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Utilizing IMS-MS to Increase Proteomic Coverage & Examine Structural Properties of Complex Biological Samples

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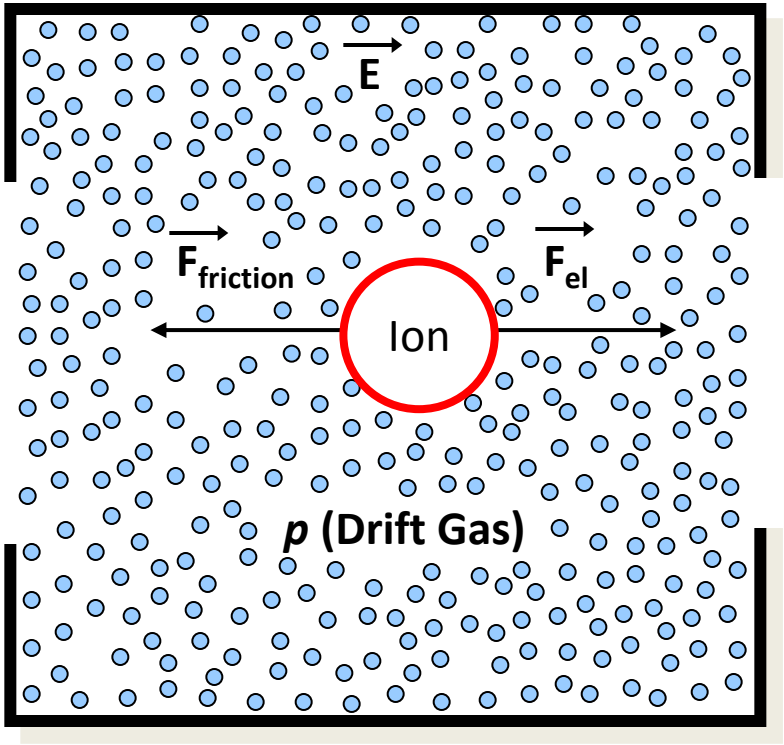
Pacific Northwest National Laboratory

