# How far can collision-induced dissociation take us in top-down characterization of monoclonal antibodies?

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**Abstract:**

Monoclonal antibodies (mAbs) are essential biotherapeutics, requiring detailed structural characterization to understand their stability, functionality, and therapeutic performance. This study explores the potential of collision-induced dissociation (CID) for top-down mAb characterization, focusing on disulfide bond cleavage and sequence coverage under varying conditions. Infliximab and other IgG mAbs were analyzed in native, partially denatured, denatured, and supercharged states using Orbitrap mass spectrometers. Preliminary results show that CID effectively cleaves interchain disulfide bonds, generating abundant intact light chain ions and high sequence coverage, particularly in complementarity-determining regions (CDRs). Optimized conditions, including in-source unfolding and higher energy collisional dissociation (HCD), significantly enhance fragmentation efficiency. Surprisingly, CID produces fragmentation patterns comparable to electron- and photon-based methods, offering a more accessible approach for disulfide bond analysis in top-down proteomics. Key instrument parameters, such as collision energy and gas pressure, influence fragmentation efficiency and product-ion abundances. Ongoing studies will expand to additional solution conditions, refining parameters to maximize sequence coverage and enhance structural insights. This work establishes new benchmarks for combining native MS with CID to study solvent effects on infliximab's structure, charge distribution, fragmentation, and light/heavy chain separation.

**Keywords:**

CID, Disulfide bonds, Light chain, Heavy chain, Sequence coverage