Practical Consideration for Acquisition and Analysis of DIA/SWATH-MS dataset

Mukul K. Midha, Ph.D.

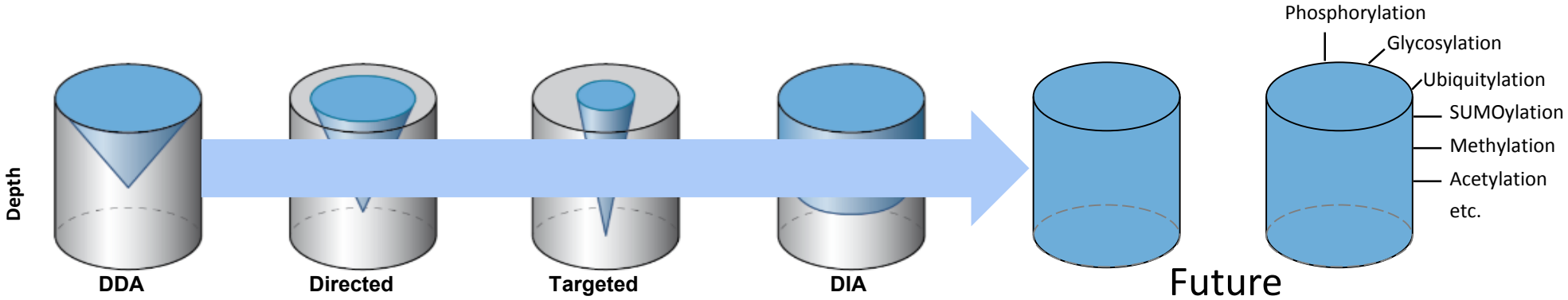
03/23/2019

Moritz group
Institute for Systems Biology, Seattle

Outline

- DIA/SWATH-MS basic concepts
- DIA/SWATH-MS objectives and workflows
- DIA/SWATH-MS analysis tools

Mass spectrometry based proteomics



Shotgun/ discovery

Shotgun with inclusion lists

Selected **R**eaction **M**onitoring

SWATH

Comprehensive
Sensitive
Reproducible

&

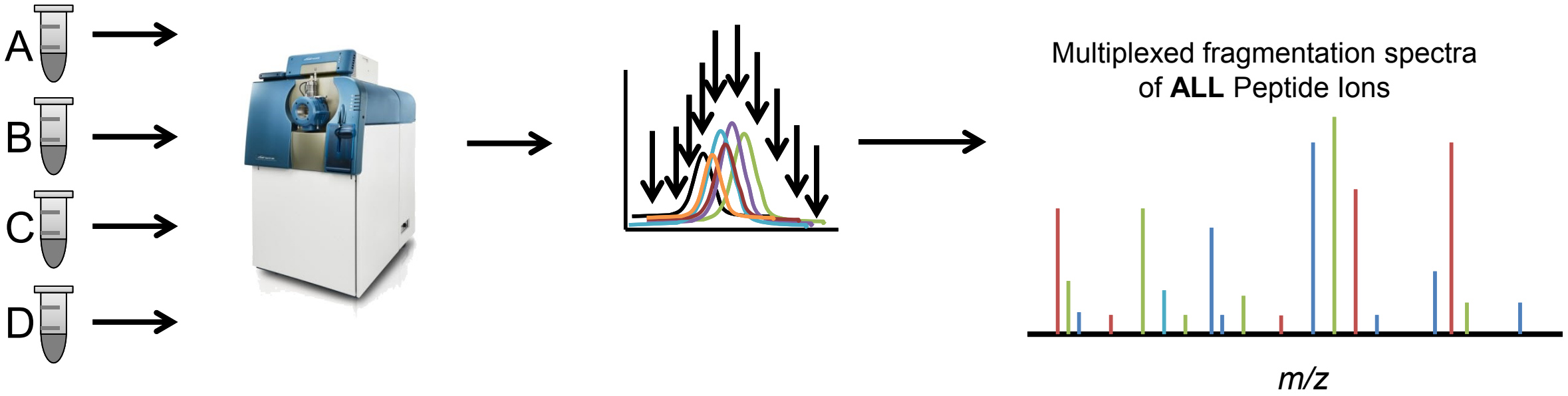
Protein Modifications

Adapted from Ariel Bensimon et al, Annu. Rev. Biochem. 2012, 81, 379.

- protein composition of a sample
- thousands of peptides
- stochastic nature of precursor selection

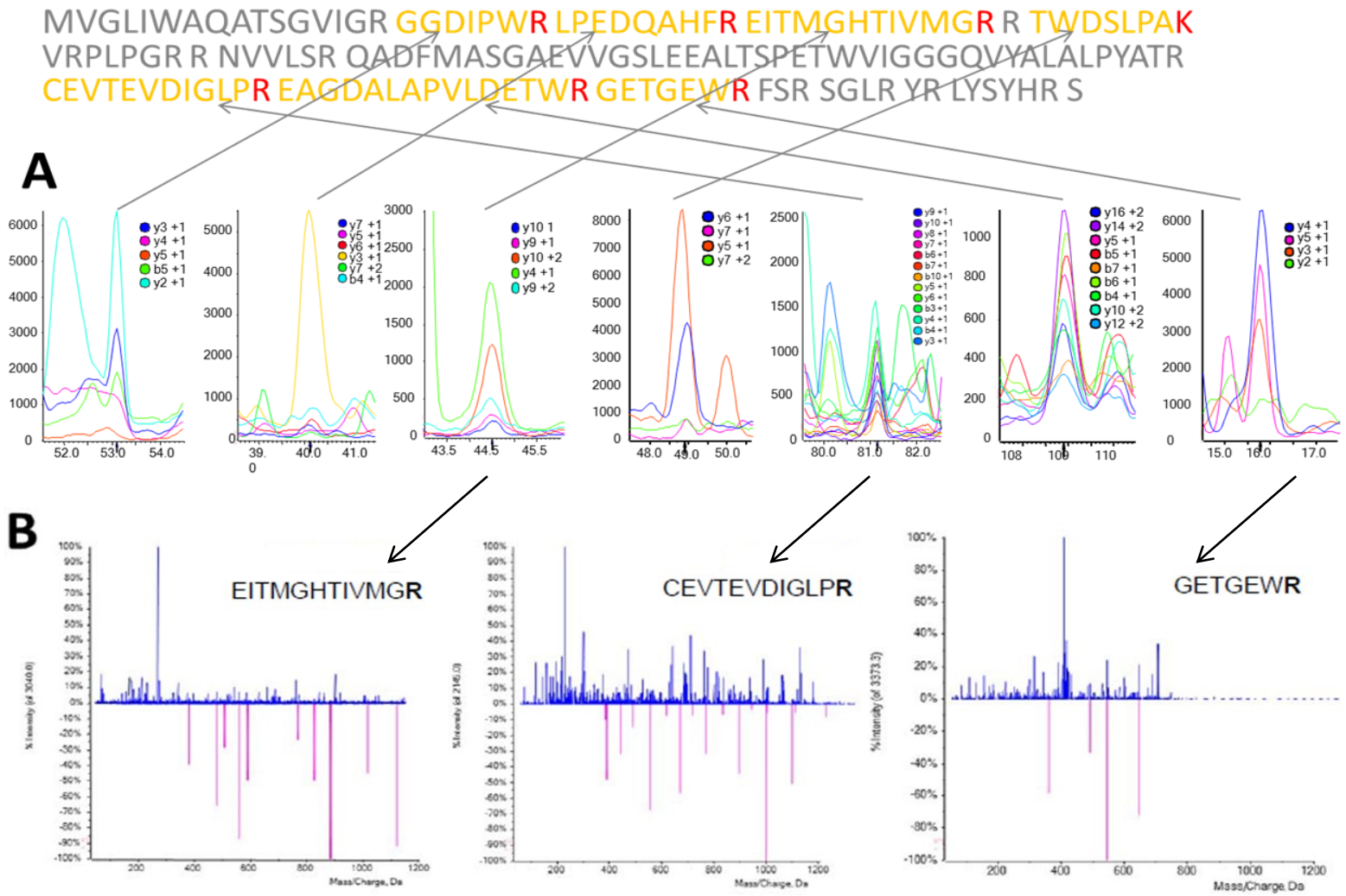
- lower limit of detection, wider dynamic range
- increased reproducibility
- limited number of peptides

