

**Rapid Quantitative Analysis of Small
Molecules Using Nano-Electrospray –
Differential Mobility Spectrometry –
Mass Spectrometry**

COSMOS

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QUANTITATIVE ANALYSIS OF BIOLOGICAL SAMPLES

Desirable Features

- **High Sensitivity**
- **Low Sample / Reagents Consumption**
- **Short Analysis Time / High Throughput**
- **Little or No Ionization Suppression**
- **Minimal Sample Cleanup**

Typical Approaches

- NanoElectrospray Ionization
- Capillary LC – NanoElectrospray MS
- Short Capillary Columns
- Direct Infusion After Sample Cleanup

Potential Drawbacks

- Longer Analysis Times by Capillary LC
(Answer: Monolithic Columns?)
- Poor Ruggedness of Capillary Columns
- Sensitivity to Contaminants

Our Proposed Answer

**Direct Infusion – Nano-ESI –
DMS - MS**

May By-Pass or Reduce
Cleanup Steps and Eliminate
LC

Where DMS Assumes Role of LC

Capillary LC-MS Analysis of DNA Adducts

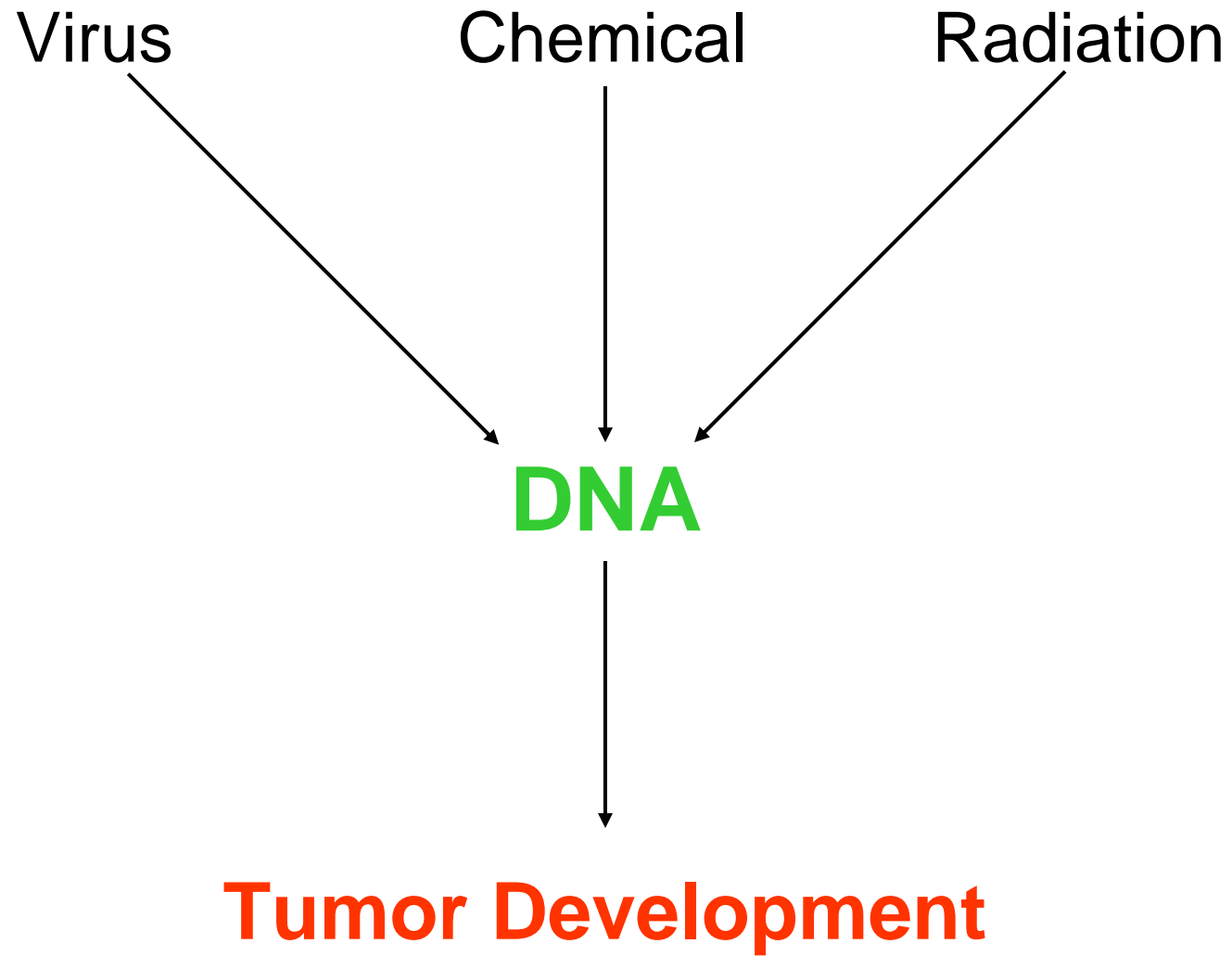
Virus

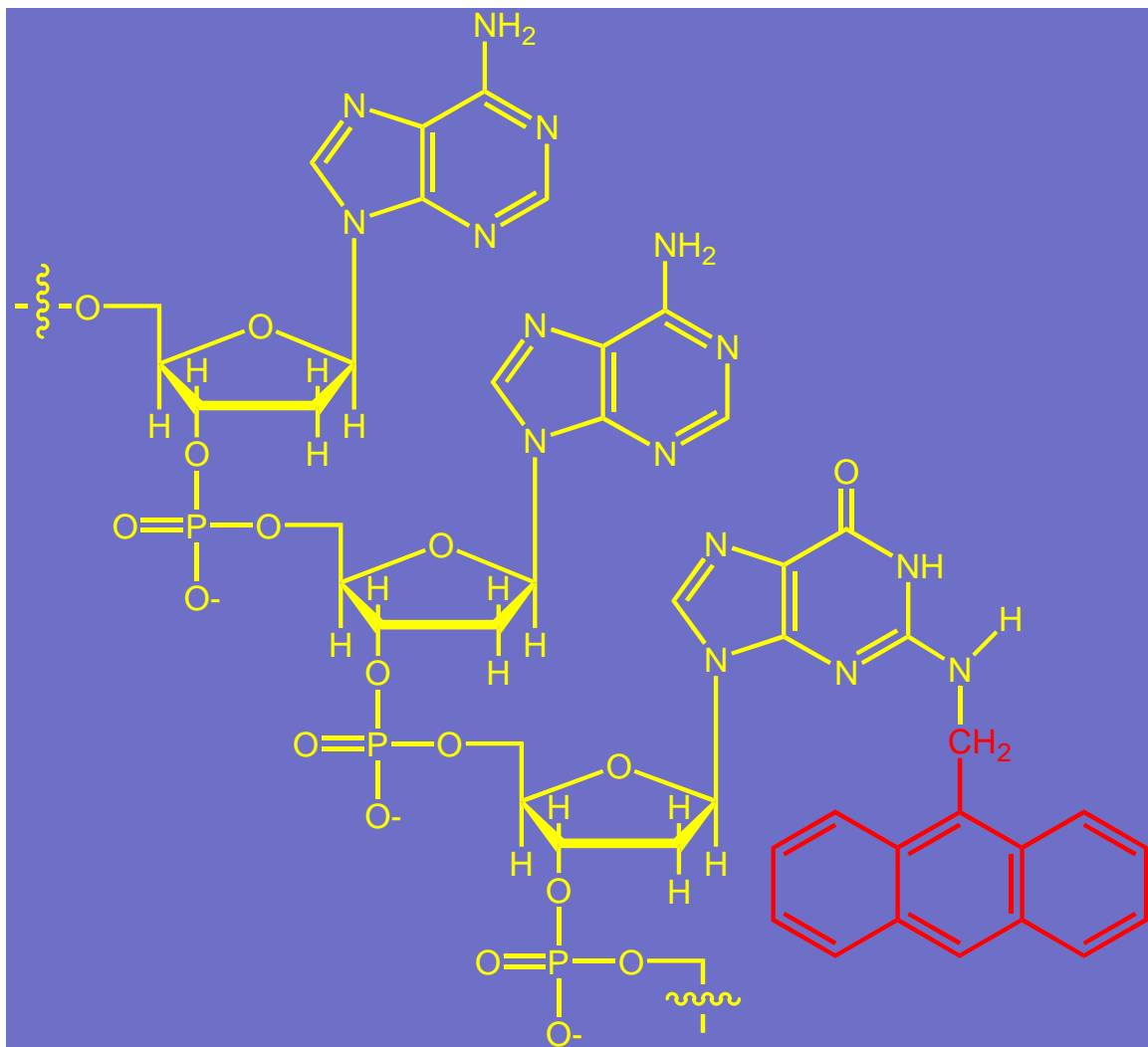
Chemical

Radiation

DNA

Tumor Development



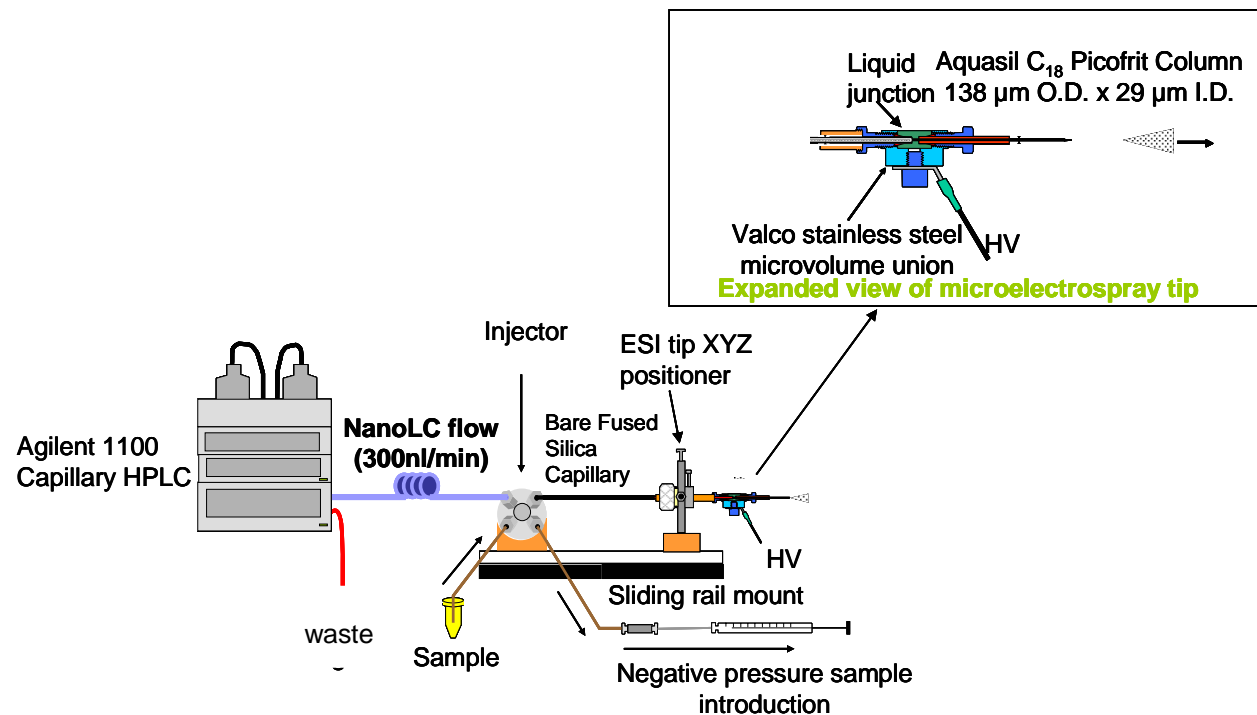


Bohr, V.A., *Carcinogenesis*, **1991**, 12, 1983-1992.

