

ABSTRACT

Mass spectrometry (MS) techniques have, over the last twenty years, found their stable place in the structural biology toolkit. They are not only employed to provide information on the protein primary sequence, but are increasingly used to probe higher orders of protein structure as well. They may not boast the atomic resolution and the ability to directly provide structural coordinates, but on the other hand suffer from very few experimental limitations as they are able to work under native conditions in solution, provide data fast, with low sample consumption and for proteins and complexes of vastly differing sizes. Perhaps most importantly, they may often be employed to study conformational dynamics of proteins and can thus complement other methods with higher spatial resolution in integrative structural biology approaches.

The main focus of this Ph.D. thesis was hydrogen / deuterium exchange coupled to MS (HXMS), which is one of the most widespread structural MS methods. Recombinantly produced aspartic protease nepenthesin-1 from *Nepenthes* pitcher plants was characterized, immobilized and extensively tested with the intention to expand the portfolio of aspartic proteases in HXMS workflow and to improve the spatial resolution of the technique.

Following successful implementation of nepenthesin-1 into the HXMS protocol, it was used in combination with rhizopuspepsin in analyses of a challenging highly-flexible cellulolytic enzyme cellobiose dehydrogenase (CDH). This biotechnologically interesting protein was studied in order to obtain structural explanation for the regulation of its activity by pH and divalent cations. For this, HXMS was complemented by native MS with ion mobility and protein surface electrostatics calculations.

Together, the structural MS and computational techniques brought some interesting observations concerning the analyses of transient protein complexes. Moreover, they provided a direct experimental proof that repulsion of negative charge patches close to interdomain interface in CDH is the key mechanism governing its functioning in solution.

Keywords: Structural mass spectrometry, nepenthesin-1, hydrogen / deuterium exchange mass spectrometry (HXMS), native mass spectrometry with ion mobility (IMMS), aspartic protease, protein immobilization, cellobiose dehydrogenase (CDH), protein surface electrostatics, flavocytochrome, direct electron transfer.