Ion mobility concept

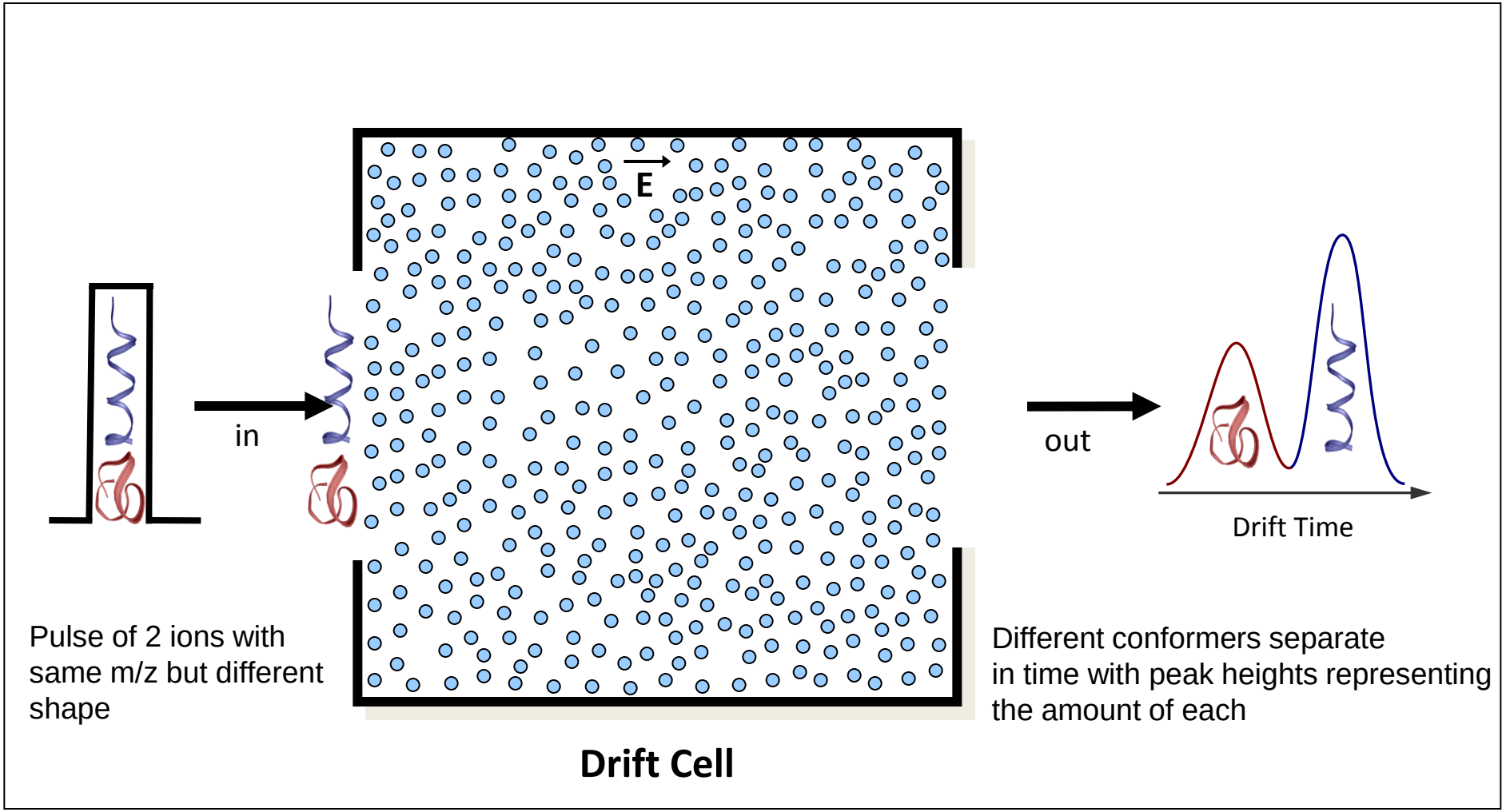


Drift Cell

velocity is constant

$v = K \vec{E}$
K = ion mobility

Ion mobility concept



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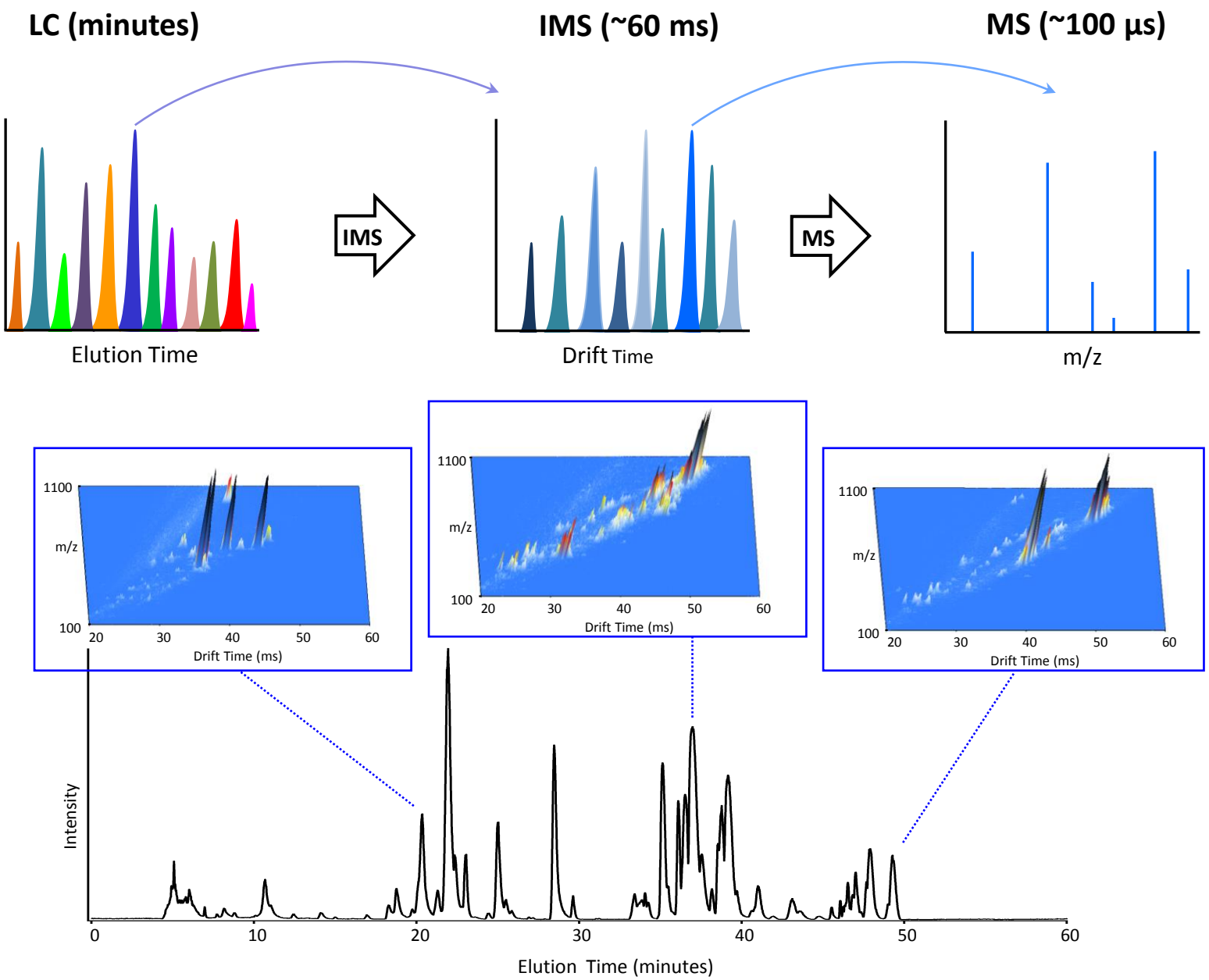
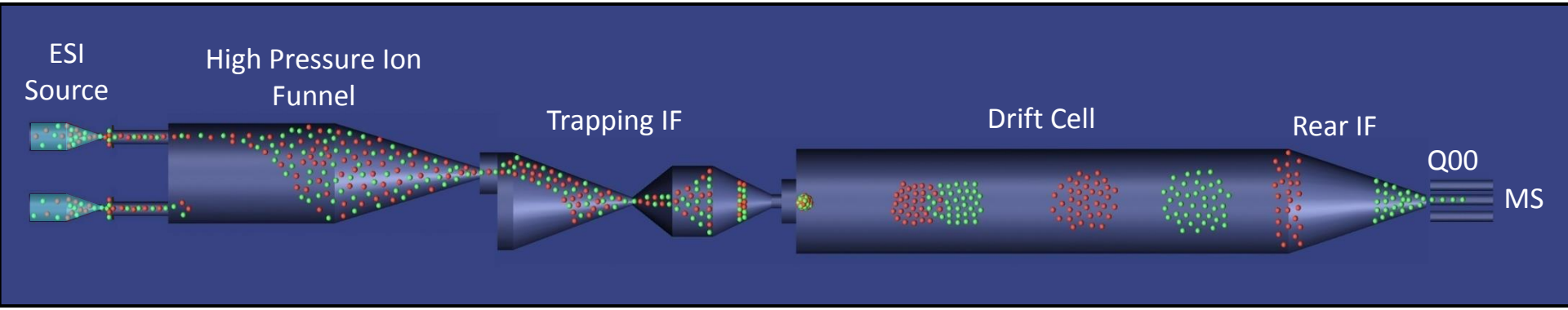


