# Impact of Divalent Metal Ions on the Proteomics S-Trap ™ Filter Digestion Protocol

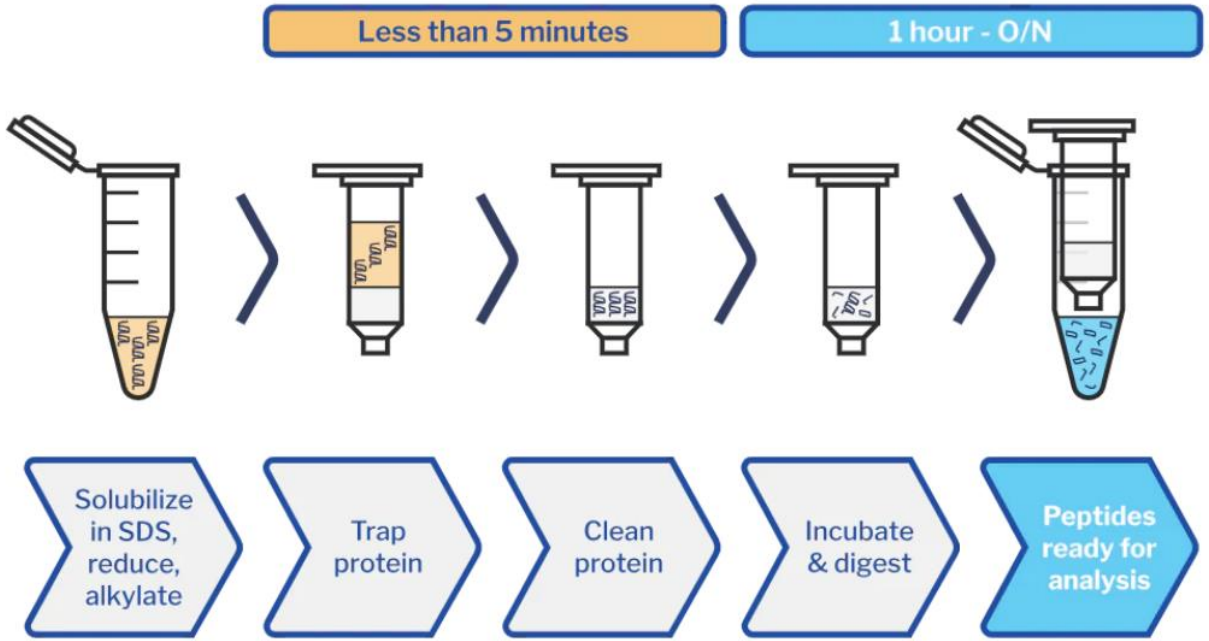
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# Background



