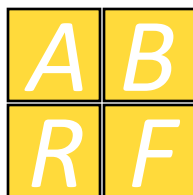




Proteomics Standards Research Group (sPRG)



sPRG2009 Prototype Sample @HSPH

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HSPH Proteomics Resource
Harvard School of Public Health

sPRG

Why Would YOU Be Interested to Analyze the sPRG'2009 Sample???

Experimental/Methodology Questions Our Lab Tried to Address:

- Do we need to look at multiple peptide charge states to quantitate protein (peptide) abundance accurately?
- Will the discovery mode of LC-MS/MS data dependent data acquisition (i.e. “gas phase fractionation” or multiple m/z MS1 range dda) work for quantitative analysis?
- Will AMT software packages designed for label-free quantitation work for quantitative analysis applying SIL internal standards?
- How the length of an LC gradient does impact protein/peptide quantitation results?

Use Your Imagination in Your Trials of the sPRG'09 Sample!!!

Experimental Conditions

1. 30-min long gradient; single mass range dd data acquisition;
2. 30-min long gradient; three mass ranges dd data acquisition;
3. 60-min long gradient; single mass range dd data acquisition;

Data Analysis:

Database Search, Identification, Validation:

Sequest-Sorcerer, Scaffold, Protein Prophet, Peptide Prophet
(Sorcerer)

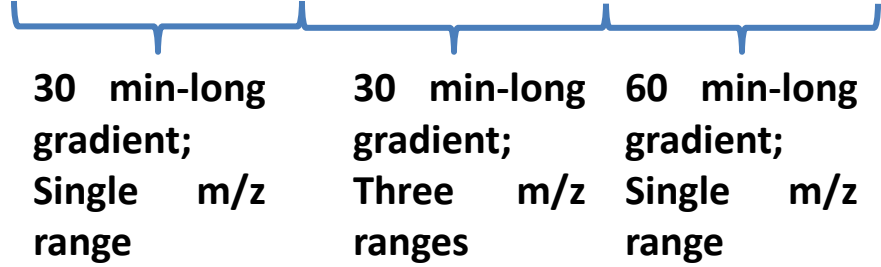
Quantitative Analysis:

1. X!Calibur, QUANT Browser (Thermo Fisher)
2. Progenesis LC-MS (Nonlinear Dynamics)

Spectral Counts

#	Visible?	Starred?	MS/MS View: Identified Proteins (5)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	
							123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	
1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Histidyl-tRNA synthetase, cytopla...P12081 SY...		57 kDa	★	240	414	224	406	200	389	202	219	218
2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Peroxiredoxin-1 - Homo sapiens (... Q06830 PR...		22 kDa	★	7	15	10	10	7	19	11	7	14
3	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ribosyldihyronicotinamide dehy... P16083 NQ...		26 kDa	★	87	145	90	147	83	123	89	84	93
4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum albumin - Homo sapiens (H...P02768 AL...		69 kDa	★	139	231	141	220	133	227	141	131	148
5	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ubiquitin - Homo sapiens (Human) P62988 UB...		9 kDa	★	42	97	45	84	44	82	39	48	48

Probability Legend:



Mean Spectral Counts

#	Identified Proteins (5)	Accession Number	Molecular Weight	Mean 30 min, Single m/z Range	Mean 30 min, Three m/z ranges	Mean 60 min, Single m/z Range
1	Histidyl-tRNA synthetase, cytoplasmic - Homo sapiens	P12081 SYHC_HUMAN	57 kDa	292.7	331.7	213.0
2	Serum albumin - Homo sapiens	P02768 ALBU_HUMAN	69 kDa	170.3	193.3	140.0
3	Ribosyldihydronicotinamide dehydrogenase [quinone] - Homo sapiens	P16083 NQO2_HUMAN	26 kDa	107.3	117.7	88.7
4	Ubiquitin - Homo sapiens	P62988 UBIQ_HUMAN	9 kDa	61.3	70.0	45.0
5	Peroxiredoxin-1 - Homo sapiens	Q06830 PRDX1_HUMAN	22 kDa	10.7	12.0	10.7

Number of Unique Peptides

#	Visible?	Starred?	MS/MS View: Identified Proteins (5)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	
							123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	
1	✓	☆	Histidyl-tRNA synthetase, cytopla... P12081 SY...		57 kDa	★	65	75	63	75	62	78	66	62	67
2	✓	☆	Peroxiredoxin-1 - Homo sapiens (... Q06830 PR...		22 kDa	★	4	10	6	7	6	11	7	5	9
3	✓	☆	Ribosyldihyronicotinamide dehy... P16083 NQ...		26 kDa	★	33	43	38	44	31	36	32	33	37
4	✓	☆	Serum albumin - Homo sapiens (H... P02768 AL...		69 kDa	★	61	74	64	66	59	71	55	56	60
5	✓	☆	Ubiquitin - Homo sapiens (Human) P62988 UB...		9 kDa	★	15	19	16	14	17	16	13	17	16

30 min-long
gradient;
Single m/z
range

30 min-long
gradient;
Three m/z
ranges

60 min-long
gradient;
Single m/z
range

Mean Numbers of Unique Peptides

#	Identified Proteins (5)	Accession Number	Molecular Weight	Mean 30 min, No Gas Phase	Mean 30 min, Gas Phase2	Mean 60 min, No Gas Phase2
1	Histidyl-tRNA synthetase, cytoplasmic - Homo sapiens	P12081 SYHC_HUMAN	57 kDa	67.7	71.7	65.0
2	Serum albumin - Homo sapiens	P02768 ALBU_HUMAN	69 kDa	66.3	65.3	57.0
3	Ribosyldihyronicotinamide dehydrogenase [quinone] - Homo sapiens	P16083 NQO2_HUMAN	26 kDa	38.0	37.0	34.0
4	Ubiquitin - Homo sapiens	P62988 UBIQ_HUMAN	9 kDa	16.7	15.7	15.3
5	Peroxiredoxin-1 - Homo sapiens	Q06830 PRDX1_HUMAN	22 kDa	6.7	8.0	7.0

