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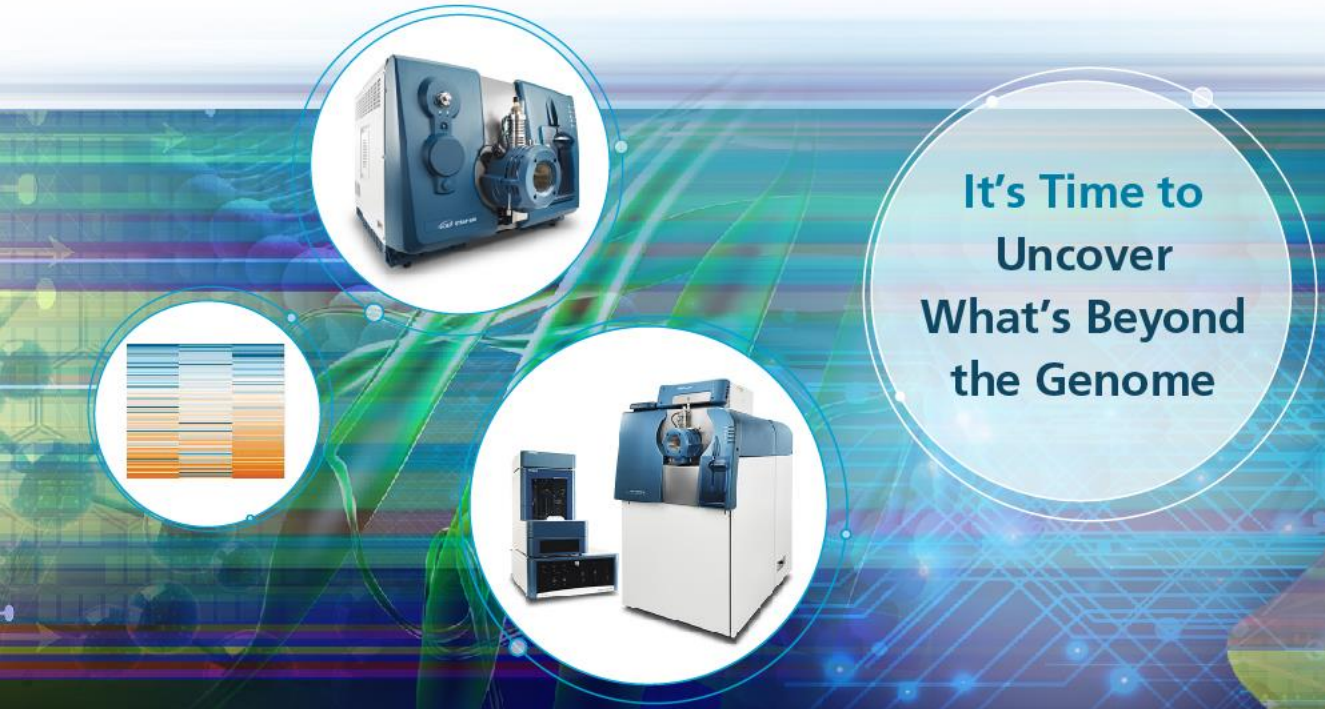


