Leveraging MS1 Data in NTA Workflows to Improve PFAS Discovery

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Outline

The Lop-Sided Nature of NTA Data Features

Case of No MS/MS data

Recent Developments in PFAS Prioritization

Kendrick, Kaufmann, and Zweigle

RT Models and Molecular Formula Decomposition

Conclusion

Non-Targeted Analyses generate results with varying confidence levels.

What is NTA?
Non-Targeted Analysis

BP4NTA - The characterization of the chemical composition of any given sample without the use of a priori knowledge regarding the sample's chemical content.

The **framework** by which a defined **chemical space** is investigated within a sample without a priori knowledge for the primary purpose of **chemical discovery**.

NTA Feature Distribution

Body of Knowledge Limited

Features in Electrospray may be chemicals, in-source fragments, or clusters/adducts

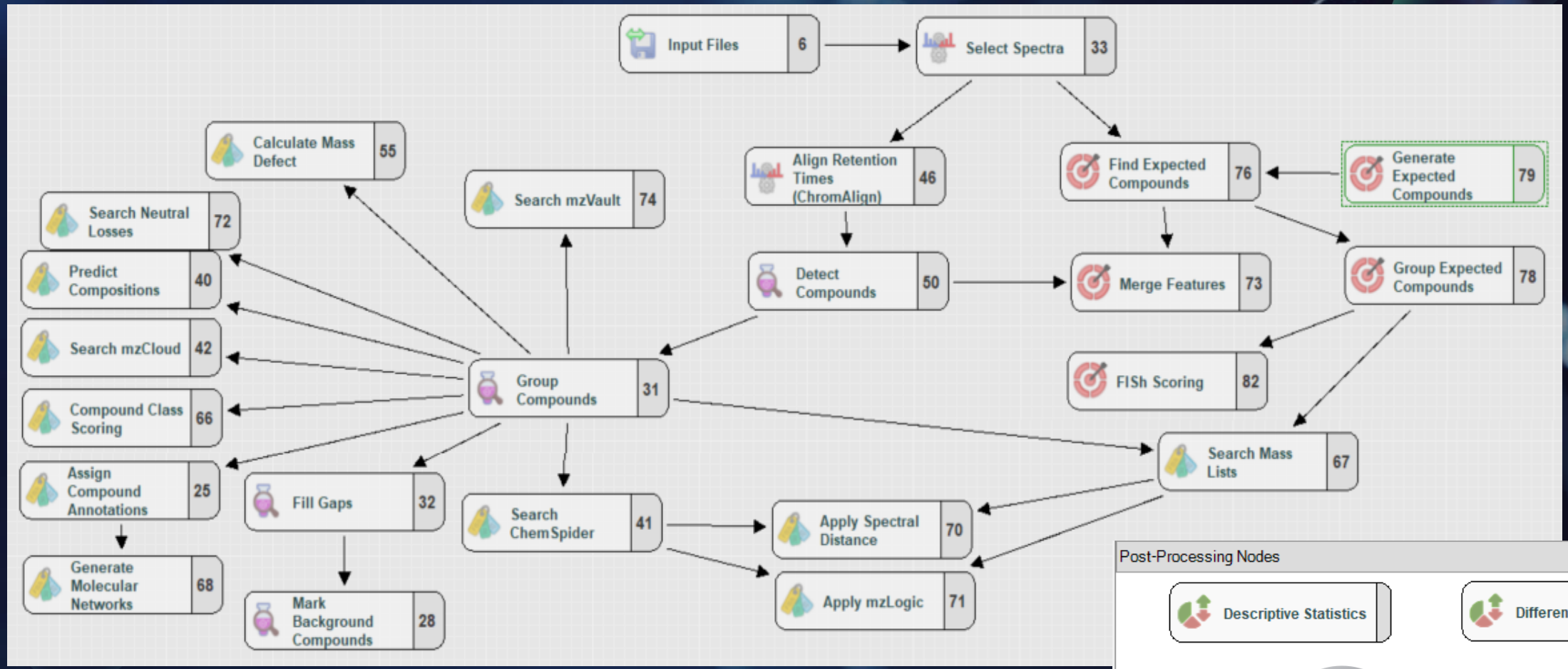
Chemical space of full scale NTA study is vast
(+9000 for drinking water)

More difficult to deconvolute In-source fragments than clusters
(once a bond breaks, the neutral is lost)

Confidence Annotation of features naturally heavily weighted on lower confidence scores
(Few level 1 and many Level 4/5)

Complete NTA Workflow

Example – Thermo Compound Discoverer 3.3



No MS/MS data
Conundrum of ddMS2 Workflows

Typical Full Scan NTA acquisition has a data-dependent MS2 component to it.

Triggered only when chemical feature is above threshold

Trade-off of ddMS2 over All ions fragmentation (AIF) is ease of deconvolution

Ultimately, many features do not have MS/MS data

Can we increase identification confidence for the many features that have no MS2 data?

Kendrick Mass / Mass Defect

Plot of Kendrick Mass Defect (KMD) vs. MW

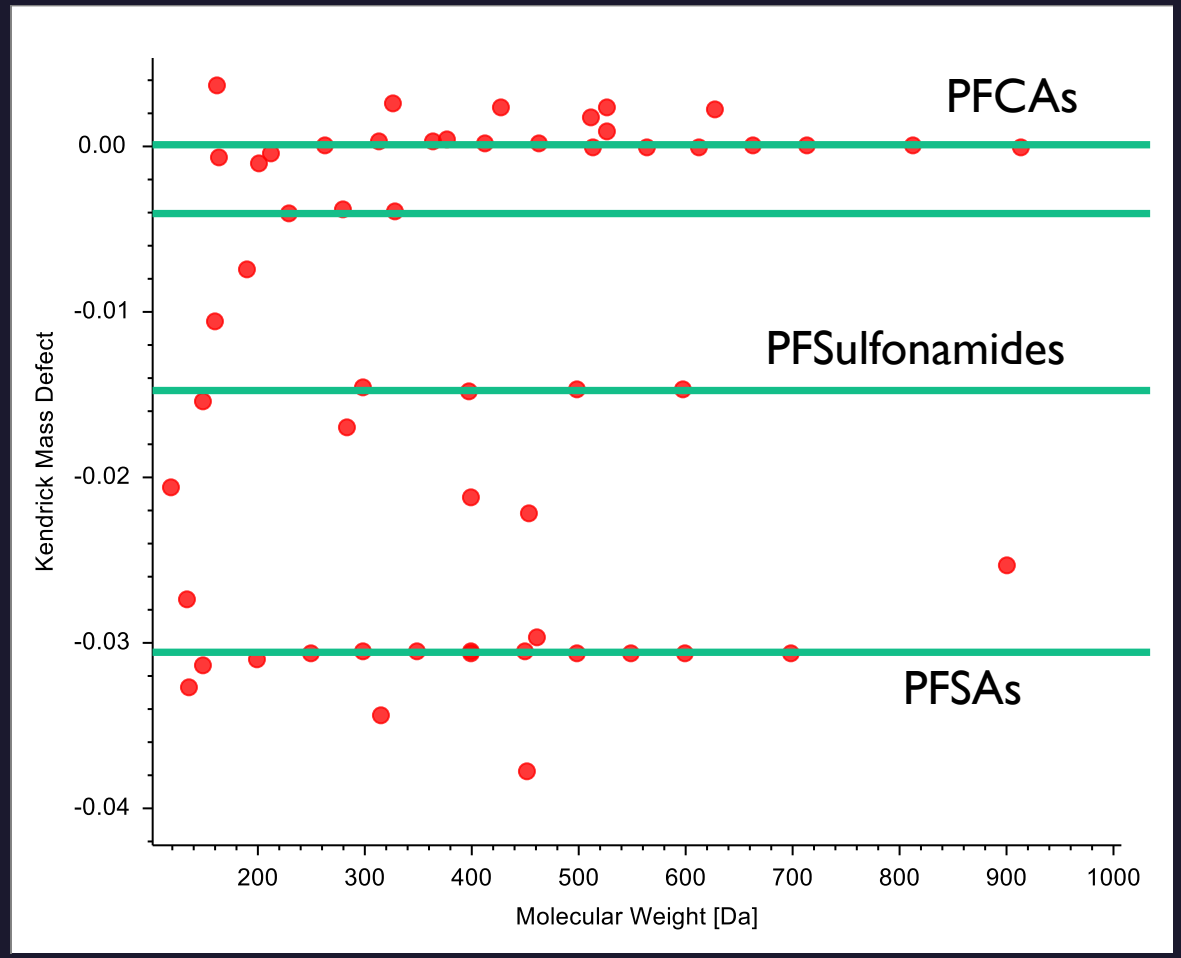
KMD(CF2) = M * round(KFM) / KFM – round(M)

Where KFM = 49.9968 and M = mass(feature)

Produces horizontal lines where CF2 homologs occur

Mass Defect = MW – **floor**(MW)

Disadvantage: Scales poorly to non CF2 homologs



What's in a M/Z

Example: [M-H]- ion for PFOA

<u>For PFAS</u>:

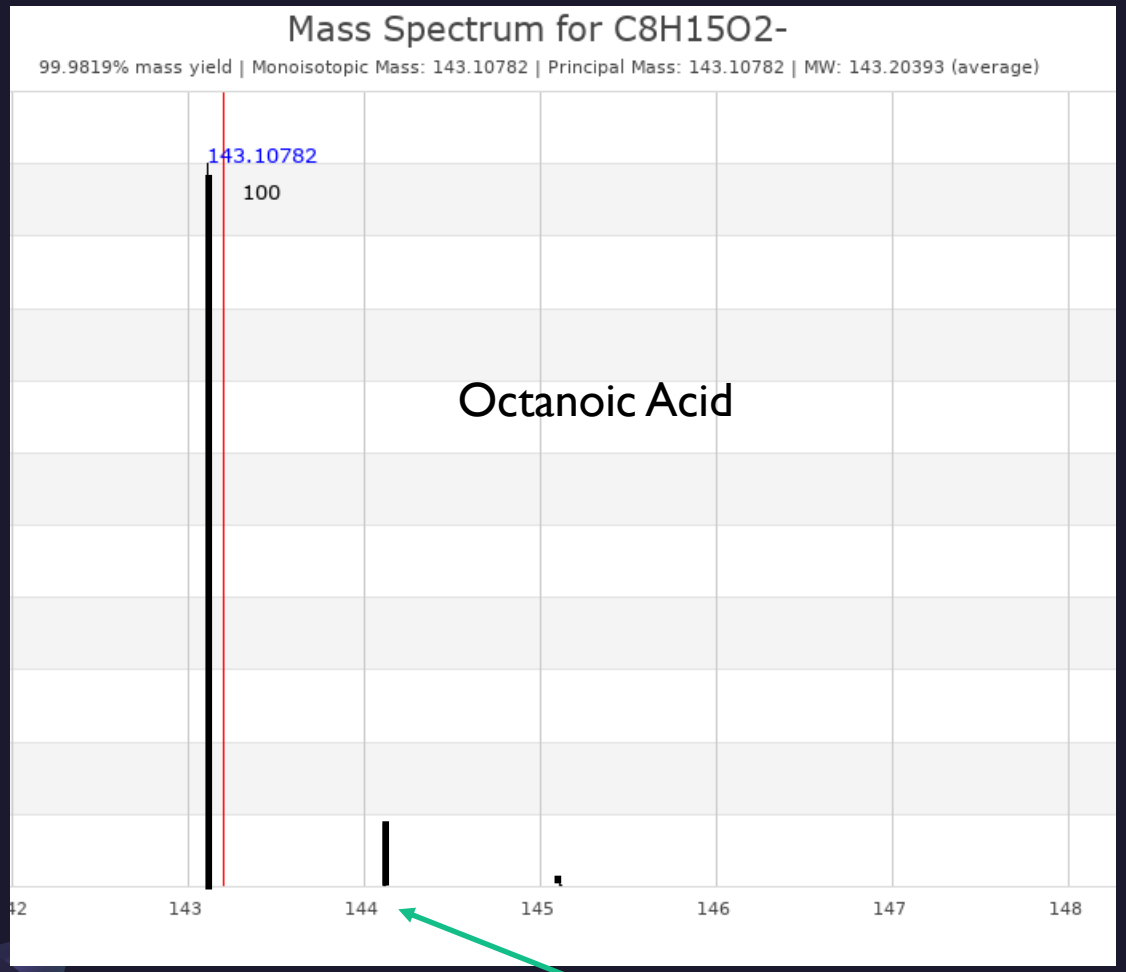
- m/z is larger because F atoms have replaced H atoms

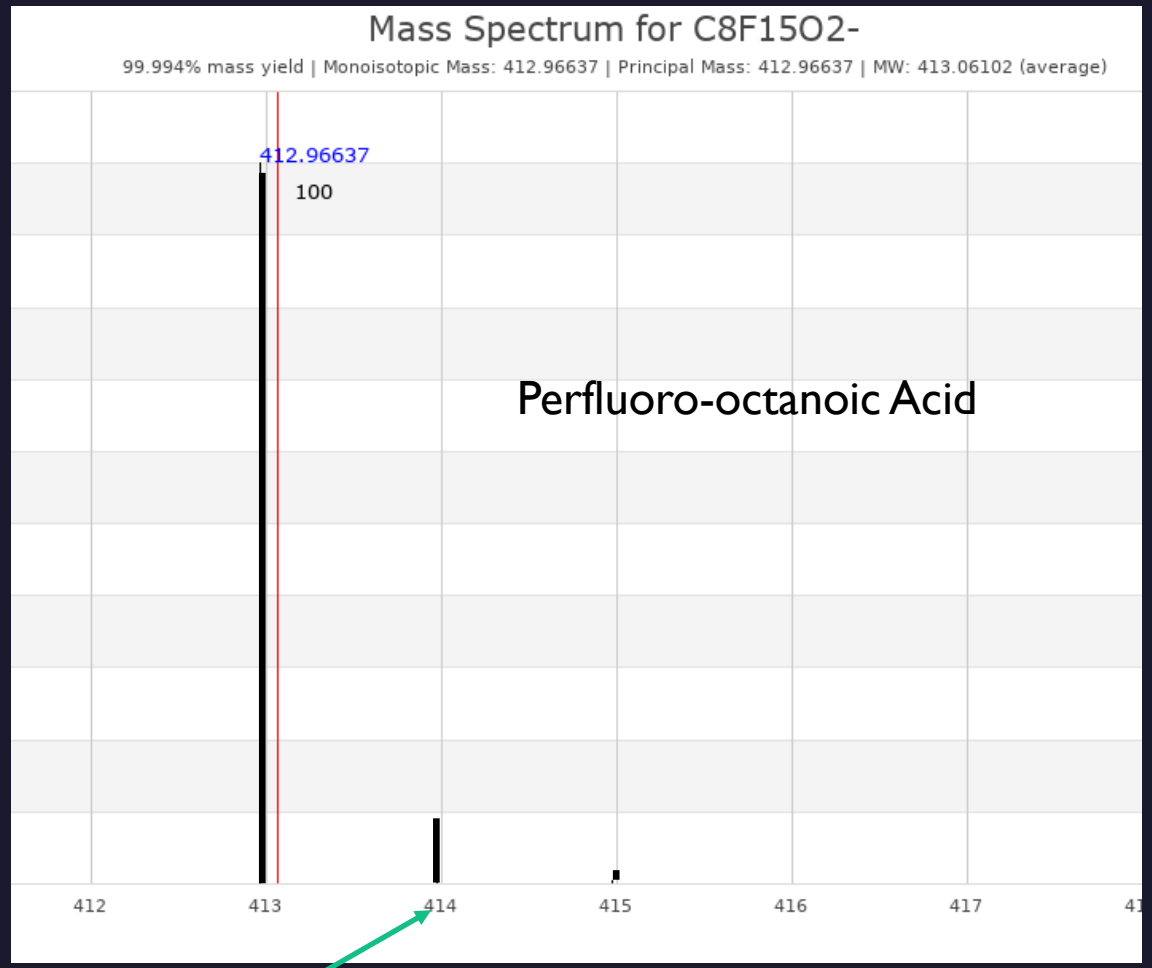
- Mass defect becomes larger as more F atoms added

412.96637

Mass Defect – Δ from integer

What's in a Mass Spectrum (MS1 Full Scan)





If Carbon 13 abundance is 8.73%, this feature has 8 carbons (independent of F atoms)

What’s in a Mass Spectrum (MS1 Full Scan)

