PBI Pressure BioSciences Inc.

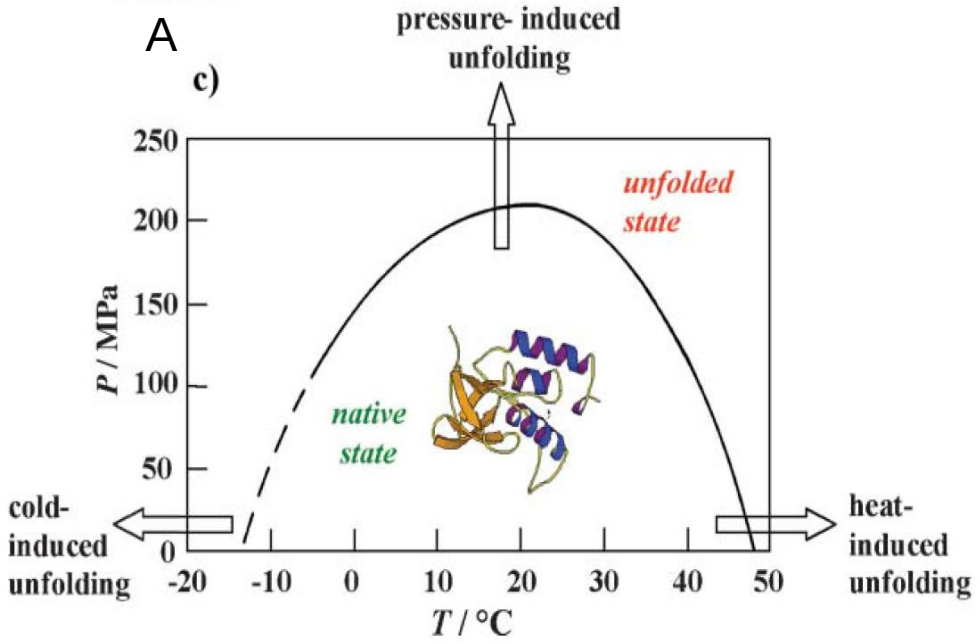
Exploring Applications of High Pressure in Biological Sample Preparation

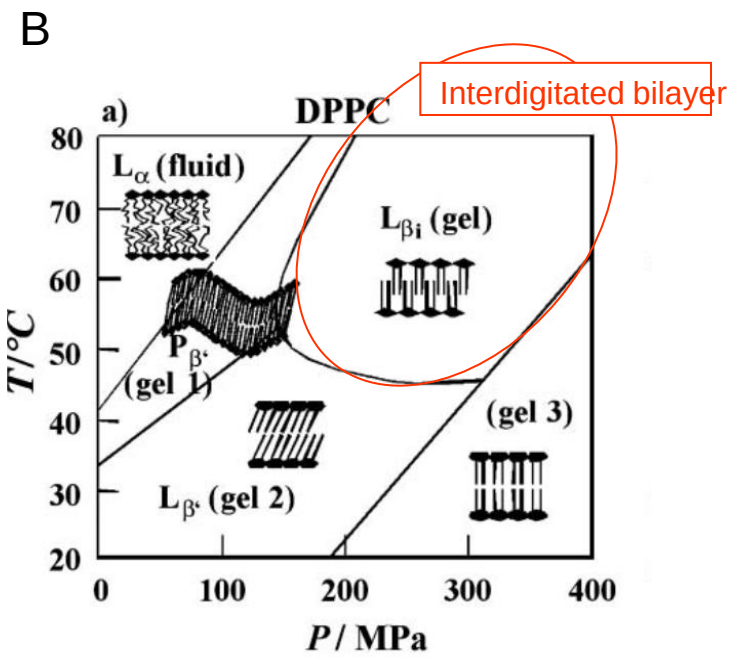
Alexander Lazarev, Ph.D.

Second Annual
SAMPLE PREP & TARGET ENRICHMENT IN MOLECULAR DIAGNOSTICS

Two Dimensions of Thermodynamics:

protein and lipid properties are perturbed by pressure



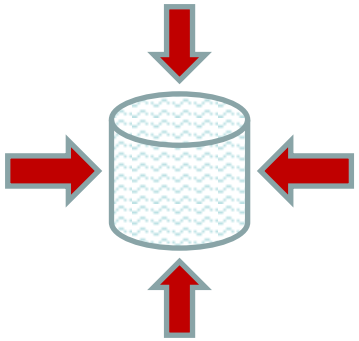


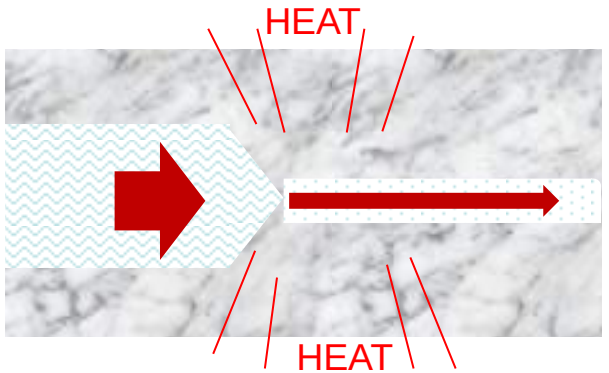
A) Phase diagram of protein stability
B) Interdigitation of lipid bilayers in an ester-ester linked DPPC bilayer: high pressure SAXS and FT-IR data.

Daniel I, Oger P, Winter R. Chem Soc Rev. 2006; 35 (10); 858-75

Hydrostatic vs. Hydrodynamic Pressure:

Energetics



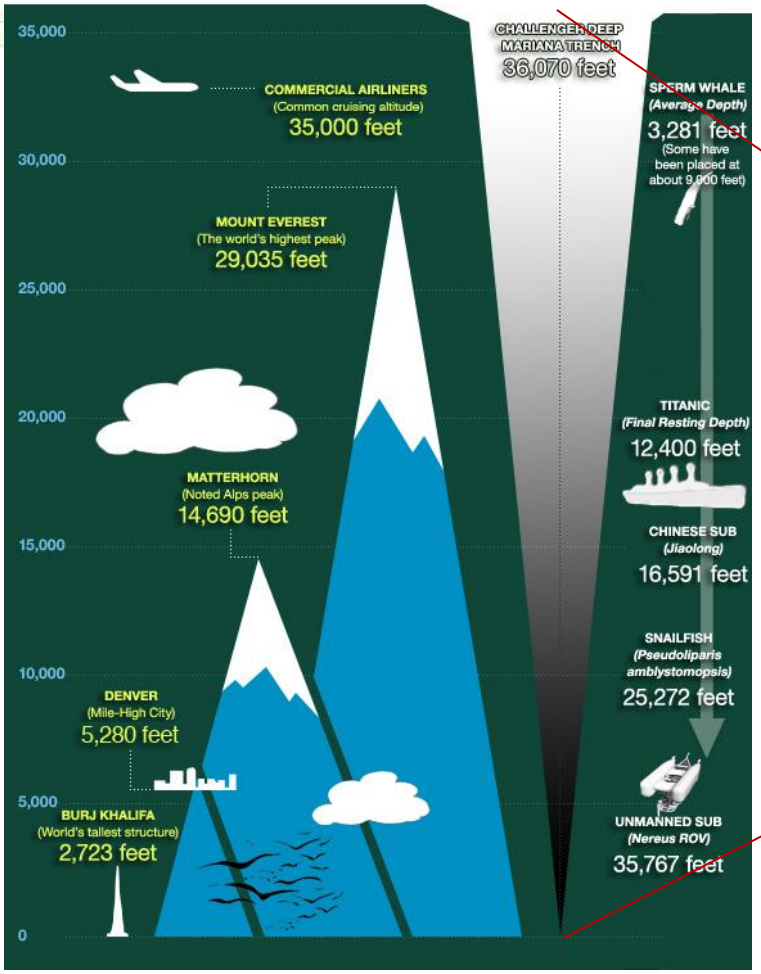


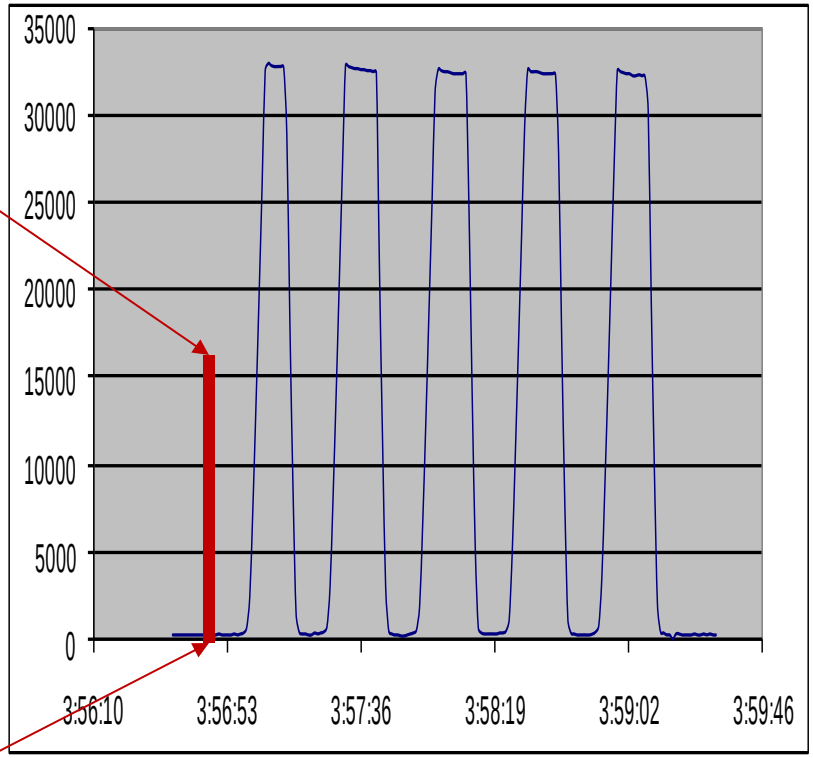
Thermodynamic: $PV = Const.* T$

Mechanical: $E_k = E_r + E_t$

Pressure Cycling Technology (PCT):

16,000 psi, Marianas Trench





PBI Equipment controls pressure up to 60,000 psi

"Cycles of hydrostatic pressure between ambient and ultra high levels used for precise control of biomolecular interactions"

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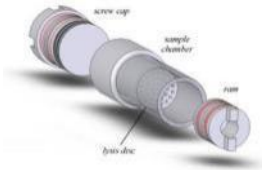
PBI Products

Barocycler™ NEP3229

Barocycler™ NEP2320

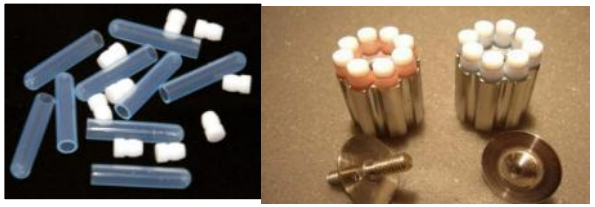
Barocycler™ HUB440

PULSE Tube FT500

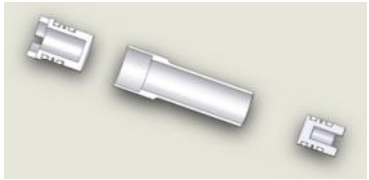


PBI Shredder

PCT MicroTubes



PULSE Tube FT500-ND



Reagent Kits

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Applications of PBI Products

Current install base – ca. 200 systems

- Biological Sample Preparation
- Forensics
- Synthetic chemistry
- Pathogen Inactivation
- Cell Biology
- Protein Dynamics
- HPLC
- Material Science and Engineering

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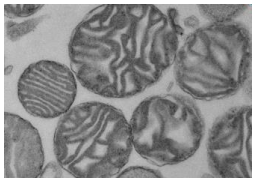
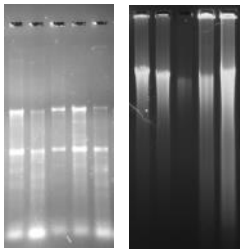
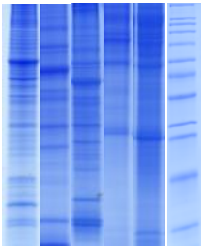
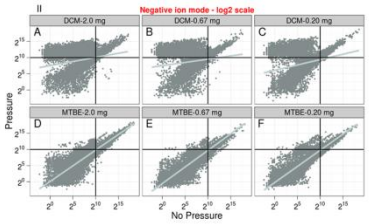
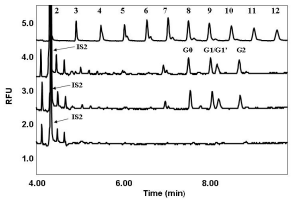
Biological Sample Prep Applications

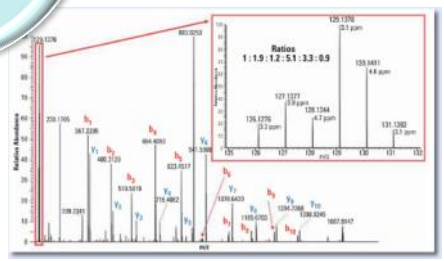












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Differential Cell Lysis - Forensics

