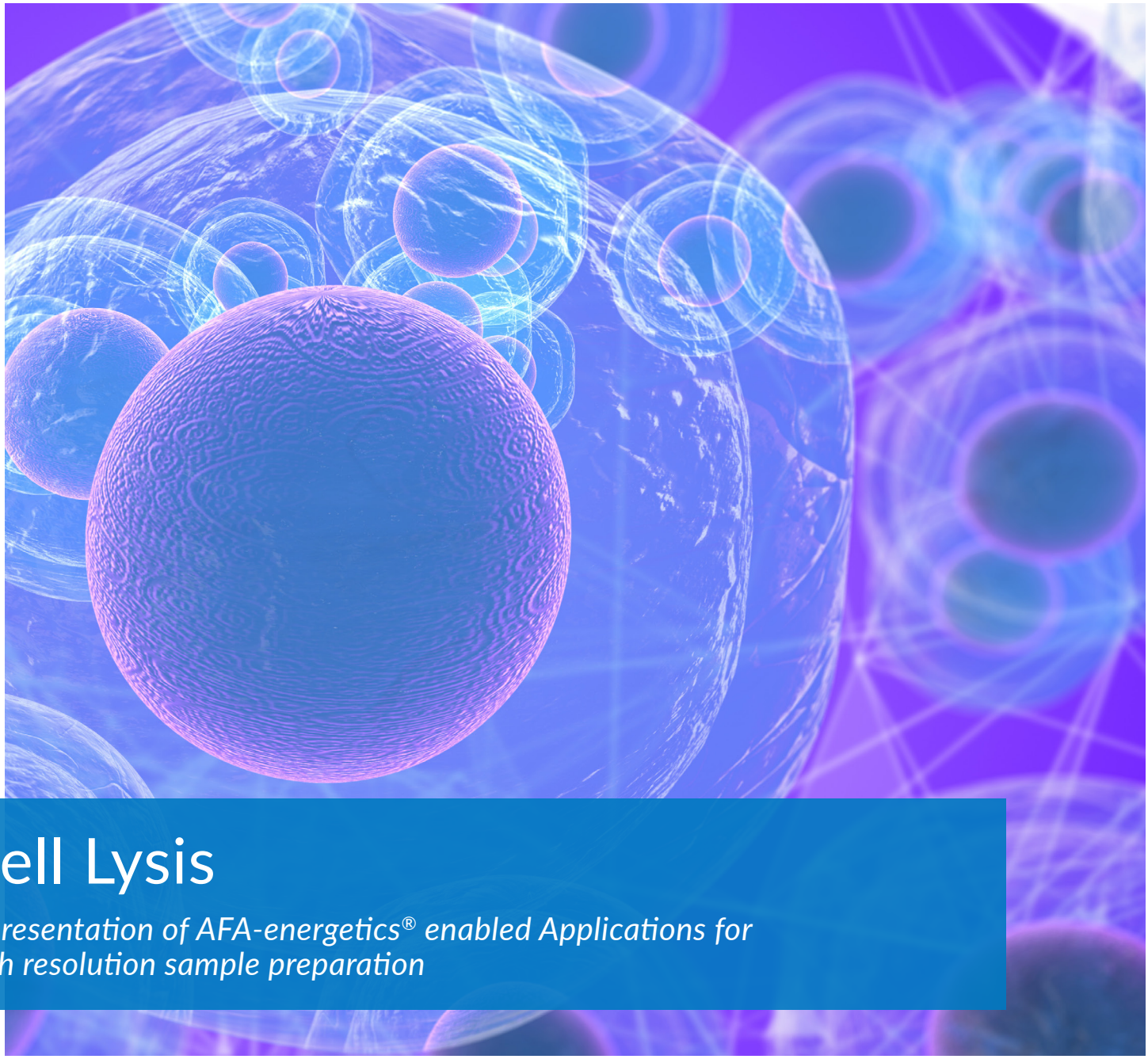

Cell Lysis

A presentation of AFA-energetics® enabled Applications for high resolution sample preparation

