MULTIPLEXED ION MOBILITY SPECTROMETRY-TIME-OF-FLIGHT MASS SPECTROMETRY

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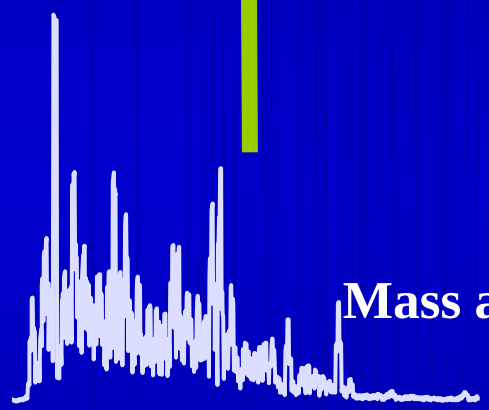
Faster separations with MS provide a basis for higher throughput measurements….

Sample size and
MS dynamic range



Mass accuracy

Separation power

Lower limit of detection

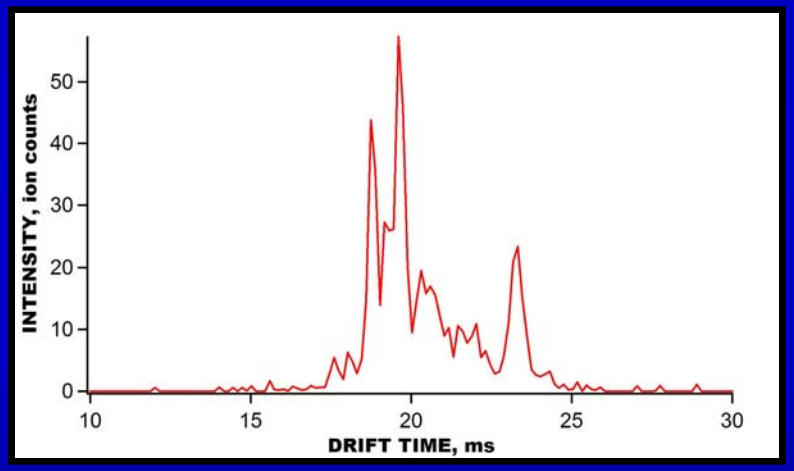
As throughput increases:

- Cost/analysis decreases
- More replicates feasible
- Complexity more addressable
- New applications enabled

ION MOBILITY SPECTROMETRY (IMS)

Operation:

Output of IMS:



$$t_{drift} = \frac{L}{k\,E}$$ **Drift time**

$$k = \frac{3}{16\sqrt{2\pi}} \frac{Ze}{\Omega_{av}\, N_density \sqrt{\mu k_b T}}$$ **Mobility**

$$\Delta t = \sqrt{t_{init}^2 + \left(\frac{t_{drift}}{R_d}\right)^2}$$ **Temporal spread**

$$R_d = \sqrt{\frac{LEZe}{16 k_b T \ln 2}}$$ **Thermal diffusion-limited maximum resolution**

LIMITATIONS OF SIGNAL AVERAGING APPROACH

- **Inefficient ion accumulation prior to IMS**

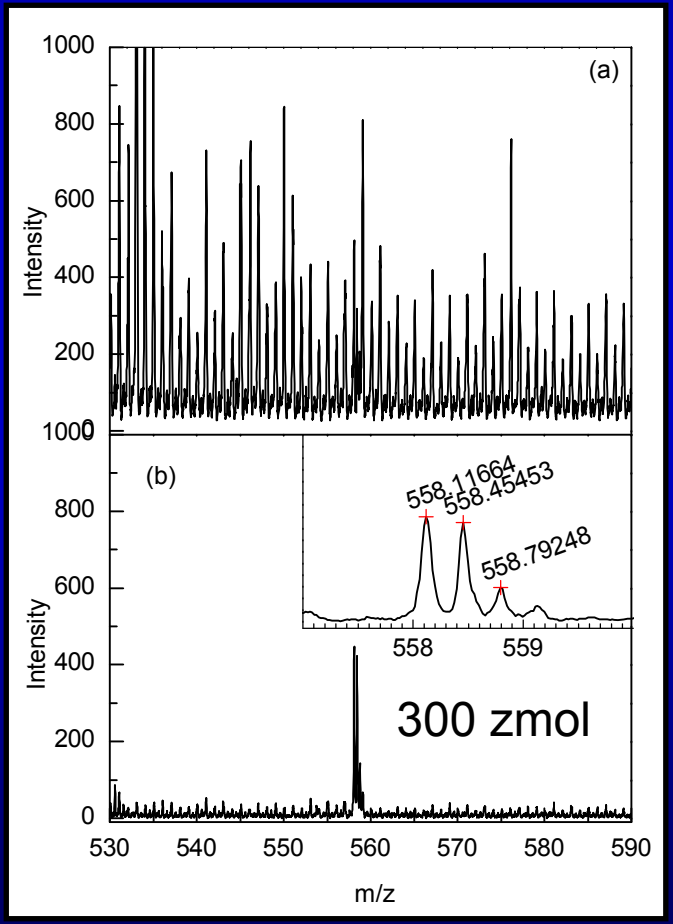
  Ibrahim, Y. *et al* WPE 082

- **Poor interfacing to TOF MS**

  High-Sensitivity Ion Mobility Spectrometry/Mass Spectrometry Using Electrodynamic Ion Funnel Interfaces. Tang, K. *et al. Anal. Chem.,* **2005**, *77* (10), 3330-3339.

- **Low duty cycle (0.1-1 %)**

  Multiplexed Ion Mobility Spectrometry-Orthogonal Time-of-Flight Mass Spectrometry
  Belov, M. *et al. Anal. Chem.,* **2007,** *79* (6), 2451-2462.



MULTIPLEXING ADVANTAGE

If there are *n* unknowns and a *Hadamard* matrix of order *n* is used as a chemical balance or (+ -1) -weighing design, then the mean square error in each unknown is reduced by a factor of *n*;

or SNR is increased by a factor of

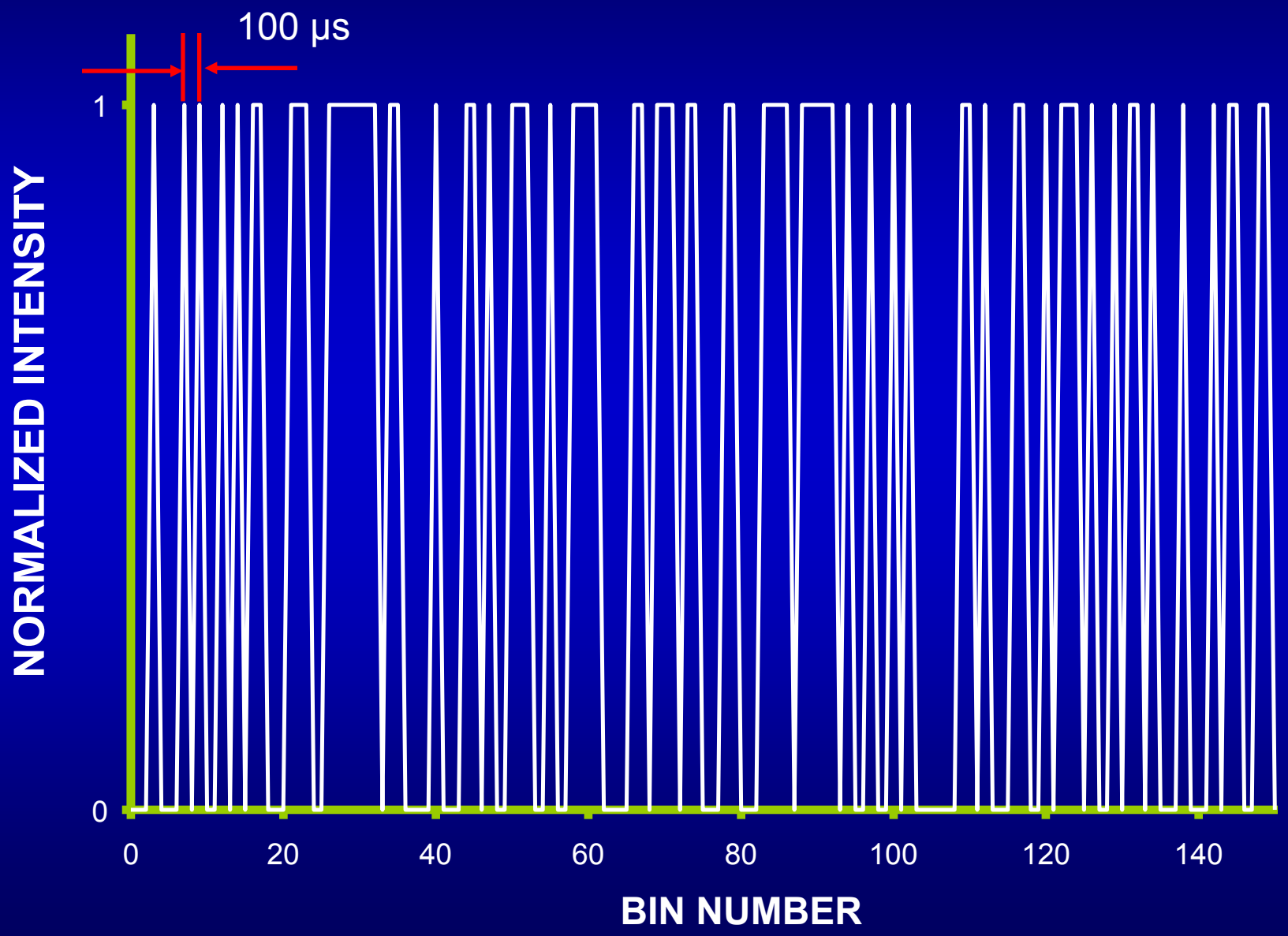
$$\sqrt{n}$$

If there *n* unknowns and an S- matrix of order *n* is used as a spring balance or (0,1) -weighing design, then the mean square error in each unknown is reduced by a factor of *n/4*; or SNR is increased by a factor $(n+1)^2/4n \sim n/4$,

