ASMS 2017 Workshop Report

Analytical Lab Managers' Interest Group

Organizers: Emily Chen, Allis S. Chien

1. Title of workshop: ***Proteomic Workflow Insider Info: Unpublished Collective Practical Knowledge***

   Speakers/Discussion moderators:
   - Emily Chen, PhD, Columbia University
   - Ben Orsburn, PhD, Thermo Fisher Scientific
   - Rosa Viner, PhD, Thermo Fisher Scientific
   - Dave Tabb, PhD, Stellenbosch University

2. Date of workshop/meeting
   Tuesday June 6, 2017
   5:45-7 pm, Room 142

3. Estimate of attendance: 100

4. Summary of program and discussion

There is no one-size-fits-all solution to the diversity of proteomic analyses that our labs handle every day. At the same time, we have to make sensible decisions on what workflows to use, balancing scientific goals and available resources. The wisdom gained through these experiences is crucial to daily operations and consistent high-quality results, yet typically goes unpublished. This unpublished knowledge can help all of us make better decisions when performing proteomic analyses. The 2017 ASMS Analytical Lab Managers' Workshop was dedicated to sharing these nuggets of practical wisdom.

Four segments of proteomic workflows were covered – sample preparation, liquid chromatography, mass spectrometry, and data analysis; slides are appended to this report.

- Sample preparation: Emily Chen (Columbia University Medical Center) introduced a decision tree of workflows and sample prep conditions for a variety of biological samples.
- Liquid chromatography: Ben Orsburn (Thermo, http://proteomicsnews.blogspot.com) examined nanoLC for proteomics, along with appropriate QCs and distinguishing between issues with the MS and LC.
- Mass spectrometry: Rosa Viner (Thermo) discussed practical considerations of TMT-based quantification.

- Data analysis: David Tabb (Stellenbosch University, https://pickingupthetabb.wordpress.com) offered important pointers on proteomic study design and bioinformatics analysis.

The panelists' expertise and passion for their topics were self-evident, and all presented practical details and recommendations that can be immediately implemented. The 48 requests for the workshop slides attest to the usefulness of the information.

Great questions from attendees. Discussion could have continued for some time past the official end of the workshop. Questions from the app Q&A and session comments section which were not answered during the session were answered in the app afterward.

Logistical notes:

- The room was too small this year! Turnout was larger (100) than initially anticipated (60).
- For the panel discussion, tall chairs (such as the ones the Young Mass Spectrometrists' group had in room 240-242) for the panelists would have been welcome – remember to ask for these next year if needed.
- In addition to Q&A, the meeting app came in extremely handy to collect the flood of slide requests.
- Recording the workshop for would have been even more valuable. In the bigger picture, with 15 simultaneous workshops each evening and meeting attendees having to make difficult choices as to which workshop to attend, the option to make workshop recordings available after the meeting would be valuable to many ASMS members.

