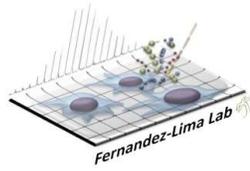


“Characterization of DOM Using TIMS-UHR-MS/MS”

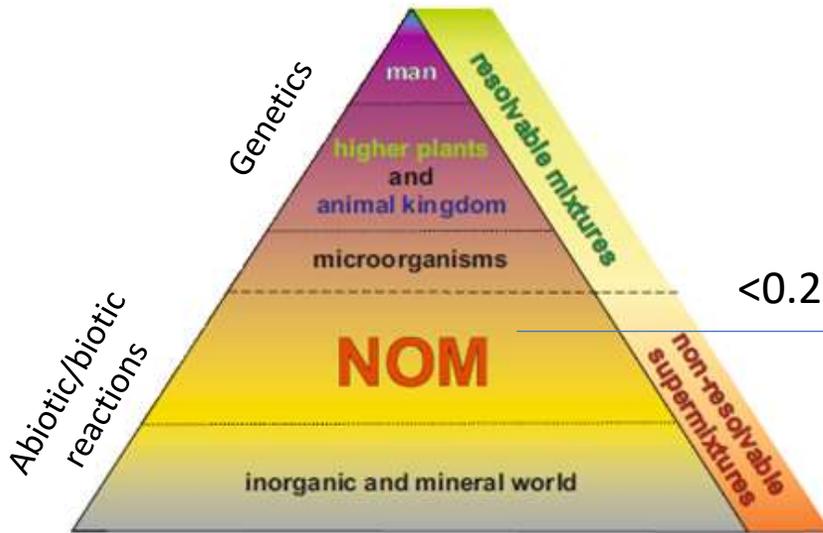
**Prof. Francisco Fernandez-Lima
Dpt of Chemistry and Biochemistry
Florida International University**

Dissolved Organic Matter



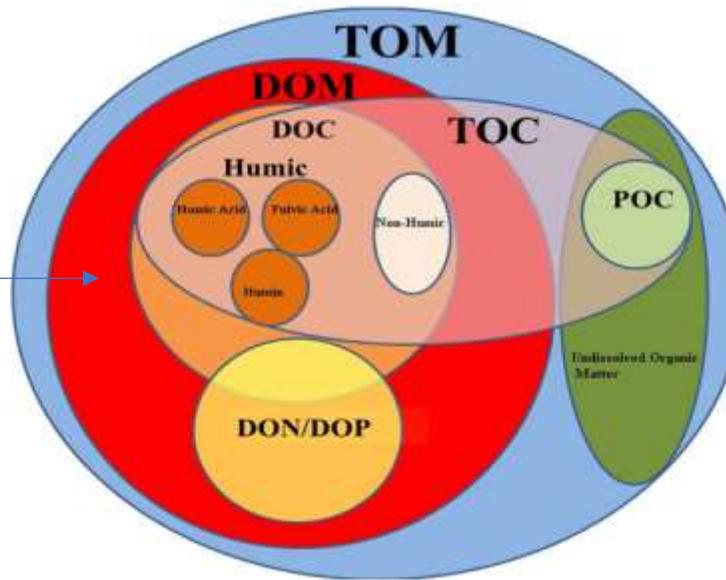
Chemical composition: CHONSP

DOM: Dissolved Organic Matter

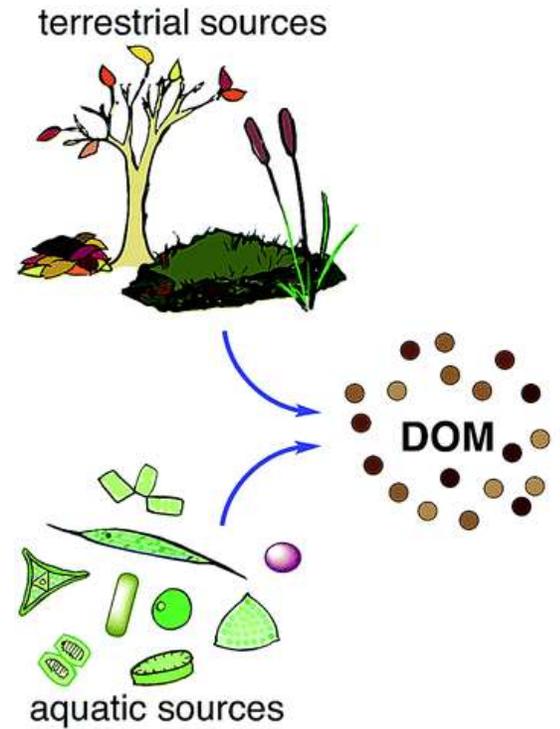


NOM: Natural Organic Matter

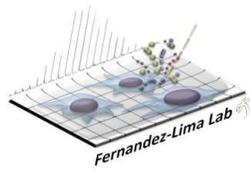
<0.2 μm



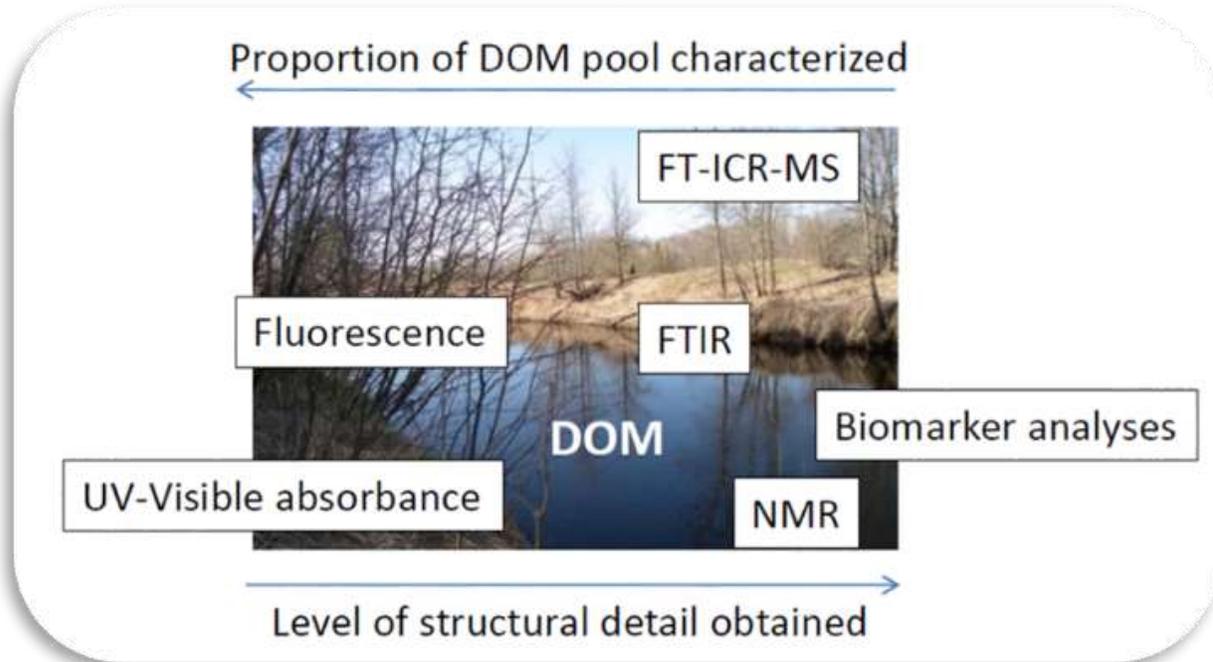
Forms of organic matter



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

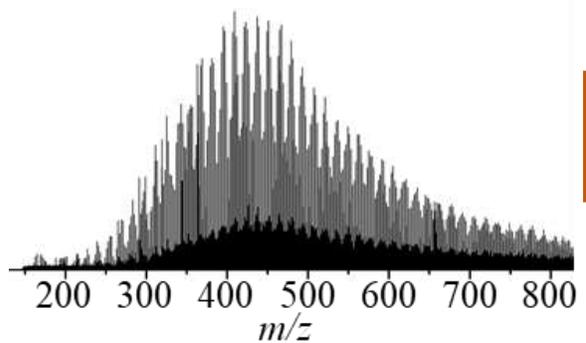
Traditional untargeted workflow for DOM analysis



Direct infusion



Broadband MS

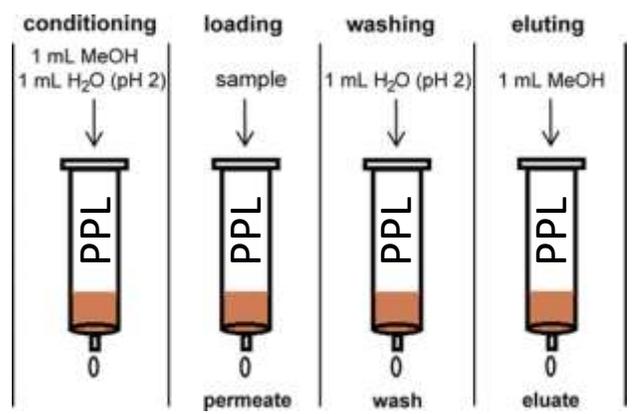


Chemical formula assignment

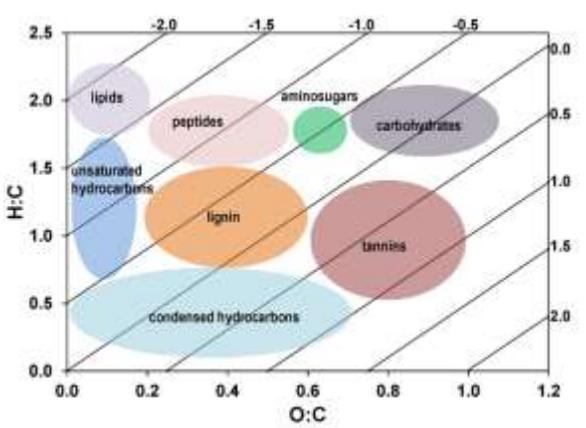
Formula constraints:

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

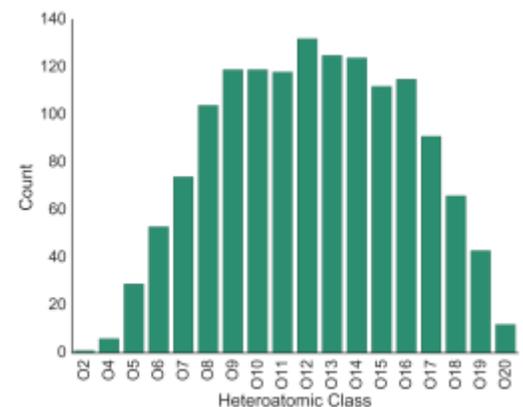
Solid phase extraction



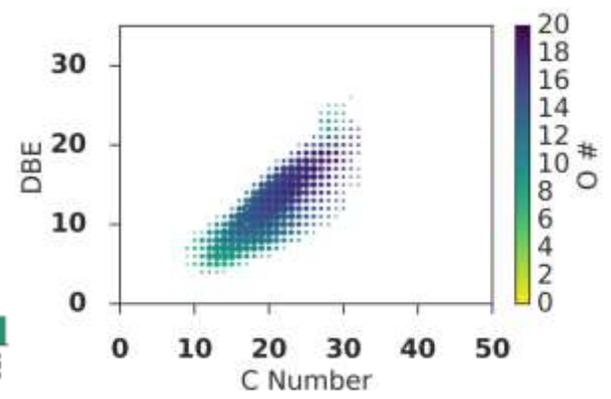
Compositional space



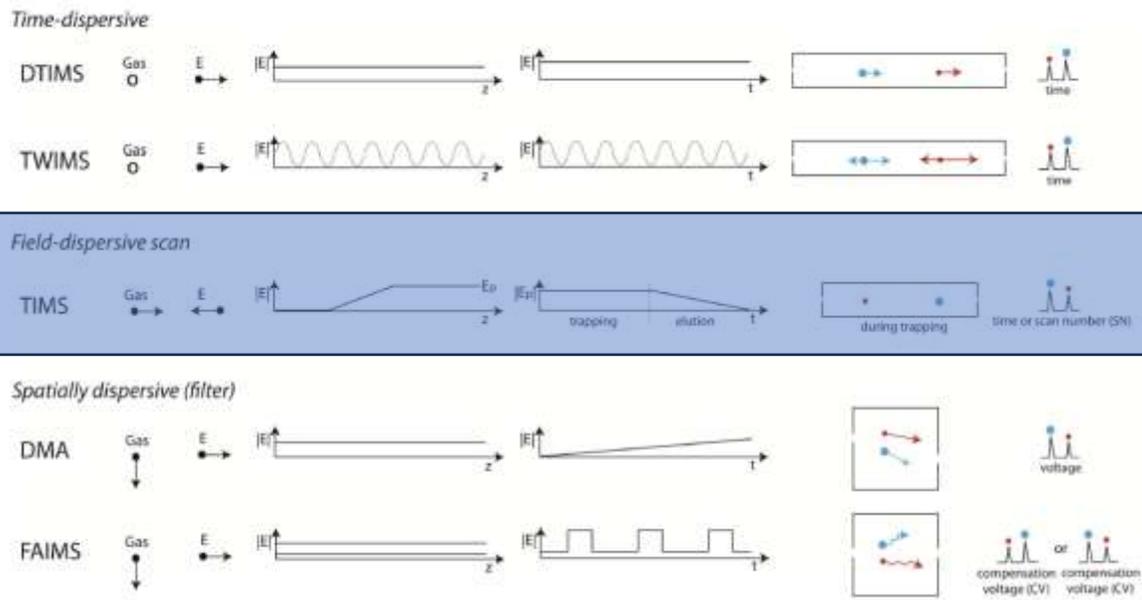
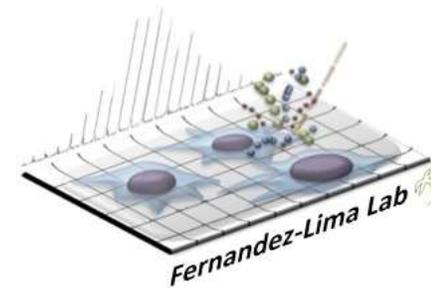
Heteroatom classes



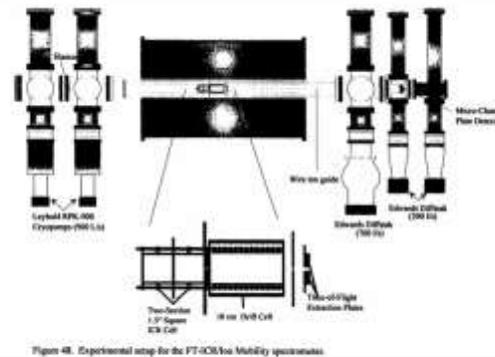
DBE vs C#



IMS and FT-ICR MS

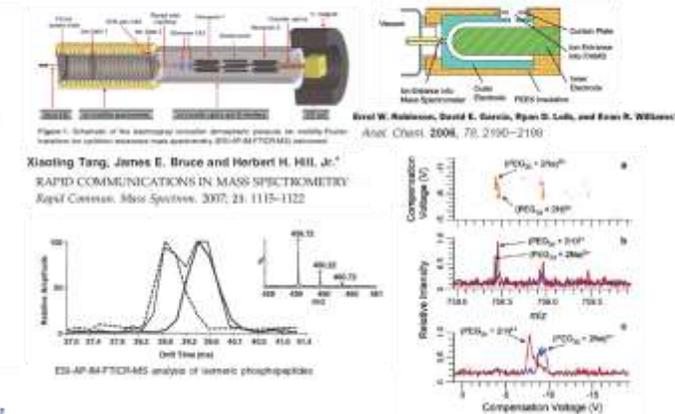


7T FT-DT-ICR-IMS-TOF MS



The development of a Fourier transform cyclotron resonance ion mobility spectrometer and studies of ion motion in a wire ion guide cell. [Gillig, Kent James](#). Texas A&M University ProQuest Dissertations & Theses, 1997. 9815740

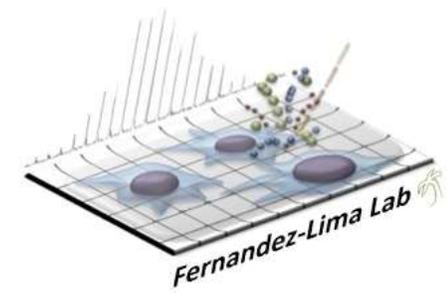
DTIMS-FT-ICR MS & FAIMS-FT-ICR MS



and other drift ICR measurements*...

