TopSpec - 829157

WP7 - Signal detection and data processing

Deliverable: D7.1 - Two Prototype FTMS Booster installed and tested - protocol

Task: Develop data acquisition systems (FTMS Booster TD) for protein top-down analysis and install the two of them at the consortium partners sides (IP and KI) on the Orbitrap mass spectrometers (SPS: the lead, performs the task using SPS infrastructure and personnel)

Content:

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1. Executive summary

- The two prototype DAQ systems, named FTMS Booster TD, have been developed and benchmarked at Spectroswiss facilities in Lausanne, Switzerland;
- **The first prototype DAQ system** was successfully interfaced to a Q Exactive Orbitrap instrument equipped with the Omnitrap in January 2021;
- **The second prototype DAQ system** was successfully interfaced to a Q Exactive HF Orbitrap at the Institute Pasteur in Paris, France in September 2021;
- The initial experimental results (unreduced time-domain transients) from the upgraded Q Exactive Orbitrap platforms have been generated for model proteins and antibodies;
- The protocols for the installation and calibration of the two prototype DAQ systems have been validated and improved during the installation procedures and user training;
- **Overall, the developed signal detection approaches have confirmed their ability to appropriately detect the unreduced signals (transients) from a TopSpec platform;**
- Further work includes optimization of the experimental set-ups, development of the data acquisition methods that benefit from the access to the unreduced data (time-domain transients), and demonstration of the value of these set-ups for the TopSpec applications.

2. Description of the action

At the initial WP7 activities step, we performed initial development, testing, implementation, and evaluation of a prototype high-performance data acquisition subsystem (DAQ) dedicated for the use in TopSpec platforms, which we refer to now as the FTMS Booster TD. The device is based on the high-performance data acquisition architecture as pioneered by Spectroswiss for data acquisition from FTMS instruments, such as Fourier transform ion cyclotron resonance mass spectrometers. In the following WP7 activities steps, we continued to work on the developments and optimization of the initial prototypes for TD MS applications.

The reach the objectives of WP7, the unreduced data in the form of time-domain transients are to be acquired from Orbitrap platforms by the means of the external DAQ systems designed specifically for top-down applications. The two types of the unreduced data in FTMS are: time-domain transients and absorption mode FT mass spectra (**Figure 1**).

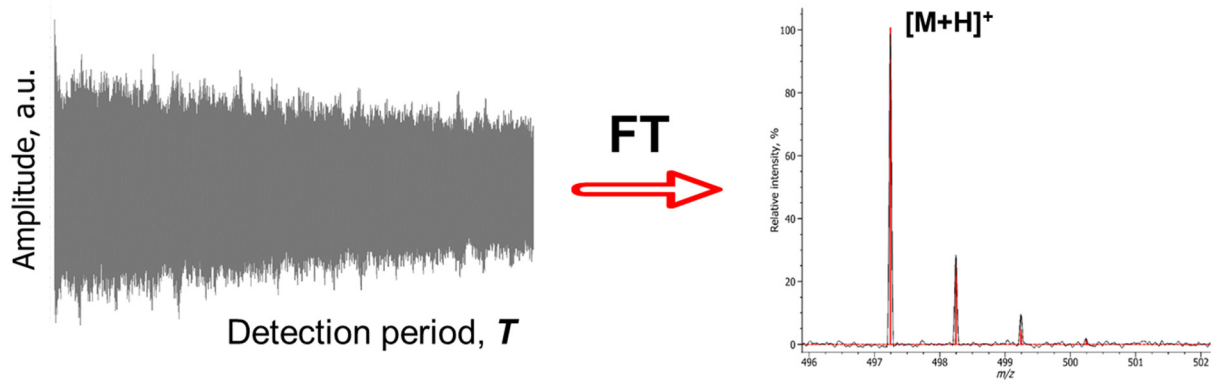

Figure 1. The two types of the unreduced data in FTMS that provide equal information output: time-domain transients (left panel) and absorption mode FT mass spectra (right panel).

To acquire the time-domain transients from the Orbitrap platforms, the prototype high-performance data acquisition systems were developed and benchmarked specifically for top-down applications. Importantly, the new generation DAQ system architecture was employed, **Figure 2**.

