

# De Novo Sequencing of an Antibody Using 157 nm Photofragmentation

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## 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 guanidium 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.

Proteolytically digested mAb was subjected to PSD and photodissociation using 157 nm light implemented in an ABI modified 4700 MALDI-TOF MS for peptide analysis

### Sample Preparation

- Sample Denaturation
- Reduction/Alkylation
- Enzymatic Digestion

### Mass Spectrometric Analysis

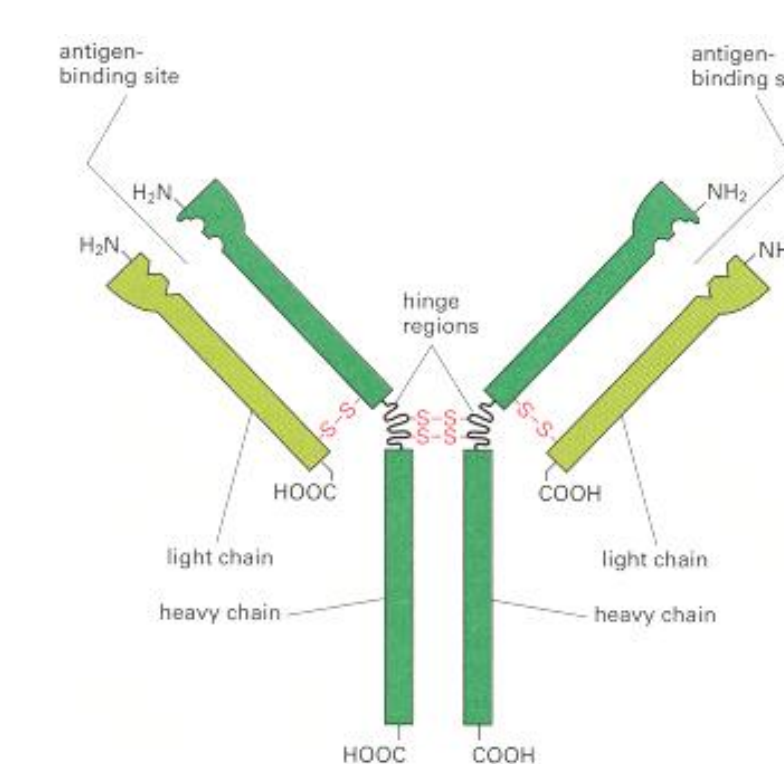
- Fraction Collection
- Photofragmentation
- Post Source Decay

