therefore MW), so powerful is that it does this with very high accuracy due to the technique's high level of resolution that can yield MW accuracy approaching a few parts per million. In addition, this is achieved with very high sensitivity (requiring a very small sample, a few picomoles or less) and with reasonably good quantitation in terms of the relative amounts of the different ionized molecular species that are observed. However, MS has another attribute that is also very important, making MS even more powerful. This additional attribute concerns the ability of mass spectrometers to fragment specifically selected molecular ions using methods such as collision-induced dissociation (CID), electron transfer dissociation (ETD), or electron capture dissociation (ECD) (Leurs et al., 2015). The ability of MS to pick ionized species (precursor ions) with specific *m*/*z* values and then fragment them at limited cleavage sites, but in a random manner to create a collection of overlapping smaller fragments that can be further separated and analyzed in terms of very accurate *m*/*z* values and relative amounts, is referred to as tandem MS, MS/MS or MS^2 (Siuzdak, 2003), or through a somewhat similar process developed by Waters called MS^e (Plumb et al., 2006; Waters Corporation, 2011).

The application of tandem MS can also be extended to the resulting smaller product fragments created from the first fragmentation process within the same run. Such a repetitive process of applying tandem MS to prior generated fragments is referred to more generally as MS^n, multiple stage, or sequential MS, and is an additional powerful attribute of MS. Although historically the utility of MS fragmentation has played an important role in the use of MS in general structural characterization, in the case of characterizing biopharmaceuticals its full capability (along with common enzymatic fragmentation outside the mass spectrometer) is realized when the fragmentation information generated is combined with the following list of prior key knowledge:

1. The known amino acid sequence of the biopharmaceutical's polypeptide chain(s).
2. The known corresponding MWs of the amino acid building blocks.
3. The known MW changes that will occur when specific PTMs are encountered.
4. The known specific points of cleavage when using various proteases or the nature of the limited number of specific cleavage points encountered when physicochemical fragmentation procedures inside a mass spectrometer are used.

Using this prior knowledge, the biopharmaceutical scientist can create a constraining database of possible MW fragments that could be generated during these fragmentation processes. This theoretical information is then employed in analyzing the actual experimentally measured array of MS-MW data generated from various fragmentation approaches. To facilitate these analytical comparisons, appropriate computer software is used to conduct informatic searches to help reconstruct and identify the initial species that were present in the injected sample. By so doing, very accurate and knowledgeable qualitative and quantitative conclusions about the primary structure of a biopharmaceutical and its heterogeneity can be achieved. Structural information that can be extracted from these MS studies include the following: