1) Introduction and method

- Proteomics analyses typically begin with sample lysis and protein extraction. This single step is responsible for the vast majority of variability in proteomics data (Fig. 1).
- Quality sample preparation is critical to the reliability of the results from any proteomics experiment. Two sources of variability are relevant to sample preparation:
  - Variability in sample extraction arises predominantly from two sources: (1) the sample matrix (e.g., tissue vs. cell culture) and (2) the method of sample extraction. Sample extraction methods range from simple homogenization to highly sophisticated techniques like AFA, which enables biological samples to be processed in a non-contact, non-invasive manner. AFA is a novel technology that combines high-frequency acoustic energy with a localized high-intensity acoustic focus to disrupt tissues and cells without mechanical force (Fig. 2). The AFA process enables biological samples to be processed in a non-contact, non-invasive manner. AFA is a novel technology that combines high-frequency acoustic energy with a localized high-intensity acoustic focus to disrupt tissues and cells without mechanical force (Fig. 2). The AFA process enables biological samples to be processed in a non-contact, non-invasive manner. AFA is a novel technology that combines high-frequency acoustic energy with a localized high-intensity acoustic focus to disrupt tissues and cells without mechanical force (Fig. 2).
- Our combined workflow is universal: the solubilization power of 5% SDS with the S-Trap™ with extremely high surface area to volume ratios. This combination of high-frequency acoustic energy and a localized high-intensity acoustic focus enables precise, efficient disruption of cells and tissues (Fig. 3). The AFA process enables biological samples to be processed in a non-contact, non-invasive manner. AFA is a novel technology that combines high-frequency acoustic energy with a localized high-intensity acoustic focus to disrupt tissues and cells without mechanical force (Fig. 2).

2) Covaris Adaptive Focused Acoustics (AFA) technology

The Adaptive Focused Acoustics (AFA) technology is a unique process to deliver controlled, non-contact sonic energy to isolated, biological samples while maintaining cell viability and fully processing the sample. The AFA process enables biological samples to be processed in a non-contact, non-invasive manner. AFA is a novel technology that combines high-frequency acoustic energy with a localized high-intensity acoustic focus to disrupt tissues and cells without mechanical force (Fig. 2).

3) ProtiFi™ S-Trap™ sample processing technology

The Suspension-Trapping (S) or S-Traps™ method is a technique to extract, solubilize, and handle all proteins in high concentrations of sodium dodecyl sulfate (SDS, ≤5%) prior to their capture, concentration, and cleaning and digestion. Proteins are captured in the submicron pores of the S-Traps™ with extremely high surface area to volume ratios. This allows them to be rapidly cleaned of SDS and contaminants including all detergents, urea, salts, glycerol, PEG, Laemmli loading buffer, and salts, etc. Proteases are then introduced into the pores where they physically congregate in high concentrations enhance protease-substrate interaction and further digestion. Rapid (<1 h) digestion follows. Capture of protein within the trap (SDS depletion, wash, and protease addition) requires just minutes. After a one-hour digest at 43 °C, peptides are eluted and ready for downstream processing.

4) Materials and methods

Mouse tissues were collected in PBS-approved facilities from male 10-week-old black mice after anesthesia, sacrifice, and sacrifice by cardiac puncture. Pancreas, brain, liver, kidney, bone, muscle, skin and bone were harvested in that order and immediately frozen in liquid nitrogen and stored at -80 °C. Prior to analysis, these samples were subject to mechanical homogenization and sonic dissection. Tissues were sonicated on ice for 90 – 120 seconds in 1% SDS, 1% Triton X-100, 50 mM KCl, 10 mM Tris-HCl, pH 7.4 with a 200 W Covaris sonicator (Covaris, Inc., Woburn, MA). After a one-hour digest at 47 °C, peptides are eluted and ready for downstream processing. Percent of total variability. Data from reference 1.

5) Extraction conditions effect on protein yield

We compared different extraction buffers using AFA and bead beating on both fresh frozen tissues (Fig. 6) and FFPE blocks (Fig. 7). 5% SDS applied with AFA consistently provided the highest protein yield for FFPE samples. Defragmentation was not required as the combined SDSAFA/S-Trap system fully dissolves FFPE samples. S-Trap sample processing then removed all paraffin without alteration. Single 10 μl samples, all extractions in triplicate.

6) Extraction conditions effect on protein identification

While aqueous buffers are often preferred in proteomics due to the traditional difficulty of detergent removal, detergents (5% SDS or TP) were absolutely necessary to resolve membrane proteins; they also increased digestion rates of membrane proteins. Membrane protein ID from aqueous buffers was >98% from both brain and pancreas. While biochemically unsurprising, this significant result was not anticipated, and proteins that were observed proteins calls into question a large number of workflows and what may have been unable to observe. Note that the yield with TP, a common aqueous detergent buffer for 2D gel work, was at least 5x lower than with 5% SDS (Figs. 6).

7) Conclusions

- The combination of 5% SDS, AFA, and S-Traps is a universal protein extraction, handling and digestion solution which makes sample-specific optimization obsolete.
- 5% SDS with AFA extraction and S-Traps proteomics preparation reproducibly samples the entire proteome and consistently identifies the highest number of proteins, even when protein loading is matched before processing to peptides.
- The combined system is fully suited to high-throughput automation with 96-well plates.
- When applied to FFPE samples, the SDSAFA/S-Trap solution is a one tube, one column solution which eliminates the need for slow and toxic deparfimization steps. It significantly increases efficiency, throughput, yield and thus protein ID rates.
- We anticipate the combined workflow of Covaris AFA and ProtiFi S-Traps sample processing will enable reproducibility in bottom-up proteomics and thus support the translation of proteomics into clinical applications.

8) References

2. Laughum J.R., and Dunn, G. Covaris Inc. 2016 Method and apparatus for co-aggregation of multiple targets using acoustic processing. U.S. Patent 9,486,756. See also https://www.covaris.com/resources/solutions/ultrasonic/. Zougman A., Gobena, H., Zougman A., Gobena, H., Zougman A., and Laugham J.R. 2013. The Suspension-Trapping (S) or S-Traps™ method is a technique to extract, solubilize, and handle all proteins in high concentrations of sodium dodecyl sulfate (SDS, ≤5%) prior to their capture, concentration, and cleaning and digestion. Proteins are captured in the submicron pores of the S-Traps™ with extremely high surface area to volume ratios. This allows them to be rapidly cleaned of SDS and contaminants including all detergents, urea, salts, glycerol, PEG, Laemmli loading buffer, and salts, etc. Proteases are then introduced into the pores where they physically congregate in high concentrations enhance protease-substrate interaction and further digestion. Rapid (<1 h) digestion follows. Capture of protein within the trap (SDS depletion, wash, and protease addition) requires just minutes. After a one-hour digest at 43 °C, peptides are eluted and ready for downstream processing.

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*ProtiFi and Covaris technologies are patented and patent pending.