Applications of Ultra-high Pressure in Biotechnology

Harvard Medical School
Joseph B. Martin Conference Center
The Rotunda, New Research Building
77 Avenue Louis Pasteur
Boston, MA 02115

May 21, 2010

Sponsored By
Pressure BioSciences, Inc. (PBI)
14 Norfolk Ave., S. Easton, MA 02375
508-230-1828
NASDAQ: PBIO
www.pressurebiosciences.com

Co-hosted By
The Laboratory for Innovative Translational Technologies (HC-LITT) and Central Laboratory (HCCL) of Harvard Catalyst | The Harvard Clinical and Translational Science Center (Harvard CTSC) led by Drs. Winston P. Kuo, Robert Distel, and Vincent Ricchiuti and the Proteomics Resource at Harvard School of Public Health led by Dr. Alexander R. Ivanov

Disclaimer
Harvard Catalyst | The Harvard Clinical and Translational Science Center, HC-LITT, HCCL, HPR, and the DoD do not endorse any company or product. The opinions expressed during this event are solely those of the speakers and of PBI, and do not represent the views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, the National Center for Research Resources, the DoD, or the National Institutes of Health.
PROGRAM

8:00 am Coffee

8:30 am Welcome Richard T. Schumacher, President and CEO, PBI

8:35 am Opening Remarks Winston Patrick Kuo, DDS, DMSC
Director, Harvard Catalyst Laboratory for Innovative Translational Technologies (HC-LITT)
Harvard Medical School Instructor
Department of Developmental Biology
Harvard School of Dental Medicine

Session I Pressure Fundamentals & Enhanced Proteolysis

Chairperson Alexander R. Ivanov, Ph.D.
Director, HSPH Proteomics Resource
Research Scientist
Harvard School of Public Health

8:50 am High Pressure Instruments for Innovation and Discovery
Edmund Y. Ting, B.S.M.E., M.Sc., Sc.D.
Pressure BioSciences, Inc., S. Easton, MA 02375

9:20 am Development of Essential Sample Preparation Techniques in Proteomics Using Ultra-High Pressure
Emily Freeman and Alexander R. Ivanov, Ph.D.
Harvard School of Public Health, HSPH Proteomics Resource, Department of Genetics and Complex Diseases, Boston, MA 2115

9:50 am High Pressure Digestion Improves Reproducibility and Differential Expression Monitoring in Proteomic Experiments
E. Bonneil1, C. Bell1, R. Biringer2, J. Saba1, and P. Thibault1
1Institute for Research in Immunology and Cancer, Université de Montréal, Canada
2Life Sciences Mass Spectrometry, Thermo Fisher Scientific, San Jose, CA 95134

10:20 am Application of High Pressure for High Performance Proteomics
Daniel López-Ferrer, Jungbae Kim, Kim K. Hixson, Karl K. Weitz, Rui Zhao, Scott Kronewitter, Mikhail E. Belov and Richard D. Smith, Pacific Northwest National Laboratory, Richland, WA

10:50 am BREAK
Session II  Pressure Enhanced Cancer Testing

Chairperson  Vincent Ricchiuti, Ph.D.
Director, Harvard Catalyst Central Laboratory (HCCL)
Associate Biochemist, Endocrinology, Diabetes &
Hypertension, Brigham and Women’s Hospital
Harvard Medical School

11:10 am  High Pressure Direct Protein Extraction from Tissue – Trypsin Digest with Pressure Cycling Technology (PCT)
Paul H. Pevsner, Jennifer Oprihory, Nicholas Sobol, Parth Kothiya, Zeineen A. Momin, Naresh Vasani, and Tiffany Remsen
Department of Pathology, University of Missouri School of Medicine, Columbia, MO

