Top Down proteomics with a 14.5 T FT-ICR mass spectrometer: Secondary ion collection in an external octopole ion trap

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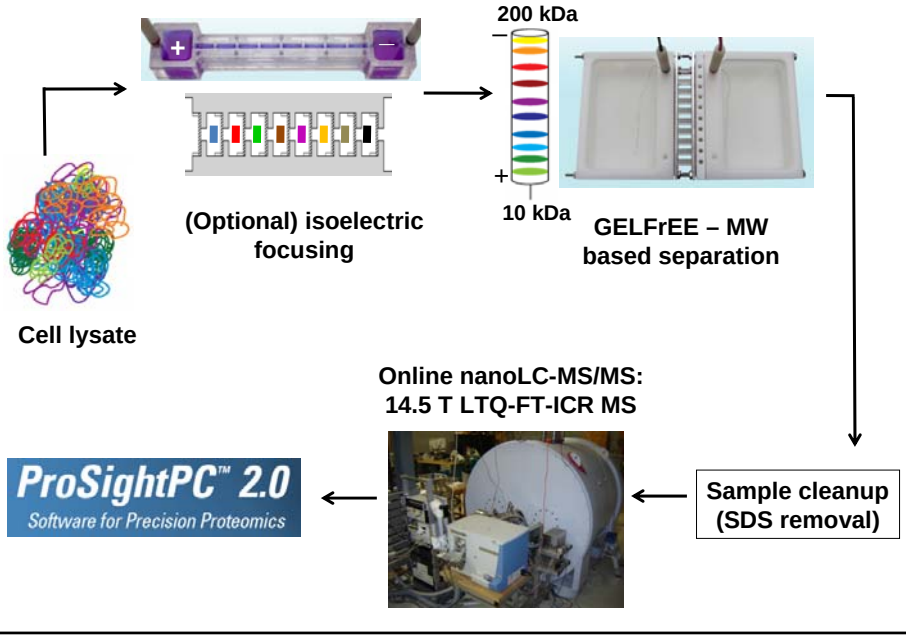
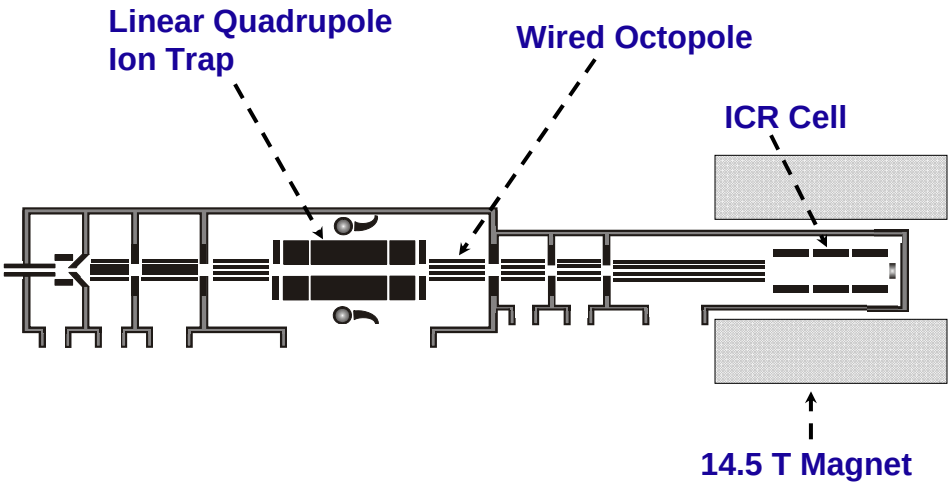
Top Down Proteomics Work Flow

