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From Variability to Standardization: Reframing Protein Analysis with Comprehensive Sample Preparation Workflows Enabled with Adaptive Focused Acoustics® (AFA®) Technology

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Abstract

Proteomics has entered an era of unprecedented analytical sensitivity, driven by advances in high resolution mass technology and computational pipelines. However, the world of proteomics or protein analysis remains constrained by a persistent and under addressed bottleneck: sample preparation variability. Unlike sequencing based omics, where library preparation has achieved increasing standardization, proteomics workflows remain highly sensitive to upstream processing conditions, particularly in heterogeneous and clinically relevant samples such as formalin fixed paraffin embedded (FFPE) tissues.

In this report, we discuss how controlled acoustic energy-based sample processing such as Adaptive Focused Acoustics (AFA) Technology, can enable a critical step toward transforming proteomics into a reproducible, scalable, and clinically translatable discipline. This white paper explains how precise control of mechanical energy enables consistent protein extraction, efficient purification, and improved digestion efficiency – all while reducing bias across diverse sample types. We further propose that standardized sample preparation platforms, and not incremental improvements in instrumentation, are the defining factor in advancing the next phase of proteomics innovation.

Introduction

The Hidden Bottleneck in Proteomics

Proteomics has achieved remarkable gains in sensitivity, throughput, and depth of coverage over the past decade using the combined technology of liquid chromatography (LC) coupled to mass spectrometry (MS).[1] Modern workflows enabled by high resolution accurate mass spec technology can ensure the ability to quantify thousands of proteins across complex biological systems. Such abilities enable a wide array of applications ranging from biomarker discovery to systems biology and drug development. However, unlike genomics, where standardized workflows for DNA extraction and library preparation have reduced variability, proteomics remains fundamentally constrained by inconsistent sample preparation.[2] Each of the steps in protein extraction, solubilization, purification, and digestion introduce significant variability, often exceeding that of downstream analytical platforms.[3] These limitations in sample preparation are particularly pronounced in:

- Formalin Fixed Paraffin Embedded (FFPE) samples, where crosslinking complicates protein recovery[4]
- Low-input clinical samples, where yield and loss are critical
- Heterogeneous tissues, where disruption or homogenization efficiency varies spatially
- Different cell types, where lysis efficiency can vary depending on the number of cells and cell types

As a result, proteomics studies frequently suffer from:

- Insufficient and inaccurate data due to:
  - Limited protein detection sensitivity
  - Insufficient peptide depth
  - Missed cleavages that negatively impact data quality
  - Inadequate post-translational modification (PTM) information
- Reduced reproducibility, reliability, and robustness, from inefficient sample preparation
- Bias against low abundance or membrane proteins

These challenges highlight a central paradox. While proteomics has achieved analytical precision, the upstream sample preparation workflows that feed it remain largely unstandardized.

The Case for Controlled and Comprehensive Sample Preparation: From Chemical to Physical Control

Sample preparation for traditional proteomics workflows rely heavily on starting with chemical lysis. Sometimes, depending on the sample type, it is combined with mechanical disruption techniques such as probe sonication or bead beating. However, these approaches are inherently variable:

- Probe sonication introduces localized heating and cavitation artifacts[5]
- Bead beating produces inconsistent mechanical forces across samples[6]
- Manual workflows amplify operator-dependent variability

In contrast, Adaptive Focused Acoustics (AFA) Technology introduces a fundamentally different paradigm by ensuring precise, controlled, tunable delivery of acoustic energy under controlled temperature conditions (**Figure 1**). In doing so, the approach enables:

- Uniform energy distribution
- Controlled matrix disruption
- Reproducible sample processing without thermal degradation

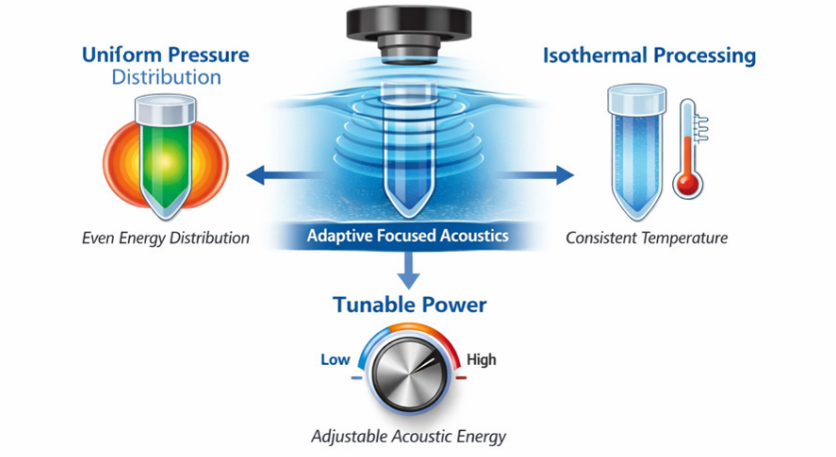

Figure 1. Critical benefits of AFA Technology: *i*) ability to distribute pressure equally; *ii*) maintaining controlled temperature condition for every sample; *iii*) ability to tune power based on sample requirements.

AFA Technology and the Standardization of Proteomics Workflows

Protein Extraction from Challenging Matrices

Efficient and unbiased protein extraction is a prerequisite for deep proteome coverage. Studies have shown that incomplete homogenization (lysis, disruption) disproportionately affects membrane and structural proteins, leading to systematic bias.[7] On the other hand, AFA Technology enables clear benefits for matrices that are typically considered challenging in the world of proteomics:

- **FFPE:** Active deparaffinization and efficient disruption of crosslinked FFPE tissues – from scrolls[8] to laser capture micro-dissected (LCM) samples[9]
- **Fresh Frozen (FF) Tissues:** Efficient homogenization of a wide array of amounts of FF tissues[10]
- **Cells:** Efficient lysis, extraction, purification, and digestion across a wide range of mammalian cell inputs – from very low cell numbers, to millions[11]

Across all matrix types, AFA Technology also enables enhanced buffer penetration and improved recovery of otherwise inaccessible protein fractions, facilitating faster purification and digestion without compromising data quality.

Compared to traditional sample preparation methods, it generates robust, reliable, and fast sample preparation workflows (**Figure 2**) for every protein type, ultimately resulting in better proteomic profiles with high reproducibility.

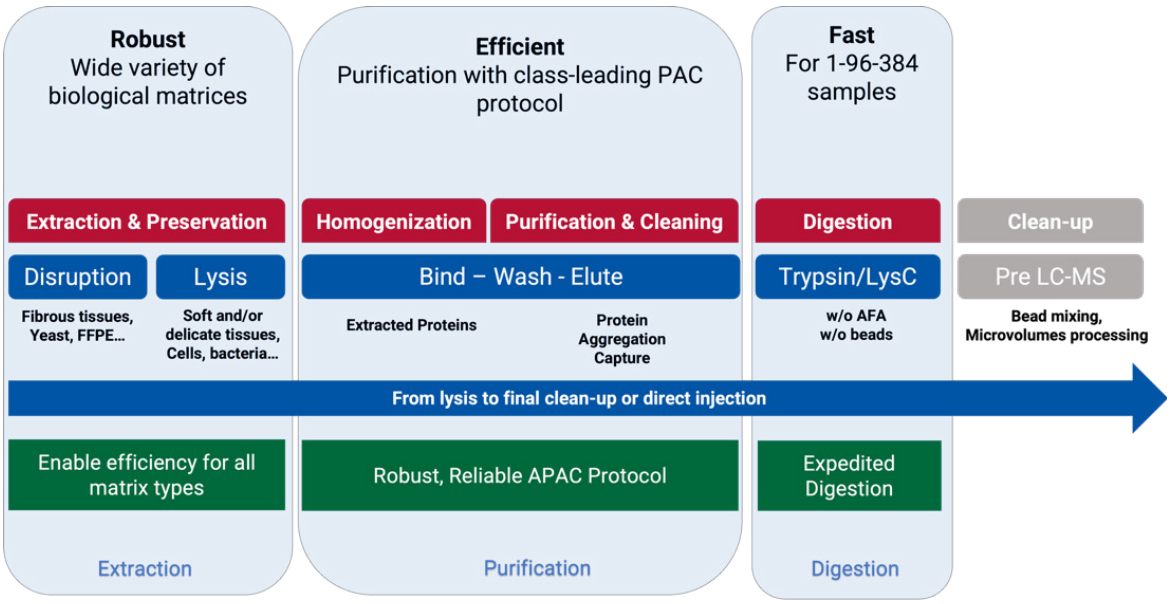

Figure 2. A comprehensive three-step sample preparation workflow for protein analysis. This workflow can be applied across all matrices, protein types, and starting amounts.

