

## SUPPLEMENTAL

Supplemental Table I. The whole model of a set of 24 LC-ESI-MS/MS experiments at the level of sample treatments, proteins, peptides and parent and fragment ion types. A model total of 48,198 log transformed parent fragment ions intensity values identified by fragment m/z values by X!TANDEM resulting in 822 observations of 302 peptides from 119 proteins separated over 24 column fractions. The probability that the transformed LC-ESI-MS/MS data fails to show significant variation apparently approaches zero. Note the differences between sample treatments and proteins achieved an F value of 1930 and 234 respectively indicating the approach shows great statistical power. **The data from 12 QA and 12 PS column pH fractions are shown.**

### Analysis of Variance log peptide or fragment intensity

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	338	22564.545	66.7590	213.1918
Error	47859	14986.598	0.3131	<b>Prob &gt; F</b>
C. Total	48197	37551.143		0.0000*

### Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
sample treatment	24	23	1930.0361	267.9774	0.0000*
Protein accession number	118	11	224.1555	65.0755	<.0001*
Peptide sequence	301	196	9243.4962	150.6055	0.0000*
ion type (peptide versus fragment)	1	1	2773.6519	8857.528	0.0000*

Supplemental Table II. The one way ANOVA tables from the 24 column fractions with 119 proteins and 302 peptides from a set of LC-ESI-MS/MS Chromatography fraction samples. From the 24 column fractions, 15 difference groups were revealed (see Panel D). From the 119 protein identified in the small experiment, 52 groups of pair wise differences were obtained (see supplemental data tables). From the 302 peptide sequences identified in the small experiment, approximately 145 groups of pair wise differences were obtained (see supplemental data tables). **The data from 12 QA and 12 PS column pH fractions are shown.**

**A, Analysis of variance of column fractions**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
sample treatment	24	6617.320	275.722	429.3792	0.0000*
Error	48173	30933.823	0.642		
C. Total	48197	37551.143			

**B, Analysis of variance of proteins**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Protein accession number	118	6863.040	58.1614	91.1213	0.0000*
Error	48079	30688.102	0.6383		
C. Total	48197	37551.143			

**C, Analysis of variance of peptides**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Peptide sequence	301	17381.928	57.7473	137.1329	0.0000*
Error	47896	20169.215	0.4211		
C. Total	48197	37551.143			

**D, Means Comparisons for all fraction pairs using Tukey-Kramer HSD**

Level											Mean
HiS4	A										5.5553271
HIS3b	A	B									5.3930296
HIS3a		B	C								5.3202714
TrypHiS5		B	C	D							5.2735733
HiQpH6.5			C	D	E						5.1510498
HiS2				D	E	F					5.0589955
HiS5.5					E	F	G				5.0266574
HiS3						F	G				4.9807248
HiQ11b							G				4.9215026
HiS6a						F	G	H			4.9103701
HiS10						F	G	H	I		4.8838077
HiS7.6							G	H	I		4.8347193
HiQpH7								H	I		4.8322354
HiQ9									I		4.7904202
HiS7								H	I	J	4.6474917
HiQ11a										J	4.6049974
HiQ_pH8b										J	4.5894854
HiQ_pH7.6b										J	4.5843135
HiS8										K	4.4403131
HiQ10											4.4319175
HiQ_pH7.6											4.4263171
HiQpH6										M	4.1793883
HiS9										M	3.9807336
HiQpH5										N	3.7337752
HiS8										N	3.5916031

Levels not connected by same letter are significantly different.

