August 2020

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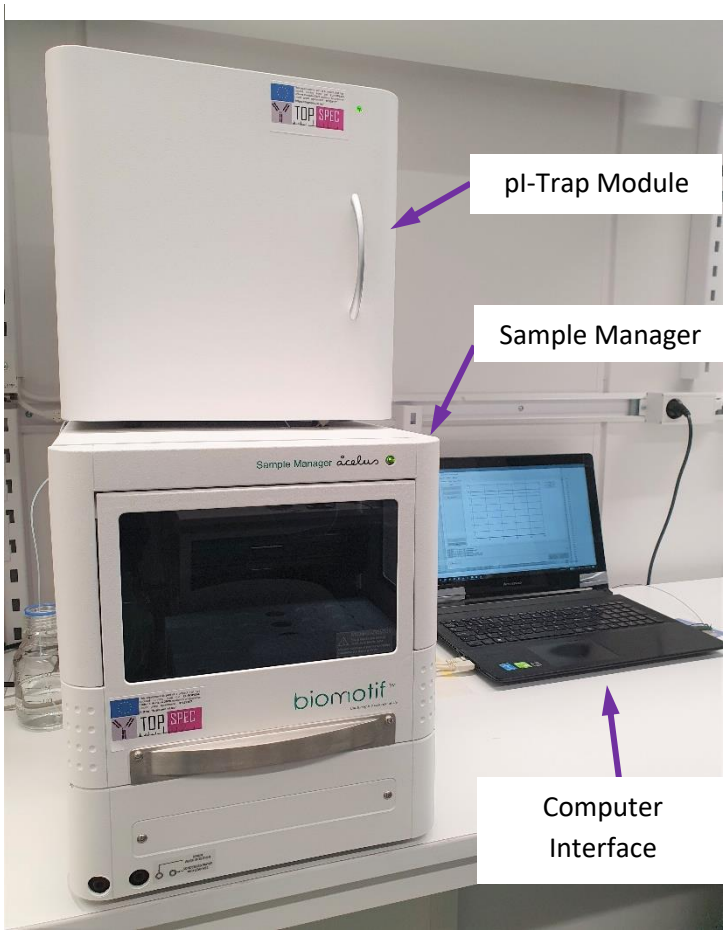
D5.1 Prototype pI-Trap ESI Installed and Tested Protocol, at Karolinska Institutet

The Omnitrap platform will benefit from an upstream separation step by decreasing the co-eluting analytes and thus reducing the spectra complexity as well as avoiding potential ion suppression effects that will undermine the overall sensitivity of the analysis.

In this context, the combination of the Omnitrap platform with isoelectric focusing (IEF) separations is envisioned as a tool to separate antibodies prior being ionized and detected by the Omnitrap Orbitrap mass spectrometer. The separation platform is an automated isoelectric focusing instrument based on a device with a series of ion-selective membranes, called pI-Trap, connected to an autosampler for unattended operation.

During the present period the manufacture and software/hardware integration of the automated pI-Trap system, -named Åcelus- have been finalized. The system consists of two interconnected units: Sample Manager and pI-Trap Modules (**Figure 1**).

