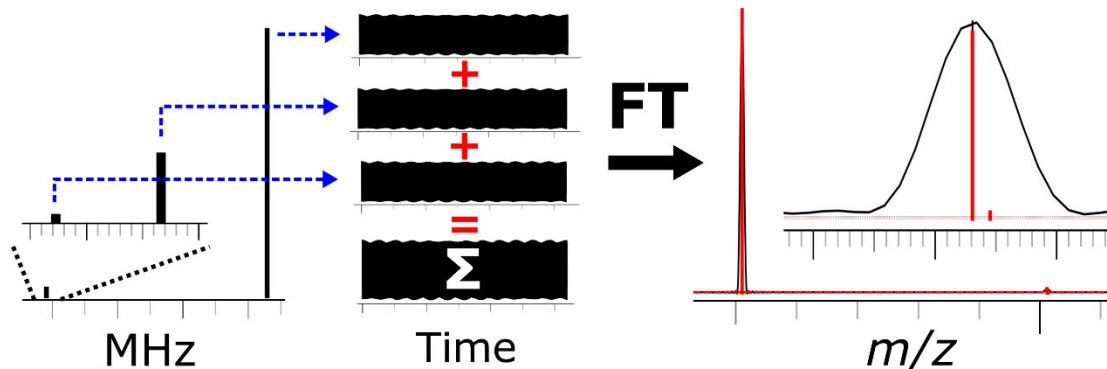


14th European Fourier Transform Mass Spectrometry Workshop

FT Mass Spectra Simulation: Fundamentals and Applications

Simulated transient

FT mass spectrum



Yury Tsybin
Spectroswiss
Lausanne, Switzerland

tsybin@spectroswiss.ch

July 12, 2022; 14:00

How & Why to Simulate the FTMS Data?

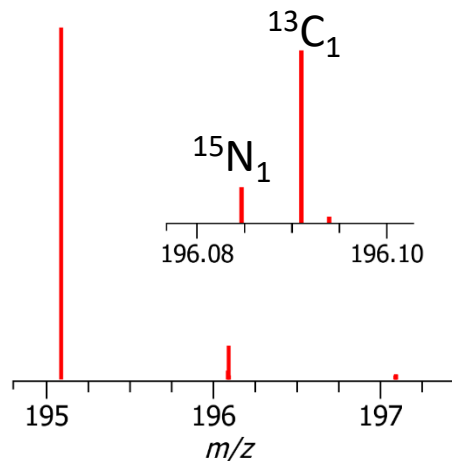
- Approaches to simulate the FTMS isotopic envelopes and mass spectra
- Teaching & training: understanding the FTMS concepts and definitions
- Reviewing & writing: manuscripts, project applications, ideas verification
- Support FTMS data interpretation and experiment design (settings selection)
- Advancing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

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)

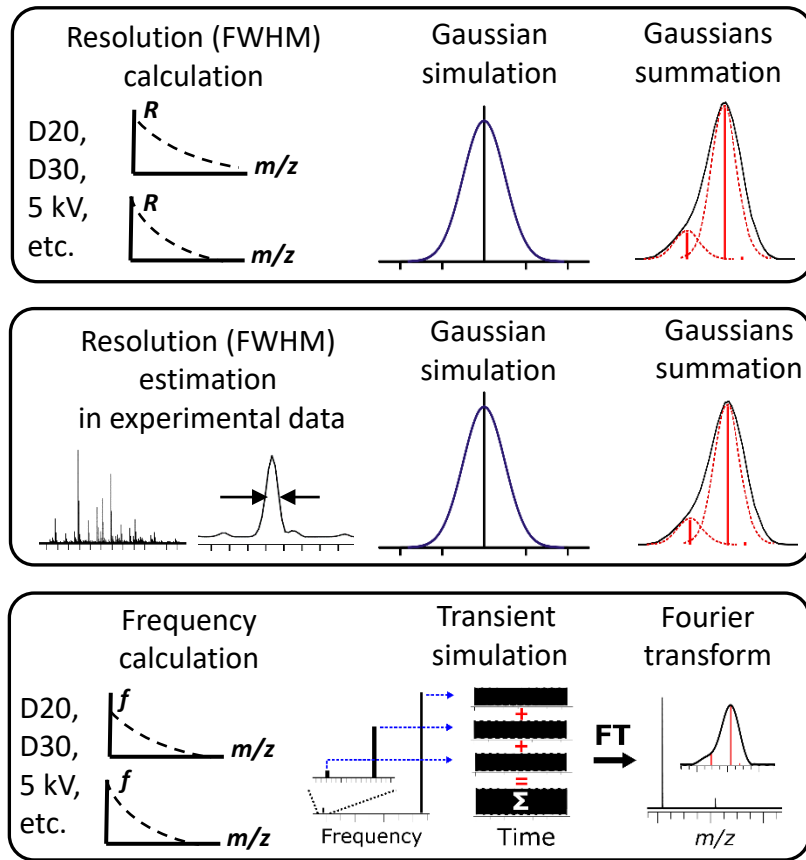
FTMS Data Simulation Approaches

Isotopic distribution calculation



ChemCalc
IsoSpec2
EnviPat

...



Protein analysis?
(isotopic beats)

Peak interference?

Computational speed?

FTMS Data Simulation via Time-Domain Transients

Elemental composition: **a**



Ionization type:

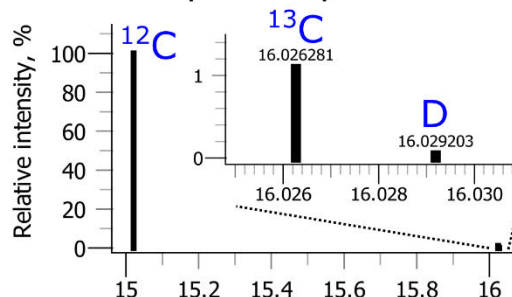
positive radical, $\text{CH}_3^{+\bullet}$

Isotopologue rank:

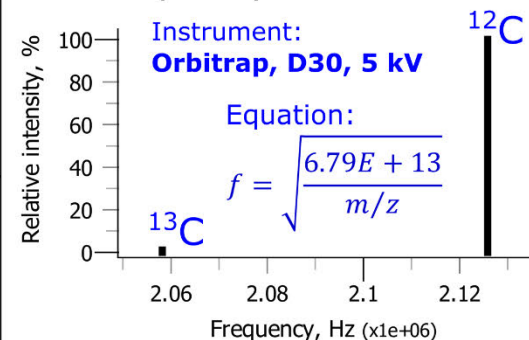
1 - 2,

relative intensity threshold

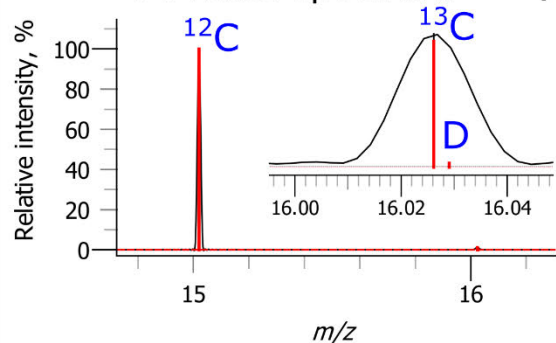
Isotopic compositions **b**



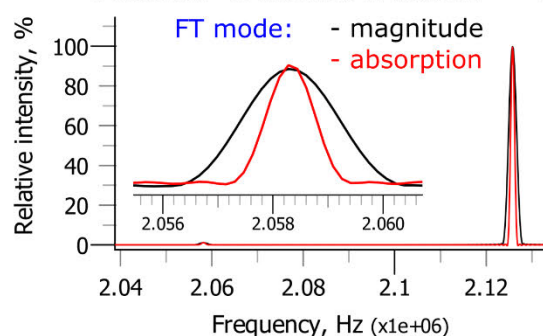
Frequency calculation **c**



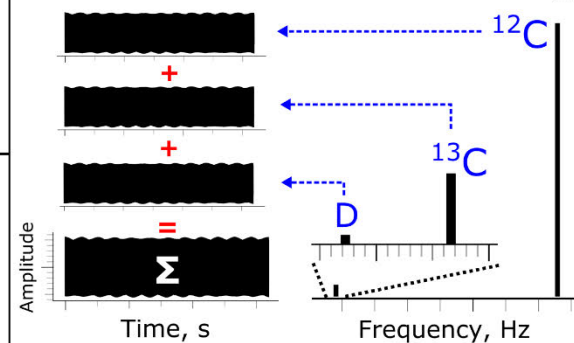
FT mass spectrum **f**



Fourier transformation **e**



Simulated transient **d**



Why to Simulate the FTMS Data?

- Approaches to simulate the FTMS isotopic envelopes and mass spectra
- Teaching & training: understanding the FTMS concepts and definitions
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The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS i

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: 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

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			
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

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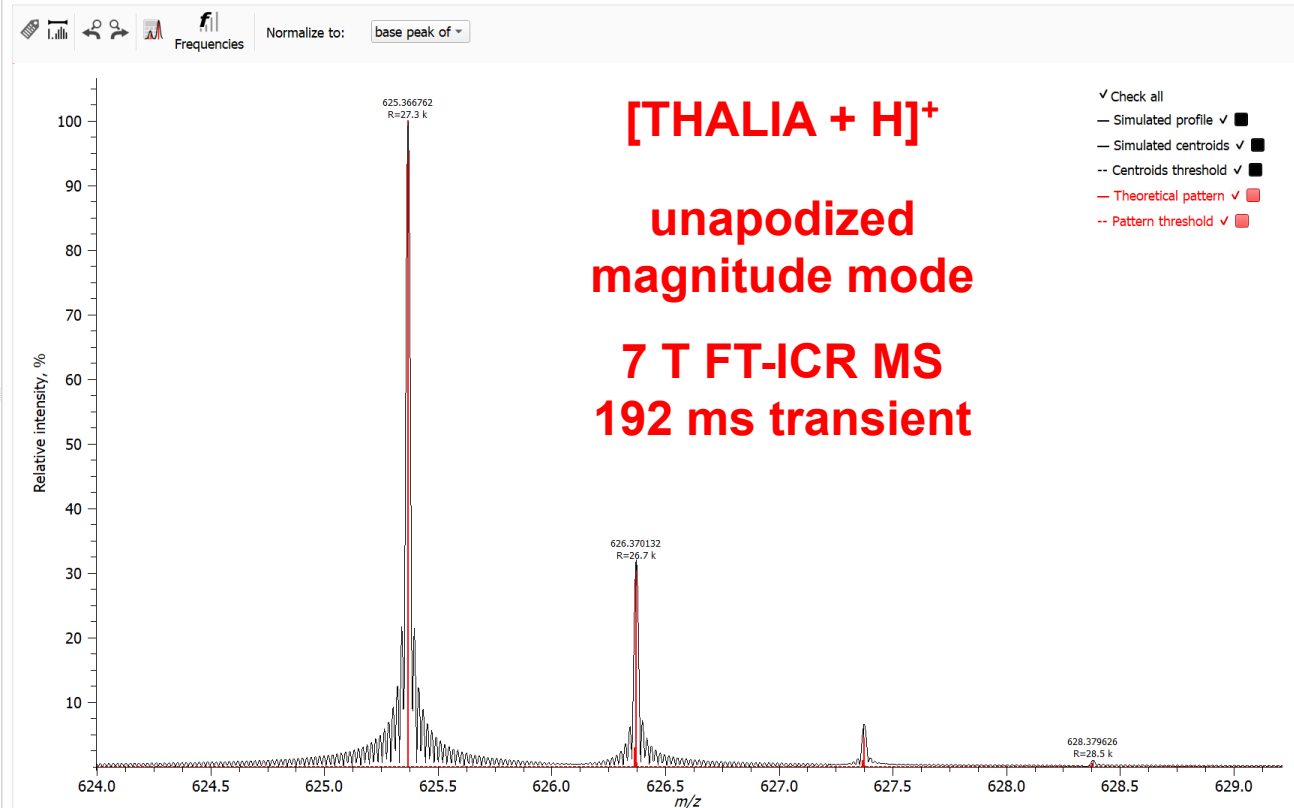
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Mass Spectrum Transient Theor. vs Exp. centroids



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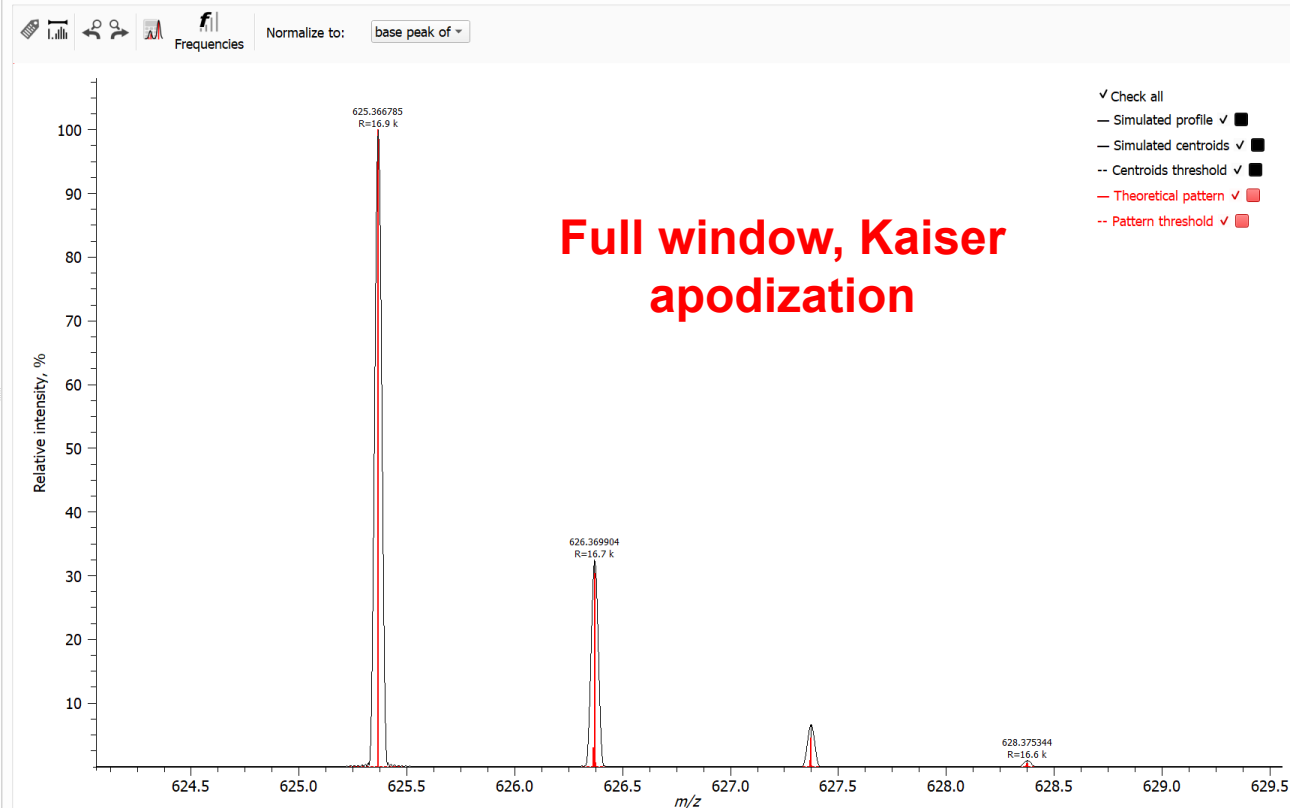
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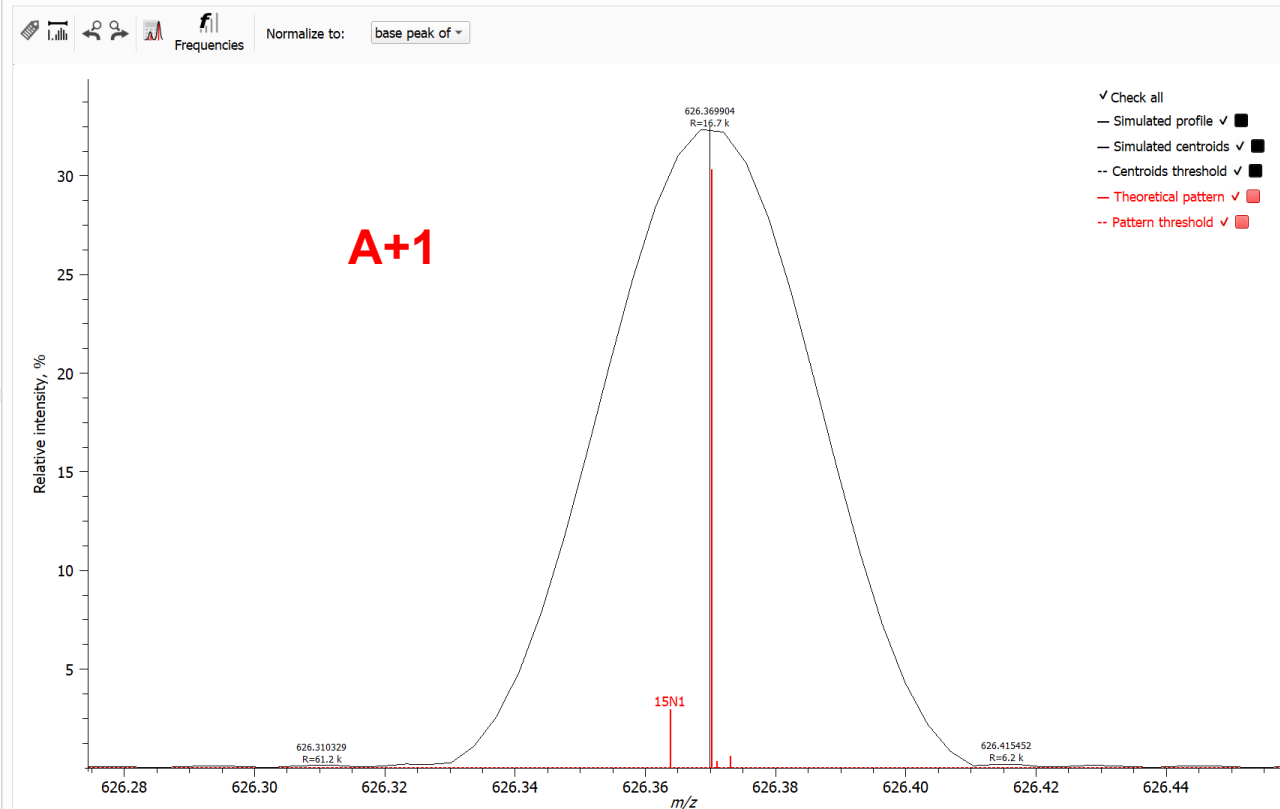
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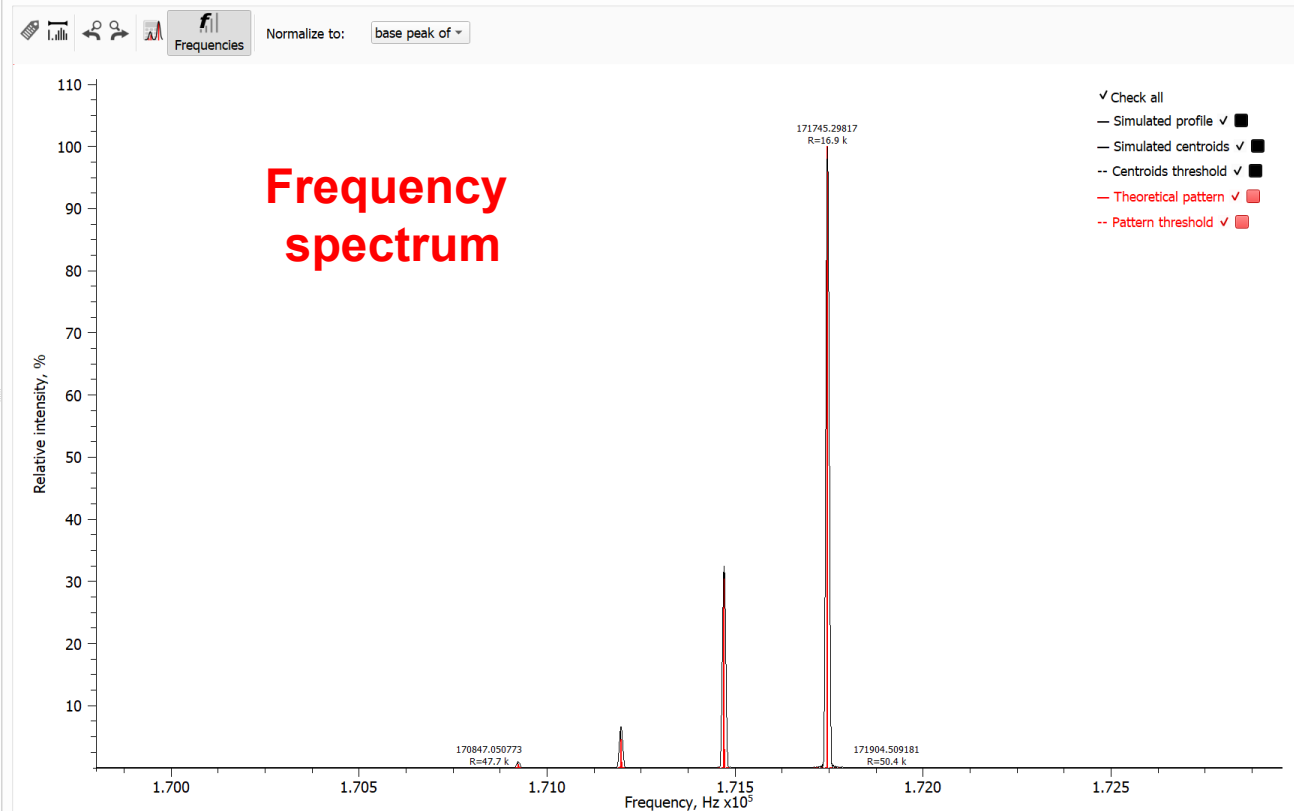
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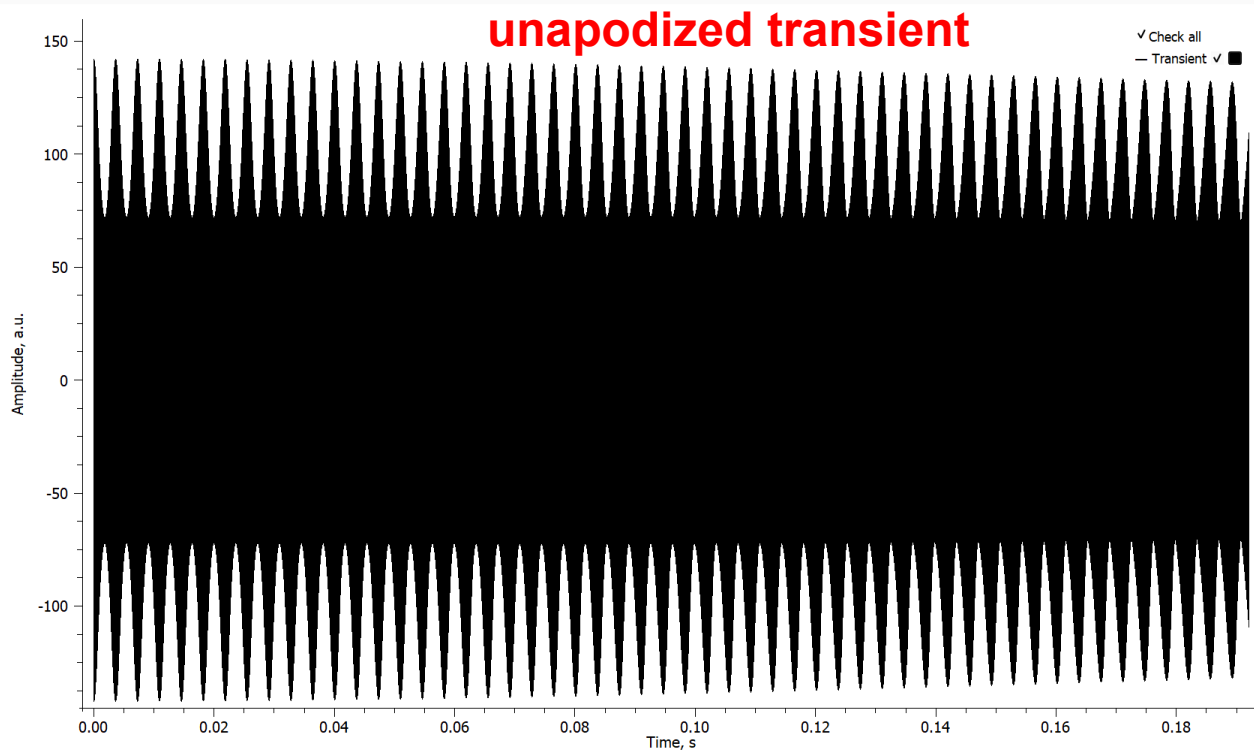
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Mass Spectrum Transient Theor. vs Exp. centroids

