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Chemical Proteomic Profiling of the Interacting Proteins of Isoprenoid Pyrophosphates

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Abstract

Isoprenoid pyrophosphates are involved in protein prenylation and assume regulatory roles in cells; however, little is known about the cellular proteins that can interact with isoprenoid pyrophosphates. Here, we devised a chemical proteomic strategy, capitalizing on the use of a desthiobiotin–geranyl pyrophosphate (GPP) acyl phosphate probe for the enrichment and subsequent identification of GPP-binding proteins using liquid chromatography-tandem mass spectrometry (LC-MS/MS). By combining stable isotope labeling by amino acids in cell culture (SILAC) and competitive labeling with low vs high concentrations of GPP probe, with ATP vs GPP acyl phosphate probes, or with the GPP probe in the presence of different concentrations of free GPP, we uncovered a number of candidate GPP-binding proteins. We also discovered, for the first time, histone deacetylase 1 (HDAC1) as a GPP-binding protein. Furthermore, we found that the enzymatic activity of HDAC1 could be modulated by isoprenoid pyrophosphates. Together, we developed a novel chemical proteomic method for the proteome-wide discovery of GPP-binding proteins, which sets the stage for a better understanding about the biological functions of isoprenoids.

Graphical Abstract

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Supporting Information

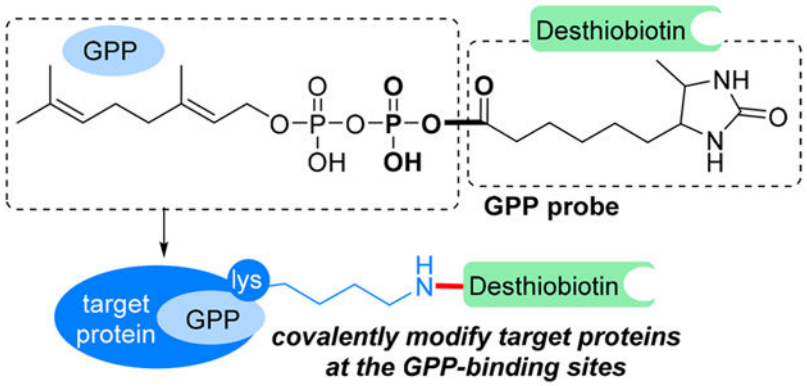
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01676.

Detailed experimental procedures, preparation of the GPP probe, full-scan MS/MS and ESI-MS, fitting curve examples of LC-MS, Western blot, and bioinformatic analysis (PDF)

Table S1: Quantitative profiling results with the GPP probe (XLSX)

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c01676

The authors declare no competing financial interest.



Isoprenoid pyrophosphates, such as geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP, Figure 1A), are intermediates involved in the biosynthesis of terpenes, terpenoids, and steroids and play important roles in cell signaling.[1,2] In the latter respect, isoprenoid pyrophosphates have been well studied for their functions in protein prenylation, which is an important type of post-translational modification for the regulation of target proteins, including GTPases and protein phosphatases.[3,4] Additionally, isoprenoids were found to regulate transcriptional and post-transcriptional events, e.g., the expression of ATP-binding cassette transporter A1 (ABCA1)[5] and Ras-related proteins.[6] Moreover, Dumelin et al.[7] discovered geranylated tRNA in bacteria, which could lead to codon bias and frameshift during translation. Recently, Lu et al.[8] identified *S*-geranylgeranyl-L-glutathione as a potent ligand for human B cell-confinement receptor P2RY8 in lymphoid tissues. Despite these established functions, isoprenoids may assume previously unrecognized roles in cellular processes. We reason that a systematic discovery of isoprenoid-binding proteins at the proteome-wide level will provide a more complete understanding about the biological functions of isoprenoids.