Covaris®

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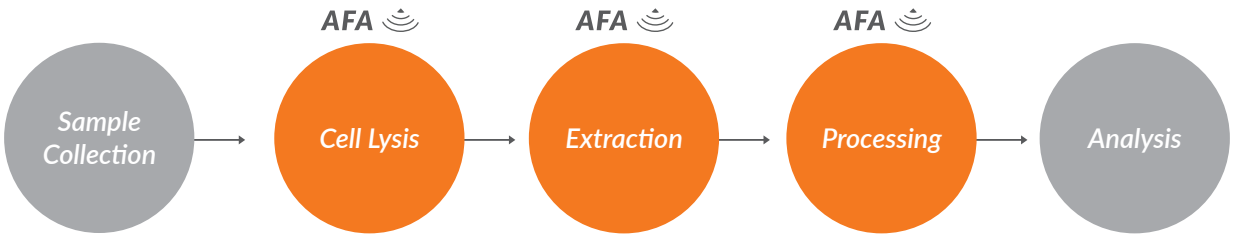
Introduction

The choice of sample-preparation method is a critical first step in proteomic and metabolomic studies because it is an essential part of chromatographic and spectroscopic analyses. It affects both the observed molecule content and the downstream biological interpretation.

An ideal sample-preparation method should:

- Be as non-selective as possible
- Prevent loss and/or degradation during the preparation procedure
- Avoid contamination
- Enable high-throughput processing
- Ensure reproducibility
- Be compatible with the downstream analytical method

The importance of these aspects emerged in the last decade with more sophisticated techniques like FASP (Filter Assisted Sample Preparation), and the use of high grade reagents in order to reduce the possible interference with the intended analysis techniques, like Liquid Chromatography-Mass Spectrometry (LC-MS). In most cases, sample preparation remains inconsistent and time consuming. Covaris Adaptive Focused Acoustics® (AFA®) can substantially reduce sample variability and overall turn-around-time to simplify the workflow for MS.



AFA Benefits:

- Standardize sample preparation
- Preserve sample heterogeneity
- High-throughput, automation ready
- Reproducible
- Compatible with downstream analysis methods
- Versatile: most sample types
- Streamline workflow

In addition to sample preparation improvements, there is a desire to reduce the sample size and increase the throughput while maintaining or achieving higher reproducibility, even when working with difficult to process samples (plants, yeast, hard mammalian tissues, FFPE blocks).

A huge variety of workflows exist to obtain the highest yield and purity dependent on the species/organisms, the sample type and the targeted molecules. Biomolecule diversity presents another challenge, for example, proteins with post translational modification can be very fragile, or membrane proteins very insoluble. This can lead to great variability in recovery and quality, especially in core labs which require a standardized workflow suitable for a wide range of biological samples.

This document highlights the recent developments of Adaptive Focused Acoustics for isolating proteins and other biomarkers from difficult-to-treat samples for a variety of inputs including complex tissue to single cell analysis, for high total protein yields as well as native proteins preservation, focused ultrasounds ensure a reproducible, non-contact and isothermal treatment of each sample, leading to higher quality extraction and biomarker preservation.

References

- Proteomic Challenges: Sample Preparation Techniques for Microgram-Quantity Protein Analysis from Biological Samples. P Feist et al., Int. J. Mol. Sci. 2015, 16, 3537-3563; DOI: 10.3390/ijms16023537
- Challenges in biomarker discovery with MALDI-TOF MS. J Hajduk et al., Clinica Chimica Acta Volume 458, 1 July 2016, Pages 84-98, DOI: 10.1016/j.cca.2016.04.033
- Integral membrane proteins in proteomics. How to break open the black box? O. Vit et al., Journal of Proteomics 153 (2017) 8–20, DOI: 10.1016/j.jprot.2016.08.006
- Modern Proteomics –Sample Preparation,Analysis and Practical Applications Advances in Experimental Medicine and Biology - pp23-62 – 2017 DOI: 10.1007/978-3-319-41448-5_4
- Selecting Sample Preparation Workflows for Mass Spectrometry-Based Proteomic and Phosphoproteomic Analysis of Patient Samples with Acute Myeloid Leukemia. Hernandez-Vallares et al., Proteomes 2016, 4, 24, DOI: 10.3390/proteomes4030024

