Fluorogenic probe for fast 3D whole-cell DNA-PAINT

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DNA-PAINT is a single-molecule localization microscopy method based on the transient binding of DNA-based fluorescent imager probes. Unlike STORM and PALM, DNA-PAINT is resistant to photobleaching and the blinking kinetics are tunable independently of the fluorophore. However, DNA-PAINT traditionally suffers from two severe limitations: i) it is very slow, taking hours for a single image, and ii) optical sectioning is necessary to reduce background which makes it largely incompatible with standard widefield illumination.

Here we present a novel fluorogenic imager probe based on a fluorophore-quencher design. On binding to its complementary sequence, probe fluorescence increases 80 fold. To optimize for fast blinking kinetics, the probe was paired to a docking sequence with partial mismatches to decrease binding affinity. Reduced background fluorescence with our new fluorogenic probe allows imaging at significantly higher frame rates (100 Hz) compared to conventional DNA-PAINT. We demonstrate that immunolabeled microtubules in fixed COS-7 cells can be resolved as hollow tubules within minutes, with an approximately 26× faster rate of blinking. Furthermore, it is now possible to perform DNA-PAINT under widefield illumination. We demonstrated this by combining DNA-PAINT with 4Pi-SMS microscopy to image mitochondria over a thickness of more than 2 microns in 3D.

The synergy between photobleaching resistance and faster imaging rate permits the collection of super-resolution volumes at a quality and sampling-density that has not been previously achievable.

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