answering the questions of when and where CPD activation takes place in the cell.

The observation that the CPD alone is more effectively labeled than the autocleavage substrate led the authors to speculate about a conformational regulation of toxin activity. This is based on the assumption that in the full length toxin the natural cleavage site is located close to the active site, reducing its accessibility, e.g., for CPD inhibitors. Hence, a clinically applicable CPD inhibitor will have to cope with the tough task of both competing with an autocatalytic substrate and reacting with a rather unreactive protease. However, Puri et al. (2010) have demonstrated that it is possible to take up this challenge. Their promising approach is likely to provide guidance for the development of a new generation of drugs targeting *C. difficile* virulence factors rather than cellular viability, thereby rendering this important bacterial pathogen innocuous (Clatworthy et al., 2007).

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Reeling in the Catch: Advancing Cleavable Linkers for Proteomics

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In global proteomic applications that focus on the characterization of proteins that have been derivatized using bioorthogonal chemistry, a challenge persists in the release of labeled proteins from supports used for purification. In this issue, Yang and coworkers (2010) present a detailed study of the diazobenzene-cleavable linker system as an effective solution for proteomic studies.

In recent years, critical technical advances have profoundly enhanced the ability to perform global analysis of biological systems, including novel bioorthogonal labeling reactions and mass spectrometry-based proteomic techniques. For the former, the ability to achieve selective labeling of biomolecules within the extremely complex environments of organisms, cells, and cell extracts has opened numerous avenues for the efficient characterization of biological systems. Here, bioorthogonal reactions, including the azide–alkyne cycloadditions, either the copper-catalyzed or copper-free reactions, as well as the Staudinger Ligation, have emerged as vital chemical tools (Sletten and Bertozzi, 2009). In examples of powerful applications, the analogs of sugars (Saxon and Bertozzi, 2000), amino acids (Link et al., 2003; Liu and Schultz, 2010), and lipids (Kho et al., 2004; Hang et al., 2007) bearing diminutive reactive tags that represent only minor structural perturbations (azide or alkyne) have been shown to effectively infiltrate biosynthetic pathways. This has been exploited to achieve the bioorthogonal labeling of resulting cell surface glycoproteins, newly synthesized proteins, and posttranslationally lipidated proteins, respectively. In addition, the strategy of activity-based protein profiling has been advanced for the collective labeling of proteins using small molecule probes that label target proteins (Speers et al., 2003; Cravatt et al., 2008).

Despite these advances, technical challenges persist that complicate such applications, particularly when the goal is to isolate and identify labeled proteins and to characterize the location of the labeling event. For this purpose, bioorthogonal chemistry is typically used to introduce biotin onto labeled proteins, followed by subsequent purification via streptavidin enrichment (Figure 1). However, a problem exists: it is challenging to achieve quantitative release of proteins from avidin supports after purification due to the high affinity of the streptavidin–biotin interaction. As a result, there has been considerable interest in devising cleavable affinity tags to release proteins following the labeling and purification stages. To be effective, this chemistry must survive all aspects of the labeling and separation processes, but must be cleanly released using mild conditions so as not to affect labeled proteins and subsequent mass spectrometry-based detection. In this issue of *Chemistry and Biology*, Yang and coworkers (2010) present a detailed study of a diazobenzene system that is shown to be highly effective for cleavage in sophisticated proteomics applications.

