124-color super-resolution imaging by engineering DNA-PAINT blinking kinetics

Orsolya K. Wade[1,2,5], Johannes B. Woehrstein[1,2,5], Philipp C. Nickels[1,2,5], Sebastian Strauss[1,2,5], Florian Stehr[2], Johannes Stein[2], Florian Schueder[1,2], Maximilian T. Strauss[1,2], Mahipal Ganji[1,2], Joerg Schnitzbauer[1,2], Heinrich Grabmayr[1,2], Peng Yin[3,4], Petra Schwille[2] & Ralf Jungmann[1,2]

[1]Department of Physics and Center for Nanoscience, Ludwig Maximilian University, 80539 Munich, Germany, [2]Max Planck Institute of Biochemistry, 82152 Martinsried near Munich, Germany, [3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts, USA, [4]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, USA. [5]These authors contributed equally to this work.

Supplementary Methods

Materials

Unmodified, dye-labeled, and biotinylated DNA oligonucleotides were purchased from MWG Eurofins or Integrated DNA Technologies. DNA scaffold strands were purchased from Tilibit (p7249, identical to M13mp18). Streptavidin was purchased from Thermo Fisher (cat: S-888). BSA-Biotin was obtained from Sigma-Aldrich (cat: A8549). Glass slides were ordered from Thermo Fisher (cat: 10756991) and coverslips were purchased from Marienfeld (cat: 0107032). Freeze 'N Squeeze columns were ordered from Bio-Rad (cat: 732-6165). PEG-8000 was purchased from Merck (cat: 6510-1KG). Tris 1M pH 8.0 (cat: AM9856), EDTA 0.5M pH 8.0 (cat: AM9261), Magnesium 1M (cat: AM9530G) and Sodium Chloride 5M (cat: AM9759) were ordered from Ambion. Ultrapure water (cat: 10977-035) was purchased from Thermo Fisher Scientific. Potassium chloride (cat: 6781.1) was ordered from Roth. Sodium hydroxide (cat: 31627.290) was purchased from VWR. Tween-20 (cat: P9416-50ML), Glycerol (cat: G5516-500ML) and Methanol (cat: 32213-2.5L) were ordered from Sigma-Aldrich. Protocatechuate 3,4-Dioxygenase Pseudomonas (PCD) (cat: P8279), 3,4-Dihydroxybenzoic acid (PCA) (cat: 37580-25G-F) and (+-)-6-Hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox) (cat: 238813-5G) were purchased from Sigma-Aldrich. SYBR Safe DNA gel stain was purchased from Invitrogen (cat: SS33102). HeLa cells were purchased from the Leibniz Institute DSMZ (cat: ACC-57). A549 cells were purchased from ATCC. Dulbecco's Modified Eagle medium (DMEM) with high glucose, GlutaMAX™ and sodium pyruvate (cat: 31966-021), Fetal Bovine Serum (FBS) (cat: 10500-064), 1× Phosphate Buffered Saline (PBS) pH 7.2 (cat: 20012-019), 10× PBS pH 7.4 (cat: 70011036), and 0.05% Trypsin–EDTA (cat: 25300-054) were purchased from Thermo Fisher Scientific. 16% (w/v) Paraformaldehyde (cat: 28906) was purchased from Thermo Fisher Scientific. Glutaraldehyde (cat: 16220) was obtained from Electron Microscopy Sciences. Bovine Serum Albumin (cat: A4503-10G) was ordered from Sigma-Aldrich. Penicillin-Streptomycin (cat: 15140-122) was ordered from Thermo Fisher Scientific. Triton X-100 (cat: 6683.1) was purchased from Roth. Glass-bottomed 8-well µ-slides (cat: 80827) were obtained from ibidi. Primary polyclonal goat anti-CHC antibody (cat: sc-6579) was purchased from Santa Cruz Biotechnology. Primary monoclonal mouse anti-PMP70 antibody (cat: SAB4200181) was purchased from Sigma-Aldrich. Secondary polyclonal antibodies (cat: 705-005-147 and 715-005-150) were purchased from Jackson ImmunoResearch. Dextran sulfate 50% solution was purchased from Merck (cat: S4030). Sheared Salmon Sperm DNA (cat: AM9680), 10× PBS (cat: AM9624), 20× SSC (cat: AM9763), Hi-Di Formamide (cat: 4440753), yeast tRNA (cat: 15401011), and UltraPure BSA (cat: AM2616) was purchased from Thermo Fisher Scientific. Ribonucleoside Vanadyl Complex (VRC) (cat: S1402S) and RNase Inhibitor, Murine (cat: M0314S) were purchased from New England Biolabs.

Buffers

Origami buffers. Four buffers were used for DNA origami sample preparation and imaging: Folding Buffer (10 mM Tris, 10 mM EDTA, 12.5 mM $MgCl_2$, pH 8); Buffer A (10 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.05 % Tween 20, pH 7.5); Buffer B (5 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 1 mM EDTA, 0.05 % Tween 20, pH 8); Origami imaging buffer (same as B, but supplemented with 1× PCA, 1× PCD, and 1× Trolox). 100× Trolox: 100 mg Trolox, 430 µL 100 % Methanol, 345 µL 1M NaOH in 3.2 ml H_2O. 40× PCA: 154 mg PCA, 10 ml water and NaOH were mixed and pH was adjusted 9.0. 100× PCD: 9.3 mg PCD, 13.3 ml of buffer (100 mM Tris-HCl pH 8, 50 mM KCl, 1 mM EDTA, 50 % Glycerol). 2× PEG-Buffer was used for PEG precipitation (15 % PEG-8000, 500 mM NaCl in 1× TE buffer, pH 8.0). RNA-FISH buffers: Wash buffer: 10% formamide in 2× SSC; Hybridization buffer: 12.5 nM of working probe solution in 2xSSC, 10% formamide, 10% dextran sulfate, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sheared salmon sperm DNA, 2 mM VRC, 0.10 mg/ml UltraPure BSA, RNase Inhibitor, Murine ~10U/µl. Blocking buffer for Immunofluorescence: 3% BSA, 0.1% Triton X-100 in PBS. Cell imaging buffer: (1× PBS pH 8, 500 mM NaCl, 1× PCA, 1× PCD, 1× Trolox).

Stochastic binding simulations

DNA-PAINT blinking traces were simulated using the stochastic reaction simulation tool COPASI[1]. Simulations were carried out as described earlier[2]. In brief, we simulated structures displaying 40 binding sites of 8 nt length, or 40 sites of 10 nt length and structures displaying 120 sites of 8 nt or 120 sites of 10 nt length. All simulation parameters were experimentally obtained. For 8 nt and 10 nt binding sites, a mean binding duration of 0.4 s and 5 s was determined, respectively. With our current imaging buffer constitution, an association rate of $2.9 \cdot 10^6 (\mathrm{Ms})^{-1}$ was used. Imager concentration was set to 80 pM, and the integration time to 30 ms with 1500 s total acquisition time. For each DNA origami design, 50 structures were simulated separately. The data from the four simulations were pooled, and clustered using the HDBSCAN algorithm, with 'min_cluster_size' set to 15, and 'min_samples' set to 1. (**Supplementary Figure 1**).

DNA origami design, assembly and purification

DNA origami structures were designed using the design module of Picasso[3]. Folding of structures was performed using the following components: p7249 M13 single-stranded DNA scaffold (0.01 µM), core staples (0.5 µM), biotin staples (0.5 µM), modified staples (each 0.5 µM), 1× folding buffer in a total of 20 µl for each sample. Annealing was done by cooling the mixture from 65 ºC to 25 ºC in 2 hours in a thermocycler. Structures were purified either using PEG-precipitation[4] (40 versus 120 binding sites and 4 corner origami), or by running the samples on a 1.5% agarose gel (1.5% agarose, 0.5× TA buffer, 12.5 mM $MgCl_2$, 1× SYBR Safe), cutting out bands containing the folded structures and purifying them using Freeze 'N Squeeze Columns (spun for 5 min at 1,000 ×g) (124 color imaging) (**Supplementary Figure 9**).

Cell culture

HeLa cells were used for CHC and PMP70 imaging. For RNA-FISH experiments A-549 cells were used. All cells were grown in high glucose (4.5 g/l) DMEM supplemented with GlutaMAX™, 1 mM sodium pyruvate and 10% FBS. Cells were seeded into 8-well-chambered cover glasses and grown to approximately 70% confluency.

Design of RNA- Fluorescence in situ Hybridization probes

RNA-FISH probes were designed against the mRNA sequence of the longest transcript variant of each gene (**Supplementary Notes 3** and **4**). FASTA sequences were taken from the NCBI Genome Browser. We used the Stellaris® Probe Designer version 4.2 with a masking level of 5 to get 40 probe strands for each target. These probes were then elongated on the 3'-end with DNA-PAINT handle sequences for DNA-PAINT imaging (**Supplementary Note 2**).

Hybridization of RNA-FISH probes

Cell media was aspirated and cells were rinsed with 1× PBS. Cells were fixed with 4% formaldehyde in 1× PBS for 10 min at room temperature, then washed two times with 1× PBS and 4 mM VRC. Permeabilization was carried out with 70% (v/v) ethanol for 6 hours at 4 ºC. Before hybridization, cells were incubated in wash buffer supplemented with 4 mM VRC for 10 minutes at room temperature. Hybridization: 300 µl of the hybridization solution containing 12.5 nM of probes was added to the cells. Hybridization was carried out in a sealed chamber at 37 ºC for 16 hours. Washing: Chambers were rinsed once then washed twice for 30 min each at 37 ºC in wash buffer.

Antibody conjugation

Antibodies were conjugated to DNA-PAINT docking sites via maleimide-PEG2-succinimidyl ester chemistry[3, 5] (see **Supplementary Table 8** for handle sequences).

