Multidimensional Protein Identification Technology

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Introduction

Before the rise of Multidimentional Protein Identification Technology (MudPIT), protein and peptide mixtures were resolved using traditional proteomic technologies such as gel-based 2D chromatography which separates proteins by isoelectric point and molecular weight. MudPIT combines column multidimensional chromatography with tandem mass spectrometry (MS/MS) hence providing higher resolution and extra sensitivity to the protein/peptide separation processes. MudPIT has become an important tool for proteome studies as in parasite-host protein discrimination, erratic post-translational protein modifications, mutated protein identification and subcellular fraction protein identification among other uses (Schirmer et al, 2009).
This work's objective is to present the technique, its advantages and emphasize its role in modern scientific research as well as to discuss its possible limitations.

Technique Overview

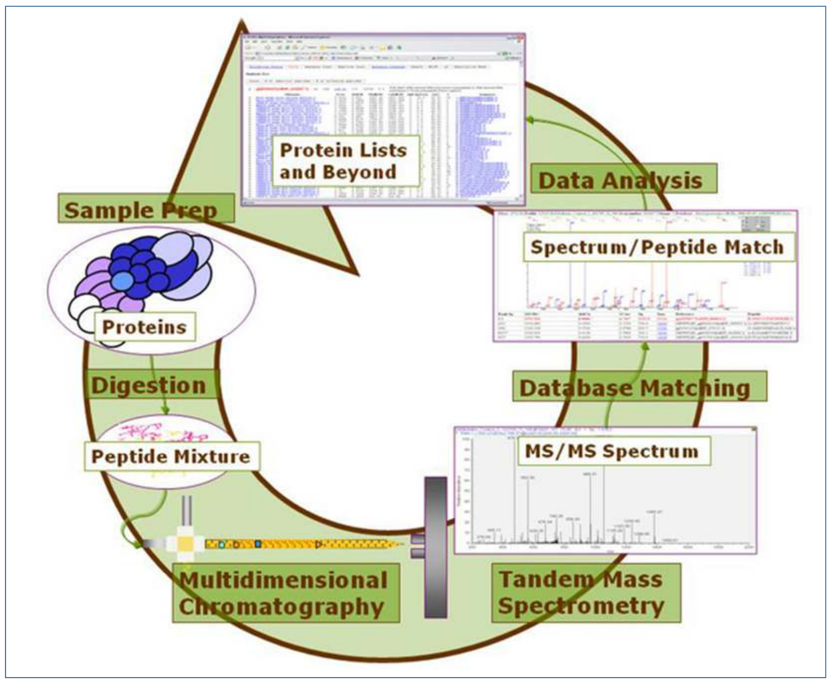

Illustration from: http://research.stowers.org/proteomics/mudpit.html

Samples. Range from tissues, cell lysates, cell organelle, purified proteins etc.
Digestion. Most proteins are amenable to Trypsin digestion which is the most used. The result are positive charged peptides in their N-terminus.
Mutidimensional Chromatography (HPLC). Can be done in biphasic or triphasic fashion. For a biphasic column, Strong Cation Exchange (SCX) and Reversed Phase (RP) are used, for the latter another RP is added previous to SCX.
Tandem Mass Spectrometry (MS/MS). HPLC elutions are coupled to a MS/MS spectrometer.
Database Matching. The results obtained from MS/MS are compared to a theoretical spectra digestion of the selected peptide from a database via software (i.e. SEQUEST®).
Data Analysis. The obtained matches are statistically analysed to avoid error. Data analysis also allows performing functional analysis using PLGEM-STM permitting differential protein expression discrimination.

Limitations

- Since a whole cell proteome might contain a few hundred different proteins, MudPIT's ''bottom-up'' approach, can yield few thousands different peptides, an amount that is hard to analyse.
- Chromatography's simple setup renders the technique rather inflexible.
- Very slow.

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FOCUS: EMERGING INVESTIGATORS: RESEARCH ARTICLE

Fast Photochemical Oxidation of Proteins Coupled to Multidimensional Protein Identification Technology (MudPIT): Expanding Footprinting Strategies to Complex Systems

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MudPIT Breakthroughs

This new technique's breakthrough lays on its higher resolution and extra sensitivity on detection and analysis.

- One peptide can be enough to identify a protein, and a small portion of sample can be sufficient to identify an entire proteome.
- Avoidance of the typical band broadening problems associated of many chromatographic steps.
- Automatization of result harvest.
- Yield of qualitative and quantitative results.

MudPIT's role in modern scientific research include 270 abstracts on PubMed among which 29 are focused on cancer.

Applications

MudPIT's extreme sensitivity opens door for a wide range of applications:

- Generation of a more global protein distribution map in subcellular compartments than with mRNAs expression arrays or immunofluorescence.
- Detection of erratic post-translational protein modification in diseased tissues.
- Study the disappearance/appearance/modification of a protein in a new strain faster than genome sequencing.
- Detection of low abundance transcriptional regulators, signaling factors, and proteins involved in cellular and physiological processes in healthy and diseased tissues during different stages of organism's development.
- Identification of proteins that are harder to isolate (i.e. insoluble proteins)

Conclusions

MudPIT's limitless applications and robust method have earned it its place as an important tool in modern proteomics.
Continuous technological and bioinformatical advances will permit to refine the technique in separation steps and data analysis.

References

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