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The recent developments and applications of the traceless-Staudinger reaction in chemical biology study

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Bioorthogonal reactions and their applications in selective protein manipulations, such as labeling, localization, and post-translational modification studies, are one of the most important themes in the current chemical biology frontier. Studies in recent years have demonstrated that the traceless-Staudinger reaction has been turned into a typical bioorthogonal reaction with versatile accessibility, high efficiency, and reliability. Many of its potential applications have been investigated in life science and chemical biology studies. Herein, the current developments and mechanism studies of traceless-Staudinger reaction are summarized. The typical usages in the chemical biology field will also be discussed, hoping to contribute to further modifications and developments *en route*.

1. Introduction

In the post-genetic era, more and more novel post-translational modifications (PTMs) of proteins have been discovered, whose regulatory functions in metabolic pathways are still unknown, but challenging to be deciphered.[1] Preparation and functional studies of proteins with PTMs are becoming very limited using traditional genetic engineering and molecular biology techniques. Therefore, the discovery and development of novel and robust bioorthogonal reactions would provide promising tools to shed light on puzzles in chemical biology.[2]

The term 'bioorthogonality' is usually used to describe a chemical reaction that can occur with high efficiency, specific selectivity, low toxicity and high biological tolerance under mild aqueous conditions.[3] These reactions are widely used to the modification of biomacromolecules such as proteins, nucleic acids, polysaccharides, and lipids. The bioorthogonality can be roughly divided into three levels according to the targets and purposes of applications: (1) bioorthogonal reactions *in vitro*, where the only requirement is no side reaction over other functional groups on the biomolecule;[4] (2) the reagents cannot react with any other components in the system for the reactions in cell lysate or localized died cells;[5] (3) the reactions have no influence on cellular metabolism conditions *in vivo*, such as in living cells,[6] tissue or even entire higher animal level.[7] To achieve those goals, bioorthogonal reactions always proceed between specially designed external reagents and certain unnatural groups incorporated into biomolecules (such as proteins, saccharides), or the specific recognition between enzymatic domains with their substrate small peptide chains.[8]

In the beginning, native amino acid residues were utilized in bioorthogonal reactions for labeling, ligation (bioconjugation formation), or functionalization of proteins *in vitro*.[9] The thiol and amino groups are widely used for nucleophilic reactions, including amide formation,[10] disulfide bond formation,[11] maleimide thiol–ene reaction,[12] *etc.* (Fig. 1a). However, these reactions mentioned above always suffered from the low chemical selectivity and couldn't achieve single-site labeling, unless all the other amino acids (such as cysteine whose natural

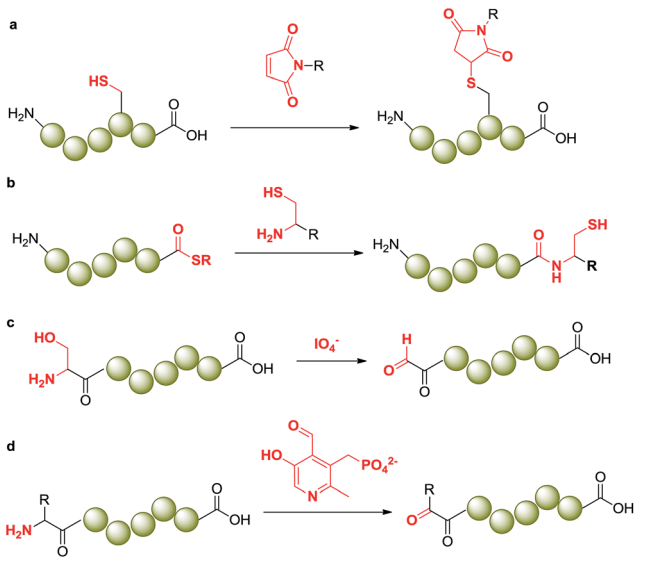

Fig. 1 Typical bioorthogonal reactions utilizing native amino acid residues for protein modifications.

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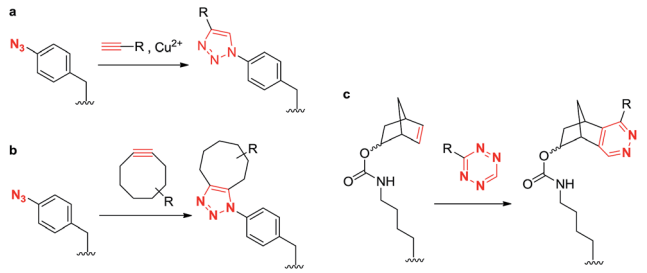

Fig. 2 Three types of ncAA-based bioorthogonal reactions.

abundance is the lowest[13]) were mutated to leave a single site for labeling.[14] An alternative method is the specific labeling on C-terminal or N-terminal by native chemical ligation (NCL) related methods[15] (Fig. 1b) or serine periodate oxidation[16] (Fig. 1c) and biomimetic transamination reaction *via* pyridoxal-5′-phosphate (PLP)[17] (Fig. 1d). Those strategies can only label one site on a single protein, but still fail to selectively label one protein in cell lysate or living cell conditions.

The desired functional groups can be introduced into proteins at any site for bioorthogonal reactions by the non-canonical amino acid incorporation (ncAA) technique.[18] These artificial groups can undergo large number of bioorthogonal reactions with high chemoselectivity, such as the (Cu-free) click reaction (Fig. 2a and b), tetrazine inverse-electron-demand Diels–Alder (iEDDA) reaction (Fig. 2c).[19,20] Those chemoselective reactions facilitate modifications of biomolecules in cell lysate or living cells.[21] The click reaction and Diels–Alder reaction have been introduced in recent reviews.[22] Herein, the recent methodology development and biological applications of traceless-Staudinger reaction will be summarized.