Amount of DNA and Tissue Required to Isolate 1ng of Adduct at Various Levels of Modification

Adduct Level (1 modification per 10^x bases)	Amount of DNA Required	Amount of Tissue Required
10^8	100 μ g	100mg
10^9	1mg	1,0g

Schematic of capillary liquid chromatography system in line with Finnigan TSQ 700 mass spectrometer.

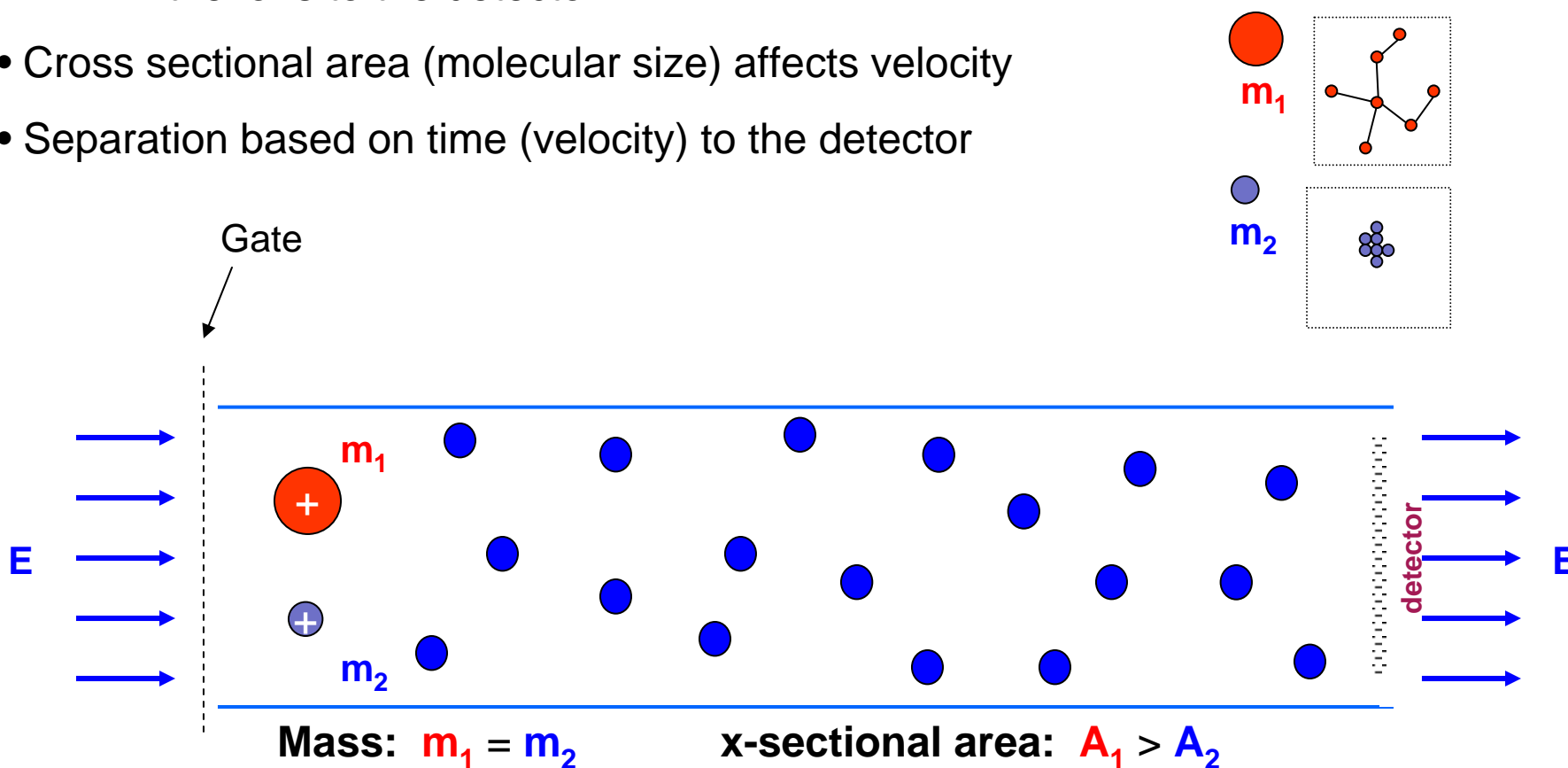


SOME FUNDAMENTAL PRINCIPLES

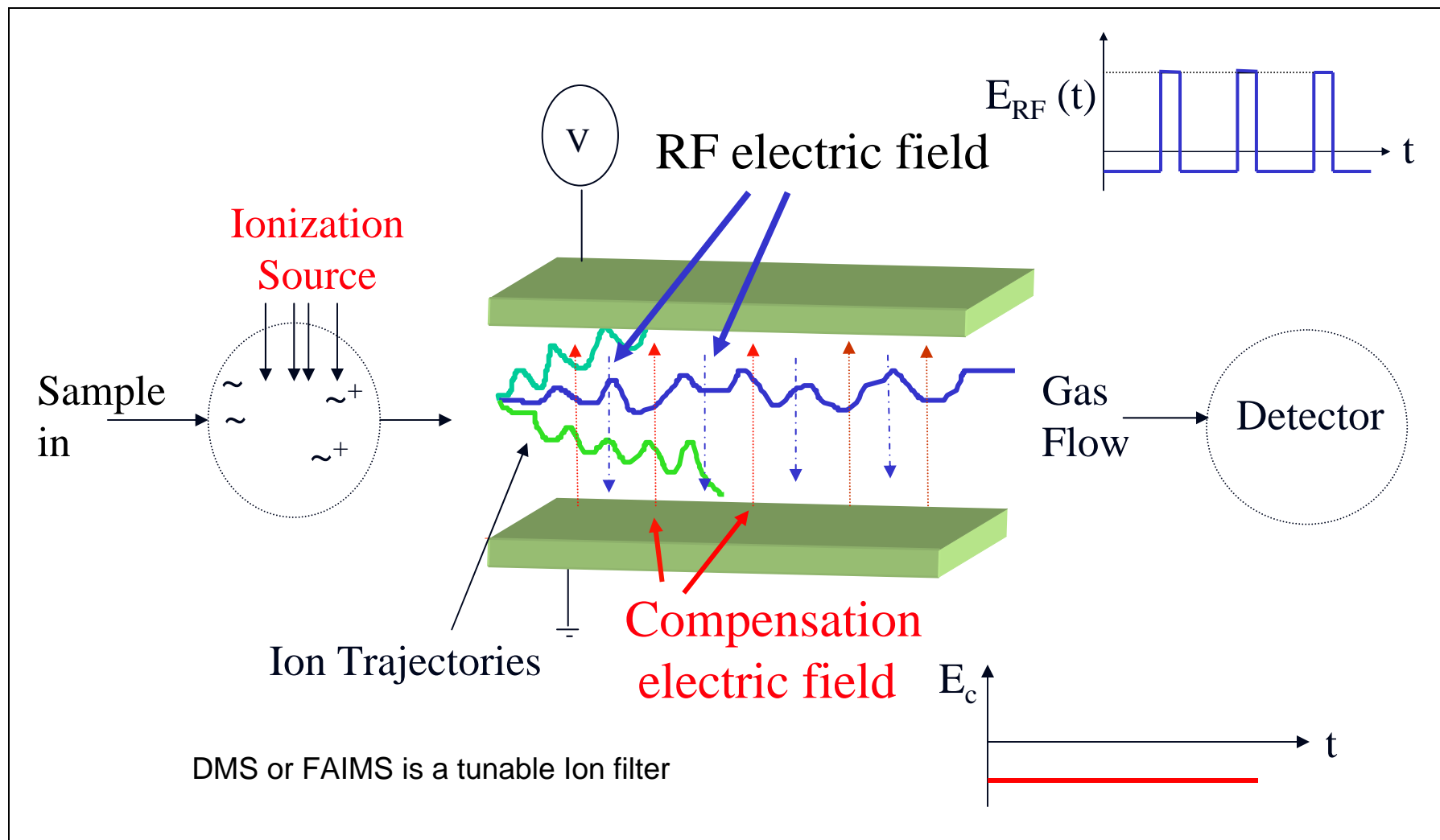
- ION MOBILITY MASS SPECTROMETRY
- DIFFERENTIAL ION MOBILITY MASS SPECTROMETRY

Ion Mobility Spectrometry

- Atmospheric pressure in flight tube, with electric field pulling the ions to the detector
- Cross sectional area (molecular size) affects velocity
- Separation based on time (velocity) to the detector

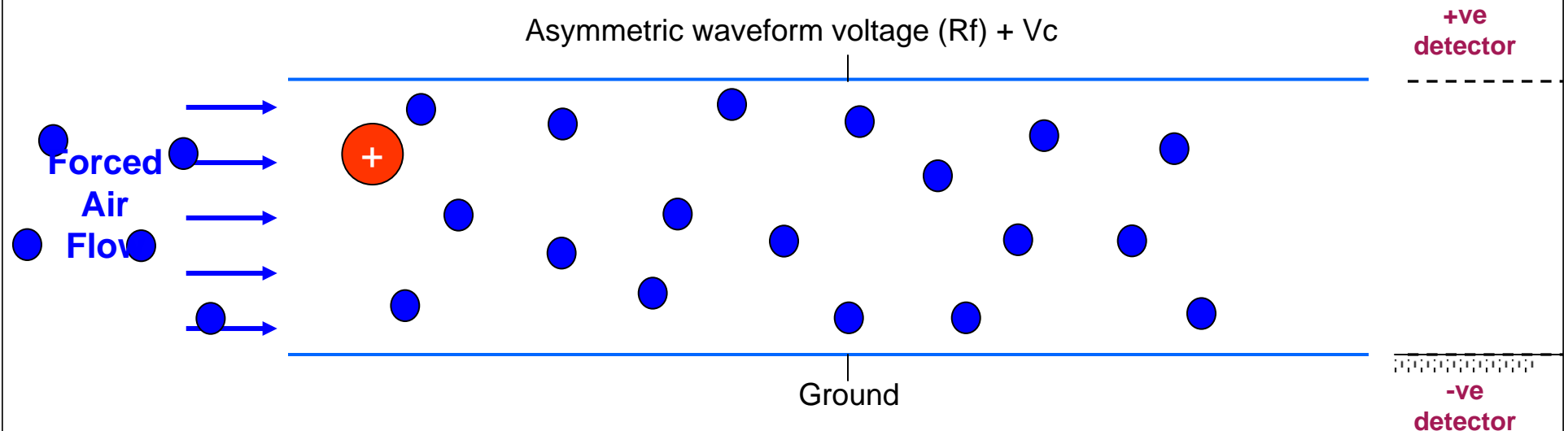


DMS Operation Principle



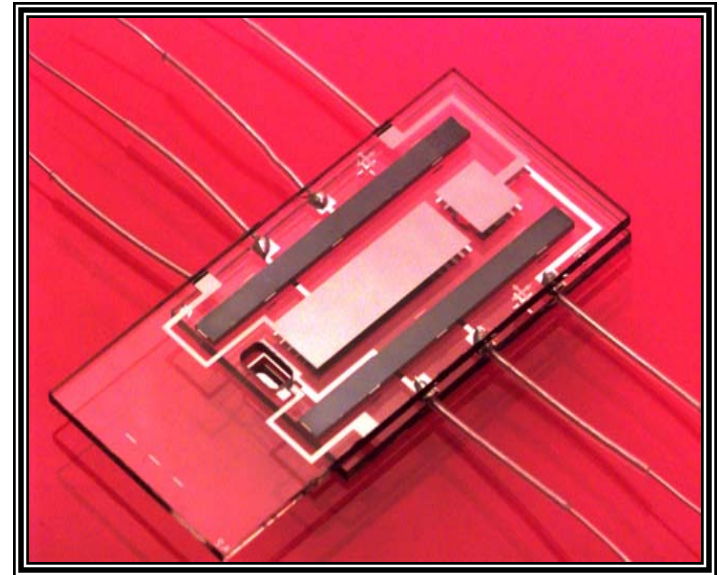
Differential Mobility Spectrometry

- Unlike traditional ion mobility, DMS requires forced air flow to pass ions through the plates to the detector.



