PBI Pressure BioSciences Inc.

TRENDS AND APPLICATIONS OF HYDROSTATIC PRESSURE IN BIOMEDICAL ANALYSIS: EXPLORING A FORGOTTEN THERMODYNAMIC DIMENSION

Alexander Lazarev

49th EHPRG Conference,
Budapest, Hungary

High pressure in life sciences

- Pressure affects lipid bilayers and proteins
- A wide range of spectroscopic techniques can be successfully used under pressure
- Pressure perturbs equilibrium without addition of excess thermal or chemical energy
- Pressure perturbation is generally reversible
- Ionic and Van-der-Waals interactions respond to pressure in significantly different ways
- Pressure drives phase transitions which are not accessible by manipulation of temperature alone.

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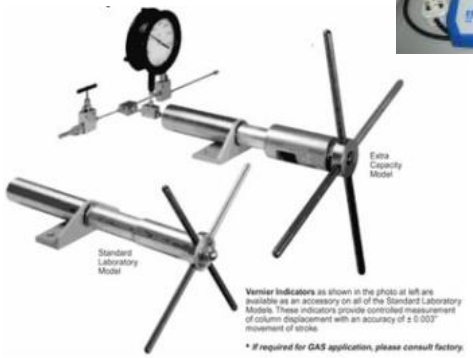
Temperature vs. pressure control equipment



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Pressure BioSciences, Inc.

Our corporate mission is to enable life science community to utilize the unique thermodynamic effects of high pressure.

We achieve this by designing compact (bench-top), easy to use high pressure equipment as well as developing new applications in the area of sample preparation for genomics and proteomics, cell biology and protein biochemistry.

Headquarters in Boston, Massachusetts, USA
NASDAQ:PBIO

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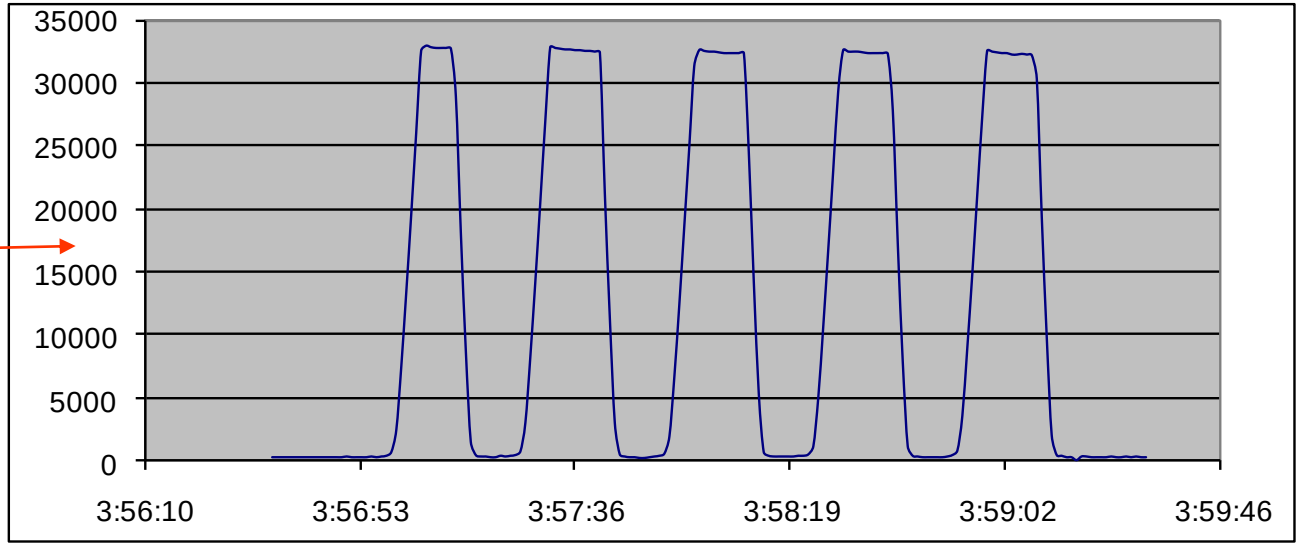
Pressure Cycling Technology (PCT):

1.1 kBar Marianas Trench



"Control of biomolecular interactions by pulses of hydrostatic pressure between ambient and high levels"

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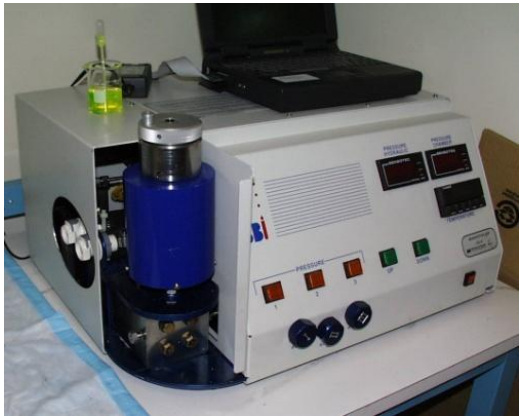
Barocycler™ product line: the history



