



TopSpec - 829157

WP1 – Omnitrap and IMS Development and Testing

Deliverable: D1.1 Two fully equipped Omnitrap traps & one IMS installed

Objectives: To develop an Omnitrap with advanced ion transfer and manipulation capabilities. **Deliverable D1.1** requires two Omnitrap platforms and one IMS unit to be fully equipped with driving electronics, gas lines, pulse valves and control software designed to be constructed and installed.

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Author: Dimitris Papanastasiou, Fasmatech

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1. Installation at Karolinska Institutet (KI)

The Omnitrap – IMS platform was installed successfully at Karolinska Institutet in October 2022. Engineers from Fasmatech and MS Vision worked jointly, and with the support provided by Karolinska personnel, to upgrade the Exploris 480 Orbitrap mass spectrometer with the latest version of the Omnitrap technology produced in Athens (**Figure 1**).

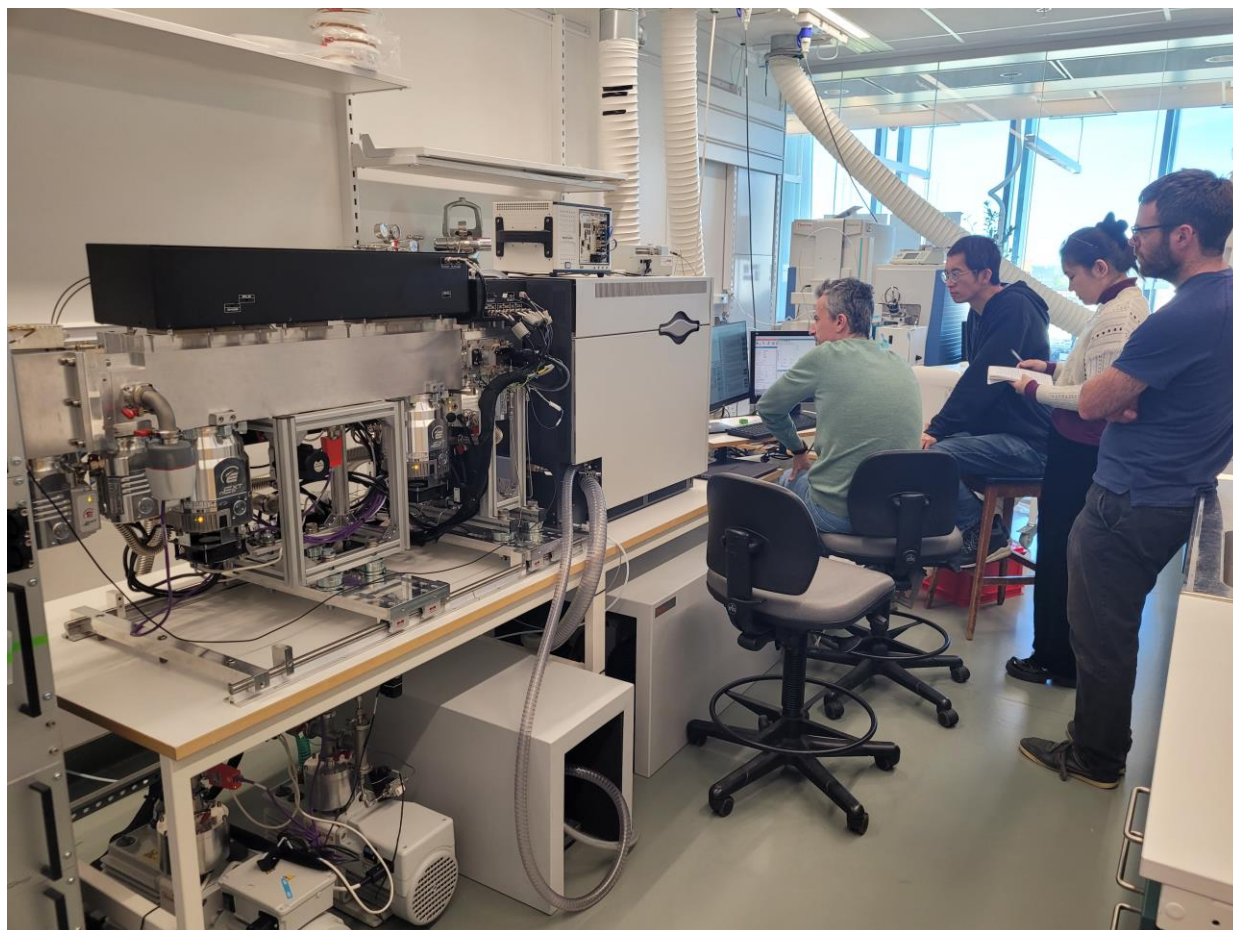


Figure 1. The Omnitrap – IMS platform coupled to the Exploris 480 Orbitrap MS was installed successfully in October 2022 at Karolinska Institutet. In the photo, Thanos Smyrnakis (Fasmatech), Zhaowei Meng (Karolinska Institutet), Hezheng Lyu (Karolinska Institutet) and Alex Langlands (MS Vision).