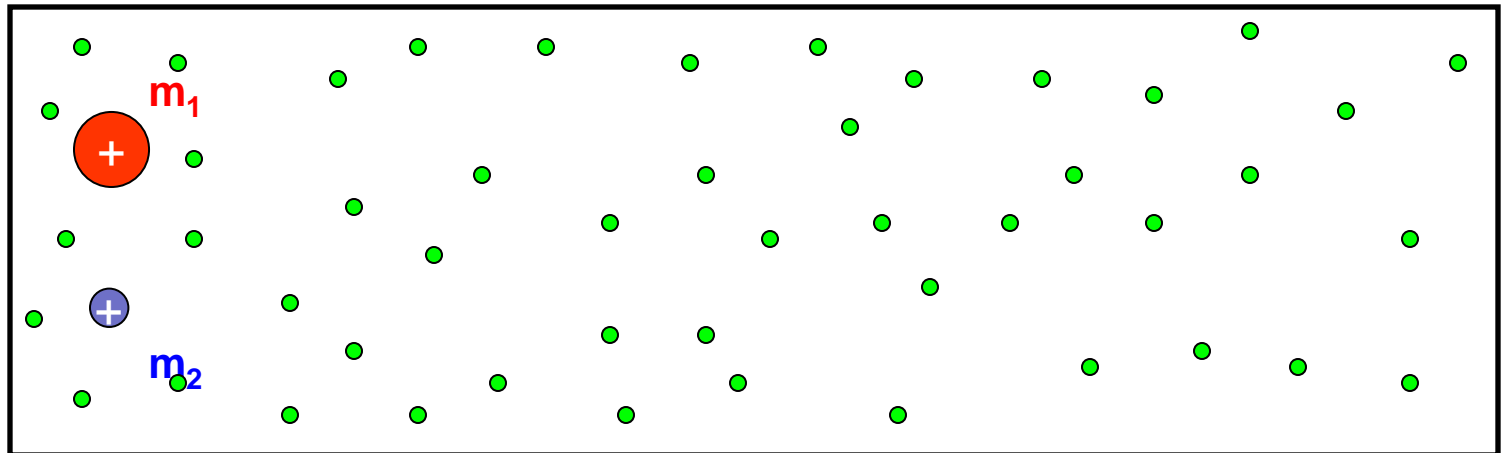
Differential Mobility separation within the Sionex DMS sensor

- Rapid separation/detection (msec)
- Internal + and – ion electrometer detectors
- 25¢ Quarter size



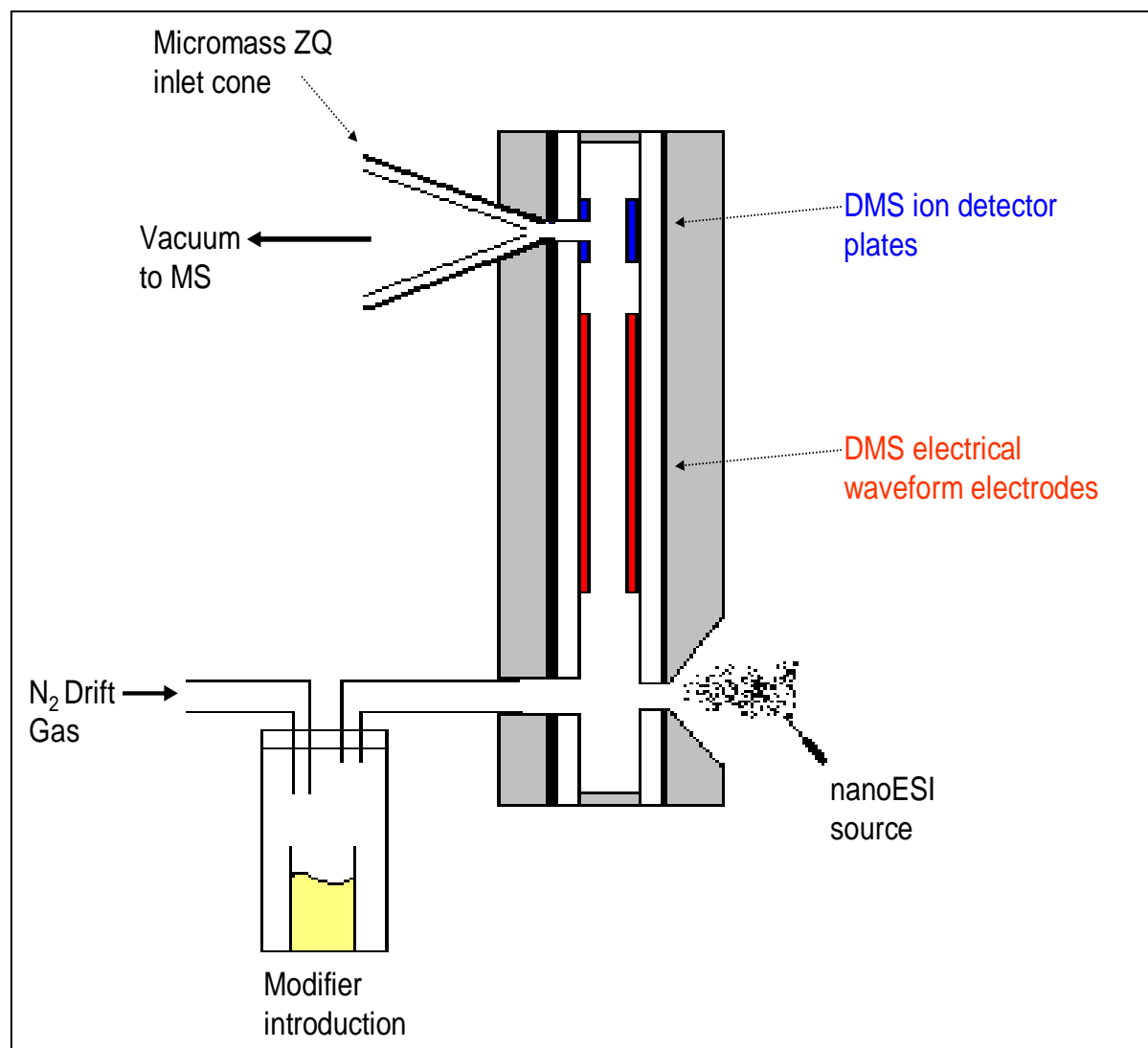
Mass: $m_1 = m_2$

Cross sectional area: $m_1 > m_2$

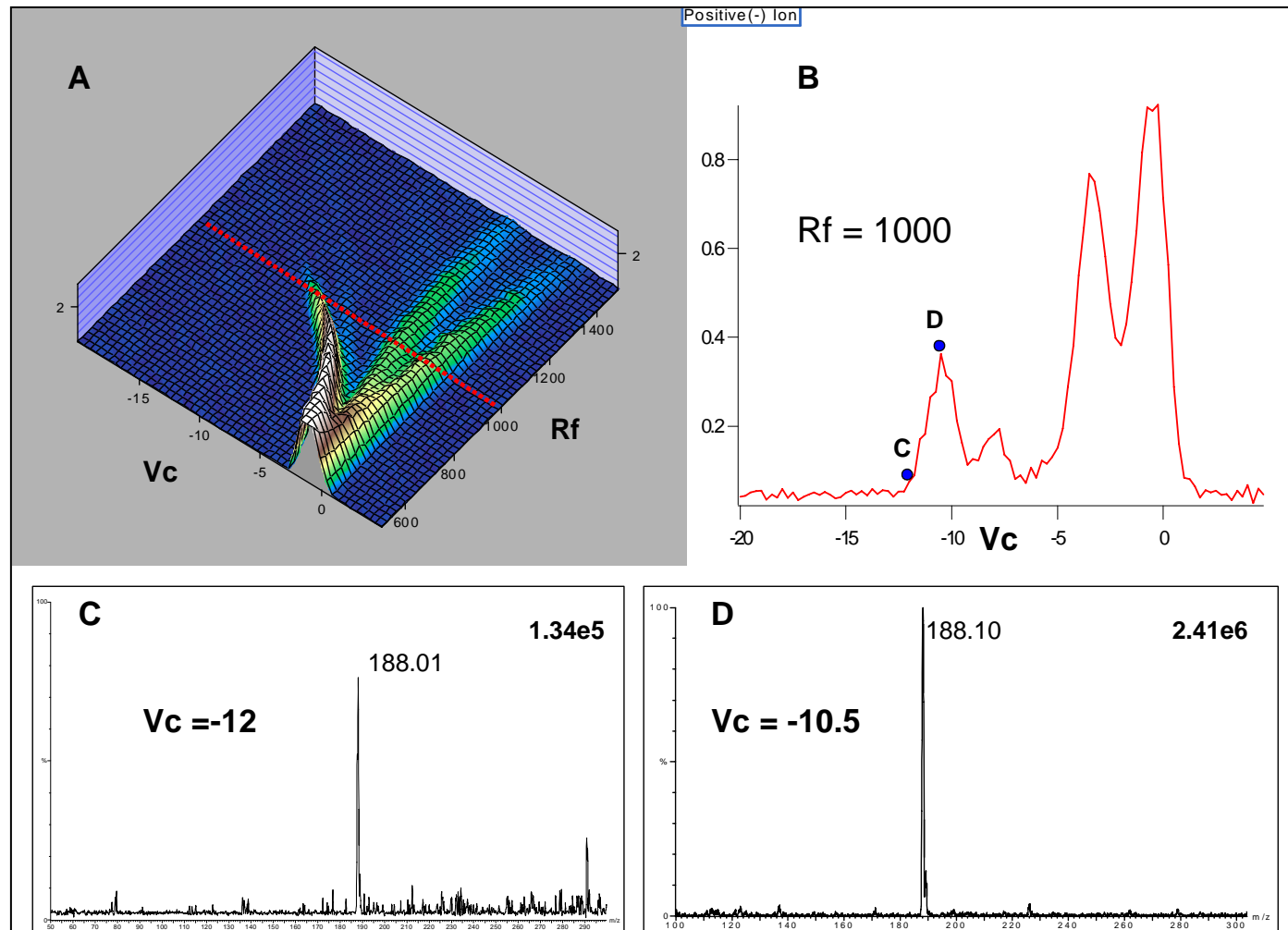


Custom nanoESI-DMS-MS system

- Incorporates a modified **Sionex Co. SDP-1** DMS sensor
- Micromass ZQ mass spectrometer
- Custom made nanospray source