Sequence Coverage

#	Visible?	Starred?	MS/MS View: Identified Proteins (5)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	Bio S...	
							123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	123008_ABRF_10percent_Halo_...	
1	✓	☆	Histidyl-tRNA synthetase, cytopla... P12081 SY...		57 kDa	★	73%	75%	80%	73%	76%	73%	72%	69%	80%
2	✓	☆	Serum albumin - Homo sapiens (H... P02768 AL...		69 kDa	★	70%	74%	74%	75%	74%	72%	75%	70%	67%
3	✓	☆	Ribosyldihydronicotinamide dehy... P16083 NQ...		26 kDa	★	89%	77%	86%	77%	84%	88%	83%	91%	92%
4	✓	☆	Ubiquitin - Homo sapiens (Human) P62988 UB...		9 kDa	★	71%	72%	79%	72%	80%	72%	79%	80%	72%
5	✓	☆	Peroxisredoxin-1 - Homo sapiens (... Q06830 PR...		22 kDa	★	27%	35%	59%	37%	46%	51%	56%	35%	54%

30 min-long
gradient; Single
m/z range

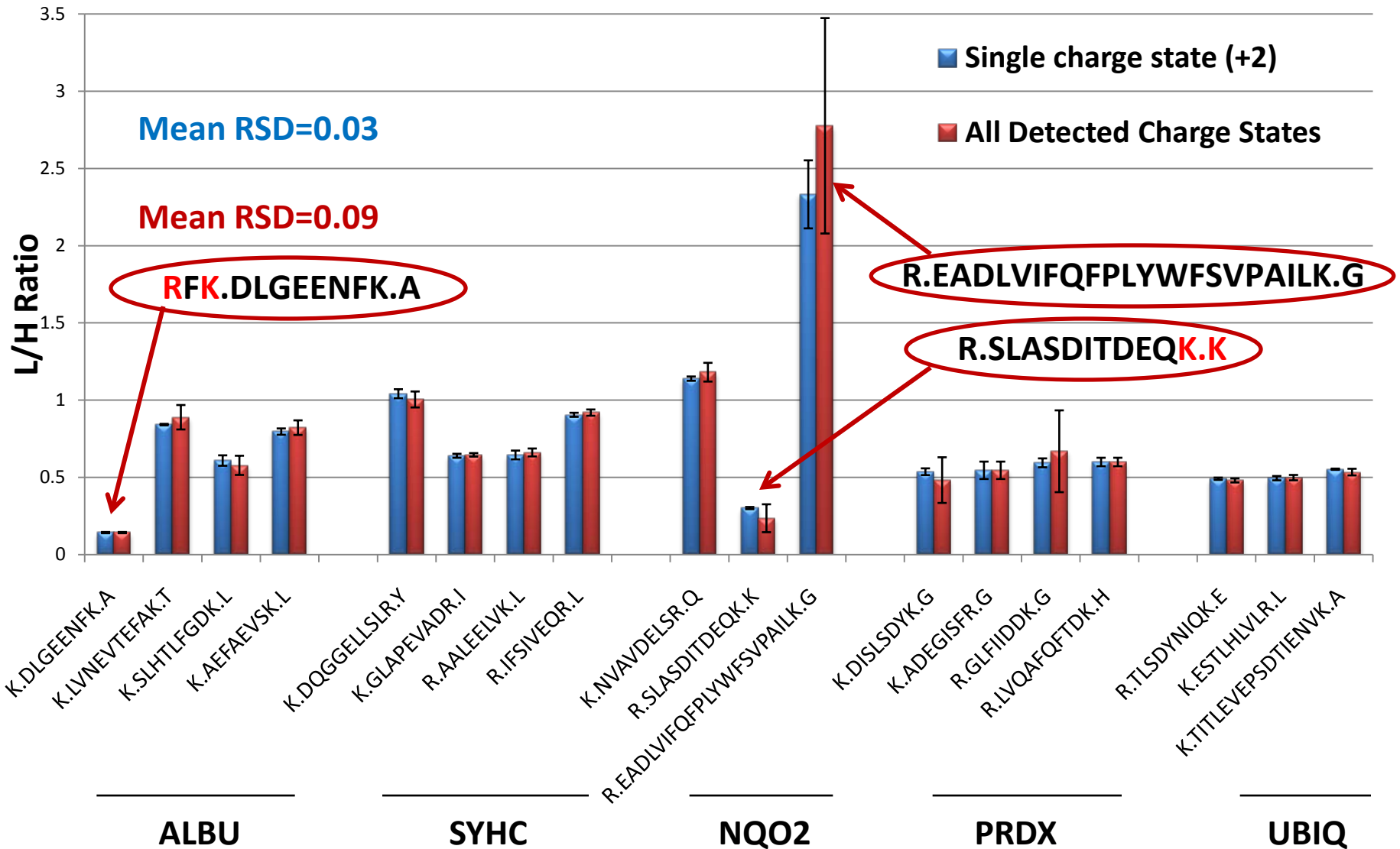
30 min-long
gradient;
Three m/z
ranges

60 min-long
gradient;
Single m/z
range

Mean Sequence Coverage

#	Identified Proteins (5)	Accession Number	Molecular Weight	Mean 30 min, No Gas Phase	Mean 30 min, Gas Phase2	Mean 60 min, No Gas Phase2
1	Histidyl-tRNA synthetase, cytoplasmic - Homo sapiens	P12081 SYHC_HUMAN	57 kDa	76.0%	74.0%	73.7%
2	Serum albumin - Homo sapiens	P02768 ALBU_HUMAN	69 kDa	72.7%	73.7%	70.7%
3	Ribosyldihydronicotinamide dehydrogenase [quinone] - Homo sapiens	P16083 NQO2_HUMAN	26 kDa	84.0%	83.0%	88.7%
4	Ubiquitin - Homo sapiens	P62988 UBIQ_HUMAN	9 kDa	74.0%	74.7%	77.0%
5	Peroxisredoxin-1 - Homo sapiens	Q06830 PRDX1_HUMAN	22 kDa	40.3%	44.7%	48.3%