The Omnitrap platform installed at KI, was developed during the course of the TopSpec project in Athens, and the first of the two Omnitrap systems to be delivered. The platform includes the electron source, the hydrogen plasma gun and the ion mobility spectrometer. All fragmentation methods were tested effectively on site, including MS3 experiments with proteins (Ubiquitin and NIST antibody). In addition, the accumulation functionality necessary for producing high quality top down data in analytical workflows for intact antibodies was performed successfully.

Training: An appreciated 3-day training course was also completed following the installation of the platform and included both hands on training and a theoretical workshop held by Dimitris Papanastasiou (Fasmatech) which was attended by both students and senior researchers (**Figure 2**).



Figure 2. Theoretical workshop held by Dimitris Papanastasiou (Fasmatech).

Workflows and software: Several experimental workflows were generated and added to the library of the latest version of the Omnitrap software. Numerous software upgrades have been introduced throughout the development period of this new technology, including upgrades for the ion mobility spectrometer and also upgrades related to additional functionality implemented for ion isolation and resonance excitation procedures. An example of the high quality ExD reactions accomplished on this novel platform is presented in **Figure 3** where the reaction time between ubiquitin ions and 35 eV electrons was scanned. Sequence coverage was calculated using the new top down analysis software developed by Fasmatech. An optimum reaction time of 30 ms

was observed and the corresponding sequence coverage produced by an automated search was 97.5%. Additional manual curation of the data increases sequence coverage to 100% and improves primary fragment ion complementarity. This is currently considered the fastest EID experiment performed to date as compared to any commercially available MS platform.

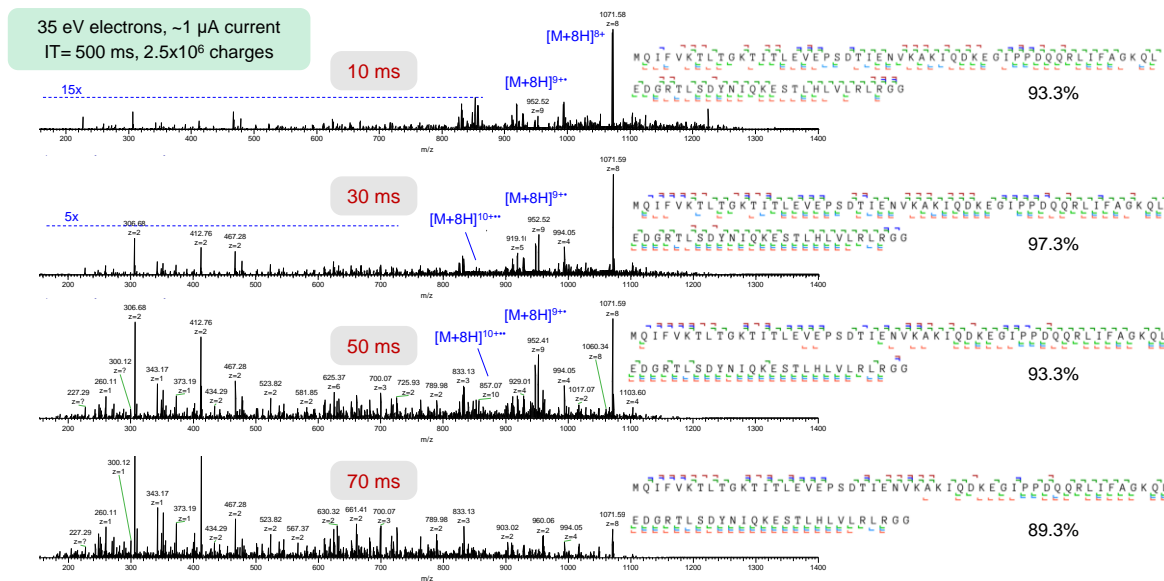


Figure 2. MS2 EID if ubiquitin ions for different reaction times and the corresponding sequence coverage results produced by the new top down analysis software developed by Fasmatech.

Going beyond the deliverables set out in the TopSpec GA, the installed instrument has access to new methods developed to enable analytical workflows in data-dependent acquisition (DDA) mode using the Omnitrap technology. **Figure 3** summarizes the instrumentation parameters and the speed accomplished in DDA mode for the analysis of a protein digest of HeLa cells. These new developed methods are able to perform MS2 EID and MS2 ECD DDA experiments at remarkable 9 and 6 Hz scan rates respectively, after optimizing the experimental and method parameters. In this workflow, ions are injected and thermalized directly to segment Q5 while all other transfer times have been minimized.

