Table 9: Structural MS - New Developments in Limited Digestion, HDX, FPOP, Charge Detection, and Ion Mobility

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Table Scope:

Structural mass spectrometry (MS) encompasses a wide variety of techniques that provide complementary information on the higher order structure (HOS) of molecules. This information can be used to gain insights on residue accessibility and reactivity, binding interfaces, epitopes, and conformational shifts all of which can be harnessed for accelerating drug-development. In this roundtable discussion we will explore the current utility and opportunities for structural MS in the various phases of drug development. In early phase development uses may include gathering data to support intellectual property (IP) claims, epitope mapping and/or drug candidate selection. MS derived HOS information may also find use in enabling phase 1 development and investigation new drug (IND) filing. Further along in development, structural MS could be used for product comparability studies that enable process changes. Post-approval, structural-MS data could support biologics license application (BLA) filings. New technological developments in sample processing and instrumentation will ensure an increasingly important role for structural MS in drug development.

Discussion Notes:

The first set of questions pertained to the adoption of structural MS techniques in early discovery and identification. Questions included: how and when are these techniques being utilized across different groups? Are people actively using these techniques in their workflows? What are the limitations? Most initial conversations pertained to MS-based footprinting techniques:

- Footprinting techniques such as HDX and FPOP are currently being utilized for epitope mapping and screening in a high-throughput manner.
- However, capturing conformational states and dynamics is difficult. All data is typically congested together, so specific conformations are difficult to measure/identify. Also, 10 – 15% of labeling is typically recommended in order to prevent the reagent from influencing the dynamics and function of the protein system.
- Often, a combination of footprinting and computational methods would provide valuable structural information, especially when a crystal structure is not readily available. Rather than overlaying data to an existing crystal structure, footprinting can offer a starting point for subsequent computational methods such as docking simulations.
- MS-based footprinting techniques are becoming especially popular right now due to the development of robust workflows that feature better reagents, software, and automation. Most importantly, footprinting techniques are the “sweet spot” between resolution and speed.
- Despite the robustness of labeling techniques, some limitations exist. For example, some molecules in the early discovery stage possesses better solubility in DMSO (e.g., 0.1%). However, DMSO is a radical scavenger, which limits the applicability of FPOP for molecules that are stored in DMSO. Within FPOP, the use of hydrogen peroxide can also cause oxidative damage, and the laser irradiation (typically 266 nm) used to photolyse the hydrogen peroxide to produce •OH can

be damaging to protein structure because it is within the absorption region of aromatic amino acids. In the context of HDX, H/D scrambling can occur during collisional activation, which can further complicate HDX data analysis.
- Carbene chemical footprinting was discussed as potential alternative that addresses the limitations of other footprinting techniques like those of FPOP and HDX described above.
- Cross-linking MS has also provided valuable information regarding protein structure and protein-protein interactions. However, the reaction needs to be optimized in order to prevent artifactual linking that is not representative of the interactions that are being probed. The ideal reagents for cross-linking are still being sought after.

The discussion then transitioned into talking about ion mobility and its use in structural MS. Most of the conversation revolved around how ion mobility could be adopted by biopharmaceutical industries. Some questions included: what kind of information can ion mobility provide you with? How does ion mobility compare to conventional separation techniques such as liquid chromatography?

- Ion mobility can achieve fast separations (ms timescale) compared to conventional liquid chromatography and can achieve separations that are often difficult to achieve (e.g., isomers).
- Ion mobility is useful in structural measurements because its output, drift time, can be converted into collision cross sections which can be compared to theoretical cross sections derived from computational workflows. These measurements can be achieved using a small amount of sample in the presence of structural heterogeneities.
- Coupled with collision induced unfolding (CIU), ion mobility can reveal subtle structural differences between molecules that possess similar cross section values but different gas-phase unfolding patterns and stabilities.
- Ion mobility and CIU have also been utilized to study and characterize the way proteins aggregate after thermal and pH stress.
- Much work in ion mobility-mass spectrometry revolves around connecting gas-phase structures to solution-phase structures. Much of this work is being performed alongside molecule dynamic simulations of proteins in the absence of bulk solvent.
- Resolving individual conformations/structures using ion mobility of large proteins is still difficult. Despite the increase in resolution in ion mobility instruments such as the Waters Cyclic platform, these are not sufficient in resolving conformation of large proteins compared to small molecules and peptides.
- However, high resolution ion mobility is very useful in separating different kinds of peptides without the need for MS^2 experiments for quantitation that is typical using conventional LC-MS workflows. The risk of ion suppression, however, would exist because peptides are not being separated prior to the electrospray process.
- Regardless of the many benefits of ion mobility in structural MS, it has its limitations as a routine methodology in the biopharmaceutical industry. Chief among this is the use of nano-electrospray ionization when performing native IM-MS experiments, which minimize the technology's throughput. Moreover, it is a technique is still being developed and validated within the context of biologic characterization and assessment in a regulated environment.

Charge detection was briefly discussed in the context of characterizing the heterogeneity of highly glycosylated proteins as well as very large supramolecular assemblies such as viral capsids.

There was minimal discussion on limited digestion. Most of the conversations revolved around how it would help crystallize certain portions of a protein as well as assisting in mapping epitopes in highly complex protein systems.