ASMS Analytical Lab Managers Interest Group

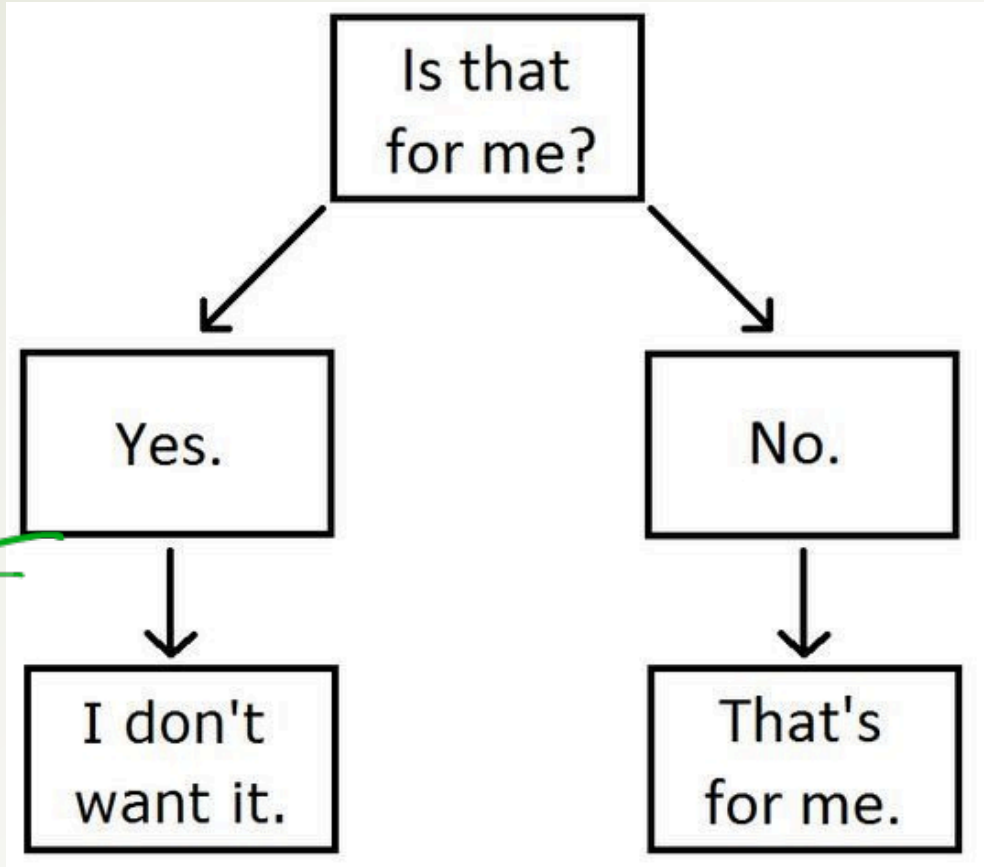
Proteomics Workflow

Practical Knowledge From Insiders

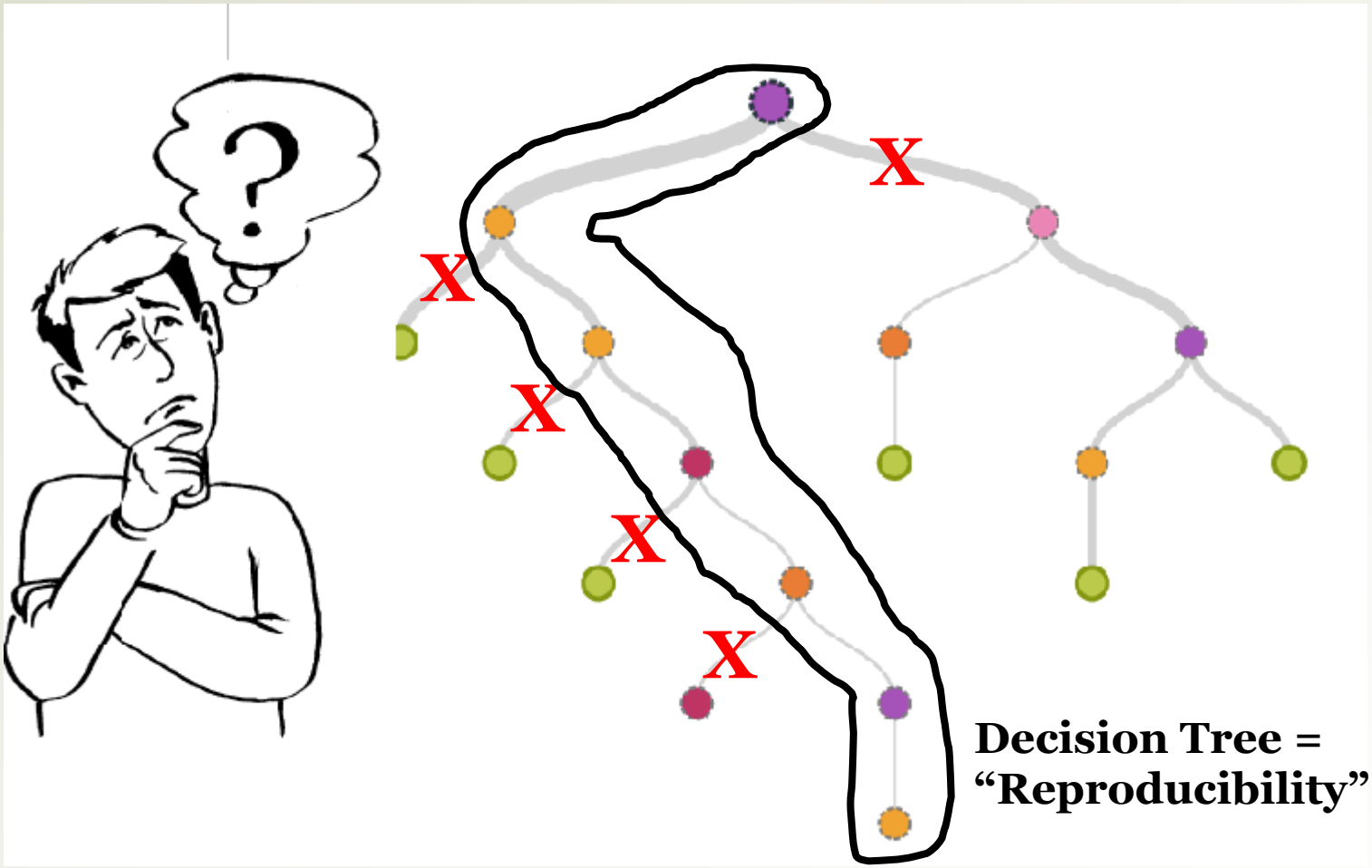
Presenter: Emily I. Chen, Ph.D

Director of HICCC Proteomics Shared Resource, Columbia University Medical Center

We Make Decisions Everyday



Negative Or Unpublished Findings Are Valuable!



Sample-Independent MS Workflow

Simple Protein Identification Workflow

Emily I. Chen
CUMC
Ben Orsburn
Thermo
Rosa Viner
David Tabb
Stellenbosch U.

Sample Preparation

Mass Spectrometry

Data Analysis

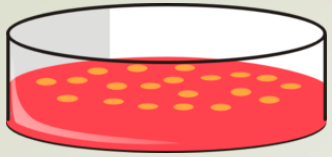
Sample Preparation For MS Workflow

1st Q : What type of samples?

2nd Q: Maximize either "total amount" of proteins or "subset" of proteins?

3rd Q: Label-free or labeled approach for MS quantification?

Sample Preparation For MS Workflow (Decision Tree)



Conditioned Media

(>70% confluency
> 48 hours conditioned)

→ **Collect with protease inhibitor cocktail + Concentrate**

Frozen Cell Pellets

(Accurate Cell Count!)

→ **PTM & total lysate** → 8M or 4M Urea

Qubit Assay

Expected yield ~ 240ug/million cells

→ **Subgroup (e.g. MP)** → Detergent-based lysis buffer

Enhanced BCA

→ SDS PAGE

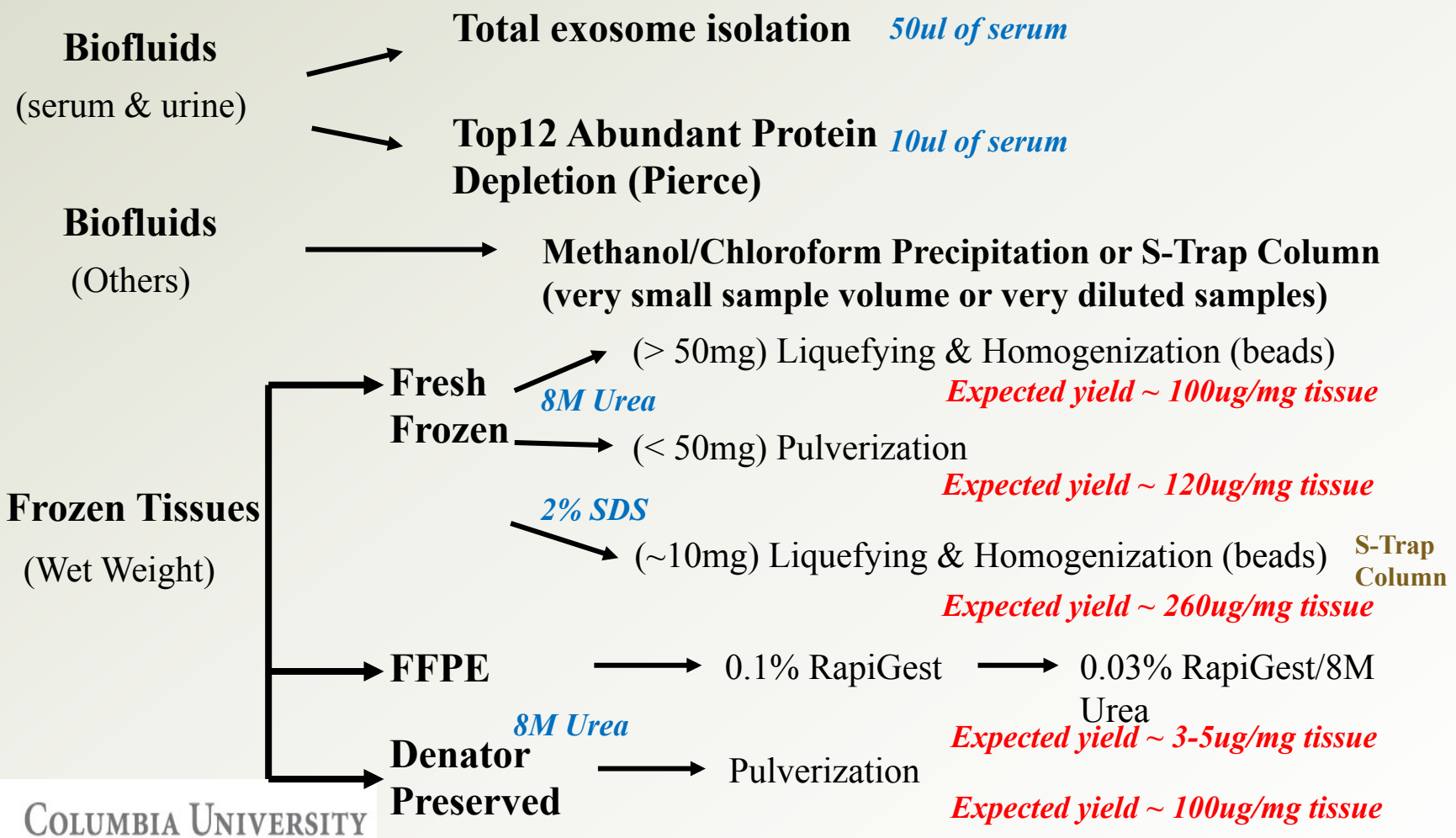
→ **Samples prepared from Users** → Detergent-based lysis buffer

Expected yield ~20% to 40% more than 8M urea

→ S-Trap Column (http://www.protifi.com/s-trap/)

- *Get rid of detergents*
- *On column proteolysis*

Sample Preparation For MS Workflow (Decision Tree)



How Do We Share The Practical Knowledge?

Professional networks/Societies

- ASMS
- HUPO
- ABRF

  Core director network
  Reproducibility Initiative
  SOP Initiative

The **devil** is in the **details!**

LIQUID CHROMATOGRAPHY FOR PROTEOMICS

Ben Orsburn, Ph.D.