2. The discovery and development of traceless-Staudinger reaction/ligation

2.1 The discovery of traceless-Staudinger reaction

Early back to 1919, Staudinger[23] found that the nucleophilic attack of the triphenylphosphine to the azide terminal nitrogen atom, followed by the release of nitrogen gas, could generate an active aza-ylide (or phosphinimine). The negative charge containing nitrogen in aza-ylide, similar to carbon–phosphine ylide, could undergo nucleophilic attack to the carboxyl group if existed, or simply got hydrolyzed (Fig. 3a).

Bertozzi *et al.* first employed Staudinger reaction for protein labeling by designing an *o*-phosphine terephthalic ester, whose adjacent carbonyl group could be attacked to generate native amide bond linkage under mild conditions. However, the relatively large triphenylphosphine structure remained in labelled proteins, which might change their native structures and affect their natural functions (Fig. 3b). To eliminate this potential influence, triphenylphosphine-*o*-carboxyl methanol ester was replaced by acylated diphenylphosphorphenol ester in which the triphenylphosphine oxide structure could be released after the ylide attacking the adjacent carbonyl group.[24] This new methodology for peptide ligation by forming amide bond was named as traceless-Staudinger reaction/ligation (Fig. 3c). It's noted that Raines group[25] also developed another type of thioester based phosphine reagents for traceless-Staudinger reaction. The phosphine reagents could form thioester by thiol exchange reaction, followed by the ligation with another N-terminal azide containing peptide (Fig. 3d).

One of the advantages of traceless-Staudinger reaction is its capacity to form native amide bond. However, the original traceless-Staudinger reaction has several limits. First, the second order rate constants (10^{-3} M^{-1} s^{-1}) of those Staudinger reactions are much lower than copper-catalyzed azide-alkyne cycloaddition (CuAAC) (10–200 M^{-1} s^{-1}), tetrazole-based Diels–Alder cycloaddition (1–10^4 M^{-1} s^{-1}), and cross-metathesis reaction (~0.3 M^{-1} s^{-1}) *etc.*[26] Second, the triphenylphosphine reagents have a relatively low solubility in aqueous buffer, and thus organic solvents such as DMF or THF are usually added with middle to high ratio (over 50%) to the reaction system. Besides, the excess amount of acylated phosphine esters may cause side reactions.[27] For example, acylated phosphine esters may react with the nucleophilic amino acids of unprotected peptides or proteins.[28] Those problems significantly limited the widespread applications of traceless-Staudinger reactions in chemical biology studies.

2.2 The development of traceless-Staudinger reaction

In order to overcome the disadvantages of Staudinger reaction mentioned above, tens of modified phosphine reagents have been developed. Bertozzi group[24] first developed the phosphine ester **1–4** (Fig. 4). The results showed that phosphine phenolic ester **1** underwent ligation smoothly but phosphine alkyl ester **2** hydrolyzed nearly in the model system. The reduced amide formation rate might due to the flexibility of the methylene group in the aza-yield intermediate, which was overridden by the hydrolysis process. To increase the reaction rate for phenolic ester **1**, they introduced imidazole phosphine into **3** to kinetically activate the carbonyl groups. Unfortunately, the reaction rate turned out to be slower than the case for **1**. This fact was probably caused by the electron-withdrawing effect of imidazole, which decreased the nucleophilicity of phosphorous atom. Additionally, the Staudinger reaction of the more activate phosphine phenolic thioester **4** also released only the hydrolysis product.

Raines group studied phosphine thioester derivatives as phosphine reagents for Staudinger reaction[25] and found that alkyl thioester **5** as phosphine reagent could give much higher ligation yield (91%) compared with phenolic thioester **4** (<10%).[29] They used this reagent to the protein localization on chips, and no obvious side-reaction was observed[24,30] compared to Bertozzi's phosphine **4**. Viola group synthesized phosphinoethane thioester **6** for ligation and found the reaction proceeded rapidly and quantitatively at Gly–Gly ligation site.[31] During the traceless-Staudinger reaction of peptides, huge differences in ligation efficiency over different C-terminal amino acid residues of peptide thioester were observed.[32,33] The ligation at C-terminal glycine residue could reach to over 90% yield, while non-glycine site or the existence of other stereo group (such as

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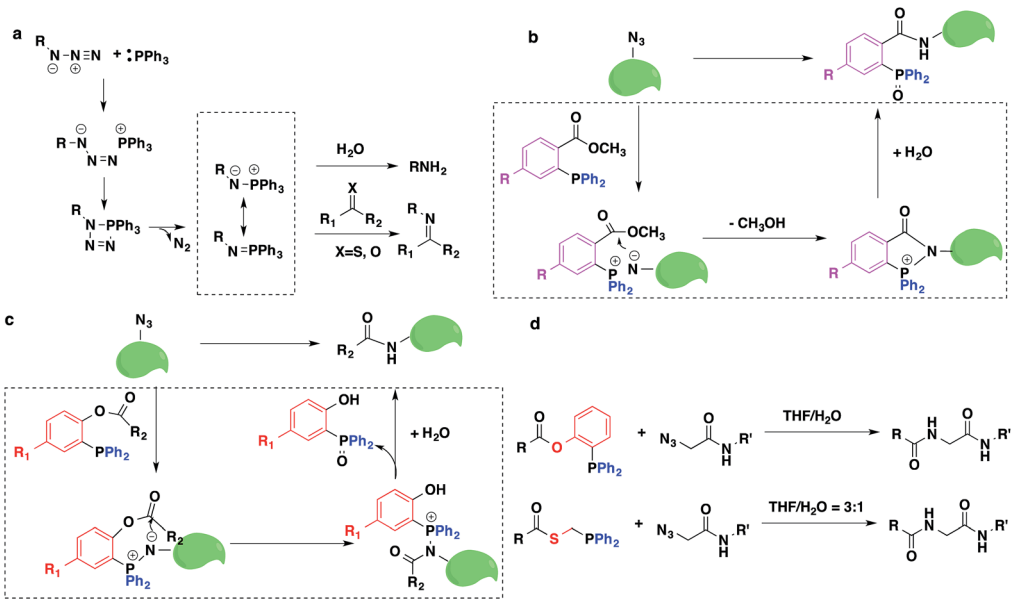

