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A Novel Selective Comprehensive Two-Dimensional Online nanoLC-CZE-MS Platform for Proteoform Characterization

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Summary

CE-MS has been used to separate proteoforms on intact protein levels, however, selectivity and sensitivity are often not sufficient for complex biological samples. We therefore developed a two-dimensional (2D) heart-cut nanoLC-CZE-MS platform and have shown that this allows the pre-separation of intact proteins from a complex matrix and a 280-fold increased sensitivity compared to a one-dimensional CZE-MS approach. Furthermore, with this 2D approach, a higher proteoform selectivity can be gained [1]. While the transfer of a peak from the first to the second dimension is efficient using our two-dimensional heart-cut approach, the characterization of multiple chromatographic peaks or partly separated proteoforms is time consuming, requires high sample amounts, and might lead to incomplete proteoform characterization. Therefore, we expanded the nanoLC-CZE-MS platform to perform selective comprehensive nanoLC-CZE-MS. There, the RPLC column is connected to a storage capillary by a 10-port valve to decouple the storage capillary from the first dimension while performing the second dimension analysis. This allows the storage of a fraction from the first dimension for subsequent 2D analysis. The volume of the stored fraction depends on the volume of the storage capillary and can be easily adjusted. The storage capillary is also connected to an 8-port nanoliter valve with four 20 nL internal loops. Hence, 20 nL fractions can be transferred from the storage capillary to the second dimension. Here, we discuss different coupling approaches for RPLC-CZE-MS and show the essential parameters of our selective comprehensive two-dimensional nanoLC-CZE-MS platform. In addition, we show initial results from the analysis of a cell lysate using our selective comprehensive two-dimensional online nanoLC-CZE-MS platform, demonstrating its highly selective and sensitive analysis of proteoforms.

References

[1] A. Stolz, C. Neusüß, Characterisation of a new online nanoLC-CZE-MS platform and application for the glycosylation profiling of alpha-1-acid glycoprotein, Anal. Bioanal. Chem. 414 (2022) 1745–1757.