THE DEVELOPMENT AND OPTIMIZATION OF AZIDE-BASED BIOORTHOGONAL REACTIONS

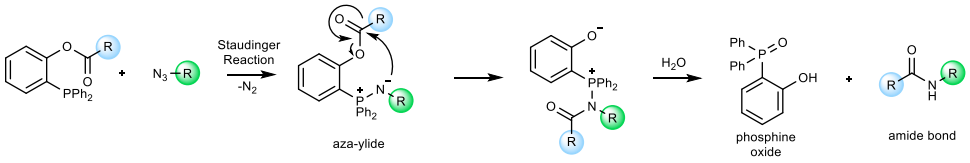
Selena Hernandez Tuesday November 17th, 2020

INTRODUCTION

The term bioorthogonal refers to any chemical reaction that can occur inside of living systems without interfering with native biochemical processes. Typically, a chemical reporter is metabolically incorporated into a biomolecule of interest using an amino acid, carbohydrate, or inhibitor. Then, a bioorthogonal reaction takes place with an appropriately functionalized molecule to enable detection. Since their introduction, bioorthogonal reactions have enabled the study of biomolecules of interest such as glycans, proteins, and lipids within their native environments. The reagents of a bioorthogonal reaction must be selective for only each other, must not disrupt or be disrupted by the biological system, have rapid reaction rates, be biocompatible, and utilize a chemical reporter that is easily metabolically incorporated. Therefore, azides are ideal reactants for bioorthogonal reactions due to their small size, kinetic stability, and absence from biological systems. Additionally, its properties as a soft electrophile allows for selective reactivity.[1]

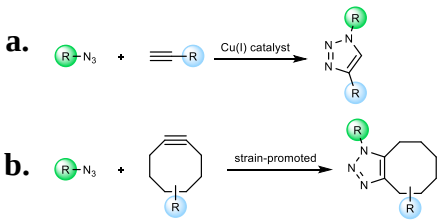
STAUDINGER LIGATION

In a seminal paper in 2000, the Bertozzi group showed that the Staudinger reaction can also be applied for the formation of an amide bond through rearrangement of the aza-ylide intermediate in a process coined the Staudinger ligation. While not the first bioorthogonal reaction, it was the first to utilize abiotic functional groups, which allowed for their use in *in vivo* models. The key to this development was the intramolecular trapping of the nucleophilic aza-ylide by an electrophilic ester, leading to a covalent amide bond via a putative pentacoordinate phosphine intermediate. To avoid the presence of a residual phosphine oxide after ligation, the traceless Staudinger ligation was developed, which now included the release of a phosphine oxide to form a native amide bond (Scheme 1). Since then, efforts have been made to tune this reaction to allow for the ligation of a variety of molecules for applications in peptide formation, drug delivery, and cell engineering.[2]



Scheme 1. The Traceless Staudinger Ligation

AZIDE-ALKYNE CYCLOADDITION



Scheme 2. The CuAAC and SPAAC reactions.

One bioorthogonal reaction that has been widely used since its inception is the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC) to form triazoles, also known as "click chemistry" (Scheme 2a). The utility of CuAAC cannot be understated, since its inception it has seen wide applications in organic synthesis, combinatorial chemistry, drug development, and materials science. However, it became clear that CuAAC has some limitations as a bioorthogonal reaction due to the cytotoxicity of the copper(I) species and potential side reactions with amino acids. A variety of copper stabilizing ligands have been developed to increase the efficiency of CuAAC, however copper or another transition metal is often a necessary component.[3] To overcome this challenge, it was shown that cyclooctynes could undergo a selective strain-promoted azide-alkyne cycloaddition (SPAAC) without the aid of a catalyst, albeit with slower reaction rates (Scheme 2b). Since this initial report, numerous cyclooctynes have been synthesized in a quest to improve the reaction's kinetic properties. Notable advancements include the installation of fluorines to lower the energy of the LUMO and promote the reaction with the azide, the introduction of aromatic rings to introduce more sp^2-hybridized carbons to increase the ring strain, and the addition of hydrophilic groups to improve solubility in aqueous solvents and decrease lipophilicity. In general, an increase in reactivity in the cyclooctyne has led to a decrease in stability and solubility, so a compromise between these properties is required. Since the optimization of the cyclooctyne reactivity, these reactions have seen promise in applications such as biomolecule imaging, advanced biomaterials, and surface functionalization.[4]

CONCLUSION AND OUTLOOK

It is clear that bioorthogonal chemistry has many exciting applications for the study and manipulation of biological systems. The challenge for organic chemists is to develop and tune these reactions to follow the requirements for bioorthogonality. Efforts in the last 20 years have shown that azide is an ideal reaction partner for these systems, and further work in this field has the potential to lead to additional discoveries.

REFERENCES

(1) Sletten, E.M. et al. *Acc. Chem. Res.* **2011**, *44*, 666. (2) Bednarek, C. et al. *Chem. Rev.* **2020**, *120*, 4301. (3) Li, L. et al. *Molecules* **2016**, *21*, 1393. (4) Dommerholt, J. et al. *Top. in Curr. Chem*, **2016**, *16*, 374.