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Chemoproteomic Profiling of Bile Acid Interacting Proteins

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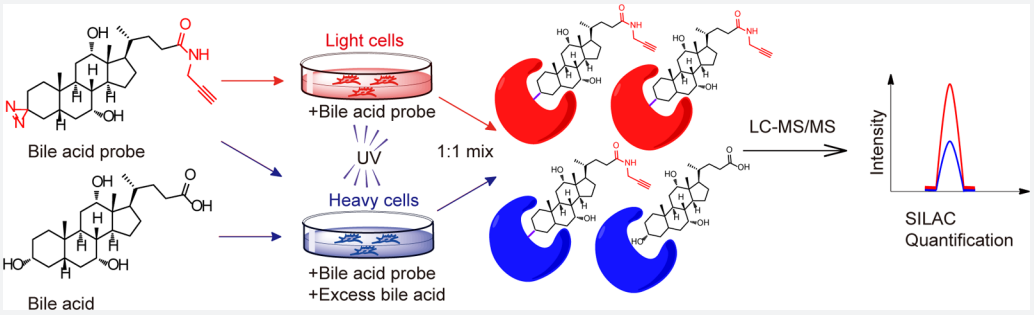
Supporting Information



ABSTRACT: Bile acids (BAs) are a family of endogenous metabolites synthesized from cholesterol in liver and modified by microbiota in gut. Being amphipathic molecules, the major function of BAs is to help with dietary lipid digestion. In addition, they also act as signaling molecules to regulate lipid and glucose metabolism as well as gut microbiota composition in the host. Remarkably, recent discoveries of the dedicated receptors for BAs such as FXR and TGR5 have uncovered a number of novel actions of BAs as signaling hormones which play significant roles in both physiological and pathological conditions. Disorders in BAs' metabolism are closely related to metabolic syndrome and intestinal and neurodegenerative diseases. Though BA-based therapies have been clinically implemented for decades, the regulatory mechanism of BA is still poorly understood and a comprehensive characterization of BA-interacting proteins in proteome remains elusive. We herein describe a chemoproteomic strategy that uses a number of structurally diverse, clickable, and photoreactive BA-based probes in combination with quantitative mass spectrometry to globally profile BA-interacting proteins in mammalian cells. Over 600 BA-interacting protein targets were identified, including known endogenous receptors and transporters of BA. Analysis of these novel BA-interacting proteins revealed that they are mainly enriched in functional pathways such as endoplasmic reticulum (ER) stress response and lipid metabolism, and are predicted with strong implications with Alzheimer's disease, non-alcoholic fatty liver disease, and diarrhea. Our findings will significantly improve the current understanding of BAs' regulatory roles in human physiology and diseases.

INTRODUCTION

Bile acids (BAs) are a class of important endogenous metabolites that consist of a steroid core and a side chain with a carboxyl group.[1] The number and position of hydroxyl groups on the steroid core determine their hydrophobicity. In human, the primary BAs are cholic acid (CA) and chenodeoxycholic acid (CDCA), which are synthesized from cholesterol in liver and are subsequently conjugated with taurine or glycine to increase their water solubility.[2] The conjugated BAs are secreted into intestine and undergo deconjugation, dehydroxylation, or isomerization by gut microbiota to form secondary bile acids, including deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA).[3] Being typical amphipathic molecules, BAs can facilitate emulsification and absorption of dietary lipid and fat-soluble vitamins. Nearly 95% of the intestinal BAs are reabsorbed in ileum and return back to liver via an active transport system called "enterohepatic circulation".[4] The homeostasis of BAs is maintained by the balance between their synthesis, secretion, and reabsorption.[2]

Since the discovery of endogenous receptors for BAs such as the nuclear farnesoid X receptor (FXR) and the plasma membrane bound G protein-coupled receptor 5 (TGR5), much effort has been devoted to understand the role of BAs as signaling molecules.[5−7] Given that the BA receptors have a broad tissue expression profile and a wide range of downstream target genes, BAs have been shown, via activating these receptors, to regulate numerous physiological processes in the body, such as lipid and lipoprotein metabolism, glucose homeostasis, energy expenditure, carcinogenesis, and intestinal inflammation.[8−11] BAs can also regulate protein functions by directly interacting with key metabolic enzymes or ion channels. For example, BAs bind to phospholipase D (PLD) to allosterically stimulate the production of endogenous anandamides,[12,13] and target the mitochondrial inner membrane protein ADP/ATP translocase (SLC25A6) to mediate direct mitochondrial destruction of toxic BAs.[14] In addition,

