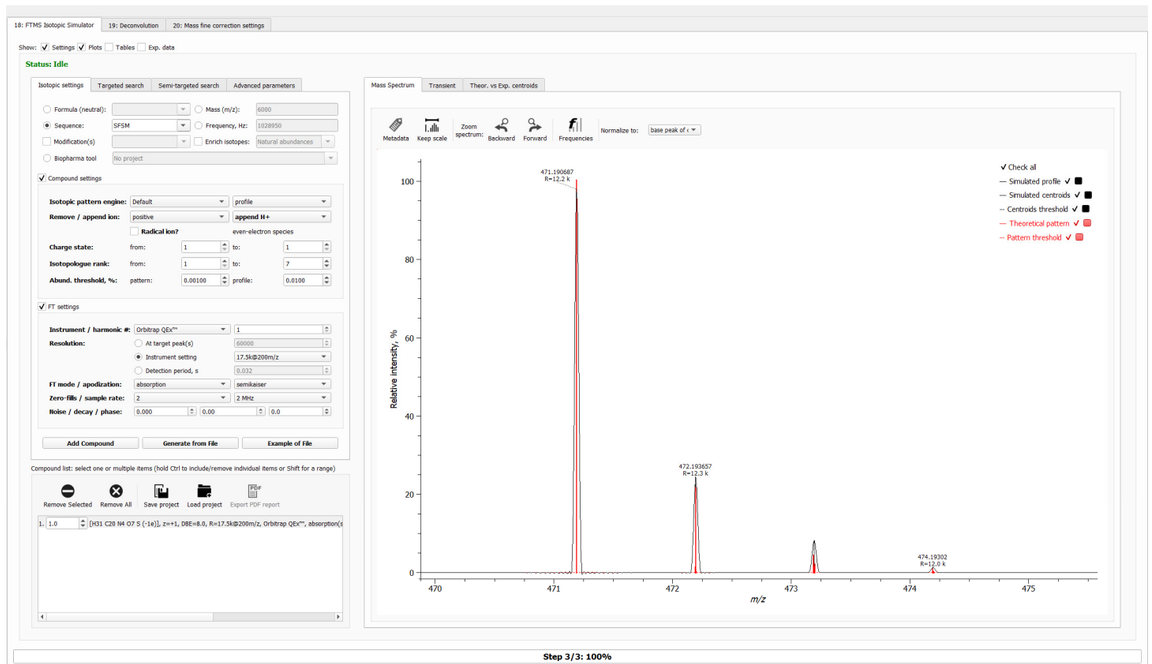
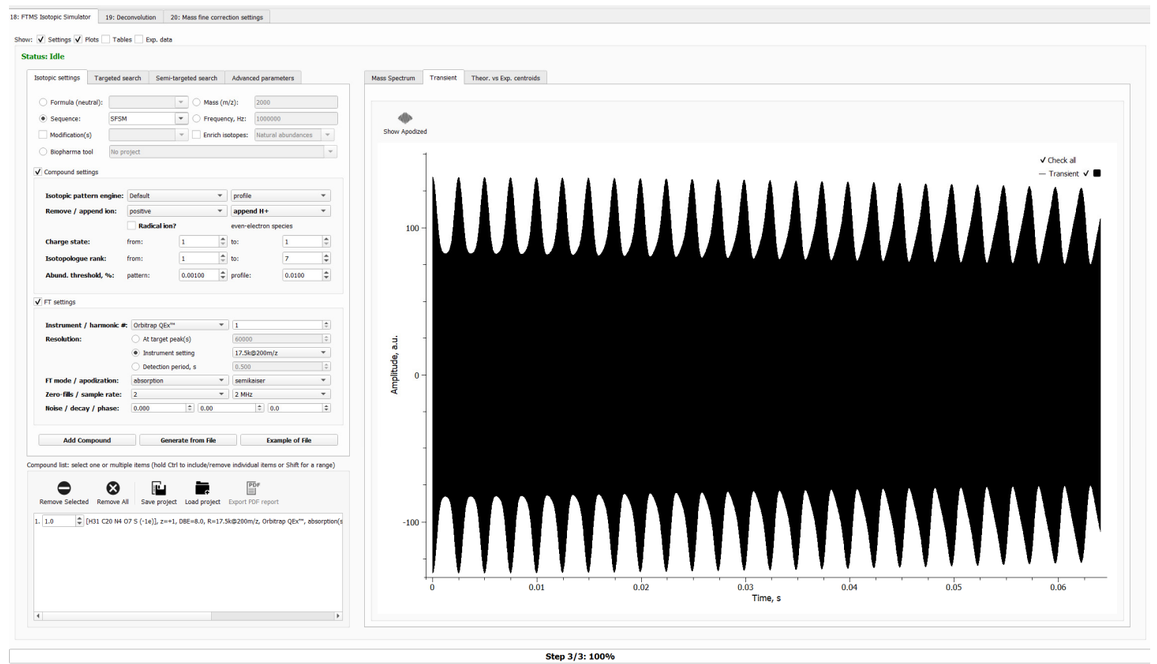
Spectroswiss
FTMS Science and Technology

Application Note #1

FTMS Simulator: Step-by-Step FTMS Data Generation





FTMS Isotopic Simulator: SFSM Peptide Analysis

18: FTMS Isotopic Simulator | 19: Deconvolution | 20: Mass fine correction settings

Show: ☑ Settings ☑ Plots ☐ Tables ☐ Exp. data

Status: Idle

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

- ○ Formula (neutral): ○ Mass (m/z): 6000
- ◉ Sequence: SFSM ○ Frequency, Hz: 1028950
- ☐ Modification(s) ☐ Enrich isotopes: Natural abundances
- ○ Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine:	Default		profile	
Remove / append ion:	positive		**append H+**	
	☐ **Radical ion?**		even-electron species	
Charge state:	from:	1	to:	1
Isotopologue rank:	from:	1	to:	7
Abund. threshold, %:	pattern:	0.00100	profile:	0.0100

☑ FT settings

Instrument / harmonic #:	Orbitrap QEx™	1	
Resolution:	○ At target peak(s)	60000	
	◉ Instrument setting	17.5k@200m/z	
	○ Detection period, s	0.032	
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

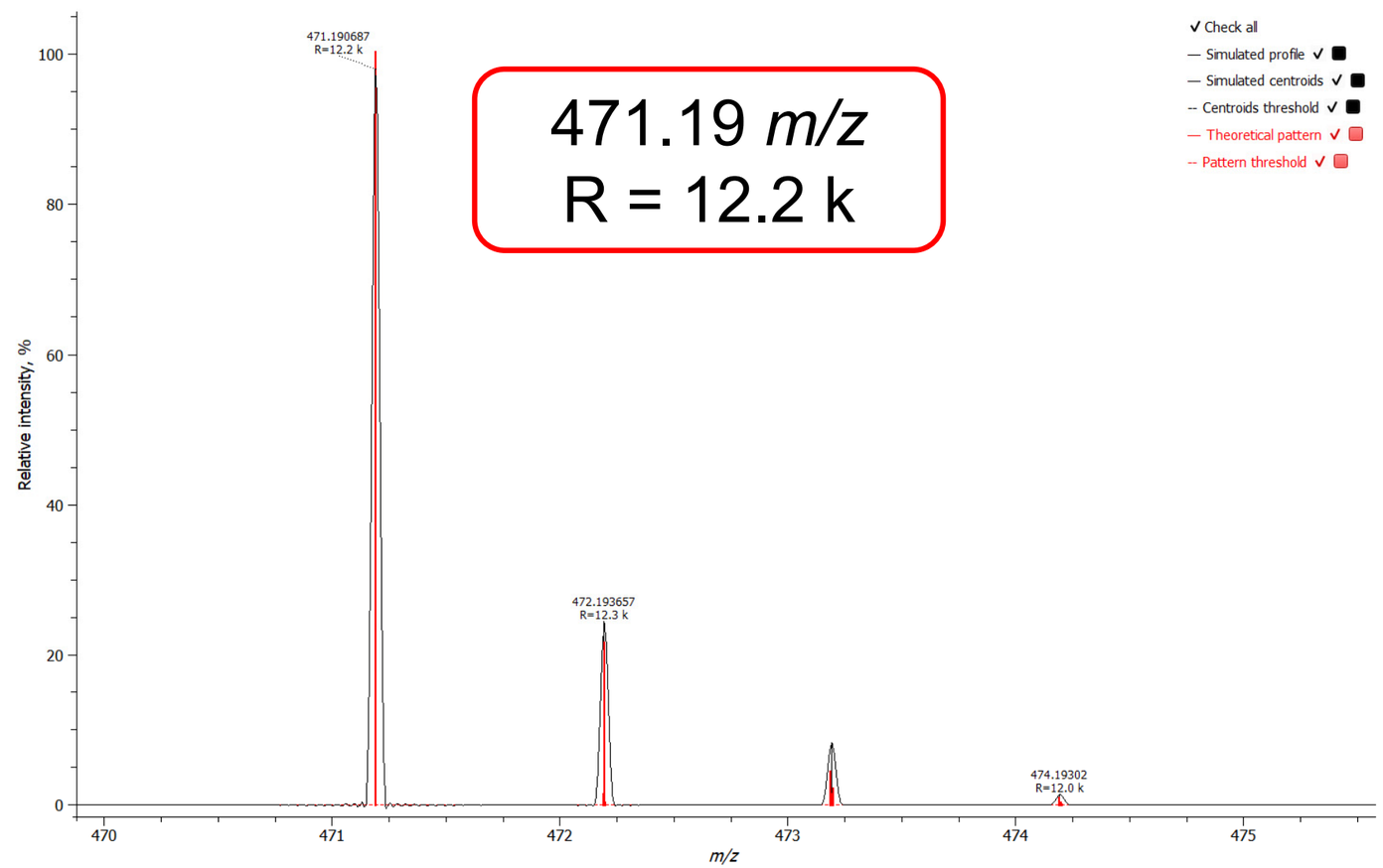
Compound list: select one or multiple items (hold Ctrl to include/remove individual items or Shift for a range)

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

1. 1.0 [H31 C20 N4 O7 S (-1e)], z=+1, DBE=8.0, R=17.5k@200m/z, Orbitrap QEx™, absorption(s

Mass Spectrum | Transient | Theor. vs Exp. centroids

Metadata | Keep scale | Zoom spectrum: | Backward | Forward | Frequencies | Normalize to: base peak of (



471.19 *m/z*
R = 12.2 k

Step 3/3: 100%

SFSM peptide analysis with FTMS: **transient**

18: FTMS Isotopic Simulator | 19: Deconvolution | 20: Mass fine correction settings

Show: ☑ Settings ☑ Plots ☐ Tables ☐ Exp. data

Status: Idle

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

- Formula (neutral): ○ Mass (m/z): 2000
- Sequence: SFSM ○ Frequency, Hz: 1000000
- Modification(s) ☐ Enrich isotopes: Natural abundances
- Biopharma tool: No project

☑ Compound settings

Isotopic pattern engine:	Default		profile	
Remove / append ion:	positive		**append H+**	
	☐ **Radical ion?**		even-electron species	
Charge state:	from:	1	to:	1
Isotopologue rank:	from:	1	to:	7
Abund. threshold, %:	pattern:	0.00100	profile:	0.0100

☑ FT settings

Instrument / harmonic #:	Orbitrap QEx™	1
Resolution:	○ At target peak(s)	60000
	◉ Instrument setting	17.5k@200m/z
	○ Detection period, s	0.500
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

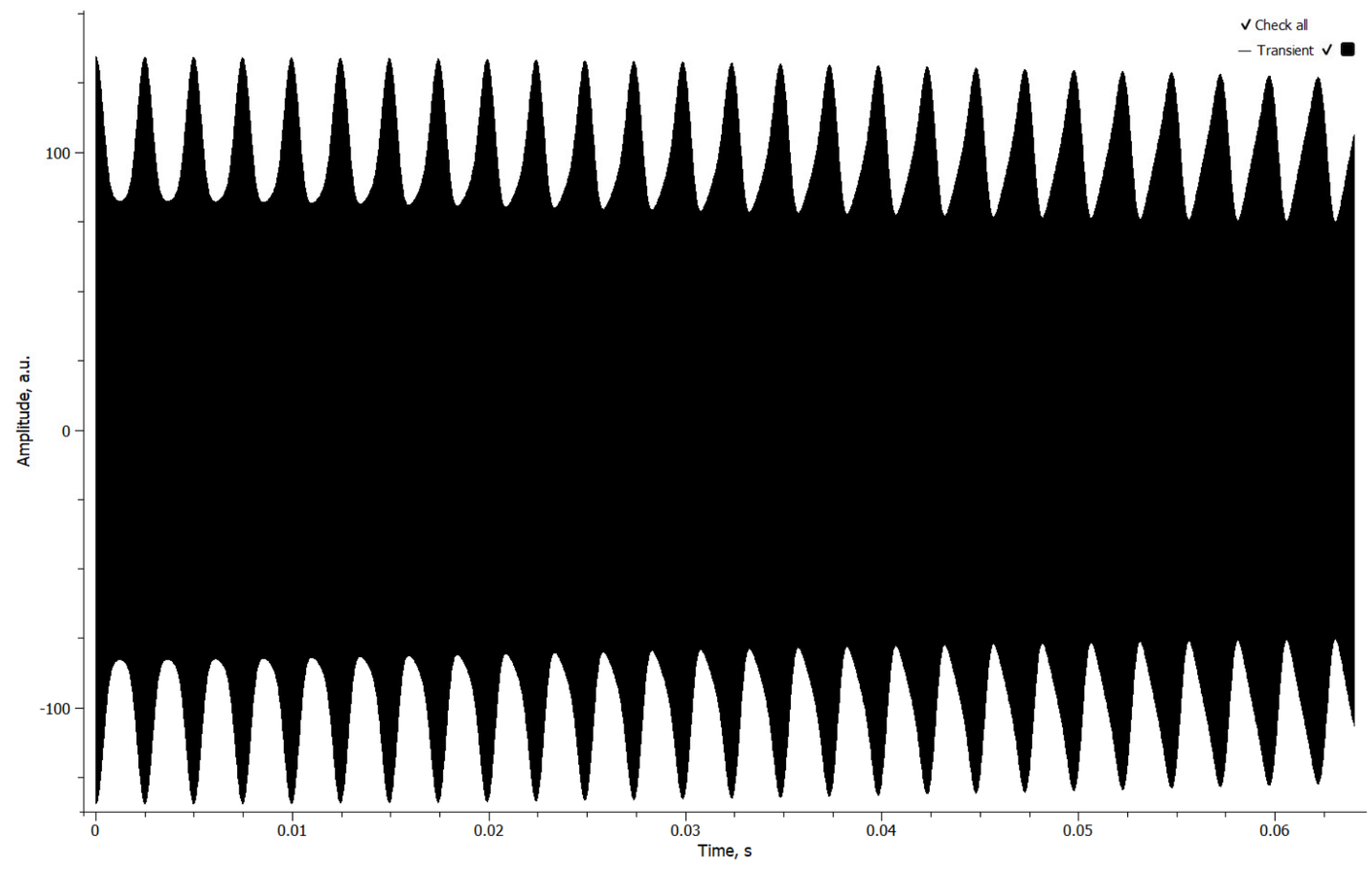
Compound list: select one or multiple items (hold Ctrl to include/remove individual items or Shift for a range)

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

1. 1.0 [H31 C20 N4 O7 S (-1e)], z=+1, DBE=8.0, R=17.5k@200m/z, Orbitrap QEx™, absorption(s

Mass Spectrum | Transient | Theor. vs Exp. centroids

Show Apodized



64 ms

Step 3/3: 100%

SFSM peptide analysis with FTMS: **frequency**

18: FTMS Isotopic Simulator | 19: Deconvolution | 20: Mass fine correction settings

Show: Settings Plots Tables Exp. data

Status: Idle

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

Formula (neutral): | Mass (m/z): 2000
Sequence: SFSM | Frequency, Hz: 1000000
Modification(s) | Enrich isotopes: Natural abundances
Biopharma tool: No project

Compound settings

Isotopic pattern engine: Default / profile
Remove / append ion: positive / append H+
Radical ion? even-electron species
Charge state: from: 1 to: 1
Isotopologue rank: from: 1 to: 7
Abund. threshold, %: pattern: 0.00100 profile: 0.0100

FT settings

Instrument / harmonic #: Orbitrap QEx™ / 1
Resolution: At target peak(s): 60000 | Instrument setting: 17.5k@200m/z | Detection period, s: 0.500
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 (hold Ctrl to include/remove individual items or Shift for a range)

