Boosting Bioorthogonal Bond-Cleavage Reactions of Tetrazines and *trans*-Cyclooctenes via Intramolecular Interactions

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The emergence of bioorthogonal reactions in recent years expanded the repertoire of heterocycles as chemical tools to enable spatiotemporal visualization and control of biological function in chemical biology, leading to a revival of "classical" heterocyclic reactions in a modern light. Among bioorthogonal elimination reactions, the tetrazine-triggered cleavage of *trans*-cyclooctene (TCO) conjugates (click-to-release) has outstanding click kinetics but the rate and efficiency of the subsequent release step vary significantly depending on the structure of the tetrazine (Tz). Consequently, only poor release has so far been obtained when using the reagents of choice considering click kinetics (i.e., highly reactive tetrazines such as bis(2-pyridyl)-1,2,4,5-tetrazines), whereas less reactive bis(alkyl)tetrazines can be used to achieve complete cleavage.

Based on our increasing understanding of the underlying mechanisms of Tz/TCO click-to-release chemistry [1] that enhancing the formation of *tautomer 1* is key to an efficient release (Fig. 1), we aimed to control post-click tautomerization by incorporation of a directing OH-group into the *trans*-cyclooctene scaffold (iTCO).

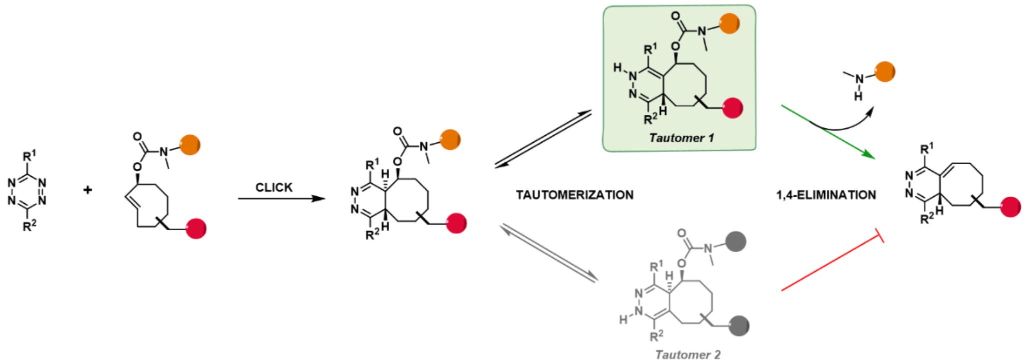

Fig. 1: Proposed mechanistical pathways of the post-click reaction, adapted from Carlson *et al.* [1]

Unexpectedly, we observed nearly instantaneous release of iTCO even after reaction with highly reactive tetrazines, which we uncovered is due to a new elimination mechanism. By the development of iTCO we have thus not only achieved directed and accelerated post-click tautomerization but moreover enhanced elimination independent from the structure of the Tz, leading to a boost of the overall reaction by three orders of magnitude compared to the current state-of-the-art.

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[1] Jonathan C. T. Carlson, Hannes Mikula, Ralph Weissleder, *J. Am. Chem. Soc.* **2018**, *140*, 3603-3612.