Pacific Northwest
NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Increasing Molecular Coverage in Complex Biological and Environmental Samples by Using IMS-MS

Erin Shammel Baker

Kristin E. Burnum-Johnson, Jon M. Jacobs, Yehia M. Ibrahim, Daniel J. Orton, William F. Danielson III, Kevin L. Crowell, Young-Mo Kim, Thomas O. Metz, Gordon A. Anderson and Richard D. Smith

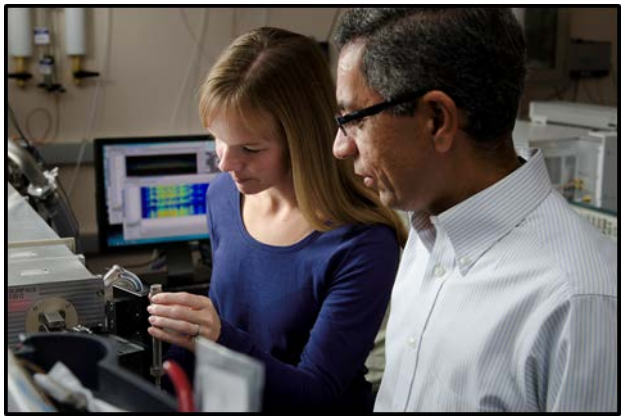
Pacific Northwest National Laboratory

Introduction

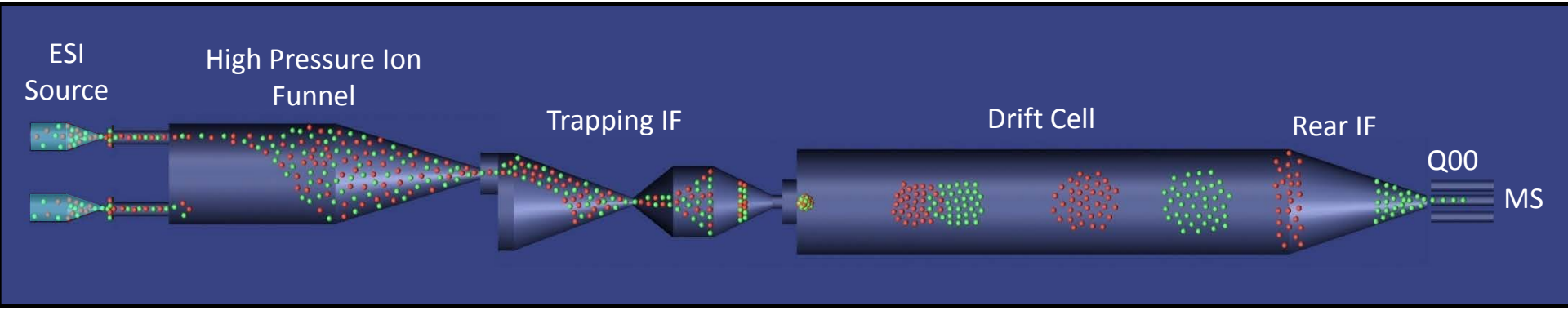
Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Why are we interested in using IMS-MS platforms?

1. IMS adds complementary information to MS measurements which helps lower false discovery rates, separates isomers and allows faster LC separations
2. IMS-TOF MS provides greater dynamic range of detection relative to trapping (e.g. Orbitrap) instruments
3. Detection of structural changes in peptides/ proteins can help characterize specific disease states (structural biomarkers)



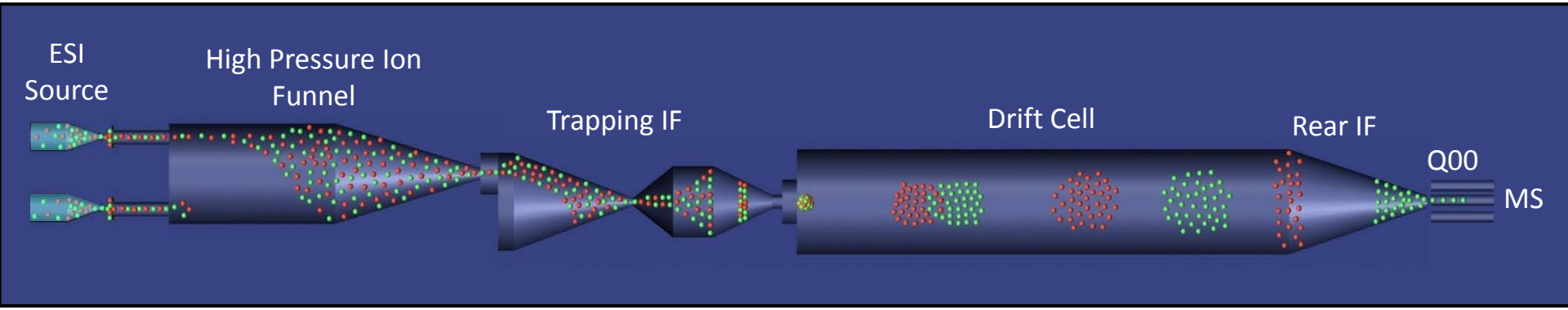
IMS-MS instrumentation



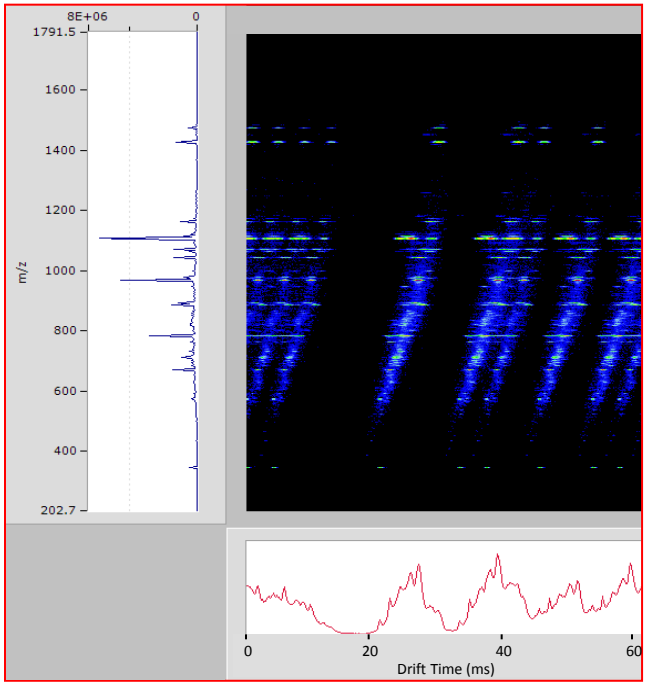
Features:

- NanoESI ion source with 2 inlets for on-the-fly calibration
- Off-axis hourglass ion funnel/accumulation trap before IMS
- Rear ion funnel after IMS
- Segmented quadrupole for CID
- High dynamic range Agilent TOF or Q-TOF MS