Fig. 3 The mechanism and development of traceless-Staudinger reaction.

glycopeptide[34]) significantly decreased the ligation efficiency. Mechanism study of thioester-based Staudinger ligation indicates that the tetrahedral thioester intermediate **17** (Fig. 5) may undergo two competitive processes: (1) the process I is that breaking the C–S bond gives a amide intermediate **18** (Fig. 5), which can further hydrolyzed to generate the product; (2) the process II is that the addition of the oxygen atom to the phosphorous center forms a bridge oxazaphosphetane **19** (Fig. 5) to give phosphonamide. For the non-glycine sites, the bulky side chains make the process II more favorable to get removed and produce undesired side product. To avoid the side reaction, the nucleophilicity of center phosphorous atom should be reduced. Based on this principle, *p*-chloro **7** and *p*-methoxyl **8** (Fig. 4) substituted derivatives were synthesized and the results showed methoxyl group could significantly improve the ligation efficiency with an 84% at phenylalanine site. Specifically, no such side product according to the process II was observed.

Another problem of Staudinger reaction is the solubility of phosphine reagents. Staudinger reactions mentioned above underwent in organic solvent, which limited their further applications in biological systems. Therefore, Raines's group

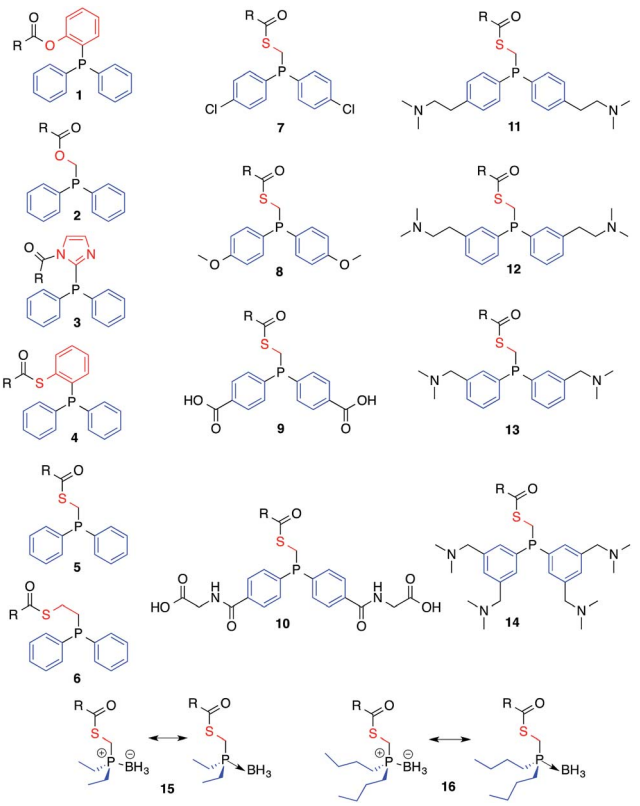

Fig. 4 Chemical structures of different phosphine reagents for traceless-Staudinger reaction.

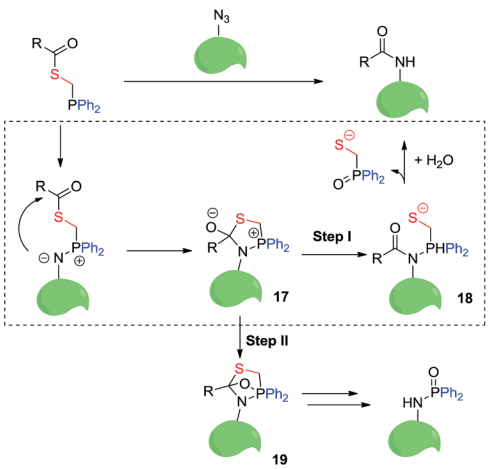

Fig. 5 The possible side reaction of traceless-Staudinger reaction.

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synthesized derivatives with *para*-water-soluble groups, such as carboxyl group **9**, carbonyl glycine **10**, aminoethyl **11** (Fig. 4). It's found the *p*-carboxyl substituted derivative was insoluble, while the *p*-carbonyl glycine derivative could dissolve in 0.4 M of phosphor buffer (pH 7.8) with a concentration up to 50 mM.

Although the increasing of pH could increase the water solubility of both compound **9** and **10**, the high pH also raised the risk of hydrolysis of peptide thioesters. Therefore, they further designed and synthesized the *p*-aminoethyl substituted thioesters **11**. The incorporation of tertiary amine can avoid the intramolecular S-to-N acyl shift. Moreover, the nitrogen atom on the aza-ylide structure contains high negative charge, which can be easily protonated under aqueous solution. Besides, the introduction of two *p*-aminoethyl groups would significantly reduce protonation level of the core nitrogen atom and decrease possible side reactions. With the help of the different versions of water-soluble phosphines, the traceless-Staudinger reaction was achieved in organic-free aqueous solutions for the first time.[35] Furthermore, Raines *et al.*[36] synthesized other aminoalkanyl-substituted phosphines as **12**, **13**, and **14** (Fig. 4). By comparing the solubility of these derivatives under different pH conditions, they found the protonated *m*-diaminomethyl phosphine could generate the largest Coulomb rejection to core nitrogen atom in aza-ylide if got protonated. For maximum efficiency, increased solubility can avoid the side reaction and increase the ligation yield in aqueous buffer.

Additionally, the phosphine reagents with two benzene rings are highly unstable. To stabilize phosphine reagents, boron-based protection groups were developed to coordinate the lone pair with the empty orbital. Bulky bases such as 1,4-diazabicyclo[2.2.2]octane (DABCO) can remove boron groups quantitatively. Kiessling group[37] first introduced this protecting method for the synthesis of thioesters **15** and **16** (Fig. 4) and further applied them into the preparation of *N*-glycopeptide.