1999: Barocycler™ v. 2.4

2002: Barocycler™ NEP 2017

Current PBI products

Barocycler™ NEP3229

30 mL chamber
5ksi – 35ksi
Hydraulic system
External t° control

Barocycler™ NEP2320

10 mL chamber
5ksi – 45ksi
Pneumatic system
External t° control

Barocycler™ HUB440

No chamber*
500 psi – 60ksi
Pneumatic system
Modular peripherals

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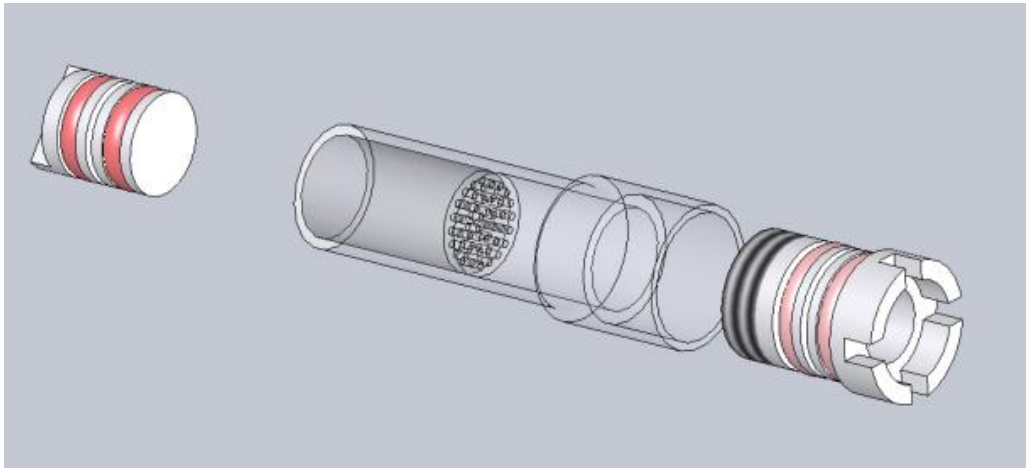
PULSE Tubes

PULSE (**P**ressure **U**sed to **L**yse **S**amples for **E**xtraction) Tubes

- Sample is always contained
- Not in contact with the instrument parts or pressure media,
- Additional functionality:
- Tissue homogenization
- Field collection
- Transportation and storage

Inert polypropylene and silicone materials



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PCT sample containers

Inert Teflon®
t° range: –240 to +205°C
Low binding surface
Variety of volumes,
Flexible workflow
Up to 48 samples/batch

Cartridge system permits pressure cycling and incubation at temperatures above boiling point

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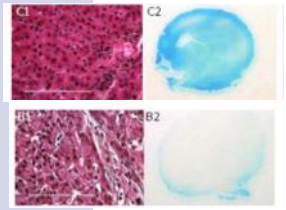
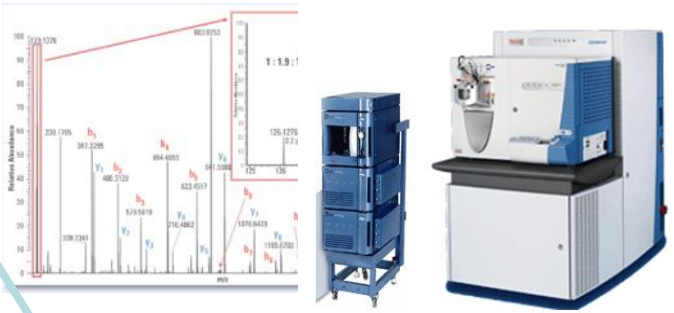
PCT sample preparation applications

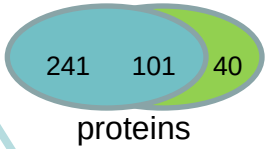
FORENSIC DNA EXTRACTION

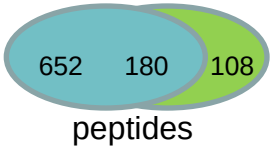
PATHOLOGY SAMPLE PREPARATION



ACCELERATED ENZYMATIC DIGESTION

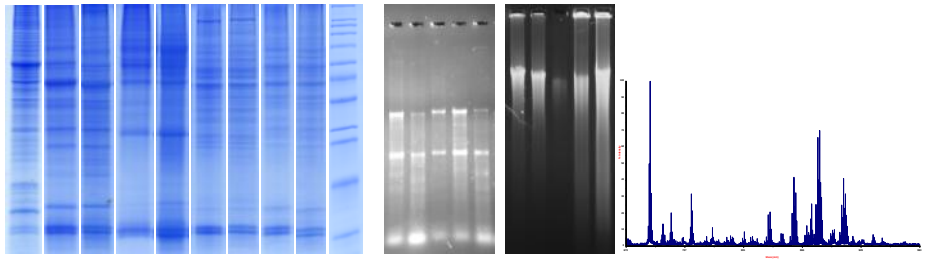




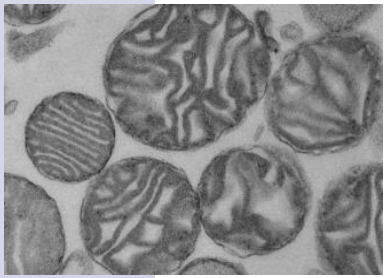


CELL AND TISSUE LYSIS

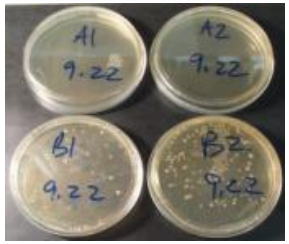
EXTRACTION AND FRACTIONATION DNA, RNA, LIPIDS AND PROTEINS



SUBCELLULAR FRACTIONATION



PATHOGEN INACTIVATION



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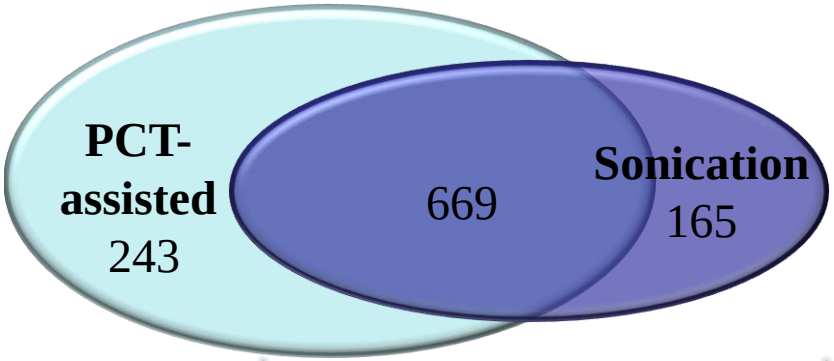
PCT-assisted cell lysis for proteomics

Grand Total: 1077

Enriched with cytosolic Proteins.