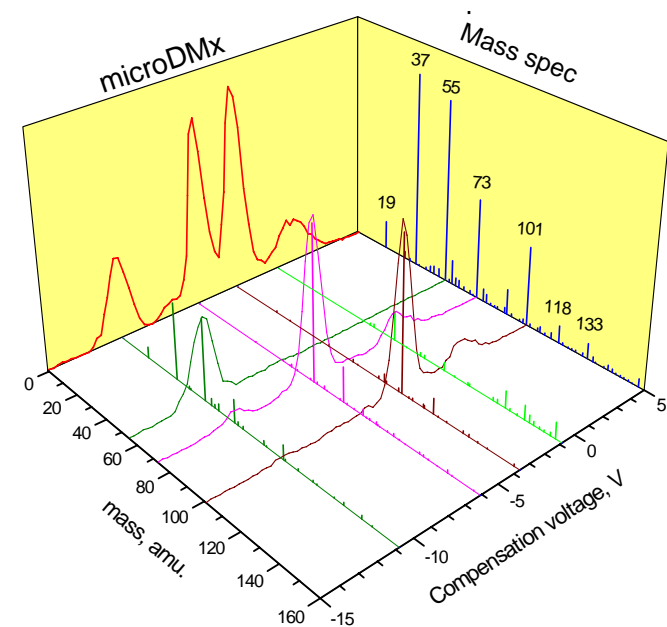


Example DMS dispersion plot (a) and extracted DMS spectra at $R_f = 1000$ V (b), selected R_f and V_c point mass spectra (c and d)



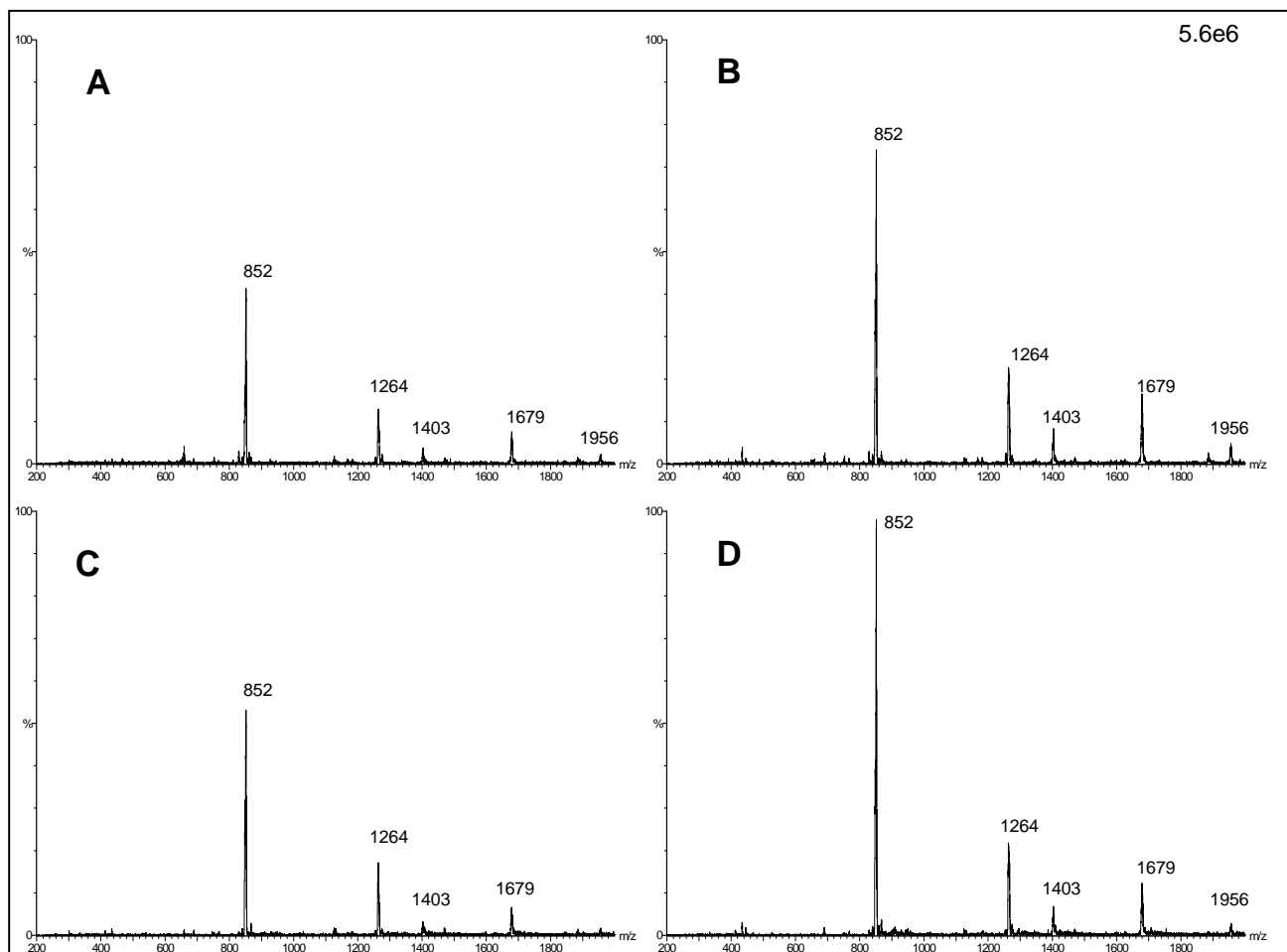
DMS-MS 3D Spectra of Butanone APCI in ambient air

- Shows orthogonality of separations
- Tune V_c and detect by MS
- E.g., m/z 73 $[M+H]^+$ and also $(H_2O)_4H^+$



Courtesy of E. Nazarov, SIONEX,

Mass spectra (DMS turned off) of (a) 0.25 mg/ml maltopentaose in 50/50 wat/meth, same but with 8000 ppm methanol drift gas modifier (b), 0.25 mg/ml maltopentaose in 50/50 wat/meth 2mM NaCl (c), same but with 8000 ppm methanol drift gas modifier (d). All spectra normalized to same ion signal



0.25 mg/ml maltopentaose in 50/50 methanol/water, selected ion DMS spectra for the m/z 1956 ion (a), m/z 1679 ion (b), m/z 1403 ion (c), m/z 1264 ion (d), and m/z 852 ion (e). Rf set to 1500 V and Vc scanned from -25 to +5. (left side without drift gas modifier, right side with 8000 ppm methanol)

