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Selective comprehensive online nanoLCxCZE-MS platform for top-down proteoform analysis

Capillary zone electrophoresis-mass spectrometry (CZE-MS) has been used to separate proteoforms on intact protein levels, however, sensitivity and selectivity are often not sufficient for complex biological samples. We therefore developed a two-dimensional (2D) heart-cut nanoLC-CZE-MS platform and showed 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[1]. While the transfer of a peak from the first to the second dimension is efficient in this setup, 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 platform to perform selective comprehensive nanoLCxCZE-MS. Here, the RPLC column is connected to a storage capillary by a 10-port valve to decouple the storage capillary from the first dimension. The volume of the stored fraction can easily be adjusted by changing the inner diameter or length of the capillary. The storage capillary is also connected to an 8-port nanoliter valve with 20 nL internal loops. Hence, 20 nL fractions can be transferred from the storage capillary to the second dimension. Here, we show the essential parameters of our selective comprehensive nanoLCxCZE-MS platform and demonstrate the high selectivity and sensitivity for the analysis of proteoforms in a human cell lysate (Caucasian colon adenocarcinoma cells). Using our selective comprehensive nanoLCxCZE-MS platform, we were able to detect up to four times more proteoforms in the stored fraction compared to 1D nanoLC-MS. In addition, we show for Histone H4 how using CZE in the second dimension provides an additional layer of information for proteoform identification and validation in the cell lysate.

[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.

User consent

yes

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