Chemical Reactions for Super-Resolution and Single-Color Multiplexing Microscopy

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Fluorescence microscopy is a powerful tool to study structure interactions in biology. Multiplex is applied to visualize these interactions in a single experiment. Yet, only a limited number of methods are frequently used to achieve multiplexing in fluorescence microscopy. Separation via physical properties, e.g. fluorescence lifetime or color of the emitted light, is the predominant method. Here we introduce a new multiplexing method to discriminate two labels of the same color in time.

We recently developed a method for switching fluorescent probes via a chemical reaction between a bright “on” and a dark “off” state by reversible coordination of Cu(II) onto a ligand, which we named CHIRON.[1-3] Here, we show that besides its application for localization-based super-resolution microscopy, the probe is also suitable for single-color multiplexing microscopy. This allows the study of interacting structures in a single experiment using only a single spectral channel, circumventing the problem of chromatic aberrations. Using reversible chemical switching and photo-bleaching, we can resolve two structures at the same wavelength in time. The method is compatible with any standard fluorescence microscope, ensuring its broad applicability but also adding an interesting perspective for super-resolution techniques, like STED.[4]

Currently, we are working on improving the probe design to best fit the needs for live-cell applications. Our focus lies on a modular system to easily incorporate commercial dyes.