Published in final edited form as:

Curr Opin Chem Biol. 2010 December ; 14(6): 790–796. doi:10.1016/j.cbpa.2010.09.020.

Chemoenzymatic Methods for Site-Specific Protein Modification

David Rabuka

Redwood Bioscience, Burlingame, CA 94010 USA

Abstract

In the past decade, numerous chemical technologies have been developed to allow the site-specific post-translational modification of proteins. Traditionally covalent chemical protein modification has been accomplished by the attachment of synthetic groups to nucleophilic amino acids on protein surfaces. These chemistries, however, are rarely sufficiently selective to distinguish one residue within a literal sea of chemical functionality. One solution to this problem is to introduce a unique chemical handle into the target protein that is orthogonal to the remainder of the proteome. In practice, this handle can be a novel peptide sequence, which forms a “tag” that is selectively and irreversibly modified by enzymes. Furthermore, if the enzymes can tolerate substrate analogs, it becomes possible to engineer chemically modified proteins in a site-specific fashion. This review details the significant progress in creating techniques for the chemoenzymatic generation of protein-small molecule constructs and provides examples of novel applications of these methodologies.

Introduction

Proteins offer several advantages over small molecules as therapeutics or as diagnostic probes including exquisite target specificity, multiplicity of function, and relatively low off-target activity. The chemical modification of proteins may extend these advantages by rendering them more potent, stable, or multimodal. One significant obstacle to the creation of a chemically altered protein therapeutic or reagent is the production of the protein in a biologically active, homogenous form. Common heterologous expression systems, such as those in *E. coli* or Chinese hamster ovary cells, often cannot recapitulate exact post-translational modifications to the recombinant proteins necessary for achieving a desired function. However, recent advances in protein engineering have overcome these difficulties by exploiting the genetic machinery of protein production, the specificity of enzymatic reactions, and the tools of synthetic organic chemistry to direct the precise and selective formation of chemical bonds.

A number of standard chemical transformations are commonly used to create and manipulate the post-translational modifications on proteins. For example, several methods are able to modify the side chains of certain amino acids selectively. Carboxylic acid side chains (aspartate and glutamate) can be targeted by initial activation with a water-soluble carbodiimide reagent and subsequent reaction with an amine. Similarly, lysine can be targeted through the use of activated esters or isothiocyanates, and cysteine thiols can be

© 2010 Elsevier Ltd. All rights reserved.

drabuka@redwoodbioscience.com .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

targeted with maleimides and α -halo-carbonyls. Although this approach is widely used in research settings, it is rarely able to produce *site-specific* modifications on proteins due to the multiple copies of each amino acid residue within the protein that possess similar reactivity. Therefore, these methods produce heterogeneous mixtures, often rendering the modified proteins unsuitable for research or therapeutic uses. As a consequence, a number of researchers have been trying to address the following challenge: how does one introduce functionality to a protein of interest that is chemically orthogonal to the rest of the proteome?

This review highlights many methods designed to introduce chemically defined, unnatural molecules into proteins site-specifically via enzymatic transformations. The methods are grouped into three categories: 1) self-labeling protein-enzyme fusions; 2) chemoenzymatic post-translational protein modification; and 3) chemoenzymatic co-translational protein modification.

Self-Labeling Protein-Enzyme Fusions

Enzyme-catalyzed reactions that proceed via irreversible conjugation with suicide substrates can be used for labeling when fused to a protein of interest. A number of pairs of enzymes and suicide substrates are available and many of these enzymes tolerate useful modifications to their substrates (Figure 1). By generating translation fusions between these enzymes and a protein of interest, it is possible to link the modified substrate to a protein of interest through a covalent bond. In general these reactions are fast, irreversible and site-specific.