2.3 The mechanistic and kinetics studies of Staudinger reaction

Bertozzi *et al.*[38] pioneered in the study of Staudinger reaction mechanism by ^{31}P-NMR. The reactant **20**, product **24**, and aza-ylide **21** was easy to identify. However, the intermediate **21** might go through two pathways (intermediate **22** or **23**) to form product **24**. The oxazaphosphetane **22** was possibly assigned to the peak at −51.47 ppm, while the intermediate **23** was not identified (Fig. 6a). Furthermore, 2D NMR data indicated the existence of the intermediate **26** with a trigonal bipyramidal configuration.[39] However, **22** was ruled out clearly because of the mismatched coupling constant. The rate-limiting step of the reaction is another issue. In general, there would be two "contradictory" steps: in step I, the phosphine reagent attacks the terminal nitrogen atom in the azide (conjugation **25**); in step II, the negatively charged nitrogen attacks back to the center phosphorous atom (conjugation **21**). In most cases, the rate-limiting step is the step I. For example, the reaction between triphenylphosphine and benzoyl azide **27** (Fig. 6d) applies second-order kinetics with a k_1 lower than k_2. However, when reacting with benzenesulfonyl azide **28** (Fig. 6d), the reaction shows a unimolecular kinetics with k'_1 higher than k'_2, caused by the dramatic electron-withdrawing effect of the sulfonyl group. This contradictory implies a potential barrier to improve the reaction rate: any trials of increasing the nucleophilicity of the phosphorous may also cause the decrease of k_2 as a side effect. The kinetic mechanism of traceless-Staudinger reaction was also studied by Raines *et al.*[40] Thioesters was used as phosphine reagents and the result found the existence of the oxazaphosphetane intermediate by using ^{13}C–^{31}P COSY NMR spectra. The oxazaphosphetane could lead to formation of phosphonamide as side reaction. However, the attempt fail to isolate the intermediate. To summarize, the mechanisms of Staudinger reaction remain to be clarified.

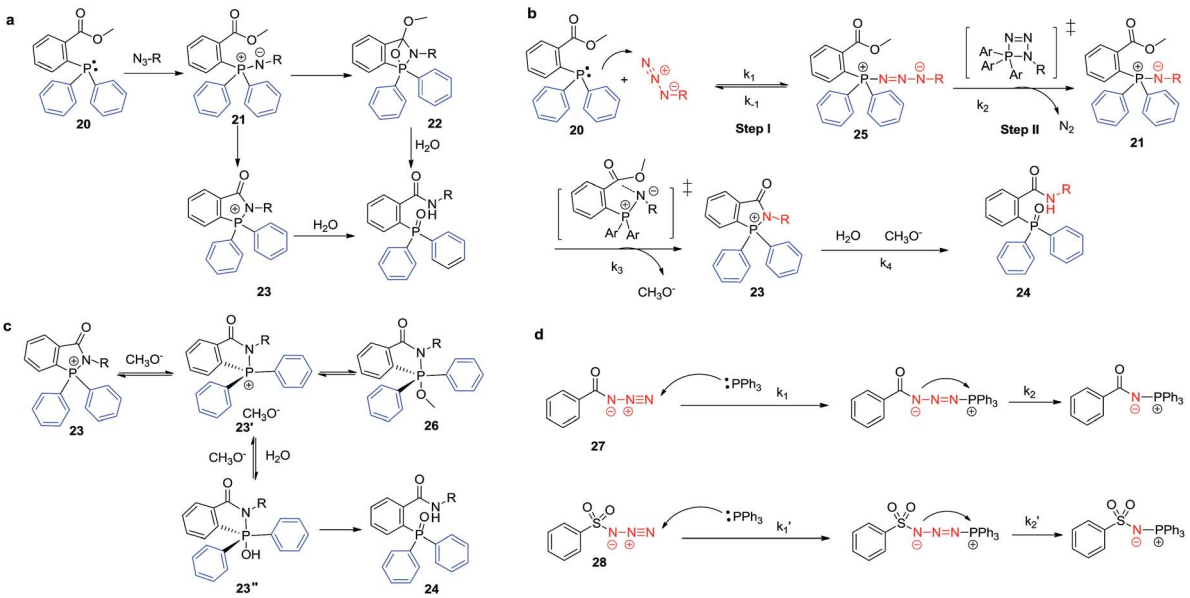

Fig. 6 Mechanism and kinetic study of traceless-Staudinger reaction.

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3. Other derivative Staudinger reaction

Besides the diverse versions of phosphine reagents, some phosphine-based reaction systems have also been developed.

3.1 Staudinger-phosphite reaction

Staudinger-phosphite reaction was introduced as a novel bio-orthogonal reaction by the Hackenberger group. Phosphite, or trialkoxyphosphine, which contains a core phosphorous atom with oxidation state, can also occur nucleophilic reaction with azide group to generate aza-ylide. The phosphite aza-ylide further transfers to phosphonamide by leaving an alkoxyl group (Fig. 7a). A photo-caged phosphite reagent was synthesized and reacted with an azidophenylalanine-containing protein[41] to generate a phosphotyrosine analog as phosphorylated protein mimic (Fig. 7b),[42] or to achieve the PEGylation of a certain target protein.[43] The PEGylation of N-terminal azide groups by Staudinger-phosphite reaction can increase the stability of peptides and proteins in living cell.[44]

3.2 Staudinger-phosphonite reaction

The reaction between azide and phosphonite group was also developed as a novel Staudinger-phosphonite reaction. The dialkoxymonoalkylphosphine, which can be regarded as highly unsymmetrical phosphite, can also nucleophilic attack the azide by the phosphorous atom. After the formation of aza-ylide, only two alkoxyl groups can be released and generate unique product with alkyl group. Owing to the high reductivity and instability, boron groups were used to protect the phosphonite for the introduction PEGylation into calmodulin (Fig. 8a).[45] Besides, Hackenberger and coworker synthesized boron-based dialkoxymonoalkynephosphine, in which the alkyne could undergo CuAAC[46] with protein component **1** (Fig. 8b green). After the removal of copper ion, the phosphonite

