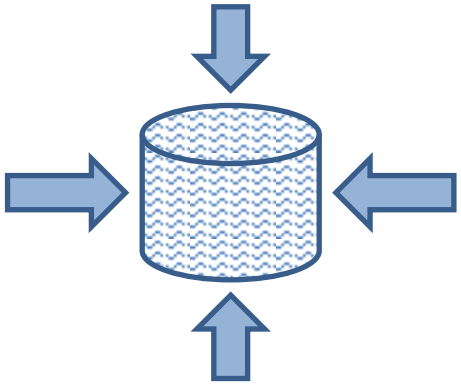
From PCT-HD to SWATH® Data Acquisition in Half a Day

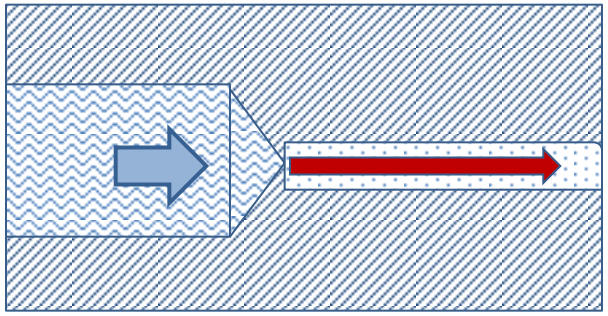
Vera Gross, Ph.D.
Senior Scientist

PBI Pressure BioSciences Inc.

Gentle Hydrostatic Pressure vs. Disruptive Hydrodynamic Pressure

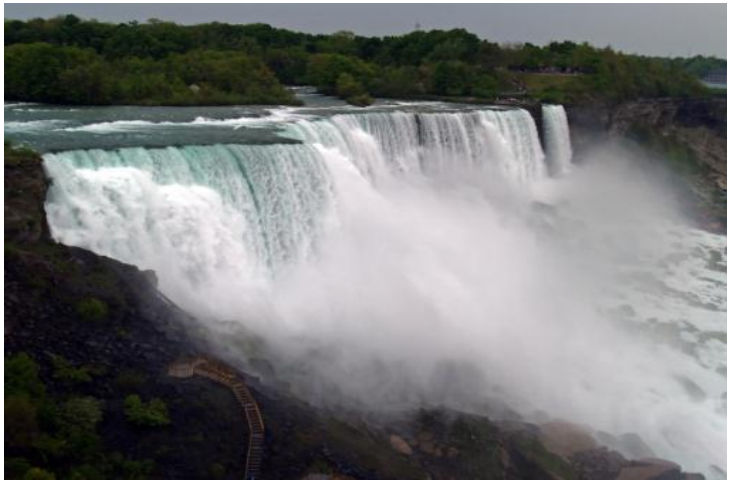


Hydrostatic pressure is associated with only small temperature changes

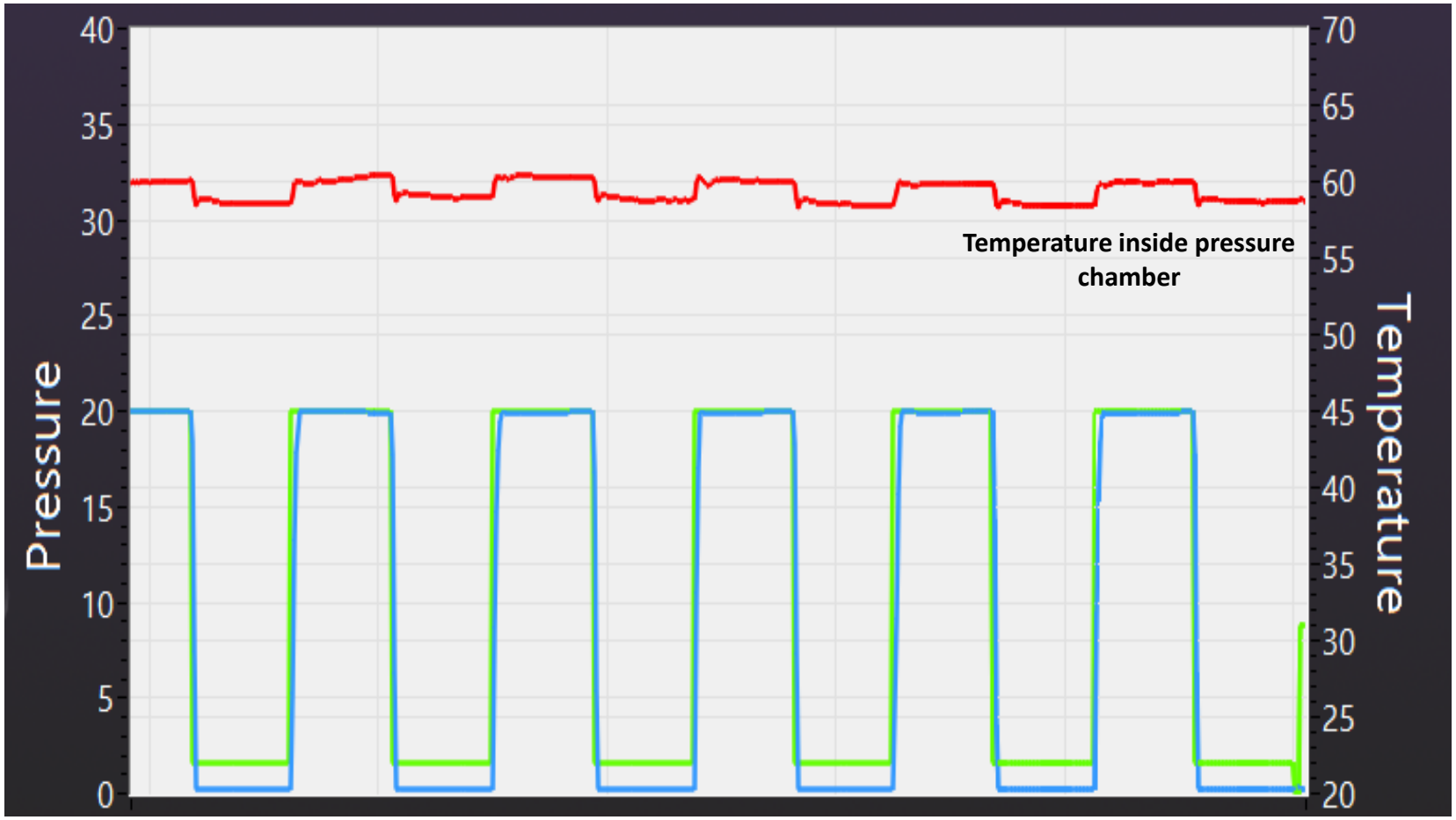


Hydrodynamic pressure transforms kinetic energy into mechanical shearing and heat

