

Pressure Cycling Technology (PCT)
Prominently Featured at
US HUPO 2008, "Proteomics & Beyond"
March 16 - 19, 2008

Dr. Alexander Ivanov

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Environmental Health Proteomics Facility,
Harvard School of Public Health

Oral presentation, Monday March 17 at 11:30am

Comprehensive Analysis of White Fat Adipose Tissue Using Detergent-Free Protein Extraction by Pressure Cycling and High Resolution Tandem Mass Spectrometry

Abstract:

Fat adipose tissue plays a key role in energy metabolism, lipid synthesis and secretion of signaling proteins linked to obesity, insulin resistance, inflammation and other physiological complications. Efficient proteomic analysis of adipose tissue is highly valuable for studies of type II diabetes, obesity, cancer and many other manifestations of metabolic syndrome. Fat adipose tissue contains up to 80-90% lipids by mass, which makes conventional detergent-based protein solubilization and extraction methods inefficient as they tend to produce highly variable results, especially affecting important hydrophobic membrane proteins localized in organelles and the plasma membrane. Abundant sample-derived lipids tend to sequester detergents into micelles, thus interfering with protein extraction. This study was enabled by the use of alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and specialized organic solvents for disruption of cells, micelles and membrane fragments and efficient protein recovery from lipid-rich adipose tissue. Adipose tissue samples from obese (ObOb) mice were used to optimize the conditions for protein extraction. When the efficiency of protein extraction was determined, it confirmed overall higher protein recovery by the novel method. Differential proteomic analysis using optimized extraction techniques followed by 1D- and 2D-SDS-PAGE and protein identification by liquid chromatography and high mass accuracy high mass resolution electrospray tandem mass spectrometry have been performed to identify adipose tissue proteins specific to several genetically distinct model animal lines. Several unique protein species were identified in the extracts of adipose tissue. Post-translational modifications including phosphorylation, acetylation and ubiquitylation of selected proteins detected in multiple isoforms were characterized. Comparative proteomic analysis of adipose tissue isolated from several genetically different murine model lines on different diets, has revealed differences in protein expression. The results of proteomic analyses were compared to transcriptomic expression profiles and submitted to functional pathway analysis. Differentially regulated energy and glucose metabolism pathways identified in this way may be useful in the mechanistic studies of obesity and associated disorders such as Type II diabetes and non-alcoholic fatty liver disease.

CALENDAR OF EVENTS

4TH ANNUAL U.S. HUPO
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SBS 14TH ANNUAL CONFERENCE & EXHIBITION
ST. LOUIS, MO
APRIL 6-10, 2008

Poster Profile

Isolation of Proteins from Subcutaneous Murine Tumor and Adipose Tissues Using a Pressure Cycling Technology

Poster Presented by Gary B. Smejkal¹; Deena Small²; Sumithra Urs³;
Ada T. Kwan¹; ¹Pressure BioSciences, Woburn, MA; ²University of
New Hampshire, Durham, NH; ³Maine Medical Center Research
Institute, Scarborough, ME

Abstract:

Extremely small and rare tissue samples must be processed with high efficiency to enable reliable proteomic analysis. For small samples of murine tumor and adipose (10-100 mg) or rat liver (0.5-10 mg), Pressure Cycling Technology (PCT) yielded more protein in 30 minutes compared to pulverization of tissue frozen under liquid nitrogen (LNP) followed by buffer extraction of the triturate for 0.5-48 hours. For four subcutaneous tumor types, protein extraction efficiency was 4.4% ± 0.3% for PCT compared to 1.5% ± 0.6% for LNP. Further, a coefficient of variation (CV) of 7.8% was observed between the four tumor types for PCT compared to 37.7% for LNP. Also, PCT extracted as much as four times more protein from small adipose tissue samples than LNP. Finally, it is demonstrated that PCT derived sufficient protein from less than 1 mg of rat liver (0.7 ± 0.3 mg) which enables highly reproducible analyses through two-dimensional gel electrophoresis (2DGE).

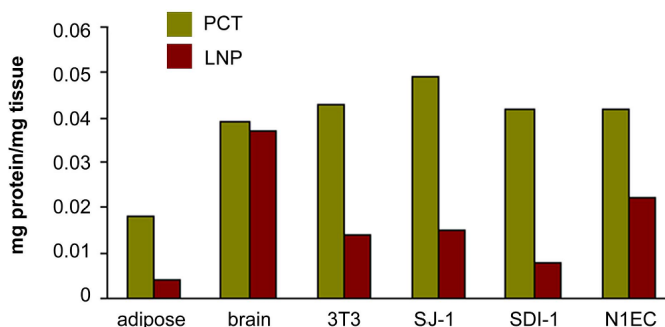


Figure 2. Comparative extraction efficiency of PCT and LNP, expressed in milligrams of protein per milligram of initial tissue mass, for adipose, brain, and four subcutaneous tumor types (3T3, SJ-1, SDI-1, and N1EC). Mean mass of SJ-1 and N1EC tumors was 21.6 ± 3.8 mg (n = 4). Mean mass of adipose and brain tissues were 72.6 ± 11.8 mg (n = 4)

