Development of an Esterase-Targeted, Cell-Trappable and Photoactivatable Diazoindanone Rhodol for Live Cell Imaging and Stochastic Optical Reconstruction Microscopy

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In this study, we discuss the photochemical properties and applications of a double-activatable fluorophore **1**, which is based on a previously reported scaffold. Upon enzymatic deacetylation and subsequent irradiation with 405 nm laser, **1** yields a major fluorescent photoproduct **2**. In the absence of the preceding enzymatic deacetylation, however, mostly non-fluorescent compound **3** is obtained. A bright signal can thus only be attained after photoactivation of **1** in the presence of intracellular carboxylesterases. Moreover, under constant light irradiation, we expect fast photoconversion of **1** immediately after deacetylation. This mechanism is useful for *in situ* mapping of enzymatic reactions and tracking of carboxylesterases with stochastic optical reconstruction microscopy (STORM). In this talk, we will furthermore discuss the development of a *steady state* technique for STORM that allows acquisition of a large number of images without intensity decay, by constantly replenishing the intracellular reserves of **1**. With this novel technique, we aim to obtain higher labeling densities and record super-resolution time-lapse sequences of slow cellular processes.

[1] V. N. Belov, C. A. Wurm, V. P. Boyarskiy, S. Jakobs, S. W. Hell, *Angew. Chem. Int. Ed.* **2010**, *49*, 3520–3523.