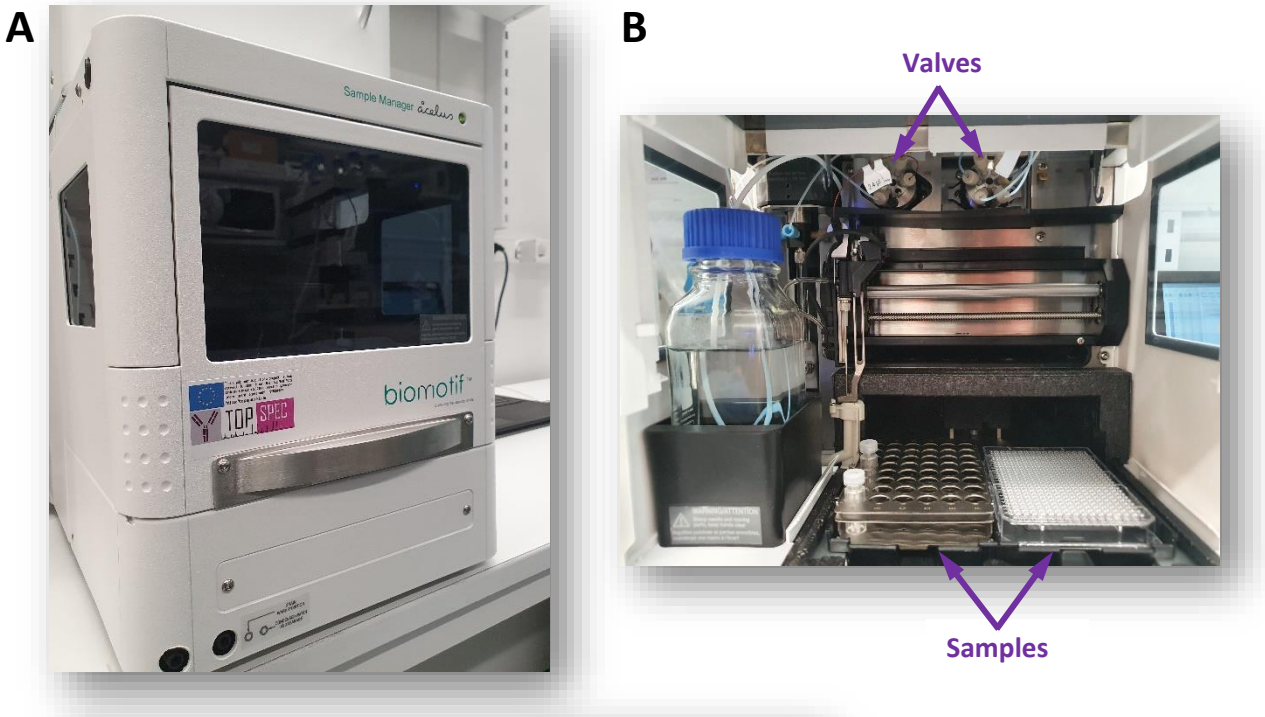
Figure 1. Automated pI-Trap Åcelus System Installed at Karolinska Institutet Module



D5.1.1 Sample Manager

The Sample Manager Module (**Figure 2A**) houses a dual 6-port valve system, a syringe pump with a 4-channel solvent selection control valve, a solvent/buffer bottle, as well as space for two racks/plates for standard LC-MS vials (8x6 racks) and 96- or 384- well plates. Vials containing the protein samples are located into this temperature controlled autosampler chamber, 4-8 °C (**Figure 2B**). In **Figure 3** a diagram depicting the component and fluidic connections of the Sample Manager module is shown.

Figure 2. Sample Manager Module, with the enclose (**A**), valves, injection needle, sample tray and well-plates (**B**)



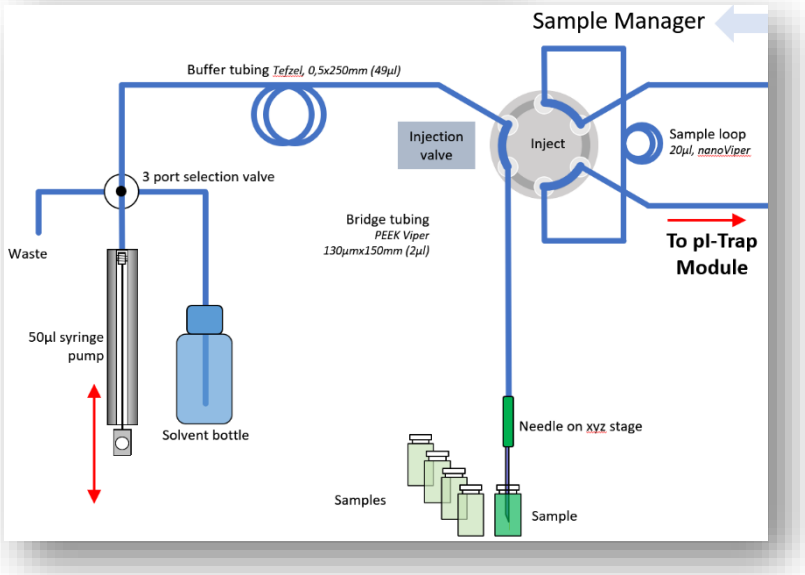


Figure 3. Diagram depicting the component and fluidic connections of the Sample Manager module.

D5.1.2 pI-Trap Module

The pI-Trap Module (**Figure 4**) contains the pI-Cell, the IEF power supply and a syringe pump with a 2-channel solvent selection control valve. The configuration of both modules was chosen to provide maximum flexibility to our consortium partners to experiment with different injection modes and pI-Cells. A typical configuration for the connection of the Åcelus system with online ESI MS can be seen in **Figure 5**.