SWATH-MS- Discovery Proteomics

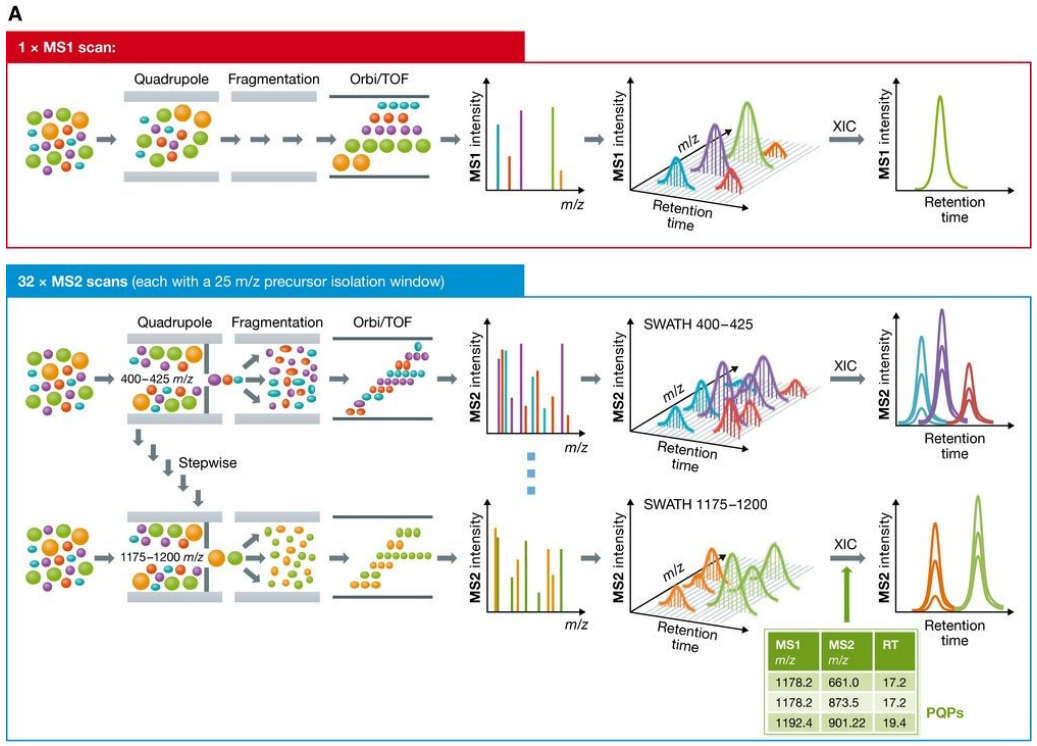


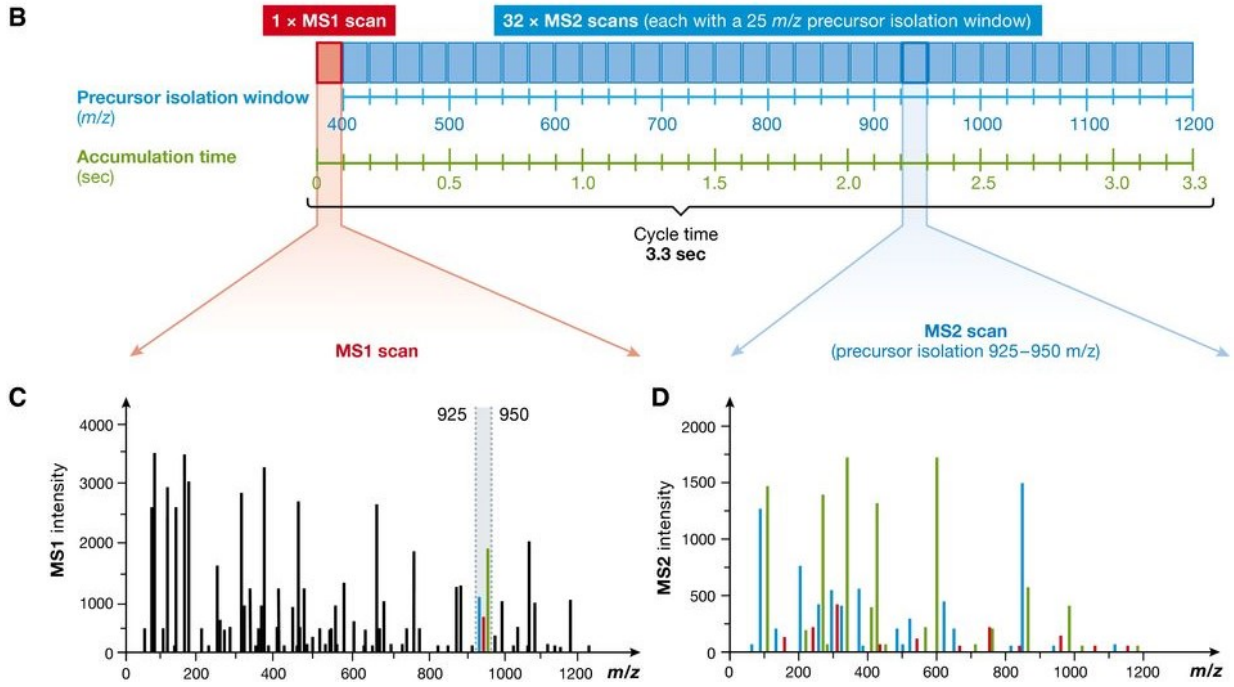
- Each sample is directly analyzed without fractionation.
- Selection of peptides is multiplexed.
- Results are multiplexed spectra to be identified by known spectral library
- Next stage is computational analysis.

SWATH-MS targeted data analysis



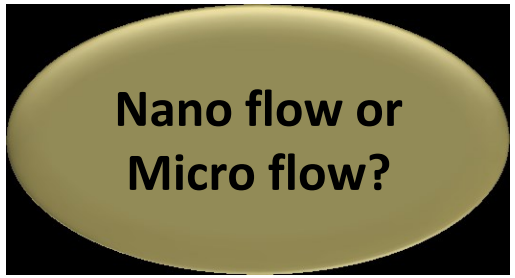
Principle of sequentially windowed acquisition in DIA/SWATH-MS

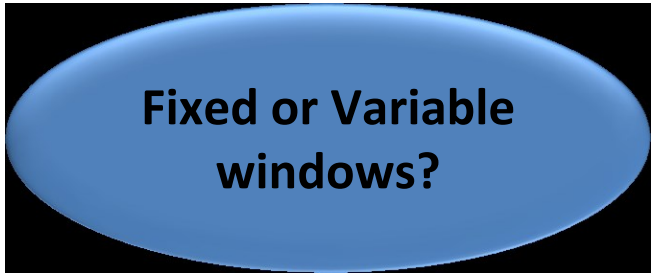




Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial, Ludwig, C. et al Molecular Systems Biology 2018

SWATH-MS/DIA experiment setup…..So much to choose from!!