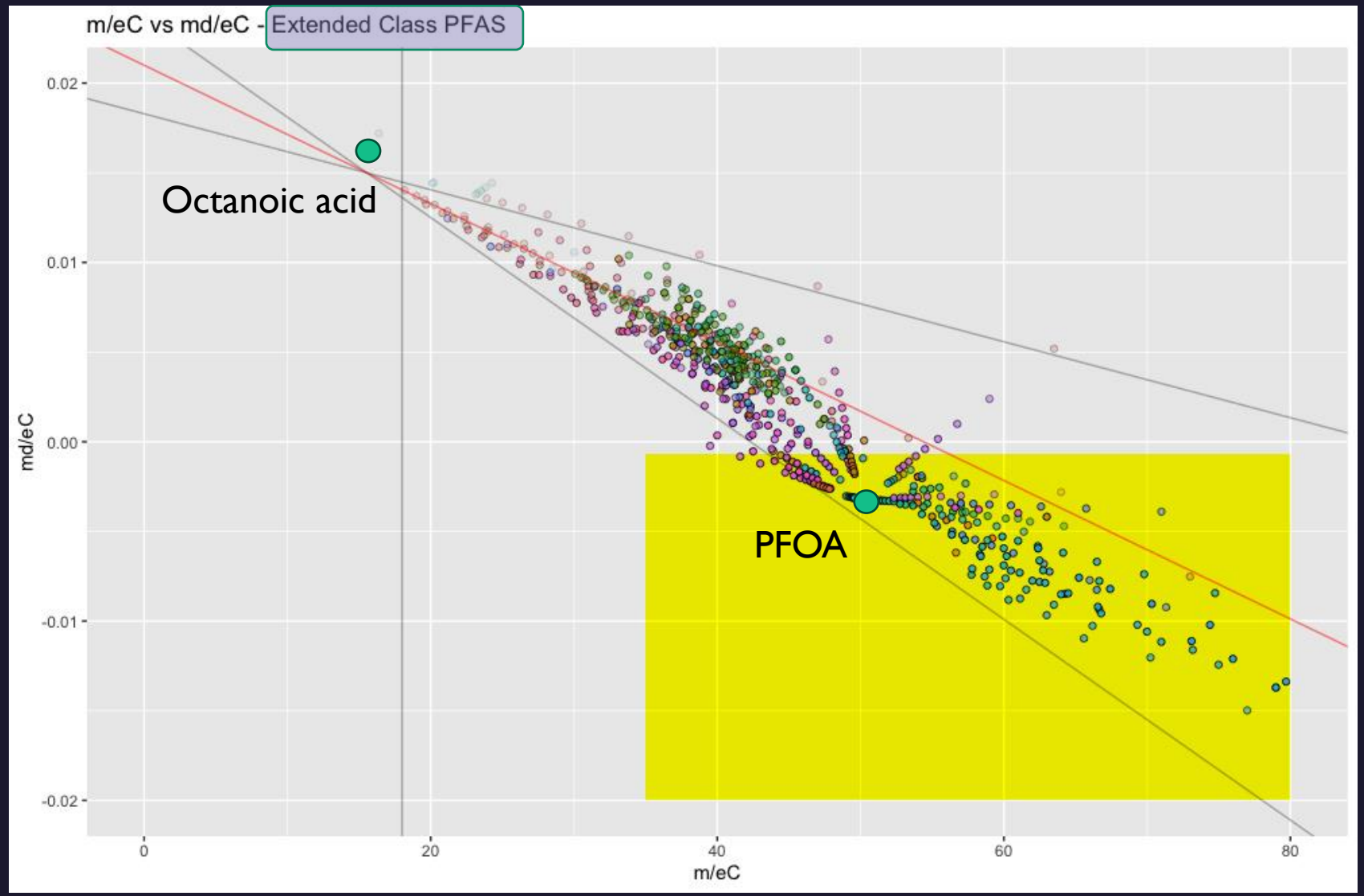
OCTANOIC ACID

- MF = C8 H16 O2
- MW ~ 143
- MW/Carbon ~ 18
- Mass Defect = 0.10782
- MD/Carbon = 0.0135

• PFOA

- MF = C8 H F15 O2
- MW ~ 413
- MW/Carbon ~ 52
- Mass Defect = -0.03363
- MD/Carbon = -0.0042

Kaufmann Plot of PFAS (md/C vs. m/C)



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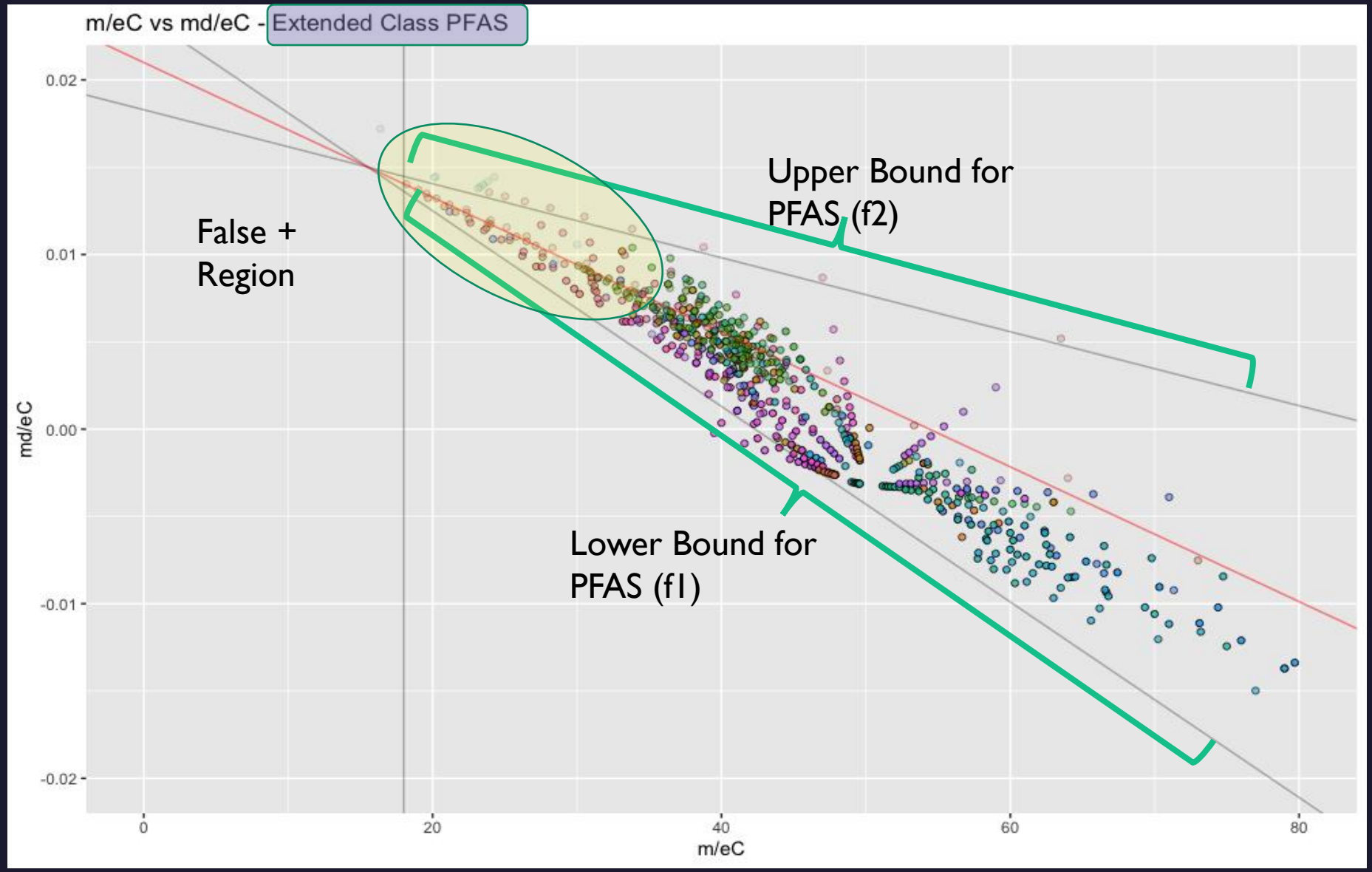


PFAS-ness – Upper and Lower Bounds

Tags	Checked	Name	Formula	RT [min]	m/z	Area (Max.)	Annot. ΔMass [ppm]	MS2	Annot. Source	mzVault Library Matches	Mass List Matches	eC	m/C	md/C	f1PFAS	f2PFAS	Calc. MW
	☑	TFMSi	C H F3 O2 S	1.246	132.95675	18576503	-6.79					1.07535	124.5772	-0.0334(	12.60436	-25.34853	133.96402
	☑	TFMS	C H F3 O3 S	1.327	148.95173	110842740	-5.65					1.09651	136.7597	-0.0373(	15.49424	-26.69815	149.95900
	☑	TFMSA	C H2 F3 N O2 S	1.767	147.96772	42968824	-5.64					1.16133	128.2793	-0.0215	26.60083	-12.64038	148.97499
	☑	PFPrA	C3 H F5 O2	2.176	162.98162	157987627	-5.32					2.80207	58.52420	-0.0040(	5.07650	-9.88992	163.98880
	☑	PFEtS	C2 H F5 O3 S	3.477	198.94889	124526386	-2.43					0.55187	362.3254	-0.0794	99.78122	-20.90803	199.95617
	☑	PFBA	C4 H F7 O2	4.393	168.98864	523352731	-2.83					3.03458	70.51583	-0.0046(	11.13253	-8.00697	213.98587
	☑	PFPrS	C3 H F7 O3 S	4.769	248.94611	172347615	-0.31					2.78246	89.83194	-0.0167	9.85090	-16.01061	249.95338
	☑	PFMPA, PFOPeA	C4 H F7 O3	4.823	228.97388	41856299	-1.02					3.93709	58.4140	-0.0047	4.22659	-10.70149	229.98116
	☑	Bistriflimide	C2 H F6 N O4 S2	4.925	279.91786	172916023	0.08					0.69323	405.2421	-0.1079	95.24803	-40.37625	280.92514
	☑	PFPeA	C5 H F9 O2	5.353	262.97598	167080174	-0.59					5.16132	51.14645	-0.0032	1.67347	-10.72550	263.98313
	☑	PFBS	C4 H F9 O3 S	5.501	298.94303	328331590	0.14					3.74778	80.03408	-0.0132(	7.86058	-14.59128	299.95031
	☑	PFMBA, PFOHxA	C5 H F9 O3									4.93940	56.68268	-0.0044	3.62880	-10.69677	279.97820
	☑	3:3-FTCA	C6 H5 F7 O2									6.48017	37.34740	0.00271	-0.07565	-7.67255	242.01756
	☑	H-PFHpA	C7 H2 F12 O2									7.61739	45.42055	-0.0018(	-0.06563	-10.47198	345.98628

eC	m/C	md/C	f1PFAS	f2PFAS
1.07535	124.5772	-0.0334(	12.60436	-25.34853
1.09651	136.7597	-0.0373(	15.49424	-26.69815
1.16133	128.2793	-0.0215	26.60083	-12.64038
2.80207	58.52420	-0.0040(	5.07650	-9.88992
0.55187	362.3254	-0.0794	99.78122	-20.90803
3.03458	70.51583	-0.0046(	11.13253	-8.00697
2.78246	89.83194	-0.0167	9.85090	-16.01061
3.93709	58.41401	-0.0047	4.22659	-10.70149
0.69323	405.2421	-0.1079	95.24803	-40.37625
5.16132	51.14645	-0.0032	1.67347	-10.72550
3.74778	80.03408	-0.0132(	7.86058	-14.59128
4.93940	56.68268	-0.0044	3.62880	-10.69677