CHC and PMP70 Immunostaining

Cell medium was aspirated and cells were fixed with 3% paraformaldehyde, 0.1% glutaraldehyde, and 0.3% Triton X-100 in PBS for 10 min at room temperature, then washed three times with PBS. Free aldehyde groups were reduced using 1 mg/ml sodium borohydride in PBS for 7 min, followed by three washing steps with PBS for 5 min. Cells were blocked and permeabilized with blocking buffer for 90 min. Cells were stained with primary antibodies, anti-CHC goat and anti-PMP70 mouse (both diluted 1:100), in blocking buffer overnight at 4 ºC. Cells were washed three times with PBS for 5 min. Cells were incubated with DNA-conjugated secondary antibodies (anti-goat-P12-8 nt and anti-mouse-P13-9 nt (**Supplementary Table 8**) diluted in blocking buffer (1:200) for 1 hour at room temperature before finally washing the cells three times in PBS for 5 min.

Super-resolution microscope setups

Custom TIRF Setup. Fluorescence imaging was carried out on an inverted Nikon Eclipse Ti microscope (Nikon Instruments) with the Perfect Focus System, applying an objective-type TIRF configuration with an oil-immersion objective (Apo SR TIRF 100×, NA 1.49, Oil). Two lasers were used for excitation: 561 nm (200 mW, Coherent Sapphire) or 640 nm (150 mW, Toptica iBeam smart). The laser beam was passed through cleanup filters (ZET561/10 or ZET642/20, Chroma Technology) and coupled into the microscope objective using a beam splitter (ZT561rdc or ZT647rdc, Chroma Technology). Fluorescence light was spectrally filtered with an emission filter (ET600/50m and ET575lp or ET705/72m and ET665lp, Chroma Technology) and imaged on an electron-multiplying charge-coupled device (EMCCD) camera (Andor iXon Ultra 897) or sCMOS camera (Andor Zyla 4.2) without further magnification, resulting in an effective pixel size of 160 nm (EMCCD) or 130 nm (sCMOS after 2×2 binning). Our custom TIRF setup was used for Figures 1d and 2f (EMCCD). Spinning Disk Confocal Setup: DNA-PAINT imaging of RNA-FISH samples was performed using an Andor Dragonfly Spinning Disk Confocal system (Andor) based on an inverted Nikon Eclipse Ti2 microscope (Nikon Instruments) with the Perfect Focus System, using an oil immersion objective (Plan Apo 100×, NA 1.45, Oil). For excitation, a 561 nm laser (2 W, MPB) was used. The laser beam was passed through a beam conditioning unit (Andor Borealis) for reshaping the beam from a Gaussian profile to a flat top profile. Next, the beam was coupled into the Andor Dragonfly spinning disk unit, passed through the multi-pinhole disk with a pinhole size of 40 µm and from there coupled into the objective lens. Excitation and emission light was spectrally split using a beam splitter (CR-DFLY-DMQD-01). Fluorescence light was spectrally filtered with an emission filter (TR-DFLY-F600-050) and imaged on an sCMOS camera (Andor Zyla 4.2 PLUS) without further magnification, resulting in an effective pixel size of 130 nm (sCMOS after 2×2 binning). The field of view was 1024 × 1024 pixels which is equivalent to 133.12 µm × 133.12 µm when taking the pixel size into account. The disk speed was set to 6000 rpm and an excitation field stop of 13.3mm × 13.3mm was applied. The Spinning Disk Confocal setup was used to acquire the image in **Figure 2b**.

Imaging conditions

Figure 1d. Imaging was carried out using an imager strand concentration of 75 pM (P3-Cy3B). 50,000 frames were acquired using the EMCCD camera at 30 ms integration time with the EM gain set to 300, the pre-amp gain set to 3, and the readout bandwidth set to 17 MHz. Laser power (@561 nm) was set to 2.5 mW (measured before the back focal plane (BFP) of the objective).

Figure 1f. Images were acquired with an imager strand concentration of 2 nM (P3-Cy3B imager). 150,000 frames were acquired using the EMCCD camera at 30 ms integration time with the EM gain set to 300, the pre-amp gain set to 3, and the readout bandwidth set to 17 MHz. Laser power (@561 nm) was set to 500 mW (measured at the back focal plane (BFP) of the objective).

Figure 2b. DNA-PAINT microscopy was carried out using 10 nM of P3-Cy3B in imaging buffer. 40,000 frames were acquired using the EMCCD camera at 200 ms integration time and a readout bandwidth of 540 MHz. Laser power (@560 nm) was set to 500 mW resulting in a power of 18.3 mW at the sample plane. This can be translated to an intensity of 103.27 W/cm^2 at the sample plane.

Figure 2f. DNA-PAINT imaging of protein samples was carried out using the following imager strands: P12-Cy3B (250 pM) and P13-Cy3B (50 pM) in imaging buffer. 80,000 frames were acquired using the EMCCD camera at 30 ms integration time with the EM gain set to 300, the pre-amp gain set to 3, and the readout mode to 17 MHz. Laser power (@561 nm) was set to 8 mW (measured before the back focal plane (BFP) of the objective).

Figure 3c. Imaging was carried out using the following imager strands: P1-Atto655 (20 nM), P2-Cy3B (20 nM) and P3- Atto488 (20 nM), in Buffer B. 15,000 frames were acquired using the EMCCD camera at 100 ms integration time with the EM gain set to 300, the pre-amp gain set to 3, and the readout mode to 17 MHz. Laser power was set to ~25 mW (@488 nm), ~30 mW (@561 nm), and ~30 mW (@642 nm, all measured before the back focal plane (BFP) of the objective).

For all imager strand sequences see **Supplementary Table 1**.

Image analysis

Raw fluorescence data was subjected to spot-finding and subsequent super-resolution reconstruction using the 'Picasso' software package[3].

Analysis of DNA origami data

Automated structure selection: After super-resolution reconstruction, structures were automatically selected using Picasso's 'Pick similar' function with the following settings: Pick radius: 320 nm; Standard deviation: 2.

Filtering: After automated selection, picked 'spots' were further processed in order to remove unspecific binding events from specific ones originating from DNA origami locations. To achieve this, we implemented a multi-step filtering procedure. First, in order to remove non-repetitive binding events (e.g. imager strands non-specifically adsorbing to the surface), we fitted the mean frame value of binding events (from all picked spots) throughout the whole image acquisition. The rationale behind this step is that repetitive, correct picks (i.e. containing DNA origami structures) will yield a mean frame value of roughly half the number of total frames in the acquisition (gaussian distributed), while non-repetitive events will in most cases not last throughout the whole image acquisition time frame, leading to a mean frame value that is outside this distribution. We chose the mean of the distribution and set a cut-off value at +/- two times the standard deviation for filtering. Next, to also filter out structures with a non-repetitive blinking behavior, but with most events occurring around the mean frame value, we plotted the standard deviation of the mean frame values and used a cut-off value of 2000, and all data below this threshold were disregarded. (see **Supplementary Figures 3** and **4** for results).

Cluster Analysis: After filtering, the data was analyzed using an HDBSCAN[6] clustering algorithm. 'Min_cluster_size' was set to 15, and 'min_samples' was set to 1, in the case of the four origami species (**Supplementary Figure 5 and 6**), and to 20 and 3, respectively, in the case of the 4-corner origami structures (**Supplementary Figures 7** and **8**).

Barcode Identification of 124 origami structures: First, all structures from all three acquisition rounds were aligned. Every structure exhibits a distinct kinetic blinking information in each of the three channels. This information was extracted for each spectral channel. The distribution of the number of binding events in each channel shows four separated clusters (see **Figure 3b**). After assigning every picked structure to one of the clusters in each channel, barcodes were identified.

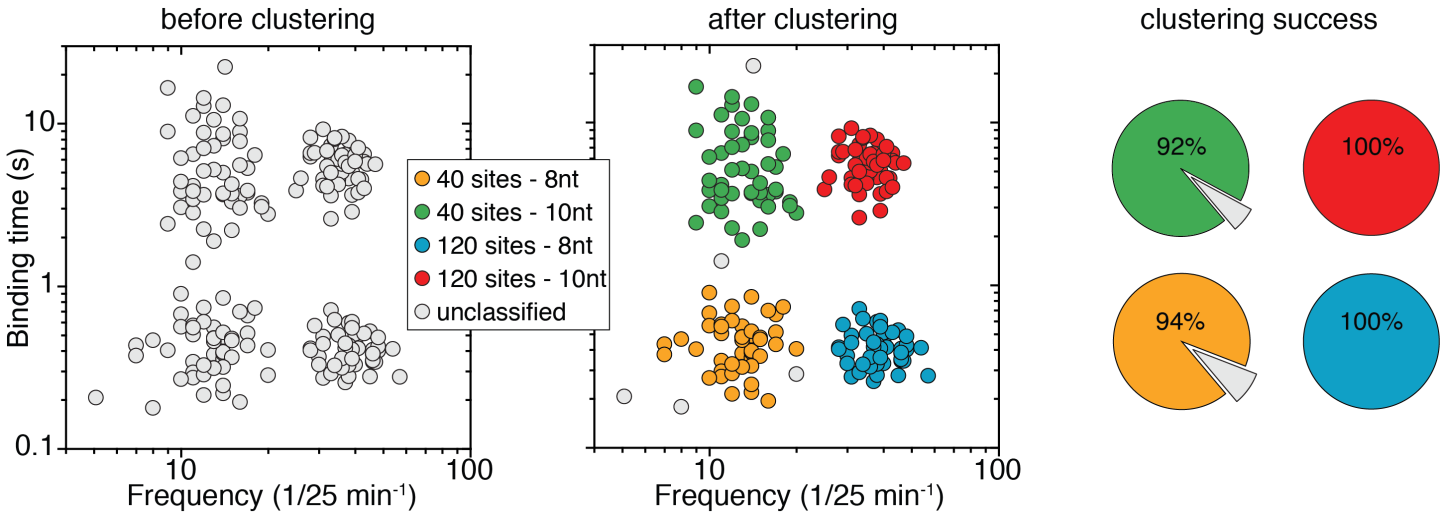
Analysis of RNA-FISH data

Single mRNA species were manually selected using Picasso's pick tool with a pick diameter of 520 nm. Kinetic information was extracted as for the DNA origami case. Localizations were linked allowing a gap size of 2 frames between localizations to obtain a list of single binding events. The total number of binding events calculated from each structure was plotted to obtain a histogram of binding frequencies.

