Top Down Proteomics at High Magnetic Field

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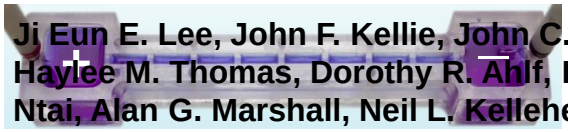
Atlanta, GA
October 7th, 2009

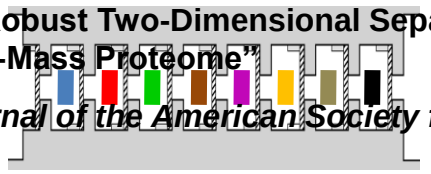
Modern Top Down Proteomics Work Flow



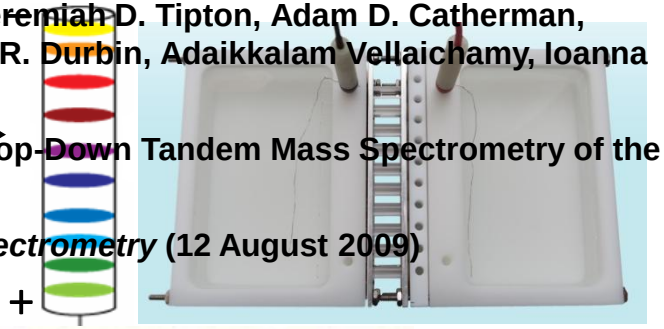
Ji Eun E. Lee, John F. Kellie, John C. Tran, Jeremiah D. Tipton, Adam D. Catherman, Haylee M. Thomas, Dorothy R. Ahlf, Kenneth R. Durbin, Adaikkalam Vellaichamy, Ioanna Ntai, Alan G. Marshall, Neil L. Kelleher

"A Robust Two-Dimensional Separation for Top-Down Tandem Mass Spectrometry of the Low-Mass Proteome"

Journal of the American Society for Mass Spectrometry (12 August 2009)

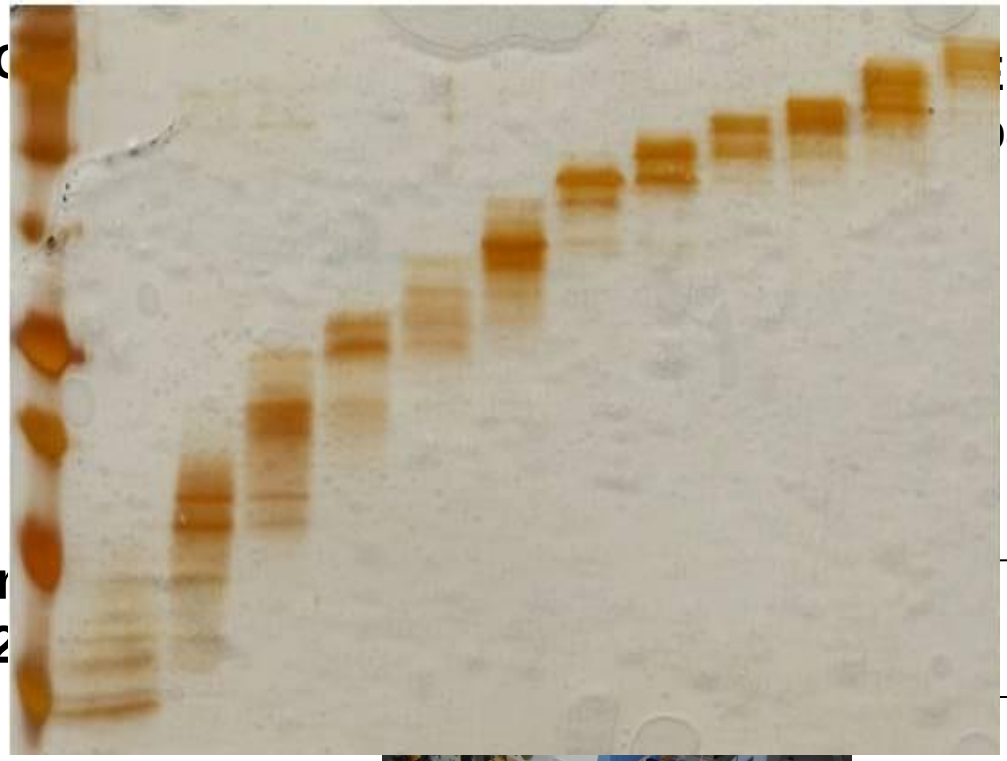


200 kDa



Cell lysate

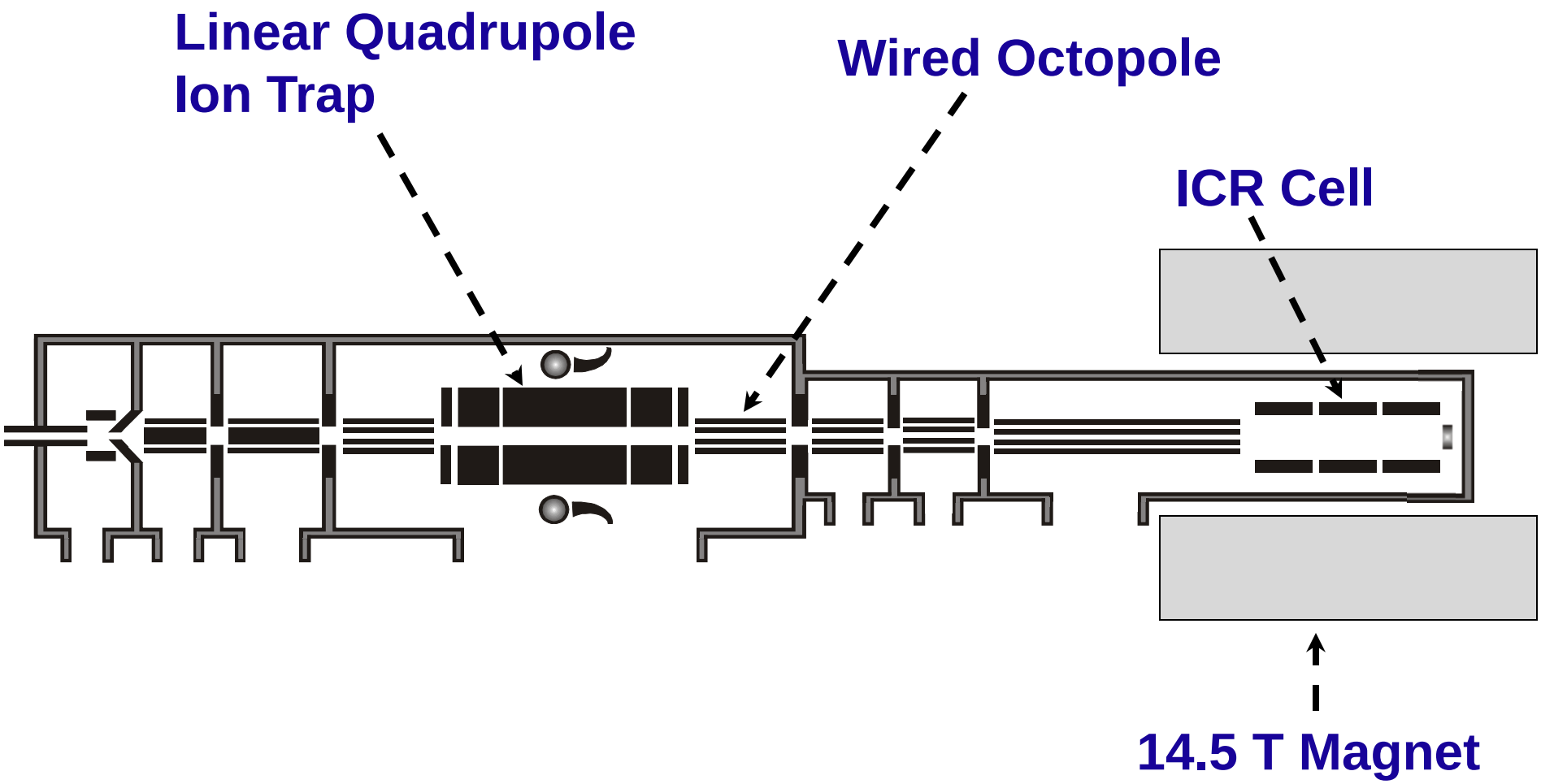
(C – MW
aration



Protein Inforr
ProSightPC 2

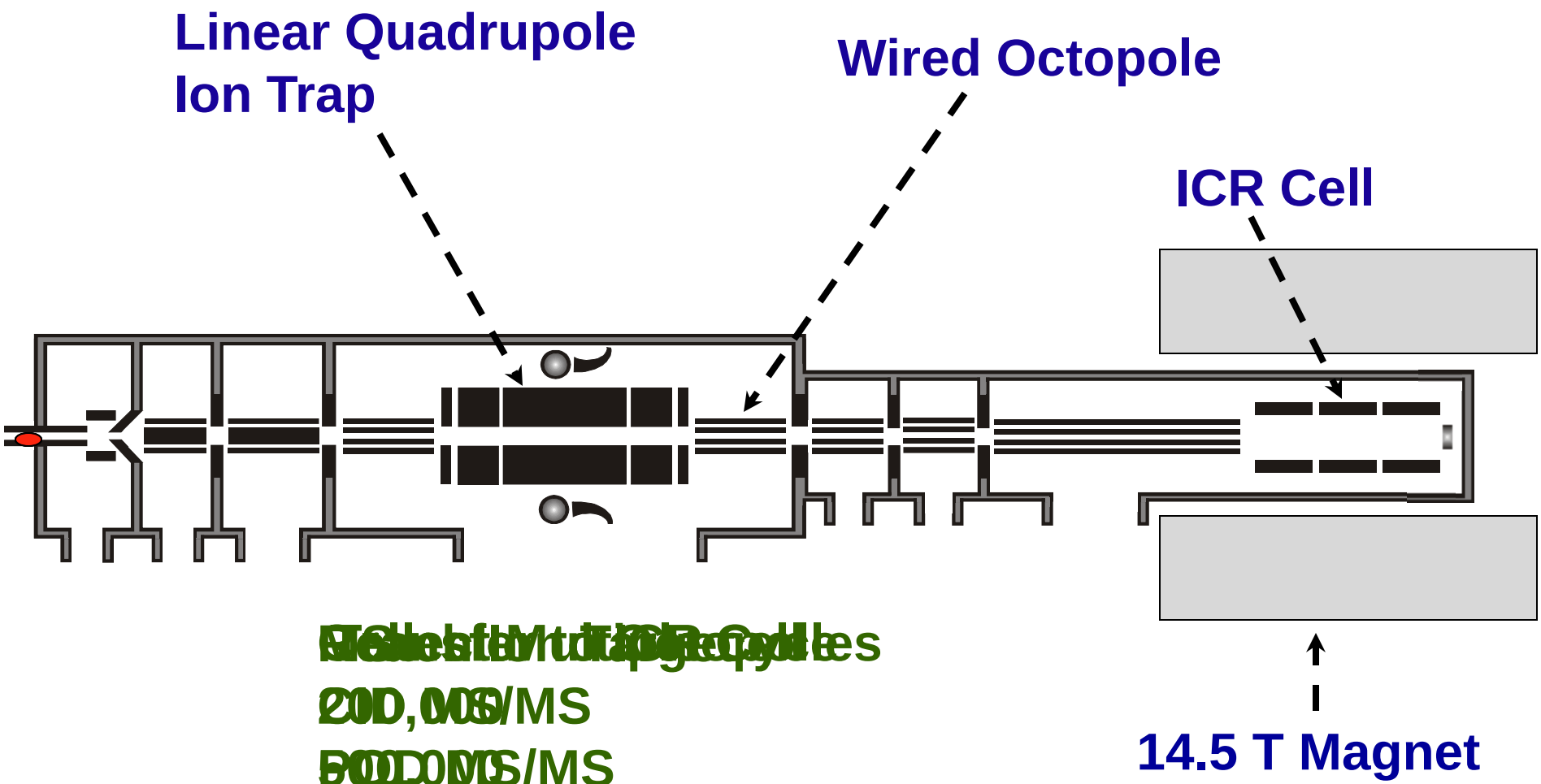
Sample cleanup
(SDS removal)