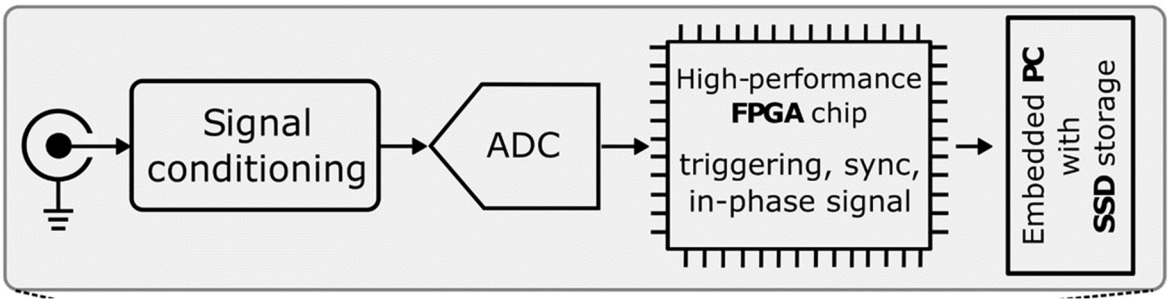

Figure 2. The new generation architecture is in the core of the high-performance DAQ system. The real-time digital signal processing takes place on the FPGA (field-programmable gate array).

The general implementation of a prototype high-performance data acquisition system (FTMS Booster TD) on an Orbitrap (any model) is presented in **Figure 3**.

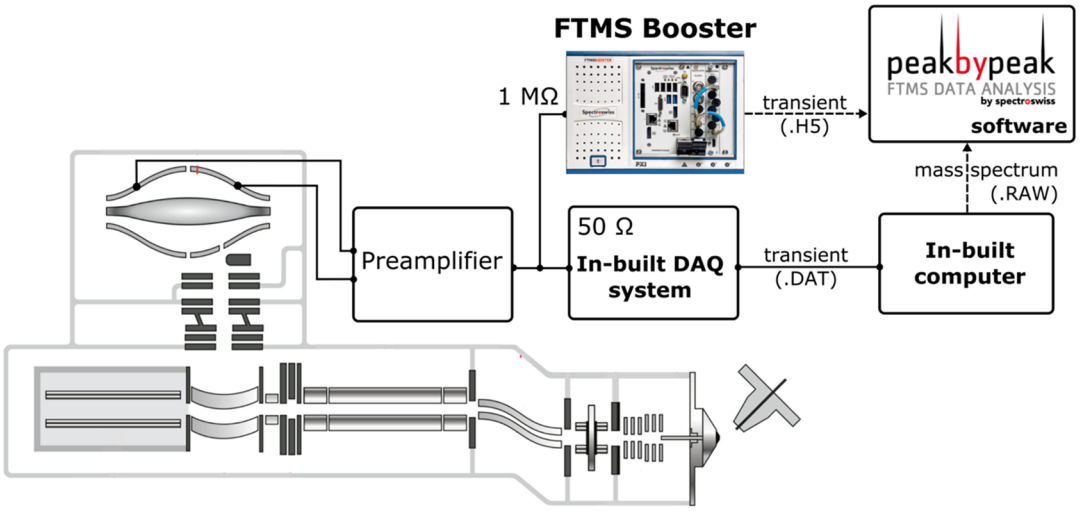

Figure 3. A prototype of a high-performance data acquisition system is connected in parallel to the original built-in data acquisition system (Thermo) to the preamplifier output. The unreduced FTMS data (time-domain transients) are thus amplified and digitized as coming out from the pre-amplifier in an analogue mode. The schematics is adapted from www.planetorbitrap.com

We installed the first prototype (aimed for the exploitation at Karolinska Institute, Stockholm, Sweden) in the labs of the Fasmatech in Athens, Greece (**Figure 4**).