11:40 am  Recovery and Immunoaffinity Enrichment of Integral Membrane Proteins from Metastatic Ovarian Cancer Tissue
Luke Schneider, Varsha Likhte, William Wright, Frances Chu, Emma Cambron, Anne Baldwin, and Jessica Krakow, Target Discovery, Inc., Palo Alto, CA

12:10 pm  Application of Pressure for Improved Proteomic Analysis of FFPE Tissue and for Improved Sample Quality of Formalin-based Tissue Histology
Carol B Fowler 1,3, Timothy J Waybright 1, Timothy D Veenstra 2, Timothy J O’Leary 3, and Jeffrey T Mason 1
1Department of Biophysics, Armed Forces Institute of Pathology, Rockville, Maryland, 2Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, Maryland, and 3Veterans Health Administration, Washington, DC

12:40 pm  LUNCH - box lunches offered at no charge just outside the Rotunda

Session III  Pressure Enhanced Extraction and Inactivation: Forensics, Biological Threat Organisms, Ticks, & 40 Million Year Old Amber

Chairperson  Robert J. Distel, Ph.D.
Research Director, Translational Research Laboratory
Center for Clinical and Translational Research
Dana-Farber Cancer Institute
Associate Director, Harvard Catalyst Laboratory for Innovative Translational Technology (HC-LITT)

1:30 pm  A Need for Improved Sample Inactivation and Extraction Methods to Support Systems Biology Analysis of Biological Threat Organisms
Bradford S. Powell 1 and Loreen L. Lofts 2
1Bacteriology Division, 2Virology Division
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702
2:00 pm  Convenient and Reliable Extraction of *Borrelia burgdorferi* from Ticks Using Pressure Cycling Technology (PCT) and the PCT Shredder  
Richard C. Tilton, Ph.D.  
Medical Diagnostics Laboratories

2:20 pm  The Real Jurassic Park: The Isolation of Proteins from Microorganisms Preserved in Amber Inclusions for 40 Million Years  
Gary B. Smejkal\(^1,2\)  
\(^1\) Harvard Catalyst Laboratory for Innovative Translational Technologies, Boston, MA.  
\(^2\) Politecnico di Milano, Department of Chemistry, Materials & Chemical Engineering, Milan, Italy

2:40 pm  Pressure Cycling Technology (PCT) Augments Sensitivity of Detection and Robustness in Forensic DNA Analyses  
Bruce Budowle, Ph.D.  
Department of Forensic and Investigative Genetics, Institute of Investigative Genetics, University of North Texas Health Science Center, Ft Worth, Texas

3:10 pm  BREAK

3:30 pm  Session IV  
Bio-therapeutics Manufacturing and Future Trends  
Chairperson  
Winston Patrick Kuo, DDS, DMSC  
Director, Harvard Catalyst Laboratory for Innovative Translational Technologies (HC-LITT)  
Harvard Medical School Instructor  
Department of Developmental Biology  
Harvard School of Dental Medicine

3:30 pm  Rapid Sample Preparation Method for Analysis of N-Linked Glycans  
Zoltan Szabo, Tomas Rejtar, Andras Guttman, and Barry L. Karger  
Barnett Institute, Northeastern University, Boston, Massachusetts 02115

3:50 pm  High-Pressure Assisted In-Gel Tryptic Digestion for Qualitative and Quantitative Characterization of Protein Mixtures  
Melkamu Getie-Kebtie and Michael Alterman  
FDA-CBER-NIH, Rockville Pike, HFM – 735 Bldg 29A rm 2820, Bethesda, MD 20892

4:10 pm  High Pressure in Life Sciences – Trends and Future Opportunities  
Alexander Lazarev, Ph.D.  
Pressure BioSciences, Inc., S. Easton, MA 02375

4:40 pm  Final Thoughts and Closing Remarks  
Winston Patrick Kuo, DDS, DMSC  
Director, Harvard Catalyst Laboratory for Innovative Translational Technologies (HC-LITT)  
Harvard Medical School Instructor  
Department of Developmental Biology  
Harvard School of Dental Medicine
ABSTRACTS

