Quantifying cancer related proteins via targeting MS assay resources

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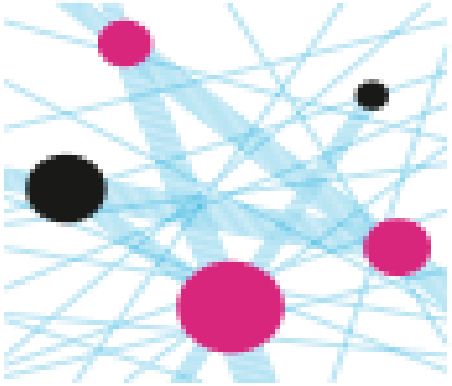


BD HPP Objectives

"The biology and disease oriented branch of the Human Proteome Project (B/D-HPP) was established by the Human Proteome Organization (HUPO) with the main goal of supporting the broad application of state-of the-art measurements of proteins and proteomes by life scientists studying the molecular mechanisms of biological processes and human disease. This will be accomplished through the generation of research and informational resources that will support the routine and definitive measurement of the process or disease relevant proteins".

The BD HPP Executive Committee, *JPR*, 2013

State-of-the-art: SRM based approach

- SRM based measurements have been shown to be reproducible between labs (CPTAC) and cover minimally 5 orders of magnitude

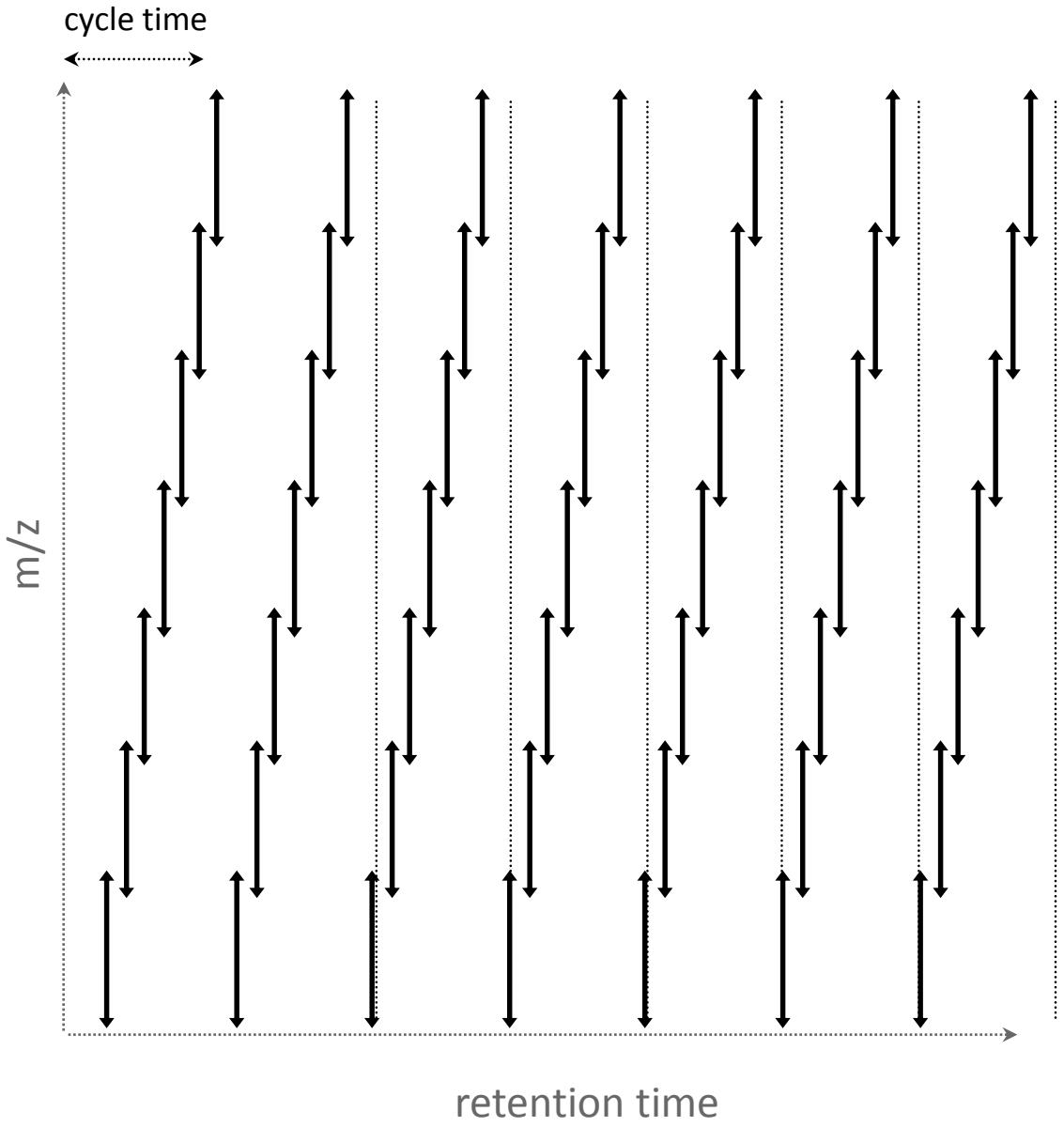
- SRM assay resources (including IT) have been established:
  - PeptideAtlas Project
  - PASSEL
  - Chorus
  - NCI SRM and immuno SRM assay resource

