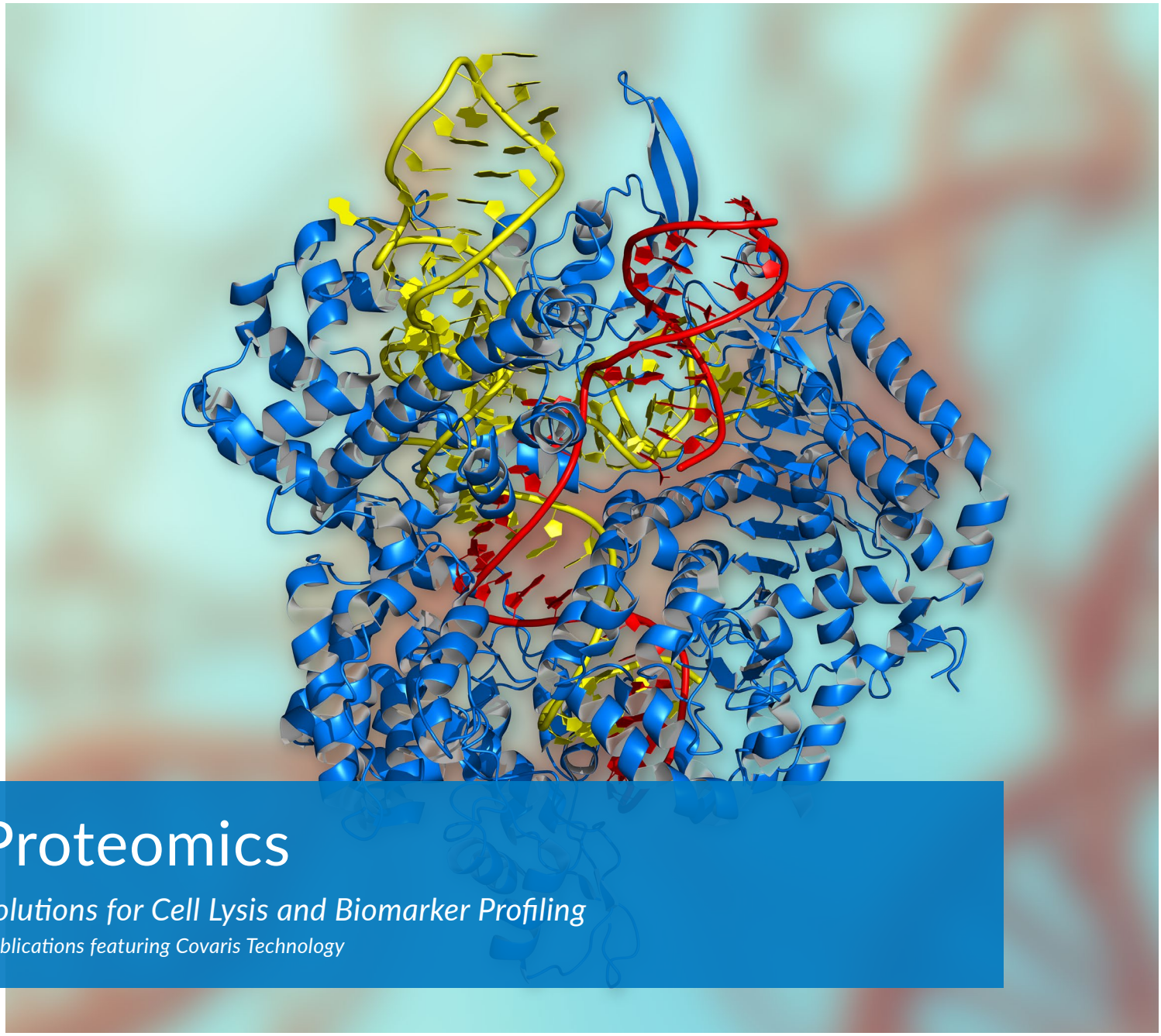

Proteomics

Solutions for Cell Lysis and Biomarker Profiling

Publications featuring Covaris Technology

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Introduction

The choice of sample-preparation method is critical 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

1. be as non-selective as possible;
2. prevent loss and/or degradation during the preparation procedure;
3. avoid contamination;
4. enable high-throughput;
5. ensure reproducibility;
6. 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 quality grade reagents, in order to reduce the possible interference with the intended analysis techniques like LC-MS. Mass spectrometry (MS) coupled with liquid chromatography (LC), or LC-MS, is the analytical technique of reference for most of the recent "omics" technologies such as proteomics, metabolomics, or lipidomics. In most cases, sample preparation remains inconsistent and time consuming: this workflow for MS comprises several steps which duration and variability can be substantially reduced with the help of Adaptive Focused Acoustics® (AFA®).

