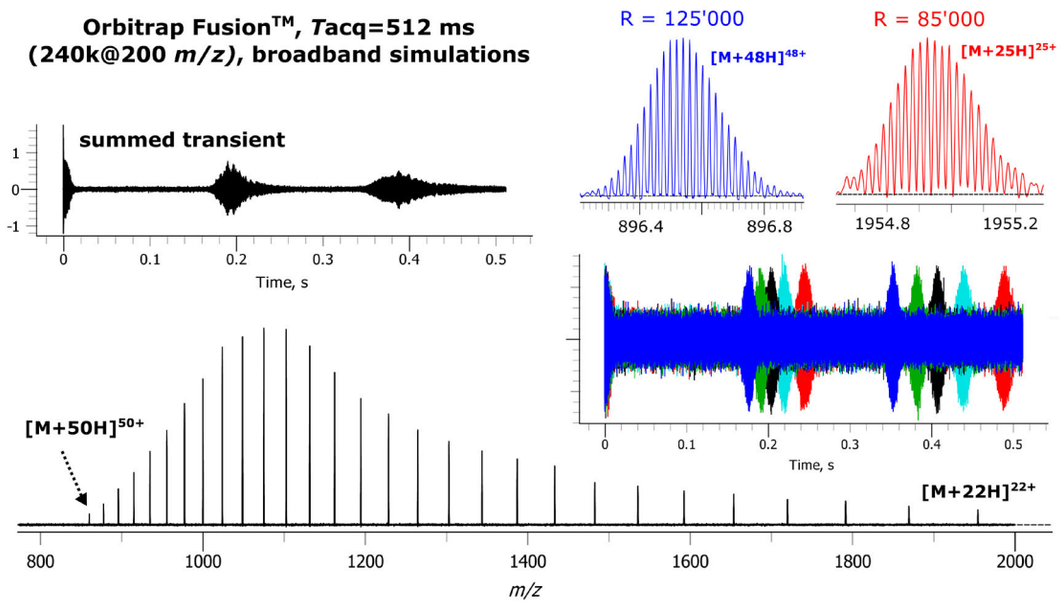
The expanded views into the corresponding isotopic patterns of the simulated profile mass spectrum show presence of both mass and intensity deviations w.r.t. the theoretical isotopic patterns.

FTMS Data Simulator: Protein Analysis

User control of the data processing parameters in FTMS Data Simulator allows to investigate accuracy of protein isotopic envelopes representation. Example shown is an *in-silico* analysis of ovalbumin (~43 kDa) using an Orbitrap™ Fusion™ with an ion detection period of 512 ms (set resolution of 240k @ *m/z* 200).



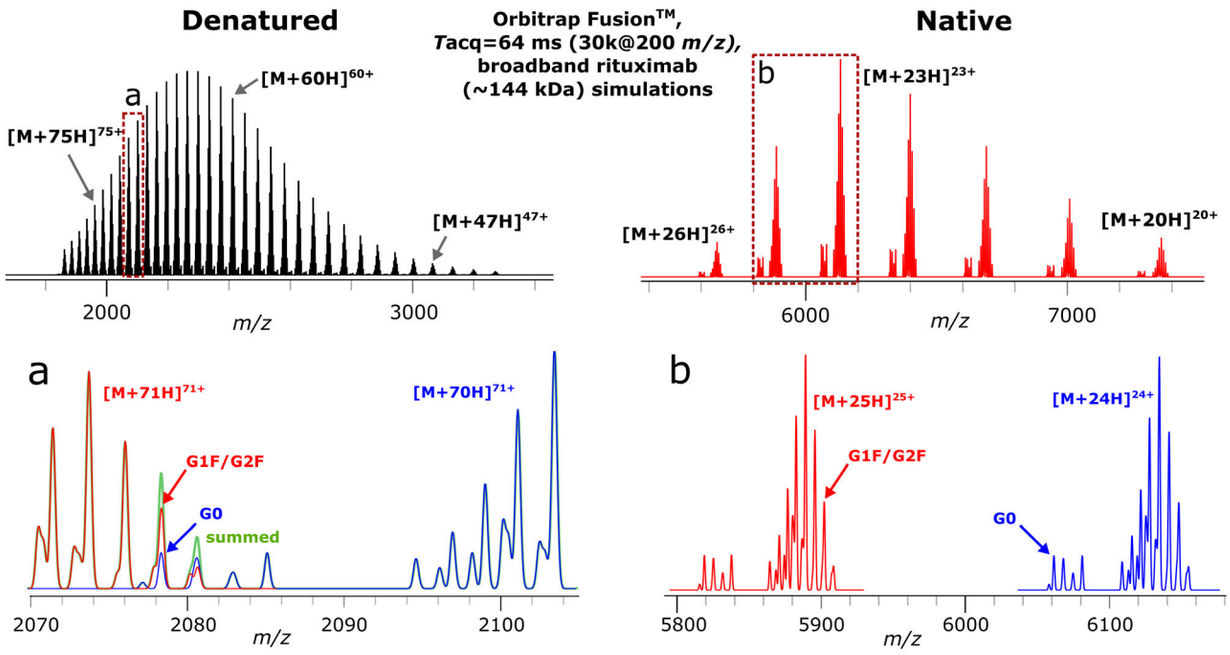
Isotopic distribution of a protein (above) indicates influence of minor isotopic fine structure peaks contributing to the integral protein isotopic distribution. Particularly heavy isotopologues can be distorted due to the extreme complexity of their isotopic fine structures. For example, number of components in a A+37 isotopologue increases significantly when intensity thresholds are reduced (see inset).