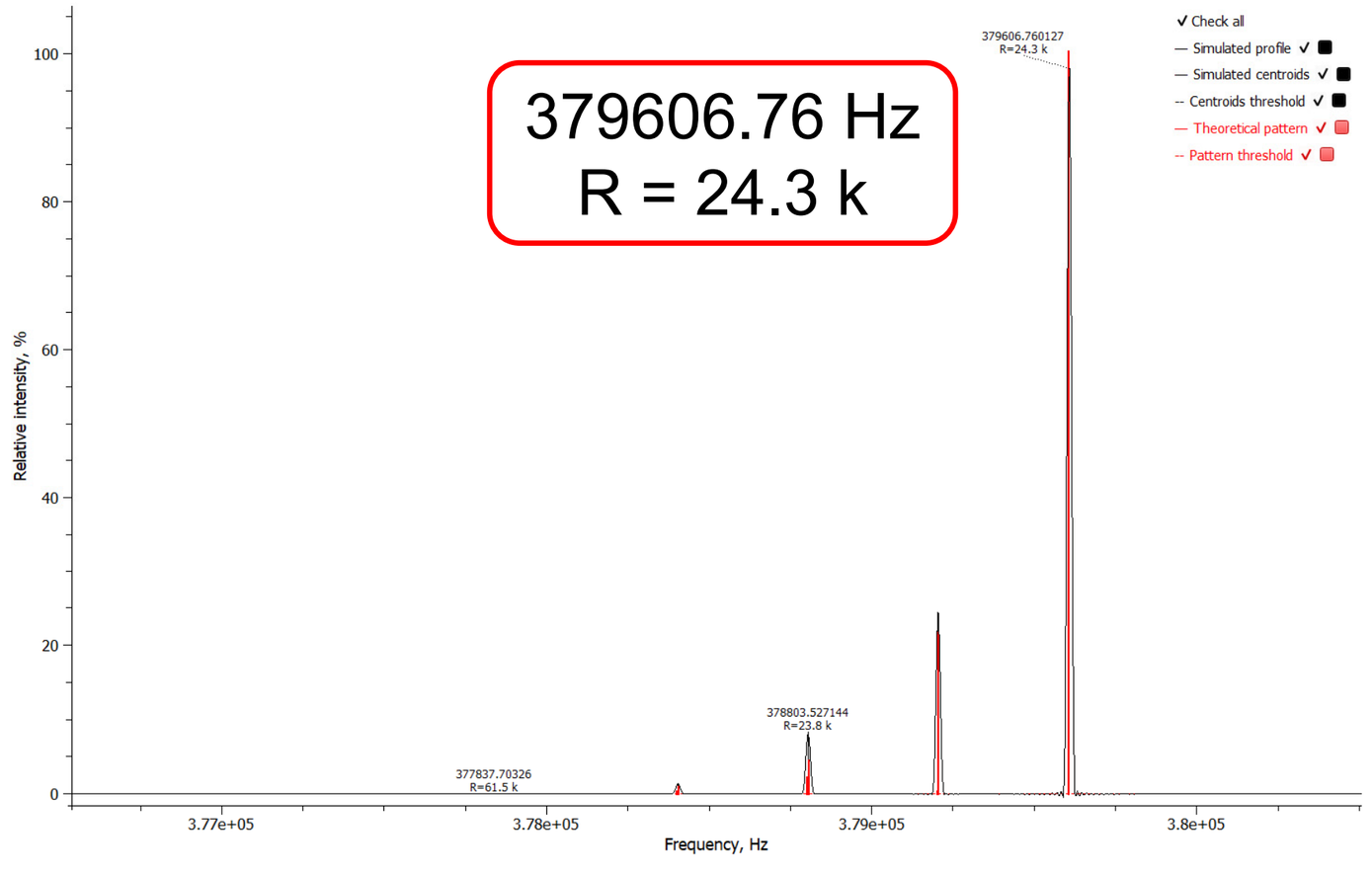
Remove Selected | Remove All | Save project | Load project | Export PDF report

1. 1.0 [H31 C20 N4 O7 S (-1e)], z=+1, DBE=8.0, R=17.5k@200m/z, Orbitrap QEx™, absorption(s

Mass Spectrum | Transient | Theor. vs Exp. centroids

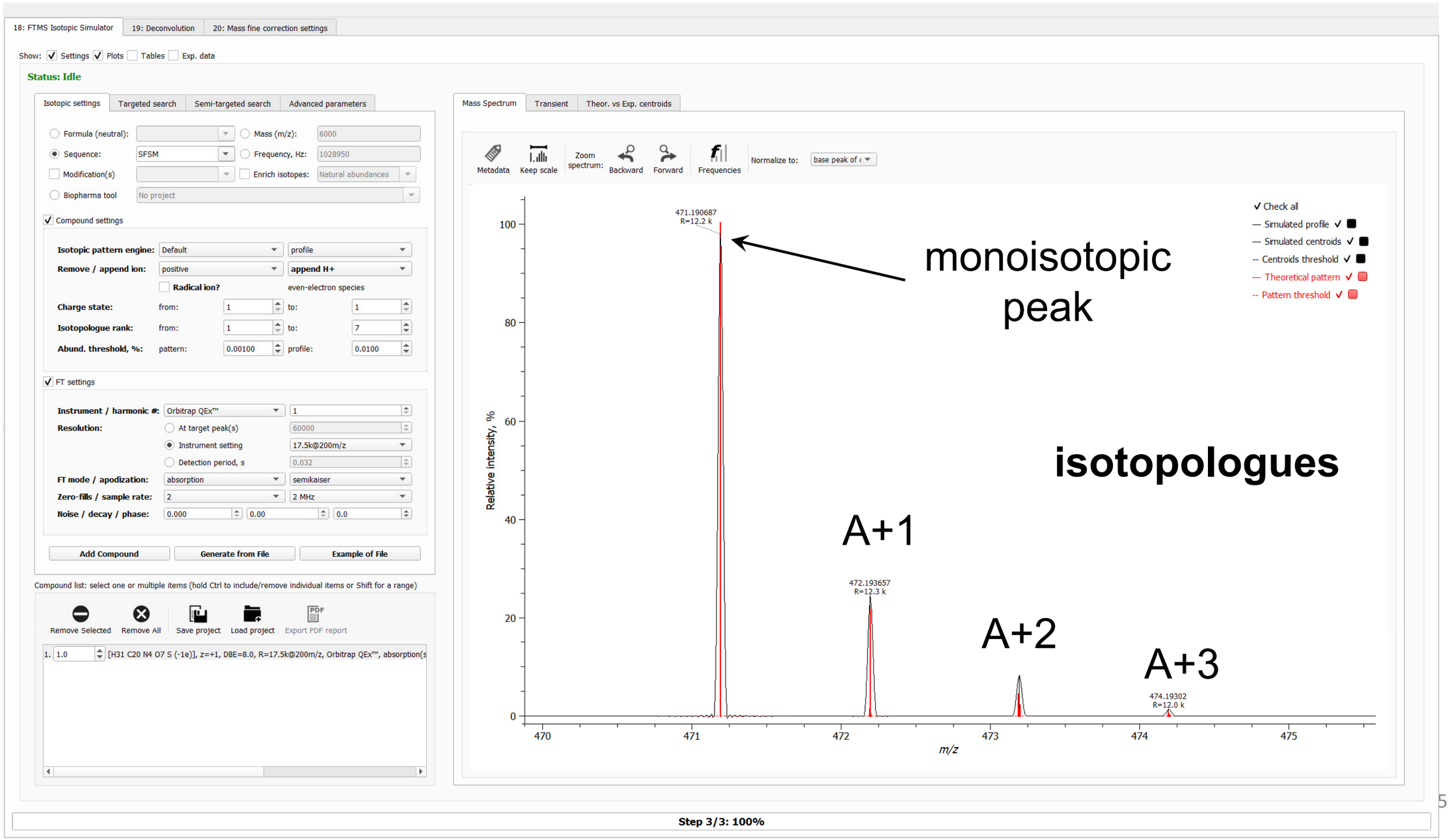
Metadata | Keep scale | Zoom spectrum: | Backward | Forward | Frequencies | Normalize to: base peak of (

379606.76 Hz
R = 24.3 k

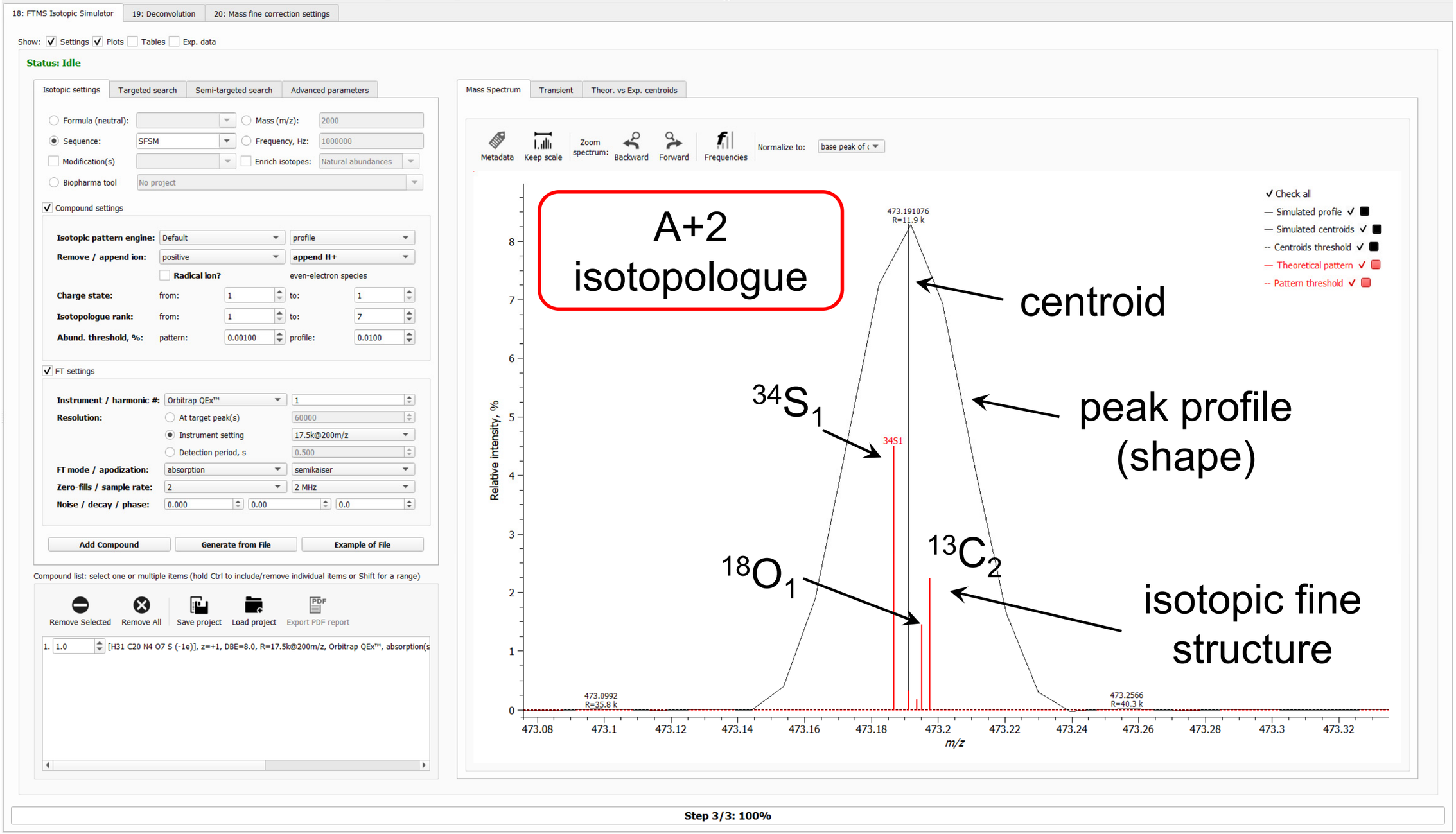


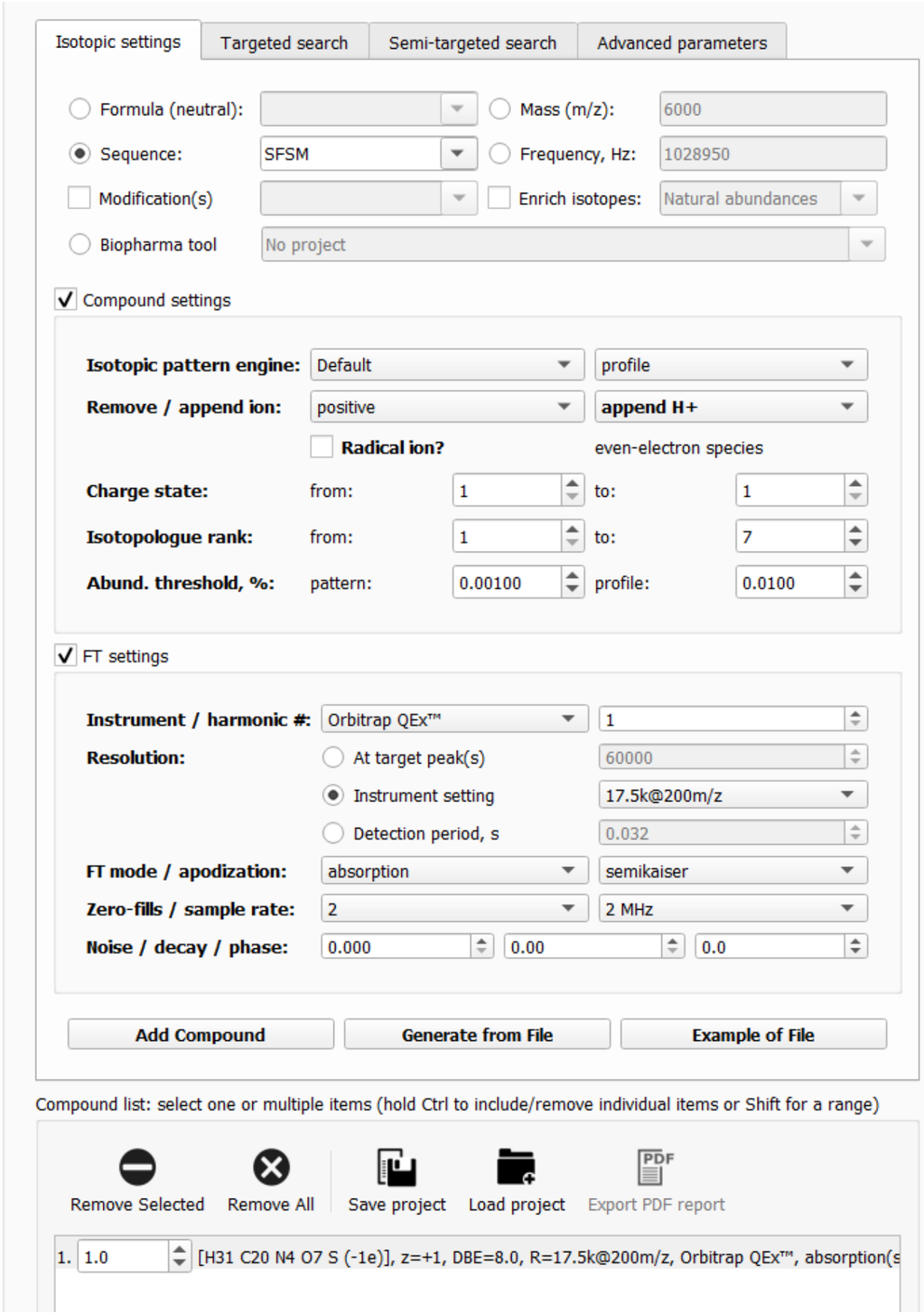
Step 3/3: 100%

SFSM peptide analysis with FTMS: ***m/z***



SFSM peptide analysis with FTMS: ***m/z***





Spectroswiss

Compound definition:

- elemental composition
- amino acid sequence
- mass (*m/z*) or frequency value

Ion (charged compound) definition:

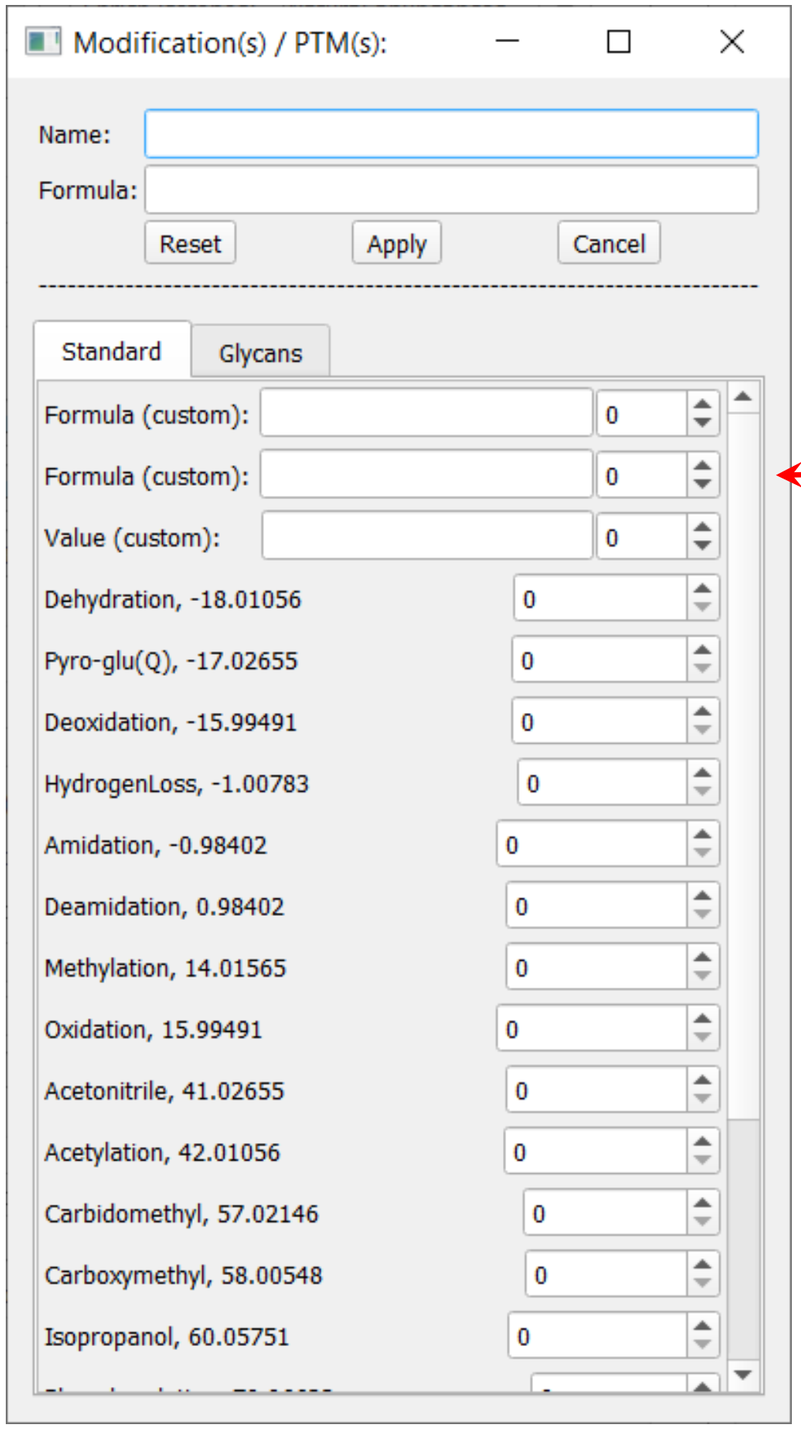
- Charge carrier: electron, H^+, K^+, Na^+, Cs^+, I^-, $HCOO^-$
- Ionization mode: positive, negative, or a neutral species
- Charge state: from the lowest to the highest
- Isotopologues: how many and which ones