Improving Efficiency – From Purification to Digestion

AFA Technology can significantly enhance purification efficiency by delivering highly controlled acoustic energy under controlled temperature conditions. Following protein extraction, (**Figure 2**), the approach promotes efficient solubilization and uniform mixing, improving protein accessibility while reducing insoluble debris and contaminant carryover. Critically, it also supports key pre-digestion chemistry steps such as disulfide bond (S–S) reduction and cysteine (Cys) alkylation by enabling rapid, homogeneous reagent distribution and consistent reaction conditions which, in turn, ensures faster completion of the reaction, while reducing variability. Integrated with bead-based purification strategies for targeted protein depletion or enrichment, AFA Technology maximizes interaction between proteins and beads (magnetic or functionalization added to the bead surface), enhancing specificity, reducing nonspecific binding, and improving recovery of target analytes. Together, these effects yield cleaner, more consistent protein samples with improved processing efficiency and reproducibility, ultimately strengthening the reliability and depth of downstream proteomic analysis (**Figure 3**). The entire purification step can either be conducted manually or in an automated environment depending on the sample type and throughput requirement.[12, 13]

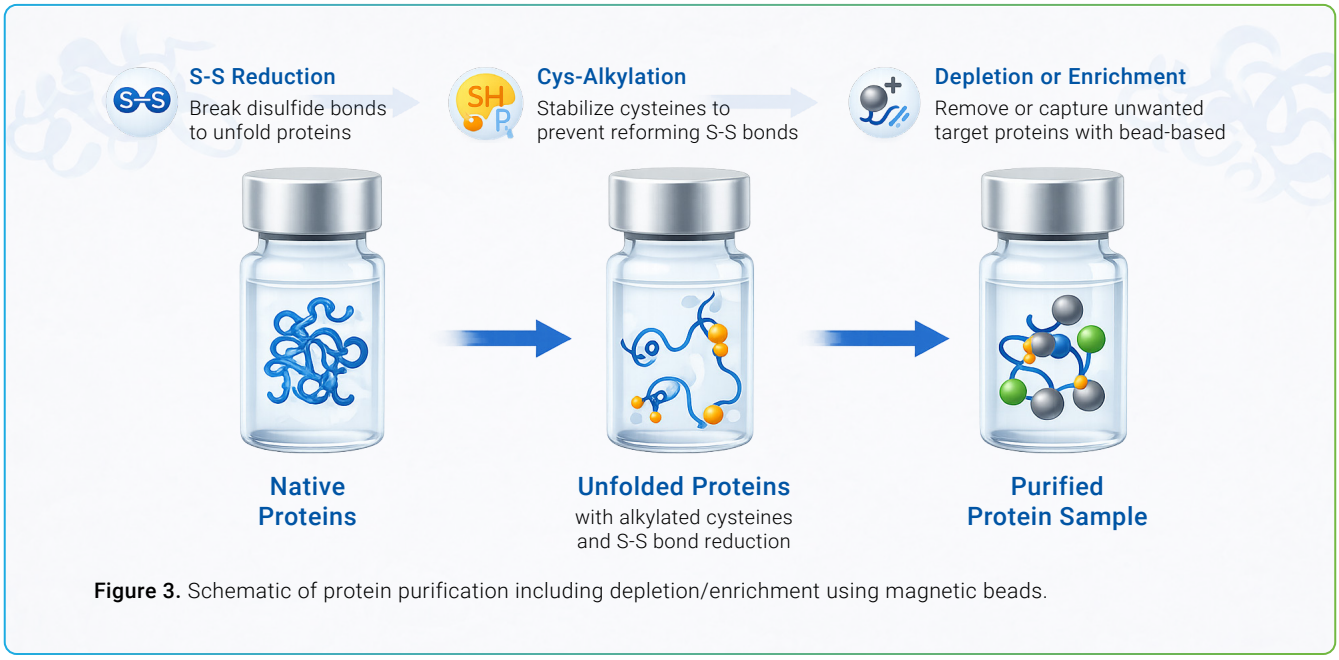

Figure 3. Schematic of protein purification including depletion/enrichment using magnetic beads.

In bottom-up proteomics, or peptide mapping or signature peptide quantitation experiments, inaccurate and irreproducible digestion remains a major source of analytical error, often leading to incomplete proteolysis, increased missed cleavages, and inconsistent peptide generation across samples.[14] These issues are typically driven by poor enzyme accessibility, suboptimal mixing, local concentration gradients, and temperature fluctuations, which ultimately result in biased peptide representation, reduced sequence coverage, and compromised quantitative accuracy. Variability in digestion efficiency can also introduce significant batch effects, undermining reproducibility in large scale studies. In contrast, AFA-accelerated digestion (**Figure 4**) overcomes these limitations by delivering controlled low power acoustic energy in a continuous scanning mode that ensures uniform mixing, consistent enzyme-to-substrate interaction, and tightly regulated and controlled temperature conditions. This results in rapid, highly reproducible digestion with improved proteolytic efficiency, generating greater peptide depth and sequence coverage without any additional missed cleavages.[9] The outcome is a more consistent and reliable peptide population, enabling higher confidence in protein identification and quantitation across samples and studies – from bottom-up proteomic assays to routine quantitative assays (which typically monitor a signature peptide of the target protein or proteins).

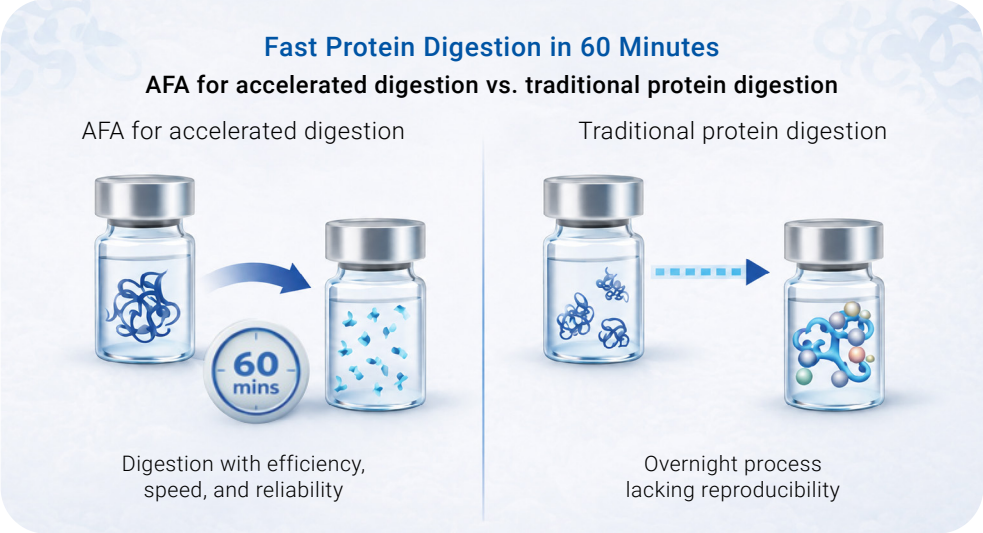

Figure 4. Comparison of protein digestion using the traditional approach (right) to that of AFA-enabled digestion (left), which offers speed, reliability, reproducibility, and robustness.