Top down Proteomics Instrumentation: Modified 14.5 T LTQ-FT



Schaub, T.M.; Hendrickson, C.L.; Horning, S.; Quinn, J.P.; Senko, M.W. and Marshall, A.G. Anal. Chem., *80*, 3985 - 3990 (2008)

Top down Proteomics Instrumentation: Modified 14.5 T LTQ-FT



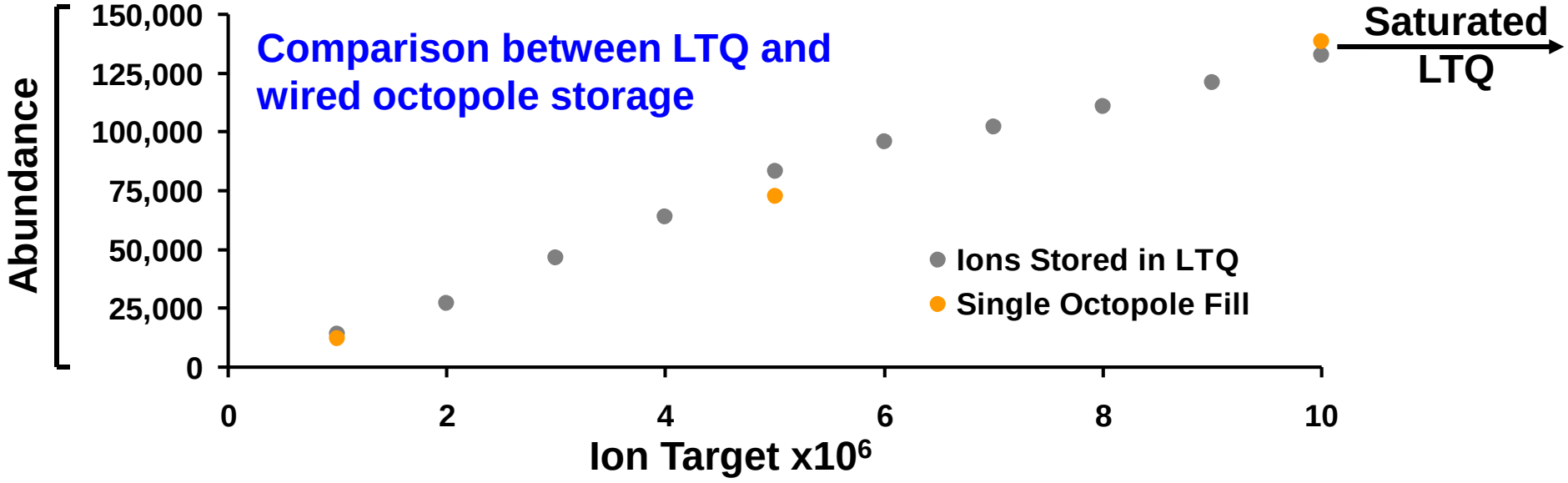
200,000

500,000

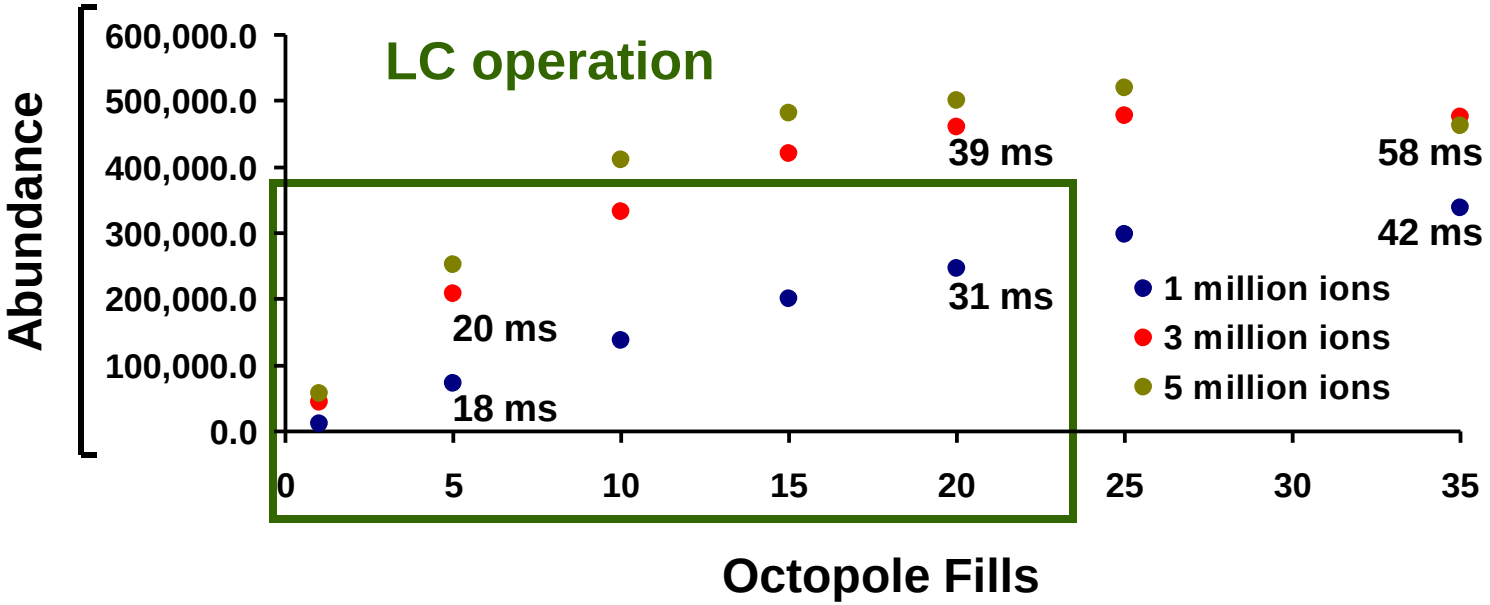
1,000,000

.....

Ion Storage Capacity: 14.5 T LTQ-FT



2 - 3 fold increase in capacity



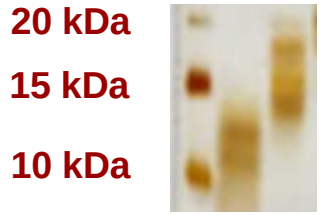
LC MS Performance: Duty Cycle

Scan Settings

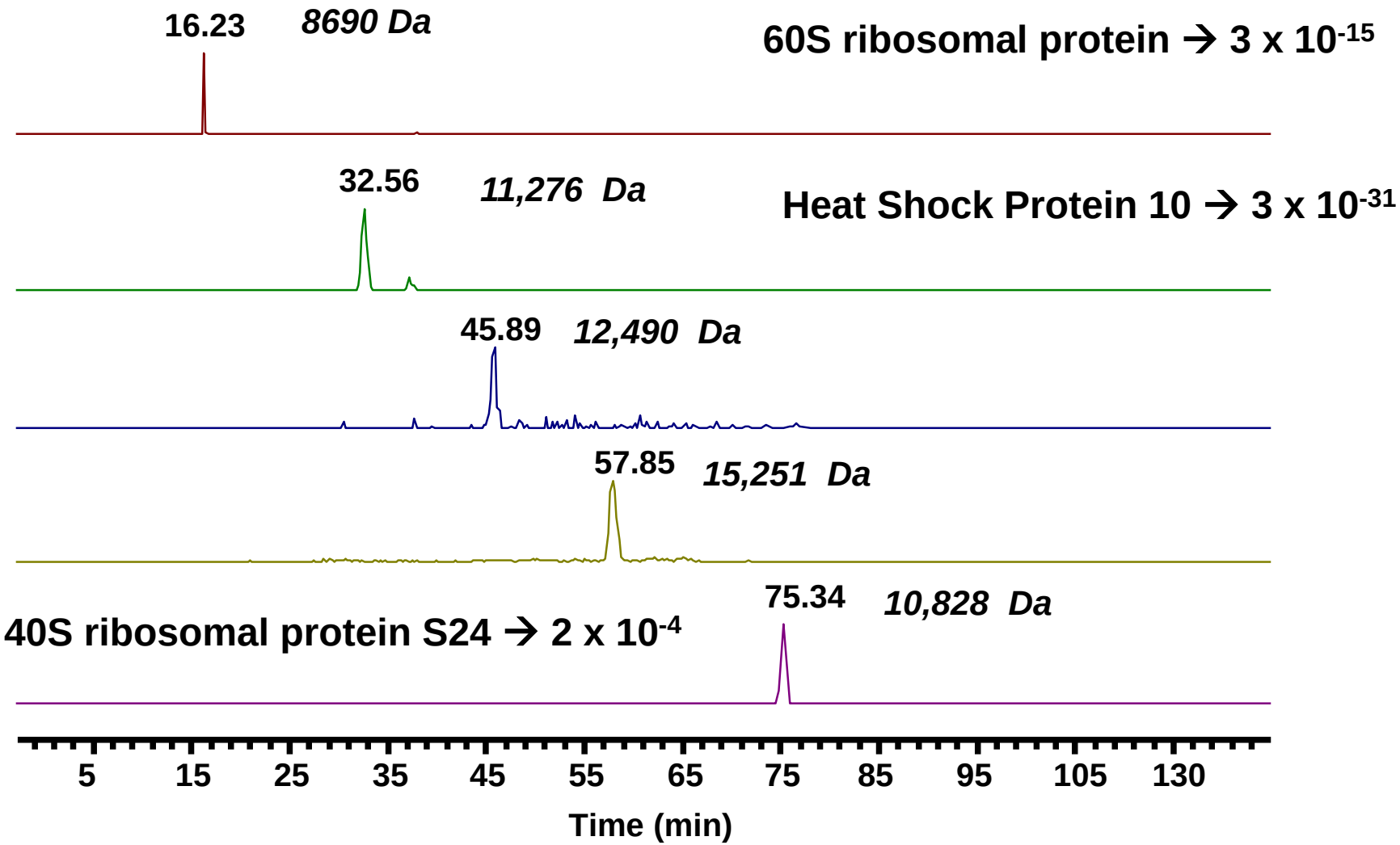
MS → 3 octopole fills – 1μscan – 1million ions → 15 - 60 ms accumulation time

