

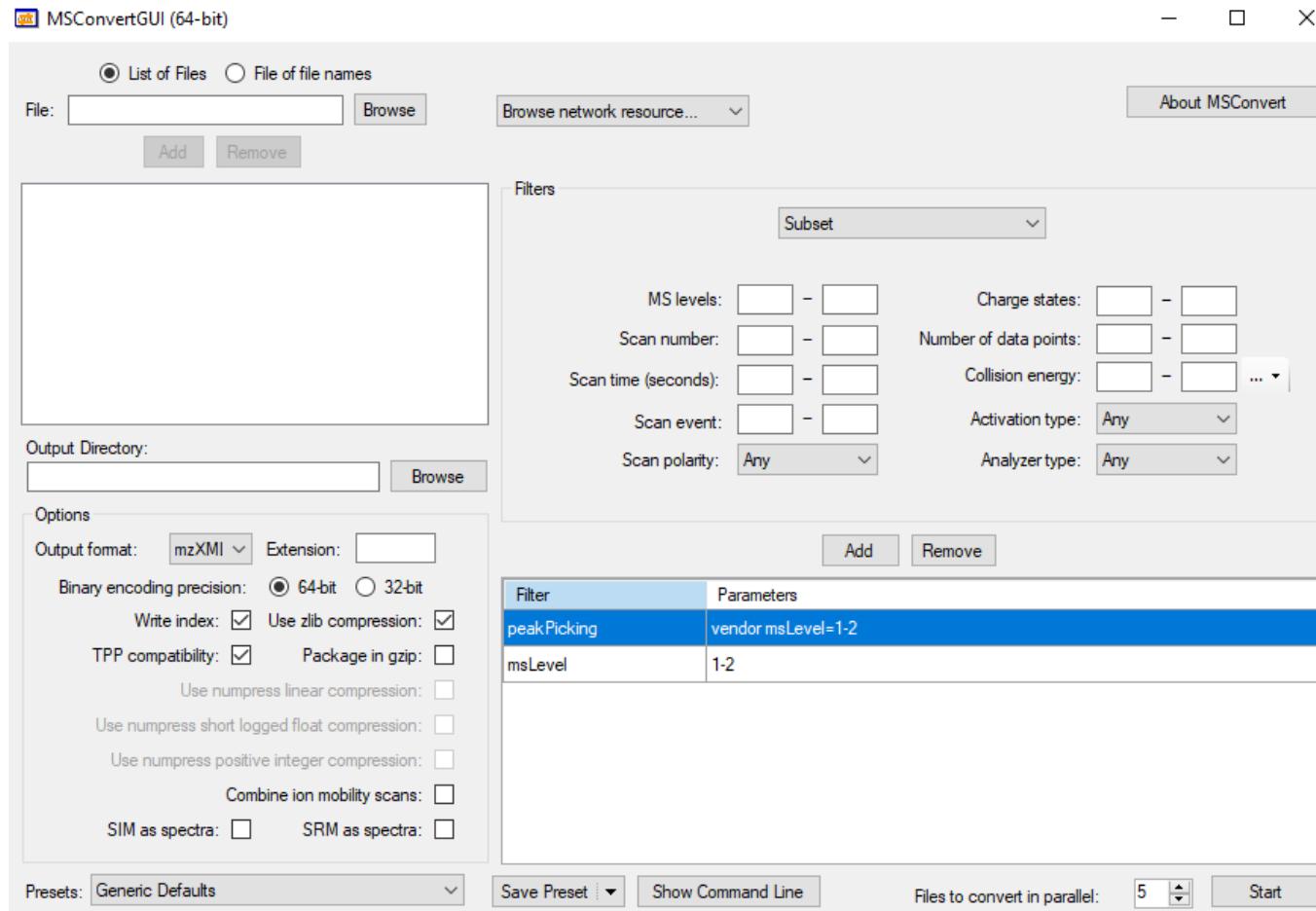
LC-MS-Cheet-Sheet



[https://github.com/plyush1993/
LC-MS-Cheet-Sheet/tree/main](https://github.com/plyush1993/LC-MS-Cheet-Sheet/tree/main)