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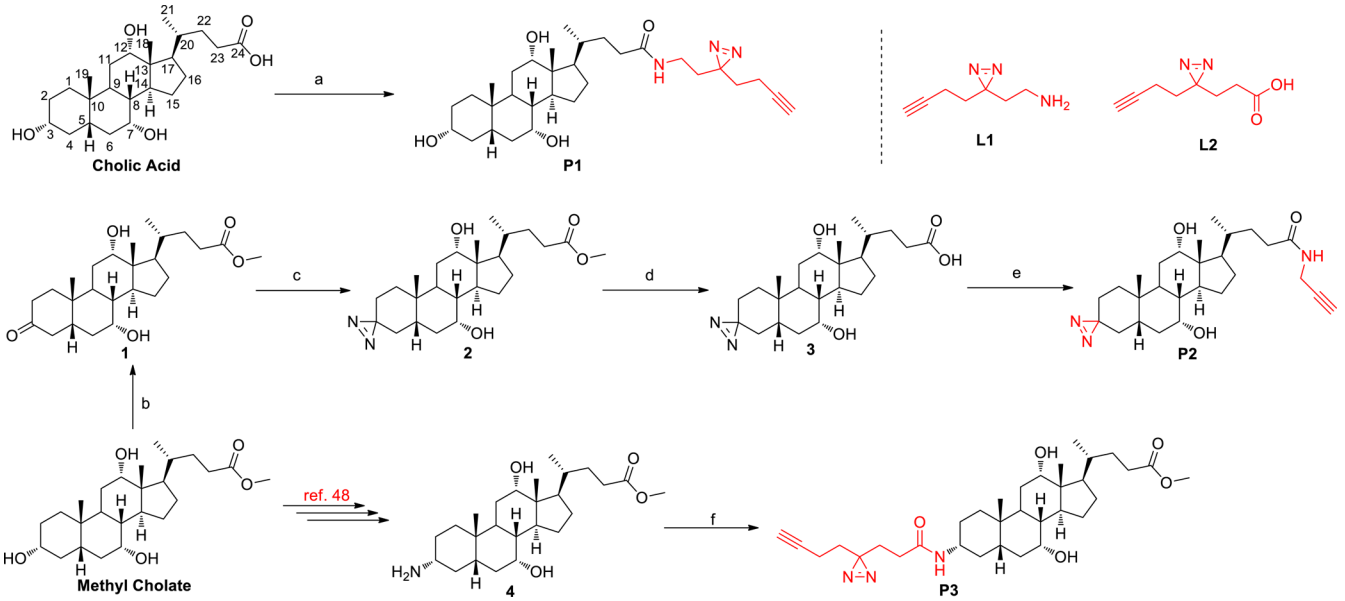
Scheme 1. Design and Synthesis of the Clickable and Photoreactive Bile Acid Probes[a]



[a]Reagents and conditions: (a) **L1**, BOP, DIPEA, DMF, 24 h, 85%. (b) Ag_2CO_3 on Celite (50 wt %), toluene, 140 °C, 36 h, 92%. (c) (1) NH_3, MeOH, 0 °C, 3 h; (2) HOSA, −15 °C, 2 h, then rt, 14 h; (3) I_2, TEA, MeOH, 30 min, 76%. (d) NaOH, MeOH, 36 h, quant. (e) 2-Propynylamine, HBTU, DIPEA, DMF, 20 h, 95%. (f) **L2**, BOP, DIPEA, DMF, 24 h, 88%.

BAs dynamically interplay with gut microbiota to impact host metabolism and the intricate mechanisms for such crosstalk remain to be unveiled.[15,16]

Consistent with the important and diverse roles of BAs, disorders in BA signaling are strongly associated with numerous human diseases, such as non-alcoholic fatty liver diseases (NAFLD), type 2 diabetes, diarrhea, and inflammatory bowel disease.[17−19] For example, studies show that FXR-deficient mice on chow diet develop steatosis, insulin resistance, and bile acid malabsorption which causes diarrhea.[20,21] Conversely, activation of FXR improves diet-induced fatty liver disease and insulin sensitivity, and significantly reduces symptoms in animal models of diarrhea.[22−24] BA receptors have therefore become attractive therapeutic targets, and several agonists for FXR and TGR5 have entered clinical trials, aiming to offer new treatment options for these diseases.[25,26] Recently, obeticholic acid (OCA), a bile acid derived FXR agonist, has been officially approved for clinical treatment of primary biliary cholangitis (PBC).[27] Moreover, BAs can also be *de novo* synthesized in brain and act as potent antagonists for NMDA and GABAA receptors in the central nervous system.[28,29] It has been reported that a secondary BA tauroursodeoxycholic acid (TUDCA) can easily go through the blood−brain barrier and is found to be a strong neuroprotective agent in treatment of Alzheimer's disease (AD).[30,31]

Though our knowledge on BA signaling has been greatly improved in the past 20 years, most of the basic research and therapeutic exploration are still focused on FXR and TGR5, the two well-characterized BA receptors. Are there any other novel endogenous receptors for BAs? What are the rest of BA-interacting proteins in cells? These important questions remain to be answered. Current approaches to study BA−protein interactions mainly rely on traditional molecular biotechnologies and genetic mouse models. While they can discover a number of individual BA−protein interactions in a case-by-case manner, the low throughput puts great limitation on gaining deeper and broader understanding of BAs' mechanism of action.[13,14,32−34]

Here we aim to perform a comprehensive characterization of BA-interacting proteins in mammalian proteomes using quantitative chemical proteomic technologies. Our approach was inspired by the previous efforts to map cholesterol-binding and lipid-binding proteins in proteomes using activity-based protein profiling (ABPP).[35,36] We synthesized multiple clickable, photoreactive BA-based chemical probes and applied them in combination with quantitative proteomics based on stable-isotope labeling by amino acids in cell culture (SILAC) to globally profile BA-interacting proteins directly in living cells. With our ABPP-SILAC strategy, we identified >600 potential BA-interacting proteins with high confidence which include known receptors, transporters, and biosynthetic enzymes of BA. More importantly, our quantitative chemoproteomic profiling uncovered a large number of novel BA-interacting proteins that are functionally enriched in critical metabolic pathways such as endoplasmic reticulum (ER) stress response and fatty acid metabolism, and are strongly linked to vital diseases including Alzheimer's disease, non-alcoholic fatty liver disease, and diarrhea. Our study provides the first global map of BA-interacting proteins in mammalian cells that will serve a valuable resource for exploring and understanding BA biology.

