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Letters

A Chemoproteomics Approach to Profile Phospholipase D-Derived Phosphatidyl Alcohol Interactions

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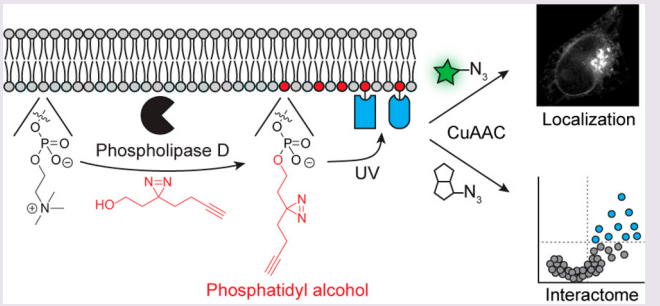
ABSTRACT: Alcohol consumption leads to formation of phosphatidylethanol (PEth) via the transphosphatidylation activity of phospholipase D (PLD) enzymes. Though this non-natural phospholipid routinely serves as a biomarker of chronic alcoholism, its pathophysiological roles remain unknown. We use a minimalist diazirine alkyne alcohol as an ethanol surrogate to generate clickable, photoaffinity lipid reporters of PEth localization and lipid−protein interactions via PLD-mediated transphosphatidylation. We use these tools to visualize phosphatidyl alcohols in a manner compatible with standard permeabilization and immunofluorescence methods. We also use click chemistry tagging, enrichment, and proteomics analysis to define the phosphatidyl alcohol interactome. Our analysis reveals an enrichment of putative interactors at various membrane locations, and we validate one such interaction with the single-pass transmembrane protein basigin/CD147. This study provides a comprehensive view of the molecular interactions of phosphatidyl alcohols with the cellular proteome and points to future work to connect such interactions to potential pathophysiological roles of PEth.



INTRODUCTION

Alcohol use is responsible for approximately 5.1% of the global burden of disease, and it is the top risk factor for deaths and disability among those 15−49 years of age.[1] It is associated with many diseases, including addiction, malnutrition, alcoholic liver disease, dementia, and several cancers.[2−4] Ethanol is itself rather inert, needing high concentrations to directly perturb membrane and protein functions. Instead, metabolites of ethanol contribute to important aspects of the pathophysiology of alcohol consumption.[5]

The phospholipid phosphatidylethanol (PEth) is one such ethanol metabolite. It is produced through the transphosphatidylation activity of ubiquitous phospholipase D (PLD) enzymes. PLDs catalyze nucleophilic substitution of the headgroup of the abundant phospholipid phosphatidylcholine (PC). It accepts either water, which generates phosphatidic acid, a lipid with important signaling functions, or short, aliphatic primary alcohols, which generates phosphatidyl alcohols. Incorporation of primary alcohols such as ethanol into phosphatidyl alcohols is a conserved function of PLDs across plants, bacteria, and animals.[6] PEth can accumulate to quite high levels (up to 0.1% of total blood phospholipids in heavy drinkers).[7] Owing to the specificity of its biosynthesis and its long half-life (∼days in human blood), PEth is widely used as a clinical biomarker of chronic alcoholism.[7] Despite the widespread prevalence of PEth, the effects of this ethanol-derived lipid metabolite on cellular function remain largely unknown.

Existing studies on the functions of phosphatidyl alcohols have relied on low-throughput, candidate-based approaches. First, some studies have investigated the effects of PEth on the in vitro, catalytic activities of enzymes known to be implicated in the pathology of alcohol use (e.g., protein kinase C, PI-specific phospholipase C).[8−11] Such studies, which found modest effects, have typically used in vitro assays at very high, nonphysiological lipid concentrations. Alternatively, application of ethanol to cells has revealed numerous pleiotropic effects, but the specificity to PEth and its physiological relevance is unclear for these approaches.[12−14] In particular, ethanol-induced phenotypes, even when they were found to be dependent upon PLD activity, were not linked to engagement of specific molecular targets by PEth. To reveal the biological mechanisms by which PEth may affect physiological function, and how such mechanisms may contribute to the pathological effects of alcohol abuse, a comprehensive view of the molecular

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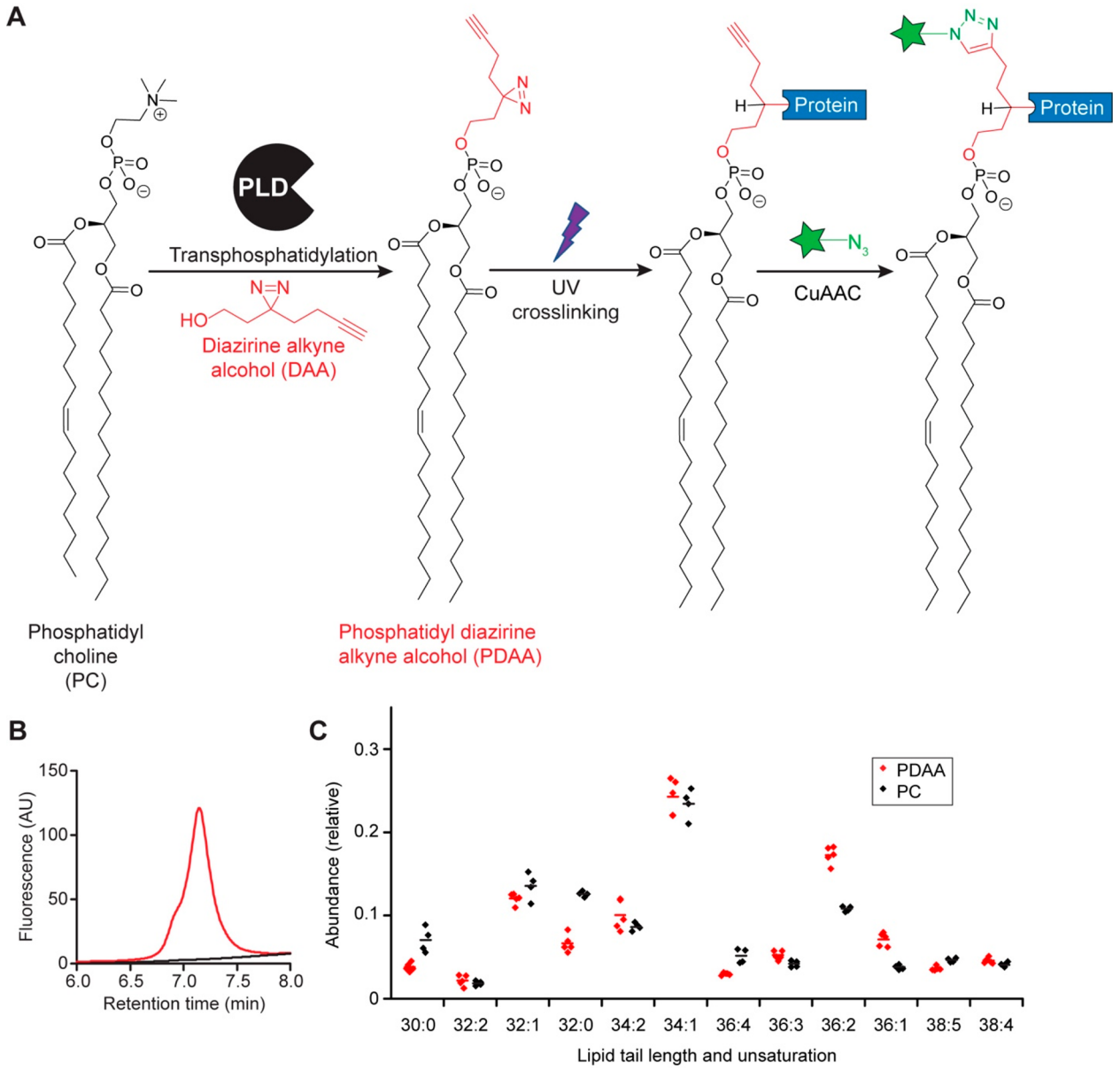