NANOLC –ESSENTIAL, BU
CHALLENGING

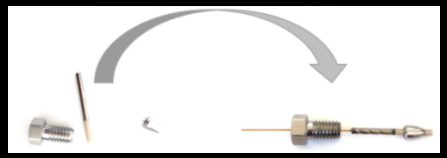
- Nanoflow ESI has been used for at least 20 years
- Despite this time and thousands of studies, it is still a challenging procedure
- The joke “it’s always the LC” may be around for a while

NANOLC IMPROVEMENT

One major weakness is the creation of low dead volume connections

Zero dead volume unions

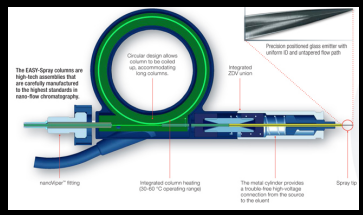
- NanoViper™
- ZircoFrit™
- ZenFit™



Integrated columns and emitters

- PicoFrit

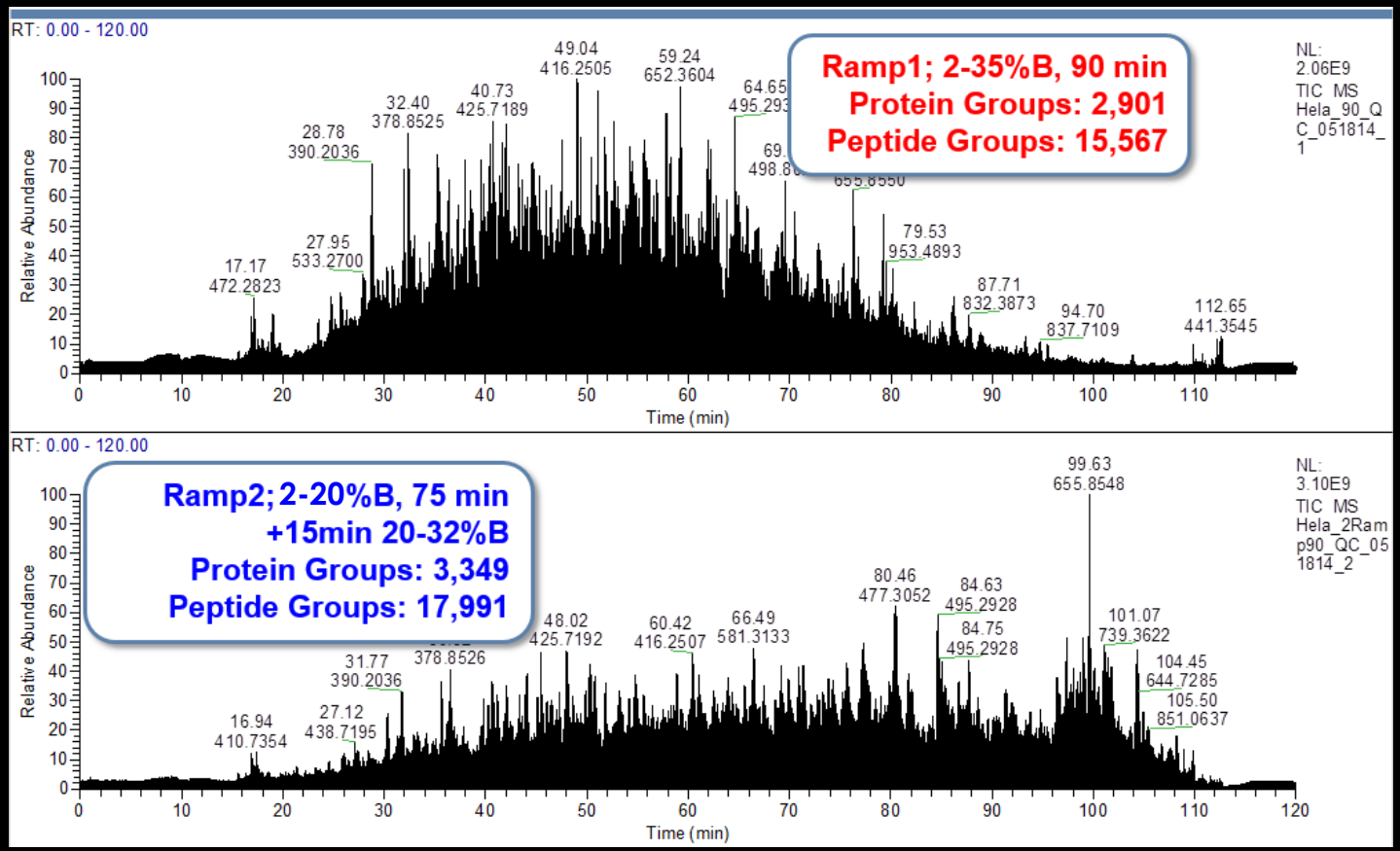
- And other vendors have followed



All these have improved peak shape, reproducibility, and peptides identified

EVOLVING C1

New C-18 material can improve separation, if used correctly



BETTER INSTRUMENTS REQUIR
BETTER QC/Q

BSA and other single protein digests are still in use in many labs for checking system health and performance.

Peak shape, intensity, and chromatographic reproducibility can be tested with this metric.

MAbs. 2015 Nov-Dec; 7(6): 1104–1117. PMCID: PMC4966392
Published online 2015 Jul 28. doi: 10.1080/19420862.2015.1074364

Performance metrics for evaluating system suitability in liquid chromatography—Mass spectrometry peptide mass mapping of protein therapeutics and monoclonal antibodies

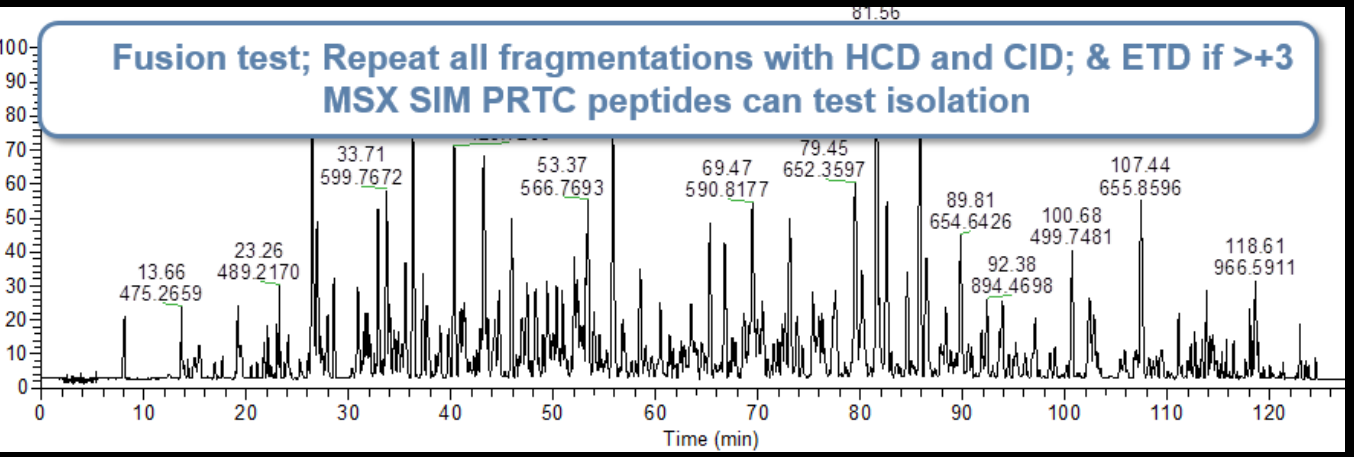
Mowei Zhou,† Ashley C Gucinski,* and Michael T Boyne, II‡