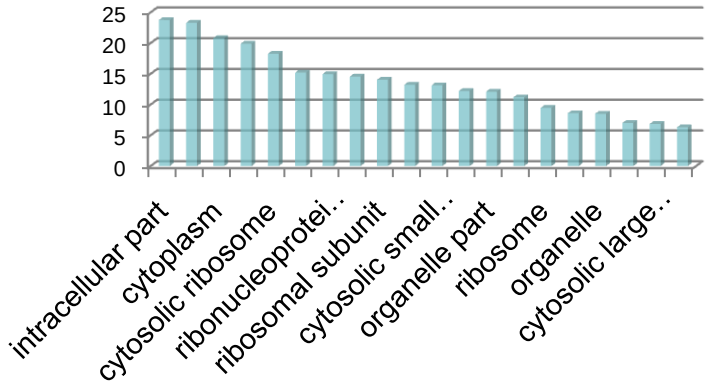
Membrane Proteins are extracted from the pellet in a second PCT extraction (data not shown)

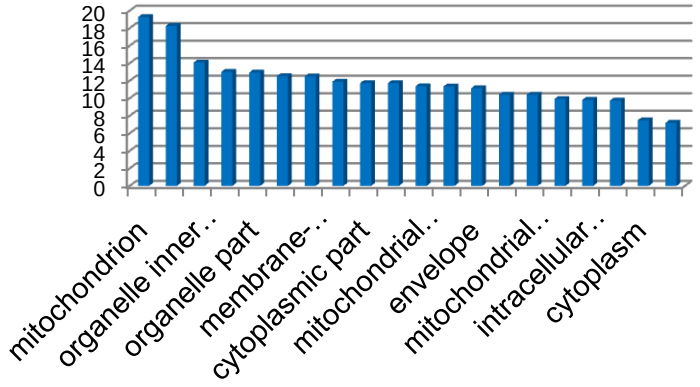
912



834

Does not permit fractionation by localization, contains some organellar and membrane-associated proteins





HepG2 proteome extracted by PCT and by sonication in 50 mM Ammonium Bicarbonate. Data: Freeman *et al., ASMS 2008*

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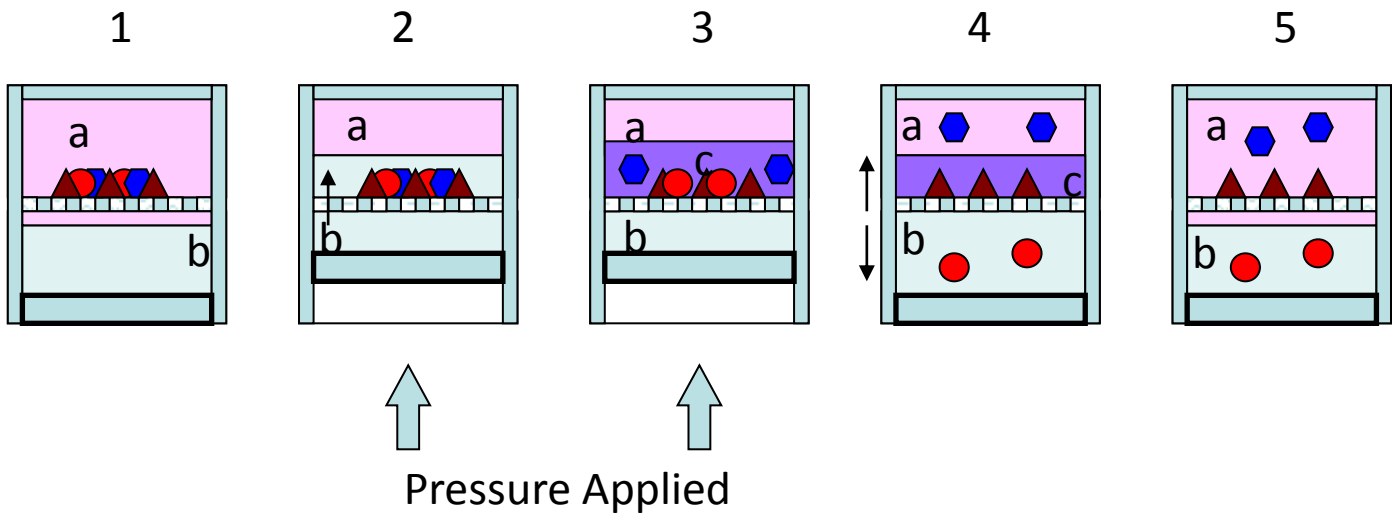
Pressure-enhanced liquid-liquid extraction



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

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

ProteoSolve-SB workflow

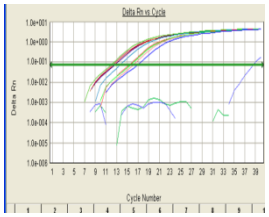
Detergent-Free Extraction of Proteins, DNA , RNA and lipids

SAMPLE + REAGENTS

CENTRIFUGATION ~12,000g for 10 min.

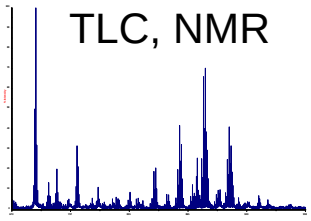
Pellet: Nucleic Acids

GENOMICS:
DNA Sequencing
SNPs
RNAi



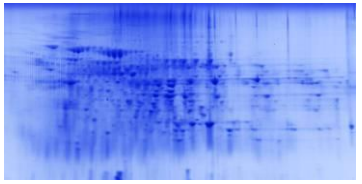
Top Phase: Lipids

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...