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

TIMS and FT-ICR MS



John Kominoski, PhD.



Dennis Leyva, PhD.



Lilian V. Tose, PhD.



Rudolph Jaffe, PhD.



Jacob Porter, PhD.



Paolo Benigni

Catherine Costello, PhD.



SA-TIMS FT-ICR
ECD MS/MS
BU (2016)

OSA-TIMS
FT-ICR MS
IRMPD
(2016)

Non-linear TIMS
(2018)

TIMS-CHEF
SORI-CID
(2020)

CASI-FTMS
Graph-DOM
(2022)



SA-TIMS
FT-ICR MS
FIU (2015)



Mark E. Ridgeway, PhD.



Melvin A. Park, PhD.



Chris J. Thompson, PhD.



Paul Spier, PhD.



Fahad Saeed, PhD.

Jeremy wolf, Steve Van Orden, Joel&Greg, Joshua, Olivier...



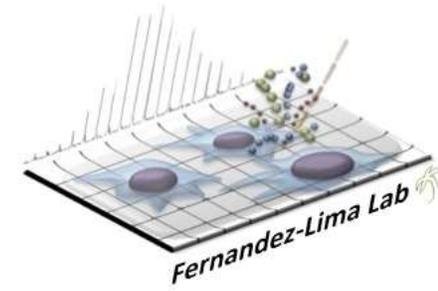
Desmond Kaplan, PhD.

TIMS-
qTOF
(2010)

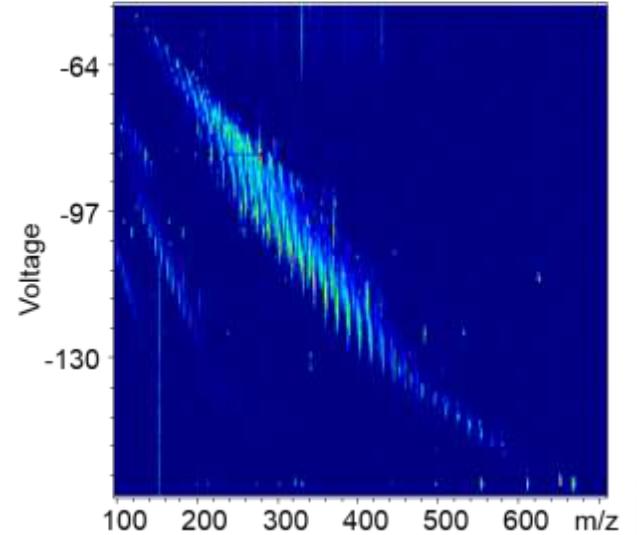
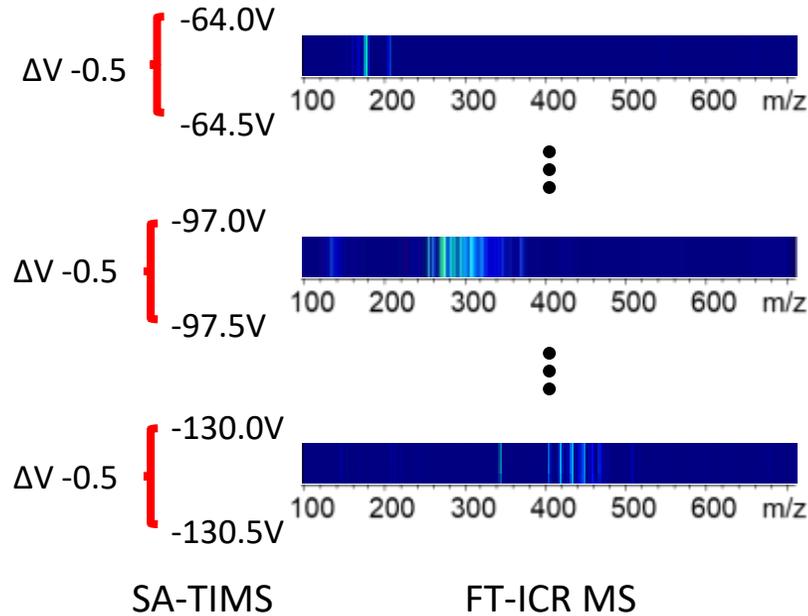
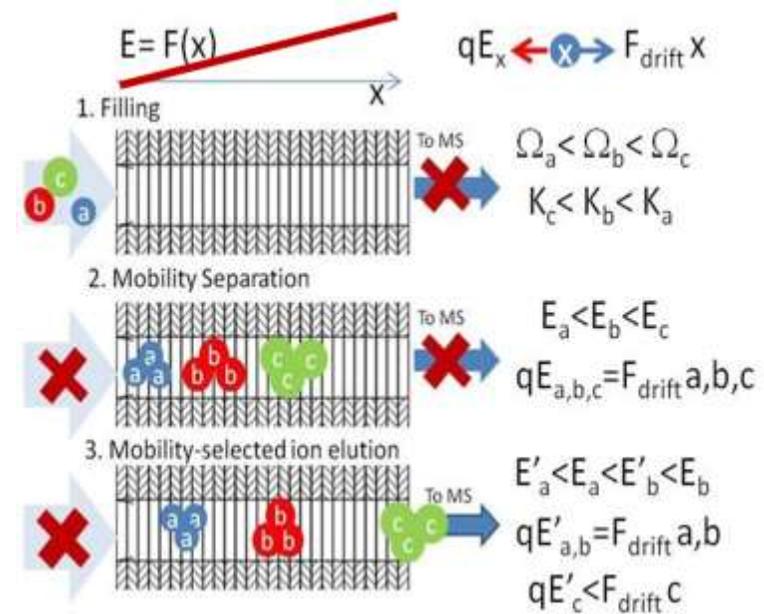
SA-TIMS
FT-ICR MS
TAMU
(2011-13)

TIMS and FT-ICR MS

SA-TIMS Principles of operation



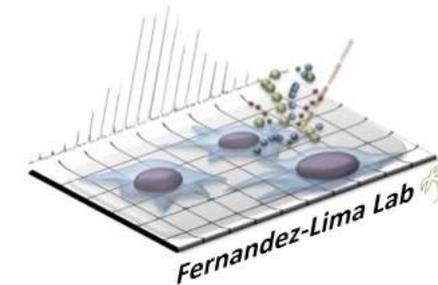
Scanning the voltage (mobility) range



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.

TIMS and FT-ICR MS

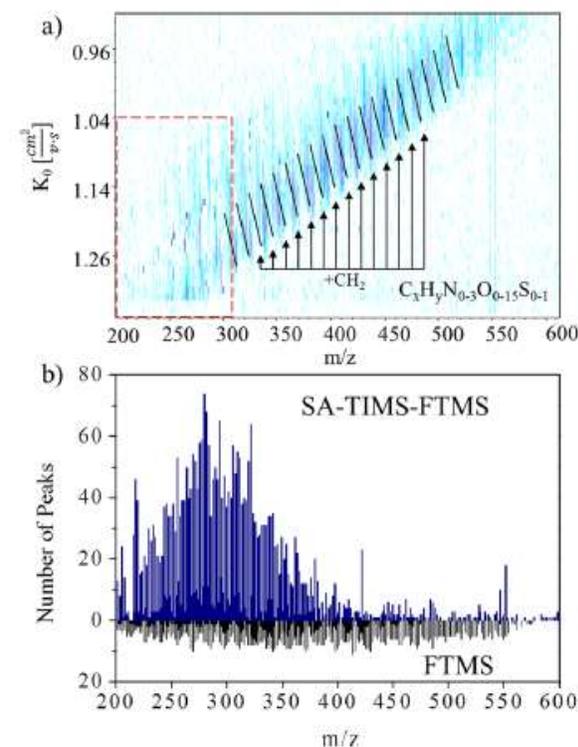
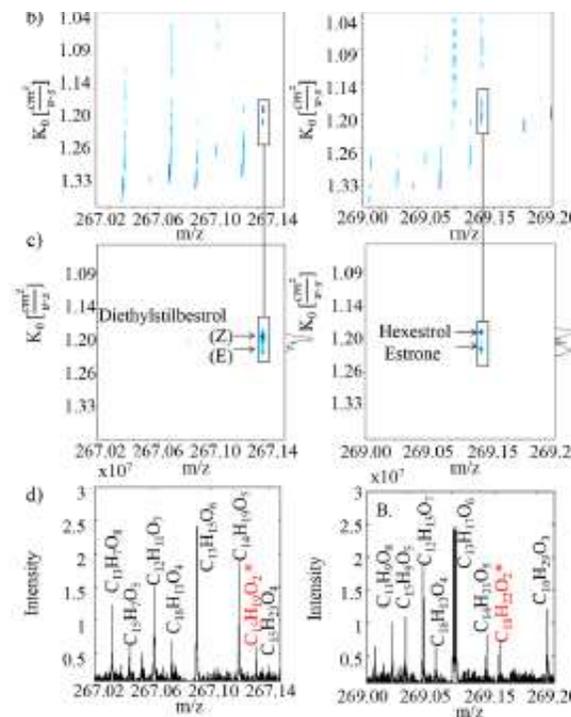
2015 SA-TIMS-q-FT-ICR MS/MS



cis/trans isomers

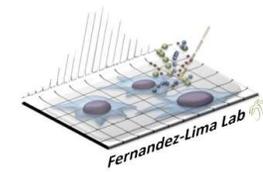


structural isomers

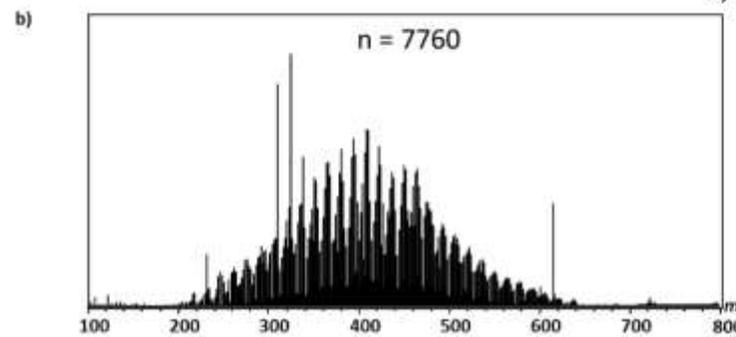
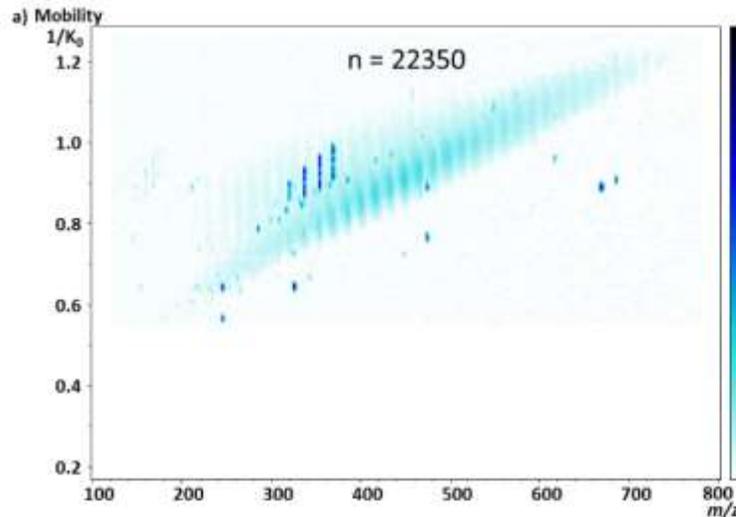


P. Benigni, C. J. Thompson, M. E. Ridgeway, P. A. Park, F. Fernandez-Lima. Targeted high resolution ion mobility separation coupled to ultra-high resolution mass spectrometry of endocrine disruptors in complex mixtures. *Anal. Chem.* 87 (8), 4321–4325, 2015.