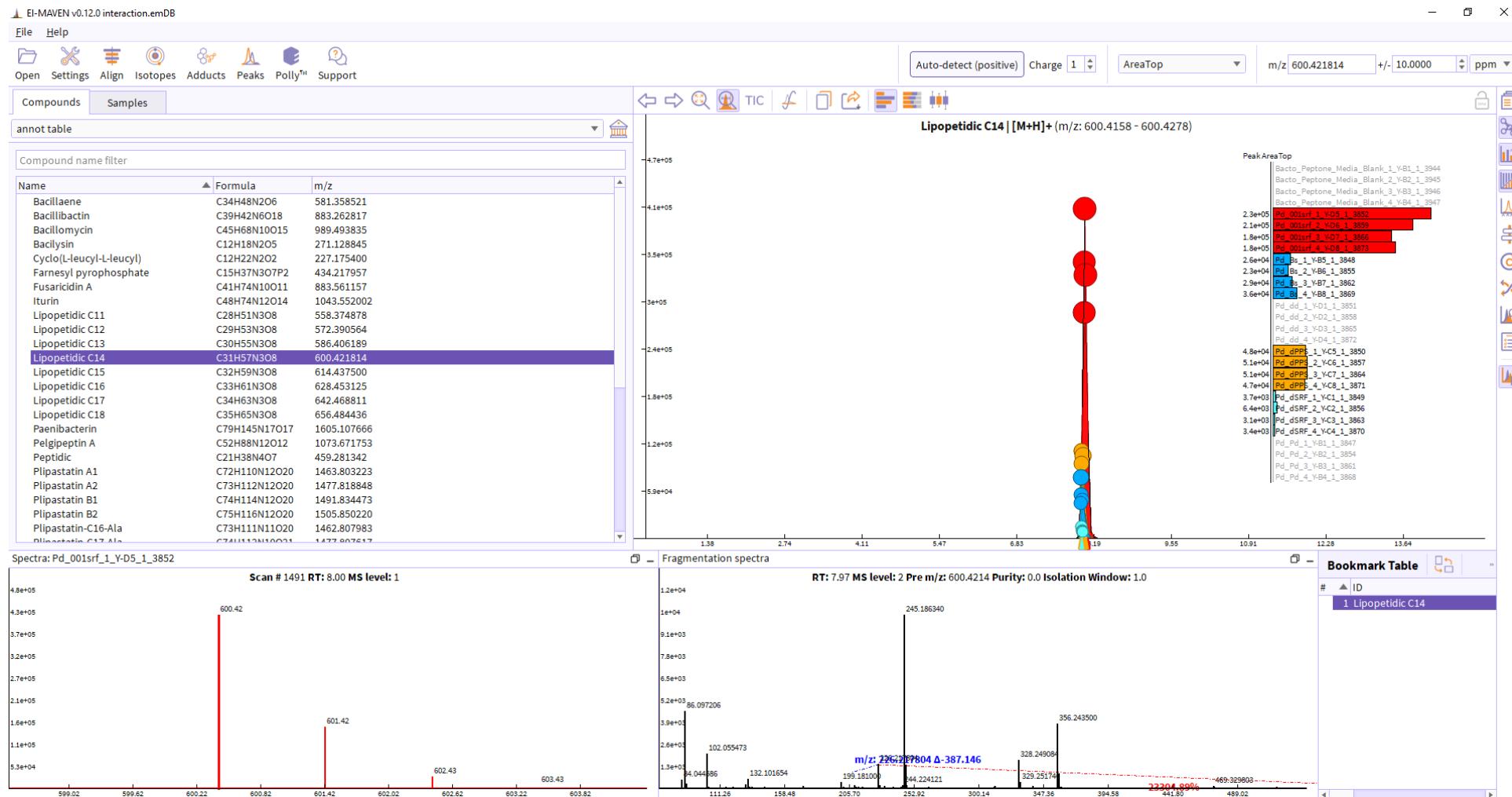
MSConvert

Convert raw LC-MS to mzXML, mzML



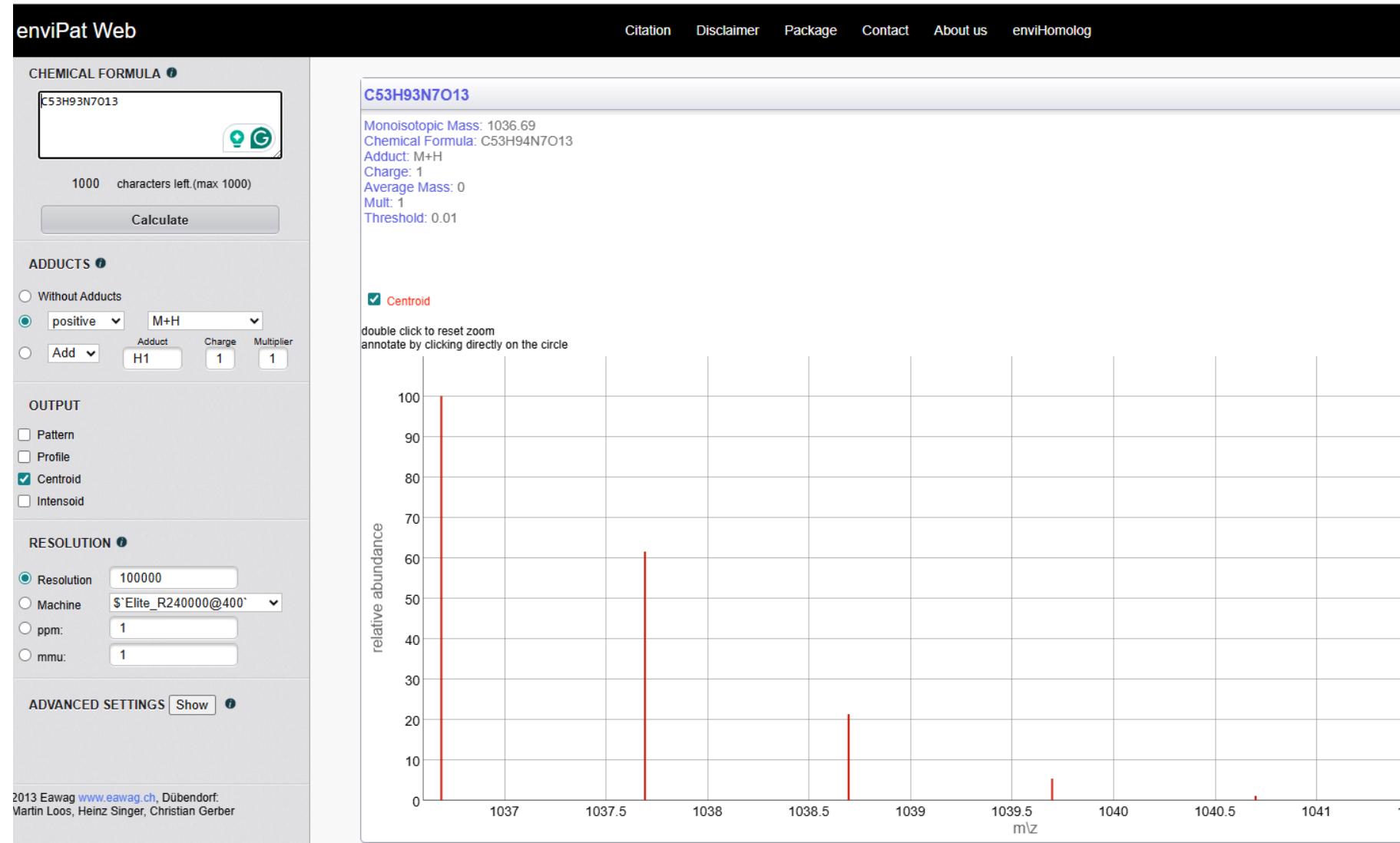
El-Maven

Open LC-MS (mzXML, mzML), get MS1-2 spectra, integrate peaks, search by database



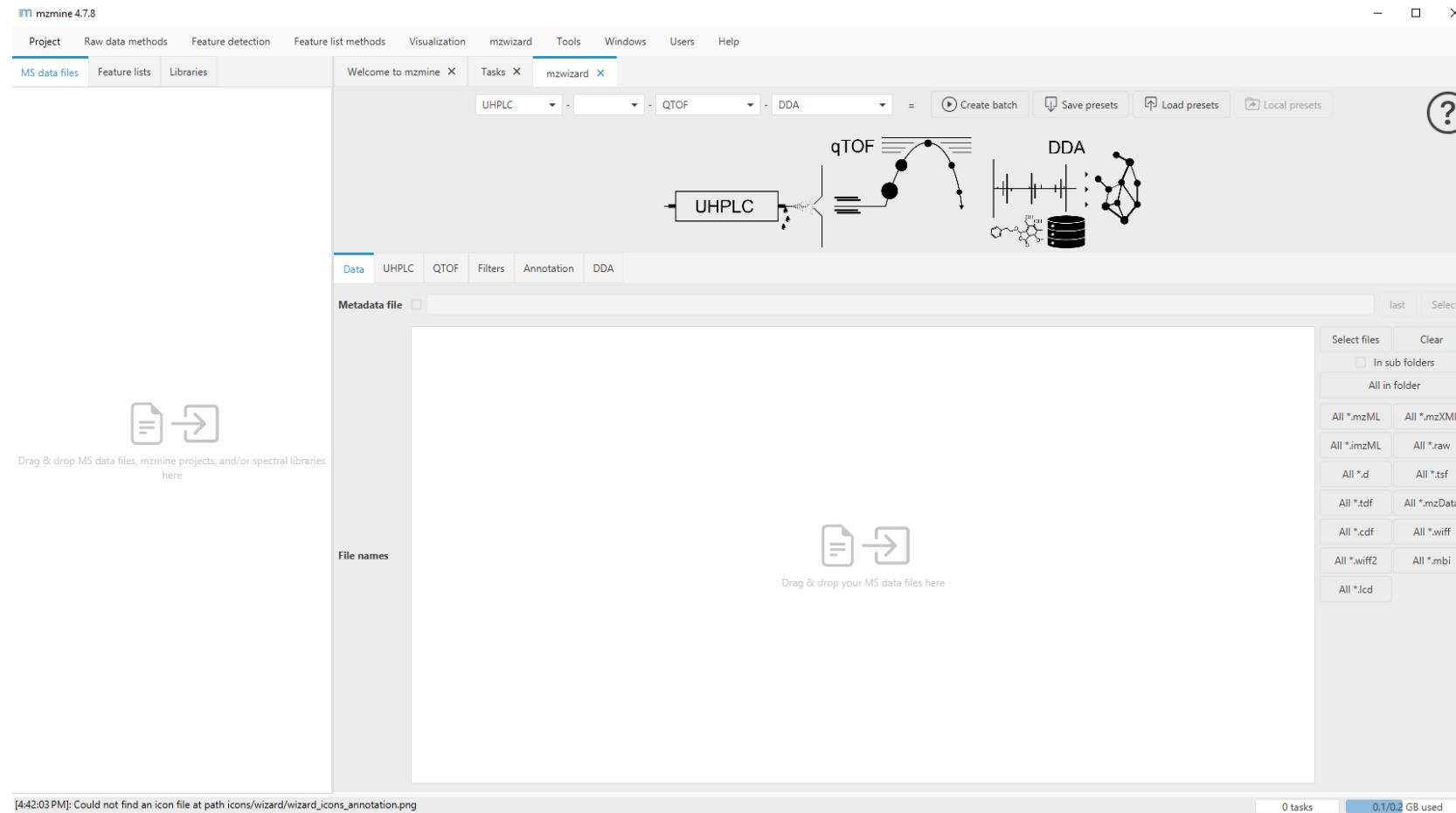
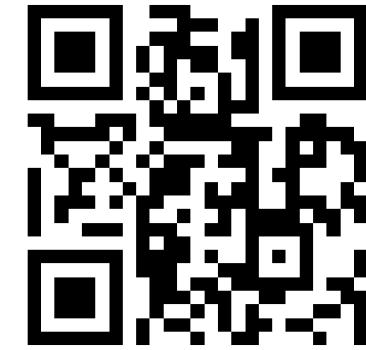
EnviPat

MS1 Isotopic Pattern Generating



MzMine

Peaks Integration (peak table)
Generating spectra files (mgf)
Annotation / Identification



SIRIUS

Annotation / Identification (by mgf from mzmine)



Compute

Preset Default Save Save as Remove Export Import Advanced

Global Configuration

Instrument Q-TOF
MS2 mass accuracy (ppm) 10

Possible Adducts: [M + H]⁺ [M + Na]⁺ [M + K]⁺ [M]⁺ [M + H₃N + H]⁺

Search DBs: test PubChem Biocyc Blood Exposome CHEBI

all none bio

Spectral Library Search

Spectral Matching Identity Search Precursor Deviation (ppm) 20 Analogue Search Perform Analogue Search

SIRIUS - Molecular Formula Identification

SIRIUS Strategy: De novo + bottom up (recommended)

Perform de novo below m/z 400

Element Filter: Apply 'allowed elements' filter to De novo Allowed elements H,C,N,O,P Re-detect

Predict properties: CSI:FingerID - Fingerprint Prediction & CANOPUS - Compound Class Prediction