IMS-MS instrumentation



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

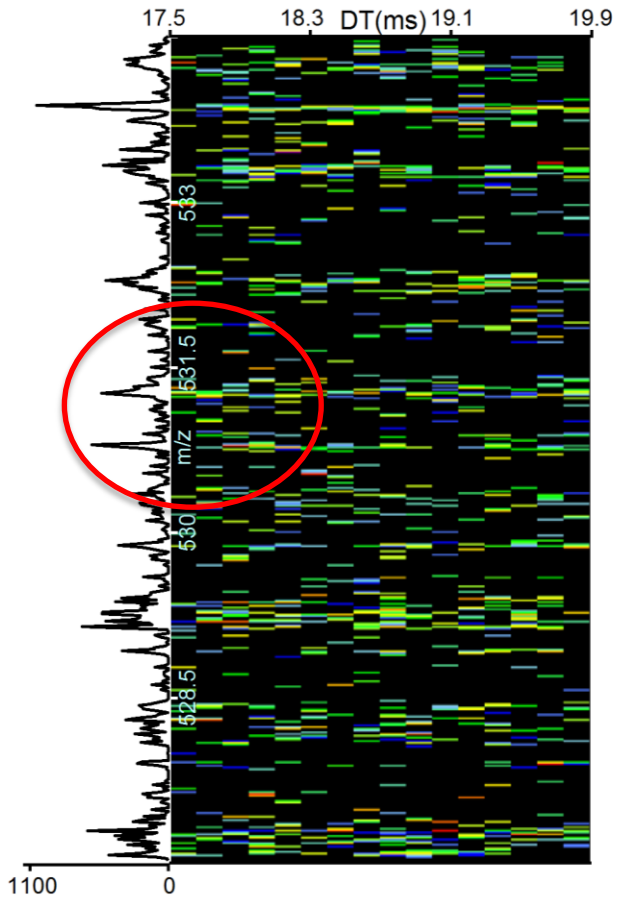
8 peptides spiked in human serum

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	✔	✔	✔

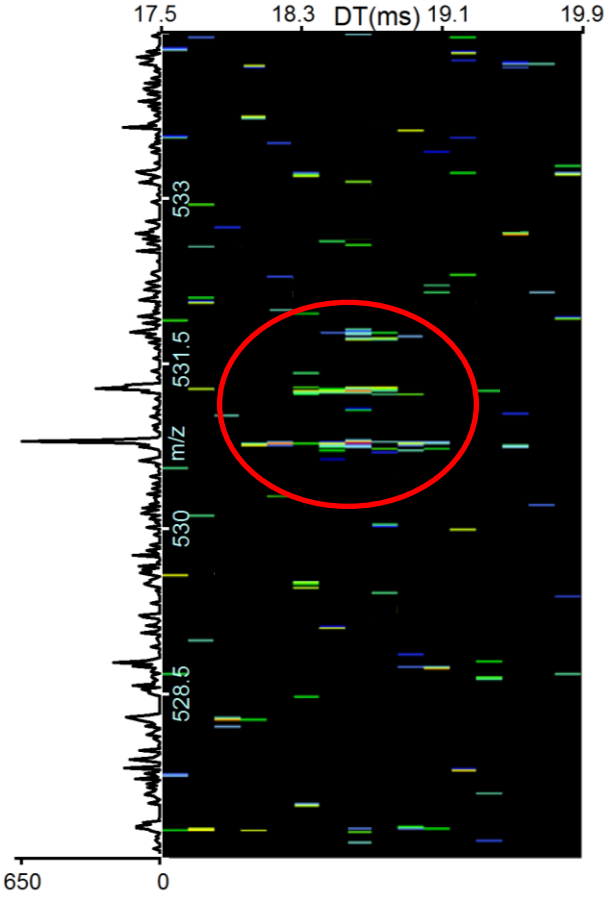
Sample analyzed using Velos-Orbitrap, TOF MS and IMS-TOF MS instruments

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)

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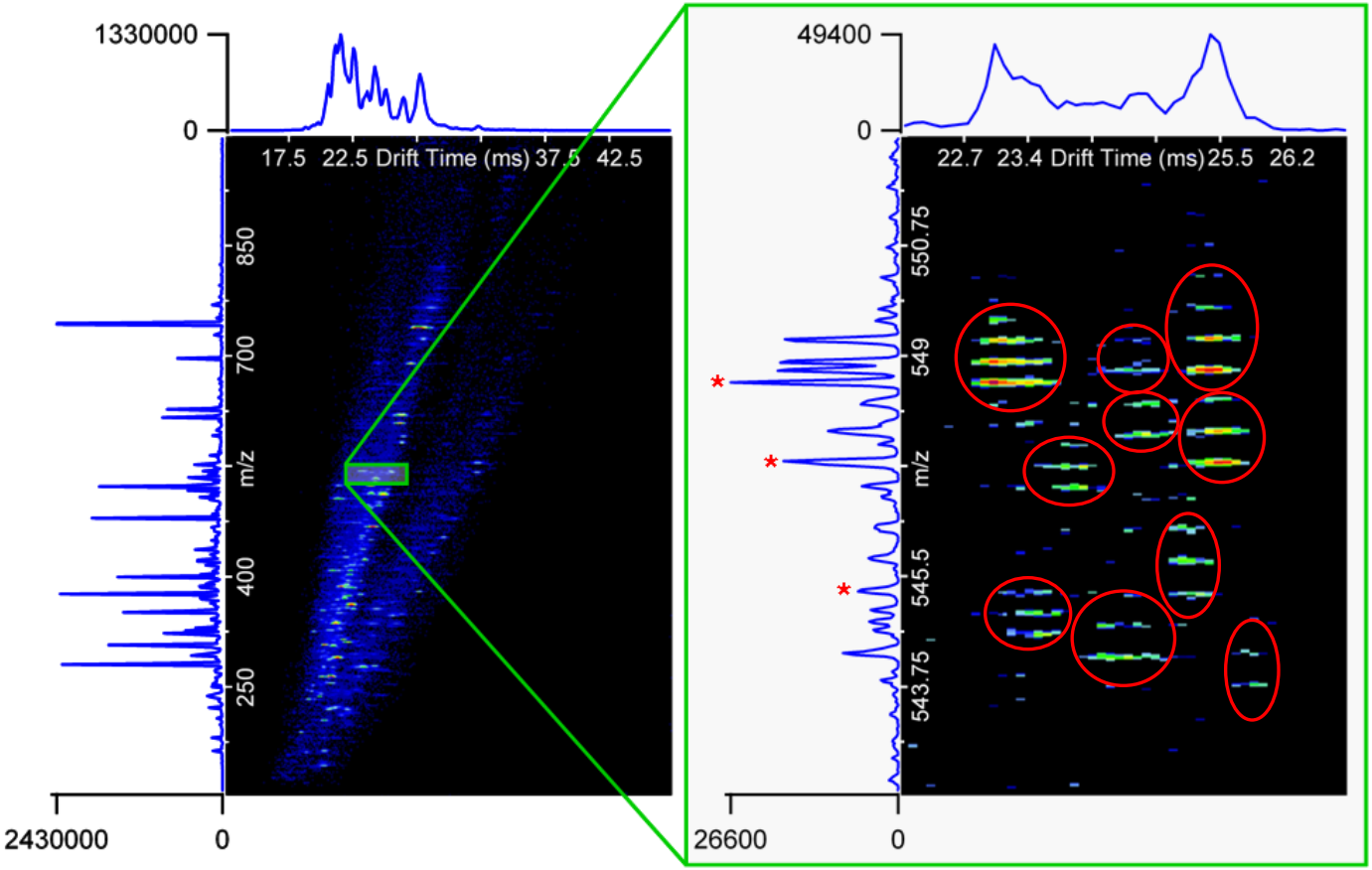
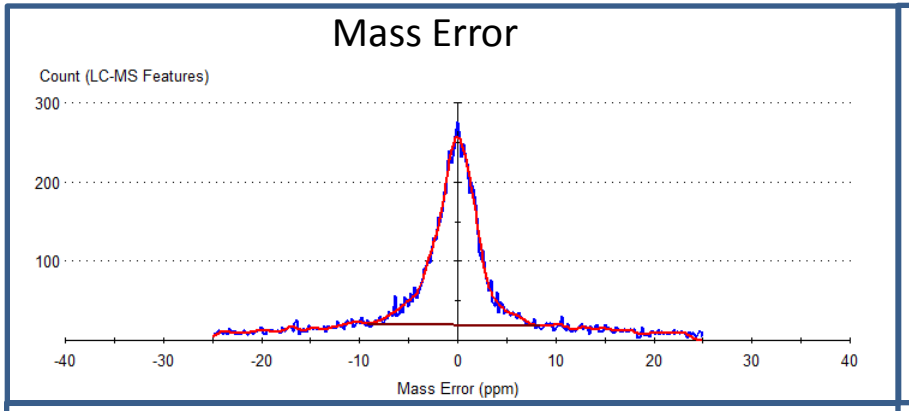
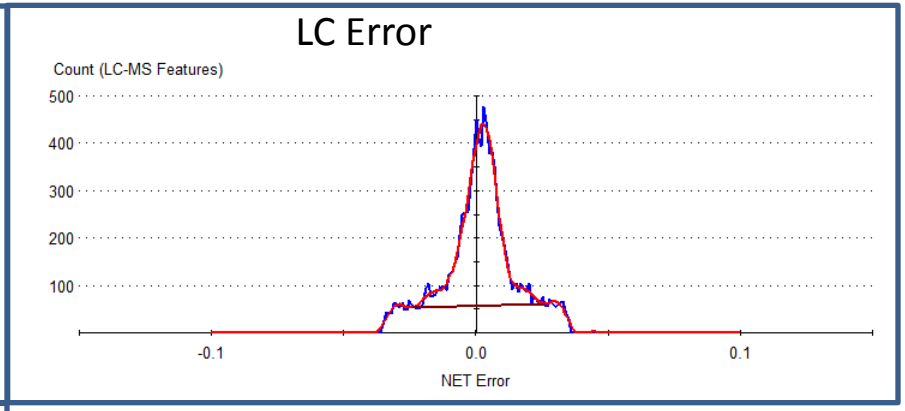