Supplemental Table III. Whole model and one way ANOVA of Complement C3 at the level of sample treatment, peptides and ion type (parent versus fragment). **The data from 12 QA and 12 PS column pH fractions are shown. Portions of the SAS output are shown without modification.**

**A, Whole model ANOVA**

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	765.42394	85.0471	529.4423
Error	1067	171.39783	0.1606	<b>Prob &gt; F</b>
C. Total	1076	936.82177		0.0000*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
FileName	3	3	197.09233	408.9852	<.0001*
PeptideSequence	5	5	37.99711	47.3085	<.0001*
parent versus fragment	1	1	58.27820	362.7983	<.0001*

**B, One way ANOVA of log transformed parent intensity values from complement C3 across sample treatments**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
FileName	3	12.518440	4.17281	32.7114	<.0001*
Error	12	1.530773	0.12756		
C. Total	15	14.049213			

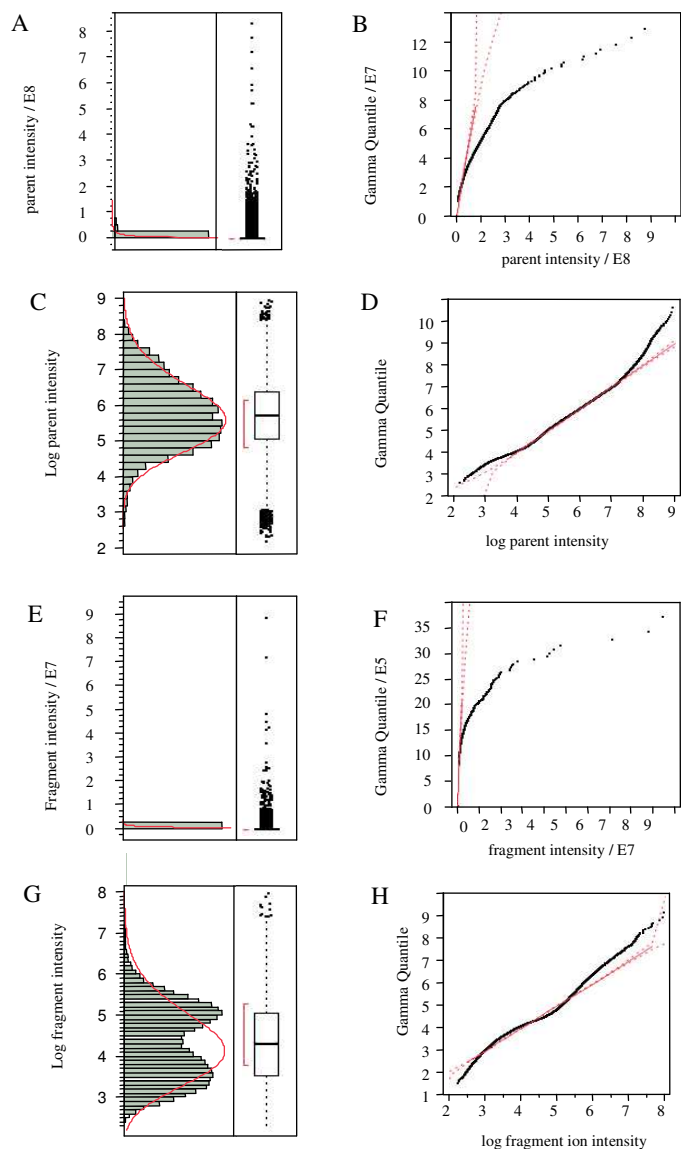
**C, One way ANOVA of log transformed parent and fragment intensity values from complement C3 across sample treatments.**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
sample treatment	3	667.46243	222.487	886.2847	<.0001*
Error	1073	269.35934	0.251		
C. Total	1076	936.82177			

