Comprehensive Sample Preparation for Proteins Enabled by AFA® Technology

Debadeep Bhattacharyya, Ph.D. & Tom O'Hare, Ph.D. | Covaris, LLC, Woburn, Massachusetts 01801

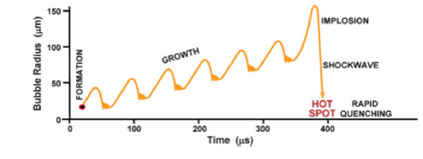
Sample preparation can significantly impact data quality derived from complex samples. This is especially important for proteomics research, owing to diversity observed in proteins, which cannot be amplified. Covaris' Adaptive Focused Acoustics® (AFA®) Technology can address a wide variety of sample types (FFPE, LCM, fresh tissue, cells, bacteria, yeast), while increasing speed, throughput, reproducibility, and reliability. AFA is compatible with other purification approaches like SP3 or S-Trap and extensively used for bottom-up proteomics to targeted quantitation of proteins in complex biological matrices.

Introduction

Covaris promotes robust, easy, and scalable sample preparation through:

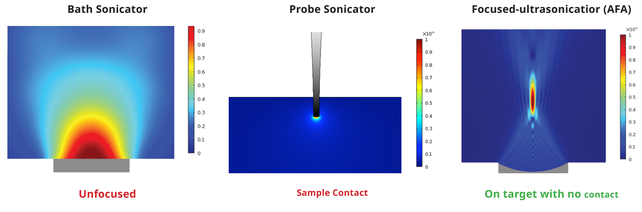
- Standardized tissue/cell disruption
  - Improved consistency and reproducibility
  - Hands-free, automated
  - Processing in different formats, from 1 tube up to 96-well plate, with same sample-to-sample quality
- Fast, non-contact, easy, cold, and reproducible extraction for all soft and hard tissue samples (includes non-mammalian samples like plants, yeast, and bacteria)
  - Use your buffer of choice
  - Scale up from single tube to 96-well format and beyond
- Active, non-toxic deparaffinization and improved rehydration of FFPE and LCM tissue
  - Enables "fresh frozen like" protein extraction from FFPE samples, without any organic solvent

Acoustic Energy is the Key

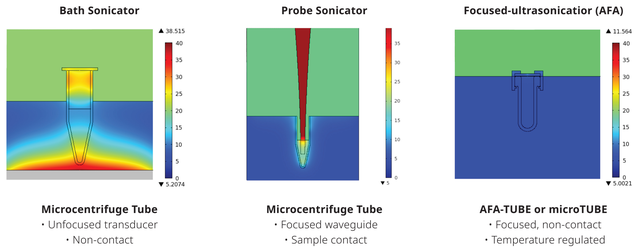


AFA energy is more closely scaled to the sample size eliminating the risk of damage from extreme heat or contamination; enabling higher quality, higher recovery, and rapid sample preparations.

Control Energy



Maintain Temperature

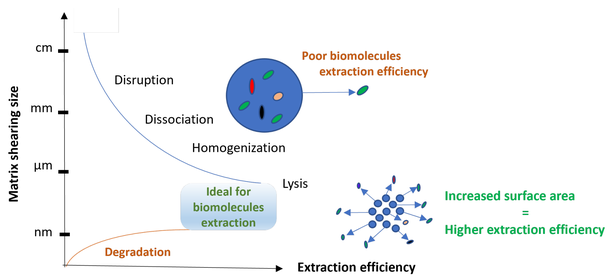


Microcentrifuge Tube
- Unfocused transducer
- Non-contact

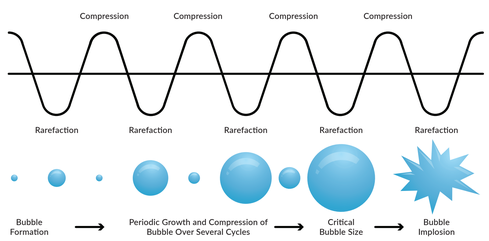
Microcentrifuge Tube
- Focused waveguide
- Sample contact