SWATH® For Quantitative Proteomics

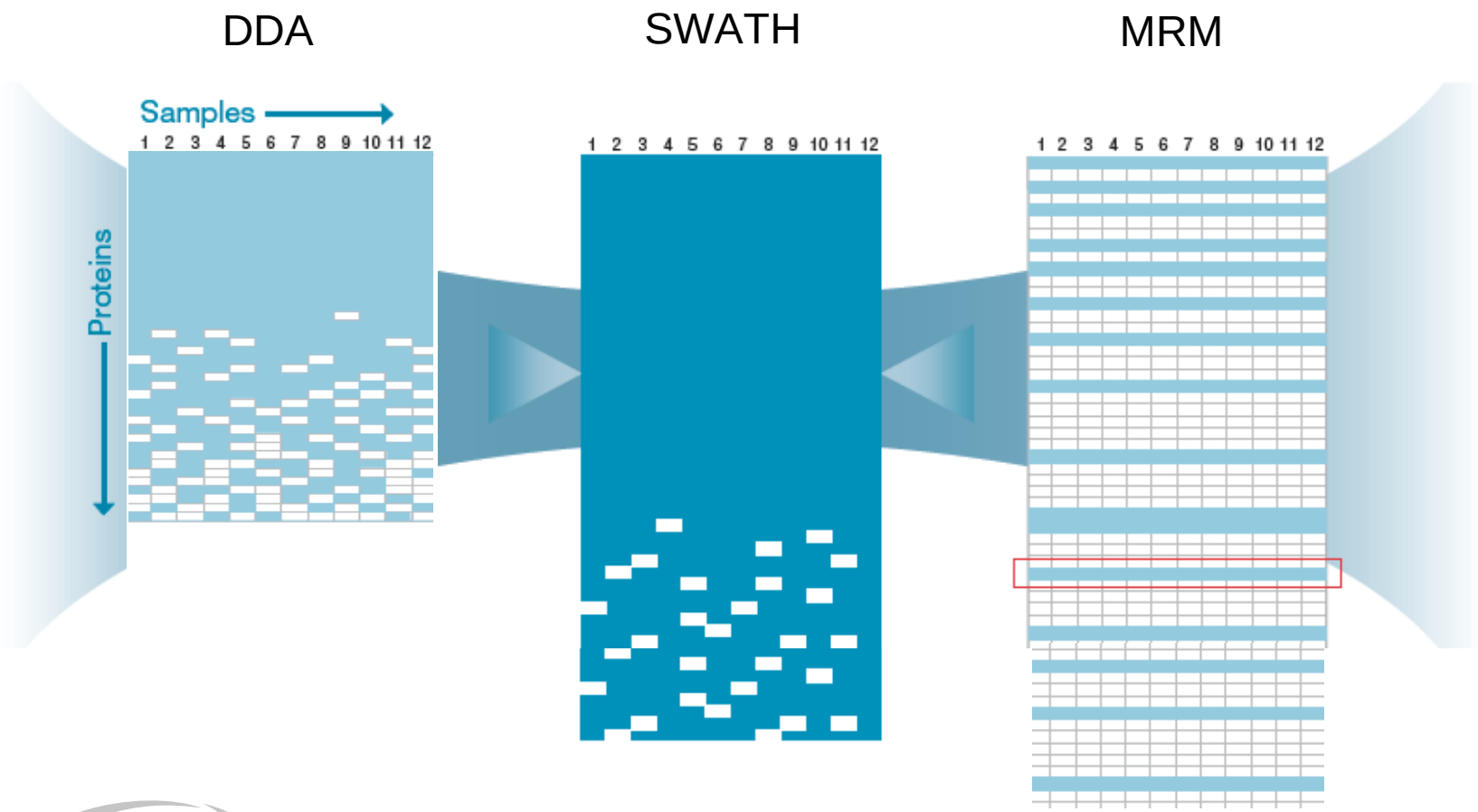
Randy Arnold, PhD – SCIEX

Pressure Biosciences ASMS Breakfast Seminar, June 6, 2017

Next-Generation Quantitative Proteomics

