Sandra Wilson[1]; Darryl J.C. Pappin[1,2]; Alexandre Zougman[3]; John Wilson[1]

PROTIFI™
Bringing precision omics to life!™

S-Trap Turbo: From Sample Prep to Analysis in Record Time

[1]ProtiFi LLC, Fairport, NY, United States; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor NY, United States; [3]University of Leeds, Leeds, England

1) Introduction

With the ability to handle extremely diverse sample types at varied operator skill levels and without the need for protocol modification, the S-Trap sample preparation system has found widespread adoption in the proteomics community.

However, recent advances in data acquisition throughput, in some cases now only minutes per sample, necessitate concomitant advances in bottom-up sample preparation.

The elimination of any and all extraneous elements in a sample preparation workflow increases both throughput and robustness. One of the more tedious (and often time-variable) steps in proteomics sample processing is post-elution sample dry-down.

Here we present the new S-Trap turbo: S-Traps that yield minimal elution volumes of highly concentrated peptides suited for immediate analysis by injection on LC-MS or spotting on MALDI.

Samples

PIXUL™

Lysis

Protein assay

Prepare

S-TRAP Turbo™

Concentrate
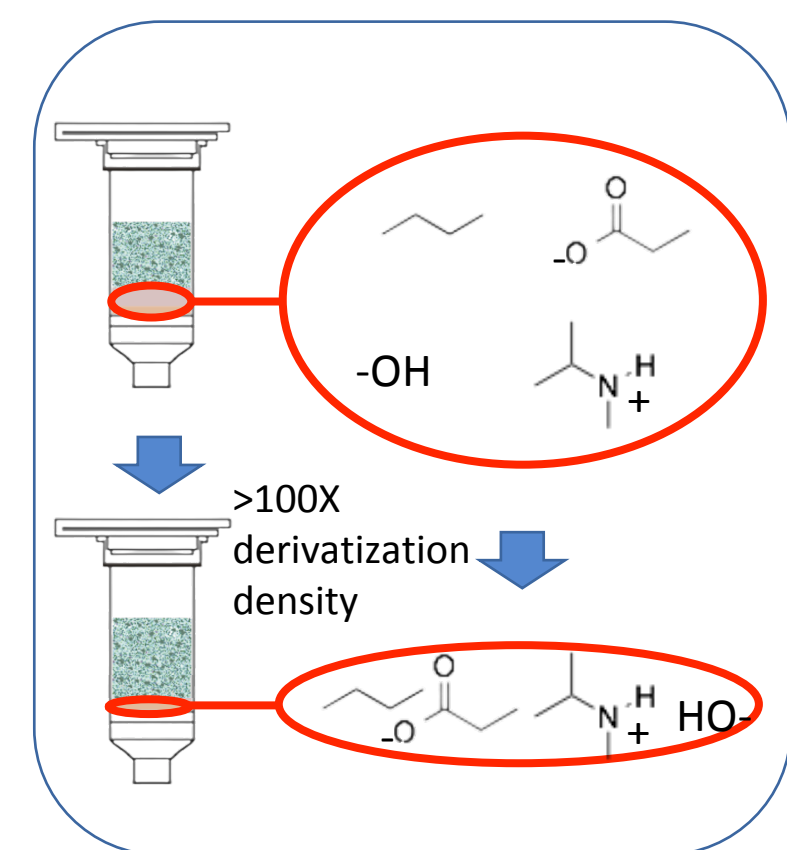
2) Methods

Novel snap-cap S-Trap turbo micro columns were constructed via plastic injection molding.
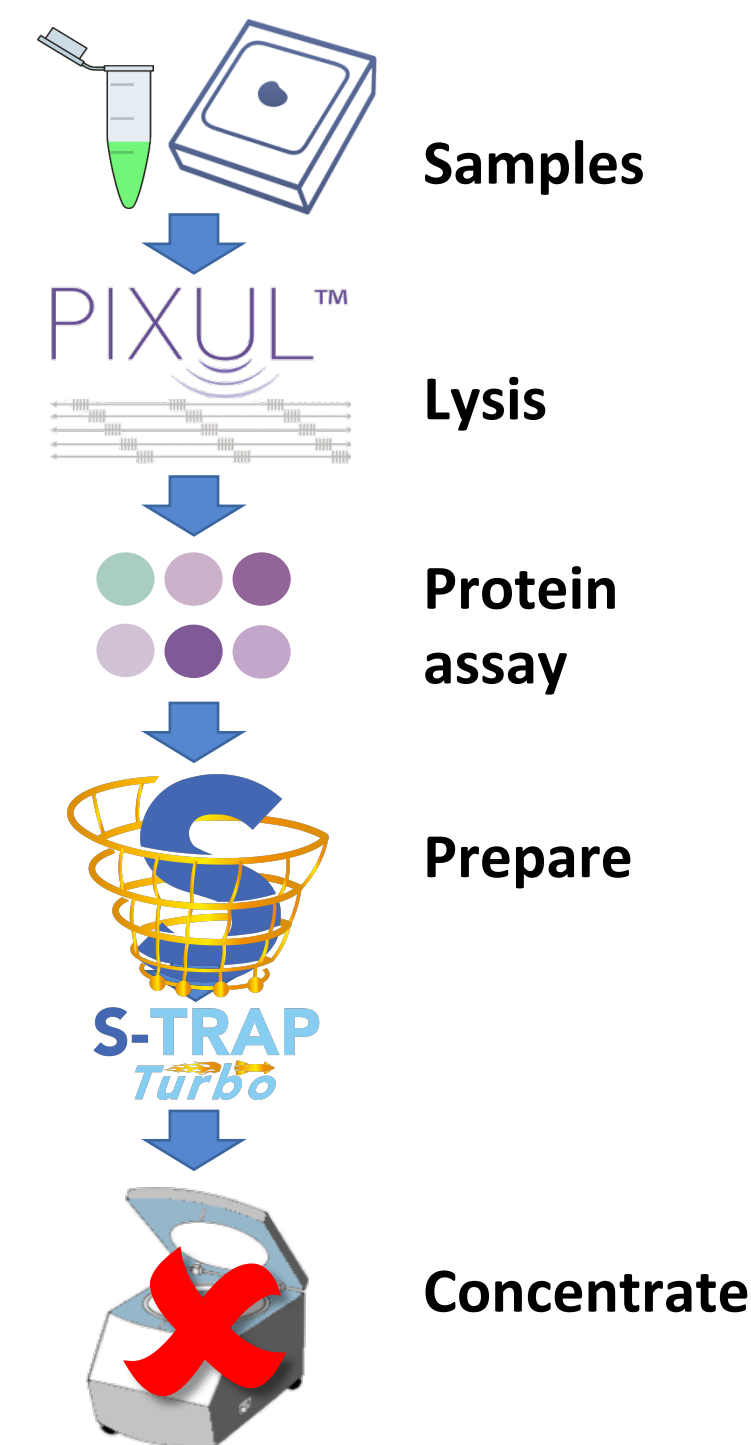
The columns incorporated newly developed, low-binding polymeric traps densely derivatized with novel surface modifications hundreds of times more dense than in traditional S-Traps

The standard S-Trap protocol steps of lysis, reduction and alkylation, denaturation, binding, washing and tryptic digestion were performed both for standard and turbo S-Traps

Samples were analyzed by LC-MS on an Agilent QTOF (6546 and/or 6550), Thermo Orbi-class instrument or Bruker timsTOF Pro.

