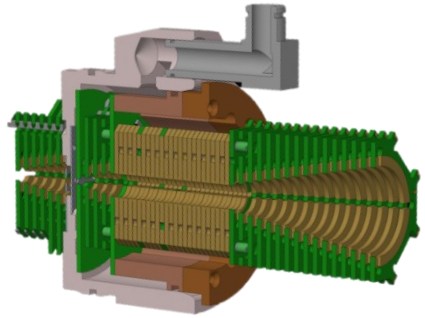
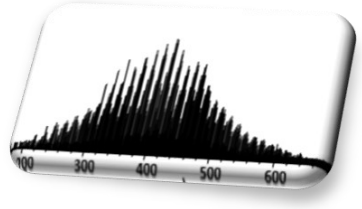
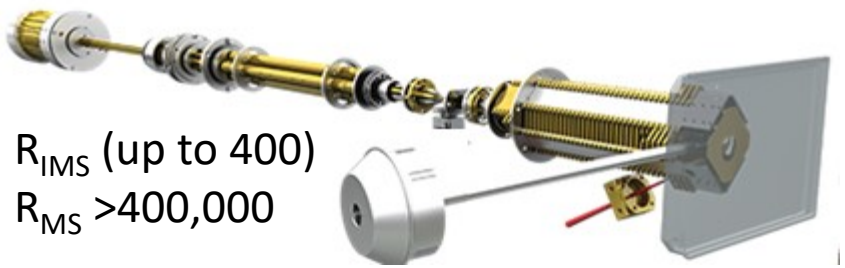
Complex mixture characterization using complementary tandem Trapped Ion Mobility Spectrometry and FT-ICR MS/MS

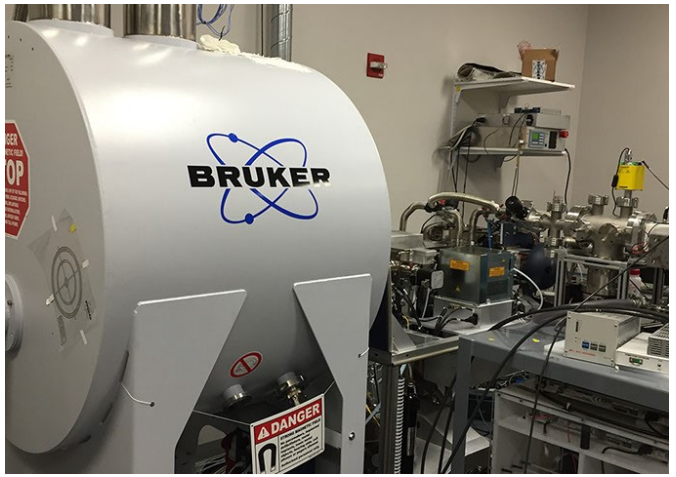


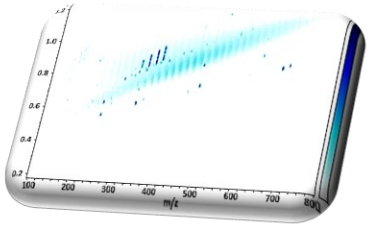
Francisco Fernandez-Lima, PhD
Professor
Department of Chemistry and Biochemistry
Florida International University, Miami, FL





R_{IMS} (up to 400)
R_{MS} >400,000





Acknowledgments

Group Members

Principal Investigator:
Dr. Francisco Fernandez-Lima

Post-doctoral fellows
Valadares Tose
*Khoa Pham**

PhD Graduate Students
Dennys Leyva
Clement Olanrewaju
Miguel Santos
Sam Miller
Cassandra Fuller

Collaborators

Dr. Rudolph Jaffe
Dr. Piero Gardinali
Dr. Fahad Saeed
Dr. John Kominoski

E-MSion Inc.
Dr. Valery G. Voinov
Dr. Joseph S Beckman

Bruker Daltonics
Dr. Mark E. Ridgeway
Dr. Melvin A. Park
Dr. Peter Sanders
Dr Desmond Kaplan*

FIU AMSF
Dr. Yarixa Cintron
Dr. Kevin J. Dit Fouque

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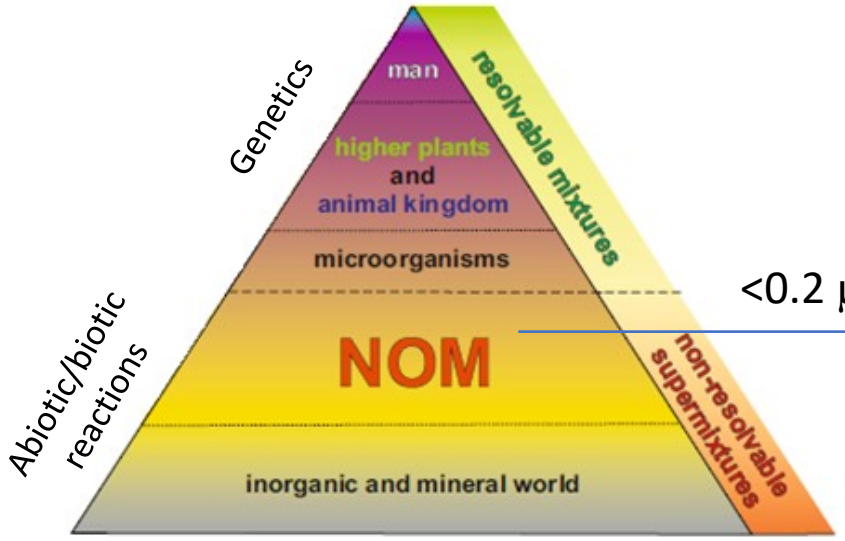
CHE-1654274
R01GM134247

Thank you for your attention!

Dissolved Organic Matter

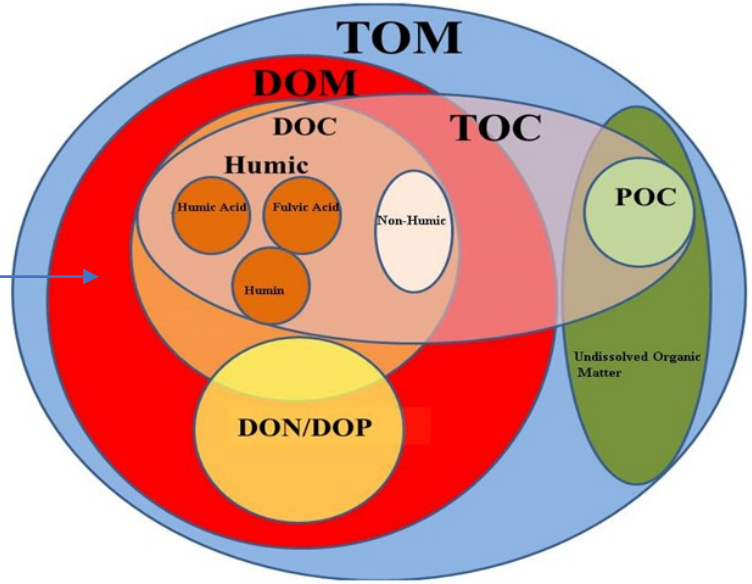
Chemical composition: CHONSP



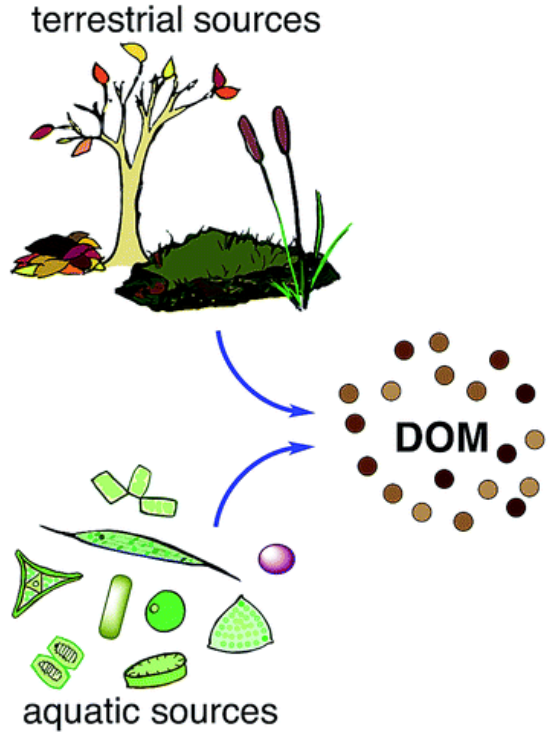
NOM: Natural Organic Matter

<0.2 μm

DOM: Dissolved Organic Matter



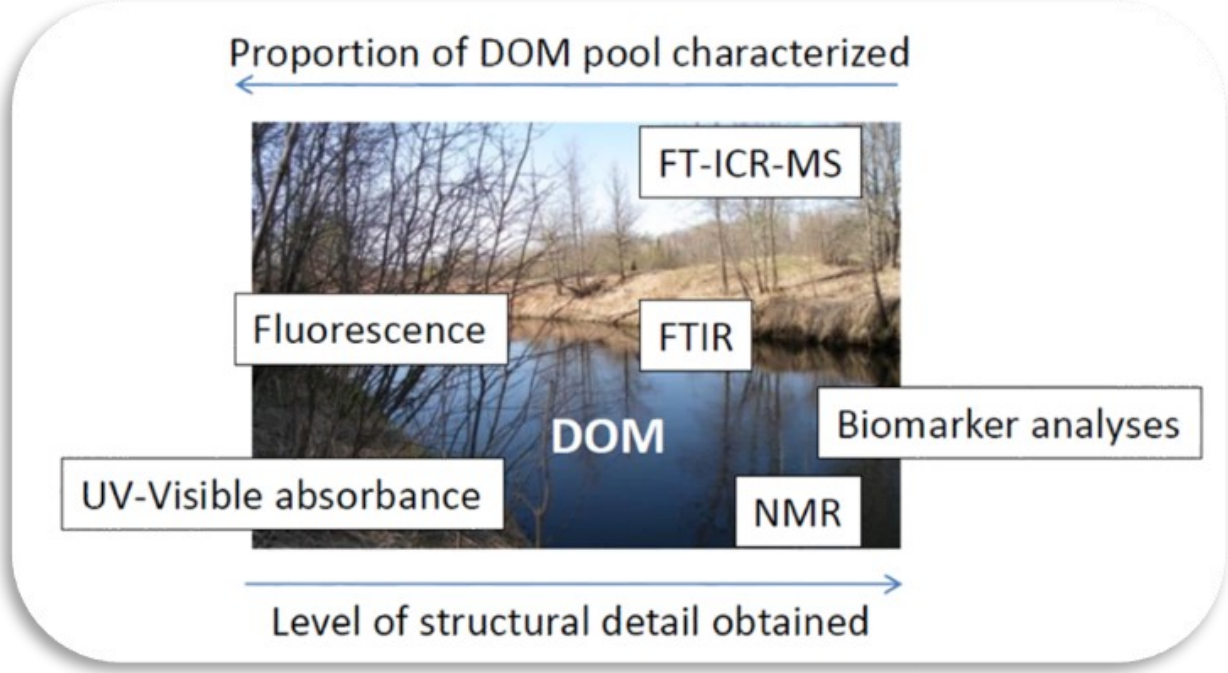
Forms of organic matter



N. Hertkorn, et.al. *Analytical and Bioanalytical Chemistry*, 2007, **389**, 1311-1327.
Pagano, T. et. al. *Water* **2014,** *6* (10), 2862
Lin, V. S. *Environmental Science: Processes & Impacts* **2015,** *17* (12), 2002-2005.

Analytical approaches

Bulk vs molecular level characterization



Challenges in DOM characterization

- High structural heterogeneity
- Wide range of molecular weights
- **Isomeric complexity**

NMR:

- Requires DOM concentration and pure samples.
- Detect some specific DOM functionalities.

Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry

- Ultra-high mass resolution (R>400, 000)
- Molecular composition
- Structural information based on MS/MS

Minor, E., et.al. *Environmental Science: Processes & Impacts* **2014,** *16* (9), 2064-2079.

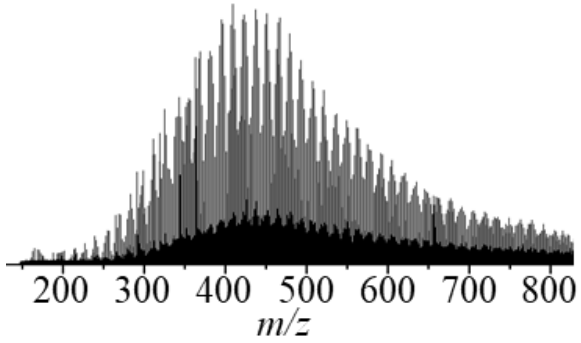
Traditional untargeted workflow for DOM analysis

Direct infusion

FT-ICR MS

Orbitrap MS

Broadband MS

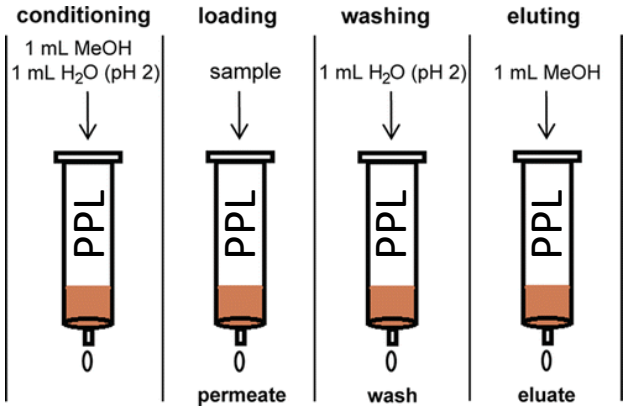


Chemical formula assignment

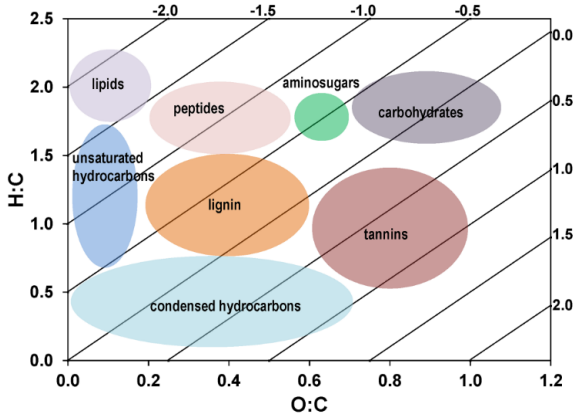
Formula constraints:

- $C_{4-50}H_{4-100}N_{0-3}O_{0-25}S_{0-2}$
- S/N>3-6
- m/z range 100-1000
- Tolerance error <1 ppm
- DBE-O <10
- 0<O/C≤2, 0.3≤H/C≤2.5
- Isotopologue peaks confirmation
- Removal of isolated assignments

Solid phase extraction

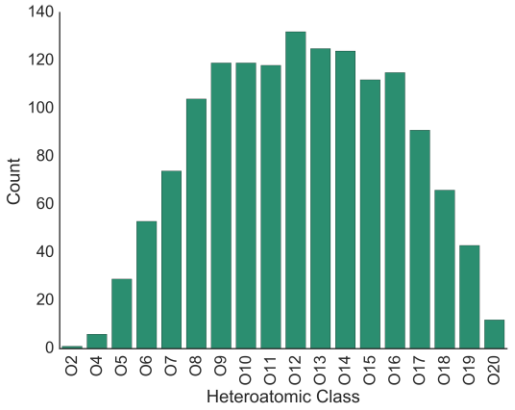


Compositional space

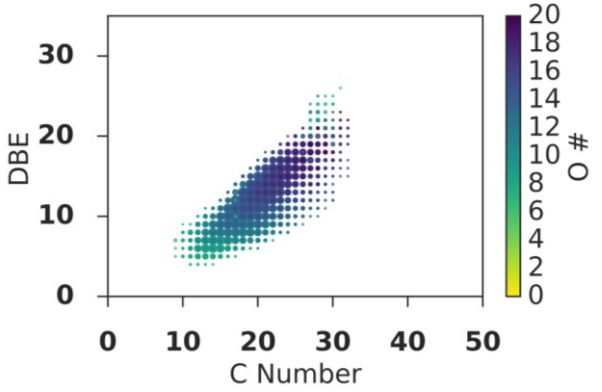


Van Krevelen diagram

Heteroatom classes

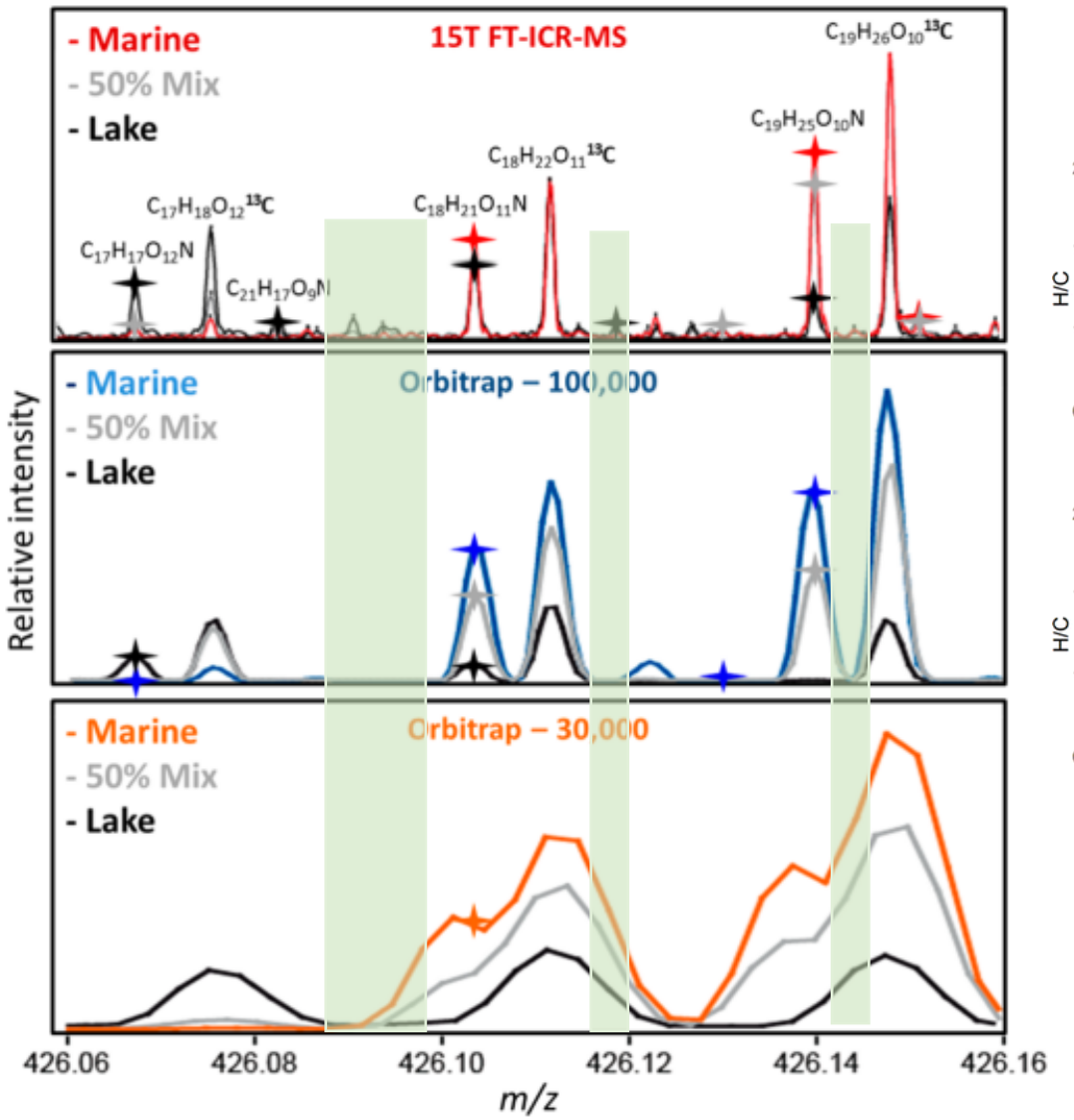


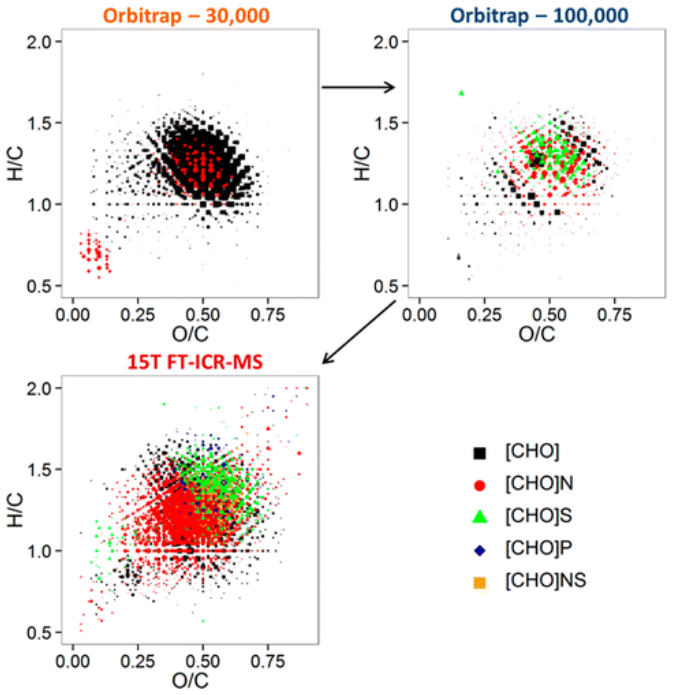
DBE vs C#

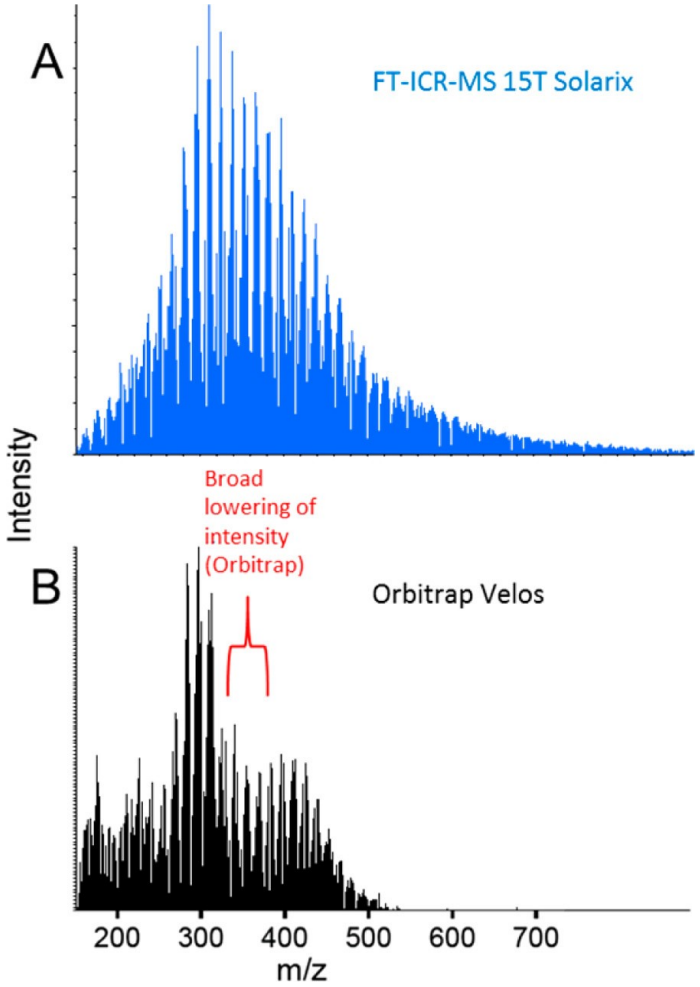


Y. Li, M. Harir, et.al, *Analytical Chemistry*, 2016, **88**, 6680-6688.
Mann BF, et.al (2015). *PLOS ONE* 10(6): e0130557

Orbitrap MS vs FT ICR MS



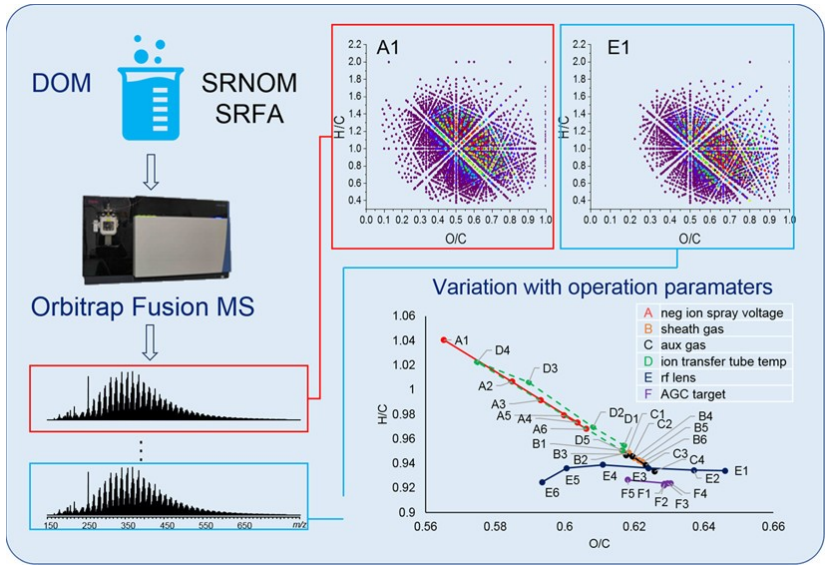




Orbitrap resolved ~ 70% of molecular formulas identified by FT-ICR MS

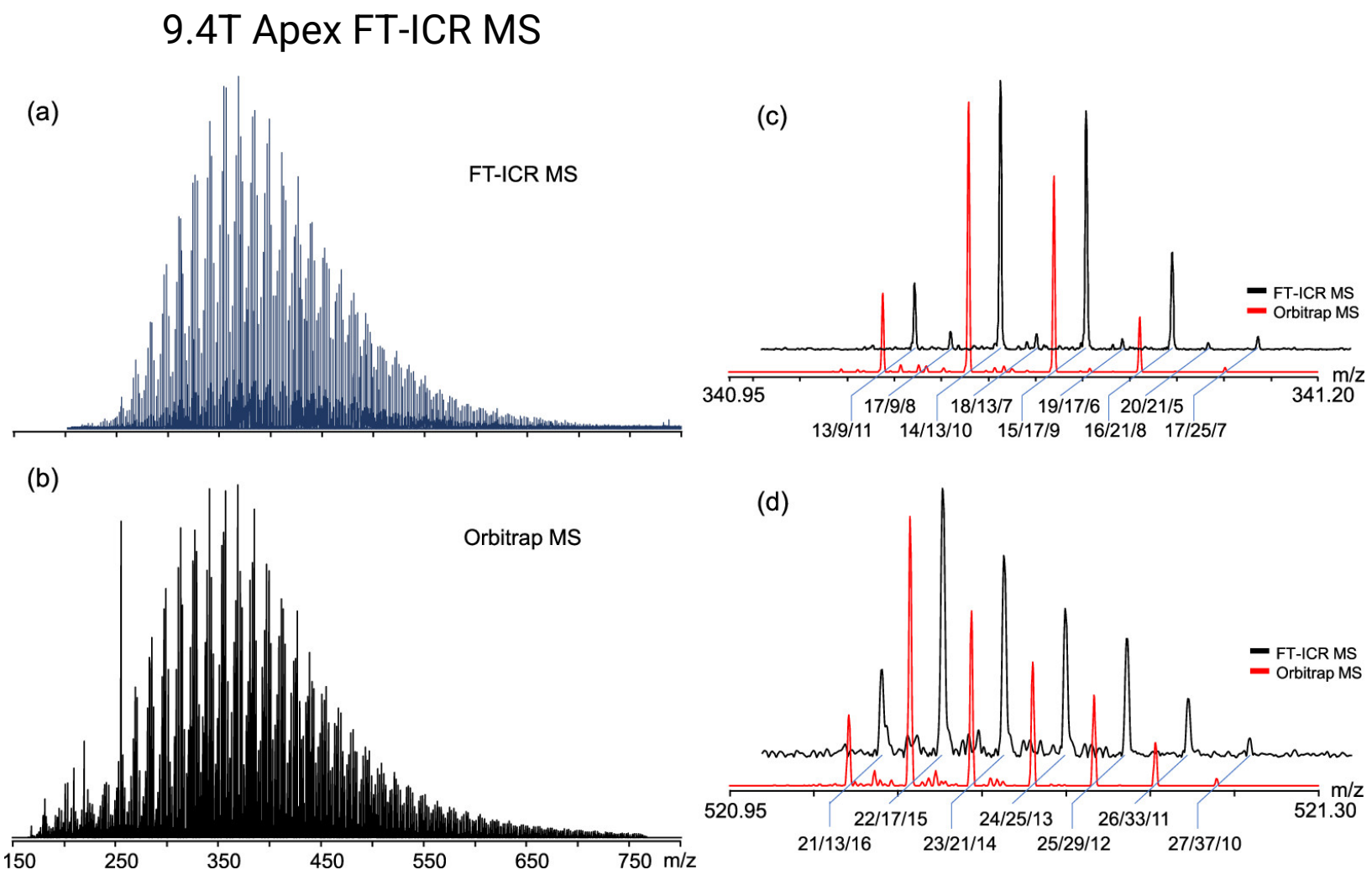
J. A. Hawkes, T. Dittmar, C. Patriarca, L. Tranvik and J. Bergquist, *Analytical Chemistry*, 2016, **88**, 7698-7704.

Orbitrap MS vs FT ICR MS



Orbitrap Fusion MS

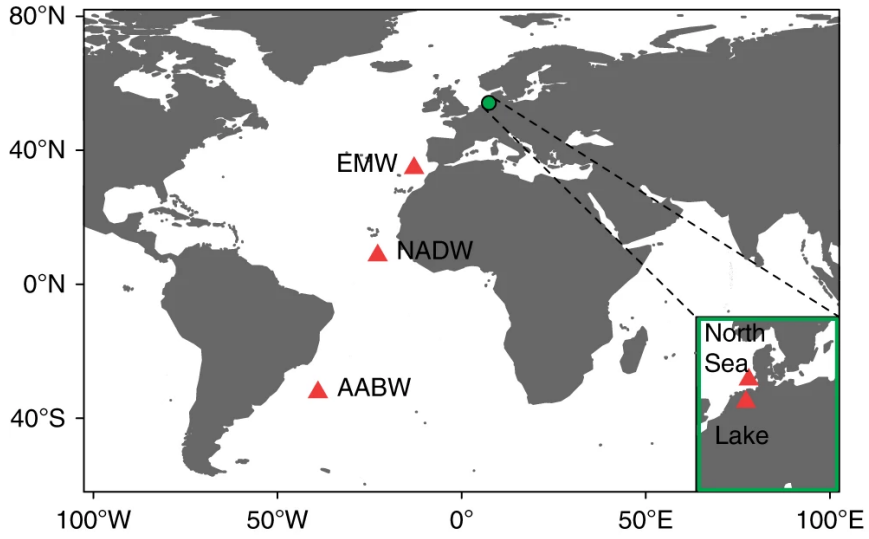
500,000 resolving power (at m/z 200)



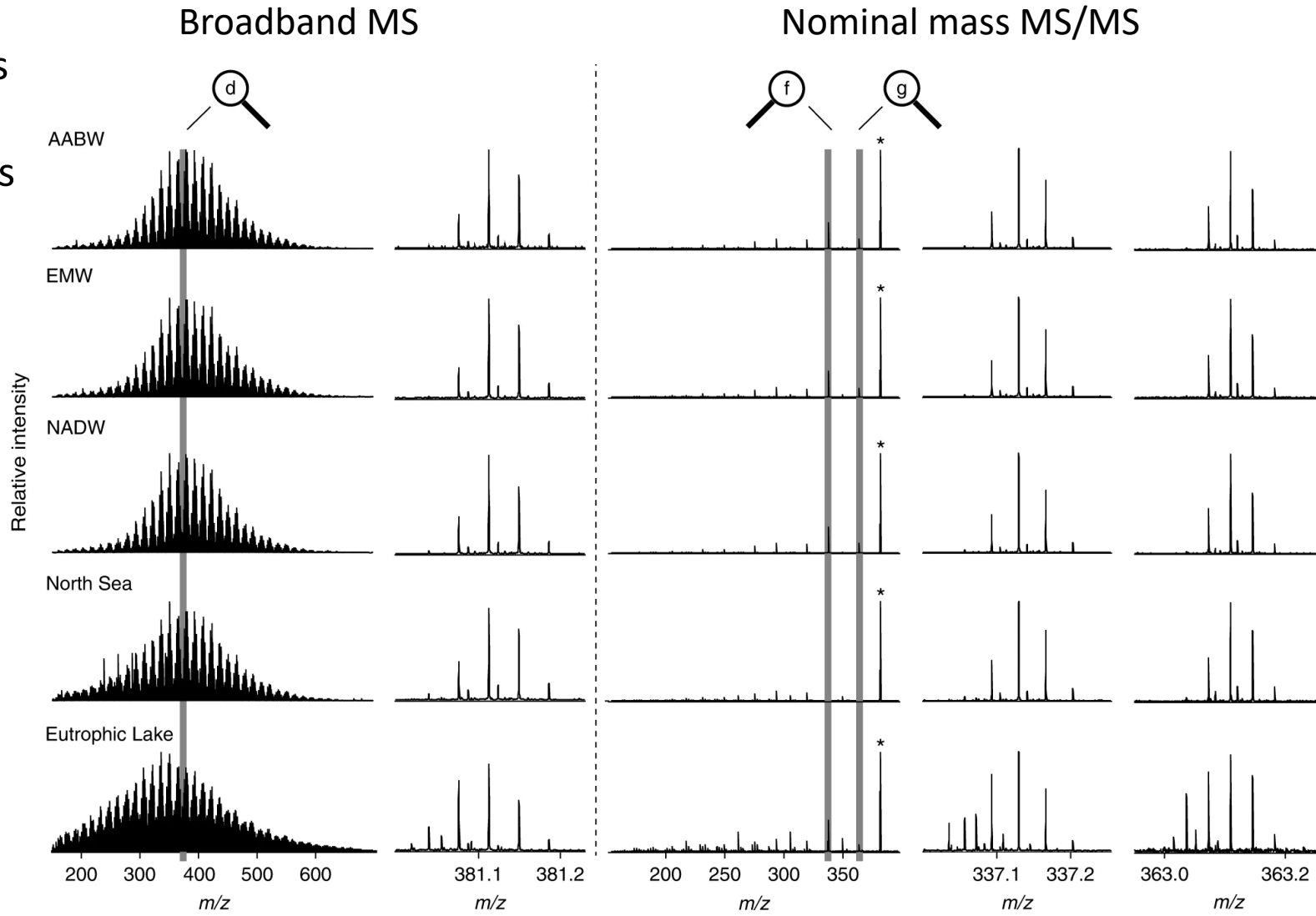
ACS Omega 2020, 5, 10, 5372–5379

DOM structural characterization using tandem MS

- A major component of DOM is ubiquitous
- Identical structural features of isomers
- Universal degradation/synthetic pathways

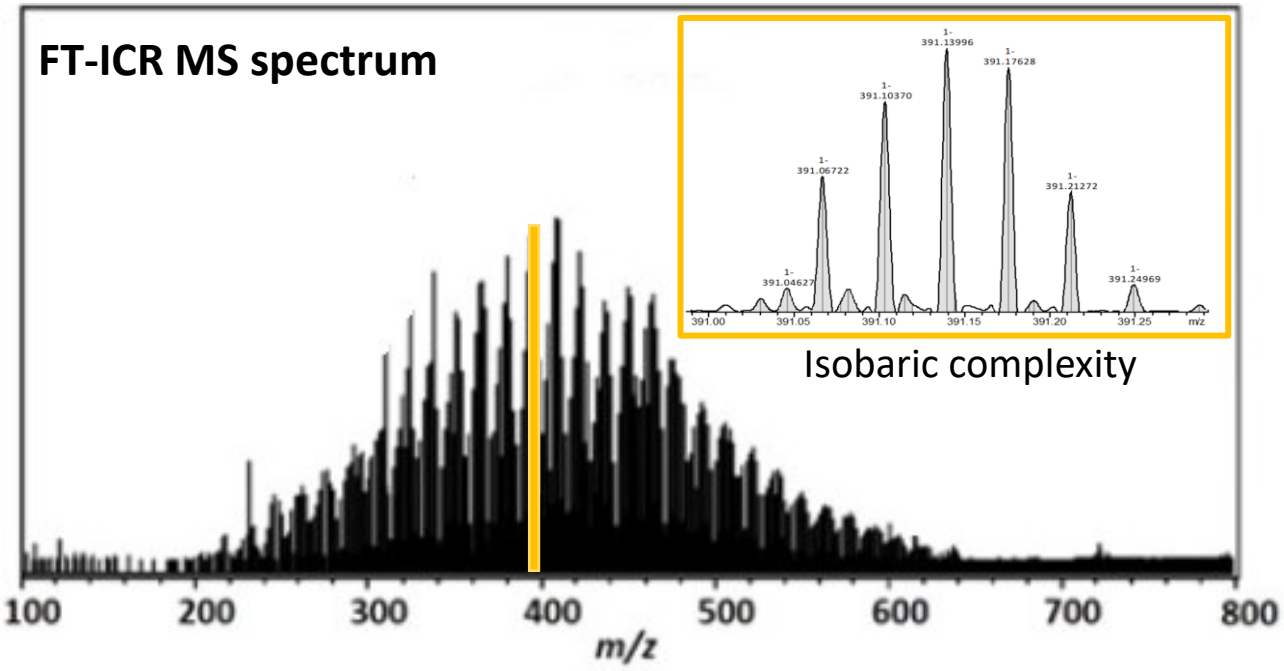


Structure of DOM components and the number of isomers behind a chemical formula remain unknown!

