Exploring crosstalk between post-translational modifications in half the time

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Video Abstract

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Abstract

A new method for identifying post-translational modifications in proteins promises to cut biomedical researchers' workload in half. Enabling multiple affinity enrichment procedures to be run in parallel, the one-pot method yields the same search results as traditional methods in less time and from less tissue. As proteomics researchers know well, identifying post-translational modifications in biological samples can be tedious. Enriching samples with target modifications, such as the attachment of acetyl, succinyl or methyl groups to amino acid residues, and matching experimental data with catalogued results involves numerous steps. And the work load is only getting bigger. With exploding interest in how multiple modifications are linked across the vast proteome, the amount of time and the amount of sample required for exploration are skyrocketing in proportion. But with the new one-pot enrichment method, that could soon change. Traditionally, probing for two possibly related modifications works like this. A sample is digested and enriched for modification A using antibodies; then, a separate portion of the digested protein sample is enriched for modification B using a different set of antibodies. Alternatively, in a serial enrichment, the flow-through obtained from enriching modification A is collected and enriched for modification B. In the one-pot scheme, the two modifications are enriched for in the same tube at the same time. Tests showed that for modifications involving the addition of an acetyl or succinyl group, the results generated by the traditional method and the new one-pot approach were quantitatively equivalent. The difference, of course, was that the one-pot method cut the sample preparation time and the required sample amount by half. What's more is that the method enabled the detection of both modifications on the same peptide, pointing to an actual protein complex where crosstalk between the two could play a regulatory function. Theoretically, the new approach can be extended to even more and different modifications. The exception is when the biochemical environments required to enrich for different modifications are incompatible and can't actually be housed in the same pot. Still, the proposed method offers researchers a way to cut time, cost and sample volume in studying post-translational modifications. And with the recent surge in exploring crosstalk between multiple modifications, the timing of this development could be just right.