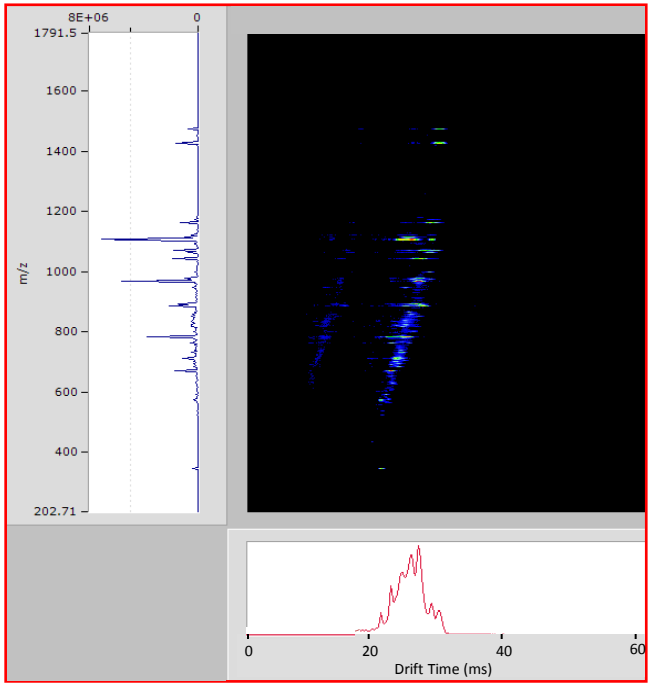
Multiplexed IMS-MS



Multiplexed IMS-MS Spectrum



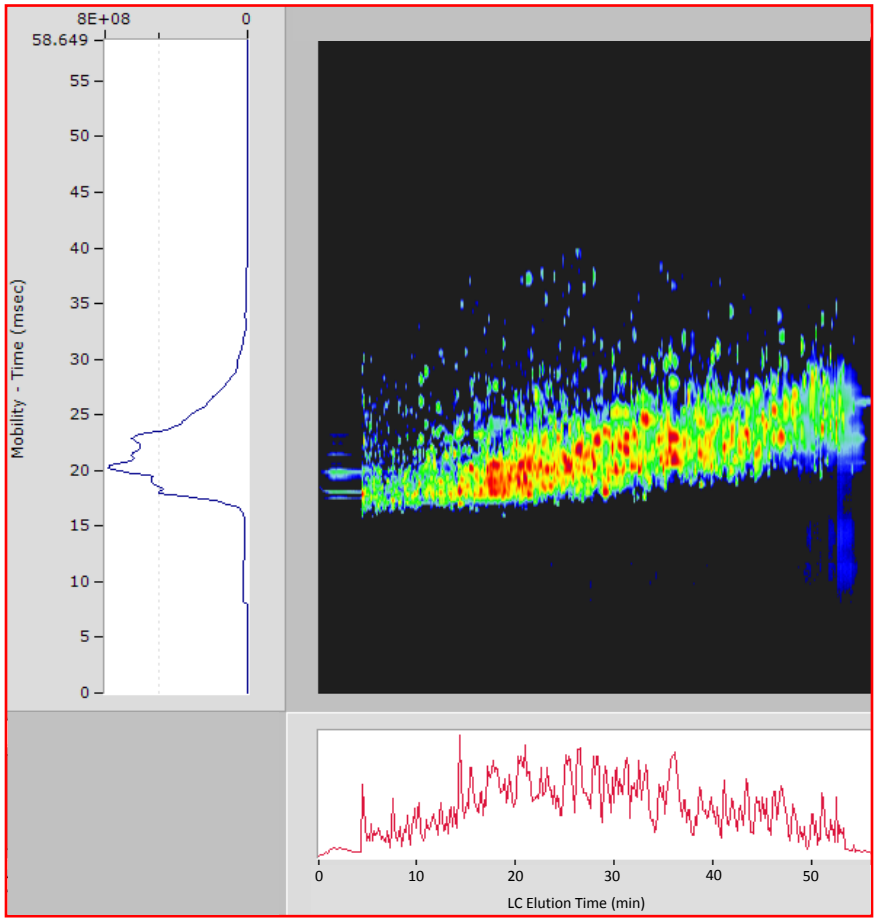
De-multiplexed IMS-MS Spectrum



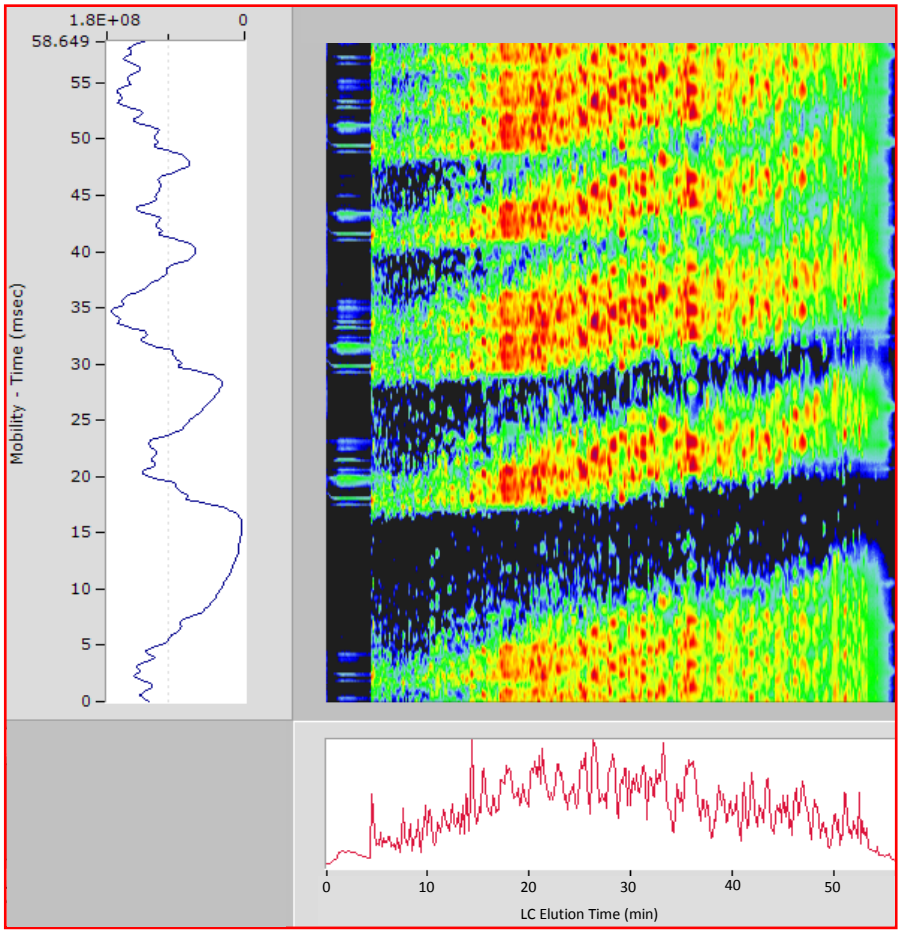
Multiplexing utilizes more drift time space and increases signal

M.E. Belov, M.A. Buschbach, D.C. Prior, K. Tang, R.D. Smith, *Anal. Chem.*, 2007, *79* (6), 2451–2462.

Multiplexed LC-IMS-MS



De-multiplexed IMS-MS spectra



Multiplexed IMS-MS spectra

Multiplexing utilizes more drift time space and increases signal

M.E. Belov, M.A. Buschbach, D.C. Prior, K. Tang, R.D. Smith, *Anal. Chem.*, 2007, *79* (6), 2451–2462.

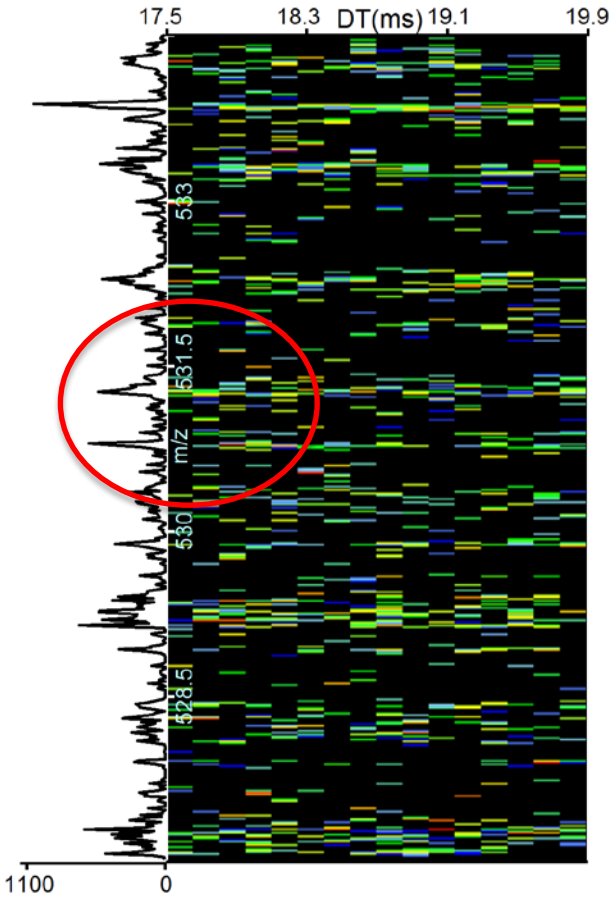
8 peptides spiked in human serum

Spiking Level	Non-Serum Peptide	60-min LC-IMS-TOF MS	60-min LC-TOF MS	100-min LC-Velos-Orbitrap
100 pg/mL	Melittin	ND	ND	ND
100 pg/mL	Dynorphin A Porcine Fragment 1-13	✔	ND	ND
1 ng/mL	Des Pro Ala Bradykinin	✔	ND	ND
1 ng/mL	Leucine Enkephalin	✔	ND	ND
10 ng/mL	3X FLAG Peptide	✔	✔	ND
10 ng/mL	Substance P	✔	✔	✔
100 ng/mL	Methionine Enkephalin	✔	✔	✔
100 ng/mL	[Ala92]-Peptide 6	✔	✔	✔

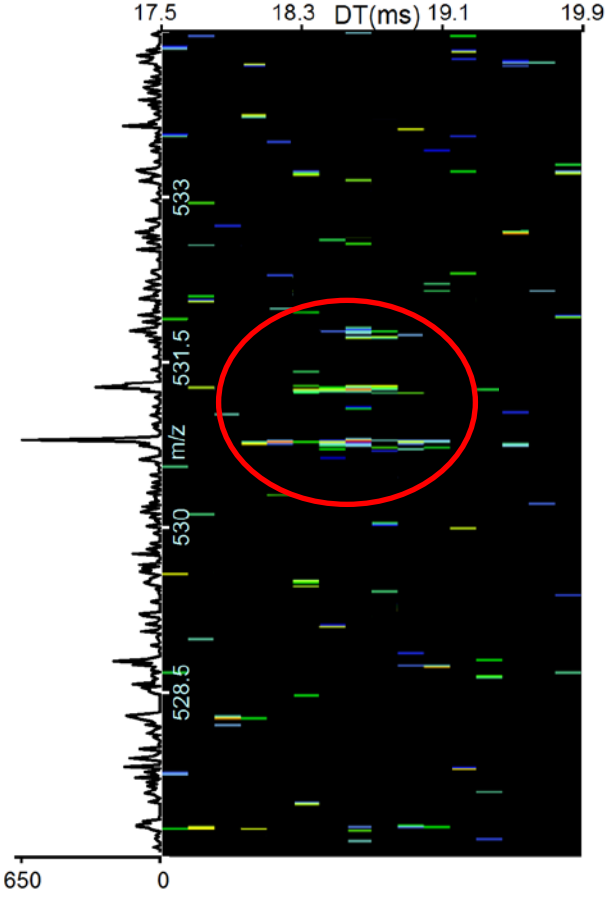
Sample analyzed using Velos-Orbitrap, TOF MS and IMS-TOF MS instruments

Benefits of IMS drift time separation

1. Improved Sensitivity & Increase Feature Detection & Confidence



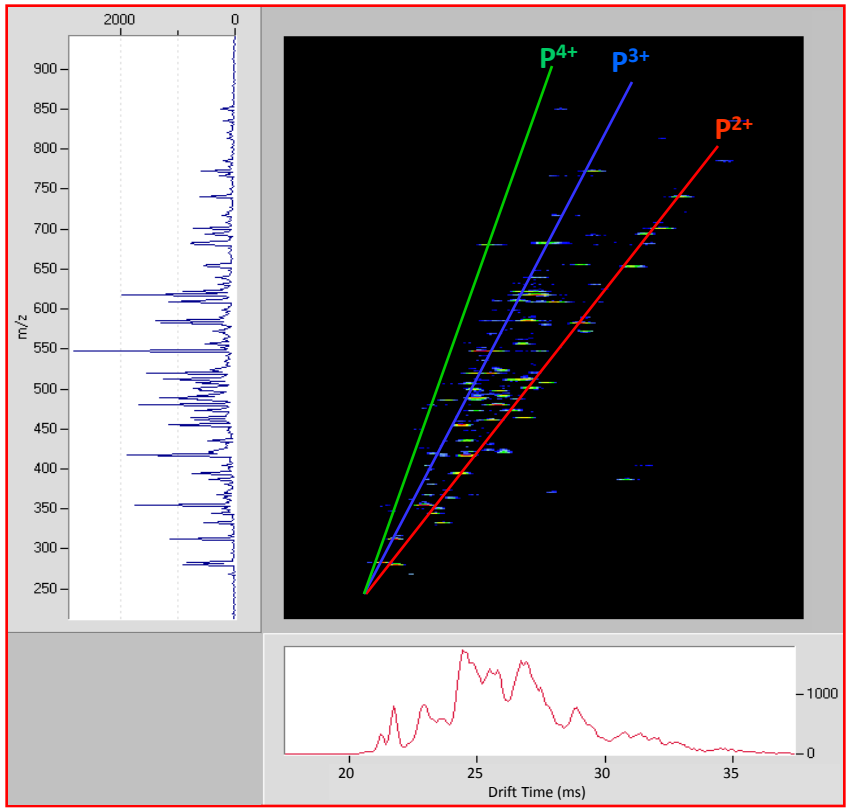
QTOF MS of Bradykinin (100 pM)