### De Novo Sequencing

- MASCOT database comparison
- *De novo* sequencing software

## 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.

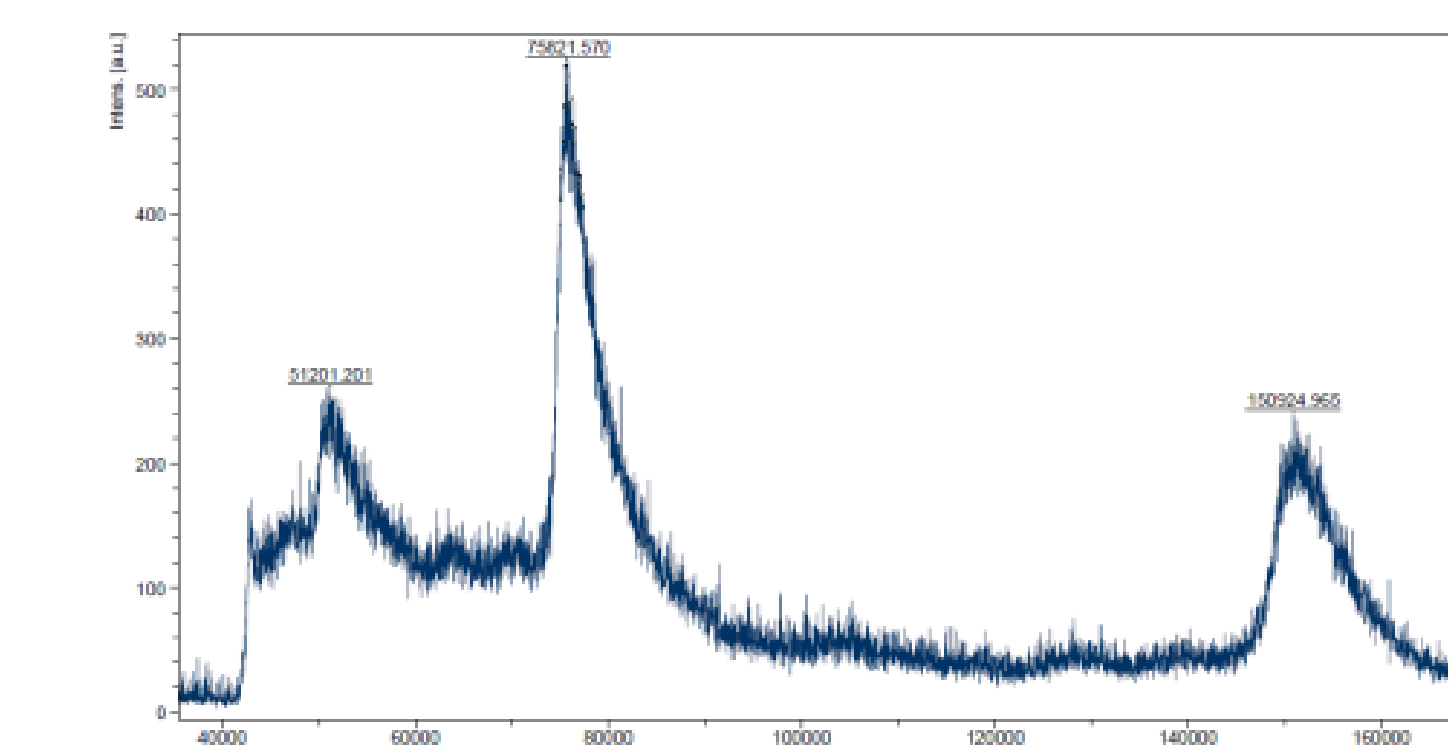


