New Proteomics capabilities based upon Structures for Lossless Ion Manipulations (SLIM) Ion Mobility-Mass Spectrometry

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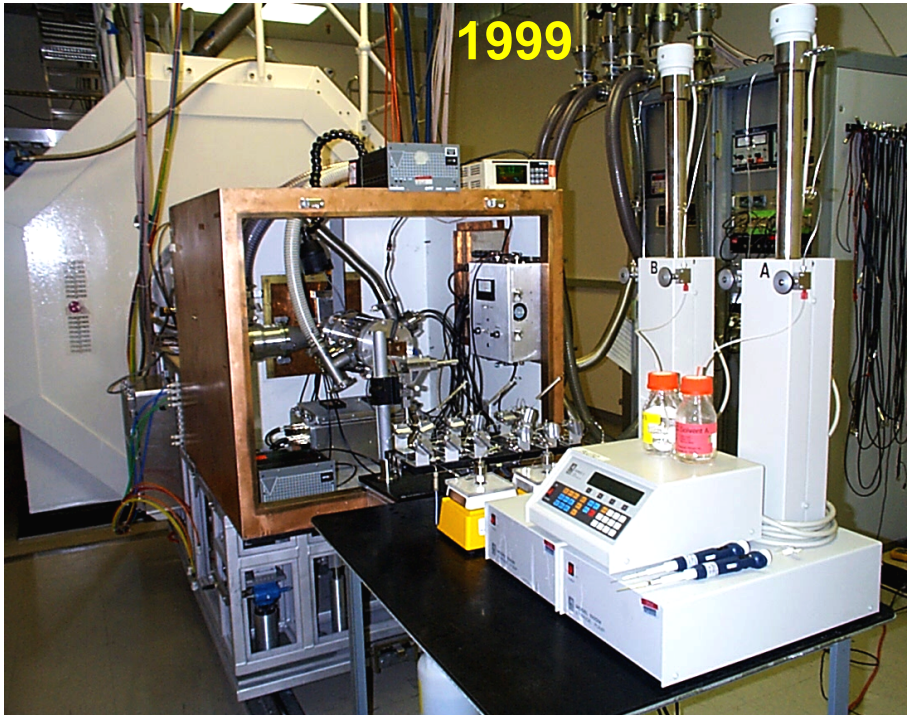
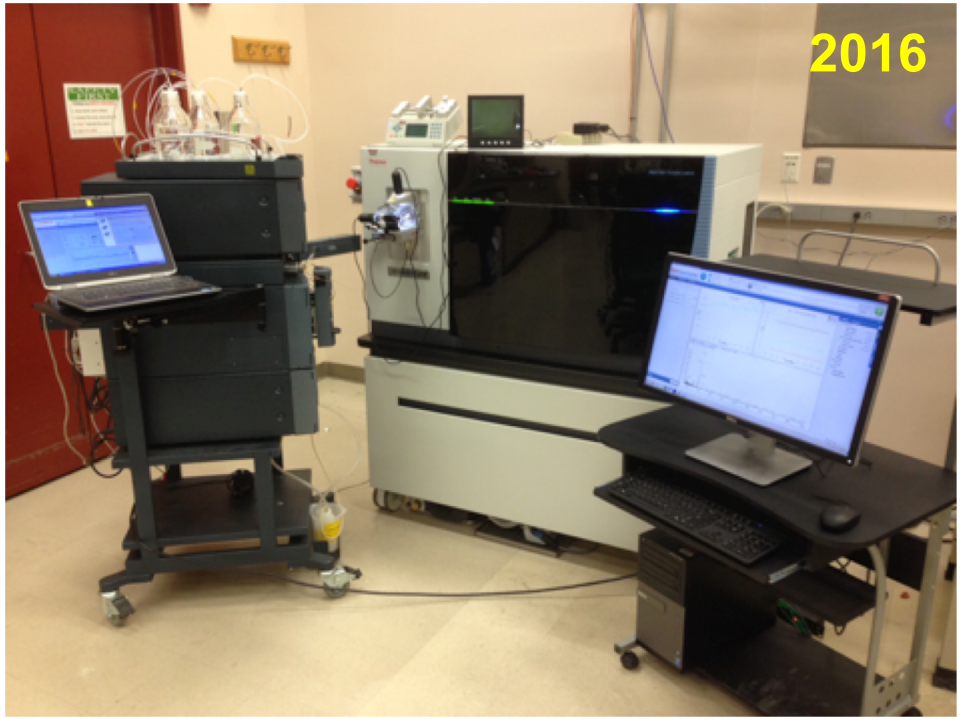
Richland, Washington

LC for MS-based proteomics is relatively mature



PNNL developed nanoLC and 11.5 tesla FTICR MS



Waters nanoLC and ThermoFisher Fusion Lumos orbitrap MS

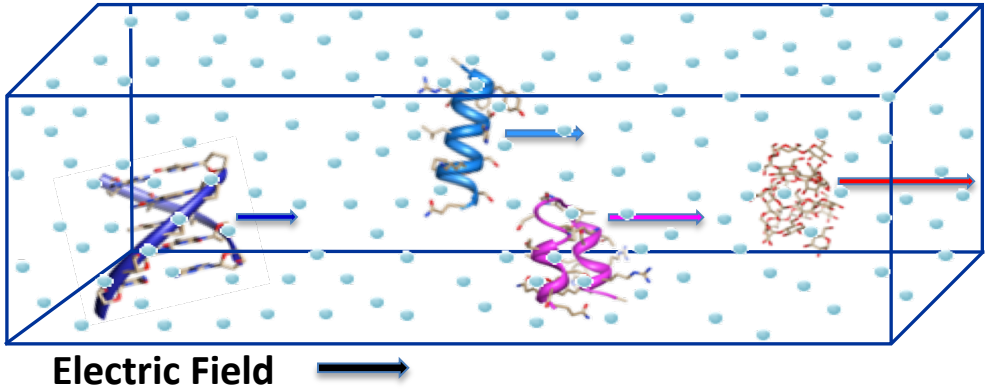
Some limitations of LC separations for MS-based proteomics

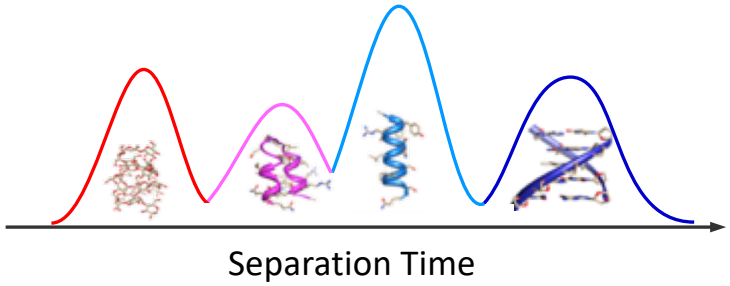
- Throughput limitations:
  - Typically ~hour per injection for LC separation
  - Multiple sample fractions used to increase proteome coverage
- Platform stability issues (e.g. variations in LC retention times) leading to increased FDRs and degraded quantification
- Insufficient sensitivity; samples too small; large coverage gaps due to limited dynamic range of measurements
- Measurement ‘blind spots’: inability detect or distinguish possibly important constituents

A potentially better path: ion mobility (IM) separations

- Generally fast (typically msec) and highly reproducible
- Can increase dynamic range and sensitivity, providing deeper coverage
- Provide ion structural information (collision cross section; CCS)
- Broadly applicable (proteomes, metabolomes, lipidomes, glycomes…..)

IM separation in drift tube





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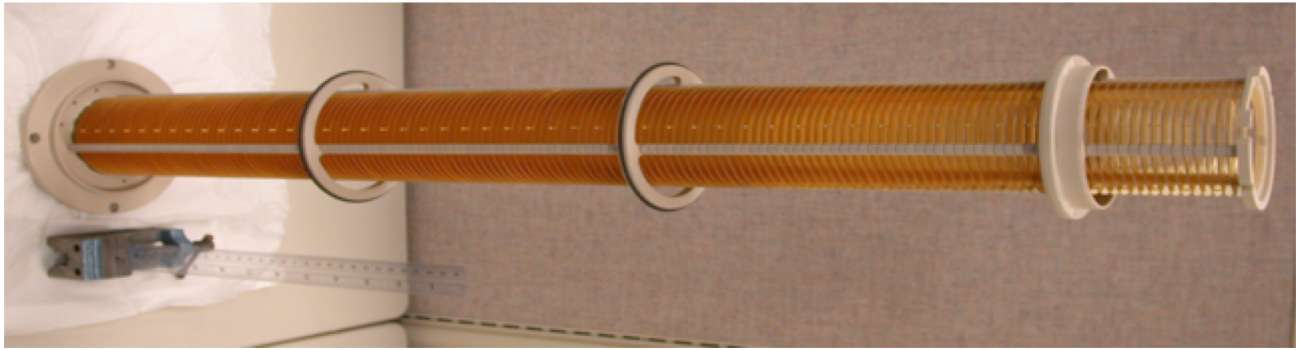
IM Drift tube (maximum length ~1 meter)



Some challenges for IM separations in MS-based proteomics

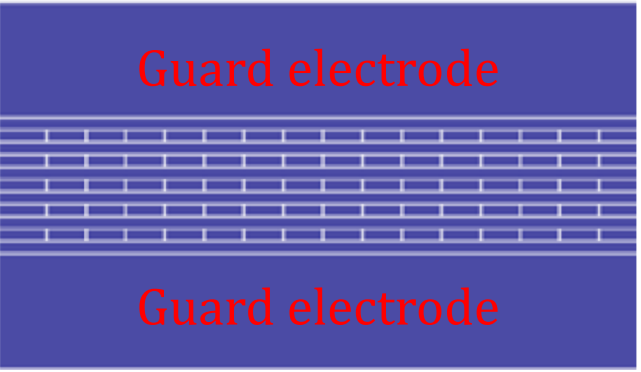
The need for:

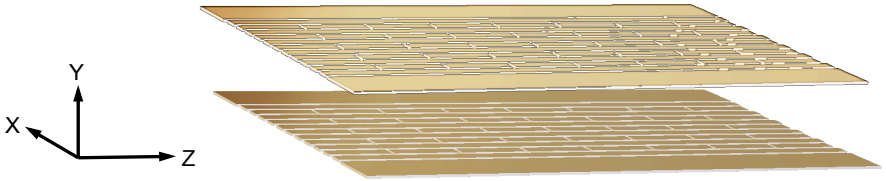
- Larger separation powers and peak capacities
- Higher speed (in some implementations)
- Greater sensitivity and dynamic range

SLIM are constructed from electric fields

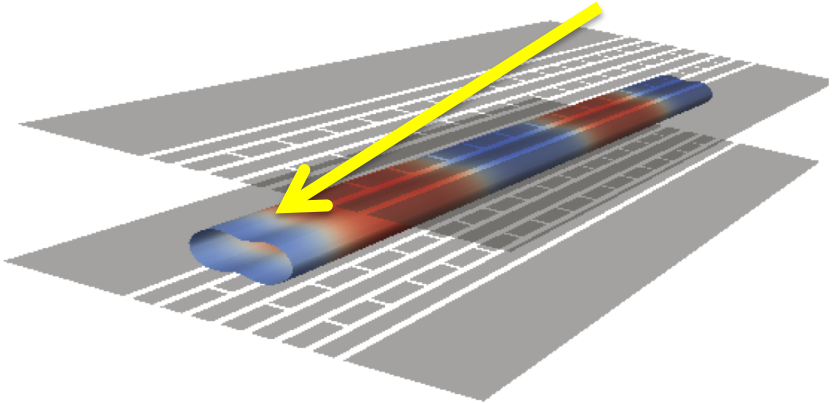
- SLIM ion conduits, switches, traps, etc. constructed from electric fields in a gas at low pressure (e.g. 4 torr N_2) in the gap between two surfaces
- Electric fields generated by applying appropriate potentials to mirror-image arrays of electrodes on the two surfaces

Typical SLIM electrode arrangement (6,5)
(6 RF electrodes separating 5 TW electrode arrays)



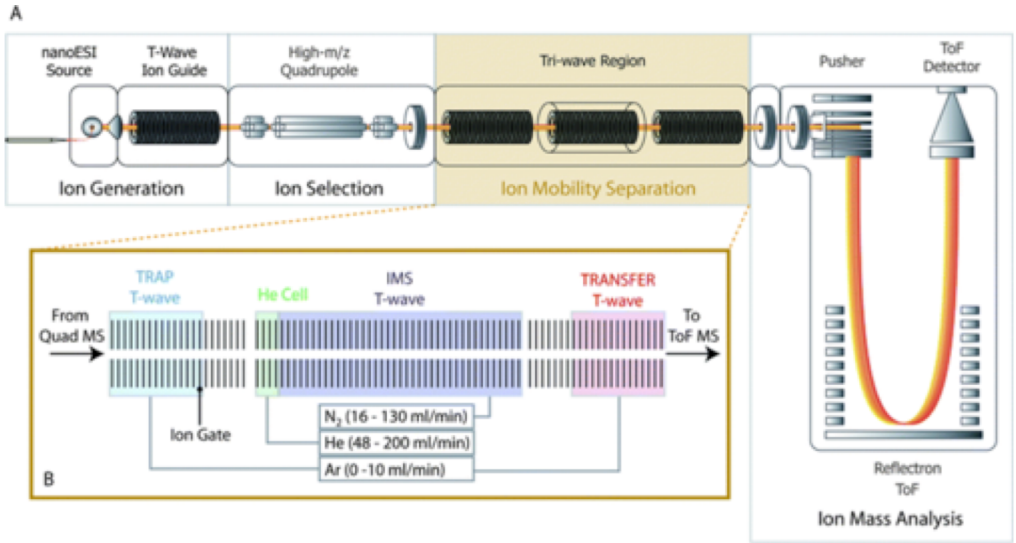


Region of ion confinement



Use of traveling waves (TW) for IM

- Waters Synapt 18 cm stacked ring ion guide TW IM-MS introduced 2006
- Higher resolution Synapt G2 with 25 cm SRIG TW IM-MS introduced 2009

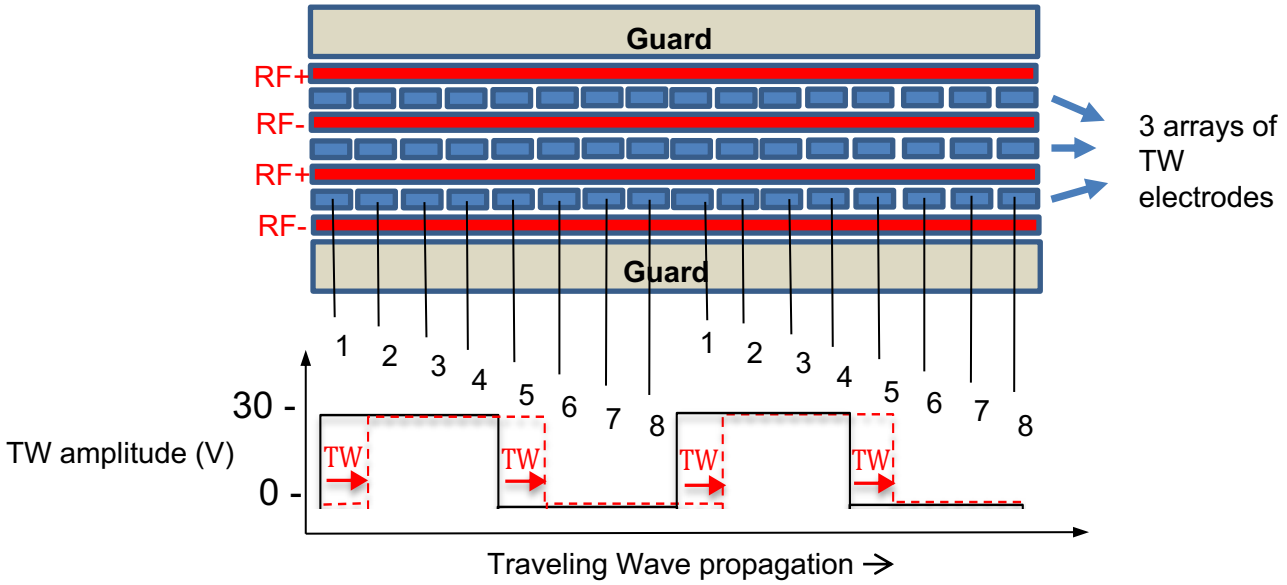


- Similar to drift tube IM, theory* indicates $R \propto (L)^{1/2}$
- Voltages needed independent of *L;* large voltage drops avoided

* Shvartsburg and Smith, Analytical Chemistry, *80*, 9689 (2008)