Quantitative Analysis: Peak Integration And Charge States

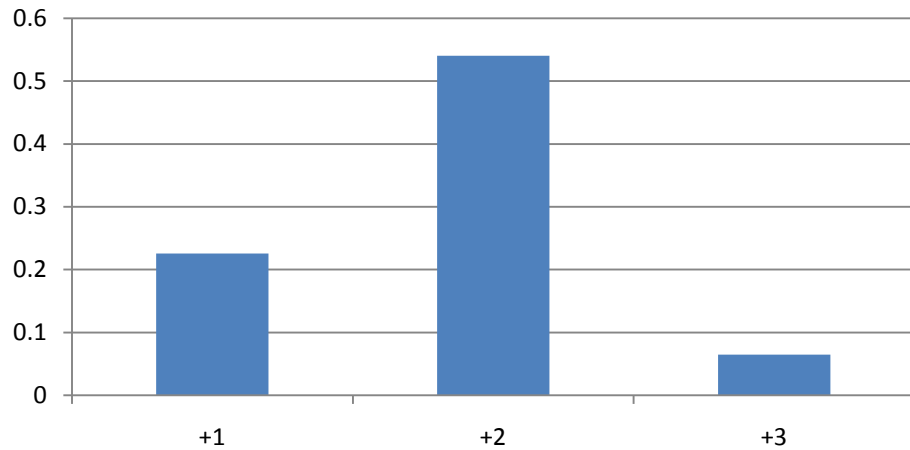


Quantitative Analysis: Potentially Misbehaving Peptides

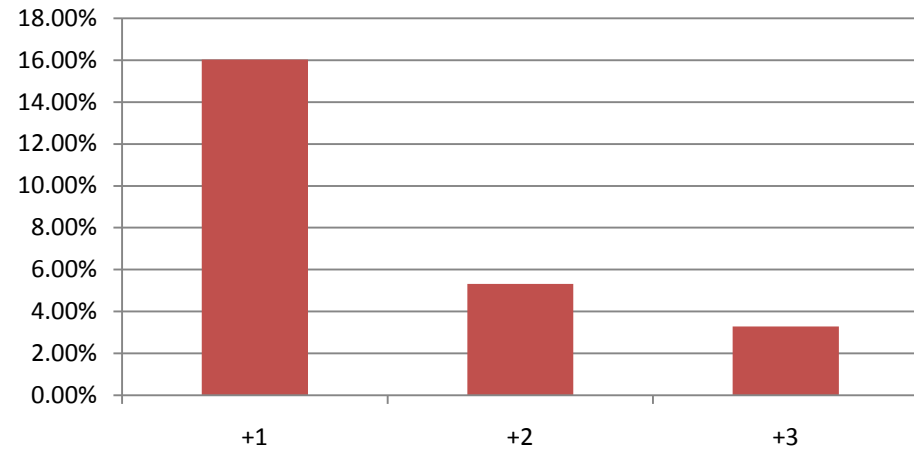
Peptide	Protein Name	Proteotypic peptide
Albu1	ALBU_HUMAN	K.DLGEENFK.A
Albu2	ALBU_HUMAN	K.LVNEVTEFAK.T
Albu3	ALBU_HUMAN	K.SLH ^H TLFGDK.L
Albu4	ALBU_HUMAN	K.AEFAEVSK.L
Syhc1	SYHC_HUMAN	K.DQGGELLSLR.Y
Syhc2	SYHC_HUMAN	K.GLAPEVADR.I
Syhc3	SYHC_HUMAN	R.AALEELVK.L
Syhc4	SYHC_HUMAN	R.IFSIVEQR.L
Nqo2_1	NQO2_HUMAN	K.NVAVDELSR.Q
Nqo2_2	NQO2_HUMAN	R.SLASDITDEQK.K
Nqo2_3	NQO2_HUMAN	R.EADLVIFQFPLY ^W F ^W SVPAILK.G
Prdx1	PRDX1_HUMAN	K.DISLSDYK.G
Prdx2	PRDX1_HUMAN	K.ADEGISFR.G
Prdx3	PRDX1_HUMAN	R.GLFIIDDK.G
Prdx4	PRDX1_HUMAN	R.LVQAFQFTDK.H
Ubiq1	UBIQ_HUMAN	R.TLSDYNIQK.E
Ubiq2	UBIQ_HUMAN	K.ESTLH ^H LVLR.L
Ubiq3	UBIQ_HUMAN	K.TITLEVEPSDTIENVK.A

Peptide Charge States in Quantitated Peptides, N=3

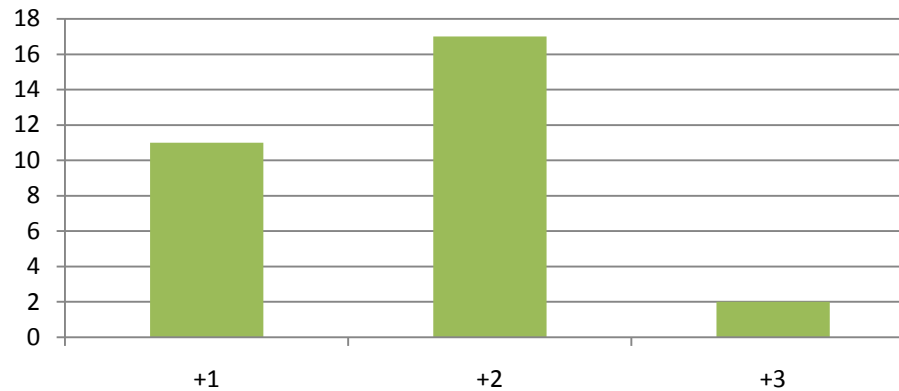
Mean RSD



Mean CV



Number of times used for quantitation



DD Data Acquisition: Single m/z MS Range vs. Three m/z MS Ranges

MS: 350-1700 mmu

MS2 MS2 MS2 MS2 MS2 MS2 MS2

MS: 350-1700 mmu

MS2 MS2 ...

VS.