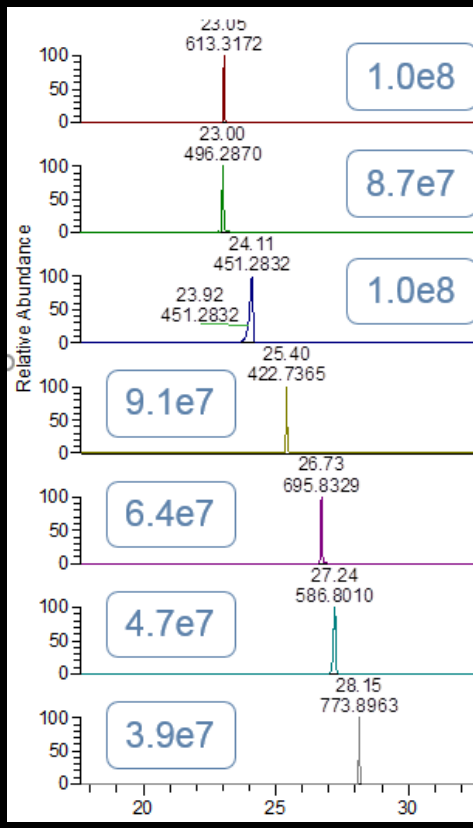
A deliberately handicapped Q Exactive Classic can still detect every BSA peptide.

use low level PRTC peptide mix spiked into HeLa lysate

HELA QC METRIC EXAMPL

Many metrics can be tested in one run





Number of IDs is less important than equipment baseline

File from Shan Randell/image Tara Schroeder

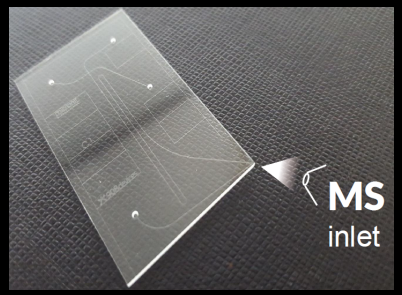
DO WE STILL NEED IT

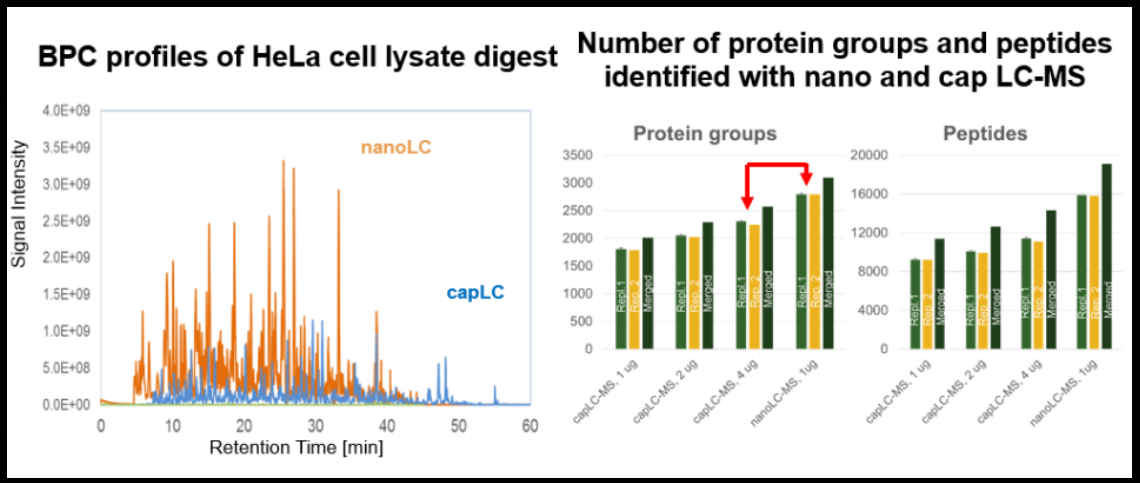
After all these mass spec improvements, do we still need nanoLC?

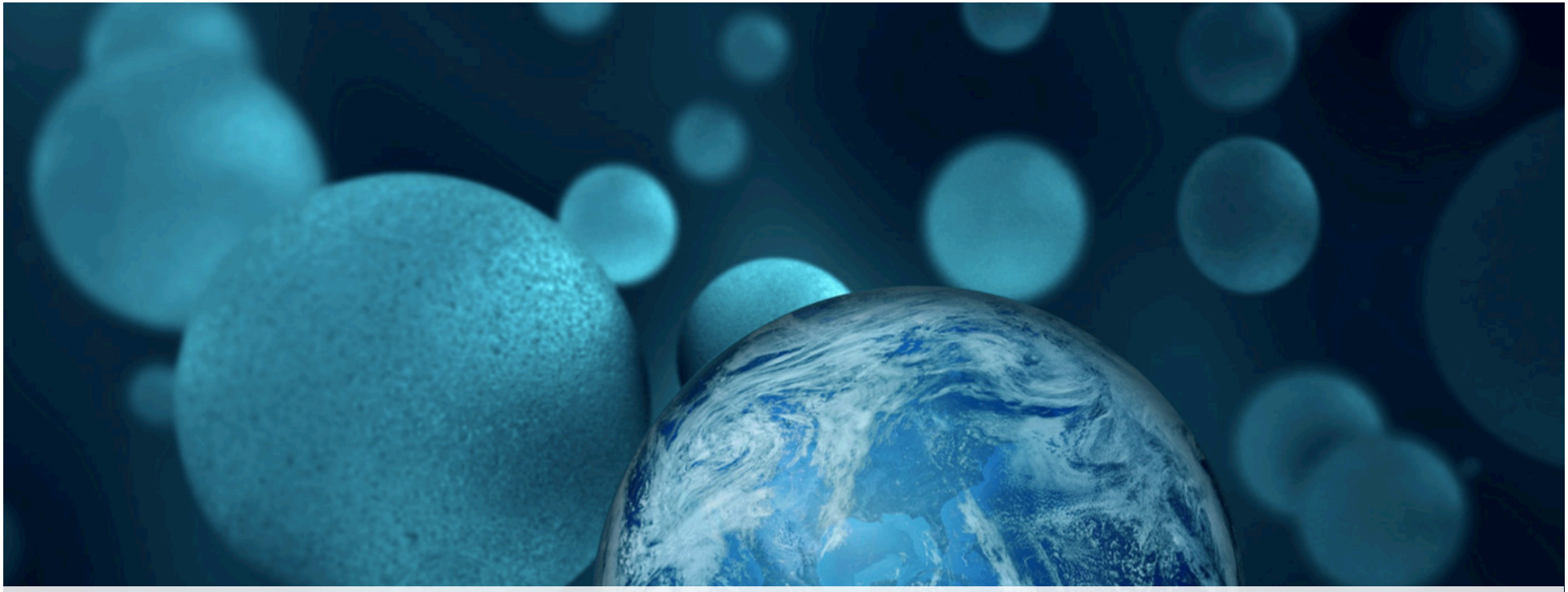
Answer – definitely for the highest complexity and most precious samples

Alternatives?

- MicroCapillary electrophoresis (ZipChip)
  - See 3 posters from Bob Cole Lab; JHU
- Capillary flow (80% nanoIDs with less hassle)







ThermoFisher
SCIENTIFIC

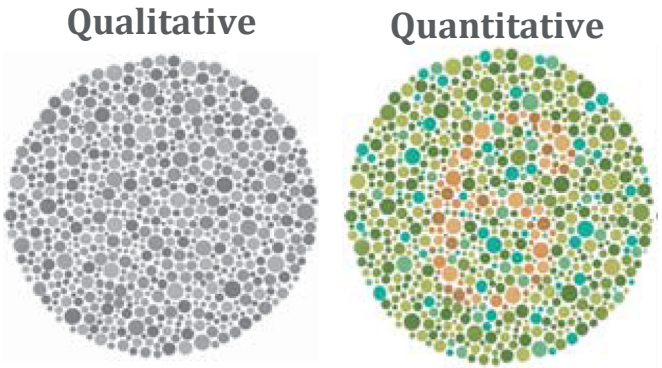
Practical considerations of TMT-based quantification: Tips and tricks on how to quantify global proteome changes using TMT-labeling approach

