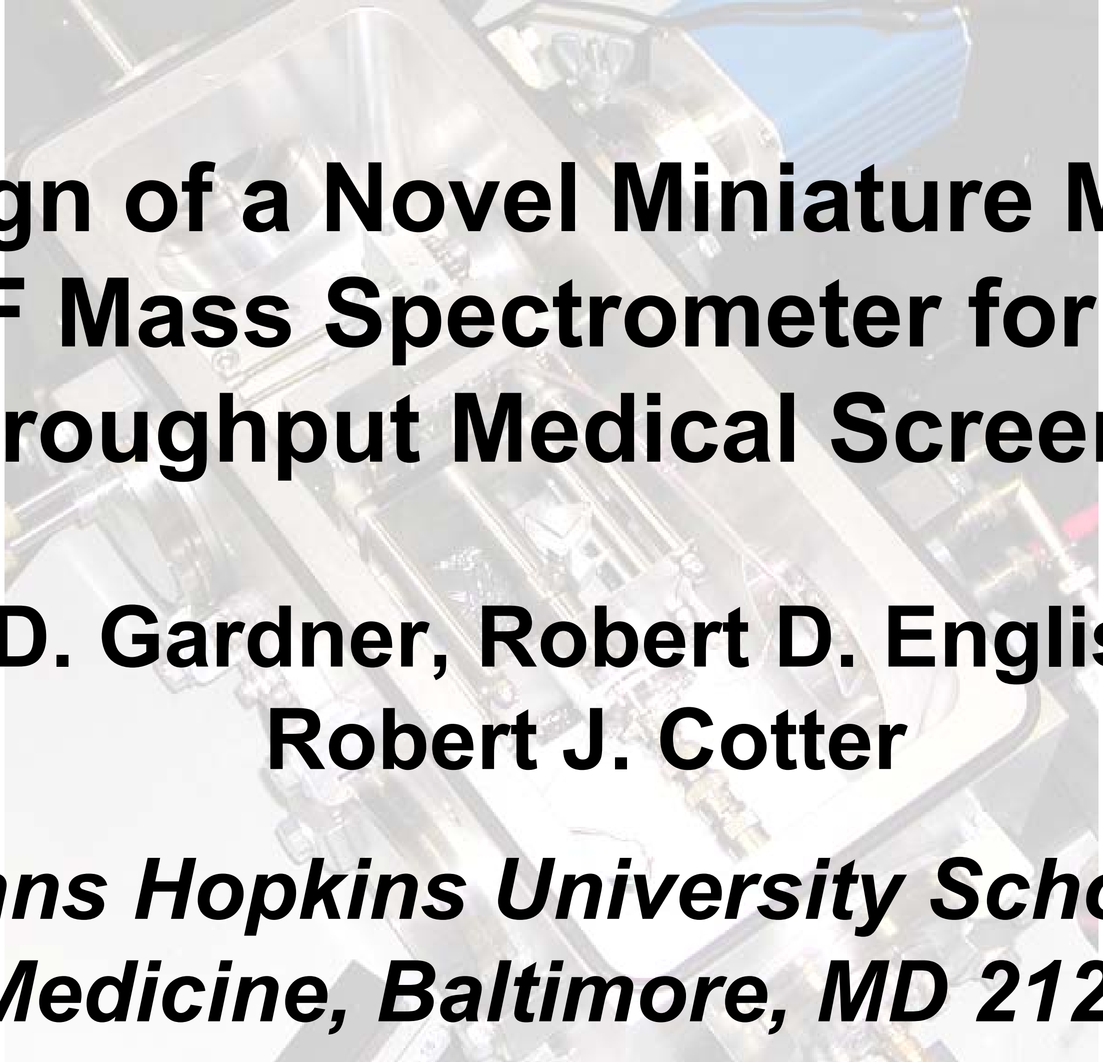
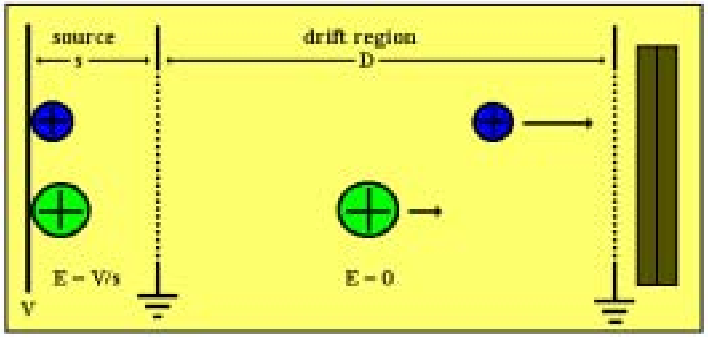


Design of a Novel Miniature MALDI-TOF Mass Spectrometer for High Throughput Medical Screening

Ben D. Gardner, Robert D. English and Robert J. Cotter

Johns Hopkins University School of Medicine, Baltimore, MD 21205

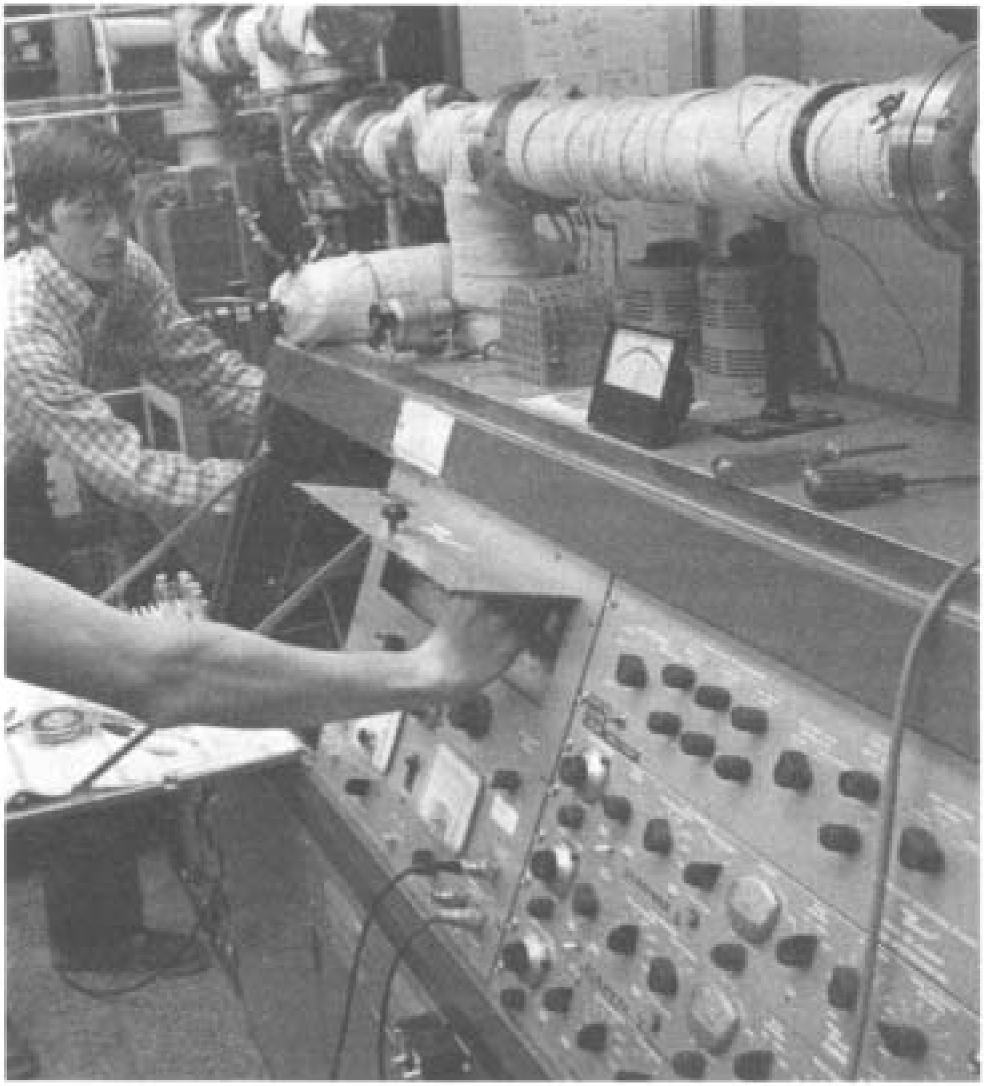
The time-of-flight mass spectrometer is a very simple instrument:



Ions formed in the ion source (s) appear at the detector with flight times through the drift region (D) proportional to the square root of their *mass/charge*:

$$t = \left(\frac{m}{2eV}\right)^{1/2} D$$

Why is it so difficult to miniaturize?



The flight time of an ion has a more complex dependence:

$$t = \overbrace{\frac{(2m)^{1/2}}{eE}\left[(U_0 + eEs)^{1/2} \pm U_0^{1/2}\right]}^{\text{time in ion source}} + \overbrace{\frac{(2m)^{1/2} D}{2(U_0 + eEs)^{1/2}}}^{\text{time in flight tube}} + t_0$$

initial kinetic energy distribution

turn-around time

distribution of initial position in the source

distribution in time of ion formation

eEs = *eV* when ion is accelerated across the whole source region

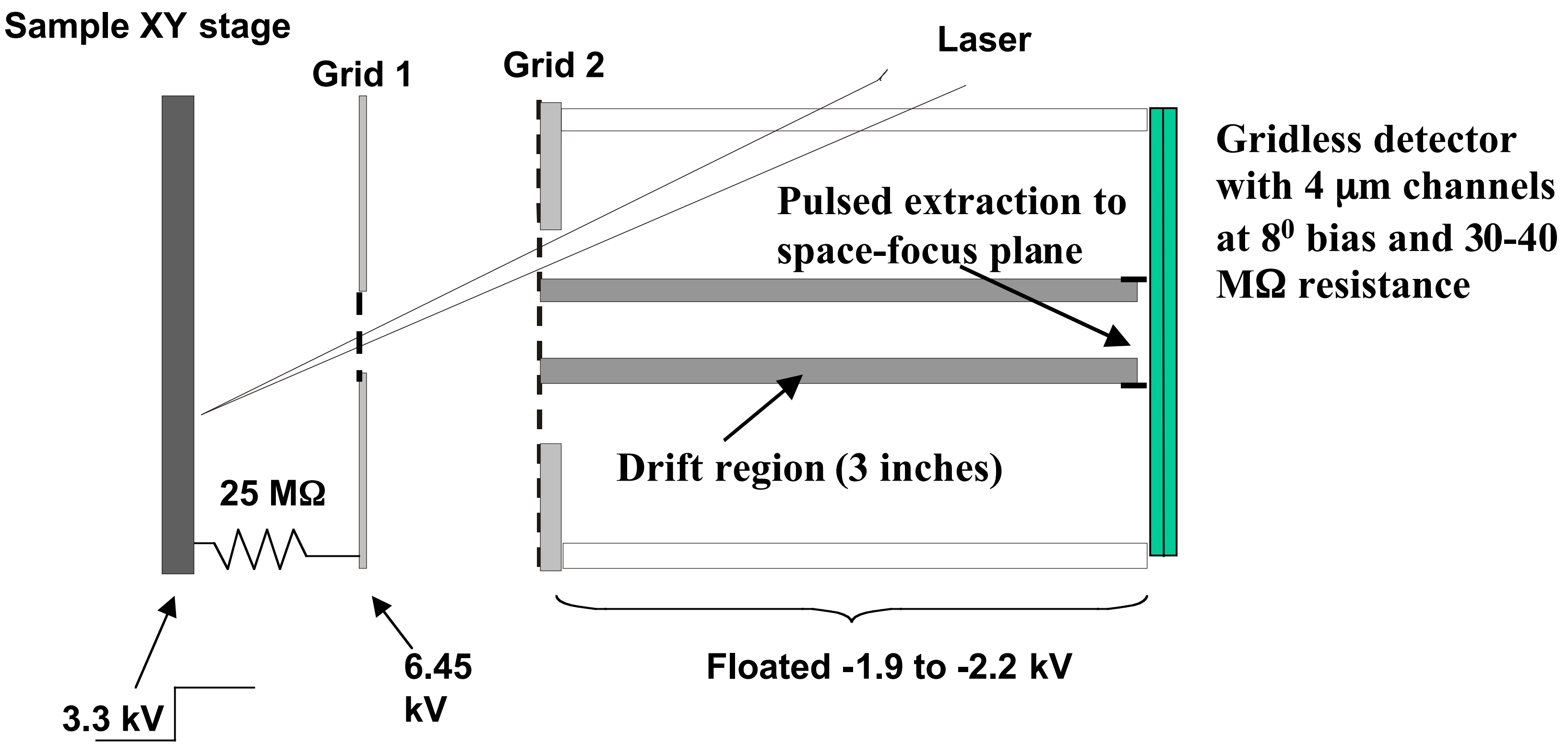
Mass resolution $m/\Delta m = t/2\Delta t$

- Δt reflects time resolution of the digitizer (need 4 Gs/s)
- Δt reflects response of the detector (< 2ns)
- Increase t by using longer flight tube
- Increase t using lower accelerating voltage
  - reduces sensitivity at high mass (post-acceleration increases Δt)
  - exacerbates effect of initial kinetic energy ($eV >> U_0$)

The dimensions and the time spent in the source cannot be neglected

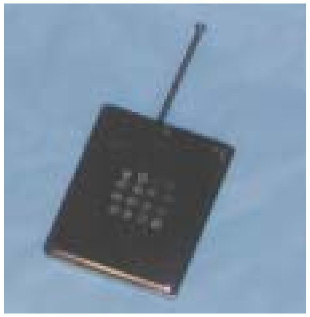
- Source cannot be shrunk proportionately and maintain HV extraction
- Time spent in the source will be different for ions of different energy
  - reflectron cannot compensate for both time and energy

Miniaturized linear, 3-inch, pulsed TOF mass spectrometer

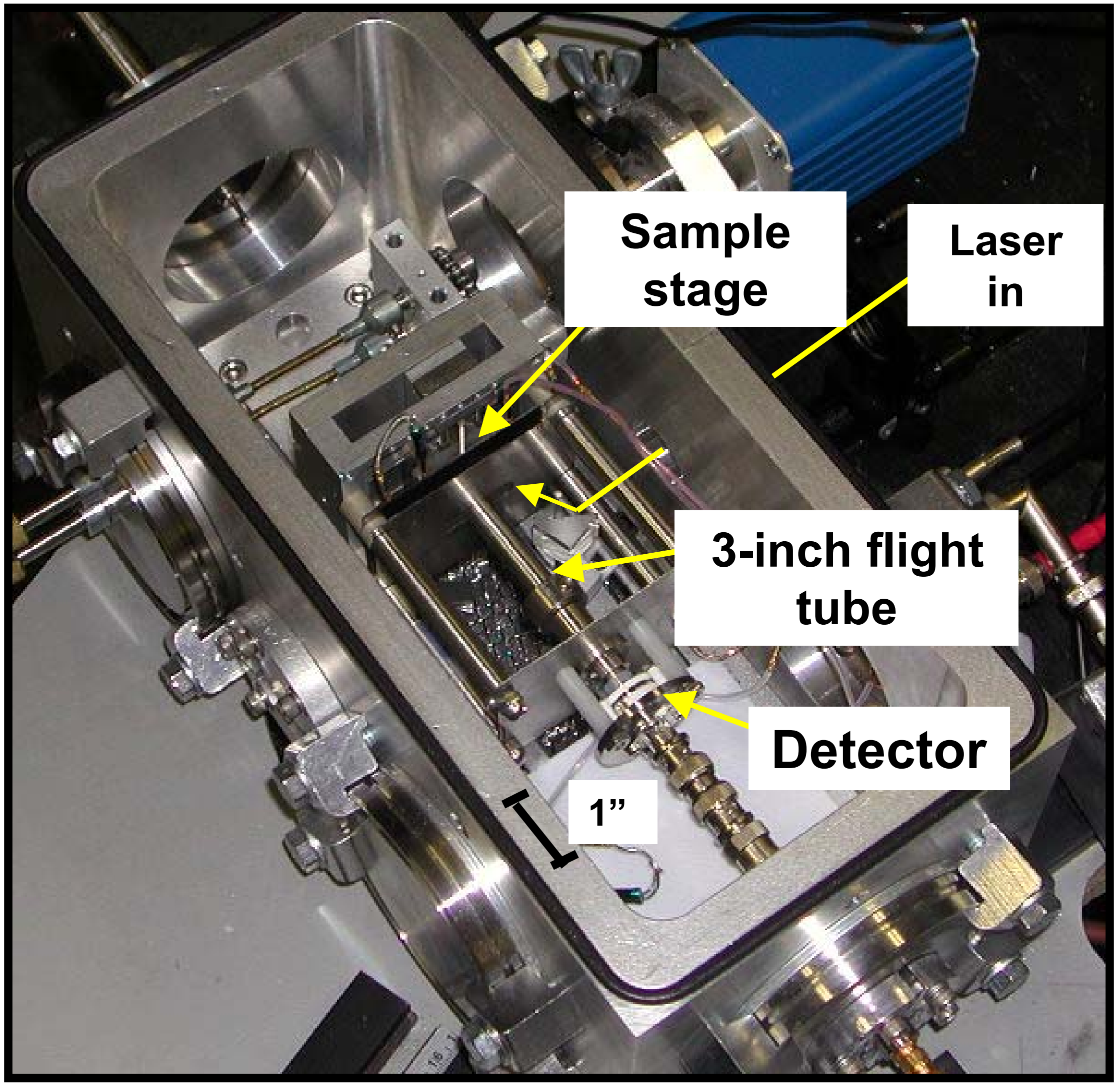


Gridless detector with 4 µm channels at 8^0 bias and 30-40 MΩ resistance

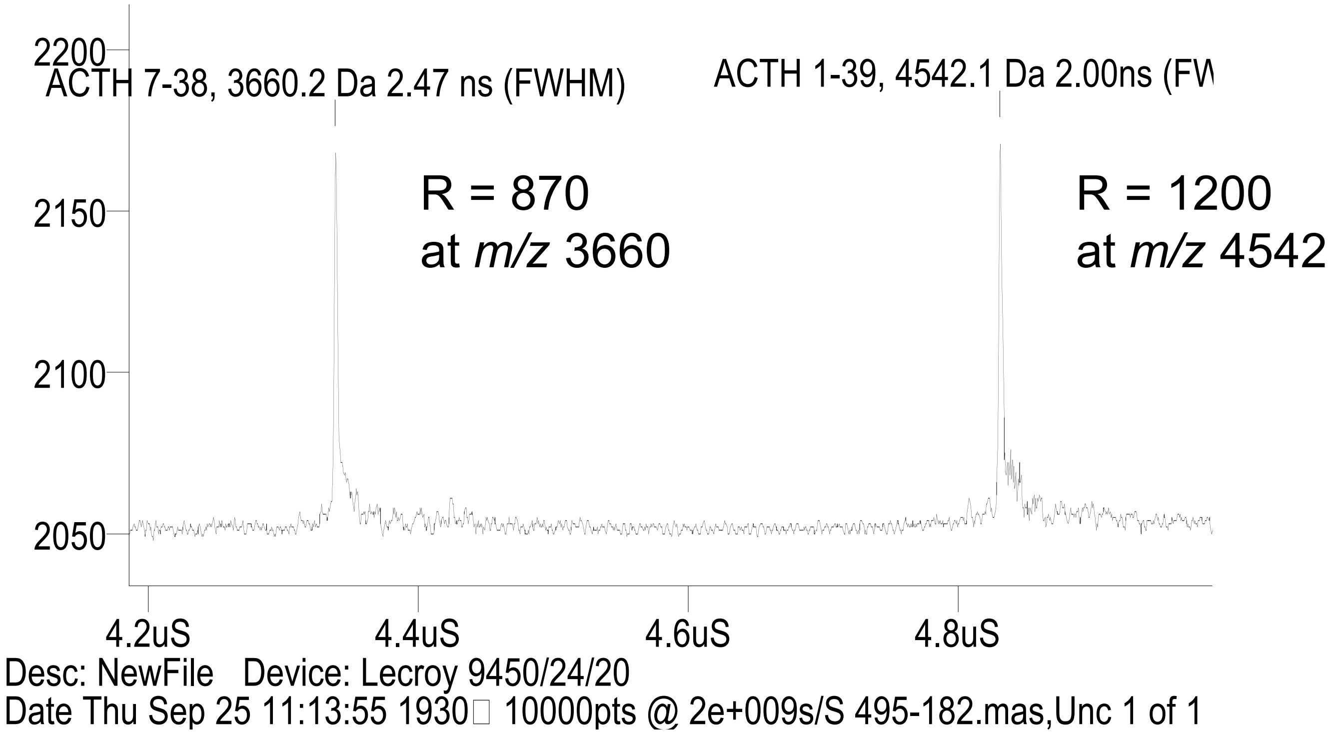
Miniaturized instruments for bioagent detection, high throughput analyses and diagnostics