High-throughput Clinical Proteomics from Cells, Fresh Frozen Tissue, and FFPE Samples

High-throughput and streamlined workflows are essential in clinical proteomics for robust, reliable, and comprehensive proteome profiling. Researchers are looking for standardized protocols to process various samples including fresh-frozen tissue, FFPE tissue, or blood. The two papers below exemplify how Covaris AFA can set new standards in sample preparation for proteomics.

Clinical practice requires reduced human intervention, and the ability to process small inputs with sufficient throughput. However, protein extraction is still largely a manual process with many steps, including lysis and homogenization of the sample for proper protein solubilization and stabilization. AFA-energetics simplifies the workflow and harmonizes protocol while enabling full automation, including integration laboratory robotics, of the process.

Automated sample preparation with SP3 for low-input clinical proteomics
T Mueller, J Krijgsveld et al. Molecular Systems Biology 16:e9111 | 2020 – DOI: 10.15252/msb.20199111
This paper is the first published automated method for protein sample preparation using Covaris. The authors demonstrated 1) the ability to work with low volumes, 2) the possibility to work on both cells and tissues, and 3) the efficiency of a single pot workflow to extract and identify proteins reproducibly and consistently.

In clinical proteomics, FFPE blocks represent one of the largest sources of archived samples. Traditionally, due to inefficient or incomplete deparaffinization and decrosslinking, FFPE analysis has suffered from poor protein recovery, lack of reproducibility, and lack of speed. The unique combination of Covaris AFA and ProtiFi™ S-Traps™ allows for a rapid, streamlined approach using one tube and one column. This novel workflow affords the highest yields of proteins, number of identifications, and the most reproducible FFPE sample processing. In addition, it is well suited for high-throughput workflows.

Keywords : FFPE, oncology, cancer research, paraffin

References

- HYPERsol: High-Quality Data from Archival FFPE Tissue for Clinical Proteomics, DM Marchione et al., 2020. DOI: 10.1021/acs.jproteome.9b00686 J. Prot. Res 2020, 19, 2, 973-983
  - The results presented in this article indicate the superiority of combining AFA/SDS based buffer/S-Trap columns (described as HYPERSOL) over traditional methods to efficiently extract proteins from different FFPE samples, including old samples stored for more than 17 years.
- US HUPO 2019 poster, Total Solubilization of FFPE samples for High Throughput Clinical Proteomics , J. Wilson, J. Wojcik et al. https://abrf2019.gorgesapps.us/node/3876
  - This work is the foundation of the HYPER-sol paper.
- HUPO 2018 poster, Universal Sample Processing of Multiple Sample Types For Reproducible Proteomic Sample Preparation J. Wilson, V. Meyyappan et al., https://covaris.com/wp-content/uploads/HUPO-2018-ProtiFi-Covaris-Poster.pdf
  - This poster presents a universal protocol for protein extraction, on difficult samples like FFPE, brain or pancreas. Data shows how efficient this protocol is to isolate proteins that can be missed by other methods.

Preserving Protein Integrity: Extraction of Native Proteins

When considering extraction, it is important to define what population of proteins is of interest, as it is nearly impossible to find conditions that will accommodate all classes of proteins with comparable efficiency. Here we focus on scientific publications/ communications describing methods that maintain the native state of the proteins. This will allow the study of their post translational modifications (PTMs) like phosphorylation or ubiquitination, or more complex downstream applications such as activity assays, as examples.