Reproducibility as a Key Metric

Sample Variance

Sample preparation is widely recognized as the largest contributor to technical variance in bottom-up proteomics, often surpassing the variability introduced by LC-MS/MS acquisition and data processing. While advancements in mass spectrometry have significantly increased sensitivity and speed, the laborious nature of sample preparation, including multiple manual liquid transfers and timed reactions inherent to sample preparation, often limit the reproducibility and accuracy of quantitative measurements. As discussed above, several factors contribute to the proteomic data variance: *i*) protein extraction/lysis efficiency (which varies significantly between matrix types); *ii*) enzymatic digestion that requires a cautious approach (several factors such as trypsin-to-substrate ratio, buffer composition, and digestion times can have a strong impact on the efficiency of peptide generation); *iii*) contaminants such as salts, detergents, or other interfering agents can impact peptide separation by causing unwarranted ion suppression (especially with LC-MS/MS).[15]

Improving Reproducibility

To minimize the impact of sample preparation on overall workflow variability, the field is moving towards:

- **Comprehensive Workflows:** Fast, simplified, one-plate workflows that can reduce the number of processing steps, thereby minimizing technical noise.
- **Early Quality Control:** Monitoring the consistency of sample preparation using quality control metrics (e.g., protein concentration, enzyme ratio optimization, peptide yield assessments) is essential for controlling data quality before MS analysis.
- **Automation:** Implementing automated platforms, such as 384-well plate systems, which significantly reduces the human error while enhancing inter- and intra-plate reproducibility.

AFA-enabled Comprehensive Sample Prep Workflows – One Solution for Every Proteomic Assay

AFA-enabled comprehensive sample preparation workflows are designed to deliver end-to-end reproducibility across proteomics applications by tightly controlling every critical variable in the workflow. The precise, tunable acoustic energy under controlled temperature conditions and appropriate, optimized workflows help eliminate variability and ensure consistent sample processing for every sample, regardless of protein type, input sample amount, and matrix complexity. Covaris' comprehensive sample preparation workflow ensures highly consistent reaction kinetics during every step starting with extraction, and continuing to reduction, alkylation, purification, and digestion. In an automated environment, the workflow ensures reproducible outcomes while offering a scalable, high-throughput process. High protein coverage,[8] consistent peptide yield,[9] reduced technical variation, and highly reproducible protein identification and quantitation follow directly from this level of process control.

Cell-based Proteomics

Cell-based proteomic assays offer a powerful window into cellular function, enabling direct measurement of protein expression, signaling dynamics, and post-translational modifications in biologically relevant systems.[16] By working with intact cells (cultured cell lines, primary cells, or organoids), researchers can capture context-specific biology that is often lost in bulk tissue analysis. This makes cell-based proteomics indispensable for drug discovery, functional genomics, and precision medicine applications, where understanding protein-level responses to perturbations is critical.

Achieving these outcomes, however, depends on overcoming a persistent challenge: in cell-based proteomics, sample preparation remains the most variable and technically demanding step in the workflow. Efficient and reproducible cell lysis must balance completeness with preservation of protein integrity and PTMs. Downstream steps—including reduction, alkylation, and cleanup—must remove interfering substances (lipids, nucleic acids, salts) without introducing losses or bias. The complexity increases further when dealing with heterogeneous cell populations, low cell counts, or sensitive signaling states, where even minor inconsistencies can distort biological interpretation.

Additionally, scalability can be a significant constraint, particularly as studies include hundreds or thousands of samples. The above-mentioned challenges for sample preparation of cells are further magnified by the inherent complexity of the cellular proteome, which spans a wide dynamic range and includes membrane proteins, protein complexes, and transient modifications.[17] Ensuring compatibility with diverse downstream detection platforms, from LC-MS for discovery proteomics to antibody based assays for targeted measurements can potentially add another layer of technical demand. As a result, robust, standardized, and detection-technology-agnostic sample preparation workflows are increasingly recognized as foundational to reliable proteomic outcomes.

Looking ahead, the field is rapidly advancing toward single-cell proteomics, where the goal is to resolve protein expression at the level of individual cells.[18] This shift introduces unprecedented sensitivity requirements and places extreme pressure on sample preparation workflows to minimize losses, contamination, and variability. While innovations in microfluidics, ultra-low-volume processing, and advanced mass spectrometry are driving progress in this space, much work has to be accomplished at the sample preparation level, primarily in the lysis and digestion stages. In this regard, Covaris' truPREP® Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion) and associated workflow imparts the ability to offer fast, reliable, and reproducible workflows for extraction, purification, and digestion of proteins from a wide range of cells (**Figure 5**).

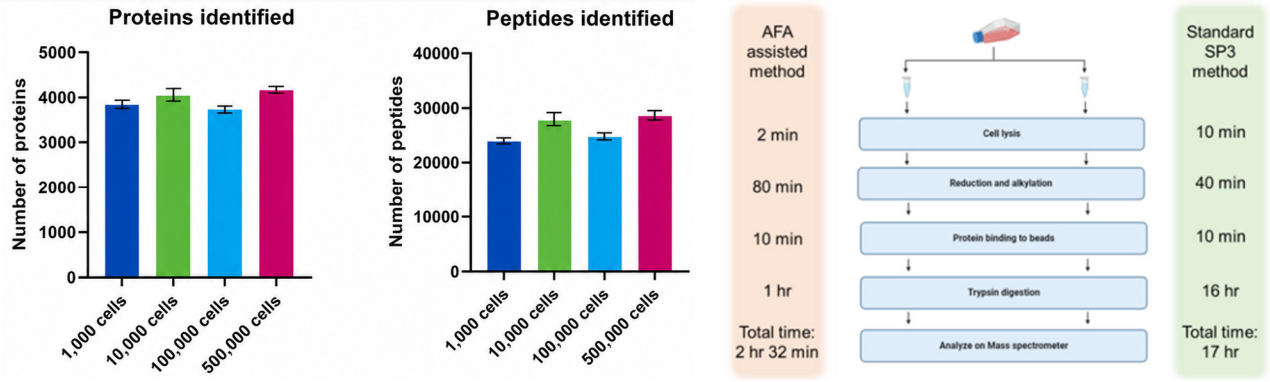

Figure 5. Comparison of the performance of Covaris' cell lysis kit with traditional approaches.

As depicted in **Figure 5**, Covaris' cell lysis kit offers several significant benefits, which can also be further understood from **Figure 6**. The workflow ensures a comprehensive coverage of a number of cells, from 1,000 up to 500,000 – essentially offering flexibility and scalability in every experimental design. Developed with the Protein Aggregation Capture (PAC) workflow, this kit is designed to address the future of cell-based proteomics. The future of single cell proteomics is expected to be defined by workflows that are not only scalable and automatable but also capable of delivering consistent, high-quality data from ever-smaller inputs; ultimately unlocking a deeper, more precise understanding of cellular heterogeneity and disease biology.

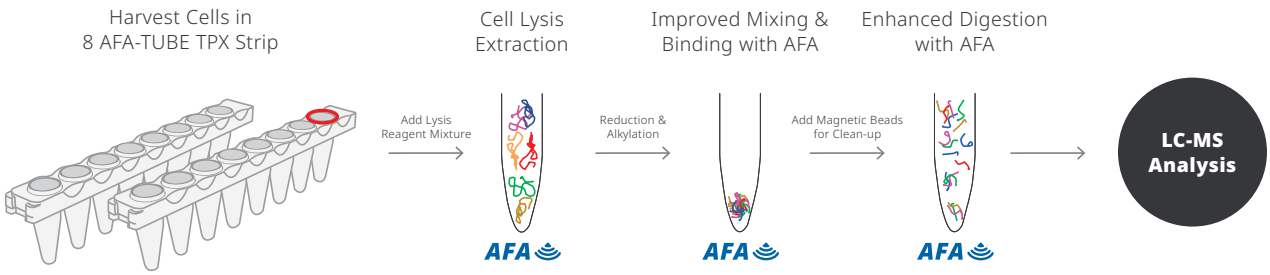

Figure 6. Workflow of cell lysis kit showing every step of the workflow starting with harvesting of cells followed by cell lysis, purification, and digestion.

