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Abstracts Book

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O52 - Boosting Proteomics at Single-Cell Level: Enhancing Peptide Recovery, Automation, Chromatographic Robustness and Performance

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Selected Keynote Lecture

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Abstract

Advances in mass spectrometry have enabled single-cell proteomics, yet peptide loss due to surface adsorption remains a key bottleneck. Using HeLa tryptic digests, we showed that adsorption follows a Langmuir model, with substantial signal loss at very low concentrations. To mitigate this, we optimized a one-pot sample preparation protocol using polymer-based vials (PMMA or PET) and nonionic detergents (PEO or DDM), resulting in up to 16-fold improvement in peptide recovery.

Building on this, we developed WellOmics, a microfluidic chip in a 384-well plate format, operated solely by centrifugation. This consumable automates the entire process, from single-cell FACS collection to LC-MS injection, while reducing manual handling and minimizing working volumes to the sub-microliter scale.

To further improve analytical performance, we developed the Trap-Refocusing-Elute (TRE) nanoLC mode, a robust approach that combines the sharp peak shapes of direct injection with the added benefits of online desalting and flexible injection volumes (1–10 μL). TRE allows for complete sample transfer and enables rapid 25-minute analysis cycles on standard UPLC platforms.

Finally, we created a high-coverage spectral library for DIA using sequential digestion and peptide fractionation, identifying 282,008 peptides and 9,588 proteins.

Together, these innovations improve peptide recovery, data quality, and scalability—marking a major step forward in high-throughput single-cell proteomics.

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