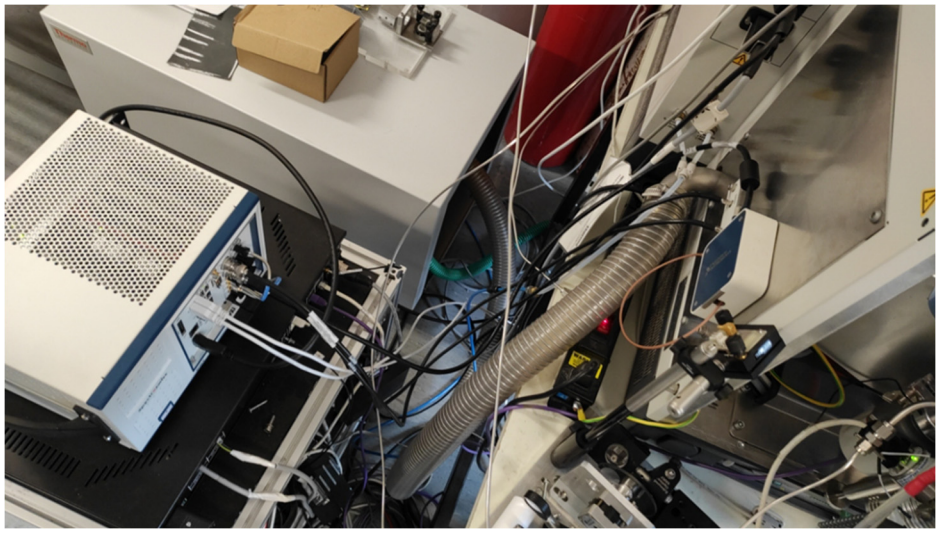

Figure 4. FTMS Booster TD on a Q Exactive Plus platform equipped with Omnitrap (installation at Fasmatech, Athens, Greece). The connection interface is shown on the right side – the two signal cables and a digital trigger cable are necessary to accurately record the time-domain transients.

The second installation was performed directly at the second site of TopSpec platform exploitation - at the Institut Pasteur in Paris, France (**Figure 5**).

Figure 5. FTMS Booster TD installed on a Q Exactive HF Orbitrap platform at the Institut Pasteur, Paris, France. The bottom panel shows the TopSpec scientists and colleagues from both Spectroswiss and Institut Pasteur. The Q Exactive HF will be equipped with Omnitrap in 2021.

3. Performance

The general objective of acquiring and processing the unreduced data (time-domain transients) is to maximize information output from Orbitraps for mAbs analysis: to deliver increased confidence and productivity, including throughput. The initial results obtained with the installed prototype DAQ systems are the following:

- Time-domain transient acquisition with an external data acquisition system is now possible
- Absorption mode FT (aFT) mass spectra can now be generated
- Extended length transients (exceed the current maximum length by 3-fold) provide the proportionally increased resolution
- The initial data acquisition methods and workflows are established

Protein analysis results: model proteins, for example ubiquitin, were successfully analyzed on the TopSpec platform using the time-domain transients (see technical report from July 2021). The following results have been reported:

- Post-processing (after the experiment is completed): transient averaging (summation) is enabled and can be performed with transients acquired in any order and in multiple technical replicates/experiments
- Transient averaging provides expected sensitivity benefits compared to spectral averaging/summation of .RAW mass spectra (reduced profile).
- Dependence of reduced profile mass spectra averaging on the noise thresholding level could be established.
- For ubiquitin analysis, transient averaging exhibits more product ion isotopic envelopes in the high mass range, as well as improved statistical representation of isotopic envelopes in general.
- Improved isotopic envelope representation yields sequence coverage increase for ubiquitin of 5-10 % for either CID or ECD data. Additional metric related to the confidence in product ion ID could be implemented.

Antibody analysis results: standard monoclonal antibodies (mAbs), for example NIST and trastuzumab, were successfully analyzed on the TopSpec platform using the time-domain transients (see technical report from July 2021). The following results have been reported:

- Analysis of herceptin, particularly CID of intact herceptin, shows that increased transient length (enabled by longer transient acquisition with the FTMS Booster TD), provides proportionally increased resolution. It allows to deconvolve complex mixtures of product ions and reduce the related peak interference. Enhanced confidence in product ion identification and increased number of identifications could be achieved, **Figure 6**.

- Specifically, for MS4 experiments, averaging of transients belonging to the non-consecutive scans provides particularly interesting alternative to spectral averaging. Results show noticeable increase in S/N for specifically low charge state product ions.
- High quality of the transients acquired with the FTMS Booster TD enabled true absorption mode FT processing (aFT). That is different with the eFT processing implemented on the commercial Orbitraps. As a result, peaks that could be identified as electronics noise are eliminated from the aFT mass spectra (out of phase), but remain present in the eFT spectra (potentially due to the magnitude mode FT processing part of the eFT).
- Further evaluation of transient and aFT processing approaches is needed to reveal the most beneficial applications of this technology in a combination with OMNI trap. That includes data averaging between multiple LC-MS and LC-MS/MS technical replicates.

