


COMPARISON OF CARTRIDGE AND BEAD-BASED SAMPLE PREPARATION STRATEGIES FOR BOTTOM-UP PROTEOME ANALYSIS OF DETERGENT-CONTAINING SAMPLES

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INTRODUCTION

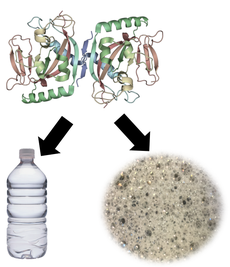
Detergents including sodium dodecyl sulfate (SDS) are becoming increasingly accepted in proteomics workflows, owing to a growing list of semi-automated approaches, designed to effectively purify and digest protein mixtures ahead of bottom up LC-MS/MS analysis. Since the advent of filter-aided sample preparation (FASP) as a cartridge-based format which simplifies SDS removal and protein digestion, numerous other strategies have been reported.[1] Among the cartridge and bead-based technologies are Suspension Trapping (S-Trap), in-StageTip (iST), Single-Pot, Solid-Phase-Enhanced Sample Preparation (SP3), and the ProTrap XG, all of whom report successful acquisition of peptide lists from SDS-containing samples.[1,2,3,4,5] Here, we directly evaluate the performance of these sample preparation strategies looking at four specific figures of merit: the protein/ peptide recovery, sample purity as judged by residual level of SDS, protein digestion efficiency, and sample throughput/ process time.

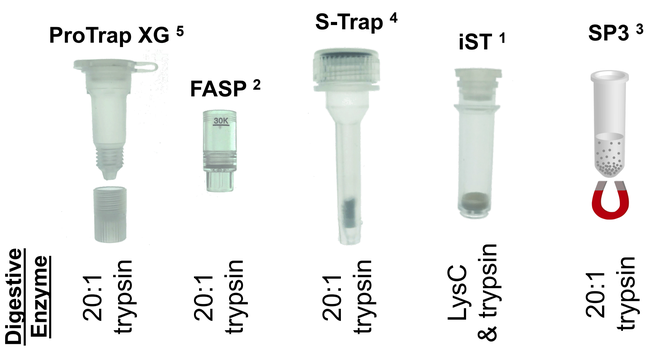
SUMMARY

PRODUCT	RECOVERY (% by mass)	PURITY (% SDS depleted)	TOP-DOWN COMPATIBLE?	DIGESTION EFFICIENCY	PREP TIME (approximate)
PROTRAP XG	* 99.9 ± 1	* > 99.9 %	✓	82 %	* 10 min + digestion
FASP	79 ± 2	99.8 ± 0.1%	✗	82 %	2 h + digestion
S-TRAP	82 ± 2	* > 99.9 %	✗	73 %	2 h
iST	71 ± 10	INCOMPATIBLE	✗	* 91 %	1.5 h (+ SDS depletion)
SP3	91 ± 6	99.9 ± 0.03 %	✗	68 %	30 min + digestion

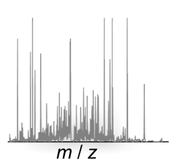
METHODS

- Yeast protein was extracted into water. Remaining fraction was extracted into 0.5% SDS_{aq} to a final concentration of 1 g/L.



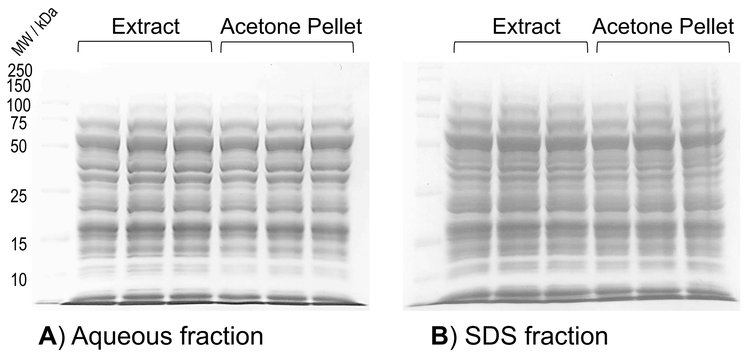


- Yeast lysates were cleaned and digested according to the protocols corresponding to each sample preparation product.
- An LC-UV assay at λ = 214 nm was used to determine peptide recovery by mass.
- Based on the determined recoveries, collected peptides were dried and re-solubilized in equal concentration, and equal peptide masses were subject to bottom-up MS analysis.
- A methylene blue active substances (MBAS) assay was used to quantify residual SDS to determine the purity achieved from each sample prep product.

