Gel-free proteomics by Combined FRActional DIagonal Chromatography - COFRADIC

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Peptide-centric proteomics tries to compensate inherent drawbacks of 2D-PAGE-based proteomics by characterizing the generally more soluble peptides obtained after digesting an isolated proteome. Clearly, the major obstacle of peptide-centric proteomics is the huge number of peptides requiring MS/MS analysis. Since the capacity of contemporary mass spectrometers is inadequate, too many peptides are not identified in a direct analysis of a complete proteome digest. In general, two different approaches are applied to compensate for this: orthogonal (IEC and RP, John Yates' MudPIT approach) chromatographic separations split the peptide mixture in as many analyzable components as possible prior to MS/MS or specific peptides are (affinity)-isolated and serve as signatures for their corresponding proteins. As the latter reduces the number of analytes, it is generally conceived that a higher part of a proteome will eventually be identified. Based upon the diagonal electrophoresis techniques described in the 1960's, our lab developed a central technology termed combined fractional diagonal chromatography (COFRADIC) that isolates predefined sets of peptides for further characterization by automated LC-MS/MS. Major differences with similar techniques is that COFRADIC does not need affinity anchors and by simply changing the sorting chemistry different sets of peptides are isolated. Procedures for isolating methionyl, cysteinyl and amino terminal peptides (1) are available and combined with differential stable isotope tagging allow in-depth analysis of proteomes and their fluxes. Other, more recently developed procedures focus on posttranslational modifications including phosphorylation and N-glycosylation (reviewed in (2)). One of the most ppopularäpplications is the analysis of protein processing in a proteomic background using the N-terminal COFRADIC technique. Examples of such analyses that will be discussed include a detailed degradomic view on apoptotic Jurkat T-lymphocytes (3) and an analysis of the substrates of the Omi protease (4). This will illustrate the unique characteristic of N-terminal COFRADIC as it is the sole protein technology that identifies protease substrates and delineates the exact processing sites simultaneously in a large, unchanged proteomic background. Another example deals with the in vitro delineation of granzyme A and granzyme B

substrates in freeze-thaw cell lysates. And, we will furthermore illustrate the versatility of COFRADIC by discussing and exemplifying ways to isolate peptides containing sialylated N-glycosylated asparagines, phosphorylated peptides and peptides encompassing ATP-binding sites in proteins.

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