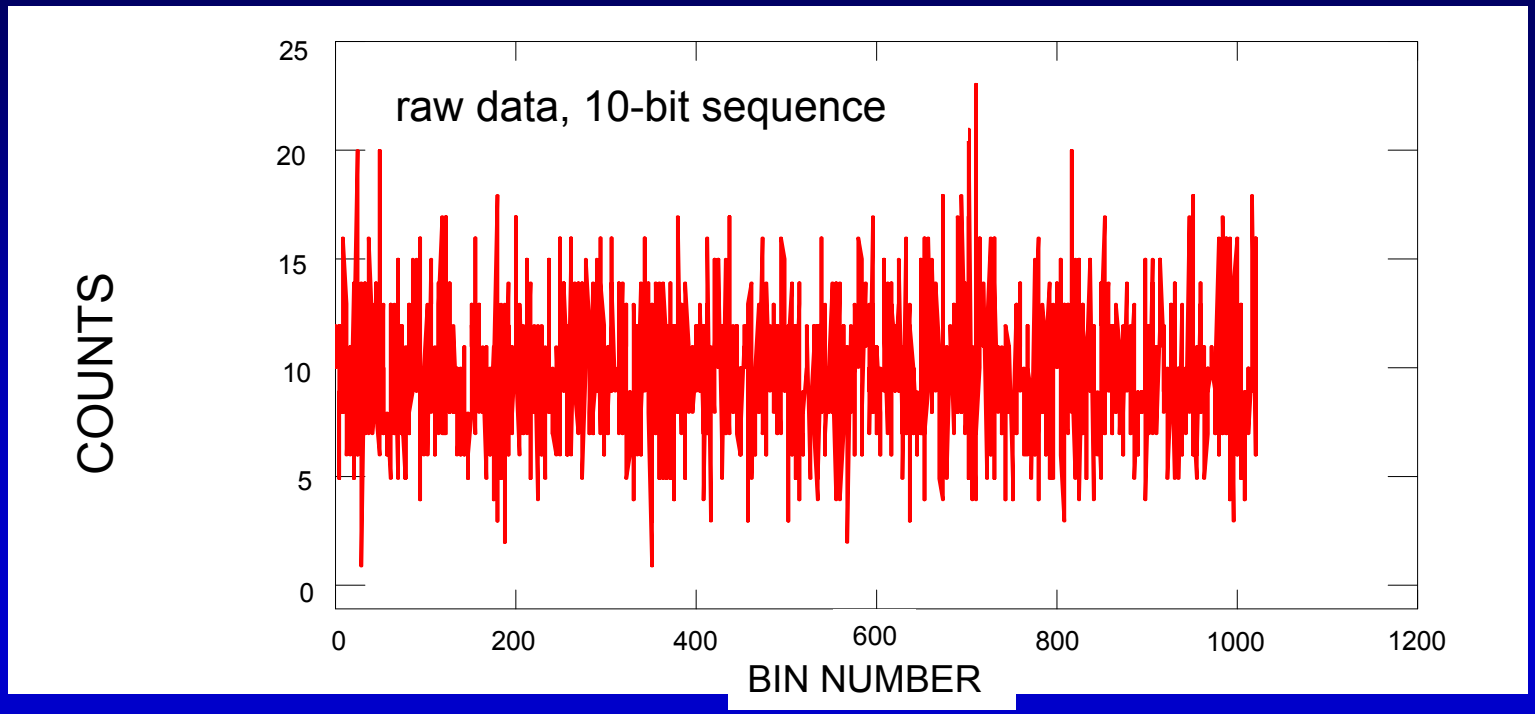
or SNR is increased by a factor of

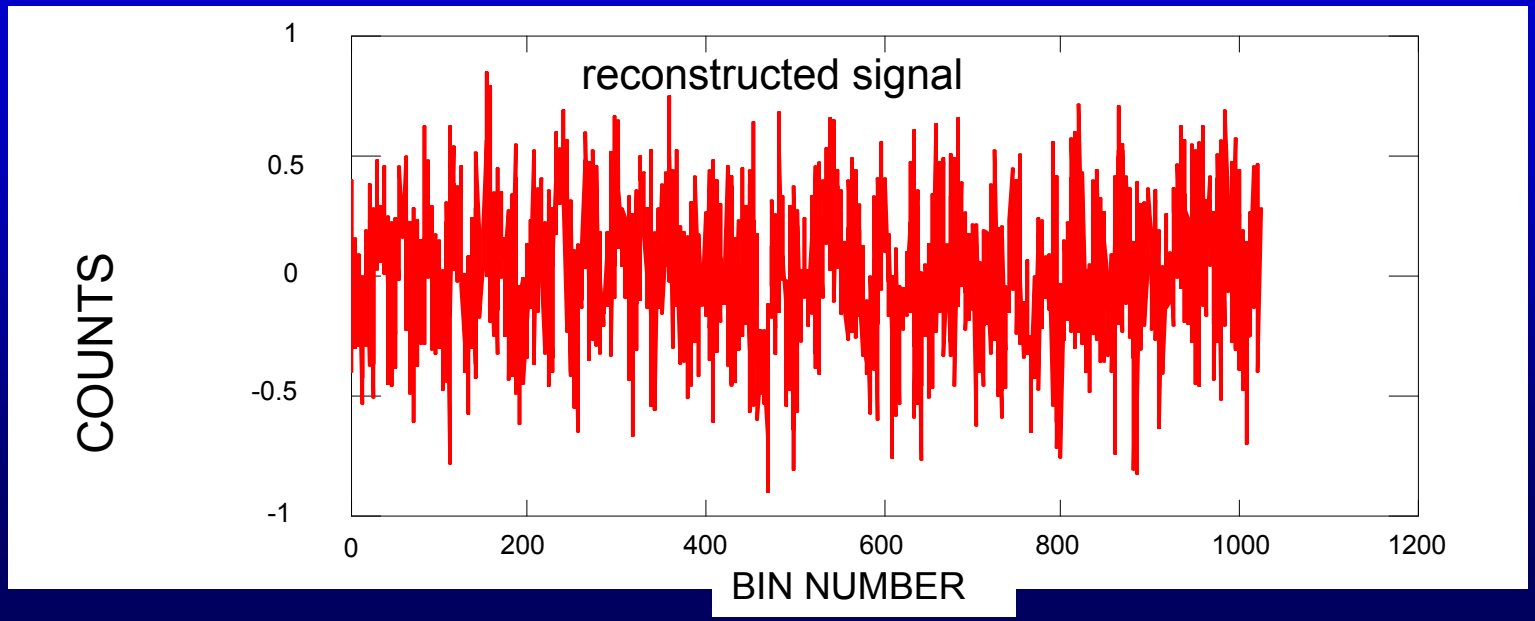
$$(n+1)/2\sqrt{n} \approx \sqrt{n}/2$$

ENCODING 10-BIT SEQUENCE IN HADAMARD TRANSFORM EXPERIMENT



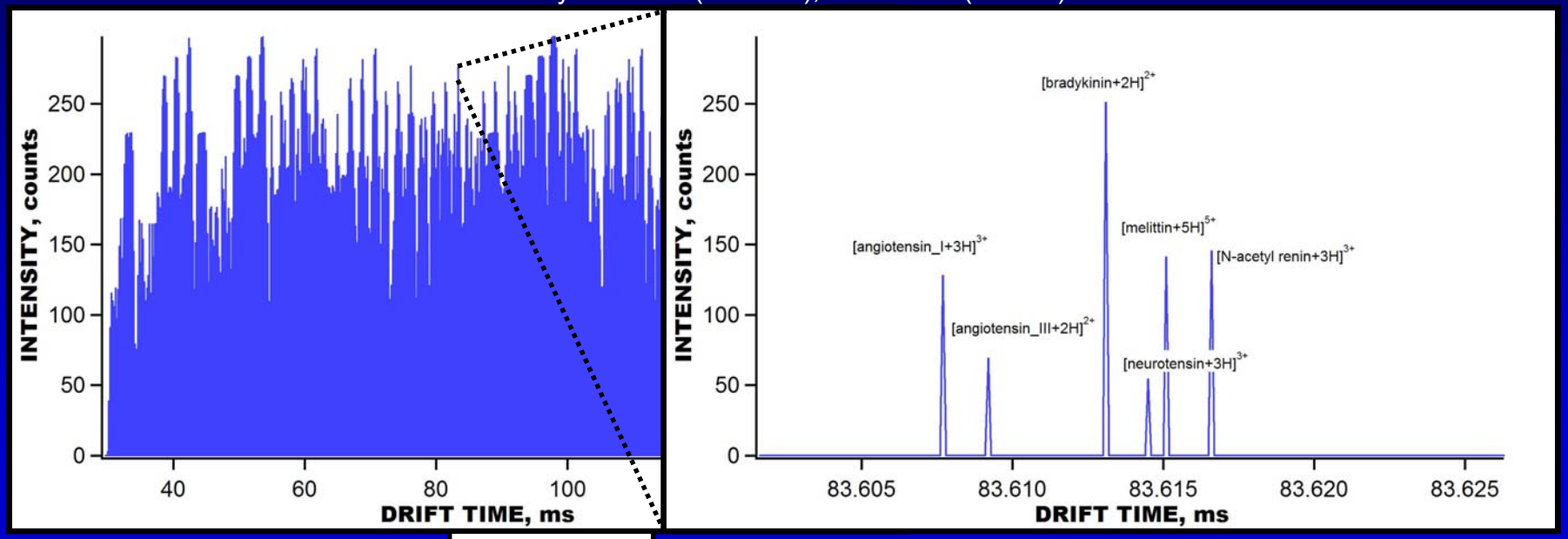
HADAMARD TRANSFORM IMS–TOF AT 4 TORR



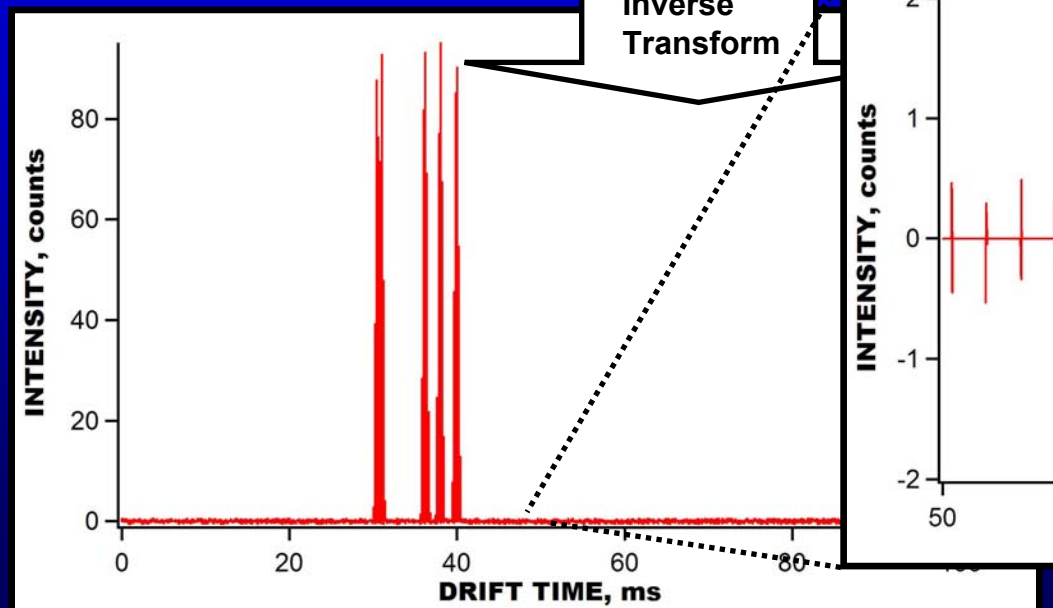


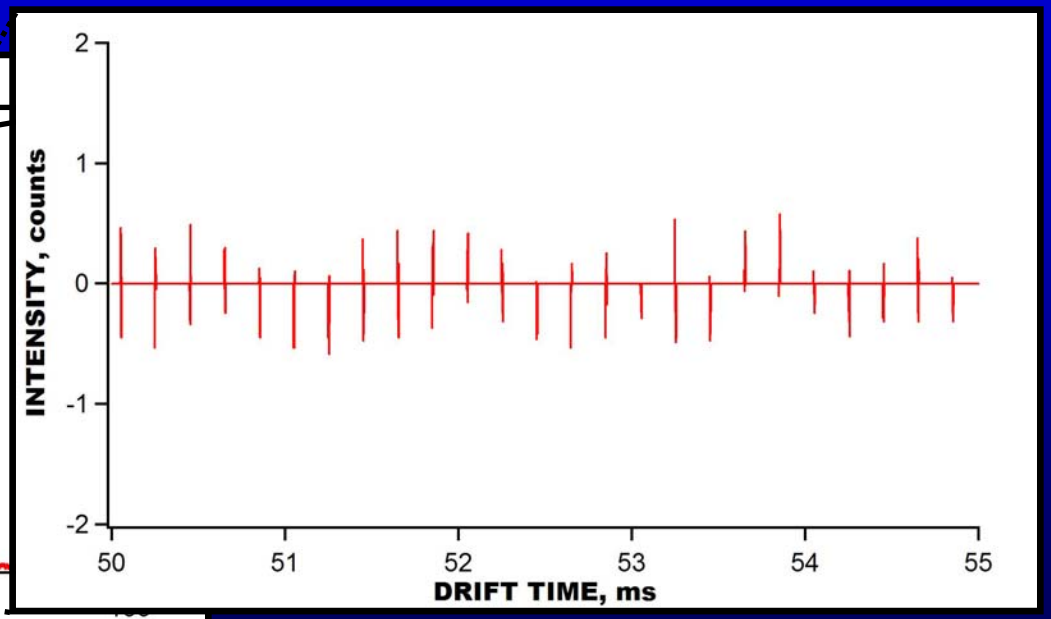
MODELED MULTIPLEXED IMS-TOF SIGNAL

Angiotensin_I +3 (432.89), Angiotensin_III +2 (459.25), Bradykinin +2 (530.78), Neurotensin +3 (558.314), N-acetyl renin +3 (600.994), Melittin +5 (570.15)