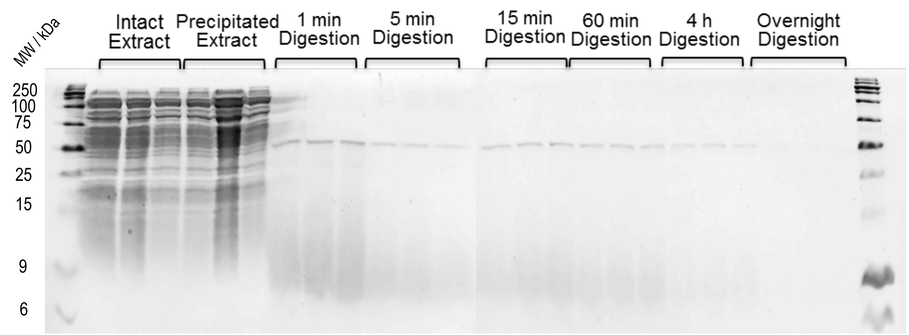


RESULTS

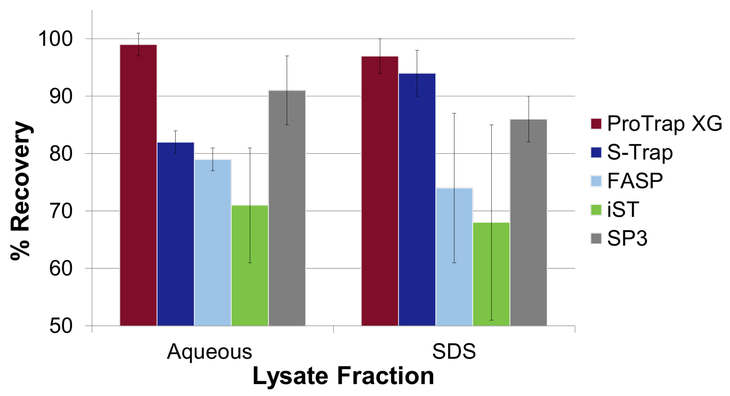
SDS PAGE Analysis of Initial Extracts and Solution Digest Control



