SimulTOF™ Mass Spectrometry for High Performance MS-MS

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SimulTOF Systems from Virgin Instruments Corp.

Biological Mass Spectrometry in the 21st Century
Electrospray(ESI) or MALDI?

- Electrospray(ESI)
  - High performance ESI widely available
  - MS dictates separation conditions
    - Low flow rates, very small columns, limited capacity and long LC runs
- MALDI-TOF
  - Very fast (full spectrum/laser shot @5 kHz)
  - Tradeoff between speed and sensitivity (avg. of multiple shots)
  - Resolution and mass accuracy inferior to best of electrospray
  - Can be interfaced with variety of separations
  - Inherently simpler, more user friendly, and easier to automate
- Can MALDI compete?

Why speed is important

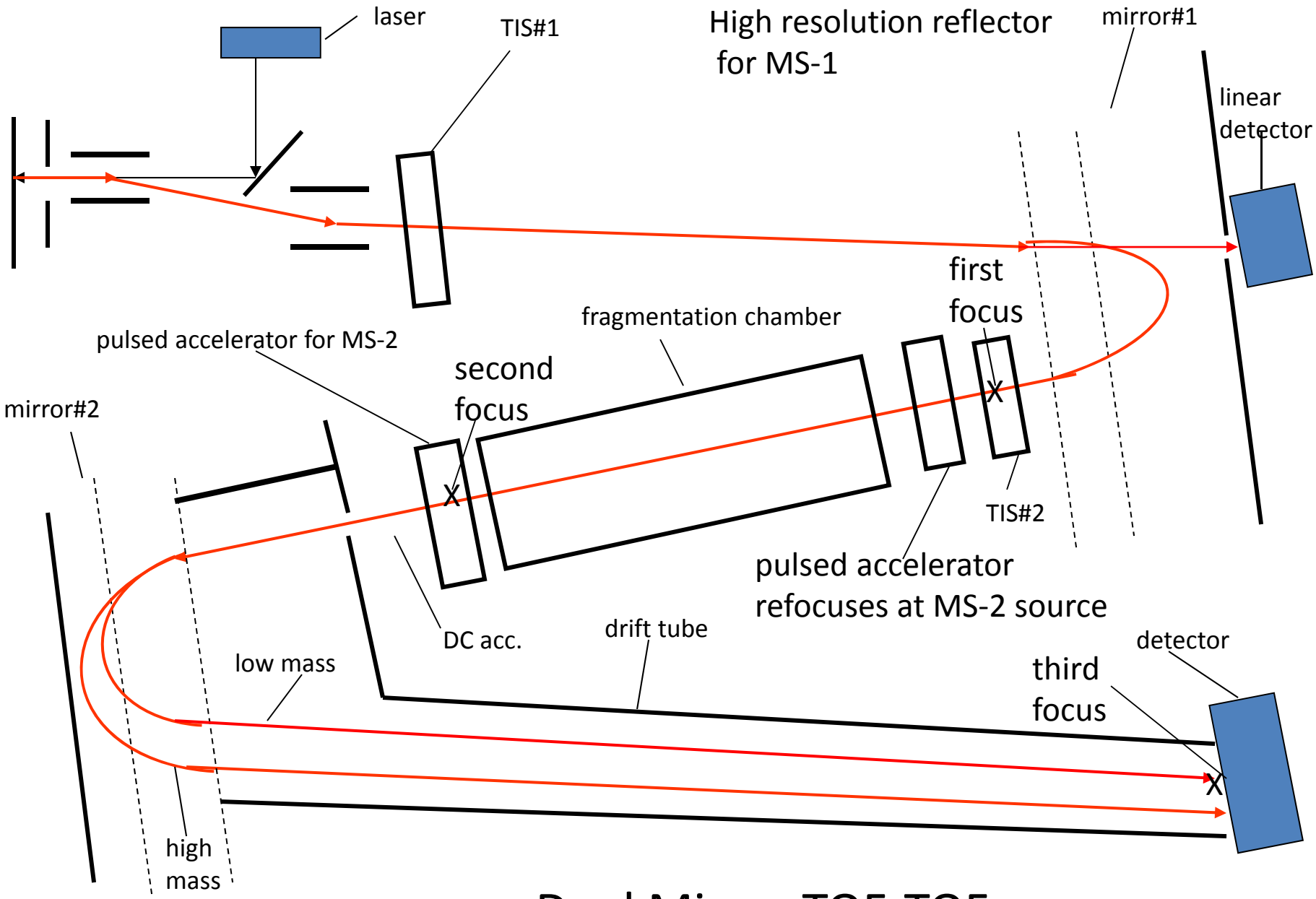
- Higher quality spectra and more spectra/sample *better use of sample*
  - S/N, dynamic range, mass accuracy
  - Improved sensitivity for low abundance peptides
- Makes applications of other features practical
  - Surface imaging
  - Precursor scanning
  - Interface to LC & Molecular Scanner, etc.
- Higher throughput
  - *more samples and more fractions*

Current Status of MALDI-TOF

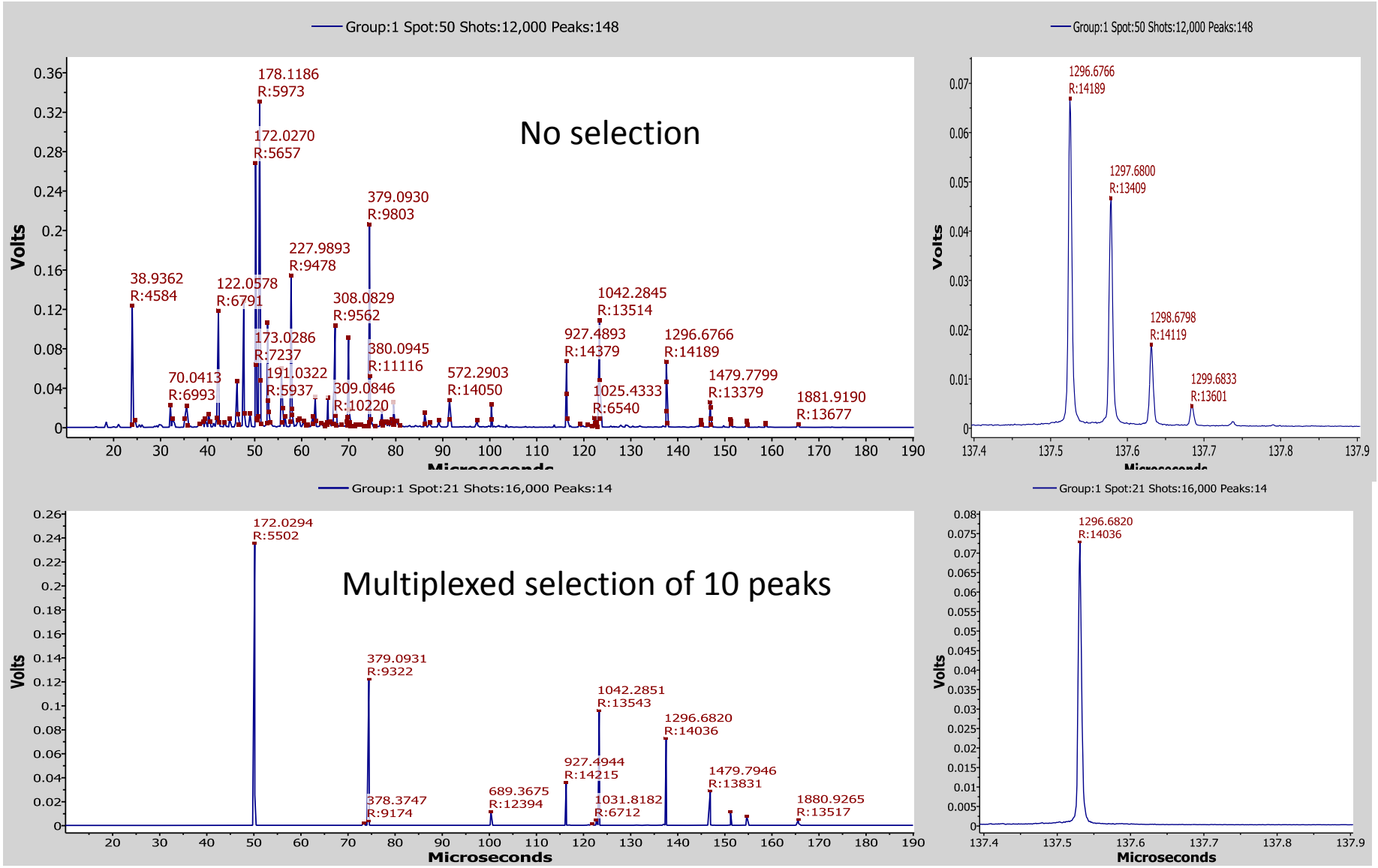
- TOF mass spectrometry has developed rapidly in response to the invention of MALDI.
- Resolving power, mass accuracy, and overall ionization, transmission, and detection efficiency are all excellent in the most recent commercial versions of MALDI-TOF and TOF-TOF instruments.
- However, current instrumentation falls well short of the requirements for efficient use in many potential applications.
  - They are too slow,
  - resolving power for precursor selection in MS-MS is inadequate
  - sample utilization is poor
  - fragmentation efficiency is low
  - spatial resolution inadequate for some imaging applications
  - automated data interpretation is unreliable
  - instruments are too complex and expensive for many laboratories.
- **Our research addresses these deficiencies**

