A new type of immobilized metal ion affinity chromatography (IMAC) adsorbent for highly specific enrichment of phosphopeptides for phosphoproteomics analysis

Phosphorylation is a key reversible modification of proteins that acts as a switch to regulate almost all biological processes. Phosphoproteomics analysis aims to identify and characterize all the protein phosphorylations presented in cells or tissues. Conventional adsorbents for phosphopeptide enrichment lack enough specificity, which seriously compromises the performance of phosphoproteome analysis. The research lab headed by Prof. Zou Hanfa in CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS) developed a new type of immobilized metal ion affinity chromatography (IMAC) adsorbent for highly specific enrichment of phosphopeptides in phosphoproteomics analysis. The schematics for this new IMAC are shown in the Figure below. The phosphate groups are covalently coupled onto the surface of polymer beads acting as the chelating ligands to coordinate the Zr(IV) or Ti(IV) ions, and then coordinated Zr(IV) or Ti(IV) ions exhibited the highly specific interaction with the phosphate groups on phosphopeptides, which could thus be specifically enriched by this new type of IMAC adsorbent.

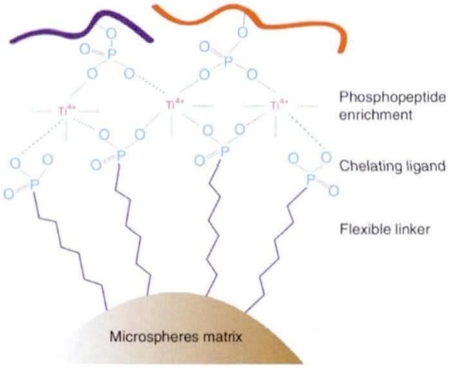

Figure The schematics of the new IMAC (reprinted from *Nat Protoc* **2013**, 8: 461—480)

The first result concerning this new type of IMAC adsorbent was reported by Zou's Lab in 2006 (*J Proteome Res* **2006**, 5: 2431—2437) by validating the proof of the concept on chemical modification of silicon chip with MALDI TOF-MS detection, then it was extended to immobilize Zr (IV) to the phosphonate-modified poly(glycidyl methacrylate- co-ethylene dimethacrylate) polymer beads for enrichment of phosphopeptides from complex proteome samples (*Mol Cell Proteomics* **2007**, 6: 1656—1665). It was demonstrated that the performance of this new type of IMAC adsorbent was much better than the conventional adsorbents. In 2008, the IMAC adsorbent with coordination of Ti(IV) ion was developed (*J Proteome Res* **2008**, 7: 3957—3967). The performance of this new type of IMAC adsorbent can be further improved by adopting new matrix materials. For example, a poly(ethylene glycol)-brush decorated magnetic polymer was prepared for immobilization of Ti(IV), an exceptionally great specificity to capture phosphopeptides from a tryptic digest of the mixture of a nonphosphorylated protein BSA and a phosphorylated protein alpha-casein with molar ratios of BSA/alpha-casein up to 2000:1 was achieved (*Chem Sci* **2012**, 3: 2828—2838). By using this new type of IMAC adsorbent, Zou's lab is able to identify more than 10,000 phosphorylation sites from a single proteome sample.

This new type of adsorbent has drawn worldwide attention. The first three published papers were cited more than 300 times. Because of the great contribution to phosphoproteome analysis, Prof. Zou was invited to contribute a perspective review paper in the *Analytical Chemistry* (*Anal Chem*, **2011**, 83, 8078—8085). Recently, Zou with his collaborators published a protocol on this new type of adsorbent in *Nat Protoc* (**2013**, 8: 461—480).

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