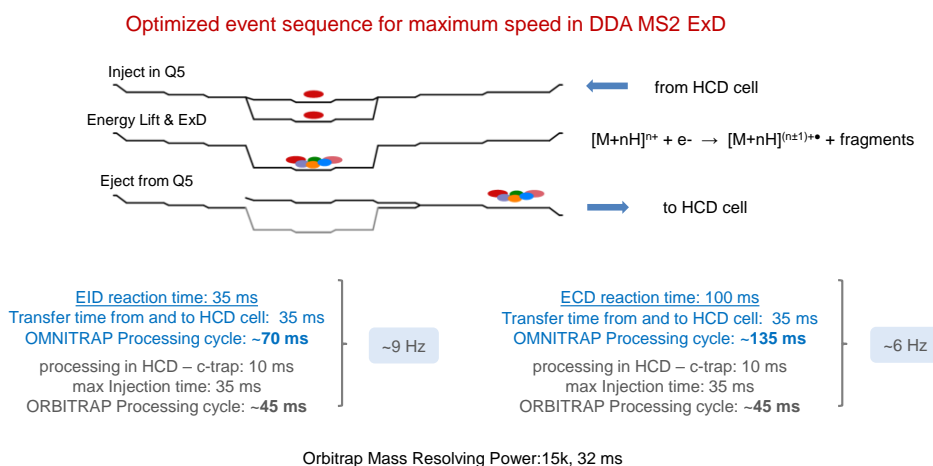


Figure 3. Optimized experimental workflow and instrument settings for MS2 ExD reactions performed in DDA mode with the first Omni, installed at KI.

Results from the analysis of the HeLa protein digest are presented in **Figure 4**. Three separate runs were performed with HCD, EID and ECD fragmentation. Despite the lower speed of EID, the number of high confidence proteins identified is ~3400, compared to ~3750 identified by HCD. The difference in the scan rate is also reflected in the number of peptide spectrum matches (PSMs) with HCD approaching 42,000 while EID produced ~23,000. ECD produced the lowest number of protein identifications due to the reduced number of MS2 scans. This limitation is attributed to the longer reaction times necessary to generate ECD fragments for low charge state ions. This first set of results highlights the potential of ExD reactions to enhance the quality of information generated in bottom up workflows.

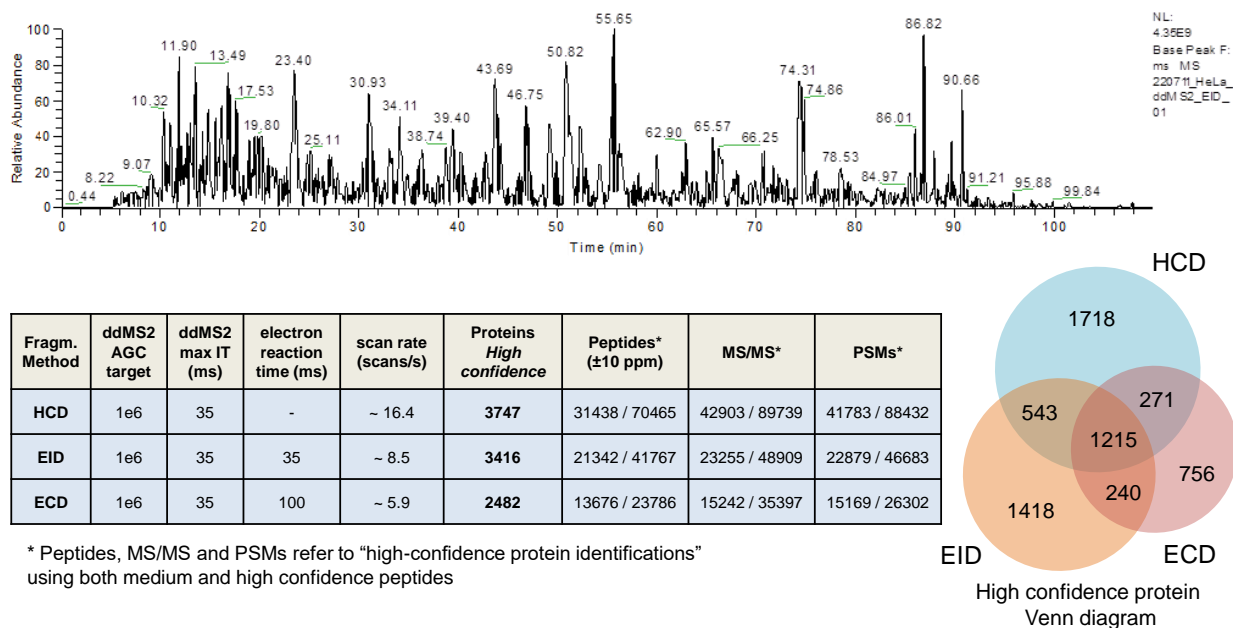


Figure 4. DDA ExD and DDA HCD results produced for the HeLa protein digest.