Powered by TripleTOF® 6600 System and SWATH® Acquisition 2.0

Targeted proteomics + Discovery proteomics



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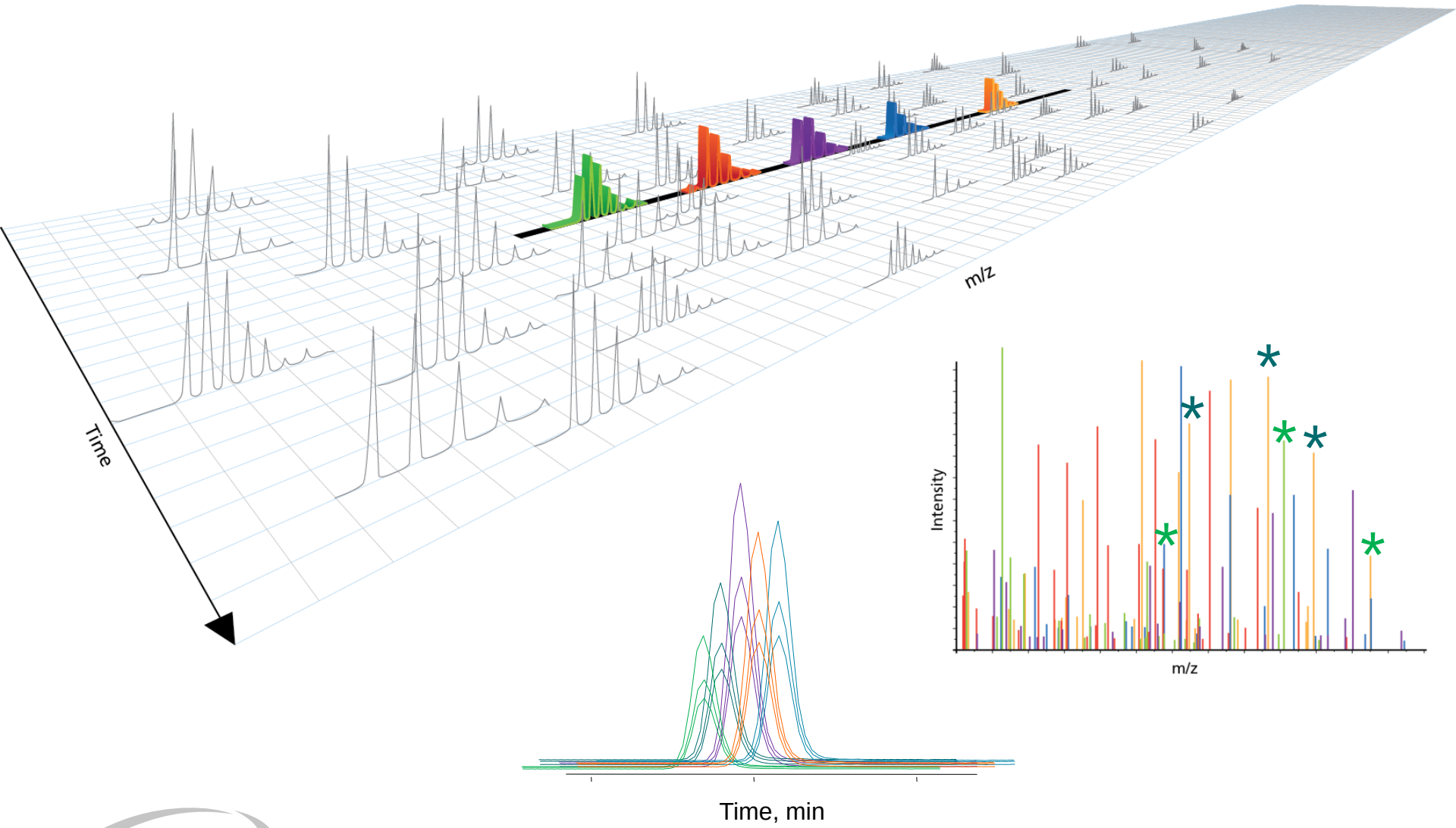
MS/MS^{ALL} with SWATH® Acquisition



