



Technical Note # TN-33

Top-Down Analysis Using the solariX-TD

Abstract

The solariX-TD™ FTMS represents a turn-key solution for top-down analysis and sets the standard for the detailed investigation of complex biological systems. Superior sensitivity, resolving power, and mass accuracy when combined with dissociation techniques such as ECD, ETD, and CID, as well as integration with LC-MS and LC-MS/MS. solariX-TD offers outstanding performance for top-down analysis of biomolecules. In this note, we demonstrate the performance of solariX-TD for the analysis of several proteins where traditional bottom-up protein analysis alone might not be effective.

Introduction

The solariX-TD is a high-end proteomics research solution that is designed to complement more traditional MS instruments. The solariX-TD can be easily integrated into a workflow that consists of other Bruker platforms such as: amaZon™ (Ion Trap), microTOF™ (ESI-qTOF), or the Flex Series axial MALDI-TOF. The focus of the solariX-TD is top-down analysis of biomolecules. The top-down concept is applicable to peptides, RNA, and carbohydrates both with and without liquid chromatographic separation. The value comes from the combination of extreme performance and versatility. This platform uniquely enables

a workflow for obtaining gross structural information or characterizing subtle modifications of biomolecules.

Structural tools

Supplementing the arsenal of conventional top-down proteomics structural fragmentation tools (ECD, Q-CID, in-cell SORI-CID) solariX-TD has now taken a quantum leap with the integration of Electron Transfer Dissociation (ETD). Adapting from Bruker's proven ETD technology, this addition to the API source facilitates rapid and facile dissociation of a wide range of molecules (Figure 1). This exciting new technique is superb for in depth analysis of proteins and peptides and their often labile, posttranslational modifications (PTMs) (Figure 2). ETD enabled analysis is fully automated, and can be used for LC-MS/MS workflows in combination with quadrupole fragmentation (Q-CID) to provide unprecedented levels of protein structural information. solariX-TD retains the ability to perform electron capture dissociation (ECD) as well. This enables detailed complimentary information to be obtained in rigorous top-down workflows. These methods can incorporate the highest "front-end" resolution for the isolation of multiple, narrow m/z ranges, such as a collection of charge states for a mixture of proteins or protein forms (Figure 3).

Performance

The solariX-TD provides a 10x improved sensitivity over the largest mass detection range available in its class by combining the patented quadrupolar, dual ion-funnel source with a unique, multiplexed RF ion transfer guide. Enhanced detection electronics and instrument control modules allows industry leading acquisition rates of high resolution data in excess of 3 Hz. Routine LC-MS/MS analysis of low-to-sub femtomolar amounts of proteolytic digests results in statistically significant protein assignments.

The addition of real-time data streaming facilitates higher resolving power for broadband data acquisition over wide mass ranges (e.g. 100-5,000 m/z). This is essential for applications such as intact protein fragmentation which can generate a complex mixture of fragments distributed over a wide m/z range (Figure 4). Data streaming is especially useful for the analysis of MALDI generated data where masses are typically distributed over a wide m/z range.

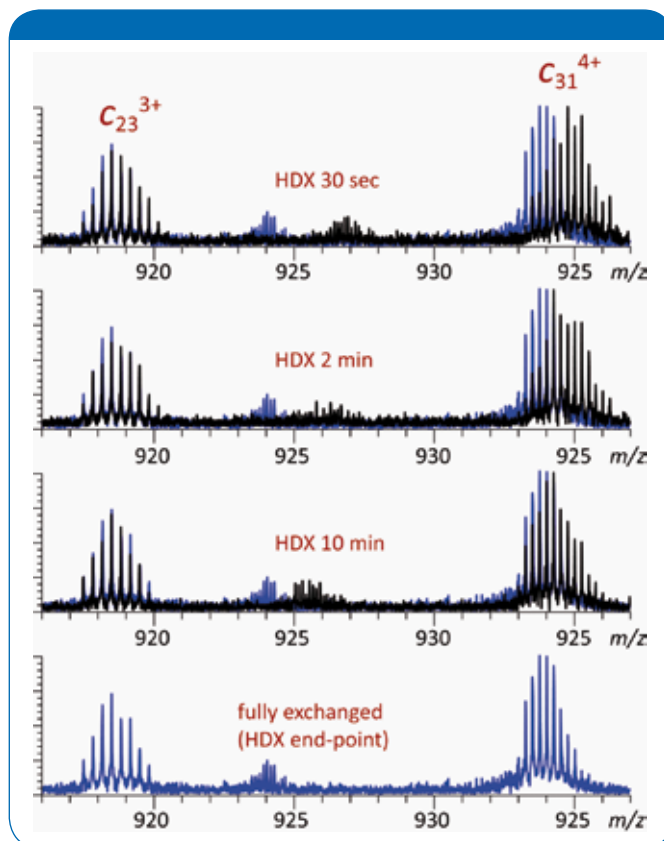


Figure 1: Determination of the structural features of the 18 kDa cellular retinoic acid binding protein I (CRABP I) using varying states of HDX with ETD.

