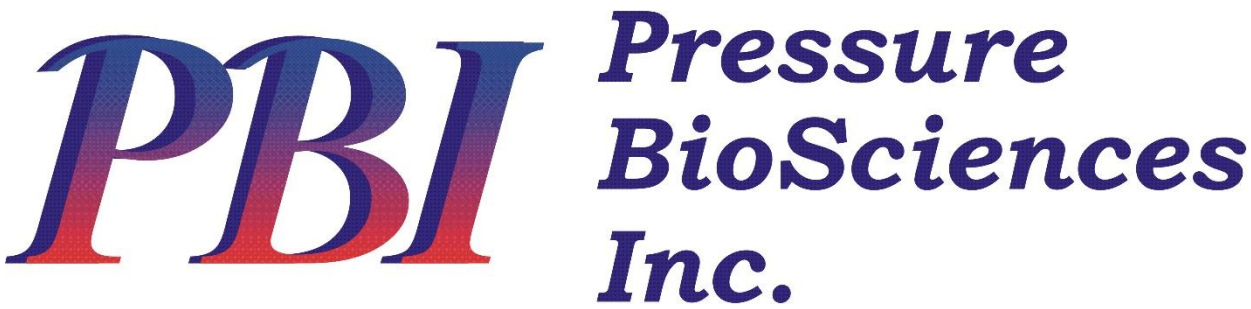


Discovery Starts With Sample Preparation™
ASMS 2017

Discovery Starts With Sample Preparation™ Breakfast ASMS 2017

Alexander Lazarev PhD – Pressure BioSciences

Randy Arnold PhD – SCIEX

Thomas Conrads PhD – Inova Schar Cancer Institute

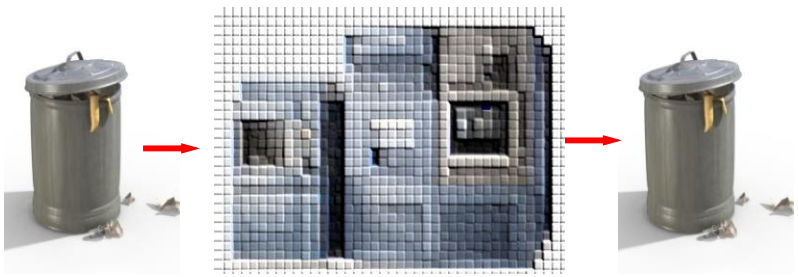
PBI Pressure BioSciences Inc.

Improved Proteomic Sample Preparation using Pressure Cycling Technology.

Alexander Lazarev, Ph.D.

June 2017

SAMPLE PREPARATION CONTINUES TO SLOW DOWN PROTEOMICS



- TWO DECADES OF PROTEOMICS RESEARCH HAVE RESULTED IN DEVELOPMENT OF EXCELLENT HIGH RESOLUTION, HIGH SPEED AND HIGH SENSITIVITY LC-MS INSTRUMENTATION.
- SAMPLE PREPARATION EXPERIENCED MUCH LESS INNOVATION AND SLOWER PROGRESS.
- TISSUE PROTEOMICS REQUIRES SPECIALIZED METHODS THAT WERE NOT CONSIDERED IN STUDIES OF BIOLOGICAL FLUIDS
- OUR GOAL WAS TO DEVELOP A CARDINALLY NEW APPROACH TO PROTEOMIC SAMPLE PREPARATION THAT WILL ENSURE HIGHEST QUALITY RESULTS

DISCOVERY STARTS WITH SAMPLE PREPARATION!

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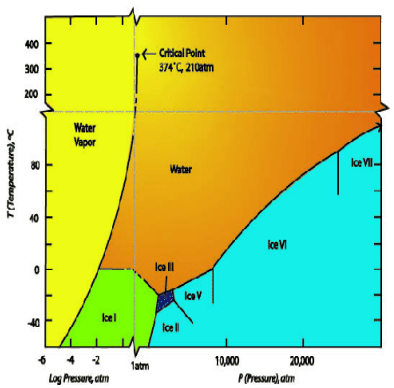
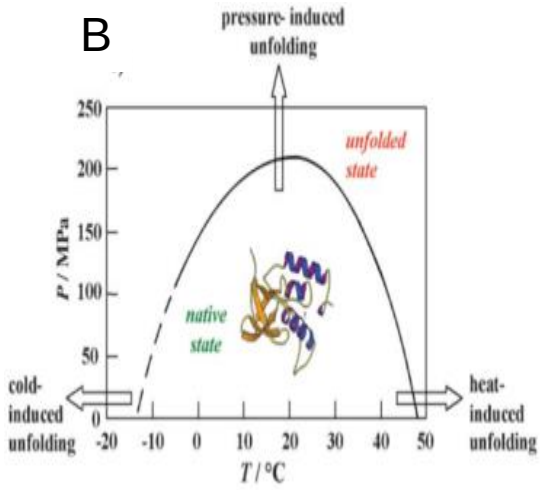
PHASE DIAGRAMS OF LIFE

A



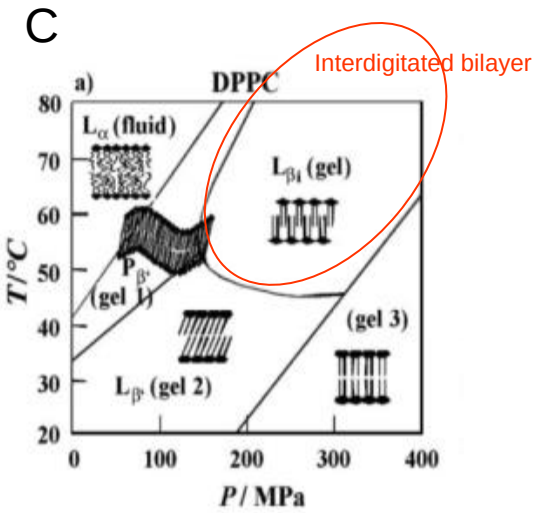
Water

B



Proteins

C



Lipids

Abundant biologically relevant molecules under pressure undergo significant phase transitions that are not accessible by manipulation of temperature alone.
Pressure as a way to directly, and often reversibly, manipulate ΔG presents unique opportunities in the laboratory.

