INTRODUCTION

- Limited proteolysis (LiP) is a recently developed peptide-centric target deconvolution approach that can identify protein targets in a complex lysate.
- LiP’s peptide-based identification of target proteins also enables a level of granularity often missing from other common target deconvolution techniques, including binding site approximation.
- Characterization of protein binding site(s) and induced conformational changes can enable better compound design.
- We present a novel proteomics-based technique termed high resolution LiP (HR-LiP) that provides peptide-specific structural information for compound targets.

RESULTS

Figure 1: HR-LiP Development with Calmodulin.

(A) Calmodulin protein coverage in HR-LiP.
(B) Peptides with high dose response correlation (> 0.6) upon addition of Ca\(^{2+}\) (n = 3).
(C) Calmodulin peptide localization from (B) in red.
(D) Mapping of peptides with high dose response correlation upon addition of CanNt peptide (blue) to Ca\(^{2+}\)-bound calmodulin (n = 3) in red.

Figure 2: HR-LiP for Analysis of Compound Binding Sites.

(A) Two compounds (BI-3802, left and JQ1, right) tested using HR-LiP.
(B) Peptides regulated by binding in BCL6 (BI-3802) and BRD4 (JQ1).
(C) BCL6 peptide mapping from (B) for BI-3802.
(D) Blue and yellow peptides from (C) overlap with published hydrogen-deuterium exchange (HDX) data.
(E) Dose response curve of top BRD4 peptide from (B).
(F) Binding site of JQ1 in BRD4.
(G) Peptide from (E) mapped to BRD4 (red). Amino acids (blue) from (F) highlighted.

CONCLUSIONS

- The ability to accurately map compound binding sites and conformational changes induced by compound binding remain a high priority in drug development.
- HR-LiP is an extension of LiP-Quant that aims to exploit peptide-specific information to predict compound binding sites, as well as sites of conformational regulation.
- Model system work in calmodulin demonstrated that HR-LiP can approximate Ca\(^{2+}\) binding sites.
- HR-LiP accurately predicts the binding site of well-characterized compounds (JQ1 and BI-3802), as well as additional sites of regulation (BI-3802) confirmed by HDX.