Creating electric traveling waves (TW) in SLIM

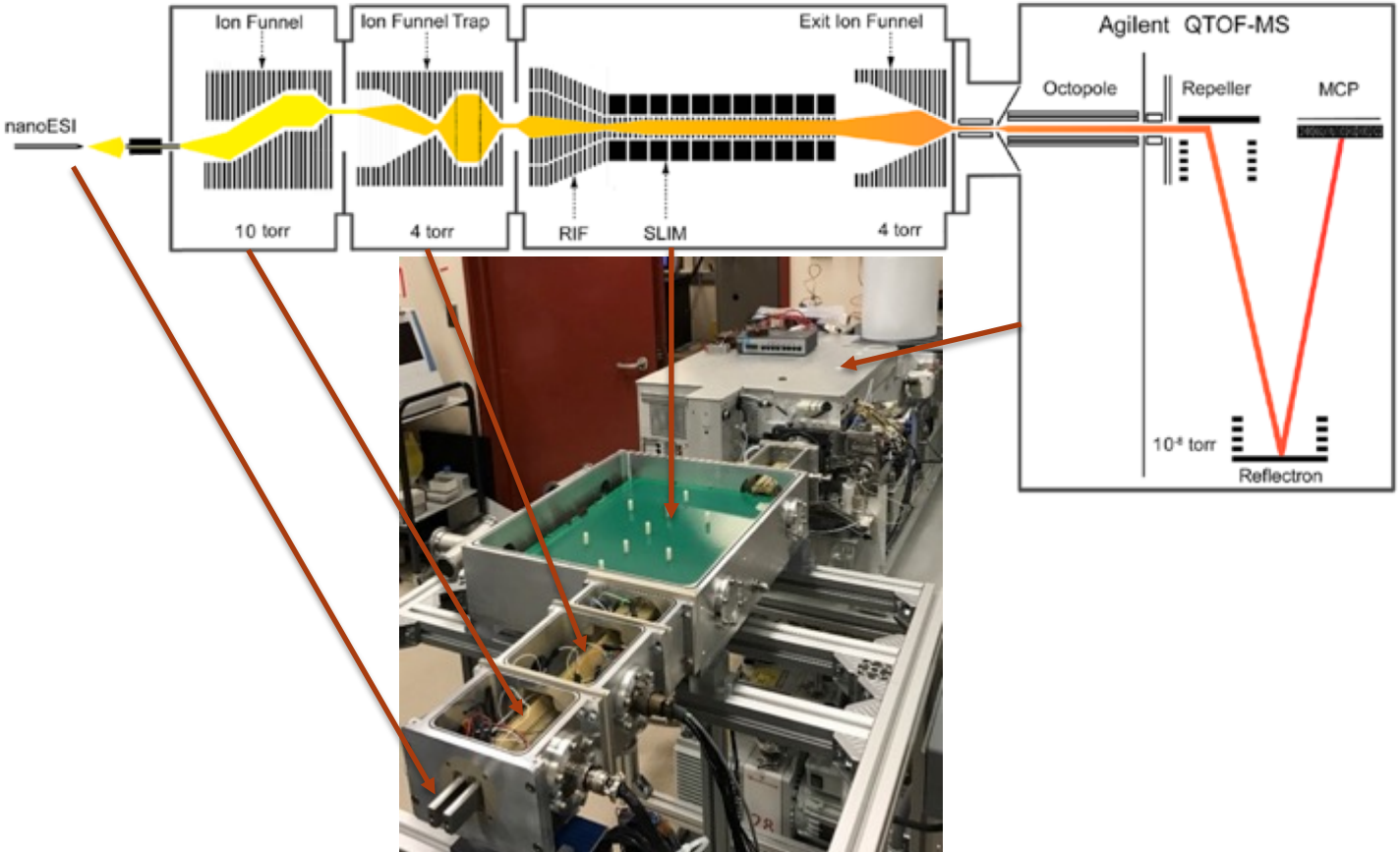
One of two SLIM surfaces with 4 RF electrodes separating 3 TW electrode arrays (4,3 arrangement)



~ 1 bin/cm; TW speed typically 20 to 200 m/s

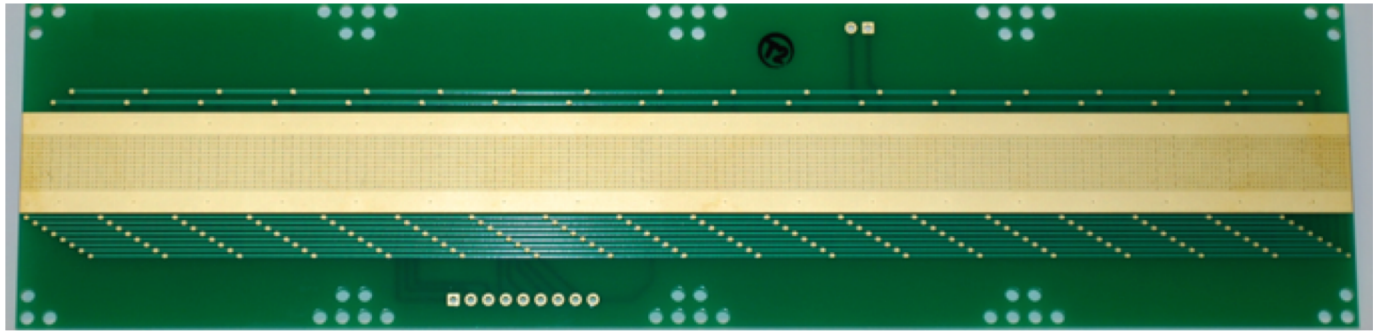
Hamid et al., Anal. Chem., *87*, 11301 (2015)

SLIM implementation with a high resolution TOF MS

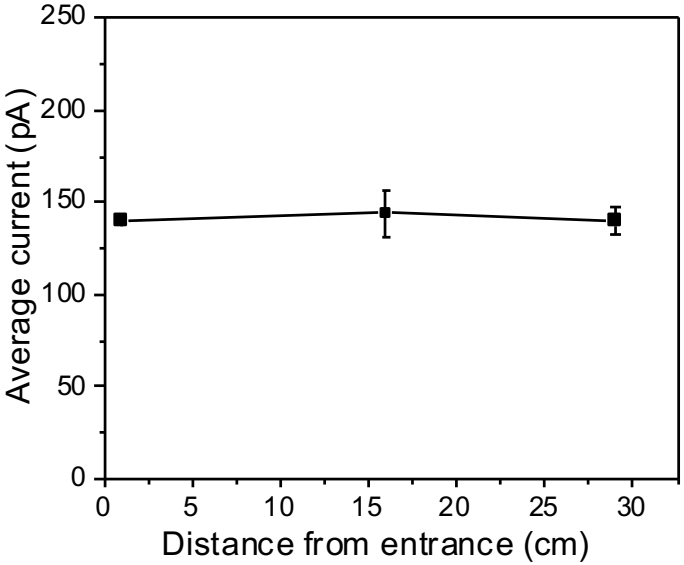


Ions losslessly transmitted through a 30-cm TW SLIM module

One of two mirror-image SLIM surfaces; 12 RF electrodes, 11 TW electrode array (12, 11) arrangement



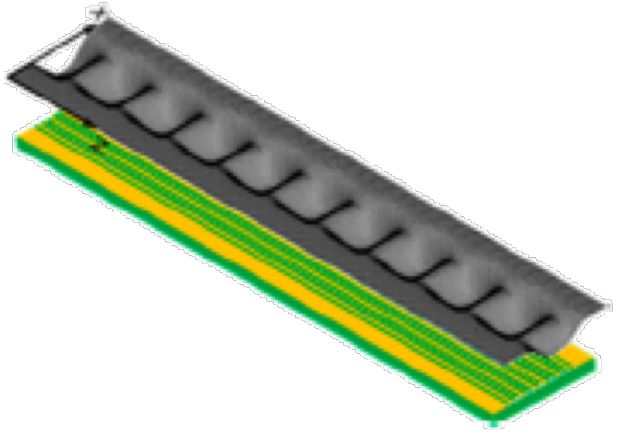
TW amplitude 30 V, speed 84 m/s
RF ~800 kHz, 200 $V_{P\text{-}P}$;
gap 5 mm; guard bias 15 V

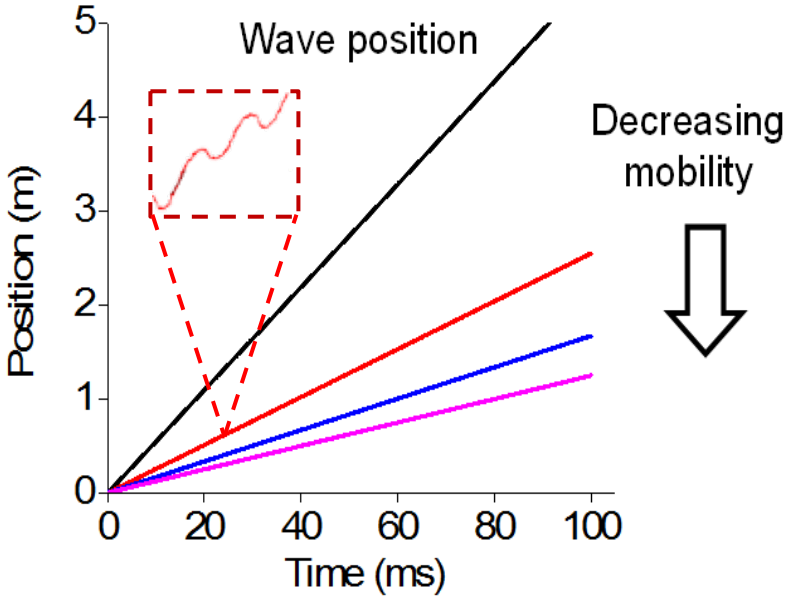


Hamid et al., Anal. Chem., *87*, 11301 (2015)

Simulation of ion mobility separation in a TW SLIM module

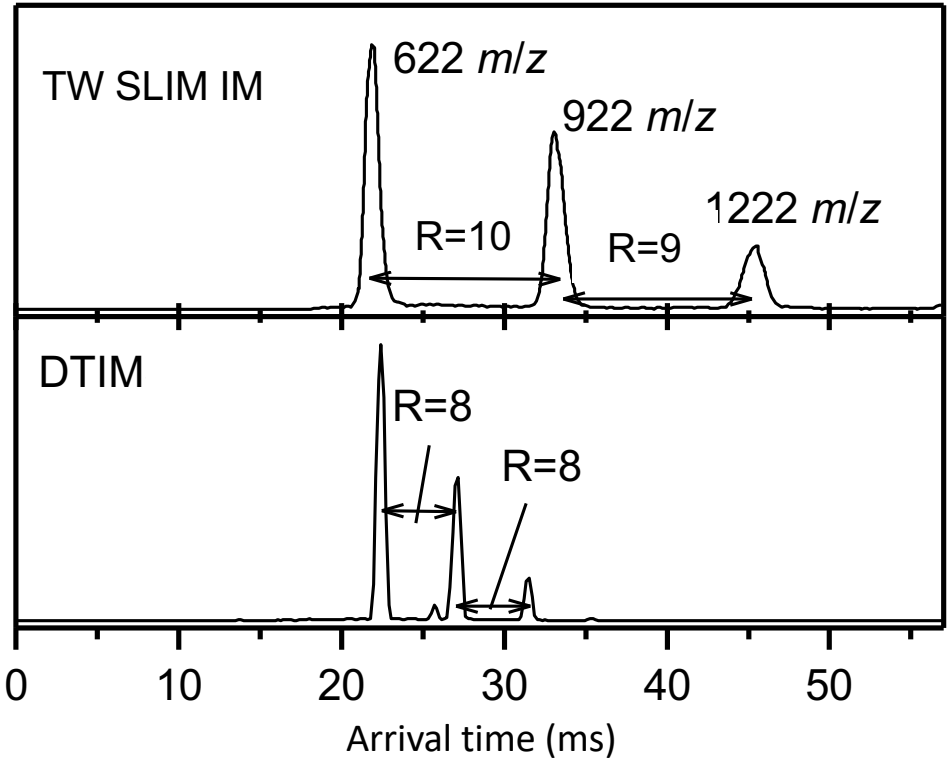
Separation occurs as lower mobility ions are passed over by a wave and fall into the following bin (4 torr Nitrogen)





IM resolution in ~1 m drift tube (DT) vs. 30 cm TW SLIM

Ion mobility resolution for Agilent 'tune mix' ions at 4 torr pressure (N_2)



Hamid et al., Anal. Chem., *87*, 11301 (2015)

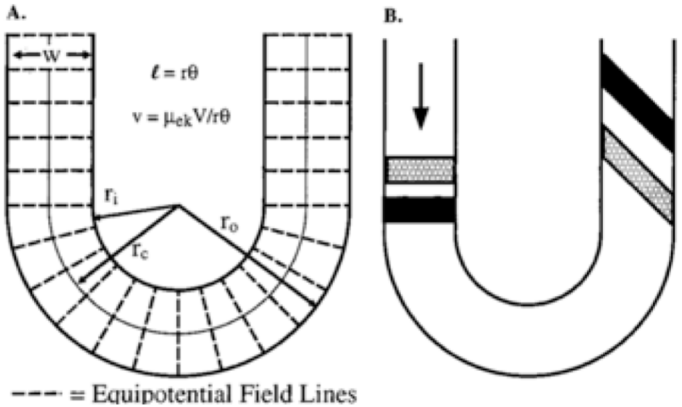
The cost of making a turn; the ‘race track’ effect

Anal. Chem. **1998,** *70,* 3781–3789

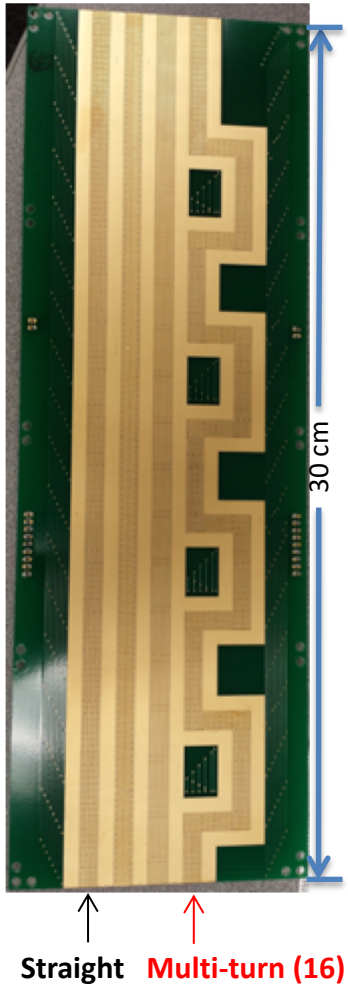
Dispersion Sources for Compact Geometries on Microchips

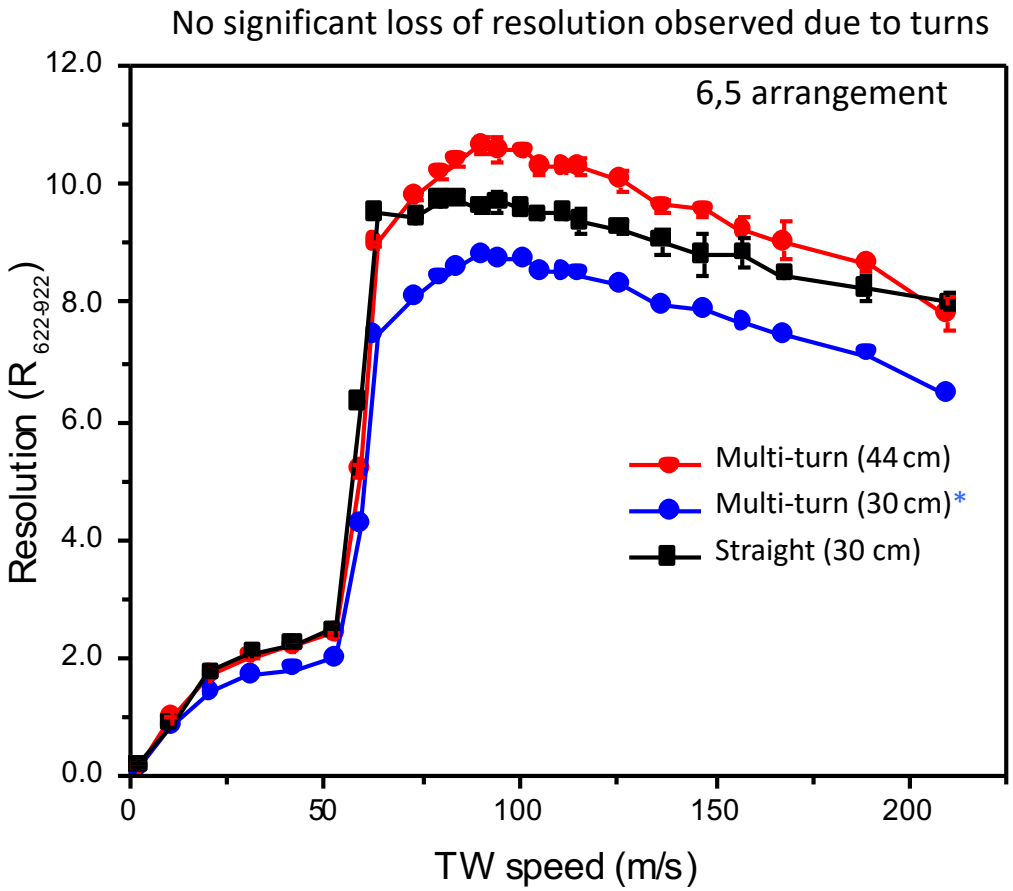
Christopher T. Culbertson, Stephen C. Jacobson, and J. Michael Ramsey*

Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831-6142



No significant loss of IM resolution observed in TW SLIM turns

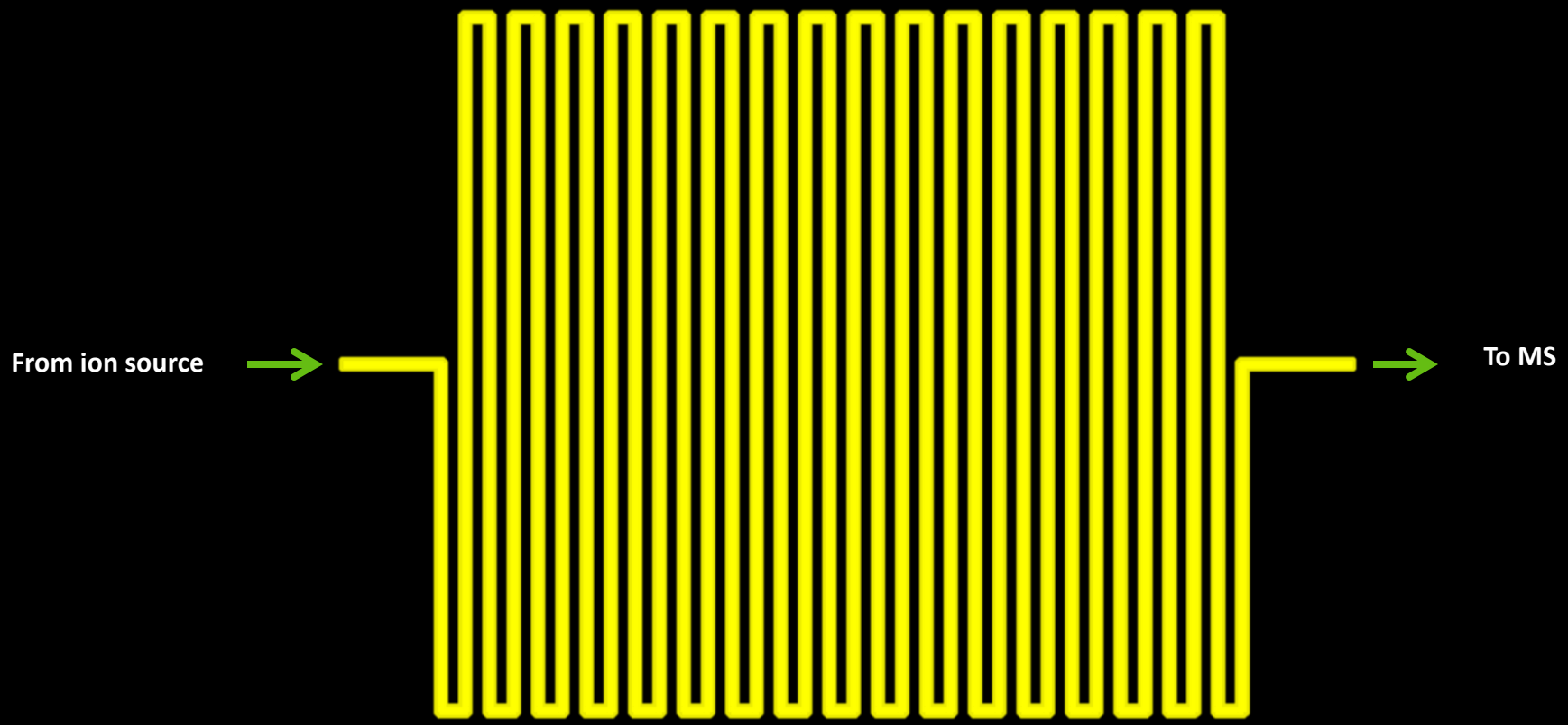




* 30 cm multi-turn path scaled from 44 cm path experimental measurements

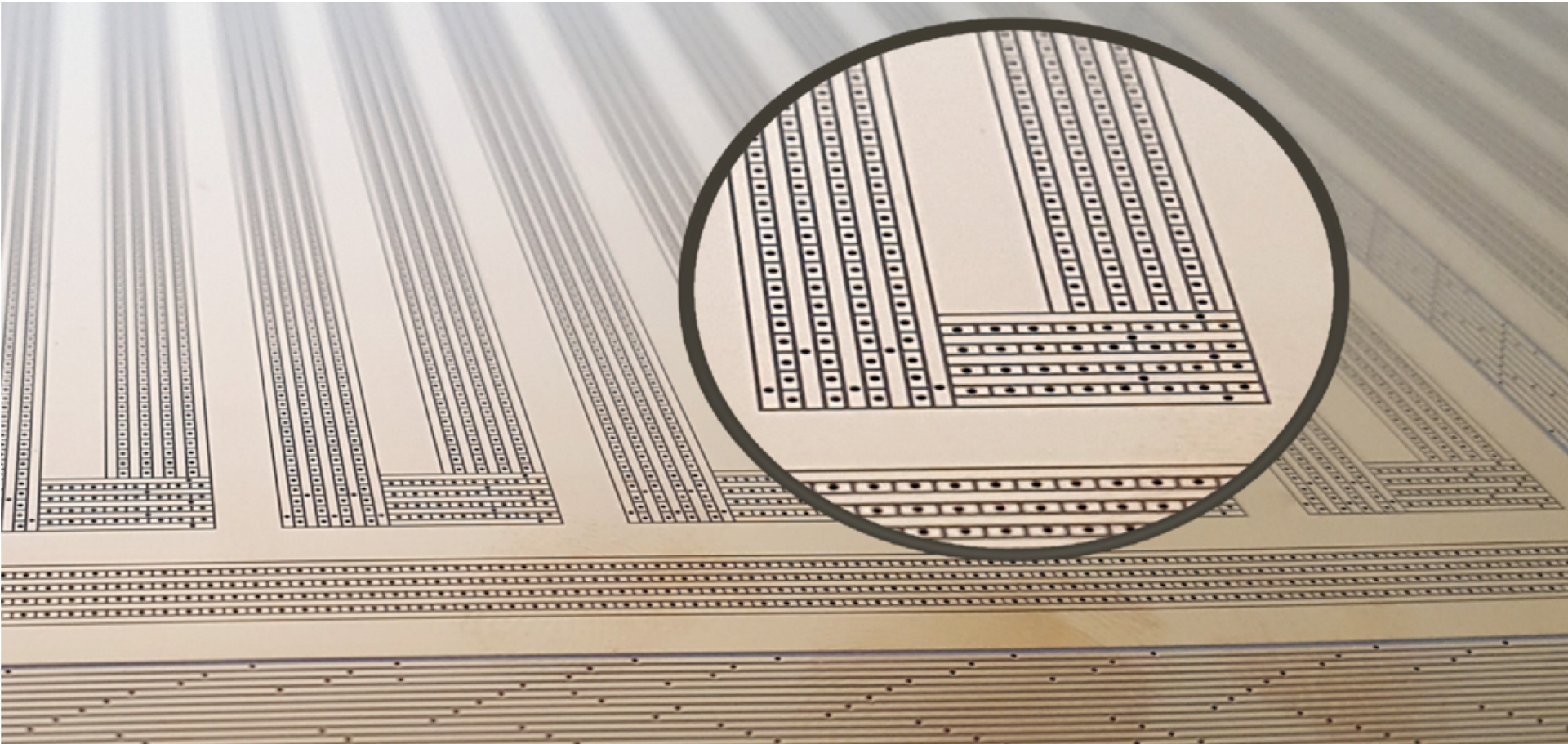
Hamid et al.; Analytical Chemistry, *88*, 8949 (2016)

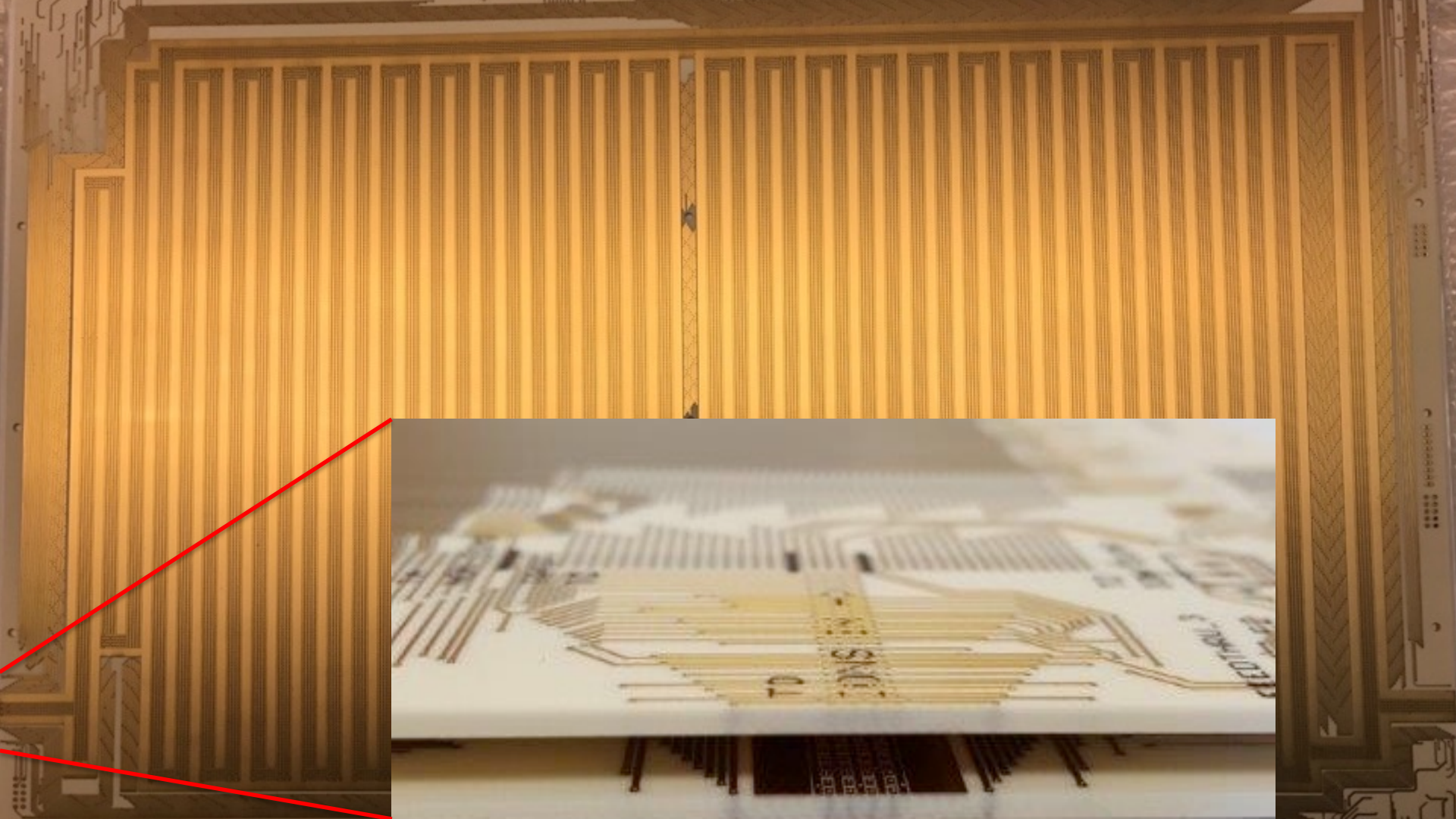
Serpentine long path SLIM design initially developed for achieving higher resolution IM separations



One surface of a long path (13 m) TW SLIM IM module

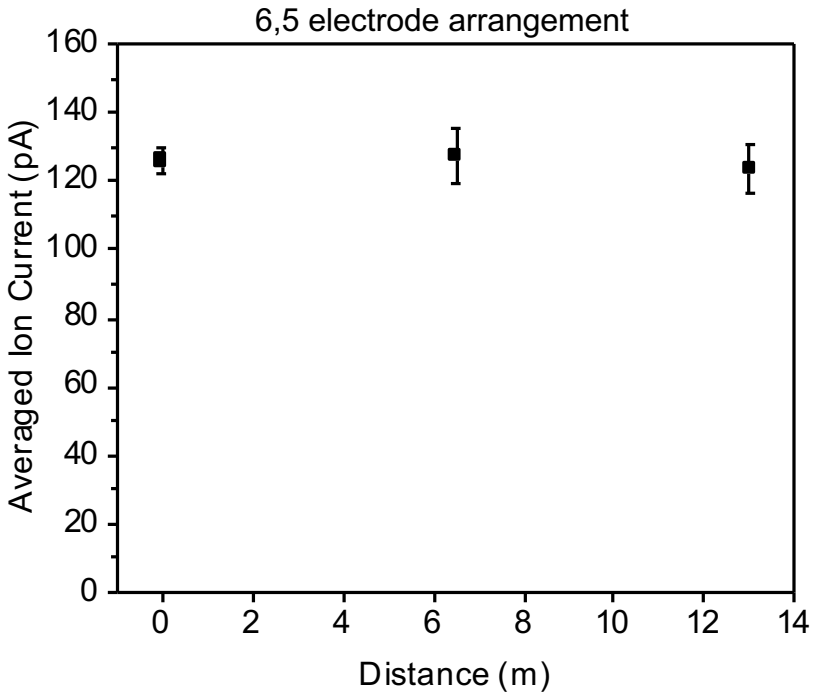
Long path IM module consists of two 14” x 18” surfaces with mirror image electrode arrays (5,4)
Each surface has 54,000 TW electrodes; two surfaces spaced by ~2.9 mm





Lossless ion transmission through a 13 m TW SLIM module

Probe inserted between SLIM surfaces to measure ion current at different locations

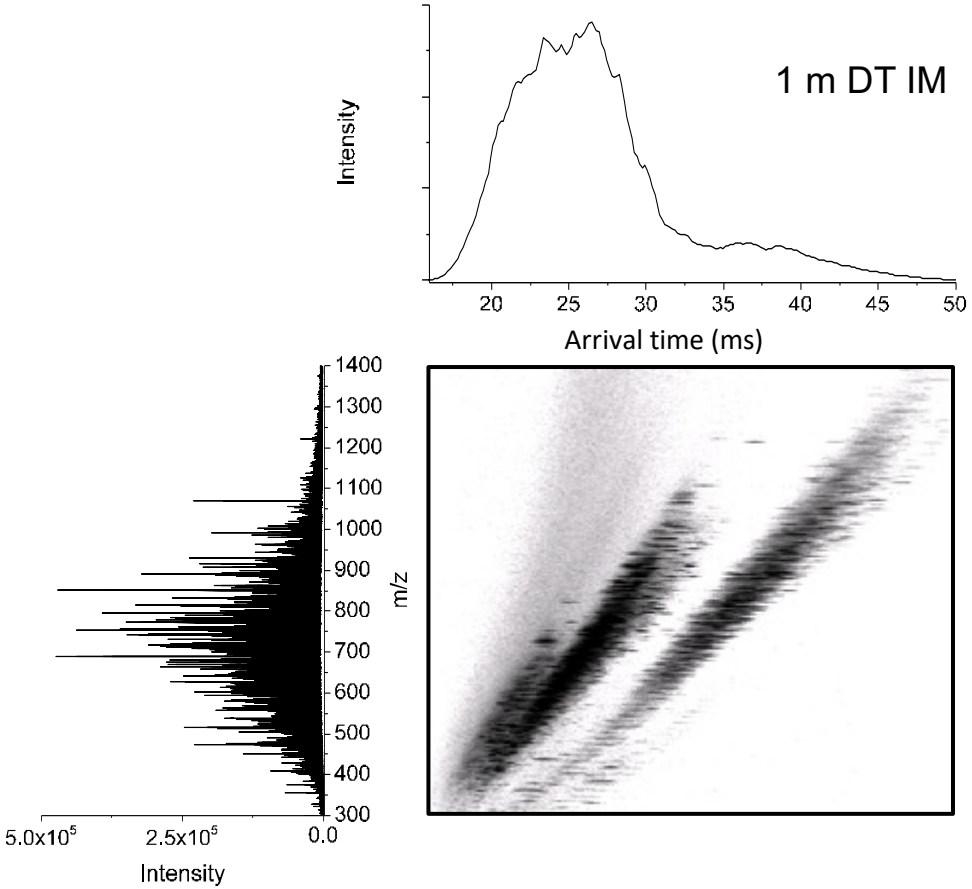


TW speed 124 m/s, TW amplitude 30 V, guard bias 5 V, RF 220 $V_{p\text{-}p}$ 650 kHz, SLIM gap 2.75 mm, 4.0 Torr N_2

Deng et al., Analytical Chemistry, *88*, 8957 (2016)

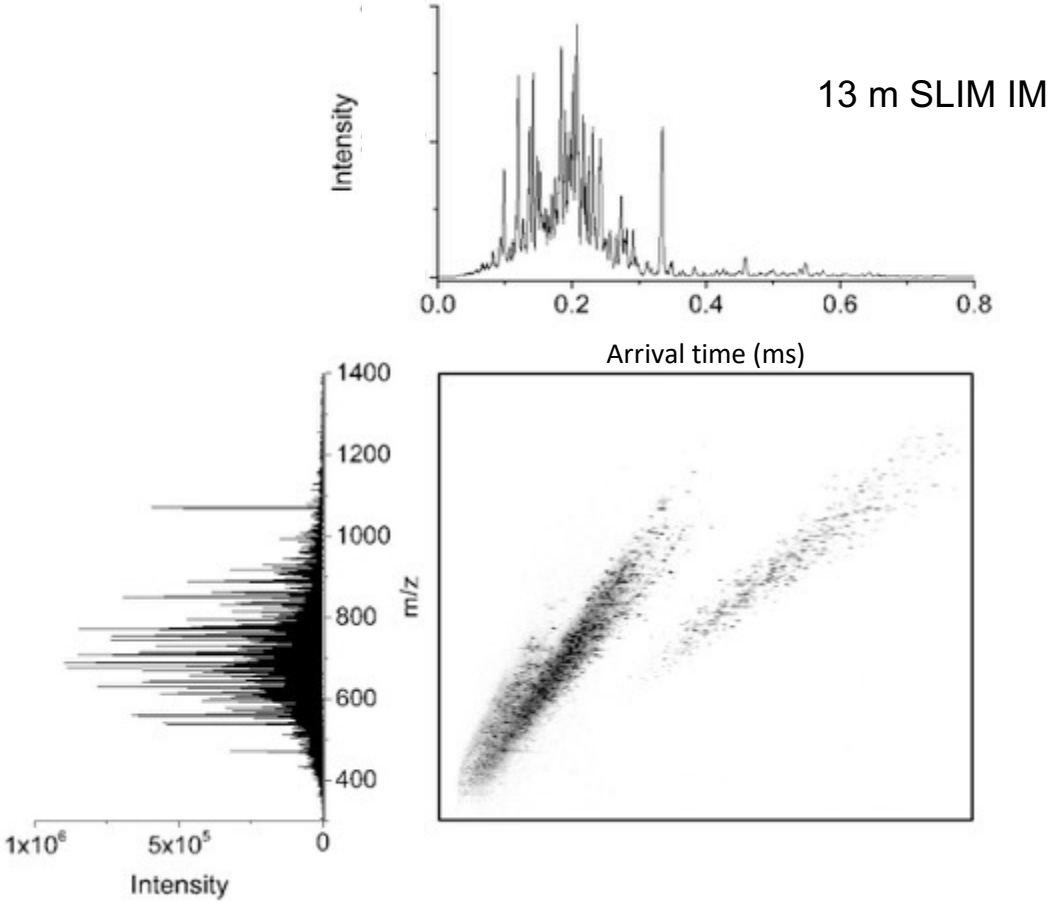
Conventional IM often provides insufficient resolution