Predict General Score threshold

CSI:FingerID - Structure Database Search

Search DBs Search strategy: PubChem as fallback Confidence mode APPROXIMATE

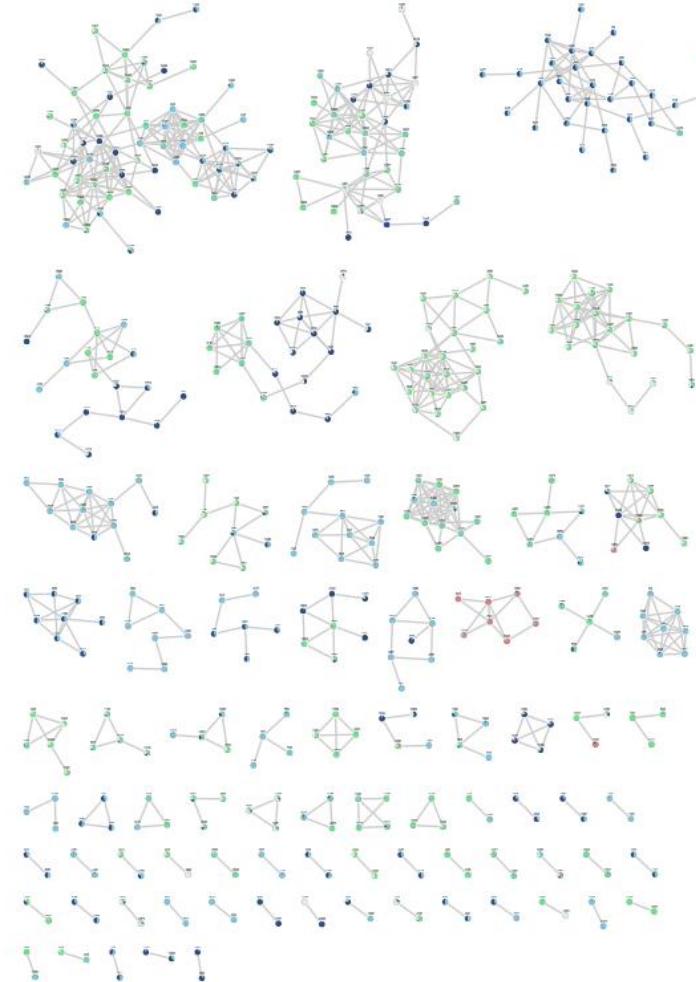
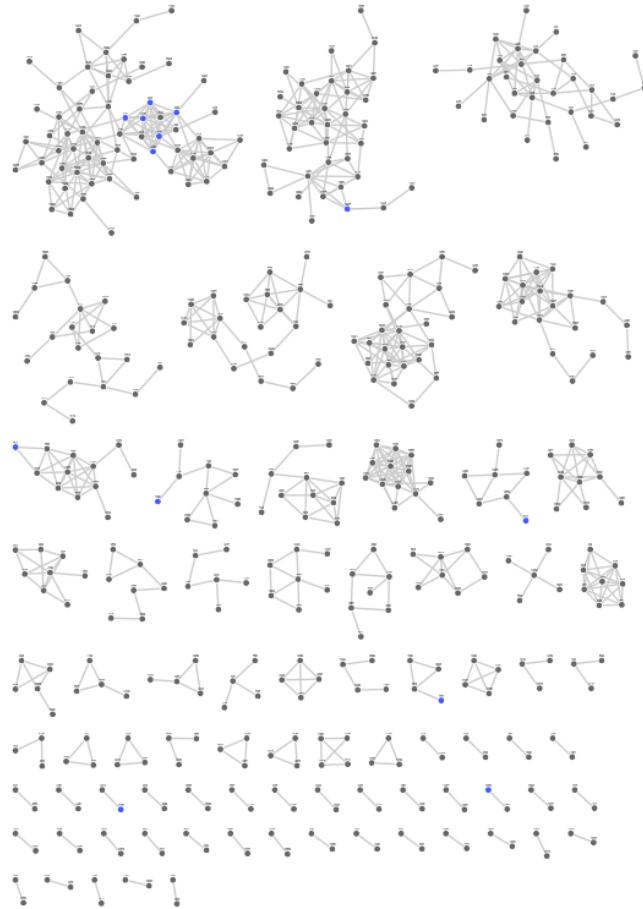
MSNovelist - De Novo Structure Generation

MSNovelist

Recompute already computed tasks? Show Command Show JSON Compute Cancel

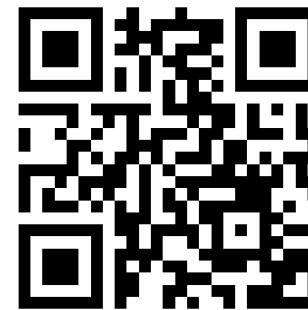
GNPS

Molecular Networking
(by mgf, peak table from mzmine)

