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Label as you fold: Methyltransferase-assisted functionalization of DNA nanostructures - SI

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Recombinant M.TaqI was produced as previously described.[1] Ado-6-azide was obtained as described before.[2] CF640R azide was obtained from Biotium, DBCO-Cy5 was bought from Jena Bioscience, DNA origami scaffolds from Tilibit nanosystems, staple strands from IDT. If not stated otherwise, chemicals were purchased from Sigma-Aldrich.

Synthesis of AdoYnCF640R

AdoYnCF640R was synthesized by coupling AdoYnYn[2,3] with CF640R azide by copper-catalyzed azide alkyne cycloaddition (CuAAC). Briefly, water (30.9 µl) and aqueous solutions of $CuSO_4$ (5.0 µl, 25.0 mM, 125 nmol, 0.5 eq.), THPTA (4.2 µl, 30.0 mM, 125 nmol, 0.5 eq.) and sodium ascorbate (20.8 µl, 500 mM, 10.4 µmol, 41.7 eq.) were mixed before the pH was adjusted to 5 with diluted sulfuric acid. Aqueous solutions of CF640R azide (89.3 µl, 2.8 mM, 250 nmol, 1 eq.) and AdoYnYn (58.2 µl, 0.01 % TFA, 4.3 mM, 250 nmol, 1 eq.) were added and the reaction was monitored by reverse-phase HPLC (Prontosil C-18 column: 250 × 4.5 mm, 5 µm, 120 Å; acetonitrile gradients 11.9–14.7% in 10 min and 14.7–70% in 20 min in aqueous 0.01% TFA; flow 1 ml/min; UV detection at 260 nm and 640 nm). After the reaction was completed (3 h at room temperature), reverse-phase HPLC (as described above) was used to isolate AdoYnCF640R (retention time of 17 min). The obtained solution was lyophilized close to dryness and an aqueous solution of TFA (0.01 %, 100 µl) was added. AdoYnCF640R was obtained with a yield of 41% (102 nmol) as determined by UV-Vis absorption spectroscopy (ε_{640} = 105 000 l mol^{-1} cm^{-1}).

Azide labeling with subsequent fluorophore attachment

A solution containing 100 nM DNA origami staples, 5 nM p7249 scaffold, 2 mM $MgCl_2$, 40 µM Ado-6-azide cofactor, 0.2 mg/ml M.TaqI in 1x CutSmart buffer (NEB) in a total volume of 50 µl is prepared. The 2 mM $MgCl_2$ are added on top of the 10 mM present in the CutSmart buffer, yielding a total final concentration of 12 mM $MgCl_2$. The mix is exposed to a temperature ramp from 65 °C to 50 °C over 1 h in a thermocycler. Another 40 µM Ado-6-azide cofactor are added and the mix is exposed again to a temperature ramp from 65 °C to 50 °C over 1 h. 2 µl of proteinase K (20 mg/ml) are added and the sample is incubated at 40 °C for 1 h. In the next step the origami is assembled by rapid heating to 80 °C, then cooling down from 80 °C to 66 °C at 2 °C/min and from 65 °C to 25 °C at 0.5 °C/min. 1.6 µl DBCO-Cy5 (10 mM) are added and the reaction is kept at 37 °C over night. The sample is transferred to an Amicon Ultra 100 kDa MWCO centrifugal filter and purified by five successive cycles of adding 400 µl 1x TAE 12 mM $MgCl_2$ buffer and centrifugation at 6000 rcf for 7 min. Taking into account that some TCGA motifs (recognition sequence of M.TaqI) are located at cross over positions in the origami triangle design and likely

not accessible to the enzyme, about 20 potential attachment sites (out of 24) are expected to remain available for labeling.

Direct labeling with fluorophores

200 nM DNA origami staples, 10 nM p7249 scaffold, 2 mM $MgCl_2$, 0.14 mM Ado-CF640R cofactor and 0.4 mg/ml M.TaqI are mixed in 1x CutSmart buffer (NEB) in a total volume of 50 µl. The 2 mM $MgCl_2$ are added on top of the 10 mM present in the CutSmart buffer, yielding a total final concentration of 12 mM $MgCl_2$. The mix is exposed to a temperature ramp from 65 °C to 50 °C over 1 h in a thermocycler. 2 µl of proteinase K (20 mg/ml) are added and the sample is incubated at 40 °C for another 1 h. In the next step the origami is assembled by rapid heating to 80 °C, then cooling down from 80 °C to 66 °C at 2 °C/min and from 65 °C to 25 °C at 0.5 °C/min. The sample is transferred to an Amicon Ultra 100 kDa MWCO centrifugal filter and purified by five successive cycles of adding 400 µl 1x TAE 12 mM $MgCl_2$ buffer and centrifugation at 6000 rcf for 7 min.