In the article, Yang and coworkers (2010) describe the design, synthesis,

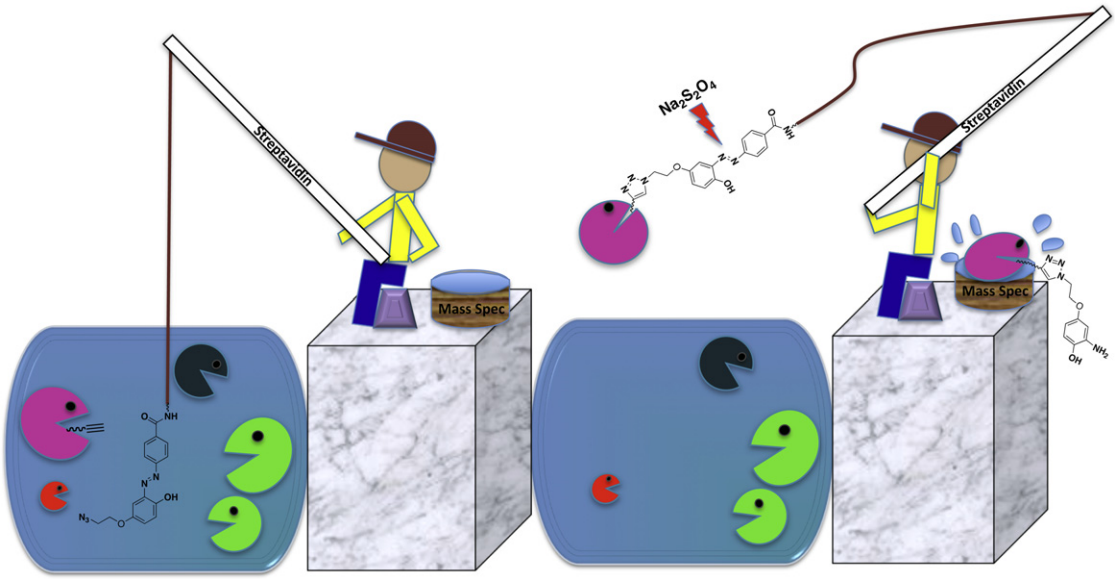

Figure 1. Cartoon Depicting the Purification of Labeled Proteins using a Diazobenzene-Cleavable Linker
Proteins that have been labeled with alkyne- (or azide-) tagged moieties are selectively derivatized using click chemistry and purified through attachment to a solid support. Cleavage of the diazobenzene linker is then used to efficiently release purified proteins to facilitate purification and analysis. Note: for a previous cartoon depicting bioorthogonal labeling using a fishing analogy, see Sletten and Bertozzi, 2009.

and analysis of multiple cleavable diazobenzene reagents to optimize cleavage resulting from treatment with the mild reducing agent sodium dithionite. Initially, a novel synthetic route was devised by which various diazobenzene derivatives could be produced in a more efficient and modular manner. In particular, two motifs were studied, one in which the diazobenzene scaffold contains a hydroxyl moiety *ortho* to the azo group, and another that lacks this substituent. It was initially identified that reagents lacking the *ortho*-hydroxyl substituent did not undergo complete cleavage, even after more than one hour of reaction, but rather became stuck in the form of partially reduced hydrazine intermediates, as evidenced by HPLC analysis. However, the introduction of the *ortho*-hydroxyl group led to complete cleavage, indicating that this group is critical for release. Interestingly, the new modular synthesis described in the article also involved the introduction of an alkoxy group in the *para*-position, which is not sufficient for enhancing cleavage. This again illustrates the particular benefit of the *ortho*-hydroxyl functionality and suggests that the enhanced cleavage may not result from a straightforward electronic effect.

Once this optimized cleavable linker was identified, this scaffold was then employed for more rigorous studies involving the labeling of unnatural amino acid residues incorporated into newly synthesized proteins. These experiments took advantage of the amino-octynoic acid (AOA) and azido-norleucine (ANL)-tagged amino acid systems (Grammel et al., 2010) for metabolic protein labeling in *S. typhimurium*. Initial studies consisting of protein labeling, streptavidin enrichment, diazobenzene cleavage, and in-gel proteomic analysis led to the identification of 456 high-confidence hits of labeled proteins, although this process did not allow for the determination of the sites of protein labeling. However, the latter was achieved using an adapted approach in which lysates were digested prior to enrichment and cleavage. This led to results that varied slightly based on the amino acid derivative used, with AOA leading to 185 peptides corresponding to 73 proteins and ANL resulting in 128 unique peptides that accounted for 65 labeled proteins. Once the described cleavable linker was shown to be effective for proteomics, another variant was designed and synthesized, bearing an added bromine atom to aid in analysis due to the identifiable isotopic patterns in the mass spectra. This system was again shown to be highly advantageous for the identification of metabolically labeled proteins and peptides.