A. Graph by Angela Tong, MIT (http://pruffle.mit.edu/3.00/Lecture_29_web/node2.html)
B. Daniel I, Oger P, Winter R. Chem Soc Rev. 2006; 35 (10); 858-75
C. Ichimori H. *et al.*, in: Adv. in High Pressure Biosci. and Biotech., Springer 1999

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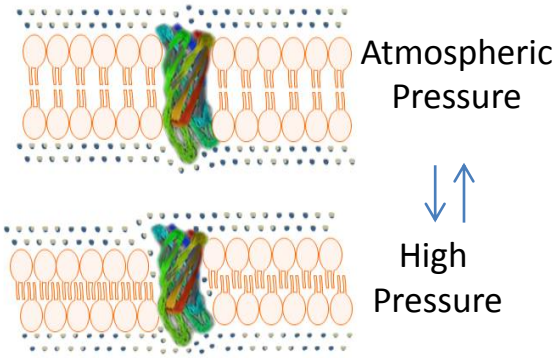
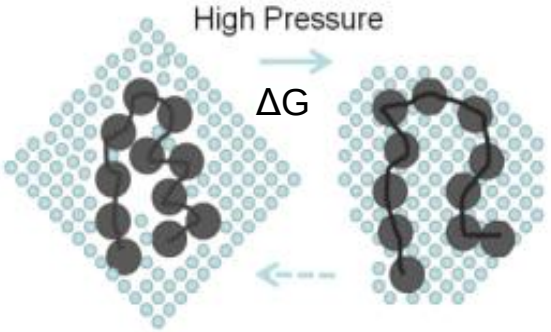
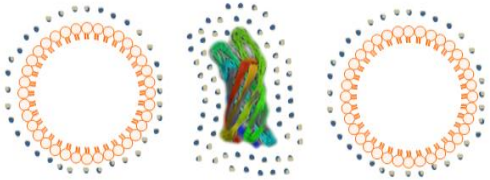
LIPID BILAYERS AND PROTEIN STRUCTURE ARE DESTABILIZED BY PRESSURE







Pressure can selectively alter protein conformation or regulate activity of certain enzymes ***without addition of thermal or chemical energy.***

*Most pressure effects can be explained by **hydration and changes in water structure***
*Pressure causes **phase transitions** in lipid bilayers and partially denatures proteins.*
*Pressure enhances **activity of several enzymes** used in proteomic and genomic workflows.*
*Pressure leads to **dissociation** of macromolecular complexes and protein aggregates.*
*Pressure acts in synergy with **chaotropic reagents and detergents***
*Higher levels of pressure cause irreversible **protein denaturation***

HIGH PRESSURE AFFECTS MOLECULAR INTERACTIONS

PBI Pressure BioSciences Inc.

PBI'S FOCUS IS BENCHTOP HIGH PRESSURE EQUIPMENT

- Developing expertize in high pressure equipment and applications
- Engaging academic collaborators to explore new applications
- Looking for industrial partners for commercialization efforts

Today:

Compact laboratory scale pressure generators and appliances

Pressure range: 0-100,000 psi (7kBar)

Temperature range: -20°C to +100°C

Research, metrology, biological sample prep.

Near Future:

Optical Spectroscopy

Higher temperature range -100°C to +300°C

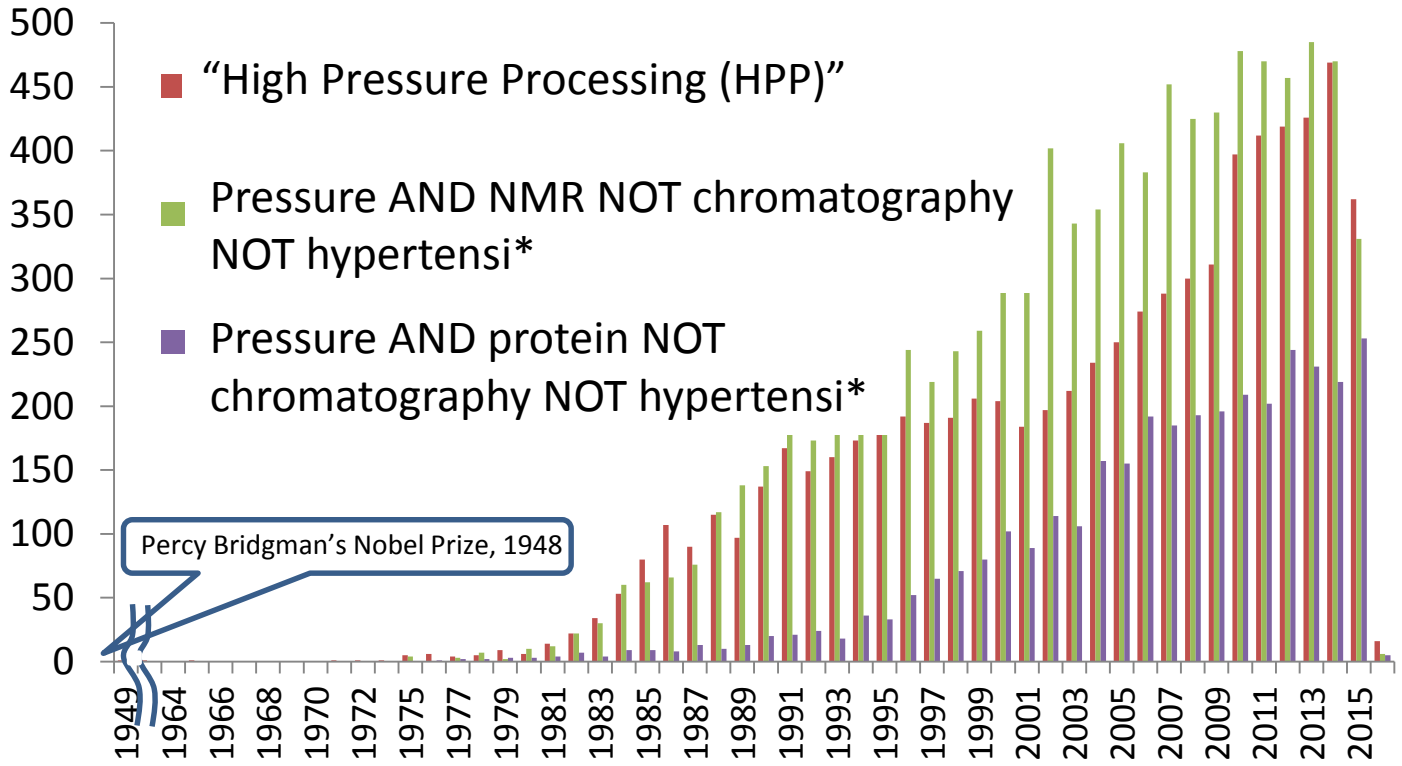
Specialized accessories

High-Shear small volume homogenizers

PBI's MISSION:

Lower the HIGH PRESSURE equipment barriers faced by scientists, so that they can make more discoveries.

GROWTH OF HIGH PRESSURE BIOSCIENCE RESEARCH



Number of PubMed-indexed research articles published per year (keywords shown) from Percy Bridgman's Nobel Prize until today. The high pressure bioscience is approaching a critical mass to become a widely accepted area of research!

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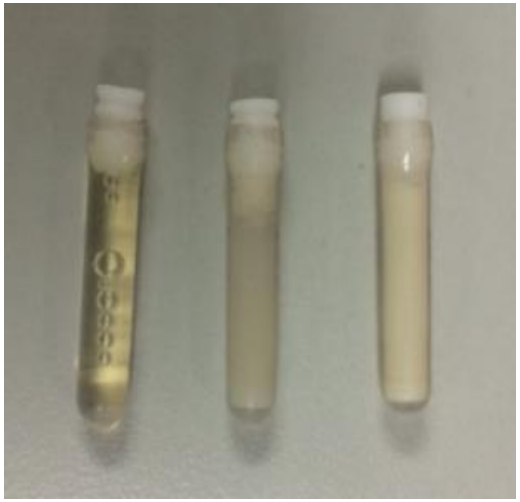
HISTORIC EXPERIMENT: COOKING AN EGG WHITE AT ROOM TEMPERATURE

Atm. 75,000 psi 90,000 psi



Atm. 75,000 psi 90,000 psi

30 minute incubation at 23°C

30 min

20 min

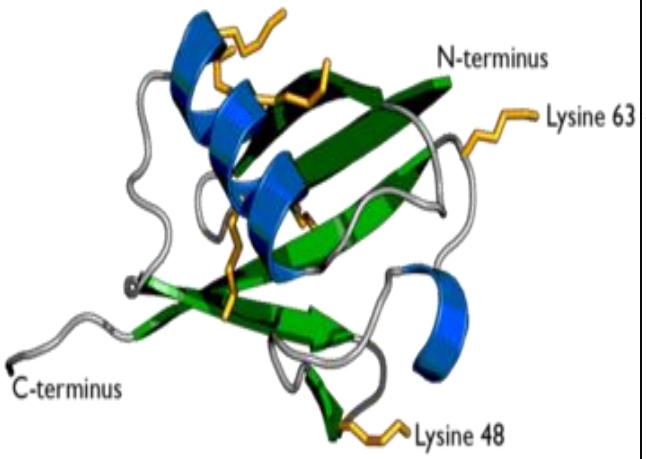
10 min

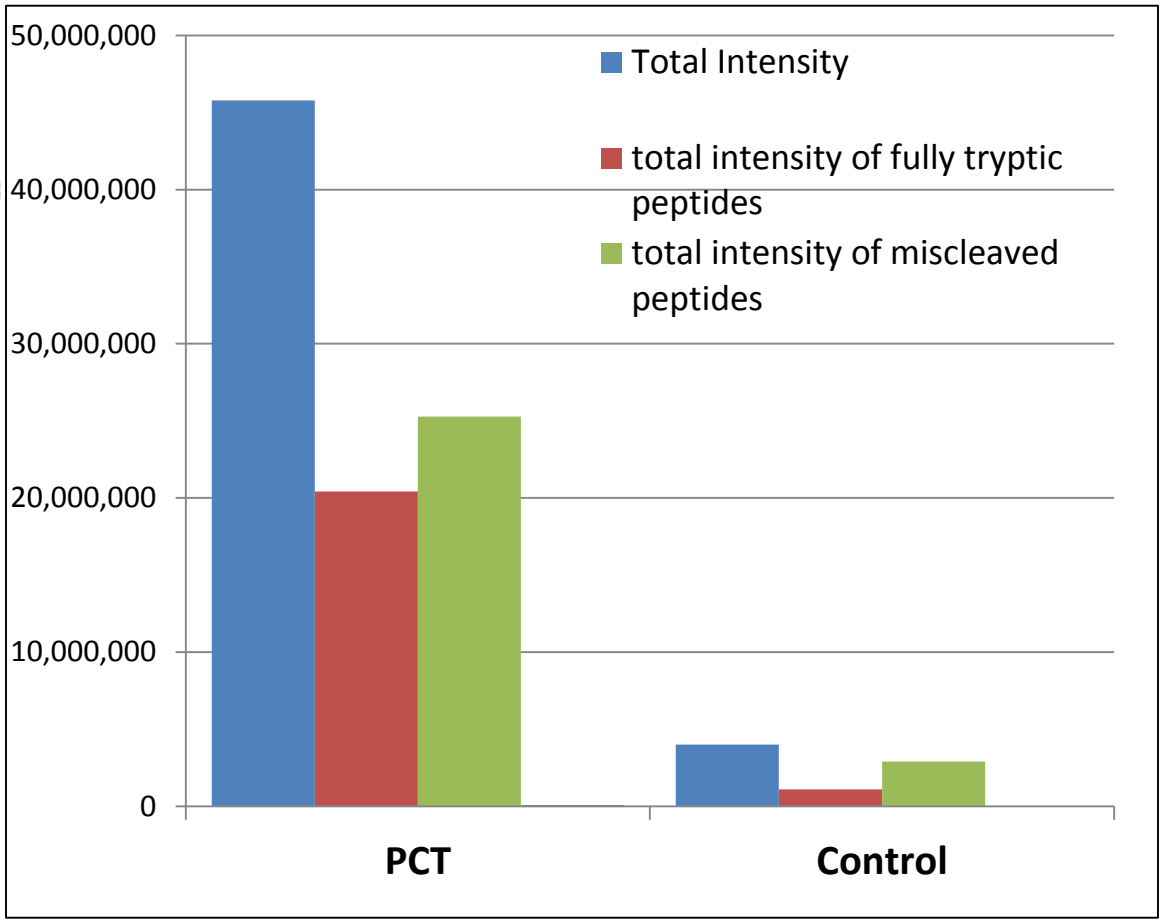
Time course at 90,000 psi

VERY HIGH PRESSURE LEADS TO PROTEIN DENATURATION

PBI Pressure BioSciences Inc.

