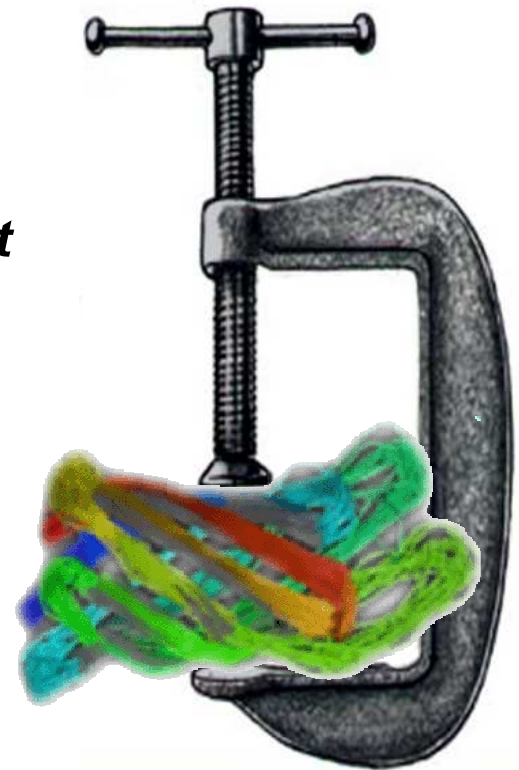




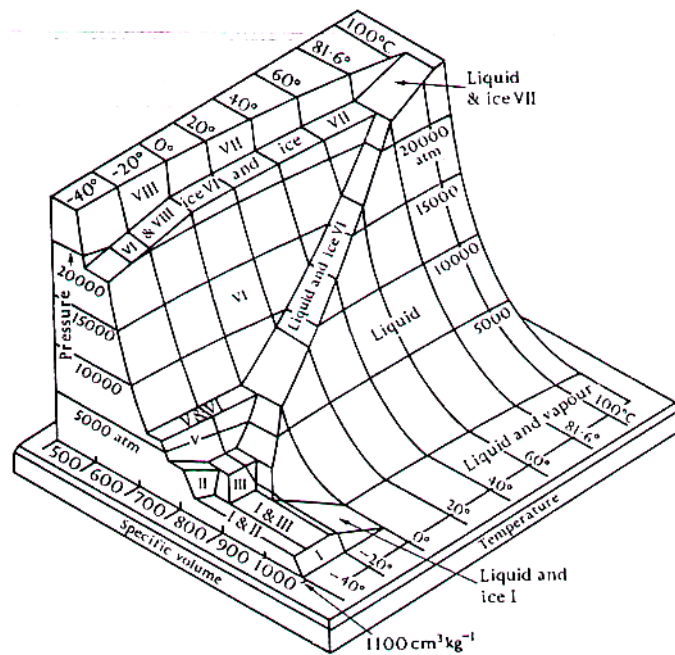
# ***Applications of Pressure Cycling Technology (PCT) in Biotechnology***

