Comparison between Sample Preparation On Chip (SPOC) and Off-Line MALDI-MS Sample Preparation Approaches for Sample Clean-Up and IMAC Enrichment

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Introduction

- Optimized sample preparation is crucial for successful and sensitive detection of peptides by MALDI-TOF MS.
- Protein identification of low femtomole amounts of analyte requires further clean-up and concentrating steps to acquire significant spectra particularly if gel workflows are approached or the sample is stabilized by detergents.
- The analysis of posttranslational modifications is even more challenging as often PTMs such as phosphorylation may occur only on at low stochiometric ratios. Hence, for phosphorylation an additional removal of background proteins and efficient isolation of phosphopeptides is necessary.
- Here we present workflows for efficient purification of digests and enrichment of phosphopeptides based on sample preparation on-chip technology (SPOC) and compare this technique to standard pipet–tip or spin-column based approaches.

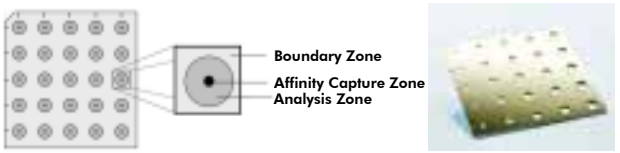

Fig. 1: Layout and on-chip sample concentration properties of Mass Spec Focus Chips

Methods

SPOC - chip based sample preparation:

For SPOC sample preparation different Mass·Spec·Focus Chips (QIAGEN) were employed. these chips contain various sample sites (Fig. 1) created by the juxtaposition of different surface chemistries in discrete concentric circular zones of wettability. After sample application the hydrophobic Boundary Zone confines the drop and prevents it from spreading (Fig 2A & 2B). The functionalized Affinity Capture Zone, which provides a IMAC surface or mimics reverse phase chemistry for sample purification, specifically retains (phospho)peptides and allows contaminants (nonphosphorylated peptides, salts, etc.) to be washed away. Consecutively, an elution step is performed and the analyte is eluted to the Analysis Zone. A drop of matrix solution is added to the well and analyte and matrix are co-crystallized in the Analysis Zone, giving a highly concentrated MALDI preparation. Spin-column and pipet-tip based IMAC and detergent removal devices were used accordingly to manufacturer manuals. MALDI analyses were carried out on a ABI 4800 TOF/TOF (Applied Biosystems) or an Axima CFR (Shimadzu) instrument.

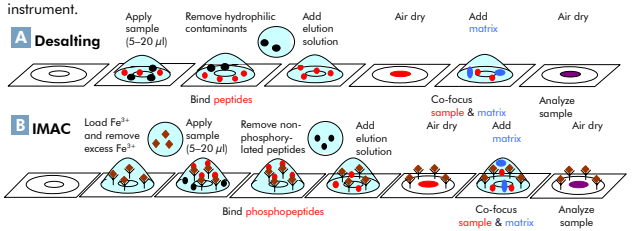

Fig. 2: SPOC workflows for A IMAC enrichment of phosphopeptides using Mass·Spec·Focus IMAC chips; B contaminant removal using Mass·Spec·Focus desalting chips.

Specificity of SPOC-IMAC

Enrichment of a p(TEY) phosphopeptide from his-tagged recombinant human MAPK1

- SPOC-IMAC allows specific phosphopeptide enrichment with low background of non-phosphorylated peptides.

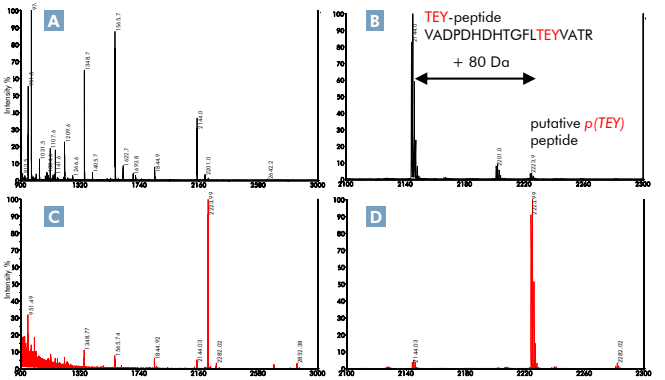

Fig. 3: Enrichment of a phosphopeptide form a tryptic digest of MAPK1 using Fe(III)-loaded Mass·Spec·Focus IMAC chips and analysis on a ABI 4800: A MALDI-MS spectrum of the crude digest without SPOC-IMAC enrichment acquired on a stainless steel target; B close-up of A around the mass of the putatively phosphorylated TEY-peptide (common MAPK phosphorylation motif); C SPOC-IMAC enrichment of the same digest; D close-up of C.

