

Pressure BioSciences, Inc.

**Pressure Cycling Technology (PCT):
Applying High Hydrostatic Pressure to
Environmental Proteomics**

**International Workshop on Environmental Proteomics
January 20, 2010
Nate Lawrence**

Discovery Starts With Sample Preparation

PBI

History of High Pressure in Life Sciences

- **1623-1662:** Blaise Pascal – described fundamental concepts of pressure and vacuum
- **1895:** H. Royer – pressure kills bacteria
- **1899:** B.H. Hite *et al.* – pressure preserves milk
- **1914:** P.W. Bridgman - pressure coagulates egg white
- **1989:** High pressure processing of food products
- **2000:** First International Conference on HPBB
- **2008:** Fifth International Conference on HPBB in the USA

Pressure Cycling Technology (PCT)

PCT is a Novel, Enabling Technology that Uses
Cycles of Hydrostatic Pressure Between
Atmospheric and Ultra-high Levels (up to
35,000 psi and greater) to Allow for the
Precise Control of Biomolecular Interactions

Understanding Hydrostatic Pressure



U.S. Navy Bathyscaphe
Trieste (1958-1963)



Marianas Trench:
38,713 ft (11,800m) deep
16,000 PSI (120MPa)

Significant portion of the Global Biosphere is
subjected to high hydrostatic pressure!

Current Extraction Methods

- Mortar & Pestle
- Chemistry
- Dounce homogenizer (glass on glass)
- Potter-Elvehjem homogenizer (Teflon on glass)
- Enzymatic Digestion
- Polytron shearing homogenizers
- Blenders
- Bead Beating
- Sonication
- Repeated Freeze/Thaw cycles
- French Press (≤ 2000 PSI)



*The PCT Sample Preparation System
A Methods Development Tool*



BarocyclerTM NEP2320



BarocyclerTM NEP3229

Why It Works

- Pressure is a Thermodynamic Process
- Compressibility of Water
- Synergy of Pressure, Temperature and Chemistry

User-Adjustable Variables

- Pressure (up to 35 kpsi)
- Number of Cycles
- Cycle Profile
- Chemistry
- Temperature

The FT500 PULSE Tube

Specially designed multi-functional tube

- Single-Use
- Versatile, works with:
 - Standard and custom reagents
 - Various sample types
 - Range of sample sizes
- Convenient
- Efficient
- Safe: closed tube, sample fully-contained