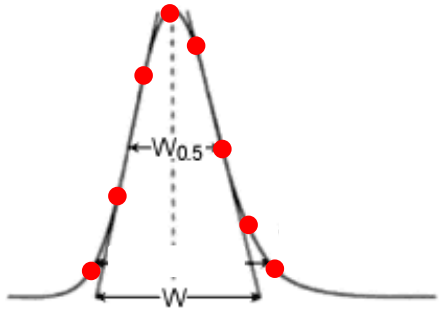




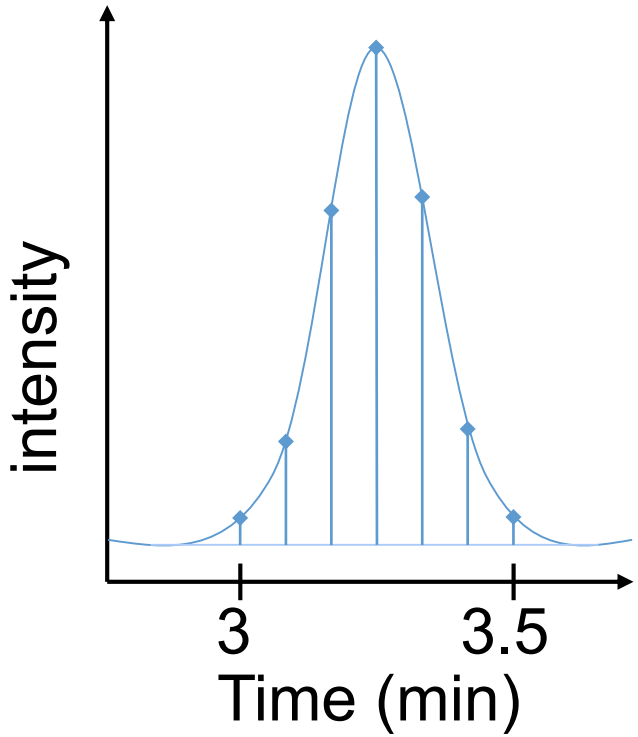
What are the critical acquisition attributes for DIA/SWATH-MS?

- ✓ High resolution MS/MS
- ✓ Cycle Time
- ✓ Q1 Isolation Windows
- ✓ Dynamic Range

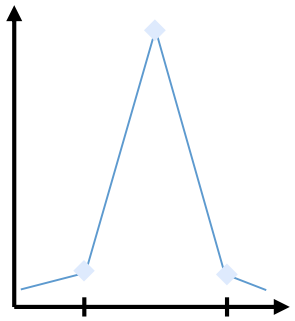
Cycle time = **# Windows** x **Accumulation time**

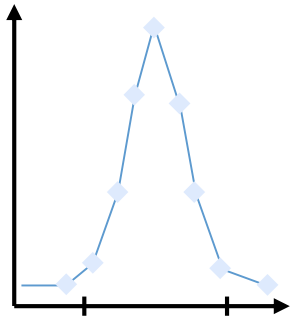


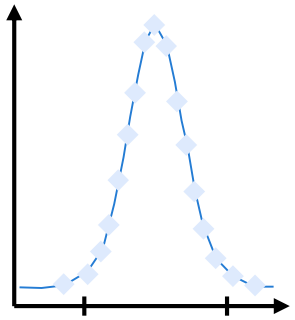
DIA Acquisition- Quantitation



ex. 30 s peak width at base
3 s cycle will collect 10 DPPP
<3 s will over sample
>3 s will under sample







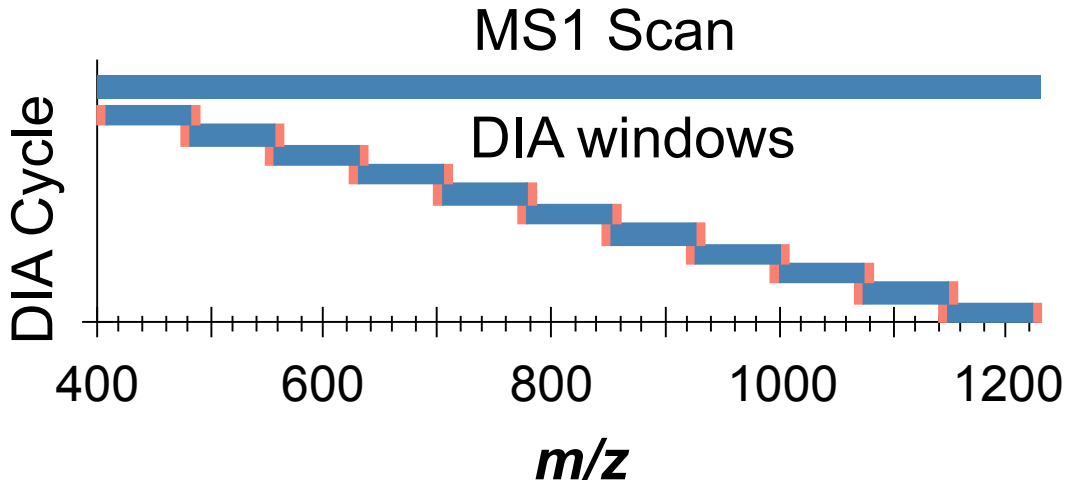
Data Points Per Peak (DPPP)