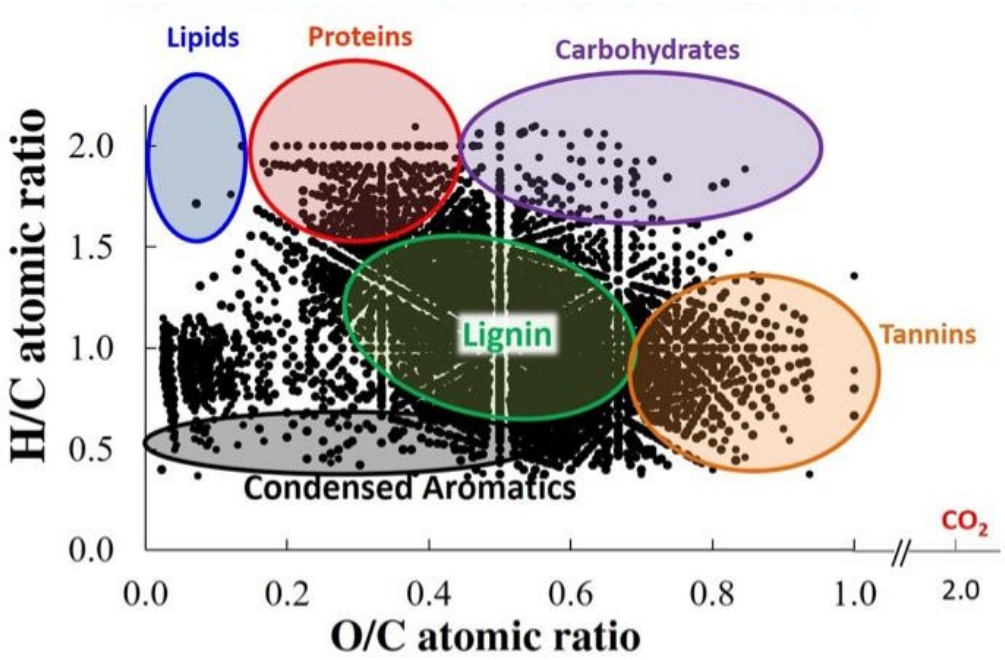


Zark M, Dittmar T. *Nature Communications*. 2018;9(1):3178.

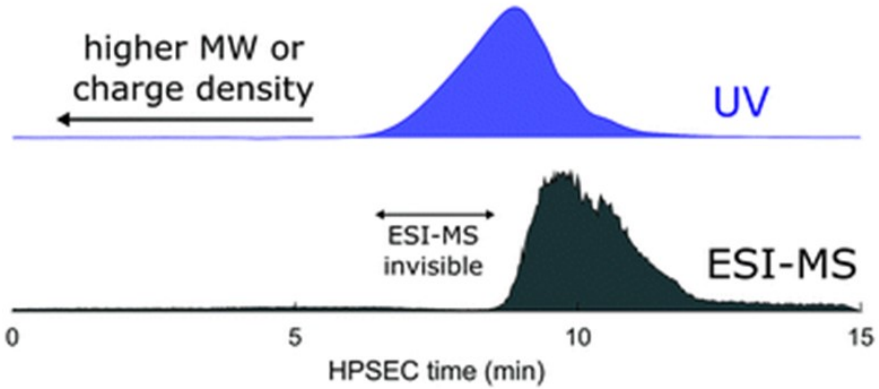
DOM molecular complexity



Diversity in compound classes



Size complexity



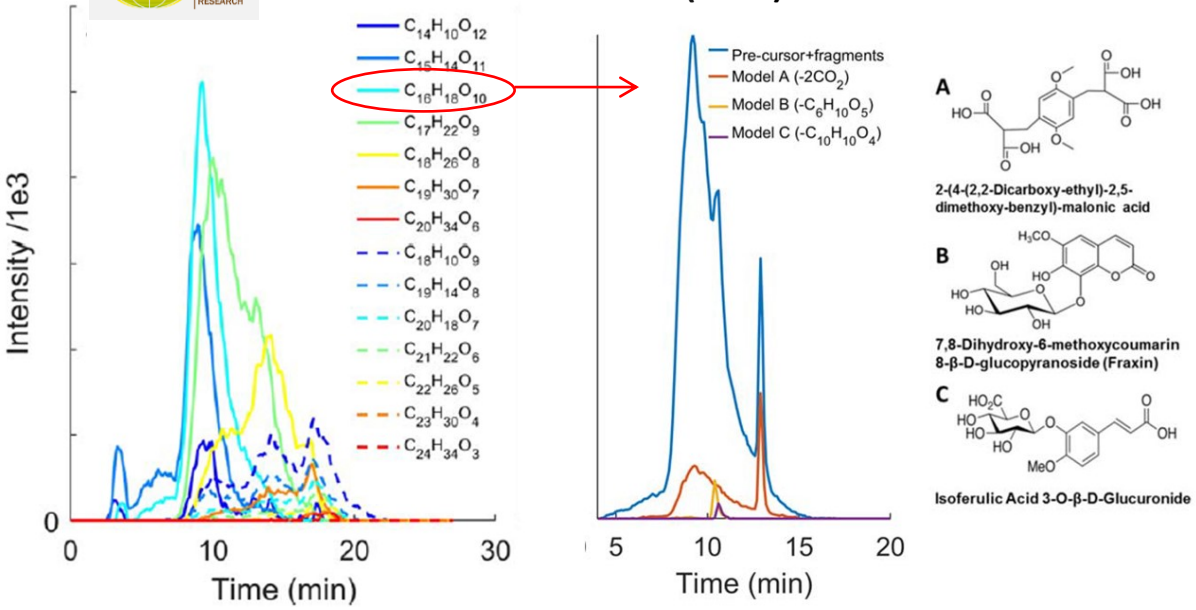
Woods, G, et. Al. *Environmental Science & Technology* **2011,** *45* (9), 3880-3886.
Hawkes, J. A. et.al. *Faraday Discussions* **2019,** *218* (0), 52-71.

Nontraditional approaches

HPLC-Orbitrap MS/MS

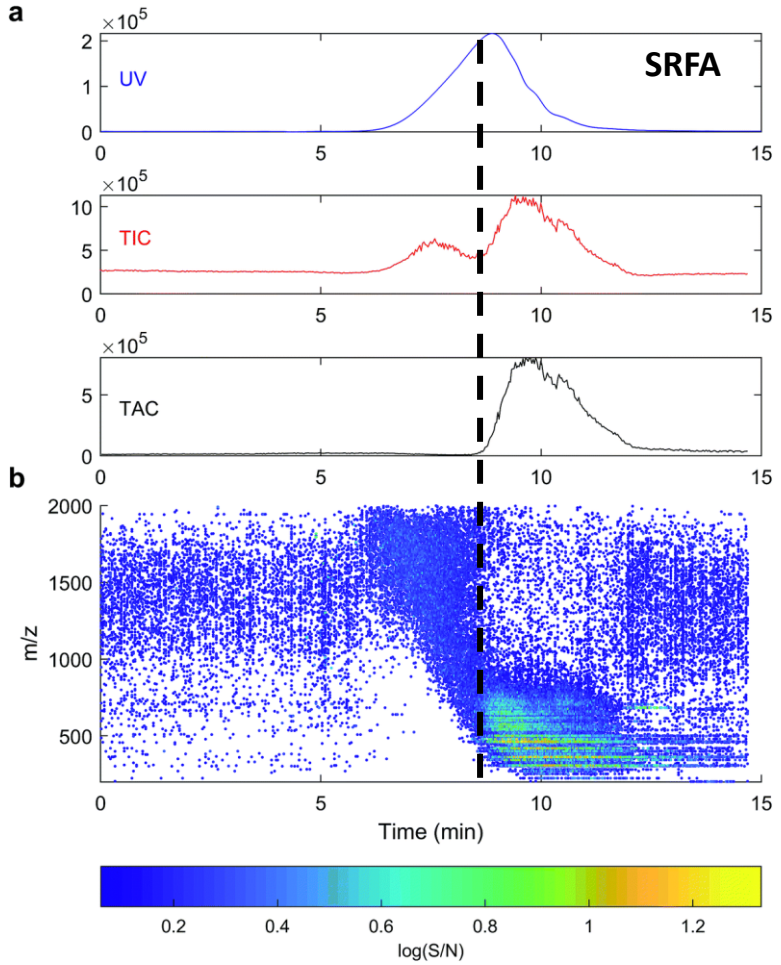
14 precursors at 369 **Da**

Suwannee river fulvic acid (SRFA)



- Liquid chromatography provides more information than direct infusion.
- It does not resolve the isomeric complexity of DOM.
- Dissimilar fragmentation patterns compared to model compounds.

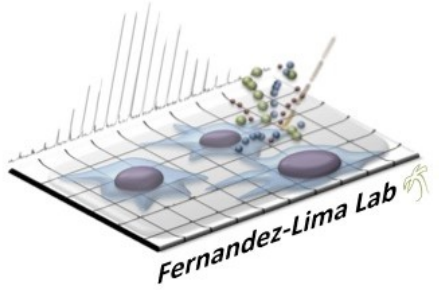
HPSEC-UV-Vis DAD and HPSEC-HESI-Orbitrap-MS

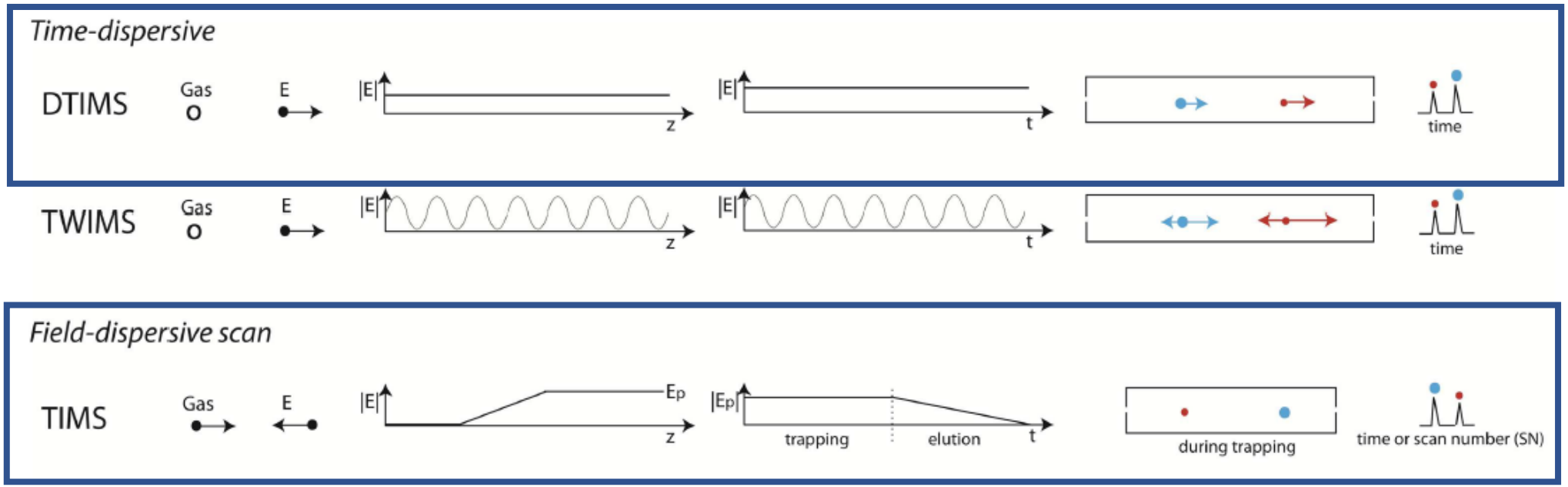


- Adding new separation dimensions reduce molecular complexity.
- Optically active compounds not efficiently ionized by ESI.

Hawkes, J. A. et al. *Limnology and Oceanography Letters* 2018, ***3*** (2), 21-30.
Hawkes, J. A. et al. *Faraday Discussions* 2019, **218**, 52-71

Ion Mobility Spectrometry

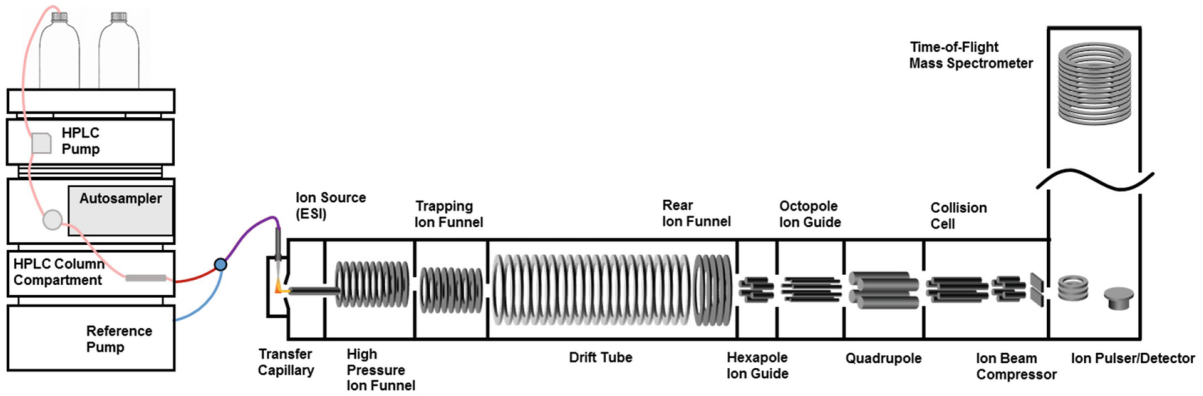




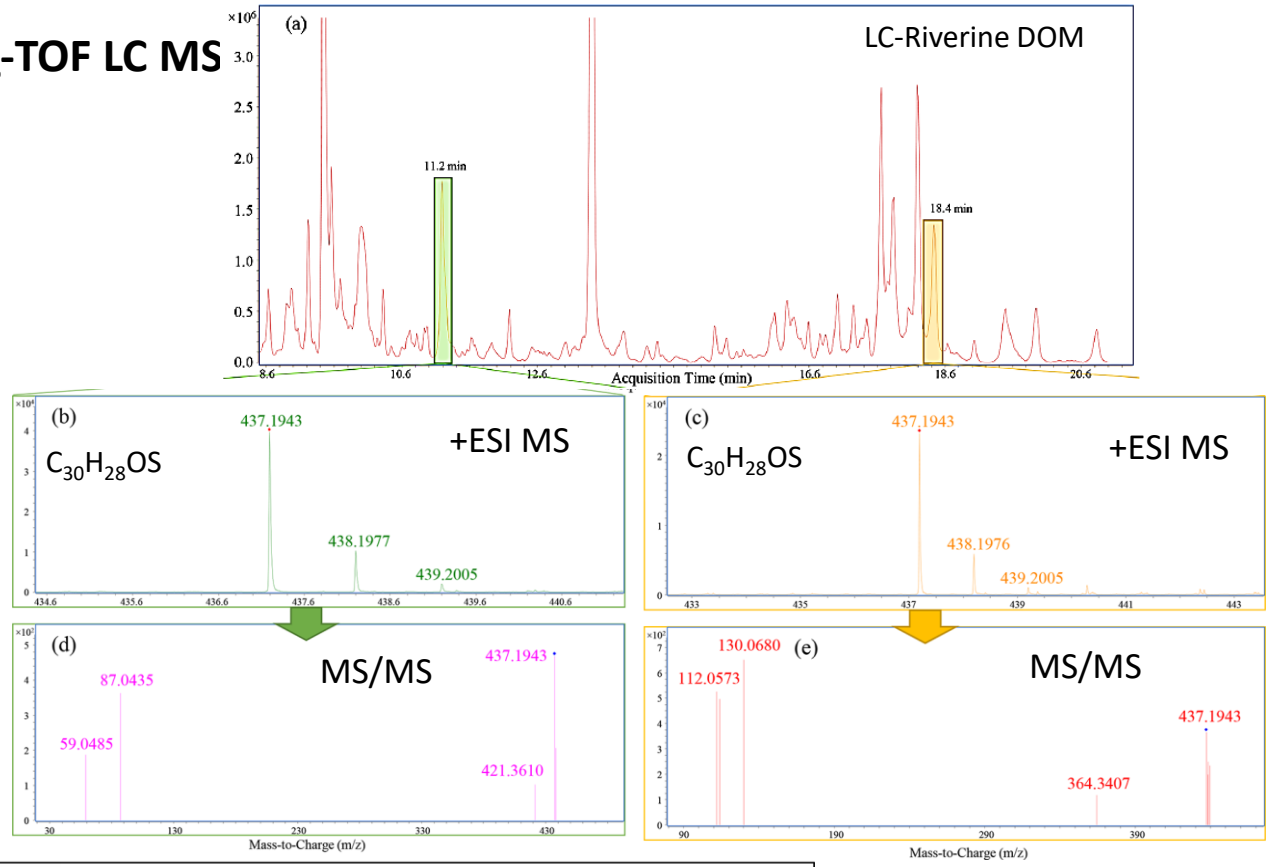
Gabelica et al., Mass Spectrometry Reviews, 2019, 38, 291–320

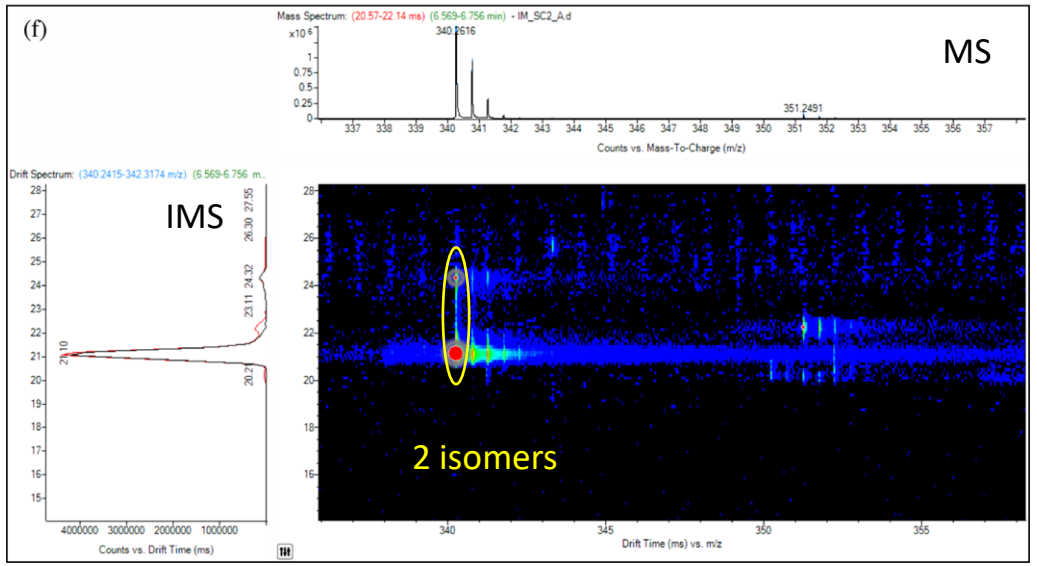
Nontraditional approaches

Drift Tube IMS coupled to q-TOF MS/MS



DT-IMS Q-TOF LC MS

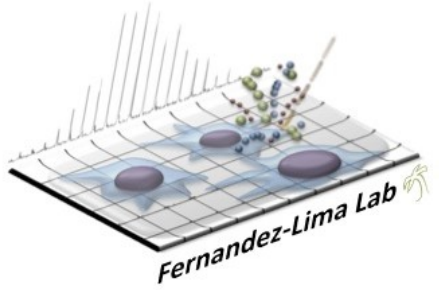




- Limited IMS resolving power (<60)
- 1-3 isomers per chemical formula

K. Lu, et.al, *Environmental Science & Technology*, 2018, **52**, 7182-7191.

Ion Mobility Spectrometry



Time-dispersive

DTIMS Gas E |E| z |E| t time

TWIMS Gas E |E| z |E| t time

Field-dispersive scan

TIMS Gas E |E| Ep z |Ep| trapping elution t during trapping time or scan number (SN)

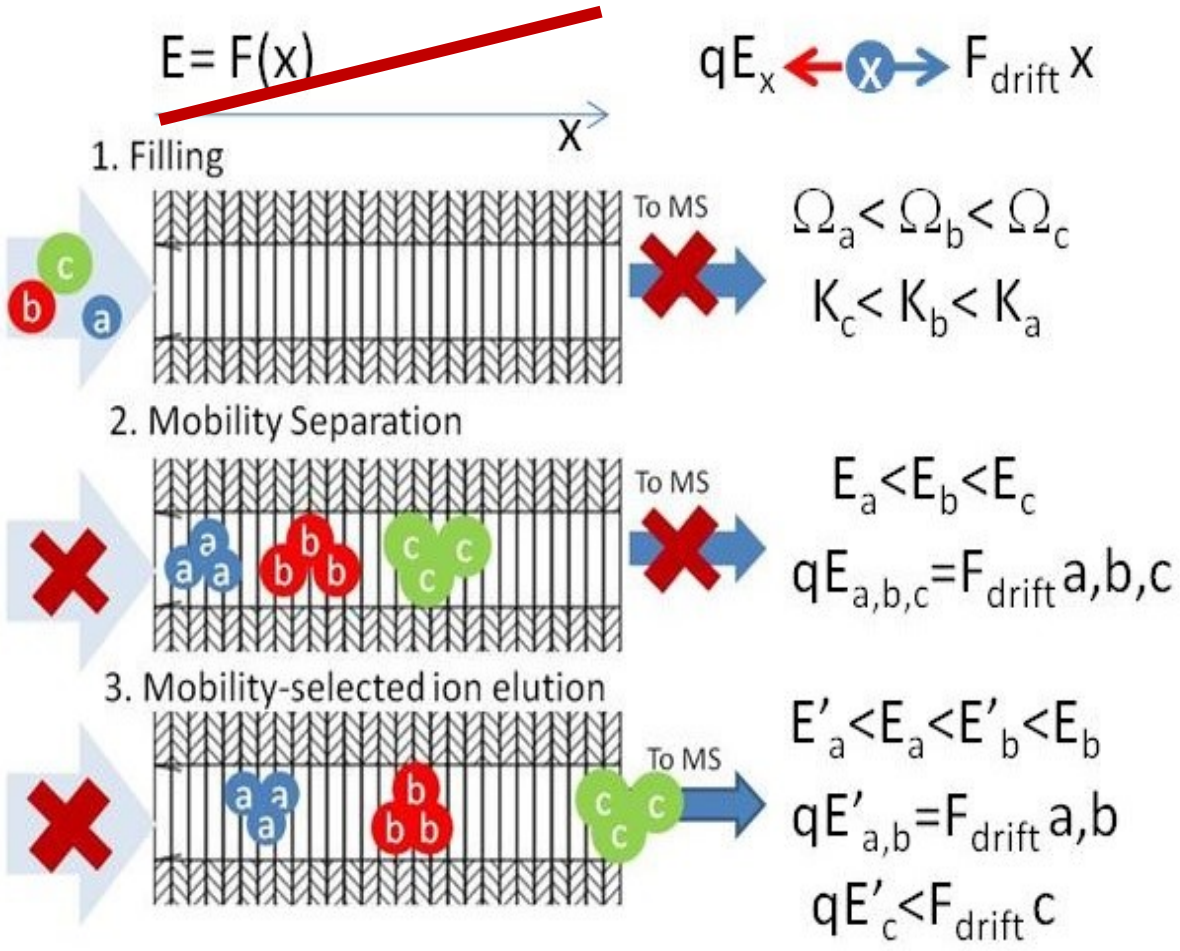
Spatially dispersive (filter)

DMA Gas E |E| z |E| t voltage

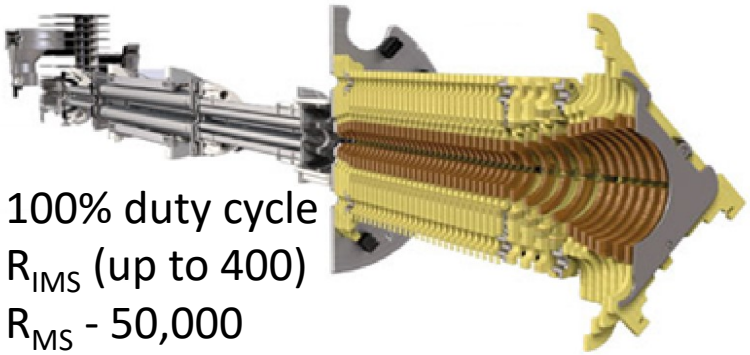
FAIMS Gas E |E| z |E| t or compensation voltage (CV) compensation voltage (CV)

Gabelica et al., Mass Spectrometry Reviews, 2019, 38, 291–320

Trapped Ion Mobility Spectrometry



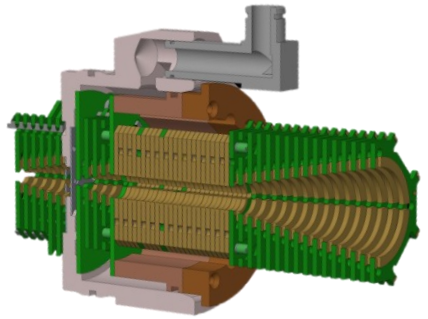
trapTIMS-q-TOF MS/MS



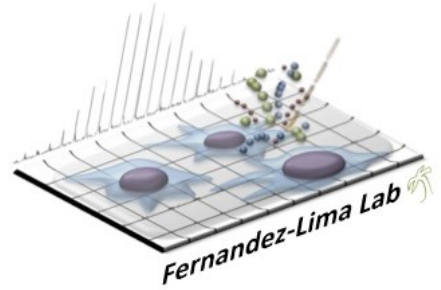
100% duty cycle
R_{IMS} (up to 400)
R_{MS} - 50,000

TIMS-q-FT-ICR MS/MS

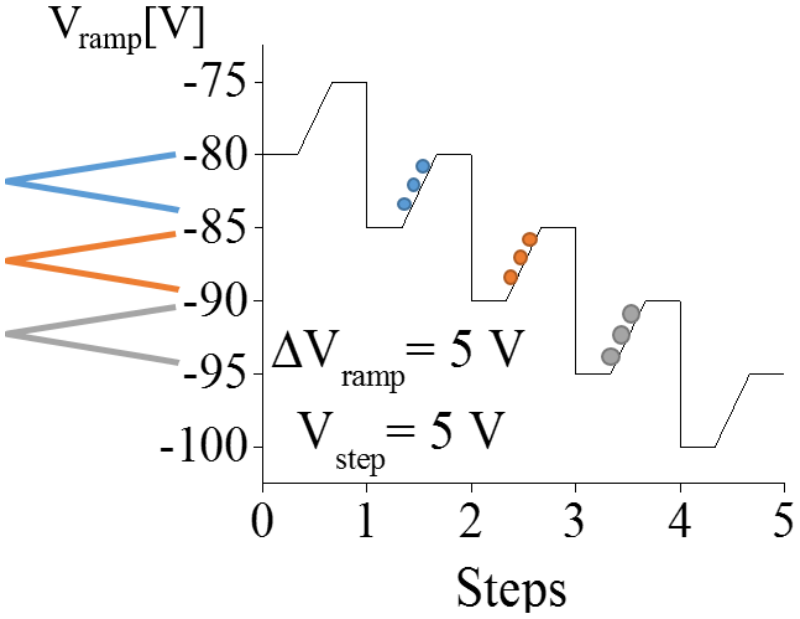
R_{IMS} (up to 400)
R_{MS} >400,000

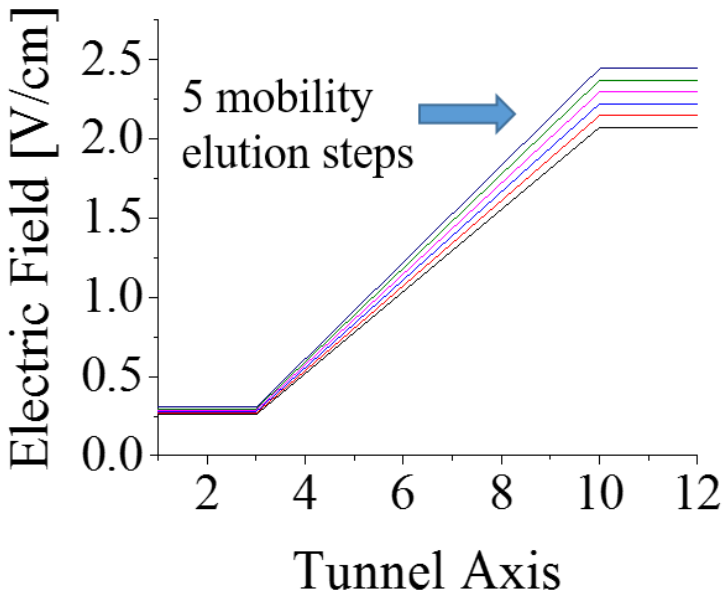


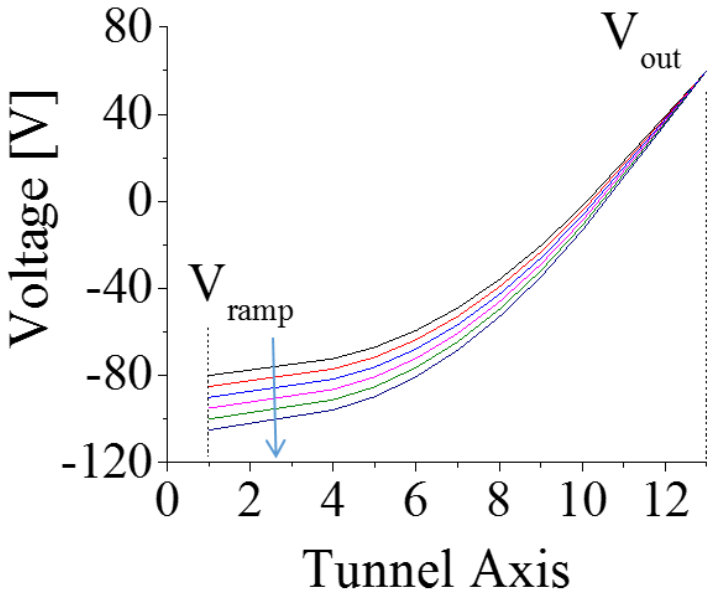
Trapped Ion Mobility Spectrometry



SA-TIMS Principles of operation



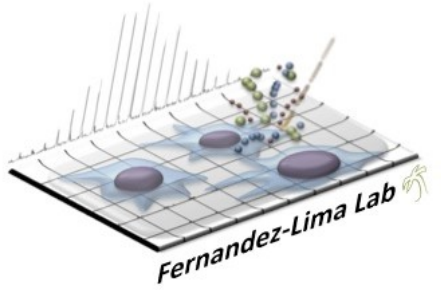




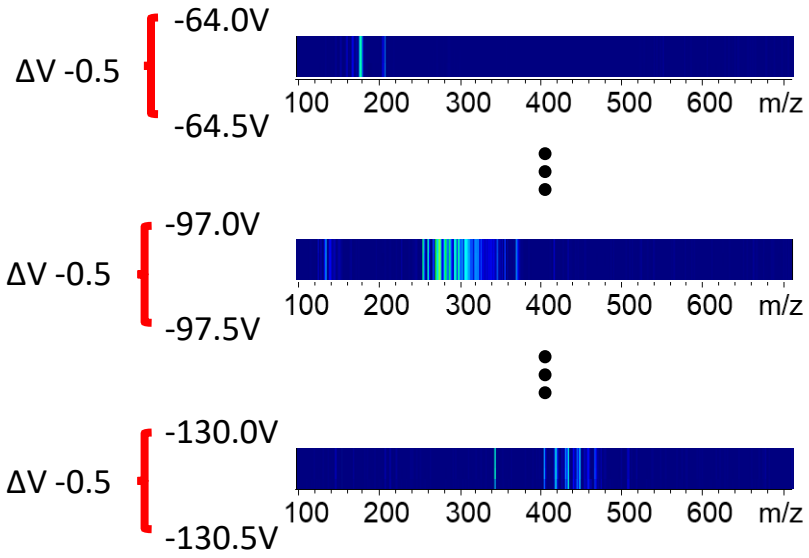
P. Benigni, F. Fernandez-Lima," Oversampling SA-TIMS coupled to FT-ICR MS: Fundamentals and Applications". Anal .Chem. 88 (14), 7404–7412, 2016.