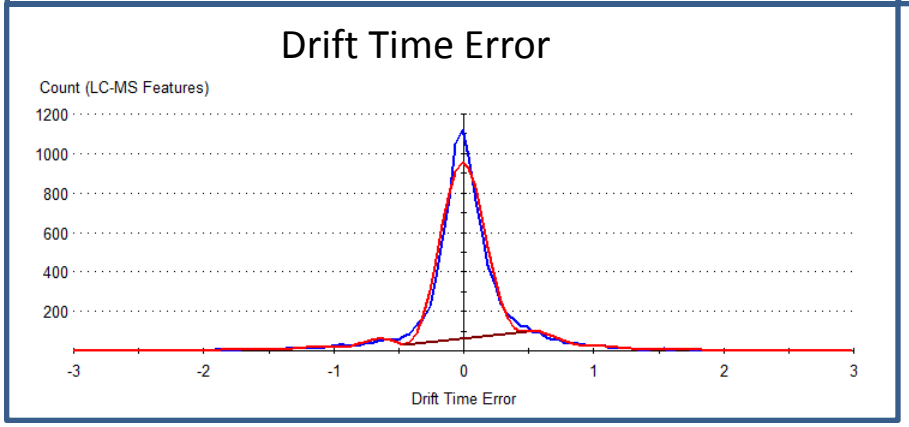
Only 3 features discerned without drift time dimension (*)

Using IMS drift time in databases

2. Reduced FDR due to added dimension







Human Serum Peptide Analysis

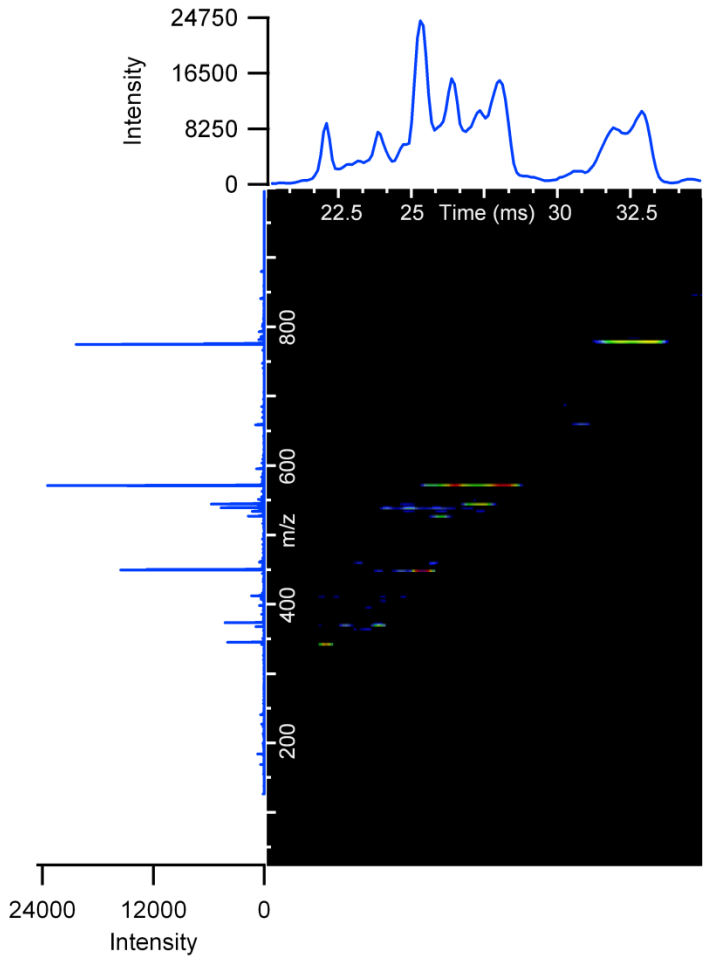
100-min LC-Velos Orbitrap
3986 Features with FDR <5%

60-min LC-IMS-MS
7519 Features with FDR <5%
If you only use LC and MS
4007 Features with FDR <5%

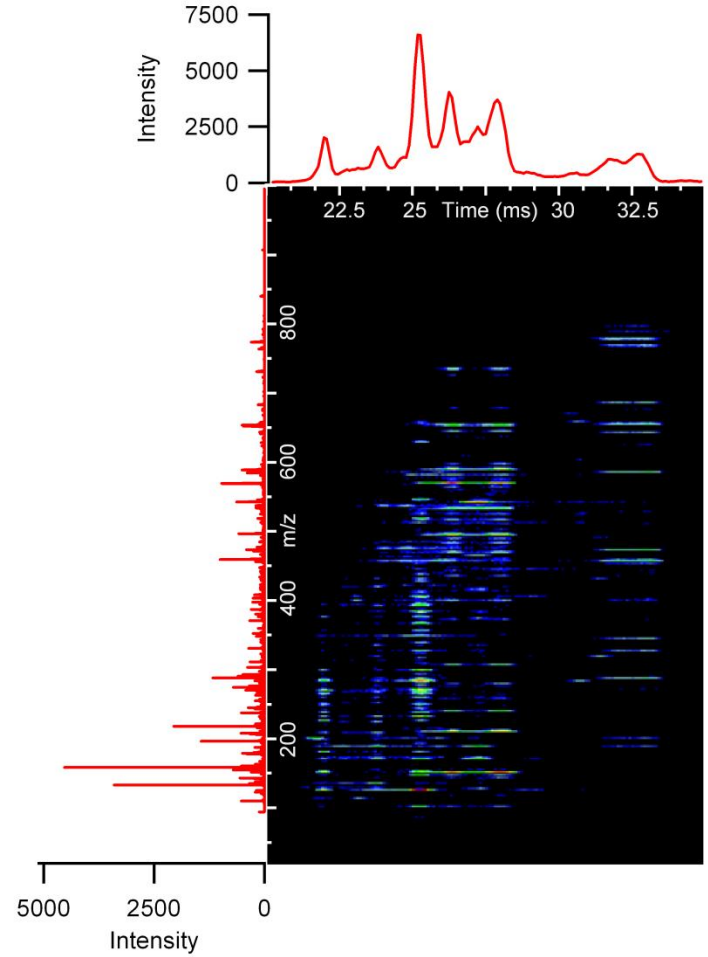
Extra dimension adds confidence to LC-IMS-MS Features Matches to AMT Tag DB

