Bioconjugate Chemistry

Communication

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Single-Stranded DNA as a Cleavable Linker for Bioorthogonal Click Chemistry-Based Proteomics

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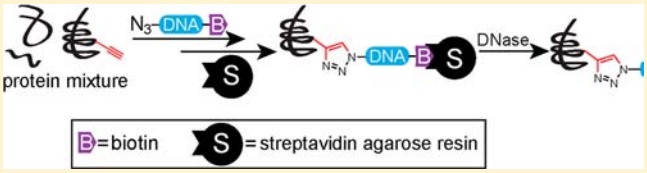
Supporting Information

ABSTRACT: In this communication, we report a new class of cleavable linker based on automatically synthesized, single-stranded DNAs. We incorporated a DNA oligo into an azide-functionalized biotin (biotin-DNA-N_3) and used the probe to enrich for alkyne-tagged glycoproteins from mammalian cell lysates. Highly efficient and selective release of the captured proteins from streptavidin agarose resins was achieved using DNase treatment under very mild conditions. A total of 36 sialylated glycoproteins were identified from the lysates of HL60 cells, an acute human promyeloid leukemia cell line. These sialylated glycoproteins were involved in many different biological processes ranging from glycan biosynthesis to cell adhesion events.



Since the discovery of the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC)[1,2] and the strain-promoted copper-free click chemistry,[3,4] bioorthogonal click chemical reactions have become indispensable tools for the identification of post-translationally modified proteins and for activity-based protein profiling (ABPP).[5] To identify proteins that are post-translationally modified, azide or alkyne tags are introduced into the target protein pools either metabolically[6−8] or via enzymatic approaches.[9,10] In the second step, affinity probes, e.g., biotin, functionalized in a complementary fashion, are ligated, allowing for affinity capture, e.g., by immobilized streptavidin, and subsequent mass spectrometry analysis. This method has been successfully applied to the identification of proteins that are glycosylated,[11] lipidated,[12] and methylated[13] among many other modifications. In the scenario of ABPP, an activity-based probe functionalized with the azide or the alkyne is used to label target proteins covalently either in living animals or in crude cell lysates.[14] The labeled proteins are then enriched and identified in an analogous manner to the former case.[15,16]

Although a number of elegant strategies have been developed to introduce azide or alkyne tags into target proteins for bioorthogonal ligation with biotin probes and affinity capture with immobilized streptavidin, relatively less effort has been devoted to the development of methods for the selective elution of the captured proteins from the resins. Due to the tight binding affinity of biotin to streptavidin ($K_d \sim 10^{-15}$ M), the release of biotinylated species from streptavidin agarose resins requires harsh, denaturing conditions, i.e., boiling the resins at 100 °C in sodium dodecyl sulfate (SDS) buffer. Under these conditions, contaminated proteins binding to immobilized streptavidin through nonspecific, hydrophobic interactions and proteins that are endogenous biotinylated are coeluted, which complicates the downstream mass spectrometry analysis. To address this issue, new probes have been designed that incorporate a cleavable linker into the azide- or the alkyne-functionalized biotin in the format of an "azide/alkyne-cleavable linker−biotin". Applying these probes to the click chemistry-based proteomics, target proteins can be selectively released from the immobilized streptavidin by cleaving the linker. A few cleavable linkers that have been developed exploit their sensitivities toward protease-,[17] pH-,[18] redox,[18,19] and photo-mediated cleavage conditions,[18,20] respectively. However, most of these strategies are complicated either by stringent cleavage conditions or multistep chemical synthesis. For example, the reduction of azobenzene-based linkers with sodium dithionite is both pH- and air-sensitive, affording low cleavage efficiency under typical reaction conditions.[18] Also, the construction of ortho-nitrobenzene-based photosensitive probes requires nine linear step synthesis, and the nitrosoaldehyde byproduct formed upon photolysis is reactive toward polypeptides.[21] Such complications set hurdles for the adaptation of these methods for routine proteomic analysis in biology-oriented laboratories.

Here, we report a new type of cleavable linker based on automatically synthesized, single-stranded DNAs and its application to bioorthogonal chemical proteomics (Scheme 1). We incorporated a DNA oligo into an azide-functionalized biotin (biotin-DNA-N_3) and used the probe to enrich for alkyne-tagged glycoproteins from mammalian cell lysates. Highly efficient and selective release of the captured proteins from streptavidin agarose resins was achieved following DNase treatment at 37 °C.

