Roundtable Session 1 – Table 9 – Mass Spectrometry & Predictive Stability

Facilitator: Steven Pomerantz, *Johnson and Johnson Innovative Medicine*

Scribe: Leah Wang, *Pfizer Inc, Chesterfield*

Abstract:

The rapid growth of protein-based therapeutics has revolutionized modern medicine, offering treatments with high specificity and efficacy for a wide range of diseases. However, the successful development, manufacturing, and long-term storage of protein drugs hinge critically on their molecular stability. Traditionally, experimental methods such as stress-testing and accelerated stability studies have been the gold standard for assessing protein stability, yet these approaches are time-consuming, resource-intensive, and often lack mechanistic insight. In recent years, the convergence of advanced analytical techniques — most notably mass spectrometry (MS) — with computational predictive models has ushered in a new era for understanding and forecasting protein stability.

Mass spectrometry offers unparalleled sensitivity and resolution for the characterization of protein therapeutics. Techniques such as native MS, hydrogen-deuterium exchange (HDX-MS), and top-down/bottom-up proteomics enable detailed mapping of structural features, post-translational modifications, degradation products, and subtle molecular conformations in complex formulations. MS-based methods have proven invaluable for detecting chemical and physical instabilities, such as oxidation, deamidation, aggregation, and fragmentation—events that can dramatically impact therapeutic efficacy and safety. Moreover, high-throughput MS approaches are increasingly being integrated into quality control workflows, providing real-time data to support process development and batch release.

Complementing experimental MS data, predictive stability modeling harnesses machine learning, molecular dynamics simulations, and bioinformatic algorithms to forecast a protein's susceptibility to degradation under various conditions. These models leverage both intrinsic sequence information and extrinsic factors, such as buffer composition and temperature, to anticipate structural changes and chemical liabilities. By incorporating MS-derived molecular fingerprints, predictive algorithms can be trained to recognize stability patterns with greater accuracy, enriching risk assessments and guiding rational formulation strategies.

The synergy between mass spectrometry and predictive stability frameworks extends into regulatory sciences as well, informing regulatory submissions and comparability assessments for biosimilars. As the biopharmaceutical industry seeks to accelerate drug development timelines, reduce failure rates, and optimize product shelf-life, the integration of MS analytics and AI-driven stability predictions emerges as a transformative paradigm.

Despite these advances, significant challenges remain. The complexity of higher-order protein structure, the possibility of unforeseen degradation pathways, and the variability inherent in manufacturing conditions present ongoing obstacles to reliable prediction. Future directions include further harmonization of MS-based data with multi-modal physical measurements, the incorporation of real-world storage data into predictive models, and the development of more transparent AI frameworks.

Discussion Questions:

This discussion seeks to address the following: Which MS methodologies are currently most impactful for protein stability assessment? How can predictive algorithms be validated and integrated into regulatory practice? Are there significant gaps in our understanding of the relationship between molecular attributes measured by MS and real-world stability outcomes? By fostering interdisciplinary collaboration between analytical chemists, biophysicists, data scientists, and regulatory experts, we can catalyze progress toward safer, more effective, and longer-lasting protein therapeutics.

Notes:

The roundtable brought together participants from both the research and development functions to discuss shared challenges and strategies in predictive stability, developability assessment, analytical techniques, data sharing, and collaboration.

1. Predictive Stability Modeling and Data Utilization

- The group discussed predictive stability modeling, highlighting the use of accelerated stability data, qualitative and quantitative aproach (mathematical modeling). They discussed the complexities of building robust training datasets and structuring large-scale data for future analysis.
- Accelerated Stability and Extrapolation: It was discussed that accelerated stability data at higher temperatures can be used by mathematical model to extrapolate and predict long-term stability at lower temperatures. There are examples of successful use of data extrapolation in regulatory filings, and this approach applies to datasets beyond just MS. It was discussed that this quantitative approach is not yet standard practice, and qualitative approach is still being used.
- The need to predict molecule stability early in research was emphasized, but some organizations lack sufficient training data for robust modeling, especially for certain molecule types.
- Data Structuring and Data Lakes: Participants noted challenges in managing large datasets, such as transitioning from historical unstructured databases to data lakes, and the need for improved correlation across data types.
- The group agreed that data scientists add significant value.

2. Analytical Techniques for Stability and Developability Assessment

- Techniques Compared: The group discussed a range of MS and biophysical techniques, including hydrogen-deuterium exchange (HDX), collision-induced unfolding (CIU), differential scanning calorimetry (DSC), nanoDSF (nano Differential Scanning

Fluorimetry), AUC, and mass photometry. They compared these methods in terms of utility, throughput, and limitations.

- CIU, nanoDSF, and DSC: Both CIU and nanoDSF require minimal material. Research groups often use nanoDSF initially and conduct DSC when more material is available. CIU provides gas-phase stability information but has lower resolution, and it is unclear if gas-phase profiles predict long-term solution stability.
- Mass Photometry: This technique was identified as a potential alternative to analytical ultracentrifugation (AUC), though its readiness for routine support is still under consideration.
- MS Limitations: Examples of challenges were noted in quantifying clipped species by MS, as it may not accurately reflect clipping percentages.
- HDX Adoption: HDX is widely used for probing higher order structure (HOS) and dynamics, but concerns remain about its complexity and throughput.
- Risk Assessment: Emphasis was placed on risk assessment in data packages, including categorizing issues by risk level. The group acknowledged that distilling complex data into a single risk score is difficult.
- Assay Coverage and Data Gaps: There are cases where reduced SPR response occurs without detectable PTMs or degradation hotspots, possibly due to HOS changes or disulfide bond scrambling. Limited resources in discovery can restrict comprehensive characterization, highlighting the need for a broader range of assays and consideration of high-throughput MS-based HOS analysis. Within the participants' organization, HOS-MS methods like HDX and FPOP are used for investigations or formulation development, in addition to epitope mapping.
- Immunogenicity Assessment: One participant mentioned that immunogenicity is assessed on a case-by-case basis for forced degraded samples, in addition to binding and activity.

3. Discovery to Development Data Handover and Risk Assessment

- It is acknowledged that the process is evolving
- Data Package Adequacy: The group discussed the typical data package transferred from discovery to development, with a focus on MS content. Development participants generally find the research data package satisfactory, recognizing differences in data depth between functions due to the broader scope in research and more focused analysis in development.
- In research MS data package, emphasis is often placed on CDRs due to fast screening and conserved Fc regions, while development conducts thorough analysis across the entire molecule, albeit for fewer candidates. This again highlights the importance of communication and collaboration between research and development. It is discussed and agreed within the group that PTMs alone are not sufficient and other attributes (e.g. fragmentation, high/low molecular weight) are important to be analyzed.
- One challenge discussed is that MS data is not always linked to bioassays in research, often due to missing bioassay data as a result of limited material or assay readiness. SPR is the common functional readout in research.
- Material Concerns: Discovery materials may be from non-CMC representative cell lines or processes, and at different scales.
- Collaboration and Knowledge Sharing: The value of ongoing communication and collaboration between research and development was emphasized, with the handover process evolving as organizations mature. Early-stage organizations may provide less data, while later-stage organizations develop more comprehensive packages. Alignment on necessary data is key to a successful transition. Meetings and collaboration efforts

between discovery and development teams are essential to align on data requirements and stress conditions. Joint meetings, harmonization initiatives, and sharing regulatory learnings from development to research were discussed and highlighted as beneficial practices.