DT IM-MS of a complex peptide mixture



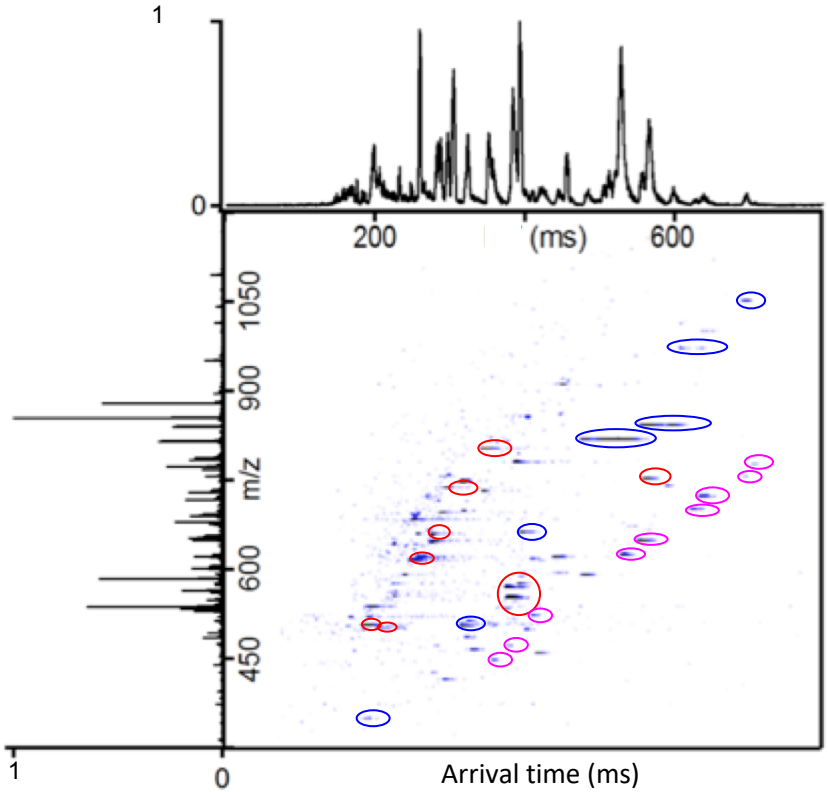
Greatly improved IM separations in 13 m SLIM

SLIM IM-MS of peptide mixture provides IM peak capacities of ~250



SLIM IM-MS of a peptide/carbohydrate/lipid mixture

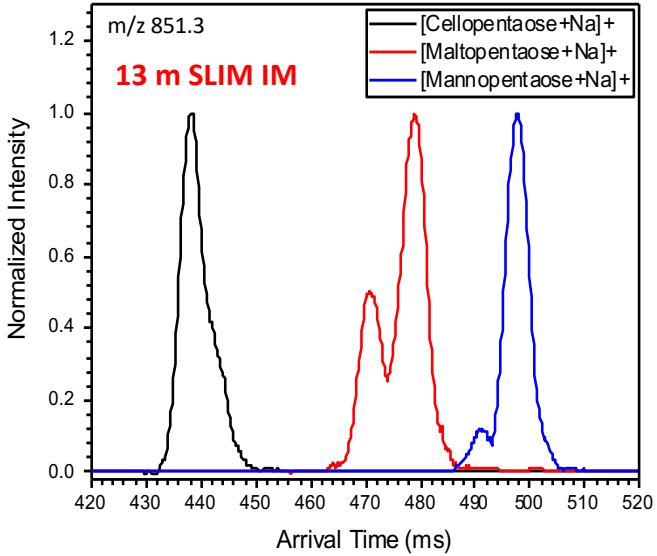
13 m path length SLIM IM-MS

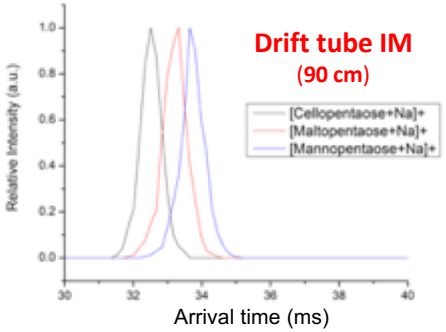


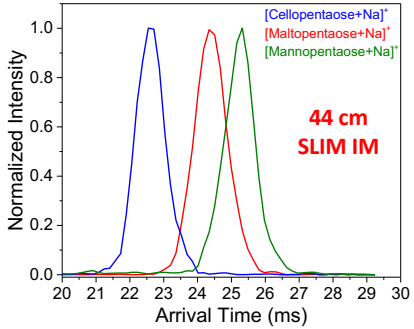
4 Torr N_2; 2.9 mm gap; TW speed 145 m/s, 30 V amplitude; 5 V guard bias; RF 628 kHz, 220 V_{pp}

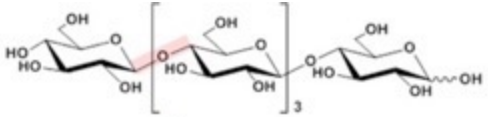
Deng et al., ChemistrySelect, *1*, 2396 (2016)

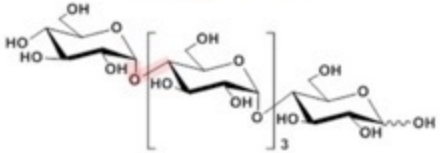
Comparison of IM separations for 3 pentasaccharides using long path SLIM, drift tube, short SLIM and Waters Synapt G2 IM-MS

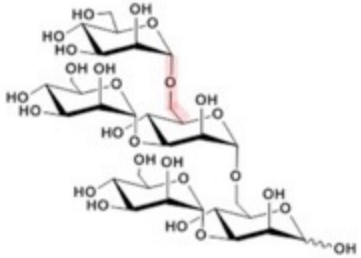


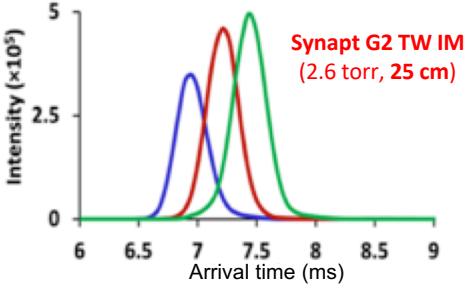








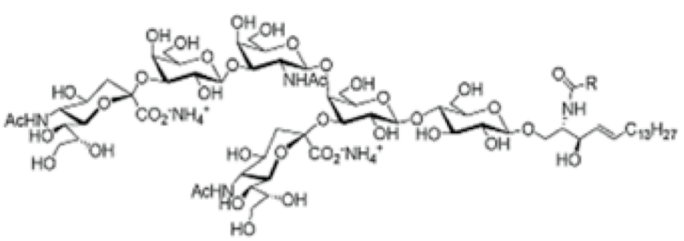




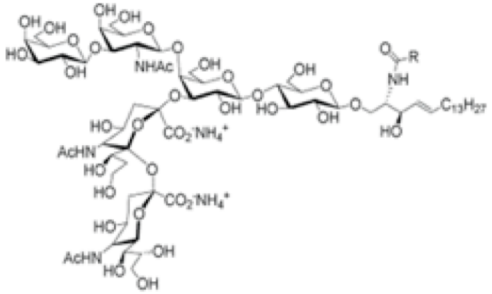
SLIM IM-MS of ganglioside isomers

GD1a and GD1b (18:1/18:0)

1. GD1a

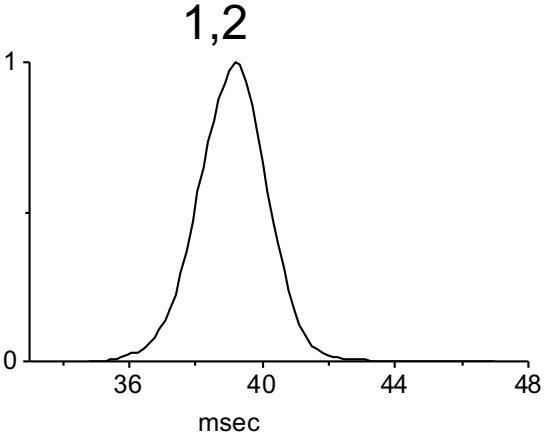


2. GD1b

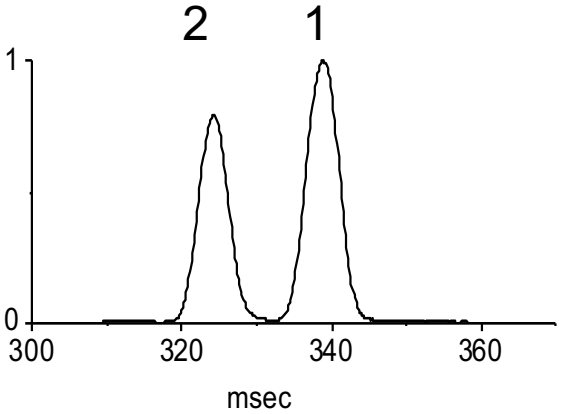


$(M-2H)^{2-}$

1 m Drift tube IM-MS

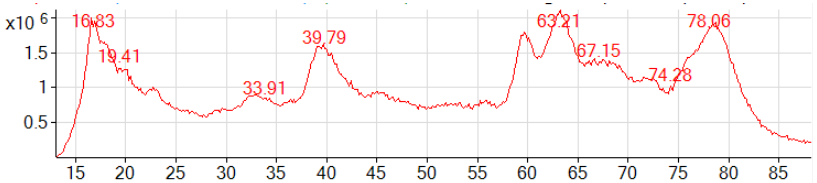


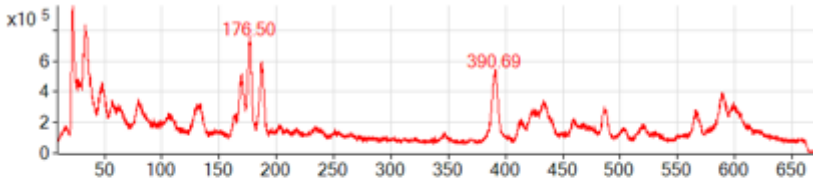
SLIM IM-MS 16 m

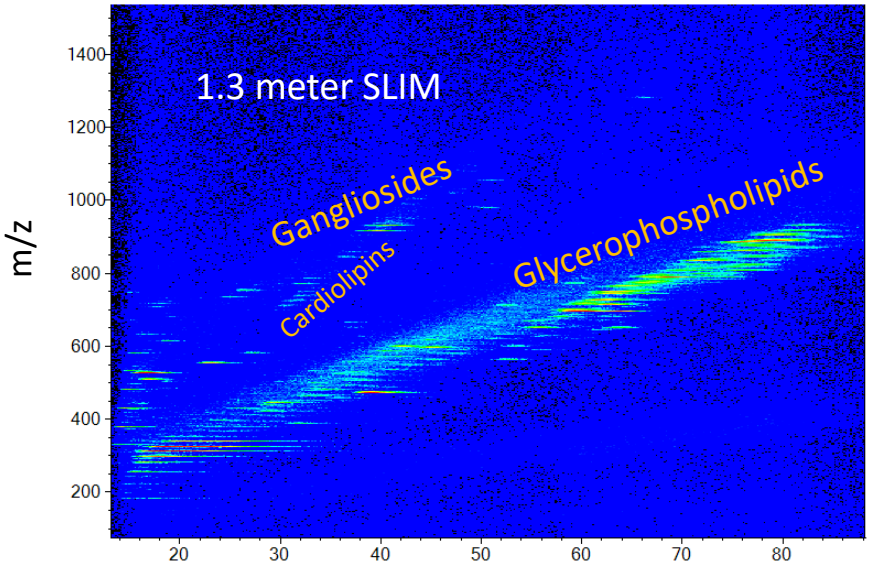


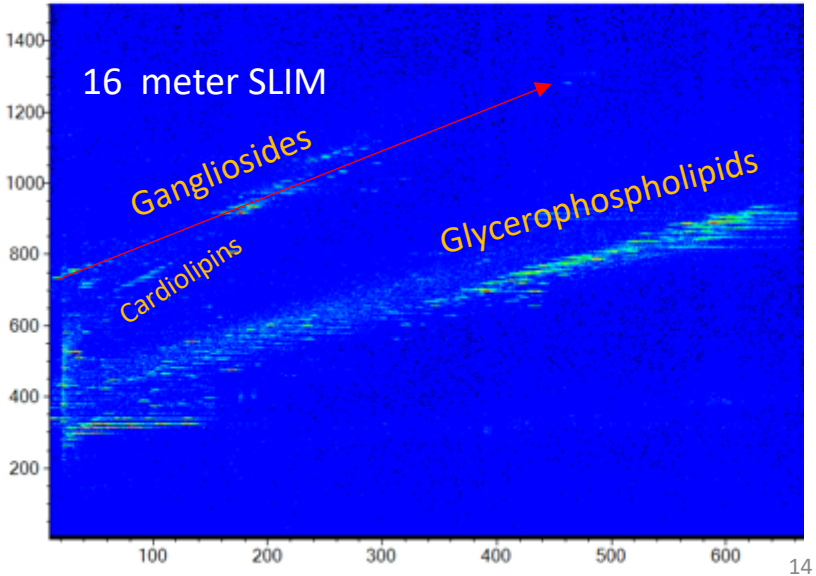
SLIM IM-MS of brain tissue polar lipid extract