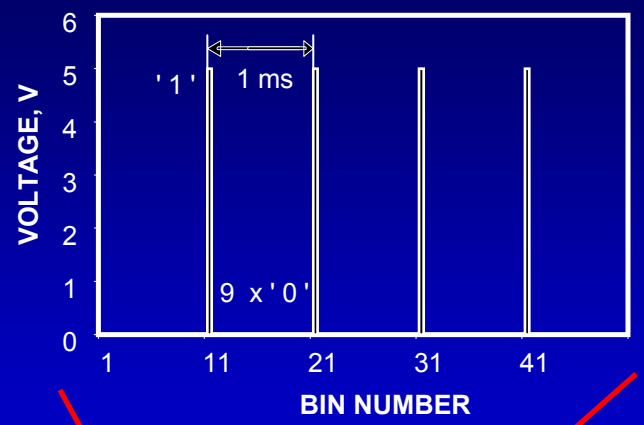


Inverse Transform

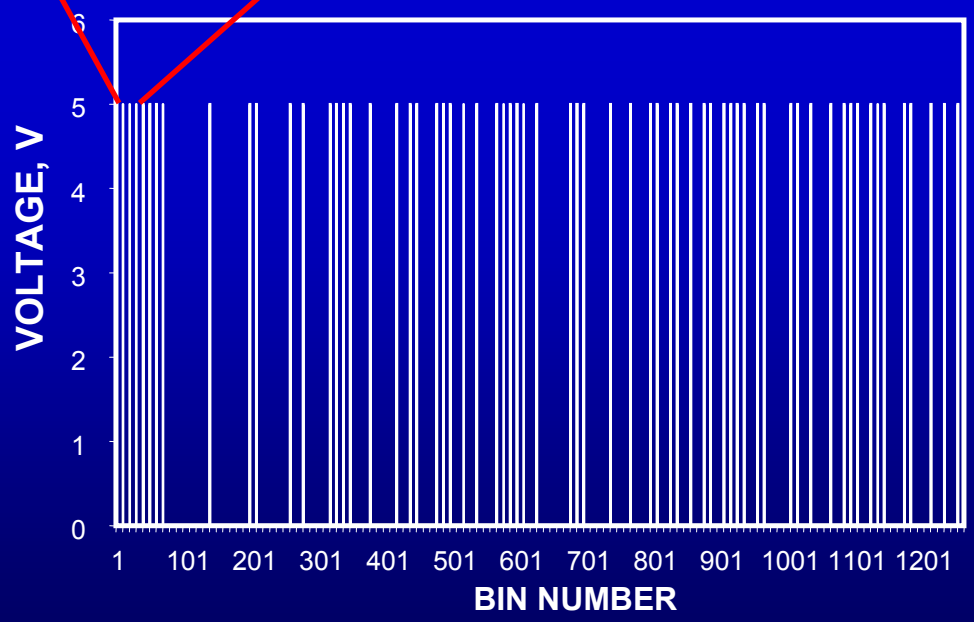




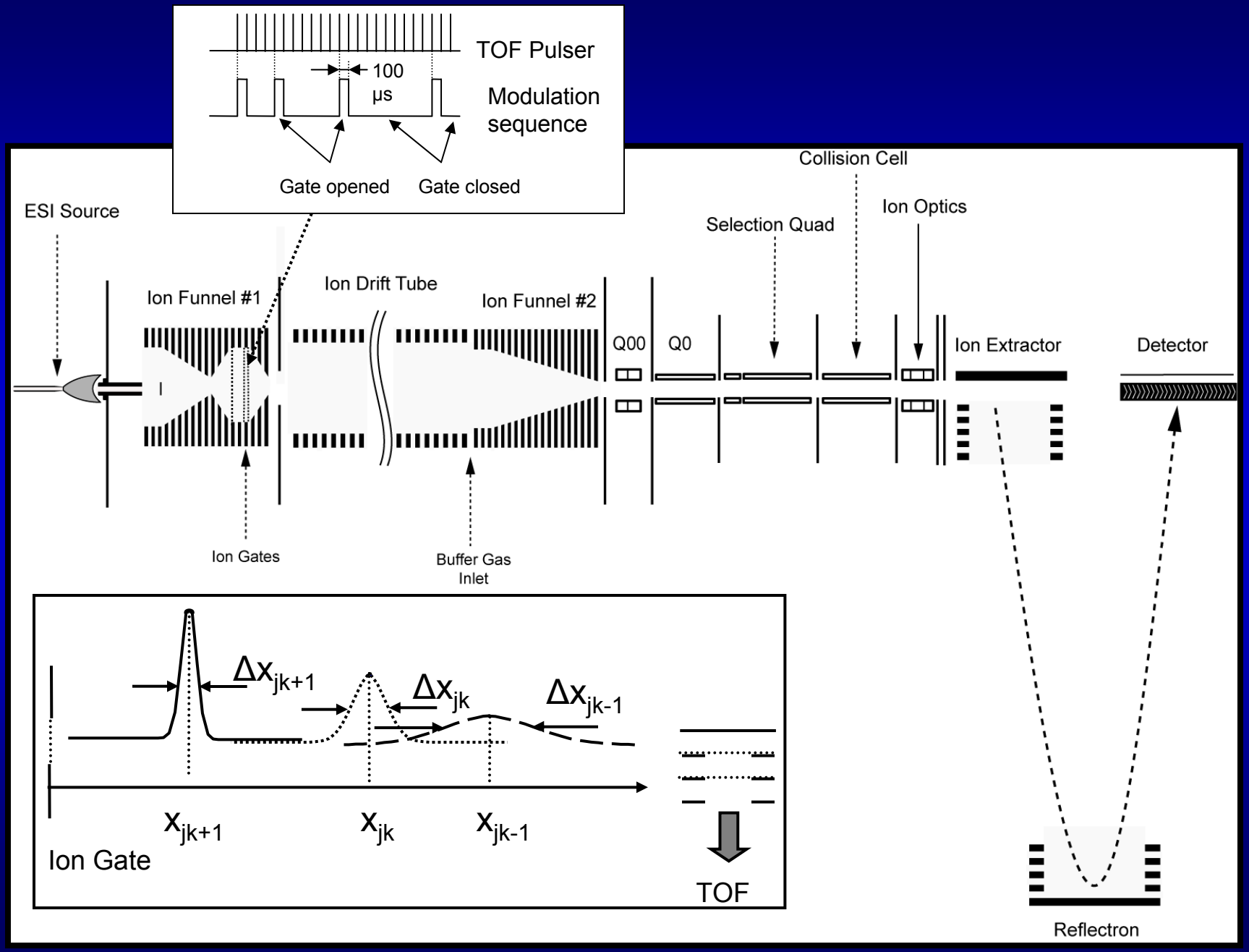
SIGNAL ENCODING AND RECONSTRUCTION



$$\begin{bmatrix}\text{ORIGINAL}\\ \text{IMS-TOF}\\ \text{VECTOR}\end{bmatrix} = \begin{bmatrix}\text{INVERSE}\\ \text{MATRIX}\end{bmatrix} \times \begin{bmatrix}\text{DETECTED}\\ \text{IMS-TOF}\\ \text{VECTOR}\end{bmatrix}$$

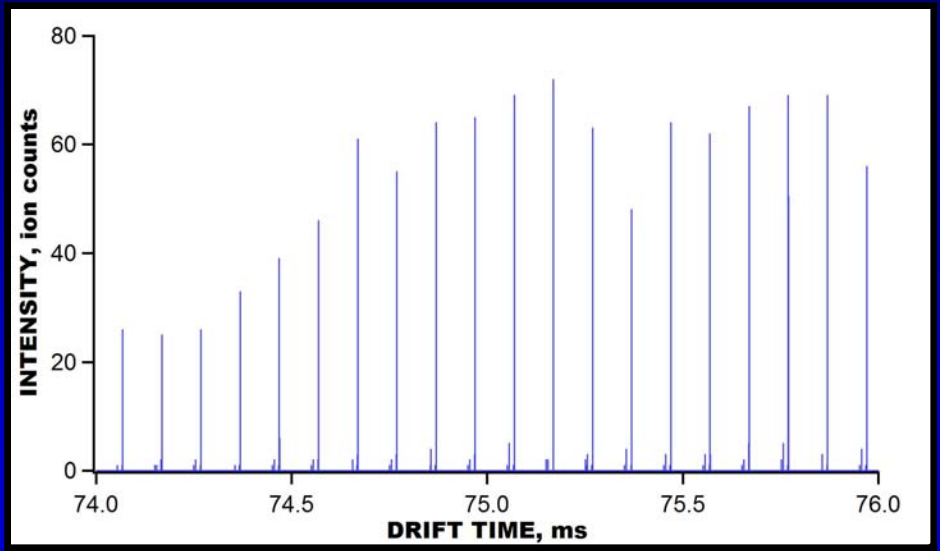


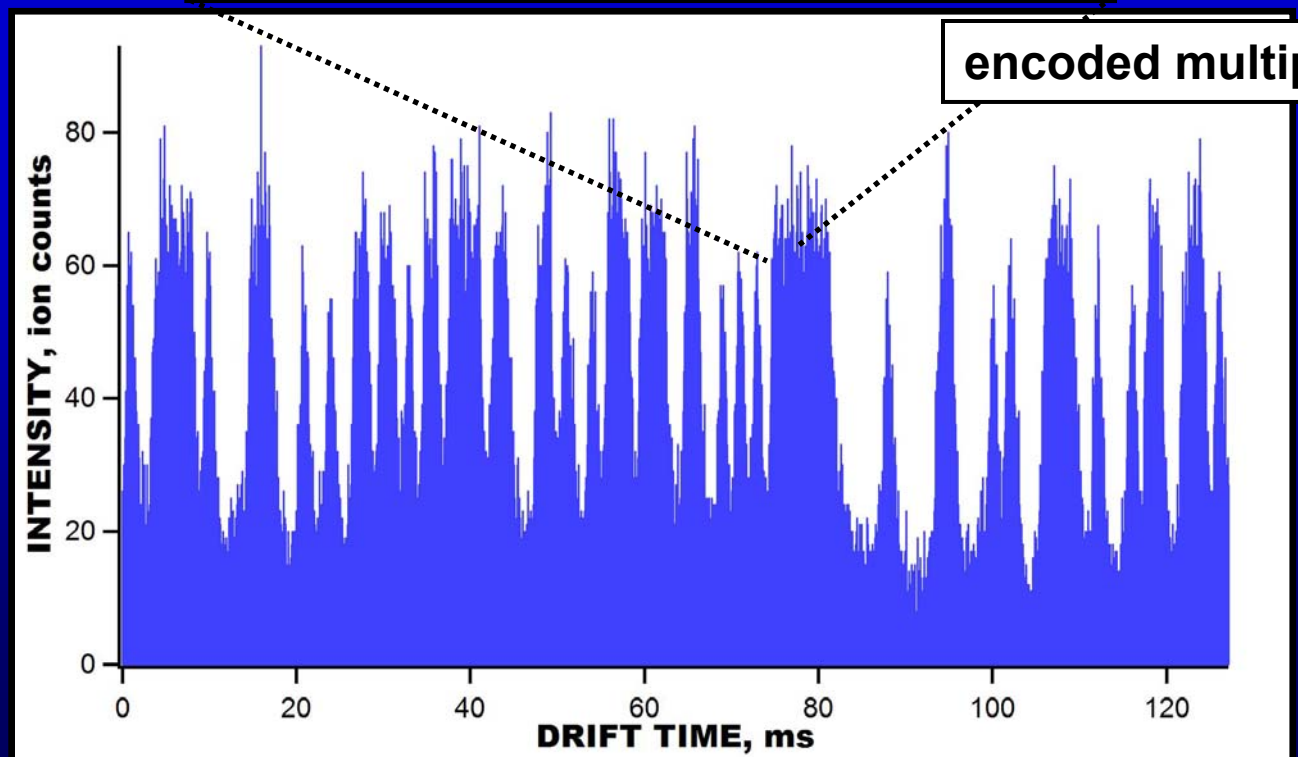
EXPERIMENTAL SETUP



MULTIPLEXED IMS-TOF (QSTAR)