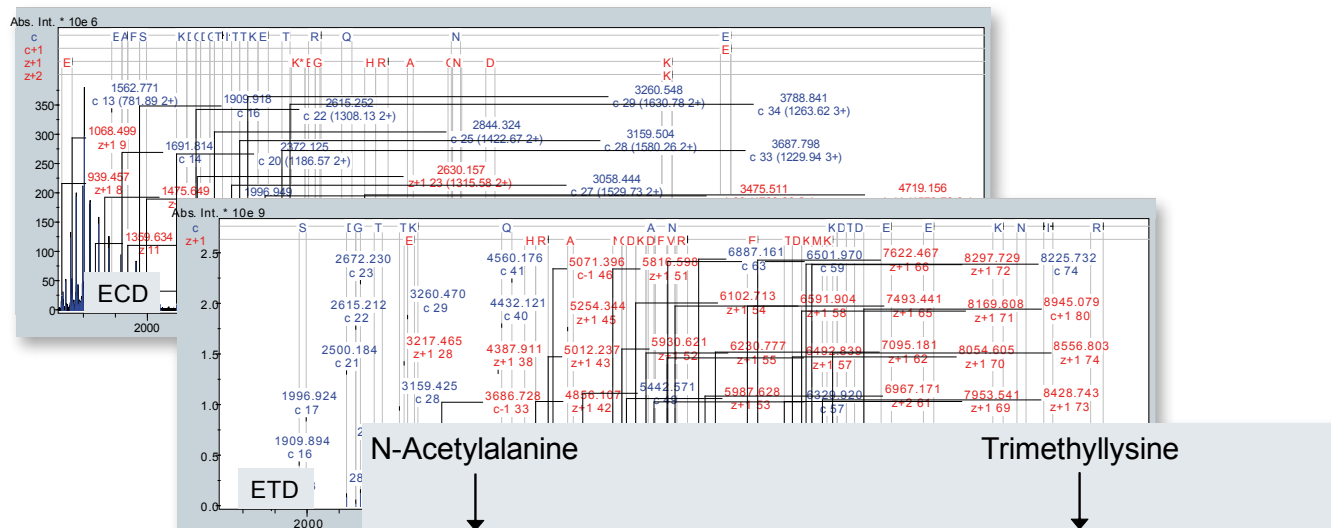


Figure 2: ECD and ETD performed on Calmodulin. As illustrated above, the expected phosphorylations are not present and the lysine at position 116 is trimethylated.