Activity-based protein profiling (ABPP) has been widely exploited for the identification and quantification of specific groups of proteins from complex proteomes.[9] Various molecular probes have been developed for the study of isoprenoidmodified proteins associated with biosynthesis[10] and prenylation[11] pathways. These isoprenoid probes contain a fluorescent group or a handle for enrichment, such as alkynyl,[12] azido,[13] and biotin[14] groups, at the terminus of the aliphatic chain of isoprenoids (Figure 1A). By employing them as isoprenyl group donors, along with inhibition of endogenous isoprenoid synthesis, the methods facilitated efficient labeling of the prenylated proteome. Although these probes are useful for studying protein prenylation, they do not provide information about cellular proteins that interact with isoprenoids. In addition, a chemical probe harboring a biotin moiety and a photoreactive benzophenone moiety linked to the terminus of a geranyl group was synthesized and employed to identify the interactome of isoprenoid pyrophosphate in yeast.[15]

Most proteins that bind to phosphate-containing small molecules harbor lysine or arginine residue(s) in their binding pockets, where electrostatic and hydrogen bonding interactions between phosphate and a lysine or arginine side chain promote ligand binding.[16] On the

basis of the nucleophilicity of lysine, nucleotide acyl phosphate probes have been successfully developed to label lysine residues and employed for the affinity-based profiling of ATP-[17,18] and GTP-binding proteins.[19,20]

In this study, we developed a desthiobiotin–GPP acyl phosphate probe for high-throughput interrogation of the interactome of isoprenoid pyrophosphate (Figure 1). In particular, the interaction between the GPP component of the probe with GPP-binding proteins promotes the nucleophilic reaction between the side chain of lysine residue(s) located at or near the GPP-binding site and the acyl phosphate, thereby installing a desthiobiotin on the side chain of the lysine(s) (Figure 1B). In conjunction with stable isotope labeling by amino acids in cell culture (SILAC) and competitive labeling with low vs high concentrations of GPP, with ATP vs GPP acyl phosphate probes, or with the GPP probe in the presence of increasing concentrations of free GPP, we uncovered a large number of candidate isoprenoid-binding proteins, including histone deacetylase 1 (HDAC1). We also examined how isoprenoid diphosphates modulate the enzymatic activity of HDAC1.

We first synthesized the desthiobiotin-tagged GPP probe with an acyl-phosphate linker and employed the probe for the labeling and enrichment of GPP-binding proteins (Figure S1). More than 800 desthiobiotin-labeled proteins were identified with single liquid chromatography-tandem mass spectrometry (LC-MS/MS) run on an LTQ-Orbitrap Velos mass spectrometer. Since the GPP probe is very reactive toward nucleophiles, many proteins could be labeled due to nonspecific interactions. To identify proteins with high binding affinity and selectivity to the GPP probe, we conducted two types of competition experiments using a SILAC-based quantitative proteomic workflow (Figure 2).

In the first competition experiment, we treated cell lysates separately with high (100 μM) and low (10 μM) concentrations of the GPP probe (Figure 2A), following previously described approaches and experimental conditions for the proteome-wide discovery of reactive cysteine[21] and nucleotide-binding proteins.[22] In this regard, nonspecific GPP–protein interactions will give rise to much higher labeling efficiency at higher than at lower probe concentrations, whereas those proteins interacting specifically with GPP will display a similar labeling efficiency at the two different probe concentrations. The quantification results obtained from forward and reverse SILAC labeling experiments are consistent (Table S1). Additionally, this competition experiment led to the identification of 348 peptides derived from 231 proteins with the ratios in labeling efficiency for high/low concentrations of the GPP probe being less than 2 (Figure 2B). These include desthiobiotin-modified peptides from two known GPP-binding proteins, farnesyl diphosphate synthase (FDPS) and geranylgeranyl diphosphate synthase 1 (GGPS1) (Figures S2 and S3), thereby validating the capability of the method in uncovering GPP-binding proteins.

Over 210 out of the 890 probe-labeled proteins are ATP-binding proteins. To ascertain whether these proteins are also GPP-binding proteins, we performed another competition experiment to evaluate the relative labeling efficiencies of the GPP probe and a previously reported ATP probe,[18] which share the same acyl phosphate linkage and desthiobiotin as the GPP probe (Figure 2C). As expected, many ATP-binding proteins, including kinases, heat shock proteins, elongation factors, etc., exhibit much higher labeling efficiencies with the

ATP than the GPP probe. Most of these ATP-binding proteins are highly abundant in cells; thus, they may be labeled with the GPP probe through nonspecific interactions. Importantly, both FDPS and GGPS1 were labeled at higher efficiencies with the GPP probe than the ATP probe (Figure 2D). This result also underscores the good selectivities in the labeling of GPP- and ATP-binding proteins with the corresponding acyl phosphate probes.