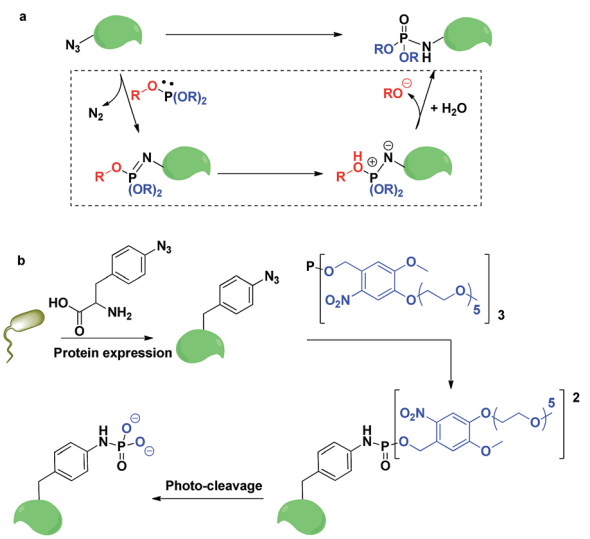

Fig. 7 The Staudinger-phosphite reaction.

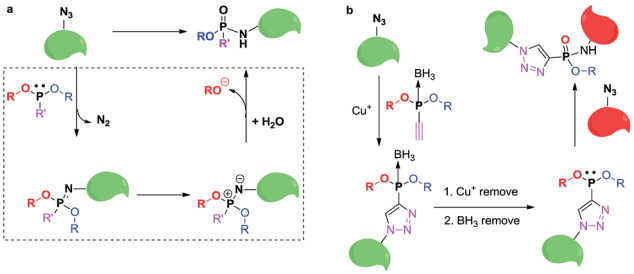

Fig. 8 The Staudinger-phosphonite reaction.

product could further react with another protein component (Fig. 8b red) and achieved the bioconjugation of two azide-containing proteins.

3.3 Intermolecular Staudinger reaction

Another more specific Staudinger reaction was known as intermolecular Staudinger reaction, which was discovered in 1921.[47] In this reaction, triphenylphosphine is used to attack the azide group to obtain aza-ylide. The aza-ylide can further nucleophilic attack to any existing electrophilic groups, such as carboxyl groups, to generate traceless native amide bond linked product. Kosiova *et al.*[48] utilized the reaction to label azide-containing nucleosides with different coumarin or ferrocene carboxylic acid and got over 50% isolation yields.

4. The applications of Staudinger reaction

Staudinger reactions possess high selectivity and good biocompatibility, and have been widely used in biological studies.

4.1 The applications of Staudinger reaction *in vitro*

One of most important applications of Staudinger reaction *in vitro* is the traceless peptide ligation of the protein.[49] The Raines group achieved the total synthesis of Robnuclease A by the combination of traceless-Staudinger reaction/ligation and NCL. In this method, diphenylphosphinomethanethiol ester dipeptide unit (110–111) was ligated with the N-terminal azide group of the third fragment (112–124) on-resin by the traceless-Staudinger reaction. Then, the ligation fragment (110–124) reacted with the expressed first segment (1–109) *via* NCL to obtain the objective protein (Fig. 9). Additionally, the traceless-Staudinger ligation strategy was employed to prepare cyclic peptides. The boron protected phosphine thioester was treated with DABCO and then underwent cyclization of peptides.[50] Besides, Staudinger-phosphite reaction was used to synthesize glycolpeptide linked by phosphonamide bond.[51]

4.2 The applications of Staudinger reaction in cell lysate systems

Traceless-Staudinger ligation has already been widely used in the cell lysate or localized living cell systems for the labelling, localization, and functionalization of certain biomolecules. In

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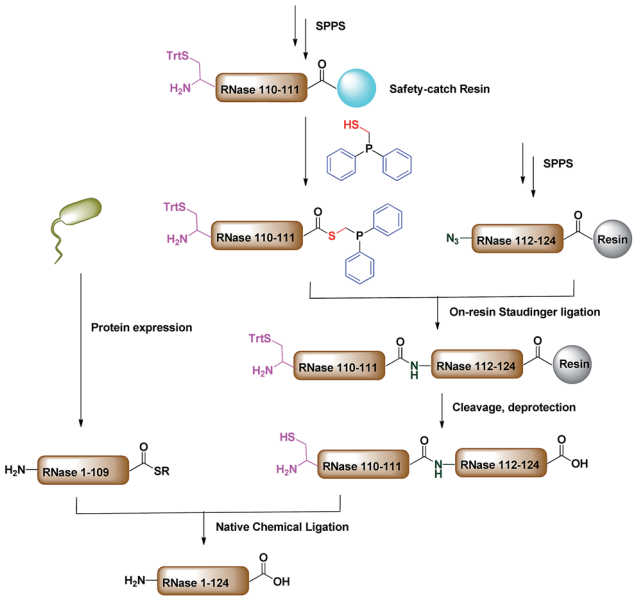

Fig. 9 The applications of traceless-Staudinger reaction *in vitro*.