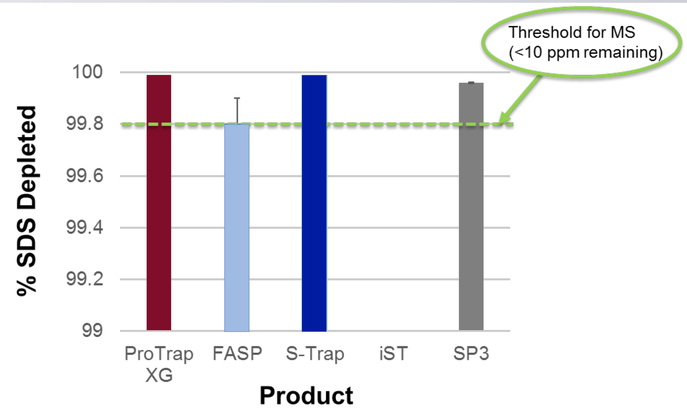
A) Aqueous fraction **B**) SDS fraction



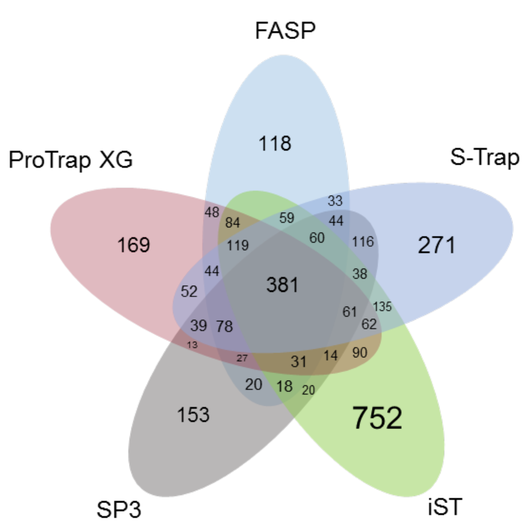
RECOVERY



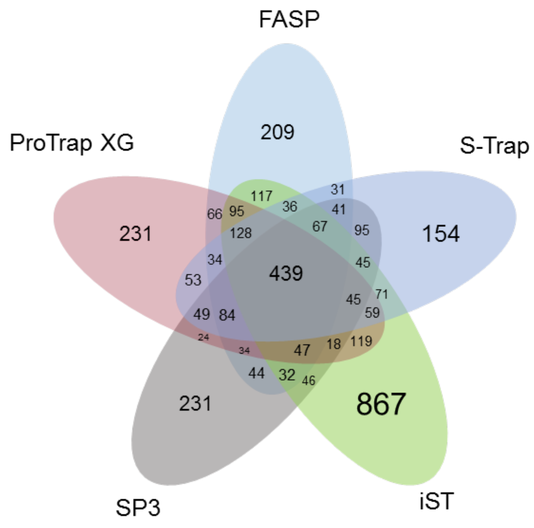
PURITY



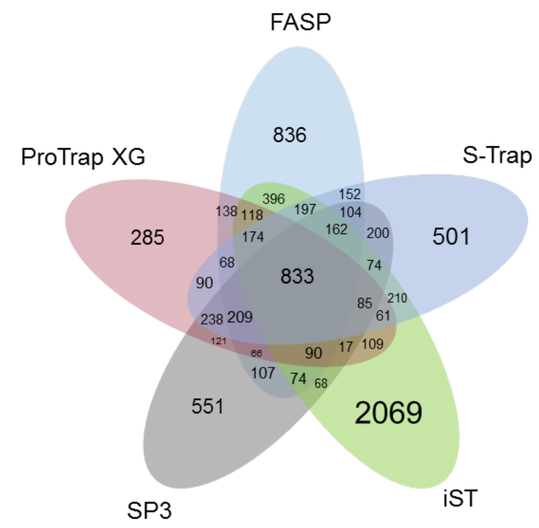
BOTTOM–UP MS ANALYSIS



A) Venn diagram of peptides identified in aqueous fraction

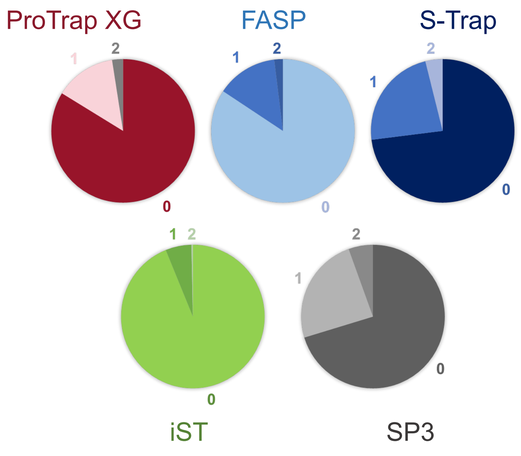


B) Venn diagram of peptides identified in SDS fraction

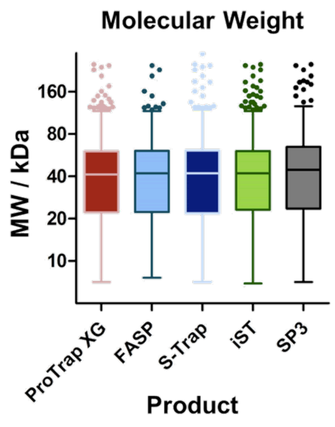


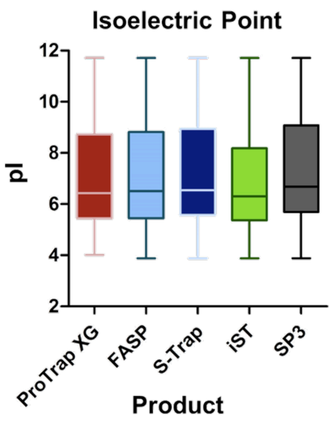
C) Venn diagram of peptides identified from High-res analysis of SDS fraction

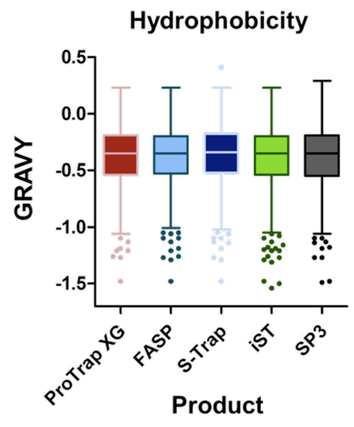
Frequency of Missed Cleavages



Proteome Coverage







CONCLUSIONS

- Complete digestion is efficiently achieved in solution, as well as in the cartridge-based strategies. Slightly less efficient digestion was observed from the bead-based method, SP3.
- The ProTrap XG gave optimal results in the most categories: recovery, purity, speed, top-down compatibility.
- The In-Stage Tip protocol resulted in the most peptide identifications by MS, which is attributed to its superior digestion efficiency. Future studies will investigate the efficiency of solution digestion with LysC and trypsin for comparison.
- All products gave homogeneous proteome coverage, without biasing recovery on molecular weight, pI, or hydrophobicity.

ACKNOWLEDGEMENTS

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REFERENCES

1. Mann et al. (2007). *Nature Protocols, 2*, 1896-1906.
2. Mann et al. (2009). *Nature Methods, 6*, 359-62.
3. Hughes et al. (2014). *Molecular Systems Biology, 10.*
4. Yu et al. (2018). *Journal of Proteome Research, 17*, 2917-2924.
5. Doucette et al. (2015). *Journal of Proteomics, 118*, 140-150.