Reversible chemistry for universal protein extraction and cleanup of whole proteins and peptides for fast, high yield proteomics sample preparation

Stephanie Biedka[1]; Jonathan Minden[1, 2]; Nolan Frey[2]; Amber Lucas[1]

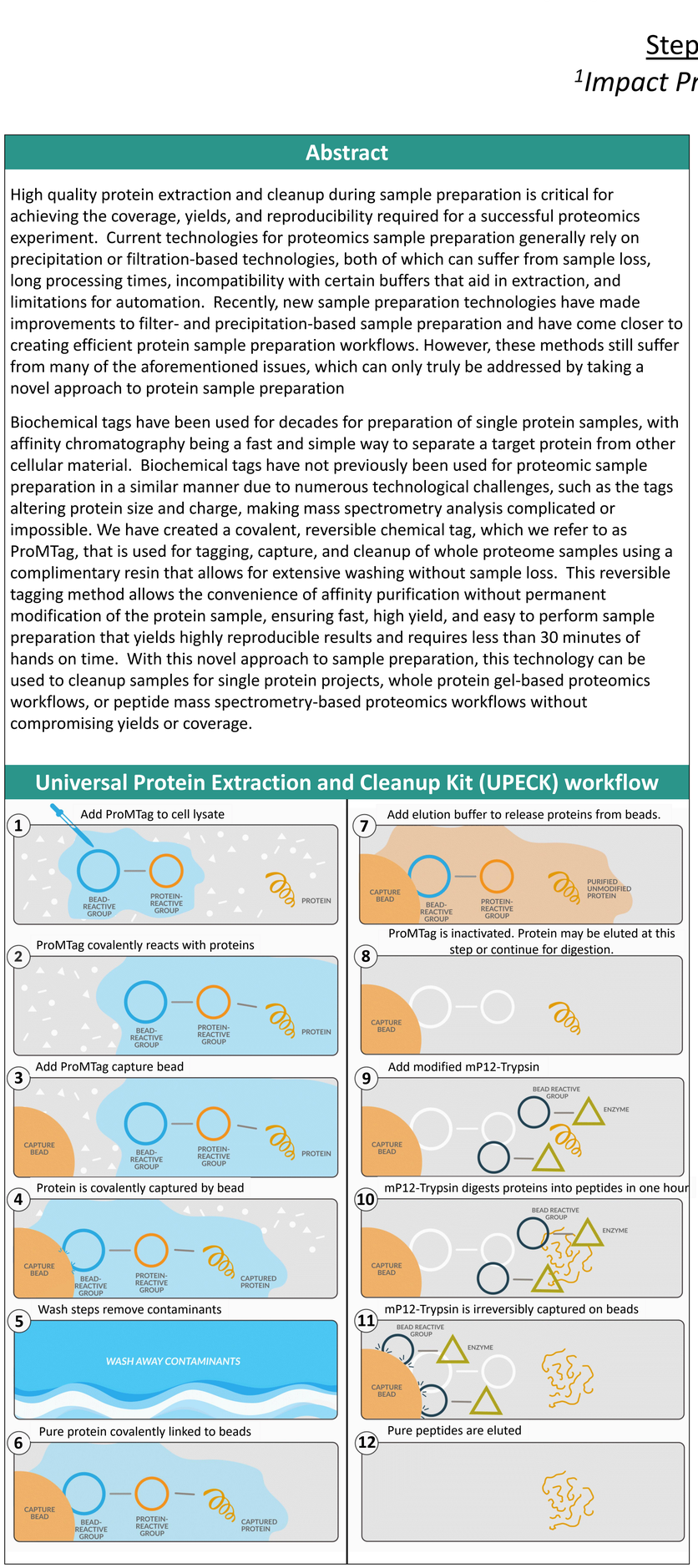
[1]*Impact Proteomics, Pittsburgh, PA;* [2]*Carnegie Mellon University, Pittsburgh, PA*

