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Implementing a high-throughput nanoflow proteomics workflow using a dual-trap single column approach with in-plate resuspension of peptides

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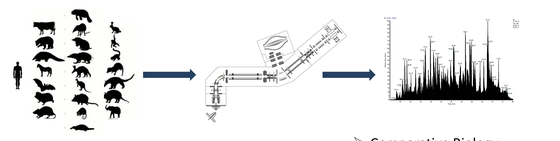
Abstract

Recent years have seen an explosion in sample sizes, not just in large consortia studies like the The SCALLOP consortium (Systematic and Combined AnaLysis of Olink Proteins) or UK Biobank Studies that use 10 000s of patient samples, but in fields like single-cell proteomics that require analyzing many 100s of samples per treatment. For this reason, there is a continuing effort to scale analyses to the 100s and 1000s (and beyond) sample scale. In mass spectrometry-based proteomics the primary limitation is how to operate the liquid chromatography system over time to avoid replacing columns, while also running fast enough to avoid re-calibration mid-run. One recent solution is the dual-trap single column approach that essentially operates LC steps in parallel such that one sample is loaded while the other sample is eluted onto the mass spectrometer. We have implemented this system at NIST using nanoflow to preserve high-sensitivity for single-cell applications. Likewise, we have demonstrated the autosampler can successfully resuspend dried peptides in wells immediately before applications. This allows our lab to receive pre-digested and cleaned samples from remote collaborators and run them with minimal effort. Though we detect approximately 50 % fewer proteins than our typical 10 samples per day method on our system (*i.e.*, 2000 instead of 4000 proteins from a HeLa digest, and 200 instead of 400 proteins from undepleted plasma), we are now able to run nearly 60 samples per day in a robust manner. Overall, this nanoflow dual trap single column setup allows for ongoing and future studies benchmarking single-cell proteomics as well as embarking on large-scale plasma proteomics studies

Background

- Continuing need to scale proteomic analysis to the 100s and 1000s (and beyond) sample scale
- Goal: operate robustly at > 40 samples per day (spd) with minimal user input



100s of species
1000s of samples
or
100s of cells (scProteomics)

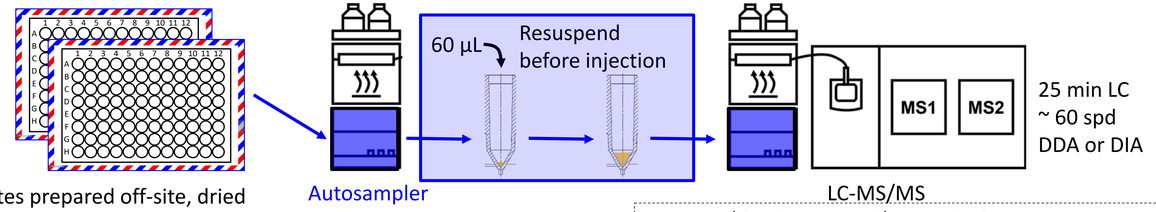
- Comparative Biology
- Detect Immunological Shifts
- Detect Viruses and Pathogens

- Implement the dual-trap single column method[1] at nanoflow[2]

[1]Kreimer, S. *et al.* Analytical chemistry **94**, 12452-12460 (2022).
[2]Kreimer, S. *et al.* Analytical chemistry **95**, 9145-9150 (2023).

Methods

Off-site processing to on-site high-throughput



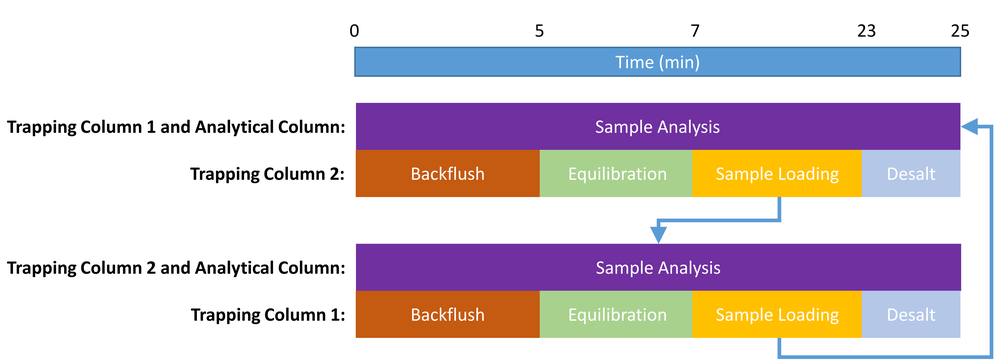
Plates prepared off-site, dried down, and clean peptides sent to NIST Charleston

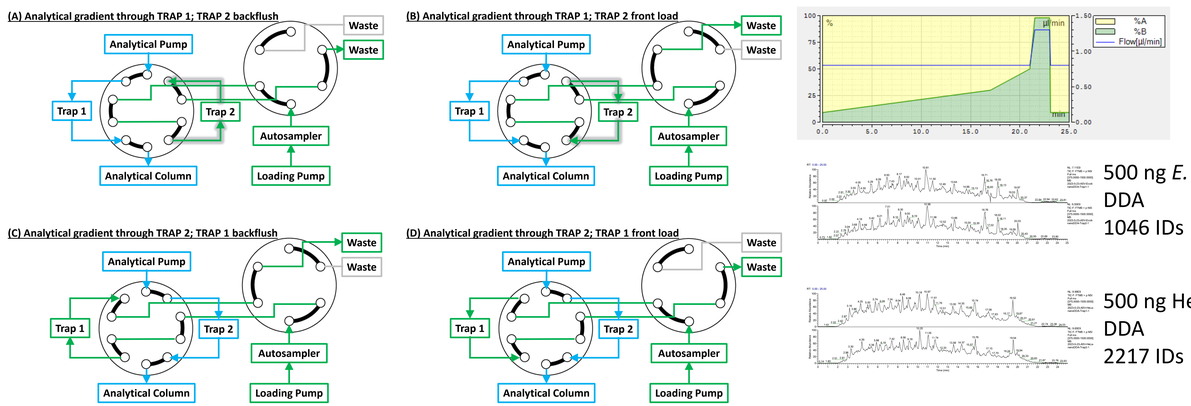
Autosampler

LC-MS/MS

Ultimate 3000 – Thermo Fusion Lumos
Trapping (at 15 µL/min): Thermo C18 PepMap 100 µ-precolumn trap cartridges (300 µm i.d x 5 mm)
Analytical (at 800 nL/min): Thermo Acclaim PepMap RSLC 2 µm C18 column (75 µm id x 15 cm)

Dual-trap single column (DTSC)



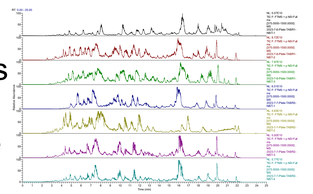


500 ng *E. coli*
DDA
1046 IDs

500 ng HeLa
DDA
2217 IDs

Performance

- Received 142 undepleted plasma processed off-site
- Plate design included external QCs and sample pool QCs, four sets per plate
- NIST SRM 1950 Human Plasma was the EQC, and showed reasonable consistency across 72 h



- Even at nanoflow, the system showed remarkable consistency and robustness

Conclusions

- 57 spd with pre-digested samples from external collaborators, minimal user input
- Need in-plate and offline EQC to monitor instrument performance across the run
- Would benefit from internal control spike into each well to provide more quality metrics and monitoring
- Ongoing work will optimize DDA and DIA methods for different sample types (high dynamic range like undepleted plasma, or less dynamic samples like tissue lysates) and quantify effects of gradient length on proteome depth