SUPPORTING INFORMATION

Hybridization and reaction-based, fluorogenic nucleic acid probes

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Commercially available chemicals of the best quality from Aldrich/Sigma/Fluka (Germany) were obtained and used without purification. Unmodified DNAs were purchased from Iba-Go GmbH (Germany). Phosphoramidites and controlled pore glass (CPG) solid support were from Glen Research (USA) and Link Technologies (UK). DNA synthesis was conducted on a K&A H-8 DNA/RNA synthesizer. MALDI-TOF mass spectra were recorded on a Bruker MicroTOF mass spectrometer. The matrix mixture (2:1 v/v) was prepared from 6-aza-2-thiothymine (ATT, saturated solution in acetonitrile) and diammonium citrate (0.1 M in water). Samples for mass spectrometry were prepared by the dried-droplet method by using a 1:2 probe/matrix ratio. Mass accuracy with external calibration was 0.1% of the peak mass, that is, $\pm$ 7.0 at m/z 7000. HPLC was performed at 22 °C on a Shimadzu liquid chromatograph equipped with a UV detector and either a Macherey–Nagel Nucleosil C4 250 x 4.6 mm column or a Macherey–Nagel Nucleosil C18 250 x 4.6 mm. Gradient of solution B (CH_3CN) in solution A (0.1 M aqueous $(NEt_3H)(OAc)$ for ODN1-4 or 5 % CH_3CN in 0.1 M aqueous $(NEt_3H)(OAc)$ for ODN5) was used for HPLC purifications. UV/Vis spectra were measured on a PerkinElmer Bio+ UV/Vis spectrophotometer by using 1 cm optical path, micro–UV-cuvettes (Brand GmbH, Germany) with a sample volume of 0.1 mL. Fluorescence spectra were acquired on a Varian Cary Eclipse fluorescence spectrophotometer by using black-wall fluorescence ultra-microcuvettes (Hellma GmbH, Germany) with a sample volume of 0.1 mL. These cuvettes were also used for photochemical experiments. Light from red laser (635 nm, 2.6 mW) was applied from the top of the cuvette via an optical fiber.

Synthesis of DNA conjugates was conducted as described elsewhere: Rotaru, A.; Mokhir, A. *Angew. Chem.* **2007**, *119(32)*, 6293-6296.

Conjugate ODN1: Yield 61 %, HPLC (C18 column, in 32 min from 0 to 40 % solution B, in 10 min to 90 % solution B): R_t = 24.0 min; MALDI-TOF MS, negative mode, calculated for $C_{240}H_{299}N_{72}O_{143}P_{22}S_2$ ($[M-H]^-$): *m/z* 7222, found 7221.

Conjugate ODN2: Yield 68 %, HPLC (C18 column, in 32 min from 0 to 40 % solution B, in 10 min to 90 % solution B): R_t = 23.1 min; MALDI-TOF MS, negative mode, calculated for $C_{232}H_{285}N_{72}O_{139}P_{21}$ ($[M-H]^-$): *m/z* 6953, found 6952.

Conjugate ODN3: Yield 63 %, HPLC (C4 column, in 32 min from 0 to 40 % solution B, in 10 min to 90 % solution B): R_t = 24.0 min; MALDI-TOF MS, negative mode, calculated for $C_{256}H_{325}N_{77}O_{146}P_{22}S_2$ ($[M-2H]^-$): *m/z* 7558, found 7560.

Conjugate ODN4: Yield 73 %, HPLC (C4 column, in 32 min from 0 to 40 % solution B, in 10 min to 90 % solution B): R_t = 23.7 min; MALDI-TOF MS, negative mode, calculated for $C_{248}H_{308}N_{77}O_{142}P_{21}$ ($[M-2H]^-$): *m/z* 7286, found 7283.

Conjugate ODN5: Yield 13 %, HPLC (C4 column, in 32 min from 1 to 70 % solution B, in 6 min to 90 % solution B, 10 min at 90 % solution B): R_t = 20.0 min; MALDI-TOF MS, negative mode, calculated for $C_{247}H_{303}N_{92}O_{127}P_{21}InCl$ ($[M-H]^-$): *m/z* 7355, found 7360.

