Developments in Data Independent MS Acquisition
Comprehensive and Reproducible Identification and Label-Free Quantification of Proteins

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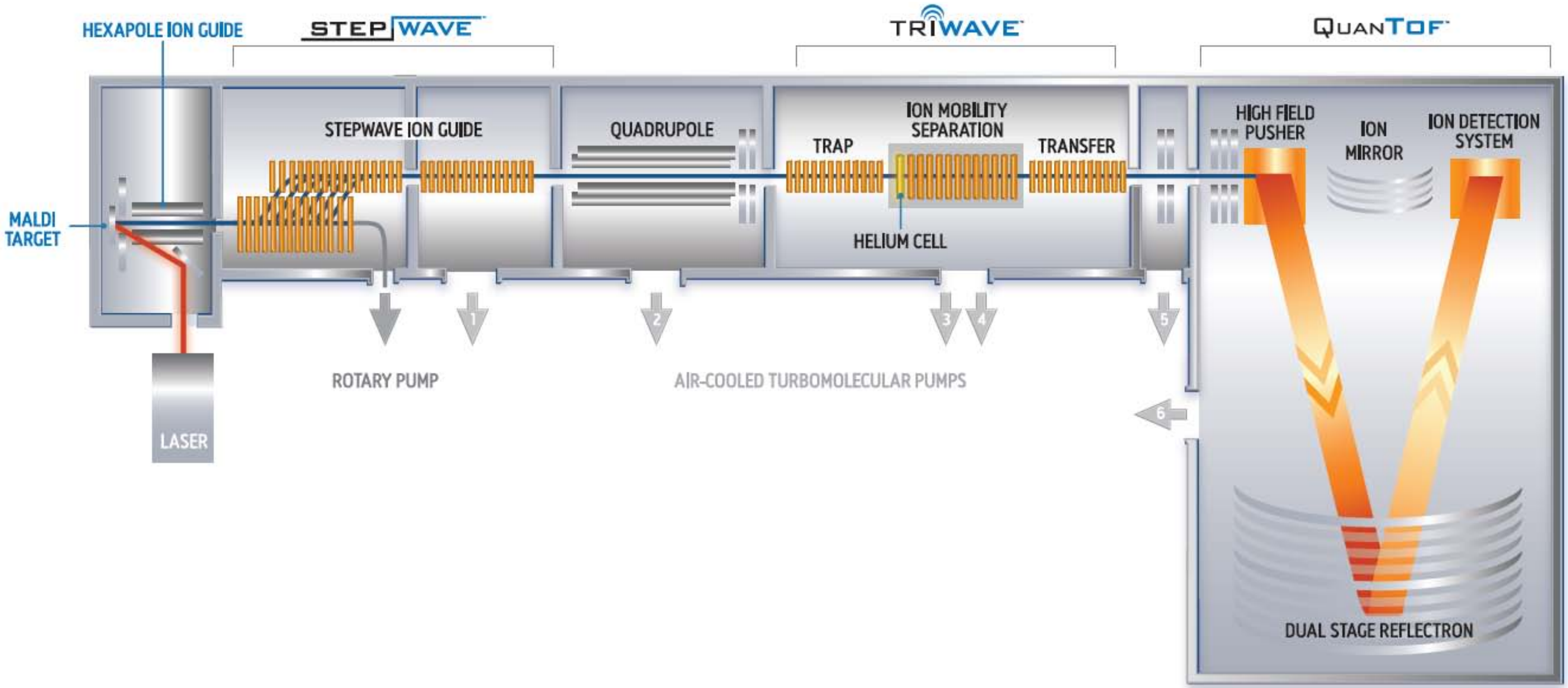
SYNAPT® G2-S

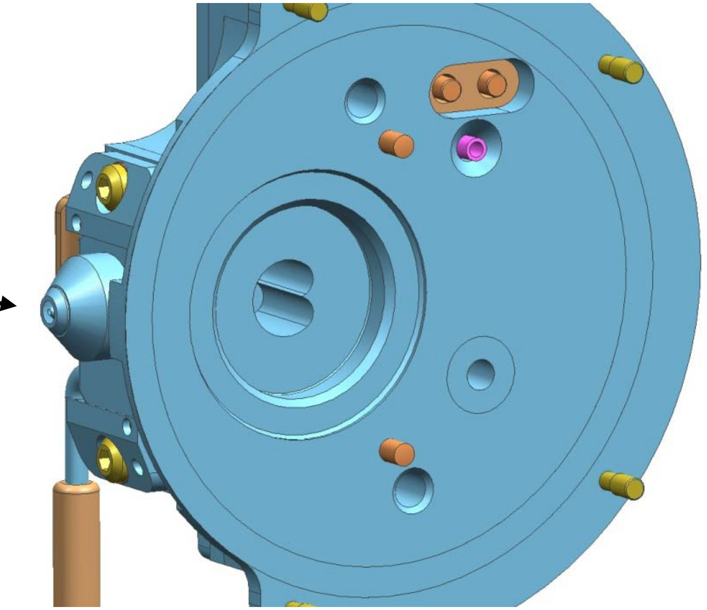
The next step in the evolution of High Definition MS

SYNAPT G2-S Geometry



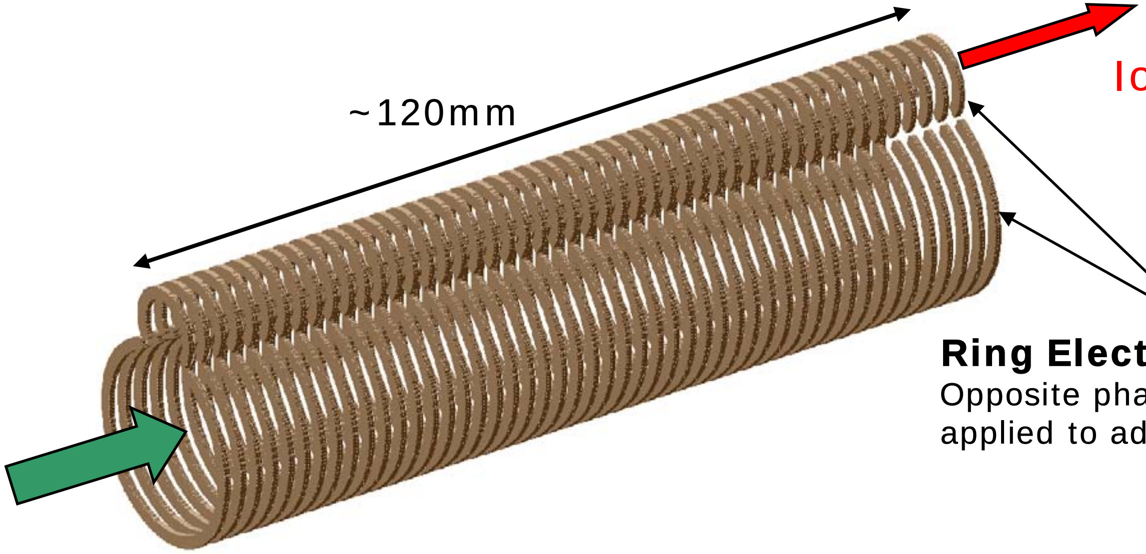
Ion Block Design

- **Sampling cone aperture**
  - — Increased to 0.8mm diameter
    - o Approx 5x increase in gas/ion flow
- **New ion block design**
  - — No supplemental pumping
  - — All ions (and gas) enter StepWave guide
    - o Approx 200x increase in gas flow
    - o <u>Up to 200x increase in ion flux.</u>



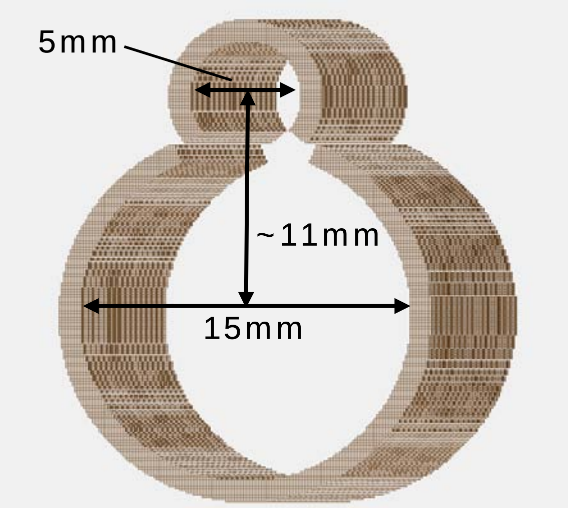
Novel StepWave ion guide design

Conjoined Ion Guide



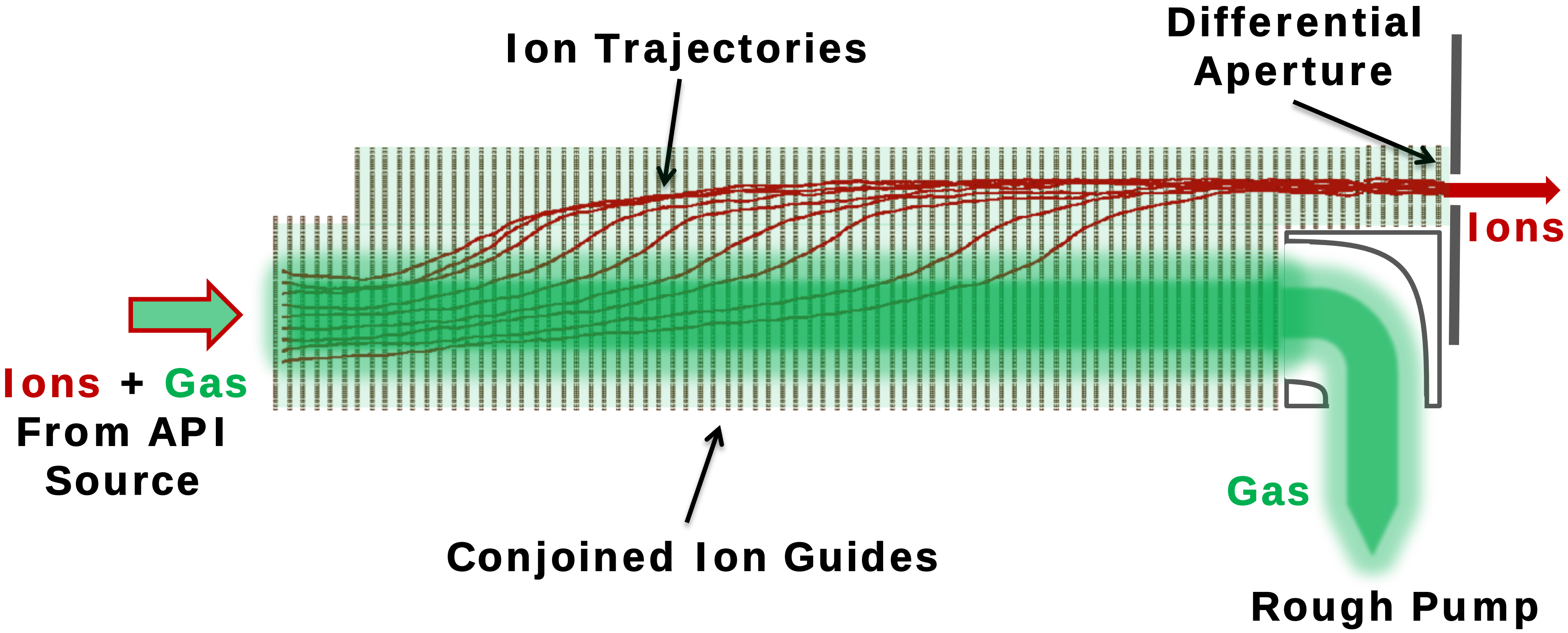
Ring Electrodes
Opposite phase of RF
applied to adjacent plates