Zweigle et al did similar work calculating a single value using a more complicated calculation

Machine Learning Approach to RT Modeling

RT Modeling – How and Why

Using R packages: rcdk (QSAR), neuralnet to generate a 7:4 MLP

Pretty good prediction +/- 1.0 min, some outliers

Not universal and not directly transferable

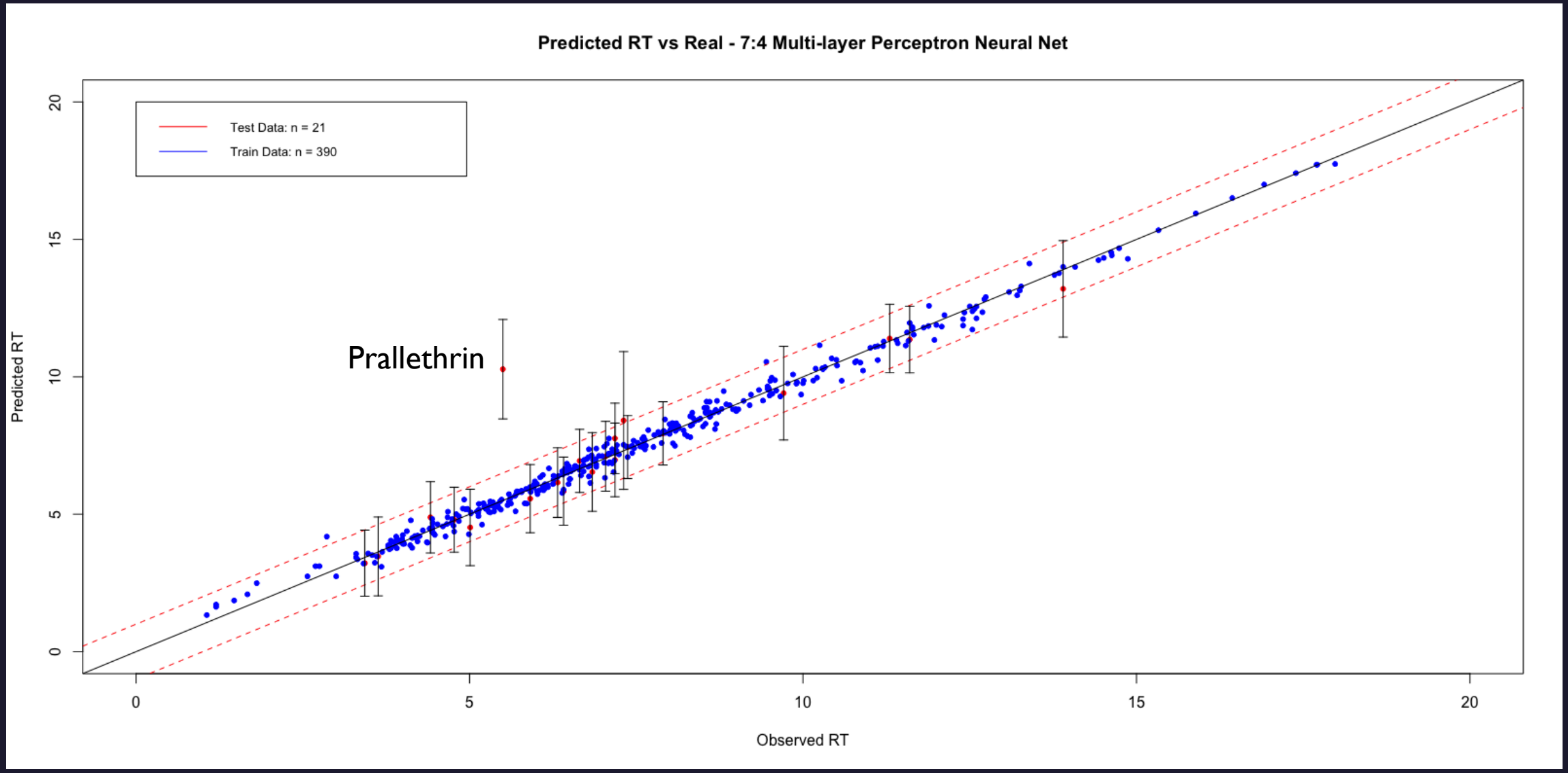
Overly trained for PFAS (80+ PFAS) but includes pesticides, CECs

Did initial Cross-Validation from 10% to 90% training, run with 5 replicates of randomized data. RMSE minimization confirmed.

Dominant QSARs that predict RT are usual suspects (eg: AlogP)

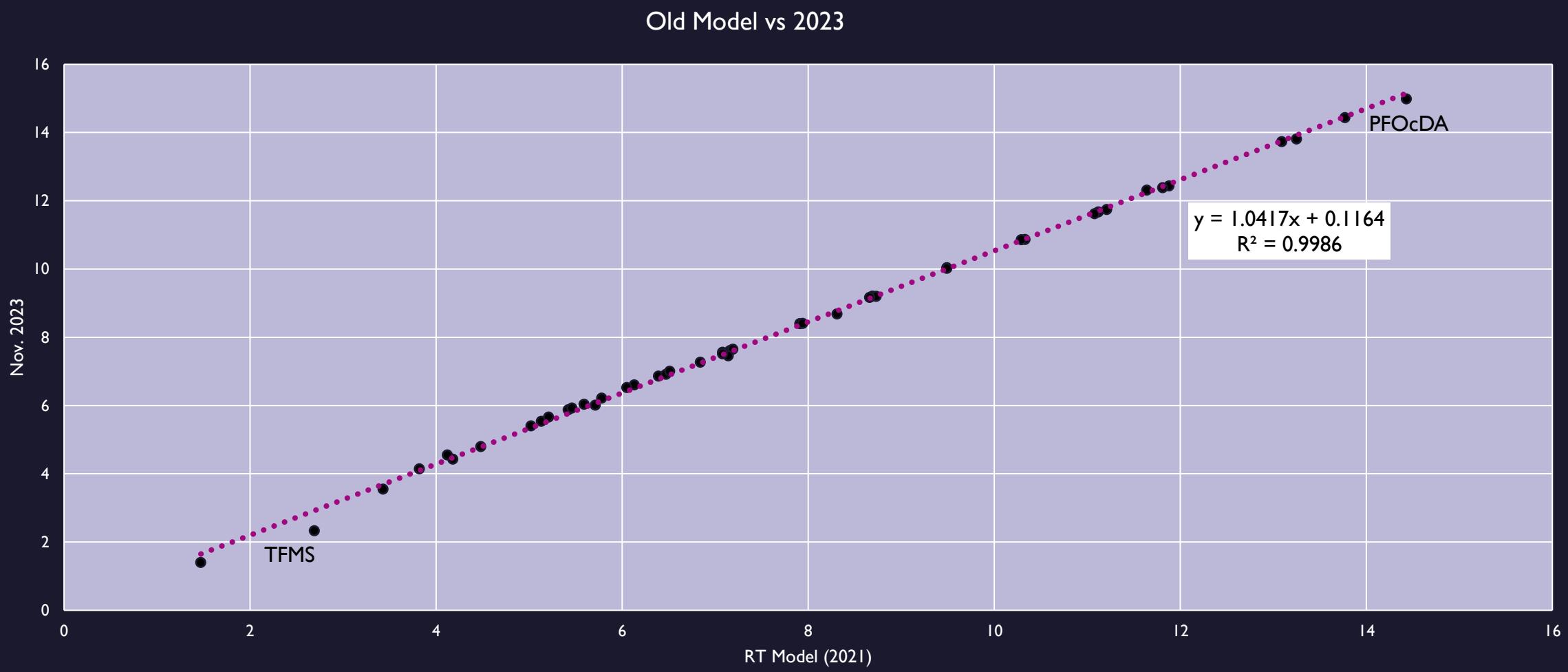
Another tool – to help increase ID confidence