Figure 1. XL-IMPACT is a strategy to generate clickable, photo-cross-linkable phosphatidyl alcohol probes in cells via phospholipase D activity. (A) Schematic of the XL-IMPACT approach, wherein the minimalist diazirine alkyne alcohol (DAA) is a PLD transphosphatidylation substrate to generate phosphatidyl DAA (PDAA) lipids that can be covalently tethered to nearby proteins after UV irradiation to enable visualization or enrichment of lipid−protein interactions following CuAAC tagging. (B) HPLC analysis of extracts from cells labeled via XL-IMPACT. HeLa cells were treated with the PLD inhibitor FIPI (black) or vehicle (red) for 30 min followed by DAA and the phorbol ester PMA for 1 h. Lipid extracts were tagged via CuAAC with a BODIPY-azide probe and then analyzed by fluorescence-coupled HPLC. Shown is the peak corresponding to the BODIPY-tagged PDAA at a retention time of ∼7 min. AU: arbitrary units. (C) LC−MS analysis of lipid extracts from cells labeled via XL-IMPACT. Cells were treated as in (B), except that the CuAAC tagging step was omitted, and lipid extracts were analyzed directly by LC−MS. PDAA and phosphatidylcholine (PC) species were quantified, identified by acyl tail composition (lipid tail length:degree of unsaturation).

interactions of PEth with endogenous biological molecules is urgently needed.

Here, we describe a chemoproteomics approach to map the protein interactors of phosphatidyl alcohols. Previously, we had discovered that PLD enzymes can accept bioorthogonally functionalized primary alcohols in transphosphatidylation reactions, leading to a method we termed **Im**aging **P**LD **A**ctivity with **C**lickable Alcohols via **T**ransphosphatidylation (IMPACT).[15−17] Building on these findings, here we used a bifunctional primary alcohol to generate a clickable photoaffinity phosphatidyl alcohol probe in situ, via the action of endogenous PLD enzymes. Chemoenzymatic production of the lipid photoaffinity probe in this manner, i.e., in live cells, enables it to have the same subcellular localizations as other phosphatidyl alcohols such as PEth, that are generated by PLD transphosphatidylation.

The photo-cross-linking diazirine group within the lipid probe enables UV light-mediated covalent labeling of protein interactors, and the terminal alkyne within the probe enables downstream detection following a click chemistry tagging step.[18−21] This method, termed cross-linking IMPACT, or XL-IMPACT, enables both visualization of sites of phosphatidyl alcohol production and identification of phosphatidyl alcohol-interacting proteins. Herein, we demonstrate both types of

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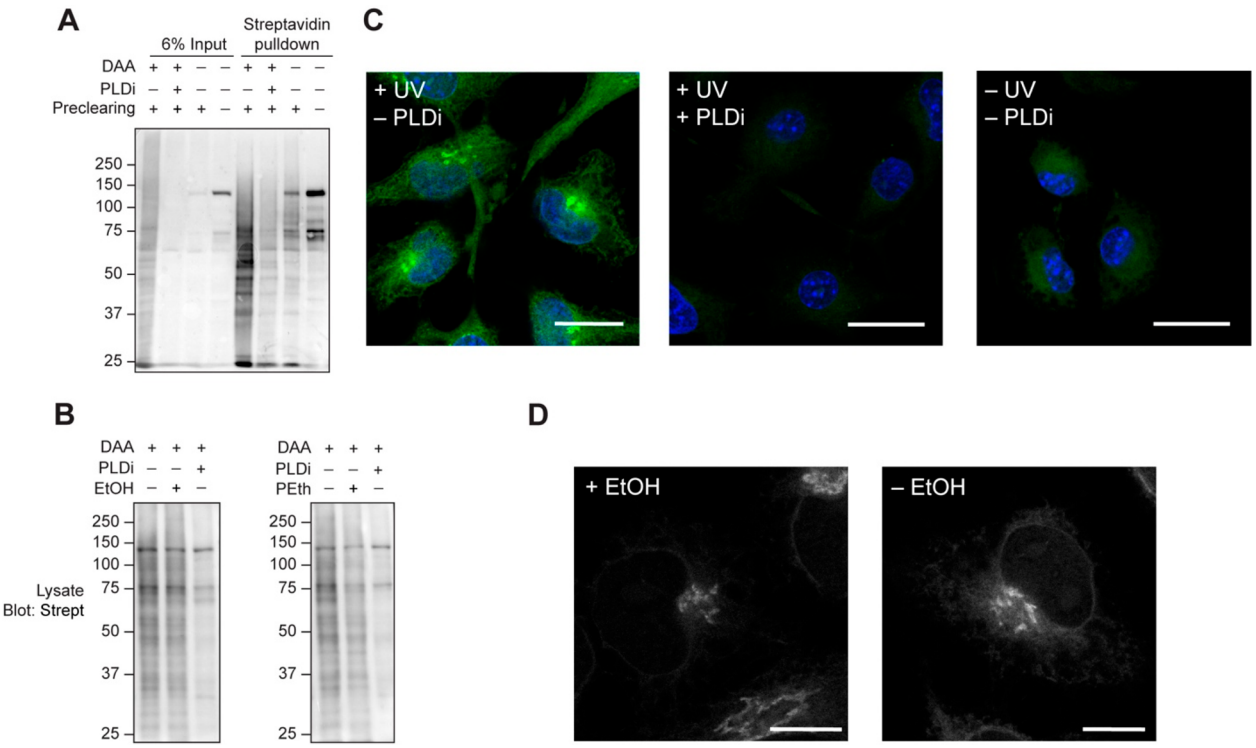

