

Analysis of Proteins in Linear Mode using an AXIMA-TOF²™

The AXIMA-TOF²™ is a new MALDI-TOF mass spectrometer, capable of high energy collision induced dissociation (CID) experiments. The instrument is based on the well established AXIMA-CFR™*plus* platform and can be operated in both linear and reflectron modes, permitting analysis over a wide mass range.

Here, we demonstrate the analysis of intact proteins by MALDI-MS in linear mode using the AXIMA-TOF²™.

Figure 1(a) shows the MALDI-MS spectrum obtained for bovine serum albumin (BSA; 10 pmol/μL (5 pmol on-target)) prepared in sinapinic acid, using a pulsed extraction value of 66,000 i.e. optimized for singly-charged BSA. Figure 1(b) shows the spectrum obtained for BSA at 500 fmol/μL (250 fmol on-target) using the same pulsed extraction value. Even at the lower concentration, good signal-to-noise is obtained demonstrating the high sensitivity of this new instrument in linear mode.

Figure 2 shows the MALDI-MS spectrum obtained for BSA at 10 pmol/μL (5 pmol on-target) using a pulsed extraction value of 150,000, clearly showing the doubly-charged ((2+) 33216 *m/z*), singly-charged ((1+) 66430 *m/z*) and dimer ((2M+) ~132860 *m/z*) peaks. Good resolution and peak shapes were obtained indicating the high performance of the instrument at higher masses.

Figure 3 shows the spectrum obtained following the analysis of a mixture of proteins (cytochrome C, myoglobin and trypsinogen (LaserBio Labs, France)). In addition to signals corresponding to singly-charged species, peaks were also detected consistent with the doubly-charged species. Such mixtures can be used for instrument calibration or to check the performance of an instrument. Figures 2 and 3 demonstrate the high performance in linear mode across a wide mass range.

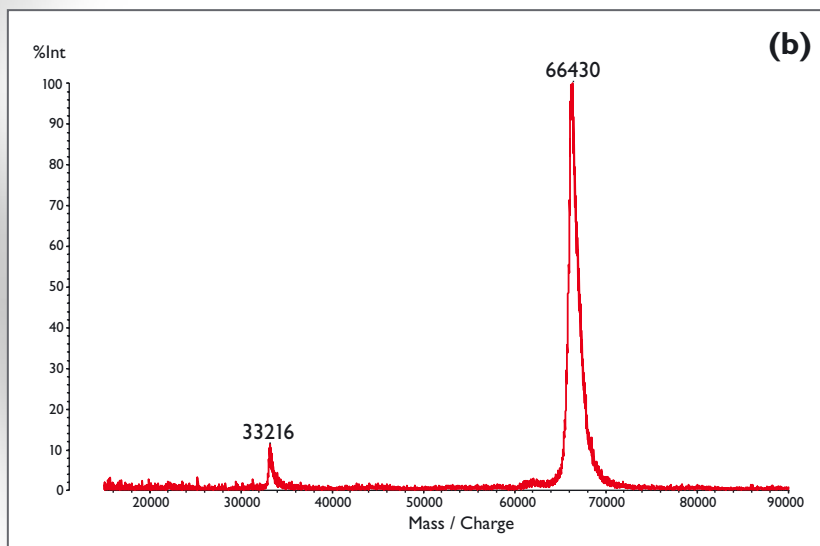
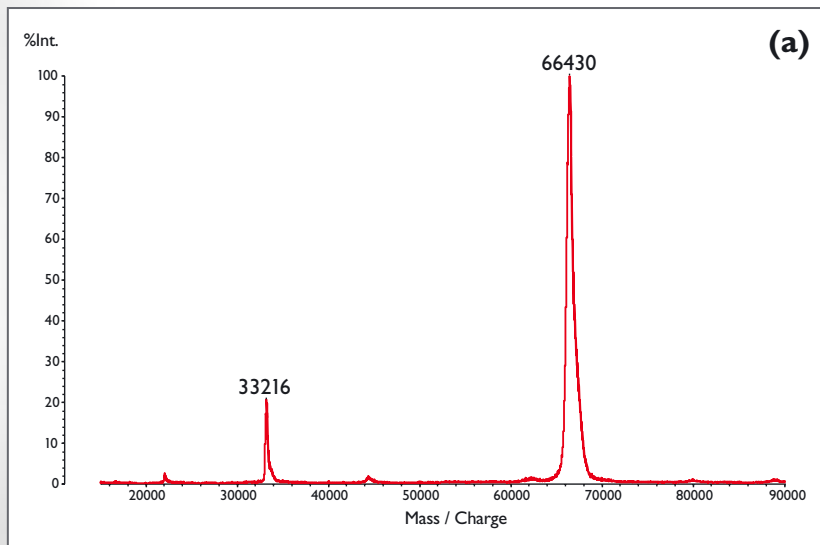


Figure 1: MALDI-MS spectra obtained for BSA in linear mode acquired using a pulsed extraction value of 66,000 (internally calibrated); (a) 10 pmol/μL (5 pmol on-target) and (b) 500 fmol/μL (250 fmol on-target).

