Kinetics of protein precipitation: optimizing recovery, purity, and throughput using the ProTrap XG

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OBJECTIVES

- Optimize the efficiency of protein precipitation
- Determine the versatility of precipitation for dilute samples
- Quantify SDS depletion; determine whether MS threshold of <10 ppm is achieved
- Determine digestion efficiency within the ProTrap XG
- Analyze proteome coverage, determining whether precipitation favors proteins of high MW, or particular pI or GRAVY score

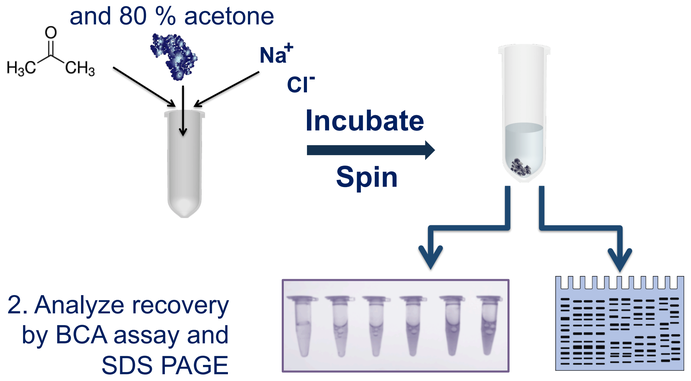
INTRODUCTION

Efficient sample preparation is key to any proteomics workflow, with three priorities at the forefront: protein recovery, purity, and sample throughput. Acetone precipitation is a common method of purifying proteins from a complex sample prior to LC-MS/MS analysis, but is sometimes set aside due to seemingly variable yields.[1] Our group previously established that the addition of 1-30 mM salt with 80% *v/v* acetone facilitates recoveries >95%.[2] In order to maximize recovery, purity and sample throughput, we investigated the kinetics of protein precipitation with respect to structural properties of proteins, including molecular weight, hydrophobicity, and charge.

METHODS

Optimize precipitation protocol

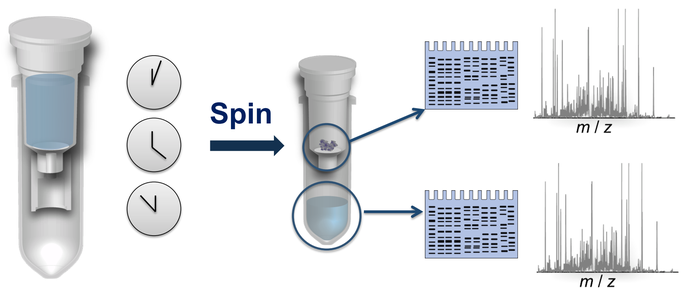
1. Precipitate protein with 0.1 - 100 mM NaCl and 80 % acetone



2. Analyze recovery by BCA assay and SDS PAGE

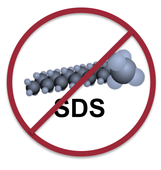
Precipitation in the ProTrap XG

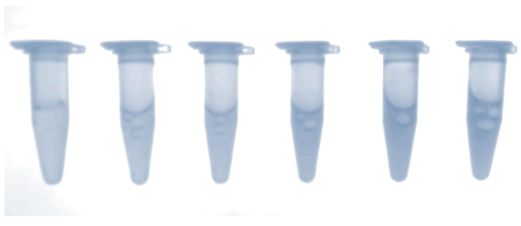
1. Precipitate yeast proteome with 20 mM NaCl and 4 volumes acetone



2. Analyze pellet and supernatant by bottom-up LC-MS/MS

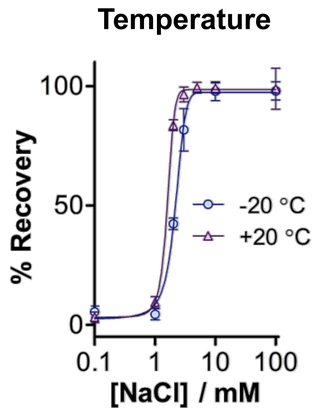
3. Determine purity by quantifying residual SDS by MBAS assay