Compact
Ion
Cloud



Additional design benefit

Robustness



Ion Trajectories

Differential Aperture

Ions

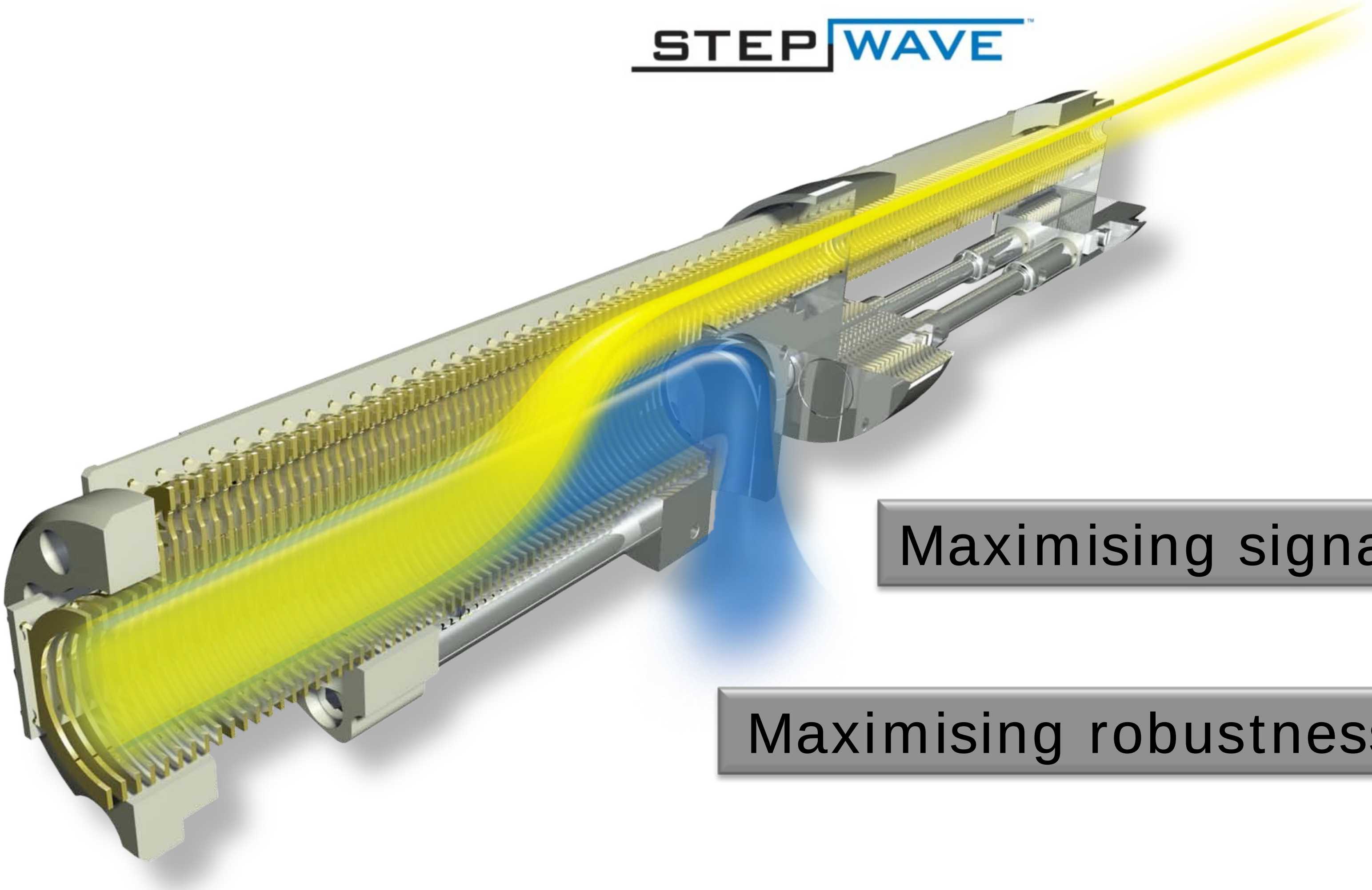
Ions + Gas From API Source

Conjoined Ion Guides

Gas

Rough Pump

Designed to deal with problems associated with a larger sampling orifice

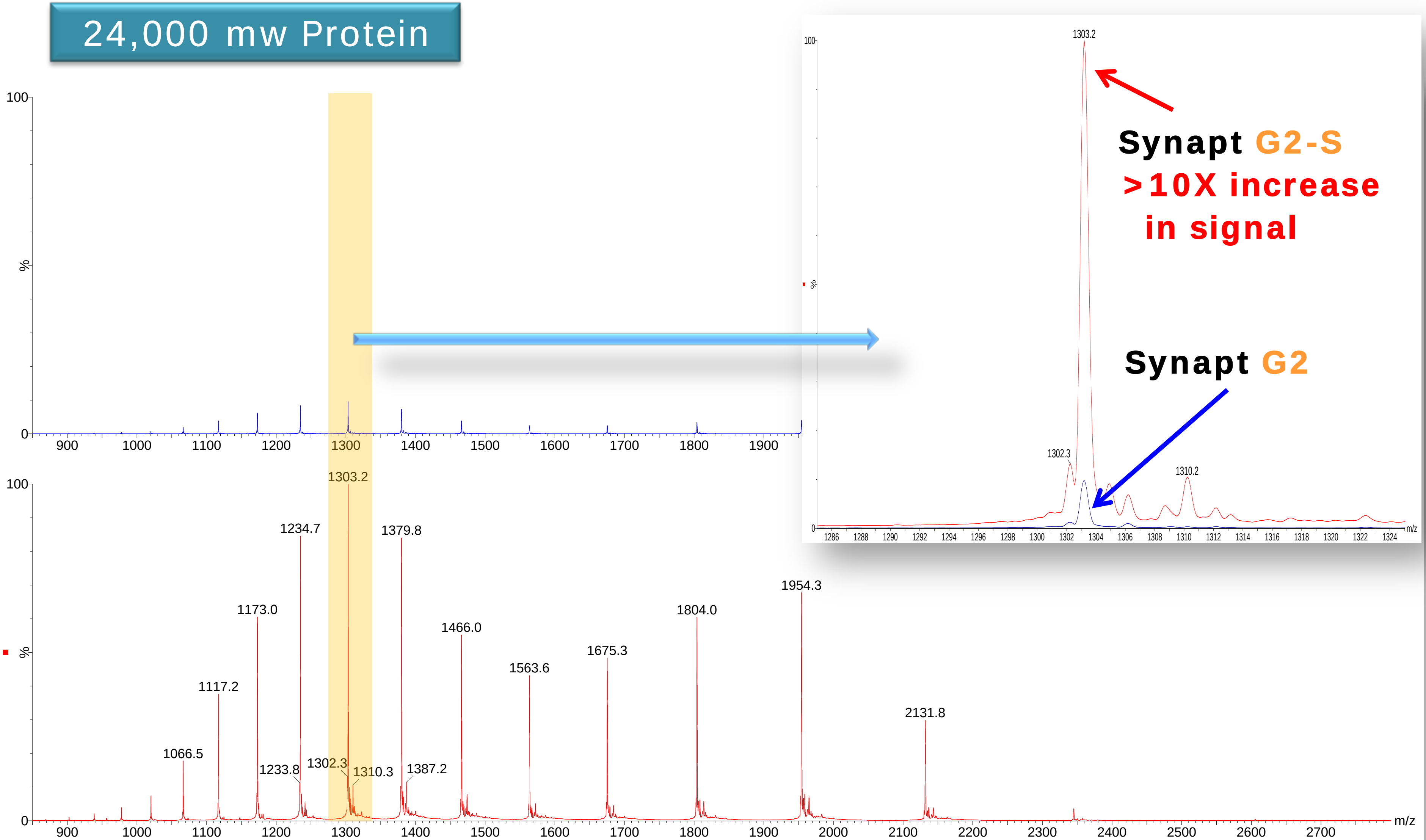


Maximising signal

Maximising robustness

ESI Positive Ion Sensitivity
Synapt G2-S

24,000 mw Protein



Synapt G2-S

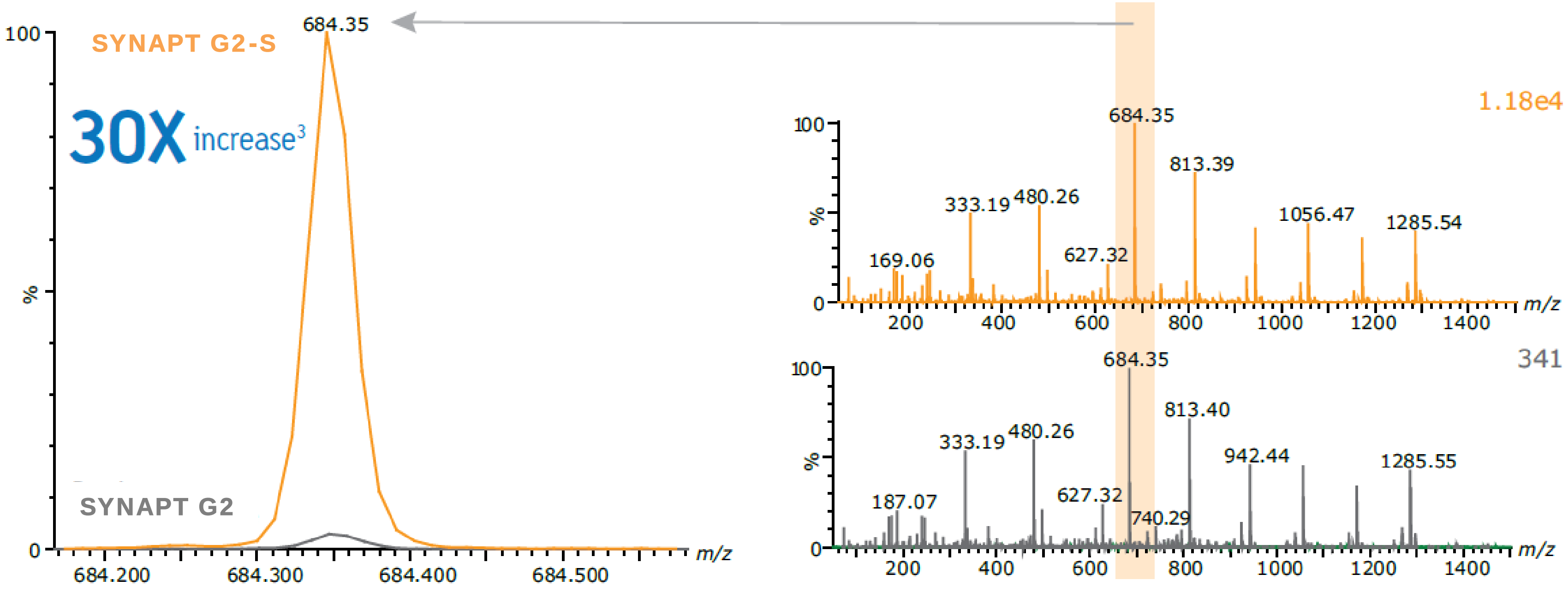
>10X increase in signal

Synapt G2

Identification

...limits of detection

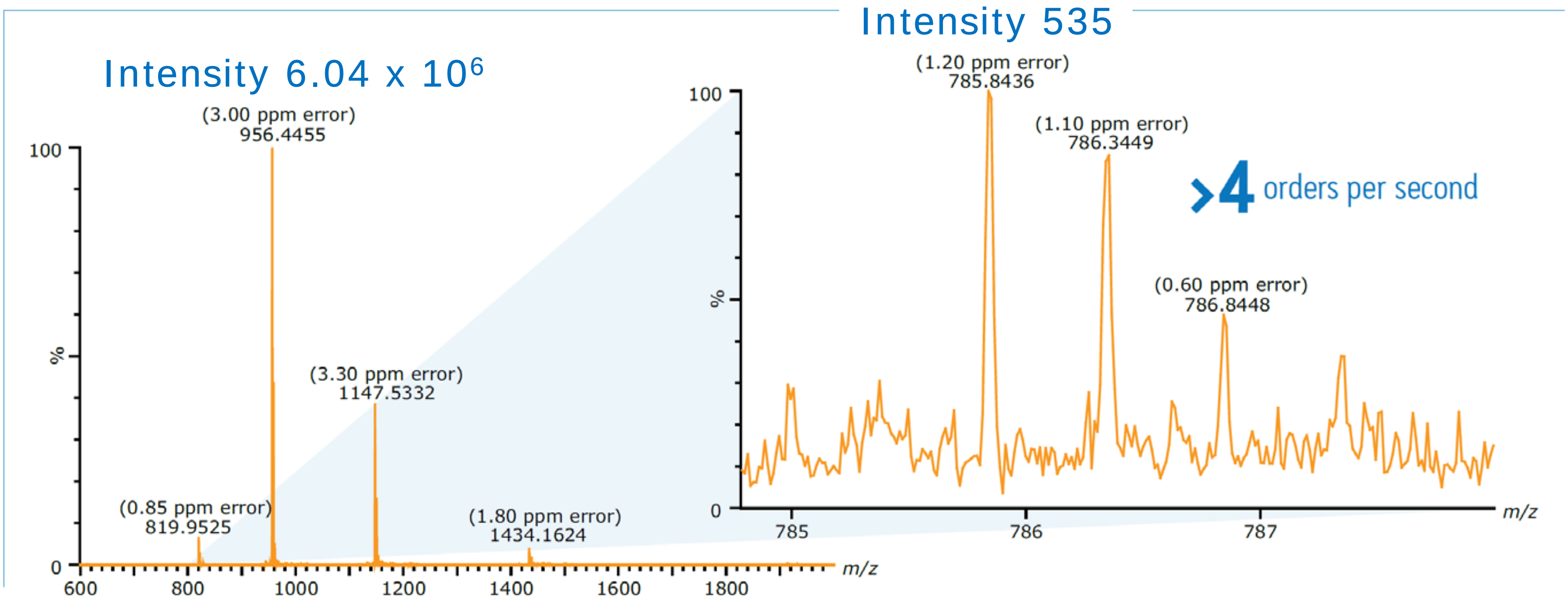
SYNAPT G2-S vs SYNAPT G2



Identification

…detection in complex mixtures

Glu-Fibrinopeptide spiked into Bovine Insulin (1/10,000)



In-Spectral Dynamic Range > 4 orders of magnitude

MS^E Synapt G2-S vs Synapt G2

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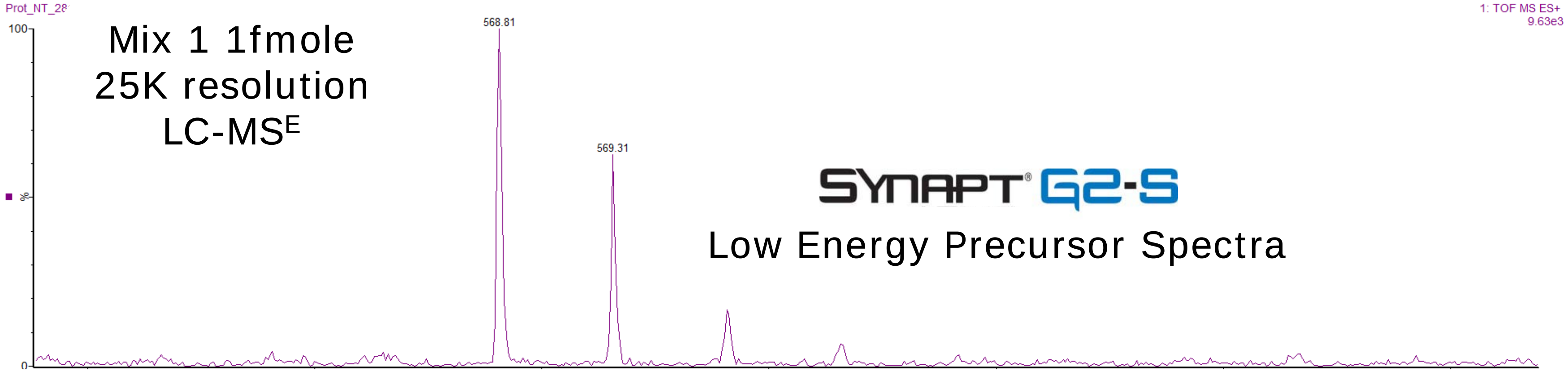
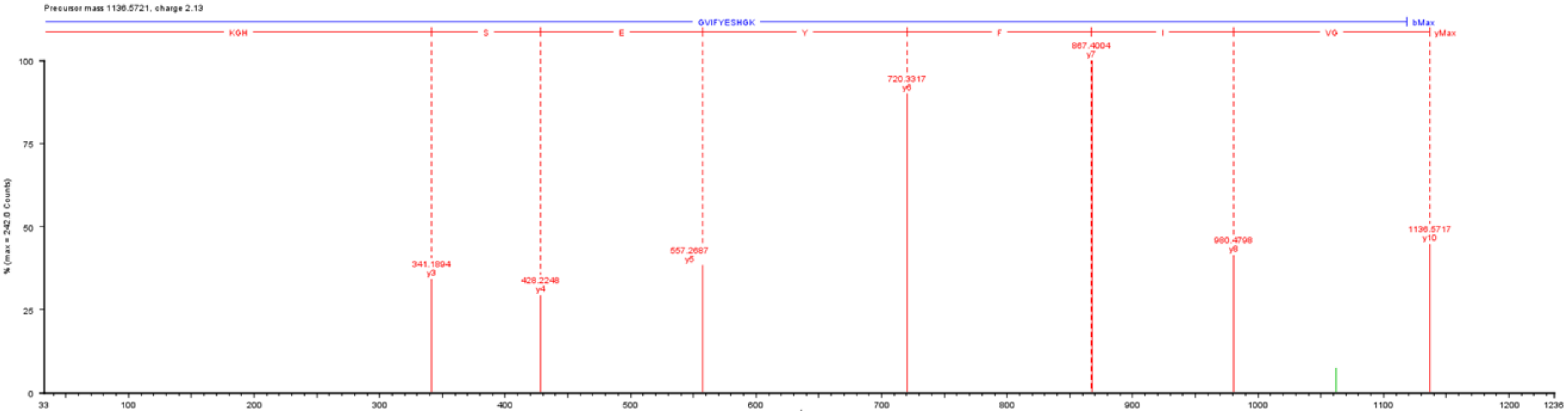