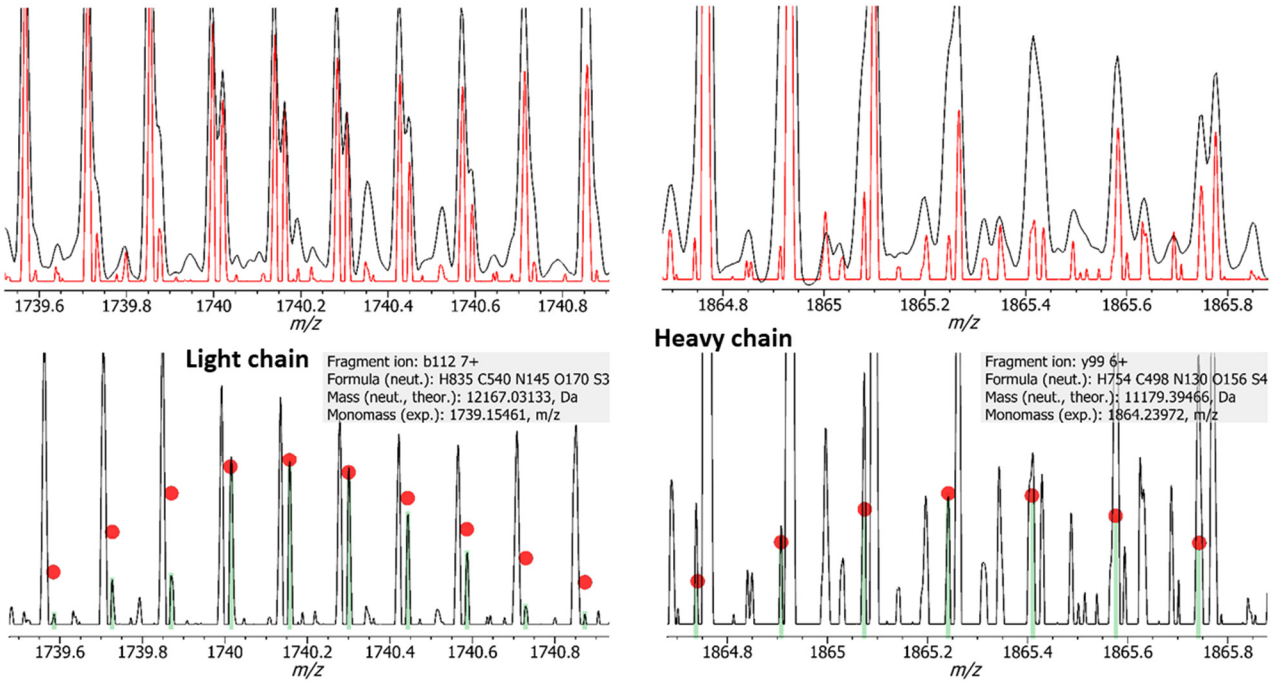

Figure 6. Resolution improvements obtained using time-domain transient acquisition from an Omnitrap-equipped Orbitrap at Fasmatech (Athens, Greece), following implementation of a prototype high-performance data acquisition system, FTMS Booster TD. Top panel data shown in black – the original RAW mass spectra, data shown in red – acquired with the FTMS Booster TD. Bottom panel shows results of data processing of time-domain data acquired with the FTMS Booster TD. Left column – light chain of Herceptin; right column – heavy chain of Herceptin. Experimental settings: sample – Herceptin mAb, transient length 3 s, maximum ion accumulation time 100 ms, gas-phase ion fragmentation by CID of an intact mAb, 300 scans averaged.

4. Protocols

The "FTMS Booster TD Acceptance Protocol" has been compiled and its content was verified during the two installations. The current version of the protocol includes the following parts:

- General information (title page)
- Product setup (completeness of delivery, visual inspection, hardware interfaces)
- Product calibration (connections calibration, transient trigger decoder calibration, and LC decoder calibration procedures)
- Product basic performance (does standard FTMS calibration pass? Does standard FTMS noise test pass? Does standard reference experiment performance match that prior to the FTMS Booster installation?)
- Special agreements (special agreements are made? List them. If a special specification supersedes one of the standard specifications, the special specification will be demonstrated during product installation only if this has been agreed upon prior to the product installation.)
- Signature page with a Warranty information (The warranty period starts as of today according to the contract.)
- Appendix A: product calibration routine description
- Appendix B: contents of delivery

The described above protocols were fulfilled at both installation sites. The on-going work includes optimization of the installation and acceptance protocols.