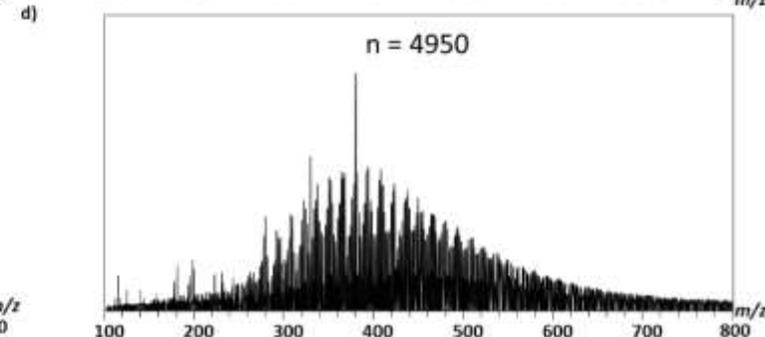
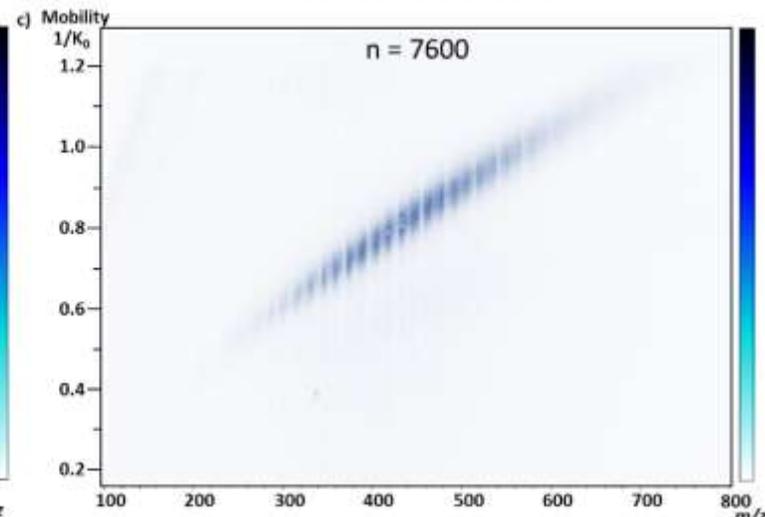
TIMS and FT-ICR MS



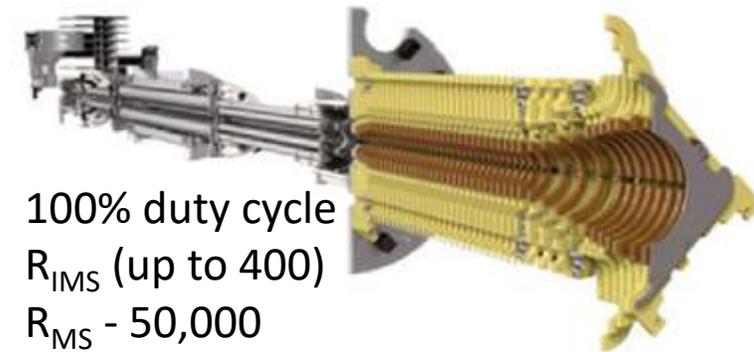
TIMS-FT-ICR MS



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



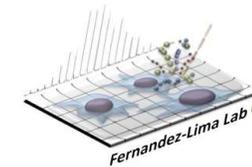
R_{IMS} (up to 400)
 R_{MS} >400,000

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

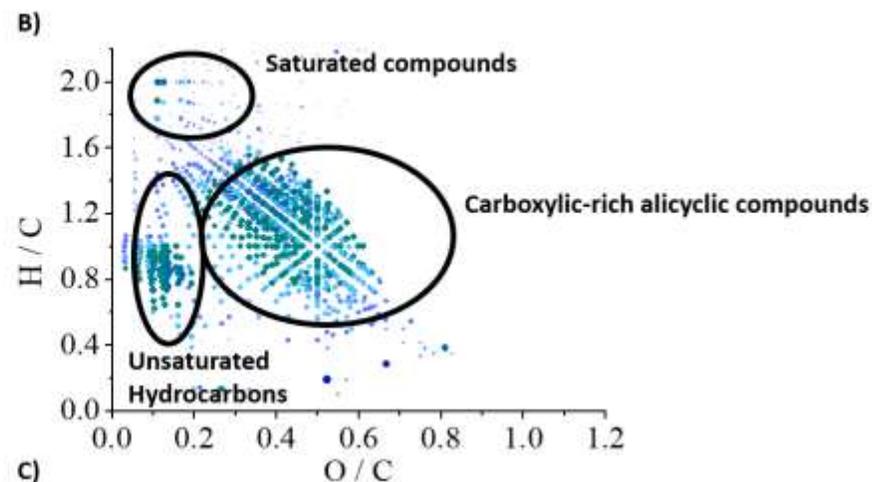
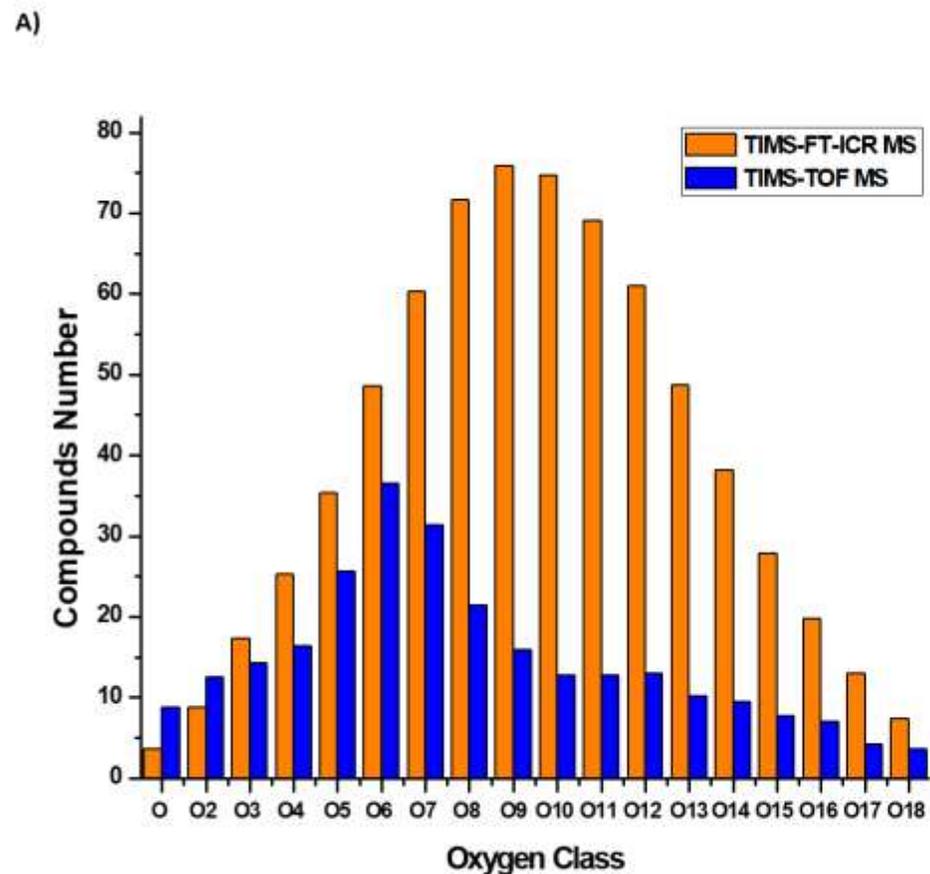


L.V. Tose, P. Benigni, D. Leyva, A. Sundberg, C. E. Ramírez, M. E. Ridgeway, M. A. Park, Wanderson Romão, R. Jaffé, F. Fernandez-Lima, "Coupling Trapped Ion Mobility Spectrometry to Mass Spectrometry: TIMS-TOF MS vs TIMS-FT-ICR MS", *Rapid Comm. Mass Spectrom.* 2018, 32, 1287– 1295.

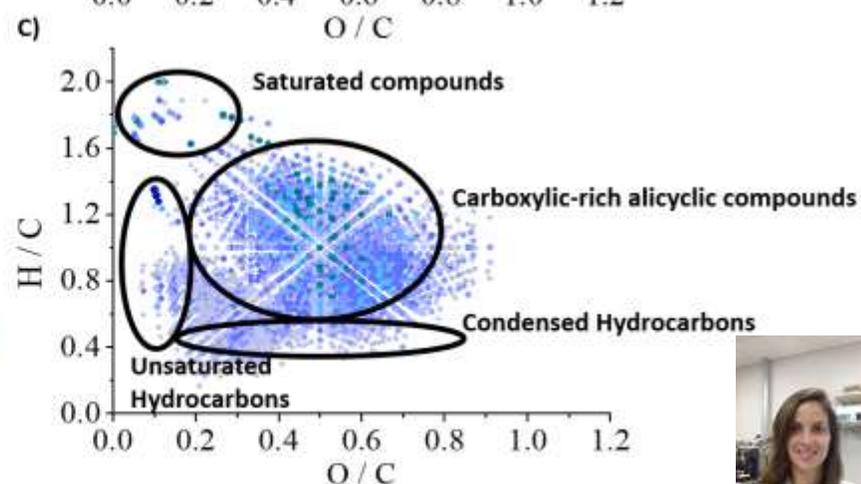
TIMS and FT-ICR MS



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



TIMS-q-TOF MS/MS



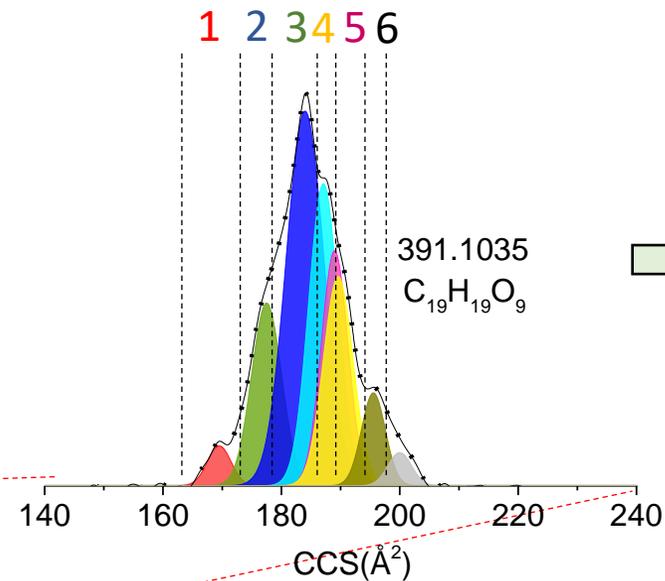
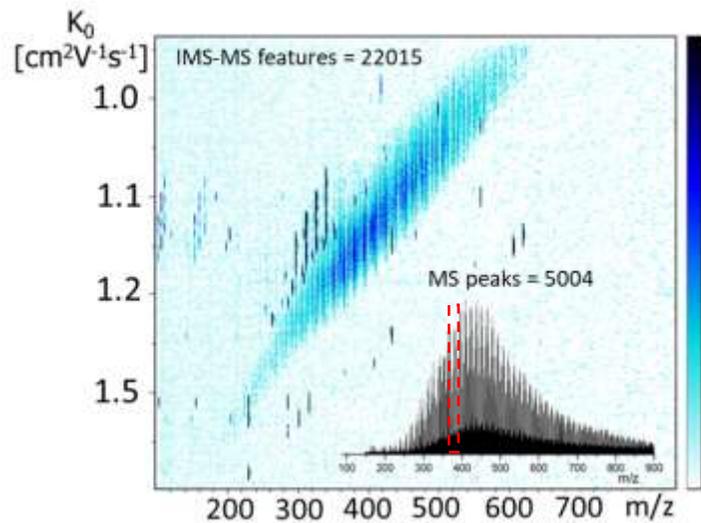
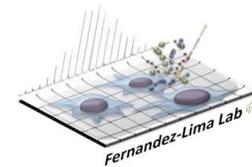
TIMS-q-FT-ICR MS/MS



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.

TIMS and FT-ICR MS

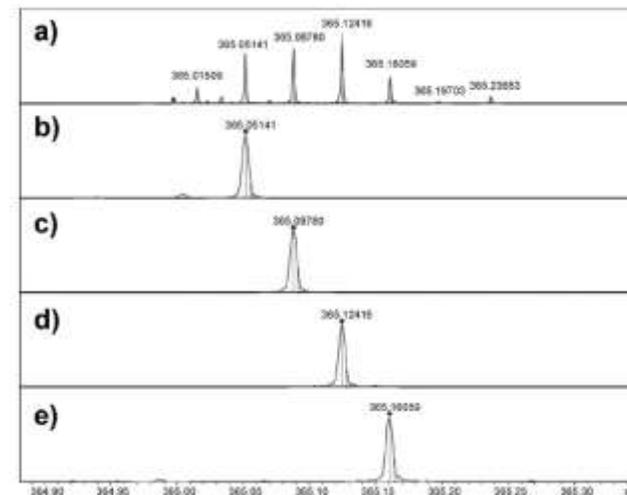
How can we improve confidence during DOM structural assignment?



IMS1 \rightarrow MS/MS₁ \rightarrow Structures (1)

IMS2 \rightarrow MS/MS₂ \rightarrow Structures (2)

IMS_n \rightarrow MS/MS_n \rightarrow Structures (n)



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

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

GEER2025



TIMS and FT-ICR MS

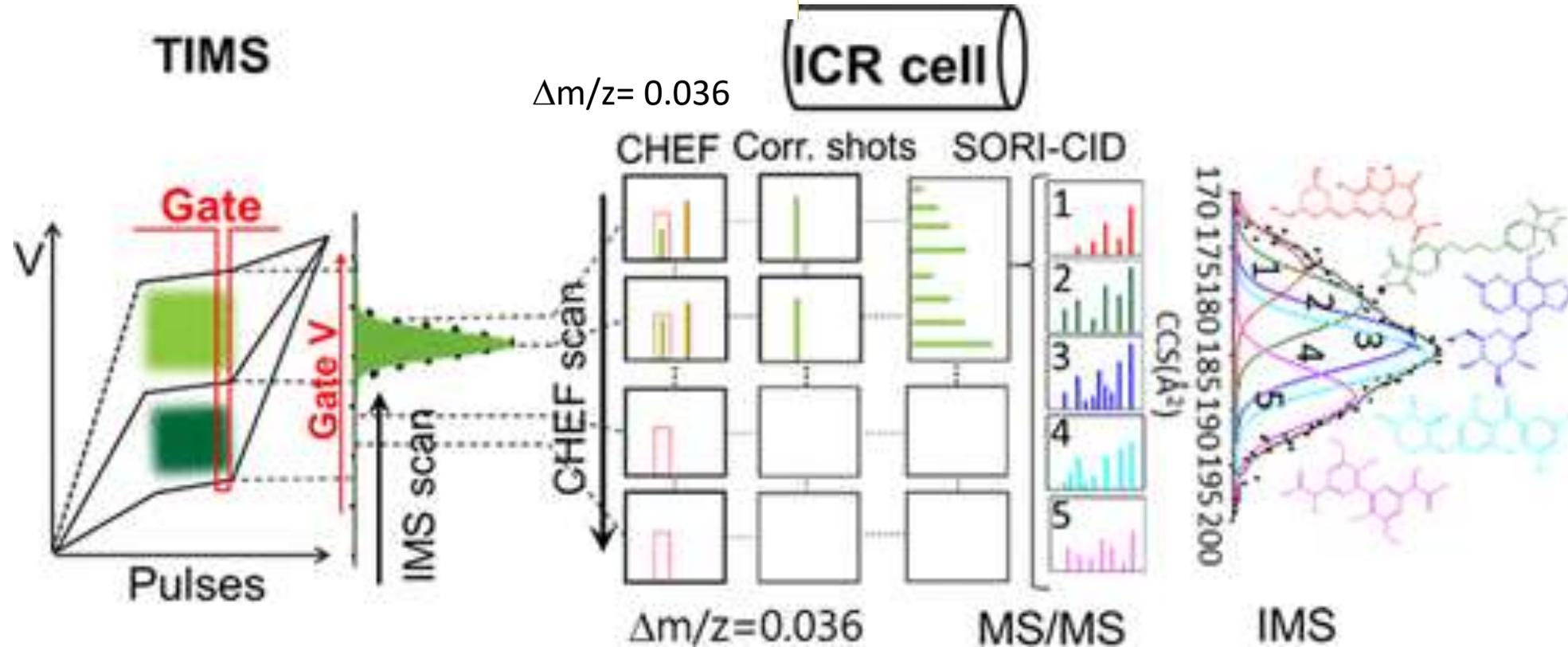
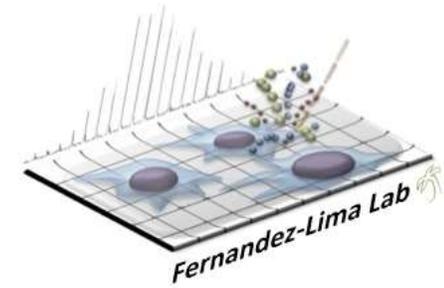
2020