<7 DPPP = under sampling

7-10 DPPP = optimal sampling

>10 DPPP = over sampling

DIA/SWATH-MS precursor isolation windows

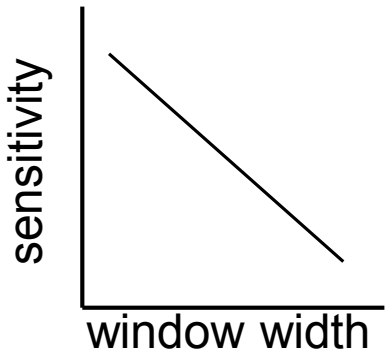


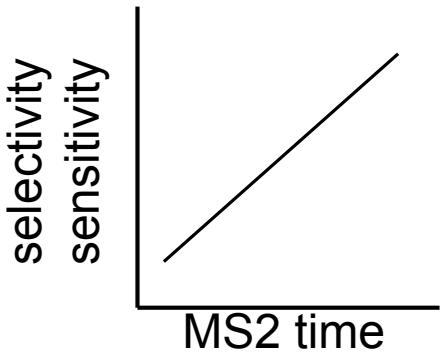
Window Strategies

- sequential segments
  - w/ or w/o overlap
  - static or variable width

windows x MS2 acquisition time = cycle time

42 (20 *m/z* width, 400-1200 *m/z*, 1 Da overlap) x 60 ms = 3.6 s

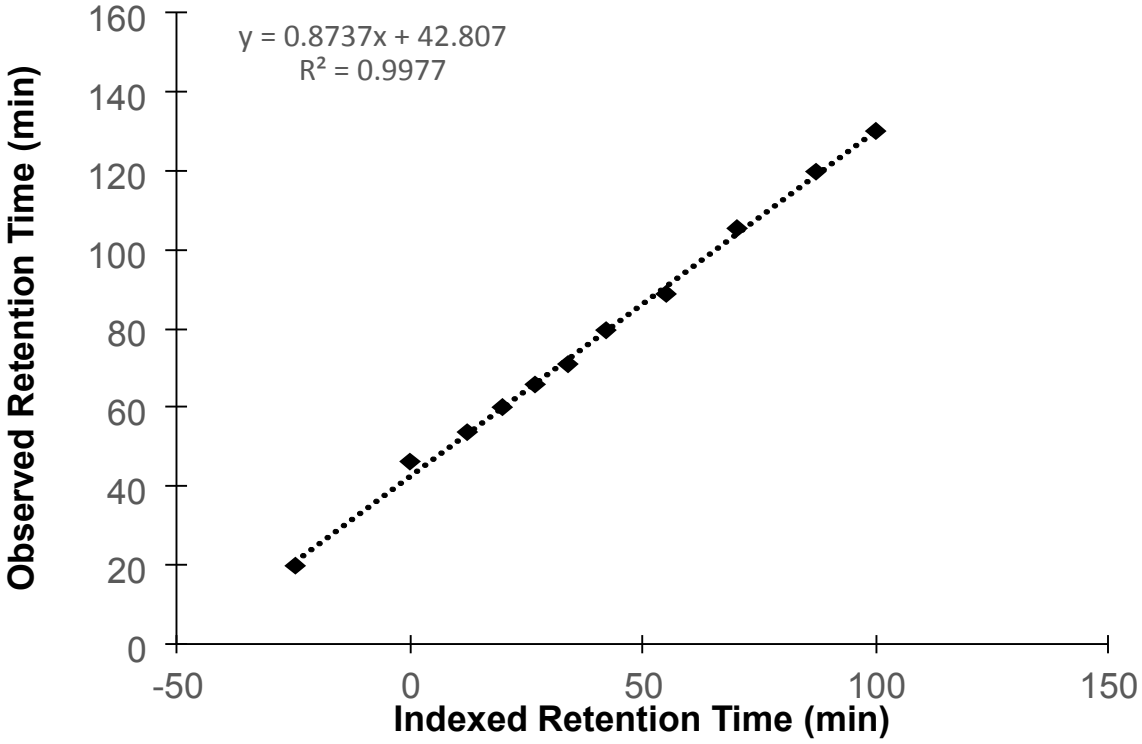


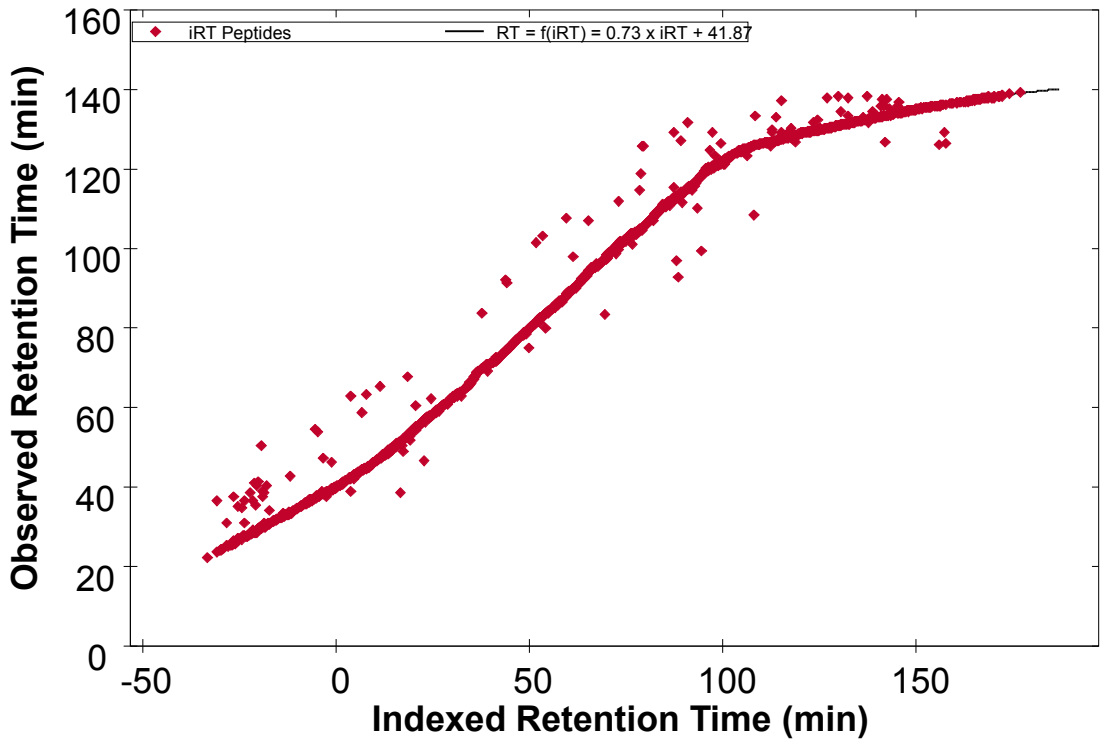


Choices are dependent on chromatography, application and platform.

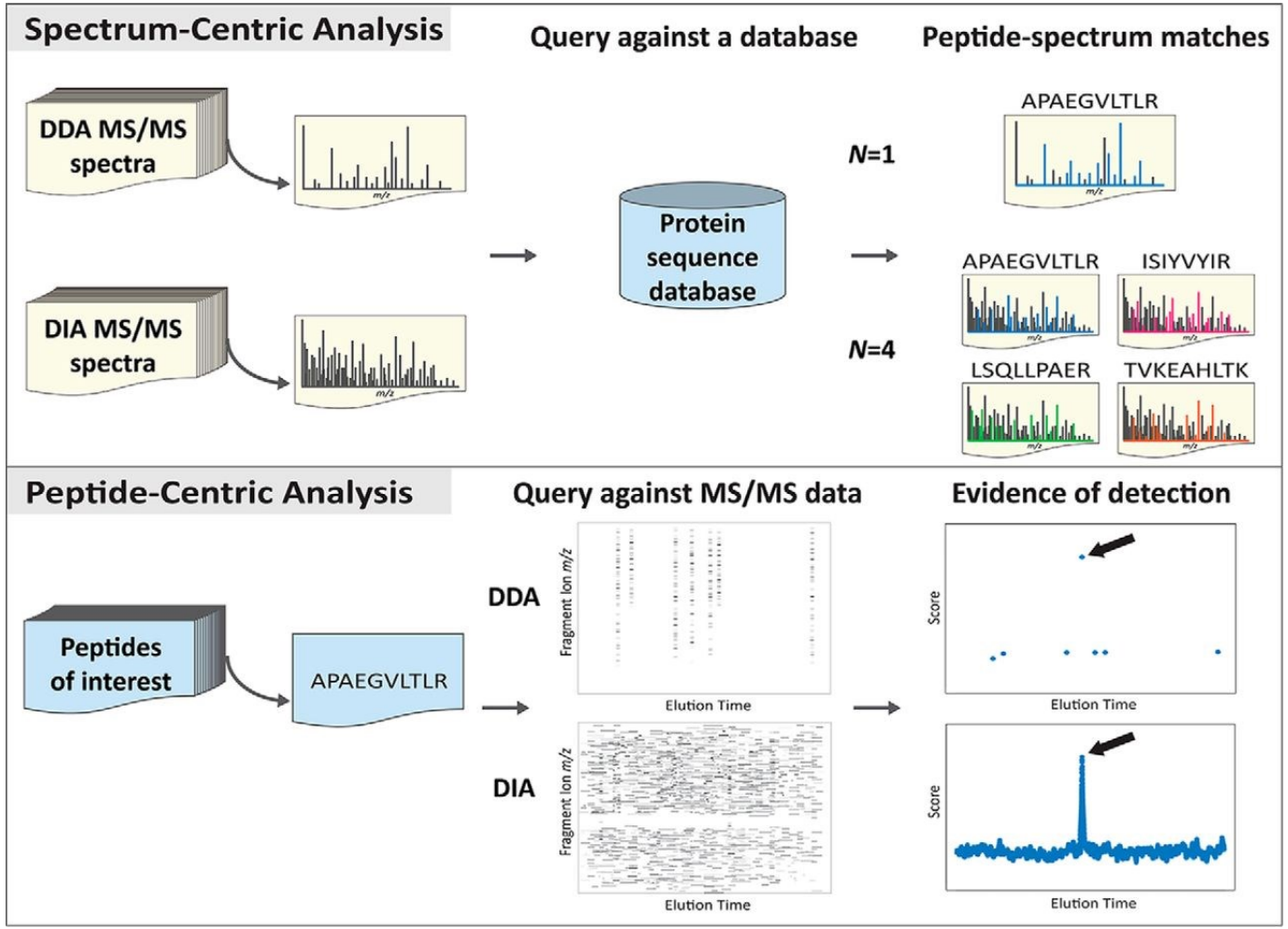
DIA data analysis – retention time normalization