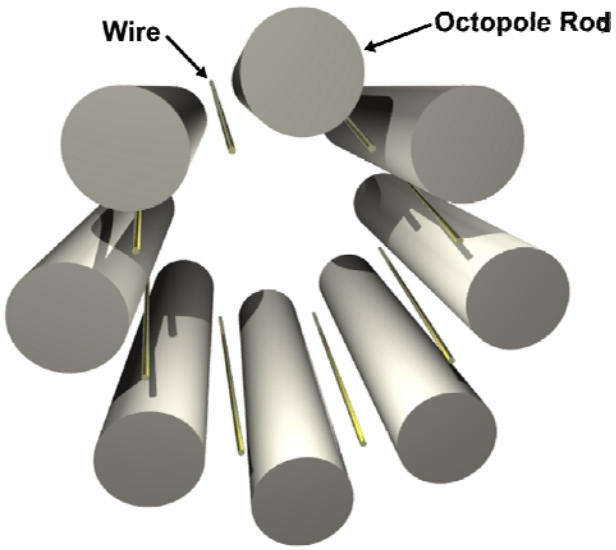


Secondary Ion Accumulation



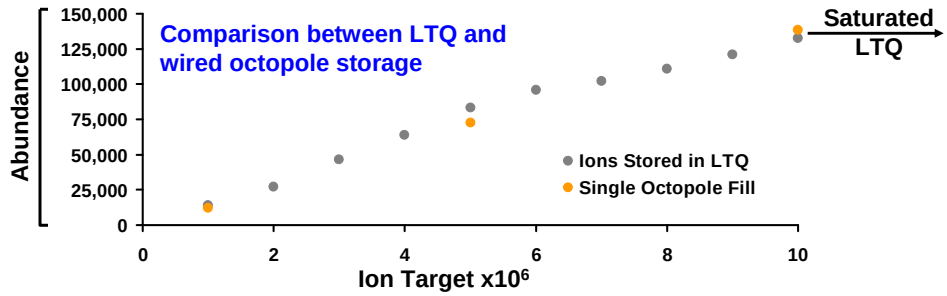
Schaub, T.M.; Hendrickson, C.L.; Horning, S.; Quinn, J.P.; Senko, M.W. and Marshall, A.G.
Anal. Chem., *80*, 3985 - 3990 (2008)

Wired Octopole

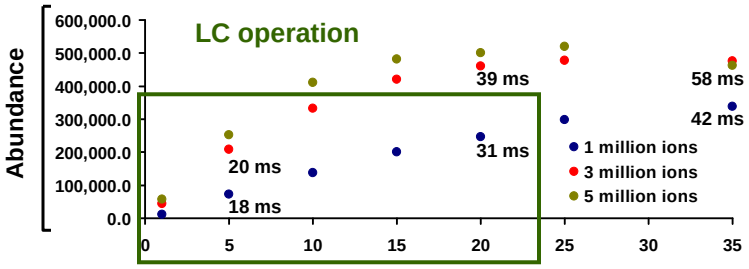


Wilcox B.E.; Hendrickson, C.L. and Marshall, A.G.
J. Am. Soc. Mass Spectrom., *13*, 1304 - 1312 (2002)