MS/MS → 15 octopole fills – 1 μscan – 0.5 million ions → 0.8 – 3 s acc. time

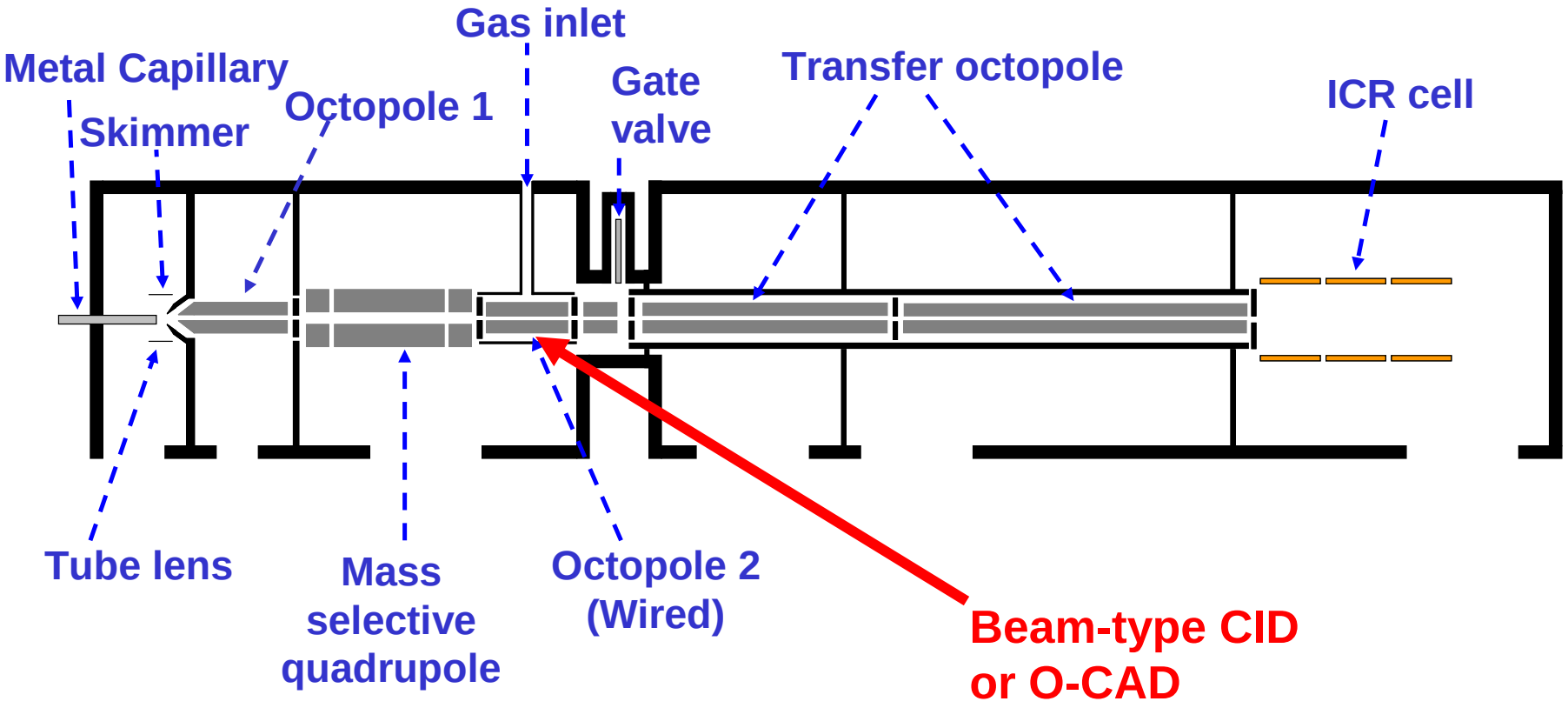
Peak width → 20 s to 1 min



Yeast Extract

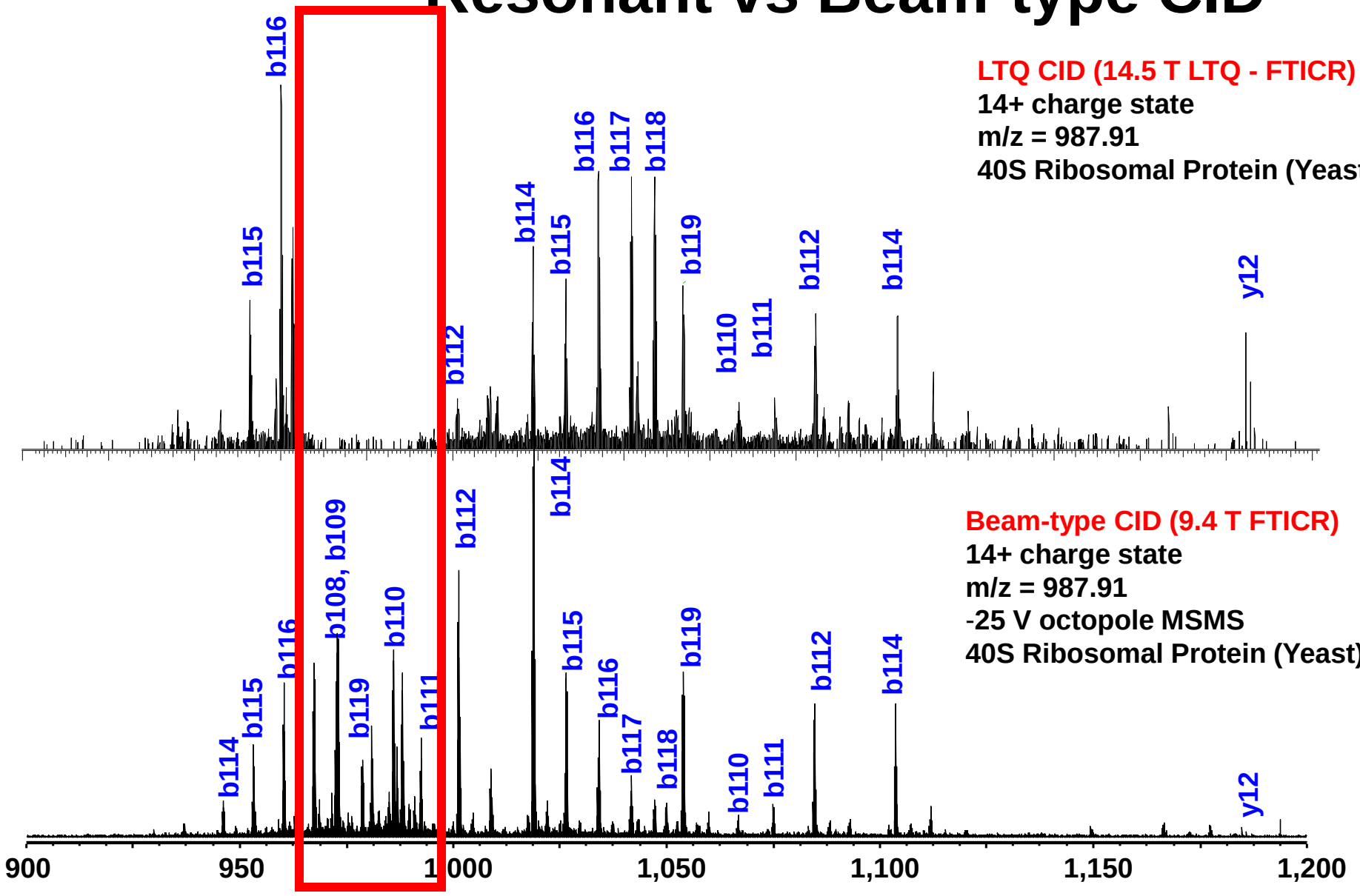


Top down Proteomics Instrumentation: Custom built 9.4 T FTICR



Kaiser, N.K.; Quinn, J.P.; Blakney, G.T.; Hendrickson, C.L. and Marshall, A.G.,
Design and Performance of a Novel 9.4 Tesla FT-ICR Mass Spectrometer for Proteome and Petroleum Analysis
57th Amer. Soc. Mass Spectrom. Annual Conf. on Mass Spectrometry & Allied Topics,
Philadelphia, PA, May 31-June 5 (2009)

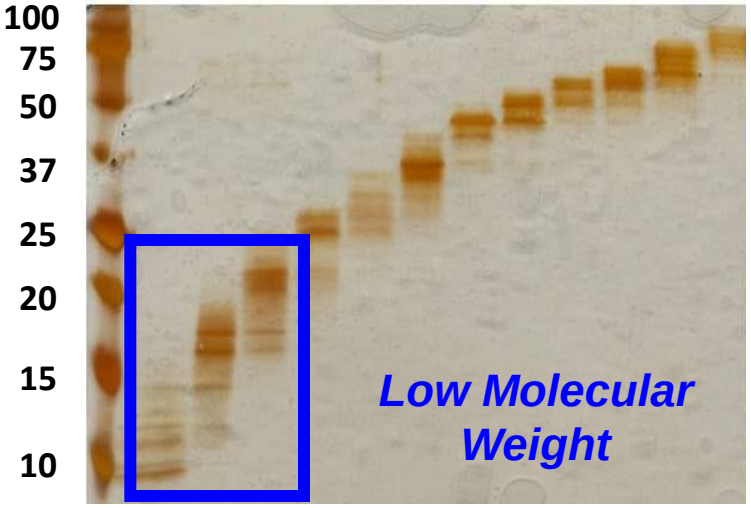
Top down Proteomics Instrumentation: Resonant vs Beam-type CID

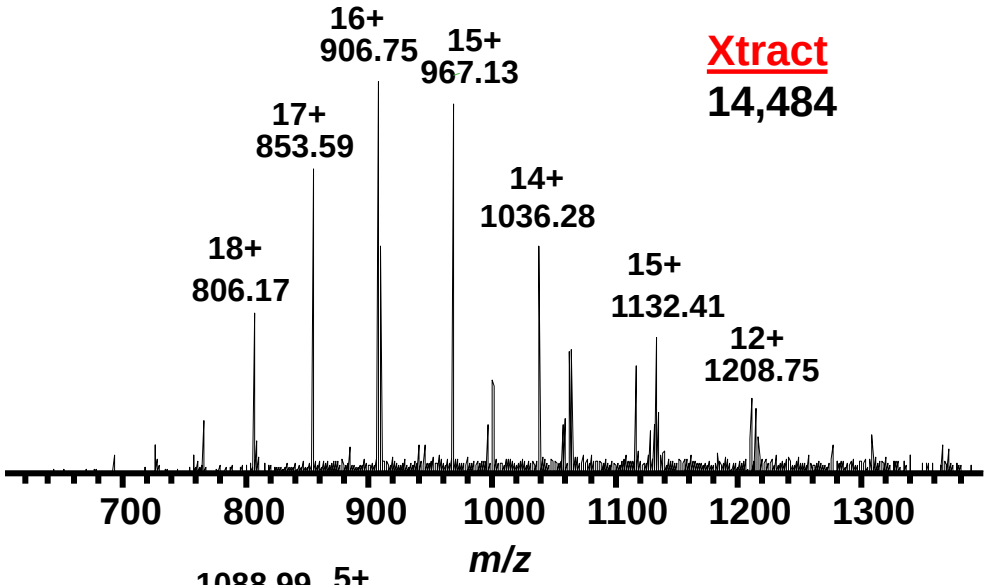


Moving up the Molecular Weight Ladder

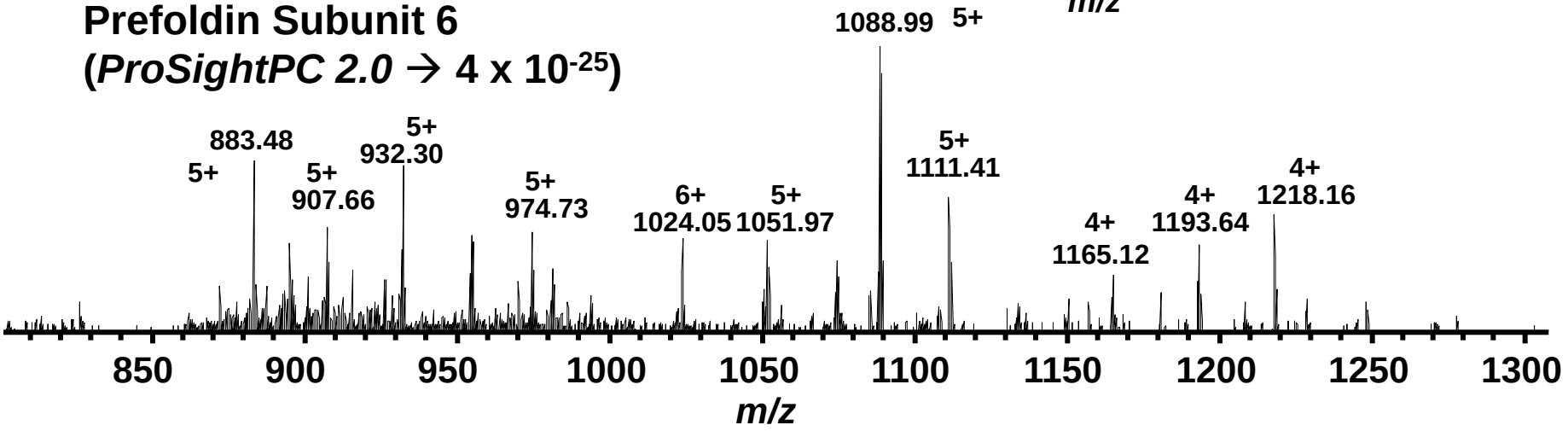
On-line nano-LC / Top-3 data dependent MS/MS