Figure 1: MALDI-TOF analysis of an intact mAb

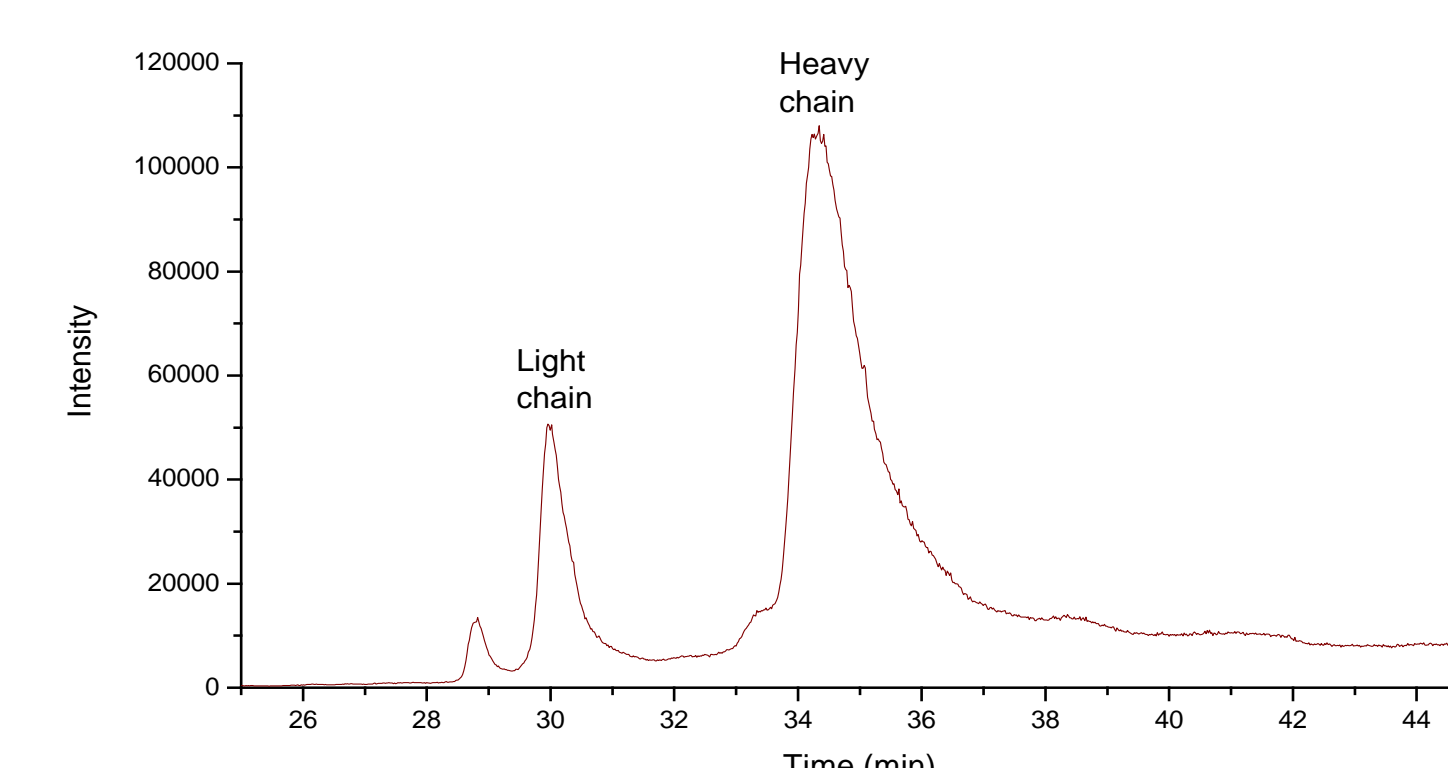


Figure 2: LC-ESI-MS analysis of a reduced and alkylated mAb

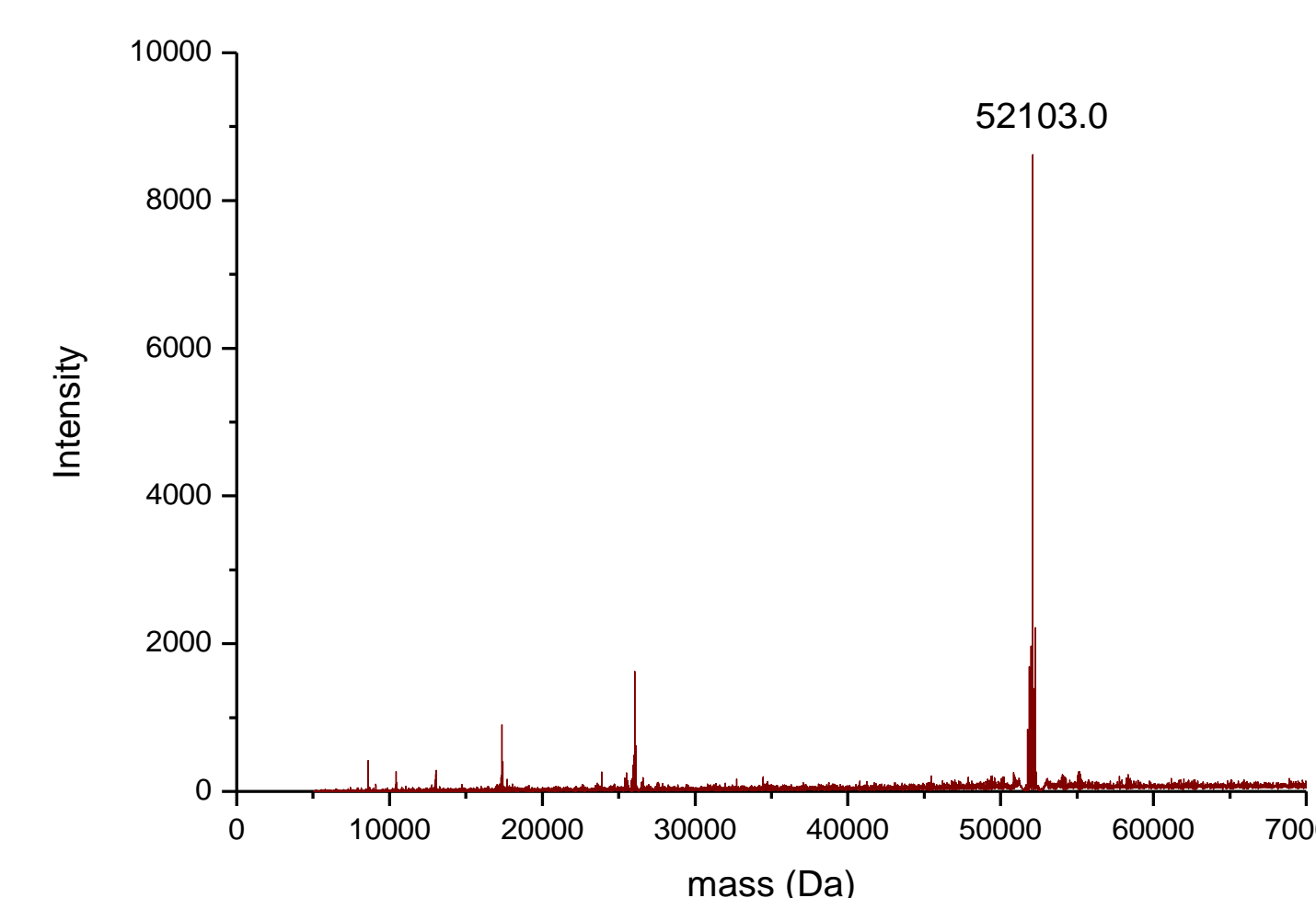