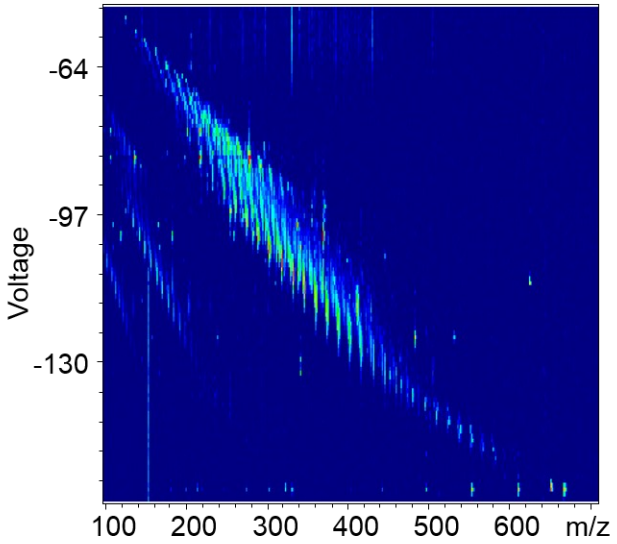
Trapped Ion Mobility Spectrometry

SA-TIMS Principles of operation



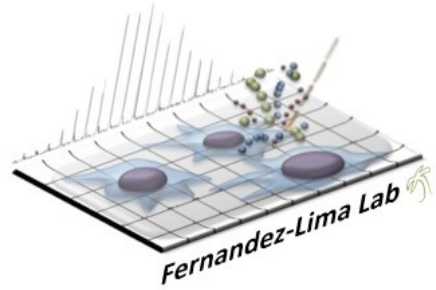
Scanning the voltage (mobility) range



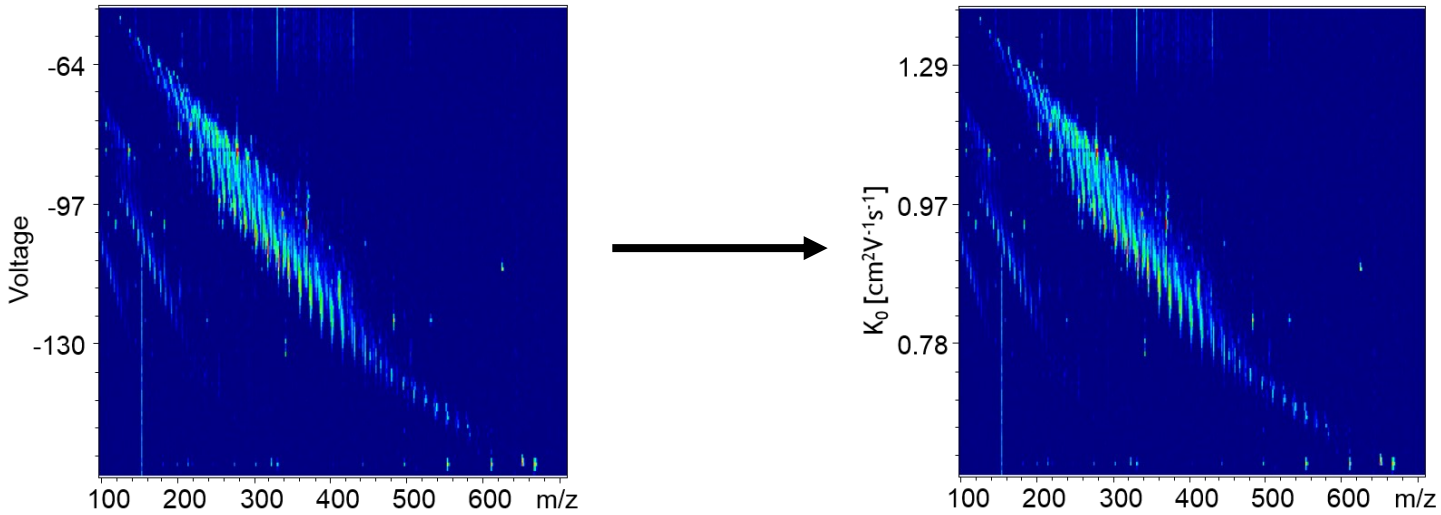


P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. Anal. Chem. 87 (8), 4321–4325, 2015.

Trapped Ion Mobility Spectrometry



SA-TIMS Principles of operation



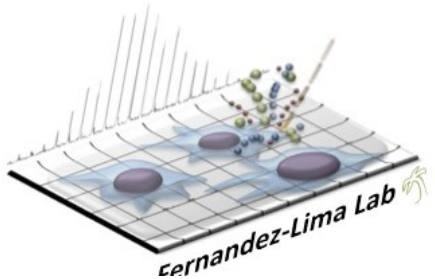
$$\underline{K} = \frac{v_g}{E} = \frac{A}{\underline{V_{elu}} - V_{base}}$$

$A \equiv Experimentally\ derived\ constant\ from\ standards$

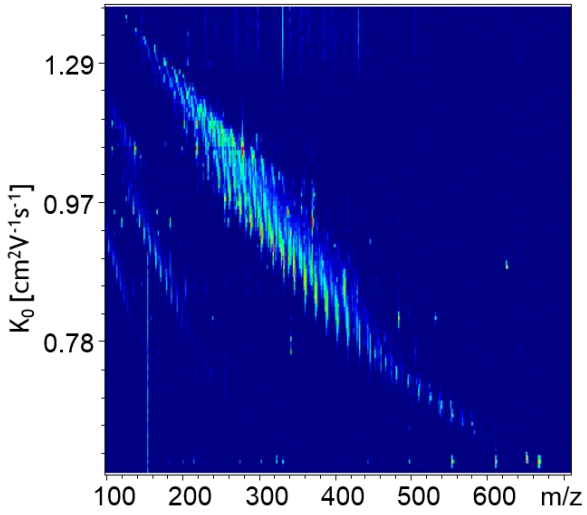
- SA-TIMS provides first-principle K measurements

P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. Anal. Chem. 87 (8), 4321–4325, 2015.

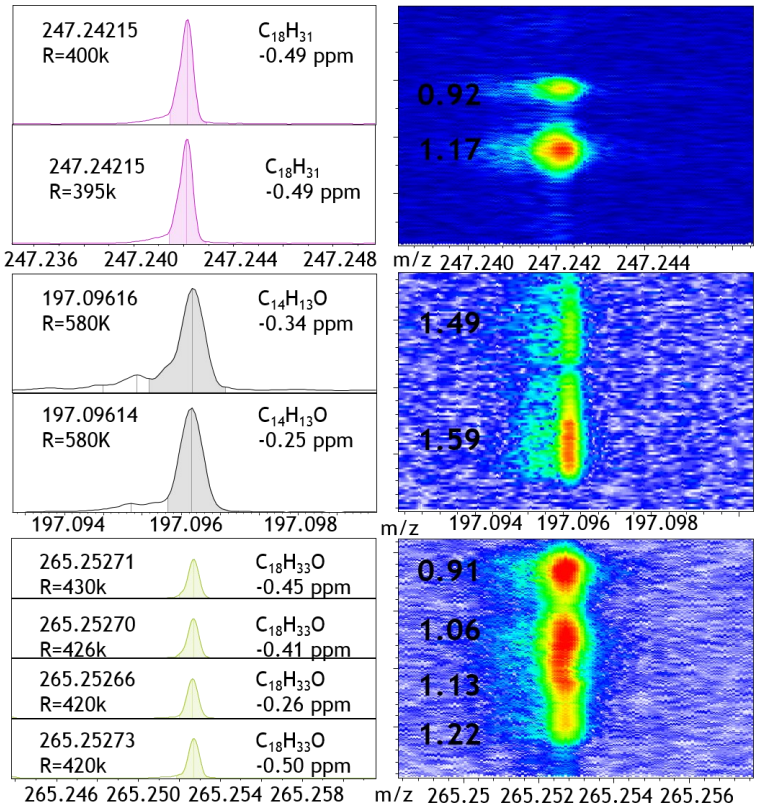
Trapped Ion Mobility Spectrometry

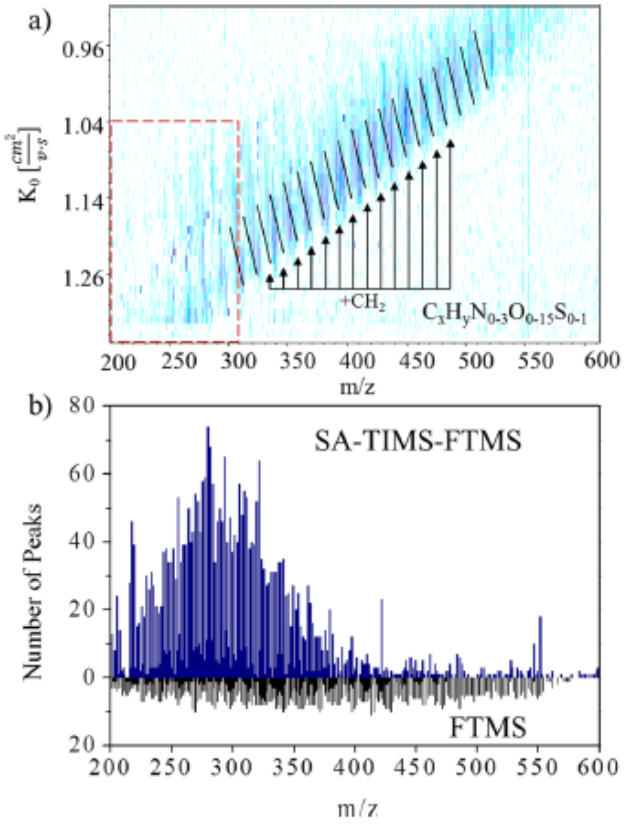


SA-TIMS Principles of operation



$$K = \frac{v_g}{E} = \frac{A}{V_{elu} - V_{out}}$$

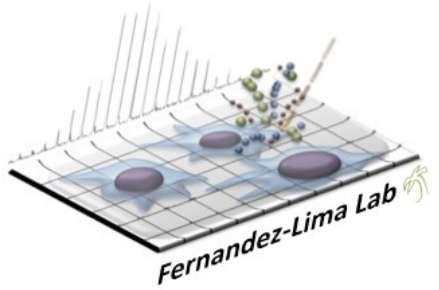




- SA-TIMS provides first-principles K measurements

P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. Anal. Chem. 87 (8), 4321–4325, 2015.

Trapped Ion Mobility Spectrometry



SA-TIMS Principles of operation

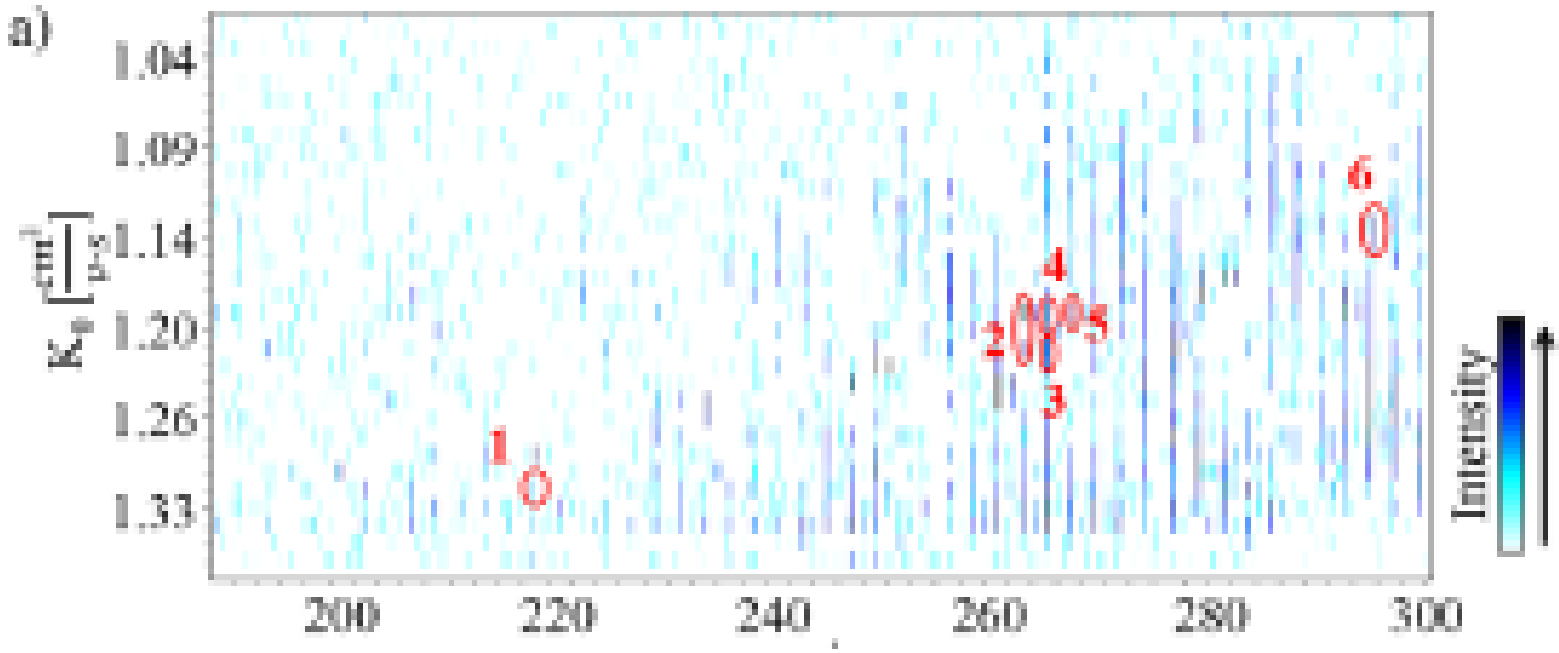


Figure. EDC compounds and SRFA standard

P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. Anal. Chem. 87 (8), 4321–4325, 2015.

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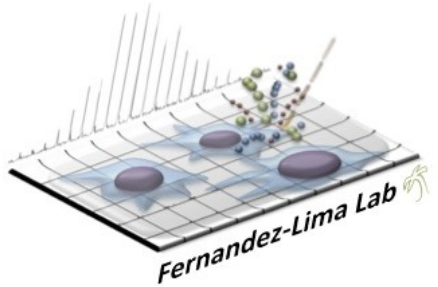
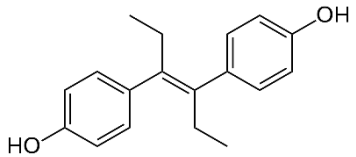
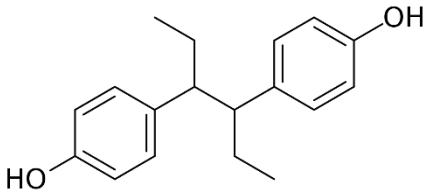


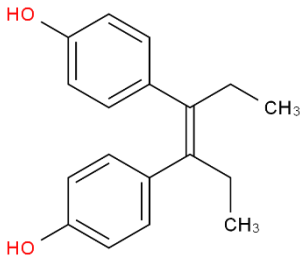
diethylstilbestrol (E)



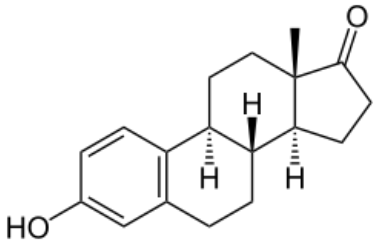
hexestrol



diethylstilbestrol (Z)



Estrone



cis/trans isomers

structural isomers

P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. Anal. Chem. 87 (8), 4321–4325, 2015.

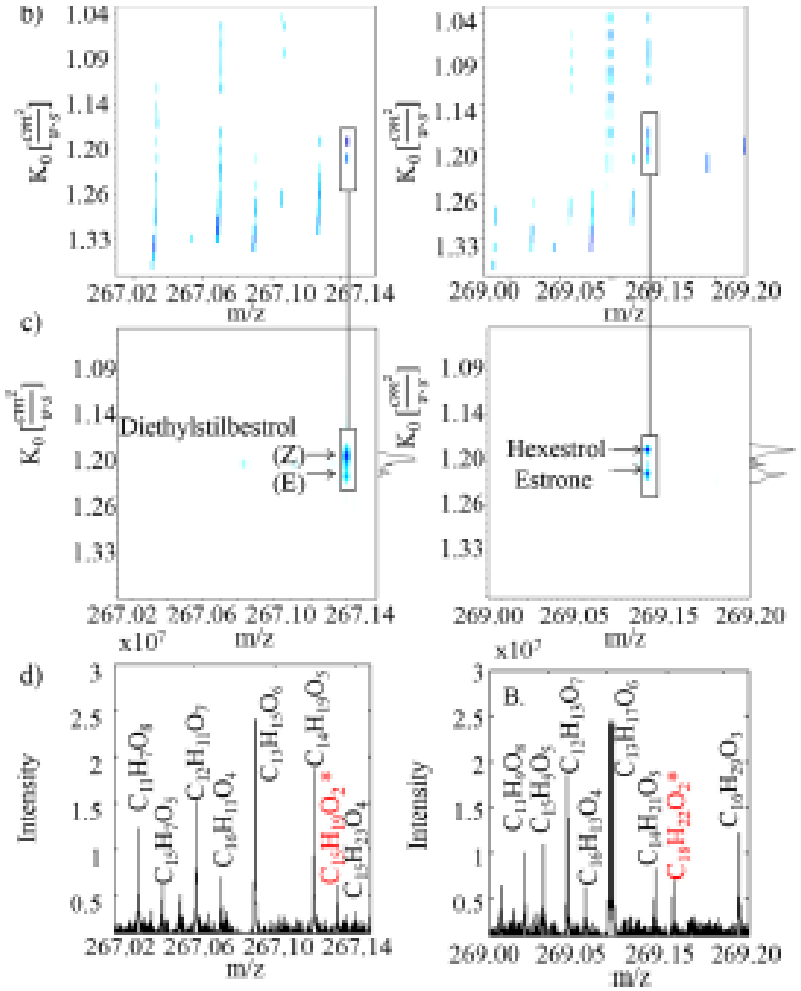
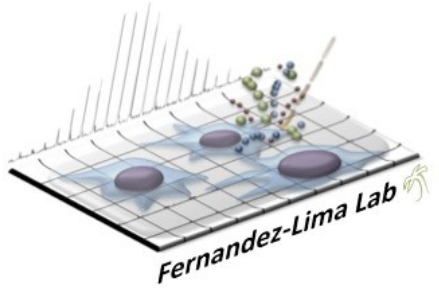
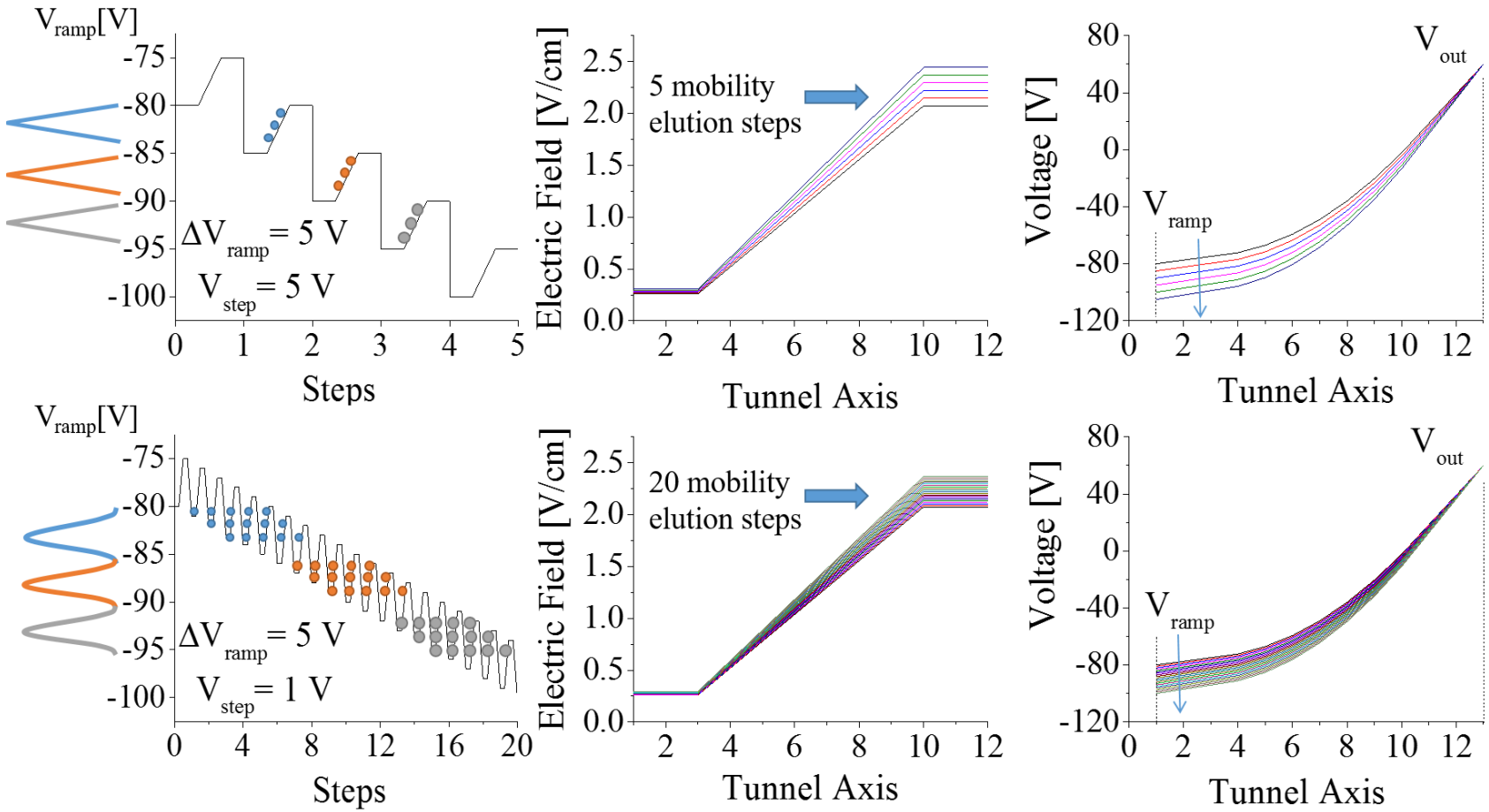


Figure. EDC compounds and SRFA standard

Trapped Ion Mobility Spectrometry

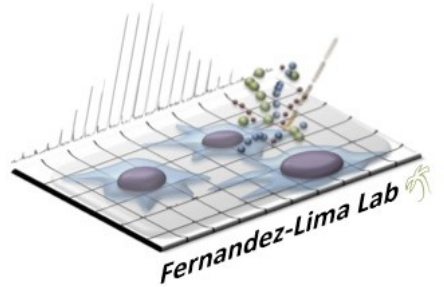
Oversampling Selective Accumulation TIMS-FT-ICR MS



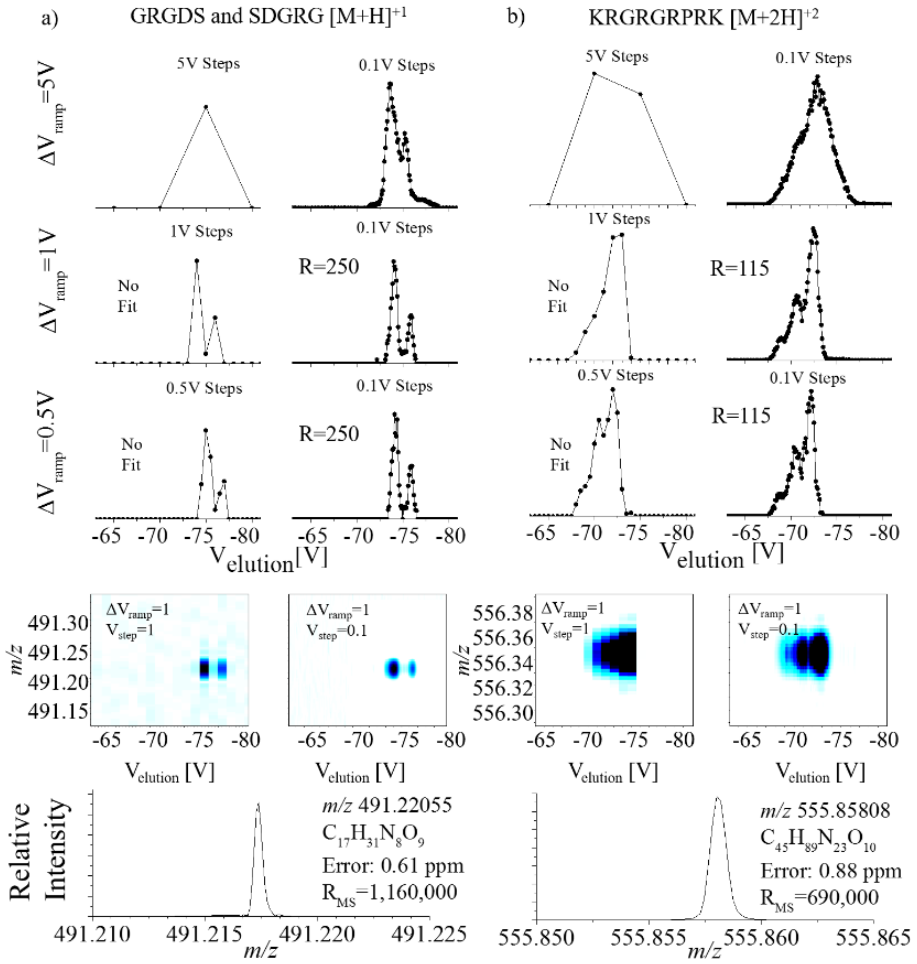


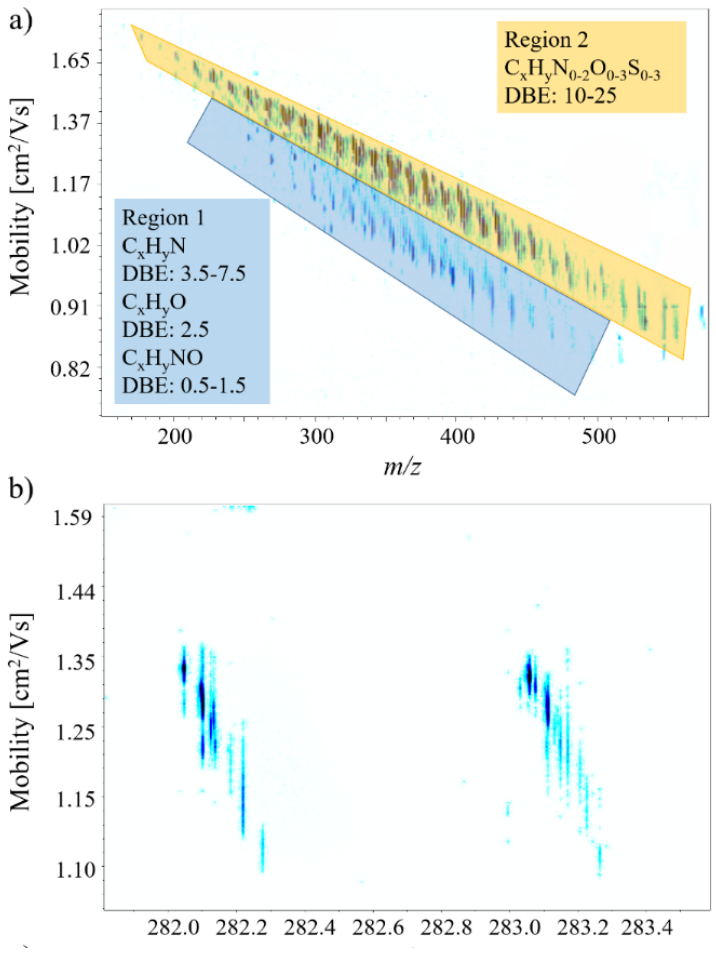
P. Benigni, F. Fernandez-Lima," Oversampling SA-TIMS coupled to FT-ICR MS: Fundamentals and Applications". Anal .Chem. 88 (14), 7404–7412, 2016.

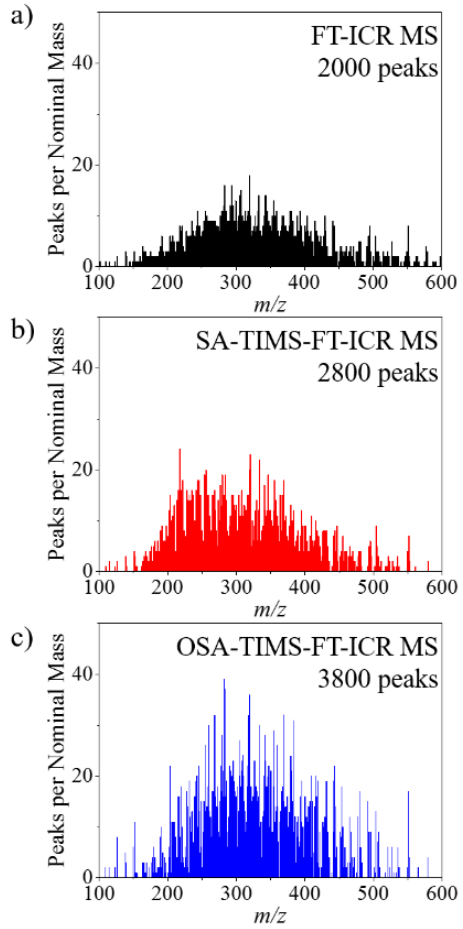
Trapped Ion Mobility Spectrometry



Oversampling Selective Accumulation TIMS-FT-ICR MS







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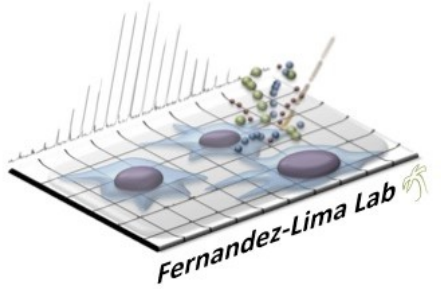
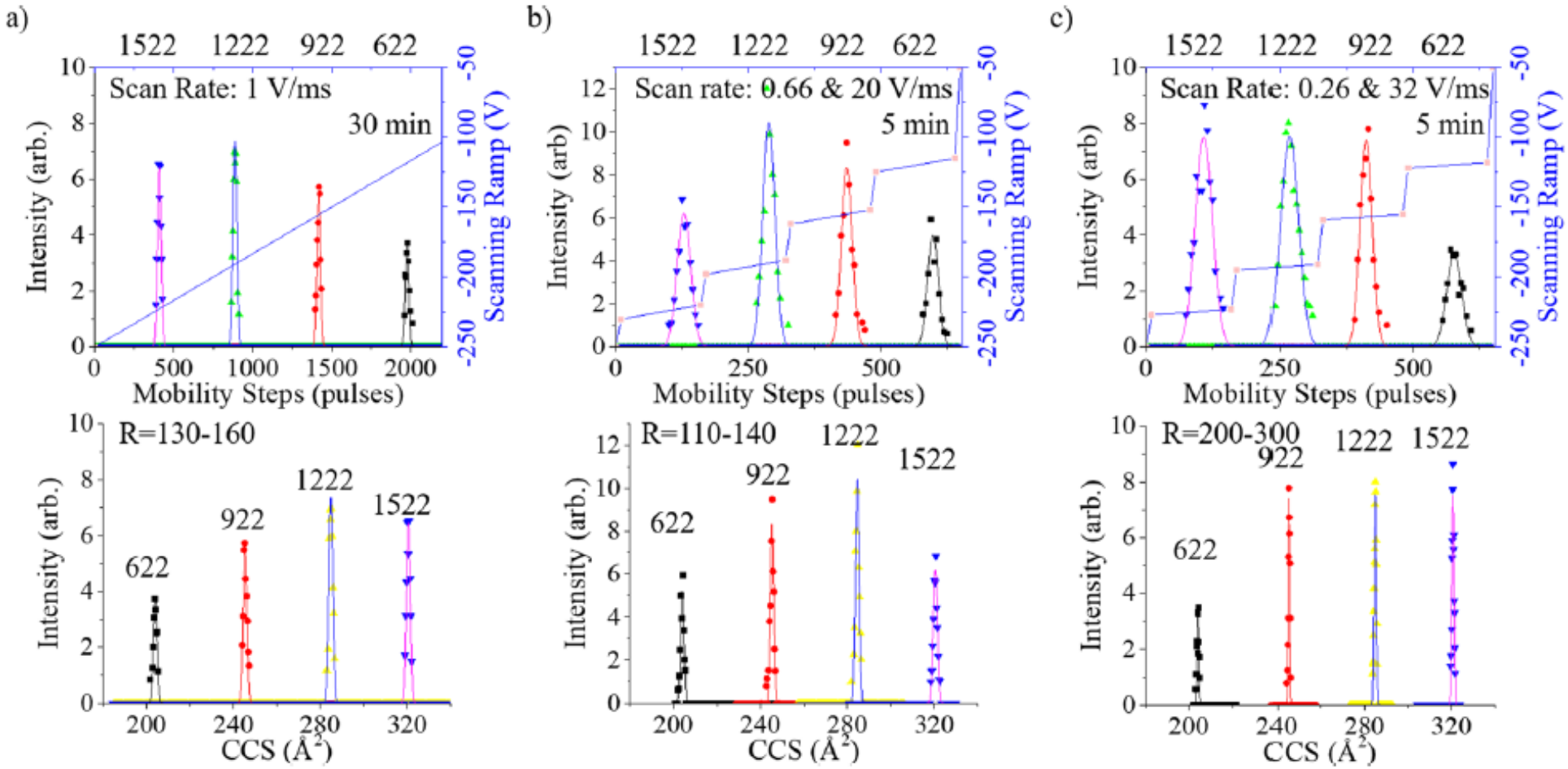
Trapped Ion Mobility Spectrometry

Non linear scan functions TIMS-FT-ICR MS

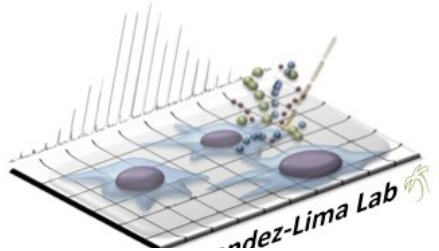


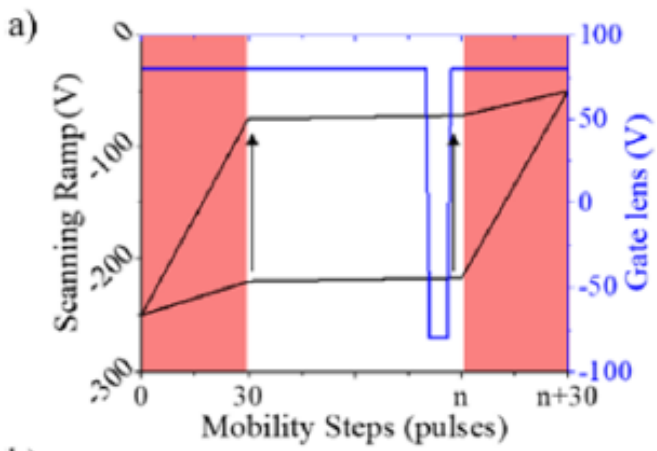


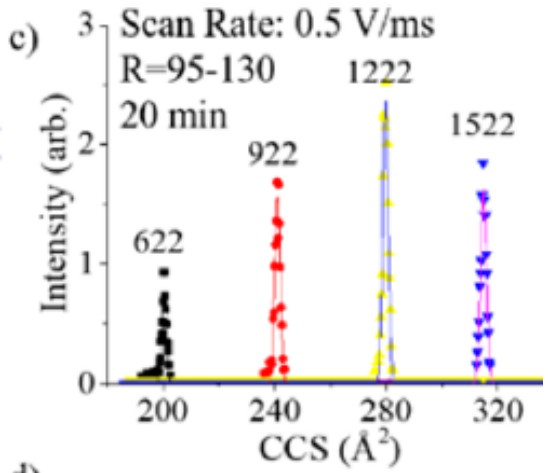
P. Benigni, J. Porter, M. E. Ridgeway, M. A. Park, F. Fernandez-Lima*; "Increasing analytical separation and duty cycle with non-linear analytical mobility scan functions in TIMS-FT-ICR MS". Anal Chem. 2018, 90 (4), 2446–2450.

