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Si-Trap™: A simultaneoushigh-throughput platform for multi-omics

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INTRODUCTION

Si-Trap™ is a high-throughput, detergent-free multi-omics sample preparation platform that enables recovery of proteins, metabolites, and lipids from a single biological sample. The workflow begins with detergent-free[1] sample dissolution, followed by neutralization and organic-solvent denaturation. Proteins are captured within derivatized Si-Trap™ pores through weak-affinity interactions, while small molecules pass through as a mass spectrometry-compatible metabolite fraction. Optional lipid collection further extends the workflow by enabling recovery of an additional molecular fraction from the same starting material. The captured proteins are then processed in situ in a 96-well format through denaturation, reduction, alkylation, and digestion with the user's protease of choice, after which peptides are eluted for LC-MS analysis[2]. By minimizing parallel preparation and sample splitting, Si-Trap™ supports a streamlined "one sample in, multiple omics out" workflow **(Fig. 1)**. This integrated approach enables reproducible, automation-ready multi-omics sample preparation while preserving proteomic depth and metabolite coverage from precious biological samples.

MOTIVATION

Multi-omics analysis provides deeper biological insight by connecting changes across proteins, lipids, and metabolites from the same biological system. However, conventional multi-omics workflows often require limited sample material to be split across separate proteomics, metabolomics, and lipidomic preparation methods. This increases handling, introduces technical variability[3], and reduces confidence that each molecular layer reflects the same starting sample. This is especially important for limited-input samples, precious clinical material, and translational studies where sample splitting can reduce data quality. An ideal multi-omics workflow should therefore recover multiple analysis-ready molecular fractions from a single input while remaining high-throughput, and cost-effective.

METHODS

The Si-Trap™ was evaluated across four sequential workflow comparisons: S-Trap™ vs. Si-Trap™ proteomics, metabolite spike-in and *Escherichia coli* (*E. coli*) metabolite recovery, UNC proteomics/metabolomics, and ProMeFa multi-omics. For proteomics-only benchmarking, matched protein inputs from tissue lysates were processed using either the standard S-Trap™ protocol or the Si-Trap™ workflow, followed by LC-MS/MS analysis on a Thermo Q-Exactive **(Fig. 2)**. In the Si-Trap™ workflow, detergent-free lysates were loaded onto the Si-Trap™ 96-well plate, where proteins were trapped, reduced and alkylated on-column, washed, digested with trypsin, and eluted for proteomic analysis.

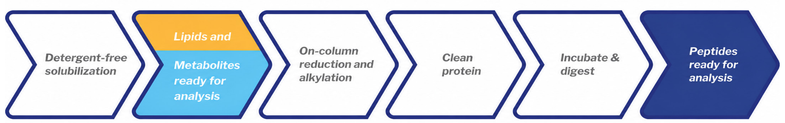

Fig. 1. Overview of the Si-Trap™ workflow

Samples are first dissolved under detergent-free conditions, followed by addition of binding solution and, when desired, an optional lipid extraction solution. Proteins are captured on the trap, while lipids and metabolites pass through for collection. Reduction and alkylation are performed in situ on the captured proteins, followed by proteolytic digestion.

Metabolite recovery was assessed using a defined 22-metabolite spike-in panel and in a separate workflow *E. coli* metabolomics. Spike-in samples contained 100 µM of each metabolite and were processed through the Si-Trap™ or prepared as a buffer-only control. For the *E. coli* comparison, QuikChange XL *E. coli* cultures were grown overnight, metabolites were extracted using either Si-Trap™ or conventional 40:40:20 methanol:acetonitrile:water extraction. Eluates were dried by SpeedVac, stored at −80 °C, and analyzed on an Agilent QTOF 6545 **(Fig. 3)**.

For the UNC comparison, MCF7 ER+ cells were processed using 80:20 methanol:water for metabolomics and urea for proteomics;These workflows were compared with the Si-Trap™. Proteomic samples were analyzed on a Thermo Vanquish Neo coupled to an Orbitrap Exploris 480, and metabolomics samples on a Thermo Vanquish Horizon coupled to an Orbitrap Exploris 240 **(Fig. 4)**.