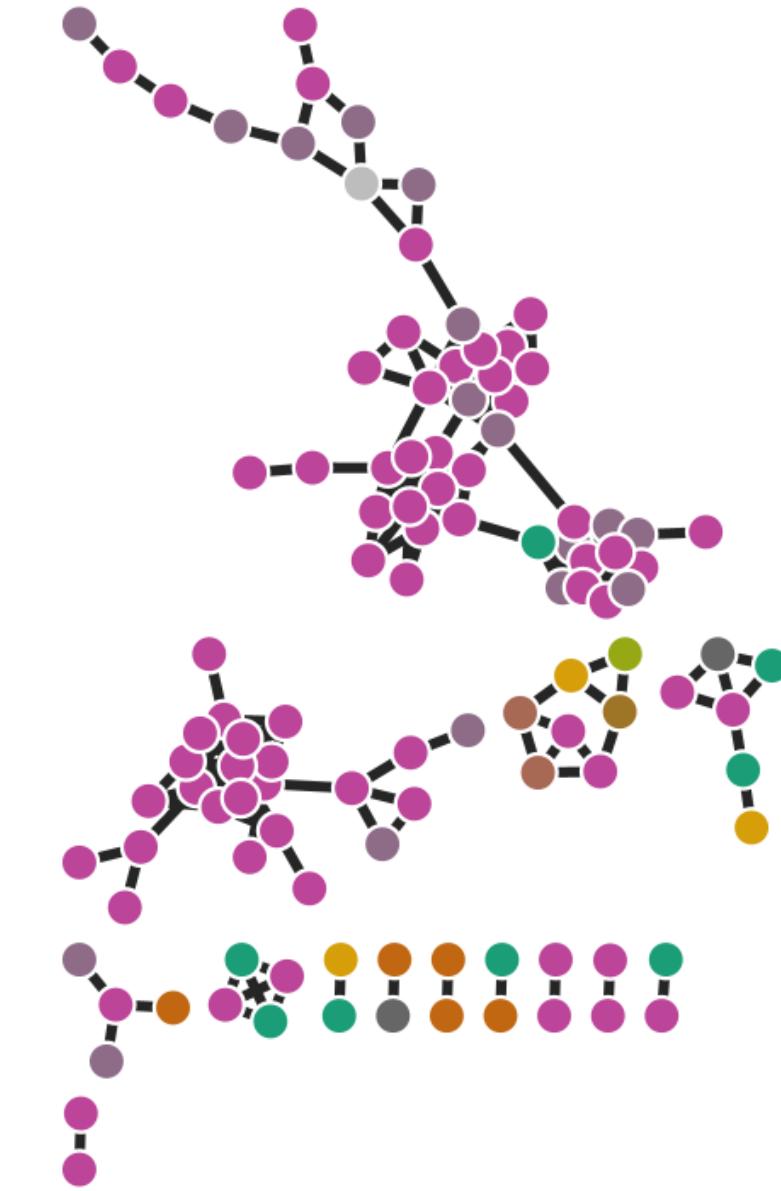


Cytoscape

Combine GNPS + Sirius



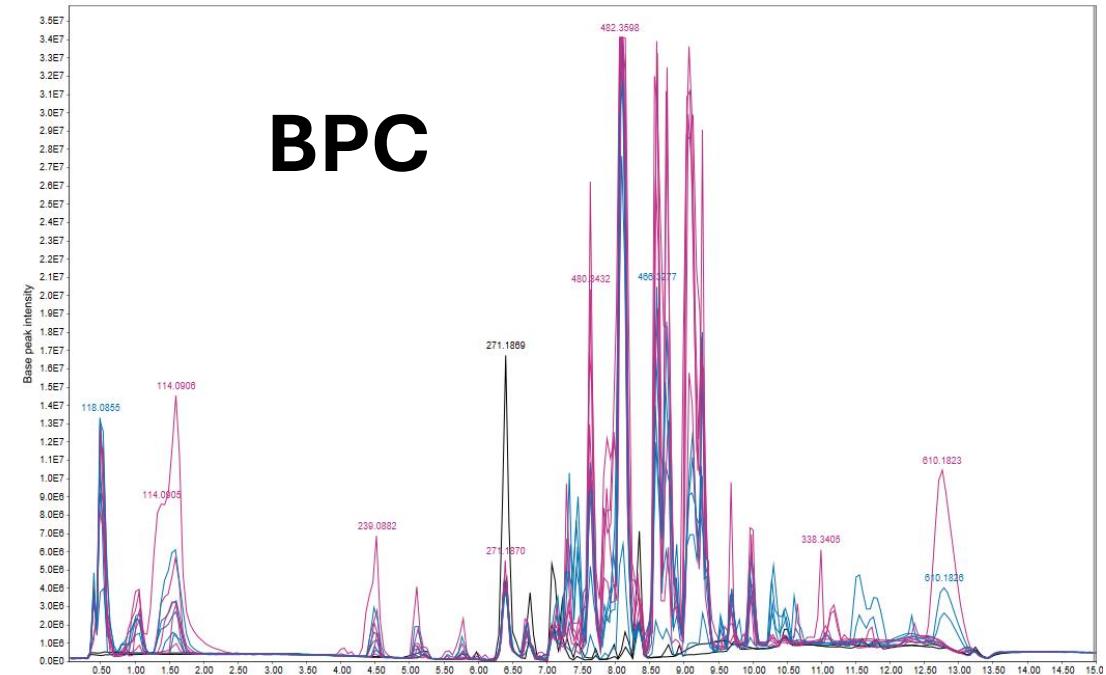
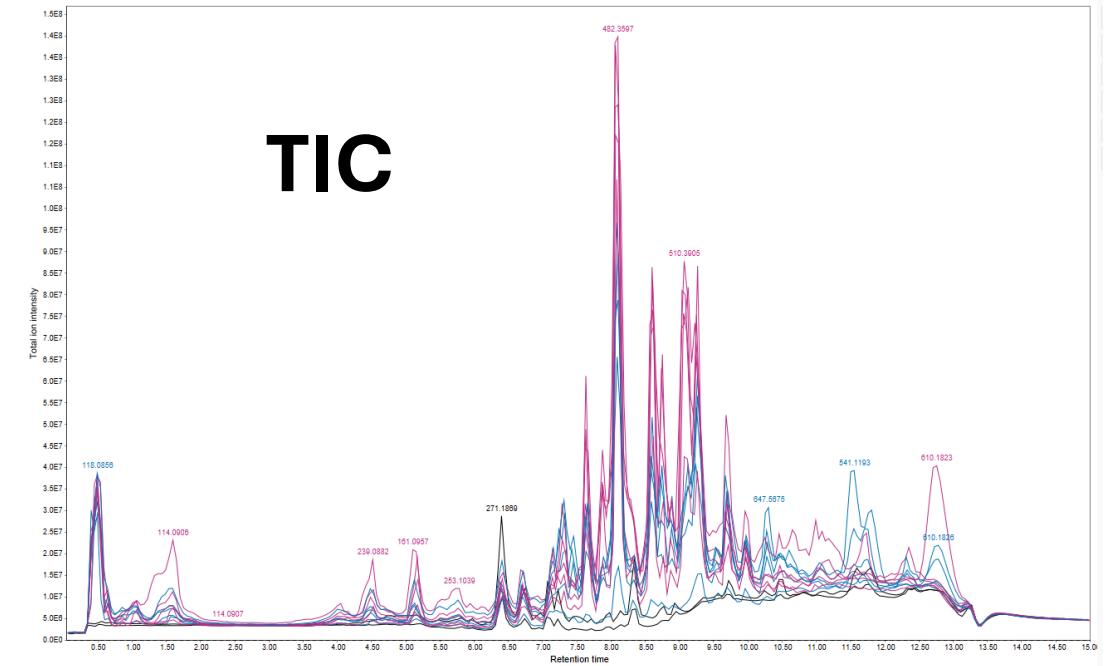
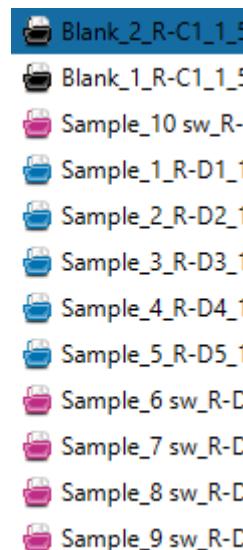
Benzeneoids	
Lipids and lipid-like molecules	
Organic Polymers	
Organic acids and derivatives	
Organic oxygen compounds	
Organohalogen compounds	
Organoheterocyclic compounds	
Organometallic compounds	
Phenylpropanoids and polyketides	



Chromatography (LC-MS) TIC & BPC

Check:

- Peak shape
- Intensity
- Number of peaks
- Noise level
- Compare with
Blanks / Replicate

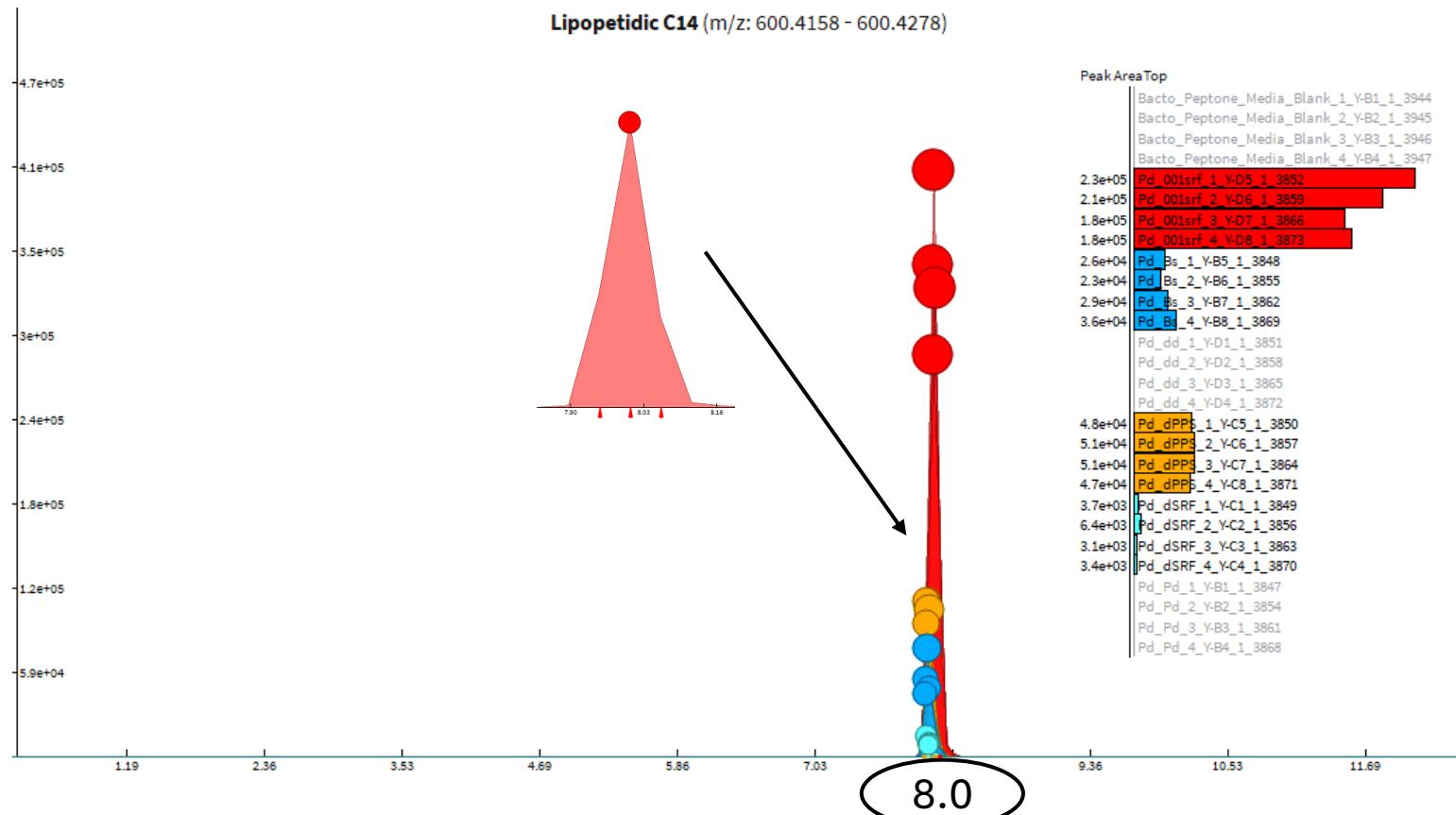


Chromatography (LC-MS) EIC (MS1)

Lipopetidic C14 C31H57N3O8 600.421814
m/z 600.42144775 +/- 10.0000 ppm

Check:

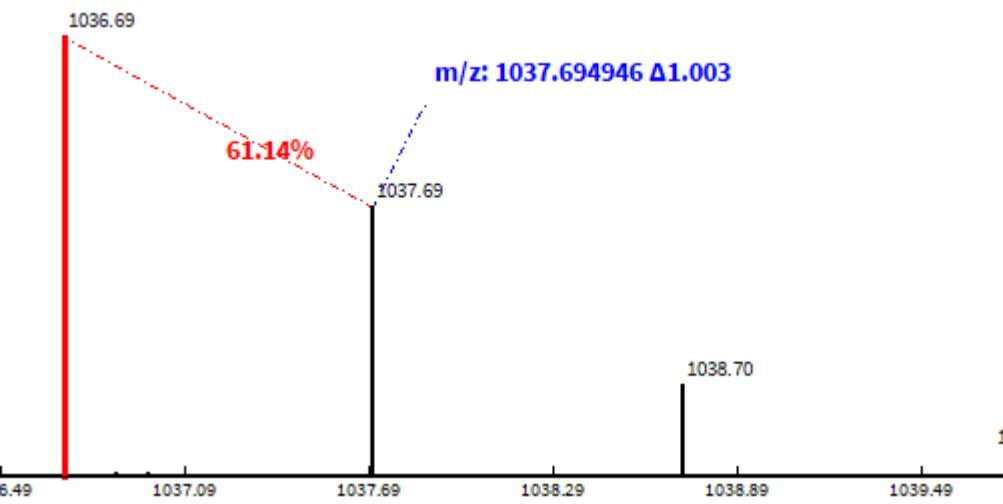
- Peak shape
- EIC in 10 ppm range
- Retention time
- Noise level
- Compare with Blanks / Replicate



Basic Spectra Interpretation

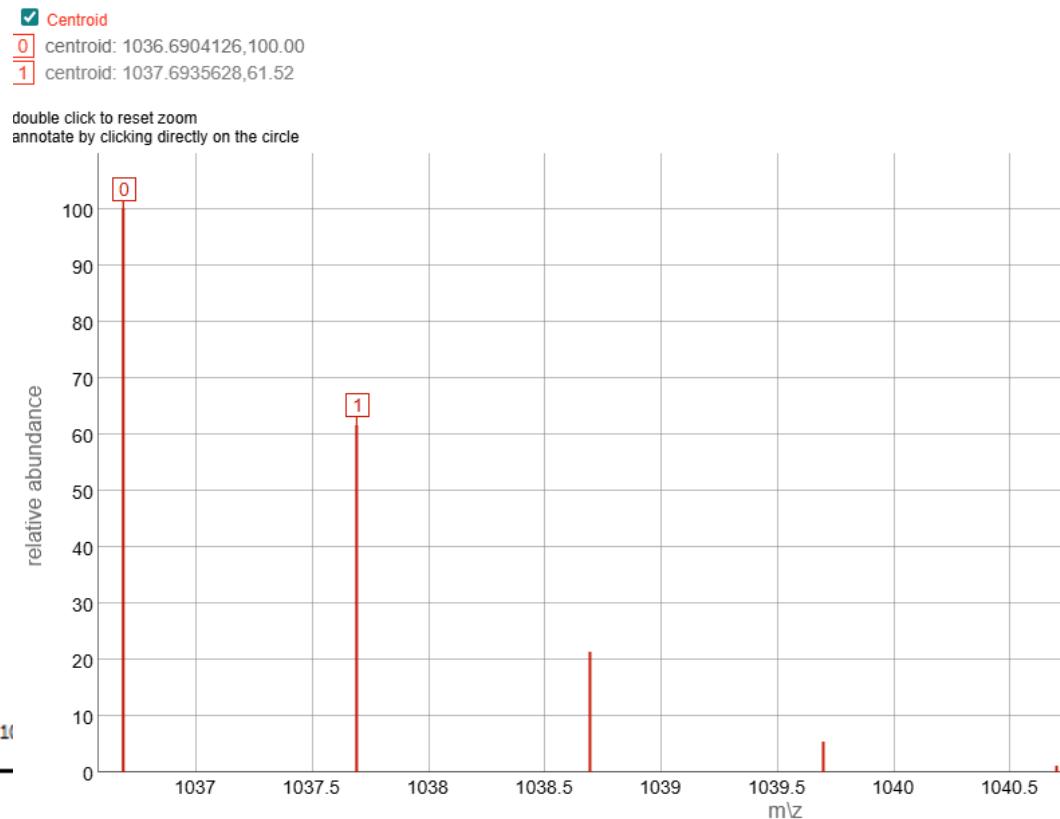
Charge z = +1

- Difference
- Ratio



Monoisotopic Mass: 1036.69
Chemical Formula: C₅₃H₉₄N₇O₁₃
Adduct: M+H
Charge: 1
Average Mass: 0
Mult: 1
Threshold: 0.01

Surfactin C15



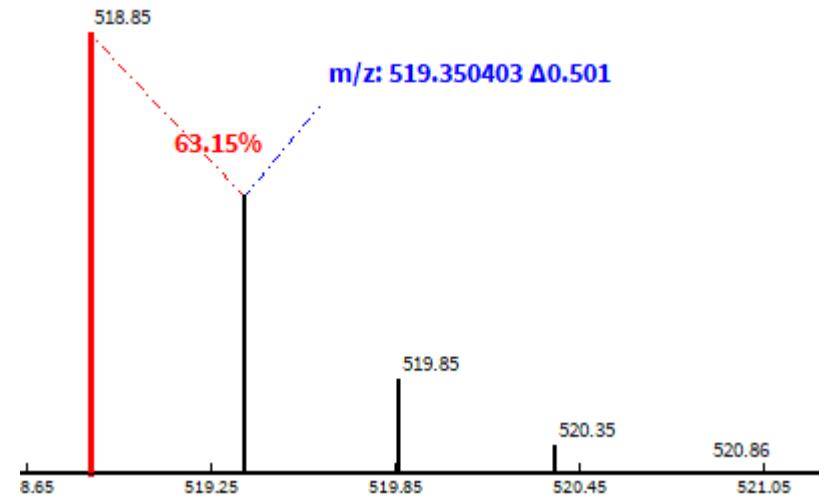
Basic Spectra Interpretation

Charge z = +2

Monoisotopic Mass: 518.8488
Chemical Formula: C₅₃H₉₅N₇O₁₃
Adduct: M+2H
Charge: 2
Average Mass: 0
Mult: 1
Threshold: 0.01

