Cost-Effective Tip Solution for High Quality Proteomics Data; S-Tip

Alia Sagatova, PhD, Tyler Scherzi, PhD, Sandra Wilson, PhD, John P Wilson, PhD

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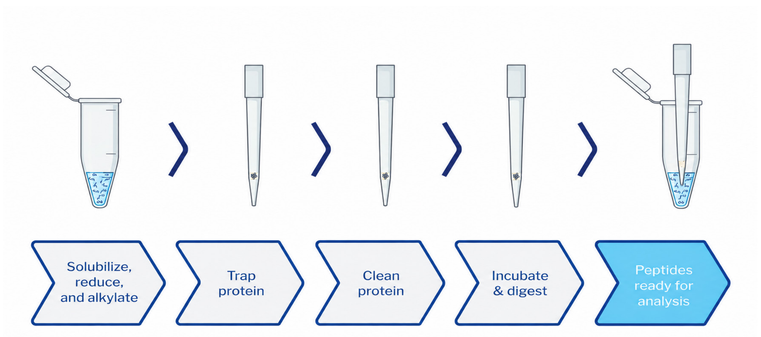
INTRODUCTION

Sample preparation for LC-MS is a critical step in proteomic mass spectrometry. This step can be time-consuming and costly. Building off our industry standard sample preparation products such as S-Trap™, S-Trap™ Turbo™ and Si-Trap™, we introduce the S-Tip, sample processing in a pipette tip that offers a solution to limited sample input as well as an immediate transition to automated platforms. The S-Tip provides a streamlined workflow reducing sample load, cost and processing time without compromising data quality.



Schematic 1

METHODS

A wide variety of polymers bearing many combinations of different functional groups were synthesized and evaluated for protein recovery and ease of pipetting (backpressure) **(Fig. 1a)**. Importantly, the S-Tip was used as a genuine tip, by pipetting up and down, not as an SPE device. Protein binding and recovery was determined using fluorescein isothiocyanate (FITC) labeled protein samples. Typically, from 5-50 µg of FITC labelled rabbit brain lysate was applied. The protein was solubilized in a 5% SDS and 50 mM TEAB and extraction in buffer A, followed by loading on to the tip. The tips were washed in buffer A, and whole protein was eluted buffer B. Fluorescence intensity was read by a Tecan Spark plate reader.

Select material formulations were further studied with bacterial and tissue lysates. Samples were solubilized in 5% SDS and 50 mM TEAB, reduced with TCEP, alkylated with chloroacetamide. In tip digestion was carried out with trypsin using 1:10 ratio of protease to protein **(Schematic 1)**. The S-Trap micro protocol was carried out in parallel using the accompanying protocol and same protein input as the tip.

Peptides were separated using 120-minute gradient on a MyMap column 25 cm, ID 75 µm packed with Reprosil Saphir 100Å 1.5 µm C18 column (Dr Maisch) and analyzed by Q Exactive HF mass spectrometer with a NanoFlex source (Thermo Fisher Scientific). Data was analyzed using DIA-NN (1).

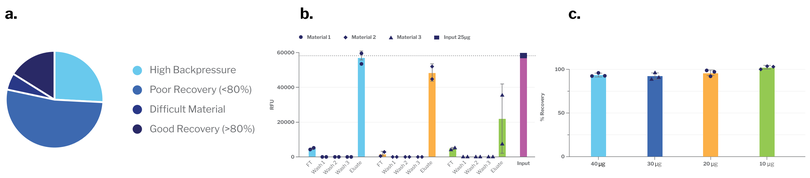


Fig. 1. S-Tip Development

a) Depending on formulation and synthesis, pore architecture and size, and surface functionalizations, polymeric formulations exhibited highly varied performance of back pressure and yield. **b)** Exemplary formulation results. Relative fluorescence units (RFU) measure protein loss and recovery from the S-Tip protocol. Fluorescence values for the flow-through (FT), wash fractions, and elution are shown. The final grey bar represents the fluorescence signal corresponding to the initial 25 µg protein input. **c)** Percent recovery of FITC-labeled protein of the optimized S-Tip. A range of protein input amounts, from 40 µg to 10 µg, was evaluated to assess recovery performance across decreasing sample amounts.

RESULTS

Tips showed excellent yield and minimal backpressure; solutions could be easily pipetted using a normal hand pipettor. The optimized S-Tip material exhibited high recovery (Fig. 1b). The S-Tip yields >90% recovery of protein input 10 µg – 40 µg **(Fig. 1c)**.

When compared to S-Trap™, the S-Tip elution yields similar protein and peptide identifications **(Figs 2 and 3)**. When using both tissue and bacterial lysate.