RESULTS AND DISCUSSION

Design and Synthesis of the Clickable and Photoreactive BA Probes. Chemoproteomic probes have emerged as powerful tools to profile the cellular targets of bioactive small molecules in native biological system.[37,38] In particular, photoreactive probes have been extensively used in combination with quantitative proteomic strategies to profile non-covalent protein−ligand interactions in cells and tissues.[39−42] In order to assess reversible BA-interacting proteins in living cells, a photoreactive group which can establish covalent binding between BA and interacting proteins under UV irradiation

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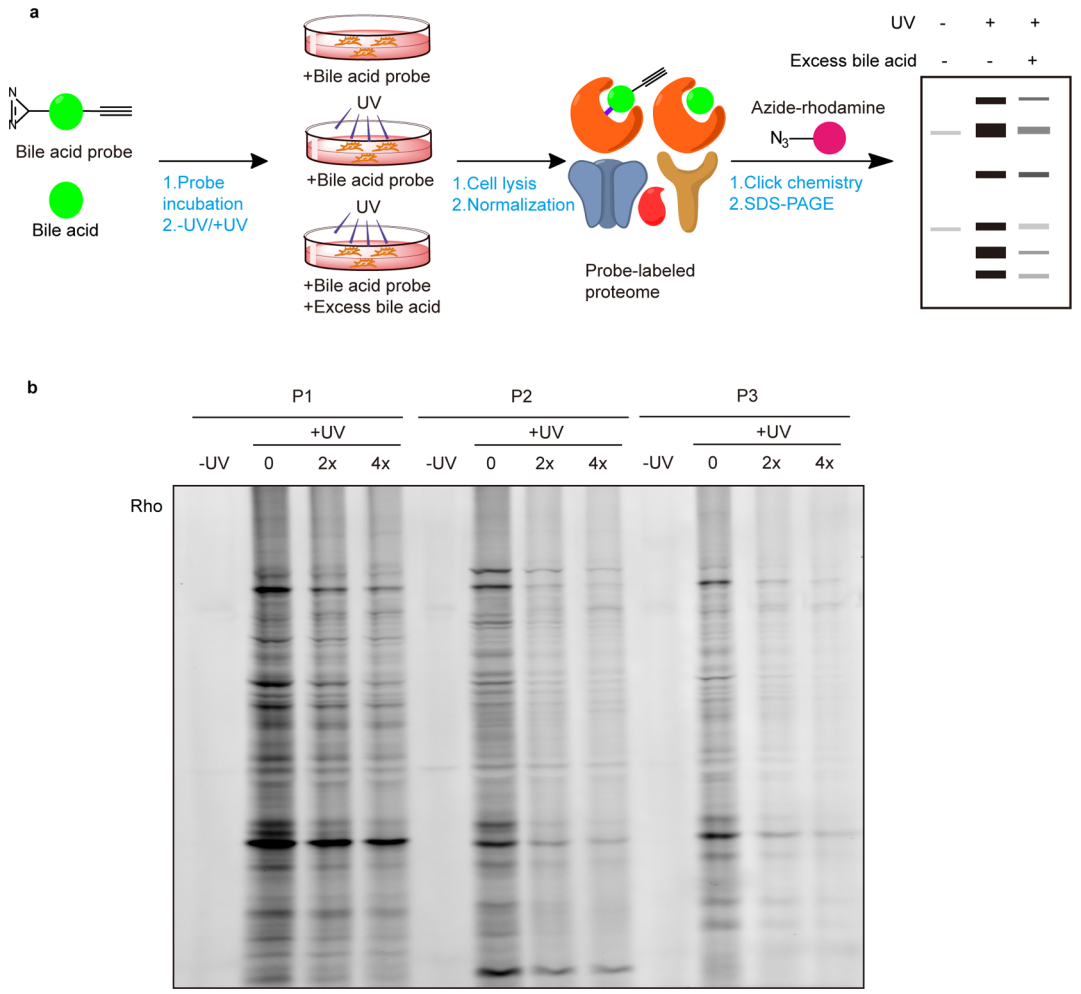

Figure 1. Gel-based profiling of BA-interacting proteins in living cells. (a) Workflow for gel-based ABPP profiling of BA-interacting proteins in living HeLa cells. (b) Evaluation of labeling efficiency of BA probes in living HeLa cells by in-gel fluorescence.

