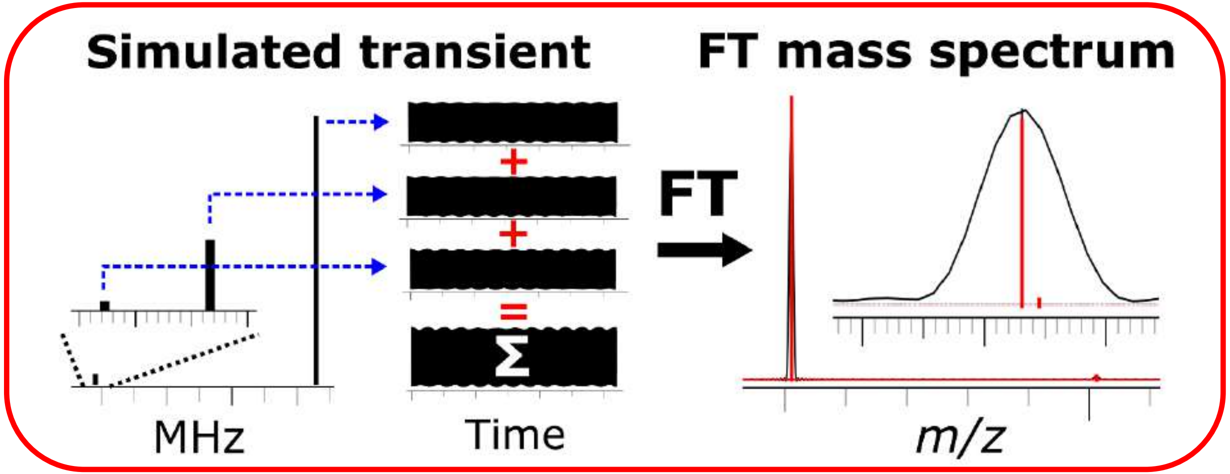
EFTMS
2022

Spectroswiss

14th European Fourier Transform Mass Spectrometry Workshop

FT Mass Spectra Simulation: Fundamentals and Applications



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Lausanne, Switzerland

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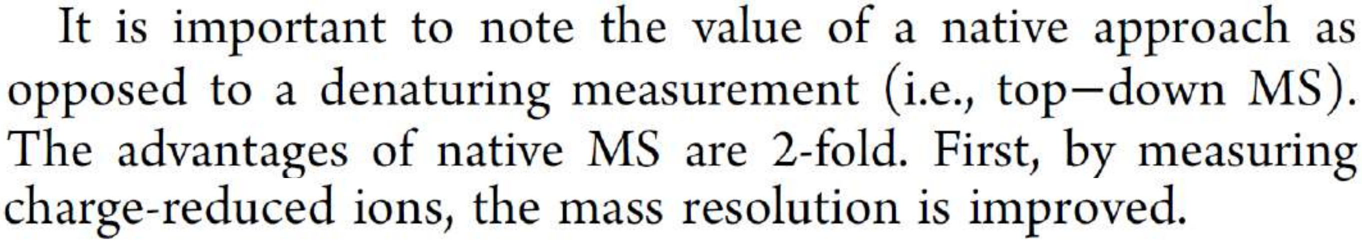
July 12, 2022; 14:00

How & Why to Simulate the FTMS Data?

- Approaches to simulate the FTMS isotopic envelopes and mass spectra
- Teaching & training: understanding the FTMS concepts and definitions
- Reviewing & writing: manuscripts, project applications, ideas verification
- Support FTMS data interpretation and experiment design (settings selection)
- Advancing the FTMS fundamentals and generating novel insights
- Support of FTMS data processing workflows (simulated vs experimental data)

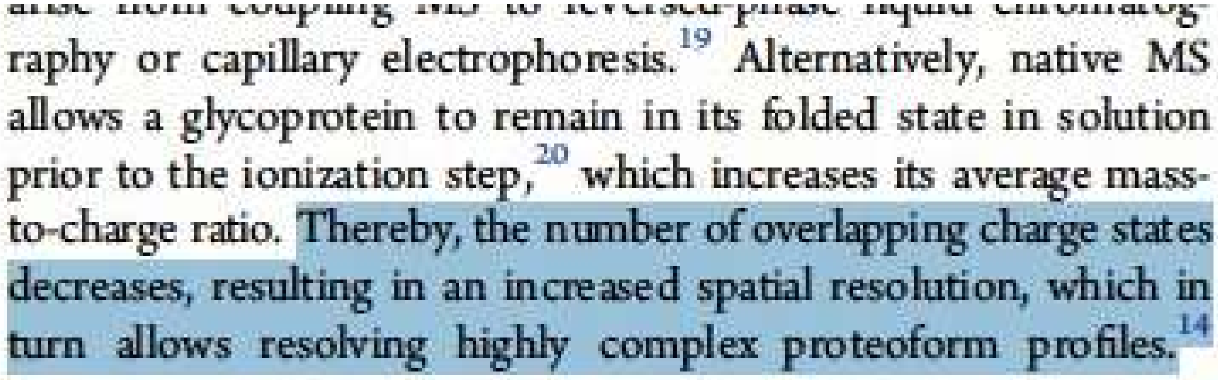
Presently, FTMS data simulations remain underused

True or False?



It is important to note the value of a native approach as opposed to a denaturing measurement (i.e., top−down MS). The advantages of native MS are 2-fold. First, by measuring charge-reduced ions, the mass resolution is improved.

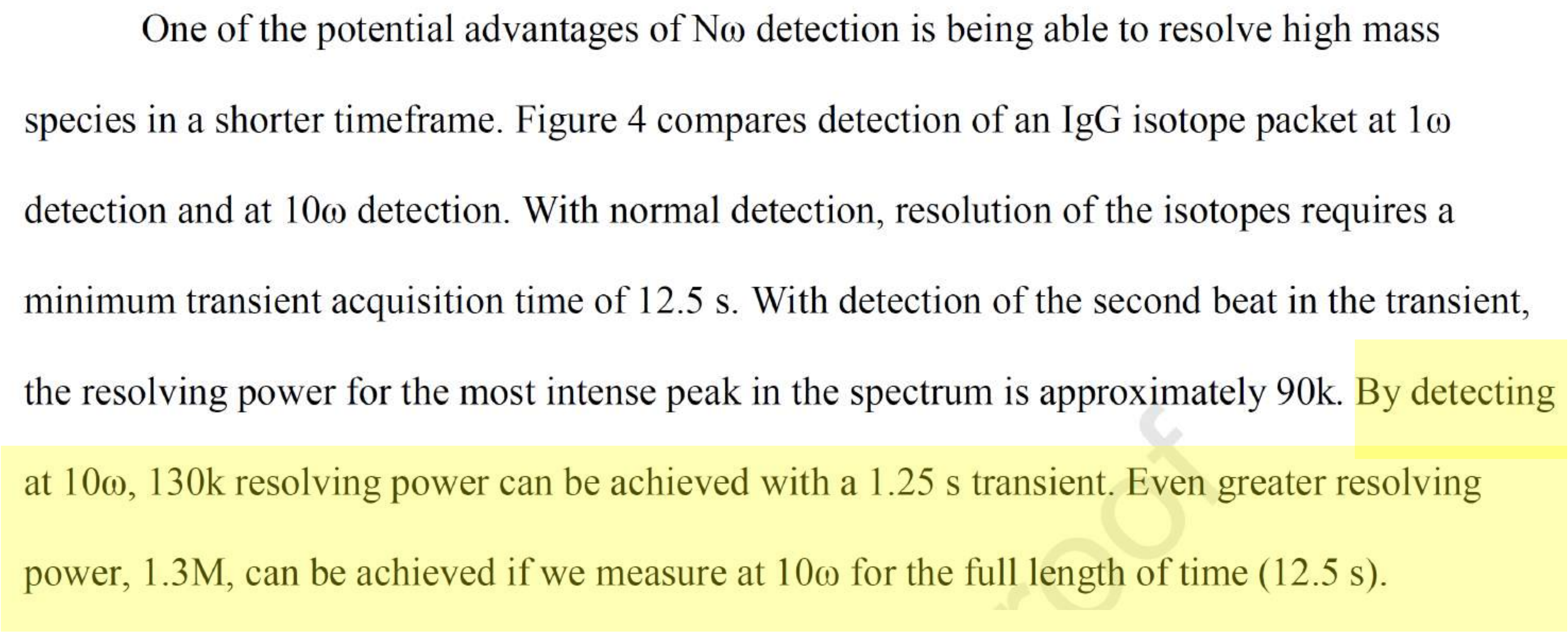
Anal. Chem., 2021



raphy or capillary electrophoresis.[19] Alternatively, native MS allows a glycoprotein to remain in its folded state in solution prior to the ionization step,[20] which increases its average mass-to-charge ratio. Thereby, the number of overlapping charge states decreases, resulting in an increased spatial resolution, which in turn allows resolving highly complex proteoform profiles.[14]

Anal. Chem., 2018

True or False?



One of the potential advantages of Nω detection is being able to resolve high mass species in a shorter timeframe. Figure 4 compares detection of an IgG isotope packet at 1ω detection and at 10ω detection. With normal detection, resolution of the isotopes requires a minimum transient acquisition time of 12.5 s. With detection of the second beat in the transient, the resolving power for the most intense peak in the spectrum is approximately 90k. By detecting at 10ω, 130k resolving power can be achieved with a 1.25 s transient. Even greater resolving power, 1.3M, can be achieved if we measure at 10ω for the full length of time (12.5 s).

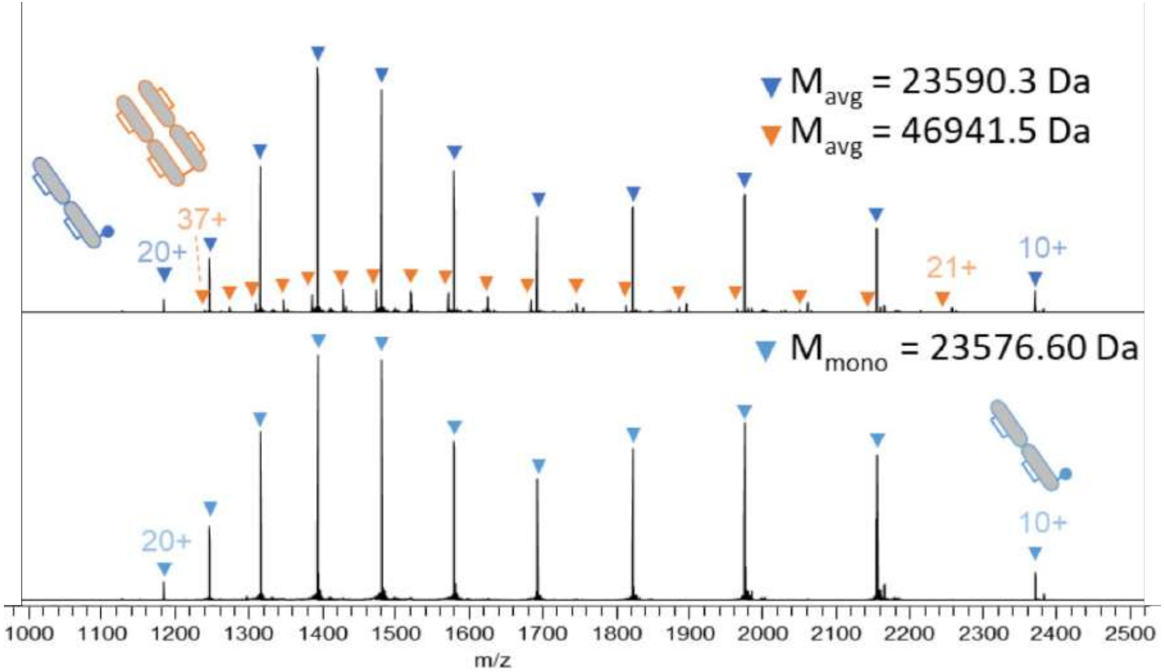
IJMS, 2020

Why is it?

Analysis of mAb light and heavy chains with a Fusion Lumos Orbitrap FTMS

15k @ *m/z* 200

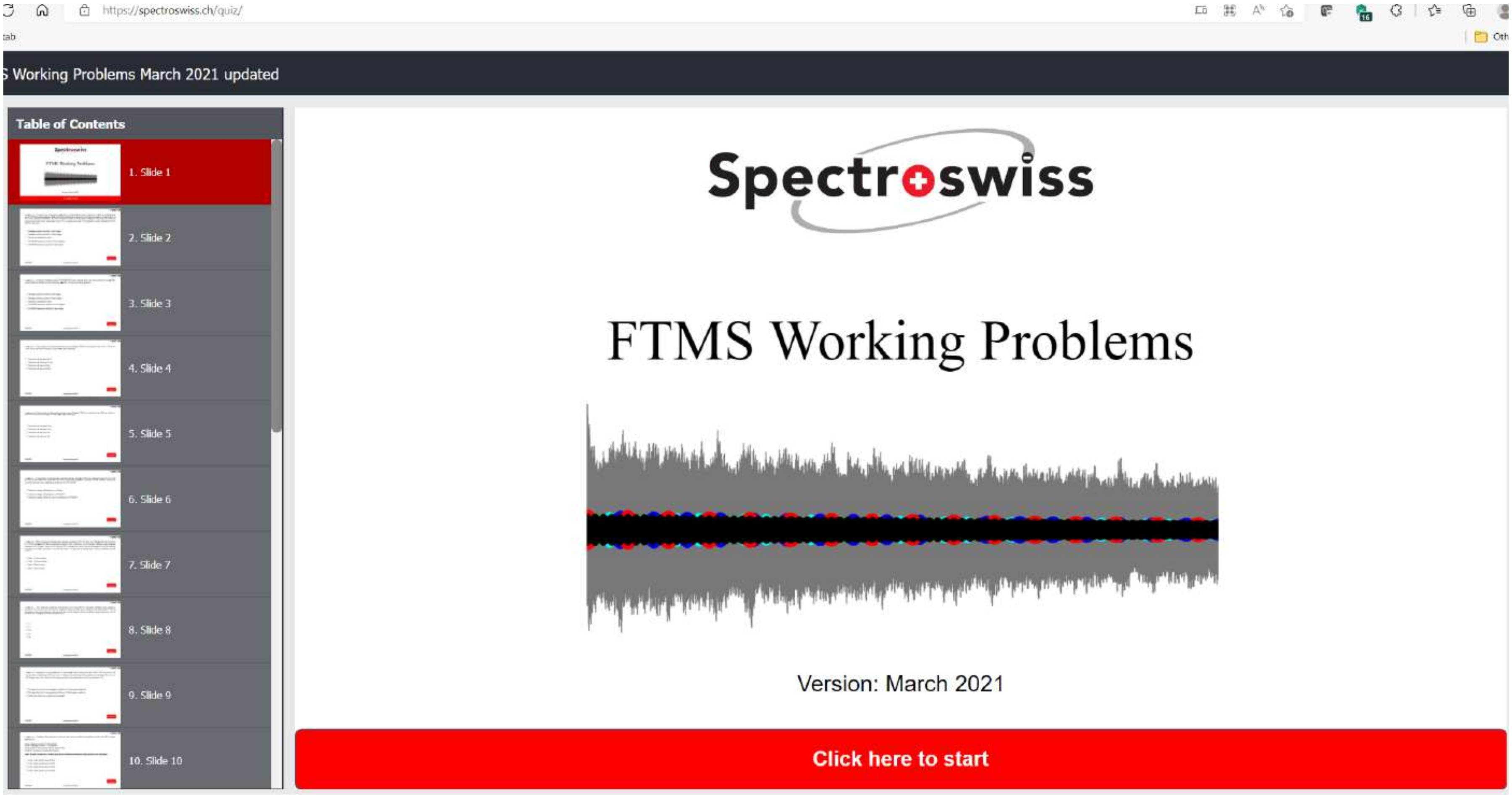
120k @ *m/z* 200



Anal. Chem., 2021

As expected, only the lowest mass is observed (Figure 2B) in the high-resolution spectrum at 23,576.60 Da (monoisotopic mass).

https://spectroswiss.ch/quiz/



Working Problems March 2021 updated

Table of Contents

1. Slide 1
2. Slide 2
3. Slide 3
4. Slide 4
5. Slide 5
6. Slide 6
7. Slide 7
8. Slide 8
9. Slide 9
10. Slide 10

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FTMS Working Problems

Version: March 2021

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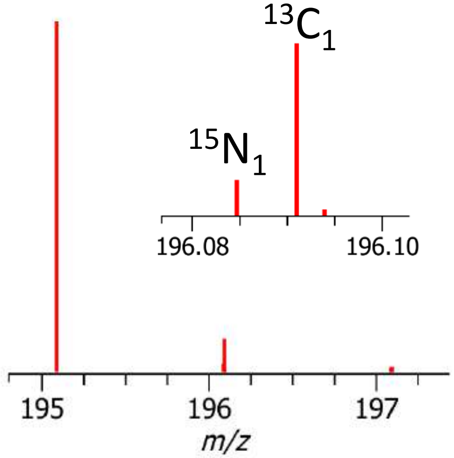
FTMS Data Simulation Approaches

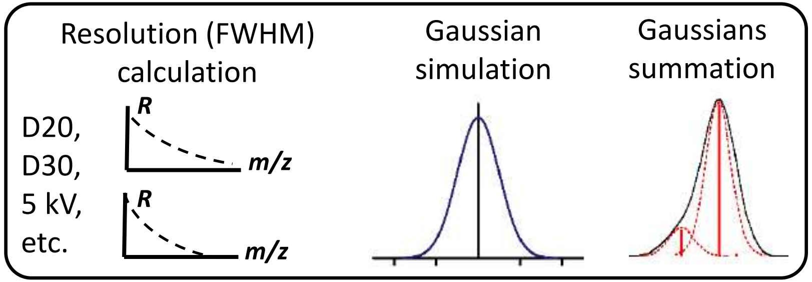
- Numerical simulations of ion motion and induced current detection
  - SIMION, Particle-in-Cell (PIC): *Amster (IJMS, 2020), Hendrickson (IJMS, 2009), etc*.
- Analytical models for estimation of ion oscillation frequencies and resolution
  - For each FTMS instrument: parameters of ion oscillations (e.g., frequency); dependency of resolution on frequency, relationship between frequency and *m/z*
    Hofstadler, IJMS IP 1994, Easterling 1999, Makarov JASMS 2009
- Empirical estimation of resolution: peak shape addition to isotopic distributions
  - Gaussian peak shapes are added using the resolution relationship with *m/z*
    https://www.envipat.eawag.ch/ - as employed in LIPIC (*Cataldi, JASMS 2021*)
    https://www.chemcalc.org/ - ChemCalc (Patiny, *J. Chem. Inf. Model* 2013)
  - The resolution values are estimated from the experimental peaks (FasmaTech, etc.)
- Accurate simulation of the FTMS data processing workflow
  - Simulation and processing of time-domain transients, for each instrument (this work)

FTMS Data Simulation Approaches

Isotopic distribution calculation



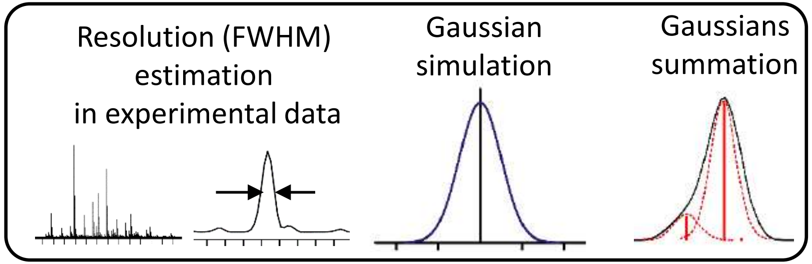
ChemCalc
IsoSpec2
EnviPat
…


Resolution (FWHM) calculation

Gaussian simulation

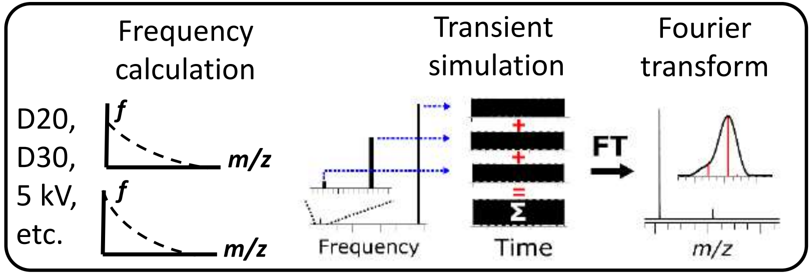
Gaussians summation

D20,
D30,
5 kV,
etc.


Resolution (FWHM) estimation in experimental data

Gaussian simulation

Gaussians summation


Frequency calculation

Transient simulation

Fourier transform

D20,
D30,
5 kV,
etc.

Protein analysis? (isotopic beats)

Peak interference?

Computational speed?

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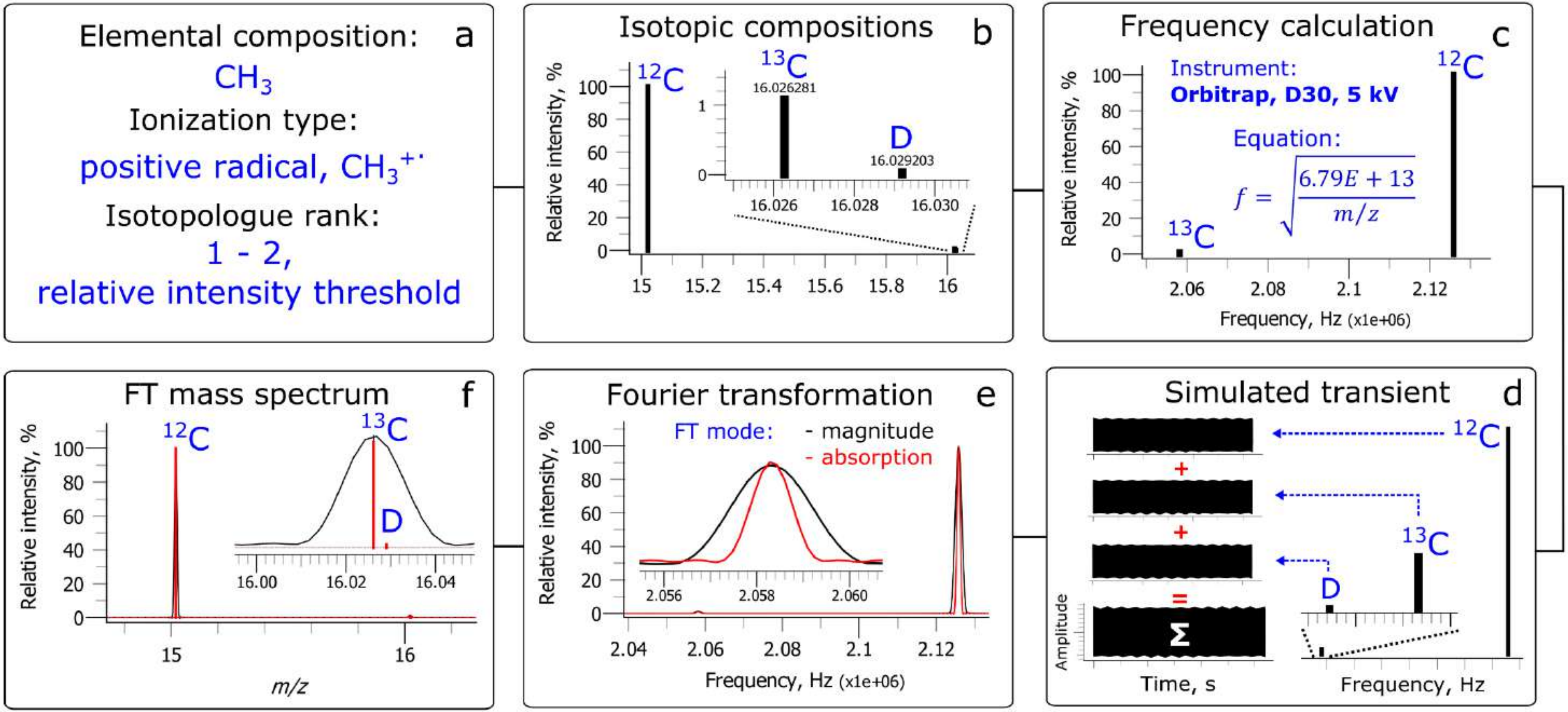
FTMS Data Simulation via Time-Domain Transients



Nagornov et al.: Small molecules: *JASMS 2020, 31, 1927–1942;* Large molecules: *JASMS 2022, 33, 1113–1125*

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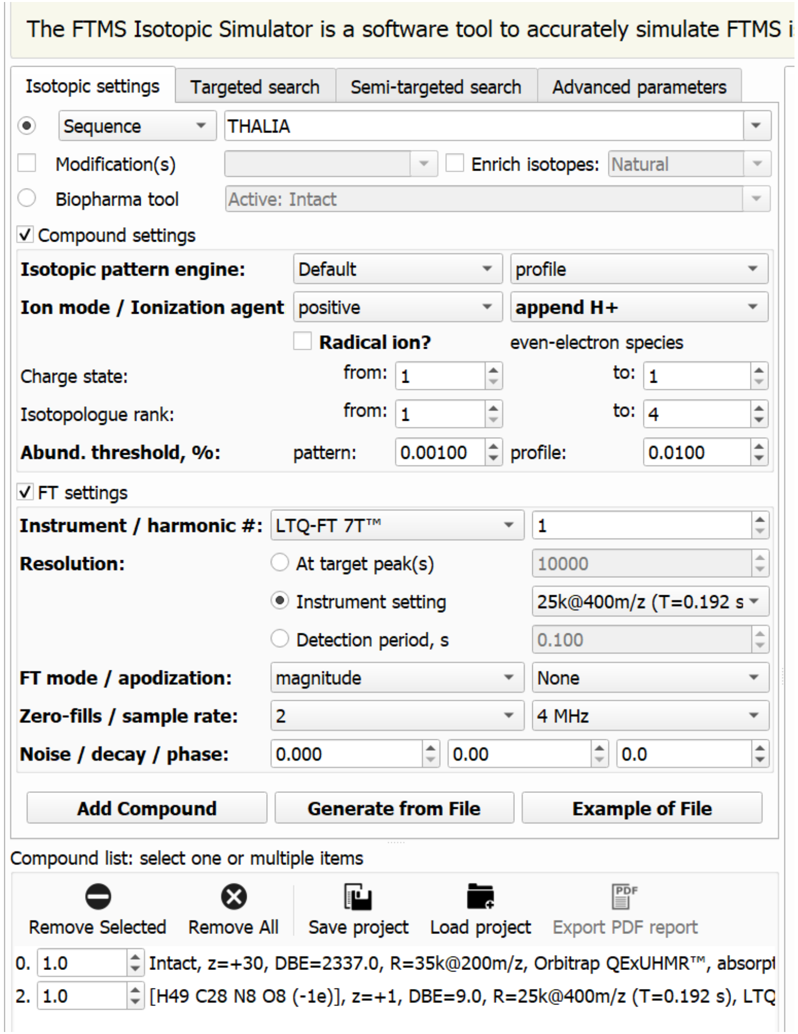


The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS i

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: Active: Intact

☑ Compound settings

Isotopic pattern engine: Default / profile

Ion mode / Ionization agent: positive / **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™ / 1

Resolution:
○ At target peak(s): 10000
● Instrument setting: 25k@400m/z (T=0.192 s
○ Detection period, s: 0.100

FT mode / apodization: magnitude / None

Zero-fills / sample rate: 2 / 4 MHz

Noise / decay / phase: 0.000 / 0.00 / 0.0

Compound list: select one or multiple items

0. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Compound definition:

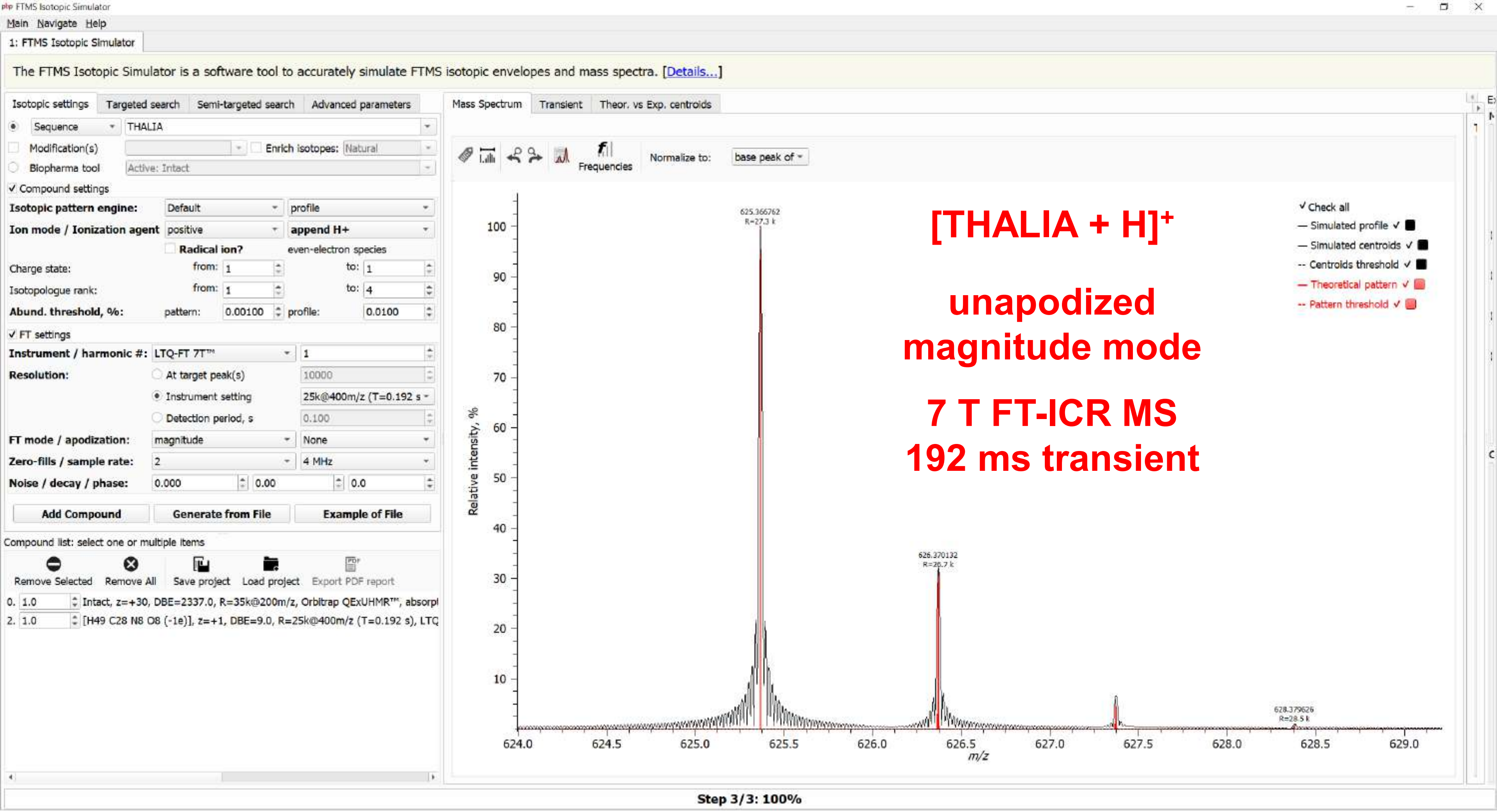
- elemental composition, isotopic labelling / enrichment
- amino acid sequence
- mass (*m/z*) or frequency value
- proteoforms – sequence and modifications (mAbs, viruses, …)

Ion (charged compound) definition:

- Charge carrier: electron, H^+, K^+, Na^+, Cs^+, I^-, $HCOO^-$
- Ionization mode: positive, negative, or a neutral species
- Charge state: from the lowest to the highest
- Isotopologues: how many and which ones

FT processing settings:

- FTMS instrument and model: ICR/MRMS, Orbitraps
- Harmonics order: fundamental and higher order harmonics
- Resolution: at target peak, instrument setting, transient length
- FT mode: absorption or magnitude
- Apodization window: none, full (Kaiser), half (semi Kaiser)
- Number of zero fills: 0, 1, 2, or 3
- Sampling rate (digitization frequency): 1, 2, 4, 6 MHz, or any
- Noise (added to the transient): noise amplitude
- Decay rate: ion signal decay rate in a transient, $e^{-(\text{decay rate})}$
- Phase: initial phase (angle) of ion detection in a transient



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s): ☐ Enrich isotopes: Natural

○ Biopharma tool: Active: Intact

✓ Compound settings

Isotopic pattern engine: Default | profile

Ion mode / Ionization agent positive | **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ | 1

Resolution:

○ At target peak(s) 10000

● Instrument setting 25k@400m/z (T=0.192 s

○ Detection period, s 0.100

FT mode / apodization: magnitude | None

Zero-fills / sample rate: 2 | 4 MHz

Noise / decay / phase: 0.000 | 0.00 | 0.0

Compound list: select one or multiple items

0. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorp

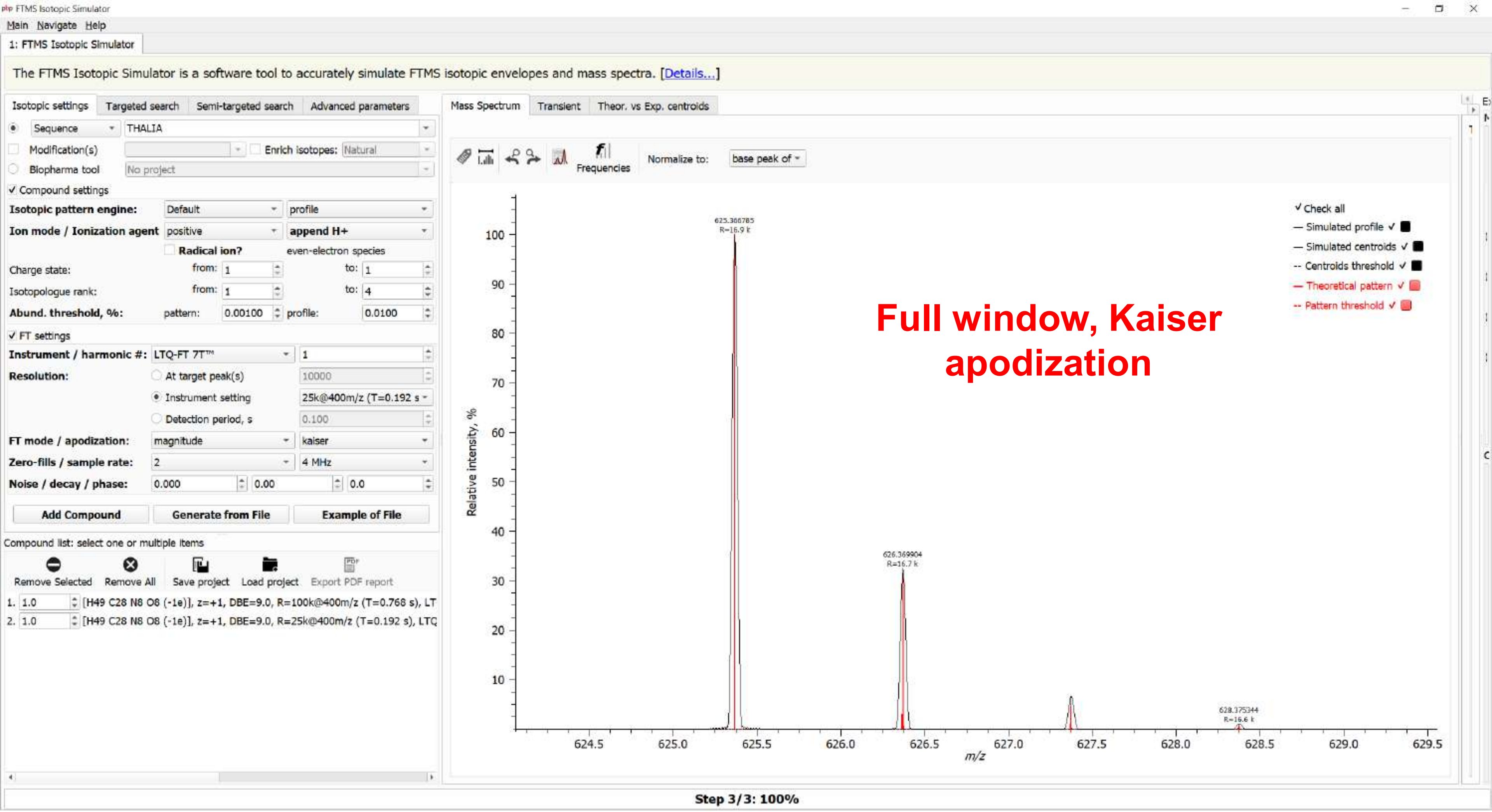
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%

$[THALIA + H]^+$

unapodized magnitude mode

7 T FT-ICR MS
192 ms transient



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

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☐ Modification(s) ☐ Enrich isotopes: Natural
○ Biopharma tool: No project
✓ Compound settings
Isotopic pattern engine: Default, profile
Ion mode / Ionization agent positive, append H+
☐ **Radical ion?** even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 4
Abund. threshold, %: pattern: 0.00100 profile: 0.0100
✓ FT settings
Instrument / harmonic #: LTQ-FT 7T™, 1
Resolution: ○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100
FT mode / apodization: magnitude, kaiser
Zero-fills / sample rate: 2, 4 MHz
Noise / decay / phase: 0.000, 0.00, 0.0

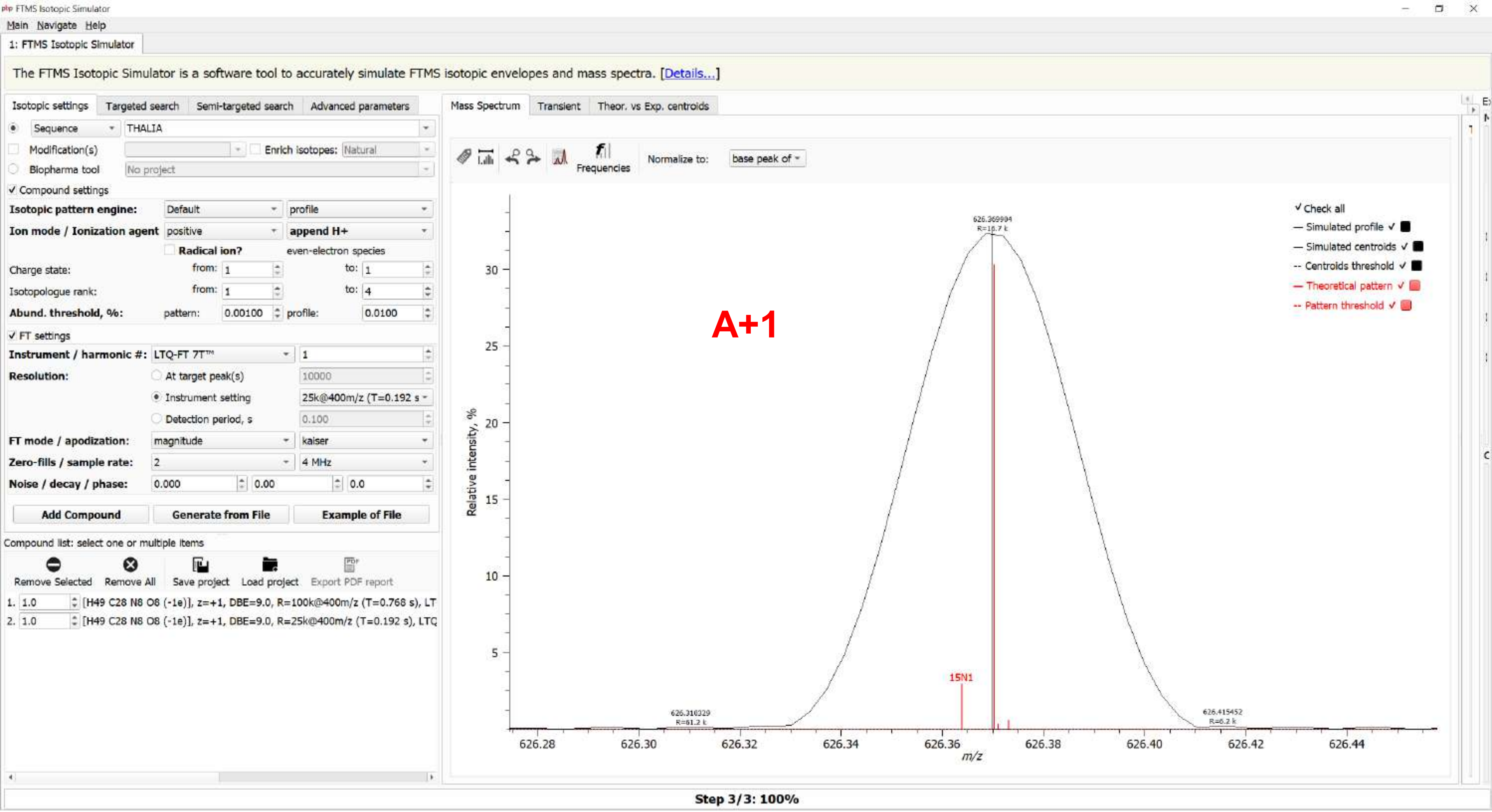
Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%

Full window, Kaiser apodization



A+1

FTMS Isotopic Simulator

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Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

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✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ / 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: magnitude / kaiser

Zero-fills / sample rate: 2 / 4 MHz

Noise / decay / phase: 0.000 / 0.00 / 0.0

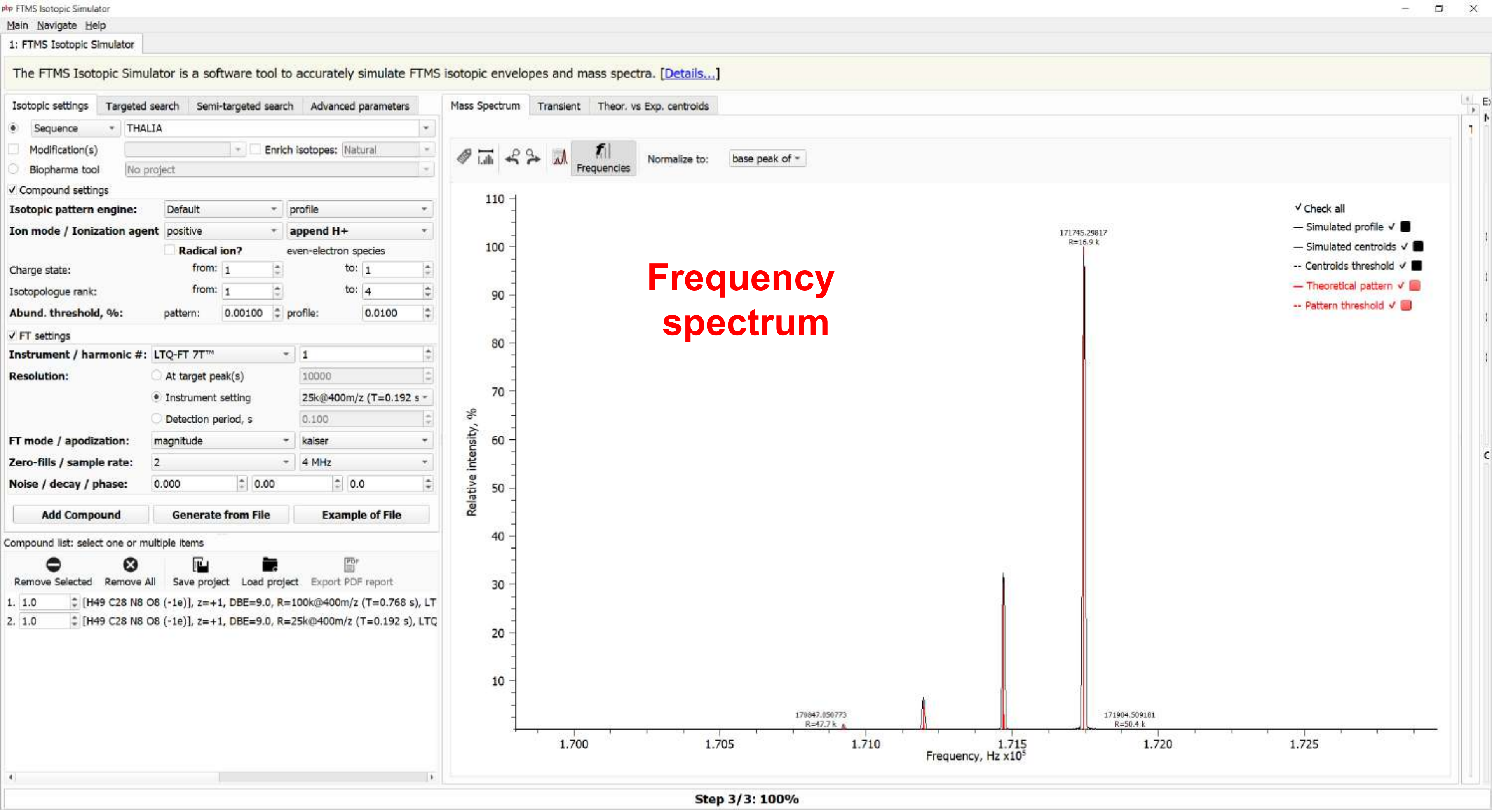
Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%

Frequency spectrum



FTMS Isotopic Simulator

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:

○ At target peak(s): 10000

● Instrument setting: 25k@400m/z (T=0.192 s

○ Detection period, s: 0.100

FT mode / apodization: magnitude, kaiser

Zero-fills / sample rate: 2, 4 MHz

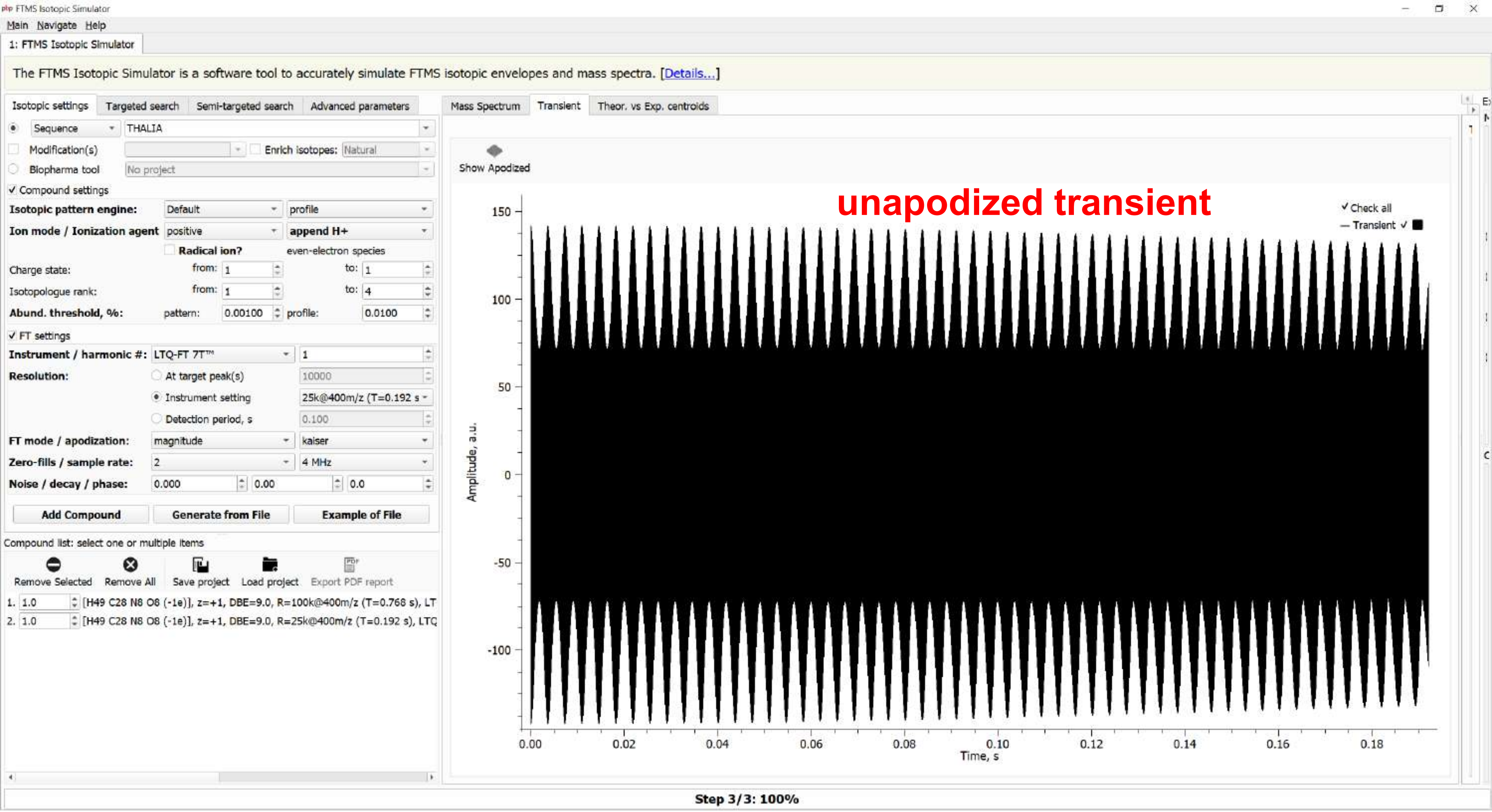
Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%



unapodized transient

FTMS Isotopic Simulator

Main Navigate Help

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

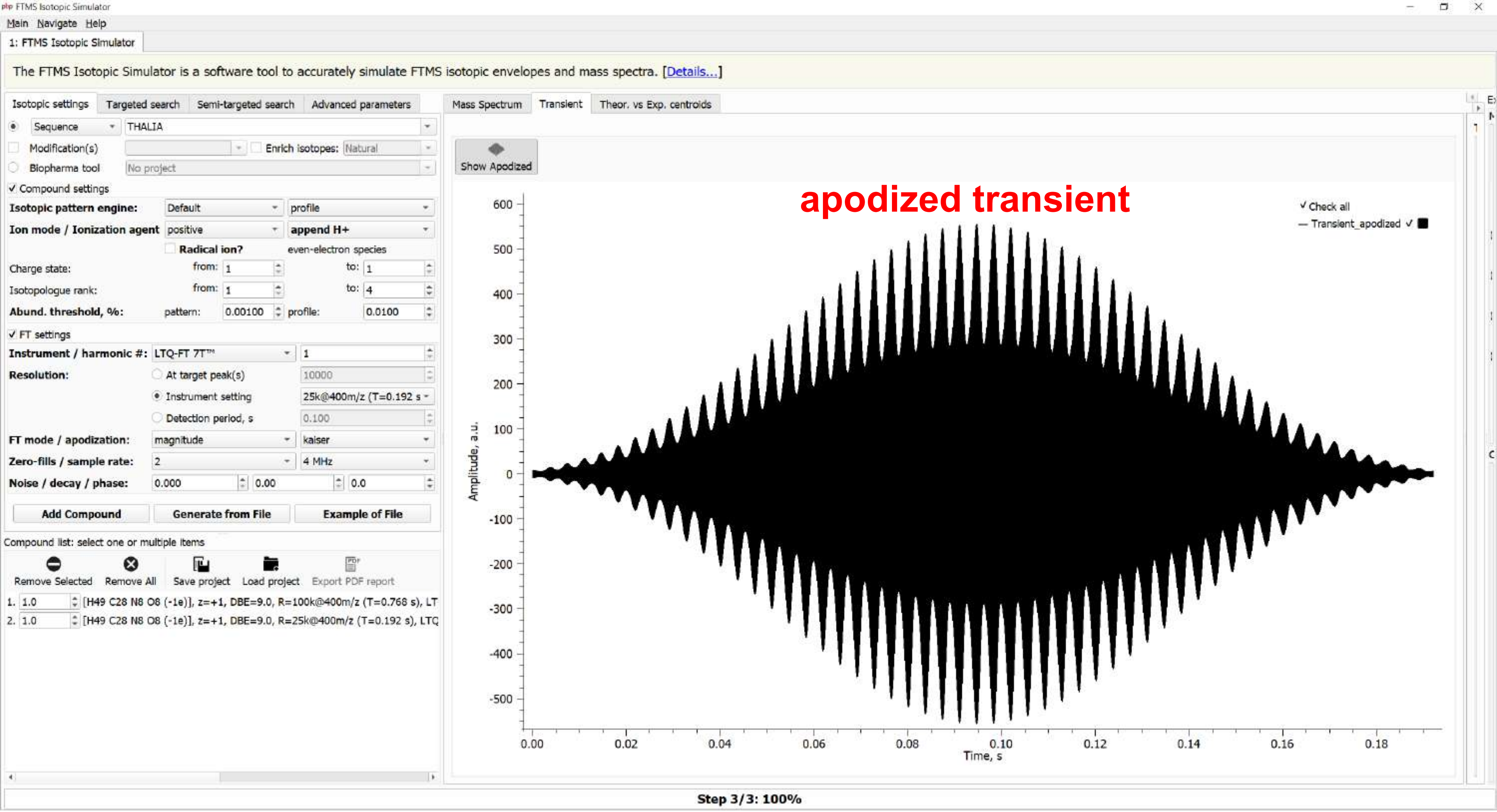
Isotopic settings | Targeted search | Semi-targeted search | Advanced parameters

● Sequence: THALIA
☐ Modification(s) ☐ Enrich isotopes: Natural
○ Biopharma tool: No project
☑ Compound settings
Isotopic pattern engine: Default | profile
Ion mode / Ionization agent positive | append H+
☐ **Radical ion?** even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 4
Abund. threshold, %: pattern: 0.00100 profile: 0.0100
☑ FT settings
Instrument / harmonic #: LTQ-FT 7T™ | 1
Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100
FT mode / apodization: magnitude | kaiser
Zero-fills / sample rate: 2 | 4 MHz
Noise / decay / phase: 0.000 | 0.00 | 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



apodized transient

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

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○ Biopharma tool: No project

☑ Compound settings
Isotopic pattern engine: Default / profile
Ion mode / Ionization agent: positive / append H+
☐ **Radical ion?** even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 4
Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings
Instrument / harmonic #: LTQ-FT 7T™ / 1
Resolution:
○ At target peak(s): 10000
● Instrument setting: 25k@400m/z (T=0.192 s
○ Detection period, s: 0.100
FT mode / apodization: magnitude / kaiser
Zero-fills / sample rate: 2 / 4 MHz
Noise / decay / phase: 0.000 / 0.00 / 0.0

Compound list: select one or multiple items

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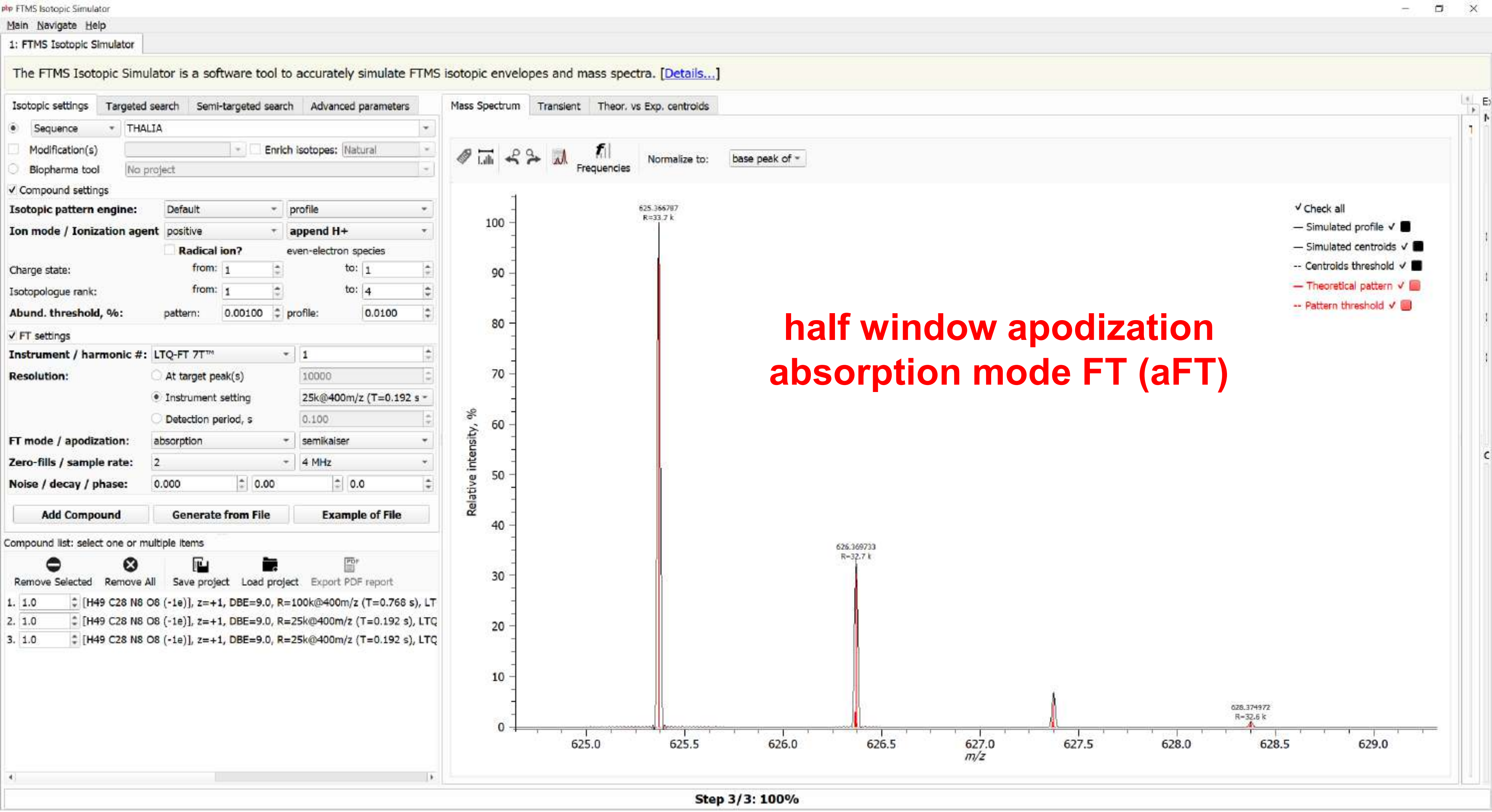
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