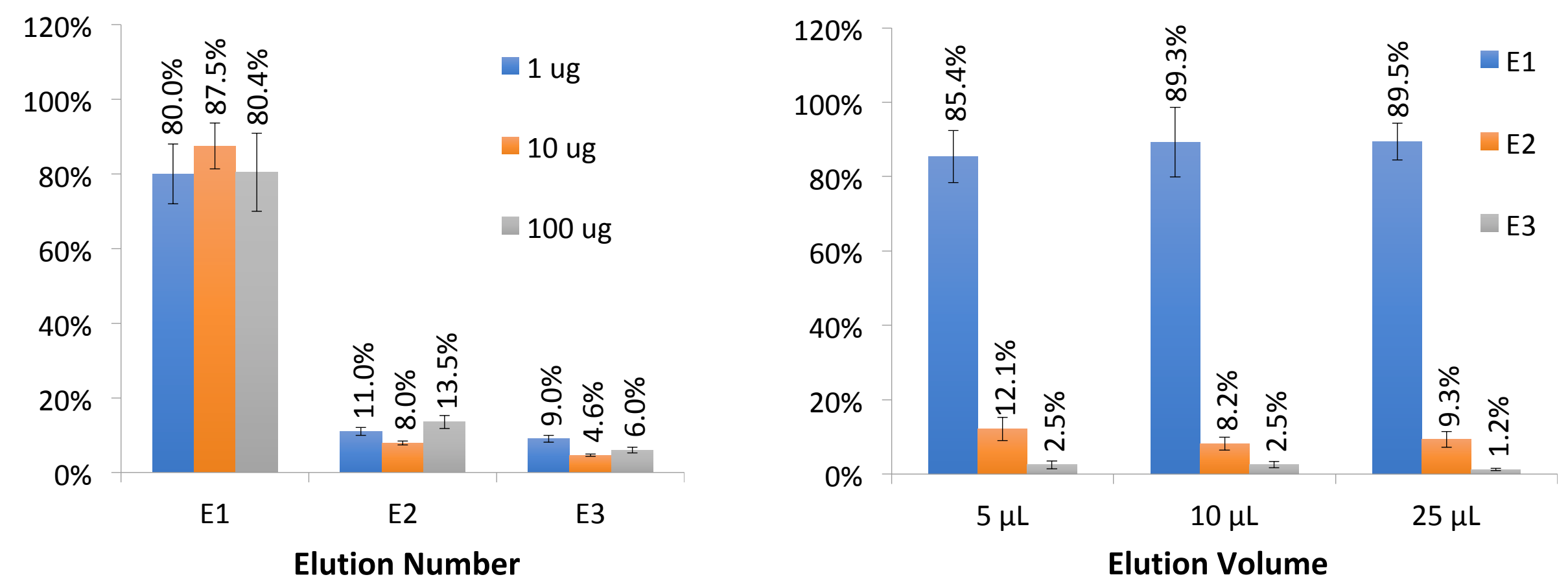Sample yield was compared and quantified using BCA and/or fluorescent assays.

Sample quality was compared by peptide and protein identification rate and reproducibility of quantifications.

-OH

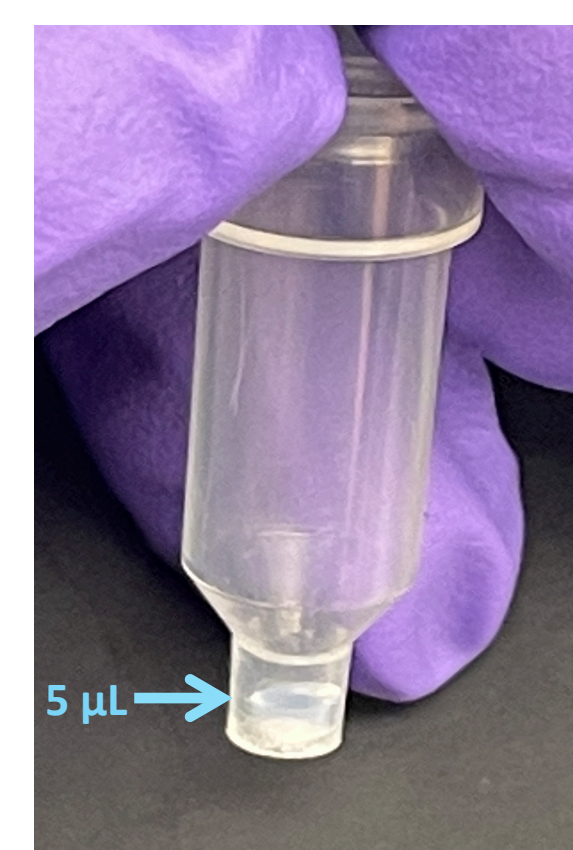>100X derivatization density

3) Small elution volumes

Turbo traps were compared to traditional S-Traps using three samples of highly varied hydrophobicity:

- Serum (most hydrophilic)
- HeLa or HEK cell lysate (both hydrophilic and hydrophobic)
- Rabbit brain acetone powder (most hydrophobic)
- Between < 1 µg to 100 µg of protein was processed

S-Trap turbo elutions as low as 5 µL were found to be reproducible and similar or equivalent to standard S-Trap digestions as judged by:
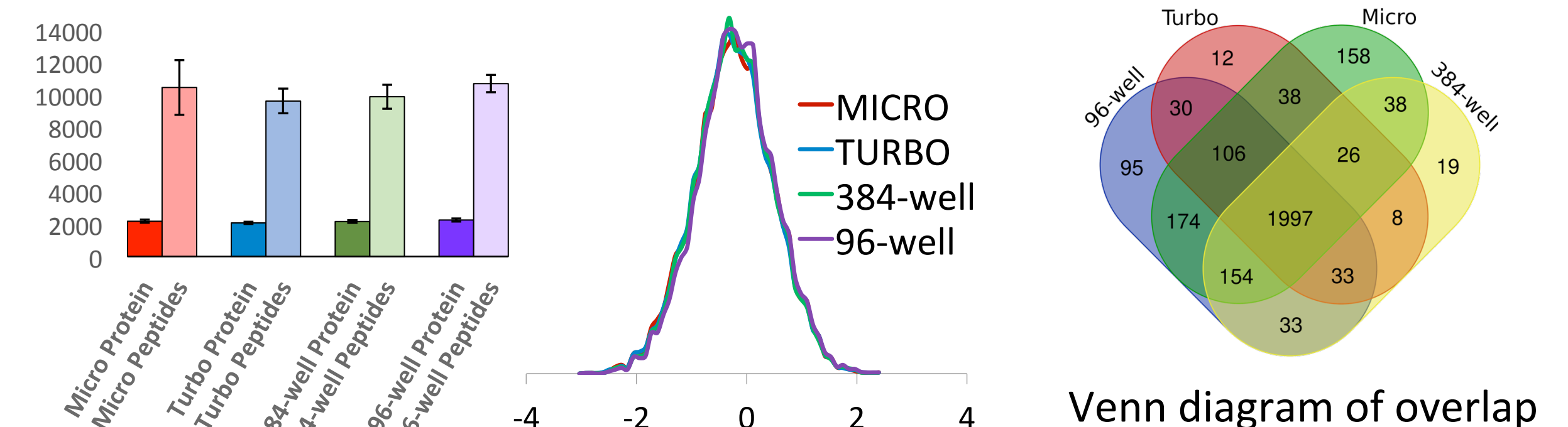
- completeness of digestion
- peptide yield and identifications
- numbers of identified peptides and proteins

S-Trap turbo elutions could be immediately loaded onto an autosampler with or without acidification

5 µL →

4) Recovery as a function of input material

From 1 µg to 100 µg of protein was applied to beta S-Trap Turbos. Three elutions were performed: the initial 5 µL digestion volume, 10 µL then 20 µL (see next panel). ~90% of sample was bound by S-Trap Turbos.

Approximately 60% of the total applied was recovered in the first elution. There was not a significant change in the behavior of the columns as a function of protein loading amount.

Legend: 1 ug (blue), 10 ug (orange), 100 ug (light blue)

Process Stage	1 ug	10 ug	100 ug
FT	12.8%	13.9%	10.7%
Washes	3.8%	2.6%	0.5%
E1	58.2%	60.7%	63.9%
E2	8.0%	5.5%	10.8%
E3	6.6%	3.2%	4.8%
Strip	10.6%	14.0%	9.3%

Key: FT = Flow Through, E = Elutions 1 through 3, Strip = SDS lysis buffer plus heat

5) Recovery with small volume

Greater than or equal to 80% of the total digested, processed peptides was eluted in the first elution fraction; this represents recovery of approximately 60% of the total applied protein in this one fraction.

