

Characterization of proteoforms of intact proteins by CE-MS and LC-CE-MS

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Electromigrative techniques are powerful tools for the separation of intact proteins and their proteoforms. However, CE-MS is still restricted by the sensitivity and ease-of-use of the interface in conjunction with low injection volumes limiting its application for biological samples. Various solutions will be presented here overcoming these shortcomings.

Initially, the power of CE-MS for the characterization of proteoforms will be presented applying the nanoCEasy interface [1]. Efficient separation of proteins and proteoforms depends strongly on the applied capillary coating. Very recently we developed efficient coatings enabling finetuning the EOF [2] and, thus, increase the separation efficiency for proteins of certain mobility. Results on the application for protein separation from biological samples will be presented.

nanoLC-CE-MS is a promising tool for targeted protein and proteoform analysis in biological samples. Initially a heart-cut nanoLC-CE-MS was setup and the performance regarding improved sensitivity as well as separation of proteoforms was demonstrated [3]. Due to the increased loadability, the nanoLC-CZE-MS setup exhibits a strongly improved increased concentration sensitivity compared to CZE-MS. The combination of high sensitivity and orthogonal selectivity enables the detailed characterisation of intact proteoforms at physiologically relevant concentrations. A novel selective comprehensive online nanoLC-CE-MS configuration will be presented and discussed in the context of targeted proteoform analysis in biological samples using proteoforms of histone as an example.

References

- [1] J. Schlecht, A. Stolz, A. Hofmann, L. Gerstung, C. Neusüss, Anal. Chem. 2021, 93, 44, 14593.
- [2] L. Dhellemmes, L. Leclercq, H. Frick, A. Höchsmann, N. Schaschke, C. Neusüss, H. Cottet. J. Chrom. A 1720 (2024) 464802.
- [3] A. Stolz, C. Neusüss, Analytical and Bioanalytical Chemistry 2022 Vol. 414 No. 5, 1745.

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