- iRT spike
  - set of non-endogenous peptides
  - Used to convert to iRT scale (linear)
- Observed peptides/features
  - High-precision iRT (non-linear)
  - Anchor points





DIA/SWATH-MS: Library based and library free approach



Peptide-Centric Proteome Analysis: An Alternative Strategy for the Analysis of Tandem Mass Spectrometry Data, Ying S. Ting et al , MCP, 2015

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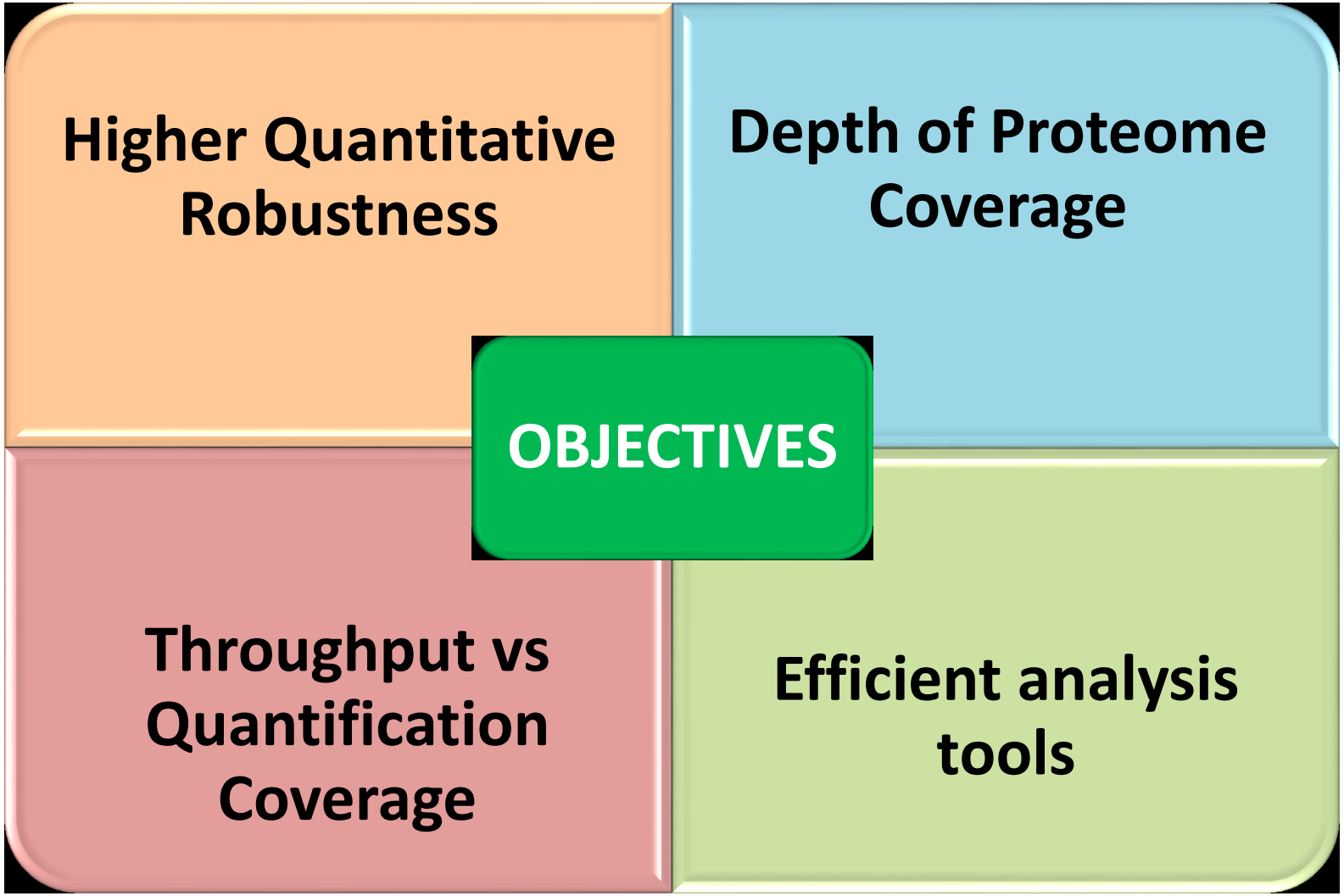
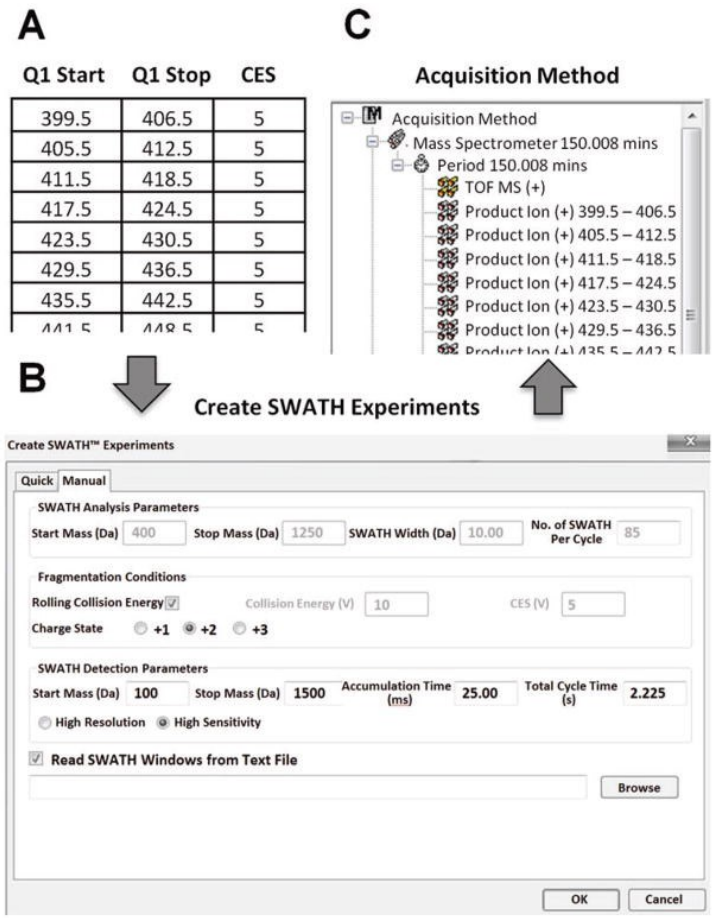

Higher Quantitative Robustness
Depth of Proteome Coverage
OBJECTIVES
Throughput vs Quantification Coverage
Efficient analysis tools