1. Installation at Institut Pasteur (IP)

The second Omnitrap platform developed during the course of the TopSpec project was installed successfully at Institut Pasteur in March 2022. Engineers from Fasmatech and MS Vision worked jointly and supported by personnel from partner IP to upgrade the Q Exactive HF Orbitrap mass spectrometer with the latest version of the Omnitrap technology produced in Athens (**Figure 5**).

The functionality of the Omnitrap platform installed at partner IP includes ExD, CID, a hydrogen gun, MSn, isolation and ion accumulation. The system will be upgraded in the near future with a VUV lamp to enable selective dissociation of disulfide bonds, providing access to all the CDR regions of antibodies. Experiments with light chain ions performed on liquid chromatographic time scales are already underway, demonstrating the enhanced capabilities of this unique platform. **Figure 6** shows an example of an MS2 ECD experiment performed with a reduced-alkylated light chain, followed by collisional activation of the charge reduced species. Near-complete sequence coverage is obtained (>90%). Data processing is currently being performed with the new top down software provided by Fasmatech. Additional experiments are currently underway to improve the current results and demonstrate for the first time that obtaining complete sequence coverage in top down MS is feasible.



Figure 5. The Omnitrap platform coupled to the Q Exactive HF Orbitrap MS was installed successfully in March 2022 at Institut Pasteur. In the photo, Tingting Fu (Institut Pasteur), Alex Langlands (MS Vision) and Thanos Smyrnakis (Fasmatech).

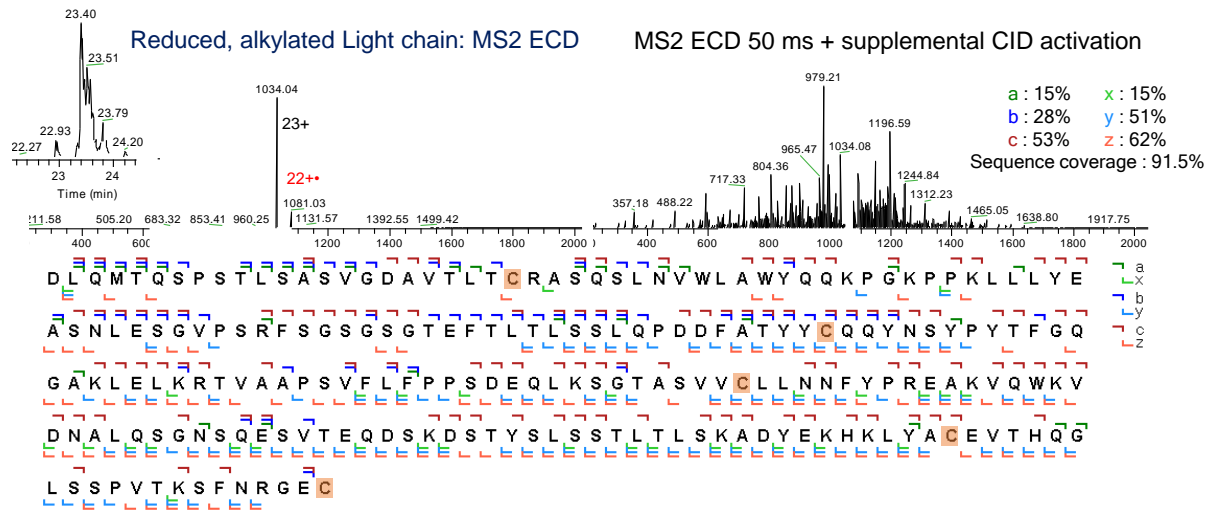


Figure 6. MS2 ECD experiment followed by collisional activation of charged reduced light chain ions performed on liquid chromatographic time scales and providing >90% sequence coverage.

Summary

To summarize D1.1, both Omnitrap platforms have been delivered to partners KI and IP and they are fully operational. Fasmatech has successfully produced a new high-end MS platform that will have a significant impact on the field of protein analysis in both top down and bottom-up workflows. The addition of the Booster technology provided by partner SPS and new software made available from all partners (FT, SPS and NTU) will push the boundaries of mAb analysis to new levels. The support of Thermo has been pivotal for the successful delivery of the two TopSpec platforms. Deeper integration between the Omnitrap platform and Orbitrap technology will continue beyond the duration of the TopSpec project, highlighting further the high level of success accomplished and the new demand created during the course of this project.