High Pressure Instruments for Innovation and Discovery
Edmund Ting, B.S.M.E., M.Sc., Sc.D.
Pressure BioSciences, Inc., 14 Norfolk Ave., S. Easton, Massachusetts 02375
eting@pressurebiosciences.com

Percy Bridgman (Harvard University) won the 1946 Nobel Prize for his work on the physics of substances under high hydrostatic pressure. A portion of his work focused on the thermodynamic effect of pressure on proteins and other biological substances. Bridgman had to invent his own high-pressure equipment. Today, scientist can readily purchase counter-top instruments that allow them to study advanced biological sciences without having to master high-pressure engineering first. High pressure is becoming a common tool for scientific investigation. In this presentation, we will review the typical components found in a high-pressure system, the methods used to seal high pressure, and the physical process of compression. We will illustrate the compression heating effect and see how this effect may (or may not) influence the experimental results achieved.

Development of Essential Sample Preparation Techniques in Proteomics Using Ultra-High Pressure
Emily Freeman and Alexander R. Ivanov
Harvard School of Public Health, HSPH Proteomics Resource, Department of Genetics and Complex Diseases, 665 Huntington Avenue, B1-409, Boston, MA 2115
aivanov@hsph.harvard.edu

Minimization of preanalytical variables and standardization of sample preparation are imperative for successful discovery-driven and translational research involving large-scale (“omics”) biomolecular profiling. Here we applied the use of high pressure to maximize efficiency of both cell lysis and enzymatic digestion while minimizing the time of protein digestion, manual involvement in sample processing, and pre-analytical variability introduced during sample preparation prior to mass spectrometry-based proteomics analysis. The sample preparation techniques were evaluated and optimized using standard proteins and cell lysates. The resulting data shows that the developed techniques can achieve superior protein recovery when compared to conventional protocols. These approaches provide a substantial gain in the identification rate of a wide range of organelle- and membrane-associated proteins and protein complexes, meanwhile obtaining up to a 24-fold increase in digestion throughput without compromising sample-to-sample reproducibility.
Mass spectrometry (MS)-based proteomics has been proven to be a powerful technique for protein identification, post-translational modification characterization and differential protein expression monitoring. The “bottom-up” strategy used in proteomics consists of in-gel or in-solution proteolytic digestion of proteins, peptide separation on a C18 column, MS/MS peptide sequencing and finally data processing. Depending on the number of liquid or gel band samples, the whole process can take several days to be completed. Two-dimensional separations and faster peptide signal processing have made sample preparation the bottleneck in proteomic throughput. High-pressure digestion has been reported to give similar peptide and protein identifications than the conventional overnight digestion in a lesser time. Subtle protein expression changes are often the hallmark of important cellular events and remain intrinsically difficult to detect in bottom up proteomics experiments due to the overwhelming sample complexity and variability associated with sample preparation. Here we report that the use of pressure-cycling technology not only fasten the proteolytic process but also increase significantly the reproducibility for gel-band, cell lysate or membrane samples enabling confident expression profiling of membrane proteins.

Application of High Pressure for High Performance Proteomics
Daniel López-Ferrer, Jungbae Kim, Kim K. Hixson, Karl K. Weitz, Rui Zhao, Scott Kronewitter, Mikhail E. Belov and Richard D. Smith
Pacific Northwest National Laboratory, Richland, WA
daniel.lopez-ferrer@pnl.gov

