

**Pressure BioSciences' Dr. Alexander Lazarev (VP of R&D) Presents  
Poster and Podium Talk Featuring the Novel & New Barocycler HUB440 at the  
49<sup>th</sup> European High Pressure Research Group (EHPRG) Conference**

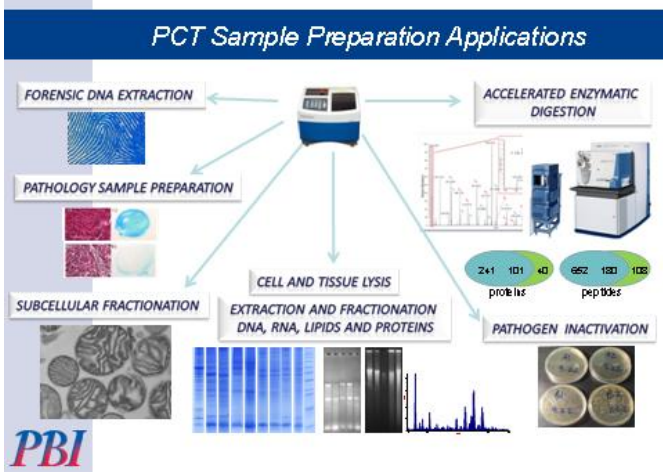
**Program also Included Presentations by Scientists Using  
Pressure Cycling Technology (PCT) from the Barnett Institute of Northeastern University,  
and from the University of Massachusetts - Boston**

**European High Pressure Research  
Group (EHPRG) Conference**

**Podium Talk**

**Dr. Alex Lazarev**

**Trends and Applications of Hydrostatic  
Pressure in Biomedical Analysis: Exploring  
a Forgotten Thermodynamic Dimension**



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PRESENTATION](#)

**European High Pressure Research  
Group (EHPRG) Conference**

**Poster Presentation**

**Pressure BioSciences**

**Development and Applications of an  
Automated, Benchtop, Dynamic  
Pressure Generator  
for High Pressure Bioscience Research**

Alexander Lazarev, Timothy Straub, James Behnke  
and Edmund Y. Ting

Pressure BioSciences, Inc. 14 Norfolk Ave.,  
South Easton, MA 02375, USA

Pressure is a significant thermodynamic parameter that is orthogonal to temperature. The work of the 1946 Nobel Prize winner Percy W. Bridgman inspired further research of high pressure thermodynamics in biological systems. However, the limitations of material science and engineering to these days have led to a prominent lack of commercial laboratory equipment suitable to adequately support high pressure research efforts in life sciences. For decades, high pressure research has been driven by do-it-yourself enthusiasts capable of combining fundamental knowledge of biology, physics and chemistry with the need to practice general plumbing and sophisticated mechanical engineering skills. Majority of the high pressure biochemistry and biophysics work has been done using primitive manual equipment and hours of mandatory physical exercise. The goal of this work is to develop a user-friendly, dynamically-controlled, automated pressure generator suitable for unattended pressure perturbation studies of protein conformation, including enzymology, kinetics of protein folding, aggregation, and self-assembly.

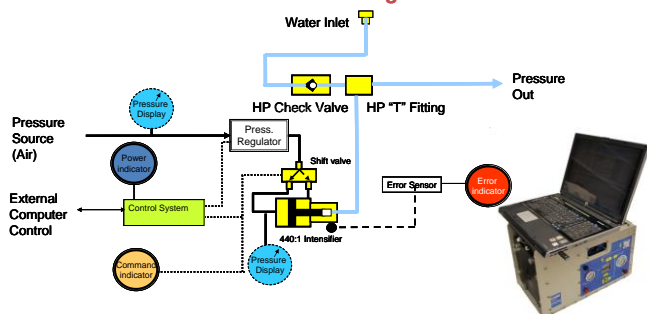
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Column 1

**PBI EVENTS**

**PBI to showcase pressure cycling technology (PCT) and its applications in forensics at the Midwestern Association of Forensics Scientists (MAFS) meeting in Lombard, IL on September 19-23, 2011. Mr. Tom Strader (Sales Director, Midwest) will present a summary of research results generated at PBI and other laboratories using PBI's Barocycler that show improvement in the extraction of DNA from forensic samples.**

*Development and Applications of the Automated Benchtop Dynamic Pressure Generator for High Pressure Bioscience Research: Continued from Page 1, Column 2*

**HUB440 Pressure Generator: Flow Diagram**



The core of the system is an electronically-controlled air-driven reciprocating pressure intensifier with a fixed ratio of 1:440, with a single stroke displacement volume of 3.6 mL and a maximum outlet pressure of 4 kBar. The intensifier is equipped with a dynamic, high pressure seal system with minimum friction, resulting in the ability to ramp pressure over time with a resolution of several Bar per second in both ascending and descending directions. Water is used as a high pressure media due to its low compressibility. An electronic regulator controls the air pressure and the direction of air flow to the front or the back of the air cylinder, resulting in a precise extension or retraction of the intensifier piston. Pressure is controlled via a manual control panel on the front of the instrument or remotely by the USB-powered Data Acquisition and Control interface. A built-in high pressure transducer permits real-time pressure feedback. The system is powered by a 24 VDC power supply. High pressure check valves prevent the back flow of water and enable rapid refill of the intensifier with water for multi-stroke operation. Open frame packaging of the instrument provides easy access to all serviceable components and enables rapid customization. A high pressure outlet is equipped with the standard high pressure type "T" fitting compatible with a wide variety of industry-standard high pressure equipment.

