

PROJECT OVERVIEW

Purpose

- To compare different cleavable detergents used for protein extraction prior to LC/MS/MS proteome analysis.

Results

- Extracts of *E. coli* were prepared using different cleavable detergents, PPS Silent™ Surfactant and Rapigest™ SF.
- The PPS Silent™ Surfactant produces two water soluble, non-surfactant molecules when cleaved.
- Analysis of the PPS Silent™ Surfactant sample yielded 571 identified proteins while 428 proteins were identified from the Rapigest™-treated sample.
- The separate digestions yielded a total of 644 identified proteins.

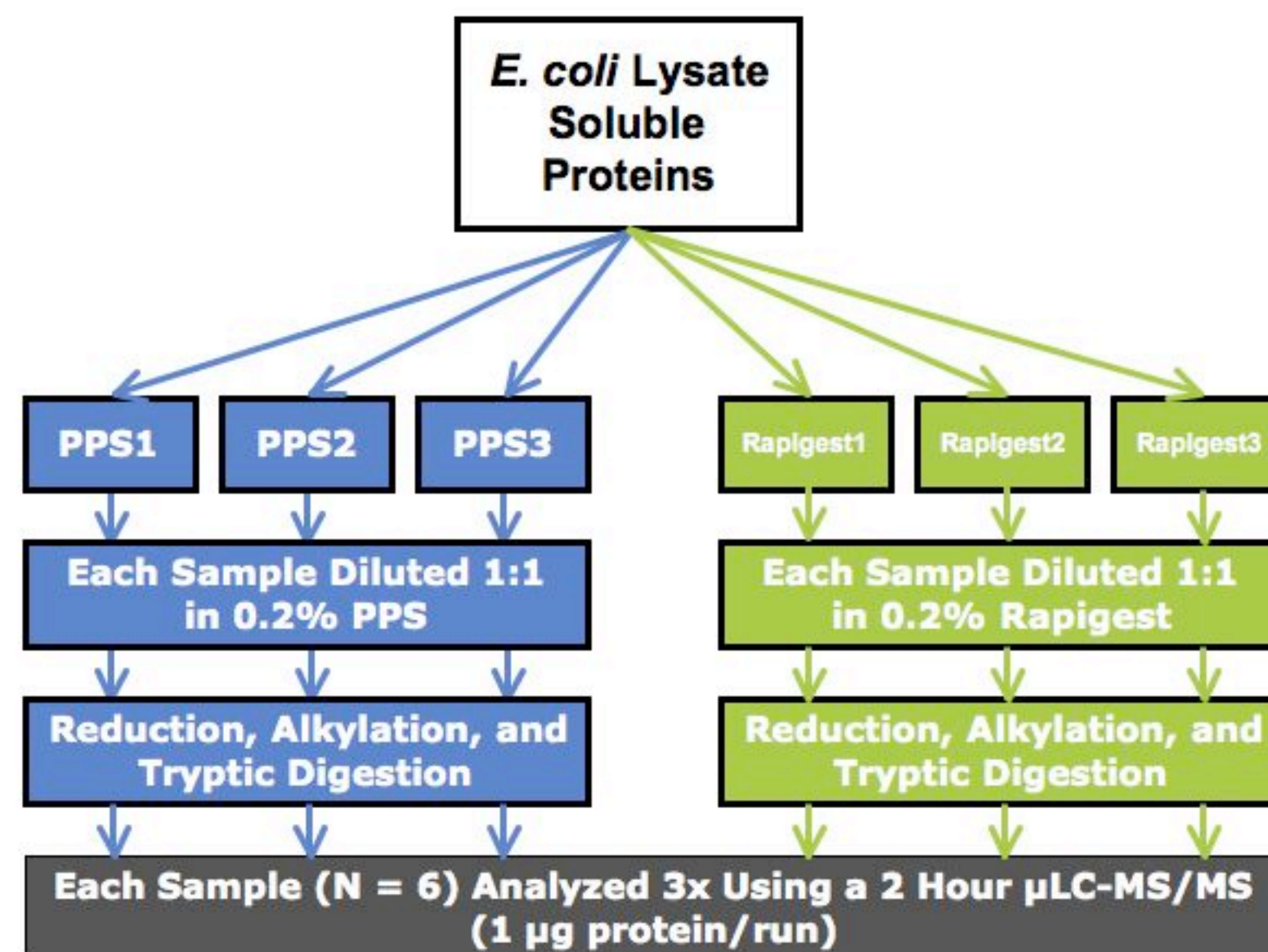
Conclusions

- PPS Silent™ Surfactant provides for the maximum numbers of identified proteins from *E. coli* cell extracts in a single experiment.
- Separate proteolytic digestions carried out using Rapigest™ and PPS Silent™ Surfactant produce two complementary sets of identified proteins.

