Fully Automated Proteomics Sample Preparation and Peptide Desalting Workflow for LC-MS/MS Analysis

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Background and Rationale

Mass spectrometry-based proteomics requires multiple preparation steps, including protein denaturation, reduction, alkylation, digestion, peptide cleanup, desalting, and final LC-MS/MS-compatible reconstitution. These steps are traditionally labor-intensive and can introduce variability across operators, batches, and sample types. To improve throughput and reproducibility, we developed an automated proteomics sample preparation workflow using the Unified Separation Technology Auto M96 and Auto M32 platforms. This workflow integrates rapid immobilized enzyme digestion, peptide cleanup, and pre-packed desalting kit-based sample preparation to generate LC-MS/MS-ready peptide samples with reduced hands-on time.

Automated Sample Preparation Workflow

The workflow was implemented using the Unified Separation Technology Auto M96 and Auto M32 automated sample preparation systems. The Auto M96 supports high-throughput processing of 1–96 samples using disposable magnetic rod sleeves and 96-well plates, with automated activation, sample loading, washing, and elution steps. The Auto M32 provides a lower-throughput complementary platform using magnetic rods for sample extraction and purification, with a valve-free and tubeless design suitable for LC-MS/MS sample preparation.

293T whole-cell lysate was used to evaluate the fully automated proteomics sample preparation workflow. Briefly, protein samples were loaded into the pre-packed sample preparation kit, and the kit plate was placed into the designated position on the Unified M96 or M32 automated processing system. After initiating the programmed workflow, all major sample preparation steps, including reagent mixing, reduction/alkylation, enzymatic digestion, peptide cleanup, washing, and elution, were performed automatically. The complete automated processing workflow required approximately 2 hours. After elution, the final peptide eluate was transferred to microcentrifuge tubes, dried using a SpeedVac, reconstituted in LC-MS/MS-compatible solvent, and directly analyzed by LC-MS/MS.

Immobilized Enzyme Design and Optimization

To enable rapid digestion in the automated workflow, a capillary-format immobilized trypsin enzyme module was developed and optimized. Porous silica-based particles were magnetically modified, incubated with trypsin, and retained in the capillary using an external magnetic field. BSA was used as a standard protein to evaluate digestion performance under 5-min digestion at 37°C. Digestion products were analyzed by MALDI-TOF-MS and searched using Mascot/Swiss-Prot, with amino acid sequence coverage and matched peptide numbers used as performance readouts.

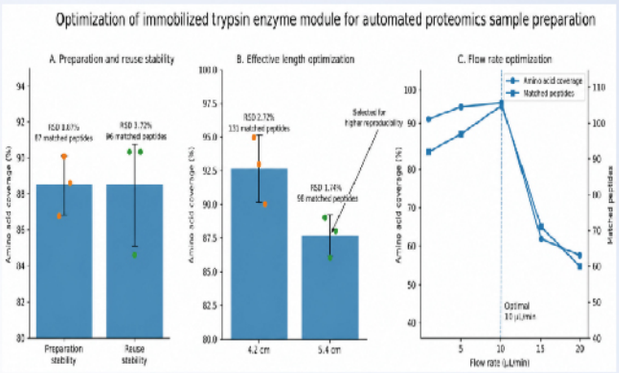

Fig. 2. Optimization of the immobilized trypsin enzyme module for rapid automated digestion.

Optimization of Immobilized Enzyme Performance

Material screening showed that magnetic modification improved digestion performance, likely by enhancing enzyme loading and protein–enzyme interaction. Effective length and flow rate were further optimized using BSA digestion. A 5.4 cm effective length and 10 µL/min flow rate provided a practical balance between digestion efficiency, matched peptide numbers, and reproducibility. Preparation and reuse tests showed stable performance, supporting this immobilized enzyme module as a robust rapid-digestion component.

Capillary Immobilized Enzyme Validation

Immobilized enzyme performance was evaluated by MALDI-TOF-MS and Mascot/Swiss-Prot database searching using amino acid coverage and matched peptide numbers as readouts. The optimized capillary immobilized enzyme format maintained strong digestion performance during reuse and across independently prepared immobilized enzyme formats, providing the method-level basis for the downstream 5-min automated digestion test in 293T whole-cell lysate.