MS — MS/MS-1 — MS/MS-2 — MS/MS-3 (High Resolving Power with FT- ICR)





Prefoldin Subunit 6
(*ProSightPC 2.0* → 4 x 10^{-25})



Moving up the Molecular Weight Ladder

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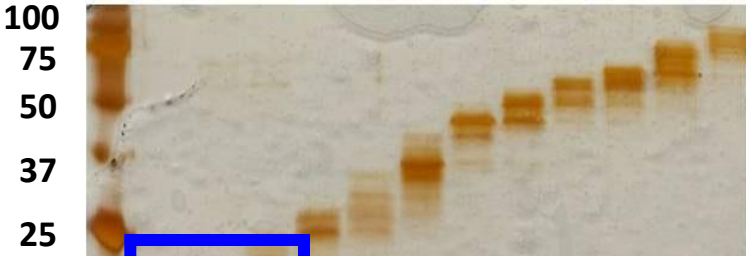
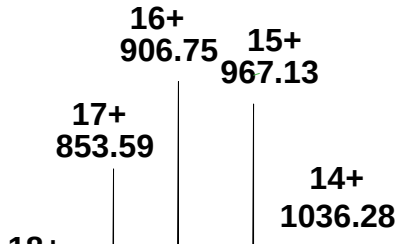
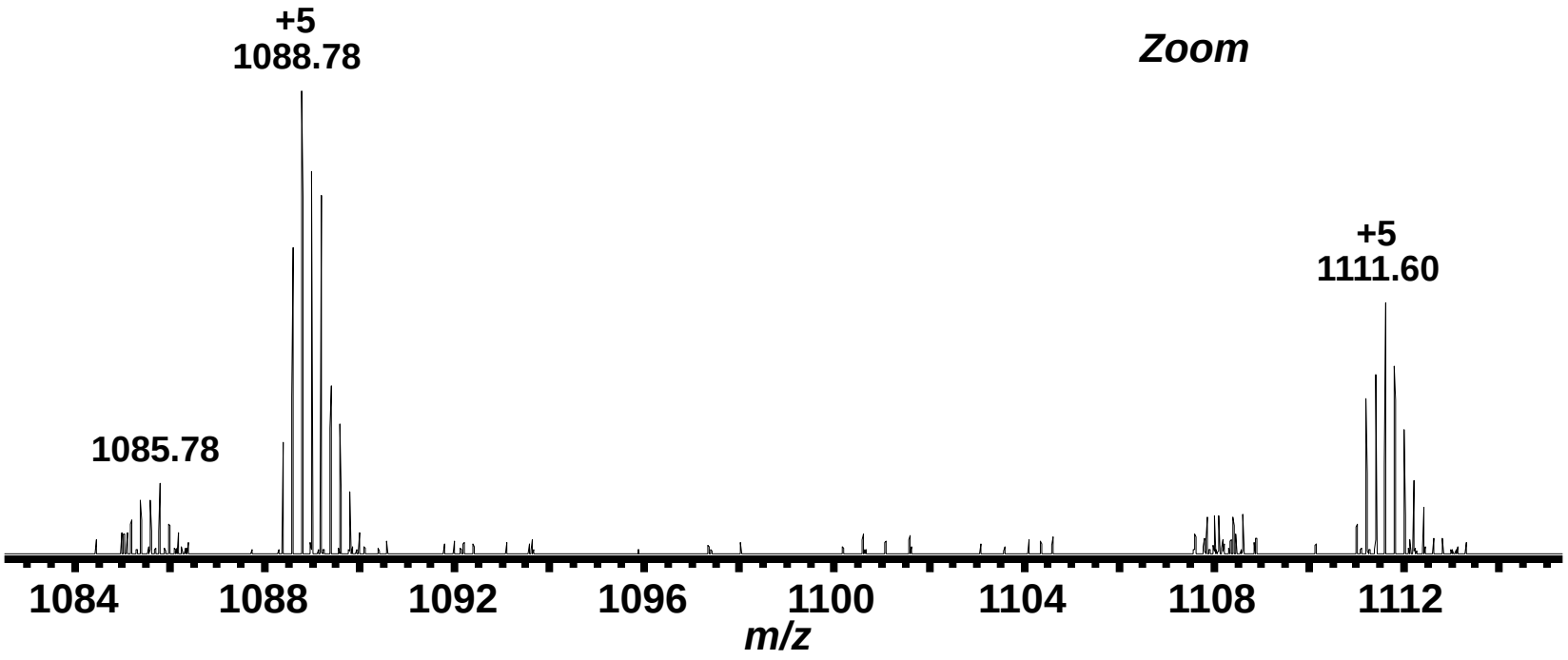




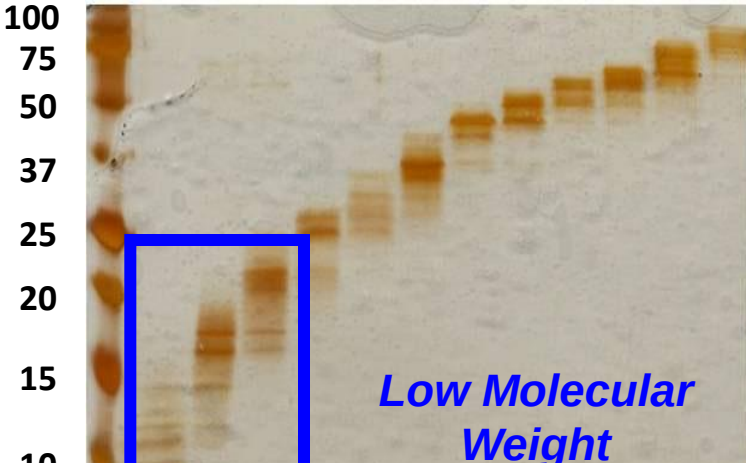
Xtract
14,484

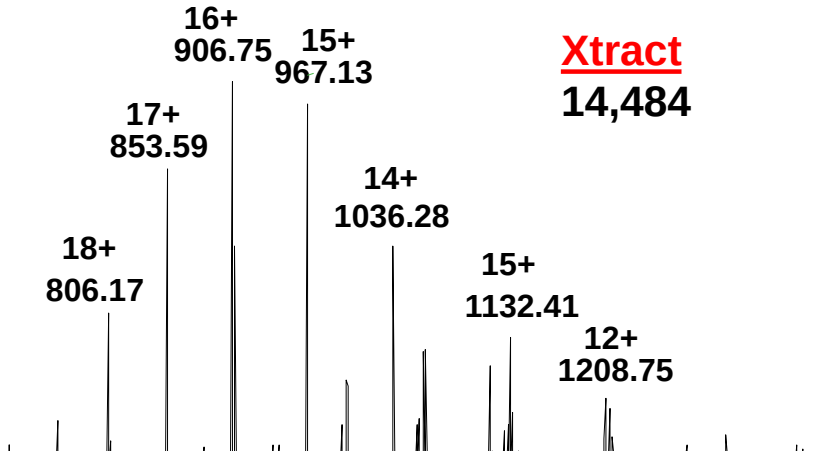


Moving up the Molecular Weight Ladder

On-line nano-LC / Top-3 data dependent MS/MS

MS — MS/MS-1 — MS/MS-2 — MS/MS-3 (High Resolving Power with FT- ICR)



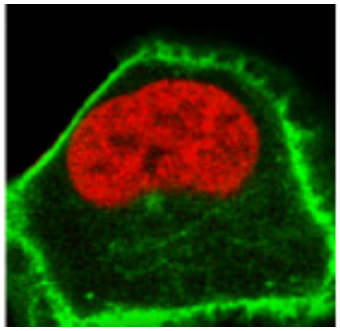


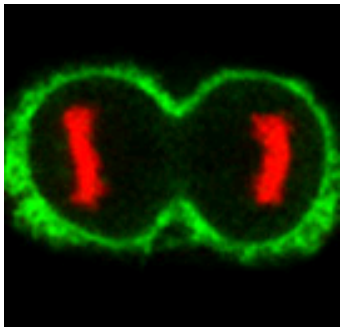
Prefoldin Subunit 6

4 x 10^{-25}

b_1	-A-E-L-I-Q-K-K-L-Q-G-E-V-E-K-Y-Q-Q-L-Q-K-D-L-S-K-S-M-S-G-R-Q-	y_{99}
b_{31}	-K-L-E-A-Q-L|T|E|N|N|I|V|K-E-E|L|A|L|L|D|G-S-N|V|V|F-K-L|L|G-	y_{69}
b_{61} |P-V-L-V-K|Q-E-L-G-E|A-R-A-T-V-G-K-R-L-D|Y-I-T-A-E|I-K-R-Y-E-		y_{39}
b_{91}	-S-Q-L-R-D-L-E-R-Q-S-E-Q-Q-R-E-T-L-A-Q-L-Q-Q-E-F-Q-R-A-Q-A|A|	y_9
b_{121}	-K-A-G-A-P-G-K-A-	y_1

Top Down Proteomics of Different Cell States





Asynchronous **Mitosis**

M phase arrest: colchicine (1 µM) for 18 h

Asynchronous HeLa cell lysate → 3 GELFrEE runs (8 fractions each) → nanoLC-MS/MS (5-25 kDa)

M phase arrest HeLa cell lysate → 4 GELFrEE runs (8 fractions each) → nanoLC-MS/MS (5-25 kDa)

Pesavento, J. J et al. *Mol. Cell. Biol.* 2008, 468-486
http://www.bc.biol.ethz.ch/people/groups/gerlichd/people/schmitzm

PTM Changes (Phosphorylation)

Asynchronous

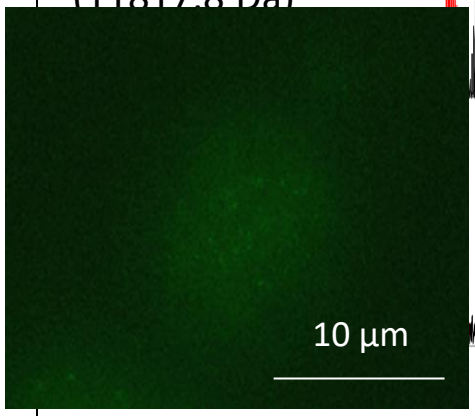
Asynchronous
M phase arrested

(■ : phosphorylation)

Histone H2A.X

Stathmin
(17202.9 Da)

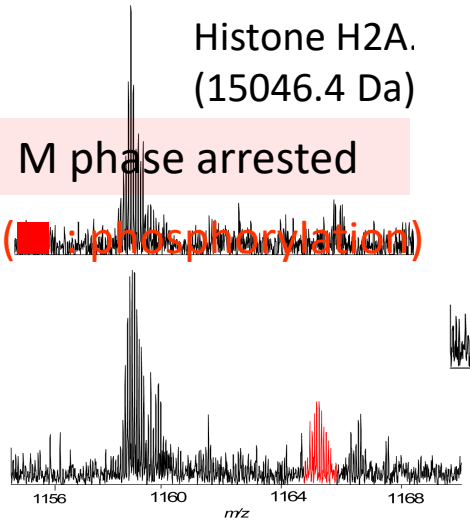
60S acidic
ribosomal protein
(11817.8 Da)