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



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

Correlated harmonic excitation field (CHEF) and shots ejection of isobaric ions



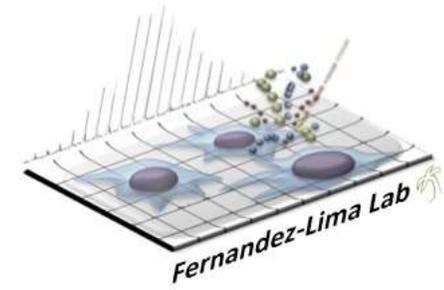
TIMS and FT-ICR MS

2020

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

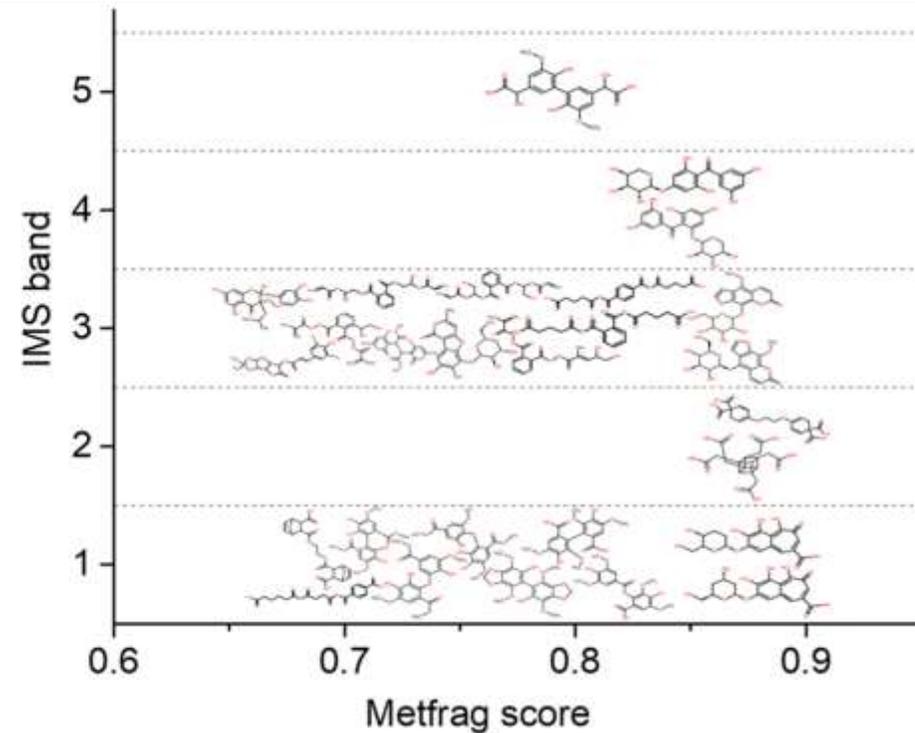
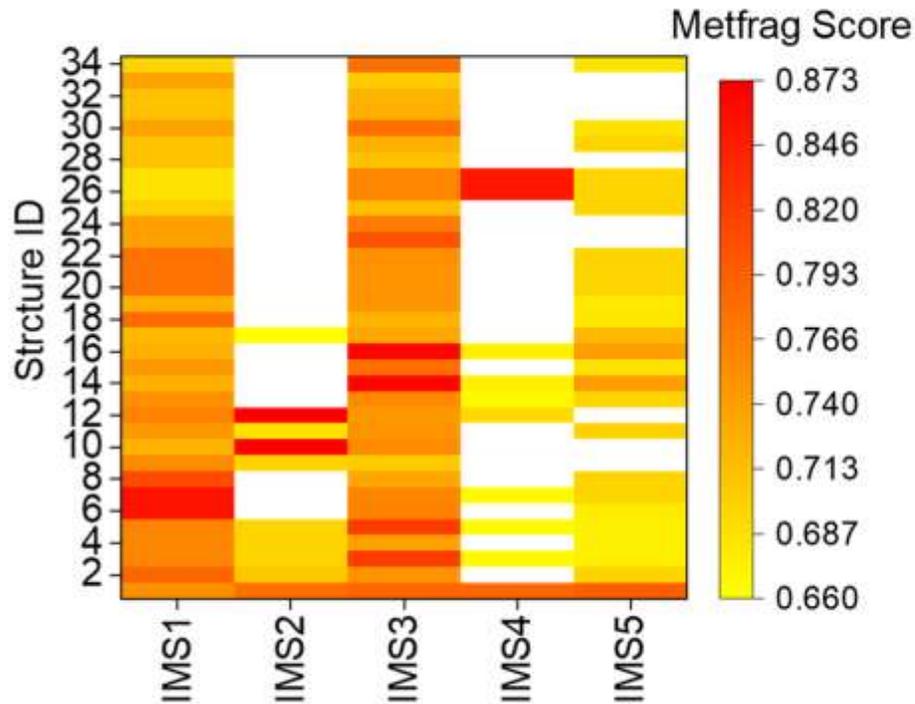


OSA-TIMS-q-FT-ICR CHEF SORI CID MS/MS



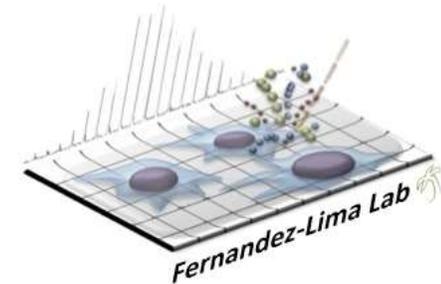
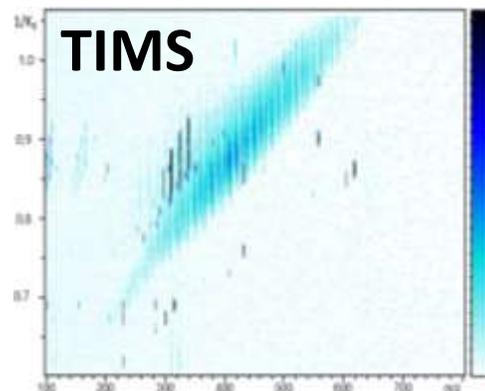
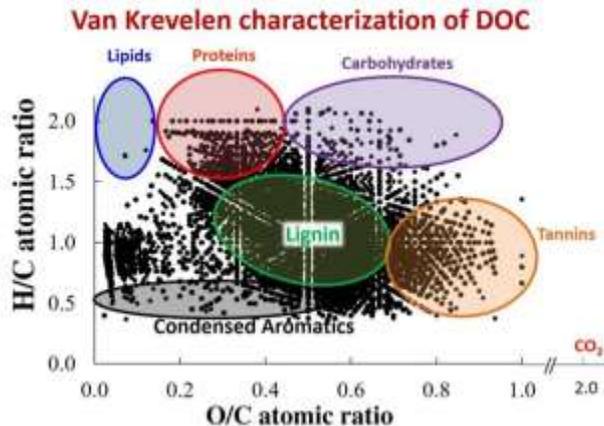
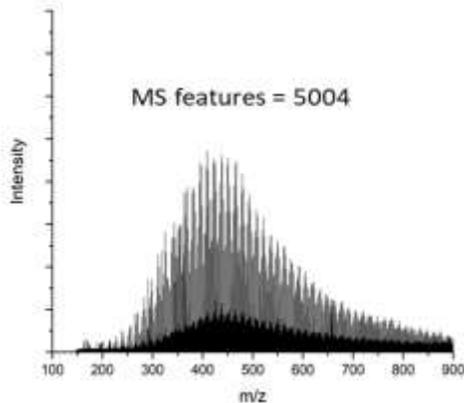
100-1000x reduction on the candidate assignment search space!!!!

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

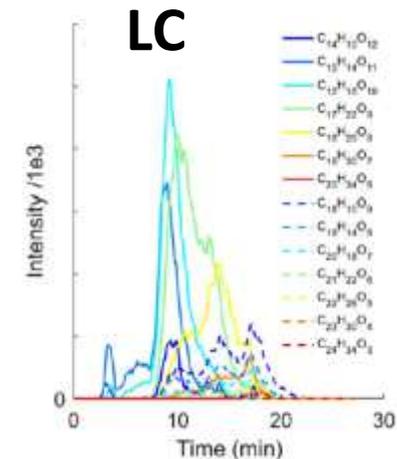
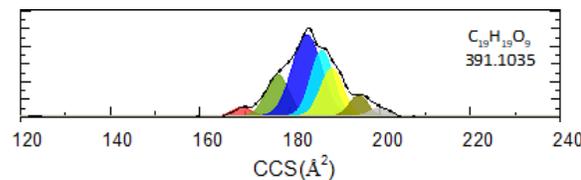


TIMS and FT-ICR MS

The analysis of DOM by UHRMS is very challenging!

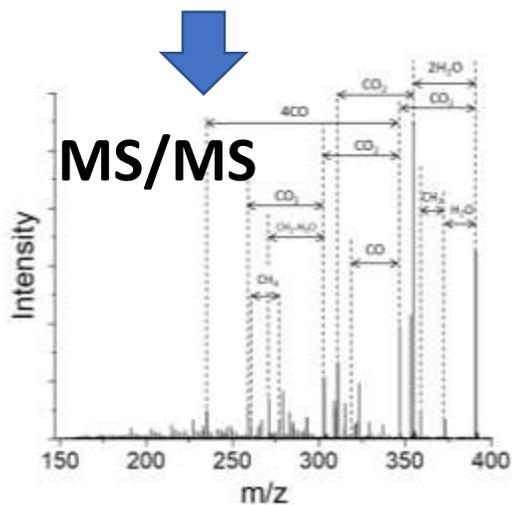


LC can not resolve DOM isomeric complexity

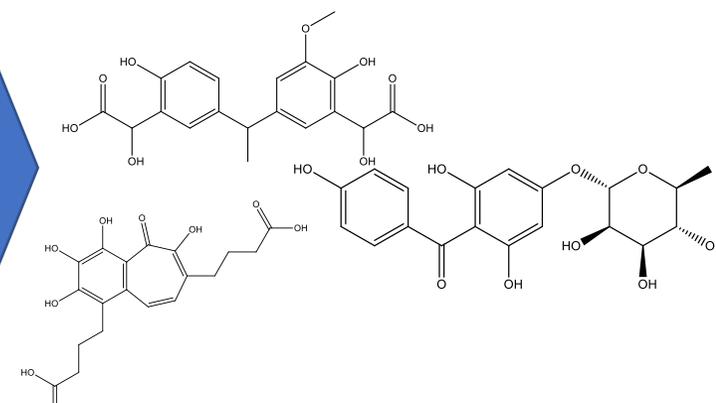
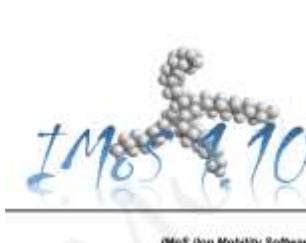


TIMS-FT ICR MS provides complementary separation and candidate structures for DOM.

