High-Throughput Proteomics Platform Based on Ion Mobility Time-of-Flight Mass Spectrometry

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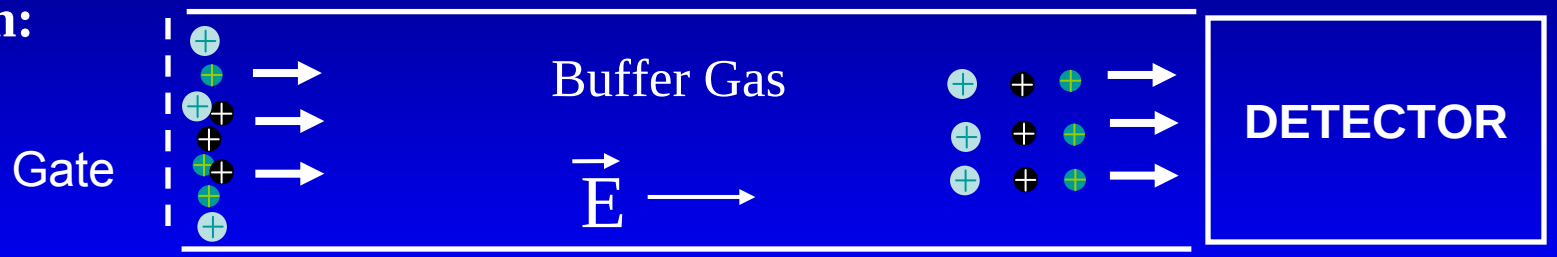
OUTLINE

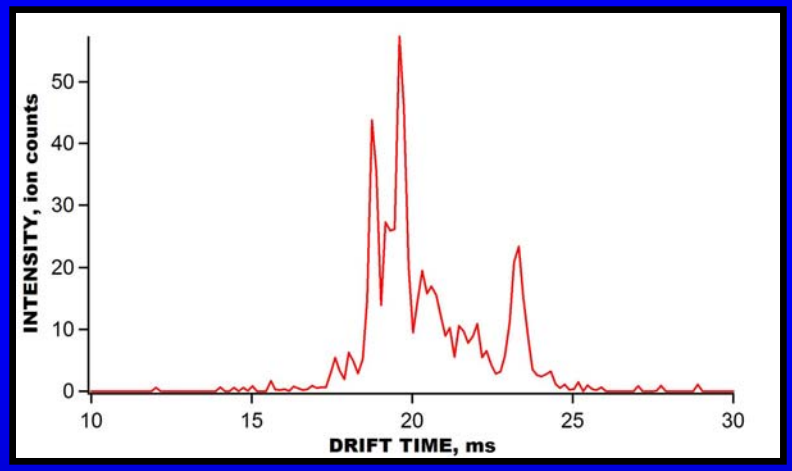
- Brief introduction
- Overview of developed technologies
- Application

ION MOBILITY SPECTROMETRY (IMS)

Operation:



Output of IMS:



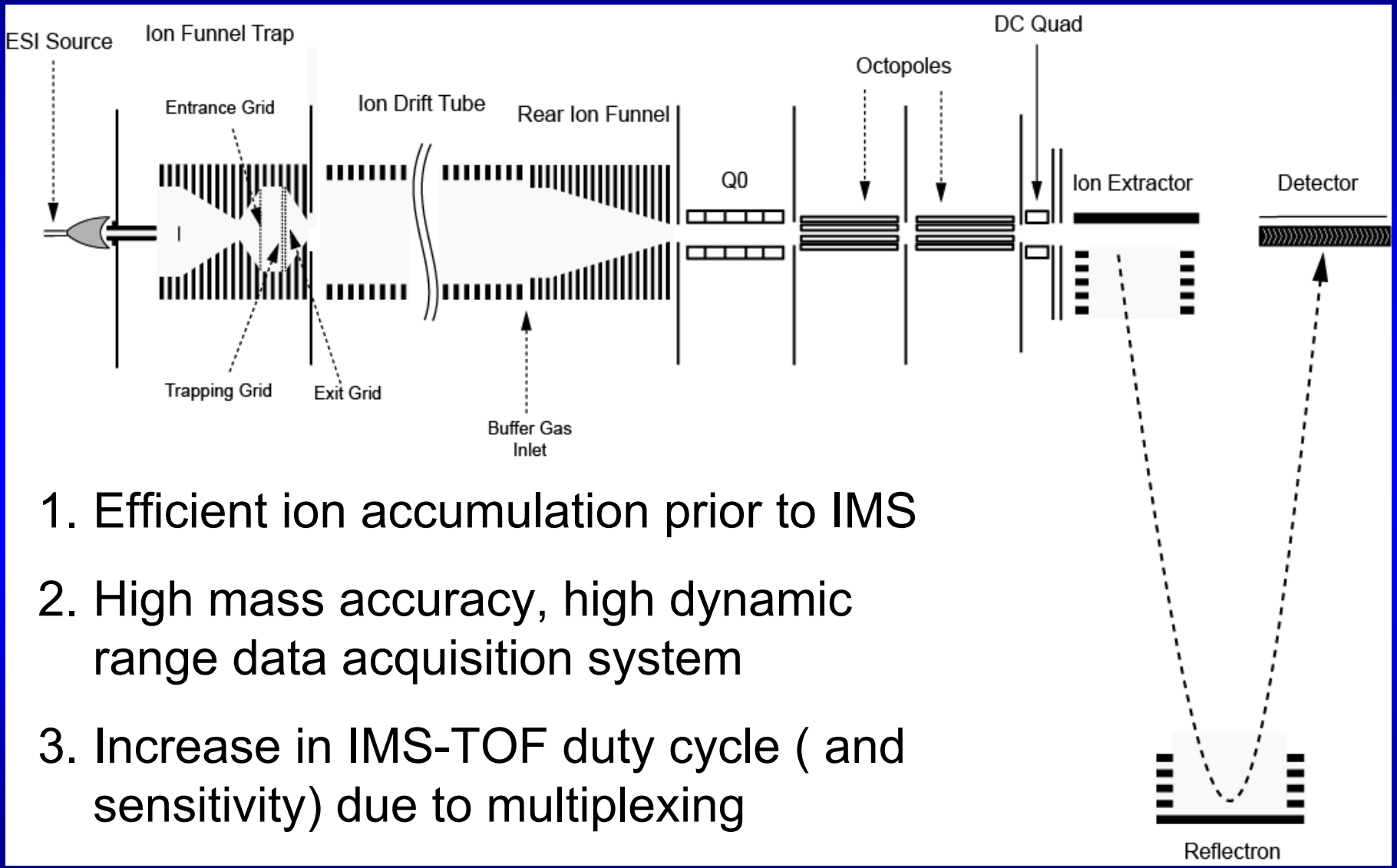
$$t_{drift} = \frac{L}{K\,E}$$ **Drift time**

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

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

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

IMS-TOFMS EXPERIMENTAL SETUP



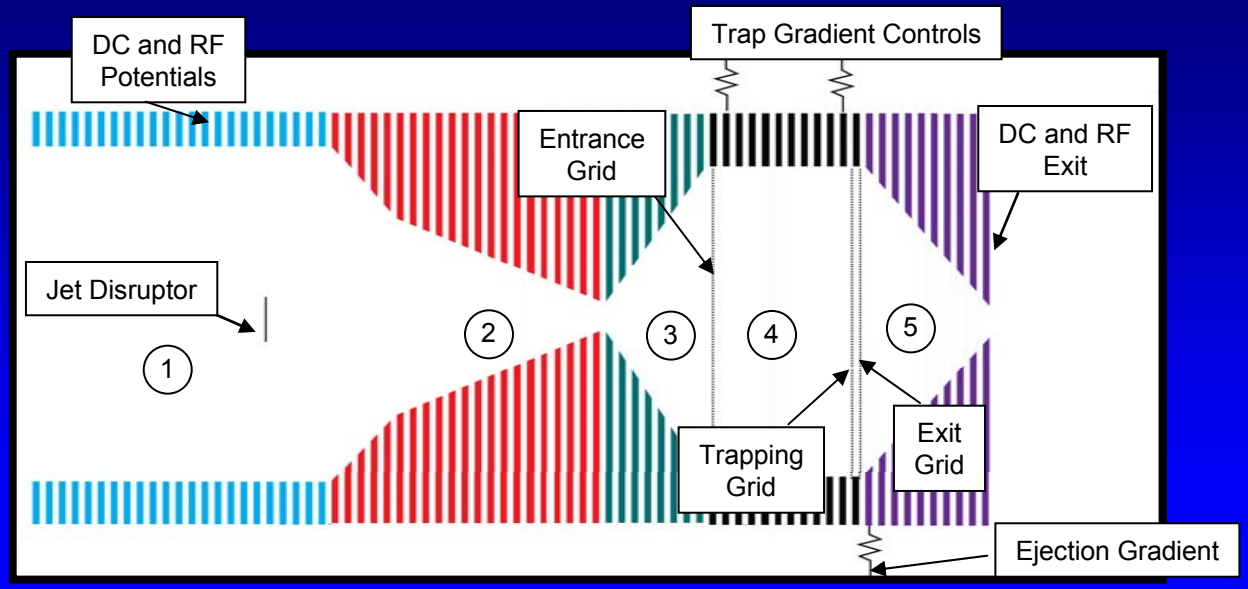
1. Efficient ion accumulation prior to IMS
2. High mass accuracy, high dynamic range data acquisition system
3. Increase in IMS-TOF duty cycle (and sensitivity) due to multiplexing

