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ARTICLE

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Broadening the Utility of Farnesyltransferase-Catalyzed Protein Labeling Using Norbornene–Tetrazine Click Chemistry

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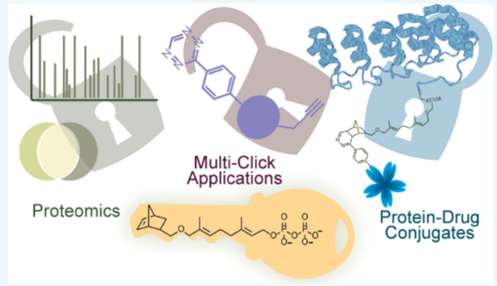
ABSTRACT: Bioorthogonal chemistry has gained widespread use in the study of many biological systems of interest, including protein prenylation. Prenylation is a post-translational modification, in which one or two 15- or 20-carbon isoprenoid chains are transferred onto cysteine residues near the C-terminus of a target protein. The three main enzymes—protein farnesyltransferase (FTase), geranylgeranyl transferase I (GGTase I), and geranylgeranyl transferase II (GGTase II)—that catalyze this process have been shown to tolerate numerous structural modifications in the isoprenoid substrate. This feature has previously been exploited to transfer an array of farnesyl diphosphate analogues with a range of functionalities, including an alkyne-containing analogue for copper-catalyzed bioconjugation reactions. Reported here is the synthesis of an analogue of the isoprenoid substrate embedded with norbornene functionality (C10NorOPP) that can be used for an array of applications, ranging from metabolic labeling to selective protein modification. The probe was synthesized in seven steps with an overall yield of 7% and underwent an inverse electron demand Diels–Alder (IEDDA) reaction with tetrazine-containing tags, allowing for copper-free labeling of proteins. The use of C10NorOPP for the study of prenylation was explored in the metabolic labeling of prenylated proteins in HeLa, COS-7, and astrocyte cells. Furthermore, in HeLa cells, these modified prenylated proteins were identified and quantified using label-free quantification (LFQ) proteomics with 25 enriched prenylated proteins. Additionally, the unique chemistry of C10NorOPP was utilized for the construction of a multiprotein–polymer conjugate for the targeted labeling of cancer cells. That construct was prepared using a combination of norbornene–tetrazine conjugation and azide–alkyne cycloaddition, highlighting the utility of the additional degree of orthogonality for the facile assembly of new protein conjugates with novel structures and functions.



INTRODUCTION

Chemical probes infixed with bioorthogonal functionality have become particularly important tools for the study of biological processes. Such probes are used for substrate identification, site-selective modification, or to give insights into enzyme function. Bioorthogonal probes have been highly useful in the study of protein prenylation, a post-translational modification involved in cellular signaling and regulation. Dysregulation of prenylation has been implicated in several diseases, including cancer, Alzheimer's disease (AD), malaria, and progeria.[1−6] During prenylation, either farnesyl or geranylgeranyl groups (Figure 1A) from the corresponding diphosphates (farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP), respectively) are transferred onto a cysteine present in a distinctive C-terminal amino acid motif. That motif is typically CaaX, CCXX, CXC, or CC, where C is the site of modification (a cysteine residue), a is generally an aliphatic amino acid, and X is a variable amino acid.[3,7] The specific sequence determines whether the protein is a substrate for farnesyltransferase (FTase), geranylgeranyl transferase I (GGTase I), geranylgeranyl transferase II (GGTase II), or geranylgeranyl transferase III (GGTase III) (Figure 1B).[8,9] The enzymes that catalyze prenylation have demonstrated structural flexibility regarding their isoprenoid substrate.[10−13] This allows for the transfer of FPP analogues with bioorthogonal functionalities to putative prenylated proteins for site-specific modification, visualization, or drug delivery.

Previous work on prenyltransferases has focused on the transfer of alkyne-functionalized isoprenoids, such as C15AlkOPP (Figure 1A), to prenylated proteins for labeling with azide-containing reagents through copper-catalyzed azide–alkyne

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