Fresh Frozen (FF) Tissue-based Proteomics

Fresh frozen tissue proteomics is critically important for capturing the true biological state of a sample, preserving native protein structures, PTMs, and signaling dynamics that are often lost or altered in other preservation methods.[20] However, sample preparation of FF tissues is one of the most critical yet challenging steps in the workflow, often limiting the accuracy, depth, and reproducibility of downstream analysis.[21] The inherent heterogeneity of tissues, variable cellular composition, and the presence of structurally complex and membrane-rich regions make efficient and uniform protein extraction difficult. Incomplete lysis, inconsistent homogenization, and co-extraction of interfering biomolecules can introduce significant variability, while traditional mechanical or chemical methods often lack the control needed to preserve protein integrity and post-translational modifications.

Covaris AFA-based workflows for FF tissues directly address the above-mentioned challenges by enabling highly-controlled and reproducible tissue disruption and protein extraction across diverse tissue types (**Figure 7**).

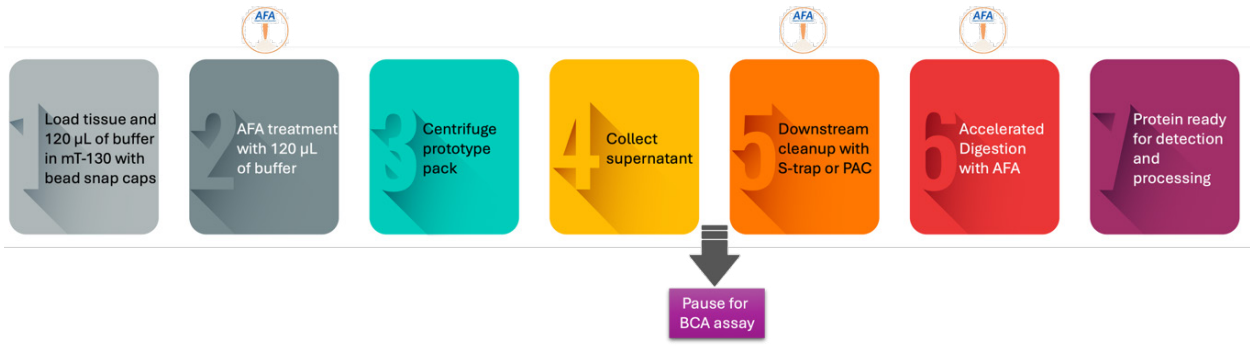

Figure 7. 7-step workflow from tissue homogenization to protein digestion for FF tissues in microtube-130 with bead snap caps (PN 500798).

The entire FF tissue workflow was optimized with a dedicated consumable, microTUBE-130 with Bead Snap-Cap (**Figure 8**), which can be used in a 96-well plate format and offers clear homogenization for a wide range of FF tissue input amounts (**Figure 8**).

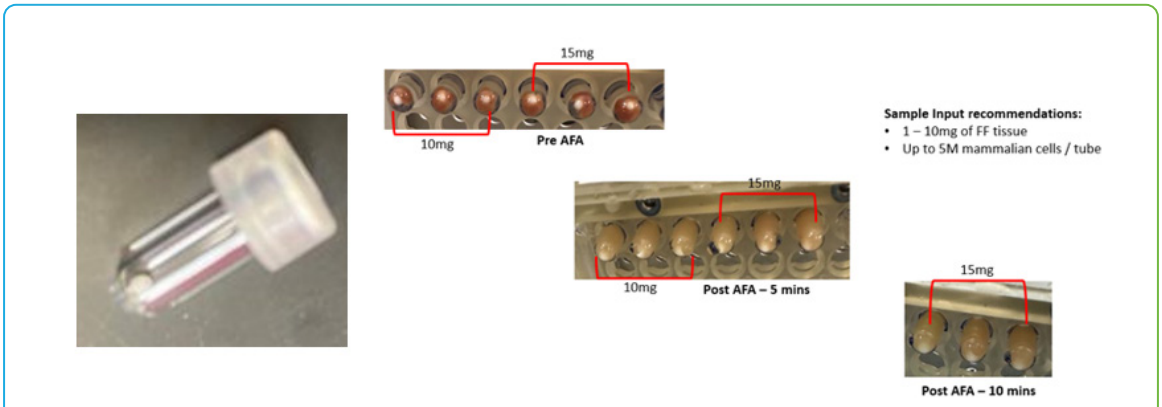

Figure 8. Picture of microTUBE-130 with Bead Snap-Cap (left) and efficient homogenization (right) of mouse liver tissues (1–15 mg) using the same consumable.

There is evidence of efficient homogenization followed by protein extraction from a host of different FF tissue types for lower inputs.[12] AFA-enabled homogenization of FF tissues from pig heart, mouse liver, and mouse kidney were found to be highly reproducible, also resulting in consistent peptide depth with very low variability. Another study reported development of a robust workflow with both FFPE and FF tissues using a robust, reliable workflow starting with efficient homogenization enabled by AFA Technology.[22]

Reliable, reproducible, and rapid sample preparation for FF tissues yields a standardized, automation-ready workflow with consistent protein and peptide inputs, enabling deeper proteome coverage and reliable performance across LC-MS, antibody-based assays, and other detection platforms.

FFPE Proteomics: A Stress Test for Workflow Robustness

FFPE samples represent one of the most valuable yet challenging resources in translational research.[23] Extraction of protein from tissues embedded in paraffin block involves *i*) efficient deparaffinization; *ii*) tissue homogenization; *iii*) extraction. Each of these steps are critical to ensure maximum protein yield and coverage that are necessary for robust, reliable analysis. Formalin fixation induces significant changes, such as protein crosslinking, chemical modifications, and reduced solubility[6]. In dealing with such samples, conventional workflows often result in low protein recovery, high variability, and incomplete proteome representation.

AFA Technology is the only method that can offer active and fast deparaffinization of FFPE samples without damaging the embedded tissue integrity. AFA-based FFPE workflows for protein analysis have demonstrated improved protein yield[8, 9, 12, 23-25] coverage and downstream peptide depth, especially unique peptide IDs (**Figure 9**).

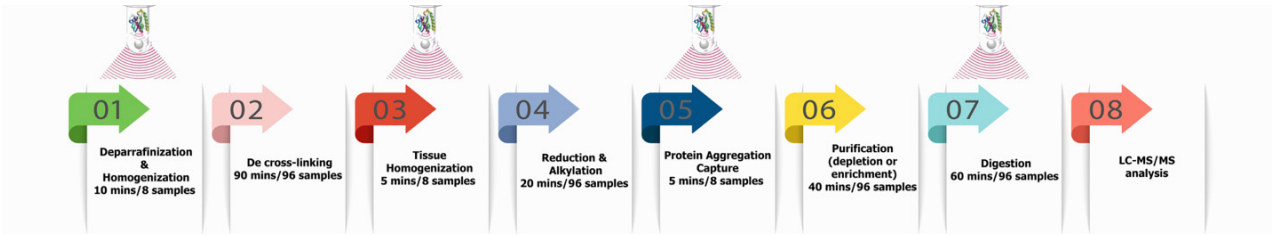

Figure 9. A comprehensive, and fast sample preparation workflow for FFPE samples enabled by AFA Technology. This one-plate workflow allows maximum recovery of proteins, resulting in exceptional protein coverage and peptide depth.

Covaris' AFA-enabled sample preparation workflow supports high-throughput proteomic analysis of FFPE tissues, including dissected samples or 10 µm scrolls, in a 96-well plate format capable of processing up to 96 samples simultaneously. Haines et al. applied this workflow, building on their prior work to demonstrate significant enhancement in protein coverage, notably including critical phosphoproteins, a valuable source of PTM information, whose recovery from FFPE tissue is typically compromised by formalin-induced crosslinking.[8]

The workflow enabled identification of 8,000–10,000 unique proteins per sample with median CVs below 20%. Applied to lung adenocarcinoma FFPE blocks using the Orbitrap™ Astral™ with 24-minute gradients, the workflow identified 11,000 fully localized phosphosites alongside up to 10,000 unique proteins, achieving deep proteome coverage and quantitative robustness comparable to tandem mass tag-based methods, demonstrating that biologically relevant phosphoproteomic results can be derived from clinically sourced FFPE tumor samples.

