INVESTIGATING THE REPRODUCIBILITY OF PROTEOMIC DATA ACROSS DIFFERENT INSTRUMENT CONFIGURATIONS AND PLATFORMS

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INTRODUCTION

In order to identify and quantify protein biomarkers in a large-scale clinical analysis, results must be consistent between instruments and across laboratories. A proteomic sample should yield the same results from different mass spectrometers, especially for the most abundant proteins in the sample. Complementary results from similar instruments are not acceptable in a clinical environment, where reproducibility is extremely important. A data independent acquisition method with a stringent protein identification protocol has the best chance of generating reproducible results from proteomic samples. In this study, results from a variety of samples run on various instruments were compared to assess qualitative and quantitative reproducibility.

METHODS

LC Systems: nanoACQUITY UPLC®
1D Chromatography: 75 µm x 15 cm, 150 µm x 10 cm, or TRIZAIC™ nanoTile™ packed with BEH C_{18} (1.7 µm)
2D Chromatography: 2D high/low pH RP/RP[1]

First dimension:
Column: 150 µm x 10 cm XBridge™ C_{18} (5 µm)
Gradient formation: discontinuous step gradient at 1 µL/min
Eluent A: 20 mM ammonium formate pH 10.0
Eluent B: Acetonitrile
Online dilution flow rate: 10 µL/min aqueous

Second dimension:
Column: 75 µm x 15 cm BEH C_{18} (1.7 µm)
Gradient: 5-40% B for 90 min at 300 nL/min
Eluent A: 0.1% formic acid in water
Eluent B: 0.1% formic acid in acetonitrile

MS Systems: Q-Tof Premier or SYNAPT™ HDMS™

MS Data Collection and Processing : MS^E data was collected for all analyses. Data was processed using ProteinLynx Global Server 2.3 with Identity^E Informatics.

Absolute Quantitation[2]: Yeast alcohol dehydrogenase was added to each sample as an internal standard. Fmol amounts of each protein were calculated using the average of the top three best ionizing peptides compared to the internal standard. Amounts were converted in to ng/µg or fmol/µg to account for differences in loading.

RESULTS

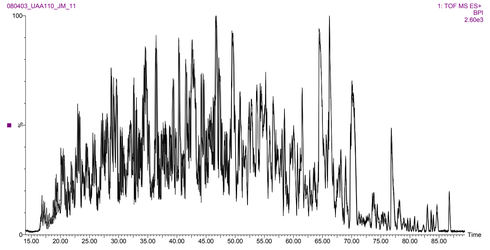

Figure 1. Overlaid triplicate injections of 1.3 µg of E. coli digest on a TRIZAIC nanoTile.

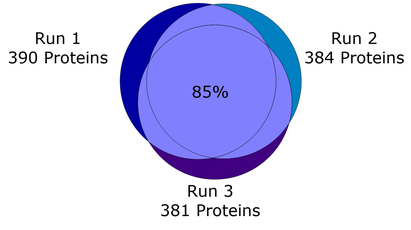

Figure 2. Venn diagram of proteins identified from triplicate runs of E. coli on the nanoTile. 349 proteins (with over 3000 peptides) were identified in at least two of three injections.

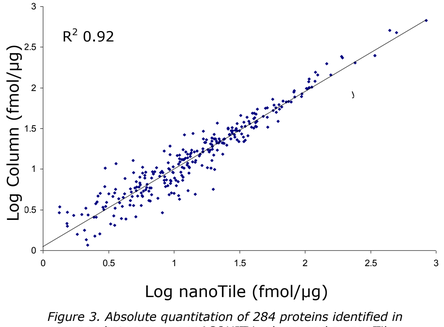

Figure 3. Absolute quantitation of 284 proteins identified in common between a nanoACQUITY column and a nanoTile.

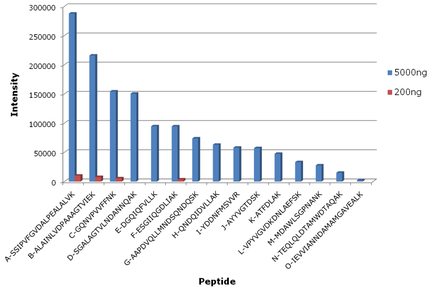

Figure 4. Peptides identified to D-galactose binding protein from E. coli. The distribution of ionization is constant despite the change in loading.

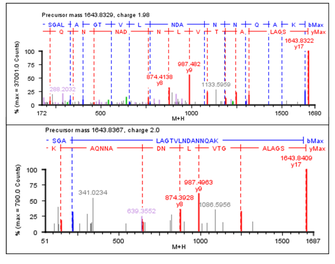

Figure 5. Reproducibility of peptide fragmentation. The same fragments with the same relative intensities are identified to this peptide from D-galactose binding protein from both the 5000 ng (top) and 200 ng analyses (bottom).

	1st Dimension		2nd Dimension	
Peptides from IF-2	Fraction Run 1	Fraction Run 2	Fraction Run 3	%RSD RT
NELGQEVLEAGPSIPVEILGLSGVPAAGDEVTVVR	4	4	4	0.7%
AAQVPVVVAVNK	3	3	3	0.8%
ADVQGSVEAISDSLLK	4	4	4	0.9%
ENELEEAVMSDR	1	1	1	1.4%
GSSLQQGFQKPAQAVNR	3.5	3.5	3	1.5%
GMASGAVIESFLDK	4	4	3	0.3%
QAEDESDREVEGGR	1	1	1	2.5%
IDKPEADPDR	1	1	1	2.7%
LVQQFADAGIR	4	4	4	1.4%
QTLIDHLNQK	3	3	3	1.1%
GDIVLCGFEYGR	3	3	3	0.6%
NELSQYGILPEEWGGESQFVHVSAK		4	4	1.3%
APVVTIMGHVDHGK	4	4	4	1.9%
DNVVIYEGELESLR	1		1	1.4%

Figure 6. Reproducibility of both dimensions using 2D high/low pH RP/RP. The peptides from initiation factor 2 from E. coli are found in the same fractions at the same retention times from triplicate analyses. For all 2D analyses, greater than 85% of the peptides were found in only one fraction.

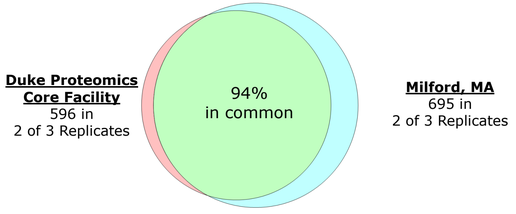

Figure 7. E. coli digest analyzed with identical 2D high/low pH RP experiments at two different locations. Data courtesy of Dr. M. Arthur Moseley and Dr. J. Will Thompson from the Duke Proteomics Core Facility. (http://www.genome.duke.edu/proteomics/)

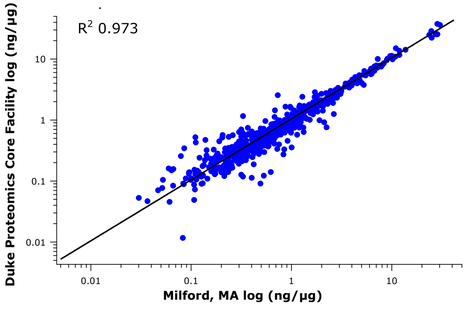

Figure 8. Absolute quantitation measurements of 558 E. coli proteins in common between laboratories. Average of 20% RSD was achieved for the absolute quantitation measurement.

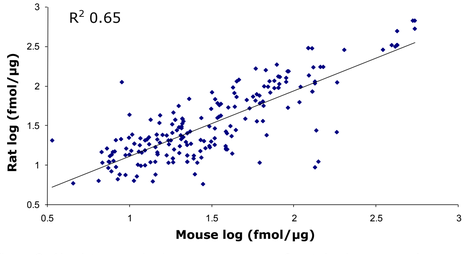

Figure 9. Absolute quantitation measurements of proteins in common between analysis of rat and mouse brain lysates. Mouse data was collected in 2007 using direct-injection with a Q-Tof Premier while the rat data was collected in 2008 with trapping on a Synapt HDMS. The average RSD was 33%.

DISCUSSION

Tryptic digests from *E. coli* were analyzed by a variety of LC configurations and mass spectrometers. From 1D LC, between 350 and 400 proteins were identified with greater than 3000 peptides. Twice as many proteins and peptides were identified using 2D chromatography. Over 90% of the proteins replicated between instruments and also between laboratories. Across instrument platforms, the relative standard deviation in absolute amount for all of the replicating proteins in *E. coli* was less than 20%. Even the comparison across species and instrument platforms yielded reproducible quantitation of proteins.

CONCLUSION

- **85% of protein identifications found in at least two of three replicate injections**
- **Absolute quantitation measurements within 20% between systems and between laboratories**
- **Measurement of absolute quantitation within 33% between rat and mouse brains**
- **Both dimensions of the 2D system reproducibly separate peptides with high resolution**
- **Reproducible peptide ionization and fragmentation**

ACKNOWLEDGEMENTS

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References

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2. J. Silva, *et.al.*, *Mol. Cell Proteomics*, **2006**, 144-156

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