Negative ion mode





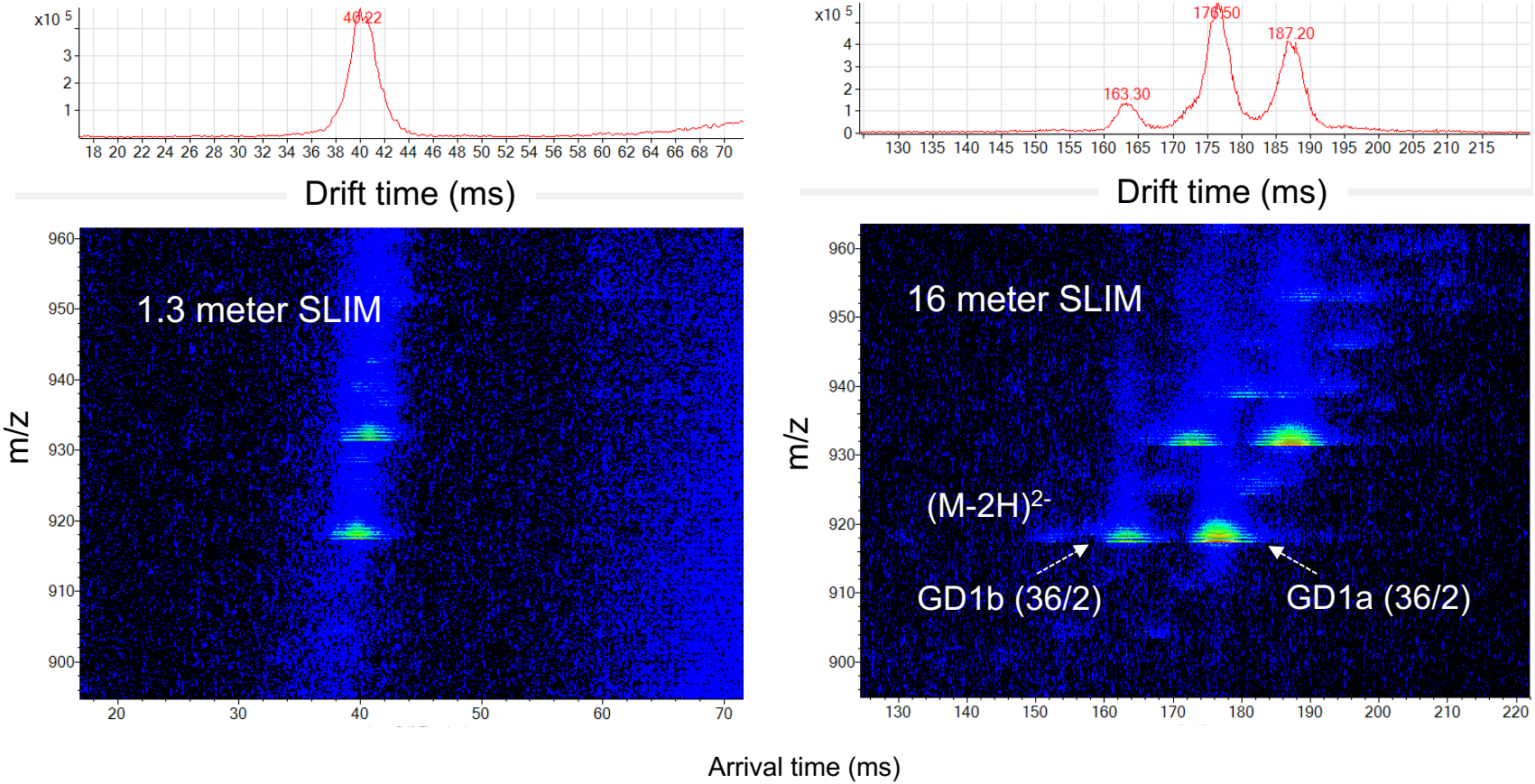




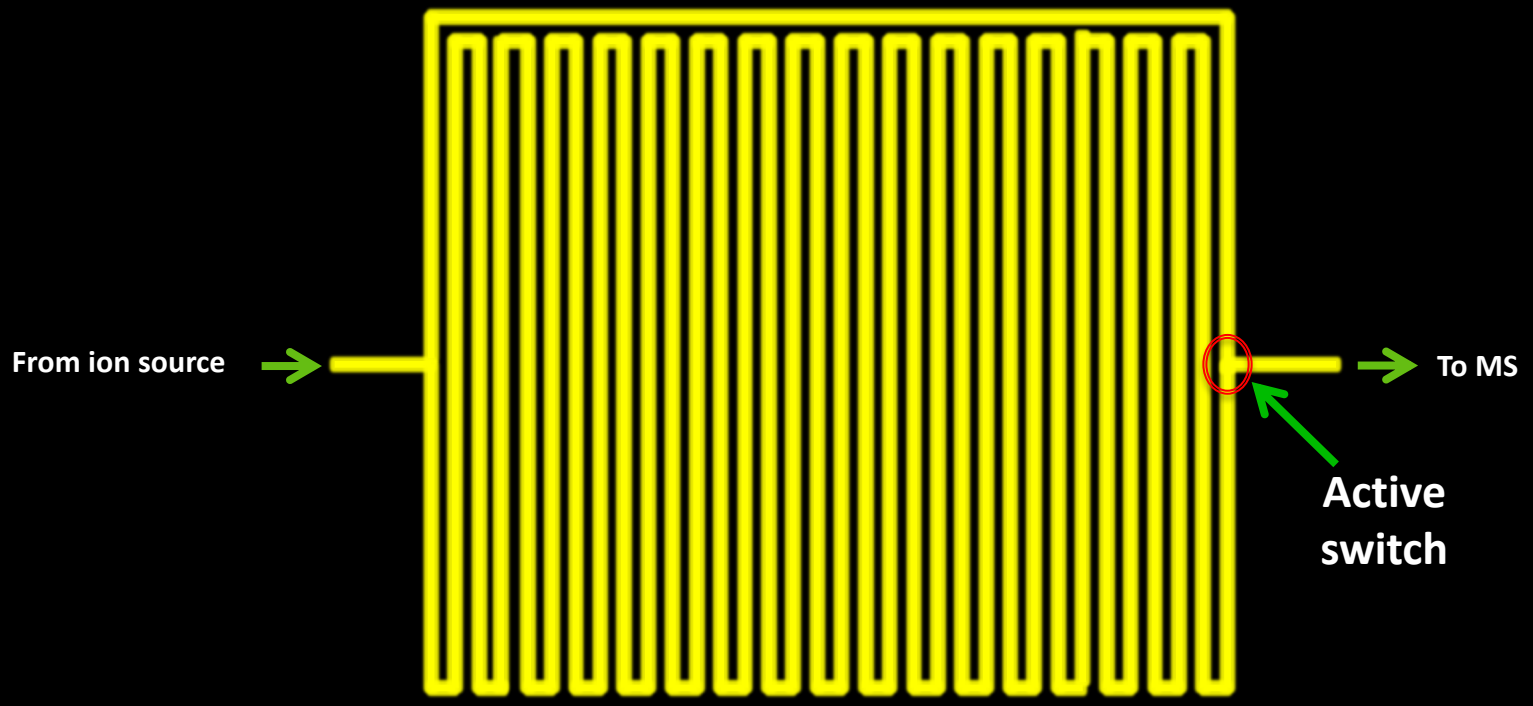
Arrival time (ms)

SLIM IM-MS of brain tissue polar lipid extract

Zoom view of window containing ganglioside GD1a and GD1b isomers

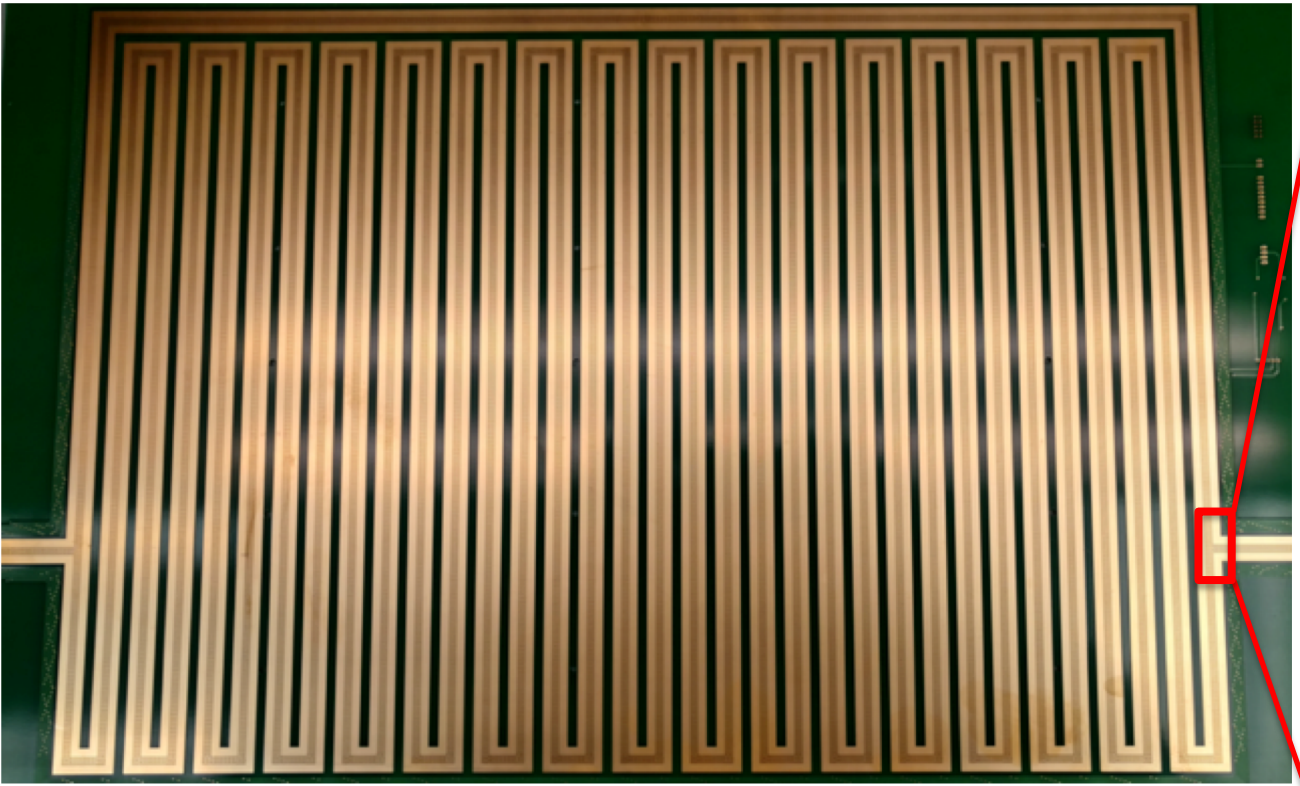


SLIM Serpentine Ultra-long Path with Extended Routing (SUPER) high resolution IM-MS

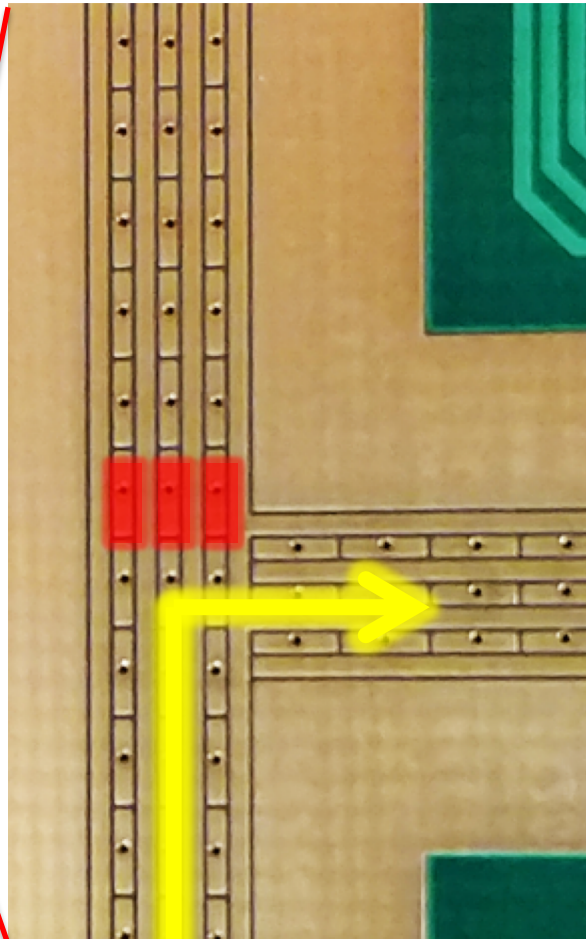


A TW SLIM SUPER IM module

When one good turn deserves another (and another….)

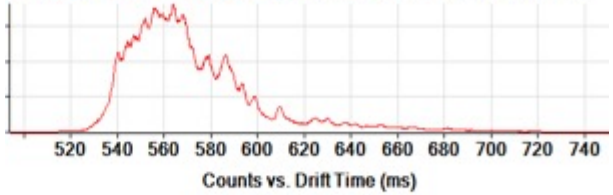


Detail of switch

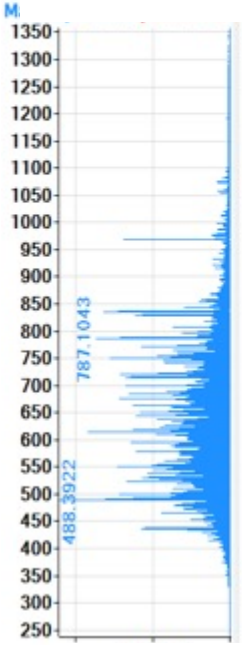


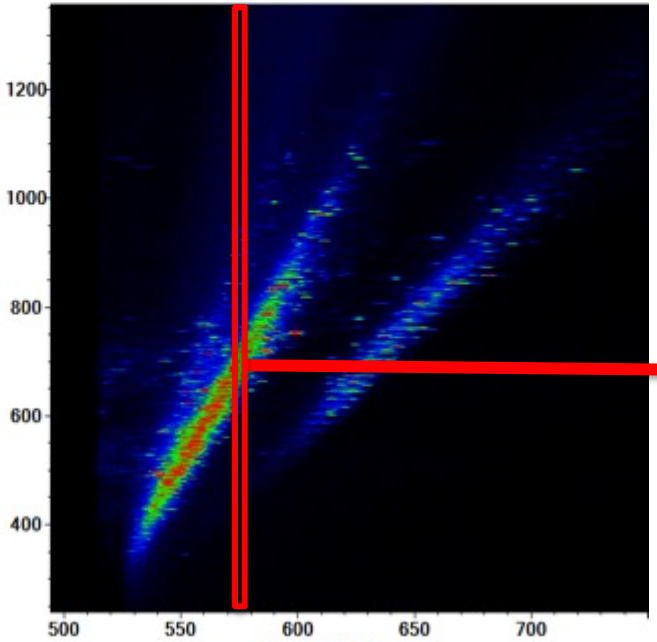
Selecting a mobility range for SLIM SUPER high resolution IM-MS

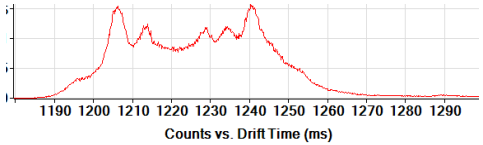
13 m SLIM IM-MS of “synthetic microbiome” peptide fraction

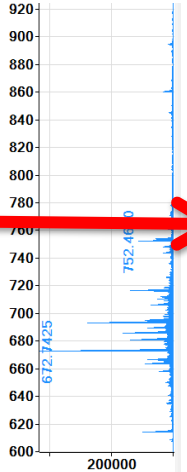


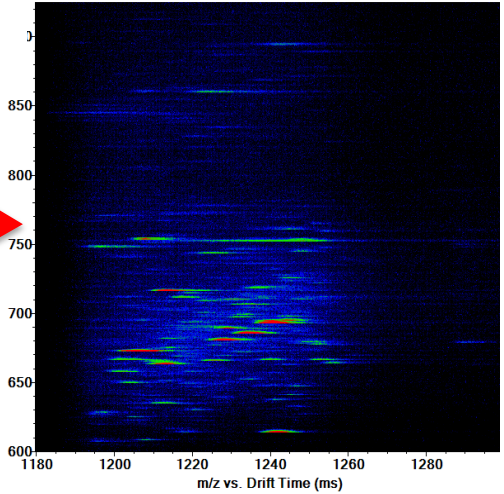
Flipping the exit switch: selecting 572-576 ms band for second pass









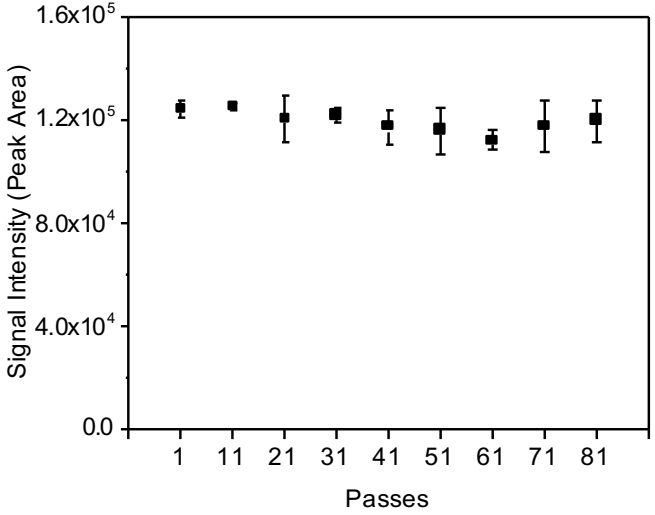


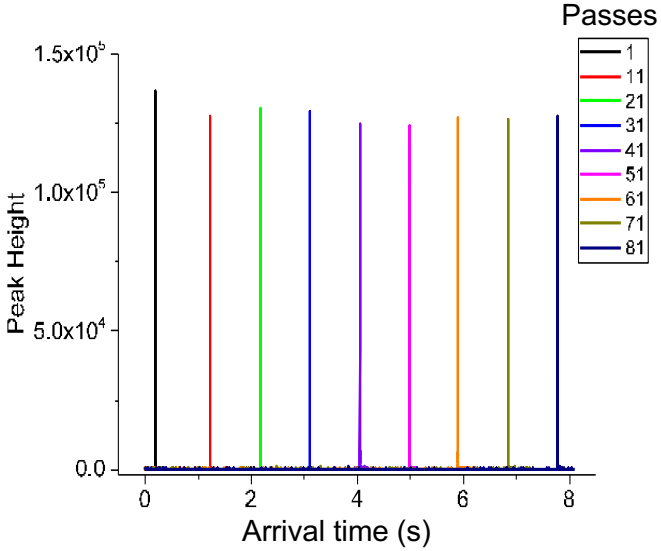
Ions can be stored or manipulated losslessly for long times

- Efficient transmission for ion populations undergoing up to 81 passes in 'traveling traps' (surfing) covering **more than 1 km**

- Much longer distances should be feasible as ions can be trapped and losslessly stored for >5 hr in SLIM

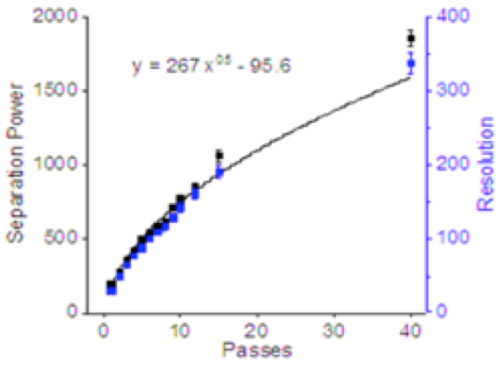
Signal for ion packets (from Agilent tune mix) vs. passes through 13 m path SLIM