For the ProMeFa comparison, HEK293 cell pellets were processed using a water/MTBE/methanol multi-omics workflow. Lipids were extracted from the apolar organic phase, metabolites from the polar aqueous phase, and proteins from the remaining pellet after resuspension in 9 M urea and FASP processing. ProMeFa lipidomics was analyzed using an Agilent UPLC C8 column coupled to a Sciex TripleTOF 5600+, metabolomics using an Agilent UPLC BEH column coupled to a Sciex TripleTOF 5600+, and proteomics using nLC-MS/MS on a Vanquish Neo coupled to a Q-Exactive. These datasets were compared with the Si-Trap™ processing for integrated protein, metabolite, and lipid recovery **(Fig. 5)**.

RESULTS

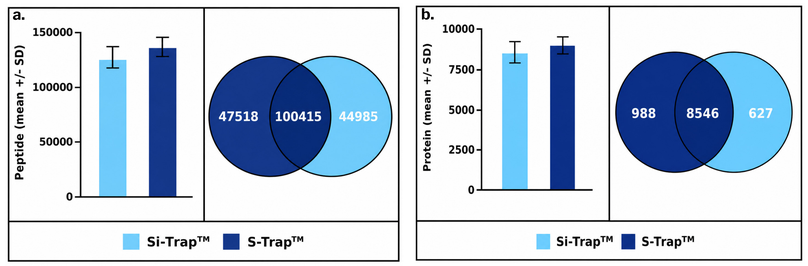

Fig. 2. Comparison of the S-Trap™ and Si-Trap™ Proteomics workflows

Peptide and protein identifications were compared between tissue lysate samples processed using the Si-Trap™ workflow and the standard S-Trap™ workflow. Si-Trap™ achieved comparable peptide identifications relative to S-Trap™ **(a)**, as well as comparable protein identifications **(b)**.

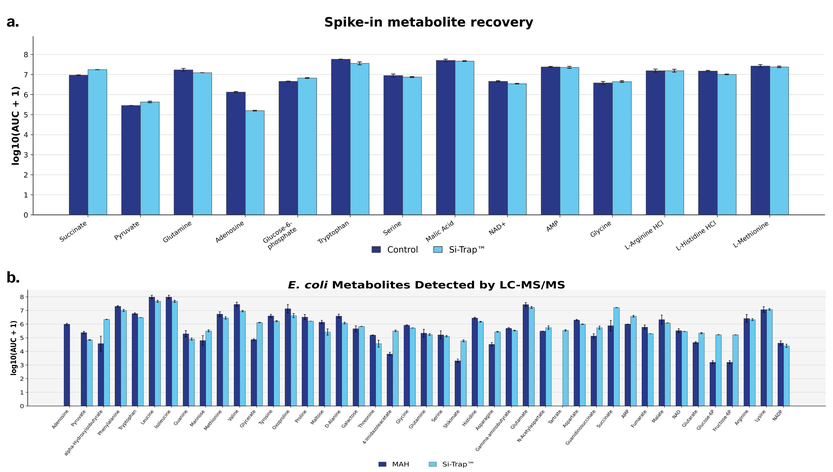

Fig. 3. The Si-Trap™ supports broad recovery of metabolites.

To assess metabolite retention, a 22-metabolite spike-in panel (100 µM each) was processed through Si-Trap™ and compared with a metabolite-elution buffer control. 14 metabolites were detected, while eight were not observed due to HILIC method limitations **(a)**. For all detected metabolites, Si-Trap™ showed recovery profiles comparable to the control, indicating strong metabolite compatibility. Recovery was also evaluated in *E. coli* samples processed by either Si-Trap™ or conventional methanol:acetonitrile:water (MAH) extraction. Across these samples, 43 metabolites were detected after excluding analytes known to be undetectable by the method. The two workflows showed broad overlap, with Adenosine detected only in MAH and Tartrate detected only in Si-Trap™ **(b)**.