Beyond these improvements, there is a desire to reduce the sample size and increase the throughput while keeping the same or even achieve a higher level of reproducibility. And some samples (plants, yeast, hard mammalian tissues) remain very difficult to process. A huge variety of workflows exist to obtain highest yield and purity, depending on the different species or organisms, the sample type or the targeted molecules. However, each biomolecule is different: 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 requires a standardized workflow suitable for a wide range of biological samples. In this context, the AFA platform also helps standardizing the sample preparation process and streamlining the entire analytical workflow to ensure comparability between different samples.

This document highlights the recent developments of AFA for isolating proteins and other biomarkers from difficult-to-treat samples: for a variety of input samples reaching from 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 - pp. 23-62 – 2017; DOI: 10.1007/978-3-319-41448-5
- 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

Preserving Protein Integrity: Extraction of Native Proteins

When considering extraction, it is important to define what population of proteins is of interest. There's often a compromise to find, as it is impossible to find conditions that will accommodate all classes of proteins with same efficiency. Here we focus on scientific publications/ communications describing methods that will keep the proteins in their native state: this will allow for instance to study their post translational modifications (PTMs) like phosphorylation or ubiquitination, or more complexs downstream applications such as activity assays.

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 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.*
- 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-requisite.*
- 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: 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.*
- 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.CAN-17-1990
  - *A third paper on the use of AFA for PDXs (see...), centered on phosphoproteogenomic to understand resistance mechanisms in breast cancer.*

Low Input Extraction

In a recent past, more and more studies have been conducted on low number of cells (<10,000). This ability to go to 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 troughput. Those low input samples must be processed in small volumes, 10 to 200 µL or even less, to maintain a sufficient concentration, while minimizing the loss between each step of the workflow. Another constraint is to ensure that every tube will be treated identically, and if possible, simultaneously, or within a short timeframe. For this reason, 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

References

- An Integrated Platform for Isolation, Processing, and Mass Spectrometry-based Proteomic Profiling of Rare Cells in Whole Blood S. Li et al., Molecular & Cellular Proteomics 14: 1672–1683, 2015; DOI: 10.1074/mcp.M114.045724
  - *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., bioRxiv; DOI: https://doi.org/10.1101/102681
  - *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 amount of parameters that can affect the efficiency of biomarker recovery. In addition, some organisms have very rigid membrane constituants, other can have a cell wall on top of their membrane, and some components insolubility can decrease drastically the quantity of desired biomolecules. AFA has shown to be efficient in a wide variety of starting materials: plants, bacteria, yeast, or hard mammalian tissue like muscle have been successfully processed with focused ultrasounds, as highlighted in the articles listed.

Keywords: yeast, plant, bacteria, cell wall, membrane

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 proteines and metabolites.*
- 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
  - *Toorchi et al. 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.*

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 demonstrate superior efficiency of AFA over classical sonication.*
- An ultra scale-down approach to study the interaction of fermentation, homogenisation 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 scale 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 scale down quantities.*

Versatility of AFA

Focused acoustics has demonstrated its efficiency to disrupt cells of great diversity and for many different objectives in the recovery of intracellular biomolecules: metabolites, antibody fragments, proteins and protein subunits, membrane proteins, lipids, all of these have been isolated with high efficiency and excellent preservation with AFA. Focused ultrasounds also provides valuable advantages to other applications as it can enhance the speed and quality of tryptic digestion as well as for hydrogels solubilization. Furthermore, for epigenomics applications, please see our related list of publications.

Keywords: lipid, metabolite, lipidomics, metabolomics, trypsin digestion

For advice, protocols, or recommended settings, please contact us at applicationsupport@covaris.com.

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- 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
  - *Authors wanted to analyze lipids contents (lipidomes) after LCM on mouse liver tissues, and used focused ultrasonication in the first preparation steps.*
- Enhanced Tryptic Digestion in under 20 minutes using AFA™ Technology. I Isaac et al., HUPO poster
  - *This poster details numerous tests comparing trypsin digestion protocols, highlighting how AFA can increase efficiency while speeding the process, down to 20 minutes.*
- Western blot analysis of cells encapsulated in self-assembling peptide hydrogels KA Burgess et al., BioTechniques 63:253-260 (December 2017); DOI: 10.2144/000114617
  - *When it comes to solubilization, AFA is the method of choice as described in this paper about vells encapsulated in SAPHs.*
- Peptidomimetic blockade of MYB in acute myeloid leukemia Ramaswamy et al., NATURE COMMUNICATIONS | (2018) 9:110; DOI: 10.1038/s41467-017-02618-6
  - *Use of AFA for sample preparation prior to western blotting and ChIP-related experiments.*
- 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.*

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Part Number: M020103 | Rev A | Edition October 2018 |