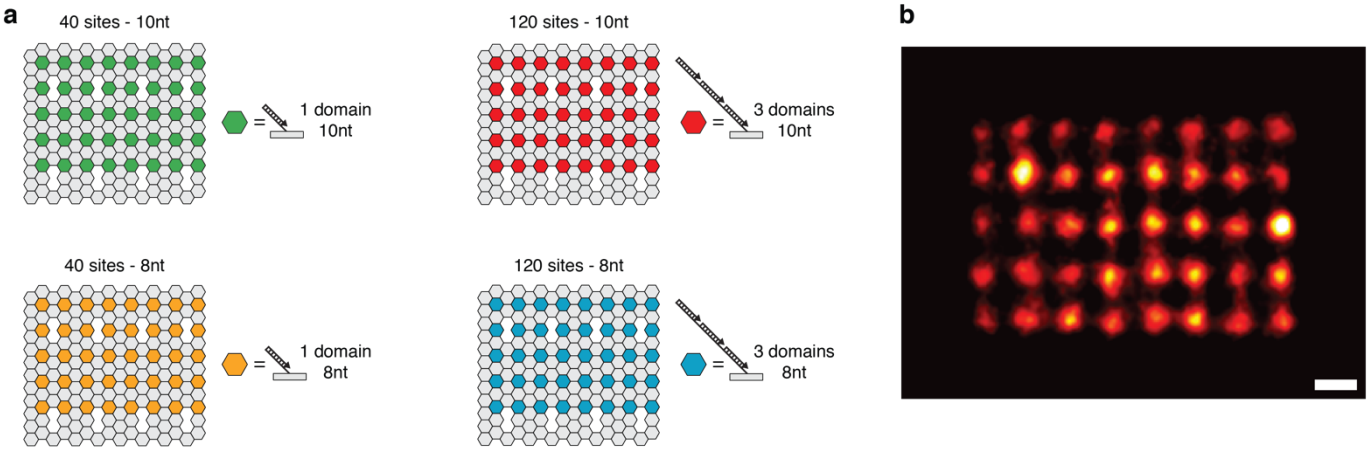
Analysis of protein data

Approximately 200 protein clusters were manually selected using Picasso's pick tool with a pick diameter of 240 nm. Kinetic information was extracted as for the DNA origami case. Localizations were linked allowing a gap size of 15 frames between localizations to obtain a list of single binding events. A mean binding time (i.e. blinking duration) was calculated from all events per pick.

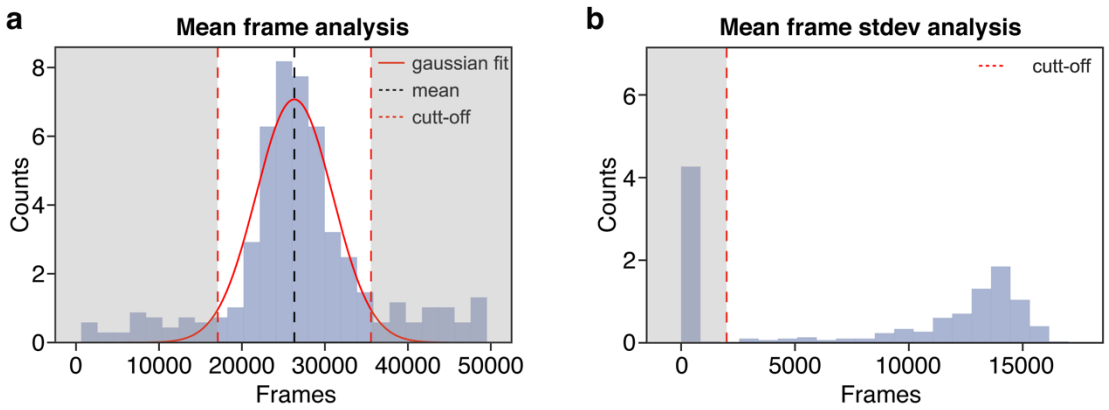
Supplementary Figures



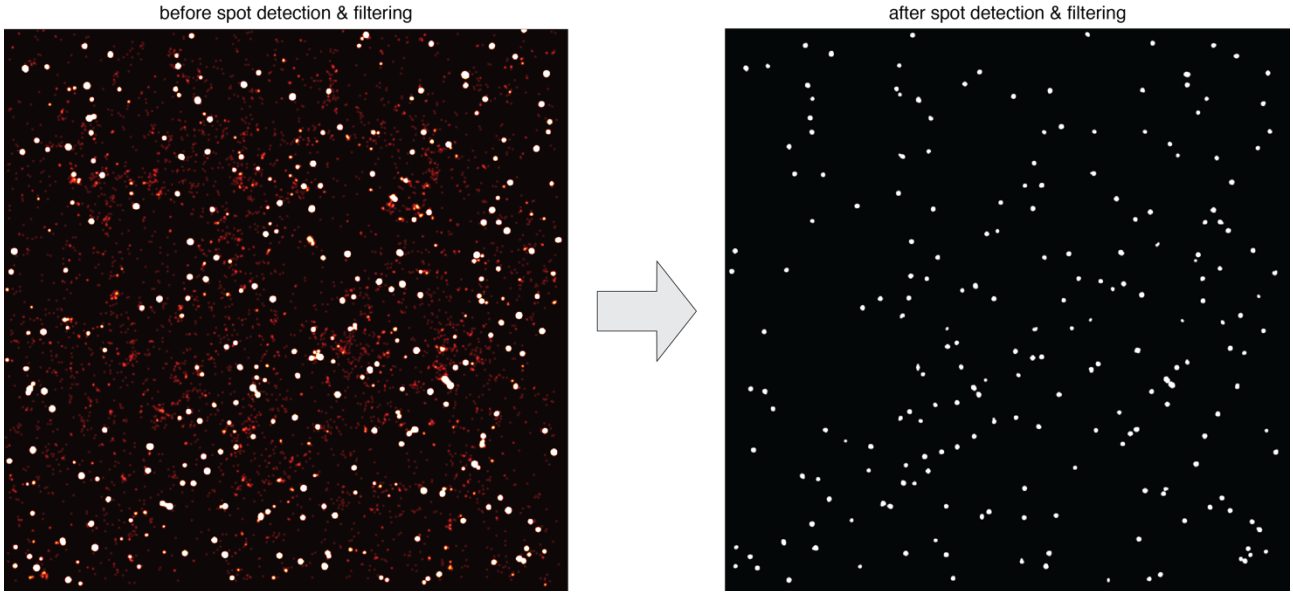
Supplementary Figure 1 | Cluster detection in simulated data. Data points from four individual stochastic binding simulations (for details see Online Methods) were clustered using HDBSCAN[6]. Data points plotted according to binding time and blinking frequency before clustering (**left**), and data plotted after clustering (**middle**). Individual colors were assigned to each of the resulting four populations. As each population was simulated individually, by comparing the results of the clustering algorithm to the original data, we were able to acquire the success rate of the clustering algorithm we used. The clustering resulted in a positive assignment rate of more than 92% in the case of all four populations (**right**).



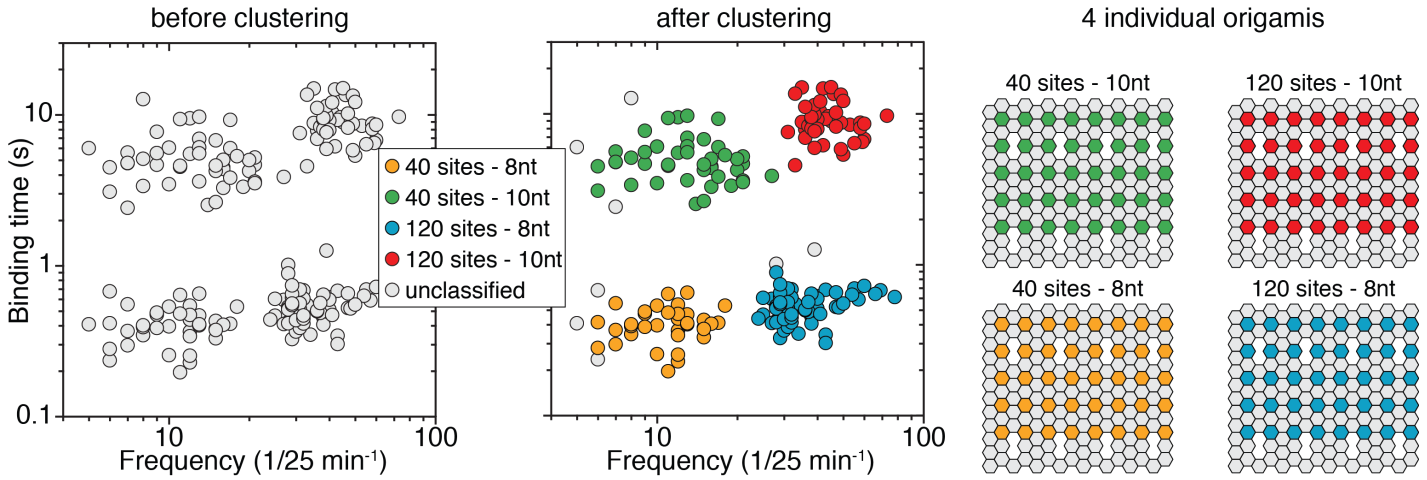
Supplementary Figure 2 | DNA origami designs for binding duration and frequency modulation. (**a**) Four DNA origami structures with different numbers and lengths of DNA-PAINT docking sites. Structures represented with green contain 40 binding domains of 10 nucleotide length each, while structures represented with yellow contain 40 binding sites, each 8 nucleotides long. Red and blue DNA origami structures were also modified at 40 positions, with each modification consisting of three sequential binding domains, resulting in a total of 120 binding sites on each structure. Red structures have 10 nucleotide long binding domains, while blue structures have 8 nucleotide long binding domains. (**b**) Super-resolved sum image of DNA origami clearly reveals the correct formation of the structures by showing all 40 binding sites, spaced 10 nm apart. Scale bar: 10 nm.