TRYPTIC DIGESTION OF UBIQUITIN





PCT conditions: 20,000psi at 55^{0}C for 1.5 hours (90 cycles) in 50mM Ammonium bicarbonate.

Control conditions: Ambient pressure at 55^{0}C for 1.5 hours in 50mM Ammonium bicarbonate.

HIGH PRESSURE HELPS TO QUANTIFY DIFFICULT TO DIGEST PROTEINS

PBI Pressure BioSciences Inc.

PCT PRODUCTS FOR BIOLOGICAL SAMPLE PREPARATION AND BIOPHYSICS

Barocycler™ 2320EXT

58,000 psi

HUB440
HUB880

HUB880 EXPLORER

58,000 psi or up to 100,000 psi

RF 1700

25,000 psi

Applications:

Homogenization/Digestion of tissue samples.

Protein Characterization

Stress response in cell biology

Pressure perturbation spectroscopy, protein dynamics

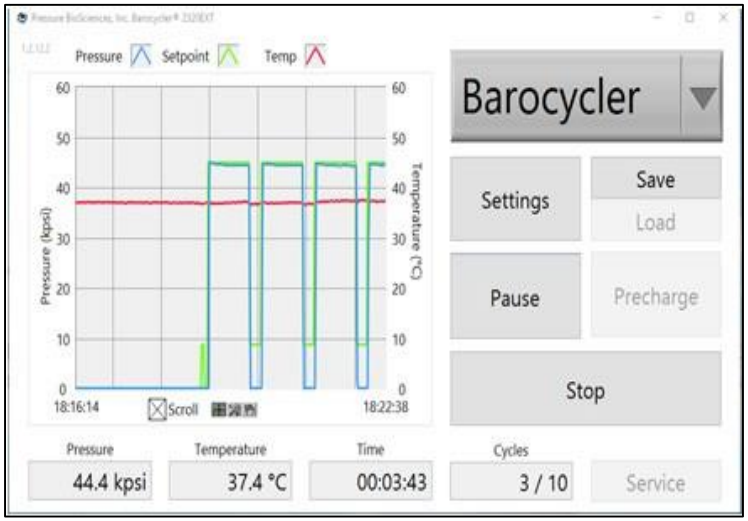
Food science, pathogen inactivation

Chromatography, UPLC column packing

PBI INSTRUMENTS MAKE HIGH PRESSURE AVAILABLE TO MANY LABS

PBI Pressure BioSciences Inc.

BAROCYCLER 2320EXT: EXTREME INSTRUMENT FOR PROTEOMIC SAMPLE PREPARATION



Specifications:

- 16 samples per batch
- Full support for MicroPestle platform
- Pressure: up to 45,000 psi (3.1 kilobar)
- Temperature: 4°C to 95°C (external chiller)
  or Ambient to 95°C (built-in electrical heater)
- Unlimited pressure programming
- Real-time graphs of pressure and temperature
- cGxP-compliant data logging and audit trails
- Enhanced security and user management
- Safe low voltage (24V DC) operation

PBI Pressure BioSciences Inc.

PBI-SCIEX PARTNERSHIP

- In 2015 PBI and SCIEX signed a co-marketing agreement to take advantage of PCT-SWATH approach pioneered by Dr. Tiannan Guo at Ruedi Aebersold's lab at ETH – Zürich.

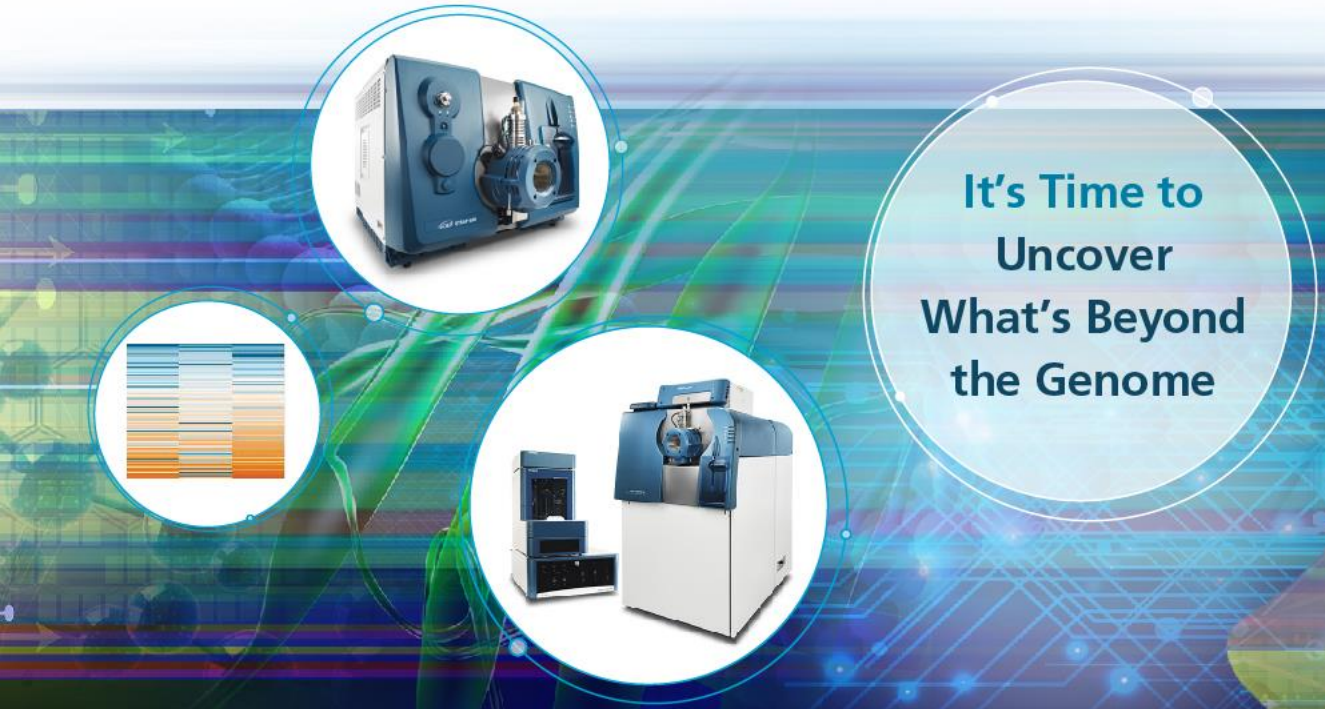
- PCT methods help to ensure reproducibility and quantitative recovery of protein signatures from a wide variety of tissue samples.