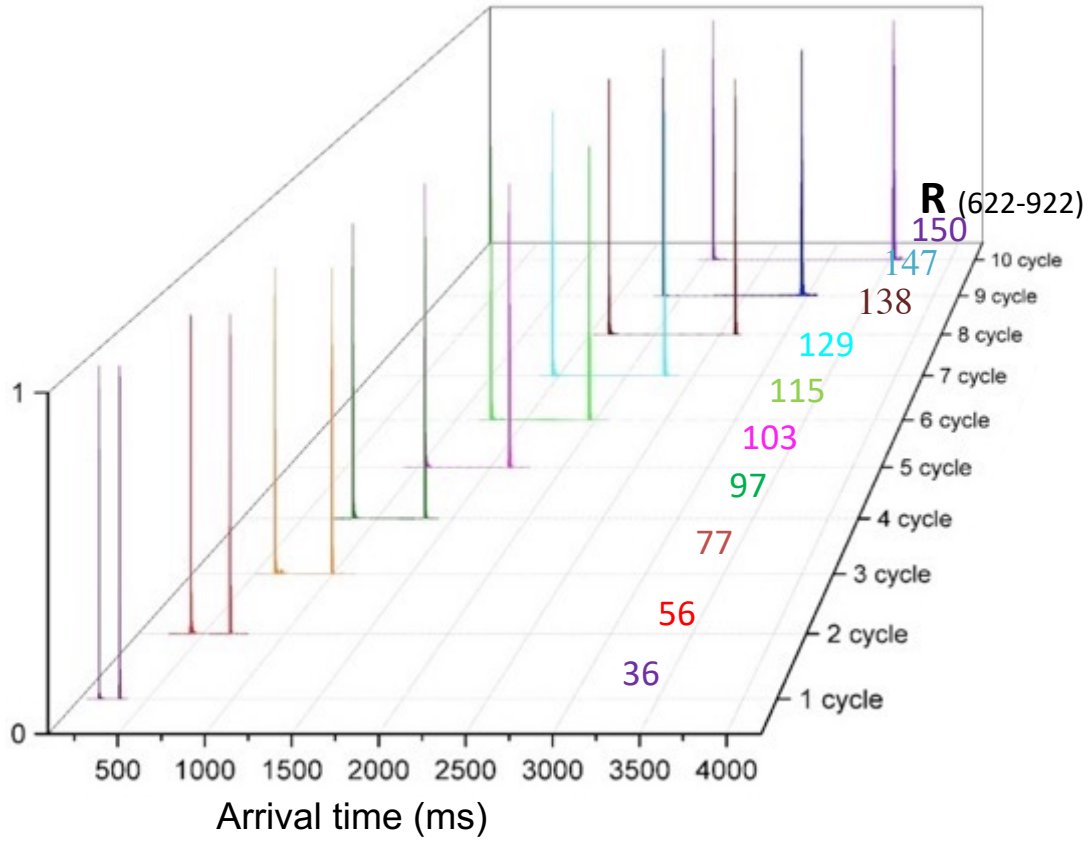


Deng et al, Analytical Chemistry, *89*, 4628 (2017)

Resolution increases with (path length)$^{0.5}$ in SLIM SUPER IM



Resolution for m/z 622 and 922
(shown corrected for peak 'lapping' beyond pass #2)



Typical performance of conventional drift tube IM:

Resolution $_{(m/z\ 622\text{-}922)}$ ~10

Resolving Power <~100

Deng et al, Analytical Chemistry, *89*, 4628 (2017)

Identifying sites of post translational modifications

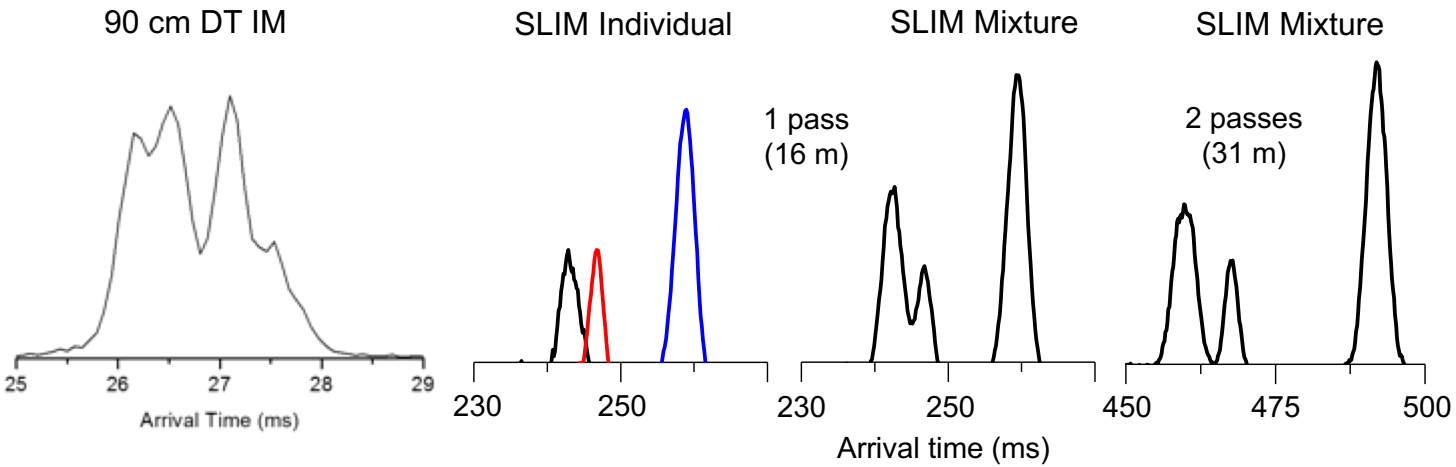
Separation of peptide phosphosite isomers in SLIM SUPER IM-MS

Peptide 1 : APLpSFRGSLPKSYVK

Peptide 2 : APLSFRGpSLPKSYVK

Peptide 3 : APLSFRGSLPKpSYVK

Time

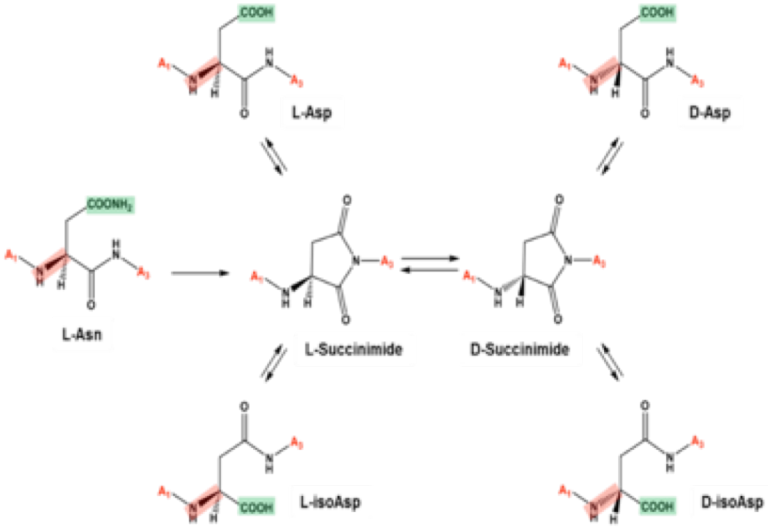


Another proteomic ‘blind spot’

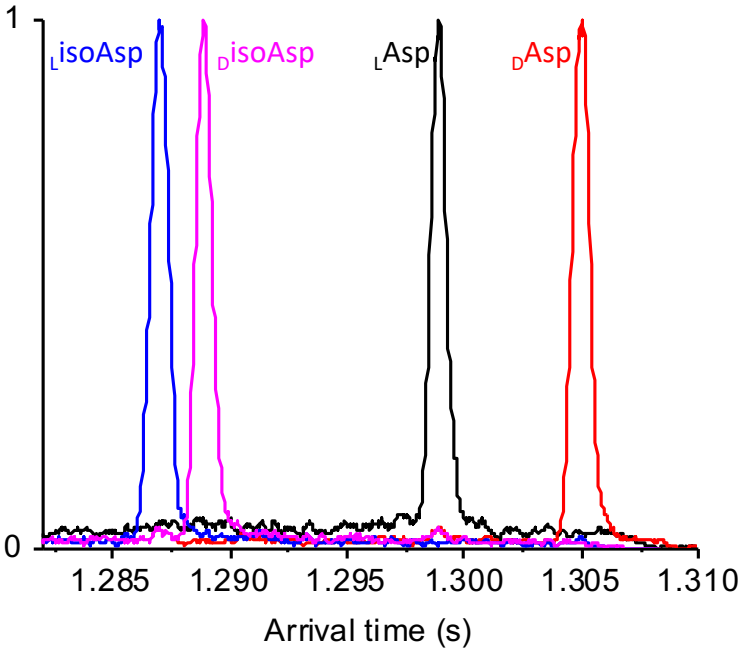
SLIM SUPER IM-MS of decapeptide epimers (varying at $_{x}$**Asp** residue)

His-$_{x}$**Asp**-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys

$_{x}$**Asp** = $_{L}$**isoAsp**, $_{D}$**isoAsp**, $_{L}$**Asp** or $_{D}$**Asp**



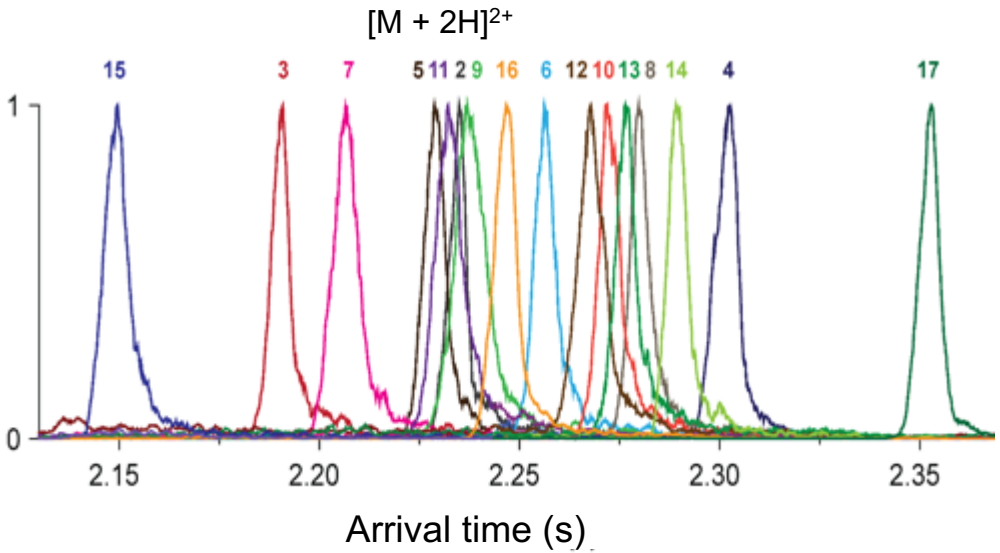
67.5 m (5 passes); $[M + 2H]^{2+}$



SLIM SUPER IM-MS of Aβ(17-28) isomers

12-mer peptide having two Asp residues subject to isomerization

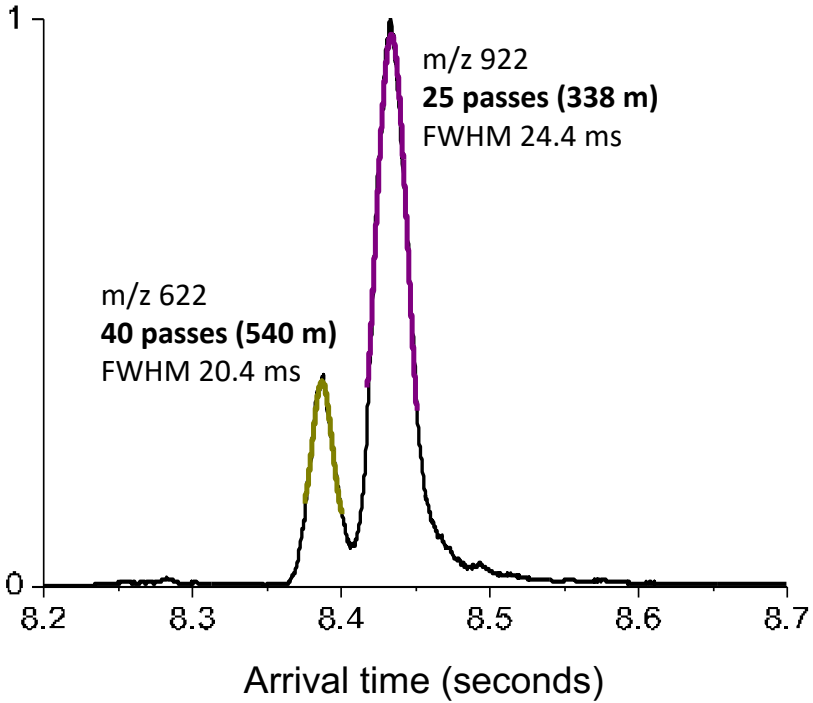
135 m (10 passes)



	Sequence	Feature	DT (s)
2	LVFFAEDVGSDK	L-Asp, L-Asp	2.234
3	LVFFAEDVGS(dD)K	L-Asp, D-Asp	2.190
4	LVFFAEDVGS(bD)K	L-Asp, L-isoAsp	2.301
5	LVFFAEDVGS(dbD)K	L-Asp, D-isoAsp	2.229
6	LVFFAE(dD)VGSDK	D-Asp, L-Asp	2.256
7	LVFFAE(dD)VGS(dD)K	D-Asp, D-Asp	2.207
8	LVFFAE(dD)VGS(bD)K	D-Asp, L-isoAsp	2.280
9	LVFFAE(dD)VGS(dbD)K	D-Asp, D-isoAsp	2.238
10	LVFFAE(bD)VGSDK	L-isoAsp, L-Asp	2.272
11	LVFFAE(bD)VGS(dD)K	L-isoAsp, D-Asp	2.232
12	LVFFAE(bD)VGS(bD)K	L-isoAsp, L-isoAsp	2.268
13	LVFFAE(bD)VGS(dbD)K	L-isoAsp, D-isoAsp	2.277
14	LVFFAE(dbD)VGSDK	D-isoAsp, L-Asp	2.289
15	LVFFAE(dbD)VGS(dD)K	D-isoAsp, D-Asp	2.149
16	LVFFAE(dbD)VGS(bD)K	D-isoAsp, L-isoAsp	2.246
17	LVFFAE(dbD)VGS(dbD)K	D-isoAsp, D-isoAsp	2.352

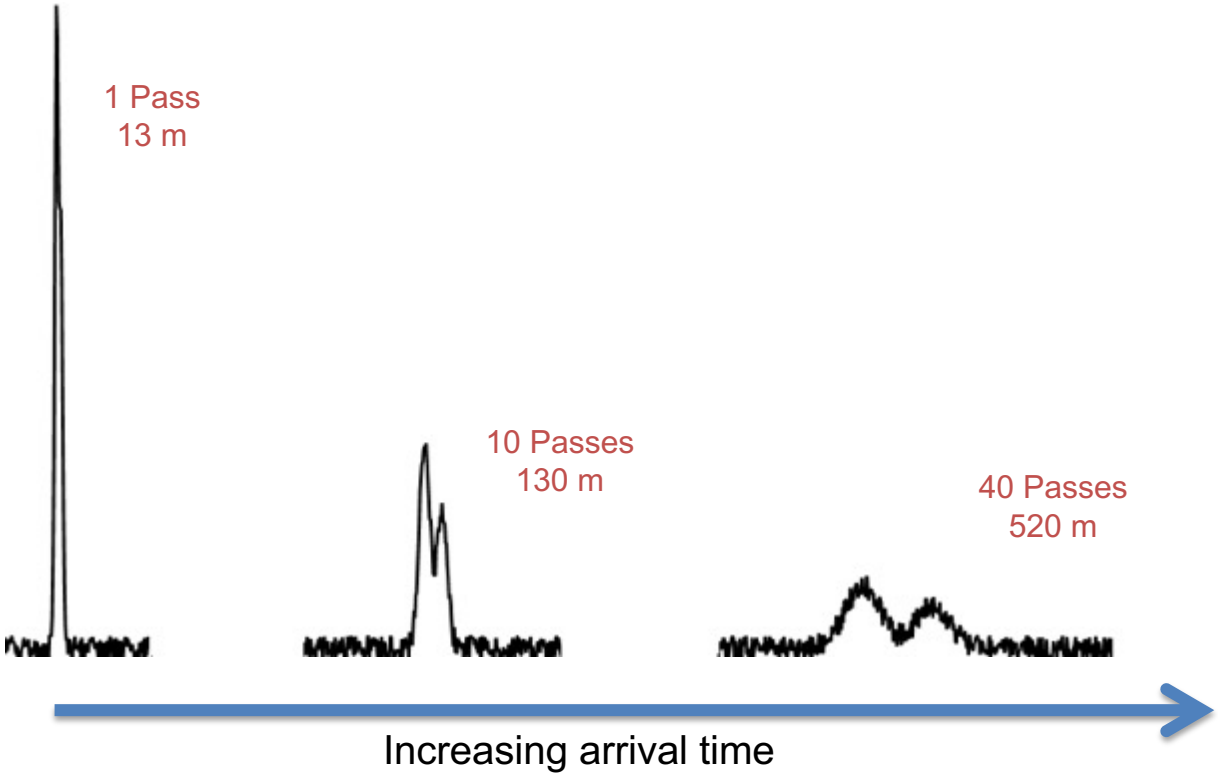
‘Peak lapping’ in multi-pass IM separations

Example of peak ‘lapping’ during IM separation in 13 m multi-pass SLIM SUPER IM module



The need to defeat diffusion

IM peak width increases due to diffusion and any detector ‘noise’ limits detection at long times



Compression Ratio Ion Mobility Programming (CRIMP)