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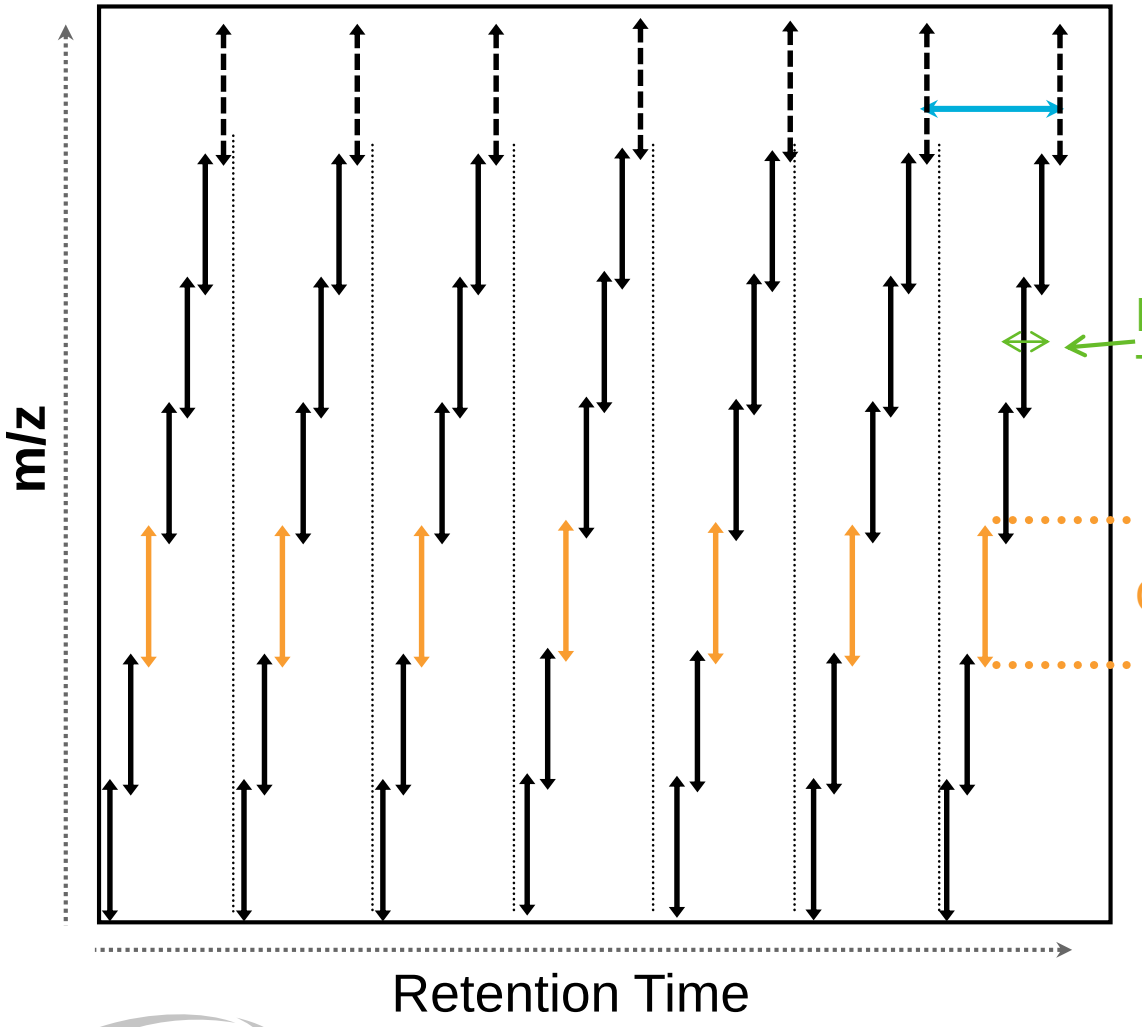
Q1 Isolation Strategy