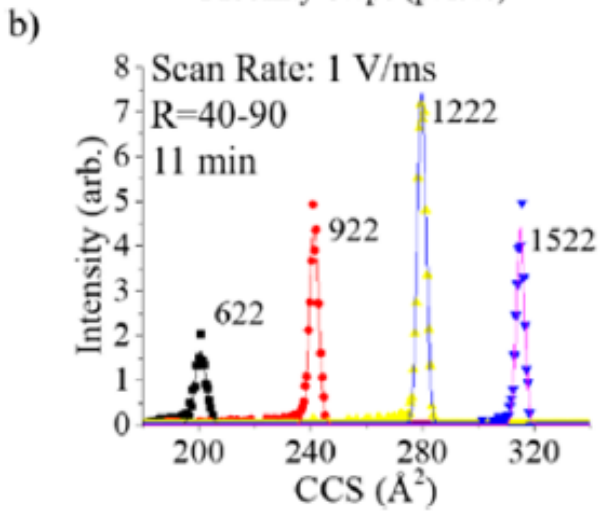
Trapped Ion Mobility Spectrometry

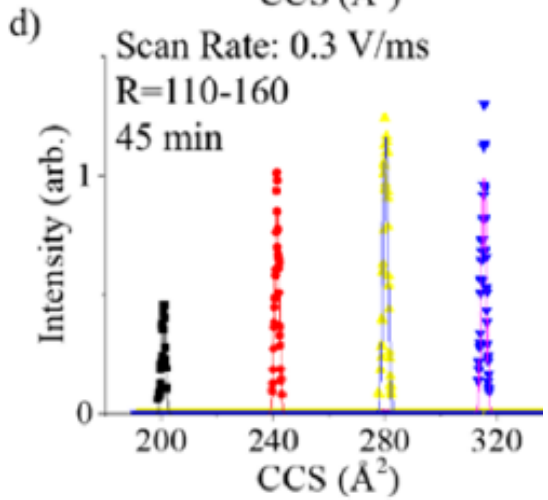
Non linear scan functions TIMS-FT-ICR MS

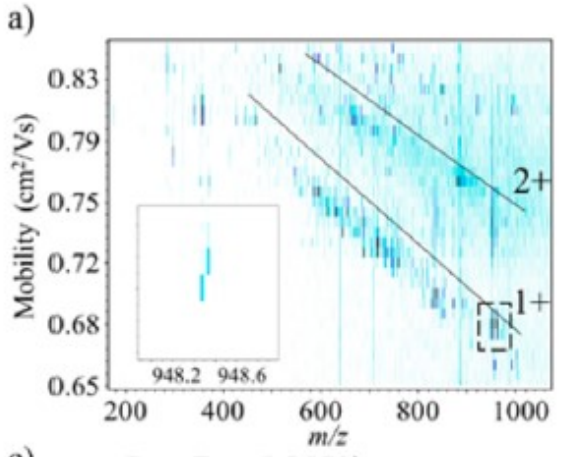


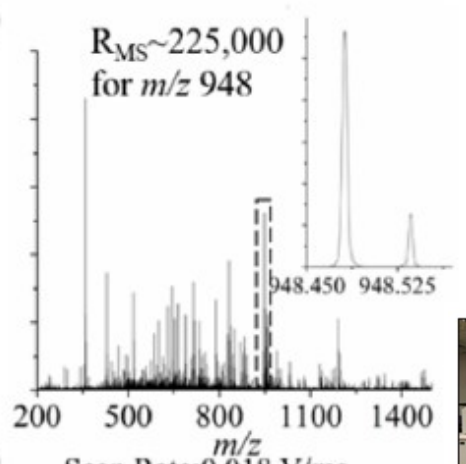


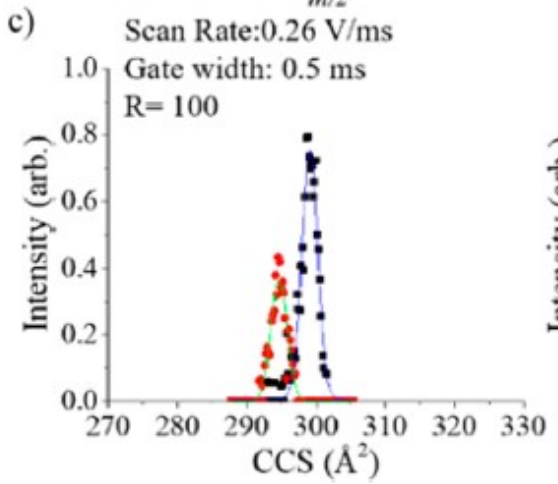


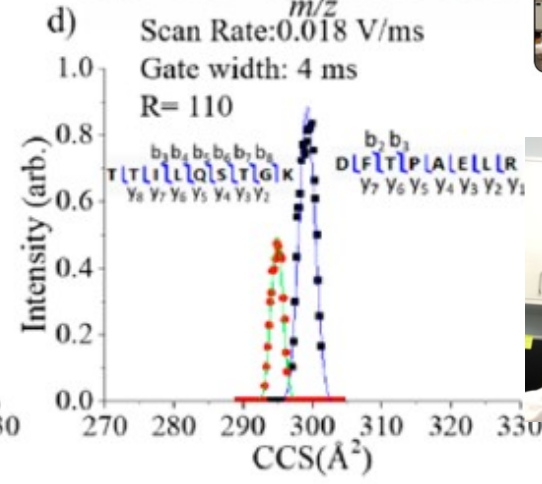


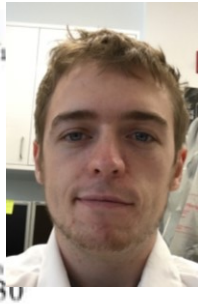












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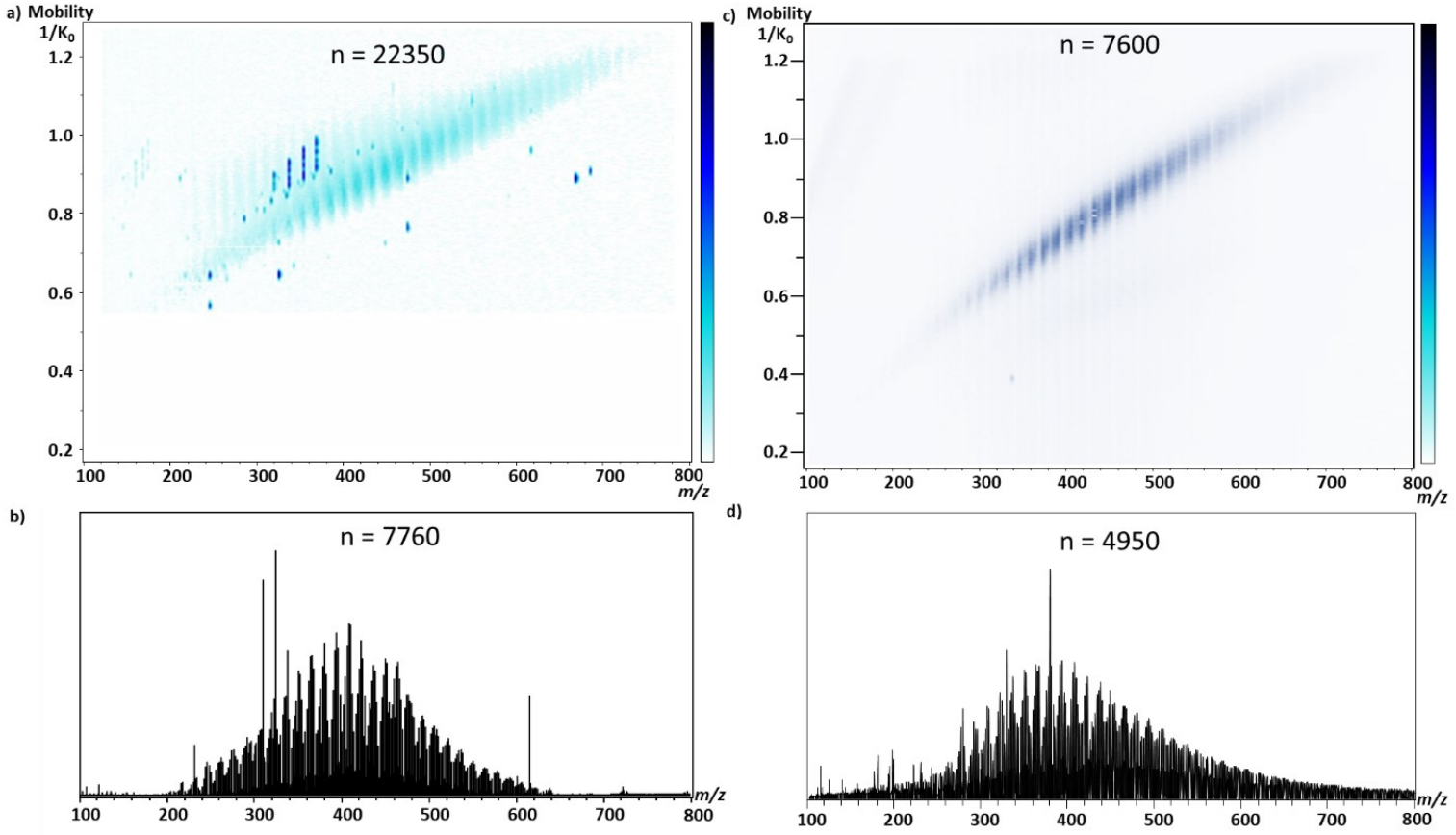
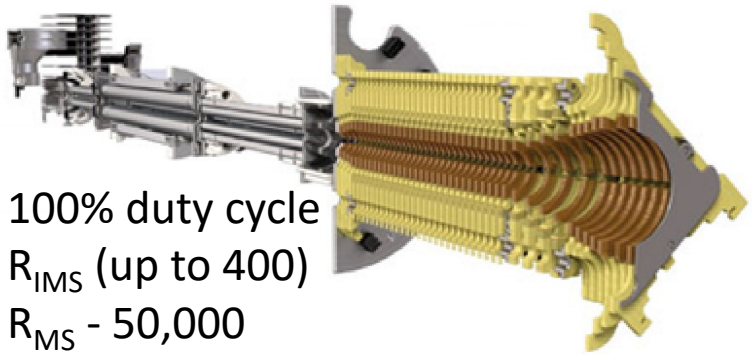
Trapped Ion Mobility Spectrometry

TIMS-FT-ICR MS



TIMS-TOF MS

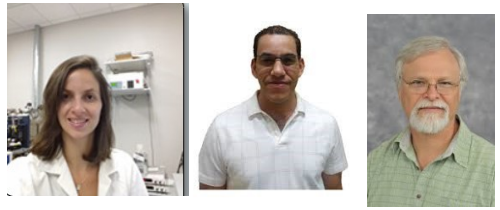
TIMS-q-TOF MS/MS



100% duty cycle
R_{IMS} (up to 400)
R_{MS} - 50,000

TIMS-q-FT-ICR MS/MS

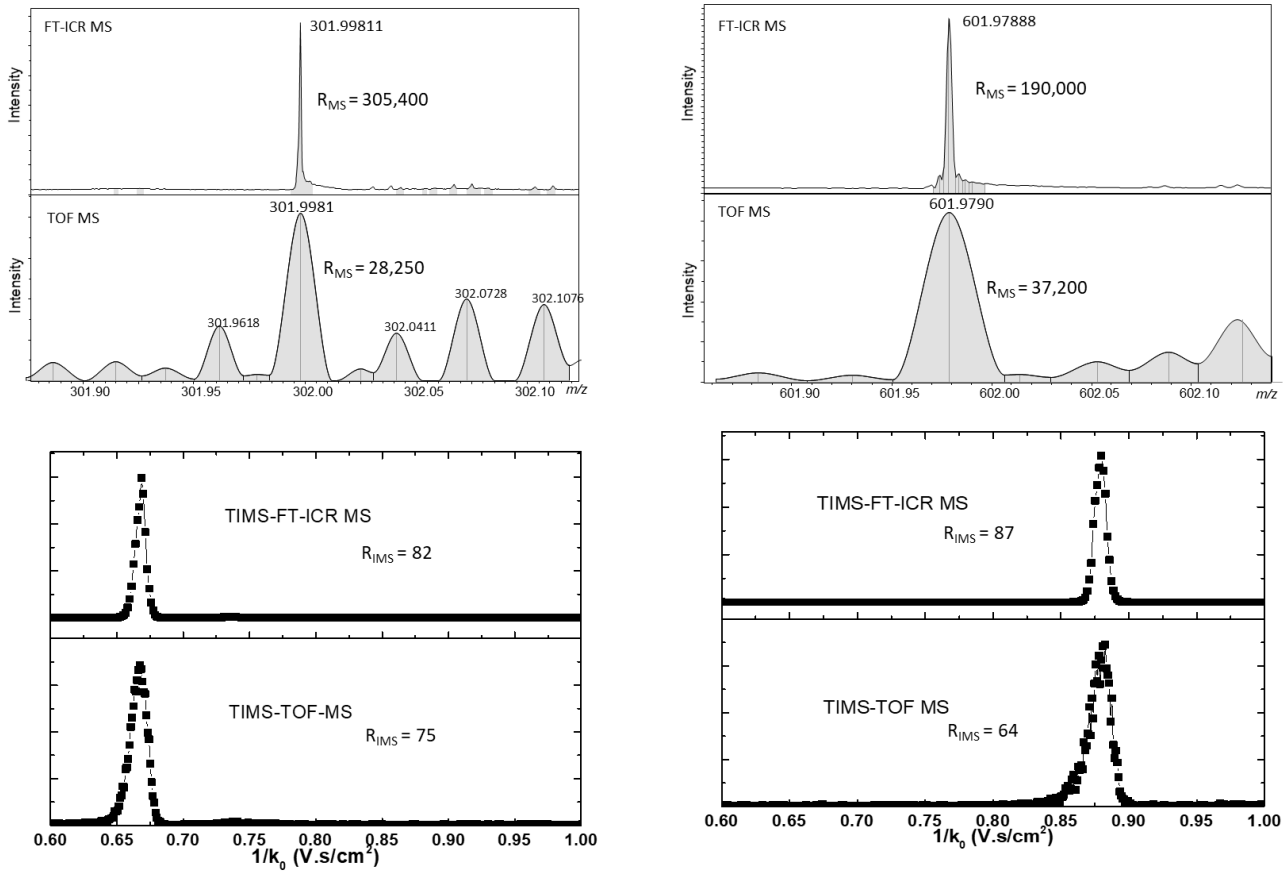
R_{IMS} (up to 400)
R_{MS} >400,000



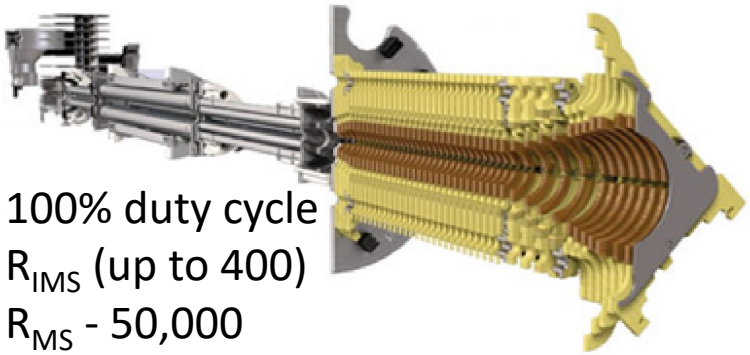
Suwannee River Fulvic Acid Standard II (SRFA) using negative ESI

L.V. Tose, P. Benigni, D. Leyva, A. Sundberg, C. E. Ramírez, M. E. Ridgeway, M. A. Park, Wanderson Romão, R. Jaffé, F. Fernandez-Lima, "Coupling Trapped Ion Mobility Spectrometry to Mass Spectrometry: TIMS-TOF MS vs TIMS-FT-ICR MS", *Rapid Comm. Mass Spectrom.* 2018, 32, 1287– 1295.

Analysis of Complex Mixtures without pre-separation



TIMS-q-TOF MS/MS



100% duty cycle
R_{IMS} (up to 400)
R_{MS} - 50,000

TIMS-q-FT-ICR MS/MS

R_{IMS} (up to 400)
R_{MS} >400,000

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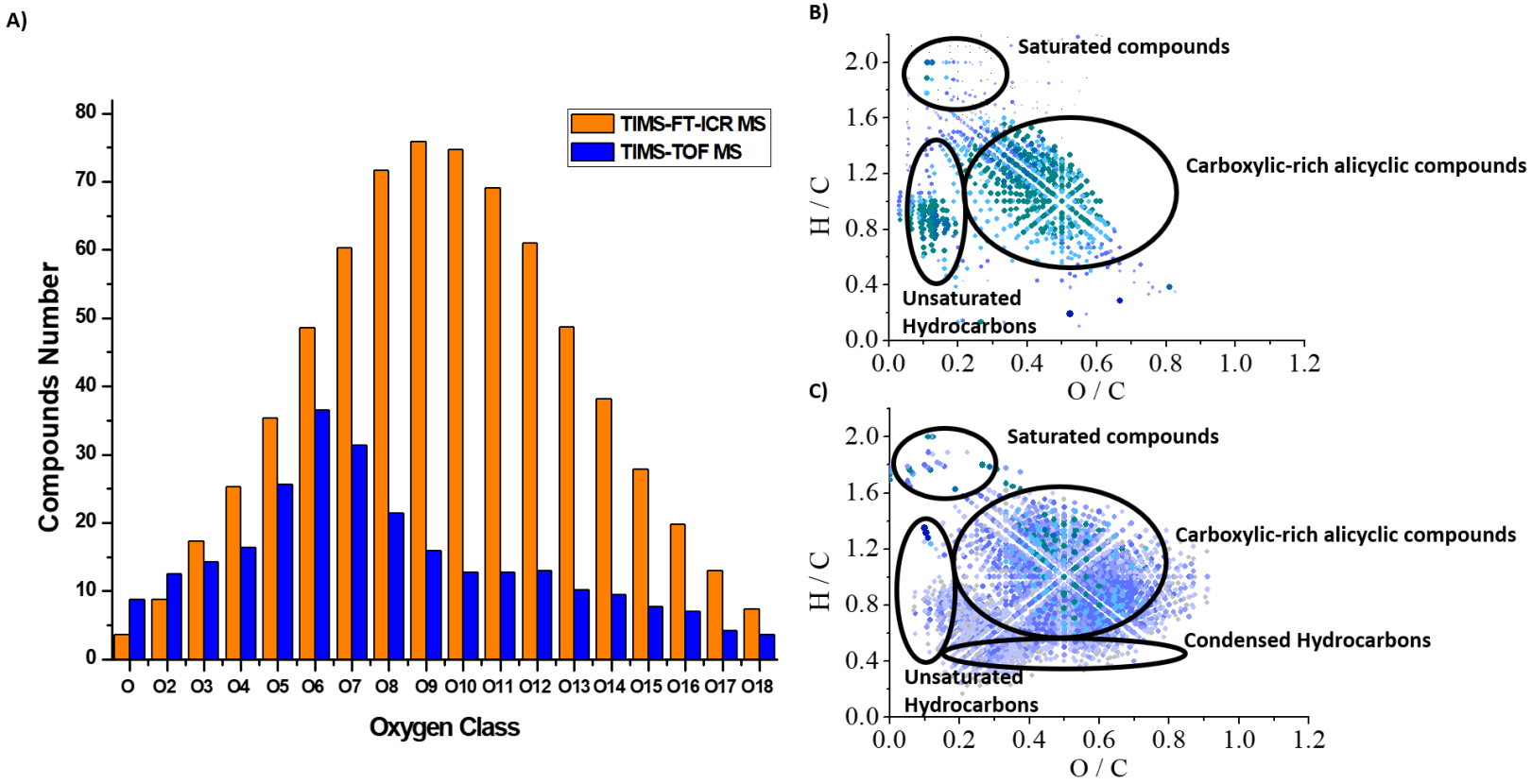
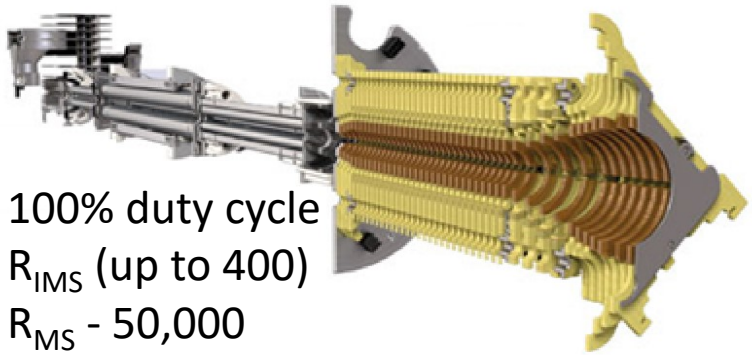


TIMS-q-TOF MS/MS



100% duty cycle
R_{IMS} (up to 400)
R_{MS} - 50,000

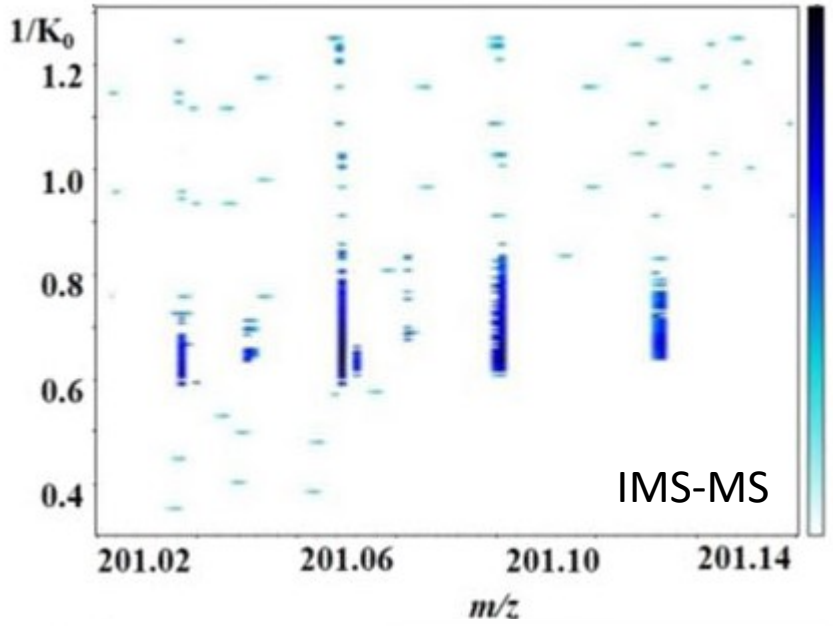
TIMS-q-FT-ICR MS/MS

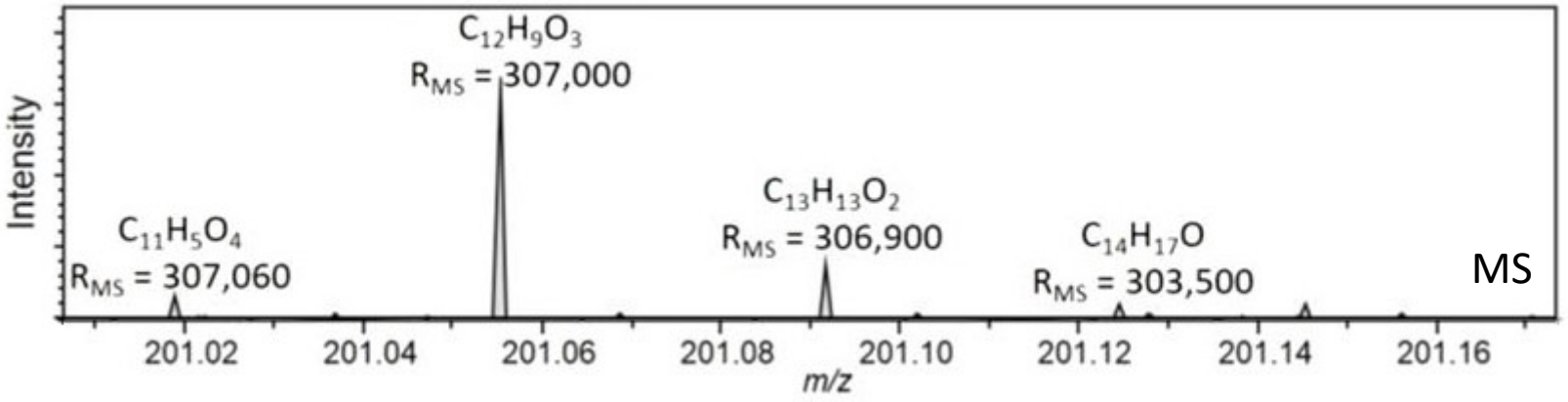
R_{IMS} (up to 400)
R_{MS} >400,000

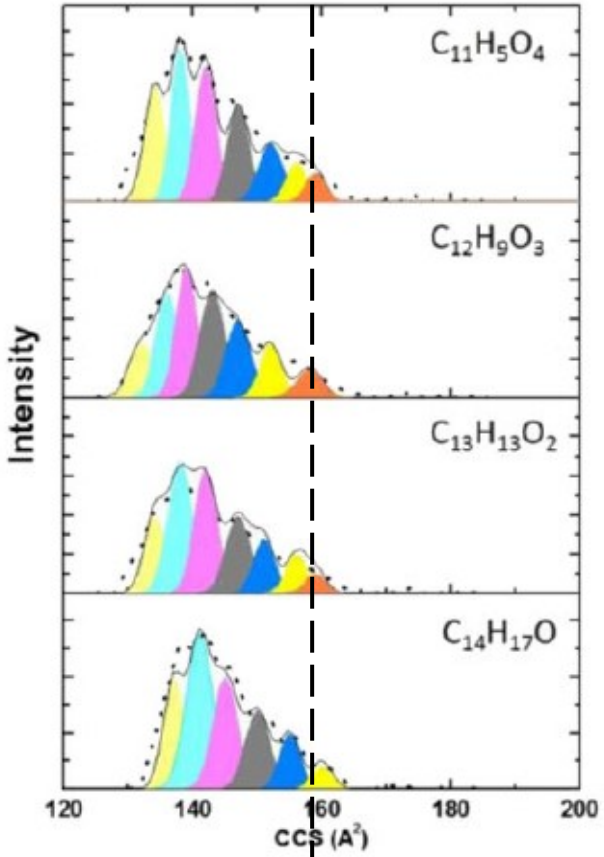
Suwannee River Fulvic Acid Standard II (SRFA) using negative ESI

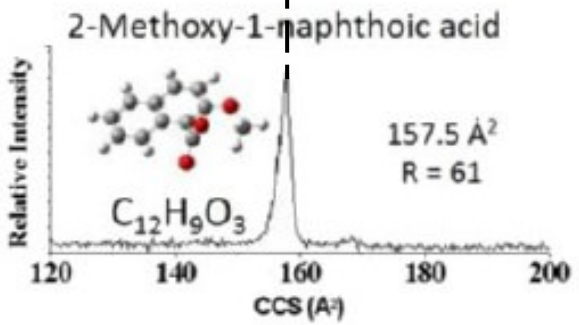
L.V. Tose, P. Benigni, D. Leyva, A. Sundberg, C. E. Ramírez, M. E. Ridgeway, M. A. Park, Wanderson Romão, R. Jaffé, F. Fernandez-Lima, "Coupling Trapped Ion Mobility Spectrometry to Mass Spectrometry: TIMS-TOF MS vs TIMS-FT-ICR MS", *Rapid Comm. Mass Spectrom.* 2018, 32, 1287– 1295.

Isomeric content in SRFA standard



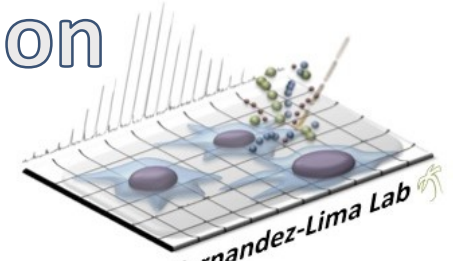




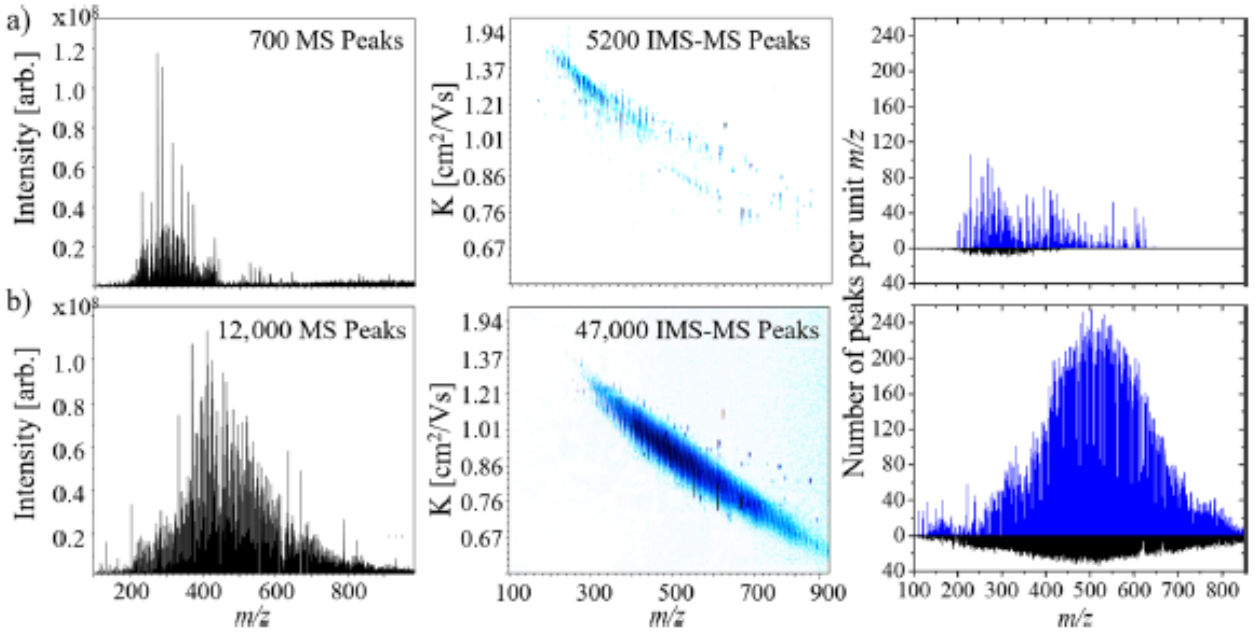


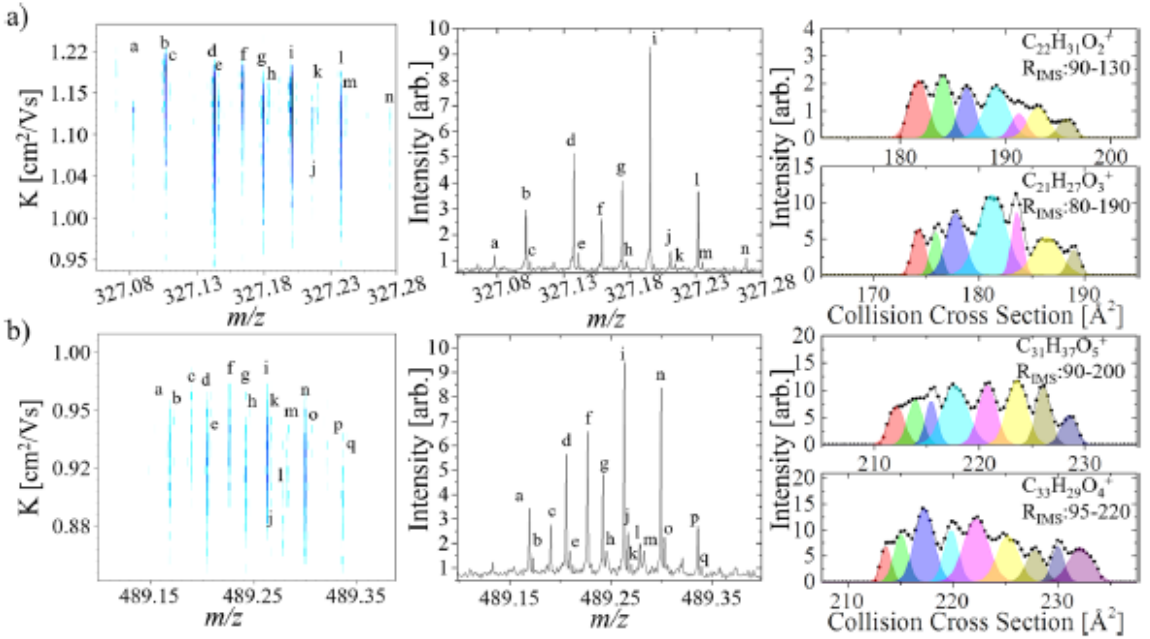
We provided a lower cutoff estimation of the number of molecular isomers in the SRFA standard

Analysis of Complex Mixtures without pre-separation



- Petroleomics (Water Accommodated Fraction)

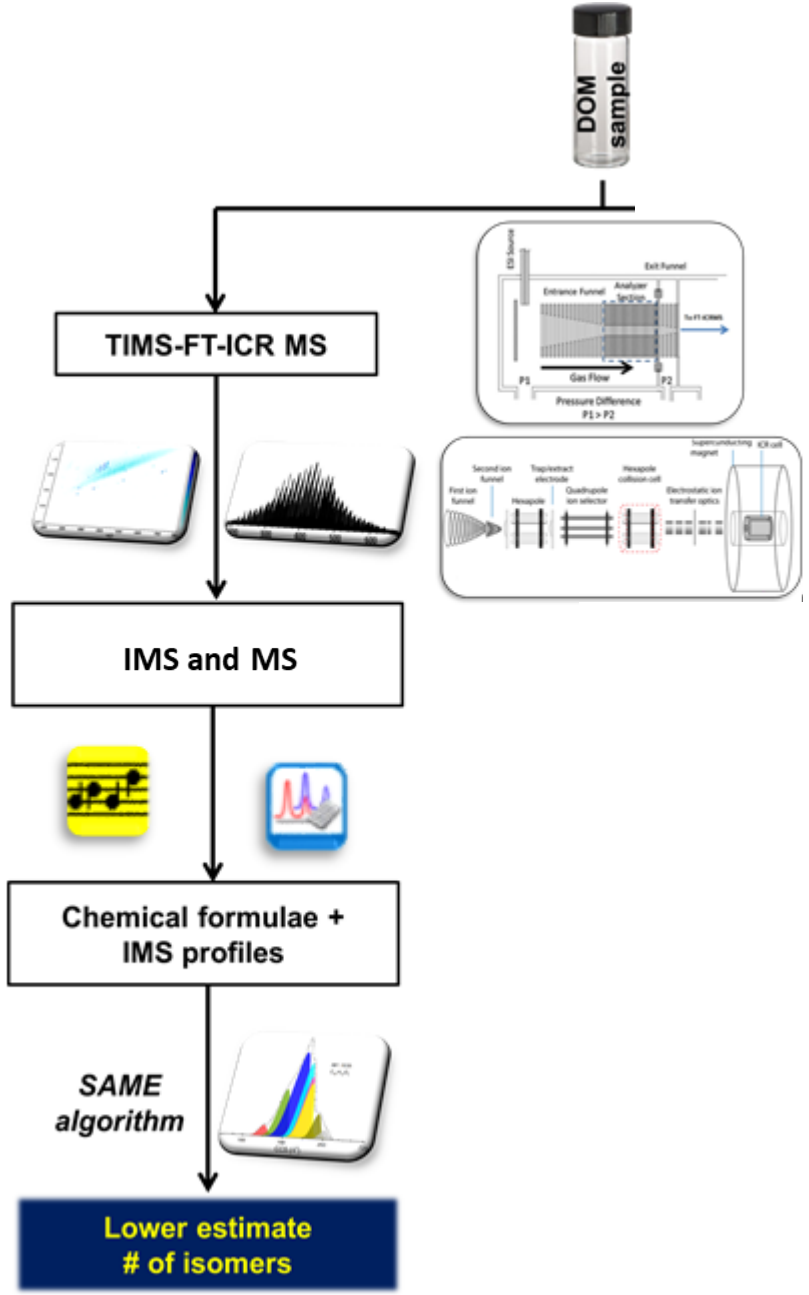




P. Benigni, K. Sandoval, C. J. Thompson, M. E. Ridgeway, M. A. Park, P. Gardinali, F. Fernandez-Lima," Analysis of Photo-irradiated Water Accommodated Fractions of Crude 2 Oils Using Tandem TIMS and FT-ICR MS", Environ. Sci. Technol., 51 (11), pp 5978–5988, 2017.