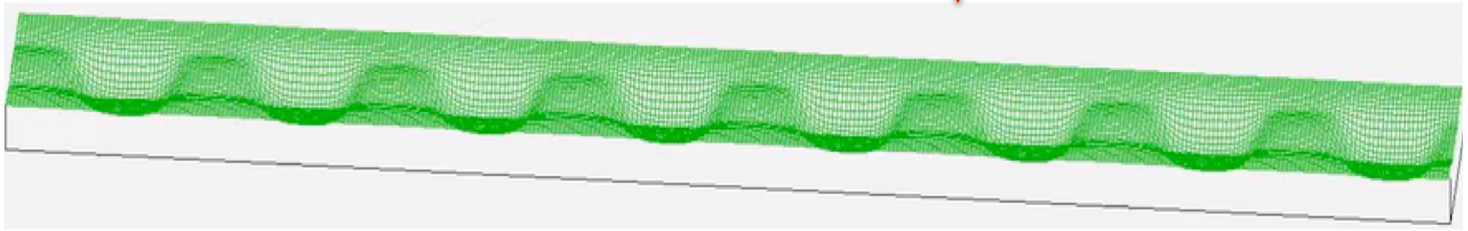
- Compression occurs at interface of traveling trap (TT) and ‘stuttering trap’ (ST) regions
- TT region runs continuously; ST region stops moving periodically
- ST region is halted half the time for CR = 2 providing **spatial** compression
- Ion distribution in ST region shifted to TT mode providing **temporal** compression

Garimella et al., Analytical Chemistry, *88*, 11877 (2016)

Defeating diffusion (sort of)

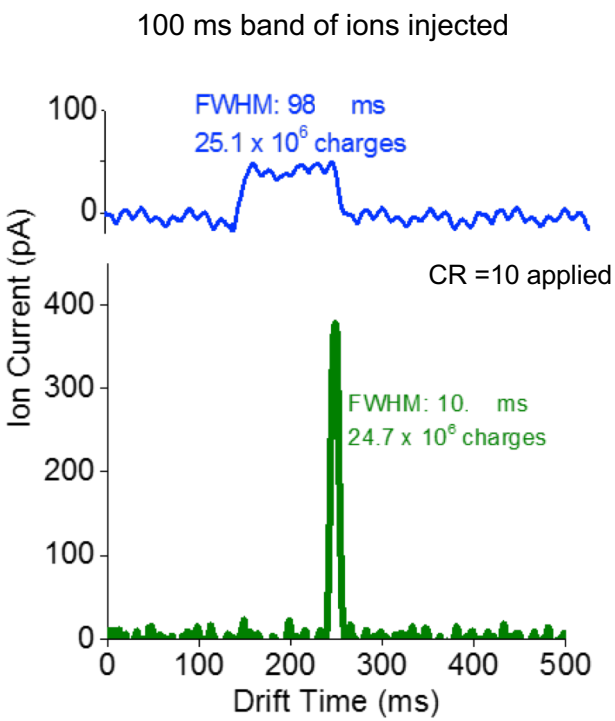
Compression Ratio Ion Mobility Programming (CRIMP)

CR = 3 applied



Simion 8.1

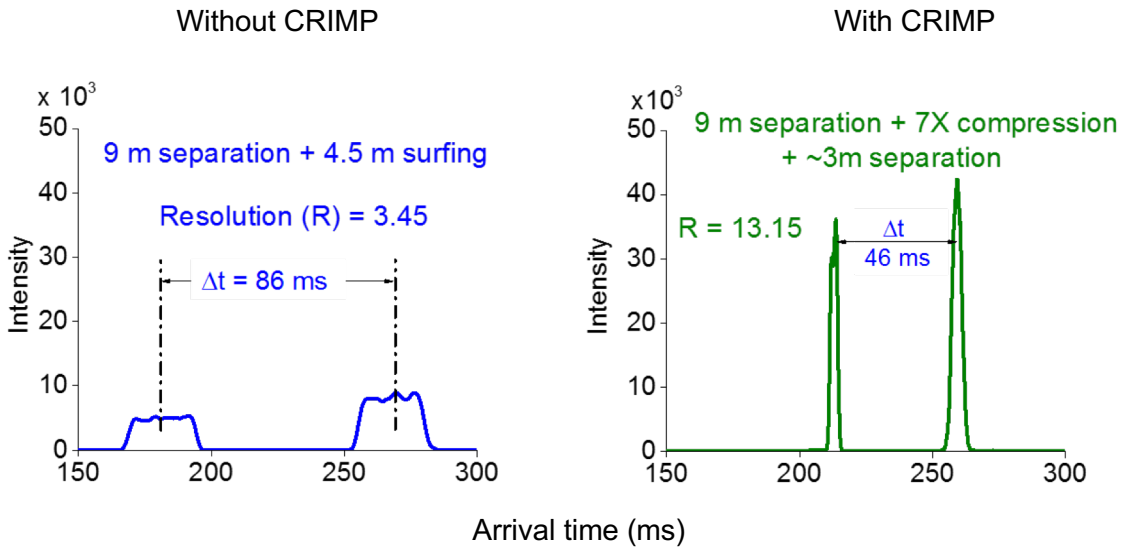
Compression Ratio Ion Mobility Programming (CRIMP)



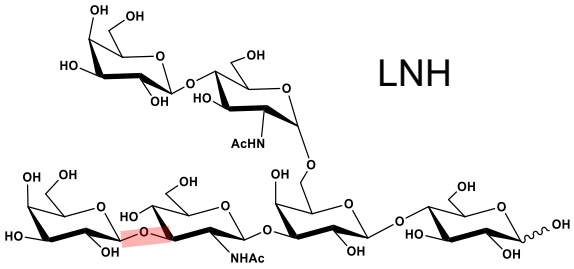
Garimella et al., Analytical Chemistry, *88*, 11877 (2016)

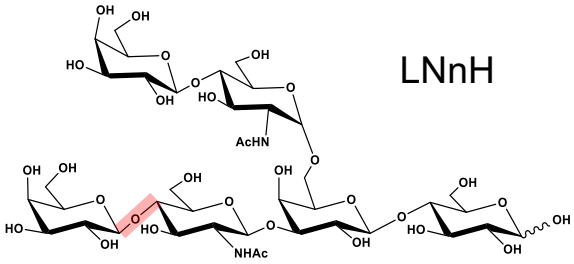
Compression Ratio Ion Mobility Programming (CRIMP)

Resolution of m/z 622 and 922 starting from 25 sec injection pulse

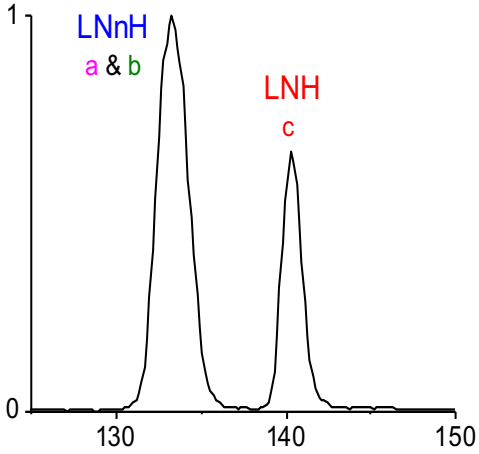


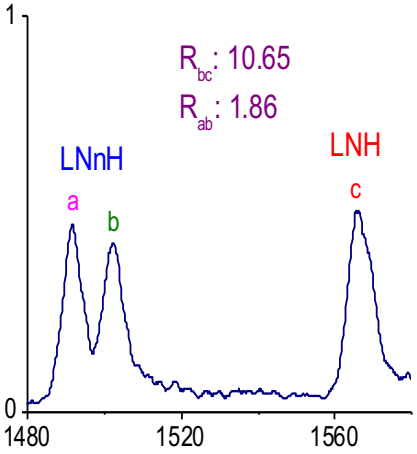
Separation of Lacto-N-hexaose (LNH) and Lacto-N-neohexaose (LNnH)

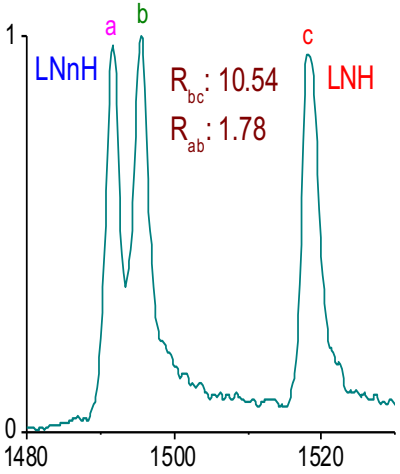




$[M+K+H]^{2+}$
m/z=1112



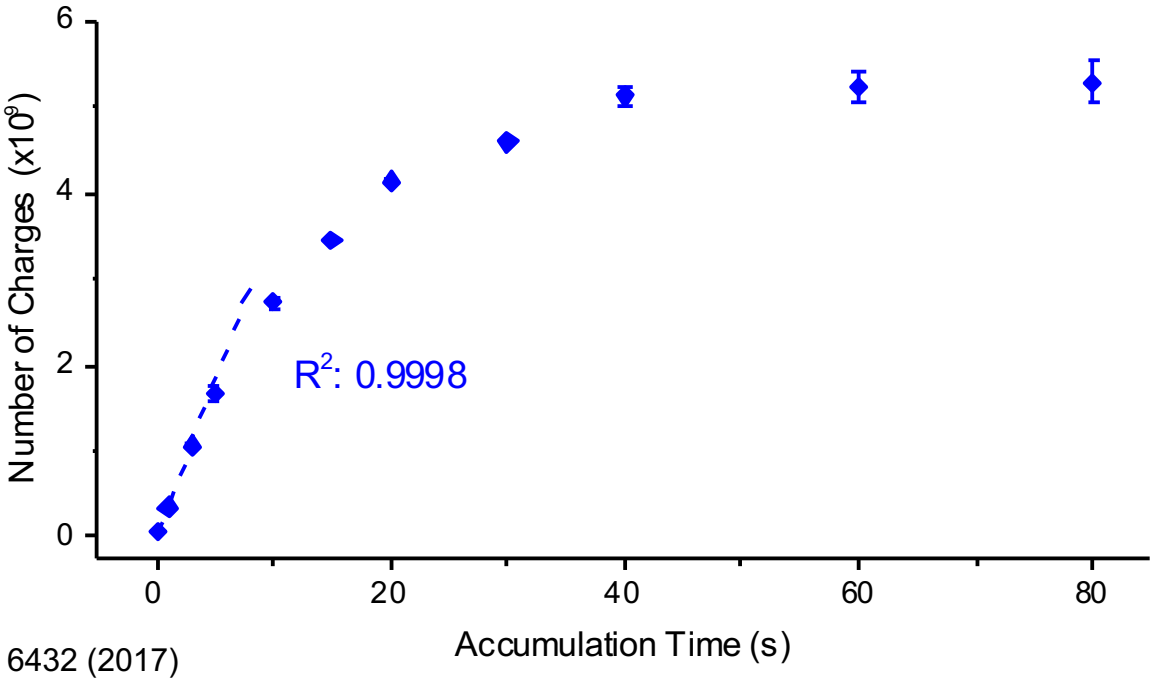




Arrival time (ms)

Accumulation and trapping of 1000-fold larger ion populations

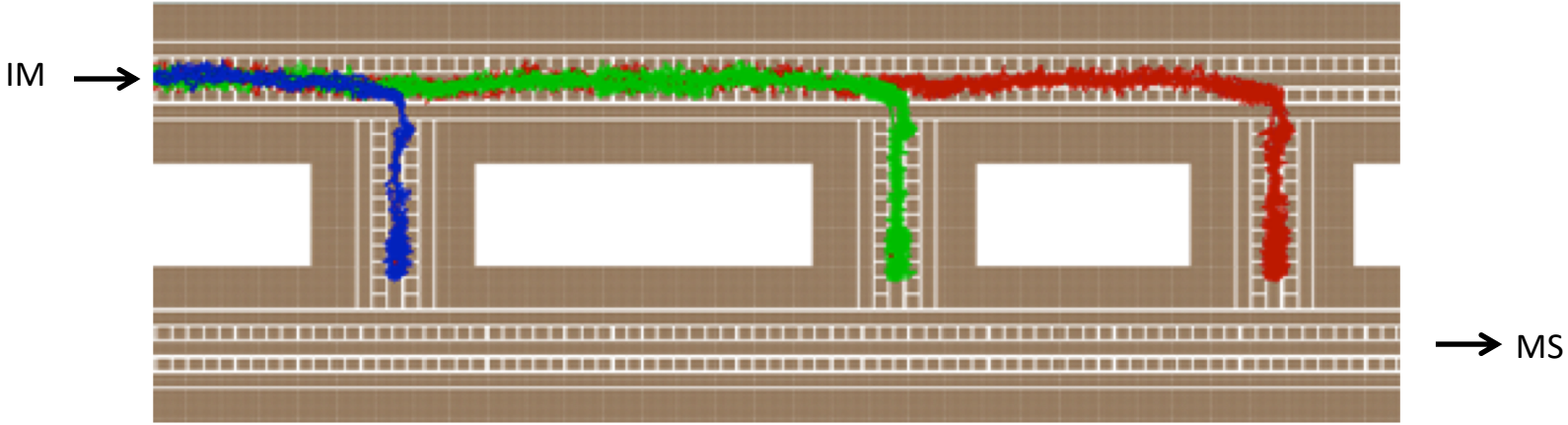
- Previously unachievable ion populations can be trapped and accumulated in selected portions of the TW SLIM ion path (limited by space charge)
- Can be a prelude to application of CRIMP for greatly increased measurement dynamic range (and coverage)



Deng et al., Analytical Chemistry, *89*, 6432 (2017)

Simulation of TW SLIM trapping and accumulation after IM separation

Screen shot of ion trajectory simulation; ions of three different mobilities first separated and shown fractionated into three different traps, stored, and selected (green) for transfer to MS



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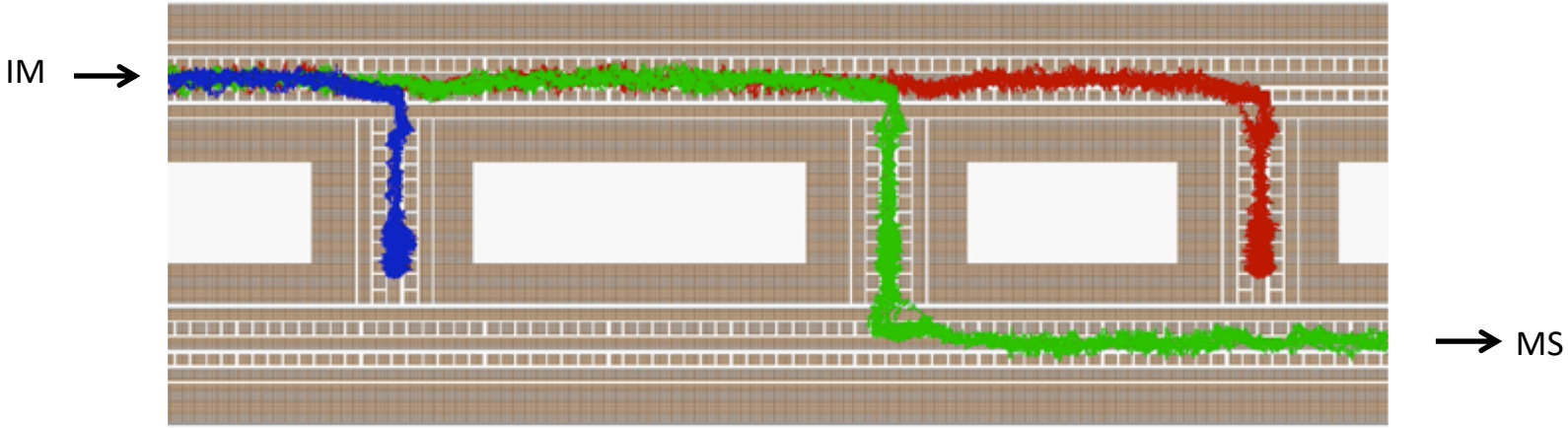
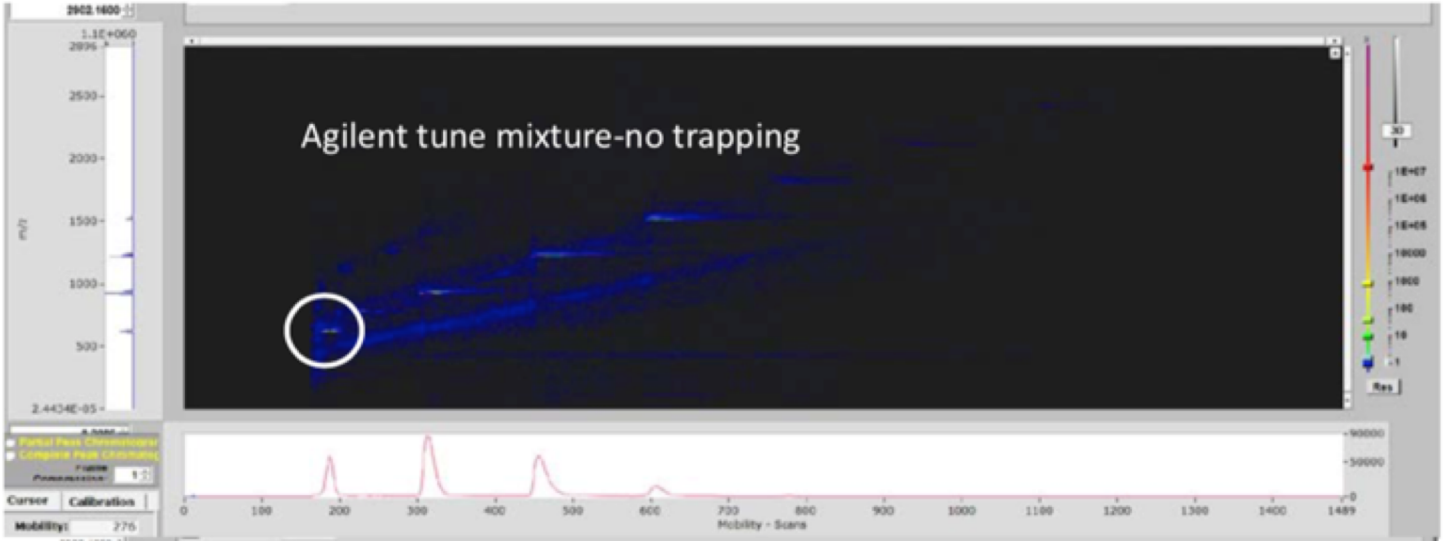
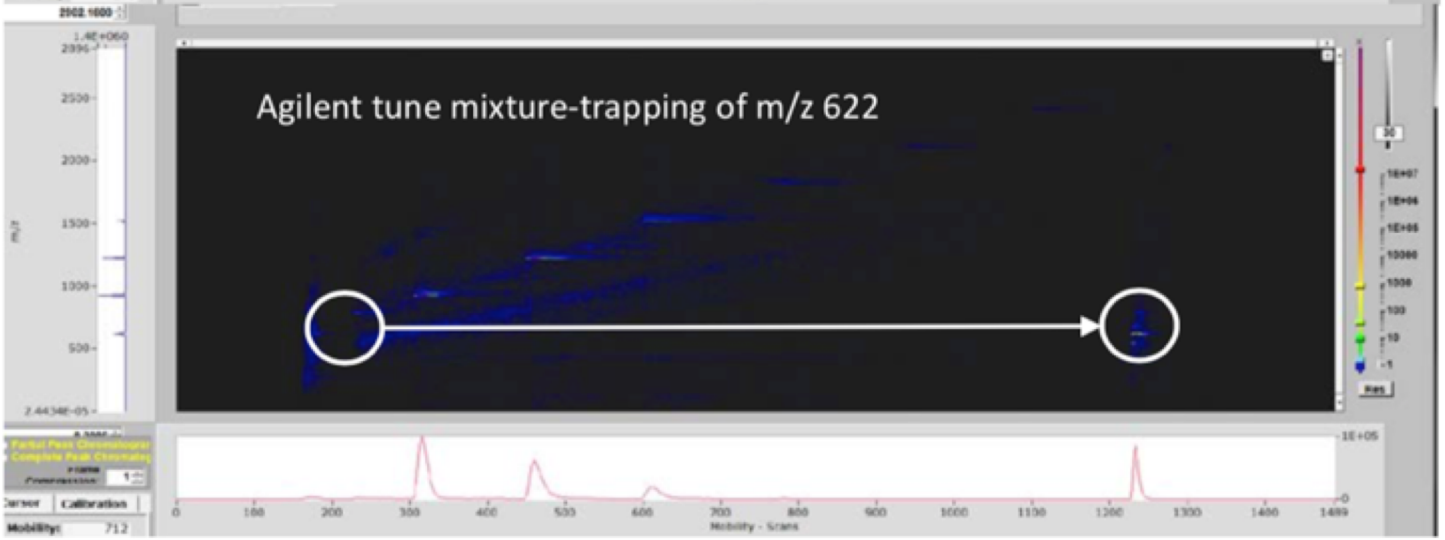
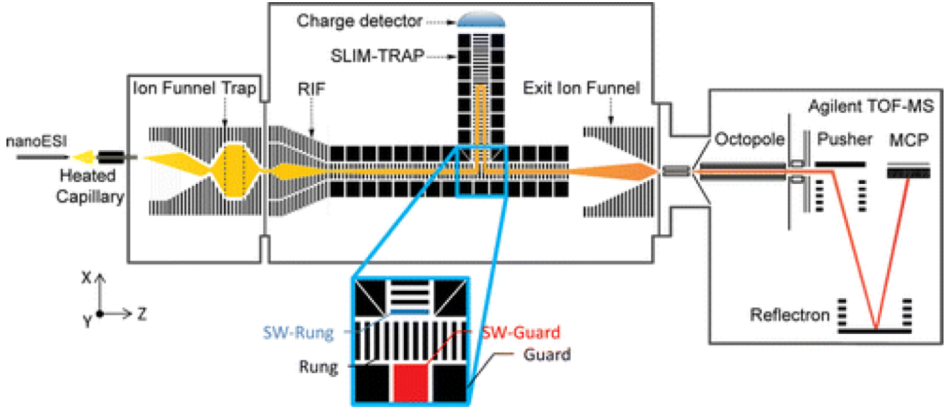