Focus of Our R&D Effort

- Develop Theory for Predicting and Optimizing the Performance of TOF Analyzers
- Apply Theory to Rational Design of Instruments for Specific Applications
  - Linear MALDI-TOF (for intact proteins)
  - High Performance Reflector MALDI-TOF (peptides and small molecules)
  - Combo Linear/Reflector with wide dynamic range
  - Multi-stage MS for isotope ratios at part-per-trillion (^{14}C, ^{41}Ca)
  - MALDI-TOF-TOF with high resolution precursor selection and multiplexed MS-MS
- High Capacity Separations Interfaced with MALDI
  - Sample Spotter
  - Separations interfaces and 3-D sample plates
  - Fully automated LC Interface (multiple sample plates)
- Software for automated instrument control and specific applications



Dual Mirror TOF-TOF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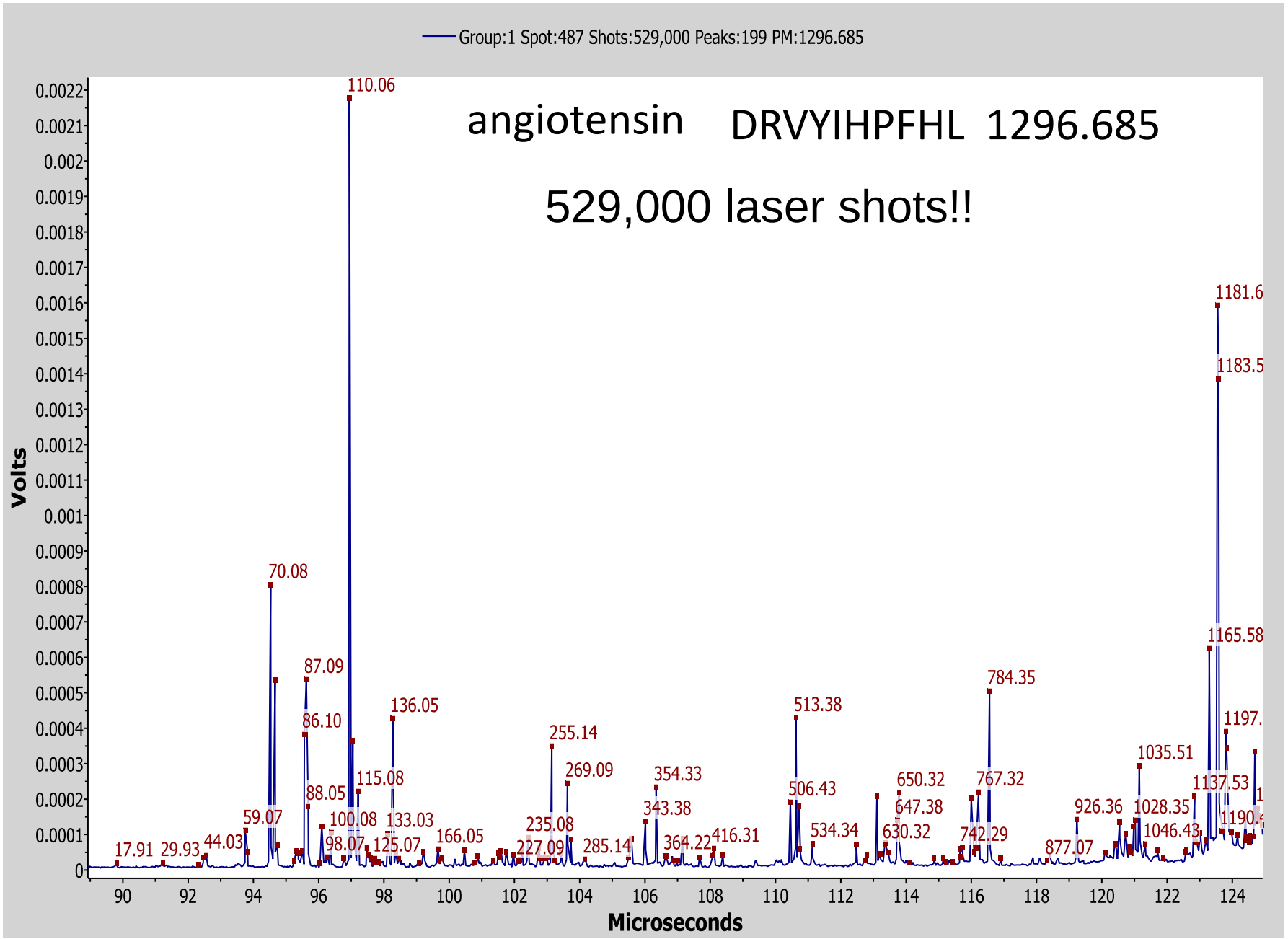
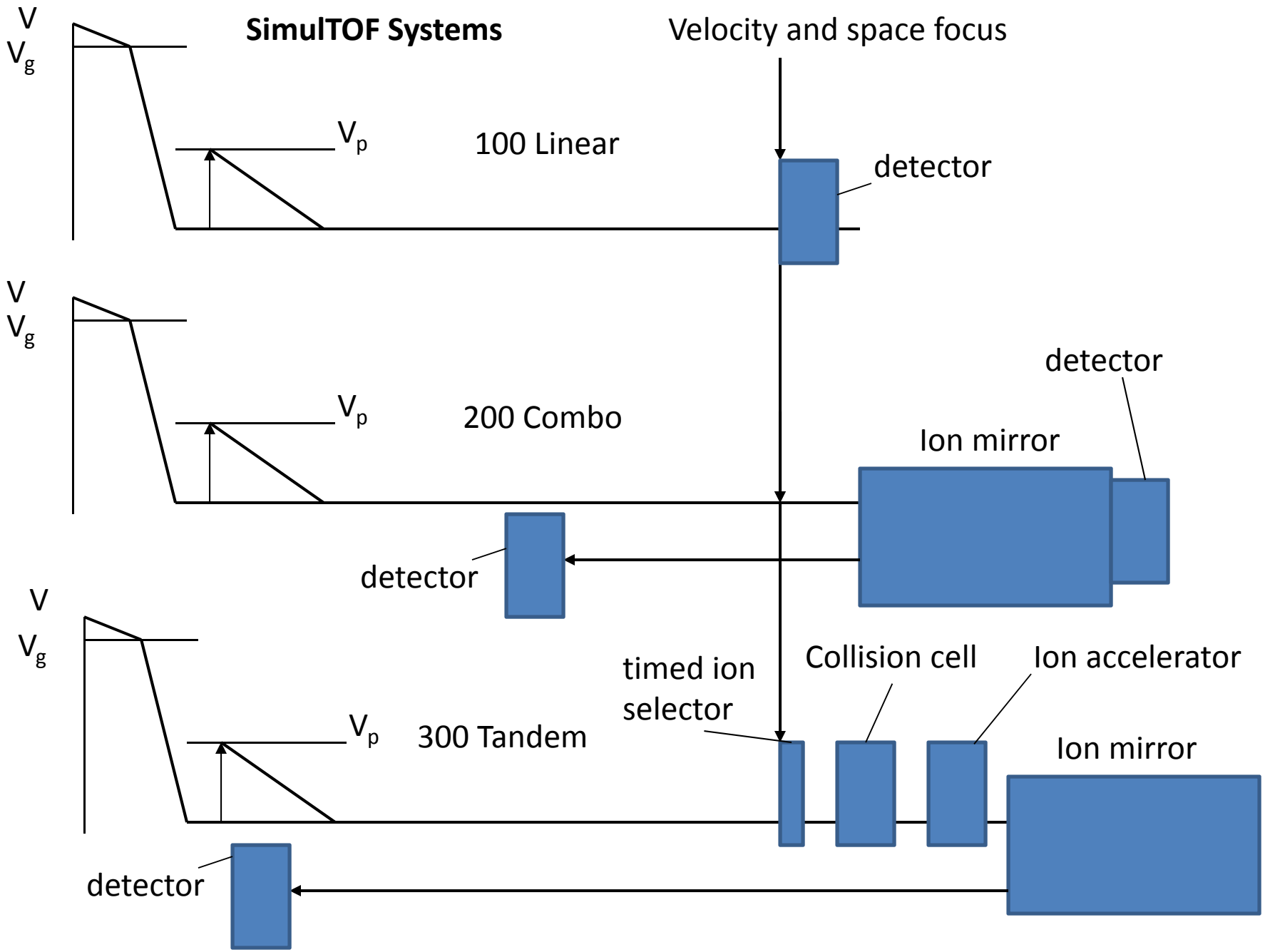
No selection
178.1186 R:5973
172.0270 R:5657
379.0930 R:9803
227.9893 R:9478
38.9362 R:4584
122.0578 R:6791
308.0829 R:9562
1042.2845 R:13514
173.0286 R:7237
927.4893 R:14379
1296.6766 R:14189
380.0945 R:11116
191.0322 R:5937
70.0413 R:6993
309.0846 R:10220
572.2903 R:14050
1025.4333 R:6540
1479.7799 R:13379
1881.9190 R:13677
Volts
Microseconds
Group:1 Spot:50 Shots:12,000 Peaks:148
1296.6766 R:14189
1297.6800 R:13409
1298.6798 R:14119
1299.6833 R:13601
Volts
Group:1 Spot:21 Shots:16,000 Peaks:14
Multiplexed selection of 10 peaks
172.0294 R:5502
379.0931 R:9322
1042.2851 R:13543
1296.6820 R:14036
927.4944 R:14215
1479.7946 R:13831
689.3675 R:12394
378.3747 R:9174
1031.8182 R:6712
1880.9265 R:13517
Volts
Microseconds
Group:1 Spot:21 Shots:16,000 Peaks:14
1296.6820 R:14036
Volts
Microseconds