Show Apodized



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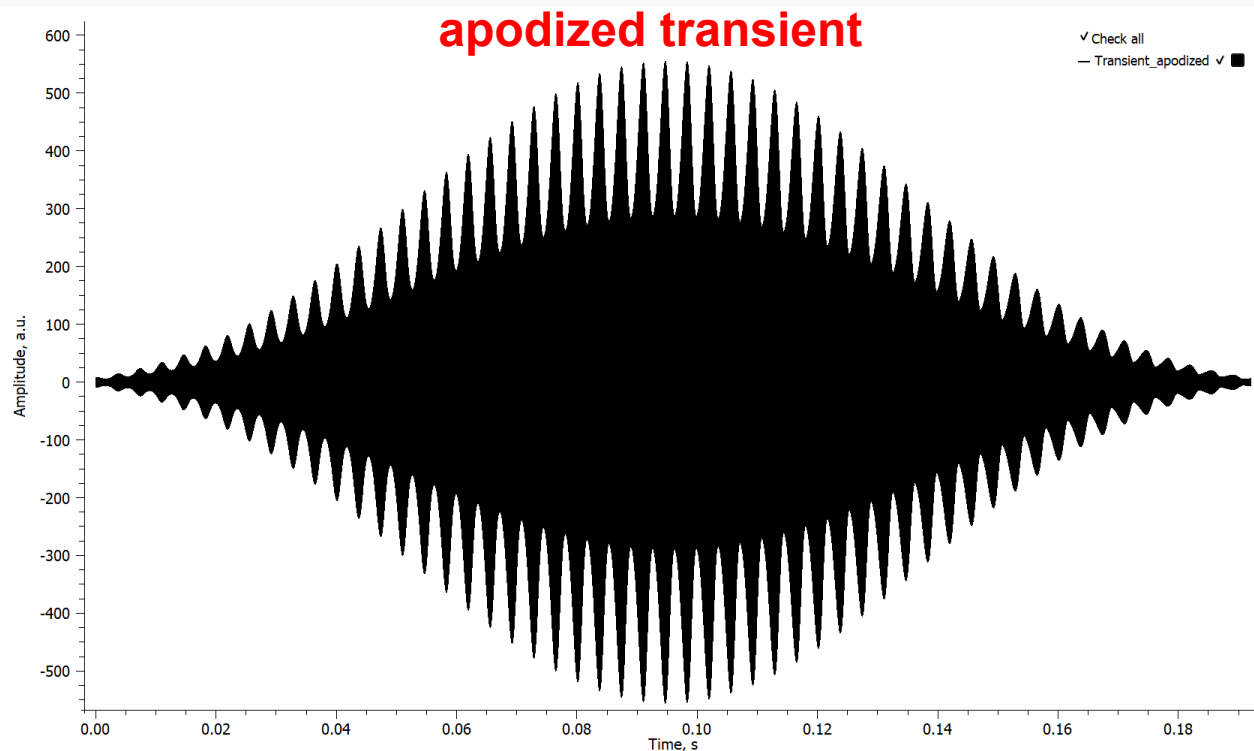
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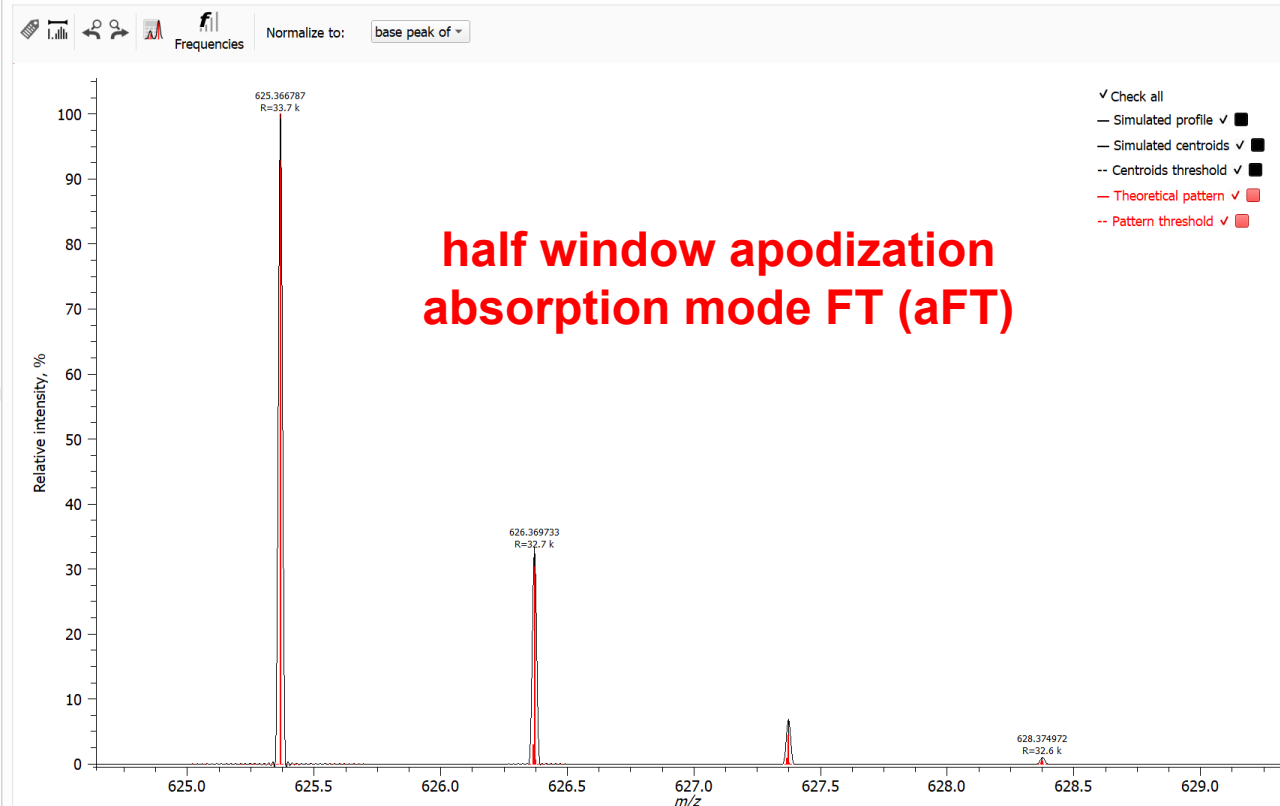
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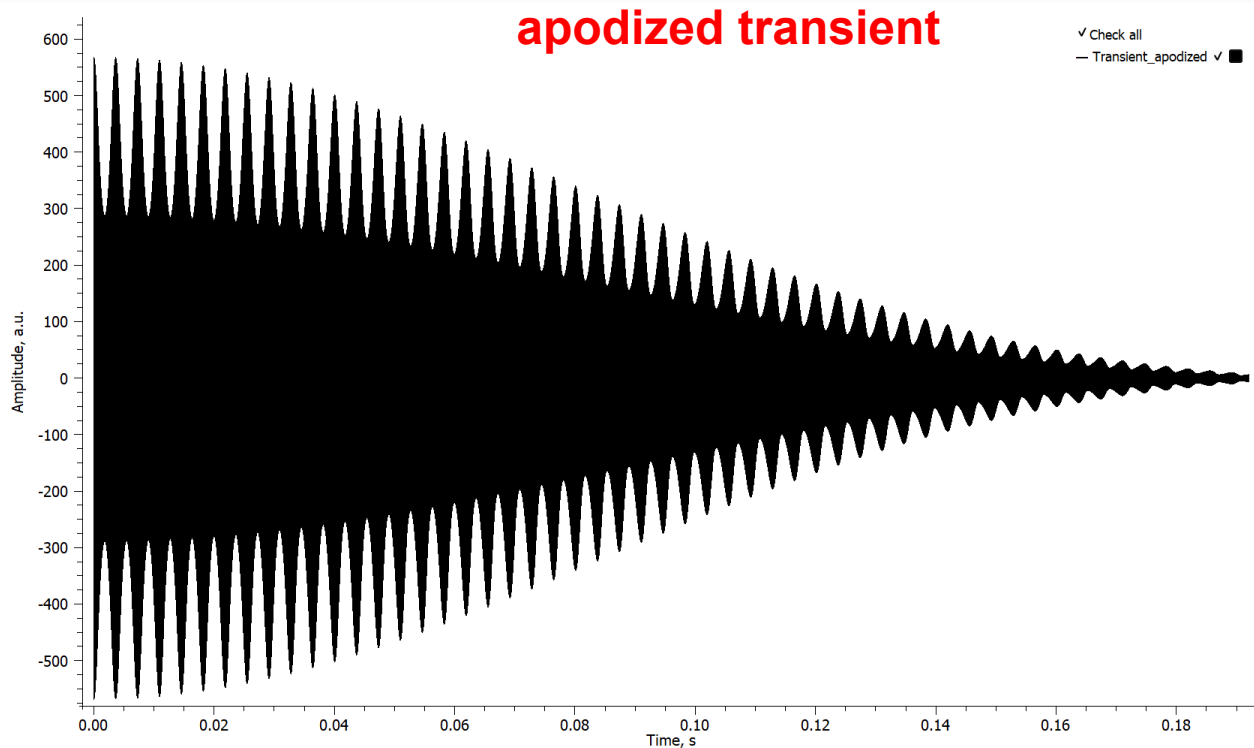
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|----|-----|--|
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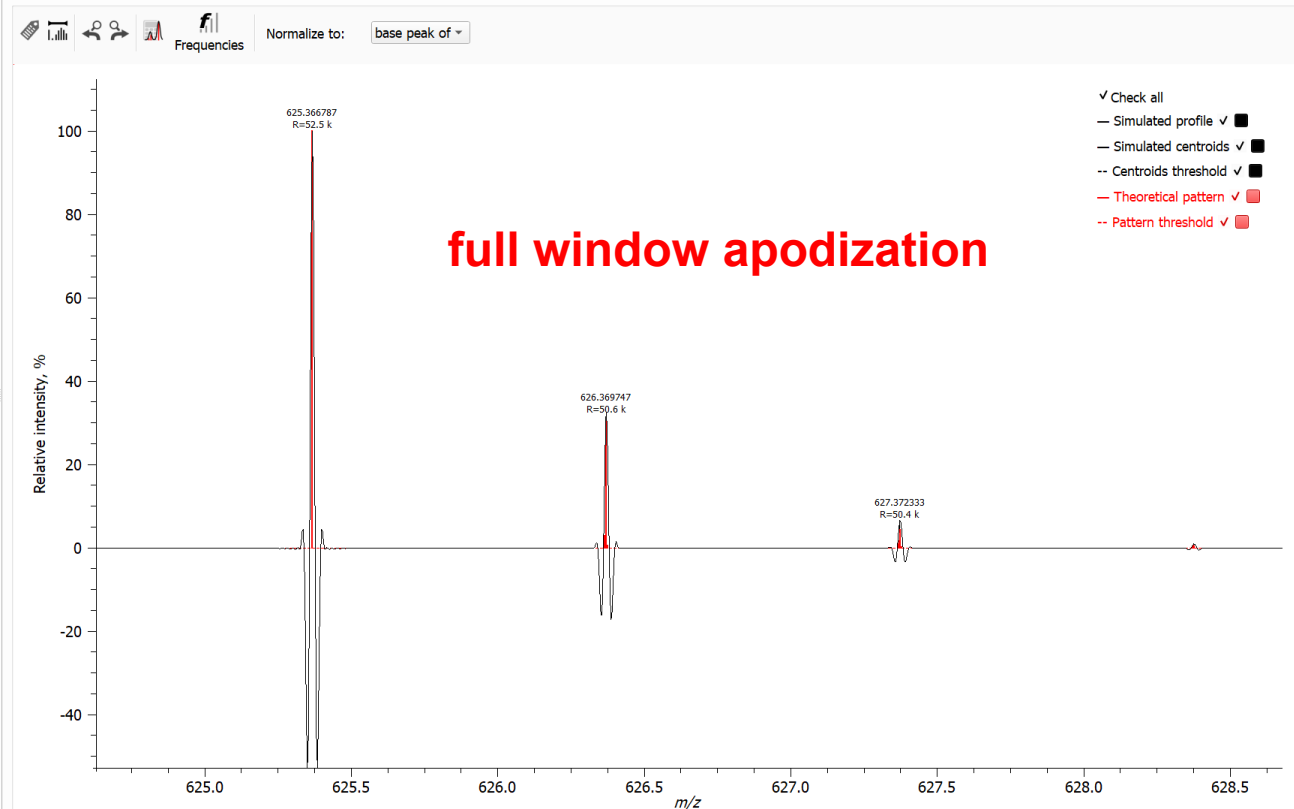
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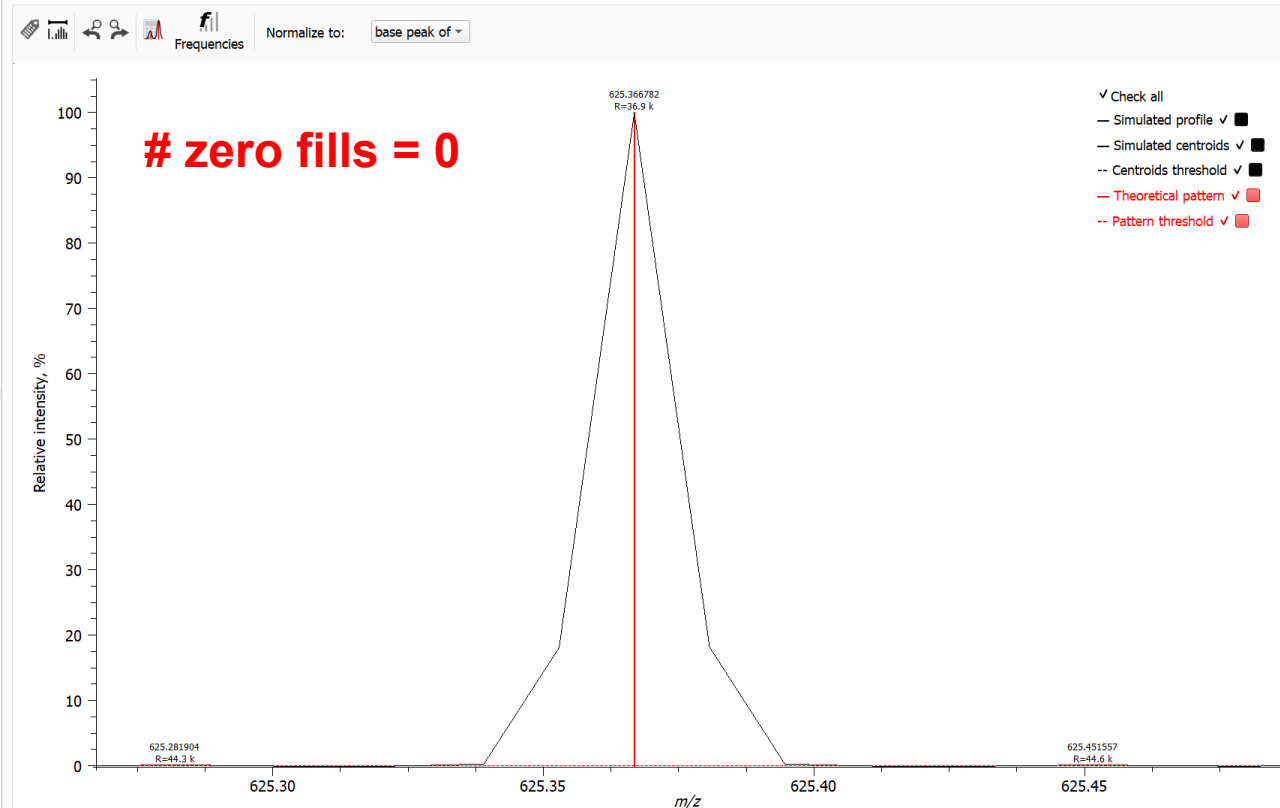
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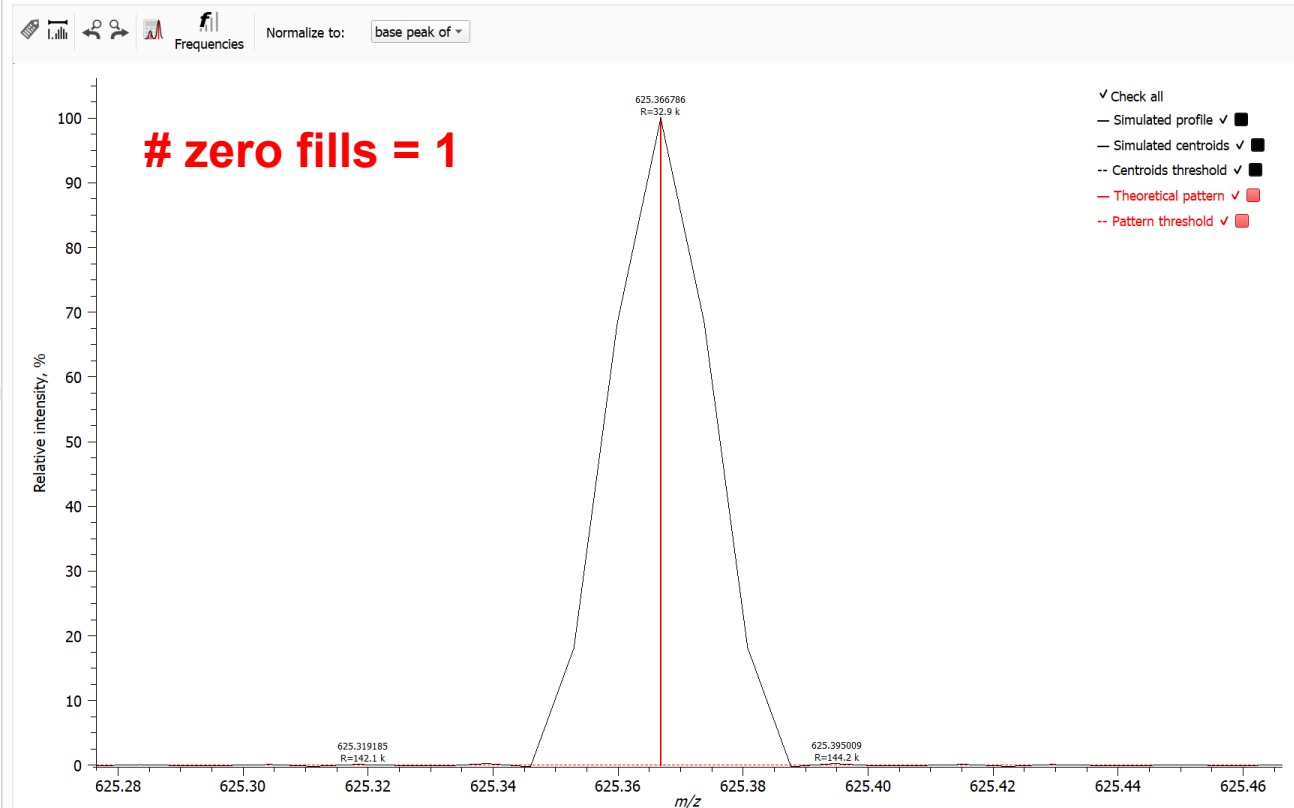
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4. 1.0	[H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ				
5. 1.0	[H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ				
6. 1.0	[H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ				