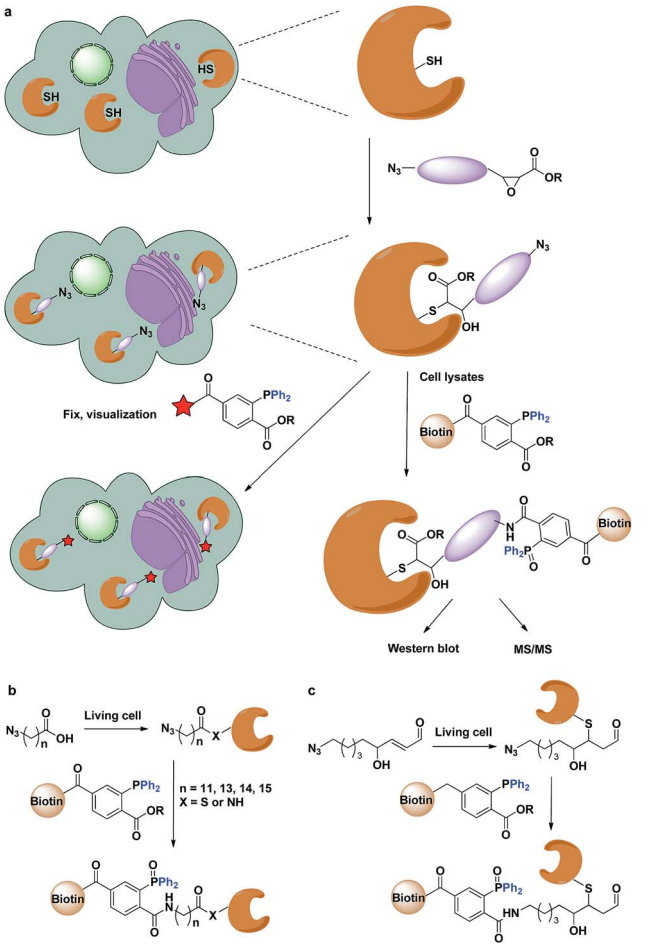

Fig. 10 The applications of traceless-Staudinger reaction in cell lysate related systems.

2006, Hang group[52] designed a group of mechanism-based probe for the cathepsin cysteine protease in tissue. The epoxyethane structure in the azide-containing small peptide probes could covalently link to cysteine proteases after entering the macrophage cells. With the help of Staudinger reaction, the proteins modified by azide group could not only react with biotinylated phosphine reagents for Western blot or MS/MS analysis, but also labeled by fluorescence dye for *in situ* visualization (Fig. 10a). One year later, Hang group[53] introduced the traceless-Staudinger reaction into the metabolic-labeling field. They first introduced ω-azide substituted long chain fatty acids into cell culture, which would be further taken into cellular metabolic process and covalently label certain proteins. By adding biotinylated phosphine reagents to the cell lysate, those proteins with long chain fatty acid PTMs could be fished out for further Western blot analysis (Fig. 10b). Similar strategy can also be applied to some products coming from lipid oxidation in cell lysate, such as 4-hydroxynonenal (HNE), which may covalently modify cellular proteins. By introducing an azide group at the terminal of HNE, the Staudinger reaction under the cell lysate conditions were used for the identification of target proteins (Fig. 10c).[54]

4.3 The applications of Staudinger reaction *in vivo*

Staudinger reaction can be performed in living cell because of its orthogonality to cellular metabolic processes. Additionally, one of advantages of Staudinger reaction is the no detected cellular toxicity. Bertozzi group[55] reported the pioneering work about the labeling of cell membrane glycoproteins. First, the azidosugar were incorporated into membrane glycoproteins. Then, biotin phosphine reagent was added to undergo Staudinger reaction with the azide group, followed by the fluorescence visualization with FITC-avidin or the analysis by flow cytometry (Fig. 11a).

By involving azidesugar into oligosaccharide chains, the Staudinger reaction can be used as a versatile tool for the modification, detection, functionalization, and remodeling of the cell surface glycoproteins. Glycosylation of membrane proteins are of great significance to the cell identification, immune reorganization, virus infection and so on. Different monosaccharides were fed to the living mice daily for a period of time, followed by the collection of the tissue cells from different organs.[56] The Staudinger reaction was applied to add a Flag-tag to the azide-containing glycoprotein, which was further used for Western blot analysis (Fig. 11b). The same strategy can also be applied to the *O*-linked glycosylation profiling in living mice,[57] and sialic acid biosynthesis and deliver studies.[58] Additionally, through the incorporation of a certain kind of monosaccharide, activity-based probes can be designed for those enzymes with specific interaction at proteome level.[59] By visualization *in situ* or western blot *in vitro* of biomolecules, Staudinger reaction provides a powerful chemical tool for the study of intercellular immune processes[60] or intracellular protein metabolism,[61] which raises its role in the current metabolic and glycomic study.[62]

Another worthy application of Staudinger reaction is turn-on fluorescence probes for living cell labeling. Unlike normal

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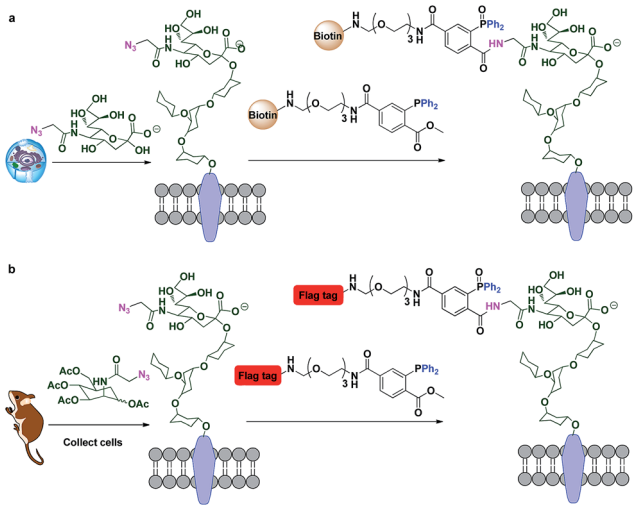

Fig. 11 The applications of traceless-Staudinger reaction in living cells/*in vivo*.

bioorthogonal reaction as A adds B to form A–B, Staudinger reaction can be abstracted as A–B adds C to form A–C with the leaving of B. Bertozzi group[63] reported the quenching effect of the lone pair of the core phosphorous atom to some coumarin dyes in 2003. The Staudinger reaction, in which the phosphorous atom got oxidized, sequentially regenerated the fluorescence (Fig. 12a). However, due to the high reductivity of phosphine reagents, the oxidation process can easily occur by air and cause serious non-specific side reactions. To overcome this problem, another fluorescein stimuli-responsive system quenched by azobenzene (Fig. 12b) was developed.[64] Both systems mentioned above were further tested on mice dihydrofolate reductase (mDHFR) with azidohomoalanine incorporated as model protein, while the fluorescein reagent was used for living HeLa cell surface labeling. For the Staudinger reaction, the azidohomoalanine can be incorporated to a protein of interest (POI) efficiently as Methionine analog.[65] Similarly, turn-off fluorescence systems would also be used for imaging in living systems (Fig. 12c).