Figure 2. XL-IMPACT enabling cross-linking to putative PDAA-binding proteins. (A) HeLa cells were treated with the PLD inhibitor FIPI (PLDi) or vehicle for 30 min followed by DAA and the phorbol ester PMA for 1 h. After a rinse, photo-cross-linking was performed with UV irradiation (365 nm) for 30 min, followed by lysis. A portion of the sample was precleared with streptavidin-agarose resin to remove endogenously biotinylated proteins, as indicated. Lysates were then tagged with biotin-azide via CuAAC, and biotinylated proteins were enriched with streptavidin-agarose resin. Shown is a streptavidin blot, with total lysate at left (input) and enriched samples at right. Molecular weight markers are in kDa. (B) HeLa cells were treated as in (A), except that during incubation with DAA, a competitor, either EtOH (50 mM) or PEth (0.5 mM), was added as indicated. Shown are streptavidin blots, with ethanol competition on the left and phosphatidylethanol (PEth) competition on the right. (C) Confocal microscopy images of HeLa cells labeled with XL-IMPACT. Cells were labeled, and UV photo-cross-linking was performed as described above. Cells were then fixed with methanol, CuAAC tagging with AFDye488-azide was carried out, and confocal microscopy was performed. Green: XL-IMPACT fluorescence; Blue: DAPI. (D) Cells were treated as (C), except ethanol (50 mM) was added along with DAA as indicated. The EtOH-treated image is shown at two brightness levels, one at levels identical to the −EtOH sample, to enable comparison of labeling intensities, and one at increased brightness, to facilitate examination of localization. Scale bars: 20 μm.

applications of XL-IMPACT. We identify hundreds of putative phosphatidyl alcohol interactors and validate the interaction with the single-pass transmembrane protein basigin/CD147. Overall, this work highlights how the permissiveness of a key lipid metabolic enzyme to functionalized, non-natural substrates enables the identification of the putative protein interactome of a disease-related lipid metabolite.

RESULTS AND DISCUSSION

We reasoned that the minimalist diazirine alkyne alcohol (DAA),[22] typically used to functionalize small-molecule probes for chemoproteomics applications, could itself be a likely transphosphatidylation substrate of PLDs, because it is an aliphatic primary alcohol similar to other PLD transphosphatidylation substrates (e.g., hexynol). The expected transphosphatidylation product, phosphatidyl diazirine alkyne alcohol (PDAA), would have the same overall charge and polarity as PEth and other phosphatidyl alcohols derived from hydrophobic, aliphatic primary alcohols, thus making it an optimal chemoproteomics probe for these species (Figure 1A).

We began by treating cells with DAA under stimulation with the phorbol ester PMA, which greatly increases endogenous PLD activity. Lipid extracts of the labeled cells were tagged via Cu-catalyzed azide−alkyne cycloaddition (CuAAC) with a BODIPY-azide fluorophore. Fluorescence-coupled HPLC analysis revealed a peak with a retention time of 7 min whose presence depended upon PLD activity, as it was absent from extracts from cells treated with the PLD inhibitor FIPI (Figure 1B). LC−MS analysis of lipid extracts further confirmed the presence of the PDAA probe. As phosphatidyl alcohols encompass a class of compounds with differing lipid tail lengths and degrees of unsaturation, we were encouraged to see a lipid tail distribution of PDAA species that closely mirrors that of other PLD transphosphatidylation products,[16] as well as the PLD substrate PC (Figure 1C).

We then assessed if PDAA could be cross-linked to proteins. After generating PDAA in cells as above, we performed cross-linking by irradiating with UV light (365 nm). Cell lysates were generated, precleared of biotinylated proteins, and tagged via CuAAC with a biotin-azide probe, followed by protein precipitation to remove excess biotin-azide and affinity enrichment using streptavidin-conjugated resin. Analysis of these samples revealed labeling of many proteins (Figure 2A). Importantly, the use of FIPI during the DAA labeling step resulted in very little labeling, indicating that the labeling was indeed due to PDAA, whose formation requires PLD activity and not free DAA. To validate that our labeling method reports on interactions with the alcoholism marker PEth, we performed competition experiments by adding either ethanol, to generate PEth in situ via PLD transphosphatidylation, or