RT Model – Observed/Predicted



RT Model (2021) vs. 2023

47 compounds – mostly PFAS

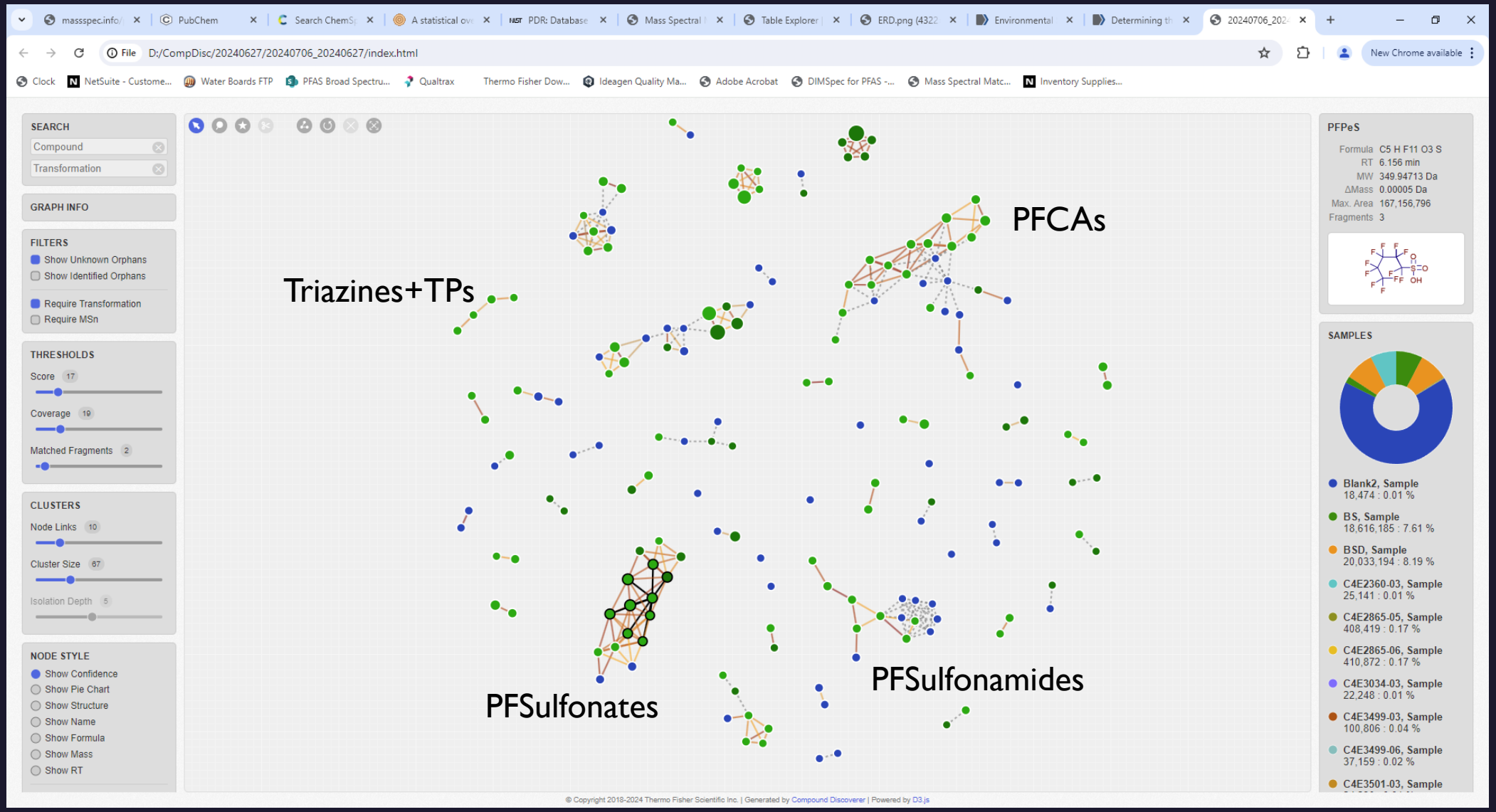


What's Required for an RT Model

- Structures of all molecules (SMILES)
- Specific Quantum Structure Activity Relationship (QSAR) calculations. Examples: ALogP, nHB Donors, nHB Acceptors, Elemental counts, polarizability)
- Train the model
- Validate the Model using various test/train ratios. Determine if RMSE goes down as train ratio increases
- Store the model
- Use model on Feature candidate lists (one feature, many possible chemicals)

Molecular Networks To Explore Feature Relationships

Molecular Networks (Thermo CD)



Informed DeNovo Molecular Formula Generation

Molecular Formula Generation

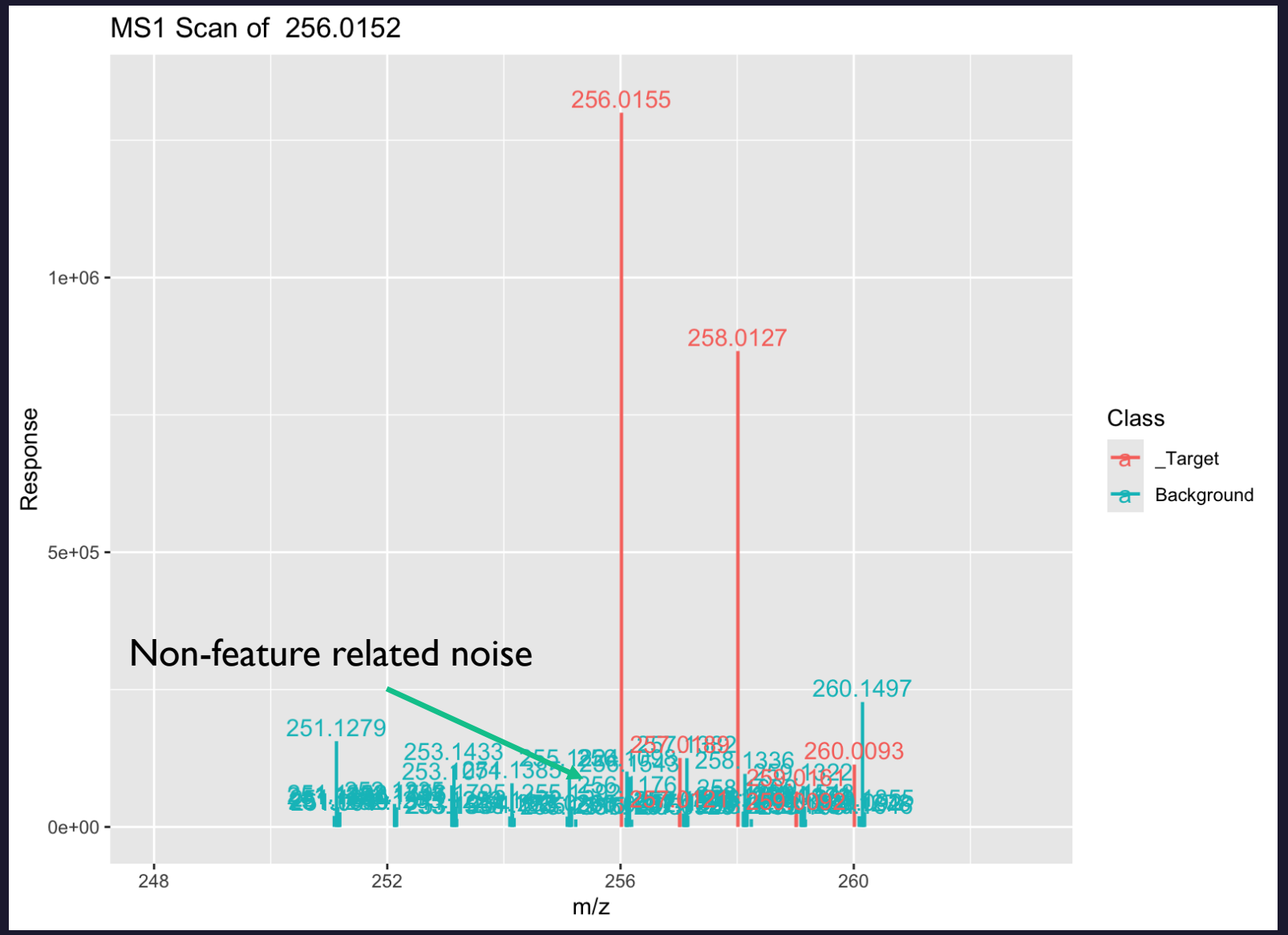
- DeNovo – "from the beginning"
- Decomposition computationally expensive / time consuming (last resort)
- Often produces more junk than useful information