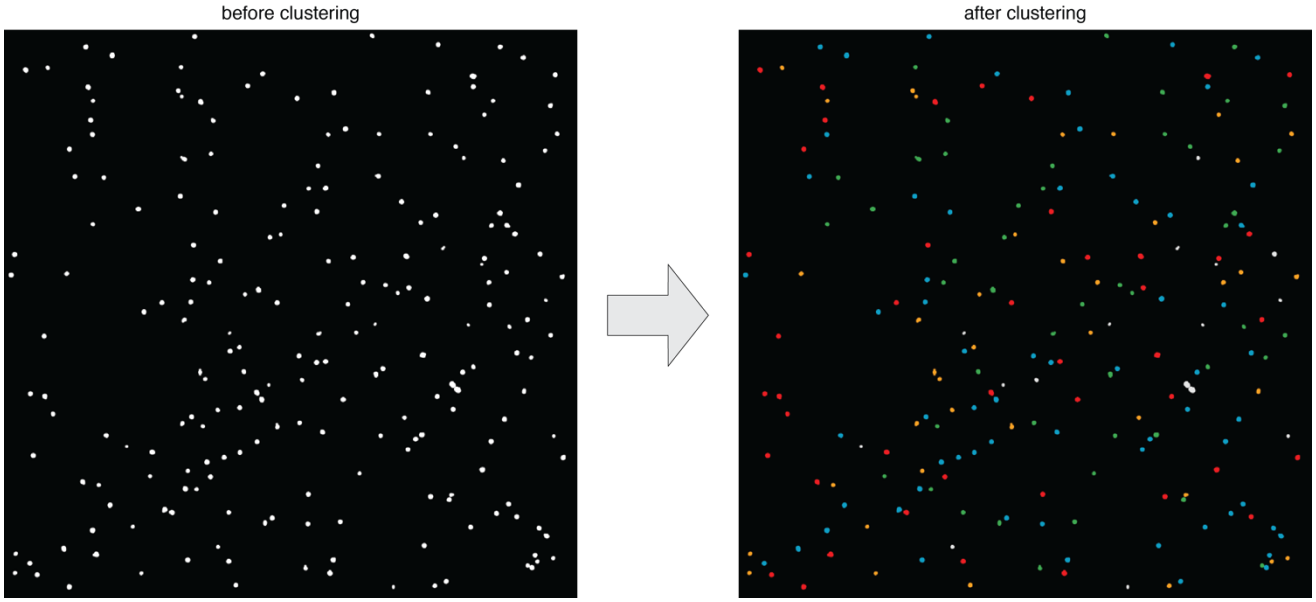
Supplementary Figure 3 | Filtering DNA origami data prior to clustering. (**a**) Resulting mean frame analysis (see Online Methods for description). Picks are rejected (gray) based on the following metric: More than 2× standard deviation of the mean. (**b**) For filtering of structures that show non-repetitive binding, however whose bindings are clustered around the mean frame, we plotted the standard deviation of the mean frame. Using a cut-off value of 2000 (red dashed line) all data below this threshold were disregarded (gray).



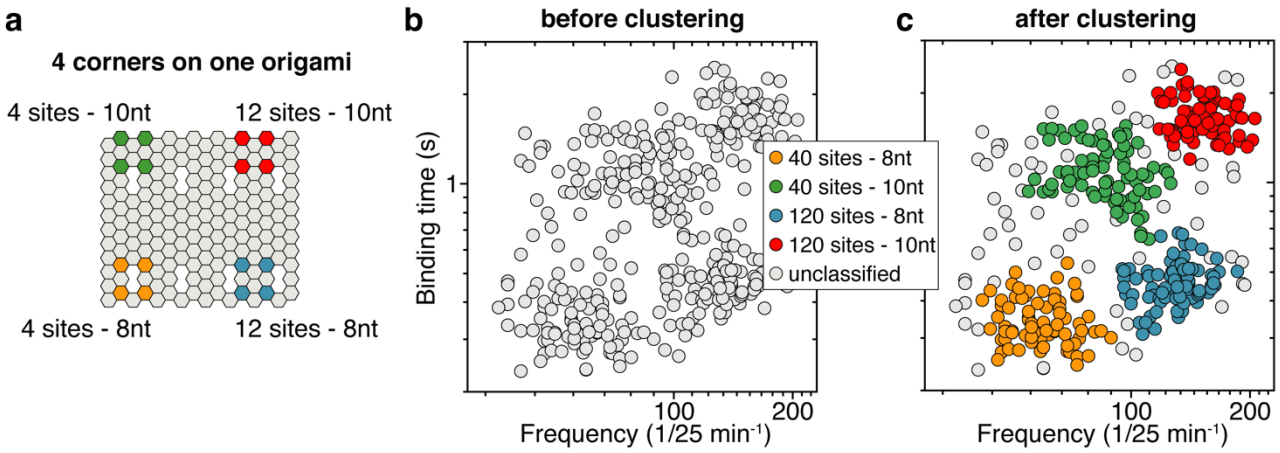
Supplementary Figure 4 | DNA-PAINT images of DNA origami before and after filtering. All initially identified localizations were rendered using Picasso Render, which was subsequently used for spot detection and picking of structures. (**left**). We rendered the same dataset after we ran all localizations from the picked spots through our filtering system (**Supplementary Figure 3** and Online Methods). Background noise and unspecific blinking events are filtered out, and no longer appear in the newly rendered image (**right**). Image size: 40.96 µm.



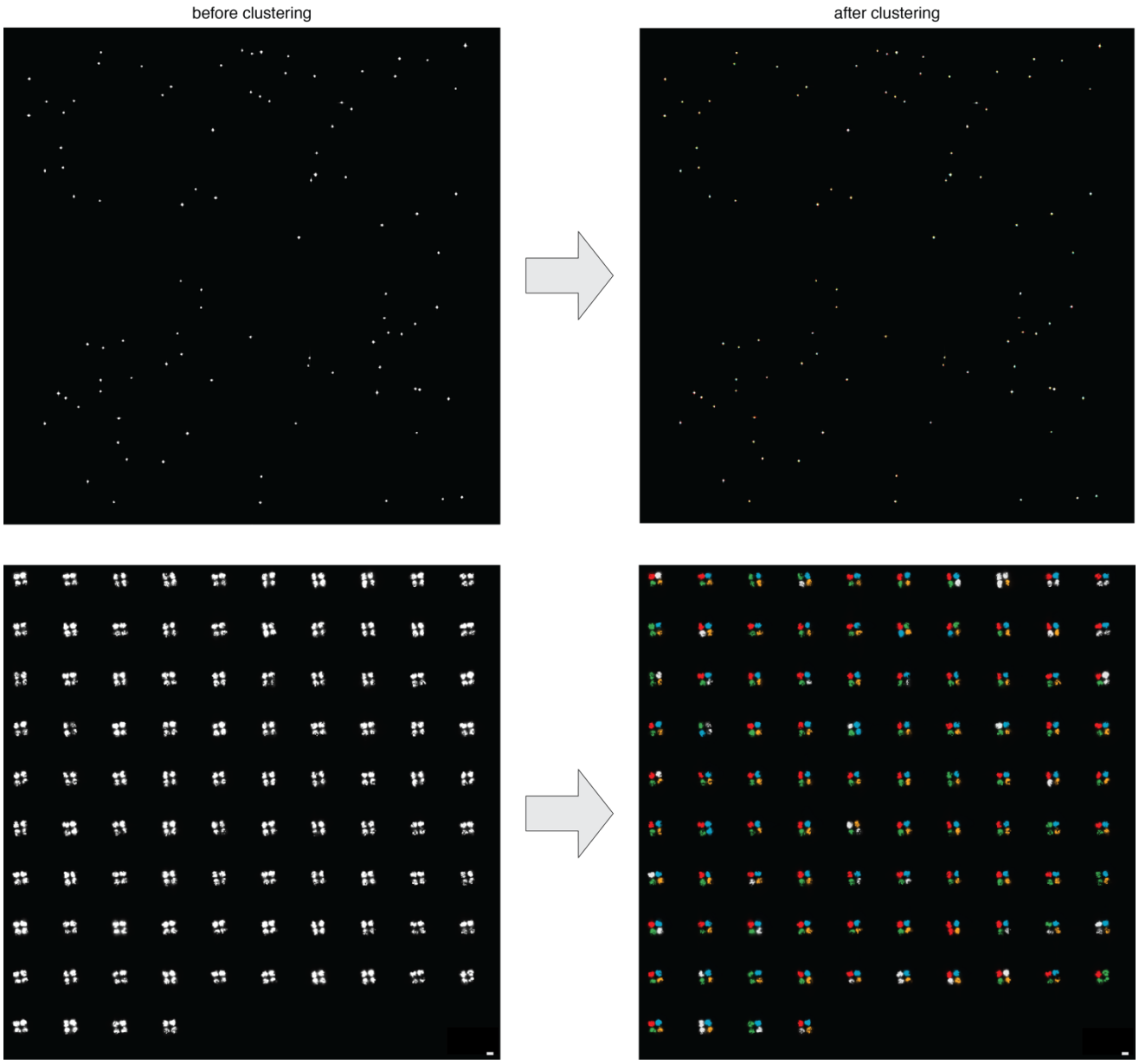
Supplementary Figure 5 | Cluster detection in DNA origami data. Data from measurements of four different DNA origami samples displaying 40 binding sites of 8 nt and 10 nt, and 120 binding sites of 8 nt and 10 nt long binding sites were acquired and plotted according to their blinking frequency and blinking duration (**left**). Clustering the data using the HDBSCAN[6] algorithm resulted in four populations, to which we assigned distinct colors (green, yellow, red and blue). Using the assigned colors, we re-plotted each data point, in the color it was assigned. Grey dots indicate structures that could not be assigned to any of the four populations (**center**). Green: 40 domains, 10 nt; Yellow: 40 domains, 8 nt; Red: 120 domains, 10 nt, Blue: 120 domains 8nt (**right**).



