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Two-Dimensional Capillary Electrophoresis for Ultrasensitive Protein Analysis

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Two-dimensional capillary electrophoresis with laser-induced fluorescence detection is used for separation of complex biological samples. Previous 2D CE separations from our lab employed a sieving dimension and a micellar electrokinetic chromatography dimension. These separation modes were not orthogonal for the separation of proteins. We have been developing a 2D CE system that would greatly improve the resolution by coupling capillary isoelectric focusing (cIEF) and capillary sieving electrophoresis (CSE). cIEF/CSE has many advantages over traditional 2D-gel electrophoresis, including higher sensitivity when coupled with laser-induced fluorescence detection, much faster analysis time, and many fewer manual manipulations. In our system, amines are labeled with Chromeo-P540, a commercially available pyrilium compound that reacts with primary amines to produce a positively charged pyridinium ion. Fluorescently labeled biomolecules are separated by 2D-CE and detected by laser-induced fluorescence excited with a 532-nm solid state laser. 2D-CE-LIF is capable of detecting zeptomole quantities of P540-labeled proteins. Work is progressing to the analysis of the protein content of a single cell using this technology.