MS: 350-700 mmu

MS2 ... MS2

Intensity: 4.91×10^6

MS: 695-1200 mmu

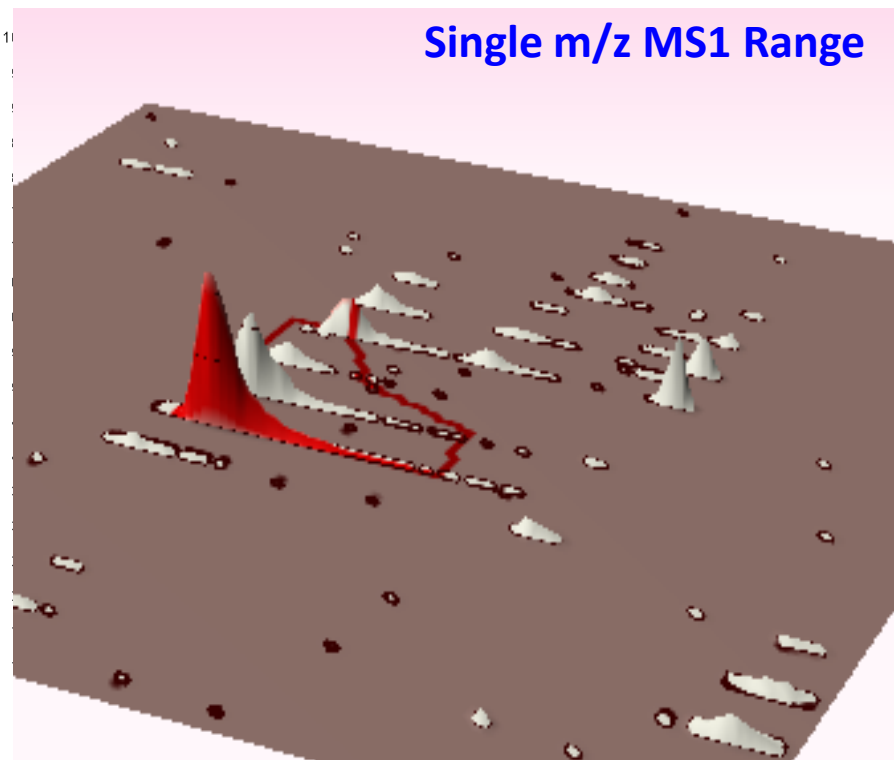
MS2 ... MS2

Intensity: 1.03×10^7

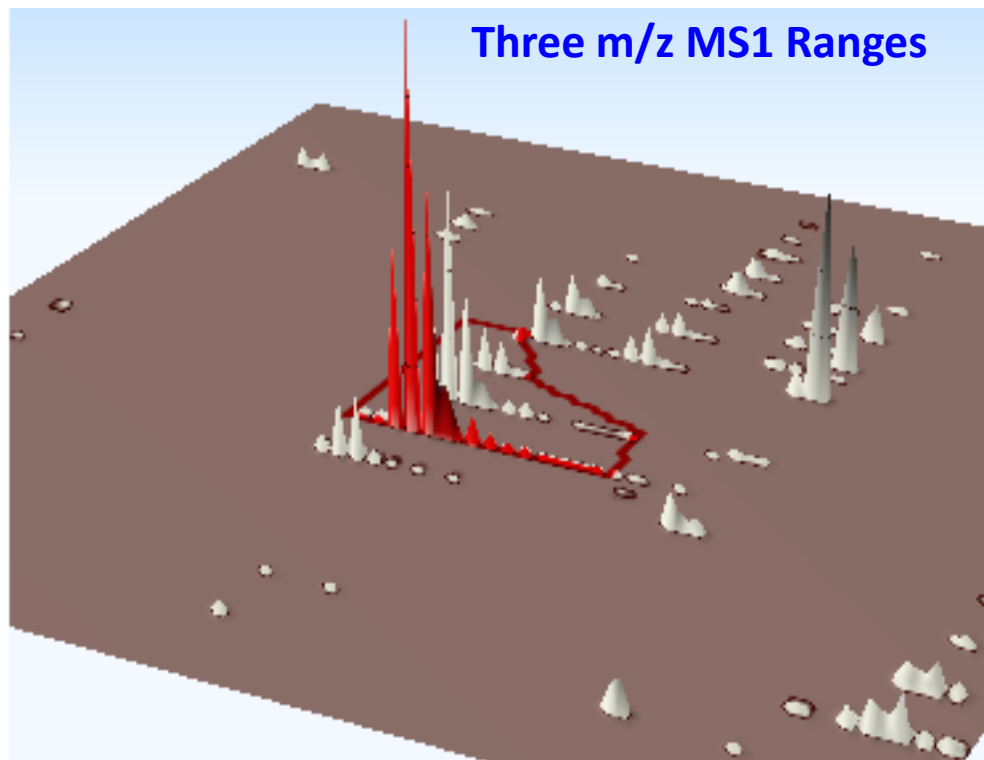
MS: 1195-1700 mmu

MS2 MS2

Single m/z MS1 Range



Three m/z MS1 Ranges



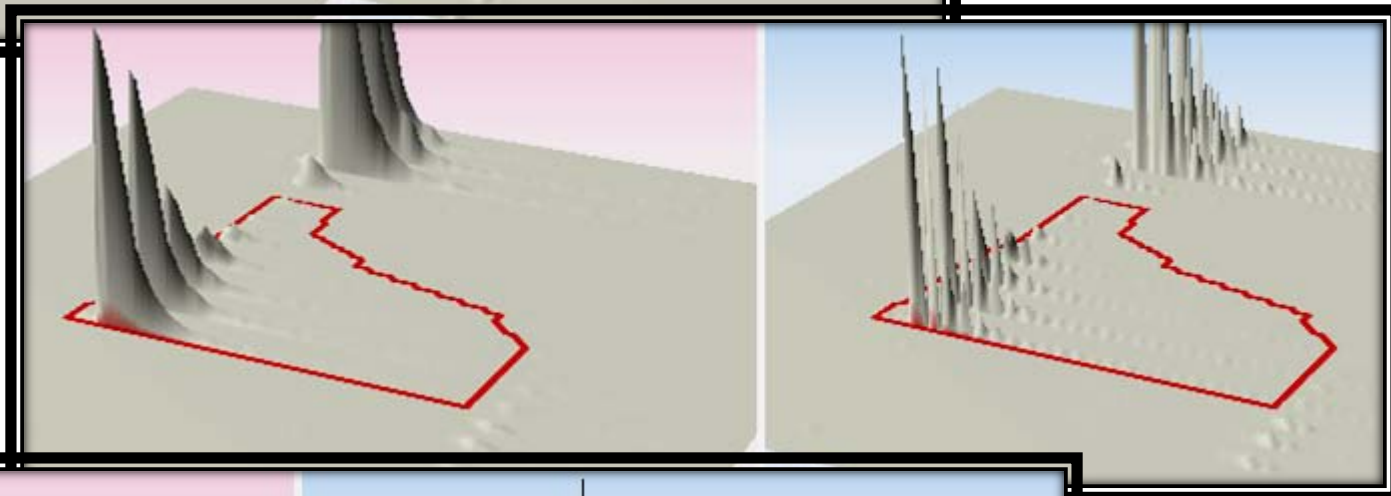
Time (min)

Time (min)