Building a Variable or Fixed Window SWATH method in Triple TOF 6600

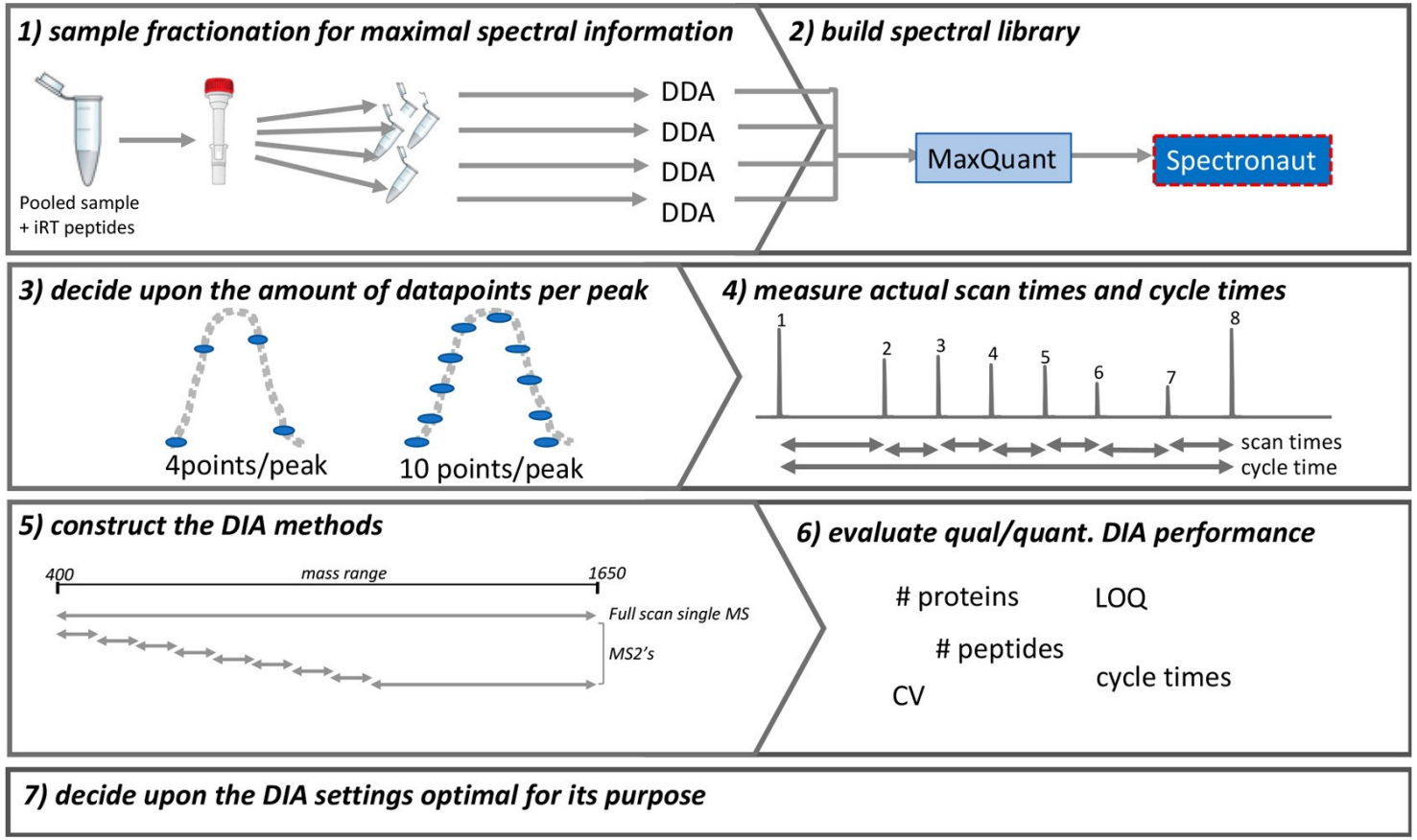
- Any window strategy can be constructed in text file format and loaded into SWATH Acquisition method editor.

- For Fixed window, manually enter the mass range, SWATH width and accumulation time for each isolation m/z window.



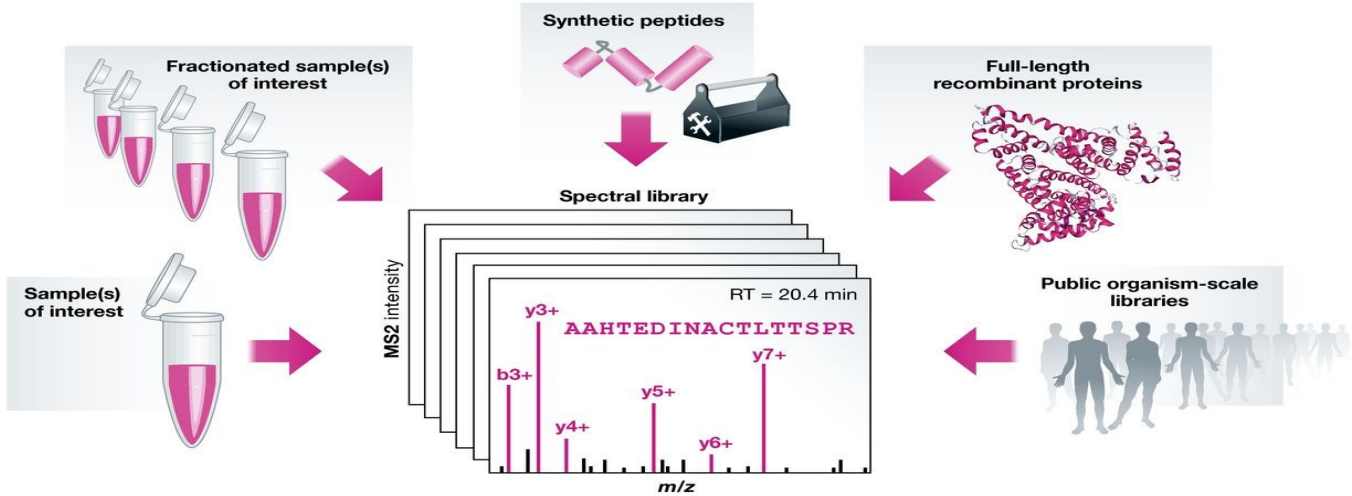
Generation of High-Quality SWATH® Acquisition Data for Label-free Quantitative Proteomics Studies Using TripleTOF® Mass Spectrometers. Schilling et al Met. Mol Bol. 2017

DIA workflow for Orbitrap Q Exactive HF



Reubsaet. L. et al, Data-Independent Acquisition for the Orbitrap Q Exactive HF: A Tutorial, Journal of Proteomics, 2019,18,3,803-813

Multiple sources of spectral ion library



Peptide query parameters (PQPs)
for target peptide AAHTEDINACTLTTSPR

Protein	Peptide sequence	Precursor ion m/z	Fragment ion m/z	Precursor charge	Fragment charge	Fragment ion type	Fragment relative intensity	Normalized retention time
Raf1	AAHTEDINACTLTTSPR	929.4416	775.4308	2	1	y7	70	45.3
Raf1	AAHTEDINACTLTTSPR	929.4416	674.3832	2	1	y6	10	45.3
Raf1	AAHTEDINACTLTTSPR	929.4416	561.2991	2	1	y5	40	45.3
Raf1	AAHTEDINACTLTTSPR	929.4416	460.2514	2	1	y4	20	45.3
Raf1	AAHTEDINACTLTTSPR	929.4416	359.2037	2	1	y3	100	45.3
Raf1	AAHTEDINACTLTTSPR	929.4416	280.1404	2	1	b3	60	45.3