Mass Spectrum Transient Theor. vs Exp. centroids



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+

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 7TTM 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

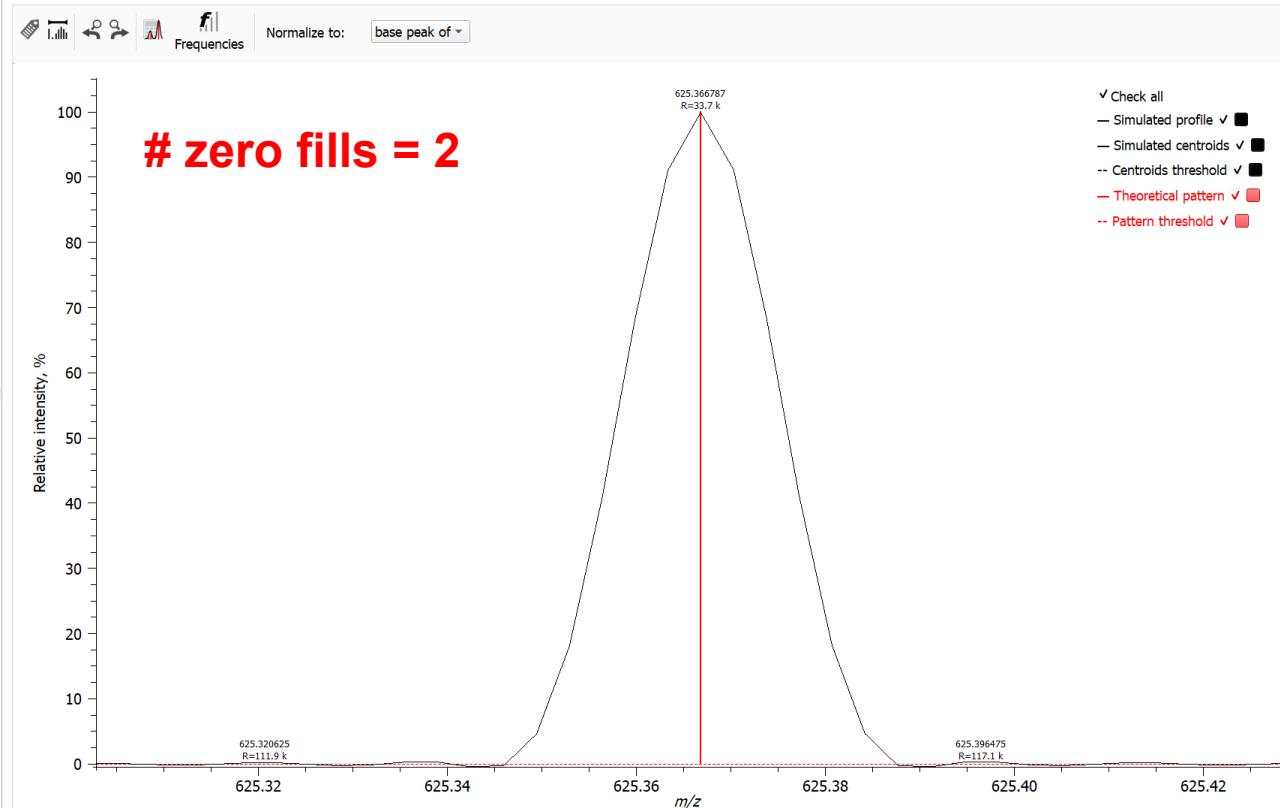
Add Compound Generate from File Example of File

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

Mass Spectrum Transient Theor. vs Exp. centroids



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+

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 7TTM 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

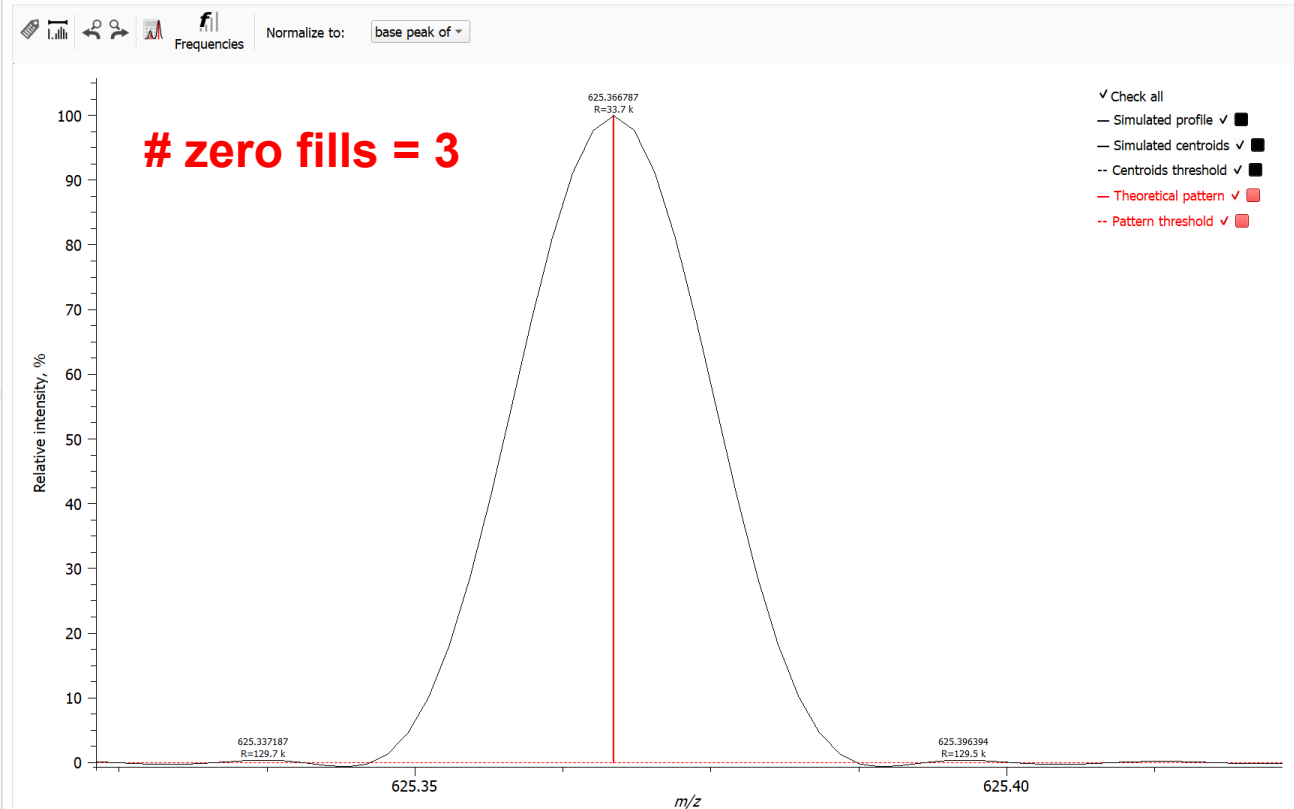
Noise / decay / phase: 0.000 0.00 0.0

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

Mass Spectrum Transient Theor. vs Exp. centroids



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 7TTM 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

Add Compound Generate from File Example of File

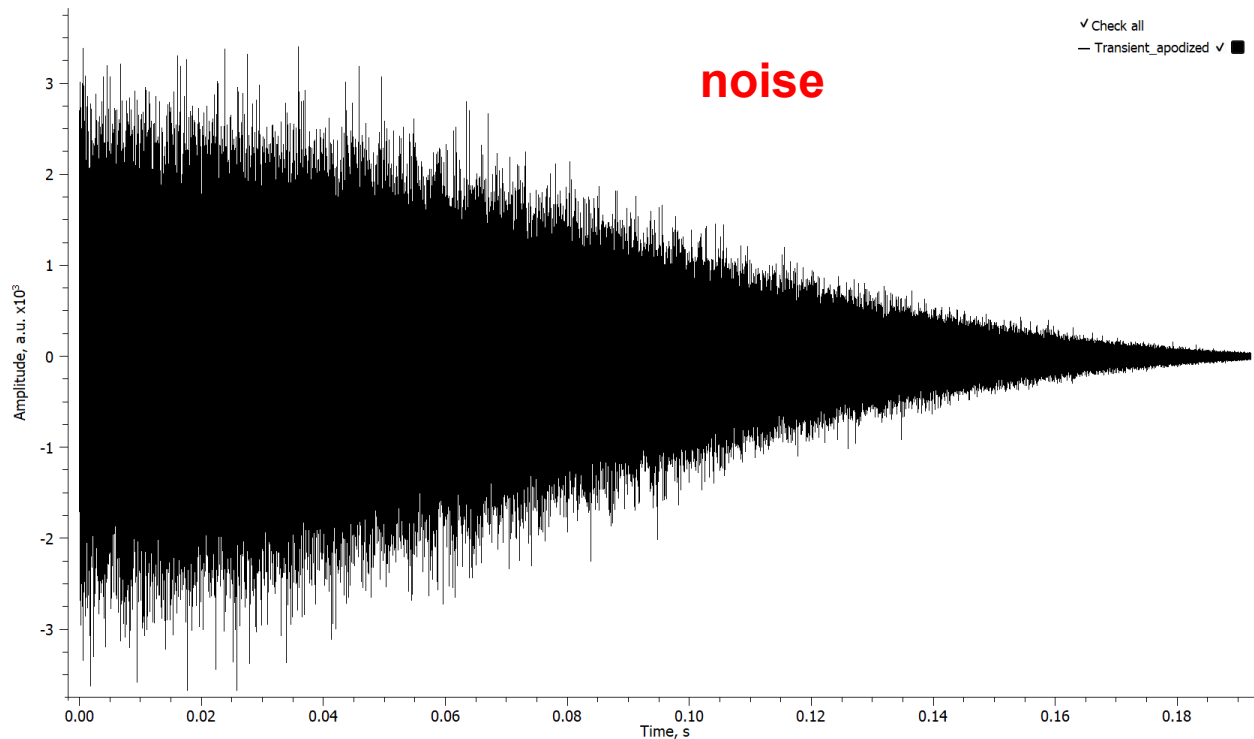
Compound list: select one or multiple items

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

Mass Spectrum Transient Theor. vs Exp. centroids

Show Apodized



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 7TTM 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

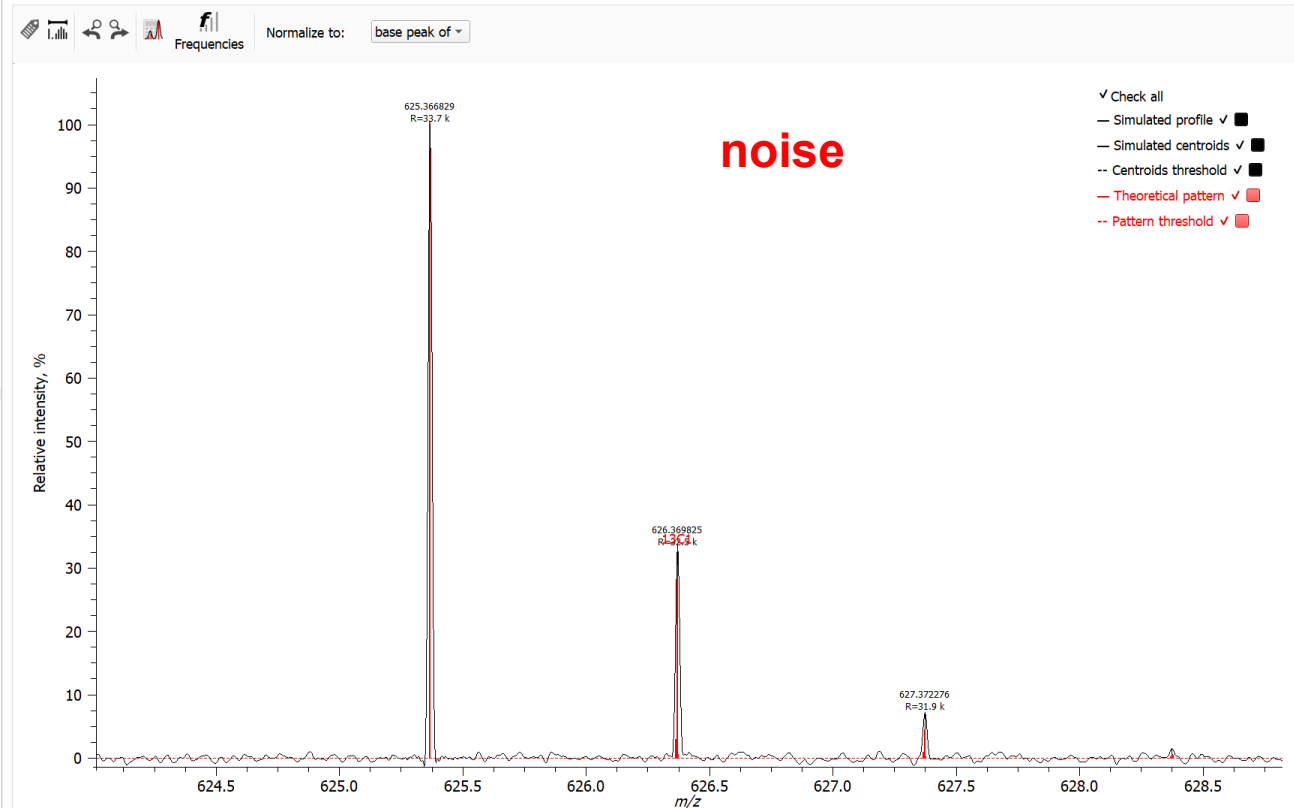
Noise / decay / phase: 2.000 0.00 0.0

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

Mass Spectrum Transient Theor. vs Exp. centroids



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 7TTM 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

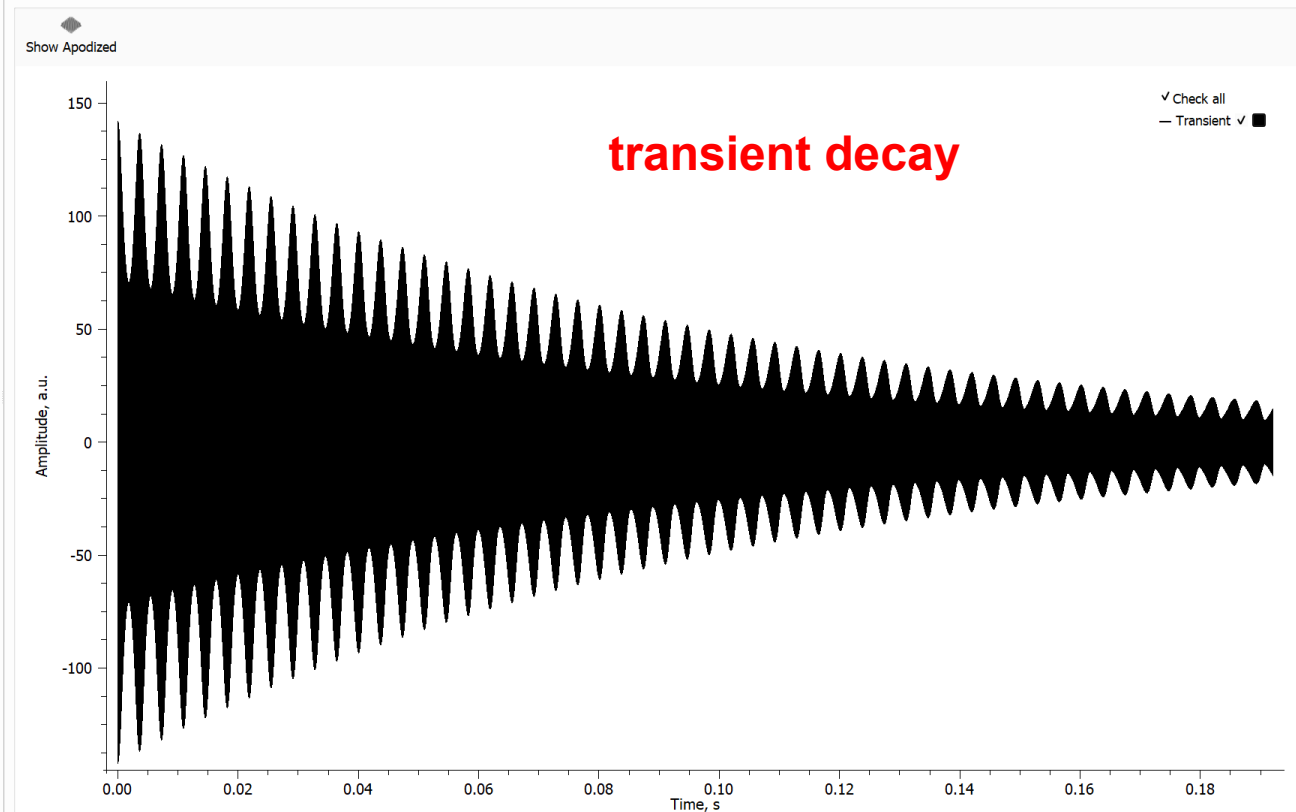
Noise / decay / phase: 0.000 2.00 0.0

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				

Mass Spectrum Transient Theor. vs Exp. centroids



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 7TTM 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

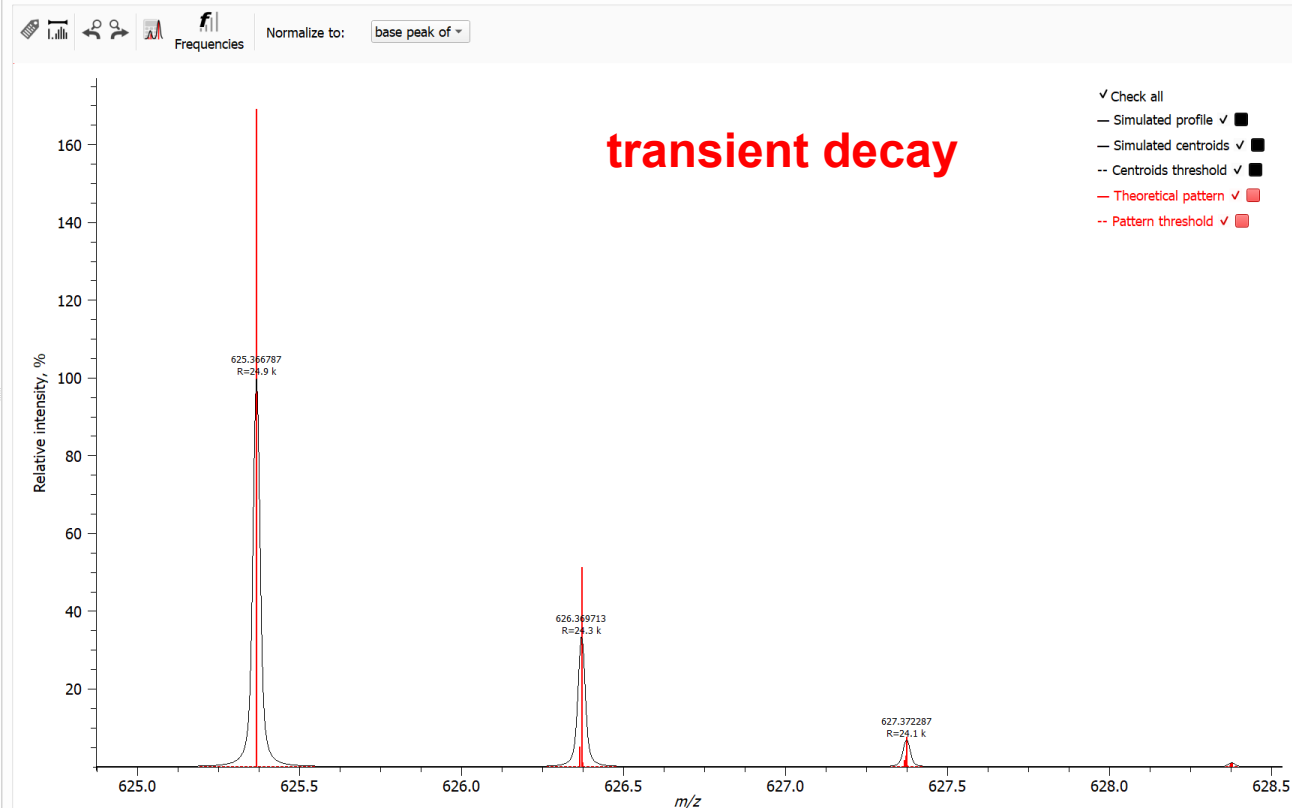
Add Compound Generate from File Example of File

