Recent Advances in Cysteine Mediated Bioconjugations

Reported by Marc Hartmann October 15th, 2024

Introduction

Chemical modification of proteins greatly expands protein function enabling them for use in probing biological systems and as powerful therapeutics. Specifically, the modification of antibodies with highly potent drug molecules, known as antibody-drug conjugates (ADCs), has emerged as a particularly promising strategy for oncology treatment.[1] Due to the high cost and sensitive three-dimensional structures of proteins, methods for their modification must be selective and extremely high yielding with fast kinetics under mild, aqueous reaction conditions. Cysteine is the most utilized residue for chemical modification due to the high reactivity of its thiol moiety and its relative low abundance in proteins.[2] Maleimides have been the gold standard of cysteine bioconjugation due to their exceptional reactivity and selectivity for cysteine.[3] However, their instability in vivo and propensity to hydrolyze increasing the heterogeneity of protein conjugates are undesirable in drug development.[3] As a result, the development of more stable, homogenous bioconjugation methods is of great interest. The seminar will cover three alternative strategies for cysteine conjugation with a particular focus on their application to next generation ADCs.

Alternative Michael Acceptors:

In 2010 and 2011, the Baker and Caddick groups respectively explored brominated electrophiles as maleimide alternatives with vastly improved stability profiles. Their ability to be difunctionalized enables their use as disulfide staplers capable of generating homogenous protein conjugates without the need for site-specific mutagenesis.[4,5] Additionally, 5-methylene pyrrolones (5MPs) have emerged as a promising maleimide replacement.[6] 5MP linkers are stable in plasma, but by design they are cleavable under intracellular reducing conditions. This cleavage is desirable in ADCs as intracellular release of the drug is often required for its function.[6] While all these methods show promise, none have been widely adopted due to difficult syntheses or lower reactivity relative to maleimides.

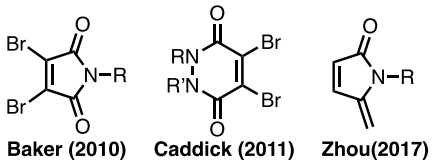

Figure 1: Alternative cysteine specific Michael acceptors

Sequence Specific Bioconjugations:

In nature, proteins' reactivity is precisely controlled by their three-dimensional structure which places amino acids in highly specialized active-site environments. Drawing inspiration from nature, several groups have explored the impact of the immediate environment of a given cysteine on its reactivity and specificity for unique electrophiles. In 1998, Tsien and coworkers discovered a

tetracysteine tag that uniquely reacts with biarsenical fluorescent dyes.[7] Taking inspiration from this, in 2016 the Pentulute group published a sequence specific "pi-clamp-mediated" cysteine bioconjugation with perfluoroaryl groups.[8] Cysteine residues had previously been shown to react rapidly with perfluoroaryl groups in organic media, but reactions in water were extremely slow. By placing the targeted cysteine within a 4AA sequence of Phe-Cys-Pro-Ph, they were able to leverage intermolecular non-covalent interactions to drastically increase the reaction rate.

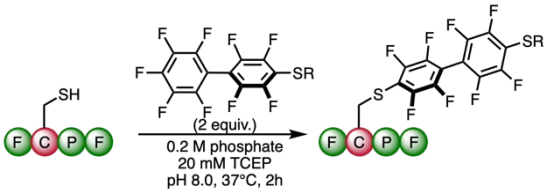

Figure 2: Π-Clamp Meditated Cysteine Conjugation

Transition Metal Mediated Methods:

Transition metals have long been utilized in organic synthesis due to their unique reactivity and high degree of chemoselectivity. In the context of bioconjugation, the chemoselectivity metals offer is desirable due to the presence of multiple nucleophilic residues and the need to functionalize them selectively. Although Pd mediated C-S bond forming processes have long been known to organic chemists, in the context of bioconjugation no Pd catalyzed C-S cross coupling had been demonstrated prior to the work of Buchwald and Pentelute in 2015[9]. They found that Pd^{II} oxidative addition complexes could be used to selectively arylate cysteine. These reactions are highly selective and efficient going to completion in 30 minutes at room temperature. Further ligand modifications have enabled these reactions to proceed in fully aqueous media. However, these Pd mediated conjugations are limited by the requirement for stoichiometric organometallic reagents.

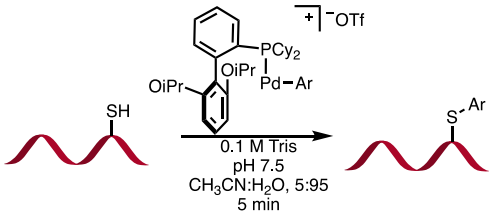

Figure 3: Arylation via Pd Oxidative Addition Complex

Outlook

Despite the promise of these methods, maleimides remain the most prominent method for cysteine bioconjugation. In general, alternative methods have been hindered by challenging syntheses, lackluster reactivity, or reliance on expensive stoichiometric organometallic reagents has stopped them from displacing the maleimide. Among these, Pd-mediated cysteine arylation has the most potential to usurp the maleimide, as they have similar kinetics, and their conjugates are vastly more stable. However, for this to happen a truly catalytic Pd-mediated system must be realized. This will require significant ligand to prevent thiol poisoning while making the catalytic cycle kinetically feasible for bioconjugation.

Reference

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(2) *J Mol Biol.* **2010** , 404(5): 902–916
(3) *Chem. Eur. J.* **2019**, *25*, 43
(4) *J. Am. Chem. Soc.* ***2010*** *132 (6), 1960-1965*)
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