Gary Sinise

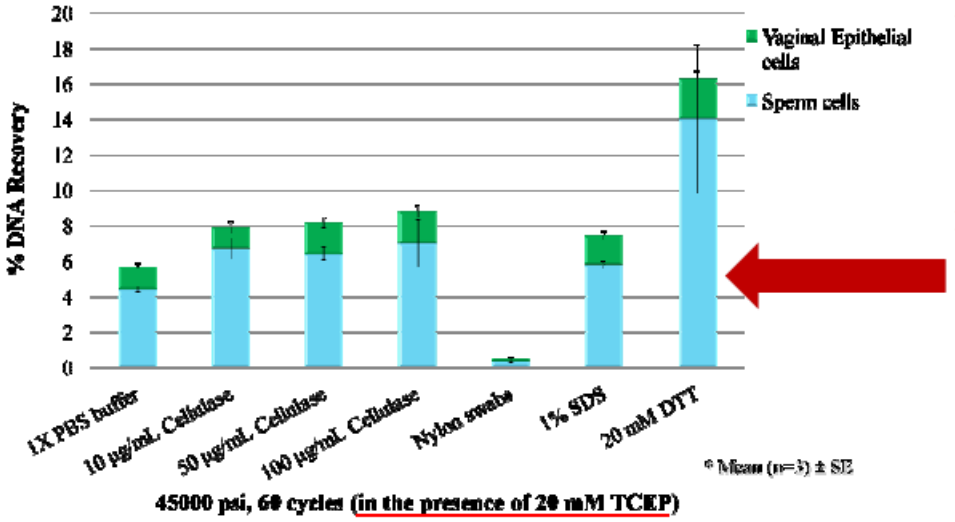
Barocycler

Barocycler highlighted in an opening episode of CSI - New York, 2012

FIU

International Forensic Research Institute

Figure 4. Effect of PCT on DNA extraction from swab (mixture of sperm and vaginal epithelial cells)



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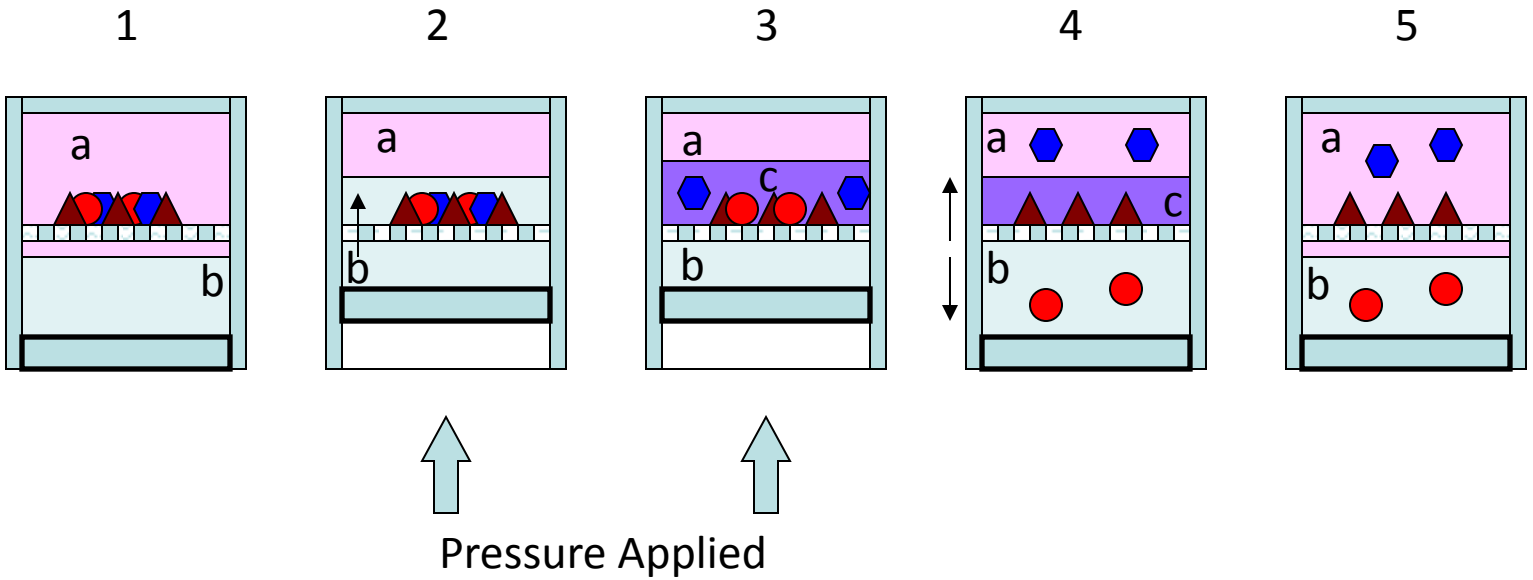
PCT-mediated liquid-liquid extraction
(*patent pending*)



Hydrophobic hydration and intermixing of poorly miscible solvents under pressure enables extraction in the homogenous solvent phase followed by partitioning under atmospheric pressure conditions.