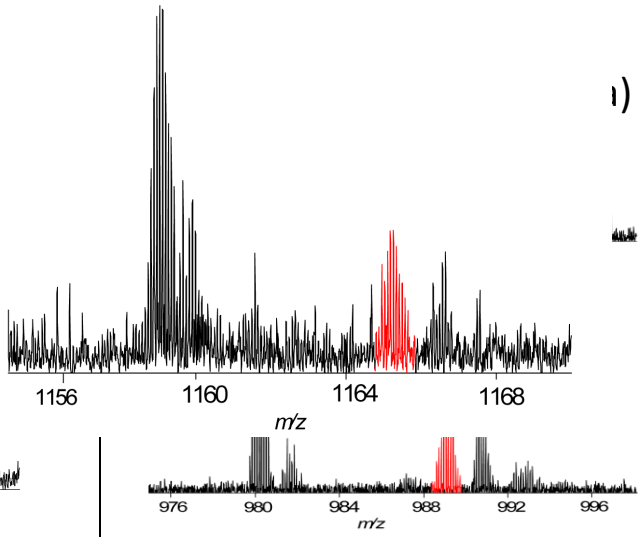


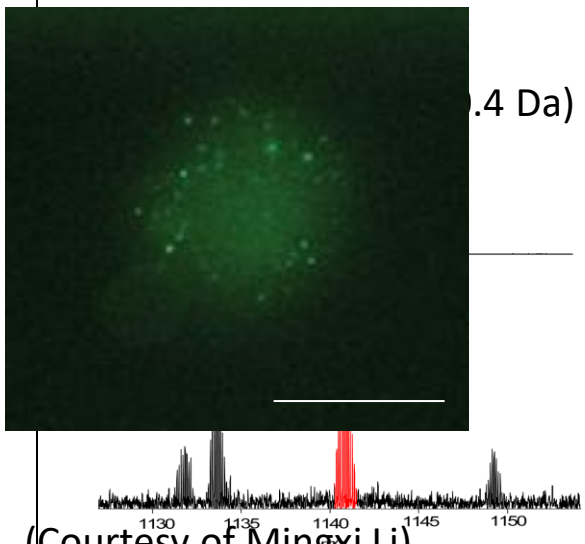
Histone H2A.
(15046.4 Da)

M phase arrested

(■ : phosphorylation)





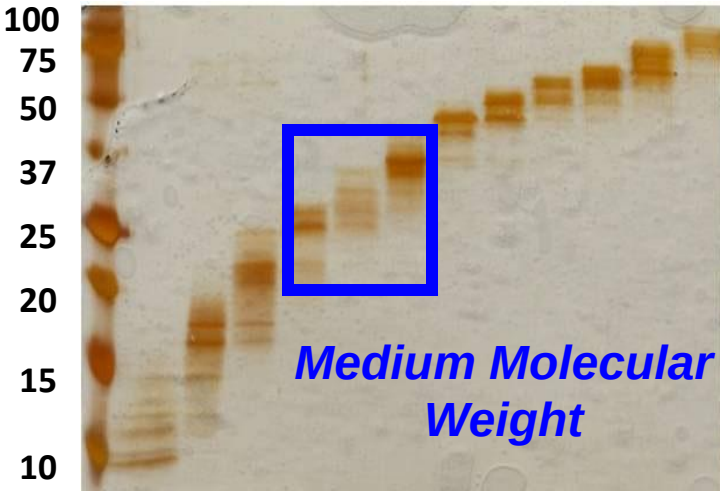


(Courtesy of Mingxi Li)

Moving up the Molecular Weight Ladder

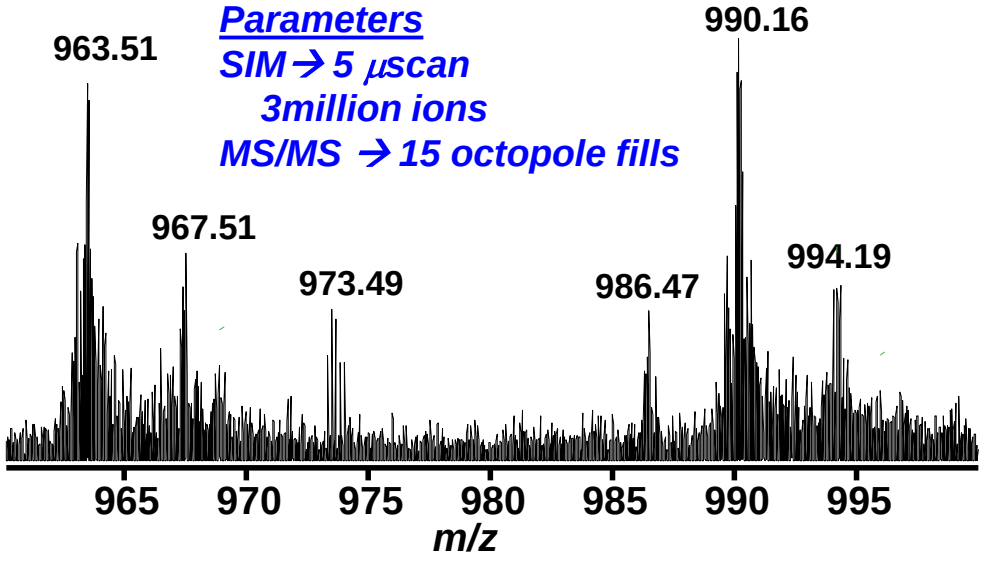
On-line nano-LC / SIM Zoom Map MS/MS

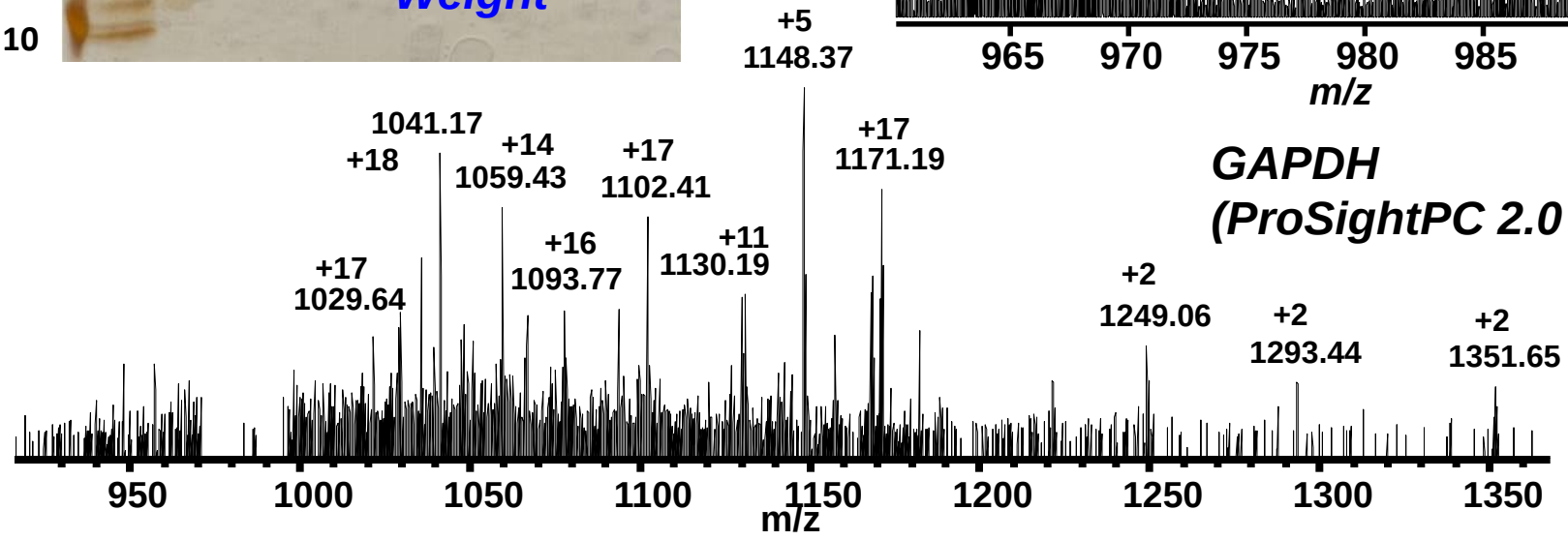
SIM *m/z* **800 – 880** → **MS/MS** → **SIM** *m/z* **880 – 960** → **MS/MS** → **……. continue**



Xtract
35,578
35,590

Parameters
SIM → 5 μscan
3million ions
MS/MS → 15 octopole fills

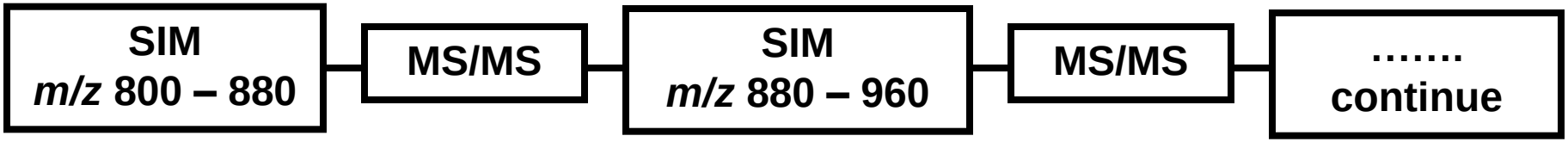




GAPDH
(ProSightPC 2.0 → 2 x 10^{-11})