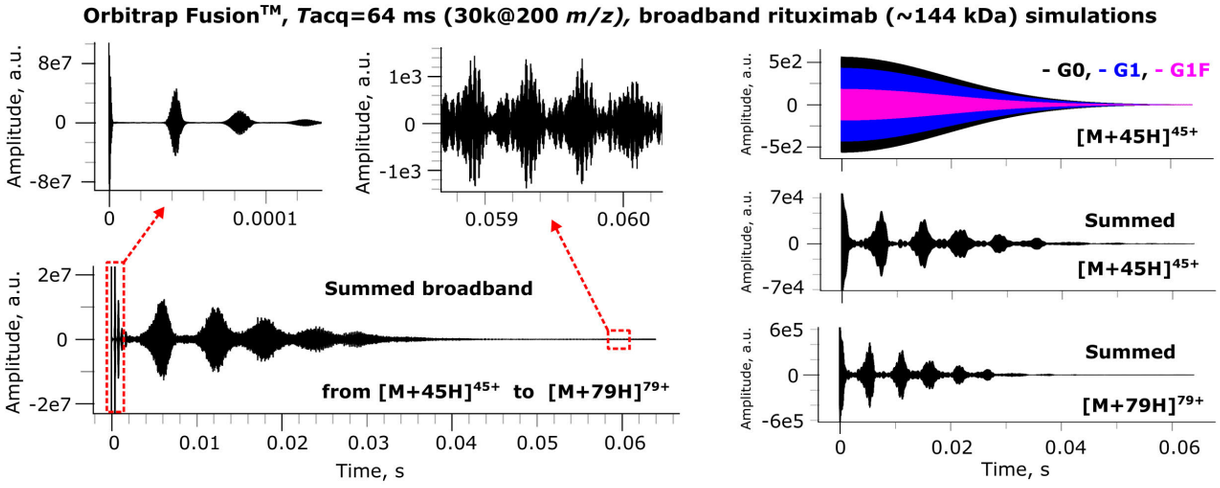
FTMS Data Simulator is capable of simulating broadband mass spectra of proteins (above). Variation of peak intensities is user-defined and can support generation of simulated mass spectra closely resembling experimental mass spectra of proteins. Charge state-specific contribution into the FTMS transients can be differentiated by color to teach FTMS basics.

FTMS Data Simulator: Antibody Analysis

FTMS Data Simulator can shed light on details of denatured and native analysis of monoclonal antibodies (mAbs). Here, analysis of a rituximab-like protein (~144 kDa) is shown as an example (employed instrument is Orbitrap™ Fusion™ with resolution setting of 30k @ *m/z* 200).