Sample plate



Mass spectrum of ACTH on the miniature linear TOF mass spectrometer

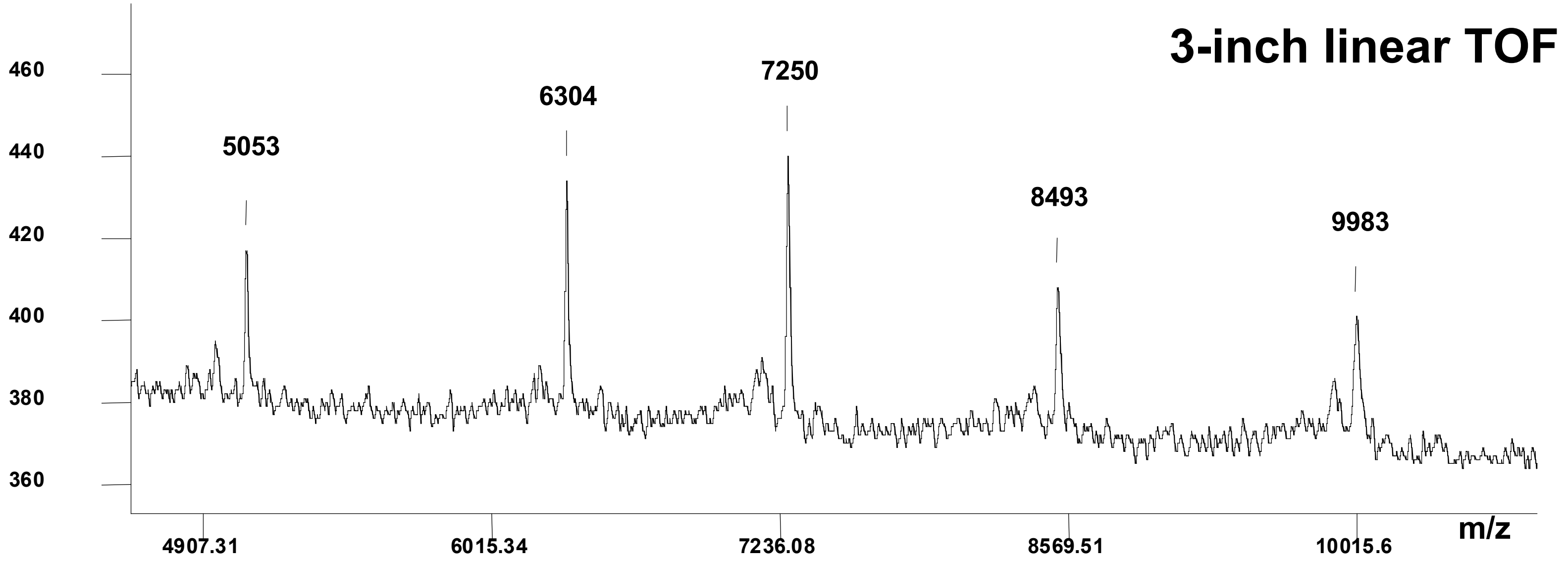


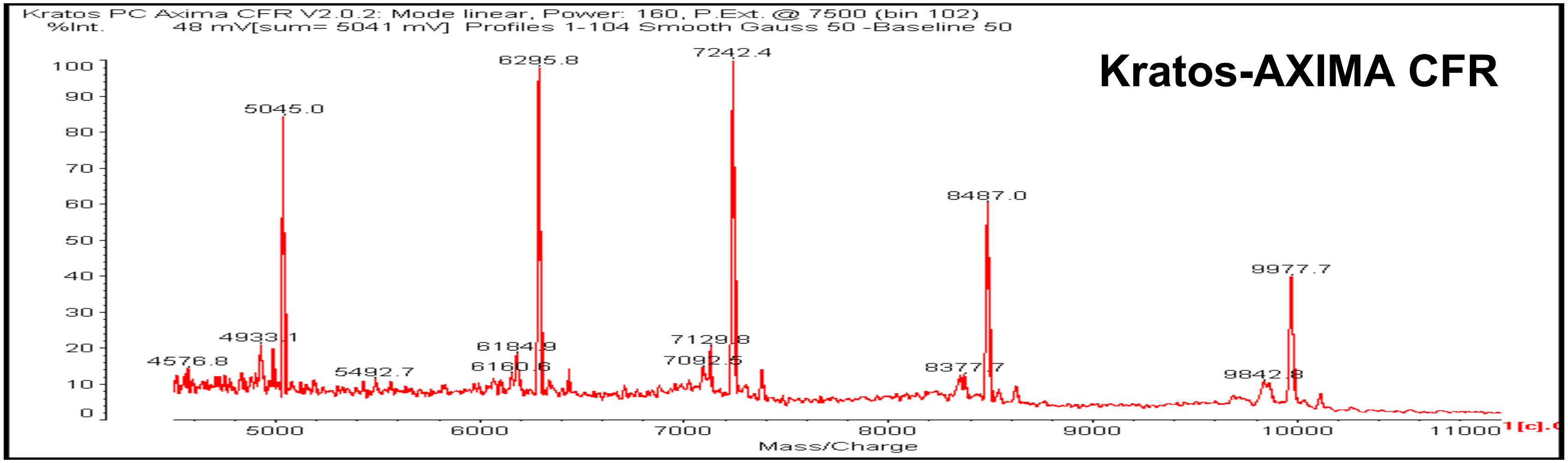
R = 870
at *m/z* 3660

R = 1200
at *m/z* 4542

Pulsed extraction is optimized for mass 4542

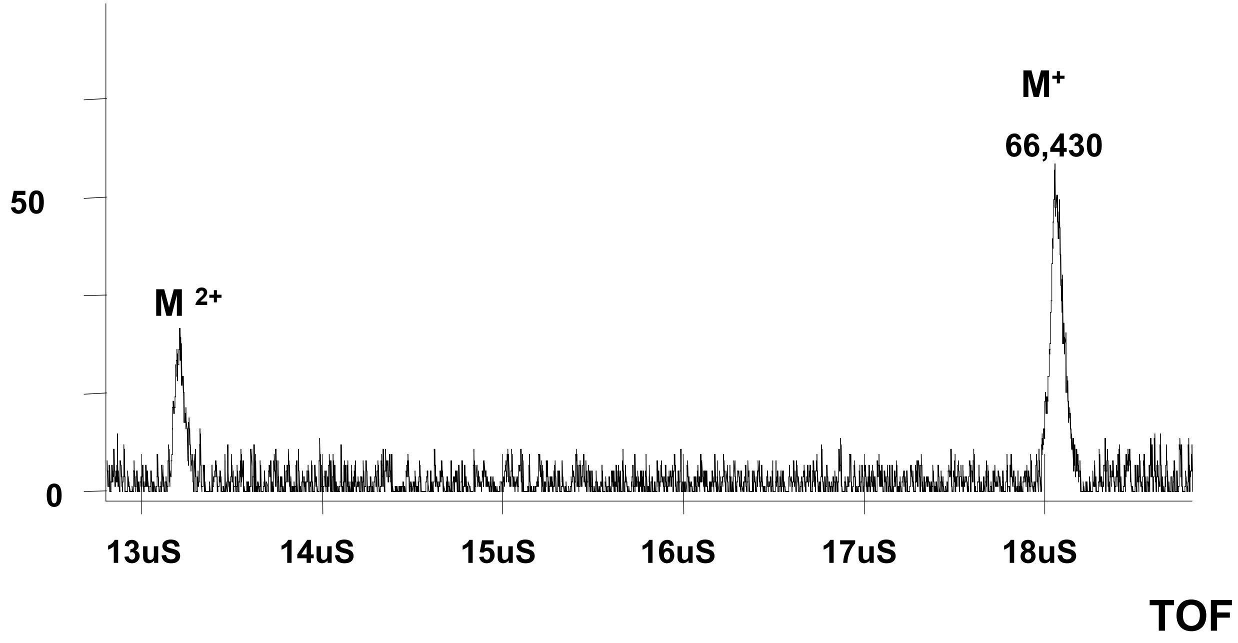
Oligonucleotide mixture



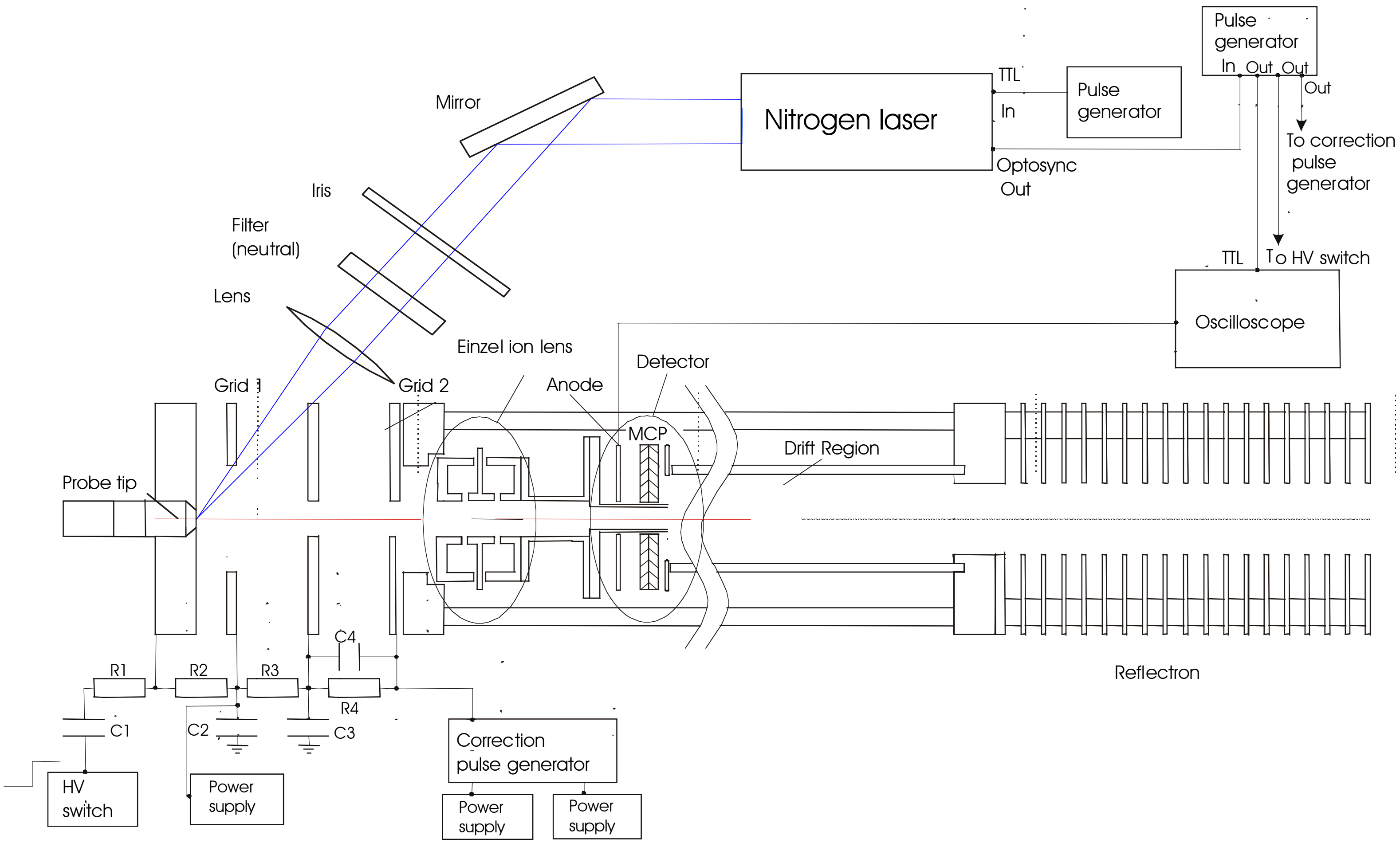


Largest mass measured to date on the 3-inch linear instrument

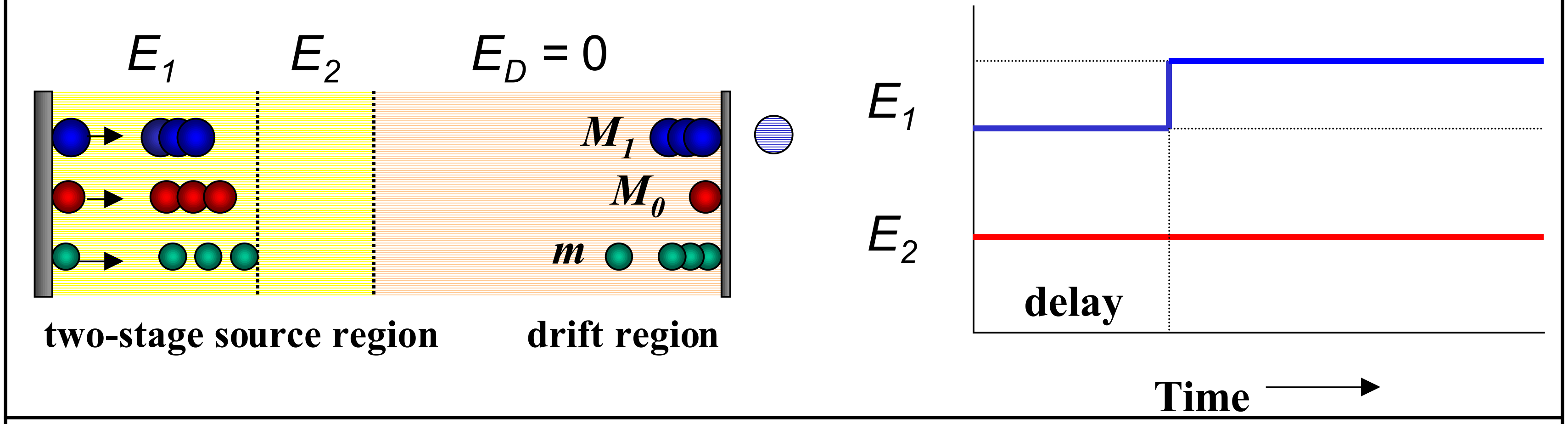
Bovine serum albumin : 66 kDa Sinapinic acid; analyte 150 pmoles



