

Table 6: New Developments in Ion Mobility and Native Mass Spectrometry

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Scope:

Mass spectrometry (MS)-based methods have become increasingly important and useful for elucidating information about biomolecular structures. Recently, there has been an increased focus on using native MS measurements to provide structural insights of intact biomolecules, non-covalent biomolecular interactions/subunit stoichiometries, and protein-ligand dynamics. Strategies for examining biomolecules in their native-like state by MS are often more challenging than denatured mass spectrometry because of need for limited solution options, gentle tuning of the mass spectrometer, and specific needs for high-mass-range instrumentation. Ion mobility (IM) has been gaining traction as a powerful tool to elucidate information molecular size and shape which can be compatible to native MS. Other techniques such as FPOP, HDX, covalent labeling and CDMS can also provide complementary structural information.

This discussion will focus on the practical application of ion mobility and native mass spectrometry as they are utilized and apply to industry including discussions over availability of technologies, utility, applications, and limitations.

Questions for Discussion:

1. What structural information can you obtain from native MS, ion mobility and other mass spectrometry techniques such as FPOP, HDX, covalent labeling and CDMS that is useful for examining proteins in their native forms?
2. What are some of the barriers for implementing native mass spectrometry and ion mobility in industry? (i.e., equipment, reagents, software, reproducibility, demand, etc.)
3. What are some of the benefits and limitations of each of the commercially available ion mobility systems as they pertain to use in biopharma?
4. Is anyone in the pharmaceutical industry including native MS or ion mobility data as part of their filing packages to health agencies? If, yes what are they including? If no, why not and do you plan to in the near future?
5. What information is obtained by running MS in native conditions vs denatured?

Discussion Notes:

1. What structural information can you obtain from native MS, ion mobility and other mass spectrometry techniques such as FPOP, HDX, covalent labeling and CDMS that is useful for examining proteins in their native forms?
 - Epitope mapping and protein-protein interaction information are generally the most common uses for these techniques.
 - HDX seems to be the most popular technique in industry. Availability of commercial instrumentation for FPOP should go a long way in making it more wide-spread.
 - Informatics for HDX and FPOP may be a major limiting factor. Some companies are making strides to make this more automated. Could take many months.
 - There are starting to be companies that offer both HDX and FPOP services on a fee-for-service basis.
2. What are some of the barriers for implementing native mass spectrometry and ion mobility in industry? (i.e., equipment, reagents, software, reproducibility, demand, etc.)
 - MS compatible mobile phases/buffers/salts is always an issue. In some cases, we may not be measuring biologically/physiologically relevant attributes if we first need to exchange into an MS compatible solution.
 - Historically low throughput, a move towards automated sample handling/analysis and ability to use well-plates might be advantageous.
 - Nano ESI is often very tedious and low throughput when having to use glass emitters.
 - Chromatographic column options for low flow rates are very limited. More robust sources that can bridge higher flow rate separations with nano ESI will help here.
 - Calibrating high m/z range is difficult and often requires manual calibration. There are also very limited options for stable high mass calibrants.
3. What are some of the benefits and limitations of each of the commercially available ion mobility systems as they pertain to use in biopharma? What do we need from vendors to improve IM?
 - Software improvements are critical in order to take advantage of the benefits of IM.
 - Balance between flexibility of the software and ease of use. Some customers want one, some want the other.
 - Analyst training to learn how to interact with the IM data and take advantage of the extra dimension of separation.

- There is a need for vendor neutral software that can be used to analyze IM-MS data from any vendor. If analysts only had to learn one software for several instruments, everyone would benefit.
4. Is anyone in the pharmaceutical industry including native MS or ion mobility data as part of their filing packages to health agencies? If, yes what are they including? If no, why not and do you plan to in the near future?
- The consensus of those at the table is that native MS and IM data are not widely included in filing packages to regulatory agencies.
 - A lot of HDX data seems to be included in intellectual property filings as epitope mapping information.
 - Collision-induced unfolding may be a promising technique that could be used for characterization of biosimilars.
 - Scientists and regulatory agencies may be comfortable with surrogate assays that indirectly measure an attribute, so little motivation to introduce methods that more directly measure that attribute.
 - There is concern that some of these methods may generate data that are difficult to explain.
5. What information is obtained by running MS in native conditions vs denatured?
- Isomerization, conformational, or folding changes that could affect potency.
 - Non-covalent aggregation. Identifying what species are actually present in the aggregate.
 - Mass information for highly heterogeneous molecules that are better resolved when acquired under non-denaturing conditions
 - Mass information for non-covalent therapeutic molecules.