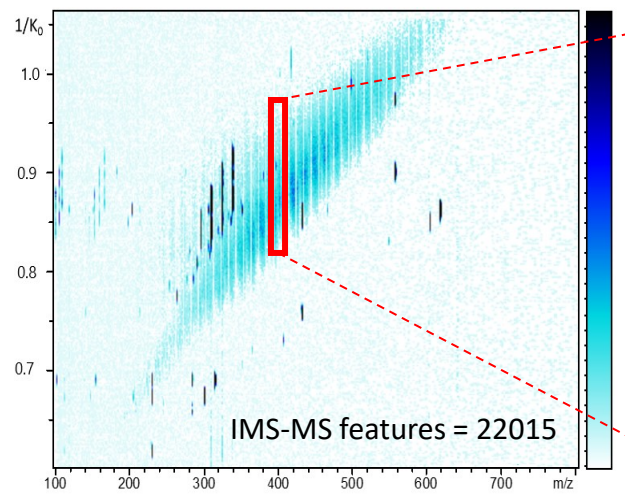
Isomeric complexity of DOM using TIMS-FT-ICR MS and MS/MS

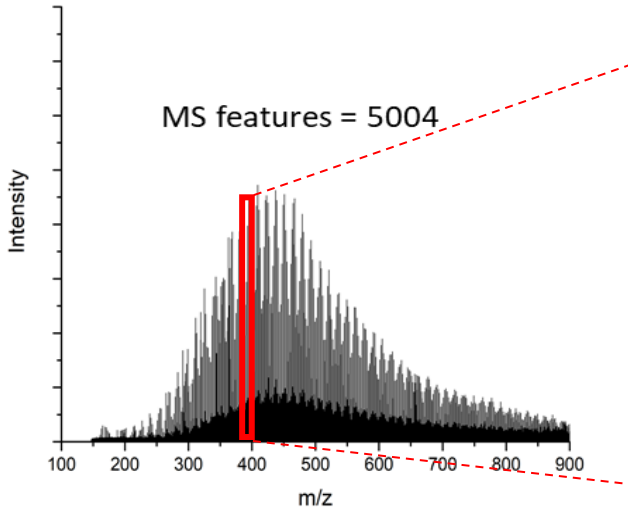
How can TIMS-FT-ICR MS help to understand the isomeric diversity of DOM?



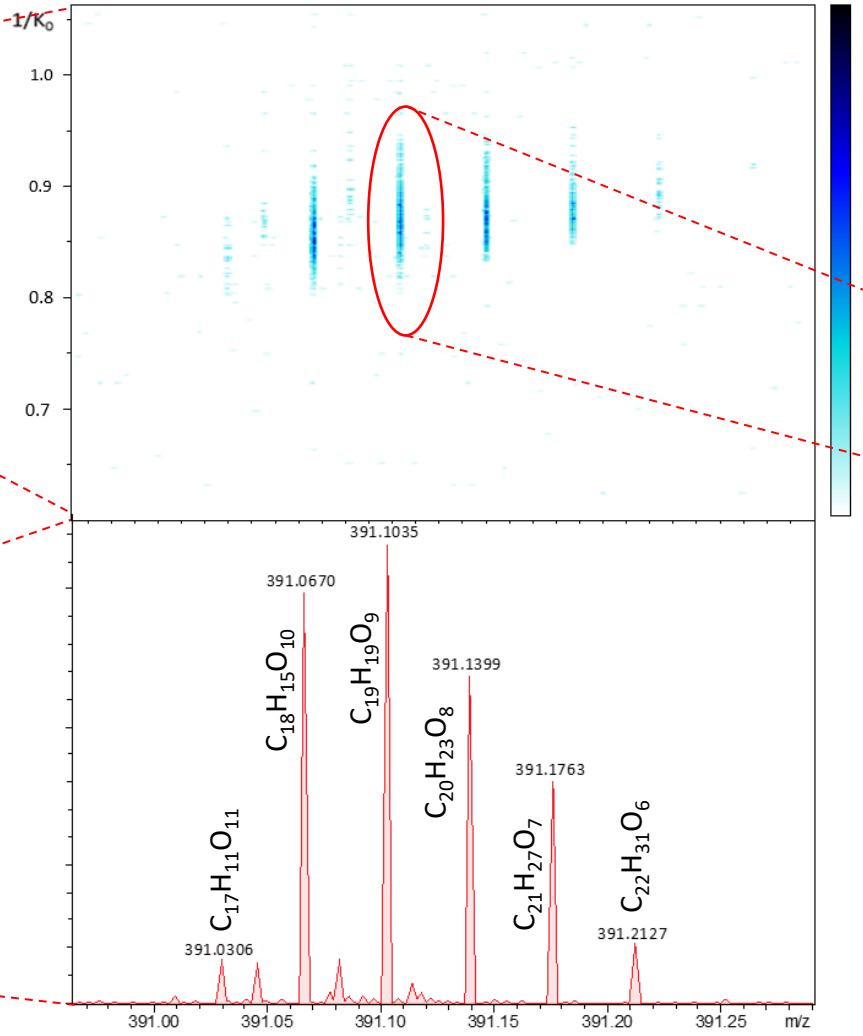
TIMS-FT ICR MS of SPE-DOM

More than 3,000 chemical formulas



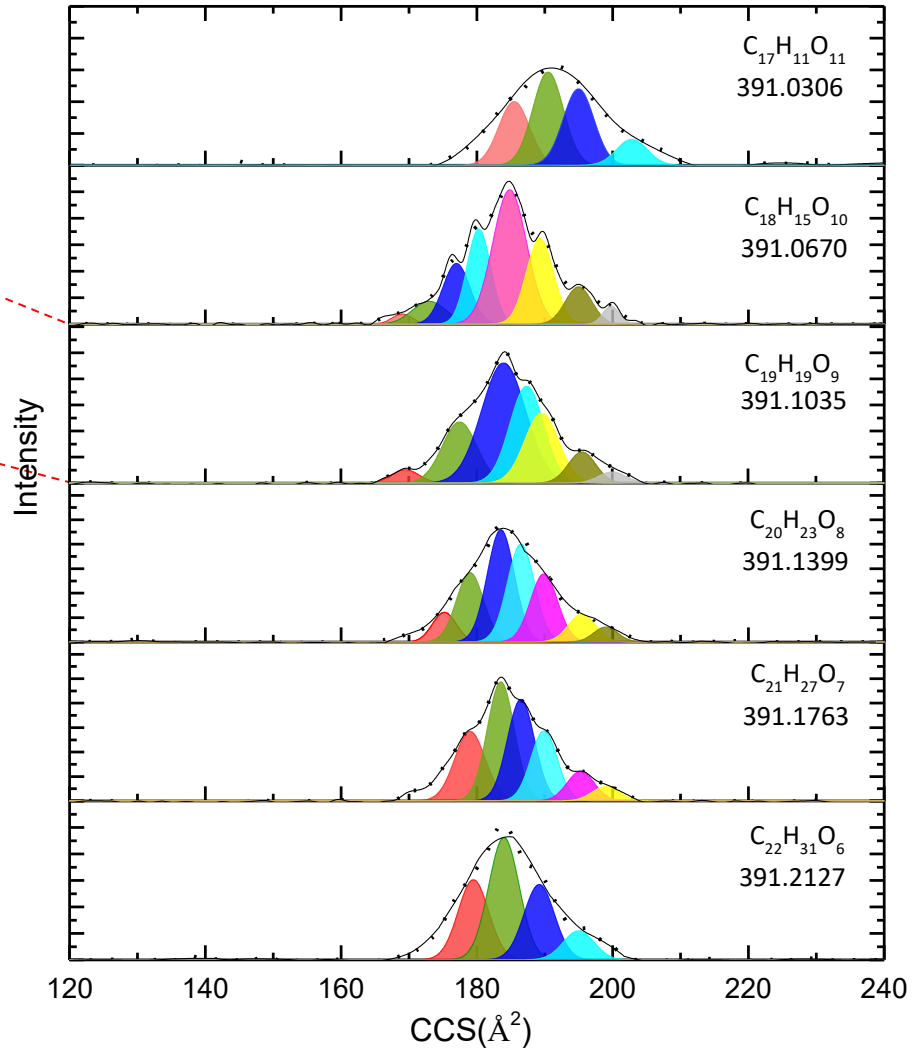


SPE-DOM from Pantanal, Brazil



2D IMS-MS and MS projection at m/z 391

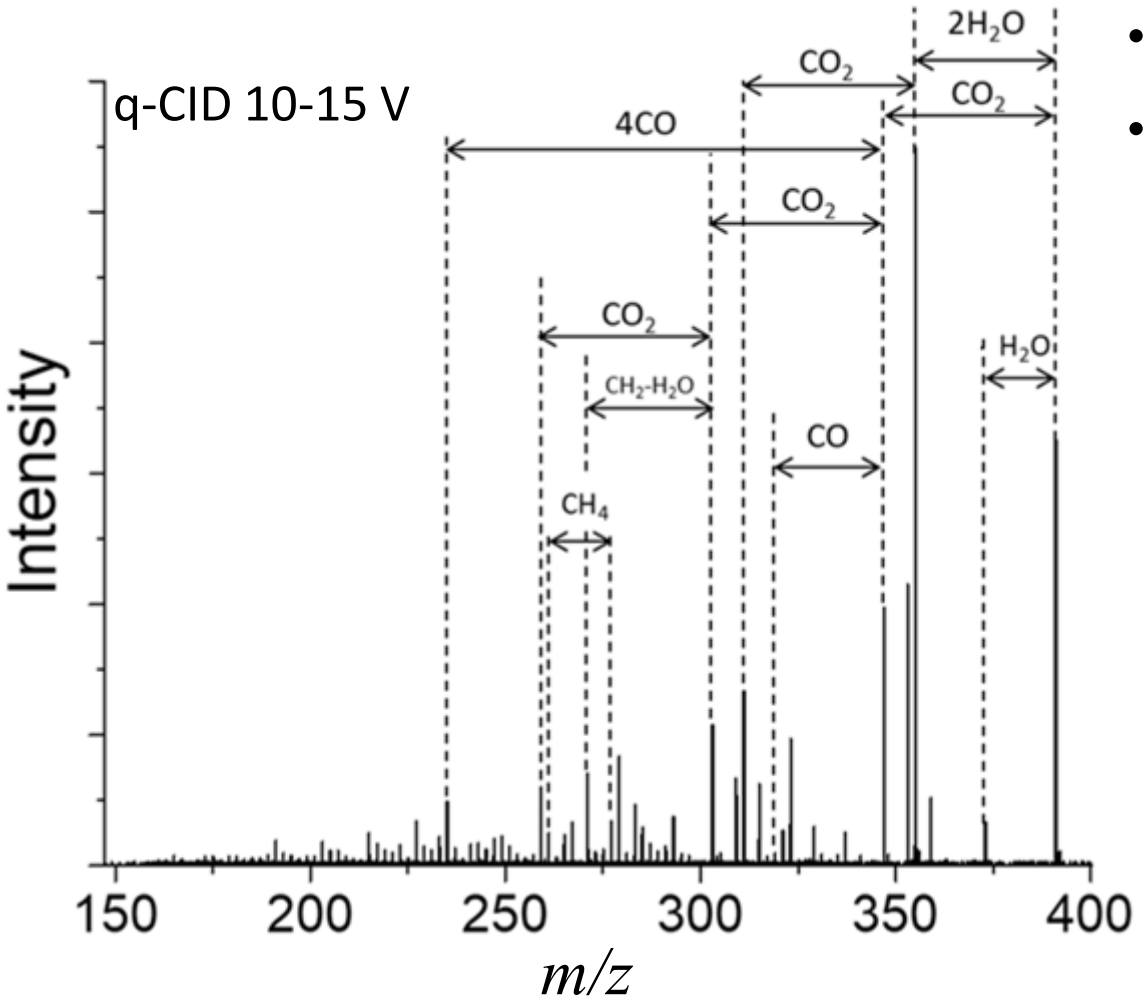
6-10 isomers per chemical formula



IMS projections of nominal mass 391

D. Leyva, et.al. *Faraday Discuss.* **2019,** *218* (0), 431-440.

MS/MS at nominal mass 391



- Neutral losses are associated with functional groups.
- The number of frag. pathways provides an estimate of the number of structural isomers.

Core fragment **Neutral loss fragments**

$[C_{19}H_{20}O_9\text{-}H]^-$

Precursor ion

m/z 391.1031

$[C_{10}H_9O_2]^- + \{5CH_2; CO; 3CO_2\}$

$[C_{12}H_7O_2]^- + \{2CH_3; O_2; CH_4; H_2O; 4CO\}$

$[C_{13}H_{11}O]^- + \{CH_4; 2H_2O; 4CO, CO_2\}$

⋮ ⋮

$[C_{10}H_3O_5]^- + \{7CH_2; O; H_2O; 2CO\}$

$[C_{13}H_5O_5]^- + \{2CH_2; 2CH_4; H_2O; CO; CO_2\}$

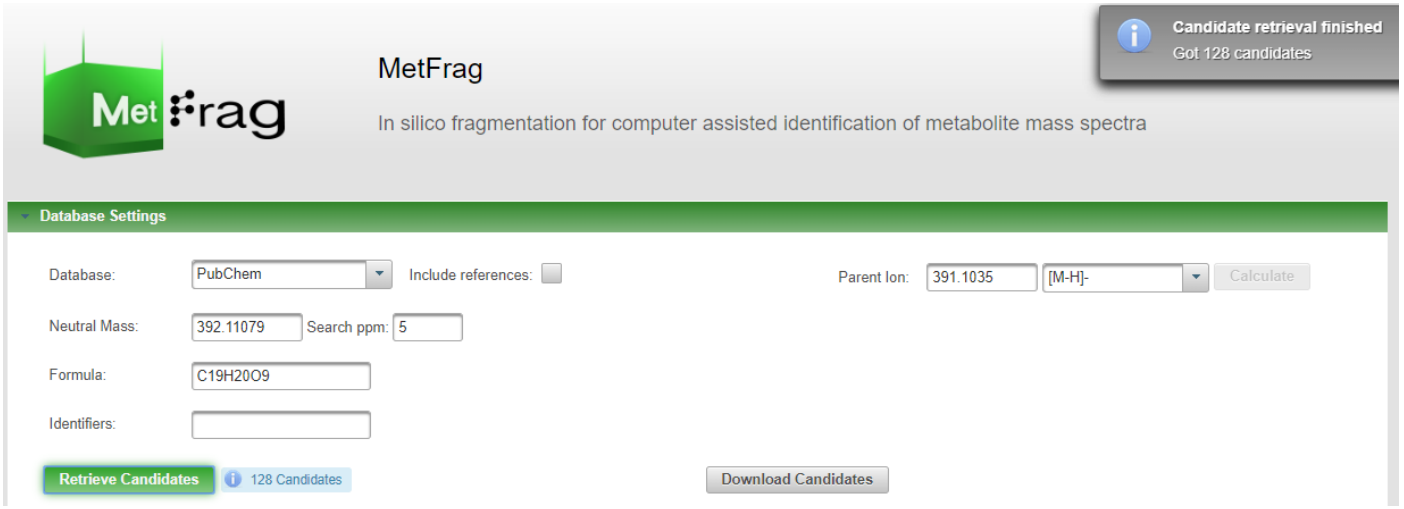
Core fragments and pathways for $C_{19}H_{19}O_9$

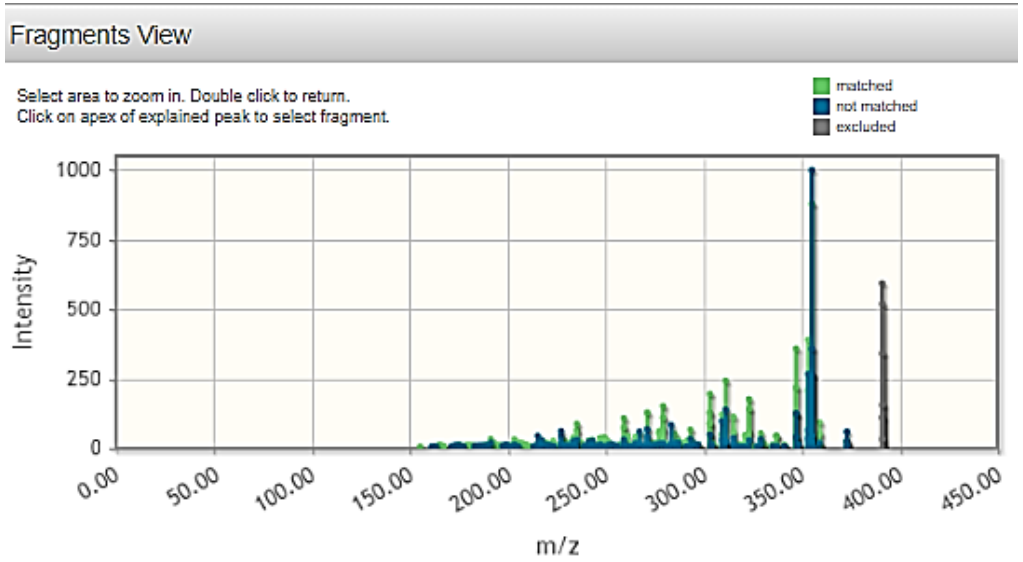
Potential neutral losses							Core fragment m/z	Number of pathways
CH_2	CH_3	O	CH_4	H_2O	CO	CO_2		
5	-	1	-	-	-	3		
2	-	-	1	1	4	1		
3	-	-	1	-	1	3		
1	2	-	-	1	4	1		
2	2	-	-	-	1	3		
4	-	-	-	1	2	2		
4	-	2	-	1	4	-		
-	-	-	2	1	6	-		
5	-	2	-	-	1	2		
-	2	1	1	-	4	1		
1	-	1	2	-	4	1		
5	-	3	-	-	2	1	173.0607 $C_{11}H_9O_2$	23
5	-	4	-	-	3	-		
1	-	-	2	-	3	2		
2	2	1	-	-	2	2		
3	-	1	1	-	2	2		
3	-	2	1	-	3	1		
2	2	2	-	-	3	1		
1	2	1	-	1	5	-		
2	-	1	1	1	5	-		
-	2	-	1	-	3	2		
3	-	-	-	2	5	-		
4	-	1	-	1	3	1		

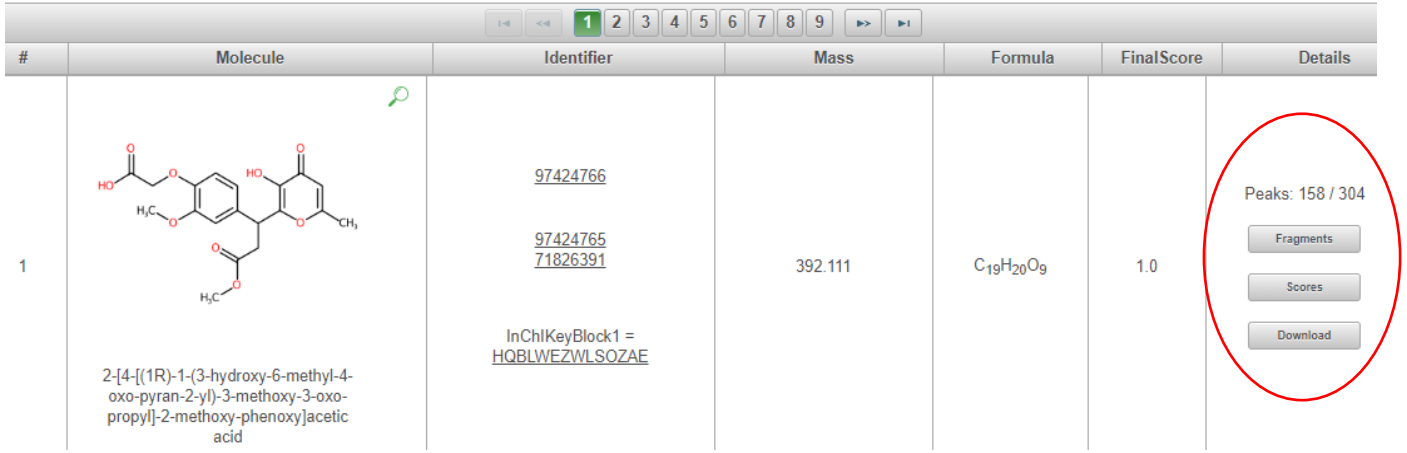
Precursor ion m/z	Core Fragment m/z	Structural isomers
391.1031 $C_{19}H_{19}O_9$	161.0607 $C_{10}H_9O_2$	13
	163.0763 $C_{10}H_{11}O_2$	7
	165.0192 $C_8H_5O_4$	3
	165.056 $C_9H_9O_3$	2
	167.0349 $C_8H_7O_4$	1
	171.0814 $C_{12}H_{11}O$	23
	173.0607 $C_{11}H_9O_2$	23
	175.0400 $C_{10}H_7O_3$	15
	183.0450 $C_{12}H_7O_2$	40
	183.0814 $C_{13}H_{11}O$	25
	185.0607 $C_{12}H_9O_2$	29
	187.0400 $C_{11}H_7O_3$	25
	201.0192 $C_{11}H_5O_4$	25
	202.9984 $C_{10}H_3O_5$	15
	205.0140 $C_{10}H_5O_5$	7
	241.0140 $C_{13}H_5O_5$	7

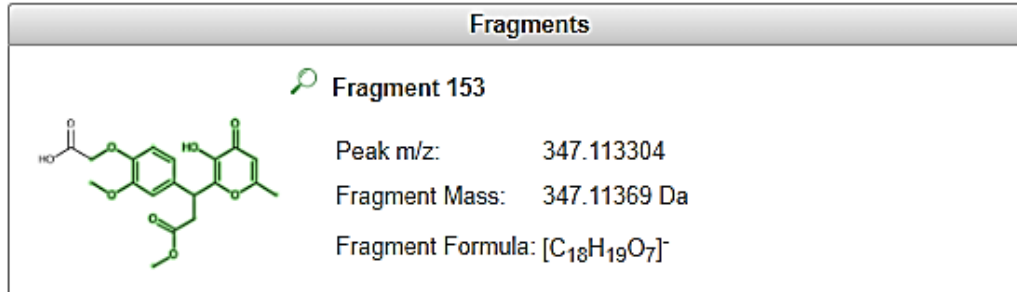
Rapid upper estimate of 260 structural isomers

In-silico fragmentation



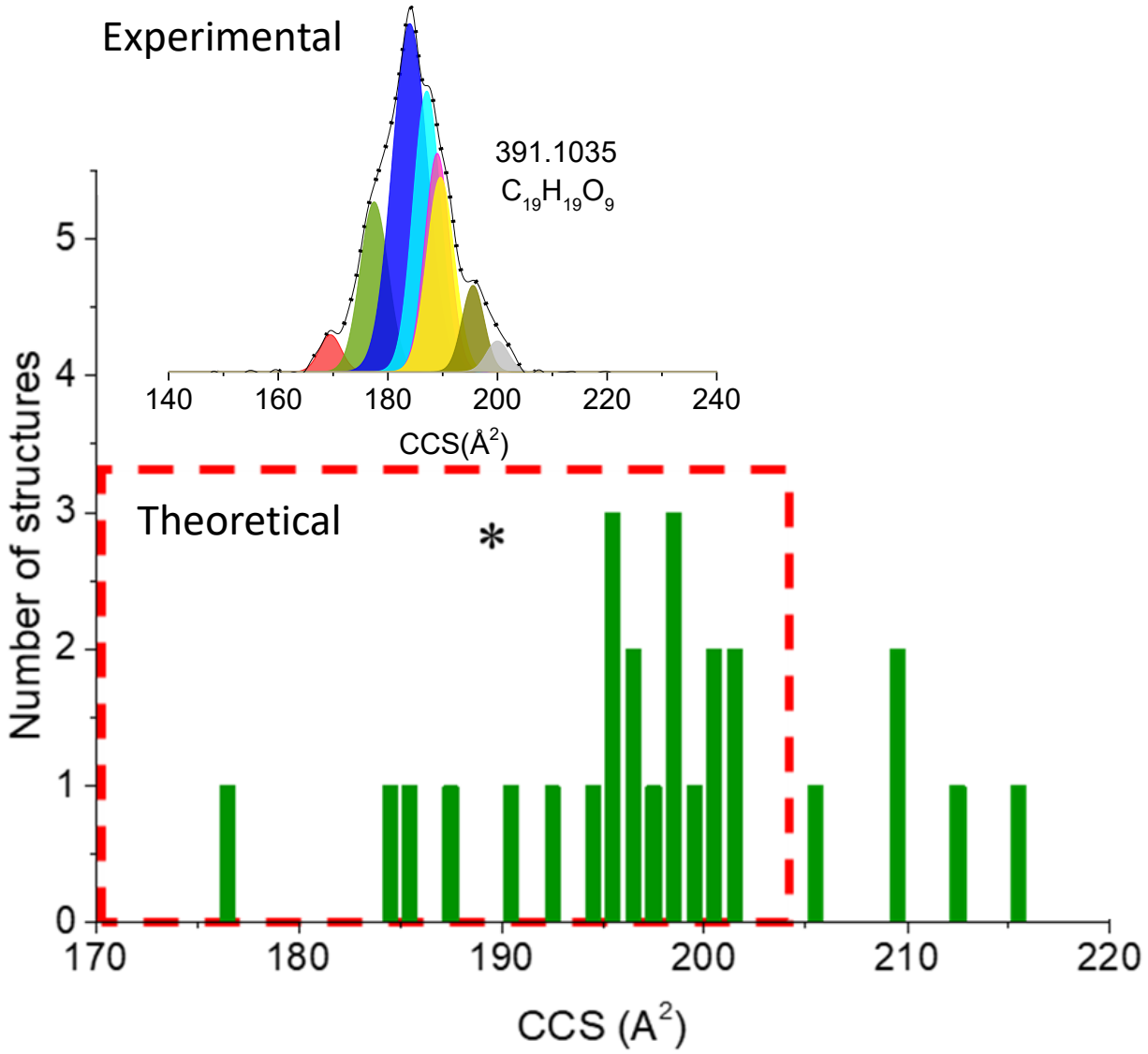


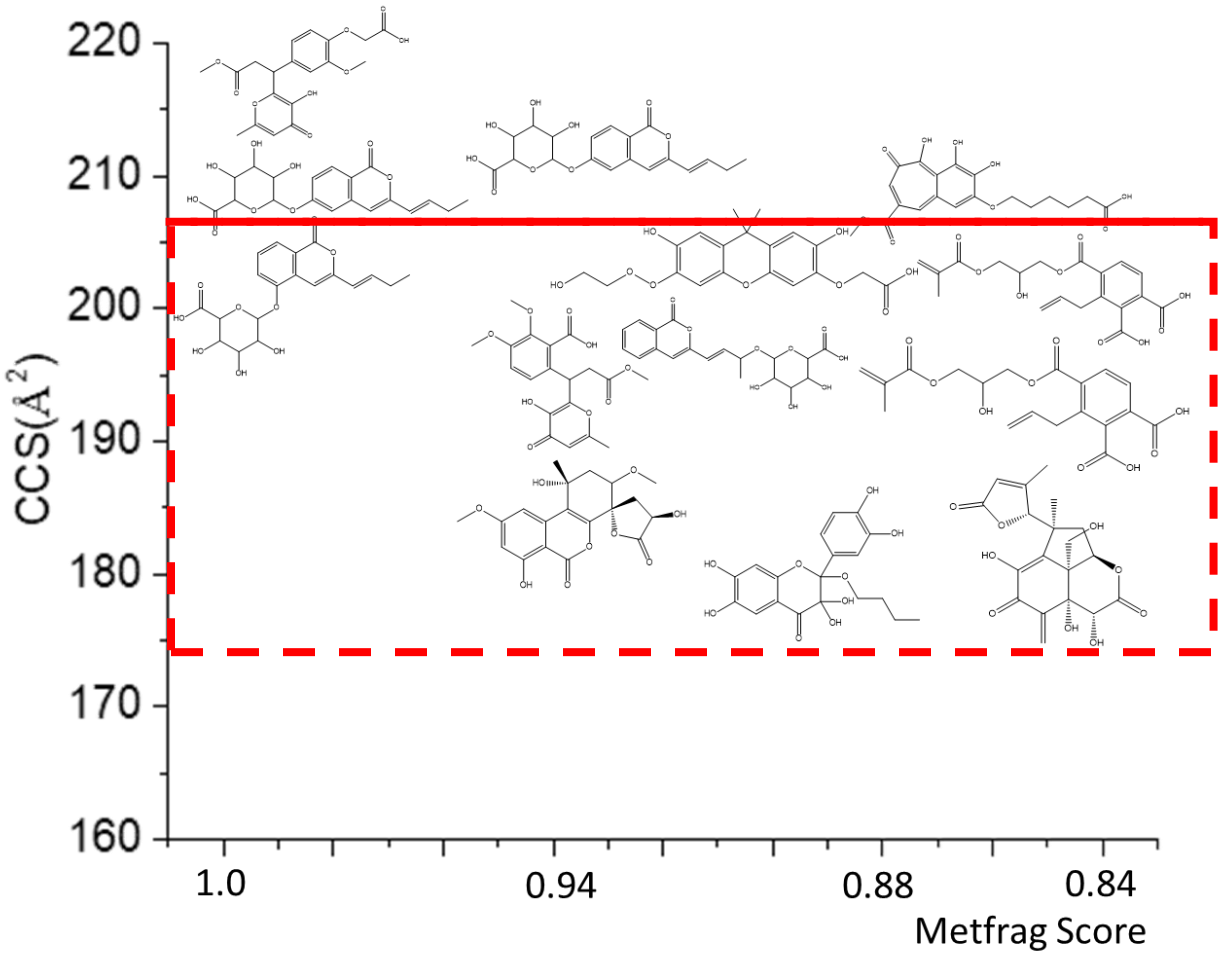




96 candidate structures matched in PubChem for $C_{19}H_{19}O_9$

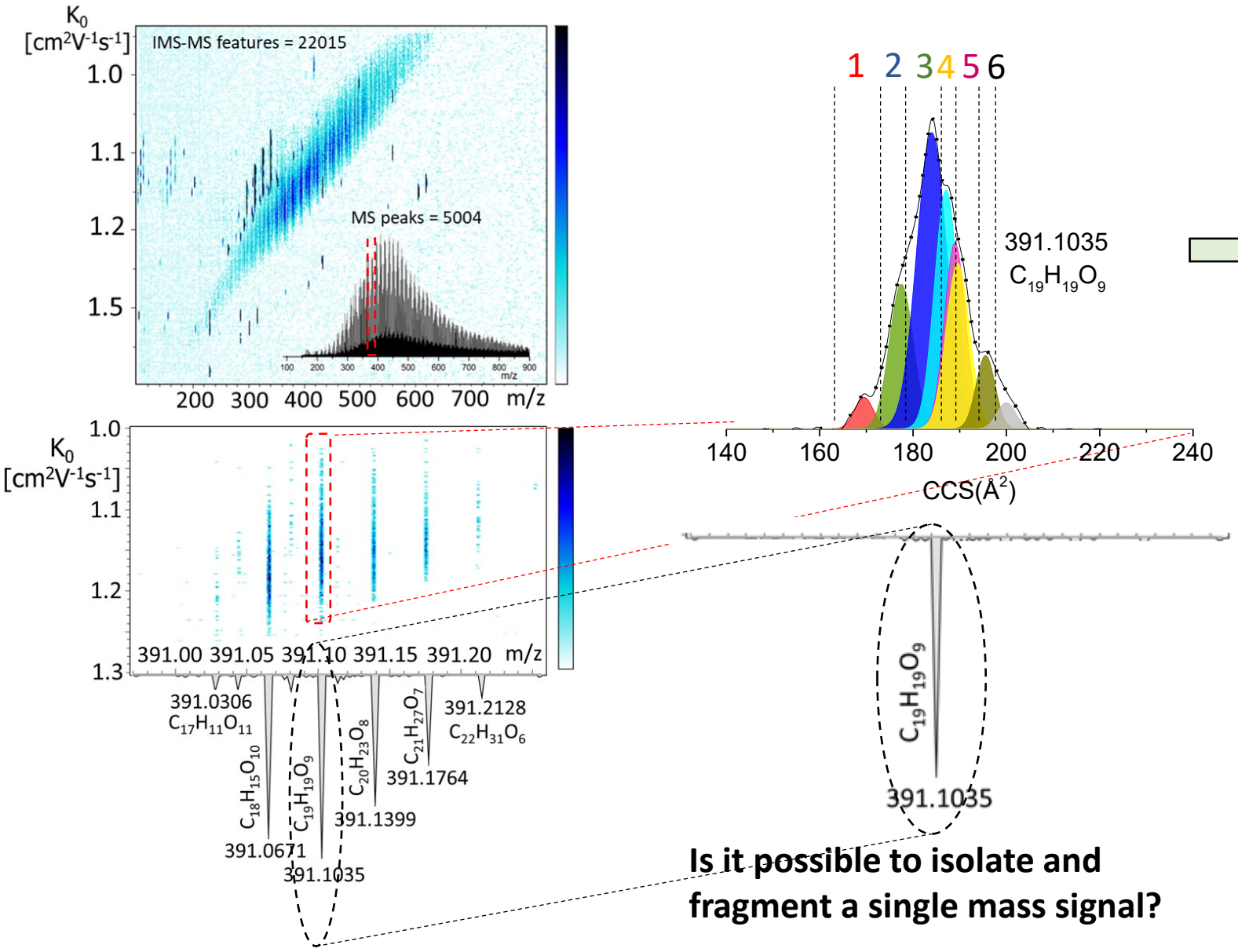
Filtering isomeric candidate structures





* Structures within 2 % of the experimental CCS range

How can we improve confidence during DOM structural assignment?