Keywords : post translational modifications (PTM), native protein, phosphoprotein, ubiquitination, glycosylation

References

- Robust pre-analytical sample preparation process preserves the accuracy and fidelity of protein phosphorylation states. Smejkal et al., HUPO 2012 - poster
  - This poster shows the efficiency of AFA to deliver over dounce homogenization with regards to protein quality and quantity.
- Combined phospho- and glycoproteome enrichment in nephrocalcinosis tissues of phytate-fed rats. T Tran et al., Rapid Commun. Mass Spectrom. 2013, 27, 2767–2776 DOI: 10.1002/rcm.6742
  - This paper stresses the importance of preserving proteins integrity during sample preparation, in particular when studying PTMs like phosphorylation and glycosylation.
- Comprehensive and sensitive proteogenomics data analysis strategy based on complementary multi-stage database search. IH Madar et al., International Journal of Mass Spectrometry, Volume 427, April 2018, Pages 11-19. DOI: dx.doi.org/10.1016/j.ijms.2017.08.015
  - Sensitivity was looked after in this proteogenomics paper studying the proteome of human cancer tissues.
- A high-efficiency cellular extraction system for biological proteomics. Dhabaria et al., J of Proteome Res. 2015 August 7; 14(8): 3403–3408, DOI: 10.1021/acs.jproteome.5b00547
  - In this paper they are looking to maximize the extraction of cellular proteins while minimizing their denaturation. AFA combined with an optimized detergent system permitted efficient native proteome extraction.
- Use of focused ultrasonication in activity-based profiling of deubiquitinating enzymes in tissue. Nanduri et al., Anal Biochem . 2016 December 15; 515: 9–13, DOI: 10.1016/j.ab.2016.09.016
  - This paper shows comparison of various sample prep methods: AFA gives the best results for follow-up of ubiquitination.
- Mapping protein signal pathway interaction in sarcoma bone metastasis: linkage between rank, metalloproteinases turnover and growth factor signaling pathways. Conti et al., Clin Exp Metastasis. 2014 Jan;31(1):15-24, DOI: 10.1007/s10585-013-9605-6
  - AFA combined with cryoPREP allowed for efficient extraction and preservation of signaling proteins further analyzed by RPPA technique.
- Integrated analysis of global proteome, phosphoproteome, and glycoproteome enables complementary interpretation of disease-related protein networks. JM Park et al., 2015, Scientific Reports | 5:18189, DOI: 10.1038/srep18189
  - Reproducible and efficient native protein extraction was key in this large-scale proteome analysis of three gastric cancer patients, integrating phospho- and glycoproteins, where both cryoPREP and AFA were used.
- Optimized cross-linking mass spectrometry for *in situ* interaction proteomics. Z Ser et al., 2018, BioRxiv, DOI : 10.1101/393892
  - AFA was used to favour extraction of native complexes while studying protein-protein interactions using cross-linking mass spectrometry (XL-MS).
- Mapping Protein Signal Pathway Interaction in Sarcoma Bone Metastasis: Linkage Between Rank, Metalloproteinases Turnover and Growth Factor Signaling Pathways Conti et al., Clin Exp Metastasis. 2014 Jan;31(1):15-24
  DOI: 10.1007/s10585-013-9605-6
  - AFA combined with the cryoPREP allowed for efficient extraction and preservation of signaling proteins further analyzed by RPPA technique