DOM chemical fingerprint is partially uncovered by FTMS



Fragmentation pathways

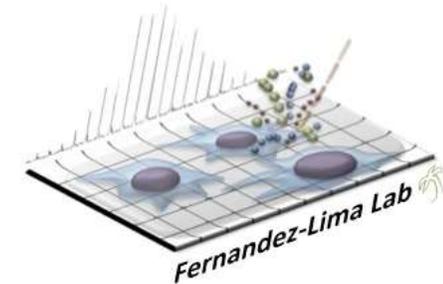


TIMS and FT-ICR MS

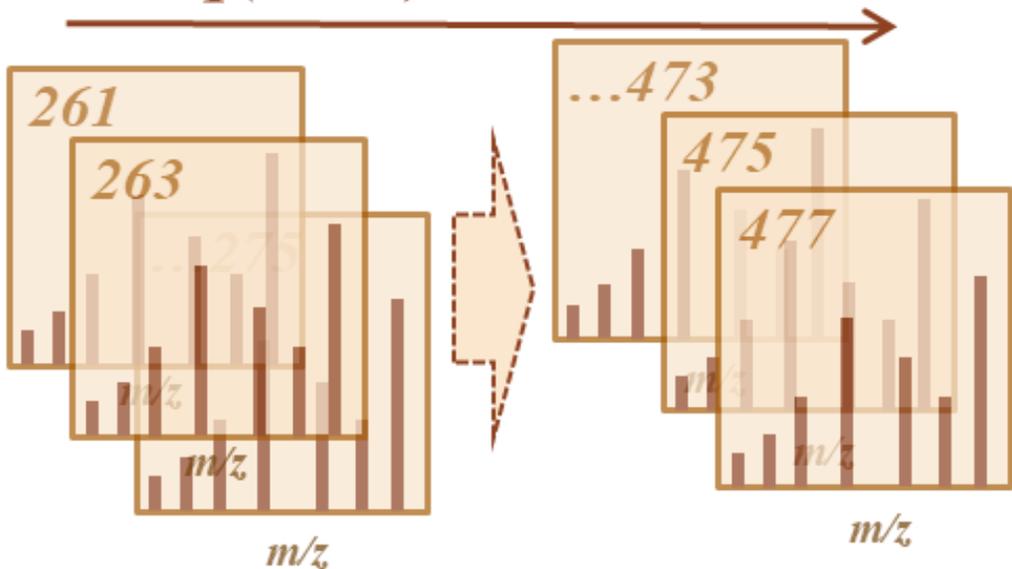
2022 R_{IMS} (up to 400)
 $R_{MS} > 400,000$



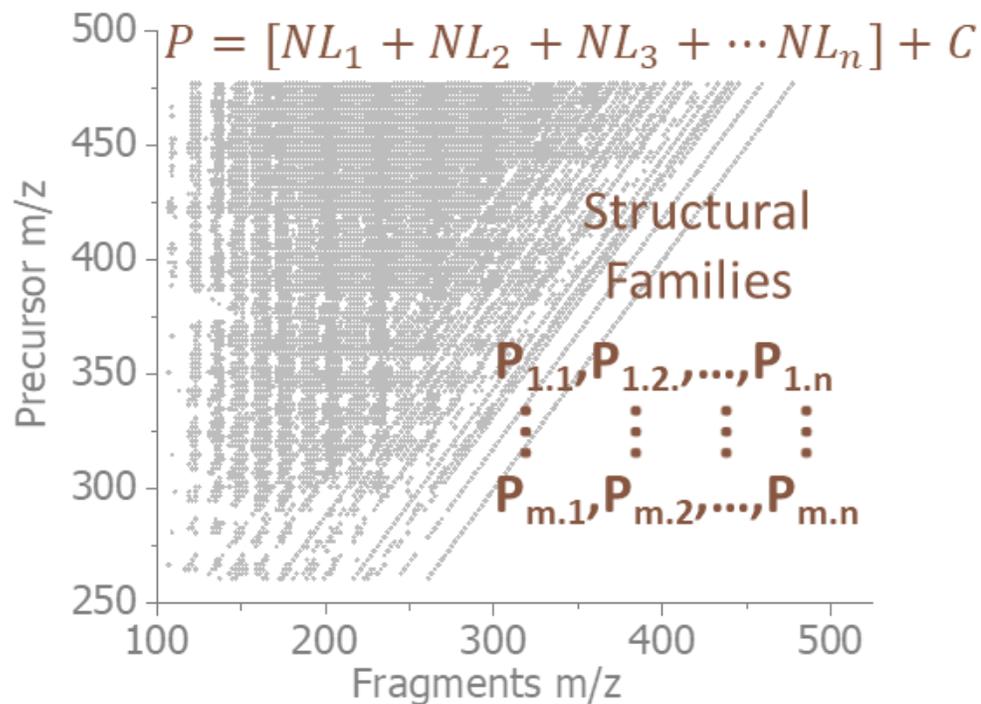
CASI-CID-FT-ICR MS/MS
 Graph-DOM



q(1Da)-CID



Fragmentation pathways



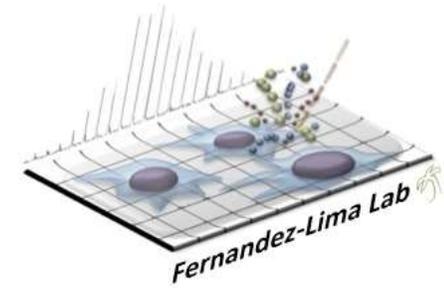
Graph-DOM

D. Leyva, M. Usman Tariq, R. Jaffé, F. Saeed, F. Fernandez-Lima*, "Description of Dissolved Organic Matter Transformational Networks at the Molecular Level", Environ. Sci. Technol. 57, 6, 2672–2681, 2023.
 D. Leyva, R. Jaffé, J. Courson, J. Kominoski, M. Usman Tariq, F. Saeed, F. Fernandez-Lima*, "Molecular level characterization of DOM along a freshwater-to-estuarine coastal gradient in the Florida Everglades", Aquatic Sci., 84 (4), 1-14, 2022.
 D. Leyva, M. U. Tariq, R. Jaffe, F. Saeed, F. Fernandez Lima*, "Unsupervised Structural Classification of Dissolved Organic Matter Based on Fragmentation Pathways", Environ. Sci. Technol., 56, 1458-1468, 2022.

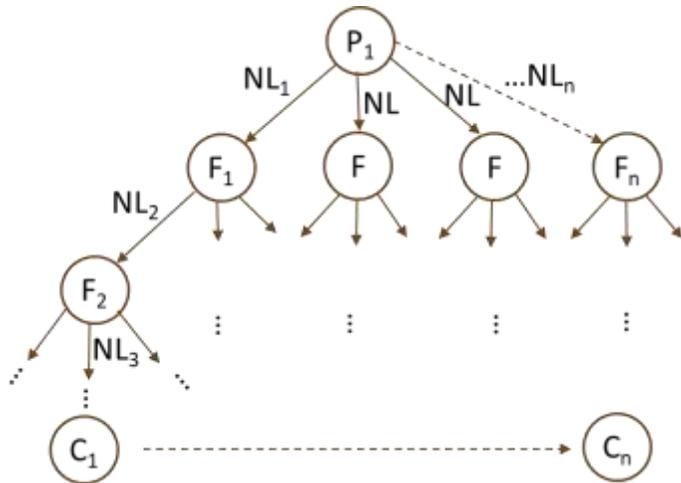
TIMS and FT-ICR MS

2022

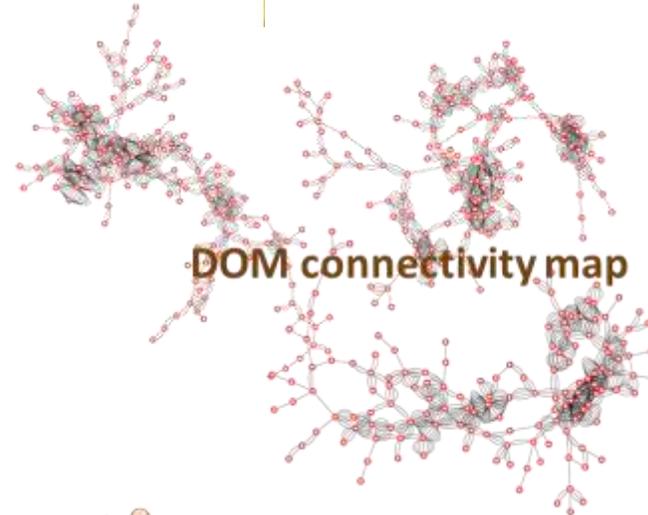
R_{IMS} (up to 400)
 $R_{MS} > 400,000$



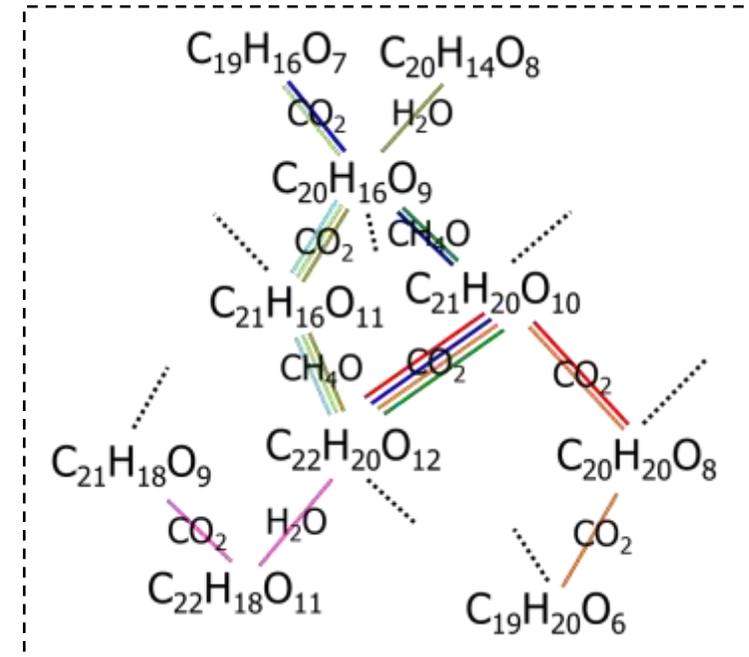
CASI-CID-FT-ICR MS/MS
 Graph-DOM



- Tree search algorithm
- NL set={O, CH₄, H₂O, CO, CH₂O, CH₄O, CO₂}
- Tol error ≤ 1 mDa

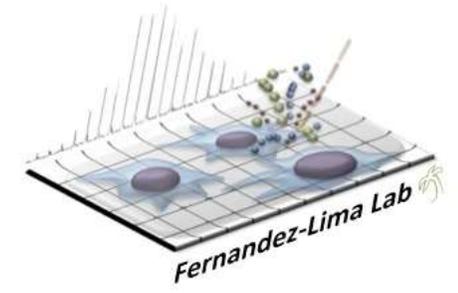


Color code	Family ID
Red	258
Green	392
Light Blue	393
Light Green	514
Dark Blue	515
Olive	841
Pink	890
Orange	999



D. Leyva, M. Usman Tariq, R. Jaffé, F. Saeed, F. Fernandez-Lima*, "Description of Dissolved Organic Matter Transformational Networks at the Molecular Level", *Environ. Sci. Technol.* 57, 6, 2672–2681, 2023.
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TIMS and FT-ICR MS



2022 R_{IMS} (up to 400)
 $R_{MS} > 400,000$

CASI-CID-FT-ICR MS/MS
Graph-DOM

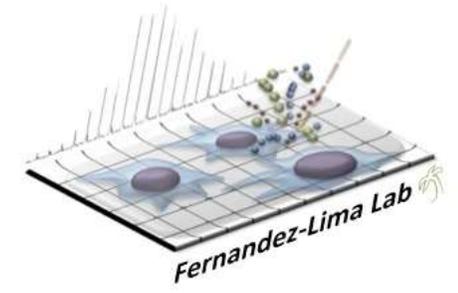
[SaeedLab - Graph-Dom](#)

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

D. Leyva, M. Usman Tariq, R. Jaffé, F. Saeed, F. Fernandez-Lima*, "Description of Dissolved Organic Matter Transformational Networks at the Molecular Level", Environ. Sci. Technol. 57, 6, 2672–2681, 2023.
D. Leyva, R. Jaffé, J. Courson, J. Kominoski, M. Usman Tariq, F. Saeed, F. Fernandez-Lima*, "Molecular level characterization of DOM along a freshwater-to-estuarine coastal gradient in the Florida Everglades", Aquatic Sci., 84 (4), 1-14, 2022.
D. Leyva, M. U. Tariq, R. Jaffe, F. Saeed, F. Fernandez Lima*, "Unsupervised Structural Classification of Dissolved Organic Matter Based on Fragmentation Pathways", Environ. Sci. Technol., 56, 1458-1468, 2022.

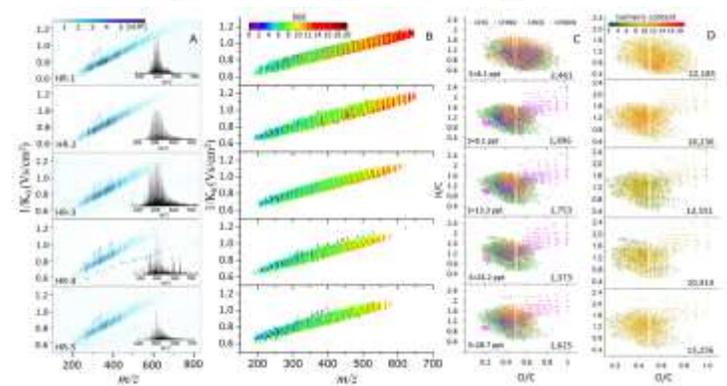
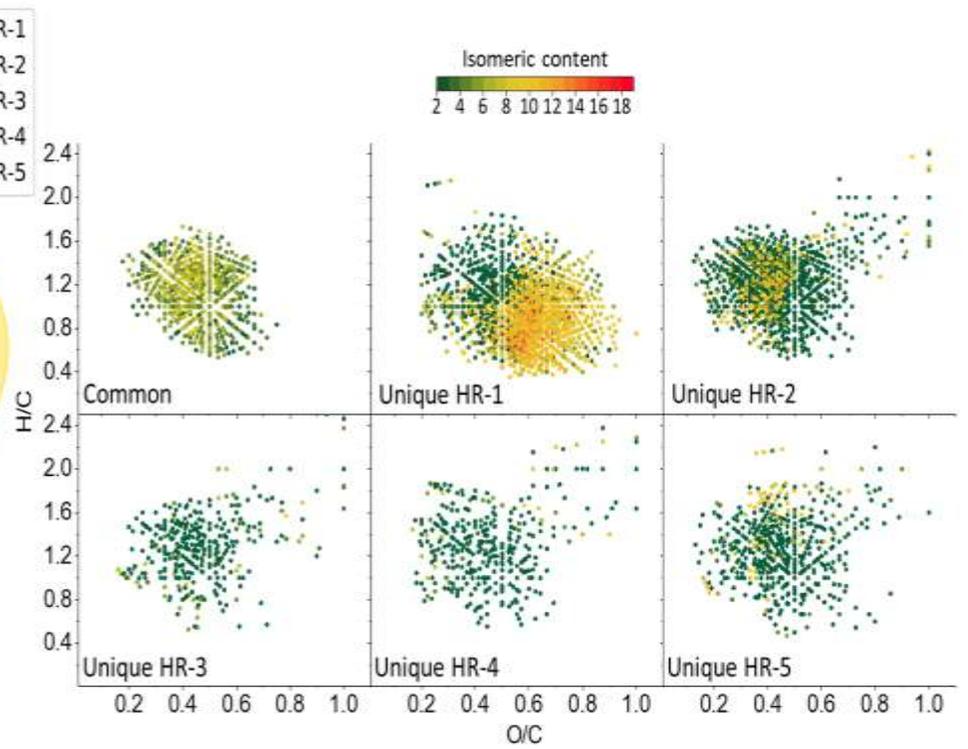
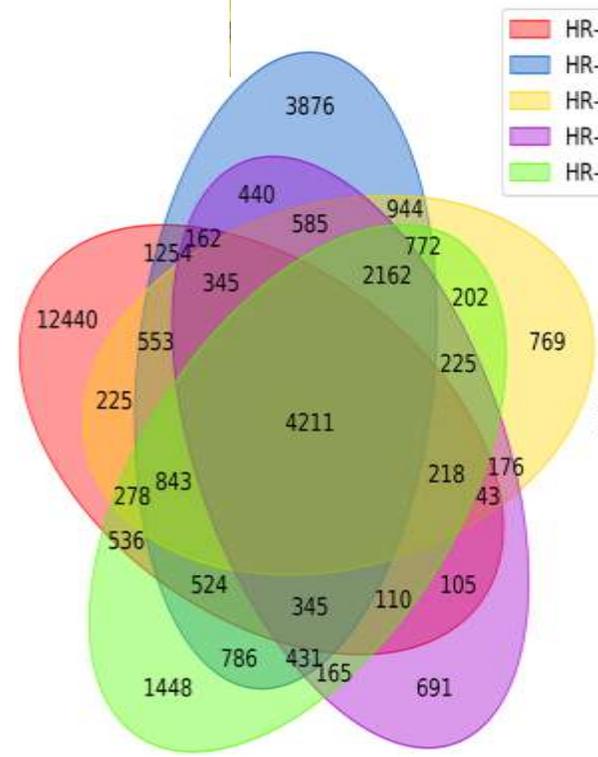
TIMS and FT-ICR MS