James Cameron in deep sea submersible for expedition to Marianas Trench in 2012 (© Natl. Geographic).
The Challenger Deep is 6.8 miles below the surface with a pressure of 15,750 psi (1000 Atm)



Pressure Cycling Technology (PCT)

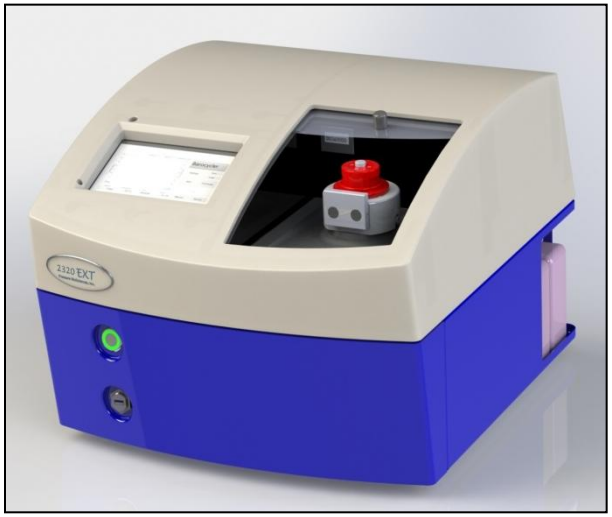


Pressure and temperature inside Barocycler pressure chamber during pressure cycling at 20 kpsi.

The higher the starting temperature, the greater the adiabatic temperature change during pressure cycling. At 60^{o}C, the ΔT is less than ± 2^{o}C.

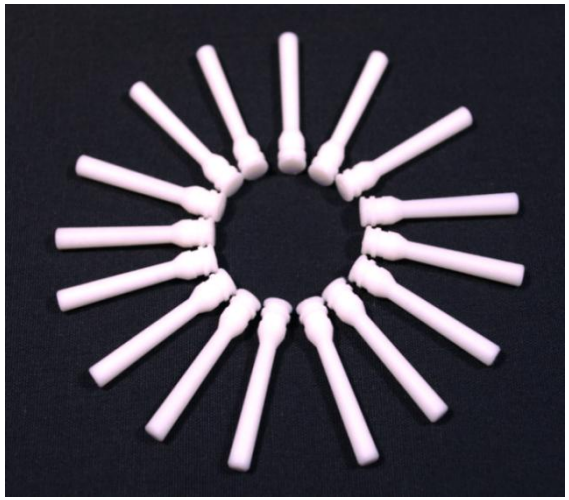
PCT-HD

Protein Extraction From Fresh or Frozen Tissues using Pressure Cycling and the PCT µPestles, Followed by Digestion with Proteolytic Enzymes such as Lys-C and/or Trypsin.



