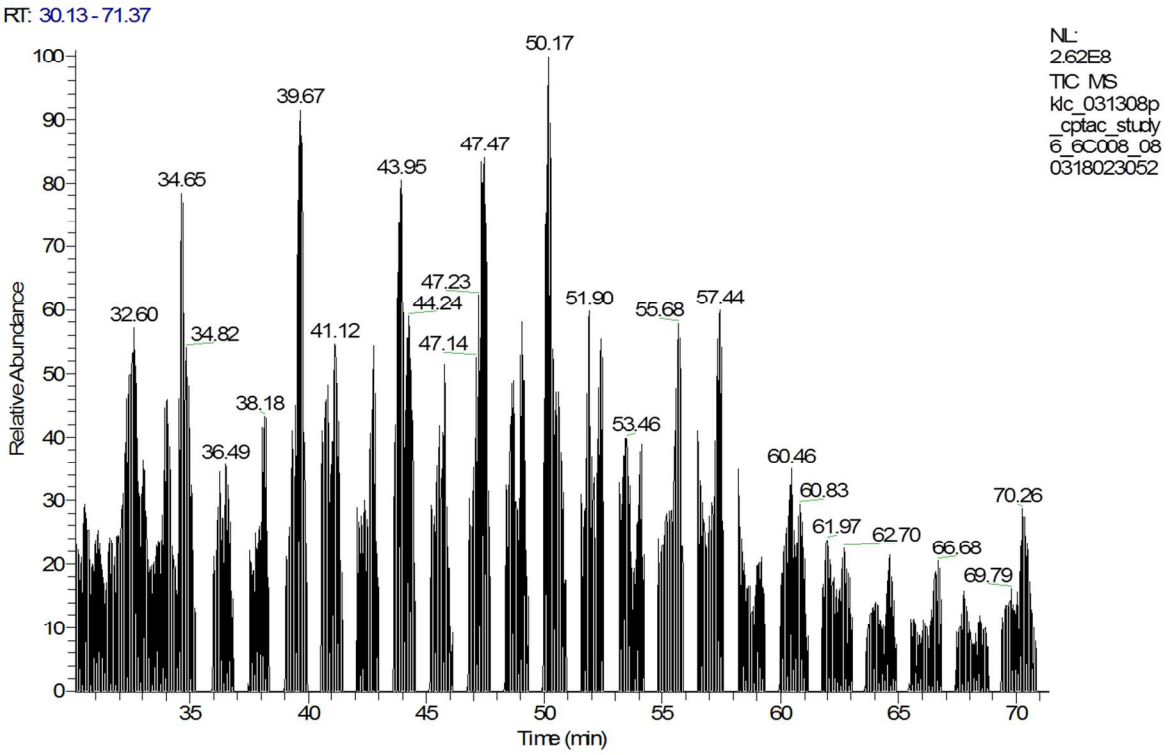
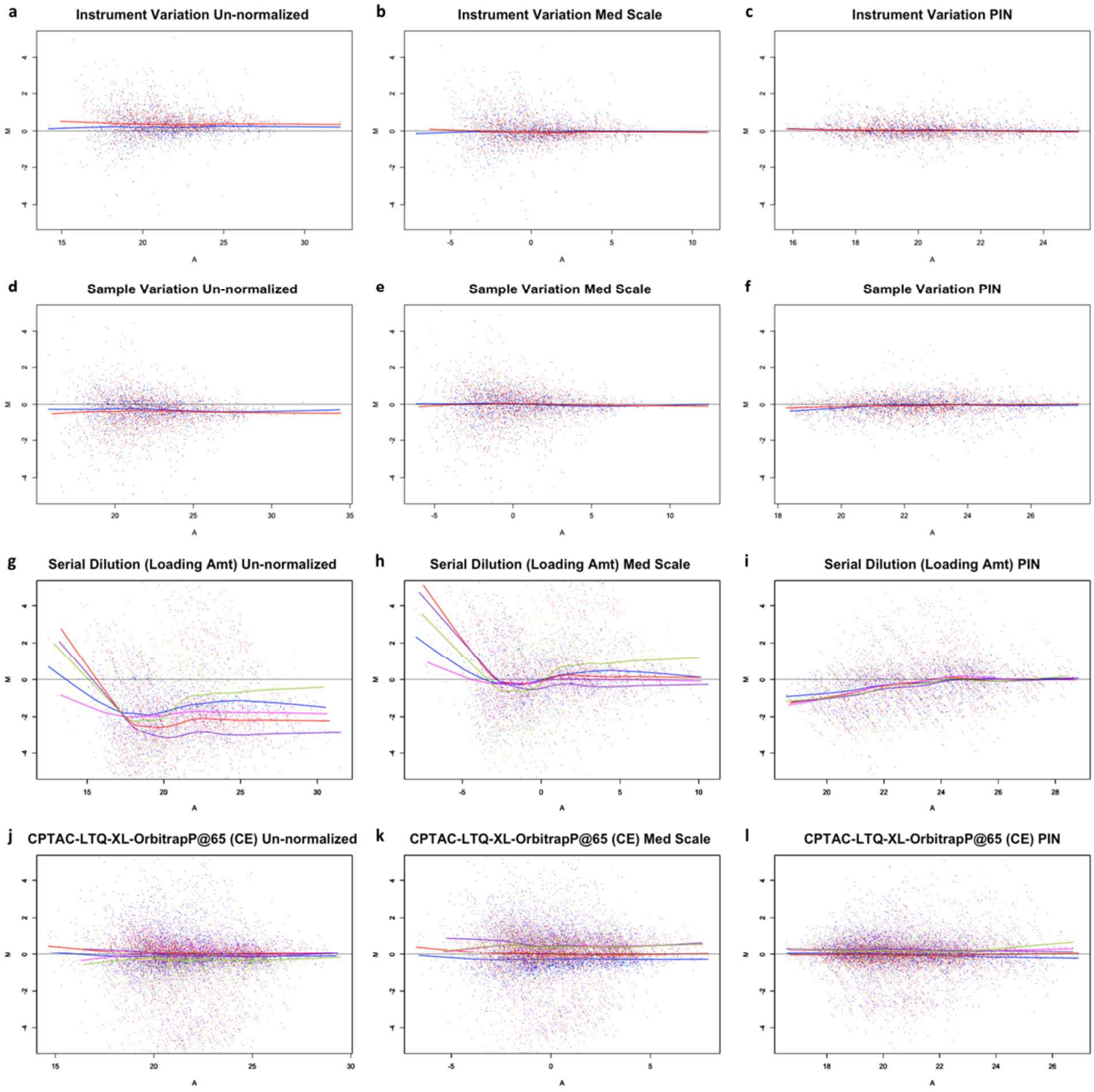
Supporting Information: This material is available free of charge via the Internet at http://pubs.acs.org.