Mix 1 1fmole
25K resolution
LC-MS^E

SYNAPT® G2-S

Low Energy Precursor Spectra

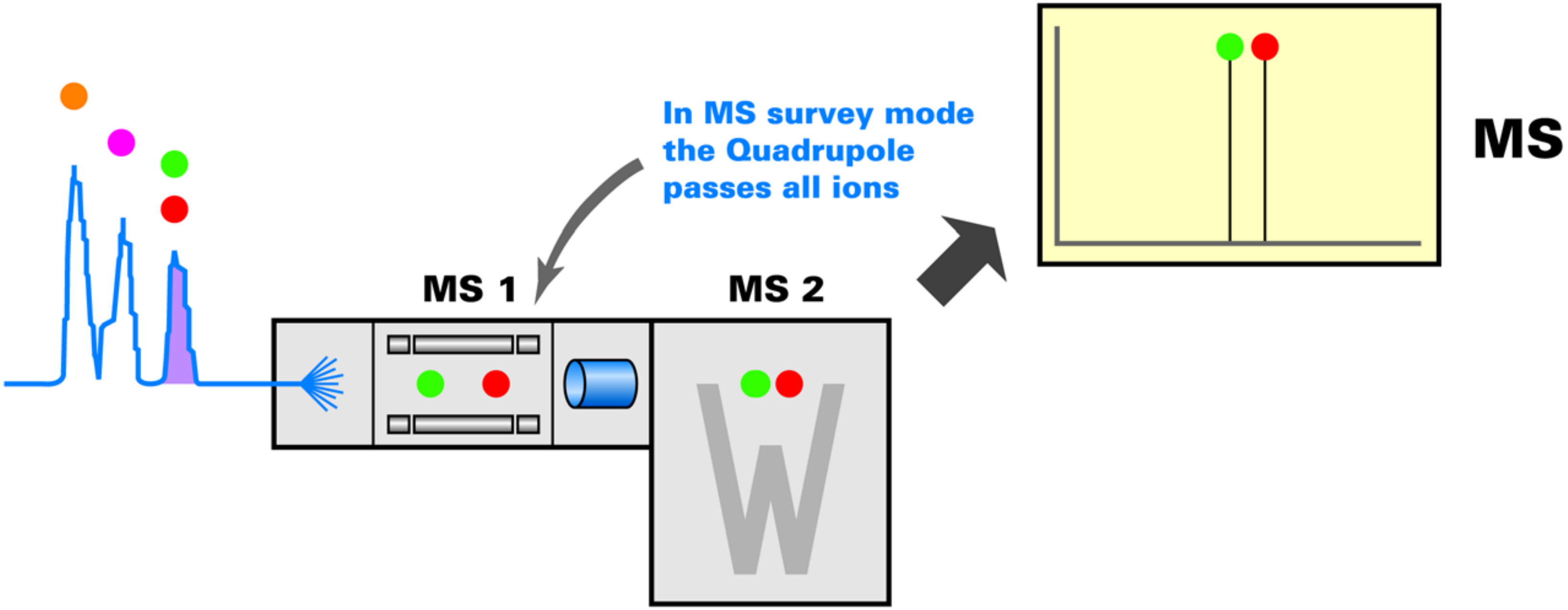


Data Dependent LC-MS/MS

vs

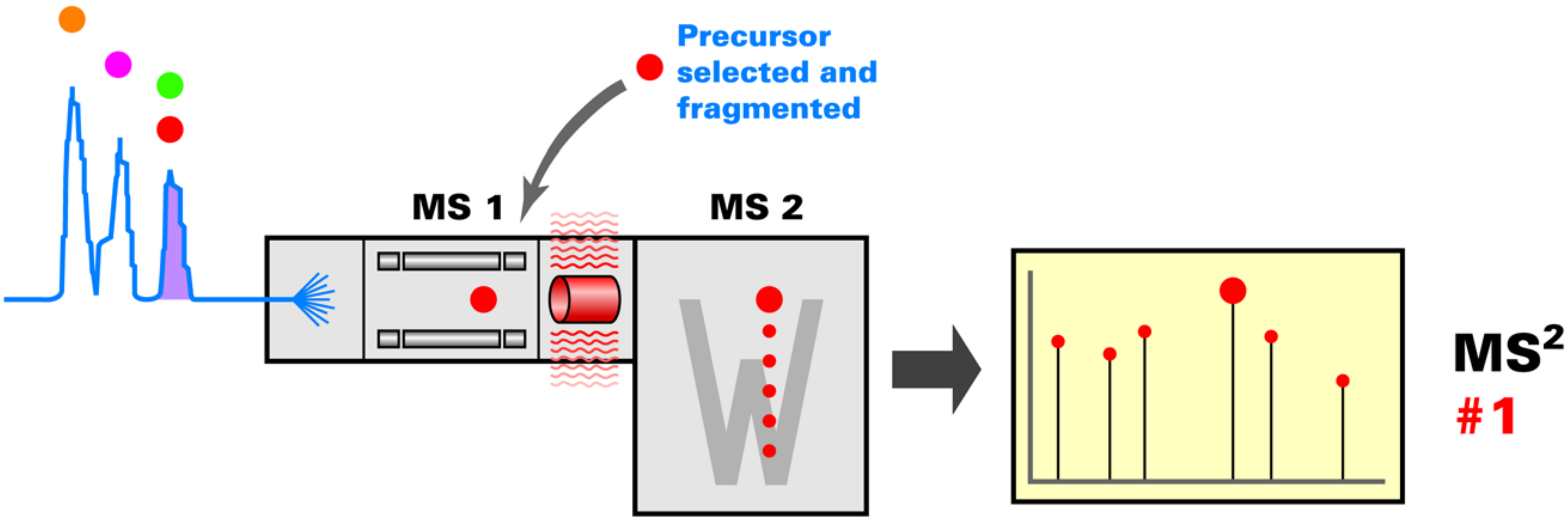
Data Independent LC-MS/MS

Conventional Data Dependent LC-MS/MS Precursor Survey Scan

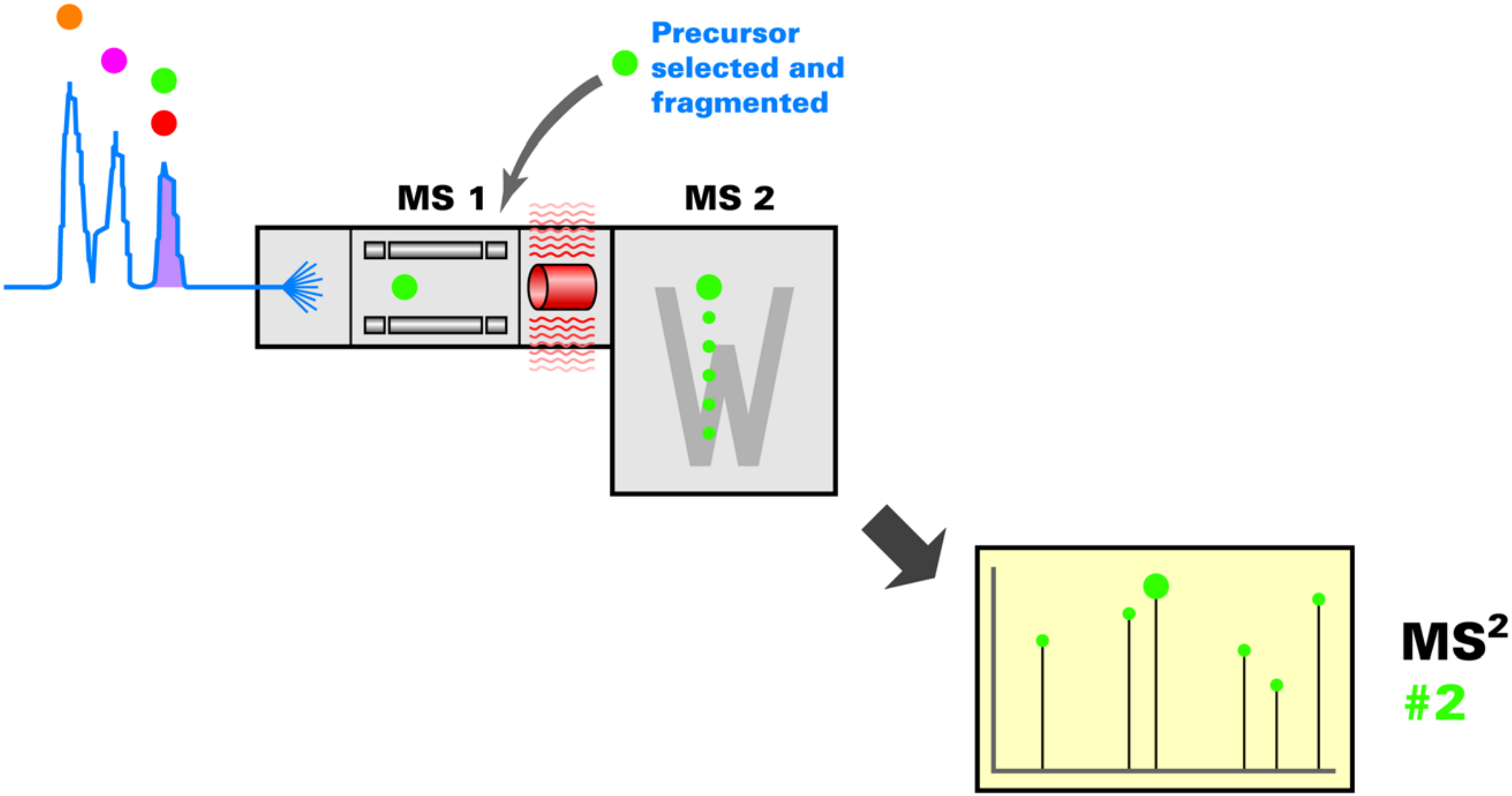


In MS survey mode the Quadrupole passes all ions

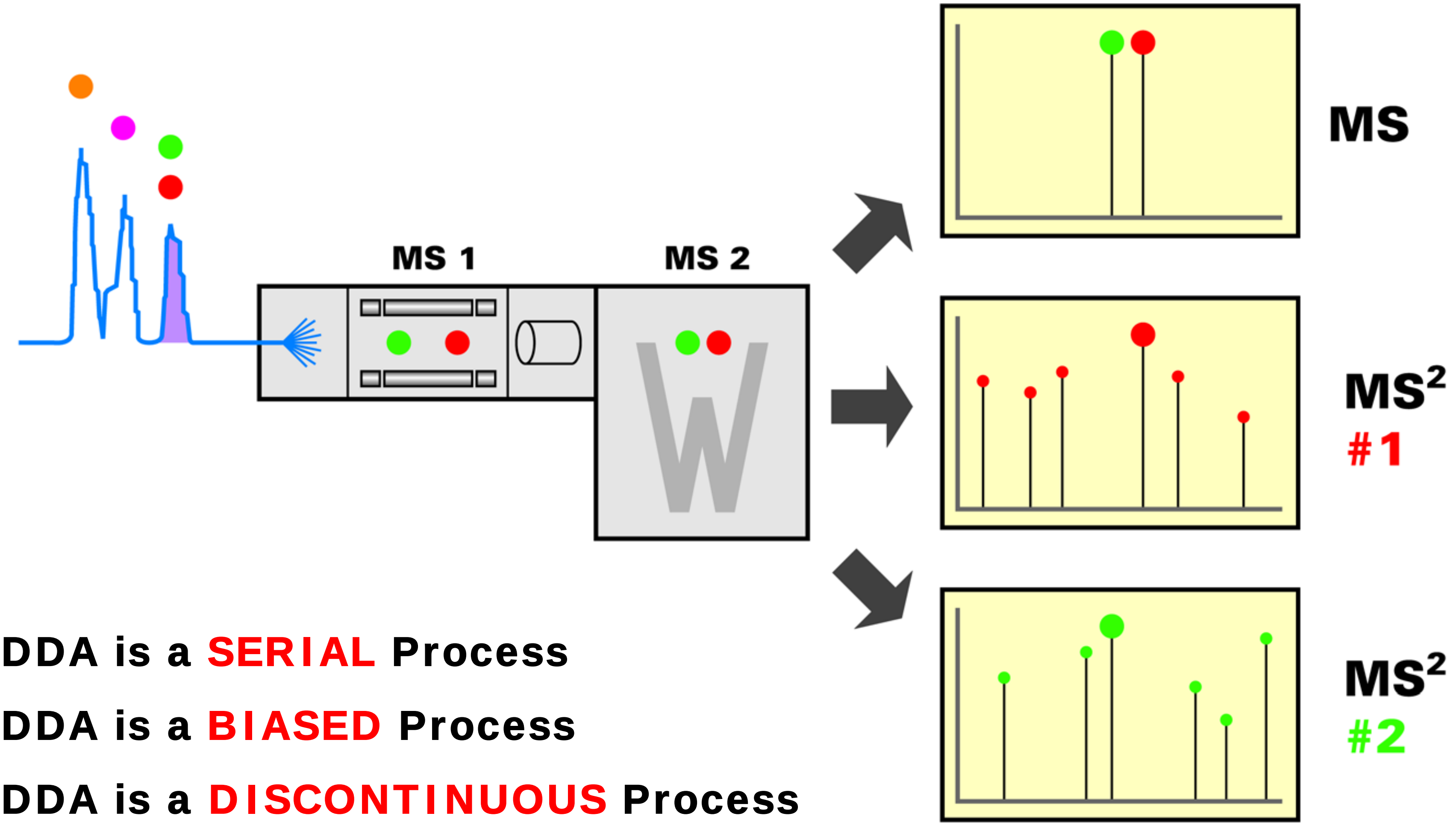
Conventional Data Dependent LC-MS/MS Product Ion Scan