Ion Storage Capacity



2 - 3 fold increase in capacity



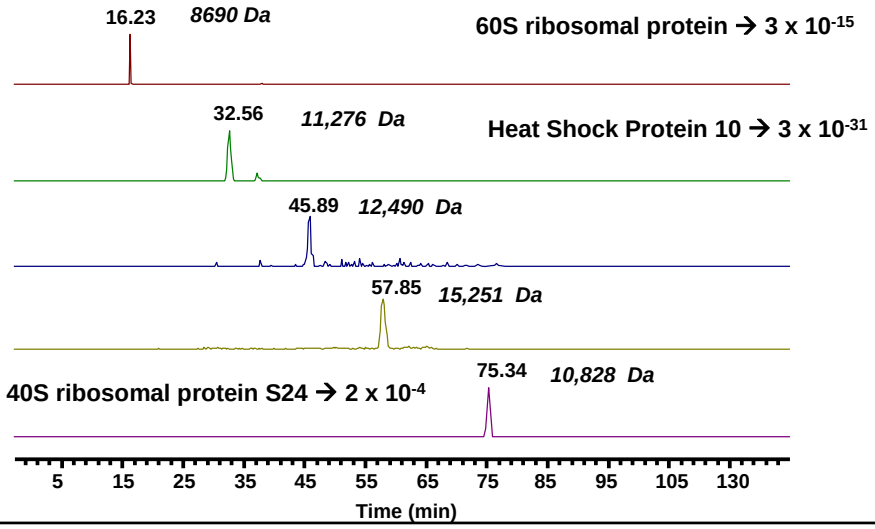
LC MS Performance: Duty Cycle

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

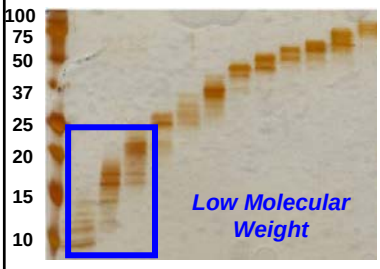
Peak width → 20 s to 1 min

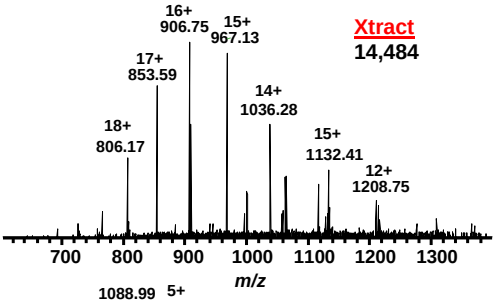


Moving up the Molecular Weight Ladder

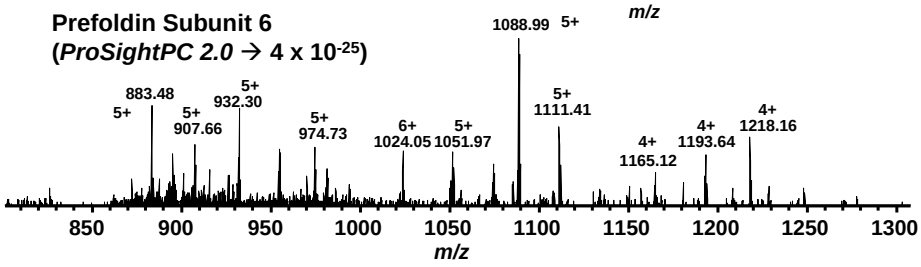
On-line nano-LC / Top-3 data dependent MS/MS

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

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

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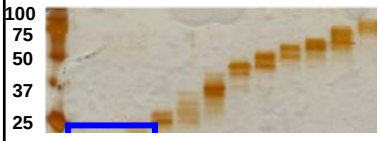
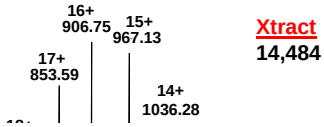
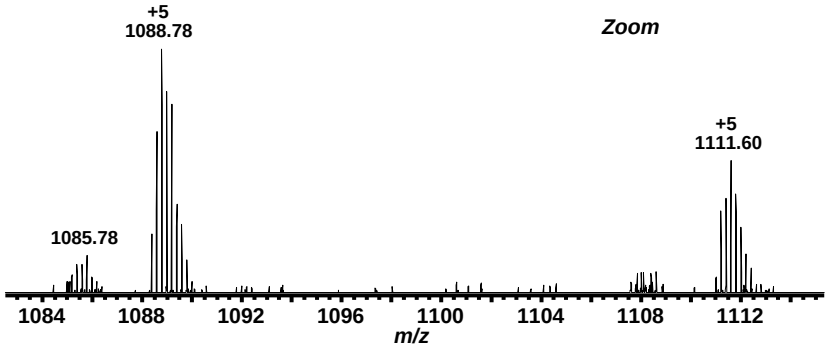


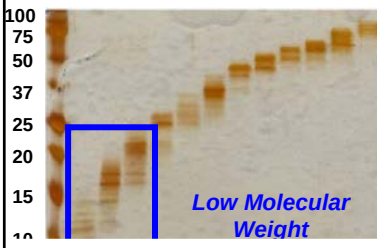


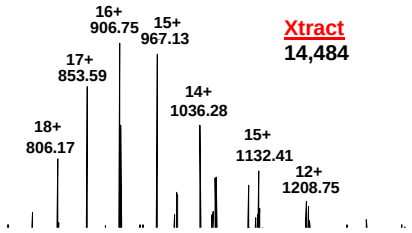


Moving up the Molecular Weight Ladder

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

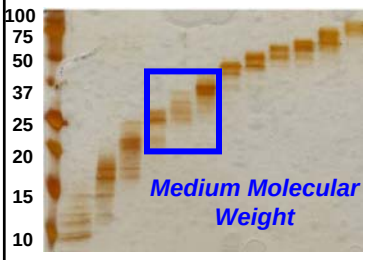
4 x 10^{-25}

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

Moving up the Molecular Weight Ladder

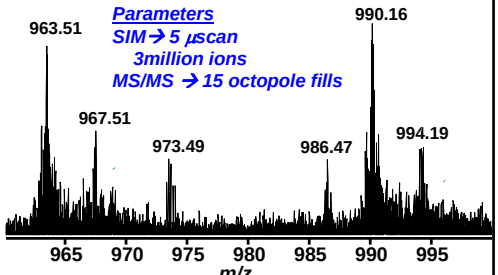
On-line nano-LC / SIM Zoom Map MS/MS

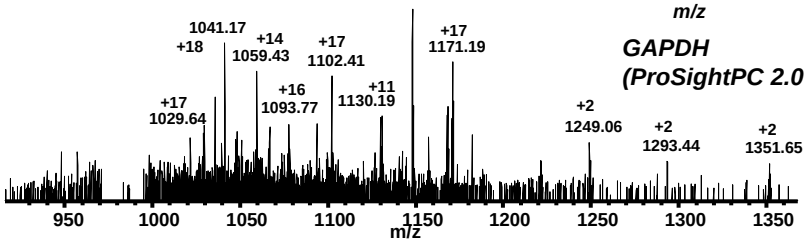
SIM *m/z* 800 – 880 → MS/MS → SIM *m/z* 880 – 960 → MS/MS → ……. continue



