

## **Abstract**

Structural proteomics combined with mass spectrometry, is a rapidly evolving scientific discipline that focuses on the characterization of the three-dimensional structures of proteins, as well as their dynamics. However, Current standard practices require a large number of separate processing steps, especially when the structural data is obtained from the bottom-up approach. Moreover, these steps are mostly performed at different times and places.

This doctoral thesis was aimed at preparation of MALDI mass spectrometry-compatible proteolytic biochips and their application in structural proteomics. These functionalized surfaces allow both the cleavage of the studied protein and the MALDI matrix crystallization followed by a mass spectrometric analysis to be performed within a single active position.

An ion-landing technique operating under ambient conditions was revealed to be optimal for the immobilization of proteolytic enzymes. This electrospray-based technique enables a direct modification of flat, smooth and conductive surfaces which are also used in the MALDI ion source. Various serine and aspartic proteases were successfully immobilized to get a wide proteolytic substrate library available for various structural proteomics applications.

A high number of active spots per biochip allowed us to perform high-throughput analysis at the time, while the low working volumes of the applied sample or quenching reagent contributed to the high reproducibility of the measurements. These parameters were very suitable for the limited proteolysis method. An analysis of hydrogen-deuterium exchange products also appeared to be very competitive to the instrumental complexity of reference LCMS detection method. However, the high level of deuterium back-exchange reaction remains a fundamental challenge of the MALDI biochip digestion and detection approach. Nevertheless, appropriate protease-modified surfaces may also be used for a rapid analysis of protein footprinting, disulfide bond mapping or verification of protein identity.

To process the large volume of measured spectra, it was necessary to develop and integrate reliable procedures for automated data evaluation and interpretation. The development of these procedures and software solutions was essential for the practical implementation of proteolytically active surfaces, and others as well, into structural proteomics workflows.

Key words:

Structural proteomics, mass spectrometry, ambient soft-landing, limited proteolysis, hydrogen deuterium exchange, MALDI, biochip, protein immobilization, data analysis.