The protein O⁶-Alkylguanine Transferase (AGT) is useful for site-specific protein labeling as it catalyzes the irreversible transfer of the alkyl group of O⁶-benzylguanine derivatives to a reactive cysteine residue within the enzyme. In cells, AGT is involved in DNA repair by stoichiometrically transferring an alkyl group from a guanine to a cysteine residue in its active site. Johnsson and colleagues pioneered the use of AGT as a novel method to site-specifically label proteins by exploiting the low specificity of the enzyme, which naturally evolved to remove numerous alkylated derivatives of O⁶-benzylguanine (BG). By fusing AGT to the C-terminus of a protein of interest, it is possible to label the fusion by irreversible conjugation with a BG analog [1]. A 20-kDa mutant of AGT known as the SNAP-tagTM has been developed with enhanced BG reactivity and decreased background endogenous DNA binding. SNAP-tagTM is resistant to degradation and has been used with great utility for time-lapse intracellular imaging experiments by conjugating the fusion to a fluorophore [2]. A derivative of the SNAP-tagTM is the CLIP-tag. The CLIP-tag catalyzes the transfer of alkyl groups from O²-benzylcytosine derivatives [3]. Combining both strategies in a single host enables FRET measurements and two color pulse chase experiments [4]. Importantly, SNAP-tagTM or CLIP-tag labeling can be accomplished both *in vitro* and *in vivo* and in multiple organisms including *E. coli*, *S. cerevisiae*, and mammalian cell lines.

The enzyme haloalkane dehydrogenase (DhaA) cleaves carbon-halogen bonds in aliphatic halogenated compounds. The natural mechanism of DhaA proceeds by an aspartate side chain displacing the halogen to generate an ester that is subsequently hydrolyzed to release the dehalogenated alkane from the enzyme as an alcohol. Researchers at Promega developed a mutant of DhaA, the Halo-tag protein (HTP) [5]. The monomeric haloalkane dehalogenase, a 33 kDa protein, contains a critical mutation that blocks hydrolysis of the covalent intermediate, resulting in a stable ester linkage between the small molecule and HTP active site. When HTP is fused to a protein of interest the nucleophilic aspartic acid will catalyze the covalent modification of the fusion with haloalkane-derivatized substrates. A variety of small molecules have been conjugated to Halo-tagged proteins, including fluorophores and quantum dots [6]. The HaloTag7, another catalytically inactive DhaA

variant, was designed to overcome issues of solubility, protein expression and detection. HaloTag7 can be an effective alternative to the more common affinity tags [7].

Cutinase is a 22-kDa serine esterase that has also been adapted as a self-modifying enzyme. Probes containing *p*-nitrophenol phosphate esters act as suicide inhibitors of the enzyme. Appropriately modified, these suicide inhibitors form covalent chemical conjugates with cutinase fusions in a site-specific fashion. Conjugation of fluorophores [8] to cutinase fusions has been achieved with the appropriate phosphate esters.

Chemically programmable antibodies (Abs) are a novel class of self-labeling enzymes that leverage the biochemical properties of antibodies and therapeutic synthetic small molecules or peptides. Pioneered by Barbas and colleagues, synthetic small molecules can be irreversibly conjugated to a catalytic monoclonal aldolase in a site-specific reaction [9]. The Abs were designed to mimic the activity of the generic fructose biphosphate aldolase enzyme that cleaves a 6-carbon fructose sugar into two 3-carbon products via a retro aldol reaction. This transformation is achieved via the formation of a Schiff base intermediate with an active site lysine residue, followed by deprotonation of a neighboring hydroxyl group on the fructose backbone. This leads to spontaneous cleavage between C3 and C4 of the sugar. A catalytic antibody, Ab 38C2, was generated that mimicked this aldolase mechanism. When exposed to suitably modified substrates, 38C2 catalyzed the selective and irreversible conjugation of the substrate to its active site lysine via amide bond formation. While natural aldolases tolerate only minor changes in the structure of the donor substrate, remarkably, the catalytic Abs can be conjugated with a wide variety of small molecules, generating a site-specifically labeled chemical-protein hybrid incorporating properties of both the small molecule and protein [10,11].