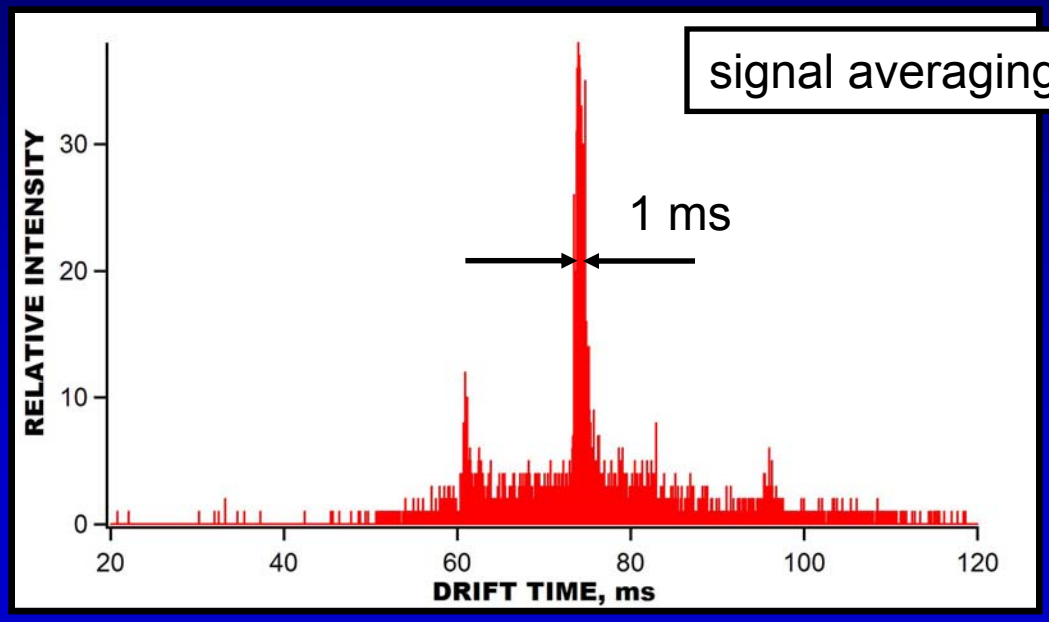
1 μM reserpine, 1000 averages

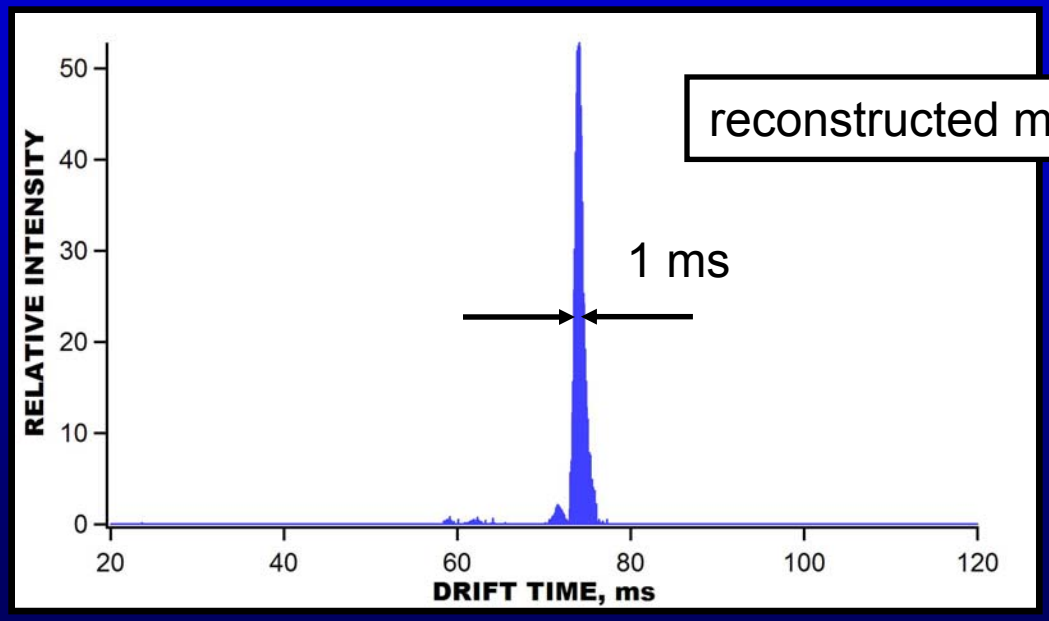




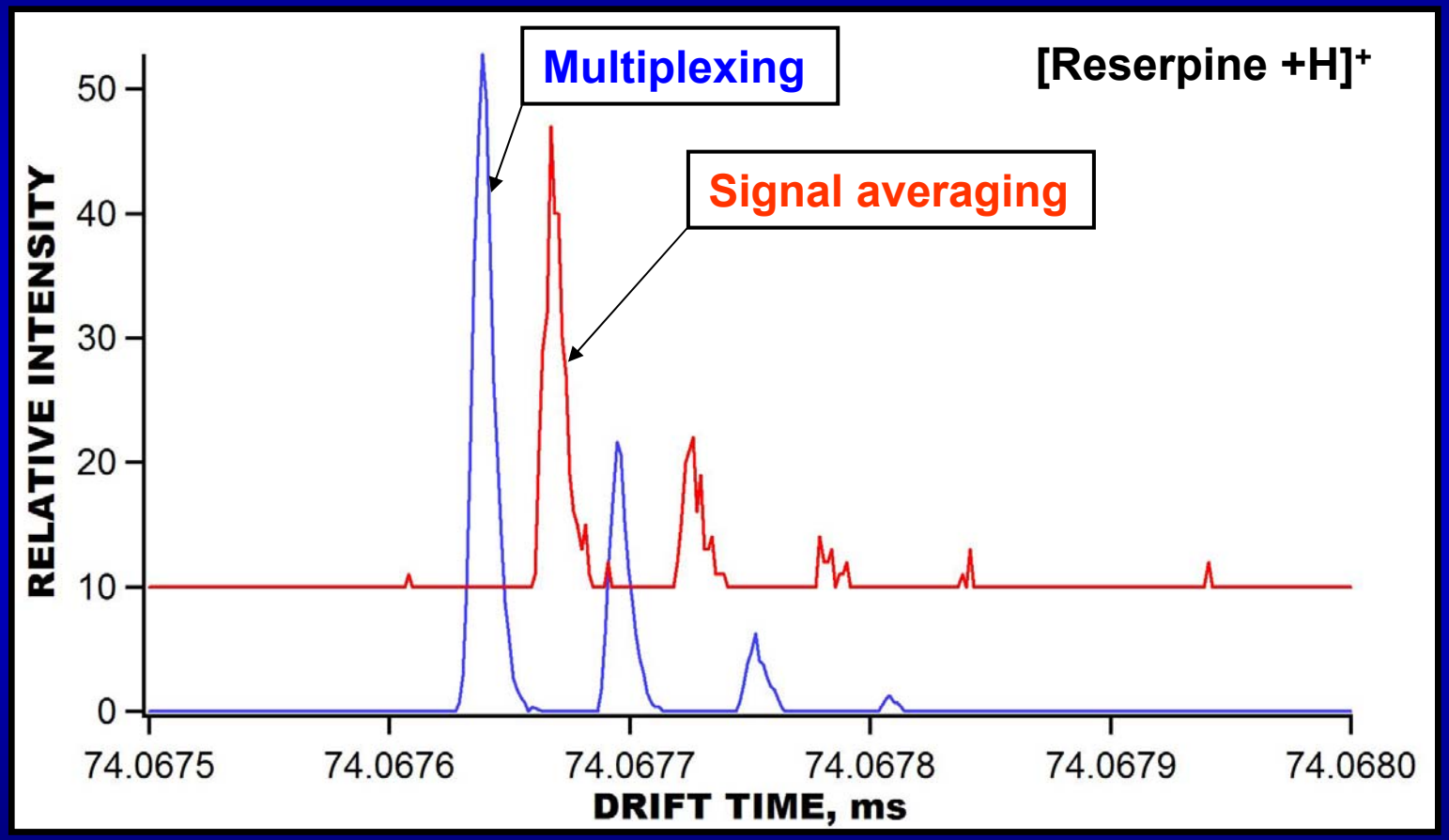
COMPARISON OF SIGNAL AVERAGING AND MULTIPLEXED IMS-TOF

1 μM reserpine, 1000 averages



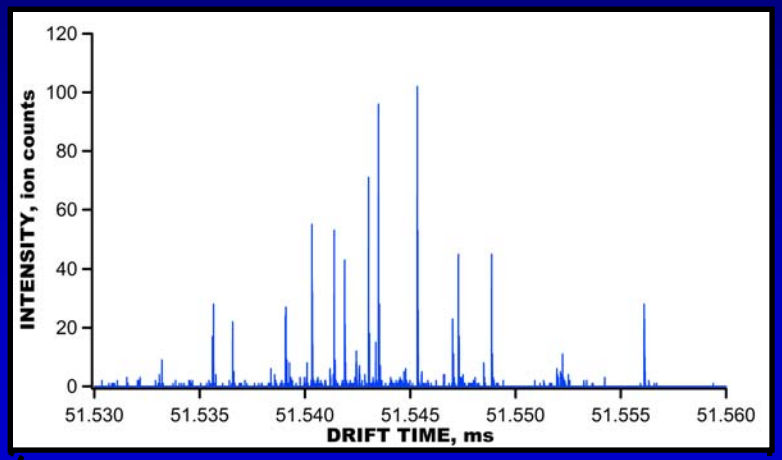


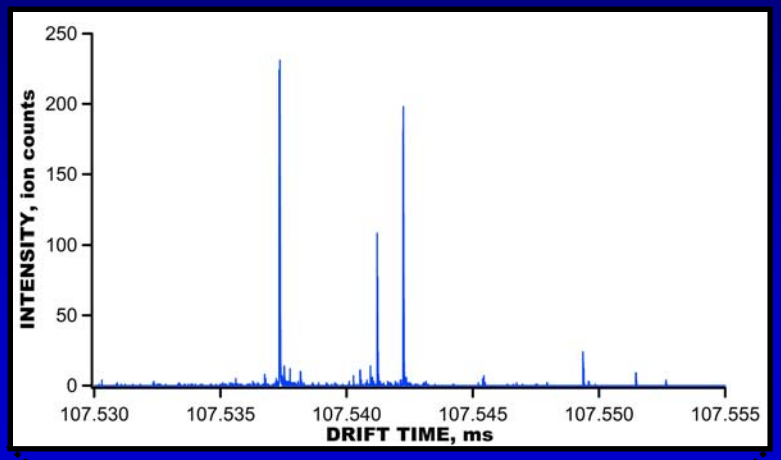
COMPARISON OF MASS SPECTRA IN MULTIPLEXED AND SIGNAL AVERAGING MODES

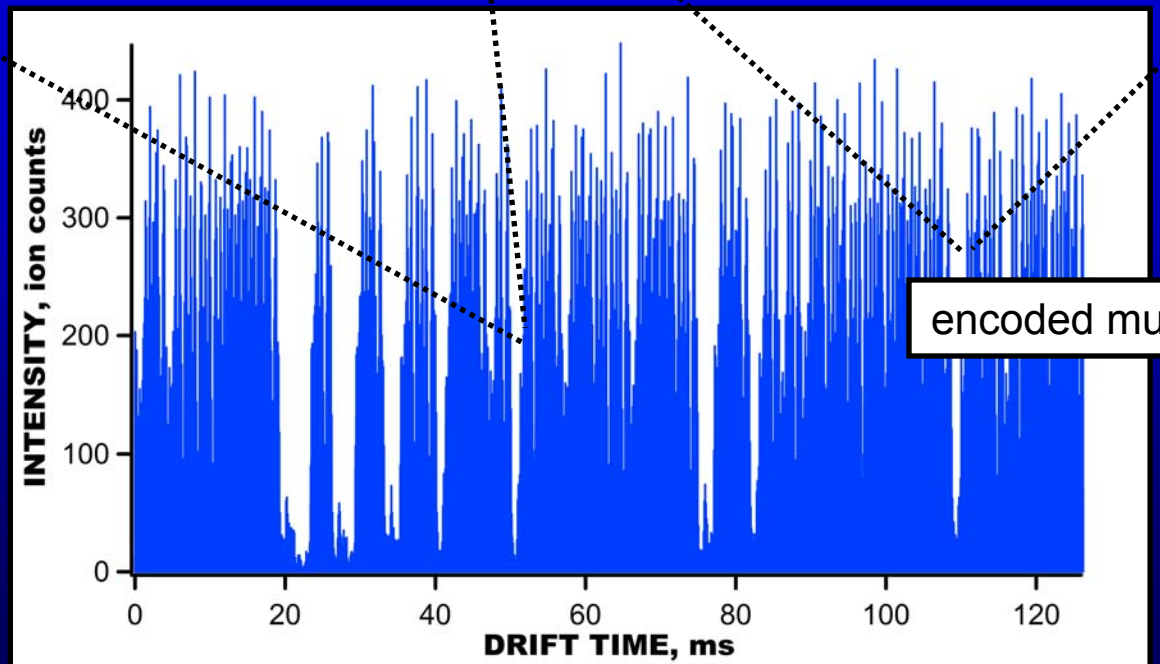


MULTIPLEXED IMS-TOF (AGILENT)

1 μM angiotensin I, bradykinin, neurotensin, fibrinopeptide A

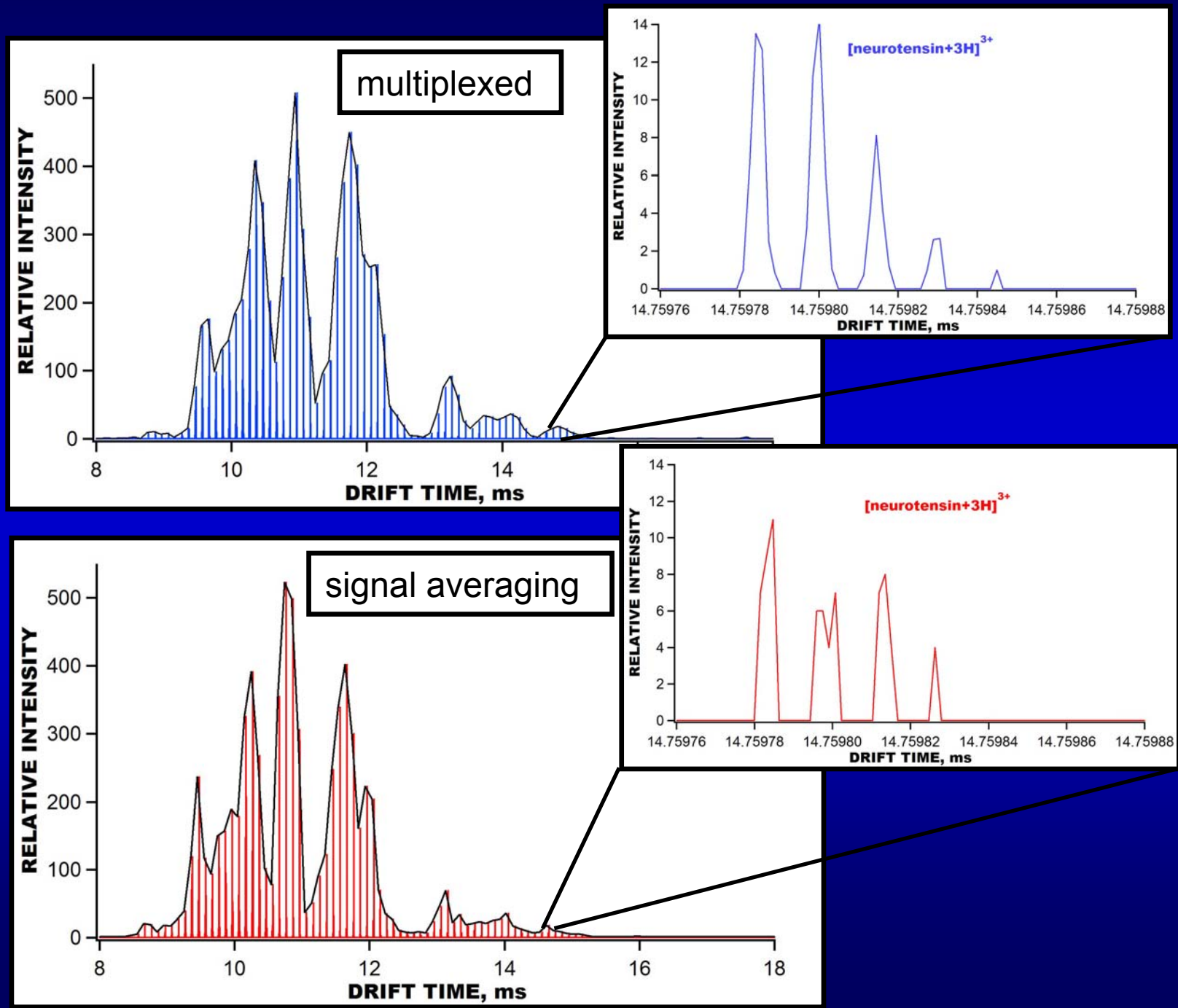






encoded multiplexed signal

COMPARISON OF MULTIPLEXED AND SIGNAL AVERAGING APPROACHES



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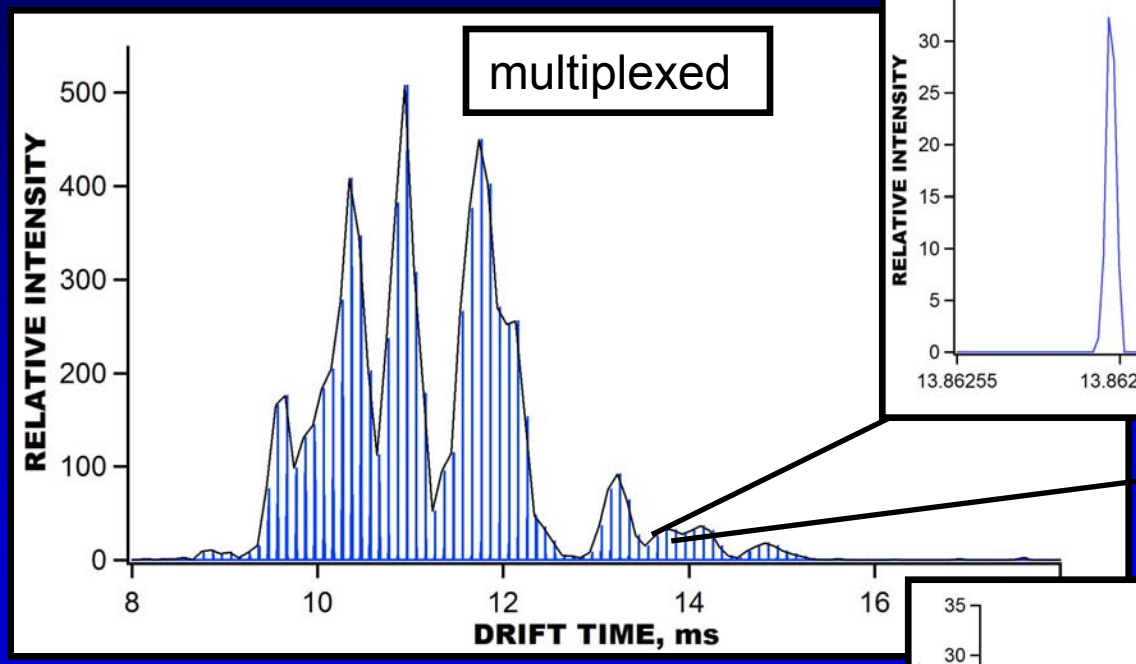