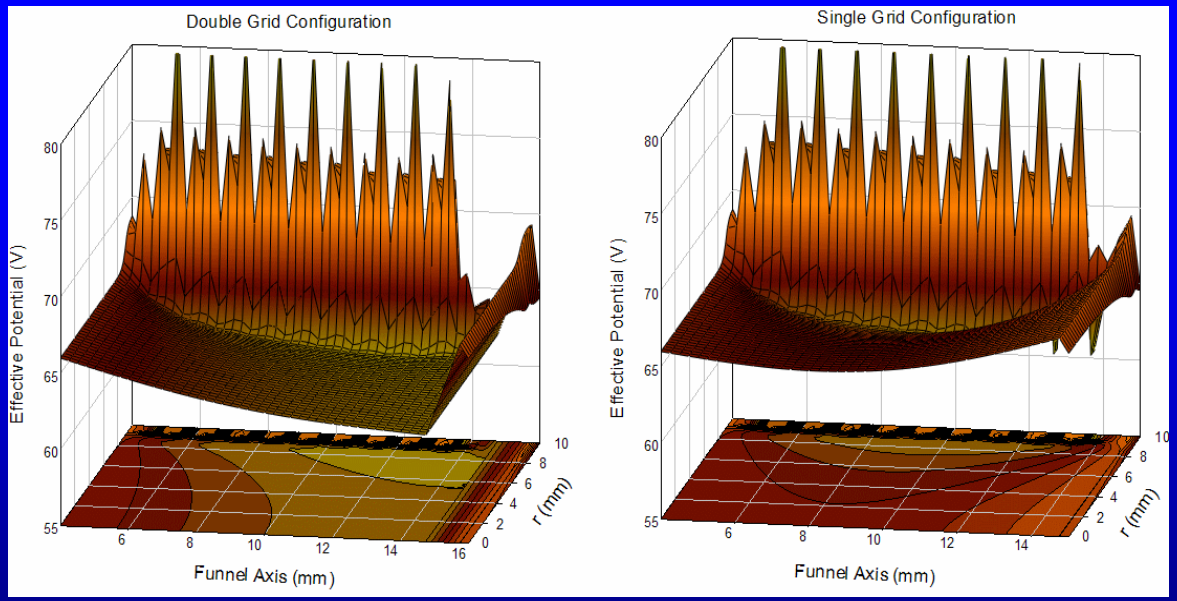
ION FUNNEL TRAP FOR IMS

CHALLENGE:

Efficiently accumulate ions at higher pressures (a few Torr) and rapidly introduce ion packets into an IMS drift tube

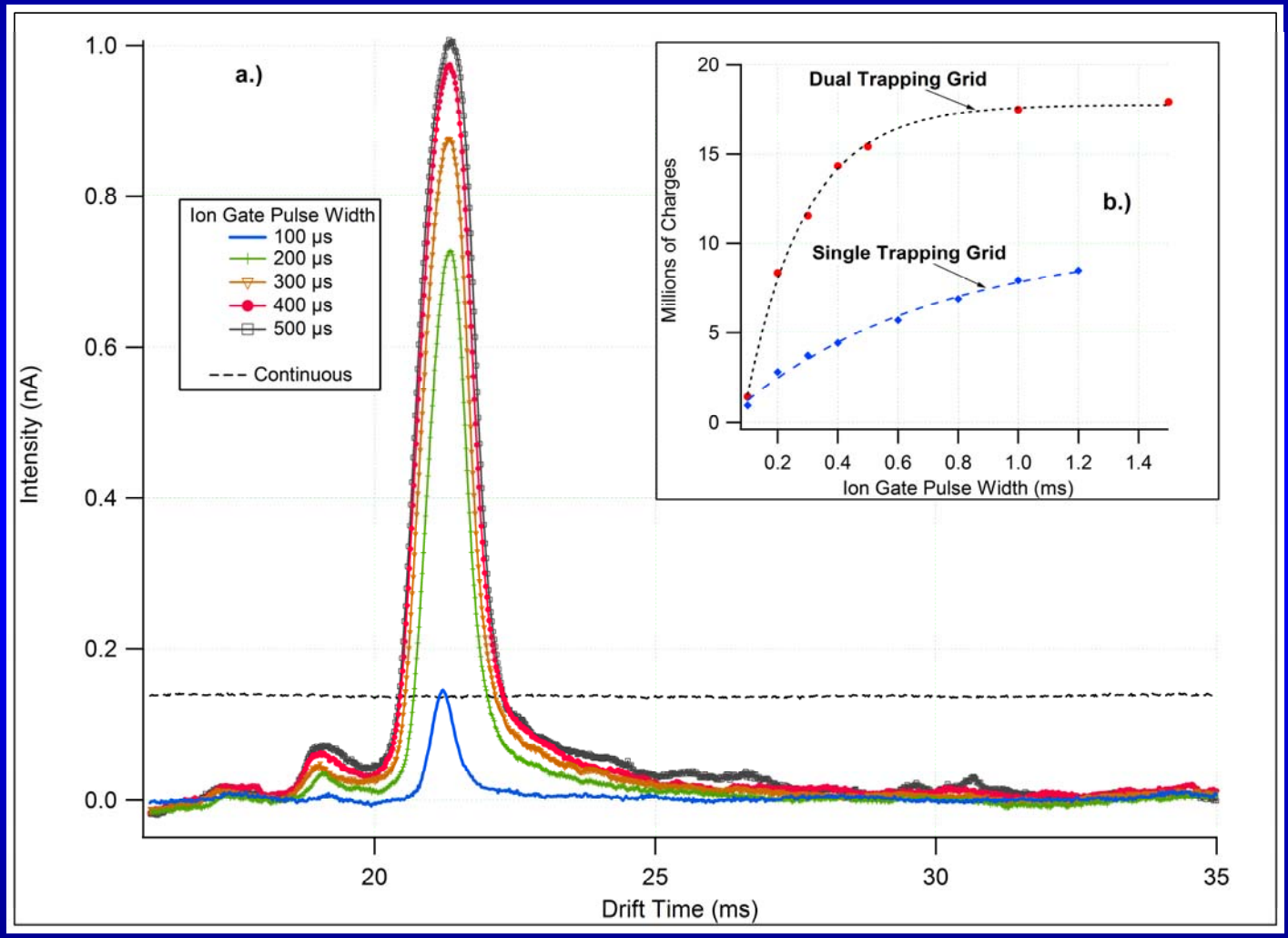
IMS ION FUNNEL TRAP DESIGN





1. Ibrahim, Y.M.; Belov, M.E.; Tolmachev, A.V.; Prior, D.C.; Smith R.D. *Anal. Chem,* 2007, 79, 7845 -7852
2. Clowers, B. H.; Ibrahim, Y. M.; Prior, D. C.; Danielson, W. F., III; Belov, M. E.; Smith, R. D. *Anal. Chem.*, 2008, 80, 612 -623

IMS-ONLY SIGNALS WITH IMPROVED ION TRAP



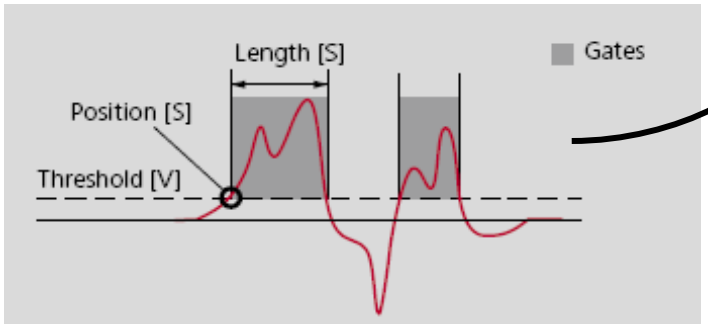
ANALOG-TO-DIGITAL DETECTION

CHALLENGES:

1. Better match increased ion packet charge density due to ion accumulation
2. Maintain high mass accuracy and mass resolution at large variations in signal intensities
3. Eliminate or drastically minimize dead time between TOF spectra acquisitions within an IMS frame and between the frames

ANALOG-TO-DIGITAL DETECTION

ANALOG-TO-DIGITAL ANALYZER AP240 SSR



1
4 MB

2
4 MB

acquisition thread

ION MOBILITY

2.5 MB

1

50 TOF scans | 50 TOF scans | 50 TOF scans
50 TOF scans | 50 TOF scans

2

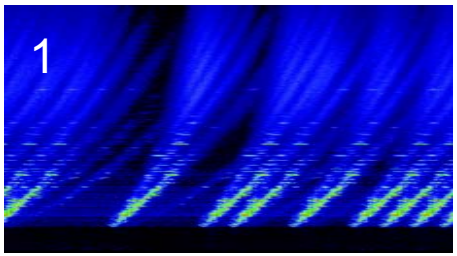
50 TOF scans | 50 TOF scans | 50 TOF scans
50 TOF scans | 50 TOF scans