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☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default / profile

Ion mode / Ionization agent positive / append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption / semikaiser

Zero-fills / sample rate: 2 / 4 MHz

Noise / decay / phase: 0.000 / 0.00 / 0.0

Compound list: select one or multiple items

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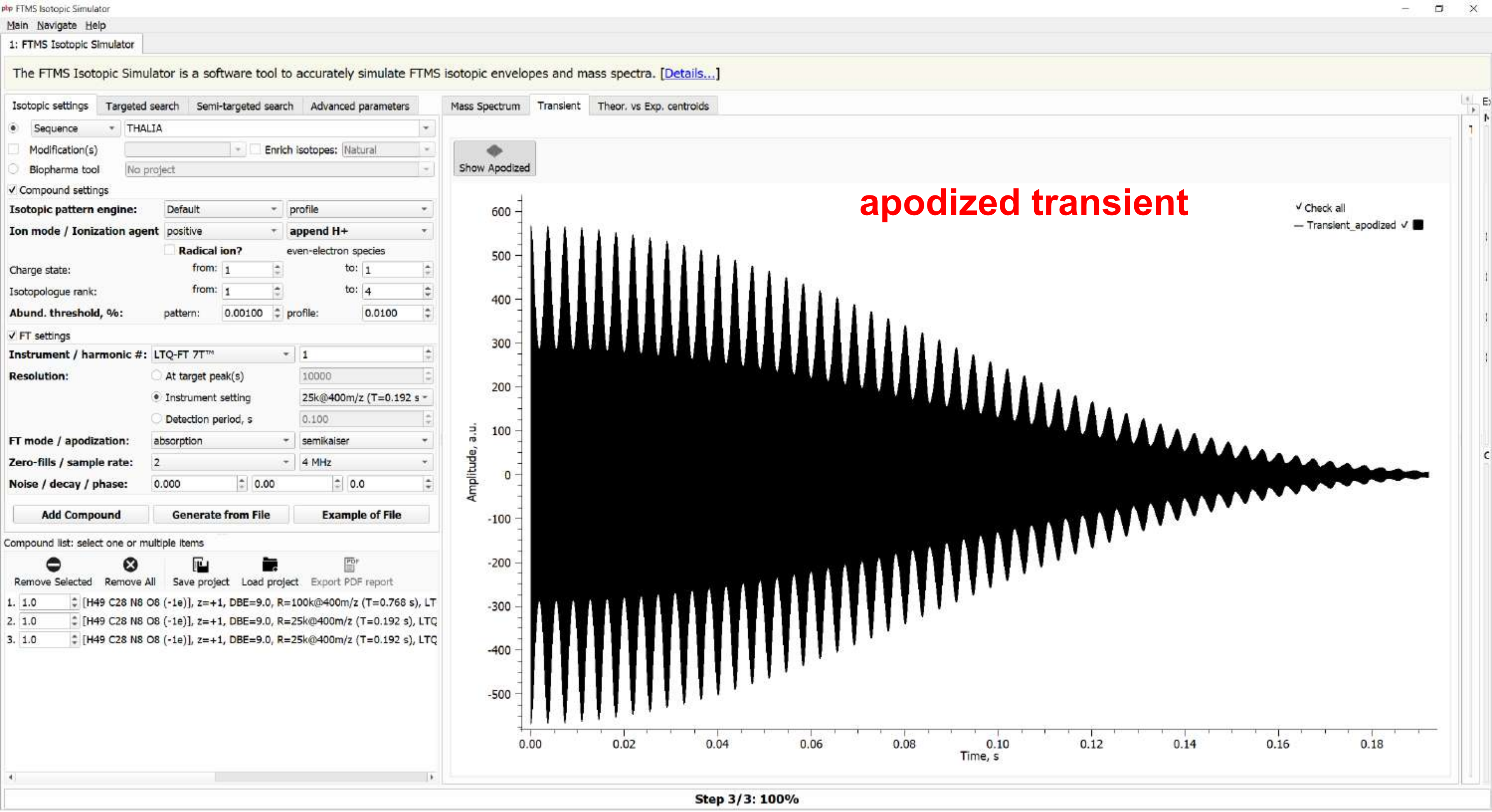
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

half window apodization
absorption mode FT (aFT)

Step 3/3: 100%



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s): ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

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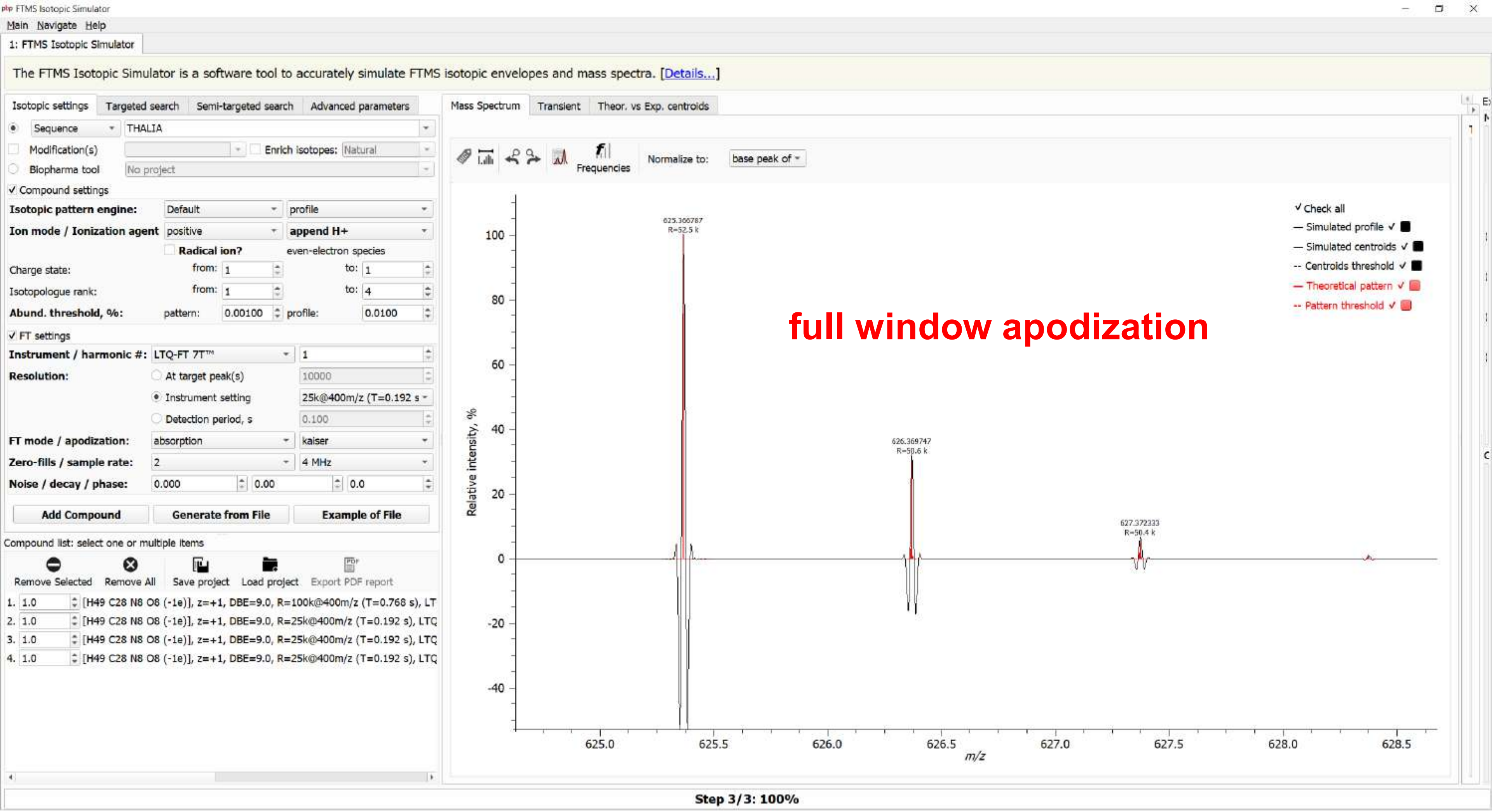
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%

apodized transient



full window apodization

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☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:
- ○ At target peak(s): 10000
- ● Instrument setting: 25k@400m/z (T=0.192 s
- ○ Detection period, s: 0.100

FT mode / apodization: absorption, kaiser

Zero-fills / sample rate: 2, 4 MHz

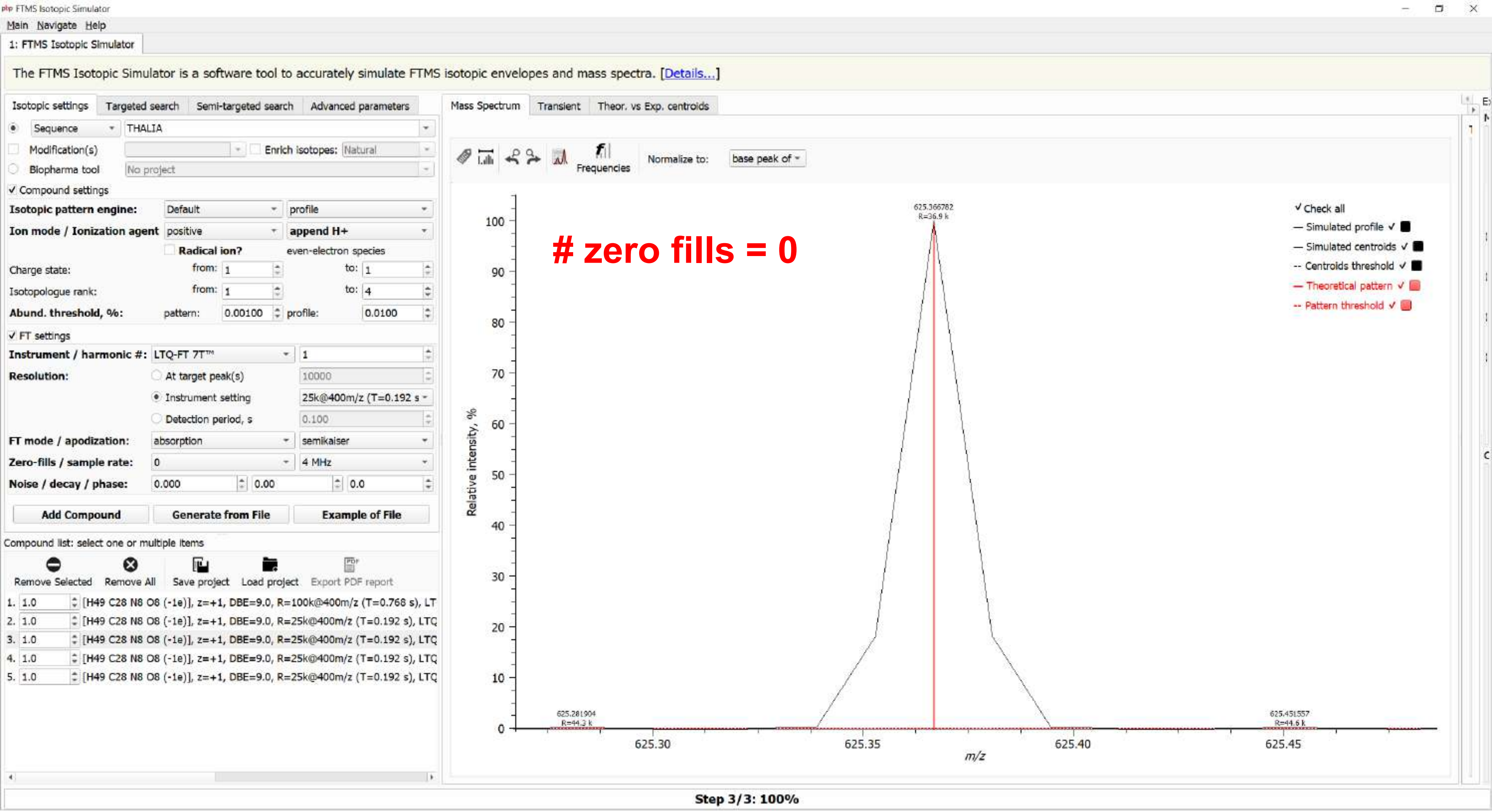
Noise / decay / phase: 0.000, 0.00, 0.0

Normalize to: base peak of

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



zero fills = 0

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA
☐ Modification(s)   ☐ Enrich isotopes: Natural
○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default / profile
Ion mode / Ionization agent positive / append H+
☐ **Radical ion?** even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 4
Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

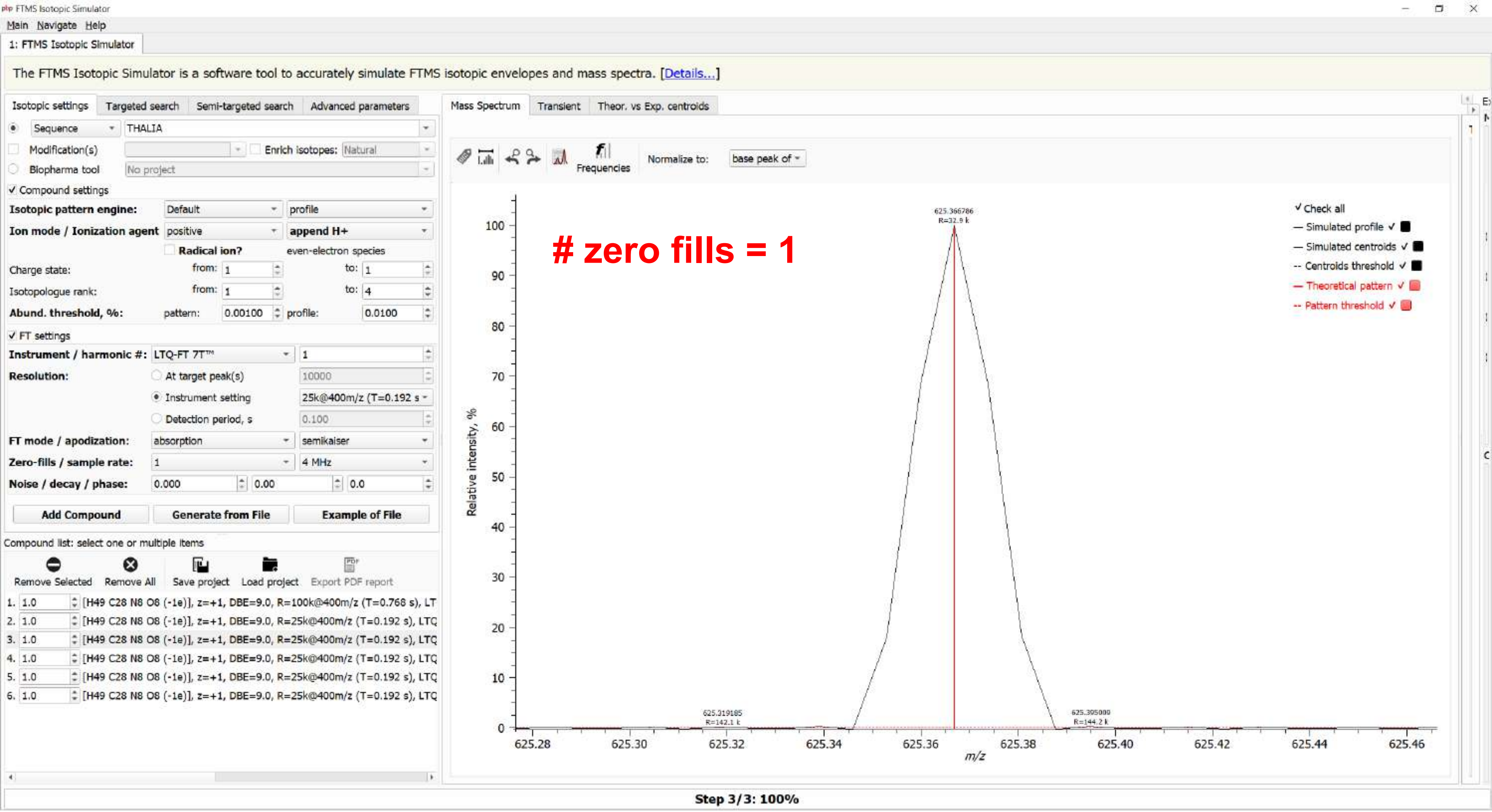
Instrument / harmonic #: LTQ-FT 7T™ / 1
Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100
FT mode / apodization: absorption / semikaiser
Zero-fills / sample rate: 0 / 4 MHz
Noise / decay / phase: 0.000 / 0.00 / 0.0

Normalize to: base peak of

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
5. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



 # zero fills = 1

FTMS Isotopic Simulator

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 1, 4 MHz

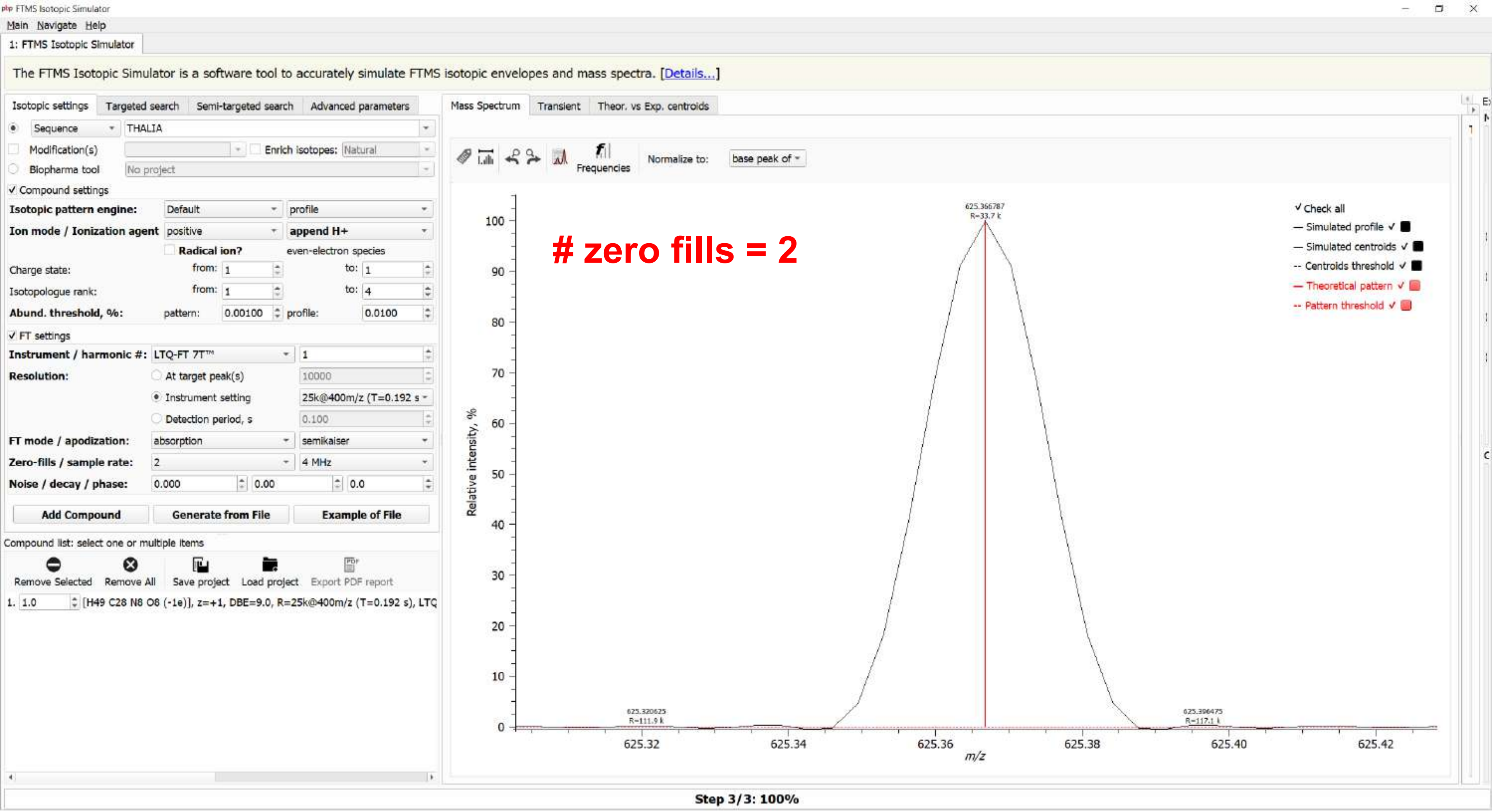
Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=100k@400m/z (T=0.768 s), LT
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
5. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
6. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%



zero fills = 2

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default / profile

Ion mode / Ionization agent positive / append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ / 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption / semikaiser

Zero-fills / sample rate: 2 / 4 MHz

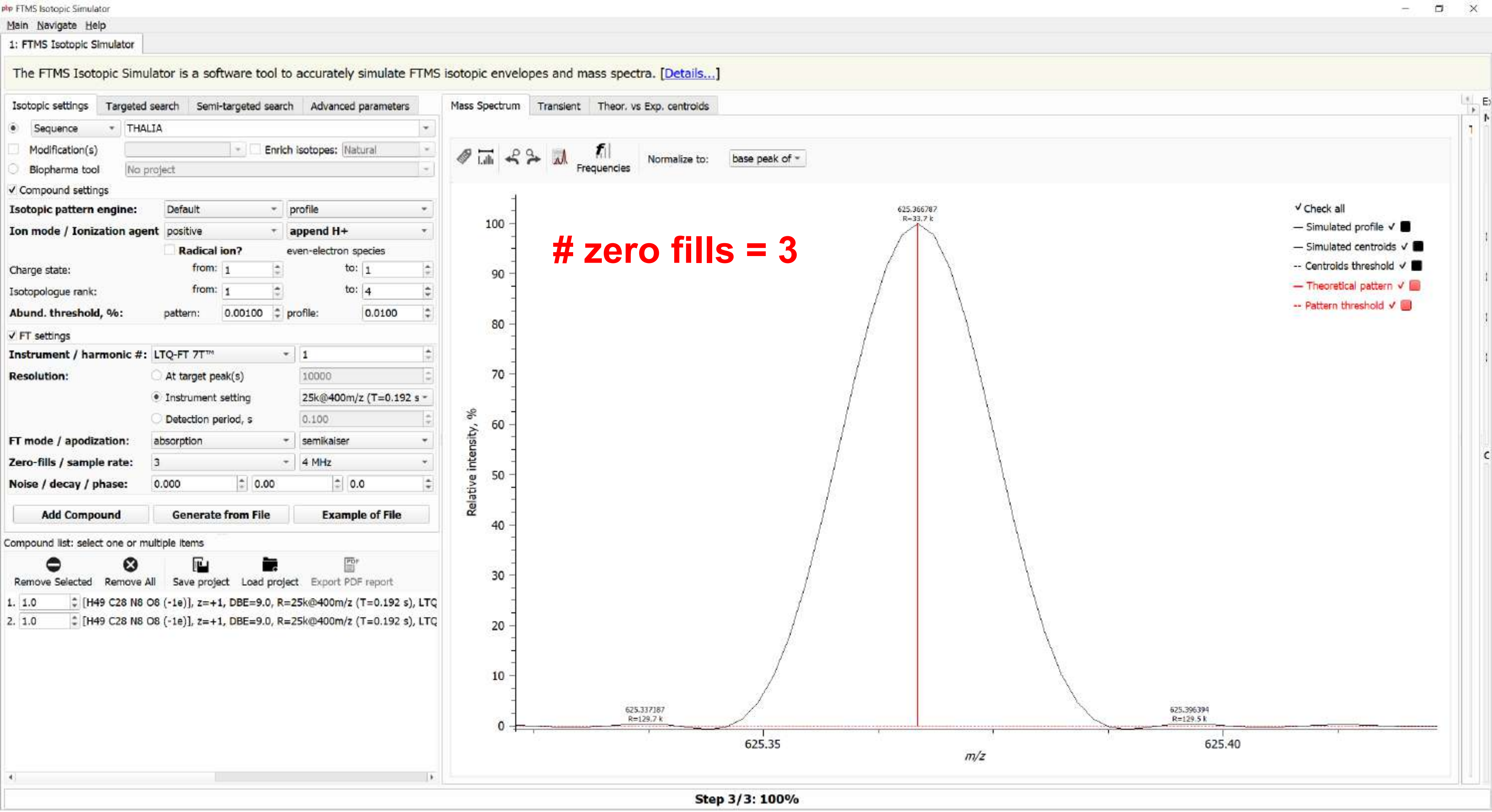
Noise / decay / phase: 0.000 / 0.00 / 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA
☐ Modification(s) ☐ Enrich isotopes: Natural
○ Biopharma tool: No project
✓ Compound settings
Isotopic pattern engine: Default, profile
Ion mode / Ionization agent positive, append H+
☐ **Radical ion?** even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 4
Abund. threshold, %: pattern: 0.00100 profile: 0.0100
✓ FT settings
Instrument / harmonic #: LTQ-FT 7T™ 1
Resolution: ○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100
FT mode / apodization: absorption semikaiser
Zero-fills / sample rate: 3 4 MHz
Noise / decay / phase: 0.000 0.00 0.0

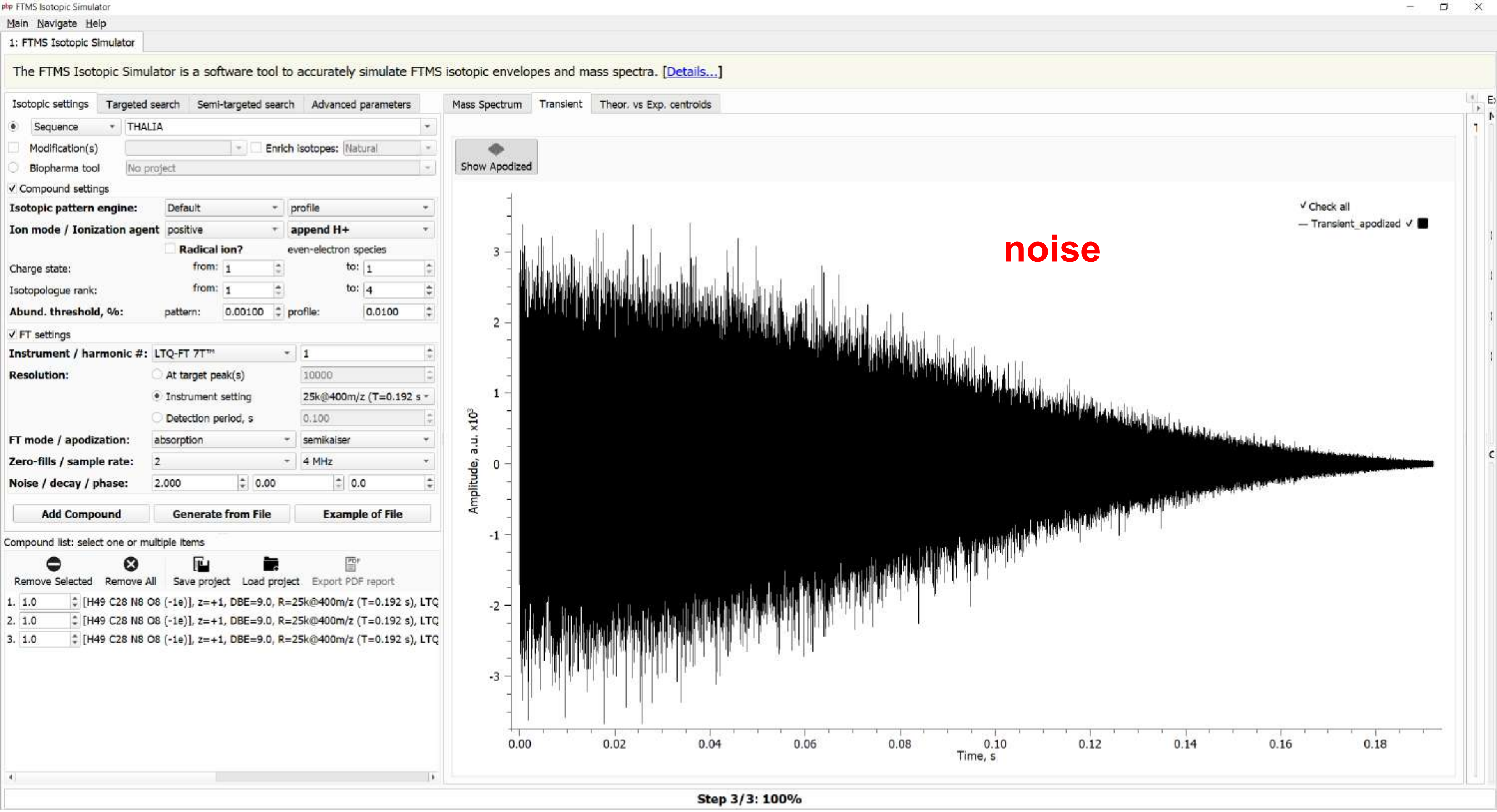
Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