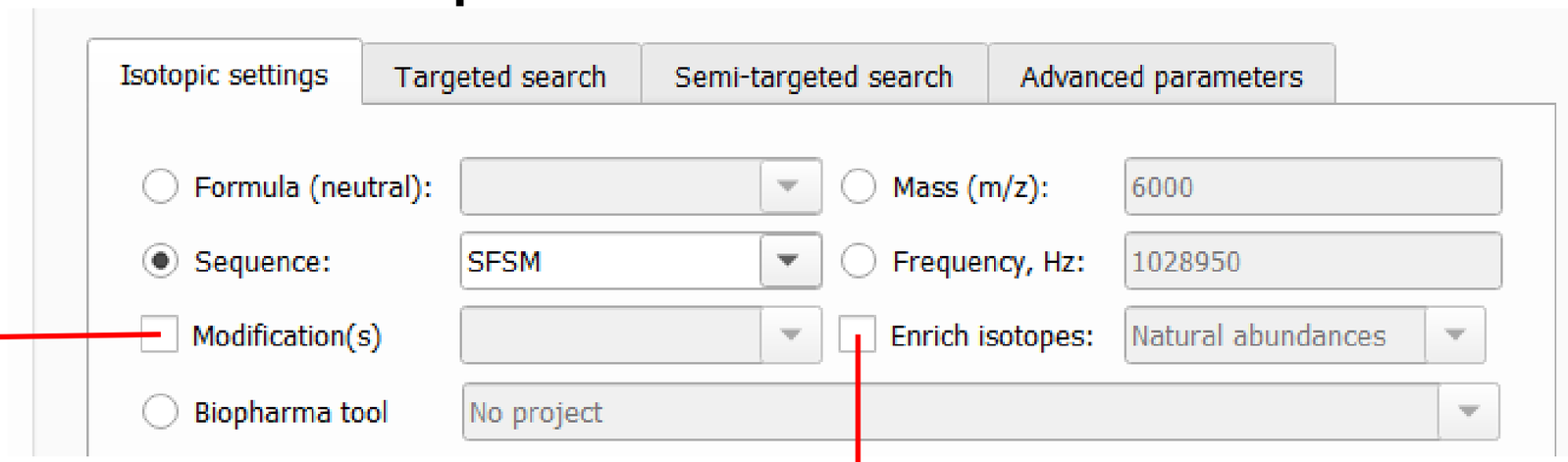
FT processing settings:

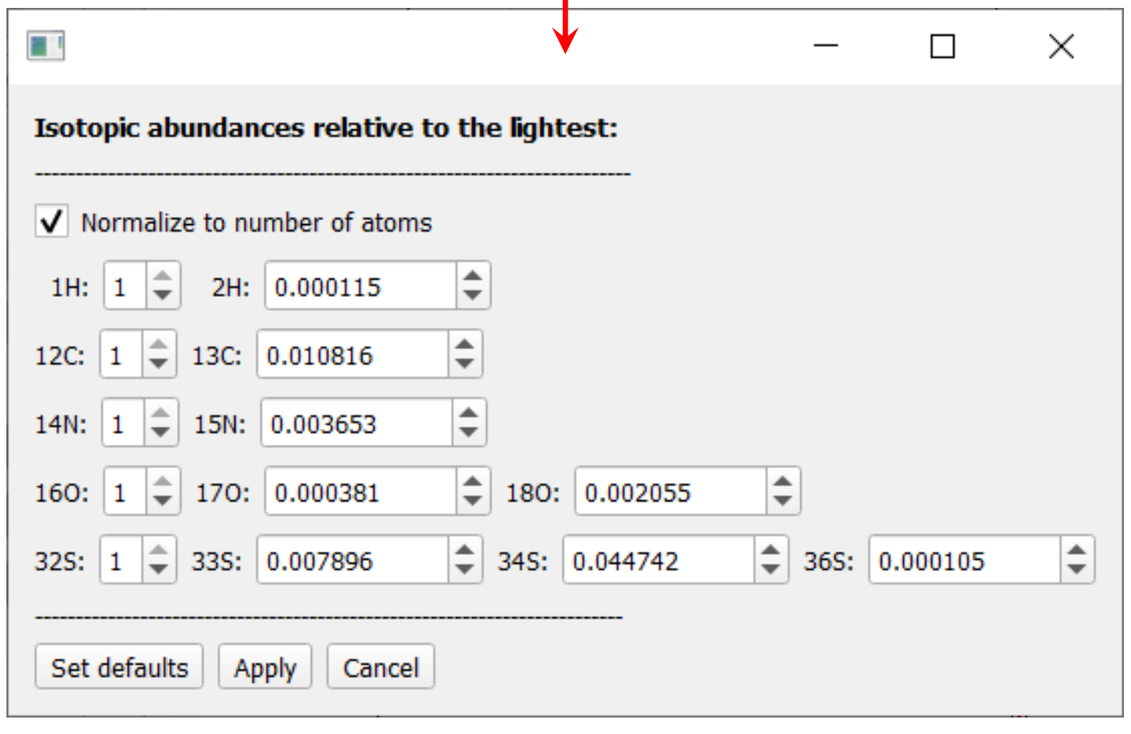
- FTMS instrument and model: ICR/MRMS, Orbitraps
- Harmonics order: which harmonic to calculate
- Resolution: at target peak, instrument setting, transient length
- FT mode: absorption or magnitude
- Apodization window: none, full (Kaiser), half (semi Kaiser)
- Number of zero fills: 0, 1, 2, or 3
- Sampling rate (digitization frequency): 1, 2, 4, or 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

Compound Definition

Spectroswiss







Compound Definition

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

Formula (neutral):

Sequence: SFSM

Modification(s)

Biopharma tool: No project

Biopharma FT simulations

Intact | Top-down

Light chain #2: H1598 C1032 N277 O335 S6, 23423.48472 Da

Light chain #1: H1598 C1032 N277 O335 S6, 23423.48472 Da

Compound list: select one or multiple items (hold Ctrl to include/remove individual items or Shift for a range)

Add modification | Remove Selected | Remove All | Protein library | Add standard proteoforms | Save project | Load project | Submit project

1. 1.0 Intact: H9972 C6460 N1724 O2014 S44, 145421.5021 Da
2. 5.0 Intact, G0/G0F: H10146 C6566 N1732 O2088 S44, 148166.027 Da
3. 30.0 Intact, 2xG0F: H10156 C6572 N1732 O2092 S44, 148312.168 Da
4. 55.0 Intact, G0F/G1F: H10166 C6578 N1732 O2097 S44, 148474.309 Da
5. 48.0 Intact, 2xG1F: H10176 C6584 N1732 O2102 S44, 148636.449 Da
6. 11.0 Intact, G1F/G2F: H10186 C6590 N1732 O2107 S44, 148798.590 Da

mAb type: IgG1

Enzyme type: None: Intact

Clear maps

Compound info: IgG1, Intact

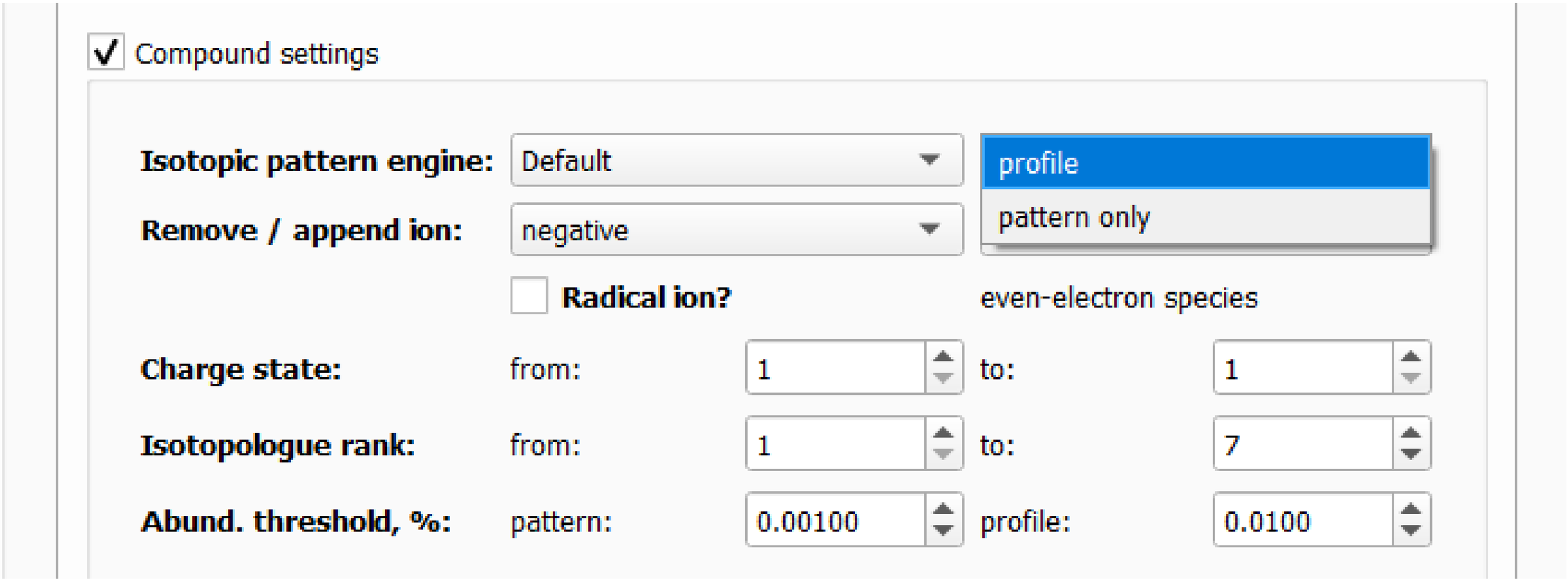
Intact: H9972 C6460 N1724 O2014 S44, 145421.5021 Da | Add

Heavy chain #2: H3388 C2198 N585 O672 S16, 49242.44526 Da

Heavy chain #1: H3388 C2198 N585 O672 S16, 49242.44526 Da

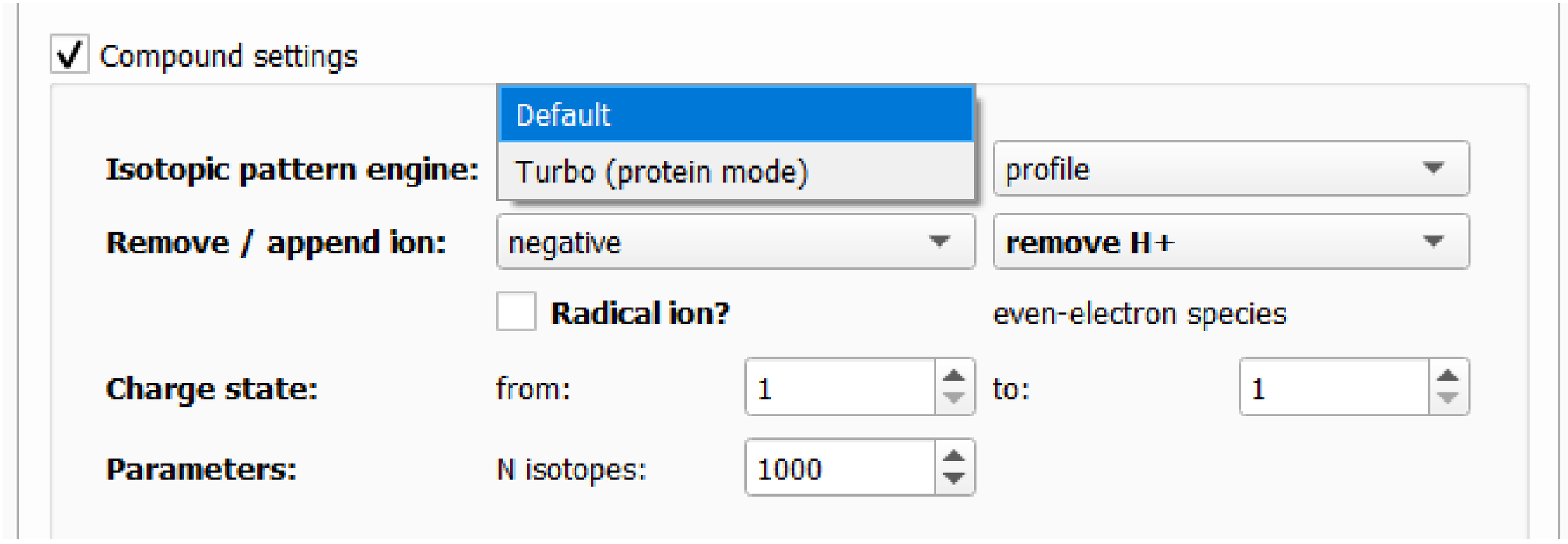
Compound Settings

- Set realistic abundance thresholds for the isotopic pattern/profile calculations
- Lower treshold provide higher accuracy simulations, but are computation-heavy

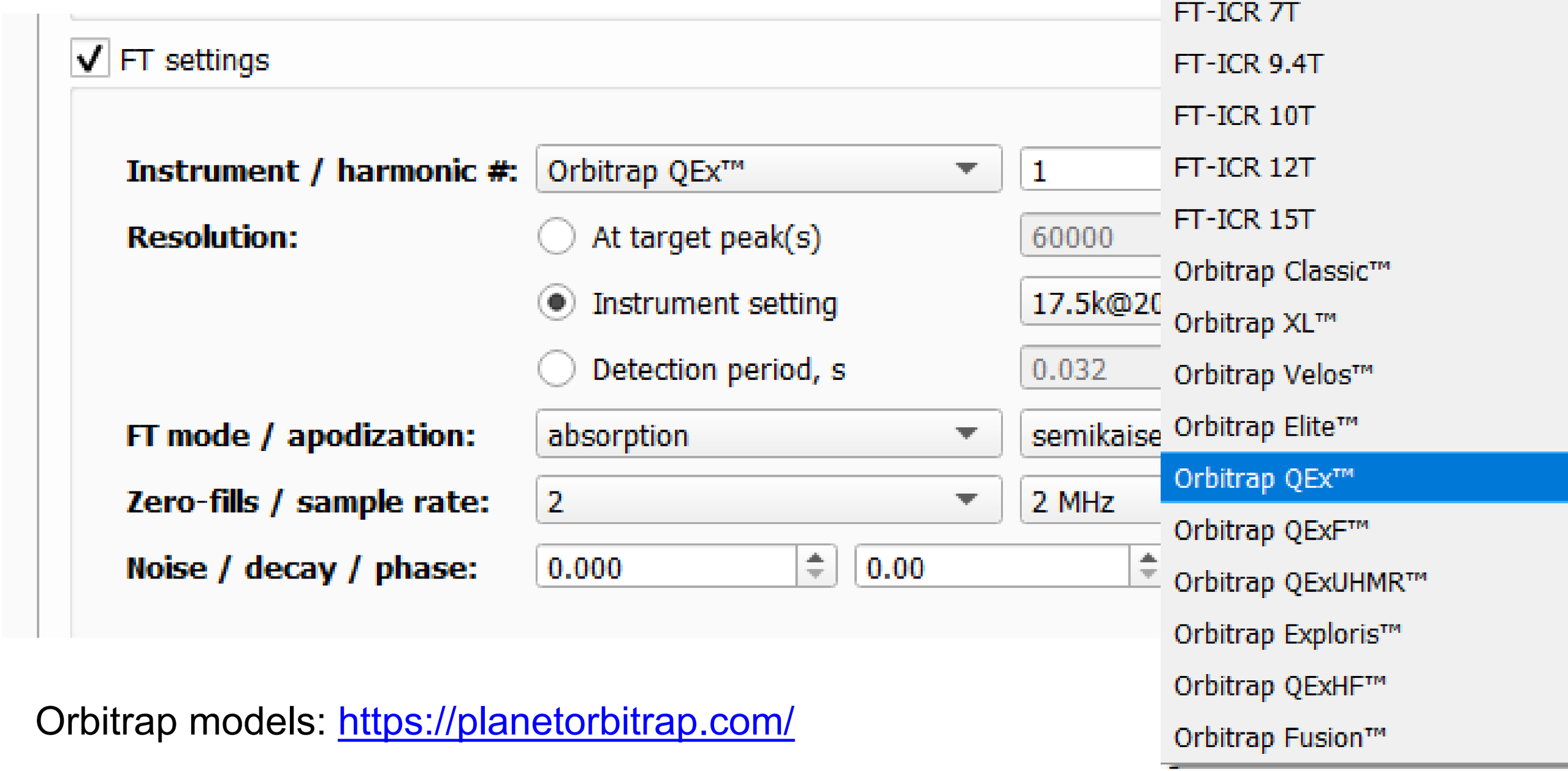


Compound Settings

- Engine: Speed vs. Accuracy; the more accurate, but slow – default mode
- Use «Turbo (protein mode)» when simulating compounds heavier than 30 kDa