Diskless PULSE Tube



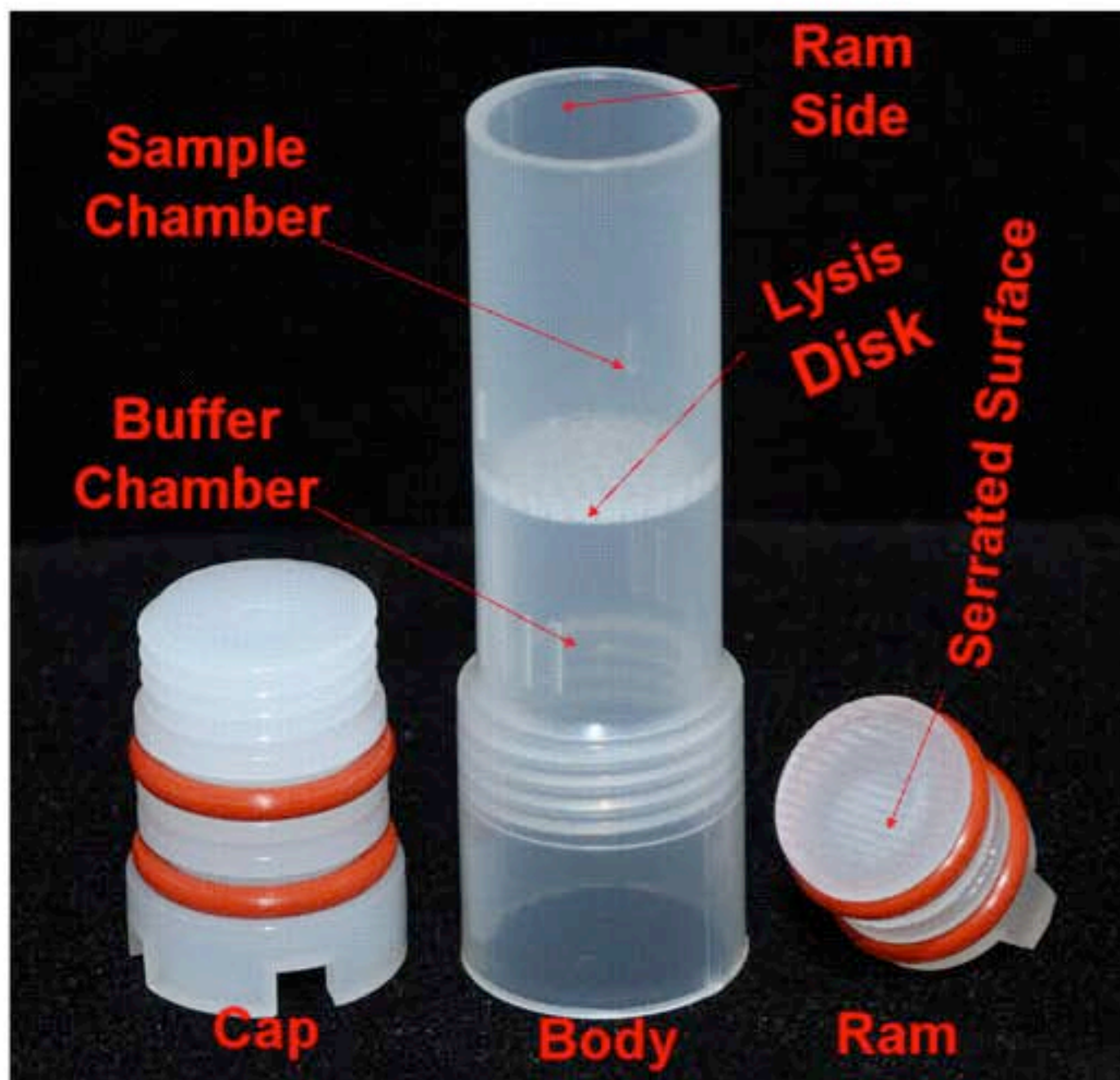
FT500-ND PULSE Tubes

Mechanical



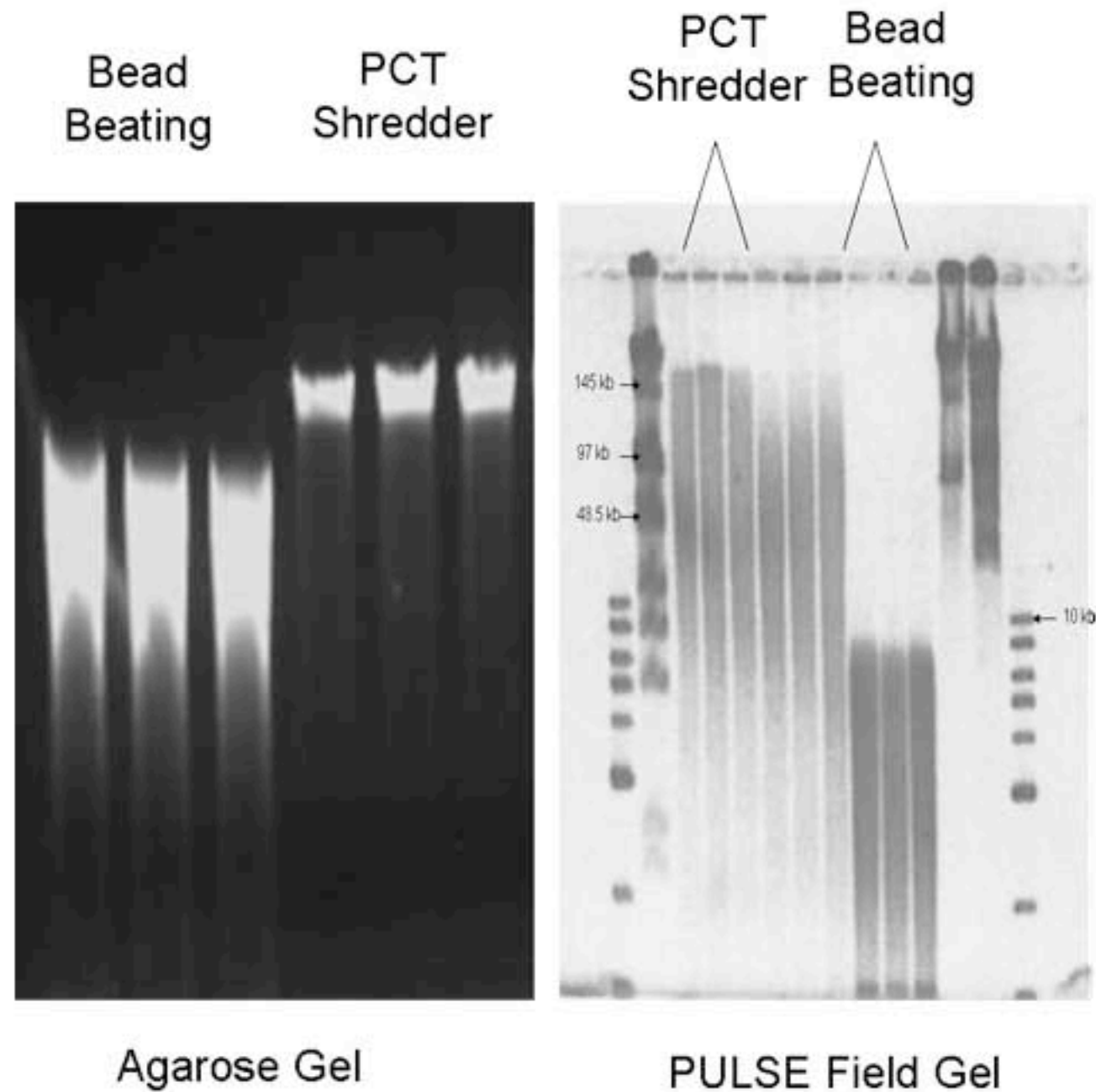
The PCT Shredder

The PCT Shredder PULSE Tube



**Release of DNA
with the
PCT Sample Preparation
System
(PCT SPS)**

DNA Extracted from Spinach Leaf



Summary Findings of Okubara et al. (USDA)

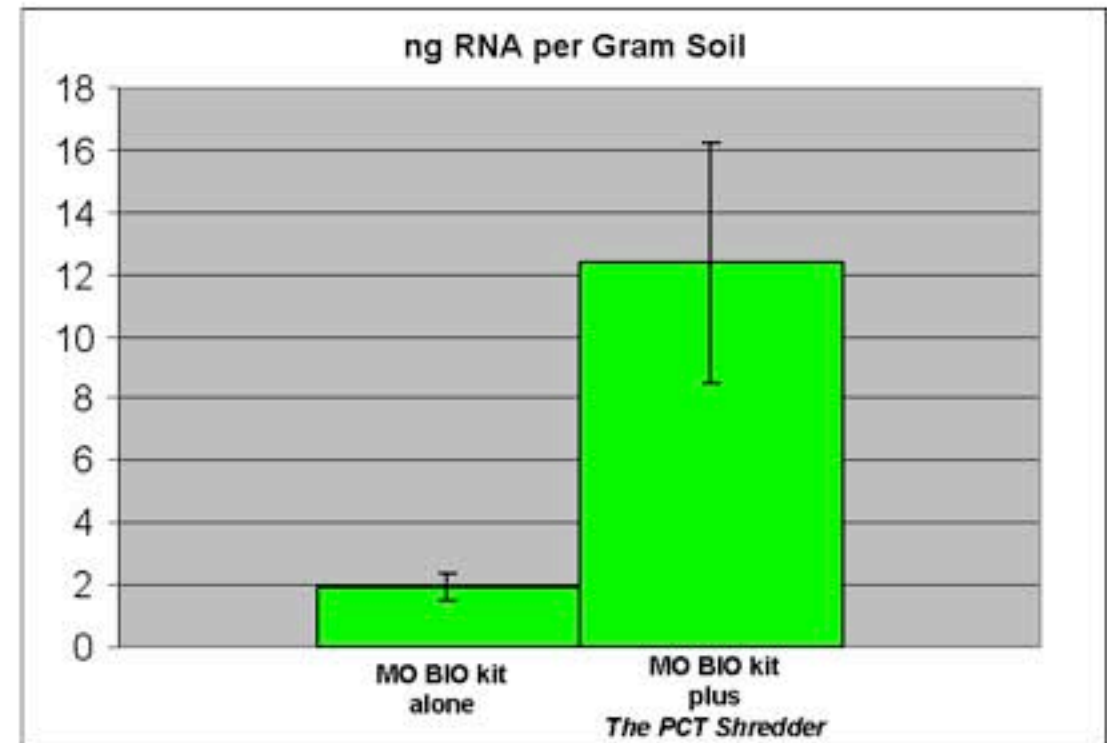
- Using a novel extraction system that uses Pressure Cycling Technology (PCT), we have obtained *Rhizoctonia* DNA from lyophilized wheat roots that were recalcitrant to homogenization.
- PCT also improved the extraction of *Rhizoctonia* and *Pythium* DNA from agricultural soils up to 16-fold compared to a bead beating extraction method.
- Furthermore, reproducibility of the extraction was so reliable that pathogen quantification generally could be derived from a single rather than triplicate extractions.