Gross, *et al*., Journal of Biomolecular Techniques (2008) 19*:187–197*

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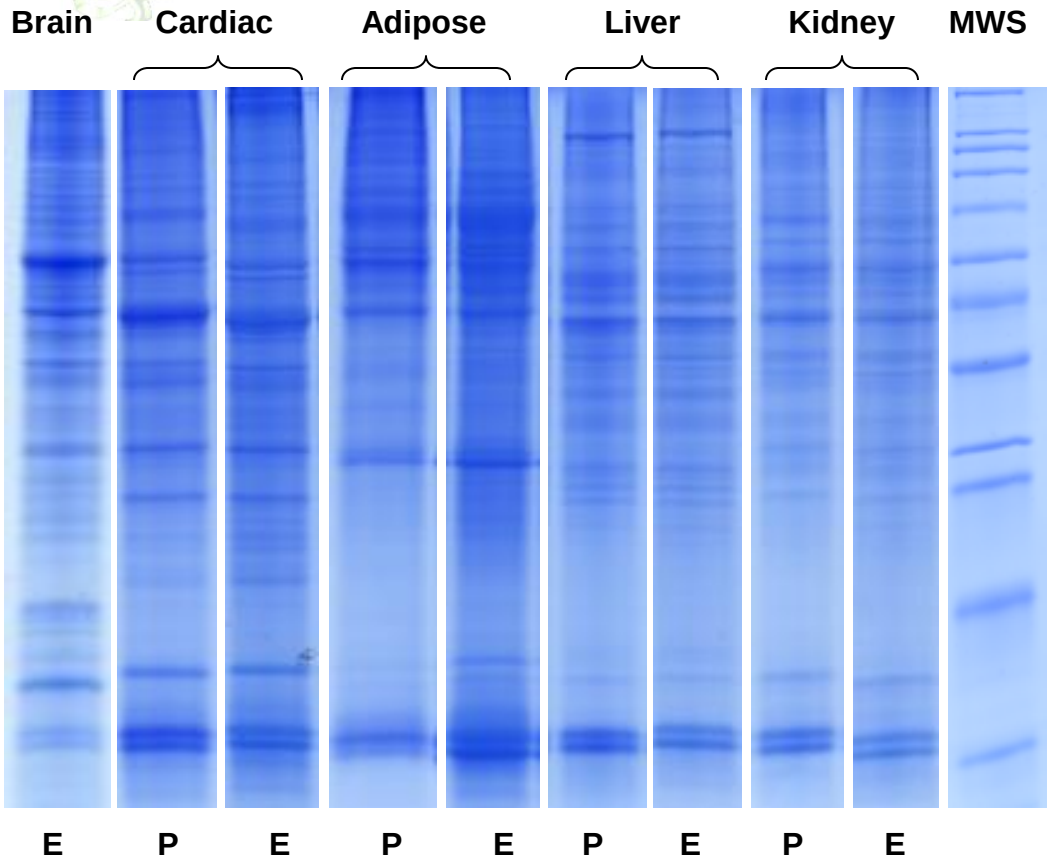
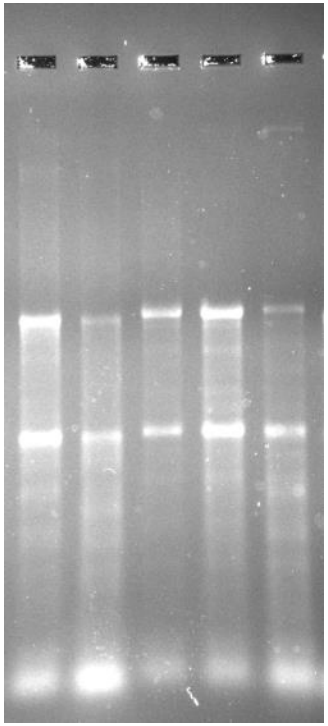
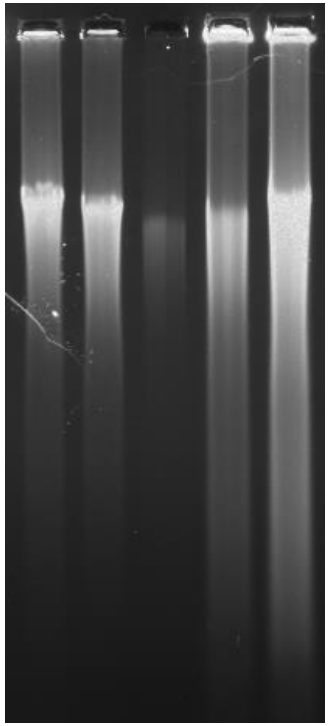
Protein, RNA and DNA Recovery From Rat Tissues

*using Barocycler and **ProteoSOLVE-SB** kit*







Solvent removed by: E – evaporation; P - precipitation

Gross, *et al*., JBT (2008) 19*:187–197*

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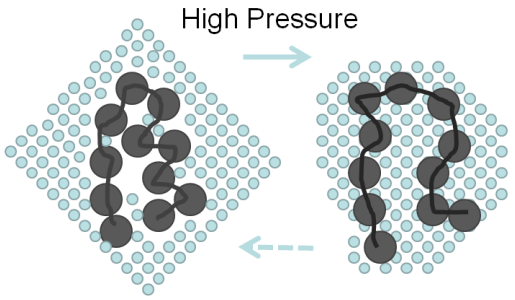
Proteins under pressure

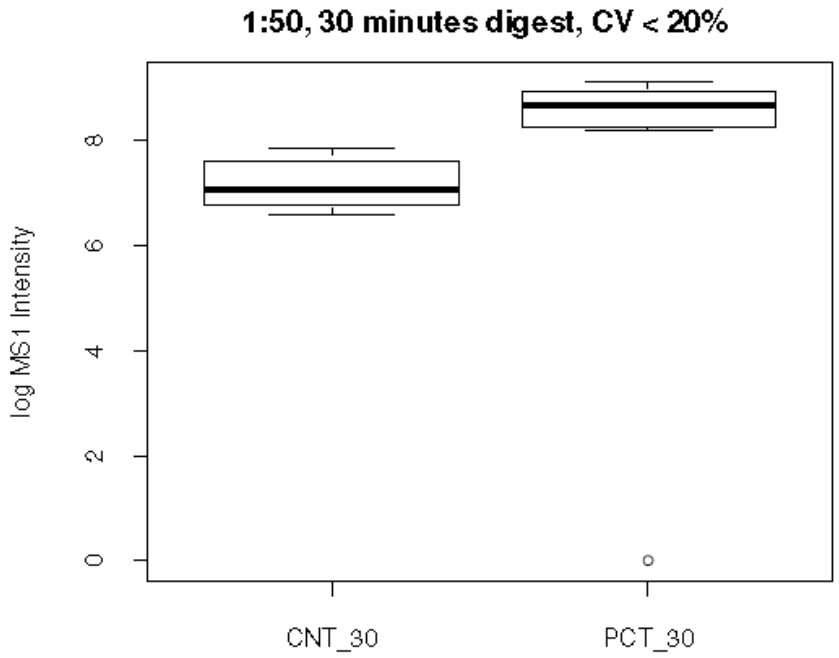


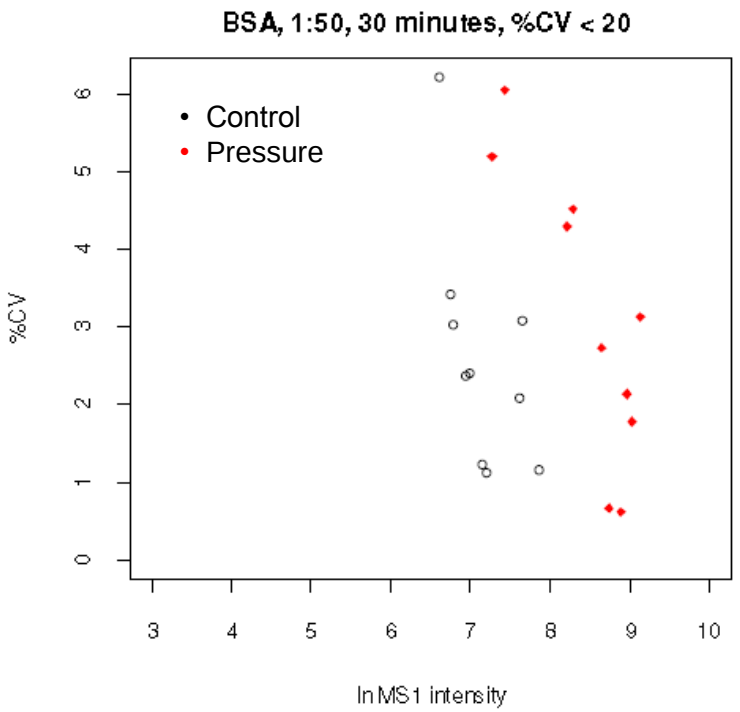
Pressure can selectively alter protein conformation or regulate activity of certain enzymes:

- *Control exonucleases - early efforts in DNA sequencing*
- *Inactivate enzymatic activity during cell lysis and extraction* (Phosphatases, Proteases, RNases, etc.)
- *Boost proteolytic digestion in proteomic sample preparation* (Trypsin, Chymotrypsin, Pepsin, Lys-C)
- *Boost lytic enzymes for cell lysis and DNA purification* (Lysozyme, Proteinase K, Zymolyase)
- *Enzymatic protein deglycosylation* (PNGase F)

BSA digest, PCT vs. atmospheric pressure

Reproducibly higher intensities for most peptides





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PCT-SWATH:
Fast conversion from biological samples to digital data

Biological samples → BIG Data → Knowledge

a reliable, sensitive, and cheap method

Pressure cycling technology (PCT)-assisted fast digestion

cm

cells or tissue biopsy
~1mg

All procedures
in single tube

Batch processing
Minimize variations

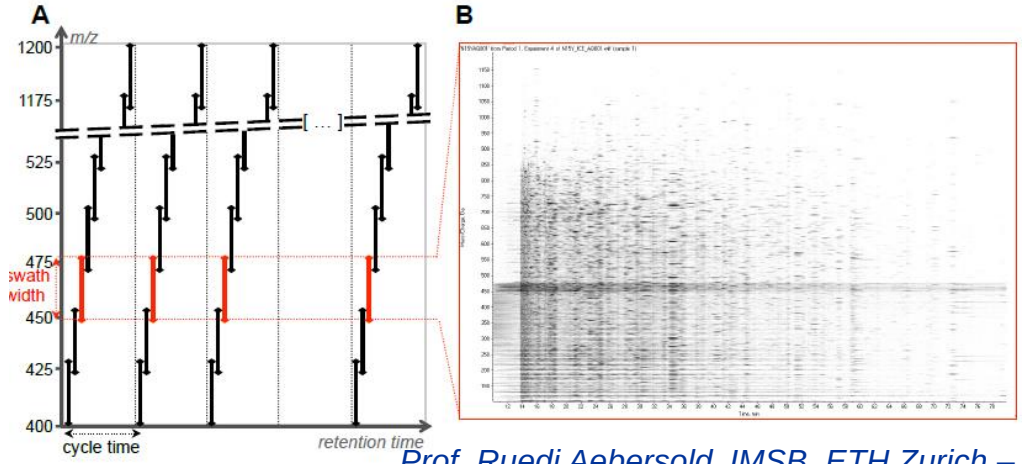
Pressure cycling technology
fast and efficient digestion

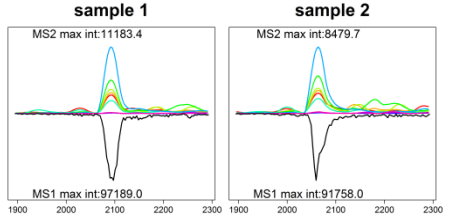
Yield:
~20ug peptides
from 1mg kidney
tissue

2ug peptides
sufficient for a
swath analysis

6~8 hrs to convert 6 pieces of tissues into clean peptides that are ready for MS analysis

Proteomic analysis using SWATH-MS





targeted data extaction

Prof. Ruedi Aebersold, IMSB, ETH Zurich – US HUPO 2013

Gillet, et al., 2012 – manuscript in preparation

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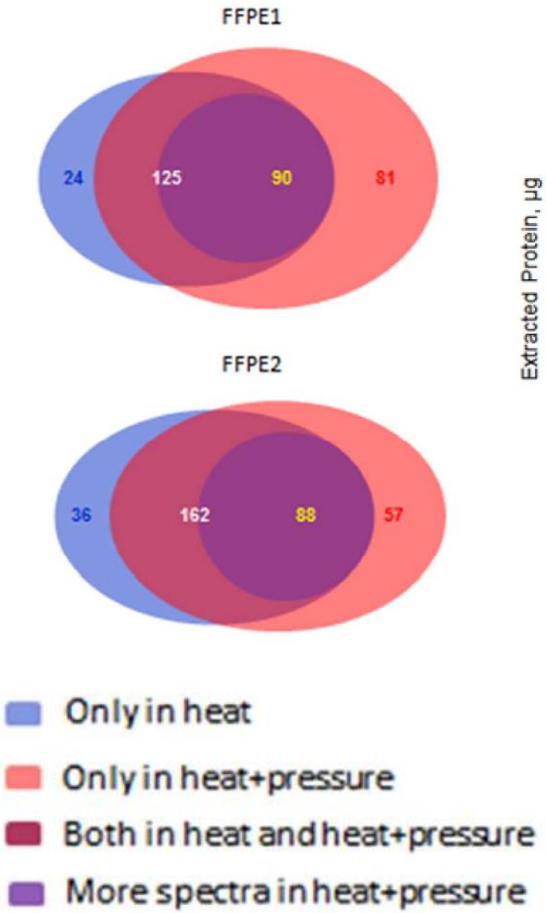
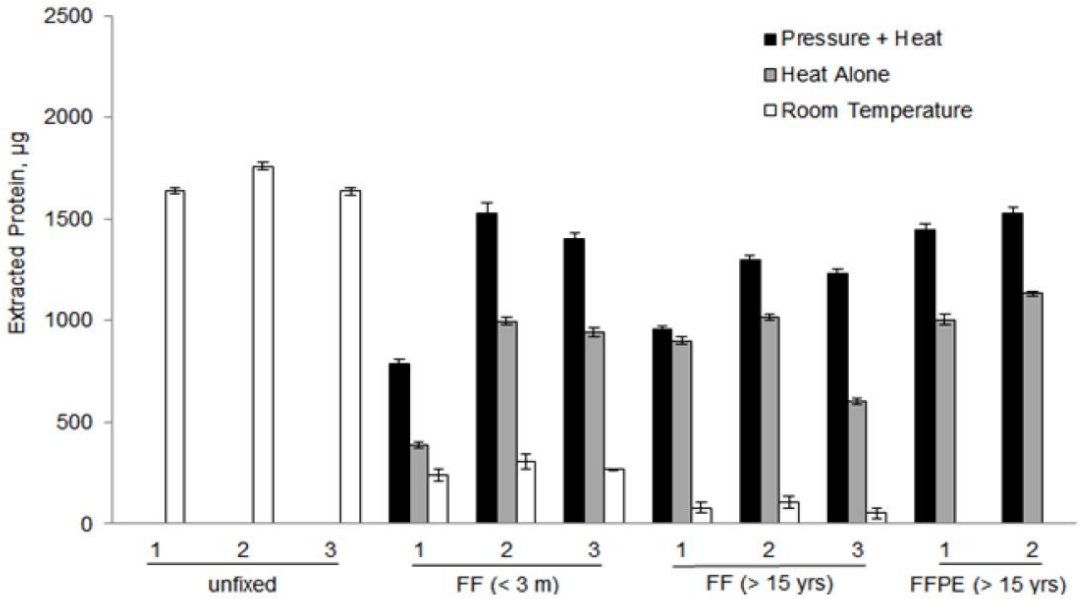
Protein Extraction From FFPE Aorta Samples

Jones Hopkins School of Medicine





.. the combination of both pressure and heat make it possible to take advantage of old archival FFPE aorta tissue for MS proteomic analysis and quantification with high protein identification confidence and sequence coverage.