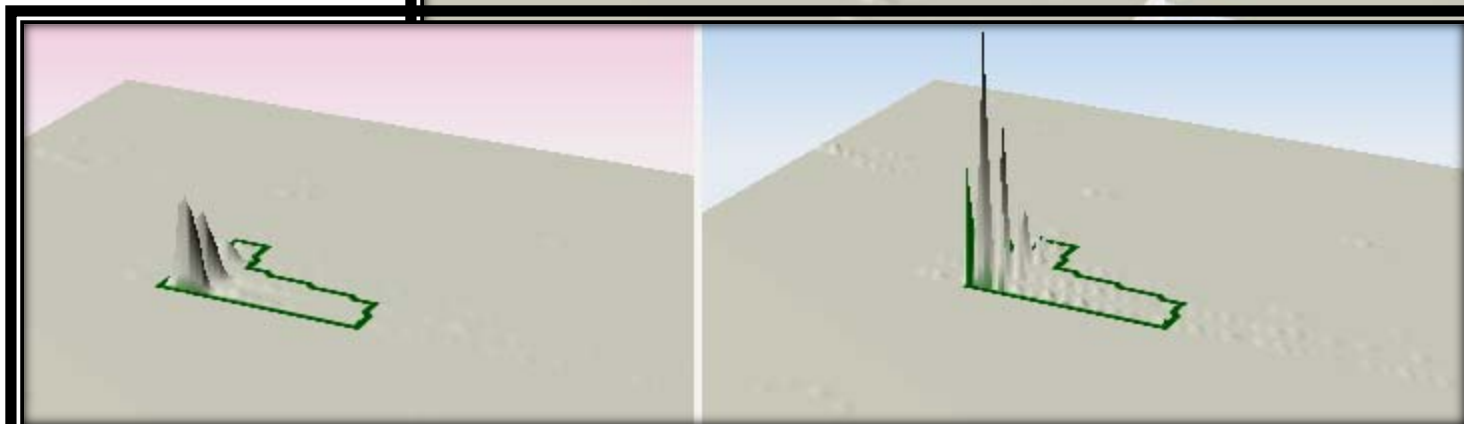
DD Data Acquisition: Single m/z MS Range vs. Three m/z MS Ranges



+1

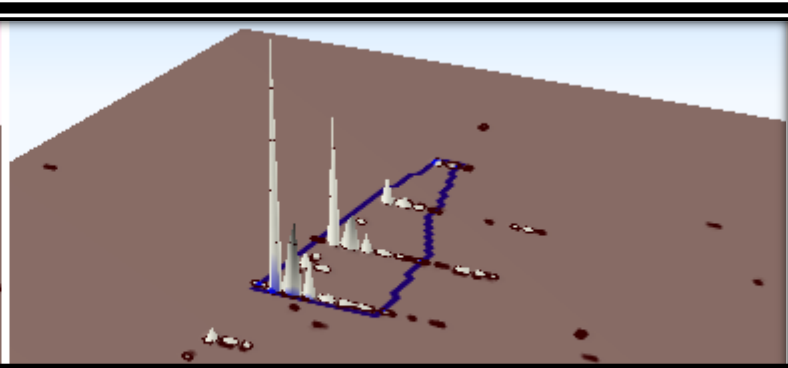
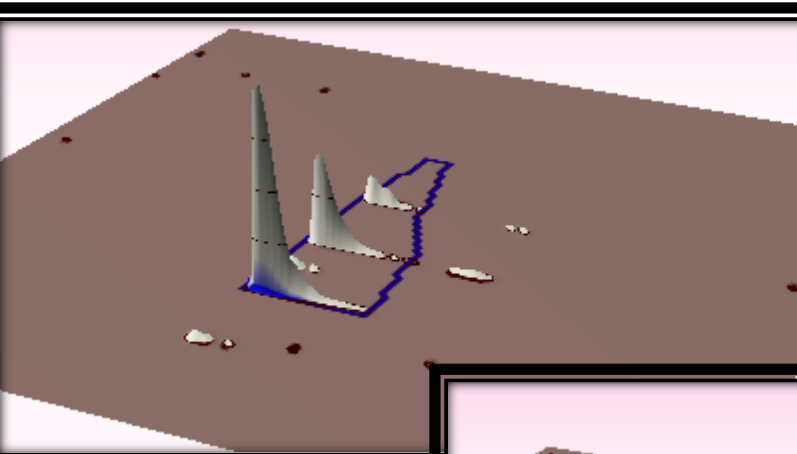