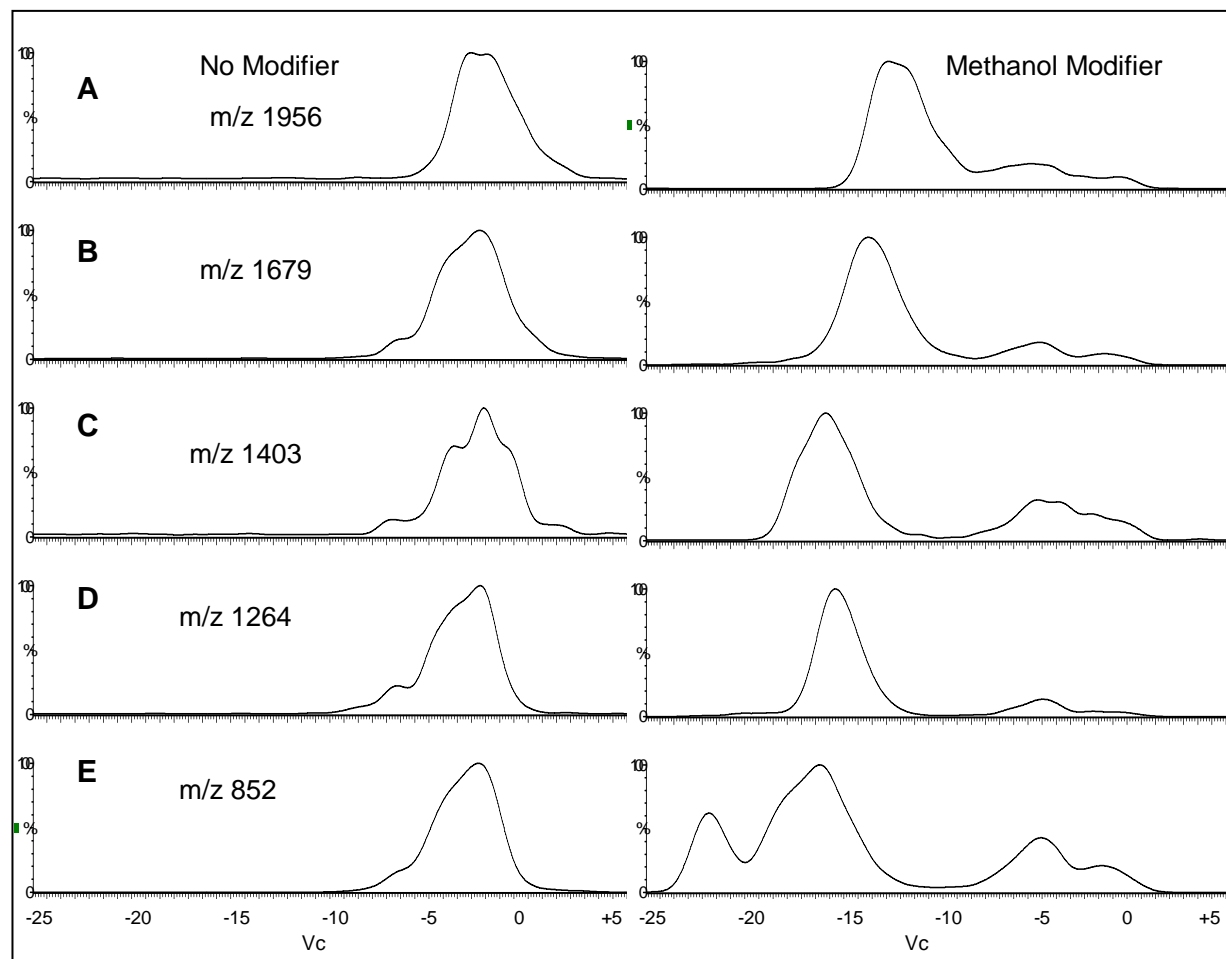
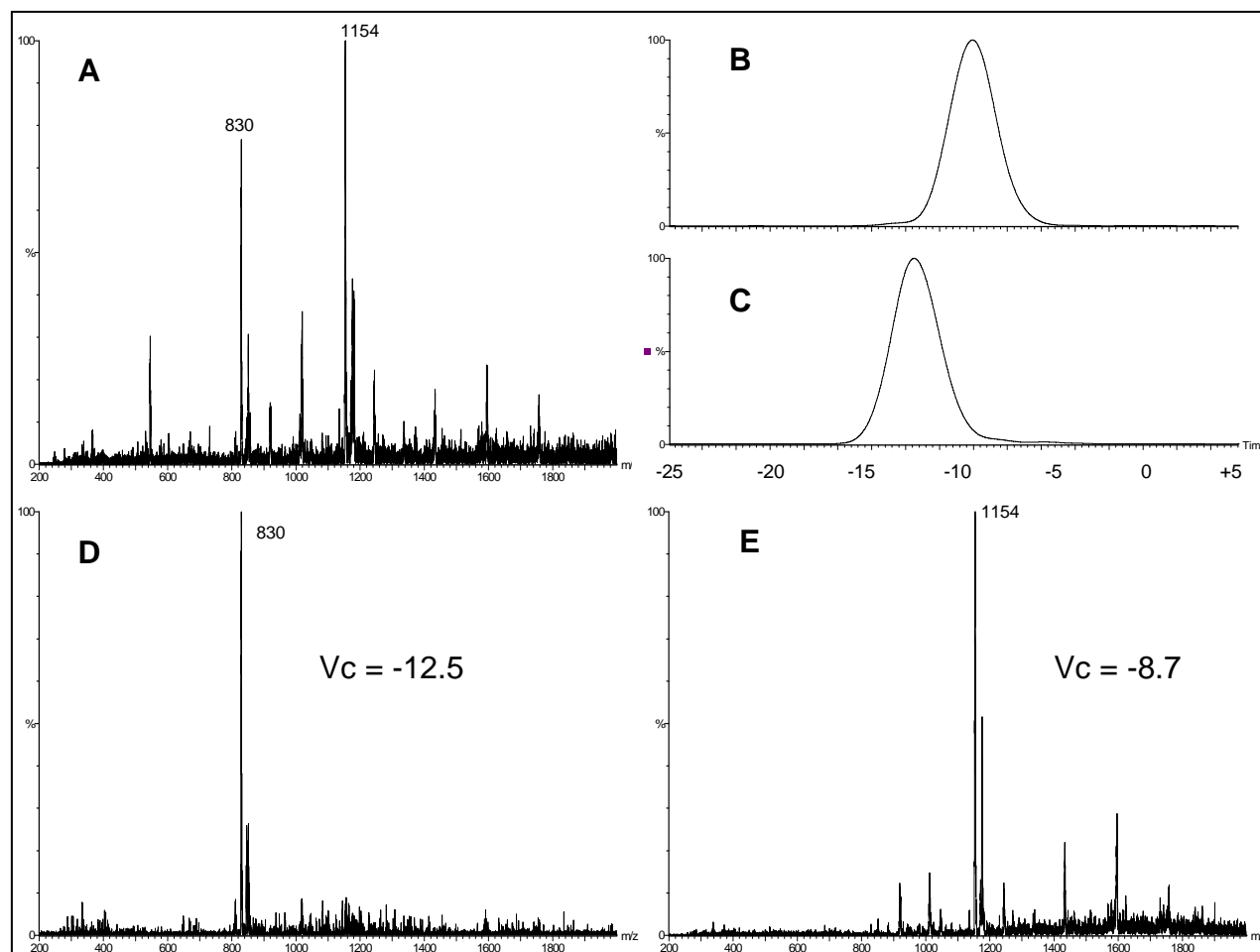
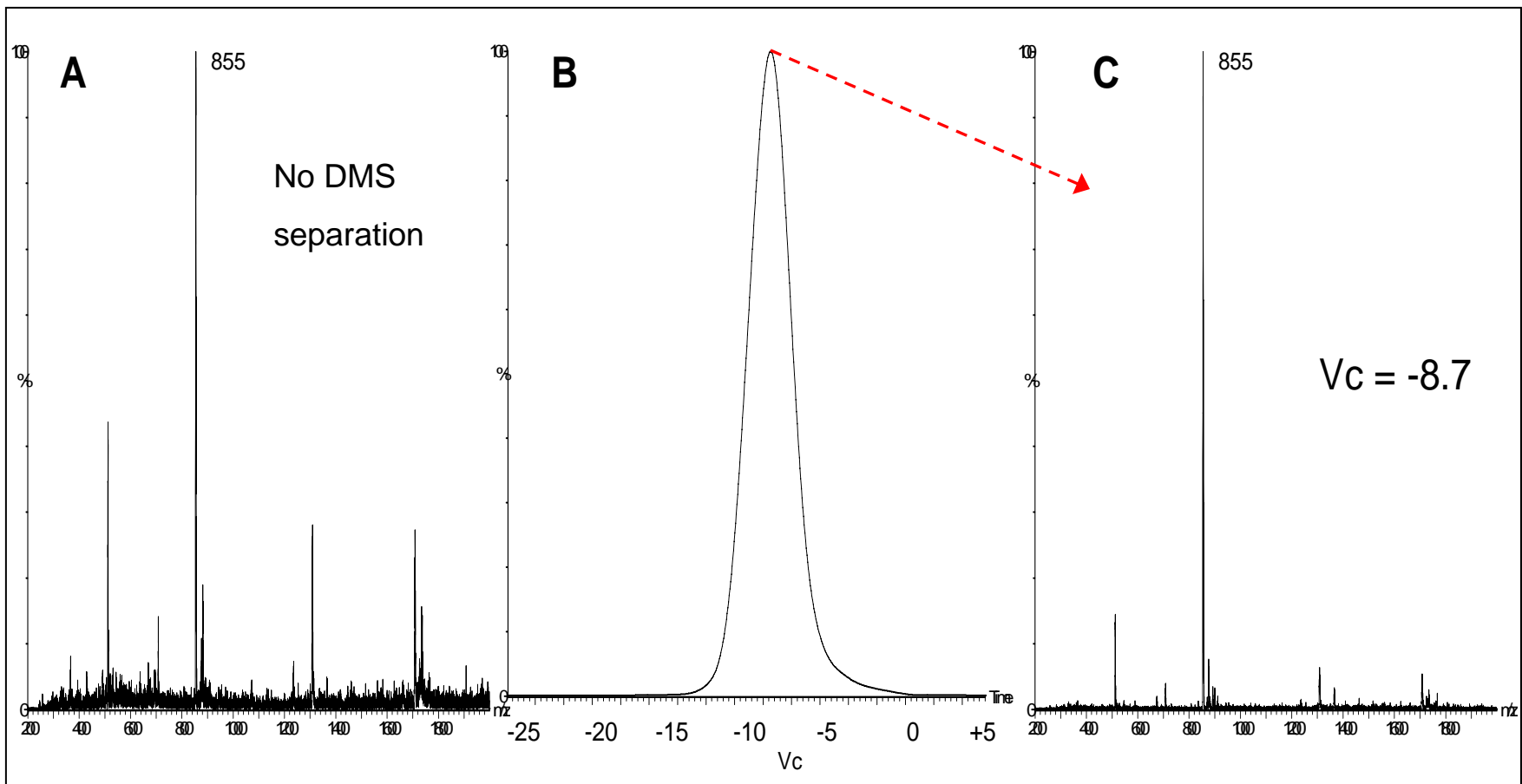


Figure 6. (a) mass spectra (DMS turned off) of 0.25 mg/ml each maltopentaose and maltoheptaose in 60/40 methanol/tetrachlorethane with the 8000 ppm methanol drift gas modifier, (b) the m/z 1154 selected ion DMS spectra at $R_f = 1250$, (c) the m/z 830 selected ion DMS spectra at $R_f = 1250$, (d) the mass spectra collected with the V_c set to -12.5 ($R_f = 1250$), and (e) the mass spectra collected with the V_c set to -8.7 ($R_f = 1250$)

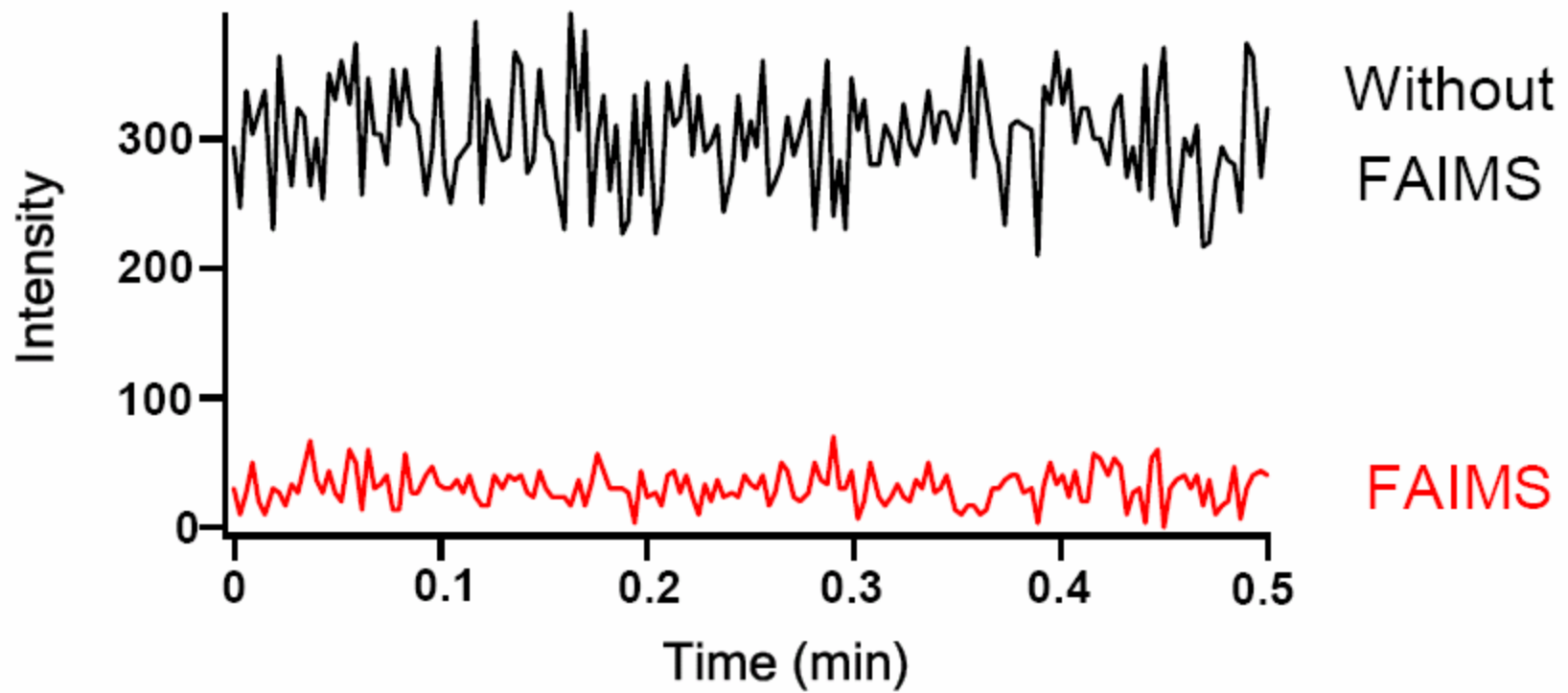


Demonstration of Mass Spectral Signal/Noise Improvement through DMS Ion Separation

- Mass Spectra of Lacto-N-fucopentaose I (LNFP I) sample (oligosaccharide with M.W. 854) with and without DMS separation