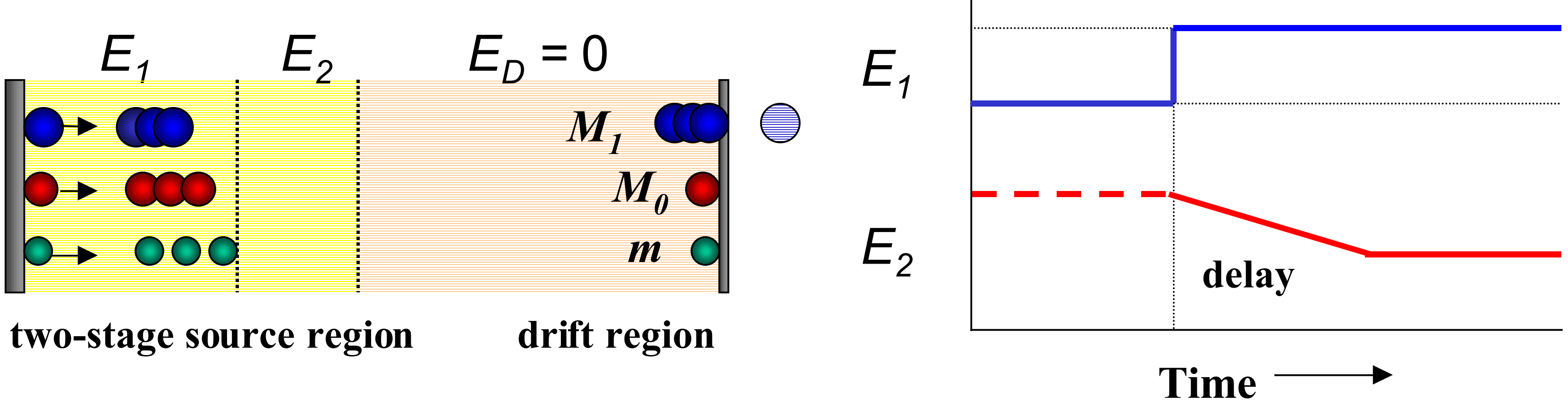
MCA MALDI re-TOFMS instrument



a) *Time-lag focusing (pulsed extraction, delayed extraction) methods.*

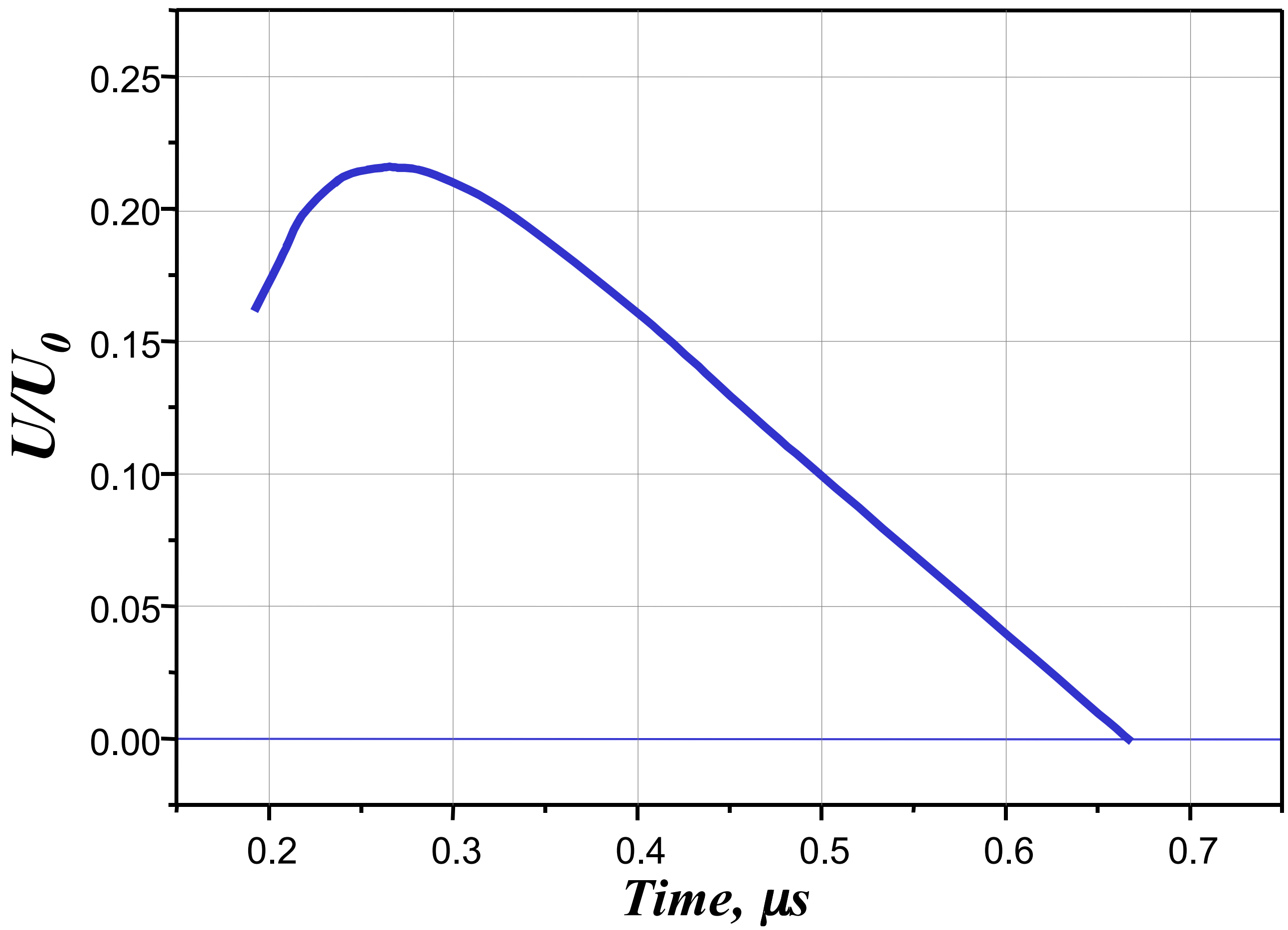


b) *Mass correlated acceleration method.*



Correction Pulse Waveform used in MCA

d_e = 0.36 cm, d_a = 3.2 cm, d_{refl} = 28.1 cm, L_0 = 62.1 cm



Comparison of pulsed extraction and *mass-correlated acceleration* on a linear TOF mass spectrometer

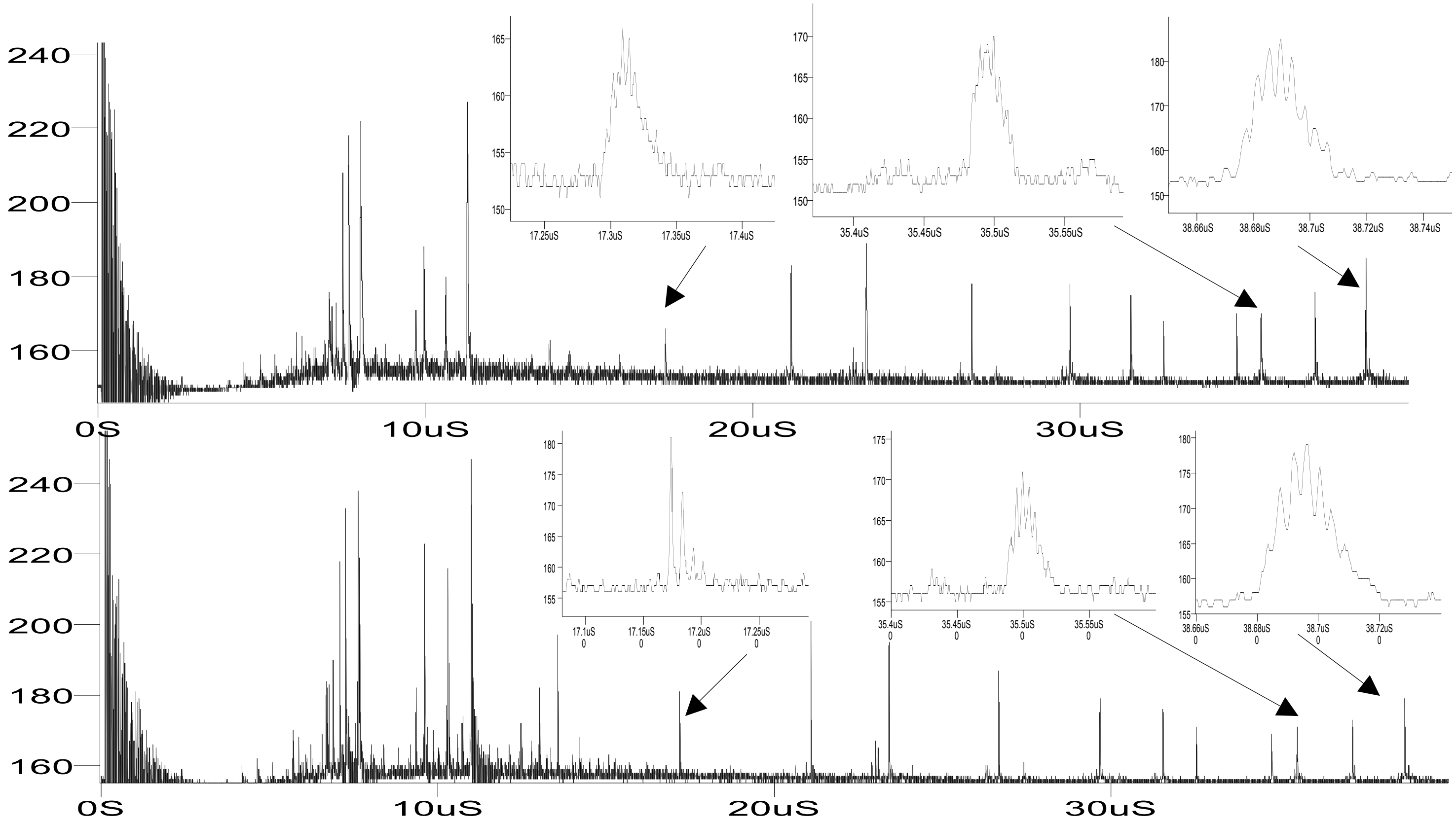


Figure 1. Averaged mass spectra of a mixture of 11 peptides obtained with normal pulsed (delayed) extraction (top) or with *mass-correlated acceleration* (bottom) in a linear TOF. Insets are shown for methionine enkephalin-Arg-Gly-Leu, 900 Da; biocytin-β-endorphin, 3819 Da; ACTH 1-39, 4541Da.

Comparison of pulsed extraction and *mass-correlated acceleration* on a reflectron TOF mass spectrometer

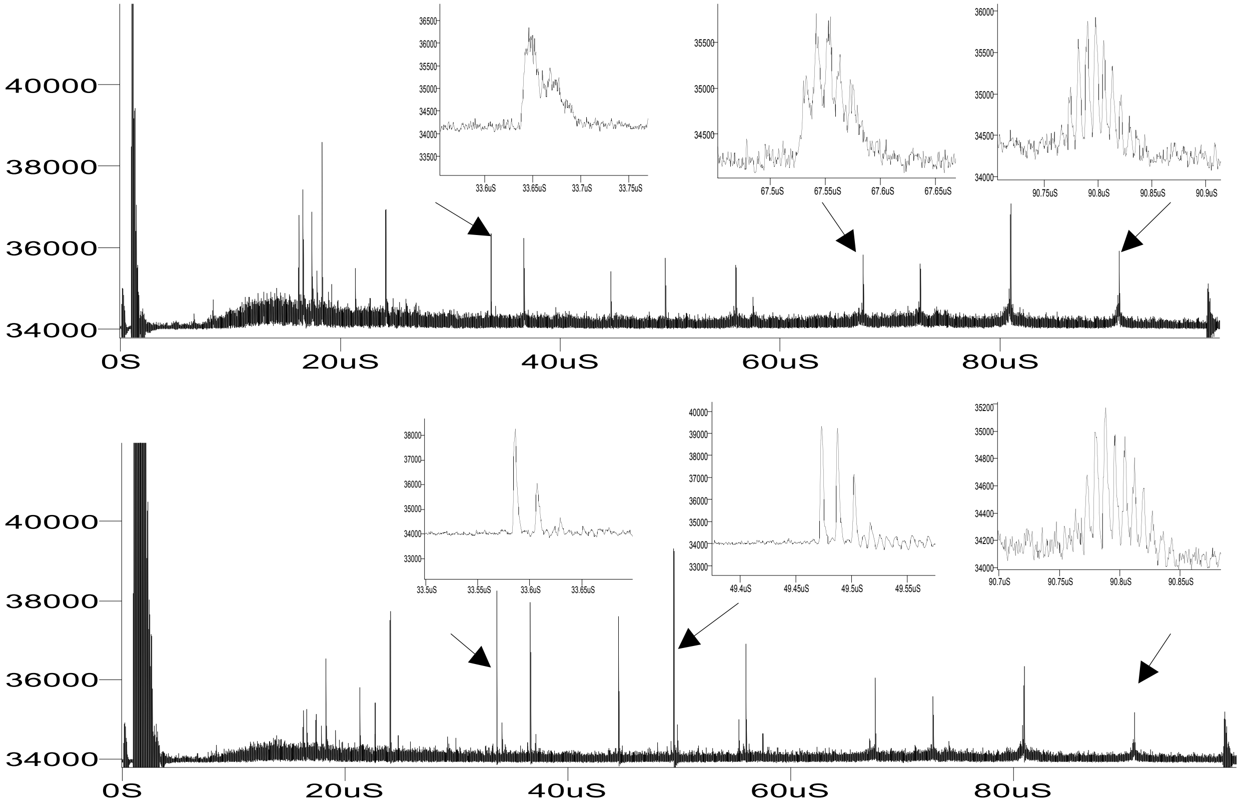
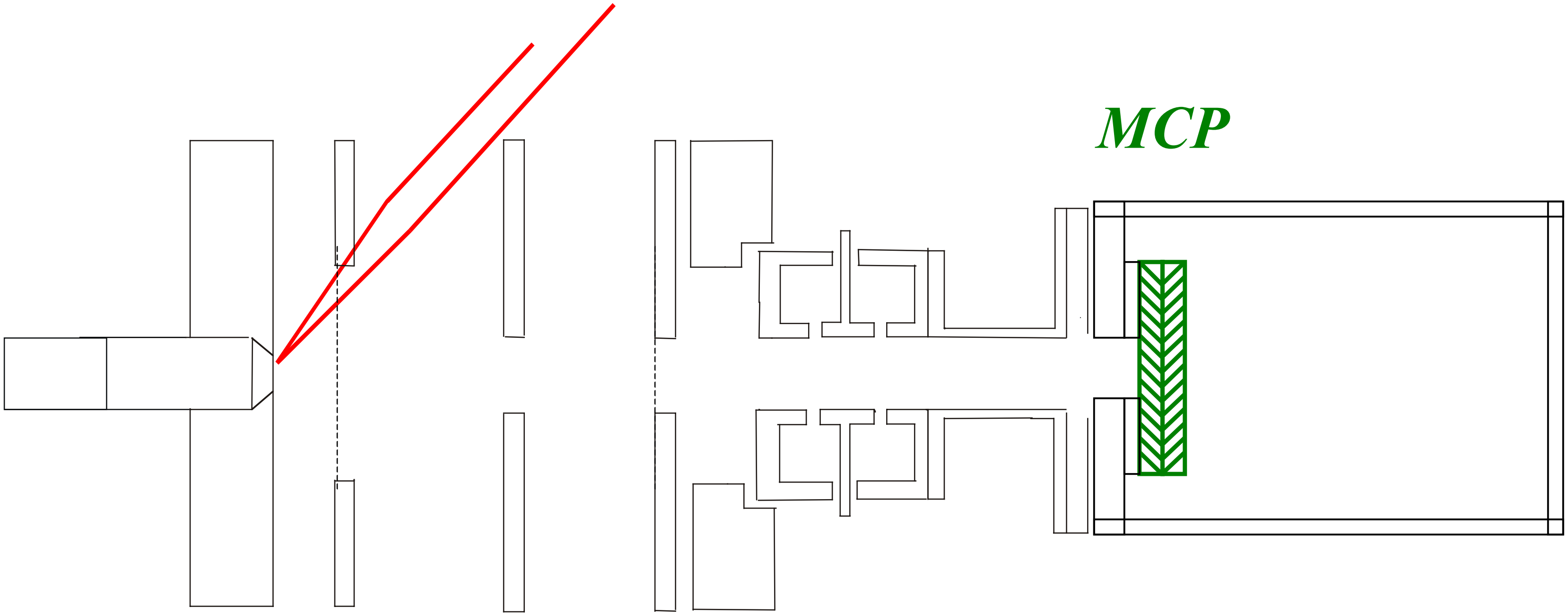


Figure 2. Averaged mass spectra of a mix of 9 peptides obtained with normal pulsed (delayed) extraction (top) or with *mass-correlated acceleration* (bottom) in a reflectron TOF. Insets are shown for bradykinin, fragment 1-7, 758 Da; neurotensin, 1674 Da; somatostatin 28, 3150 Da; insulin, 5734 Da.

