Table 12: Trending MS Topic - Analytical Mass Spectrometry Challenges with New Modalities

Facilitator: Anders Lund, *Synlogic, Inc., Cambridge, MA USA*
Scribe: Jason Rouse, *Pfizer, Inc., Andover, MA USA*

Discussion Notes:

A small group of industry and vendor partner colleagues discussed the application of mass spectrometry techniques and methods for two newer therapeutic modalities: Adeno-associated viruses (AAVs) for gene therapy applications and messenger RNA (mRNA) for vaccine applications.

AAV Characterization:
AAVs are large megadalton molecules that contain one linear, single-stranded deoxyribonucleic acid (ssDNA) genome enclosed by an outer protein shell. The outer shell of AAV contains sixty VP1, VP2 and VP3 capsid protein subunits in a ~1:1:10 ratio, respectively. The capsid subunits have posttranslational and potential chemical modifications that may modulate infectivity and potency. The roundtable attendees discussed (at a high level) the different mass spectrometry methods involved in the characterization of AAV. Analysis of the capsid proteins via LC-MS/MS - peptide mapping and LC/MS – intact/subunit methods is relatively straightforward. The group primarily discussed charge detection mass spectrometry (CDMS) analysis of AAV and compared/contrasted it to analytical ultracentrifugation (AUC), which is the current industry standard for evaluating the ratio of empty capsids, partially packaged capsids and fully packaged capsids. Both techniques can resolve the low level, partially packaged capsids amid the empty and full capsids of greater abundance. CDMS appears to have slightly better resolution over AUC. As a watch out, the partially packaged capsids can slide under the main peak in AUC, which is something that CDMS can help ensure doesn't happen (as an orthogonal technique). The group believed that AUC requires higher amounts of sample than CDMS, which is an important consideration for gene therapy products since AAV material amounts are typically very limited. CDMS instruments are not commercially available for purchase, but samples can be submitted to academic or vendor labs under a fee-for-service agreement at the current time.

Oligonucleotide and mRNA Characterization:

LC-MS/MS analysis of synthetic oligonucleotides is required by health authorities to confirm the intended primary sequence. Complete sequencing of oligonucleotides with 20 residues is doable with current MS technologies but it remains challenging. Usually, multiple charge states need to be fragmented in multiple acquisitions to gain full sequence coverage. As well, significant optimization of the collisional activation conditions and instrument parameters are required. It was mentioned that the new zeno trap and electron activated dissociation (EAD) technologies in the new SCIEX ZenoTOF 7600 system may boost low level fragment ion signals and enhance the fragmentation patterns, respectively, for more efficient elucidation of oligonucleotide sequence. In contrast, mRNA molecules are comprised of 1000-10000+ nucleotide residues. For characterization of mRNA primary structure, enzymatic digestion and oligonucleotide mapping via LC-MS/MS is performed (similar to proteolytic peptide mapping of proteins). Here, each oligonucleotide is identified by accurate mass and the unique fragmentation pattern that correlates with the respective sequence. With mRNA, one of the major challenges is unambiguously identifying all oligonucleotide fragments after digestion. Some oligonucleotides will have the same nucleotide composition and accurate mass but a slightly different sequence. Having a rich fragmentation pattern and contiguous 5’ and 3’ gas-phase fragment ion series for multiple charge states is very important for finding both single and multiple nucleotide sequence differences for accurate identification of each enzymatically derived oligonucleotide in mRNA. It would be highly beneficial to industry colleagues if academic and vendor groups could investigate improved new mass spectrometric fragmentation techniques and instrument-specific methods/conditions for enhanced oligonucleotide sequencing.