To integrate a single-stranded DNA into biotin-N_3 to form a cleavable probe, we used biotin-modified controlled pore glasses (CPG) as the solid support to introduce nucleotide

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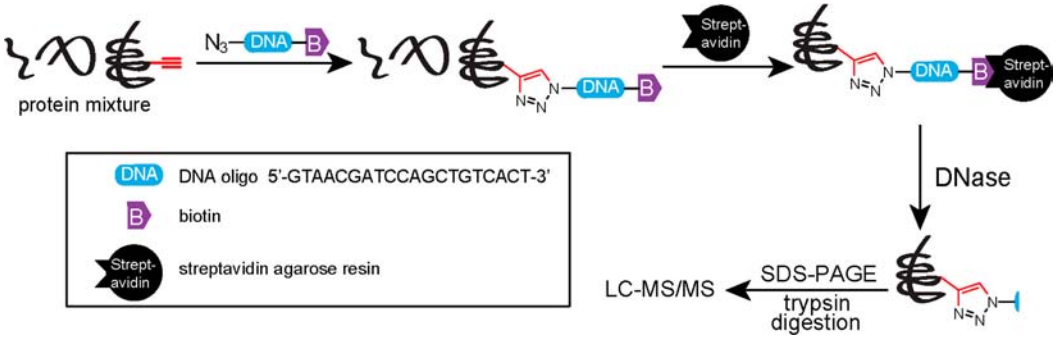
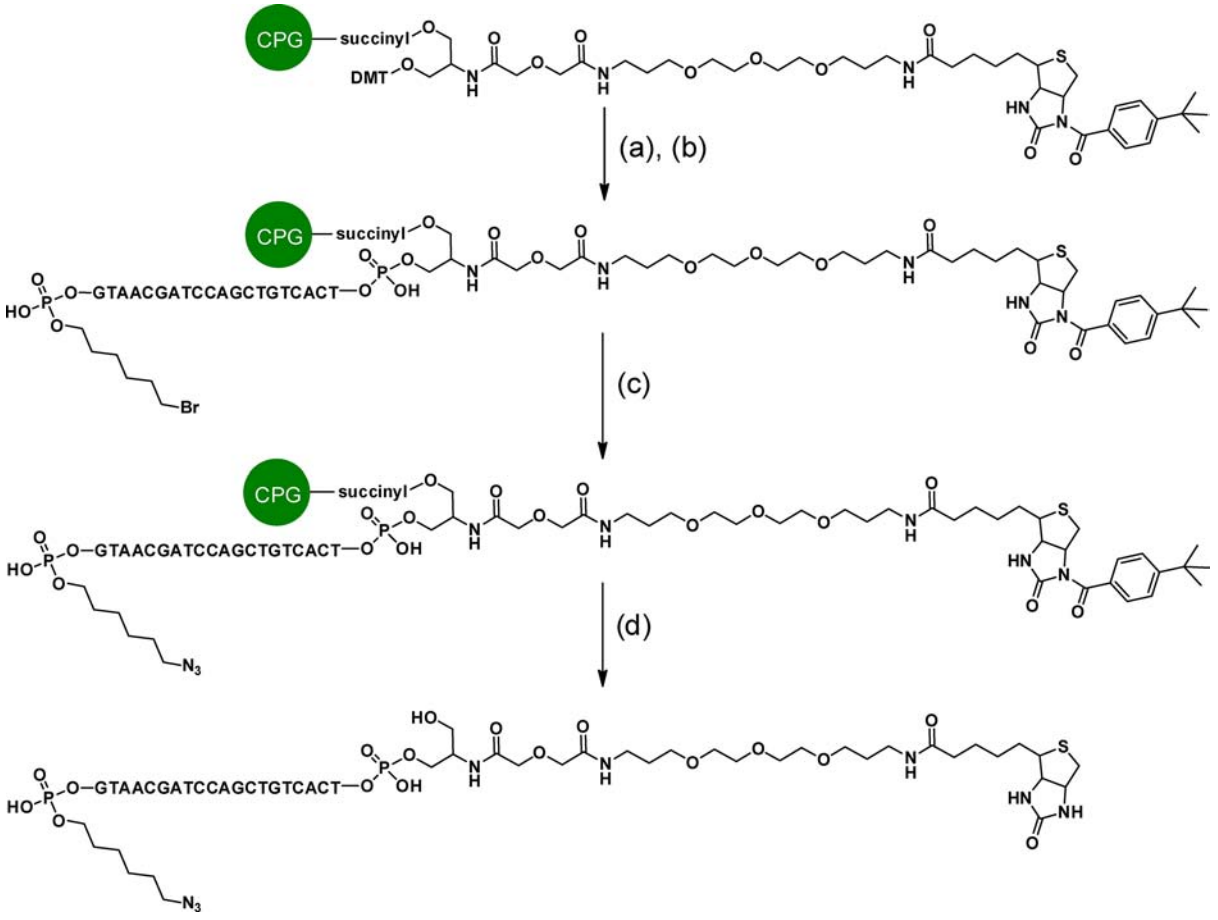
Scheme 1. DNA Oligo-Based Cleavable Linker for the CuAAC-Based Bioorthogonal Proteomics