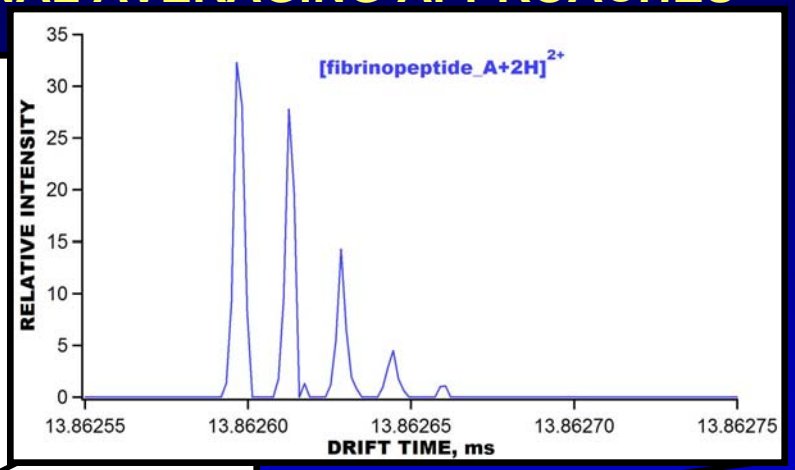

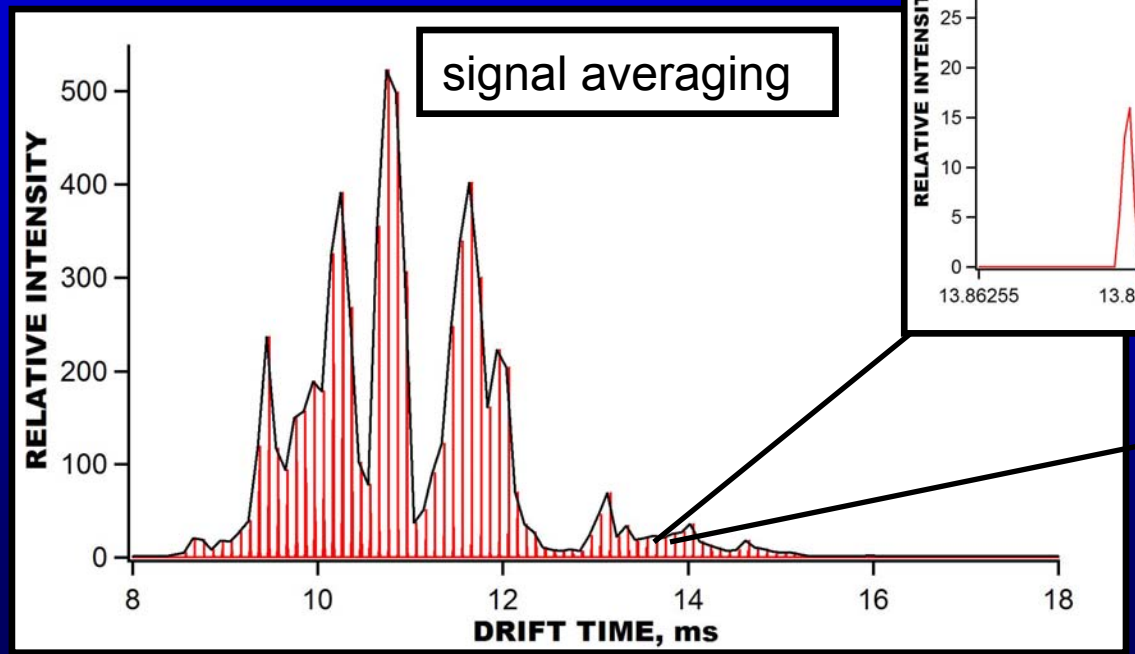

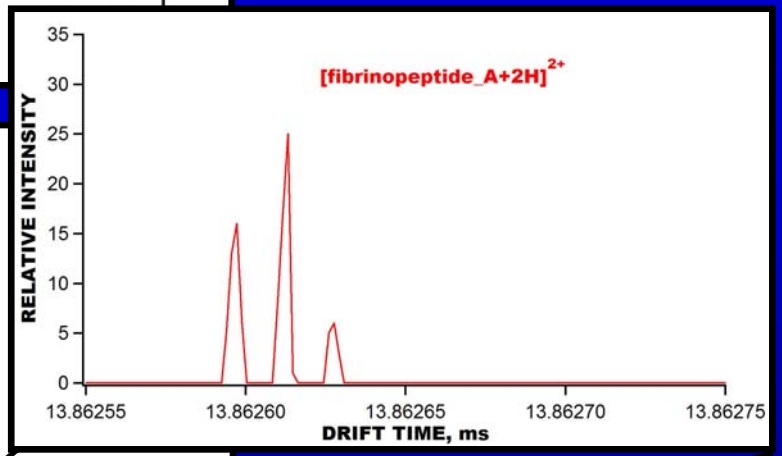

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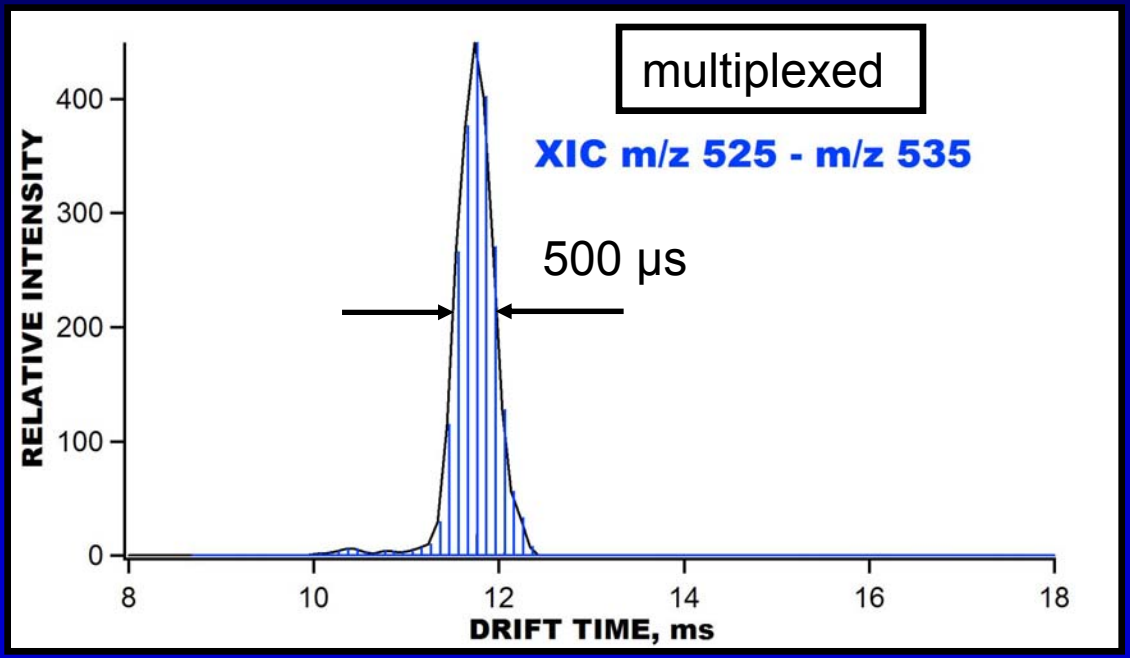
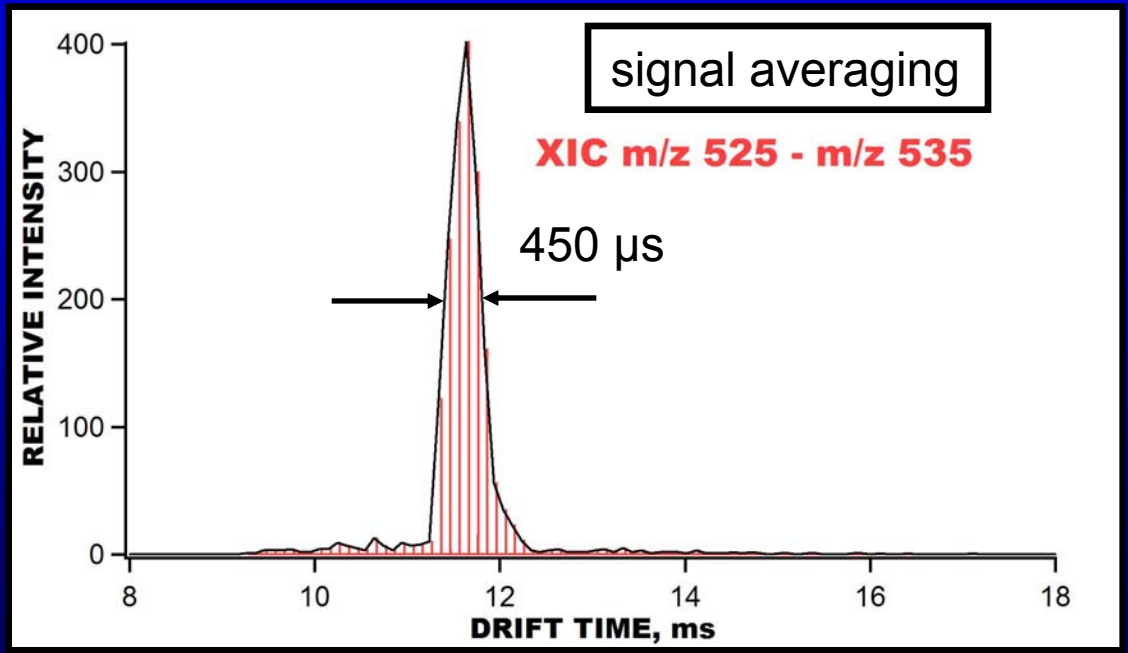
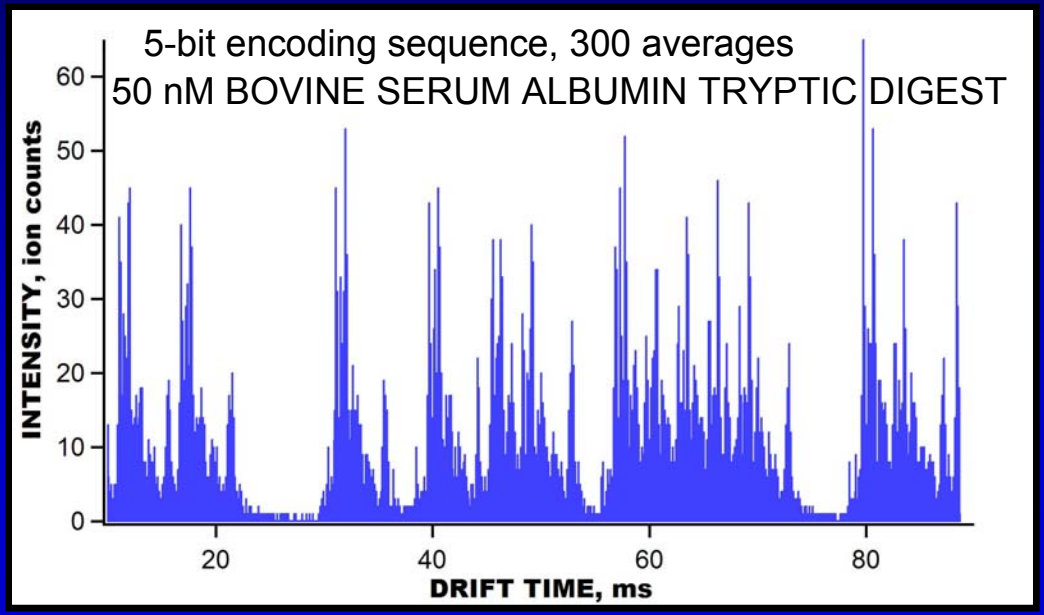
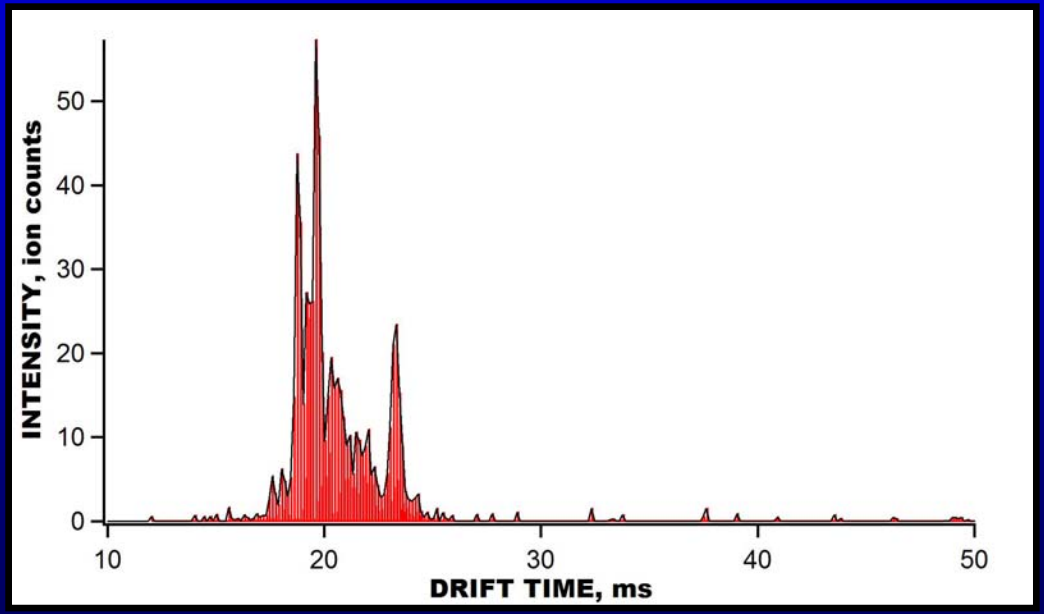


