SWATH® ACQUISITION PRIMER

The value of SWATH® Acquisition for proteomics

Customer Situation & Problems

1. Most discovery proteomics done today use a DDA (data-dependent acquisition) workflow, so called shotgun proteomics. High Resolution MS typically achieves today ~3000 identifications in a 3 hours nanoflow run for complex samples e.g. human. And more and more, researchers need to quantify the changes in the proteome to really understand the biology they are studying, e.g. normal versus diseased.

2. When there's a quantitation part from this DDA workflow, it's done either in label free at MS1 level (Orbitrap favorite workflow) or with chemical tags (e.g. iTRAQ and TMT at MS2 level, Silac at MS1). One of the limitation of MS1 quantitation workflows is less specificity and worse S/N. But the main drawback of all DDA workflows is poor data completeness (the missing value issue) across many injections. It's due to stochastic nature of DDA that relies on peptide detection in the MS1 survey scan to trigger MS/MS. In complex samples, where many peptides are co-eluting all the time, not all the same peptides are triggered for MS/MS inj after inj. Typically, if you inject 5x the same complex sample, you find only ~70% of common peptides in the 5 injections. And it's getting worth as number of injections increases. Data completeness (~inter samples reproducibility) is finding repetitively the same peptides in all injections. This is a key point to do a quant study for a big number of samples (e.g. human clinical proteomics). Indeed, you can do quantitative conclusions only on peptides you find in all injections/samples.

3. Targeted quantitation by MRM or MRMHR/PRM is the gold-standard for quantitation and provides high data completeness and excellent specificity and sensitivity. However it is limited in the number of proteins (~100) that can be quantified per run and is therefore better suited for downstream biomarker validation studies.

4. Some samples are irreplaceable so you only get one shot at analysis. You must get the most of it.

5. There's a shift toward high throughput (HT) analysis for clinical human proteomics (industrialized proteomics), like NGS did for genomics. Large cohorts of 100s to 1000s of samples will need to be analyzed in life science research to serve precision medicine. The low throughput and robustness of nanoflow MS is a challenge and creates a bottleneck.

6. There's a market trend toward integration of omics data (genomics/proteomics/metabolomics).

SWATH® Acquisition 30 seconds Elevator Pitch… the take home message!

"SWATH® Acquisition is a game-changing technique that essentially acts as protein micro-array and is the most comprehensive way to generate comprehensive identification and quantitation of the entire proteome. It generates a digital record that can be mined retrospectively for the years to come without the need of reinjecting the samples."

Leroy HOOD - President of the Institute of System Biology - USA

Customer Needs

There is a critical need for a simple proteomics workflow that provides higher quality quantitation of a very large number of species in the same run, and gets the same results on large numbers of samples. For customers working on clinical proteomics, high throughput proteomics will become a must to have.

SWATH® Acquisition figures of merit

1 DIA (data independent analysis) method for comprehensive detection and quantitation of 1000s proteins

- **Highly reproducible across many samples:** SWATH® Acquisition fragments ALL detectable peptides, solving the "missing value" issue of DDA
- **SWATH® Acquisition** outperforms DDA: 5000 proteins quantified in 1 hour!

2 High quality quantitation – 'MRM like'

- Label free quantitation in MS/MS at High Resolution
- Improved sensitivity and specificity vs MS1 label free quant
- High dynamic range ~4+ orders

3 Easy quantitation with no method development

4 Permanent digital record of the sample in one injection

- Re-inspection w/o re-injection as new biological questions arise

5 Micro flow SWATH® Acquisition fits for HT clinical proteomics

- 5000 proteins quantified at CV<20% in 1 hour injection!
- 150 proteomes a week for industrialized proteomics

6 OneOmics™ solution for greater biologic insights

- Combines SWATH® Acquisition data and NGS Illumina data into the cloud

Why SWATH® Acquisition is the best of class of all DIA workflows?

1 Very high sensitivity and spectra quality in MS/MS for TripleTOF® systems

- low amol on column

2 TripleTOF® systems are the fastest High Resolution systems

- Up to 100Hz in MS/MS to use the largest number of small windows for highest specificity
- Patented Variable Windows acquisition for increased specificity vs other DIA workflows
- Up to 200 SWATH® Acquisition Variable Windows per cycle on the 6600 (100 VW on 5600+)

3 TripleTOF® systems have High Resolution in MS/MS independent of scan speeds and m/z

- ~30,000 in MS/MS for better selectivity and spectral quality

5 High dynamic Range up to ~5 orders

- To detect low abundant peptides in a complex mixture

SWATH® Acquisition is all about MS/MS performance. All at the same time without any compromise.

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