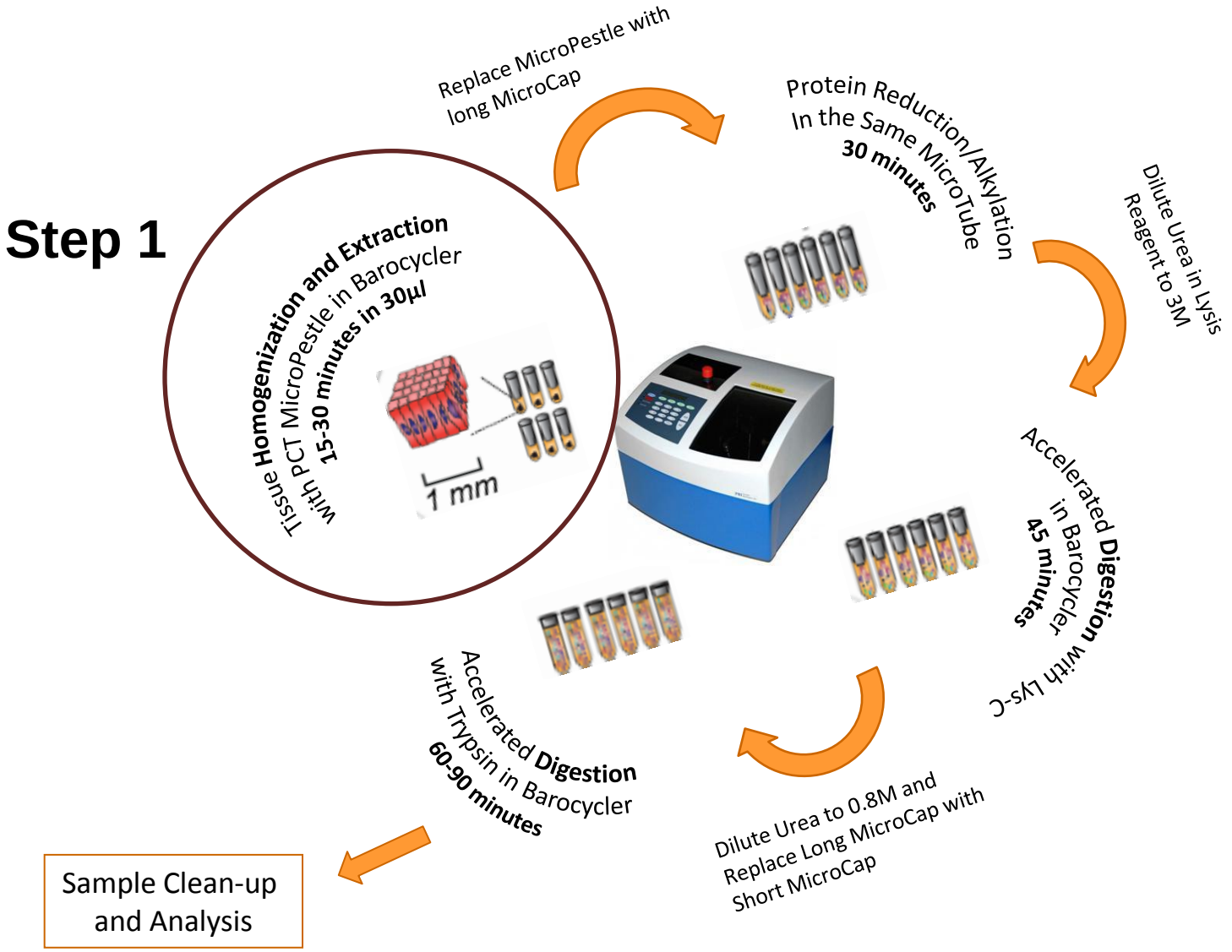
Barocycler 2320EXT

PCT MicroTubes

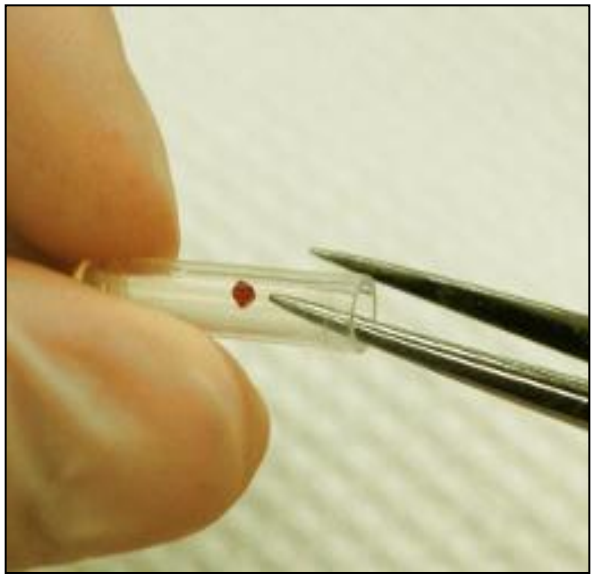


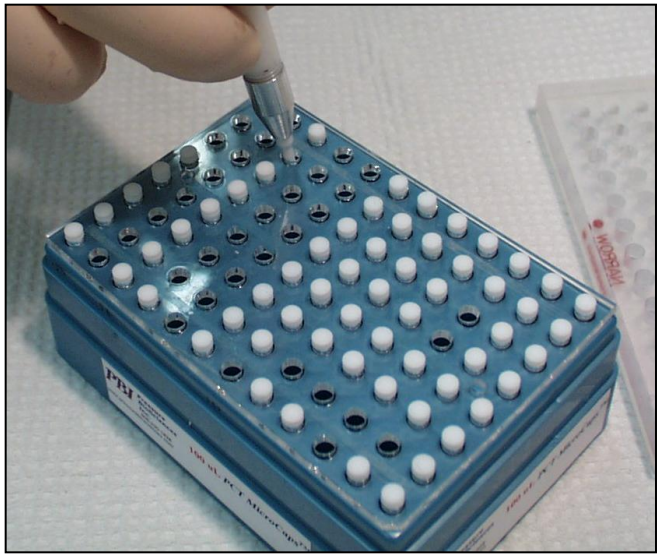
PCT µPestles

PCT-HD
Tissue Homogenization



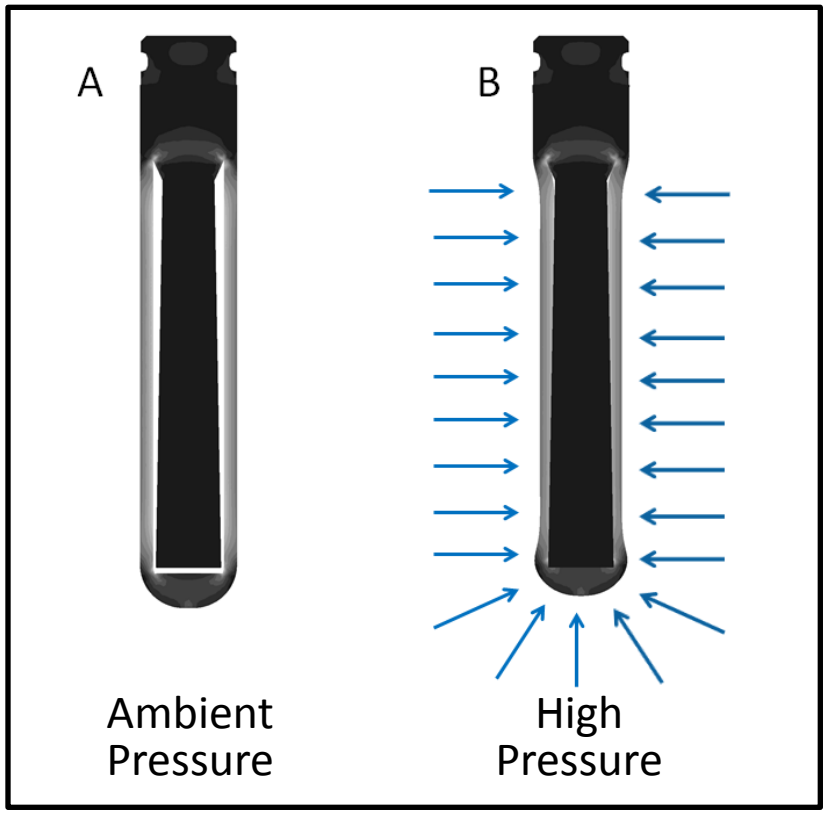
Convenient and Efficient Tissue Homogenization of up to 16 Samples Simultaneously