Is it possible to isolate and fragment a single mass signal?

MetFrag

PubChem

IMS1→ MS/MS$_1$ → Structures(1)

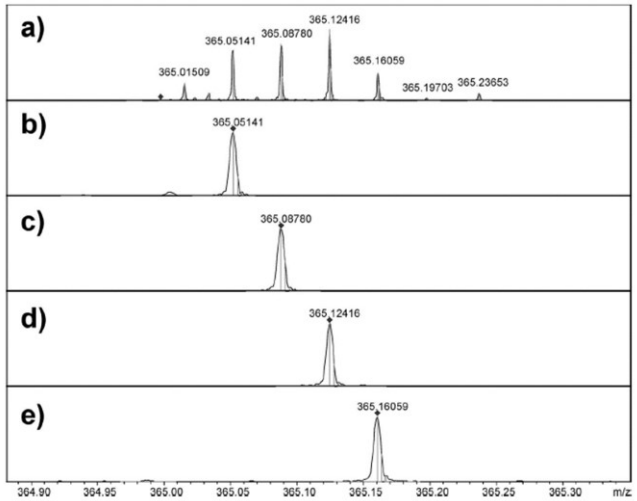
IMS2 → MS/MS$_2$ →Structures (2)

.

.

.

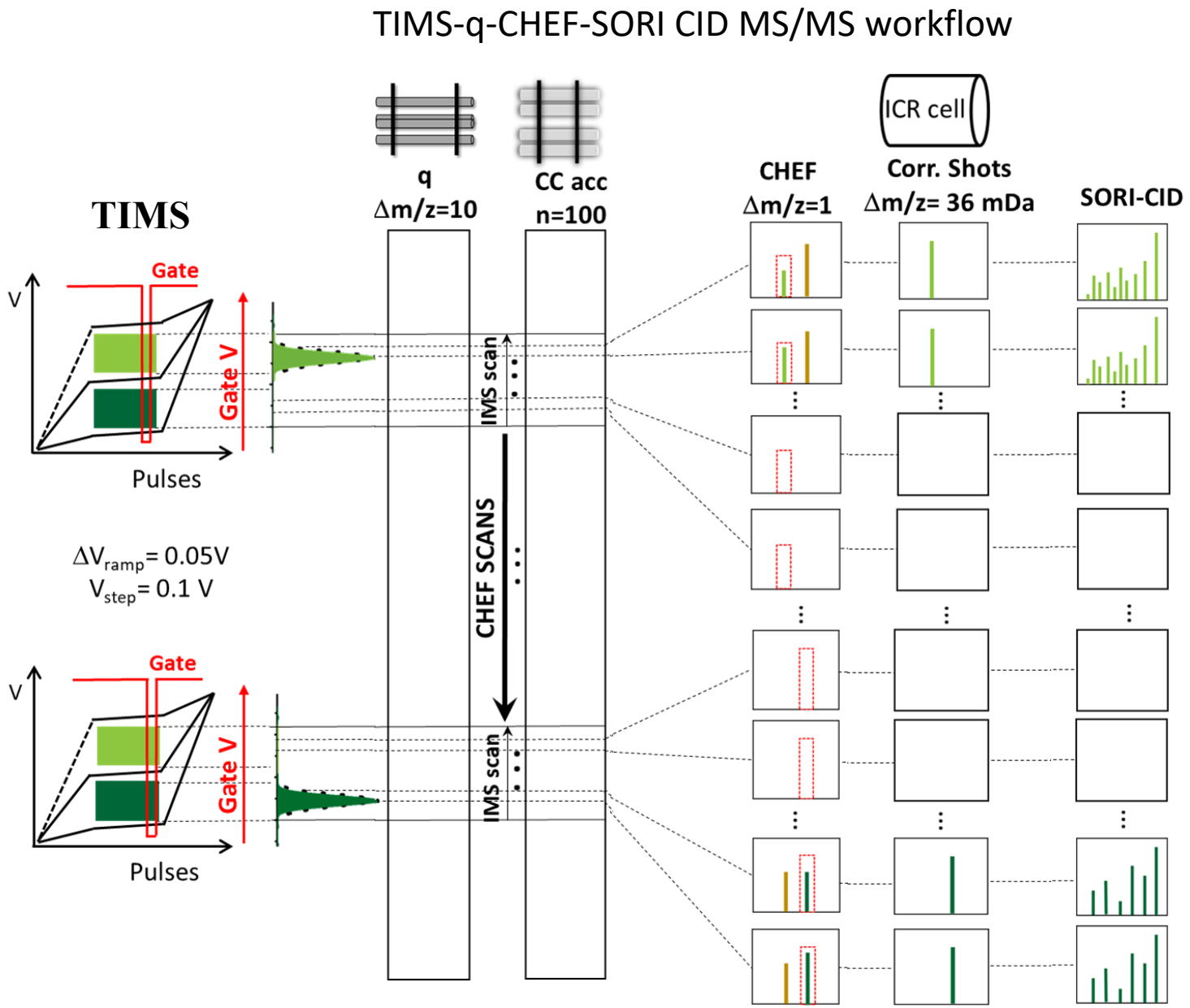
IMS$_n$ →MS/MS$_n$ →Structures (n)



M. Witt, J. Fuchser and B. P. Koch, *Anal. Chem.*, 2009, **81**, 2688-2694.

D. Leyva, et.al. *Faraday Discuss.* **2019,** *218* (0), 431-440.

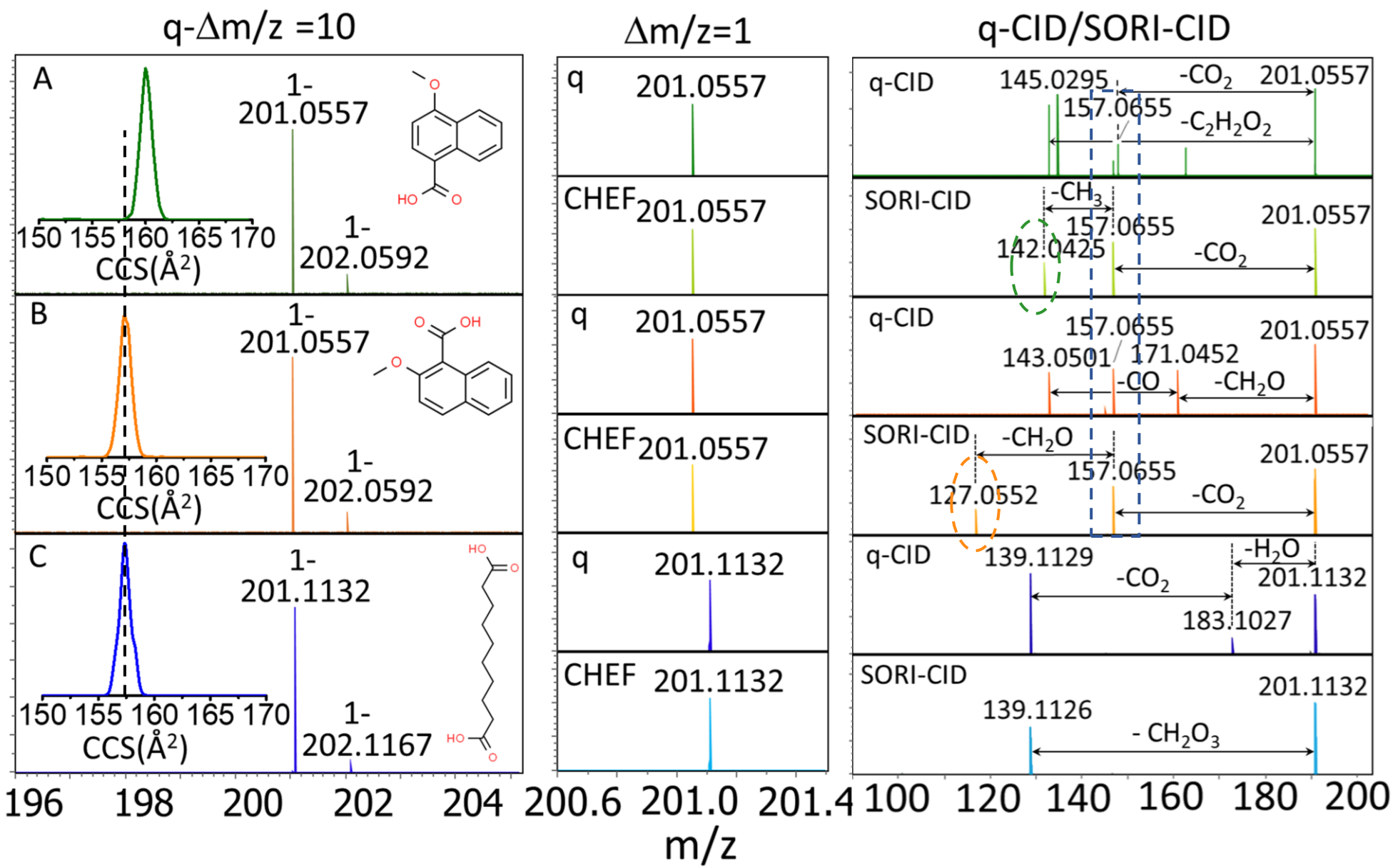
TIMS-FT-ICR CHEF-SORI-CID MS/MS



- IMS resolution ~100
- Mass resolution~ 200,000
- correlated harmonic excitation field (CHEF)
- SORI-CID power: 1-2%

D. Leyva, et.al., *Anal. Chem.* **2020,** *92* (17), 11960-11966.

q-CID vs CHEF-SORI-CID)of standards A, B and C

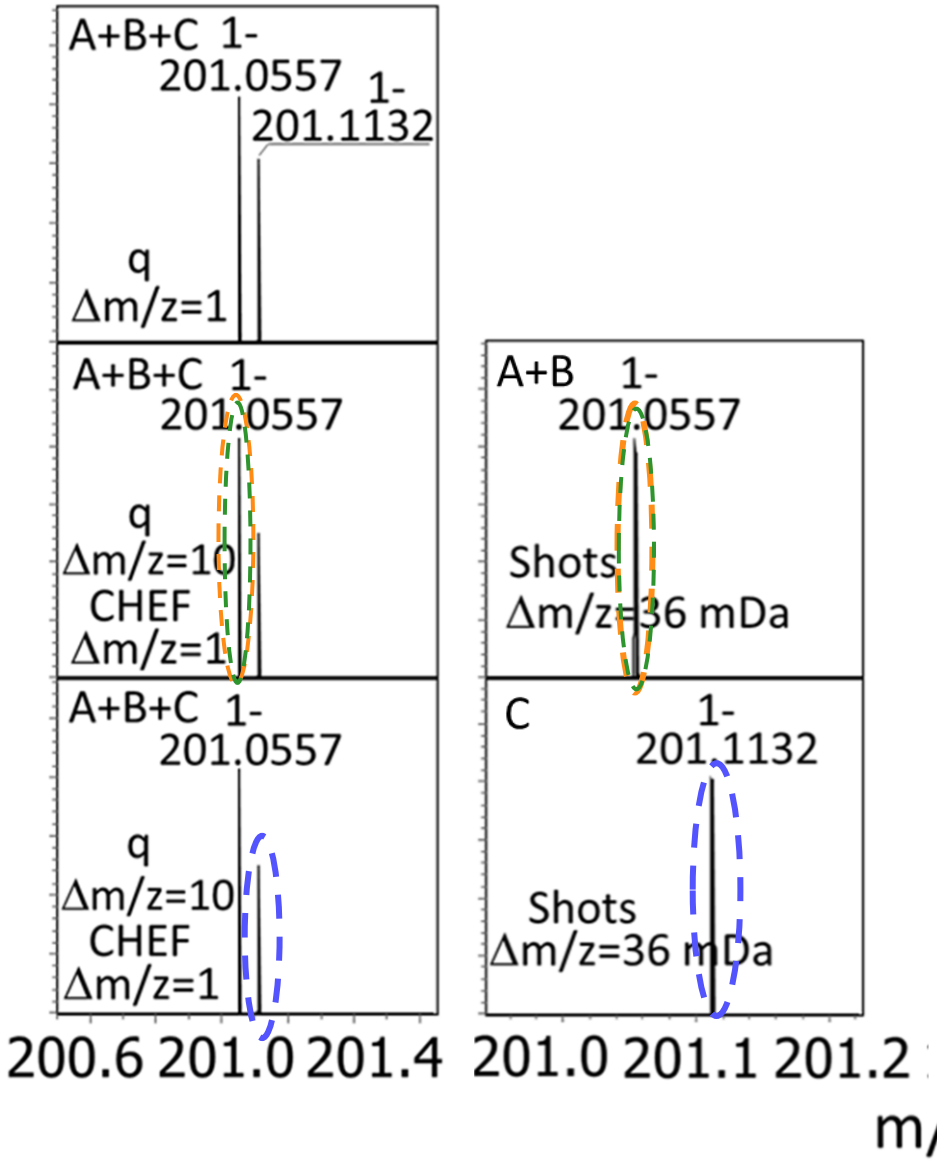


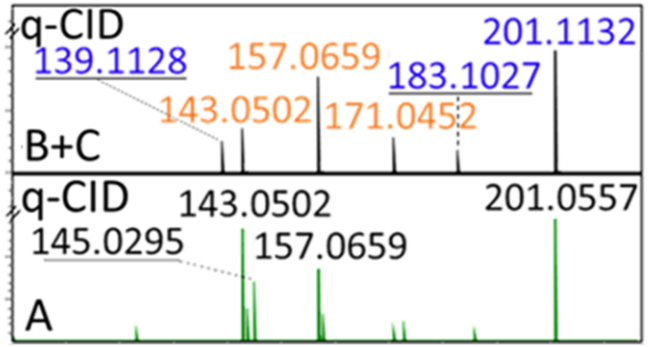
Characteristic fragments!

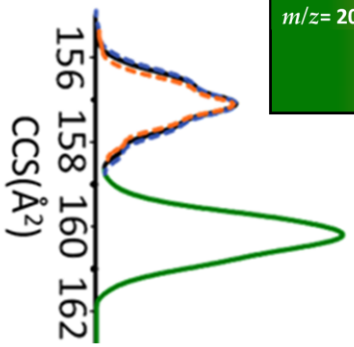
Characteristic fragments!

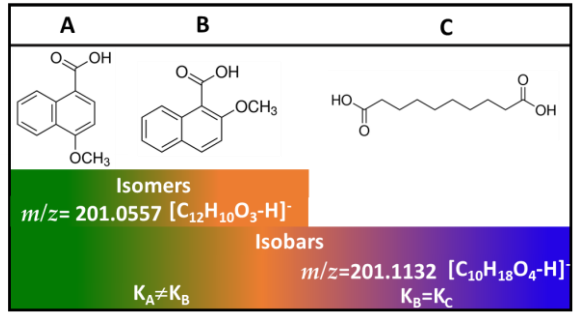
D. Leyva, et.al., *Anal. Chem.* **2020,** *92* (17), 11960-11966.

q-CID vs CHEF-SORI-CID of the standards mixture



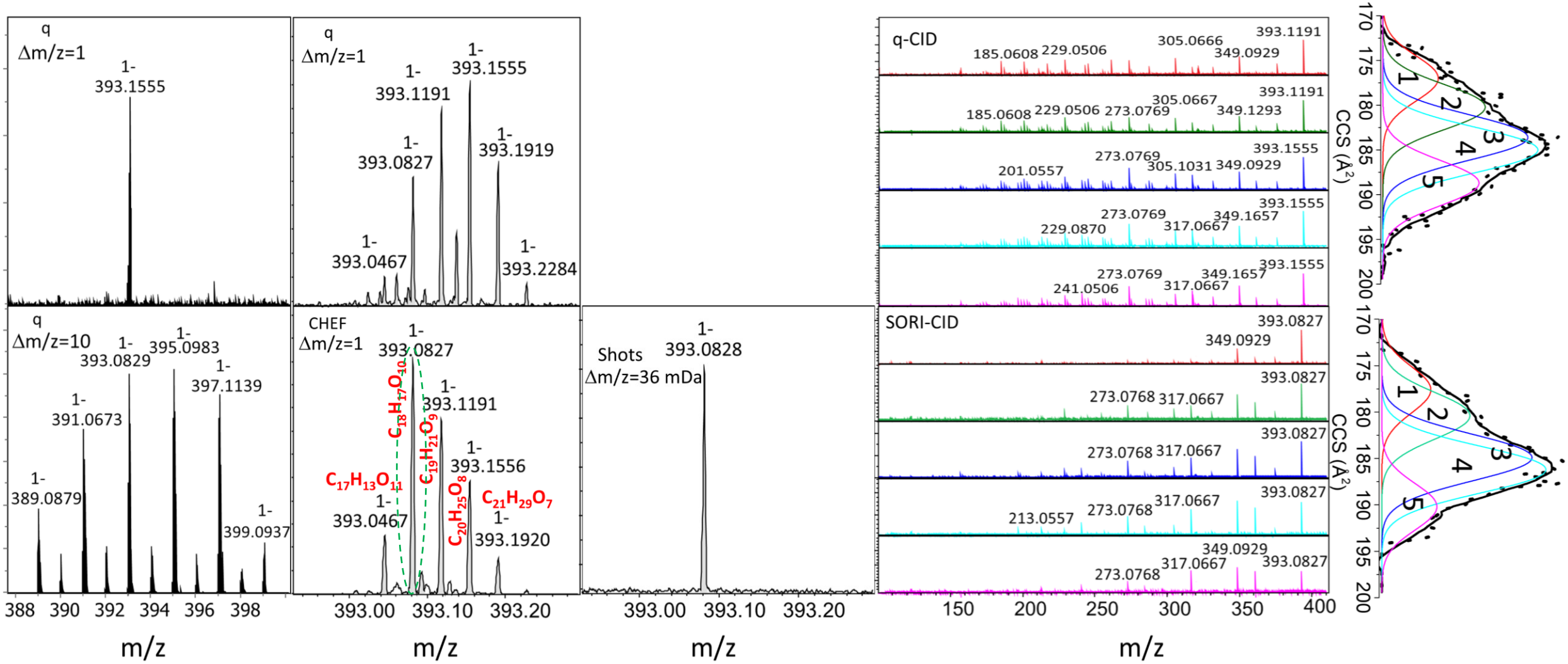






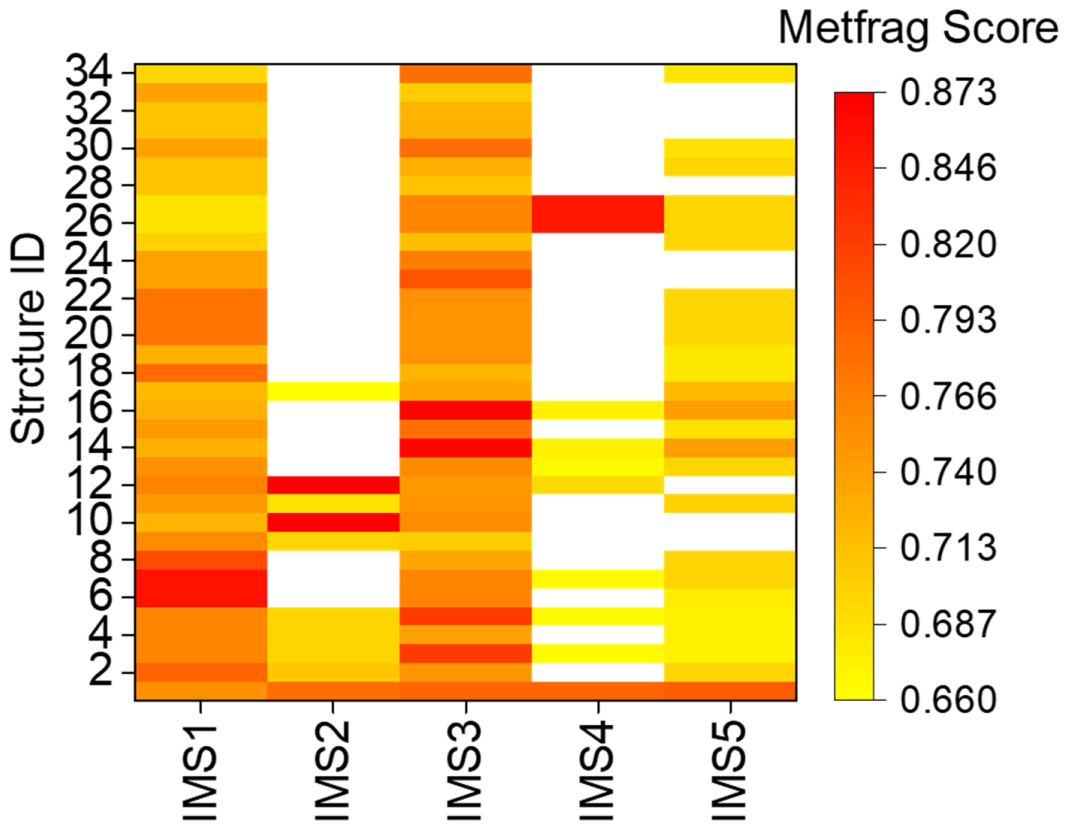
D. Leyva, et.al., *Anal. Chem.* **2020,** *92* (17), 11960-11966.

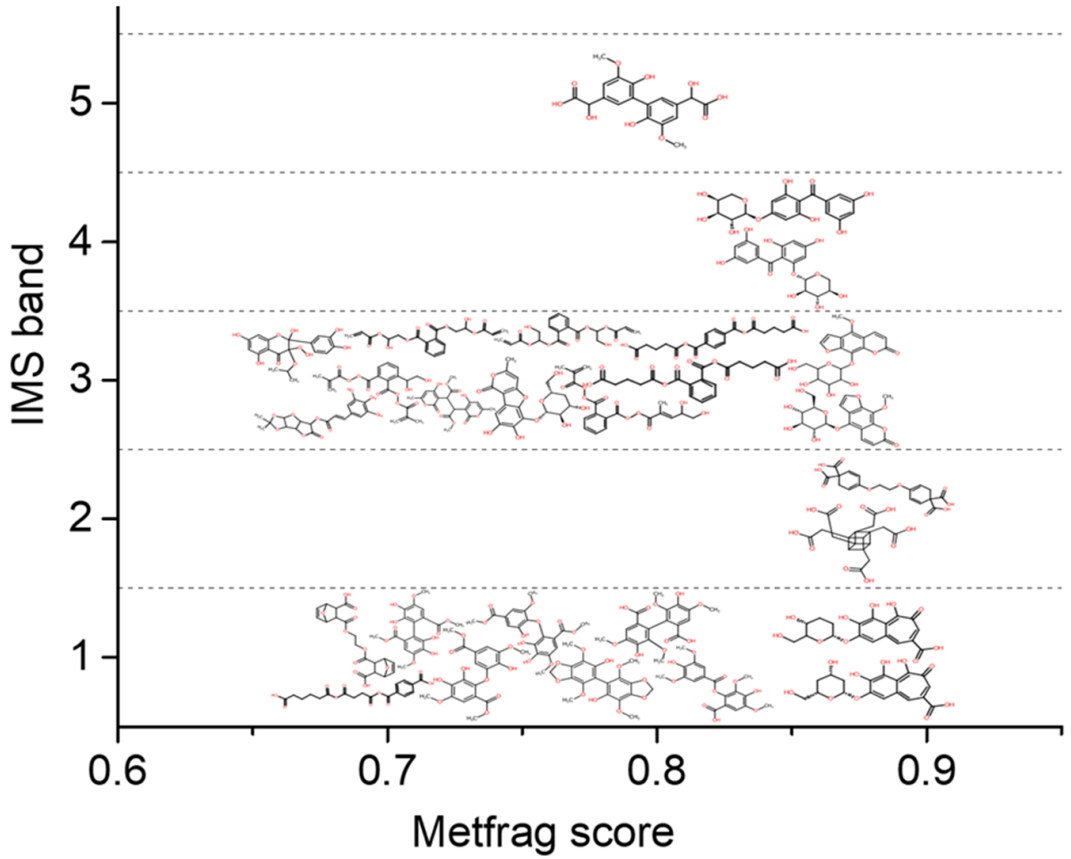
q-CID and CHEF-SORI-CID of Pantanal SPE-DOM sample



D. Leyva, et.al., *Anal. Chem.* **2020,** *92* (17), 11960-11966.

Candidate isomeric structures for $[C_{18}H_{18}O_{10}\text{-}H]^-$ filtered by Metfrag scores and IMS



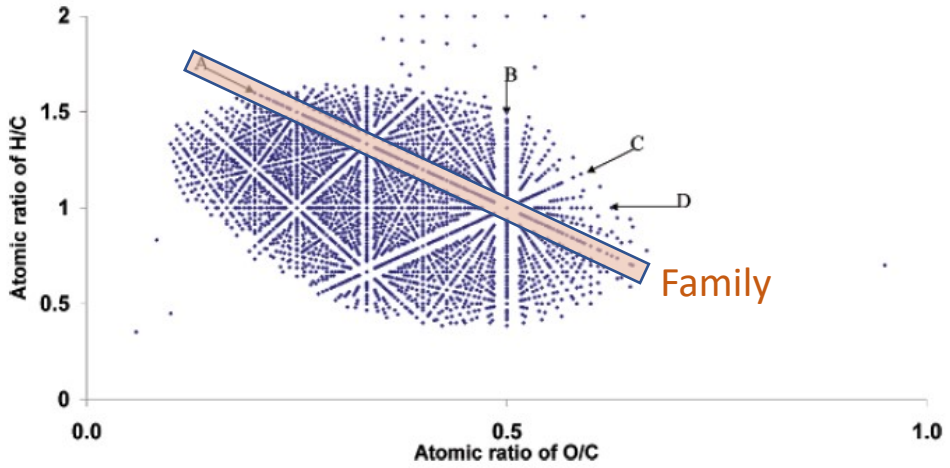


IMS1 and IMS3 bands grouped most of the assigned structures

D. Leyva, et.al., *Anal. Chem.* **2020,** *92* (17), 11960-11966.

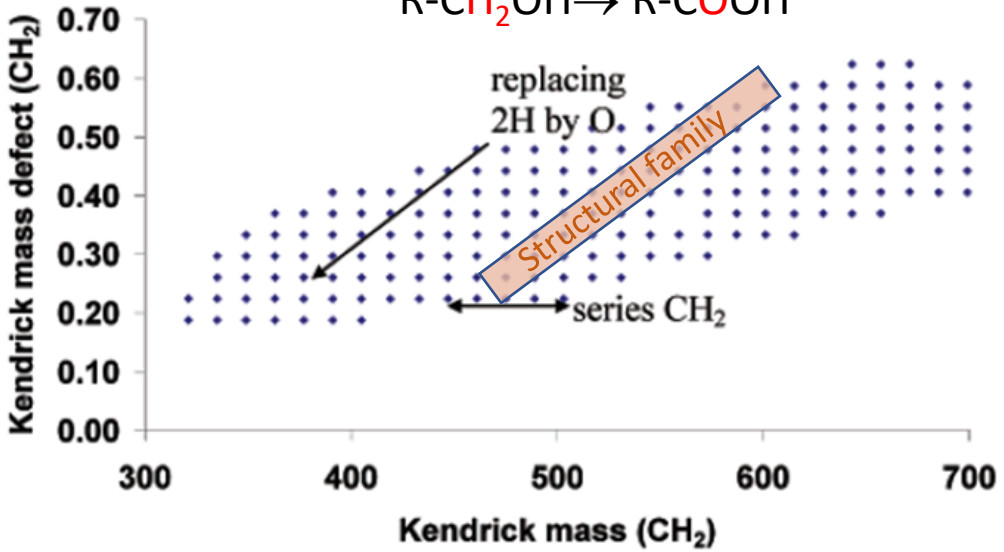
DOM "structural" trends from broadband FT-ICR MS

van Krevelen plot



A: Methylation, demethylation, or alkyl chain elongation
B: Hydrogenation/dehydrogenation
C: Hydration/condensation
D: Oxidation/reduction, decarboxylation.

R-CH_2OH→ R-COOH



$$Kendrick\ mass(CH_2) = experimental\ mass\frac{14}{14.015650}$$

$$Kendrick\ Mass\ Deffect(CH_2) = nominal\ mass - Kendrick\ mass(CH_2)$$

Compositional change	Structural change
$-H_2$	Side chain oxidation
$+O_2$	Aromatic ring openings
CH_4 vs O	C_2H_5 vs CHO

An accurate structural assignment of DOM families cannot be solely predicted from chemical formulas

P. G. Hatcher, H. E. Lerch, A. L. Bates and T. V. Verheyen, *Org. Geochem.*, 1989, **14**, 145-155.
S. Kim, R. W. Kramer and P. G. Hatcher, *Anal. Chem.*, 2003, **75**, 5336-5344.
Stenson, A. C.; Marshall, A. G.; Cooper, W. T. *Anal. Chem.,* **2003,** *75* (6), 1275-1284.

Similarities of DOM molecules based on common neutral losses

selected precursors				neutral losses									
m/z	DBE	AI	molecular formula	H_2O	CH_4O	H_4O_2	CO_2	CH_2O_3	HNO_3	$C_2H_4O_3$	CH_3NO_3	SO_3	C_2O_4
293.1397	6	0.1	$C_{16}H_{22}O_5$	x	x	x	x	x		x			
295.1554	5	0	$C_{16}H_{24}O_5$			x	x						
309.1346	6	0	$C_{16}H_{22}O_6$	x	x		x	x					
311.1505	5	0	$C_{16}H_{24}O_6$				x	x					
325.1662	5	0	$C_{17}H_{26}O_6$				x	x					
341.1245	6	0	$C_{16}H_{22}O_8$	x	x		x	x		x			x
341.1609	5	0	$C_{17}H_{26}O_7$	x	x		x	x		x			x
343.1401	5	0	$C_{16}H_{24}O_8$	x			x	x		x			x
353.1611	6	0	$C_{18}H_{26}O_7$	x	x	x	x	x		x			x
355.1767	5	0	$C_{18}H_{28}O_7$	x	x		x	x		x			x

Table modified from J. P. LeClair, J. L. Collett and L. R. Mazzoleni, Environ. Sci. Technol., 2012, 46, 4312-4322.

Analogous neutral loss patterns suggest similar structures differing by specific chemical units.

How can we identify structural families of DOM compounds?

1. Comprehensive fragmentation is needed to understand DOM structural commonalities.
2. Effective data mining approach is key for deconvoluting DOM *"chimeric"* MS/MS spectra.

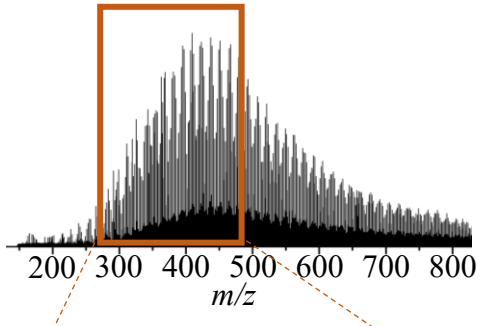
J. P. LeClair, J. L. Collett and L. R. Mazzoleni, *Environ. Sci. Technol.*, 2012, **46**, 4312-4322.

FT-ICR MS q-CASI-CID MS/MS

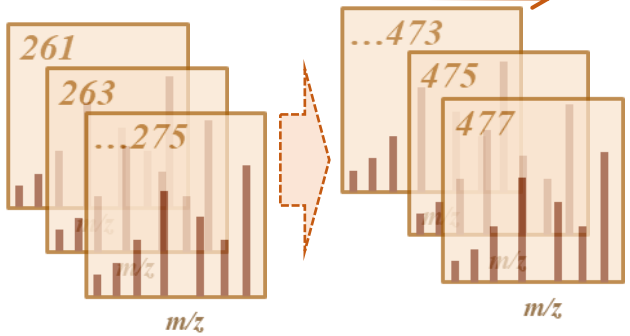
(-)ESI-FT-ICR MS

1.

Pantanal, Brazil



q(1Da)-CID



MF assignment

Bruker 7T Solarix FT-ICR MS

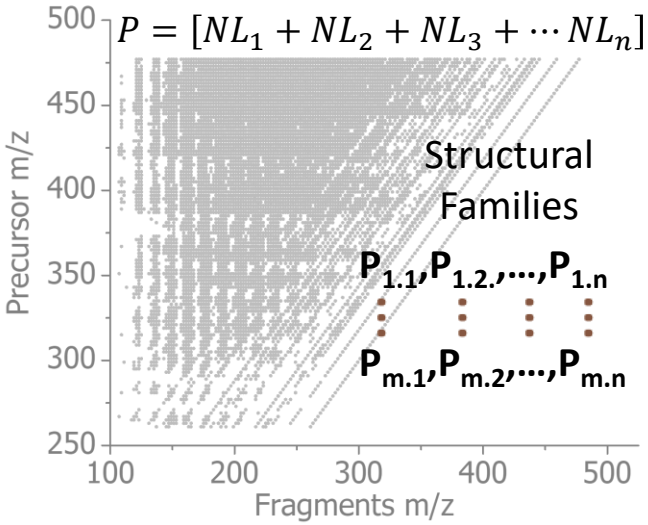
- R=4x10^6 (m/z 400)
- Tailored CID energies 15-27 V
- 70-100 average scans/segment
- 5-8 MS^2 segments

(-)ESI-FT-ICR CASI-CID MS/MS

2.