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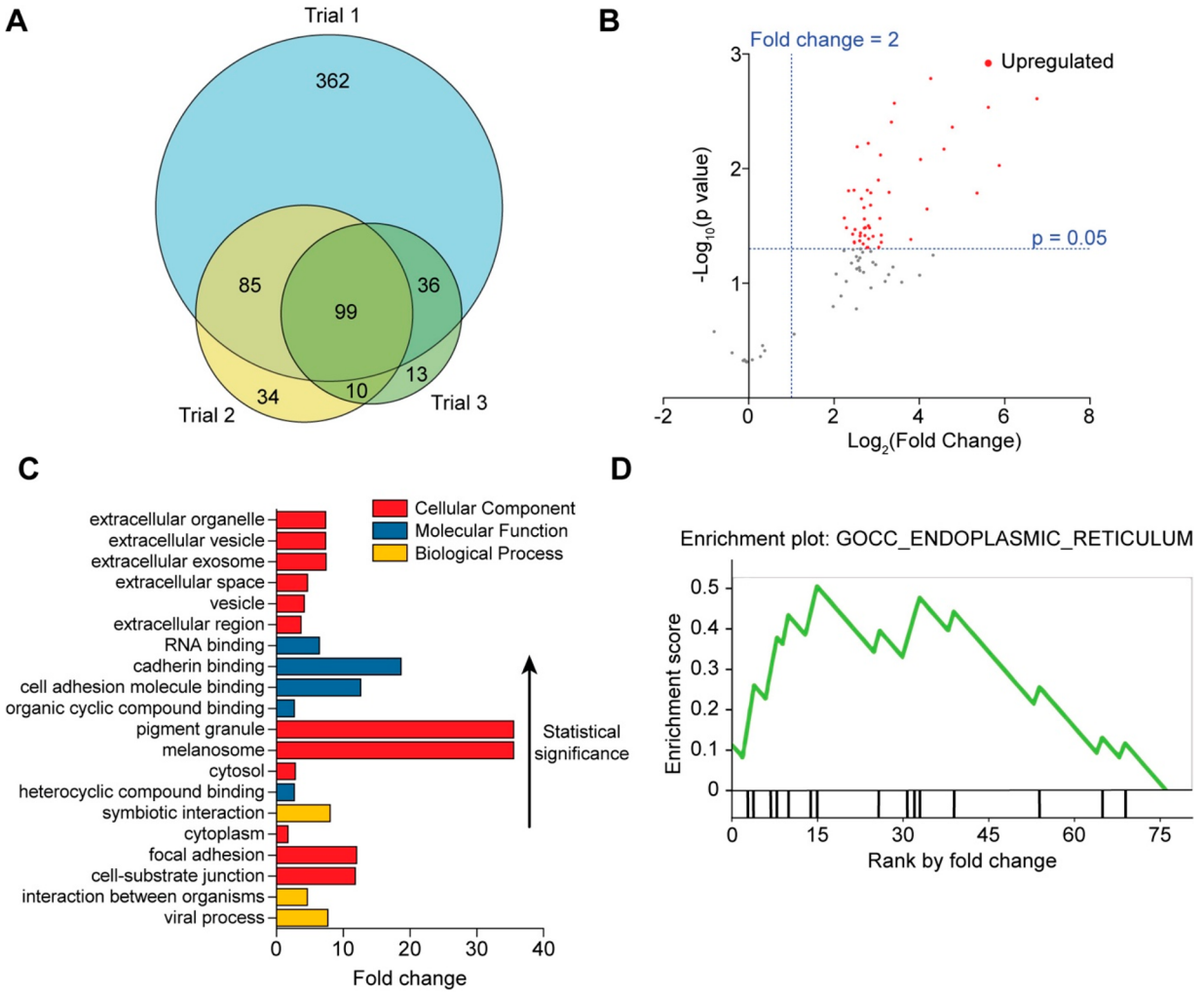

Figure 3. Identification of PDAA-binding proteins using XL-IMPACT and bottom-up proteomics. (A) Venn diagram showing all proteins identified in the proteomics analysis across three replicate trials. (B) Volcano plot of proteins that were detected in all three replicate experiments. The *x*-axis shows the $\log_2$(fold change), where fold change represents the ratio of protein abundance in the DAA vs DAA+PLDi (negative control) samples, and the *y*-axis shows the $-\log_{10}$(*p* value) ($n = 3$). (C) Gene ontology (GO) analysis of proteins detected in all three replicates, showing the top 20 groups, ordered by statistical significance (*p* value), with the highest significance at the top and with *x*-axis showing the fold change, indicating the extent of enrichment of proteins from that data set compared to the entire proteome. (D) Gene set enrichment analysis (GSEA) of ER-localized proteins detected in all three replicates, with higher fold change to the left, indicating that, among all the PDAA interactors identified, those that localize to the ER are among the most highly enriched in our data set.

PEth itself (Figure 2B). Importantly, we found that ethanol addition at the doses used does not suppress the PLD-mediated formation of PDAA (Figure S4). Critically, in both cases, the extent of PDAA labeling decreased when the competition reagent was added, suggesting that PDAA and PEth have shared preferences in their protein binding properties.

Though the key motivation for the development of XL-IMPACT was the identification of phosphatidyl alcohol interactomes, it is also a useful variant of IMPACT for visualization of the localization of these lipids in situ. The localization of signaling lipids can play important roles in determining their effects on signaling pathways.[23,24] Yet, a challenge with visualizing lipids is their dynamic behavior, both from diffusion within a bilayer and trafficking between different membranes.[25] Even standard cell fixation methods, which rely on cross-linking of primary amines or treatment with organic solvents, do not covalently immobilize most lipids, leading to artifacts or loss of signal when imaging lipid localization in fixed samples.[26] By design, XL-IMPACT involves covalent tethering of the target lipids to nearby proteins, opening up the possibility of using traditional cell fixation procedures, compatible with detergent permeabilization for immunofluorescence labeling of intracellular epitopes, to visualize the localization of PDAA lipids.

We found that labeling cells via XL-IMPACT followed by fixation and permeabilization led to a retention of XL-IMPACT fluorescence on intracellular organelles. Here, we treated cells with DAA and PMA as before, followed by rinsing, UV irradiation, fixation with methanol, and CuAAC tagging with a green fluorophore (AFDye488-azide). Critically, methanol fixation typically removes most lipids, but using XL-IMPACT, we observed fluorescence at several locations, including the ER and nuclear envelope and the Golgi complex (Figure 2C). Again, the fluorescence was dependent upon PLD activity, as it was eliminated by treatment with FIPI during the DAA labeling step and also depended upon UV cross-linking. We obtained similar results with paraformaldehyde fixation