Rosa Viner

June 5 2017

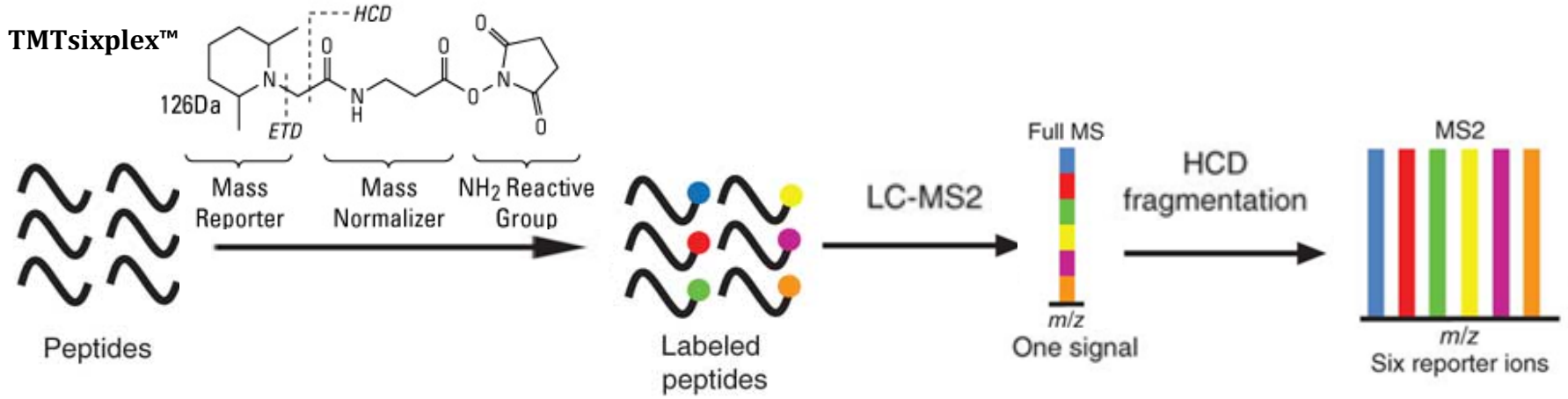
Moving Beyond Qualitative Proteomics

Problem: Quantitative information about expression level of a protein is essential to understanding its biological role in response to change or disease.



Add another dimension to any experiment by determining the relative abundance of each identified protein

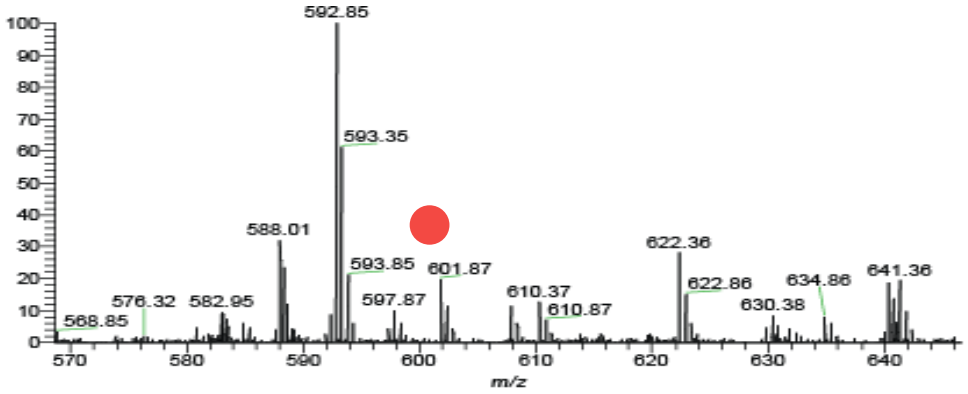
Isobaric Labeling/Tandem Mass Tags™ (TMT)*



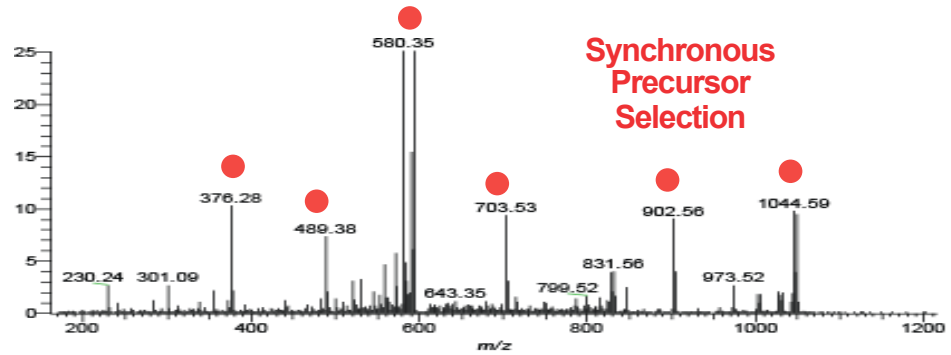
* Ting, L. et al. 2011. Nature Methods 8: 937-940

Synchronous Precursor Selection (SPS) for Accurate Quantification

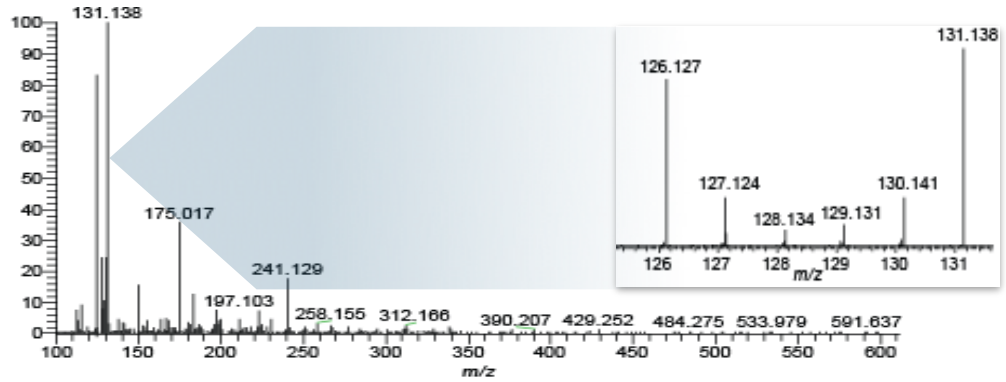
Precursor Ion



CID MS^2, Ion Trap



HCD MS^3, Orbitrap



- Available on the Thermo Scientific™ Orbitrap Fusion™ MS and Thermo Scientific™ Orbitrap Fusion™ Lumos ™ MS
- Select multiple MS^2 precursors using a single fill and notched isolation waveform
- Improves the ratio accuracy and at the same time dramatically boosts sensitivity

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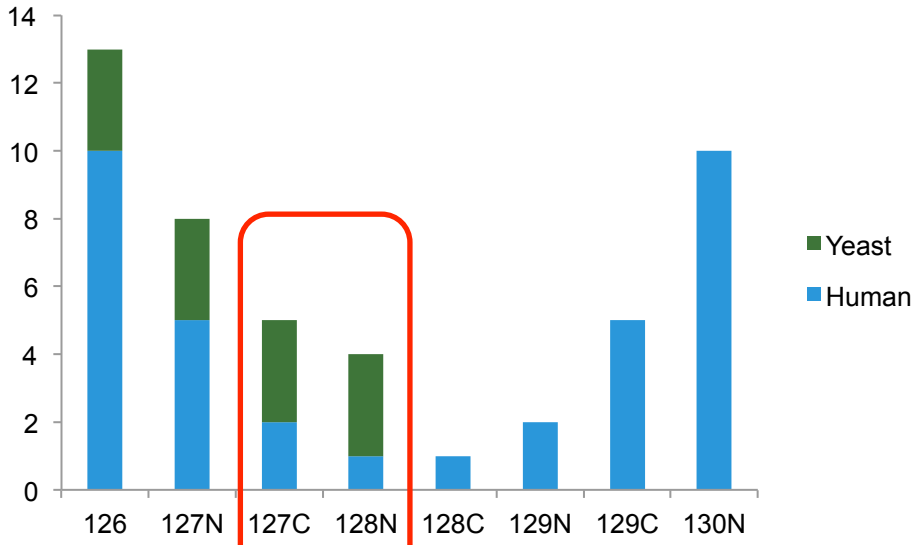
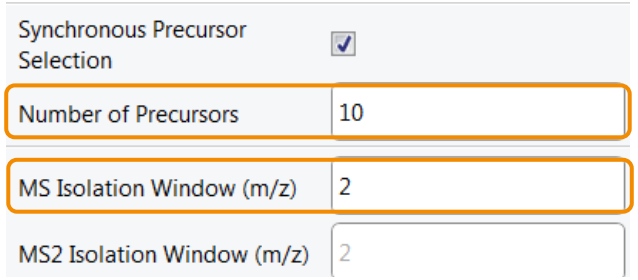
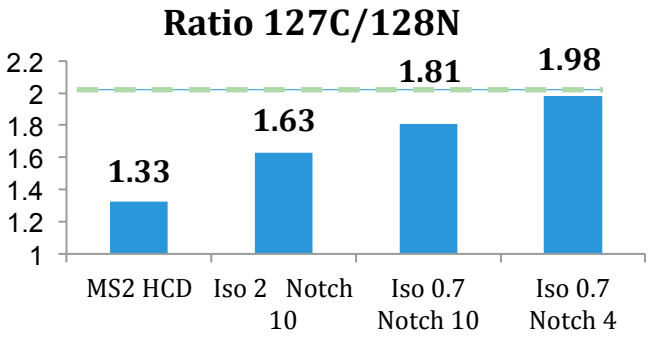
Multinotch MS^3 quantification is accurate and sensitive