Moving up the Molecular Weight Ladder

On-line nano-LC / SIM Zoom Map MS/MS

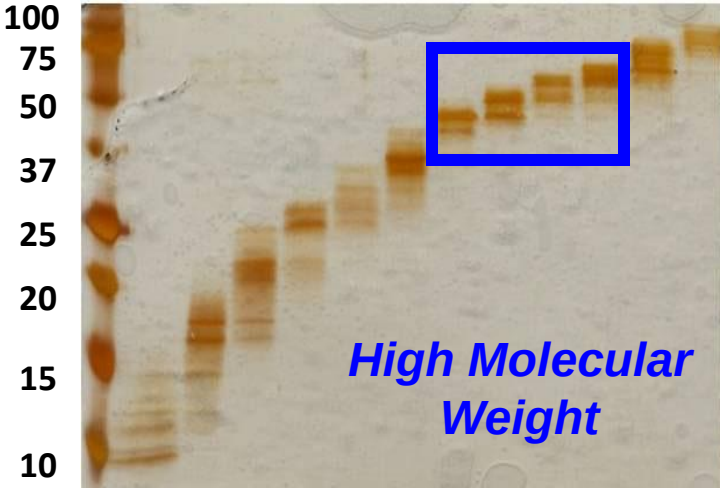


GAPDH
2 x 10^{-11}

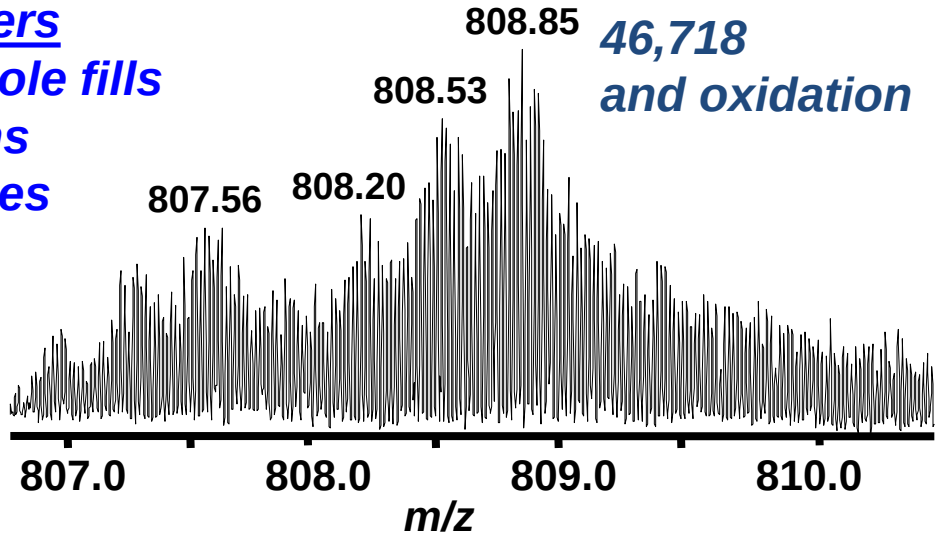
b_{1}	V-R-V-A-I-N-G-F-G-R-I-G-R-L-V-M-R-I-A-L-S-R-P-N-V-E-V-V-A-L	y_{302}
b_{31}	N-D-P-F-I-T-N-D-Y-A-A-Y-M-F-K-Y-D-S-T-H-G-R-Y-A-G-E-V-S-H-D	y_{272}
b_{61}	D-K-H-I-I-V-D-G-K-K-I-A-T-Y-Q-E-R-D-P-A-N-L-P-W-G-S-S-N-V-D	y_{242}
b_{91}	I-A-I-D-S-T-G-V-F-K-E-L-D-T-A-Q-K-H-I-D-A-G-A-K-K-V-V-I-T-A	y_{212}
b_{121}	P-S-S-T-A-P-M-F-V-M-G-V-N-E-E-K-Y-T-S-D-L-K-I-V-S-N-A-S-C-T	y_{182}
b_{151}	T-N-C-L-A|P-L-A-K-V-I-N-D-A-F-G-I|E-E-G-L-M-T-T-V-H-S-L-T-A	y_{152}
b_{181}	T-Q-K-T-V-D-G-P-S-H-K-D|W-R-G-G-R-T-A-S-G-N-I-I|P-S-S-T-G-A	y_{122}
b_{211}	A-K-A-V-G-K-V-L-P-E-L-Q-G-K-L-T-G-M-A-F-R-V-P-T-V-D-V-S-V-V	y_{92}
b_{241}	D-L-T-V-K-L-N-K-E-T-T-Y-D-E-I-K-K-V-V-K-A-A-A-E-G-K-L-K-G-V	y_{69}
b_{271}	L-G-Y-T-E|D-A|V|V|S|S|D-F-L-G-D-S-H-S-S-I-F-D-A-S-A-G-I-Q-L	y_{39}
b_{301}	S-P-K-F-V-K-L-V|S-W|Y-D-N-E-Y-G-Y-S-T-R-V-V-D-L-V-E-H-V-A-K	y_{9}
b_{331}	A	y_{1}

Moving up the Molecular Weight Ladder

On-Line LC with Fraction collection



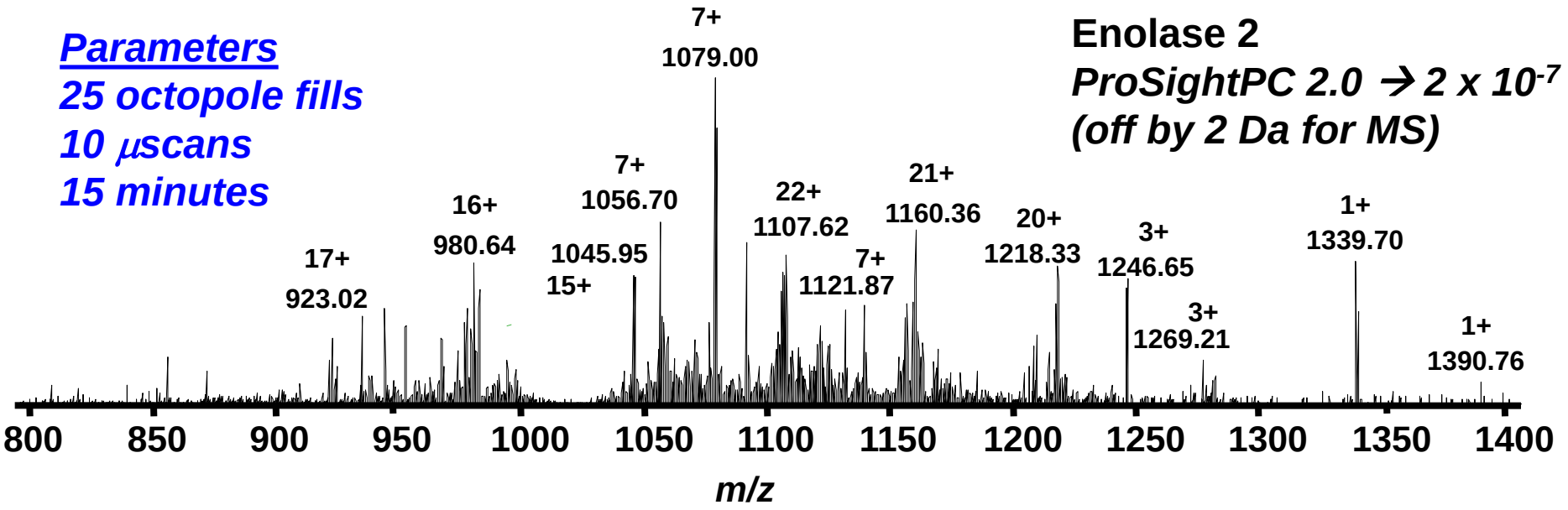
Parameters
15 octopole fills
15 μscans
20 minutes



46,718 and oxidation

Parameters
25 octopole fills
10 μscans
15 minutes

Enolase 2
ProSightPC 2.0 → 2 x 10^{-7}
(off by 2 Da for MS)



