Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary information

Green-to-red primed conversion of Dendra2 by blue and red lasers

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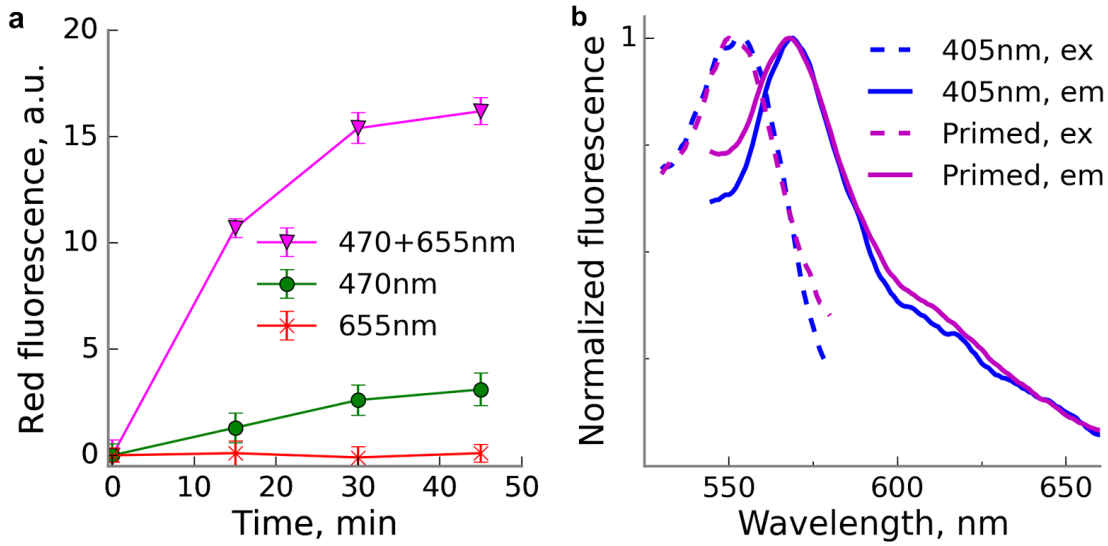

Figure S1. Photoconversion of Dendra2 in a cuvette by LEDs. (**a**) Change of red fluorescence (excitation at 555 nm, emission at 600 nm) intensity upon illumination with 470 nm (green curve), 655 nm (red curve), or 470 + 655 nm (magenta curve). (**b**) Fluorescence spectra of photoconverted Dendra2. Excitation and emission spectra of Dendra2 after *in vitro* primed (470 + 655 nm; magenta lines) or 405-nm photoconversion (blue lines).

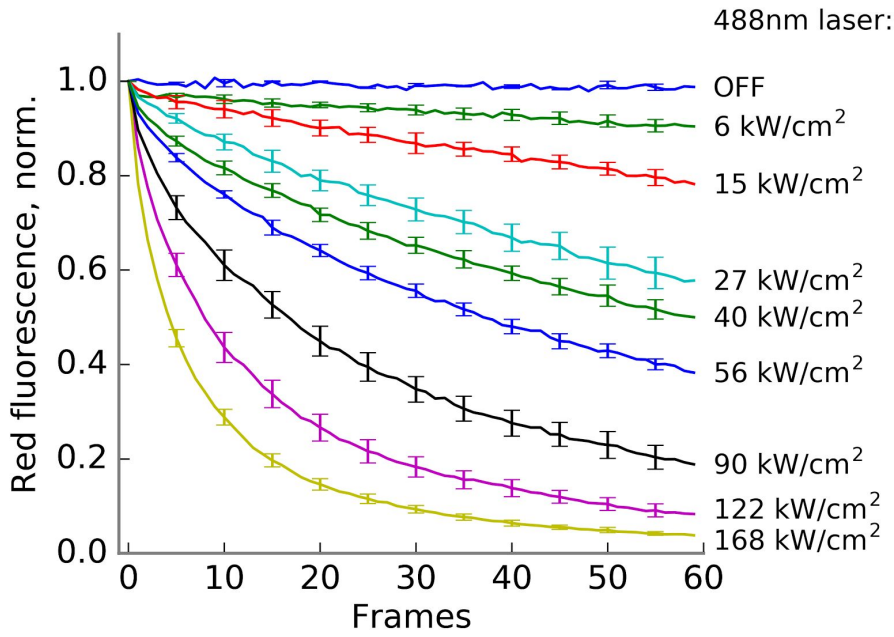

Figure S2. Bleaching of the red form of Dendra2 by 488 nm laser. Dendra2 immobilized on beads was activated with 405 nm light and then illuminated with 488 nm laser of the designated intensities. Data are mean ± s.d. (error bars) for n = 25 beads.

