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Chemical proteomics: ligation and cleavage of protein modifications

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Bioorthogonal ligation and cleavage methods are of major importance in the field of chemical biology. Recently, there has been significant progress in the improvement of classic ligation procedures as well as in the establishment of new ligation methodologies. Furthermore, the design of cleavable linkers for protein enrichment has lately received much attention. These techniques equip researchers with a wealth of tools suitable for proteomic applications. In order to ease navigation through these diverse systems, we here provide a comprehensive overview of methods that are useful for chemical proteomics.

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Introduction

The attachment of molecular modifications onto proteins for various applications has been a central challenge in the emerging field of chemical proteomics in recent years. As a consequence, the demand for procedures to attach or cleave modifications has been on a significant rise as well. In this review, we intend to provide a brief comparison between current bioorthogonal ligation methods for protein modification and recent cleavage procedures of these moieties with a focus on protein purification and activity-based protein profiling applications (cf. Scheme 1).

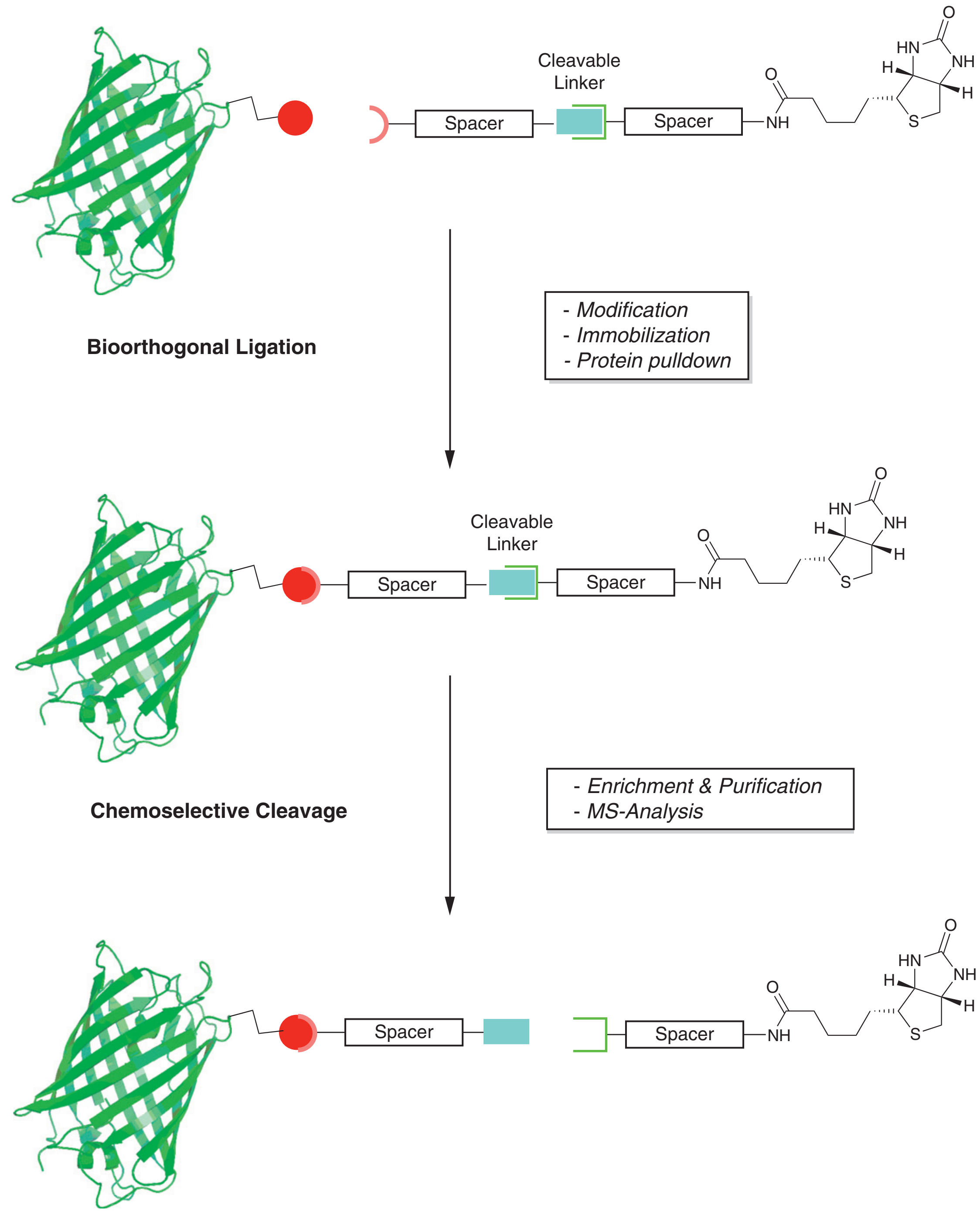
Innovative and relevant bioconjugation methods