Group:1 Spot:487 Shots:529,000 Peaks:199 PM:1296.685
angiotensin DRVYIHPFHL 1296.685
529,000 laser shots!!
Volts
Microseconds
110.06
70.08
87.09
86.10
136.05
115.08
88.05
59.07
100.08
133.03
98.07
125.07
166.05
17.91
29.93
44.03
255.14
269.09
235.08
227.09
285.14
354.33
343.38
364.22
416.31
513.38
506.43
534.34
650.32
647.38
630.32
784.35
767.32
742.29
877.07
926.36
1035.51
1028.35
1046.43
1137.53
1165.58
1181.6
1183.5
1197.

Primary focus shifted to Sensitivity, Dynamic Range, Throughput, and Reproducibility

- Ion production
  - MALDI 5 kHz operation
- Time focusing to reduce effect of initial position and initial velocity
  - Pulsed extraction
  - Simultaneous space and velocity focusing (SimulTOF)
- Ion optics for efficient ion transmission of ions of interest and mass gate and filtering to remove ion noise
- Detector for efficient detection of broad range of masses with fast response
  - Destroy the myth that high mass singly charged ions cannot be detected by conventional detectors

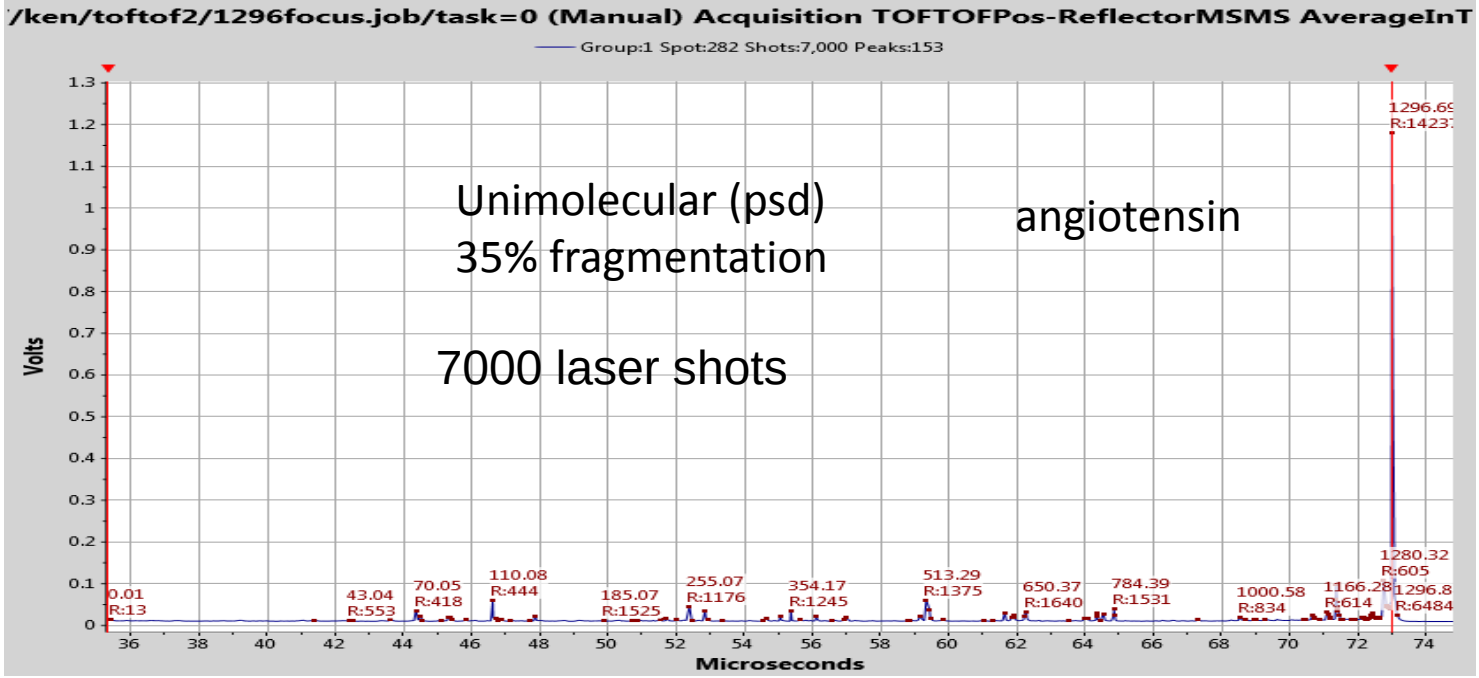
Note: All data presented are "raw" directly from the digitizer.
No data processing, smoothing, or baseline correction has been employed.

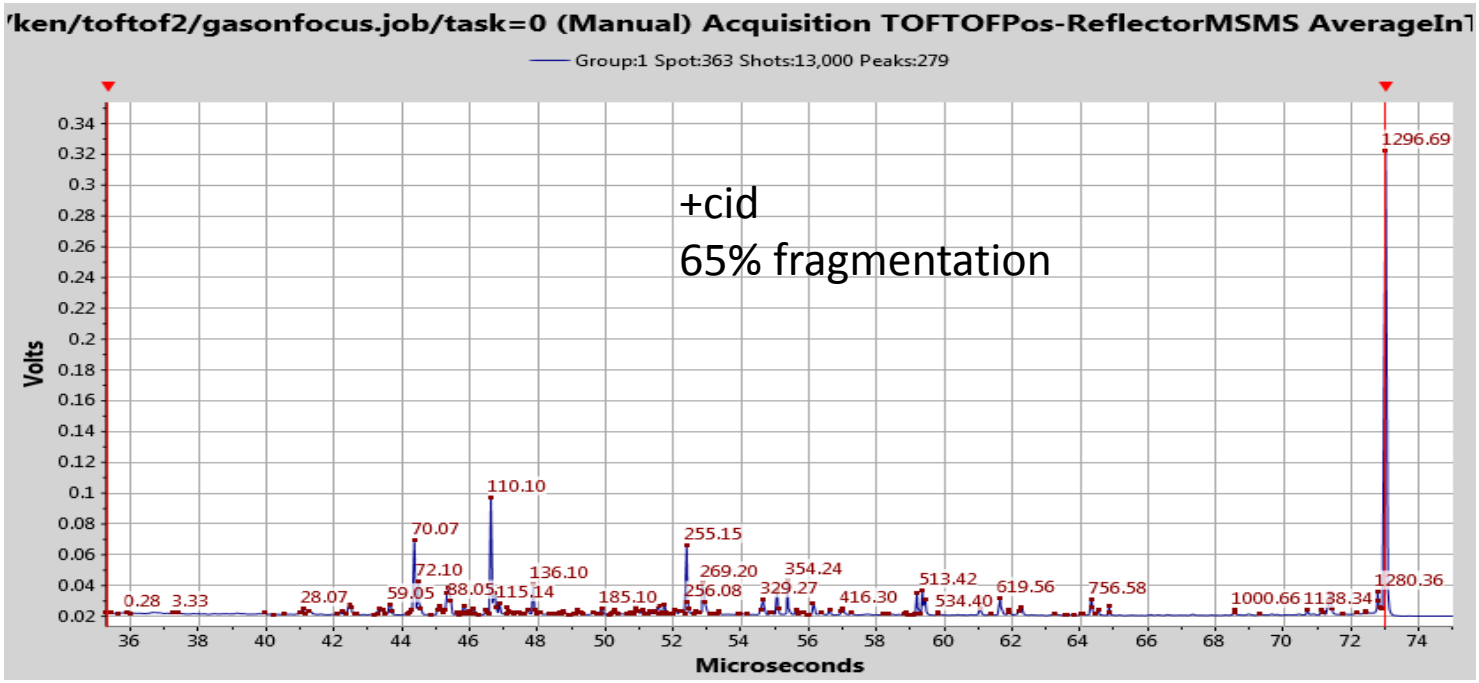

V
V_g
SimulTOF Systems
Velocity and space focus
V_p
100 Linear
detector
V
V_g
V_p
200 Combo
detector
Ion mirror
detector
V
V_g
timed ion
selector
Collision cell
Ion accelerator
V_p
300 Tandem
Ion mirror
detector