Supplementary Figure 6 | DNA origami data before and after cluster identification. After spot detection and filtering, all DNA origami structures (seen as white spots in the left image) were clustered according to their blinking behavior (**Supplementary Figure 5**) (**left**). We then re-rendered the image assigning a pseudo-color for each spot, according to the population it belonged to - green, yellow, red or blue (**right**). Image size: 40.96 µm.



Supplementary Figure 7 | Clustering results for four-corner DNA origami. (**a**) We designed a DNA origami structure that contained all four previously used binding site designs, one in each corner. Four DNA origami staples were modified at each corner, resulting in the following designs: 4 single domains with a 10 nt binding site, (green), 4 single domains with an 8 nt binding site, 4 staples with 4×3 (12) domains with 10nt binding sites (red), and 4 staples with 4×3 (12) domains with 8 nt binding sites (blue). (**b**) These origami structures were imaged using DNA-PAINT, and the binding times and binding frequencies were plotted, after filtering, as in the case of the four separate origami species. (**c**) We then clustered the data using HDBSCAN and re-plotted them, marking the points with the color of the cluster they were assigned to. Grey dots were not assigned to any cluster.



Supplementary Figure 8 | Four-corner DNA origami structures before and after cluster identification. Images of four corner DNA origami structures on coverslip before and after clustering of the four corners of each structure (**top**). A higher magnification image of picked origami structures rendered in ordered succession, before and after clustering (**bottom**). Each corner was then picked individually, and all localizations were run through the HDBSCAN algorithm. Results of the clustering algorithm can be seen on the bottom right image, with the identified corners shown in color. As expected we were able to assign the four corners to the four different kinetic populations according to their blinking behavior, even with the lower number of binding sites. Scale bars: 40 nm.

Supplementary Figure 9 | Agarose gels of the 124 individual DNA origami structures. Fluorescent scan of the agarose gels of the 124 individual frequency barcodes (1.5% agarose, 1×TAE buffer + 10mM $MgCl_2$, 1× SybrSafe stain). All individual monomer bands (monomer band for barcode ID = 1 is indicated by red arrow) were physically extracted from the gel and the structures purified using Freeze'N'Squeeze spin columns. L: Ladder, Sc: Scaffold strands.

Supplementary Tables

Supplementary Table 1 | DNA-PAINT imager strands

Name	Sequence	Dye on 3′-end
P1	5′-CTAGATGTAT-3′	Atto655
P2	5′-TATGTAGATC-3′	Cy3B
P3	5′-GTAATGAAGA-3′	Atto488 or Cy3B
P12	5′-GCTCTAACTA-3′	Cy3B
P13	5′-CCTTCTCTAT-3′	Cy3B

Supplementary Table 2 | Core staple strands for rectangular DNA origami

Position	Name	Sequence
A1	21[32]23[31]BLK	TTTTCACTCAAAGGGCGAAAAACCATCACC
B1	23[32]22[48]BLK	CAAATCAAGTTTTTTGGGGTCGAAACGTGGA
C1	21[56]23[63]BLK	AGCTGATTGCCCTTCAGAGTCCACTATTAAAGGGTGCCGT
D1	23[64]22[80]BLK	AAAGCACTAAATCGGAACCCTAATCCAGTT
E1	21[96]23[95]BLK	AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC
F1	23[96]22[112]BLK	CCCGATTTAGAGCTTGACGGGGAAAAAGAATA
G1	21[120]23[127]BLK	CCCAGCAGGCGAAAAATCCCTTATAAATCAAGCCGGCG
H1	21[160]22[144]BLK	TCAATATCGAACCTCAAATATCAATTCCGAAA
I1	23[128]23[159]BLK	AACGTGGCGAGAAAGGAAGGGAAACCAGTAA
J1	23[160]22[176]BLK	TAAAAGGGACATTCTGGCCAACAAAGCATC
K1	21[184]23[191]BLK	TCAACAGTTGAAAGGAGCAAATGAAAAATCTAGAGATAGA
L1	23[192]22[208]BLK	ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG
M1	21[224]23[223]BLK	CTTTAGGGCCTGCAACAGTGCCAATACGTG
N1	23[224]22[240]BLK	GCACAGACAATATTTTTGAATGGGGTCAGTA
O1	21[248]23[255]BLK	AGATTAGAGCCGTCAAAAAACAGAGGTGAGGCCTATTAGT
P1	23[256]22[272]BLK	CTTTAATGCGCGAACTGATAGCCCCACCAG
A2	19[32]21[31]BLK	GTCGACTTCGGCCAACGCGCGGGGTTTTTC
B2	22[47]20[48]BLK	CTCCAACGCAGTGAGACGGGCAACCAGCTGCA
D2	22[79]20[80]BLK	TGGAACAACCGCCTGGCCCTGAGGCCCGCT
E2	19[96]21[95]BLK	CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC
F2	22[111]20[112]BLK	GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT
H2	19[160]20[144]BLK	GCAATTCACATATTCCTGATTATCAAAGTGTA
I2	22[143]21[159]BLK	TCGGCAAATCCTGTTTGATGGTGGACCCTCAA
J2	22[175]20[176]BLK	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA
L2	22[207]20[208]BLK	AGCCAGCAATTGAGGAAGGTTATCATCATTTT

M2	19[224]21[223]BLK	CTACCATAGTTTGAGTAACATTTAAAATAT
N2	22[239]20[240]BLK	TTAACACCAGCACTAACAACTAATCGTTATTA
P2	22[271]20[272]BLK	CAGAAGATTAGATAATACATTTGTCGACAA
A3	17[32]19[31]BLK	TGCATCTTTCCCAGTCACGACGGCCTGCAG
B3	20[47]18[48]BLK	TTAATGAACTAGAGGATCCCCGGGGGGTAACG
D3	20[79]18[80]BLK	TTCCAGTCGTAATCATGGTCATAAAAGGGG
E3	17[96]19[95]BLK	GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC
F3	20[111]18[112]BLK	CACATTAAAATTGTTATCCGCTCATGCGGGCC
H3	17[160]18[144]BLK	AGAAAACAAAGAAGATGATGAAACAGGCTGCG
I3	20[143]19[159]BLK	AAGCCTGGTACGAGCCGGAAGCATAGATGATG
J3	20[175]18[176]BLK	ATTATCATTCAATATAATCCTGACAATTAC
L3	20[207]18[208]BLK	GCGGAACATCTGAATAATGGAAGGTACAAAAT
M3	17[224]19[223]BLK	CATAAATCTTTGAATACCAAGTGTTAGAAC
N3	20[239]18[240]BLK	ATTTTAAAATCAAAATTATTTGCACGGATTCG
P3	20[271]18[272]BLK	CTCGTATTAGAAATTGCGTAGATACAGTAC
A4	15[32]17[31]BLK	TAATCAGCGGATTGACCGTAATCGTAACCG
B4	18[47]16[48]BLK	CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA
C4	15[64]18[64]BLK	GTATAAGCCAACCCGTCGGATTCTGACGACAGTATCGGCCGCAAGGCG
D4	18[79]16[80]BLK	GATGTGCTTCAGGAAGATCGCACAATGTGA
E4	15[96]17[95]BLK	ATATTTTGGCTTTCATCAACATTATCCAGCCA
F4	18[111]16[112]BLK	TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC
G4	15[128]18[128]BLK	TAAATCAAAATAATTCGCGTCTCGGAAACCAGGCAAAGGGAAGG
H4	15[160]16[144]BLK	ATCGCAAGTATGTAAATGCTGATGATAGGAAC
I4	18[143]17[159]BLK	CAACTGTTGCGCCATTCGCCATTCAAACATCA
J4	18[175]16[176]BLK	CTGAGCAAAAATTAATTACATTTTGGGTTA
K4	15[192]18[192]BLK	TCAAATATAACCTCCGGCTTAGGTAACAATTTCATTTGAAGGCGAATT
L4	18[207]16[208]BLK	CGCGCAGATTACCTTTTTTAATGGGAGAGACT
M4	15[224]17[223]BLK	CCTAAATCAAAATCATAGGTCTAAACAGTA
N4	18[239]16[240]BLK	CCTGATTGCAATATATGTGAGTGATCAATAGT
O4	15[256]18[256]BLK	GTGATAAAAAGACGCTGAGAAGAGATAACCTTGCTTCTGTTCGGGAGA
P4	18[271]16[272]BLK	CTTTTACAAAATCGTCGCTATTAGCGATAG
A5	13[32]15[31]BLK	AACGCAAAATCGATGAACGGTACCGGTTGA
B5	16[47]14[48]BLK	ACAAACGGAAAAGCCCCAAAAACACTGGAGCA
C5	13[64]15[63]BLK	TATATTTTGTCATTGCCTGAGAGTGGAAGATT
D5	16[79]14[80]BLK	GCGAGTAAAAATATTTAAATTGTTACAAAG
E5	13[96]15[95]BLK	TAGGTAAACTATTTTTGAGAGATCAAACGTTA
F5	16[111]14[112]BLK	TGTAGCCATTAAAATTCGCATTAAATGCCGGA
G5	13[128]15[127]BLK	GAGACAGCTAGCTGATAAATTAATTTTTGT