Step 3/3: 100%

zero fills = 3



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

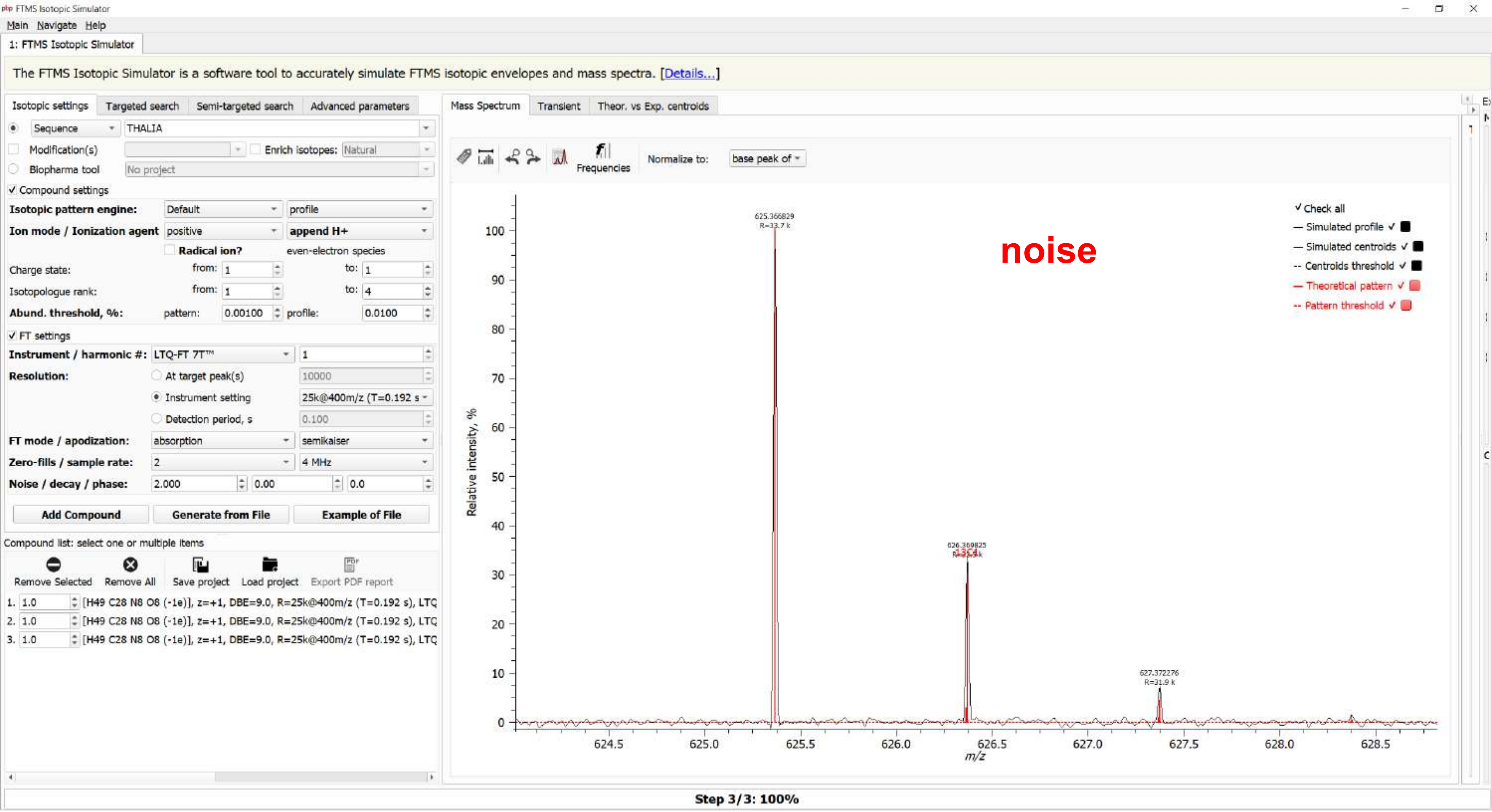
Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 2.000, 0.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



noise

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:

○ At target peak(s) 10000

● Instrument setting 25k@400m/z (T=0.192 s

○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

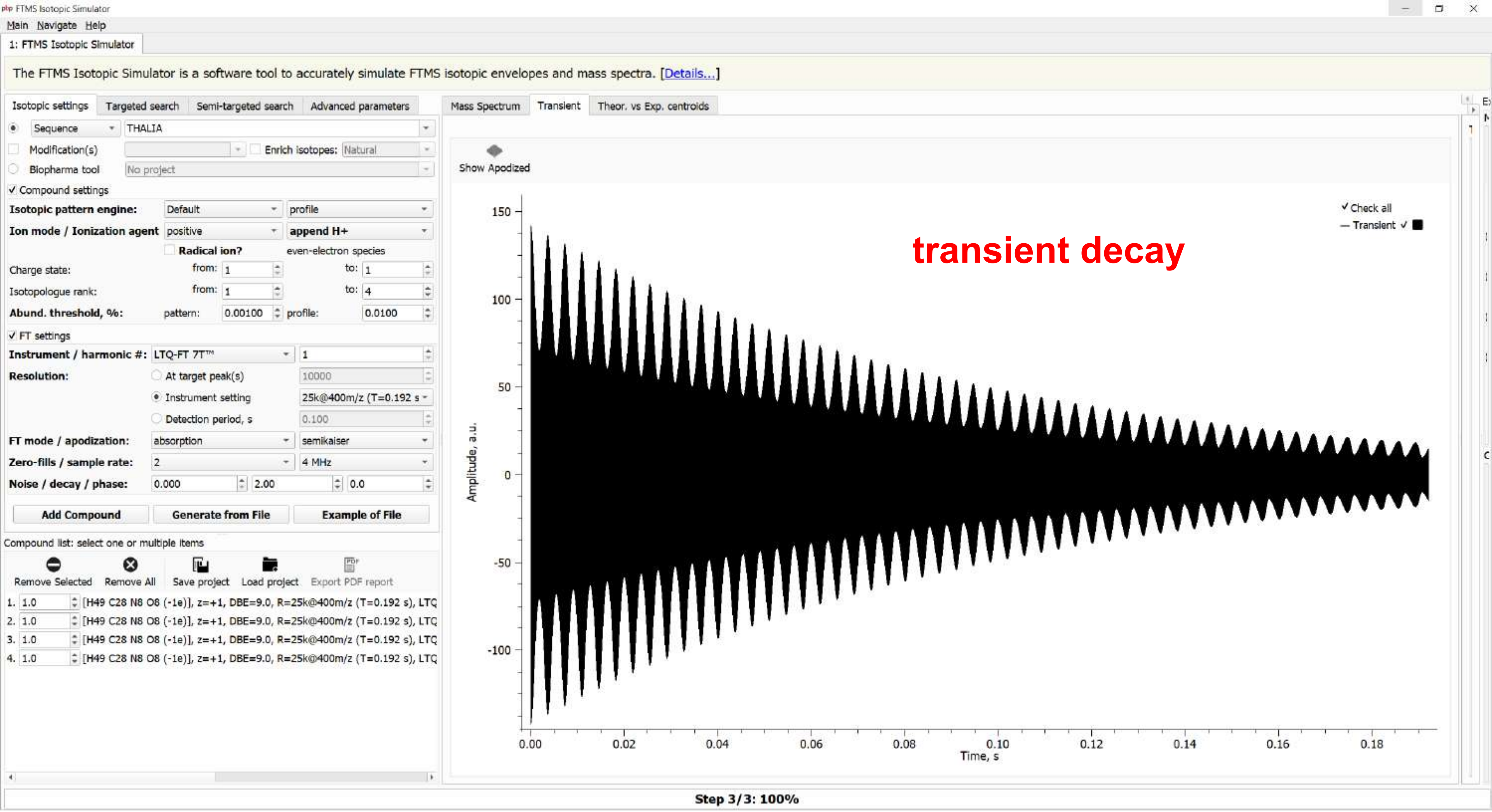
Noise / decay / phase: 2.000, 0.00, 0.0

Normalize to: base peak of

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:

○ At target peak(s) 10000

● Instrument setting 25k@400m/z (T=0.192 s

○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

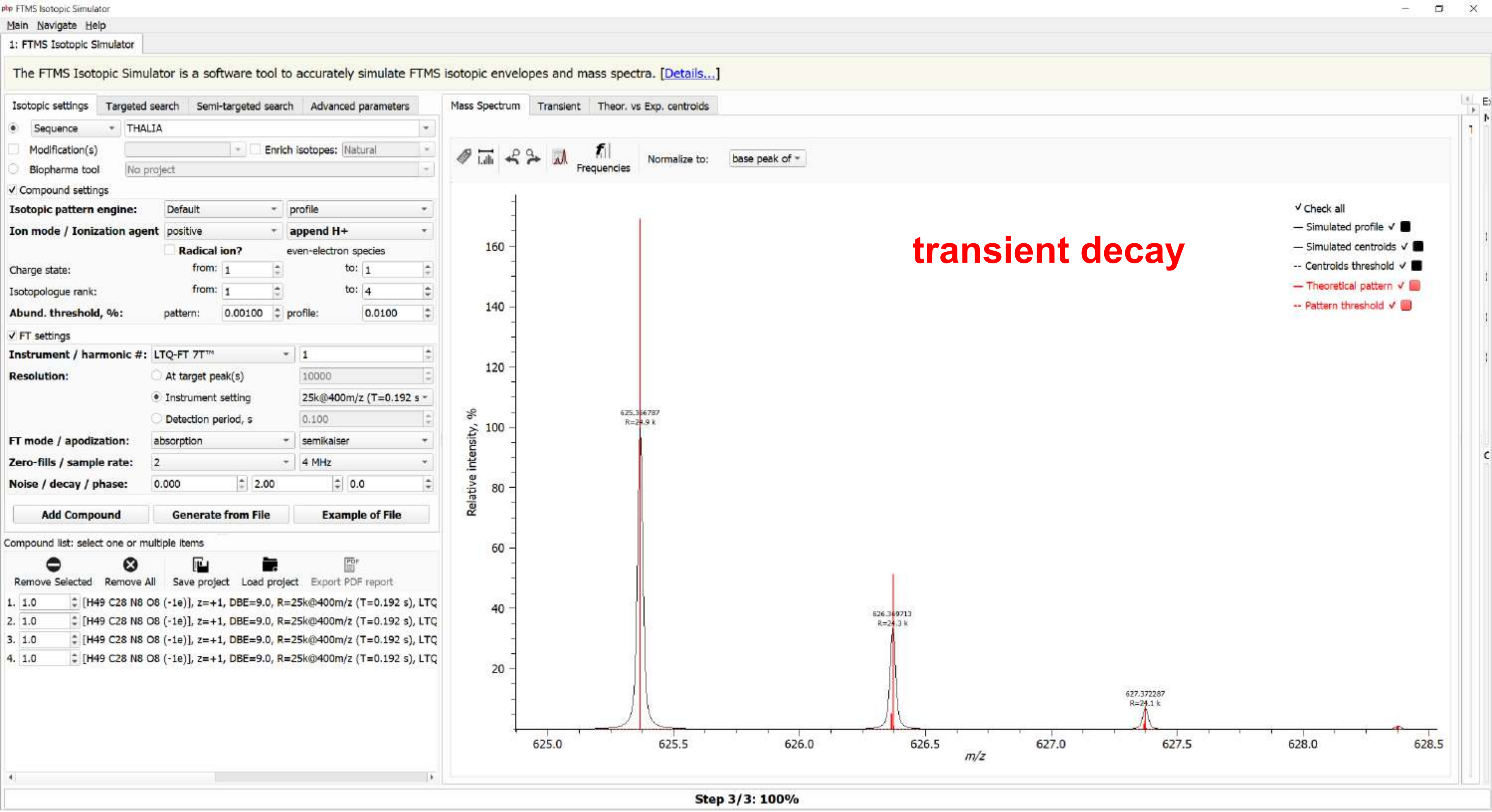
Noise / decay / phase: 0.000, 2.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%

transient decay



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

✓ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

✓ FT settings

Instrument / harmonic #: LTQ-FT 7T™ 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 0.000, 2.00, 0.0

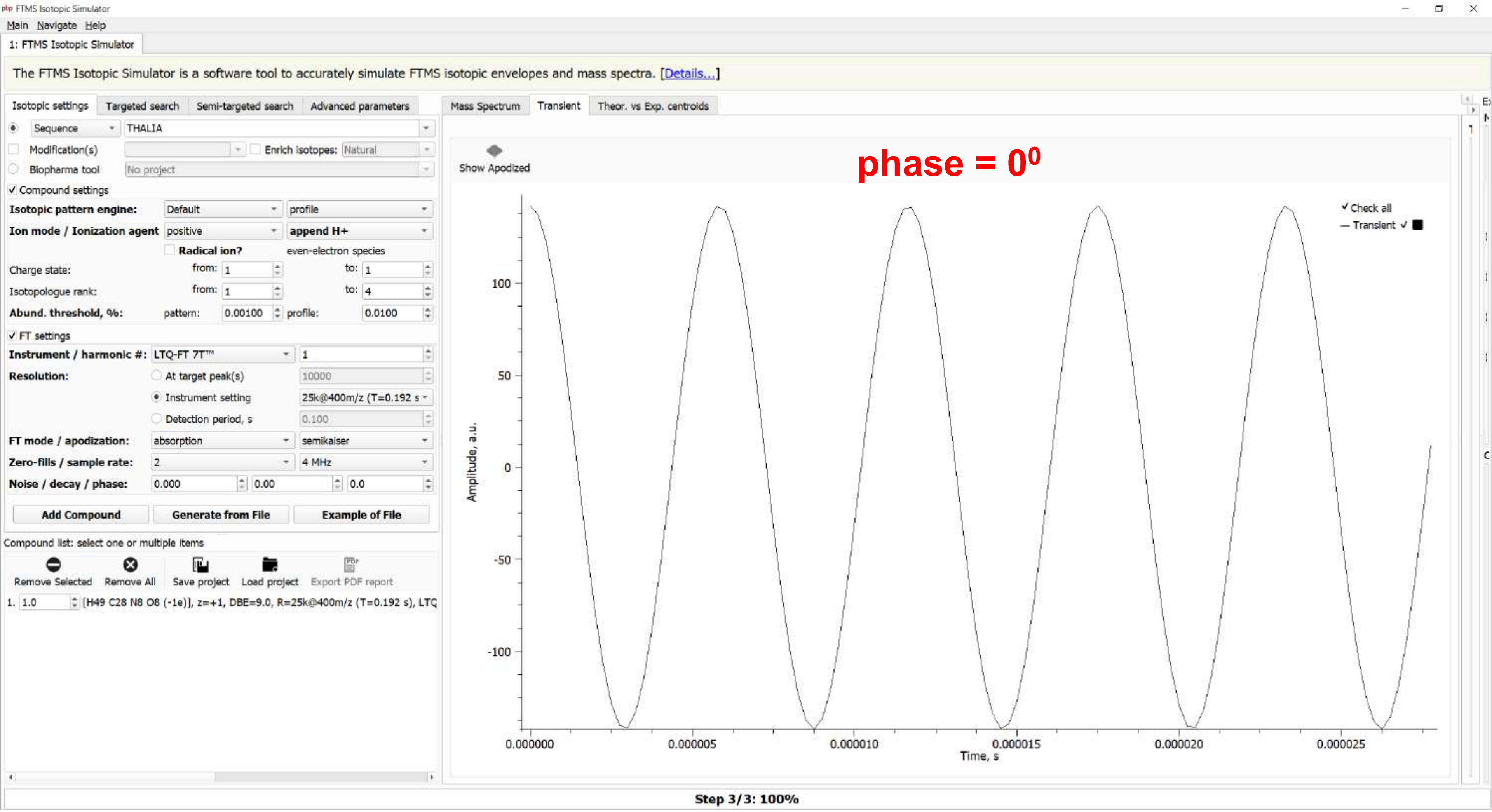
Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Normalize to: base peak of

transient decay

Step 3/3: 100%



phase = 0^0

FTMS Isotopic Simulator

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:

○ At target peak(s) 10000

● Instrument setting 25k@400m/z (T=0.192 s

○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

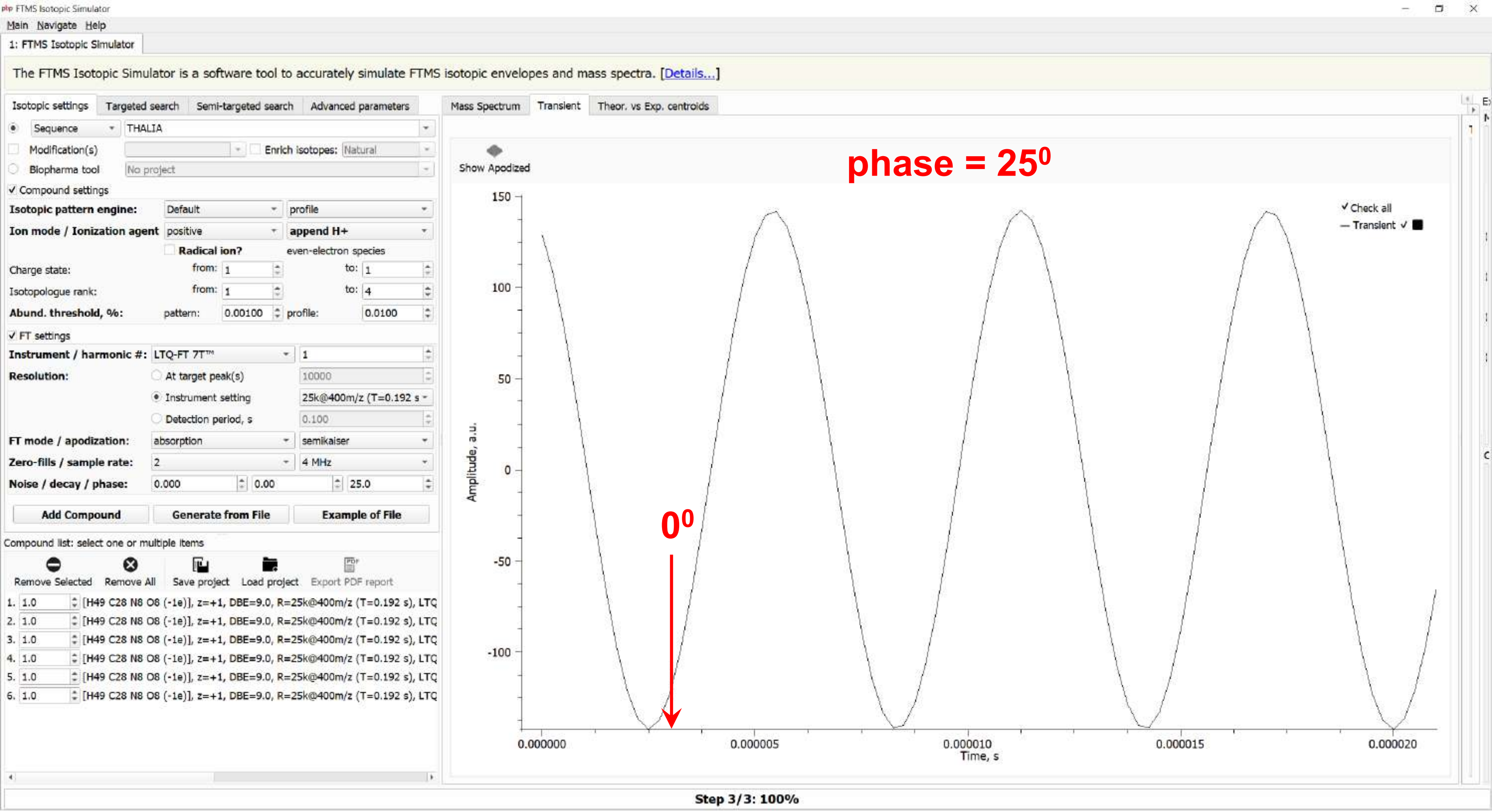
Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



FTMS Isotopic Simulator

Main Navigate Help

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

Isotopic settings | Targeted search | Semi-targeted search | Advanced parameters

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:
○ At target peak(s): 10000
● Instrument setting: 25k@400m/z (T=0.192 s
○ Detection period, s: 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 0.000, 0.00, 25.0

Add Compound | Generate from File | Example of File

Compound list: select one or multiple items

Remove Selected | Remove All | Save project | Load project | Export PDF report

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
5. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
6. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Mass Spectrum | Transient | Theor. vs Exp. centroids

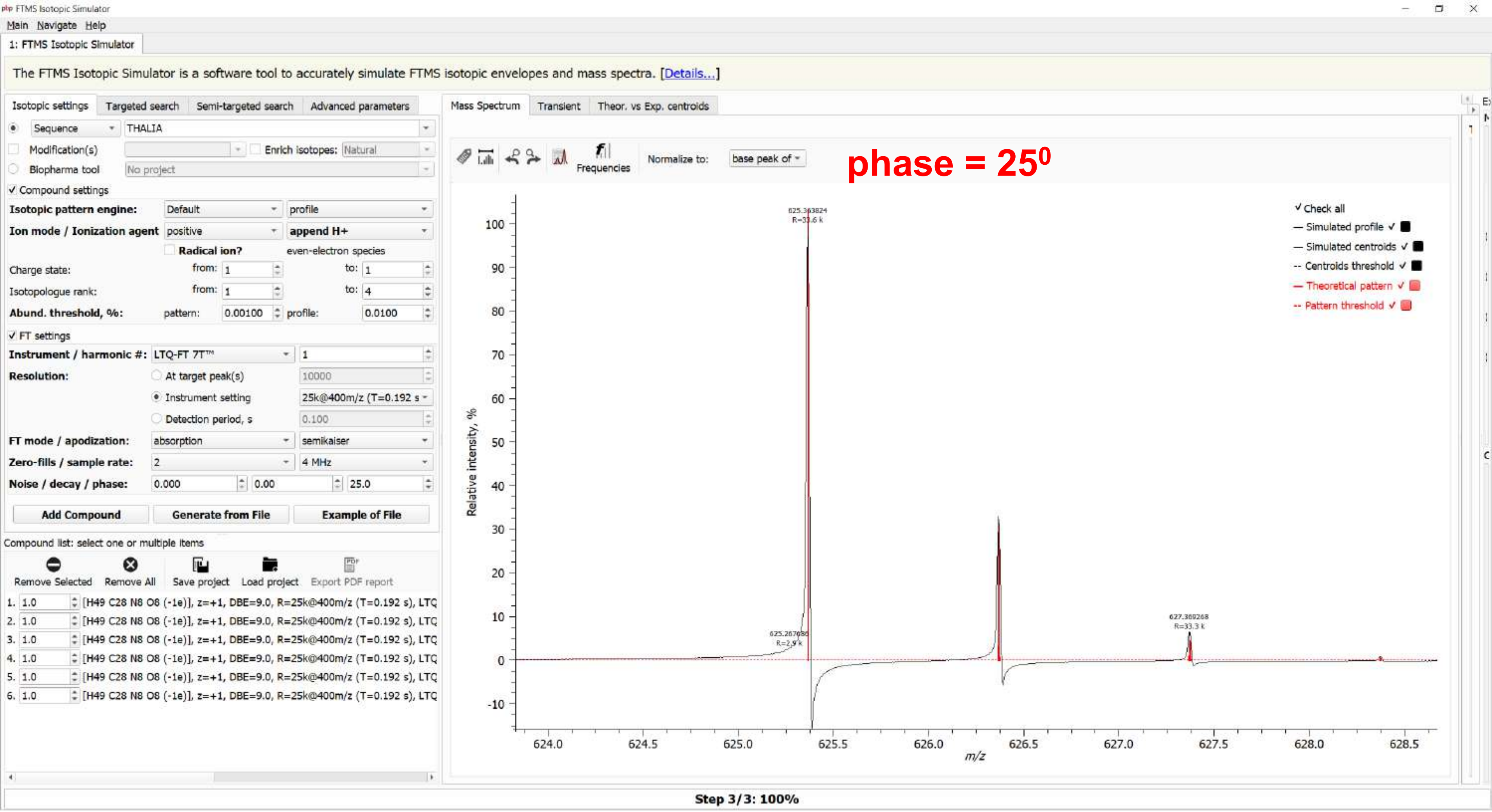
Show Apodized

phase = 25^0

0^0

Step 3/3: 100%

phase = 25^0



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent: positive, **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 25k@400m/z (T=0.192 s
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 4 MHz

Noise / decay / phase: 0.000, 0.00, 25.0

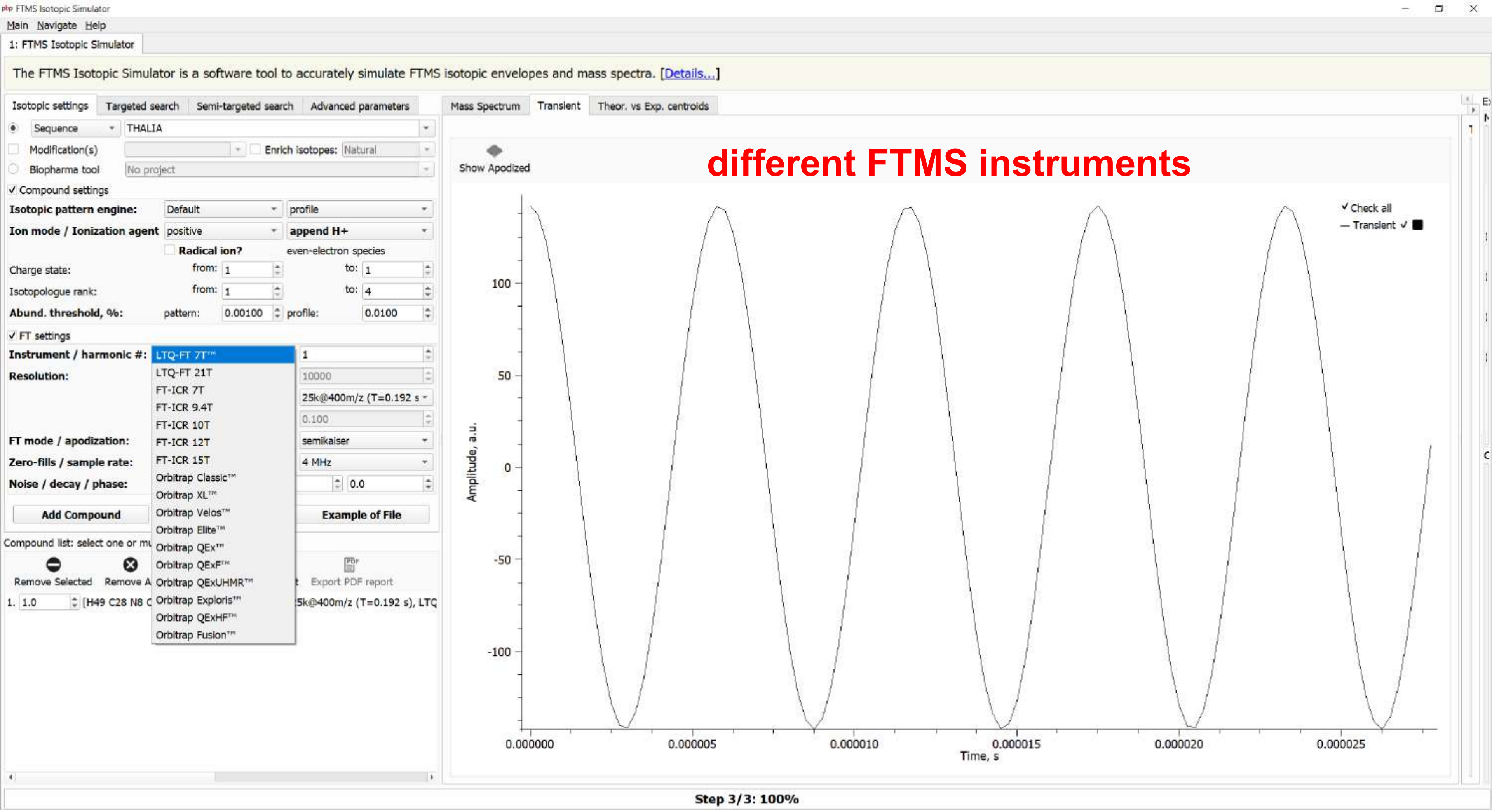
Normalize to: base peak of

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
2. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
3. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
4. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
5. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
6. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%

different FTMS instruments



FTMS Isotopic Simulator

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

● Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine: Default, profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 1 to: 1