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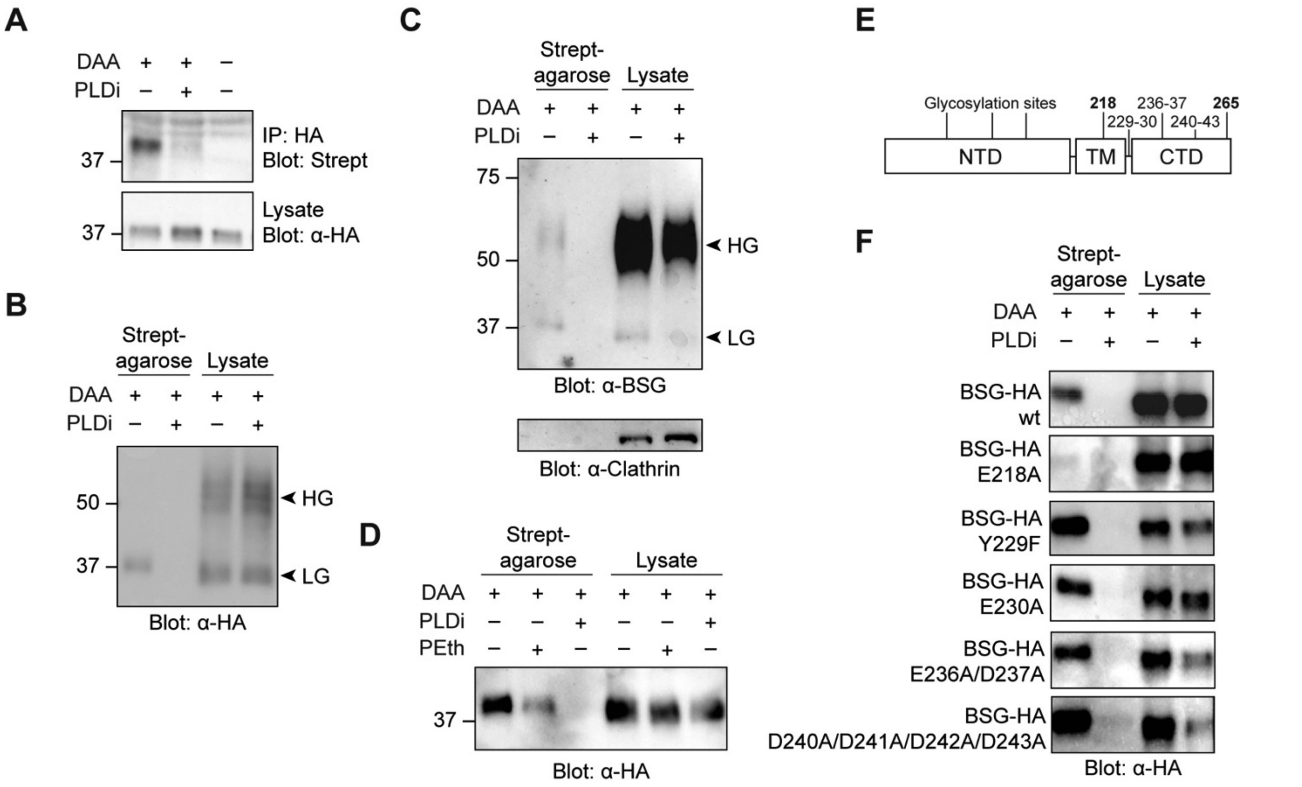

Figure 4. Basigin (BSG/CD147) interacts with the phosphatidyl alcohol probe PDAA. Cells were transfected with BSG-HA (A–B, D), mutants of BSG-HA (F) or not transfected with any plasmids (C), and XL-IMPACT was performed by incubation with PLDi (FIPI) or vehicle for 30 min, followed by DAA and PMA for 1 h, rinsing, UV cross-linking, and CuAAC tagging with biotin-azide. Extracts were then enriched using either anti-HA agarose (A) or streptavidin-agarose (B–D, F). Shown in (A) is a streptavidin blot of anti-HA-enriched proteomes, showing that XL-IMPACT labels BSG-HA. An anti-HA blot of whole-cell lysate indicates similar levels of BSG-HA in all samples. Shown in (B) is reciprocal anti-HA blot of the streptavidin-enriched proteome, showing that XL-IMPACT predominantly labels the ~37 kDa form of overexpressed BSG-HA. Shown in (C) is an anti-BSG blot of streptavidin-enriched proteomes, demonstrating that XL-IMPACT can label both the ~55 kDa (high glycosylation, HG) form and ~37 kDa (low glycosylation, LG) forms of endogenous BSG, with a preference for the LG form. An anti-clathrin blot is included as a loading control for whole cell lysates. Molecular weight markers are in kDa. Shown in (D) is an anti-HA blot of streptavidin-enriched proteome, after XL-IMPACT was performed with PEth competition as indicated. Shown in (E) is the domain structure of BSG, depicting its glycosylated N-terminal domain (NTD), transmembrane domain (TM), and C-terminal domain (CTD), with residues indicated that are mutated in (F). Shown in (F) is an anti-HA blot of streptavidin-enriched proteome after XL-IMPACT labeling of cells transfected with either wild type BSG-HA or the indicated mutant form of BSG-HA.

followed by permeabilization with Triton X-100, albeit with slightly higher levels of background, potentially due to incomplete rinse-out of unreacted probe or disruption of lipid bilayers by paraformaldehyde fixation (Figure S1).

Because this protocol enables lipid visualization in permeabilized cells, it is in principle compatible with antibody-based immunofluorescence labeling of intracellular epitopes. Therefore, we performed colocalization studies with markers of different organelles after XL-IMPACT labeling to demonstrate the feasibility of this approach and to establish the localizations of XL-IMPACT fluorescence (Figures S2 and S3). We found that XL-IMPACT fluorescence substantially colocalized with markers of the ER and the Golgi complex and to a lesser extent with other components of the endomembrane system such as exosomes, endosomes, and lysosomes. Adding ethanol to compete with DAA during the XL-IMPACT labeling did not change the localization of XL-IMPACT-derived fluorescence (Figure 2D), though, consistent with the Western blot-based competition experiments (Figure 2B), it did reduce the extent of XL-IMPACT fluorescence (Figure 2D). These results highlight the utility of using a bifunctional tagging approach with clickable and photo-cross-linking functionalities to enable visualization of lipid probes using methods optimized for protein visualization, as has been demonstrated previously for several classes of endogenous lipids.[27−30]

To identify proteins that interact with phosphatidyl alcohols, we performed bottom-up proteomics analysis of XL-IMPACT-labeled proteomes enriched as described above (Figure 2A). We identified more than 500 proteins across three biological replicates, including 99 proteins that were identified in all three replicates (Figure 3A). Normalized protein abundances were log-transformed, and Student's *t* tests were performed to check statistical significance. Of the 99 proteins, virtually all were enriched in the XL-IMPACT group over the FIPI control, and 50 of these were statistically significantly enriched based on a cutoff of $p < 0.05$ on a Student's *t* test and at least a 2-fold enrichment in the XL-IMPACT vs FIPI-treated samples (Figure 3B).