In recent years there has been an emphasis on improving automation and increasing throughput for proteomics research. To achieve these goals, sample preparation steps need to be enhanced to enable more rapid processing. We have studied the role of elevated pressures in enzymatic digestions on-line and off-line for LC-MS purposes. The off-line application used a Barocycler™, which uses pressure cycling technology (PCT) in the range of 0 to 35 kpsi. To further enhance methods for high-throughput applications, an on-line digestion system was developed which carries out cell lysis, protein derivatization and protein digestion all at once. We also explored the possibility of integrating glycomics and proteomic studies within the same system. This new pressurized solvent method looks promising for ultra-high-throughput applications, i.e., “omics on the fly.”
High Pressure Direct Protein Extraction from Tissue – Trypsin Digest with Pressure Cycling Technology (PCT)
Paul H. Pevsner, Jennifer Oprihory, Nicholas Sobol, Parth Kothiya, Zeineen A. Momin, Naresh Vasani, and Tiffany Remsen
Department of Pathology, University of Missouri School of Medicine, Columbia, MO pevsner1@earthlink.net

Trypsin belongs to a class of serine peptidases of proteolytic enzymes used to digest proteins into various-sized peptides for scientific analysis. Current methods of enzymatic digestion often use heat and prolonged digest intervals. BSA and Cytochrome-C were used to demonstrate proof of principle that trypsin digest of these proteins could be achieved in minutes at ambient temperature. Trypsin digestion with PCT cycling at short intervals of high and low pressure produce rapid trypsin digestion comparable to longer intervals at ambient pressure and elevated temperature. Long digest intervals have been cited as a major disadvantage in protein studies and prompted a search for viable time-saving alternatives, such as PCT. Samples digested in minutes with PCT give virtually the same results as those digested for one hour at 55°C.

Recovery and Immunoaffinity Enrichment of Integral Membrane Proteins from Metastatic Ovarian Cancer Tissue
Luke Schneider; Varsha Likhte; William Wright; Frances Chu; Emma Cambron; Anne Baldwin; and Jessica Krakow
Target Discovery, Inc., Palo Alto, CA luke_schneider@targetdiscovery.com

Integral membrane proteins play key biological roles in cell signaling, transport, as well as pathogen invasion. However, quantitative clinical assays for this critical class of proteins (e.g., immunosorbant assays) remain elusive, and are generally limited to serum-soluble extracellular fragments. Furthermore, classic proteomic approaches to membrane protein analysis involve digestion of the generally soluble intra- and extra-cellular domains from the generally-insoluble transmembrane regions. Such peptidization separates the intracellular, extracellular, and transmembrane components of membrane proteins, resulting in significant informational loss. In this presentation we describe the development of a new method for the quantitative extraction of intact integral membrane proteins (including GPCRs) from solid metastatic ovarian tumors using pressure cycling technology in combination with a new buffer system. The TD buffer system is fully compatible with immunoaffinity methods (e.g., ELISA and immunoaffinity chromatography), as well as conventional proteomic techniques (e.g, 2-D gels, Western blots, and mass spectrometry). We demonstrate near quantitative recovery of the membrane proteins EDG2, EDG4, FAS, KDR, and LAMP-3 by Western blots. Both cytosolic and integral membrane proteins are recovered together in this buffer which is demonstrated in 2-D gels, allowing a more complete picture of the cellular proteome. We also demonstrate how to adapt existing commercial ELISAs for serum-soluble fragments (e.g., sVEGFR2) to measure the tissue titers of the intact membrane proteins from which they originate. Using immunoaffinity enrichment / mass spectrometry we also demonstrate mass spectrometric characterization of
proteins recovered from solid tumors with this new method for biomarker validation and isoform characterization.

