NEW MICROFLUIDIC CHIP TARGETING PHOSPHOPROTEOMES

Dayin Lin, Martin Vollmer and Tom van de Goor

Agilent Technologies, Inc.

Protein phosphorylation is one of the most important post-translational modification (PTM) events among mechanisms of regulating protein function in cells. Myriad biological processes, including cell proliferation, migration, and apoptosis involve phosphorylation steps. One of the major efforts in proteomics is devoted to the identification and understanding of phosphoproteomes in cells. Nevertheless, comprehensive identification of sites of protein phosphorylation remains a challenge, best left to experienced proteomics experts. In order to achieve selective enrichment of phosphorylated proteins and peptides most commonly used technologies are currently immobilized metal affinity chromatography (IMAC), anti-phosphotyrosine antibodies, and titanium dioxide prior to LC/MS (liquid chromatography and mass spectrometry) analysis. Recent advances in HPLC chip technology have created an environment to allow automation of such a workflow with increased ease of use and confidence of analysis. The new microfluidic chip is a re-usable HPLC nano-flow rate chip with titanium dioxide particles (TiO_2) based phosphopeptide enrichment. The chip is a multilayer polyimide laminate that contains an enrichment section with TiO_2 beads flanked on both sides with C18 reversed phase material. The 3 section sandwich is separated from each other by micro-fabricated frits. This enrichment section is connected to a reversed phase separation column ending in an integrated electro-spray tip by a micro valve in direct contact with the chip surface providing a zero dead volume high pressure seal. The chip is used with a HPLC-chip/MS instrumentation using the HPLC-chip cube interface combined with a Mass Spectrometer. The unique sandwich configuration of the enrichment section provides researchers three modes of peptide analysis: (1) standard peptide analysis, (2) phosphopeptide analysis only, and (3) combined peptide and phosphopeptide analysis. This approach will offer non-expert proteomics researchers a reliable way in phophoproteome analysis.