H5	13[160]14[144]BLK	GTAATAAGTTAGGCAGAGGCATTTATGATATT
I5	16[143]15[159]BLK	GCCATCAAGCTCATTTTTTAACCACAAATCCA
J5	16[175]14[176]BLK	TATAACTAACAAAGAACGCGAGAACGCCAA
K5	13[192]15[191]BLK	GTAAAGTAATCGCCATATTTAACAAAACTTTT
L5	16[207]14[208]BLK	ACCTTTTTATTTTAGTTAATTTCATAGGGCTT
M5	13[224]15[223]BLK	ACAACATGCCAACGCTCAACAGTCTTCTGA
N5	16[239]14[240]BLK	GAATTTATTTAATGGTTTGAAATATTCTTACC
O5	13[256]15[255]BLK	GTTTATCAATATGCGTTATACAAACCGACCGT
P5	16[271]14[272]BLK	CTTAGATTTAAGGCGTTAAATAAAGCCTGT
A6	11[32]13[31]BLK	AACAGTTTTGTACCAAAAACATTTTATTTC
B6	14[47]12[48]BLK	AACAAGAGGGATAAAAATTTTTAGCATAAAGC
C6	11[64]13[63]BLK	GATTTAGTCAATAAAGCCTCAGAGAACCCTCA
D6	14[79]12[80]BLK	GCTATCAGAAATGCAATGCCTGAATTAGCA
E6	11[96]13[95]BLK	AATGGTCAACAGGCAAGGCAAAGAGTAATGTG
F6	14[111]12[112]BLK	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA
G6	11[128]13[127]BLK	TTTGGGGATAGTAGTAGCATTAAAAGGCCG
H6	11[160]12[144]BLK	CCAATAGCTCATCGTAGGAATCATGGCATCAA
I6	14[143]13[159]BLK	CAACCGTTTCAAATCACCATCAATTCGAGCCA
J6	14[175]12[176]BLK	CATGTAATAGAATATAAAGTACCAAGCCGT
K6	11[192]13[191]BLK	TATCCGGTCTCATCGAGAACAAGCGACAAAAG
L6	14[207]12[208]BLK	AATTGAGAATTCTGTCCAGACGACTAAACCAA
M6	11[224]13[223]BLK	GCGAACCTCCAAGAACGGGTATGACAATAA
N6	14[239]12[240]BLK	AGTATAAAGTTCAGCTAATGCAGATGTCTTTC
O6	11[256]13[255]BLK	GCCTTAAACCAATCAATAATCGGCACGCGCCT
P6	14[271]12[272]BLK	TTAGTATCACAATAGATAAGTCCACGAGCA
A7	9[32]11[31]BLK	TTTACCCCAACATGTTTTAAATTTCCATAT
B7	12[47]10[48]BLK	TAAATCGGGATTCCCAATTCTGCGATATAATG
C7	9[64]11[63]BLK	CGGATTGCAGAGCTTAATTGCTGAAACGAGTA
D7	12[79]10[80]BLK	AAATTAAGTTGACCATTAGATACTTTTGCG
E7	9[96]11[95]BLK	CGAAAGACTTTGATAAGAGGTCATATTTCGCA
F7	12[111]10[112]BLK	TAAATCATATAACCTGTTTAGCTAACCTTTAA
G7	9[128]11[127]BLK	GCTTCAATCAGGATTAGAGAGTTATTTTCA
H7	9[160]10[144]BLK	AGAGAGAAAAAAATGAAAATAGCAAGCAAACT
I7	12[143]11[159]BLK	TTCTACTACGCGAGCTGAAAAGGTTACCGCGC
J7	12[175]10[176]BLK	TTTTATTTAAGCAAATCAGATATTTTTTGT
K7	9[192]11[191]BLK	TTAGACGGCCAAATAAGAAACGATAGAAGGCT
L7	12[207]10[208]BLK	GTACCGCAATTCTAAGAACGCGAGTATTATTT
M7	9[224]11[223]BLK	AAAGTCACAAAATAAACAGCCAGCGTTTTA

N7	12[239]10[240]BLK	CTTATCATTCCCGACTTGCGGGAGCCTAATTT
O7	9[256]11[255]BLK	GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA
P7	12[271]10[272]BLK	TGTAGAAATCAAGATTAGTTGCTCTTACCA
A8	7[32]9[31]BLK	TTTAGGACAAATGCTTTAAACAATCAGGTC
B8	10[47]8[48]BLK	CTGTAGCTTGACTATTATAGTCAGTTCATTGA
C8	7[56]9[63]BLK	ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG
D8	10[79]8[80]BLK	GATGGCTTATCAAAAAGATTAAGAGCGTCC
E8	7[96]9[95]BLK	TAAGAGCAAATGTTTAGACTGGATAGGAAGCC
F8	10[111]8[112]BLK	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT
G8	7[120]9[127]BLK	CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA
H8	7[160]8[144]BLK	TTATTACGAAGAACTGGCATGATTGCGAGAGG
I8	10[143]9[159]BLK	CCAACAGGAGCGAACCAGACCGGAGCCTTTAC
J8	10[175]8[176]BLK	TTAACGTCTAACATAAAAACAGGTAACGGA
K8	7[184]9[191]BLK	CGTAGAAAATACATACCGAGGAAACGCAATAAGAAGCGCA
L8	10[207]8[208]BLK	ATCCCAATGAGAATTAACTGAACAGTTACCAG
M8	7[224]9[223]BLK	AACGCAAAGATAGCCGAACAAACCCTGAAC
N8	10[239]8[240]BLK	GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA
O8	7[248]9[255]BLK	GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA
P8	10[271]8[272]BLK	ACGCTAACACCCACAAGAATTGAAAATAGC
A9	5[32]7[31]BLK	CATCAAGTAAAACGAACTAACGAGTTGAGA
B9	8[47]6[48]BLK	ATCCCCCTATACCACATTCAACTAGAAAAATC
D9	8[79]6[80]BLK	AATACTGCCCAAAAGGAATTACGTGGCTCA
E9	5[96]7[95]BLK	TCATTCAGATGCGATTTTAAGAACAGGCATAG
F9	8[111]6[112]BLK	AATAGTAAACACTATCATAACCCTCATTGTGA
H9	5[160]6[144]BLK	GCAAGGCCTCACCAGTAGCACCATGGGCTTGA
I9	8[143]7[159]BLK	CTTTTGCAGATAAAAACCAAAATAAAGACTCC
J9	8[175]6[176]BLK	ATACCCAACAGTATGTTAGCAAATTAGAGC
L9	8[207]6[208]BLK	AAGGAAACATAAAGGTGGCAACATTATCACCG
M9	5[224]7[223]BLK	TCAAGTTTCATTAAAGGTGAATATAAAAGA
N9	8[239]6[240]BLK	AAGTAAGCAGACACCACGGAATAATATTGACG
P9	8[271]6[272]BLK	AATAGCTATCAATAGAAAATTCAACATTCA
A10	3[32]5[31]BLK	AATACGTTTGAAAGAGGACAGACTGACCTT
B10	6[47]4[48]BLK	TACGTTAAAGTAATCTTGACAAGAACCGAACT
D10	6[79]4[80]BLK	TTATACCACCAAATCAACGTAACGAACGAG
E10	3[96]5[95]BLK	ACACTCATCCATGTTACTTAGCCGAAAGCTGC
F10	6[111]4[112]BLK	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC
H10	3[160]4[144]BLK	TTGACAGGCCACCACCAGAGCCGCGATTTGTA
I10	6[143]5[159]BLK	GATGGTTTGAACGAGTAGTAAATTTACCATTA