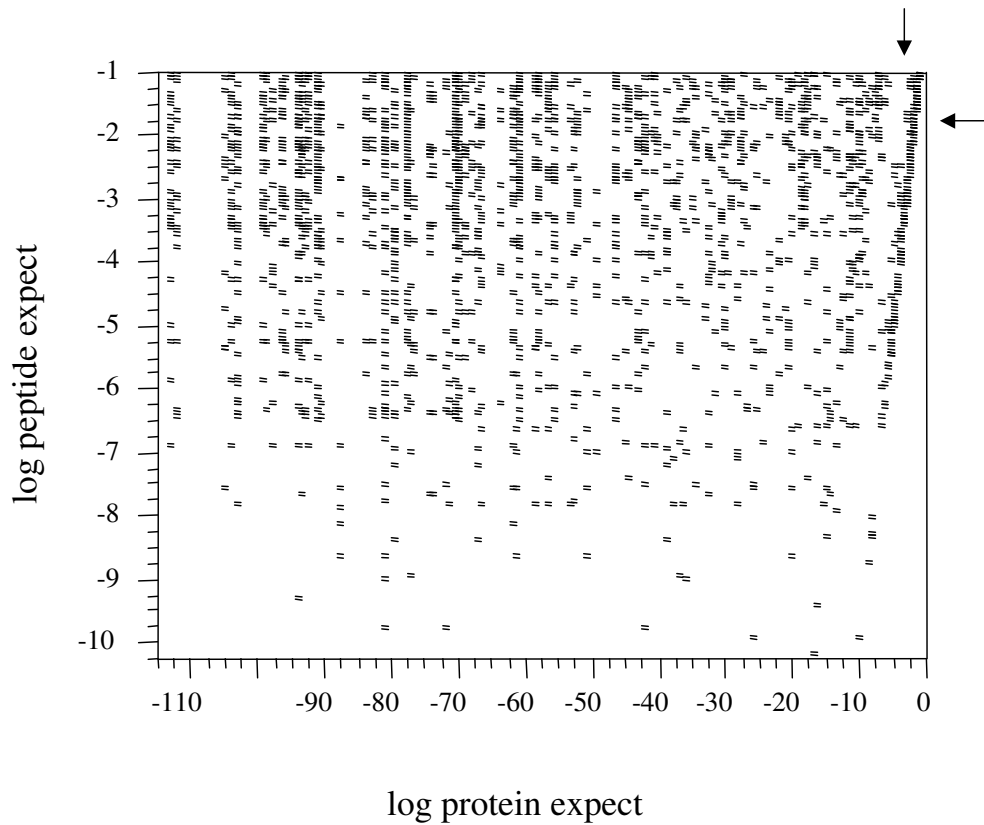
**D, One way ANOVA of log transformed fragment intensity values from the many complement C3 peptides across sample treatments.**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
sample treatment	3	655.64324	218.548	1113.874	0.0000*
Error	1057	207.38875	0.196		
C. Total	1060	863.03199			

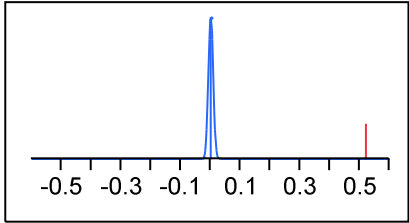
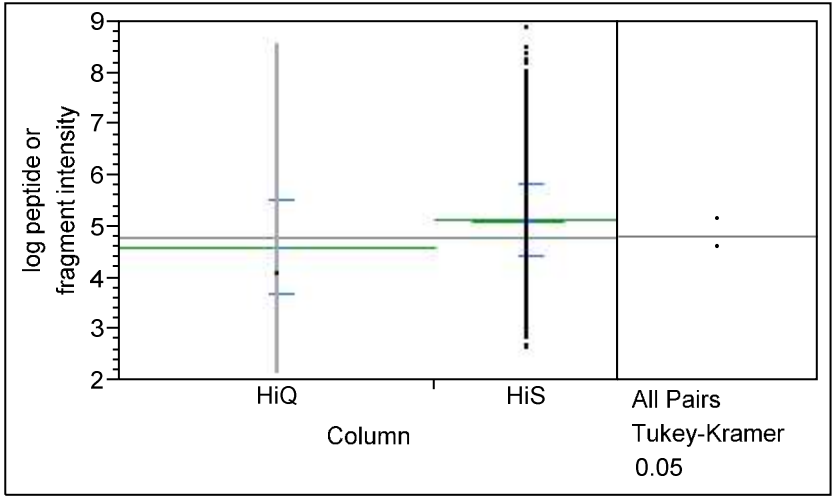
Figure Legends