PBI in the News

Pressure BioSciences, Inc. Announces Move to New Corporate Offices

Pressure BioSciences, Inc. (Nasdaq: [P BIO](#)) announced that it has moved its corporate offices to 14 Norfolk Avenue in South Easton, Massachusetts. This move was necessary to accommodate the Company's transition from an early-stage, research and development company to a fully commercial operation. Richard T. Schumacher, PBI's Founder, President, and CEO commented: "We now have approximately 5,500 square feet, with sufficient office, conference, engineering lab, and storage space to allow us to better develop our infrastructure to support our PCT product commercialization. In addition, the 18 month lease, with equal term extension, gives us the financial flexibility we require as we continue to grow our business and carefully manage our financial resources". The Company's primary research and development facility continues to be located at 6 Gill Street in Woburn, MA.

Corporate address: Pressure BioSciences, Inc. 14 Norfolk Avenue South Easton, MA 02375 (t) (508) 230-1828 (f)(508) 230-1829	Research and Development Center: Pressure BioSciences, Inc. 6 Gill Street, Suite H Woburn, MA 01801 (t) (781) 932-9477 (f) (781) 932-9294
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Matthew B. Potter Joins Pressure BioSciences, Inc. as Vice President of Sales

Pressure BioSciences, Inc. (Nasdaq: [P BIO](#)) announced that Matthew B. Potter has joined PBI's senior executive management team as Vice President of Sales. Mr. Potter brings with him nearly twenty years of experience in sales and sales management at a number of leading life sciences companies. Mr. Potter will oversee PBI's seven US-based, regional sales directors and will be responsible for driving the Company's commercialization of its novel and patented pressure cycling technology (PCT) product line.

Mr. Richard T. Schumacher, PBI's Founder, President, and CEO stated: "This is an important and exciting time for our Company, as our efforts in 2008 are clearly focused on both the development of market awareness for PCT and on the expansion of our small but growing customer base. To this end, we believe that Matt's background and management style is a not only a great fit with our team, but should also help to accelerate our PCT commercialization plan. We consider ourselves very fortunate to have someone of Matt's caliber join our management team."

Mr. Potter commented: "In over 19 years of selling products into the research marketplace for the analysis of nucleic acids, proteins, and small molecules, one issue -- how to achieve high quality extraction of bio- molecules prior to analysis -- nearly always rose to the top. This was always a key issue, and to that end, I believe strongly that the PCT Sample Preparation System answers many of the sample preparation problems that researchers encounter, and offers unique advantages to customers looking to extract DNA, RNA, proteins, and small molecules from a wide variety of biological samples. PBI has a dedicated and talented group of people throughout the organization, and I look forward to working with them."

Poster Profile

Unified Sample Preparation Approach Using Hydrostatic Pressure Cycling: Simultaneous Isolation of Proteins, Nucleic Acids and Lipids from a Single Sample

**Poster Presented by Vera S. Gross; Greta Carlson;
Gary B. Smejkal; Ada T. Kwan; Timothy Straub;
Alexander V. Lazarev
Pressure BioSciences, Inc., Woburn, MA**

Abstract:

Systems biology studies require the incorporation of methods used in the fields of genomics, transcription profiling and proteomics, as well as the rapidly emerging area of metabolomics. While powerful and sensitive techniques are available for the individual isolation and analysis of nucleic acids, proteins, lipids, and small molecules, major bottlenecks arise because multiple sample replicates are usually required if all of these cellular components are to be analyzed in the same sample. Current sample preparation techniques rely upon mutually incompatible sample preparation methods and solvents to isolate nucleic acids, proteins, lipids, and small molecules from cells and tissues. Moreover, the strong detergents and chaotropic agents often used to solubilize samples tend to interfere with subsequent separation and downstream analyses. We have developed a novel detergent-free sample preparation technique which allows concurrent isolation and fractionation of protein, nucleic acids, and lipids from samples as diverse as cell cultures, brain tissue, adipose tissue and liver. This novel method relies on a synergistic combination of cell disruption by alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and optimized reagents that dissolve and partition distinct classes of molecules into separable fractions. We report rapid simultaneous isolation of nucleic acids (DNA and/or RNA), lipids, proteins, and small molecules from samples of cultured human fibroblasts, PC-12 cells, and several types of mammalian tissues. Gel electrophoresis and real-time PCR confirm that high recovery of intact genomic DNA and good yields of intact RNA are obtained using this novel technique. Small molecule recovery has been confirmed by HPLC. Total protein fractions have been analyzed by SDS-PAGE and 2D-PAGE, confirming excellent reproducibility of the new method and high recovery of total protein. Several proteins extracted with significantly higher yields by the novel method *versus* conventional detergent-based techniques have been identified by MALDI-TOF mass spectrometry.