

Sección temática: Proteómica y Metabolómica

Multi-dimensional Protein Identification Technology

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Before the rise of the Multidimensional Protein Identification Technology (MudPIT), protein and peptide mixtures were resolved using traditional proteomic technologies like the gel-based 2D chromatography that separates proteins by isoelectric point and molecular weight. This technique was tedious and limited, since the characterization of single proteins required isolation of protein gel spots, their subsequent proteolysis and analysis using Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. The very importance of MudPIT lays in the automatization of result harvest from initial samples such as tissues and cellular content (Schirmer, 2003). MudPIT substituted gel-based chromatography with High-Performance liquid chromatography (HPLC), a liquid multidimensional chromatography that uses biphasic or triphasic columns hence contributing to a better accuracy on peptide separation. In addition, MudPIT substituted MALDI-TOF with tandem mass spectrometry permitting peptide fragmentation using collision-induced dissociation cells as well as mass determination of the consequent products using a computational algorithm like SEQUEST (Paoletti, 2004). This new technique's breakthrough lays on the higher resolution and extra sensitivity it provides to the protein/peptide separation processes. Therefore, one peptide can be enough to identify a protein, and a small portion of sample can be sufficient to identify an entire proteome while avoiding the typical band broadening problems associated of many chromatographic steps. MudPIT's extra sensitivity renders it a powerful tool for parasite-host protein discrimination, erratic post-translational protein modifications, mutated protein identification and subcellular fraction protein identification among other uses. This work's objective is to present the technique and its advantages, emphasize its role in modern scientific research and discuss its possible limitations.

References:

- Paoletti et al. (2004). *Principles and applications of multidimensional protein identification technology*. Expert review of proteomics.
- Schirmer EC et al. (2003). *Mudpit: a powerful proteomics tool of discovery*. Discovery medicine.