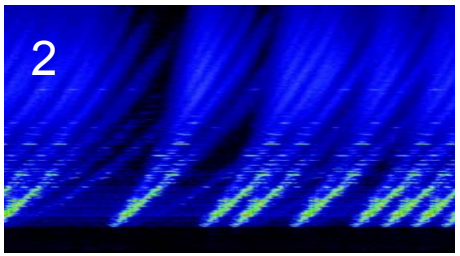
2.5 MB

processing thread

240 MB

240 MB



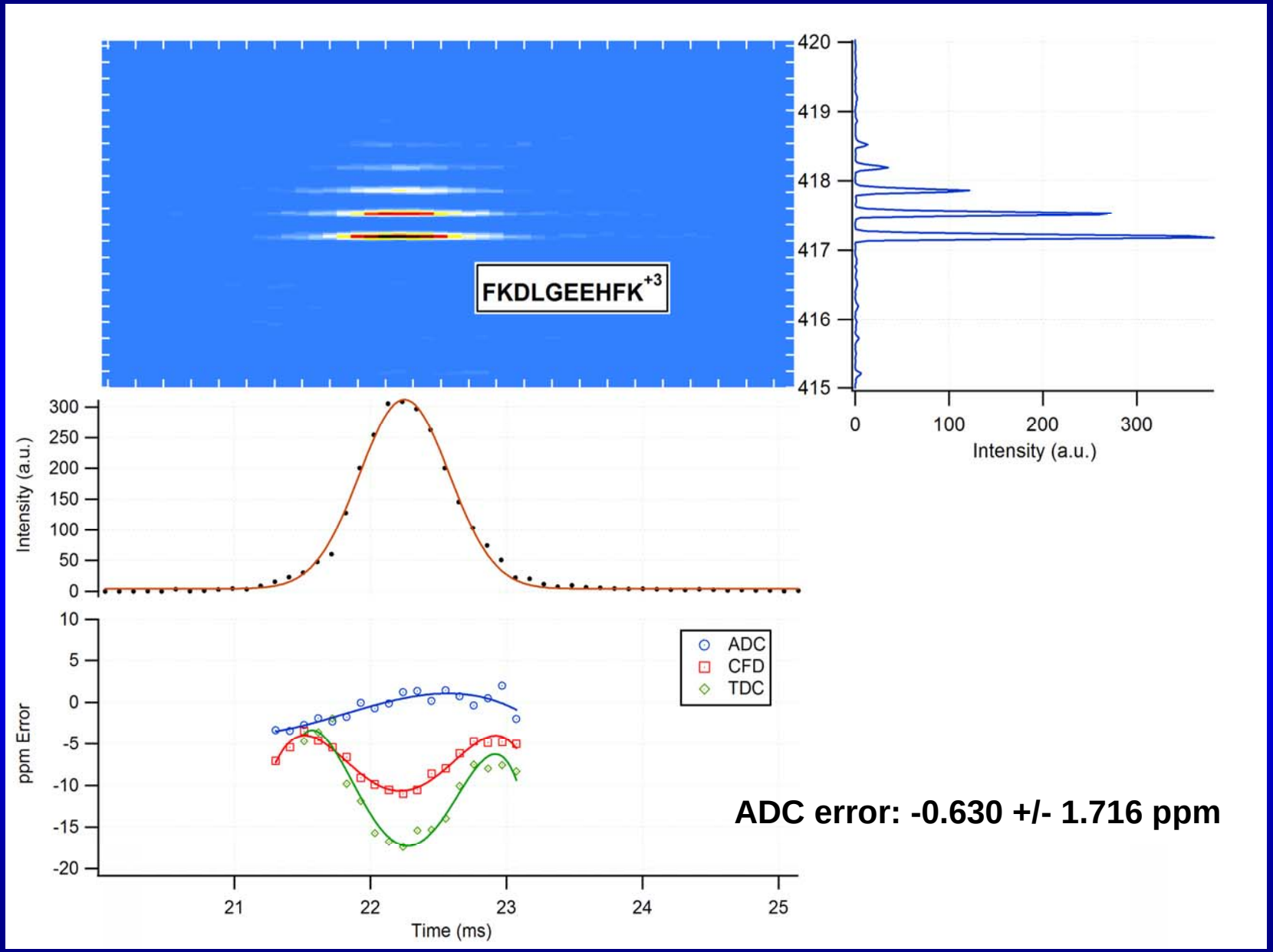


Ion Mobility Frames

saving thread

HARD DRIVE

PEPTIDE-LEVEL ADC vs TDC COMPARISON



ADC error: -0.630 +/- 1.716 ppm

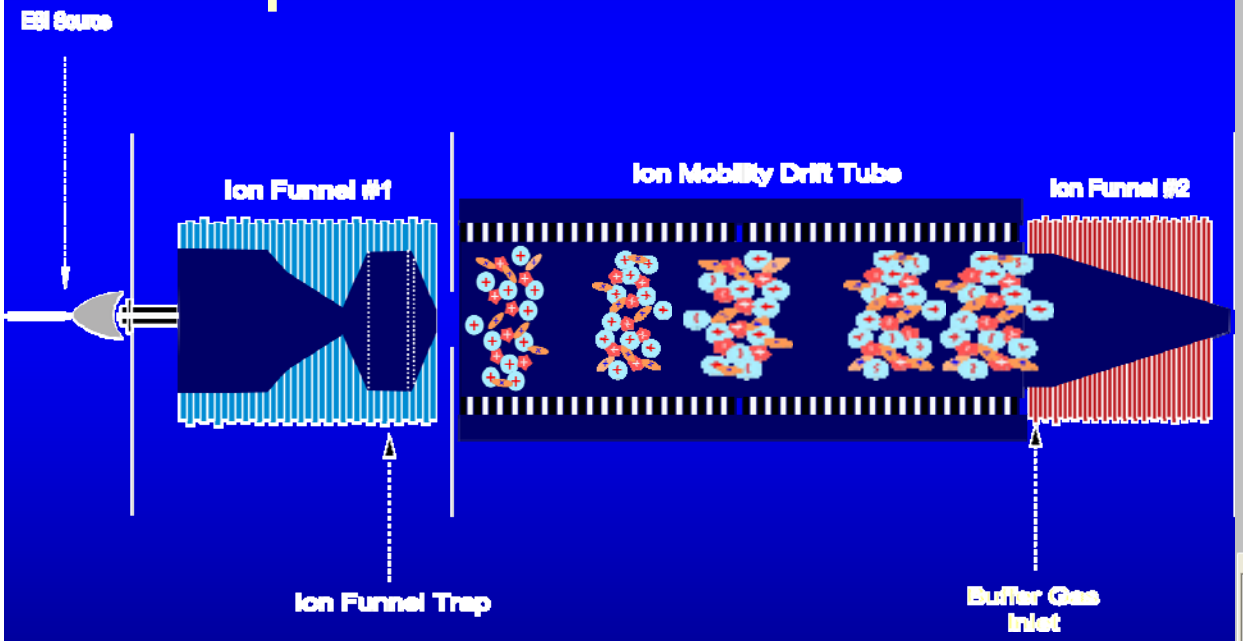
B.H. Clowers; M.E. Belov ; D.C. Prior ; W. F. Danielson ; R. D. Smith “Mass Accuracy and Dynamic Range in Ion Mobility-Mass Spectrometry Measurements: ADC vs. TDC “, WP 040

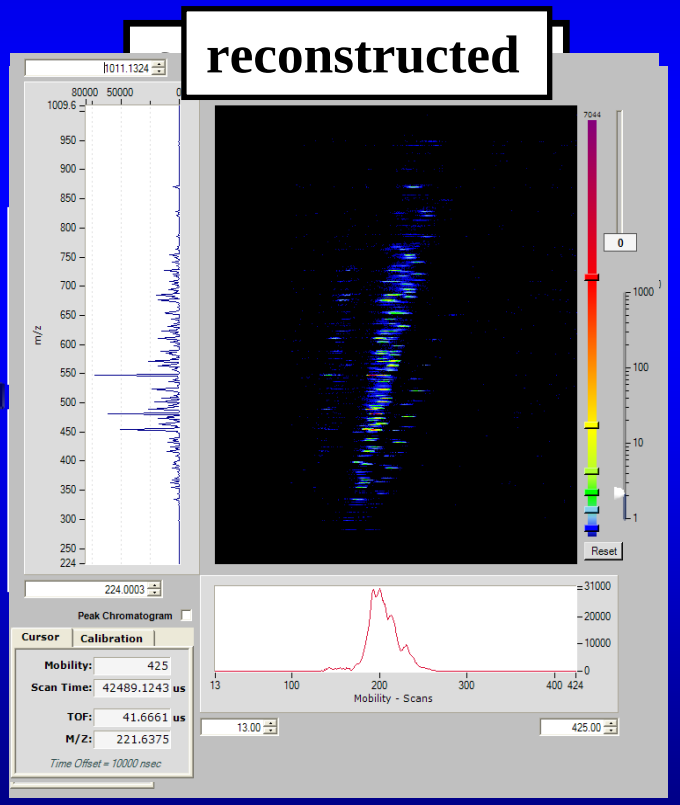
MULTIPLEXING WITH IMS-TOFMS

CHALLENGE: drastically increase duty cycle of IMS-TOFMS without affecting IMS and TOFMS resolution

SIGNAL AVERAGING VS. MULTIPLEXED IMS-TOF

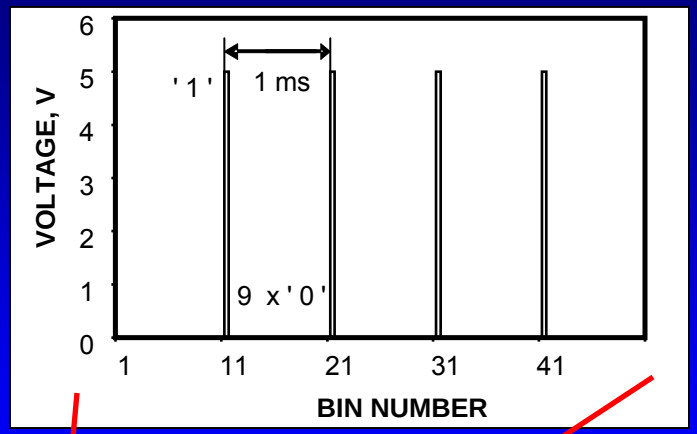
- **Signal averaging: ion accumulation between IMS separations; limited by ion trap capacity**
- **Multiplexed: multiple ion packets per single IMS separation**





M.E. Belov, M. Buschbach, D.C. Prior, K. Tang, R.D. Smith. *Anal Chem*., 2007, 79, 2451-2462