Conventional Data Dependent LC-MS/MS Product Ion Scan



Conventional Data Dependent LC-MS/MS
- Weaknesses -



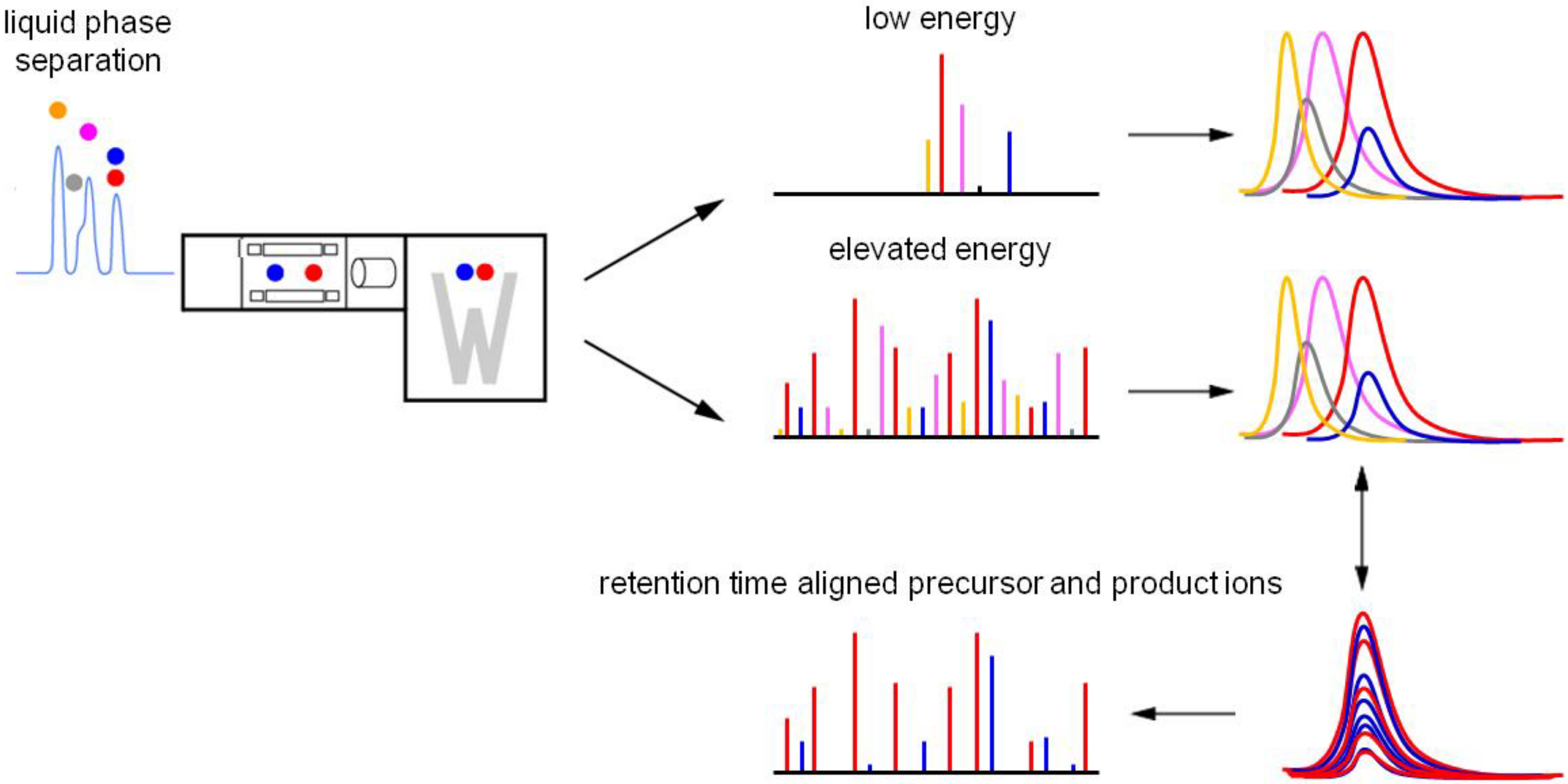
DDA is a SERIAL Process

DDA is a BIASED Process

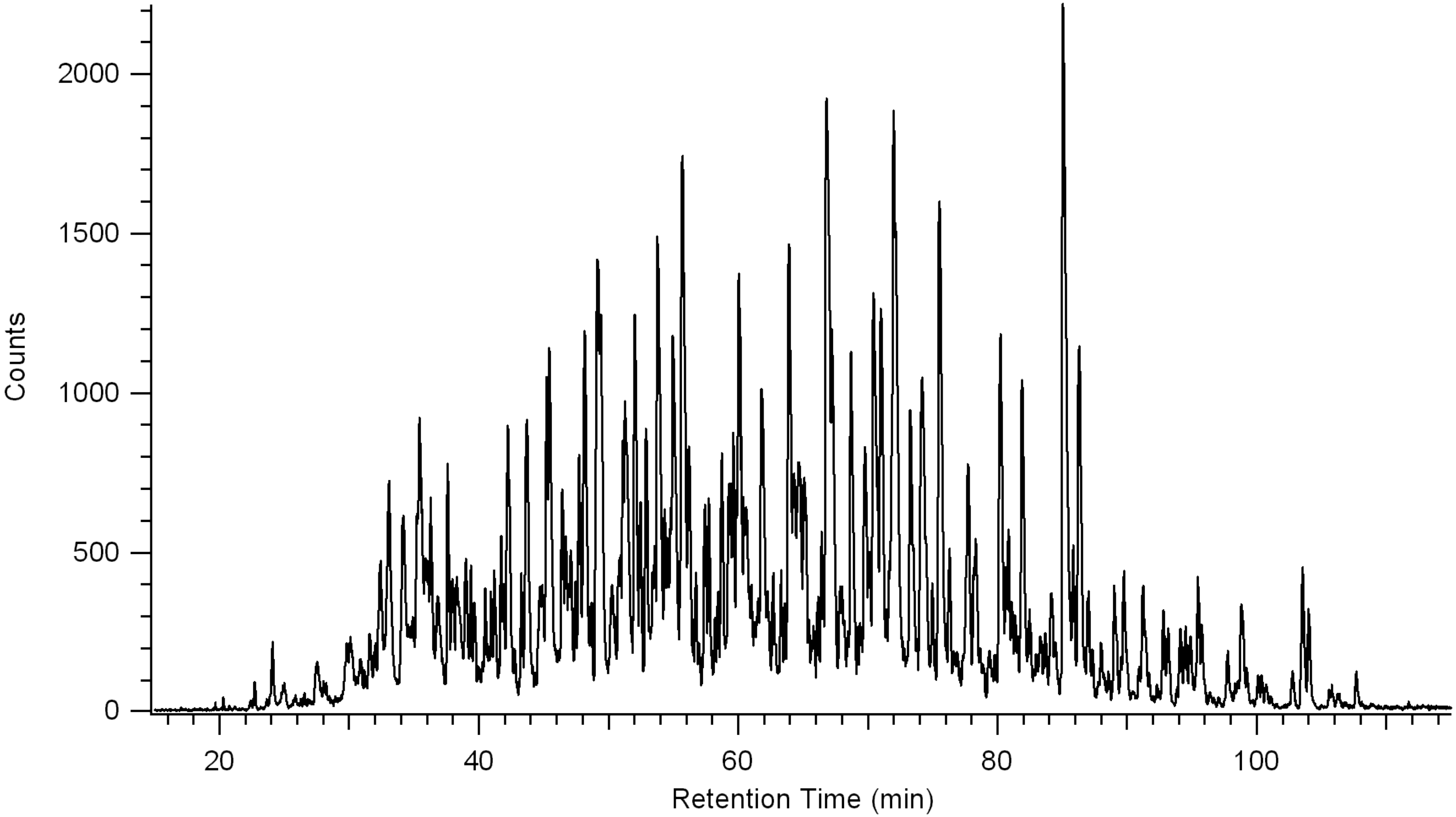
DDA is a DISCONTINUOUS Process

DDA is subject to INTERFERENCES

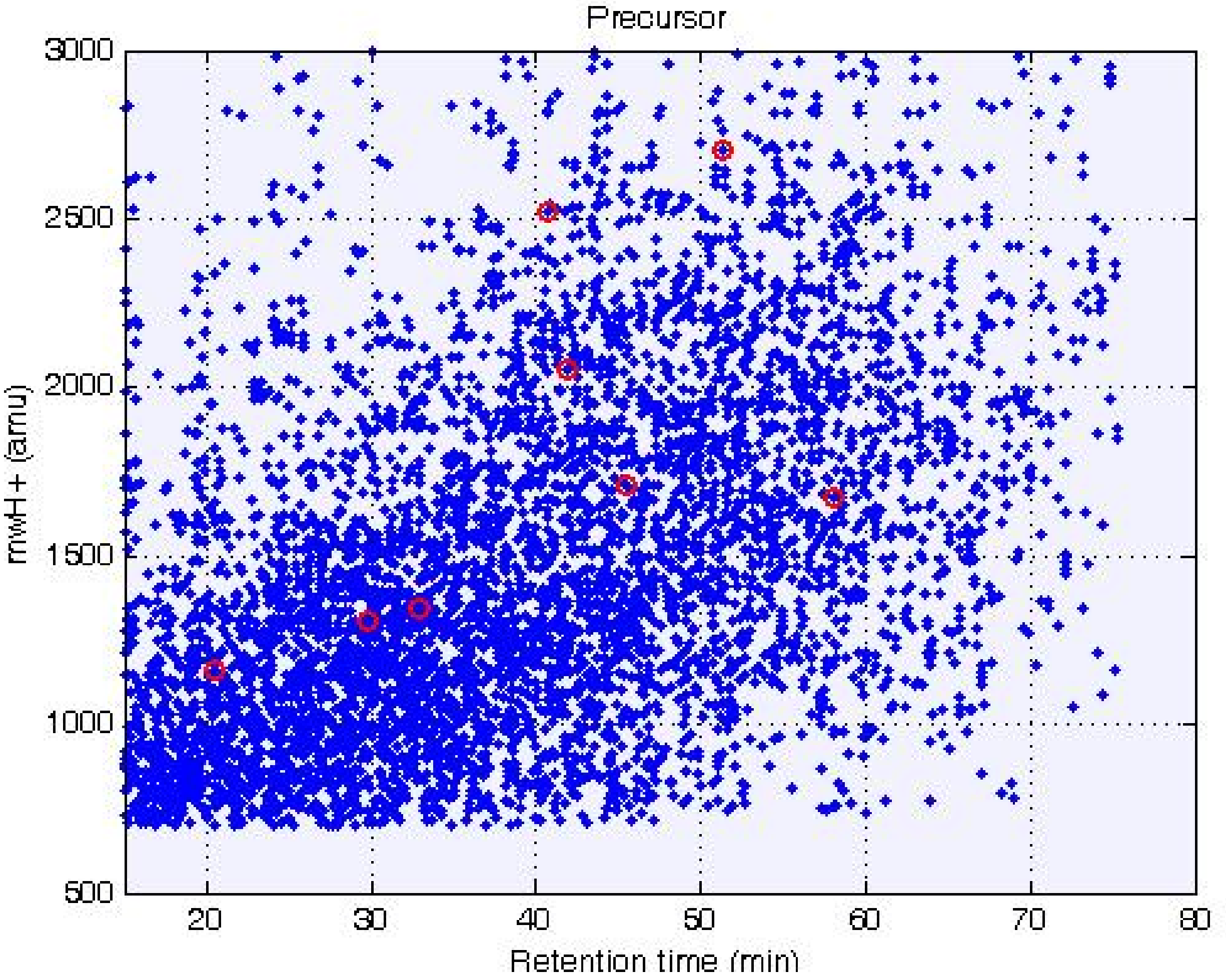
Data Independent LC-MS/MS (LC-MS^E)



