Intracellular Retargeting *via* Bioorthogonal Substitution

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Bioorthogonal bond cleavage reactions have recently emerged as a thriving area of chemical research. The fastest known bioorthogonal reaction, an inverse electron demand Diels-Alder reaction between a tetrazine and a trans-cyclooctene (TCO) has recently been modified to allow a click-to-release approach. In this controlled reaction, upon click the release of a molecule is triggered, maintaining a fixed linkage to a moiety (*Fig*. 1a) [1]. Using a further functionalized tetrazine leads to formal exchange of a ligand in a bioorthogonal substitution (*Fig*. 1b). This highly modular approach opens up an extended application scope for chemical biology.

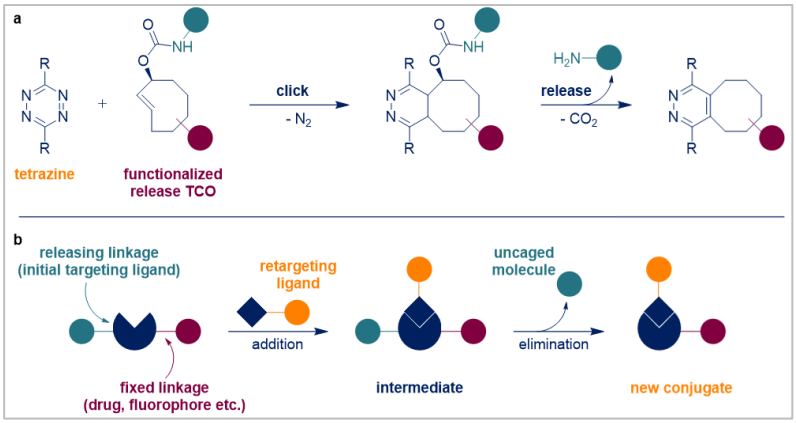

Fig. 1 a) *Click-to-release chemistry.*

b) *Concept of bioorthogonal substitution for intracellular retargeting*

In a proof of concept study, a modified ligand-TCO-fluorophore conjugate and a tetrazine modified with a retargeting ligand were evaluated for their potential to perform intracellular cleavage and retargeting by *in vitro* experiments using a model system.

Experiments were carried out using the fibrosarcoma cell line HT1080, that was modified to overexpress Bruton's Tyrosine Kinase (BTK), which is selectively and irreversibly targeted with Ibrutinib, a small molecule FDA approved drug [2].

Preliminary experiments show promising results for intracellular retargeting, which can be used for a variety of applications in biomedical research, including *in vivo* imaging and targeted drug delivery.

[1] Angew. Chemie Int. Ed. 52, 14112–14116 (2013)

[2] Recent Results Cancer Res. 201, 259–267 (2014)