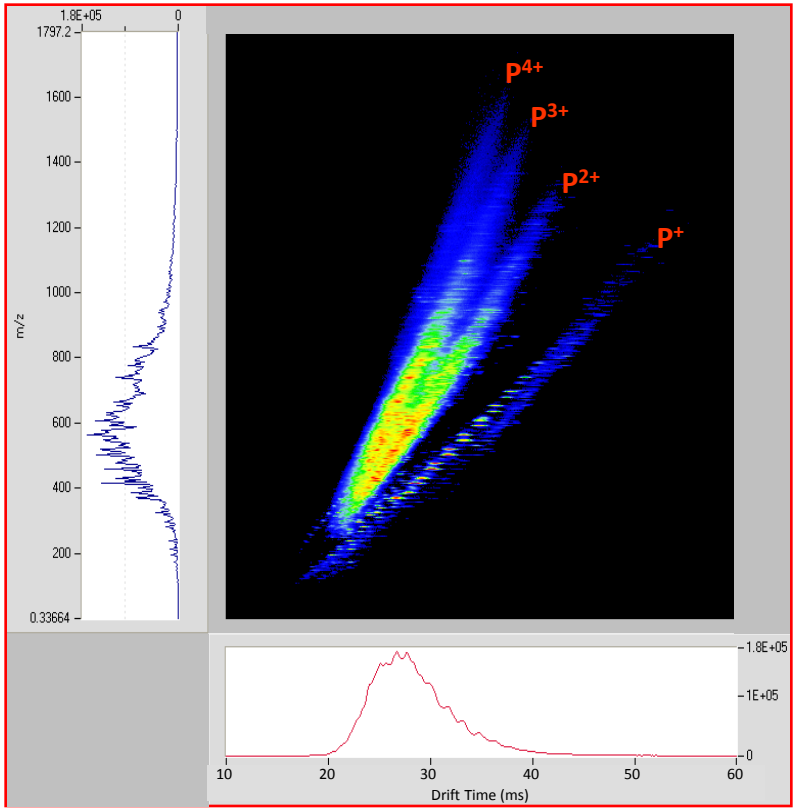
IMS-QTOF MS of Bradykinin (100 pM)

Benefits of IMS drift time separation

2. Separates by Shape and Charge State



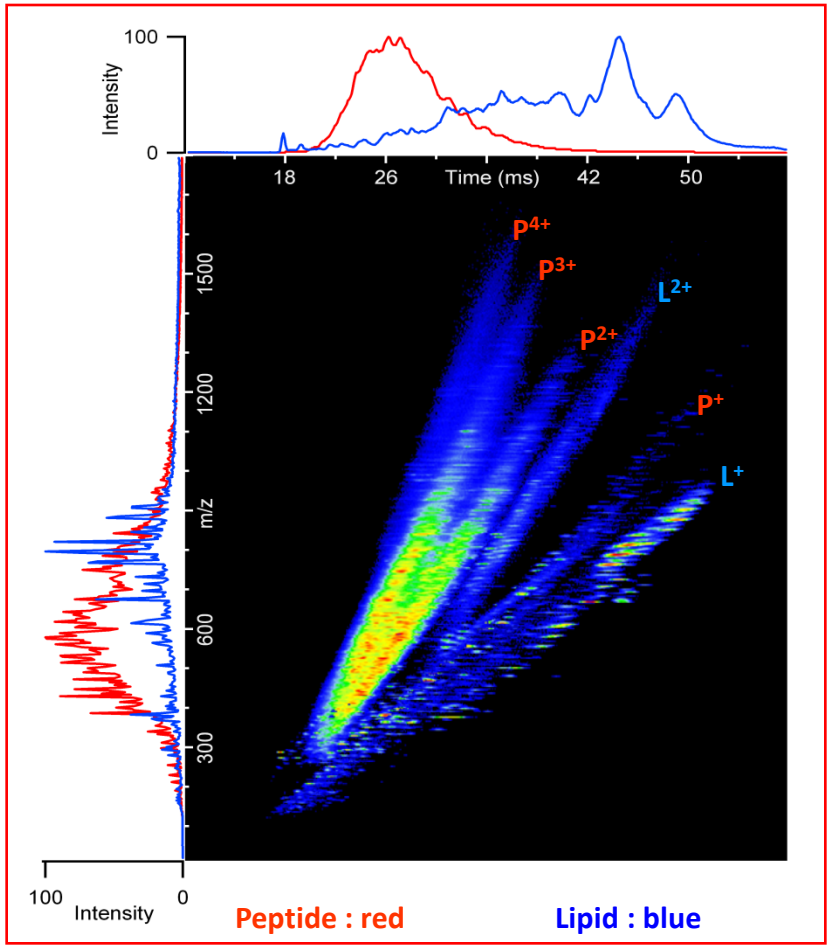
BSA tryptic digest (25 µg/mL) (5 sec acquisition)

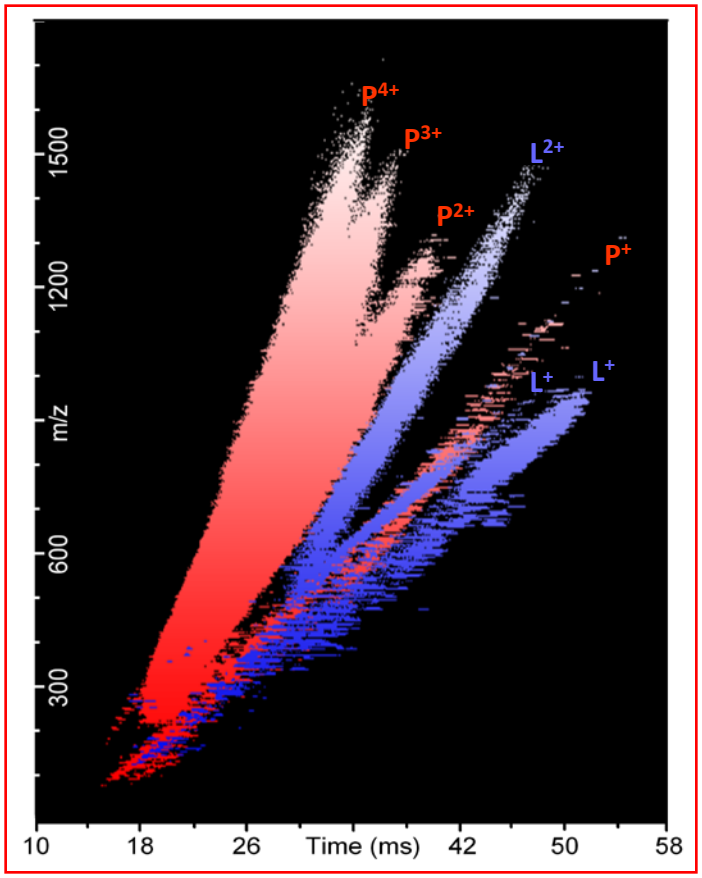


Human Plasma tryptic digest (0.5 mg/mL) (summed LC run for 50 minutes)

