Table 12: Intact Analysis/Top Down Analysis

**Facilitator:** Srikanth Kotapati, *Bristol-Myers Squibb Company*

**Scribe:** David Passmore, *RubrYc Therapeutics Inc.*

**Scope:**
Mass spectrometry provides critical information for the characterization and identification of proteins. Top down analysis is increasingly being preferred over conventional peptide based and sub-fragment based approaches of protein characterization, since the protein is introduced into the mass spectrometer in its “native” state. However, there are several challenges associated with top down analysis such as protein separation, sensitivity and data complexity. This roundtable aims to discuss the applications, challenges and recent advances in Intact/Top Down Analysis.

**Questions for Discussion:**
1. What are the traditional and novel applications of Top Down Analysis?
2. What are the challenges of Top Down analysis, specifically in non-covalent protein complexes and Protein conformation analysis?
3. What are the recent advances in Top Down analysis in terms of separation techniques, instrumentation and data analysis?
4. Can Top Down approach be used for quantitation/bioanalysis?

**Discussion Notes:**
- The table was well attended with a mix of attendees from BioPharma, Biotech, and Vendor companies. Active participation from several of the attendees.
- Standard applications of top down include QC of protein identity by intact and sub-unit analysis.
- Native mass spectrometry was used by many of the table participants. Applications included analysis of non-covalent aggregates, multi-subunit protein complexes, and ADC lacking intra-chain di-sulphides.
- New trends in Native mass spectrometry include very high-resolution mass spectrometers that allow for better deconvolution of raw data, and several people were utilizing charge-stripping techniques such as “ion sponge” functionality developed by Thermo.
- There was an in-depth discussion of best practice around deconvolution of raw data files with the sliding window method cited as an ideal solution for identifying poorly resolved proteins.
- Several people at the table were using top-down methods for bioanalysis, however, few were attempting absolute quantitation by top-down methods.
- Overall summary is that top-down methods were frequently utilized as QC and identity testing applications, with growing use of native mass spec to characterize non-covalent protein complexes.