3847 | TARGET IDENTIFICATION OF MULTI-PASS TRANS-MEMBRANE G-PROTEIN COUPLED RECEPTOR USING LIP-MS

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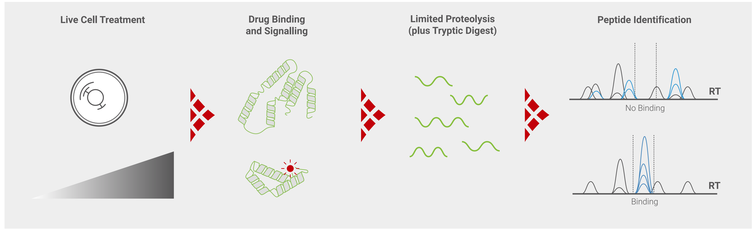
INTRODUCTION

Target identification (ID) in drug discovery is key to understanding a compound's mechanism of action. Membrane proteins are particularly challenging for a host of biological reasons (e.g. low abundance). Limited proteolysis coupled to mass spectrometry (LiP-MS) is a label-free, peptide-centric structural approach to target ID that has successfully characterized numerous cytosolic drug targets in lysate. Here we present a modified workflow in live cells designed to both enrich membrane protein identifications and also characterize downstream signalling cascades induced by drug binding. Collectively, this approach improves detection of multi-pass membrane proteins, as well as provides a system-level view of downstream perturbations that together provide a clearer picture of how a compound functions.

Reference:
Piazza & Beaton et al. *Nat Commun* **11**, 4200 (2020).

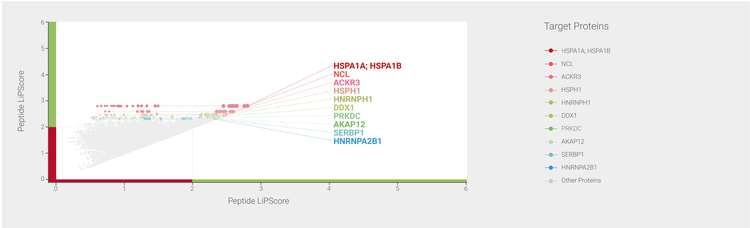


CONCLUSIONS

- **LiP-MS is a robust target deconvolution methodology that can be used in both lysate and live-treated cells**
- **LiP-MS is able to identify the GPCR (ACKR3) target of a tool compound known to bind this target**
- **LiP peptides identified for ACKR3 map to the known binding site of the compound**
- **Analysis of additional LiP peptides identified indicates structural perturbations in many intracellular pathways known to be downstream of ACKR3**
- **Preliminary analysis suggests that intracellular LiP peptides localize to known sites of structural functionality**

RESULTS

Figure 1: LiP Protein Identifications
Over 8,200 proteins were identified using live cell LiP with the tool compound IAX16840. Amongst these proteins 82 proteins had one or more LiP peptide above the threshold LiP score of 2. The third highest ranking hit, ACKR3, is the known G-protein coupled receptor of the control compound.



(A)

Gene ID	Peptide ID	R^2	LiP Score
ACKR3	_TVMCPNMPNK_	0.916	2.49
ACKR3	_SFYPEHSIK_	0.873	2.35

(B)

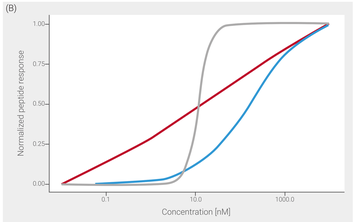


Figure 2: ACKR3 LiP Peptides
(A) 2 of 17 peptides quantified for ACKR3 have calculated LiP scores above our threshold score (LiP score > 2). **(B)** A dose response corrleation was plotted for 3 ACKR3 peptides, however only 2 peptides (red and blue) had correlations robust enough to pass score thresholds for consideration as a positive hit.

Figure 3: ACKR3 Peptide Mapping
Two peptides from ACKR3 that pass the LiP threshold score are mapped to the protein's structure. A 2D model shows that both peptides are from the extracellular portion of the protein (left, protter.com), while a 3D structure (right, structural model: AF-P25106-F1) shows that the peptides flank the compound's known binding site.

INTERAX

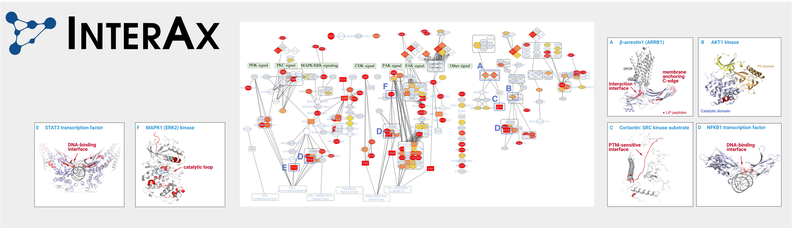


Figure 4: LiP Peptides mapped to ACKR3/ CXCR4 Signalling Pathways
82 proteins were identified with at least one LiP peptide scoring above 2. These proteins are enriched in the chemokine GPCR signaling networks (coloured shapes) known to be downstream of ACKR3. Preliminary analysis suggests that many of the LiP peptides localize to known functional sites (inset structures).