Combining these two sets of quantitation data led to the identification of more than 100 proteins displaying ratios of <2 in the high vs low GPP probe concentration experiment and ratios of >2 in the GPP vs ATP probe experiment. (The quantification results for all the peptides and proteins are shown in Table S1.) Among these proteins, HDAC1 is a well-studied epigenetic modulator that regulates gene expression through deacetylation of lysine residues in core histone proteins. MS/MS results for the desthiobiotin-conjugated tryptic peptide of HDAC1 are shown in Figure S4.[23,24]

To further elucidate the labeling selectivity of the GPP probe and to narrow down the list of potential GPP-binding proteins, the probe labeling efficiency was tested using SILAC-labeled protein lysates without GPP pretreatment or with pretreatment with increasing concentrations of free GPP (Figure 3A). Because of the competitive binding of GPP with target proteins, the labeling efficiencies of GPP-binding proteins by the GPP probe would decrease with increasing concentrations of free GPP. For instance, when treating the heavy cell lysate with GPP prior to probe labeling, the detected signal for the heavy-labeled peptide of FDPS is much lower than the cell lysates without GPP pretreatment (Figure 3B).

We incubated the heavy cell lysate individually with five different concentrations of GPP (i.e., 50, 100, 200, 500, and 1000 μM) prior to labeling with the GPP probe. MaxQuant analysis of the six sets of LC-MS/MS data together led to the identification and quantification of more than 2000 desthiobiotin-labeled peptides from 1814 proteins. Among them, 284 proteins were quantified in all 6 experiments with PEP scores being less than 1×10^{-4} (Table S1; representative ESI-MS data are shown in Figures S5–S7). To efficiently identify the proteins with a GPP-concentration-dependent decrease in labeling efficiency, we employed a simple IC_{50} model (i.e., the concentration of GPP that gives rise to a 50% attenuation in labeling with the GPP probe) to estimate the inhibitory effect of free GPP on probe labeling by plotting the ratios of heavy/light-labeled peptide against the $\log_{10}$[GPP].[25] After calculating IC_{50} using linear regression (representative fitting curves are shown in Figure S8), we selected proteins with $R^2 > 0.4$ and ranked them according to increasing IC_{50} values (Figure 3C and Table S1). Both FDPS and GGPS1 are among the top on the list of proteins with small estimated IC_{50} values, suggesting that this quantitative proteomic method could be employed for identifying new GPP-binding proteins.

An advantage of the probe labeling method resides in its ability to identify the lysine residues that are involved in binding with the phosphate moiety of isoprenoids (Figures S2–S4). Among the identified labeling sites, the probe-labeled lysine for the tryptic peptide derived from FDPS is located in the isopentyl pyrophosphate (IPP)-binding site, and it interacts directly with the phosphate group of IPP based on a previously reported crystal structure (Figure 4A).[26] The labeling efficiency of the GPP probe decreased with increasing

concentration of free GPP in the heavy cell lysates (Figures 4C and S5–S7), suggesting the capability of the method in evaluating the affinity between GPP and target proteins.

The probe-labeled lysine identified for GGPS1 is near the binding site of geranylgeranyl pyrophosphate; the lysine side chain, however, points toward the protein surface rather than the binding pocket, as shown in a previously published crystal structure (Figure 4B).[27] This may account for the high ratio observed for this lysine-labeled peptide in the probe concentration competition experiment (Figure 2A). The signal for the detected heavy-labeled peptide decreased at a higher GPP concentration when compared with FDPS, again indicating a weaker binding with GPP (Figure 4D).

