

Abstract:

Post-translational modifications (PTMs) mediated by ubiquitin and other ubiquitin-like (UbL) proteins are essential for proper development, and their dysregulation can contribute to diseases, including rare disorders. The specificity of ubiquitination is governed by E3 ligases, yet one of the field's major challenges lies in distinguishing their bona fide substrates from non-specific interacting proteins. Studying these modifications presents significant hurdles due to the low cellular abundance of modified proteins, the transient nature of many PTMs, and the fact that these modifications often trigger rapid protein degradation. To overcome these obstacles, we developed biotin-based enrichment strategies that enable the identification of UbL-modified protein interactomes and the precise mapping of E3 ligase substrates. We leverage these tools to investigate the role of UbL pathways in rare diseases and to probe how pharmacological interventions may rewire E3 ligase specificity—an approach with direct relevance for advancing targeted protein degradation strategies.