Benefits of IMS drift time separation

3. Distinguish different classes of compounds

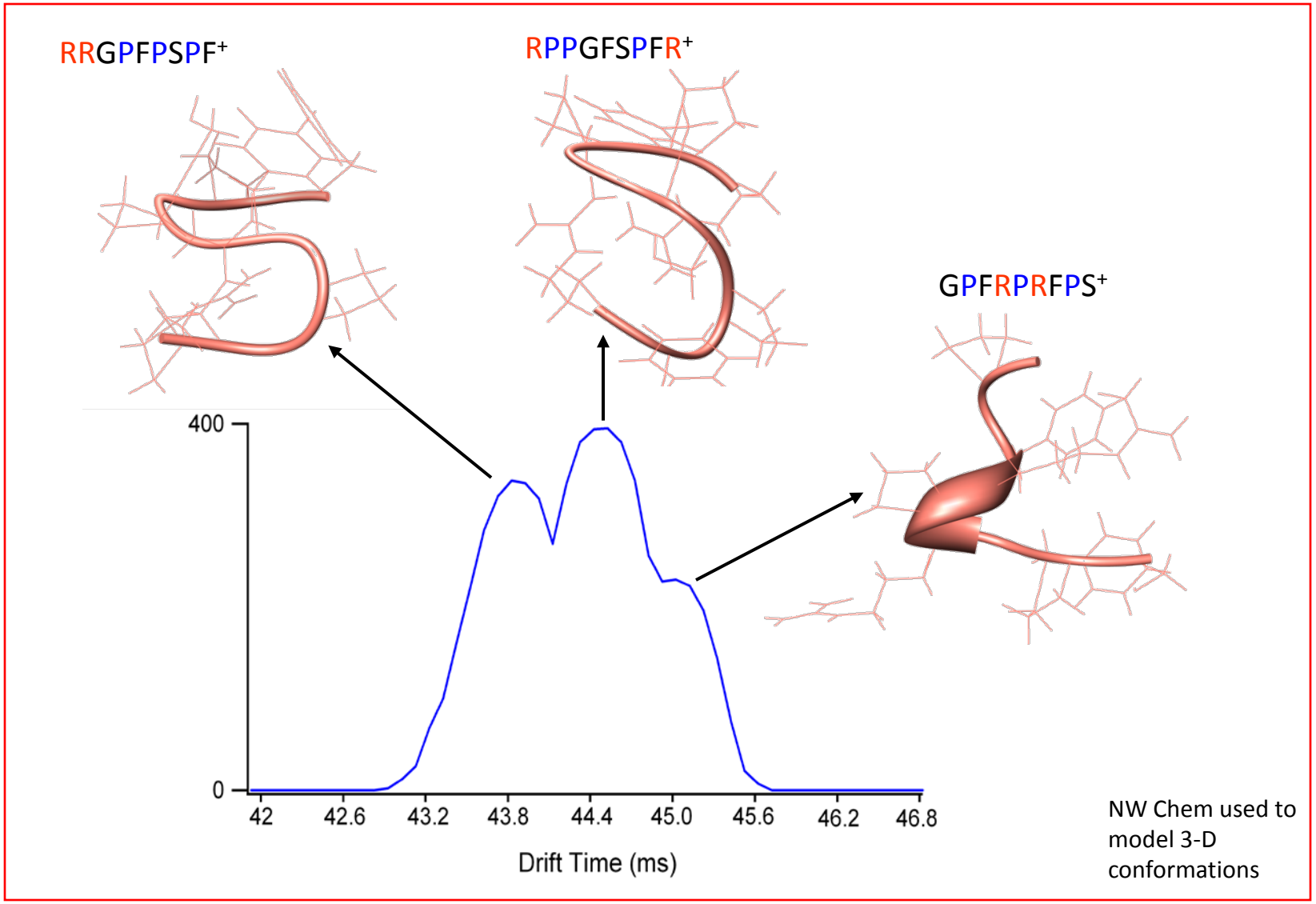




Peptides and lipids are easily distinguished

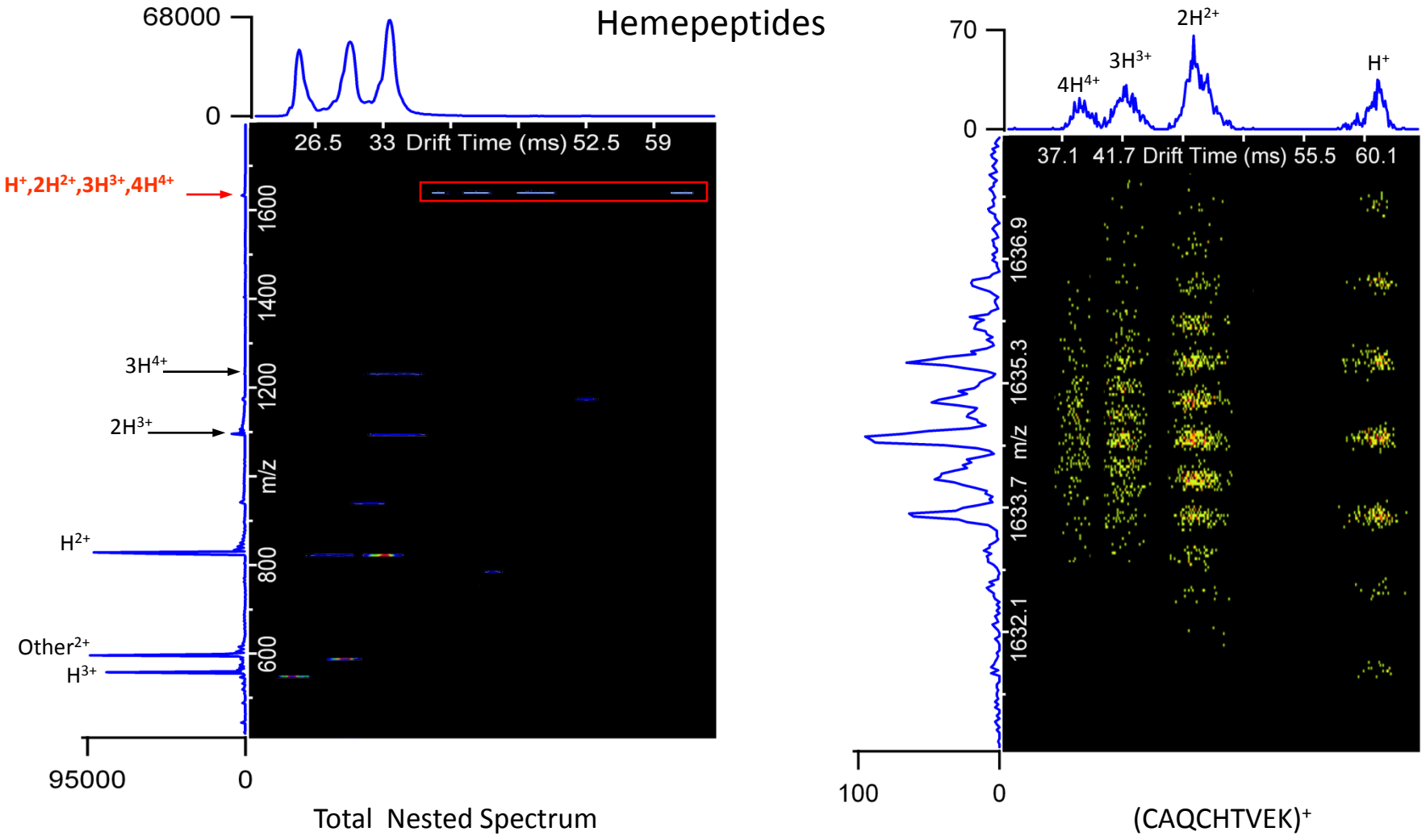
Benefits of IMS drift time separation

4. Distinguish sequence isomers



Benefits of IMS drift time separation

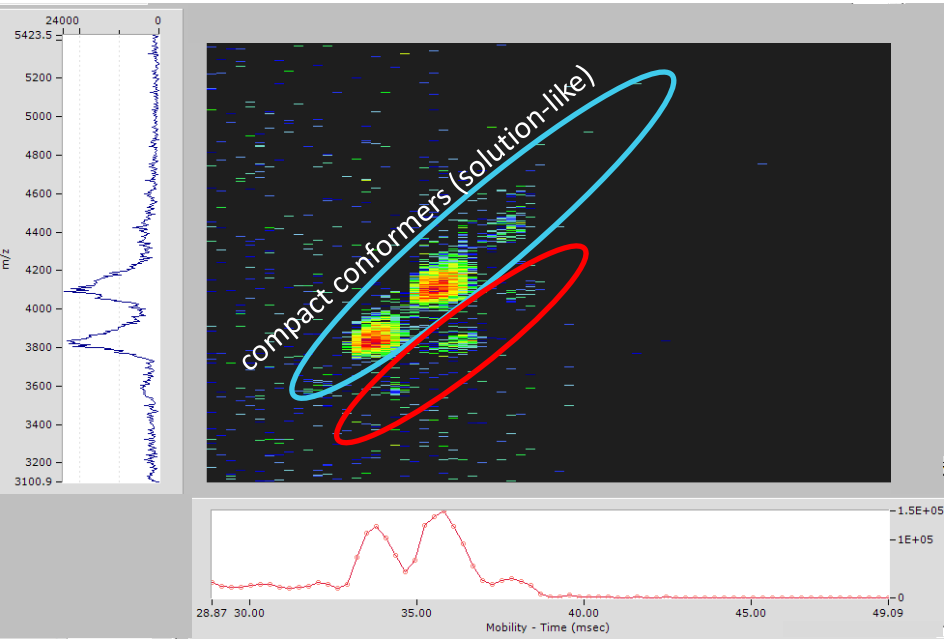
5. Characterize Aggregation Levels & Analyze Interactions