Example above shows simulated mass spectra of (left) denatured and (right) native rituximab-like protein "acquired" in the broadband (wide) mass range spreading its typical charge states: denatured - from 45 to 79; native - from 20 to 26. Each charge state includes major glycoforms, e.g., G0, G0F, G1, G1F, G2, G2F and their combinations, including addition of sialic acid (SA) and losses of GlcNAc (HexNAc) moiety. Benefits of native FTMS are apparent.

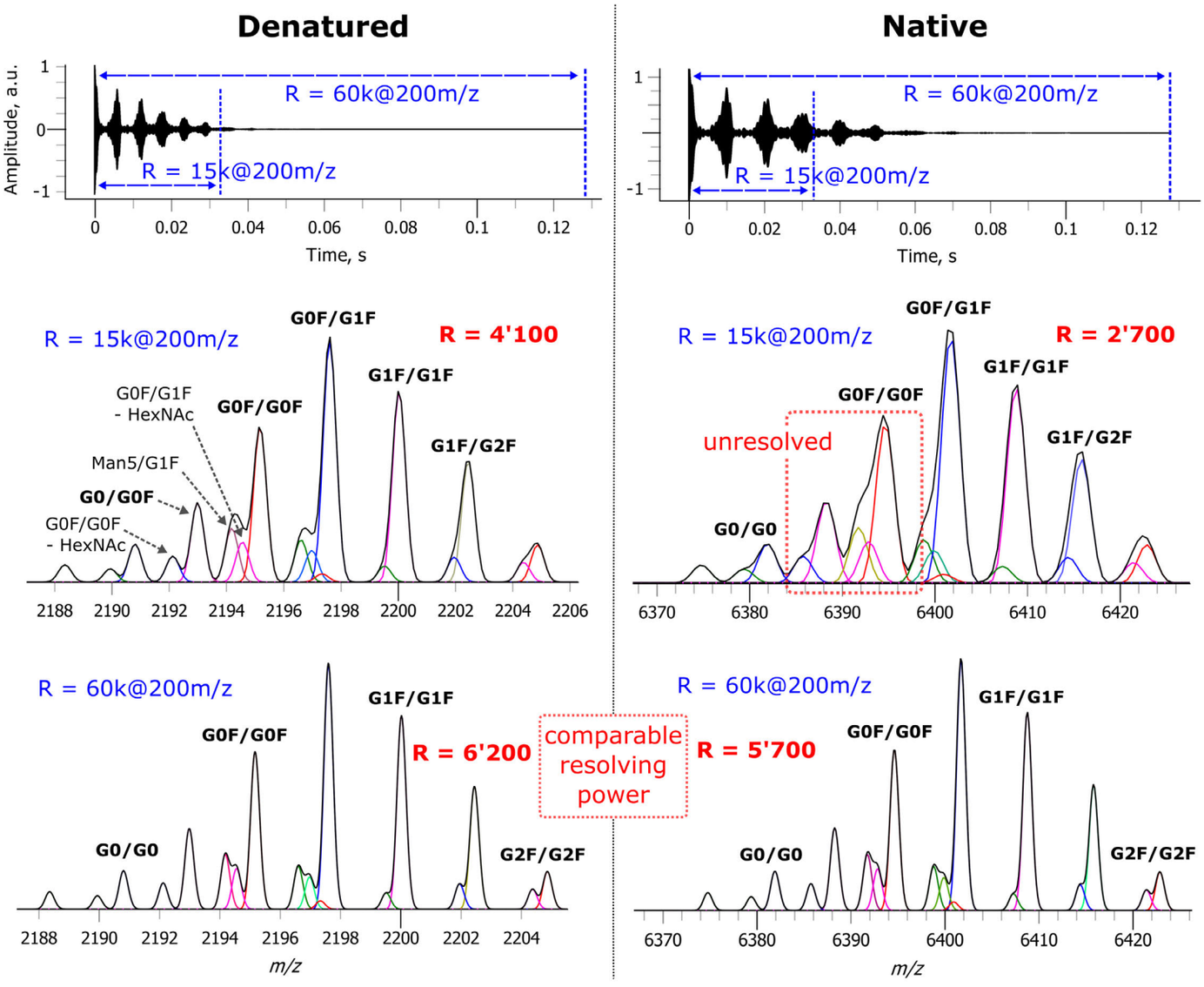


Simulated transient structure of the denatured rituximab-like protein acquired in the broadband mass range (left panel) shows a strong transient decay due to the interferences between components with different frequencies (collisions with gas molecules are not considered).