Transcriptomics

Release of RNA with the PCT Sample Preparation System (PCT SPS)

RNA Extracted from Soil

ng RNA/gm Soil MO BIO Protocol Alone	ng RNA/gm Soil MO BIO Kit Plus <i>The PCT Shredder</i>
1.6	14.5
1.9	14.5
1.7	6.5
2.5	14.0



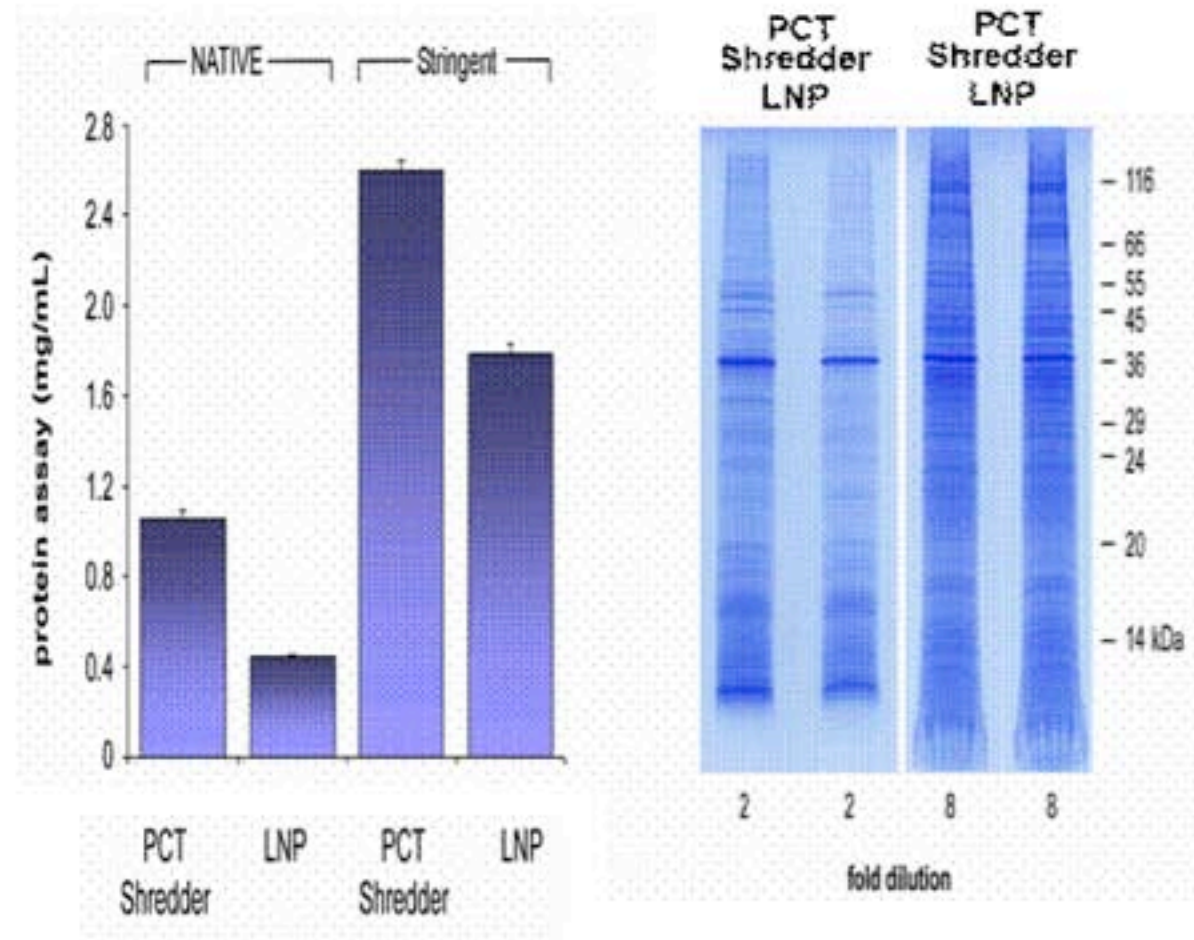
Proteomics

Proteins Under Pressure:

Applications of Pressure Cycling Technology in Proteomics and Protein Biochemistry