What is the level of Complexity?
E coli UPLC Separation

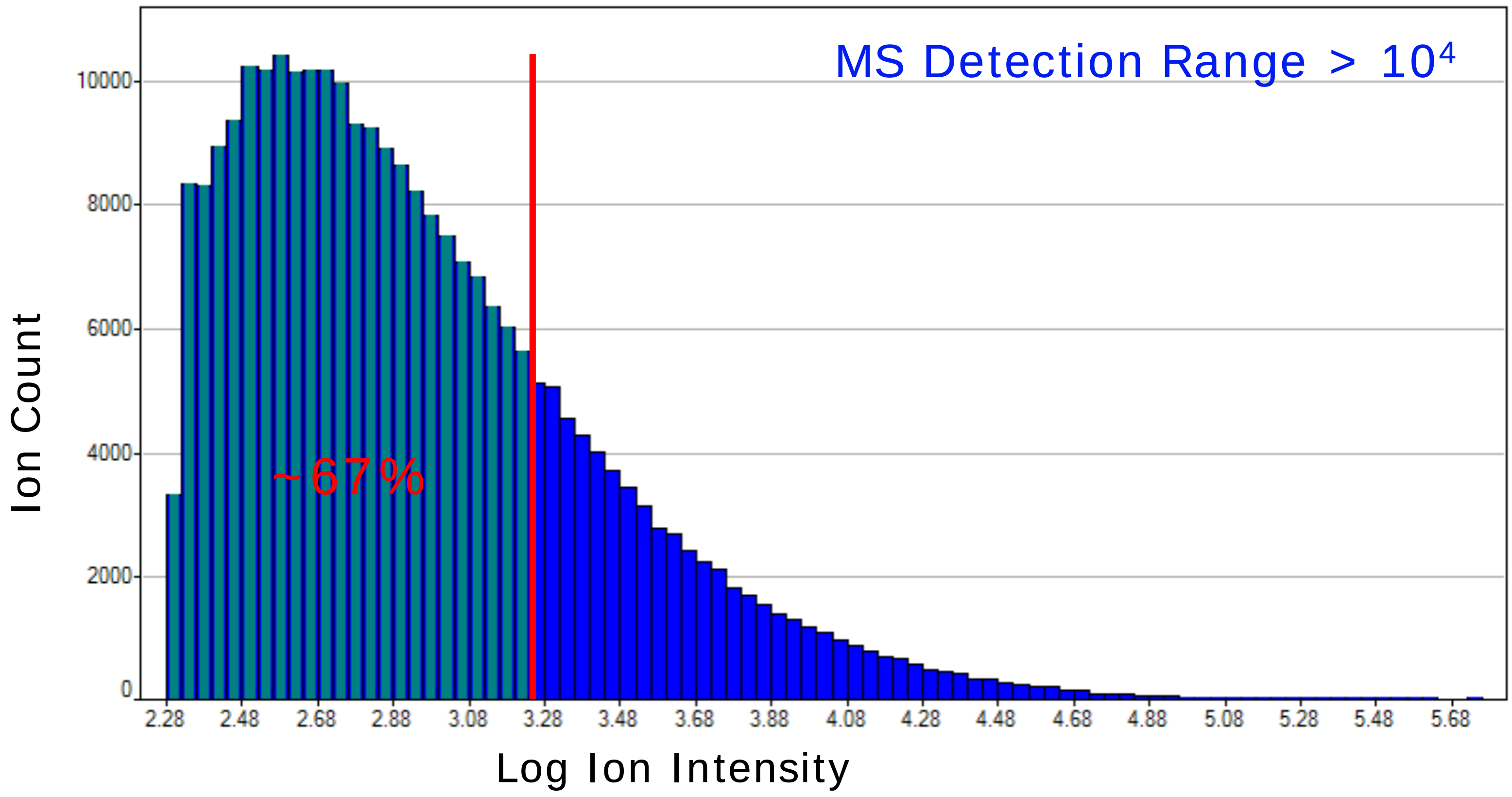


E coli Precursor MH+ Density



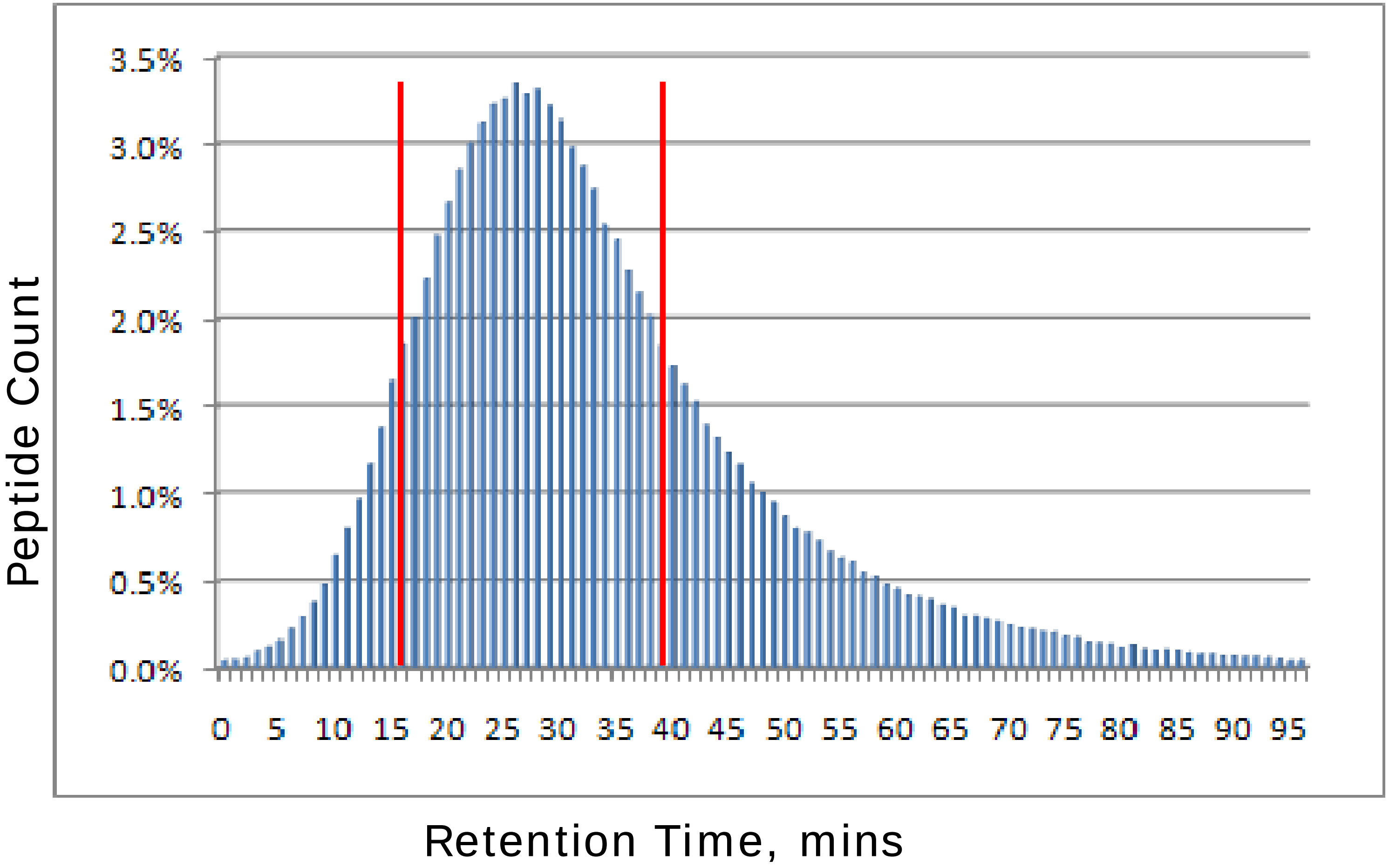
~450K Ion Detections = ~36K EMRT's = ~10K ID'd peptides = 697 ID'd Proteins

Intensity distribution of all ion detections in an E coli lysate digest



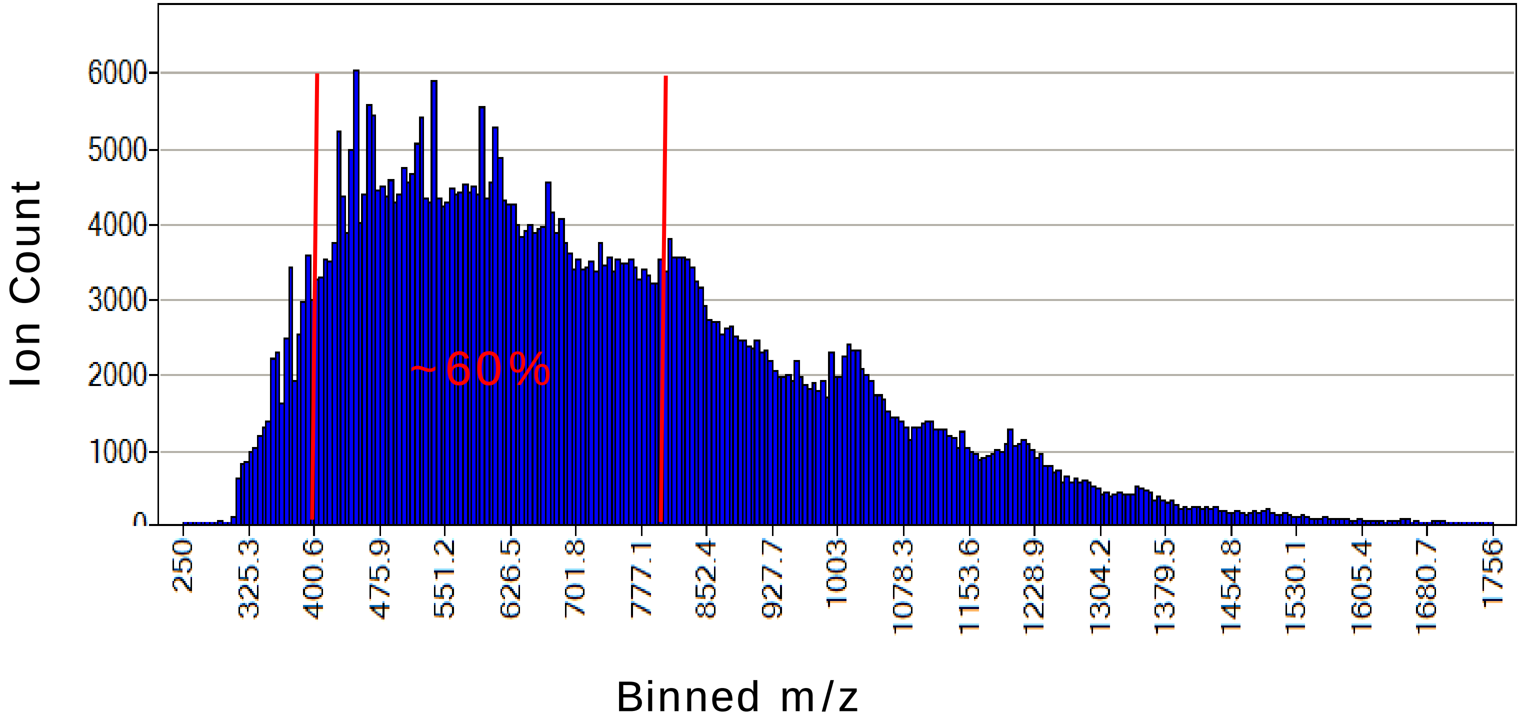
~2/3 of all precursor ion detections are less than 2 orders of magnitude in intensity of the most abundant ion detections.

Peptide Retention Time Distribution



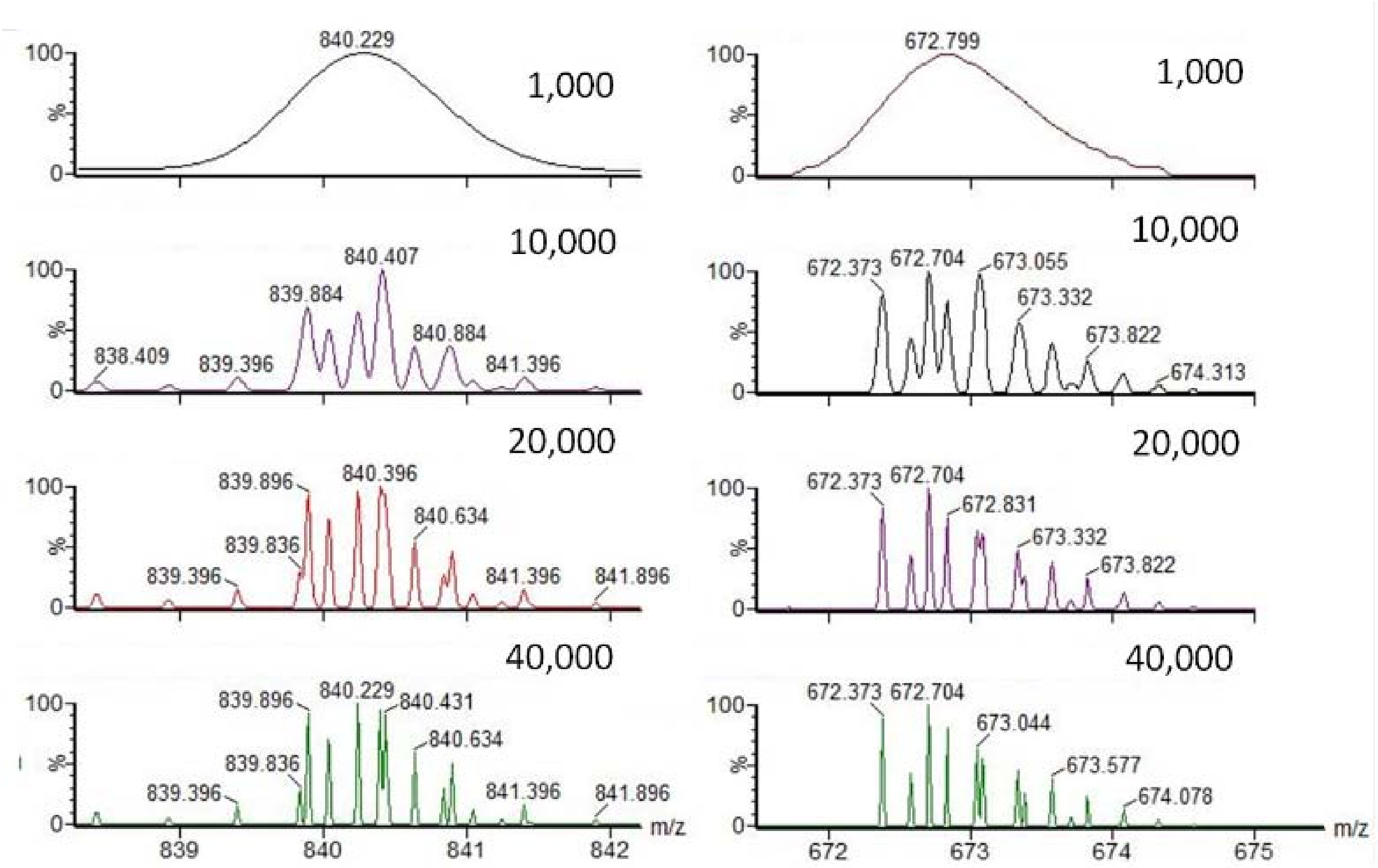
Approximately 50% of all tryptic peptides elute in 20% the chromatographic gradient elution time