A major challenge for ligation methods of molecular entities onto proteins is to facilitate high selectivity within a complex substrate mixture containing a vast array of functionalities. As a consequence, reactions suitable for ligation in chemical biology require chemoselectivity, excellent reliability and biocompatibility. Because of the small amounts of probe and protein present in a typical sample, high yielding processes and mild reaction conditions are essential for addressing fundamental biomolecular applications. Reviewing relevant bioconjugation methods, it is mandatory to start mentioning briefly the historical important Staudinger ligation, a truly bioorthogonal azide–phosphine conjugation with numerous applications in chemical biology. Nevertheless, as this article focuses on recent developments of highly innovative methods in chemical proteomics, the huge area of Staudinger ligation chemistry is recommended to be reviewed elsewhere [1,2].

Modern copper-catalyzed azide–alkyne cycloadditions

The copper-catalyzed azide–alkyne 1,3-dipolar cycloaddition, or CuAAC, has been applied extensively for bioorthogonal purposes since its independent discovery by both Meldal and coworkers [3] and Sharpless *et al.* [4] (Table 1). The acceptance and usage as the prototypical click chemistry reaction in countless applications can be reasoned due to two particular benefits. First, both partners of the CuAAC reaction — alkyne and azide — are readily synthetically available, do not typically occur in a cellular environment and are sterically benign. Secondly, the actual conversion of alkyne and azide to the triazole species proceeds with rapid reaction kinetics, high chemoselectivity and remarkable reliability on basis of robust catalysts. However, the major drawback of CuAAC is its toxicity and denaturing effects in physiological environments caused by the copper(I)-mediated generation of reactive oxygen species (ROS) from solubilized oxygen [5]. One approach to address this issue is the optimization of the conditions for the catalytic system, its additive reagents and their amounts as evaluated by Finn and coworkers in a broad screening [6]. A more fundamental attempt to improve biocompatibility has been established through introduction of catalytic copper systems based on customized copper(I) ligands. Highly water-soluble multidentate *tris*-triazolyl ligands enhance the rate of cycloaddition notably, thereby lowering the necessary concentration of cytotoxic copper(I). Furthermore, these ligands can act additionally as sacrificial reductants for reactive oxygen species. Prominent developments in this CuAAC ligand design, presented in Table 1, are Sharpless' and Fokin's *tris*-(hydroxypropyltriazolylmethyl)amine (THPTA) [7], and more recently Wu and coworkers' bis[(*tert*-butyltriazolyl)methyl]-[(2-sulfatylethyltriazolyl)methyl]-amine (BTTES) [8] as well as bis[(*tert*-butyltriazolyl)methyl]-[(2-carboxymethyltriazolyl)methyl]-amine (BTTAA) [9••]. Both the BTTES and BTTAA ligands show excellent activities with rate increases orders of magnitude higher [10] than present

Scheme 1



strained cyclooctynes (cf. below) [11] while revealing high biocompatibility. For instance, labeling live cells on the surface via metabolic incorporated azides was demonstrated successfully by this method while outperforming competitive methods [9••].

A different approach to increase CuAAC activity while decreasing copper-concentration was more recently established by Ting and coworkers [12••]. Herein, the key step has been the raise of the effective copper(I) concentration at the reaction site via pre-coordination of the catalytic metal centre by a chelating azide substrate (Table 1). Remarkably high reaction rates both *in vitro* and *in vivo* with only 40–100 μM. $CuSO_4$ have been achieved. However, the limited availability of azide substrates featuring the essential pyridine donor yet