Isotopologue rank: from: 1 to: 4

Abund. threshold, %: pattern: 0.00100 profile: 0.0100

☑ FT settings

Instrument / harmonic #: LTQ-FT 7T™, 1

Resolution: 10000, 25k@400m/z (T=0.192 s, 0.100

FT mode / apodization: semikaiser

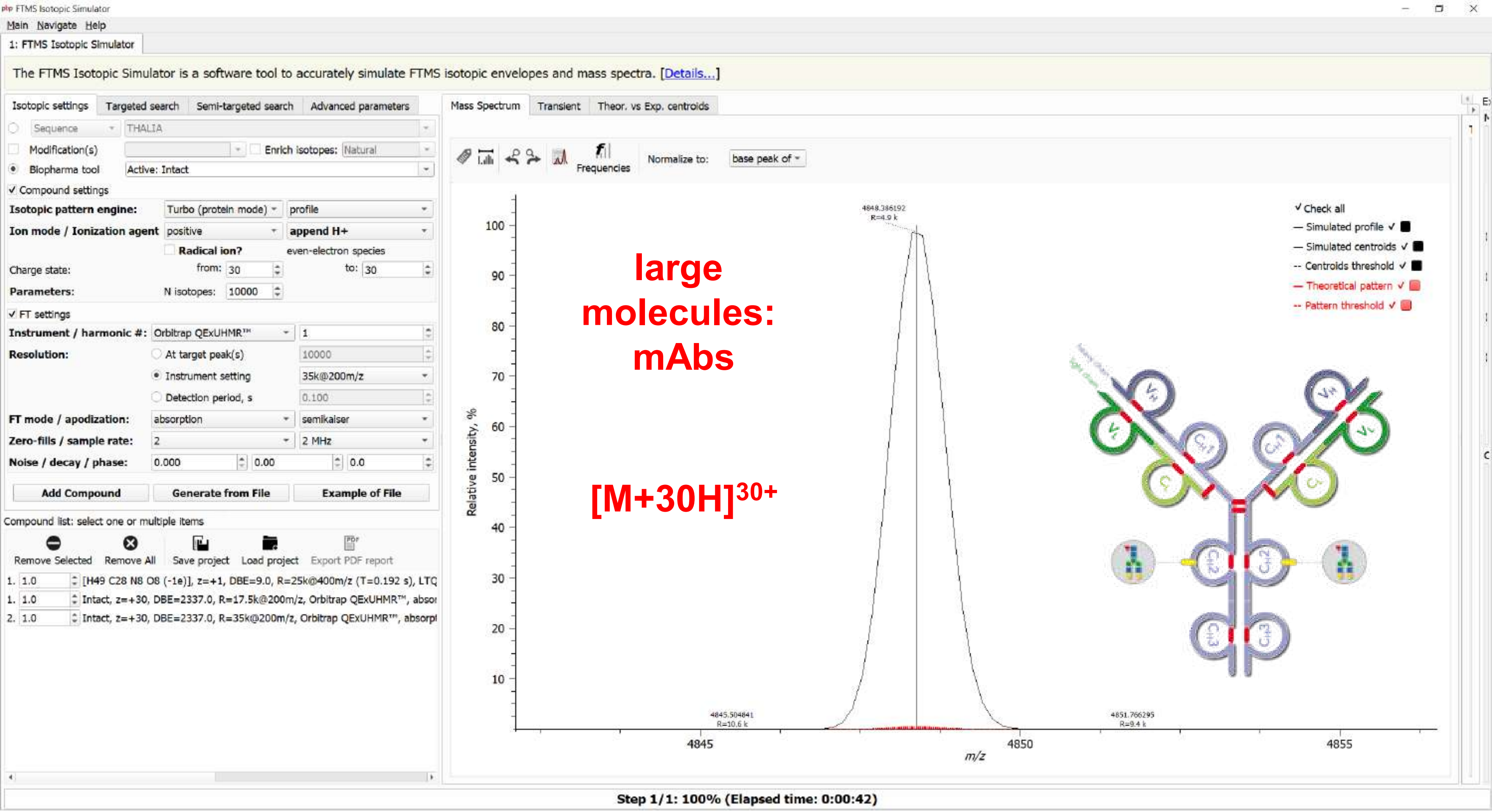
Zero-fills / sample rate: 4 MHz

Noise / decay / phase: 0.0

Compound list: select one or mu

1. 1.0 [H49 C28 N8 C … 5k@400m/z (T=0.192 s), LTQ

Step 3/3: 100%



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

○ Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

● Biopharma tool: Active: Intact

✓ Compound settings

Isotopic pattern engine: Turbo (protein mode) | profile

Ion mode / Ionization agent positive | append H+

☐ **Radical ion?** even-electron species

Charge state: from: 30 to: 30

Parameters: N isotopes: 10000

✓ FT settings

Instrument / harmonic #: Orbitrap QExUHMR™ | 1

Resolution:

○ At target peak(s): 10000

● Instrument setting: 35k@200m/z

○ Detection period, s: 0.100

FT mode / apodization: absorption | semikaiser

Zero-fills / sample rate: 2 | 2 MHz

Noise / decay / phase: 0.000 | 0.00 | 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
1. 1.0 Intact, z=+30, DBE=2337.0, R=17.5k@200m/z, Orbitrap QExUHMR™, absor
2. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

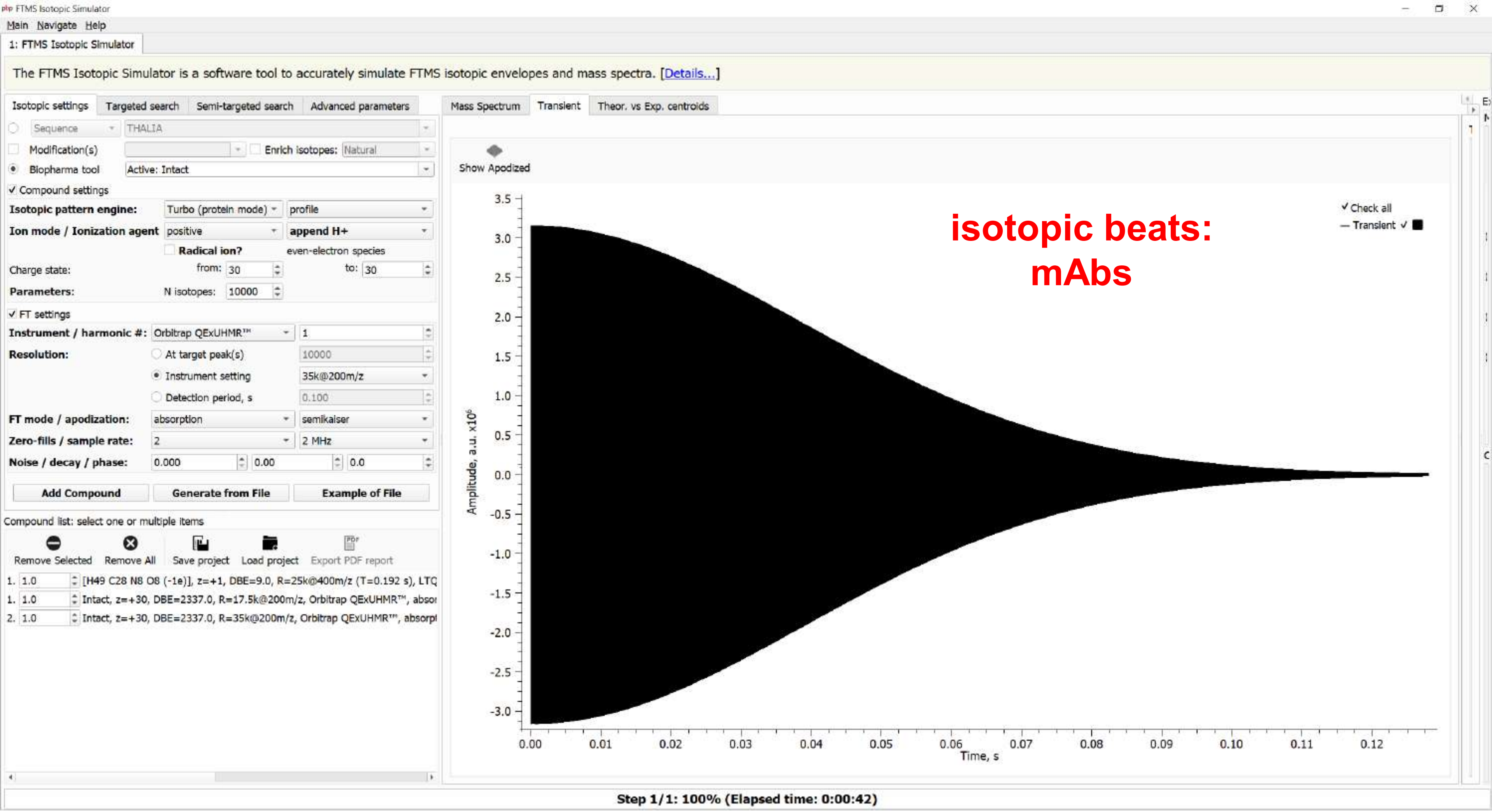
Normalize to: base peak of

large molecules: mAbs

$[M+30H]^{30+}$

Step 1/1: 100% (Elapsed time: 0:00:42)

isotopic beats: mAbs



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

○ Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

● Biopharma tool: Active: Intact

✓ Compound settings

Isotopic pattern engine: Turbo (protein mode), profile

Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 30 to: 30

Parameters: N isotopes: 10000

✓ FT settings

Instrument / harmonic #: Orbitrap QExUHMR™, 1

Resolution:

○ At target peak(s): 10000

● Instrument setting: 35k@200m/z

○ Detection period, s: 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 2 MHz

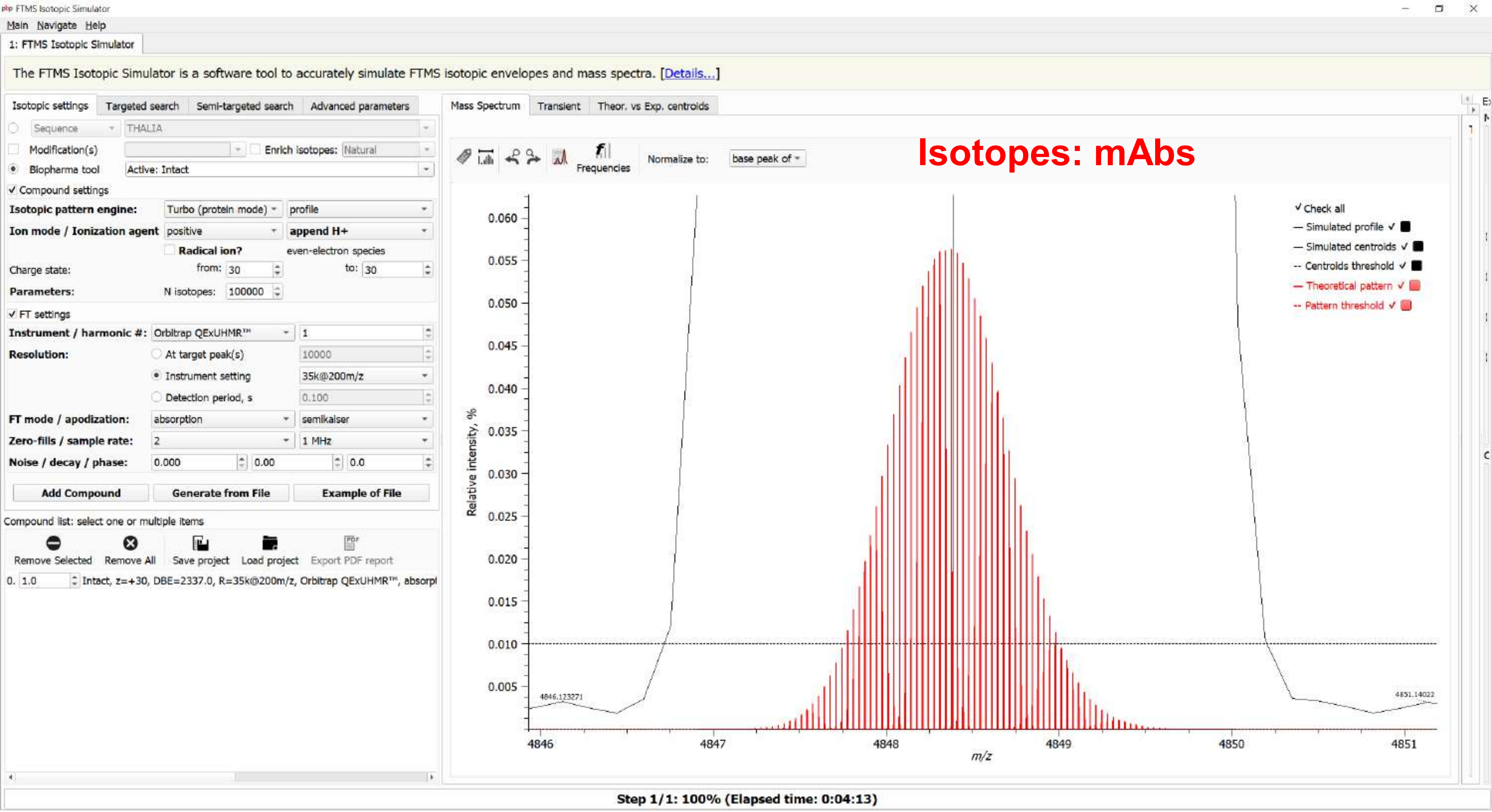
Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ
1. 1.0 Intact, z=+30, DBE=2337.0, R=17.5k@200m/z, Orbitrap QExUHMR™, absor
2. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

Step 1/1: 100% (Elapsed time: 0:00:42)

Isotopes: mAbs



FTMS Isotopic Simulator

Main Navigate Help

1: FTMS Isotopic Simulator

The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

Isotopic settings | Targeted search | Semi-targeted search | Advanced parameters

○ Sequence THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

● Biopharma tool Active: Intact

☑ Compound settings

Isotopic pattern engine: Turbo (protein mode) profile

Ion mode / Ionization agent positive **append H+**

☐ **Radical ion?** even-electron species

Charge state: from: 30 to: 30

Parameters: N isotopes: 100000

☑ FT settings

Instrument / harmonic #: Orbitrap QExUHMR™ 1

Resolution:

○ At target peak(s) 10000

● Instrument setting 35k@200m/z

○ Detection period, s 0.100

FT mode / apodization: absorption semikaiser

Zero-fills / sample rate: 2 1 MHz

Noise / decay / phase: 0.000 0.00 0.0

Add Compound **Generate from File** **Example of File**

Compound list: select one or multiple items

Remove Selected Remove All Save project Load project Export PDF report

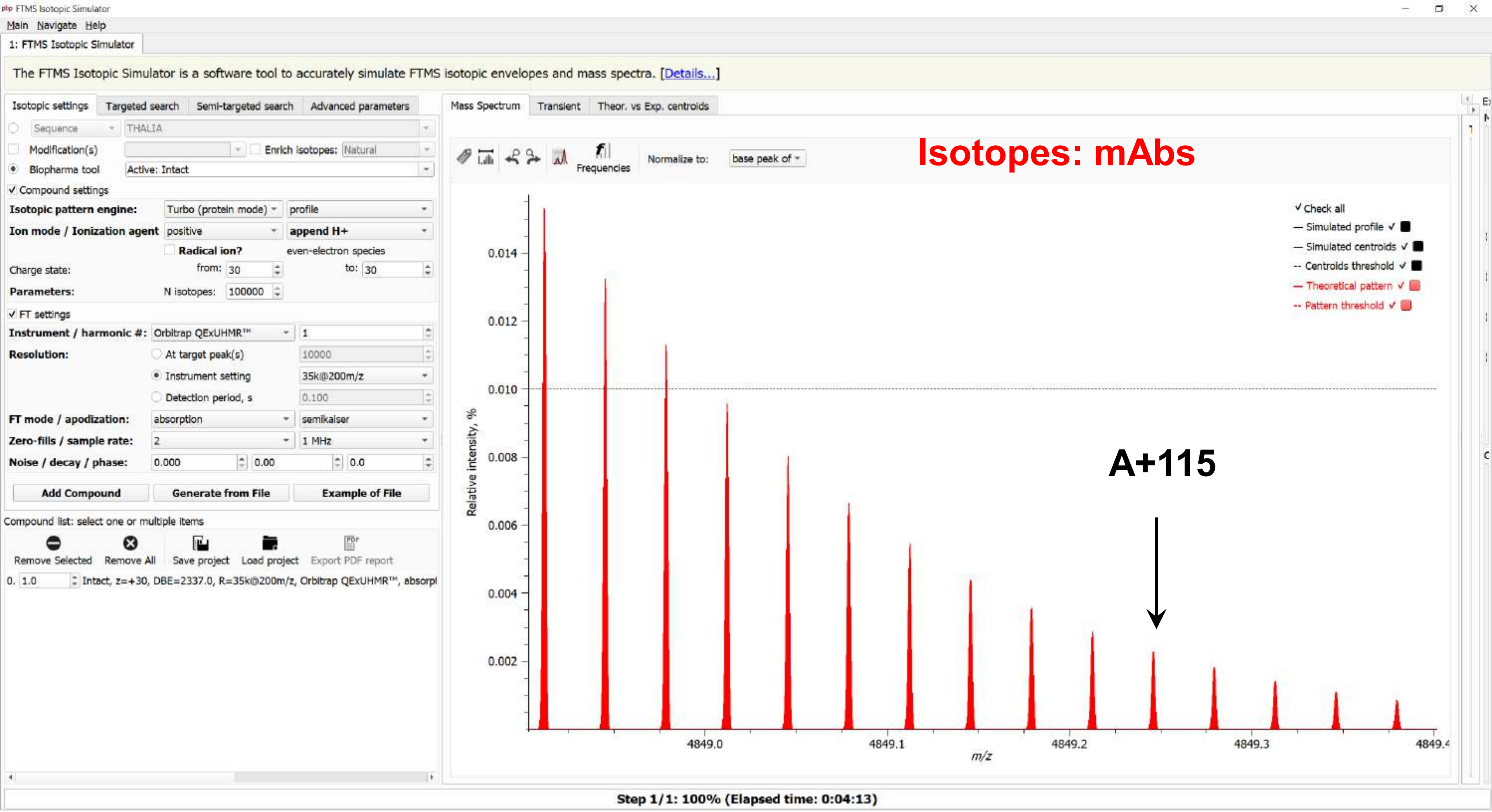
0. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

Mass Spectrum | Transient | Theor. vs Exp. centroids

Normalize to: base peak of

Step 1/1: 100% (Elapsed time: 0:04:13)

Isotopes: mAbs



FTMS Isotopic Simulator

1: FTMS Isotopic Simulator

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Isotopic settings | Targeted search | Semi-targeted search | Advanced parameters

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Ion mode / Ionization agent positive, append H+

☐ **Radical ion?** even-electron species

Charge state: from: 30 to: 30

Parameters: N isotopes: 100000

☑ FT settings

Instrument / harmonic #: Orbitrap QExUHMR™, 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 35k@200m/z
○ Detection period, s 0.100

FT mode / apodization: absorption, semikaiser

Zero-fills / sample rate: 2, 1 MHz

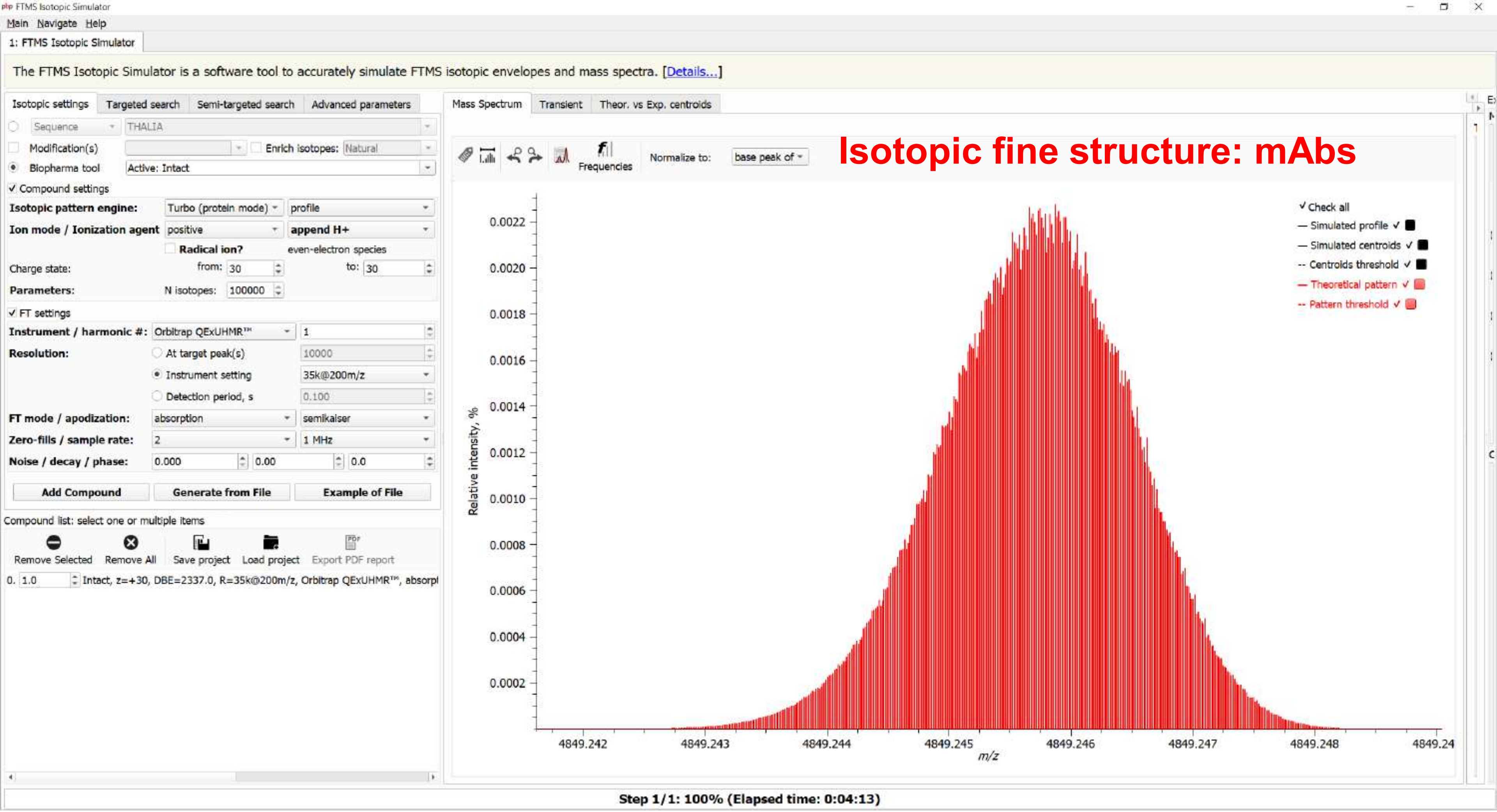
Noise / decay / phase: 0.000, 0.00, 0.0

Compound list: select one or multiple items

0. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

Step 1/1: 100% (Elapsed time: 0:04:13)

Isotopic fine structure: mAbs



The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

○ Sequence: THALIA

☐ Modification(s) ☐ Enrich isotopes: Natural

● Biopharma tool: Active: Intact

☑ Compound settings

Isotopic pattern engine: Turbo (protein mode) / profile

Ion mode / Ionization agent positive / append H+

☐ **Radical ion?** even-electron species

Charge state: from: 30 to: 30

Parameters: N isotopes: 100000

☑ FT settings

Instrument / harmonic #: Orbitrap QExUHMR™ / 1

Resolution:
○ At target peak(s) 10000
● Instrument setting 35k@200m/z
○ Detection period, s 0.100

FT mode / apodization: absorption / semikaiser

Zero-fills / sample rate: 2 / 1 MHz

Noise / decay / phase: 0.000 / 0.00 / 0.0

Compound list: select one or multiple items

0. 1.0 Intact, z=+30, DBE=2337.0, R=35k@200m/z, Orbitrap QExUHMR™, absorpt

Normalize to: base peak of

Step 1/1: 100% (Elapsed time: 0:04:13)

How & Why to Simulate the FTMS Data?

- Approaches to simulate the FTMS isotopic envelopes and mass spectra
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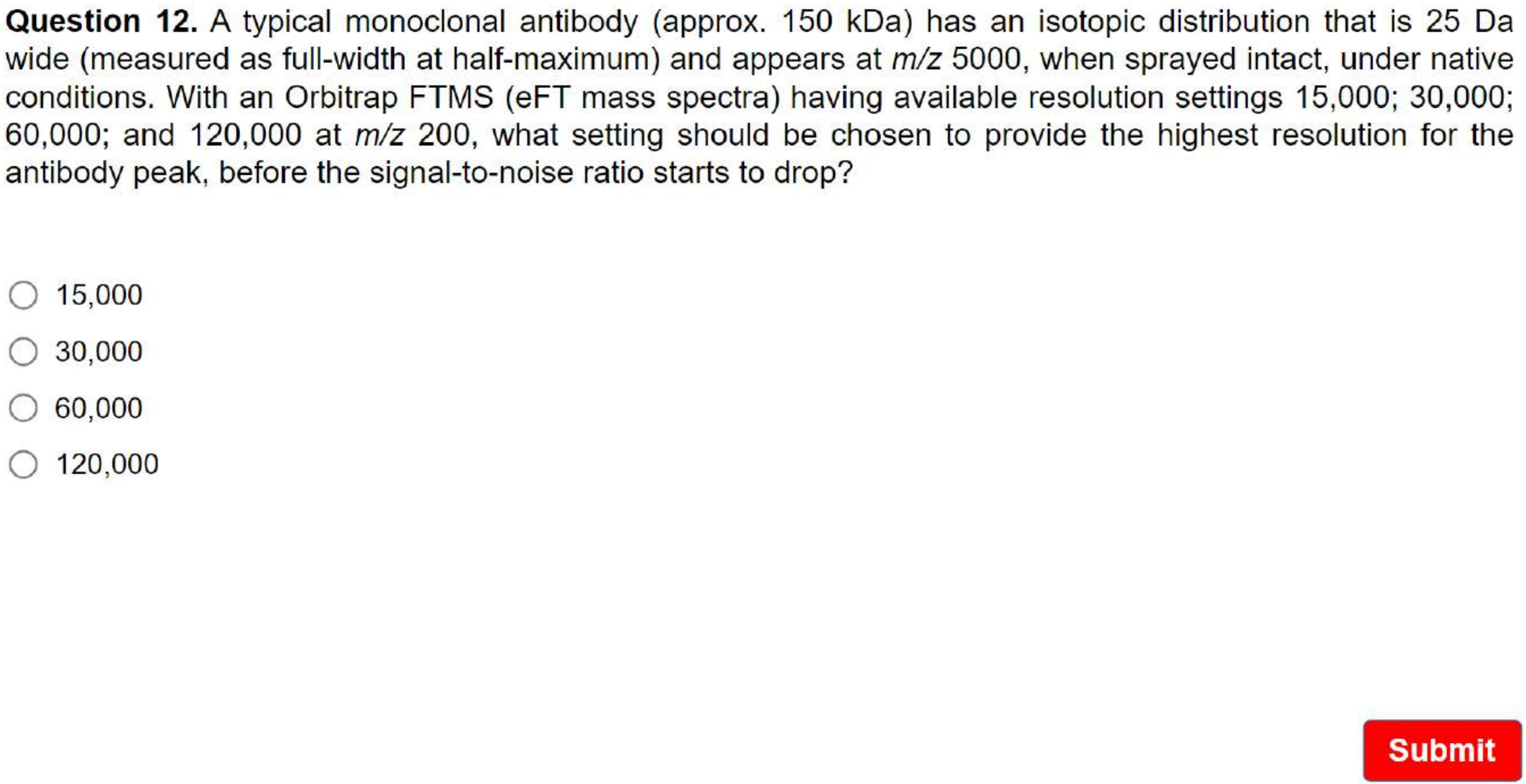
https://spectroswiss.ch/quiz/



Question 12. A typical monoclonal antibody (approx. 150 kDa) has an isotopic distribution that is 25 Da wide (measured as full-width at half-maximum) and appears at *m/z* 5000, when sprayed intact, under native conditions. With an Orbitrap FTMS (eFT mass spectra) having available resolution settings 15,000; 30,000; 60,000; and 120,000 at *m/z* 200, what setting should be chosen to provide the highest resolution for the antibody peak, before the signal-to-noise ratio starts to drop?