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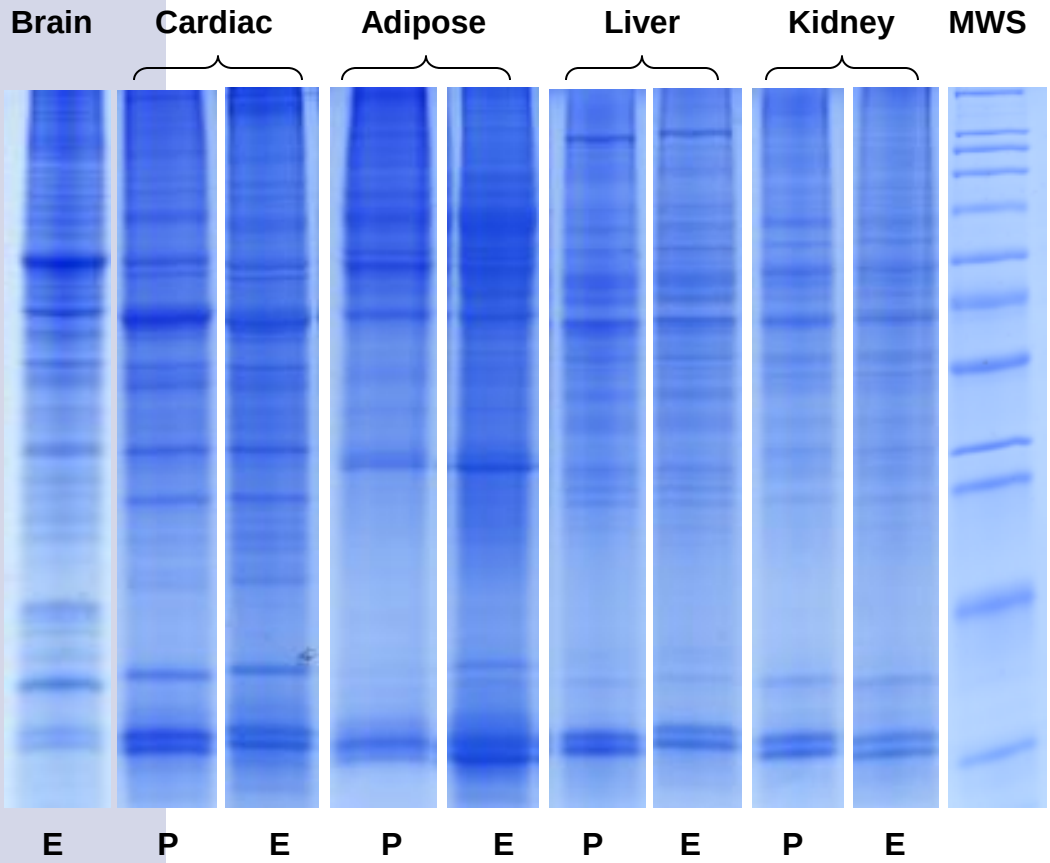
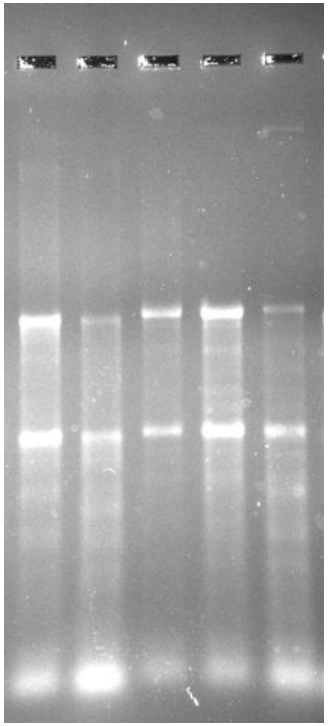
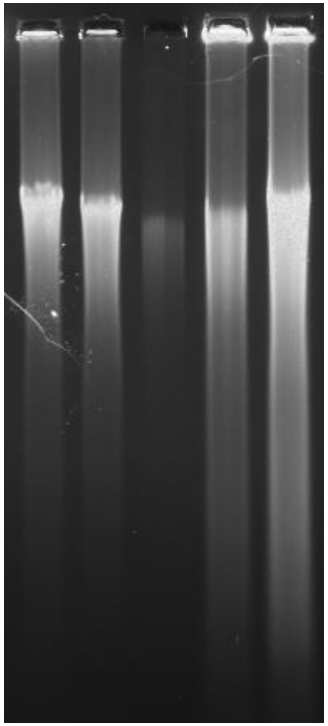
Gross et al, JBT, 2008 19:189–199 ABRF: "Outstanding article of the year"