needs to be incorporated into a BA scaffold to generate the corresponding probe. Additionally, an alkyne group should be appended, which allows further modification with azide-containing biotin or fluorescein by copper-catalyzed azide–alkyne cycloaddition (CuAAC).[43,44] Conceivably, the carboxylic acid moiety at the C-24 position could be modified based on the fact that the endogenous BAs are also conjugated with taurine or glycine at the C-24 position to generate bile salts. A previous report has shown that fluorescein-labeled taurocholate at the C-3 position would retain its original biological activity.[45] As ester linkage may be cleaved by endogenous esterases,[35] the more stable amide linkage may be adopted in probe design. According to these considerations, ultimately, we designed and prepared a set of structurally diverse BA probes derived from cholic acid structure belonging to primary BAs, each of which contains an alkyne group and a photoreactive diazirine group (Scheme 1). **P1** was directly prepared in 85% yield from cholic acid with the minimalist linker **L1** developed by Yao and coworkers.[46] The synthesis of **P2** commenced with the selective oxidation of the 3-hydroxy group of methyl cholate to afford ketone **1** in 92% yield.[47] Then the ketone moiety was further transformed to a diazirine moiety using the standard conditions (NH_3/HOSA/I_2) to furnish compound **2**. Hydrolysis of methyl ester followed by amidation with 2-propynylamine smoothly afforded the desired **P2** in 95% yield. According to the literature reported protocol,[48] we could also transform methyl cholate to 3-amino methyl cholate (**4**). Then amidation of **4** with the minimalist linker **L2** generated **P3** in 88% yield. Another two analogues of **P1** and **P3** containing ester linkage were also synthesized and evaluated. Unfortunately, they failed to label proteins in lysate owning to the unstable ester bond.

Gel-Based ABPP Profiling of BA-Interacting Proteins in Living Cells. With these BA probes in hand, we first evaluated their labeling efficiency in living cells by in-gel fluorescence (Figure 1a). HeLa cells were treated with 50 μM of each of the BA probes and shed with 365 nm UV light for 5 min to induce photo-cross-linking. The labeled cell lysates were conjugated with azide-rhodamine via CuAAC and separated by SDS–PAGE. In addition to a negative control that was done without UV cross-linking, two "competition" samples were prepared in parallel where cells were cotreated with 2× and 4× more methyl cholic acids during the probe labeling step. The in-gel fluorescence revealed clear and distinct labeling profiles for each probe (Figure 1b), which suggested that difference in positions of installing the diazirine and alkyne groups indeed affect the probe–protein interactions in different ways. As expected, the labeling events were UV-dependent, confirming that most of the probe–protein interactions are noncovalent and these interactions could be competed off by excessive natural BAs in a dose-dependent manner (100 μM or 200 μM). Among the three BA probes tested, **P1** showed the strongest labeling profile overall, however, its labeling was less sensitive to

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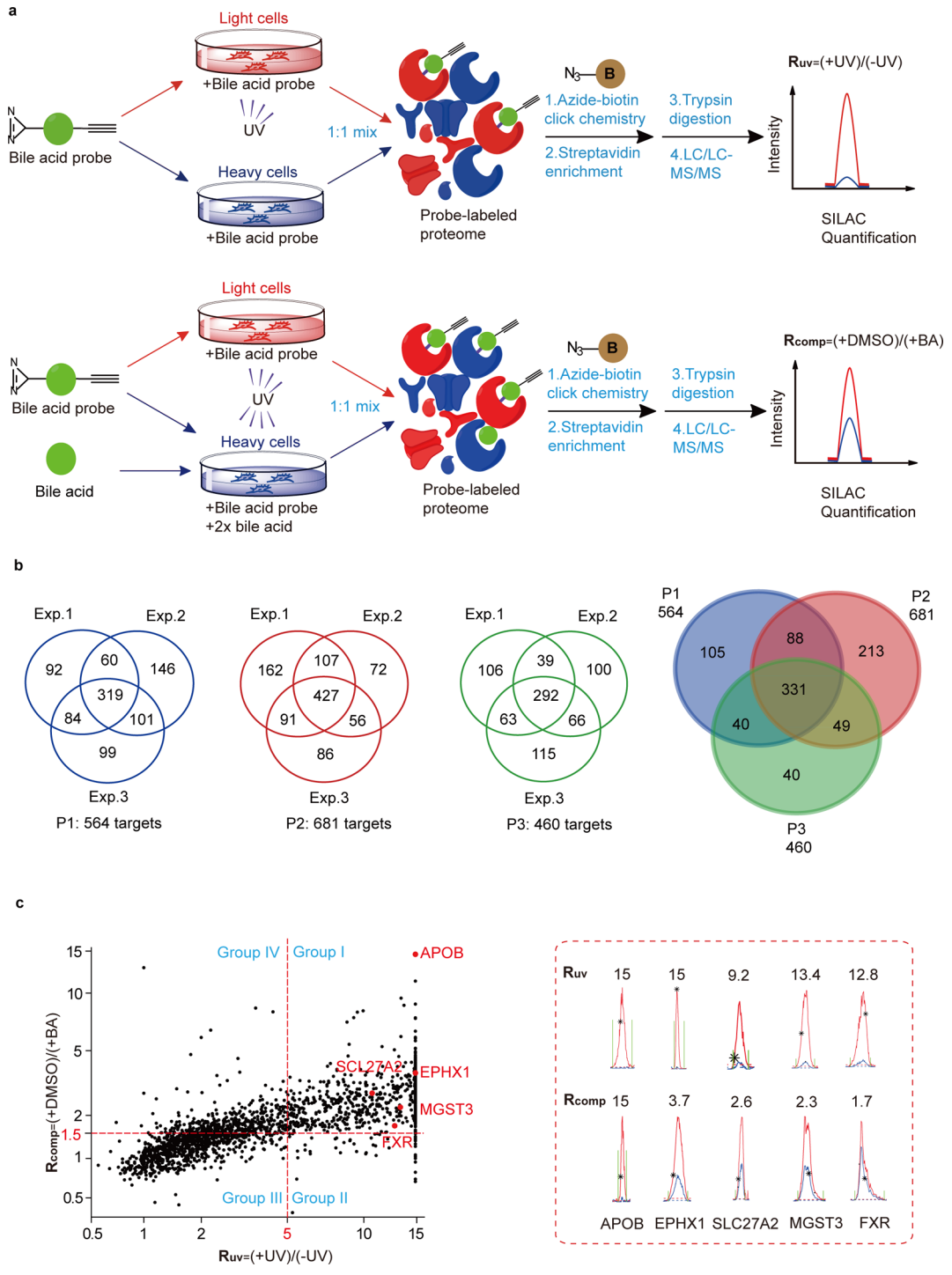