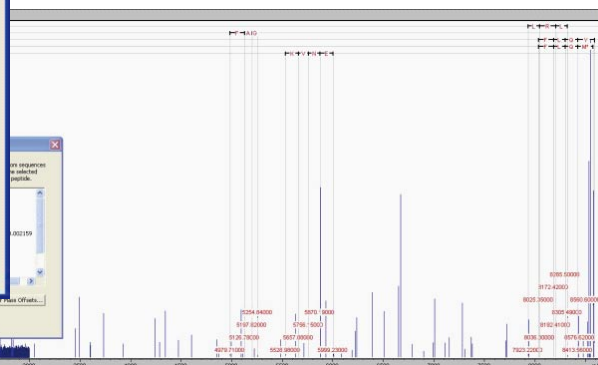
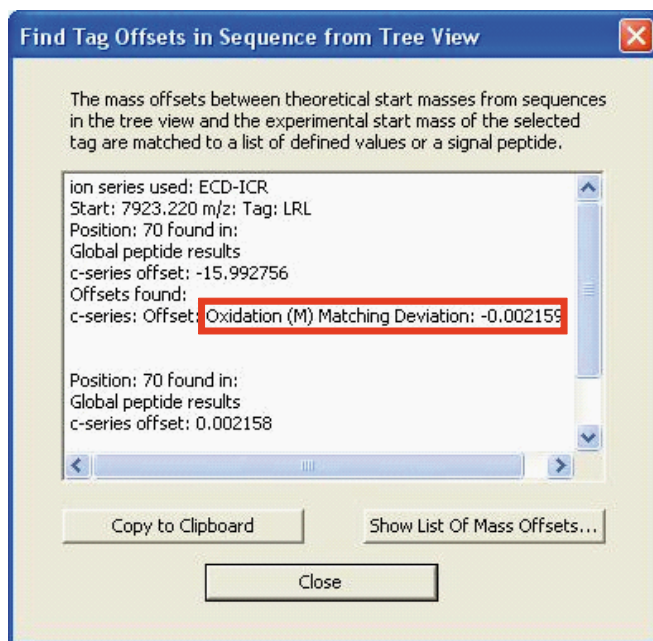
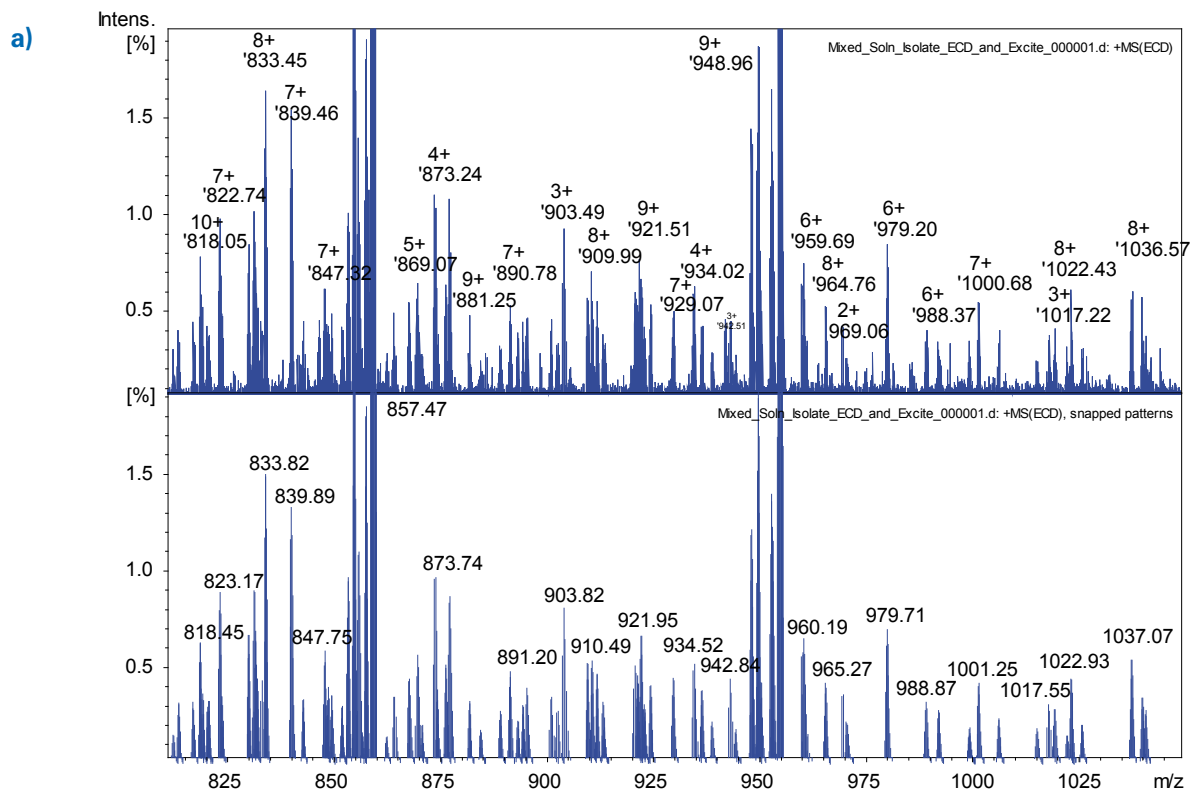


Figure 3: ECD analysis of modified ubiquitin. In-cell isolation (3a) is used to isolate all detected charge states of the oxidized form of ubiquitin from the wild type and ECD is performed on the isolated charge states and the data (3b) is processed with BioTools™, where the sequence tags are compared to the putative sequence and automatically corrected for the oxidative addition.