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

Mass Spectrum Transient Theor. vs Exp. centroids



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 7TTM 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

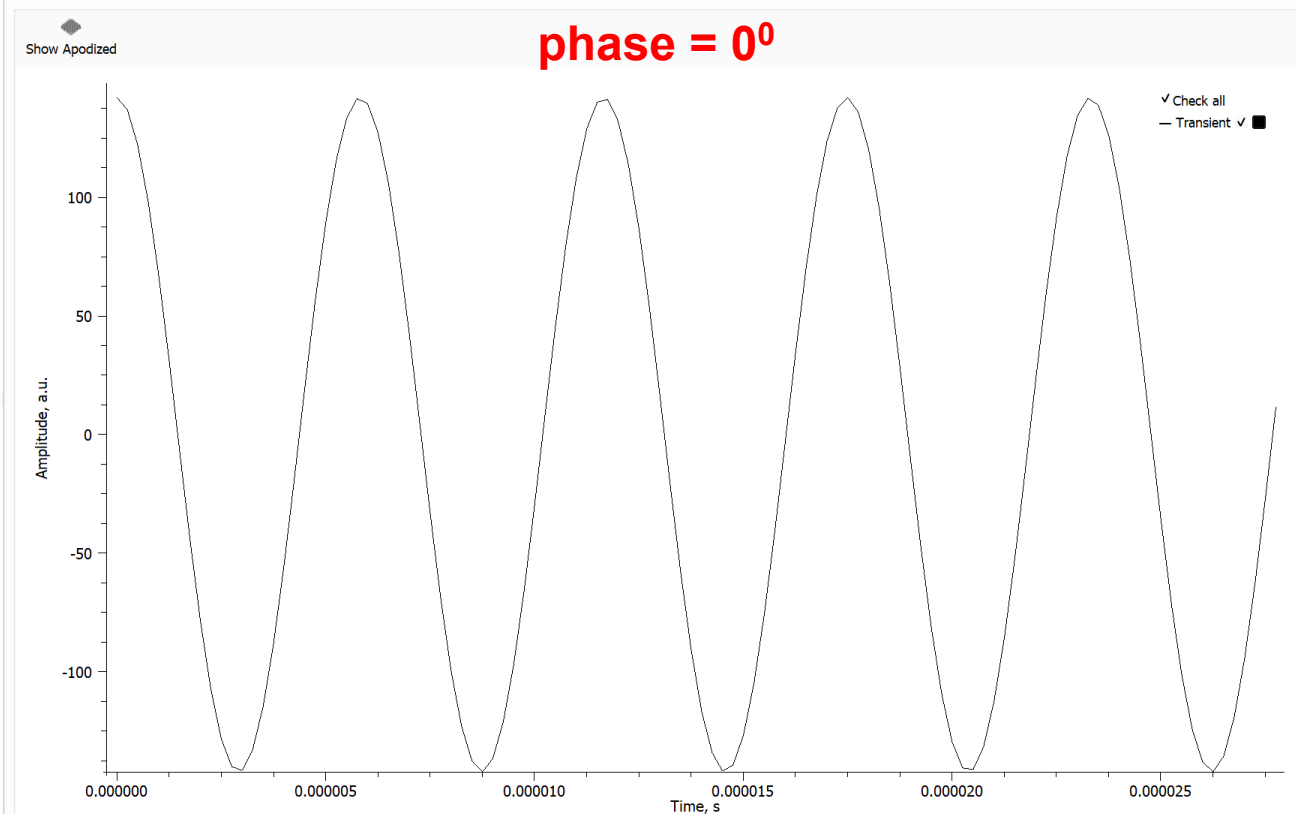
Add Compound Generate from File Example of File

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

Mass Spectrum Transient Theor. vs Exp. centroids



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 7TTM 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

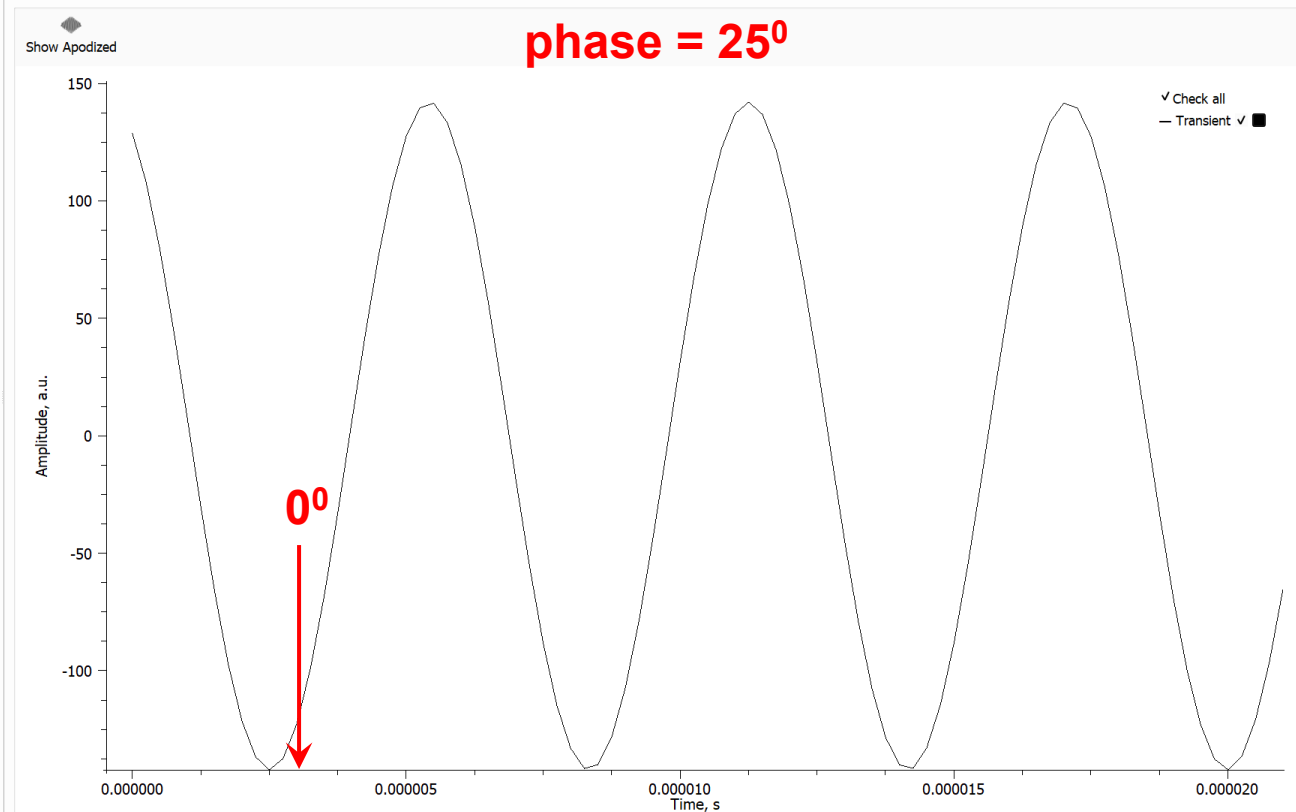
Noise / decay / phase: 0.000 0.00 25.0

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



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+

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 7TTM 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

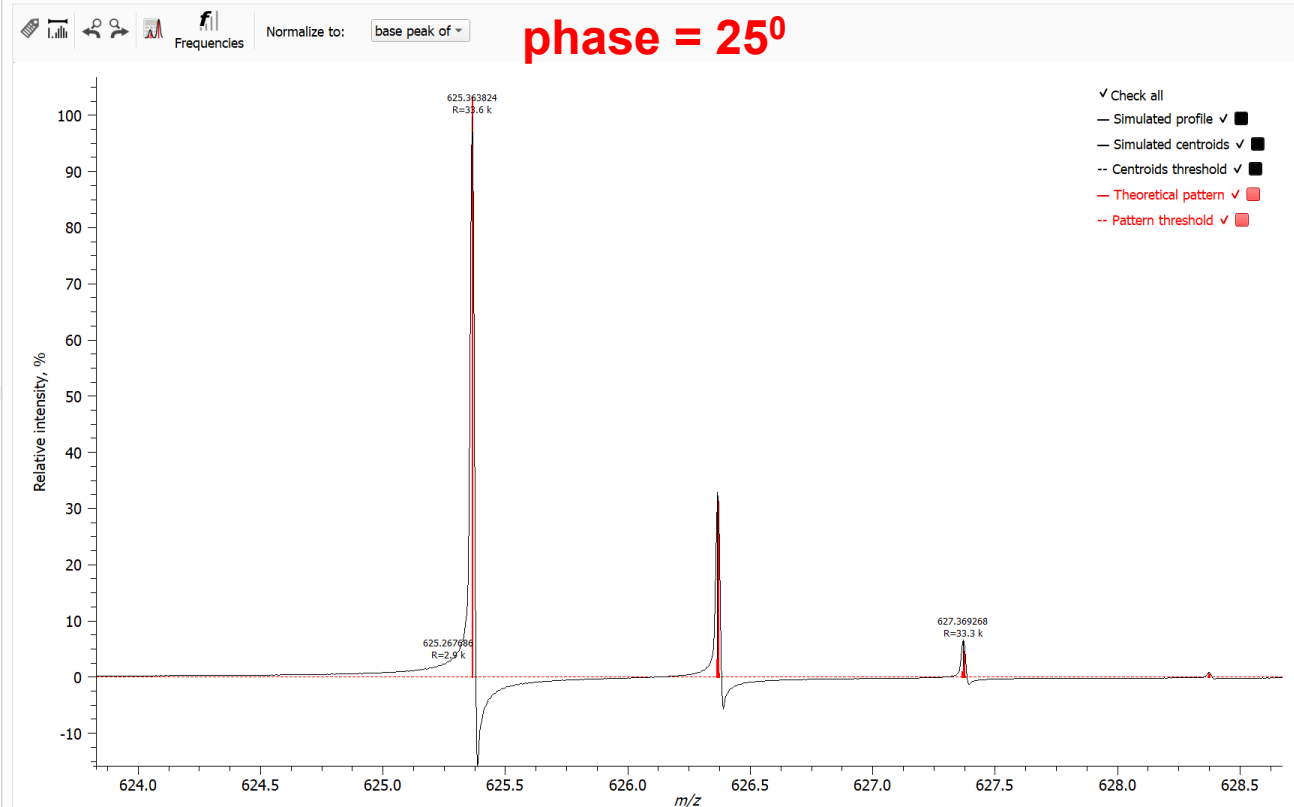
Noise / decay / phase: 0.000 0.00 25.0

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



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 7TTM 1

Resolution: LTQ-FT 21T 10000

FT-ICR 7T 25k@400m/z (T=0.192 s)

FT-ICR 9.4T 0.100

FT-ICR 10T semikaiser

FT-ICR 12T 4 MHz

FT-ICR 15T

Orbitrap ClassicTM 0.0

Orbitrap XLTM

Orbitrap VelosTM

Orbitrap EliteTM

Orbitrap QExTM

Orbitrap QExFTM

Orbitrap QExUHMRTM

Orbitrap ExplorisTM

Orbitrap QExHFTM

Orbitrap FusionTM

Compound list: select one or more

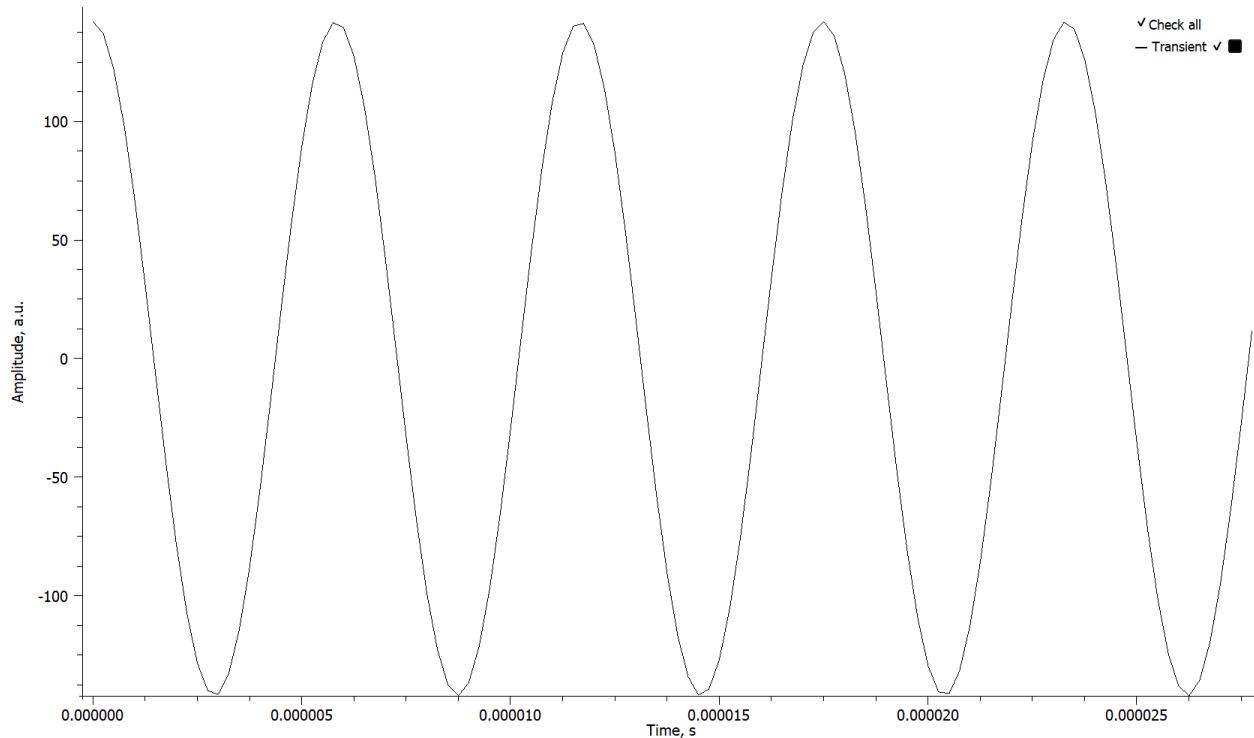
Remove Selected Remove All

1. 1.0 [H49 C28 N8 C]

Mass Spectrum Transient Theor. vs Exp. centroids

Show Apodized

different FTMS instruments



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: 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

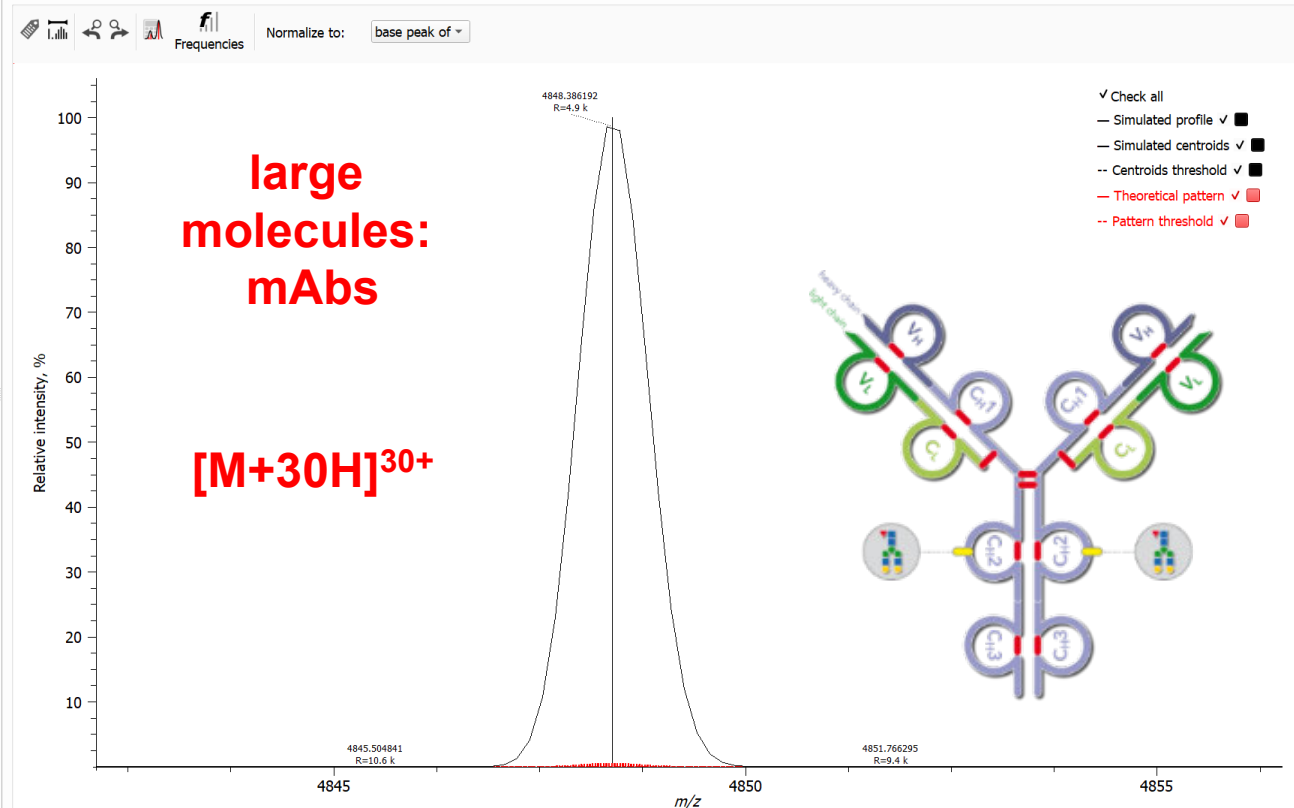
Noise / decay / phase: 0.000 0.00 0.0

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
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™, absorp

Mass Spectrum Transient Theor. vs Exp. centroids



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: 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

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

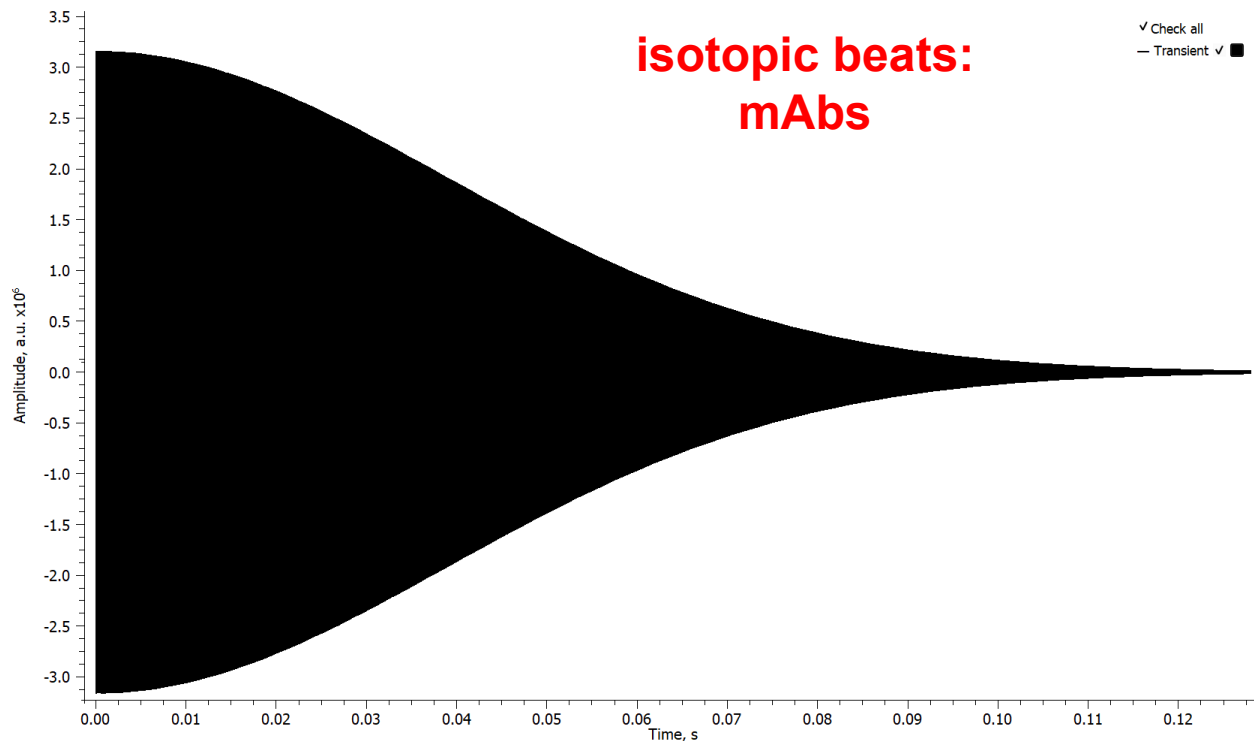
1. 1.0 [H49 C28 N8 O8 (-1e)], z=+1, DBE=9.0, R=25k@400m/z (T=0.192 s), LTQ