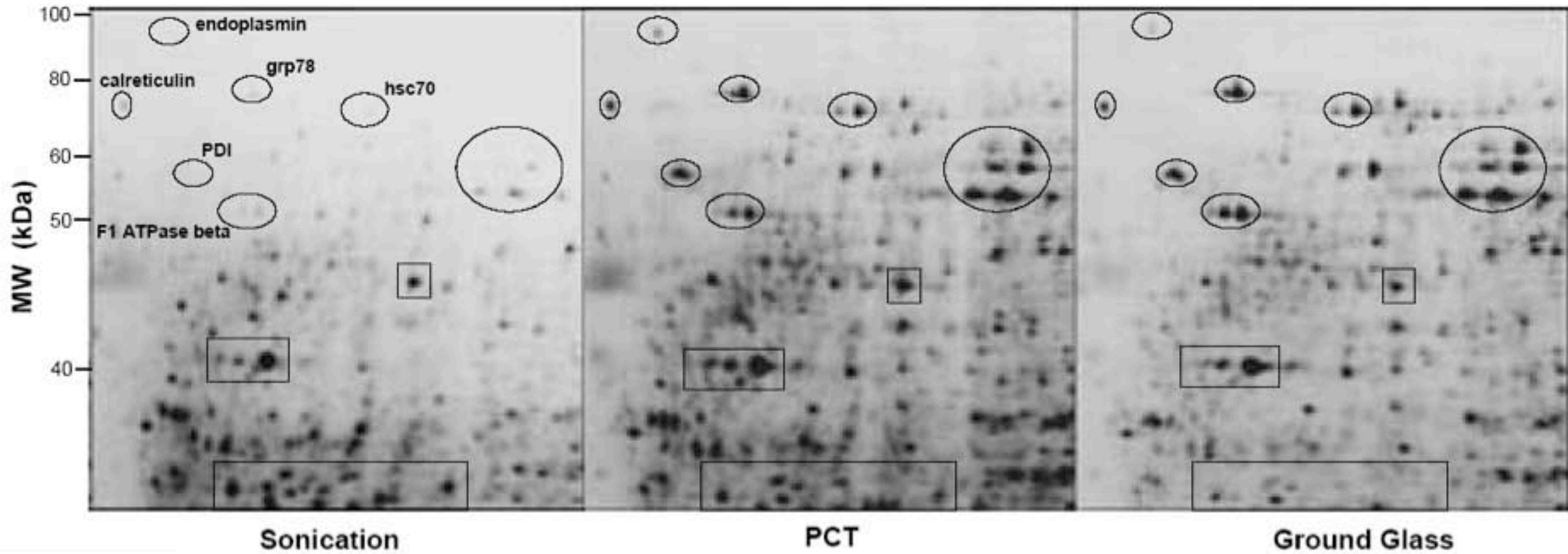


Protein Extracted from *C. elegans*

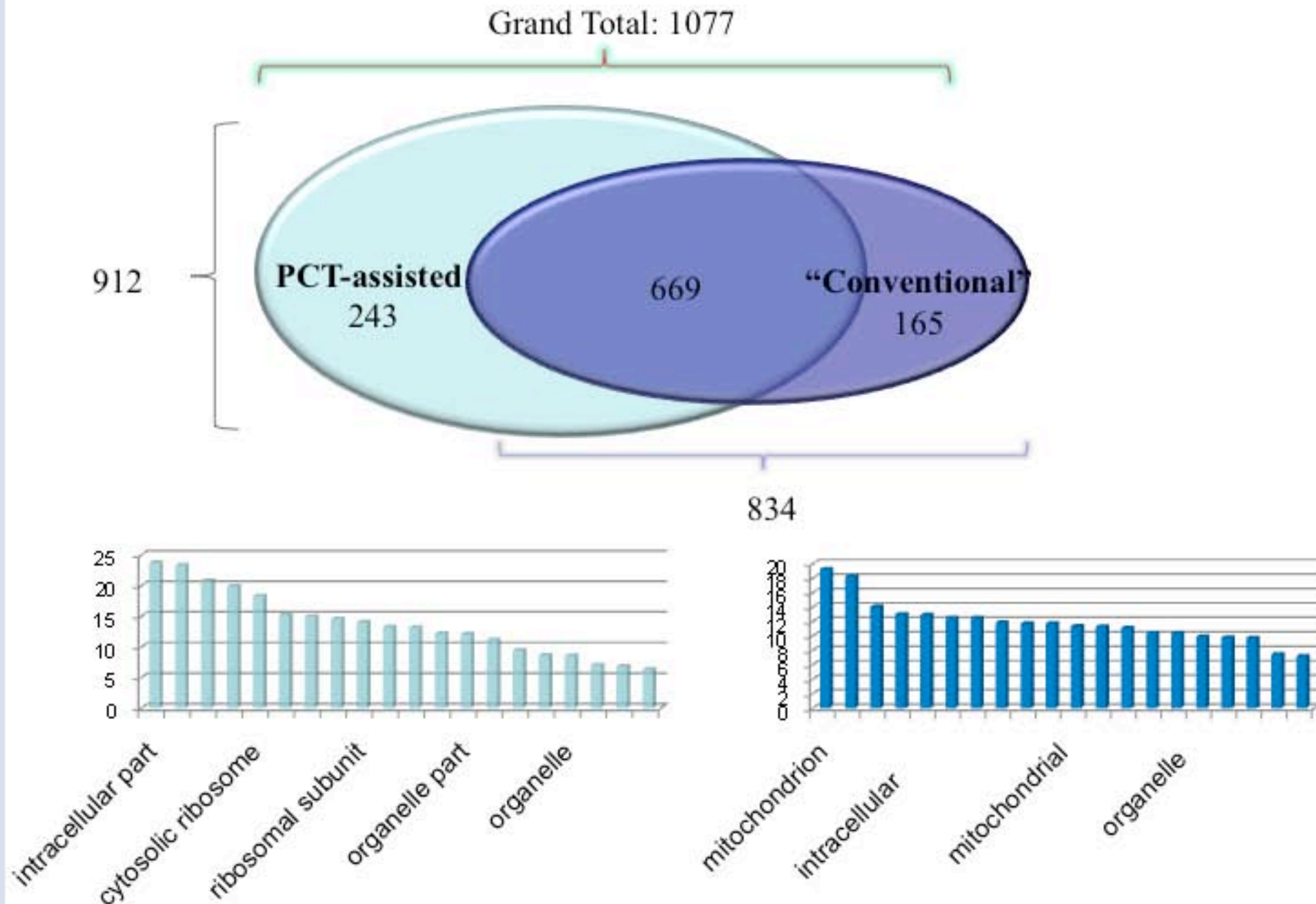


Extraction of Protein from *C. elegans* Using The PCT Shredder and PBI's ProteoSolve-CE Native and Stringent Kits ("FAST" Method) Results in Greater Yield of Protein than Extraction with Mortar and Pestle with Liquid Nitrogen (LNP).