Mass correlated acceleration on a miniature MALDI TOF mass spectrometer



Original reflectron instrument, length to detector: 2 meters.
Miniaturized (linear mode) length to detector: ***13 cm (~5.1 in).***

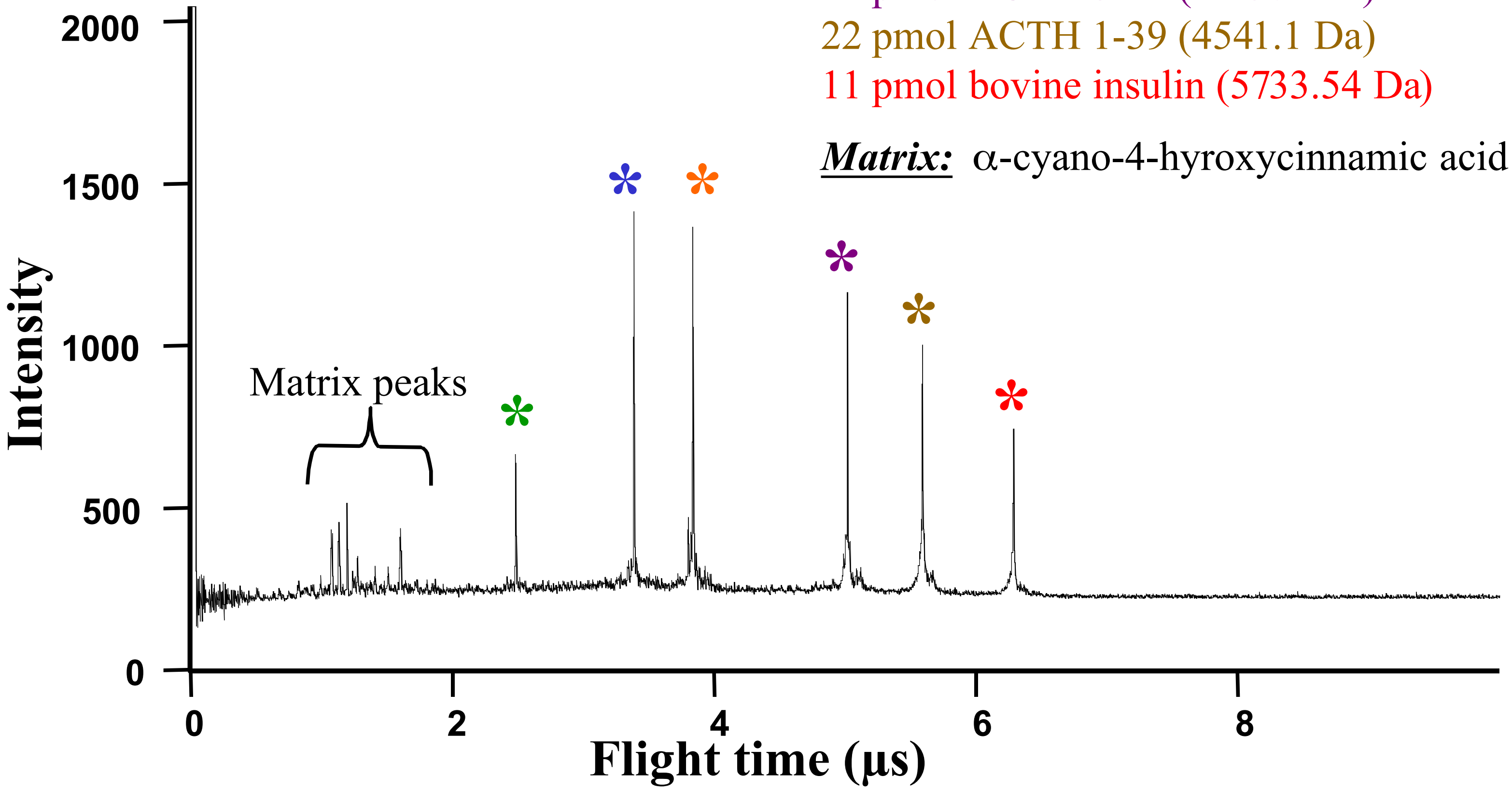
V_{acc} = 17.144 kV, Einzel lens = 1.0 kV, DE pulse voltage = 3.0 kV,
Detector = -2.2 kV, Laser shots = 50 (@ 10 Hz)

Normal pulsed extraction mode

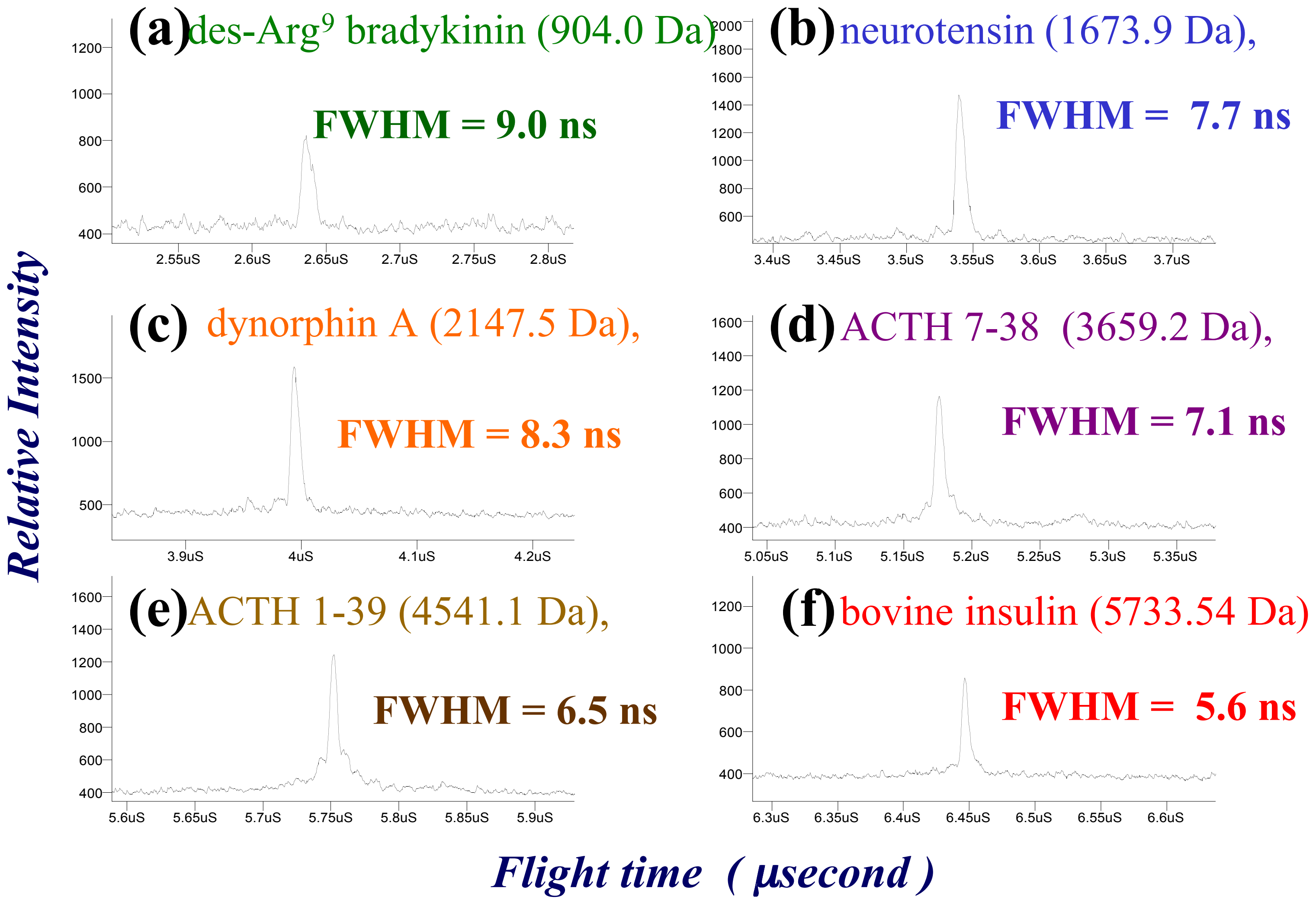
Peptide mixture:

22 pmol des-Arg^9 bradykinin (904.0 Da)
22 pmol neurotensin (1673.9 Da)
11 pmol dynorphin A (2147.5 Da)
32 pmol ACTH 7-38 (3659.2 Da)
22 pmol ACTH 1-39 (4541.1 Da)
11 pmol bovine insulin (5733.54 Da)

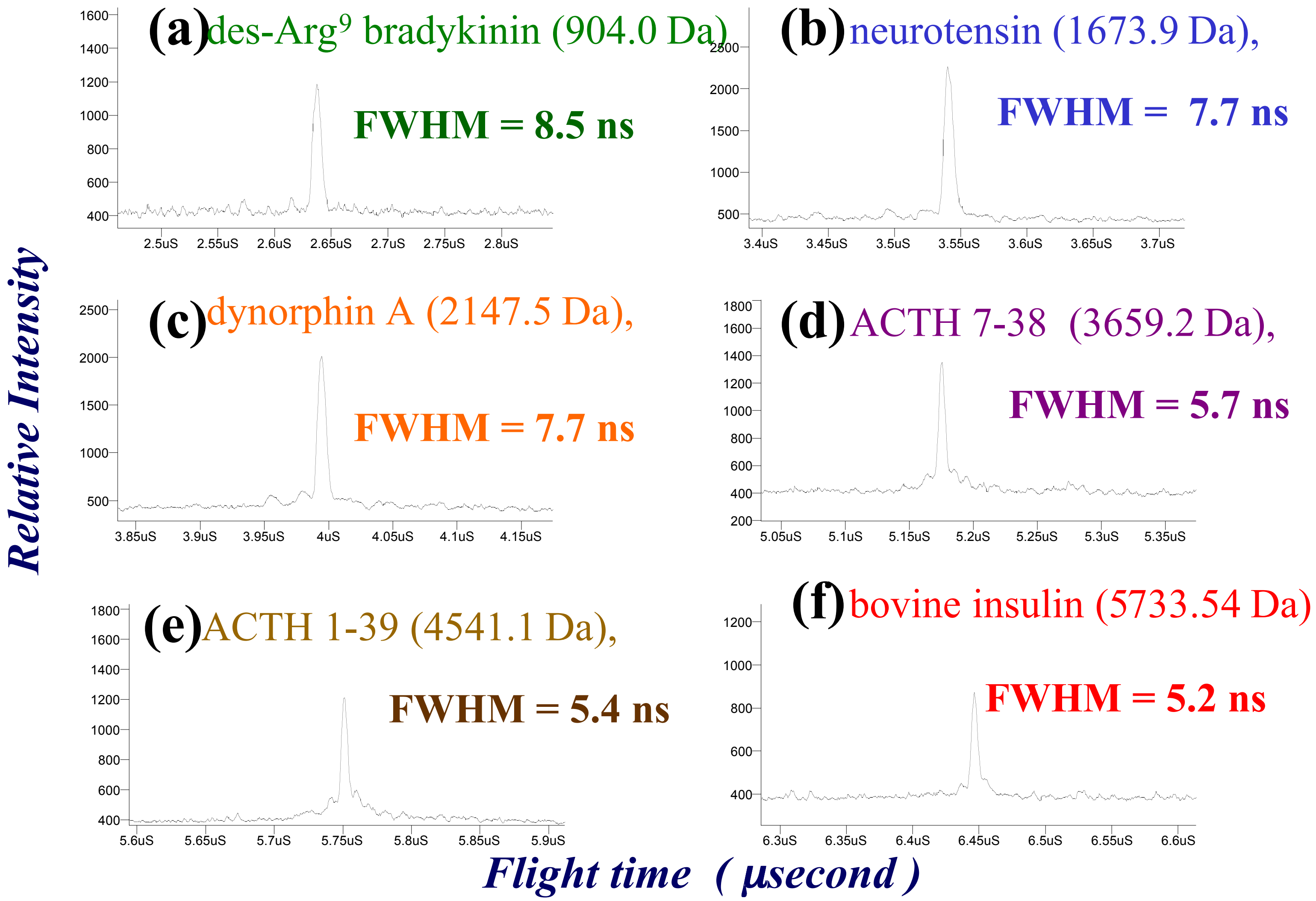
Matrix: α-cyano-4-hyroxycinnamic acid



Pulsed Extraction (no MCA)



Mass-correlated acceleration



(a) des-Arg^9 bradykinin (904.0 Da)

FWHM = 8.5 ns

(b) neurotensin (1673.9 Da),

FWHM = 7.7 ns

(c) dynorphin A (2147.5 Da),

FWHM = 7.7 ns

(d) ACTH 7-38 (3659.2 Da),

FWHM = 5.7 ns

(e) ACTH 1-39 (4541.1 Da),

FWHM = 5.4 ns

(f) bovine insulin (5733.54 Da)

FWHM = 5.2 ns

Relative Intensity

Flight time (μsecond)

Conclusions

- It is possible to obtain mass resolution in excess of 1/1000 on a 3-inch mass spectrometer.
- Dynamic non-linear fields can be used to compensate for the initial ion starting conditions.
- Mass-correlated acceleration provides wider mass range focusing than normal pulsed extraction.

Acknowledgements

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Slava Kovtoun

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National Institutes of Health

[redacted]

In order to maintain good performance for a miniaturized instrument:

1. **Developed a TOF mass spectrometer with a 3-inch mass analyzer in which pulsed extraction is used to focus ions at the detector surface to high order**

   Source does not have to be reduced proportionally

   Detector grid is removed and flight tube floated to channelplate voltage

   Extraction pulse delay time is mass dependent

- **Developed *mass-correlated acceleration* (MCA) to provide broad mass range focusing at a single extraction voltage and delay time**

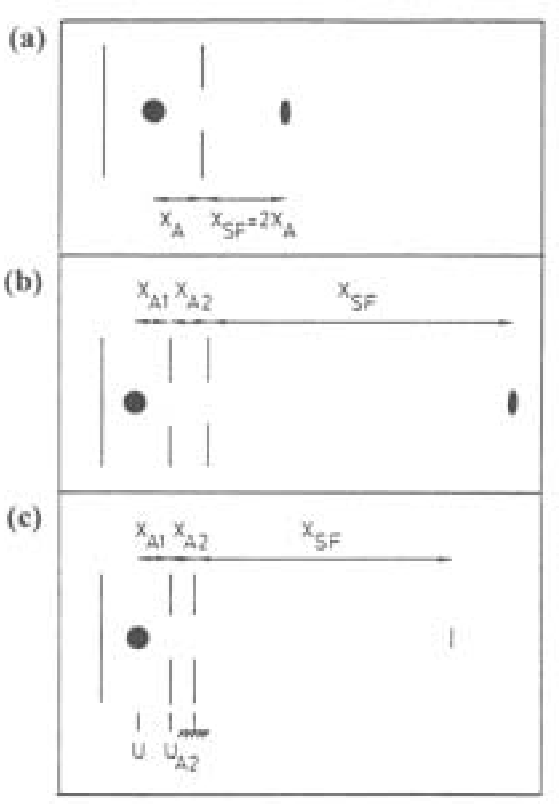
  Comparison with normal pulsed extraction

  Application to tryptic digests

3. **Currently developing MCA on a miniaturized instrument**

Source does not have to be reduced proportionally

Lessons from the “space focusing” problem.



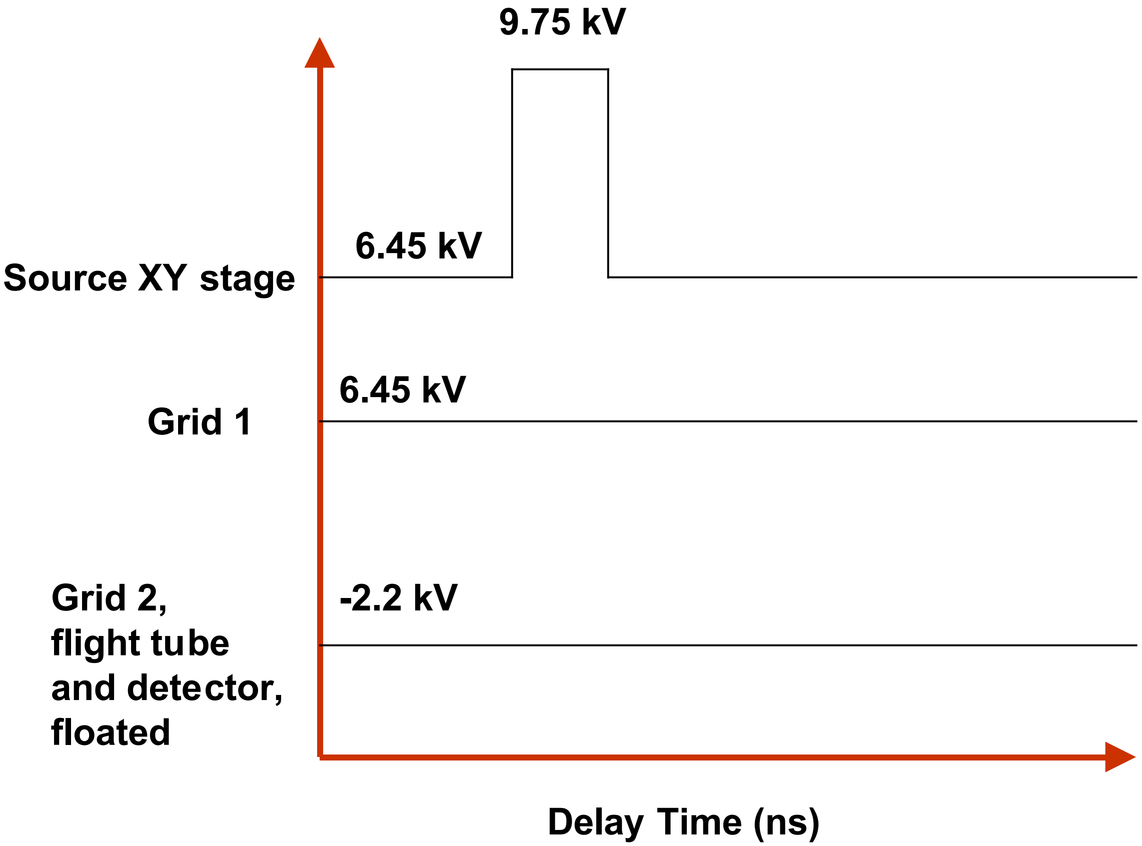
Single-stage, first-order focusing: ions focused at 2X

Dual-stage, first order focusing: can move space focus plane to a much longer distance

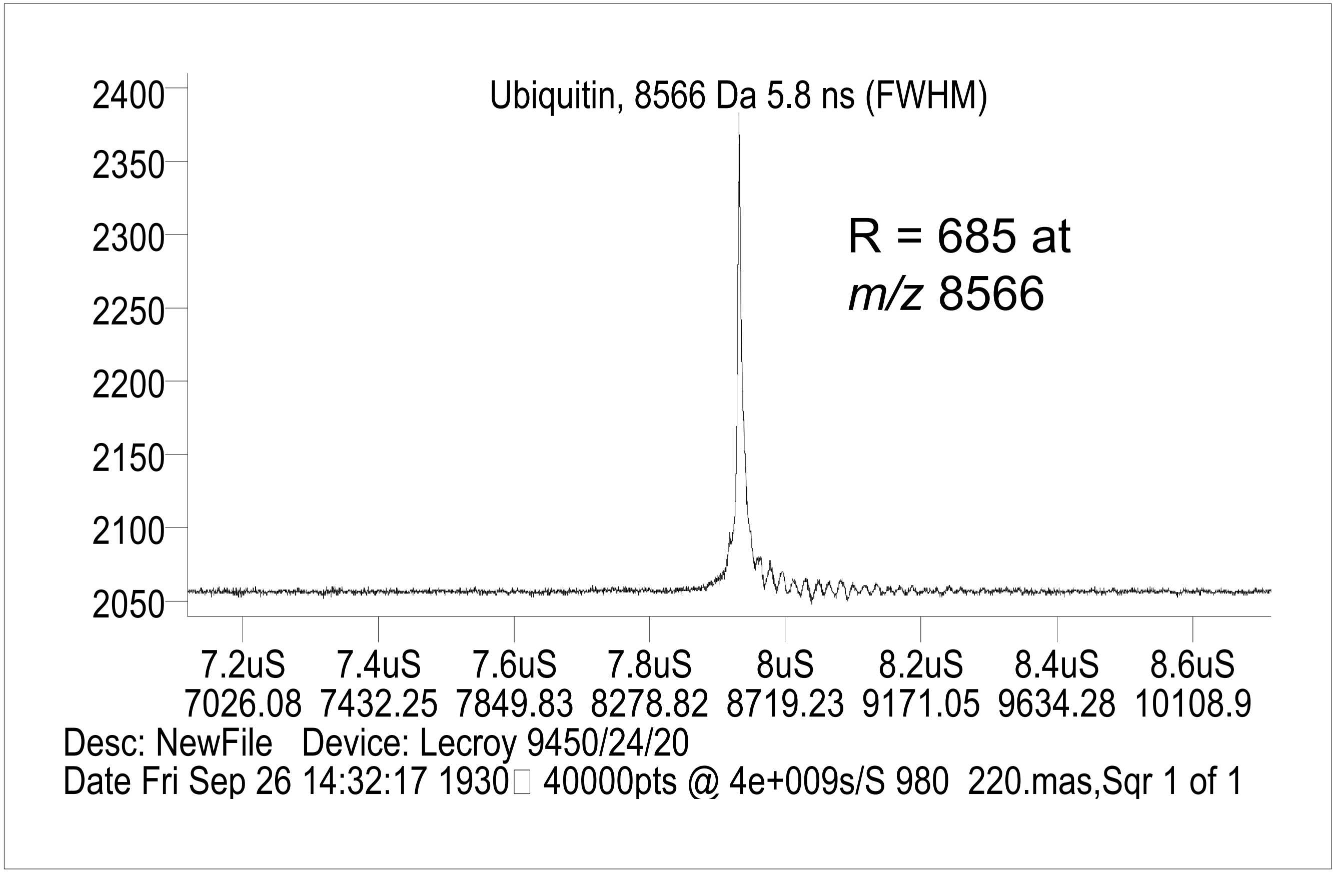
Dual stage, second order focusing: better focusing achieved at a particular distance that is relatively short

Boesl, U.; Weinkauf, R.; Schlag, E.W., Int J. Mass Spectrom. Ion Processes 112 (1992) 121-166.

