Pretargeting intracellular oncogenic proteins for click-to-release

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Systemically administered chemotherapy in the form of cytotoxic agents like doxorubicin is often accompanied by severe side effects. In a similar way to antibody drug conjugates, small molecule drug conjugates (SMDCs) aim to localize the treatment by combining a targeting ligand with a cytotoxic moiety. The localizing part of an SMDC must be selective for a target associated or specific to the cancer tissue in question. For our system we chose the epidermal growth factor receptor (EGFR) a receptor kinase which is overexpressed in several types of cancer, including lung and colorectal cancer, and afatinib a covalent inhibitor targeting the kinase domain of this protein.[1]

The inverse electron-demand Diels-Alder (IEDDA) reaction between tetrazines and trans-cyclooctenes (TCOs) is an emerging biorthogonal reaction. Since its introduction in 2008[2] it has found application in the traditional sense of click reactions of connecting two scaffolds, but moreover it can also act in a dissociative manner called click-to-release,[3] when a suitable leaving group is placed next to the alkene. A biologically active compound can be rendered inactive by placing a bulky TCO on a suitable heteroatom. It will only be activated upon reaction and subsequent release with a tetrazine.

By way of connecting the activating tetrazine to the EGFR targeting afatinib, we aim to localize the cargo release to cells with overexpressed levels of EGFR. Following this treatment, TCO protected MMAE can be introduced which will only be activated in cells with elevated levels of the tetrazine.

[1] Li et al., *Oncogene*, **2008**, *27*, 4702-4711.
[2] Melissa L. Blackman, Maksim Royzen, Joseph M. Fox, *J. Am. Chem. Soc*., **2008**, *130*, 13518-13519.
[3] Versteegen et al., *Angew. Chem. Int. Ed.*, **2013**, *52*, 14112-14116.