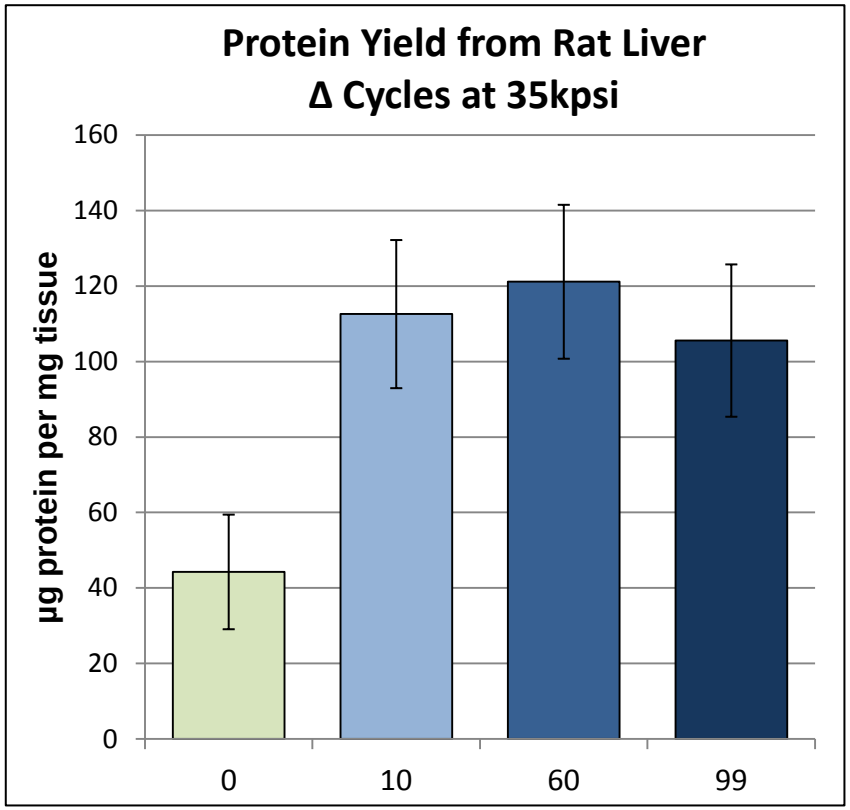




Place tissue (up to 3mg) into MicroTube → Add 30µl of lysis reagent (eg, 4M urea) → Pick up µPestle using the Capper Tool and insert it into the MicroTube → Place tubes into Barocycler cartridge → drop cartridge into pressure chamber→ PCT 60 cycles (~30 minutes).

MicroPestle Homogenization by PCT

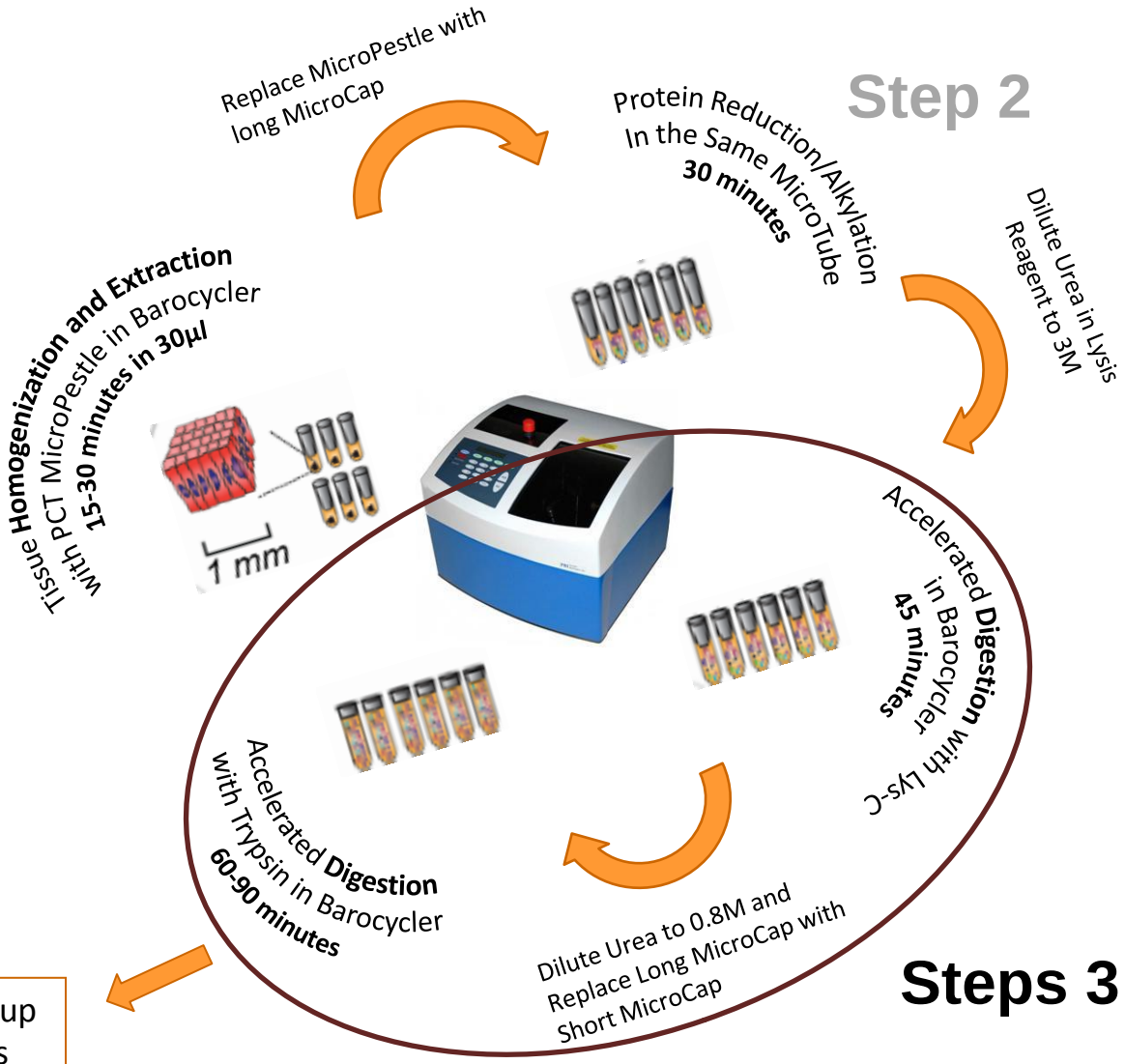




At each pressure cycle the tube is compressed, causing it to narrow and shorten.

This mechanical action repeatedly squeezes the tissue through the narrow space around the pestle tip, resulting in a Dounce homogenizer-like effect and leading to efficient, hands-off homogenization.