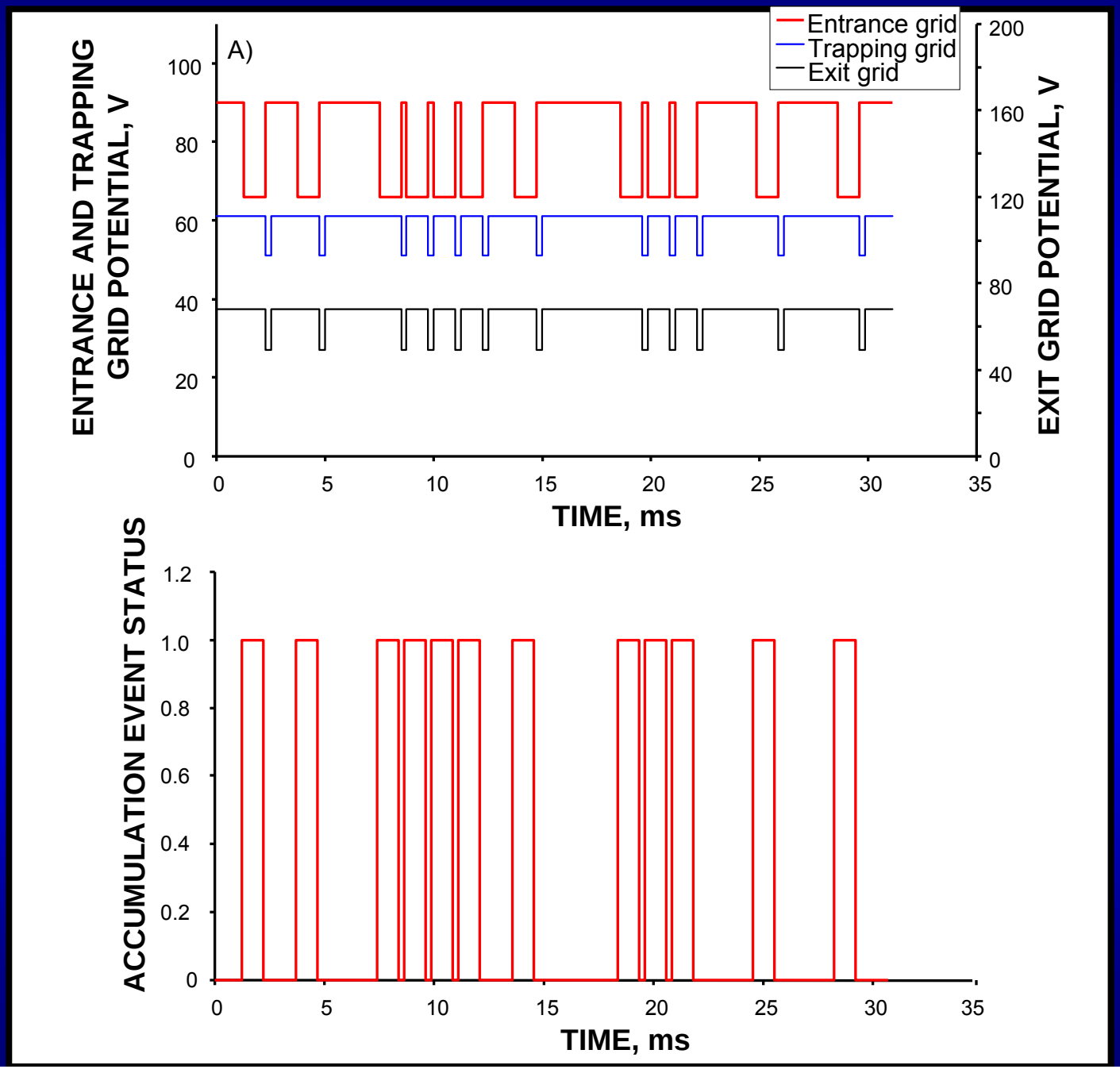
SIGNAL ENCODING AND RECONSTRUCTION



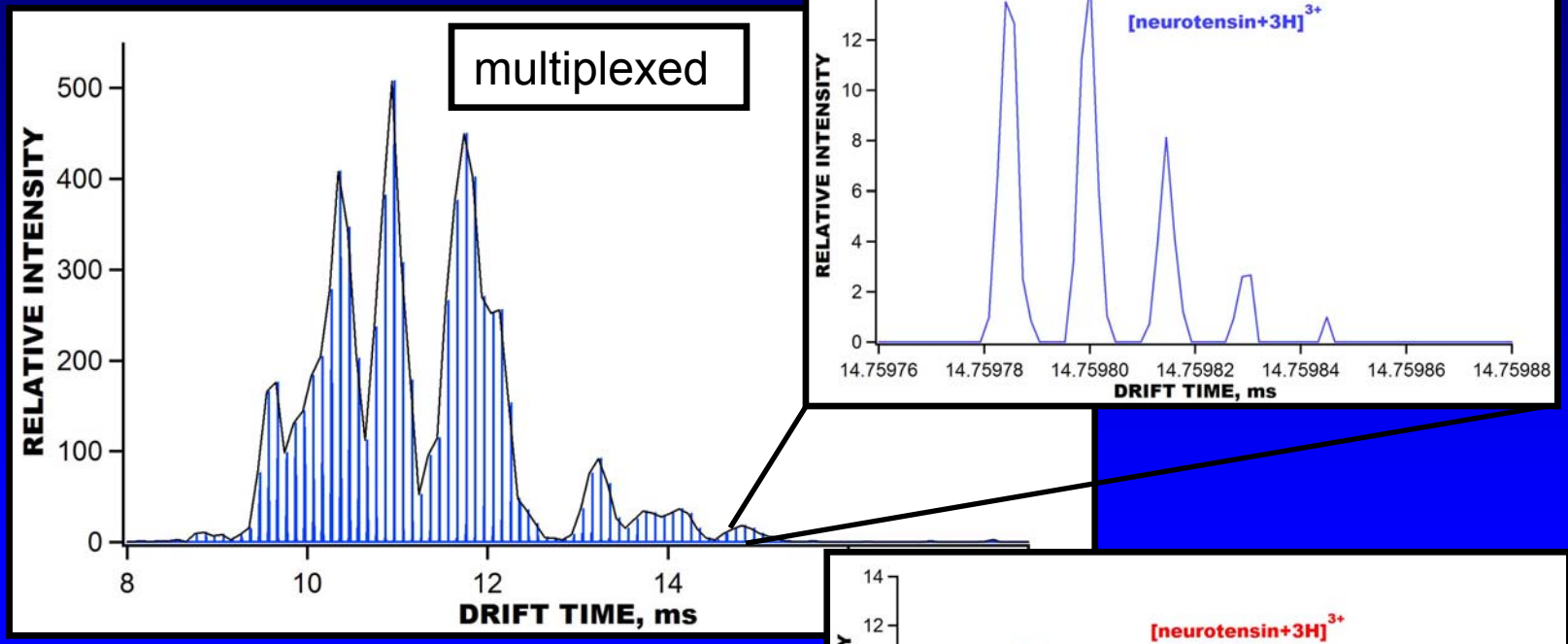
$$\begin{bmatrix}\text{ORIGINAL}\\ \text{IMS-TOF}\\ \text{VECTOR}\end{bmatrix} = \begin{bmatrix}\text{INVERSE}\\ \text{MATRIX}\end{bmatrix} \mathbf{x} \begin{bmatrix}\text{DETECTED}\\ \text{IMS-TOF}\\ \text{VECTOR}\end{bmatrix}$$

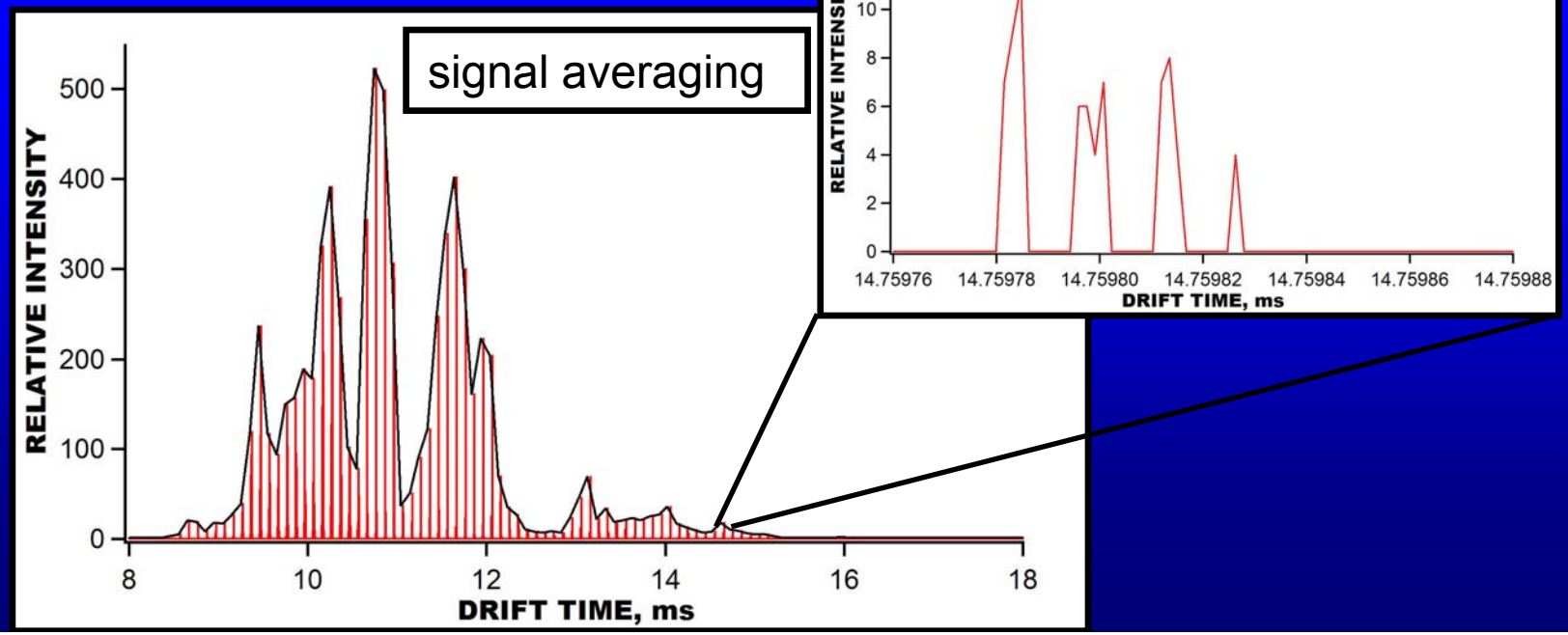
1. Mitigate detrimental effects due to thermal diffusion and space charge repulsion upon signal reconstruction
2. Accumulate ions between adjacent releases in the ion funnel trap
3. Provide constant and short ion ejections into the IMS drift tube to maintain high IMS resolution