2022 R_{IMS} (up to 400)
 $R_{MS} > 400,000$



DOM isomeric complexity along a salinity

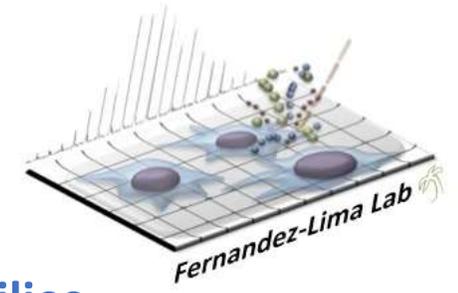
CASI-CID-FT-ICR MS/MS
 Graph-DOM



D. Leyva, R. Jaffé, J. Courson, J. Kominoski, M. Usman Tariq, F. Saeed, F. Fernandez-Lima*, "Molecular level characterization of DOM along a freshwater-to-estuarine coastal gradient in the Florida Everglades", *Aquatic Sci.*, 84 (4), 1-14, 2022

TIMS and FT-ICR MS

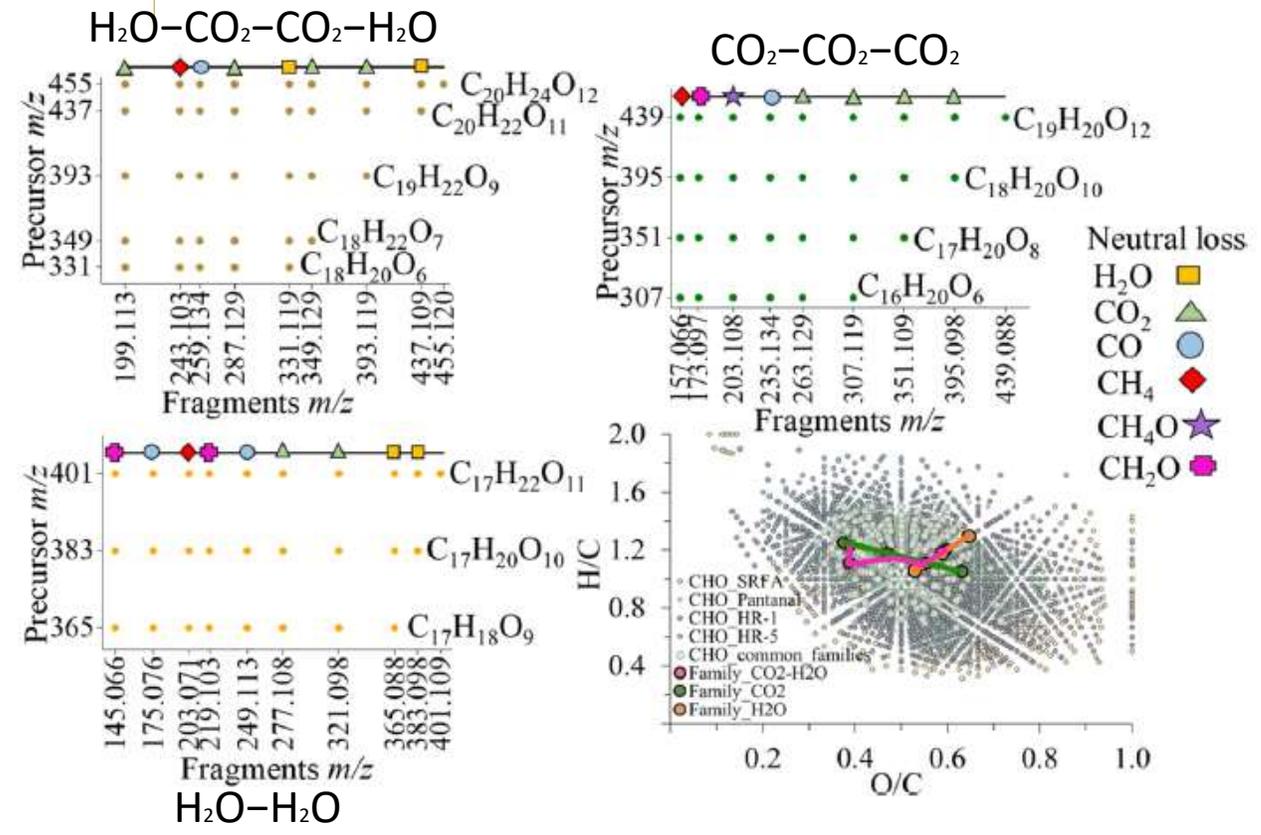
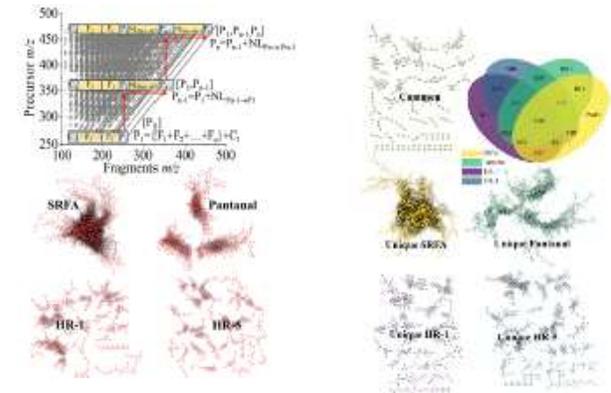
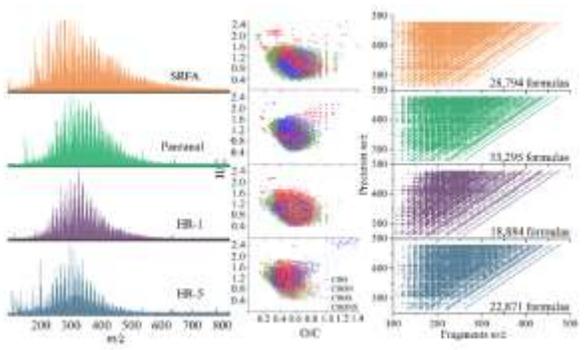
2022 R_{IMS} (up to 400)
 $R_{MS} > 400,000$



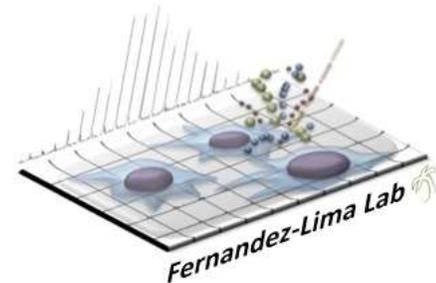
Fernandez-Lima Lab

Representation of three common CHO families

CASI-CID-FT-ICR MS/MS
 Graph-DOM



D. Leyva, M. Usman Tariq, R. Jaffé, F. Saeed, F. Fernandez-Lima*, "Description of Dissolved Organic Matter Transformational Networks at the Molecular Level", Environ. Sci. Technol. 57, 6, 2672–2681, 2023.



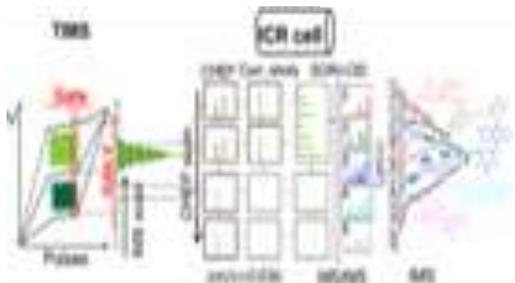
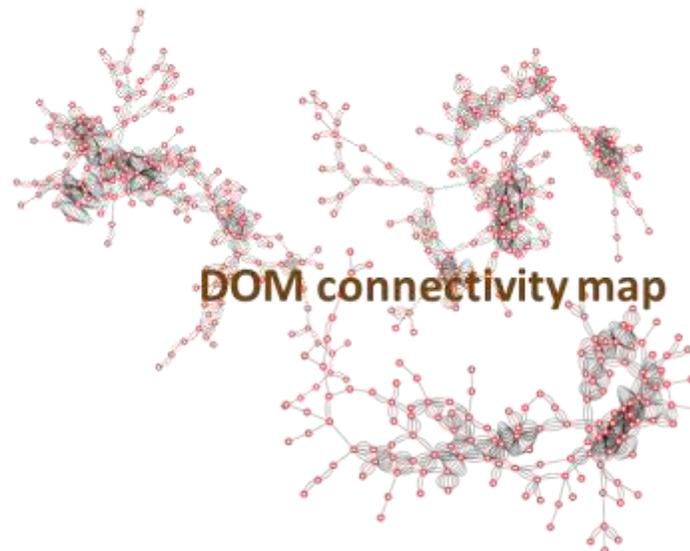
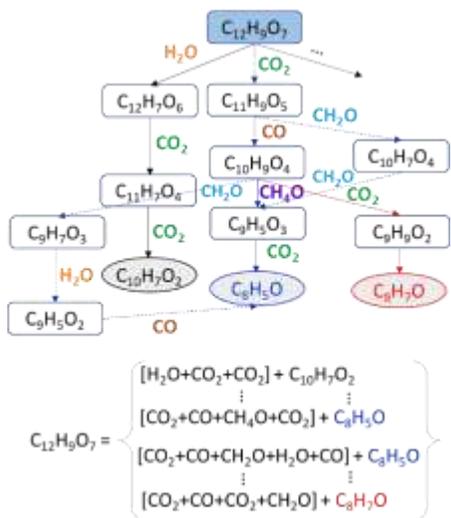
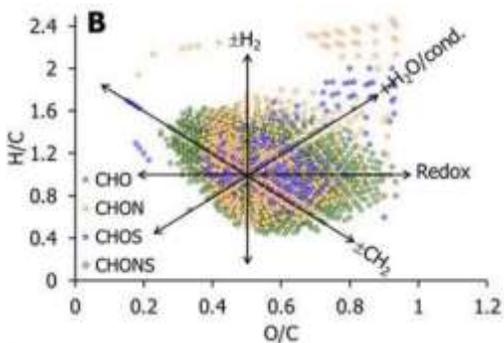
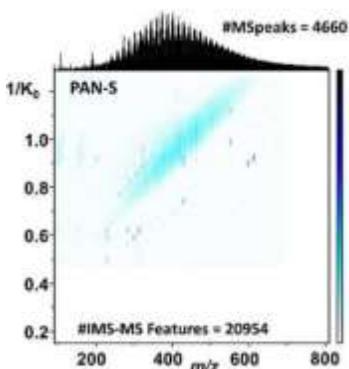
Development of Multidimensional IMSⁿ-FT-ICR MSⁿ Tools for the Characterization of Complex Mixtures

GEOMASS

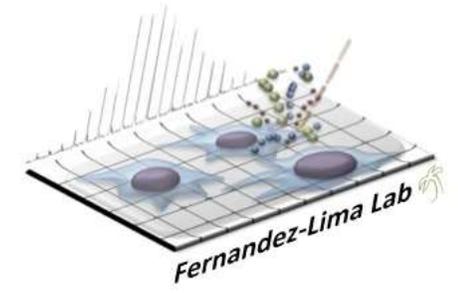
"MS experience for all"

Winter School
Analysis of Complex Mixtures
Spring Break Week
Florida International University MMC,

"Following environmental transformations at the molecular level"

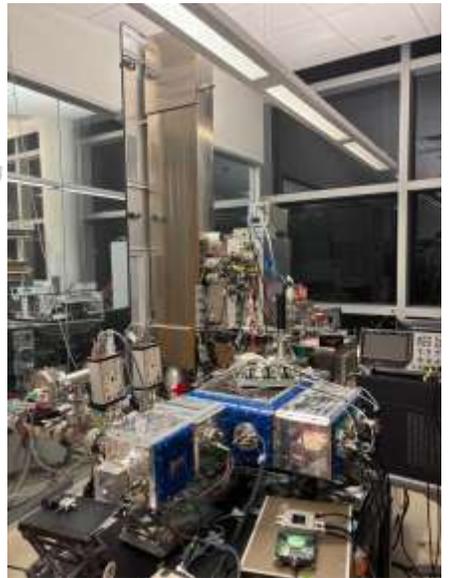
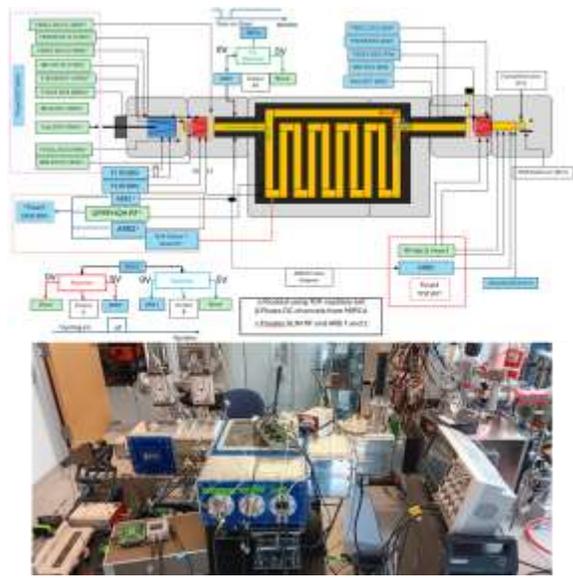
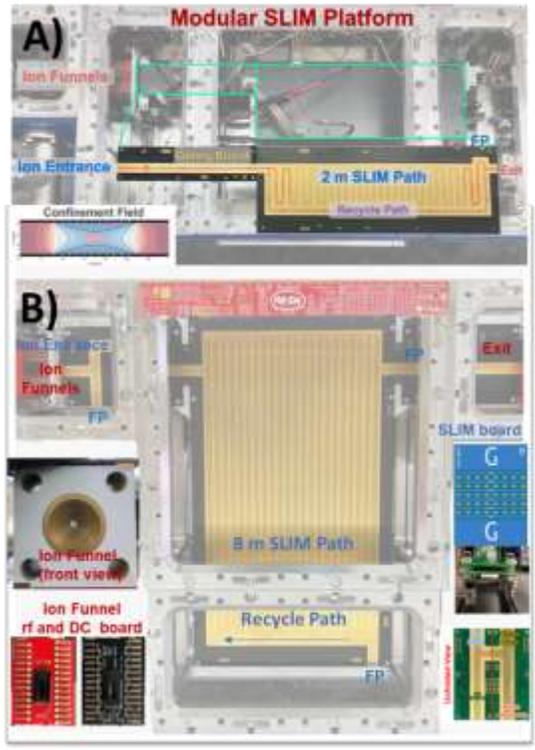
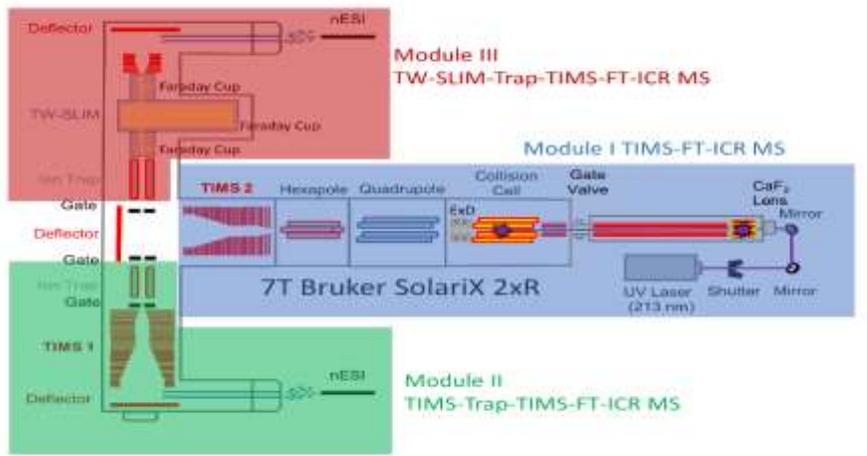