THANK YOU!

PBI Pressure BioSciences Inc.

SCIEX

Answers for Science.
Knowledge for Life.™



SWATH® For Quantitative Proteomics

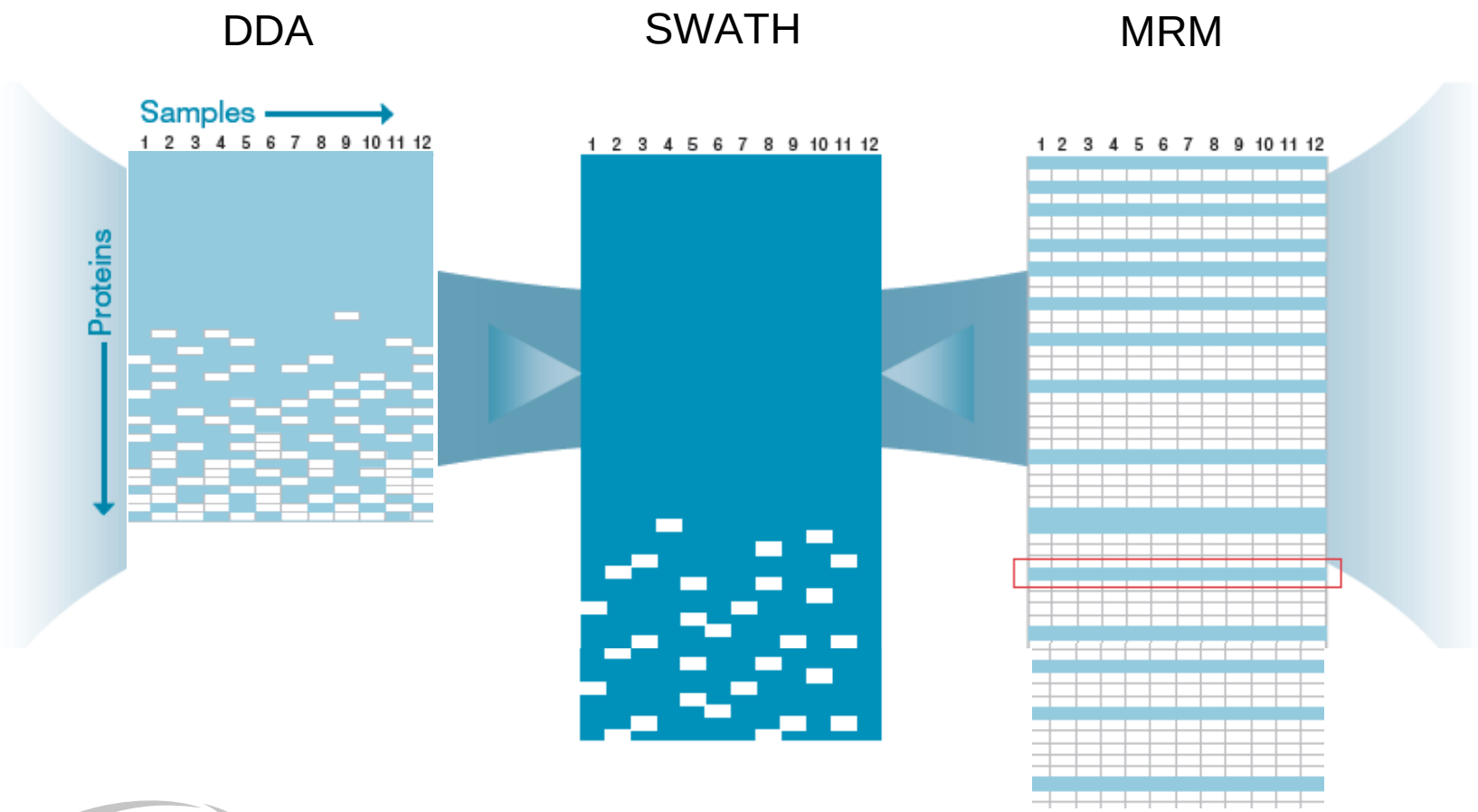
Randy Arnold, PhD – SCIEX

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