ION GATES ENCODING AND ION ACCUMULATION



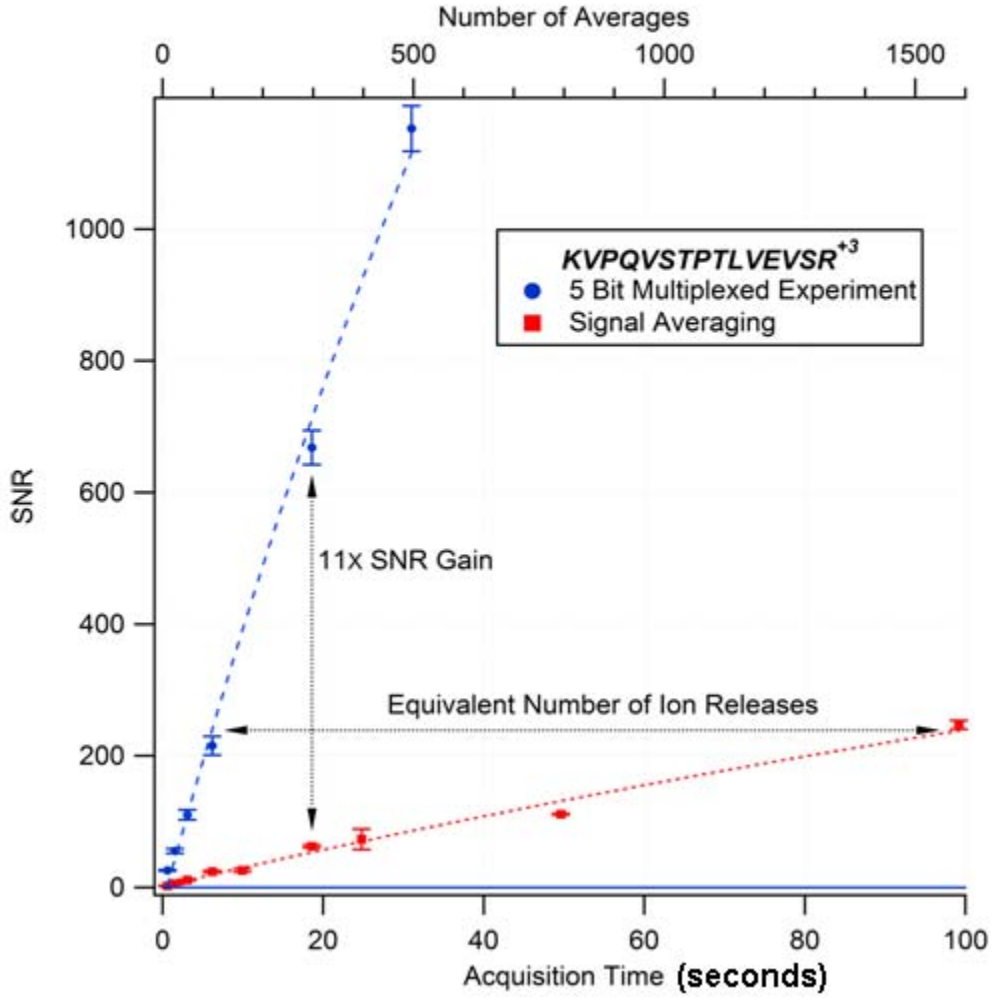
COMPARISON OF MULTIPLEXED AND SIGNAL AVERAGING APPROACHES

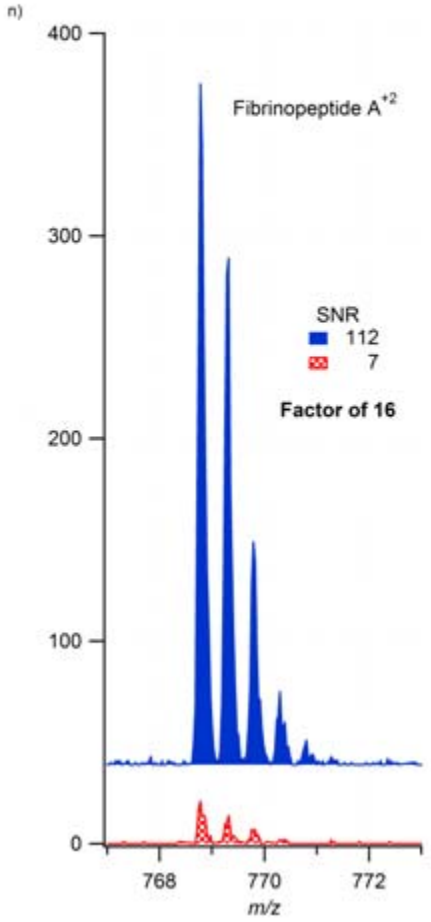




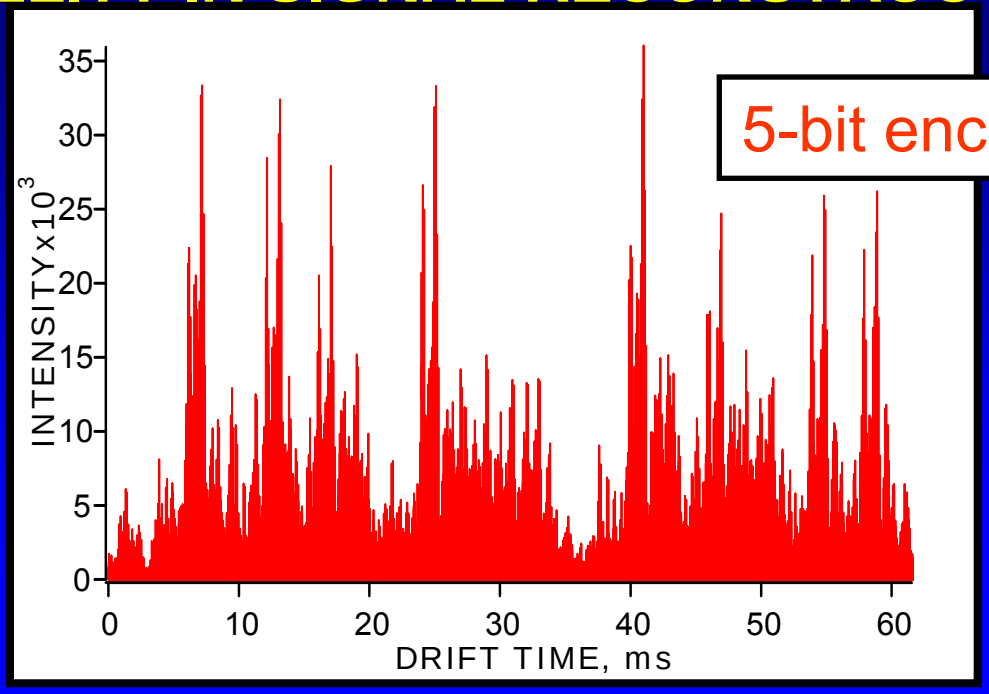
SIGNAL/NOISE IMPROVEMENTS DUE TO MULTIPLEXING

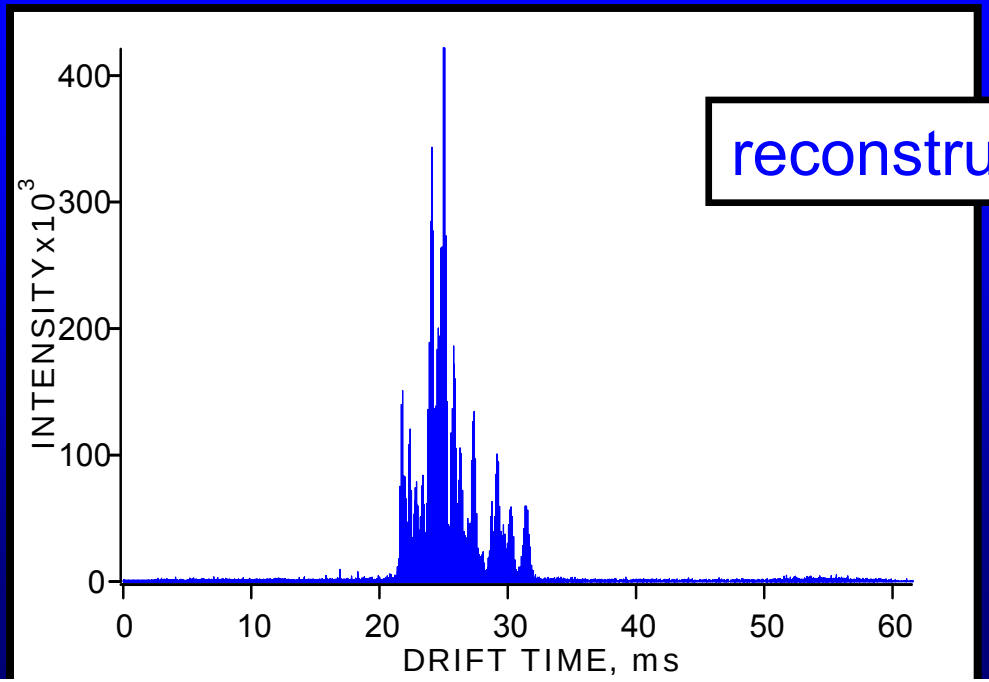
- **Equivalent S/N obtained >10 times faster**