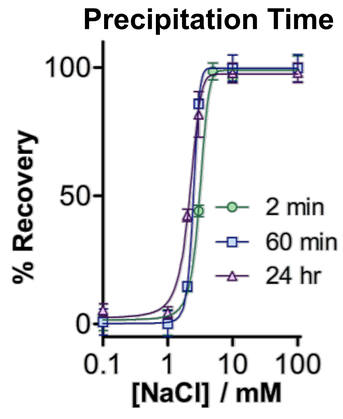


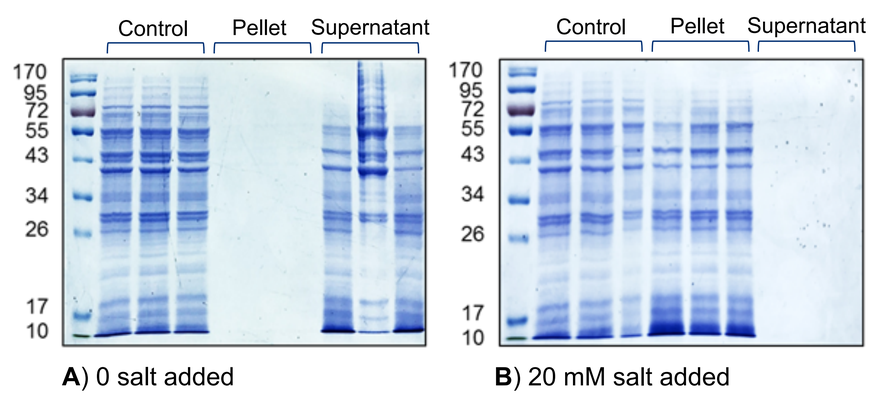


RESULTS

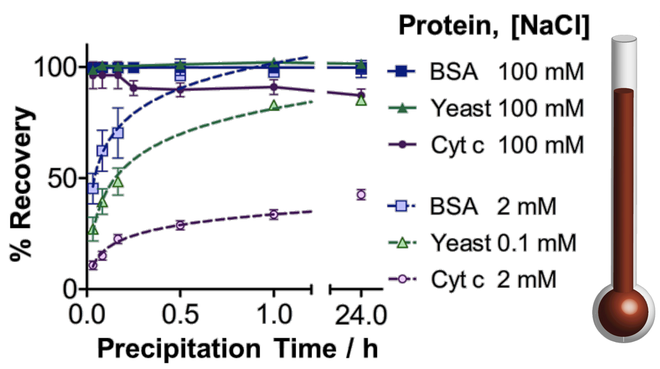
High salt is critical for high recovery in 2 min



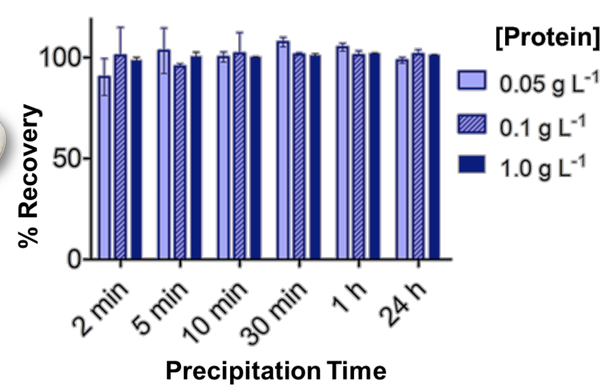




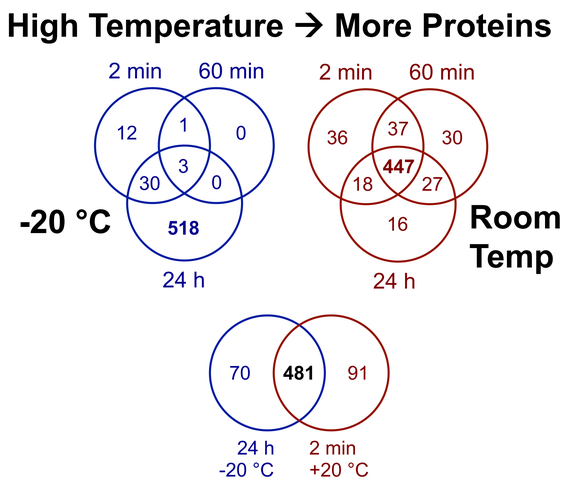
High salt improves precipitation kinetics