Fu Z., et al., Proteomics: Clinical Applications. 2013. [Epub ahead of print]

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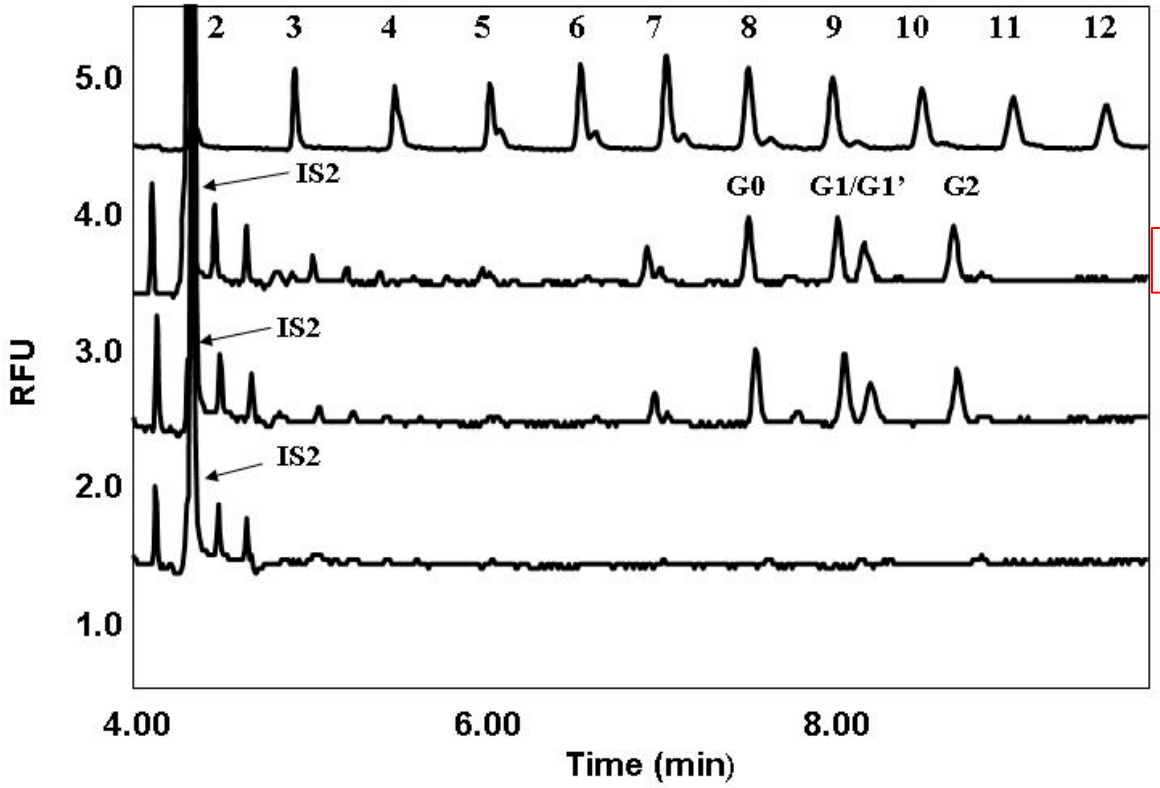
Deglycosylation of IgG with PNGase F

accelerated sample preparation for glycoprotein characterization



Oligosaccharide Ladder

IgG glycans, 2041 Atm. 5 min.

IgG glycans, 1 Atm. 3 hours.

IgG glycans, 1 Atm. 5 min.

Szabo, Z., et al., Analytical Chemistry, 82 (6), pp. 2588-2593, 2010

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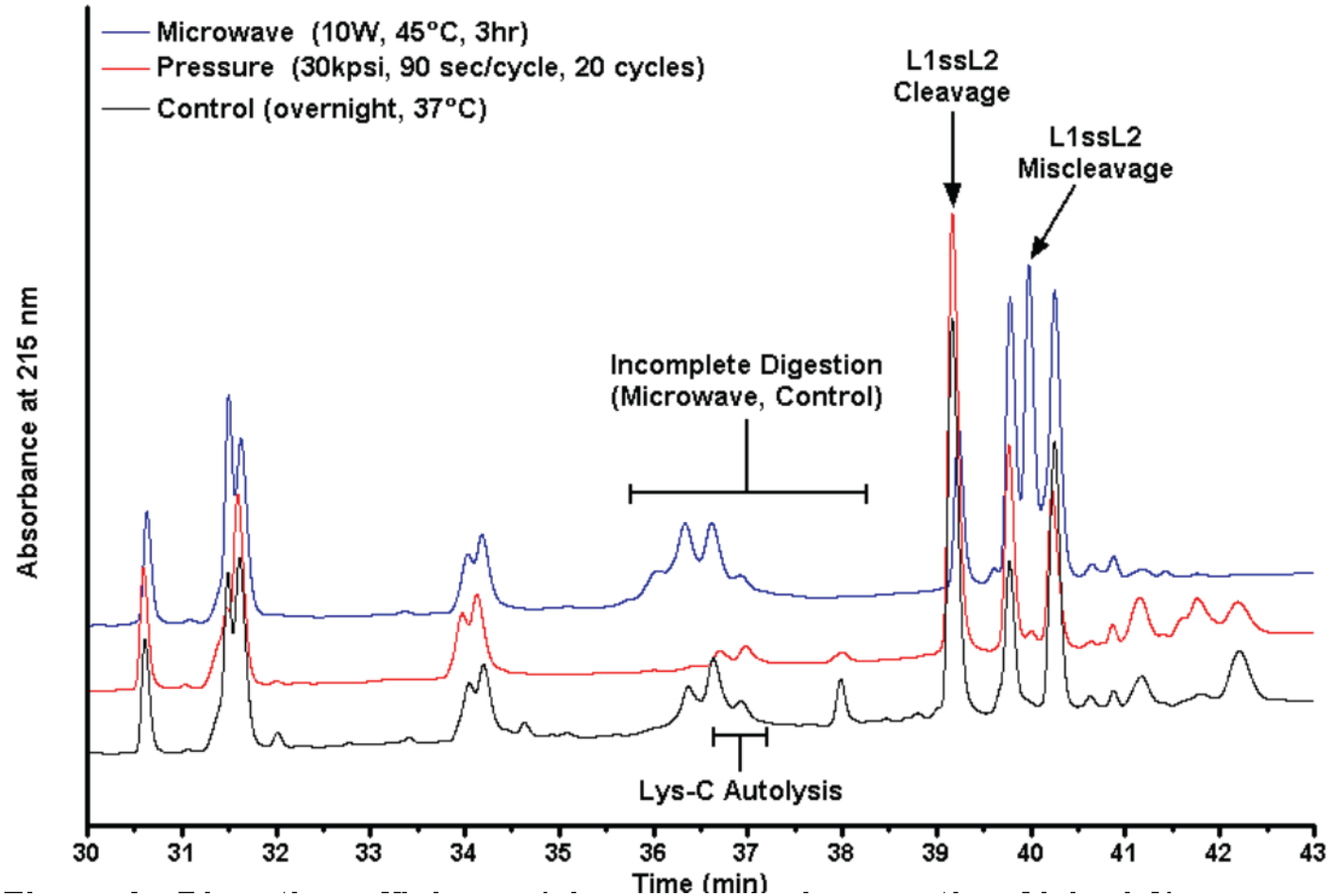
PCT vs. microwave digestion:
unreduced IgG + Lys-C