Xtract
35,578
35,590

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

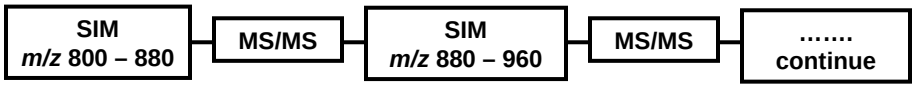




GAPDH
(ProSightPC 2.0 → 2 x 10^{-11})

Moving up the Molecular Weight Ladder

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

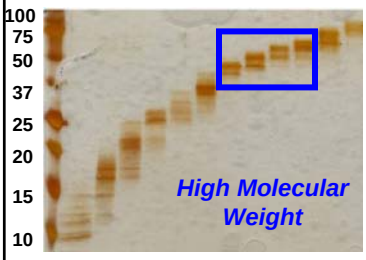


GAPDH
2 x 10^{-11}

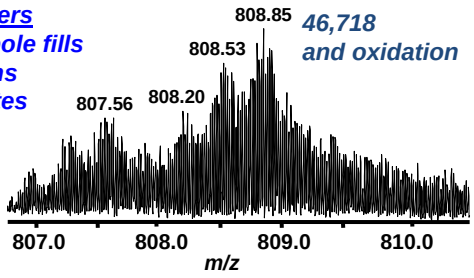
b_1	V-R-V-A-I-N-G-F-G-R-I-G-R-L-V-M-R-I-A-L-S-R-P-N-V-E-V-V-A-L	y_{302}
b_{31}	N-D-P-F-I-T-N-D-Y-A-A-Y-M-F-K-Y-D-S-T-H-G-R-Y-A-G-E-V-S-H-D	y_{272}
b_{61}	D-K-H-I-I-V-D-G-K-K-I-A-T-Y-Q-E-R-D-P-A-N-L-P-W-G-S-S-N-V-D	y_{242}
b_{91}	I-A-I-D-S-T-G-V-F-K-E-L-D-T-A-Q-K-H-I-D-A-G-A-K-K-V-V-I-T-A	y_{212}
b_{121}	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

