

## TARGETED PROTEOMICS: A POWERFUL APPROACH PROVIDING NEW INSIGHTS IN BIOLOGY.

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Major concerns in the development of proteome technologies have been 1) protein coverage; 2) correct protein identification; 3) quantitative aspects and 4) identification and localization of post-translational modifications. Since the introduction of 2D-gels in the mid seventies, different strategies have been designed to accommodate these important issues. These involved new concepts in protein/peptide chemistry, improvements in chromatography technologies and robotics connected with high throughput and high sensitivity mass spectrometry. These technical improvements went hand in hand with the creation of new tools in bioinformatics, able to link mass-spec data with predicted protein sequence data bases and assisting in the interpretation of complex data. All these developments fundamentally altered the way biological questions could be addressed.

I will summarize how our laboratory has contributed to different aspects of contemporary proteomics. We developed the COmbined FRActional DIagonal Chromatography (COFRADIC) technology, allowing targeted sorting for biologically relevant aspects in highly complex peptide mixtures. This not only considerably improved sample coverage and lead to focused analyses, but it also provided means for a global analysis of post-translational modifications which were previously not accessible. Our COFRADIC approach created the necessity to develop particular bioinformatics tools not only to steer the work-flow but also to improve protein identification confidence. This plethora of tools was recently supplemented with a strategy for automated differential quantitative global sample analysis. At the end of the proteomics pipeline highly complex data emerge: the result of global cellular systems. The different aspects of our work will be illustrated using beta-actin KO cells and organisms; proteolytic processing and cleavage events as cell regulators and methionine oxidation resulting from oxidative stress.