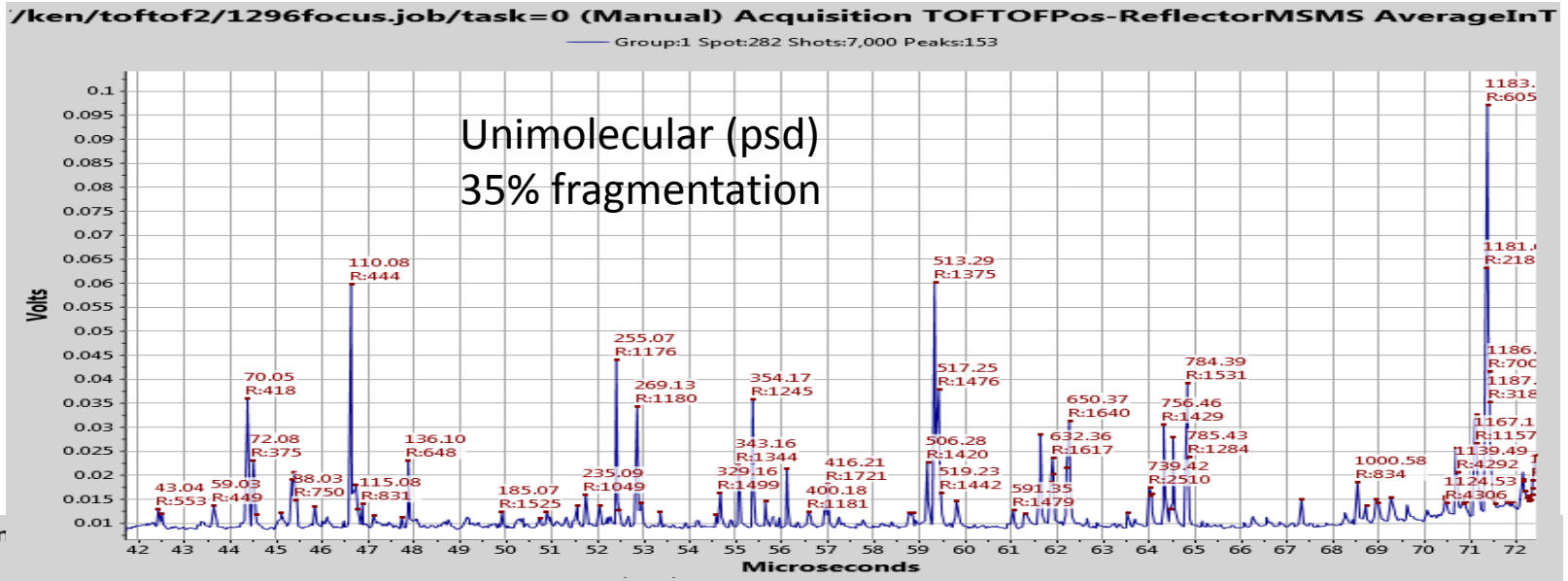
SimulTOF Systems

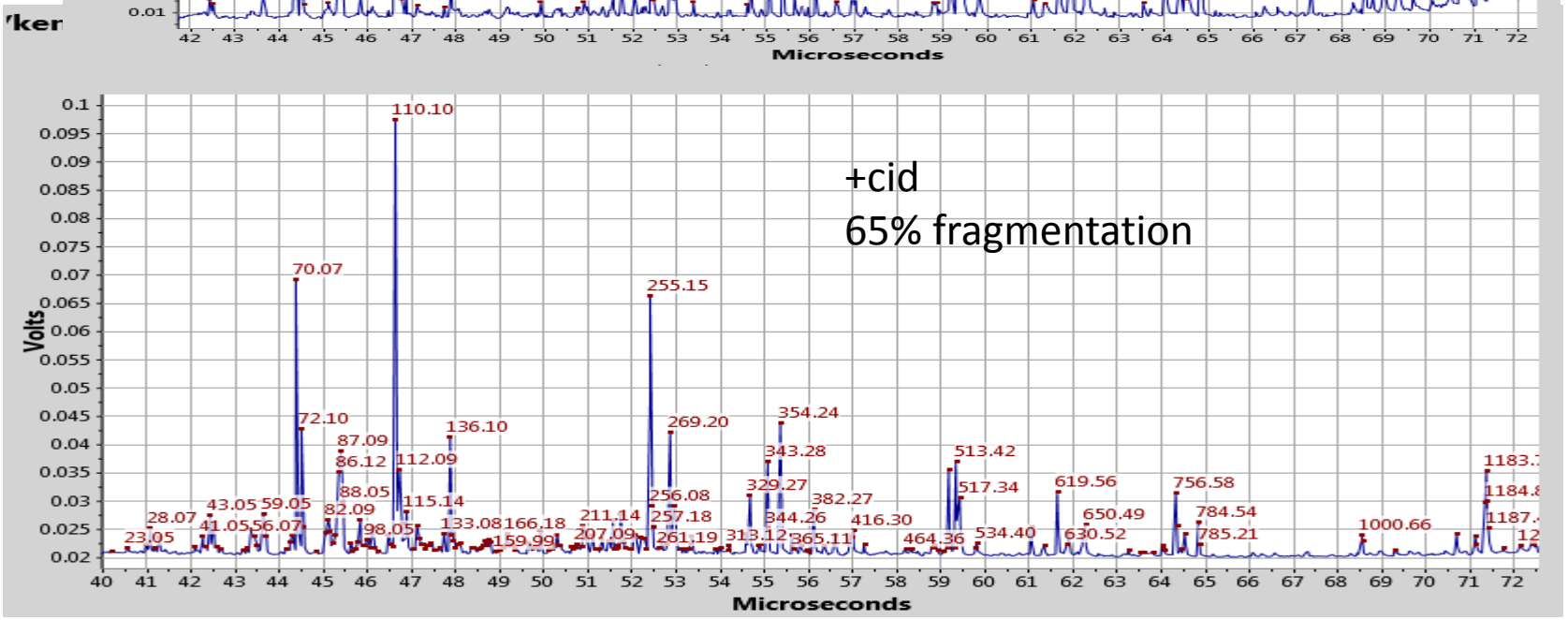
Guiding Innovations in Science for Life

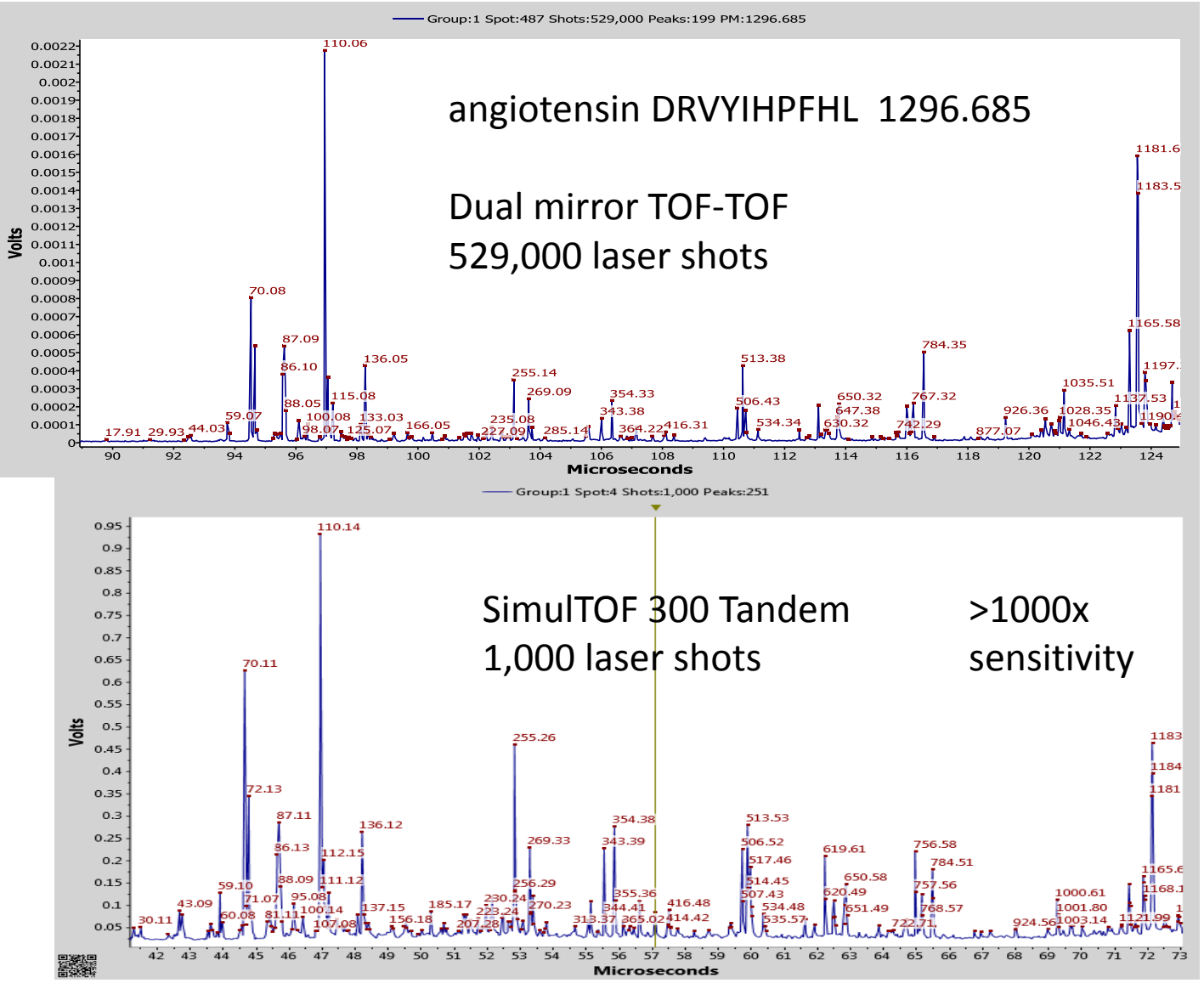
Commercial products introduced at ASMS 2012

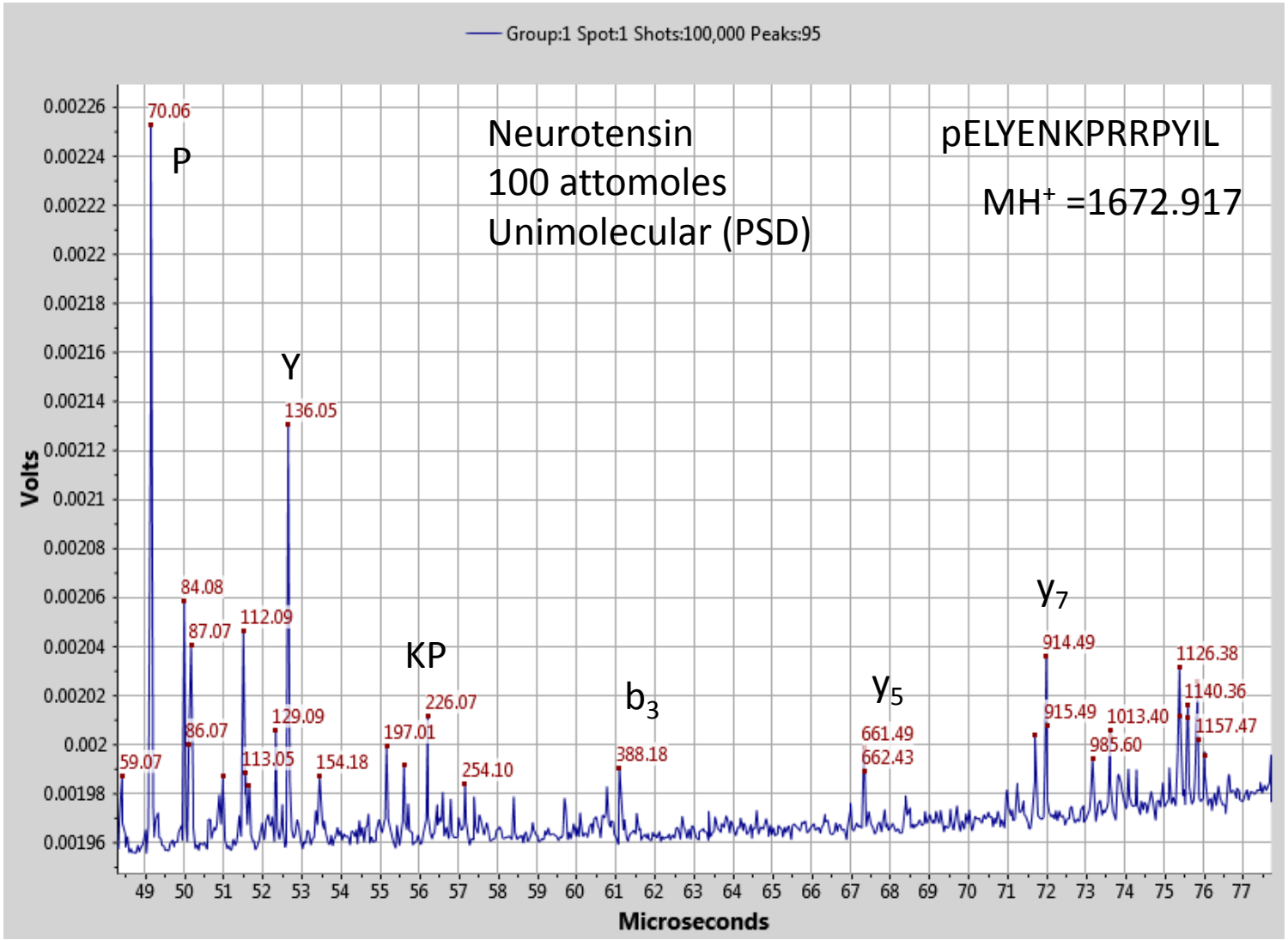

/ken/toftof2/1296focus.job/task=0 (Manual) Acquisition TOFTOFPos-ReflectorMSMS AverageInT
Group:1 Spot:282 Shots:7,000 Peaks:153
Unimolecular (psd)
35% fragmentation
7000 laser shots
angiotensin
Volts
Microseconds
0.01 R:13
43.04 R:553
70.05 R:418
110.08 R:444
185.07 R:1525
255.07 R:1176
354.17 R:1245
513.29 R:1375
650.37 R:1640
784.39 R:1531
1000.58 R:834
1166.28 R:614
1280.32 R:605
1296.8 R:6484


/ken/toftof2/gasonfocus.job/task=0 (Manual) Acquisition TOFTOFPos-ReflectorMSMS AverageInT
Group:1 Spot:363 Shots:13,000 Peaks:279
+cid
65% fragmentation
Volts
Microseconds
0.28
3.33
28.07
59.05
70.07
72.10
88.05
110.10
115.14
136.10
185.10
255.15
256.08
269.20
329.27
354.24
416.30
513.42
534.40
619.56
756.58
1000.66
1138.34
1280.36
1296.69