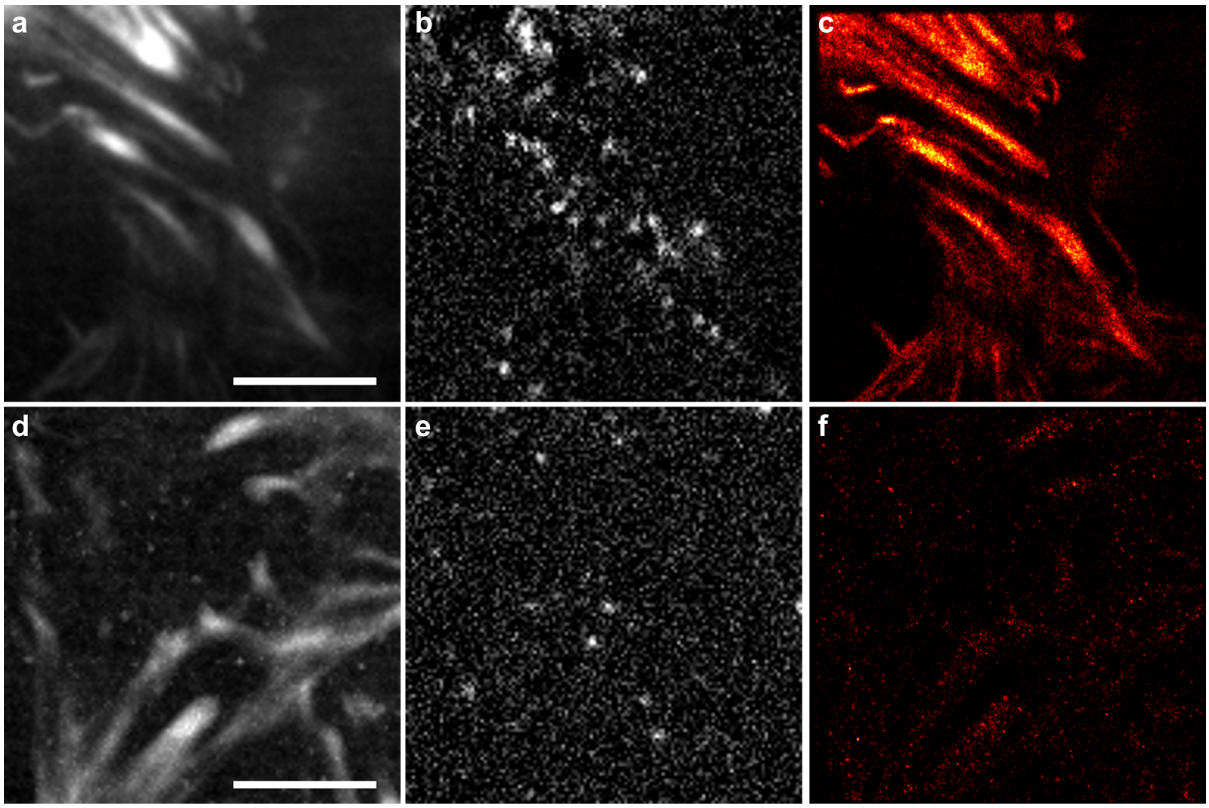

Figure S3. Performance of primed and 488nm-induced photoconversion of Dendra2 for single-molecule localization microscopy. Live HeLa Kyoto cells expressing Dendra2-Lifeact were analyzed. Top row (**a-c**): primed photoconversion by 488 and 640 nm lasers; bottom row (**d-f**): photoconversion by 488 nm laser of the same intensity. (**a**,**d**) Widefield images, (**b**,**e**) Representative frames from the time series (median-subtracted); (**c**,**f**) Super-resolved images of the same regions (total of 5000 frames). Scale bars 5 μm.

Supplementary Methods

Photoconversion by LEDs. For protein expression, the pDendra2-pQE plasmid was transformed into *Escherichia coli* XL1 Blue strain (Invitrogen, USA). Bacterial cultures were grown overnight at 37°C at darkness. The cells were pelleted by centrifugation, resuspended in phosphate-buffered saline and lysed by sonication. The protein was purified by immobilized metal-affinity chromatography using TALON resin (Clontech Laboratories, USA). The cuvette with a Dendra2 solution was illuminated for 30 min with focused light of red (1.2 mW/cm^2) and blue (45 μW/cm^2) ultra-bright light-emitting diodes (LUXEON Rebel high power LEDs LXM3-PD01, 655 nm; LXML-PB01-0040, 470 nm, Philips Lumileds Lighting Company, USA). For conventional photoconversion, Dendra2 solution was illuminated by 405 nm light (~1 W/cm^2 from X-Cite XLED1 light source (Excelitas Technologies, USA). Varian Cary Eclipse Fluorescence spectrophotometer was used to measure excitation and emission spectra.

