Abstract

Therapeutic Monoclonal Antibodies (mAbs) offers great versatility treatment areas, and are the fastest growing subset of the biopharmaceutical industy. In fact, mAbs are used to treat a variety of diseases and disorders, such as cancer and inflammation and autoimmune disorders. As therapeutic agents, mAbs presents a great potential, as they belong to a group of proteins, which already exists in the body. During treatment, therapeutic mAbs can utilize the immune system to combat their target antigen.

Several requirements are imposed by authorities, such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA). They demand an extensive characterization of structural, physicochemical and purity features of a mAb product, before it can be approved for clinical trials and subsequently released for patient treatment. Thus, fast and cost-efficiant methods for mAb characterization are important for the further development of mAbs and all biopharmaceuticals.

In this project we set out to improve Mass Spectrometry (MS)-based approaches for mAb characterization. We chose to focus on improving the approach for *de novo* sequencing and to perform a structural analysis of Immunoglobulin Gamma (IgG) molecules.
We have characterized proteases for the use in *de novo* sequencing. One of these proteases turned out to be a potential alternative to Arg-C. Furthermore, we developed a novel method to confidently discriminate between the isobaric residues Leucine and Isoleucine by Multiple-stage Mass Spectrometry (MS^n) analysis. Distinguishing between these residues poses a challenge in *de novo* sequencing and previous approaches has relied on Edman degradation for full primary sequence coverage. Finally, we discovered a confirmational change in native IgG molecules by using chemical crosslinking and Tandem Mass Spectrometry (MS/MS) analysis.

The findings in this study has made a considerable contribution to the improvement in the complete sequencing of mAb. Furthermore, the discovery of conformational changes in IgG structure upon recognizing an antigen may contribute to an optimization in the future design of mAb and Antibody-Drug Conjugates (ADCs).