Right panel shows transients (top) generated independently (with a user-defined intensity ratio between the glycoforms) for a number of single glycoforms, including G0, G1 and G1F at z=45, (middle and bottom) show transients generated with a glycosylation pattern at charge states 45 and 79 acquired separately. Total number of glycoforms is 30 for the summed transients.

FTMS Data Simulator: Antibody Analysis

In-silico comparing resolution performance in denatured and native analysis of a monoclonal antibody, rituximab-like protein (~144 kDa), using an Orbitrap™ Fusion™ with two resolution settings: 15k @ *m/z* 200 and 60k @ *m/z* 200.



Example shows simulated transients and mass spectra of (left) denatured (z=67) and (right) native (z=23) rituximab analysis. Each charge state includes major glycoforms, G0, G0F, G1, G1F, G2, G2F and their combinations, including addition of sialic acid (SA) and losses of GlcNAc (HexNAc).

Results indicate that the resolution performance is better for the denatured mAb analysis in comparison with the native one at the short detection period of 32 ms (R = 15k @ *m/z* 200). Serendipitously, the resolution performance becomes comparable at the longer detection period of 128 ms (R = 60k @ *m/z* 200). Presumably, the reason is the faster transient decay (top panel) due to the interference of higher frequencies in the denatured analysis compared to the native one, where frequencies are lower (1.7 times, 312 kHz vs. 180 kHz).

FTMS Data Simulator: Experimental Data Search

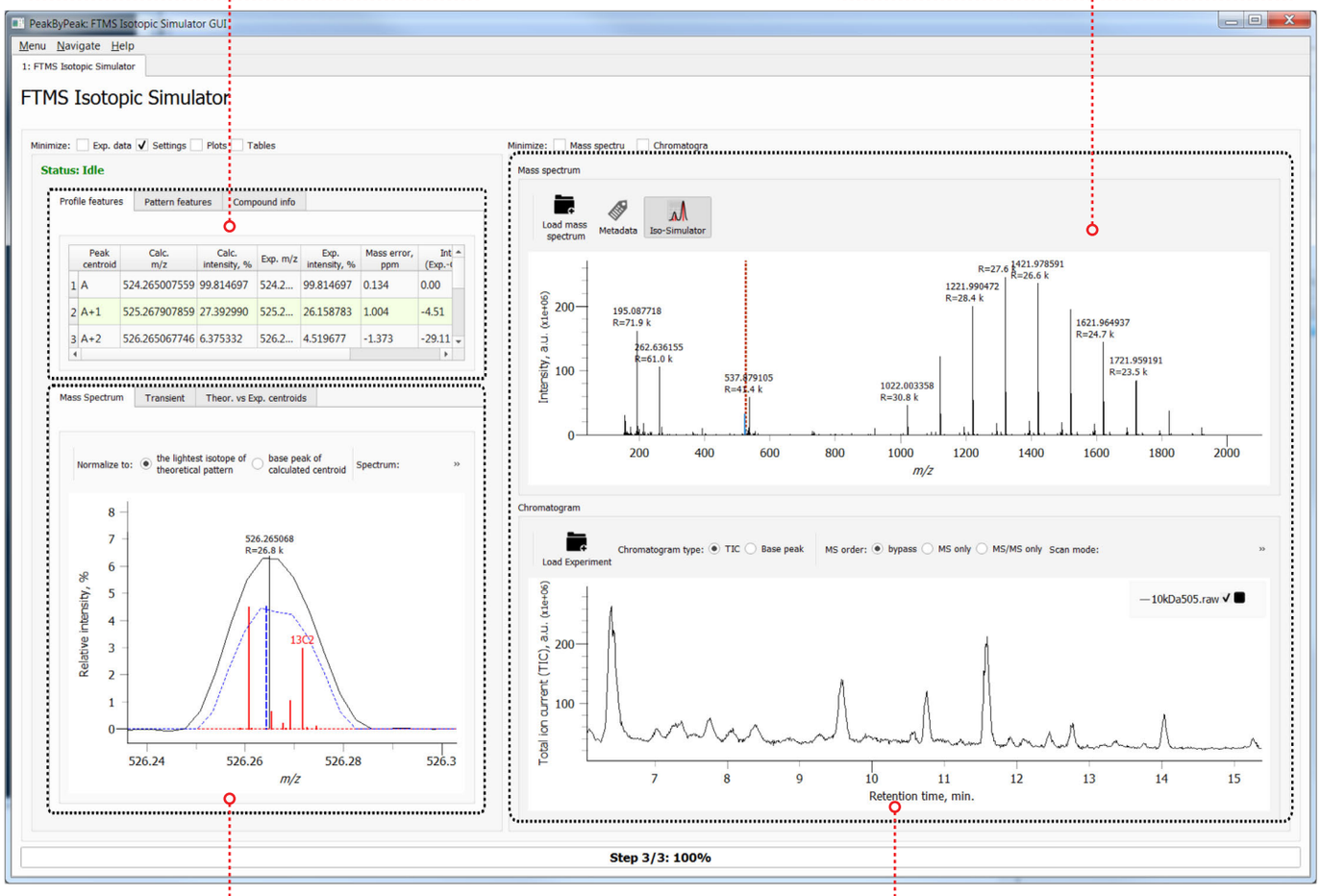
Extension of FTMS Data Simulator allows to match simulated FTMS isotopic envelopes with the experimental mass spectra (.RAW) and rank the results.