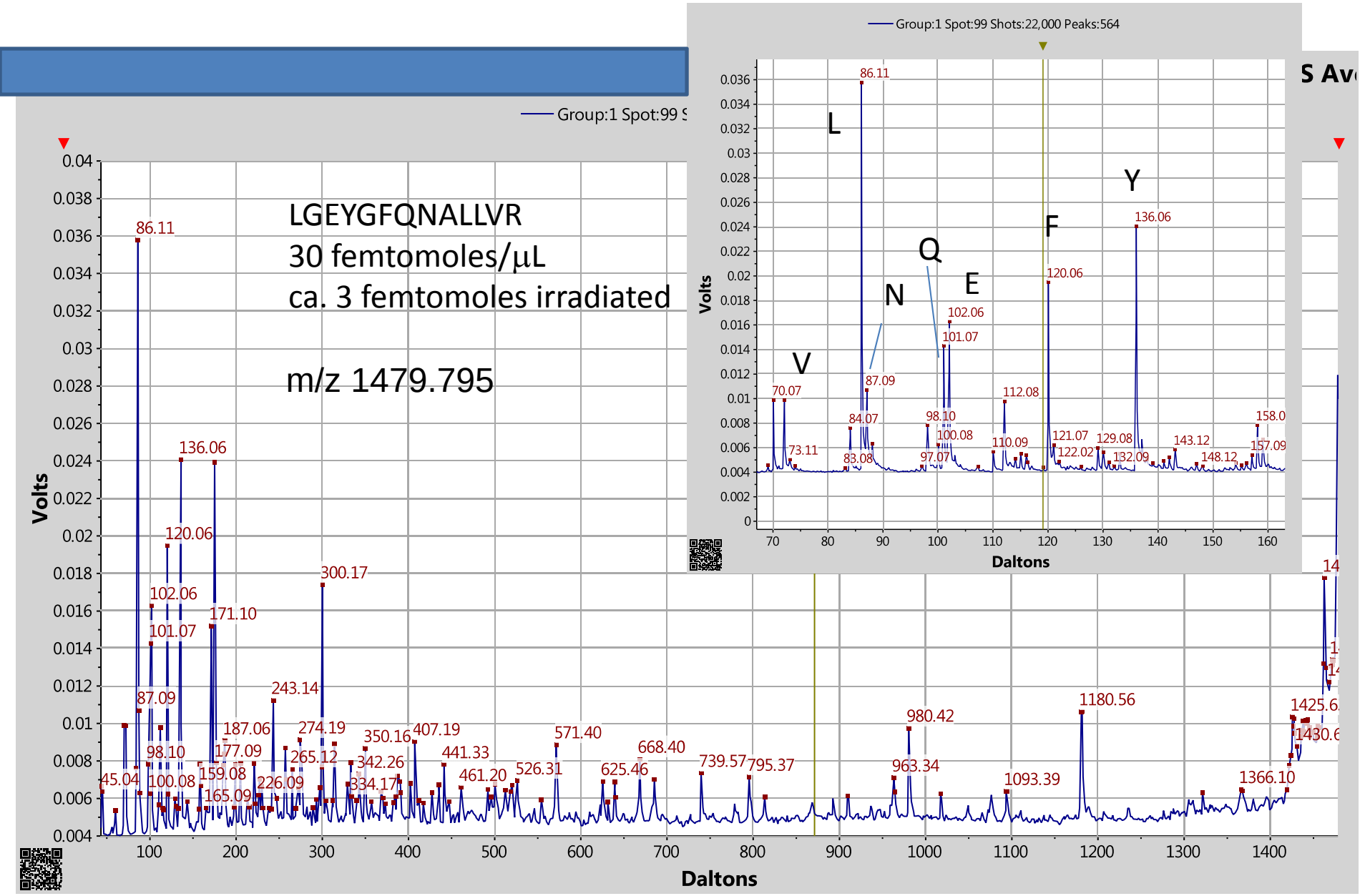

/ken/toftof2/1296focus.job/task=0 (Manual) Acquisition TOFTOFPos-ReflectorMSMS AverageInT
Group:1 Spot:282 Shots:7,000 Peaks:153
Unimolecular (psd)
35% fragmentation
Volts
Microseconds

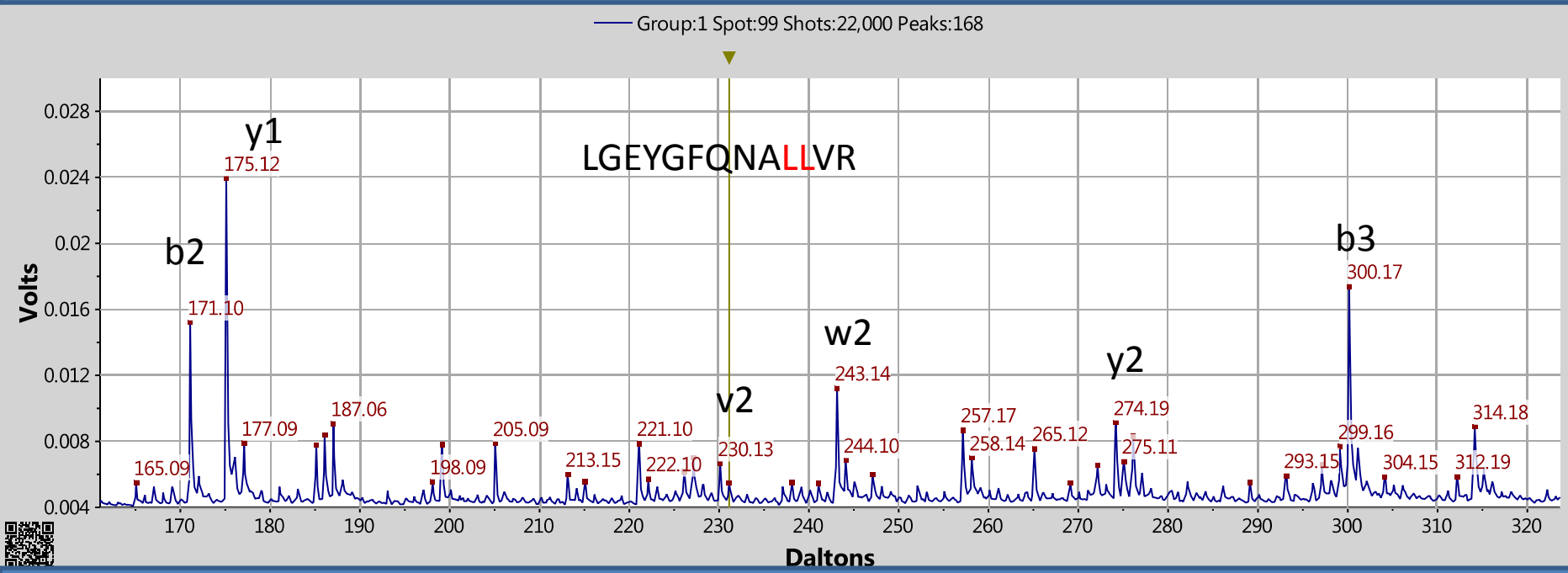

+cid
65% fragmentation
Volts
Microseconds

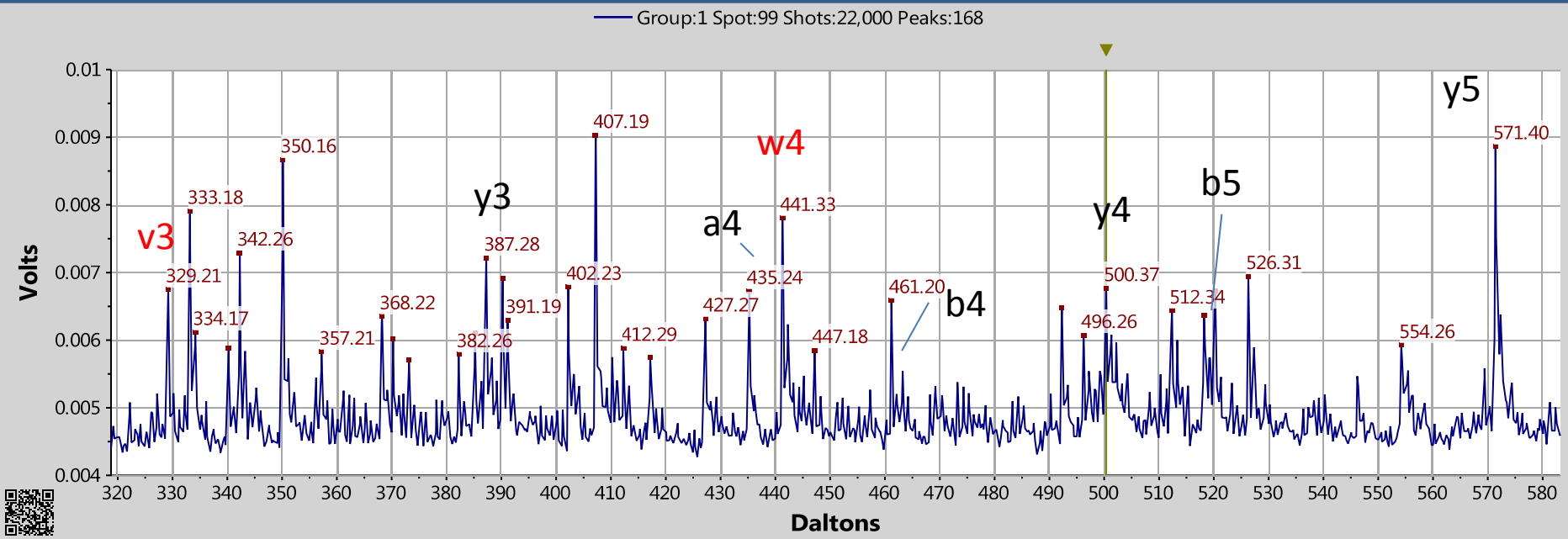

Group:1 Spot:487 Shots:529,000 Peaks:199 PM:1296.685
angiotensin DRVYIHPFHL 1296.685
Dual mirror TOF-TOF
529,000 laser shots
Volts
Microseconds
Group:1 Spot:4 Shots:1,000 Peaks:251
SimulTOF 300 Tandem
1,000 laser shots
>1000x
sensitivity
Volts
Microseconds

