Cross-dressing proteins by olefin metathesis

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Chemists have established numerous methods for performing protein conjugations, but metathesis catalysts have largely remained absent from this toolkit. Evidence that proteins bearing allylsulfides undergo cross-metathesis with chosen alkenes in aqueous conditions will allow chemists to harness the power of metathesis catalysts for modifying biomolecules and other water-soluble compounds.

The covalent modification of biopolymers in living systems typically occurs with extraordinary chemoselectivity and regioselectivity. These reactions allow nature to 'accessorize' biopolymers at precise sites with an array of chemical functionalities that may be absent in the limited repertoire of monomer units available to the biosynthetic machinery. Bioorganic chemists have been remarkably successful in establishing efficient synthetic protocols for standard biopolymer chains. The current challenges are to emulate the precision with which nature performs conjugation reactions and to create artificial macromolecular structures not offered by the natural biosynthetic machinery. Two recent manuscripts[1,2] from Davis *et al.* outline new approaches for decorating proteins with chosen appendages. Significantly, their work expands protein modification reactions to include olefin metathesis.

General strategies for bioorthogonal protein modification have included the site-specific incorporation and subsequent reactions of aldehydes, ketones and azides[3]. Chemists are now exploring suitable approaches to use these reactions for elaboration of biomolecular structure in complex milieus. However, bioorthogonality and biocompatibility are not necessarily synonymous. For instance, although Cu(I) can enhance the rate of azide-alkyne cycloaddition reactions, the use of this reaction for *in vivo* applications has been hampered by the toxicity of copper. To overcome this potential limitation, strained alkyne substrates have been explored to enable copper-free azide-alkyne cycloaddition reactions in living systems[4]. This example highlights the importance of developing new bioconjugation methods for researchers seeking chemical tools to probe complex biological systems.

The cross-metathesis reaction is a key part of the organic chemist's arsenal—and an alkene handle would be bioorthogonal—but its use in bioconjugation has remained limited. Typical alkenes have proven to be difficult substrates for the cross-metathesis reaction in water, although these substrates have undergone the related ring-closing metathesis reaction with greater success[5,6]. The studies by Davis *et al.* describe the conversion of a cysteine residue (**Fig. 1**, A) into dehydroalanine on protein surfaces (**Fig. 1**, B)[2]. The α,β-unsaturated amide can undergo a conjugate addition reaction with a thiol to provide a functionalized protein (**Fig. 1**, C). Alternatively, reaction of the dehydroalanine with allylmercaptan affords an allylsulfide group (**Fig. 1**, D), which can undergo cross-metathesis with an alkene in the presence of the Hoveyda-Grubbs metathesis catalyst (HG II)[7,8] to obtain the functionalized protein E (**Fig. 1**)[1]. Davis and colleagues find that allylsulfides are superior substrates for the cross-metathesis reaction in water compared with other modified alkenes, including vinyl and homoallylsulfides, potentially owing to the optimum sulfur coordination to the ruthenium center. The two key developments presented in these studies are generation of the dehydroalanine group as a Michael acceptor on protein surfaces and cross-metathesis of proteins under aqueous conditions, which makes a powerful reaction in organic and polymer chemistry potentially available for the elaboration of biopolymers.

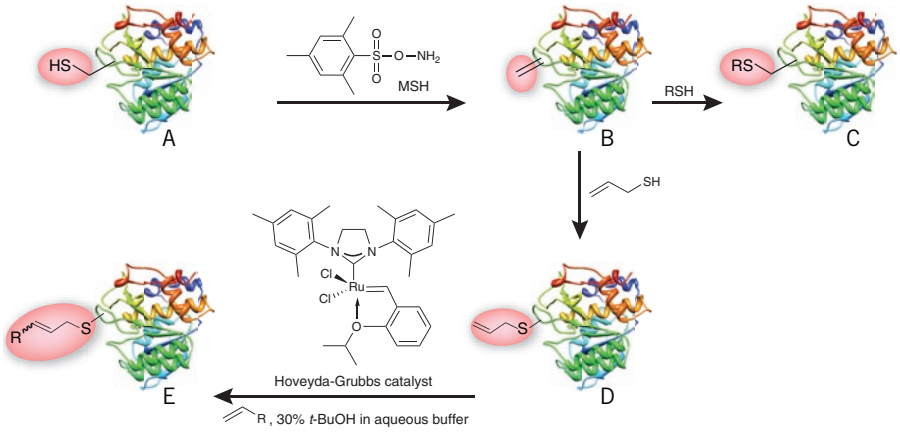

Figure 1 Two approaches for site-specifically adorning proteins with desired functionalities. A cysteine group on the protein surface (A) is eliminated with *O*-mesitylenesulfonylhydroxylamine (MSH) to provide a dehydroalanine residue (B), which can undergo conjugate addition reactions with thiols to afford a functionalized protein (C). Alternatively, the dehydroalanine-protein may be treated with allylmercaptan to obtain an allylsulfide group on the protein surface (D). Allylsulfide groups were found to be effective substrates for the cross-metathesis reaction in the presence of the commercially available Hoveyda-Grubbs II catalyst under aqueous conditions. These studies surmount critical obstacles limiting the use of cross-metathesis reactions in bioconjugate chemistry.