***Dr. Vera Gross, Senior Scientist  
Pressure BioSciences, Inc.***

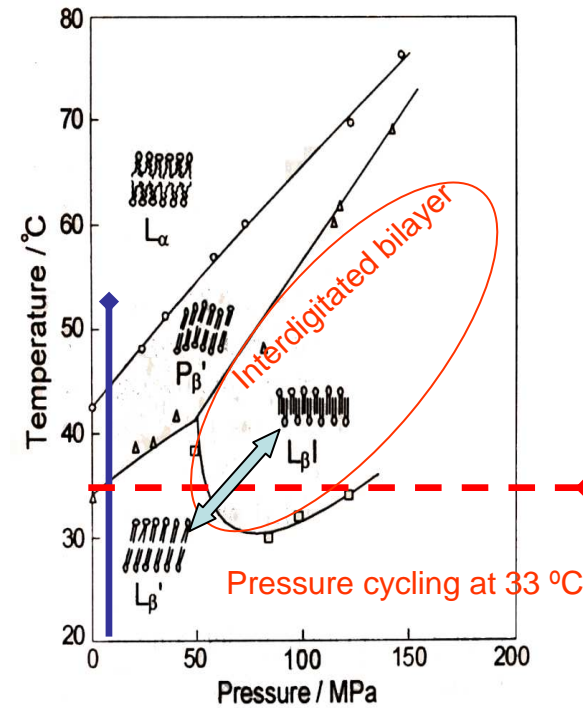
*Presentation at MSACL 2011*



# Two Dimensions of Thermodynamics: Temperature and Pressure



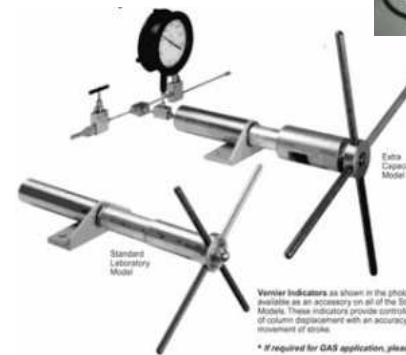
Water



Lipids\*

1 Atm = 0.1MPa  
2380 Atm = 35,000psi = 241MPa

# Temperature vs. Pressure Control Equipment



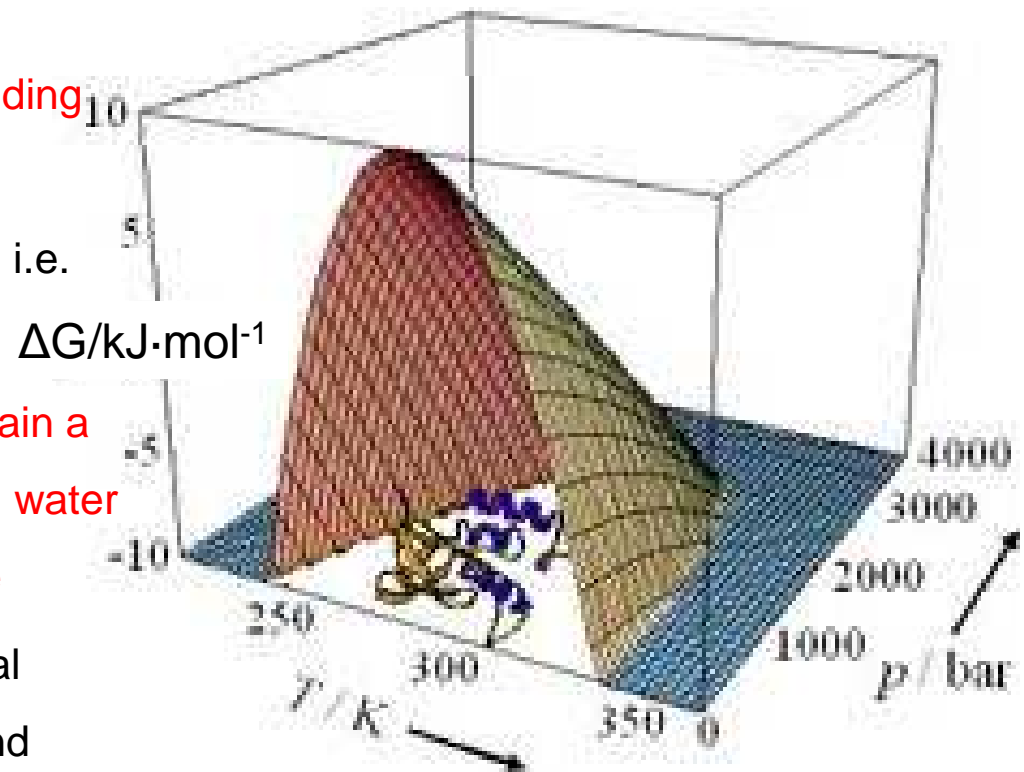
Vernier indicators as shown in the photo at left are available as an accessory on all of the Standard Laboratory Models. These indicators provide controlled measurement of column displacement with an accuracy of a 0.002 movement of stroke.  
\* If required for GAS application, please consult factory.



# Proteins under Pressure

- Pressure promotes **dissociation** of oligomeric proteins
- Pressure promotes protein **unfolding and re-folding**
- Unfolding is driven by **hydration**, i.e. volume reduction
- Pressure-denatured proteins **retain a relatively compact structure with water molecules penetrating their core**
- Pressure-induced conformational change is highly **reproducible** and generally **reversible**