m/z Distribution of All Ions in an E coli Lysate Digest



Approximately 60% of all ion detections are between m/z 400-800

Data Complexity
Mass Resolution and Accuracy is Critical



Mass Resolution

Precursor m/z

Product m/z

Ion mobility enhanced UPLC-MS^E

Synapt G2-S Schematic

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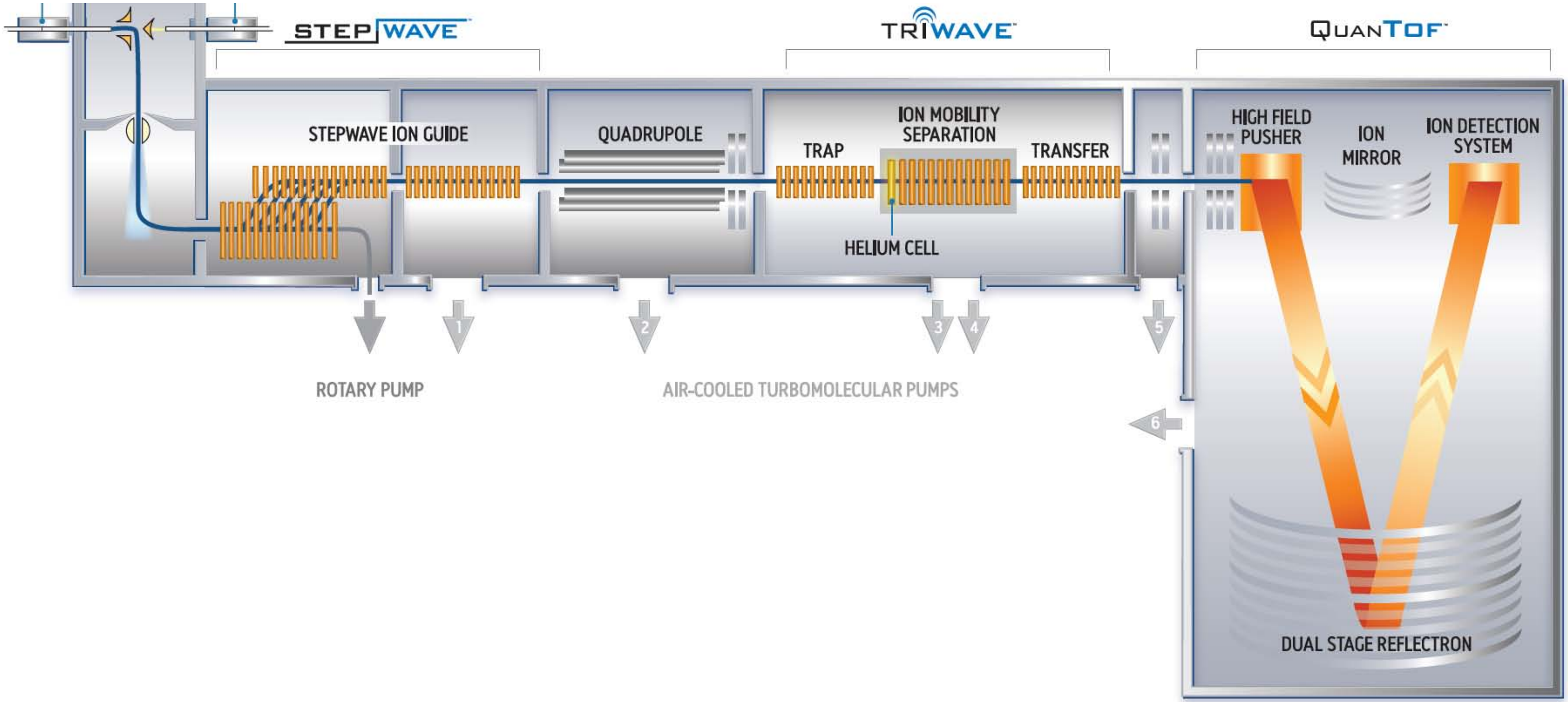
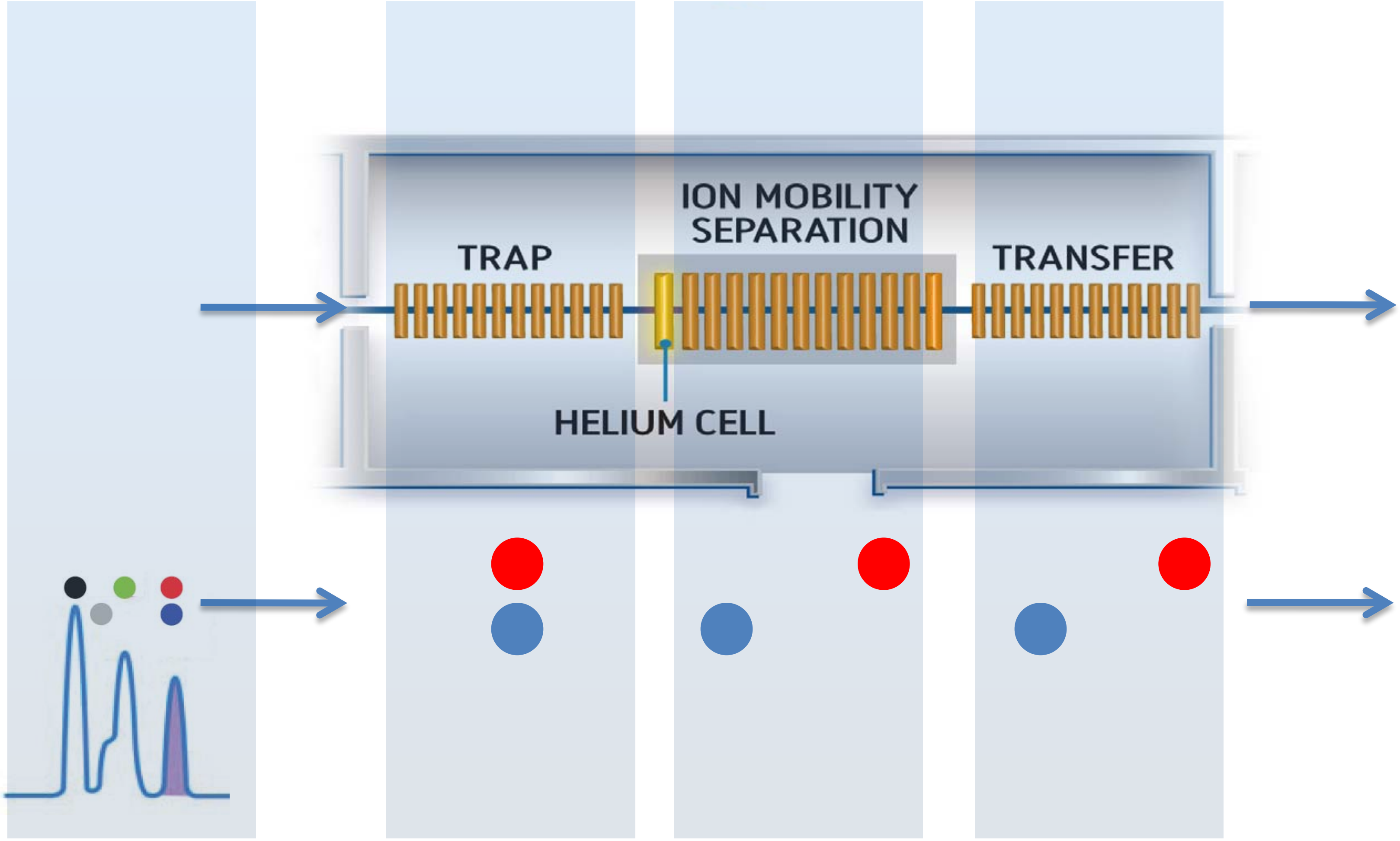


