Cy3-Cy5 Covalent Heterodimers for Super-Resolution Imaging

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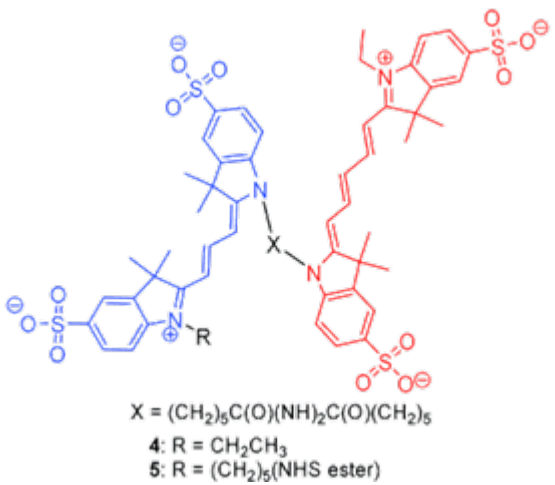
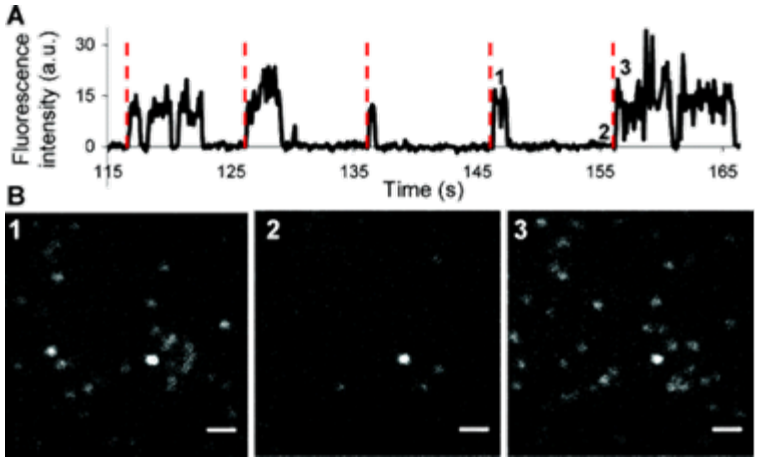
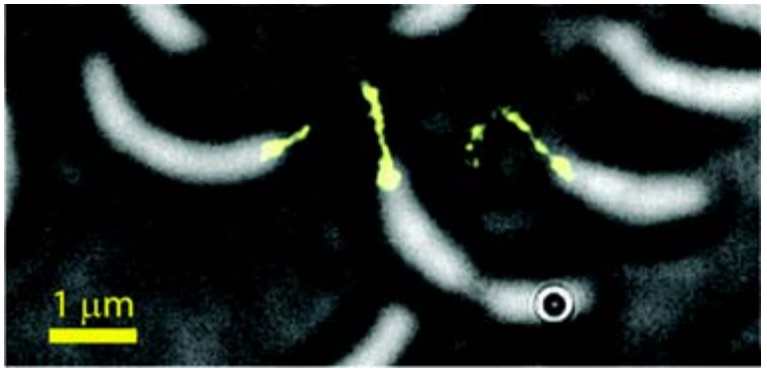


Cy3-Cy5 covalent heterodimers (top left) behave as single-molecule photoswitches. Excitation with 633 nm light produces fluorescence emission, or, in the presence of thiol, eventually causes the Cy5 to enter into a meta-stable dark state. Emission can be restored with a short pulse of very low intensity (5 W cm^{-2}) 532 nm light, absorbed by the Cy3.

The Cy3-Cy5 covalent dimer retains this photoswitching behavior, even when covalently attached to bovine serum albumin protein (bottom left). The red dashed line in the fluorescence time trace denotes each 532 nm reactivation pulse. Fluorescence images are shown that correspond to the time positions labeled 1-3 in panel A.

Super-resolution fluorescence images of the thin, narrow stalks of *Caulobacter crescentus* cells (bottom right, yellow) can be obtained using the Cy3-Cy5 covalent heterodimer and stochastic optical reconstruction microscopy (STORM).





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