TIMS and FT-ICR MS



Hardware Upgrades:

SLIM –TIMS integration



CHE-2304837
Sept 2023-Aug 2026

<http://www.clowersresearch.com/main/open-source-ims-initiative/>



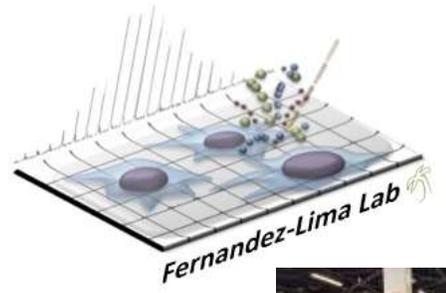
Dr. Brian Clowers
GAA electronics

TIMS and FT-ICR MS

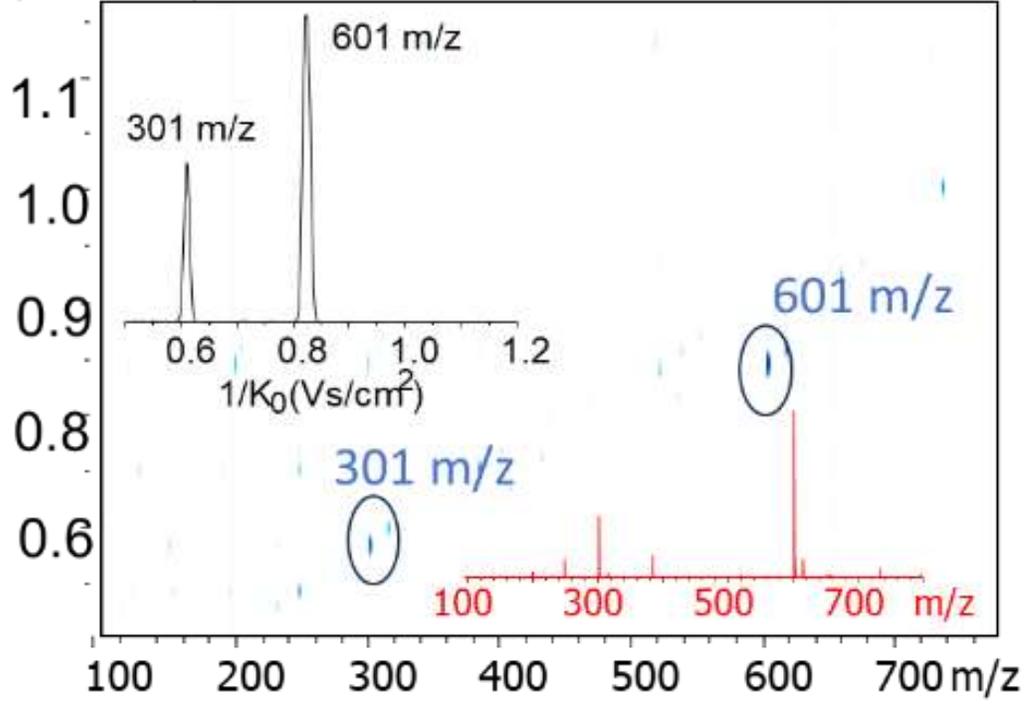
2024

9.4T OSA-TIMS-FT-ICR MS

“direct” Absorption mode



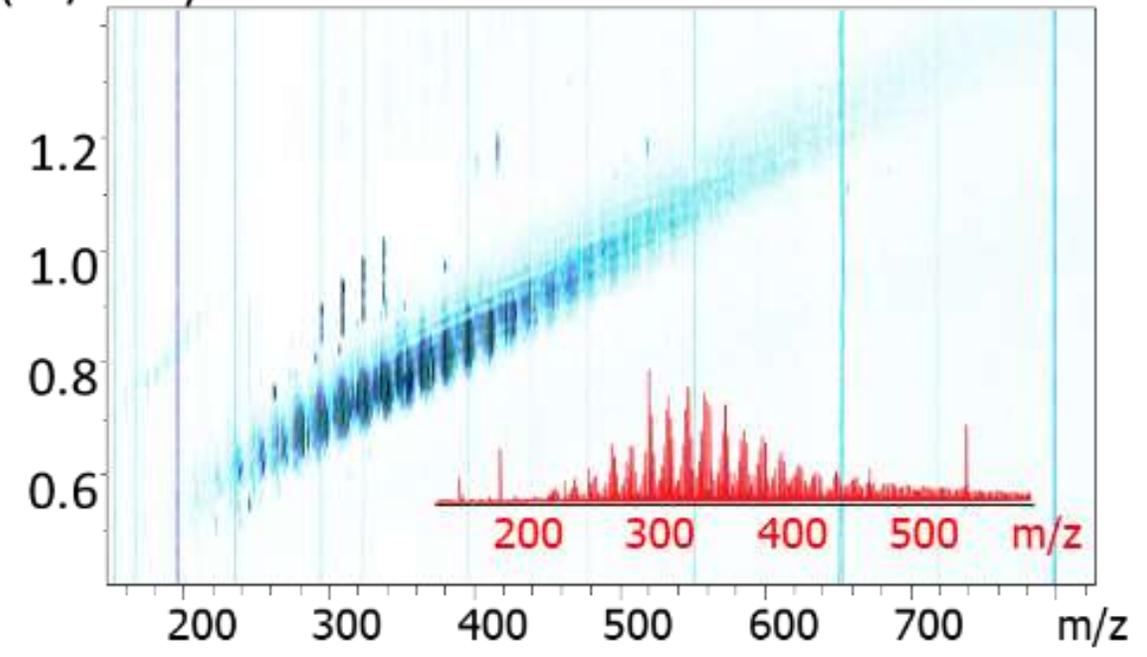
1/Ko
(Vs/Cm²)



1/Ko
(Vs/Cm²)

DOM

Harney River, Everglades National Park (HR1),



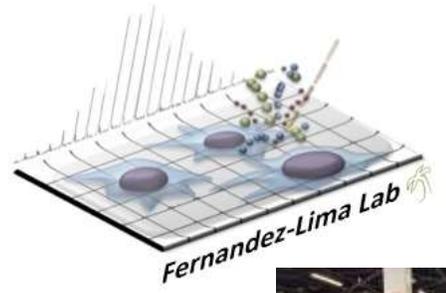
P. R B. Oliveira, D. Leyva, L. V. Tose, C. Weisbrod, A. N. Kozhinov, K. O. Nagornov, Y. O Tsybin, F. Fernandez-Lima*, “Revisiting Dissolved Organic Matter Analysis Using High-Resolution Trapped Ion Mobility and FT-ICR Mass Spectrometry”, *J. Am. Soc. Mass Spectrom.* 2024, 35, 10, 2400–2407.

TIMS and FT-ICR MS

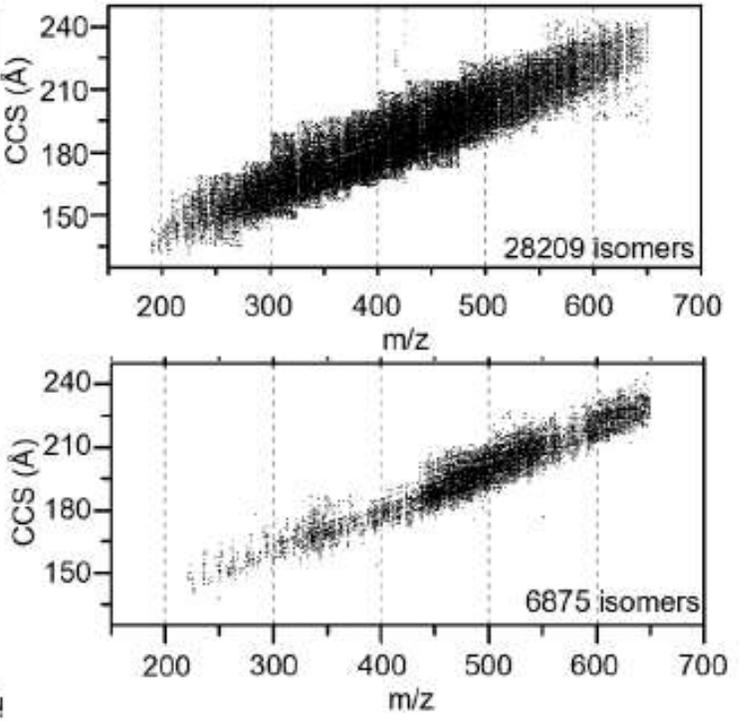
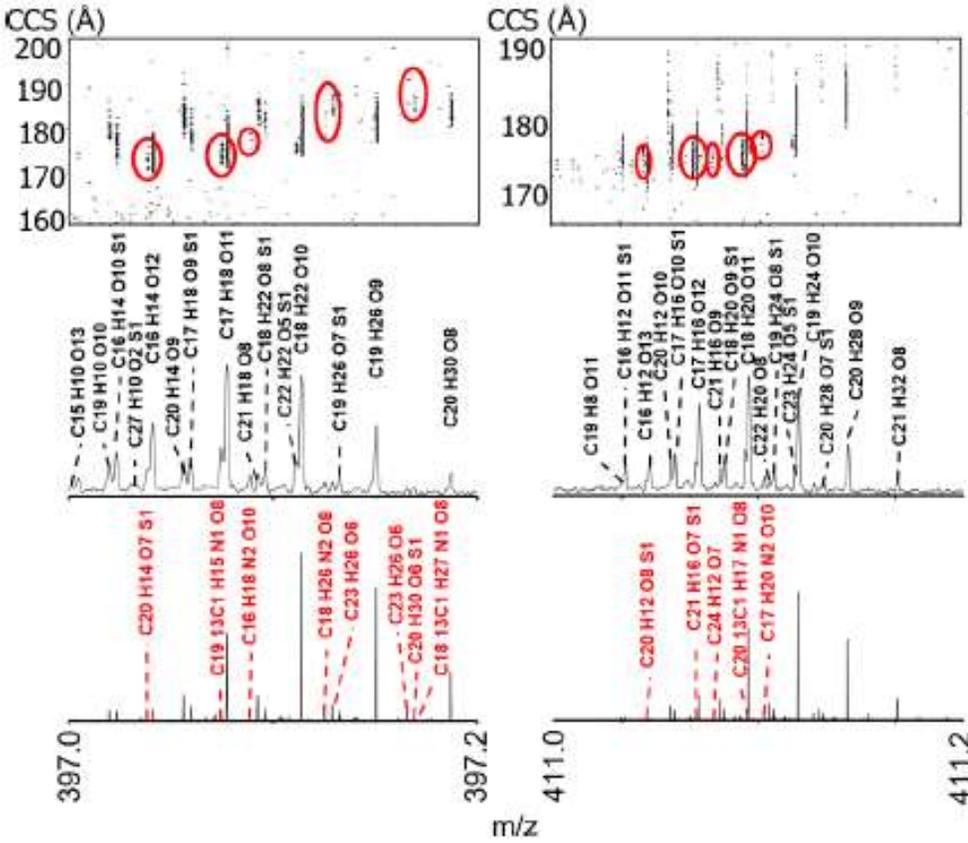
2024

9.4T OSA-TIMS-FT-ICR MS

“direct” Absorption mode



2D Peak detection enhancement



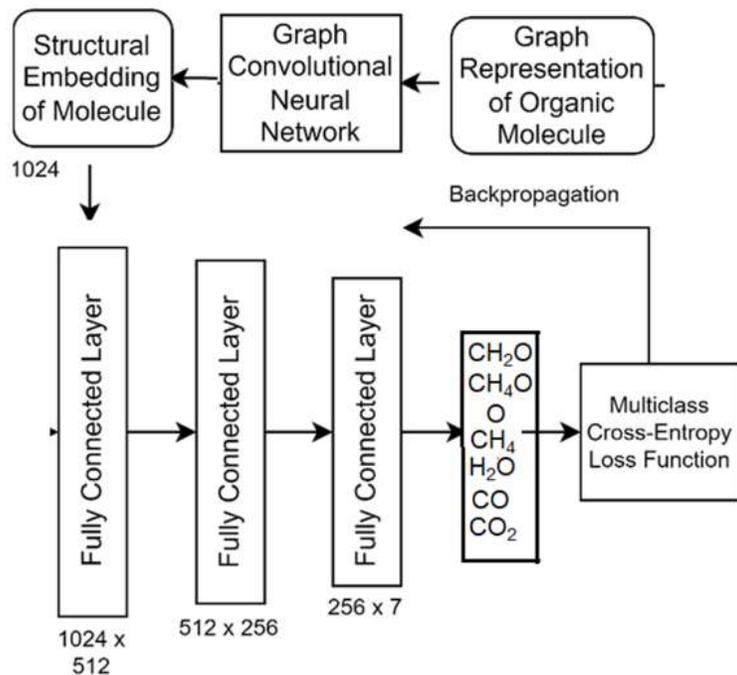
24% increase

newly detected formulas and isomers



P. R B. Oliveira, D. Leyva, L. V. Tose, C. Weisbrod, A. N. Kozhinov, K. O. Nagornov, Y. O Tsybin, F. Fernandez-Lima*, “Revisiting Dissolved Organic Matter Analysis Using High-Resolution Trapped Ion Mobility and FT-ICR Mass Spectrometry”, *J. Am. Soc. Mass Spectrom.* 2024, 35, 10, 2400–2407.