Similarly, Adachi, Y *et al.* reported robust proteomic profiles obtained from a wide number of FFPE samples, some of which were more than 30 years old.[23] FFPE samples of hepatocellular carcinoma (HCC) and adjacent non-tumor liver (NTL) tissues from 19 HCC patients from 1988–1992 were evaluated in which the AFA-enabled workflows offered efficient protein extraction combined with SP3 cleanup and data-independent acquisition (DIA). The workflow identified and quantified approximately 7,000 proteins, with excellent reproducibility, where proteomic profiles clearly distinguished between HCC and NTL tissues, and identified 630 differentially expressed proteins (467 upregulated in HCC, 163 upregulated in NTL). Pathway analysis revealed expected biological differences: HCC showed enrichment in proliferation/genomic maintenance pathways (e.g., ribosome biogenesis, DNA replication), while NTL showed enrichment in metabolic pathways (e.g., cytochrome P450), consistent with known biology and validated by COSMIC database. This validated Covaris workflow unlocks the potential of these historically invaluable specimens for powerful retrospective studies, contributing to a clear and comprehensive understanding of cancer such as HCC.

Reproducibility and Reliability in LCM-FFPE Samples

Pujari, G. *et al.* reported[9] that for LCM samples, not only did they see an equivalent performance in the number of proteins but the AFA-enabled workflow reduced their total processing time from 20 hours to 3 hours, without compromising data quality. In this study, four replicates were processed under different conditions (**Figure 10**). All samples were first collected in tubes and then manually transferred to the 96-well plates for AFA processing. After digestion, the samples were cleaned and analyzed by mass spectrometry. Protein and peptide identification numbers, reproducibility, and processing time were compared across all workflows.

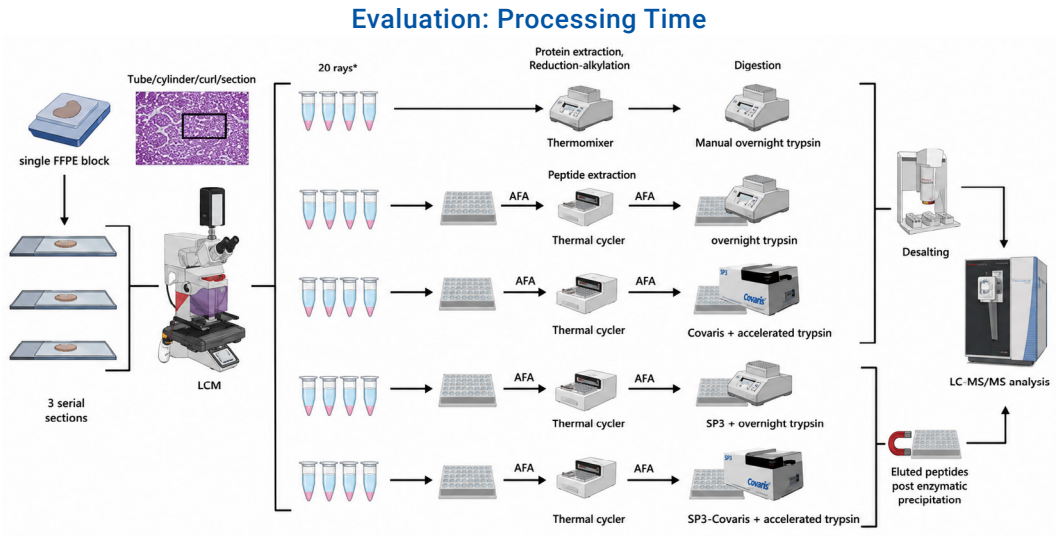

Figure 10. Comparison of sample preparation workflows for LCM-FFPE tissue processing.

Protein and peptide numbers in AFA-accelerated workflows were comparable to standard workflow with the mass spectrometry results showing that both AFA-accelerated methods are similar to the standard tube-based method in terms of protein and peptide identification numbers (**Figure 11**).

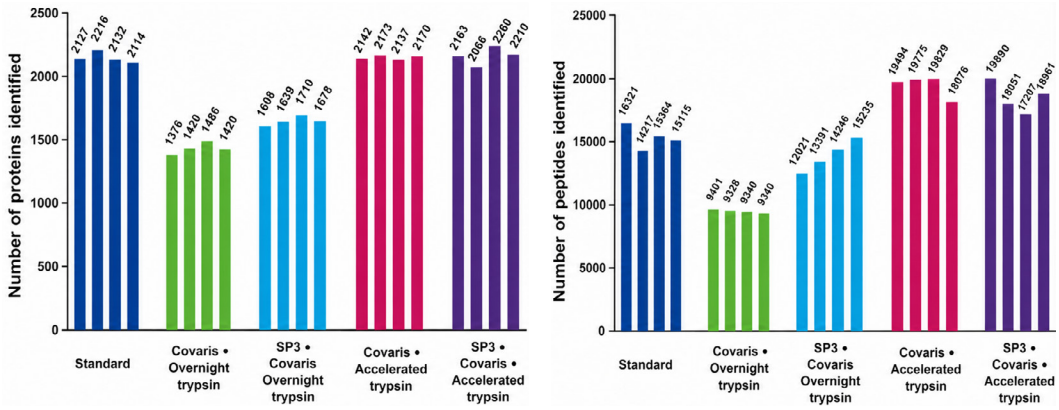

Figure 11. Both AFA-accelerated methods yielded protein and peptide numbers comparable to the standard method. Using SP3 did not add significant benefits.

The workflow is compatible with the full range of LC-MS/MS platforms, from high-resolution accurate mass instruments for total proteome discovery to triple quadrupole systems for sensitive targeted quantitative analysis.

Multi-Organ Proteomics: All in the Same Plate

Multi-organ proteomics presents significant sample preparation challenges due to the inherent biological diversity across tissues, ranging from from highly vascularized organs like liver, to dense, fibrous structures such as heart or tumor tissue. Each organ differs in cellular composition, protein abundance, lipid content, and extracellular matrix complexity, requiring tailored lysis and extraction conditions that are difficult to standardize. Variability in sample preservation methods (e.g., fresh frozen vs. FFPE) further complicates protein recovery and integrity. Additionally, inconsistent homogenization, inefficient protein solubilization, and incomplete removal of contaminants can introduce bias, reduce reproducibility, and limit proteome depth. As multi-organ studies scale, the need for robust, uniform, and automatable workflows becomes critical to ensure cross-tissue comparability and high-quality downstream mass spectrometry data.[26]

AFA-enabled sample preparation is critical for multi-organ proteomics because it delivers a single, standardized workflow capable of handling the diverse physical and biochemical properties of different tissues. These workflows ensure efficient and reproducible tissue disruption, protein extraction, and homogenization across soft, fibrous, and lipid-rich organs alike—minimizing variability introduced during sample prep. This consistency ensures comparable protein recovery and digestion efficiency across organs, which is essential for accurate cross-tissue analysis.[27, 28]

The last few years have witnessed several publications highlighting the value of robust sample preparation leading to high quality multi-organ proteomics data. Xiong et al. applied AFA-assisted proteolysis as part of a high-throughput sample preparation workflow to enable rapid and comprehensive analysis of complex proteomes across large sample sets, advancing the potential of systems biology research. In this study, a high-throughput MS-based proteomics method was integrated into narrow-window data-independent acquisition (nDIA) with short-gradient micro-flow chromatography, enabling profiling of >240 samples per day. This approach identified 6,201 and 7,466 human proteins with 1- and 2-min gradients, respectively, and analyzed 507 samples composed of 13 different tissues from mice treated with the enzyme-drug L-asparaginase (ASNase) or its glutaminase-free Q59L mutant, generating a quantitative profile of 11,472 proteins following drug treatment. In summary, the integration of AFA Technology to optimize this high-throughput proteomics method accelerates systems-level analysis of a preclinical model to generate biological insights and clinically actionable hypotheses.[27]