The studies described by Yang and coworkers (2010) put forth the diazobenzene cleavable linker as an invaluable tool for global proteomic analysis of complex samples containing labeled proteins. This approach, in addition to other recent advances employing protease-, pH-, photolysis-, and redox-based cleavable linkers, will facilitate large scale proteomic analysis and thus further advance this technically challenging but rewarding endeavor.

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Making *E. coli* an Erythromycin Production Plant

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The production of bioactive compounds in heterologous hosts has become a valuable tool for the investigation of biosynthetic pathways and their rational engineering. In this issue of *Chemistry & Biology*, Zhang et al. (2010) report the manipulation of *E. coli* for the production of erythromycin A.

Many bioactive compounds, including clinically important antibiotics such as erythromycin, are synthesized by microorganisms that often are only poorly accessible by molecular genetic techniques. Thus, genetic engineering of these biosynthetic pathways requires substantial efforts as basic protocols for gene transfer, DNA isolation, and mutagenesis have to be individually developed for each producing strain. Therefore, the expression of complete biosynthetic pathways in heterologous hosts is a promising way to engineer and improve such compounds. Heterologous expression of small gene clusters encoding biosynthesis of aromatic polyketides has already resulted in good yields (see Lopez et al., 2010) and allowed the manipulation of the pathways by standard methodology. Using this approach, derivatives of the natural compounds could be generated in a much easier way, when compared with engineering the original producing strains. Nevertheless, these studies mostly used closely related expression hosts.

However, basic knowledge of growth, cultivation properties, metabolism and physiology, and the availability of molecular tools are scarce in these heterologous production hosts in comparison with the model organism of choice for molecular biology, *Escherichia coli.* Therefore, many efforts have been undertaken to make *E. coli* available as a host for the heterologous production of secondary metabolites (for review, see Gao et al., 2010); thus far, only few success stories have been reported. These include the production of amorpha-4,11-diene, a precursor of artemisinin (Martin et al., 2003), 2,6-dimethyl-3,5,7-trihydroxy-7-(3′-amino-5′-hydroxyphenyl)-2,4-heptadienoic acid, a precursor of rifamycin (Watanabe et al., 2003), epothilone B and C (Mutka et al., 2006) or the production of several fungal and plant polyketides (for review, see Gao et al., 2010).

In 2001, the biosynthesis of 6-deoxyerythronolide B (6-dEB), the macrocyclic core of the antibacterial macrolide erythromycin A, in a rationally engineered *E. coli* strain was described (Pfeifer et al., 2001). The *E. coli* strain BAP1 was constructed via the expression of the *Bacillus subtilis* phosphopantetheinyl transferase Sfp, and BAP1 was now able to attach the phosphopantetheinyl prosthetic group essential for the activity of the erythromycin polyketide synthase. In addition, its metabolism was optimized to provide the 6-dEB building blocks in sufficient amounts by deleting genes involved in propionate catabolism and by introducing genes for propionyl-CoA and *(2S)*-methylmalonyl-CoA biosynthesis. Initially, yields of 20 mg × l^{-1} of the biologically inactive erythromycin precursor 6-dEB were obtained in the BAP1 host, which could be increased by process optimization up to 1.1 g × l^{-1} (Lau et al., 2004).

In the following years, further steps were described to reconstitute the complete erythromycin biosynthetic pathway in *E. coli*. The combination of two tailoring genes and 14 genes involved in deoxysugar biosynthesis and the attachment of the macrolide megalomicin biosynthetic gene cluster in the *E. coli* strain producing 6-dEB resulted in the heterologous production of erythromycin D and erythromycin C at low titers (Peirú et al., 2005). However, with this approach, the heterologous production of the biosynthetic end product erythromycin A had not been achieved.

In this issue of *Chemistry & Biology*, Zhang et al. (2010) report the reconstruction of the erythromycin A biosynthetic pathway of *Saccharopolyspora erythraea* in *E. coli* BAP1. In addition to the erythromycin PKS genes, the authors assembled 17 genes responsible for deoxysugar biosynthesis, macrolide tailoring, and resistance of *S. erythraea* into two operons, placing *eryBI-BVII and ermE* and *eryCI-CVI, eryF, eryG, eryK* under the control of T7 promoters. In a first approach, a two host strategy was applied: the precursor 6-dEB was produced in the previously described