- Kinetic energy (velocity) distribution is correlated with the spatial distribution so that one can achieve similar higher order focusing
- However, focusing will be mass-dependent

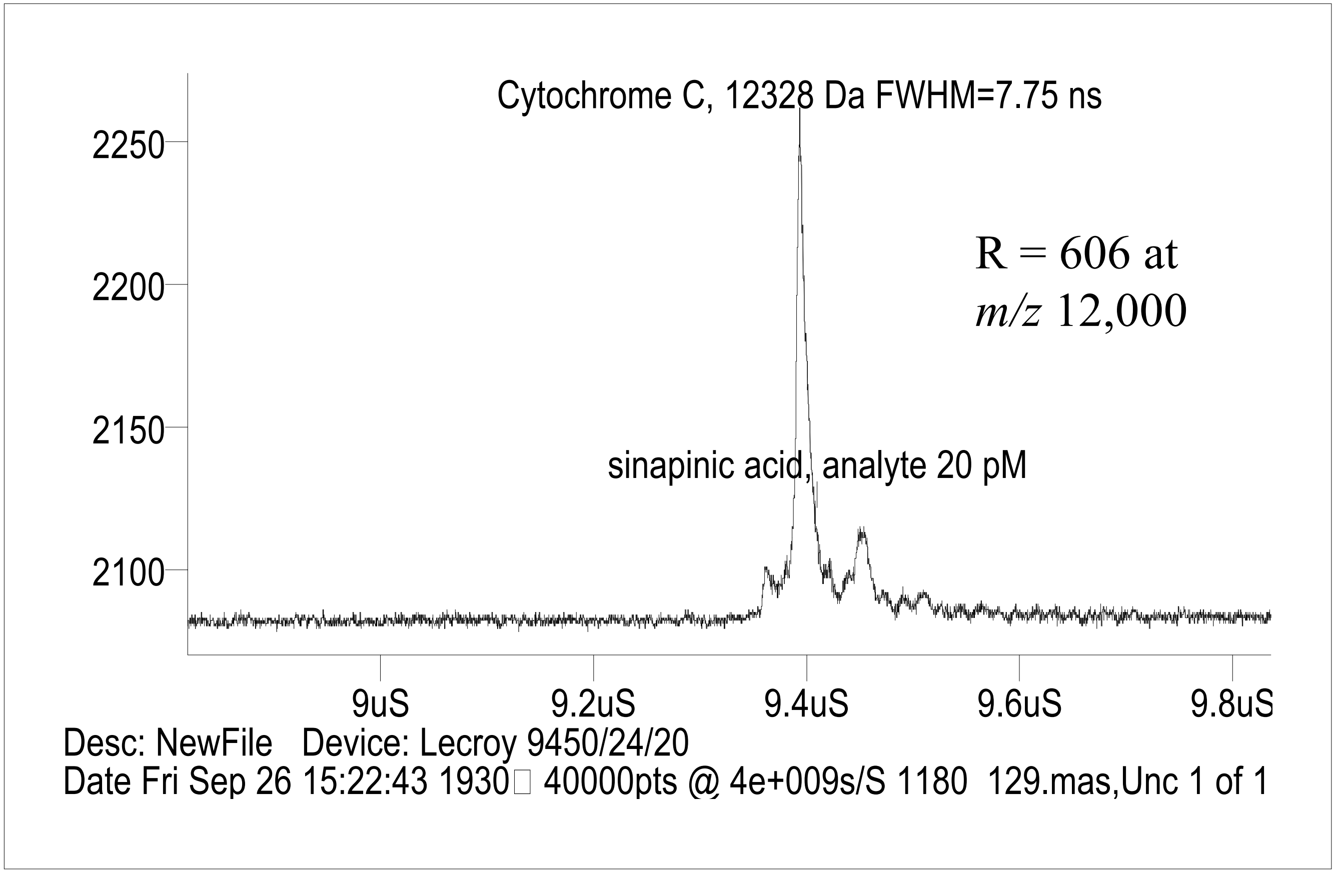


Mass spectrum of ubiquitin on the miniature linear TOF mass spectrometer



R = 685 at
m/z 8566

Mass spectrum of cytochrome C on the miniature linear TOF mass spectrometer

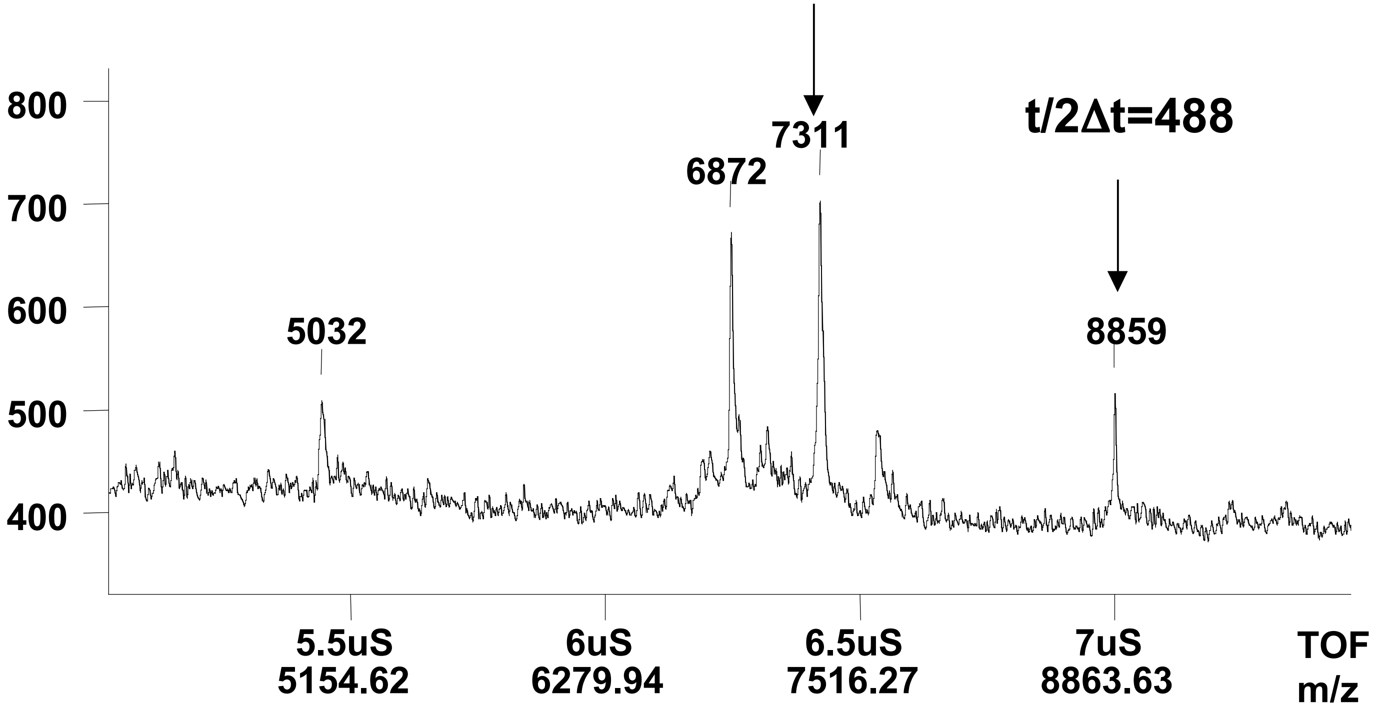


R = 606 at
m/z 12,000

Desc: NewFile   Device: Lecroy 9450/24/20
Date Fri Sep 26 15:22:43 1930 40000pts @ 4e+009s/S 1180 129.mas,Unc 1 of 1