- **Blank extracted mouse plasma**
- **Monitor morphine SRM transition**

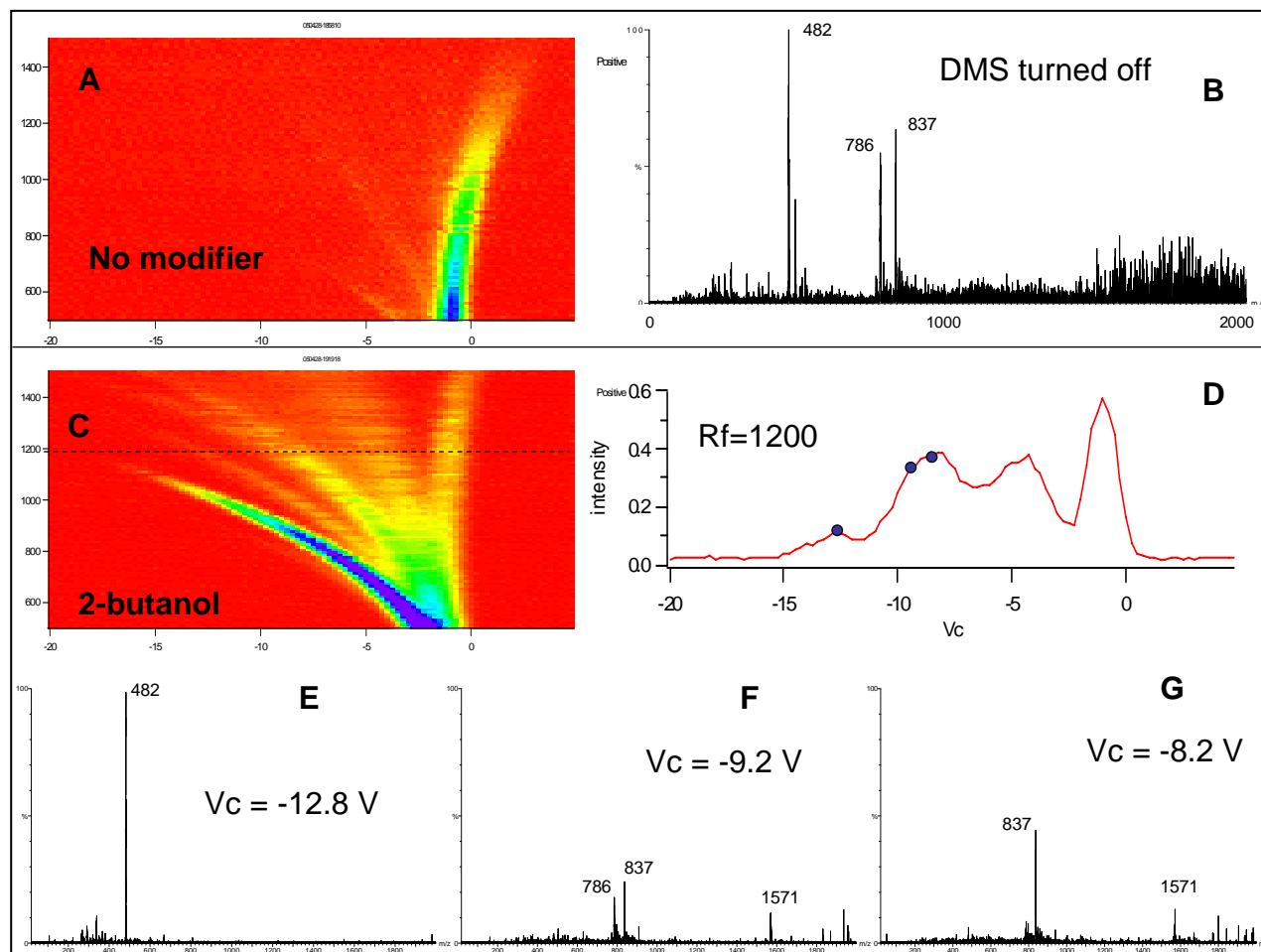


Study 1: Investigation into rapid peptide separation and quantitation capabilities via direct infusion nanoESI-DMS-MS

- DMS peptide separation?
- Can drift gas modifiers improve separation?
- Hurdle: peptide aggregate/cluster ion formation
 - nanoESI formation of 17mer⁴⁺ and 19mer⁵⁺ aggregate ions of leucine enkephalin in high abundance.¹
 - ESI formation of heteroaggregate ions in addition to homoaggregate ions from peptide mixture ^{2,3}
 - May contribute to high m/z background ions observed in ESI-MS peptide analysis ²

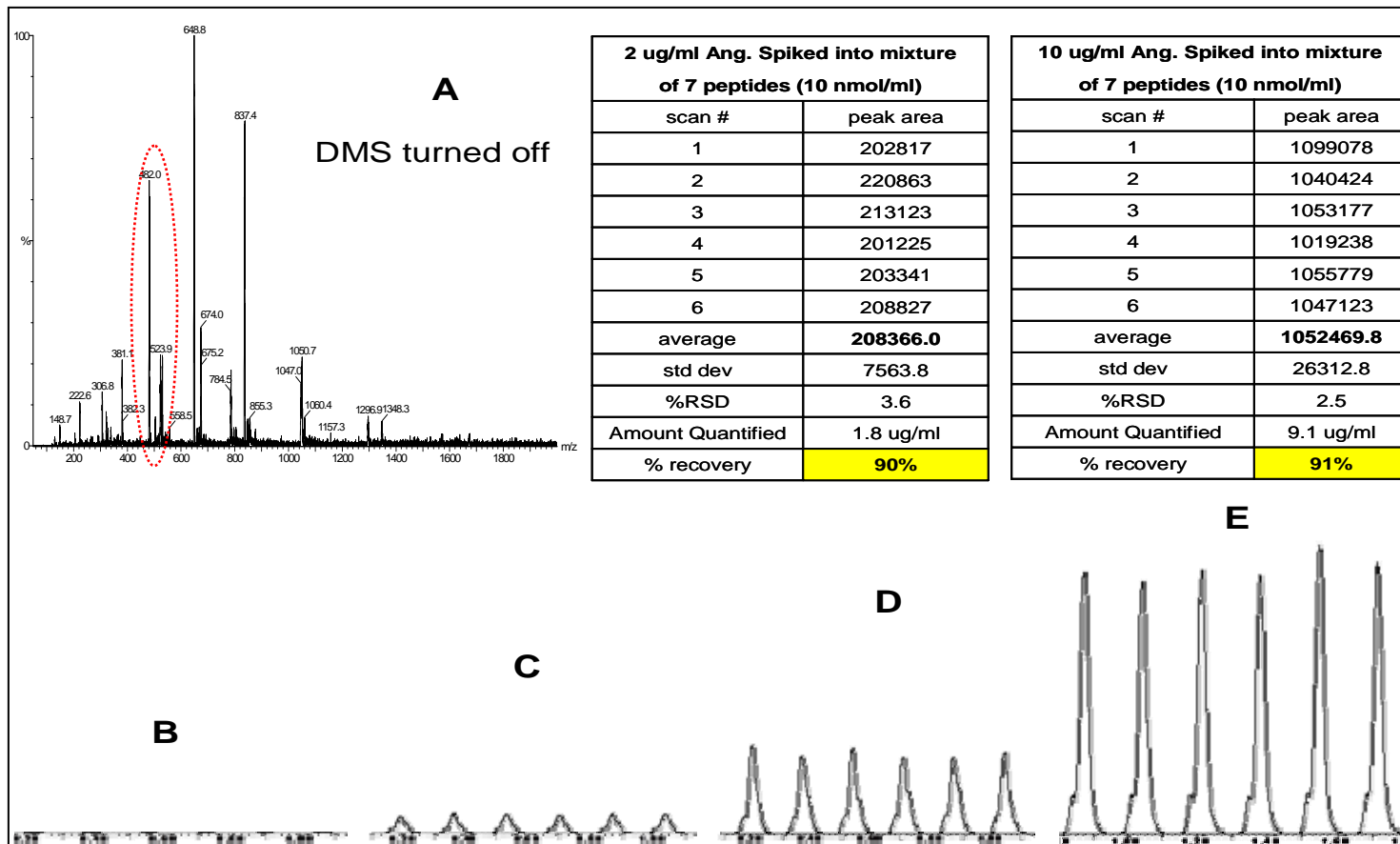
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1. Jurchen, J. C., Garcia, D. E., Williams, E. R., Gas-Phase Dissociation Pathways of Multiply Charged Peptide Clusters. *J Am Soc Mass Spectrom.* **2003**, 14, 1373-1386.
 2. Counterman, A. E., Hilderbrand, A. E., Srebalus Barnes, C. A., Clemmer, D. E. Formation of Peptide Aggregates during ESI: Size, Charge, Composition, and Contributions to Noise. *J Am Soc Mass Spectrom.* **2001**, 12, 1020-1035.
 3. Lee, S. W., Beauchamp, J. L. Fourier Transform Ion Cyclotron Resonance Study of Multiply Charged Aggregates of Small Singly Charged Peptides Formed by Electrospray Ionization. *J Am Soc Mass Spectrom.* **1998**, 10, 347-351.

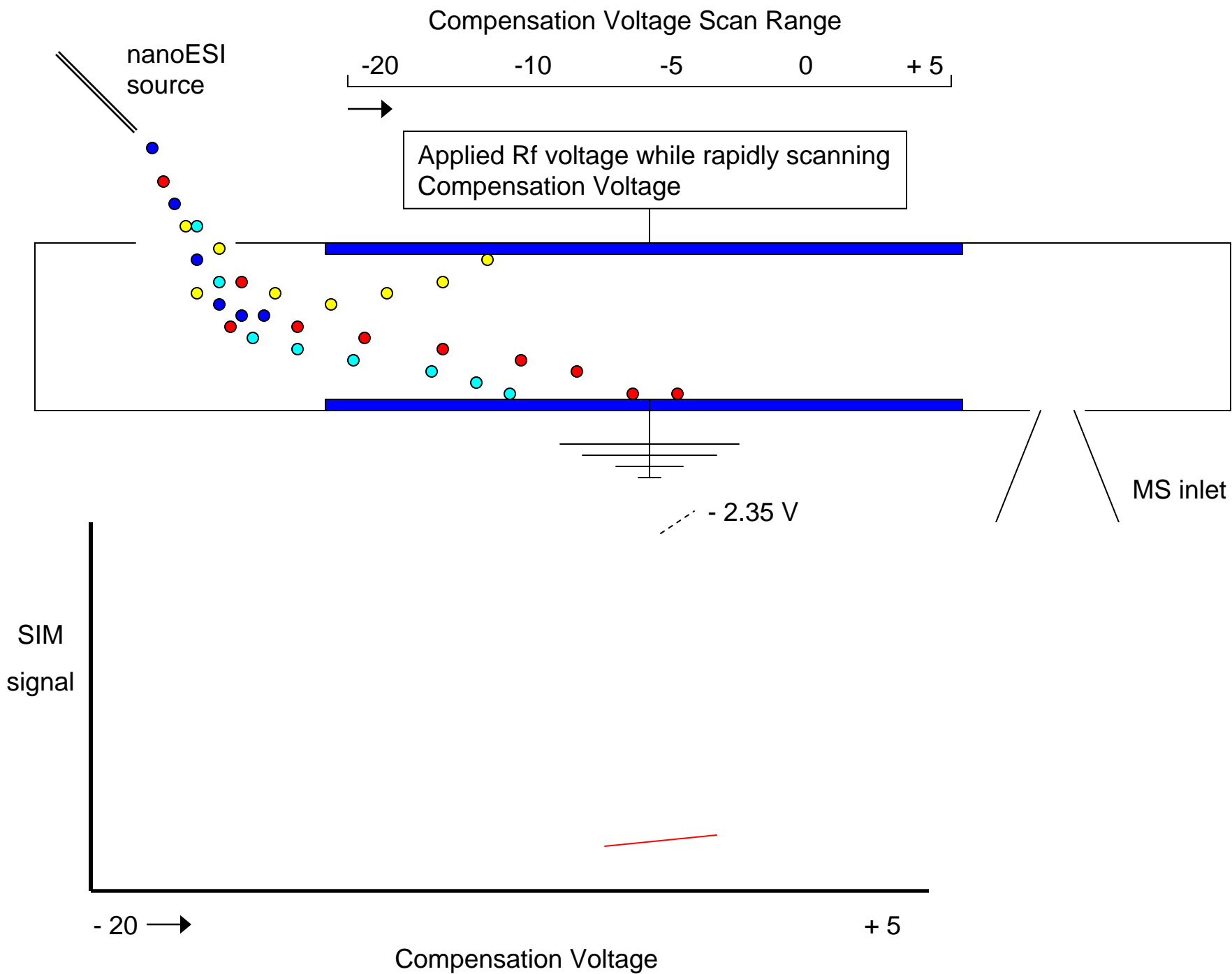
Mixture of Angiotensin, Glu-Fib B, and Neurotensin 0.05 mg/ml each
A,B - DMS off, no modifier; C-G 8000 ppm 2-butanol
E - G Mass spectra at selected Vc points (Rf = 1200)