INTRODUCTION

This study describes a new reagent used to prepare hydrophobic samples for analysis by mass spectrometry. PPS Silent™ Surfactant is a cleavable detergent that was developed to improve the sensitivity of MALDI analysis of hydrophobic proteins. Recently, application of this reagent has been extended to include analysis of complex protein mixtures using microcapillary LC/MS/MS. The advantages of using such a reagent will be highlighted and the reagent will be compared to a similar reagents, RapiGest™ SF (Waters Corp., Milford, MA).

SAMPLE PREPARATION

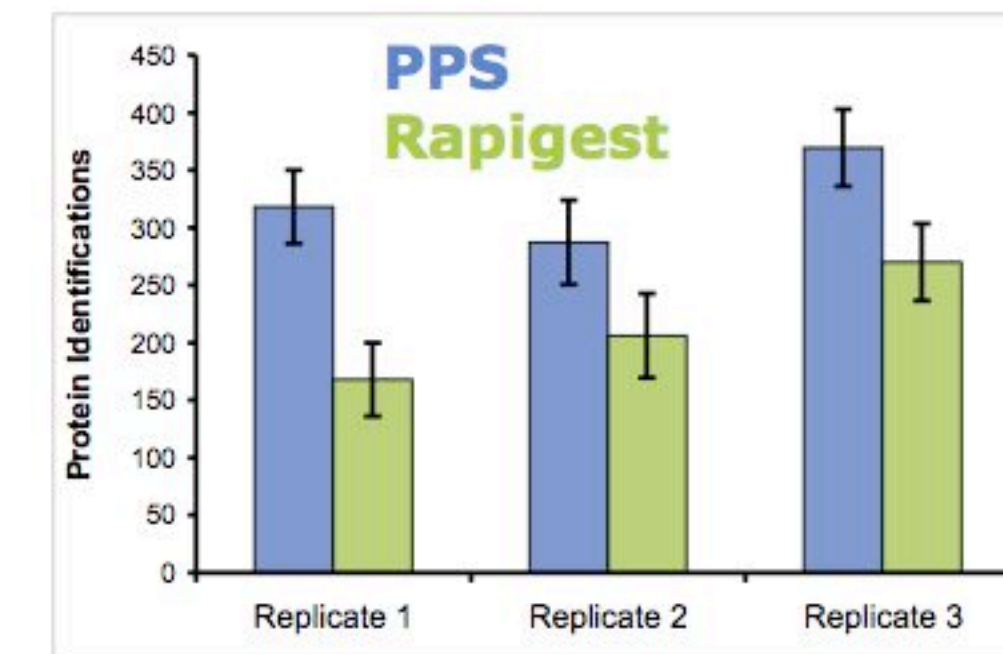


Complex mixtures of proteins derived from *E. coli* were analyzed using PPS Silent™ Surfactant. Cell extracts are performed using 0.1% detergent in buffer (pH 7.8). Proteins were reduced and alkylated prior to digestion using trypsin. Prior to mass spectrometry run, HCl was added to cleave the detergent. Tryptic peptides were analyzed using LC/MS/MS with an ion trap mass spectrometer. The number of peptides and proteins identified were compared to evaluate and compare reagent performance. All experiments were repeated in triplicate.