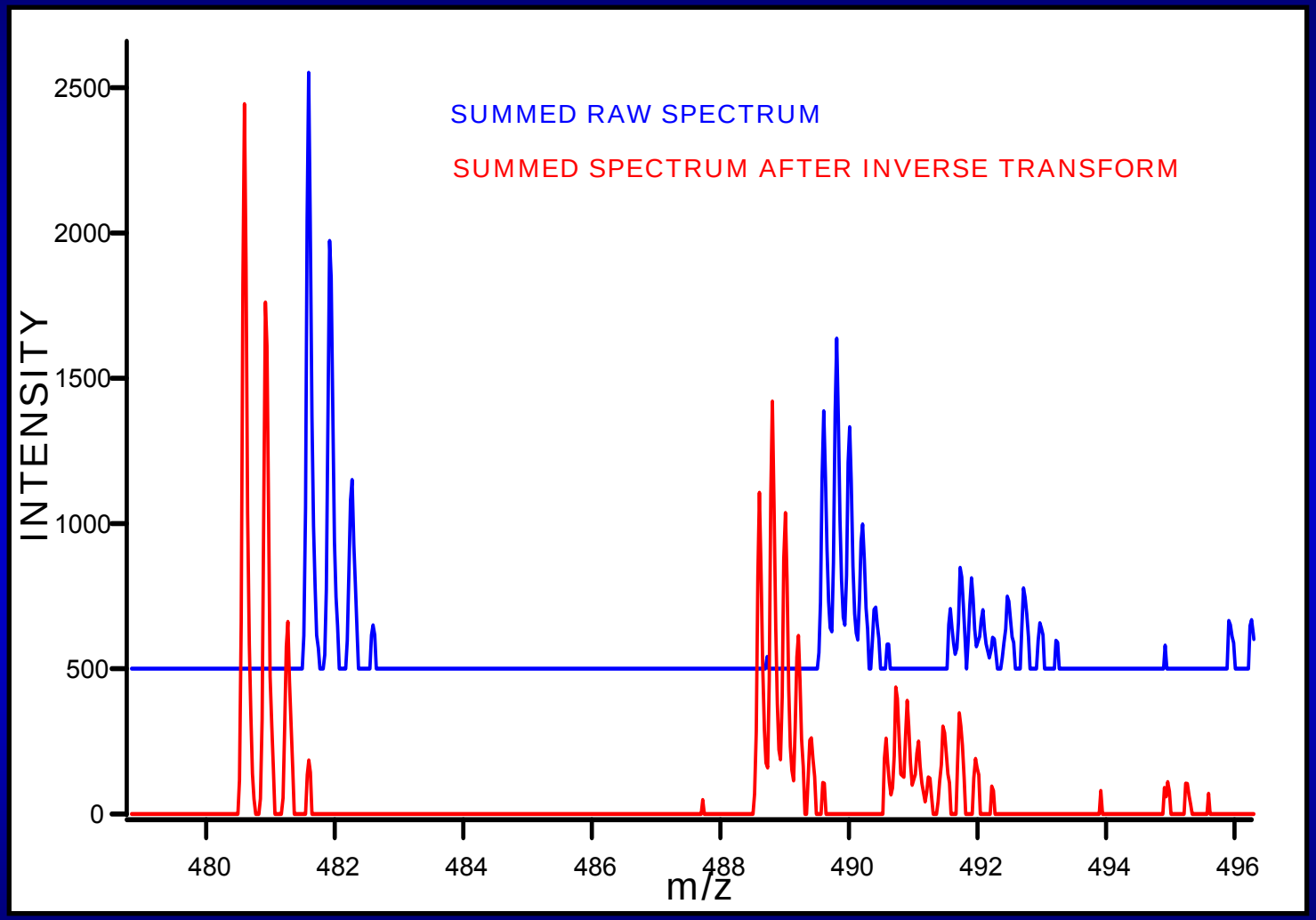


COMPARISON OF MASS SPECTRA FOR ENCODED AND DECODED SIGNALS



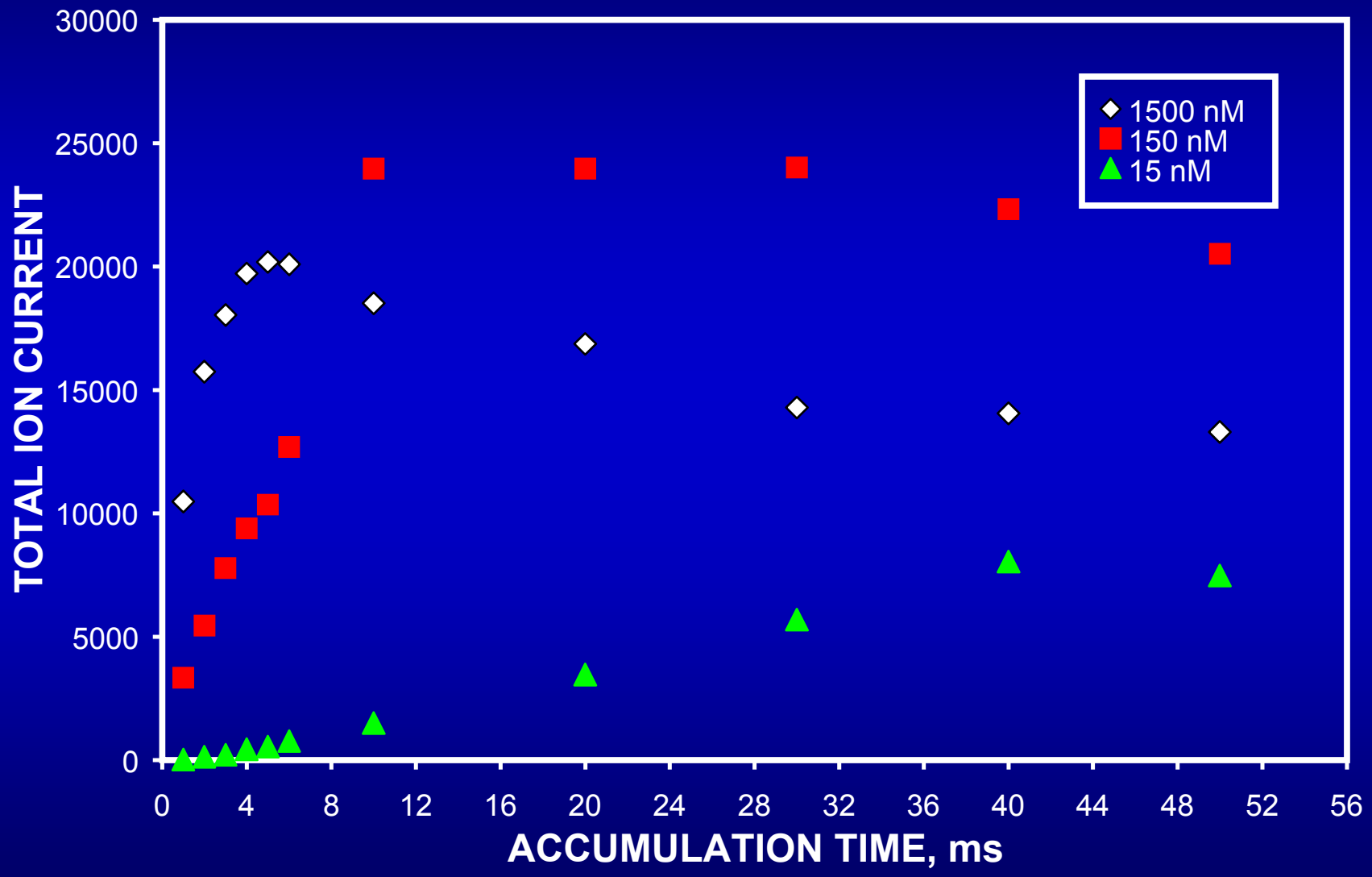


COMPARISON OF MASS SPECTRA FOR ENCODED AND DECODED SIGNALS



ACCUMULATION EFFICIENCY AT DIFFERENT CONCENTRATIONS

BOVINE SERUM ALBUMIN TRYPTIC DIGEST



DYNAMIC MULTIPLEXING

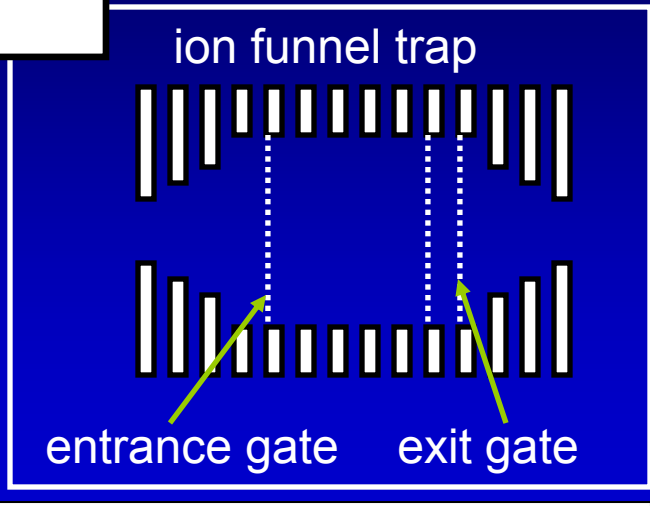
CONCEPT: IMS-TOF automatically adjusts to signals from the source to ensure maximum sensitivity and dynamic range

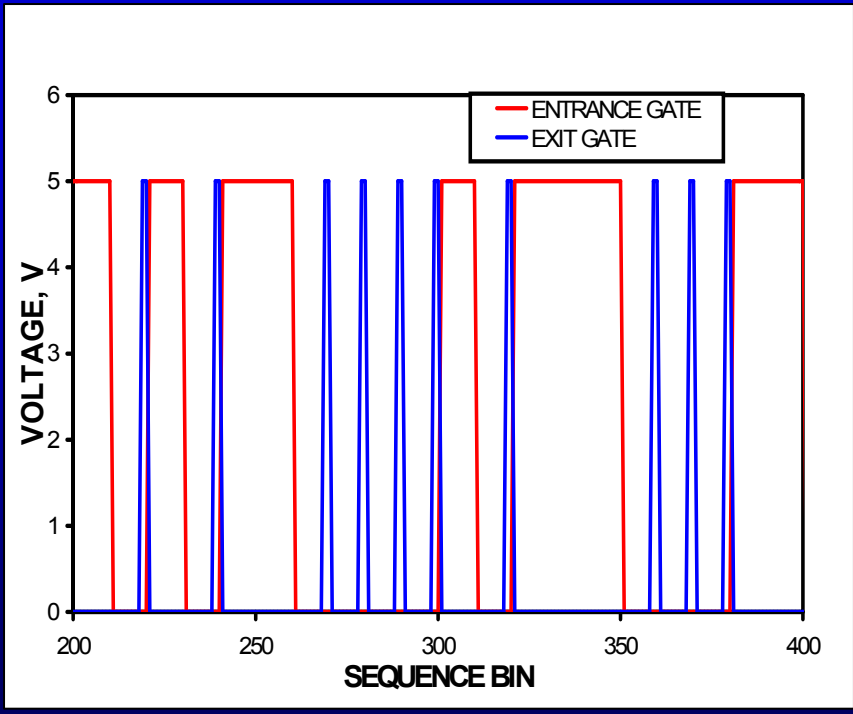
select LC fraction

run short IMS-TOF prescan at fixed accumulation time (4 ms)