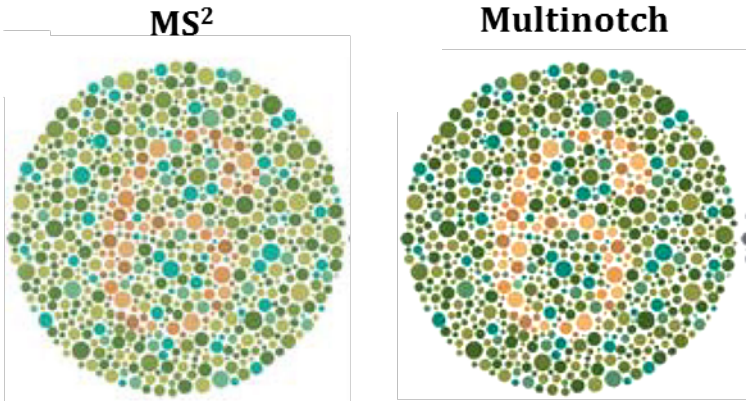


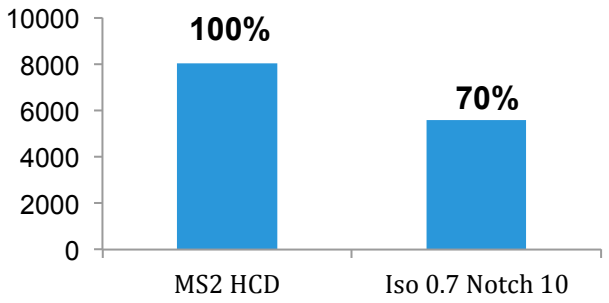
Human 10 : 5 : 2 : 1 : 1 : 2 : 5 : 10

Yeast 1 : 1 : 1 : 1









* TMT MS^3 Peptides-Quan template in Method Editor

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Relative Protein Quantitation Experiment Requirements

- Reproducibility and accuracy
- Sensitivity

How can we achieve this?

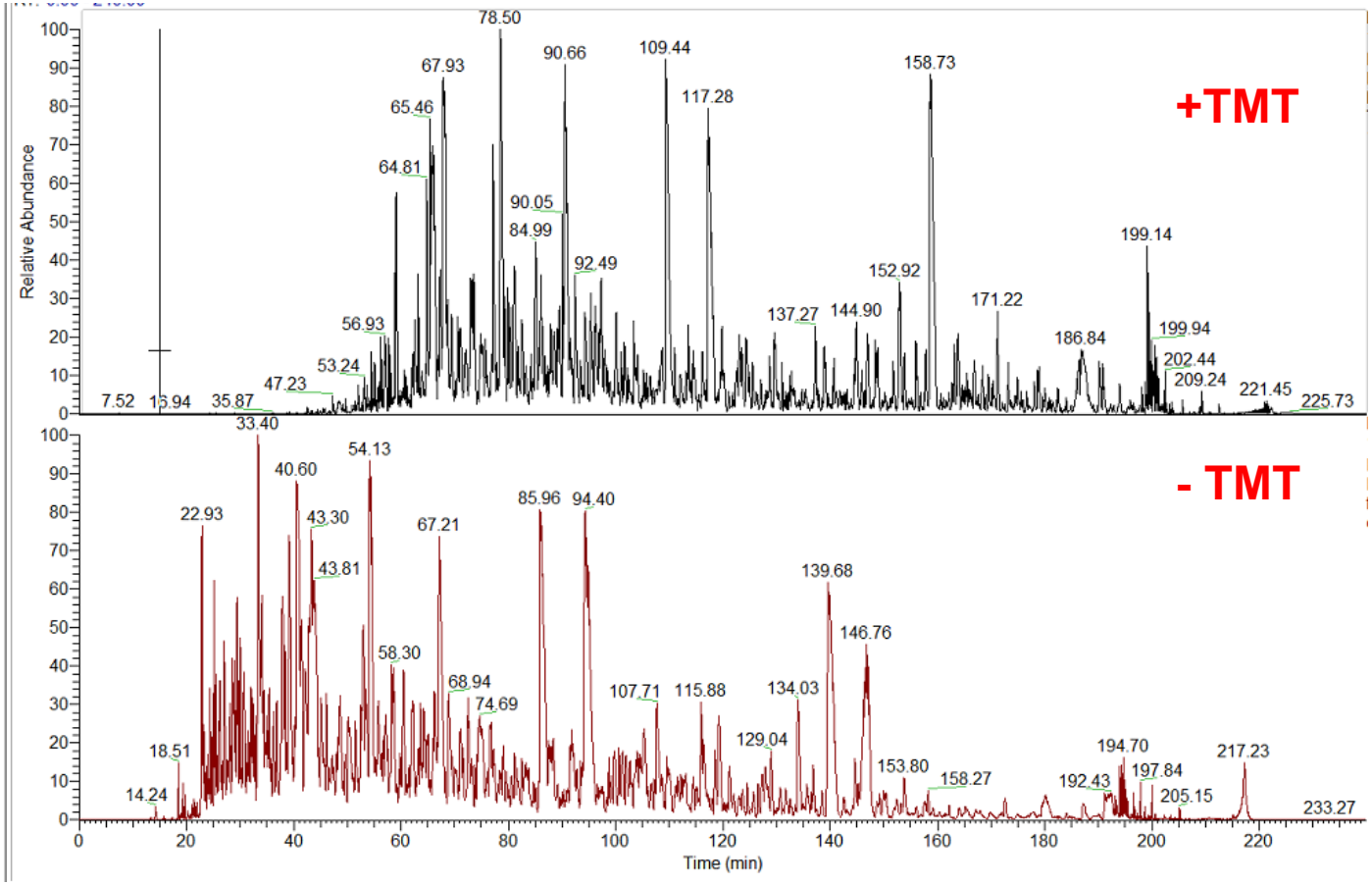
How can we achieve this?