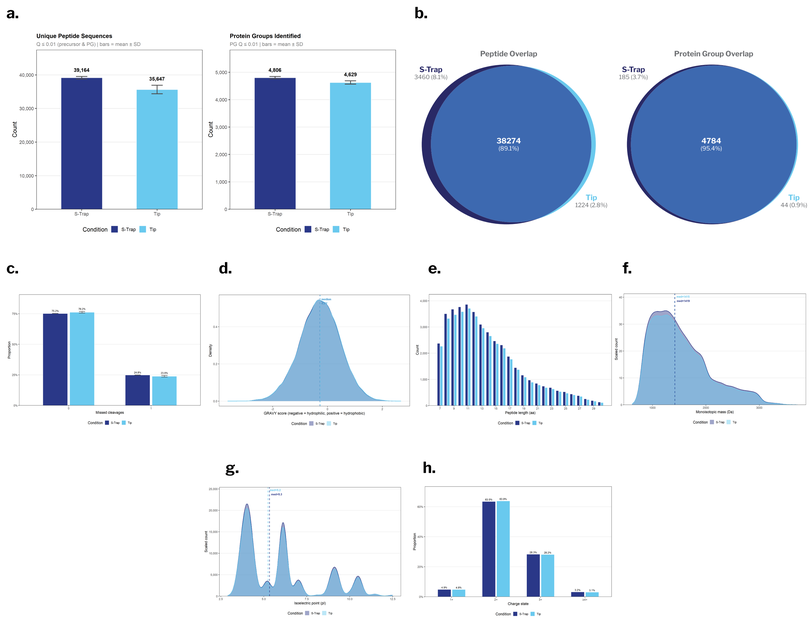


Fig. 2. S-Tip and S-Trap performance on tissue lysates

a) Bar graphs showing the number of unique peptide sequences (left) and protein groups (right) identified per condition. 30 µg rabbit brain lysate was prepared using S-Trap (dark blue) or S-Tip (light blue). Error bars represent SEM (n = 3). 1 µg of peptides was injected in triplicate for LC-MS analysis. **b)** Venn diagrams showing the overlap in unique peptide sequences (left) and protein groups (right). **c)** Frequency of missed cleavages for each condition. **d)** Distribution of GRAVY (Grand Average of Hydropathicity) scores for peptide sequences identified calculated using the Kyte-Doolittle hydropathy scale. Negative scores indicate hydrophilic peptides; positive scores indicate hydrophobic peptides. Dashed vertical lines indicate the median GRAVY score per condition. **e)** Bar chart showing the number of unique peptide sequences identified per peptide length (in amino acids) for S-Trap and S-Tip sample preparation methods. **f)** Density plot showing the distribution of monoisotopic peptide masses for unique peptide sequences identified by S-Trap and S-Tip. **g)** Density plot showing the distribution of theoretical isoelectric points for unique peptide sequences identified by S-Trap and S-Tip. pI values were calculated using the bisection method with standard amino acid pKa values. **h)** Bar chart showing the proportion of unique precursors assigned to each charge state (1+ to 4+) for S-Trap and S-Tip sample preparation methods.

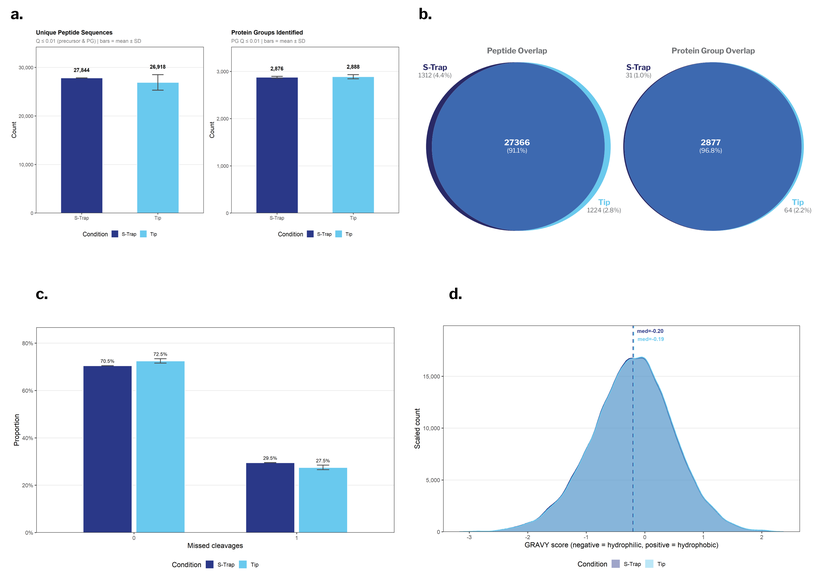


Fig. 3. S-Tip and S-Trap performance on *E. coli* lysates

a) Bar graphs showing the mean number of unique peptide sequences (left) and protein groups (right) identified per condition. 30 µg bacteria lysate was prepared using S-Trap (dark blue) or S-Tip (light blue). Error bars represent SD (n = 2). 1 µg of peptides was injected in triplicate for LC-MS analysis. **b)** Venn diagrams showing the overlap in unique peptide sequences (left) and protein groups (right). **c)** Frequency of missed cleavages for each condition. **d)** Distribution of GRAVY (Grand Average of Hydropathicity) scores for peptide sequences identified calculated using the Kyte-Doolittle hydropathy scale. Negative scores indicate hydrophilic peptides; positive scores indicate hydrophobic peptides. Dashed vertical lines indicate the median GRAVY score per condition.

CONCLUSIONS

We developed a new novel sample preparation system, the S-Tip, which allows standard pipettors to efficiently recover proteins and peptides. The S-Tip was developed to have excellent recovery, low cost and very high ease of use. The S-Tip and S-Trap yield comparable numbers of protein and peptide identifications with >95% overlap in identified protein groups. Peptides generated using both workflows exhibited statistically identical properties in terms of missed cleavages, hydrophobicity (GRAVY scores), peptide length and mass, and isoelectric point. Collectively, these findings demonstrate the potential of the S-Tip to be a cost-effective approach to proteomic sample preparation.

REFERENCES

1. Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. Nat Methods. 2020 Jan;17(1):41-44. doi: 10.1038/s41592-019-0638-x. Epub 2019 Nov 25. PMID: 31768060; PMCID: PMC6949130.