The work by Davis and colleagues raises two follow-up questions regarding the versatility of the metathesis approach and its implementation under physiological conditions. The present study tests metathesis of proteins on a relatively unhindered position. The authors successfully perform cross-metathesis on an allyl substrate placed on a flexible loop of the serine protease subtilisin (**Fig. 1**). It will be valuable to determine whether an alkene group placed on a more sterically demanding position, such as in the middle of a helix, can also interact with the metathesis catalyst. Second, the authors used an alcohol-water mixture to solubilize the metathesis catalyst and the protein. These conditions will likely destabilize many folded proteins. Several water-soluble analogs of the metathesis catalysts, including the Hoveyda-Grubbs catalyst, have been described

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previously, and use of these catalysts may allow metathesis on proteins under completely aqueous conditions[6].

If the metathesis catalyst can be fully operational under physiological conditions, the cross-metathesis reaction may be extended to *in vivo* applications. One strategy for biopolymer modification *in vivo* uses the biosynthetic machinery to introduce new amino acids or saccharides incorporating non-natural functionality, permitting their conjugations through the Staudinger ligation[9] or azide-alkyne cycloaddition reactions[4,10]. The direct incorporation of allylsulfides into protein structures may facilitate the use of the metathesis reaction for *in vivo* applications. In this regard, it is notable that the authors report some incorporation of *S*-allylcysteine into proteins expressed in *Escherichia coli*, evidently as an analog of methionine. Presumably, the suitable positioning of *S*-allylcysteine with this approach would permit direct modification of such proteins through cross-metathesis.

Until now, the goal of chemists has been to identify any rudimentary bioorthogonal methods suitable for labeling proteins. With the increased ability to use different conjugating strategies, we can now be more discriminating in the choice of linkers available for protein conjugation. The protein chemist now has a choice, for example, between using a polar triazole ring obtained by azide-alkyne cycloaddition or a nonpolar alkene group available from cross-metathesis. These tools for protein tailoring permit chemical biologists to accept biopolymers as they are provided 'off the rack' or to outfit them with an increasingly sophisticated set of accessories.

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20S ways to apoptosis

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Chemical inhibitors of the proteasome have received substantial attention owing to the success of bortezomib in the treatment of multiple myeloma. A recent whole-cell screen identified the proteasome inhibitor argyrin A and suggests a new role for p27^{Kip-1} in regulating apoptosis.

The proteasome is a large protease that functions as one of the two major routes of bulk protein degradation in cells[1]. Although the concept was initially received with strong scientific skepticism, proteasome inhibition is now a validated therapeutic approach in cancer[2]. The peptide boronate proteasome inhibitor bortezomib (Velcade, formerly PS-341) has unmatched antitumor activity in people with multiple myeloma (MM)[3]. Interestingly, for reasons that are not entirely clear, preclinical studies have concluded that bortezomib and other proteasome inhibitors kill MM cells via distinct mechanisms, and so combining the two drugs leads to synergistic cell killing, even in MM cells that are bortezomib-resistant[4]. A recent natural-product screen for compounds that promote p27 accumulation identified argyrin A as a new proteasome inhibitor that blocks tumor cell growth via a mechanism distinct from that of bortezomib[5]. The implication of p27 accumulation will cause many in the field to reconsider their ideas about how proteasome inhibition causes cell killing.

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Two general hypotheses have been advanced to explain the cytotoxic effects of proteasome inhibitors. One posits that proteasome inhibition causes the accumulation of particular proteins that induce cell death, while the other suggests that the general accumulation of junk protein that occurs when the proteasome is blocked triggers apoptosis. For example, some previous studies have implicated stabilization of IκBα, the physiological inhibitor of the inflammation-associated transcription factor NFκB[6], expression of Myc[7], or accumulation of proapoptotic members of the BCL-2 family (Bim, Bik and Noxa)[7,8] in bortezomib-induced apoptosis, whereas others concluded that the drug kills cells by stimulating the unfolded protein response (UPR) and endoplasmic reticular (ER) stress.

The p27 protein blocks cell cycle progression by inhibiting cyclin-dependent kinases[9]. Although p27 is rarely inactivated by mutation, its expression level is often reduced in cancers, and Nickeleit *et al.*[5] have predicted that small molecules that restore p27 expression in cancers would have antitumor activity. To test this hypothesis, they developed a high-throughput whole-cell assay to screen a focused natural-product library of myxobacterial metabolites at a relatively high level of stringency (70 nM). Their screen identified argyrin A, and subsequent functional studies demonstrated that it promotes p27 accumulation by inhibiting the proteasome. Intraperitoneal administration of the compound produced strong proteasome inhibition *in vivo* and decreased tumor growth in preclinical models. Importantly, it was efficacious not only in tumors derived from cells that were killed by the drug *in vitro* but also in tumors derived from bortezomib-resistant cells. Mechanistic studies demonstrated that all of the effects of argyrin A are dependent on accumulation of p27 (**Fig. 1**). However, experiments with a mutant form of p27 revealed that its familiar function as a cyclin-dependent kinase inhibitor was not involved, which indicates that some new, unidentified mechanism is at play.

Several independent lines of evidence support their conclusion that argyrin A is a more specific inhibitor of the proteasome than bortezomib. Like argyrin A, proteasome subunit knockdown induced apoptosis via a p27-dependent mechanism, whereas bortezomib did not. Furthermore, gene expression profiling studies demonstrated that the effects of argyrin A are more similar to proteasome subunit knockdown than bortezomib's effects are. Finally, the authors ruled out stabilization of IkBα and Myc accumulation in argyrin A's effects, and their gene expression profiling results did not support a role for ER stress in