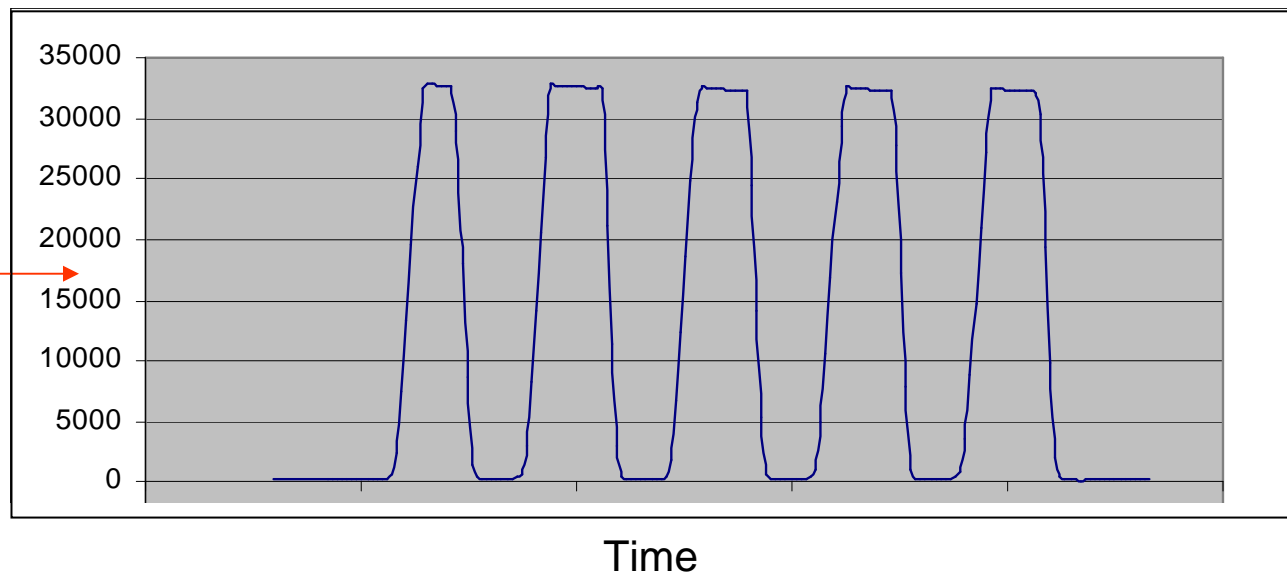
Protein "Phase Diagram"



## Pressure Cycling Technology (PCT):

“Cycles of hydrostatic pressure between ambient and ultra high levels, which allow for the precise control of biomolecular interactions”

~16,000 PSI  
(~1000 Atm)  
Marianas  
Trench



User controlled variables:

Number of cycles, Maximum pressure (up to 45,000 psi), Time at high pressure, Time between pressurizations, and Temperature

# PBI Products

Barocycler™ NEP3229



Barocycler™ NEP2320



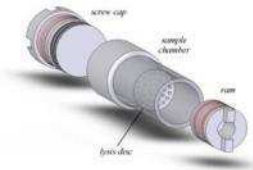
Barocycler™ HUB440



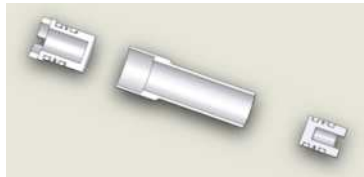
PCT Shredder and The Shredder SG3

PCT MicroTubes

PULSE Tube FT500



PULSE Tube FT500-ND



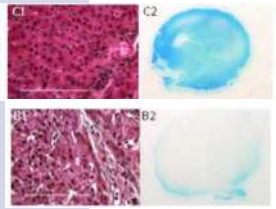
Reagent Kits

# PCT Applications

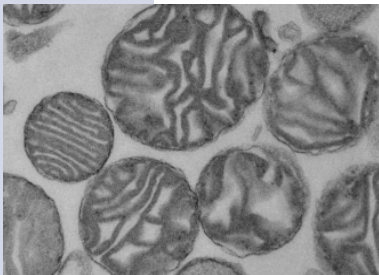
**FORENSIC DNA EXTRACTION**



**PATHOLOGY SAMPLE PREPARATION**

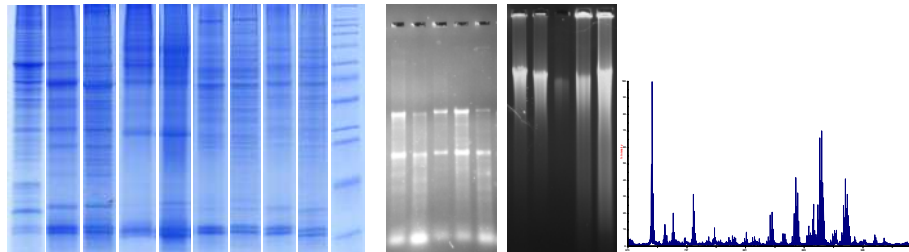


**SUBCELLULAR FRACTIONATION**

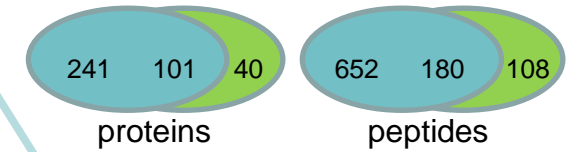
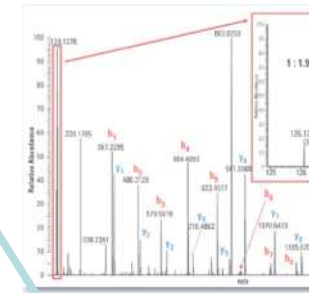