Preparation of stable fluorescent beacons

200 nM DNA origami staples, 10 nM p7249 scaffold, 2 mM $MgCl_2$, 0.14 mM Ado-6-azide cofactor and 0.4 mg/ml M.TaqI are mixed in 1x CutSmart buffer (NEB) in a total volume of 50 µl. The 2 mM $MgCl_2$ are added on top of the 10 mM present in the CutSmart buffer, yielding a total final concentration of 12 mM $MgCl_2$. The mix is exposed to a temperature ramp from 65 °C to 50 °C over 1 h in a thermocycler. 2 µl proteinase K (20 mg/ml) are added and the sample is incubated at 40 °C for another 1 h. In the next step the origami is assembled by rapid heating to 80 °C, then cooling down from 80 °C to 66 °C at 2 °C/min and from 65 °C to 25 °C at 0.5 °C/min. 3.3 µl of 1x TAE with 120 mM $MgCl_2$ are added to the DNA origami solution to reach a final $MgCl_2$ concentration of 20 µM. 50 µm of the solution are placed in a 96 well plate and exposed to UV radiation for 6 h (UVP 3UV-36 lamp, 302 nm setting). A control sample was meanwhile stored in the dark. 3.2 µl DBCO-Cy5 (10 mM) are added and the reaction is kept at 30 °C over night. The solution is transferred to a 100k MWCO Amicon centrifugal filter and purified by five cycles of addition of 400 µl 1x TAE with 12 mM $MgCl_2$ and centrifugation at 6000 rcf for 7 min. To test the heat stability, 12 µl of the purified origami solution are kept at 65 °C for 30 min in a thermocycler.

Fluorescence microscopy sample preparation

1 µl of the purified and labeled origami solution (2 to 5 ng/µl) and 0.4 µl YOYO-1 (20 µM) are added to 49 µl imaging buffer (6 mM $MgCl_2$, 400 mM DTT, 1x TE) and incubated at room temperature for at least 30 min. 10 µl were loaded into the gap between a microscopy slide and a chemically activated coverslip.[4] Adsorption of remnant, free-floating Cy5 molecules to the glass (for Figure S4) was facilitated by an imaging buffer with increased magnesium concentration (12 mM $MgCl_2$).

Fluorescence microscopy imaging

Fluorescence microscopy was carried out on an epi-fluorescence microscope from TILL Photonics with a 100x oil immersion objective (UPlanSApo,∞/0.17/FN26.5/ N.A. 1.4, Olympus), an EMCCD camera (Ixon3, Andor) and the following filter sets: ex485/20, em525/30 (YOYO-1) and ex640/14, em684/24 (Cy5 / CF640R). The fluorescence intensities in the histograms were normalized to match a maximum at roughly 250 a.u.

Attempt at labeling pre-assembled DNA origami

Before developing the in situ labeling strategy, we tested directly labeling already assembled DNA origamis: 1 nM assembled DNA origami, 40 µM Ado-CF640R cofactor and 7.8 µg/ml M.TaqI are mixed in 1x CutSmart buffer (NEB) in a total volume of 25 µl. The solution is kept at 37 °C for 2 h. 2 µl of proteinase K (20 mg/ml) are added and the sample is incubated at 45 °C for another 2 h. The sample is transferred to an Amicon Ultra 100 kDa MWCO centrifugal filter and purified by four successive cycles of adding 400 µl 1x TAE 12 mM $MgCl_2$ buffer and centrifugation at 6000 rcf for 7 min. Figure S1 shows fluorescence microscopy images that demonstrate the (unsuccessful) labeling results.

