Selective Activation DNA-PAINT Imaging with Reductive Caging

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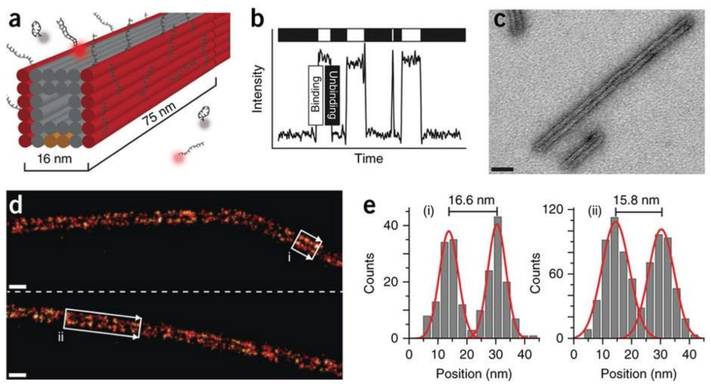
Abstract

Advances in super-resolution microscopies enable us to observe sub-diffraction-limit organization of biological samples, such as intracellular distribution or structures in cells or tissues. However, super-resolution microscopies have limitations such as photobleaching of fluorescent dyes during prolonged light exposure. DNA-PAINT (Point accumulation in nanoscale topography) overcomes the photobleaching limit by transient binding of short DNAs (docking strands that label the target and imager strands conjugated with fluorescent dye that is complementary to docking strands). The transient binding of short DNAs produces movies of blinking single molecules, resulting in localization-based super-resolution images. However, DNA-PAINT imaging is still limited due to high background from the unbound imaging strands in solution that slows down the acquisition speed. To enable background-free DNA-PAINT imaging, we combined DNA-PAINT with “reductive caging” in which fluorophores are turned into transient dark states by reducing agents and activated back to the fluorescent state by weak UV illumination. The reduced, caged dyes attached to the imager strands can significantly reduce the background from unbound image strands. Also, an evanescent field or light sheet of UV can selectively activate caged fluorophores near the substrate or any plane of interest, enabling optical control on the turn-on rate. The combination of reductive caging and selective activation can enable fast image acquisition and optical sectioning on a widefield microscope.

DNA-PAINT

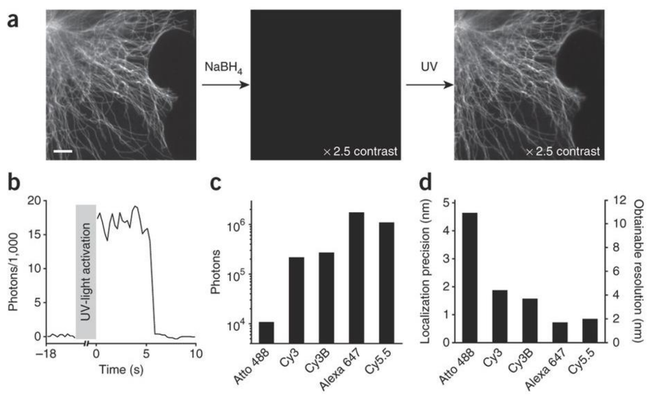


- *Fluorescence switch ON/OFF:* Transient biding/unbinding of short DNA strands
- *Advantage:* Enable multi-color imaging with single fluorophore
- *Disadvantage:* Limit in data acquisition time due to low imager concentration

Fig.1 (**a**) A microtubule-like DNA origami polymer is decorated with single-stranded extensions (docking strands) on two opposite faces (red) spaced ~16 nm apart. Complementary fluorescent imager strands transiently bind from solution to docking strands. (**b**) Transient binding between imager and docking strands produces fluorescence blinking, allowing stochastic super-resolution imaging. (**c**) TEM image of origami polymers with a measured width of 16 ± 1 nm (mean ± s.d.). (**d**) DNA-PAINT super-resolution images obtained using Cy3b-labeled imager strands (15,000 frames, 5-Hz frame rate). Two distinct lines are visible. (**e**) Cross-sectional histograms of the boxed areas i and ii in **d** both reveal a distance of ~16 nm as designed (FWHM of each distribution is ~7–10 nm). Scale bars, 40 nm

Ralf Jungmann, et al. Nature Methods , 11,313-318 (2014)

Reductive Caging



- *Fluorescence switch OFF:* Reducing agent (e.g. $NaBH_4$) converts fluorophores to a stable dark state.
- *Fluorescence switch ON:* Reduced dyes return to fluorescent on-state under weak UV illumination.
- *Advantage:* Orders of magnitude more photons than thiol-based switching.