Several previously published studies suggested potential functional roles of isoprenoid pyrophosphates in the nuclei. For instance, prior studies revealed the nuclear localization of FDPS[28] and the presence of farnesylated proteins in the nuclei.[29] In addition, the elevated expression of FDPS could contribute to increased levels of misshapen nuclei, which confers augmented senescence in human fibroblasts.[30] Moreover, the enzymatic activity of the HDAC complexes was previously shown to be allosterically modulated through binding with inositol phosphates.[31] Although HDAC1 was not included in the selected proteins in Figure 3C due to some interference peaks in the mass spectrum, the probe labeling efficiency of HDAC1 decreases with increasing GPP concentrations (Figure S7). On the basis of the reported crystal structure of HDAC1, the identified GPP probe-labeled lysine in the tryptic peptide derived from HDAC1 is situated in the allosteric pocket where inositol phosphate binds (Figure 5A).[32] Hence, we next asked whether isoprenoids could modulate the enzymatic activity of HDAC1. Toward this end, we employed calf thymus core histones as substrates to examine how the in vitro enzymatic activity of HDAC1 is influenced by the presence of GPP. Our results showed that preincubation of recombinant HDAC1 with GPP led to elevated efficiency in the deacetylation of H4K16Ac, demonstrating that GPP could stimulate the enzymatic activity of HDAC1 (Figure 5B–C). Similar findings were made for GGPP in the low concentration range (<100 μM, Figure 5D). Pretreatment of HDAC1 with higher concentrations of GGPP, nonetheless, resulted in diminished HDAC1 enzymatic activity. The exact mechanism underlying the suppression of HDAC1 activity at high concentrations of GGPP is unclear and warrants further investigation. Along this line, it is of note that the acetylation level is also tested with Ac-histone H4 antibody and Ac-Lys antibody, which yielded similar results as those obtained for H4K16Ac (Figure S9).

In summary, we developed a chemical proteomic strategy for interrogating systematically the interactome of isoprenoid pyrophosphates in cultured human cells. Our results led to the identification of previously known GPP-binding proteins, including FDPS and GGPS1, where the labeled lysines are located in the isoprenoid binding sites. Compared to the previously reported photoreactive probe,[15] our probe does not carry any modifications on the isoprenoid component; thus, our probe mimics better the native interactions between isoprenoid pyrophosphate and their binding proteins. It is worth noting that, because of the nature of the probe design, our method does not allow for labeling and, hence, detection of those isoprenoid pyrophosphate-binding proteins that do not carry a lysine (i.e., with an arginine instead) at the binding pocket.

Importantly, our method resulted in the identification of many proteins whose abilities in GPP binding were not previously documented (Table S1). The results from gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of all the probe-labeled proteins are shown in Figure S10. Among them, histone deacetylase HDAC1 is an important enzyme in epigenetic regulation through controlling acetylation levels of lysine residues in histone tails. Our in vitro histone deacetylase assay with purified HDAC1 showed that GPP and GGPP could stimulate the enzymatic activity of HDAC1. These results suggest that isoprenoids may modulate histone epigenetic signaling by regulating HDAC1 activity. It will be important to characterize biochemically the interaction between HDAC1 and GPP/GGPP (e.g., determining the binding constants) in the future.