Scheme 2. Synthesis of Biotin-DNA-N_3 on the Solid Phase CPG[a]



[a]Reagents: (a) solid phase synthesis using Ac-dC-CE (β-cyanoethyl) Phosphoramidite, dA-CE Phosphoramidite, dT-CE Phosphoramidite, dG-CE Phosphoramidite; (b) solid phase synthesis using 5′-Bromohexyl Phosphoramidite; (c) NaN_3/NaI; (d) CH_3NH_2/NH_4OH.

building blocks sequentially via a DNA oligonucleotide synthesizer (Scheme 2). The DNA oligo was designed to bear no predictable secondary structure and to have a length of 20 nucleotides, ensuring efficient cleavage by DNase when the biotin probe was captured on streptavidin agarose resins. The oligonucleotide was then capped by reacting with 5′-bromohexyl phosphoramidite. Substitution of the bromide with an azide was realized by reacting with NaN_3. The resulting biotin-DNA-N_3 was then eluted from the CPG solid support and purified with a NAP-10 column (Figure S1 in the Supporting Information).

With the cleavable biotin-DNA-N_3 probe in hand, we first tested its compatibility with CuAAC coupling conditions. It is well-known that reactive oxygen and nitrogen species generated from the canonical CuAAC system, i.e., $CuSO_4{\cdot}5H_2O$ + tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) + sodium ascorbate, could induce the breakdown of polynucleotides.[22,23] Therefore, we adopted the new CuAAC catalyst system, i.e., $CuSO_4{\cdot}5H_2O$ + 3-(4-((bis((1-*tert*-butyl)-1*H*-1,2,3-triazol-4-yl)-methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)propan-1ol (BTTP) ([BTTP]:[Cu] = 4:1) + sodium ascorbate, recently developed in our laboratory.[24] The new catalyst system showed excellent activity in protein labeling studies, especially in conjugate reactions with negatively charged species (e.g., FLAG peptide−DYKDDDDK). Based on this precedent, we reacted the biotin-DNA-N_3 probe with an alkyne-modified Fluor 488

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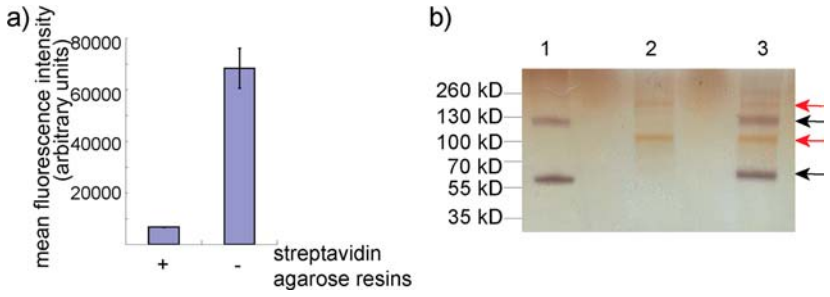

