Identification of Nuclear Protein Complexes Using Open Tube Capillary Chromatography

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Affinity chromatography is becoming an increasingly important component in proteomics and is more widely employed as a technology to explore PTM, protein-protein interactions and part of a chemical strategy for functional studies within the proteome. An emerging area for proteomics technology development is miniaturerization and the use of affinity chromatography as part of multidimensional chromatographic procedures.

Open-tube capture of proteins involves the covalent attachment of affinity ligands to the inner walls of a capillary tube (typically 1 meter in length, with capacities on the order of 10-100 µg total protein), and do not involve any packed resin materials. The proteins targeted for capture are trapped on the tube's inner surface, and are eluted into a segment of eluent where the volume of eluent can be less than the internal volume of the capillary, and is substantially smaller than the volume of starting sample. Unlike standard separation resin beds that are fundamentally characterized by non-uniform flowpaths and pore structures of variable accessibility, open-tube capillary separations are characterized by highly uniform separation surfaces. This results in a high degree of control and reproducibility of separation conditions over the entire separation surface, as well as possessing no unwashed volumes. This results in high levels of target protein enrichment, but also unparalleled separation selectivity and specificity.

Immobilized-metal affinity chromatography (IMAC) in conjunction with open-tube capillary columns were used to study the binding and interacting partners from mammalian nuclear cell extracts. The results demonstrate the ability of the capillaries to efficiently capture and purify a subset of nuclear proteins that bind to immobilized metal ions. Following identification of the proteins using LC-ESI-MS/MS, components of a nuclear protein complex were identified. The identification of such complexes will enable further elucidation of protein network pathways involving nuclear protein complexes.