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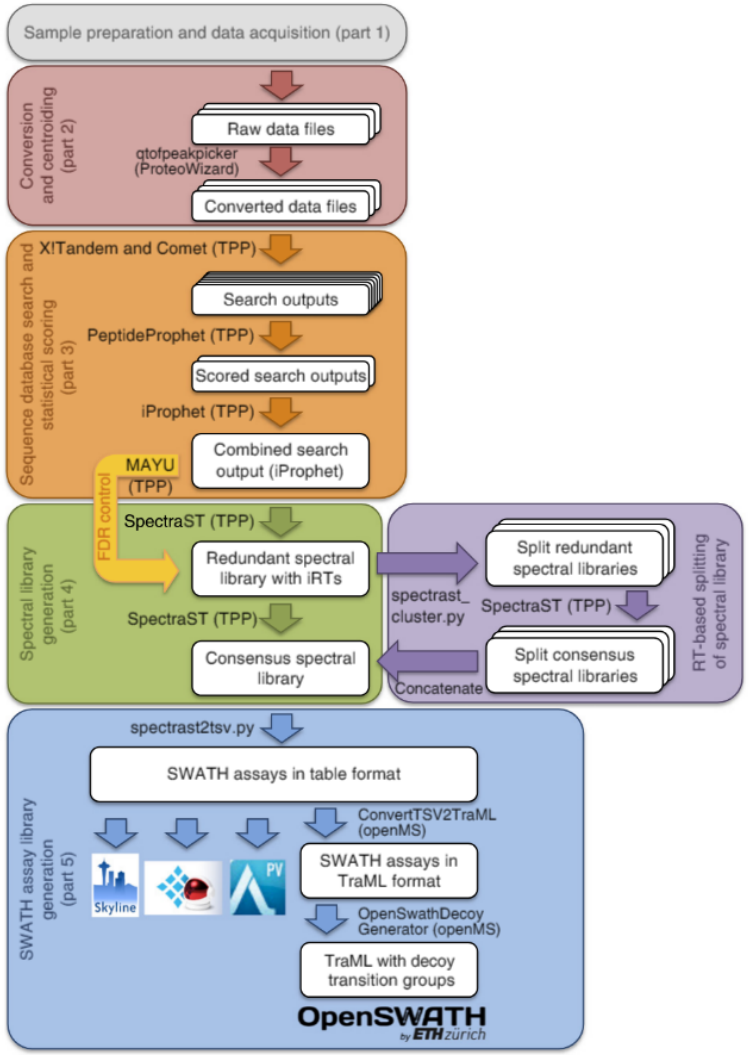
Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial, Ludwig, C. et al Molecular Systems Biology 2018

Workflow for DIA/SWATH assay library generation



Trans Proteomic Pipeline

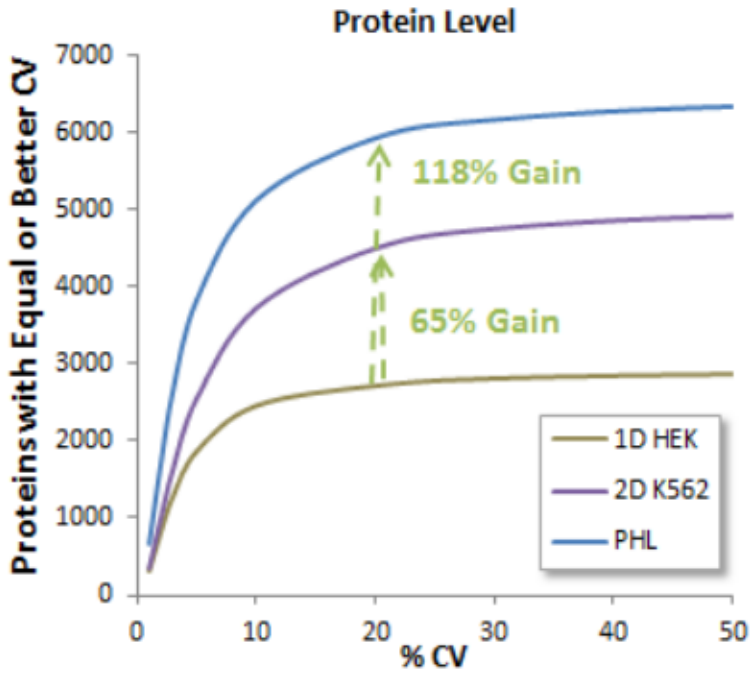
MQ

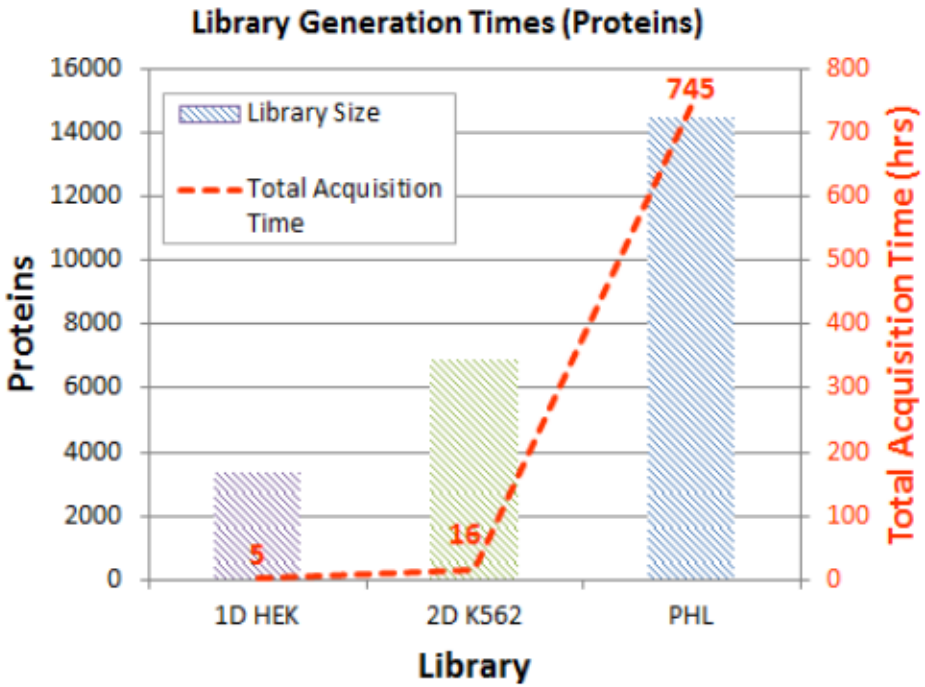
PP ProteinPilot™ Software

Skyline

Building high-quality assay libraries for targeted analysis of SWATH MS data. Schubert Olga et al, Nature Protocol, 2015

Impact of Deeper Ion Libraries on Extraction of Quantitative Data from Human Cell Lysate SWATH

