Cedars Sinai

WP 241: Maximizing Throughput with Parallelization in Large Cohort and Single Cell Proteomic Analysis

When: Wednesday, June 8th; 10:30am – 2:30pm CT

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Introduction

Single-cell and large cohort studies of disease require high throughput to appropriately capture population heterogeneity over thousands of samples or cells. Depth of analysis can be traded for throughput, but the time spent on loading the sample, flushing the column(s), and re-equilibrating the system reduces the ratio of time spent acquiring meaningful data to total operation time (instrument utilization, IU). We maximize IU in rapid analysis (15 min/sample) of single cells and blood plasma using a novel dual-trap one-column configuration which parallelizes trapping column cleaning, equilibration, and sample loading with analysis of the previous sample. We achieved 90% IU in low micro-flow analysis of plasma and based on preliminary data can achieve 80% IU in nano-flow analysis of single cardiomyocytes.

Methods

The dual-trap one-column configuration can be assembled on any LC system with a 10-port 2-position valve, a second 2-position valve with at least 6 ports, an analytical pump, and a loading pump (e.g. Ultimate 3000 (Thermo)). The analytical gradient is directed through one of the trapping columns installed on the 10-port valve on to the analytical column. Loading pump flow is directed through the auto-sampler into the second valve which controls the direction of flow through the second trapping column to allow back-flushing and forward-loading of the subsequent sample. In the next method the 10-port valve is switched to analyze the sample on the second trapping column, while the first trapping column is cleaned and loaded with the following sample.

Preliminary Data

Our plasma platform uses two 50 x 0.3 mm columns packed with 5 um Kinetex C8 particles (Phenomenex) for trapping peptides and CapLC column (Thermo) for analytical separation. The separation is carried out at 9.5 uL/min and a Newomics 8-nozzle emitter electrosprays the peptides into TIM-TOF Pro (Bruker). We optimized sample loading, ion accumulation, ramp times, and PASEF-DIA windows to achieve the highest possible identification of proteins and peptides in 15 minutes of analysis. The acquired data were analyzed with the alpha version of the Bruker modified DIA-NN. The platform reproducibly quantified 600-700 proteins and 3000-4000 peptides and peptide elution spanned 1-14.5 minutes, thus achieving 90% IU.Our single cell pipeline was benchmarked with 32 cardiomyocyte cells isolated by CellenOne into a 384-well plate. The cells were lysed in DDM (n-dodecyl b-D-maltoside) and digested with trypsin in a sub microliter volume. The low volume dried rapidly and the Ultimate 3000 auto-sampler was used to re-suspend and inject the cells from the isolation plate. In preliminary evaluation, direct injection unto a 15 cm x 75 um internal diameter PepSep column packed with 1.9 um C18 phase was used. Data were acquired with PASEF-DDA on the SCP-TIMS-TOF (Bruker) and analyzed with FragPipe 17.1. A total of 1374 proteins, 15654 peptides were identified from all cells, with an average of 920 proteins per cardiomyocyte.Method run-time was 30 minutes with 7 minutes to inject the 5 uL sample loop at 800

nL/min, 18 minute analytical gradient at 300 nl/min, and 5 minutes of high organic flush and equilibration at 800 nL/min. With the dual-trap one-column configuration and optimization of the gradient, it is feasible to reduce the run-time to 15 minutes with an allowance of 3 minutes for peptide elution through the trap and column for 80% IU without sacrificing depth of analysis.

Novel Aspect

The dual-trap one-column configuration and high throughput analysis of individual cardiomyocytes are novel.