	mz	MF	charge	RdisopScore	unsat	parity	error	nrule	ppm	SENIOR3	HtoC	NtoC
1	371.1009	C6H31O7N2S4	1	9.183108e-02	-7.5	e	-2.288724e-05	Valid	-0.0616739	3	5.1666667	0.3333333
5	371.1006	C13H23O10S	1	5.182191e-02	2.5	e	-2.393812e-04	Valid	-0.6450570	9	1.7692308	0.0000000
7	371.1006	C11H11N14S	1	5.010691e-02	13.5	e	-2.500732e-04	Valid	-0.6738686	45	1.0000000	1.2727272
8	371.1006	H150O10N2S	1	4.081205e-02	-73.0	e	-3.119322e-04	Valid	-0.8405591	-140	Inf	In
10	371.1012	C8H146O8	1	4.587981e-02	-64.0	e	3.399638e-04	Valid	0.9160953	-128	18.2500000	0.0000000
12	371.1005	C5H19O13N6	1	2.704001e-02	-0.5	e	-4.223192e-04	Valid	-1.1380173	5	3.8000000	1.2000000
14	371.1005	C3H7O3N20	1	2.590080e-02	10.5	e	-4.330112e-04	Valid	-1.1668289	41	2.3333333	6.6666667
17	371.1013	C6H23O8N6S2	1	3.147397e-02	-1.5	e	4.460708e-04	Valid	1.2020202	11	3.8333333	1.0000000
21	371.1014	CH154O5N2S3	1	2.005891e-02	-74.0	e	5.564578e-04	Valid	1.4994784	-134	154.0000000	2.0000000
23	371.1015	C14H27O5S3	1	1.442732e-02	1.5	e	6.290088e-04	Valid	1.6949805	15	1.9285714	0.0000000
26	371.1017	C7H35O2N2S6	1	4.583770e-03	-8.5	e	8.455028e-04	Valid	2.2783636	9	5.0000000	0.2857142
27	371.1000	C5H27O12N2S2	1	2.348783e-03	-6.5	e	-8.912772e-04	Valid	-2.4017114	-3	5.4000000	0.4000000
29	371.1000	C3H15O2N16S2	1	2.191293e-03	4.5	e	-9.019692e-04	Valid	-2.4305230	33	5.0000000	5.3333333
31	371.1018	C6H15O9N10	1	3.007650e-03	4.5	e	9.150288e-04	Valid	2.4657143	19	2.5000000	1.6666666
34	371.0999	C18H11O2N8	1	1.755212e-03	17.5	e	-9.355252e-04	Valid	-2.5209459	43	0.6111111	0.4444444
37	371.0999	C5H138O2N10	1	1.063501e-03	-58.0	e	-1.008076e-03	Valid	-2.7164480	-106	27.6000000	2.0000000
39	371.1019	CH146O6N6S	1	1.459270e-03	-68.0	e	1.025416e-03	Valid	2.7631725	-126	146.0000000	6.0000000