Moving up the Molecular Weight Ladder

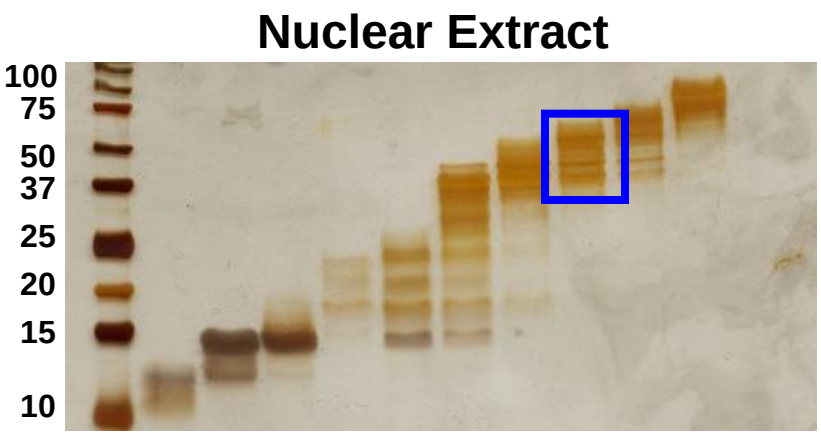
On-Line LC with Fraction collection

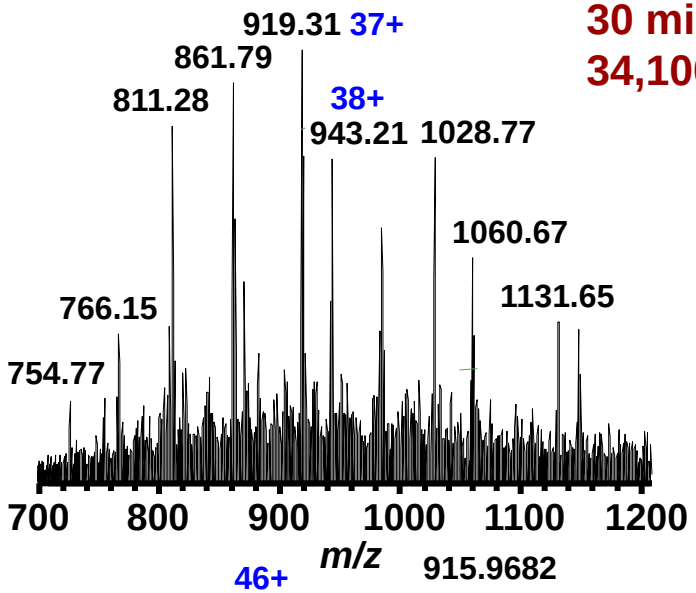
Enolase 2

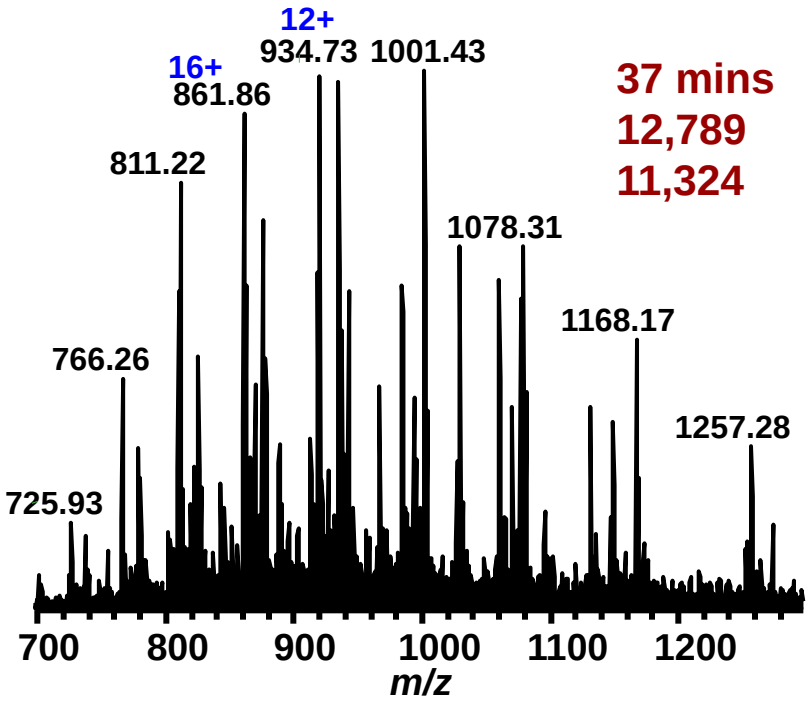
ProSightPC 2.0 → 2 x 10^{-7}

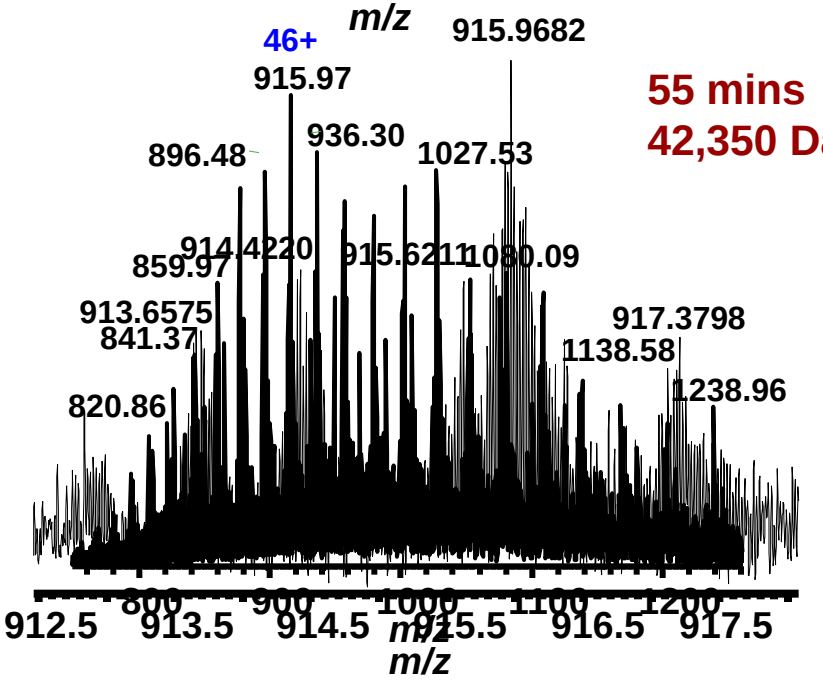
b_{1}	A-V-S-K-V-Y-A-R-S-V-Y-D-S-R-G-N-P-T-V-E-V-E-L-T-T-E-K-G-V-F	y_{407}
b_{31}	R-S-I-V-P-S-G-A-S-T-G-V-H-E-A-L-E-M-R-D-E-D)K-S-K-W-M-G-K-G	y_{377}
b_{61}	V-M-N-A-V-N-N-V-N-N-V-I-A-A-A-F-V-K-A-N-L-D-V-K-D-Q-K-A-V-D	y_{347}
b_{91}	D-F-L-L-S-L-D-G-T-A-N-K-S-K-L-G-A-N-A-I-L-G-V-S-M-A-A-A-R-A	y_{317}
b_{121}	A-A-A-E-K-N-V-P-L-Y-Q-H-L-A-D-L-S-K-S-K-T-S-P-Y-V-L-P-V)P-F	y_{287}
b_{151}	L-N-V-L-N-G-G-S-H-A-G-G-A-L-A-L-Q-E-F-M-I-A-P-T-G-A-K-T-F-A	y_{257}
b_{181}	E-A-M-R-I-G-S-E-V-Y-H-N-L-K-S-L-T-K-K-R-Y-G-A-S-A-G-N-V-G-D	y_{227}
b_{211}	E-G-G-V-A(P-N-I-Q-T-A-E-E-A-L-D-L-I-V-D-A-I-K-A-A-G-H-D-G-K	y_{197}
b_{241}	V-K-I-G-L-D-C-A-S-S-E-F-F-K-D-G-K-Y-D-L-D-F-K-N-P-E-S-D-K-S	y_{167}
b_{271}	K-W-L-T-G-V-E-L-A-D-M-Y-H-S-L-M-K-R-Y-P-I-V-S-I-E-D-P-F-A-E	y_{137}
b_{301}	D-D-W-E-A-W-S-H-F-F-K-T-A-G-I-Q-I-V-A-D-D-L-T-V-T-N-P-A-R-I	y_{107}
b_{331}	A-T-A-I-E-K-K-A-A-D-A-L-L-L-K-V-N-Q-I-G-T-L-S-E-S-I-K-A-A-Q	y_{77}
b_{361}	D-S-F-A-A(N(W(G-V-M-V-S-H-R-S-G-E-T-E-D-T-F-I-A-D-L-V-V-G-L	y_{47}
b_{391}	R-T-G-Q-I-K-T-G-A-P-A-R-S-E-R-L-A-K-L-N-Q-L-L-R-I-E-E-E-L-G	y_{17}
b_{421}	D-K-A-V-Y-A-G-E-N-F-H-H-G-D-K-L	y_{1}

Asynchronous and M Phase HeLa Cells High Molecular Weight

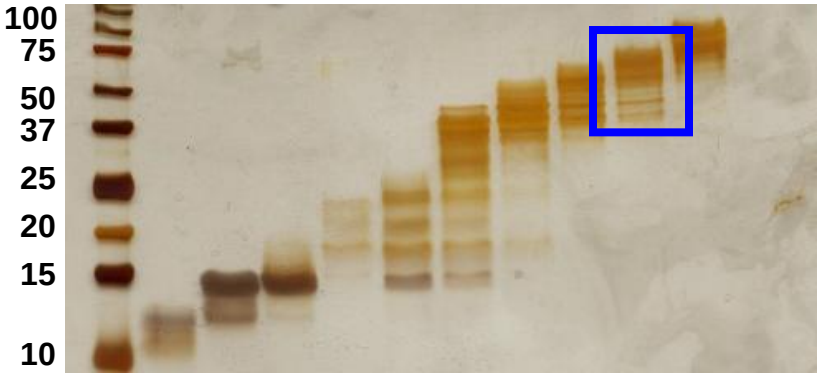


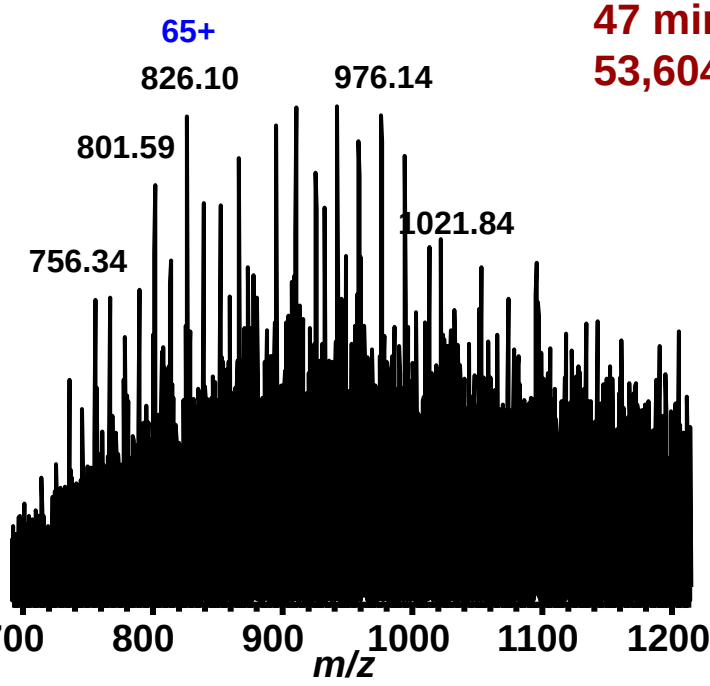


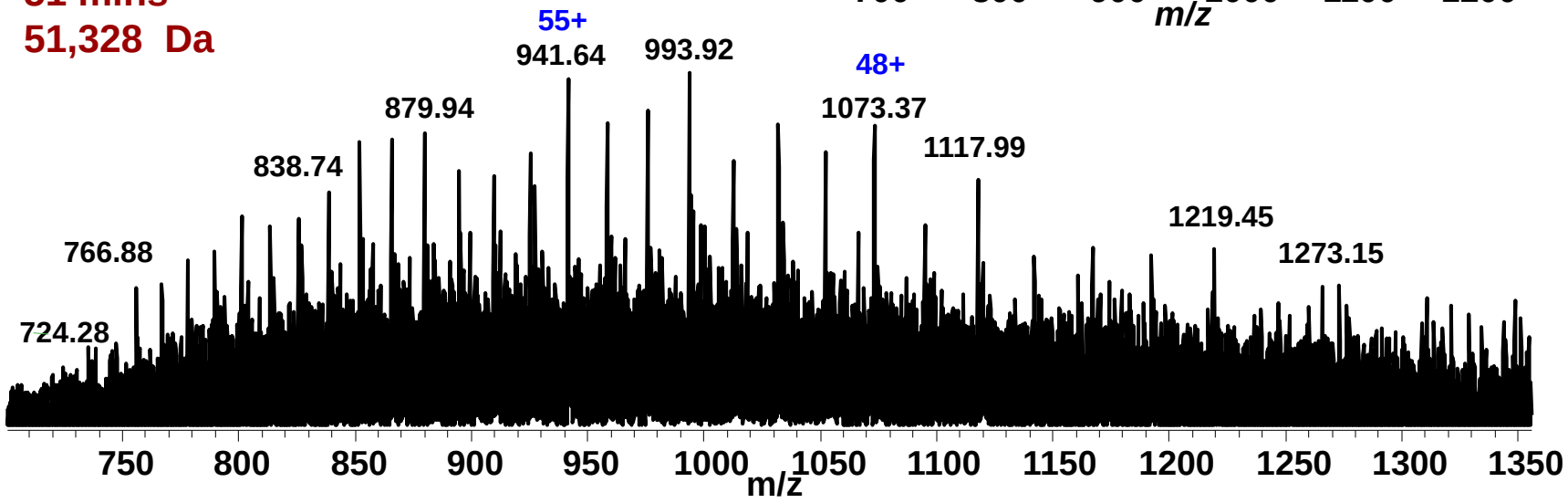




Asynchronous and M Phase HeLa Cells High Molecular Weight







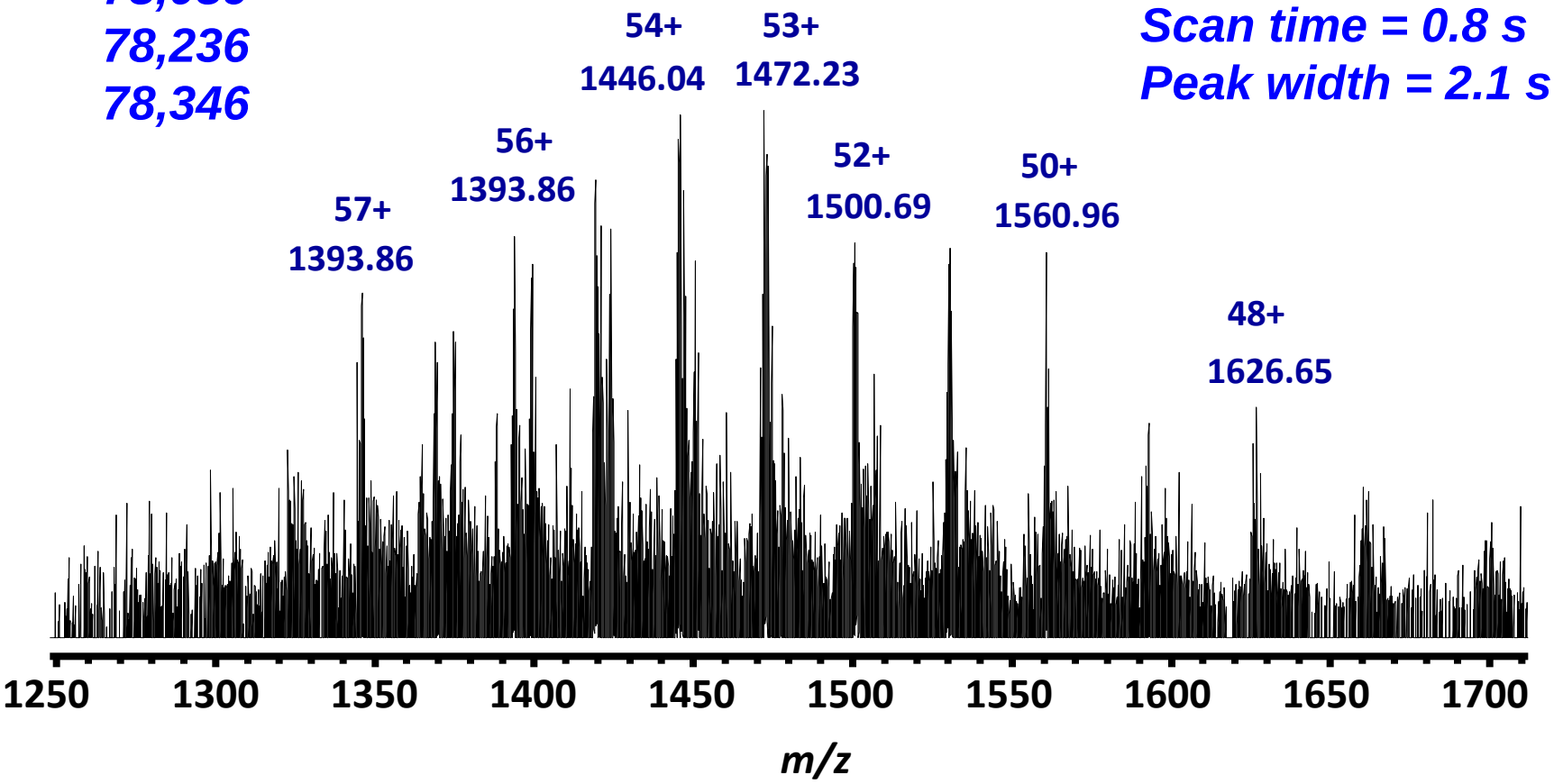
On-line nano-LC Benchmark: MS detection 1.2 pmol on column – C_4 75 μm i.d.

