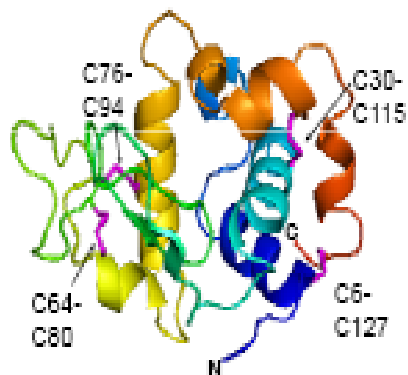
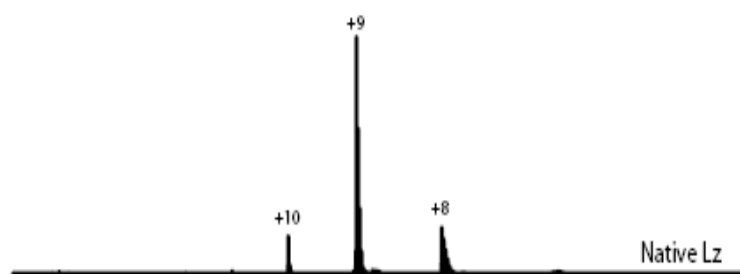


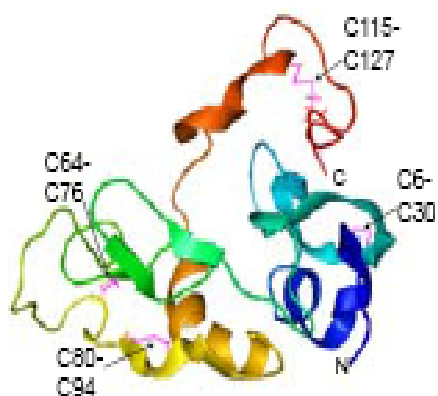
Natively Oxidized Lysozyme



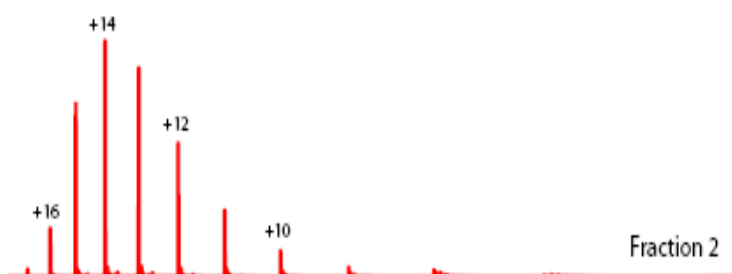
ESI Charge Envelope of Natively Oxidized Lysozyme



Lysozyme Disulfide Scrambled Species



ESI Charge Envelope of Lysozyme Disulfide Scrambled Species



In the pharmaceutical industry it is necessary to continuously monitor each drug throughout the development process. There are various methods for monitoring the quality of protein biopharmaceuticals, including well-developed mass spectrometry methods for detecting unwanted post-translational modifications (i.e. oxidation & deamidation). These modifications rely on mass spectrometry to detect a mass shift upon occurrence. On the other hand, the mass remains the same when disulfide scrambling occurs, yet a protein's native conformation can be severely compromised. If this occurs, it can lead to the drug's inefficacy, protein aggregation, and/or an immune response. Currently, there is no simple way to detect disulfide scrambling in protein biopharmaceuticals, which should be monitored throughout the development process and throughout the drug's shelf life. A combination of proteolysis, liquid chromatography and mass spectrometry is used today to map the disulfide bonds of protein biopharmaceuticals.

We propose a method that would create a way to continuously monitor a protein biopharmaceutical for disulfide scrambling. First, we generate various disulfide scrambled species, map their disulfide connectivity, and acquire a profile of their charge envelope using mass spectrometry. As a result, we have a reference of each possible species when testing samples for stability over time. Second, there is difficulty when dealing with regions of a protein with a high density of Cys residues within its' sequence. We approach this problem with a fairly new fragmentation technique, electron capture dissociation (ECD), combined with various proteolytic steps and reverse phase chromatography to map the disulfide bonds within these Cys rich regions.