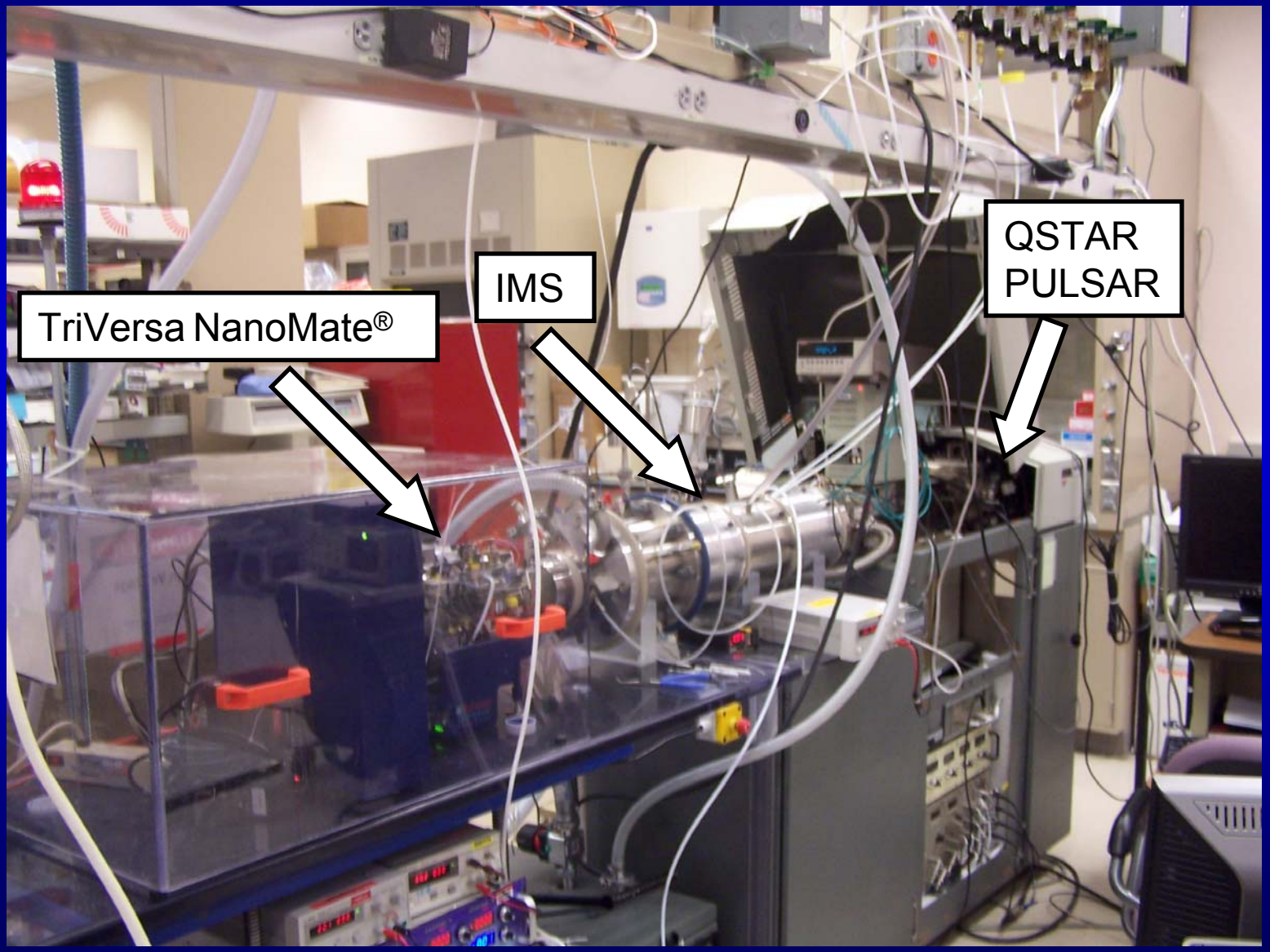
calculate total ion current and select the ***optimum pseudo-random sequence*** based on a calibration function

acquire data with the ***optimum pseudo-random sequence***, decode raw signal





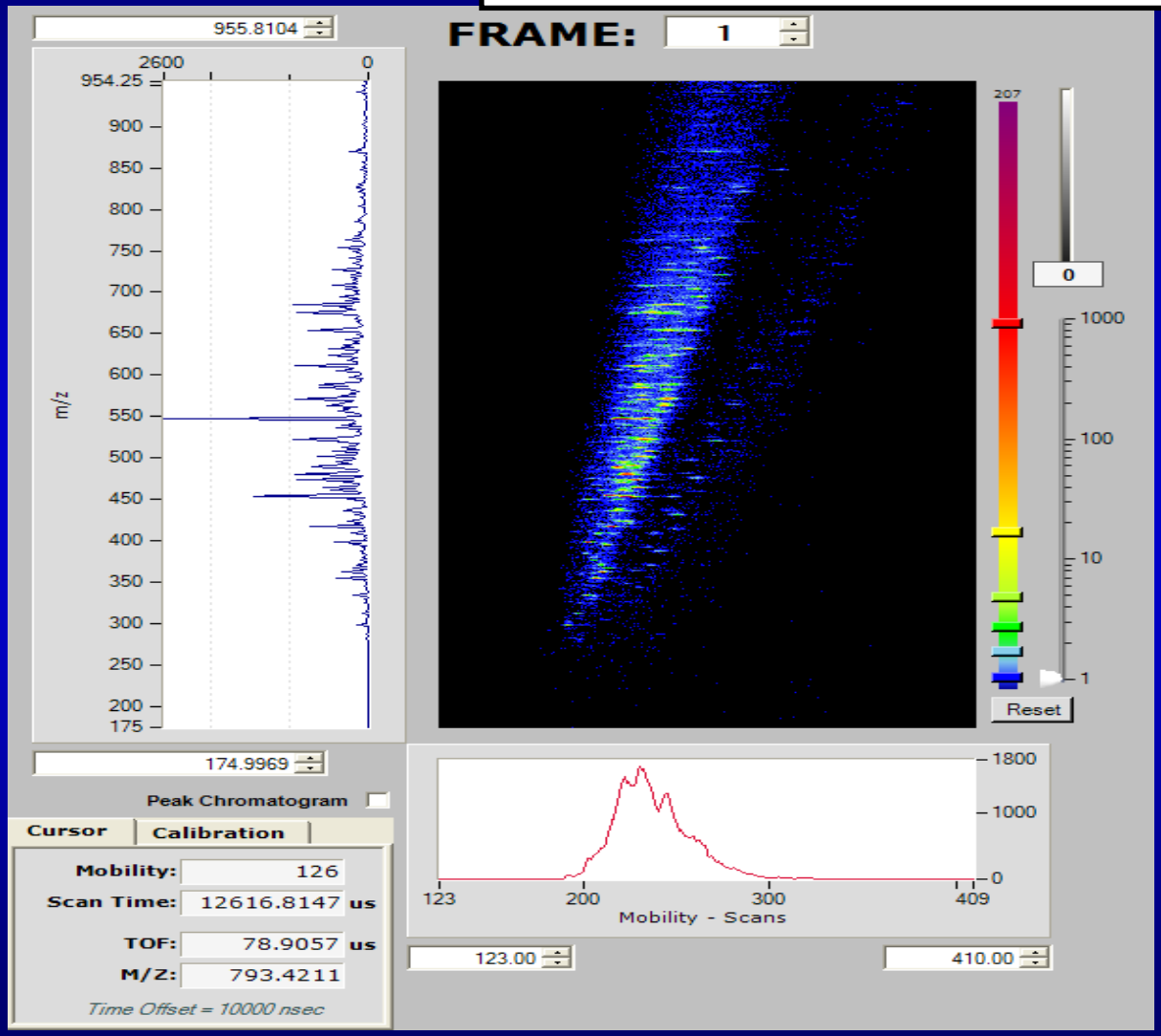
EXPERIMENTAL SETUP



AUTOMATED DYNAMIC MULTILEXING

1.5 µM BSA TRYPTIC DIGEST

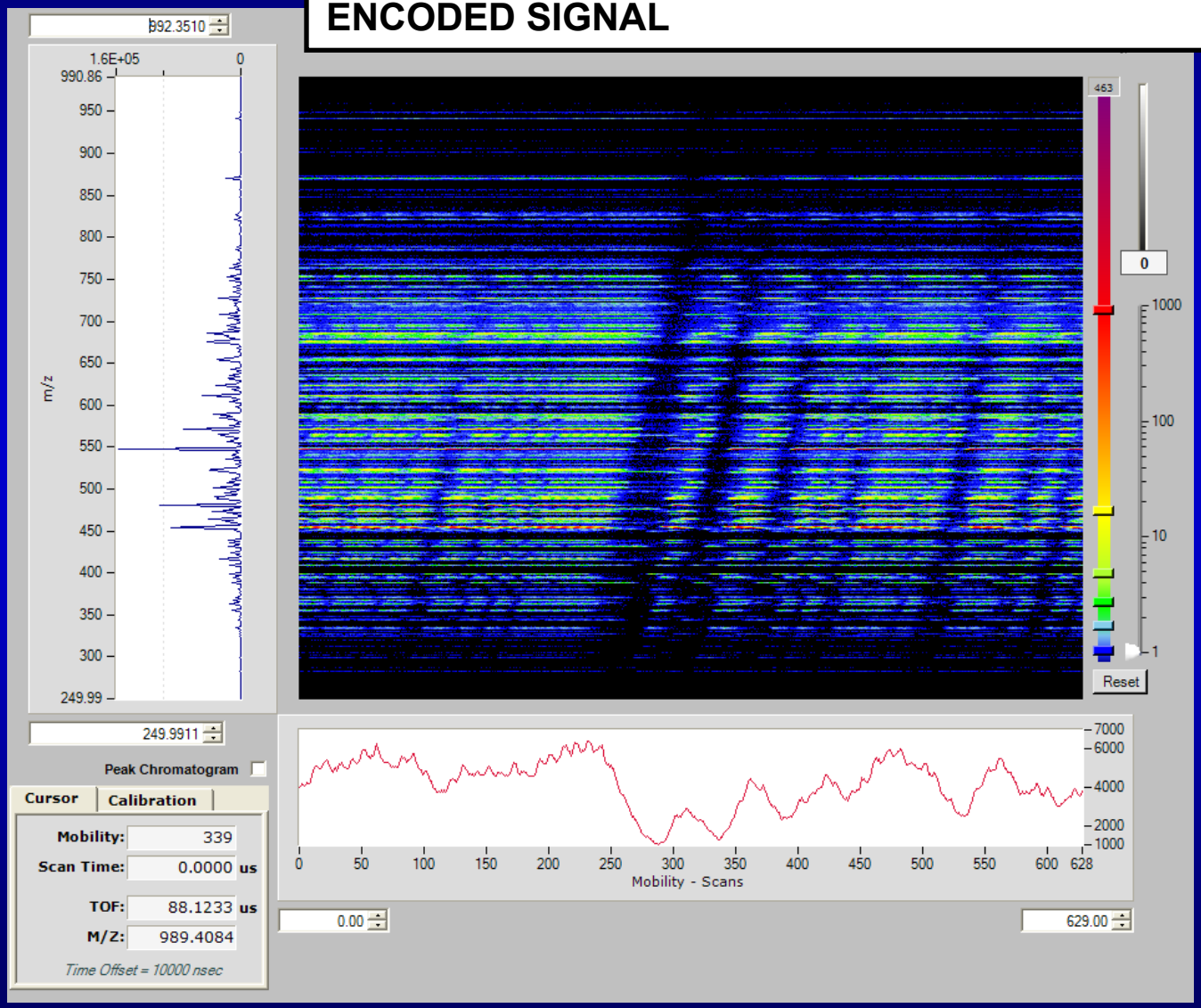
PRESCAN, 4 ms accumulation, 50 averages



AUTOMATED DYNAMIC MULTILEXING

1.5 µM BSA TRYPTIC DIGEST

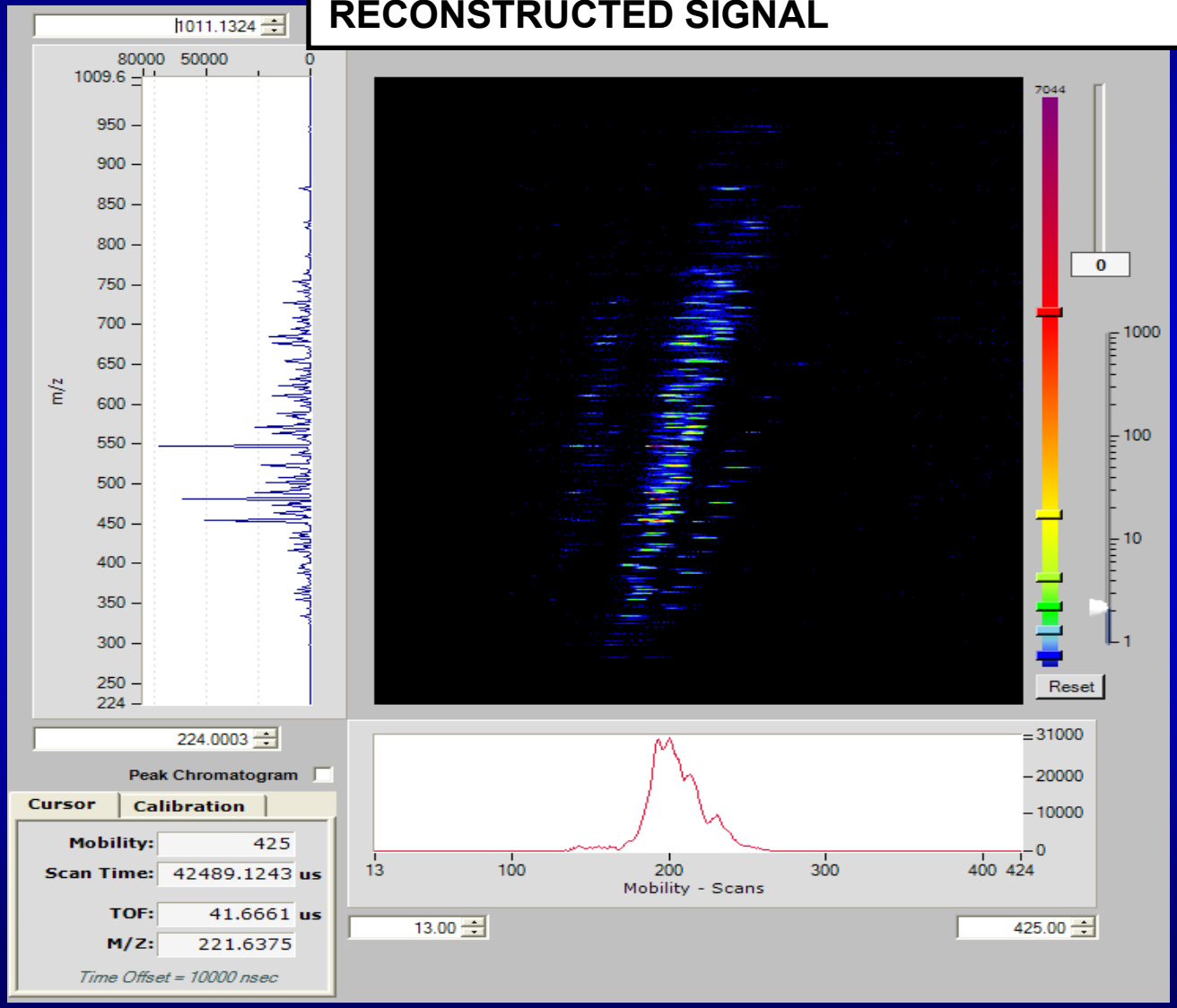
FULL SCAN, 6-bit encoding sequence, 500 averages, **ENCODED SIGNAL**