Chemoenzymatic Post-Translational Protein Modification

Many post-translational protein modifications can be harnessed for the site-specific chemoenzymatic modification of proteins. Typically, this is accomplished by fusing a novel tag (typically a short peptide derived from the consensus sequence of the enzyme substrate) to recombinant proteins (Figure 2). Subsequent reaction with the enzyme specific for the tag and its small molecules substrate generates a covalent product.

Ting and co-workers have highlighted the utility of this strategy using *E. coli* biotin ligase (BirA) to modify recombinant cell surface proteins [12]. BirA is an enzyme responsible for the posttranslational modification of a small number of bacterial proteins with the cofactor biotin. The enzyme also recognizes and biotinylates an engineered 15-residue “acceptor peptide” (AP) sequence, which can be fused to the N- or C-terminus of any target protein. Importantly, *E. coli* BirA does not biotinylate any mammalian proteins, rendering it “orthogonal” to the mammalian proteome. Using a synthetic ketone-containing biotin isostere (“keto-biotin”) as a substrate, Ting and co-workers demonstrated that BirA could be used as a “ketone ligase.” For example, BirA and keto-biotin were used to modify epidermal growth factor receptors (EGFR) containing the 15-residue BirA substrate consensus sequence. The modified protein was expressed on the surface of HeLa cells and keto-biotin was conjugated using BirA and ATP as a cofactor. The ketone, a unique chemical functional group in the context of a cell surface, was then chemically conjugated to a fluorescein hydrazide reagent and the trafficking of the product was imaged by fluorescence microscopy.

The same lab recently developed *E. coli* lipoic acid ligase (LplA) to attach lipoic acid analogs to the LplA acceptor peptide. LplA could transfer a panel of alkynyl and azido probes to the acceptor peptide with varying efficiencies. These substrates participate in Cu-catalyzed and strain-promoted [3+2] cycloadditions, which react more rapidly and with

greater orthogonality than ketone condensation reactions. Given their orthogonal reactivity, Ting used BirA and LplA (followed by respective secondary chemistries) to simultaneously label and image two different cell-surface proteins [13]. Recently mutant LplA enzymes were generated that accepted novel probes consisting of a coumarin moiety for live cell imaging of LplA tagged proteins [14].

Ting and coworkers have also explored the substrate specificity of transglutaminases (TGases), widely expressed enzymes that catalyze amide bond formation between glutamine and lysine side chains. They found that guinea pig liver transglutaminase (gpTGase) tolerates structural analogs of the amine-containing substrate. Through the introduction of a “Q-tag” (PKPQQFM), recombinant proteins were site-specifically modified by TGases [15]. Substrate analogs incorporating biotin, Alexa568 or a benzophenone photoaffinity probe were all efficiently transferred to the Q-tag. Additionally, TGase can site-specifically PEGylate tagged therapeutic proteins to enhance their serum half-lives [16].

Sortases (SrtA) are transpeptidases that may also be repurposed for site-specific protein modification. *Staphylococcus aureus* or *Streptococcus pyogenes* use sortases to anchor proteins to their cell walls through a covalent linkage. SrtA cleaves a short peptide sequence (LPXTG) on a protein substrate generating an acyl-enzyme intermediate. The substrate is then transferred to a polyglycine linker of a co-substrate forming an amide bond. The LPXTG modification can be introduced to either terminus of a fusion protein and these constructs [17] have been successfully used for the *in vitro* modification of proteins with novel glycoforms and lipids [18] as well as live cell labeling [19].