J10	6[175]4[176]BLK	CAGCAAAAGGAAACGTCACCAATGAGCCGC
L10	6[207]4[208]BLK	TCACCGACGCACCGTAATCAGTAGCAGAACCG
M10	3[224]5[223]BLK	TTAAAGCCAGAGCCGCCACCCTCGACAGAA
N10	6[239]4[240]BLK	GAAATTATTGCCTTTAGCGTCAGACCGGAACC
P10	6[271]4[272]BLK	ACCGATTGTCGGCATTTTCGGTCATAATCA
A11	1[32]3[31]BLK	AGGCTCCAGAGGCTTTGAGGACACGGGTAA
B11	4[47]2[48]BLK	GACCAACTAATGCCACTACGAAGGGGGTAGCA
C11	1[64]4[64]BLK	TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGAGGTCAATC
D11	4[79]2[80]BLK	GCGCAGACAAGAGGCAAAAGAATCCCTCAG
E11	1[96]3[95]BLK	AAACAGCTTTTTGCGGGATCGTCAACACTAAA
F11	4[111]2[112]BLK	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA
G11	1[128]4[128]BLK	TGACAACTCGCTGAGGCTTGCATTATACCAAGCGCGATGATAAA
H11	1[160]2[144]BLK	TTAGGATTGGCTGAGACTCCTCAATAACCGAT
I11	4[143]3[159]BLK	TCATCGCCAACAAAGTACAACGGACGCCAGCA
J11	4[175]2[176]BLK	CACCAGAAAGGTTGAGGCAGGTCATGAAAG
K11	1[192]4[192]BLK	GCGGATAACCTATTATTCTGAAACAGACGATTGGCCTTGAAGAGCCAC
L11	4[207]2[208]BLK	CCACCCTCTATTCACAAACAAATACCTGCCTA
M11	1[224]3[223]BLK	GTATAGCAAACAGTTAATGCCCAATCCTCA
N11	4[239]2[240]BLK	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT
O11	1[256]4[256]BLK	CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGGGAACCAG
P11	4[271]2[272]BLK	AAATCACCTTCCAGTAAGCGTCAGTAATAA
A12	0[47]1[31]BLK	AGAAAGGAACAACTAAAGGAATTCAAAAAAA
B12	2[47]0[48]BLK	ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT
C12	0[79]1[63]BLK	ACAACTTTCAACAGTTTCAGCGGATGTATCGG
D12	2[79]0[80]BLK	CAGCGAAACTTGCTTTCGAGGTGTTGCTAA
E12	0[111]1[95]BLK	TAAATGAATTTTCTGTATGGGATTAATTTCTT
F12	2[111]0[112]BLK	AAGGCCGCTGATACCGATAGTTGCGACGTTAG
G12	0[143]1[127]BLK	TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA
H12	0[175]0[144]BLK	TCCACAGACAGCCCTCATAGTTAGCGTAACGA
I12	2[143]1[159]BLK	ATATTCGGAACCATCGCCCACGCAGAGAAGGA
J12	2[175]0[176]BLK	TATTAAGAAGCGGGGTTTTGCTCGTAGCAT
K12	0[207]1[191]BLK	TCACCAGTACAAACTACAACGCCTAGTACCAG
L12	2[207]0[208]BLK	TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG
M12	0[239]1[223]BLK	AGGAACCCATGTACCGTAACACTTGATATAA
N12	2[239]0[240]BLK	GCCCGTATCCGGAATAGGTGTATCAGCCCAAT
O12	0[271]1[255]BLK	CCACCCTCATTTTCAGGGATAGCAACCGTACT
P12	2[271]0[272]BLK	GTTTTAACTTAGTACCGCCACCCAGAGCCA

Supplementary Table 3 | Biotinylated staple strands

No	Pos	Name	Sequence	Modification
1	C02	18[63]20[56]BIOTIN	ATTAAGTTTACCGAGCTCGAATTCGGGAAACCTGTCGTGC	5'-BT
2	C09	4[63]6[56]BIOTIN	ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	5'-BT
3	G02	18[127]20[120]BIOTIN	GCGATCGGCAATTCCACACAACAGGTGCCTAATGAGTG	5'-BT
4	G09	4[127]6[120]BIOTIN	TTGTGTCGTGACGAGAAACACCAAATTTCAACTTTAAT	5'-BT
5	K02	18[191]20[184]BIOTIN	ATTCATTTTTGTTTGGATTATACTAAGAAACCACCAGAAG	5'-BT
6	K09	4[191]6[184]BIOTIN	CACCCTCAGAAACCATCGATAGCATTGAGCCATTTGGGAA	5'-BT
7	O02	18[255]20[248]BIOTIN	AACAATAACGTAAAACAGAAATAAAAATCCTTTGCCCGAA	5'-BT
8	O09	4[255]6[248]BIOTIN	AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	5'-BT

Supplementary Table 4 | Modified staple strands for DNA origami. The underlined 3'-end sequence (same for all the modified staples) is specific for the structures with 40 domains of 8nt length. Supplementary Table 5 contains the sequences modifications corresponding to the other three DNA origami species used in the experiments.

No	Pos	Name	Sequence
1	A1	P3(40,8)_1_B3	TTAATGAACTAGAGGATCCCCGGGGGGTAACG **TTTCTTCATT**
2	A2	P3(40,8)_1_B5	ACAAACGGAAAAGCCCCAAAAACACTGGAGCATTTCTTCATT
3	A3	P3(40,8)_1_B7	TAAATCGGGATTCCCAATTCTGCGATATAATGTTTCTTCATT
4	A4	P3(40,8)_1_B9	ATCCCCCTATACCACATTCAACTAGAAAAATCTTTCTTCATT
5	A5	P3(40,8)_1_B11	GACCAACTAATGCCACTACGAAGGGGGTAGCATTTCTTCATT
6	A6	P3(40,8)_1_D3	TTCCAGTCGTAATCATGGTCATAAAAGGGGTTTCTTCATT
7	A7	P3(40,8)_1_D5	GCGAGTAAAAATATTTAAATTGTTACAAAGTTTCTTCATT
8	A8	P3(40,8)_1_D7	AAATTAAGTTGACCATTAGATACTTTTGCGTTTCTTCATT
9	A9	P3(40,8)_1_D9	AATACTGCCCAAAAGGAATTACGTGGCTCATTTCTTCATT
10	A10	P3(40,8)_1_D11	GCGCAGACAAGAGGCAAAAGAATCCCTCAGTTTCTTCATT
11	A11	P3(40,8)_1_F3	CACATTAAAATTGTTATCCGCTCATGCGGGCCTTTCTTCATT
12	A12	P3(40,8)_1_F5	TGTAGCCATTAAAATTCGCATTAAATGCCGGATTTCTTCATT
13	B1	P3(40,8)_1_F7	TAAATCATATAACCTGTTTAGCTAACCTTTAATTTCTTCATT
14	B2	P3(40,8)_1_F9	AATAGTAAACACTATCATAACCCTCATTGTGATTTCTTCATT
15	B3	P3(40,8)_1_F11	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATTTCTTCATT
16	B4	P3(40,8)_1_H3	AGAAAACAAAGAAGATGATGAAACAGGCTGCGTTTCTTCATT
17	B5	P3(40,8)_1_H5	GTAATAAGTTAGGCAGAGGCATTTATGATATTTTTCTTCATT
18	B6	P3(40,8)_1_H7	AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTTTCTTCATT
19	B7	P3(40,8)_1_H9	GCAAGGCCTCACCAGTAGCACCATGGGCTTGATTTCTTCATT
20	B8	P3(40,8)_1_H11	TTAGGATTGGCTGAGACTCCTCAATAACCGATTTTCTTCATT
21	B9	P3(40,8)_2_B3	ATTATCATTCAATATAATCCTGACAATTACTTTCTTCATT
22	B10	P3(40,8)_2_B5	TATAACTAACAAAGAACGCGAGAACGCCAATTTCTTCATT
23	B11	P3(40,8)_2_B7	TTTTATTTAAGCAAATCAGATATTTTTTGTTTTCTTCATT
24	B12	P3(40,8)_2_B9	ATACCCAACAGTATGTTAGCAAATTAGAGCTTTCTTCATT

25	C1	P3(40,8)_2_B11	CACCAGAAAGGTTGAGGCAGGTCATGAAAGTTTCTTCATT
26	C2	P3(40,8)_2_D3	GCGGAACATCTGAATAATGGAAGGTACAAAATTTTCTTCATT
27	C3	P3(40,8)_2_D5	ACCTTTTTATTTTAGTTAATTTCATAGGGCTTTTTCTTCATT
28	C4	P3(40,8)_2_D7	GTACCGCAATTCTAAGAACGCGAGTATTATTTTTTCTTCATT
29	C5	P3(40,8)_2_D9	AAGGAAACATAAAGGTGGCAACATTATCACCGTTTCTTCATT
30	C6	P3(40,8)_2_D11	CCACCCTCTATTCACAAACAAATACCTGCCTATTTCTTCATT
31	C7	P3(40,8)_2_F3	ATTTTAAAATCAAAATTATTTGCACGGATTCGTTTCTTCATT
32	C8	P3(40,8)_2_F5	GAATTTATTTAATGGTTTGAAATATTCTTACCTTTCTTCATT
33	C9	P3(40,8)_2_F7	CTTATCATTCCCGACTTGCGGGAGCCTAATTTTTTCTTCATT
34	C10	P3(40,8)_2_F9	AAGTAAGCAGACACCACGGAATAATATTGACGTTTCTTCATT
35	C11	P3(40,8)_2_F11	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTTCTTCATT
36	C12	P3(40,8)_2_H3	CTCGTATTAGAAATTGCGTAGATACAGTACTTTCTTCATT
37	D1	P3(40,8)_2_H5	CTTAGATTTAAGGCGTTAAATAAAGCCTGTTTTCTTCATT
38	D2	P3(40,8)_2_H7	TGTAGAAATCAAGATTAGTTGCTCTTACCATTTCTTCATT
39	D3	P3(40,8)_2_H9	AATAGCTATCAATAGAAAATTCAACATTCATTTCTTCATT
40	D4	P3(40,8)_2_H11	AAATCACCTTCCAGTAAGCGTCAGTAATAATTTCTTCATT

Supplementary Table 5 | List of DNA-PAINT handles

Name	Sequence added to 3′ ends of core staples
P3-40-8nt	5′-TT-TCTTCATT-3′
P3-40-10nt	5′-TT-TCTTCATTAC-3′
P3-120-8nt	5′-TT-TCTTCATT-TT-TCTTCATT-TT-TCTTCATT-3′
P3-120-10nt	5′-TT-TCTTCATTAC-TT-TCTTCATTAC-TT-TCTTCATTAC-3′