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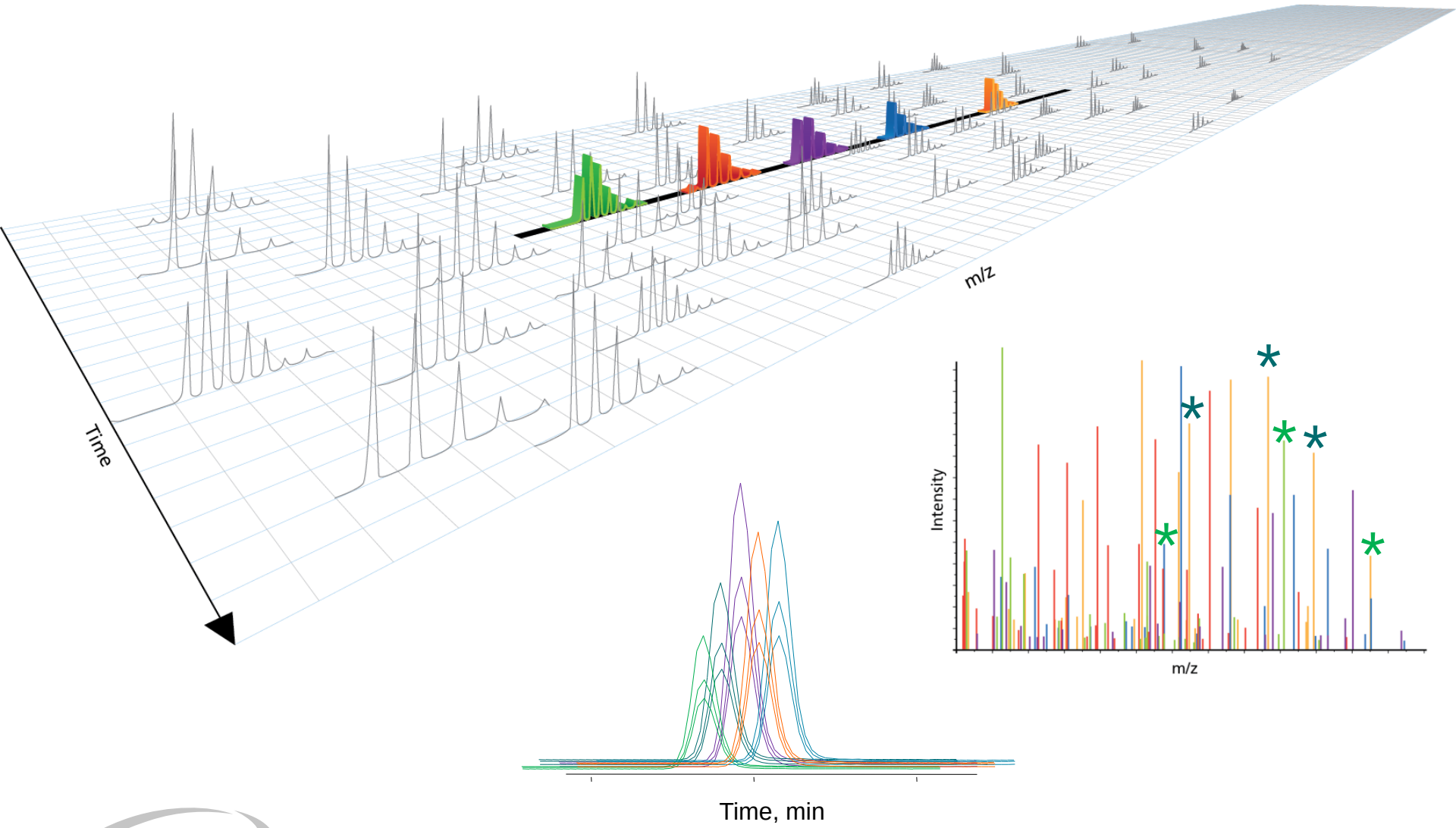
MS/MS^{ALL} with SWATH® Acquisition



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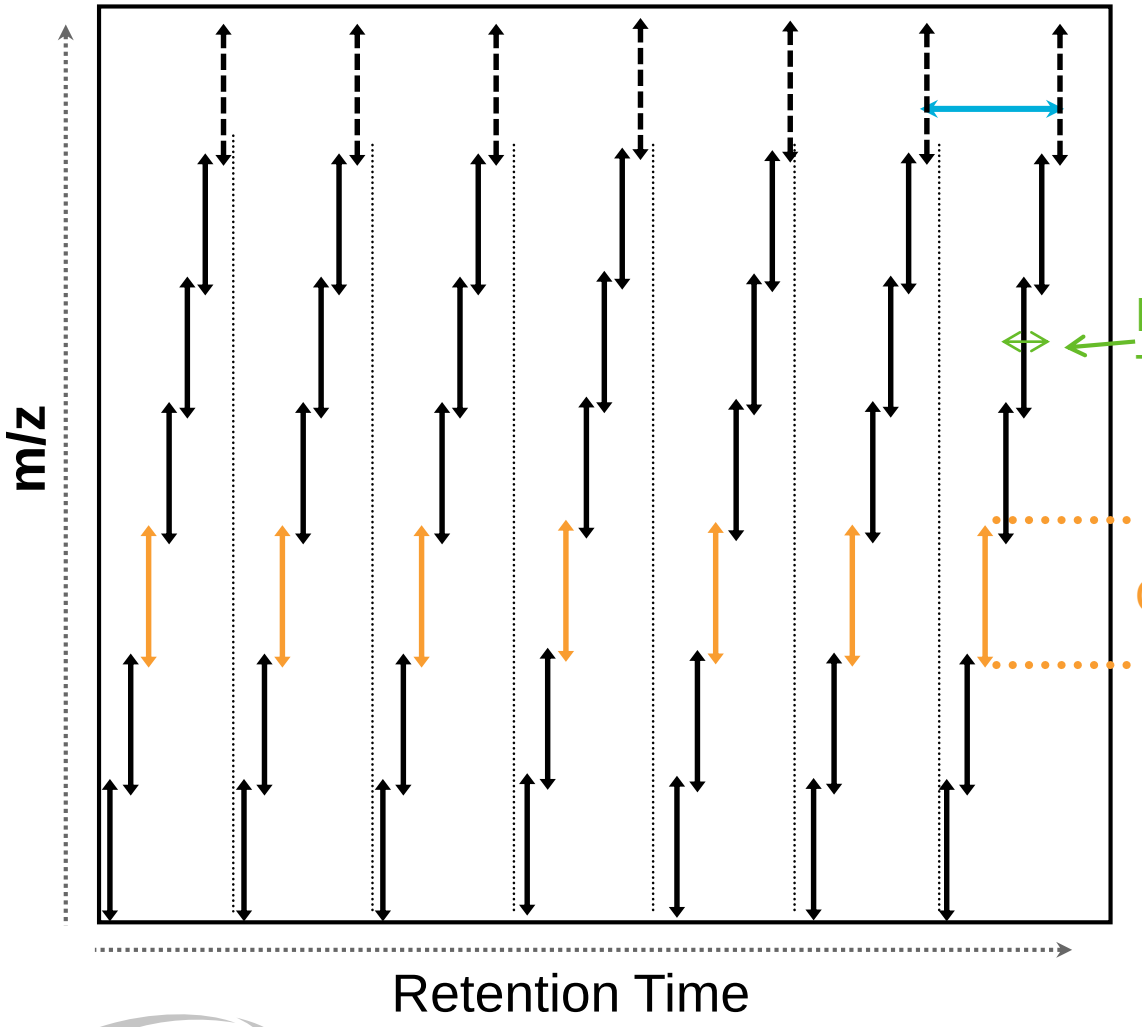
Q1 Isolation Strategy