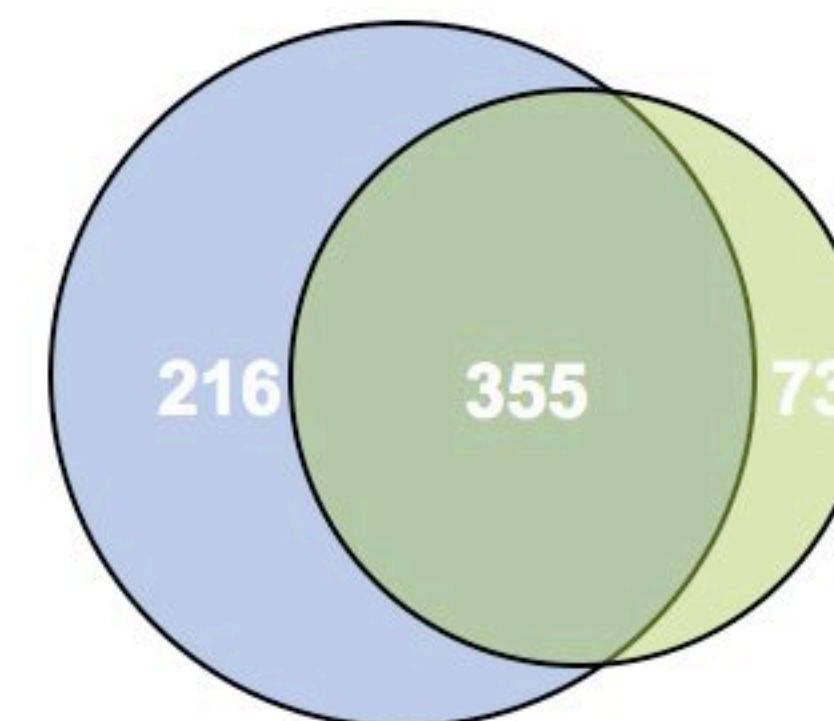
RESULTS AND CONCLUSIONS

PPS Silent™ Surfactant shows an increase in the overall number of proteins identified from *E. coli*.

Results shown are the number of proteins identified in replicate analyses of *E. coli* extract using LC/MS/MS. Proteins were identified with <1% false discovery rate.



Different Cleavable Detergents Provide Complementary Information:

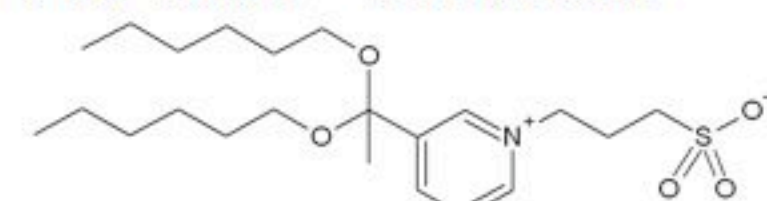


Nonredundant Protein Identifications from 9 PPS Runs and 9 Rapigest Runs

PPS = 571 Protein IDs
 Rapigest = 428 Protein IDs
 Total = 644 Protein IDs

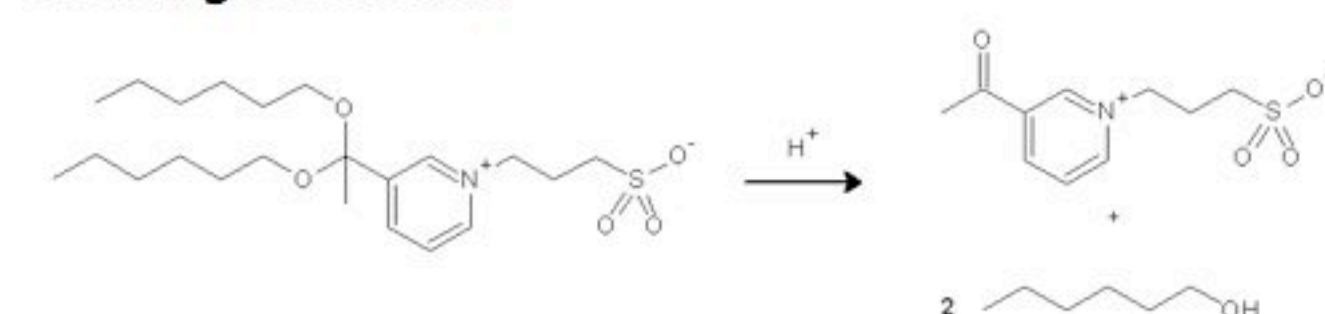
CONTROLLED ELIMINATION OF PPS SILENT™ SURFACTANT

PPS Silent™ Surfactant



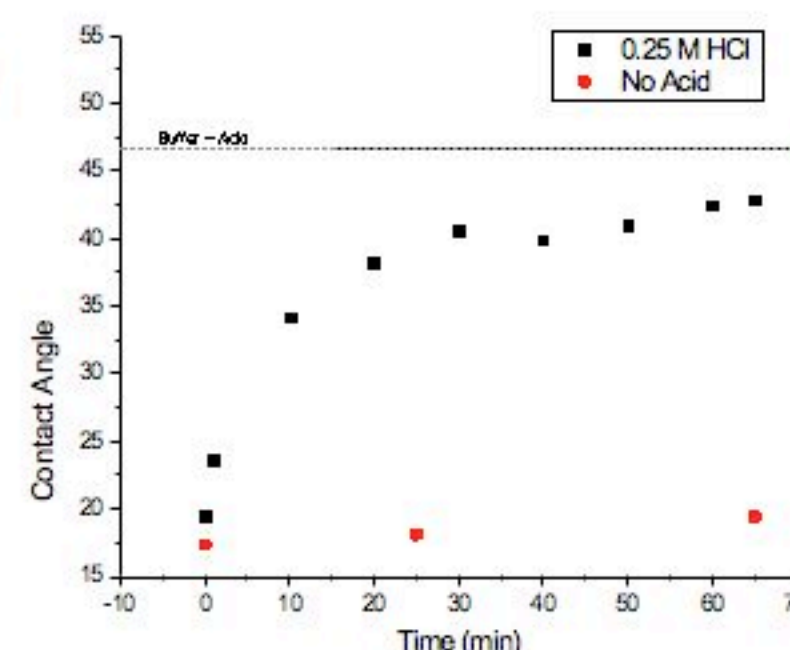
3-[3-(1,1-bisalkoxyethyl)pyridin-1-yl]-propane-1-sulfonate