**Application of Pressure for Improved Proteomic Analysis of FFPE Tissue and for Improved Sample Quality of Formalin-based Tissue Histology**

Carol B Fowler1,3, Timothy J Waybright2, Timothy D Veenstra2, Timothy J O’Leary3, and Jeffrey T Mason3

1Department of Biophysics, Armed Forces Institute of Pathology, Rockville, Maryland, 2Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, Maryland and 3Veterans Health Administration, Washington, DC
carol.fowler1@us.army.mil

High-throughput genomic and proteomic methods hold great promise for developing knowledge of the molecular characteristics of cancer, which can be translated into practical interventions for the diagnosis, treatment, and prevention of this disease. When fresh or frozen tissue is used for proteomic analyses, the results cannot be related directly to the clinical course of diseases. If routinely fixed and embedded archival tissues could be used for standard proteomic methods such as 2-D gel electrophoresis and mass spectrometry (MS) these powerful proteomic techniques could be used to both qualitatively and quantitatively analyze large numbers of tissues for which the clinical course has been established. However, the analysis of archival Formalin-Fixed Paraffin-Embedded (FFPE) tissues by high-throughput proteomic methods has been hampered by the adverse effects of formalin fixation, specifically formaldehyde-induced protein adducts and cross-links that are formed during tissue fixation and subsequent histological processing.

We have recently demonstrated the use of high hydrostatic pressure as a method for efficient protein recovery from FFPE tissues. High-pressure experiments were conducted at 65–100°C under a pressure of 45,000 psi. Complete reversal of formaldehyde-induced protein adducts and cross-links was observed by SDS-PAGE when lysozyme tissue surrogates, model tissue plugs formed by treating one or more cytoplasmic proteins with formaldehyde, were extracted at 45,000 psi and 80–100°C in Tris-HCl pH 4 containing 2% sodium dodecyl sulfate1. More recently, we have found that the application of pressure, when combined with high temperature, improves protein extraction efficiency and protein quality in more complex model surrogates and FFPE tissue. When multi-protein tissue surrogates were extracted under elevated pressure, total protein extraction efficiency was 4-fold greater than for non-pressure extracted tissue surrogates. Mass spectrometry (MS) of the FFPE surrogates retrieved under elevated pressure also showed that both the low and high-abundance component proteins were identified with sequence coverage comparable to the native, unfixed surrogate mixture. Non-pressure-extracted surrogate samples yielded few positive peptide identifications and a high number of false peptide identifications by MS (42-100%). When FFPE mouse liver was extracted at elevated pressure at pH 4, 7, or 9, there was an almost 2-fold increase in the number of unique protein identifications by MS compared to FFPE tissue extracted at the same temperature and length of time, but without pressure. More importantly, the number of unique peptides and proteins identified from FFPE mouse liver were virtually identical to
matched fresh tissue. Finally, we have extended the application of elevated pressure to improve the quality of formalin-based tissue histology. Our preliminary results show that the application of pressure increases the rate of formaldehyde penetration into tissue by more than 7-fold while preserving tissue morphology.

More recently, we have found that the application of pressure, when combined with high temperature, improves protein extraction efficiency and protein quality in more complex model surrogates and FFPE tissue. When multi-protein tissue surrogates were extracted under elevated pressure, total protein extraction efficiency was 4-fold greater than for non-pressure extracted tissue surrogates. Mass spectrometry (MS) of the FFPE surrogates retrieved under elevated pressure also showed that both the low and high-abundance component proteins were identified with sequence coverage comparable to the native, unfixed surrogate mixture. Non-pressure-extracted surrogate samples yielded few positive peptide identifications and a high number of false peptide identifications by MS (42-100%). When FFPE mouse liver was extracted at elevated pressure at pH 4, 7, or 9, there was an almost 2-fold increase in the number of unique protein identifications by MS compared to FFPE tissue extracted at the same temperature and length of time, but without pressure. More importantly, the number of unique peptides and proteins identified from FPPE mouse liver were virtually identical to matched fresh tissue. Finally, we have extended the application of elevated pressure to improve the quality of formalin-based tissue histology. Our preliminary results show that the application of pressure increases the rate of formaldehyde penetration into tissue by more than 7-fold while preserving tissue morphology.