UPLC/HDMS^E

…deconvoluting chimericy



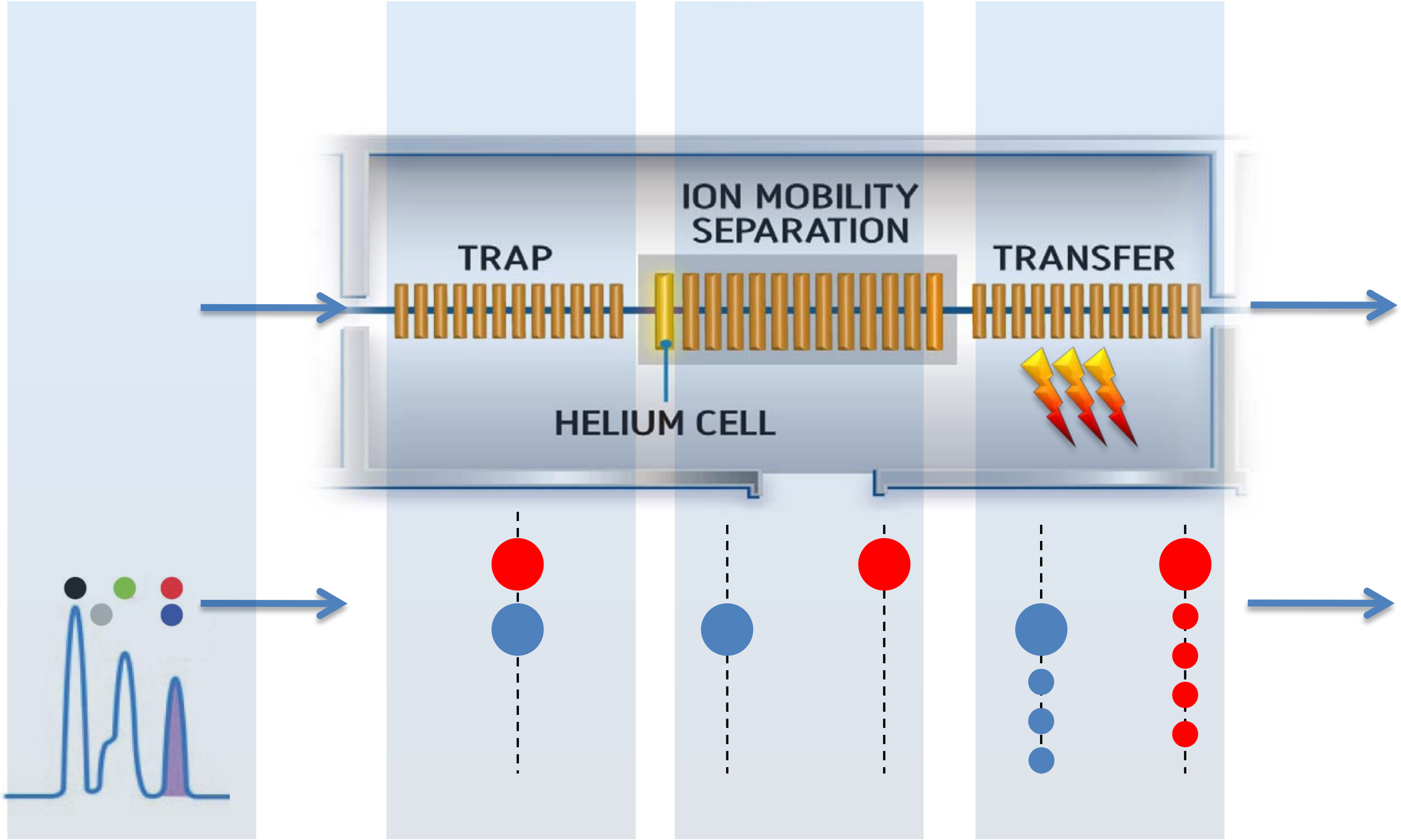
Co-Eluting Peptides

Ionized Precursors

Precursors Transferred to TOF MS

UPLC/HDMS^E

…deconvoluting chimericy



Co-Eluting Peptides

Ionized Precursors

Precursors & Products Time Aligned

UPLC-HDMS^E

Mobility Drift Time + Retention Time Alignment

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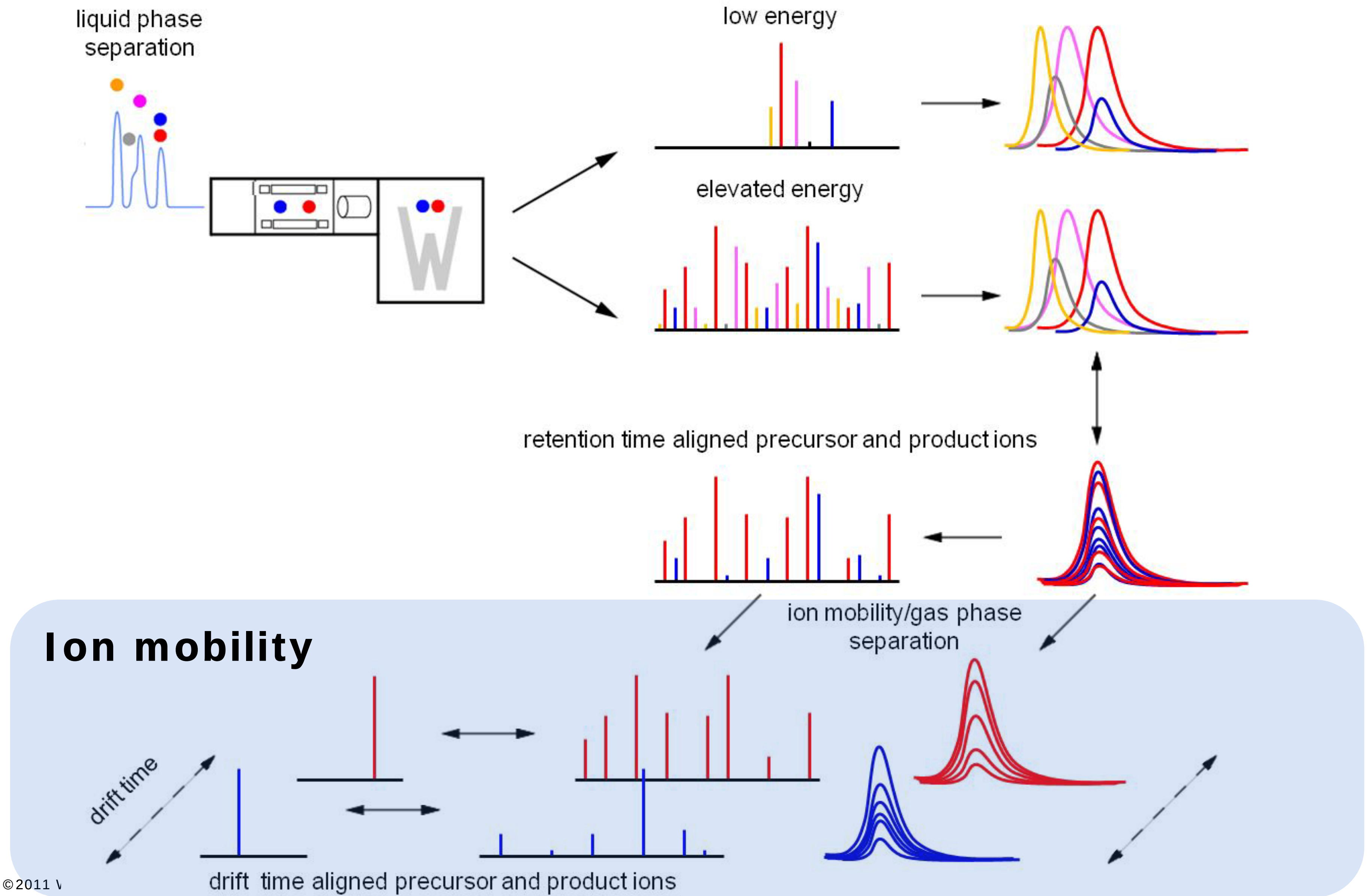
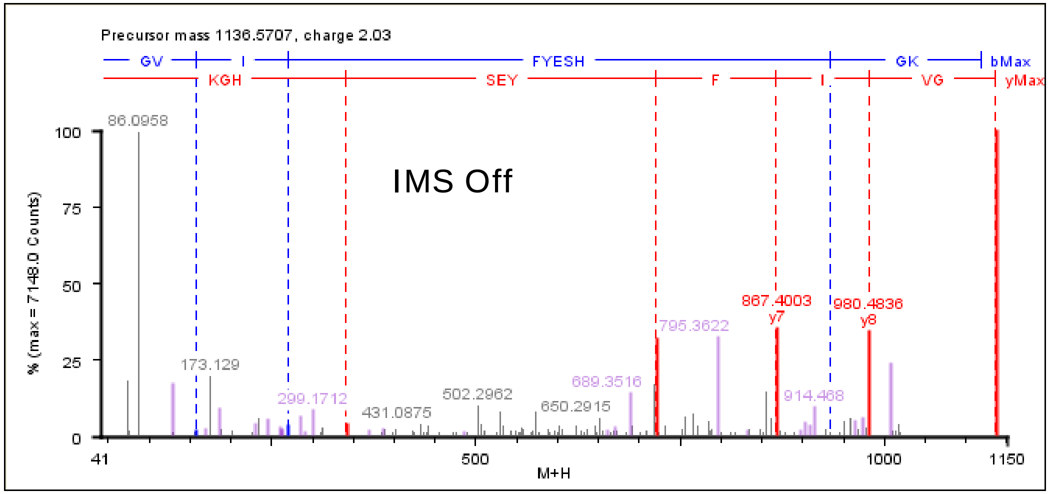
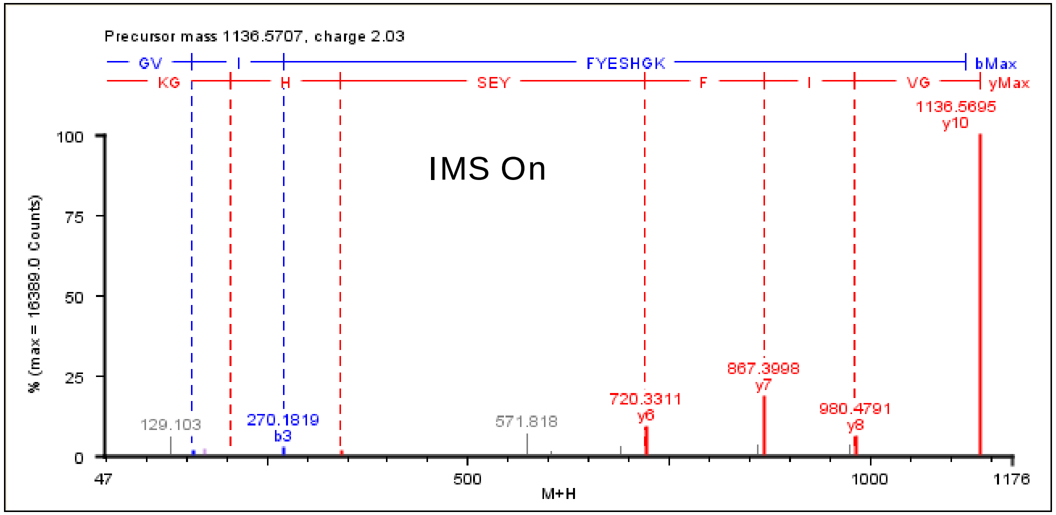


Impact of Ion Mobility Enhanced UPLC-MS^E Precursor/Product Drift Time Alignment



Without Drift Time Alignment
254 product ions tentatively associated with one precursor



With Drift Time Alignment
35 product ions tentatively associated with one precursor

How Does Ion Mobility Improve Protein Identification Rate and Quality

- Increases analytical peak capacity/ separation power by approximately 7-10 fold

- Reduces chimeric interferences & distraction
  - Precursor and product ions are mobility drift time aligned as well as chromatographic retention time aligned .
    - Increased specificity, less distracting product ions per peptide
  - Improved precursor charge state recognition
    - Isotopes for each charge state of each peptide precursor have the same mobility drift time
  - More precursor ions detected because of reduced interference from chemical noise
  - Improved measurement & confidence in peptide monoisotopic accurate mass

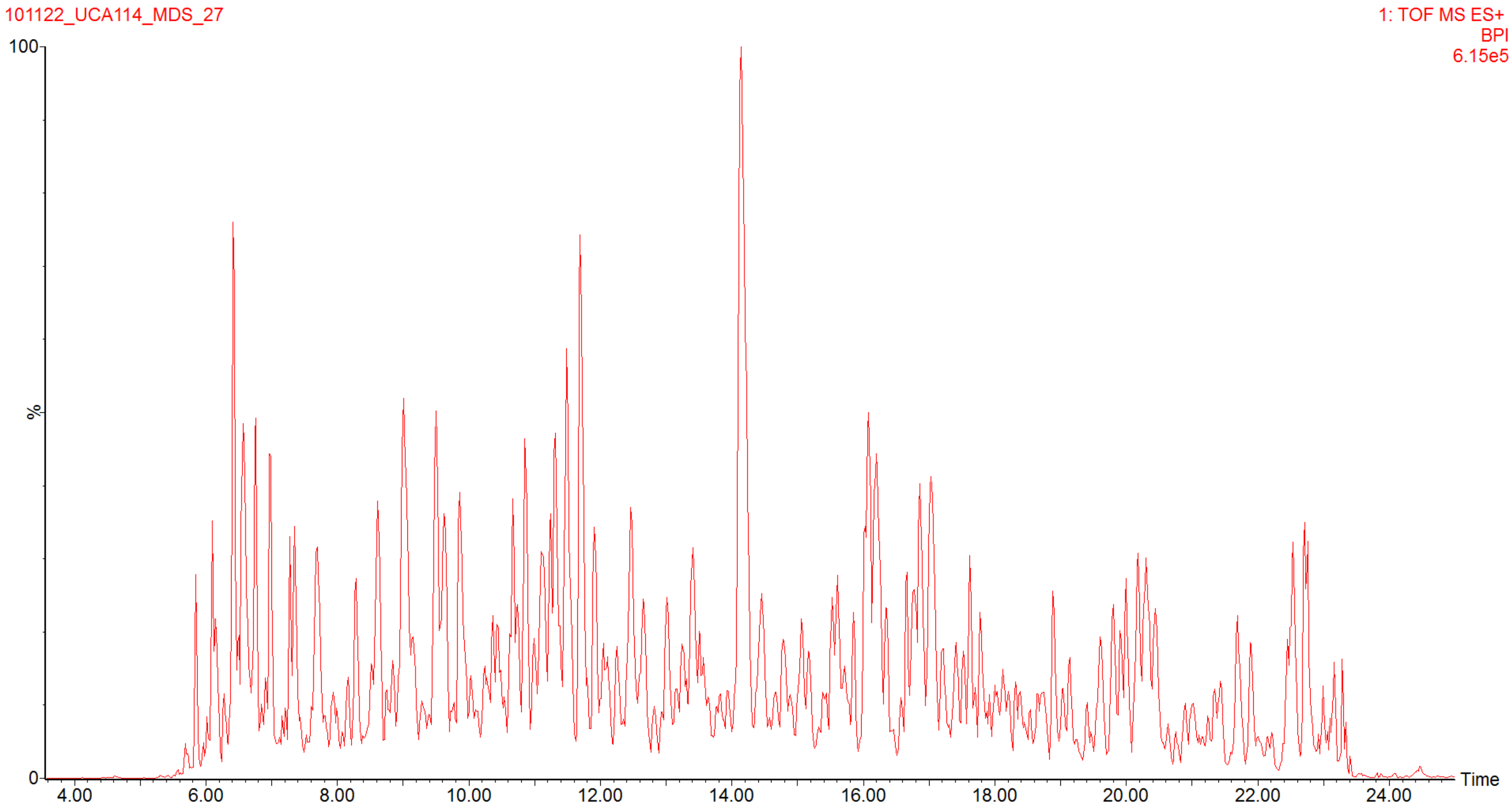
- Adds an additional dimension of separation without increasing analysis time