PCT-HD
Pressure-Enhanced Protein Digestion



Convenient and Efficient Protein Digestion of up to 16 Samples Simultaneously

50µl

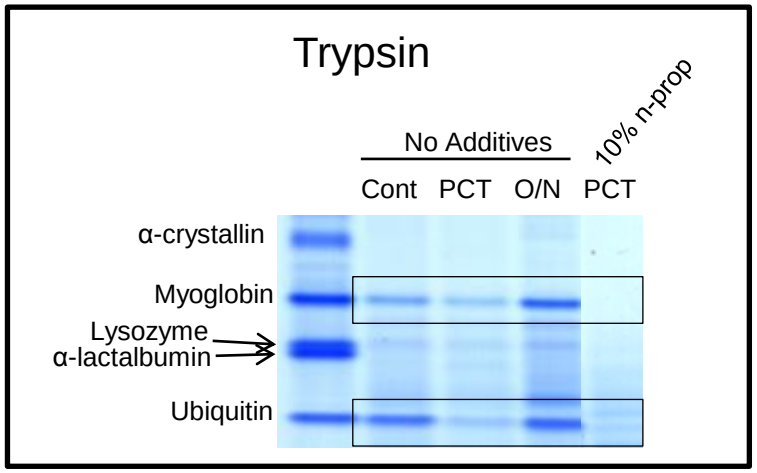
100µl

150µl

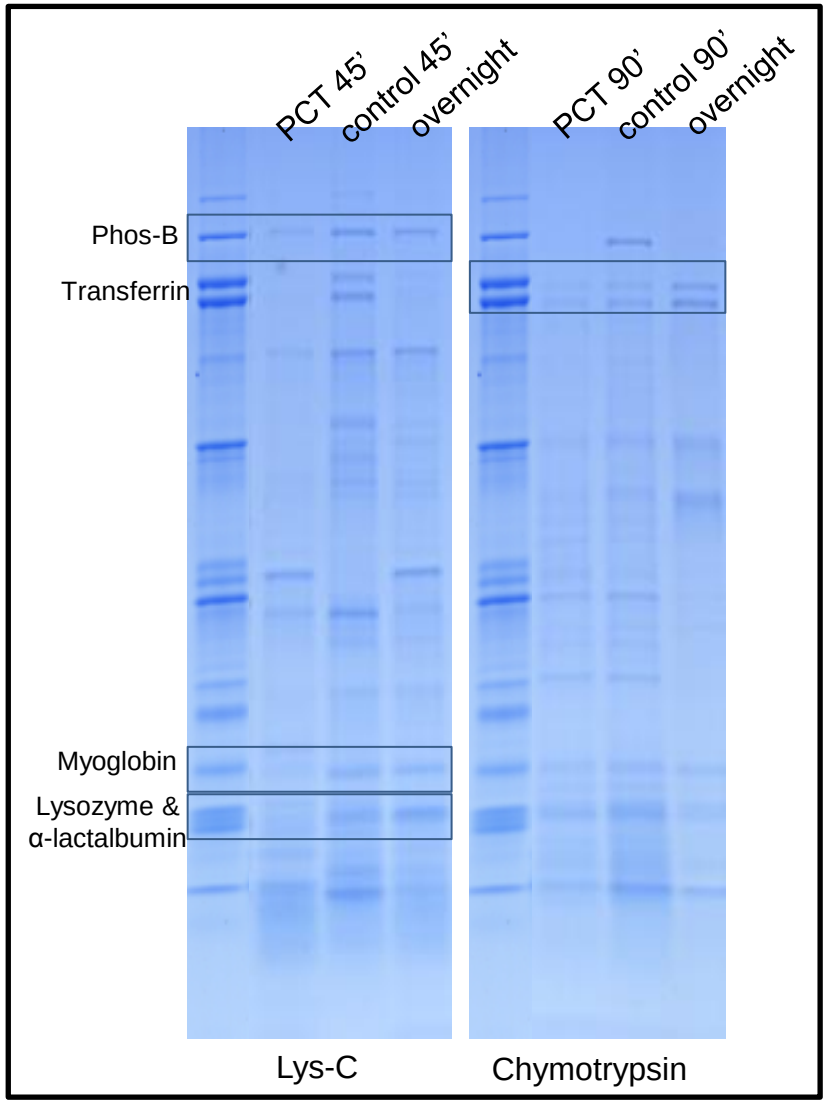
Discard MicroPestle → Add appropriate reagents/enzymes → Seal MicroTube with MicroCap → Perform PCT at desired temperature and pressure.

MicroCaps of different lengths are available for use with small (~50ul), medium (~100ul) and large (150ul) sample volumes.

Digestion Under Pressure:
Better Digestion of Difficult-to-Digest Proteins

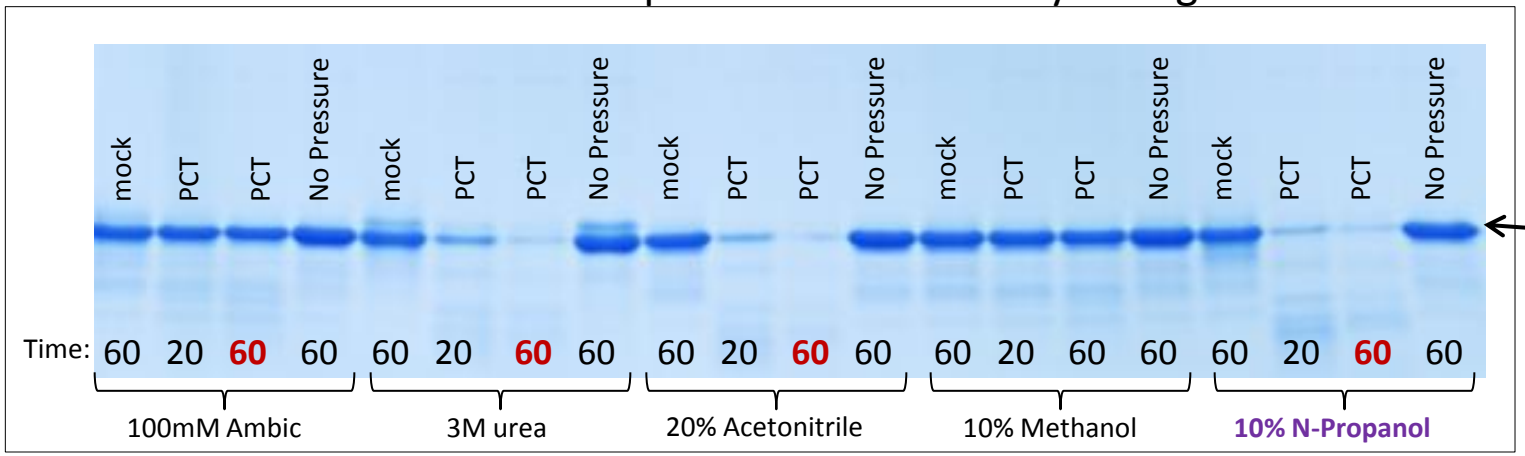


A mixture of 15 model proteins was digested with or without pressure cycling at 20,000kpsi (trypsin) or 45,000psi (lys-C and chymo). Positive control digests were incubated overnight at 35-37°C with shaking. Selected proteins that were poorly digested in the overnight digests are indicated.