A Need for Improved Sample Inactivation and Extraction Methods to Support Systems Biology Analysis of Biological Threat Organisms

1Bradford S. Powell and 2Loreen L. Lofts
1 Bacteriology Division, 2 Virology Division
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702
BRADFORD.S.POWELL@US.ARMY.MIL

Biological threat agents (BTA), when considered as samples for detailed molecular analysis, present the unique risk of hazardous infectivity during collection and processing. Although traditional chemical and physical methods of biological inactivation are useful for the purpose of decontamination, these approaches are not suitable for the pre-treatment of sample material that is intended for inspection by detailed analytical methods such as are employed in systems biology. Most notably, inactivation by oxidizers, cross-linking agents, pressurized steam, or irradiation can substantially modify or destroy covalent bond structure and irretrievably change biological molecules, thereby diminishing the utility of their study by sophisticated instrumentation and methodology. Importantly, new approaches should support a growing need to safely transfer an analytical sample outside of the biological containment environment or to ship material from remote locations, preferably without cold chain custody. Beyond agent inactivation, a more universal method for molecular extraction would better support multiplex analyses and leverage the use of limited sample. Popular methods tailored for nucleic acid extraction typically denature or chemically modify proteins, affecting
proteomics or other analysis requiring native structure. Current methods are also specific for the class of BTA and can differ substantially between sample comprising virus, vegetative bacteria or spore. New approaches for BTA inactivation and extraction are needed to address these opposing requirements of dependable inactivation and preservation of analyte molecular integrity. Discussion will include results from two new technology platforms being explored to pre-treat BTA samples for safe handling and unimpaired protein analysis.

Convenient and Reliable Extraction of *Borrelia burgdorferi* from Ticks Using Pressure Cycling Technology (PCT) and the PCT Shredder

Richard C. Tilton, Ph.D.
Medical Diagnostics Laboratories
RCTILTON@comcast.net

A rapid and reliable DNA extraction approach was developed for the downstream detection and identification of the Lyme disease pathogen *Borrelia burgdorferi* and other bacterial pathogens in ticks and other arthropods. The approach uses a novel sample preparation platform combining mechanical homogenization (*The PCT Shredder*) and pressure cycling technology (PCT). Samples were placed in specialized disposable sample containers (PULSE Tubes) compatible with both stages of sample preparation, permitting processing without the requirement for manual sample transfer. Each tick or pooled sample was homogenized in a PULSE Tube using *The PCT Shredder* in Tris buffer (50 mM Tris-HCl, 25 mM EDTA, 500 mM NaCl, 1% NP 40, pH 8.0) to rupture the tough exoskeleton of the arthropod, followed by proteinase K treatment and lysis of bacterial cells, both under cycling pressure at 56°C and treatment with CTAB at 65°C. DNA was extracted by a standard phenol-chloroform method and precipitated with isopropanol. Real time PCR was performed to detect *Borrelia burgdorferi* specific sequences versus total bacterial load. Three out of five *Ixodes scapularis* (deer ticks) and 2 out of 4 dog ticks were identified as positive for presence of the *B. burgdorferi* 23S rRNA gene, while bacterial-specific 16S rDNA gene amplification gave strongly positive results for all the tick DNA preps isolated by *The PCT Shredder*. The sensitivity of the PCR test for *B. burgdorferi* was assessed by spiking serial dilutions of purified *B. burgdorferi* DNA into tick DNA preps that were confirmed negative by prior PCR tests. The detection limit of the real-time PCR test for *B. burgdorferi* was shown to correspond to approximately one genome copy. Reported results indicate that a combination of mechanical homogenization by *The PCT Shredder* followed by extraction by PCT in a Barocycler allows for the efficient and reproducible detection of *B. burgdorferi* DNA in a complex biological matrix of tick biomass. The suggested workflow eliminates the current procedure of slicing the arthropod with a razorblade or grinding it with mortar and pestle, reduces the risk for sample cross-contamination and potential nosocomial infection, and emphasizes reproducibility and operator safety. Single-use sample containers can be used not only for extraction, but for field sample collection and transportation to the central laboratory, including shipment by mail.
The Real Jurassic Park: The Isolation of Proteins from Microorganisms Preserved in Amber Inclusions for 40 Million Years