Example UPLC-HDMS^E data

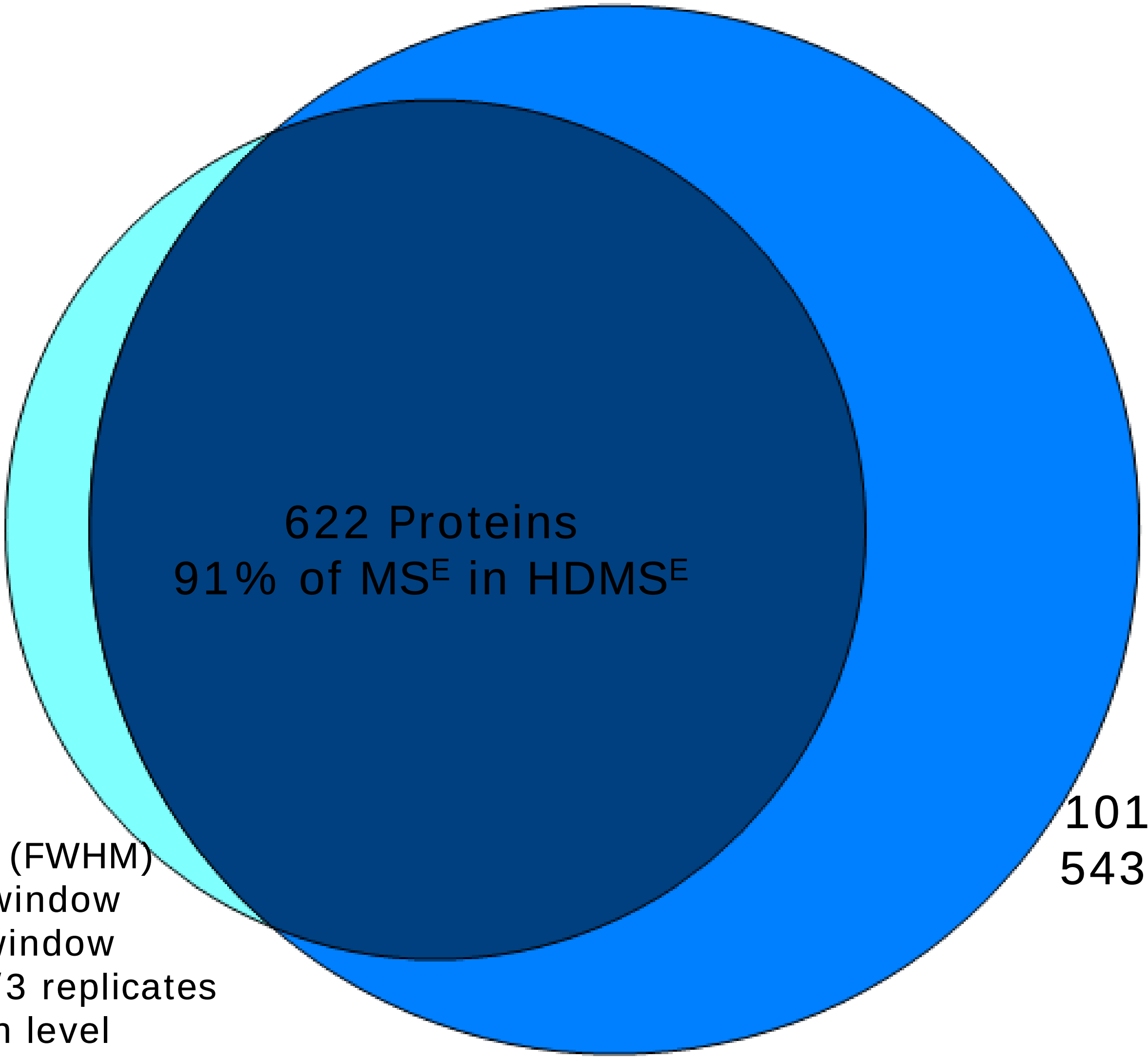
The Impact of Ion Mobility

Synapt G2 HDMS

Application 1:
High Throughput analysis of Shewanella



Increased number of proteins/ peptides identified



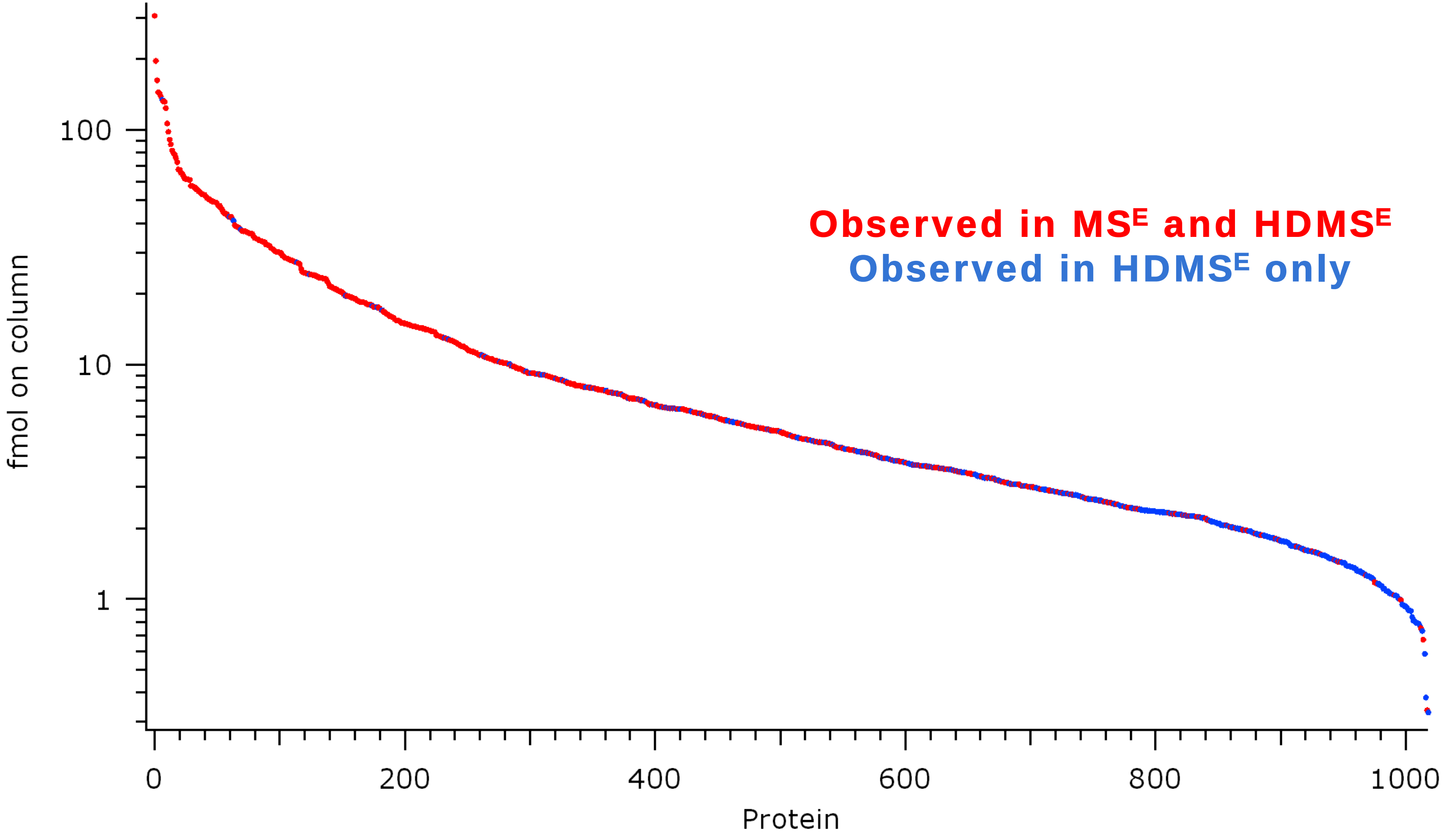
MS^E
684 Proteins
4337 peptides

622 Proteins
91% of MS^E in $HDMS^E$

$HDMS^E$
1019 Proteins
5432 peptides

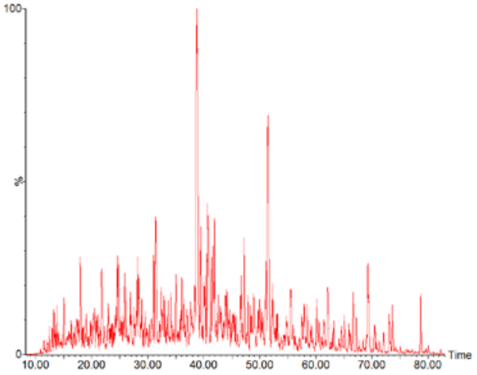
Resolution >20,000 (FWHM)
5 ppm precursor window
12 ppm product window
Proteins had to be in 2/3 replicates
2% FPR at protein level
6 peptides/protein

Absolute Quantitation of Proteins from *Shewanella Oneidensis*

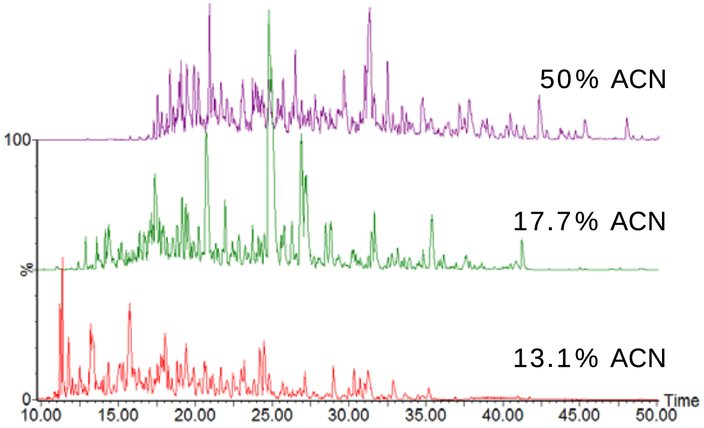


Application 2: Increased Proteome Coverage of *Caenorhabditis elegans*

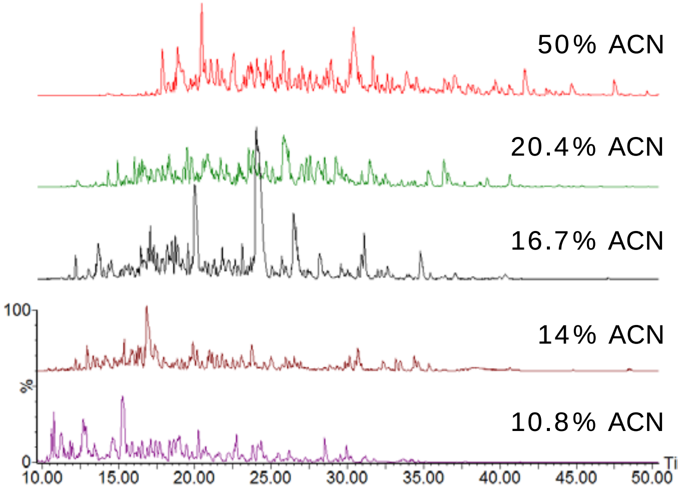
1D Chromatography
1 µg *C. elegans*
< 2 hours



2D-3 Fraction
1.5 µg *C. elegans*
< 4 hours



2D-5 Fraction
2.5 µg *C. elegans*
< 6 hours



Increasing Number of Identified Proteins with Increase in Peak Capacity

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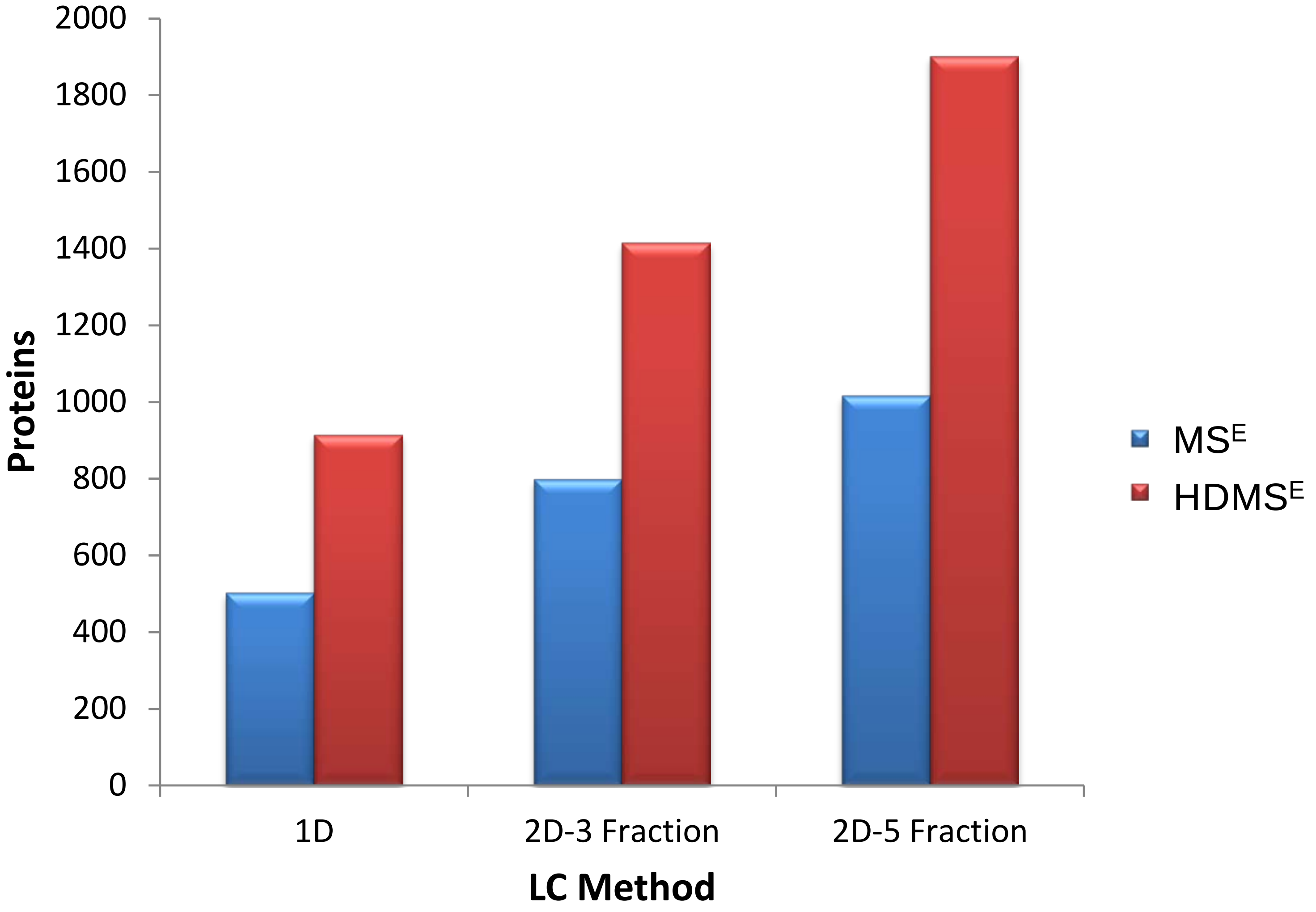
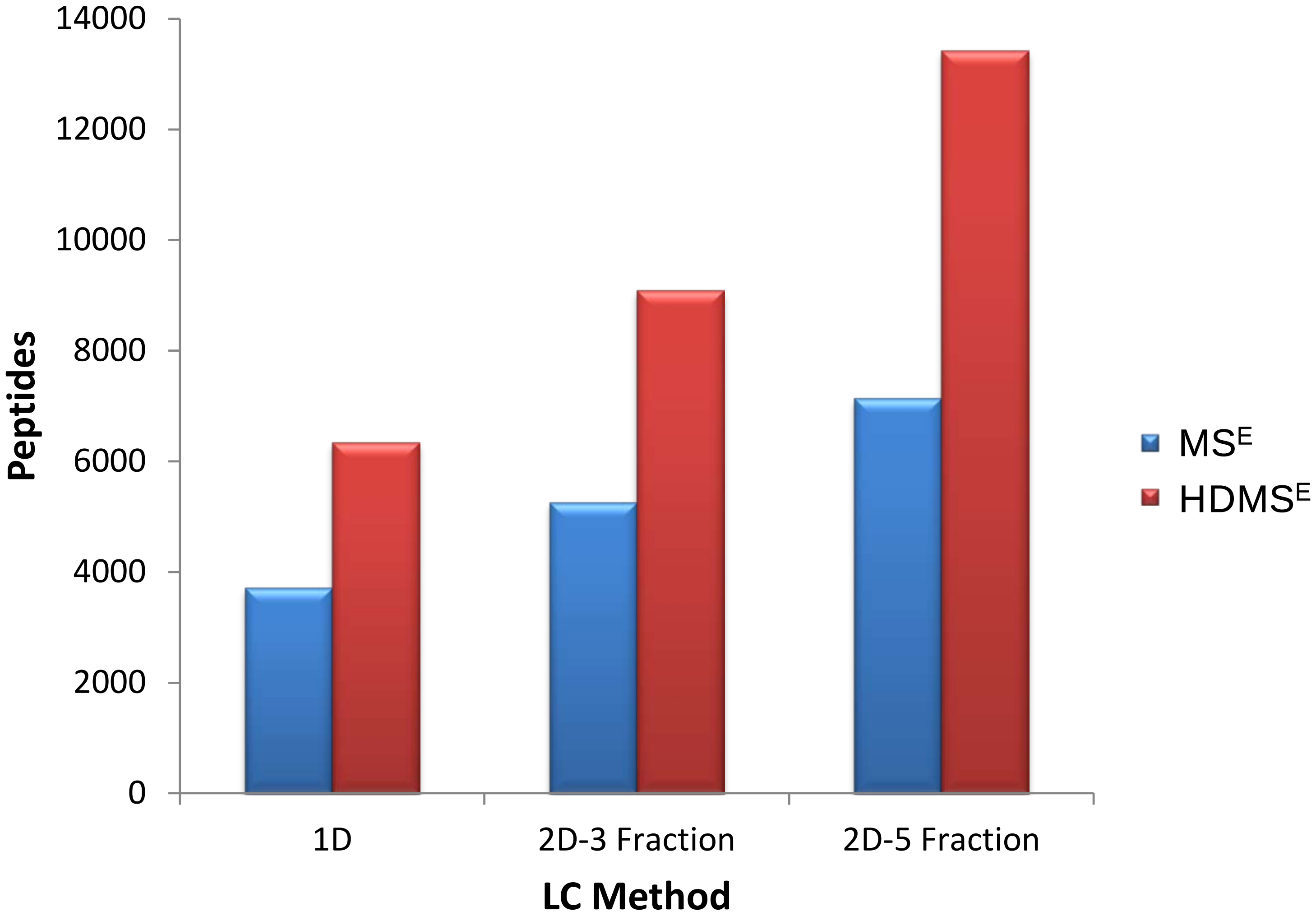


Increasing Number of Identified Peptides with Increase in Peak Capacity



Acknowledgements

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