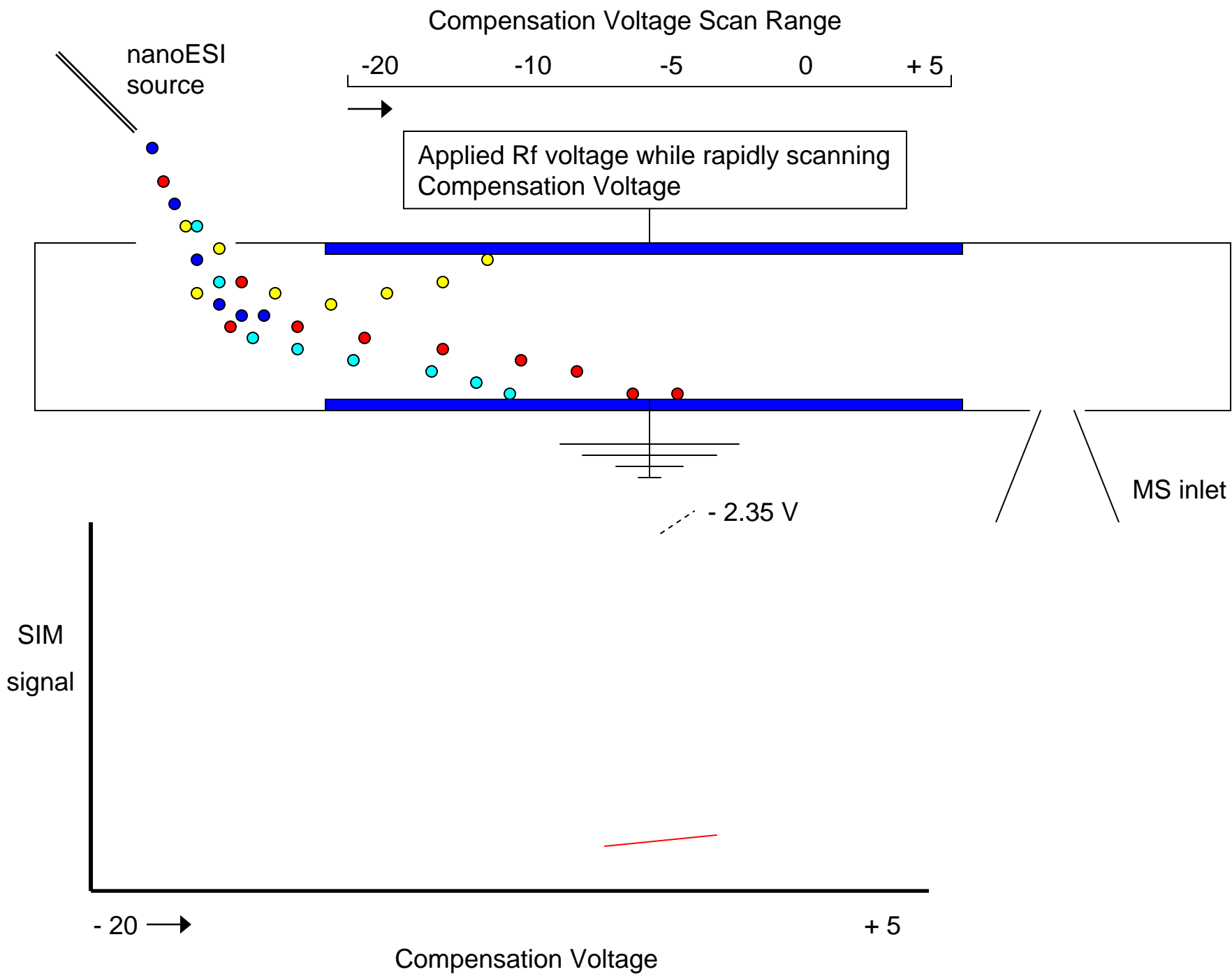


Ultra-Rapid Quantitation via Selected Ion-Rapid Vc Scanning Platform

- Rf held constant, and Vc rapidly scanned (<10 sec.) and selected MS ion signal monitored
- Match peak apex Vc to standard for specificity and integrate peak area for quantification
- repeat injections of six-peptide mixtures containing 0, 2, 10 and 25 µg/ml angiotensin

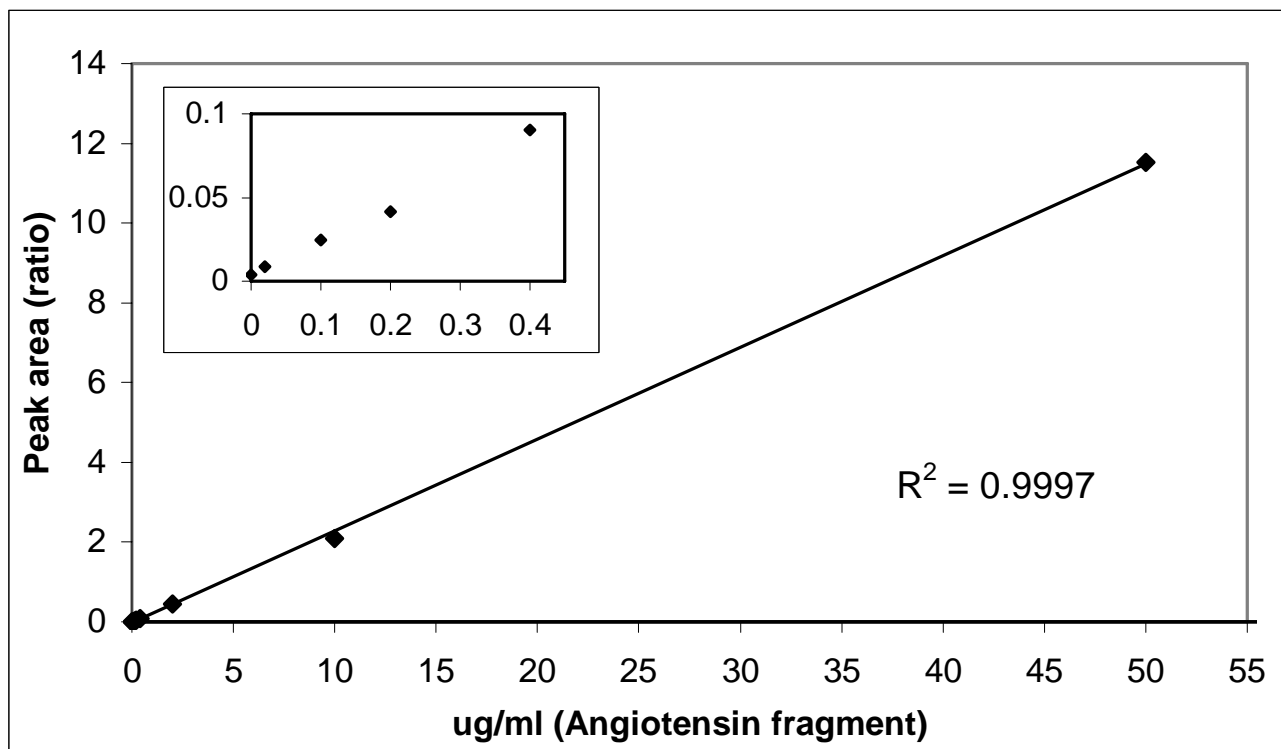






Linear regression of angiotensin concentration ($\mu\text{g/ml}$) vs. response (peak area ratio of angiotensin/internal std.) spiked into six peptide mixture. Inlay plot shows zoomed-in view of the four low concentration and blank solution data points. Scan time: 10 sec/point

angiotensin fragment concentration (ug/ml)	peak area ratio (angiotensin / internal std.)
0	0.0040
0.02	0.0086
0.1	0.0248
0.2	0.0417
0.4	0.0906
2	0.4510
10	2.0944
50	11.5238



Using this platform for ultra-high throughput quantitative analysis

- 0.5 – 5 sec. sample analysis times appear reasonable to achieve
- Incorporate an automated sample handling/nanospray system to improve quantitative reproducibility by:
 - Eliminating carryover
 - Reducing capillary clogging
 - Providing reproducible flow and tip position
- Nanospray conditions provide:
 - Improved sensitivity
 - Reduced ion suppression effects
 - Reduced sample consumption