Gary B. Smejkal1, 2
1 Harvard Catalyst, Laboratory for Innovative Translational Technologies, Boston, MA.
2 Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milan, Italy.
smejkal.gary@gmail.com

Recent reports of peptides isolated from the mineralized skeletal elements and alleged “soft tissues” from Tyrannosaurus rex have spurred controversy over whether proteins can be preserved over millions of years. However, soft tissues are not replaced by minerals in amber, which otherwise occurs during lithification. Organisms engulfed in terpenous resins within minutes or seconds were rapidly dehydrated, a prerequisite to preservation, thus retarding any chemical reaction requiring water.

Dominican Republic amber from the Oligo-Miocene epoch, 30-40 million years ago, was interrogated for residual proteins. Proteins were extracted from amber triturates by repetitive cycling between 0 and 35,000 psi using a pressure cycling technology (PCT). Exclusion of the protein isolates from polyacrylamide gel indicated molecular masses in the multi-million Dalton range, and failure of these aggregates to penetrate these gels proved to be an effective means for further concentrating trace proteins from paleontological samples. Tandem mass spectrometry of trypsin digests led to the identification of 86 peptides from 20 Saccharomyces proteins.

Pressure Cycling Technology (PCT) Augments Sensitivity of Detection and Robustness in Forensic DNA Analyses

Bruce Budowle, Department of Forensic and Investigative Genetics, Institute of Investigative Genetics, University of North Texas Health Science Center, Ft Worth, Texas 76107
Bruce.Budowle@unthsc.edu

Forensic DNA typing is a robust and valid methodology for the characterization of forensic biological. The exquisite sensitivity of the DNA typing assays permits very minute quantities of DNA to be typed. There is a trend today, however, to increase the sensitivity of the assays further to type exceedingly challenging samples, i.e., those that are highly degraded and very limited in quantity (less than 200 pg total DNA). These assays have been called low copy number (LCN) typing and many focus on “touch samples.” However, when processing a small number of input DNA template molecules during the PCR as is done with LCN typing, exaggerated stochastic sampling effects will occur. Therefore the LCN methodology does not yield reproducible/reliable results. There are different approaches to addressing the vagaries of LCN typing. Most have attempted to address the post-analysis interpretation phase. However, the old adage “garbage in/garbage out” tells us that no amount of statistical manipulations will improve the assay. It is desirable to focus on ways to obtain more DNA in the pre-analytical and analytical phases of the analysis. The yield of DNA during extraction of forensic materials is low and inefficient. PCT offers a sound approach to improving the LCN typing by improving recovery of DNA from challenging forensic samples and from the matrices of routine collection devices. Preliminary work on bone, hair and swabs demonstrate increased DNA yield via pressure. With
more yield there is a downstream effect of providing more template molecules for the PCR. The more DNA that is placed in the PCR, the more robust is the DNA results (within saturation limits) and more forensic cases may be typed. This presentation will show results of forensic research studies to date and suggest approaches to enhance yield further.

