Roundtable Session 1 – Table 5 – Mass Spec for Higher Order Structure in Biologics Development

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Abstract:

Determination of protein higher order structure (HOS) is a key component in defining critical quality attributes (CQAs) for any biological product. It has been widely accepted that changes in HOS can impact quality, safety, and efficacy of biopharmaceutical products with increased potential for immunogenicity and loss of biological function. Historically, HOS of biological products has been characterized and monitored by various traditional biophysical approaches that provide either global or local HOS information. These approaches include CD, IR, DLS, DSC, as well as techniques that afford higher resolution, including NMR and X-ray.

Recently, mass spectrometry has emerged as an important analytical approach to study HOS of biomolecules. Over the past decades, MS-based protein footprinting technologies have become widely used in structural and functional biology due to ease of use, ability to provide rich information with relatively shorter turnaround time, as well as technological advancement (including sample labeling technology, separation/MS hardware, as well as data processing software).

This roundtable will focus on multiple MS-based protein footprinting technologies, as well as strategies and challenges during such applications to enhance product and process understanding.

Discussion Questions:

1. What are the most common MS-based protein footprinting technologies used during biopharmaceutical discovery and development?

    a. What are the advantages and disadvantages of each technology?

2. Rationales to employ MS-based protein footprinting technologies (i.e., compared to traditional biophysical technologies)

3. What are the major challenges in implementing MS-based protein footprinting technologies in your organization?

4. What is the future of this area?

Notes:

Our roundtable discussed a specific HOS case study involving a novel antibody construct, the single-chain variable fragment (scFv). The scFv construct is a fusion protein with the variable regions from the heavy (VH) and light chains (VL) of immunoglobulins connected with a short linker peptide. The particular discovery-phase scFv construct discussed in the roundtable was prone to aggregation (J&J, mAbs, 2023). The roundtable group debated the importance of understanding this aggregation in terms of collecting experimental HOS data as well as analyzing the protein sequence and/or crystal structure with specialized software to understand if there were distinct molecular features inducing the aggregation. A computational assessment of HOS can be helpful in terms of predicting charge and hydrophobic patches to facilitate enhanced understanding of possible aggregation mechanisms. The table participants felt that site-specific experimental HOS data was needed to best understand the contact points so that the novel construct could be further engineered with confidence to prevent aggregation. Relying on software alone has the potential to reveal too many possible sites – of which may or may not be credible – thereby hampering subsequent protein engineering of the construct. MS-based techniques such as fast photochemical oxidation of proteins (FPOP) and hydrogen-deuterium exchange (HDX) were discussed as key, site-specific HOS experimental technologies to better understand the aggregation contact points. For HDX, there is a revised version of HDX MS that involves sub-zero temperatures. The colder temperatures allow for the use of extended gradients for better peptide separation, detection and quantification without having increased back-exchange effects.