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™, absorp

Mass Spectrum Transient Theor. vs Exp. centroids

Show Apodized



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

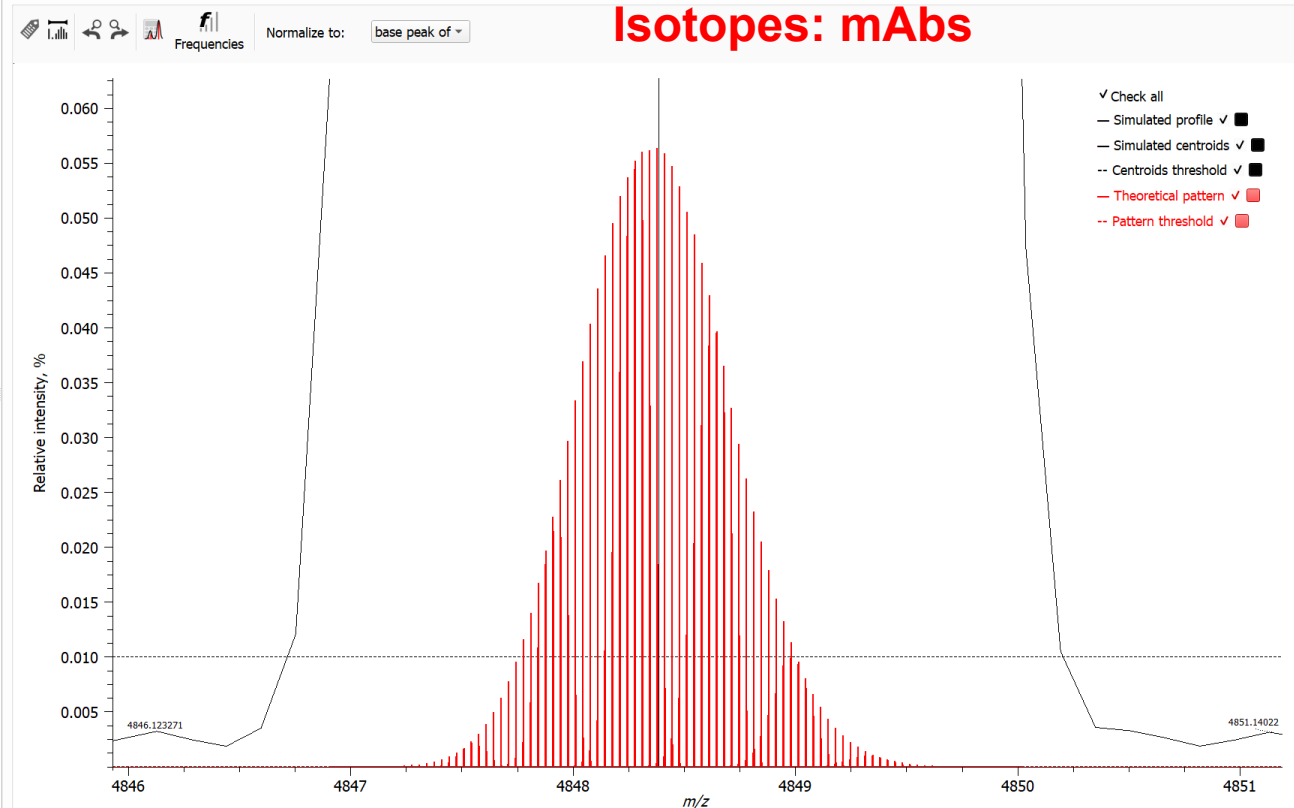
Add Compound Generate from File Example of File

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



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

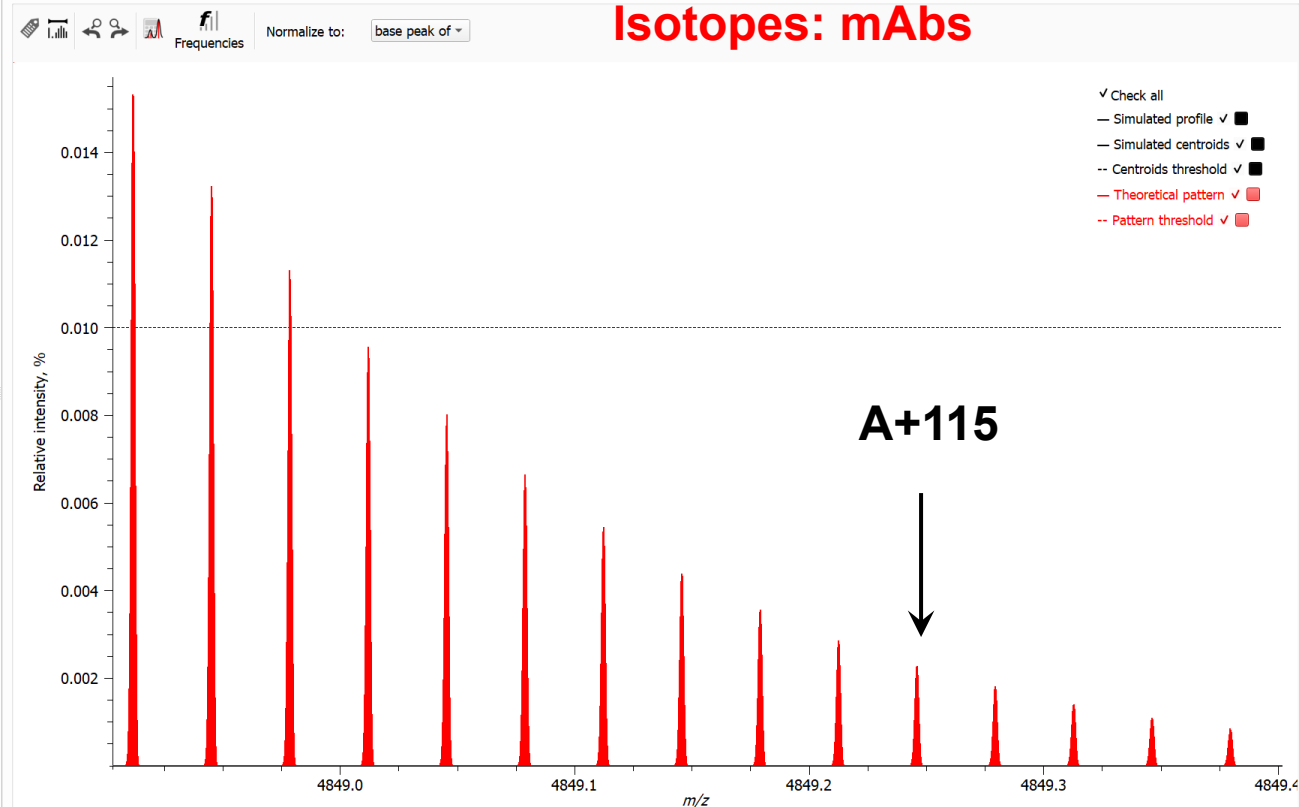
Add Compound Generate from File Example of File

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



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

☐ 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

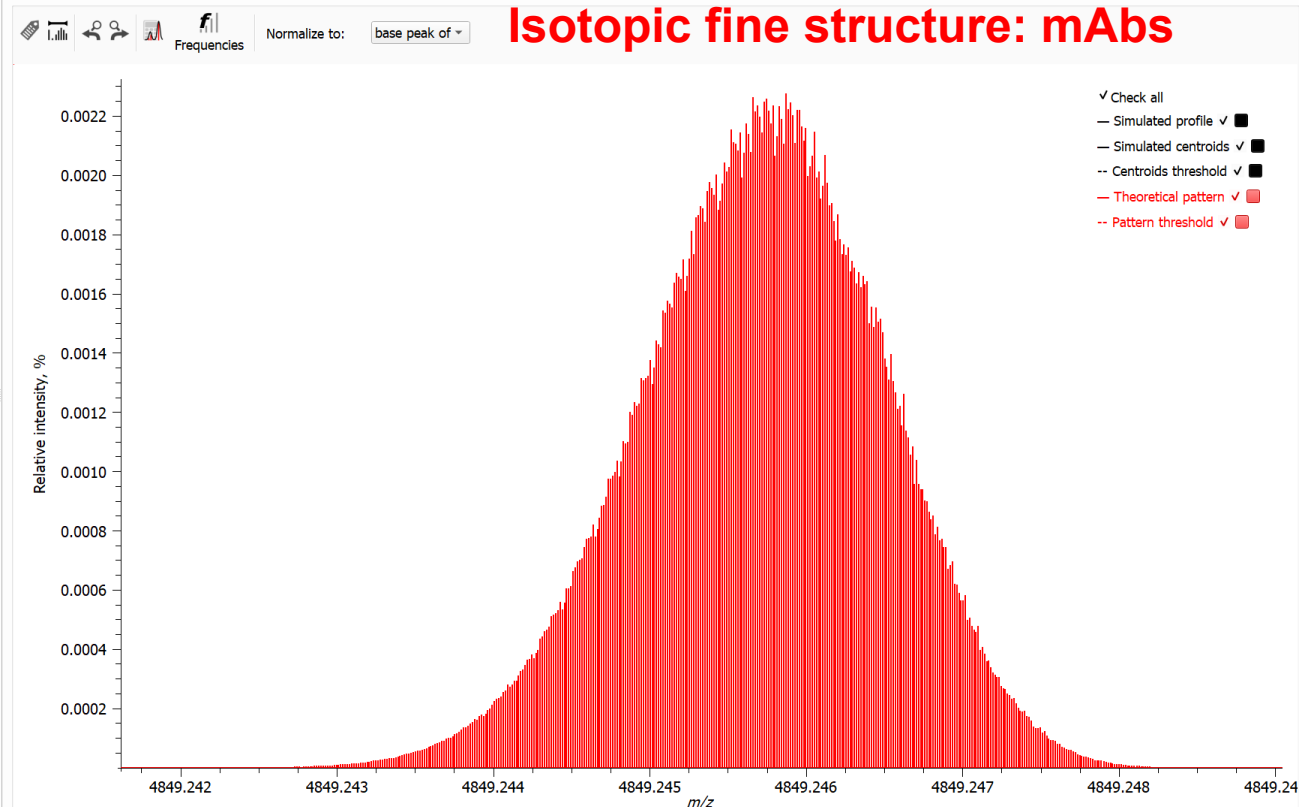
Zero-fills / sample rate: 2 1 MHz

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

Mass Spectrum Transient Theor. vs Exp. centroids



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)

Why to Simulate the FTMS Data?

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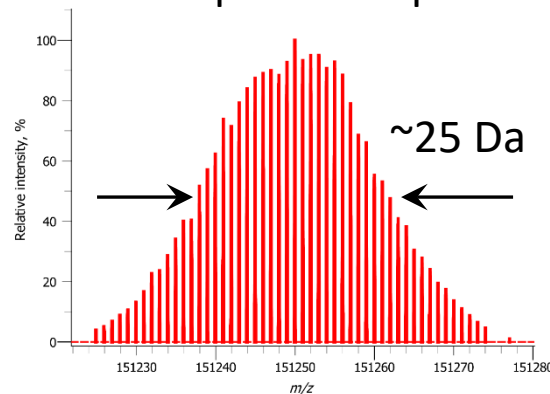
Question 12. A typical monoclonal antibody (approx. 150 kDa) has an isotopic distribution that is 25 Da wide (measured as full-width at half-maximum) and appears at m/z 5000, when sprayed intact, under native conditions. With an Orbitrap FTMS (eFT mass spectra) having available resolution settings 15,000; 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

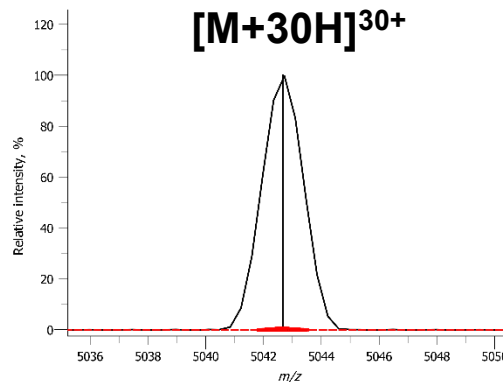
Submit

Monoclonal Antibody (mAb) Analysis

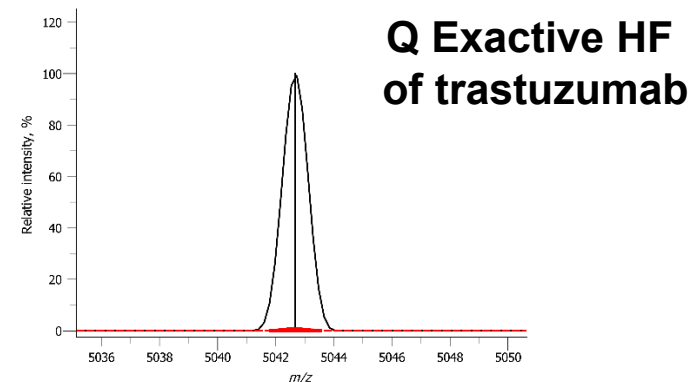
Isotopic Envelope



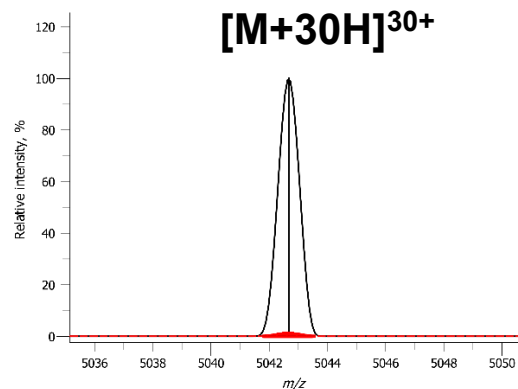
15k @ m/z 200: 3'100



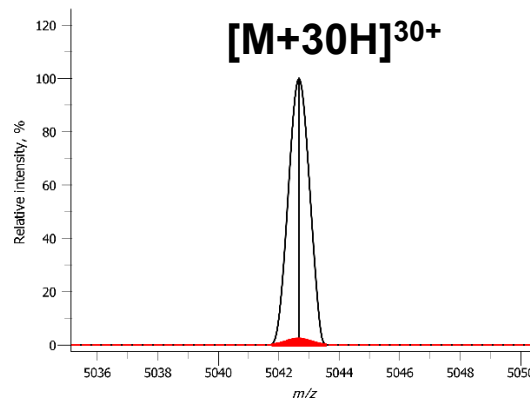
30k @ m/z 200: 4'900



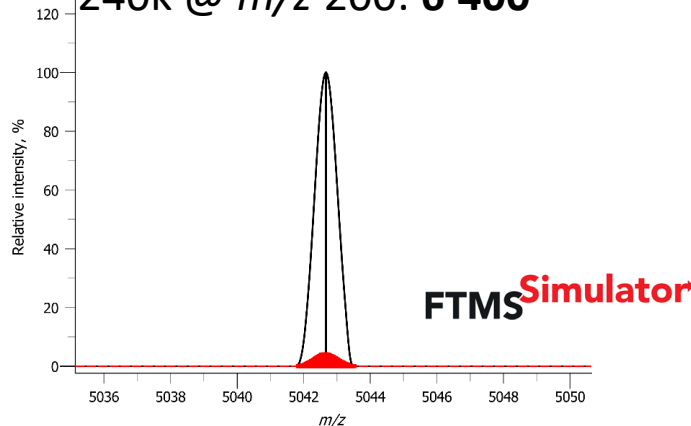
60k @ m/z 200: 6'000



120k @ m/z 200: 6'300

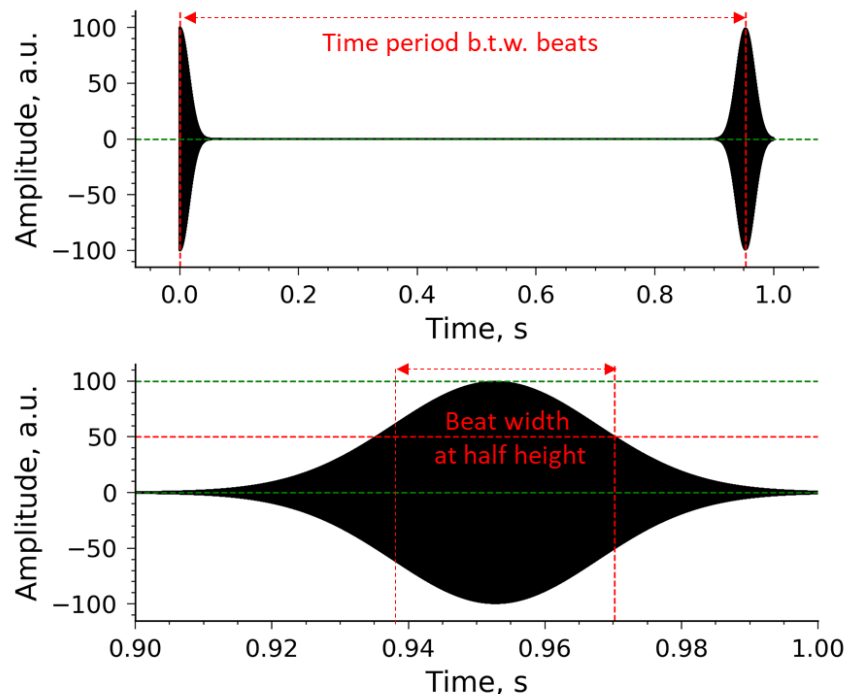


240k @ m/z 200: 6'400



Isotopic Beats in FTMS Transients

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



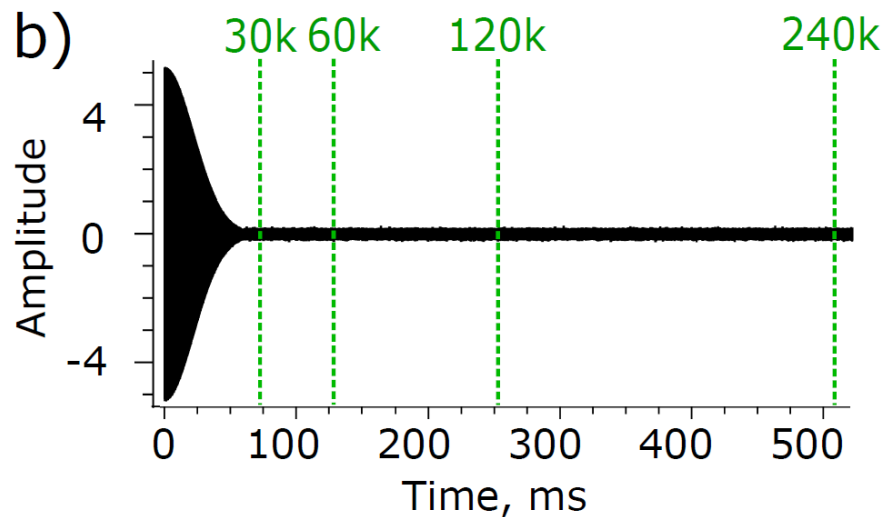
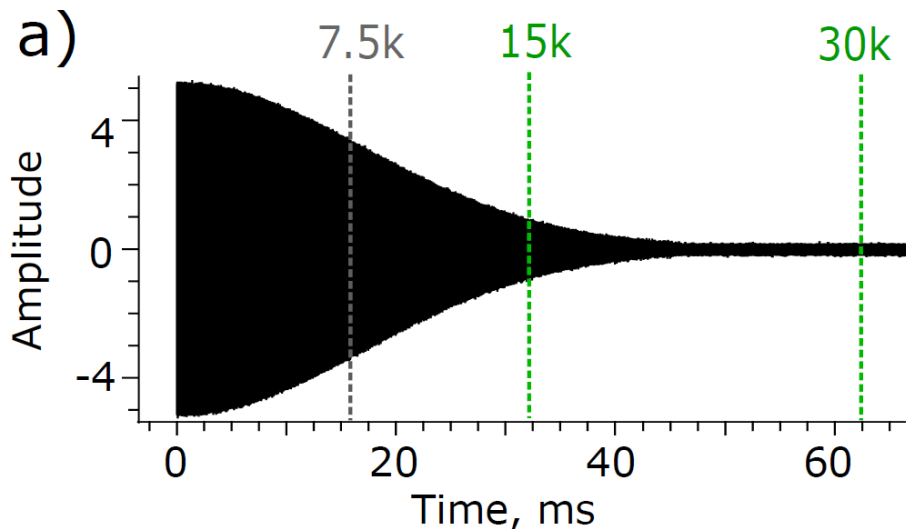
Hofstadler, S. A.; Bruce, J. E.; Rockwood, A. L.; Anderson, G. A.; Winger, B. E.; Smith, R. D. **Isotopic beat patterns in Fourier transform ion cyclotron resonance mass spectrometry: implications for high resolution mass measurements of large biopolymers.** *IJMS and Ion Processes* 1994, 132, 109-127