AFA-TUBE or microTUBE
- Focused, non-contact
- Temperature regulated

Shearing Size vs. Extraction Efficiency

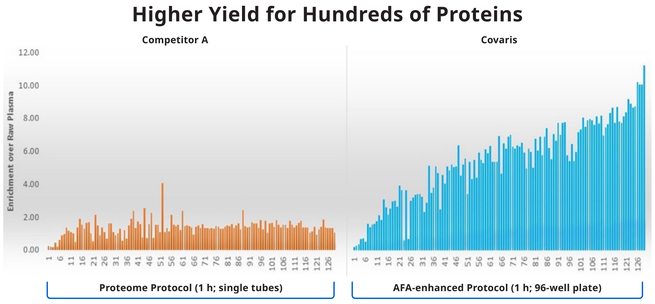


Compression and Refraction



- Acoustic waves pass through liquid solution
- Localized pressure fluctuations
- Dissolved gases forms microscopic bubbles
- Grow, oscillate and collapse

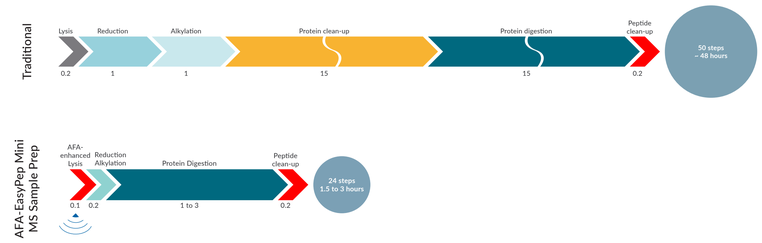
Higher Yield for Hundreds of Proteins



1. Raw plasma (25 µL) was subjected to high abundance protein (albumin, immunoglobulin) depletion process via the PureProteome™ matrix
2. Treating the plasma (2 µL) /matrix mix with AFA (blue bars) significantly enhances retention of > 130 biomarkers as compared to the passive mixing protocol (orange bars) and enriches to levels that are in many instances exceeding the expected (4 to 5-fold) enrichment based on measured albumin / immunoglobulin removal.

Faster and Efficient Tagging Protocols

A host of different protocols were developed across different platforms and matrix types with different throughput capabilities. For FFPE samples in particular, AFA was used on samples along with Covaris Tissue Lysis Buffer (TLB) for emulsification of paraffin without addition of any organic solvent. Post-emulsification of paraffin, SP3 was used for protein depletion, digestion, and clean-up. The entire workflow can be carried out in the same 8-tube strip or 96-well plate (depending on throughput requirements). Robust sample preparation for TMT comes with a set of challenges including efficient and reproducible lysis, hands-on time/number of steps, and sample viscosity. TMT labeling studies were found to be significantly faster (reduction from traditional workflows of 48 hours to 1.5 - 3 hours) and easier (50 steps to 24 steps).



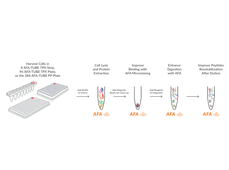
For every bottom-up proteomic and protein quantitation assay using AFA, significant increase in the number of proteins and peptides were observed. In addition, increased efficiency in binding and digestion were also observed for some of the assays. AFA ensures increased confidence in protein analysis data from challenging samples, regardless of the nature and type of proteins (phosphoproteins, membrane proteins, hydrophobic peptides, etc.). In addition, AFA can address a wide range of throughput capabilities

Single Plate Sample Prep for Every Matrix

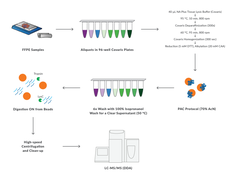
Same Plate from Lysis to Final Clean—up or Direct Injection

Disruption	Lysis	Homogenization	DNA Shearing/ Shredding	Purification Cleaning	Digestion	Resuspension
Fibrous tissues, Yeast, FFPE, and more	Soft and/or delicate tissues, Cells, Bacteria	Plasma protein distribution		SP3, S-Trap, and EasyPEP	Temperature control and Gentle mixing	