Figure 2. MS-based profiling of BA-interacting proteins in living cells by ABPP-SILAC. (a) Scheme for ABPP-SILAC experiments in which UV-dependent labeling and competition by 2× natural BA are quantified, respectively. (b) Venn diagrams showing the number of BA-interacting proteins identified specifically with each BA probe and commonly with all three probes. (c) Representative SILAC ratio plot for all BA-interacting proteins identified with **P2**. The *x*-axis is the SILAC ratio quantified in the UV-dependent labeling experiment. The *y*-axis is the SILAC ratio quantified in the BA-competition experiment. Select examples of known BA-interacting proteins are colored in red, and their extracted ion chromatograms are shown on the right.

competition of native BA compared to that of **P2** and **P3**. We also separated the probe-labeled lysates into soluble and membrane fractions by ultracentrifugation before conjugation with the fluorescent reporter, and the results showed stronger labeling signals in the membrane fraction (Figure S1), which is consistent with the high lipophilicity of these three BA probes.

MS-Based ABPP Profiling of BA-Interacting Proteins.

We next applied these BA probes in a quantitative chemical proteomic platform to identify BA-interacting proteins in living cells. The technology combines ABPP with SILAC-based quantitative proteomics,[49] which allows us to identify and quantify probe-labeling protein targets by liquid chromatography−tandem mass spectrometry (LC−MS/MS). HeLa cells

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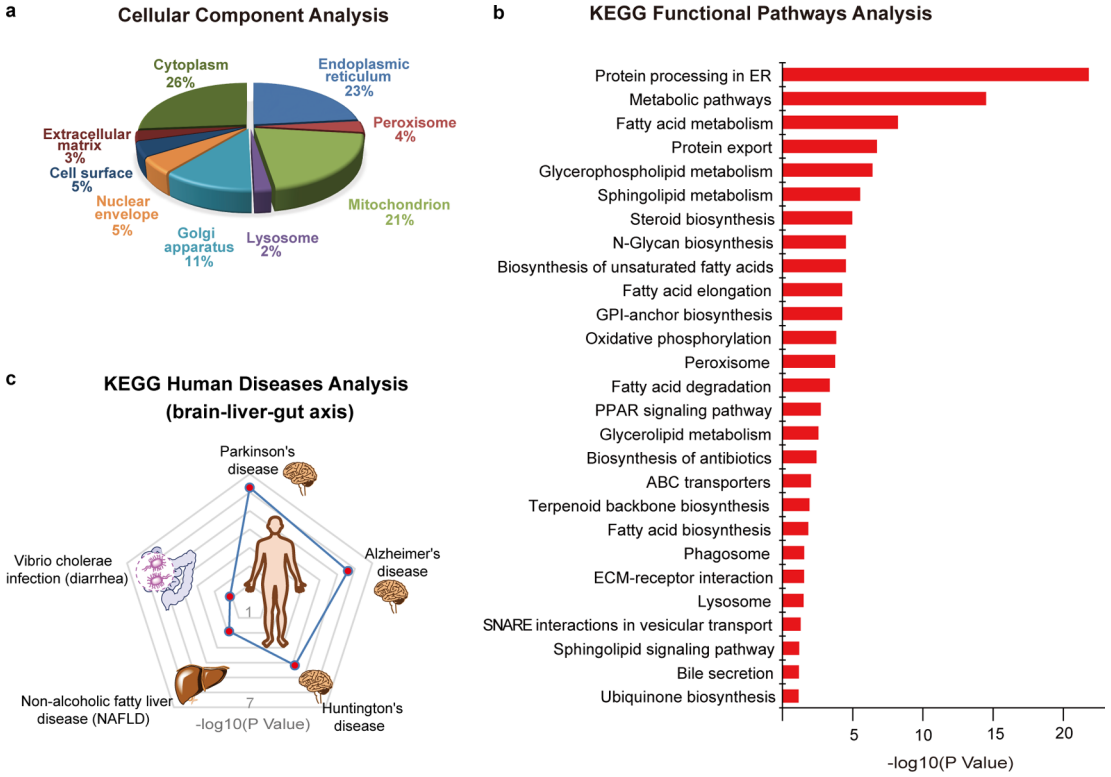

