Introduction

Proteomics technologies are an integral part of biological and clinical research. Significant contributions by the proteomics field are driven by the diverse and advanced analytical approaches employed to comprehensively characterize proteins, including quantitative analysis of proteome variations, modifications, and interactions. The ABRF Proteomics Standards Research Group (sPRG) focuses on designing and developing standards and reagents for mass spectrometry-based proteomics applications. The sPRG is currently conducting a study focused on validating a large set of standards for interassay, intersite, and interlaboratory normalization in label-free as well as in quantitative stable isotope labeled-based analyses. The standard has been formulated as two mixtures: 1,000 stable isotope 13C5N2-labeled synthetic tryptic peptide cores and peptides mixed with a tryptic digest from HEK 293 cell lysate.

Methods

Sequences of synthetic peptides were derived from approximately 550 proteins, conserved across proteomes of commonly analyzed species, Homo sapiens, Mus musculus, and Rattus norvegicus. Peptides represent a wide range of hydrophilic and hydrophobic points and total points of typical complex proteomic samples. The 1,000 isotope-labeled peptides were spot-synthesized by gpi.com. Individual peptides were reconstituted, combined, and digested by solid-phase extraction. HEK cell lysates were prepared by RIPA lysis and sonication, and proteins were methanol precipitated, neutralized, and digested with Lys-C and trypsin. The HDX proteolytic peptide mixture was digested by solid-phase extraction. For the combined formulation, 1 µg of synthetic peptide was added to 5 µg of the HDX peptide mixture. The synthetic peptide mixture and the combined synthetic and HDX-derived peptide mixture were characterized individually by sPRG members representing multiple LC-MS-based instrument platforms.

Characterization of Peptide Standards

![Characterization of Peptide Standards](image)

Label-Free Peptide Dilution Experiment

![Label-Free Peptide Dilution Experiment](image)

Protein Identification of ABRF Peptide Standards

![Protein Identification of ABRF Peptide Standards](image)

Data Processing and Analysis

50 Data Dependent (IDA) and 6 Data Independent (SWATH) LC-MS runs were performed using 4 types of instruments at 6 laboratories. Mascot mgf files were generated from all 50 IDA files using MSConvert (ProteomeSoftware) on Thermo Scientific. A new file and All Scans MS Data Consumer 1.3 software. Protein identification was performed using Mascot 2.4 and a concatenated ABRF peptide sequence and the human Swiss-Prot database. Mascot.dat files were generated with X! Tandem and uploaded to the Proteome Experimenter Laboratory Free Precursor Ion Analysis was performed using Skyline 1.4. Briefly, Mascot.dat files were imported to generate a spectral library. Raw files were integrated and the peak area results were exported to csv file which were imported into Excel and R 2.14.2 for downstream bioanalytical analysis.

Protein ID of HEK

All seventeen HEK LC-MS IDA runs were combined and searched with Mascot against the human SwissProt database. The search results were filtered to 1% FDR on the peptide level and on the protein level resulting in 4,171 distinct proteins from 128,170 distinct peptide identifications greater than identity.

Conclusion

The Proteomics Standards Research Group reports the progress of its efforts to develop a comprehensive proteome normalization standard, designed to represent proteins of various concentrations spanning three orders of magnitude. 1,000 isotope labeled peptides were synthesized and analyzed in the laboratories of sPRG members with various instruments and fragmentation methods. Greater than 99% of the proteins were identified during the validation runs. In peptide dilution experiments, the majority of peptides behaved as expected with a linear retention time retention time (R²) of 0.9838. The peptides were spiked into a HEK digest and demonstrated retention time retention time retention time variability in both retention time and peak area.

Acknowledgements

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Participate in the sPRG2013 Study and have fun with quantitation and refining your analytical approaches! Visit www.abrf.org/sprg