It will be important to validate the binding capabilities and explore the biological functions of other identified candidate isoprenoid-binding proteins. Along this line, our quantitative proteomic experiment also led to the identification of Ataxia telangiectasia and Rad3 related (ATR) as a GPP-binding protein (Figure 3C), and it will be interesting to assess how GPP binding modulates the functions of ATR in DNA damage response signaling in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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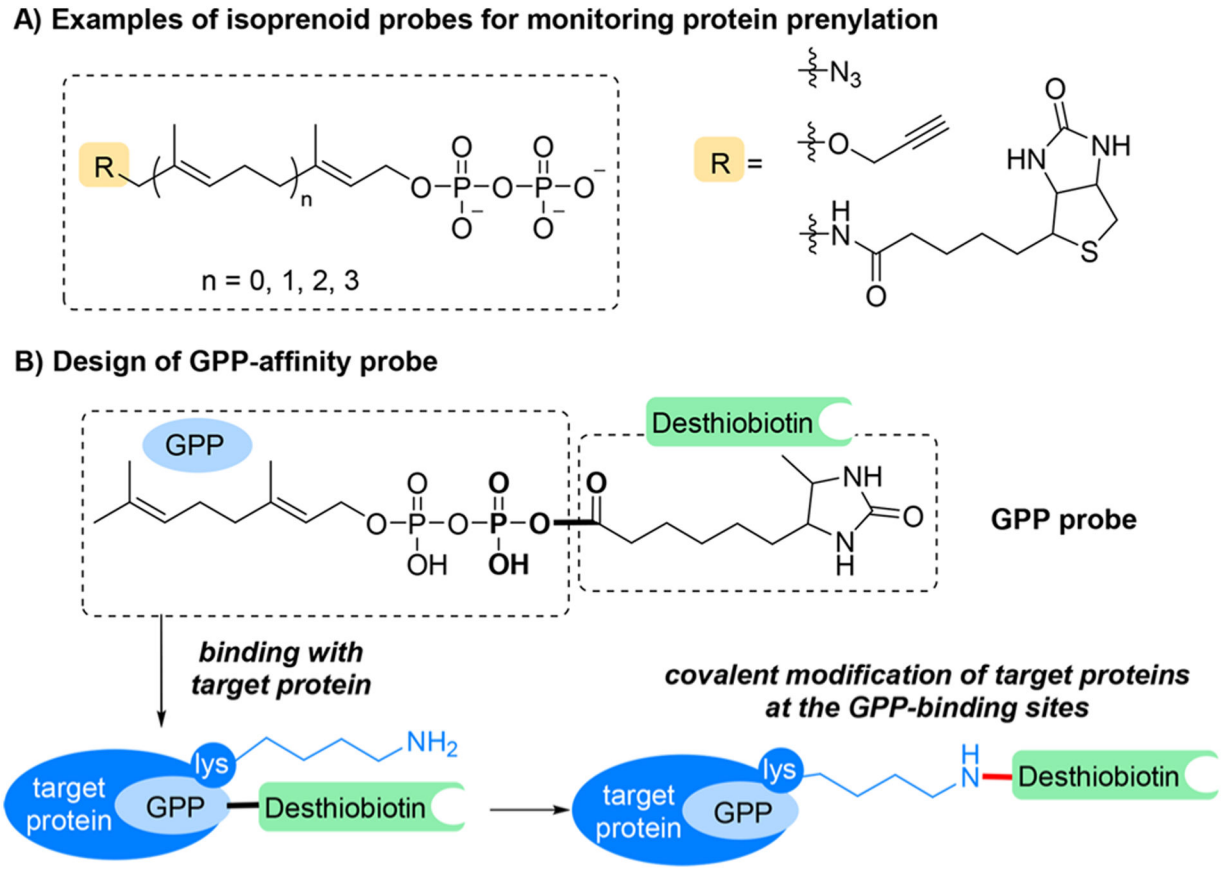

Figure 1.

Isoprenoid probes for studying protein prenylation (A) and design of the affinity-based probe for the covalent labeling and enrichment of GPP-binding proteins and their component peptides (B).

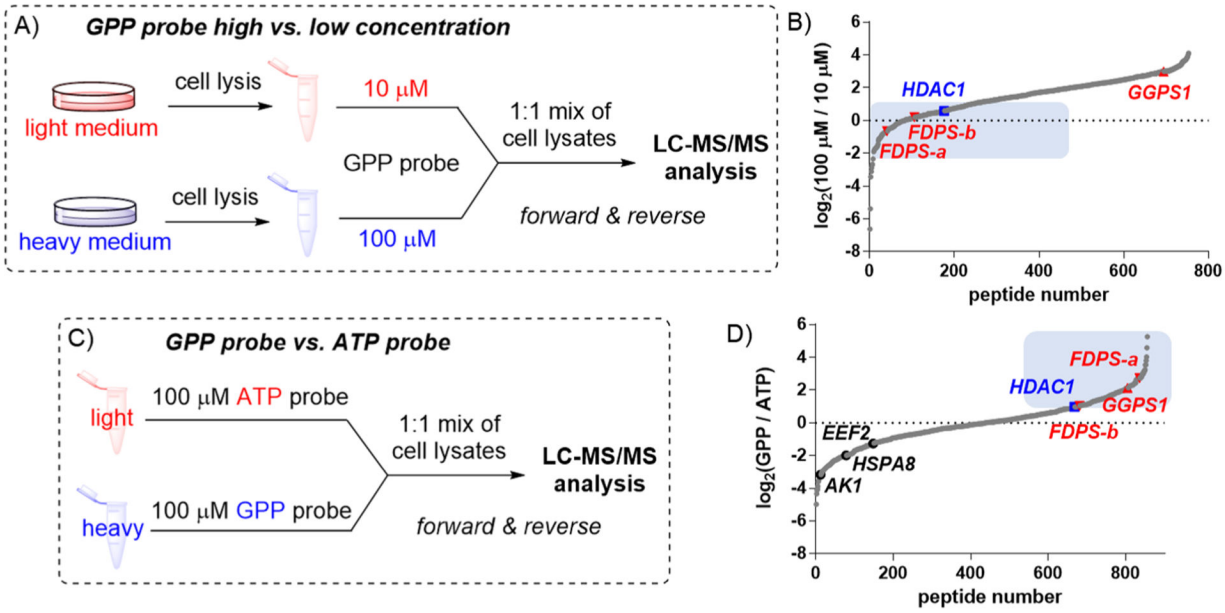

Figure 2.