Abstract

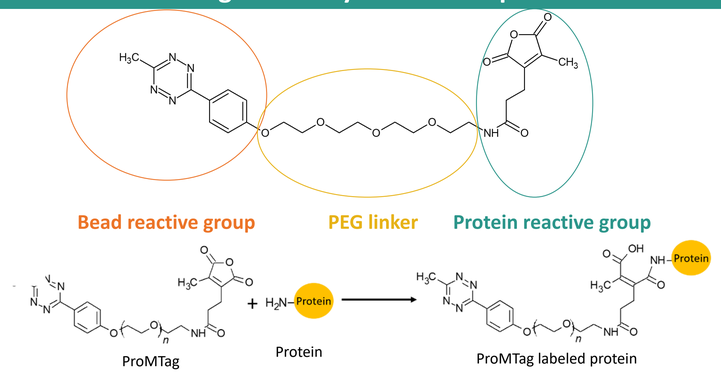
High quality protein extraction and cleanup during sample preparation is critical for achieving the coverage, yields, and reproducibility required for a successful proteomics experiment. Current technologies for proteomics sample preparation generally rely on precipitation or filtration-based technologies, both of which can suffer from sample loss, long processing times, incompatibility with certain buffers that aid in extraction, and limitations for automation. Recently, new sample preparation technologies have made improvements to filter- and precipitation-based sample preparation and have come closer to creating efficient protein sample preparation workflows. However, these methods still suffer from many of the aforementioned issues, which can only truly be addressed by taking a novel approach to protein sample preparation

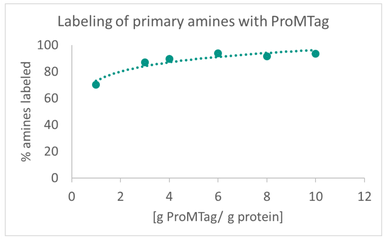
Biochemical tags have been used for decades for preparation of single protein samples, with affinity chromatography being a fast and simple way to separate a target protein from other cellular material. Biochemical tags have not previously been used for proteomic sample preparation in a similar manner due to numerous technological challenges, such as the tags altering protein size and charge, making mass spectrometry analysis complicated or impossible. We have created a covalent, reversible chemical tag, which we refer to as ProMTag, that is used for tagging, capture, and cleanup of whole proteome samples using a complimentary resin that allows for extensive washing without sample loss. This reversible tagging method allows the convenience of affinity purification without permanent modification of the protein sample, ensuring fast, high yield, and easy to perform sample preparation that yields highly reproducible results and requires less than 30 minutes of hands on time. With this novel approach to sample preparation, this technology can be used to cleanup samples for single protein projects, whole protein gel-based proteomics workflows, or peptide mass spectrometry-based proteomics workflows without compromising yields or coverage.

Universal Protein Extraction and Cleanup Kit (UPECK) workflow



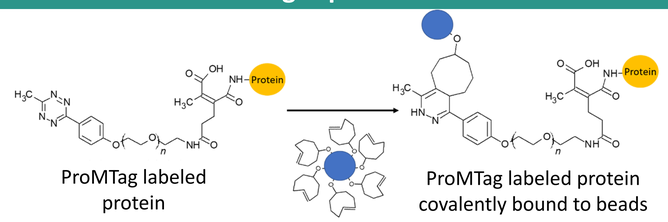
ProMTag reversibly reacts with proteins



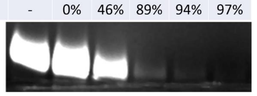


Proteins were labeled with increasing amounts of ProMTag and labeling efficiency was assessed using a fluorescamine assay. We found that a 1X by mass labeling of the protein sample labeled 70% of the amines in the sample. This jumped to 86% at 2X by mass labeling and plateaued at 93% at 6X by mass labeling. Since only one amine per protein needs to be labeled for this workflow to be successful, these labeling efficiencies were more than enough to ensure thorough protein labeling

ProMTag labeled protein can be captured on ProMTag capture beads

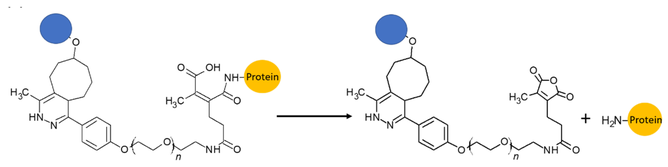


Incubation time (min)	Load	0	5	15	30	60
% bound	-	0%	46%	89%	94%	97%

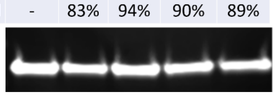


Proteins labeled with ProMTag and Cy5 were incubated with ProMTag capture beads over the course of 60 min and assessed for binding by running the supernatant solution on a polyacrylamide gel. Protein bands were quantified for their fluorescence intensity and compared to the load to determine percent of protein bound to the beads. Binding leveled off around 15 min, with incremental improvements to reach 97% bound after 60 min.