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

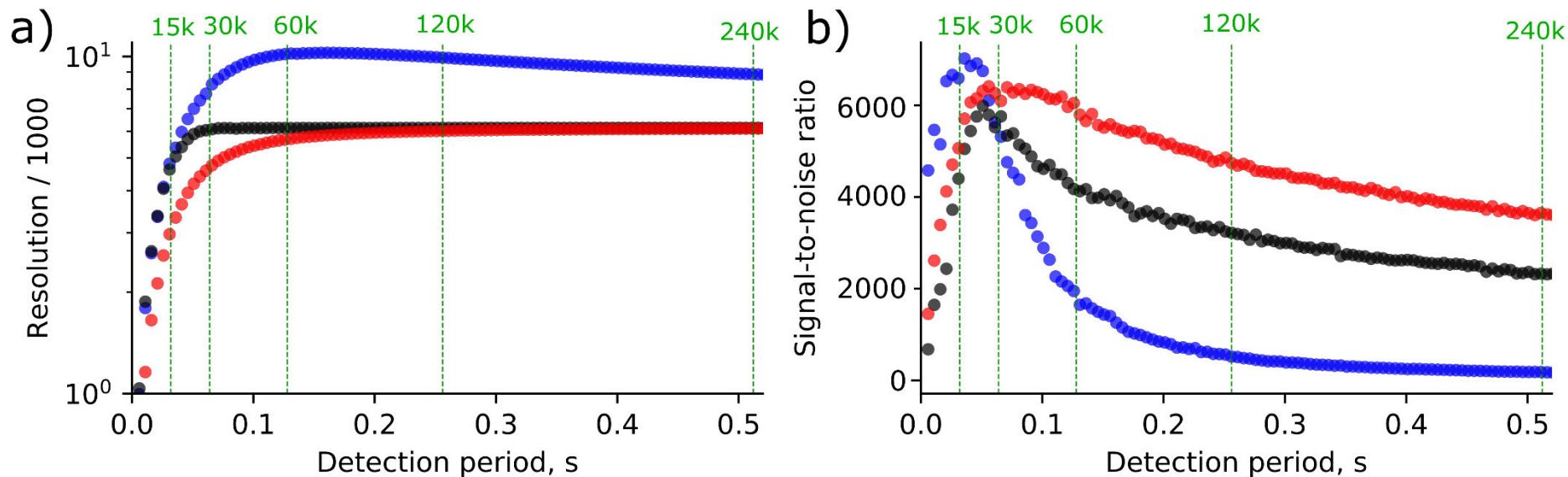
- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation with FTMS Simulator



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

Resolution and SNR Dependencies: mAbs

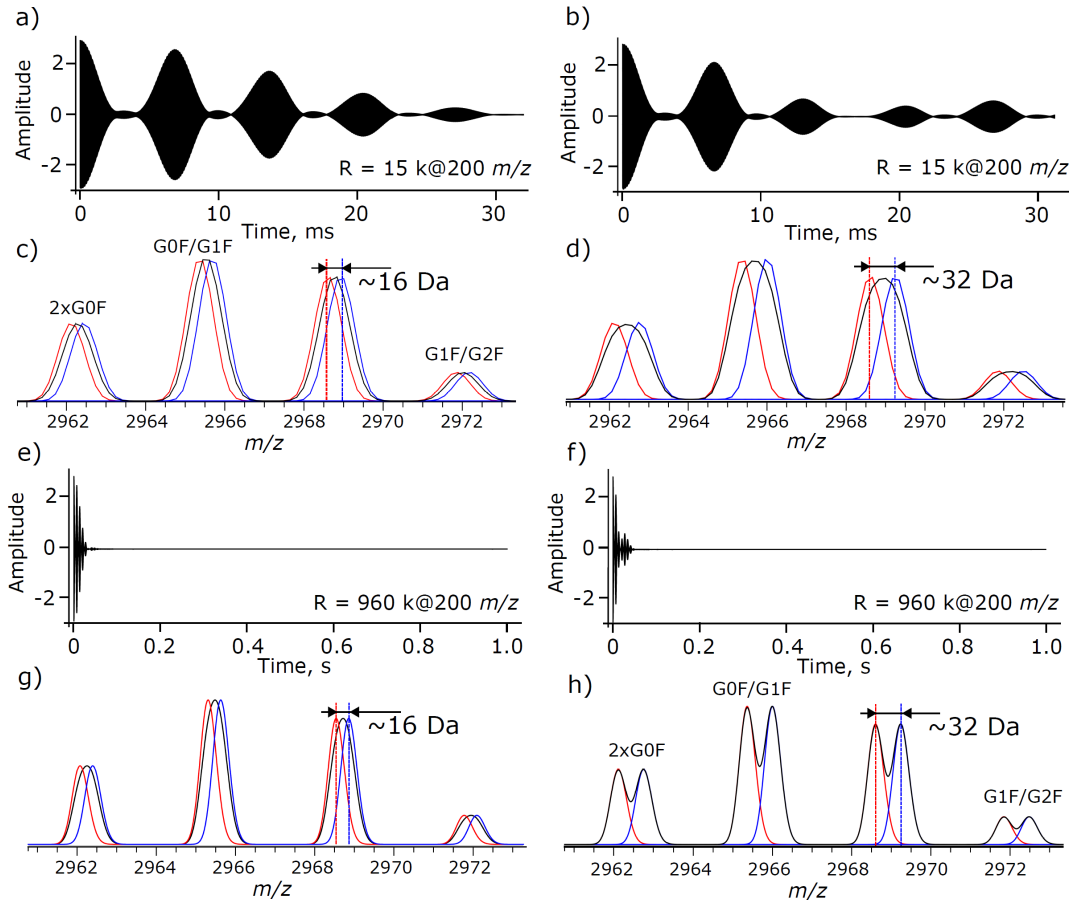
- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, $[M+30H]^{30+}$
- Transient simulation and data processing with FTMS Simulator



- Colors: unapodized aFT; half window apodized aFT; full window apodized aFT

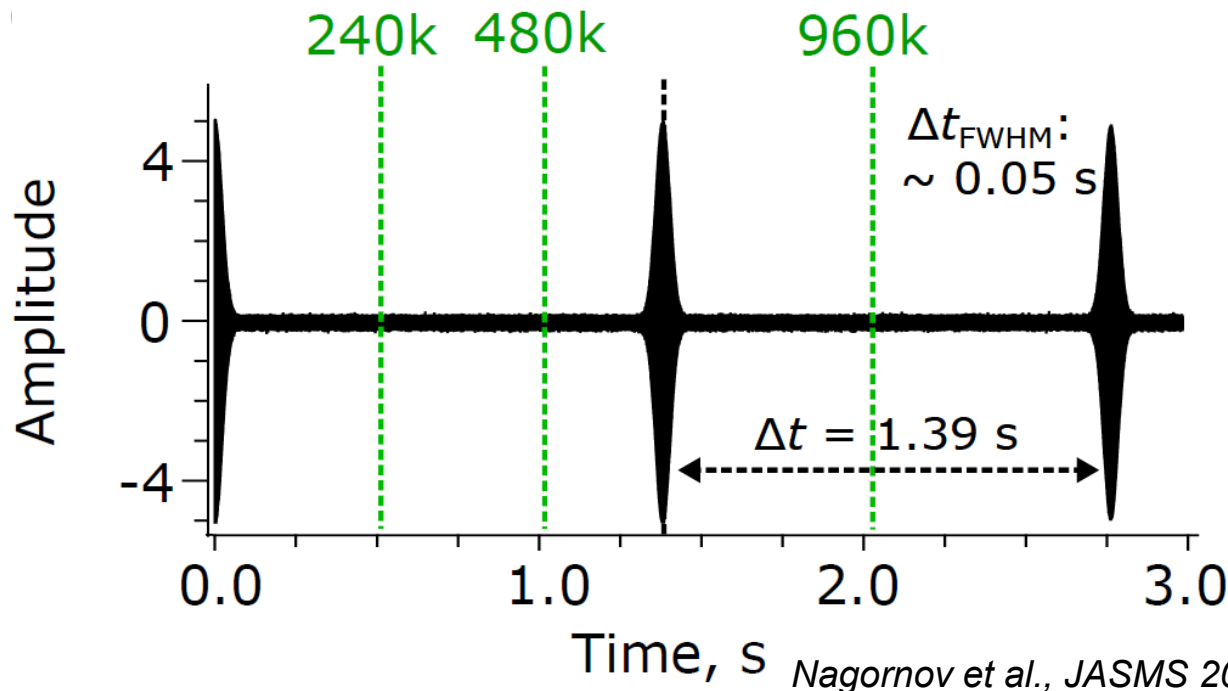
Consequences for mAb Analysis

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



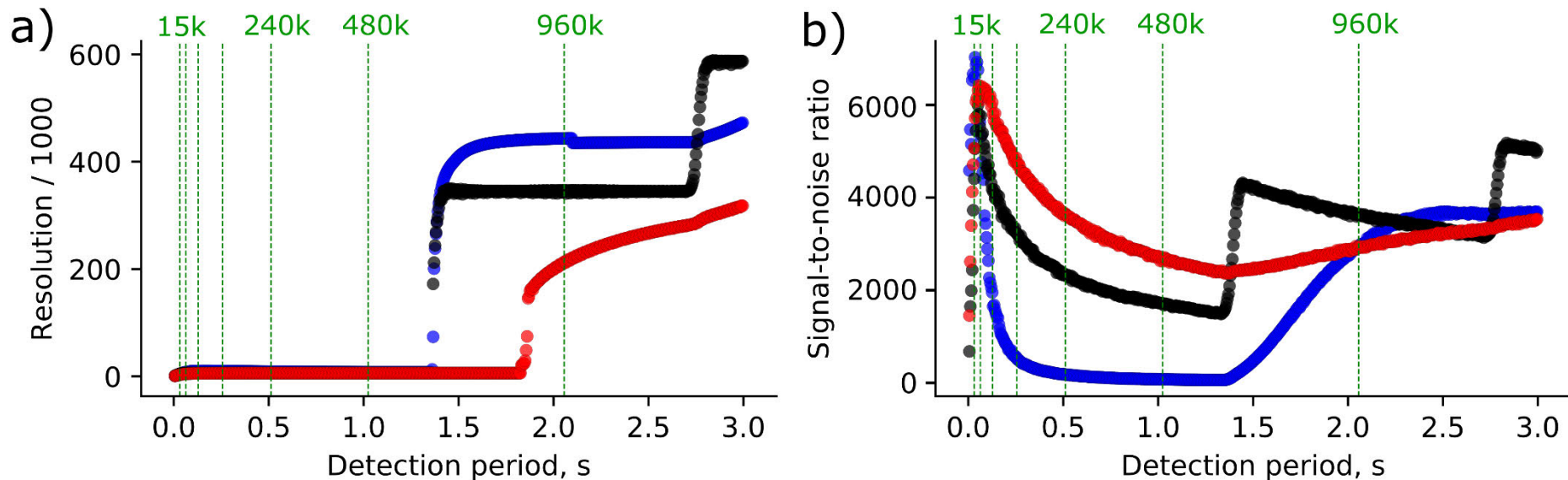
Isotopic Beats in **High-Resolution** FTMS: mAbs

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



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



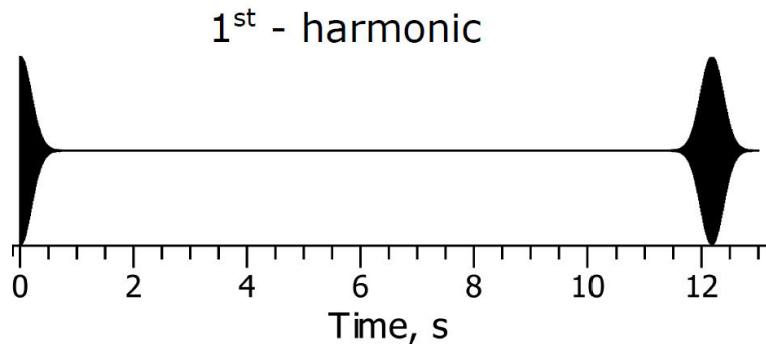
- Colors: unapodized aFT; half window apodized aFT; full window apodized aFT

How & Why to Simulate the FTMS Data?

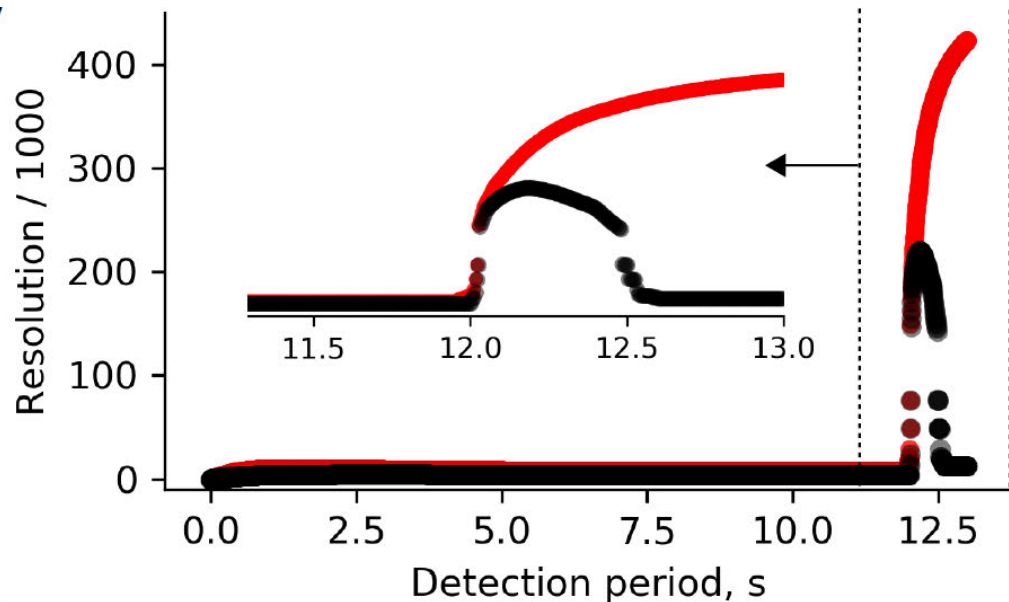
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Resolution «Resonance» in the mFT ICR

- Sample: a biopolymer with an average mass of 150 kDa, $\text{H}_{10282}\text{C}_{6646}\text{N}_{1724}\text{O}_{2141}\text{S}_{44}$
- Instrument: a 12 T FT-ICR MS, $[\text{M}+10\text{H}]^{10+}$, full window (Kaiser-type) apodization
- Simulations: FTMS Simulator



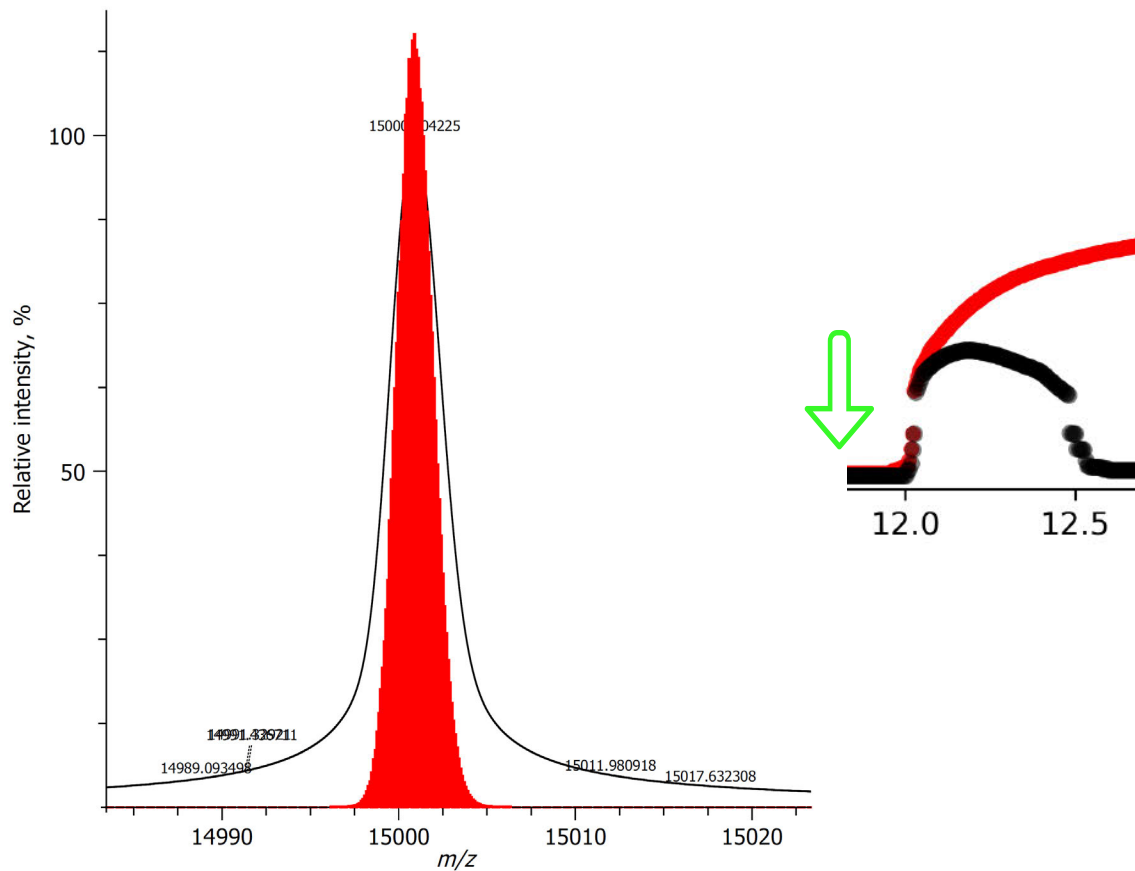
To be verified experimentally!



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

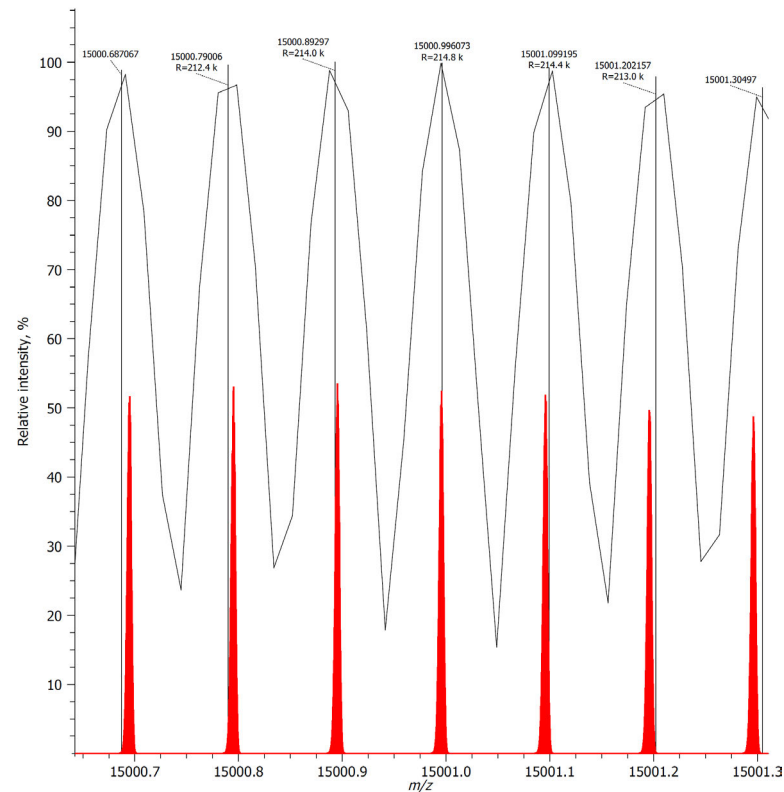
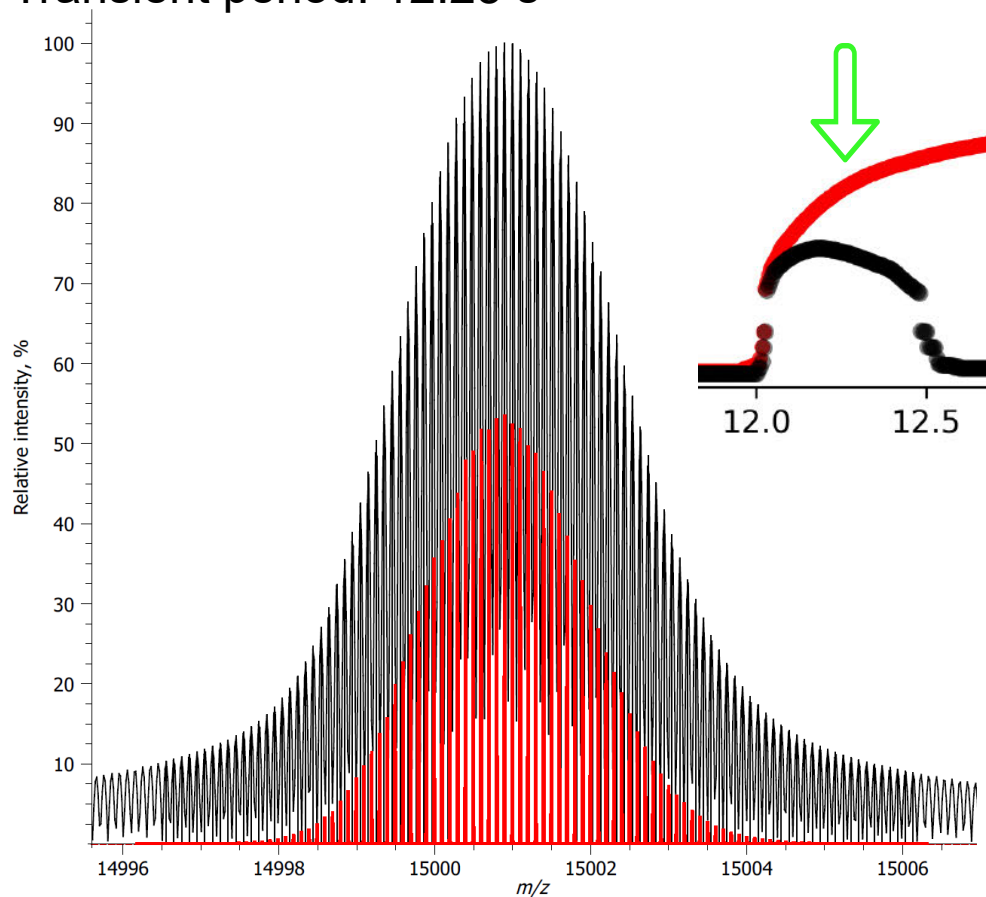
The 2nd Beat Challenge: Full Window Apodization