Bacillus globigii spores

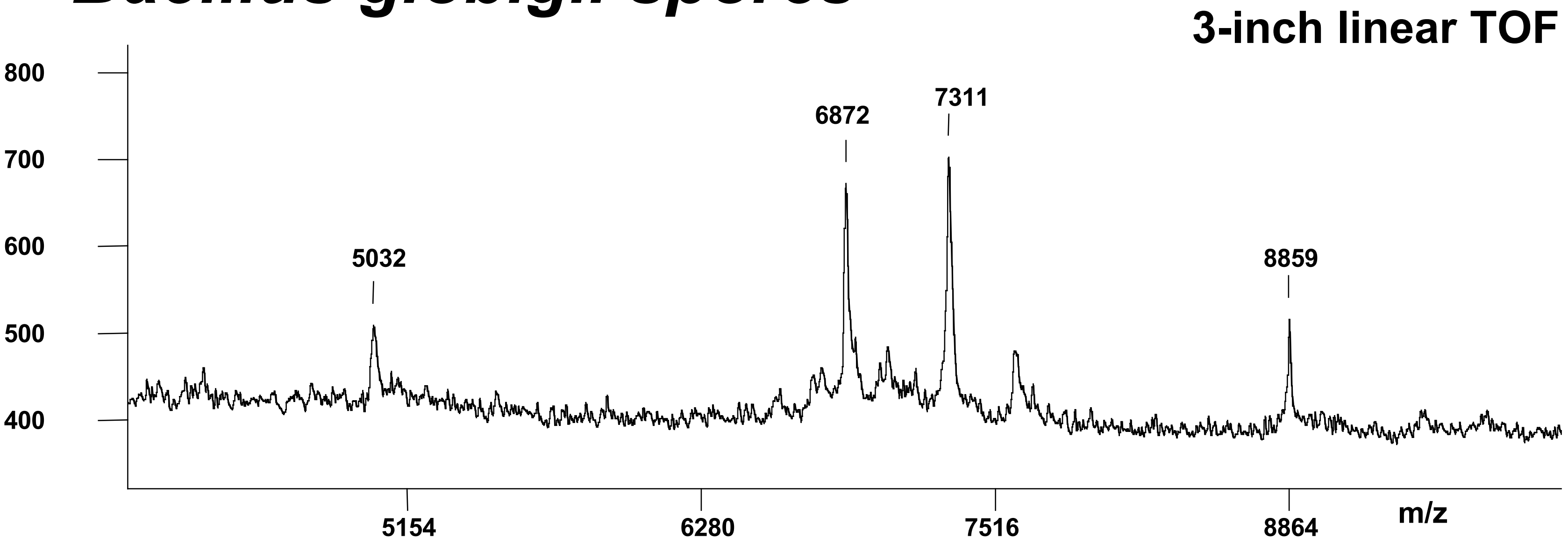
3mg/ml (25% TFA)
saturated α-cyano

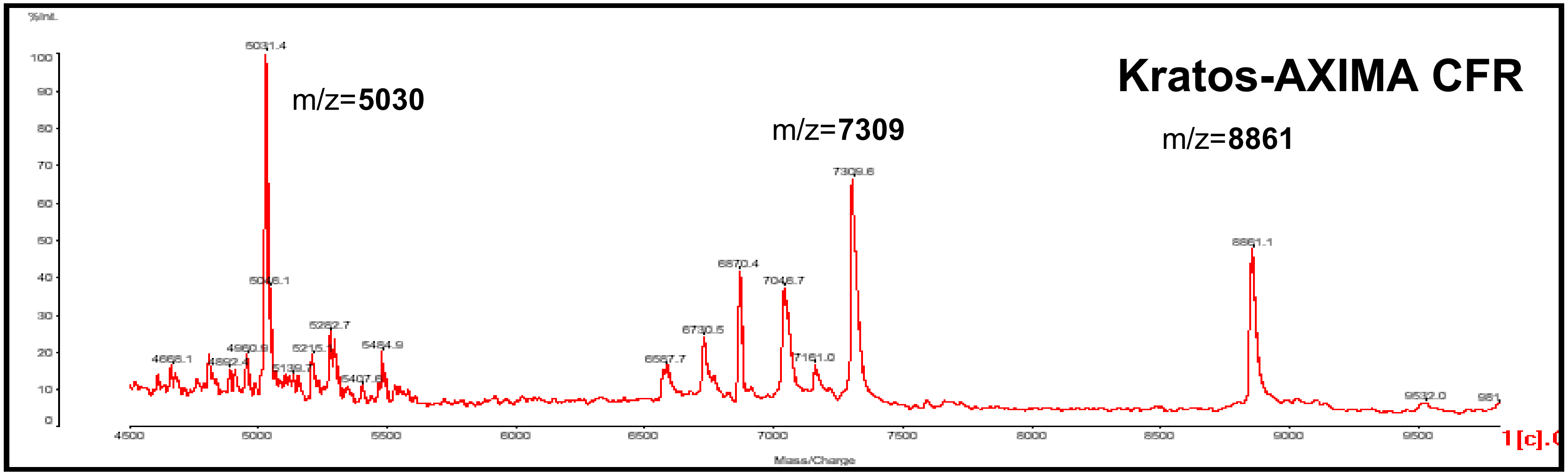


t/2Δt=360

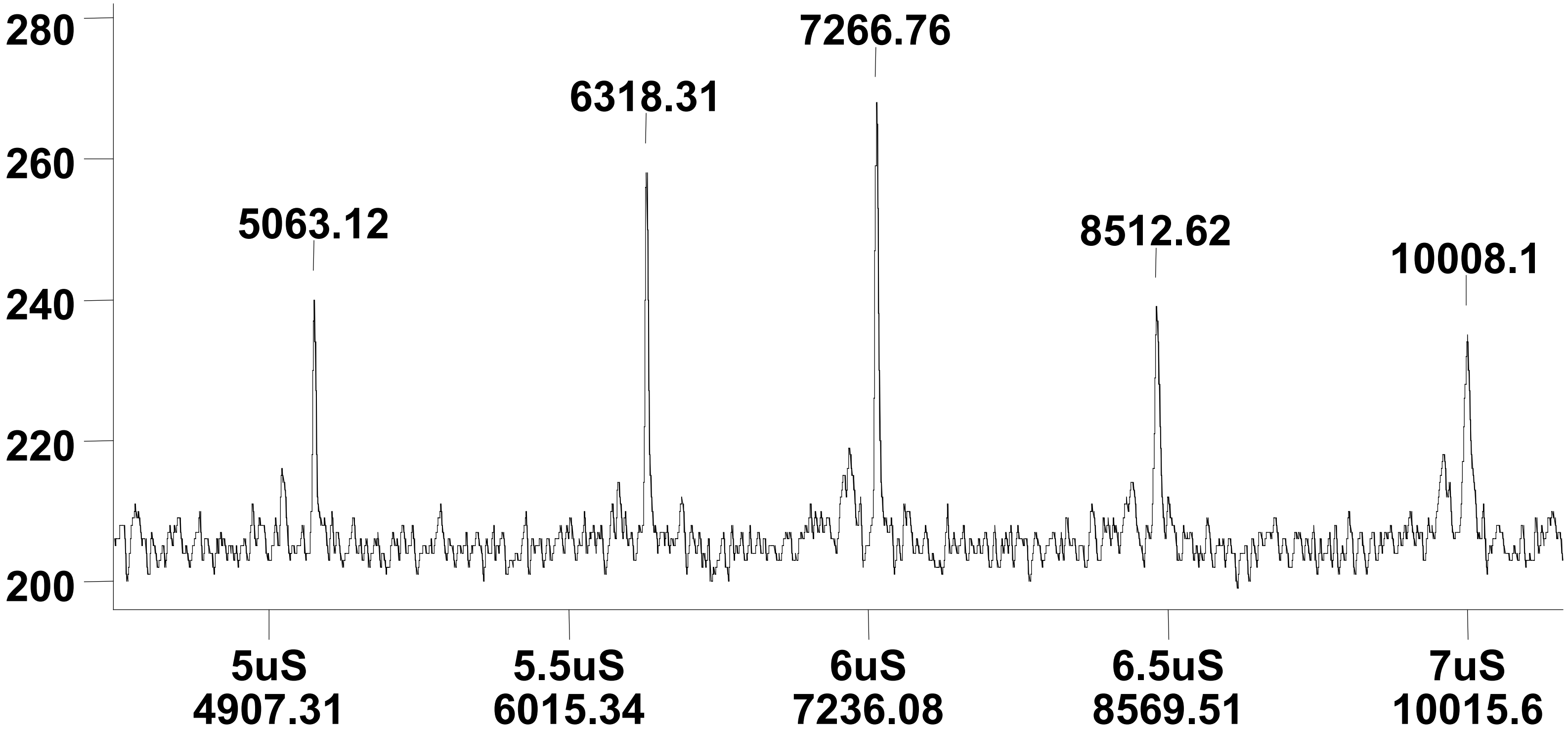
t/2Δt=488

Bacillus globigii spores

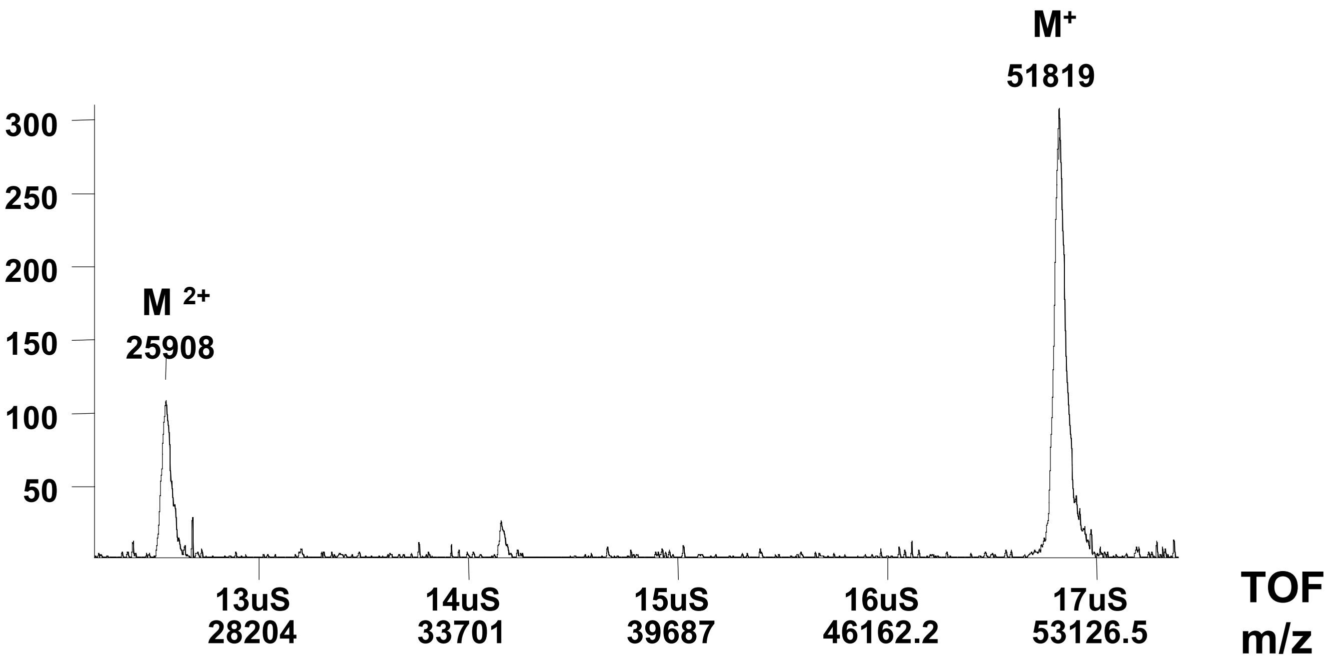




Mass spectrum of an oligonucleotide mixture on the miniature linear TOF MS

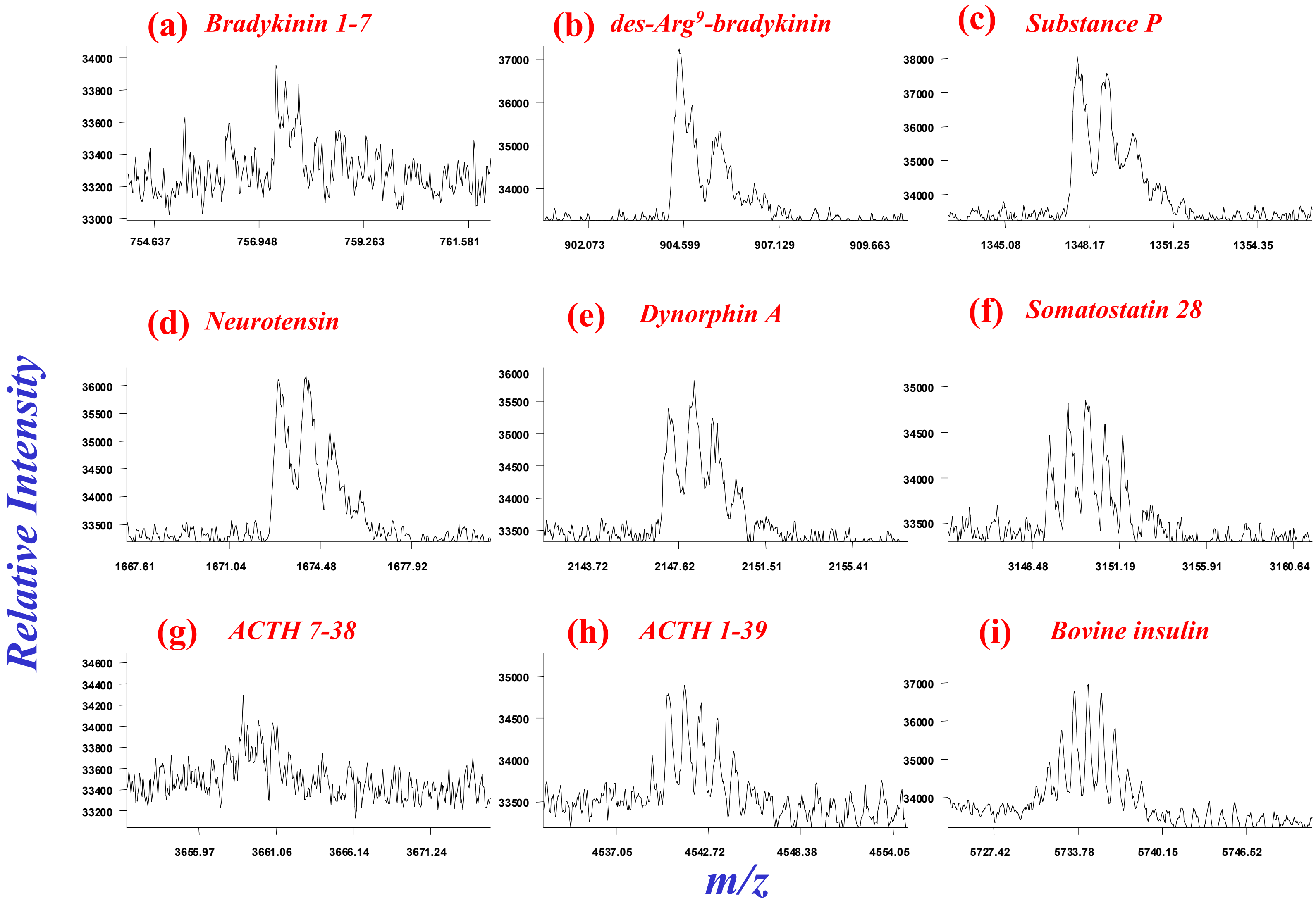


C fragment of tetanus toxin: 52 kDa on the 3-inch linear TOF mass spectrometer

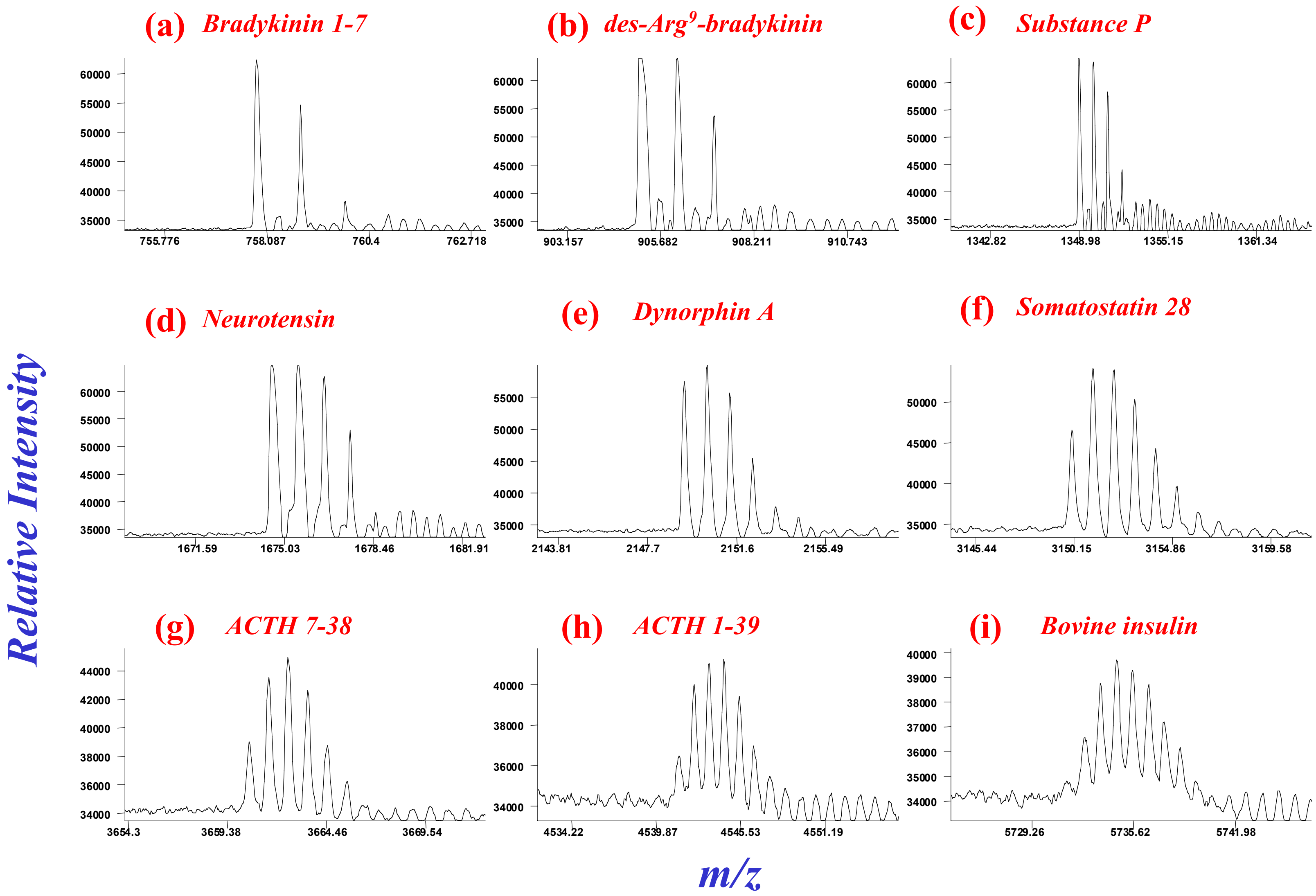


***Mass-correlated acceleration* brings the multiplex advantage to pulsed extraction methods, allowing ions to be focused at high resolution across a broad mass range.**

Pulsed Extraction (no MCA)



With MCA



(a) *Bradykinin 1-7*

(b) *des-Arg^9-bradykinin*

(c) *Substance P*

(d) *Neurotensin*

(e) *Dynorphin A*

(f) *Somatostatin 28*

(g) *ACTH 7-38*

(h) *ACTH 1-39*

(i) *Bovine insulin*

Components of a peptide mixture analyzed in a MCA re-MALDI TOF mass spectrometer

Peptide Name	**Monoisotopic $(M+H)^+$**	**Peak Width (ns)**	**Mass Resolution**
Bradykinin, frag. 1-7	757.40	2	8395
des-Arg^9-bradykinin	904.47	2	9154
Substance P	1347.74	2	11124
Neurotensin	1672.92	3	8245
Dynorphin A	2147.20	3	9323
Somatostatin 28	3147.47	3	11257
ACTH 7-38	3657.93	3	12120
ACTH 1-39	4539.27	3	13487
Insulin (bovine)	5730.61	3	15132

Excellent mass resolution is maintained over a broad mass range

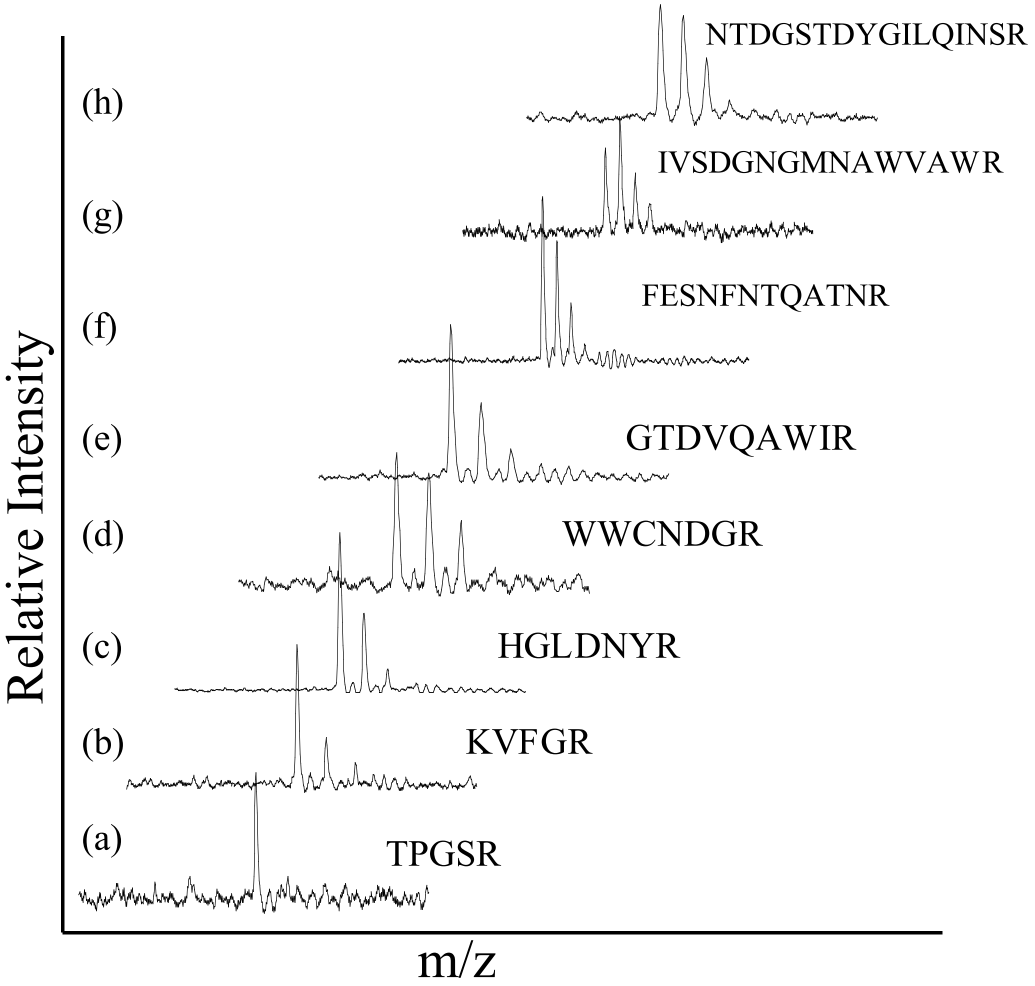
Linear two-point calibration

Peptide Name	**Calibration 1**		**Calibration 2**		**Calibration 3**	
	$(M+H)^+$	Δm	$(M+H)^+$	Δm	$(M+H)^+$	Δm
Bradykinin, frag. 1-7	calibrant	---------	756.9	-0.5	754.8	-2.6
des-Arg^9-bradykinin	904.6	+0.1	904.1	-0.4	902.0	-2.5
Substance P	1348.2	+0.5	calibrant	---------	1345.6	-2.1
Neurotensin	1673.6	+1.0	1673.2	+0.3	1671.1	+1.8
Dynorphin A	2148.2	+0.7	2147.8	+0.6	2146.0	-1.2
Somatostatin 28	3148.5	+1.0	3148.2	+0.7	3147.2	-0.3
ACTH 7-38	3658.7	+0.8	3658.6	+0.7	calibrant	---------
ACTH 1-39	calibrant	---------	calibrant	---------	4539.5	+0.2
Insulin (bovine)	5728.8	-1.8	5729.1	-1.5	calibrant	---------

In subsequent instruments a non-linear instrument function will be used for 2 point calibration

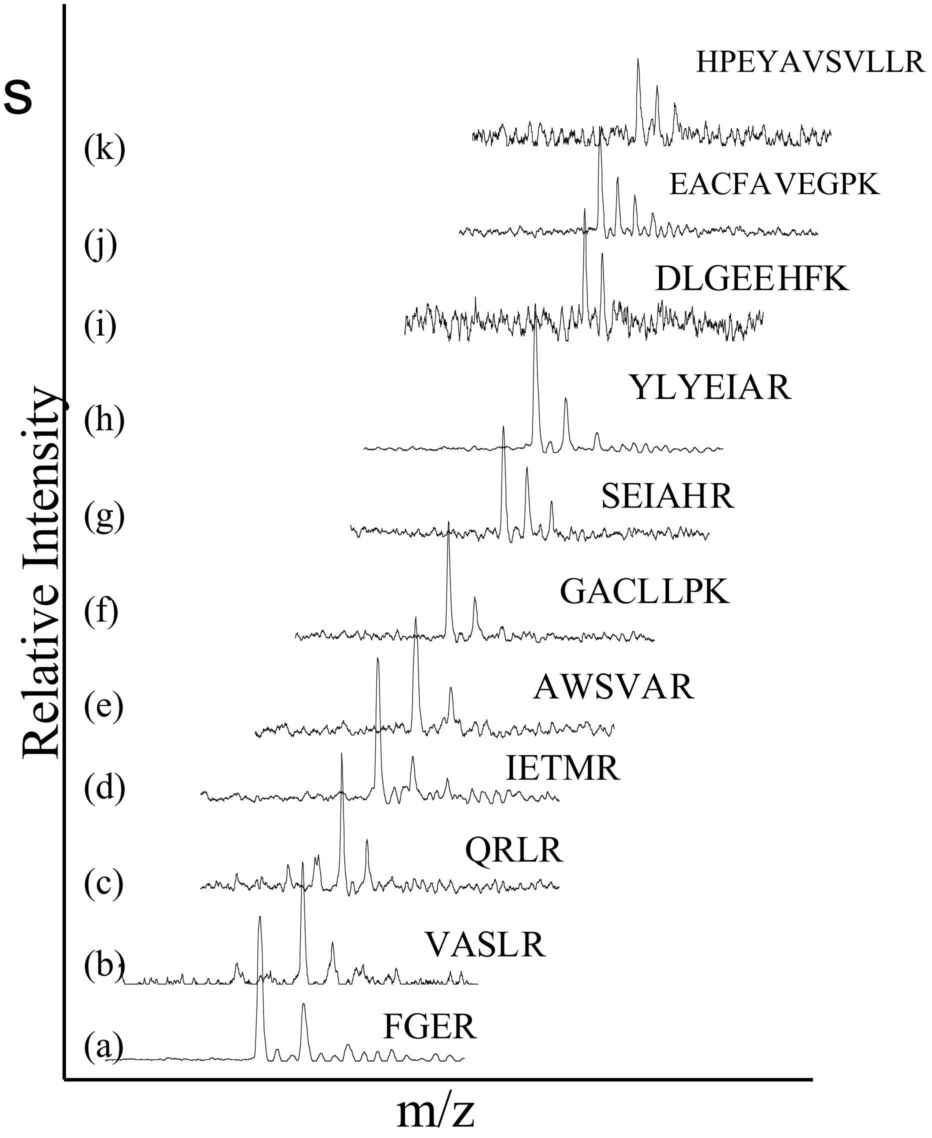
Using *mass-correlated acceleration*, peptides in a lysozyme tryptic digest are all focused