Isotopic pattern/profile features:

- compound info;
- theoretical vs experimental
- mass/intensity error;
- similarity score

Experimental data section single spectrum:

- load single spectrum;
- visualize theor. vs exp.
- mass shift correction



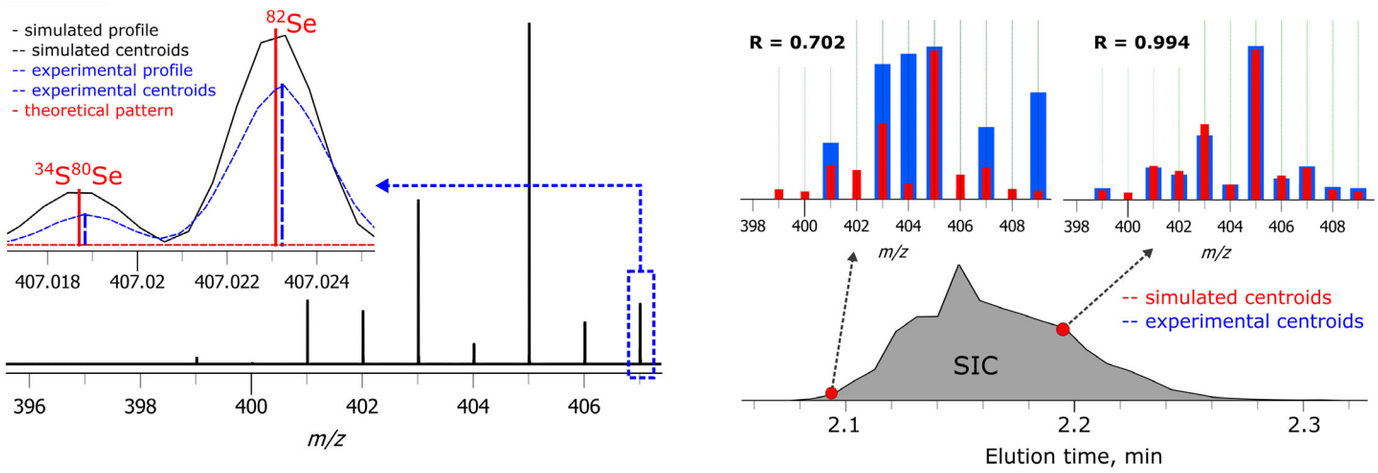
Data visualization section:

- compare simulated and experimental profile/centroid;
- mass or frequency spectra;
- centroids similarity graph.