SILAC-based competition assay for assessing GPP-binding proteins at the entire proteome scale. (A) The workflow for the competition assay. (B) Quantitative comparisons of the labeling efficiencies with the use of 100 vs 10 μM GPP probe. The data were obtained from 2 forward and 2 reverse labeling experiments; FDPS-a and FDPS-b represent two desthiobiotin-labeled peptides identified from FDPS. (C) Workflow for the competition between GPP and ATP acyl phosphate probes. (D) Quantification results obtained from the GPP/ATP competition assay with 2 forward and 2 reverse SILAC labeling experiments.

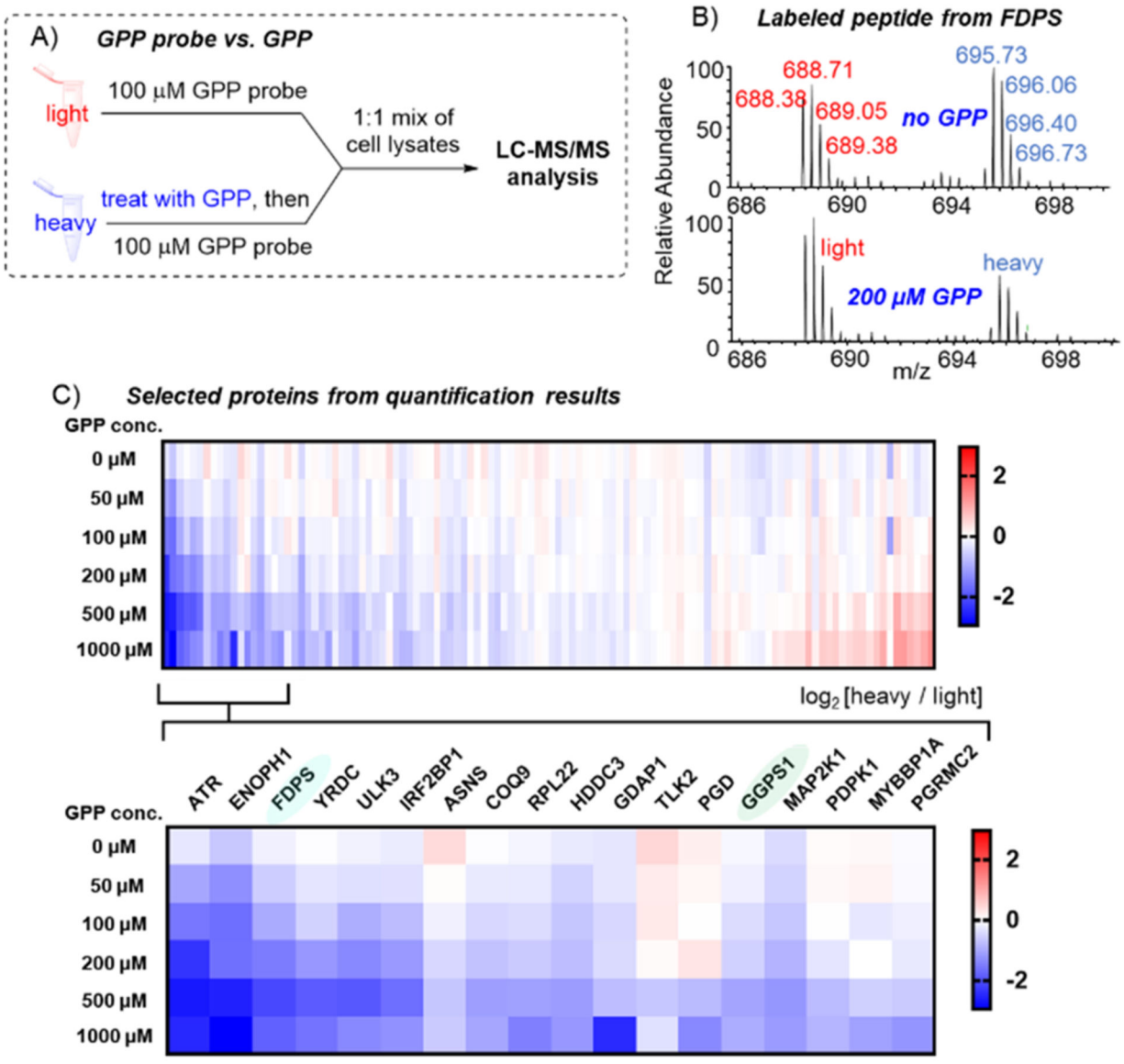

Figure 3.

Quantitative analysis for GPP inhibition on probe labeling efficiency. (A) Workflow for the competition experiment. (B) Full-scan ESI-MS of the light- and heavy-labeled tryptic peptide LKEVLEYNAIGGKYNR from FDPS in the presence or absence of free