Peptide mixture from C18 column

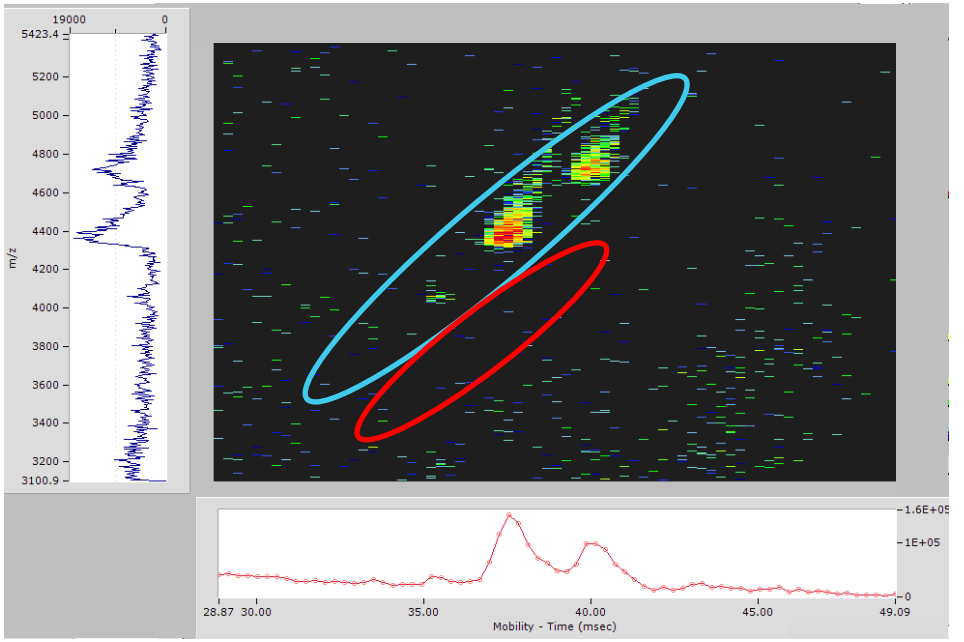
Benefits of IMS drift time separation

5. Characterize Aggregation Levels & Analyze Interactions



Transthyretin (TTR) Tetramer

Both compact and extended conformers



TTR Tetramer with Drug Bound

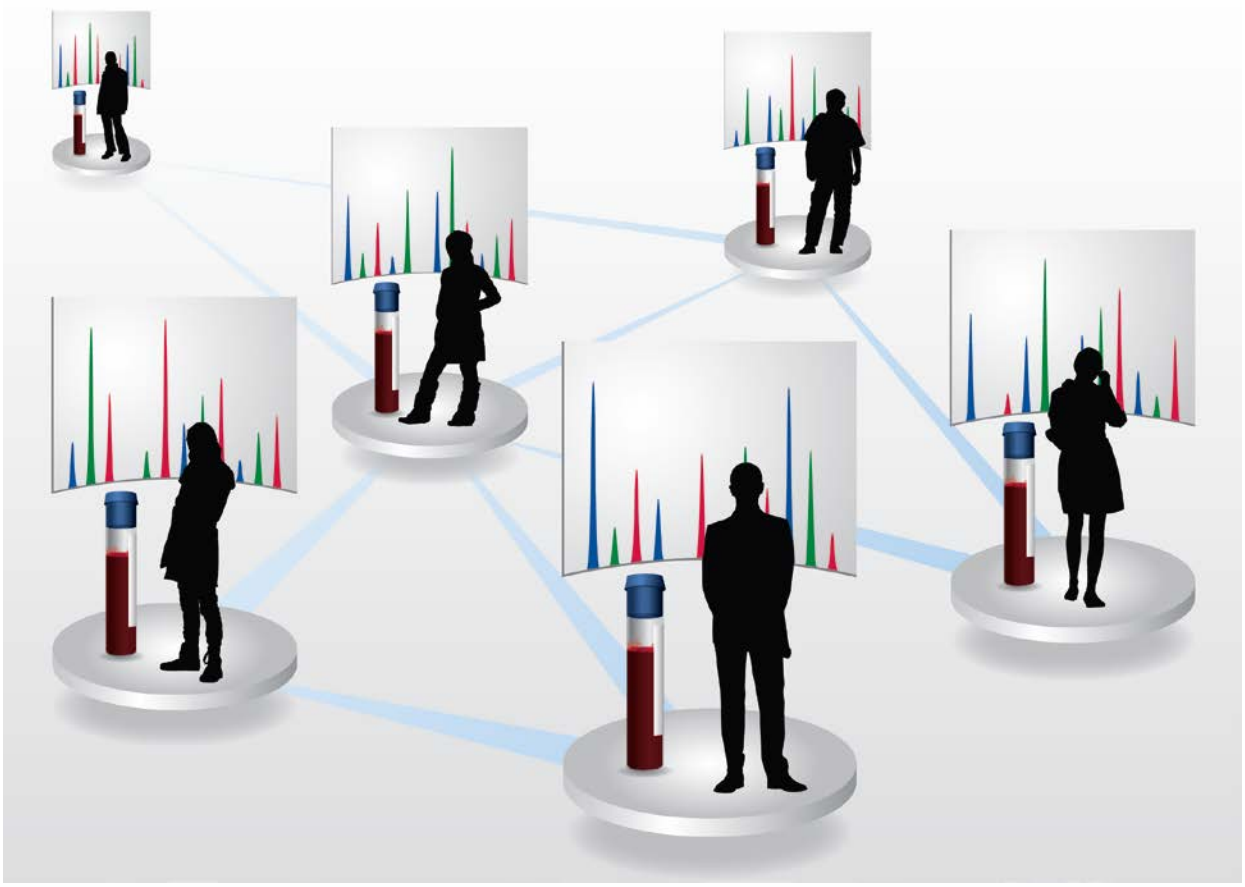
No extended conformers

Drug stabilizes compact (solution phase) structure

Samples from Catherine Costello

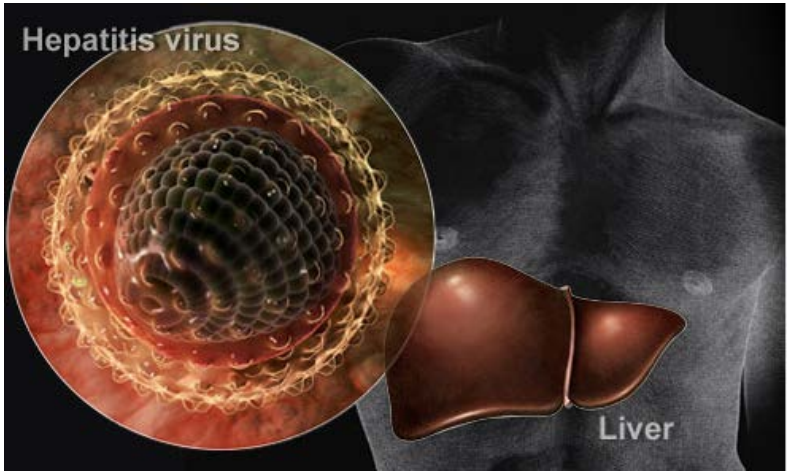
Biological diversity studies

- Thousands of samples need to be analyzed to understand the diversity in a population
- IMS-MS allows for faster analysis of many samples with high sensitivity

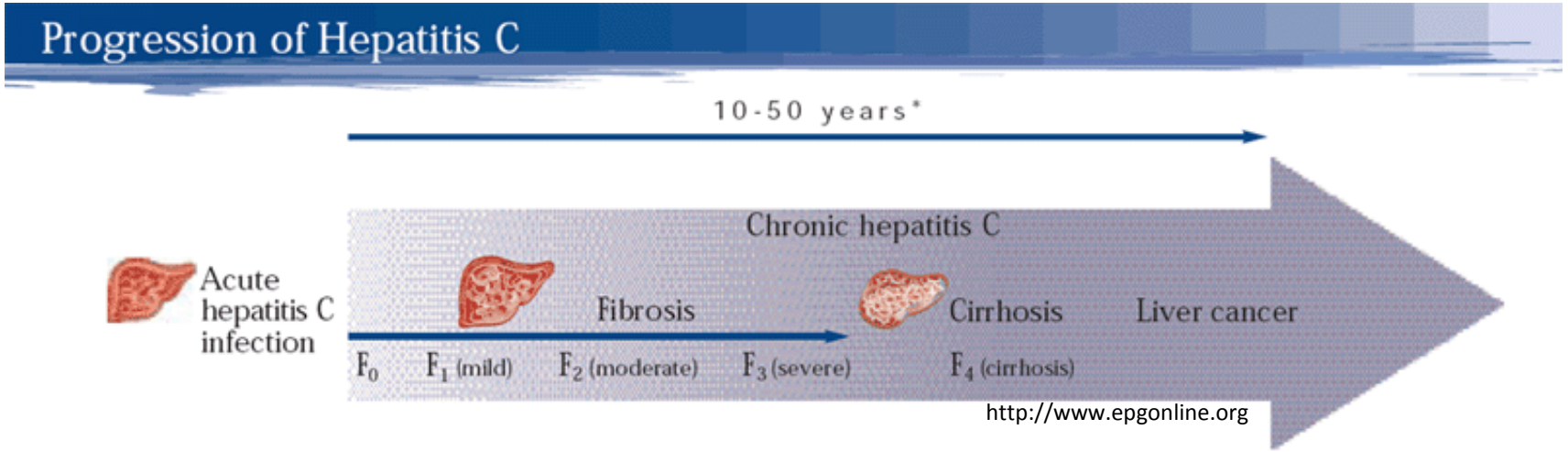


Chronic liver disease

- **Multiple Factors**
  - **Hepatitis (A,B,C)**
  - Alcohol (ALD)
  - Diabetes
  - Various autoimmune and recessive conditions



MedicalRF.com/John M. Daugherty/Photo Researchers Inc

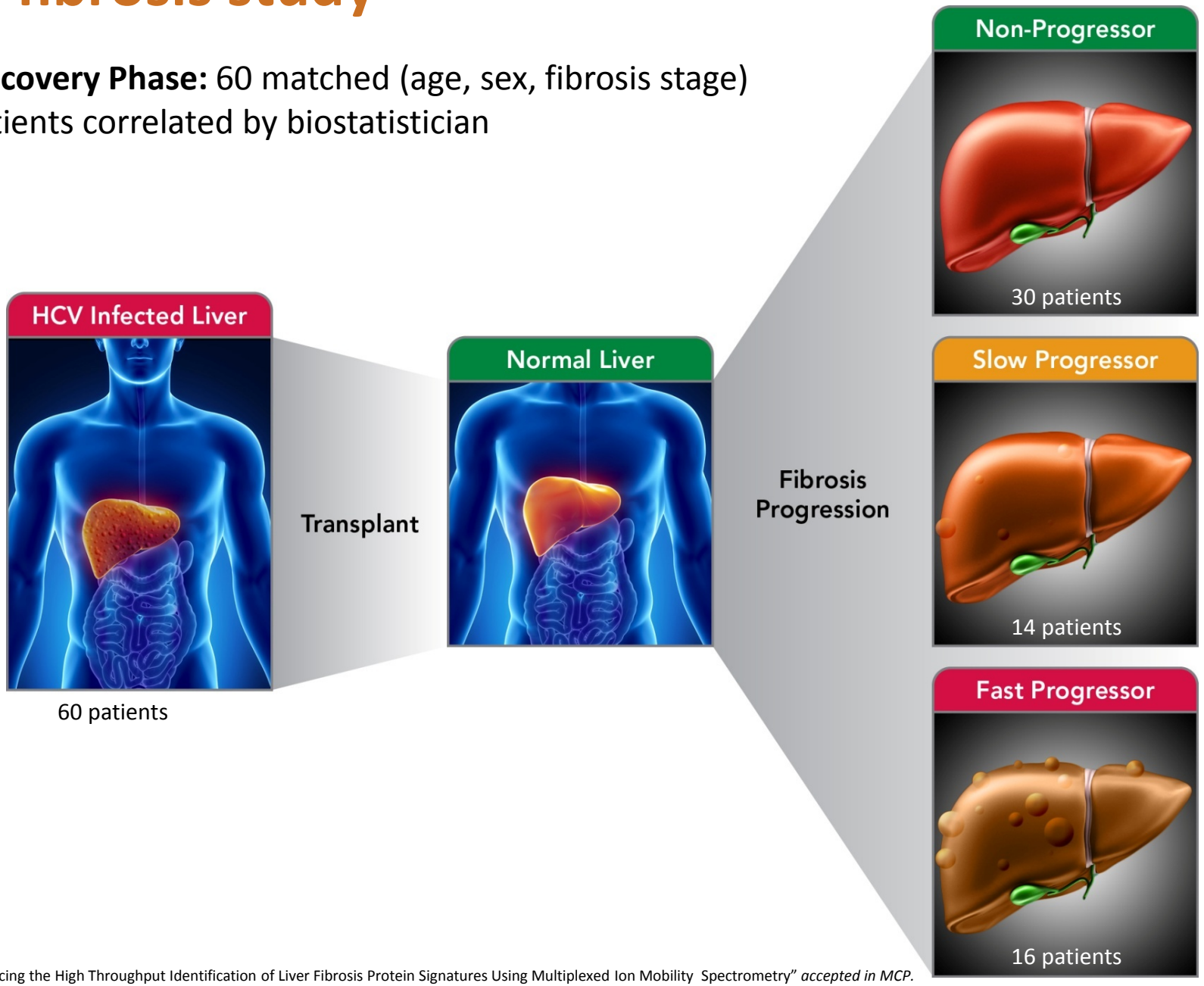


http://www.epgonline.org

- Estimated 130 million people world-wide have HCV
- Blood borne pathogen with no vaccine