To understand broad trends for this large putative PDAA interactome, we performed gene ontology (GO) analysis (Figure 3C). Encouragingly, among the 20 most significant GO groups, most are locations or functions enriched in membranes. This result is also consistent with recent reports indicating a preference for diazirine-based probes for labeling membrane-proximal residues.[31,32] Interestingly, more than half

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of the labeled proteins are related to exosomes, which is reinforced by the partial colocalization of XL-IMPACT fluorescence with a marker of this organelle (Figure S2). Whereas the ER, a major site of XL-IMPACT labeling (Figures 2C and S3) does not appear among the most significantly enriched GO groups, gene set enrichment analysis (GSEA) of the proteomics data revealed that ER-residing proteins are significantly more likely to be the most highly enriched proteins among the detected interactome (Figure 3D). This result suggests that, whereas the number of ER-residing proteins are not significantly overrepresented compared to a random sample of proteins, the proteins that are detected have relatively high fold enrichment, accounting for the strong ER-localized XL-IMPACT-derived fluorescence in our imaging experiments.

We next selected one of the most highly enriched proteins as a candidate for detailed examination to validate the findings from the high-throughput proteomics results. We focused our attention on basigin (BSG/CD147) for several reasons: it was one of the top hits, it can function in processes occurring in the ER and in exosomes,[33,34] and it has been rarely detected by others in similar streptavidin-pulldown proteomics negative control samples,[35] meaning that it is more likely to be a specific interactor. BSG has important physiological functions, including regulation of lactate transport and chemotaxis. It is also an important host factor in infectious disease, as it is required for *Plasmodium falciparum* infection of erythrocytes in malaria and was recently proposed as an alternative entry mechanism for SARS-CoV-2. We first expressed HA-tagged BSG in cells, carried out XL-IMPACT labeling with biotin-azide tagging, and then performed anti-HA immunoprecipitation to enrich BSG-HA. Gratifyingly, a streptavidin blot of these samples indicated that BSG-HA was biotinylated (Figure 4A). When we instead enriched the XL-IMPACT-labeled proteome using streptavidin-conjugated agarose and then performed an anti-HA blot, we again observed biotinylation of BSG-HA (Figure 4B). In both experiments, XL-IMPACT labeling of BSG-HA was FIPI-sensitive and dependent upon UV irradiation. We performed a similar streptavidin-agarose enrichment of nontransfected, XL-IMPACT-labeled cells and were able to detect biotinylation on endogenous BSG (Figure 4C). Importantly, when PEth was added in a competition experiment, we detected a drop in BSG labeling by XL-IMPACT (Figure 4D), suggesting that BSG is a likely target of PEth as well as PDAA.

BSG is glycosylated in an N-terminal domain, and it appears as two different glycosylated forms, a high-glycosylation form with a molecular weight of ∼50−75 kDa and a low-glycosylation form with a molecular weight of ∼37 kDa.[36] The high-glycosylation form is considered to be the mature form, residing on post-Golgi compartments (predominantly at the cell surface). By contrast, the low-glycosylation form is considered to be the immature form, and it has a largely ER-based, intracellular localization. Interestingly, XL-IMPACT preferentially labels the low-glycosylation form, revealed by examination of the extent of labeling of the enriched forms of the protein compared with its levels in lysate (Figures 4B−C). In the BSG-HA experiment, XL-IMPACT labeling was visible only on the low-glycosylation form, which is present at nonphysiologically high amounts due to BSG-HA overexpression, that is, roughly equivalent to the high-glycosylation form (Figure 4B). In the endogenous BSG experiment, the high-glycosylation form was detected to a much greater extent than the low-glycosylation form in lysate, but XL-IMPACT labeled each form roughly equivalently, again indicating a preference for the low-glycosylation form (Figure 4C). These data are consistent with the primarily intracellular localization pattern of PDAA (Figure 2C) and other similar phosphatidyl alcohols, as we have demonstrated previously.[15−17]

Finally, we set out to map potential PDAA/PEth binding sites on BSG by mass spectrometry. BSG is a single-pass transmembrane protein that, in addition to its glycosylated, luminal/extracellular N-terminal domain, contains a transmembrane helix and a cytoplasmic C-terminal domain (Figure 4E). We overexpressed BSG-FLAG in HEK 293T cells and performed XL-IMPACT labeling by treatment with DAA and PMA followed by rinsing and UV cross-linking. Enriched BSG-FLAG was then labeled with an isotopically coded, cleavable picolyl azide probe,[37] trypsinized, and analyzed by MS/MS. Encouragingly, we detected modification on Q265 with a glycerophosphate-linked DAA group (Figure S5 and Table S2). Because this modification corresponds to a deacylated form of PDAA that retains the glycerol moiety, it could only have been generated from a PLD transphosphatidylation product and not cross-linking of the free alcohol, DAA, to the protein. Q265 appears at the C terminus of BSG, within its disordered cytoplasmic domain. Considering that our probe is generated on the cytoplasmic side of membranes, this labeling is consistent with the expected localization of PDAA lipids.

We had also hypothesized that residues within the transmembrane helix and in membrane-proximal regions might also comprise part of a lipid binding site on BSG. However, no modified peptides corresponding to these portions of BSG were detected in site MS mapping experiments, perhaps due to inefficient trypsin cleavage in this internal region of the protein. Therefore, to explore the possibility of other residues that might participate in PDAA binding, we performed a targeted mutagenesis study. We generated mutations in BSG-HA at several residues within the transmembrane helix and membrane-proximal regions of the C-terminal domain that are known to have a preference for binding with diazirine (e.g., Asp, Glu, and Tyr)[32] and performed XL-IMPACT labeling to assess whether mutation of these residues would diminish PDAA cross-linking. Remarkably, the E218A mutation eliminated the majority of PDAA labeling, suggesting that E218 is important for binding to PDAA lipids (Figure 4F). As a control, we verified that the ability of PDAA to cross-link to other proteins in cells expressing BSG(E218A) was not diminished (Figure S6). E218 is an acidic residue within the BSG transmembrane helix that is highly conserved across species, and previous studies have shown that this residue is important for the chaperone function of BSG.[38] Given the results of these mapping studies and the competition experiments with PEth, we suggest that a binding site (or separate binding sites) involving E218 and Q265 could function in the recognition of PEth.

In sum, these studies validate BSG as a bona fide interactor of PDAA, and likely of PEth, and highlight the power of XL-IMPACT for coupling the visualization of PLD-derived phosphatidyl alcohol lipids to the identification of interactomes of these lipids using chemoproteomics. Further, they point to the possibility that engagement of BSG and other proteins may be a mechanism by which ethanol-derived PEth lipids affect cell physiology.