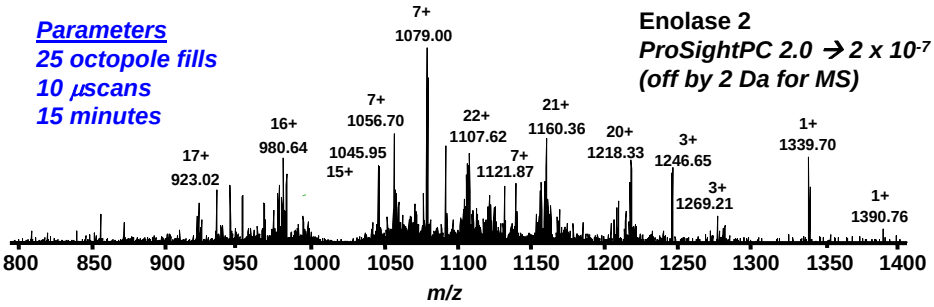


Parameters
15 octopole fills
15 µscans
20 minutes



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

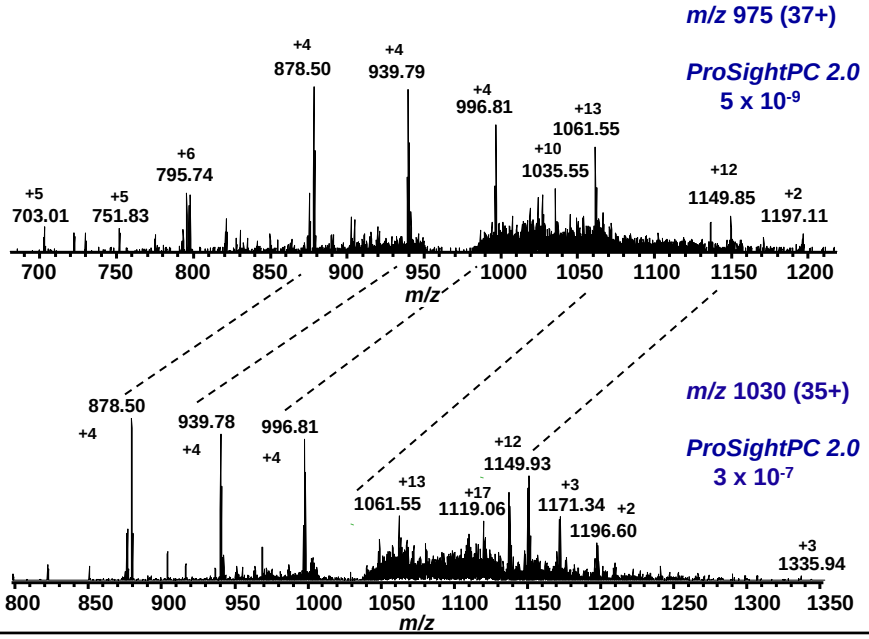
On-Line LC with Fraction collection

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

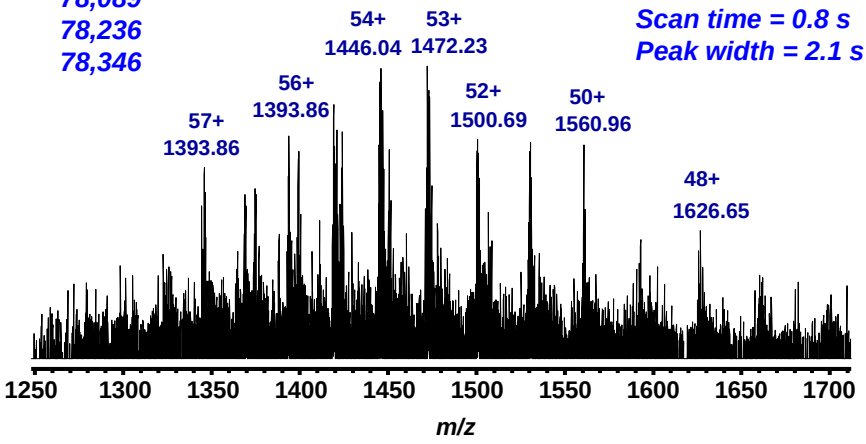
Fragmentation of Different Charge States



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

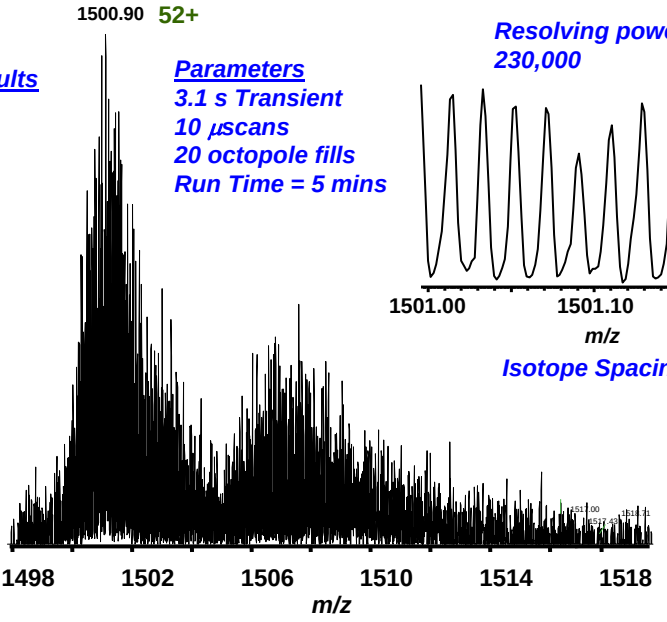
Parameters
MS: 5 octopole fills
6 scans
1.6 s transient
Scan time = 0.8 s
Peak width = 2.1 s