Considering Quantitation



Cycle time
= # steps X accum time

MS/MS Accumulation Time

Balance Specificity with Sensitivity

Q1 window

Total mass range
= Q1 width x # steps

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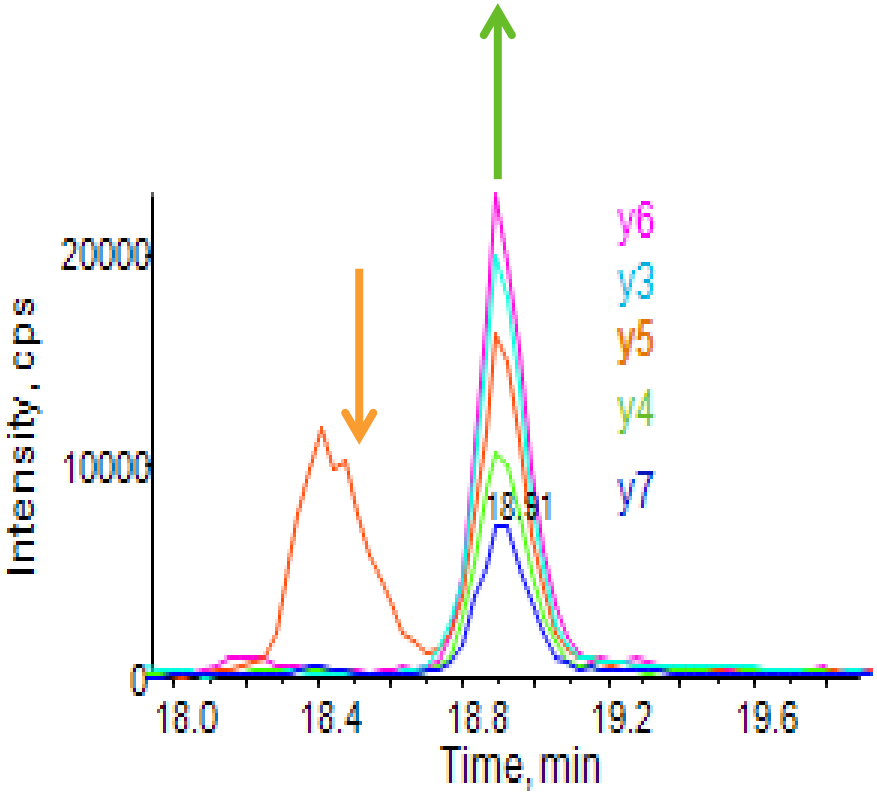
Extending Depth of Coverage

Signal / Noise

- S/N key to digging deeper in a highly complex sample

1. Increase specificity – decrease chemical noise/interferences
   - Smaller Q1 window
   - Variable windows
   - Longer gradients
2. Increase signal
   - Loading more sample
     - Chromatography, MS LDR
3. Deeper ion libraries
   - Large whole proteome libraries



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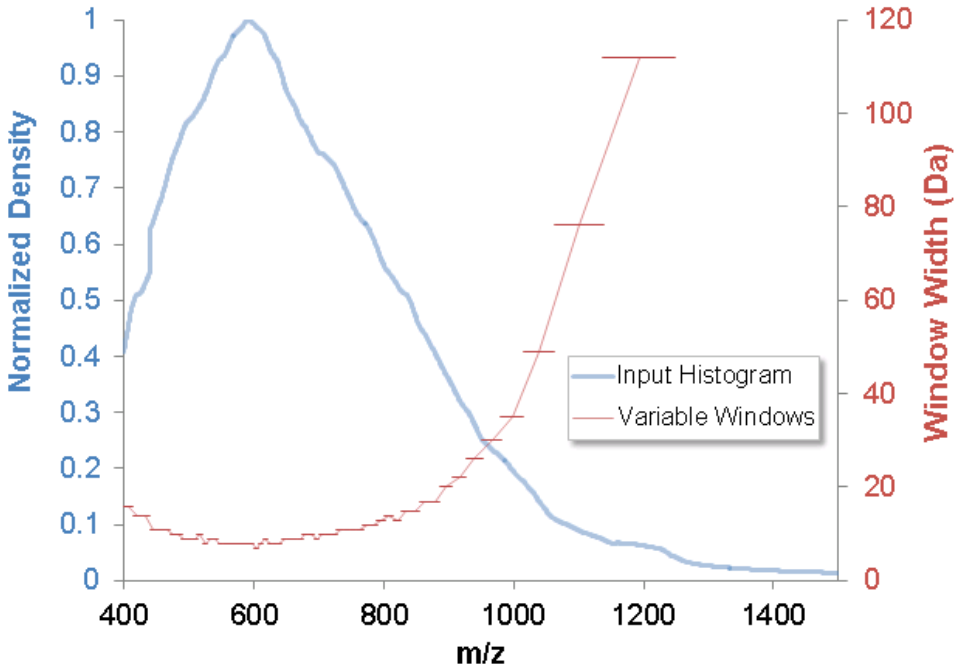
Variable Window SWATH® Acquisition

- Adjust Q1 selection window to maintain a roughly constant number of peptides to maintain specificity
  - Narrower window in m/z dense regions
  - Optimal cycle time maintained by adjusting accumulation time
- Reduce number of precursors in window for increased specificity of quantification
- Data acquisition supported in Analyst® Software TF 1.7 or later