Analysis of Mouse Liver Lysates by 2DGE: Comparison of PCT, Sonication, and Ground Glass Tissue Grinder



PCT-assisted Cell Lysis in Detergent-free Buffer



HepG2 proteomes extracted either by PCT or by sonication in 50 mM AmBic

Some Advanced Applications

Differential Lysis: Exploiting The Pressure Profile

- Extract Mitochondria

Systems Biology: Exploiting The Synergy of Pressure and Chemistry

- ProteoSolve_{SB} Kit

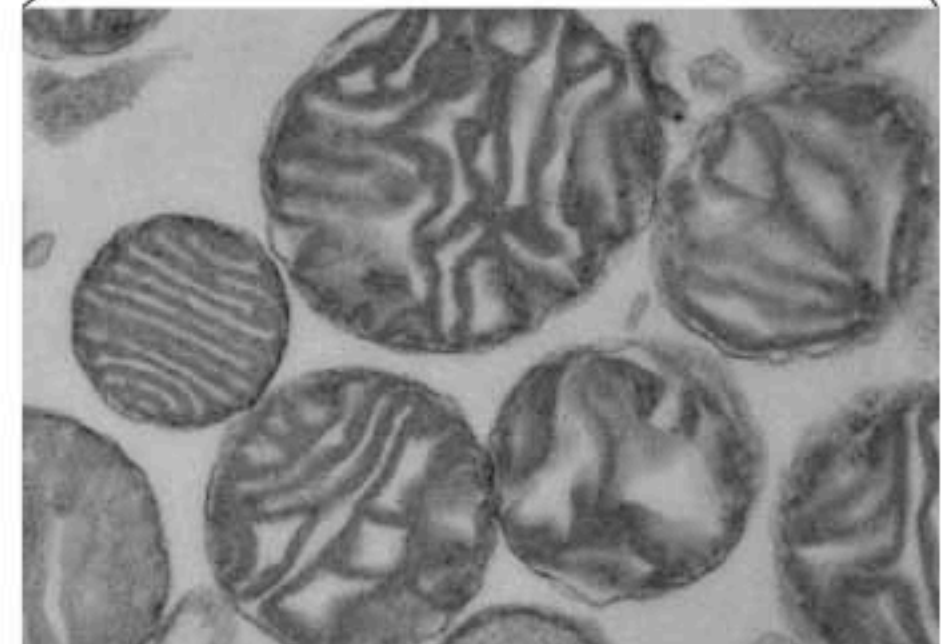
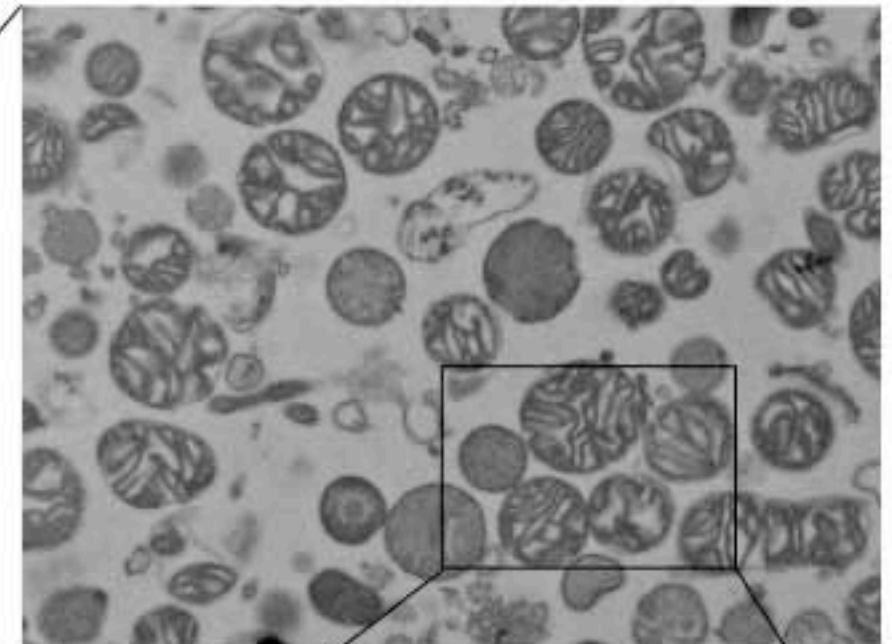
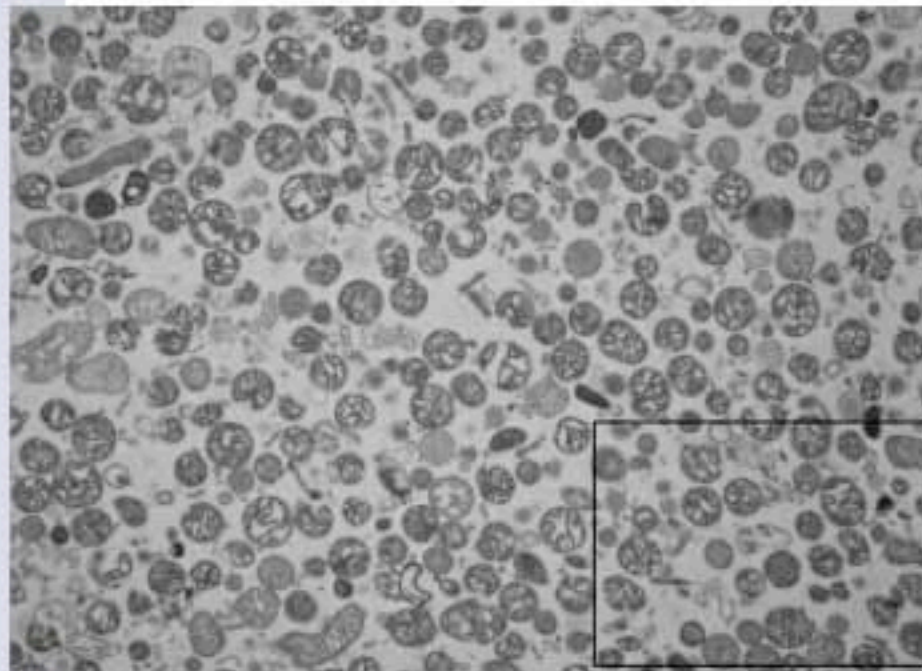
Pressure-dependant, detergent-free extraction of proteins, lipids and nucleic acids

Bacteria, Animal Tissue (Especially effective for lipid-rich tissues)

Standardization of Mass Spectrometry

- Pressure-Enhanced Enzymatic Proteolysis

“Live” Kidney Mitochondria Isolated by PCT



ProteoSolve-SB

Tissue Fractionation by Hydrostatic Pressure Cycling Technology: The Unified Sample Preparation Technique for Systems Biology Studies