+2



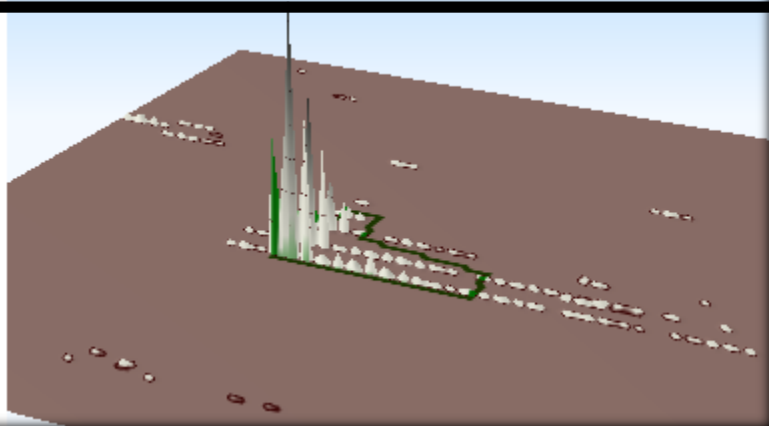
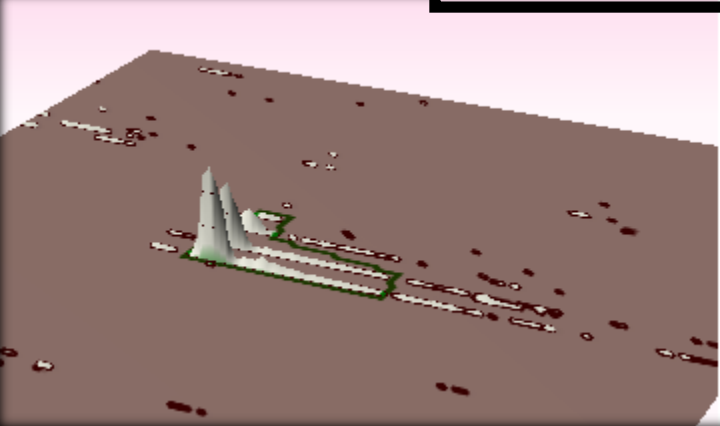
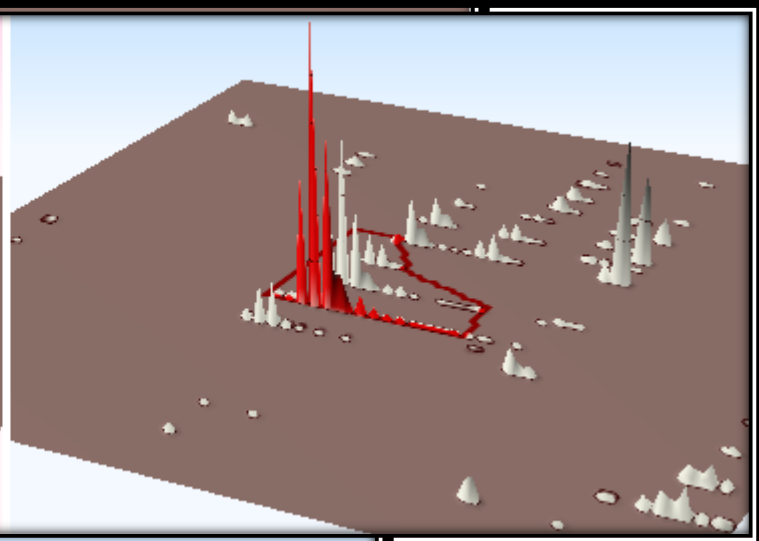
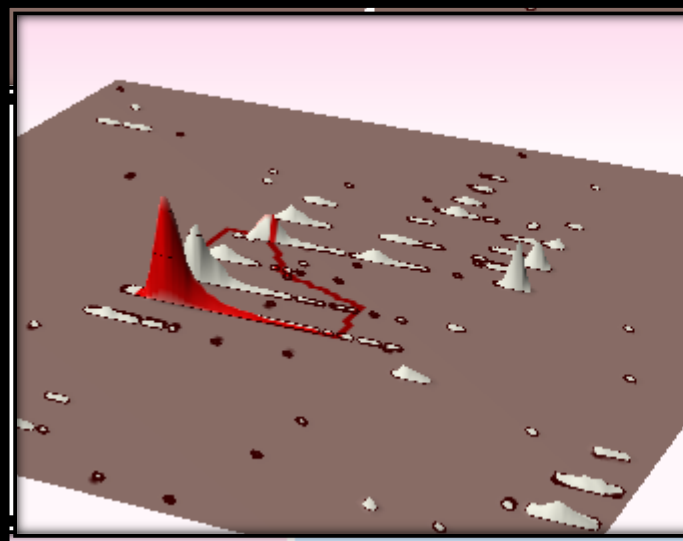
+3