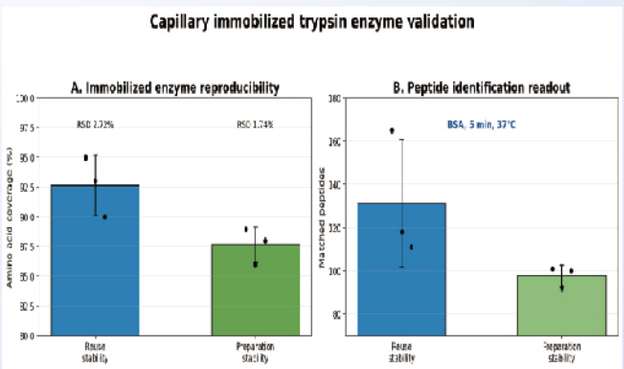

Fig. 3. Validation of the capillary-format immobilized enzyme module using BSA digestion.

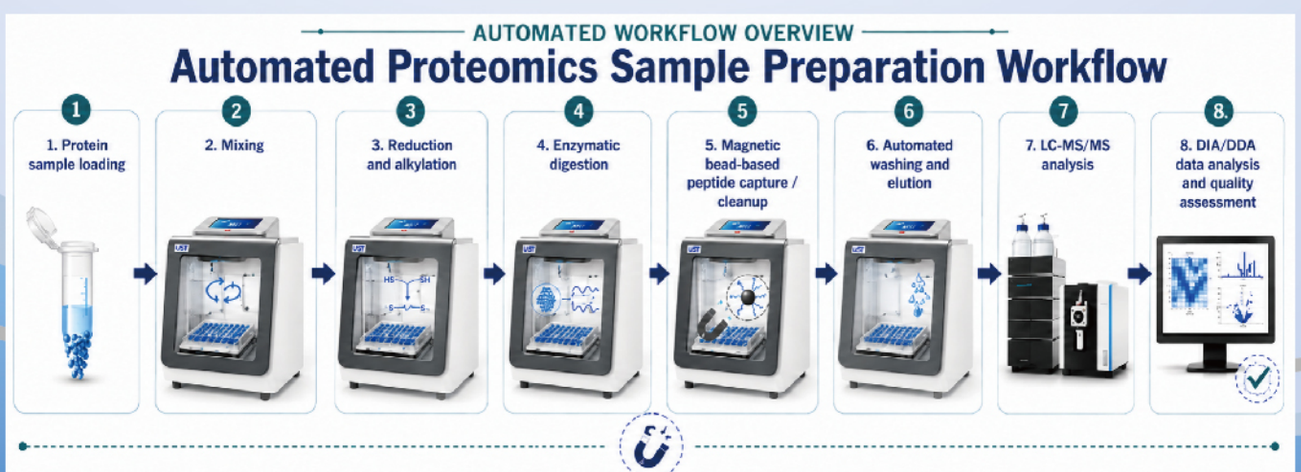

Fig. 1. Automated proteomics sample preparation workflow on the UST platform.

Rapid 5-min digestion maintains high peptide identifications and low unspecific cleavage

To evaluate the automated digestion workflow in a complex proteomics sample, 293T whole-cell lysate was processed across a 5–25 min digestion time course. Total identified peptides and unspecific cleavage rates were plotted together to assess both digestion depth and specificity. Across all tested digestion times, peptide identifications remained high while unspecific cleavage stayed low. Notably, 5-min digestion already generated high peptide identifications with minimal unspecific cleavage, supporting rapid automated digestion for whole-cell proteomics sample preparation.

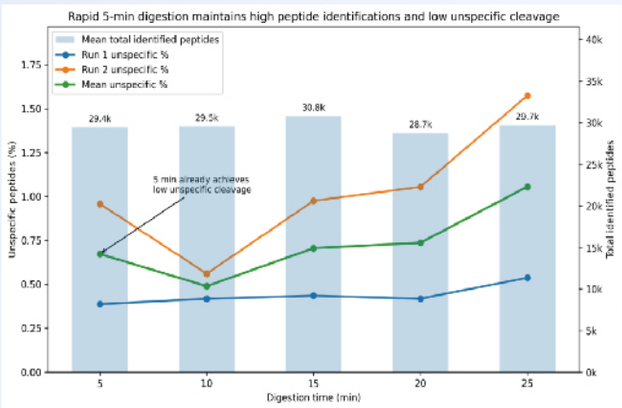

Fig. 4. Rapid 5-min digestion maintains high peptide identifications and low unspecific cleavage in 293T whole-cell lysate.