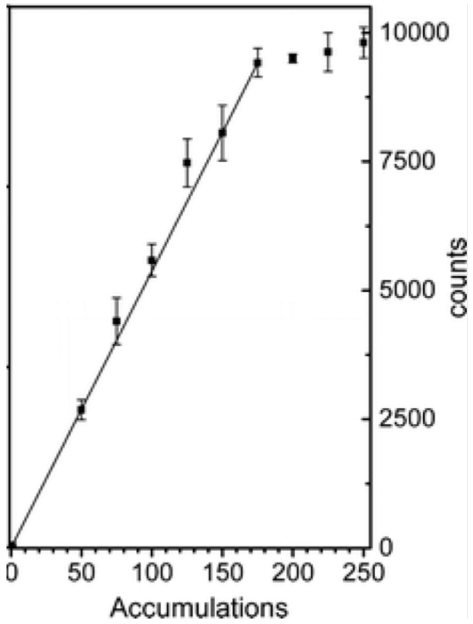

Agilent tune mixture-no trapping
Mobility - Scans


Agilent tune mixture-trapping of m/z 622
Mobility - Scans

SLIM IM peak selection, trapping and accumulation

Trapped ion population size increases linearly with accumulation number until limited by trap capacity (space charge effects)





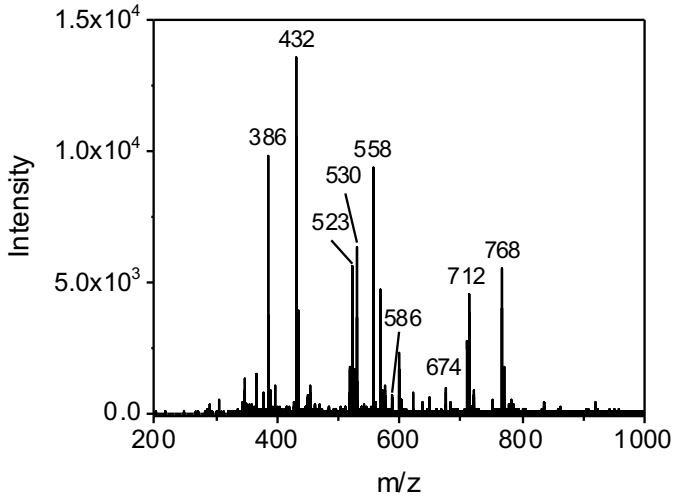
Chen et al., Anal. Chem., *88*, 1728 (2016)

CRIMP ion accumulation

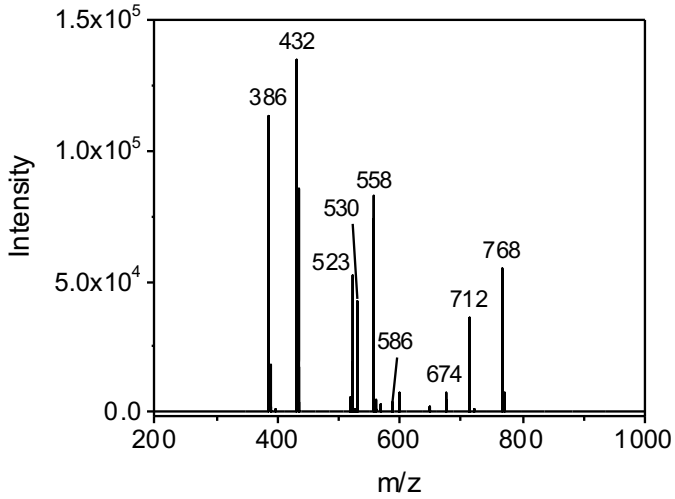
- Greater intensities
- No evident fragmentation
- Reduced ‘chemical noise’ with ESI

Comparison of mass spectra from ESI of peptide mixture

Continuous ion introduction



After CRIMP accumulation

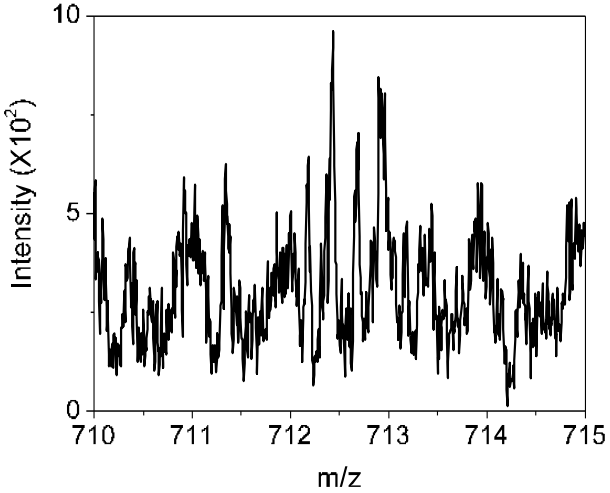


CRIMP ion accumulation

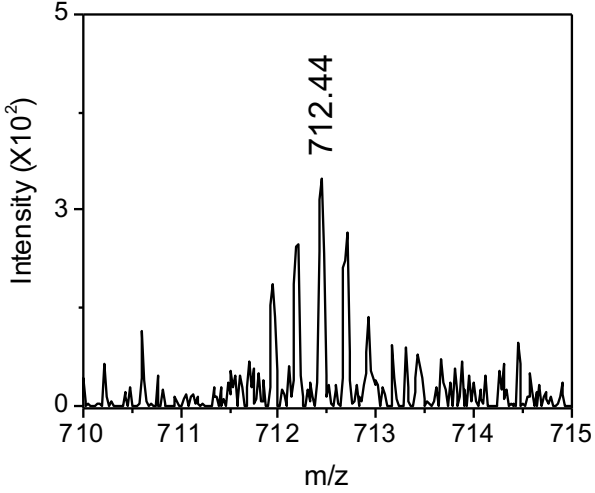
- Greater intensities
- No evident fragmentation
- Reduced ‘chemical noise’ with ESI

Comparison of MS using nanoESI (60 nL/min) of 1 pM melittin solution

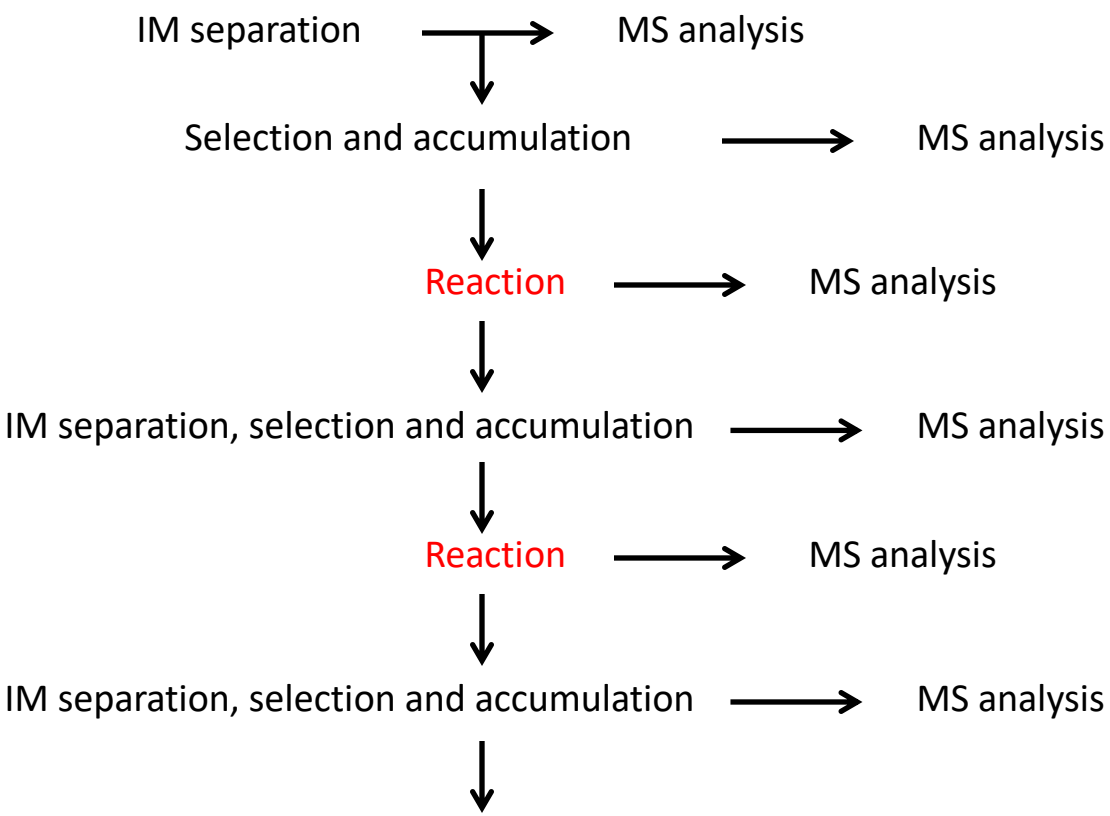
Continuous ion introduction



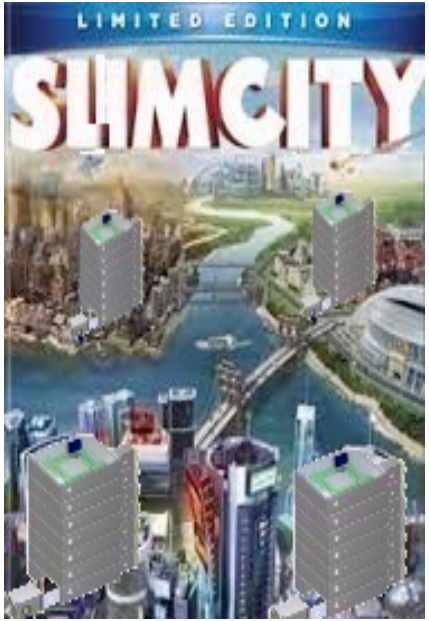
After CRIMP accumulation



Combining TW SLIM IM separation and reaction modules provides broad flexibility

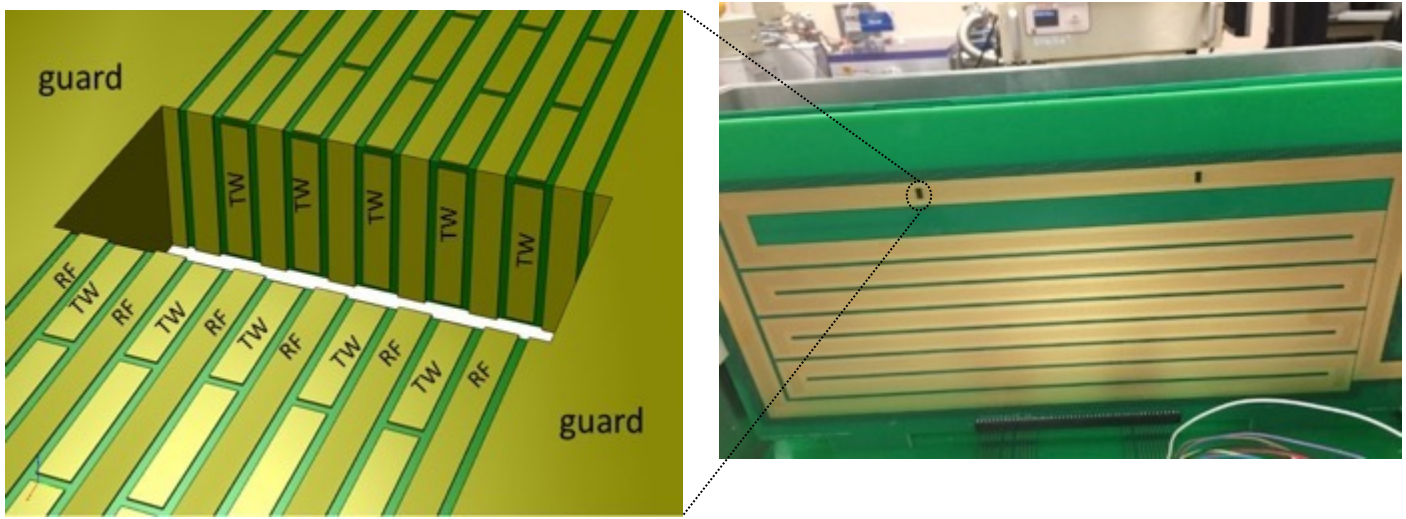


Building up in SLIM city; multi-level devices



Building up in SLIM city; multi-level devices

Moving ions between different levels of stacked SLIM modules



- Much longer path (~500 m) multi-level SLIM under development aiming to provide separation IM peak capacities of >5000

- Projected ability to trap and accumulate ion populations >10^{10} in <10 s, to provide high dynamic range SLIM SUPER IM-MS analyses in <1 min

Summary

- Traveling wave SLIM IM-MS provides resolution and peak capacities already nearly comparable to conventional HPLC
- Previously indistinguishable isomers separated using SLIM SUPER IM-MS
- CRIMP with SLIM allows compression and use of much larger ion populations, greatly increasing sensitivity and dynamic range
- Potential for much longer path length and multi-level SLIM for e.g. much higher resolution IM and IM-CID-IM

Acknowledgements

SLIM and IM-MS
Yehia Ibrahim
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Erin Baker

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Spencer Prost
Randy Norheim

Informatics/bioinformatics
Sam Payne
Matt Monroe
Aivett Bilbao

Previously with SLIM
Liulin Deng
Ahmed Hamid
Tsung-Chi Chen

Gordon Anderson

Agilent Technologies
MOBILion Inc.

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DOE Office of Biological and Environmental Research
NIGMS P41 Biomedical Technology Proteomics Research Resource