Confocal microscopy. LSM 510 Meta inverted microscope with 63X 1.4NA oil objective (Carl Zeiss, Germany) was used for confocal microscopy of Dendra2 immobilized on TALON beads. Green and red fluorescence before and after local protein photoconversion was detected at zoom 1 with 488 nm and 561 nm laser lines, respectively. Photoconversion was achieved by illumination of a region of the beads at zoom 16 (single 512x512 px frame, 80 μm^2, pixel dwell time 1.6 μs) with 488 nm laser of varied intensities (8 - 190 kW/cm^2) either alone or in combination with 633 nm laser (450 kW/cm^2). Light intensities were measured at the sample position by a Laser Power Meter LP1 (Sanwa, Japan). The power density was calculated from laser power measurements, wavelength, and the objective' NA, as previously described.[1]

Genetic constructs, cell culture and transient transfection. pDendra2-LifeAct[2] and pDendra2-actin vectors were from Evrogen. HeLa Kyoto cells (EMBL collection, Mycoplasma free, PCR-based detection) were grown in Dulbecco's Modified Eagle Media (DMEM) containing 10% fetal bovine serum, 50 U/ml penicillin, 50 μg/ml streptomycin and 4 мM L-glutamine at 37°C and 5% CO_2. For transient transfection and imaging 35 mm Fluorodish™ cell culture dishes (WPI Inc.) were used. Transfections were performed with X-tremeGENE 9 DNA transfection reagent (Roche) according to the manufacturer's protocol. The cells were imaged in Minimum Essential Medium Eagle (MEM) (Sigma) without serum.

Live cell imaging, **single-molecule localization microscopy.** Imaging was carried out on Eclipse Ti N-STORM microscope (Nikon, Japan) with NIS-Elements Software and/or the Micro-Manager software.[3] The samples were focused using 100X

oil-immersion objective (Apo TIRF/1.49, Nikon) and PFS (perfect focus system), C-NSTORM QUAD filter cube (Nikon), and a 1.5x lens to the effective pixel size 107 nm of the EM-CCD camera (iXon3 DU-897, Andor, UK). The camera was operated at 10 MHz readout rate, 14-bit, with an EM gain set to 200 and pre-amplifier gain set to 5.1 (12.15 electrons per A/D count).

For power dependence measurements of primed photoconversion, summed in Fig. 2, the total internal reflection mirror was set to neutral position, resulting in unfocused beam of laser light coming straight out of the objective. The power of 488 nm and 640 nm laser lines was adjusted in the range of 0.05 - 4.5 W/cm^2 and 0.76 - 250 W/cm^2, respectively, and applied as 300ms-long pulses. The sample was continuously illuminated with a green laser (561 nm) at 120 W/cm^2, with EM-CCD exposure time of 50 ms. To address the possible errors arising from non-uniform illumination or microscope focusing, a full set of the measurements of the effect of various laser powers on photoconversion was obtained from the same cells in the following manner. A bleaching step (90 sec illumination with 561 nm laser, full power) preceded each acquisition in order to bleach any pre-activated Dendra2 molecules. Then the normalization measurement was taken (4.5 W/cm^2 488 nm, no red light for Fig. 2a, 1 W/cm^2 488 nm, no red light for Fig. 2b), followed by the experimental data points. At the end of the series the normalization measurement was repeated.

All cell culture experiments were triplicated to address the variation arising from cell heterogeneity (7-8 randomly chosen cells in each experiment; no cells were excluded from consideration). The total number of samples (cells) was limited to 23, sufficient for estimation of the population standard deviation from the sample standard deviation with an error no greater than 30% with a confidence level of 95%, assuming normally distributed values. The expected magnitude of the photoconversion enhancement (more than an order of magnitude for previously published primed conversion with 700-850 nm light) well exceeded possible margin for error with chosen sample size. None of the collected data points showed strong deviation from normality (Shapiro-Wilk test, $p > 0.05$). The Shapiro-Wilk test, as well as the two-tailed, unequal variance t-test (Fig.2) were performed with the SciPy software (http://scipy.org/).

For single-molecule localization microscopy, the angle of the TIRF mirror of the microscope was adjusted to allow for total internal reflection. The sample was illuminated at 405 nm (0.22 W/cm^2) versus 488 nm (1 W/cm^2) and 640 nm (240 W/cm^2) laser beams, with pulses of 50 ms. The running median filtering was applied to the time series to improve the consequent localization.[4] Sparse images of individual fluorophores were fitted in ThunderStorm[5] plugin for Fiji.[6]

Supplementary References

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