

Protein prefractionation by magnetic bead based technologies

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The trend towards the analysis of highly complicated analyte mixtures using minute sample preparations presents an enormous challenge for current analytical instrumentation. One of the most challenging attempts is the direct analysis of human serum or plasma, presenting a dynamic range in protein abundance spanning at least 10-11 orders of magnitude. Since there is so far no instrumentation available, which is able to handle such a combination of complexity and dynamic range, preseparation of the samples is mandatory for subsequent analysis. Currently, in a proteomics experiment this is typically done by one- or two-dimensional gel electrophoresis or one- or two-dimensional liquid chromatography. While both types of technology have driven proteomics for a number of years, both have also drawbacks. While a gel is an excellent fraction collector, there are significant difficulties with very small or large proteins, very hydrophobic and very basic ones. LC separation on the other hand provides excellent separation on the peptide level but often rather poor separation on the protein level which makes it especially suitable for bottom-up analyses.

However, despite their benefits, both technologies are unselective in a way that the proteins of interest can hardly be enriched for a targeted type of analysis. This can be overcome by combination of gel- or LC-based separations with the use of magnetic particle based prefractionation. Magnetic beads have several advantages in their use as a separation medium:

- They are available in a high number of surface modifications
- They are usually solid particles which makes them much less prone to unspecific adsorption than agarose- or sepharose-based beads
- They are scalable

The eluates can be analyzed directly by ESI or MALDI mass spectrometry or further separated (typically) by LC. The combination of the two technologies allows for a targeted analysis of specific subproteomes. The use of beads as a prefractionation tool with medium to high specificity alone or in combination with further separation prior to mass spectrometric analysis will be discussed. Examples will be presented from the areas of

phosphopeptide as well glycoprotein analysis. For the latter we will present data from the analysis of the human serum glycoproteome.