**CELL AND TISSUE LYSIS**

**EXTRACTION AND FRACTIONATION  
DNA, RNA, LIPIDS AND PROTEINS**



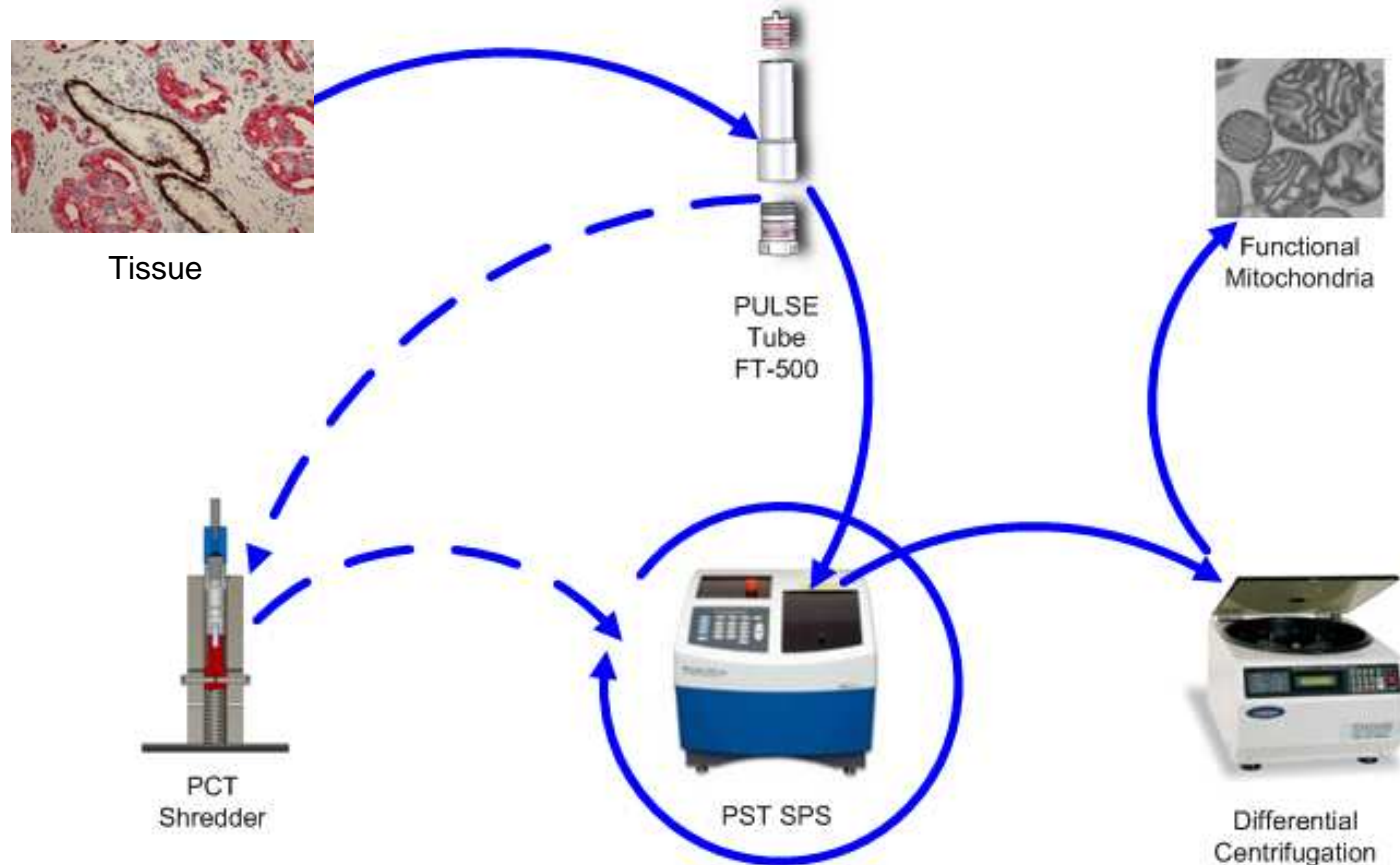
**ACCELERATED ENZYMATIC  
DIGESTION**



**PATHOGEN INACTIVATION**



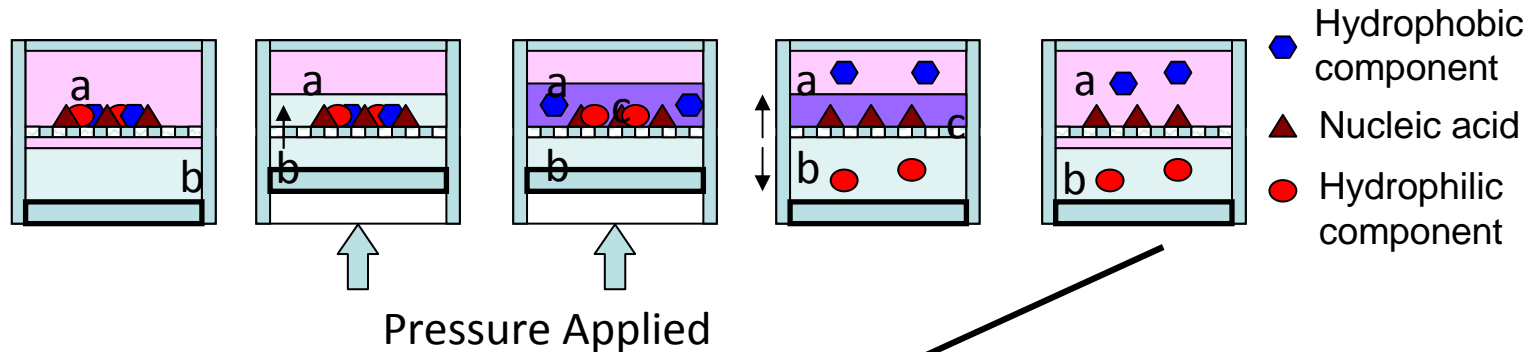
## *Differential lysis by PCT: Extraction of Intact Functional Mitochondria*



Pressure cycling at 10,000-20,000 psi is used to disrupt the plasma membrane for gentle cell lysis and release of intact and functional mitochondria, which are then purified by standard differential centrifugation.

# PCT-mediated liquid-liquid extraction

## ProteoSolve-SB workflow



*Gross et al, JBT, 2008 19:189–199*  
ABRF: "Outstanding article of the year" Award

### CENTRIFUGATION

**Pellet: Nucleic Acids**

**GENOMICS and TRANSCRIPTOMICS:**  
DNA Sequencing,  
PCR, RT-PCR,  
SNPs, RNAi

**Top Phase: Lipids**

**LIPIDOMICS:**  
GC-MS, LC-MS,  
MALDI/TOF, TLC,  
NMR

**Bottom Phase: Proteins**

**PROTEOMICS**  
SDS-PAGE, 2D PAGE,  
Western blotting,  
LC-MS/MS,  
MALDI-TOF, etc...

# Summary

High pressure represents a rich opportunity for bioscience discovery. Engineering advances continue to make laboratory high pressure equipment more convenient and affordable.

Pressure Cycling Technology has a variety of applications:

\* Initial steps of sample preparation for genomics, transcriptomics and proteomics

Compatible with a wide variety of reagents, including organic solvents and many commercially available kits

*ProteoSolve-SB:*

Efficient extraction and fractionation of proteins, lipids and nucleic acids

Improved recovery of membrane-associated and other hydrophobic proteins

Differential lysis by pressure cycling:

Efficient and reproducible tissue disruption; Automation reduces user-to-user variability;

Gentle lysis yields intact and functional mitochondria

\* Enhanced enzymatic digestion for Mass Spectroscopy and other applications

\* Pathogen inactivation, pathology sample preparation, forensic DNA recovery



# Thank You!



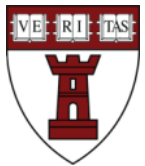
BIDMC



HSPS



BWH



HSDM



Pacific Northwest  
NATIONAL LABORATORY



Northeastern University



JOHNS HOPKINS  
MEDICINE

UCLA



National Institute of  
General Medical Sciences

*SBIR-II GM079059*



National Institute of Allergy and Infectious Diseases  
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

*SBIR-I AI081518*

**PBI**