Protein prenylation is a post-translational modification characterized by the addition of lipids, either a farnesyl (C15) or a geranylgeranyl (C20) group, near the C terminus. Protein Farnesyl Transferase (PFTase) and Protein Geranylgeranyl Transferase (PGGTase) recognize a short peptide motif, CAAX where A is an aliphatic amino acid and X is either A or M for farnesylation or L for geranylgeranylation. By adding this motif to a protein of interest it is possible to introduce site-specific lipidation. Both of these enzymes can tolerate unnatural, synthetic analogs of the native substrate diphosphate lipid donors, including fluorophores, alkynes, and azides. The enzymes are also tolerant of varying isoprenoid units, including small single unit moieties [20]. The small modification minimizes alternations to protein solubility and function.

Proteins have also been selectively elaborated chemically when fused to an acyl carrier protein (ACP) containing a phosphopantetheine (Ppant) prosthetic group. Ppant is covalently attached to the ACP by an enzyme named phosphopantetheine transferase (PPTase). PPTases use coenzyme A (CoA) as the source of Ppant and can tolerate various chemical moieties attached to the CoA molecule making it a good candidate for chemoenzymatic protein labeling. Numerous chemically modified CoA analogs have been used to incorporate biotin affinity tags or various small molecule probes in ACP-fusion proteins on cell surfaces *in vivo* [21]. However, the significantly larger size of the fusion relative to other tags is a potential liability. In order to address this problem, Walsh and colleagues used phage display to isolate two minimal peptide epitopes for two different PPTase subfamilies. The selected 12 amino acid peptides are inserted into a protein of interest where they are site-specifically conjugated with CoA derivatives [22]. Subsequent efforts further minimized the tag to eight residues. PPTase recognized the reduced sequence both *in vitro* and *in vivo* [23].

The *in vitro* repurposing of enzymes to elaborate proteins post-translationally has been an invaluable tool for generating synthetic glycans and is now considered a routine procedure [24]. These reactions are catalyzed by glycosyltransferases, the majority of which transfer a glycosyl donor to a sugar or amino acid acceptor. These transferases generally utilize

nucleotide diphosphate sugar donors (e.g., UDP-GlcNAc) with occasional exceptions such as sialyltransferases, which require CMP-sialic acids. A number of glycosyltransferases tolerate chemically modified analogs of their natural substrates.

Sialyltransferases are commonly used in a protein-engineering context to generate site-specifically modified biomolecules. Sialyltransferases can tolerate numerous chemical modifications to a CMP-sialic acid nucleotide donor at either the C-9 or C-5 positions of the sugar ring. Such modifications may even include large water-soluble polymers. GlycoPEGylation is a novel strategy designed by the scientists at Neose Technologies Inc to generate PEGylated proteins in a site-specific manner. An initial enzymatic transfer of a GalNAc residue to a threonine or serine residue on the protein backbone is followed by the addition of a synthetic sialic acid chemically elaborated with PEG. This strategy was used to develop G-CSF and INF- α 2b, currently in clinical use [25].

Additional glycosyltransferases can be used for site-specific protein engineering. Both wild type and mutant β -1,4-galactosyltransferase will transfer modified galactose and *N*-acetyl galactosamine donors containing chemical handles at the C2 position of the sugar ring for further elaboration. Glycan moieties incorporating a keto group have been added to monoclonal antibodies to generate site-specifically labeled Abs containing cross-linking agents, novel imaging agents for proteomic analysis [26], or cytotoxic compounds for targeted drug delivery systems [27].