Xtract Results

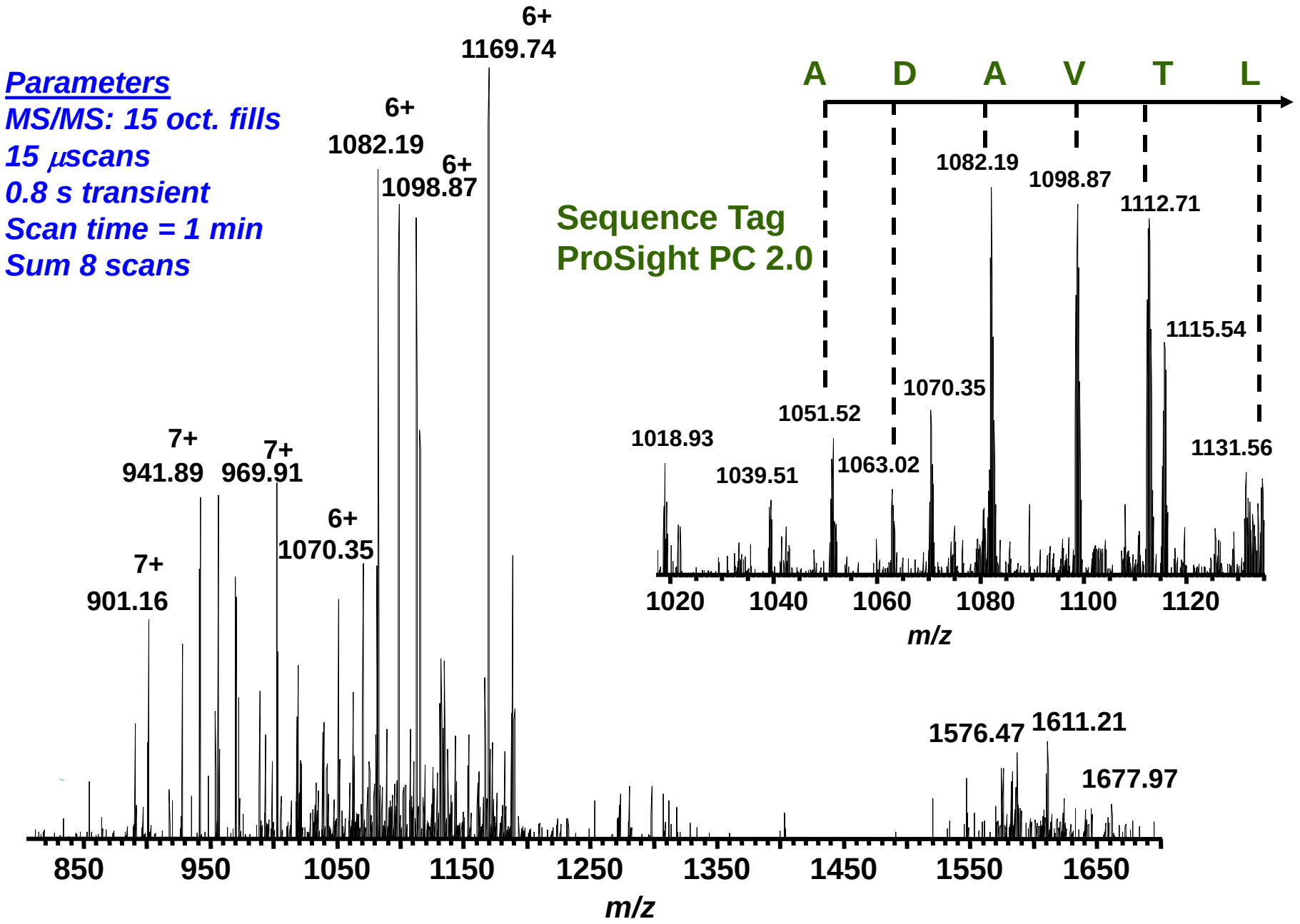
77,946
77,966
78,089
78,236
78,346

Parameters

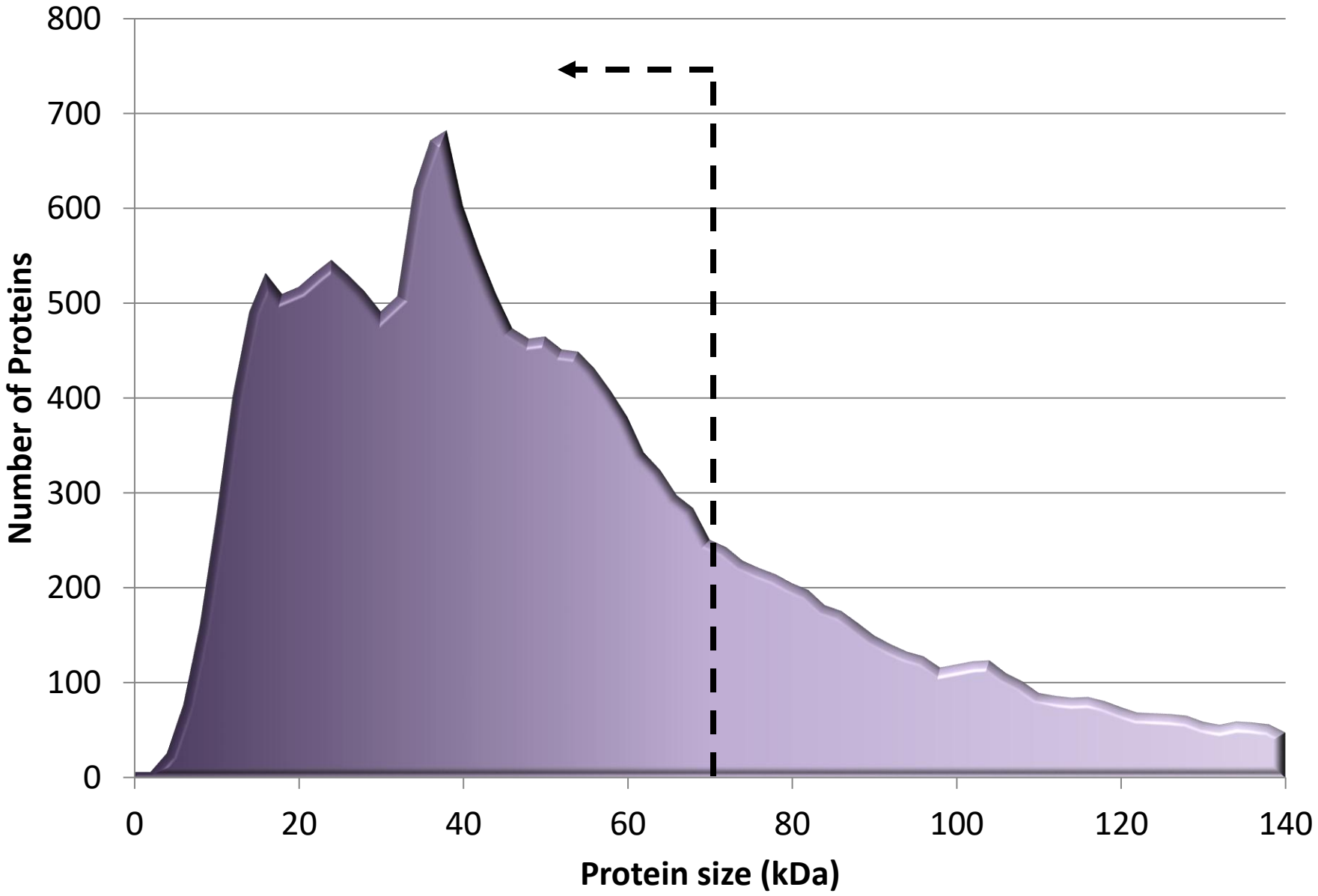
MS: 5 octopole fills
6 scans
1.6 s transient
Scan time = 0.8 s
Peak width = 2.1 s



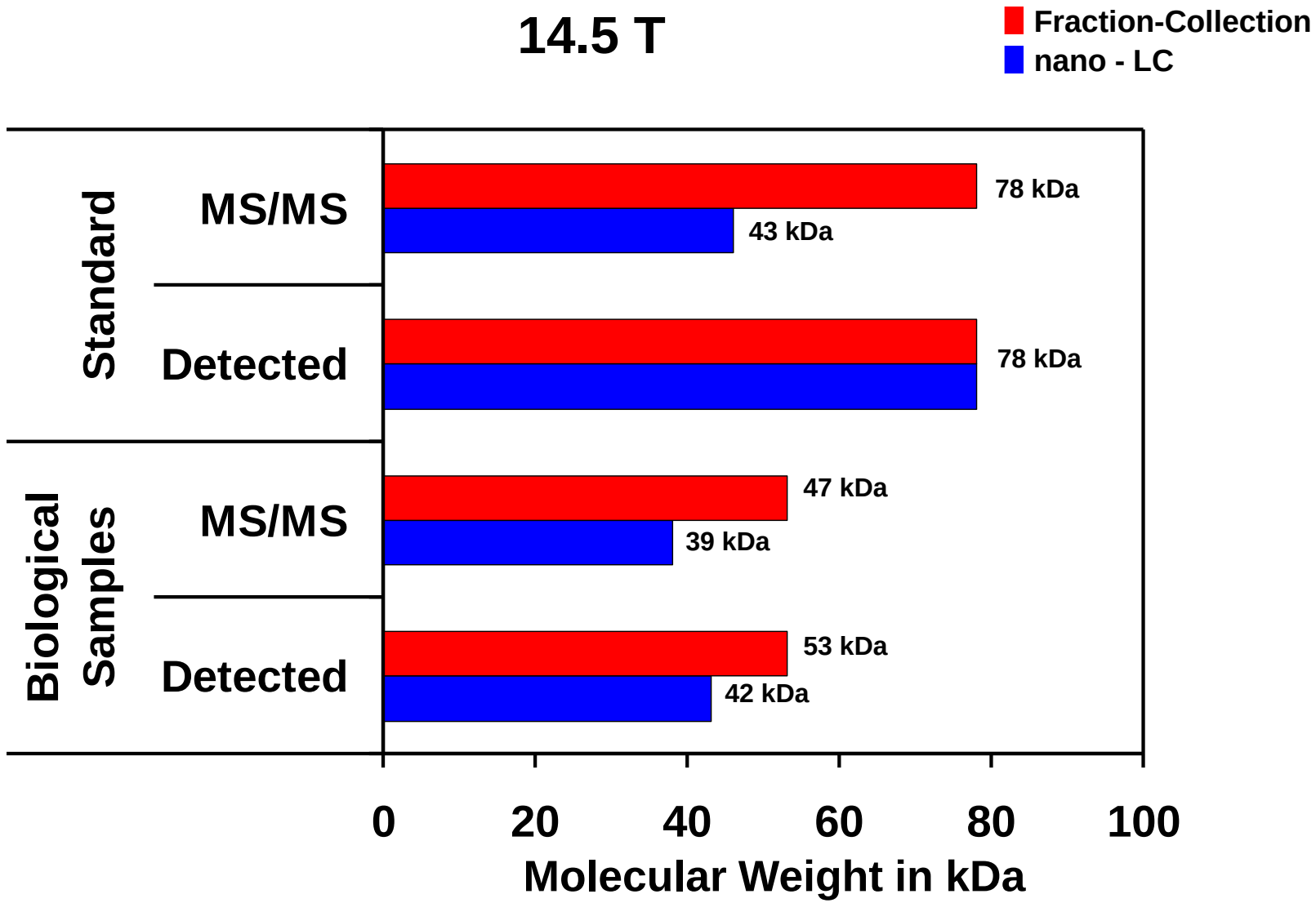
Off-line Fraction Collection – MS/MS



Human Proteome



High Throughput Top Down Proteomics
High Mass Range



Conclusions

Orthogonal separation, IEF – GELFrEE – RP, provides improved separations to deal with sample complexity.

Different RP chromatography conditions yield improved results based on the molecular weight of the proteins found in the sample.

Different molecular weight fractions dictate the instrument scan mode and tune parameters (LTQ-FT).

Beam-type CID provides increased information over Resonante-type CID.

Clean solvents and chromatography material are NEEDED!

Acknowledgements