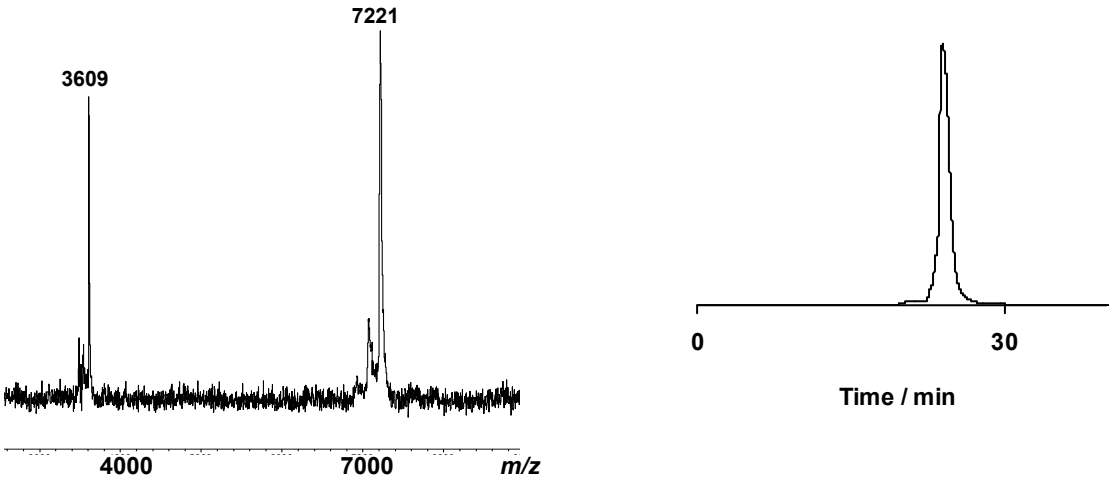

Figure S1. MALDI-TOF mass spectrum (left plot, *m/z* values for $[M-2H]^{2-}$ and $[M-H]^{-}$ peaks are indicated) and HPLC profile (right plot) of HPLC purified conjugate ODN1

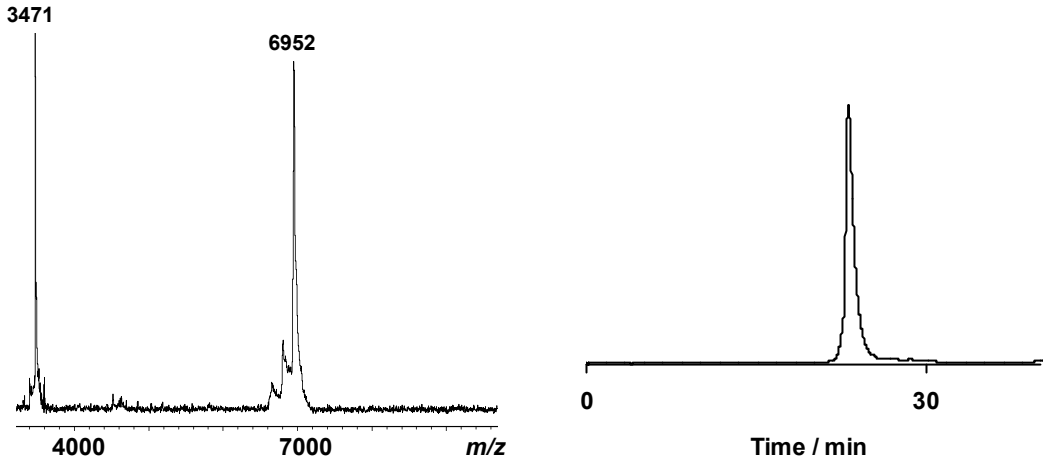

Figure S2. MALDI-TOF mass spectrum (left plot, *m/z* values for $[M-2H]^{2-}$ and $[M-H]^{-}$ peaks are indicated) and HPLC profile (right plot) of HPLC purified conjugate ODN2

