

Improved Ion Gate for High Resolution Precursor Ion Selection

The introduction of 'soft' ionisation techniques such as electrospray and MALDI coupled with mass spectrometry have facilitated molecular weight determination of fragile/non-volatile compounds. The low internal energy of the ions generated minimises fragmentation and promotes the formation of intact species which can be used to confirm molecular weight and, in the case of peptide mass fingerprint (PMF) analyses, used to infer protein identity.

Equally important is the confirmation of structure through the activation and dissociation of precursors. In the case of a sample containing a single component, the precursor ions can simply be activated and the resulting fragments mass analysed. As the sample only contains a single component, the origin of all the fragment ions is known. More commonly, however, the sample being analysed contains several precursors and simultaneous activation of all the precursors would result in a complicated fragment ion spectrum, with a loss of connectivity between precursor and fragment ions. Put simply, it would not be clear which fragment ions originated from which precursor.

To alleviate this problem, an ion selection device can be used to 'isolate' a single precursor from a mixture of ions. With the selection of a single precursor prior to activation, the connectivity between the precursor and resulting fragment ions is restored. The degree of resolution of the ion selection device is critical as this determines the complexity of sample which can be successfully analysed. If the resolution of the ion selection device is low, it may not be possible to isolate a single component and additional fractionation of the sample may be required prior to analysis.

The AXIMA-TOF²™, a MALDI TOF-TOF system, features a novel, dual ion gate capable of high resolution precursor ion selection (see Figure 1). The ion gate operates using a high voltage pulser which rapidly decreases the voltages applied to the gates to coincide with arrival of the precursor ion(s) of interest. The short time of flight separation which occurs between the ion source and the ion gate, combined with the speed of the high voltage pulser, results in a precursor ion selection resolution of ~400. The ion gate is set by the user in the LAUNCHPAD™ software, using cursors to define the start and end of the required ion gate 'window'.

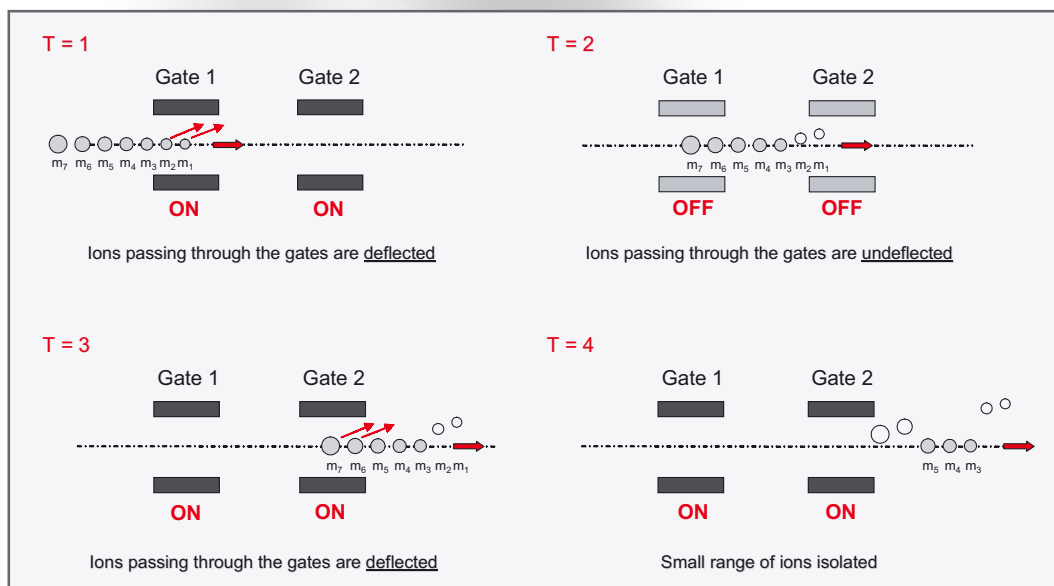


Figure 1. Operation of the dual ion gate for high resolution precursor ion selection on the AXIMA-TOF²™

The example below demonstrates how the ion gate can be used to isolate and subsequently fragment closely related peptides in a protein ID application. The sample (conalbumin tryptic digest (Michrom Bioresources)) was prepared and analysed in MS mode. Three different peptides of similar mass (1329, 1331 and 1334 m/z) were detected with overlapping isotopic distributions and were matched to different peptide sequences in a Mascot® Peptide Mass Fingerprint search. The three peptides were subsequently isolated, in turn, and individual MS/MS spectra acquired. The data from the three MS/MS spectra were used to create a single MS/MS mass list containing fragment ion and precursor ion masses for the three peptides which was used to perform a MS/MS ions search (Mascot®).