DD Data Acquisition: Single m/z MS Range vs. Three m/z MS Ranges



+1

+2

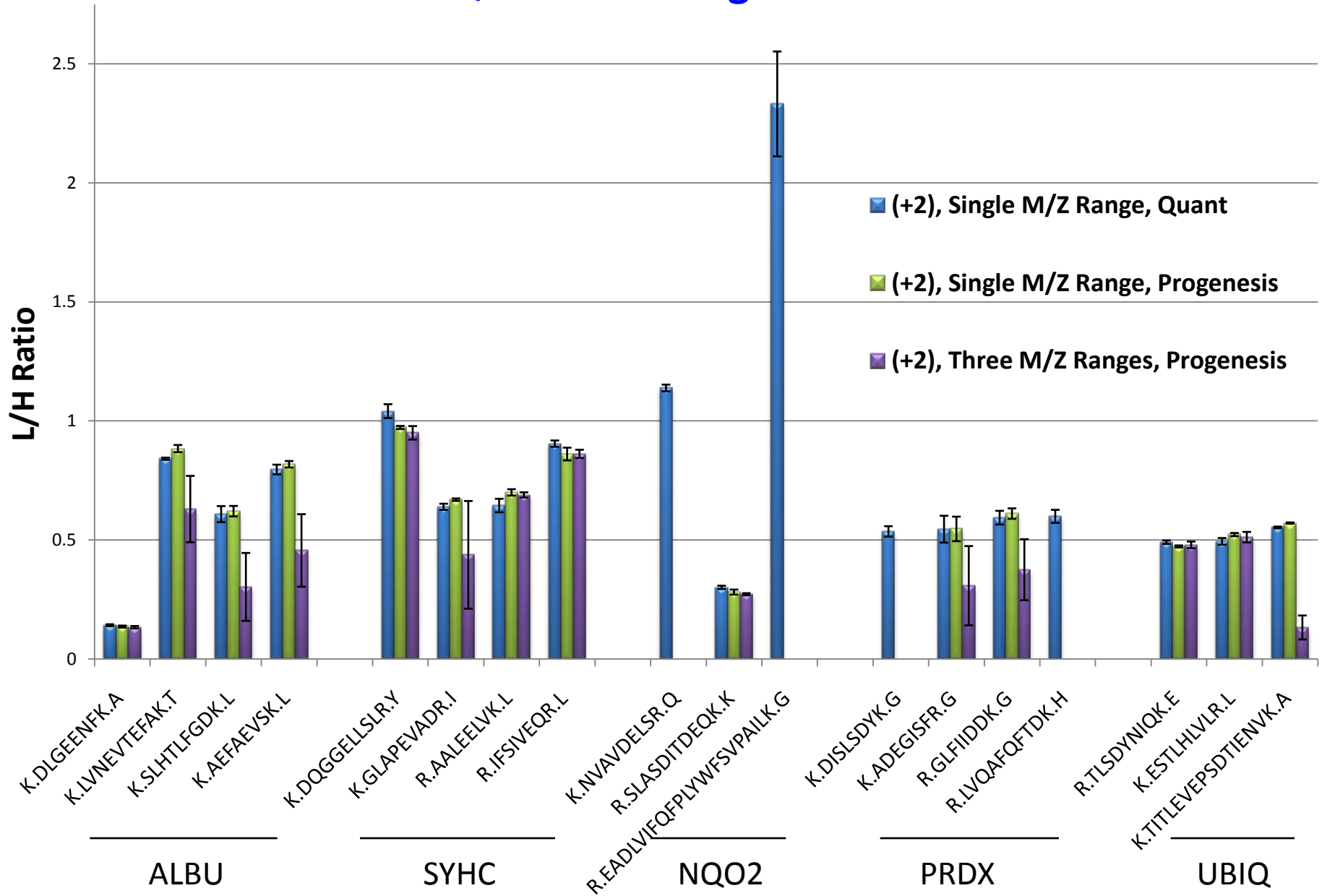


+3

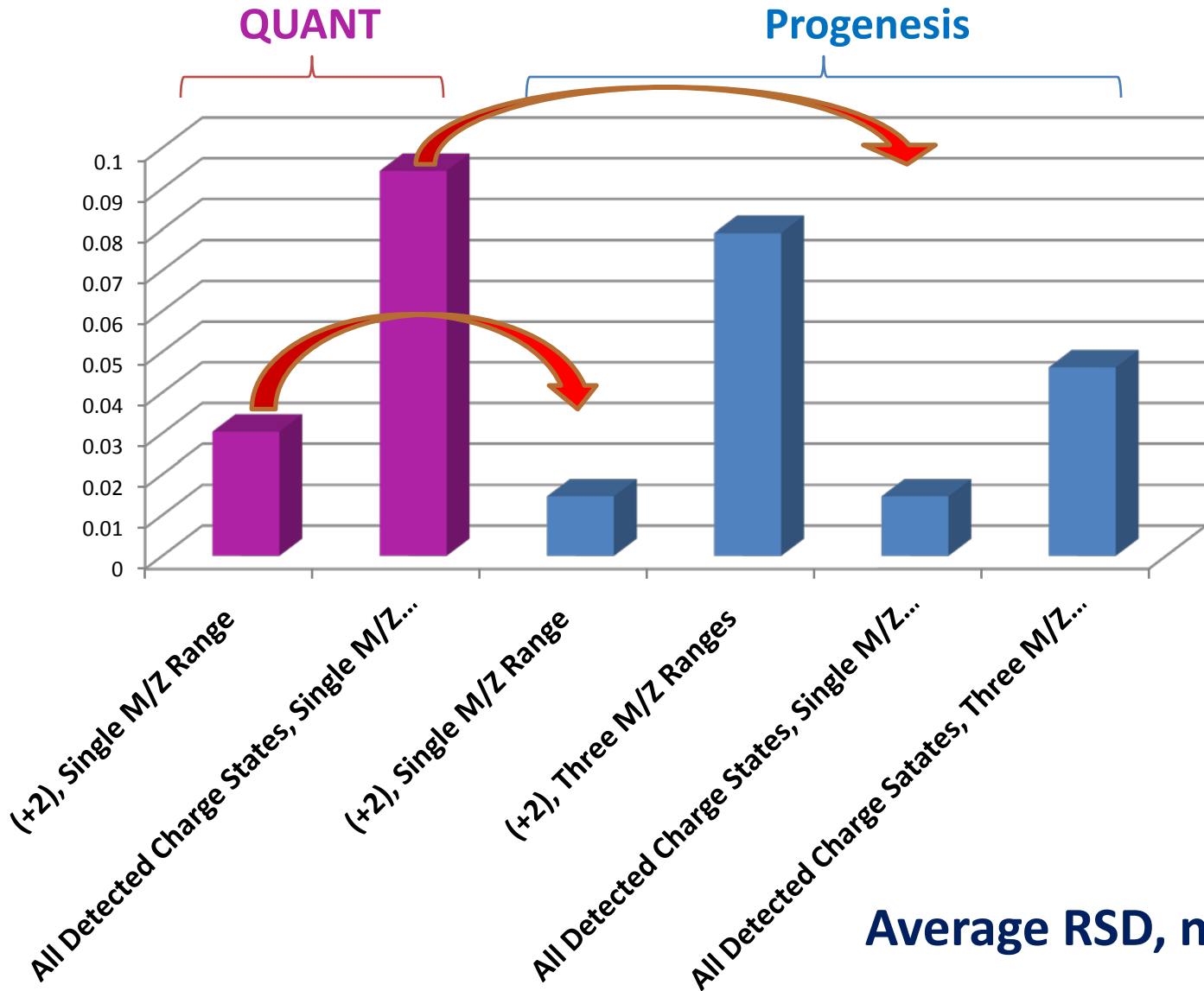
DD Data Acquisition: Single m/z MS Range vs. Three m/z MS Ranges. Peak Area Ratio.

Accession #	Ratio, Summed Peptide Peak Area Three m/z Ranges / Single m/z Range
P12081 SYHC_HUMAN	2.16
P02768 ALBU_HUMAN	2.04
P16083 NQO2_HUMAN	2.93
P62988 UBIQ_HUMAN	3.06
Q06830 PRDX1_HUMAN	1.98
Mean	2.43±0.52

DD Data Acquisition: Single m/z MS Range vs. Three m/z MS Ranges. QUANT vs. Progenesis