Off-line Fraction Collection – MS SIM

Xtract Results
77,887
77,904
77,966
77,989
78,006
78,023
.....
78,400

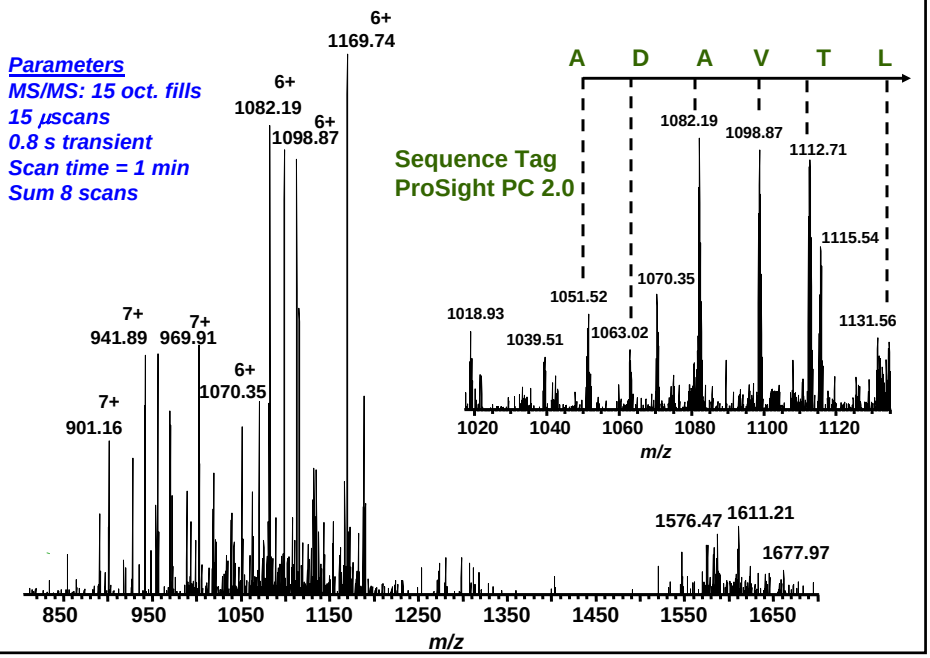
Parameters
3.1 s Transient
10 μscans
20 octopole fills
Run Time = 5 mins