Benefits of IMS drift time separation

3. IMS-(CID)-MS Multiplexed Fragmentation



Parent Spectrum

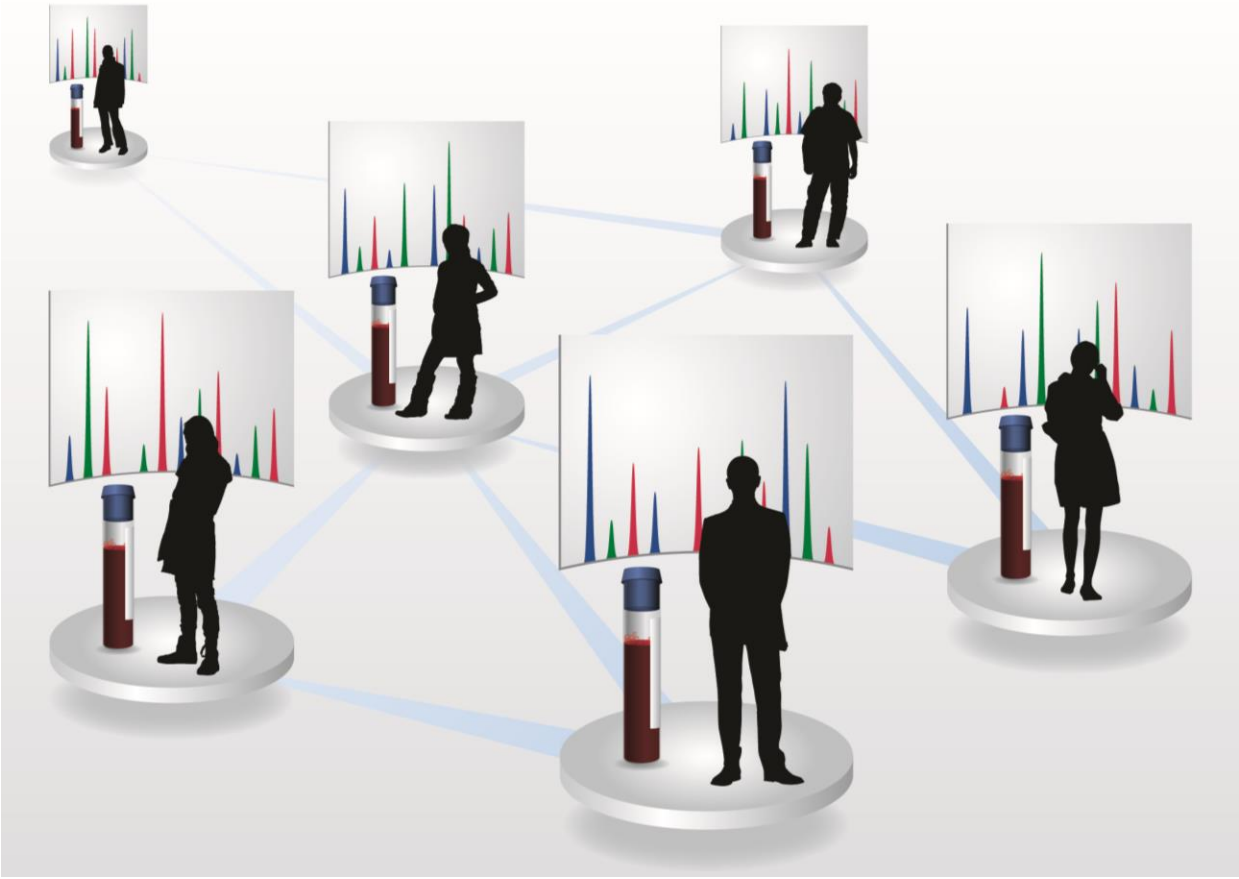


Fragmentation Spectrum

Fragments have the same drift time as precursors

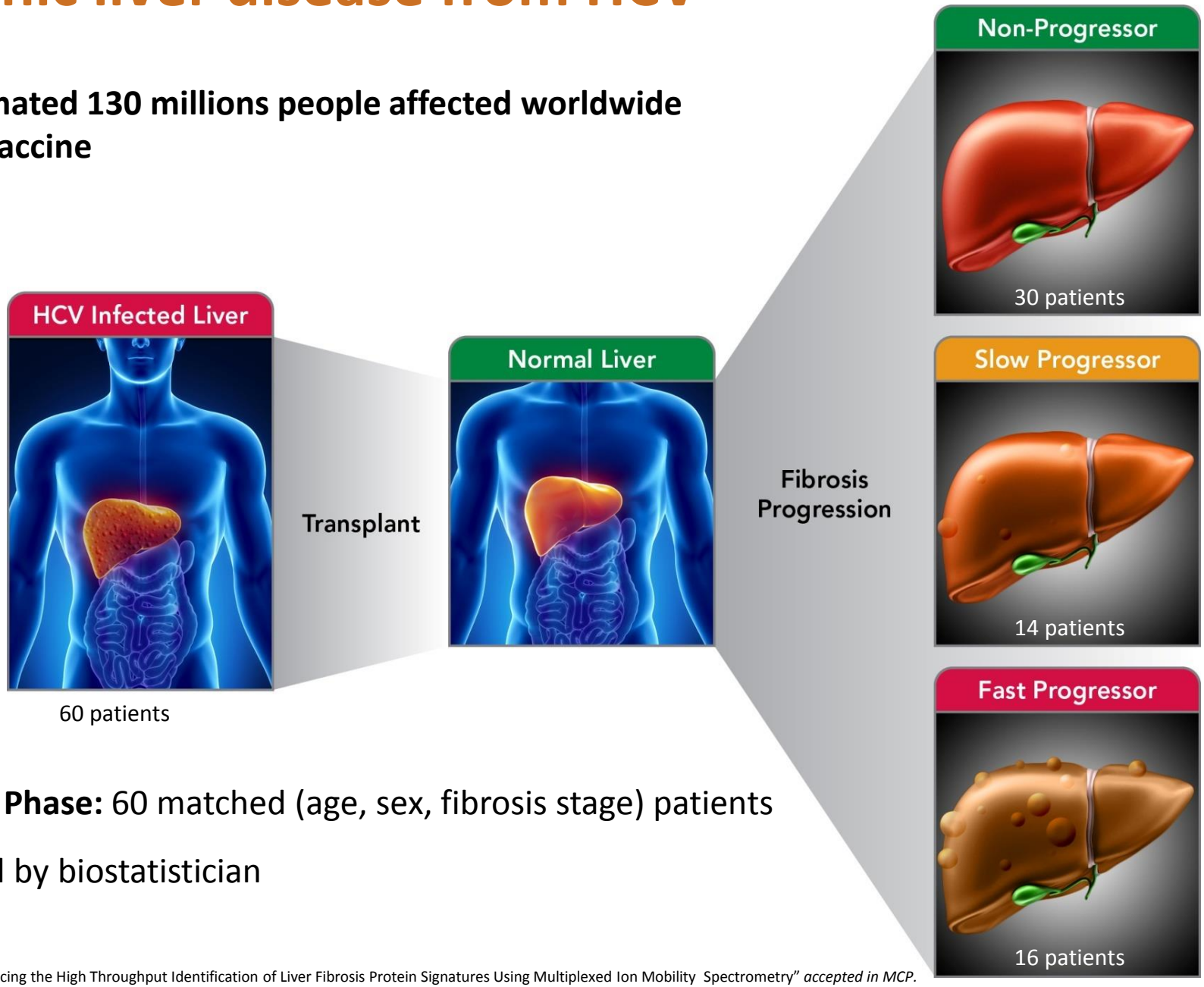
Biological diversity studies

- 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 from HCV

- **Estimated 130 millions people affected worldwide**
- **No vaccine**



Discovery Phase: 60 matched (age, sex, fibrosis stage) patients