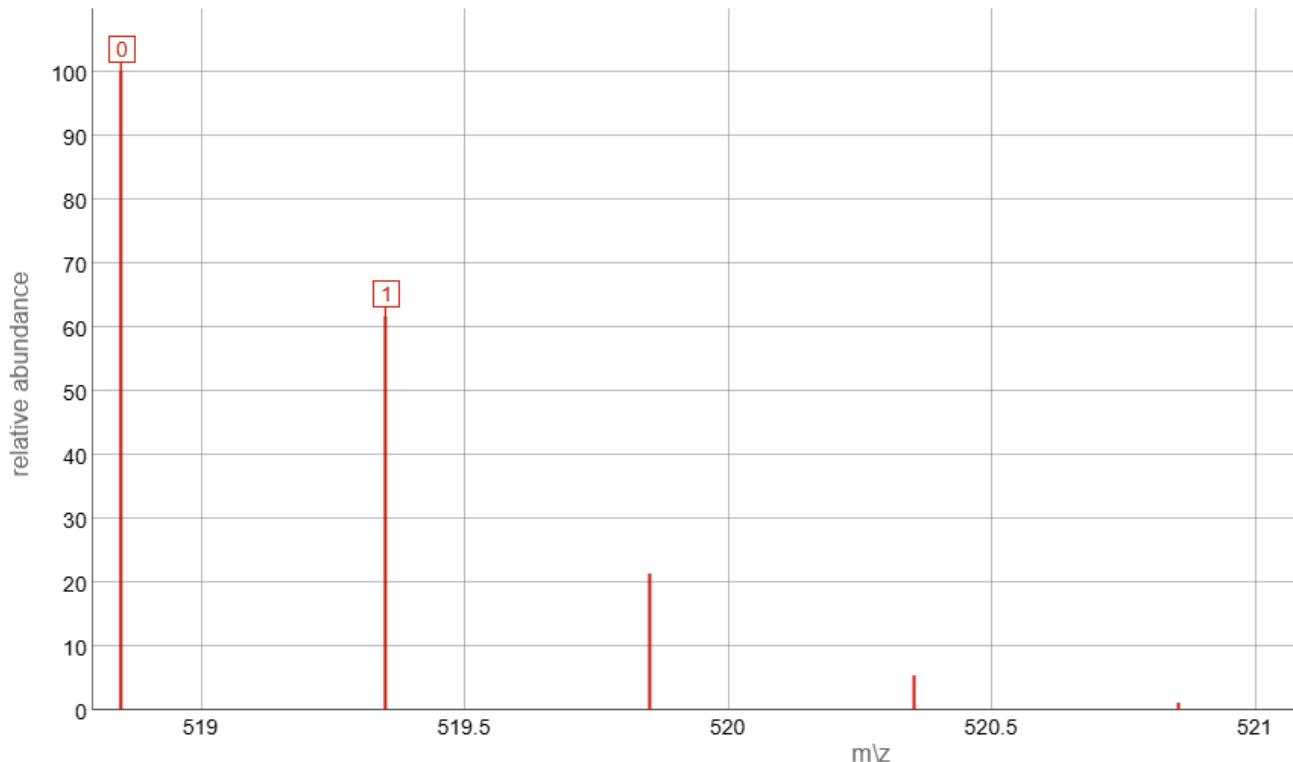
Surfactin C15

- Difference
- Ratio



Centroid
0 centroid: 518.8488445,100.00
1 centroid: 519.3504199,61.53

double click to reset zoom
annotate by clicking directly on the circle

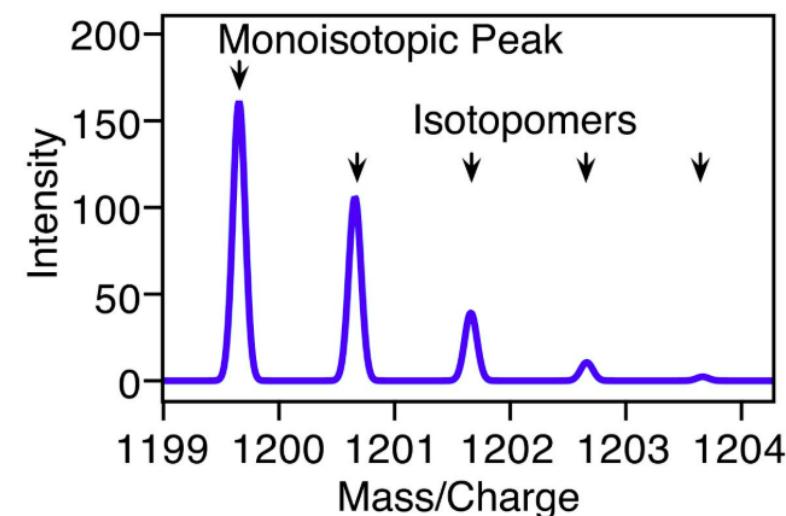
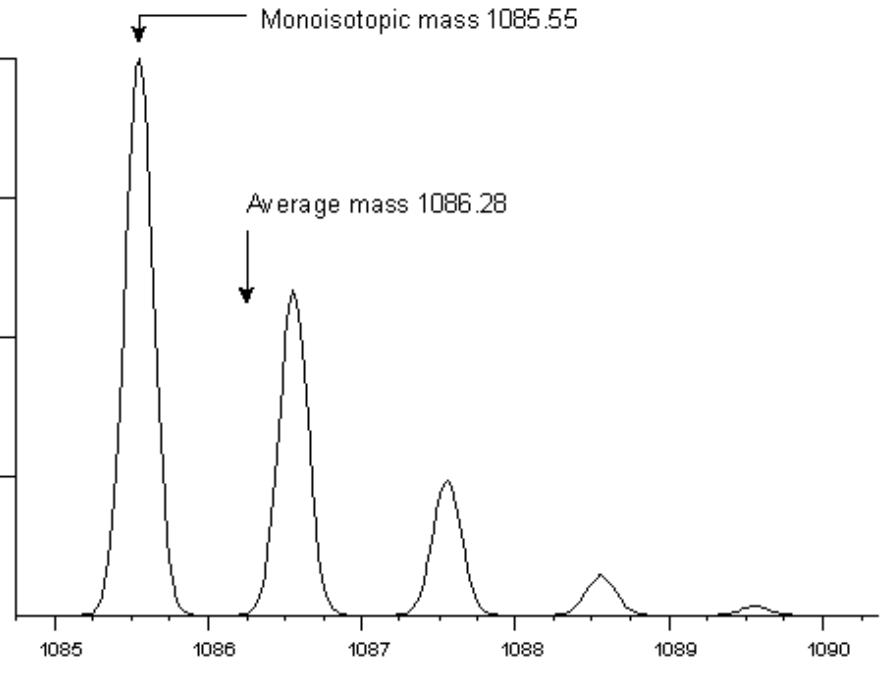


Basic Spectra Interpretation

Monoisotopic Mass

Monoisotopic mass is the exact mass of a molecule or ion, calculated by summing the masses of the most abundant, naturally occurring stable isotope

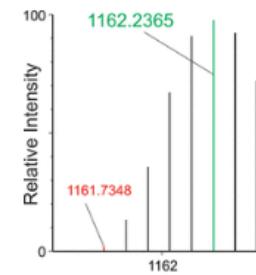
Peptide HLKTEAEMK



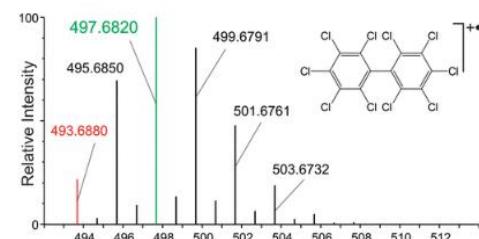
Usually follows such a decreased pattern with a Max int for **MIM** and lowest m_z for a small molecules

Exceptions:

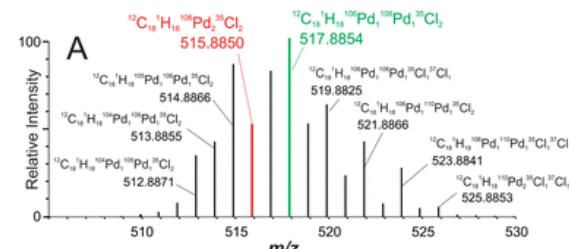
Large Peptide/Protein



Halogenated molecules



Metals

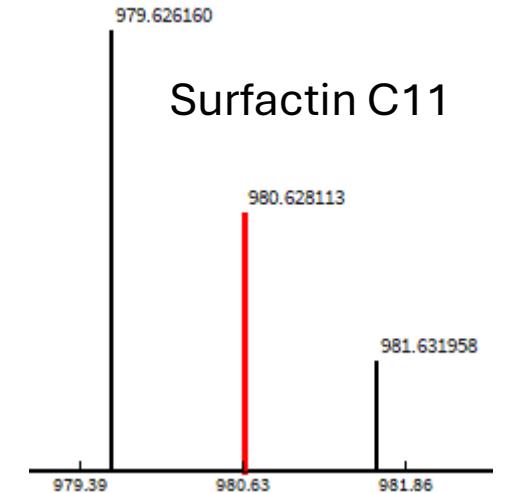


Basic Spectra Interpretation

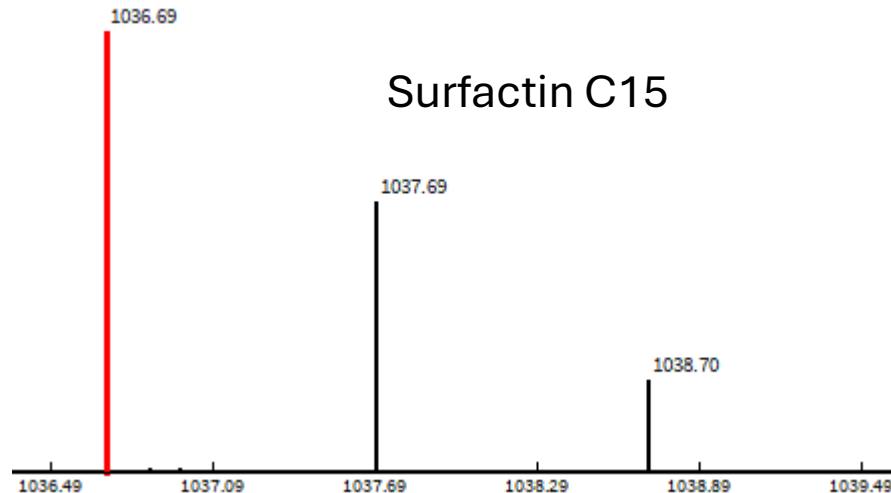
Monoisotopic Mass

- Search by Monoisotopic Mass (with a specific adduct) by default $[M+H]^+$ or $[M-H]^-$ and $Z = \pm 1$
- Compare with Theoretical Pattern (specific adduct, Z)
- Somehow prove structure by Isotopic Pattern
- The most intense in MS1 spectra if clean, or not if fragments, other peaks co-eluted
- The lowest mass in isotopic pattern usually
- The highest m/z value in MS1 (excluding isotopic pattern) if clean peak w/o adducts

