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Going Big: Non-Denaturing HRMS- Hyphenated Separations Unleash the Analysis of Complex Proteoform Mixtures Over 100 kDa

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Summary

In the last decade, significant progress has been made in native mass spectrometry (MS), enabling the characterization of large proteins and protein complexes in application areas such as biopharmaceutical and structural biology studies. Yet, to date, many studies that apply native MS use purified samples and direct infusion with nanospray sources, reducing the application potential of this technique.

Hyphenating native separations to native MS allows the measurement of complex samples, resolving proteoforms according to specific mechanisms (and therefore aiding identification) and increasing the dynamic range of the measurement. However, the approaches to perform native-MS hyphenated separations are currently geared towards analyzing biotechnological protein products available in relatively large amounts and need to be more sensitive for biological studies. In our research, we aimed to extend the application of native separation methods to study intact proteins and complexes in microscale format to allow for the analysis of biological samples. The separations were developed at nanoflows, facilitating desolvation during electrospray ionization and increasing MS detection sensitivity.

In this presentation, we will discuss our results obtained using non-denaturing capillary zone electrophoresis (in collaboration with Aalen University), nanoflow size exclusion chromatography, and nanoflow ion-exchange chromatography, and their hyphenation to MS. Focus of the talk will be in particular the use of nanoflow cation exchange chromatography. Results from the analysis of reference proteins between 10 and 150 kDa, a model cell lysate, and serum immunoglobulin G by a salt-mediated pH gradient with volatile additives will be discussed. Proteins presented non-denatured mass spectra, and low detection limits were achieved (0.22 pmol of monoclonal antibodies). Excellent chromatographic separations were obtained, including the resolution of different proteoforms for large proteins (over 140 kDa). The proposed native hyphenated separations setup shows great potential for analyzing diverse proteins in native top-down proteomics and provides unprecedented opportunities for clinical applications.