Comparison of IMAC Methods

Chip based versus spin-column based enrichment approaches

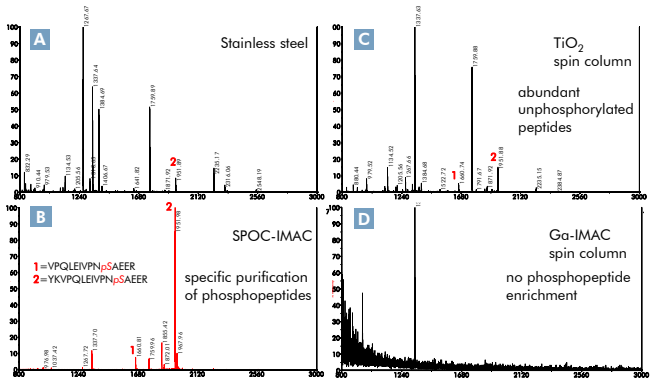

Fig. 4: MALDI-MS spectra of tryptic alpha-S1-casein digest with different IMAC methods, A crude digest on stainless steel target; B SPOC-IMAC enrichment using Mass·Spec·Focus IMAC Chip; C a Ga-IMAC based spin-column system; D a TiO_2 based spin-column system; instrument ABI 4800 TOF/TOF.

Comparison of Sample Clean-Up Methods

Chip based vs micro-column based peptide purification & concentration

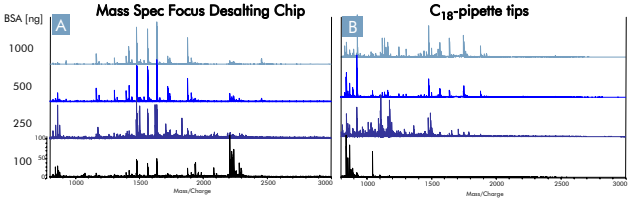

Fig. 5: MALDI-MS spectra for different amounts of BSA in-gel digests after purification on A a Mass Spec Focus Desalting Chips or B after processing with C_{18}-resin packed pipette tips; instrument Shimadzu Axima CFR.

BSA [ng]	Peptide masses matched to sequence		Mascot score	
	SPOC Desalting	C_{18} tips	SPOC Desalting	C_{18} tips
1000	21	19	152	105
500	16	16	58	50
250	10	10	64	48 (Keratin 1st)
100	11	1	54	not identified

Tab. 1: Comparison of peptide masses matching to BSA sequence and corresponding Mascot scores after purification on Mass·Spec·Focus Desalting Chips and after processing with C18-resin packed pipette tips

Summary:

- The SPOC approach provides efficient purification and enables sensitive detection of peptides.
- For phosphoproteomics the SPOC-IMAC method allows specific enrichment of phosphopeptides from complex mixtures. The chip-based approach outperformed in our experiments spin-column based methods particularly with respect to a reduced non-specific binding of non-phosphopeptides.
- For sample clean-up of tryptic peptides the comparison of a SPOC desalting method and a pipet-tip based C18 micro-column showed similar relative sequence coverages for tryptic digests, although the SPOC approach showed less low mass peptides but more higher mass peptides than the C18 micro-column indicating rather a C10-C14 characteristic. However, the strong concentrating effect of the SPOC method resulted in a lower limit of detection and based on the higher MASCOT scores also an higher significance in protein identification.
- Chip based approaches are particularly suited for more parallel or even fully automated workflows, as all protocols are based on basic sequential liquid handling steps. Spin-column or pipet-tip based approaches in contrast need more sophisticated liquid handling technology due to the back-pressure of resins or the necessity for sequential centrifugation and pipetting for spin-columns. Also spin columns typically do not allow elution with low µl volumes directly to a MALDI plate.