Report: Top Down Proteomics and Top Down Mass Spectrometry: Emerging Technologies

Date/Time: November 2nd, 2021, 5:45 – 7 PM EST

Location: Philadelphia, PA, and virtual (via Zoom)

Presiding: Joe Cannon, Bristol-Meyers Squibb (In-person); Caroline DeHart, Frederick National Laboratory for Cancer Research (virtual); Frederik Lermyte, Technische Universität Darmstadt (virtual)

Estimated Attendance: 76 (in-person and virtual)

List of Presenters/Panelists:

Tian Xu, Michigan State University

Luca Fornelli, University of Oklahoma

John McGee, Northwestern University

Lloyd Smith, University of Wisconsin - Madison

Following a brief summary of recent publications, new collaborative projects, and ongoing outreach initiatives of the Consortium for Top-Down Proteomics by Paul Danis, the workshop agenda comprised four 5-7 minute talks followed by up to 10 minutes of subsequent discussion. The first talk, presented by Tian Xu from Liangliang Sun's group at Michigan State University, provided an overview of capillary electrophoresis-mass spectrometry (CE-MS) applied to qualitative and quantitative top-down proteomic analysis of two colorectal cancer cell lines on a Q-Exactive HF mass spectrometer. Highlights included the identification of 23,000 human proteoforms within these two backgrounds, the largest number identified by a single study to date, and several marked proteoform-level differences between the two cancer contexts, both in terms of PTM profile and relative abundance. The second talk, presented by Luca Fornelli from the University of Oklahoma, detailed the method and technology development underlying high-throughput proton-transfer charge reduction MS3 fragmentation via SIM walking on Orbitrap Fusion Lumos and Orbitrap Eclipse mass spectrometers. Highlights included the benefit of the new HCD fragmentation algorithm and higher S/N ratios available on the Eclipse, which provided significantly improved data across a broad MW range in fewer LC-MS runs, along with commercially available instrument methods and software to allow easier translation of the optimized PTCR-Eclipse workflow to audience members' own laboratories. The third talk, presented by John McGee from Neil Kelleher's group at Northwestern University, outlined the principles underlying multiplexed individual ion mass spectrometry (I2MS) on Orbitrap-based platforms for enhanced targeted proteoform detection and characterization. Highlights included the identification of over 500 proteoforms from a single GELFrEE fraction, far exceeding the identifications provided from that sample by state-of-the-art denaturing top-down proteomics methods, and a broad range of ongoing studies directly facilitated by I2MS (e.g. patient Sars-CoV-2 antibody surveys, combination with nano-DESI for spatially-resolved proteoform identification from mouse brain tissue). The final talk, presented by Lloyd Smith of the University of Wisconsin-Madison, covered both the inherent challenges and required future innovations for bioinformatic analysis of proteoform-level data. Each subsequent Q&A session led to a lively discussion, meaning that the workshop audience remained fully engaged until the end of the allotted time. Particular topics of interest

included improved sensitivity for proteoform detection, accuracy of top-down quantitation methods, and ease of translation of specialty methods to other laboratories for broader adoption. These are currently under consideration for themes of the next Top Down Proteomics Workshop, to hopefully be held in Minneapolis, MN, in June 2022.