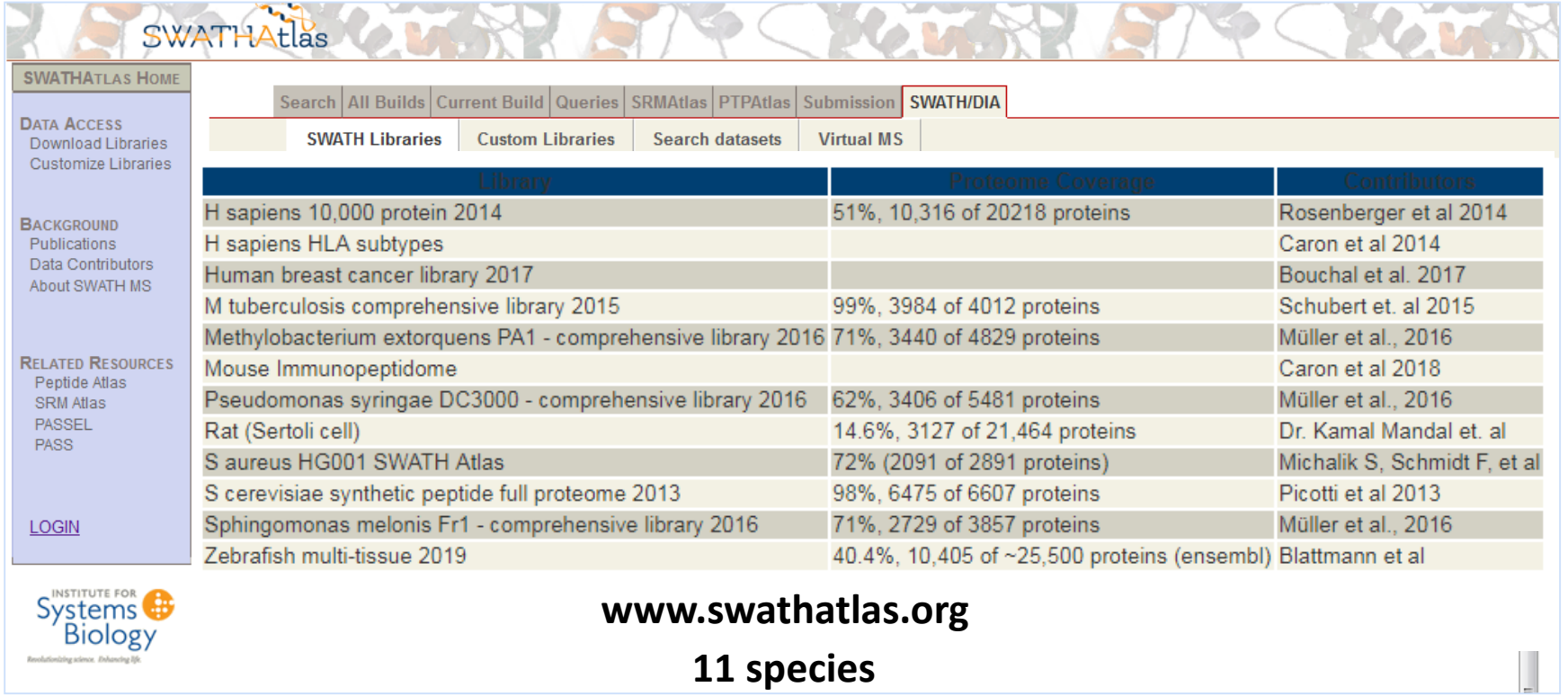




Extending Depth of Coverage with SWATH® Acquisition Using Deeper Ion Libraries, SCIEX, Technical note

SWATH Atlas

Repository for SWATH-MS spectral libraries and results



www.swathatlas.org

11 species

DIA/SWATH- Prior Knowledge and Consistency

- Relative product ion abundance
  - Spectral ion libraries
- Retention time Information
  - iRT libraries
- Powerful enough to be used cross-lab/ cross experiment.
- More powerful run to run compared to DDA.

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DIA/SWATH-MS Data Analysis tools

Commercial

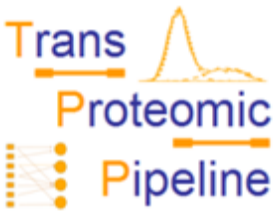
Sciex

Biognosys

Proteome Software

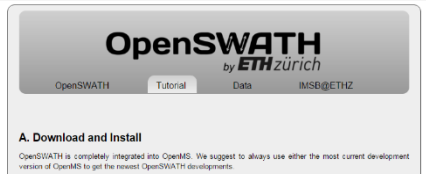
Bioinformatics Solutions

OPEN SOURCE



Institute for Systems Biology

Data Independent Signal Correlator
ISB



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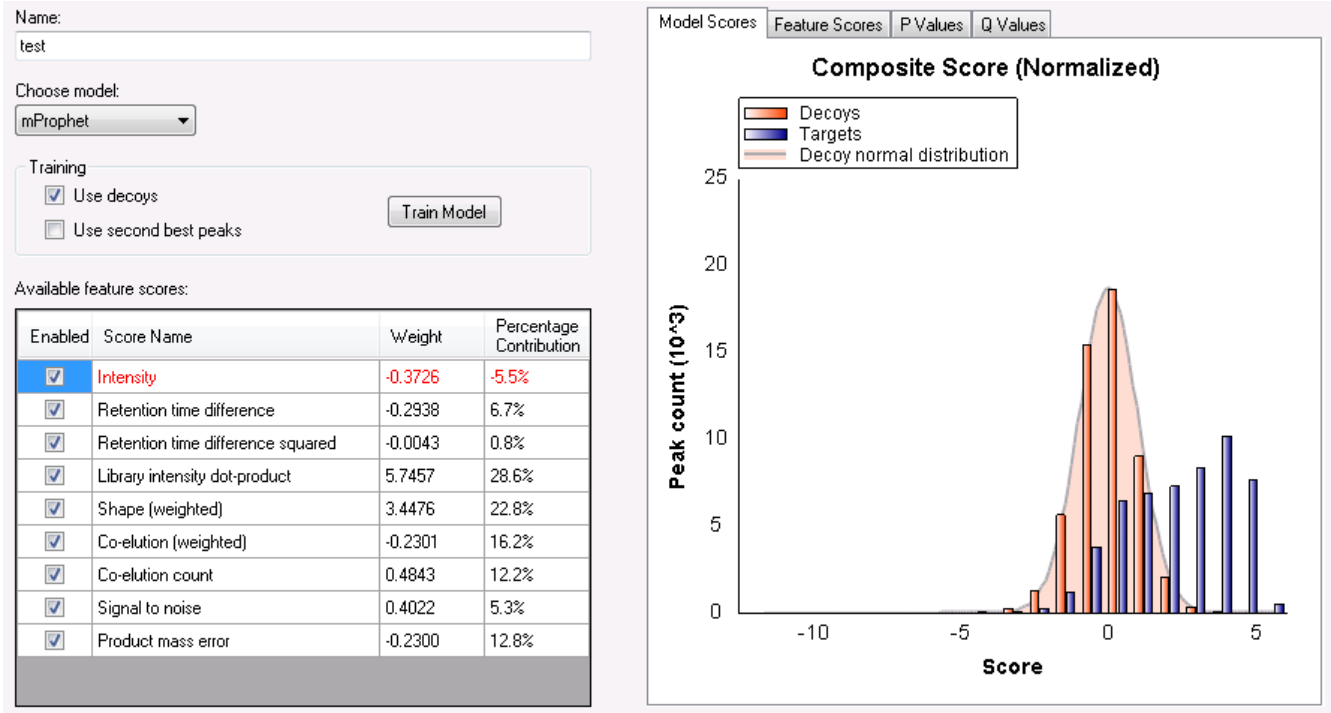
Peak Scoring and Picking Models

- **mProphet algorithm is implemented in OpenSWATH, Skyline and Spectronaut.**
- **PeakView scoring is based on spectral (correct isotope, m/z error, MS2 quality) and chromatographic attributes (RT, Peak width).**
- **Available feature scores are different that contribute to discriminant (d-score) or Composite (c) score.**



Acknowledgements

Proteomics Research Group (PRG)

Mission

The Proteomics Research Group (PRG) is a volunteer scientific organization dedicated to sharing knowledge about the analysis of proteins. The PRG aims to assist protein scientists and resource facilities to achieve their highest potential by sponsoring annual research studies that examine current techniques and capabilities. Through the promotion of broad participation and scientific excellence, the PRG aims to raise awareness, knowledge and education about modern methods of protein analysis.

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