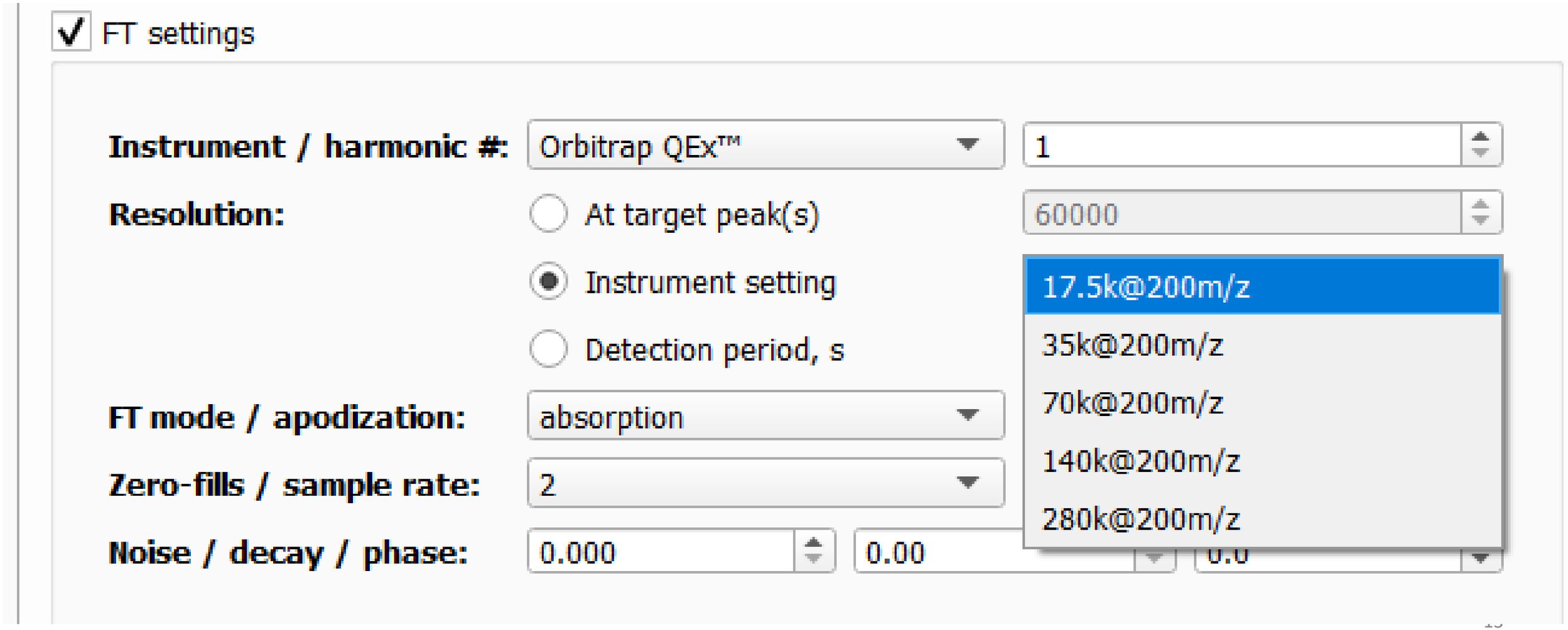
FT Processing Settings: **Frequency**



Orbitrap models: https://planetorbitrap.com/

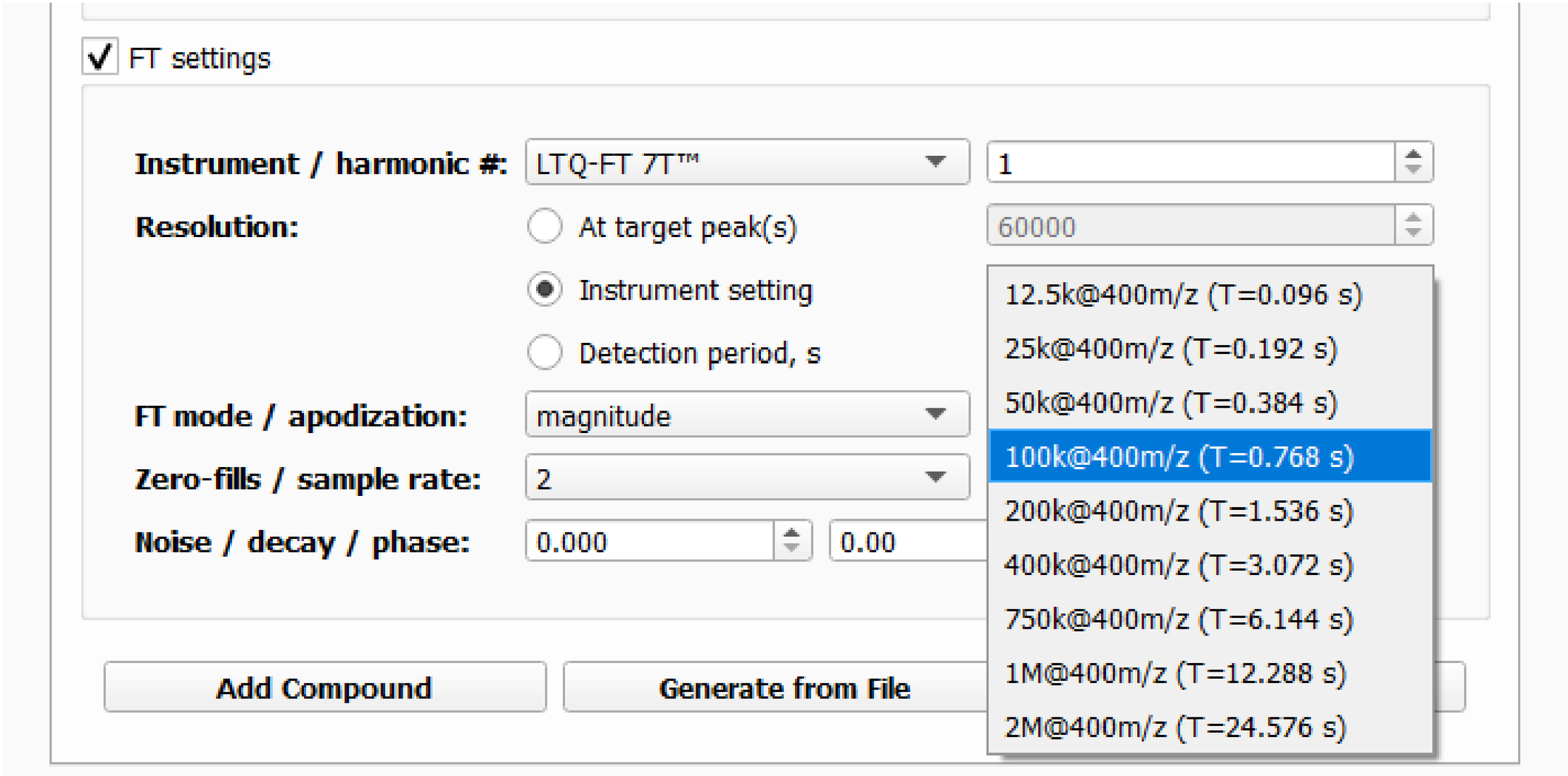
FT Processing Settings: **Resolution**

- **Orbitrap** resolution settings are typically estimated at *m/z* 200 (eFT mode)
- The original LTQ Orbitrap models estimate resolution at *m/z* 400 (mFT or eFT)



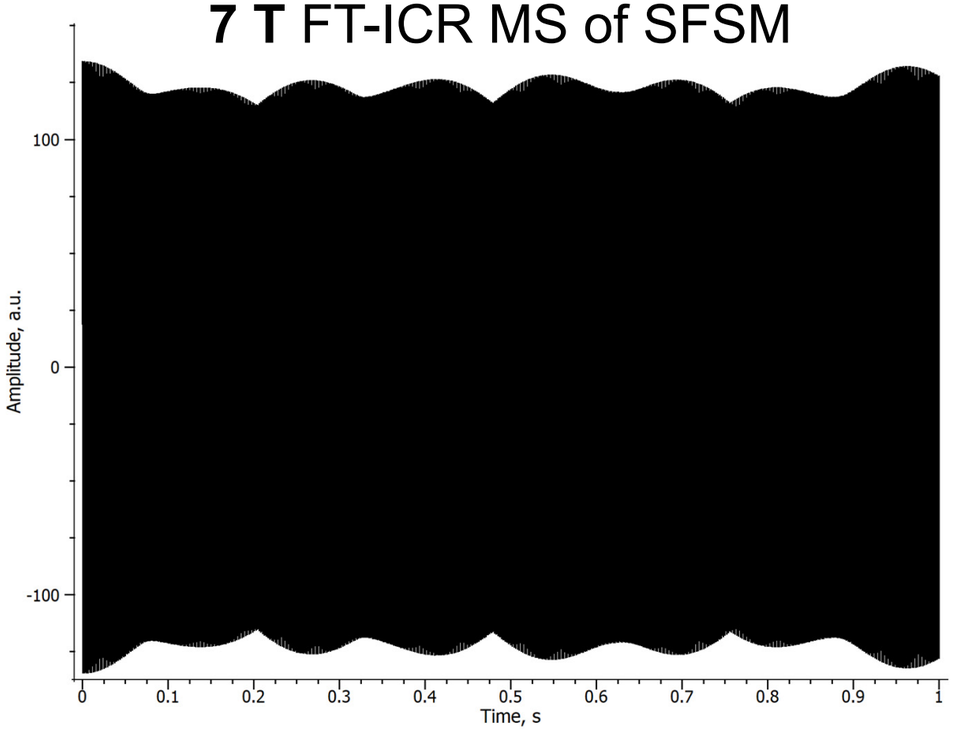
FT Processing Settings: **Resolution**

- **LTQ FT ICR MS** resolution settings are estimated at *m/z 4*00 (mFT mode)



FT Processing Settings: **Decay**

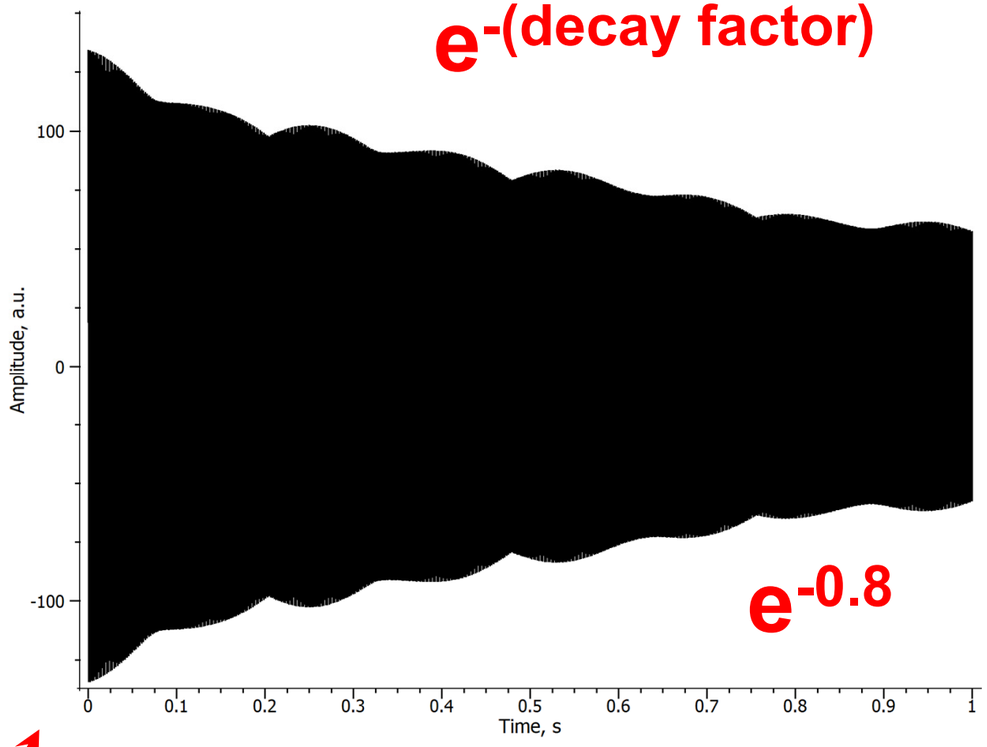
7 T FT-ICR MS of SFSM



space charge

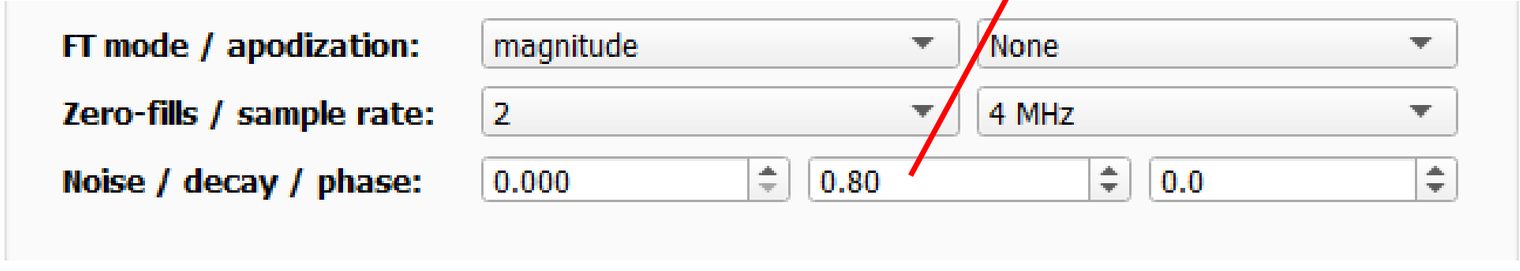
de-phasing

e-(decay factor)

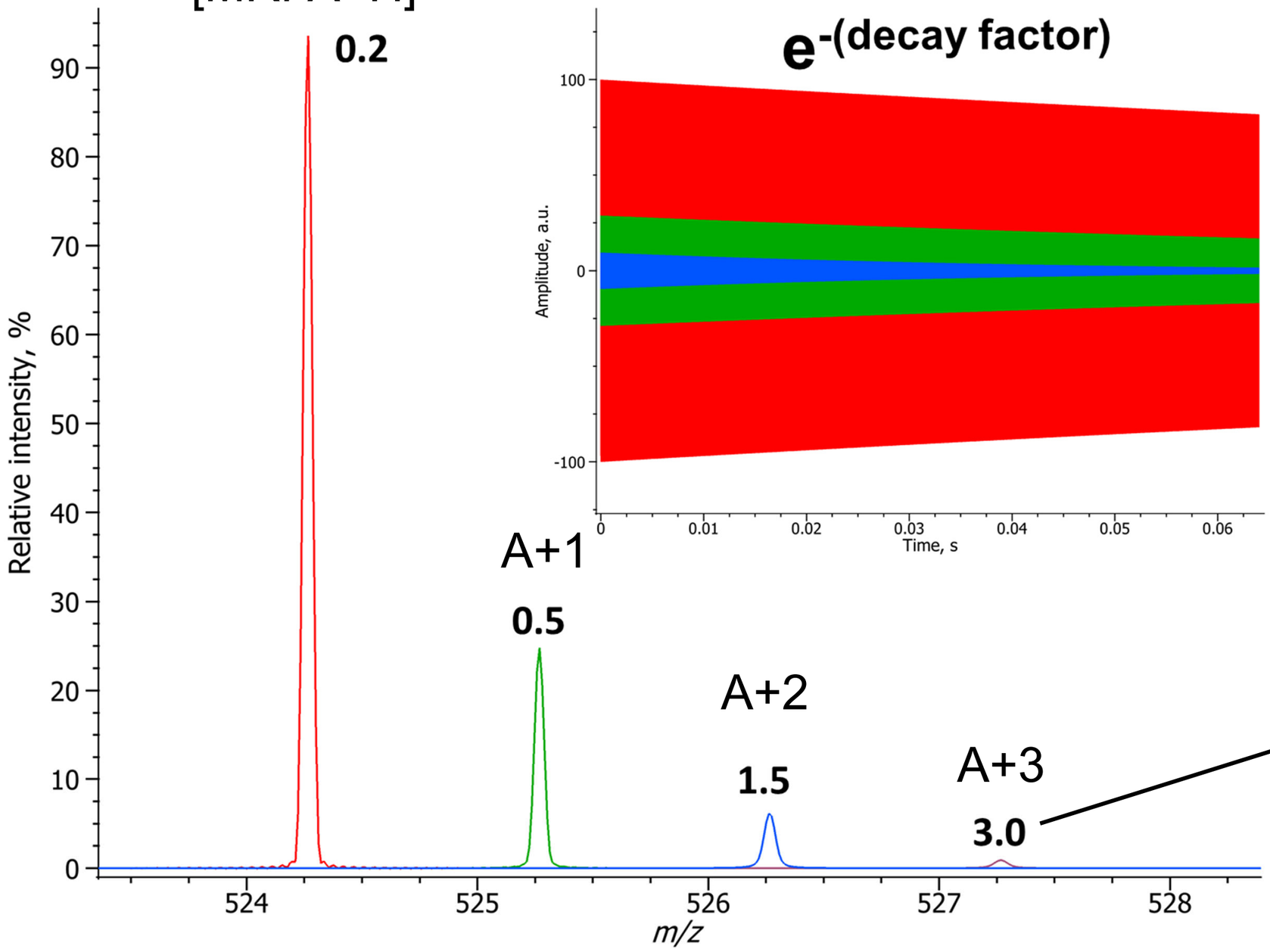


- Raw transient
- No decay

- Resulting transient with decay

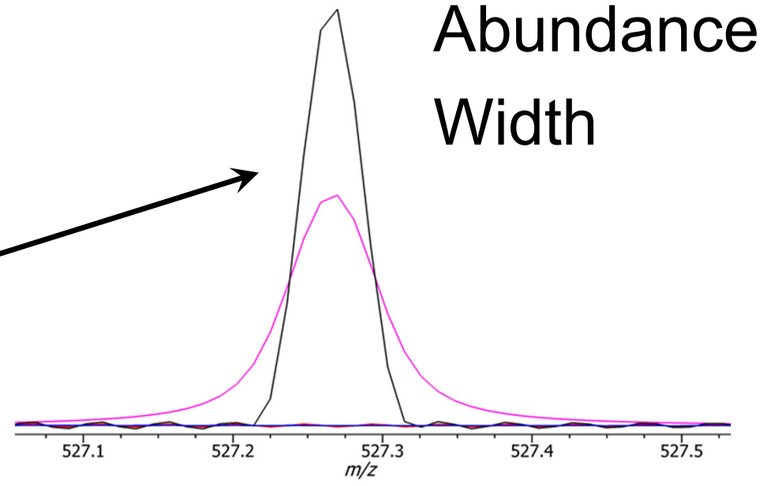


FT Processing Settings: **Decay**



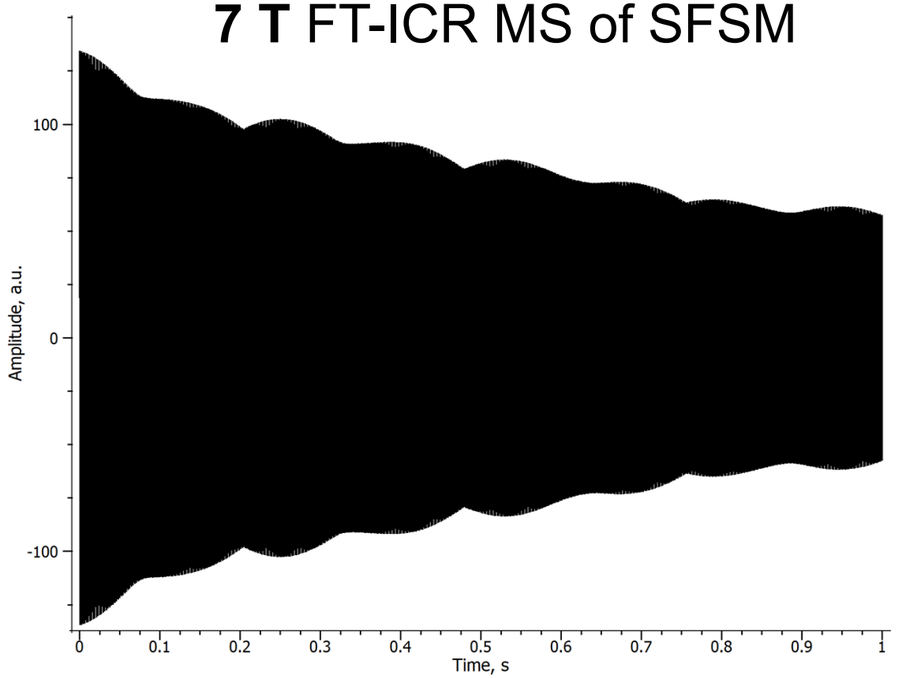
- Example: MRFA peptide *in-silico* analysis with a Q Exactive Orbitrap™
- Decay factors for isotopologues are S/N dependent