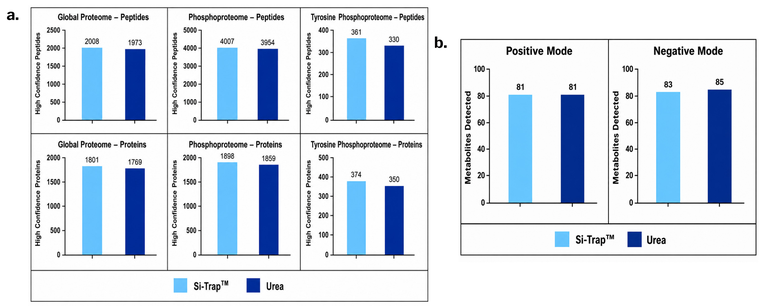

Fig. 4. Si-Trap™ Maintains Proteomic Depth While Preserving Broad Metabolite Coverage

Proteomic and metabolomic performance were compared between Si-Trap™ and conventional UNC sample preparation workflows using MCF7 ER+ breast cancer cells. For proteomics, Si-Trap™ produced comparable or slightly higher identification counts across global proteome, phosphoproteome, and tyrosine-phosphoproteome analyses relative to the urea-based workflow **(a)**. For metabolomics, Si-Trap™ maintained broad metabolite coverage in both positive and negative ionization modes, with detection levels similar to the conventional methanol-based extraction workflow **(b)**.

RESULTS (CONT.)

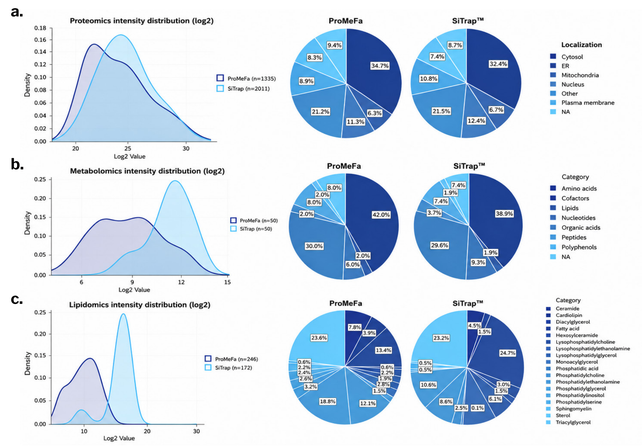

Fig. 5. The Si-Trap™ preserves broad multiomic coverage and signal recovery across metabolomics, proteomics, and lipidomics

Feature intensity distributions and molecular class representation were compared between the ProMeFa multi-omics workflow and Si-Trap™ using HEK293 cell samples. Proteomics **(a)**, metabolomics **(b)**, and lipidomics **(c)** outputs were evaluated across the two workflows. Density plots show global feature intensity distributions, while pie charts summarize the composition of detected features by protein localization, metabolite category, or lipid class. In this comparison, Si-Trap™ maintained broad molecular-class coverage across all three omics layers and produced higher-intensity profiles in metabolomics and lipidomics, with a modest rightward shift in proteomics.

CONCLUSION

Our results demonstrate that the Si-Trap™ delivers a unified, LC-MS-ready sample preparation workflow capable of recovering proteins, metabolites, and lipids from a single biological input, reducing the need for separate extraction protocols while minimizing hands-on time, variability, and sample loss.

The Si-Trap™ matched the depth and performance of the standard S-Trap™ workflow, demonstrating that multi-omics capability does not compromise proteomic quality.

In both spike-in and *E. coli* experiments, the Si-Trap™ delivered metabolite recovery comparable to conventional extraction methods, with broad overlap and only minor method-specific differences, confirming efficient retention and elution of small molecules while maintaining compatibility with standard LC-MS metabolomics pipelines.

In the UNC and ProMeFa comparisons, the Si-Trap™ supported strong recovery across proteomics, metabolomics, and lipidomics, maintaining broad molecular-class coverage and high-intensity signals.

Multi-omics sample preparation should be simple, reproducible, scalable, and practical for real-world laboratories. Together, these findings position the Si-Trap™ as a scalable, high-performance solution for generating richer, more integrated datasets from limited or precious biological material: one sample in, multiple molecular classes and integrated biology out.

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