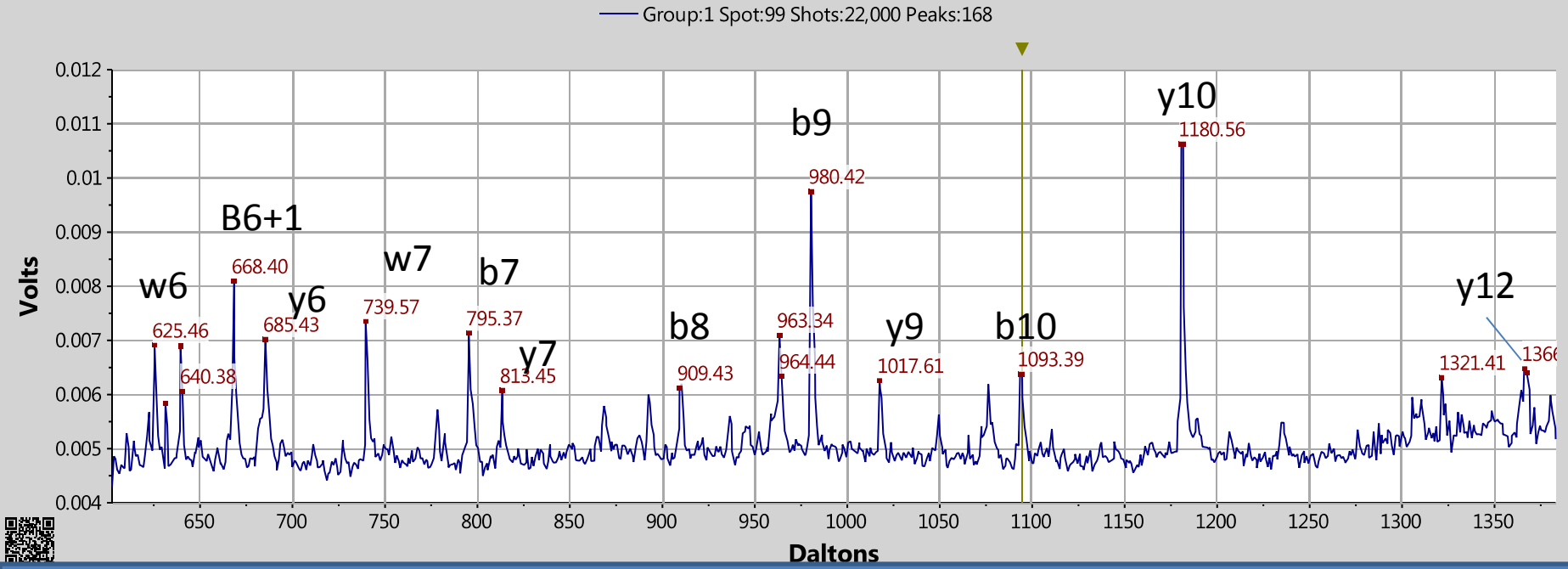


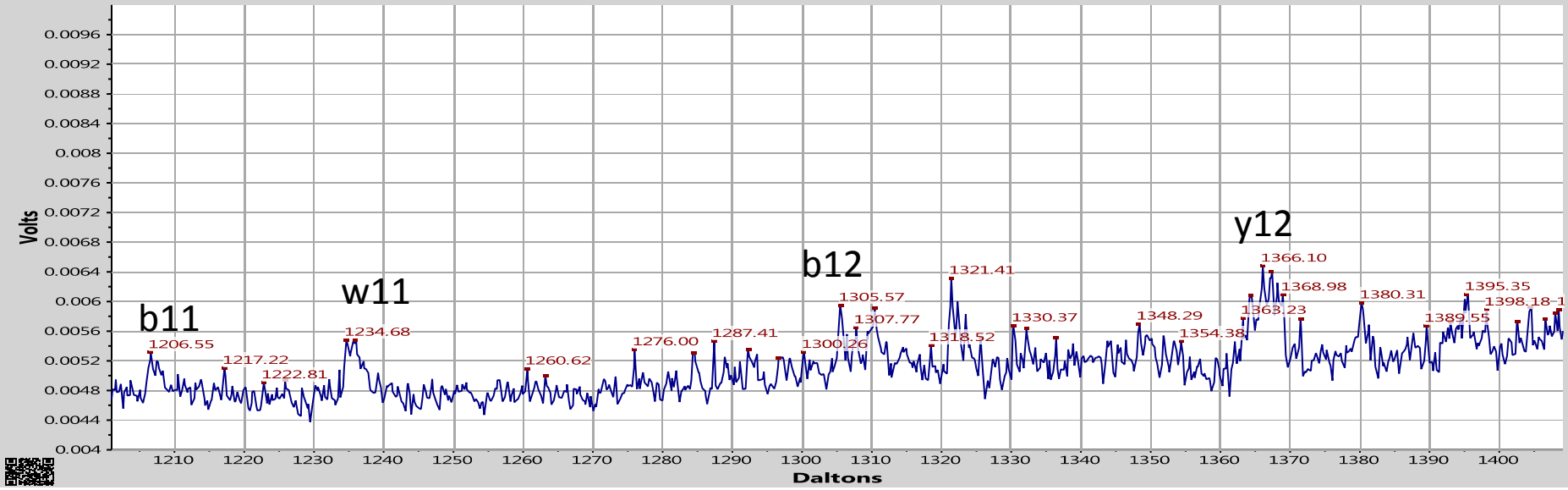
MS-MS at 100 attomoles/μL on 2.5 mm spot. 100,000 shots, 20 s acquisition at 5 kHz
19 of 30 most intense peaks matched neurotensin using Protein Prospector