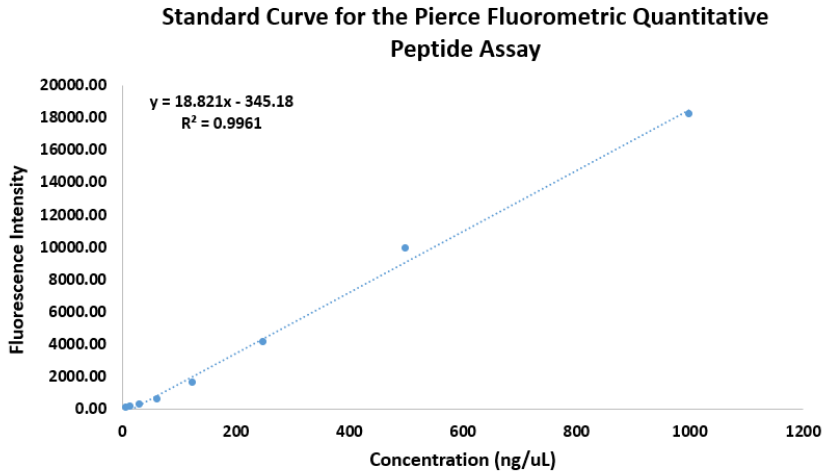


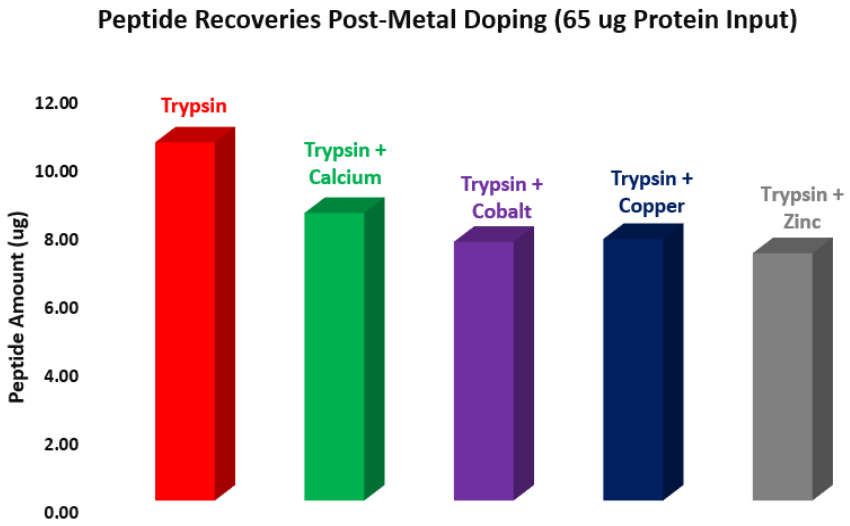

**Credit due to Protifi LLC**

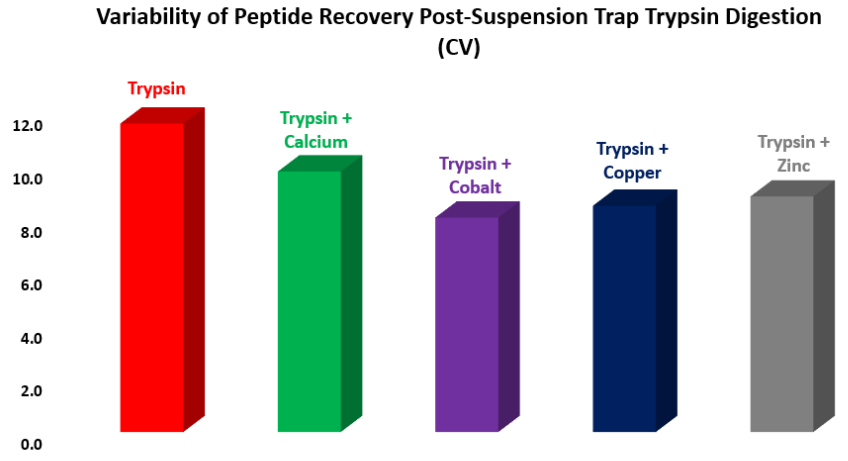
# Experimental Design

- Aliquoted 65 ug of Promega MS Compatible Intact Yeast Protein Extract™ per sample
- Spiked triplicates of each sample condition- blank trypsin, calcium, cobalt, copper, zinc
- Performed digestion on S-trap™ micro filters - added sub-stoichiometric metal ratios to trypsin- 1% catalyst loading
- Ran Pierce™ fluorometric peptide quant assay to assess peptide recoveries

# Results


Standard Curve for the Pierce Fluorometric Quantitative Peptide Assay
y = 18.821x - 345.18
R² = 0.9961
Fluorescence Intensity
20000.00
18000.00
16000.00
14000.00
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
0
200
400
600
800
1000
1200
Concentration (ng/uL)


Peptide Recoveries Post-Metal Doping (65 ug Protein Input)
Peptide Amount (ug)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Trypsin
Trypsin + Calcium
Trypsin + Cobalt
Trypsin + Copper
Trypsin + Zinc


Variability of Peptide Recovery Post-Suspension Trap Trypsin Digestion (CV)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Trypsin
Trypsin + Calcium
Trypsin + Cobalt
Trypsin + Copper
Trypsin + Zinc

# Conclusions and Discussion

- Peptide recoveries low overall but metal-doped samples were consistently slightly lower in all conditions- will increase catalyst loading
- Paramagnetic species quench fluorescence- maybe residual free metal ions?
- S-trap ™ micro variability- should run experiment on S-trap ™ plates

# Future Work

- Optimize assay conditions- protein input, enzyme-substrate ratio, metal stoichiometry, modified trypsin, etc.
- Mass spectrometry experiments to assess peptide and protein IDs
- Post-optimization, experiment with complex proteomes- human, mouse, etc.

# Acknowledgments

SUMS Staff

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ABRF Education Committee