Liver fibrosis study

- **Discovery Phase:** 60 matched (age, sex, fibrosis stage) patients correlated by biostatistician

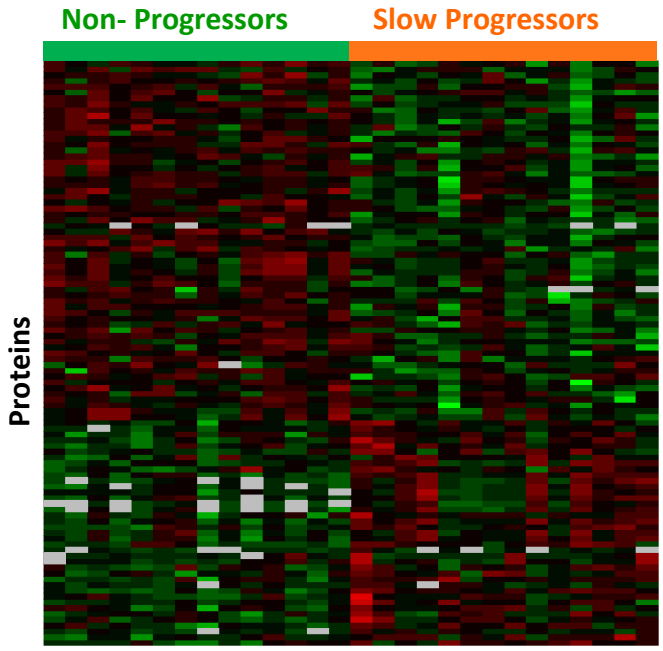


E. S. Baker, et al. "Advancing the High Throughput Identification of Liver Fibrosis Protein Signatures Using Multiplexed Ion Mobility Spectrometry" *accepted in MCP.*

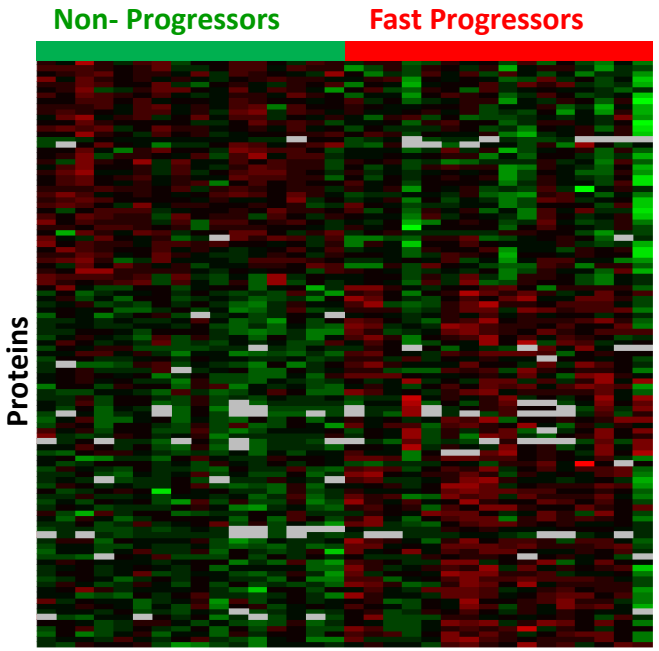
Liver fibrosis study

Discovery Phase

- Analyzed 60 post-liver transplant patients with LC-IMS-MS
- At least 2 unique peptides were required to identify a protein; significant peptides have p and q values <0.05
- Statistical analysis identified 136 proteins that distinguish between conditions



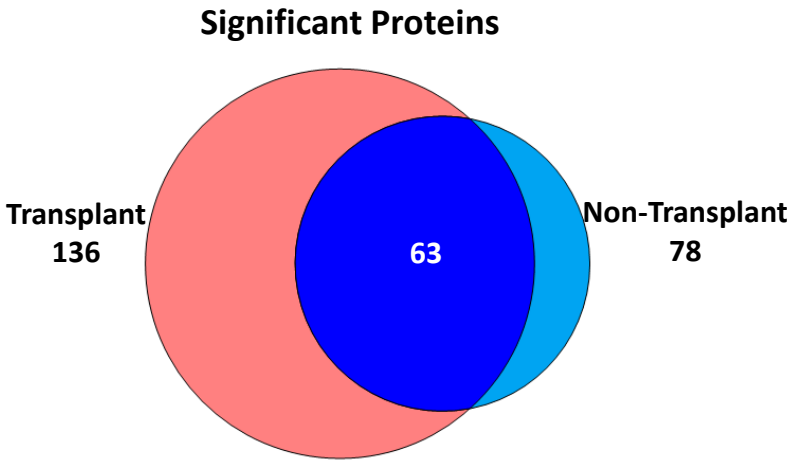
Decrease = Green
Increase = Red

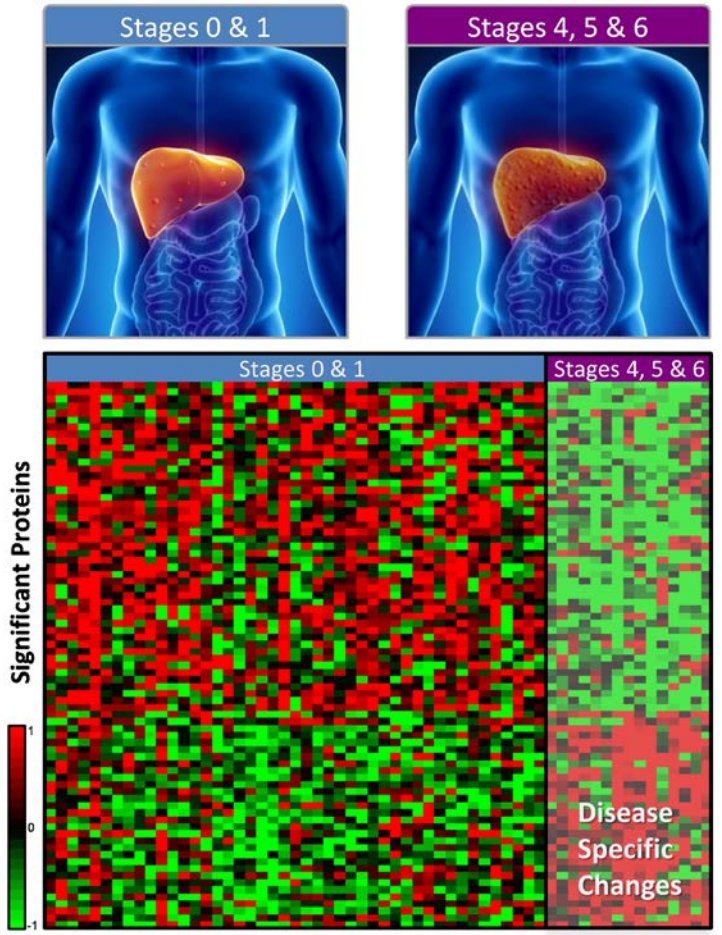


Liver fibrosis study

Non-transplant Comparison

- Analyzed 60 non-transplant patients with Ishak score 0-1 versus 4-6
- At least 2 unique peptides were required to identify a protein; significant peptides have p and q values <0.05
- 63 statistically significant proteins between conditions





Liver fibrosis study

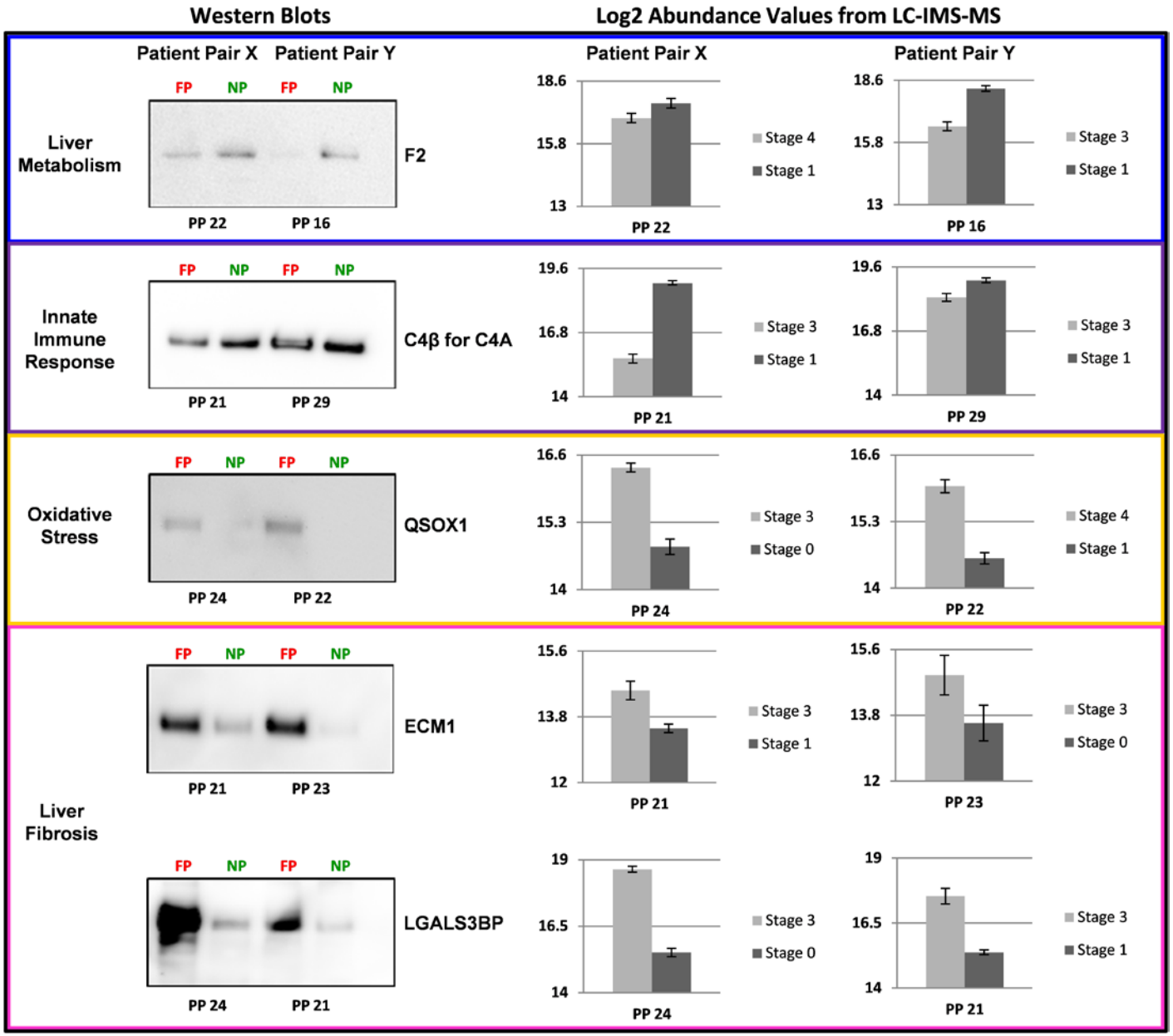
19 Example Proteins

- Classified into 4 groups: liver metabolism, immune response, oxidative stress and liver architecture
- Overall trends with increasing fibrosis
  - Liver metabolism decreases
  - Oxidative stress increases
  - Extracellular matrix proteins (within liver architecture) increase in fast progressors
  - Differences between slow and fast progressor liver architecture proteins observed

