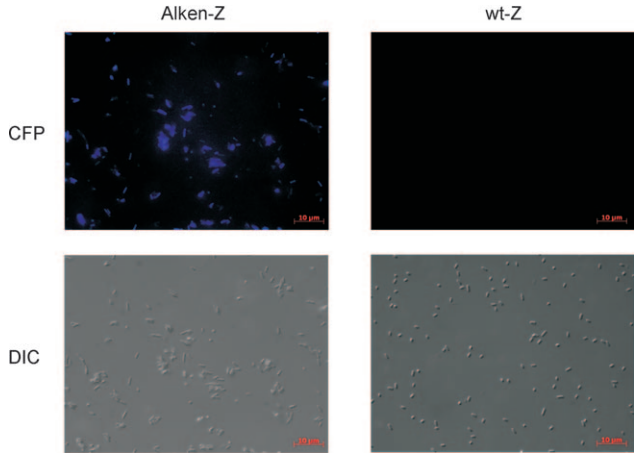

Figure 1. Selective functionalization of an alkene-containing Z-domain protein with a tetrazole (see Scheme 1 a) in *E. coli* cells: CFP-channel (top) and DIC-channel images (bottom) of bacterial cells expressing either the alkene-containing or wild-type (wt) Z-domain protein after treatment with the tetrazole (100 μM). Reproduced with permission from Ref. [8]. CFP = cyan fluorescent protein, DIC = differential interference contrast.

reaction with acrylamide derivatives.[8] By way of explanation, the authors suggested that the lower LUMO energy of the acrylamide led to a better overlap with the HOMO of the nitrile imine (LUMO = lowest occupied molecular orbital; HOMO = highest occupied molecular orbital). The selection of an appropriate nitrile imine/alkene pair might hold the potential for further optimization.

The special feature of the reaction presented herein is clearly its light dependence. This property opens the way for the precise spatial and temporal control of the ligation event and could thus provide a formidable tool for cell biology. However, it will also restrict the use of the ligation reaction to media and biological material that are transparent at the applied wavelength. By fine-tuning of the substituents on the diaryl tetrazole, it was already possible to shift the absorption maximum so that the reaction could be triggered with light with a wavelength of 365 nm, which is less harmful to cells.[33]

In summary, recent research efforts have yielded important developments for the selective modification of proteins and for bioorthogonal ligation. These developments broaden the scope of application of these reactions. Two further recent examples are also based on the reaction of an alkene moiety: The research group of Davis has started to exploit the potential of cross-metathesis for the modification of proteins functionalized with an allyl sulfide (Scheme 1 c),[34] whereas Fox and co-workers reported the extremely fast Diels–Alder reaction of tetrazines with *trans*-cyclooctenes for protein modification (Scheme 1 d).[35] There seems to be a bright and exciting future ahead for selective chemical protein modification.

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