This proportion was not significantly changed either by a change in the amount of loaded protein nor by increasing the elution volume from 5 µL to 25 µL. A second and third elution ultimately recover an additional ~10% of the total applied protein, a proportion that does not necessarily warrant speed-vaccing.

Legend: 1 ug (blue), 10 ug (orange), 100 ug (gray)

Elution Number	1 ug	10 ug	100 ug
E1	80.0%	87.5%	80.4%
E2	11.0%	8.0%	13.5%
E3	9.0%	4.6%	6.0%

Legend: E1 (blue), E2 (orange), E3 (gray)

Elution Volume	E1	E2	E3
5 µL	85.4%	12.1%	2.5%
10 µL	89.3%	8.2%	2.5%
25 µL	89.5%	9.3%	1.2%

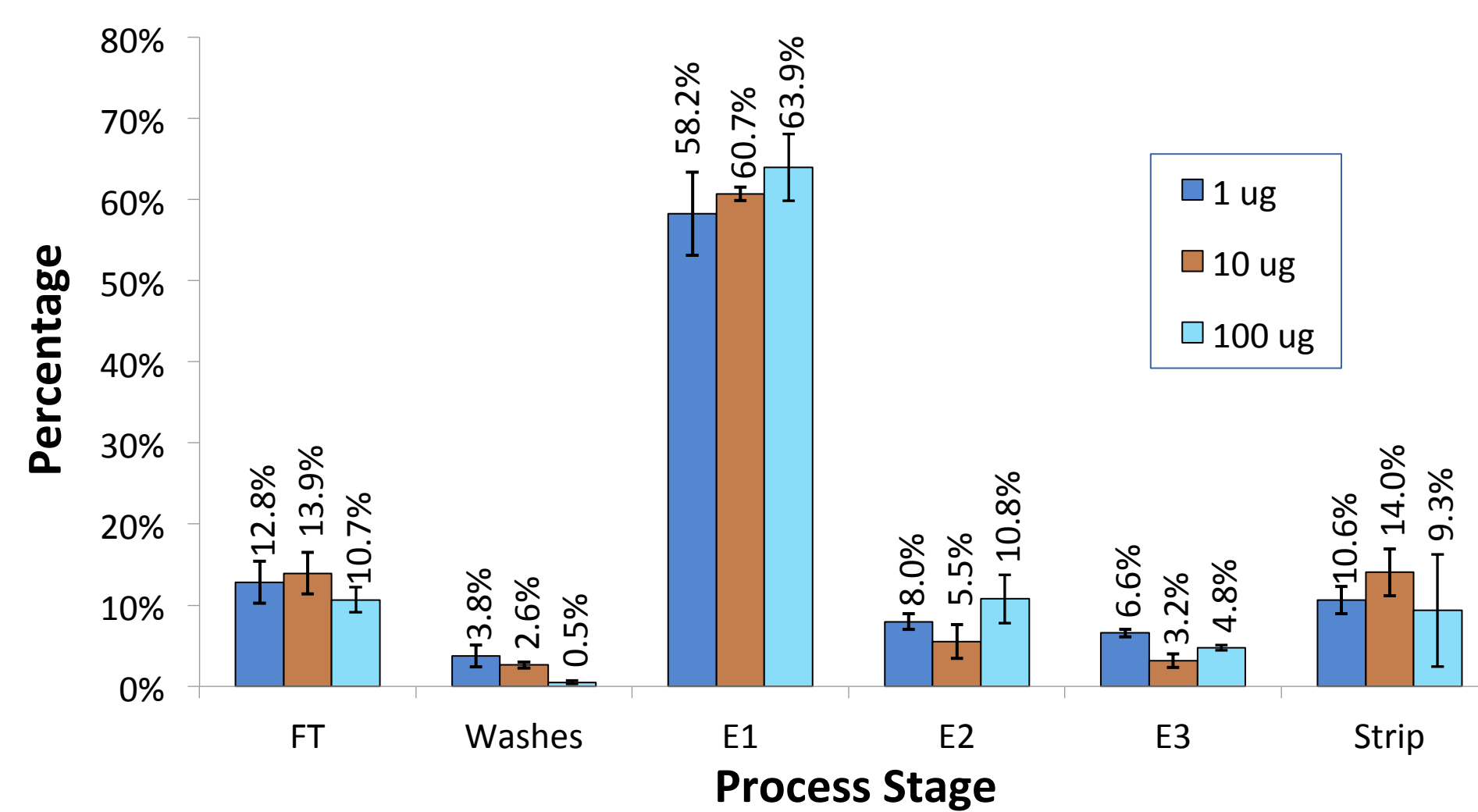**S-TRAP Turbo**

6) Equivalence across S-Trap platforms

As measured by identification rates, rates of missed cleavage and GRAVY distributions, the different S-Trap format are statistically indistinguishable.

Bar chart categories: Micro Protein, Micro Peptides, Turbo Protein, Turbo Peptides, 384-well Protein, 384-well Peptides, 96-well Protein, 96-well Peptides

Distribution legend: MICRO, TURBO, 384-well, 96-well

Venn diagram of overlap (Turbo, Micro, 96-well, 384-well): 12, 158, 30, 38, 38, 106, 26, 19, 95, 174, 1997, 8, 154, 33, 33

7) Time savings

By offering an "elute and shoot" solution, the S-Trap turbo affords a further increase in the efficiency of proteomics sample processing.

