

Top-Down Mass [Spectrometry](#): Towards Protein Sequencing and Elucidation of Post-translational Modifications

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Abstract

In the growing [application](#) of mass spectrometry to clinical analyses, detection and characterization of protein variants by mass spectrometry has a long history. For proteins such as hemoglobins and transthyretin, characterization of amino acid substitutions, that can define genetic point mutations, and post-translational modifications, are integral to the determination of the pathogenesis of the diseases and is necessary for precise diagnosis. In our methodology development efforts for using MS to obtain complete protein sequence coverage and identify sites and occupancy of post-translational modifications at the protein level we have focused on top-down approaches using MALDI-reISD and multiplexed ESI-HCD MS/MS of proteins.

Using MALDI-reISD as a rapid means of sequence determination of hemoglobins variants, we observed that fragmentation roughly covered the first 50-60 amino acids on the N- and C-terminus of both the alpha and beta forms in a single experiment. The expected complexity resulting from the presence of two different proteins does not appear to present insurmountable difficulties for data interpretation.

We also explored ESI-HCD MS/MS and multiple precursor selection using the newly released Q Exactive mass spectrometer. Our initial results indicated that from the charge envelope of the intact form of the beta chain of human hemoglobin, five different charge states could be “multiplexed” or sequentially accumulated in the HCD collision cell to undergo simultaneous HCD. These summed ions MS/MS were observed to yield fragment ion mass spectra representative of all charge states HCD fragment ion tandem mass spectra obtained independently. The advantage of “multiplexing” the charged states was a considerable [savings](#) in time and the data obtained offered more complete sequence coverage than what could be obtained by using a single charge state

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