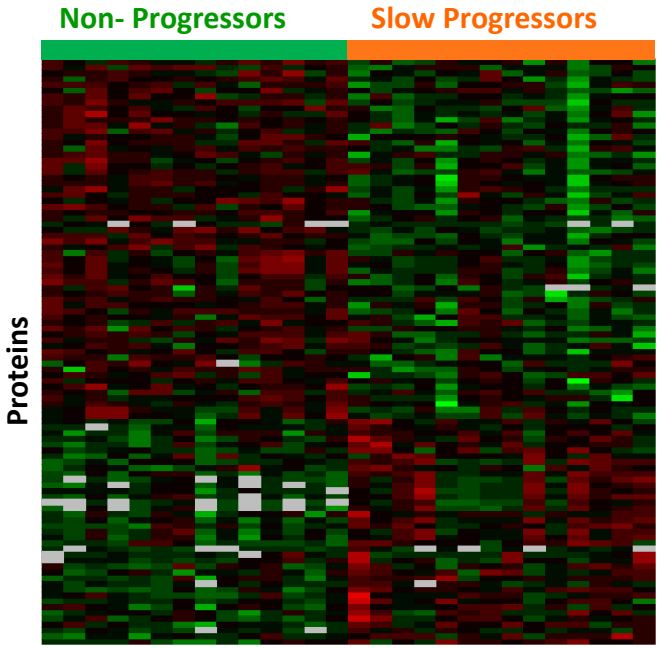
Correlated by biostatistician

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

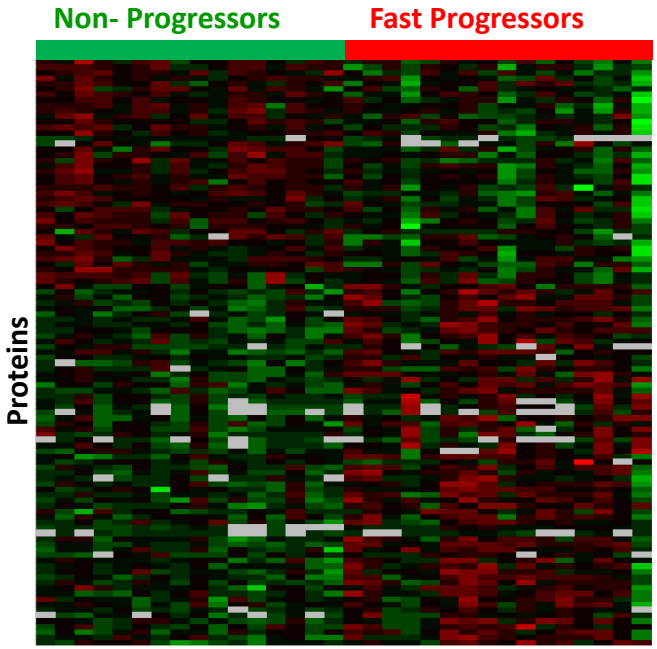
Liver fibrosis study

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



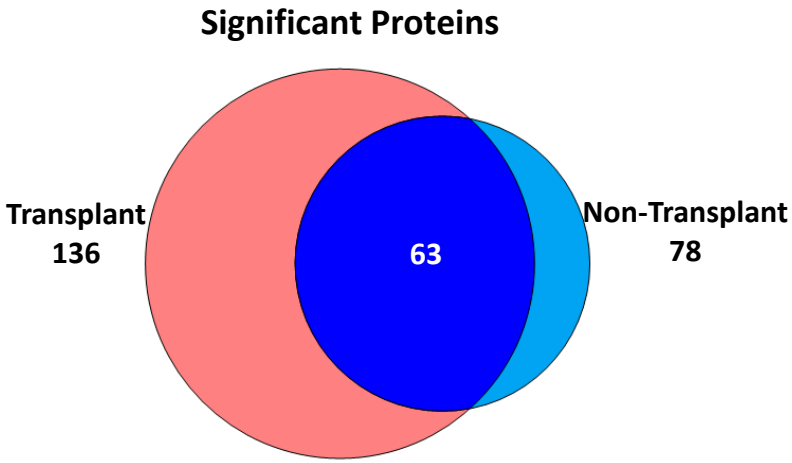
Decrease = Green
Increase = Red

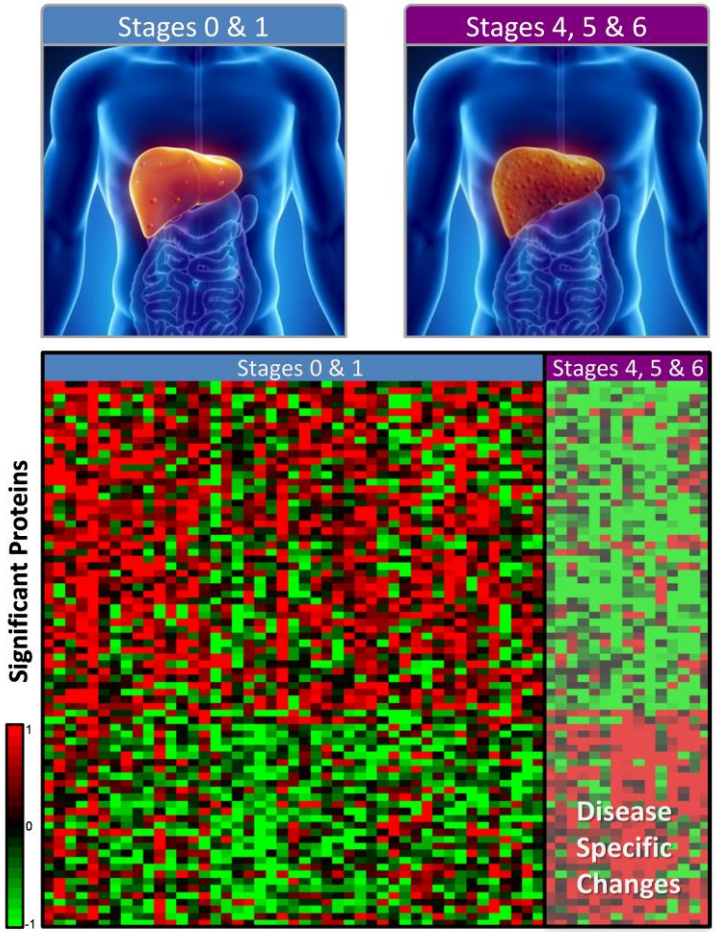


Liver fibrosis study

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

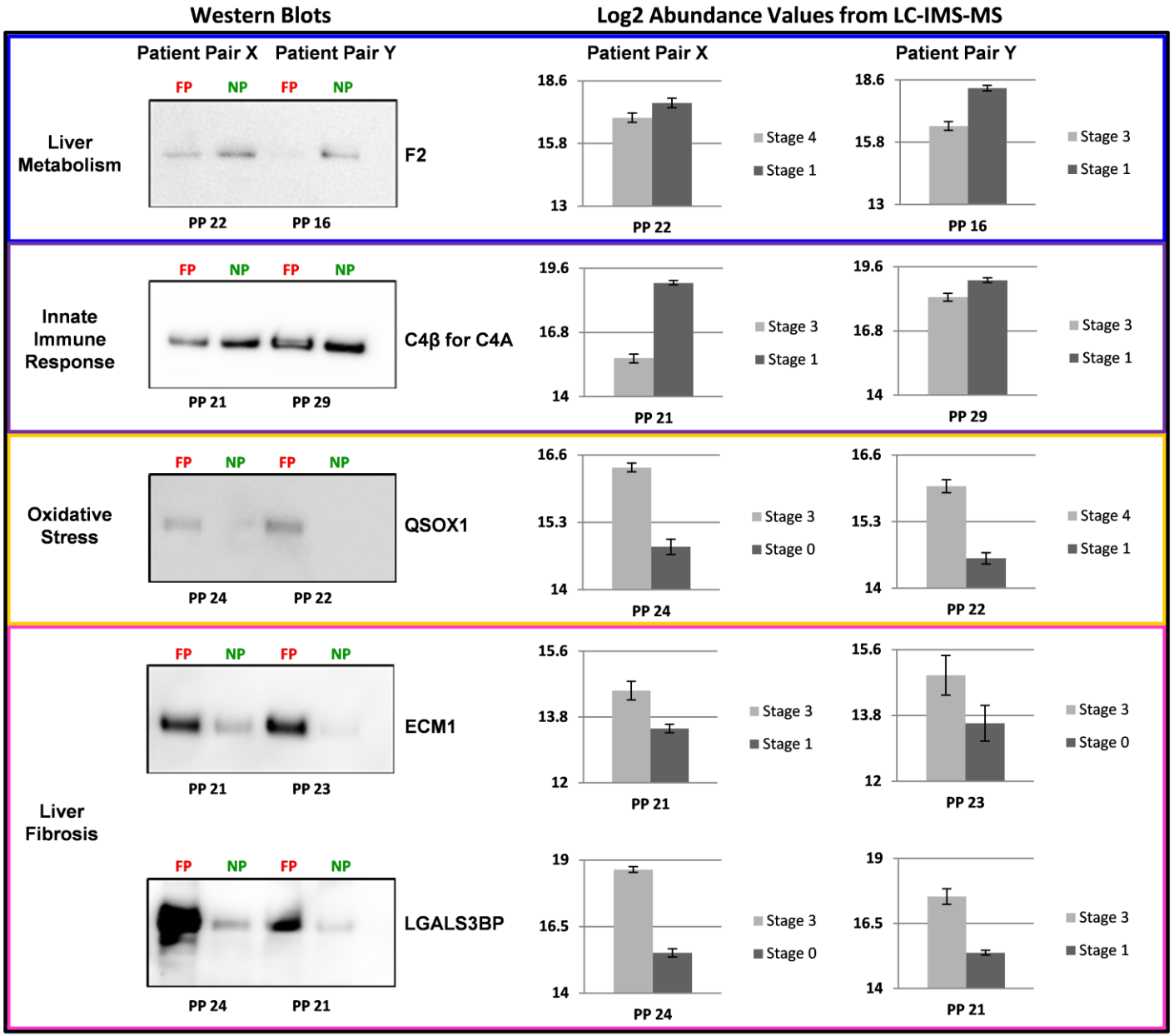