- 6-Phosphogluconate Dehydrogenase Links Cytosolic Carbohydrate Metabolism to Protein Secretion via Modulation of Glutathione Levels H Li et al., 2019 - Cell Chemical Biology 26, 1306–1314 – DOI: 10.1016/j.chembiol.2019.05.006
    - Reproducible cell lysis was performed on cell pellets using AFA for LC-MS analysis.
- High sensitivity quantitative proteomics using automated multidimensional nanoflow chromatography and accumulated ion monitoring on quadrupole-Orbitrap linear ion trap mass spectrometer. P Cifani et al., Mol Cell Proteomics. 2017 Nov;16(11):2006-2016 DOI: 10.1074/mcp.RA117.000023
    - Authors sought to increase sensitivity of detection, including modified proteins. Improved sample preparation was one of the pre-requisites.
- Probing the global kinome and phosphoproteome in Chlamydomonas reinhardtii via sequential enrichment and quantitative proteomics. E Werth et al. The Plant Journal (2017) 89, 416–426 DOI: 10.1111/tpj.13384
    - The authors were looking for a method being effective for disrupting Chlamydomonas cells and improve native protein extraction. They had the objective of maximizing yield to accommodate the requirement for high amounts of protein in the kinome and phosphoproteome enrichment steps used downstream.
- The phosphorylated redox proteome of *Chlamydomonas reinhardtii*: Revealing novel means for regulation of protein structure and function. McConnell et al., Redox Biology Volume 17, July 2018, Pages 35-46 DOI: doi.org/10.1016/j.redox.2018.04.003
    - The Hicks lab (see Werth et al.) describes demonstration of protein-level enrichment with AFA of reversibly oxidized proteoforms in Chlamydomonas reinhardtii with subsequent phosphopeptide analysis to determine the extent of phosphorylation in the redox thiol proteome.
- Investigating the effect of target of rapamycin kinase inhibition on the Chlamydomonas reinhardtii phosphoproteome: from known homologs to new targets. E werth et al., New Phytologist (2018) 221: 247–260, DOI: 10.1111/nph.15339
    - Using AFA for extracting phosphoproteins, Wert et al. achieved extensive coverage of the TOR-modulated phosphoproteome in Chlamydomonas using a quantitative label-free approach.
- Mass Spectrometry–Based Proteomics Reveals Potential Roles of NEK9 and MAP2K4 in Resistance to PI3K Inhibition in Triple-Negative Breast Cancers. Mundt et al., Cancer Res. 2018 May 15;78(10):2732-2746 DOI: 10.1158/0008-5472
    - Another paper on the use of AFA for PDXs (see papers from Wang and Ntai), centered on phosphoproteogenomics to understand resistance mechanisms in breast cancer.

Low Input Extraction

Recently, more and more studies have been conducted on low number of cells, <10,000. The ability to reach the individual cell level can yield essential details to distinguish between cell types and decipher their signaling activities. It is also a requirement to be able to work with high-throughputs. Those low input samples must be processed in small volumes, 10 to 200 µL or less, to maintain a sufficient concentration, while minimizing the loss between each step of the workflow. Another constraint is ensuring that every tube will be treated identically, and if possible, simultaneously or within a short timeframe. To ensure these parameters are met, researchers have developed high-throughput protocols using 96 well plates. Furthermore, in certain protocols, the combination of steps in so called "one pot" reactions reduced the complexity of the workflows and allows for better standardization.

Keywords : low cell extraction, low input cell lysis, single cell

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  - With controlled extraction parameters, the authors achieved zeptomole detection sensitivity, resulting in identification of 4000 proteins from the equivalent of 100 to 200 cells.
- Mass-spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. B. Budnik et al., *Genome Biology*, 2018, 19:161, DOI: 10.1186/s13059-018-1547-5
  - AFA was used to ensure minimal loss of proteins and obviate chemicals that may undermine peptide separation and ionization, or sample clean up that may incur significant losses.
- Integrated microscale analysis system for targeted liquid chromatography mass spectrometry proteomics on limited amounts of enriched cell populations. JG Martin et al., Anal Chem. 2013 Nov 19;85(22):10680-5 DOI: dx.doi.org/10.1021/ac401937c
  - This paper is showing AFA use in a context of low cell/low input extraction (< 5,000 cells).
- Lymphatic exosomes promote dendritic cell migration along guidance cues. M. Brown et al., J Cell Biol. 2018 Jun 4;217(6):2205-2221, DOI: 10.1083/jcb.201612051
  - Gentle extraction with protein conservation led to the identification of > 1,700 proteins in exosome-rich endothelial vesicles (EEVs), to understand what drives the release of EEVs by lymphatic endothelial cells.
- High Sensitivity Microproteomic Analysis of Rare Samples by Porous Layer Open Tubular (PLOT) Columns Coupled with Mass Spectrometry. S Li et al., poster – ASMS 2013
  - Another example showing the upsides of using AFA when working with low number of cells, compared to other traditional extraction techniques.