AUTOMATED DYNAMIC MULTILEXING

1.5 µM BSA TRYPTIC DIGEST

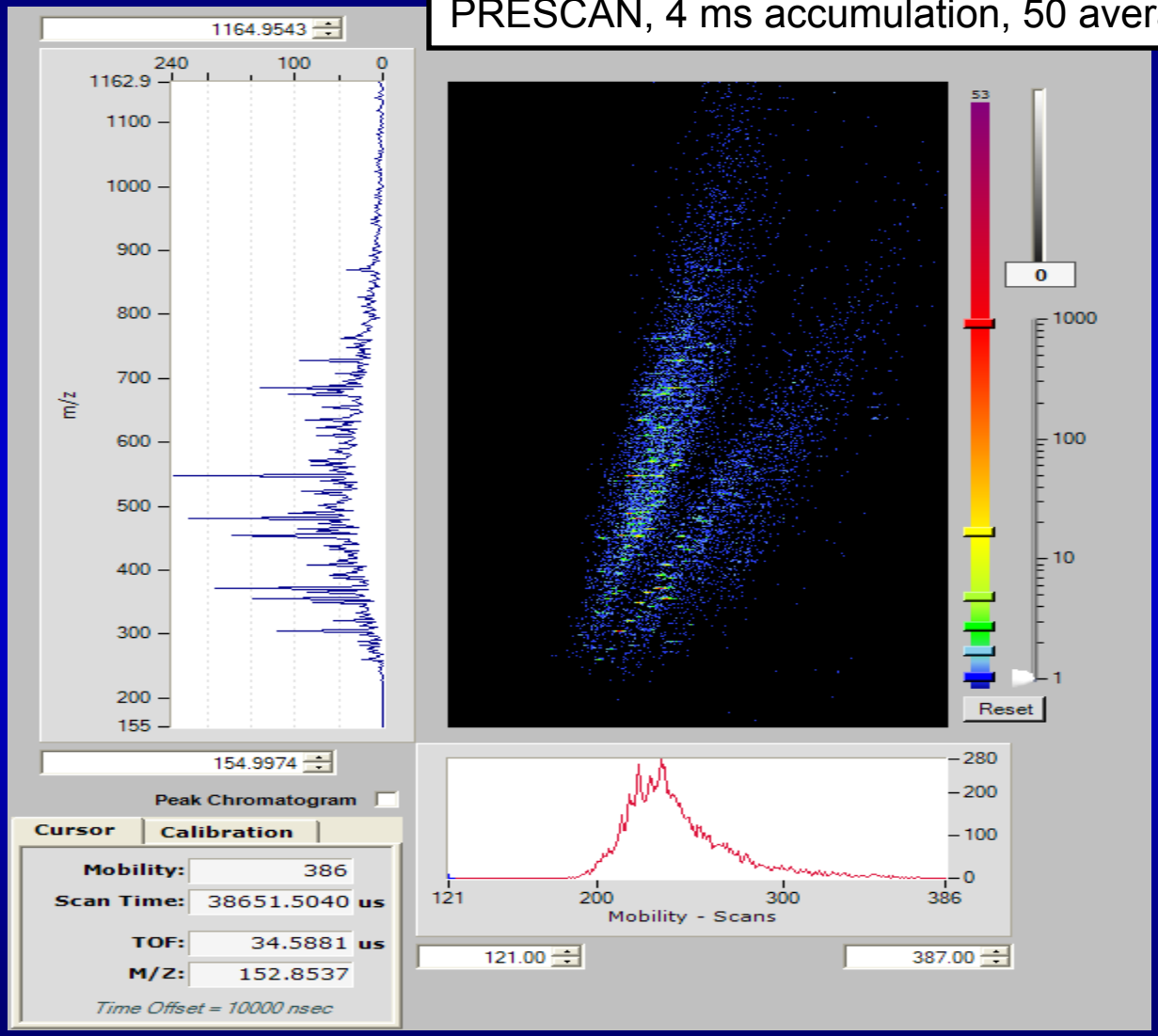
FULL SCAN, 6-bit encoding sequence, 500 averages, **RECONSTRUCTED SIGNAL**



AUTOMATED DYNAMIC MULTILEXING

15 nM BSA TRYPTIC DIGEST

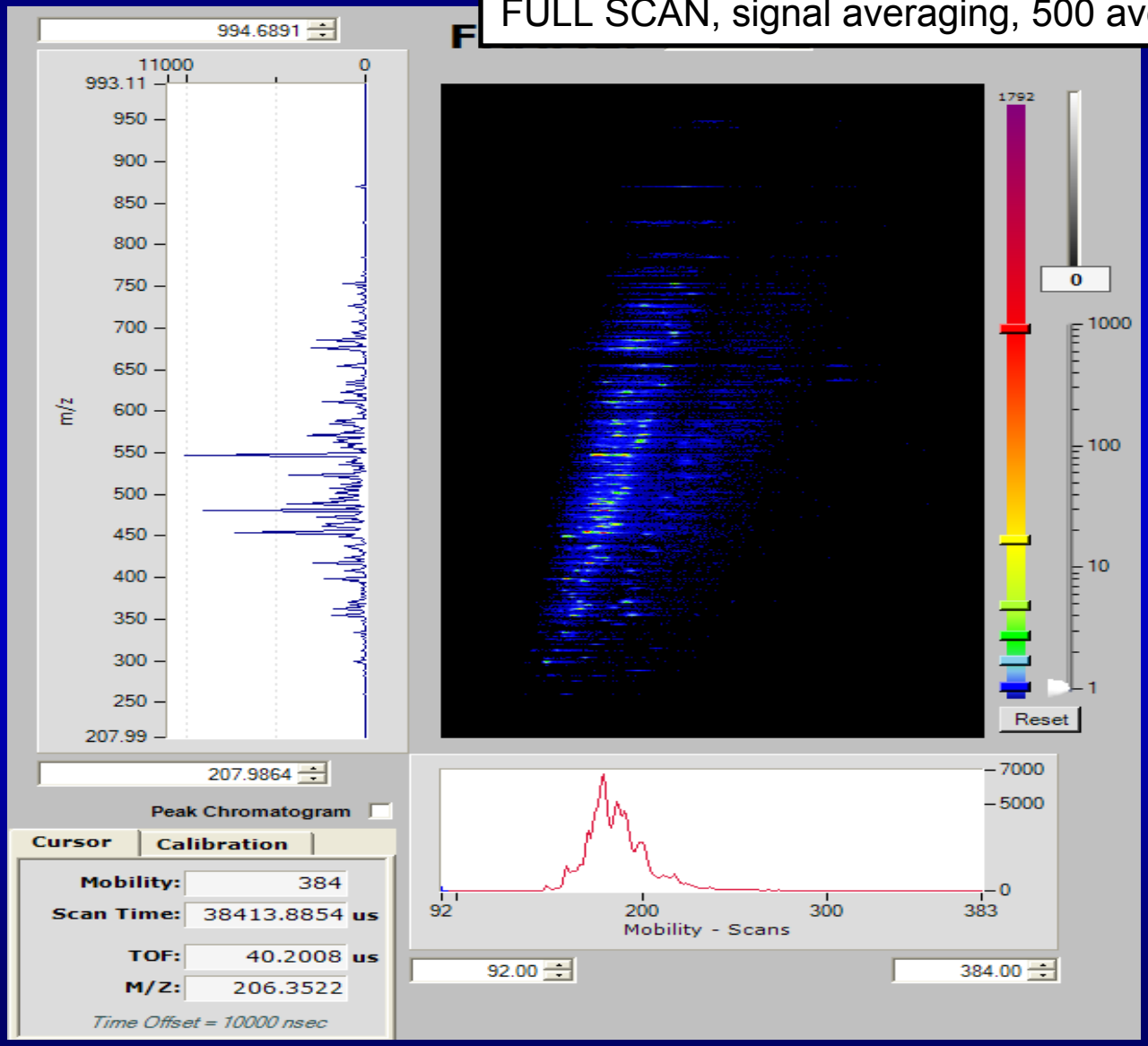
PRESCAN, 4 ms accumulation, 50 averages



AUTOMATED DYNAMIC MULTILEXING

15 nM BSA TRYPTIC DIGEST

FULL SCAN, signal averaging, 500 averages



BOVINE SERUM ALBUMIN COVERAGE

1.5 μM, 6-bit pseudo-random sequence, 500 averages

124 fragments at 20 ppm

mkwvtfisll llfssaysrg vfrr**DTHKSE IAHRFKDLGE**
EHFKGLVLIA FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD
ESHAGCEKSL HTLFGDELCK vaslretygd madccek**QEP**
ERNECFLSHK DDSPDLPKLK PDPNTLCDEF KADEKKFWGK
YLYEIARRhp yfyapellyy ank**YNGVFQE CCQAEDKGAC**
LLPKIETMRe kvlassarqr **LRCASIQKFG ERALKAWSVA**
RLSQKFPKAE FVEVTKLVTD LTKVHKECCH GDLLECADDR
ADLAKYICDN QDTISSKLKE CCDKPLLEKS HCIAEVEKDA
IPENLPPLTA DFAEDKDVCK NYQEAKDAFL GSFLYEYSRR
HPEYAVSVLL RLAKEYEATL EECCAKddph acystvfdk**L**
KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ
VSTPTLVEVS RSLGKVGTRC CTKPESERMP CTEDYLSLIL
NRLCVLHEKT PVSEKVTKCC TESLVNRRPC FSALTPDETY
VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP
KATEEQLKTV MENFVAFVDK ccaaddkeac favegpk**LVV**
STQTALA

Coverage: 83.5% (507 / 607)

BOVINE SERUM ALBUMIN COVERAGE

1.5 µM, signal averaging, 500 averages, 5 ms accumulation

35 fragments at 20 ppm

mkwvtfisll llfssaysrg vfrr**DTHKSE IAHRFKDLGEEHFK**glvlia
fsqylqqcpf dehvk**LVNEL TEFAKTCVADESHAGCEKSL**
HTLFGDELCK vaslretygd madccekqepernecflshk ddspdlpk**LK**
PDPNTLCDEF KADEKKFWGKYLYEIARrhp yfyapellyy
ankyngvfqe ccqaedkgacllpkietmre kvlassarqr lrcasiqkfg
er**ALKAWSVARLSQKFPKAE FVEVTKLVTD LTKVHKECCH**
GDLLECADDRADLAKYICDN QDTISSKLKE CCDKPLLEKs
hciaevekdaipenlpplta dfaedkdvck nyqeak
DAFLGSFLYEYSRRHPEYAVSVLL RLAKEYEATL
EECCAKDDPHACYSTVFDKLKHLVDEPQNLIKqncdqfek**LGEYG**
FQNAL IVRytr**KVPQVSTPTLVEVS R**slgkvgtrc ctkpesermp
ctedylslilnr**LCVLHEKT PVSEKVTKCC TESLVNRRPC**
FSALTPDETYVPKAFDEKLF TFHADICTLP DTEKQIKKQT
ALVELLKHKPKateeqlktv menfvafvdk ccaaddkeac
favegpk**LVVSTQTALA**

Coverage: 56.7% (344 / 607)

CONCLUSIONS

- Advanced signal processing methods have been developed to encode and decipher extremely complex signals from IMS-TOF MS analyses of biopolymers
- Multiplexed IMS-TOF was shown to provide 6-10 fold greater SNR than signal averaging approach
- Dynamic multiplexed IMS-TOF approach has been developed and evaluated with protein digests varying in concentrations by 3 orders of magnitude
- The method is characterized by an IMS duty cycle of > 50%

ACKNOWLEDGEMENTS

- David Prior
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