Fig.2 (**a**) Left, initial fluorescence signal of a fixed cell immunolabeled for microtubules with Cy3B. Center, fluorescence signal after reduction with $NaBH_4$. Right, fluorescence signal after illumination with UV light, showing approximately 40% recovery of the initial fluorescence. The image contrast in the center and right subpanels is 2.5 times that of the left subpanel. Scale bar, 5 µm. (**b**) Fluorescence trace of a single Cy3B molecule recovering to a bright state after reduction and subsequent photoactivation (gray box). (**c**) Mean number of photons detected from individual Atto 488, Cy3, Cy3B, Alexa 647 and Cy5.5 molecules after photoactivation. (**d**) Mean localization precision (left axis) and potentially obtainable resolution (right axis) determined from at least 100 molecules for each dye.

Joshua C Vaughan, et al. Nature Methods , 9, 1181-1184 (2012)

DNA-PAINT with Reductive Caging

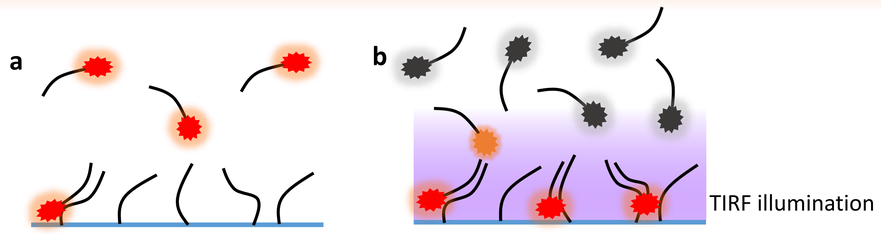


Fig.3 (a) Scheme of conventional DNA-PAINT. Freely diffusive imager strands (black strands with red star) stochastically bind to docking strands (black strand attached on the substrate in blue). All imager strands are fluorescent, so the unbound strands in solution could be recognized as background during imaging. **(b)** Scheme of DNA-PAINT with reduced fluorophores. Reduced fluorophores stay in stable dark state (show as black star), while fluorophores under UV illumination convert back to fluorescent state and emit fluroscence (red star). TIRF illumination (pale blue box) converts fluorophores near the coverslips. Other fluorophores are stay in dark state.

Conventional DNA-PAINT	Fast DNA-PAINT with Reductive Caging
Fluorescent imager	Reduced imager
Low imager concentration	High imager concentration
Excitation	Excitation + UV
Slow data acquisition (at least 30~40min)	Fast data acquisition (within 5min)

Methods

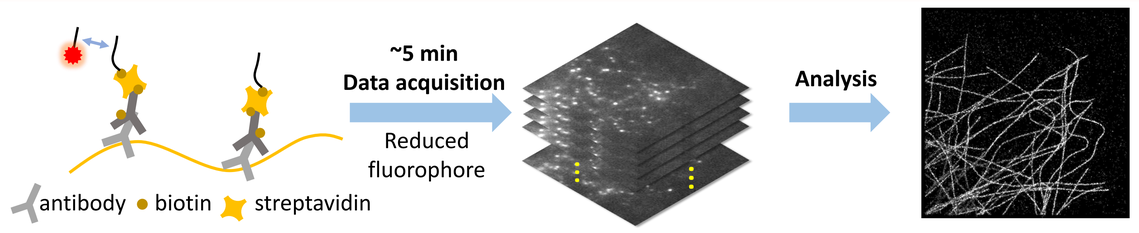


Fig.4 Left: Labeling scheme of a target of interest in cells. Docking strands are labeled using avidin-biotin interaction. Middle: Data acquisition with imager strands conjugated with reduced fluorophores. Reduced form of fluorophore enable DNA-PAINT imaging with high concentration of imager strands, therefore the data acquisition time is reduced within 5 min. Right: image after analysis. Each blinking point in raw data is localized by Gaussian distribution.