Figure 4. Diagram depicting the component and fluidic connections of the Sample Manager module and the pI-Trap Module

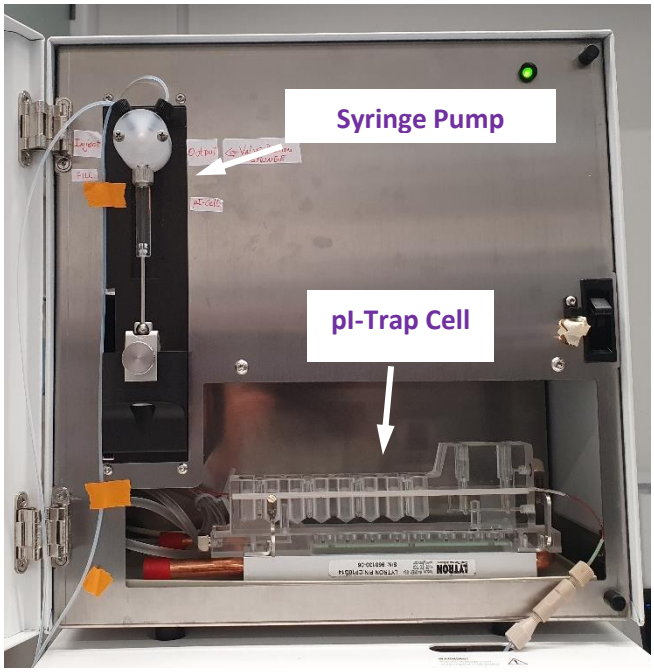
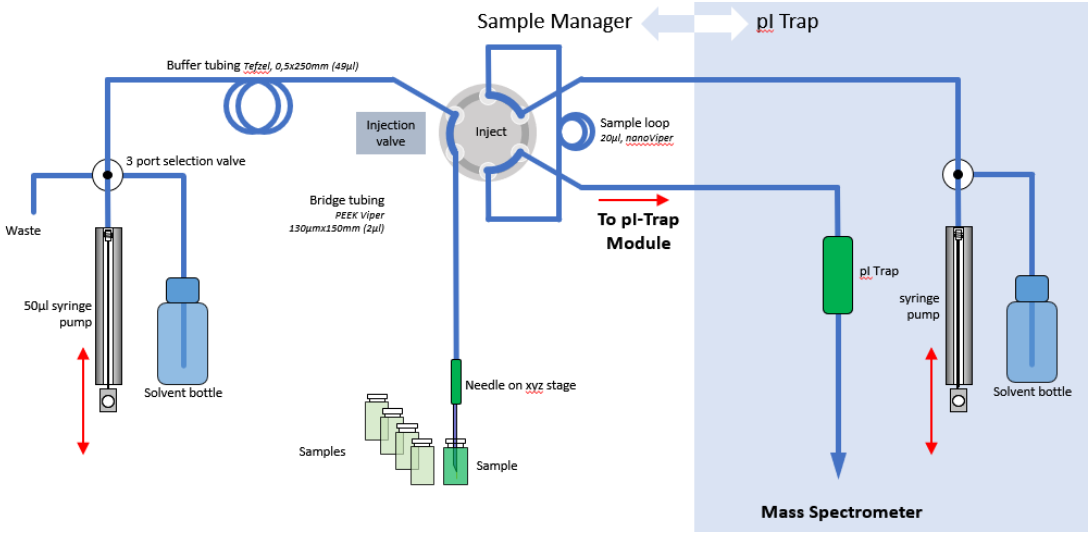


Figure 5. Diagram depicting the component and fluidic connections of the Sample Manager module and the pI-Trap Module. This configuration will be used for Åcelus ESI Omnitrap System

