

## 1. PUBLISHABLE SUMMARY

### **Summary of the context and overall objectives of the project (For the final period, include the conclusions of the action)**

A growing challenge in EU health system is cost of drugs/targeted therapies. By reducing the time to develop therapies, costs are also reduced. To reduce development time, it is imperative to understand how we defend our self against diseases. To do this, the biggest mystery and challenge is the immune system; characterizing and understanding the expressed sequence repertoire of antibodies (Abs) and identifying the antigens, remains as a problem that needs to be solved. The aim of TopSpec is to be the first to solve this challenge - opening extraordinary opportunities in medical research and drug development. We aim to create a technology that will revolutionize proteomics, and advance development of next generation Ab- and other protein-based therapeutics. Abs are the most sophisticated line of natural defense against disease. Knowing which Abs are produced in response to a given disease will enable us to understand how antibodies are optimized, to recognize targets and so lead to next-generation cures via personalized therapeutic Abs. A key, current, rate-limiting step is to efficiently and effectively sequence large protein molecules (e.g. Abs). Here, experts in radical-assisted protein sequencing team up with the best developers in ultrahigh-resolution MS, and top European scientists in protein separation and data processing. We will develop a ground-breaking TOP-down tandem mass SPECTrometry (MS/MS) platform designed to provide a significant improvement in our ability to sequence Abs, and so to solve this current bottleneck problem. Achieving the seemingly impossible, we will create, test and validate a platform capitalizing on our ground-breaking innovations, enable scientists from academia and industry to access the Ab repertoire, enhancing our ability to explore new effective cures for global diseases.

### **Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far (For the final period please include an overview of the results and their exploitation and dissemination)**

The TopSpec platform includes a pI-trap for sample separation prior to the MS, a high-performance Orbitrap MS, a versatile ion trapping device (omnitrap) for gas-phase ion activation, dissociation, manipulation and separation, an ion mobility (IM) drift cell for separating fragment ions, a high-performance data acquisition system and data processing and data analysis software tailored for Ab analysis (Figure).

MS: The complex hardware for the omnitrap are now generating high-quality top-down spectra of Abs. Effort is still required to optimize the versatility of the device. While many details of the Omnitrap have been finalized, developments of hyperthermal hydrogen atom bombardment (HHAB), IM separation of fragment ions and software continues. The original plan of producing two Omnitrap for the Q Exactive HF MS series has been revised. Instead, two platforms, the original one (Q Exactive) and one compatible with the new Exploris 480 MS, have been designed. This latter system will exploit enhanced sensitivity and mass resolution capabilities. Subsequent developments include testing the revised IM device and HHAB gun. The electron source design and investigating Coulomb explosion dissociation effects will also be undertaken.

PI-trap: Work to connect the pI-Trap system with electrospray ionization (ESI) is progressing well, with separation of protein mixtures. A small-molecule based ampholyte has been selected, providing improved detection. Efforts have been made to balance concentration of isoelectric focusing additives whilst maintaining a stable spray. The pI-Trap instrument design has been finalized and mechanical

parts fabricated. The software is under development. The final configuration of the interface between pI-Trap Cell, buffer exchanger and ESI is in progress. The most recent implementation involves integration of a nanospray needle, electrode and buffer exchanger into a single assembly.

Data acquisition hardware/processing software: The first versions of hardware tools tailored for top-down analysis are ready to be integrated into the TopSpec platforms. The core software tools (data processing and analysis) have been developed and tested on reference data from Orbitrap MS-instruments equipped with ultra-high resolution, native MS, as well as HCD, ECD and UVPD MS/MS. The core tools are ready for TopSpec data processing and analysis. Advanced processing modules will be added, providing bespoke data processing analysis capabilities, to augment the existing capability, once the performance envelope of the full Omnitrap/IMS/Orbitrap MS systems can be explored. Further hardware, method and software tools development are envisioned.

### **Progress beyond the state of the art, expected results until the end of the project and potential impacts (including the socio-economic impact and the wider societal implications of the project so far)**

Along project plan: Major achievements are superior performance of the Omnitrap electron source and enhanced ExD ion activation capabilities. Ion-electron energy interactions are performed with high efficiency over an extended electron energy range, superior to commercially available instruments. Another innovation is the slow heating CID method, that produces unique and information rich, high mass Abs fragments. Methods to utilize this benefit, by generating Ab subunits to be isolated and processed further are in progress. Prospectively, this offers superior top down analysis of large molecular ions and assemblies. A unique feature is IM separation capabilities performed on complex fragment ion populations. It will be exploited to reduce spectral congestion and facilitate de novo sequencing of Abs. Additionally, a thermal hydrogen atom source has been developed and several unique experiments performed. A method for transient level deconvolution of top-down data using asymmetric apodization function has been developed. For validation, nanoLC-MS/MS middle- and top-down workflows on Orbitrap platforms to sequence reference Ab chains has been developed. The complementary of MS/MS activation techniques on 25 and 50 kDa subunits as well as effect of the number of selected precursors for MS/MS on sequence coverage has been evaluated.

Ahead of project plan: Of note is on-going development of a method for processing Top-down protein datasets involving isotopically enriched or depleted samples. Furthermore, development of a novel algorithm for deconvolution of complex top-down spectra by deriving transient decay constant of individual peaks has been accomplished.

Beyond project plan: A deuterium lamp has been integrated with the omnitrapp and a series-simulations to investigate surface induced dissociation are finalized. We have also developed a method for charge state deconvolution of intact Abs, but still maintaining absorption mode type peak profiles - something that no other exiting method can offer. Software for accelerating manual processing of complex spectra with implementation of de novo sequencing algorithms are being developed. Progress in preparing growth media for expressing mAbs in monoisotopic media has been made. A novel method of determining which Ab binds to a given antigen without antigen mobilization and pull-down, but based on thermal profiling has been outlined.

Impact and implications: If successful, TopSpec will provide a new tool that can be used to expand our knowledge of the immune system and have a dramatic impact on personalized, precision medicine. Another significant impact will be in the field of MS instrument design. Specific impacts: 1) Increase in the speed of diagnosis and drug development. 2) Increase knowledge on an individual's Ab response to disease. 3) Expand scientific research around proteomics. 4) Create new business opportunities within and outside the project.

**Address (URL) of the project's public website**

<https://topspec.ki.se/>