Isotopic ratios errors



FT Processing Settings: **Apodization**

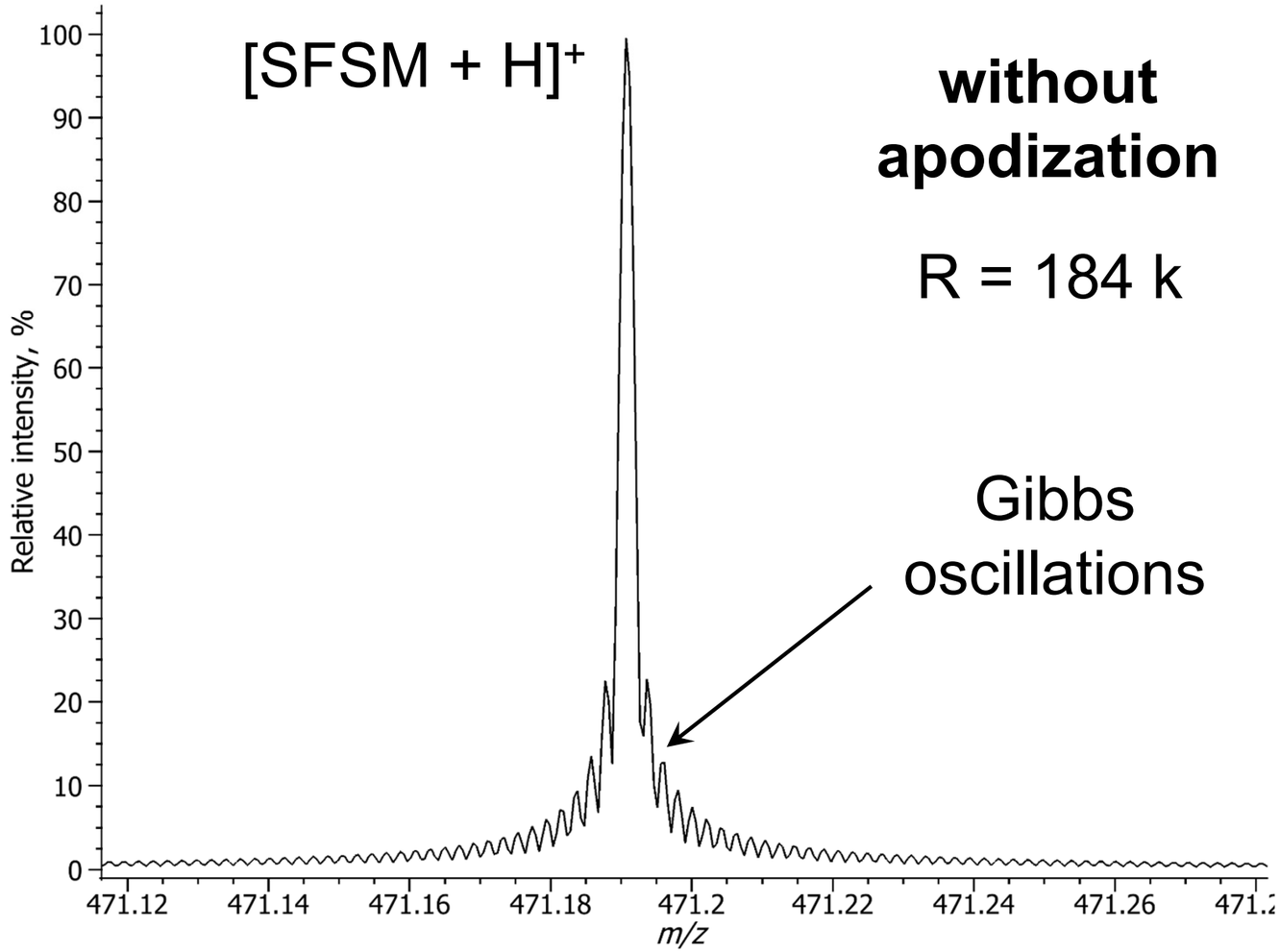
7 T FT-ICR MS of SFSM



mFT

- Raw transient
- Squared off ends of transient cause multiple false peaks in spectrum

[SFSM + H]$^+$

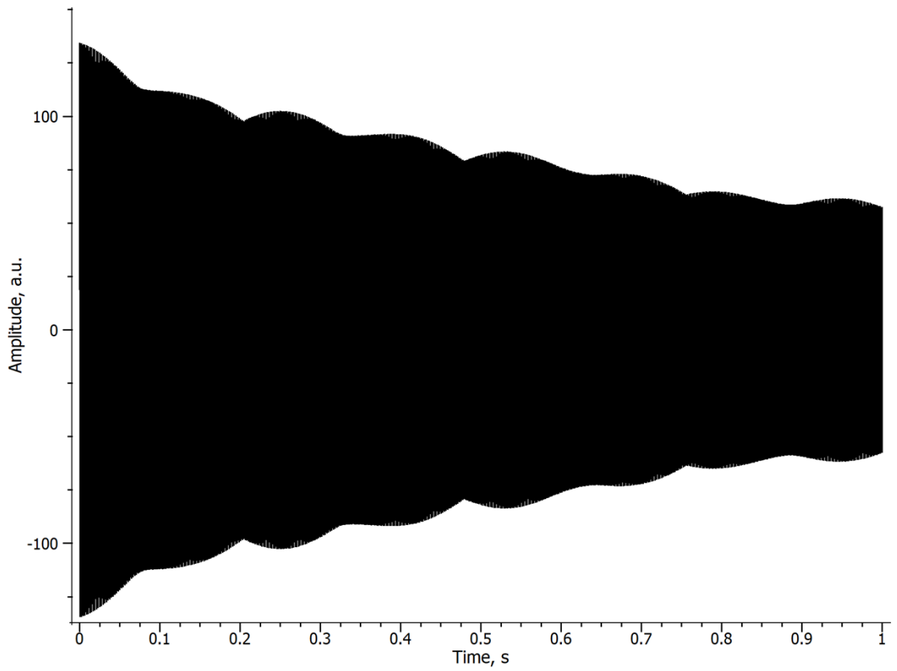


without apodization

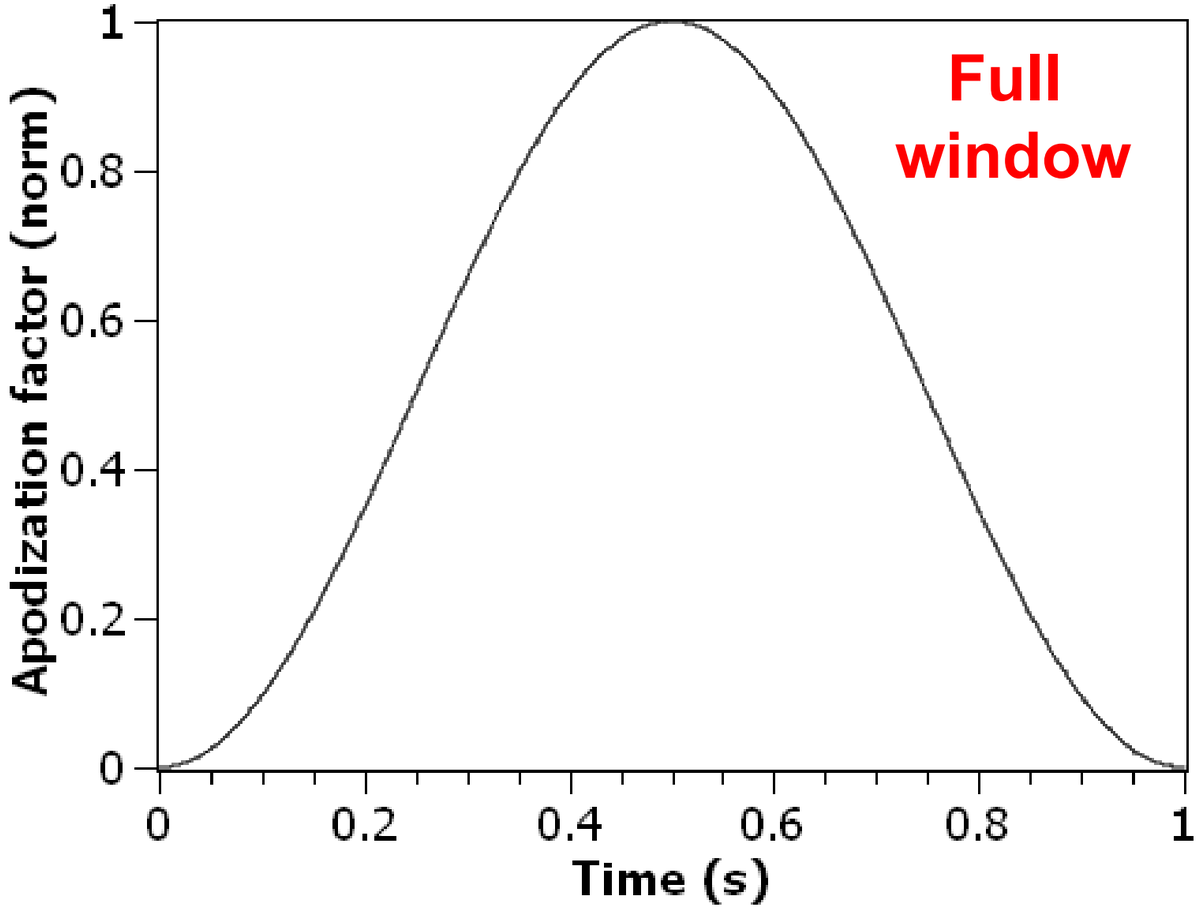
R = 184 k

- Resulting mass spectrum
- Zoomed onto a single peak

FT Processing Settings: **Apodization**



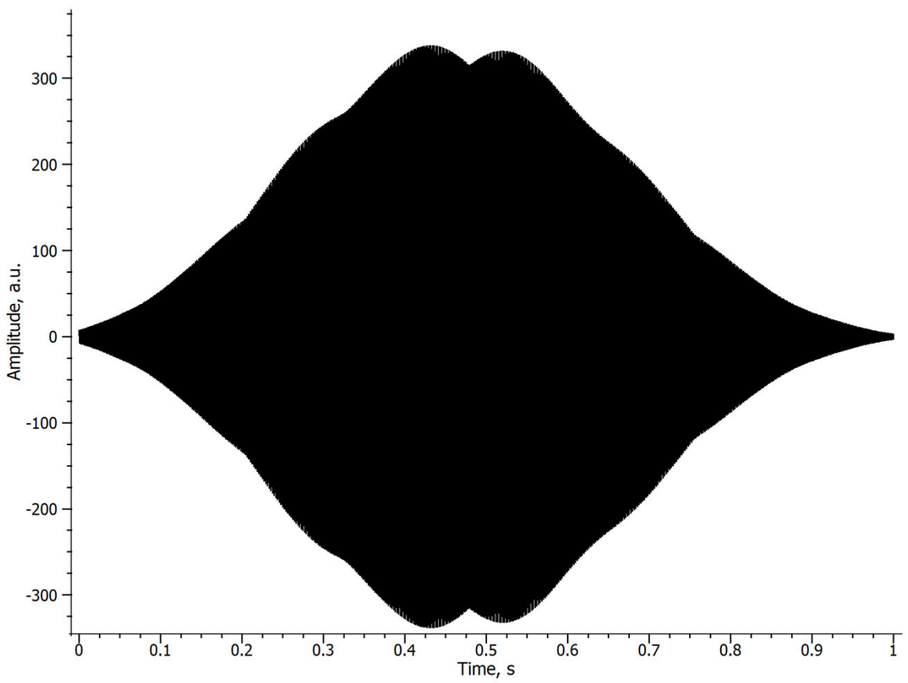
- Raw transient
- Squared off ends of transient cause multiple false peaks in spectrum



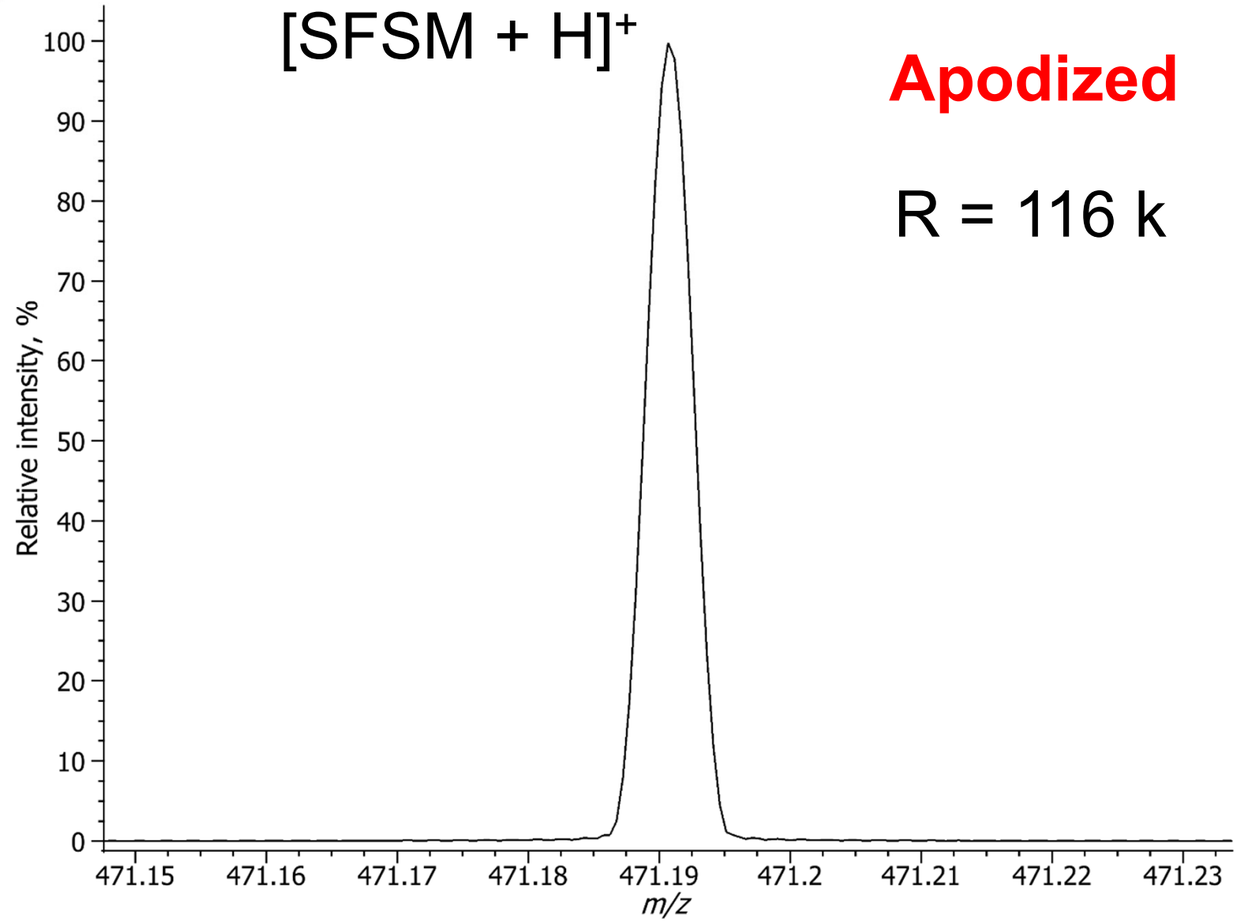
- Multiply transient by apodization function