- Transient period: 11.5 s



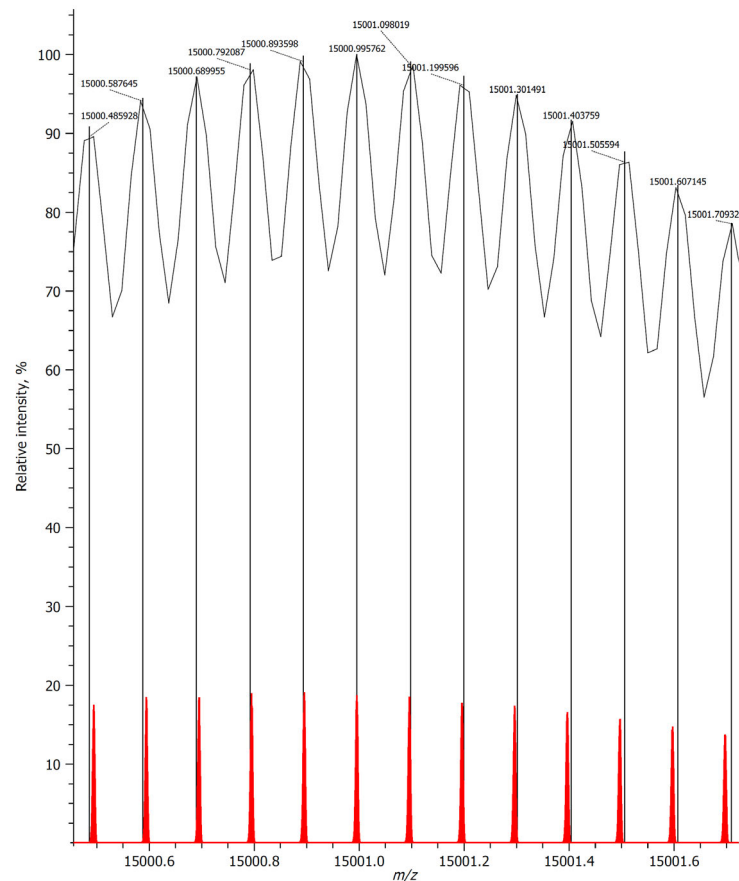
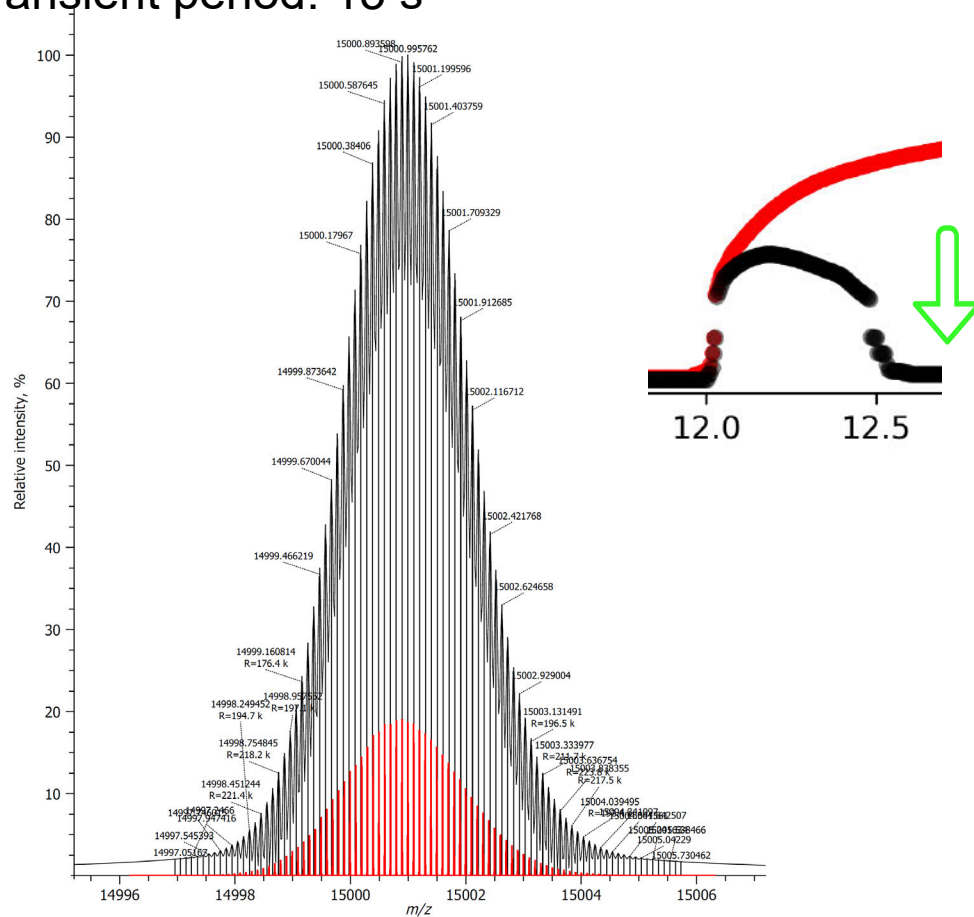
The 2nd Beat Challenge: Full Window Apodization

- Transient period: 12.25 s



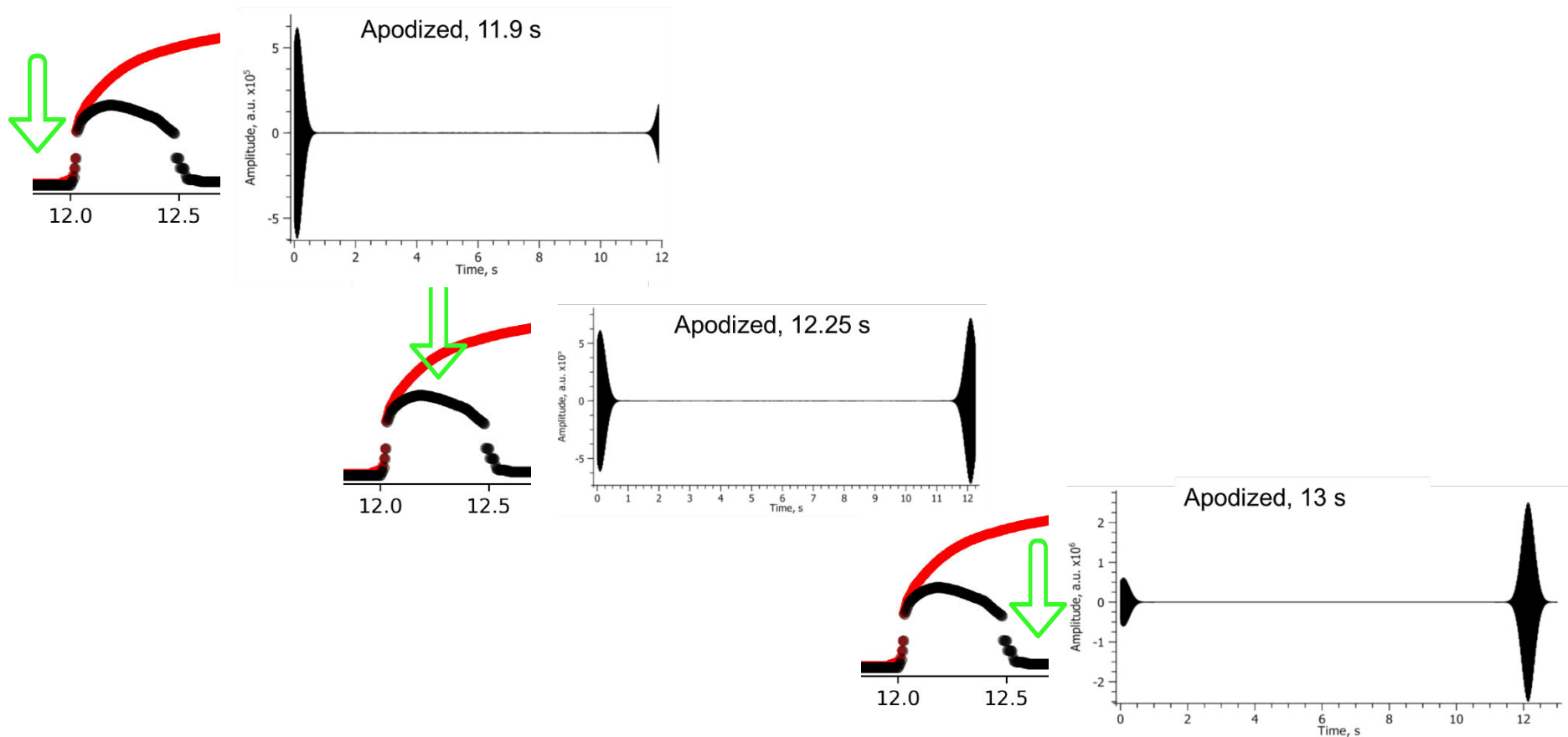
The 2nd Beat Challenge: Full Window Apodization

- Transient period: 13 s



The 2nd Beat Challenge: Full Window Apodization

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

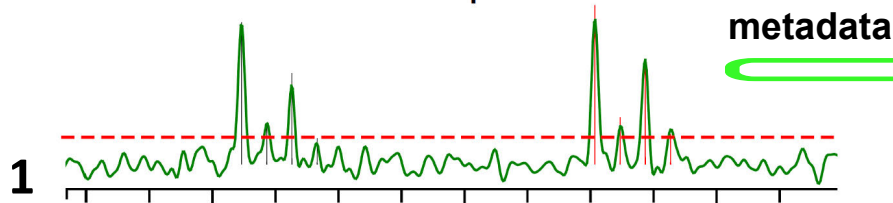


How & Why to Simulate the FTMS Data?

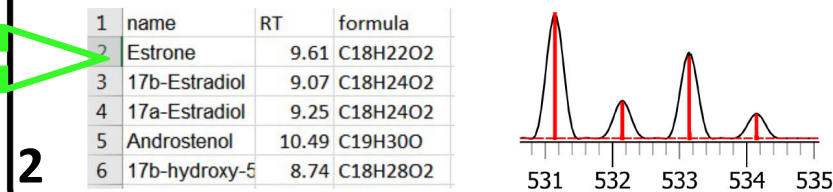
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FTMS Workflows Embedding Data Simulation

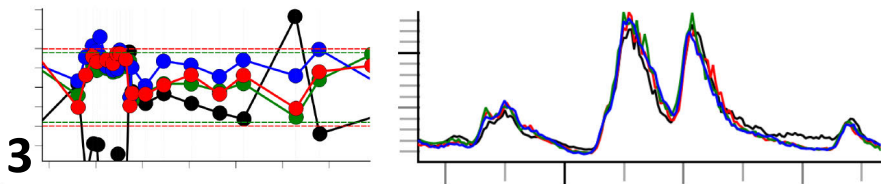
Calculate mass spectral features



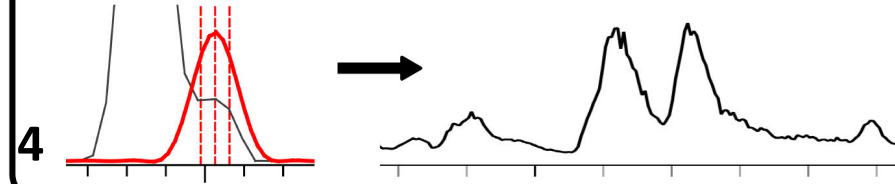
Simulate reference profile library



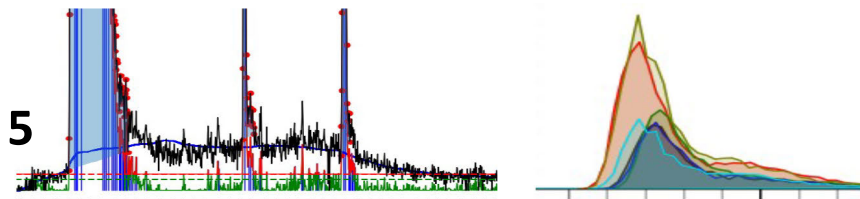
Align experiments (targeted/untargeted)



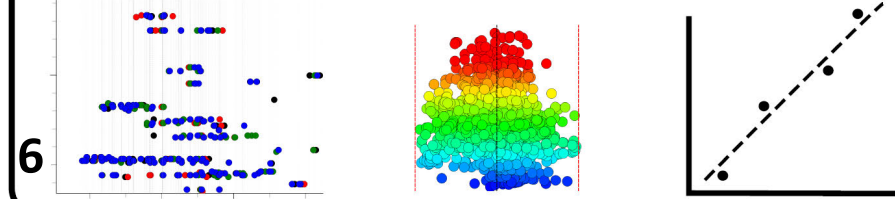
Extract SIC(s)



Process SIC(s) and detect features



Data analysis (mass recalibrate, quantify)



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 I Q S P S S L S A S V G D 1/2

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 102

103 K V E I K R T V A A P S V F I F P 119

120 P S D F O L K S G T A S V V C L L 136

mAb type: IgG1

Enzyme type: None: Intact

Clear maps

Heavy chain #2:

N E V Q L V E S G G G L V Q P G G S 1/2

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 102

103 G F Y A M D Y W G Q G T L V T V S 119

120 S A S T K G P S V F P L A P S S K 136

137 S T S G G T A A L G C L V K D Y F 152

154 P E P V T V S W N S G A L T S G V 171

171 H T F P A V L Q S S G L Y S L S S 188

188 V V T V P S S S L G I Q T Y I C N 204

205 V N H K P S N T K V D K K V E P K 222

222 S C D K T H T C P P C P A P E L L 239

Light chain #1:

N D I Q M T I Q S P S S L S A S V G D 1/2

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 102

103 K V E I K R T V A A P S V F I F P 119

120 P S D F O L K S G T A S V V C L L 136

Compound info:

Add

Heavy chain #1:

N E V Q L V E S G G G L V Q P G G S 1/2

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 102

103 G F Y A M D Y W G Q G T L V T V S 119

120 S A S T K G P S V F P L A P S S K 136

137 S T S G G T A A L G C L V K D Y F 152

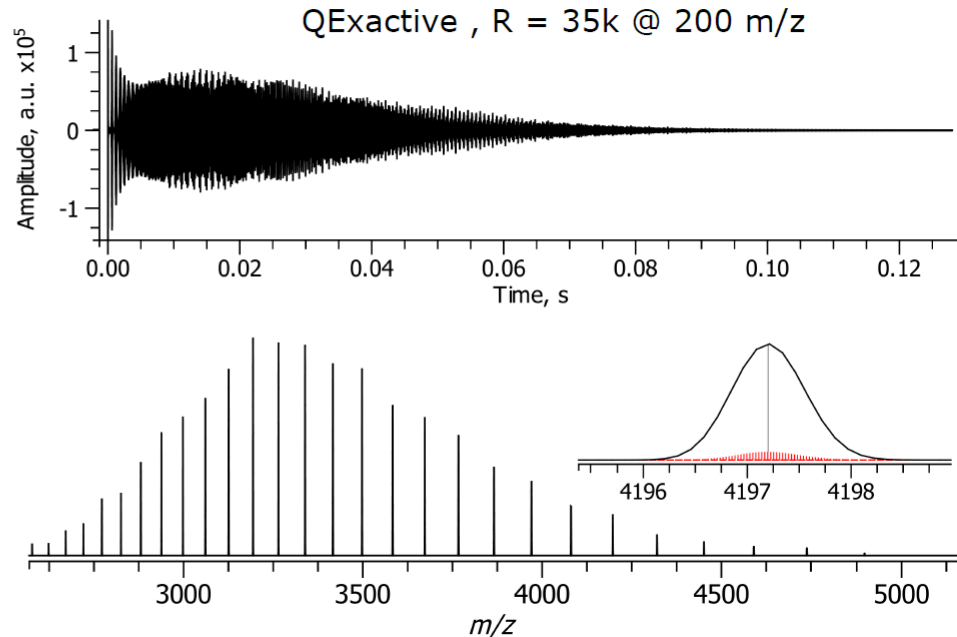
154 P E P V T V S W N S G A L T S G V 171

171 H T F P A V L Q S S G L Y S L S S 188

188 V V T V P S S S L G I Q T Y I C N 204

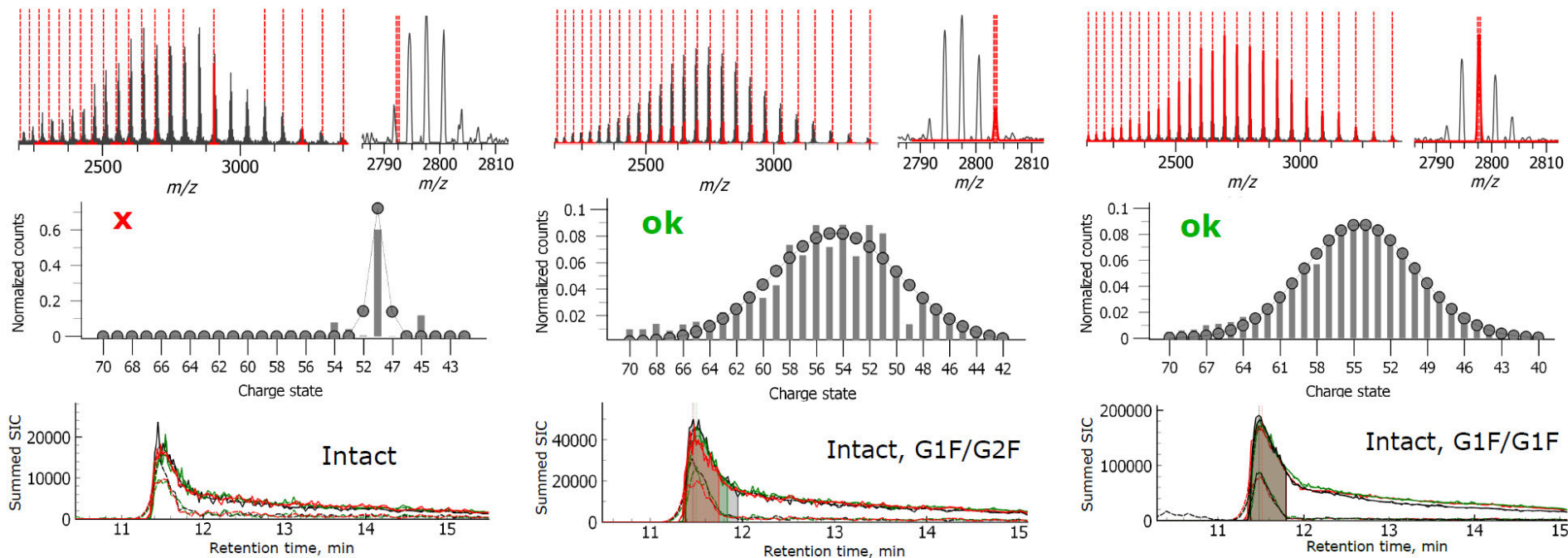
205 V N H K P S N T K V D K K V E P K 222

222 S C D K T H T C P P C P A P E L L 239



Correlate Experimental and Simulated Data

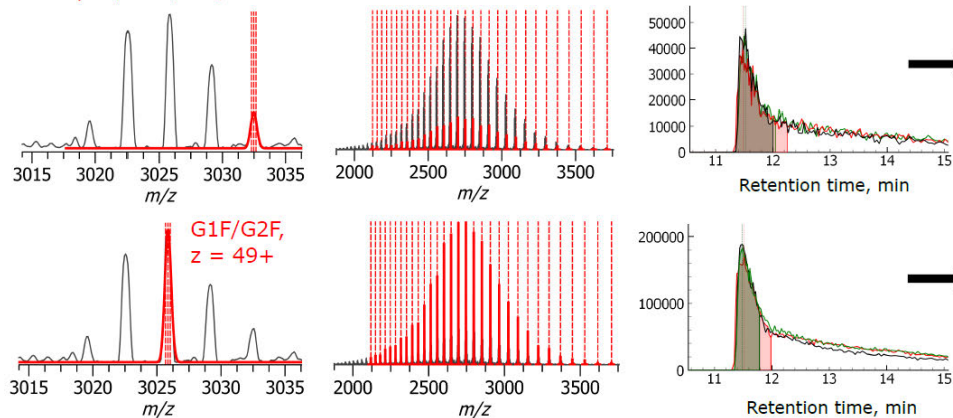
- Isotopic envelopes are compared for each charge state (no deconvolution)
- Charge state distributions are used to filter out the false positives
- Selected Ion Current (SIC) chromatograms show proteoform-specific elution periods