Supplementary Figure 1: Portion of the CPTAC Study 6C experiment's chromatogram. The distinctive saw tooth pattern is indicative of electrospray ionization inefficiency.

Supplementary Note 1- Minus vs. Average Reveals Systematic Bias

Proteomics researchers employ minus vs. average (MA) plots to visually detect systematic bias. They were originally designed to analyze systematic bias R/G channels in microarray channels[2] but later adapted to analyze systematic bias and its correction in quantitative proteomics[3, 4] (Online Methods). MA plots are essentially a ratio versus intensity ordinate system rescaled and rotated 45 degrees to allow easier observations of both linear and non-linear trends[3]. In Figure 3, locally weighted regression lines are added to the ordinate systems, deviations from M=0 indicate systematic bias.



Supplementary Figure 2: Minus vs. Average plots for four experiments: Instrument Variability, Sample Variability, Serial Dilution, and CPTAC Study 6 C vs. E.

Each row contains three MA scatter plots with locally weighted regression lines (solid lines) for an experiment. For each experiment, the first MA plot is the un-normalized data, the second MA plot is the data normalized by median scale, and the third scatter plot is the data normalized by

PIN. (a-c) MA plots for Instrument Variability experiments. (d-f) MA plots for sample variability experiments. (g-i) MA plots for Serial Dilution experiments (j-l) MA plots for CPTAC C vs. E experiments.

Supplementary Note 2- Variables and Equations Used in Analysis

Description	*Variable / Equation*	*Eq. #*
Peptide signal index	i	
Run index	j	
Predicted peptide ratio	m_i^*	
Total # of runs (replicates)	n	
Total # of peptide signals in run	p	
Measured peptide intensity	x_i	
Normalized peptide intensity	x_i'	
Average (A in MA transformation)	$a_i = (\log_2 x_{ij=1} - \log_2 x_{ij=2})/2$	Eq. 1
Minus (M in MA transformation)	$m_i = \log_2 x_{ij=1} - \log_2 x_{ij=2}$	Eq. 2
MA convoluted peptide intensity	$m_i' = m_i - m_i^*$	Eq. 3
MA de-convolution	$x'_{ji=1} = 2^{(m_i'+2a_i)/2}$, $x'_{ji=2} = 2^{(m_i'-2a_i)/2}$	Eq. 4
Inter-run Mean	$\mu_i = \frac{\sum_{j=1}^{n} x_{ji}}{n}$	Eq. 5
Intra-run Mean	$\mu_j = \frac{\sum_{i=1}^{p} x_{ji}}{p}$	Eq. 6
Inter-run Variance	$\sigma_i^2 = \frac{\sum_{j=1}^{n} x_{ji}^2}{n} - \left(\frac{\sum_{j=1}^{n} x_{ji}}{n}\right)^2$	Eq. 7
Intra-run Variance	$\sigma_j^2 = \frac{\sum_{i=1}^{p} x_{ji}^2}{p} - \left(\frac{\sum_{i=1}^{p} x_{ji}}{p}\right)^2$	Eq. 8
Standard Deviation	$\sigma = \sqrt{\sigma^2}$	Eq. 9
Pooled Estimate of Variance	$PEV = \frac{(n_1 - 1)\sigma_{m_1}^2 - (n_2 - 1)\sigma_{m_2}^2}{(n_1 - 1) + (n_2 - 1)}$ ...	Eq. 10
Coefficient of Variation	$CV = \frac{\sigma}{\mu}$	Eq. 11

REFERENCES

1. Bellew, M. et al. A suite of algorithms for the comprehensive analysis of complex protein mixtures using high-resolution LC-MS. *Bioinformatics* **22**, 1902-1909 (2006).
2. Dudoit, S., Yang, Y.H., Callow, M.J. & Speed, T.P. Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. *Statistica Sinica* **12**, 111-139 (2002).
3. Callister, S. et al. Normalization approaches for removing systematic biases associated with mass spectrometry and label-free proteomics. *Journal of Proteome Research* **5**, 277-286 (2006).
4. Kultima, K. et al. Development and Evaluation of Normalization Methods for Label-free Relative Quantification of Endogenous Peptides. *Mol Cell Proteomics* **8**, 2285-2295 (2009).