Fragmentation pathways

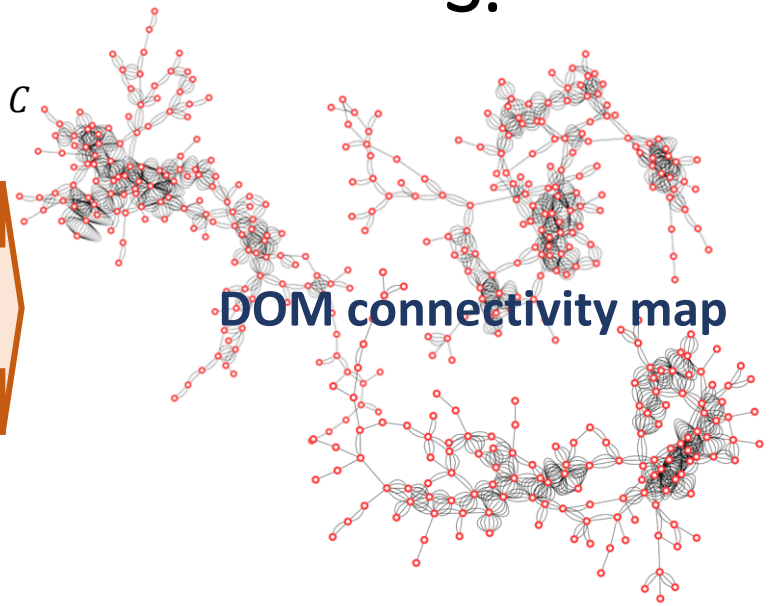
$$P = [NL_1 + NL_2 + NL_3 + \cdots NL_n] + C$$



python

Graph-DOM

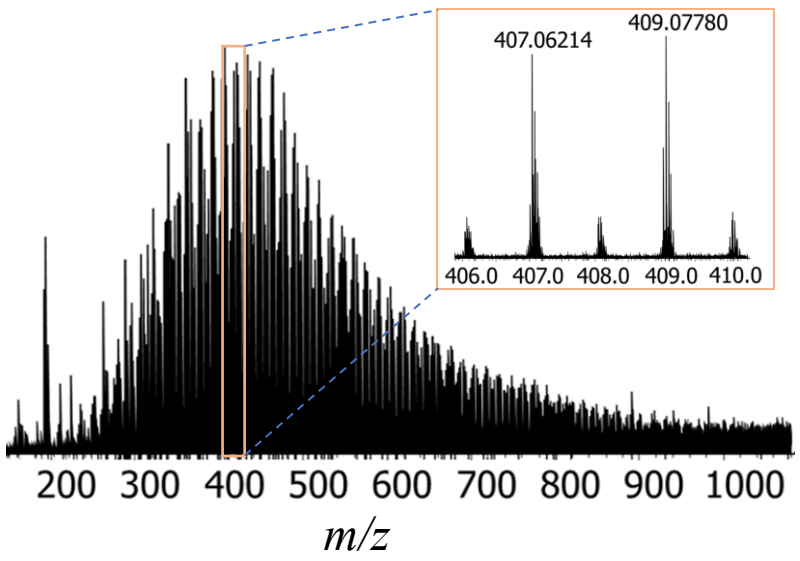
3.

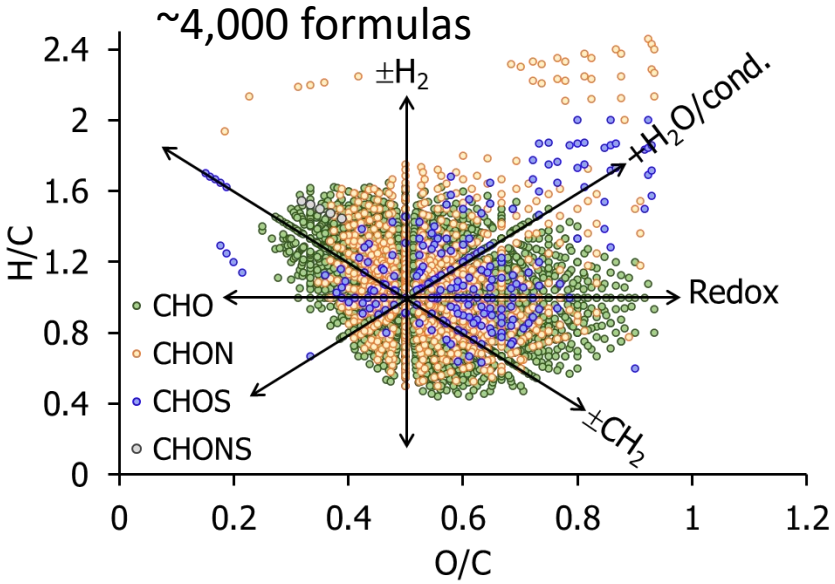


Cytoscape

D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

DOM compositional characteristics



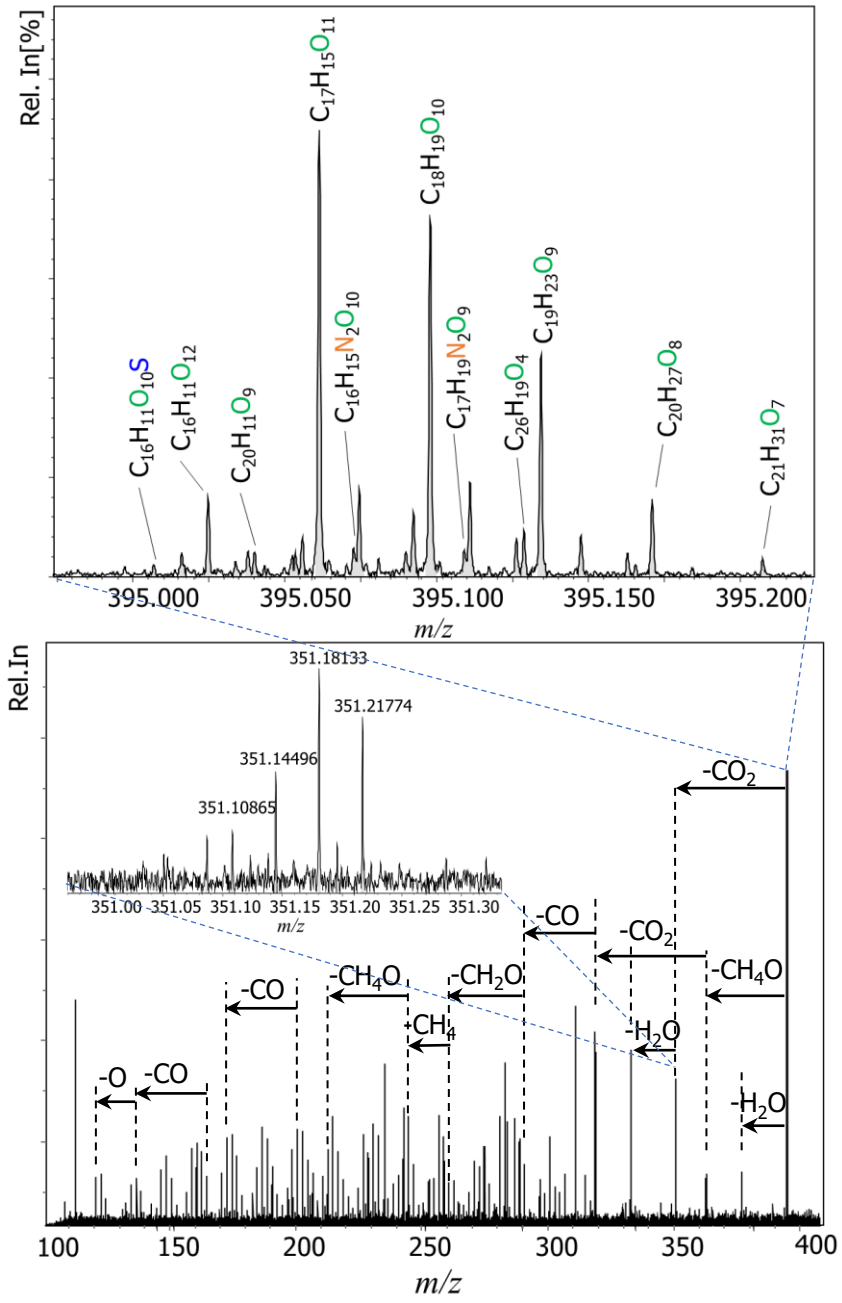


CASI-CID MS/MS

- **110 MS² spectra**
- **~ 1500 precursor ions from MS1**

Formula constrains

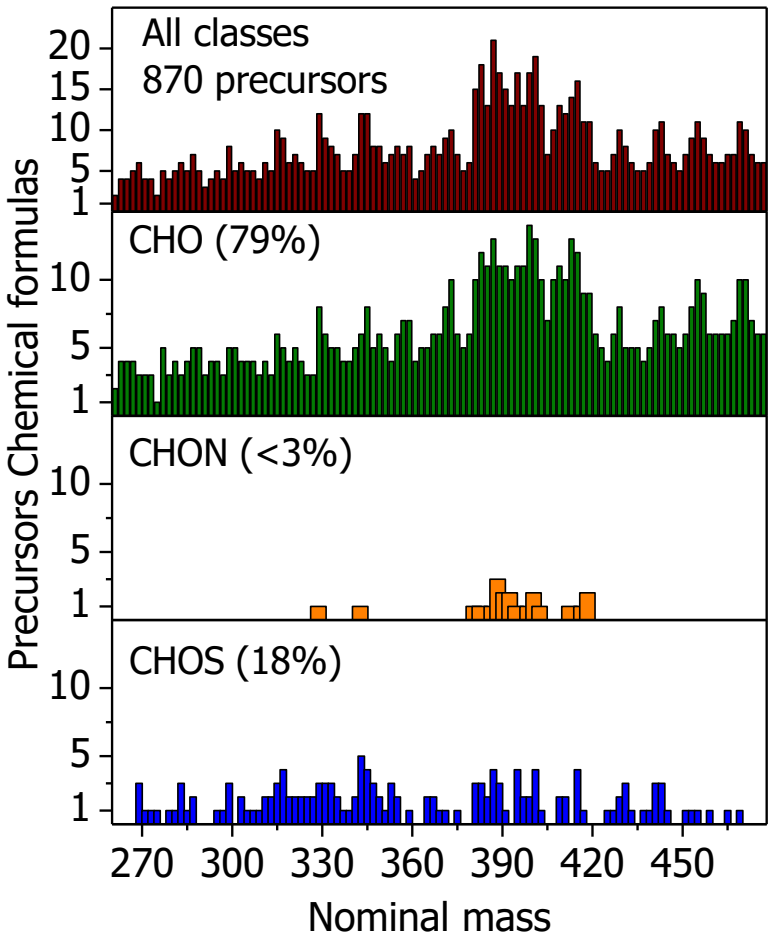
- $C_{4\text{-}50}H_{4\text{-}100}N_{0\text{–}3}O_{0\text{–}25}S_{0\text{–}2}$
- Tol. Error< 1 ppm
- DBE-O ≤ 10



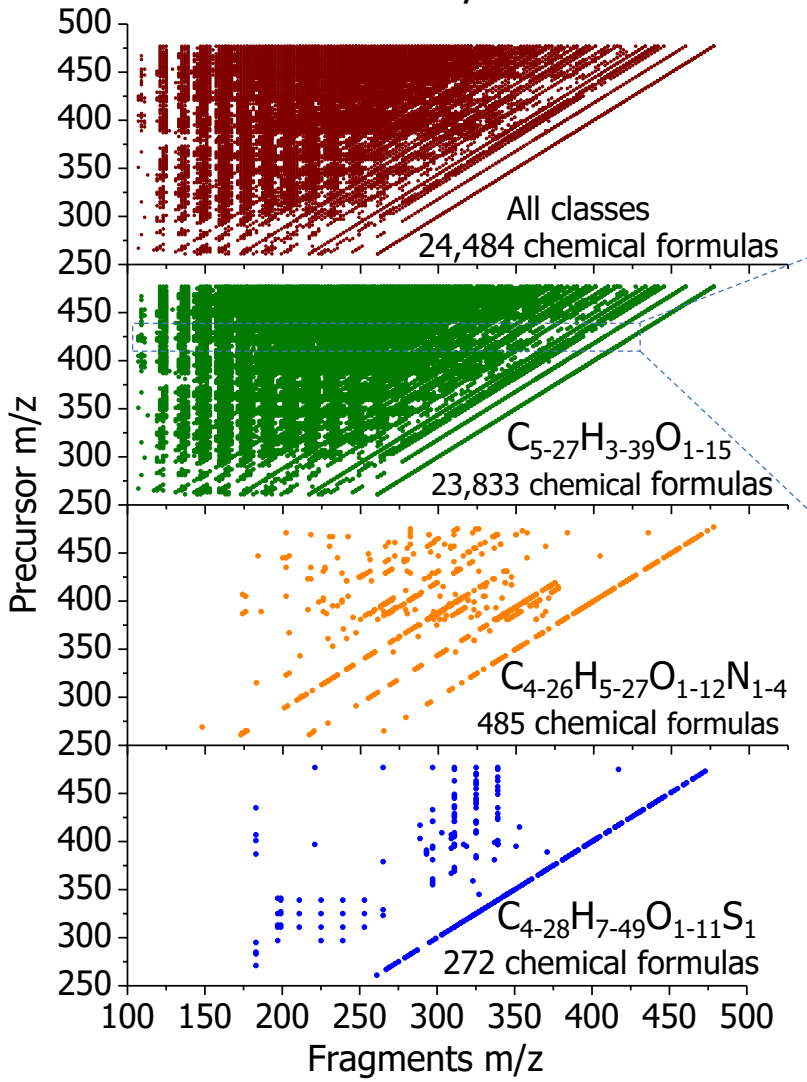
D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

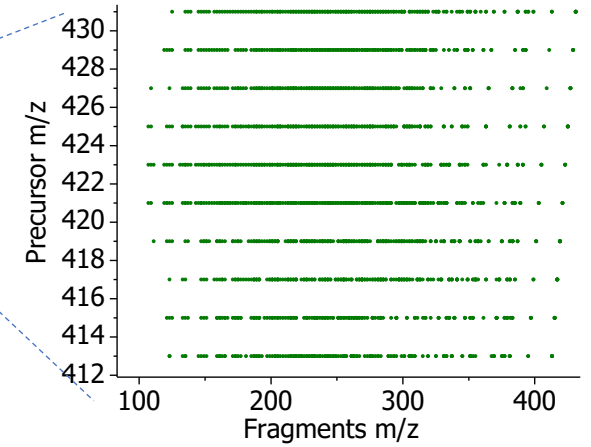
ESI-FT ICR CASI-CID MS/MS

Precursors assigned per nominal mass



2D MS/MS

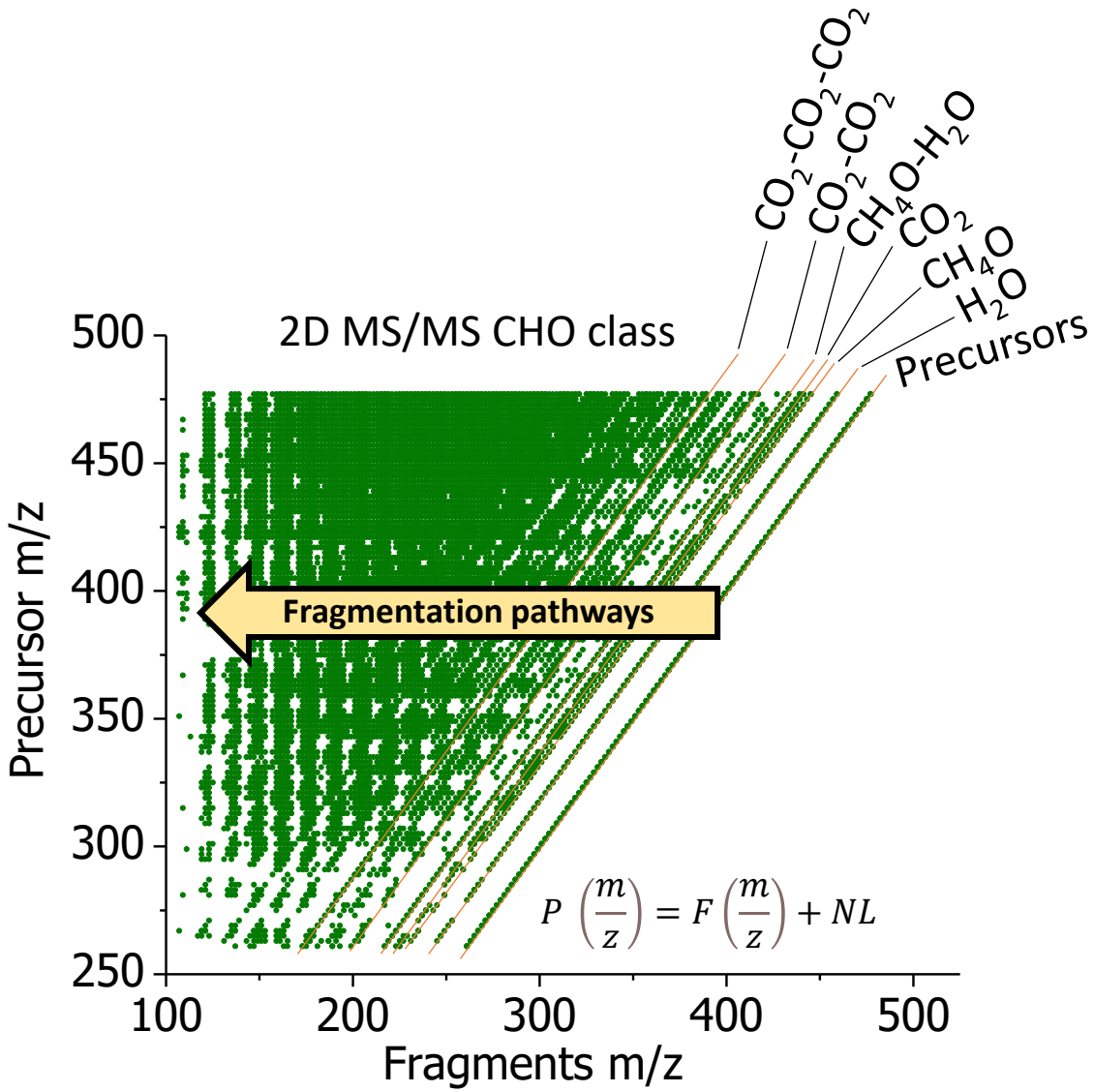




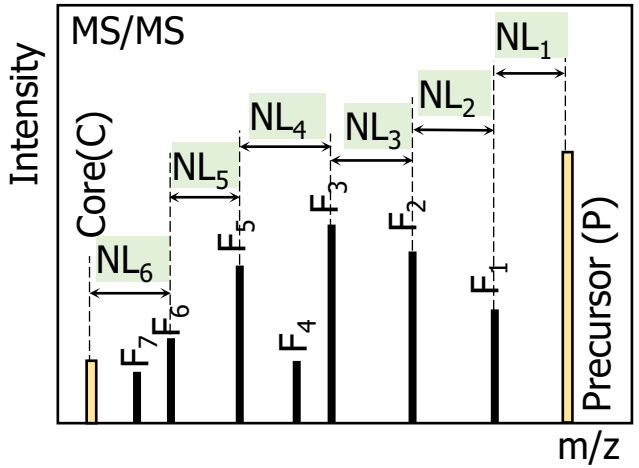
Systematic occurrence of fragmentation patterns

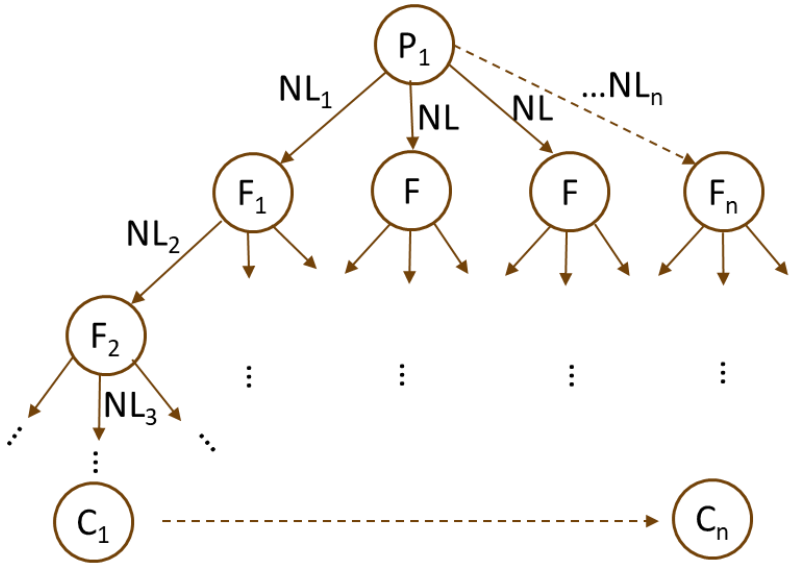
D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

Data mining at the 2D MS/MS level



Computing fragmentation pathways





- Tree search algorithm
- NL set={O, CH_4, H_2O, CO, CH_2O, CH_4O, CO_2}
- Tol error≤ 1 mDa

Pathway: $P_1 = [NL_1 + NL_2 + NL_3 + \cdots NL_n] + C_1$

Loop over all precursor formulas

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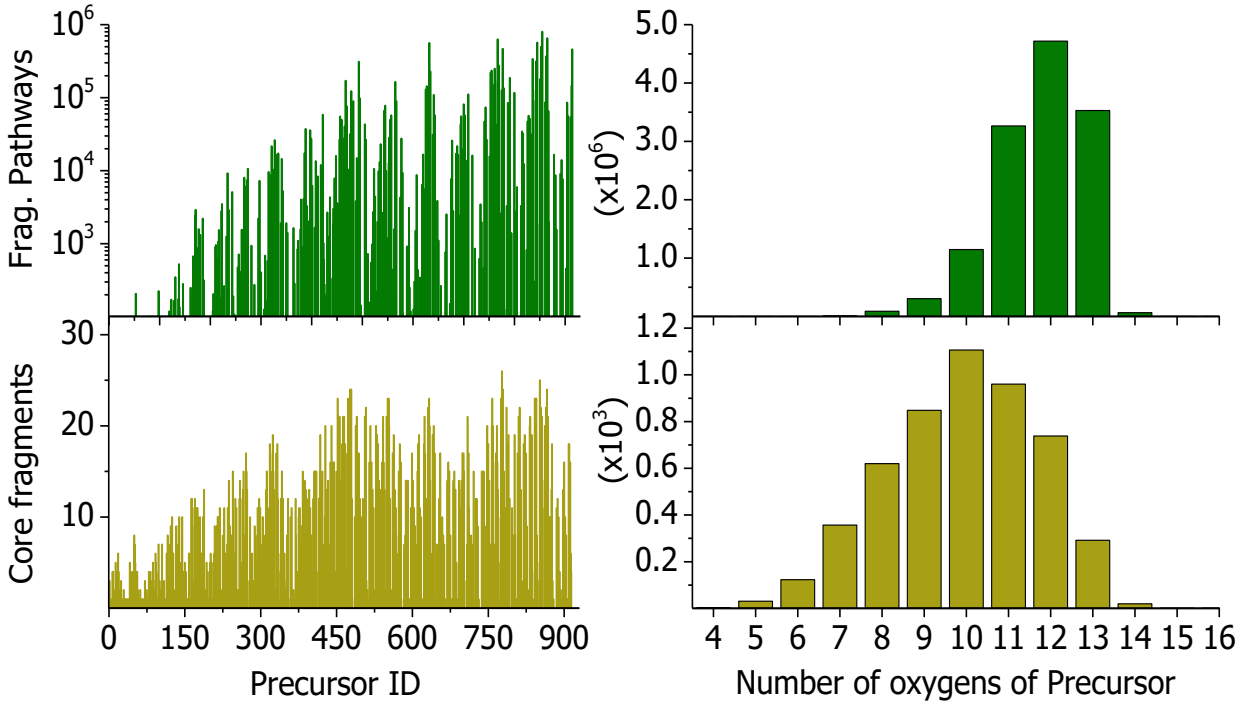
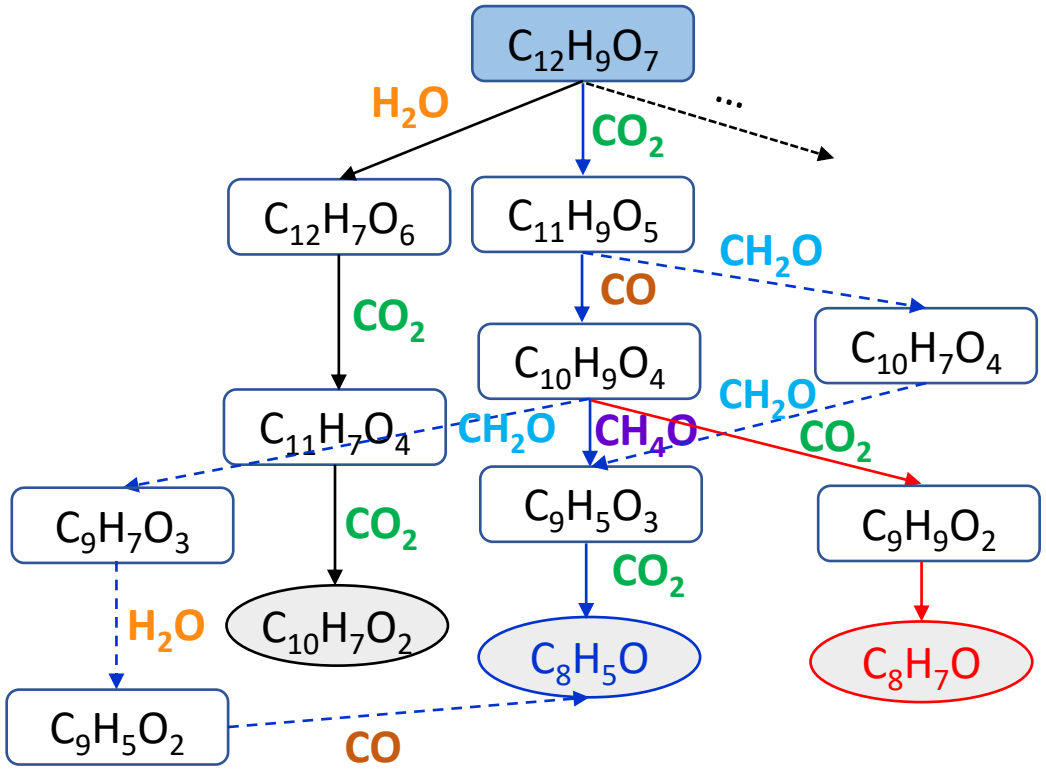
Fragmentation pathways for the CHO class





Over 10^8 ordered fragmentation pathways

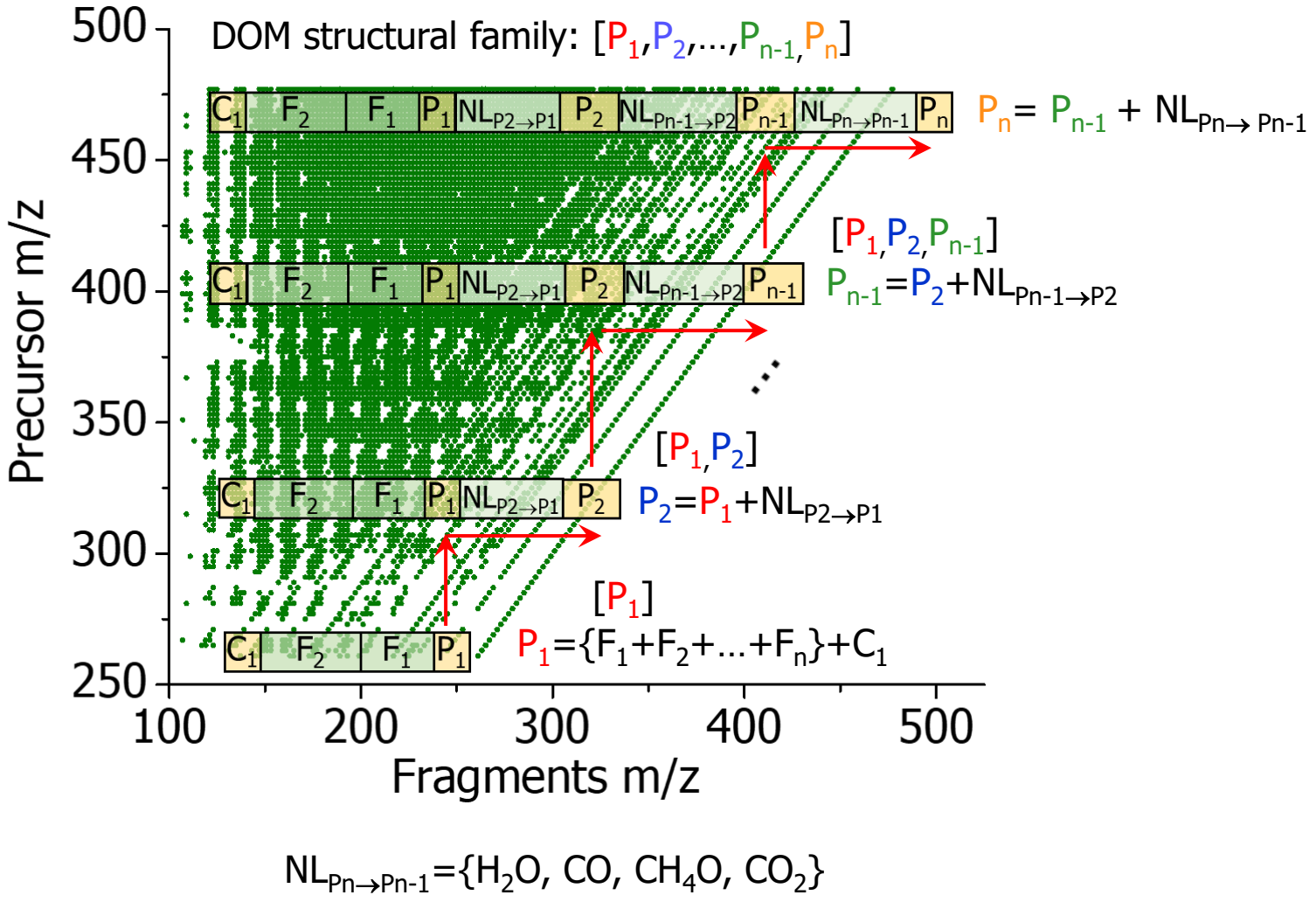
Trends

- Single precursor sharing the same core fragment.
- Multiple precursors sharing the same pathway.

$$C_{12}H_9O_7 = \begin{cases} [H_2O+CO_2+CO_2] + C_{10}H_7O_2 \\ \vdots \qquad\qquad \vdots \\ [CO_2+CO+CH_4O+CO_2] + C_8H_5O \\ [CO_2+CO+CH_2O+H_2O+CO] + C_8H_5O \\ \vdots \qquad\qquad \vdots \\ [CO_2+CO+CO_2+CH_2O] + C_8H_7O \end{cases}$$

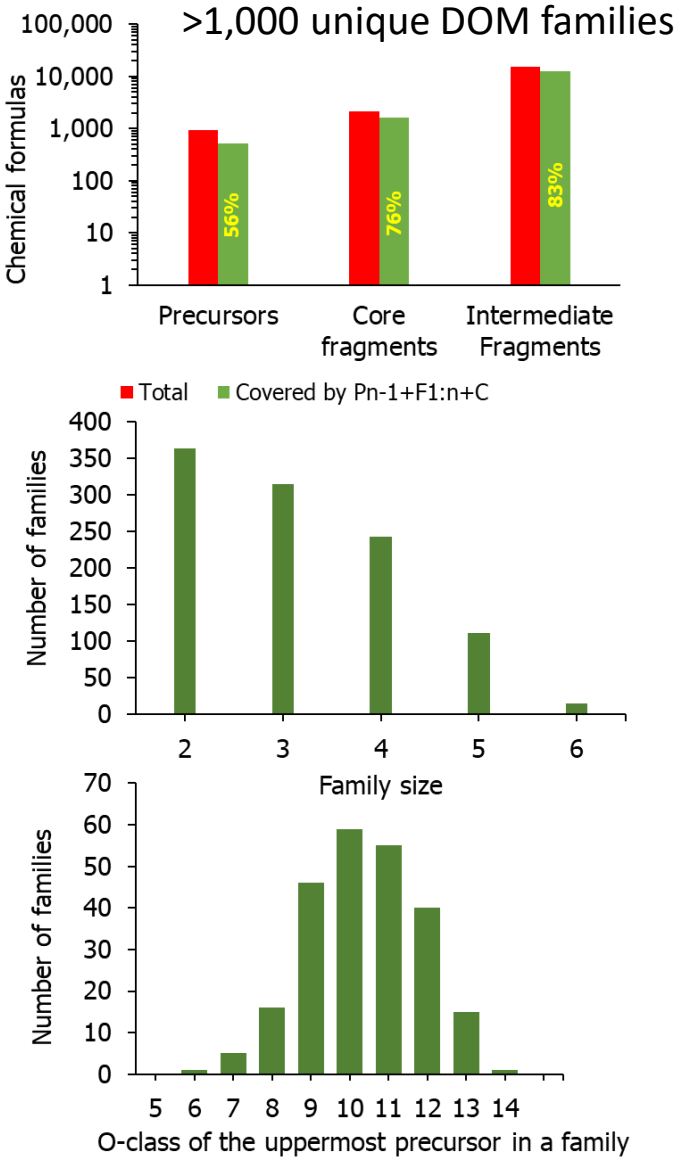
D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