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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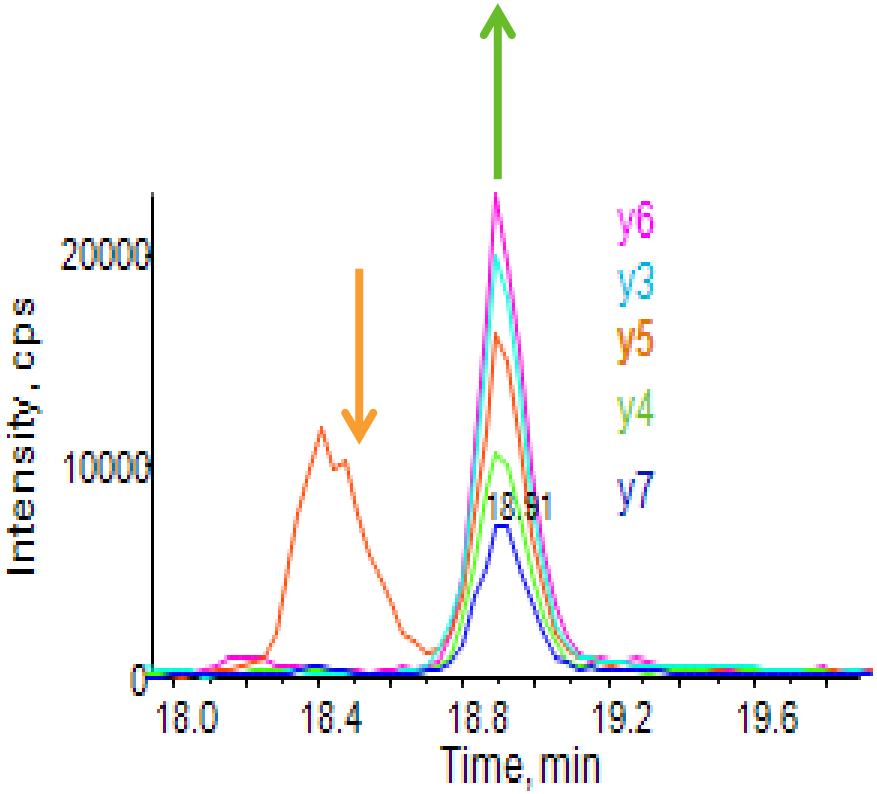
Extending Depth of Coverage

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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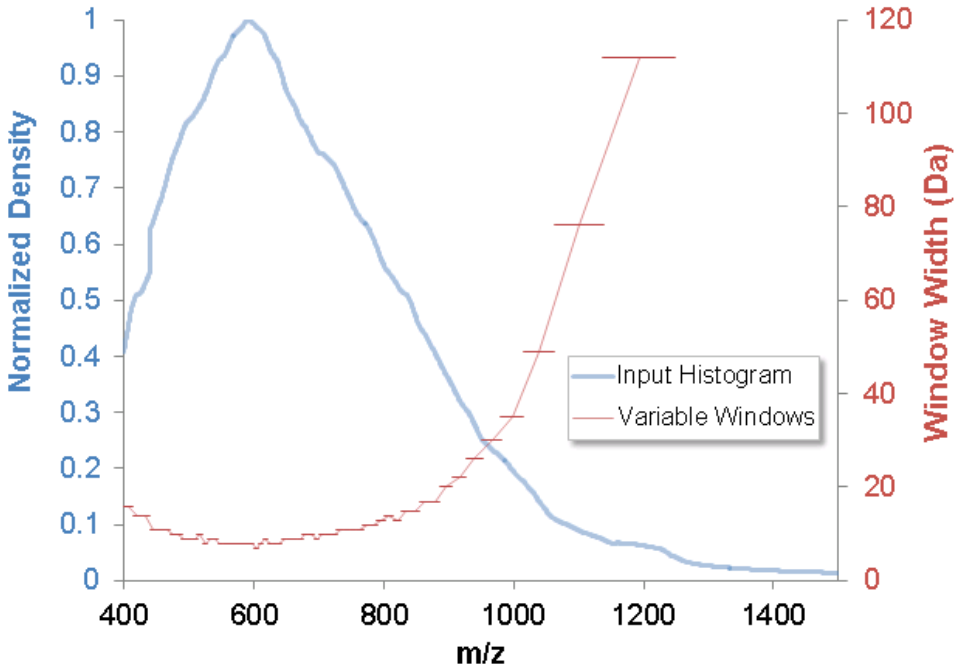
Variable Window SWATH® Acquisition