Advanced ion injection system

Advanced multipole ion injection optics enable the delivery of a wide spread of m/z values to the ICR cell. The solariX-TD incorporates a series of switchable frequencies on each stage of the multipole guide system. Equipped with intelligent control firmware, the transfer system automatically decides on the optimal frequency ranges, and applies the correct settings in the background before the acquisition begins. As a result, switching from measuring small metabolites to large protein complexes is

effortless (Figure 5). This ability to measure high m/z ions is unmatched in the FTMS arena. Ion isolation is based on an advanced, highly flexible quadrupole geometry that can facilitate time-dependant isolation windows with widths up to the full m/z range of detection. This advantage, combined with advanced data streaming technology, enables high performance over an unprecedented m/z range.

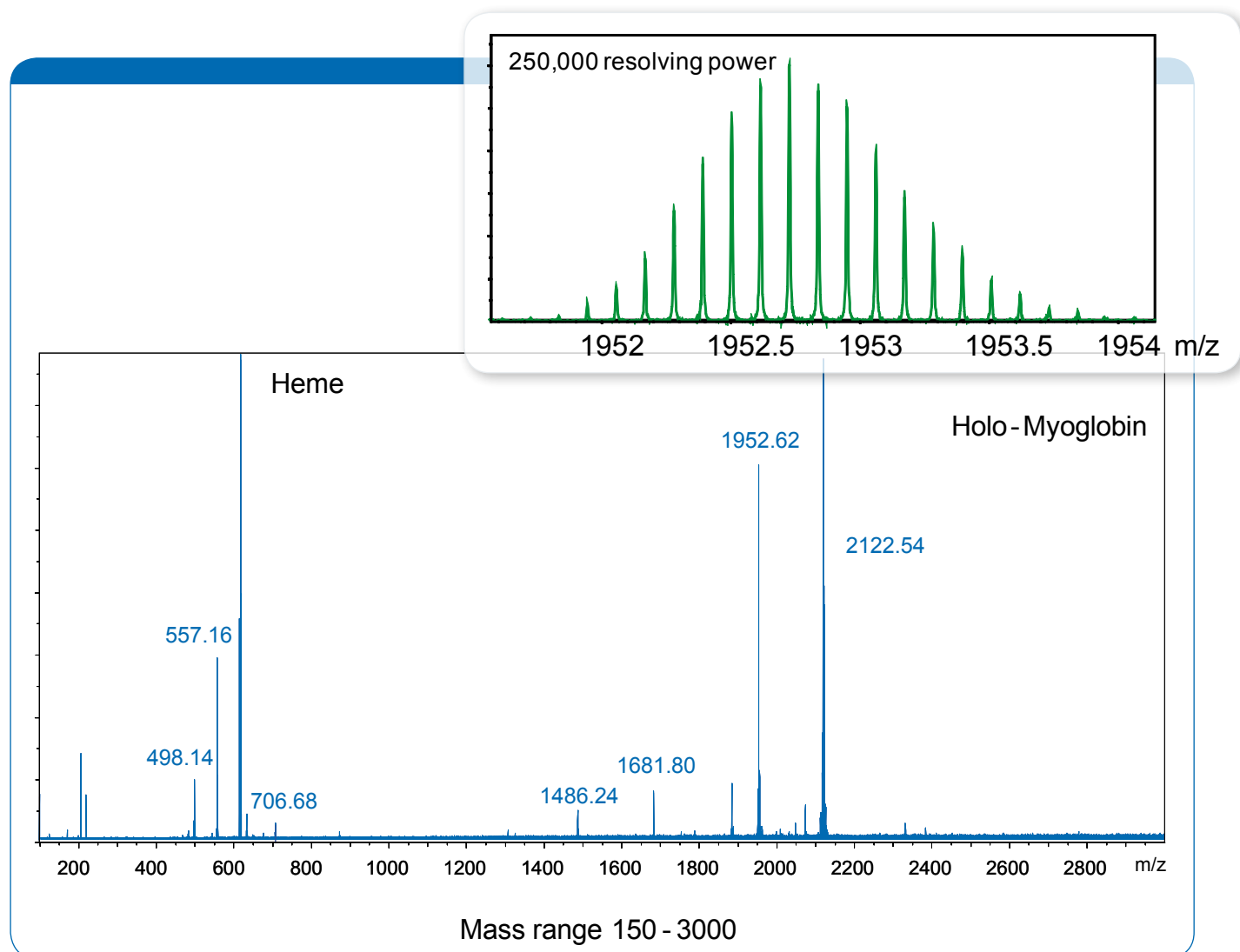


Figure 4: Broadband acquisition of myoglobin under non-denaturing solution conditions. Application of gentle CID to the protein yields several low mass fragments that are detected simultaneously with the precursor at high m/z .