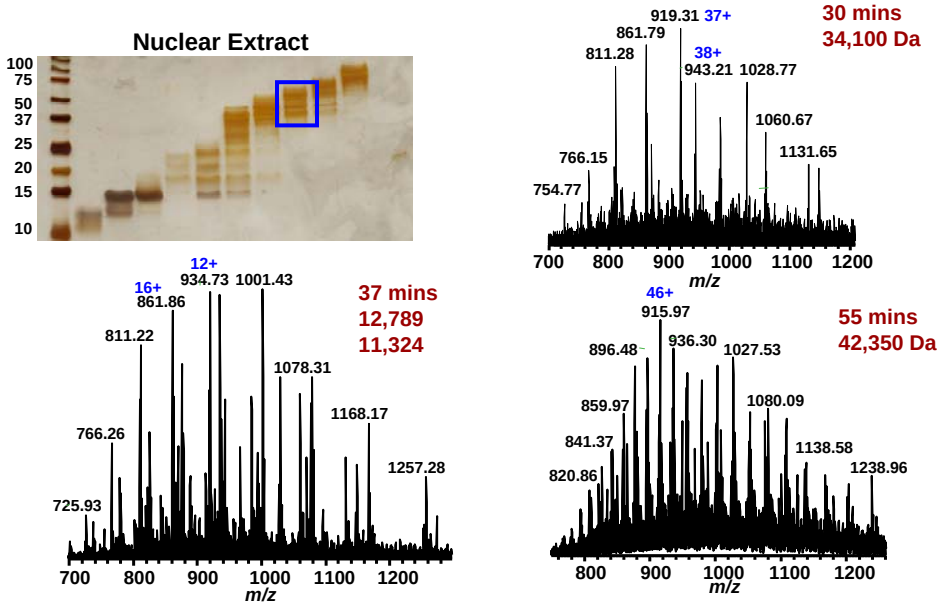
Resolving power
230,000

Isotope Spacing = 0.0192

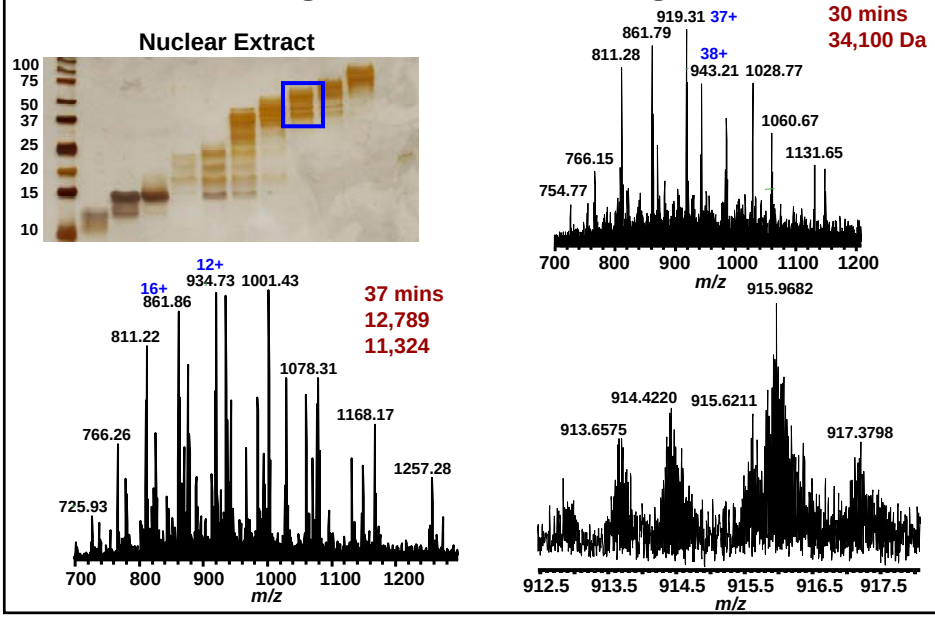
Off-line Fraction Collection – MS/MS



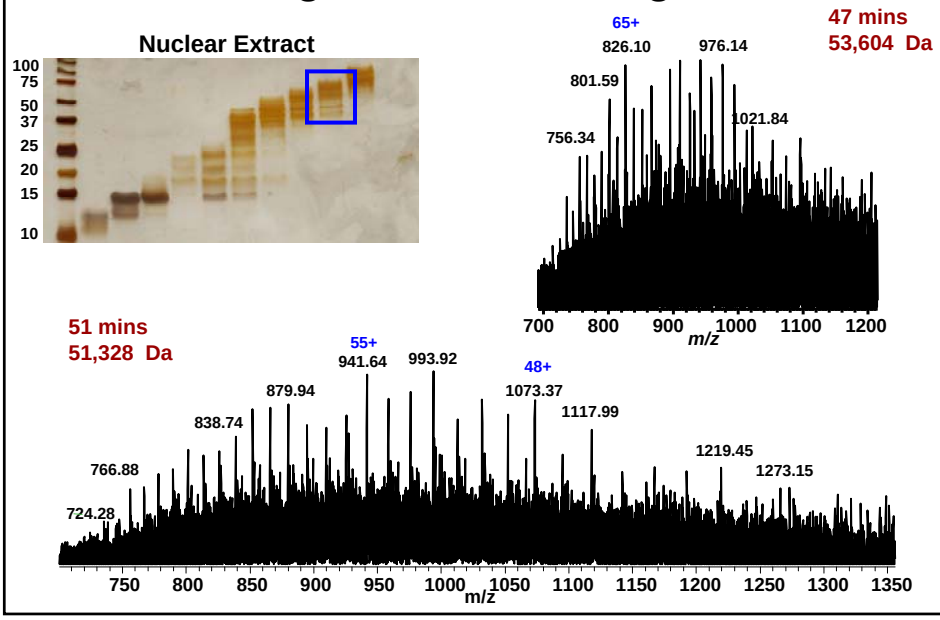
Asynchronous and M Phase HeLa Cells High Molecular Weight

