

A novel strategy to enhance membrane protein identification by reversed-phase protein pre-fractionation and HPLC-Chip/MS

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It is predicted that more than one third of the open reading frames in the human genome are encoded with membrane proteins. Membrane proteins are involved in many crucial biological processes and scores of examples have shown a direct link to human diseases, thus there is an increasing interest to study membrane function and apply the knowledge to drug discovery. However, analysis of membrane proteins represents a major challenge in proteomics due to their amphipathic nature, and despite their biological importance they remain an underrepresented subset of studied proteins within proteomics.

We have developed a strategy for enhancing membrane protein identifications by two dimensional pre-fractionation and microfluidic nano-chip-LC/MS analysis. Specifically, we have used a novel high-recovery macroporous reversed-phase (RP) C18 column to pre-fractionate intact HeLa membrane proteins under high temperature conditions. Collected RP fractions were further resolved by SDS-PAGE, in-gel digested and the resulting tryptic peptides analyzed by HPLC-Chip/MS. Alternatively, RP fractions were in-solution digested and tryptic digests analyzed via 2D HPLC-Chip/MS.

To date, we have identified more than 900 proteins with more than half of the identifications predicted or verified as integral membrane proteins. This robust and highly reproducible workflow has demonstrated utility for increasing membrane protein identifications from a highly complex membrane protein sample. Optimized conditions for the RP separation permitted total protein recoveries greater than 95%, enhanced peak resolution and showed excellent reproducibility.