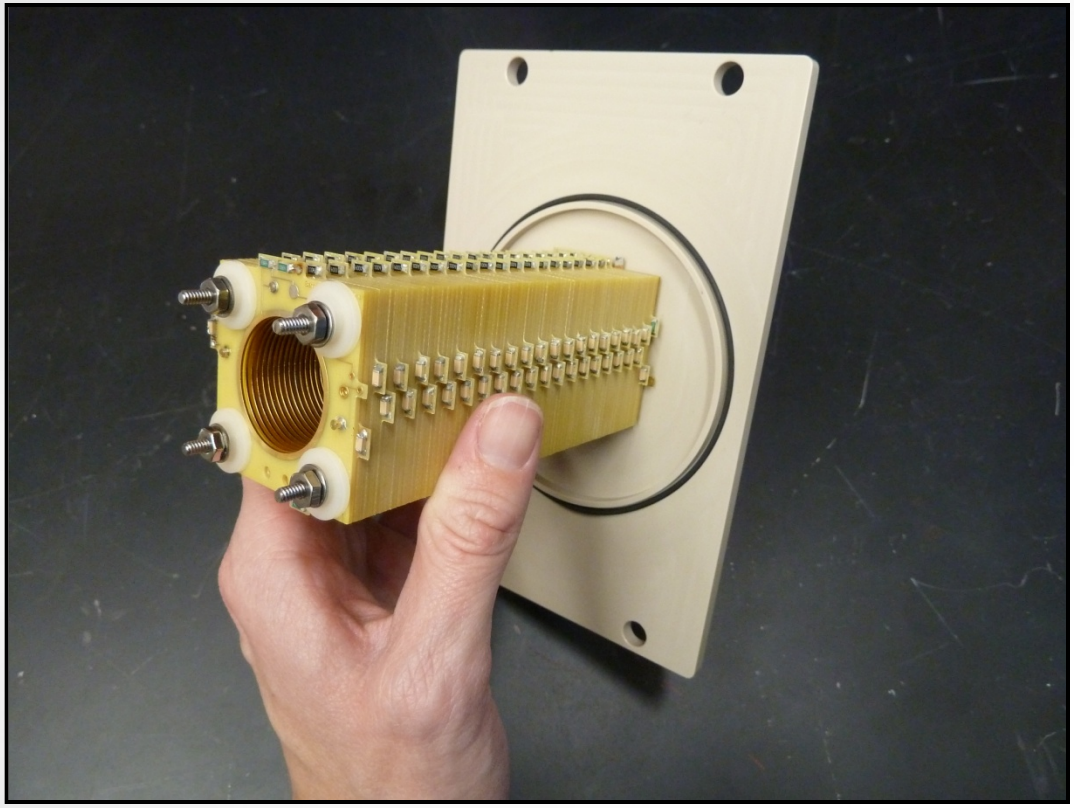
Function	Gene	SP_Descriptions	Change (SP/ NP)	Change (FP/NP)
Liver Metabolism	F2	Prothrombin		
	BCHE	Cholinesterase		
	RBP4	Retinol-binding protein 4		
	TTHY	Transthyretin		
	IGFALS	Insulin-like growth factor-binding protein complex acid labile subunit		
	IGFBP3	Insulin-like growth factor-binding protein 3		
Immune Response	C4A	Complement C4-A		
	CD14	Monocyte differentiation antigen CD14		
Oxidative Stress	QSOX1	Sulfhydryl oxidase 1		
	GPX3	Glutathione peroxidase 3		
Liver Architecture	ECM1	Extracellular matrix protein 1		
	LGALS3BP	Galectin-3-binding protein		
	ACTB	Actin, cytoplasmic 1		
	FA12	Coagulation factor XII		
	TGFBI	Transforming growth factor-beta-induced protein ig-h3		
	FA10	Coagulation factor X		
	CO5	Complement C5		
	VTN	Vitronectin		
	LUM	Lumican		

Decrease = Green
Increase = Red

Liver fibrosis study



Future Projects & Directions



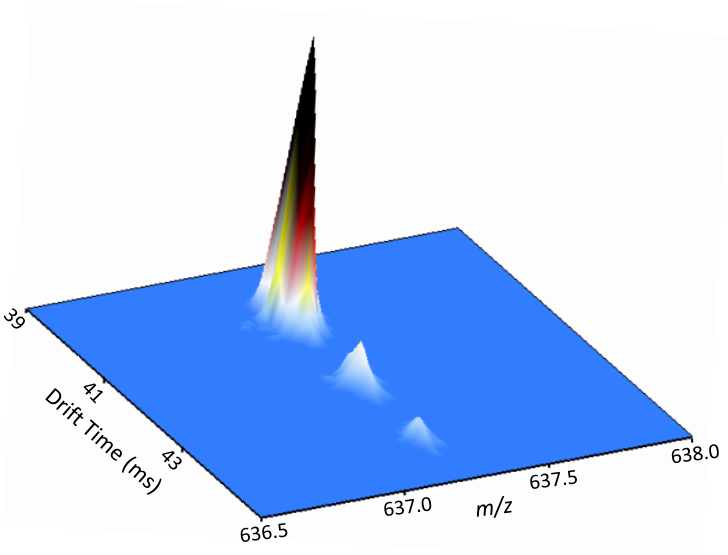
Fast lipidomic/metabolomic analyses

	IMS-MS		QTOF-MS	
	Direct Infusion	LC	Direct Infusion	LC
Lipid Extract from Plasma	463	736	253	513

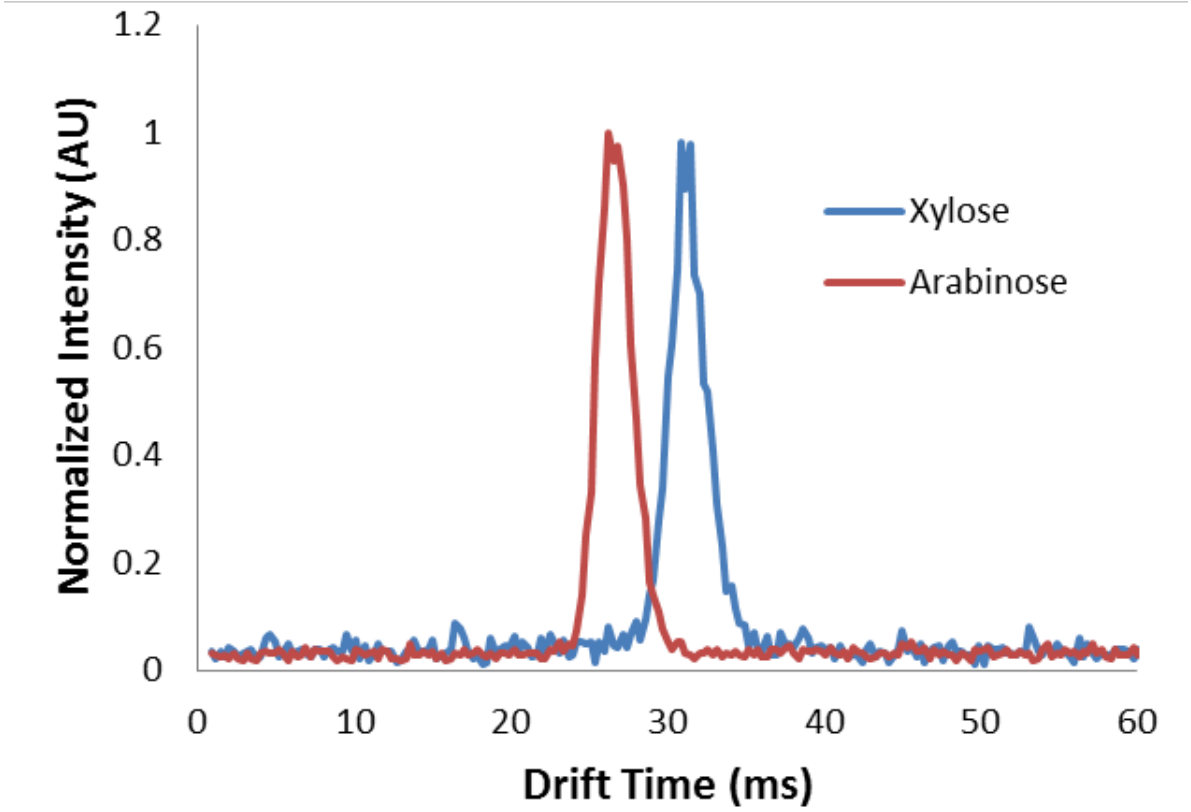
50-min LC Gradient

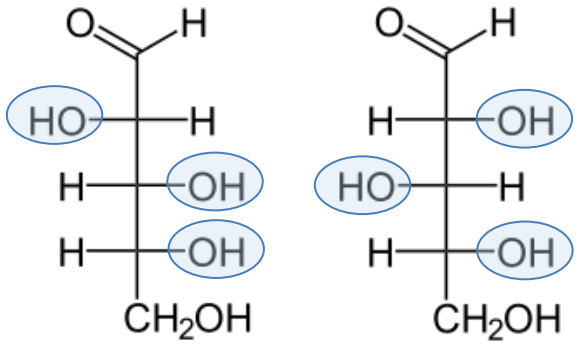
IMS cannot completely counter the ion suppression from direct infusion, but more features are observed with LC-IMS-MS than LC-QTOF MS alone