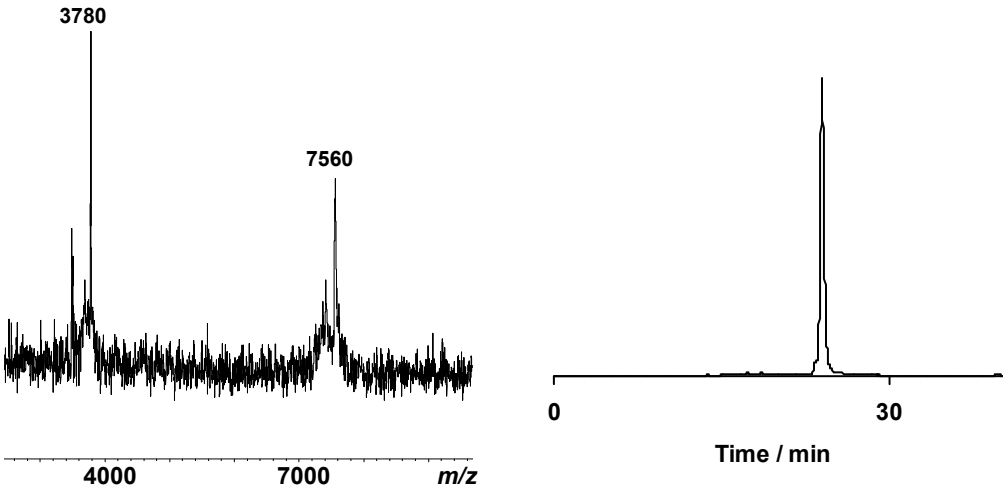

Figure S3. MALDI-TOF mass spectrum (left plot, *m/z* values for $[M-2H]^{2-}$ and $[M-H]^{-}$ peaks are indicated) and HPLC profile (right plot) of HPLC purified conjugate ODN3

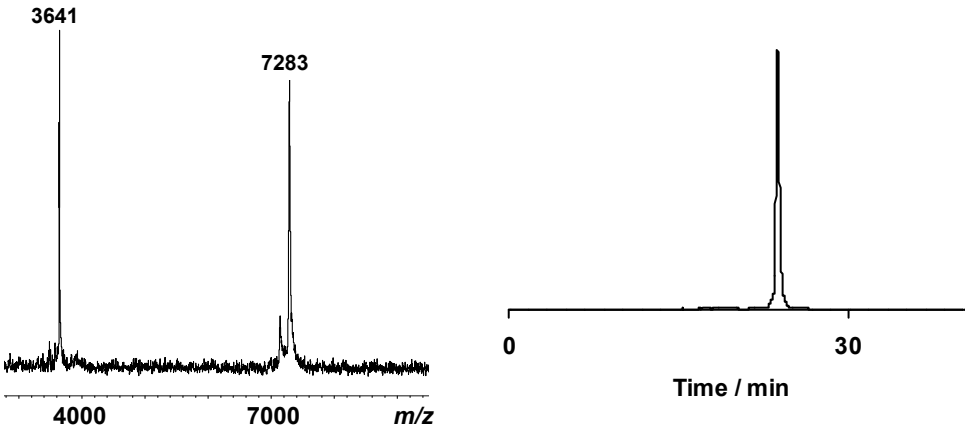

Figure S4. MALDI-TOF mass spectrum (left plot, *m/z* values for $[M-2H]^{2-}$ and $[M-H]^{-}$ peaks are indicated) and HPLC profile (right plot) of HPLC purified conjugate ODN4

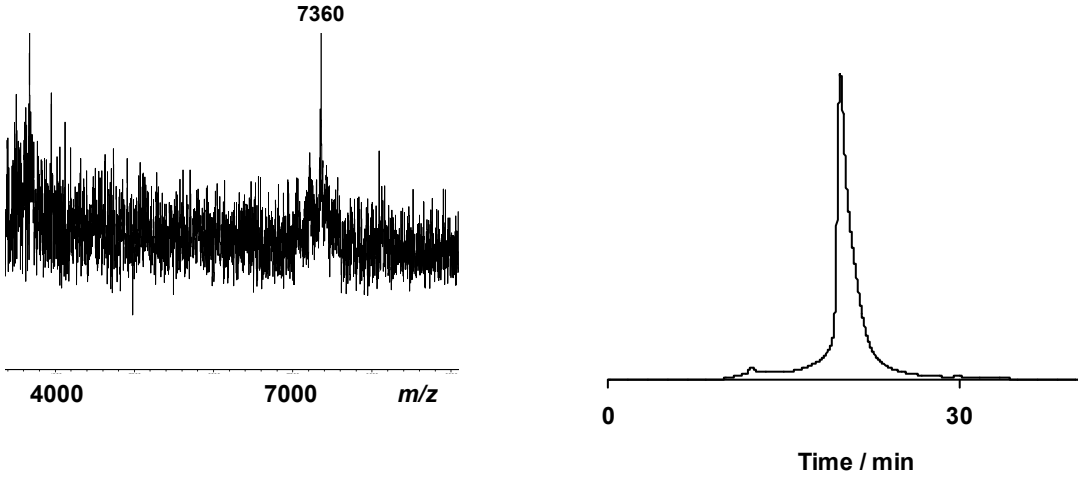

Figure S4. MALDI-TOF mass spectrum (left plot, *m/z* value for $[M-H]^-$ peak is indicated) and HPLC profile (right plot) of HPLC purified conjugate ODN5