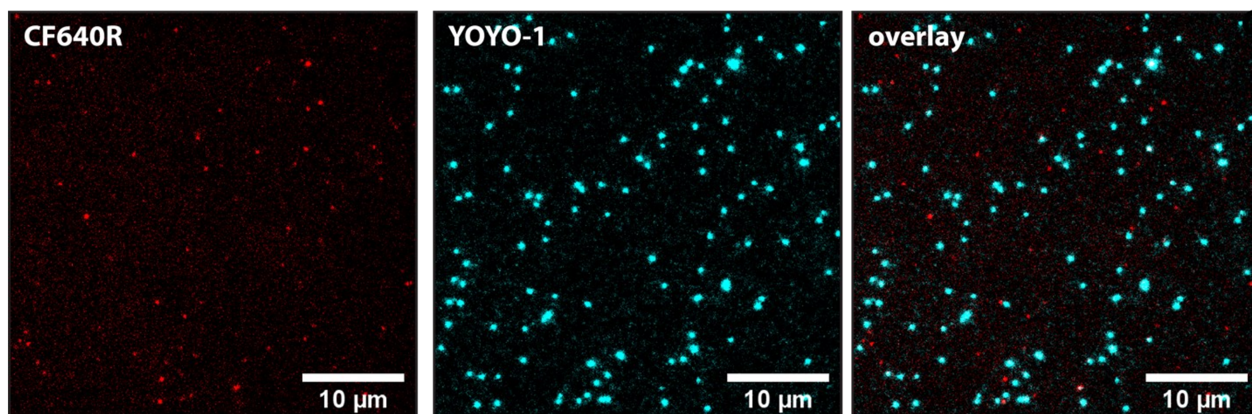

Figure S1. Fluorescence microscopy images of the attempts to label DNA origamis after their assembly (background corrected). (left) CF640 channel, covalent labels; (middle) YOYO-1 channel, intercalating DNA stain; (right) overlay.

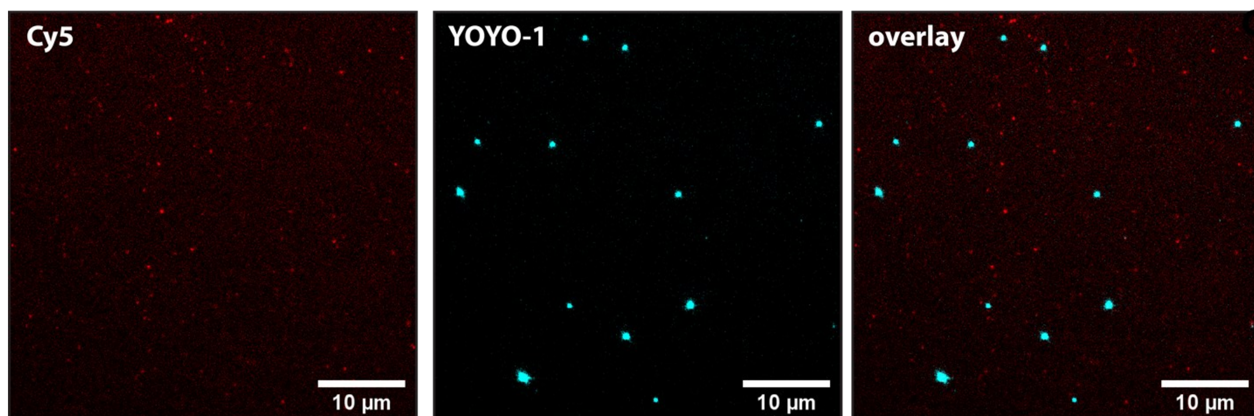

Figure S2. Fluorescence microscopy images of DNA origamis that were subjected to the same labeling procedure as the ones for Figure 1B, but without any M.TaqI having been added. (left) Cy5 channel; (middle) YOYO-1 channel; (right) overlay.

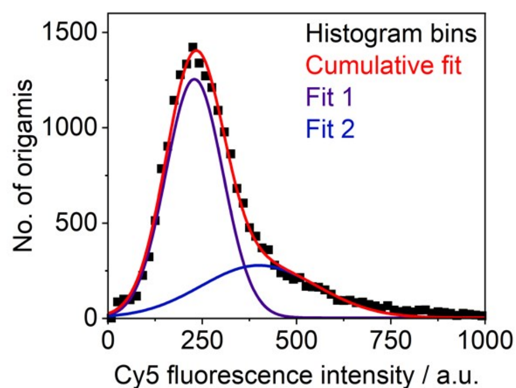

Figure S3. Data from Figure 1D, fitted by two Gaussians. The centers of the two peaks are at (229 ± 1) a.u. (Fit 1) and (400 ± 22) a.u. (Fit 2), respectively.