On a completely different application perspective, Hendricks *et al.* reported development of a comprehensive proteomics workflow for biofluids, cells, and tissues. AFA Technology was used for cells and tissue lysis to ensure robust and reproducible sample preparation in a high-throughput manner, and the whole workflow was automated with the detergent-compatible single-pot, solid-phase-enhanced sample Preparation (SP3) method for protein digestion. The synergy of these advanced methodologies facilitated a robust and high-throughput approach for cell and tissue analysis, an important consideration in translational research.[10]

Toward Scalable and Automated Proteomics

As proteomics studies expand in scale and complexity – drawing from large clinical cohorts, multi-site collaborations, and high-throughput biomarker discovery programs, the need for scalable, automated sample preparation workflows becomes increasingly critical to ensure efficiency and reproducibility. Recent examples highlight the value of automating sample preparation workflows for proteomics assays to minimize or eliminate data inaccuracies resulting from manual operation and handling.[12, 29] The future of proteomics lies in the ability to scale-up, which includes population level studies, clinical diagnostics, and drug discovery pipelines. However, scaling proteomics requires automation-ready workflows with standardized inputs and reduced hands-on time. Current sample preparation workflows can be fragmented between different approaches and technology, which makes them difficult to automate seamlessly, not only for higher throughput, but also to ensure reproducibility, reliability, and robustness of the methods.[30] Therefore, a robust and reliable proteomics sample preparation workflow delivering equal efficiency in both manual or automated modes would benefit every laboratory – from small-scale operations to those processing hundreds of samples daily.

AFA Technology transforms traditionally variable, labor-intensive sample preparation into a controlled, reproducible, and automation-ready workflow that integrates seamlessly with modern liquid handling platforms. Spanning protein extraction across any biological matrix through reduction, alkylation, bead-based purification, and accelerated digestion, the workflow reduces manual intervention and ensures uniform processing to support parallel preparation of dozens to hundreds of samples in a 96-well plate format with consistent, predictable performance. The result is a robust, end-to-end sample preparation solution that scales with study demands while safeguarding the reproducibility and data quality required for confident mass spectrometry analysis and large-scale biomarker discovery.

Multiomics Convergence: A Unified Sample Preparation Paradigm[31]

Multiomics sample preparation is often constrained by fragmented workflows, inconsistent processing methods across analyte types, and limited sample availability, especially when working with precious clinical specimens like FFPE tissue or small volume blood samples.[28, 32] These typical sample preparation challenges can introduce variability between genomic, transcriptomic, and proteomic datasets, complicating data integration and reducing confidence in biological insights. Additionally, the lack of standardized, automatable workflows creates bottlenecks in scaling studies and maintaining reproducibility across large cohorts.

AFA Technology addresses the above-mentioned challenges by providing a unified, highly controlled approach that aligns analyte workflows seamlessly. Because acoustic energy delivery is precisely tunable, AFA Technology can be optimized independently for each analyte class. This enables the sequential extraction of proteins, nucleic acids, and metabolites from the same sample without compromising the integrity of any downstream analysis. As an example, a concurrent metabolomics and proteomics workflow was recently published highlighting the benefit of using AFA Technology for rapid extraction and removal of metabolites followed by processing the same sample for proteomic assays (**Figure 12**).[25]

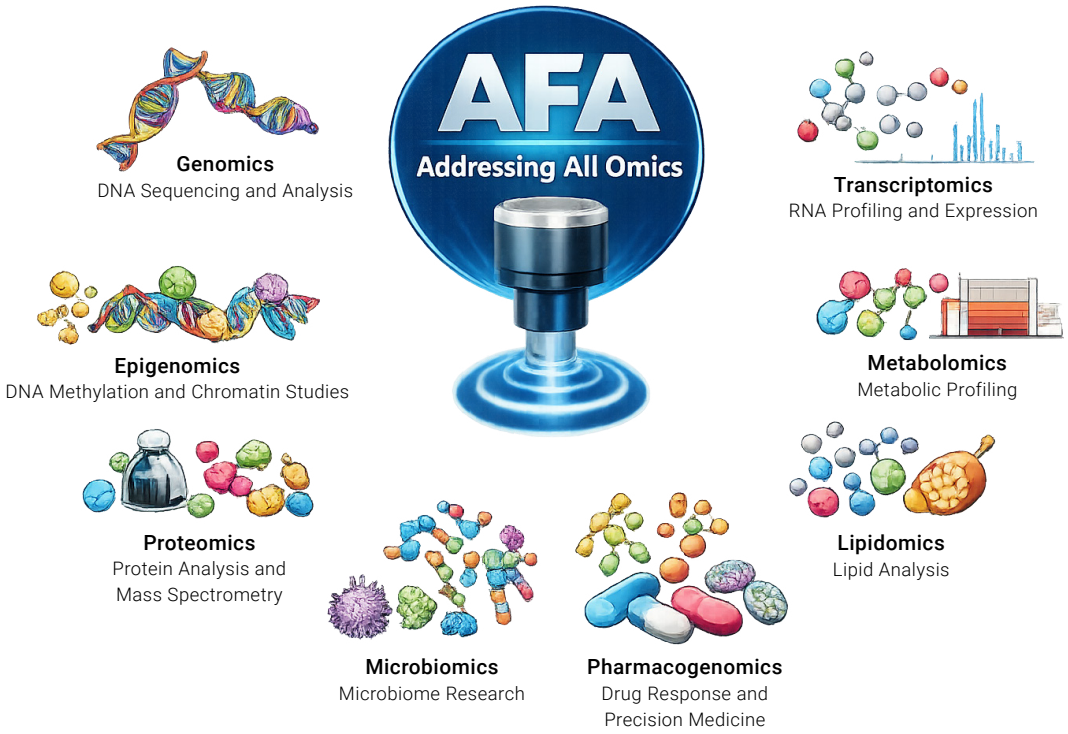

Figure 12. Multi-dimensional application of AFA across all forms of omics that can either be facilitated from the same sample or on separate parts of a sample.

The ability to start from a consistent point followed by harmonized multiomics analysis of a given sample enables laboratories (especially those focused on precision medicine or translational research studies) to generate matched datasets with minimal or no variability, which are typically introduced during preparation. When combined with automation, these robust workflows can support a scalable, end-to-end multiomics solution that improves data concordance, reduces workflow complexity, and accelerates the discovery of integrated biological insights.

Sample Preparation – Agnostic of End Detection Technology

Historically, proteomics innovation has focused on instrumentation (primarily mass spectrometers) with additional focus on data analysis algorithms. Sample preparation is often overlooked, yet itcontinues to be one of the most significant challenges in protein analysis. Inconsistent extraction, inefficient purification, and variable processing conditions introduce irreproducibility that no analytical platform can recover from. Covaris' AFA-enabled protein analysis sample preparation workflows directly address this critical weakness by delivering a detection-technology-agnostic, highly-controlled, and reproducible upstream process. With reproducible, reliable and efficient workflows, AFA Technology ensures isolation of clean and consistent proteins and peptides that integrate seamlessly with any downstream technology, from high-resolution LC-MS for deep proteomic profiling, to antibody-based assays like ELISA for targeted quantification, or other emerging analytical platforms. Laboratories gain a standardized, automation-ready workflow that removes upstream variability, preserves sample integrity, and ensures the full performance of any detection technology is realized — future-proofing proteomics strategies while maximizing data quality and return on investment.

Conclusion

Proteomics stands at an inflection point. While analytical technologies have matured, sample preparation remains the dominant source of variability and inefficiency. The ability to establish a robust, reliable, consistent sample preparation workflow is further challenged by a proliferation of approaches for every step – starting from homogenization or extraction of proteins to purification to digestion. Amid this complexity, AFA Technology has emerged as a transformative solution, enabling rapid, reproducible sample preparation for proteomics regardless of sample type, input amount, analytical objective, and user expertise.