Hard-to-lyse Samples

Sample preparation is always about optimization; there is a significant number of parameters that can affect the efficiency of biomarker recovery. Some organisms have very rigid membrane constituents while others can have a cell wall on top of their membrane, and the insolubility of some components can drastically decrease the quantity of desired biomolecules. AFA has shown to be efficient in processing a wide variety of starting materials including plants, bacteria, yeast, or hard mammalian tissue like muscle.

References

Cell Lysis in Eukaryotes

- Dihydrolipoyl dehydrogenase as a potential UVB target in skin epidermis; using an integrated approach of label-free quantitative proteomics and targeted metabolite analysis. Moon et al., Journal of Proteomics, Volume 117, 18 March 2015, Pages 70-85 DOI: dx.doi.org/10.1016/j.jprot.2014.12.016
  - AFA was used to disrupt difficult-to-lyse skin samples while ensuring good recovery of proteins and metabolites.
- High-Throughput Simultaneous Analysis of RNA, Protein, and Lipid Biomarkers in Heterogeneous Tissue Samples. Reiser et al., Clinical Chemistry 57:11 1545-1555, 2011, DOI: 10.1373/clinchem.2010.157743
  - The authors efficiently extracted several types of biomarkers from difficult tissue (atherosclerotic plaque and tumor tissue) using cryoPREP for tissue pulverization and AFA method for successful protein extraction.
- A rapid, standardized protein extraction method using adaptive focused acoustics for identification of mycobacteria by MALDI-ToF MS. LT Adams et al., Diagnostic Microbiology and Infectious Disease 86 (2016) 284–288 DOI: 10.1016/j.diagmicrobio.2016.06.001
  - This paper evaluates AFA to rapidly extract mycobacterial peptides and also for its ability to inactivate quickly all species of mycobacteria.
- Plasma membrane proteome in Arabidopsis and rice. S. Komatsu, Proteomics 2008, 8, 4137–4145 DOI: 10.1002/pmic.200800088
  - A review highlighting the advantages of acoustic techniques to homogenize protein pellets from various plant tissues.
- A Microscale Yeast Cell Disruption Technique for Integrated Process Development Strategies. MD Wenger et al., Biotechnol. Prog. 2008, 24, 606–614, DOI: 10.1021/bp070359s
  - In this yeast study, AFA non-contact approach was key to lyse efficiently high quantities of cells despite a very rigid cell wall.
- Peptidomics analysis of transient regeneration in the neonatal mouse heart. Y Fan et al., J Cell Biochem. 2017 Sep;118(9):2828-2840, DOI: 10.1002/jcb.25933
  - Use of AFA for peptidomics (the bridge between proteome and metabolome) on mouse heart tissue.
- Development of a high-throughput microscale cell disruption platform for *Pichia pastoris* in rapid bioprocess design. Blaha et al., Biotechnol Prog. 2018 Jan;34(1):130-140 DOI: 10.1002/btpr.255
  - Objective was to develop an automated, miniaturized, high-throughput, non-contact, scalable platform based on Adaptive Focused Acoustics (AFA) to disrupt *P. pastoris* and recover intracellular heterologous protein. Conclusion shows that AFA can be used very efficiently in a wide range of applications.
- Acoustic Technology for High-Performance Disruption and Extraction of Plant Proteins. M Toorchi et al., Journal of Proteome Research 2008, 7, 3035–3041, DOI: 10.1021/pr800012c
  - Authors describe how AFA performs far better on plant samples than water bath sonication by producing high-quality 2D gels and minimizing the processing time required for high-throughput proteomics research.
- Soybean Proteomics for Unraveling Abiotic Stress Response Mechanism. Z Hossain et al., J. Proteome Res. 2013, 12, 11, 4670-4684, DOI: 10.1021/pr400604b
  - Analyzing different preparation methods, the authors describe Covaris processing as resulting, “In a clearer protein pattern than the other conventional methods”.