- 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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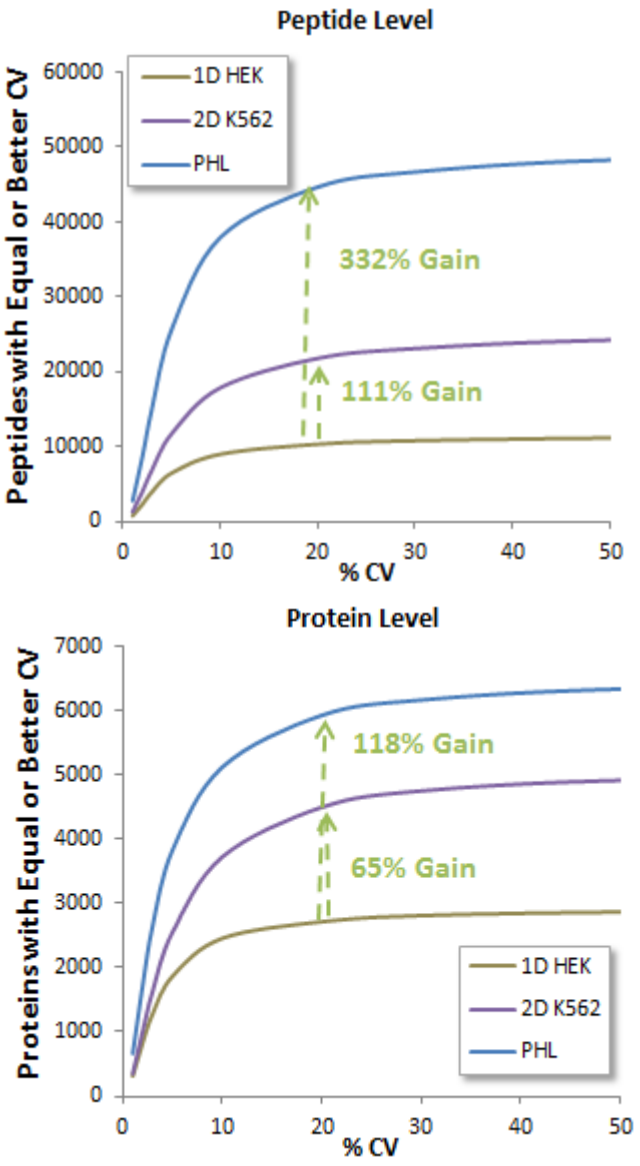
Extraction of Quantitative SWATH® Data from Human Lysate

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.



SCIEX Answers for Science. Knowledge for Life.™

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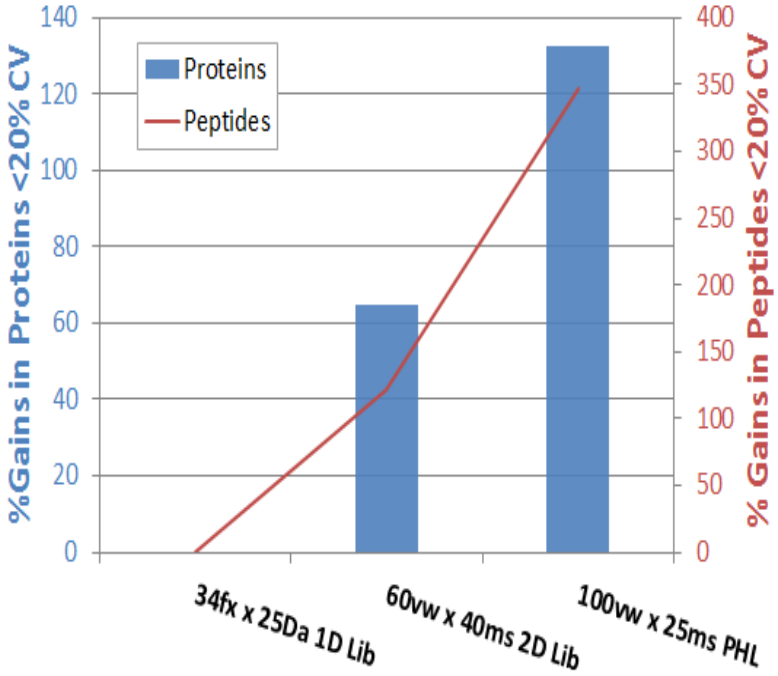
Gains in Quantified Proteins and Peptides Quantified

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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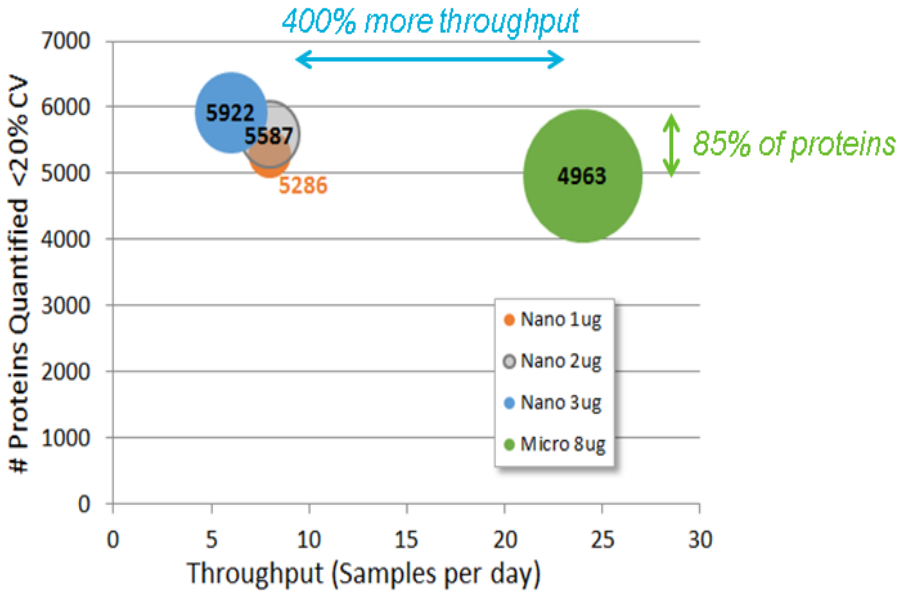
Industrialized Quantitative Proteomics

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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Knowledge for Life.™

Questions?

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THANK YOU !

www.pressurebiosciences.com

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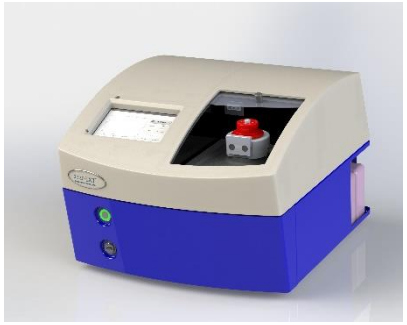

ICE CREAM & CAR RACES

8-11pm Suite 313