Figure 3. Bioinformatic analysis of BA-interacting proteins. (a). Cellular component analysis of BA-interacting proteins by gene ontology (GO). (b) Functional pathways analysis of BA-interacting proteins by Kyoto Encyclopedia of Genes and Genomes (KEGG). The enriched pathways are ranked based on their *P* values. (c) Human diseases analysis of BA-interacting proteins by KEGG. The top 5 enriched disease pathways are shown, and their *P* values are marked with red dots on the contour plot.

were cultured in "light" and "heavy" SILAC media for several passages, and in order to test whether the metabolic incorporation of heavy amino acids is complete, we combined the light and heavy cells in a 1:1 ratio and digested the mixed proteome for LC−MS/MS analysis. The distribution of the SILAC ratios showed a median value of 1.02 (Figure S2), indicating complete incorporation of the heavy amino acids and readiness of these cells for use in ABPP-SILAC experiments.

We performed two types of ABPP-SILAC experiments with each of the three BA probes in living cells. In a "UV-dependent labeling" experiment (Figure 2a, top panel), we treated light HeLa cells with 50 μM of probe for 1 h and irradiated the cells with UV light for 5 min, while the heavy cells were labeled with the same conditions except that no UV cross-linking was performed. The light and heavy cells were collected and lysed, and their proteomes were mixed with a 1:1 ratio and conjugated to an azide-biotin tag by CuAAC. After enrichment with streptavidin and on-bead digestion by trypsin, we analyzed the digested peptides with LC−MS/MS and quantified SILAC ratios for each protein. We used a light/heavy ratio (R_{uv}) of 5.0 as the cutoff to exclude any targets due to nonspecific binding to streptavidin and defined the remaining ones as specific "probe-binding" proteins. In parallel, we performed a "BA competition" experiment (Figure 2a, bottom panel) in which cells were cotreated with excessive natural BAs during the probe labeling step. More specifically, we treated light and heavy cells with 50 μM of the BA probe in the presence of either DMSO or 100 μM of methyl cholic acid, respectively. The lysed proteomes were equally combined and then subjected to ABPP-SILAC analysis as described above. We used a competition light/heavy ratio (R_{comp}) of 1.5 as the cutoff to define the "BA-sensitive proteins" group, which excluded proteins that could potentially bind to the photoaffinity portion of the probe and therefore fail to be competed by excessive natural BA. In the end, we designated those common targets from both profiling experiments with $R_{uv} \geq 5.0$ and $R_{comp} \geq 1.5$ as "BA-interacting proteins".

For each of the **P1**, **P2**, and **P3** probes, we performed three biological repeats for both types of profiling experiments, and in order to minimize the variation introduced by the stochastic nature of data-dependent acquisition by LC−MS/MS, only the protein targets that are identified in at least two out of the three replicates are considered for further analysis. The final lists include 564, 681, and 460 proteins for each of the **P1**, **P2**, and **P3** probes, respectively, and all three probes shared 331 common protein targets (Figure 2b, Table S1). Among these three probes, **P2** possessed the greatest number of specific protein targets, likely because the diazirine moiety is installed at the C-3 position on the BA backbone so that **P2** can more effectively capture the potential interactions close to the binding pocket (compared to **P1**) but is less protruding (compared to **P3**). Consistent with this hypothesis, FXR, one of the best-studied BA receptors, is captured only by **P2**, but not by the other two probes, suggesting that **P2** was a better BA derivative to simulate its interaction with protein targets.

We therefore focused on the "BA-interacting proteins" captured by **P2** for further analysis. A quick survey on the list reveals that it indeed includes quite a few proteins that are known to be involved in the pathway of BA metabolism in addition to FXR (Figure 2c, Figure S3, Table S2). For example, the integrin β-1 (ITGB1) is a known TUDCA sensor on the plasma membrane.[50] The very long chain acyl-CoA synthetase

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(SLC27A2)[51] is a critical enzyme in the BA biosynthetic pathway, and the epoxide hydrolase 1 (EPHX1)[52] is a bifunctional enzyme whose transporter function is responsible for the sodium-dependent uptake of BAs into cells. Furthermore, glutathione *S*-transferase (MGST3)[53] and serum lipoprotein (APOB)[54] have been reported to bind BA in order to facilitate their detoxification and transportation. We extracted the SILAC ion chromatographic traces of these proteins, and all of them showed clear UV-dependent and BA-competitive labeling by the probe (Figure 2c). Collectively, these known examples of BA-interacting proteins in our profiling data indicate that the photoaffinity BA probes are capturing physiologically relevant protein−BA interactions in living cells.

Interestingly, we also identified a set of BA−protein interactions which are sensitive to excessive BA competition but show UV-independent probe labeling (group IV in Figure 2c). This suggests that covalent interactions between BA and proteins may exist in cells and will be the subject of further investigation.

