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Electrokinetic protein extraction from polyacrylamide gels with in-line microfluidic digestion and integrated mass-spectral analysis

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) remains a commonly used method in proteomics and provides for the detection of changes in protein isoforms as well as comparison and relative quantification of thousands of intact proteins. In conjunction with differential Zdye labeling, spots on the nanogram scale can be detected, but the more dilute samples are subject to losses and contamination over standard multistep preparation procedures, which may lead to failed identifications by mass spectroscopy. We are developing a system that combines the preparatory steps necessary for mass spectral analysis into a fully automated, microfluidic in-line system. The system identifies dilute proteins by the targeted electroextraction of SDS-complexed proteins, followed by in-line tryptic digestion and direct analysis on an Agilent chip-LC.