100 variable windows
Current optimal method
Available on SCIEX User Community

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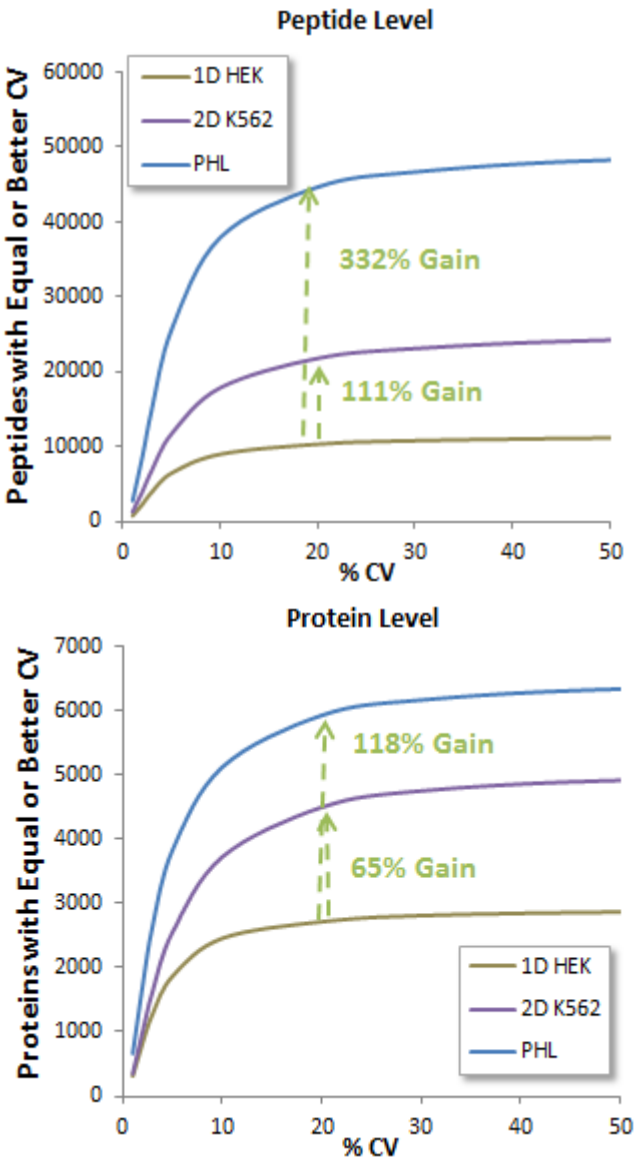
Extraction of Quantitative SWATH® Data from Human Lysate

Impact of Deeper Ion Libraries

- SWATH replicates from HEK cell lysate (3hr gradient, 3µg load)
  - Proteins/peptides quantified with <1% FDR peptides and CVs were plotted.

- **1D HEK library** - 2714 proteins and 10310 peptides were quantified
- **2D K562 library** - 111% gain in peptides and 65% gain in quantified proteins
  - 4493 proteins and 21778 peptides total
- **Pan Human Library PHL** - significant gain in quantified peptides and proteins (332% and 118%, respectively)
  - 5922 proteins and 44634 peptides quantified at <20% CV.