GPP. (C) A heat map showing the quantification results with increasing concentrations of GPP, where the proteins are ranked on the basis of the calculated IC_{50} values of the labeling efficiencies of the GPP probe.

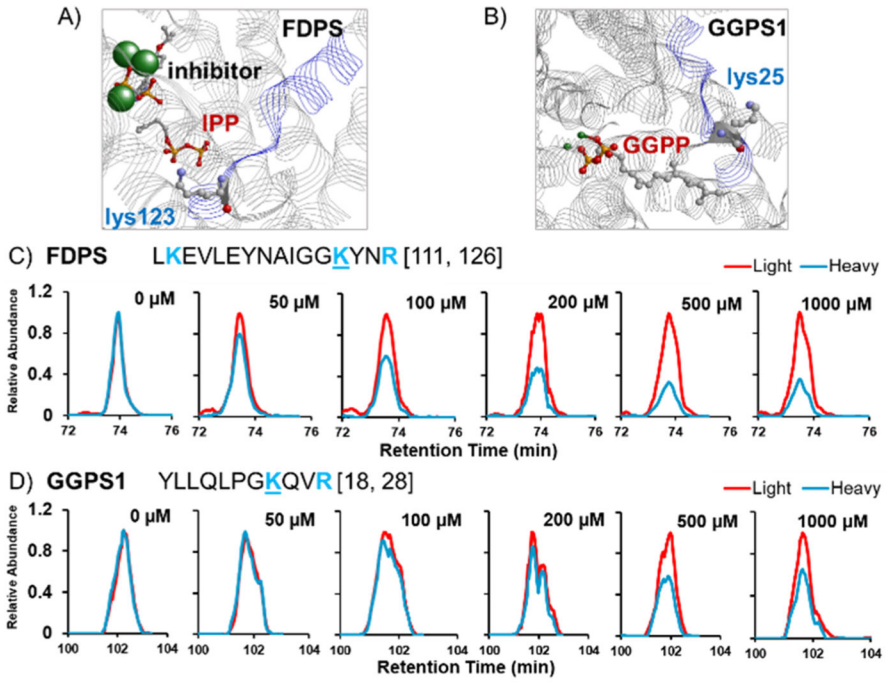

Figure 4.

Labeling sites for known GPP-binding proteins. (A) Diagrams showing the interaction between the Lys123 and the bound IPP in FDPS (PDB 4H5E); the probe-labeled Lys123 corresponds to Lys57 in the reported crystal structure, and the blue-colored strand represents the labeled peptide identified from LC-MS/MS. (B) A crystal structure of GGPS1 (PDB 2Q80) showing the GGPP-binding pocket and the location of the probe-labeled Lys25. (C, D) Comparison of the detected intensity for the light and heavy peptides for FDPS, GGPS1, and HDAC1 with increasing concentrations of GPP (from left to right: 0, 50, 100, 200, 500, and 1000 μM).