Figure 1. (a) Biotin-DNA-N_3 was reacted with 488-alkyne in the presence of BTTP/Cu(I), followed by incubation with or without (control) streptavidin agarose resins. After resins were removed, supernatant was transferred into a 96-well microtiter plate to quantify the residual fluorescence using a fluorescence plate reader (excitation 495 nm/emission 520 nm). (b) Silver-stained gel image showing specific release of the captured BSA from streptavidin agarose resins. Jurkat cell lysates containing the alkyne-modified BSA were reacted with biotin-DNA-N_3 or biotin-N_3 in the presence of the BTTP/Cu(I) catalyst. Following incubation with streptavidin agarose resins, BSA was released from resins by benzonase (lane 1) or by boiling resins in SDS buffer (lane 3). After the bezonase treatment, resins were boiled in SDS buffer to release residual proteins for SDS-PAGE analysis (lane 2). The black arrows indicate the dimeric and monomeric BSA (BSA dimerization is irreversible between pH 4.2 and 7.0 even after treatment with DTT).[26] The red arrows indicate contaminated proteins binding to immobilized streptavidin through nonspecific, hydrophobic interactions or proteins that are endogenously biotinylated.

dye (488-alkyne) in an 8:1 ratio in the presence of the BTTP-Cu(I) catalyst, mimicking the condition of labeling biomolecules with limited quantities. After one hour, the reaction mixtures were divided into three portions. Portion 1 was subjected to HPLC analysis, which showed near-quantitative dye-conjugation without breakdown of the DNA strand (Figure S2 in the Supporting Information). Subsequently, portion 2 was incubated with streptavidin agarose resins. Following centrifugation to precipitate the resins, the supernatant was transferred to a 96-well microtiter plate to measure the residual fluorescence of the uncaptured dye. As shown in Figure 1a, the reaction mixture that was treated with streptavidin agarose resins only showed ~10% fluorescence intensity as compared to that of the control (portion 3), suggesting that 90% of 488-alkyne was successfully reacted with biotin-DNA-N_3 and captured by streptavidin agarose resins. Therefore, the CuAAC condition has no apparent adverse effects on the DNA linker.

We then analyzed the site-specific cleavage of the DNA linker by DNase. We captured biotin-DNA-488, the reaction product of the biotin-DNA-N_3 with 488-alkyne, on streptavidin agarose resins, and performed on-bead digestion with benzonase, a commercially available recombinant endonuclease. The cleaved product of biotin-DNA-488 was subjected to LC/MS analysis. Two major peaks (a1 and a2) were observed in the LC trace showing absorbance at 488 nm corresponding to the two regioisomers of the 488 dye (Figure 2a). Their masses were consistent with the mass of 488−5′GT-3′, suggesting that the majority of the cleaved product of biotin-DNA-488 by benzonase bears two nucleotides "GT" (Figure 2b,c). Since the digestion of DNAs by benzonase is not sequence-dependent, a small fraction of the cleaved product contains nucleotide fragments other than "GT", as shown in Figure 2a (a3 488−5′GTAA-3′ and a4 488−5′GTA-3′). Because digestion of DNAs by a restriction endonuclease would guarantee a higher specificity of cleavage, we then performed on-bead digestion of biotin-DNA-488 with the restriction endonuclease PvuII that recognizes the CAGCTG sequence in the DNA linker after annealing biotin-DNA-488 with its complementary DNA strand. LC/MS analysis showed the site-specific cleavage and the formation of two regioisomers of 488−5′GTAACGATCCAG-3′ (Figure 2d−f).

Next, we designed a proof-of-principle experiment to test the efficiency of the biotin-DNA-N_3 probe to capture a single alkyne-tagged protein from a complex protein mixture. We introduced a single terminal alkyne group into cysteine34 of bovine serum albumin (BSA)[25] and mixed it with the lysates of Jurkat cells, a human T lymphocyte cell line, in a 1:6 weight ratio as a model system. The protein mixture was reacted with the biotin-DNA-N_3 probe in the presence of the BTTP/Cu(I) catalyst, followed by the enrichment with streptavidin agarose resins. The captured BSA was then released from resins by incubation with benzonase in Tris buffer (pH 7.5) for two hours at 37 °C. After three washes, the resins were further boiled in SDS buffer to release residual proteins that were resistant to the benzonase cleavage. In parallel, the same protein mixture was reacted with biotin-N_3 and then incubated with streptavidin agarose resins. The captured BSA was released from resins by boiling in SDS buffer. The samples released from both experiments were collected and resolved by SDS polyacrylamide gel electrophoresis (SDS-PAGE). As shown in Figure 1b, comparable levels of BSA were detected in eluents obtained from benzonase cleavage and boiling-only treatment with significantly more contaminant proteins detected in the boiling-only eluents.[26] Notably, no residual BSA was detectable in the fraction obtained from benzonase-followed-by-boiling double treatment, indicating the elution with benzonase was complete (Figure 1b and Figure S3 in the Supporting Information). Taken together, this experiment demonstrated that biotin-DNA-N_3 is an efficient and selective probe to enrich alkyne-functionalized proteins from complex mixtures and the cleavage of this probe can be achieved under mild conditions.