Automated Peptide Desalting

Peptide desalting is a critical step before LC-MS/MS analysis, but conventional C18 tip-based manual desalting is labor-intensive, operator-dependent, and difficult to scale for high-throughput proteomics. To integrate desalting into the automated workflow, we developed a pre-packed peptide desalting kit-based module suitable for automated operation and parallel sample processing.

Principle / Theoretical Model

The desalting process can be described as an adsorption–elution equilibrium. Peptides are captured by the pre-packed peptide desalting kit during sample loading, while salts and polar contaminants remain in solution and are removed during washing. The enriched peptides are then eluted into a small volume of organic solvent for LC-MS/MS analysis. Theoretical modeling indicates that peptide recovery depends on desalting material amount, sample volume, elution volume, and peptide concentration, providing guidance for kit design and operating conditions.

$$c/c_0 = \frac{V_0 k_1 / S_0 m_0}{(V_0 k / S_0 + m_0)(V_1 k_1 / S_0 + m_0)}$$

$$c_0/c = Dm_0 + \frac{E}{m_0} + F$$

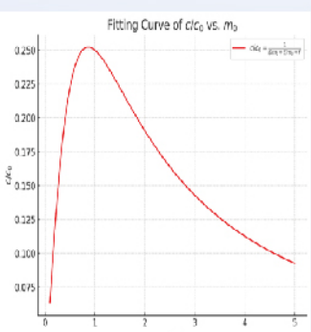

Fig. 5. Theoretical model for optimizing pre-packed peptide desalting kit performance.

Comparable performance

The pre-packed peptide desalting kit-based workflow achieved peptide cleanup performance comparable to conventional manual desalting. U2OS cell lysate testing showed similar protein identification and signal intensity profiles between automated and manual desalting, supporting its use as an integrated cleanup module for automated proteomics sample preparation.

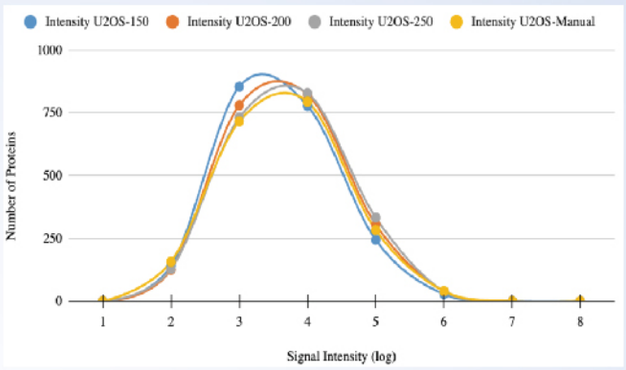

Fig. 6. Automated pre-packed peptide desalting shows comparable performance and broad input compatibility.

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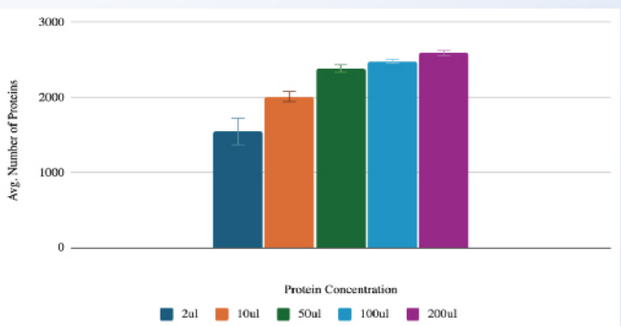

Fig. 5. Theoretical model for optimizing pre-packed peptide desalting kit performance.

Conclusions and Future Applications

We established a fully automated proteomics sample preparation workflow integrating rapid immobilized enzyme digestion, peptide cleanup, and pre-packed peptide desalting. The immobilized enzyme module enabled efficient 5-min digestion after BSA-based optimization and maintained high peptide identifications with low unspecific cleavage in 293T whole-cell lysate. The pre-packed desalting kit provided automated peptide cleanup with performance comparable to manual desalting, broad input compatibility, and stable storage performance for more than four months. Together, this integrated workflow reduces hands-on time, improves reproducibility, and supports scalable LC-MS/MS proteomics sample preparation.