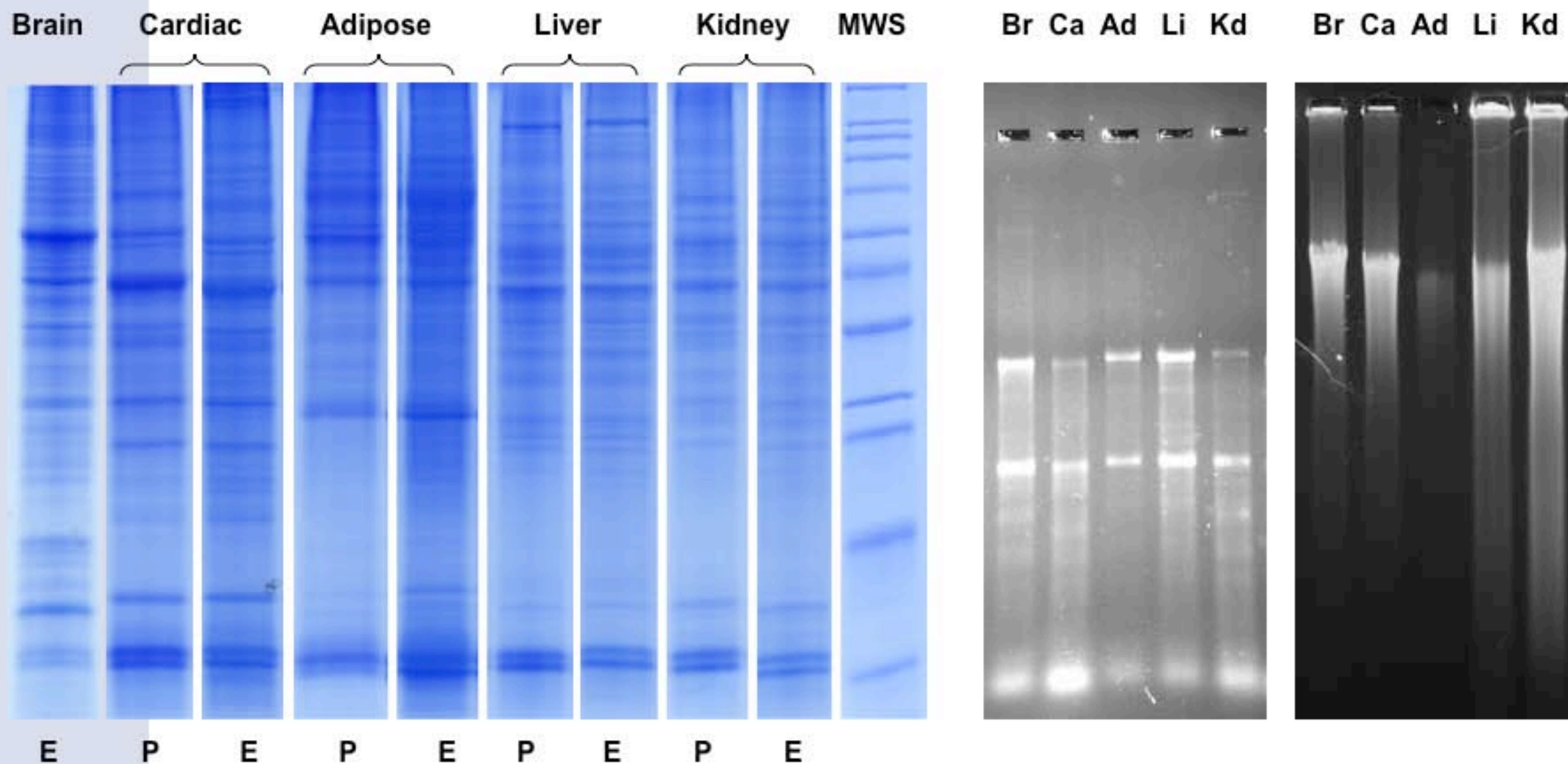
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*Winner of the The Journal of Biomolecular Techniques (JBT) Award
for Outstanding Research Article*

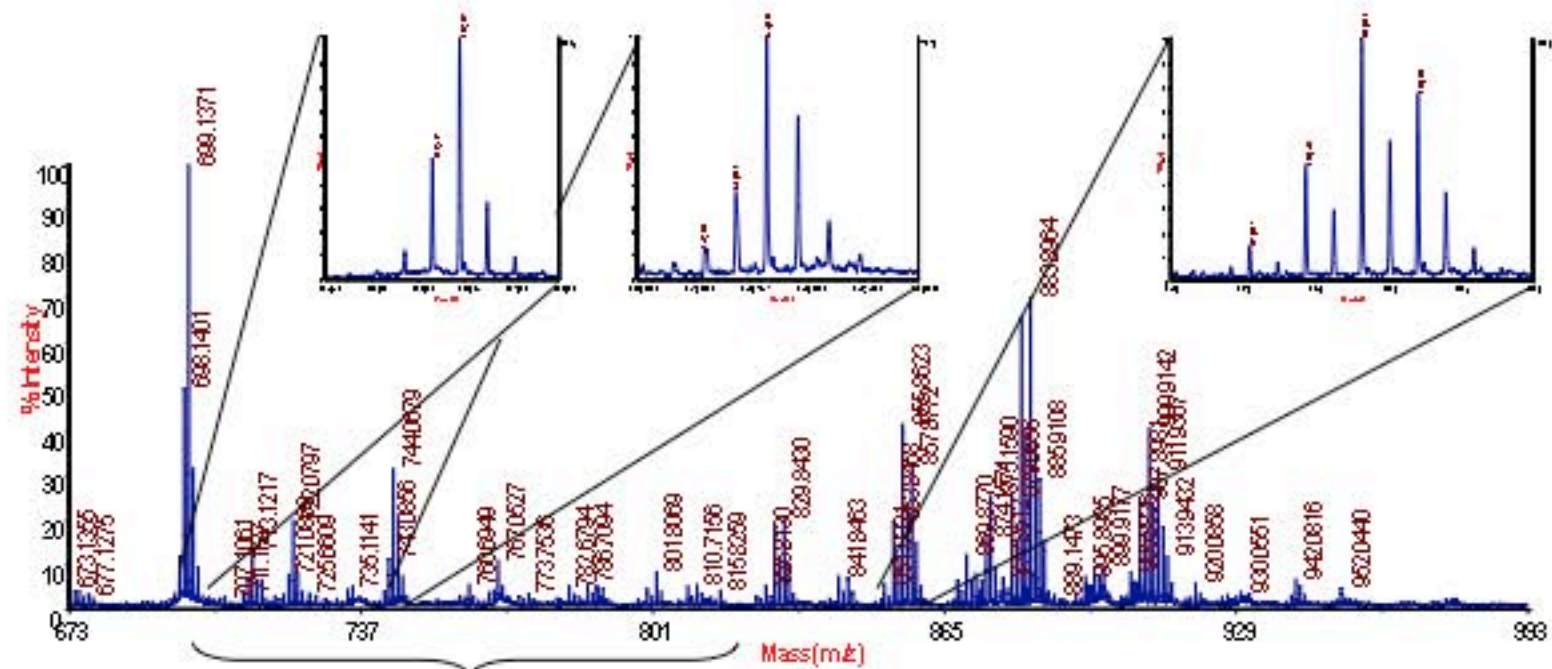
Protein, RNA and DNA Recovery From Rat Tissues