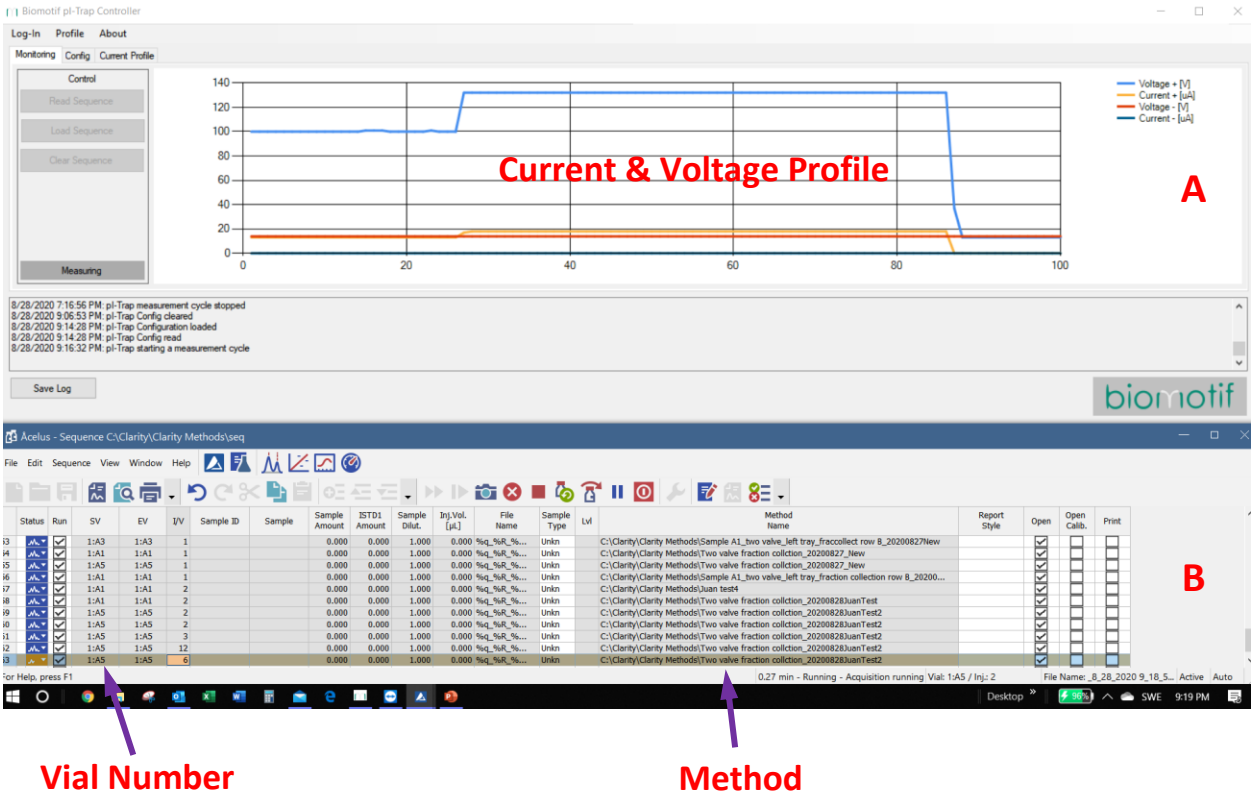


D5.1.3 Åcelus Software

The Åcelus software architecture allows the continuous monitoring and control of all the components related to sample loading (valve positions), elution, washing and contact closure signals to the pI-trap module or to the mass spectrometer (**Figure 6**).

The software allows the automated injection of one (or a sequence) of samples into the Åcelus System and communicates to mass spectrometer via contact closure signal. The scripting for controlling the valves and the injection procedures is made by a friendly interface which does not required knowledge computer programming, thus giving us (and our partners) freedom to perform a wide range of different analytical workflows during the method development of a particular sample.

Figure 6. Åcelus Software Interface including the pI-Trap GUI (**A**) and the Sample Manager Sample list (**B**).



D5.1.4 Protocols & Testing

The system has been installed at Karolinska Institutet, Sweden (**Figure 1**), and together with Prof. Roman Zubarev, we have developed and tested protocols and software configurations to provide the fastest method development environment for experimenting with different separation modes and hardware configurations. For example, additionally from the configuration shown in **Figure 5**, we have setup an automated method to collect fractions into 384-microtiter well plates. The later configuration will allow rapid method development that will be later transferred to the Åcelus ESI Omnitrap System towards the implementation of the Milestone 8 "Interfacing pI-Trap-Orbitrap".

Extensive testing and software debugging were performed especially regarding the communication between the Åcelus Sample Manager/pI-Trap Modules and successful triggering of the mass spectrometer for smooth and automated sample acquisition.

D5.1.5 Summary and Outlook

A fully automated Åcelus system has been delivered to Karolinska Institutet. An initial set of two configuration methods have been implemented to provide the maximum flexibility for the final method development step. The availability of an automated system will dramatically speed up the method development, as well as improving the overall reproducibility of the separations. Considering that there is a 6-months window towards the Milestone 8 ("Interfacing pI-Trap-Orbitrap", in February 2021) and the data provided in the previous report related to the "Successful connection of the pI-Trap to ESI MS", we believe that Milestone 8 will be completed on time.

We want to mention that although the current COVID-19 pandemic negatively affected our work, we were able to deliver a fully functional pI-Trap System to Karolinska Institutet (Prof. Roman Zubarev). Now, since both partners are located in the same city (Stockholm, Sweden), we expect our work to accelerate towards the successful implementation of the next Milestone.