After verifying the specificity and the compatibility of the biotin-DNA-N_3 probe with the optimized CuAAC conditions, we then applied the probe to the enrichment of sialylated glycoproteins from HL60 cells for their identification. The cultured HL60 cell is an acute human promyeloid leukemia cell line that has been widely used for the study of the molecular events of myeloid differentiation and the impact of pharmacologic and virologic agents on this process.[27] Due to well-documented roles of the negatively charged sialic acid in cellular adhesion and the regulation of the growth and differentiation of hematopoietic progenitor cells, HL60 cells have also been used extensively for the study of sialylated glycoconjugates in these processes.[28] To our surprise, however,

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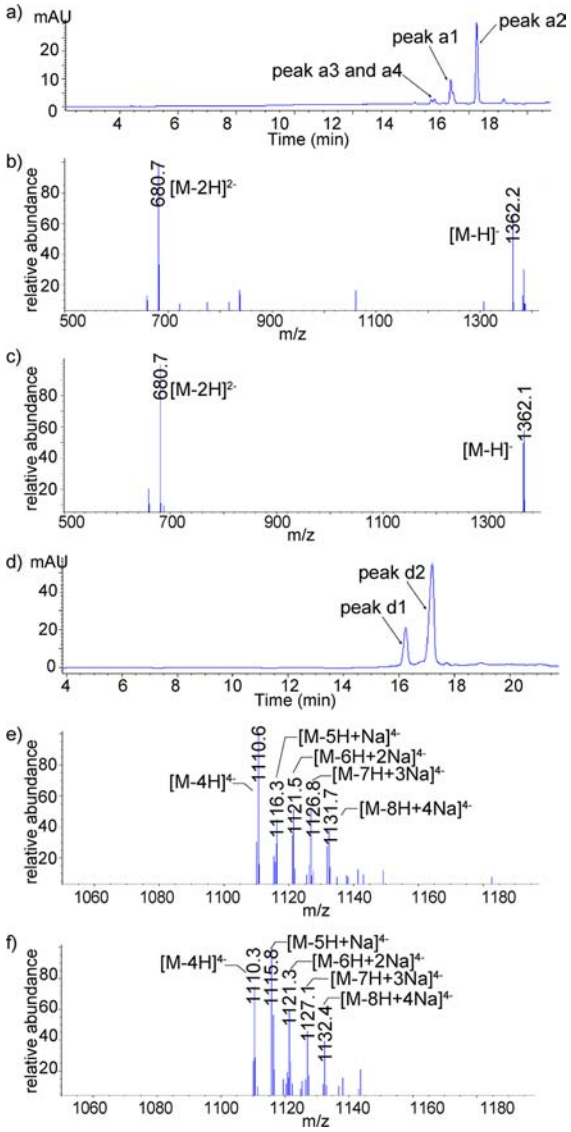

Figure 2. LC/MS analysis of the cleaved products of biotin-DNA-488 by benzonase (a–c) and PvuII (d–f). (a) HPLC trace with absorbance at 488 nm. Two major peaks (a1 and a2) and two minor peaks (a3 and a4) were observed as indicated with arrows. (b) MS spectrum of peak a1. (c) MS spectrum of peak a2. The MS spectra of both peak a1 and peak a2 indicate that the major cleaved product of biotin-DNA-488 is 488–5′GT-3′ (Chemical Formula: $C_{58}H_{71}N_{13}O_{22}P_2$; molecular weight 1364.22). (d) HPLC trace with absorbance at 488 nm. Two major peaks (d1 and d2) were observed as indicated with arrows. (e) MS spectrum of peak d1. (c) MS spectra of peak d2. The MS spectra of both peak d1 and peak d2 show that the cleaved product of biotin-DNA-488 is 488–5′GTAACGATCCAG-3′ (Chemical Formula: $C_{156}H_{194}N_{54}O_{78}P_{12}$; molecular weight 4445.25).

the proteomic-wide profiling of sialylated glycoproteins in this cell line has never been pursued.