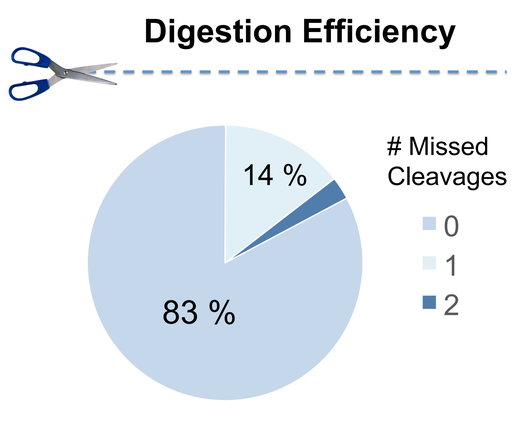


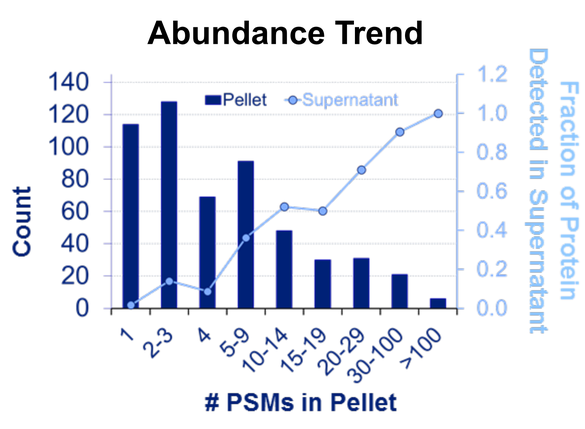
Dilute samples precipitate in 2-5 min

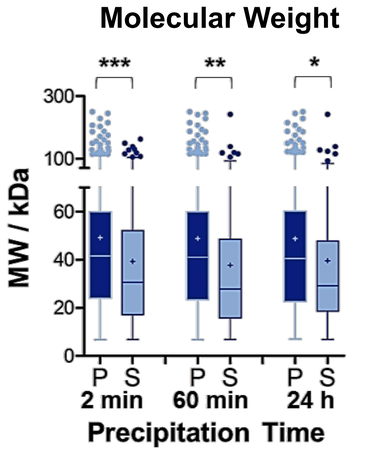


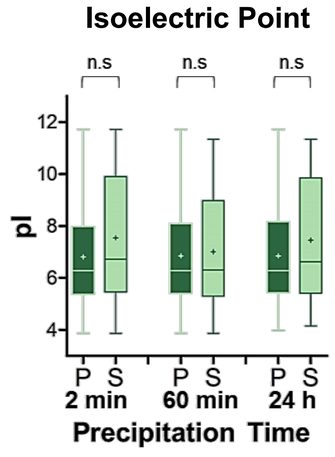
Bottom-up analysis of pellets and supernatants

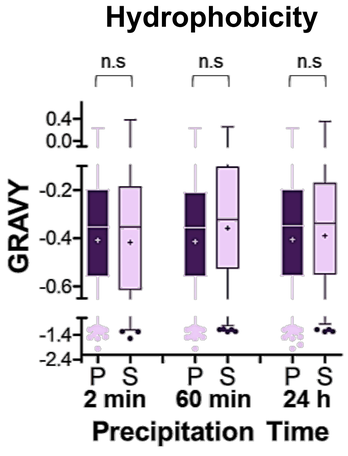












Sample Prep Efficiency of ProTrap XG

Recovery	99 ± 1 %
Purity (SDS Depleted)	> 99.9 %
Digestion Efficiency (0 Missed Cleavages)	82 %
Processing Time	15 min + digestion

CONCLUSIONS

- With >10 mM NaCl and 80% acetone, standard proteins and complex proteome mixtures can be quantitatively precipitated in 2 minutes at room temperature
- Dilute samples can be precipitated with equal efficiency to more concentrated samples
- The presence of a protein in the supernatant strongly correlates to its abundance. Most proteins identified in the supernatant are identified in the pellet with much greater abundance.
- There is no structural bias in which proteins are present in the pellet, though the recovery of low molecular weight proteins increases over time

ACKNOWLEDGEMENTS

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Dalhousie's Chemistry Graduate Student Society

REFERENCES

1. Crowell, A., Wall, M., Doucette, A. (2013). Maximizing recovery of water-soluble proteins through acetone precipitation. *Analytica Chimica Acta, 796*, 48-54.

2. Crowell, A., Maclellan, D., Doucette, A. (2015). A two-stage spin cartridge for integrated protein precipitation, digestion and SDS removal in a comparative bottom-up proteomics workflow. *Journal of Proteomics, 118*, 140-150.