Protein, RNA and DNA Recovery From Rat Tissues







Solvent removed by: E – evaporation; P - precipitation

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

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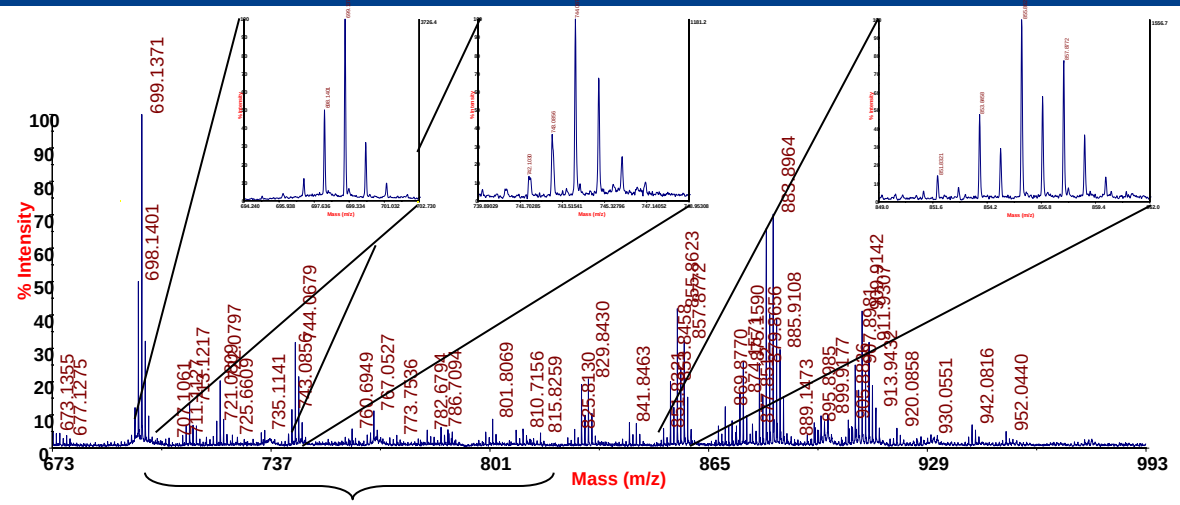
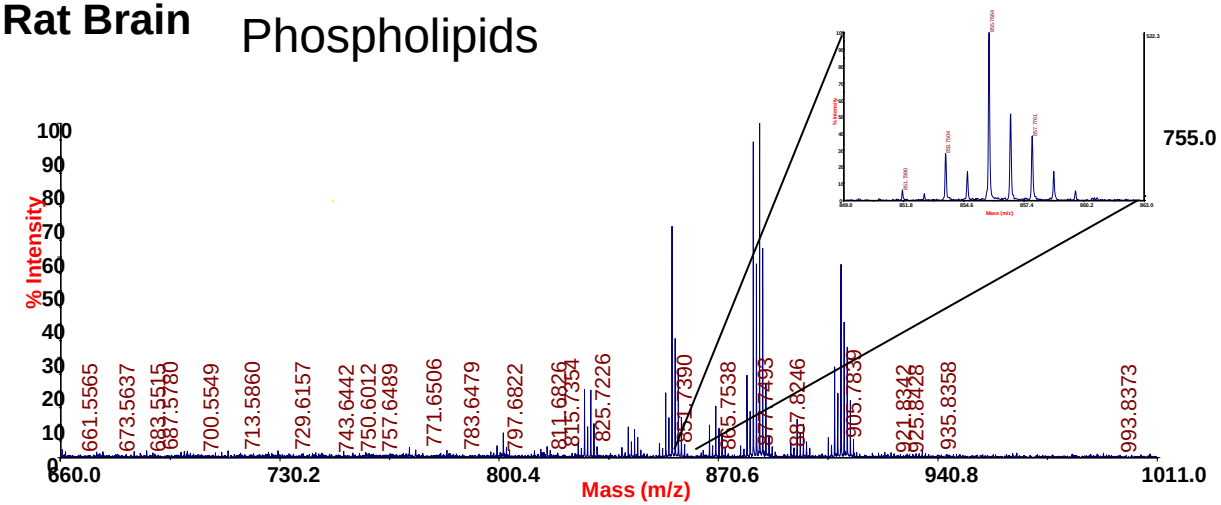
Direct Lipid Profiling by MALDI-TOF MS



Rat Brain Phospholipids



Beef pericardial fat Triacylglycerides

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

PCT: differential lysis concept

- Pressure affects various objects to a different extent, depending on compressibility of their components and energetics of molecular interactions involved.

These factors include:

- Lipid composition in a biological membrane
- Number and type of transmembrane proteins
- Structure and composition of the cell wall
- Predominant type of molecular interactions involved:

  e.g., hydrophobic interactions are weakened by pressure

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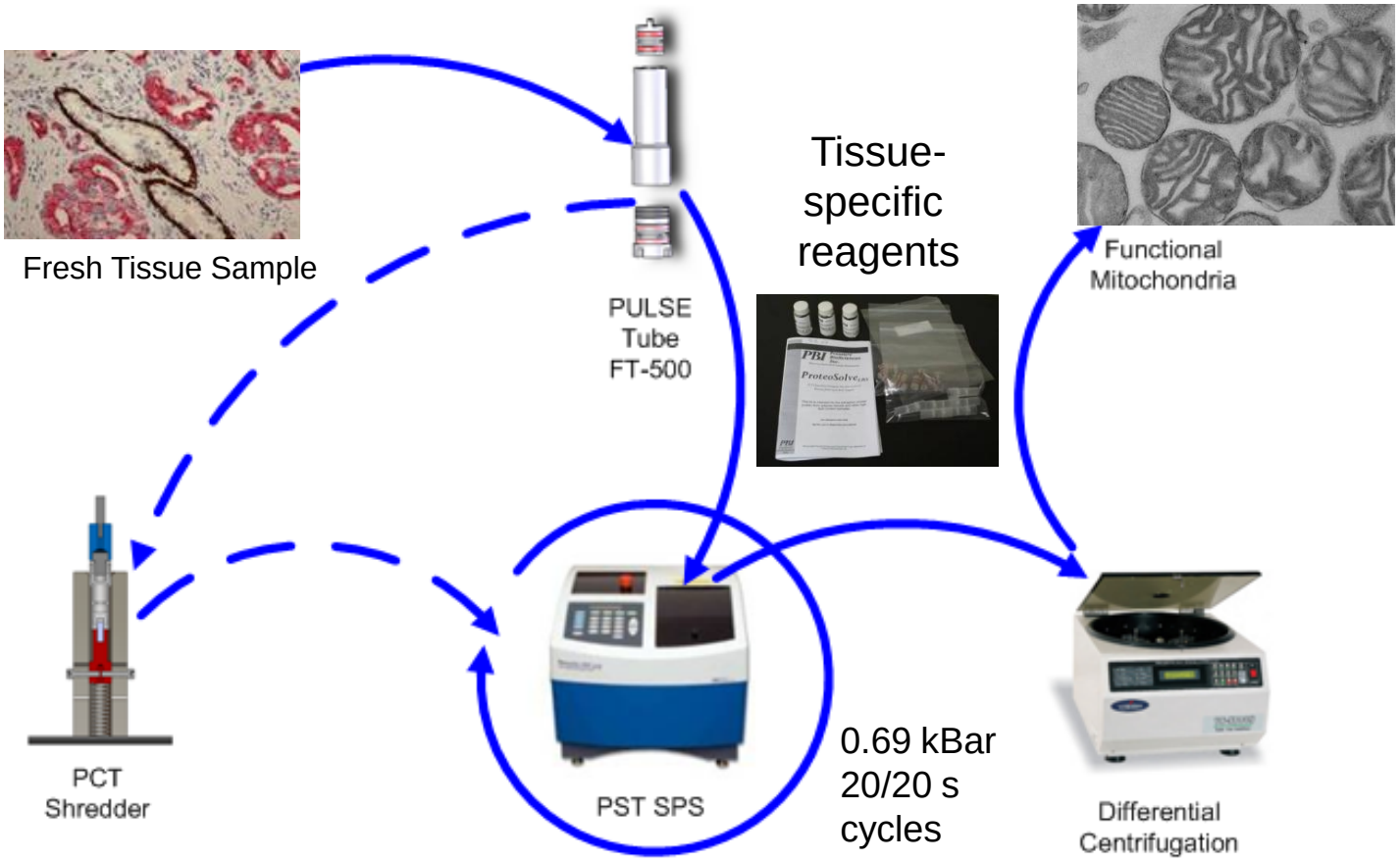
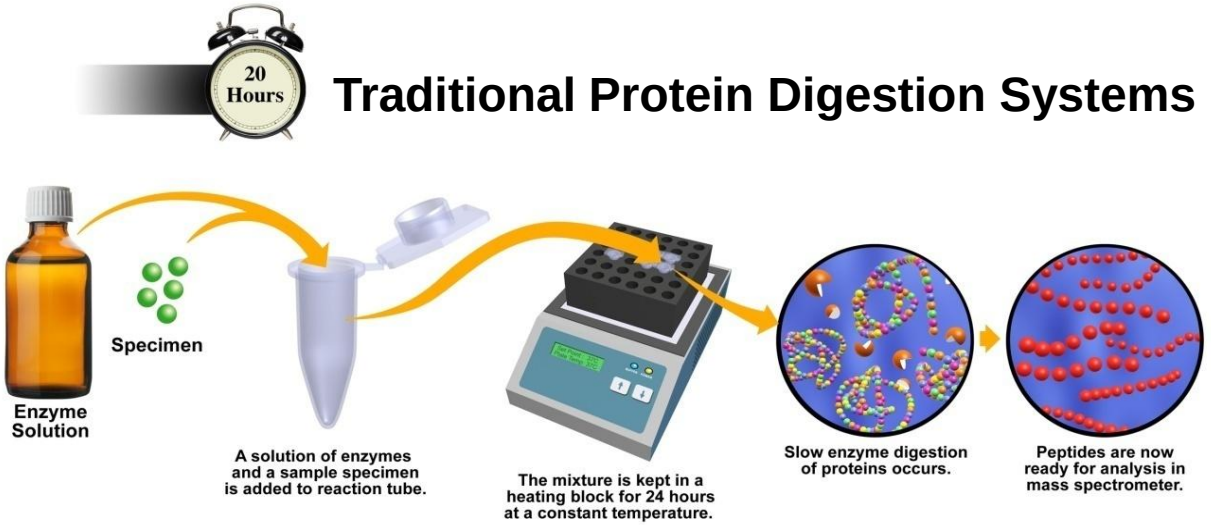
PCT: Extraction of intact functional mitochondria

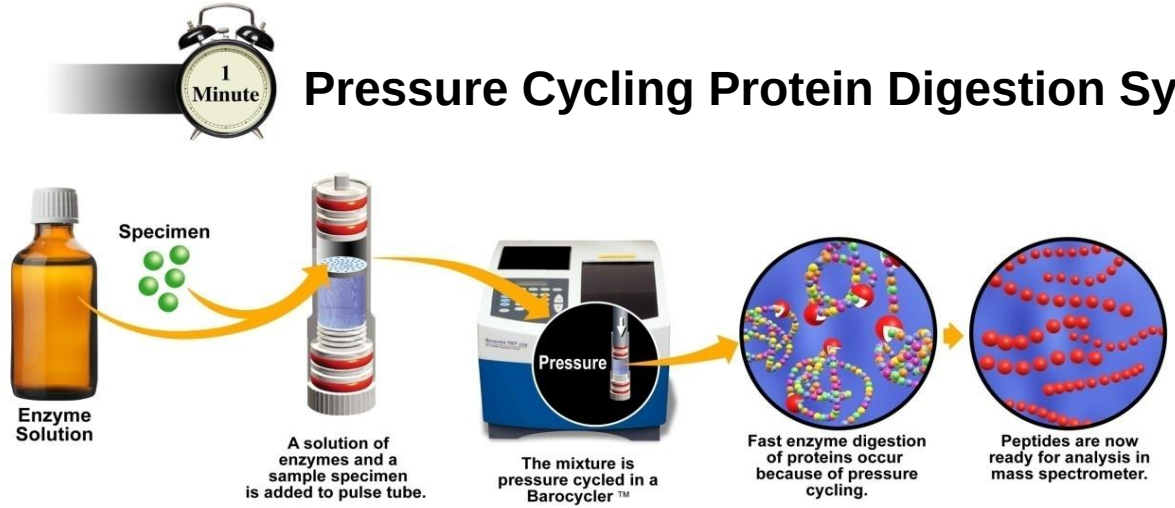


The PCT Shredder and PCT SPS replace manual homogenization tools such as scissors and Potter-Elvehjem homogenizers.

