

# AXIMA-TOF<sup>2</sup>™

Protein Identification of Complex  
Mixtures using LC-MALDI on  
AXIMA-TOF<sup>2</sup>™

- LC-MALDI is a fully automated technique permitting the identification of proteins from complex mixtures
- Multiple peptides are identified for most proteins present generating highly confident search results
- High energy CID MS/MS enhances the fragment ion spectra aiding database searching

# Protein Identification of Complex Mixtures using LC-MALDI on Axima-TOF<sup>2</sup>™

## Introduction

The aim of proteomics is to separate, quantify, identify, and characterize individual proteins of interest. When dealing with complex biological mixtures such as tissue lysates, serum or plasma samples, there is a need to identify as many proteins as possible when often the most interesting components are present at mid to low concentrations. To analyze such samples requires a high sensitivity technique which can accurately and confidently identify as many proteins as possible.

LC-MALDI is a procedure that can easily and effectively deal with complex biological samples. The experimental technique combines an LC separation with online fractionation directly onto a MALDI target. Fractionation and matrix addition is performed automatically by a spotting robot such as the Accuspot™. The spotting probe simultaneously delivers matrix solution and the LC eluent, optimizing the mixing of the two solutions and minimizing matrix 'hot spots'. MALDI mass spectrometry analysis is then performed offline. The analysis can be performed fully automatically on the novel MALDI TOF-TOF mass spectrometer - the AXIMA-TOF<sup>2</sup>™ using purpose written, dedicated LC-MALDI software (see Figure 1).

Analysis of complex un-fractionated digest mixtures may result in signal suppression and overlapping signals which can ultimately lead to a reduction in sequence coverage. The incorporation of an LC separation of the digest mixture prior to analysis allows concentration and desalting of the sample in a single step. Not only are peptides of the same nominal mass afforded temporal separation but also signal suppression is reduced due to separation of low- and high-abundance peptides. Importantly, the time available to obtain sequence information from the sample analyzed by LC-MALDI is greatly increased when compared with ES LC-MS/MS analyses. Also, significantly, the unique LC-MALDI peak picking algorithm promotes MS/MS of selected ions at the apex of the eluting chromatographic peak to allow the most efficient data acquisition. This is often not the case with ES LC-MS/MS where MS/MS acquisition can be taken on the rising edge of the eluting chromatographic peak.

Here, we investigate the value of the AXIMA-TOF<sup>2</sup>™ for protein identification by MS and MS/MS using a complex mixture of human proteins and the dedicated LC-MALDI software functionality.

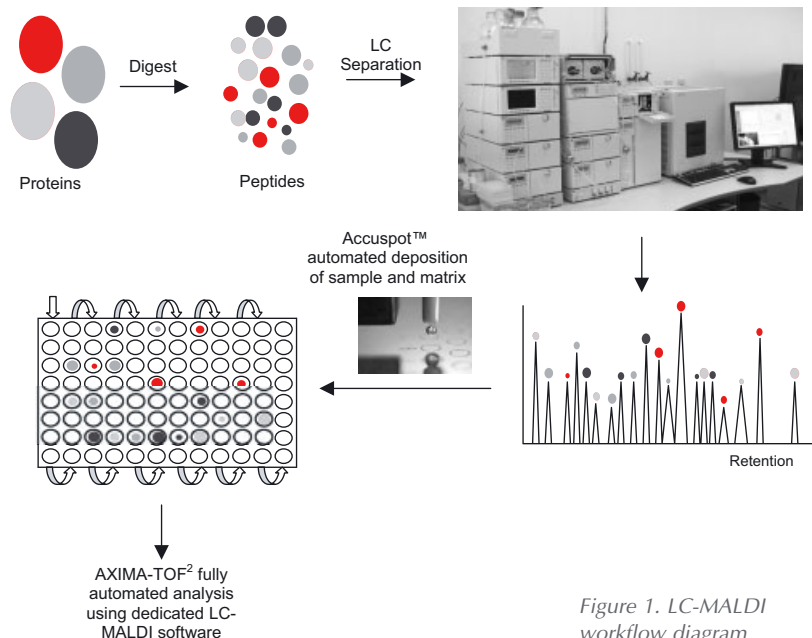


Figure 1. LC-MALDI workflow diagram

## Methods

**Materials:** A complex mixture of intact human proteins, purified from their biological source or recombinantly expressed, was analyzed. 5 pmol aliquots of each component had been combined and lyophilized in an eppendorf tube. An in-solution tryptic digestion under gentle conditions was undertaken overnight at 37°C in ammonium bicarbonate pH 8 containing DTT, EDTA, CaCl<sub>2</sub> and modified porcine trypsin added at 1:25 ratio (w:w).

**LC and Accuspot™ conditions:** A 5 µl aliquot of the sample diluted in mobile phase A (500 fmol per protein) was loaded directly onto the column (300 µm i.d. x 15 cm, 3 µm, Pepmap C<sub>18</sub> (LC Packings)) and separated using one of two 2-stage linear gradients: (A = 5% acetonitrile + 0.05% TFA, B = 80/20 acetonitrile + 0.05% TFA)

The flow rate of the system was set at 0.2 ml/min pre-split, giving a flow rate of ~5 µl/min post-split. The eluent passed through a UV/Vis detector (220 nm) and was mixed with α-cyano 4-hydroxycinnamic acid matrix (CHCA; 5 mg/ml in 50/50 acetonitrile/0.1% TFA) and deposited onto a stainless steel MALDI target using the Accuspot™ LC-MALDI deposition robot. Depending on the gradient used spotting occurred over 1- or 2- x 384-well MALDI targets. Samples were deposited at 6 sec/spot producing a spot volume of ~1 µl/spot.

Figure 5 shows a second CID enhanced MS/MS spectrum produced when a peptide ion in spot number 302 was selected and fragmented. Again a high degree of peptide fragmentation is observed giving high confidence Mascot® scores when database searching. This peptide was identified as a fragment of the protein catalase with the amino acid sequence of GAGAFGYFEVTHDITK, with a Mascot® score of 93.