Figure 3: ESI-MS of mAb heavy chain

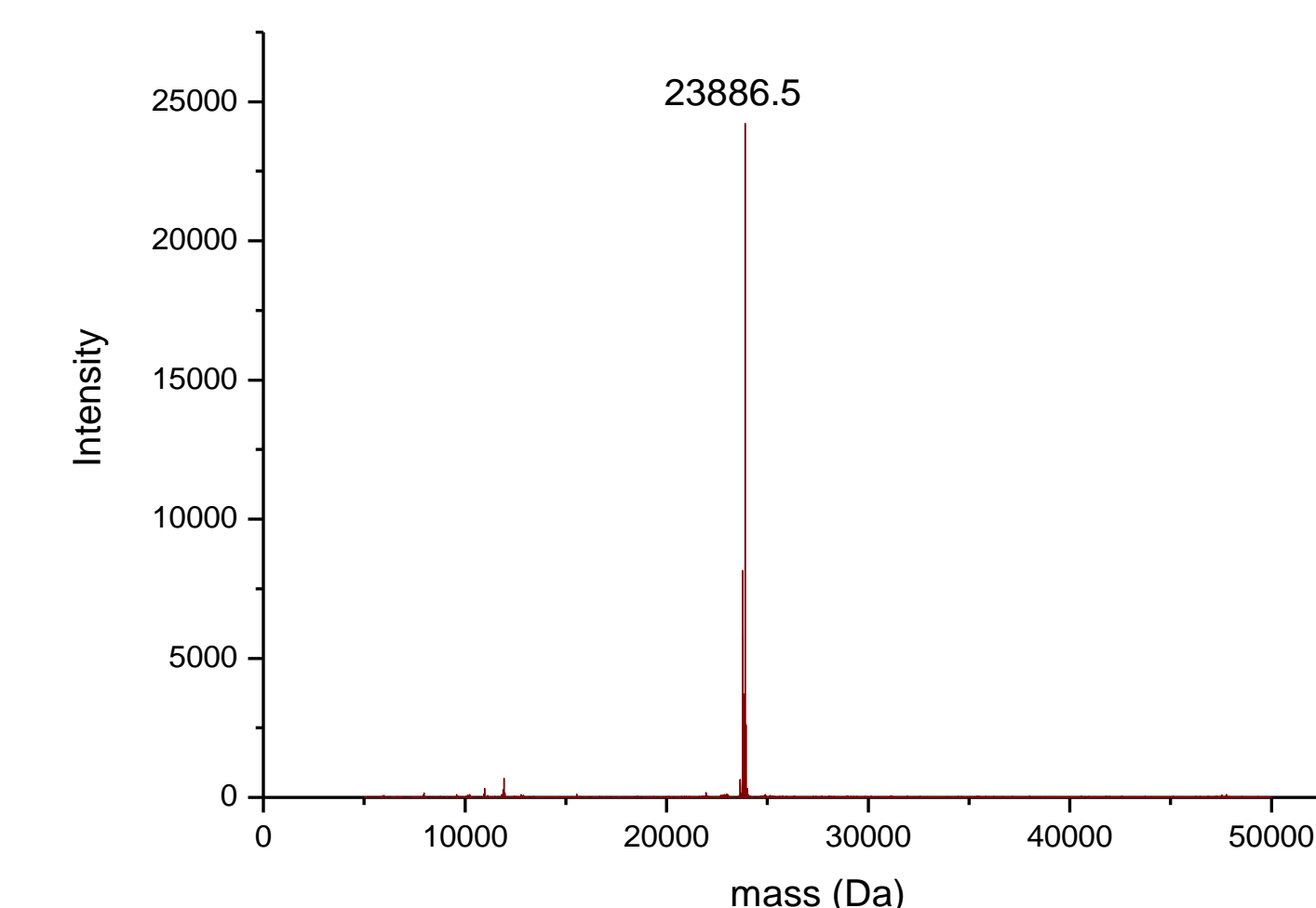


Figure 4: ESI-MS of mAb light chain

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.