FT Processing Settings: **Apodization**

Full window



mFT



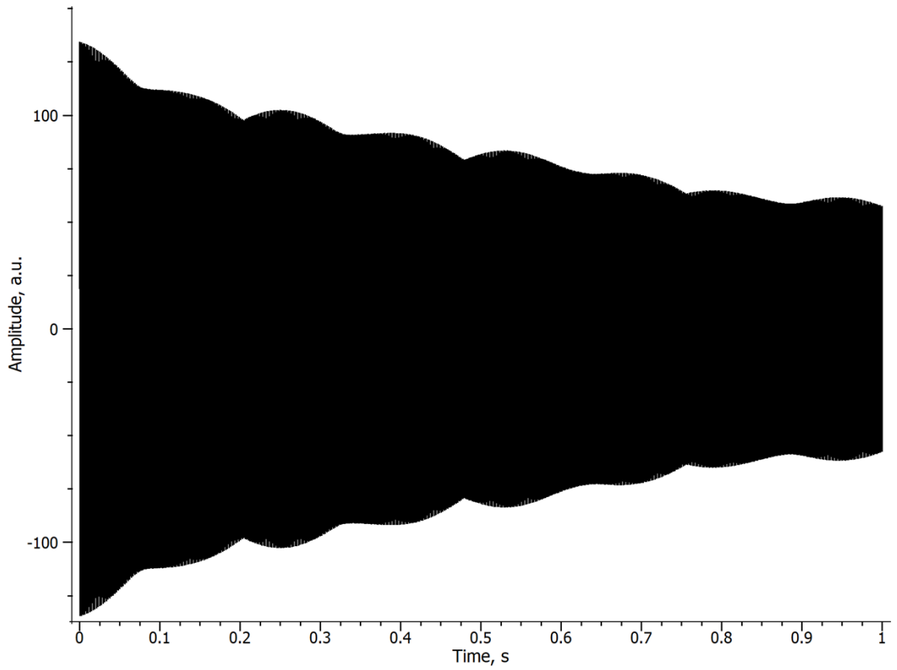
Apodized

R = 116 k

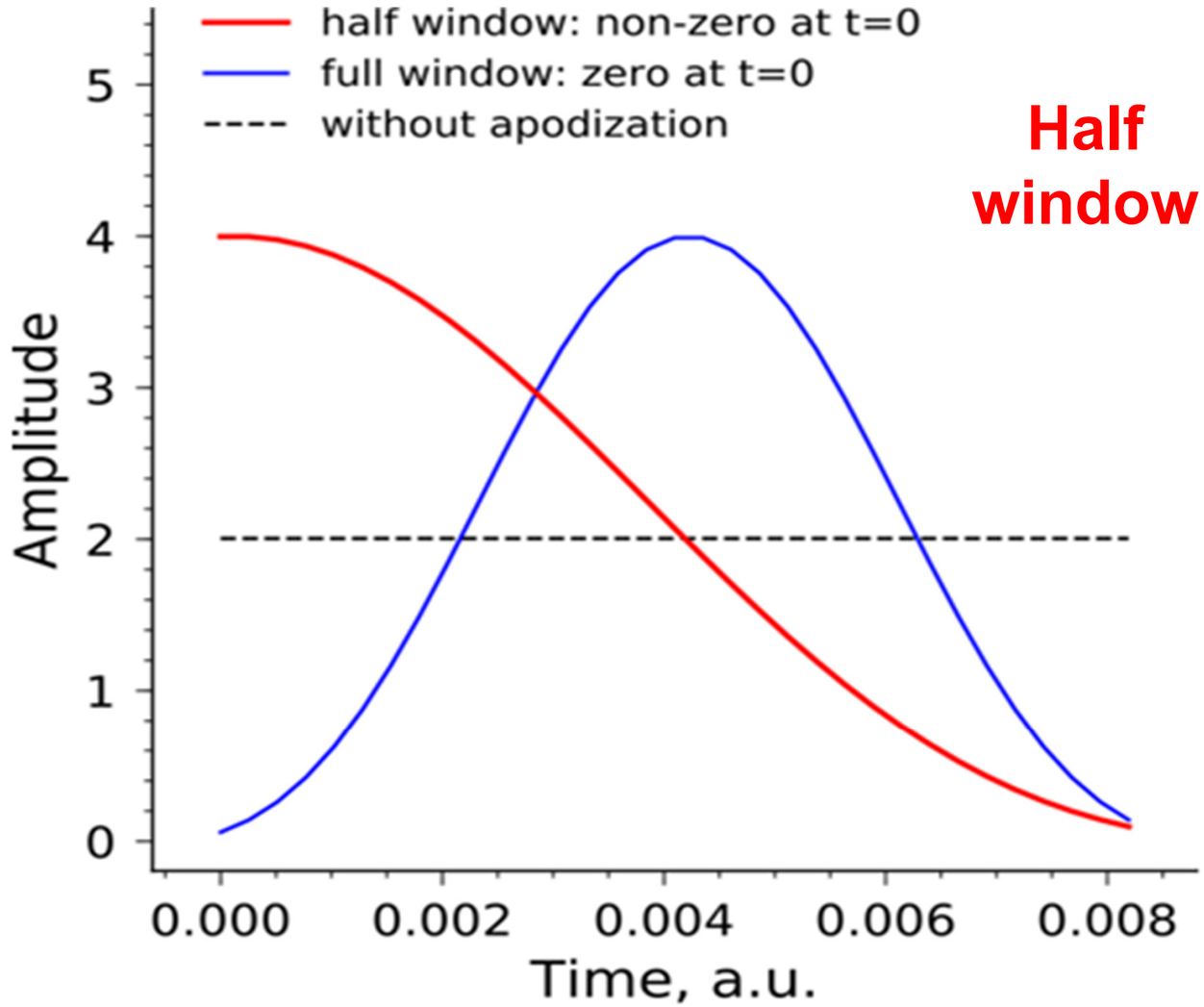
- Apodized transient
- Full window (Kaiser)
- No sharp edges
- No Gibbs oscillations

- But..
- Slight loss in resolution
- And loss in sensitivity

FT Processing Settings: **Apodization**



- Raw transient
- Squared off ends of transient cause multiple false peaks in spectrum

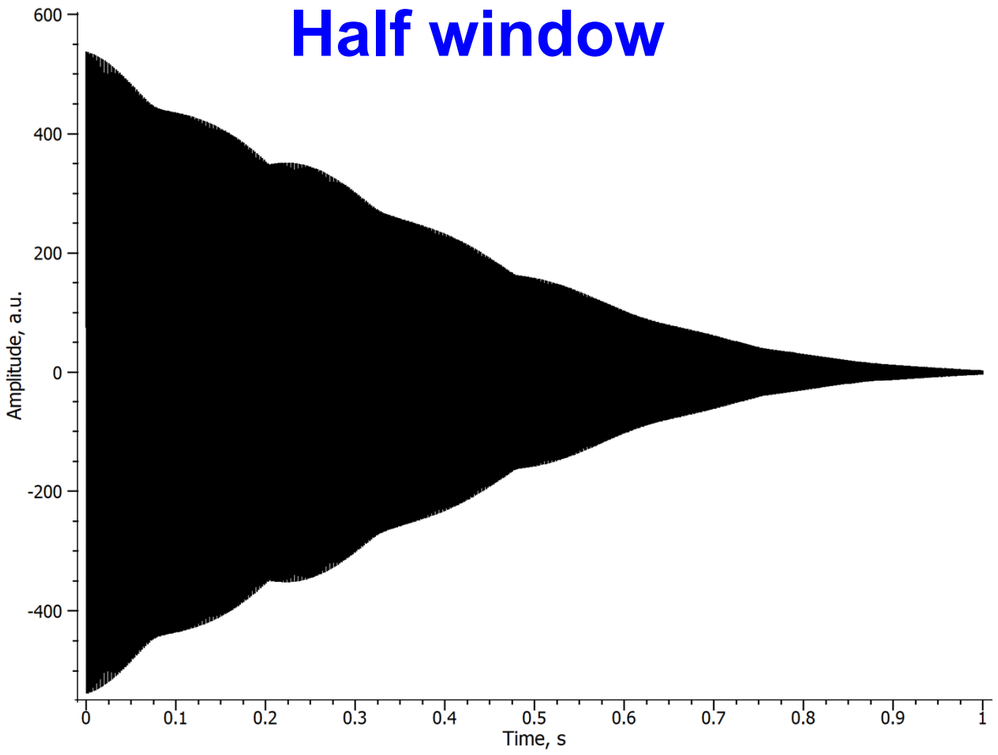


Half window

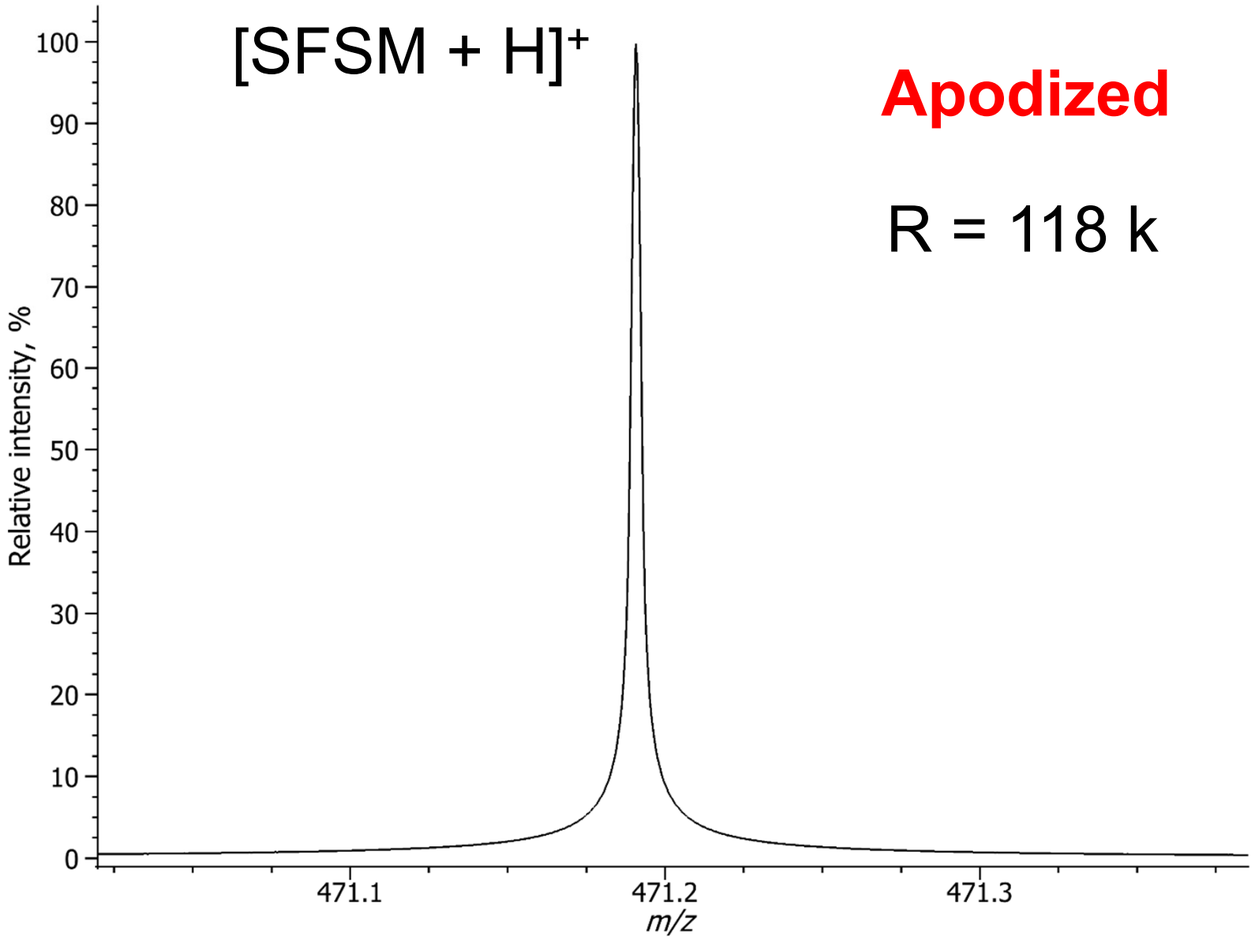
- Multiply transient by apodization function

FT Processing Settings: **Apodization**

Half window



mFT



Apodized

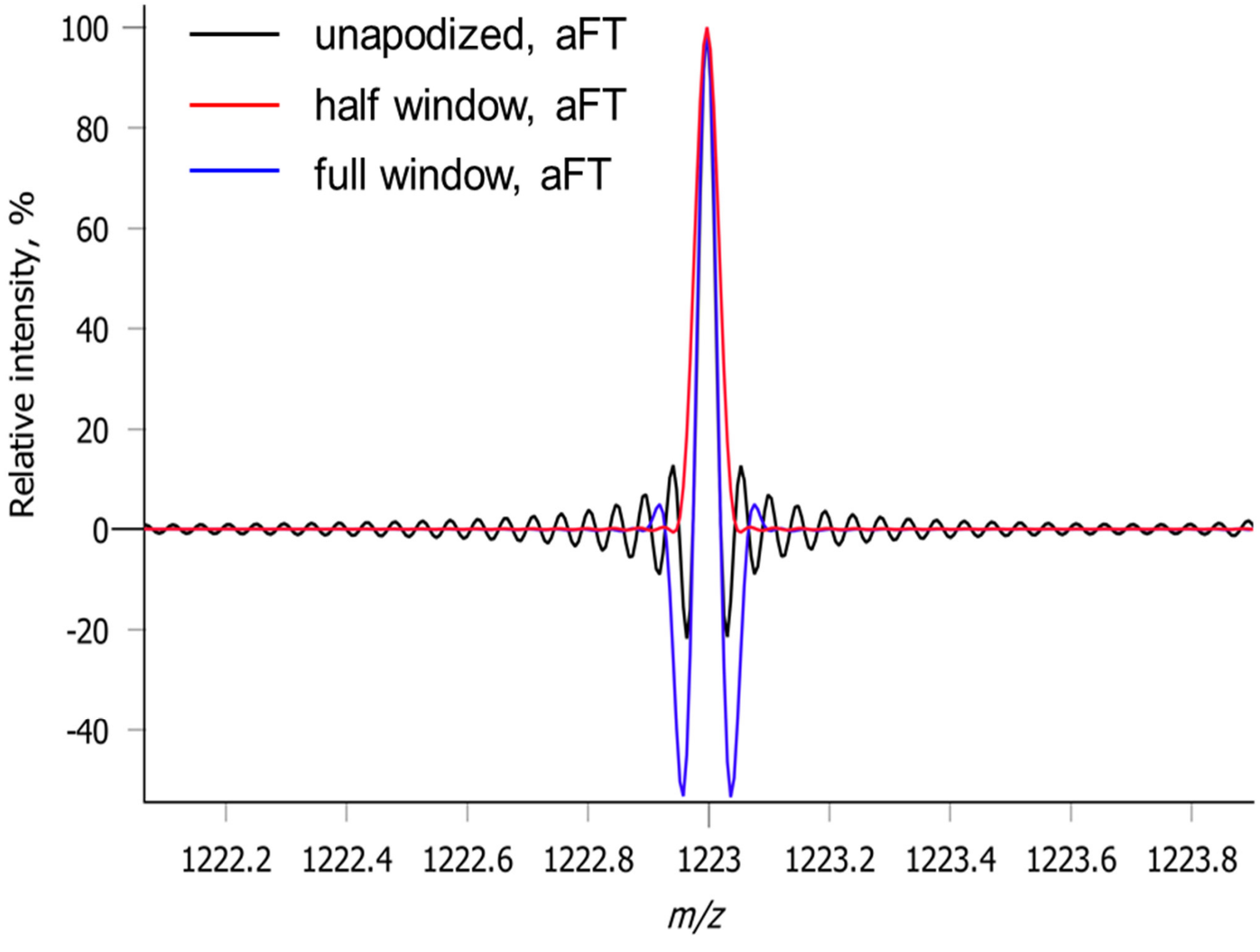
R = 118 k

- Apodized transient
- Half window (Kaiser)
- No sharp edges
- No Gibbs oscillations

- But..
- Slight loss in resolution
- And loss in sensitivity

FT Processing Settings: **Apodization**

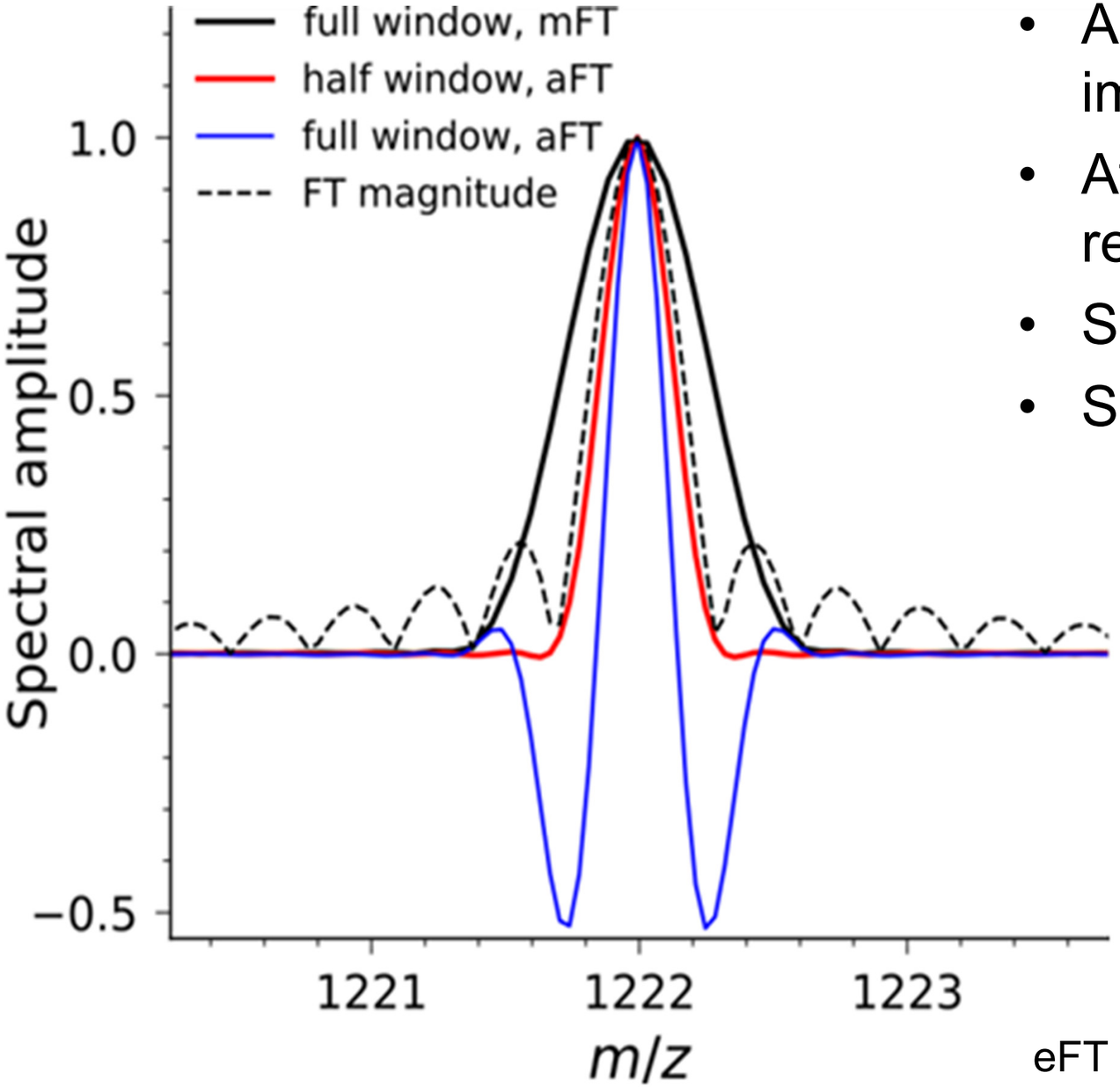
Absorption mode FT (aFT)



- Apodization in aFT improves peak shape and removes artefacts, but
- Also slightly reduces peak abundance and resolution
- **Full** window apodization produces characteristic negative side lobes
- Technically it is harder to generate aFT mass spectra with **half** window apodization

For details see: http://www.kilgourlab.com/absorption-mode-for-ft-ms/

FT Processing Settings: **Apodization**



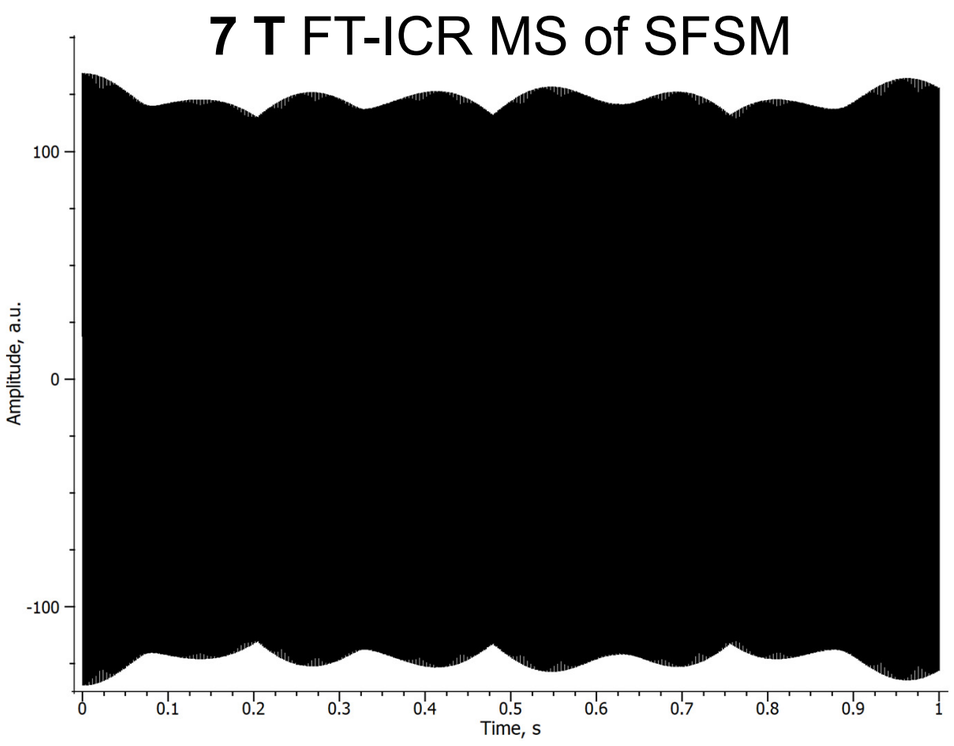
- Apodization reduces resolution, but improves peak shape, reduces artefacts
- After apodization, mFT provides twice lower resolution than aFT
- Suggested apodization window for mFT: **full**
- Suggested apodization window for aFT: **half**

eFT

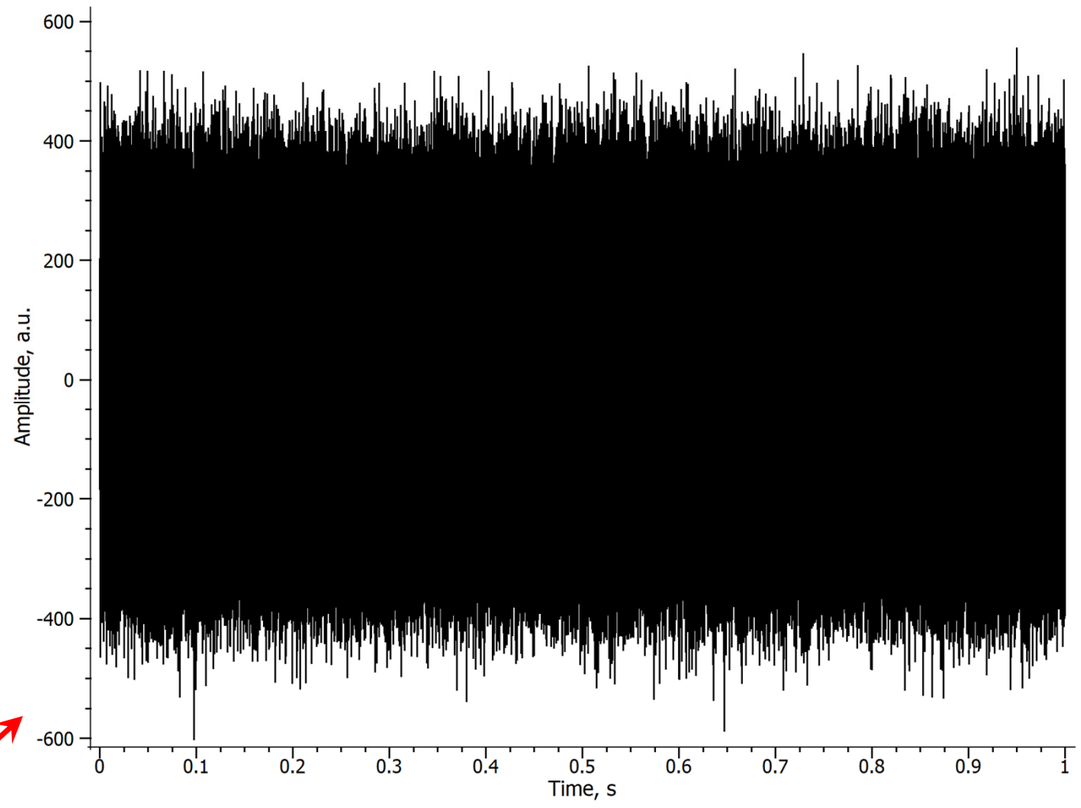
- Enhanced FT (eFT) combines mFT/aFT
- Peak bottom (50%): **unapodized** mFT
- Peak top (50%): half window aFT
- Artefacts are smoothed/removed

eFT principles: Lange, Makarov, *et al. IJMS* 369 (2014) 16–22

FT Processing Settings: **Noise**

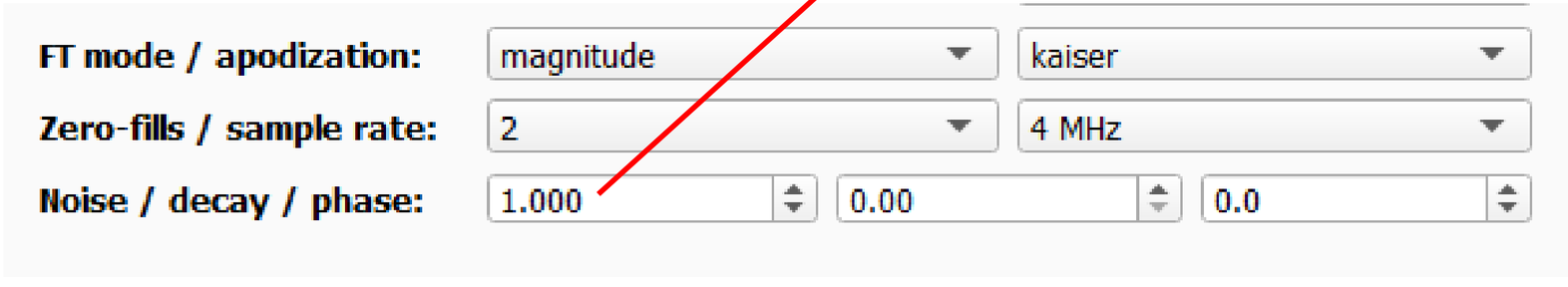


+noise

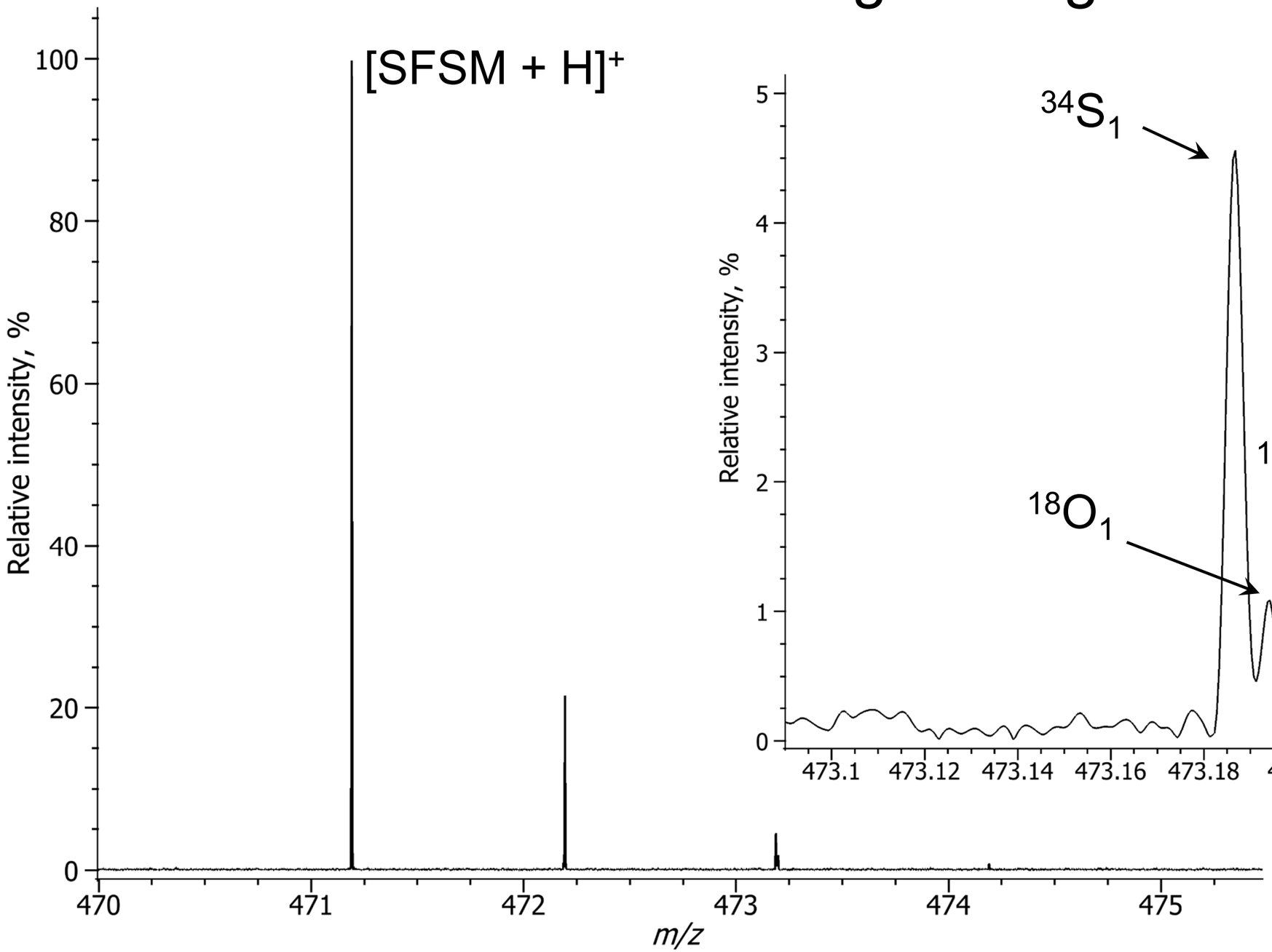


- Raw transient
- No noise

- Resulting transient with noise



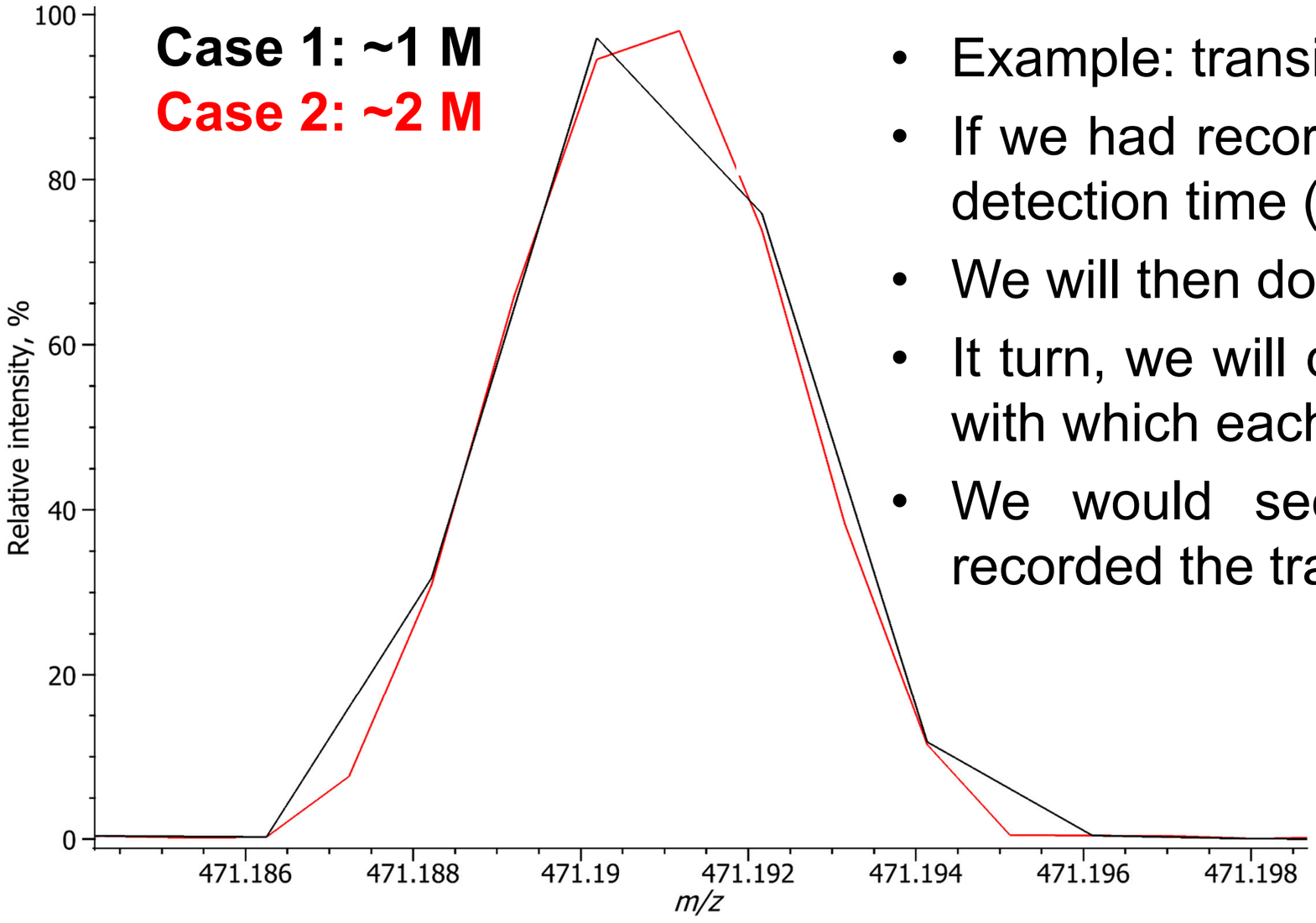
FT Processing Settings: **Noise**



A+2 isotopologue

FT Processing Settings: **Zero-fills**

- How to improve peak digitization for enhanced resolution and mass accuracy?

