

# Chemically modified antibodies

Characterised using liquid chromatography-mass spectrometry

The recognition of specific proteins by antibodies is used to an increasing extent to visualise certain proteins in the human body or to design specific detection assays. This requires tailoring of the antibody, for example via local chemical modification. By nature, several reactive groups are present on the protein that can be targets for modification. Non-specific alteration of certain amino acids in the protein sequence can compromise the functionality of the antibody. This is of course unfavourable for subsequent applications. Therefore, the reaction efficiency of chemical modifications has been monitored using liquid chromatography-mass spectrometry (LC-MS).

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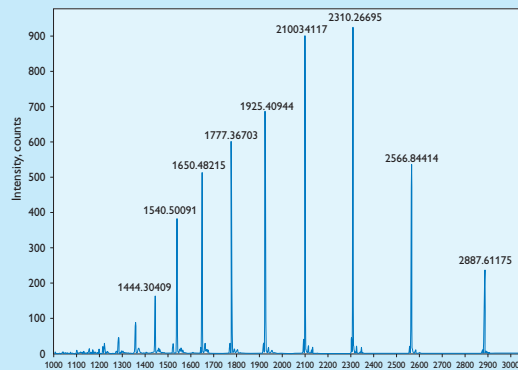


Fig. 1: ESI-ToF Mass Spectrum of an unmodified light chain.

## Introduction

The front page shows a schematic drawing of a modified antibody. To determine whether a modification has been successful, two parameters need to be investigated: firstly, which specific sites on the antibody have been modified, and secondly, what percentage of these sites has reacted? Acquiring mass spectra of the entire antibody would not provide unambiguous information on these questions. Therefore, analysis starts with the sectioning of the antibody into well-defined fragments. These fragments will be characterized individually and compared to those of unmodified antibodies.

## The number of labels present on an antibody

In a first step, the disulfide bonds in the antibody are disrupted (see front page graphic) splitting it up into two light and two heavy chains. After separation using reversed phase LC, the (modified) protein molecules are ionised in the electrospray ion source and their mass-over-charge ( $m/z$ ) ratio is determined using time-of-flight mass spectrometry (ToF-MS). In this way, the molecular mass and degree of labelling can be calculated.



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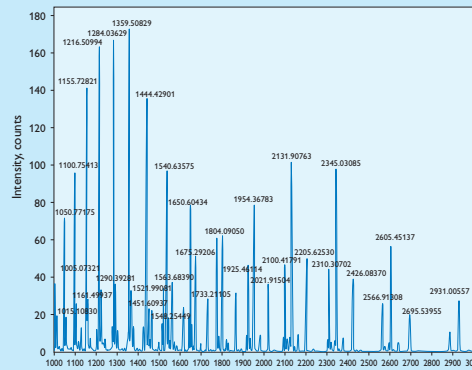


Fig. 2: ESI-ToF Mass Spectrum of a modified light chain.

Figure 1 shows the mass spectrum of an unmodified light chain. The set of signals at different  $m/z$  ratios is called a charge envelope and represents the same protein. Due to the presence of multiple protonatable sites in protein, a varying number of charges can be accommodated.

The charge envelope of the chemically modified light chain is presented in Figure 2. In this particular case, two sequential chemical modifications were performed on the protein: first a linker molecule was attached, and subsequently a reporter group was coupled to the linker (symbolized by the light bulbs in the front page graphic). As can be seen in Figures 2 and 3, such (sequential) chemical modifications result in a heterogeneous population of (partially) modified protein molecules that is explained in Table 1.

From this analysis it is clear that there is still a substantial amount of unmodified protein present and that the coupling of the reporter group to the linker molecule does not run to completion. Basically the same procedure is applicable to the heavy chain and since each antibody consists of two light and two heavy chains, the average number of linker/reporter groups per antibody can be calculated. For this LC-MS-based method, only a few micrograms of antibody is required.

## The position of reporters in the primary sequence of an antibody

To study a modified antibody in more detail, the protein can be cleaved into smaller pieces, called peptides, by a protease such as

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## Application Note 25

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elastase (see front page graphic). These peptides can be analysed individually, thereby providing insight into the positions of the amino acids that have been modified. Thus, both reaction efficiency and labelling position could be characterised, demonstrating the power of LC-MS as a protein characterisation technique.

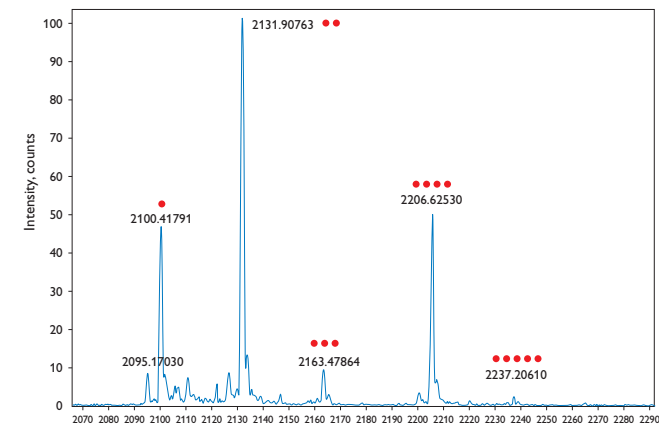


Fig. 3: detail of Figure 2. The interpretation of this spectrum is given in Table 1.

modification	$m/z$	$z$	mass (Da)	$\Delta m$
• Unmodified light chain (LC)	2100.41791	11	23093.60	
•• LC + 1 linker molecule	2131.90763	11	23439.98	346.4
••• LC + 2 linker molecules	2163.47864	11	23787.27	693.7
•••• LC + 1 linker + 1 reporter molecule	2205.62530	11	24250.88	1157.3
••••• LC + 2 linkers + 1 reporter molecule	2237.20610	11	24598.27	1504.7

Table 1: Explanation of the signals of the (partially) modified light chain seen in Figure 3.