Retrieving DOM structural families: Model Pn-1+F1:n+C

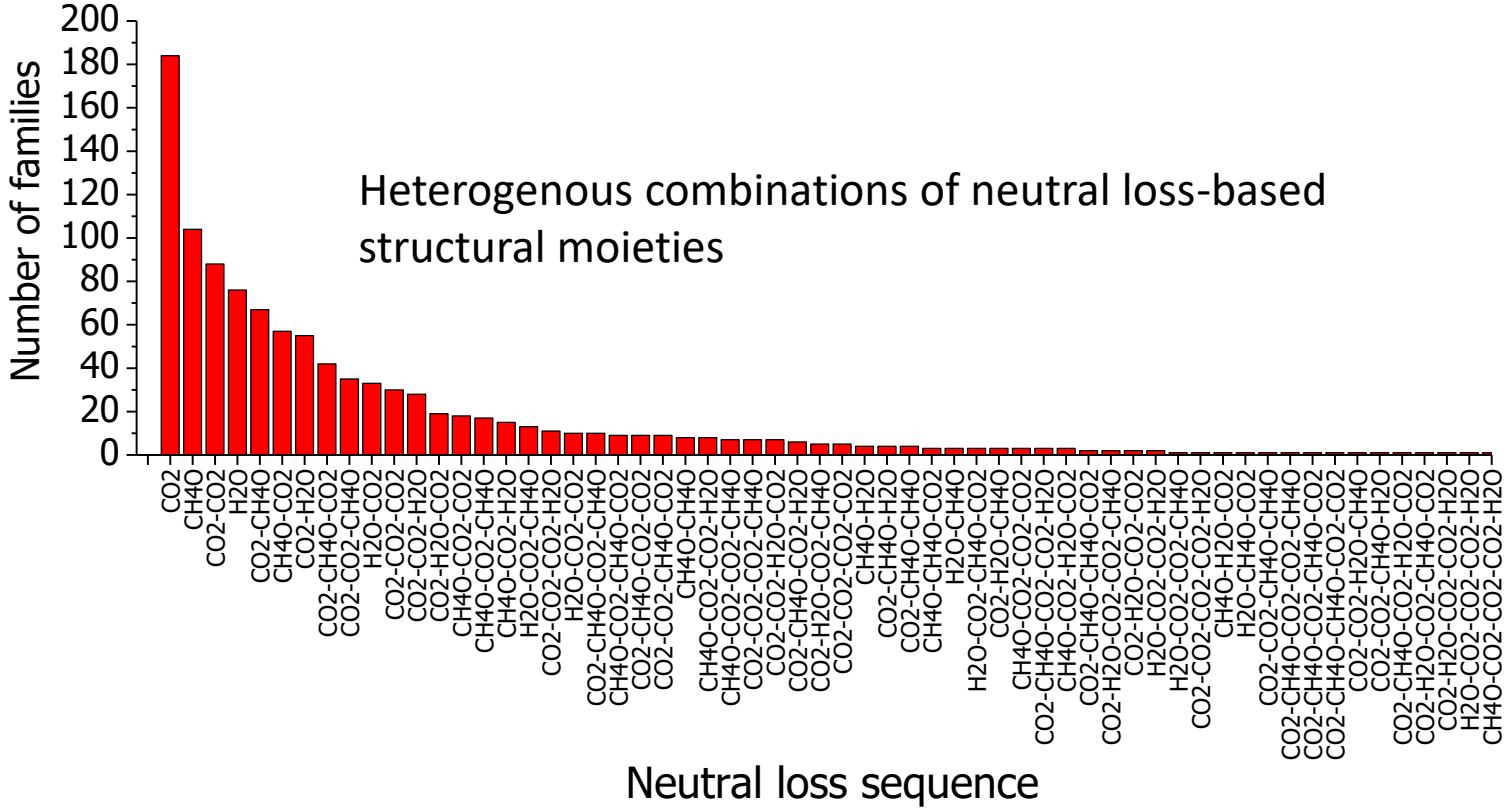


$NL_{Pn \rightarrow Pn-1} = \{H_2O, CO, CH_4O, CO_2\}$

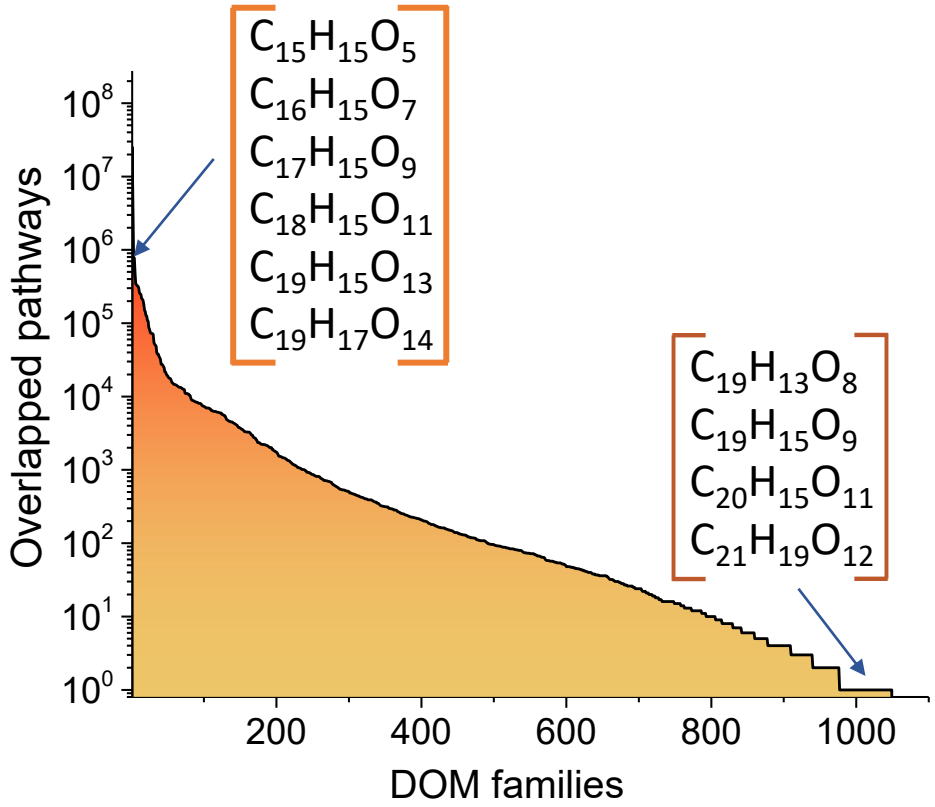
Graph-DOM performance



Structural characteristics of DOM families



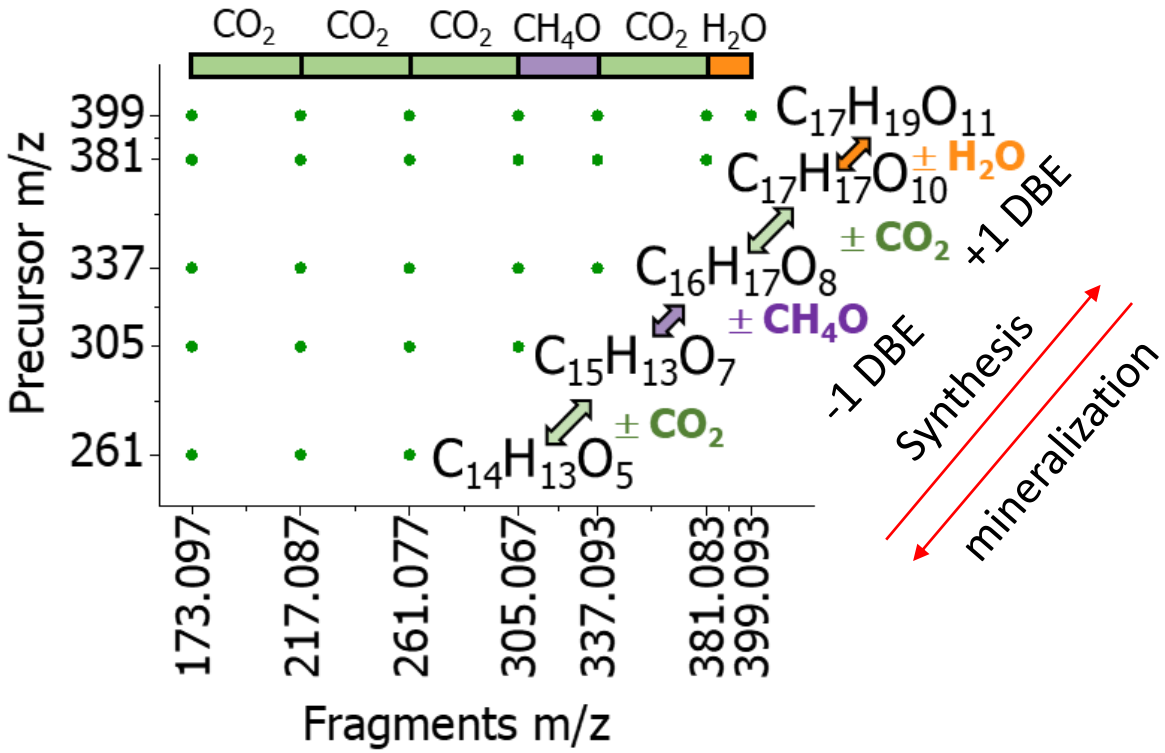
Structural transformation driven by oxygenation/deoxygenation mechanisms.



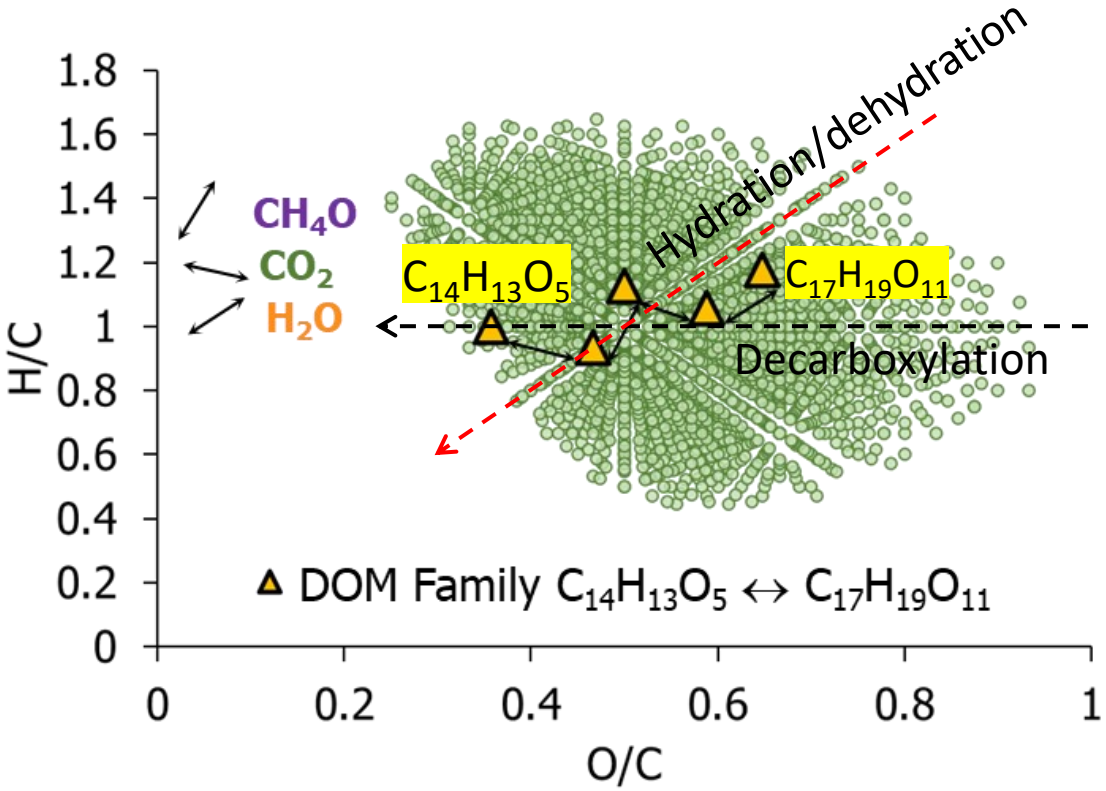
Confidence level based on overlap of fragmentation pathways.

D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

Compositional trends of DOM families



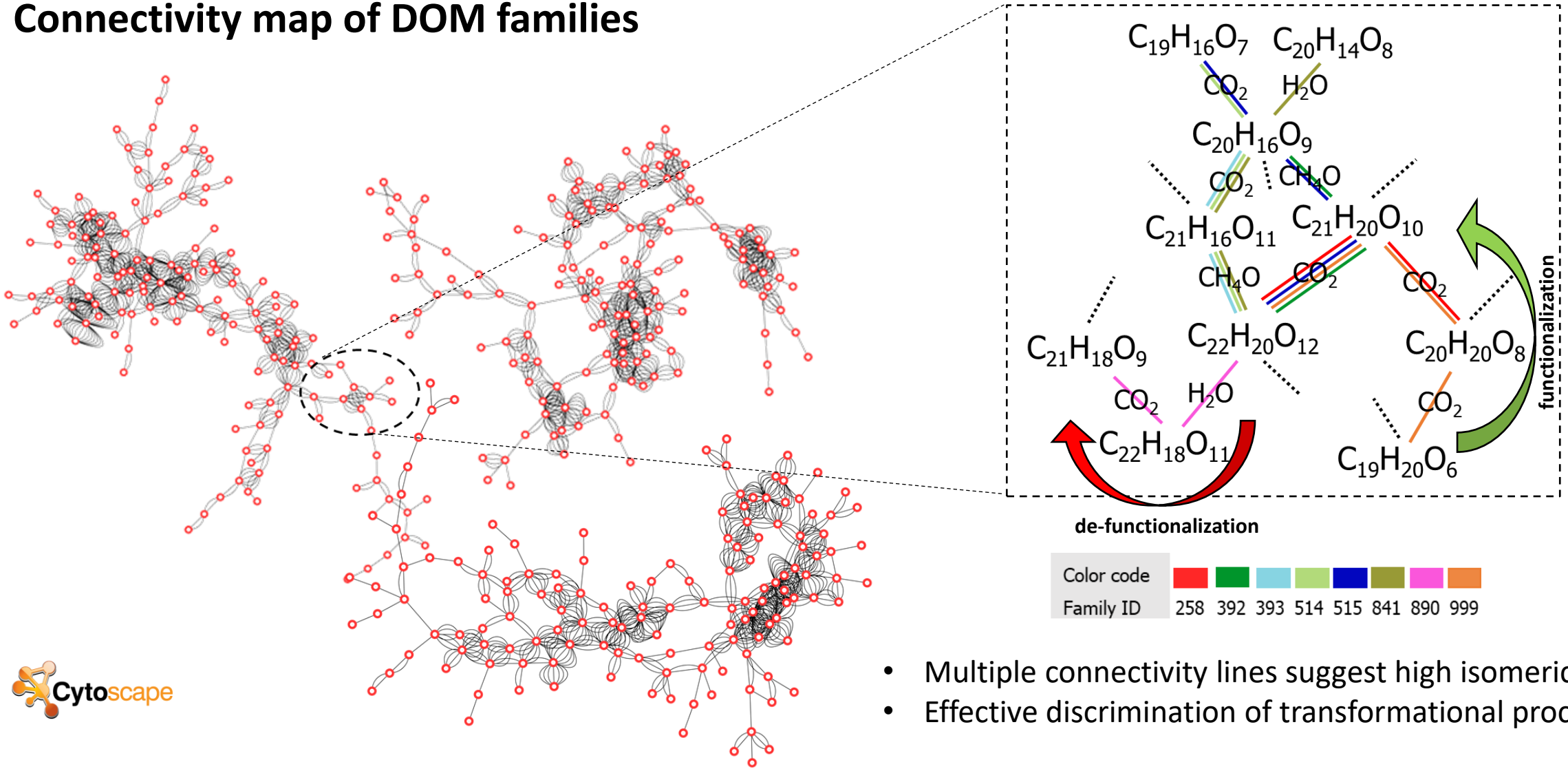
Our model is useful to explain transformational mechanisms.



DOM biogeochemical transformation mechanisms are more complex than traditionally described at the precursor level.

D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

Connectivity map of DOM families



Cytoscape

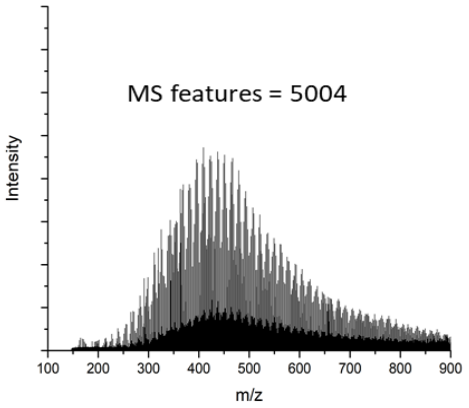
- Multiple connectivity lines suggest high isomeric content.
- Effective discrimination of transformational processes.

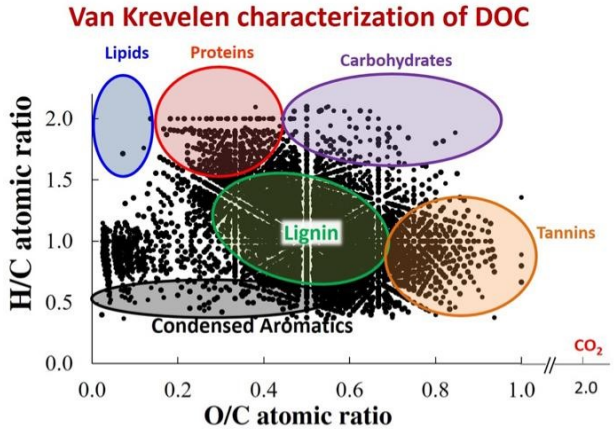
Complex assembly of interconnected molecules

D. Leyva, et.al, *Environmental Science & Technology*, 2022, **56**, 1458-1468

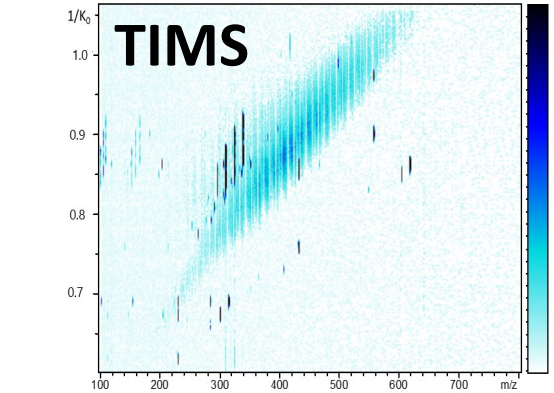
Conclusions

The analysis of DOM by UHRMS is very challenging!

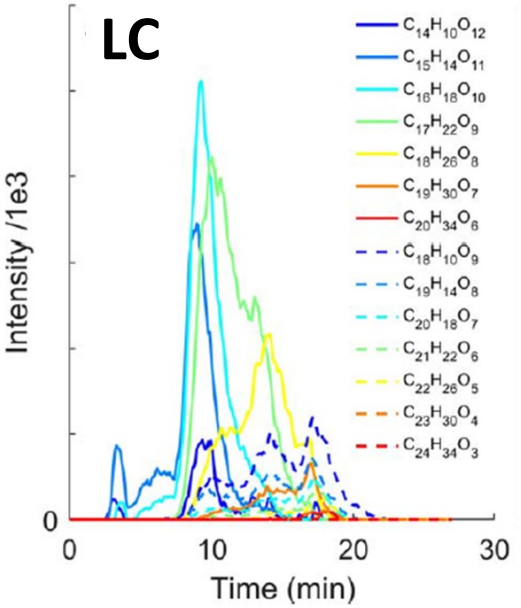




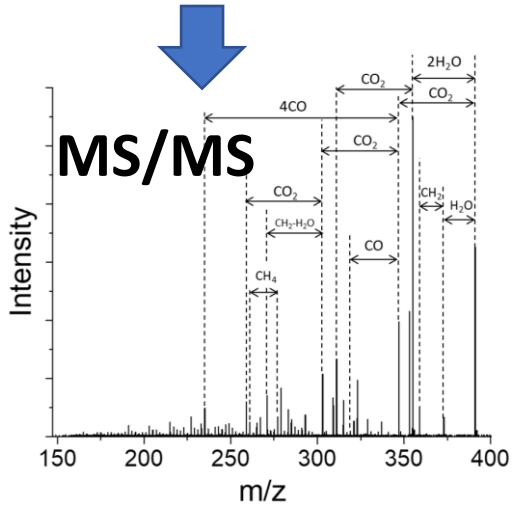
DOM chemical fingerprint is partially uncovered by FTMS



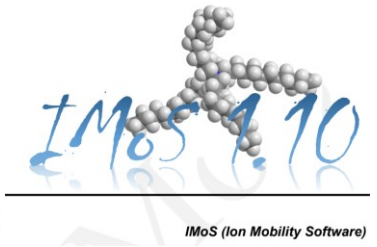
LC can not resolve DOM isomeric complexity

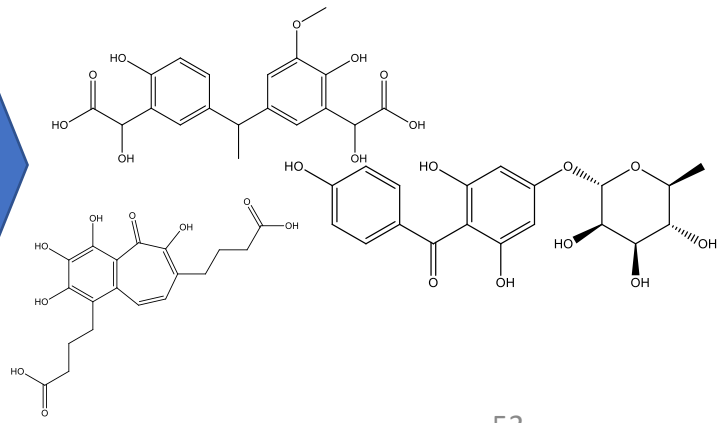


TIMS-FT ICR MS provides complementary separation and candidate structures for DOM.



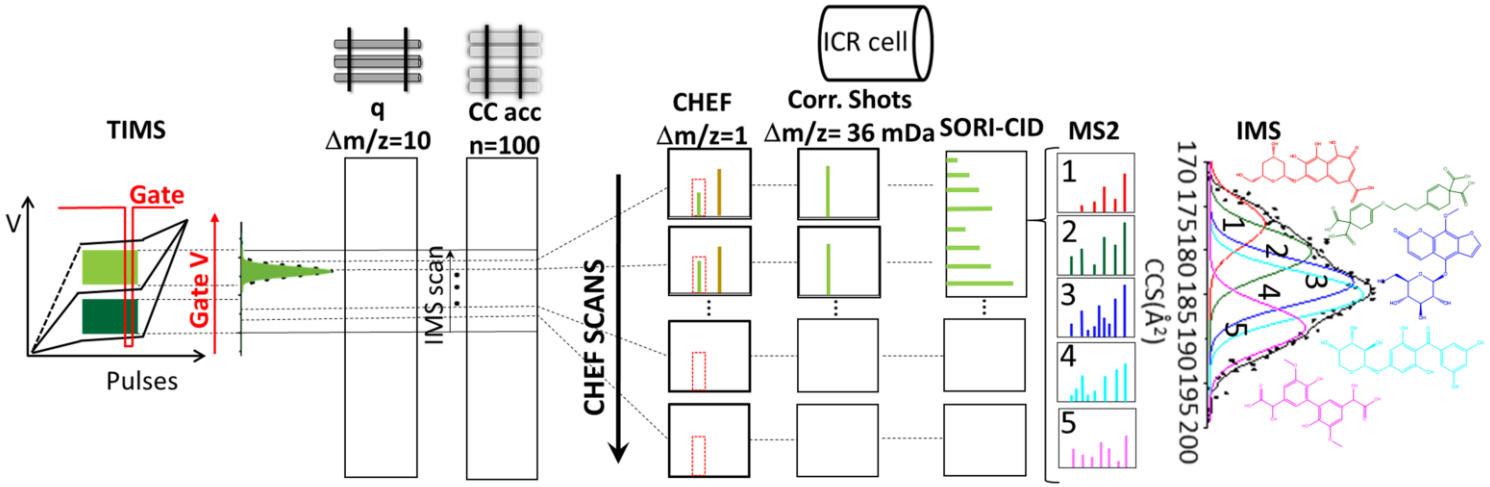
Fragmentation pathways





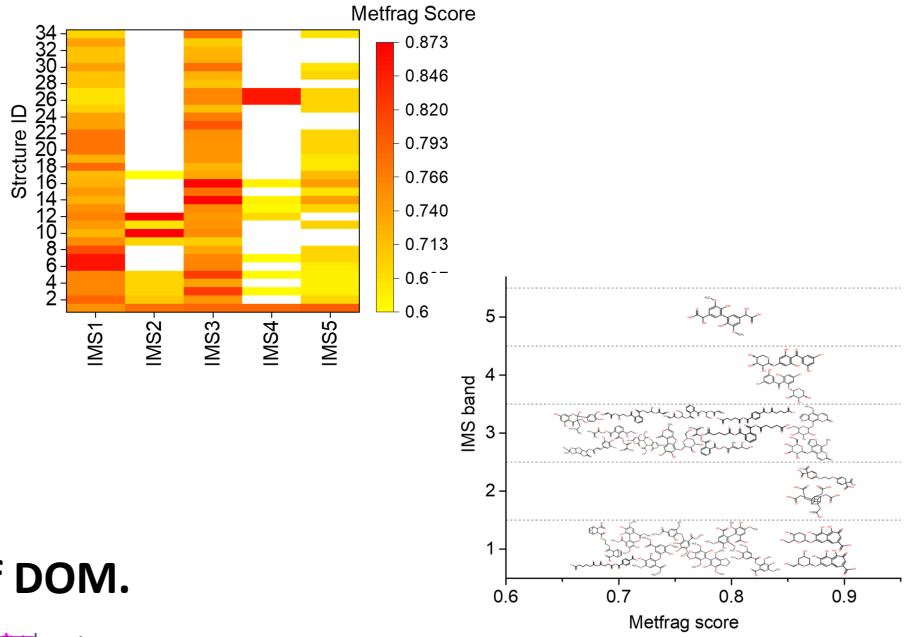
Conclusions

Increased confidence in the structural assignment of DOM.

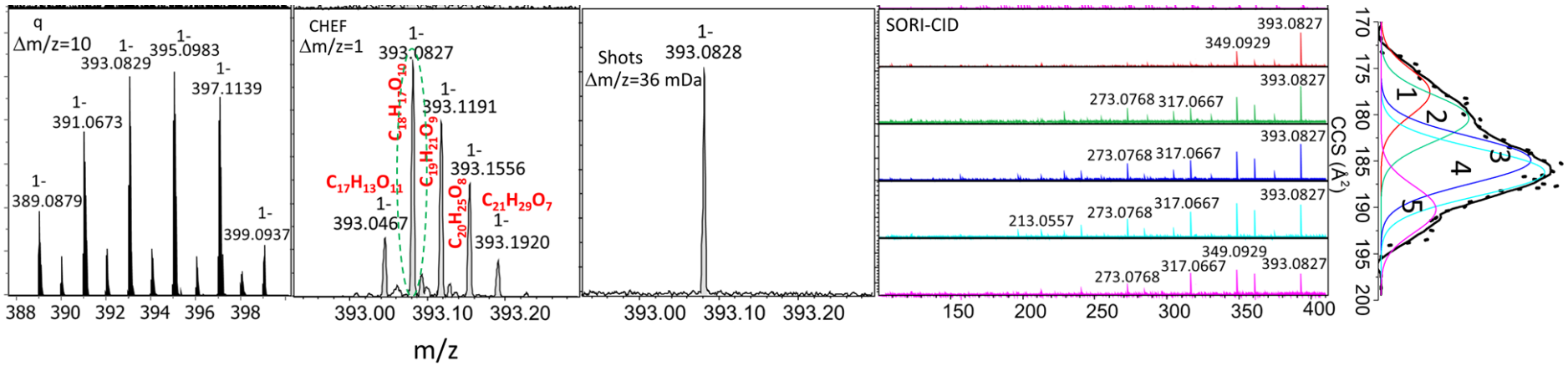


TIMS-FT-ICR MS/MS (CHEF-SORI-CID) workflow

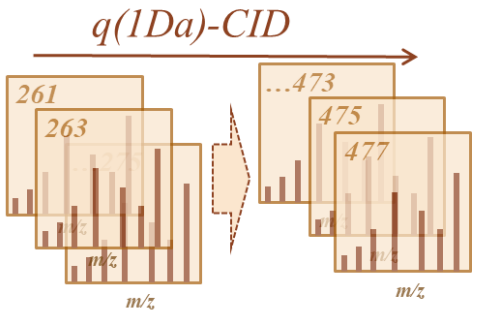
This workflow narrows down the pool of isomeric candidate structures for DOM.



We can mobility select (high res) and fragment single chemical formulas of DOM.



Conclusions



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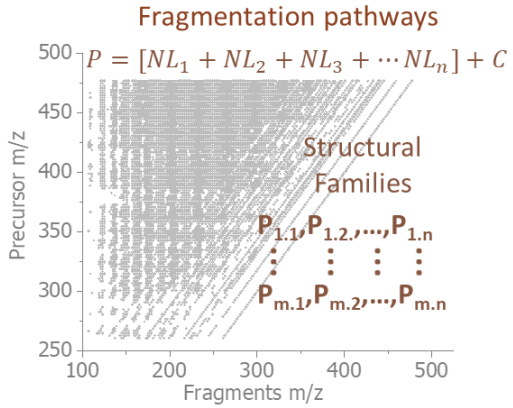

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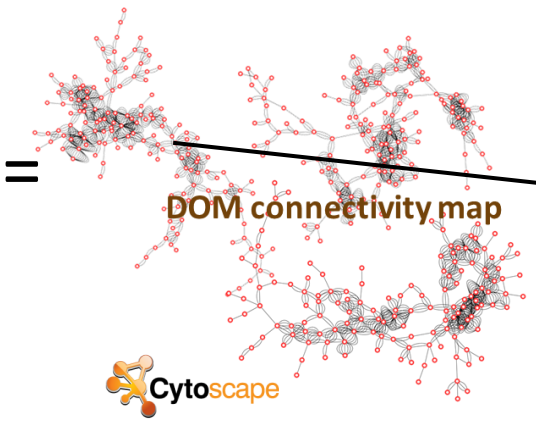


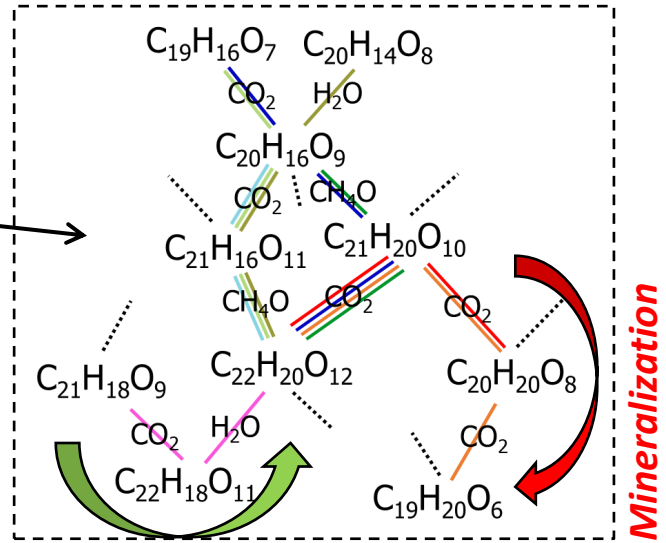


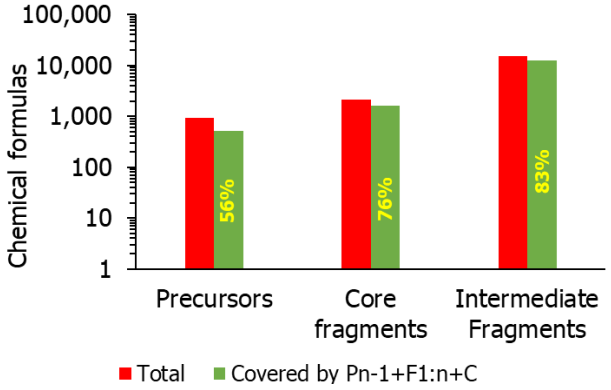
Photo-oxidation

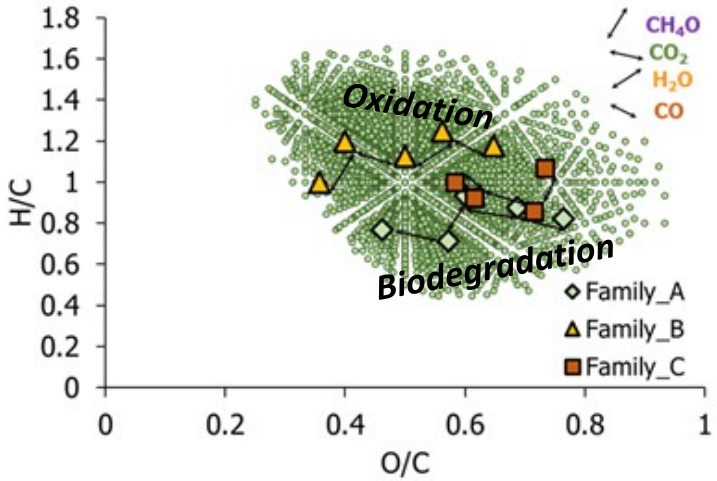
(-)ESI-FT-ICR CASI-CID MS/MS

Graph-DOM

Novel approach to classify DOM structural families

~1,100 structural families identified





DOM families are more complex than traditionally described.

Useful for understanding DOM transformational mechanisms.

Graph-DOM

Freely available at:
https://github.com/Usman095/Graph-DOM

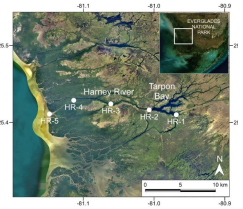
Future work

Chemical fingerprinting of DOM using CASI-CID FT-ICR MS/MS

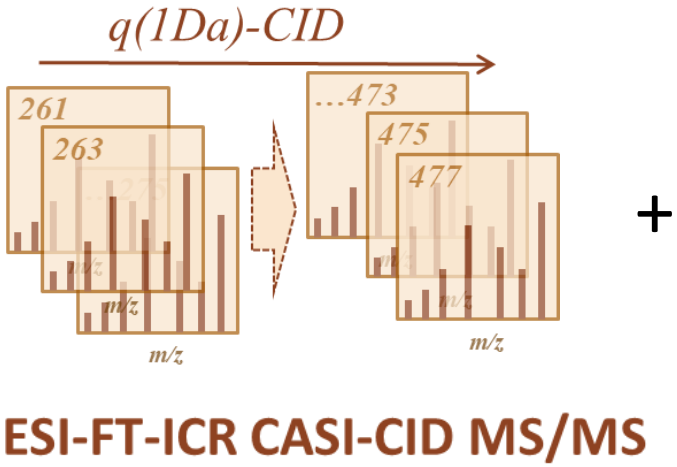
SRFA

Wetland

Freshwater



Estuary



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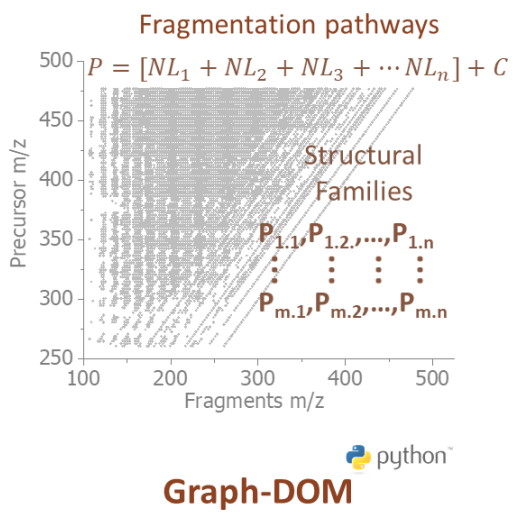

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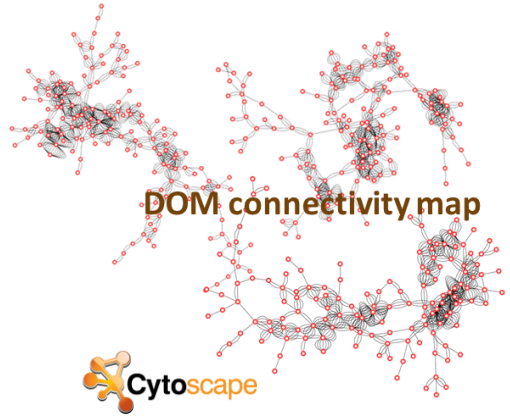
Common structural families



Unique structural families

Future work

DOM isomeric complexity along a salinity transect