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Novel bridged hybrid monolithic columns combined with mass spectrometry for top-down proteomic analysis

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Top-down proteomics could offer comprehensive investigations of proteoforms. However, it is greatly challenged by the co-elution of intact proteins which results in overlapped mass spectra. Hence, highly effective protein separation to reduce the co-elution of intact proteins from complex samples is of vital importance.
The ethane-bridged hybrid monolithic column with homogeneous macropores of 1.1 μm and large mesopores of 24 nm was prepared for protein separation with high peak capacity of 646 within 240-min gradient. Based on MS/MS analysis, 959 proteoforms corresponding to 263 proteins could be unambiguously identified from E. coli lysates in a single 240-min run. Furthermore, 347 large proteoforms with Mw higher than 30 kDa were detected in the single 75-min run. Besides, 6264 proteoforms corresponding to 885 proteins were identified from THP-1 cells induced by LPS.
The amine-bridged hybrid monolithic column with unique macropores was prepared and coupled to MS for analysis of membrane proteoforms. Due to its unique macroporous structure and secondary amino groups in the framework, the column possessed fast mass transfer, low non-specific adsorption, and electrostatic repulsion to membrane proteins, thus greatly reducing peak broadening and outperforming traditional reversed-phase columns in top-down characterization of membrane proteoforms. With this column, a total of 3100 membrane proteoforms were identified in the mouse hippocampus. The proteoform information was integrated into the interaction network of membrane protein complexes involved in oxidative phosphorylation processing, uncovering more detailed molecular basis and interaction in the biological processes.
Furthermore, highly sensitive top-down proteomic analysis of LCM slices was developed based on the narrow-bore amine-bridged hybrid monolithic column with low non-specific adsorption. Integrated with MALDI MSI, high-throughput spatially resolved proteoform analysis were achieved, yielding 366 annotated proteoform images from the mouse brain and revealing 14 differential proteoforms in the subiculum region associations with amyloid-β pathology in AD.

User consent

yes

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