Chemoenzymatic Co-Translational Protein Modification

The Bertozzi research group has reported the use of a pentapeptide consensus sequence (CXPXR) found in sulfatase active sites as a genetically encoded tag for chemical modification of proteins [28]. The formylglycine-generating enzyme (FGE) recognizes and acts specifically on this motif, oxidizing the cysteine to an unusual aldehyde-bearing formylglycine residue. The aldehyde produced by FGE on the target protein can be selectively reacted with α -nucleophiles, such as aminooxy- and hydrazide-bearing compounds. The Bertozzi group has demonstrated efficient conversion to FGly when the sequence is inserted into heterologous proteins at either terminus or internally. Importantly, this conversion is accomplished co-translationally by co-over expression of FGE, obviating the need for the addition of isolated and purified recombinant enzyme. A schematic illustrating both the modification and the method of chemical elaboration is shown in Figure 3. The native FGE of both prokaryotic and eukaryotic cells recognizes the aldehyde tag and performs the conversion of the cysteine to the aldehyde during protein translation. As a consequence, the aldehyde tag is easily moved between numerous expression systems, including mammalian cells [29]. The Bertozzi group has also demonstrated *in vivo* cell surface labeling with appropriately functionalized fluorophores and *in vitro* modifications with small molecules, peptides, PEG and imaging agents on a number of different proteins including human growth hormone, CD4 and monoclonal IgG [30].

Conclusions

Over the past years the use of enzymes to introduce site-specific, post-translational modifications in proteins has emerged as one of the most promising approaches to develop novel biologics. Combining the power of molecular biology with the precision and flexibility of synthetic chemistry, this methodology enables the generation of new classes of homogenous, enhanced proteins both *in vivo* and *in vitro*. Given the growing number of options available to the chemical biology community for site-specific chemoenzymatic protein modification, the techniques highlighted here will continue to play an essential role

in expanding our understanding of cellular behavior, in advancing biomedical engineering, and in developing new therapeutics.

Acknowledgments

The author would like to thank Zev Gartner and Martin Forstner for critical reading of this manuscript and the National Institutes of Health for financial support (1RC1EB010344-01).

References

- of special interest
 - of outstanding interest
1. Keppler A, Gendreizig S, Gronemeyer T, Pick H, Vogel H, Johnsson K. A general method for the covalent labeling of fusion proteins with small molecules in vivo. *Nat Biotechnol* 2003;21:86–89. [PubMed: 12469133] • The authors present a method for labeling proteins with fluorophores in mammalian cells.
 2. Keppler A, Pick H, Arrivoli C, Vogel H, Johnsson K. Labeling of fusion proteins with synthetic fluorophores in live cells. *Proc Natl Acad Sci U S A* 2004;101:9955–9959. [PubMed: 15226507]
 3. Gautier A, Nakata E, Lukinavicius G, Tan KT, Johnsson K. Selective cross-linking of interacting proteins using self-labeling tags. *J Am Chem Soc* 2009;131:17954–17962. [PubMed: 19916541]
 4. Mao S, Benninger RK, Yan Y, Petchprayoon C, Jackson D, Easley CJ, Piston DW, Marriott G. Optical lock-in detection of FRET using synthetic and genetically encoded optical switches. *Biophys J* 2008;94:4515–4524. [PubMed: 18281383]
 5. Los GV, Encell LP, McDougall MG, Hartzell DD, Karassina N, Zimprich C, Wood MG, Learish R, Ohana RF, Urh M, et al. HaloTag: a novel protein labeling technology for cell imaging and protein analysis. *ACS Chem Biol* 2008;3:373–382. [PubMed: 18533659] •• Los et al. introduce a novel fusion protein method for protein modification. The utility of the system is highlighted and a number of applications for the probe discussed.
 6. Zhang Y, So MK, Loening AM, Yao H, Gambhir SS, Rao J. HaloTag protein-mediated site-specific conjugation of bioluminescent proteins to quantum dots. *Angew Chem Int Ed Engl* 2006;45:4936–4940. [PubMed: 16807952]
 7. Ohana RF, Encell LP, Zhao K, Simpson D, Slater MR, Urh M, Wood KV. HaloTag7: a genetically engineered tag that enhances bacterial expression of soluble proteins and improves protein purification. *Protein Expr Purif* 2009;68:110–120. [PubMed: 19464373]
 8. Bonasio R, Carman CV, Kim E, Sage PT, Love KR, Mempel TR, Springer TA, von Andrian UH. Specific and covalent labeling of a membrane protein with organic fluorochromes and quantum dots. *Proc Natl Acad Sci U S A* 2007;104:14753–14758. [PubMed: 17785425]
 9. Rader C, Sinha SC, Popkov M, Lerner RA, Barbas CF 3rd. Chemically programmed monoclonal antibodies for cancer therapy: adaptor immunotherapy based on a covalent antibody catalyst. *Proc Natl Acad Sci U S A* 2003;100:5396–5400. [PubMed: 12702756]
 10. Gavriluk JJ, Wuellner U, Barbas CF 3rd. Beta-lactam-based approach for the chemical programming of aldolase antibody 38C2. *Bioorg Med Chem Lett* 2009;19:1421–1424. [PubMed: 19181522]
 11. Goswami RK, Huang ZZ, Forsyth JS, Felding-Habermann B, Sinha SC. Multiple catalytic aldolase antibodies suitable for chemical programming. *Bioorg Med Chem Lett* 2009;19:3821–3824. [PubMed: 19428247]
 12. Chen I, Howarth M, Lin W, Ting AY. Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. *Nat Methods* 2005;2:99–104. [PubMed: 15782206]
 13. Fernandez-Suarez M, Baruah H, Martinez-Hernandez L, Xie KT, Baskin JM, Bertozzi CR, Ting AY. Redirecting lipoic acid ligase for cell surface protein labeling with small-molecule probes. *Nat Biotechnol* 2007;25:1483–1487. [PubMed: 18059260] •• Two labeling methods are used in tandem for cell surface protein labeling, including a novel lipoic acid ligase, which selectively couples a chemical handle to a protein to an acceptor peptide tag.