- **Limitation: the number of proteins that can quantified per analysis (level of multiplexing)**

Extending the level of multiplexing of quantitatively accurate MS measurements by SWATH-MS

- Principle
- Resources
- Performance
- Towards the future

SWATH-MS Acquisition Principle

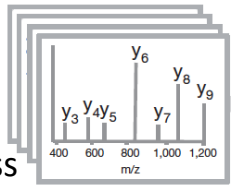


All precursors are Fragmented in every sample

SWATH-MS principle: “Targeted” -data extraction

OpenSWATH: Automatic, targeted analysis of SWATH maps

SRM Atlas

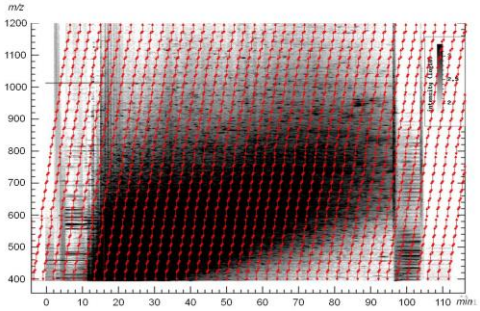


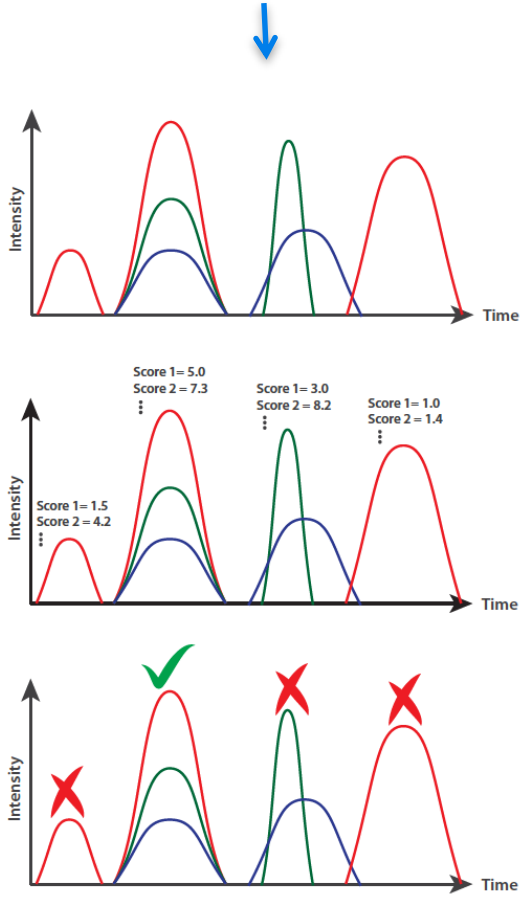
Ref. Mass spectrometric map

Proteins of interest

SWATH Coordinates :
Best fragment ion signals,
Relative intensities,
Retention time