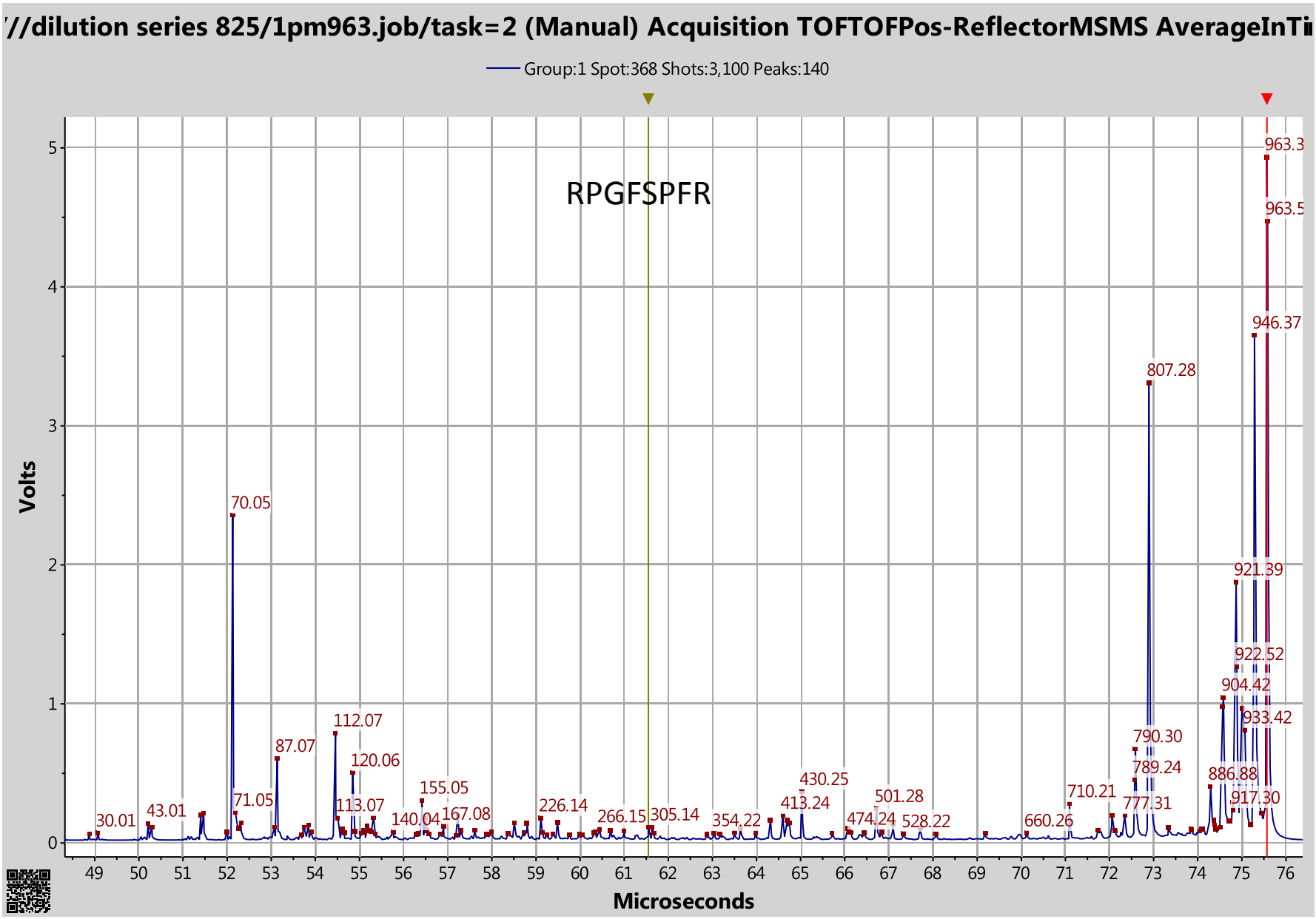

LGEYGFQNALLVR
30 femtomoles/µL
ca. 3 femtomoles irradiated
m/z 1479.795
Group:1 Spot:99 Shots:22,000 Peaks:564
Volts
Daltons
L
V
N
Q
E
F
Y
86.11
136.06
120.06
102.06
101.07
87.09
171.10
300.17
243.14
274.19
187.06
177.09
265.12
350.16
407.19
342.26
441.33
461.20
526.31
571.40
625.46
668.40
739.57
795.37
963.34
980.42
1093.39
1180.56
1366.10
1425.6
1430.6
45.04
98.10
100.08
159.08
165.09
226.09
334.17
70.07
73.11
84.07
83.08
97.07
98.10
100.08
110.09
112.08
121.07
122.02
129.08
132.09
143.12
148.12
157.09
158.0


Group:1 Spot:99 Shots:22,000 Peaks:168
LGEYGFQNALLVR
y1
175.12
b2
171.10
177.09
187.06
165.09
198.09
205.09
213.15
221.10
222.10
v2
230.13
w2
243.14
244.10
257.17
258.14
265.12
y2
274.19
275.11
b3
300.17
299.16
293.15
304.15
312.19
314.18
Volts
Daltons


Group:1 Spot:99 Shots:22,000 Peaks:168
v3
329.21
333.18
334.17
342.26
350.16
357.21
368.22
y3
382.26
387.28
391.19
402.23
407.19
412.29
a4
427.27
w4
435.24
441.33
447.18
461.20
b4
y4
496.26
500.37
512.34
b5
526.31
554.26
y5
571.40
Volts
Daltons


Group:1 Spot:99 Shots:22,000 Peaks:168
Volts
Daltons
w6
625.46
640.38
B6+1
668.40
y6
685.43
w7
739.57
b7
795.37
y7
813.45
b8
909.43
b9
980.42
963.34
964.44
y9
1017.61
b10
1093.39
y10
1180.56
y12
1321.41


Volts
Daltons
b11
1206.55
1217.22
1222.81
w11
1234.68
1260.62
1276.00
1287.41
b12
1300.26
1305.57
1307.77
1318.52
1321.41
1330.37
1348.29
1354.38
y12
1363.23
1366.10
1368.98
1380.31
1389.55
1395.35
1398.18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Group:1 Spot:368 Shots:3,100 Peaks:140
RPGFSPFR
Volts
Microseconds
963.3
963.5
946.37
807.28
921.39
922.52
904.42
933.42
790.30
789.24
886.88
917.30
777.31
710.21
660.26
501.28
430.25
413.24
474.24
528.22
354.22
305.14
266.15
226.14
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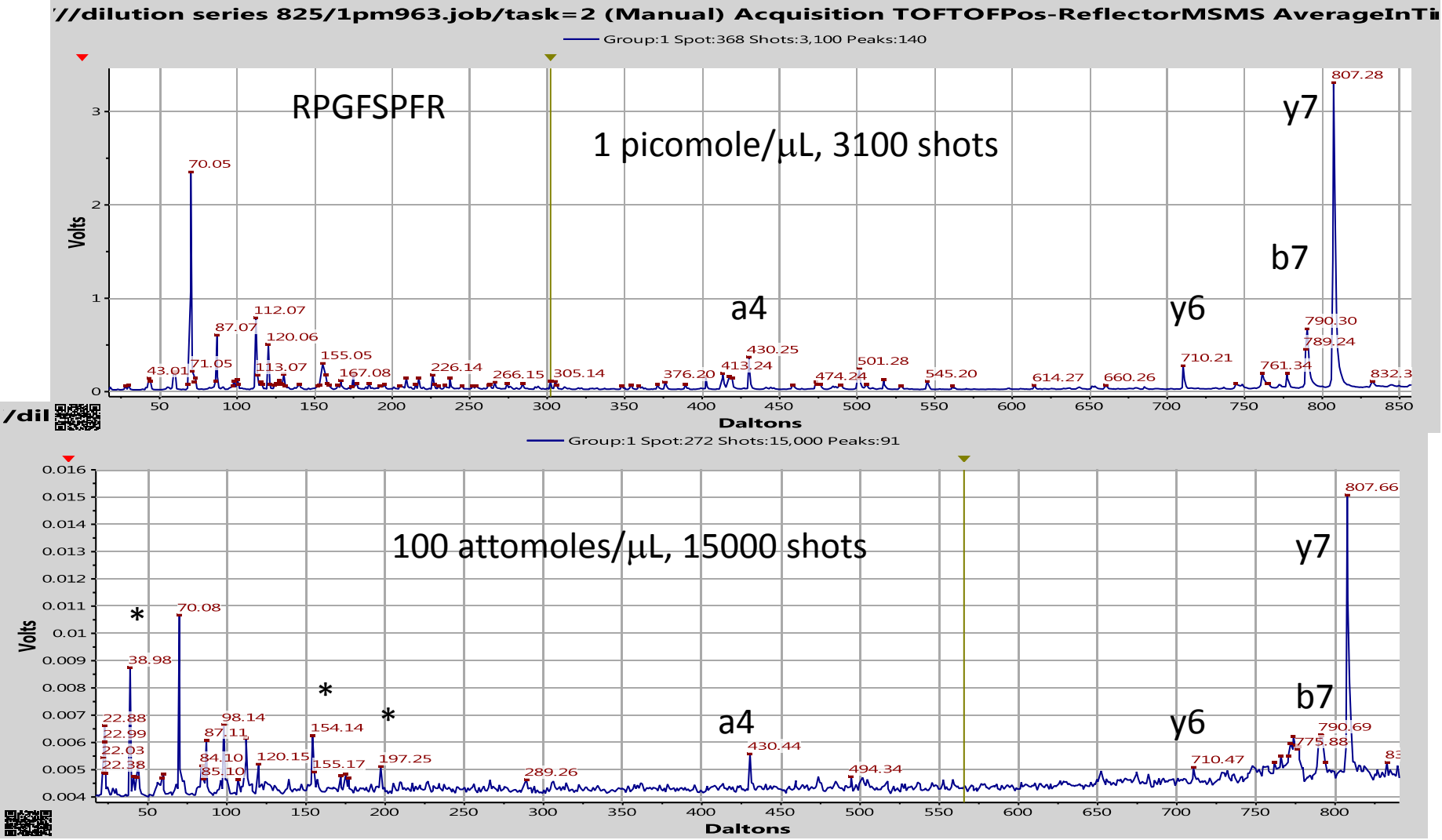
70.05
43.01
30.01



Minimum useful concentration limited by chemical noise rather than ion production rate. Improved by higher resolution precursor selection And cleaner matrices.

Present status

- Overall efficiency ca. 1000 times better with SimilTOF linear first stage rather than reflector
- Precursor resolving power in present version

  500 FWHM with sharp cutoff
- Database searchable fragment spectra generally produced from <100 attomoles of sample on surface
- Modification to provide>1500 resolving power for precursor selection under construction
- 10x Multiplexing included in hardware, further SW improvements required for full implementation

Acknowledgements

- Financial Support
  - NIGMS and NCRR of NIH
  - Christina Hsieh Vestal and the Hsieh Family
- The entire staff at Virgin Instruments
  - Kevin Hayden my alter ego
  - Steve Gabeler, Mark Dahl, Joe Valentine electronics
  - George Mills, Matt Gabeler-Lee software
  - Roger Voyer machinist
  - Steve Hattan, Ken Parker scientists
  - Christina Vestal, Bill Gibbs management
- Peter Roepstorff our friend and colleague for more than 30 years