Improved immunoaffinity enrichment methods for tyrosine phosphorylated, lysine acetylated, and lysine succinylated peptides with high sensitivity and specificity.

Mass Spectrometry

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Posttranslational modifications (PTMs) regulate and are associated with diverse biological processes, for instance tyrosine phosphorylation (pY) with cell signaling, lysine acetylation (AcK) with epigenetic regulation, and lysine succinylation (SuccK) with metabolic flux. Immunoaffinity purification (IAP) is an established technique for the study of PTMs, especially when coupled with liquid chromatography-mass spectrometry (LC-MS). Recently we introduced a magnetic bead-based IAP method that enables identification and quantification of sites of lysine ubiquitination with improved sensitivity, specificity, and ease of handling over preexisting agarose bead-based methods. Here we extend the repertoire of PTM types by presenting three novel magnetic bead-based IAP methods that enable the identification and quantification of pY, AcK, and SuccK sites, all with similar improvements over their respective agarose bead-based methods. We believe these new methods will facilitate investigations into the function and regulation of pY, AcK and SuccK modifications, especially in disease contexts with limited and challenging samples.