Finally, Figure 4 shows the MALDI-MS spectrum obtained for a monoclonal antibody sample (sample supplied courtesy of Dr. S. Kulkarni (Murex Biotech Ltd., UK)). The sample was supplied in sodium phosphate buffer containing sodium chloride. Therefore, prior to analysis, the sample was dialyzed against HPLC grade water in an attempt to reduce the concentration the salts present in the sample which may interfere with the MALDI process. Subsequently, an aliquot of the dialyzed sample solution was prepared for analysis using sinapinic acid as matrix and was analyzed using a pulsed extraction value of 150,000. The heterogeneous nature of such high mass proteins makes it virtually impossible to accurately determine the mass of such compounds. In the example shown (Figure 4), BSA was used as calibration standard and the singly-charged, doubly-charged and dimer peaks were used to calibrate the instrument. The calibration was subsequently extrapolated to cover the mass range of interest. As a result, in Figure 4 the mass of the intact antibody has been rounded to the nearest 100 Da reflecting the approximate average mass. In order to determine a more accurate mass assignment, multiple analyses of the same sample should be performed and statistics used to determine the mean value.

Conclusions

- High performance **linear mode** acquisitions are possible using a new MALDI-TOF mass spectrometer (AXIMA-TOF²™)
- Good resolution and high sensitivity can be achieved across a wide mass range (> 100,000 *m/z*)
- The software-controlled pulsed extraction feature simplifies optimization of instrumental parameters associated with peak resolution

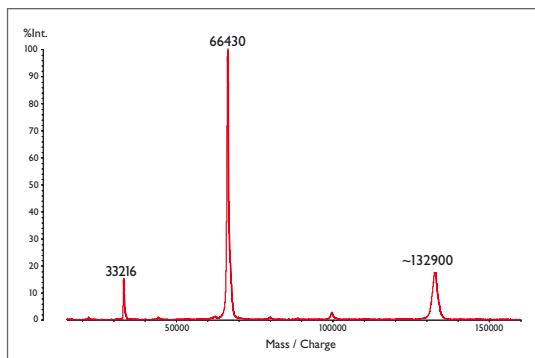


Figure 2: MALDI-MS spectrum obtained for BSA (10 pmol/μL (5 pmol on-target)) in linear mode, acquired using a pulsed extraction value of 150,000 (internally calibrated).

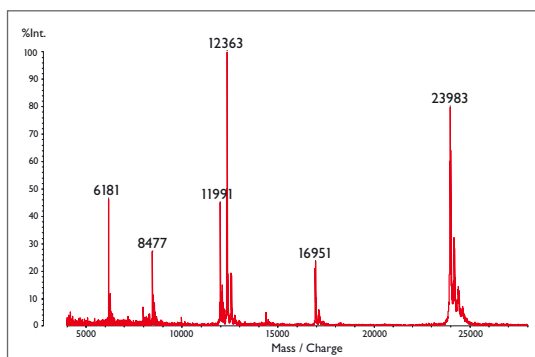


Figure 3: MALDI-MS spectrum obtained for a mixture of proteins (cytochrome C, myoglobin and trypsinogen (LaserBio Labs, France)) in linear mode, acquired using a pulsed extraction value of 20,000 (internally calibrated), demonstrating high performance in linear mode at lower masses.

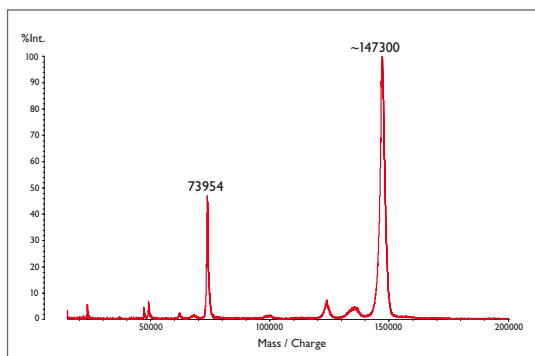


Figure 4: MALDI-MS spectrum obtained for a monoclonal antibody sample in linear mode, acquired using a pulsed extraction value of 150,000, demonstrating high performance in linear mode at higher masses. Sample supplied courtesy of Dr S Kulkarni (Murex Biotech Ltd, UK)