- Prepare your sample in LC/MS friendly way- RIPA buffer,3%SDS,8M Urea or excess of TMT are not mass spec friendly clean up required
- Calibrate your instrument as recommended by manufacturer:

- Optimize LC separation
- Use recommended instrument methods- start from templates if possible
- Perform data analysis correctly
- Check performance regularly using QC tools

Optimize LC separation

- LC gradient for unlabeled and labeled samples need to be different
- Try not to overload the column to maintain quan.reproducibility
- Perform sample clean up/use trap to preserve column performance



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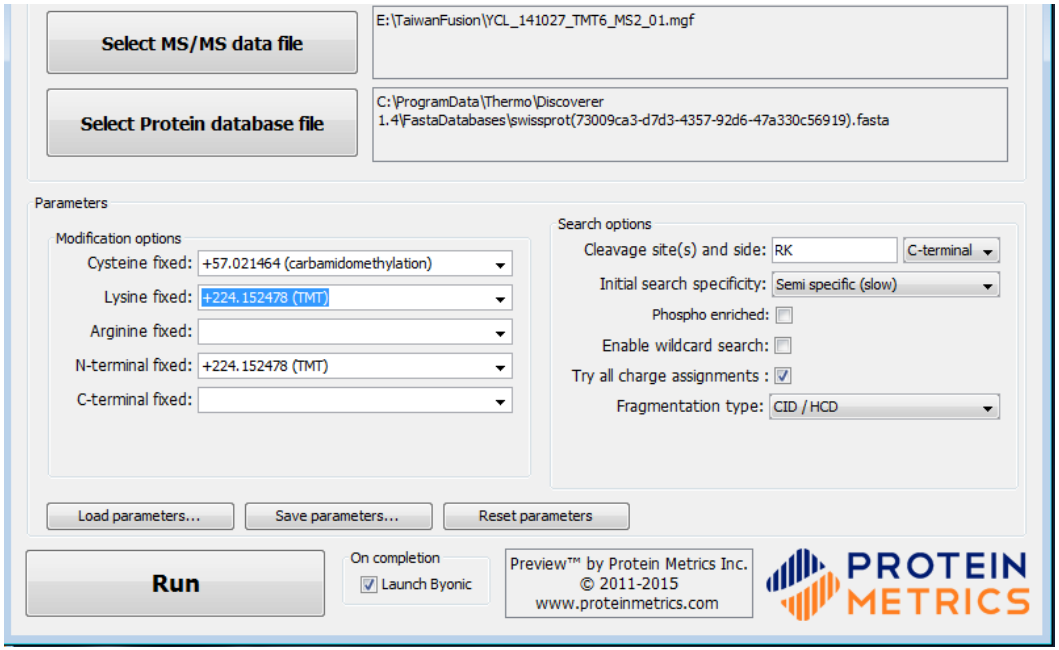
Check performance regularly using QC tools in Proteome Discoverer

- Mass accuracy
- MS2/MS3 injection time
- Average and single tag S/N
- LC profile
- Sample quality – Byonic Preview node from Protein Metrics



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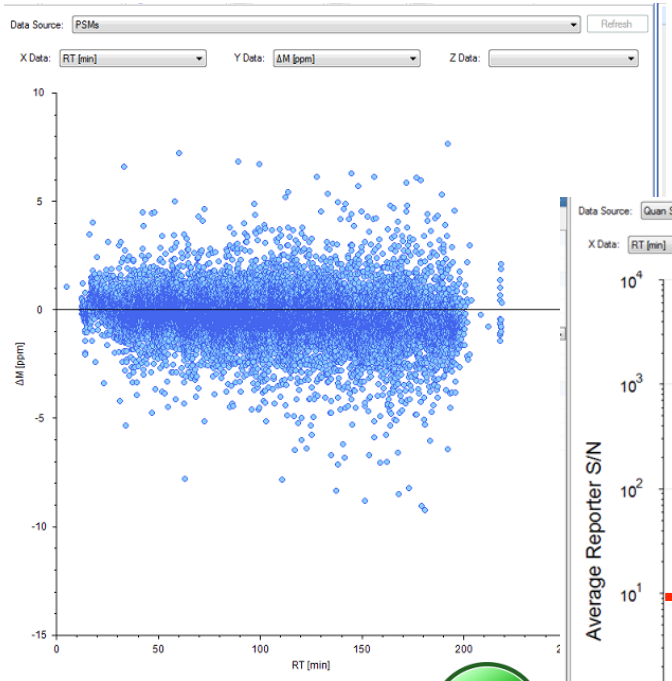
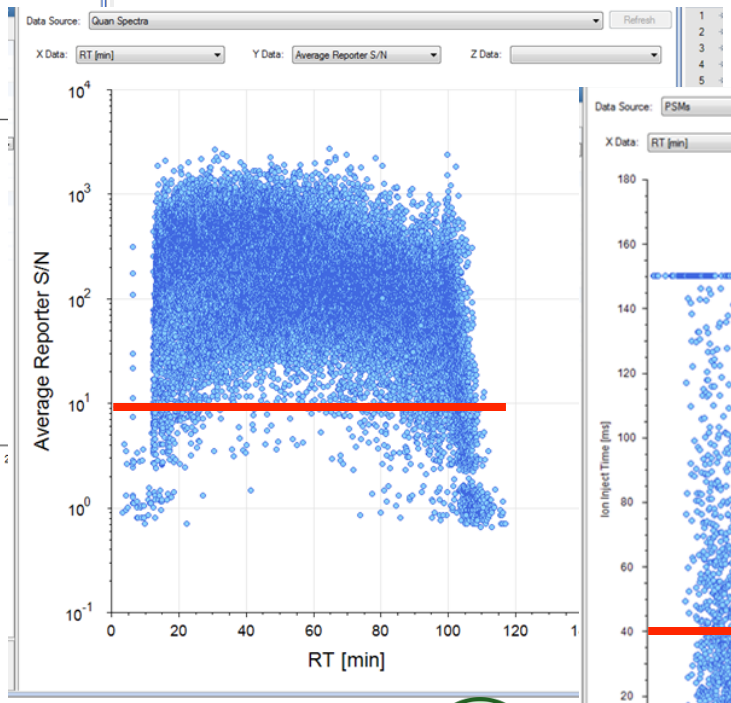
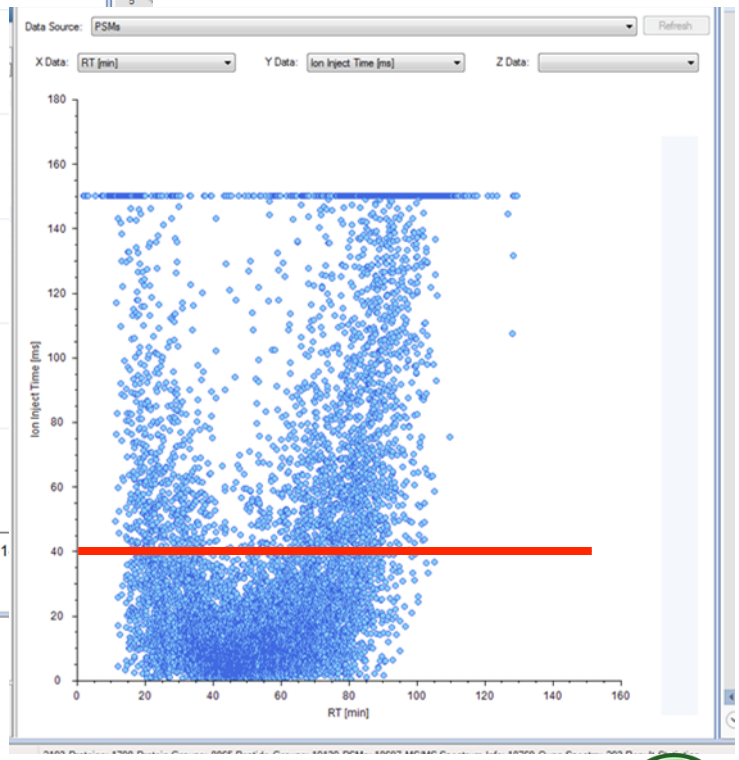
Check performance regularly using QC tools in Proteome Discoverer