14. Uttamapinant C, White KA, Baruah H, Thompson S, Fernandez-Suarez M, Puthenveetil S, Ting AY. A fluorophore ligase for site-specific protein labeling inside living cells. *Proc Natl Acad Sci U S A* 107:10914–10919. [PubMed: 20534555]
15. Lin CW, Ting AY. Transglutaminase-catalyzed site-specific conjugation of small-molecule probes to proteins in vitro and on the surface of living cells. *J Am Chem Soc* 2006;128:4542–4543. [PubMed: 16594669] • Lin et al. demonstrate selective cell surface protein labeling using transglutaminase. Importantly, the substrates are synthetical very tractable and versatile.
16. Fontana A, Spolaore B, Mero A, Veronese FM. Site-specific modification and PEGylation of pharmaceutical proteins mediated by transglutaminase. *Adv Drug Deliv Rev* 2008;60:13–28. [PubMed: 17916398]
17. Antos JM, Chew GL, Guimaraes CP, Yoder NC, Grotenbreg GM, Popp MW, Ploegh HL. Site-specific N- and C-terminal labeling of a single polypeptide using sortases of different specificity. *J Am Chem Soc* 2009;131:10800–10801. [PubMed: 19610623] • Antos et al. significantly expand the utility of sortagging by a selective tagging of either the C or the N terminus of a protein of interest.
18. Antos JM, Miller GM, Grotenbreg GM, Ploegh HL. Lipid modification of proteins through sortase-catalyzed transpeptidation. *J Am Chem Soc* 2008;130:16338–16343. [PubMed: 18989959]
19. Tanaka T, Yamamoto T, Tsukiji S, Nagamune T. Site-specific protein modification on living cells catalyzed by Sortase. *Chembiochem* 2008;9:802–807. [PubMed: 18297670]
20. Wollack JW, Silverman JM, Petzold CJ, Mougous JD, Distefano MD. A minimalist substrate for enzymatic peptide and protein conjugation. *Chembiochem* 2009;10:2934–2943. [PubMed: 19856367]
21. Zou Y, Yin J. Phosphopantetheinyl transferase catalyzed site-specific protein labeling with ADP conjugated chemical probes. *J Am Chem Soc* 2009;131:7548–7549. [PubMed: 19441828]
22. Zhou Z, Cironi P, Lin AJ, Xu Y, Hrvatin S, Golan DE, Silver PA, Walsh CT, Yin J. Genetically encoded short peptide tags for orthogonal protein labeling by Sfp and AcpS phosphopantetheinyl transferases. *ACS Chem Biol* 2007;2:337–346. [PubMed: 17465518]
23. Zhou Z, Koglin A, Wang Y, McMahon AP, Walsh CT. An eight residue fragment of an acyl carrier protein suffices for post-translational introduction of fluorescent pantetheinyl arms in protein modification in vitro and in vivo. *J Am Chem Soc* 2008;130:9925–9930. [PubMed: 18593165] • Zhou et al. describe a method of protein modification using a minimized 8 amino acid peptide epitope that is selectively recognized by PPTases. The tag can be placed at either terminus of the protein of interest or internally without any notable perturbation to the protein structure.