Maheu L. et al, Analytical Sciences, Amgen Inc., FACSS 2008

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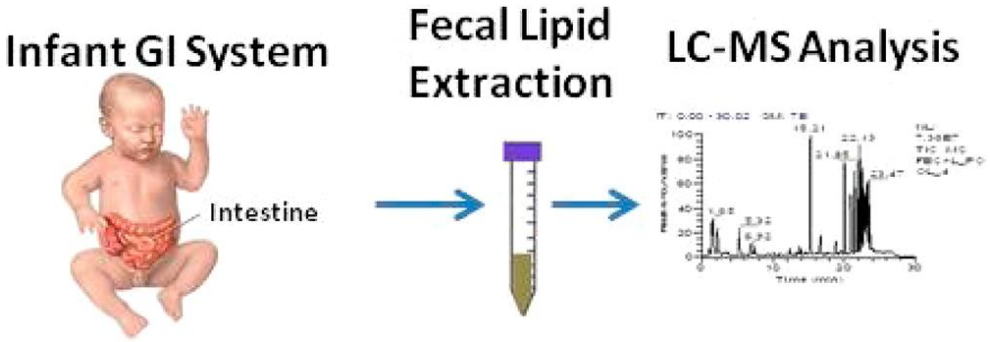
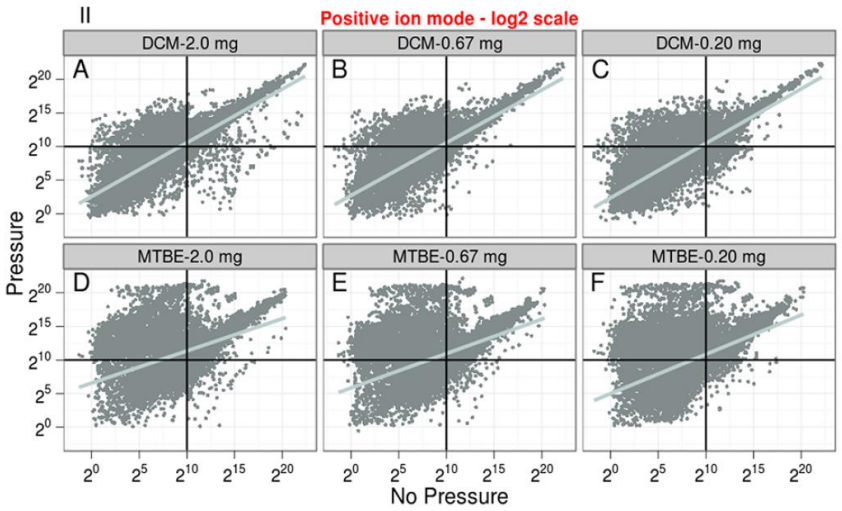
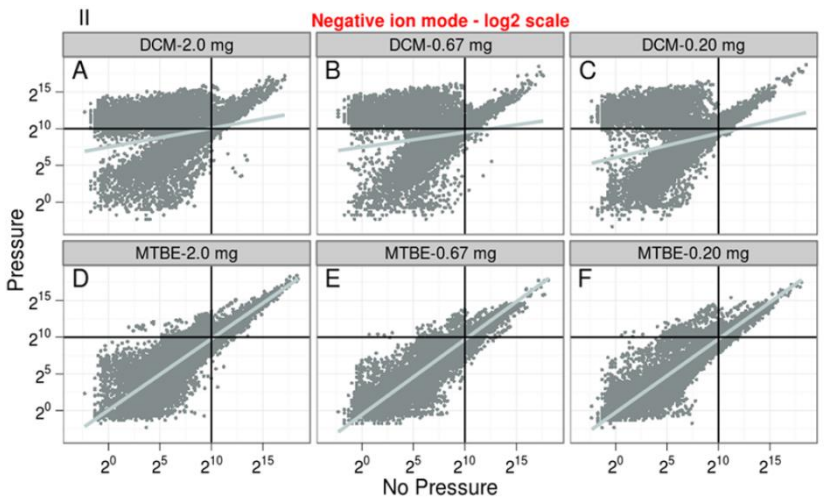
Neonatal Fecal Lipidomics

in search for markers of NEC







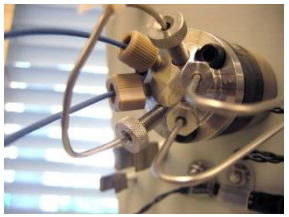
- *Gregory et al., Analytical Chem. 2013; 85(2):1114-23*

Barocycler HUB Series –
modular high pressure equipment solution

Sample Preparation,
Chemical Synthesis

Barocycler HUB160
Barocycler HUB440

HPLC Column packing



Component testing

Magnetic resonance and optical spectroscopy

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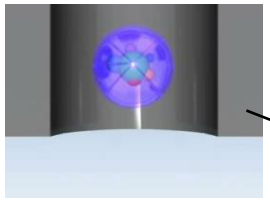
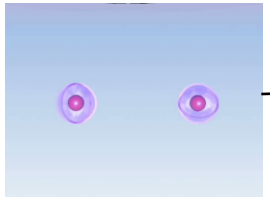
Shearing Homogenization Technology:

pressure accelerates sample through a small orifice.