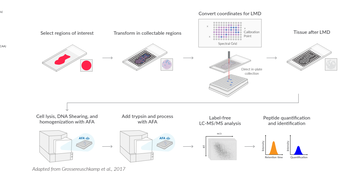
- AFA is efficient in a wide variety of downstream analytical methods with different starting materials
- The controlled and precise energy delivery makes AFA an optimal sample prep for analyses of proteins
- Address a wide array of matrices – cells, tissues, plasma, whole blood, etc.
  - AFA also shows high efficiency in processing complex matrices such as, plants, bacteria, yeast



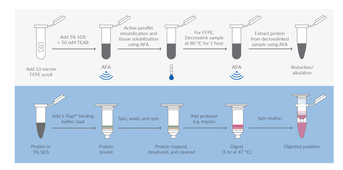
Single Pot Extraction using SP3 Clean-up: Workflow using mammalian cells and fresh frozen tissues



FFPE Scrolls with AFA and SP3 Magnetic Beads: Workflow optimized with AFA-TUBE TPX plate



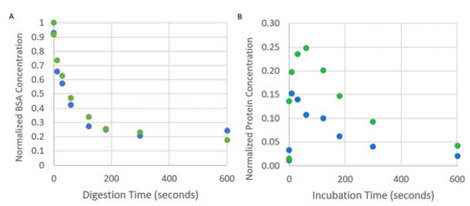
Automated Collection and Processing of LCM: Workflow optimized with AFA-TUBE TPX plate/strips and is compatible with SP3 (if needed)



FFPE Scrolls using AFA and S-Trap: truXTRAC FFPE for DNA, RNA and protein extraction. Combination of AFA and SDS results in streamlined analysis.

Ensuring Digestion Efficiency without Compromising Data Quality

1. **Goal**: expediting enzymatic hydrolysis of proteins
2. **Achievement**: ensuring high efficiency for tryptic digestion of proteins; ability to address critical high throughput requirements
3. **What it does**: combines reliable products in a streamlined workflow in flexible formats
4. **Scalability and throughput**: protocols available and can be automated in 96-well plates; enhanced to 384-well plates
5. **Additional benefits**: In addition to existing genomic protocols, AFA can now capture RNA, DNA and proteome from FFPE tissues



AFA Offers Several Benefits Towards a One-plate Sample prep Solution

1. Robust, reliable, reproducible sample prep workflows – from targeted protein quantitation to Proteomics
2. Addressing matrix complexity with ease – Unique, non-invasive and proven technology for faster, reliable extraction of proteins from any matrix (tissues, cell lysis, FFPE, whole blood cells, plasma, etc.)
3. Rapid growth in MultiOmics – Already considered a gold standard for DNA shearing by Next Generation Sequencing (NGS) laboratories, AFA is now used across a wide variety of Omics – from RNA extraction, purification to Proteomics to Metabolomics
4. Wide applicability within protein analysis – AFA has been successfully used for sample preparation of biotherapeutics to biomarkers to proteomics assays

US & APAC: +1 781.932.3959 | EU: +44 (0)845 872 0100 | Service and Instrumentation: techsupport@covaris.com | Solutions: applicationsupport@covaris.com | US: customerservice@covaris.com | EU/UK Customer Service: emeacustomerservice@covaris.com | APAC Customer Service: APACcustomerservice.com

References: Hughes et al, Nat Protoc. 2019 Jan;14(1):68-85; Hughes et al, Mol Syst Biol. 2014 Oct 30;10(10):757; Mueller et al, Mol Syst Biol. 2020, 16:e9111; Schweitzer et al, Covaris Application Note M020141; Covaris Application Note M020154; Staeber et al, Covaris Application Note M020155; Herrera et al, Clin. Proteom. 2020, 17:24; Mueller et al, Mol. Syst. Biol. 2020,16:e9111; Herrera et al, Clin. Proteom. 2020, 17:24; Stukalov et al, bioRxiv 2020 ; Niu et al, bioRxiv 2020, Covaris Application Note M020149.

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