Supplemental Figure 1. Fit of the gamma function to parent and fragment intensity values with and without Log transformation. Panels: A, the gamma function fitted to the distribution of the raw parent intensity values; B, the distribution of the parent intensity data plotted along the gamma function probability plot; C, the gamma function fitted to the log transformed parent intensity distribution; D, the distribution of the transformed parent intensity data plotted along the gamma function probability plot; E, the gamma function fitted to the distribution of the raw fragment intensity values; F, the distribution of the fragment intensity data plotted along the gamma function probability plot; G, the gamma function fitted to the log transformed fragment intensity distribution; H, the distribution of the transformed fragment intensity data plotted along the gamma function probability plot.



Supplementary Figure 2. Cartesian plot of peptide and protein expectation scores showing the distribution of peptides that are associated with expectation values of E-2 ( $p \leq 0.01$ ). The arrows indicate the location of the E-2 values for peptides and proteins. data outside the small enclose space near the origin have expectation values of  $p \leq 0.01$  or less.

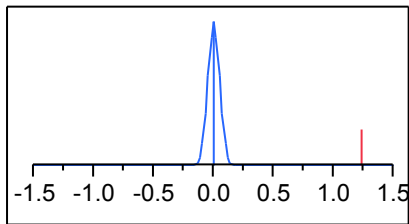
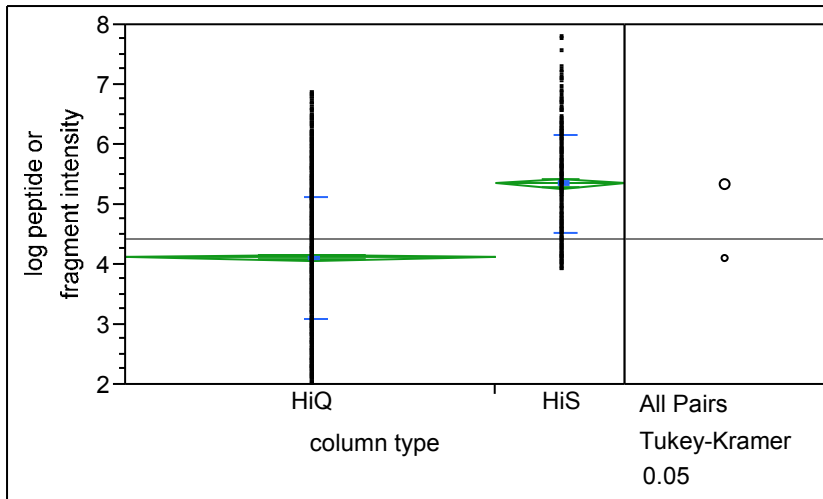


**Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column	1	3035.421	3035.42	4238.507	<.0001*
Error	48196	34515.722	0.72		
C. Total	48197	37551.143			

Supplemental Figure 3. Oneway Analysis of all log peptide or fragment intensity by Column resin. A portion of the unmodified SAS output for all peptides compared over the QA and PS chromatography is shown.





**Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
column type	1	601.8619	601.862	642.4175	<.0001*
Error	2041	1912.1522	0.937		
C. Total	2042	2514.0140			

Supplemental Figure 4. Oneway Analysis of all Apolipoprotein AI log peptide or fragment intensity by column resin. A portion of the unmodified SAS output for all Apolipoprotein AI peptides by column is shown.

See also supplemental data tables for one-way ANOVA results with Tukey-Kramer HSD test for nominal proteins, peptides or ordinal M+H values and the table of peptides and proteins.