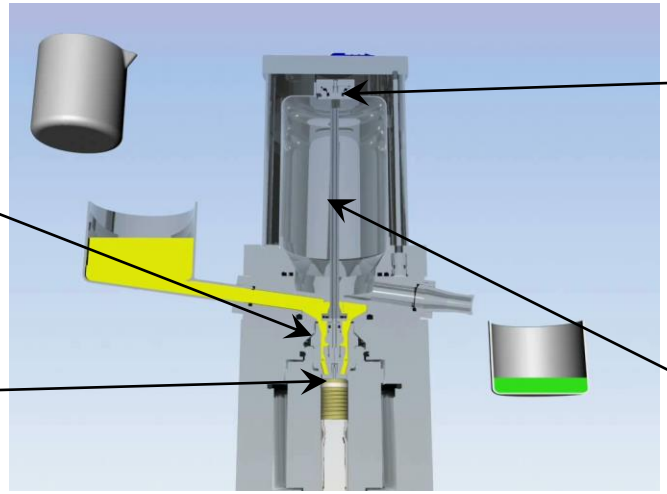
- Constant Pressure during entire process
- High energy mechanical disruption, independent of chemistry
- Ruptures covalent bonds



Acceleration

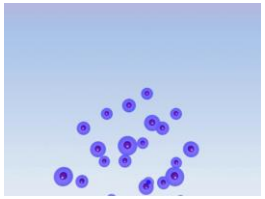


Liquid Shear



The Process

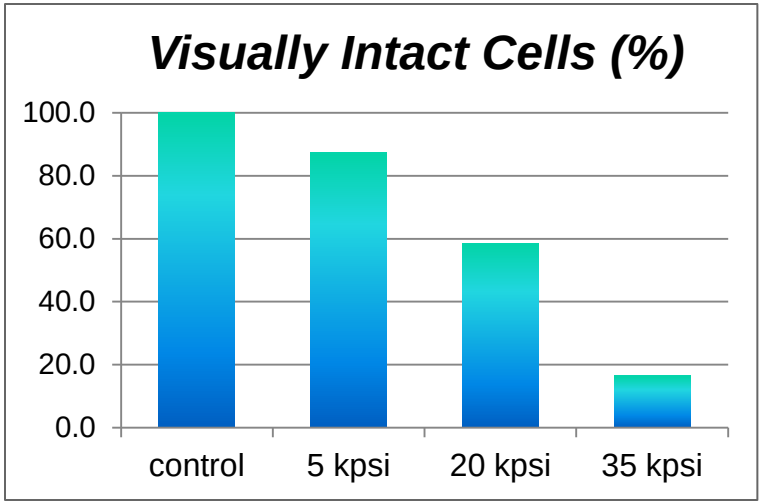
Impingement

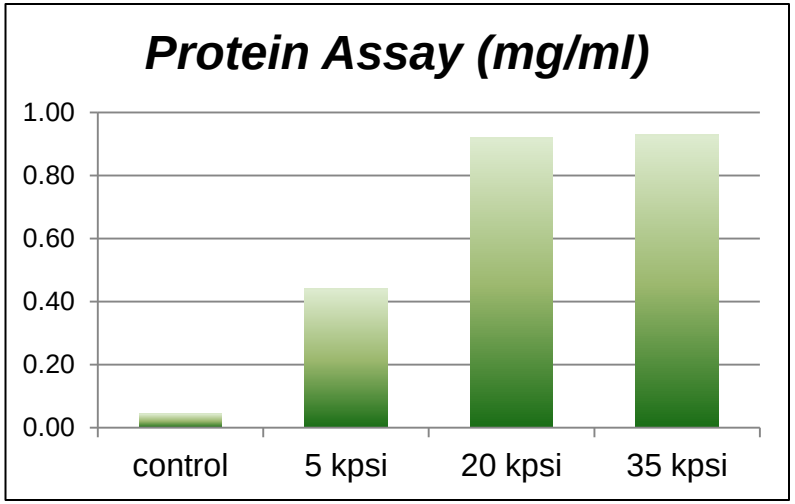


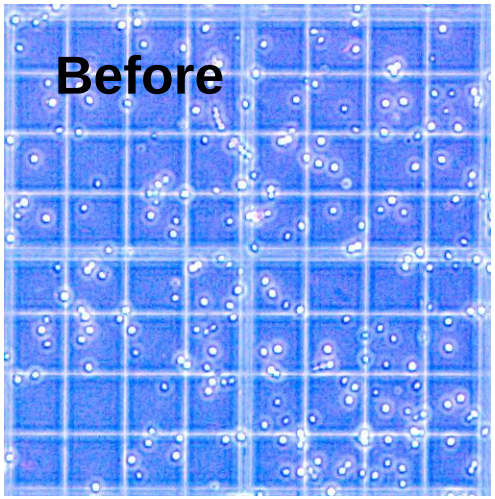
Rapid Depressurisation

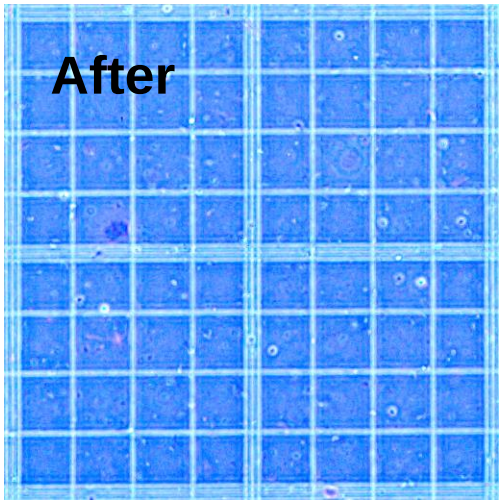
Yeast Cell Disruption

Single pass process results in over 82% disruption of *S. cerevisiae* cells.









Constant Systems Cell Disruption

1,000-40,000 psi

B/C Series
(100-565 mL/min)

TS Series Cabinet
Sample Cooling
(190-565 mL/min)

HAIVA
Viscous Samples
(40-825 mL/min)

One Shot
Non-Flowing Samples
(1-8 mL)

TS Series Benchtop
Sample Cooling
(40-255 mL/min)

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Conclusions

- Sample preparation frequently present a bottleneck in molecular analysis.
- High pressure equipment offers unique and relatively unexplored thermodynamic conditions for life science research.
- PBI and CS offer a complementary suites of high pressure-based sample preparation tools covering a broad range of specific applications in biomarker discovery and diagnostics.

Acknowledgements

Pacific Northwest NATIONAL LABORATORY
Mass spectrometry, proteomics

BWH Brigham and Women's Hospital

BOSTON COLLEGE

JOHNS HOPKINS MEDICINE

TargetDiscovery
Cancer Biomarkers

UCLA

MGH 1811 Massachusetts General Hospital

USAMRIID United States Army Medical Research Institute of Infectious Diseases
Biodefense

US FDA CBER

Northeastern University
Biopharmaceuticals

UMASS BOSTON

NATIONAL INSTITUTES OF HEALTH

SBIR R43-GM079059, R43-AI081518, R43HG007136

DEPARTMENT OF DEFENSE

SBIR-II W81XWH-08-0247

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