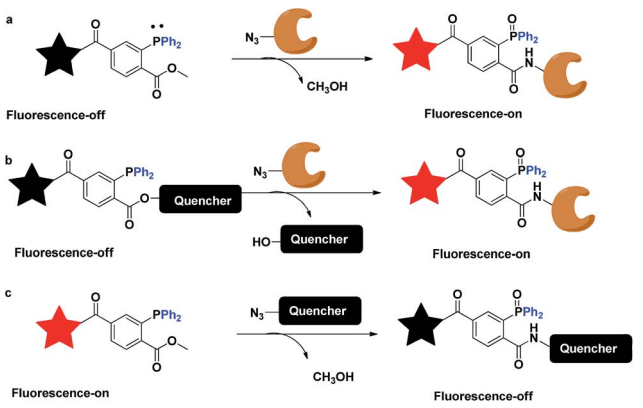

Fig. 12 The application of traceless-Staudinger reaction for turn-on fluorescence labeling.

5. Conclusion

The traceless-Staudinger reaction is one of the most promising and useful bioorthogonal reaction.[66] Using chemical synthesis,[67] ncAA technique or bio-engineering technology,[68] azide groups can be incorporated easily into target biomacromolecules.[69] The azide-containing peptides/proteins then reacts with phosphine reagents to generate native amide bond linkage by nucleophilic attacking and the intramolecular rearrangement. The reaction can undergoes with high chemoselectivity, low toxicity, and good bio-compatibility *in vitro* and *in vivo*. These outstanding characters make the traceless-Staudinger reaction as a versatile chemical tool in biology to solve increasing number of important and challenging biology problems.[70]

Acknowledgements

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Notes and references

1 J. A. Latham and S. Y. R. Dent, *Nat. Struct. Mol. Biol.*, 2007, **14**, 1017.
2 Z.-P. Wang, Y.-H. Wang, G.-C. Chu, J. Shi and Y.-M. Li, *Curr. Org. Synth.*, 2015, **12**, 150.
3 E. M. Sletten and C. R. Bertozzi, *Acc. Chem. Res.*, 2011, **44**, 666.
4 P. Agarwal and C. R. Bertozzi, *Bioconjugate Chem.*, 2015, **26**, 176.
5 X. Fang, Y. Fu, M. J. C. Long, J. A. Haegele, E. J. Ge, S. Parvez and Y. Aye, *J. Am. Chem. Soc.*, 2013, **135**, 14496.
6 J. A. Prescher and C. R. Bertozzi, *Nat. Chem. Biol.*, 2005, **1**, 13.
7 P. Agarwal, B. J. Beahm, P. Shieh and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2015, **127**, 11666.
8 Z. Wang, X. Ding, S. Li, J. Shi and Y. Li, *RSC Adv.*, 2014, **4**, 7235.
9 N. Stephanopoulos and M. B. Francis, *Nat. Chem. Biol.*, 2011, **7**, 876.
10 E. W. Kovacs, J. M. Hooker, D. W. Romanini, P. G. Holder, K. E. Berry and M. B. Francis, *Bioconjugate Chem.*, 2007, **18**, 1140.
11 G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. El Oualid, D. W. C. Timothy and G. D. Benjamin, *Angew. Chem., Int. Ed.*, 2008, **47**, 2244.
12 Y. M. Li, M. Pan, Y. T. Li, Y. C. Huang and Q. X. Guo, *Org. Biomol. Chem.*, 2013, **11**, 2624.
13 S. B. H. Kent, *Chem. Soc. Rev.*, 2009, **38**, 338.
14 Y.-W. Tan and H. Yang, *Phys. Chem. Chem. Phys.*, 2011, **13**, 1709.
15 J.-S. Zheng, S. Tang, Y.-C. Huang and L. Liu, *Acc. Chem. Res.*, 2013, **46**, 2475.
16 K. F. Geoghegan and J. G. Stroh, *Bioconjugate Chem.*, 1992, **3**, 138.

