1,2,4,5-TETRAZINE-BASED CLICK REACTIONS IN BIOORTHOGONAL CHEMISTRY

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INTRODUCTION

Over the last decade, bioorthogonal reactions, a subclass of click reactions whose components are inert under physiological conditions, have been widely used in chemical biology.[1] Despite various applications in biomolecule imaging, established reactions have several limitations, including the toxicity of the reagents, slow kinetics, and often laborious synthesis of the click components. An ideal bioorthogonal reaction should exhibit "turn-on" fluorescence with minimal background signals, as well as fast kinetics that enable rapid labeling at low reagent concentration. The inverse-electron-demand Diels-Alder reaction of the 1,2,4,5-tetrazine unit and strained alkenes has many of these desired features and has merged as an excellent candidate in the field (Figure 1).

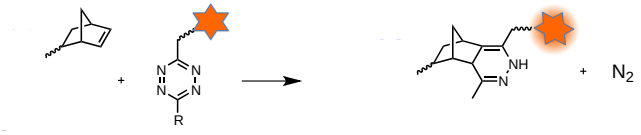

Figure 1: Tetrazine-based molecular imaging

CHEMISTRY OF THE TETRAZINE LIGATION

The irreversible reaction of tetrazine with alkene and alkyne containing compounds produces a stable dihydropyridazine and pyridazine, respectively.[2] The reaction proceeds by an inverse-electron-demand Diels-Alder reaction of the electron-poor tetrazine and electron-rich dienophiles, followed by a retro-Diels-Alder reaction with the loss of nitrogen. Kinetic study in organic solvents by Sauer et al. reveals that tetrazines react with strained alkenes with a second-order rate constant of up to 10^4 M^{-1} s^{-1},[2,3] which is several order of magnitude higher than that of existing bioorthogonal reactions. Recently, it was found that the tetrazine–*trans*-cyclooctene reaction is orthogonal to the azide–dibenzocyclooctyne reaction, which enabled a simultaneous imaging of two different biomolecules in a cellular setting.[4]

It was also discovered that the tetrazine, whose absorption maximum is at 530 nm, is able to quench the fluorescence of several green-emitting dyes, including BODIPY FL, BODIPY TMR-X, Oregon Green 488, and TAMRA-X.[5] The fluorescence quenching is attributed to the resonant energy transfer mechanism.[6] Interestingly, the fluorescence of the probe is turned-on upon the cycloaddition of the tetrazine with the dienophiles (Figure 2). This important feature

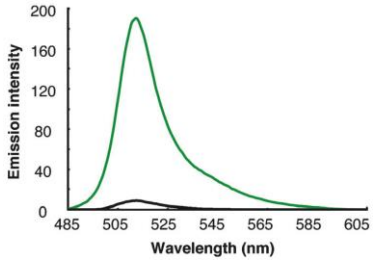

Figure 2: Emission spectra of tetrazine-BODIPY FL before (black) and after (green) reaction with cyclopropene

provides an improved fluorescent background for cellular imaging.

APPLICATIONS IN BIOORTHOGONAL CHEMISTRY

The tetrazine bearing probes can label cancer cells pre-targeted with antibodies containing strained alkenes.[4] Because of the rapid kinetics of the tetrazine-based reaction, this approach requires concentrations of the labeling agent in the nanomolar range. Taking advantage of this technique, simultaneous imaging of two different cancer cell lines was performed using the bioorthogonal reaction pair, the reaction of tetrazine with *trans*-cyclooctene and the reaction of azide with dibenzocyclooctyne. In 2010, Weissleder and co-workers expanded the use of the tetrazine-based reaction to intracellular imaging of small molecules by using a cell-permeable labeling agent.[5a]

In addition, Chin *et al.* demonstrated that norbornene-, *trans*-cyclooctene-, and cyclooctyne-bearing amino acids can be genetically encoded into desired proteins in *E. coli* and mammalian cells using the appropriate pyrrolysyl-tRNA synthetase/$tRNA_{CUA}$ pair.[5b,7] The efficient and selective protein labeling was achieved within minutes, whereas the labeling of unnatural amino acid using a copper-catalyzed click reaction requires higher fluorophore concentration and 18 hours to reach the completion.

The reaction of tetrazine and ^{18}F-containing *trans*-cyclooctene provides a new approach for the incorporation of the short half-life isotope into small molecules.[8a] Furthermore, the development of a magnetic *trans*-cyclooctene-scavenger resin offers a straightforward purification to remove the unreacted tetrazine-containing small molecules.[8b]

SUMMARY

Despite the relatively large size of the clickable components, the reaction of tetrazine and strained alkene offers valuable features to chemical biology, such as rapid kinetics, fluorogenic response. Extension of this approach to tagging other biomolecules in living organisms is desirable.

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