Considering the structural similarity between BA and cholesterol, we also compared the BA-interacting proteins identified in our study with those cholesterol-interacting proteins identified in a previous study performed by the Cravatt group[35] (Figure S4, Table S3). Both data sets share a reasonable overlap with 146 common targets including a BA biosynthetic enzyme SLC27A2, which is consistent with the fact that cholesterol serves as the precursor for BA biosynthesis. In addition, our study also identified 535 specific BA-interacting targets, among which are well-known BA receptors (FXR, ITGB1), transporters (EPHX1, ABCB1, ABCD3), and binding proteins (PLD, APOE, ALB, etc.), suggesting the high selectivity of BA probe. Further in-depth analysis of these data sets may help delineate functional pathways that are commonly and specifically regulated by the two steroids in cells.

Bioinformatics Analysis of BA-Interacting Proteins. We performed the gene ontology (GO) analysis on these BA interacting proteins regarding their subcellular distributions (Figure 3a).[55,56] Major subcellular organelles enriched with BA target proteins are endoplasmic reticulum (23%), mitochondrion (21%), Golgi apparatus (11%), nuclear envelope (5%), cell surface (5%), and peroxisome (4%). The membrane-dominant distribution is consistent with the results from the gel-based profiling that shows stronger labeling signals in the membrane fraction of the proteomes. Furthermore, the diversified subcellular locations of BA-interacting proteins are echoed by the fact that the complete biosynthesis of BA requires 17 individual enzymes and several transporters residing in multiple intracellular compartments.[1]

We next queried the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to categorize these BA-interacting proteins based on the functional pathways involved (Figure 3b).[52,53] The clustering analysis showed that the most functionally enriched pathways include protein processing in ER, lipid metabolisms (including fatty acid, glycerophospholipid, and sphingolipid metabolisms), *N*-glycan biosynthesis and oxidative phosphorylation, etc., many of which have been reported to be regulated by BA in a receptor-dependent manner.[57−60] It is also satisfying to see that the bile secretion pathway is enriched as several known BA transporters are captured by our BA probes in the profiling.

The top functionally enriched pathway, "protein processing in ER", contains 44 BA-interacting proteins. Gene ontology and functional protein association network analysis revealed that 20 of them are involved in ER stress response caused by accumulation of unfolded or misfolded proteins in ER lumen, and 7 of them are with functions of chaperone binding (Figure S5). It has been reported that a secondary BA, TUDCA, can serve as a chemical chaperone to improve ER folding capacity and reduce ER stress, which resulted in alleviation of hyperglycemia and restoration of glucose homeostasis and insulin sensitivity in livers of diabetic mice.[61] However, no exact targets of TUDCA have been reported as participating in the ER stress response. Our profiling data provide a set of potential interacting proteins of TUDCA that could serve as the ER stress responders.

BAs have been known to play important roles in regulating lipid metabolism.[62] For example, carnitine palmitoyl transferase 1A (CPT1A), a rate-limiting enzyme of fatty acid metabolism, was reported to be transcriptionally upregulated by BA through the activation of FXR.[63] Our profiling data identified a direct interaction between CPT1A and BA, which suggests that the protein function could be also regulated in a post-translational way. Phospholipase D (PLD) is another BA-interacting protein that was identified by our ABPP-SILAC profiling, and the finding is consistent with a previous study showing that BAs can allosterically activate PLD through direct interaction to enhance its dimer assembly.[12] ADP-dependent glucokinase (ADPGK) converts glucose to glucose-6-phosphate and plays an essential role in the glycolysis and pentose phosphate pathway.[64] Our chemoproteomic profiling study identified ADPGK as a BA-interacting protein and may provide a novel link for BA in direct regulation of glucose metabolism.

Disease Analysis of BA-Interacting Proteins. We analyzed these BA-interacting proteins based on their annotations recorded in the KEGG disease database[65] and found that they are strongly implicated with neurodegenerative diseases, non-alcoholic fatty liver diseases, and diarrhea (Figure 3c).

Dysmetabolism of BA has been postulated as a factor for the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson disease (PD).[66−68] AD is characterized by accumulation of amyloid-β (Aβ) peptides in brain that leads to progressive cognitive deficit.[69] TUDCA has been found to be a strong neuroprotective agent for AD, and chronic TUDCA treatment of AD mice for 6 months could significantly reduce amyloid-β deposition and ameliorate memory deficit.[31] However, the therapeutic potential of TUDCA in AD pathology has not been fully explored due to the lack of knowledge of exact molecular targets involved. Our chemoproteomic data revealed direct interaction between BA and several proteins in this pathway, including Aβ precursor protein (APP), β-site APP-cleaving enzyme 2 (BACE2), and the lipid-metabolism mediator apolipoprotein E (APOE). It is possible that TUDCA may exert its neuroprotective function by interacting with these proteins to affect APP processing and amyloid-β deposition. In addition, catechol-*O*-methyltransferase (COMT) is another potential link because this BA-interacting protein plays a prominent role in controlling the metabolism of catecholamine neurotransmitters and the inhibitors of this enzyme can block amyloid-β fibrils.[70]

Disorders in BA metabolism can lead to non-alcoholic fatty liver diseases (NAFLD), which is strongly associated with obesity, diabetes mellitus, and dislipidemia.[71] Our chemo-

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proteomic profiling identified several direct interactions between BA and key proteins that are known to be involved with the development and pathogenesis of NAFLD, including sterol regulatory element binding transcription factor 1 (SREBF1),[72] transforming growth factor beta 1 (TGFB1),[73] and multiple subunits of NADH:ubiquinone oxidoreductase[74] and cytochrome *c* oxidase.[75] Further investigation on how BA binds to and affects functions of these proteins as well as those in lipid metabolism pathways may provide novel links between BA metabolism and NAFLD.