Mass	Resol.
517.5	4693
606.7	5067
874.9	6049
937.0	6254
1046.1	6598
1429.4	7684
1676.8	8310
1754.8	8495

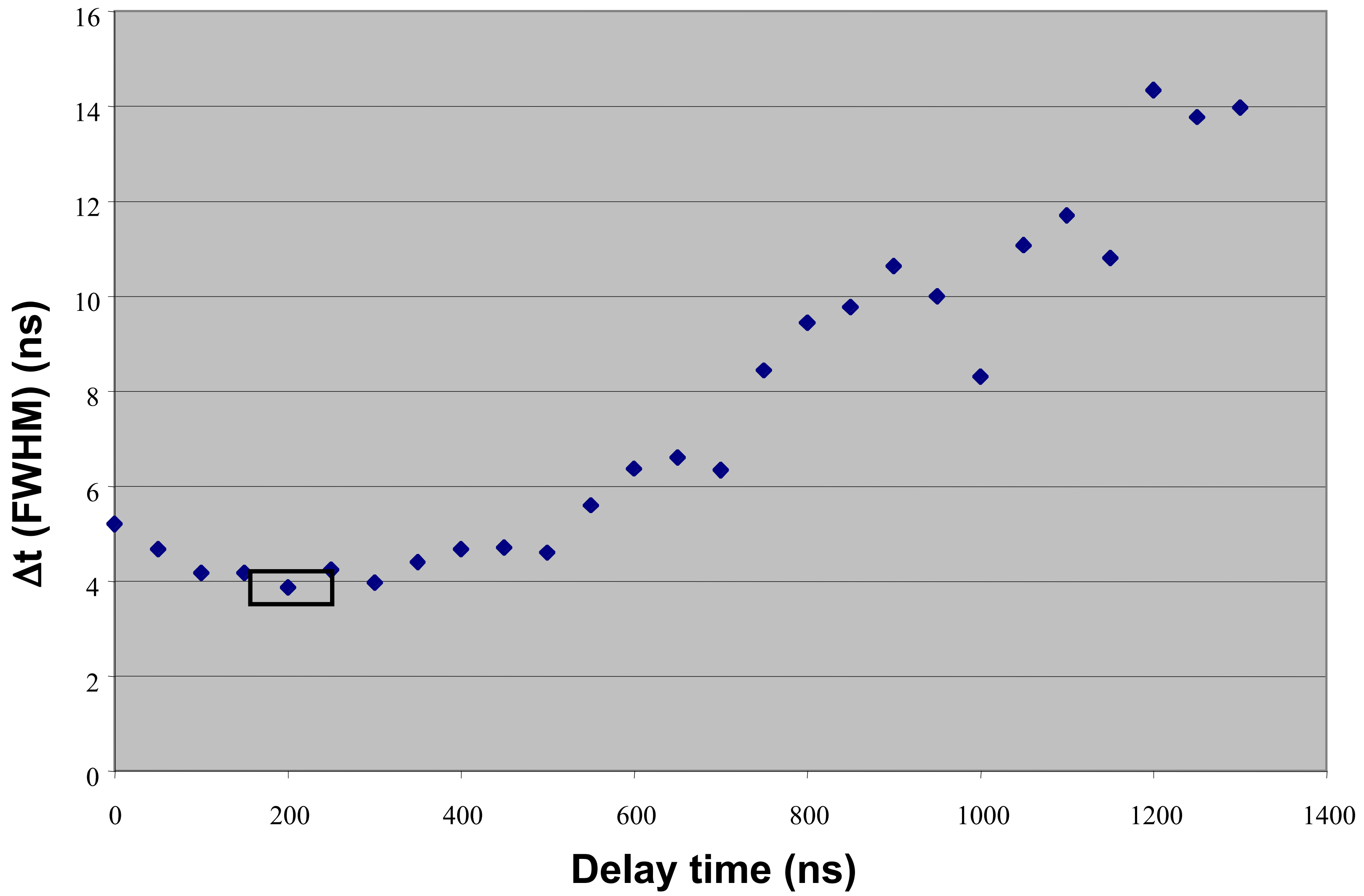


Using *mass-correlated acceleration*, tryptic peptides from BSA are all focused:

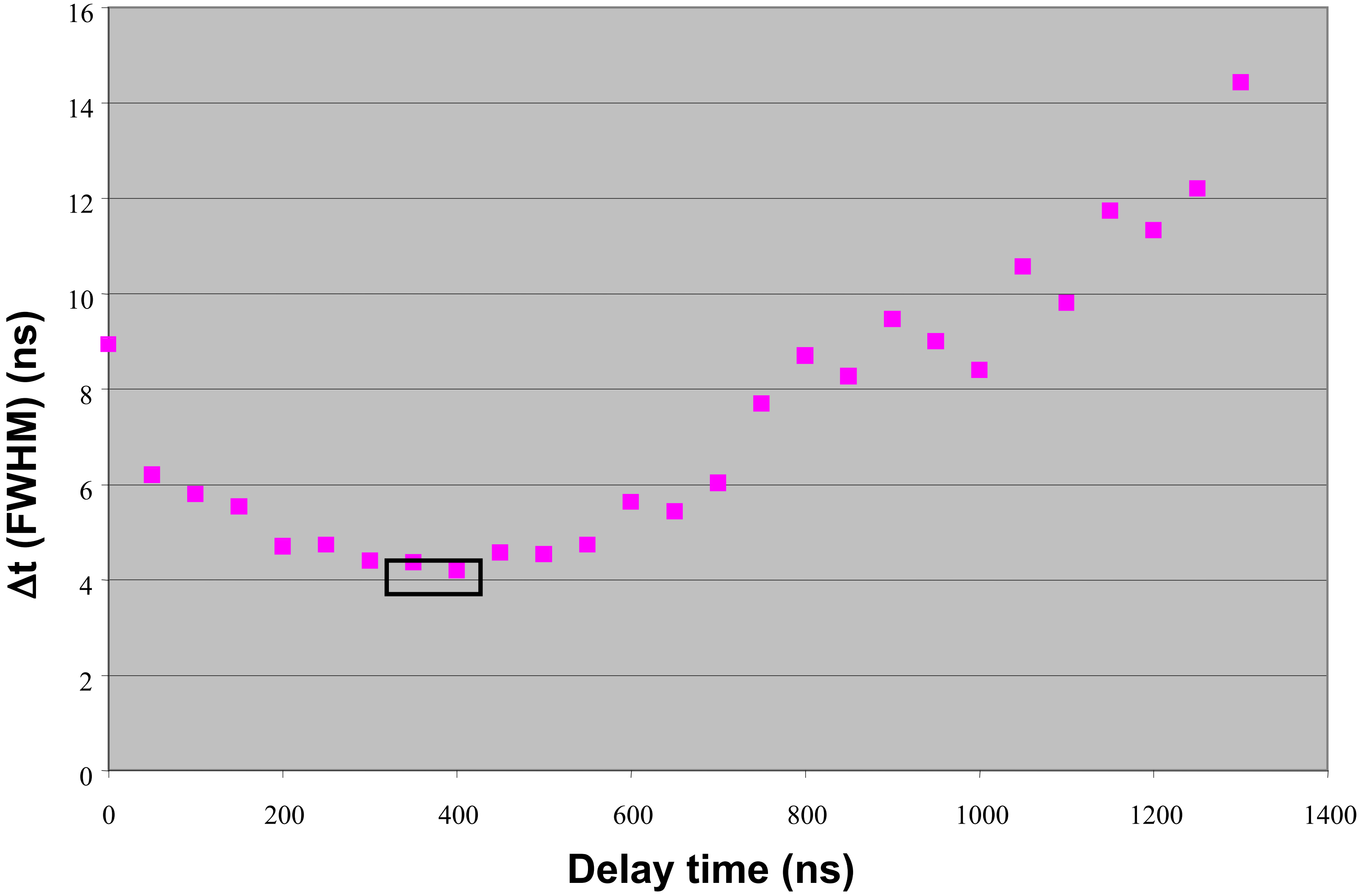
Mass	Resol.
508.5	4653
545.6	4813
572.6	4927
649.6	5237
689.7	5391
701.9	5436
712.7	5477
928.0	6225
975.0	6376
1051.2	6613
1284.5	7293



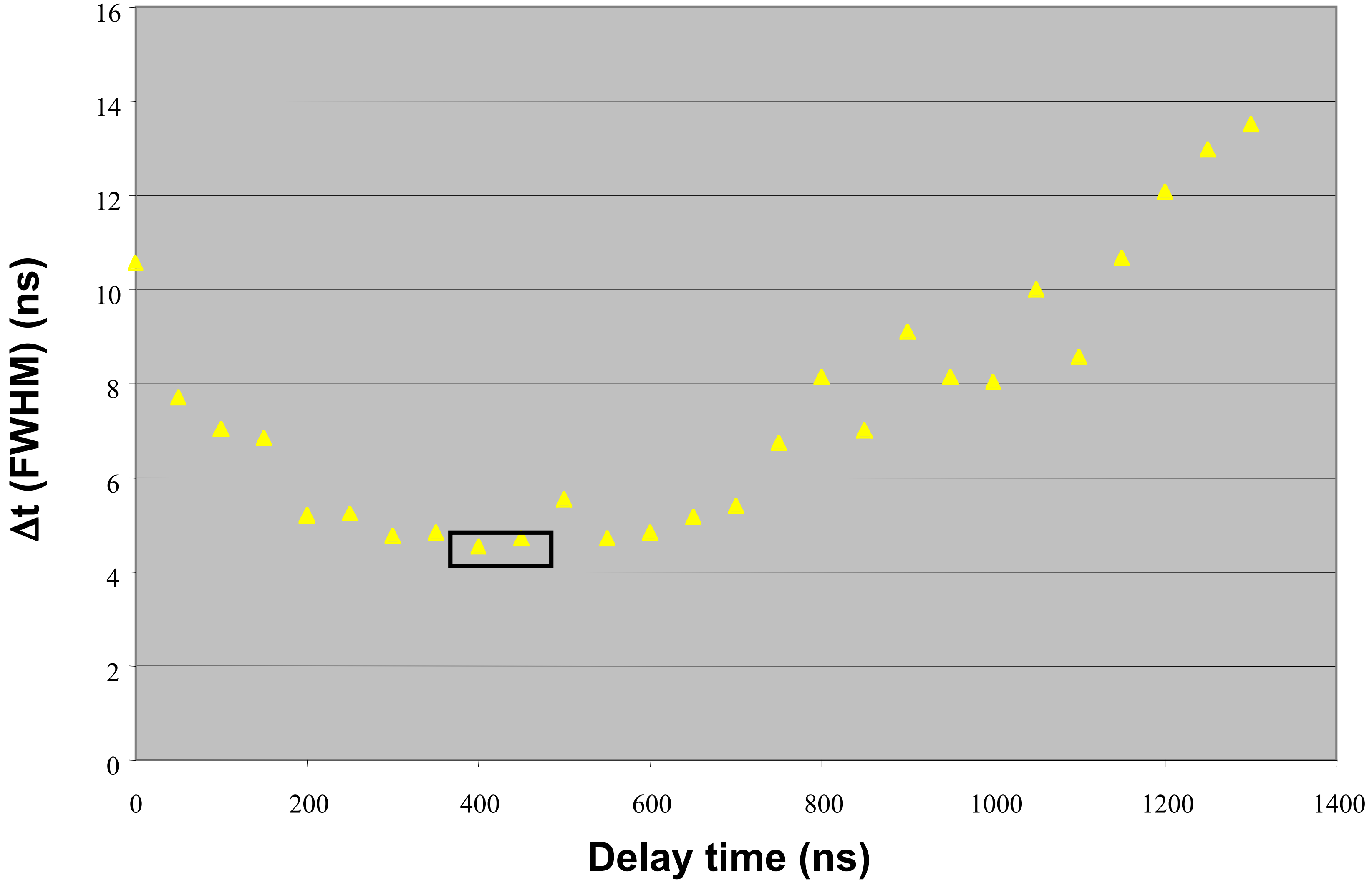
des-Arg^9-bradykinin (904.0 Da)



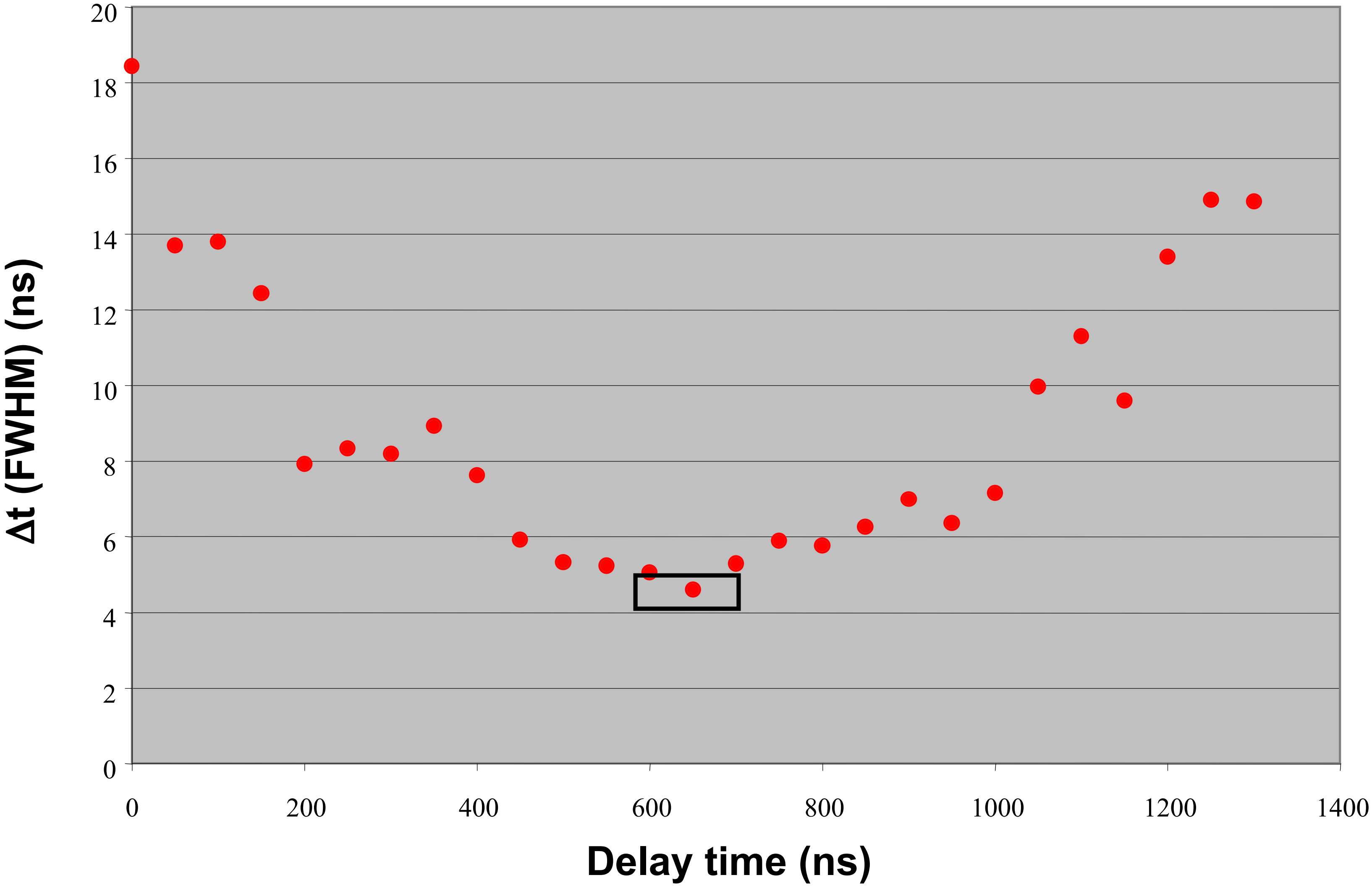
Neurotensin (1673.9 Da)