As proteomics continues to advance toward large-scale multiomics and clinically actionable applications, AFA-enabled workflows are uniquely positioned to meet these demands by introducing:

- Precision energy control
- Reproducible processing conditions across any sample type
- Expedited workflows that ensure high quality data while supporting increased productivity and profitability
- Compatibility with automation and high-throughput

By reframing sample preparation as a controlled, standardized process rather than an artisanal step, proteomics can now achieve clinical-grade reproducibility, large-scale deployment, and true multiomics integration.

References

(1) Aebersold, R.; Mann, M. Mass-spectrometric exploration of proteome structure and function. *Nature* **2016**, *537*, 347–355. Cox, J.; Mann, M. Quantitative, High-Resolution Proteomics for Data-Driven Systems Biology. *Annual Review of Biochemistry* **2011**, 80, 27. Bekker-Jensen, D. B.; Kelstrup, C. D.; Batth, T. S.; Larsen, S. C.; Haldrup, C.; Bramsen, J. B.; Sørensen, K. D.; Høyer, S.; Ørntoft, T. F.; Andersen, C. L.; *et al*. An Optimized Shotgun Strategy for the Rapid Generation of Comprehensive Human Proteomes. *Cell Systems* **2017**, *4* (6), 13.

(2) Giansanti, P.; Tsiatsiani, L.; Low, T. Y.; Heck, A. J. R. Six alternative proteases for mass spectrometry–based proteomics beyond trypsin. *Nature Protocols* **2016**, *11*, 14.

(3) Piehowski, P. D.; Petyuk, V. A.; Orton, D. J.; Xie, F.; Moore, R. J.; Ramirez-Restrepo, M.; Engel, A.; Lieberman, A. P.; Albin, R. L.; Camp, D. G.; *et al.* Sources of technical variability in quantitative LC-MS proteomics: human brain tissue sample analysis. *Journal of Proteome Research* **2013**, *12* (5), 10.

(4) Shi, S.-R.; Liu, C.; Balgley, B. M.; Lee, C.; Taylor, C. R. Protein Extraction from Formalin-fixed, Paraffin-embedded Tissue Sections: Quality Evaluation by Mass Spectrometry. *Journal of Histochemistry & Cytochemistry* **2006**, *54* (6), 5.

(5) Varnavides, G.; Madern, M.; Anrather, D.; Hartl, N.; Reiter, W.; Hartl, M. In Search of a Universal Method: A Comparative Survey of Bottom-Up Proteomics Sample Preparation Methods. *Journal of Proteome Research* **2022**, *21* (10), 15. Stathopulos, P. B.; Scholz, G. A.; Hwang, Y.-M.; Rumfeldt, J. A.; Lepock, J. R.; Meiering, E. M. Sonication of proteins causes formation of aggregates that resemble amyloid. *Protein Science* **2004**, *13* (11), 11. Shen, L.; Pang, S.; Zhong, M.; Sun, Y.; Qayum, A.; Liu, Y.; Rashid, A.; Xu, B.; Liang, Q.; Ma, H.; *et al*. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrasonics Sonochemistry* **2023**, *101*.

(6) Nicora, C.; Gritsenko, M.; Lipton, A.; Wahl, K. L.; Burnum-Johnson, K. E. Proteomic Sample Preparation Techniques: Toward Forensic Proteomic Applications. *ACS Symposium Series* **2019**, *1339*, 18.

(7) Wiśniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M. Universal sample preparation method for proteome analysis. *Nature Methods* **2009**, *6*, 4.

(8) Haines, M.; Thorup, J. R.; Gohsman, S.; Ctortecka, C.; Newton, C.; Rohrer, D. C.; Hostetter, G.; Mani, D. R.; Gillette, M. A.; Satpathy, S.; *et al*. High-Throughput Proteomic and Phosphoproteomic Analysis of Formalin-Fixed Paraffin-Embedded Tissues. *Molecular & Cellular Proteomics* **2025**, *24* (9).

(9) Pujari, G. P.; Mangalaparthi, K. K.; Madden, B. J.; Bhat, F. A.; Charlesworth, M. C.; French, A. J.; Sachdeva, G.; Daviso, E.; Thomann, U.; McCarthy, P.; *et al*. A High-Throughput Workflow for FFPE Tissue Proteomics. *Journal of the American Society for Mass Spectrometry* **2023**, *34* (7), 1225–1229.

(10) Hendricks, N. G.; Bhosale, S. D.; Keoseyan, A. J.; Ortiz, J.; Stotland, A.; Seyedmohammad, S.; Nguyen, C. D. L.; Bui, J. T.; Moradian, A.; Mockus, S. M.; *et al*. An Inflection Point in High-Throughput Proteomics with Orbitrap Astral: Analysis of Biofluids, Cells, and Tissues. *Journal of Proteome Research* **2024**, *23* (9), 4163–4169. DOI: 10.1021/acs.jproteome.4c00384

(11) Seyedmohammad, S.; Rivas, A.; Zhgamadze, M.; Haghani, A.; Kreimer, S.; Bharadwaj, A.; Sundararaman, N.; Vasantgadkar, S.; Pal, K.; Daviso, E.; *et al*. High-Throughput Workflow for Detergent-free Cell-Based Proteomic Characterization. *Journal of Proteome Research* **2025**. Aug 1;24(8):3762-3773. DOI: 10.1021/acs.jproteome.4c00892

(12) Müller, T.; Kalxdorf, M.; Longuespée, R.; Kazdal, D. N.; Stenzinger, A.; Krijgsveld, J. Automated sample preparation with SP3 for low-input clinical proteomics. *Molecular Systems Biology* **2020**. Jan;16(1):e9111. DOI: 10.15252/msb.20199111

(13) Borteçen, T.; Müller, T.; Krijgsveld, J. An integrated workflow for quantitative analysis of the newly synthesized proteome. *Nature Communications* **2023**, *14* (1), 8237. DOI: 10.1038/s41467-023-43919-3

(14) Brownridge, P.; Beynon, R. J. The importance of the digest: Proteolysis and absolute quantification in proteomics. *Methods* **2011**, *54* (4), 10. León, I. R.; Schwämmle, V.; Jensen, O. N.; Sprenger, R. R. Quantitative Assessment of In-solution Digestion Efficiency Identifies Optimal Protocols for Unbiased Protein Analysis. *Molecular & Cellular Proteomics* **2013**, *12* (10), 14. Dupree, E. J.; Jayathirtha, M.; Yorkey, H.; Mihasan, M.; Petre, B. A.; Darie, C. C. A Critical Review of Bottom-Up Proteomics: The Good, the Bad, and the Future of This Field. *Proteomes* **2020**, *8* (3). DOI: 10.3390/proteomes8030014. Domon, B.; Aebersold, R. Challenges and Opportunities in Proteomics Data Analysis. *Molecular & Cellular Proteomics* **2006**, *5* (10), 6. DOI: 10.1074/mcp.R600012-MCP200

(15) Keller, B. O.; Sui, J.; Young, A. B.; Whittal, R. M. Interferences and contaminants encountered in modern mass spectrometry. *Analytica Chimica Acta* **2008**, *627* (1), 11. Feist, P.; Hummon, A. B. Proteomic Challenges: Sample Preparation Techniques for Microgram-Quantity Protein Analysis from Biological Samples. *International Journal of Molecular Sciences* **2015**, *16* (2), 27. DOI: 10.3390/ijms16023537. Tubaon, R. M.; Haddad, P. R.; Quirino, J. P. Sample Clean-up Strategies for ESI Mass Spectrometry Applications in Bottom-up Proteomics: Trends from 2012 to 2016. *Proteomics*. **2017**, 17 (20), article e1700011. DOI: 10.1002/pmic.201700011

(16) Guo, T.; Steen, J. A.; Mann, M. Mass-spectrometry-based proteomics: from single cells to clinical applications. *Nature* **2025**, *638*, 11.