BAs have been known to interplay with microbiota in the intestine to establish a healthy gut environment for the host.[76,77] Conversely, pathogenic bacteria such as *Vibrio cholerae* have evolved sophisticated mechanisms to utilize BAs as nutrients for survival and infection,[78,79] causing severe diarrhea.[80] Two ER proteins, the ER oxidoreductase ERO1A and the protein transport channel SEC61, are critical host components hijacked by the bacteria to process cholera toxin (CTX) in ER and release it back to cytosol to exert toxicity. Our profiling data reveal that BAs interact with these two proteins and therefore provide a potential link between BA and the *Vibrio cholerae* infection process.

Validation of BA−Protein Interactions. To confirm these BA−protein interactions identified in our profiling data, we choose six proteins for further validation by Western blotting. Three of these proteins (NTCP, SLC25A6, PLD3) have been previously reported to physically interact with BA, and the other three (CPT1A, ADPGK, COMT) are novel BA binders discovered by our study which have potential functional implications in various diseases as mentioned above. Moreover, each of these six proteins has its distinct subcellular location as annotated according to the knowledge database (Figure 4).

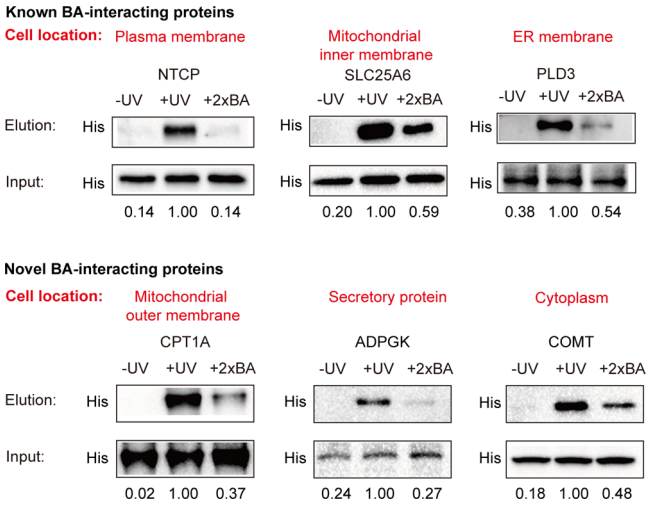

Figure 4. Experimental validation of BA−protein interactions by immunoblotting. Each protein is transiently overexpressed in HeLa cells with a His tag, and the cells are labeled with **P2**. The labeled proteins are enriched and then eluted for immunoblotting with an anti-His antibody ("Elution"). The samples without UV cross-linking or with 2× BA competition are used as control. Equal loading is validated by immunoblotting of input samples before enrichment ("Input"). Top panel: NTCP (a BA transporter), SLC25A6, and PLD3 are known BA−protein interactions that have been reported before. Bottom panel: CPT1A, ADPGK, and COMT are novel BA−protein interactions discovered in this study. The normalized intensities are shown below each band.

Each protein was recombinantly overexpressed with a 6× His epitope sequence in HeLa cells. The cells were treated *in situ* with the **P2** probe, followed by conjugation with an azide-biotin tag and enrichment of the probe-labeled proteins on streptavidin. Immunoblotting of the enriched proteins showed that all of them can be captured by the **P2** probe in a UV-dependent manner and these BA−protein interactions can be competed by excess native BA molecules by at least 1.69-fold.

CONCLUSION

Here we designed and efficiently prepared a number of structurally diverse, clickable, and photoreactive BA probes based on the cholic acid scaffold, and applied them in systematically mapping BA-interacting proteins directly in living cells. These BA probes showed good specificity for known BA receptors, transporters, and biosynthetic enzymes via noncovalent binding. Combined with SILAC-based quantitative proteomic technologies, we successfully identified a large number of novel BA-interacting proteins including chaperone binding proteins involved in ER stress response, key metabolic enzymes in diverse pathways of lipid metabolism, and functional targets strongly associated with metabolic syndrome, neurodegenerative diseases, and diarrhea. These novel BA−protein interactions will provide a rich resource for guiding new functional studies of BA signaling and expand the current understanding of BA-mediated pathways in human metabolism and disease.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.7b00134.

Additional figures and tables, methods, spectroscopic data, and ^{1}H and ^{13}C NMR spectra (PDF)

List of BA-interacting proteins identified by ABPP-SILAC (XLSX)

Comparison of BA-interacting protein targets (this study) and cholesterol-interacting protein targets (previous study[1]) (XLSX)

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Notes

The authors declare no competing financial interest.

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