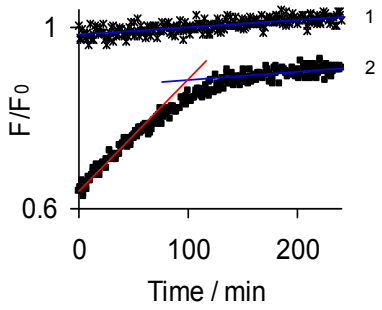

Figure S5. Changes of fluorescence intensity (F, λ_{ex}= 540 nm, λ_{em}= 580 nm) as a function of irradiation time (635 nm, 2.6 mW), F_0 – fluorescence of corresponding mixtures in the absence of the complementary nucleic acid TEMP at the time point t=0; curve 1: ODN3 (100 nM), ODN5 (1 eq); curve 2: ODN3 (100 nM), ODN5 (1 eq), TEMP (1 eq); blue and red straight lines label kinetic regions of constant cleavage rate

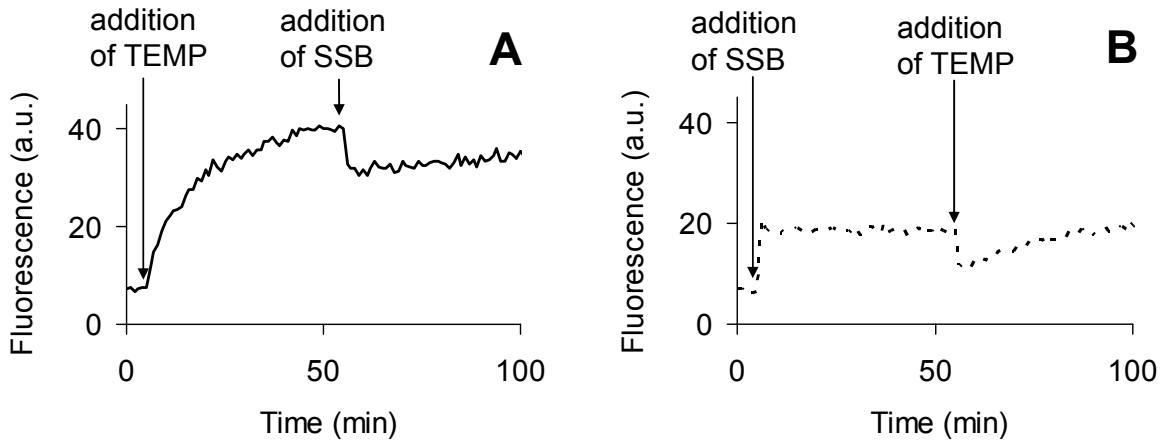

Figure S6. Changes of fluorescence (λ_{ex}= 495 nm, λ_{em}= 520 nm) of molecular beacon **MB** (100 nM) upon addition of either a nucleic acid template **TEMP** (150 nM) or SBB (150 nM); buffer: MOPS, 10 mM, pH 7, NaCl 150 mM.

