Table 10: HDX-MS/Sample Prep

Facilitators – Kyle Anderson, *NIST* and Malcolm Anderson, *Waters Corporation*

Scope:
Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is becoming more widely used to characterize higher order structure and conformational dynamics of proteins. HDX-MS is well-suited to assess biosimilarity, binding sites, and stability. However, HDX-MS does present unique measurement challenges that have inhibited the adoption or full potential of the method in various stages of the product development life cycle. This round table will aim to generate discussion among participants to better understand challenges in HDX-MS and how best to overcome them.

Questions for Discussion

1. Where in your product development life cycle is HDX being used? Where would you like to see it be used?
2. What difficulties discourage you from implementing HDX-MS analysis in your work?
3. How is reproducibility? What are the main sources of error affecting reproducibility and what steps have you taken to reduce them?
4. What formulations, preparations, and modalities are problematic for HDX-MS and limit applicability?
5. What HDX software limitations exist and need to be addressed?
6. Would standards for HDX be helpful? What types of standards would be helpful or what information would you like to get from standards?

Discussion Notes:

- Participants would like to see a full dataset of an antibody, like the NIST mAb reference material. Proteins commonly used to test protease digestion efficiency (e.g. phosphorylase B) are not representative HDX references for antibodies and other biopharmaceuticals. Companies tend to use proprietary materials for reference sets in-house, but this data is not always shareable for external discussions.
- Commercial glycosidase columns to remove glycosylation prior to protease digestion would be very helpful for many biopharmaceuticals.
- Internal standards and instrumentation advancements to reduce back-exchange would be beneficial for comparisons between laboratories/instruments.
- Throughput is a concern for prospective and current HDX-MS users. HDX-MS can be labor intensive for acquisition, data procession, and data analysis. Vendors are making faster instruments with better separation (e.g. cyclic IMS), more efficient chromatography, and software better at correctly identifying isotopic distributions.