CE combined with MS or SPR for the assessment of protein heterogeneity, conformation and affinity

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Over the last decades, capillary electrophoresis (CE) has demonstrated to be an excellent technique for the analysis of intact proteins and their proteforms. CE has the intrinsic capacity to produce narrow peaks for macromolecules and the selectivity to separate closely-related protein variants and isoforms. Moreover, it can provide proteoform resolution under near-physiological conditions, while maintaining protein affinity and conformational integrity. Changes in protein three-dimensional structure – e.g. as a result of modification or unfolding – can be reflected in the protein electrophoretic mobility. Thanks to recent technological developments in MS-interfacing, combination of CE with native MS has been made possible, offering further information on aggregation, complexation and folding states. Moreover, when combined with affinity-specific detectors such as surface plasmon resonance (SPR) binding to target biomolecules can be selectively monitored.

In this lecture methods will be presented for the coupling of (native) CE with MS and SPR for the assessment of the heterogeneity, conformation and affinity of proteins in complex samples. The performance of the CE-MS and CE-SPR systems will be demonstrated by instructive examples. Assessment of protein heterogeneity of various proteins of clinical and pharmaceutical relevance using different CE-MS approaches will be shown. CE-MS also showed good potential for revealing unfolding intermediates and conformers of amyloidogenic and pharmaceutical proteins. A microfluidic CE-SPR flow cell was developed and has demonstrated to allow selective binding assessment of mixture components, as illustrated by affinity profiling of heterogeneous enzymes and antibodies to immobilized inhibitors and antigens.