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CONCLUSION

In this study, we developed a chemoproteomics approach for probing the direct protein interactors of phosphatidyl alcohol lipids. By taking advantage of the substrate tolerance of PLD enzymes, our approach uses in situ, chemoenzymatic synthesis to generate clickable, photoaffinity lipid analogues that mirror the environment of phosphatidyl alcohols such as PEth that are produced by PLDs following ethanol consumption. Because it involves covalent cross-linking of lipid analogs to nearby proteins, XL-IMPACT enables both the visualization of lipid localizations compatible with standard fixation and permeabilization methods and the identification of protein interacting partners of phosphatidyl alcohol lipids. These results set the stage for future studies to unravel mechanisms underlying how interactions of these alcohol-derived lipids with cellular proteins may mediate pathophysiological effects in diseases associated with alcohol abuse.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.1c00584.

Materials and methods, XL-IMPACT imaging with paraformaldehyde fixation (Figure S1), colocalization of XL-IMPACT with organelle markers (Figures S2 and S3), mass spectrometry quantification of PDAA in EtOH-competed sample (Figure S4), spectral assignment of an XL-IMPACT-labeled BSG-FLAG peptide (Figure S5), and streptavidin blot of the mutagenesis screen (Figure S6). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the data set identifier PXD029749 (PDF)

Raw shotgun proteomics data (Table S1) (XLSX)

List of peptide spectrum matches of associated with the peptide modified by a deacylated form of PDAA by LC−MS/MS (Table S2) (XLSX)

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Author Contributions

W.Y. performed all experiments, except Z.L. performed LC−MS/MS site mapping experiments. W.Y. and J.M.B. conceived of the project idea, designed experiments, analyzed data, and wrote the manuscript. Z.L. and C.M.W. designed and analyzed the LC−MS/MS site mapping experiments and provided edits on the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) World Health Organization (WHO). *Global status report on alcohol and health 2018*; WHO: 2019.

(2) Barve, S.; Chen, S. Y.; Kirpich, I.; Watson, W. H.; McClain, C. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. *Alcohol Res.* **2017**, *38* (2), 289−302.

(3) Room, R.; Babor, T.; Rehm, J. Alcohol and public health. *Lancet* **2005**, *365* (9458), 519−530.

(4) Ridley, N. J.; Draper, B.; Withall, A. Alcohol-related dementia: an update of the evidence. *Alzheimer's Res. Ther.* **2013**, *5* (1), 3.

(5) Wallner, M.; Olsen, R. W. Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of GABAA receptors as an opportunity for drug development? *Br. J. Pharmacol.* **2008**, *154* (2), 288−98.

(6) Yu, C. H.; Liu, S. Y.; Panagia, V. The transphosphatidylation activity of phospholipase D. *Mol. Cell. Biochem.* **1996**, *157* (1−2), 101−105.

(7) Viel, G.; Boscolo-Berto, R.; Cecchetto, G.; Fais, P.; Nalesso, A.; Ferrara, S. D. Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. *Int. J. Mol. Sci.* **2012**, *13* (11), 14788−812.

(8) Asaoka, Y.; Kikkawa, U.; Sekiguchi, K.; Shearman, M. S.; Kosaka, Y.; Nakano, Y.; Satoh, T.; Nishizuka, Y. Activation of a brain-specific protein kinase C subspecies in the presence of phosphatidylethanol. *FEBS Lett.* **1988**, *231* (1), 221−224.

(9) Omodeo-Sale, F.; Lindi, C.; Palestini, P.; Masserini, M. Role of phosphatidylethanol in membranes. Effects on membrane fluidity, tolerance to ethanol, and activity of membrane-bound enzymes. *Biochemistry* **1991**, *30* (9), 2477−82.

(10) Nishida, A. Phosphatidylethanol inhibits phosphatidylinositol-phospholipase C activity in a competitive manner with phosphatidylinositol-4,5-bisphosphate. *Drug Alcohol Depend.* **1997**, *44* (2−3), 117−122.

(11) Chung, H. W.; Petersen, E. N.; Cabanos, C.; Murphy, K. R.; Pavel, M. A.; Hansen, A. S.; Ja, W. W.; Hansen, S. B. A Molecular Target for an Alcohol Chain-Length Cutoff. *J. Mol. Biol.* **2019**, *431* (2), 196−209.

(12) Kotter, K.; Klein, J. Ethanol inhibits astroglial cell proliferation by disruption of phospholipase D-mediated signaling. *J. Neurochem.* **1999**, *73* (6), 2517−23.

(13) Guizzetti, M.; Thompson, B. D.; Kim, Y.; VanDeMark, K.; Costa, L. G. Role of phospholipase D signaling in ethanol-induced inhibition of carbachol-stimulated DNA synthesis of 1321N1 astrocytoma cells. *J. Neurochem.* **2004**, *90* (3), 646−53.