SWATH MAP





3) Chromatogram Extraction

4) Feature Scoring

5) Statistical Analysis (e.g. mProphet)

Roest H & Rosenberger G et al, *Nature Biotechnol 2014*

SWATH-MS: A HIGHLY MULTIPLEXED TARGETING STRATEGY

Principles of Targeted mass Spectrometry:

- Generate an highly specific mass spectrometric assay for each targeted peptide (wet lab analogy: generate an antibody)

- Generate a complete digital fragment ion map of all ionized peptides **per sample**

- Use the MS assay to detect and quantify the targeted peptide in a sample (wetlab analogy: western blot, ELISA)

In essence: Mass spectrometry equivalent to 10 exp4 to 10exp5 ELISA/WB tests per hour and sample

Lange V et al. Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol (2008)
Domon B and Aebersold R, (2011) Nature Biotechnol., Picotti et at Nature Methods, 2012; Picotti et al Nature 2013,

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PCT-SWATH: Fast conversion from biological samples to digital data

Biological samples → BIG Data → Knowledge

Pressure cycling technology (PCT)-assisted tissue lysis and digestion

tissue biopsy
~1mg

All procedures
in single tube

Batch processing
Minimize variations

Pressure cycling technology
fast and efficient digestion

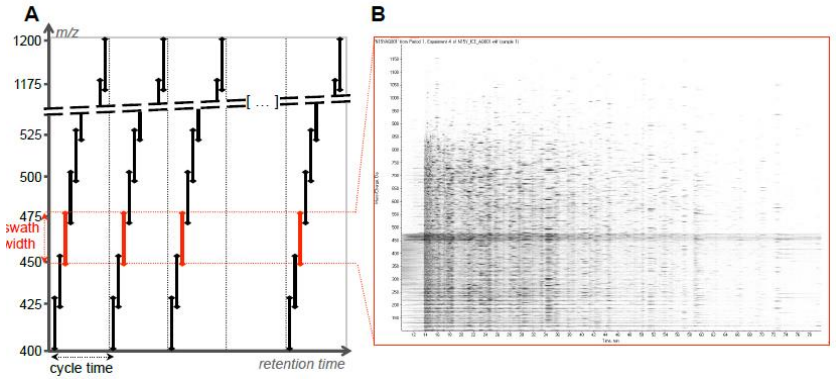
Yield:

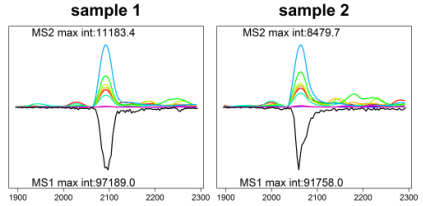
~50 ug peptides from
1mg mouse liver or
human kidney tissue

<1ug peptides
sufficient for a
swath analysis

6~8 hrs to convert 6 pieces of tissues into clean peptides that are ready for MS analysis

Proteomic analysis using SWATH-MS





targeted data extaction

<12hr

Gillet, et al. 2012.

www.nature.com/scientificdata

SCIENTIFIC DATA

OPEN

SUBJECT CATEGORIES

» Systems biology

» Proteomics

A repository of assays to quantify 10,000 human proteins by SWATH-MS

George Rosenberger[1,2], Ching Chiek Koh[1,3], Tiannan Guo[1], Hannes L. Röst[1,2], Petri Kouvonen[1], Ben C. Collins[1], Moritz Heusel[1,4], Yansheng Liu[1], Etienne Caron[1], Anton Vichalkovski[1], Marco Faini[1], Olga T. Schubert[1,2], Pouya Faridi[1,5], H. Alexander Ebhardt[1], Mariette Matondo[1], Henry Lam[6], Samuel L. Bader[7], David S. Campbell[7], Eric W. Deutsch[7], Robert L. Moritz[7], Stephen Tate[8] & Ruedi Aebersold[1,9]