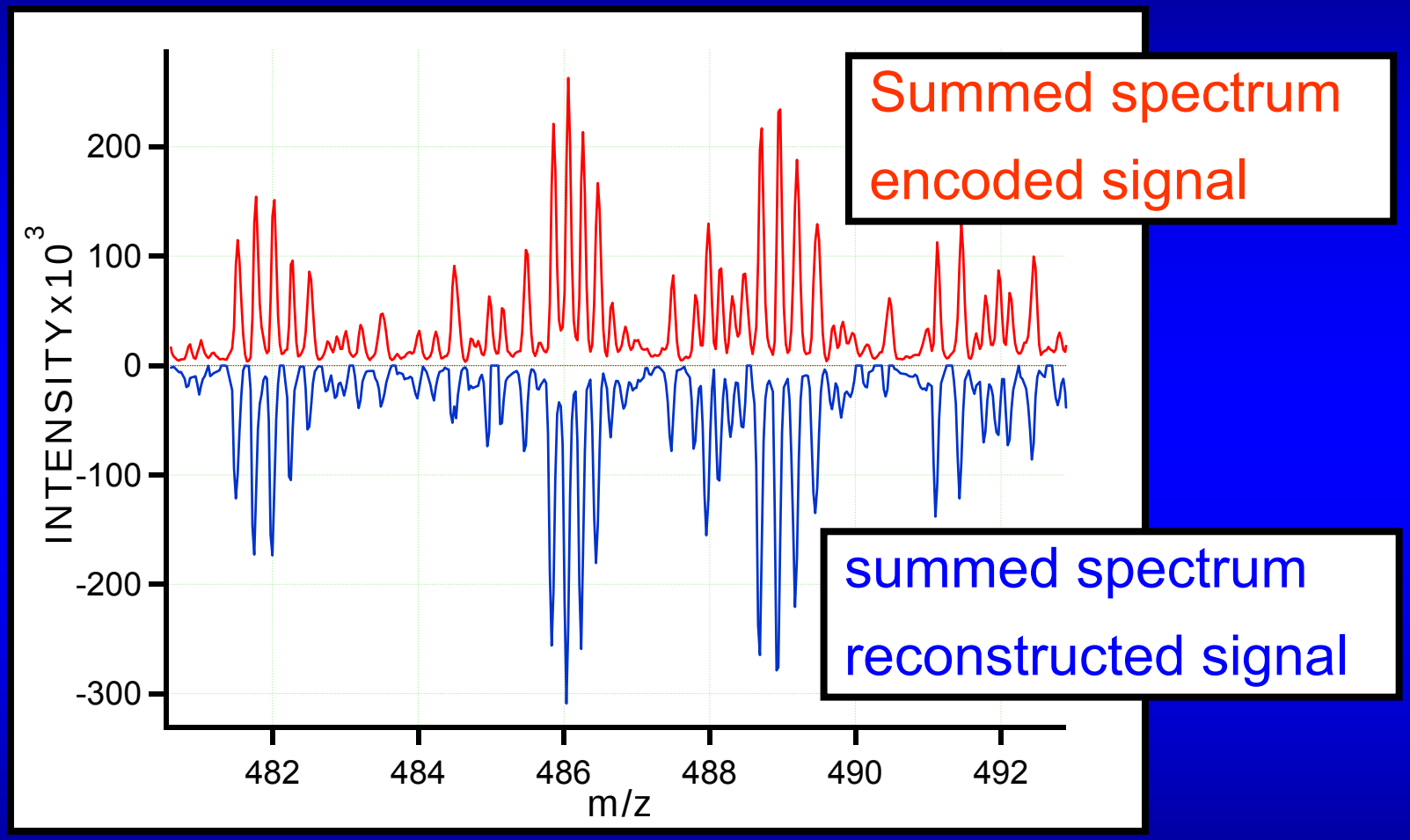


FIDELITY IN SIGNAL RECONSTRUCTION



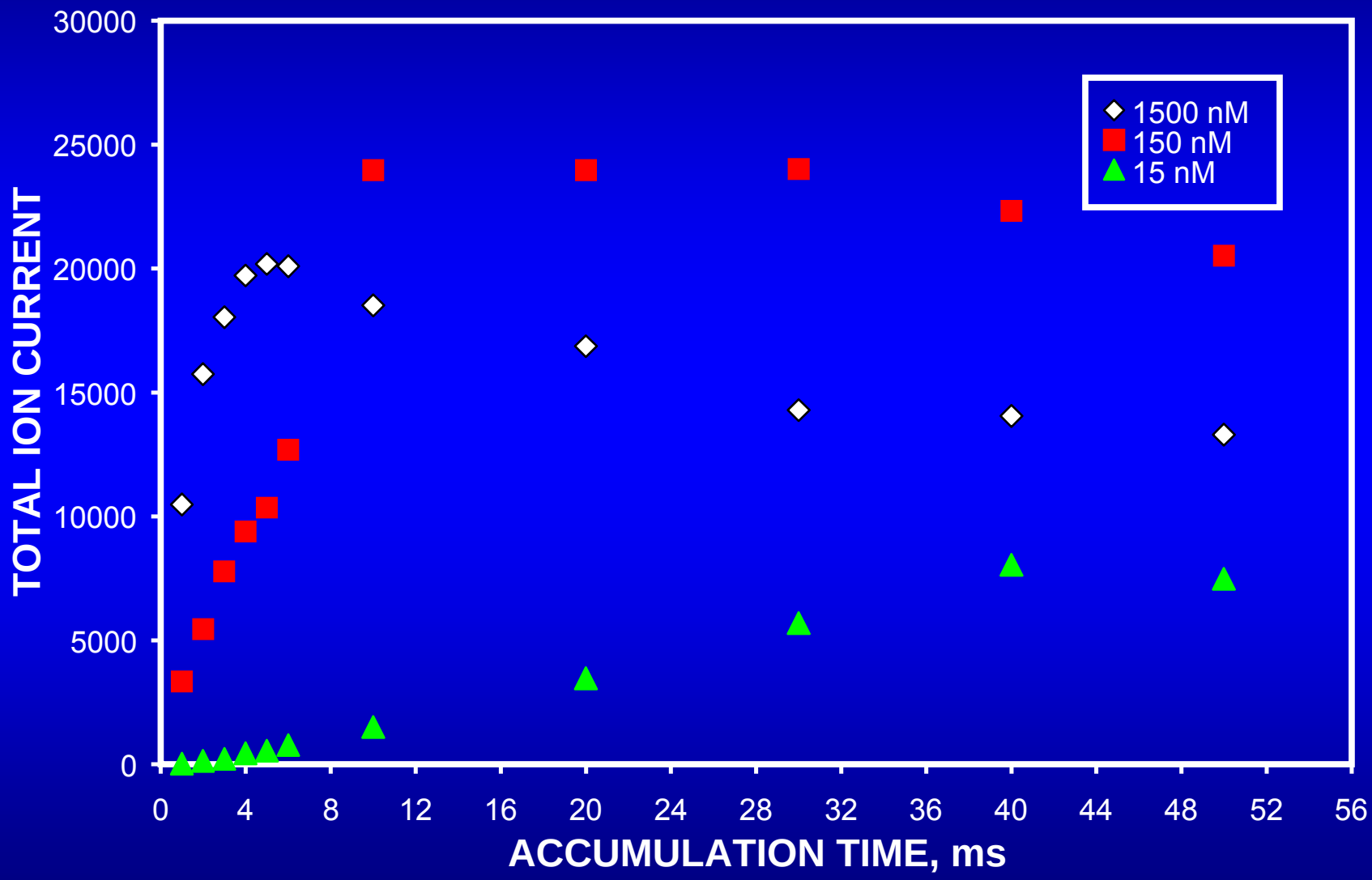


FIDELITY IN SIGNAL RECONSTRUCTION



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 (~ 2 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

n = 7 bit; $N_{bins} = 2^n-1 = 127$;

each bin is 0.5 ms, IMS is 63.5 ms

n = 5 bit; $N_{bins} = 2^n-1 = 31$;

each bin is 2 ms, IMS is 62 ms

LC-IMS-TOFMS APPLICATIONS

EXPERIMENTS WITH DEPLETED HUMAN BLOOD PLASMA

Sample: Control human plasma from Sigma-Aldrich

Depletion: GenWay Pre-packed Seppro mixed IgY12 LC5 Flow-Through

Concentration: Amicon 15 mL/5K MWCO

Digestion: 8M urea, 10 mM DTT, 40 mM iodoacetamide, trypsin (1:50 trypsin:protein)

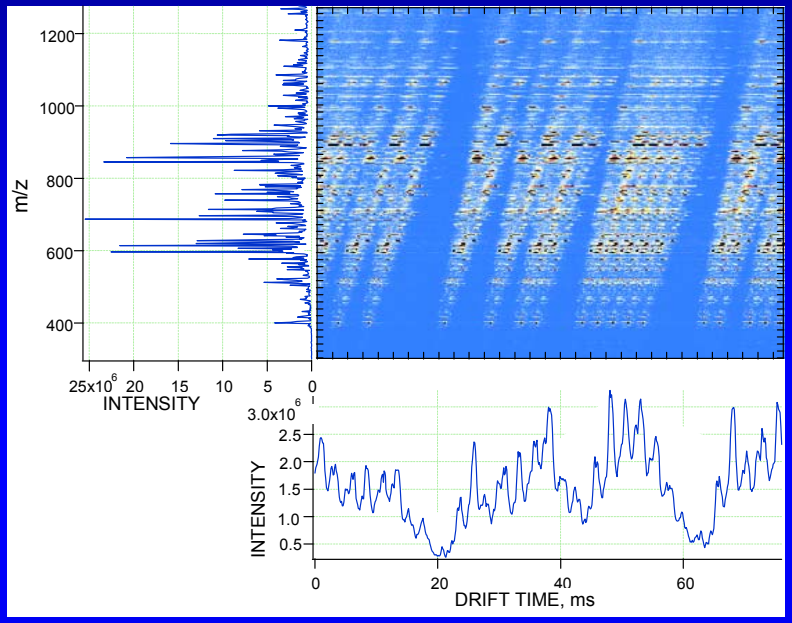
Cleanup: Discovery C18 (1 mL/100 mg)

OFF-LINE RPLC:
RP fractionation: Phenomenex reverse-phase column, Jupiter 5 µm C18 300 Å, 250 x 2 mm 5 µM, 25 fractions
Fraction delivery system: Tri-Versa NanoMate™ (Advion Biosciences)
Number of runs: 10

ON-LINE RPLC:
4-column system, 15 min separation, 10,000 psi, 15 cm, 50 µm i.d., 3 µm C18
Number of runs: 12 total, 3 runs per column

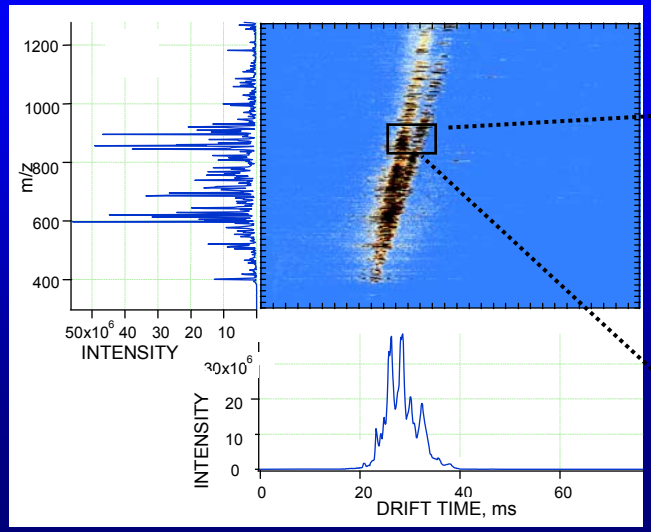
DEPLETED HUMAN BLOOD PLASMA

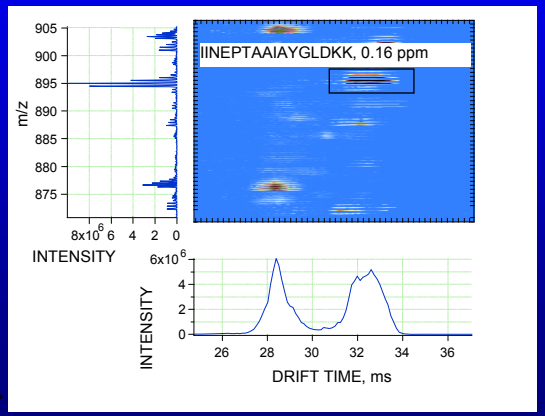
offline RPLC-multiplexed IMS-TOF, run 4, fraction 14



FRACTION	PRS, bits	MATCHES
9	4	13
10	4	95
11	4	139
12	5	140
13	6	64
14	5	118
15	6	58
16	6	39
17	6	33
18	6	13
19	5	12
20	5	18
21	5	8
22	5	4