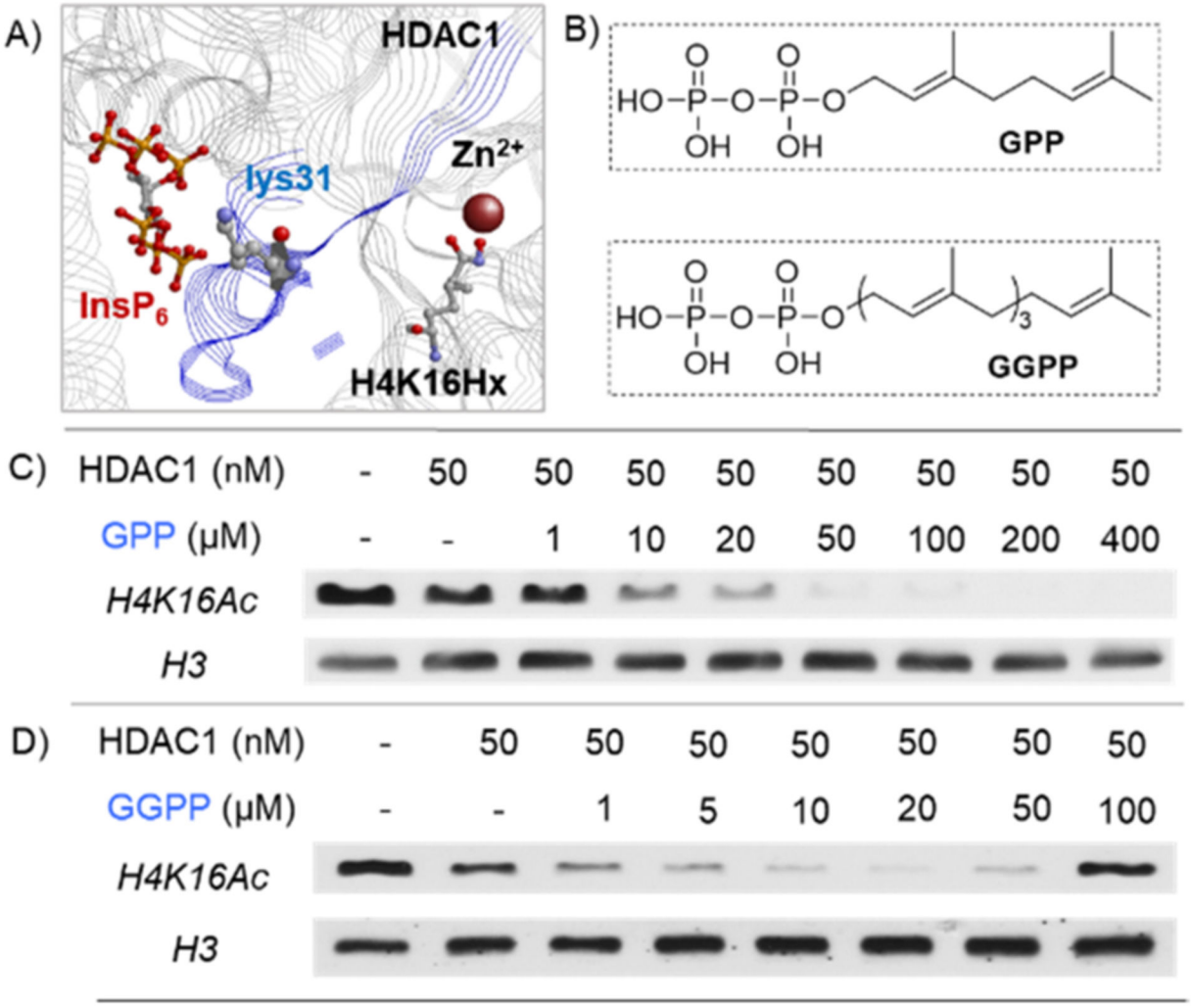

Figure 5.

GPP and GGPP could modulate the enzymatic activity of HDAC1 in vitro. (A) A crystal structure of HDAC1 (PDB 5ICN) showing the interaction of Lys31 with the bound inositol hexaphosphate in the allosteric pocket; H4K16Hx is an inhibitor for HDAC1 and occupies the active site through coordination with Zn^{2+}. (B) The chemical structures of GPP and GGPP. (C, D) Western blot results for monitoring the enzymatic activity of HDAC1 in the presence of different concentrations of GPP or GGPP, where calf thymus core histones were used as the substrate.