Showing 1 to 17 of 64 entries, 22 total columns

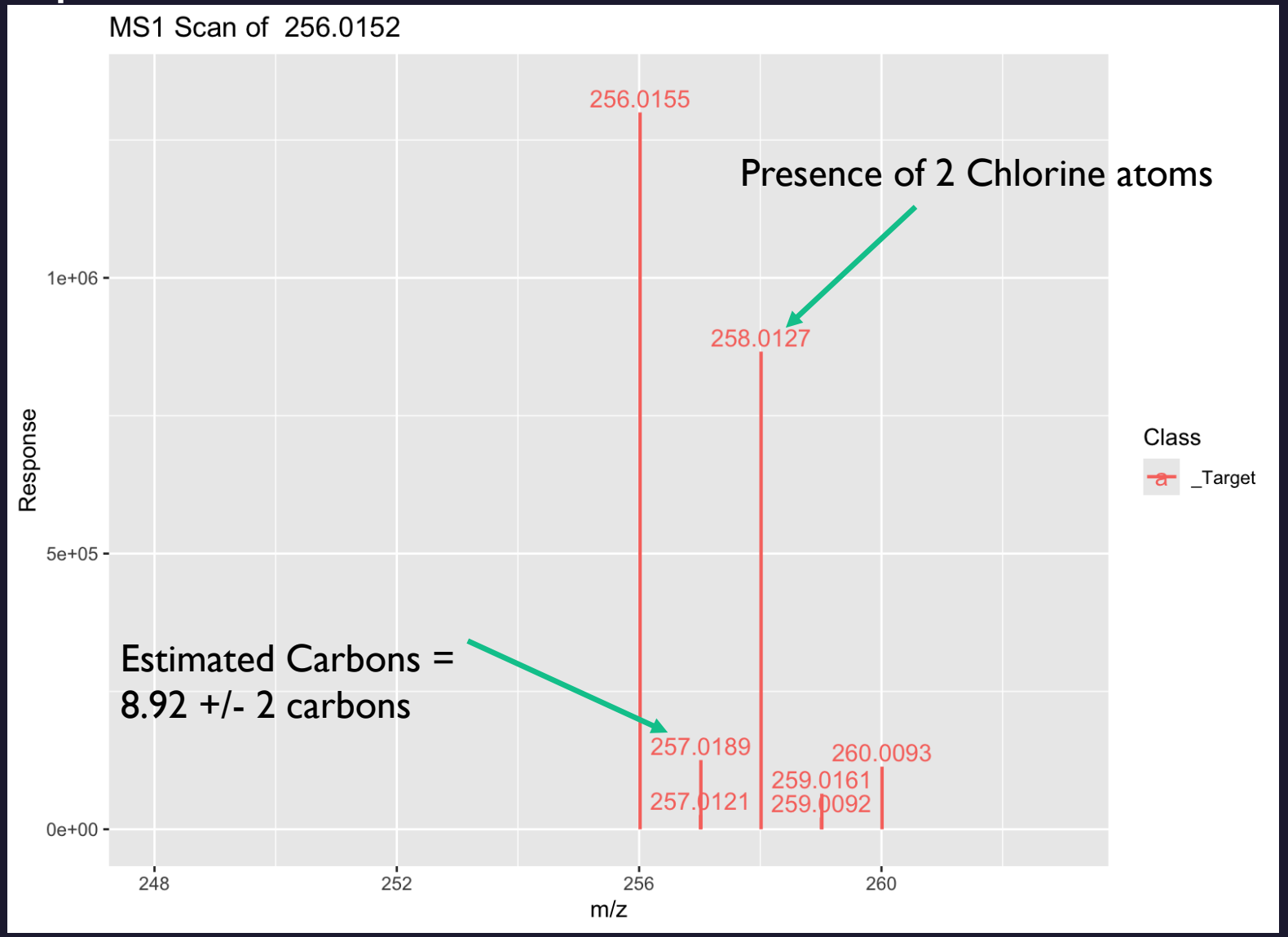
Molecular Formula Elemental Bounds

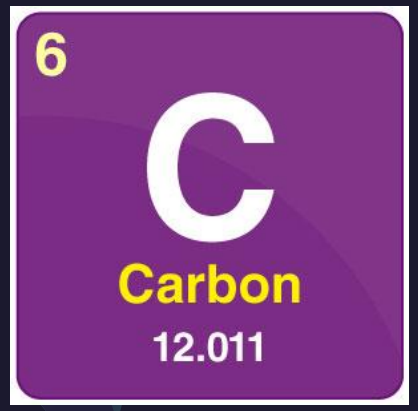
Raw MS1 Spectrum

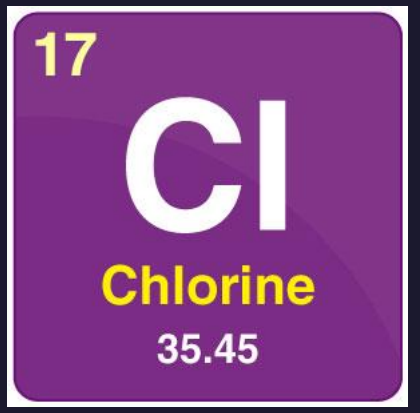


Molecular Formula Elemental Bounds

Filtered MS1 Spectrum







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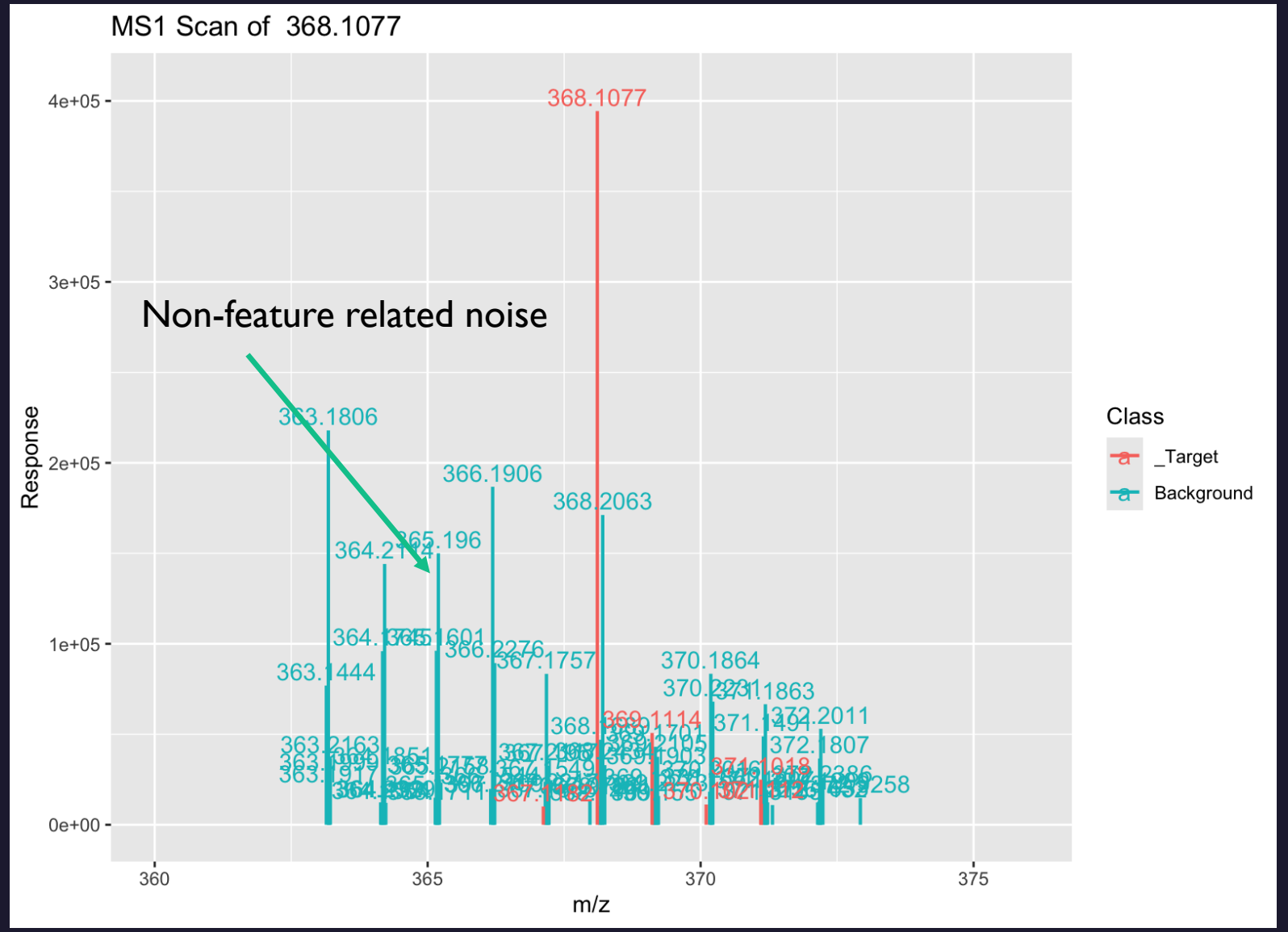

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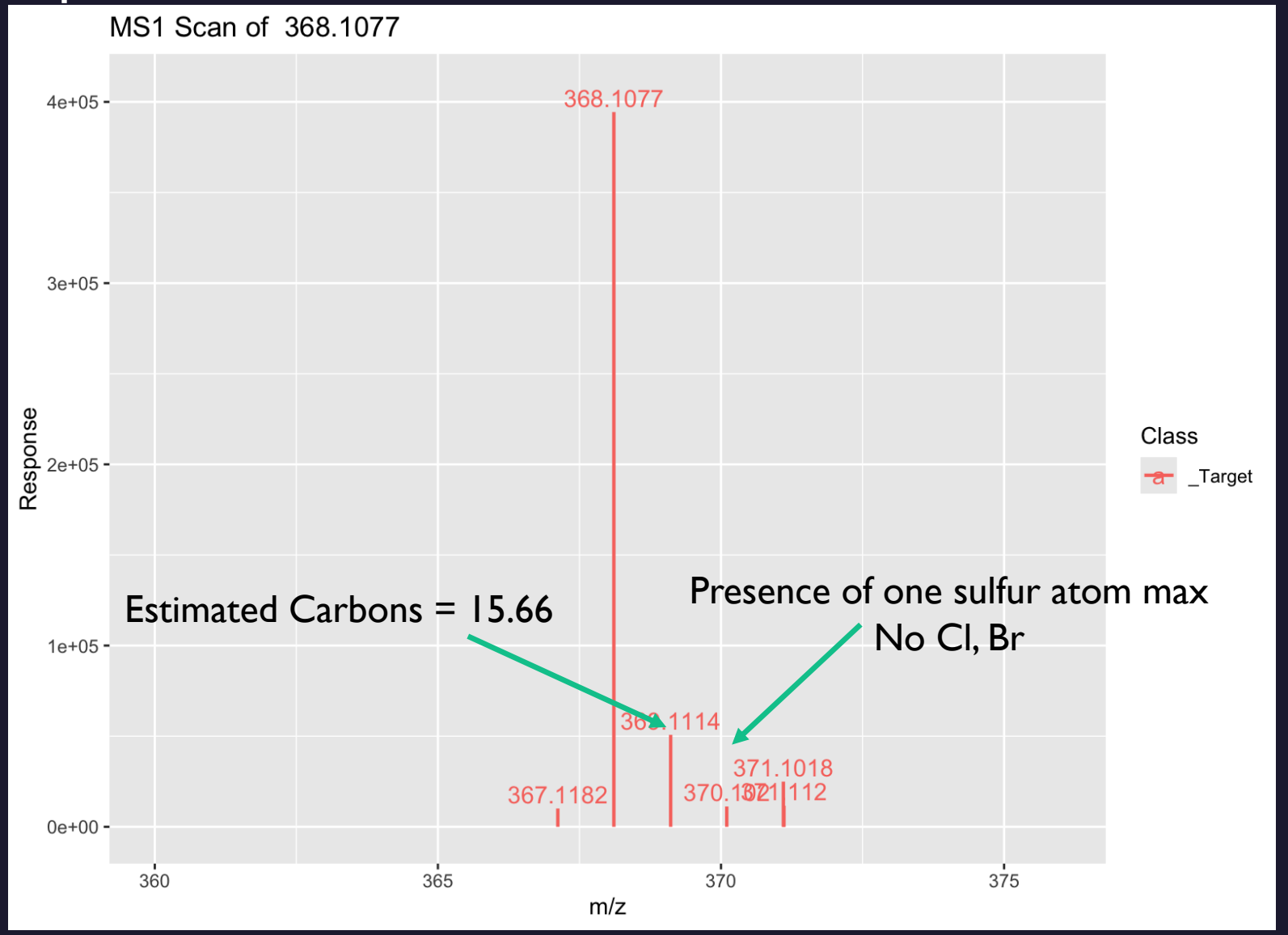

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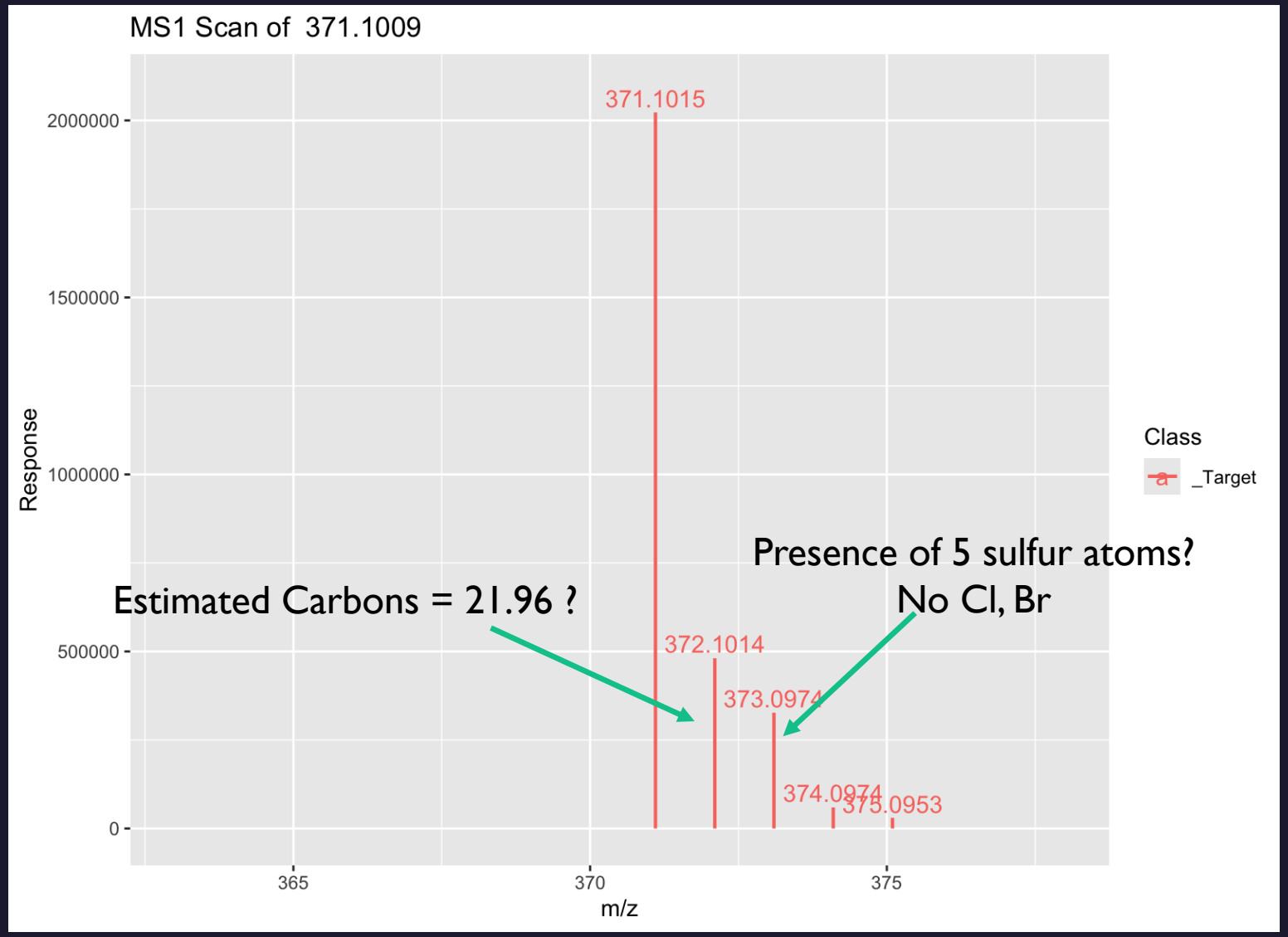