Results: fast DNA-PAINT

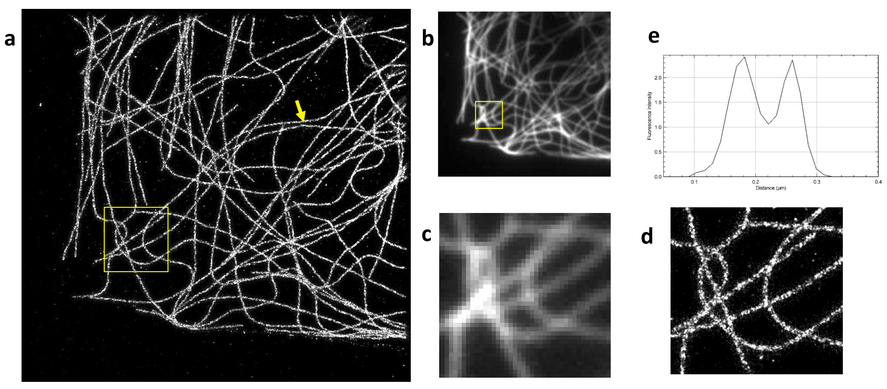


Fig.5 (a) Fast DNA-PAINT image of microtubule with reduced imager strands(20nM). **(b)** Conventional widefield image. **(c)** zoom-in of box in (b). **(d)** zoom-in of box in (a). **(e)** transverse profile of two microtubules highlighted by a yellow arrow in (a).

Results: effect of UV

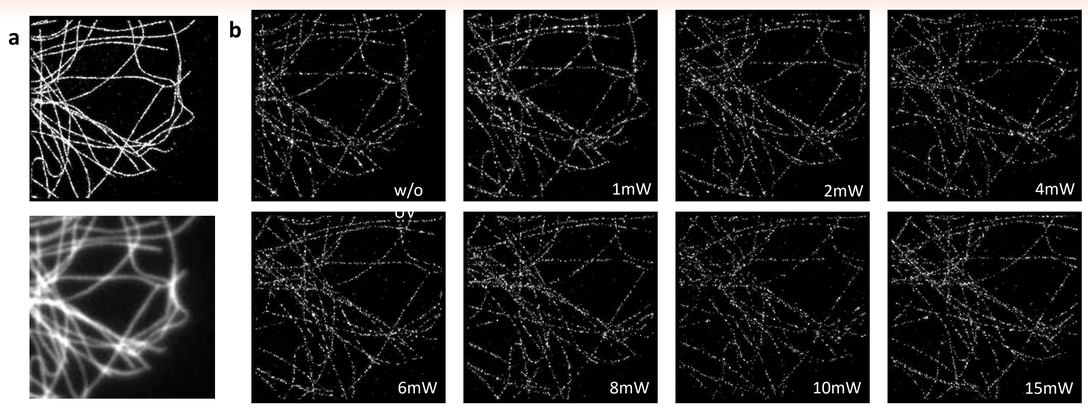
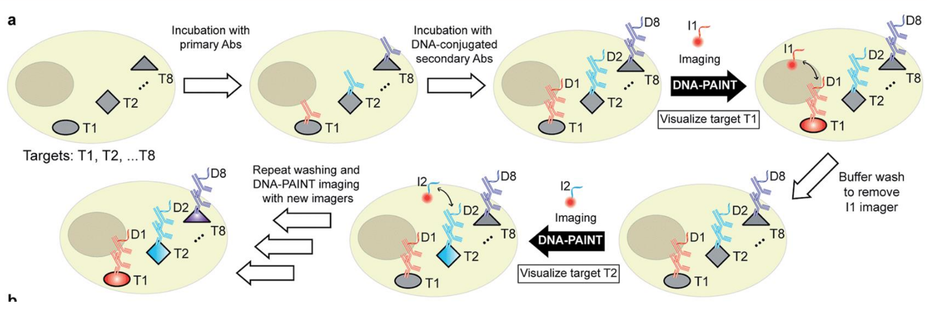


Fig.6 (a) Top: Tubulin in cells, imaged with reduced imager solution (10,000 frames). Bottom: widefield image of (a) **(b)** DNA-PAINT image of same view with (a). 1,000 frames were analyzed under various power of UV. Each UV power level is shown in the bottom right corner of the image. It seems ~10% of UV converts dark state fluorophore to bright state. However, fluorophores under higher power of UV illumination seem to be bleached.

Future Plan



- Direct conjugation of docking strands to antibody to reduce linking distance
- Multi-color imaging with single dye using difference sequence of docking and imager pairs
- Optical sectioning using various angle of incident light

Sarit S.Agasti, et al. Chem.Sci, 8, 3080-3091 (2017)