○ 15,000

○ 30,000

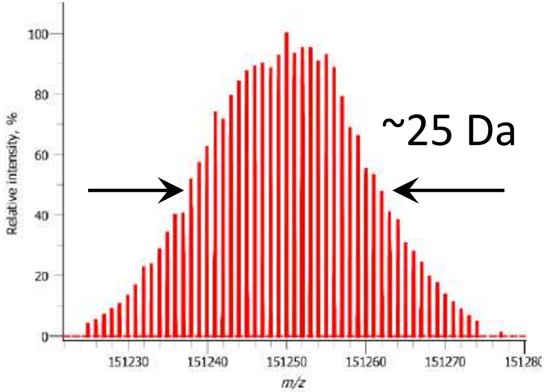
○ 60,000

○ 120,000

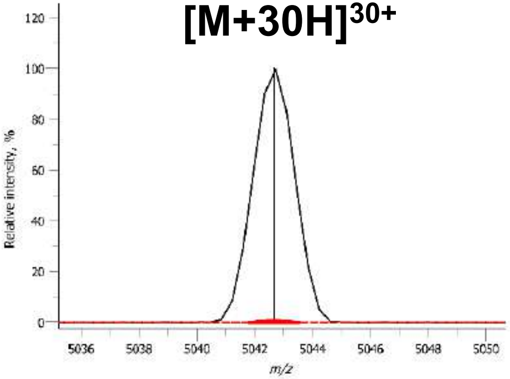
Spectroswiss

Monoclonal Antibody (mAb) Analysis

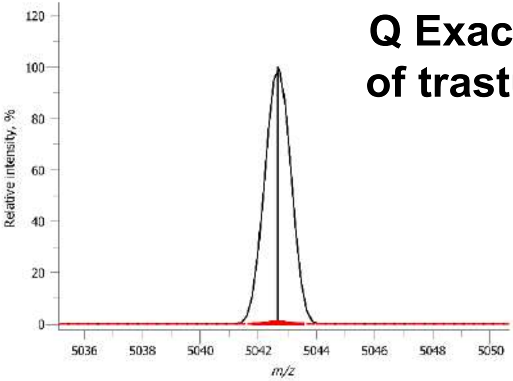
Isotopic Envelope



15k @ *m/z* 200: **3’100**

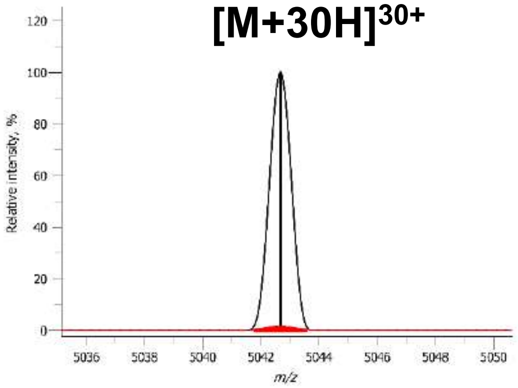


30k @ *m/z* 200: **4’900**

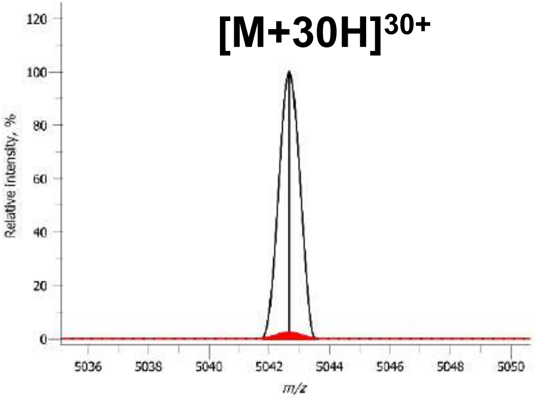


Q Exactive HF of trastuzumab

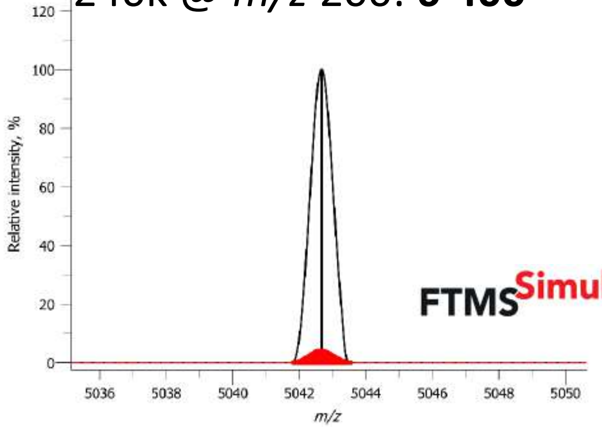
60k @ *m/z* 200: **6’000**



120k @ *m/z* 200: **6’300**



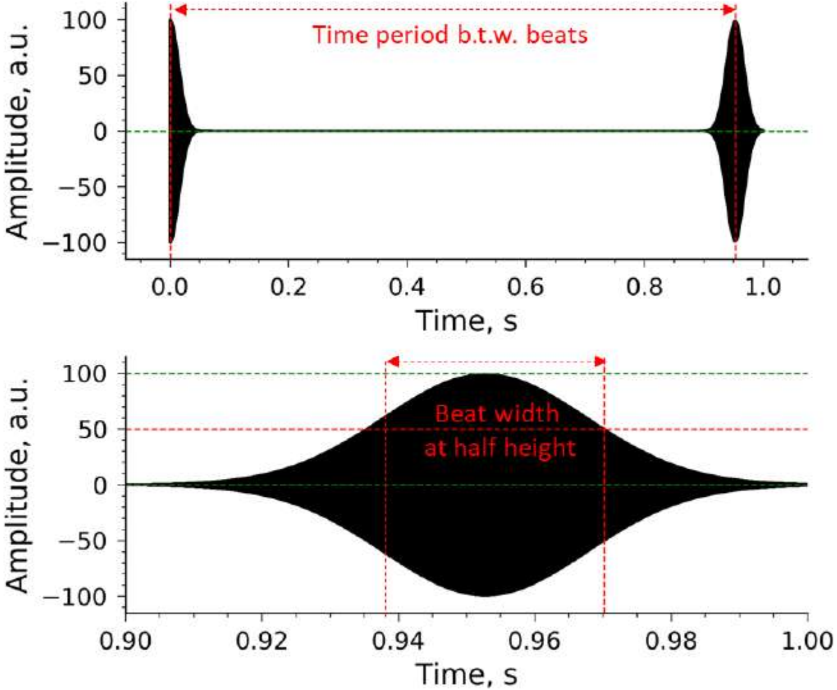
240k @ *m/z* 200: **6’400**



FTMS Simulator

Isotopic Beats in FTMS Transients

The constructive and destructive interferences between close frequency ion signals - beats



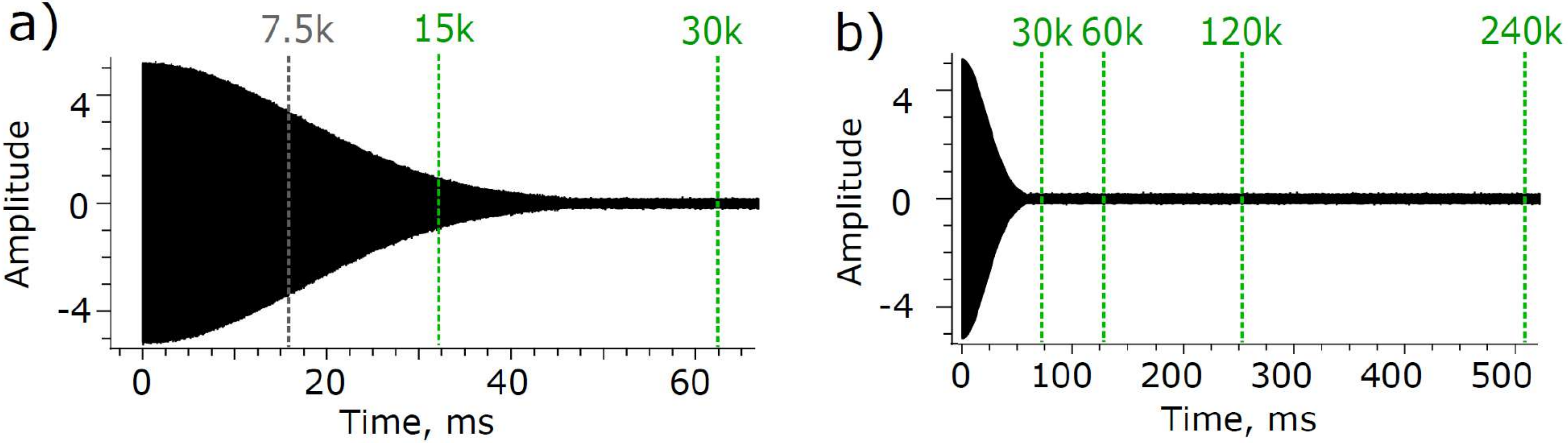
Hofstadler, S. A.; Bruce, J. E.; Rockwood, A. L.; Anderson, G. A.; Winger, B. E.; Smith, R. D. **Isotopic beat patterns in Fourier transform ion cyclotron resonance mass spectrometry: implications for high resolution mass measurements of large biopolymers.**
IJMS and Ion Processes 1994, 132, 109-127

Easterling, M. L.; Amster, I. J.; van Rooij, G. J.; Heeren, R. M. A. **Isotope beating effects in the analysis of polymer distributions by Fourier transform mass spectrometry.**
JASMS 1999, 10, 1074-1082

Makarov, A.; Denisov, E. **Dynamics of ions of intact proteins in the Orbitrap mass analyzer.**
JASMS 2009, 20, 1486-1495

Isotopic Beats in FTMS Transients: mAbs

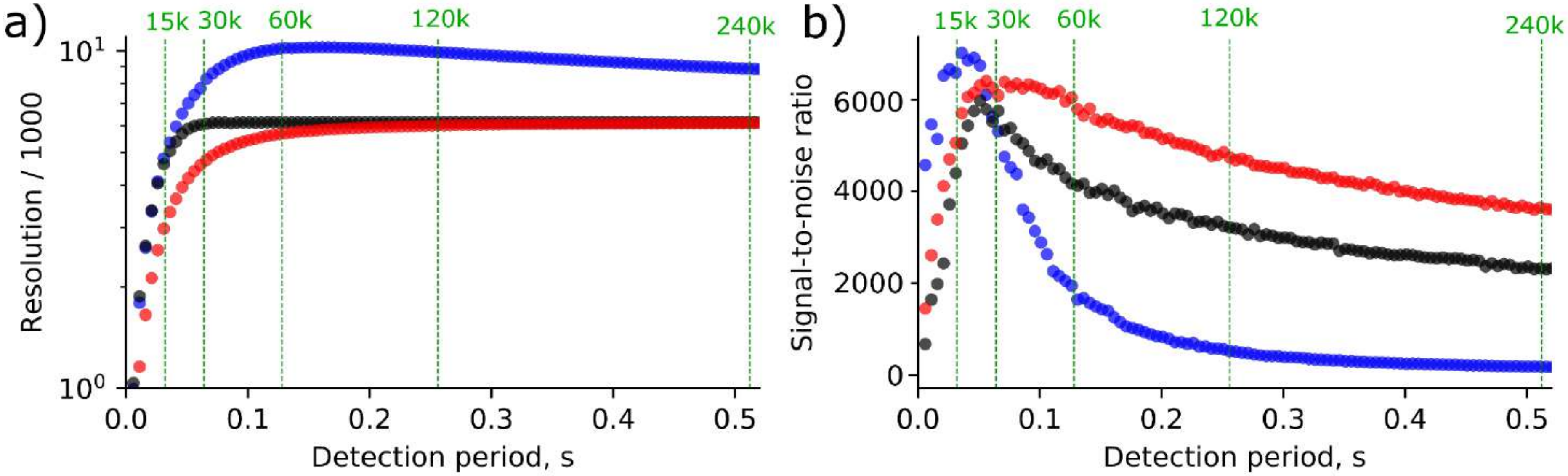
- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation with FTMS Simulator



Nagornov et al., JASMS 2022, 33, 1113–1125

Resolution and SNR Dependencies: mAbs

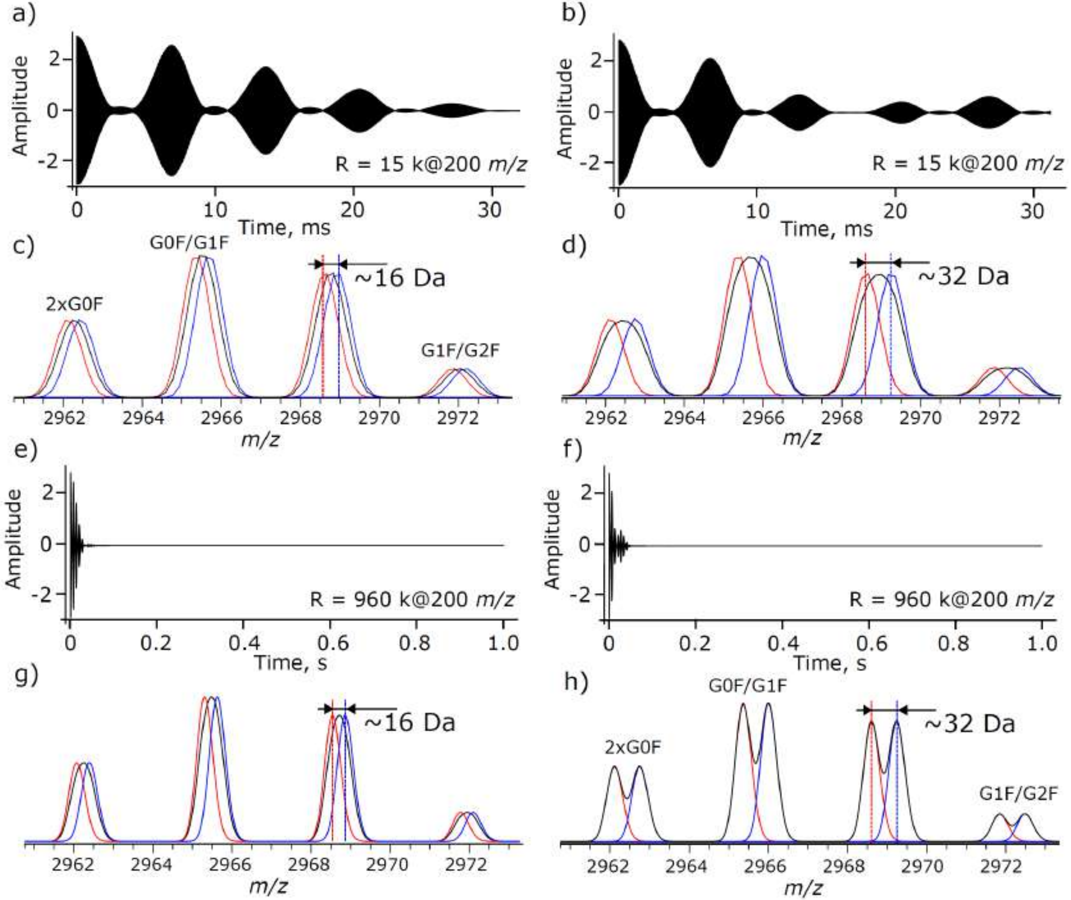
- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation and data processing with FTMS Simulator



- Colors: u**napodized aFT**; **half window apodized aFT**; **full window apodized aFT**

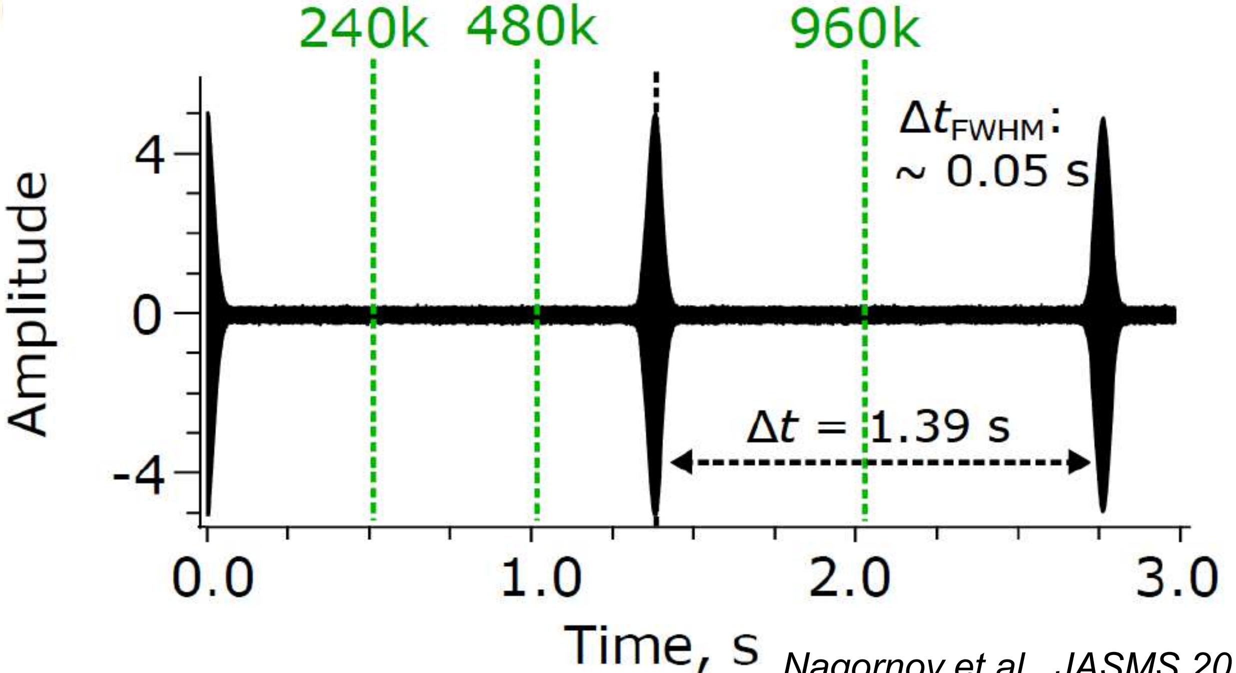
Consequences for mAb Analysis

- Only proteoforms with > 25 Da mass difference can be resolved (regular settings)



Isotopic Beats in **High-Resolution** FTMS: mAbs

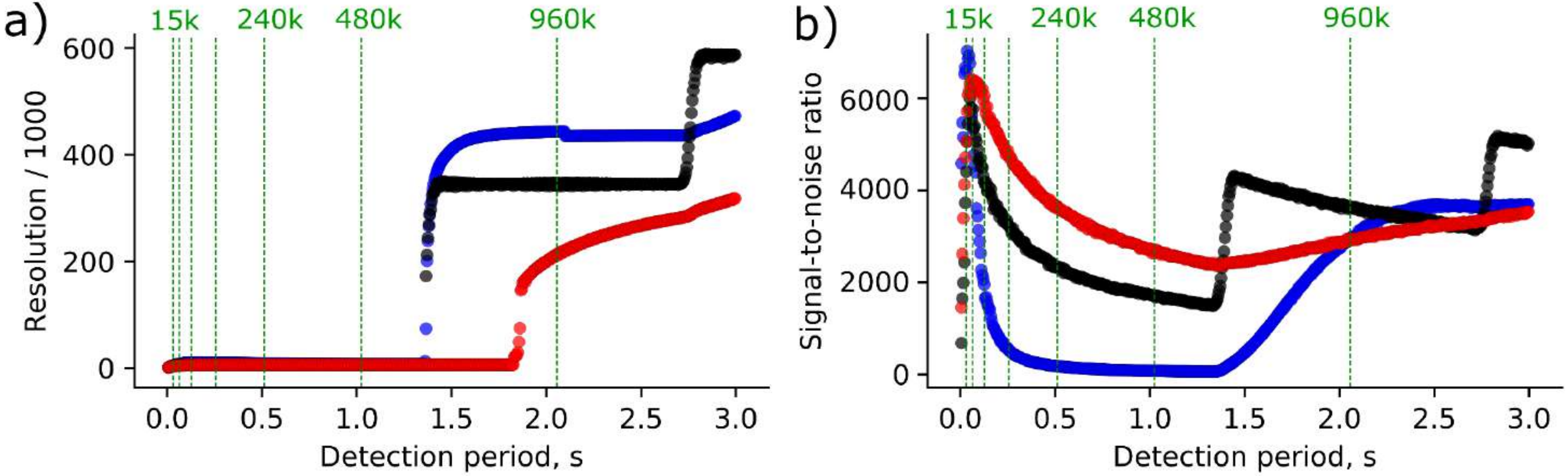
- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation with FTMS Simulator



Nagornov et al., JASMS 2022, 33, 1113–1125

Resolution & SNR Dependencies in HR FTMS: mAbs

- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation and data processing with FTMS Simulator



- Colors: u**napodized aFT**; **half window apodized aFT**; **full window apodized aFT**

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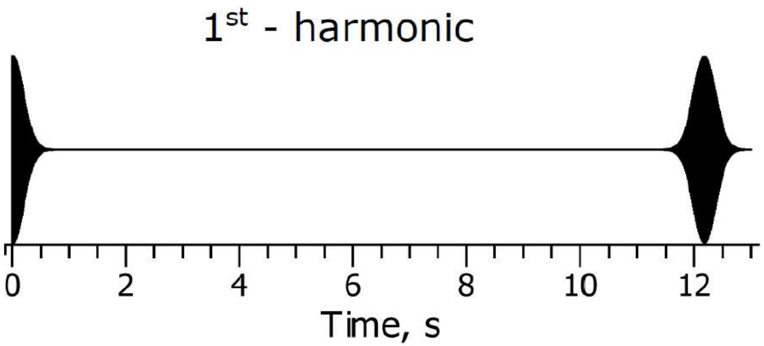
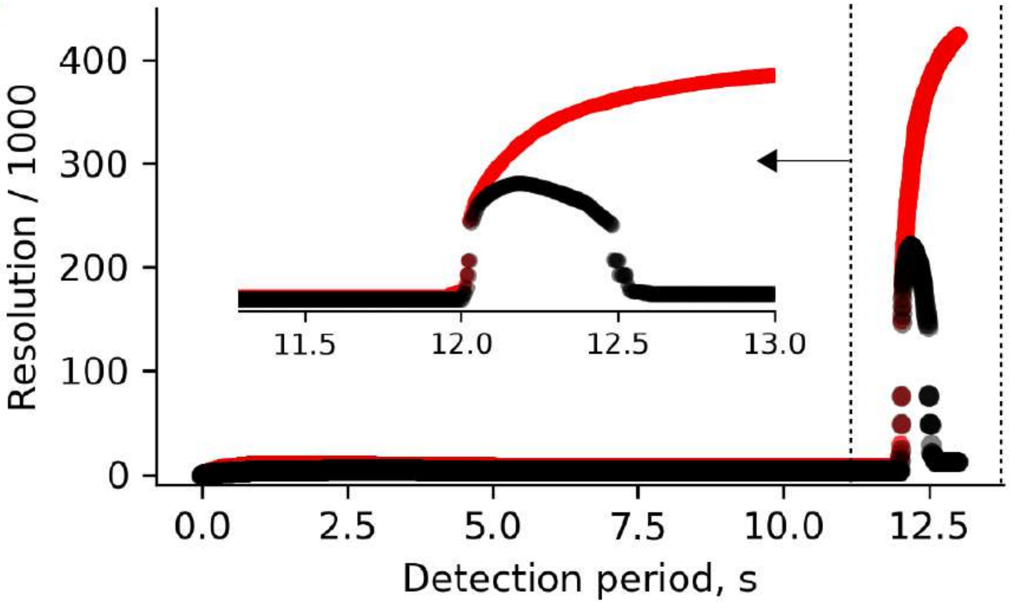
Resolution «Resonance» in the mFT ICR

- Sample: a biopolymer with an average mass of 150 kDa, $H_{10282}C_{6646}N_{1724}O_{2141}S_{44}$
- Instrument: a 12 T FT-ICR MS, $[M+10H]^{10+}$, full window (Kaiser-type) apodization
- Simulations: FTMS Simulator





To be verified experimentally!

