Development of control peptides for immunoaffinity-based enrichment of posttranslationally modified peptides

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Posttranslational modifications (PTMs) are important elements of many biological processes. A key aspect to experiments that aim to identify and quantify PTM sites is the usage of appropriate techniques, such as immunoaffinity purification (IAP) or immobilized metal affinity chromatography (IMAC), to enrich for PTMs prior to liquid chromatography-mass spectrometry (LCMS) analysis. To facilitate IAP and IMAC workflows, we sought to develop a series of synthetic peptides that contain both a specific PTM and a stable heavy isotope. Such synthetic peptides can be spiked into biological samples prior to PTM enrichment and assayed using LCMS as an internal control of PTM enrichment performance and reproducibility. We based the control peptide sequences on naturally occurring PTM sites, and selected those peptides that displayed the best long-term storage stabilities. We successfully synthesized, purified, and validated multiple control peptides for the enrichment of ubiquitin remnant (K-GG), acetyl-lysine (Ac-K), phospho-tyrosine (pY), phospho-serine/threonine (pS/pT), succinyl-lysine (Succ-K), methyl-lysine (Kme1, Kme2, Kme3), methyl-arginine (Rme1, Rme2s, Rme2a), and beta-linked N-acetyl-glucosamine (Oglcnac) peptides. In conclusion, we believe incorporating these synthetic controls into IAP or IMAC based workflows should facilitate the LCMS analysis of endogenous PTM sites.