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Digest paper

Bioorthogonal chemistry: Optimization and application updates during 2013–2017

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ABSTRACT

Bioorthogonal chemical groups to tag naturally occurring biomolecules in their native setting is a powerful tool for studying and manipulating biological processes, whereby unique functional groups incorporated into target biomolecules can be detected in a second step by using selective partners. On the other hand, bioorthogonal cleavage reactions enables chemically controlled spatiotemporal activation of intracellular proteins and prodrugs. Considerable attention has been focused on the bioorthogonal reactions, not only optimizing the known bioorthogonal reagents to gain fast reaction kinetics, high stability of the reagents and the products, but also extending new applications as well as developing new types of bioorthogonal reactions.

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Introduction

Bioorthogonal chemistry has emerged as a new powerful tool that facilitates the study of structure and function of biomolecules and cellular manipulations within living systems.[1–4] A handful of bioorthogonal reactions that can proceed selectively and efficiently under physiologically relevant conditions are now available, featured with fast kinetics, tolerance to aqueous environment, high selectivity and compatibility with naturally occurring functional groups.[5,6] In the past decade bioorthogonal reactions have been widely used for imaging, detection, diagnostics, drug delivery, and biomaterials.

Imaging living organism in real time has become viable with the discovery of the green fluorescent protein (GFP), greatly extending our understanding of biological processes.[7] However, the huge size of GFP might interfere with the normal function of the target protein and the site-specific labeling is challenging. Furthermore, the GFP is not amenable to labeling those non-genetically encoded biomolecules, such as lipids, glycans, nucleic acids as well as the

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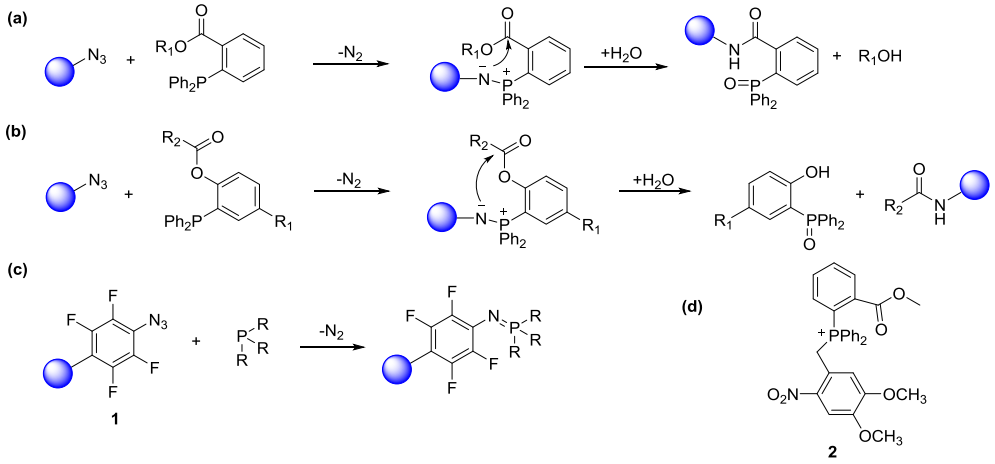

Scheme 1. The mechanism and development of Staudinger reaction. (a) Staudinger ligation. (b) Traceless Staudinger ligation. (c) PFAA Staudinger reaction. (d) Photocaged phosphines **2**.

posttranslational modifications (PTM) of proteins. To this end, bioorthogonal reactions are developed to be a powerful compliment to study an array of biomolecules in their native environment.[8] Ideal bioorthogonal reactions must be selective over other potential reactive functional groups present in biomolecules, and they should occur efficiently under physiological conditions. So far, there are many methods to introduce various bioorthogonal reactive groups into biomolecules in living systems, especially it has become available recently to incorporate unnatural amino acids bearing functional groups into proteins produced in cells. In general, the biomolecule can be selectively labelled by a bioorthogonal reaction with a complementary reagent bearing a fluorescent probe or affinity handle.[1] Some bioorthogonal reactions can be further applied to animal tissue imaging, drug delivery, and biomaterials.[9] This review will highlight the most recent development of bioorthogonal reactions, especially the new progress within the recent five years, focused on the optimization on the bioorthogonal reagents to improve the reaction rate, stability of the substrate and the biocompatibility, developing new reaction types and new applications in the biological system and therapeutics.