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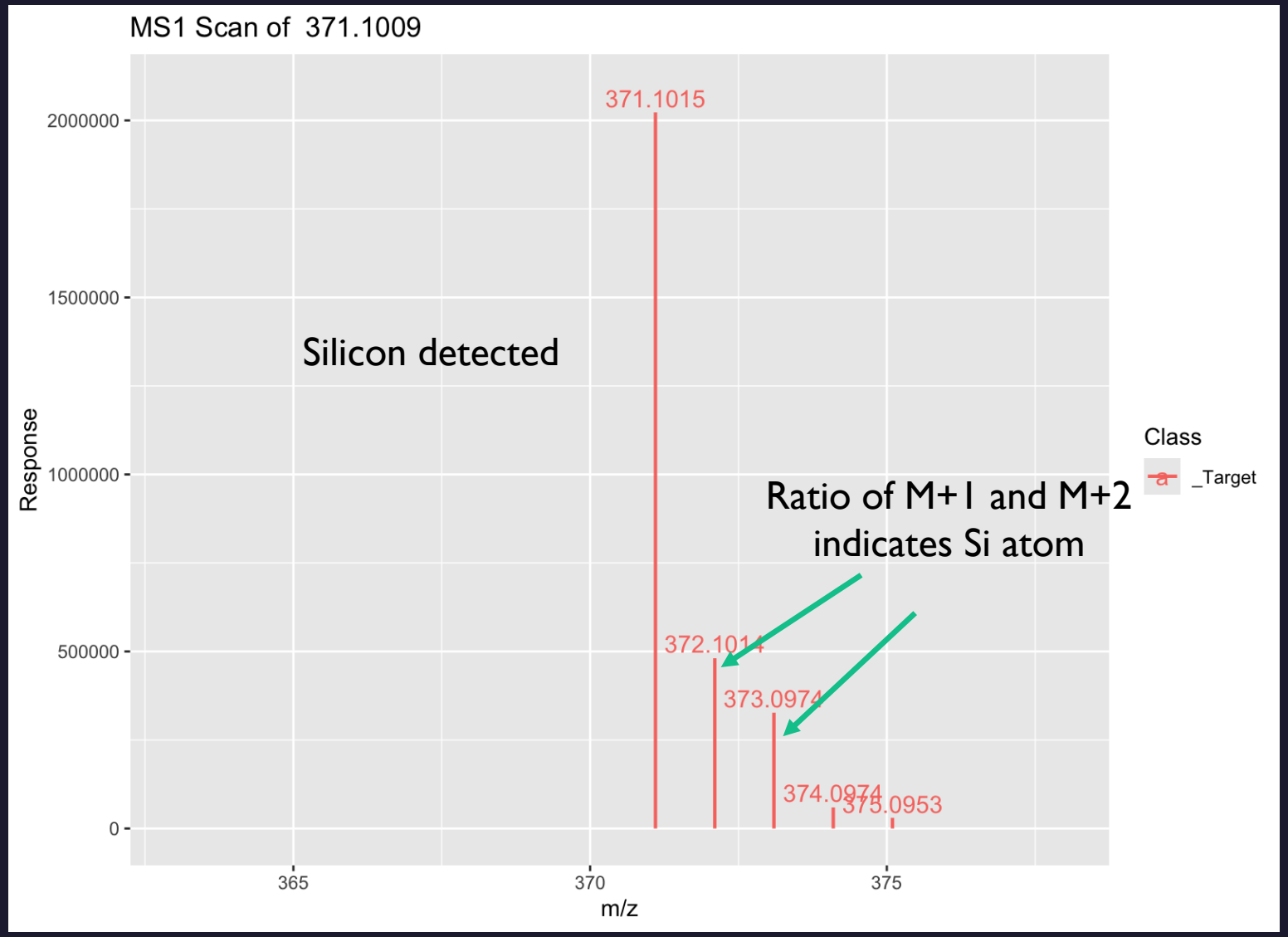

Presence of Silicon throws off M/Carbon ratio and easily identified

Conclusions

- In the absence of MS/MS data, MS1 spectra can be interrogated for more information
- Using High Res Mass Spec intrinsic values like m/z, mass defect, and [13]C ratios can be used to calculate "PFAS-ness" and can be prioritized.
- Retention time prediction models provide an orthogonal technique to confirm or reject potential features
- Plotting data with intrinsically determined values is very useful for PFAS prioritization (Kendrick or Kaufmann)
- Informed Molecular Formula predictions that properly decompose MS1 spectra with elemental bounds more effective than agnostic ones

Thank you

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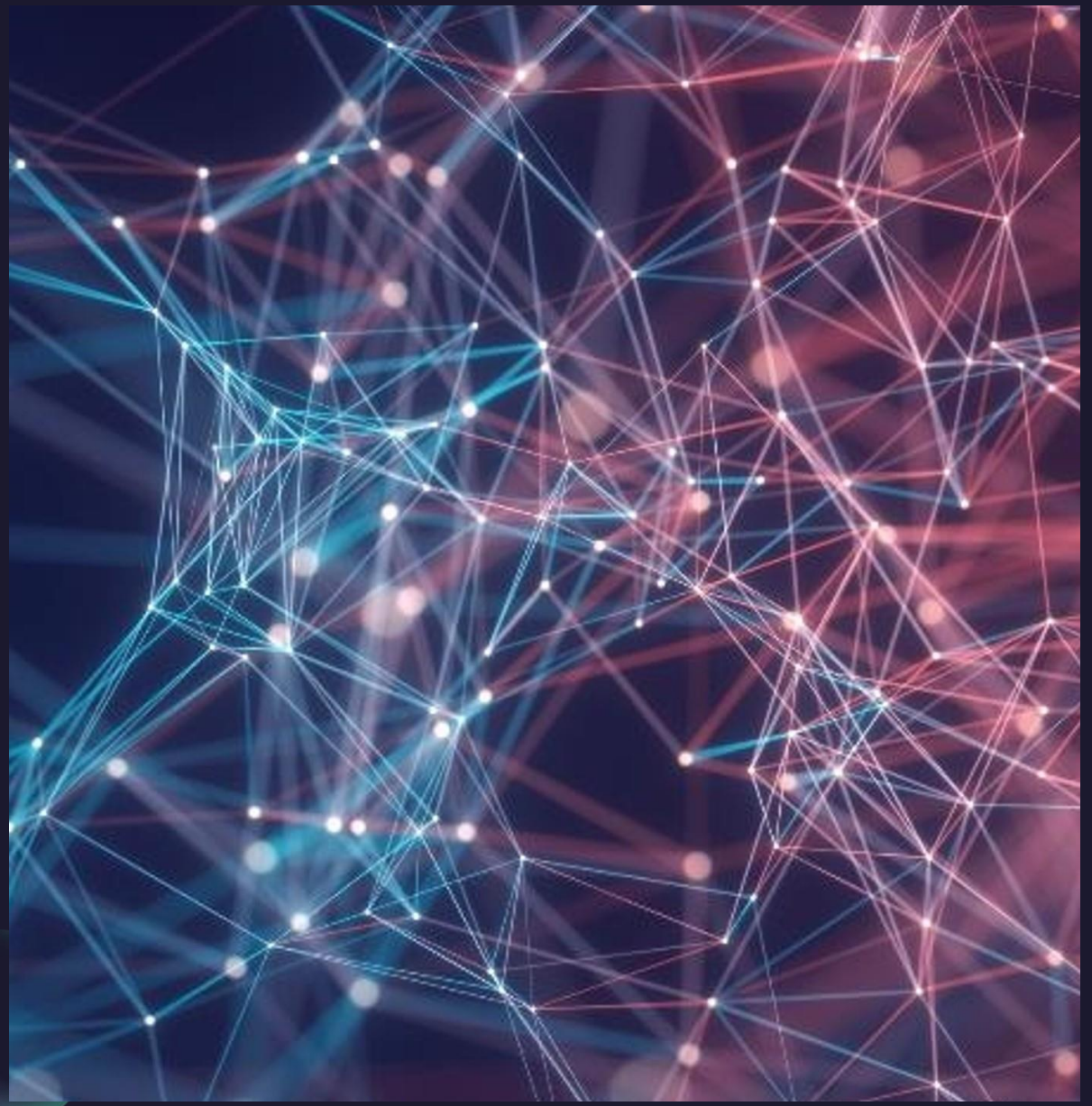

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