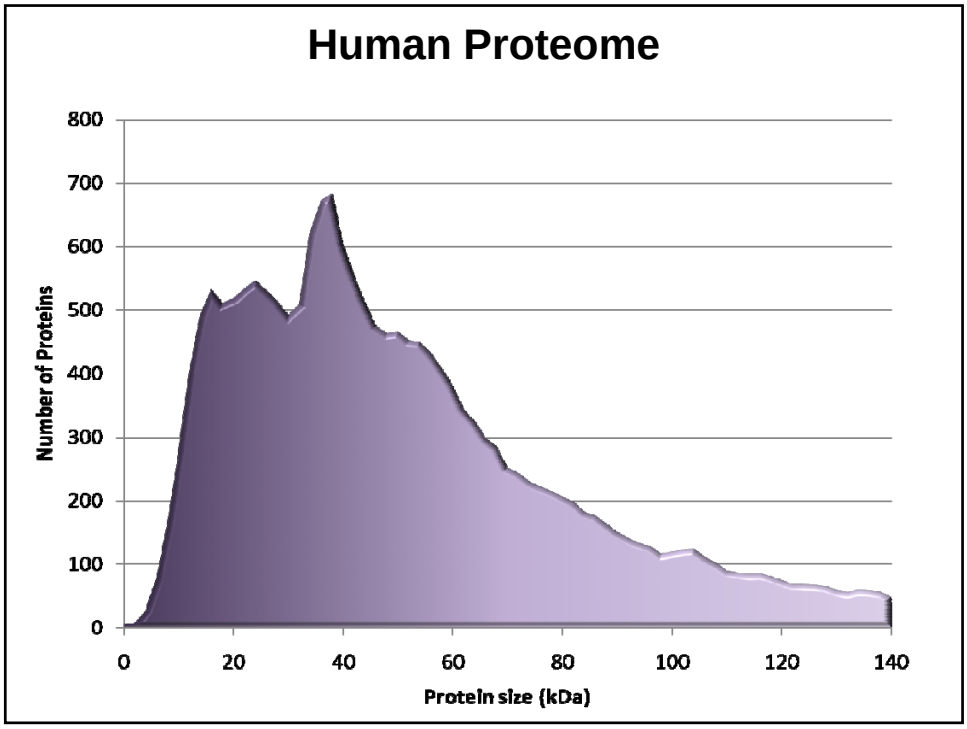


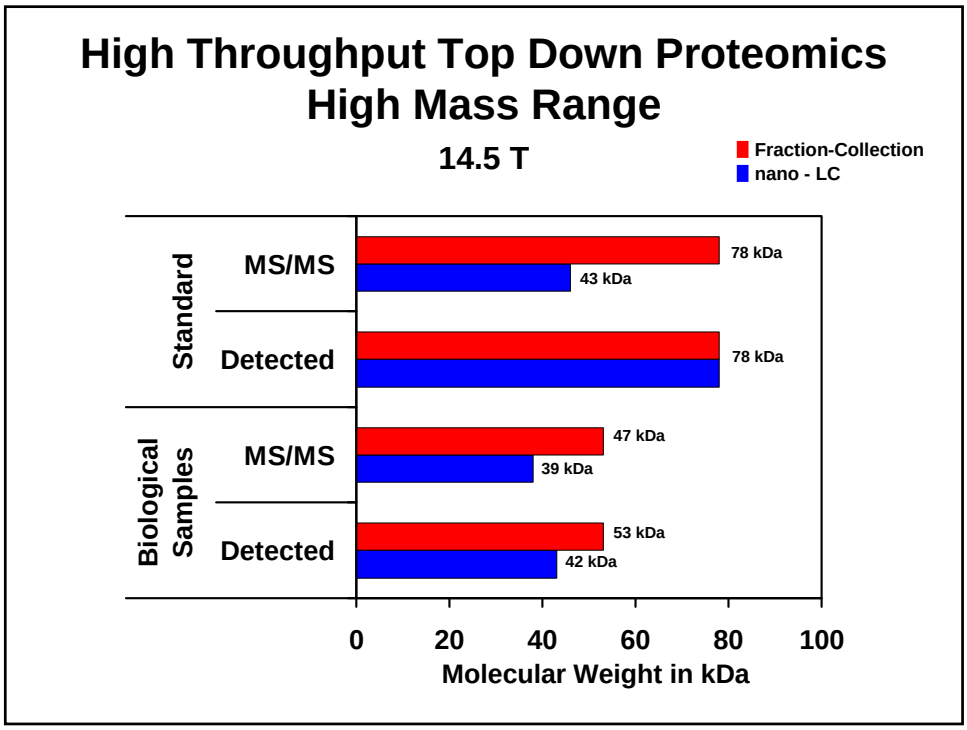
Asynchronous and M Phase HeLa Cells
High Molecular Weight



Asynchronous and M Phase HeLa Cells
High Molecular Weight




Human Proteome
800
700
600
500
400
300
200
100
0
Number of Proteins
0
20
40
60
80
100
120
140
Protein size (kDa)


High Throughput Top Down Proteomics
High Mass Range
14.5 T
Fraction-Collection
nano - LC
Standard
MS/MS
78 kDa
43 kDa
Detected
78 kDa
Biological Samples
MS/MS
47 kDa
39 kDa
Detected
53 kDa
42 kDa
0
20
40
60
80
100
Molecular Weight in kDa

Conclusions

Orthogonal separation, IEF – GELFrEE – PR, 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.

Recently, over 35 proteins have been detected from off-line fraction collection of Asynchronous and M Phase HeLa Cells. Time to automate for characterization!

ProSightPC 2.0 has high throughput capability: validation of increased number of proteins.

Clean solvents and chromatography material are NEEDED!

Acknowledgements

Alan Marshall Group

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NIH (GM – 078359; GM – 067193), NSF (DMR – 0654118)
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The State of Florida

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