# Identification of proteomic landscape of drug-binding proteins in living cells through proximity-dependent target identification

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The direct identification of proteins targeted by small molecules holds significant potential for disease diagnosis, prevention, and drug development. However, technical limitations persist in elucidating the direct interactors of small molecules. In this study, we introduce a novel target identification system called PROximity-based Compound binding protein IDentification (PROCID). By combining our direct analysis workflow of proximity-labeled proteins (Spot-ID) with the highly efficient HaloTag system, PROCID offers an innovative approach to efficiently identify the dynamic proteomic landscape of drug-binding proteins.

PROCID integrates the Spot-ID workflow, a direct analysis approach for proximity-labeled proteins, with the HaloTag system. This synergistic combination enables the efficient labeling of drug-binding proteins in live cells. Proximity labeling is achieved by covalently attaching drug derivatives to the HaloTag protein, facilitating the labeling of proteins in close proximity to the drug. The resulting protein complexes are then subjected to mass spectrometry analysis.

Utilizing PROCID, we successfully identified well-known dasatinib-binding proteins, including ABL1 and ABL2, in a live chronic myeloid leukemia cell line. Remarkably, PROCID also revealed the presence of unapproved dasatinib-binding kinases, such as BTK and CSK. Intriguingly, PROCID further identified the DNA helicase protein SMARCA2 as a novel dasatinib-binding protein. Through proximity ligation assay (PLA) and in cellulo biotinylation assay, we confirmed that the ATPase domain serves as the binding site of dasatinib on SMARCA2. This comprehensive characterization showcases the power of PROCID in identifying unknown drug-interacting proteins in live cells and expediting our understanding of drug modes of action.

PROCID emerges as a robust method for the systematic identification of drug-interacting proteins in live cells. By combining the Spot-ID workflow with the HaloTag system, PROCID overcomes existing technical limitations and offers a comprehensive view of the dynamic proteomic landscape of drug-binding proteins. The successful identification of well-established dasatinib-binding proteins, as well as the discovery of previously unknown interactors like SMARCA2, highlights the potential of PROCID in advancing our knowledge of drug mechanisms.

In summary, PROCID represents a significant advancement in the field of target identification and provides a valuable tool for understanding drug-protein interactions. By unveiling the proteomic landscape of drug-binding proteins in live cells, PROCID contributes to the development of effective therapies and offers critical insights into disease mechanisms.