NIGMS SBIR-II GM079059

Pressure-enhanced trypsin digestion





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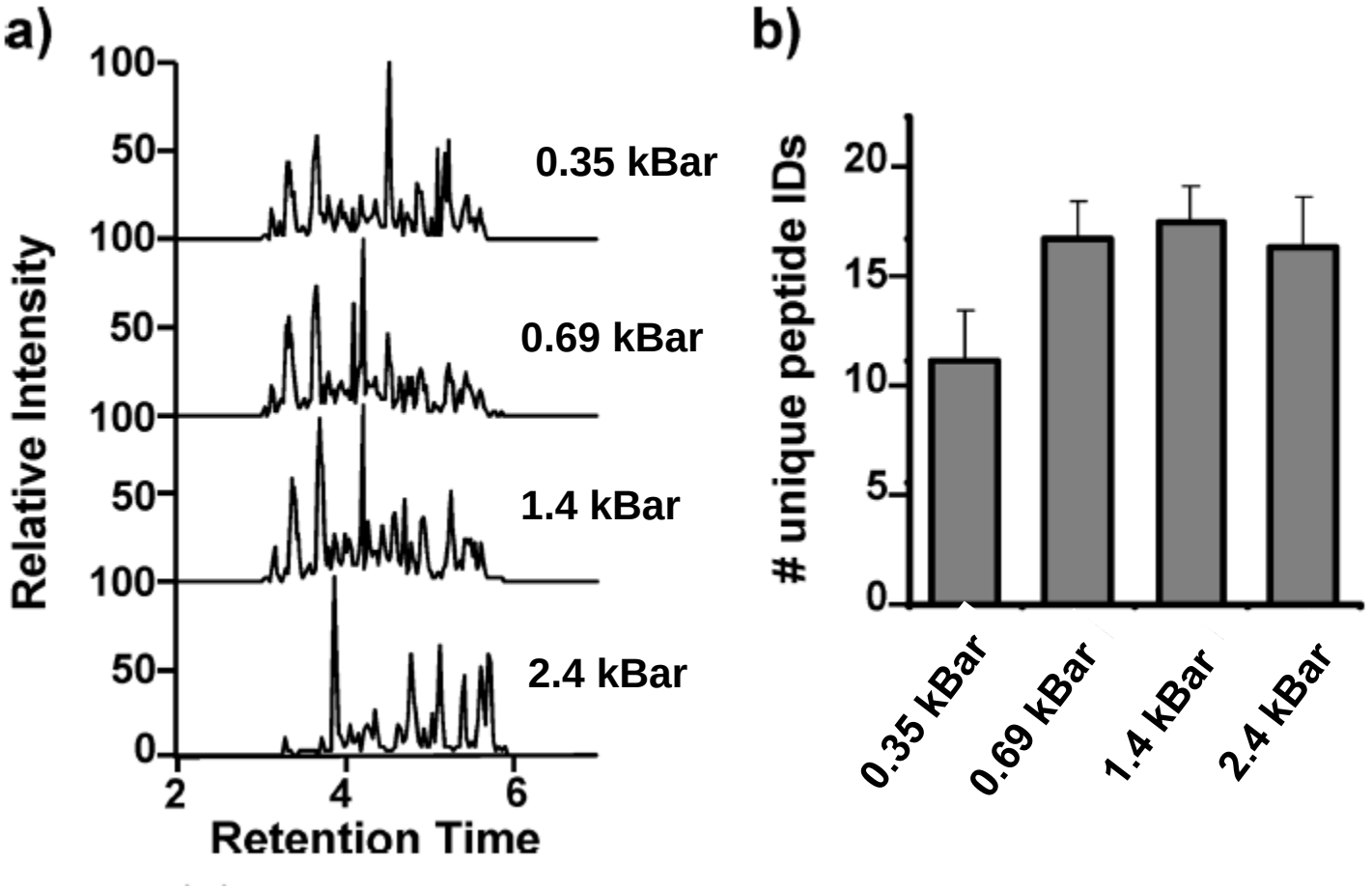
Pacific Northwest NATIONAL LABORATORY

Tryptic digestion of BSA

1 minute, 20% methanol v/v in 25 mM NH_4HCO_3, reduced and alkylated BSA , room temp. 1:50 enzyme/substrate ratio



Lopez-Ferrer. D., et al., JPR (2008) 7(8):3276-81.

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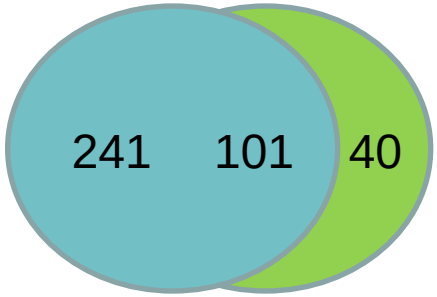
Pacific Northwest NATIONAL LABORATORY

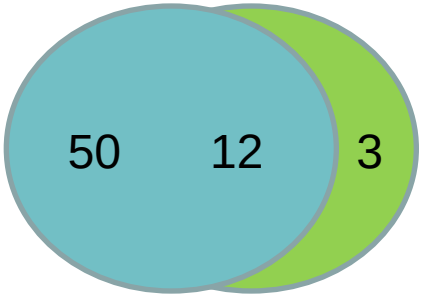
Tryptic digestion of membrane proteins

	Barocycler, 1h	Thermomixer, 18 h
Unique Peptides	**832**	288
Unique Proteins	**342**	141
Integral Membrane Proteins	**62**	15