(17) Zubarev, R. A. The challenge of the proteome dynamic range and its implications for in-depth proteomics. *Proteomics* **2013**, 13 (5), 723–726. DOI: 10.1002/pmic.201200451. Corthals, G. L.; Wasinger, V. C.; Hochstrasser, D. F.; Sanchez, J.-C. The dynamic range of protein expression: A challenge for proteomic research. *Electrophoresis* **2000**, *21* (6), 12. Hortin, G. L.; Sviridov, D. The dynamic range problem in the analysis of the plasma proteome. *Journal of Proteomics* **2010**, *73* (3), 8.

(18) Kelly, R. T. Single-cell Proteomics: Progress and Prospects. *Molecular & Cellular Proteomics* **2020**, *19* (11), 10.

(19) Eshghi, A.; Xie, X.; Hardie, D.; Chen, M. X.; Izaguirre, F.; Newman, R.; Zhu, Y.; Kelly, R. T.; Goodlett*, D. R. Sample Preparation Methods for Targeted Single-Cell Proteomics. *Journal of Proteome Research* **2023**, *22* (6), 14. Gatto, L.; Aebersold, R.; Cox, J.; Demichev, V.; Derks, J.; Emmott, E.; Franks, A. M.; Ivanov, A. R.; Kelly, R. T.; Khoury, L.; *et al*. Initial recommendations for performing, benchmarking and reporting single-cell proteomics experiments. *Nature Methods* **2023**, *20*, 12. Johnston, S. M.; Webber, K. G. I.; Xie, X.; Truong, T.; Nydegger, A.; Lin, H.-J. L.; Nwosu, A.; Zhu, Y.; Kelly, R. T. Rapid, One-Step Sample Processing for Label-Free Single-Cell Proteomics. *Journal of the American Society for Mass Spectrometry* **2023**, *34* (8), 7. Ahmad, R.; Budnik, B. A review of the current state of single-cell proteomics and future perspective. *Analytical and Bioanalytical Chemistry* **2023**, *415*, 11.

(20) Dapic, I.; Baljeu-Neuman, L.; Uwugiaren, N.; Kers, J.; Goodlett, D. R.; Corthals, G. L. Proteome analysis of tissues by mass spectrometry. *Mass Spectrometry Reviews* **2019**, *38* (4-5), 39. Espina, V.; Mueller, C.; Edmiston, K.; Sciro, M.; Petricoin, E. F.; Liotta, L. A. Tissue is alive: New technologies are needed to address the problems of protein biomarker pre-analytical variability. *Proteomics Clinical Applications* **2009**, *3* (8), 9. Holfeld, A.; Valdés, A.; Malmström, P.-U.; Segersten, U.; Lind, S. B. Parallel Proteomic Workflow for Mass Spectrometric Analysis of Tissue Samples Preserved by Different Methods. *Analytical Chemistry* **2018**, *90* (9), 9.

(21) Becker, K.-F. Using tissue samples for proteomic studies—Critical considerations. *Proteomics Clinical Applications* **2015**, *9* (3-4), 11.

(22) Schweizer, L.; Coscia, F.; Muller, J.; Doll, S.; Wierer, M.; Mann, M. AFA-sonication Followed by Modified Protein Aggregation Capture (APAC) Enables Direct, Reproducible and Non-toxic Sample Preparation of FFPE Tissue for Mass Spectrometrybased Proteomics. *Covaris Application Note* **2020**, *M020141*, 6.

(23) Adachi, Y.; Ishii, M.; Noguchi, R.; Hiraoka, N.; Yokoo, H.; Yoshimatsu, Y.; Ohtsuki, S.; Kondo, T. Time-Enduring Proteomic Fidelity in Over 30-Year-Old FFPE Tissues: Distinct Proteomic Signatures of Hepatocellular Carcinoma and Adjacent Non-Tumor Liver Tissue. *Proteomics* **2026**, *26* (1), 11.

(24) Mitsa, G.; Guo, Q.; Goncalves, C.; Preston, S. E. J.; Lacasse, V.; Aguilar-Mahecha, A.; Benlimame, N.; Basik, M.; Spatz, A.; Borchers, C. H. A Non-Hazardous Deparaffinization Protocol Enables Quantitative Proteomics of Core Needle Biopsy-Sized Formalin-Fixed and Paraffin-Embedded (FFPE) Tissue Specimens. *International Journal of Molecular Sciences* **2022**, *23*. DOI: 10.3390/ijms23084443

(25) Gegner, H. M.; Naake, T.; Aljakouch, K.; Dugourd, A.; Kliewer, G.; Müller, T.; Schilling, D.; Schneider, M. A.; Kunze-Rohrbach, N.; Grünewald, T. G. P.; *et al*. A single-sample workflow for joint metabolomic and proteomic analysis of clinical specimens. *Clinical Proteomics* **2024** Jul 5;21(1):49. DOI: 10.1186/s12014-024-09501-9

(26) Chandramouli, K.; Qian, P.-Y. Proteomics: Challenges, Techniques and Possibilities to OvercomeBiological SampleComplexity. *Human Genomics & Proteomics* **2009**. DOI: 10.4061/2009/239204

(27) Xiong, Y.; Tan, L.; Chan, W.-k.; Zhu, D.; Zhang, H.; Yin, E. S.; Donepudi, S. R.; Ding, J.; Wei, B.; Tran, B.; *et al*. High-throughput multi-organ proteomics workflow for drug efficacy and toxicity analysis. *Nature Communications* **2026**. DOI: 10.1038/s41467-026-69471-4

(28) Woodland, B.; Farrell, L. A.; Brockbals, L.; Rezcallah, M.; Brennan, A.; Sunnucks, E. J.; Gould, S. T.; Stanczak, A. M.; O'Rourke, M. B.; Padula, M. P. Sample Preparation for Multi-Omics Analysis: Considerations and Guidance for Identifying the Ideal Workflow. *Proteomics* **2025**, 25 (21-22), 76–101.

(29) Alexovic, M.; Sabo, J.; Longuespee, R. Automation of single-cell proteomic sample preparation. *Proteomics* **2021**, *21* (23-24), 2100198. King, C. D.; Kapp, K. L.; Arul, A. B.; Choi, M. J.; Robinson, R. A. Advancements in automation for plasma proteomics sample preparation. *Molecular Omics* **2022**, *18* (9), 12. DOI: 10.1039/D2MO00122E

(30) More, D.; Khan, N.; Tekade, R. K.; Sengupta, P. An Update on Current Trend in Sample Preparation Automation in Bioanalysis: strategies, Challenges and Future Direction. *Critical Reviews in Analytical Chemistry* **2024**, *55* (7), 25. DOI: 10.1080/10408347.2024.2362707. Fu, Q.; Murray, C. I.; Karpov, O. A.; Eyk, J. E. V. Automated proteomic sample preparation: The key component for high throughput and quantitative mass spectrometry analysis. Mass Spectrom Rev. 2023 Mar;42(2):873-886. DOI: 10.1002/mas.21750. Epub 2021 Nov 16.

(31) Fatou, M.; Kornobis, E.; Douché, T.; Druart, K.; Puchot, N.; Matondo, M.; Monot, M.; Bourgouin, C. High-quality proteins and RNAs extracted from exact same samples for proteomics and RNA-Seq analyses. *bioRxiv* **2026**, *17*. DOI: 10.64898/2026.01.16.699903

(32) Luo, Y.; Zhao, C.; Chen, F. Multiomics Research: Principles and Challenges in Integrated Analysis. *BioDesign Research* **2024**, *6*. Zhao, P.; Feng, Y.; Wu, J.; Zhu, J.; Yang, J.; Ma, X.; Ouyang, Z.; Zhang, X.; Zhang, W.; Wang, W. Efficient Sample Preparation System for Multi-Omics Analysis via Single Cell Mass Spectrometry. *Analytical Chemistry* **2023**, *95* (18), 8.

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