Staudinger ligations

Bertozzi and co-workers first modified the triarylphosphine derivatives by introducing ester groups on the aromatic rings for the Staudinger ligation. An aza-ylide intermediate was generated with the release of N_2 then underwent a nucleophilic attack to the ester group to yield a stable amide linkage when exposed to water (Scheme 1a). Shortly after the report on Staudinger ligation, a new methodology for forming amide bond was described, namely traceless Staudinger ligation, which adopted acylated diphenylphosphorphenol ester to prevent relatively large phosphine oxide fragment from remaining in the final labelled proteins (Scheme 1b).[10] The Staudinger reaction requiring no catalyst and exhibiting exquisite chemoselectivity has been widely used in biological studies in *vitro* and in *vivo*. However, one main drawback of the Staudinger ligation is its slow kinetics. The second-order rate constant of the reaction is just $\sim 10^{-3}$ M^{-1} s^{-1} with high concentrations of the reagents. Another limitation is the phosphines liable to oxidation. Recently, two groups reported optimized Staudinger reagents to address this issue. Yan and co-workers developed a Staudinger ligation using perfluoroaryl azides (PFAAs, **1**) to react with aryl phosphines, generating stable iminophosphoranes under ambient conditions with a much higher rate constant of 18 M^{-1} s^{-1} (Scheme 1c),[11] than the classic Staudinger ligation reaction. This reaction successfully labelled A549 cells by expressing the PFAAs groups on the cell surface, then reacting with phosphines containing fluorescein isothiocyanate (FITC). Carrico and co-workers reported a 4,5-dimethoxy-2-nitrobenzyl-caged phosphine (**2**) that is inert to oxidation and efficiently produced reactive phosphines under UV light. This photocaged phosphines can efficiently undergo Staudinger ligation to label the target protein in HEK293 cells and on live zebrafish.[12] But the slow reaction rate may still remain an obstacle for extensive biological applicability.

Further effort to improve the reaction rate was to make use of the small and reactive cyclopropenone as the bioorthogonal reagent. Prescher group reported a ligation between cyclopropenones and phosphines.[13,14] Although the reaction mechanism is different from that of Staudinger ligation, they share the phosphine reagent in common, therefore we still classify this type of reaction here. This reaction yielded a ketene-ylide intermediate by Michael-type addition of triarylphosphine to the cyclopropenone, followed by a nucleophilic attack to give the α,β-unsaturated ligation product rapidly (Scheme 2a). While monosubstituted cyclopropenones were observed to react robustly with phosphines, they were susceptible to reaction with cysteine and other biological thiols at pH >7, which limits their intracellular labeling and other utilities. The optimized disubstituted cyclopropenones were less prone to thiol attack, gaining improved stability and maintaining robust reactivity with bioorthogonal phosphines. Importantly, the stabilized dialkyl cyclopropenone scaffold was suitable for recombinant protein production via genetic code expansion, then used for sequential labeling in intracellular environments, since the products of the cyclopropenone ligation were also amenable to traceless Staudinger ligations (Scheme 2b).

Carbonyl ligations

The aldehydes and ketones can be easily introduced into cell surface by several methods, making them a powerful tool for ligation in biological systems. The formation of oximes or hydrazones with α-effect nucleophiles such as hydroxylamines or hydrazines has been successfully applied to cell surface protein labeling. However, this conjugation needed acidic conditions to achieve an appreciable reaction rate (Scheme 3a). Since some types of proteins can't tolerate acidic conditions, the conjugation reaction needs proceeding at physiological pH, in which the conjugation rate is very slow. In addition, the condensation is reversible as the product can undergo hydrolysis in aqueous media. As a result,