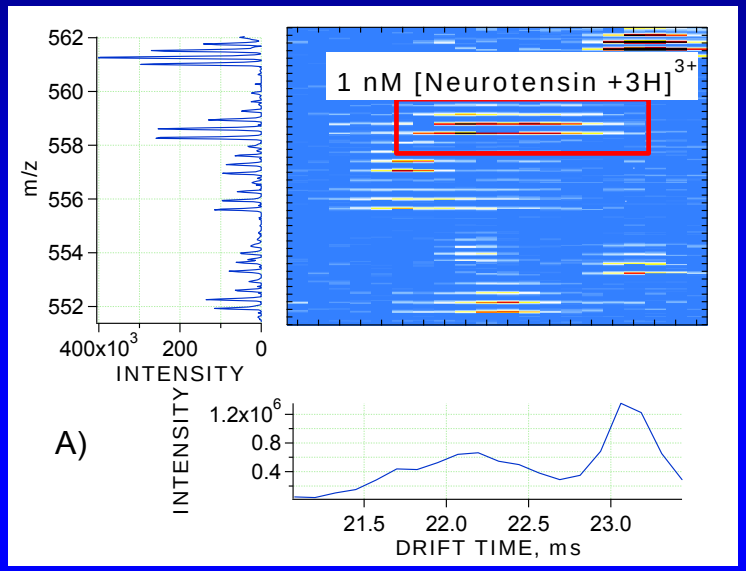
15 min

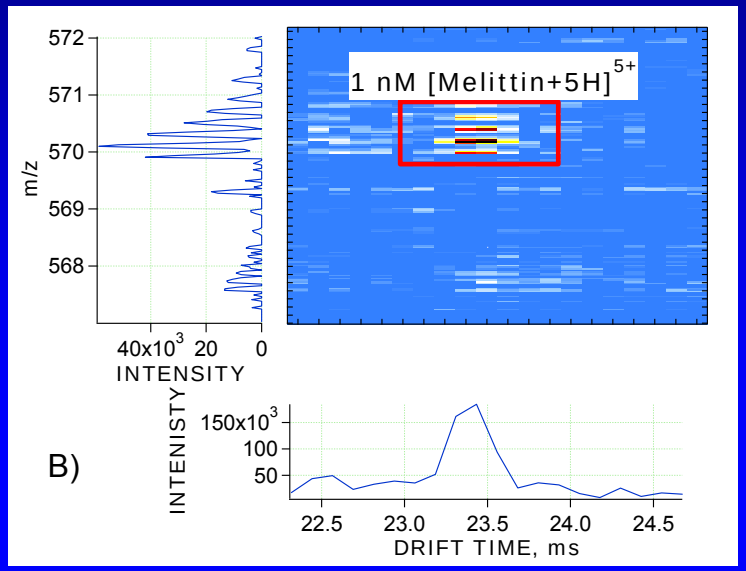


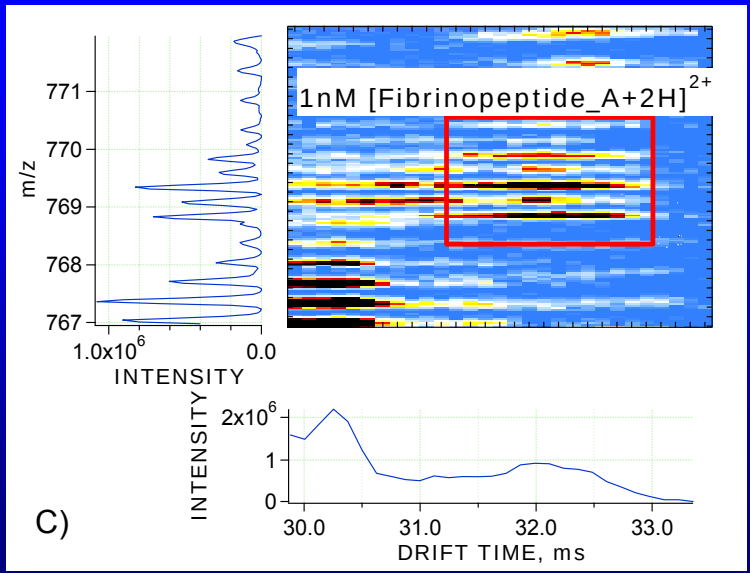


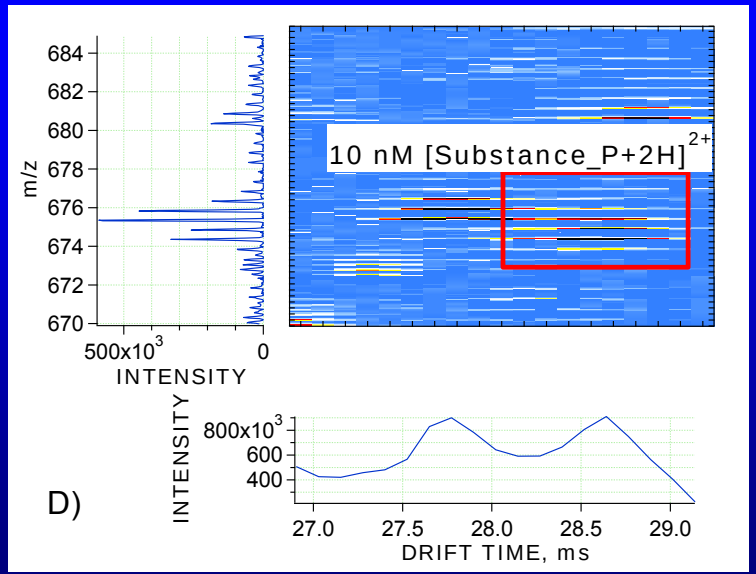
DEPLETED HUMAN BLOOD PLASMA

offline RPLC-multiplexed IMS-TOF, reconstructed signals

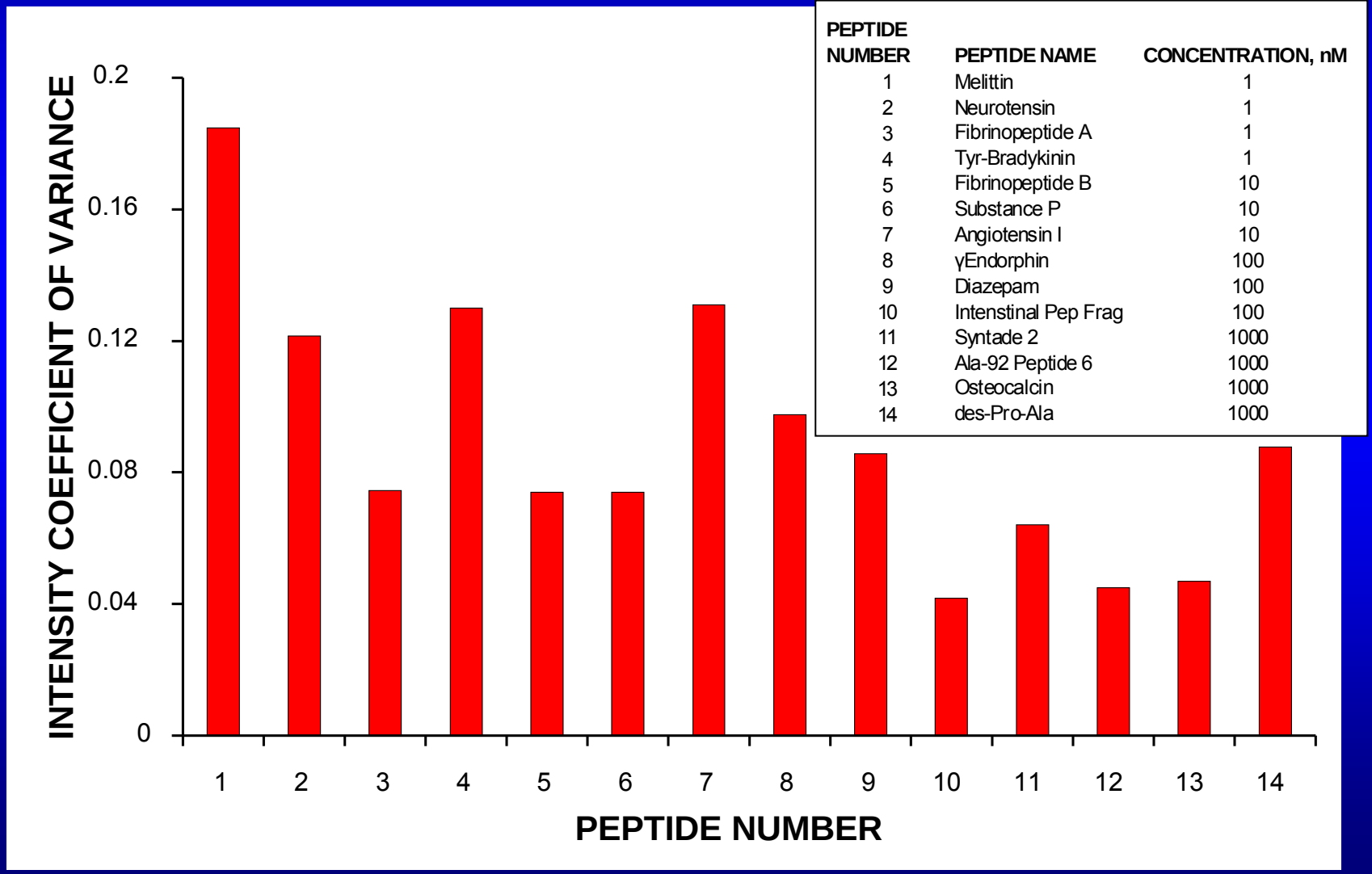








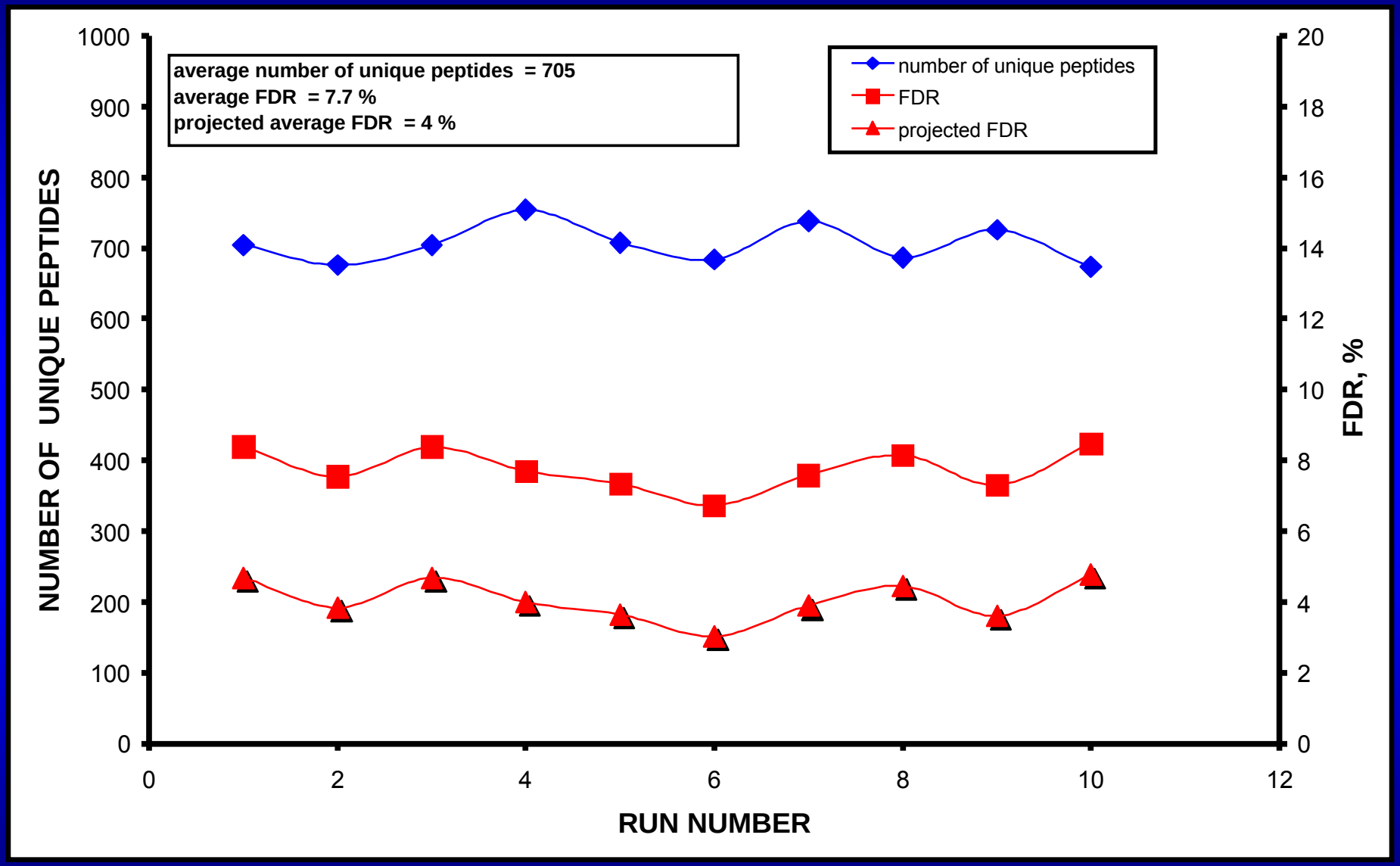
INTENSITY CVs FOR PEPTIDES SPIKED IN DEPLETED HUMAN PLASMA



PEPTIDE NUMBER	PEPTIDE NAME	CONCENTRATION, nM
1	Melittin	1
2	Neurotensin	1
3	Fibrinopeptide A	1
4	Tyr-Bradykinin	1
5	Fibrinopeptide B	10
6	Substance P	10
7	Angiotensin I	10
8	γEndorphin	100
9	Diazepam	100
10	Intenstinal Pep Frag	100
11	Syntade 2	1000
12	Ala-92 Peptide 6	1000
13	Osteocalcin	1000
14	des-Pro-Ala	1000