Proteins

Peptides

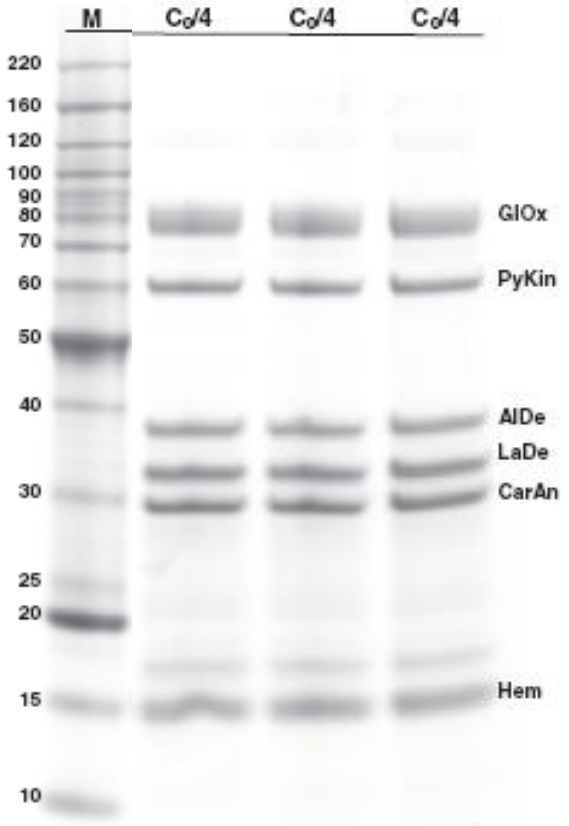


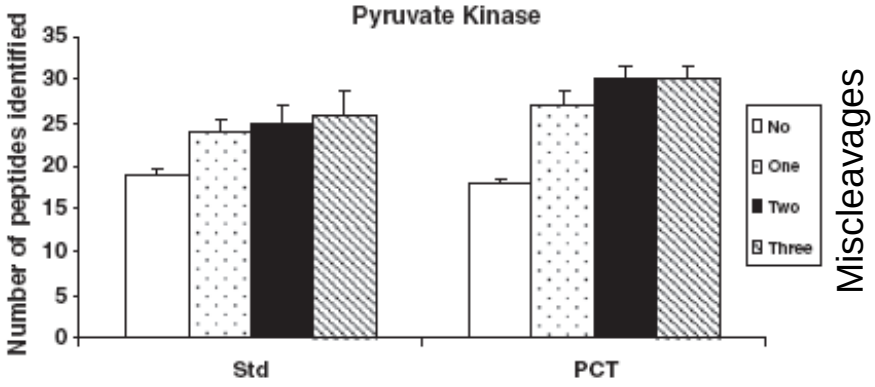
IMPs

1.4kBar Atm.

Data: Eric Bonneil and Pierre Thibault, University of Montréal, 2010

Quantitative in-gel tryptic digestion



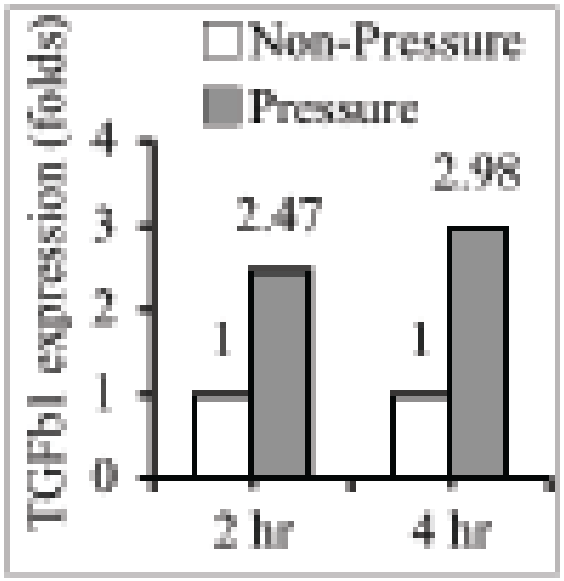


Protein	Lane 1 (spot-to-spot repeatability)			
	$C_0/32$		C_0	
	Average quantification (fmol)	%CV	Average quantification (fmol)	%CV
AlDe	8.3	5.7	405.0	4.5
CarAn	2.9	4.6	280.2	1.0
GlOx	5.0	7.2	322.5	5.1
Hem	1.4	1.4	332.9	7.4
LaDe	3.6	0.9	241.4	4.0
PyKin	2.7	2.5	154.6	1.8

Note: %CV, percentage coefficient of variation.

Getie-Kebtie *et al*., Analytical Biochemistry 409 202-212, 2011

Pressure-shock stimulates cellular stress response



0.35 kBar

Figure 3. TGF-β1 mRNA in chondrocytes was increased by ~3-fold after 2 and 4-hrs of hydrostatic pressure treatment.

Data: Prof. Yefu Li, HSDM – Poster at ORS 2011, Long Beach, CA

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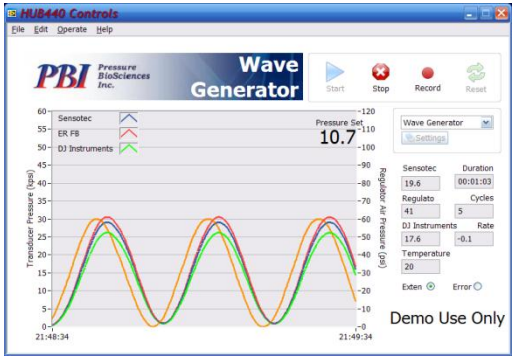
Barocycler HUB440 – programmable pressure generator

Programmable Bench-top High Pressure Generator (500 – 60,000 psi)

- Direct air-driven intensifier
- USB-powered computer interface
- Precise control pressure vs. time
- Data logging
- Interface with spectroscopy, HPLC, and other analytical instrumentation



Poster P29, Tuesday

Applications

Allows precise control of high pressure for structural biology, magnetic resonance and optical spectroscopy, chemical synthesis, HPLC and material science.

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Conclusions

• High pressure represents a thermodynamic “gold mine” for bioscience applications.

•Proteins and lipids are the most compressible biomolecules, expected to show pronounced pressure-driven effects

•Development of user-friendly high pressure equipment helps to raise awareness to pressure in many areas of research

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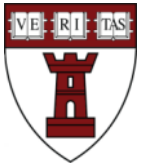
Acknowledgements

Beth Israel Deaconess Medical Center

Harvard School of Public Health

Brigham and Women's Hospital



Harvard school of Dental Medicine

SBIR-II GM079059

SBIR-I AI081518

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