ProteoSolve-SB

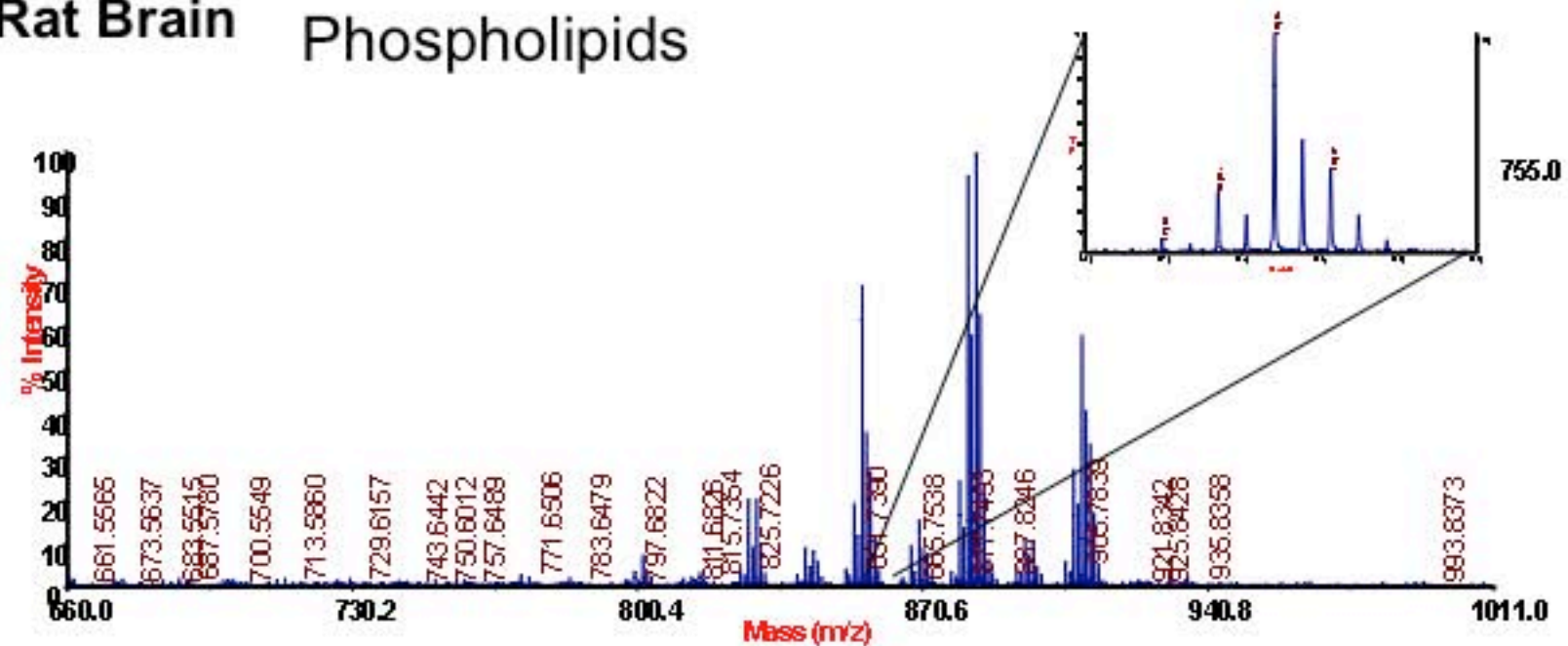


Solvent removed by: E – evaporation; P - precipitation

Direct Lipid Profiling by MALDI-TOF MS



Rat Brain Phospholipids



Beef pericardial fat

Triacylglycerides

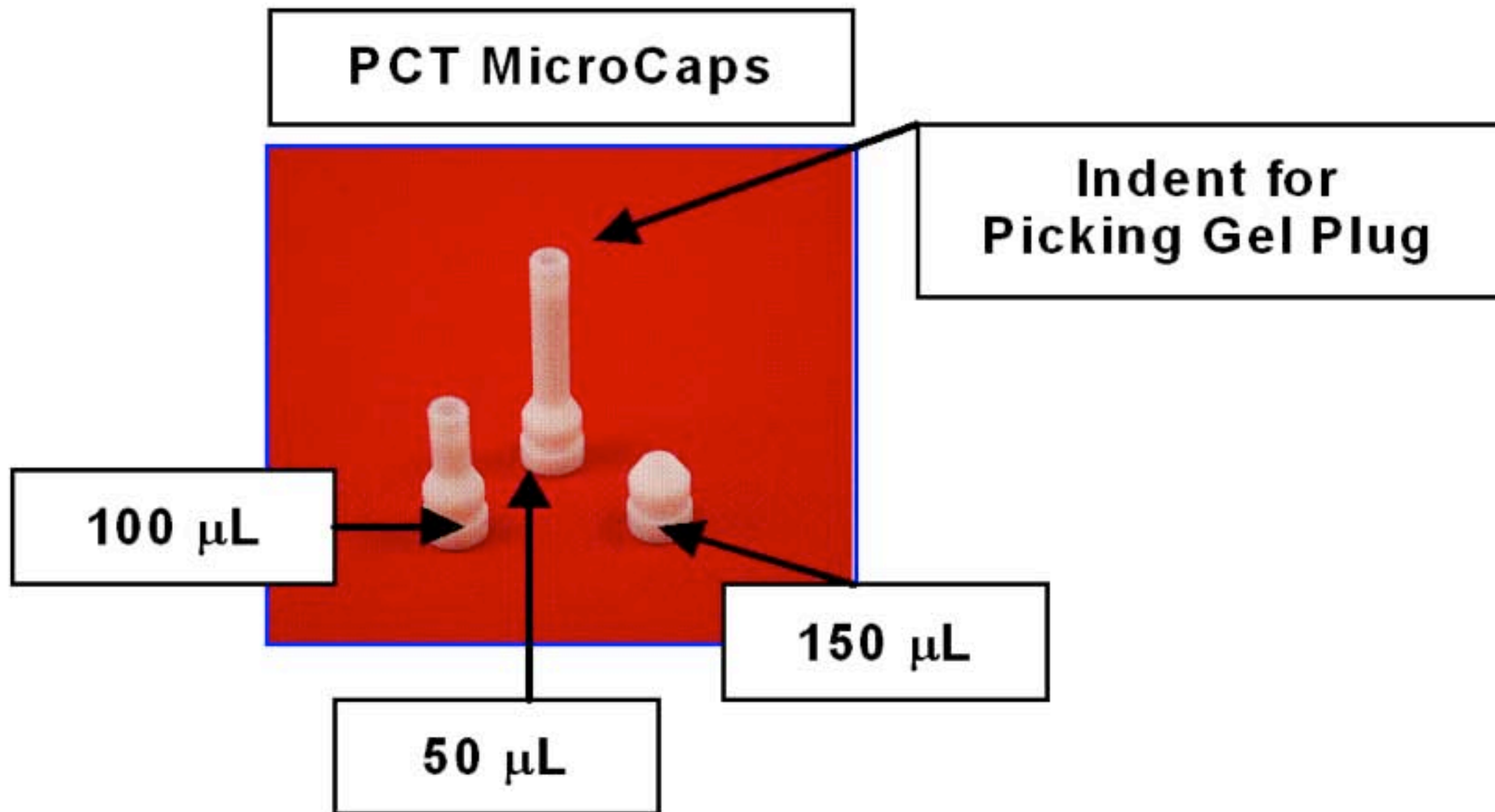
Applications in Mass Spectrometry

Pressure-Enhanced Enzymatic Proteolysis

PCT MicroTube



PCT MicroCaps



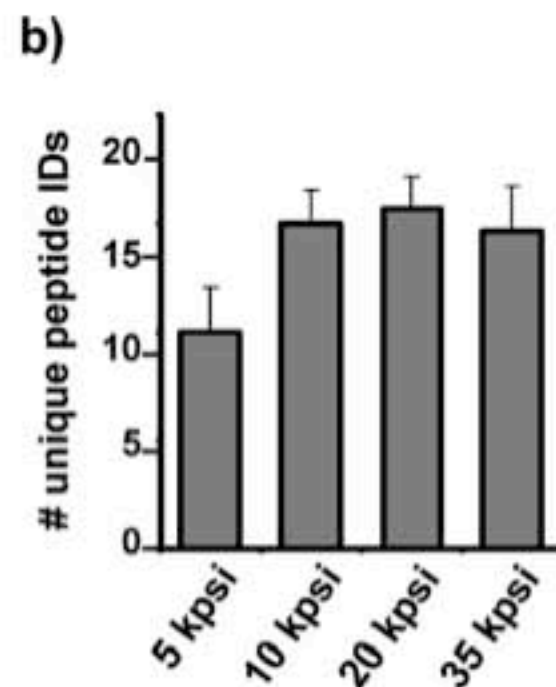
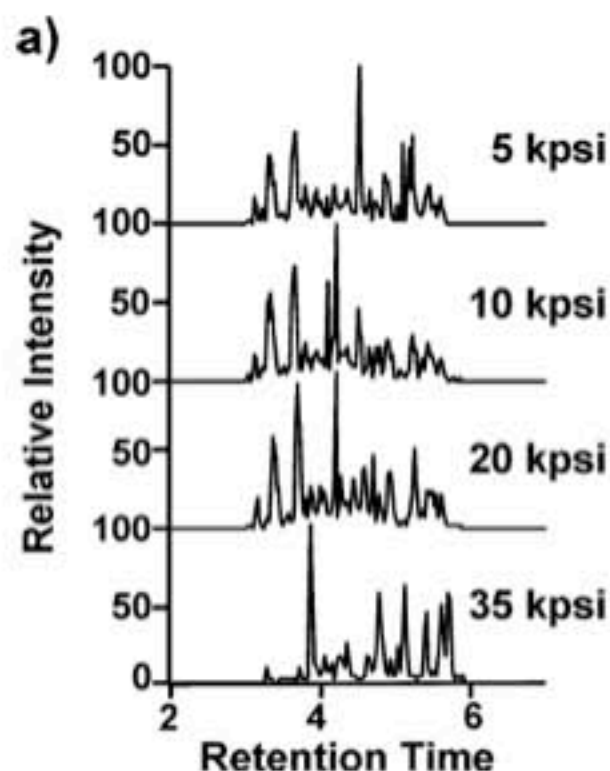
PCT-Enhanced Tryptic Digestion of BSA

Pacific Northwest National Laboratories:

Application of Pressurized Solvents for Ultrafast Trypsin Hydrolysis in Proteomics: Proteomics on the Fly

Increase pressure can dramatically increase the rate of the enzymatic digestion.

PCT simplified sample preparation compared with other newer rapid digestion methods, such as MAPED and HIFU technologies.



Successful in-solution digestions of single proteins and complex protein mixtures were achieved in 60 seconds

PCT-assisted Lys-C Digestion of Monoclonal Antibodies

AMGEN:

A Comparison of Methods for Efficient Digestion of Protein Therapeutics

Conclusion:

This study demonstrated that pressure cycling provided the most effective method for digesting monoclonal antibodies. Complete digestion can be obtained in a short period of time without inducing modifications such as methionine oxidation.

While the microwave technique has established applicability in a proteomics setting, the more stringent requirements of the biopharmaceutical arena suggest limitations of the technique with respect for characterization of protein primary structure

Summary

**To Fully Elucidate the Soil Proteome
It Is Essential to
Use More Than One Extraction Technology
and
More than One Detection Method**