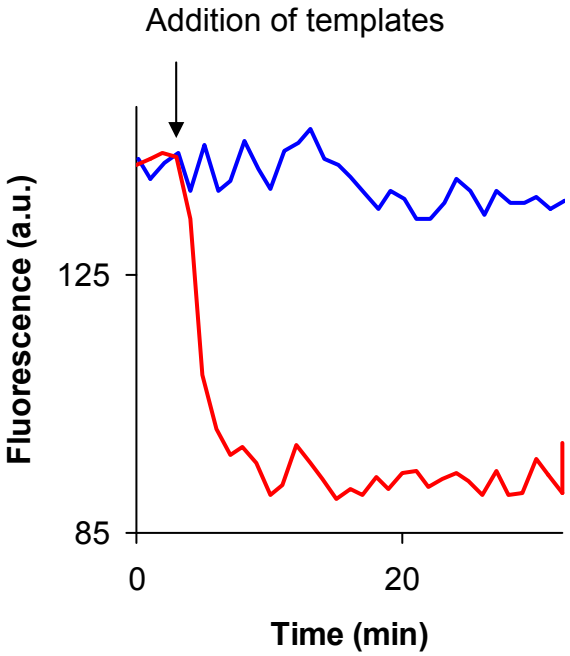

Figure S7. Change of fluorescence (λ_{ex}= 540 nm, λ_{em}= 580 nm) of a mixture of ODN3 (50 nM) and truncated ODN5 (ODN5*, 1 eq: 3'-InPPa-AGT TCT AGT AAC GA) upon addition of either a fully matched template (TEMP, red trace) or an optimized mismatched template (TEMP_26C, blue trace, see Table S1 for its sequence). Buffer: MOPS, 10 mM, pH 7, NaCl 150 mM; 37 °C

Table S1. Optimization of the position of the mismatch in nucleic acid templates used in the photochemical templated cleavage of ODN3 in the presence of ODN5*

DNA template (5'→3')	**F(+template)/ F(-tamplate)[i]**
TEMP	0.63
TEMP_23A: TGGCACCCAGCACAATGAAGAT*A*AAGATCATTGCT	0.67
TEMP_24C: TGGCACCCAGCACAATGAAGATC*C*AGATCATTGCT	0.94 (selected position)
TEMP_26C: TGGCACCCAGCACAATGAAGATCAA*C*ATCATTGCT	0.77
TEMP_29A: TGGCACCCAGCACAATGAAGATCAAGAT*A*ATTGCT	0.81

[i] F(+template)/F(-template): fluorescence (λ_{ex}= 540 nm, λ_{em}= 580 nm) of ODN3 (100 nM), ODN5* (100 nM) mixtures in the presence (F(+template)) and in the absence (F(-template) of the templates indicated in the table; Buffer: MOPS, 10 mM, pH 7, NaCl 150 mM; 37 °C

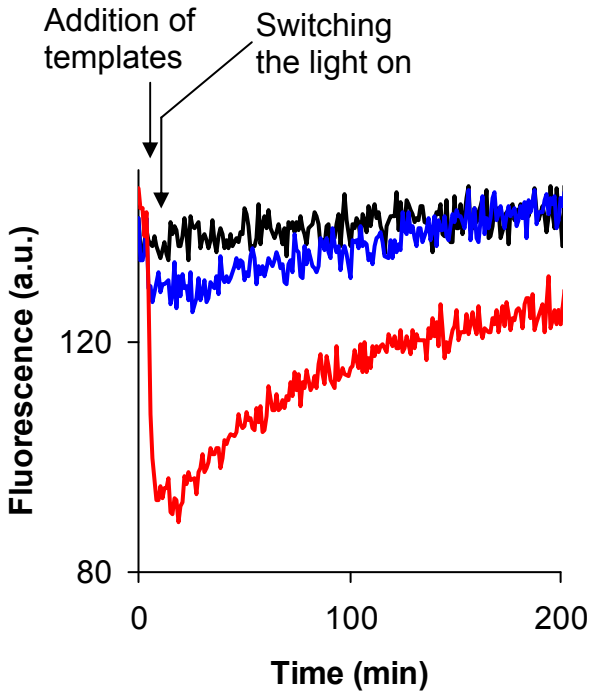

Figure S8. Change of fluorescence (λ_{ex}= 540 nm, λ_{em}= 580 nm) of a mixture of ODN3 (50 nM) and truncated ODN5 (ODN5*, 1 eq: 3'-InPPa-AGT TCT AGT AAC GA) upon exposure to light (635 nm, 2.6 mW): in the absence of any template (black trace), in the presence of a fully matched template (TEMP, red trace) or in the presence of the optimized mismatched template (TEMP_26C, blue trace, see Table S1 for its sequence). The templates were added at the time indicated with an arrow on the plot. Irradiation was started at the later time indicated with an arrow on the plot. Buffer: MOPS, 10 mM, pH 7, NaCl 150 mM; 37 °C