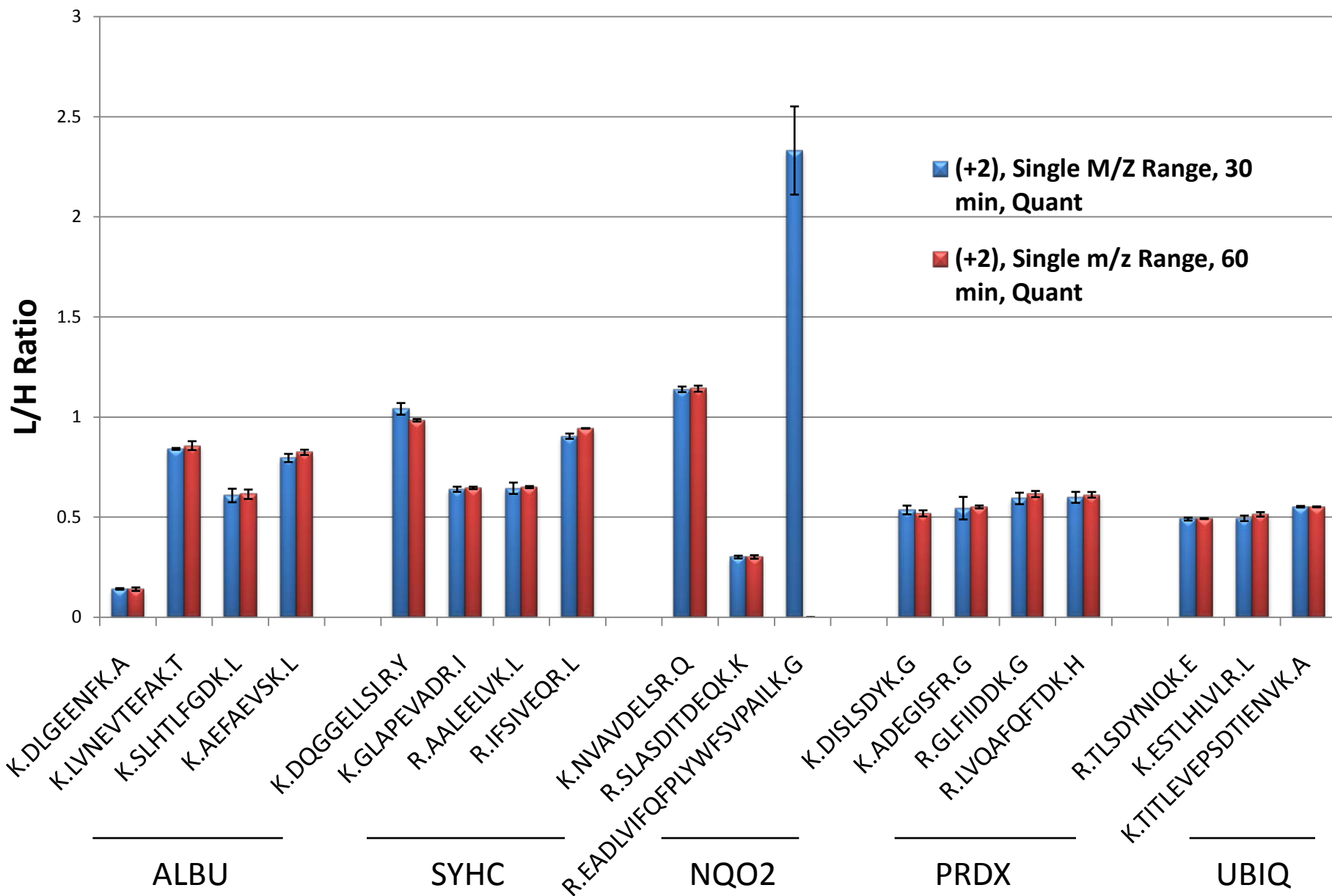


DD Data Acquisition: Single m/z MS1 Range vs. Three m/z MS1 Ranges. QUANT vs. Progenesis.



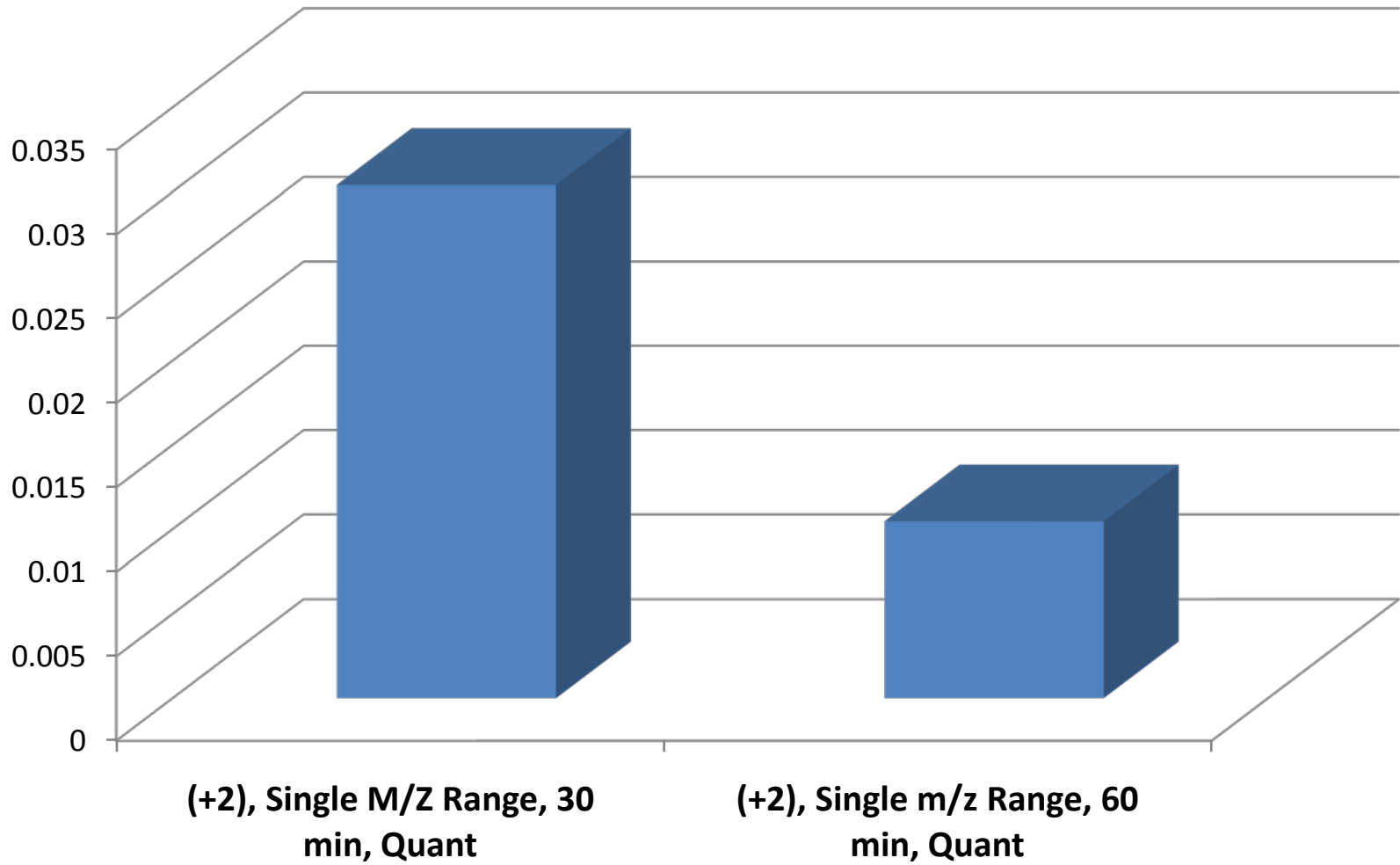
Average RSD, n=3

Length of LC Gradient



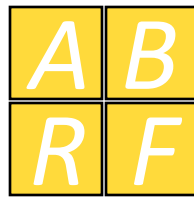
Length of LC Gradient

Mean RSD



Preliminary Observations/Conclusions

- Quantitative analysis using a single charge state provides similar results to those acquired multiple states with lower variability.
- Data dependent data acquisition mode using multiple m/z MS1 ranges (gas fractionation) can be used for quantitative analysis. Higher variability is the payoff for better sensitivity.
- AMT software packages designed for label-free quantitation with automated feature selection, alignment, and matching do work for quantitative analysis applying SIL internal standards.
- The length of an LC gradient does not impact quantitation results for peptides above the noise level. LOQ is setup- and analyte-dependent.



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