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17 M. Gilmore, R. A. Scheck, A. P. Esser-Kahn, N. S. Joshi and M. B. Francis, *Angew. Chem., Int. Ed.*, 2006, **118**, 5433.
18 Z. Hao, S. Hong, X. Chen and P. R. Chen, *Acc. Chem. Res.*, 2011, **44**, 742.
19 K. Lang, L. Davis, S. Wallace, M. Mahesh, D. J. Cox, M. L. Blackman, J. M. Fox and J. W. Chin, *J. Am. Chem. Soc.*, 2012, **134**, 10317.
20 N. K. Devaraj, R. Weissleder and S. A. Hilderbrand, *Bioconjugate Chem.*, 2008, **19**, 2297.
21 Y.-J. Lee, Y. Kurra, Y. Yang, J. Torres-Kolbus, A. Deiters and W. R. Liu, *Chem. Commun.*, 2014, **50**, 13085.
22 P. Shieh and C. R. Bertozzi, *Org. Biomol. Chem.*, 2014, **12**, 9307.
23 H. Staudinger and J. Meyer, *Helv. Chim. Acta*, 1919, **2**, 635.
24 E. Saxon, J. I. Armstrong and C. R. Bertozzi, *Org. Lett.*, 2000, **2**, 2141.
25 B. L. Nilsson, L. L. Kiessling and R. T. Raines, *Org. Lett.*, 2000, **2**, 1939.
26 K. Lang and J. W. Chin, *ACS Chem. Biol.*, 2014, **9**, 16.
27 R. Merkx, D. T. S. Rijkers, J. Kemmink and R. M. J. Liskamp, *Tetrahedron Lett.*, 2003, **44**, 4515.
28 B. L. Nilsson, M. B. Soellner and R. T. Raines, *Annu. Rev. Biophys. Biomol. Struct.*, 2005, **34**, 91.
29 B. L. Nilsson, L. L. Kiessling and R. T. Raines, *Org. Lett.*, 2001, **3**, 9.
30 M. B. Soellner, K. A. Dickson, B. L. Nilsson and R. T. Raines, *J. Am. Chem. Soc.*, 2003, **125**, 11790.
31 S. Han and R. E. Viola, *Protein Pept. Lett.*, 2004, **11**, 107.
32 M. B. Soellner, A. Tam and R. T. Raines, *J. Org. Chem.*, 2006, **71**, 9824.
33 A. Tam, M. B. Soellner and R. T. Raines, *Org. Biomol. Chem.*, 2008, **6**, 1173.
34 L. Liu, Z. Y. Hong and C. H. Wong, *ChemBioChem*, 2006, **7**, 429.
35 A. Tam, M. B. Soellner and R. T. Raines, *J. Am. Chem. Soc.*, 2007, **129**, 11421.
36 A. Tam and R. T. Raines, *Bioorg. Med. Chem.*, 2009, **17**, 1055.
37 Y. He, R. J. Hinklin, J. Chang and L. L. Kiessling, *Org. Lett.*, 2004, **6**, 4479.
38 E. Saxon, S. J. Luchansky, H. C. Hang, C. Yu, S. C. Lee and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2002, **124**, 14893.
39 F. L. Lin, H. M. Hoyt, H. van Halbeek, R. G. Bergman and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2005, **127**, 2686.
40 M. B. Soellner, B. L. Nilsson and R. T. Raines, *J. Am. Chem. Soc.*, 2006, **128**, 8820.
41 J. W. Chin, S. W. Santoro, A. B. Martin, D. S. King, L. Wang and P. G. Schultz, *J. Am. Chem. Soc.*, 2002, **124**, 9026.
42 R. Serwa, I. Wilkening, G. Del Signore, M. Mühlberg, I. Claußnitzer, C. Weise, M. Gerrits and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2009, **48**, 8234.
43 R. Serwa, P. Majkut, B. Horstmann, J.-M. Swiecicki, M. Gerrits, E. Krause and C. P. R. Hackenberger, *Chem. Sci.*, 2010, **1**, 596.
44 N. Nischan, A. Chakrabarti, R. A. Serwa, P. H. M. Bovee-Geurts, R. Brock and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2013, **52**, 11920.
45 M. R. J. Vallée, P. Majkut, I. Wilkening, C. Weise, G. Müller and C. P. R. Hackenberger, *Org. Lett.*, 2011, **13**, 5440.
46 J. E. Hein and V. V. Fokin, *Chem. Soc. Rev.*, 2010, **39**, 1302.
47 H. Staudinger and E. Hauser, *Helv. Chim. Acta*, 1921, **4**, 861.
48 I. Kosiova, A. Janicova and P. Kois, *Beilstein J. Org. Chem.*, 2006, **2**, 23.
49 A. Tam and R. T. Raines, *Methods Enzymol.*, 2009, **462**, 25.
50 R. Kleineweischede and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2008, **47**, 5984.
51 D. s. M. M. Jaradat, H. Hamouda and C. P. R. Hackenberger, *Eur. J. Org. Chem.*, 2010, **2010**, 5004.
52 H. C. Hang, J. Loureiro, E. Spooner, A. W. M. van der Velden, Y.-M. Kim, A. M. Pollington, R. Maehr, M. N. Starnbach and H. L. Ploegh, *ACS Chem. Biol.*, 2006, **1**, 713.
53 H. C. Hang, E.-J. Geutjes, G. Grotenbreg, A. M. Pollington, M. J. Bijlmakers and H. L. Ploegh, *J. Am. Chem. Soc.*, 2007, **129**, 2744.
54 A. Vila, K. A. Tallman, A. T. Jacobs, D. C. Liebler, N. A. Porter and L. J. Marnett, *Chem. Res. Toxicol.*, 2008, **21**, 432.
55 E. Saxon and C. R. Bertozzi, *Science*, 2000, **287**, 2007.
56 J. A. Prescher, D. H. Dube and C. R. Bertozzi, *Nature*, 2004, **430**, 873.
57 D. H. Dube, J. A. Prescher, C. N. Quang and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4819.
58 S. J. Luchansky, S. Goon and C. R. Bertozzi, *ChemBioChem*, 2004, **5**, 371.
59 K. A. Stubbs, A. Scaffidi, A. W. Debowski, B. L. Mark, R. V. Stick and D. J. Vocadlo, *J. Am. Chem. Soc.*, 2008, **130**, 327.
60 S. Han, B. E. Collins, P. Bengtson and J. C. Paulson, *Nat. Chem. Biol.*, 2005, **1**, 93.
61 Y. Tanaka and J. J. Kohler, *J. Am. Chem. Soc.*, 2008, **130**, 3278.
62 S. T. Laughlin and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 12.
63 G. A. Lemieux, C. L. de Graffenried and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2003, **125**, 4708.
64 M. J. Hangauer and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2008, **120**, 2428.
65 K. L. Kiick, E. Saxon, D. A. Tirrell and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 19.
66 C. I. Schilling, N. Jung, M. Biskup, U. Schepers and S. Bräse, *Chem. Soc. Rev.*, 2011, **40**, 4840.
67 Y.-K. Qi, H.-N. Chang, K.-M. Pan, C.-L. Tian and J.-S. Zheng, *Chem. Commun.*, 2015, **51**, 14632.
68 A. Dumas, L. Lercher, C. D. Spicer and B. G. Davis, *Chem. Sci.*, 2015, **6**, 50.
69 E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974.
70 X. Chen, S. Tang, J.-S. Zheng, R. Zhao, Z.-P. Wang, W. Shao, H.-N. Chang, J.-Y. Cheng, H. Zhao, L. Liu and H. Qi, *Nat. Commun.*, 2015, **6**, 7220.