Nanomate

by Advion Biosciences



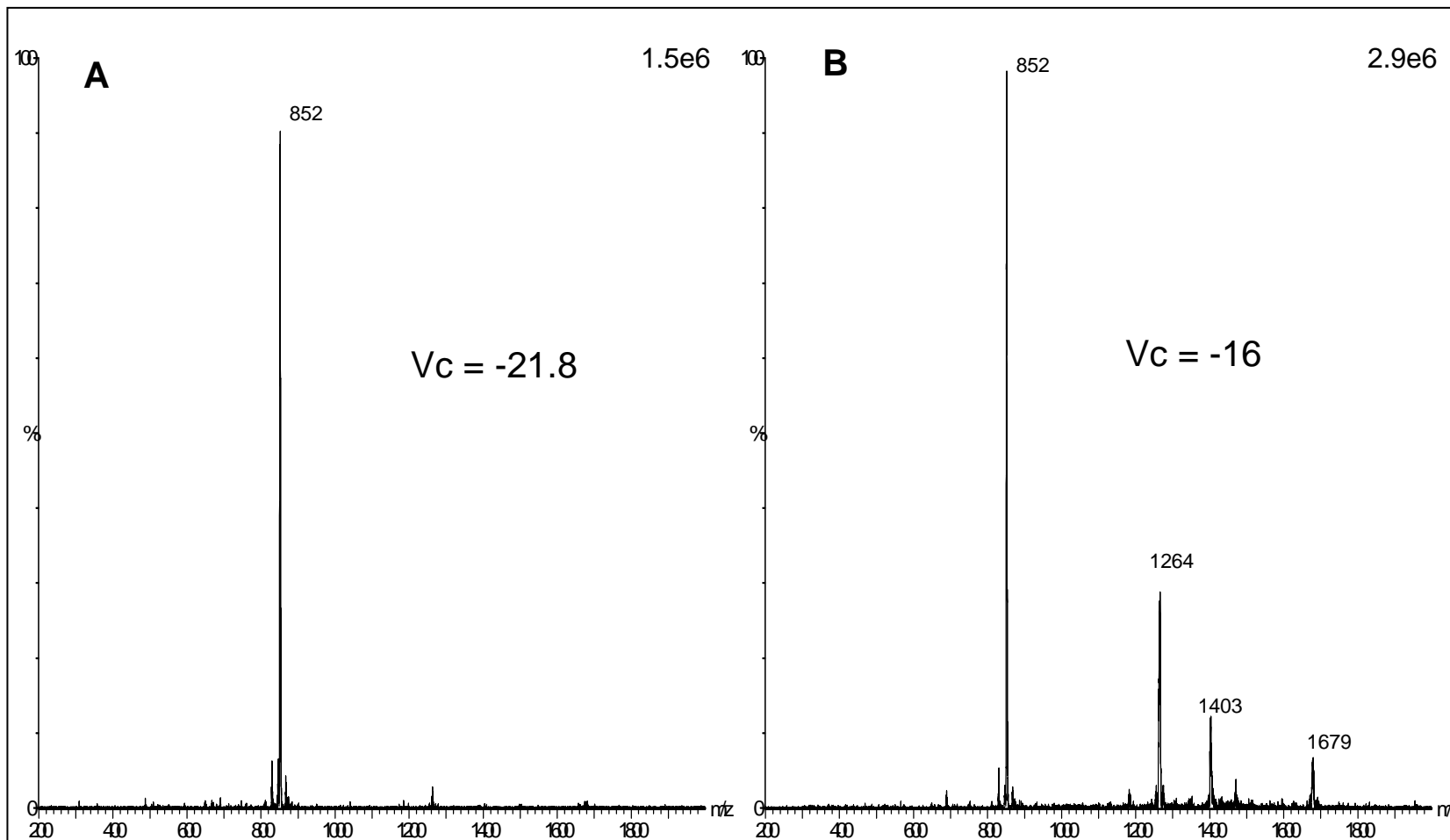
Conclusions

- Demonstration of improved DMS peptide and glycan separation via selected drift gas modifier
- Mechanistic insight into peptide aggregate ion de-clustering and improved DMS separation
- Demonstrated feasibility of selected ion-rapid Vc scanning quantitation platform for high-throughput applications
- Future work to combine an automated sample handling/nanospray device with DMS-MS system for improved accuracy and throughput

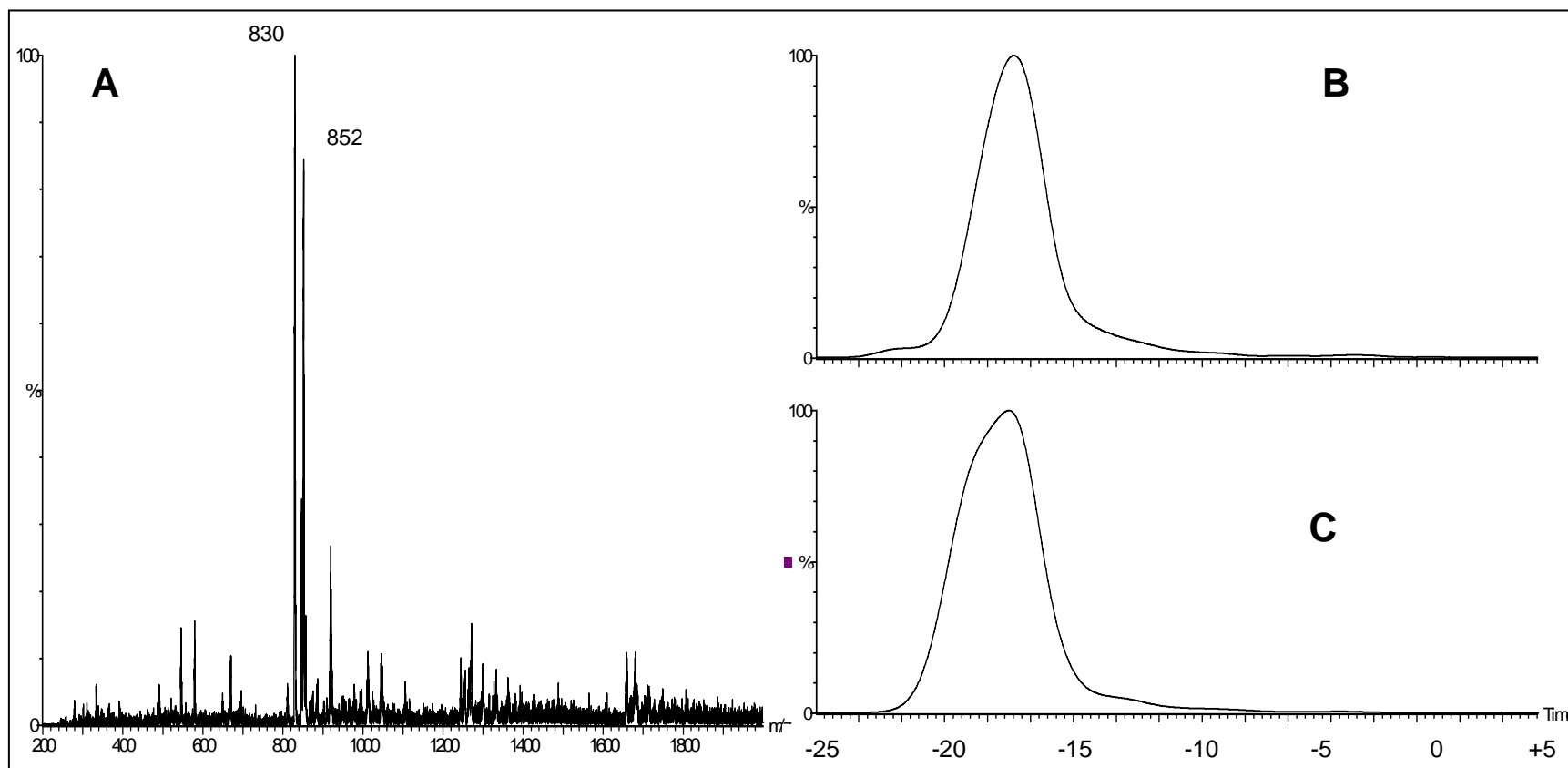
Acknowledgments

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- **Department of Chemistry and Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA**
- **Present Address: GSK North Carolina*
 - **Erkinjon G. Nazarov (Chief Scientist)**
 - **Raanan A. Miller (Chief Technology Officer, Founder)**
 - **James C. Morris (Principal Investigator)**
- **Sionex Corporation, Bedford, MA**

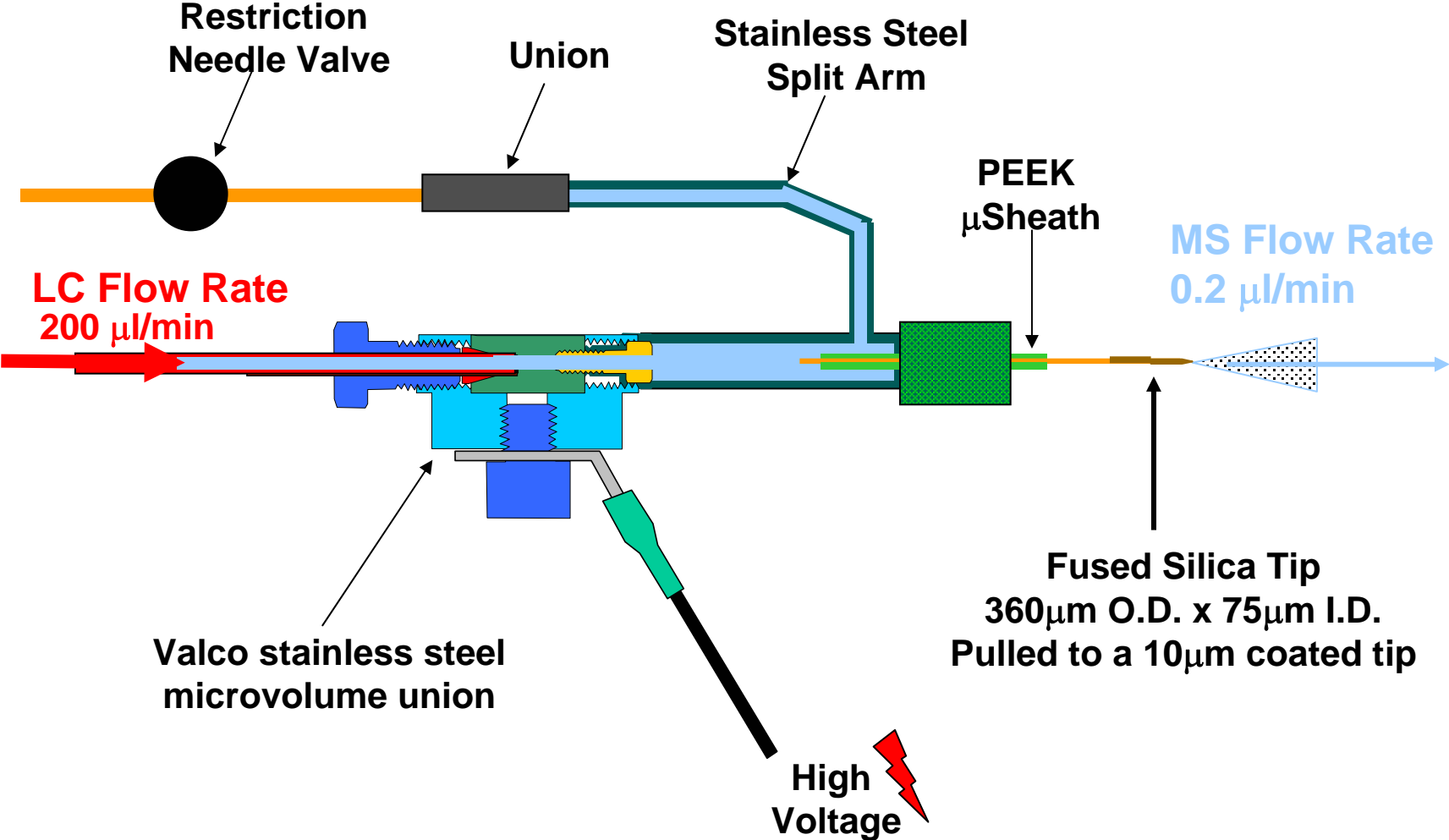
mass spectra for 0.25 mg/ml maltopentaose in 50/50 methanol/water at selected compensation voltages from analysis in Figure 3 (with methanol drift gas modifier), -21.8 V (a), and -16 V (b) with Rf set to 1500 V.



(a) mass spectra of 0.25 mg/ml maltopentaose in 60/40 meth/tetrachloroethane, with 8000 ppm methanol dopant (DMS turned off), (b) m/z 852 selected ion DMS spectra at Rf = 1500, and (c) m/z 830 selected ion DMS spectra at Rf = 1500.



Detailed Schematic of Nanosplitter Interface



Why Do We Think Concentric Splitting Works?