3 peaks observed for m/z = 637.31 all with the same elution time



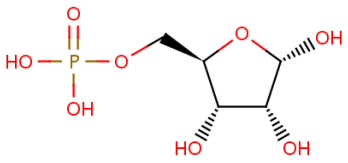
Na^+ Isomer separations with IMS



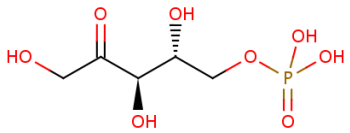


MW = 150.05

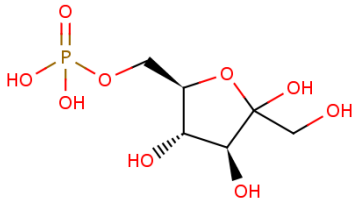
Isomer separations difficult with HILIC



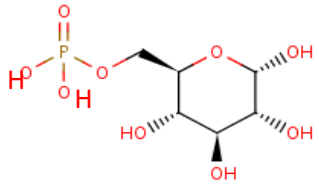
α-D-Ribose 5-phosphate (r5p)



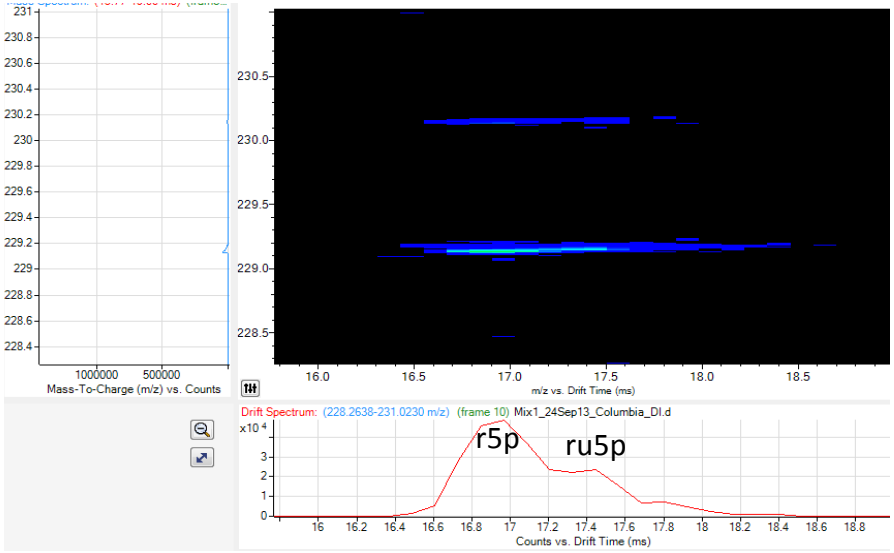
D-Ribulose 5-phosphate (ru5p)

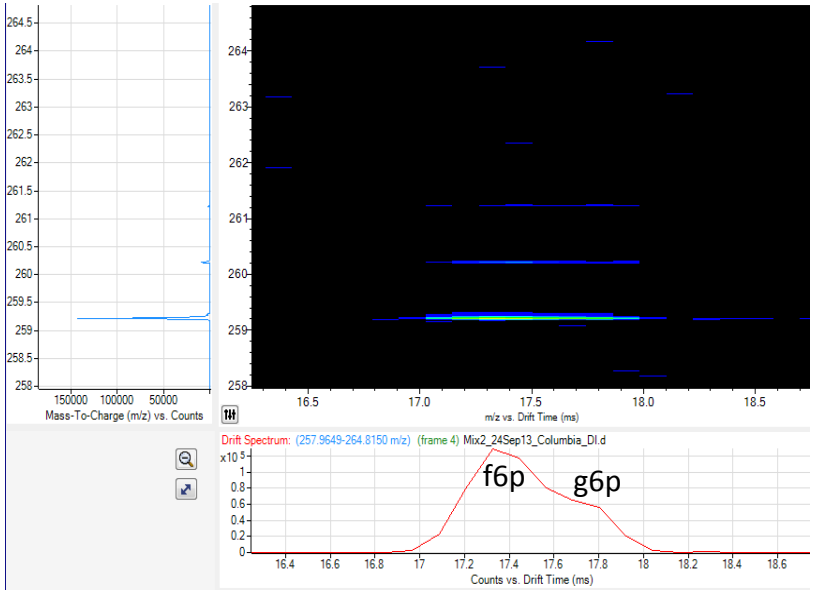


D-Fructose-6-phosphate (f6p)

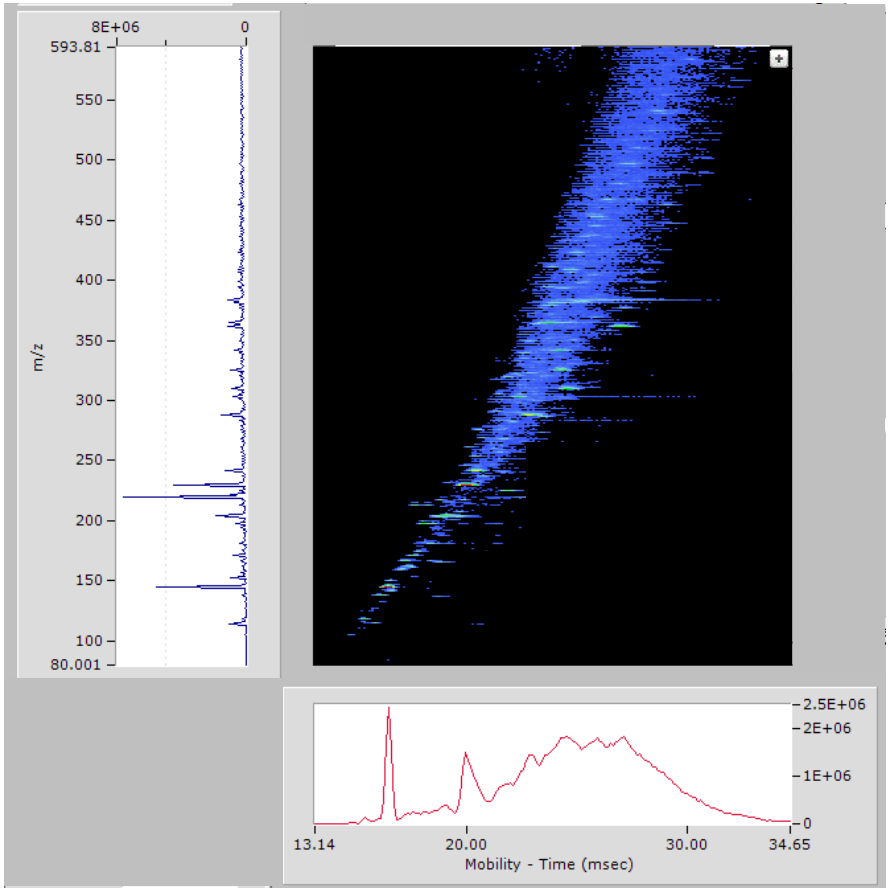


D-Glucose-6-phosphate (g6p)

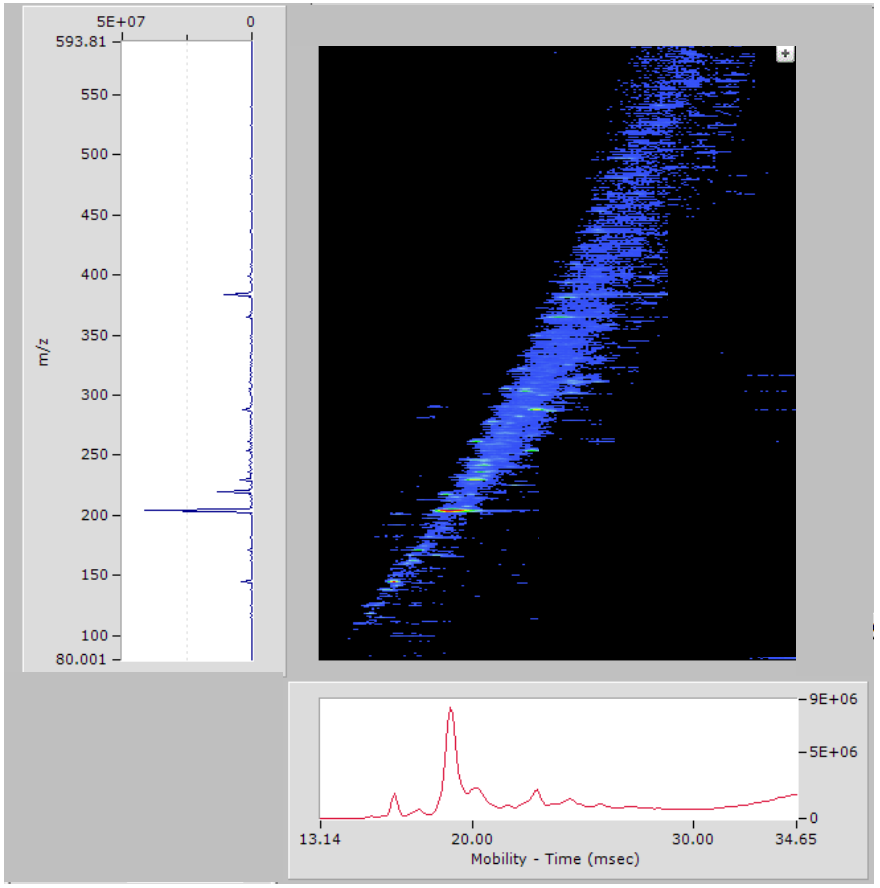




Preliminary IMS-MS analysis of urine



Positive ESI, 5 µL of pooled urine prepared



Positive ESI, 5 µL of pooled urine + 15 mM sugars (glucose, xylose, mannose and galactose) prepared

Conclusions

IMS-MS:

- Increase the throughput of sample analysis, while still detecting lower level species
- Analyze difficult samples and obtain information that was previously not detected
- Start running biological diversity studies to evaluate peptide/protein markers in hundreds to thousands of patients

Future directions:

- Perform fast lipidomic and metabolomic analyses

Acknowledgements

- National Institute of Environmental Health Sciences of the NIH (R01ES022190)
- NIH: General Medical Sciences Proteomics Center at PNNL (2 P41 GM103493-11), National Institute of General Medical Sciences and National Cancer Institute
- PNNL Laboratory Directed Research and Development Program
- Environmental Molecular Sciences Laboratory