Proteins eluted from ProMTag capture beads in 15 minutes

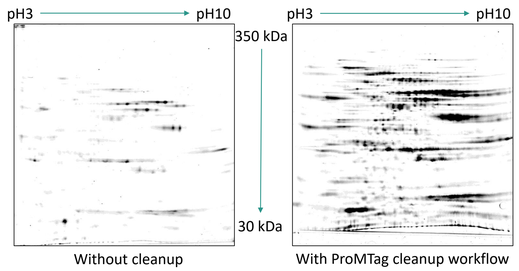


Elution time (min)	Load	15	30	60	120
% yield	-	83%	94%	90%	89%



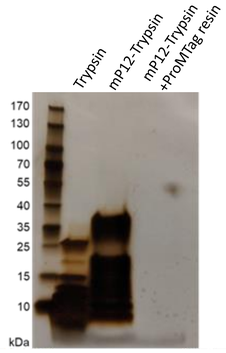
ProMTag and Cy5 labeled proteins were released from ProMTag Capture beads over the course of two hours and were assessed for release by running the supernatant solution on a polyacrylamide gel. Protein bands were quantified for fluorescence intensity and compared to the load to determine percent of protein released from the beads.

Whole protein proteome cleanup using UPECK

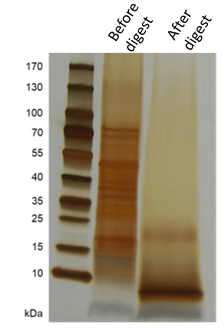


With all To test the ProMTag's ability to tag, capture, cleanup, and release proteins for proteomic analysis, we performed two-dimensional gel electrophoresis for two yeast proteome samples: one with no cleanup and one that was processed using UPECK. Not only were recoveries above 80% for UPECK, the removal of contaminants and salts in the sample allowed for the resolution and visualization of many more proteins in the cleaned up sample than in the untreated sample.

Protein digestion and Trypsin removal using mP-12 Trypsin

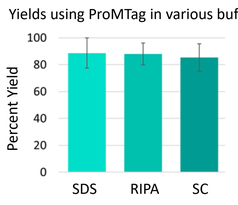


To prevent Trypsin contamination and allow for fast digestion, a modified version of Trypsin was created that can be removed by the ProMTag capture resin used in the ProMTag cleanup workflow. First, trypsin was modified to make mP12-Trypsin and a shift in molecular weight was observed as expected. When this mP12-Trypsin was incubated with ProMTag capture resin, no mP12-Trypsin remained in solution. The gel was over-developed to ensure no mP12-Trypsin was still in the sample.



To show that mP12-Trypsin can successfully digest an entire proteome in one hour, we incubated mP12-Trypsin with a whole yeast proteome for one hour and assessed the completion of digestion by imaging on a gel. After one hour digestion was successful as indicated by the disappearance of large molecular weight protein bands and the appearance of a large, small molecular weight band.

Yields and mass spectrometry of UPECK prepared samples



Yields for peptides generated using UPECK were assessed using the Pierce™ Quantitative Fluorometric Peptide Assay in various popular buffer systems. All buffer systems averaged yields around 85% for 20 µg of starting sample.

	Proteins identified	Unique Proteins identified
UPECK	147	24
Precipitation	13	0
Commercial kit 1	114	10
Commercial kit 2	111	4
	False Discovery Rate	**Average protein coverage**
UPECK	0.70%	51.60%
Precipitation	1.20%	32.10%
Commercial kit 1	1.20%	45.90%
Commercial kit 2	1.30%	43.10%

Yeast proteomes were cleaned up using four different techniques and were sent for mass spectrometry analysis. UPECK showed more identifications of proteins overall and more unique protein identifications. Additionally UPECK showed a lower false discovery rate and higher protein coverage than the other techniques. Note: Because of Covid-19, we were only able to perform this once. This needs to be repeated for statistical significance.

Acknowledgements

We would like to acknowledge and thank the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University Mass Spectrometry for perform mass spectrometry analysis for us. This work was supported in part by NIH P30 CA124435 utilizing the Stanford Cancer Institute Proteomics/Mass Spectrometry Shared Resource. This work was also supported by NSF SBIR Phase I award 1843332.