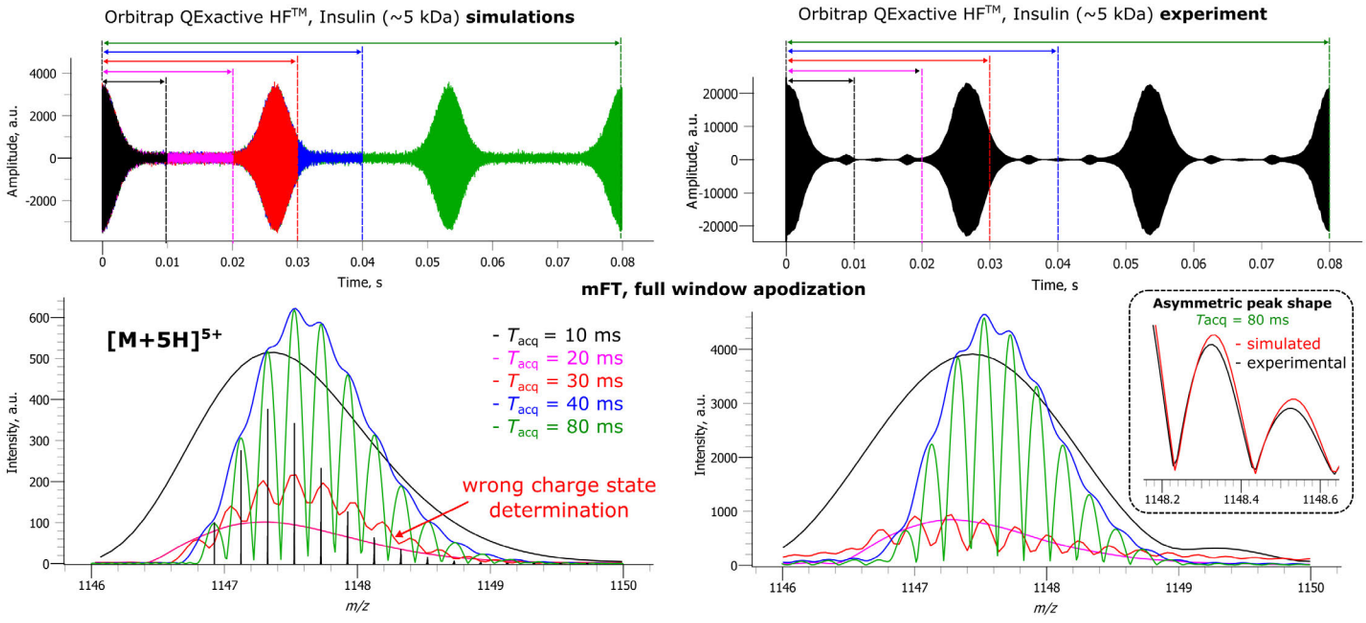
Experimental data section (experiment):

- load .RAW/.H5 experiment;
- select chromatogram type;
- spectral averaging;
- scan filtering.

FTMS Data Simulator : Experimental Data Search



Experimental data search of selenium-containing metabolite compounds in LC-MS experiment (Orbitrap™ Fusion™ Lumos™) using FTMS Data Simulator. Left panel shows comparison of simulated and experimental data (eFT, .RAW). Right panel shows example of a similarity score calculation and visualization at different elution times.



Peak interference and FT processing parameters influence in FTMS : comparison of simulations and experimental measurements of a small (~5 kDa) protein using a Q Exactive™ HF™ Orbitrap™ FTMS. A quintopoly charged insulin was (left panel) simulated and (right panel) measured with different detection periods. Both results (simulated & experimental) show distorted isotopic envelopes in mass spectra produced by magnitude mode FT of transients with the detection periods in the range of 15-35 ms. Apodization window (Kaiser-type) presumably provides the main contribution to the appearance of the isotopic envelopes.

Experimental FTMS transients for this example were acquired from a Q Exactive™ HF™ Orbitrap™ using an external high-performance data acquisition system (FTMS Booster X2, Spectroswiss). Data processed using Peak-by-Peak.

How to get FTMS Data Simulator:

If you are interested to learn more about FTMS Data Simulator or want to get your free evaluation license, you are most welcome to contact us at info@spectroswiss.ch.

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