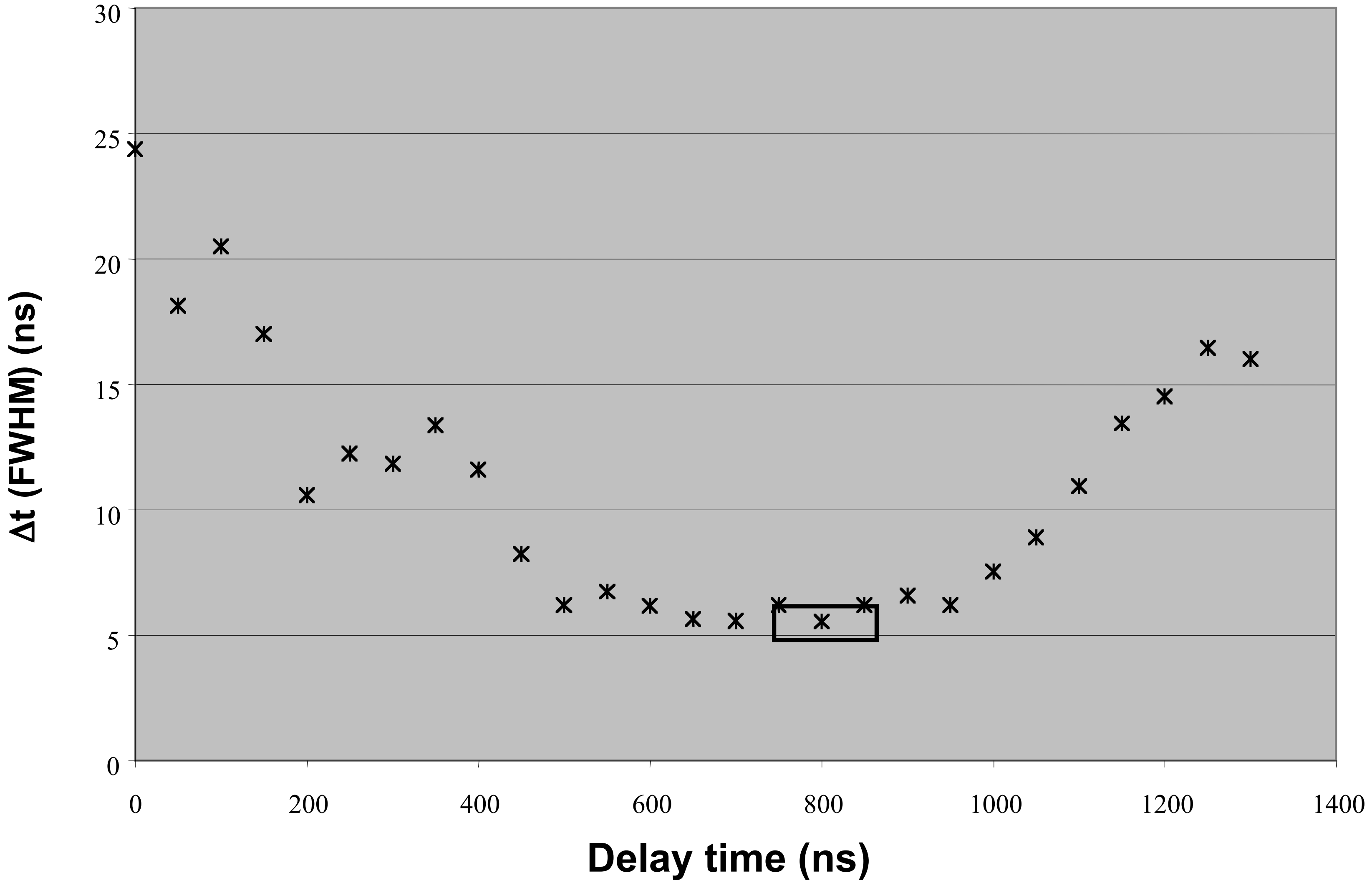
Dynorphin A (2147.5 Da)



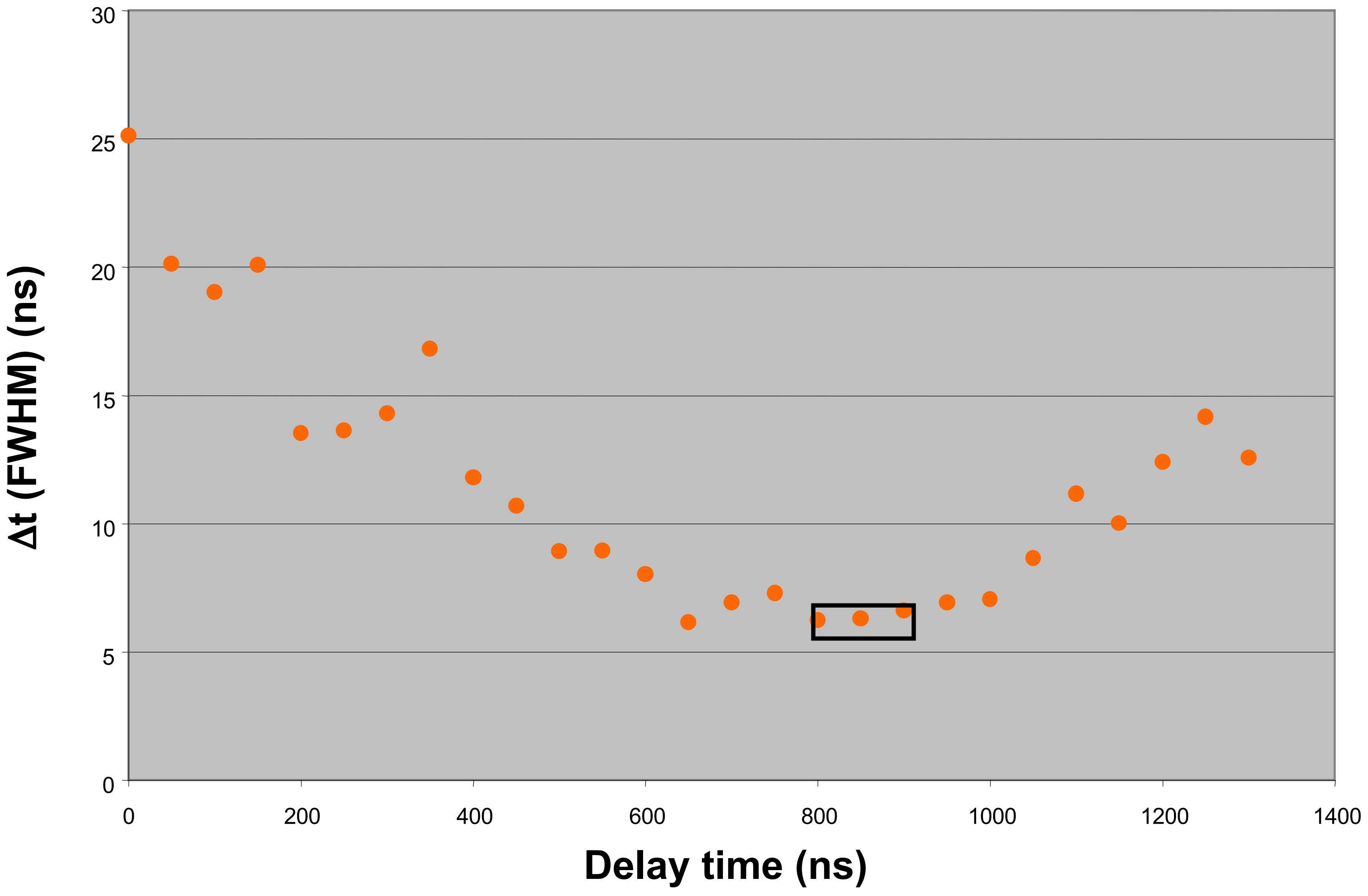
ACTH 7-38 (3659.2 Da)



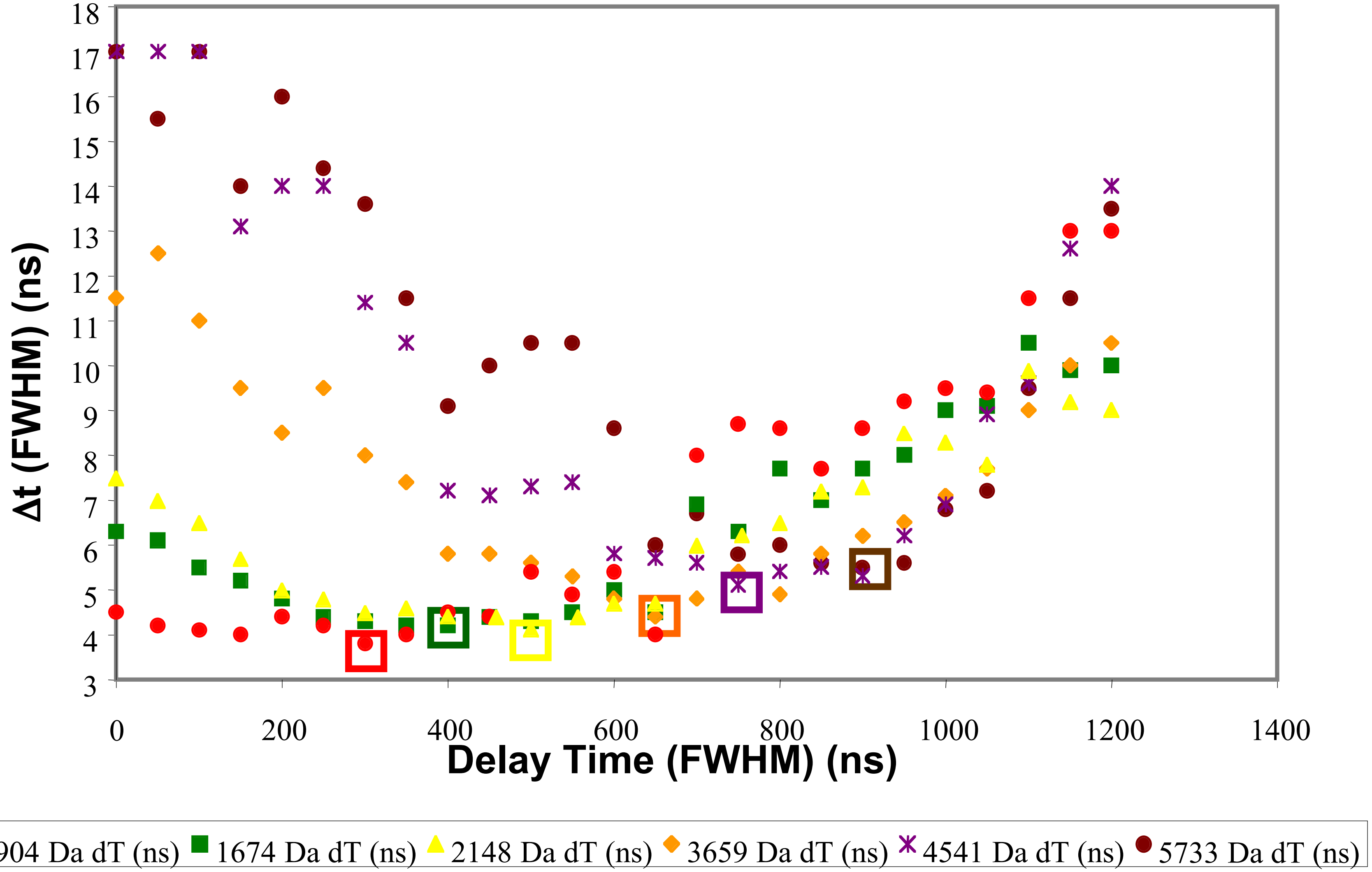
ACTH 1-39 (4541.1 Da)



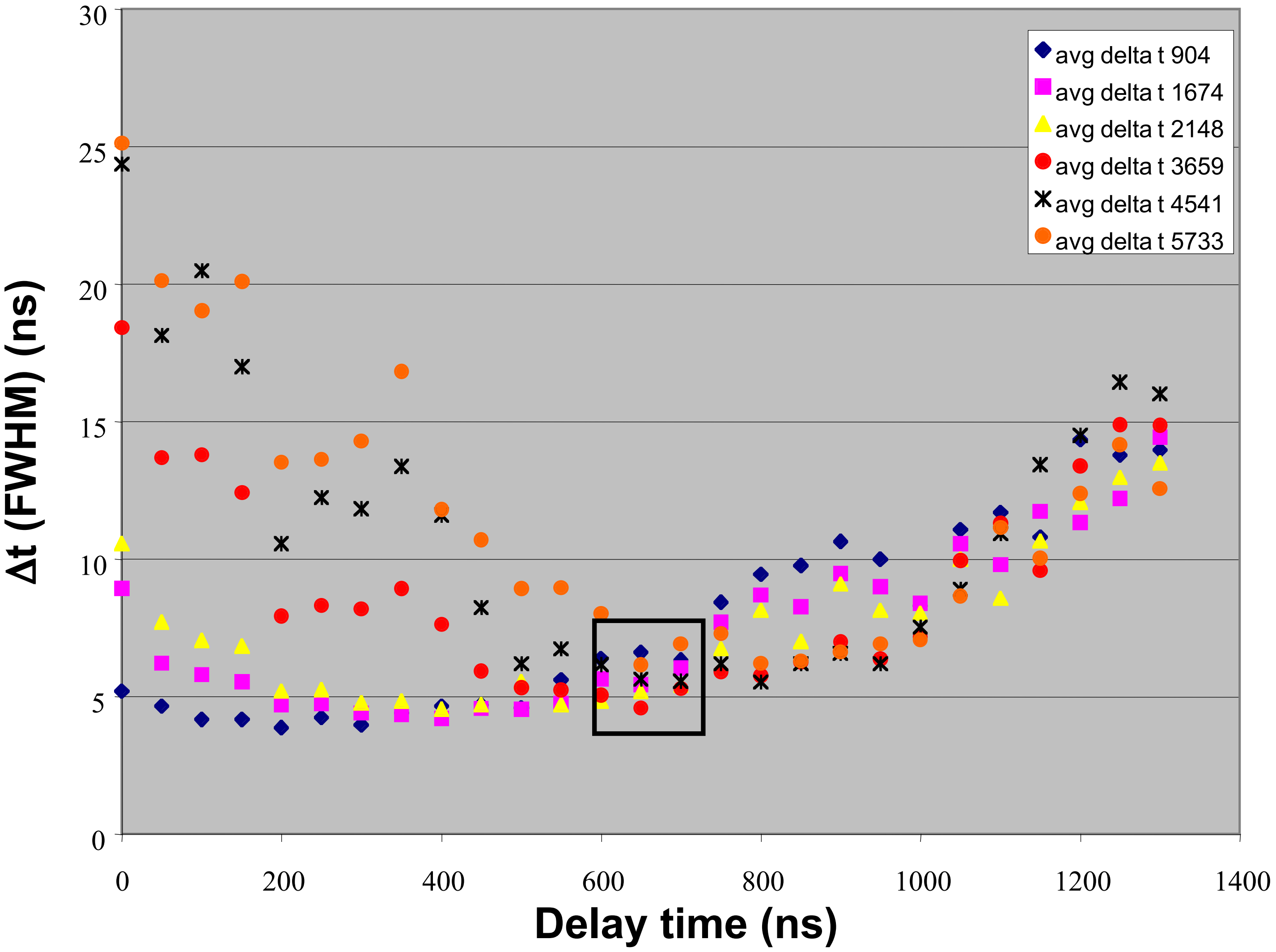
Bovine insulin (5733.54 Da)



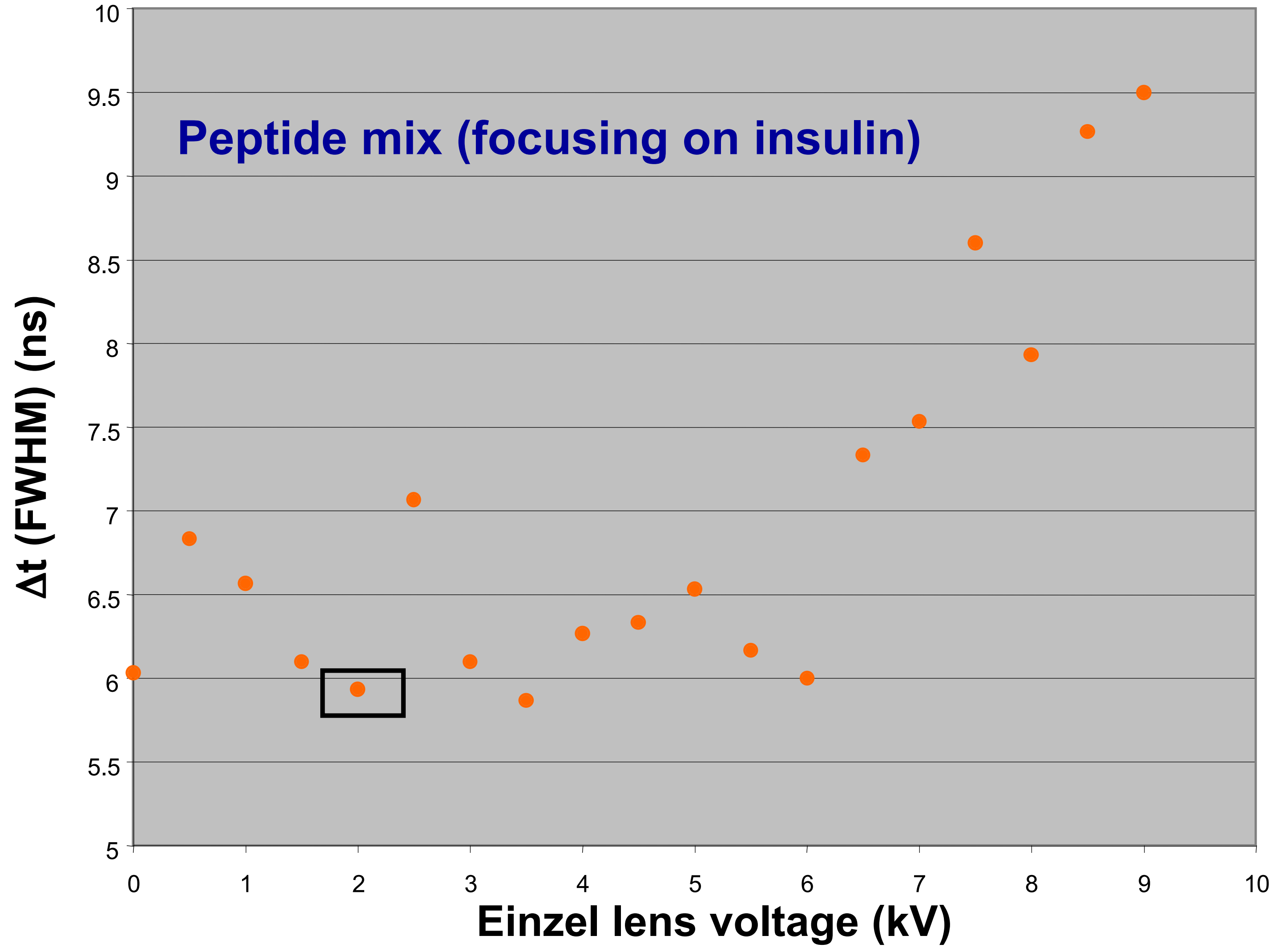
Summary for 6 Peptide Mix + HCCA



Possible to use a single delay time



Resolution dependence on Einsel lens focusing



Comparison of peak widths between PE and MCA*

M+H+	PE	MCA
904	9.0 ns	8.5 ns
1674	7.7 ns	7.7 ns
2148	8.3 ns	7.7 ns
3659	7.1 ns	5.7 ns
4541	6.5 ns	5.4 ns
5733	5.6 ns	5.2 ns

* tuned on m/z 5733