PEPTIDE FRACTIONATION FOR PROTEOMIC STUDIES.

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Proteomics has evolved towards shotgun strategies based on multidimensional chromatography and mass spectrometry analysis (LC-MS/MS) of peptide mixtures derived from cell extracts. However, very complex peptide mixtures are obtained, limiting the detection of many of those peptides which therefore, impedes the identification of several proteins. Fractionation at protein or peptide level has been found to enhance protein identification. Following this approach, we have developed three complementary methods (SCAPE) for selective isolation of peptides based on the derivatization of abundant functional groups (a and e amino groups) to modulate the presence of positive charges (at acidic pH) and further separation by cation exchange chromatography or affinity chromatography. The first method allows the selective isolation of either Lys-C or tryptic peptides that do not contain Arg or His (known as **nHnR**). The second method achieves the selective isolation of multiply charged peptides (containing Arg + His > 1, known as **RH**). The third method is based on the tryptic digestion of biotinylated Lys-C peptides followed by affinity chromatography to isolate selectively peptides called as **RRnK** because they are flanked by Arg residues (RR) and do not contain lysine residues (nK). These procedures have shown to be complementary, allowing high protein coverage. All of them have been developed for quantitative proteomics in combination with differential isotopic labeling. Particularly, **SCAPE-RH** was implemented to analyze four proteomes simultaneously. Peptides labeled with four acetic anhydrides (normal, ^{13}C1, ^{13}C2, ^{2}H3) are unambiguously quantified by Isotopica software. In addition we have developed a procedure for peptide fractionation by SDS-free polyacrylamide gel electrophoresis. Complex protein extracts separated by SDS-PAGE are trypsin digested and peptides further fractionated by PAGE (SDS free) to improve substantially peptide detection and hence, protein identification. The use of SDS for protein fractionation allows analysis of highly hydrophobic proteins and minimal protein losing. These procedures have been applied to different proteomics studies. A membrane protein extract from Neisseria meningitides allowed the identification of underrepresented proteins. A novel proapoptotic synthetic peptide inhibiting the phosphorylation of the Casein Kinase 2 has been studied for better understanding of its molecular mechanism of action. Results of these two applications are presented.