# DOCKING SITE-MEDIATED PHOTOSTABILIZATION FOR SINGLE-MOLECULE AND SUPER-RESOLUTION IMAGING

Cindy Close[1], Michael Scheckenbach[1], Alan Szalai[2], Julian Bauer[1], Lennart Grabenhorst[1], Fiona Cole[1], Thorben Cordes[3], Philip Tinnefeld[1], Viktorija Glembockyte[1]

[1]Department of Chemistry and Center for NanoScience Ludwig-Maximilians-University Munich
[2]CIBION-CONICET Buenos Aires, Argentina
[3]Department of Biology and Center for NanoScience Ludwig-Maximilians-University Munich
Cindy.Close@cup.lmu.de

DNA-PAINT is a single-molecule localization microscopy technique, relying on transient hybridization of fluorescently labeled single-stranded DNA imager strands to complementary docking strands on target molecules [1]. During acquisition, docking sites are imaged over the course of multiple binding, dissociation and photobleaching events. Through constant imager strand exchange, the limited photon budget of a single fluorophore is circumvented, making it possible to extract super-resolution images at high laser illumination intensities. Over long periods of continuous high-duty cycle excitation of fluorophores, DNA-PAINT binding sites can, however, be depleted [2]. Fluorophores in triplet excited states may generate singlet oxygen and downstream reactive oxygen species (ROS), damaging the docking sites and labeled target structures (**Figure 1a**). The use of triplet state quenchers (TSQ) and enzymatic scavenging systems is further limited to systems insensitive to pH change or high additive concentration. Inspired by fluorophore regeneration and self-repair mechanisms, we link the TSQ cyclooctatetraene to a DNA sequence [3], [4]. This photostabilizer strand binds directly next to the imager at the docking site, thereby allowing for self-regeneration and programmed exchange (**Figure 1b**). The presented contribution shows how this approach can increase the accessible photon budget. The method is characterized in a DNA origami model structure and applied to image microtubules in fixed cells. The improved longevity of DNA-PAINT docking sites is shown and the impact of photostabilizer strand regeneration is explored. The ability to mix and match optimal photostabilizer/dye pairs in this modular approach could be beneficial e.g., for multi-color measurements in structural biology, which often require multiple rounds of imaging, while preserving structural integrity of the sample.(**Figure 1c,d**)

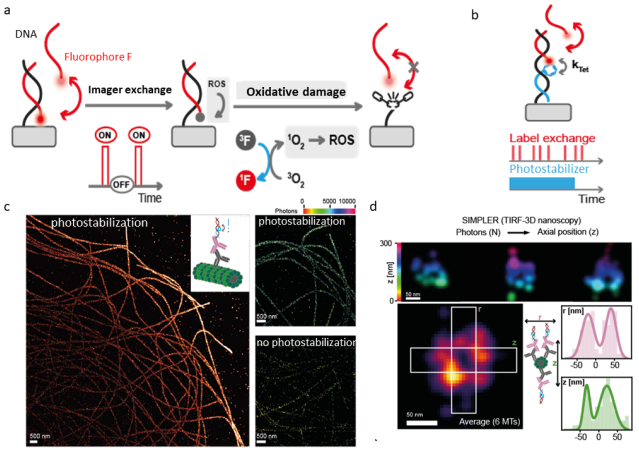


Fig. 1. a) Principle of DNA-PAINT and docking site damage via reactive oxygen species (ROS). b) Docking-site mediated photostabilization to prevent this damage. c) Impact of photostabilization on super-resolution measurements of cytoskeleton (microtubules) in cells. d) Demonstrating that, using the photostabilizer/fluorophore system, microtubules can be resolved in 3D.

[1] R. Jungmann et al., Nano Letters 2010, 10, 4756.
[2] P. Blumhardt et al., Molecules 2018, 12, 3165.
[3] M. Scheckenbach et al., Angew. Chem. Int. Ed. 2020, 60, 4931.
[4] L. Zhang et al., Angew. Chem. Int. Ed. 2022, 134 (19).