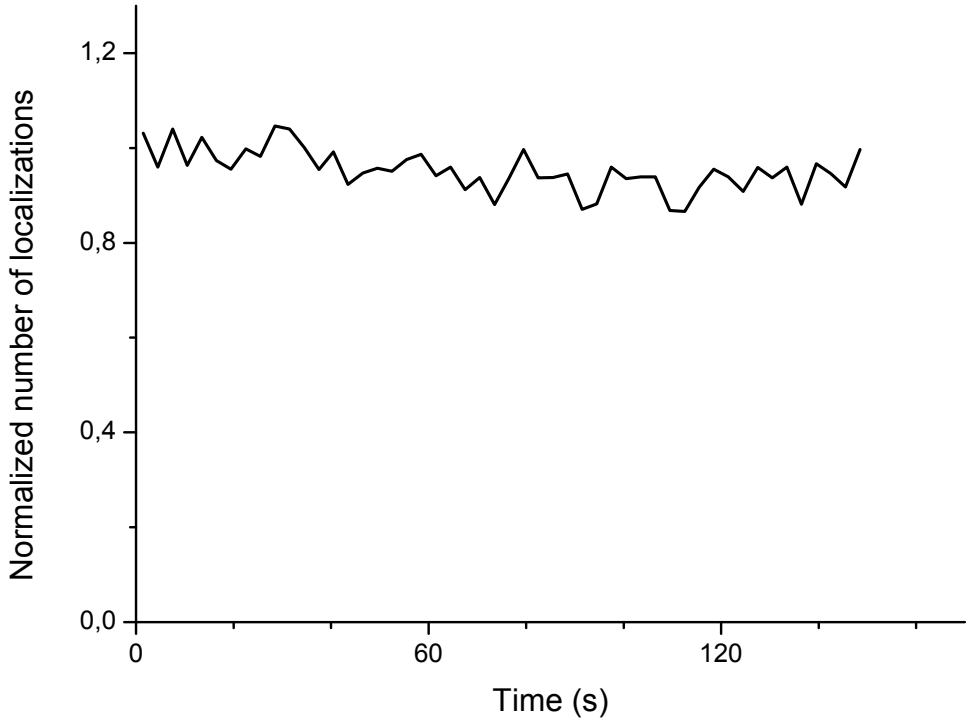

Figure S9. SM fluorescence imaging of ODN3; ODN5* was used as a photocatalyst and TEMP_26C (Table S1) - as a template. The latter oligonucleotide was bound to a glass surface; the mixture was irradiated with the red laser (647 nm). Tetramethylrhodamine from ODN3 was excited at 514 nm and monitored as described below. In contrast to the reaction in the presence of the fully matched template (Figure 4 of the main text of the paper, red trace), no cleavage of the SCH=CHS linker was observed in this case. These data are in agreement with the data shown in Figures S7 and S8 obtained with dye assemblies.

Single-molecule fluorescence imaging.

Single-molecule fluorescence microscopy was performed on a custom-built microscope.[1] Briefly, a multi-line laser (Innova 70C, Coherent, USA) was coupled into an inverted microscope (IX71, Olympus, Japan) equipped with an oil-immersion objective (60x NA1.45, Olympus, Japan). The fluorescence signal was detected using an electron-multiplying charge-coupled device (EMCCD) (Ixon, Andor, Ireland) and appropriate filters and dichroic mirrors (AHF, Tübingen, Germany).

Single-molecule fluorescence microscopy was performed in 8-well chambered LabTeks (Nunc, Germany) and in phosphate buffered saline (PBS, pH7.4, Sigma). For the actual experiment, a target DNA strand (5'-TACGTATGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCTCCTG-3') (IBA-Go

GmbH, Göttingen, Germany) carrying a biotin on the 5' end was immobilized on a glass surface that has been treated with bovine serum albumin (BSA)/BSA-biotin (1:10) (Sigma) and streptavidin (Sigma). Tetramethylrhodamine was excited with 514 nm (1-2 kW/cm^2 10 mW) and imaged at a frame rate of 4 Hz. The photocleavage of the linker was induced with 647 nm (0.5-1 kW/cm^2 5 mW). Data analysis was performed with the rapidSTORM software package.[2] Appropriate intensity thresholds were used to identify single fluorophores.

1. M. Heilemann, S. van de Linde, M. Schuttpelz et al., *Angew Chem Int Ed Engl* **47** (33), 6172 (2008).
2. S. Wolter, M. Schuttpelz, M. Tscherepanow et al., *J Microsc* **237** (1), 12 (2010).