An experimental strategy for the identification of AMPylation targets from complex protein samples

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AMPylation is a posttranslational modification (PTM) that has recently caught much attention in the context of bacterial infections as pathogens were shown to secrete Fic proteins that AMPylate Rho GTPases and thus interfere with host cell signaling processes. Although Fic proteins are widespread and found in all kingdoms of life, only a small number of AMPylation targets are known to date. A major obstacle to target identification is the limited availability of generic strategies allowing sensitive and robust identification of AMPylation events. Here, we present an unbiased MS-based approach utilizing stable isotope-labeled ATP. The ATP isotopes are transferred onto target proteins in crude cell lysates by in vitro AMPylation introducing specific reporter ion clusters that allow detection of AMPylated peptides in complex biological samples by MS analysis. Applying this strategy on the secreted Fic protein Bep2 of Bartonella rochalimae, we identified the filamenting protein vimentin as an AMPylation target that was confirmed by independent assays. Vimentin represents a new class of target proteins and its identification emphasizes our method as a valuable tool to systematically uncover AMPylation targets. Furthermore, the approach can be generically adapted to study targets of other PTMs that allow incorporation of isotopically labeled substrates.
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