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**European High Pressure Research Group (EHPRG) Conference**

**Podium Presentation**

**Barnett Institute,**

**Northeastern University**

**HIGH PRESSURE MEDIATED SAMPLE PREPARATION FOR CAPILLARY ELECTROPHORESIS ANALYSIS OF N-LINKED GLYCANS**

András Guttman<sup>1, 2</sup>, Zoltán Szabó<sup>1</sup> and Barry L. Karger<sup>1</sup> *1 Barnett Institute, Northeastern University, Boston, MA 02115 2 HLBS, University of Debrecen, Hungary*

**Effect of the PNGase F concentration on N-deglycosylation efficiency under PCT vs. atmospheric reaction conditions**

| PNGase F : Substrate ratio     | 1:10000 | 1:5000 | 1:2500 | 1:666 | 1:1250 |
|--------------------------------|---------|--------|--------|-------|--------|
| PCT (% intact RNase B)         | 83      | 71     | 7      | 7     | 7      |
| Atmospheric (% intact RNase B) | 84      | 69     | 20     | 7     | 8      |

Pressure cycle level:20 kPsi, Reaction time:20 min., Temperature:37°C.

**Advantages of pressure cycling technology (PCT) assisted enzymatic N-deglycosylation**

- The high pressure facilitates conformation changes of the target glycoprotein, increasing the accessibility of the endoglycosidase to the cleavage sites.
- 1:2500 enzyme:substrate molar ratio at 30 kPsi and 37°C quantitatively released the asparagine linked glycans in minutes.
- Pressure cycling apparently did not lead to any loss of sialic acid residues.
- The microliter scale reaction volume alleviated possible precipitation related issues.
- PCT offers simultaneous processing of 12 samples.

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## European High Pressure Research Group (EHPRG) Conference

### Podium Presentation

#### University of Massachusetts Boston CENTER FOR GREEN CHEMISTRY

#### Environmentally Benign Reduction of Organic Compounds in Water under High Hydrostatic Pressure

Anna Tomin<sup>1</sup>, Alexander Lazarev<sup>2</sup>, and Bela Torok<sup>1</sup>  
UMASS Boston<sup>1</sup> and Pressure BioSciences, Inc.<sup>2</sup>.

Green Chemistry works toward sustainability by:

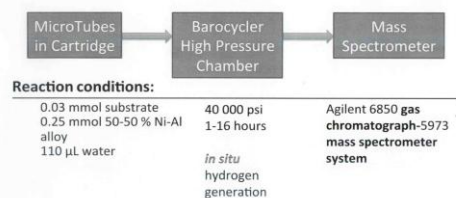
- Making chemical products that **do not harm** either our health or the environment,
- Using industrial processes that reduce **or eliminate** hazardous chemicals, and
- Designing more efficient processes that **minimize** the production of waste materials.

*P.J. Dunn, A.S. Wells, M.T. Williams,  
Green Chemistry in the Pharmaceutical Industry,  
2010., Wiley--VCH*

#### Our aims

- Develop an **environmentally benign** process:
- Reduction of ketones using 50-50% Ni-Al alloy in Water under high hydrostatic pressure (**40,000 psi**)
- **Extend** the process for a broad scope of ketones

#### Pressure enhanced processes



49th EHPRG Conference, Budapest, 2011.

#### Environmentally Benign Reduction of Organic Compounds in Water under High Hydrostatic Pressure: Continued from Page 3, Column 1

#### Conclusion:

- Development of “**green**” reduction method for broad variety of ketones
- Raney Ni-Al alloy, water, ketone & **high hydrostatic pressure**
- ***In situ*** generated hydrogen – remained in the solution
- Corresponding products (alcohols, hydrocarbon, alkene) – **good yield & selectivity**

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## Barocyler HUB440

*The Hub that Powers Your Laboratory's High Pressure Needs!*

### State-of-the-Art High Pressure Generator for Bioscience



1. Open frame design for easy reconfiguration
2. Repositionable carry handles
3. Syringe injection Luer port for system fluid
4. Rear connector for external control
5. External control cable provided
6. 9/16"-18 standard 1/4" tubing high pressure port
7. Intensifier air pressure gauge
8. System error indicator light
9. Control pressure LED display
10. Inlet pressure gauge
11. Valve position indicator
12. Input power indicator light
13. Recessed manual control panel

Intensifier Ratio: 440:1  
Intensifier Displacement: 0.22 in<sup>3</sup> (3.6 mL)  
Compact and Portable (17" x 12" 9", 55 lbs)  
Rapid Pressure Change (full pressure in seconds)  
Pressure Jump mode: sub-millisecond pressure rise or drop  
Maximum Operating Pressure: 4,000 Bar (~58,000 psi)  
Built-in or External Control by Data Acquisition System  
Air Pressure and 24VDC operated (suitable for mobile field use)

|                                       |                               |
|---------------------------------------|-------------------------------|
| EPR, NMR and optical spectroscopy     | Deep sea biology              |
| Thermodynamic phase shift experiments | Material science              |
| High Pressure Freezing                | Solvent extraction            |
| Chemical reaction kinetics            | Pathogen inactivation         |
| Organic synthesis                     | Chromatography                |
| Protein aggregation studies           | Protein conformation analysis |
| Control of enzymatic activity         | High pressure food science    |
| Compressibility measurements          | Cell and tissue lysis         |
| Pressure-jump enzyme kinetics         | Spore germination studies     |