To profile the sialylated glycoproteins in HL60 cells, we cultured the cells in media supplemented with peracetylated *N*-(4-pentynoyl) mannosamine (Ac_4ManNAl), an alkyne-tagged metabolic precursor of sialic acid (Figure 3a).[7] The unnatural monosaccharide allows the metabolic incorporation of the alkyne tag into membrane sialylated glycoproteins (Figure S4 and S5 in the Supporting Information). After three days, cells were lyzed. Cell lysates were reacted with the biotin-DNA-N_3 probe before incubation with streptavidin agarose resins for

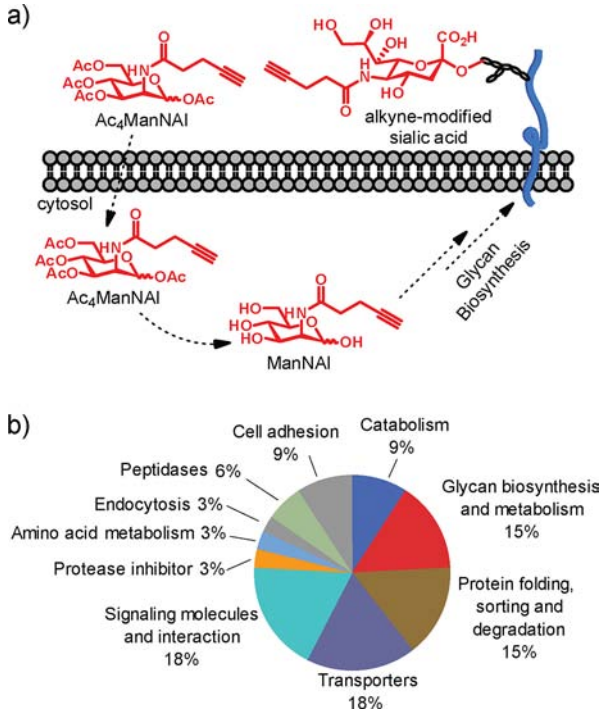

Figure 3. (a) Metabolic labeling of sialic acid using the unnatural sugar Ac_4ManNAl. Cells were cultured in medium containing Ac_4ManNAl. Ac_4ManNAl was taken up by cells, processed by a series of cellular enzymes, and converted to the alkyne-modified sialic acid and incorporated into cell-surface sialylated glycoproteins. (b) Classification of the 36 sialylated glycoproteins identified in HL60 cells.

affinity enrichment (Scheme 1). To elute the captured glycoproteins, the resins were incubated with benzonase in Tris buffer (pH 7.5) at 37 °C for eight hours. The eluted proteins were resolved using SDS-PAGE. Following in-gel trypsin digestion, the resulting peptide fragments were analyzed using LC-MS/MS.

Data analysis from two sets of repeated experiments revealed a total of 36 hits from lysates obtained from the cells treated with Ac_4ManNAl but not those from untreated counterparts (Table S1 in the Supporting Information). Among these proteins, many are known to be sialylated, including integrin, intercellular adhesion molecule 3, and leukosialin among a few others. We discovered that eight identified proteins are known to reside in the membrane of the endoplasmic reticulum or Golgi apparatus and are involved in the biosynthesis of N-linked glycans or in glycan metabolism (Table 1). Most other proteins are located in the plasma membrane; they either serve as cell-surface receptors or transporters or participate in metabolic processes (Figure 3b).

In summary, we discovered here that single-stranded DNAs serve as a new type of cleavable linkers for the CuAAC-based proteomic analysis. When integrated into biotin probes, linker cleavage and release of captured proteins was accomplished with high efficiency in very mild conditions. Using this new probe, we were able to identify 36 sialylated glycoproteins from the lysates of HL60 cells that are involved in many different biological processes ranging from glycan synthesis to adhesive events. By replacing benzonase with a restriction endonuclease, site-specific cleavage of the probe was realized, which is crucial for the integration of this new technique into proteomics studies that require the identification of post-translational-

