ALL PROTEOMIC ANALYSES ARE CREATED EQUAL AND EXHIBIT AN ION DENSITY THAT CHALLENGES CONVENTIONAL BOTTOM UP LC/MS ANALYSES

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SUMMARY

- A variety of biological samples (bacteria, yeast, human cells, human tissue, and Top6 depleted serum) were digested and analyzed by LC/Q-Tof MS using a standardized methodology.
- The resulting data shows that, while these samples produced vastly different sets of tryptic peptides, the analytical behavior of these complex peptide mixtures was barely distinguishable.
- Peptide ions were well distributed over the course of an LC/MS analysis, and the majority of peptide ions were identified in a narrow m/z range that shifts upwards during the analysis.
- Peptide intensity distributions were consistent across an LC/MS analysis, and independent of the sample source.
- Overall, the ion density around the majority of detected ions will commonly result in multiple ions being selected for "targeted" peptide MS/MS analysis, which will produce negative impacts for the identification and quantification of low and moderate intensity peptides.

METHODS

- Sample lysates were digested using a common methodology employing RapiGest™ SF to achieve efficient sample digestion.
- Digests (500 ng) were loaded on a 75 µm x 150 mm nanoACQUITY™ UPLC Atlantis® dC18 3.5 µ column, and resolved using a 90 min acetonitrile gradient (5 to 45% in 0.1% FA) on a Waters nanoACQUITY UPLC™ system.
- Mass spectra were acquired on a Waters Q-Tof Premier™ operated using the MS^E data acquisition methodology, where accurate mass data was collected for both intact peptides and the products of multiplexed peptide fragmentation in the same run.
- Work presented here will focus on the intact peptide MS data, which has been reduced by informatics tools to a set of apex retention times, accurate masses, and volumetric ion intensity measurements.
- Further details of this overall analysis methodology can be found in the following publications:
  - Chakraborty et al. RCMS. 2007;21(5):730-44.
  - Silva et al. Mol Cell Proteomics. 2006 Apr;5(4):589-607.
  - Hughes et al. J Proteome Res. 2006 Jan;5(1):54-63.
  - Silva et al. Mol Cell Proteomics. 2006 Jan;5(1):144-56.
  - Silva et al. Anal Chem. 2005 Apr 1;77(7):2187-200.

Integrated nanoscale system for LC/MS^E analysis:

- nanoACQUITY UPLC
- Waters Q-Tof Premier
- nanoACQUITY Atlantis column
- Controlled by MassLynx 4.1

MANY SAMPLES = SIMILAR RESULTS

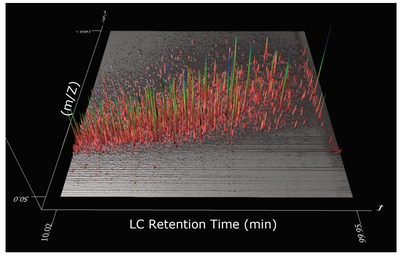


Figure 1: Typical LC/MS Profile of a Global Tryptic Digest (LC/MS Data from an E. coli digest analysis is shown)

	E. coli	Myc. Bovis	Yeast	HeLa Cell	Glioma	Top6 Serum
Ion Detections	98760	88168	119676	122183	121011	123353
Useful Detections # (%Total) (%Ion Intensity) [a]	45739 (46%) (89%)	40031 (45%) (91%)	52691 (44%) (85%)	46552 (38%) (85%)	43615 (36%) (83%)	43666 (35%) (79%)
Accurate Mass Retention Tags [b]	8808	8486	8615	8484	8794	6799
AMRT (Max Intensity)	814162	654288	824714	781530	894929	744040
AMRT (Min Intensity)	Threshold 1000	Threshold 1000	Threshold 1000	Threshold 1000	Threshold 1000	Threshold 1000
Effective Dynamic Range	814	654	825	782	895	744
AMRT in Mass Sufficient Regions # (%) [c]	8368 (95%)	7786 (92%)	7926 (92%)	7424 (88%)	8003 (91%)	6051 (89%)

Figure 2: When analyzed using standard instrument and sample preparation methods, similar results are generated for a variety of biological samples. On average, half of the 100,000 isotopic detections contribute to an AMRT (peptide detection), but these represent over 90% of total detected ion intensity during a run, and more than 90% fall into mass sufficient regions.

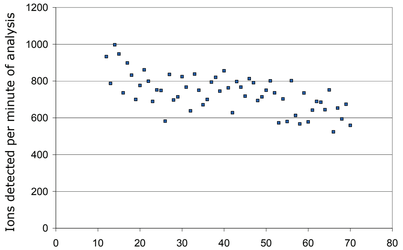


Figure 3: The flux of detected ions varies only slightly across an LC/MS experiment. Data for E. coli is shown (~720 ion detections/min, corresponding to ~ 140 detected peptides/min). Data is consistent for all samples studied.

TRYPTIC PEPTIDES DEMONSTRATE COMMON PROFILES ACROSS SAMPLES

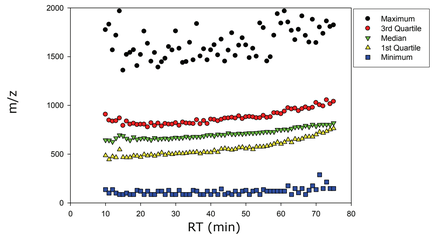


Figure 4: Data from E. coli shows that the median ion m/z increases linearly over the course of a chromatographic run. Half of ion detections (~360/min, those between 1st and 3rd quartiles) fall into a narrow m/z span only 300 - 400 m/z wide.

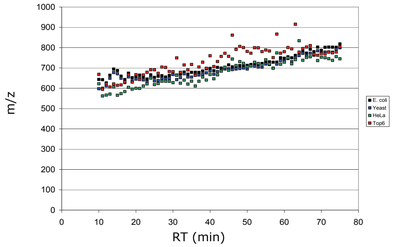


Figure 5: The median m/z changes similarly over a wide range of biological samples analyzed by LC/MS. Although the peptides are different, the resulting overall population of peptide ions is similar between samples.

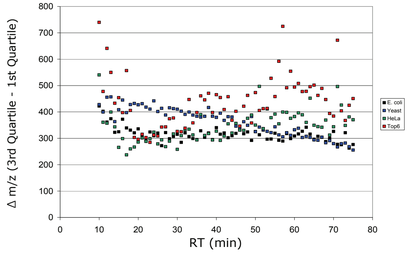


Figure 6: The range for this middle half (m/z Q3 - m/z Q1) of useful ions typically varies between 300 and 400 m/z (365 ±30 m/z) during any minute of the analysis. So, not only is the number of ions consistent across a LC/MS run (Fig 3), but the density of ions is similar over the course of an analysis.

TRYPTIC PEPTIDE INTENSITY PROFILES ARE COMMON ACROSS SAMPLES

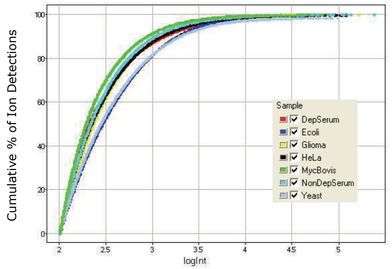
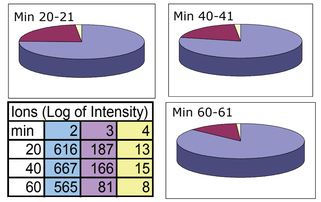


Figure 7: The intensity profile of ions is similar for a variety of biological samples. Independent of sample, we observe the most intense peptide ion is on the order of ~800,000 counts, less than 5% of ions are in the top intensity log, ~15% in the middle intensity log, and ~80% of ions are in the third log of intensity.



Ions (Log of Intensity)			
min	2	3	4
20	616	187	13
40	667	166	15
60	565	81	8

Figure 8: This ion intensity distribution pattern is consistent across an LC/MS analysis, as shown here for results from an E. coli LC/MS analysis.

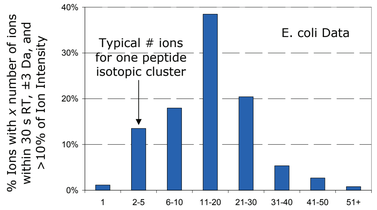
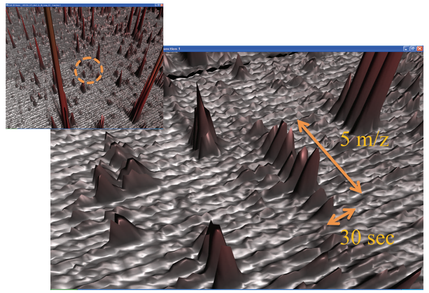


Figure 9: The majority of ions, most with low intensity (Figures 7 & 8) are identified within a narrow and shifting m/z band (Figures 4 - 6). When selecting peptide ions for MS/MS analysis, multiple peptide ions are often transferred to the collision cell. For intense ions, this has a negligible effect on peptide identification, but as peptide ion intensity decreases, the likelihood of a significantly interfering ions (>10% of intensity) within the MS/MS transmission window significantly increases.

VISUALIZING THE ION DENSITY ISSUE FOR LOWER INTENSITY IONS



CONCLUSIONS

- A variety of biological samples were analyzed using a standardized sample preparation and LC/MS^E methodology.
- The resulting data show that, while these samples generated vastly differing sets of tryptic peptides, the analytical behavior of these complex peptide mixtures is barely distinguishable.
  - The loading limitations of capillary LC, and practical dynamic range of mass detectors result in comparable numbers of detectable ions, isotopic distributions, and peptides across diverse sample classes.
  - The concept of a "universal" proteomic analysis methodology for global digests should be a clearly achievable goal.
  - Peptide ions appear evenly distributed over the heart of an LC/MS analysis.
  - A majority of peptide ions were found in a narrow m/z band that shifted upwards over the course of an LC/MS analysis.
  - Peptide intensity distributions are consistent across an LC/MS analysis, and appear independent of the sample source.
- Overall, ion density surrounding the majority of detected ions will commonly result in multiple peptide ions being selected for "targeted" MS/MS analysis.
  - This results in generation of "Chimera" MS/MS spectra that can raise both false+ and false- peptide identification rates.
  - Improved chromatographic separations can reduce but not eliminate such events.
  - These negative effects will increase as the peptide intensity decreases, and methods such as data-dependent analysis (DDA) and fragmentation-based labeling schemes e.g. iTRAQ should become noticeably less reliable with decreasing peptide intensity.
  - The use of alternative data acquisition strategies such as LC/MS^E analysis, and multiplex fragmentation friendly search algorithms should permit deeper analysis of complex peptide mixtures.

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