**Overview**

- Potentially fast and facile method for sequencing of monoclonal antibodies
- Method should allow for distinguishing of isobaric amino acids

**Introduction**

Monoclonal antibodies (mAbs) have been shown to be effective treatments for various inflammatory diseases and autoimmune diseases. Determining the sequences of these proteins is critical for troubleshooting their performance and monitoring their molecular integrity. Unfortunately, currently available sequencing methods are time consuming, require significant amounts of sample, and rely heavily on the use of tandem mass spectrometric sequencing algorithms that often have low accuracies. Development of an accurate and efficient method for de novo sequencing antibodies is therefore worth pursuing. Here, we address shortcomings of previous methods by using 157 nm photofragmentation data along with fragmentation data obtained from post source decay to de novo sequence antibodies.

**Methods**

Monoclonal antibodies (mAbs) were purchased and denatured using a 6M guanidinium chloride solution. Subsequently, reduction and alkylation of the antibody was performed by incubating the antibody in 10 mM dithiothreitol at 37°C for 1 hour followed by incubation in 50 mM iodoacetamide for 30 minutes in the dark. Following this, LC-MS analysis was performed and mAb light and heavy chain masses were obtained as seen in Figure 2, thus confirming reduction of disulfide bonds.

An entire tryptic digest of mAb was placed on a MALDI spot for analysis. Large numbers of peptides per spot caused the signal intensity of each to diminish. Next, fractions were collected from the digest and spotted. With fewer peptides per spot, the signal from each was enhanced.

**Results**

Immunoglobulin antibodies are multi-chain complexes weighing roughly 150 kDa. Each IgG is composed of 2 heavy chains and 2 light chains. Each IgG has two antigen binding sites that are part of the variable region of the antibody.

To simplify sequencing analysis, antibodies can be reduced and alkylated to yield light and heavy chains. These chains can be separated and thus purified using C4 RPLC for subsequent enzymatic digestion.

**Future Work**

Various proteases such as trypsin, papain, pepsin, chymotrypsin and Glu-C will be used to digest the mAb for subsequent protein or peptide analysis via 157 nm photofragmentation and post source decay (PSD). Based on a molecular weight of 152 kDa, we estimate that there will be roughly 70 peptides. Following liquid chromatography, fractions will be spotted.

**Conclusions**

Using data obtained from 157nm photofragmentation and post source decay spectra, our algorithm can identify x-type ions and derive peptide sequences. The confidence of amino acid assignments is evaluated by observing complementary y-, v-, and w-type ions that provide additional constraints to sequence identification.

**References**