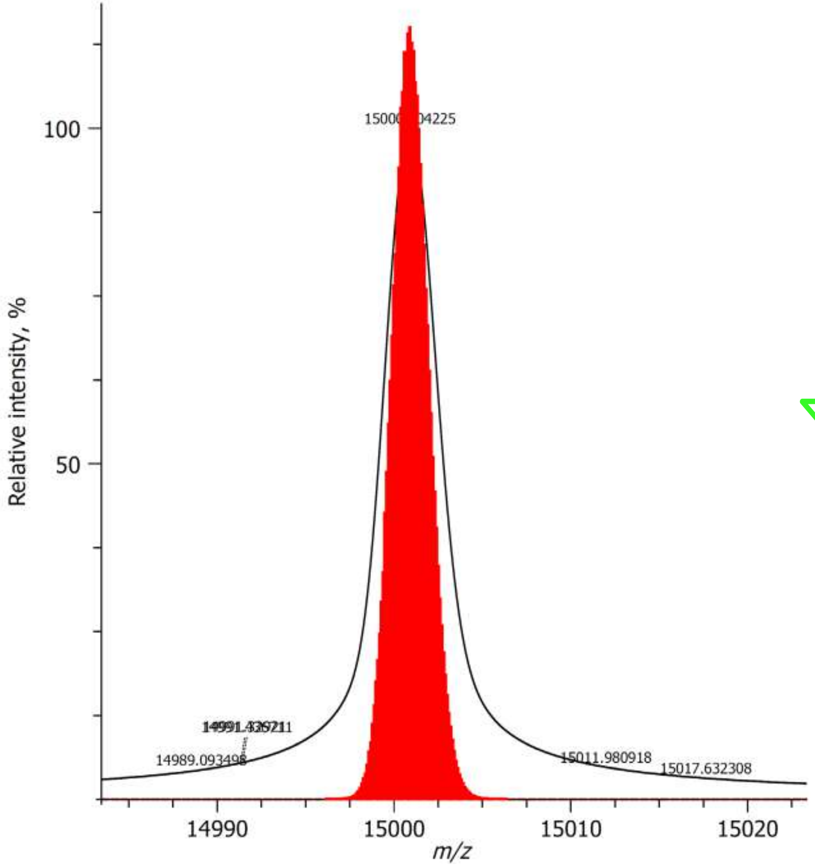
- Color coding: **magnitude mode FT**; **absorption mode FT**

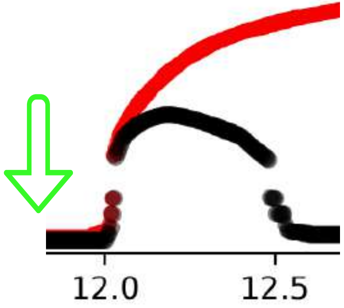
Nagornov et al., JASMS 2022, 33, 1113–1125

The 2nd Beat Challenge: Full Window Apodization

Spectroswiss

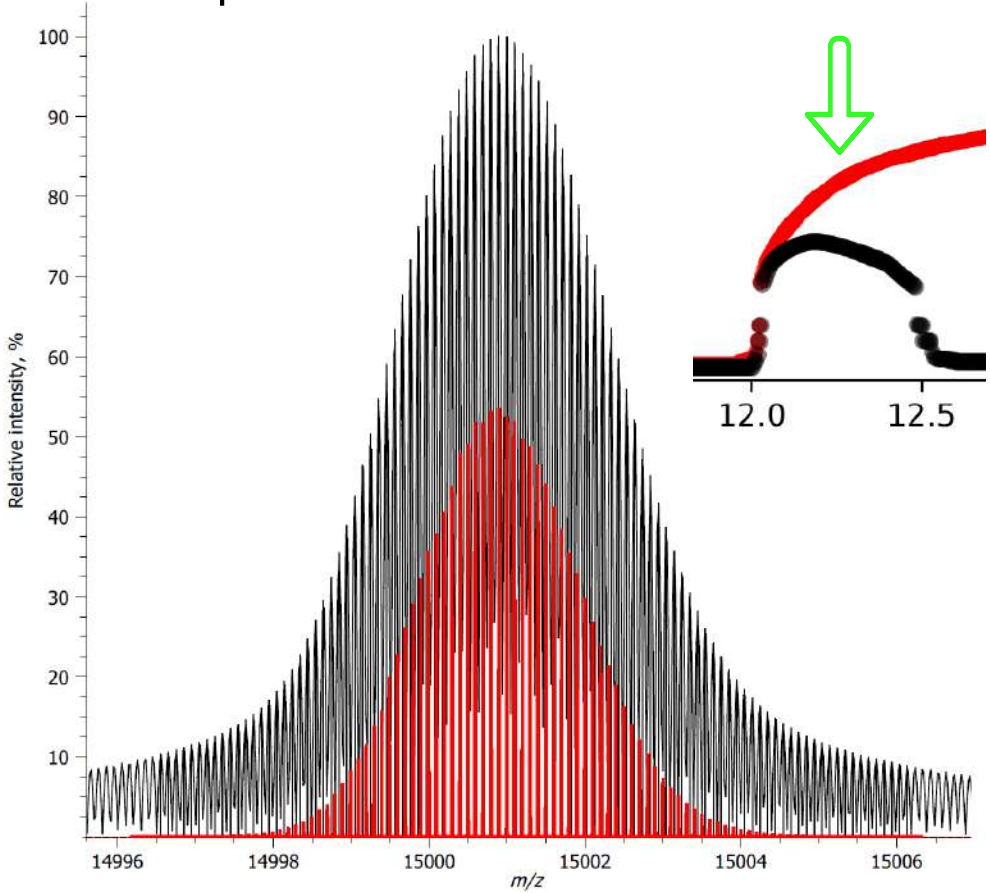
- Transient period: 11.5 s

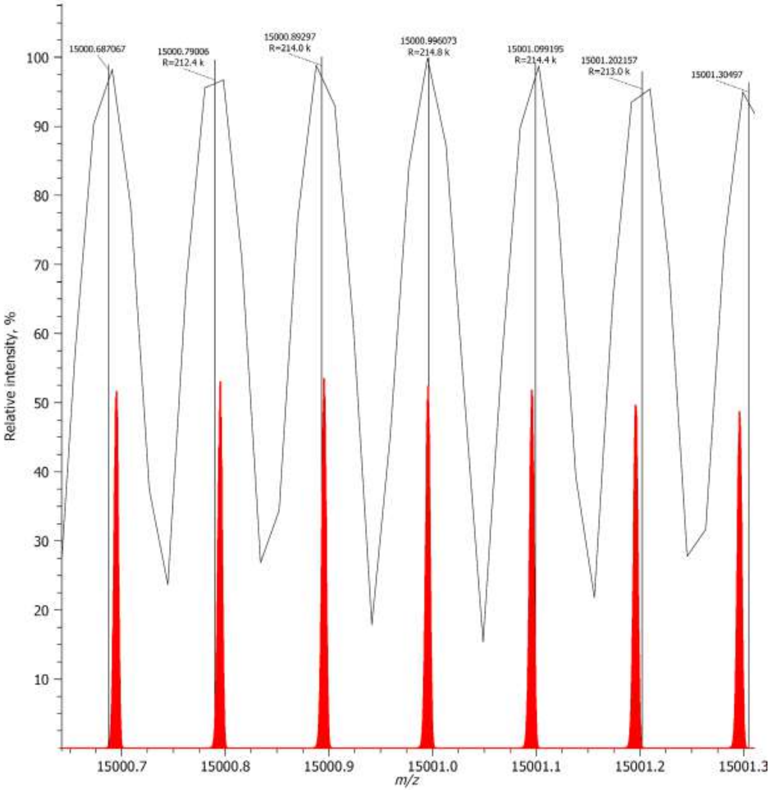




The 2nd Beat Challenge: Full Window Apodization

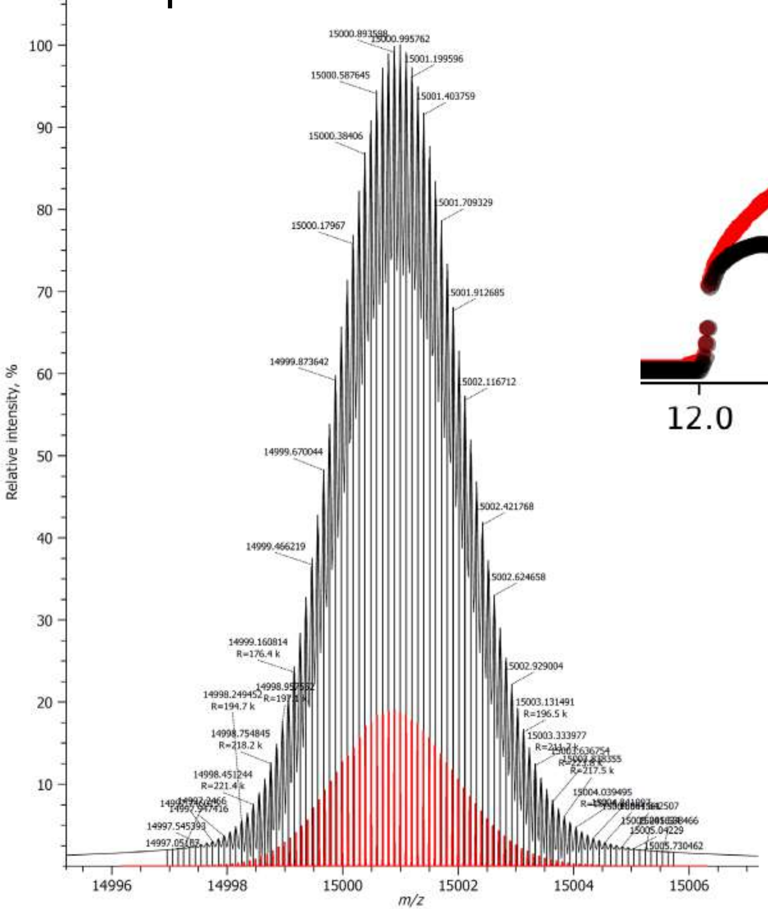
- Transient period: 12.25 s

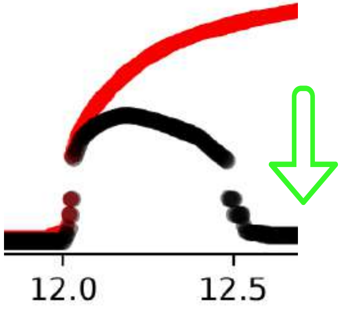


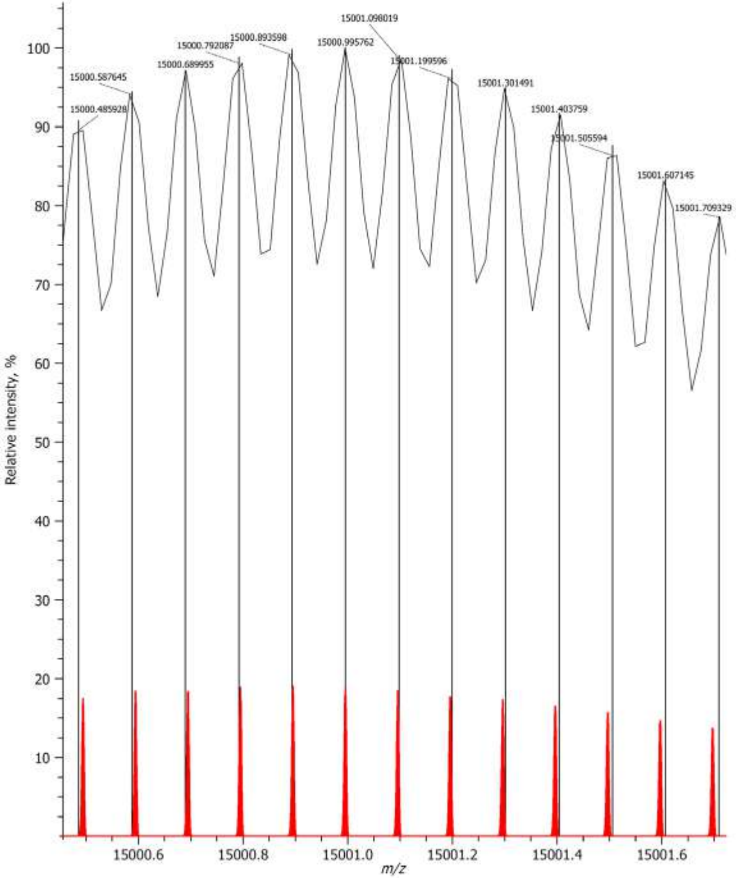


The 2nd Beat Challenge: Full Window Apodization

- Transient period: 13 s

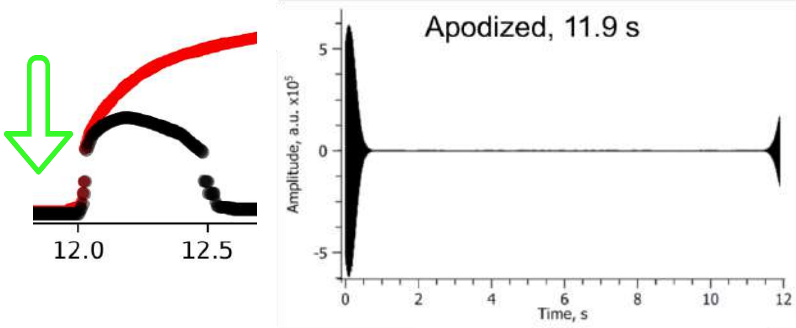


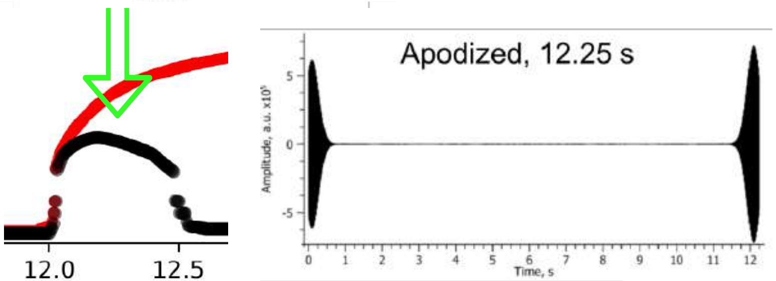


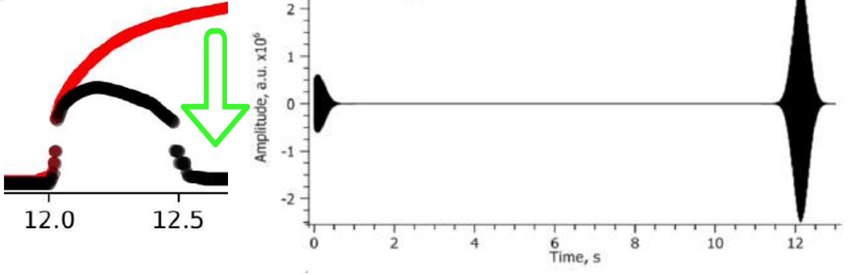


The 2nd Beat Challenge: Full Window Apodization

- Isotopic envelopes are correlated for each charge state (no deconvolution)







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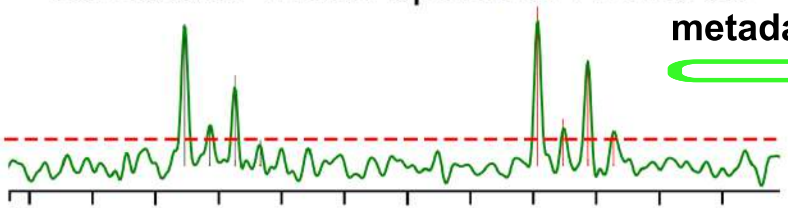
FTMS Workflows Embedding Data Simulation

Spectroswiss

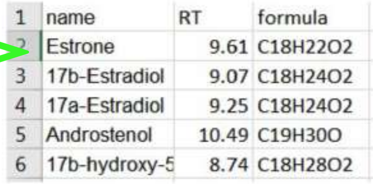
Calculate mass spectral features



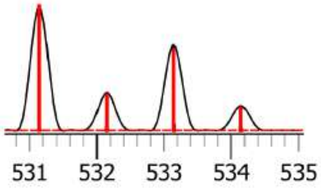
1

metadata

Simulate reference profile library

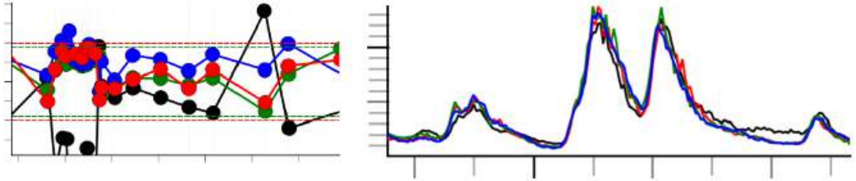


1	name	RT	formula
2	Estrone	9.61	C18H22O2
3	17b-Estradiol	9.07	C18H24O2
4	17a-Estradiol	9.25	C18H24O2
5	Androstenol	10.49	C19H30O
6	17b-hydroxy-5	8.74	C18H28O2



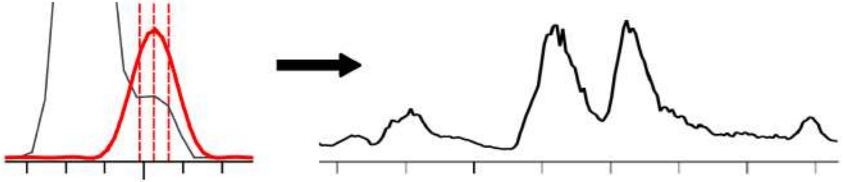
2

Align experiments (targeted/untargeted)



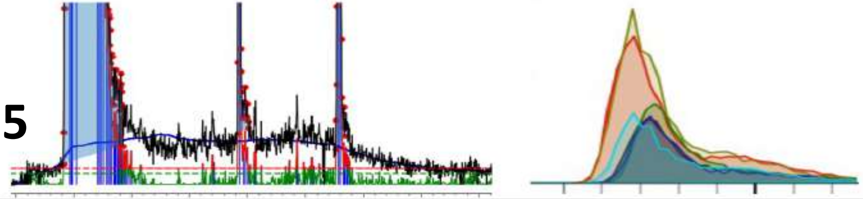
3

Extract SIC(s)



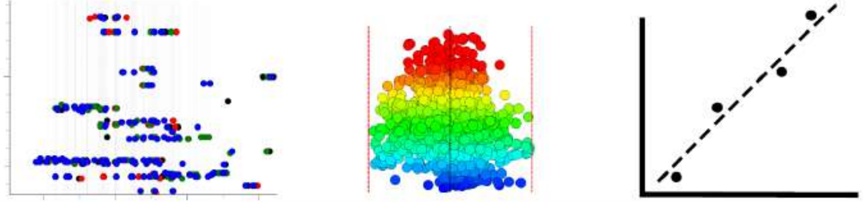
4

Process SIC(s) and detect features



5

Data analysis (mass recalibrate, quantify)



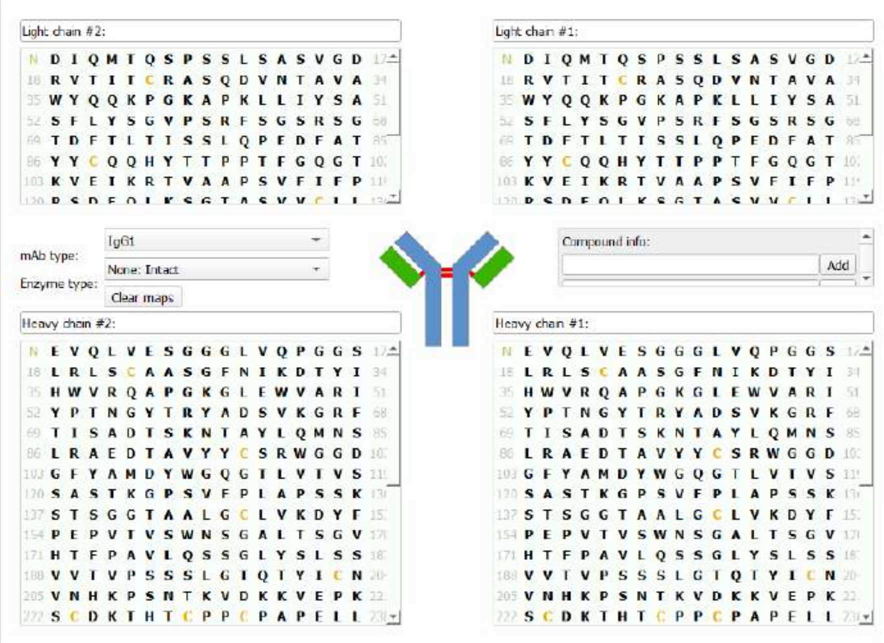
6

Nagornov et al., ASMS 2022, poster

Profile Library Simulations: Low Resolution

- Automatically determine FTMS instrument model and settings from metadata
- Specify a database of target compounds (from small molecules to proteins)
- Suggest adducts, modifications, charge carriers, and charge states

SIMULATE



Light chain #2:

```
N   D I Q M T Q S P S S L S A S V G D  17
18  R V T I T C R A S Q D V N T A V A  34
35  W Y Q Q K P G K A P K L L I Y S A  51
52  S F L Y S G V P S R F S G S R S G  68
69  T D F T L T I S S L Q P E D F A T  85
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
```

Light chain #1:

```
N   D I Q M T Q S P S S L S A S V G D  17
18  R V T I T C R A S Q D V N T A V A  34
35  W Y Q Q K P G K A P K L L I Y S A  51
52  S F L Y S G V P S R F S G S R S G  68
69  T D F T L T I S S L Q P E D F A T  85
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
```

mAb type: IgG1

Enzyme type: None: Intact

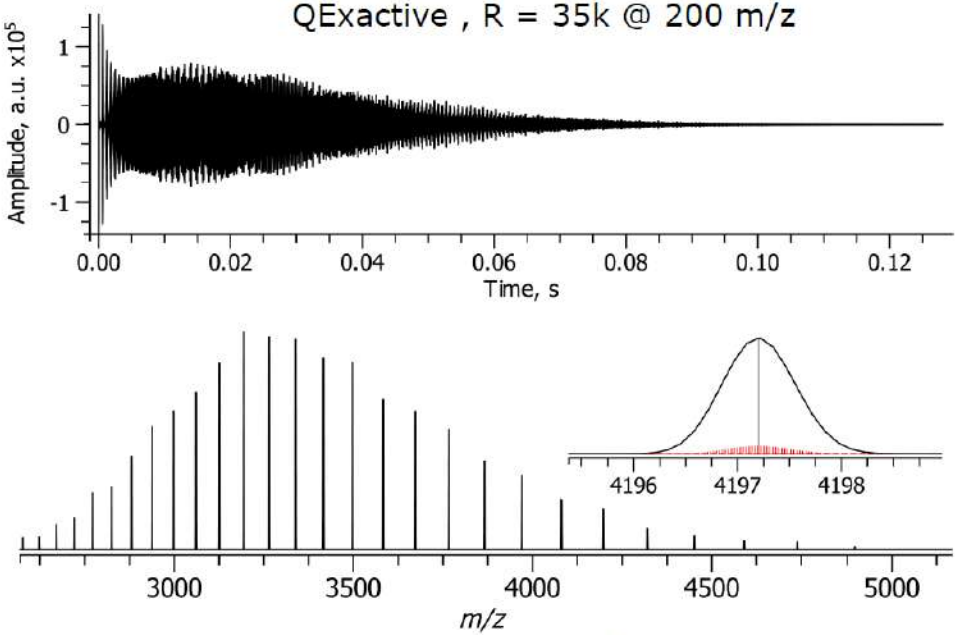
Compound info:

Heavy chain #2:

```
N   E V Q L V E S G G G L V Q P G G S  17
18  L R L S C A A S G F N I K D T Y I  34
35  H W V R Q A P G K G L E W V A R I  51
52  Y P T N G Y T R Y A D S V K G R F  68
69  T I S A D T S K N T A Y L Q M N S  85
86  L R A E D T A V Y Y C S R W G G D
103 G F Y A M D Y W G Q G T L V T V S
120 S A S T K G P S V F P L A P S S K
137 S T S G G T A A L G C L V K D Y F
154 P E P V T V S W N S G A L T S G V
171 H T F P A V L Q S S G L Y S L S S
188 V V T V P S S S L G T Q T Y I C N
205 V N H K P S N T K V D K K V E P K
222 S C D K T H T C P P C P A P E L L
```

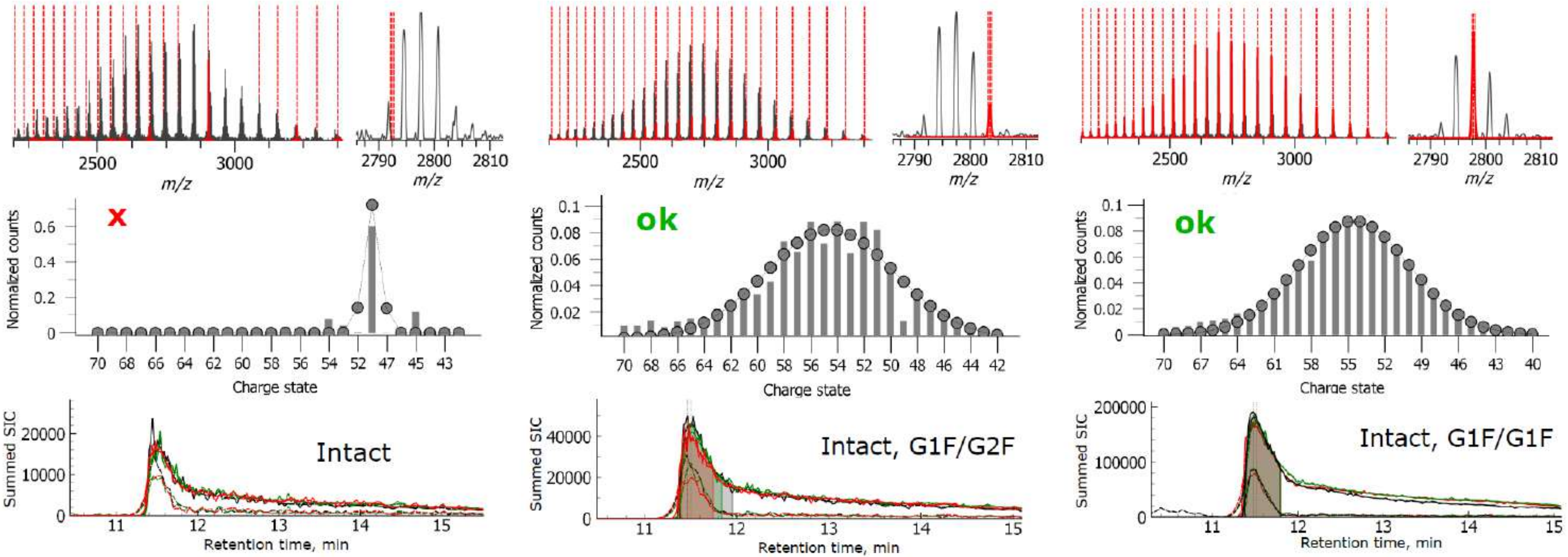
Heavy chain #1:

```
N   E V Q L V E S G G G L V Q P G G S  17
18  L R L S C A A S G F N I K D T Y I  34
35  H W V R Q A P G K G L E W V A R I  51
52  Y P T N G Y T R Y A D S V K G R F  68
69  T I S A D T S K N T A Y L Q M N S  85
86  L R A E D T A V Y Y C S R W G G D
103 G F Y A M D Y W G Q G T L V T V S
120 S A S T K G P S V F P L A P S S K
137 S T S G G T A A L G C L V K D Y F
154 P E P V T V S W N S G A L T S G V
171 H T F P A V L Q S S G L Y S L S S
188 V V T V P S S S L G T Q T Y I C N
205 V N H K P S N T K V D K K V E P K
222 S C D K T H T C P P C P A P E L L
```

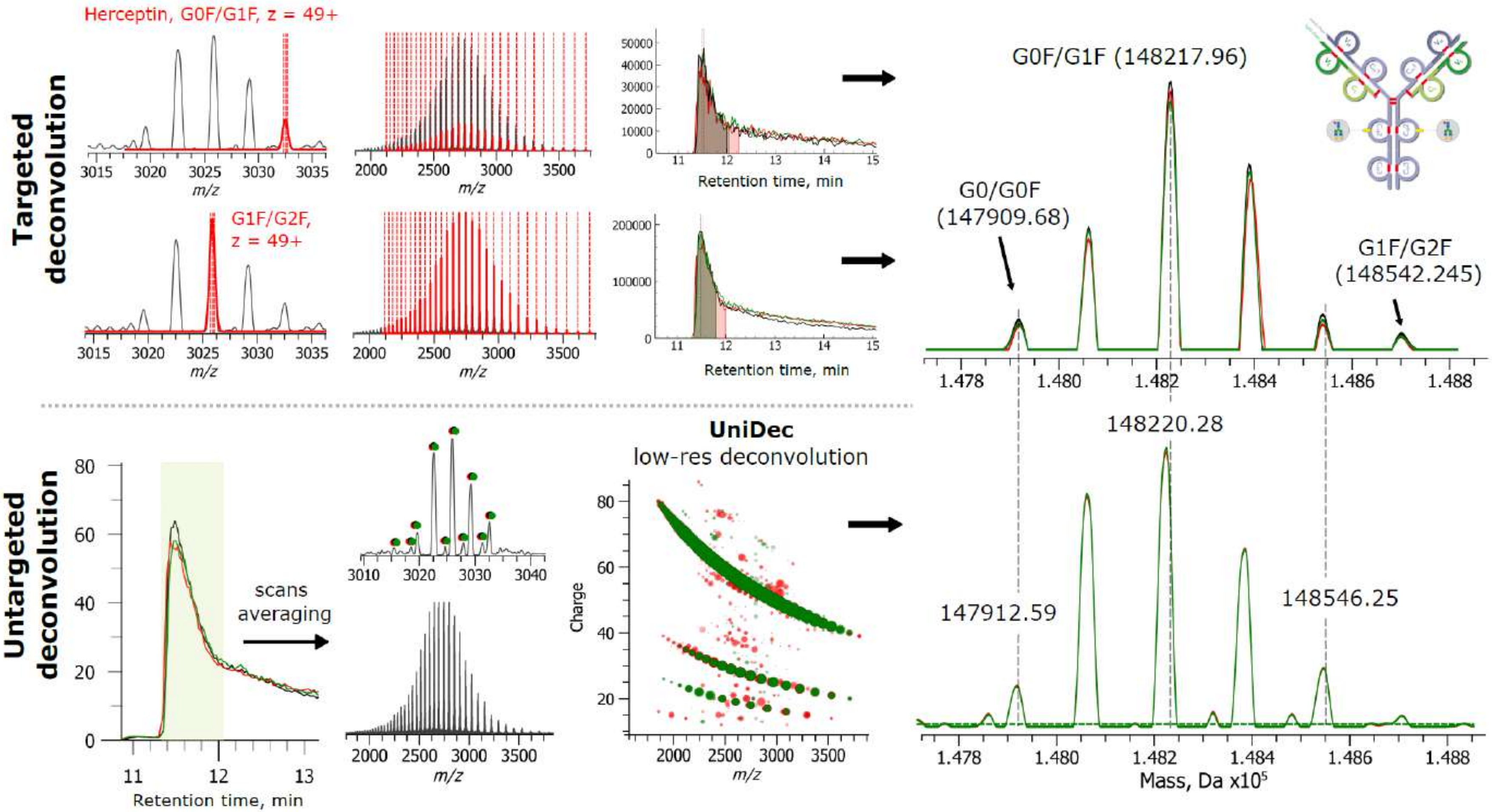


Correlate Experimental and Simulated Data

- Isotopic envelopes are compared for each charge state (no deconvolution)
- Charge state distributions are used to filter out the false positives
- Selected Ion Current (SIC) chromatograms show proteoform-specific elution periods