Cell Lysis of Patient Derived Xenografts (PDXs)

AFA is very efficient for xenografts. Along with the paper from Mundt et al. To study phosphoproteins, other teams have used it for this purpose.

- Breast tumors educate the proteome of stromal tissue in an individualized but coordinated manner. X Wang et al., Sci Signal. 2017 Aug 8;10(491), DOI: 10.1126/scisignal.aam8065
  - Studying heterogeneity between tumors requires a high degree of sensitivity and good quality protein extraction, as shown here on breast xenografts.
- Integrated Bottom-Up and Top-Down Proteomics of Patient-Derived Breast Tumor Xenografts. I Ntai, Molecular & Cellular Proteomics 15: 10.1074, DOI: 10.1074/mcp.M114.047480
  - Authors describe the first large-scale integration of genomic, bottom-up and top-down proteomic, measuring differential expression of proteins and proteoforms.

Cell Lysis in Prokaryotes

- The Role of Cadaverine Synthesis on Pneumococcal Capsule and Protein Expression. MF Nakamya et al., Med Sci (Basel). 2018 Jan 19;6(1), DOI: 10.3390/medsci6010008
  - Use of AFA to disrupt *S. pneumoniae* capsule.
- Use of Focused Acoustics for Cell Disruption to Provide Ultra Scale-Down Insights of Microbial Homogenization and its Bioprocess Impact— Recovery of Antibody Fragments from rec *E. coli.* Q Li et al., Biotechnology and Bioengineering, Vol. 109, No. 8, August, 2012 DOI: 10.1002/bit.24484
  - This study demonstrates superior efficiency of AFA over classical sonication.
- An ultra scaled-down approach to study the interaction of fermentation, homogenization and centrifugation for antibody fragment recovery from rec *E. coli.* Q Li et al., Biotechnology and Bioengineering, 2013 Aug;110(8):2150-60 DOI: 10.1002/bit.24891
  - In this study, authors apply AFA (defined as their method of choice in the upper paper) to *E. coli* for homogenization and disruption purpose in the context of ultra scaled down optimization.
- Assessment of the Manufacturability of Escherichia coli High Cell Density Fermentations. MA Perez-Pardo et al., Biotechnol. Prog., 27: 1488–1496, 2011, DOI: 10.1002/btpr.644
  - AFA helped in assessing the best physiological and biological conditions for fermentation, starting from ultra scaled down quantities.

Versatility of AFA

Covaris AFA has demonstrated its efficiency to disrupt cells of great diversity and for many different objectives in the recovery of intracellular biomolecules including metabolites, antibody fragments, proteins and protein subunits, membrane proteins, and lipids. All of these have been isolated with high efficiency and excellent preservation with AFA. This proved to be of particular interest for proteogenomics studies. AFA also provides valuable advantages as compared to other applications as it can enhance the speed and quality of tryptic digestion and for hydrogels solubilization.

Keywords: high throughput, label free, trypsin digestion, stem cells, western blotting, proteogenomics, cross linked MS (XL-MS)

