Table 8: Trending MS Topic: Analytical Mass Spectrometry Challenges with New Modalities, Charge Detection MS

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Scope:

Mass spectrometry (MS) has been a dominating tool for supporting development of Biotherapeutics in the Biopharma industry. However, in recent years, as novel modalities emerge with increasing complexity, such as bi-specific mAbs, vaccines, and gene therapies, challenges within existing mass spectrometry techniques have come to the forefront, especially when it comes to the characterization of large molecules and heterogenous mixtures. One promising approach to tackle these limitations is with Charge Detection Mass Spectrometry (CDMS), a single particle MS based technology. As novel modalities are increasing in market share, CDMS is becoming more attractive as a viable tool for its ability to characterize both large molecules and heterogeneous mixtures, for example: highly glycosylated proteins, aggregates, and genome packed viruses like adeno-associated virus (AAVs). As an emerging technology, CDMS is still in the earlier stages of application to these novel modalities, but as hardware and applications are further developed and refined, it is anticipated to have significant contributions to the field.

Discussion Topics:

This roundtable will include a discussion of existing mass spectrometry techniques and current challenges pertaining to analysis of these novel modalities in addition to discussions about CDMS technologies and their adaptation into this space. How CDMS technology is currently employed, the general perception of how the technology will evolve, advantages and disadvantages to the use of this technology in characterization of complex biotherapeutics, and tips and tricks for implementation into the laboratory will be discussed. We are all making an effort towards a common goal: to develop and adapt the most efficient and effective technology to ensure development of more complex therapeutics that are safe, effective, and have good critical quality attributes.

Discussion Notes:

- In what areas do we see CDMS having key value?
    - Full, empty, partially full capsids, AAV
        - HPLC separate empty and full, not separating partial
        - Need CDMS to see partial, determine ratio, charge variation
        - Still immature, in early stages
    - Intact viruses, VLPs, etc.

- Are there any CDMS systems that are commercially available?
    - Yes, Thermo Q-Exactive UHMR, Direct Mass Technology (DMT) available as an add-on, that is the only one *currently* commercially available
    - There is a spinoff company from Prof. Martin Jarrold – MegaDalton (electrostatic IT), accepting samples as fee for service
    - Waters is working on CDMS using Prof. Jarrold's technology, still not commercially available
- What is the accuracy of quantitating partial vs. full capsids? Where do you draw the line of partial vs. full?
    - Mapping expected genome mass, look at peak width/distribution for partially full capsids. That can help with relative quantitation for partial vs. full, but it is still good to compare against AUC for partially full capsids.
- What is CDMS sensitivity like?
    - Because you are trying to look at single ions, you want to work with samples that are low concentration. Sensitivity is great since you are looking at single ion events
    - Heterogeneity is a consideration
- Where do we see the position of CDMS in process development?
    - When looking to tune, driving towards more towards full capsid
    - Sample consumption is the advantage here. Others options when looking at empty vs full capsids include AUC, mass photometry
- Electrostatic interactions of HCPs on capsids: is it possible to look at those using CDMS?
    - In such low concentrations, this may be tricky
    - 5 MDa vs. 50-100 kDa seeing shift may be difficult
    - Even going back to therapeutics, antibodies. Express impurities to study protein-protein interactions
- Robustness of CDMS:
    - For those who have used it, the primary challenge to robustness will always be spray; typically using nESI so getting spray started, keeping spray stable may be a challenge. This will be sample dependent and often having some sort of native MS background/experience is critical here. This is not to say that it can't be improved or there are not alternatives, but this is a current challenge that frequently appears regarding robustness of the technique, but is not really about the technique of CDMS itself/inherently.
- When working with large ions, sending to C-trap is there a space charge effect:
    - AGC always optimizing ions in the C-trap. AGC off for CDMS, sending very few ions to Orbitrap at one time
- Is any additional cleaning needed?
    - No additional cleaning needed, since using such little amounts of sample
- Non-large molecules, is it being used for other heterogeneous species, e.g. glycans, etc.?

    - Yes. Advantages of CDMS are not limited to large ions. It can also be quite useful looking at highly heterogeneous species (e.g., spike protein)
- What needs to be done before this technology becomes more widely adopted by industry?
    - Online analysis would help less experienced users, improve robustness
- If there is a mixture in the sample, it is possible to measure in the same spectra
- What does sample prep look like?
    - Very analyte dependent
    - Simple buffer exchange for a lot of analyses
    - Considerations include removing adducts: options for this include extra buffer exchange, using in-source trapping for desolvation, etc. but this is also going to be analyte dependent