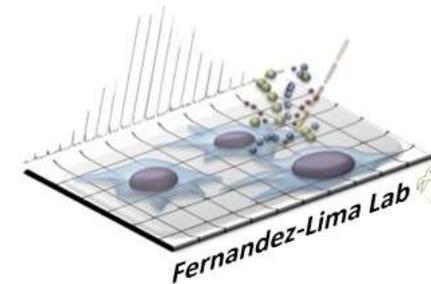
Development of Multidimensional IMSⁿ-FT-ICR MSⁿ Tools for the Characterization of Complex Mixtures



Graph convolutional neural network for predicting neutral loss from a given fragment embedding

M. U. Tariq, D. Leyvay, F. A. F. Limaz and F. Saeed, "Graph Theoretic Approach for the Analysis of Comprehensive Mass-Spectrometry (MS/MS) Data of Dissolved Organic Matter," *2021 IEEE International Conference on Bioinformatics and Biomedicine (BIBM)*, Houston, TX, USA, 2021, pp. 3742-3746, doi: 10.1109/BIBM52615.2021.9669289.

Machine Learning model

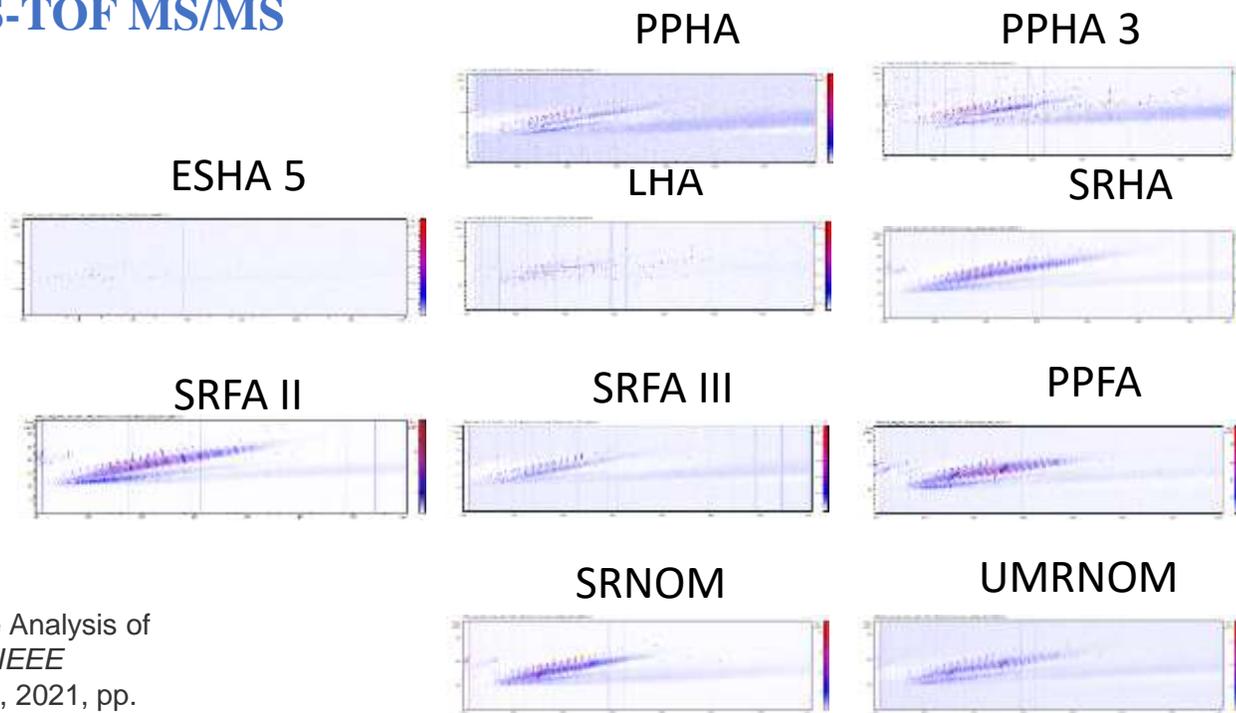


DOM Pathway Scoring

CASI TIMS-FT-ICR MS/MS -> Nominal mass $q(1\text{Da})$ CID / UVPD

TIMS-CHEF FT-ICR MS/MS->36mDa SORI CID/UVPD

TIMS-TOF MS/MS

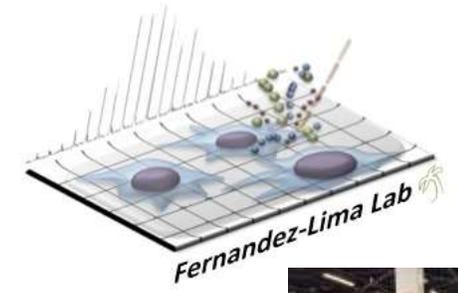


TIMS and FT-ICR MS

2024

9.4T OSA-TIMS-FT-ICR MS

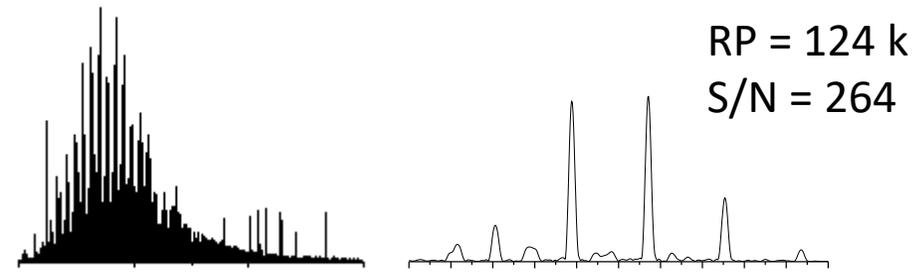
“direct” Absorption mode



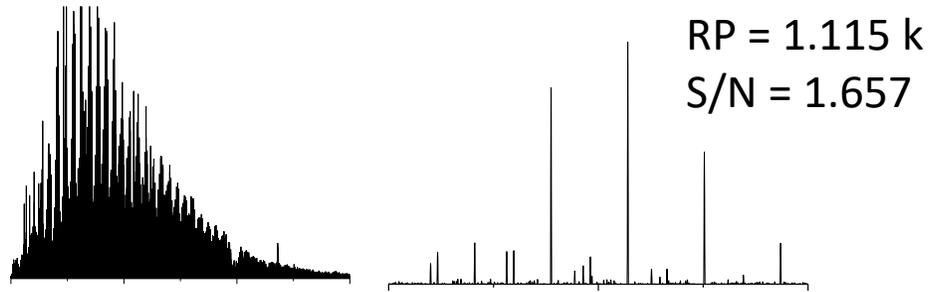
**45% increase in
Chem Formulas**



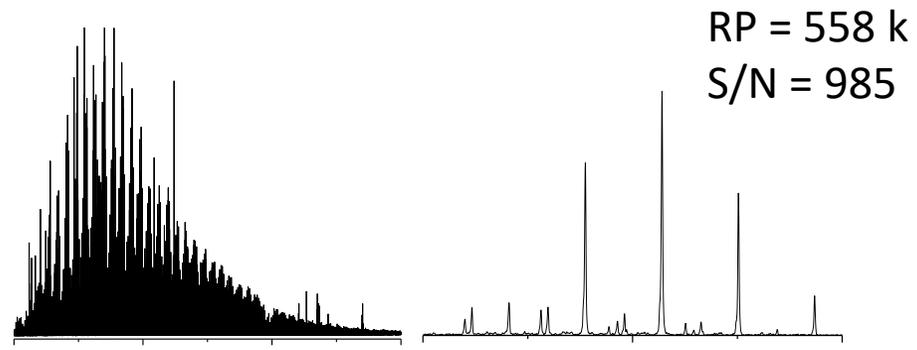
FT-ICR 7 T – Infinity cell



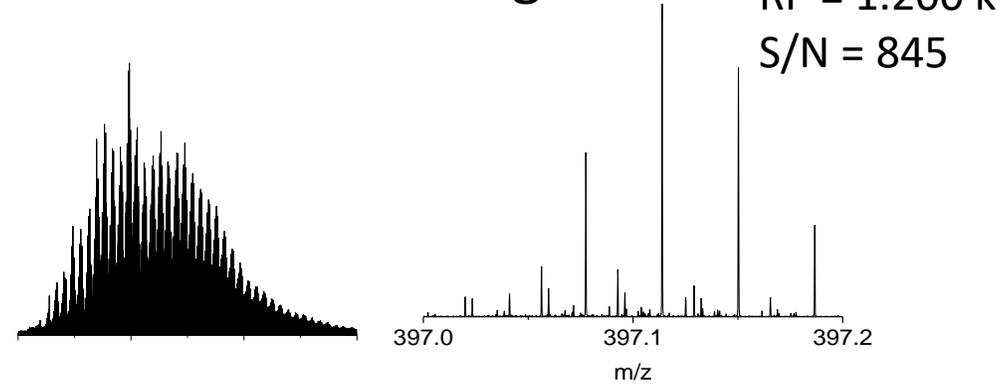
FT-ICR 9.4 T – Booster Full



FT-ICR 9.4 T - Paracell



FT-ICR 21 T - Maglab



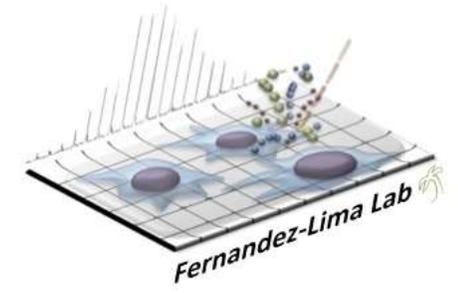
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TIMS and FT-ICR MS

2025

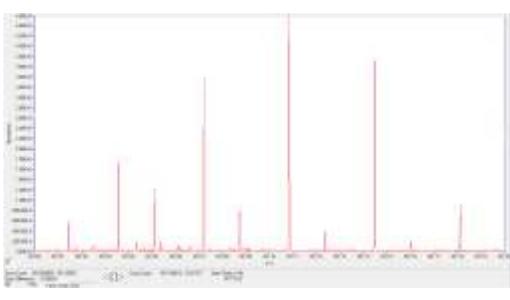
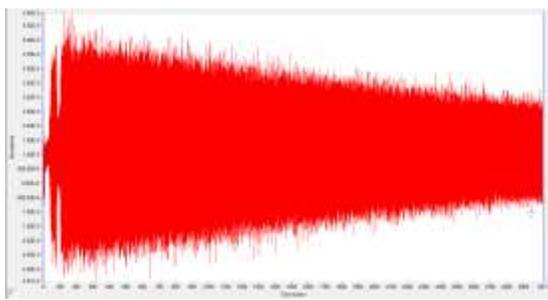
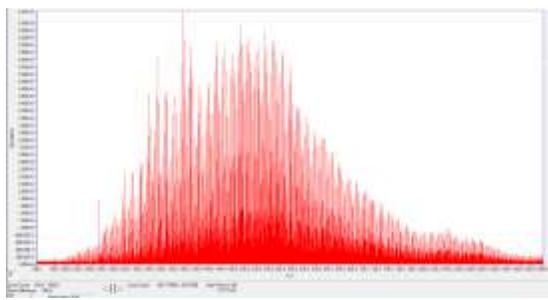
9.4T OSA-TIMS-FT-ICR MS

“direct” Absorption mode



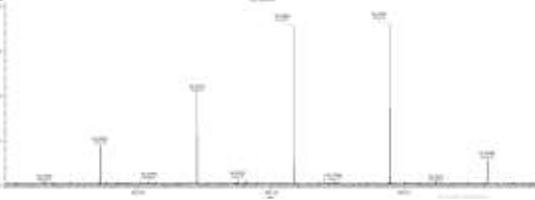
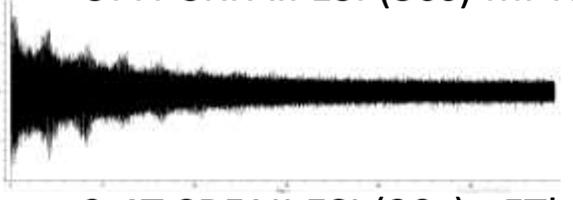
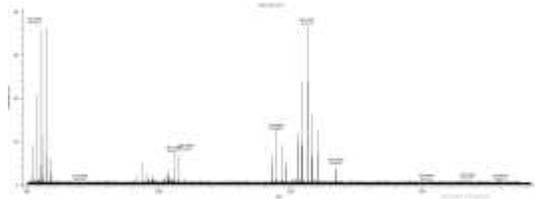
Broadband vs narrowband

21 T SRFAII ESI (3s) mFTk



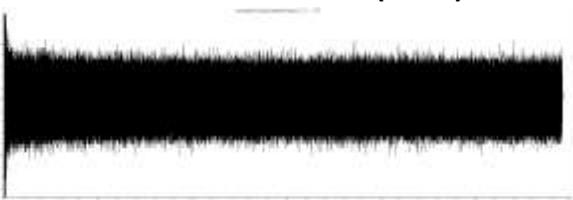
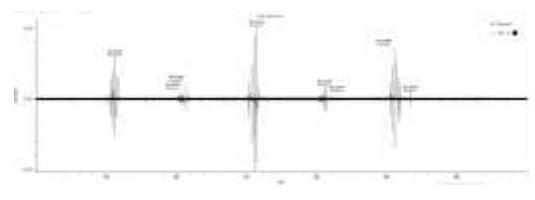
RP = 1.2M

9.4T SRFAII ESI (30s) mFTk



351.10 m/z
RP ~ 6M

9.4T SRFAII ESI (30s) aFTk



351.10 m/z
RP ~ 14 M



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Acknowledgements

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Cassandra Fuller, PhD
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Andrew Forero
Marissa Carter
Andrea Ramirez-Torres
Moshfiqur Rahman



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CHE- 2304837



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Thanks for your
attention