✗ Misidentification



✓ Identification



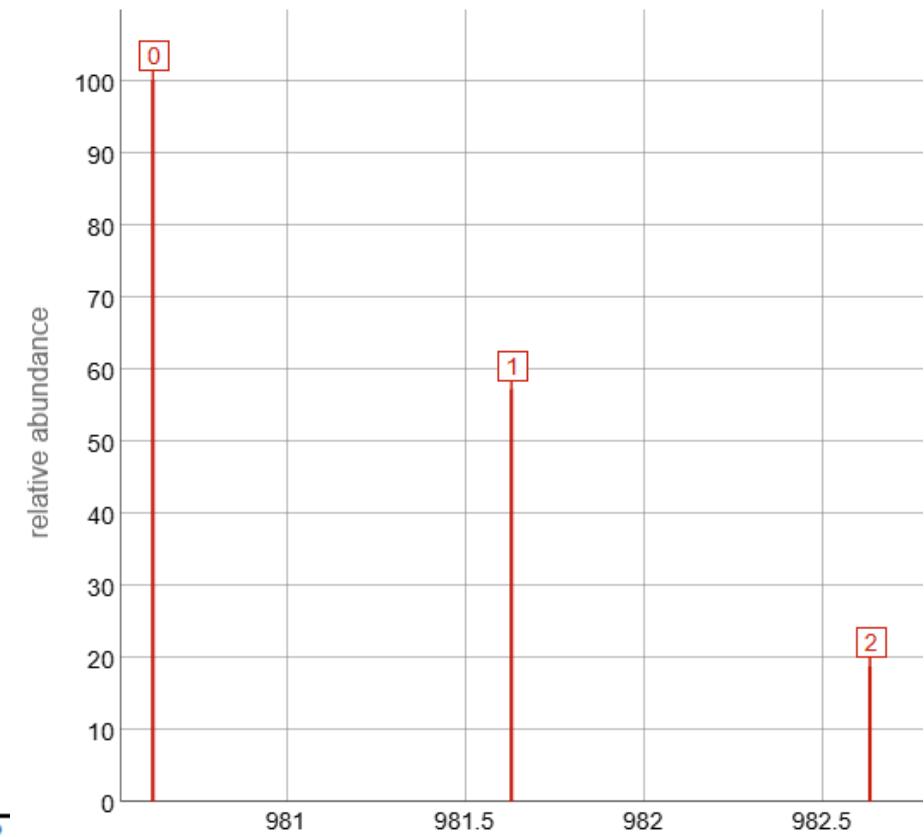
C49H85N7O13

Monoisotopic Mass: 980.6278
Chemical Formula: C49H86N7O13
Adduct: M+H
Charge: 1
Average Mass: 0
Mult: 1
Threshold: 0.01

Centroid

0 centroid: 980.6278123,100.00
1 centroid: 981.6309419,57.10
2 centroid: 982.6337762,18.67

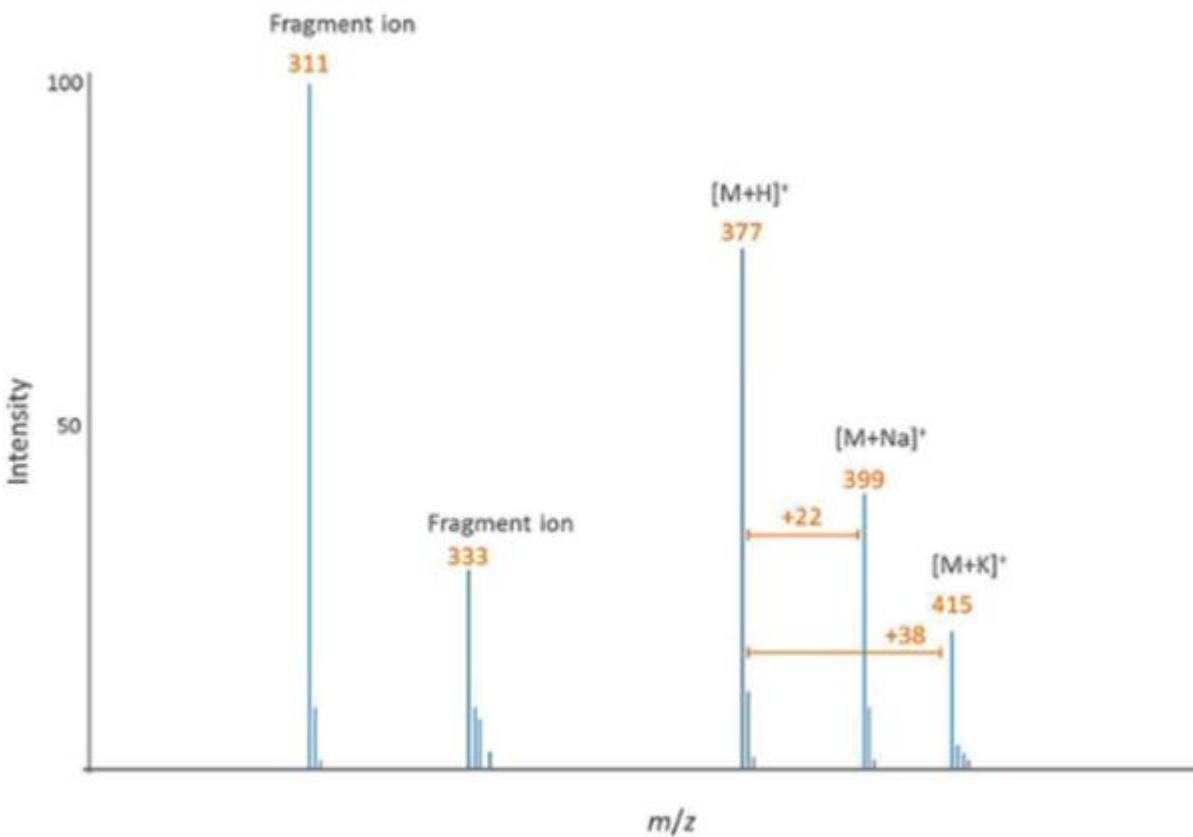
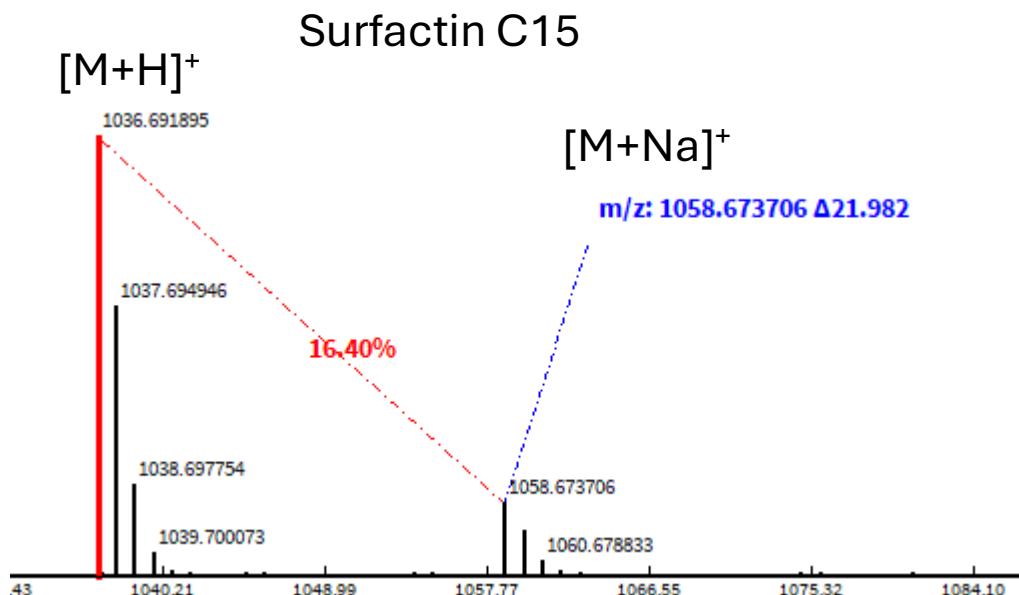
double click to reset zoom
annotate by clicking directly on the circle



Basic Spectra Interpretation

Adducts

- Usually $[M+H]^+$ or $[M-H]^-$
- Adducts with Na/K and double charged commonly occurred for peptides
- With NH_3 , Organic Acids depending on Mobile Phase