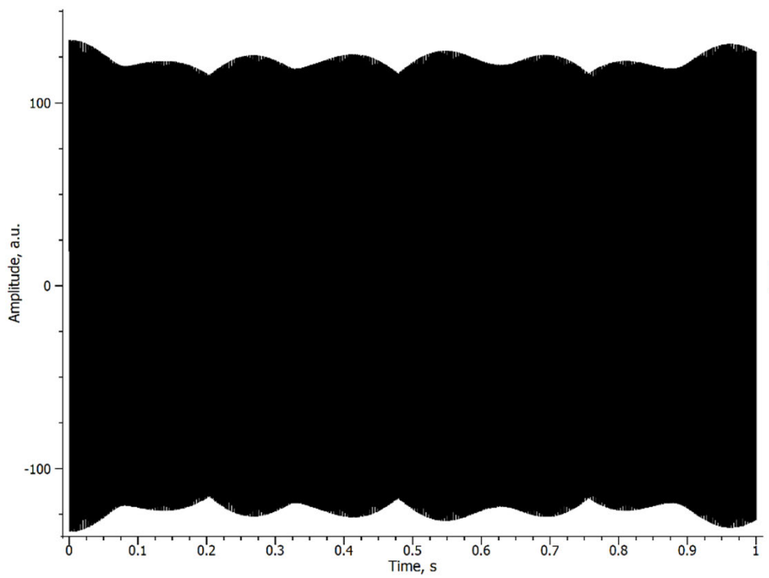


- Example: transient contains 1 M data points
- If we had recorded ~2 M points in the same ion detection time (transient length)
- We will then double the transient digitization rate
- It turn, we will double the number of data points with which each mass spectral peak is defined
- We would see the same effect if we had recorded the transient for twice as long

An alternative: zero-fills (pads)

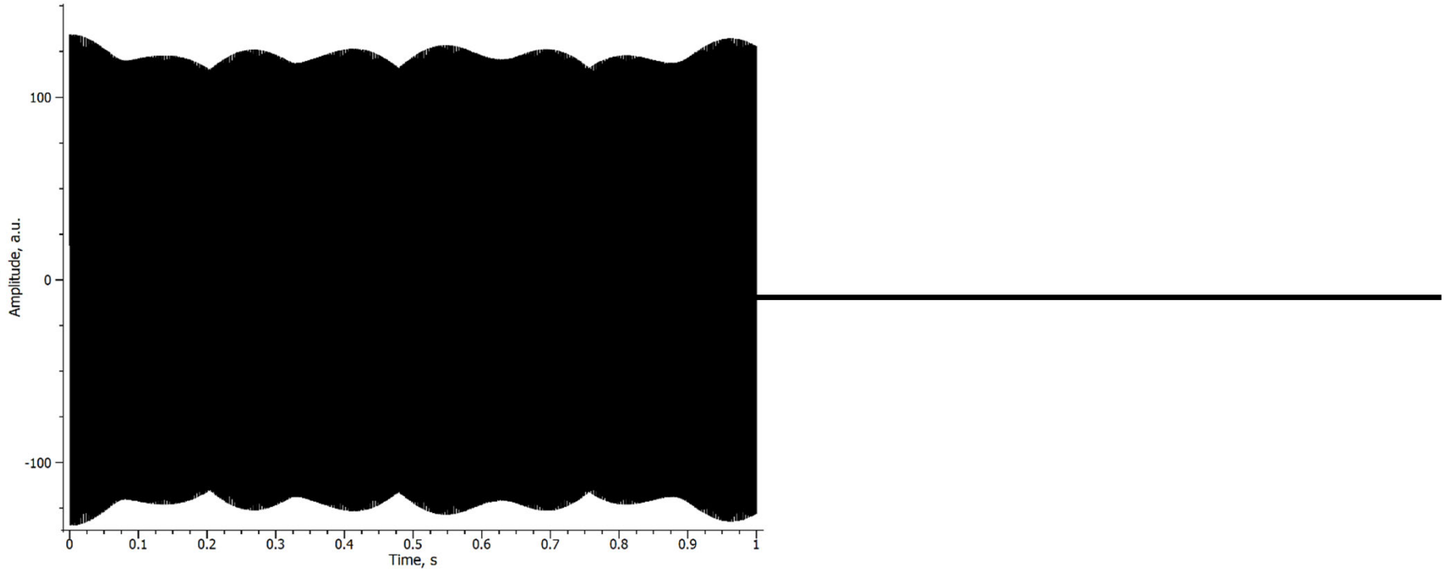
FT Processing Settings: **Zero-fills**

7 T FT-ICR MS of SFSM



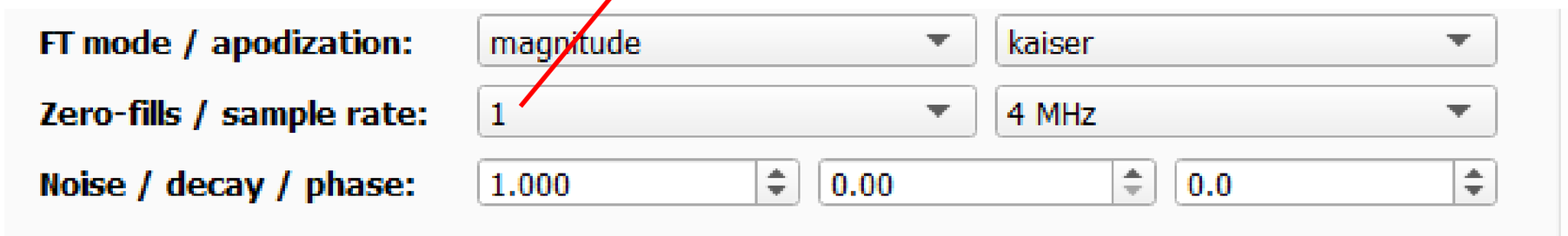
add zeroes

~1 M numbers **~1 M zeroes**

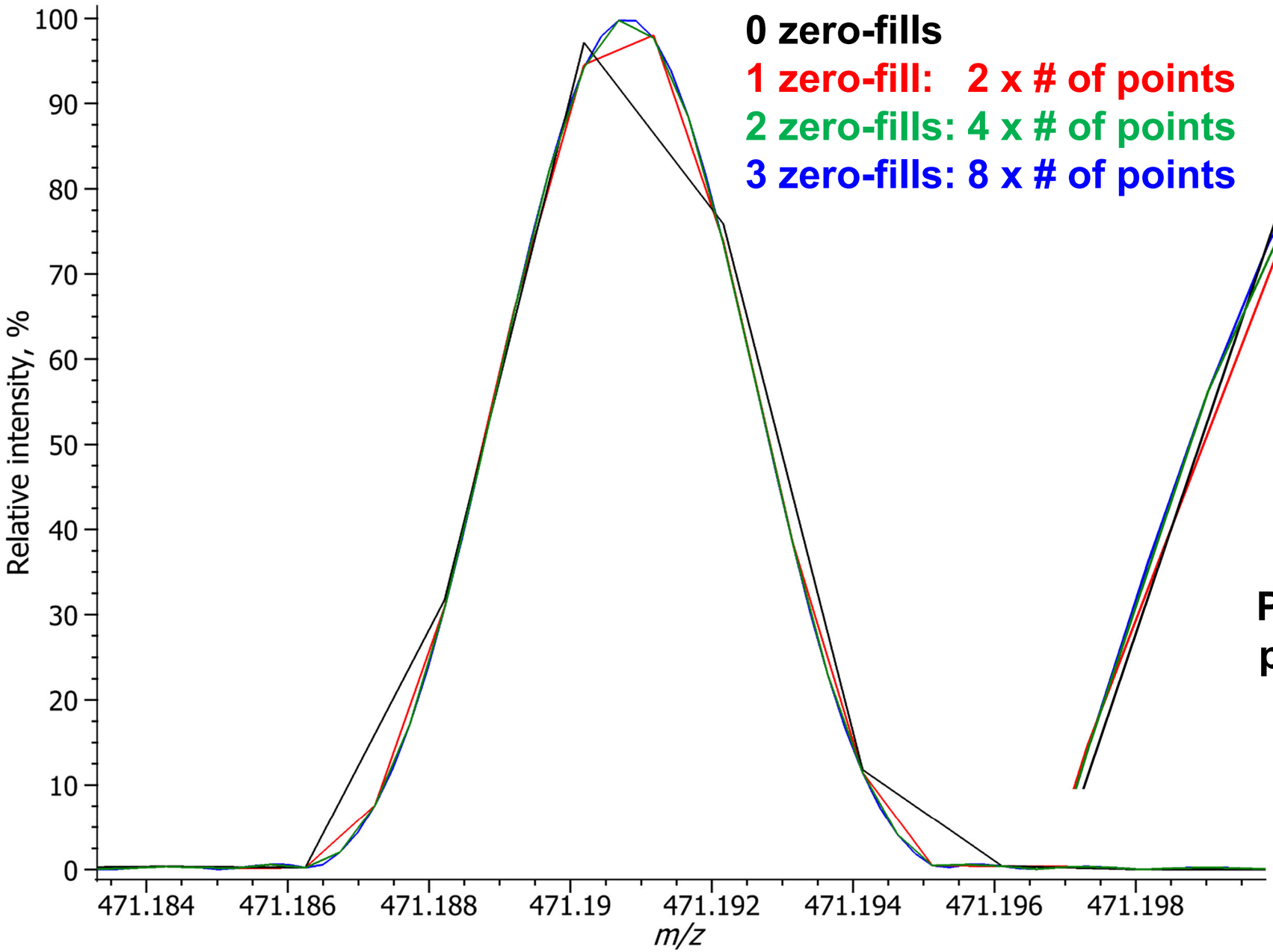


- Raw transient

- Transient with one zero-fill (pad)
- Each zero-fill doubles the length of a transient (including prior zeroes)



FT Processing Settings: **Zero-fills**



Peak picking: a 3-point parabolic interpolation

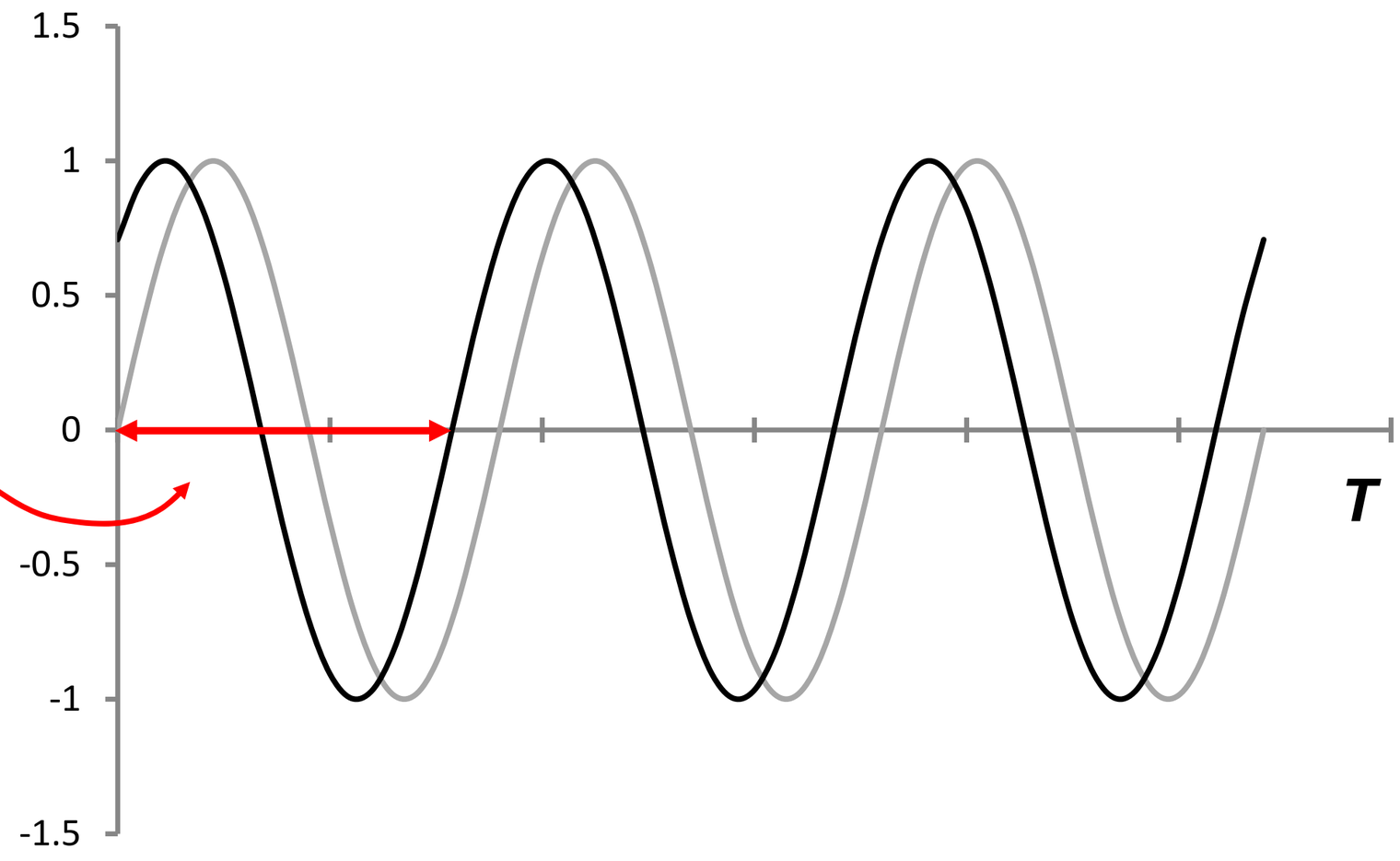
Typically in FTMS: 1-2 zero-fills

FT Processing Settings: **Phase (Angle)**

Time-Domain Ion Signal: Transient

Three components to define a wave:

- Frequency
- Magnitude (measure of amplitude)
- Phase (angle)



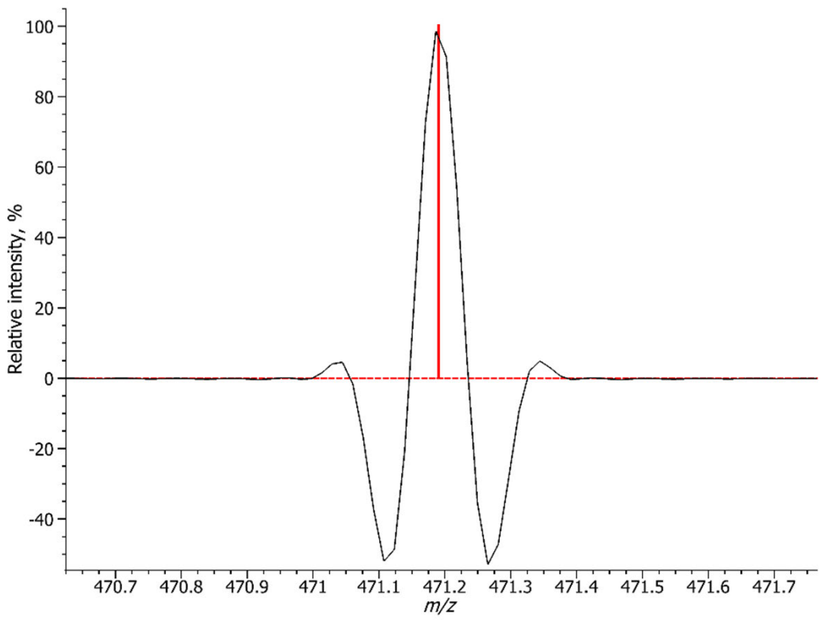
- example: two waves of the same frequency and amplitude, but different phase

For details see: http://www.kilgourlab.com/absorption-mode-for-ft-ms/

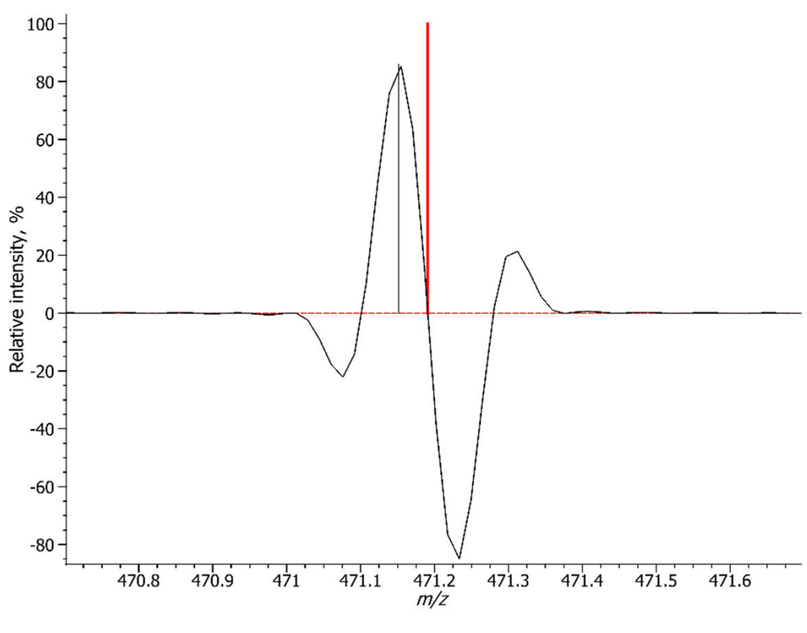
FT Processing Settings: **Phase (Angle)**

- Example: 7 T FT-ICR MS of SFMS, absorption mode FT, full window (Kaiser)
- Phase shift results in peak artefacts: peak position (*m/z*) and abundance
- Accurate phasing is crucial for artefact-free aFT mass spectra

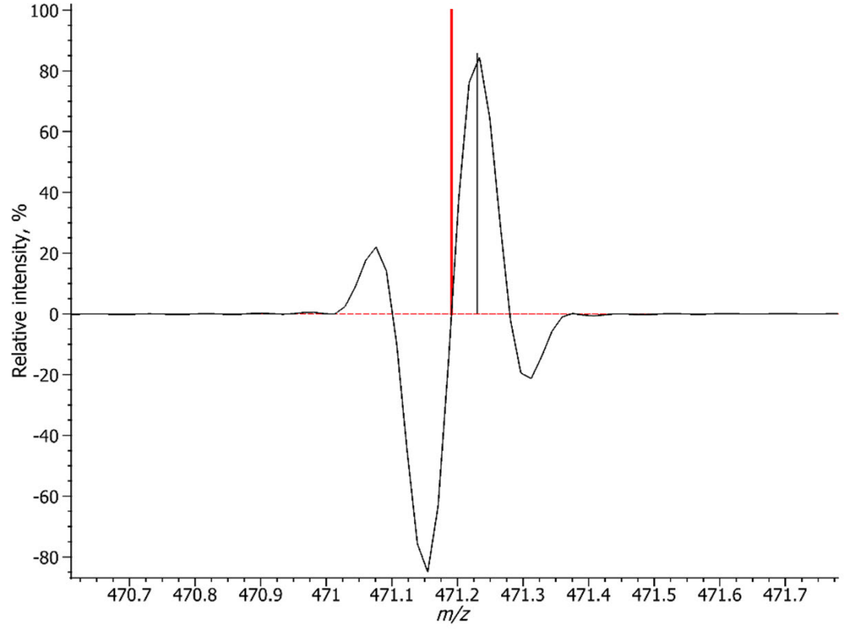
Phase: 0 degrees



Phase: 90 degrees



Phase: 270 degrees



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