Mass accuracy
LC separation



Average S/N> 10



Average injection time< 40 msec

100 ng TMT0 HeLa load

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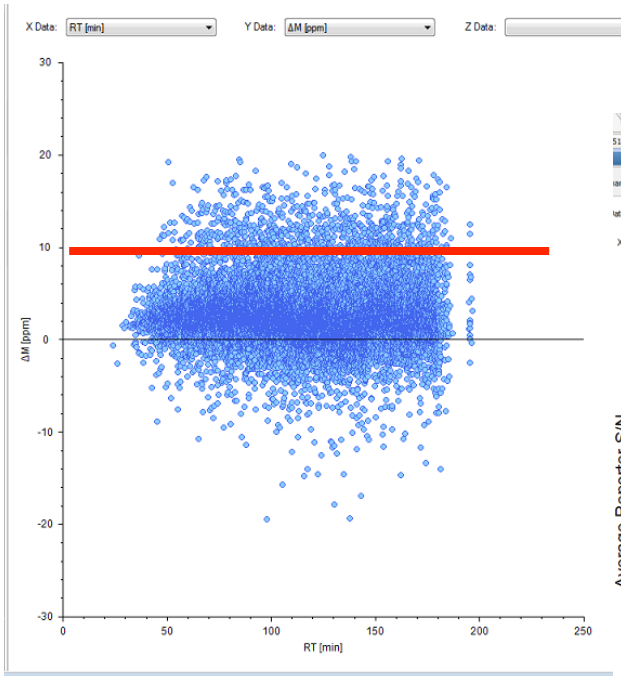
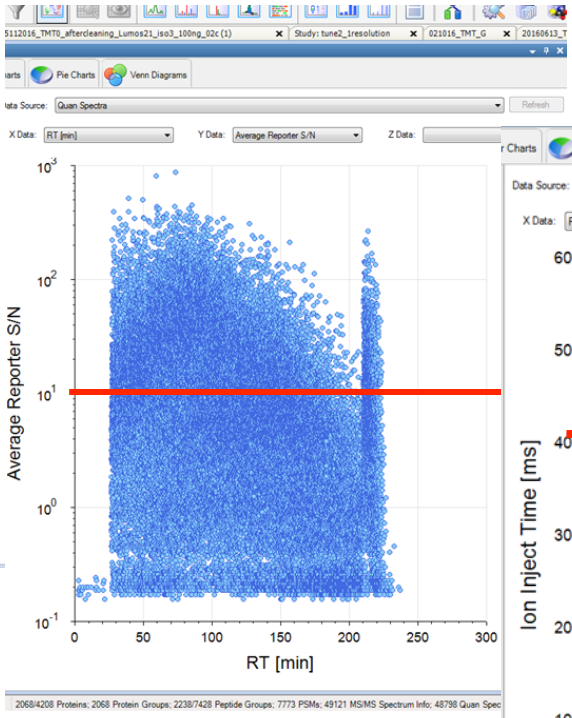
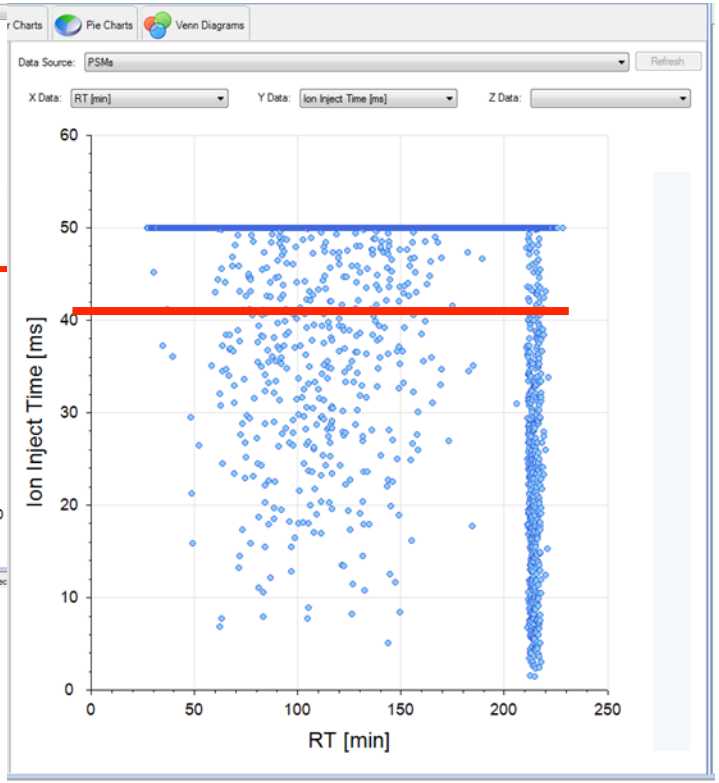
Check performance regularly using QC tools in Proteome Discoverer







Mass accuracy X
LC separation

Average S/N< 10 X

500 ng TMT0 HeLa load

Average injection time> 40 msec X

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Coming Soon from Pierce- TMT QC standard for the whole workflow

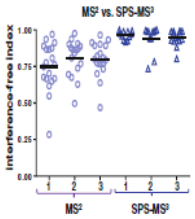
J. Am. Soc. Mass Spectrom. (2016)
DOI: 10.1007/s13361-016-1434-9

RESEARCH ARTICLE

A Triple Knockout (TKO) Proteomics Standard for Diagnosing Ion Interference in Isobaric Labeling Experiments

Joao A. Paulo, Jeremy D. O'Connell, Steven P. Gygi

Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA



Abstract. Isobaric labeling is a powerful strategy for quantitative mass spectrometry-based proteomic investigations. A complication of such analyses has been the co-isolation of multiple analytes of similar mass-to-charge resulting in the distortion of relative protein abundance measurements across samples. When properly implemented, synchronous precursor selection and triple-stage mass spectrometry (SPS-MS3) can reduce the occurrence of this phenomenon, referred to as ion interference. However, no diagnostic tool is available currently to rapidly and accurately assess ion interference. To address this need, we developed a multiplexed tandem mass tag (TMT)-based standard, termed the triple knockout (TKO). This standard is comprised of three yeast proteomes in triplicate, each from a strain deficient in a highly abundant protein (Met6, Pfk2, or Ura2). The relative abundance patterns of these proteins, which can be inferred from dozens of peptide measurements can demonstrate ion interference in peptide quantification. We expect no signal in channels where the protein is knocked out, permitting maximum sensitivity for measurements of ion interference against a null background. Here, we emphasize the need to investigate further ion interference-generated ratio distortion and promote the TKO standard as a tool to investigate such issues.

Keywords: MS standard, MuiltiNotch, TMT, Orbitrap Fusion, Lumos, Ion interference, SPS-MS3

Received: 12 April 2016/Revised: 30 May 2016/Accepted: 31 May 2016

Paulo J. & Gygi S. ASMS 2017 MP 462

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Bioinformatics

DAVID L. TABB, PH.D.
STELLENBOSCH UNIVERSITY
CAPE TOWN, SOUTH AFRICA

Two thoughts on study design

- Liebler Rule: “Five is the new three.”
  - More replicates yield more discrimination!

- Tabb Dictum: “If you want a quantitative result, run a quantitative experiment.”
  - SRM/PRM yields a much lower CV than does shotgunning.

Look further than SWISS-PROT

- UniProt Reference Proteomes include TREMBL proteins; so should you!
- Proteogenomics is better with a genome-derived database (like RefSeq / Ensembl).
- Dept. of Energy Joint Genome Institute is an underused resource for microbiomes.

Is new software the answer?

- Experience in an “old” search algorithm may outperform inexperience with a “new” one.
- Multiple search and spectral libraries are most valuable with low-resolution MS/MS.
- Exotic search methods tend to recover more spectra *from proteins you have already seen*.
- PTM discovery via blind search may be the most underused technique needed by cores.

My pet peeve

- Published numbers of proteins are generally incredible; too many factors impact counts.
- Reporting numbers of distinct peptides identified from experiments is more reliable.
- Reporting the fraction of identified spectra can be useful in evaluating information yield.

See also http://www.psidev.info/quality-control