Software

The release of solariX-TD ushers in a new era in FTMS acquisition software. solariX-TD provides automated control and measurement of virtually every instrument function. The intuitive graphical user interface also facilitates inspired experimental design. After data acquisition, the user can immediately access an almost limitless number of workflows via Bruker's Compass™ suite of processing software. Many of these workflows have a number of protein-specific tools such as SNAP, which is used for determining monoisotopic masses from complex mixtures, as shown in Figure 3a. Compass permits any analytical approach ranging from data processing and extraction to making intelligent decisions about subsequent experiments. This processing environment is common to the full line of instrumentation offered by Bruker Daltonics, providing close collaboration between FTMS generated data and that collected with other Bruker platforms.

Speed

Asynchronous data transfer and the new IPSO-Nanobay digital control architecture provide lightning fast data acquisition speeds. SolariX-TD offers the fastest duty cycles in the industry for full resolution data, which translates to lower sample consumption for top-down infusion analysis and greatly reduced duty cycles for LC-MS applications.

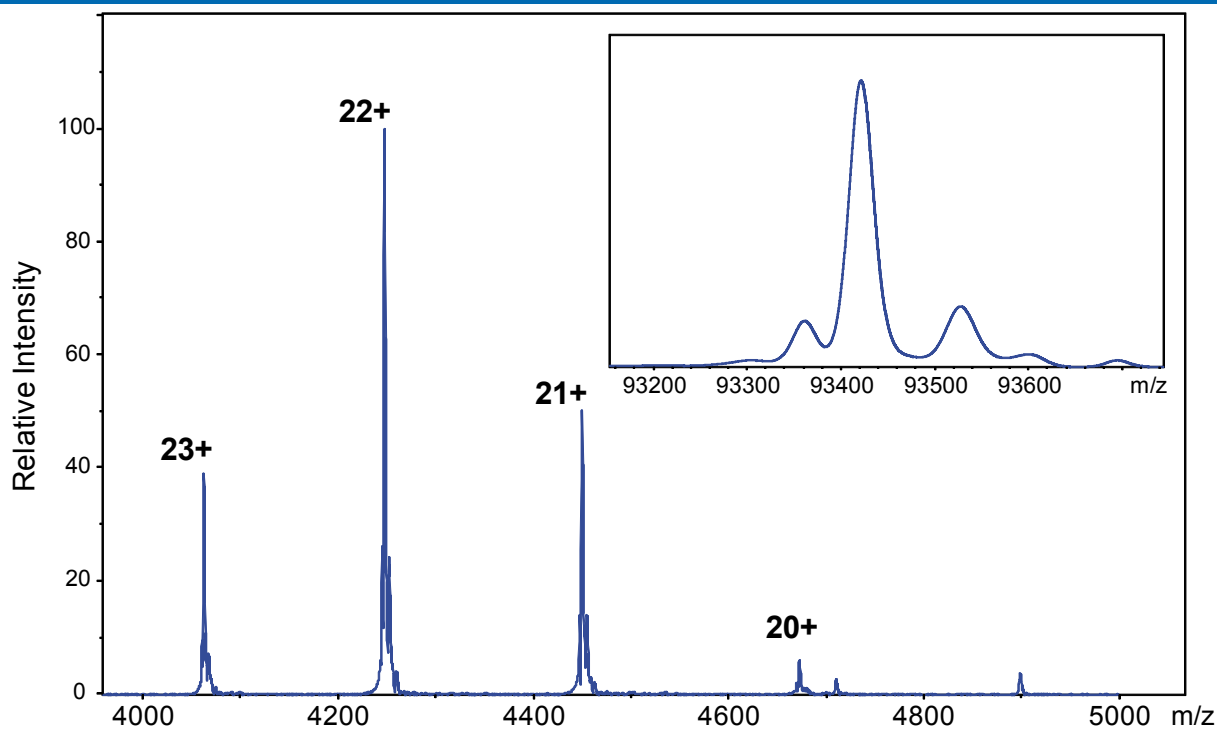


Figure 5: Enolase dimer under non-denaturing solution conditions. The advanced multipole ion injection system provides transmission of ions up to 10,000 m/z. The inset shows the data after Maximum Entropy deconvolution with the neutral mass of the molecule near ~93.4 kDa.

References

[1] Abzalimov, R.R.; Kaplan, D.A.; Easterling M.L.; Koltashov, I.A.; JASMS (in press)

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Instrumentation & Software

solarix-TD
amaZon
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Flex series
Compass
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SNAP
Mascot-TD