Summary

Affinity based diagnostics depend on biologically derived affinity reagents (e.g. antibodies or antibody fragments, receptors or aptamers) due to their ability to effectively recognize and bind specific biomarkers in competitive media for assay, sensor or LC-MS based readout of the biomarker concentrations. In spite of these benefits these binders can be either hard to get due to high costs or poor availability, or they may not be compatible with denaturing conditions required during the sample pretreatment. Such problems are partly hampering the development of diagnostic methods of neurodegenerative disorders and cancer.

The aim is to prepare molecularly imprinted polymers (MIPs) capable of recognizing peptidic biomarkers in nonphysiological media e.g. acetonitrile-buffer mixtures or in denaturing media. The peptide MIPs will be used as capture phase for solid phase extraction of biological samples.

Derivatives of a diagnostic nonapeptide biomarker NLLGLIEAK resulting from tryptic digestion of the well established protein biomarker for small cell lung cancer ProGRP, were used as templates. With the objective of finding a polymer that, as stationary phase, would retain and rebind the NLLGLIEAK from the matrix components and quantitatively elute it in a small volume, a combinatorial MIP library has been synthesized. The combinatorial MIP libraries for NLLGLIEAK have been prepared by using high-throughput synthesis of MIPs at a reduced scale (mini-MIPs), to rapidly generate 96 imprinted and their corresponding non-imprinted polymers by bulk polymerization. A careful optimization of the synthesis molecularly imprinted polymers has been achieved. The parameters which have been screened and modified are templates functional monomers, crosslinkers, percentage of crosslinking, and porogen.

To test these polymers, a rebinding step was preformed, by comparing the amount of target peptide which was bound in imprinted polymer and a blank nonimprinted polymer, the polymer which gave high imprinting factor was scaled up for separation application.

The MIPs developed in this work were proven potent receptors for their target biomarkers. The MIPs targeting the ProGRP signaling peptides were capable of selectively capturing the peptide from tryptic digests promising to significantly reduce the detection limit in the LC-MS-based assay.

Various parameters affecting the extraction efficiency of the polymer have been evaluated to achieve the selective preconcentration of the NLLGLIEAK from aqueous samples and to reduce nonspecific interactions. The imprinted polymer was evaluated for use as a SPE sorbent, in tests with aqueous standards; by comparing recovery data obtained using the

imprinted form of the polymer and a non-imprinted form (NIP). Extraction from the aqueous solutions resulted in more than 80 % recovery. A range of linearity for NLLGLIEAK between 1.5 and 50 mg/mL was obtained by loading 1 mL aqueous sample spiked with NLLGLIEAK at different concentrations in HEPES buffer of pH 7.0. The intra-day coefficient of variation (CV) and inter-day CV was below 7%.

In the end the bulk format was transferred to grafting of MIP films which showed excellent affinity and selectivity to NLLGLIEAK and was therefore suitable for the application in SPE.

A working protocol for the development of MIPs targeting biomarker peptides compatible with denaturing conditions and organic solvents i.e. common protein analysis conditions, have been developed. This promises to significantly expand the scope of the technology in proteomics and diagnostics.