Rapid discovery of differentially expressed proteins in T2D plasma samples using improved UHPLC chromatography and pSMART data acquisition

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Translational clinical proteomics links global protein discovery to targeted quantification with the ultimate goal of identifying and verifying clinically relevant disease biomarkers. When mass spectrometry (MS) is used for global quantitative protein profiling, the initial discovery phase typically results in a long list of differentially abundant proteins between sample groups. This initial list is subsequently filtered with a variety of parameters including statistical, ROC and pathway analysis tools to produce a short list of putative biomarkers for verification using targeted MS.

To increase global protein profiling efficiency and quantification, we implemented developments in the chromatographic and MS data acquisition steps. Incorporation of ultra-high performance liquid chromatography (UHPLC) using wider-bore columns provided greater loading capacities, gradient efficiencies, peak capacity and superior robustness relative to nanoflow. Recently, we introduced a new hybrid data acquisition scheme, pSMART, that facilitates reproducible MS and narrow data independent acquisition (DIA) with fast cycle times enabling better global profiling.

We applied these new workflows to investigate protein differences associated with type 2 diabetes (T2D). Experiments were performed on a large-scale plasma cohort of 134 donor samples in 4 different sample groups (obtained with full donor and IRB approval). Prior to pSMART data acquisition, the plasma samples were not submitted to any fractionation or

depletion procedures. A Q Exactive Plus and Orbitrap Fusion MS were used for data acquisition using two different methods, standard data dependent (DDA) for spectral library creation and pSMART data acquisition for quantification and peptide ID confirmation.

The results demonstrated extremely robust chromatographic precision with retention time shifts of 1% or less. A total of 581 (grouped) proteins and 5566 peptides were quantified. The peak area %CV of technical replicates (from blood tube to analysis) was ca 5%. Abundance ratios and ROC AUC scores were calculated and the data were imported into Ingenuity Pathways Analysis (IPA) for biological evaluation.