The speed at which samples can be concentrated depends on many factors including the number of samples and their volume, the kind of solvent (percentage organic content), the temperature, the efficiency of solvent removal or trapping, which drives the process, and the extent of vacuum, which itself is a function of the pump and the factors above.

Thus, speed-vaccing times can range from minutes to many, many hours. Additionally, researchers frequently encounter the situation in which a single stubborn sample refuses to dry down, sometimes even in the case of identically aliquoted tubes, necessitating additional time, temperature or both, and adding additional uncertainty to experimental planning.

The S-Trap turbo directly solves these issues by yielding clean peptides, ready to inject, with the same efficiency as other formats that require dry-down.
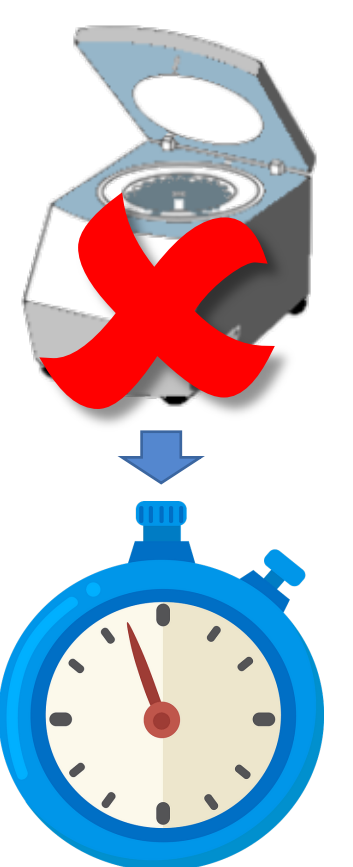
8) Conclusions

- No significant loss of hydrophobic peptides between the standard S-Trap protocol and S-Trap turbo was observed, an observation likely explained by the use of aqueous or mostly-aqueous buffer A to solubilize peptides (followed by hard centrifugation) and the traps having matched hydrophilicity.
- Sample processing time on S-Trap Turbos can be further reduced to mere minutes through the use of sonication.
- S-Trap turbo allows proteomics researchers to go from complex samples to ready-to-inject peptides in record time, with minimal steps and equipment.
- We anticipate they will enable the broader use of proteomics techniques.

UNIVERSITY OF LEEDS