**Rapid Sample Preparation Method for Analysis of N-Linked Glycans**

*Zoltan Szabo, Tomas Rejtar, Andras Guttman, and Barry L. Karger*

Barnett Institute, Northeastern University, Boston, Massachusetts 02115

T.Rejtar@neu.edu

Determination of protein glycosylation is one of the most important tasks in protein characterization in the biotech industry. Typical sample preparation protocol for analysis of N-linked glycans by CE or LC requires long deglycosylation times (several hours to overnight) and an additional cleanup step after labeling with high excess of labeling reagent. In this presentation, we report on significant improvements in sample preparation of N-linked glycans. We applied Pressure Cycling Technology (PCT) to increase the speed of N-linked glycan release by PNGase F. Pressure cycling from atmospheric pressure to as high as 30 kPsi lead to rapid (minutes) and complete (95%) release of N-linked glycans for model glycoproteins including bovine ribonuclease B, human transferrin and polyclonal human immunoglobulin. In addition, we developed a new protocol for fast labeling of the released carbohydrates with 8-aminopyrene-1,3,6-trisulfonic-acid, (APTS) label suitable for CE separation. Importantly, by replacing the conventionally used acetic acid catalyst with citric acid, as low as 1 to 10 glycan to fluorophore molar ratio (vs. the typical >100 fold excess) maintained greater than 95% derivatization. This reduced level of APTS simplified the removal of excess reagent prior to separation, which is particularly important in CE-LIF (injection bias) and CE-MS (ion suppression). Importantly, the developed protocol does not lead to desialylation, readily supports large scale analysis and high sensitivity glycan analysis of biopharmaceuticals.

**High-Pressure Assisted In-Gel Tryptic Digestion for Qualitative and Quantitative Characterization of Protein Mixtures**

*Melkamu Getie-Kebtie and Michael Alterman*

FDA-CBER-NIH, 8800 Rockville Pike, HFM – 735 Bldg 29A rm 2B20, Bethesda, MD 20892

Melkamu.Getiekebtie@fda.hhs.gov

A robust and efficient in-gel digest of electrophoretically separated proteins is central to the development of successful gel-based quantitative approach. The conventional overnight in-gel digestion approach, which is currently used as a standard technique in many proteomics labs is not only slow, but also, yields reportedly inconsistent results. This talk presents an application of pressure cycling technology (PCT) to tryptic in-gel digestion of proteins and evaluation of application of this method to label-free quantification of proteins separated by 1DE. Our study on SDS PAGE of three purified proteins demonstrated that the PCT approach performed at least as well, and sometimes better, than the conventional approach in parameters, such as number of peaks detected; number of peptides identified, and sequence coverage. For example, PCT provided sequence coverage ranging from 49% (±4) to 66% (±1) as compared to 38% (±4) to 59% (±4) obtained with conventional digestion. In addition the PCT approach reduced the digestion time required for conventional digestion, i.e., ~16 h to ~45 min. The quantification
aspect of our study revealed a good spot-to-spot and lane-to-lane coefficient of variations of less than 10% and 25%, respectively, from a simple mixture of six proteins. We also demonstrated that the method can be used in determining the relative quantities of co-migrating proteins using a more complex influenza virus preparation. The decrease in-gel digestion time without loss of downstream protein identification and quantification information for the generation of same-day proteomic data is highly desirable.

High Pressure in Life Sciences – Trends and Future Opportunities
Alexander Lazarev, Ph.D., Pressure BioSciences, Inc., 14 Norfolk Ave., S. Easton, Massachusetts 02375
alazarev@pressurebiosciences.com

High pressure is a fundamental thermodynamic parameter which, together with temperature, determines the state of matter, molecular conformation and the chemical reactivity. For centuries, temperature control equipment has been considered essential for every laboratory in the World. Pressure control, however, has been notoriously ignored, mainly due to the lack of engineering solutions to generate and maintain high levels of pressure on a laboratory bench, leaving the exploration of high pressure to very few enthusiasts.

Commercialization of the Barocycler platform brings availability of pressure-control instrumentation to an average laboratory, promising to open up many opportunities for understanding of high-pressure thermodynamics in synthetic chemistry, catalysis, structural biology, and biomarker discovery and drug development. This presentation will cover the future potential research opportunities in an emerging field at the interface of high pressure thermodynamics and biology. High pressure provides fine control of protein conformation and interactions of proteins with biological membrane, enabling real-time studies of excited states of proteins by spectroscopic techniques such as Electron Paramagnetic Resonance. Several other examples of applications where high pressure could make remarkable difference in Life Science research will be discussed.