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Table 1. Examples of Identified HL60 Proteins That Are Known to Be Sialylated or Found in the ER or Golgi

protein	accession number	protein function
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 precursor	IPI00025874	N-oligosaccharyl transferase (ER)
Integrin beta-2	IPI00291792	ICAM receptor
Isoform 1 of UDP-glucose:glycoprotein glucosyltransferase 1	IPI00024466	reglucosylation of misfolded proteins (ER)
Peptidyl-prolyl cis−trans isomerase B	IPI00646304	cis−trans isomerization of proline imidic peptide bonds (ER)
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A	IPI00297492	N-glycosylation (ER)
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B	IPI00152377	N-glycosylation (ER)
Isoform 2 of Neutral alpha-glucosidase AB	IPI00011454	glycan metabolism (ER/Golgi)
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	IPI00028635	N-glycosylation (ER)
Endoplasmin	IPI00027230	Molecular chaperone (ER)
Leukosialin (CD43)	IPI00027430	Presents carbohydrate ligands to selectins
Isoform Beta-1C of Integrin beta-1	IPI00217561	laminin receptor
Intercellular adhesion molecule 3	IPI00031620	laminin receptor

modification sites. Taking advantage of the automatic and commercially available synthesis of single-stranded DNAs, the integration of this cleavable linker into other affinity probes for proteomics, e.g., FLAG probes, would be a straightforward application.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures and proteomics data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CuAAC, Cu(I)-catalyzed azide−alkyne cycloaddition; ABPP, activity-based protein profiling; SDS, sodium dodecyl sulfate; CPG, controlled pore glasses; TBTA, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine; BTTP, 3-(4-((bis((1-*tert*-butyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)-propan-1ol; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin; 488-alkyne, alkyne-modified Alex Fluor 488 dye; Ac_4ManNAl, peracetylated *N*-(4-pentynoyl) mannosamine

■ REFERENCES

(1) Rostovtsev, V. V., Green, L. G., Fokin, V. V., and Sharpless, K. B. (2002) A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem., Int. Ed. 41*, 2596−2599.

(2) Tornoe, C. W., Christensen, C., and Meldal, M. (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem. 67*, 3057−3064.

(3) Baskin, J. M., Prescher, J. A., Laughlin, S. T., Agard, N. J., Chang, P. V., Miller, I. A., Lo, A., Codelli, J. A., and Bertozzi, C. R. (2007) Copper-free click chemistry for dynamic in vivo imaging. *Proc. Natl. Acad. Sci. U.S.A. 104*, 16793−16797.

(4) Ning, X., Guo, J., Wolfert, M. A., and Boons, G. J. (2008) Visualizing metabolically labeled glycoconjugates of living cells by copper-free and fast huisgen cycloadditions. *Angew. Chem., Int. Ed. 47*, 2253−2255.

(5) Speers, A. E., and Cravatt, B. F. (2004) Chemical strategies for activity-based proteomics. *ChemBioChem 5*, 41−47.

(6) Hanson, S. R., Hsu, T. L., Weerapana, E., Kishikawa, K., Simon, G. M., Cravatt, B. F., and Wong, C. H. (2007) Tailored glycoproteomics and glycan site mapping using saccharide-selective bioorthogonal probes. *J. Am. Chem. Soc. 129*, 7266−7267.

(7) Chang, P. V., Chen, X., Smyrniotis, C., Xenakis, A., Hu, T., Bertozzi, C. R., and Wu, P. (2009) Metabolic labeling of sialic acids in living animals with alkynyl sugars. *Angew. Chem., Int. Ed. 48*, 4030−4033.

(8) Yang, Y. Y., Ascano, J. M., and Hang, H. C. (2010) Bioorthogonal chemical reporters for monitoring protein acetylation. *J. Am. Chem. Soc. 132*, 3640−3641.

(9) Rexach, J. E., Clark, P. M., and Hsieh-Wilson, L. C. (2008) Chemical approaches to understanding O-GlcNAc glycosylation in the brain. *Nat. Chem. Biol. 4*, 97−106.

(10) Zheng, T., Jiang, H., Gros, M., del Amo, D. S., Sundaram, S., Lauvau, G., Marlow, F., Liu, Y., Stanley, P., and Wu, P. (2011) Tracking N-acetyllactosamine on cell-surface glycans in vivo. *Angew. Chem., Int. Ed. 50*, 4113−4118.

(11) Rouhanifard, S. H., Nordstrom, L. U., Zheng, T., and Wu, P. (2013) Chemical probing of glycans in cells and organisms. *Chem. Soc. Rev. 42*, 4282−4296.