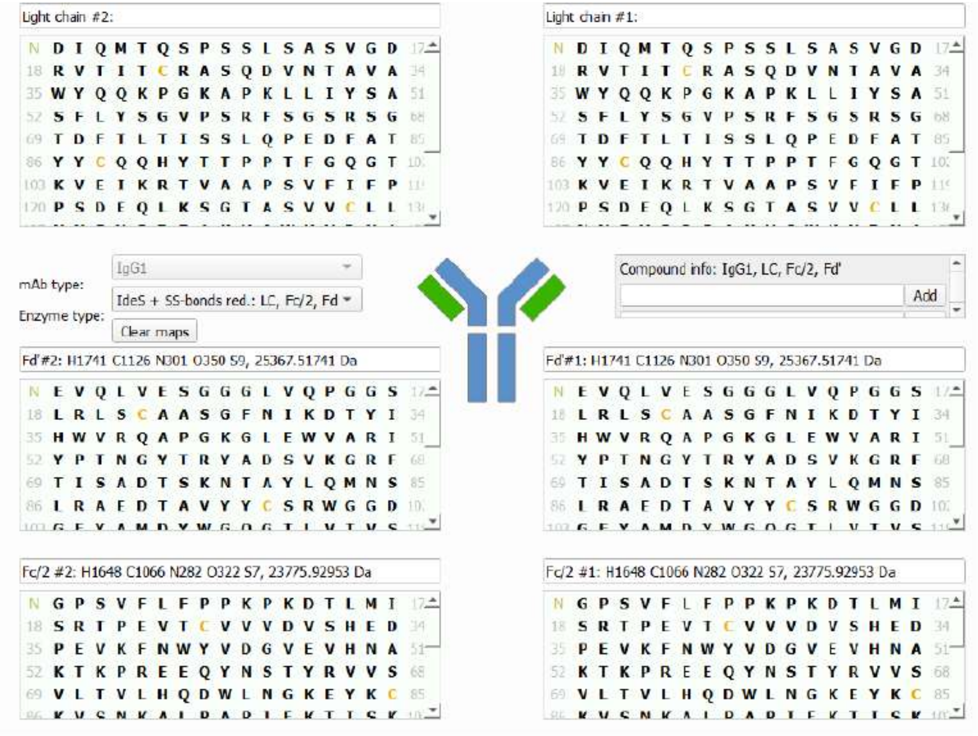
Targeted & Untargeted Deconvolution: Low Resolution



Profile Library Simulations: High-Resolution

- Isotopically resolved envelopes

SIMULATE



Light chain #2:

N D I Q M T Q S P S S L S A S V G D 17
18 R V T I T C R A S Q D V N T A V A 34
35 W Y Q Q K P G K A P K L L I Y S A 51
52 S F L Y S G V P S R F S G S R S G 68
69 T D F T L T I S S L Q P E D F A T 85
86 Y Y C Q Q H Y T T P P T F G Q G T 10
103 K V E I K R T V A A P S V F I F P 11
120 P S D E Q L K S G T A S V V C L L 13

Light chain #1:

N D I Q M T Q S P S S L S A S V G D 17
18 R V T I T C R A S Q D V N T A V A 34
35 W Y Q Q K P G K A P K L L I Y S A 51
52 S F L Y S G V P S R F S G S R S G 68
69 T D F T L T I S S L Q P E D F A T 85
86 Y Y C Q Q H Y T T P P T F G Q G T 10
103 K V E I K R T V A A P S V F I F P 11
120 P S D E Q L K S G T A S V V C L L 13

mAb type: IgG1

Enzyme type: IdeS + SS-bonds red.: LC, Fc/2, Fd

Compound info: IgG1, LC, Fc/2, Fd'

Fd'#2: H1741 C1126 N301 O350 S9, 25367.51741 Da

N E V Q L V E S G G G L V Q P G G S 17
18 L R L S C A A S G F N I K D T Y I 34
35 H W V R Q A P G K G L E W V A R I 51
52 Y P T N G Y T R Y A D S V K G R F 68
69 T I S A D T S K N T A Y L Q M N S 85
86 L R A E D T A V Y Y C S R W G G D 10

Fd'#1: H1741 C1126 N301 O350 S9, 25367.51741 Da

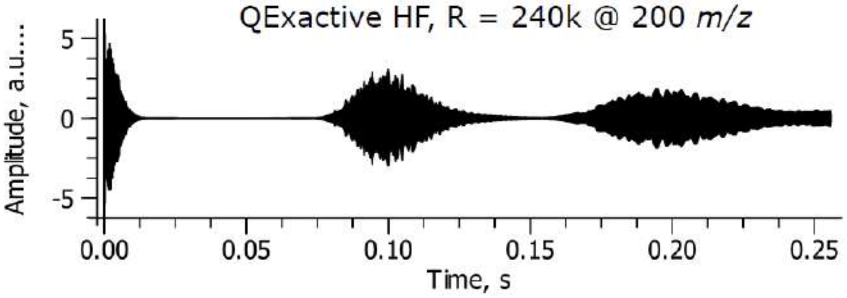
N E V Q L V E S G G G L V Q P G G S 17
18 L R L S C A A S G F N I K D T Y I 34
35 H W V R Q A P G K G L E W V A R I 51
52 Y P T N G Y T R Y A D S V K G R F 68
69 T I S A D T S K N T A Y L Q M N S 85
86 L R A E D T A V Y Y C S R W G G D 10

Fc/2 #2: H1648 C1066 N282 O322 S7, 23775.92953 Da

N G P S V F L F P P K P K D T L M I 17
18 S R T P E V T C V V V D V S H E D 34
35 P E V K F N W Y V D G V E V H N A 51
52 K T K P R E E Q Y N S T Y R V V S 68
69 V L T V L H Q D W L N G K E Y K C 85

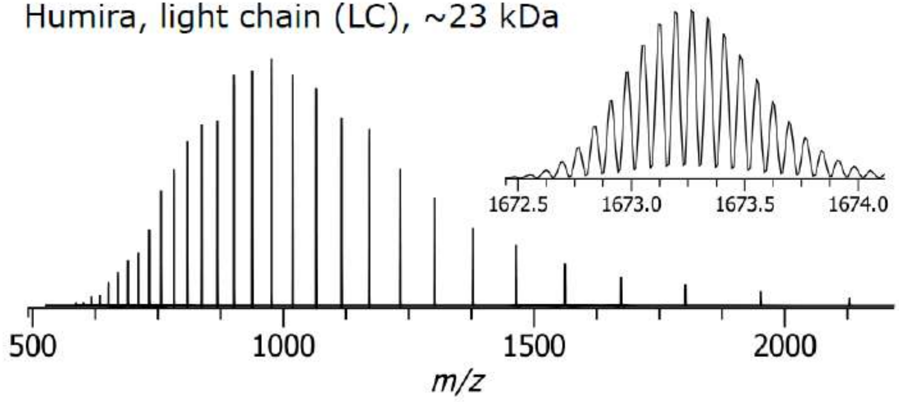
Fc/2 #1: H1648 C1066 N282 O322 S7, 23775.92953 Da

N G P S V F L F P P K P K D T L M I 17
18 S R T P E V T C V V V D V S H E D 34
35 P E V K F N W Y V D G V E V H N A 51
52 K T K P R E E Q Y N S T Y R V V S 68
69 V L T V L H Q D W L N G K E Y K C 85



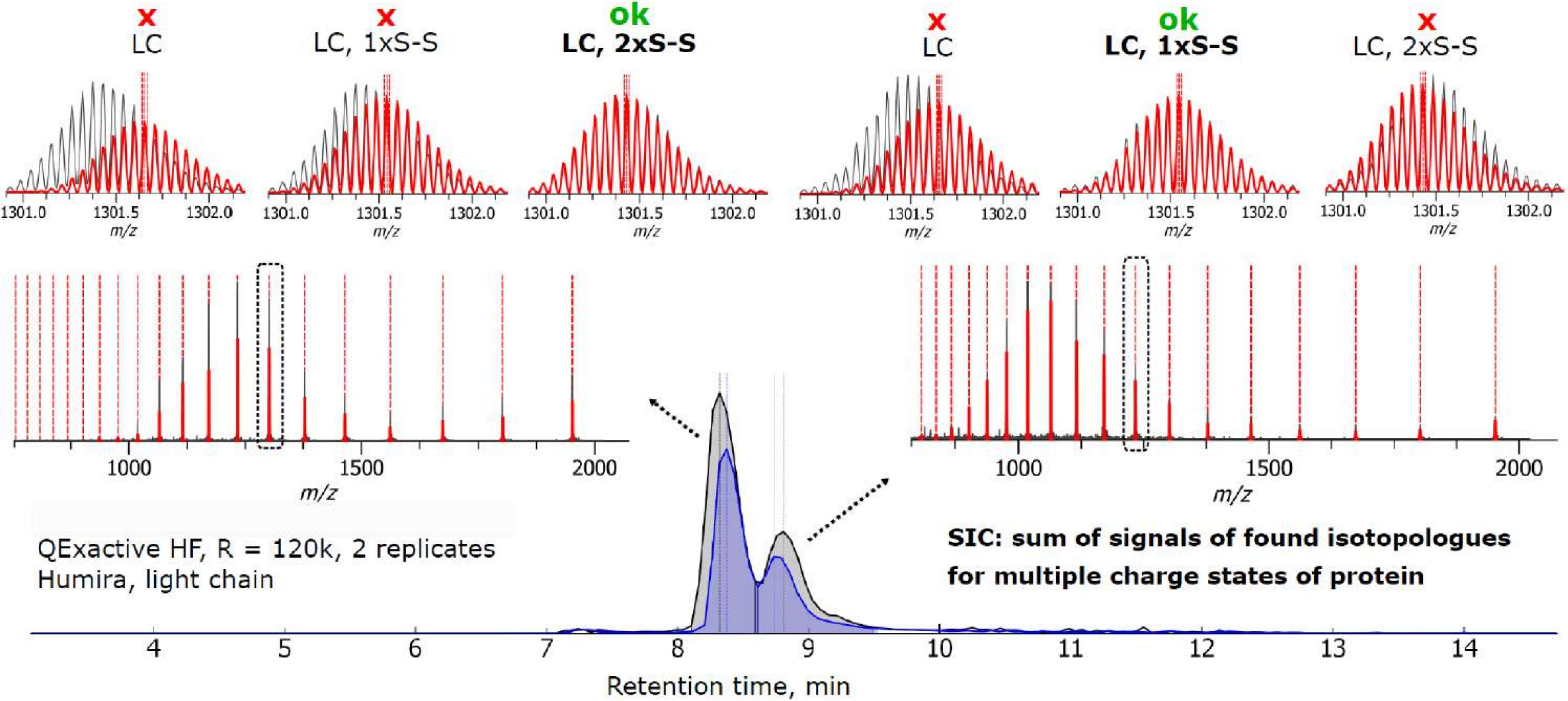
QExactive HF, R = 240k @ 200 *m/z*

Humira, light chain (LC), ~23 kDa



Targeted Deconvolution: High Resolution

- Analysis of monoclonal antibodies subunits (LC – light chains, 25 kDa)

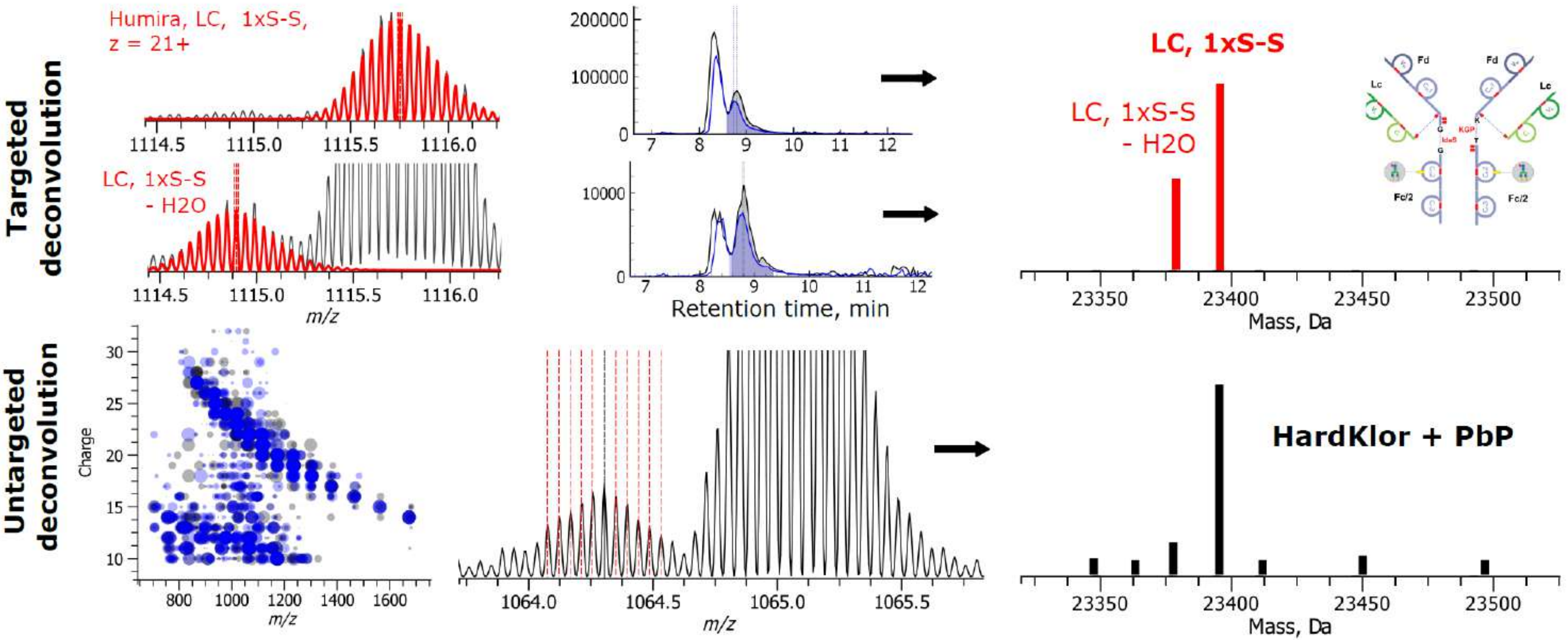


QExactive HF, R = 120k, 2 replicates
Humira, light chain

SIC: sum of signals of found isotopologues for multiple charge states of protein

Targeted & Untargeted Deconvolution: High Resolution

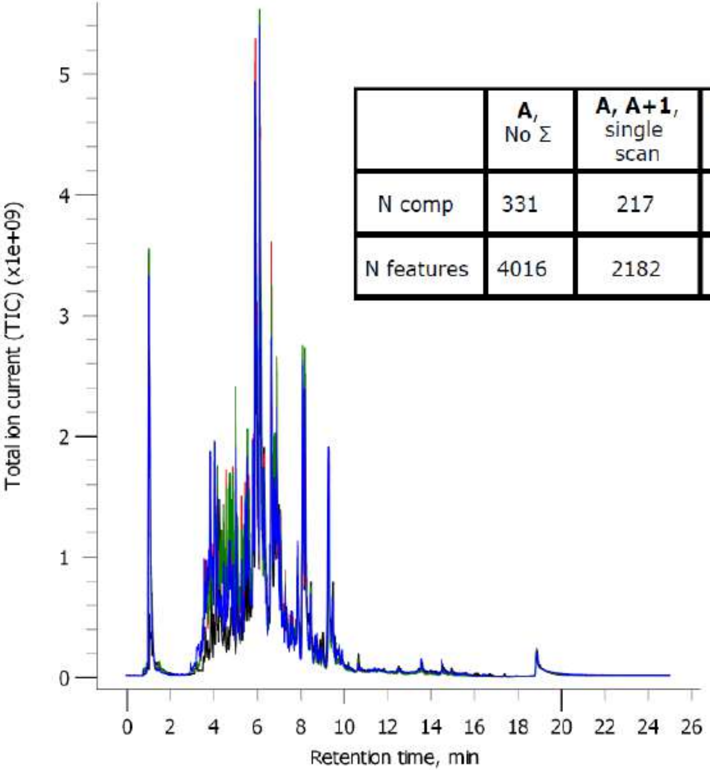
- Deconvolution approaches for isotopically-resolved data: FLEXDeconv, Hardklor, ...



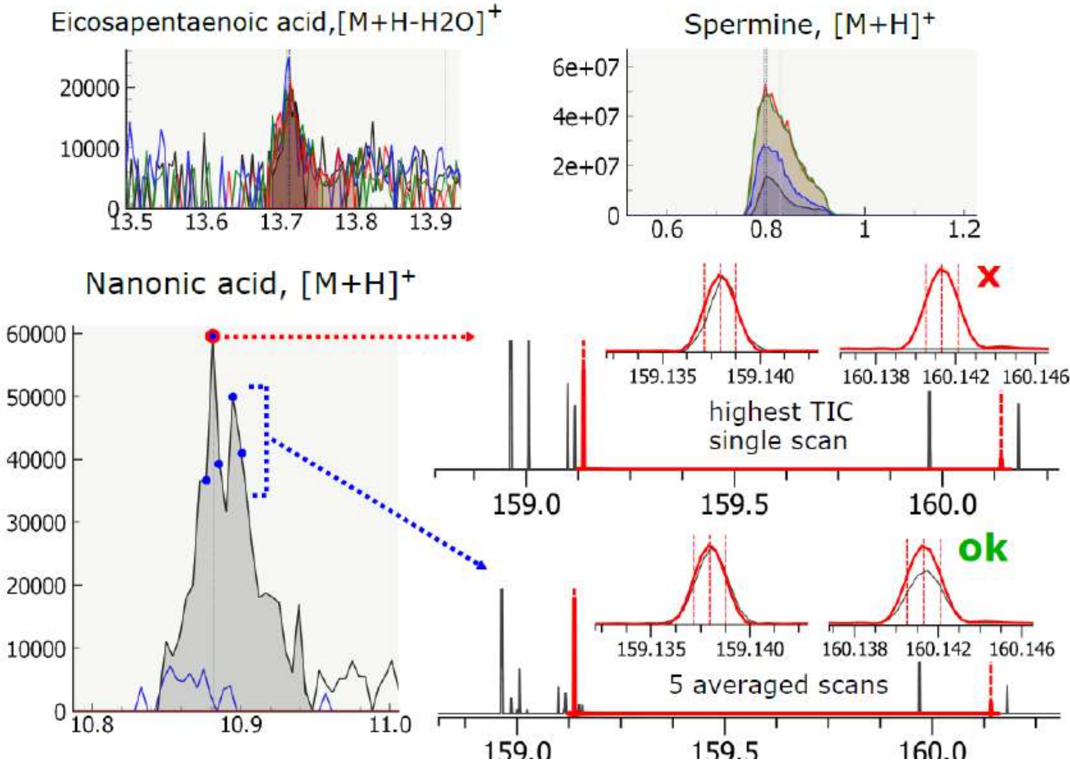
Targeted Deconvolution: Small Molecule Analysis

Spectroswiss

- Analysis of steroids in human seminal fluid with a Q Exactive Focus
- Data annotation using 789 steroids database and 5 ppm mass tolerance
- Efficient feature extraction with reduced artifacts introduction and false positives

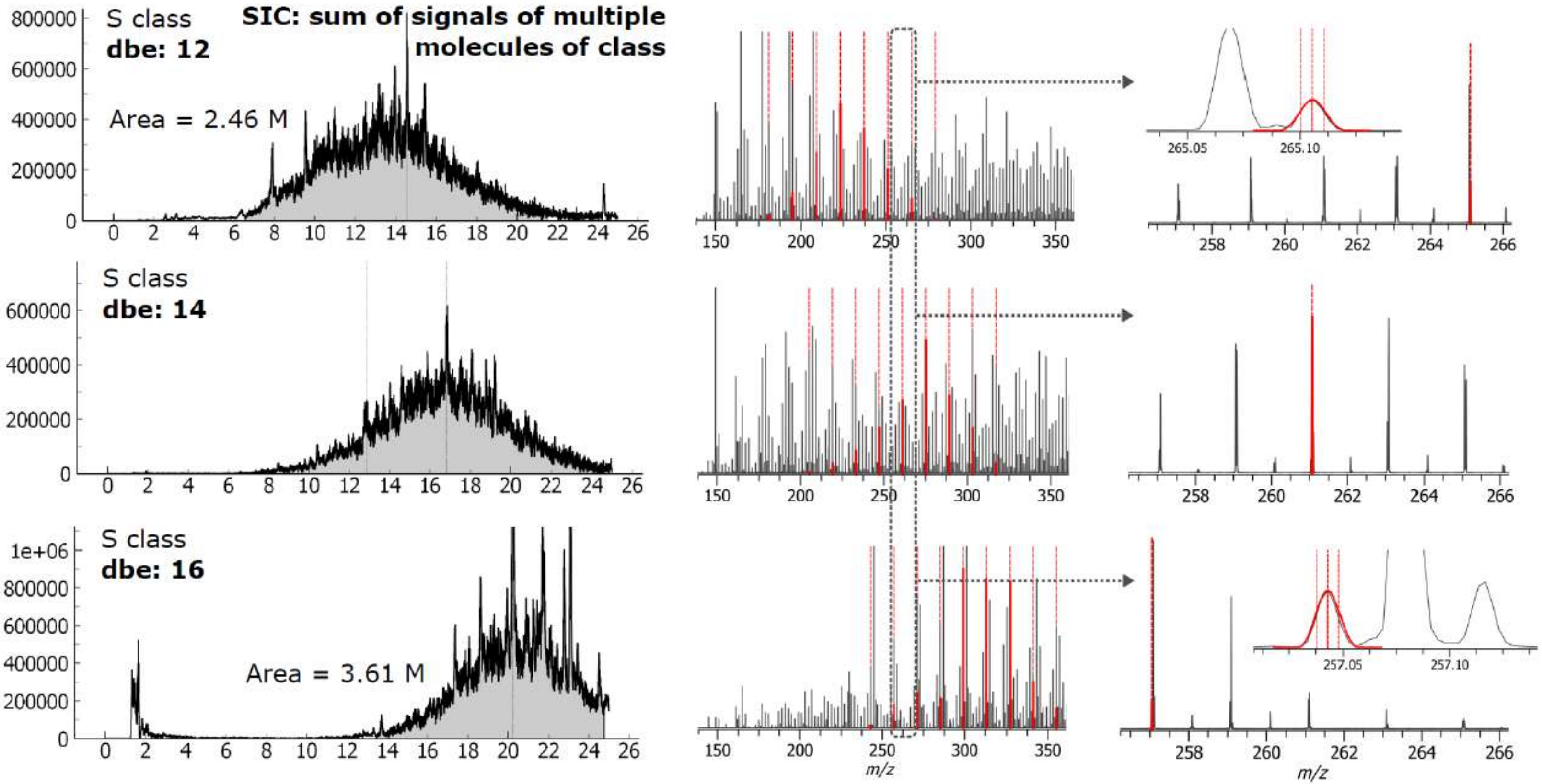


	A, No Σ	A, A+1, single scan	A, A+1, 5 Σ scans
N comp	331	217	248
N features	4016	2182	2502



Targeted Deconvolution: Complex Mixture Analysis

- LC-MS analysis of a complex mixture on a 7 T LTQ FT Ultra
- Feature extraction of compound classes: LC/GC-MS complex mixture analysis



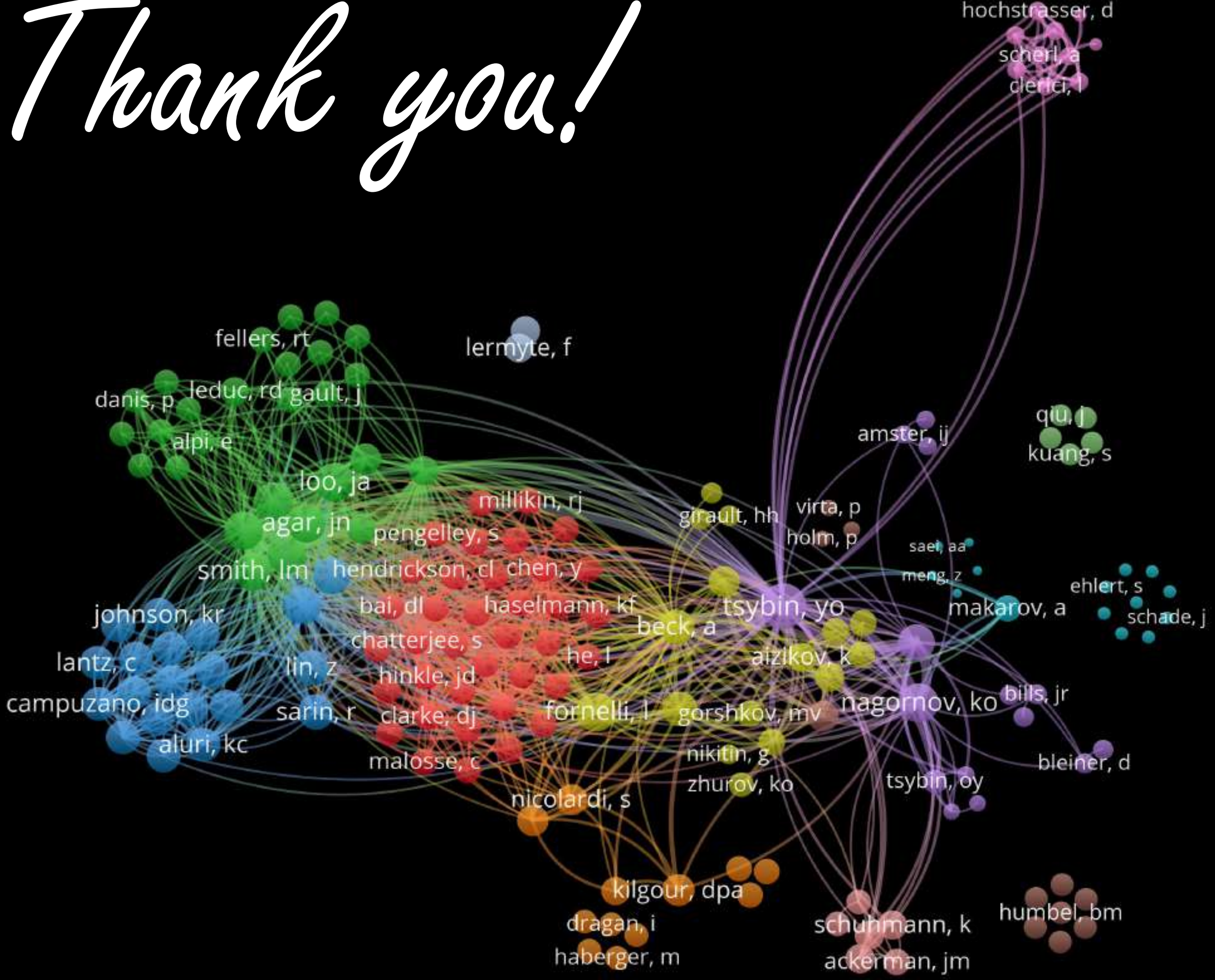
Conclusions:

Why to Simulate FTMS Data via Time-Domain Transients?

- Resolution dependence on mass, charge state, instrument model, etc.
- Peak interference and unresolved peaks – artifacts in resolution estimation
- Peak shape dependence on the FT processing – apodization, zero fills, etc.
- Computationally, data processing speeds are comparable with peak overlay methods
- Step-by-step visualization of the FT processing workflow – teaching & training
- Understanding the FTMS data and hypothesis verification (w/out experimental data)
- Revealing novel insights into FTMS, with subsequent experimental verification

Disclaimer: FTMS Simulator is only an example tool – it validates the approach

Thank you!



Spectroswiss

Sergey Girel

TOP SPEC

Project number: 829157

EFTMS 2022