Cleavage Reaction



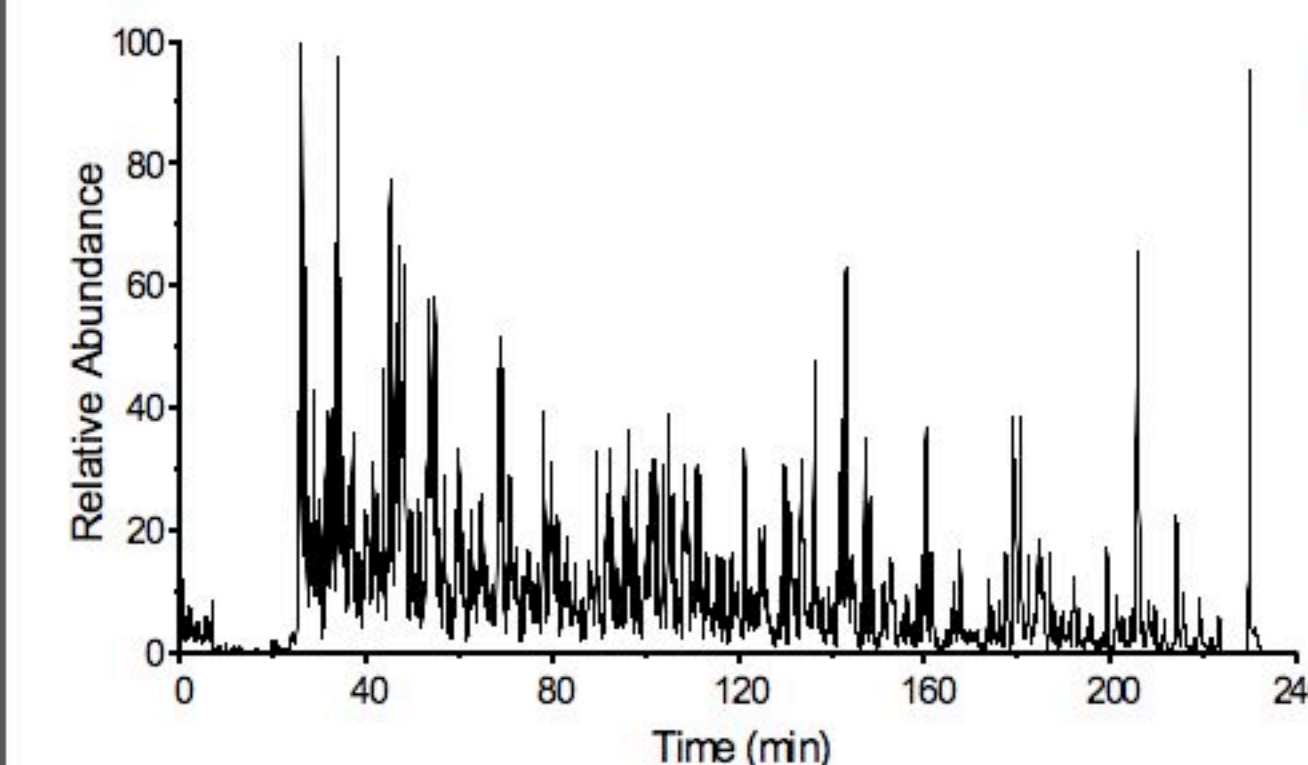
PPS Cleavage Protocol

- Prior to mass spectrometry run, add HCl to a final concentration of 250mM.
- Incubate at 37°C for 45 minutes.
- Spin sample at 14K, 4°C for 10 minutes.



The surfactant properties of PPS Silent™ Surfactant are eliminated after treatment with acid.

PPS Silent™ Surfactant allows one to identify large numbers of proteins in a single experiment. Example base peak chromatogram from the analysis of 5 µg of yeast lysate prepared using PPS Silent Surfactant. Yeast lysate was analyzed using a 4 hour gradient and a long column (60 cm).



Run #	Proteins	False Positives
1	1261	9
2	1292	1
3	1249	10
4	1270	8