(12) Hang, H. C., Wilson, J. P., and Charron, G. (2011) Bioorthogonal chemical reporters for analyzing protein lipidation and lipid trafficking. *Acc. Chem. Res. 44*, 699−708.

(13) Luo, M. (2012) Current chemical biology approaches to interrogate protein methyltransferases. *ACS Chem. Biol. 7*, 443−463.

(14) Sletten, E. M., and Bertozzi, C. R. (2009) Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. *Angew. Chem., Int. Ed. 48*, 6974−6998.

(15) Cravatt, B. F., Wright, A. T., and Kozarich, J. W. (2008) Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. *Annu. Rev. Biochem. 77*, 383−414.

(16) Uttamchandani, M., Li, J., Sun, H., and Yao, S. Q. (2008) Activity-based protein profiling: new developments and directions in functional proteomics. *ChemBioChem 9*, 667−675.
(17) Speers, A. E., and Cravatt, B. F. (2005) A tandem orthogonal proteolysis strategy for high-content chemical proteomics. *J. Am. Chem. Soc. 127*, 10018−10019.
(18) Szychowski, J., Mahdavi, A., Hodas, J. J., Bagert, J. D., Ngo, J. T., Landgraf, P., Dieterich, D. C., Schuman, E. M., and Tirrell, D. A. (2010) Cleavable biotin probes for labeling of biomolecules via azide-alkyne cycloaddition. *J. Am. Chem. Soc. 132*, 18351−18360.
(19) Yang, Y. Y., Grammel, M., Raghavan, A. S., Charron, G., and Hang, H. C. (2010) Comparative analysis of cleavable azobenzene-based affinity tags for bioorthogonal chemical proteomics. *Chem. Biol. 17*, 1212−1222.
(20) Kim, H. Y., Tallman, K. A., Liebler, D. C., and Porter, N. A. (2009) An azido-biotin reagent for use in the isolation of protein adducts of lipid-derived electrophiles by streptavidin catch and photorelease. *Mol. Cell. Proteomics 8*, 2080−2089.
(21) Mayer, G., and Heckel, A. (2006) Biologically active molecules with a "light switch". *Angew. Chem., Int. Ed. 45*, 4900−4921.
(22) Wang, Q., Chan, T. R., Hilgraf, R., Fokin, V. V., Sharpless, K. B., and Finn, M. G. (2003) Bioconjugation by copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition. *J. Am. Chem. Soc. 125*, 3192−3193.
(23) Hong, V., Presolski, S. I., Ma, C., and Finn, M. G. (2009) Analysis and optimization of copper-catalyzed azide-alkyne cycloaddition for bioconjugation. *Angew. Chem., Int. Ed. 48*, 9879−9883.
(24) Wang, W., Hong, S., Tran, A., Jiang, H., Triano, R., Liu, Y., Chen, X., and Wu, P. (2011) Sulfated ligands for the copper(I)-catalyzed azide-alkyne cycloaddition. *Chem. Asian J. 6*, 2796−2802.
(25) Dirks, A. J., van Berkel, S. S., Hatzakis, N. S., Opsteen, J. A., van Delft, F. L., Cornelissen, J. J., Rowan, A. E., van Hest, J. C., Rutjes, F. P., and Nolte, R. J. (2005) Preparation of biohybrid amphiphiles via the copper catalysed Huisgen [3 + 2] dipolar cycloaddition reaction. *Chem. Commun.*, 4172−4174.
(26) Brahma, A., Mandal, C., and Bhattacharyya, D. (2005) Characterization of a dimeric unfolding intermediate of bovine serum albumin under mildly acidic condition. *Biochim. Biophys. Acta 1751*, 159−169.
(27) Birnie, G. D. (1988) The HL60 cell line: a model system for studying human myeloid cell differentiation. *Br. J. Cancer Suppl. 9*, 41−45.
(28) Schwartz-Albiez, R., Merling, A., Martin, S., Haas, R., and Gross, H. J. (2004) Cell surface sialylation and ecto-sialyltransferase activity of human CD34 progenitors from peripheral blood and bone marrow. *Glycoconj. J. 21*, 451−459.

■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on April 29, 2013, with errors in the Supporting Information. The corrected version was reposted on May 28, 2013.