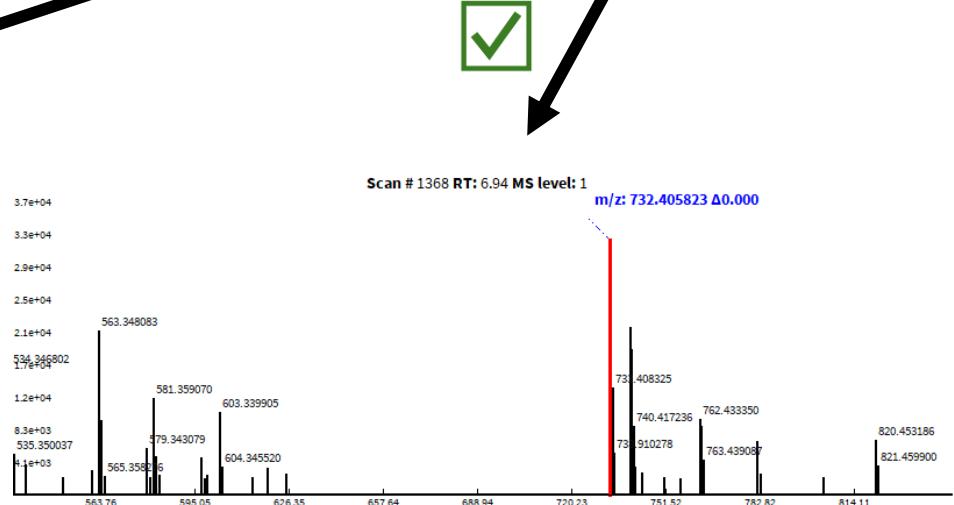
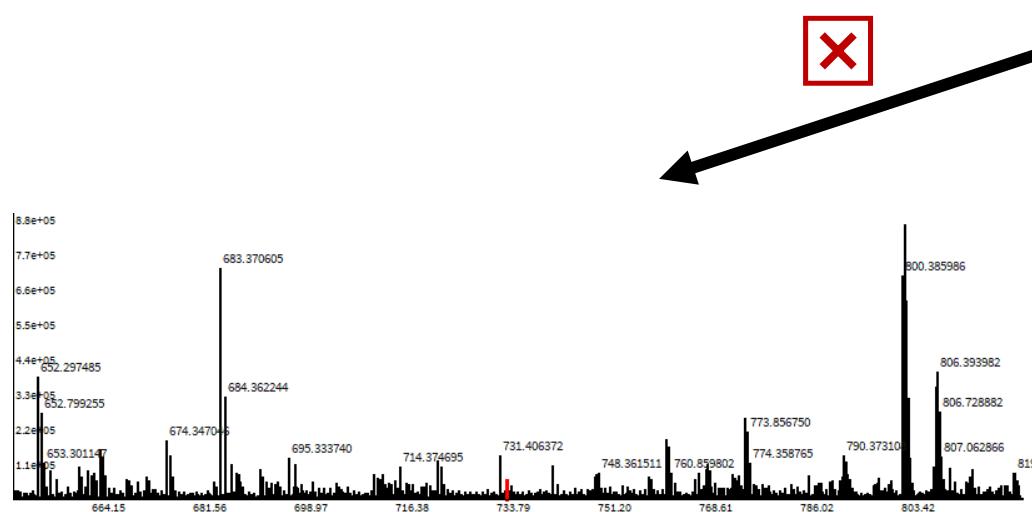
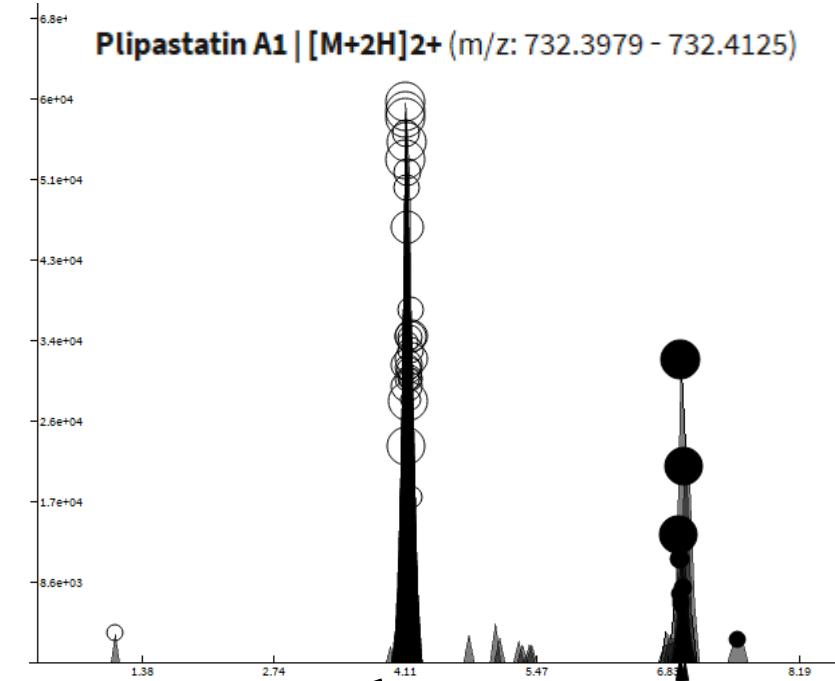
Common Adducts:

$[M+H]^+$; $[M+Na]^+$; $[M+K]^+$; $[M+NH_4]^+$
 $[M+2H]^{2+}$; $[M+2Na]^{2+}$; $[M+H+Na]^{2+}$
 $[M-H]^-$; $[M+Cl]^-$; $[M+HCOO]^-$; $[M+CH_3COO]^-$

Basic Spectra Interpretation

Abundance

- Higher than noise
- Expected isotopic pattern
- The highest signal if clean peak and no intense fragments
- The highest m/z value (excluding isotopic pattern) if clean peak w/o adducts / fragments / coeluting peaks

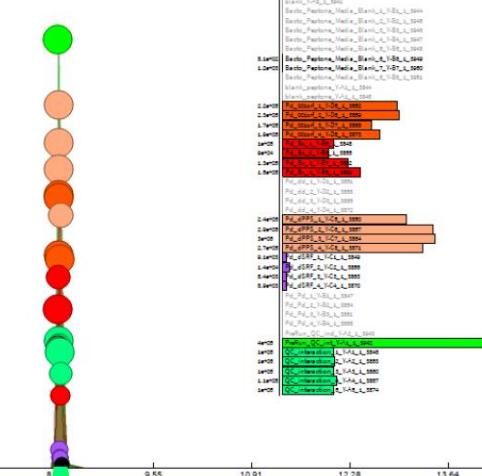


Basic Spectra Interpretation

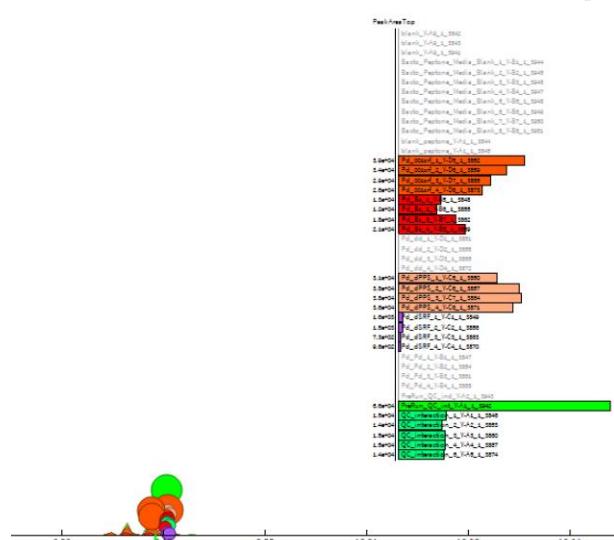
In-Source Fragments

- Perfect correlation by rt, intensity
- Present in MS2

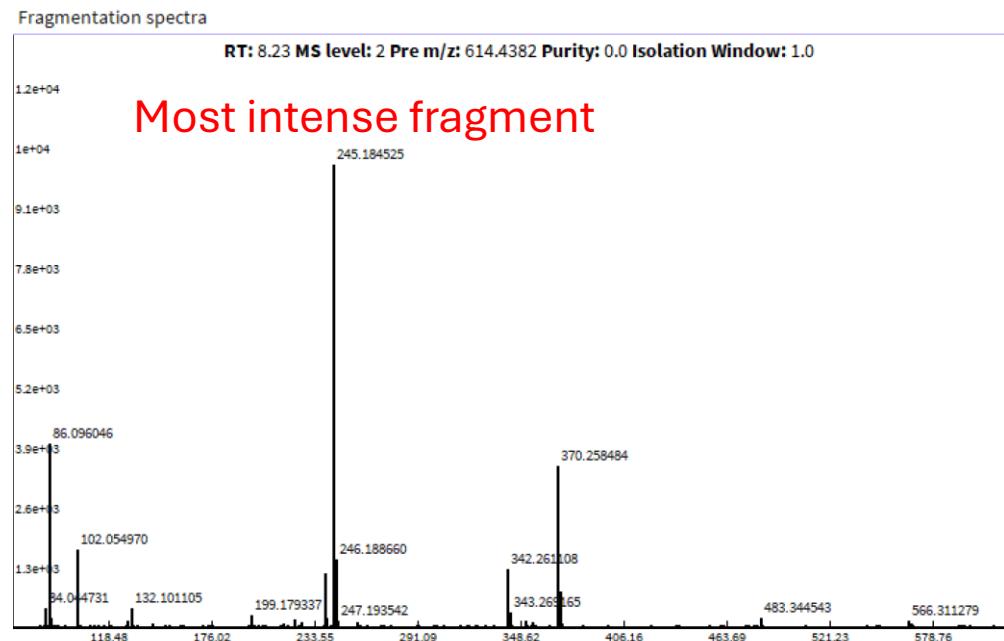
Lipopeptidic C15 product
EIC $614.4832 \pm 10\text{ppm}$



Most intense fragment
EIC $245.1825 \pm 10\text{ppm}$



MS2 Fragmentation Spectra



Basic Spectra Interpretation

MS2 (Fragmentation)

- Presence/Absence of Precursor Ion
- Spectra composition: abundance over the noise, isotopic pattern for fragments
- Used in SIRIUS / GNPS