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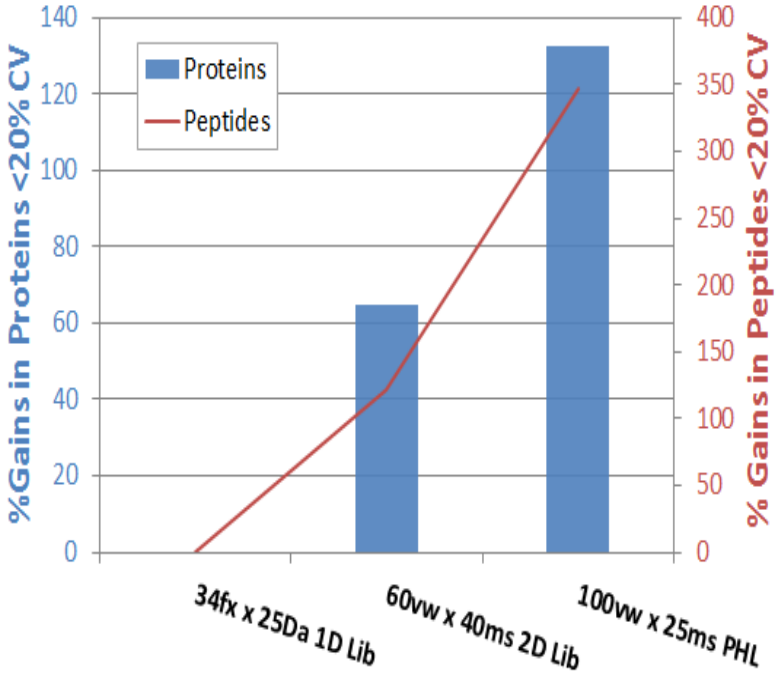
Gains in Quantified Proteins and Peptides Quantified

Through SWATH® Acquisition Innovations

- Most parameters held constant
- Impact of original SWATH method with a small library vs an intermediate approach and today's best strategy of using 100 variable windows with a large library
- Gains of **300% peptides** and **120% proteins** quantified across replicates are observed



Library	# of Proteins	# of Peptides
1D – HEK[4]	*3371*	*12809*
2D – K562[4]	*6889*	*62329*
Pan Human Library PHL[5]	*14425*	*197585*

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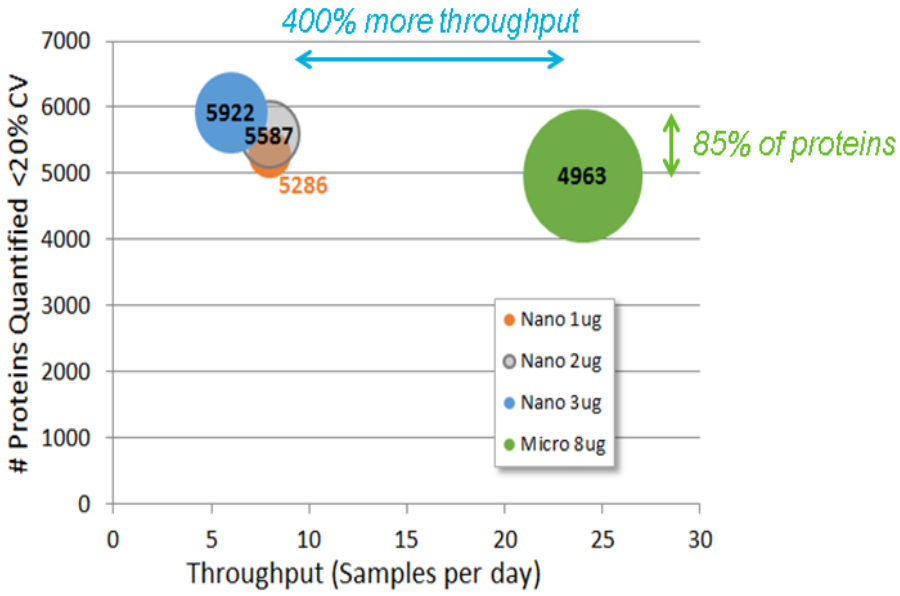
Industrialized Quantitative Proteomics

Microflow SWATH® Acquisition

- Demonstrated throughput enabling **~150 proteomes per week**
  - 1 hour total run time for up to 24 samples / day
  - Microflow LC provides higher throughput and robustness needs

- Quantified **~5000 proteins** with CV <20%
  - SWATH® Acquisition provides robust high quality quantitation
  - 100 variable Q1 windows
  - High resolution MS/MS
  - Require loading ~4x more protein on column



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Conclusions

- SWATH® Acquisition provides high quality quantitative data on complex proteomes
- Variable window isolation with many small windows provides large gains in proteins quantified
- Microflow SWATH® Acquisition provides high throughput and protein quantification consistency for large sample set
  - 150 samples per week – 1 hour per sample
- SWATH® Acquisition as a technology has evolved substantially over the last 5 years

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