19 Example Proteins

- 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		

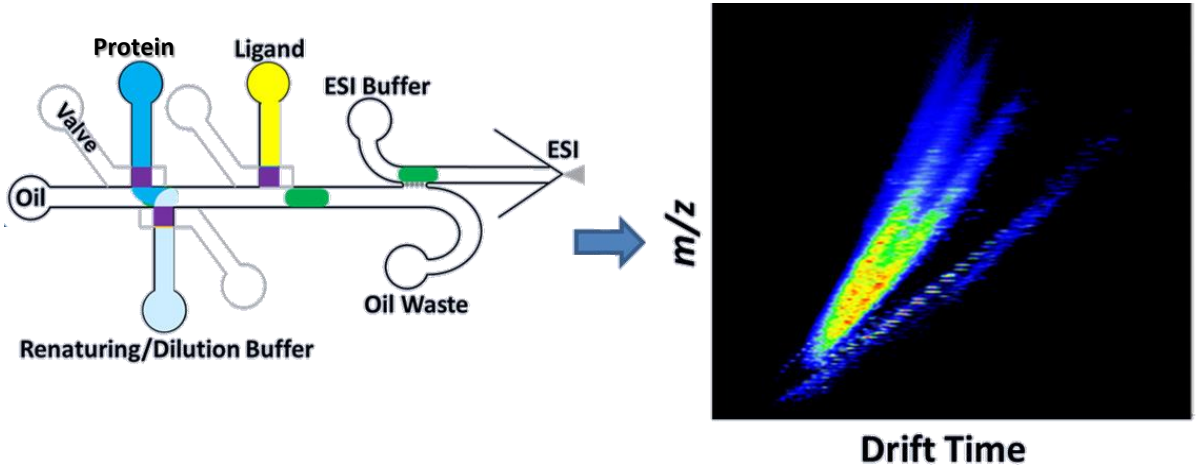
Decrease = Green
Increase = Red

Liver fibrosis study



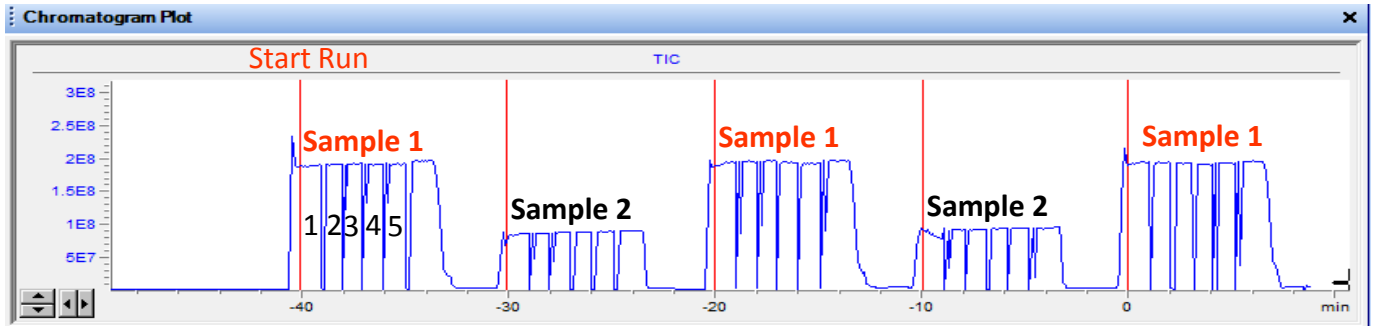
High Throughput Protein Studies

Droplet-Based Microfluidic Chip

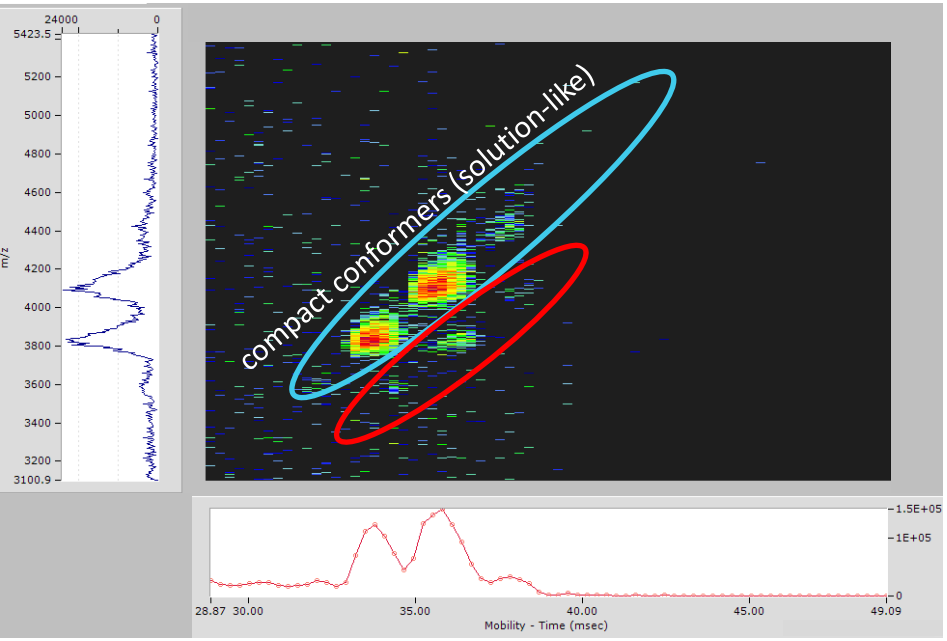


Direct Inject System

10 µm id capillary column with adjustable flow rate

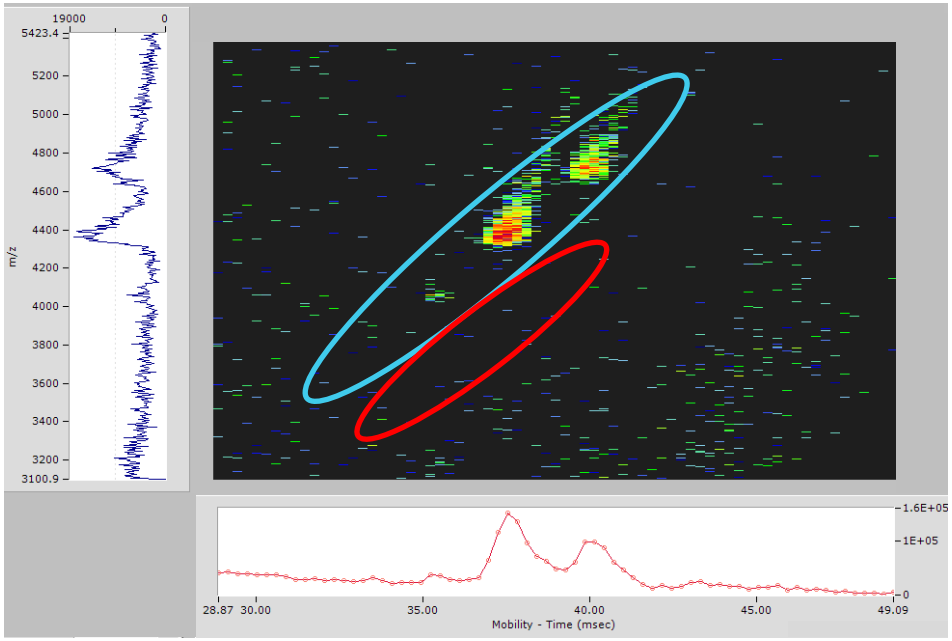


Protein Complexes



Transthyretin (TTR) Tetramer

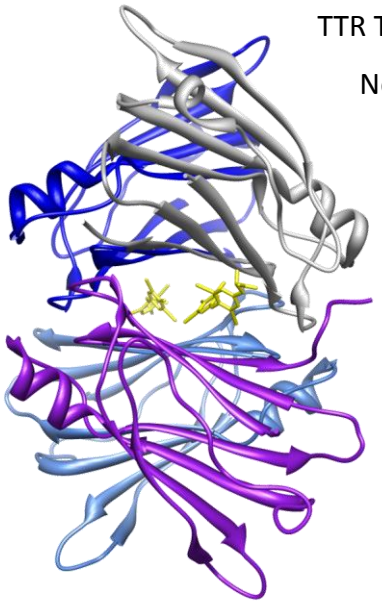
Both compact and extended conformers



TTR Tetramer with Diflunisal

No extended conformers

Drug stabilizes compact (solution phase) structure



Samples from Catherine Costello

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
- Interest in analyzing drug and metabolite effects on protein complexes

Acknowledgements

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- Environmental Molecular Sciences Laboratory