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- ProteomeGenerator: A Framework for Comprehensive Proteomics Based on de Novo Transcriptome Assembly and High-Accuracy Peptide Mass Spectral Matching Zifani et al., J. Proteome Res. 2018, 17, 11, 3681-3692 DOI : 10.1021/acs.jproteome.8b00295
  - Covaris AFA-assisted extraction is used for genome-scale and quantitative measurements of biological proteomes (proteogenomics) as allowed by modern mass spectrometry.
- PGBD5 promotes site-specific oncogenic mutations in human tumors AG Henssen et al., Nature GeNetics VOLUME 49 | NUMBER 7 | JULY 2017, DOI: 10.1038/ng.3866
  - Studying genomic rearrangements of PGBD5 which they were able to define as an oncogenic mutator, Kentsis and collaborators used AFA for efficient and reproducible cell lysis, protein extraction and chromatin shearing.
- Acoustic technology-assisted rapid proteolysis for high-throughput proteome analysis. Kim et al., ANALYTICAL SCIENCE& TECHNOLOGY Vol. 24, No. 6, 510-518, 2011, DOI: 10.5806/AST.2011.24.6.510
  - This paper shows how controlled acoustics wavelength allows for faster and more efficient digestion of proteins with trypsin.
- Enhanced Tryptic Digestion in under 20 minutes using AFA™ Technology. I Isaac et al., HUPO poster - https://www.covaris.com/wp/wp-content/uploads/2020/07/ASMS_2020_Poster.pdf
  - This poster details numerous tests comparing trypsin digestion protocols, highlighting how AFA can increase efficiency while speeding the process, down to 20 minutes.
- Development of an Automated, High-throughput Sample Preparation Protocol for Proteomics Analysis Arul et al., BULLETIN OF THE KOREAN CHEMICAL SOCIETY, Volume36, Issue7 July 2015 ; 1791-1798, DOI: 10.1002/bkcs.10338
  - The authors optimized the clean up steps downstream of protein extraction made using cryoprep and AFA acoustic ultrasonication
- Label-free quantitative proteomic analysis of human periodontal ligament stem cells by high-resolution mass spectrometry Han et al., J Periodont Res. 2018;1–10. DOI : 10.1111/jre.12604
  - AFA is used in this paper to gently process various stem cell populations
- Assessment of adaptive focused acoustics versus manual vortex/freeze-thaw for intracellular metabolite extraction from Streptomyces lividans producing recombinant proteins using GC-MS and multi-block principal component analysis Kassama et al., Analyst. 2010 May;135(5):934-42. DOI: 10.1039/b918163f
  - This study compares the efficiency of ultrasonic AFA and manual vortex/freeze-thaw extraction techniques for comparative metabolite profiling of mouse tumour necrosis factor alpha (mTNF-a) expression in S. lividans.
- Shotgun Lipidomics Combined with Laser Capture Microdissection: a Tool to Analyze Histological Zones in Cryosections of Tissues O Knittelfelder et al., Anal Chem. 2018 Jul 30 DOI: 10.1021/acs.analchem.8b02004
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preparation steps.

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- A Non-catalytic Function of SETD1A Regulates Cyclin K and the DNA Damage Response T Hoshii et al., 2018, Cell 172, 1007–1021, DOI: 10.1016/j.cell.2018.01.032
  - The authors used AFA for cell lysis prior to western blotting and chromatin shearing in ChIP experiments.
- Peptidomimetic blockade of MYB in acute myeloid leukemia Ramaswamy et al., NATURE COMMUNICATIONS | (2018) 9:110 DOI: 10.1038/s41467-017-02618-6
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- Direct Measurement of Intracellular Compound Concentration by RapidFire Mass Spectrometry Offers Insights into Cell Permeability LJ Gordon et al., J Biomol Screen. 2016 Feb;21(2):156-64. DOI: 10.1177/1087057115604141
  - AFA was used to lyse cells within a larger assay intended for improving drug development.
- Comparison of biochemical and biological effects of ML858 (salinosporamide A) and bortezomib Williamson et al., Mol Cancer Ther 2006;5(12):3052–61, DOI: Mol Cancer Ther 2006;5(12):3052–61
  - Authors study complex natural products that have antibiotic and antiproliferative activities, like salinosporamide A, which effect is linked to its ability to inhibit the proteasome. Biochemical and biological activities are assessed compared to a known molecule (bortezomib) using cell-based reporter stabilization assays. Tumor and brain tissues are used as models.

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