Received: 11 June 2014

Accepted: 06 August 2014

phl002.TraML	Proteotypic / Unique
Proteins	10 980
Peptides	150 389

Complete libraries have also been generated for:

- S. cervisiae
- e.coli
- Mtb

Extending the level of multiplexing of quantitatively accurate MS measurements by SWATH-MS

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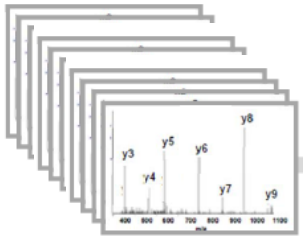
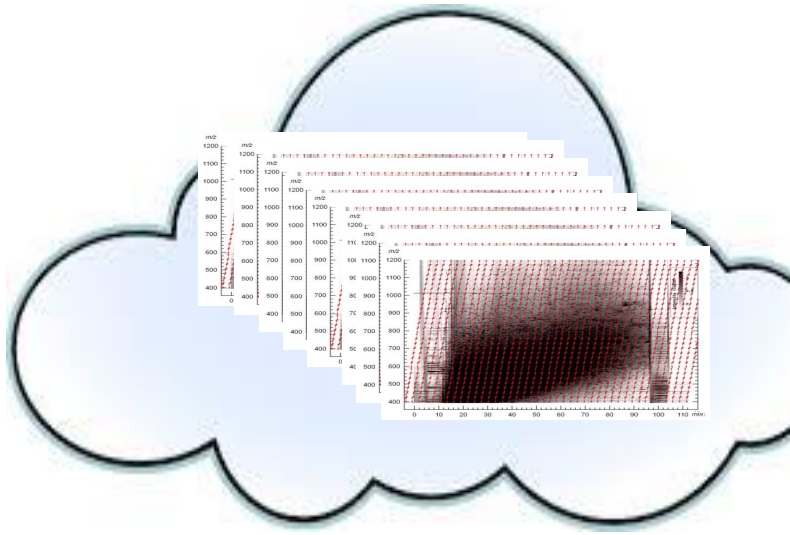
Democratizing Proteomics

Public Spectral Library
Representing whole proteomes



SRM Atlas

Public SWATH Datasets



OpenSwath
Search tool

- Queries across proteotype datasets
- Re-Mine data
- Digital biobanks
- Discover new features

OneOmics™ in the Cloud

- Next-gen sequencing
  - Fast and inexpensive
  - Highly accurate and repeatable
  - Comprehensive

- Cloud computing
  - Simple and scalable
  - Universal access to data
  - Fast processing

- Next-gen proteomics
  - Quantify thousands of proteins
  - Excellent repeatability and quantitation
  - Data completeness >98%

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AB SCIEX

Illumina® BaseSpace®

Omics? There are Apps for that.

BaseSpace®

- Web-based data management and analysis
- Eliminates need for onsite storage and computing power
- Tools for collaboration and sharing
- **NEW** Up to 50x faster than desktop processing of SWATH Proteomics

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AB SCIEX

Acknowledgements

Swath development:
Ludovic Gillet
Pedro Navarro
Steve Tate ,AB/Sciex
Ron Bonner, AB/Sciex
Hannes Roest
George Rosenberger

NCI-60:
Tiannan Guo
Julio Rodrigues-Saez (EBI)

BrightSpark
Slides courtesy Aaron Hudson (AB/Sciex)

Swiss National Science Foundation
ETH Zurich
SystemsX.ch
European Research Council, ERC
AB/Sciex
EDRN

Software
Lukas Reiter, Manfred Claassen,
Hannes Roest, George Rosenberger
Pedro Navarro, Brendan MacLean,
(Skyline)

Statistics
Olga Vitek, Purdue
Meena Chang Purdue