Targeted & Untargeted Deconvolution: Low Resolution

Herceptin, G0F/G1F, $z = 49+$

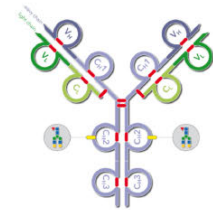
Targeted
deconvolution



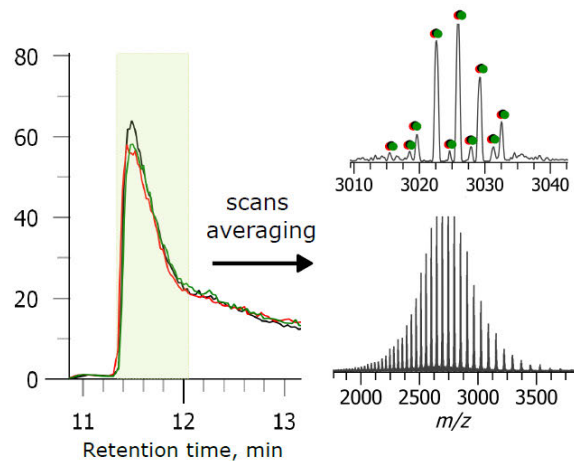
G0F/G1F (148217.96)

G0/G0F
(147909.68)

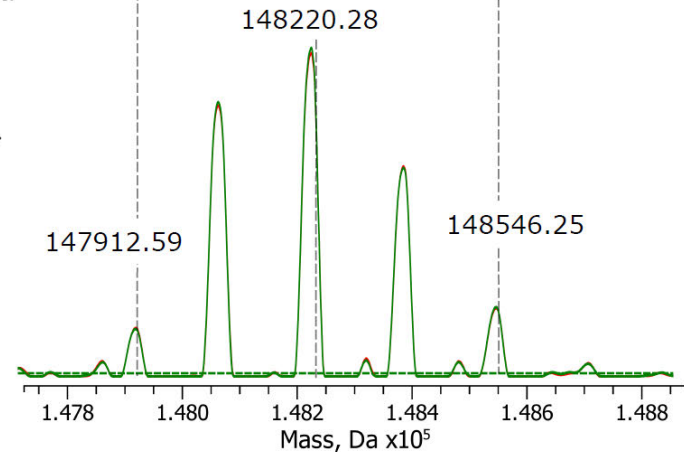
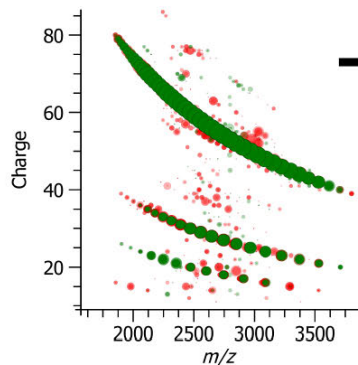
G1F/G2F
(148542.245)



Untargeted
deconvolution



UniDec
low-res deconvolution



Profile Library Simulations: High-Resolution

SIMULATE

- Isotopically resolved envelopes

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

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103 K V E I K R T V A A P S V F I F P 119

120 P S D E Q L K S G T A S V V C L L 136

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N D I Q M T Q S P S S L S A S V G D 17

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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 102

103 K V E I K R T V A A P S V F I F P 119

120 P S D E Q L K S G T A S V V C L L 136

mAb type: IgG1

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

Clear maps

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

Add

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 102

103 G E Y A M D Y W G O G T L V T V S 119

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 102

103 G E Y A M D Y W G O G T L V T V S 119

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

86 K V S N K A I P A P T E K T I S K 102

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

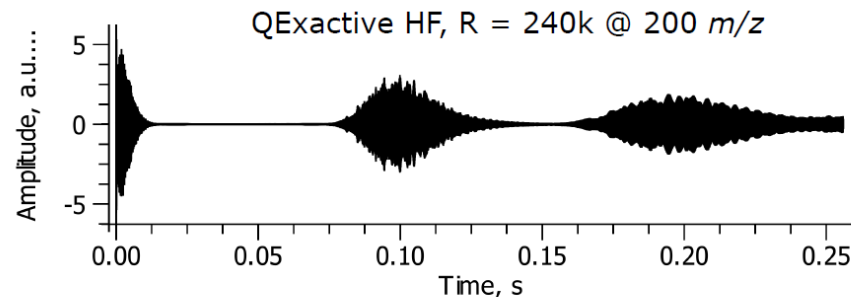
18 S R T P E V T C V V V D V S H E D 34

35 P E V K F N W Y V D G V E V H N A 51

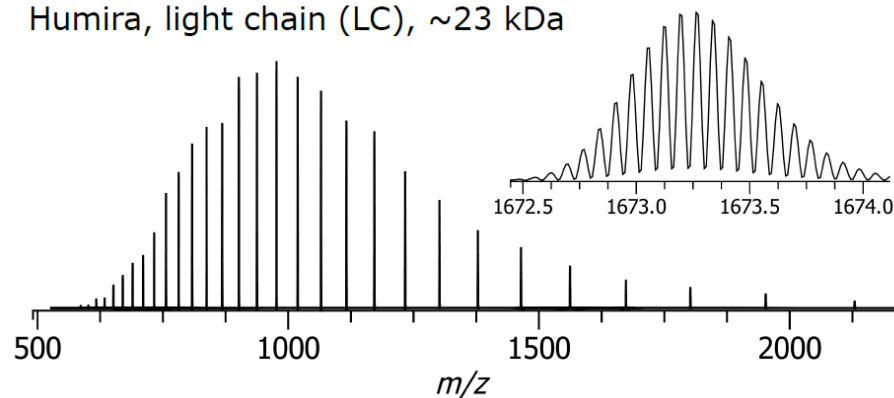
52 K T K P R E E Q Y N S T Y R V V S 68

69 V L T V L H Q D W L N G K E Y K C 85

86 K V S N K A I P A P T E K T I S K 102

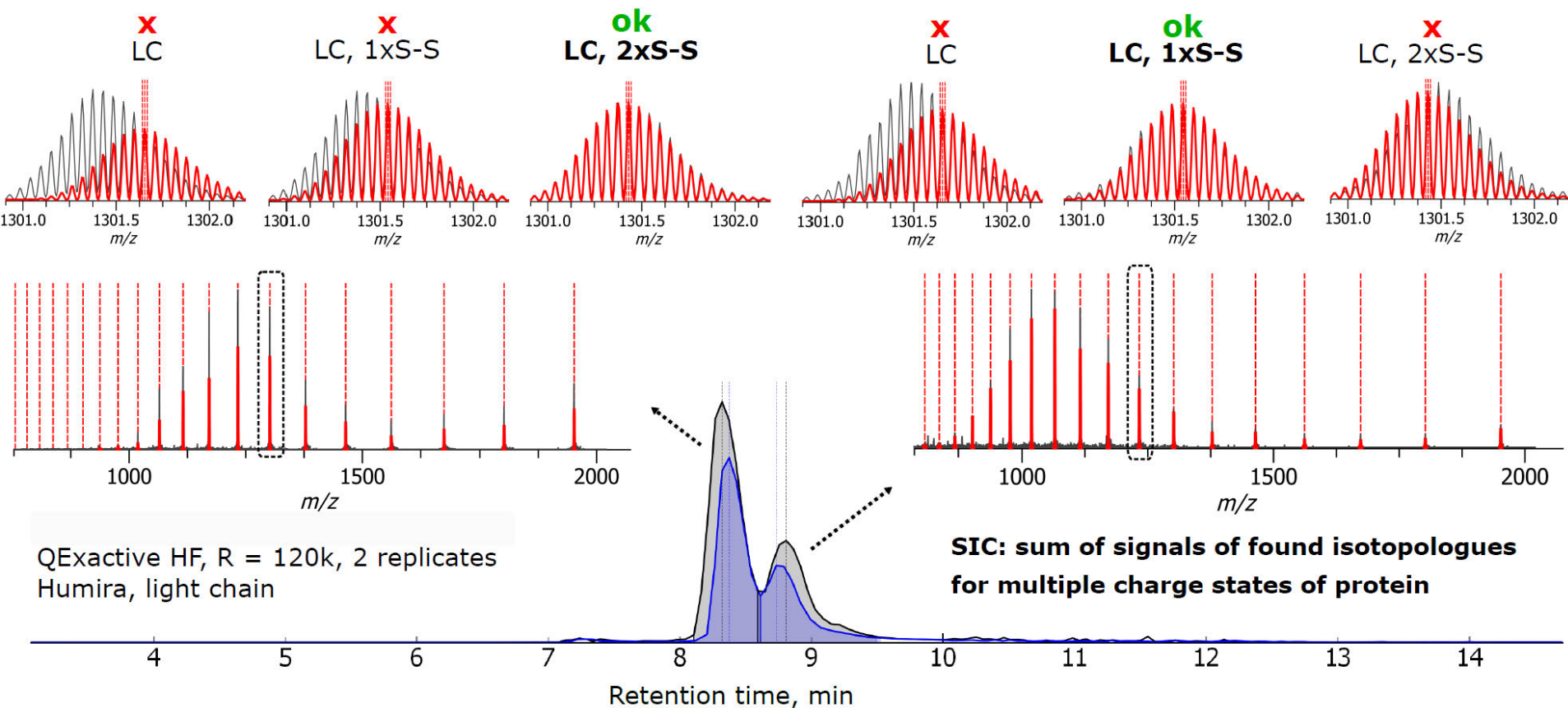


Humira, light chain (LC), ~23 kDa



Targeted Deconvolution: High Resolution

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



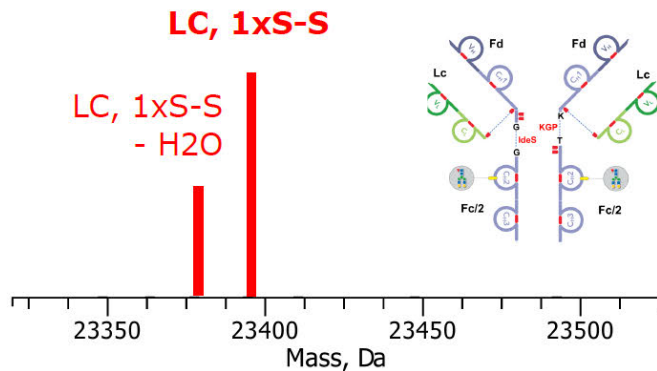
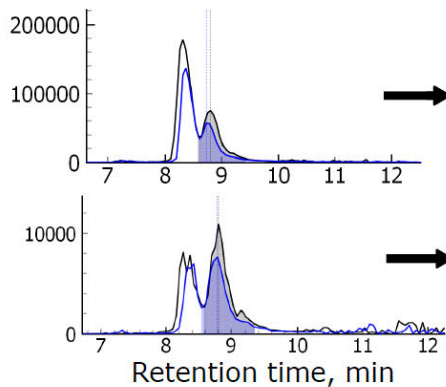
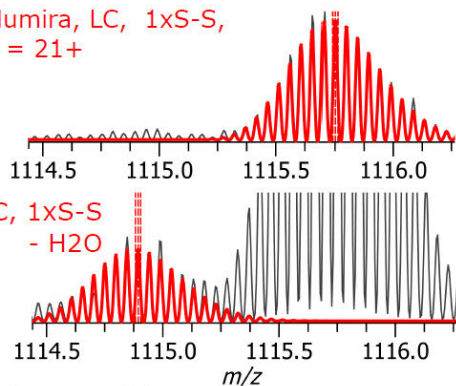
Targeted & Untargeted Deconvolution: High Resolution

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

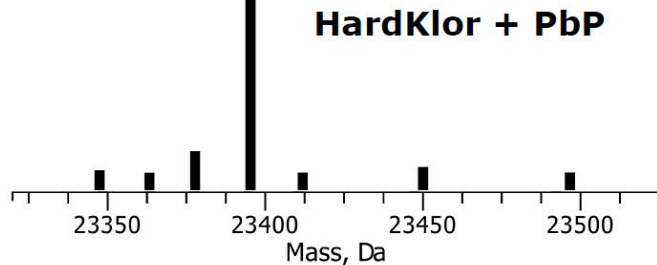
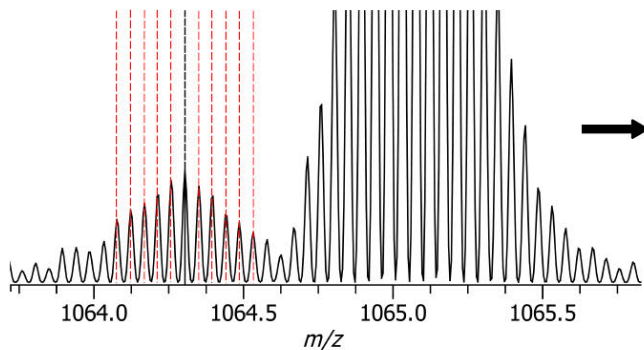
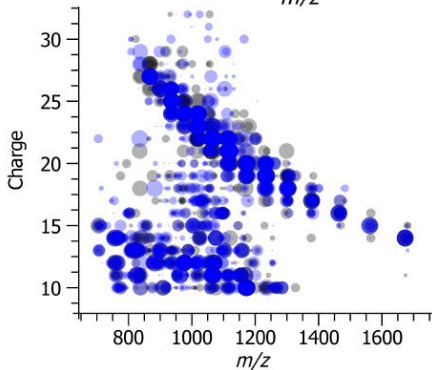
Targeted
deconvolution

Humira, LC, 1xS-S,
 $z = 21+$

LC, 1xS-S
- H₂O

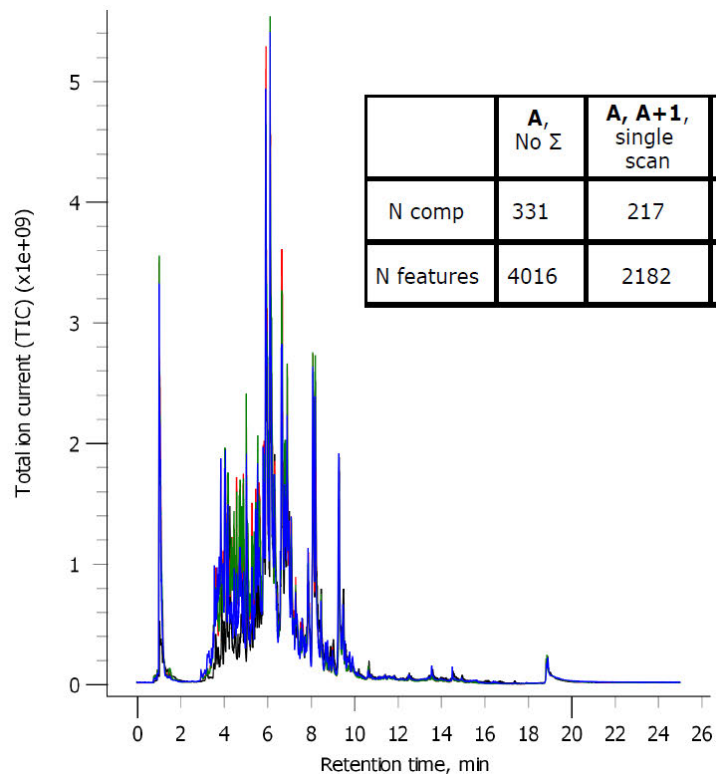


Untargeted
deconvolution



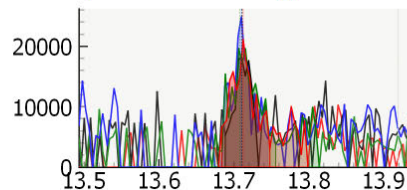
Targeted Deconvolution: Small Molecule Analysis

- 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

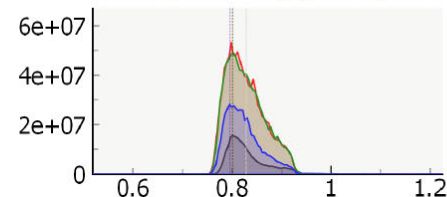


	A, No Σ	A, A+1, single scan	A, A+1, 5 Σ scans
N comp	331	217	248
N features	4016	2182	2502

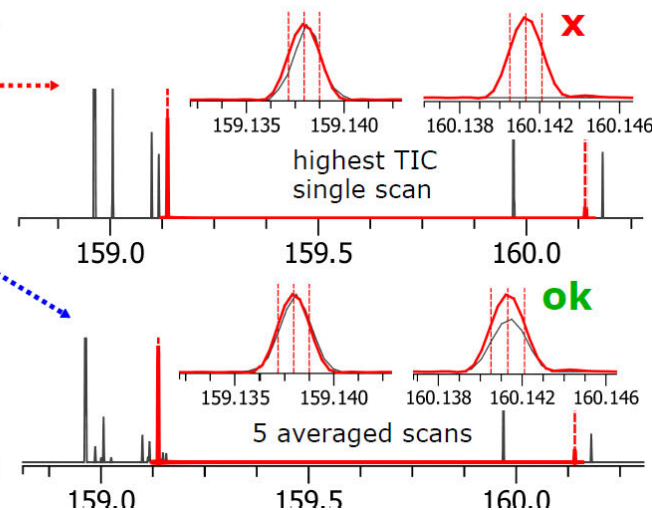
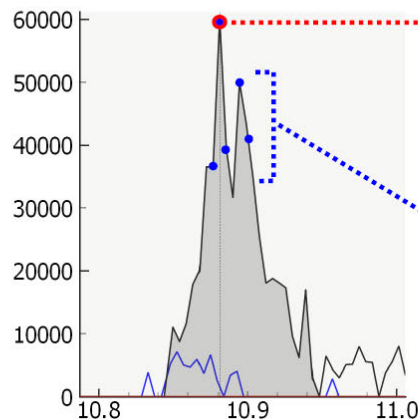
Eicosapentaenoic acid, $[M+H-H_2O]^+$



Spermine, $[M+H]^+$

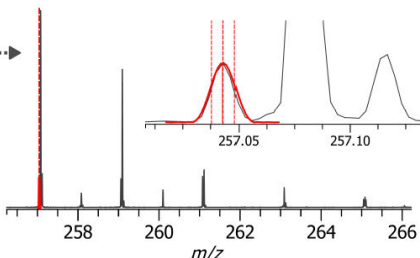
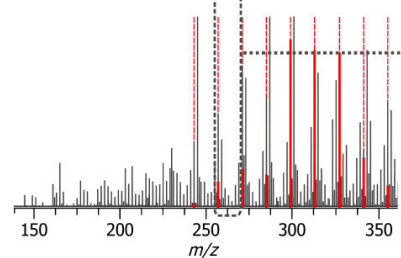
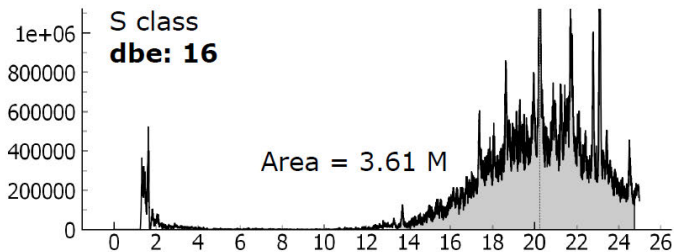
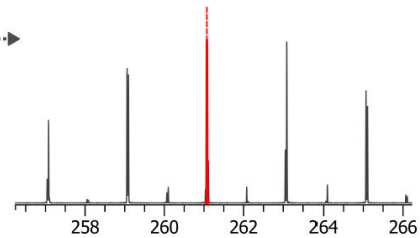
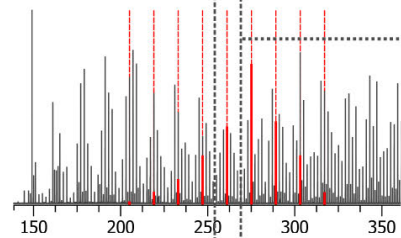
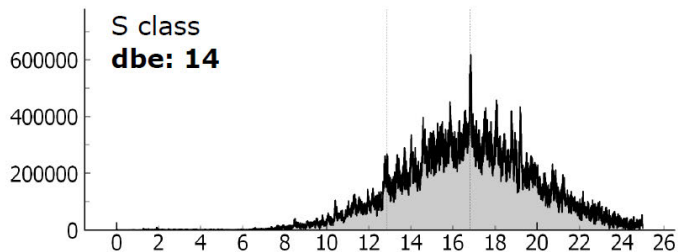
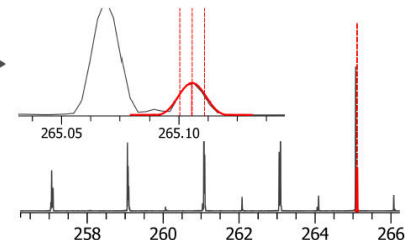
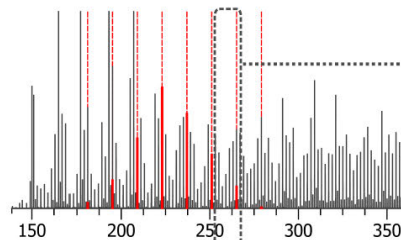
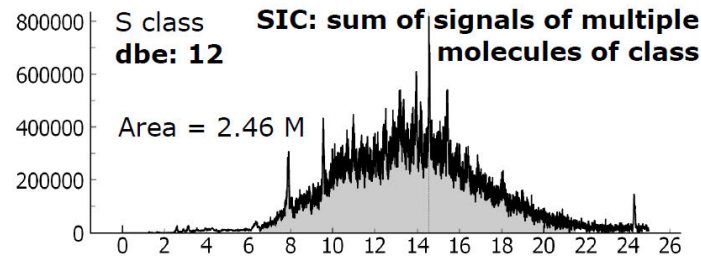


Nanonic acid, $[M+H]^+$



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