Using Molecular Switching to Achieve Super-Resolution Microscopy

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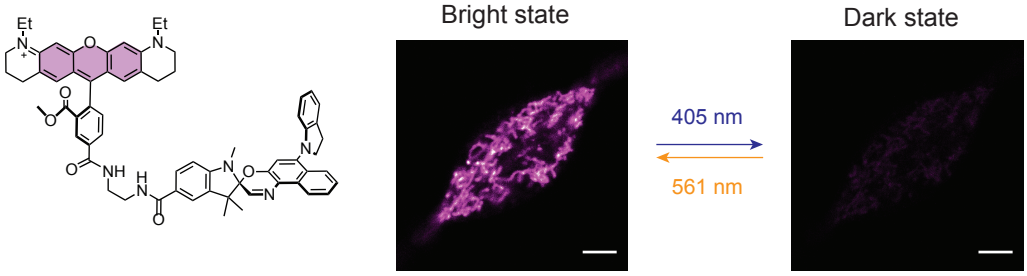
Microscopy is currently one of the most useful tools in life sciences. Optical and in particular fluorescent microscopies have completely revolutionized the way we explore the numerous processes of life. Yet, this technology, as any, has fundamental limitations like the limited temporal and spatial resolution. The latter is a strict physical limitation known as the diffraction limit. Nevertheless, starting in 1994, a number of techniques circumventing the diffraction limit were introduced leading to the field being recognized by the Nobel Prize for the so-called "super-resolution microscopy". At the core of this new technology are *molecular switches* in the form of (reversible) photo-activatable dyes and proteins.



The advantages of using small-molecules dyes – like better brightness, smaller size, faster kinetics – are usually overlooked when considering the advantages of fluorescent proteins (e.g. labelling specificity of a genetically encoded dye). This is particularly true for fast super-resolution techniques like RESOLFT (REversible Saturable OpticaL Fluorescence Transitions) which are, as a consequence, mainly available using genetically modified organisms.

During this talk, we will discuss our approach towards a functional small-molecule switchable dye meeting the requirements to be used in RESOLFT microscopy i.e., high switching contrast, strong fatigue resistance, and compatibility with living systems. To achieve a suitable dye/switch, we have explored the construction of a dyad composed of a bright rhodamine fluorophore and a molecular switch acting as fluorescence quencher.[1] Among several families of molecular switches that we have explored (e.g. spiropyrans, diarylethenes), spirooxazines give the best results and are suitable for RESOLFT microscopy.

1. Xiong, Y.‡; Vargas Jentzsch, A.‡; Osterrieth, J. W. M.; Sezgin, E.; Sazanovich, I. V.; Reglinski, K.; Galiani, S.; Parker, A. W.; Eggeling, C.; Anderson, H. L. *Chem. Sci.* **2018**, *9*, 3029.