24. Gamblin DP, Scanlan EM, Davis BG. Glycoprotein synthesis: an update. *Chem Rev* 2009;109:131–163. [PubMed: 19093879] • This review highlights many recent advances in glycoprotein synthesis, including chemical glycan assembly strategies and the use of chemoenzymatic methods, such as glycosyltransferases, for glycan synthesis.
25. DeFrees S, Wang ZG, Xing R, Scott AE, Wang J, Zopf D, Gouty DL, Sjoberg ER, Panneerselvam K, Brinkman-Van der Linden EC, et al. GlycoPEGylation of recombinant therapeutic proteins produced in *Escherichia coli*. *Glycobiology* 2006;16:833–843. [PubMed: 16717104]
26. Boeggeman E, Ramakrishnan B, Pasek M, Manzoni M, Puri A, Loomis KH, Waybright TJ, Qasba PK. Site specific conjugation of fluorophores to the remodeled Fc N-glycans of monoclonal antibodies using mutant glycosyltransferases: application for cell surface antigen detection. *Bioconjug Chem* 2009;20:1228–1236. [PubMed: 19425533]
27. Ramakrishnan B, Boeggeman E, Manzoni M, Zhu Z, Loomis K, Puri A, Dimitrov DS, Qasba PK. Multiple site-specific in vitro labeling of single-chain antibody. *Bioconjug Chem* 2009;20:1383–1389. [PubMed: 19507852]
28. Rush JS, Bertozzi CR. New aldehyde tag sequences identified by screening formylglycine generating enzymes in vitro and in vivo. *J Am Chem Soc* 2008;130:12240–12241. [PubMed: 18722427]
29. Carrico IS, Carlson BL, Bertozzi CR. Introducing genetically encoded aldehydes into proteins. *Nat Chem Biol* 2007;3:321–322. [PubMed: 17450134] • Carrico et al. describe the site-specific protein modification using formyl glycine generating enzyme to generate aldehydes on protein surfaces that are further chemically modified with a variety of small molecules, including short peptides, fluorophores and PEG.

30. Wu P, Shui W, Carlson BL, Hu N, Rabuka D, Lee J, Bertozzi CR. Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag. *Proc Natl Acad Sci U S A* 2009;106:3000–3005. [PubMed: 19202059] • Wu et al. demonstrate, for the first time, that the aldehyde tagging method for protein modification is compatible with mammalian expression systems. A number of proteins are shown to tolerate the addition of the aldehyde tag, including a monoclonal IgG that is site-selectively modified at the C-terminus and further chemically elaborated.

Figure 2.

Examples of chemoenzymatic post-translational, site-specific protein modification. The final product is created through the insertion of novel tags into a recombinant heterologous protein of interest and its subsequent reaction with an appropriate enzyme tolerant of substrate analogs.

Figure 3.

Co-translation protein modification to generate a site-specific modification on a protein of interest. The consensus sequence is cloned into a heterologous protein and, upon expression, the FGE converts the cysteine into a FGly. Nucleophile-bearing labels then react with the FGly site-specifically.