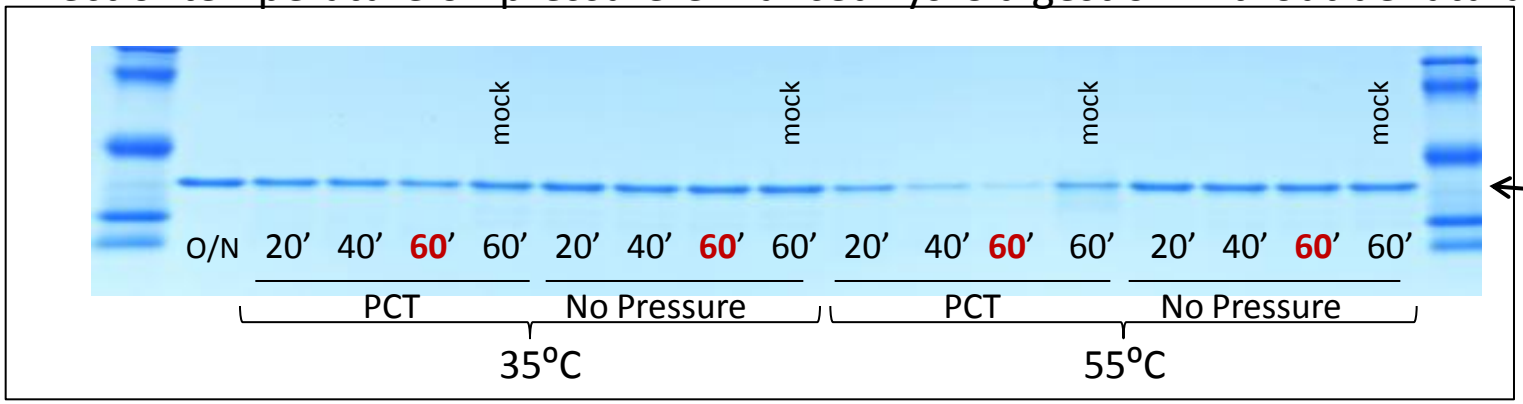


Chemistry, Temperature and Pressure: Synergistic Effects on Digestion Efficiency

Effect of various denaturants on pressure-enhanced Lys-C digestion at 35°C

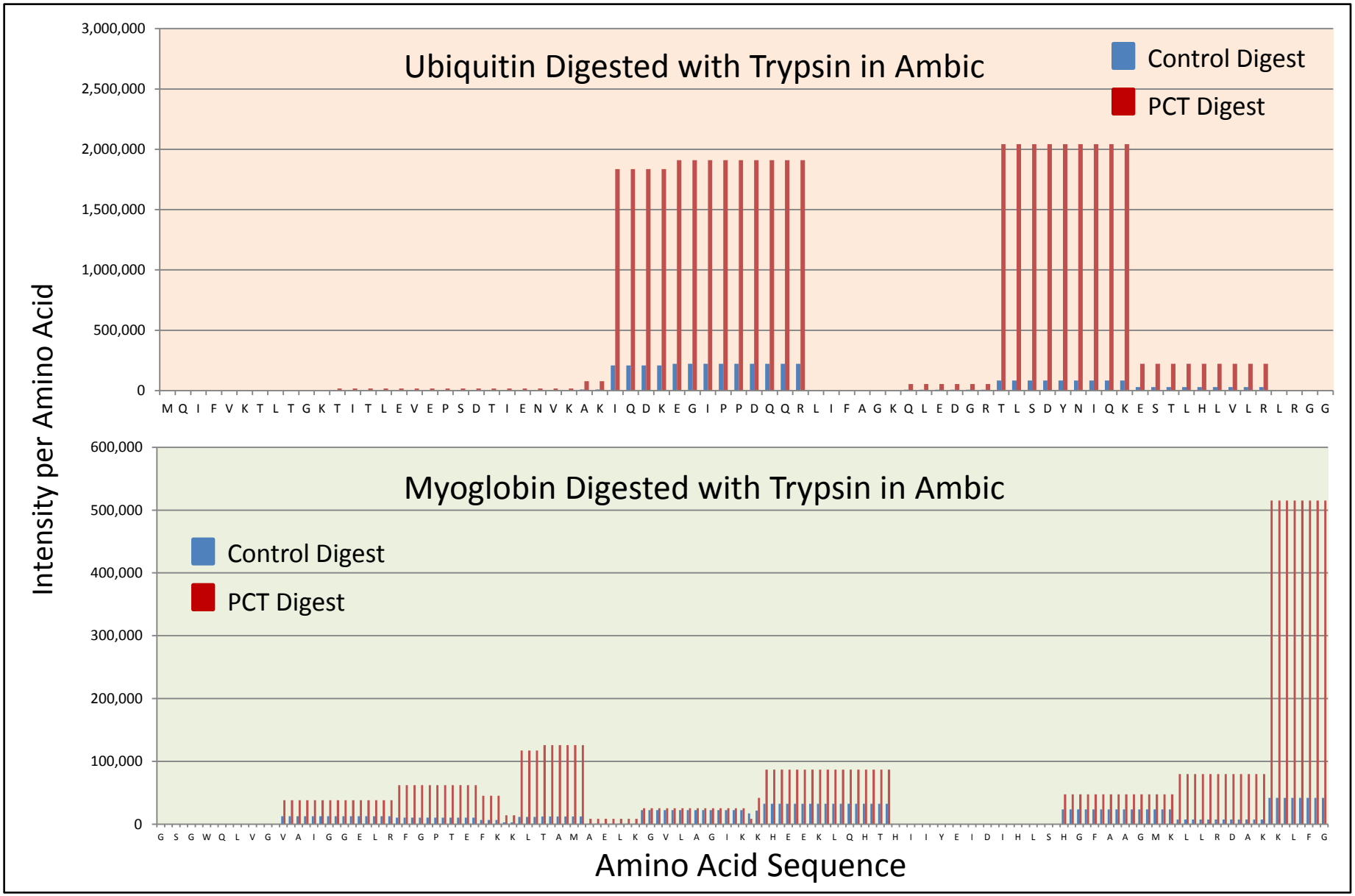


Effect of temperature on pressure-enhanced Lys-C digestion without denaturants

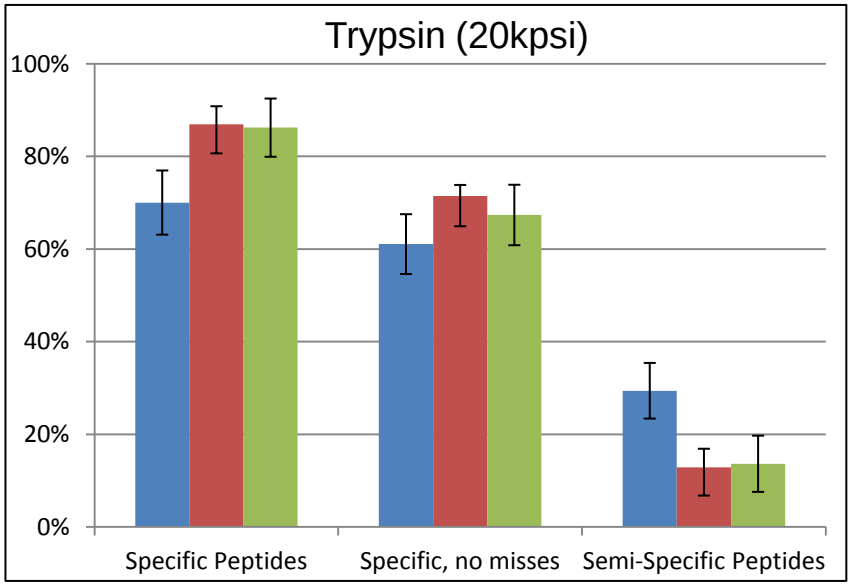


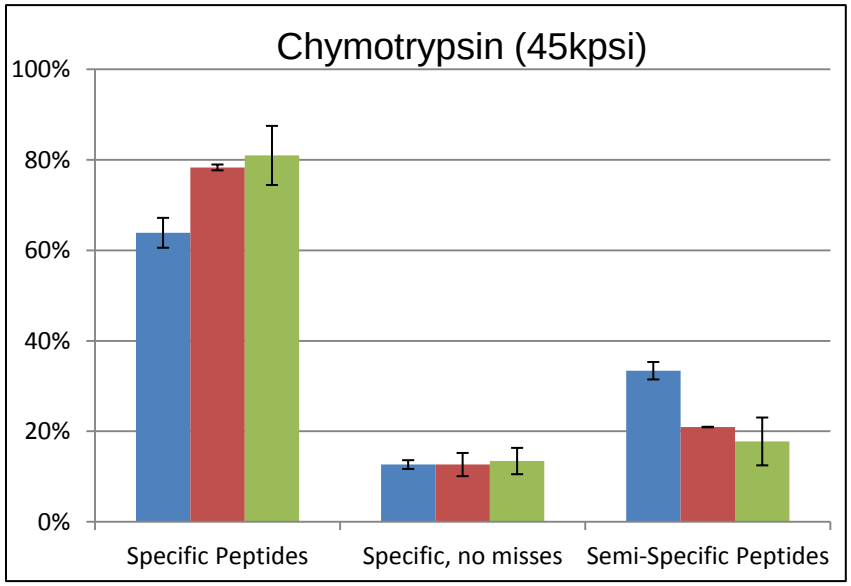
One Word of Warning: The synergy of pressure and chemistry can combine to denature not only substrate proteins, but enzymes as well. It is important to test enzyme activity at pressure when incorporating new reagents (solvents, detergents, etc) into the PCT-HD protocol.

Trypsin Digestion Under Pressure: More Peptide Intensity and Better Sequence Coverage



Accelerated Digestion Under Pressure: Specificity and Miscleavage Rates are Comparable to, or Better Than, Unpressurized Controls

