

# Chromatographic Separation Strategies for Proteomics Analysis

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Multidimensional liquid chromatography (MDLC) is a valuable technique for both bottom-up and top-down workflows in proteomics. Various approaches of MDLC, e.g. MudPit, have been described for the separation of peptides and MDLC is increasingly applied in top-down approaches. In a proteomics field such as biomarker discovery different stages can be distinguished, that all have their own requirements on the analysis strategy. MDLC allows for the flexibility to meet these requirements.

One of the arguments against MDLC is the increase in workload and analysis time with each dimension. The development of monolithic columns during the past years has brought a great improvement in the field of protein separations. Their macroporous structure leads to fast mass transfer, low backpressure and high resolution, resulting in short analysis times and efficient separations. When this is combined with automation features the throughput of a MDLC separation can be maximized.

Here we present the variation one has with MDLC by discussing both monolithic and packed columns, an MDLC workflow is demonstrated, using the ProSwift monolithic columns. A weak anion exchange column in the first dimension is combined with a Reversed Phase column in the second. Intact proteins from a bacterial cell lysate are separated in two dimensions, keeping the total analysis time below 5 hours. Besides this example, the effect of hardware and software tools on the speed and automation of the entire workflow will be demonstrated.