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Microfluidic Automation of Sample Preparation Techniques for Proteomics

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Summary

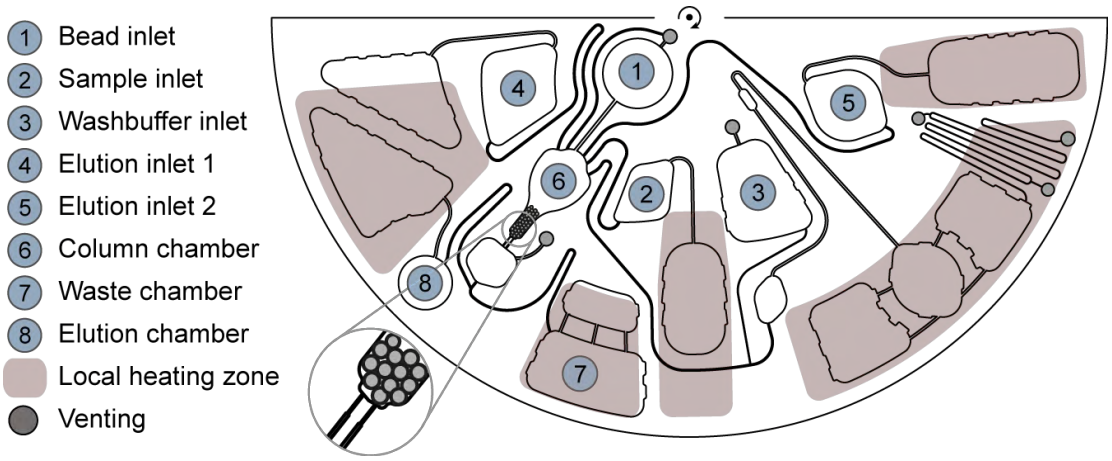

Figure 1: Schematics of the microfluidic LabDisk for solid phase extraction. Fluid propulsion is realised by a combination of centrifugal force and thermopneumatic pumping by local heating zones under the LabDisk.

The performance of the sample preparation significantly affects the downstream analysis via LC-MS/MS for bottom-up proteomics and is considered to be a major bottleneck with regard to reproducibility [1]. This is due to the numerous steps that are necessary to generate highly pure peptide samples from complex samples such as cell lysates or formalin-fixed paraffin-embedded (FFPE) tissue. Essential elements of the sample preparation process, such as homogenization of crude samples, on-bead digestion and enrichment/purification of peptides often rely on bead handling. Bead based processes, including solid phase extraction (SPE), can efficiently be implemented using centrifugal microfluidics. Efficient separation of liquid and solid phases by the artificially created gravity field and the reduction of reaction volumes enable less adsorptive losses and higher reproducibility compared to manual workflows. We present automated SPE on the centrifugal LabDisk platform demonstrated by peptide desalting of tryptic HEK-293 digestions. The microfluidic approach featured more precise results for peptide quantification (median CV of 9.3% versus median CV of 12.6% for manually desalted samples). Future work aims to transfer the microfluidic SPE concept to additional workflow steps such as on-bead (SP3) digestion and phosphor peptide enrichment. Integration of this concept with centrifugal microfluidic bead-based FFPE tissue homogenization promises ultrasonic free, highly reproducible sample preparation for FFPE tissue.

Acknowledgement

Financial support by the BMBF within the project ESTHER (project number 13GW0603D) and by the German Research Foundation (DFG) within the project MiniNter (PA2807/3-1)) is gratefully acknowledged.

References

[1] X. Ye, J. Tang, Y. Mao, X. Lu, Y. Yang, W. Chen, X. Zhang, R. Xu, R. Tian, Integrated proteomics sample preparation and fractionation: Method development and applications, TrAC Trends Anal. Chem. 120 (2019) 115667.