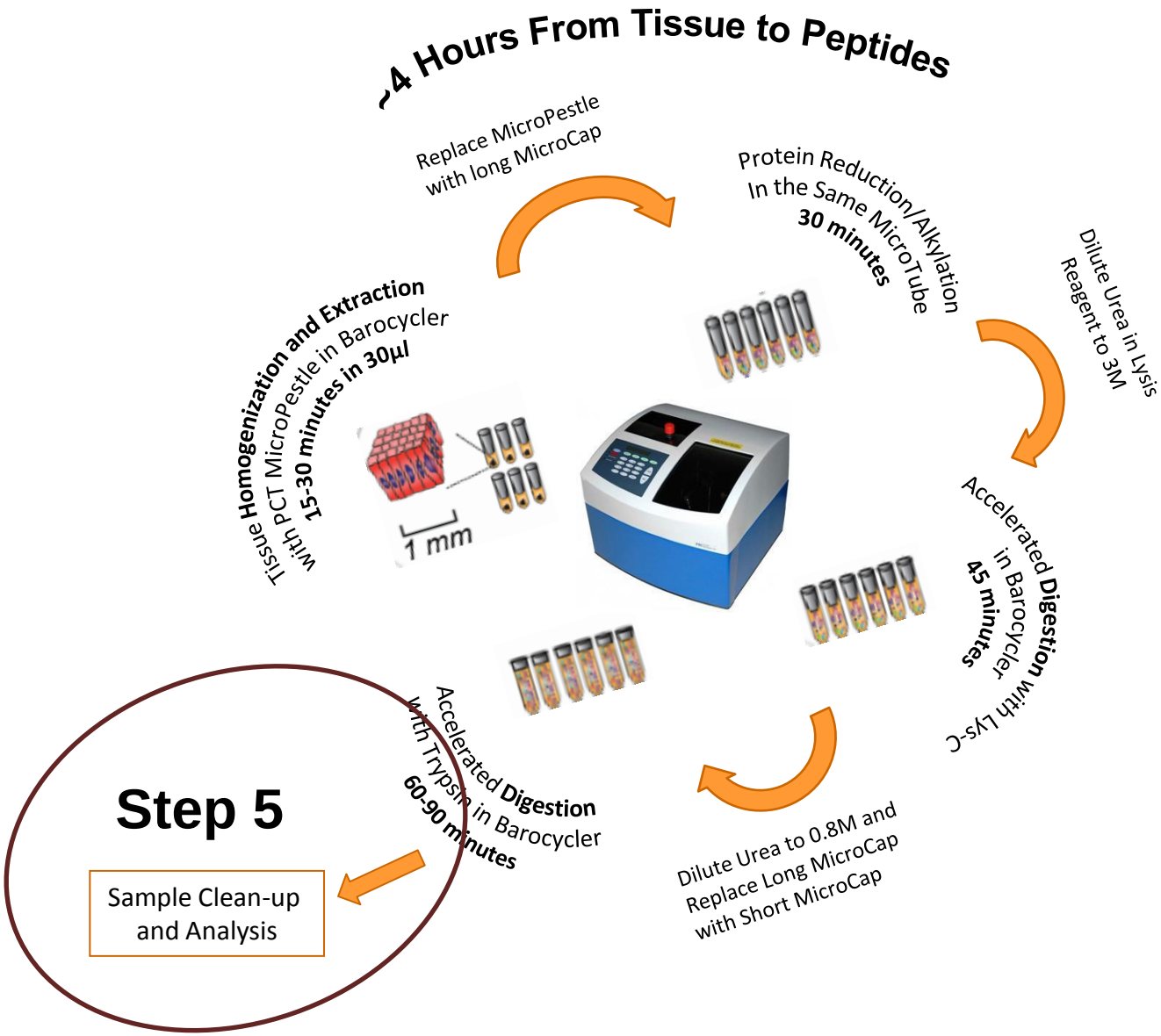




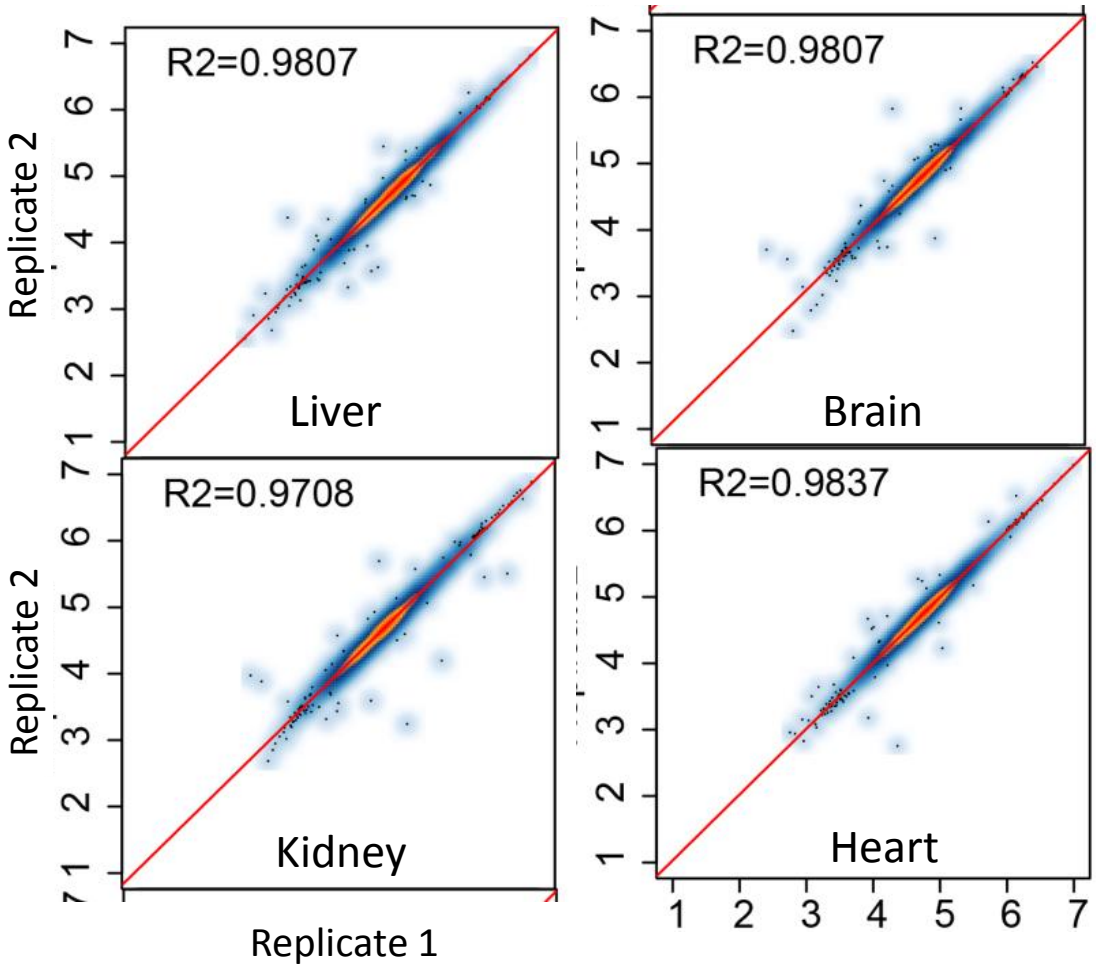
Overnight Digest PCT Digest Atm Digest control

A mixture of 15 model proteins was digested with the indicated enzyme for 90 minutes ±PCT or overnight.

PCT-HD Sample Preparation Protocol



Combining PCT-HD with SWATH-MS
Excellent Sample-to-Sample Reproducibility



Proteins were extracted from liver, kidney, brain and heart tissues and digested with lys-C followed by trypsin using the PCT-HD workflow. Peptides were analyzed by SCIEX SWATH-MS. Duplicate biological replicates were compared and demonstrate excellent sample-to-sample reproducibility (log intensity shown on X and Y axes).

Data kindly provided by Dr. T. Guo at ETH Zurich and Dr. S. Shao at Huazhong University of Science & Technology, Wuhan ,PRC.

Benefits of PCT-HD for Proteomic Applications

- Convenient and effective, hands-off tissue homogenization and protein extraction of up to 16 samples simultaneously.
- Improved recovery of hydrophobic proteins (membranes).
- Single-tube workflow minimizes sample loss and cross-contamination.
- Digestions with Trypsin and other enzymes are accelerated by pressure cycling.
  - Lys-C, Chymotrypsin, Glu-C, PNGase F, Lysozyme, and others.
- Reduced concentrations of harsh denaturants such as urea and guanidine-HCl simplify peptide clean-up.
- Better digestion of difficult-to-digest proteins.
- No increase in non-specific cleavage.
- No increase in peptide modifications like oxidation and deamidation (preliminary data).

Thank You

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