Key Features:

- Novel, high specification dual ion gate design for precursor selection
- High resolution precursor ion selection (~400)
- Facilitates the identification of closely related species

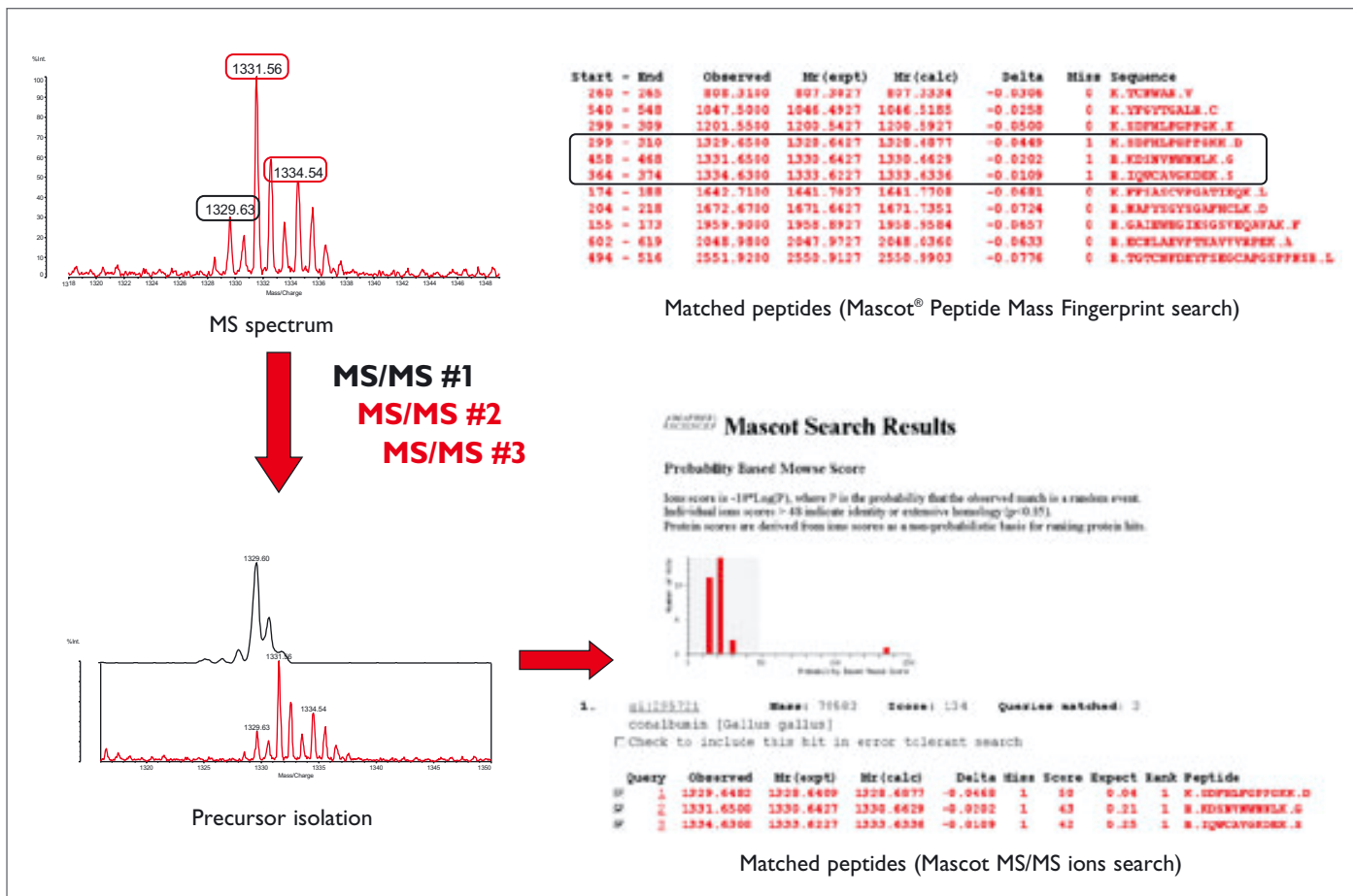


Figure 2. Use of the ion gate in a protein ID application