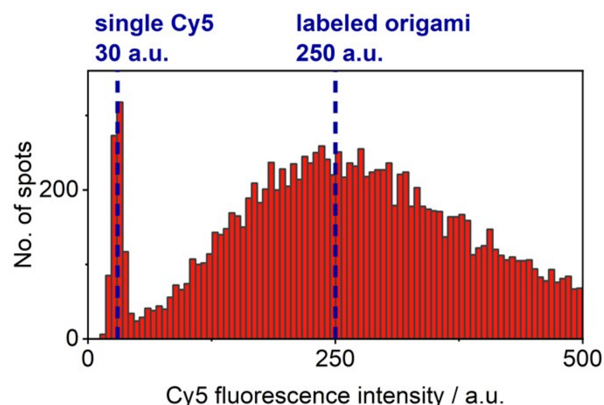

Figure S4. Fluorescence intensity distribution of a Cy5-labeled DNA origami sample where the microscopy sample was prepared with 12 mM $MgCl_2$, so that remnant individual Cy5 molecules would also adsorb to the glass surface.

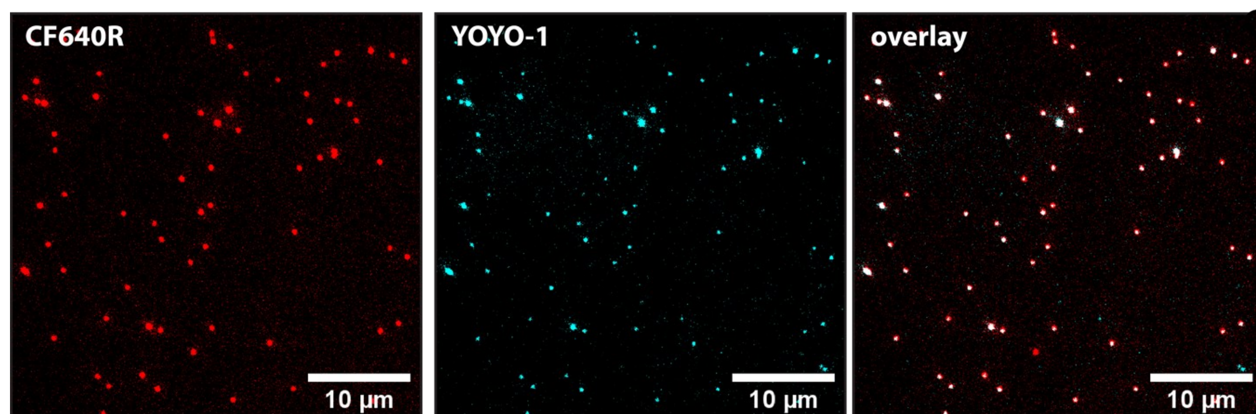

Figure S5. Exemplary fluorescence microscopy images of DNA origamis directly labeled with the dye CF640R (background corrected). (left) CF640R, (middle) YOYO-1, (right) overlay.

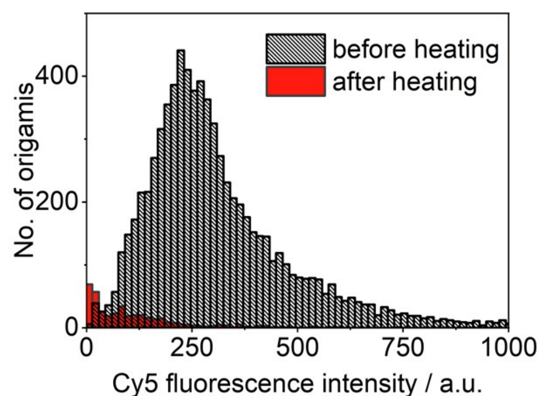

Figure S6. Fluorescence intensity distribution of a DNA origami triangle sample that has not been crosslinked by UV, before and after exposure to 65 °C for 30 min.

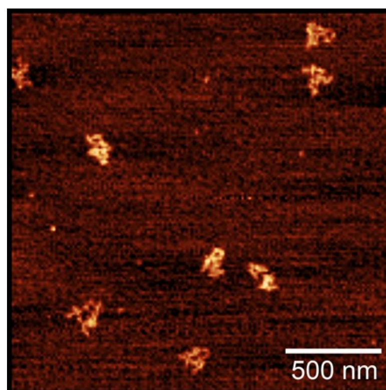

Figure S7. AFM image of UV-crosslinked fluorescent beacons after they have been heated to 65 °C for 30 min.

References

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