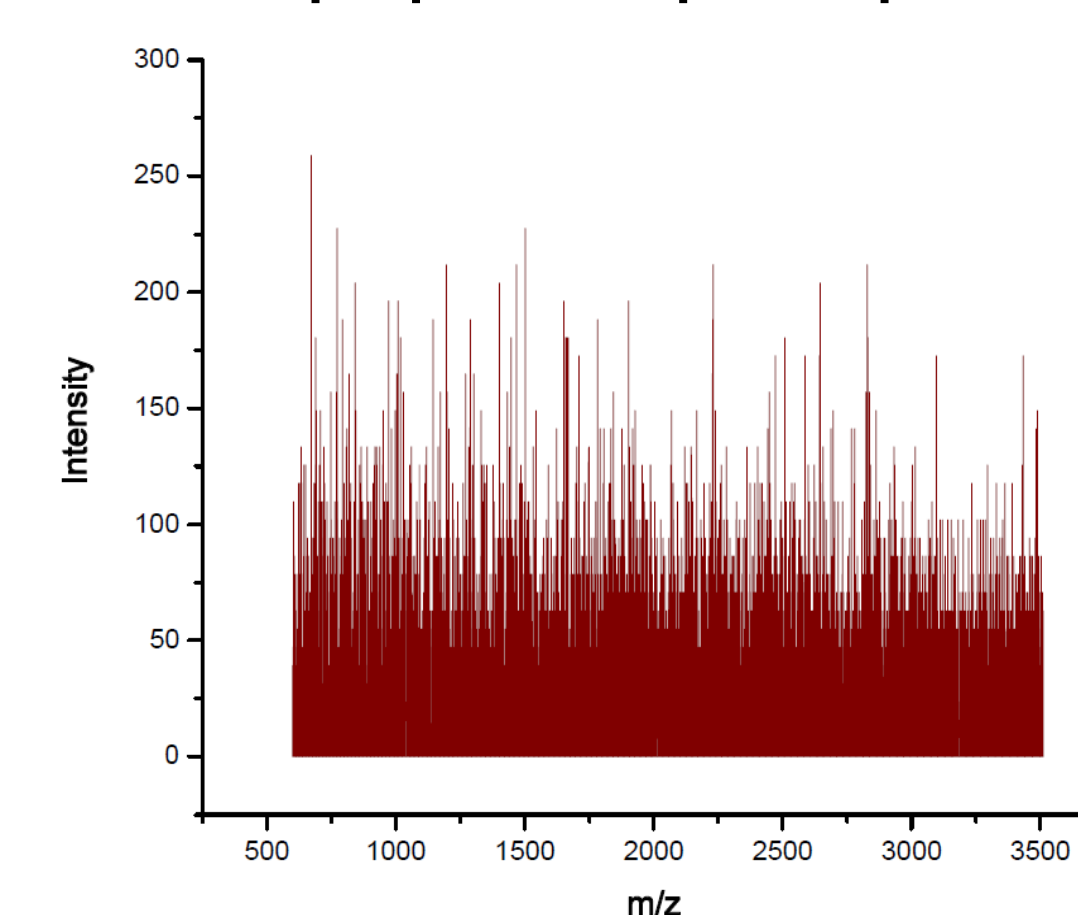


Figure 5: MALDI-TOF spectrum of entire mAb tryptic digest

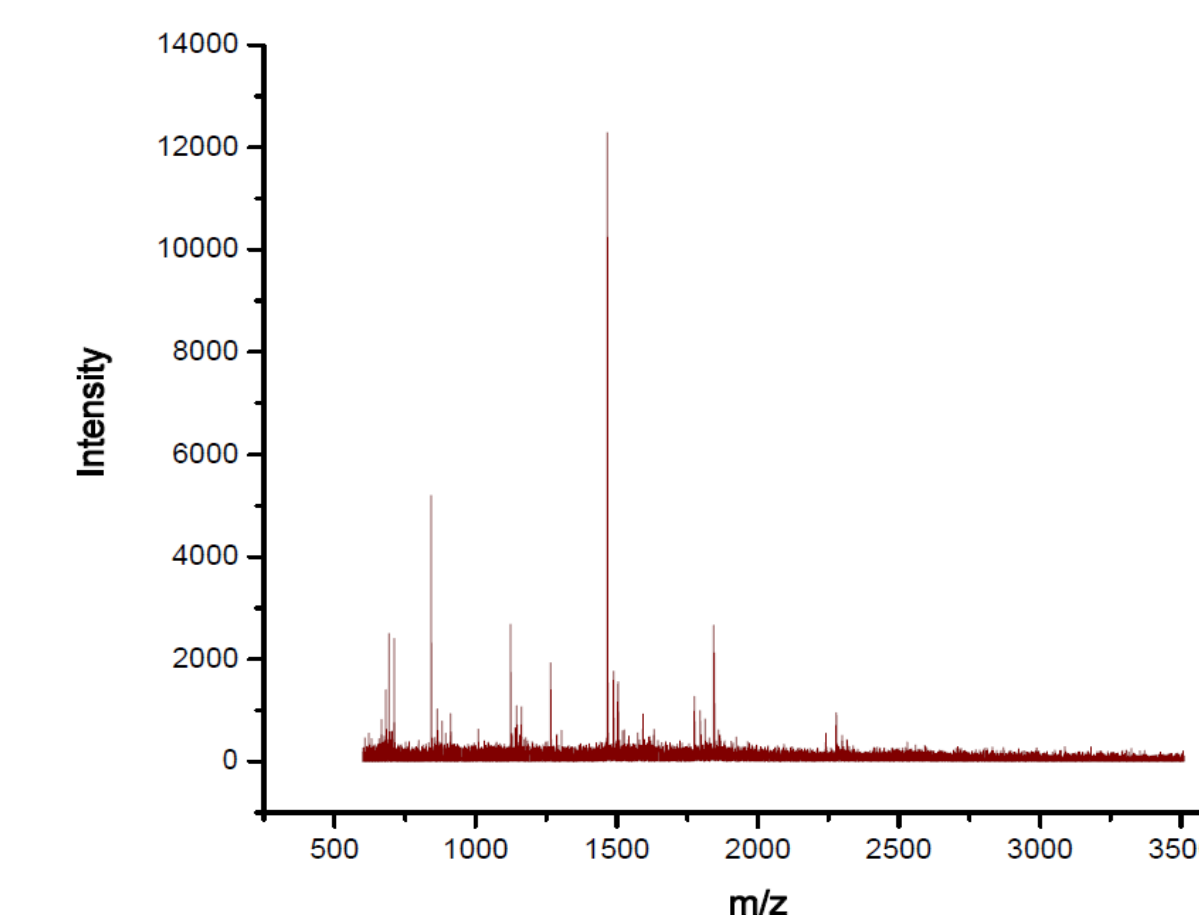


Figure 6: MALDI-TOF spectrum of a fraction of mAb tryptic digest

## 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.

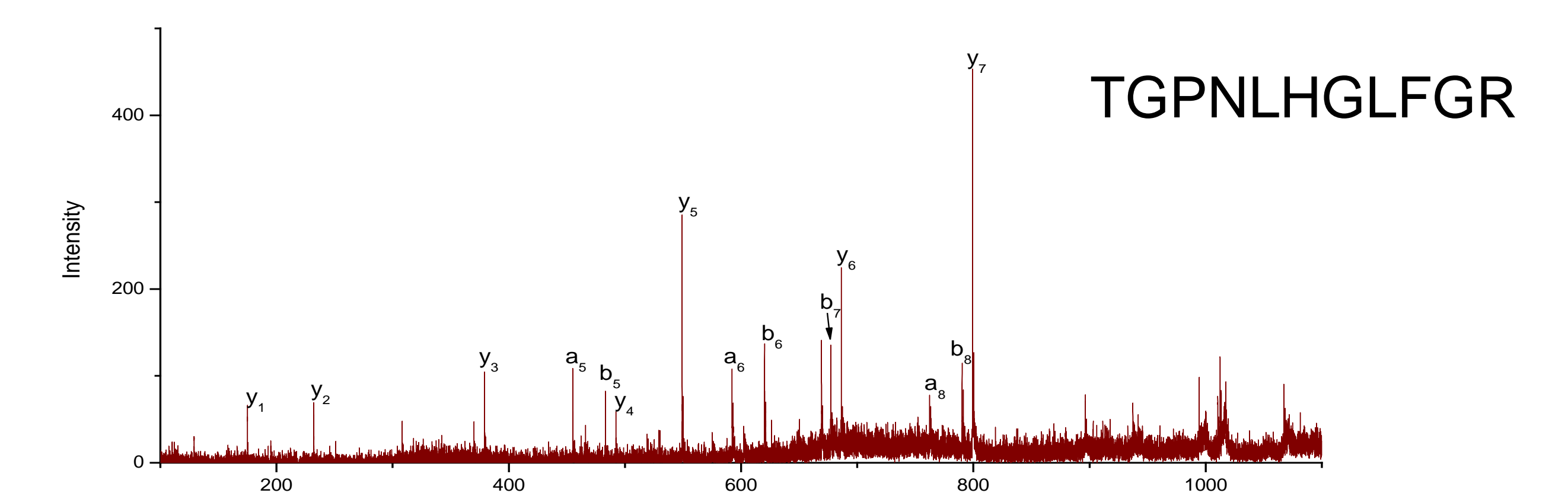


Figure 7: Post source decay of a tryptic peptide(TGPNLHGLFGR)

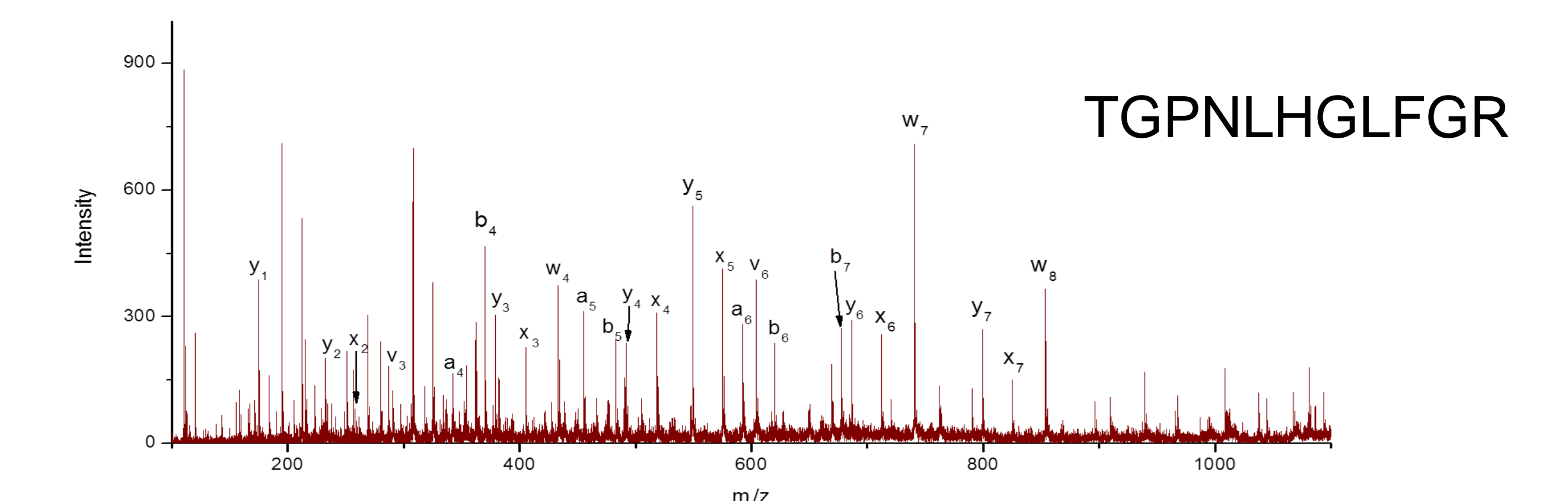


Figure 8: Photofragmentation MS/MS spectrum of a tryptic peptide

By interpreting both photodissociation 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.

## Conclusions

Using data obtained from 157nm photofragmentation and post source decay, *de novo* sequencing of an antibody will be performed. Various enzymatic digestion procedures can be used to confirm sequence assignments and the summation of peptide masses obtained for larger protein fragments may also be used for sequence confirmation.

## References

1. Zhang, L., Reilly, J. P., *Analytical Chemistry* 2010, 82 (3), 898-908.
2. Bandeira, N., Pham, V., Pevzner, P., Arnott, D., Lill, J., *Nature Biotechnology* 2008, 26 (12), 1336-8.
3. Castellana, N., McCutcheon, K., Lill, J., *Proteomics* 2011, 11, 395-405.