PEPTIDE IDENTIFICATIONS WITH OFF-LINE RPLC-MULTIPLEXED IMS-TOF

0.5 mg/mL DEPLETED HUMAN PLASMA, 25 RP FRACTIONS



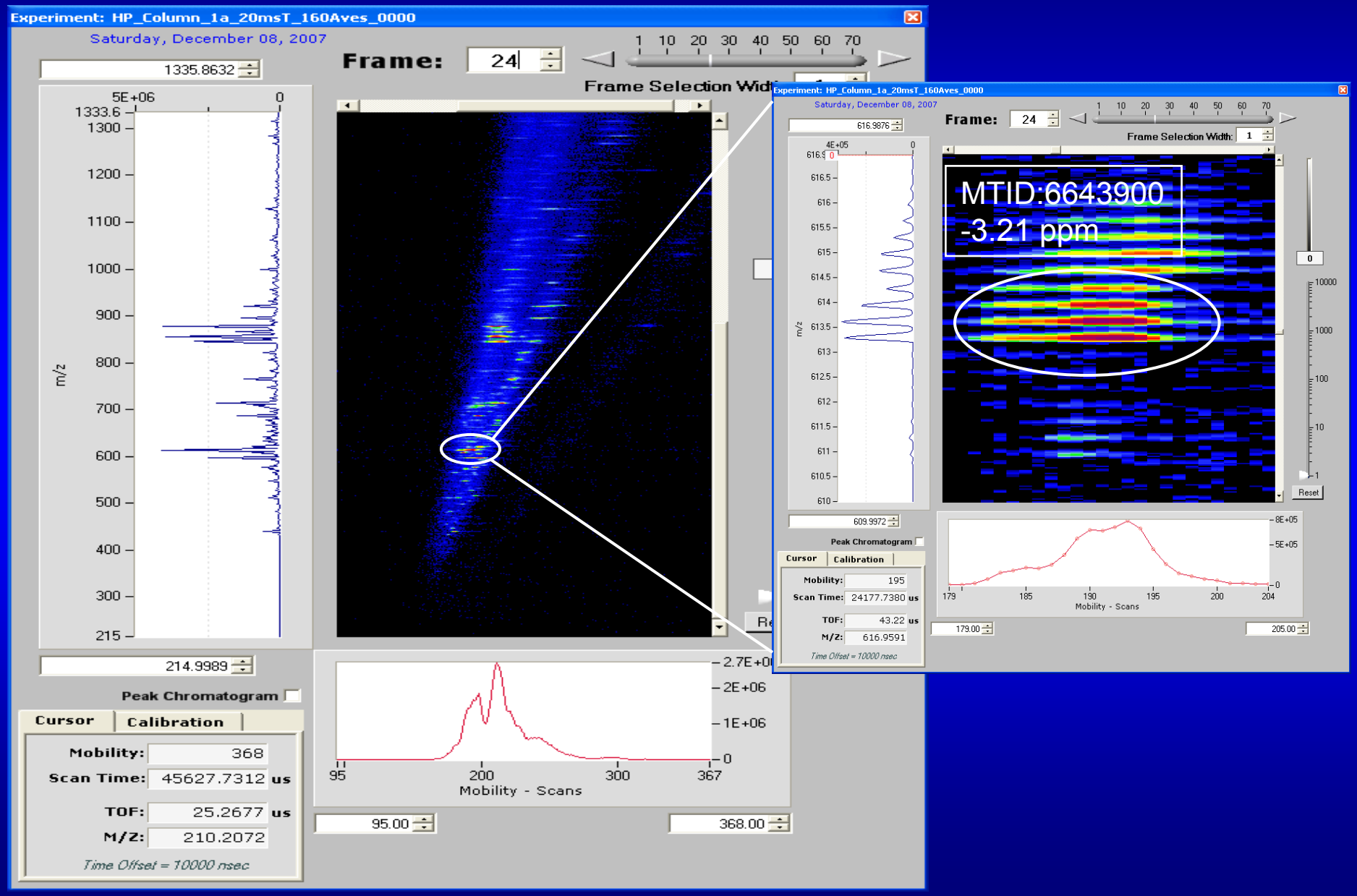
AMT tag approach: R.D.Smith, G.A. Anderson, M.S.Lipton, L.PAsa-Tolic, Y.F.Shen, T.P. Conrads, T.D. Veenstra, H.R.Udseth. *Proteomics,* 2002, *2*, 513-523

ONLINE LC-IMS-TOFMS

Fully automated 4- column dual mixer fast capillary LC-MS system

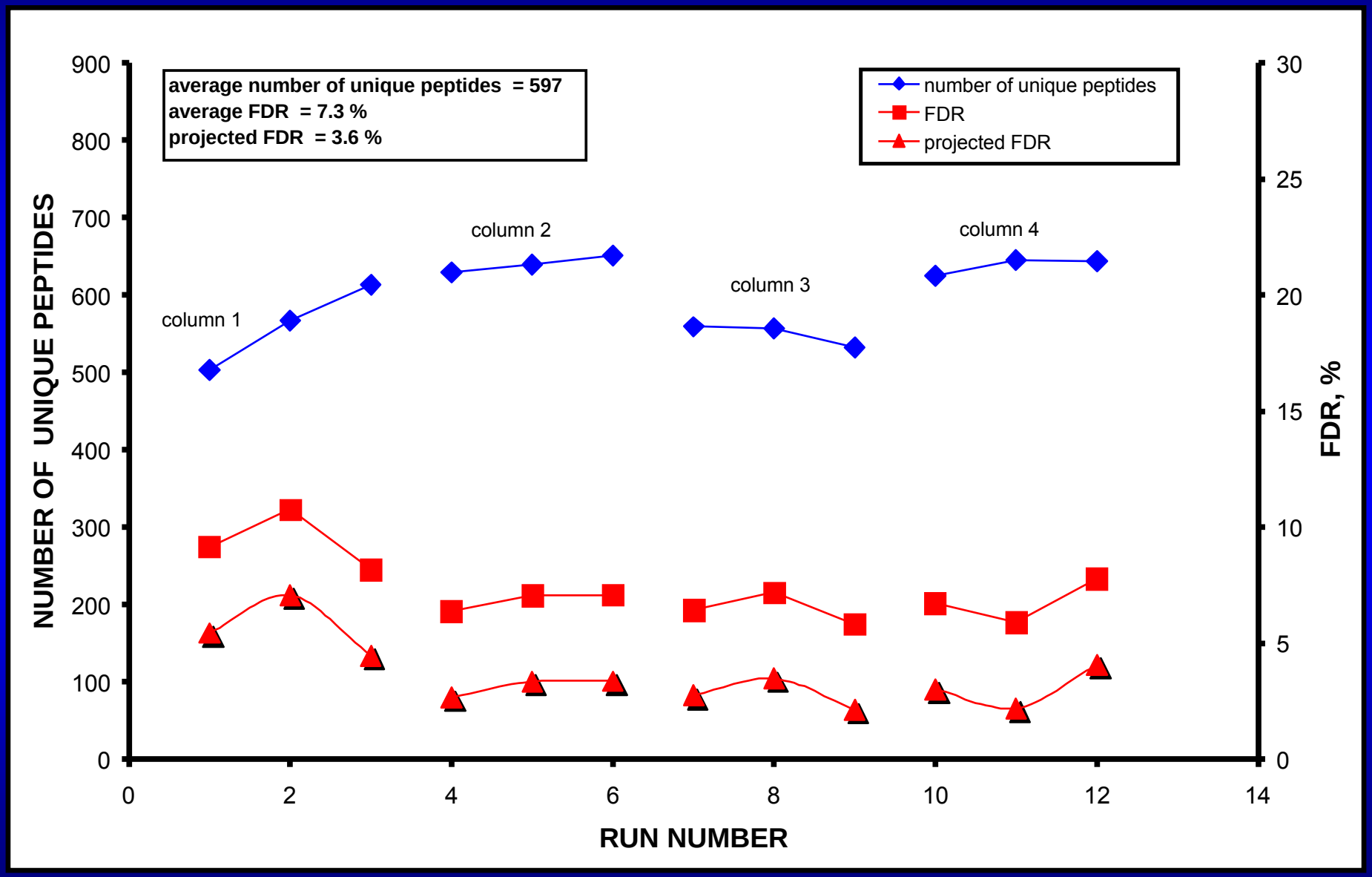
DEPLETED HUMAN BLOOD PLASMA

Online-RPLC-IMS-TOF, frame 24



PEPTIDE IDENTIFICATIONS WITH ON-LINE LC-IMS-TOF

0.5 mg/mL DEPLETED HUMAN PLASMA



CONCLUSIONS

- A novel dynamic multiplexing approach with an IMS-TOFMS instrument has been developed and rigorously evaluated in analysis of reverse-phase fractions of depleted human blood plasma.
- High throughput LC-IMS-TOFMS analysis of a depleted human blood plasma sample is accomplished in 15 min and provides a combined LC/IMS peak capacity of > 2500, mass resolution of ~ 8000 and mass accuracy of 5 ppm.
- Per single experiment, the average number of identified unique human plasma peptides was ~ 700 at a false discovery rate (FDR) of 7.5 %. When accounting for ion mobility information, a projected FDR of ~ 4% was estimated.

ACKNOWLEDGEMENTS

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