(14) Pannequin, J.; Delaunay, N.; Darido, C.; Maurice, T.; Crespy, P.; Frohman, M. A.; Balda, M. S.; Matter, K.; Joubert, D.; Hollande, F.; et al. Phosphatidylethanol accumulation promotes intestinal hyperplasia by inducing ZONAB-mediated cell density increase in response to chronic ethanol exposure. *Mol. Cancer Res.* **2007**, *5* (11), 1147−57.
(15) Bumpus, T. W.; Baskin, J. M. A Chemoenzymatic Strategy for Imaging Cellular Phosphatidic Acid Synthesis. *Angew. Chem., Int. Ed.* **2016**, *55* (42), 13155−13158.
(16) Bumpus, T. W.; Baskin, J. M. Clickable Substrate Mimics Enable Imaging of Phospholipase D Activity. *ACS Cent. Sci.* **2017**, *3* (10), 1070−1077.
(17) Liang, D.; Wu, K.; Tei, R.; Bumpus, T. W.; Ye, J.; Baskin, J. M. A real-time, click chemistry imaging approach reveals stimulus-specific subcellular locations of phospholipase D activity. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (31), 15453−15462.
(18) Lum, K. M.; Sato, Y.; Beyer, B. A.; Plaisted, W. C.; Anglin, J. L.; Lairson, L. L.; Cravatt, B. F. Mapping Protein Targets of Bioactive Small Molecules Using Lipid-Based Chemical Proteomics. *ACS Chem. Biol.* **2017**, *12* (10), 2671−2681.
(19) Niphakis, M. J.; Lum, K. M.; Cognetta, A. B., 3rd; Correia, B. E.; Ichu, T. A.; Olucha, J.; Brown, S. J.; Kundu, S.; Piscitelli, F.; Cravatt, B. F.; et al. A Global Map of Lipid-Binding Proteins and Their Ligandability in Cells. *Cell* **2015**, *161* (7), 1668−80.
(20) Hulce, J. J.; Cognetta, A. B.; Niphakis, M. J.; Tully, S. E.; Cravatt, B. F. Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. *Nat. Methods* **2013**, *10* (3), 259−64.
(21) Peng, T.; Hang, H. C. Bifunctional fatty acid chemical reporter for analyzing S-palmitoylated membrane protein-protein interactions in mammalian cells. *J. Am. Chem. Soc.* **2015**, *137* (2), 556−9.
(22) Li, Z.; Hao, P.; Li, L.; Tan, C. Y.; Cheng, X.; Chen, G. Y.; Sze, S. K.; Shen, H. M.; Yao, S. Q. Design and synthesis of minimalist terminal alkyne-containing diazirine photo-crosslinkers and their incorporation into kinase inhibitors for cell- and tissue-based proteome profiling. *Angew. Chem., Int. Ed.* **2013**, *52* (33), 8551−6.
(23) Balla, T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol. Rev.* **2013**, *93* (3), 1019−137.
(24) Tei, R.; Baskin, J. M. Spatiotemporal control of phosphatidic acid signaling with optogenetic, engineered phospholipase Ds. *J. Cell Biol.* **2020**, *219* (3), e201907013.
(25) Wong, L. H.; Gatta, A. T.; Levine, T. P. Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. *Nat. Rev. Mol. Cell Biol.* **2019**, *20* (2), 85−101.
(26) Tanaka, K. A.; Suzuki, K. G.; Shirai, Y. M.; Shibutani, S. T.; Miyahara, M. S.; Tsuboi, H.; Yahara, M.; Yoshimura, A.; Mayor, S.; Kusumi, A.; et al. Membrane molecules mobile even after chemical fixation. *Nat. Methods* **2010**, *7* (11), 865−6.
(27) Haberkant, P.; Raijmakers, R.; Wildwater, M.; Sachsenheimer, T.; Brugger, B.; Maeda, K.; Houweling, M.; Gavin, A. C.; Schultz, C.; Holthuis, J. C.; et al. In vivo profiling and visualization of cellular protein-lipid interactions using bifunctional fatty acids. *Angew. Chem., Int. Ed.* **2013**, *52* (14), 4033−8.
(28) Frank, J. A.; Yushchenko, D. A.; Hodson, D. J.; Lipstein, N.; Nagpal, J.; Rutter, G. A.; Rhee, J.-S.; Gottschalk, A.; Brose, N.; Trauner, D.; et al. Photoswitchable diacylglycerols enable optical control of protein kinase C. *Nat. Chem. Biol.* **2016**, *12* (9), 755−762.
(29) Haberkant, P.; Stein, F.; Hoglinger, D.; Gerl, M. J.; Brugger, B.; Van Veldhoven, P. P.; Krijgsveld, J.; Gavin, A. C.; Schultz, C. Bifunctional Sphingosine for Cell-Based Analysis of Protein-Sphingolipid Interactions. *ACS Chem. Biol.* **2016**, *11* (1), 222−30.
(30) Hoglinger, D.; Nadler, A.; Haberkant, P.; Kirkpatrick, J.; Schifferer, M.; Stein, F.; Hauke, S.; Porter, F. D.; Schultz, C. Trifunctional lipid probes for comprehensive studies of single lipid species in living cells. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (7), 1566−1571.
(31) Conway, L. P.; Jadhav, A. M.; Homan, R. A.; Li, W.; Rubiano, J. S.; Hawkins, R.; Lawrence, R. M.; Parker, C. G. Evaluation of fully-functionalized diazirine tags for chemical proteomic applications. *Chem. Sci.* **2021**, *12* (22), 7839−7847.
(32) West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. Labeling Preferences of Diazirines with Protein Biomolecules. *J. Am. Chem. Soc.* **2021**, *143* (17), 6691−6700.
(33) Thakur, A.; Qiu, G.; Xu, C.; Han, X.; Yang, T.; Ng, S. P.; Chan, K. W. Y.; Wu, C. M. L.; Lee, Y. Label-free sensing of exosomal MCT1 and CD147 for tracking metabolic reprogramming and malignant progression in glioma. *Sci. Adv.* **2020**, *6* (26), No. eaaz6119.
(34) Tyler, R. E.; Pearce, M. M.; Shaler, T. A.; Olzmann, J. A.; Greenblatt, E. J.; Kopito, R. R. Unassembled CD147 is an endogenous endoplasmic reticulum-associated degradation substrate. *Mol. Biol. Cell* **2012**, *23* (24), 4668−78.
(35) Mellacheruvu, D.; Wright, Z.; Couzens, A. L.; Lambert, J. P.; St-Denis, N. A.; Li, T.; Miteva, Y. V.; Hauri, S.; Sardiu, M. E.; Nesvizhskii, A. I.; et al. The CRAPome: a contaminant repository for affinity purification-mass spectrometry data. *Nat. Methods* **2013**, *10* (8), 730−6.
(36) Bai, Y.; Huang, W.; Ma, L. T.; Jiang, J. L.; Chen, Z. N. Importance of N-glycosylation on CD147 for its biological functions. *Int. J. Mol. Sci.* **2014**, *15* (4), 6356−77.
(37) Miyamoto, D. K.; Flaxman, H. A.; Wu, H. Y.; Gao, J.; Woo, C. M. Discovery of a Celecoxib Binding Site on Prostaglandin E Synthase (PTGES) with a Cleavable Chelation-Assisted Biotin Probe. *ACS Chem. Biol.* **2019**, *14* (12), 2527−2532.
(38) Manoharan, C.; Wilson, M. C.; Sessions, R. B.; Halestrap, A. P. The role of charged residues in the transmembrane helices of monocarboxylate transporter 1 and its ancillary protein basigin in determining plasma membrane expression and catalytic activity. *Mol. Membr. Biol.* **2006**, *23* (6), 486−498.

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