Supplementary Table 6 | RNA-FISH probe set targeting MKI67 mRNA variant 2

No.	Name	Sequence
1	MKI67_P3Plus_120_1	gccagaagcaaatttacaactc-TT-TCTTCATTAGCG TT-TCTTCATTA-TT-TCTTCATTA
2	MKI67_P3Plus_120_2	cagtaagttgagtataatccgtTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
3	MKI67_P3Plus_120_3	tttgcaatgttgttttgacacaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
4	MKI67_P3Plus_120_4	aattatgtaatattgcctcctgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
5	MKI67_P3Plus_120_5	aataacagacccatttacttgtTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
6	MKI67_P3Plus_120_6	tagttattacatctccatgtttTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
7	MKI67_P3Plus_120_7	gactttcattttcatacctgaaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
8	MKI67_P3Plus_120_8	gagaagctagatcttgagacacTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
9	MKI67_P3Plus_120_9	tattaggaggcaagttttcatcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
10	MKI67_P3Plus_120_10	cattaccagagactttcttttgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
11	MKI67_P3Plus_120_11	tgatagacactctctttgaaggTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
12	MKI67_P3Plus_120_12	ttgcaacaatcagatttgcttcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA

13	MKI67_P3Plus_120_13	taaattgactgtgaacttcgccTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
14	MKI67_P3Plus_120_14	tacttttcagtatgagctttcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
15	MKI67_P3Plus_120_15	aatgaagttgttgagcactctgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
16	MKI67_P3Plus_120_16	gaaagatcttccttaaagtccaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
17	MKI67_P3Plus_120_17	gtcttgaacatttcagctattcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
18	MKI67_P3Plus_120_18	agaacacatttcctccaaaactTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
19	MKI67_P3Plus_120_19	gtttccattttctctaatacacTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
20	MKI67_P3Plus_120_20	cagagaagtcattttgtaggtgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
21	MKI67_P3Plus_120_21	tgtatattcctgaactctgtagTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
22	MKI67_P3Plus_120_22	tattggttctggttgtaatgacTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
23	MKI67_P3Plus_120_23	tattttggtagttttctcatcaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
24	MKI67_P3Plus_120_24	aagaattcttcctctacatctgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
25	MKI67_P3Plus_120_25	gagttcccataaatgctttaatTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
26	MKI67_P3Plus_120_26	cgaagaattcttcttctacgtcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
27	MKI67_P3Plus_120_27	aatgcgtagatgtttttctcacTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
28	MKI67_P3Plus_120_28	cagttttatcgttagtcattgaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
29	MKI67_P3Plus_120_29	agactccataaatgctttcatgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
30	MKI67_P3Plus_120_30	gtagtttttcgttagtcattgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
31	MKI67_P3Plus_120_31	tgtctggaaaagctctctgaagTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
32	MKI67_P3Plus_120_32	aaatgtgttgatgtctttctctTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
33	MKI67_P3Plus_120_33	gatacttctgtgattttgtcatTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
34	MKI67_P3Plus_120_34	ctattttggtagttttctcatgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
35	MKI67_P3Plus_120_35	tattttggtagttttctcatcaTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
36	MKI67_P3Plus_120_36	ctgagtgctaaaaattcttcctTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
37	MKI67_P3Plus_120_37	tgtctggaagagttctttgaagTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
38	MKI67_P3Plus_120_38	ttttgtcatcagtcattgattcTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
39	MKI67_P3Plus_120_39	ttaaacgctttgatgctcttacTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA
40	MKI67_P3Plus_120_40	acgttgcttcaatactttgatgTTTCTTCATTAGCGTTTCTTCATTATTTCTTCATTA

Supplementary Table 7 | RNA-FISH probe set targeting TFRC mRNA variant 4

No.	Name	Sequence
1	TFRC-P3+_P1+_40-1	cttatcaactatgatcaccgagTTTCTTCATTAGCGTTTTATACATCTACGG
2	TFRC-P3+_P1+_40-2	cagatgagcatgtccaaagaatTTTCTTCATTAGCGTTTTATACATCTACGG
3	TFRC-P3+_P1+_40-3	tgattgaaggaagggaatccagTTTCTTCATTAGCGTTTTATACATCTACGG
4	TFRC-P3+_P1+_40-4	actacaacatagtgatctggttTTTCTTCATTAGCGTTTTATACATCTACGG
5	TFRC-P3+_P1+_40-5	agaccatatctgagaacatctgTTTCTTCATTAGCGTTTTATACATCTACGG
6	TFRC-P3+_P1+_40-6	cactccaactggcaaagataatTTTCTTCATTAGCGTTTTATACATCTACGG
7	TFRC-P3+_P1+_40-7	cctttaaatgcagggacgaaagTTTCTTCATTAGCGTTTTATACATCTACGG
8	TFRC-P3+_P1+_40-8	attcaacatcatgggttagtttTTTCTTCATTAGCGTTTTATACATCTACGG
9	TFRC-P3+_P1+_40-9	cacaaatgaaagcagttggctgTTTCTTCATTAGCGTTTTATACATCTACGG
10	TFRC-P3+_P1+_40-10	aatacagccactgtaaactcagTTTCTTCATTAGCGTTTTATACATCTACGG
11	TFRC-P3+_P1+_40-11	ttattttgtttacgcagtttcaTTTCTTCATTAGCGTTTTATACATCTACGG

12	TFRC-P3+_P1+_40-12	taaaactcattgtcaatgtcccTTTCTTCATTAGCGTTTTATACATCTACGG
13	TFRC-P3+_P1+_40-13	taccaagatgatgggatggaatTTTCTTCATTAGCGTTTTATACATCTACGG
14	TFRC-P3+_P1+_40-14	tatctaccctgtattaaaagctTTTCTTCATTAGCGTTTTATACATCTACGG
15	TFRC-P3+_P1+_40-15	attccatcatggacatttttaTTTCTTCATTAGCGTTTTATACATCTACGG
16	TFRC-P3+_P1+_40-16	acaacaacaggaaagaggcagtTTTCTTCATTAGCGTTTTATACATCTACGG
17	TFRC-P3+_P1+_40-17	aactggtttctgacattttcatTTTCTTCATTAGCGTTTTATACATCTACGG
18	TFRC-P3+_P1+_40-18	aggattcagagagatcattcacTTTCTTCATTAGCGTTTTATACATCTACGG
19	TFRC-P3+_P1+_40-19	atggaaaggcttagatctcattTTTCTTCATTAGCGTTTTATACATCTACGG
20	TFRC-P3+_P1+_40-20	gaatgaggaaaccagctacattTTTCTTCATTAGCGTTTTATACATCTACGG
21	TFRC-P3+_P1+_40-21	tttggcagcatattattctttaTTTCTTCATTAGCGTTTTATACATCTACGG
22	TFRC-P3+_P1+_40-22	cttagcaacccctaattaaattTTTCTTCATTAGCGTTTTATACATCTACGG
23	TFRC-P3+_P1+_40-23	tcactgcatttaggaaaaccagTTTCTTCATTAGCGTTTTATACATCTACGG
24	TFRC-P3+_P1+_40-24	gcctttaagtgacattgatttaTTTCTTCATTAGCGTTTTATACATCTACGG
25	TFRC-P3+_P1+_40-25	accttggataaactgagctataTTTCTTCATTAGCGTTTTATACATCTACGG
26	TFRC-P3+_P1+_40-26	tacagacactgtggtaggtaaaTTTCTTCATTAGCGTTTTATACATCTACGG
27	TFRC-P3+_P1+_40-27	gaaacactgttcccgataattaTTTCTTCATTAGCGTTTTATACATCTACGG
28	TFRC-P3+_P1+_40-28	gttgggatacatgttagatactTTTCTTCATTAGCGTTTTATACATCTACGG
29	TFRC-P3+_P1+_40-29	attaagtagaggacctggagaaTTTCTTCATTAGCGTTTTATACATCTACGG
30	TFRC-P3+_P1+_40-30	ttaaaacttgtccgcactaagtTTTCTTCATTAGCGTTTTATACATCTACGG
31	TFRC-P3+_P1+_40-31	ctctgctttaagtcaaaaggtcTTTCTTCATTAGCGTTTTATACATCTACGG
32	TFRC-P3+_P1+_40-32	ttaattgatcaccacgaatgggTTTCTTCATTAGCGTTTTATACATCTACGG
33	TFRC-P3+_P1+_40-33	cagctgatcatcacgtttataaTTTCTTCATTAGCGTTTTATACATCTACGG
34	TFRC-P3+_P1+_40-34	cacattcaagtgaggctgtaaaTTTCTTCATTAGCGTTTTATACATCTACGG
35	TFRC-P3+_P1+_40-35	atttaagtacgtgtgcgtaacaTTTCTTCATTAGCGTTTTATACATCTACGG
36	TFRC-P3+_P1+_40-36	ttatacgatgaacatgccacatTTTCTTCATTAGCGTTTTATACATCTACGG
37	TFRC-P3+_P1+_40-37	aagtaactcaaccctaactgtaTTTCTTCATTAGCGTTTTATACATCTACGG
38	TFRC-P3+_P1+_40-38	tgtcactagtctgatatttcatTTTCTTCATTAGCGTTTTATACATCTACGG
39	TFRC-P3+_P1+_40-39	atctccttaacgagaagacatcTTTCTTCATTAGCGTTTTATACATCTACGG
40	TFRC-P3+_P1+_40-40	ctaacacagtaaaggtcatgcaTTTCTTCATTAGCGTTTTATACATCTACGG

Supplementary Table 8 | Docking sites conjugated to secondary antibodies

Antibody	Docking site	Docking site sequence
Secondary-Goat	P12-8	5′-TT-TAGTTAGA-3′
Secondary-Mouse	P13-9	5′-TT-ATAGAGAGG-3′

Supplementary Table 9 | Staple strands used for 124 color DNA origami structures. All staple strands are included, except for biotinylated staples (empty rows). Core staple strands were extended with either P1, P2 or P3 handle sequences.

Plate Position	Oligo Name	Sequence
A1	21[32]23[31]BLK	TTTTCACTCAAAGGGCGAAAAACCATCACC
A2	19[32]21[31]P2	GTCGACTTCGGCCAACGCGCGGGGTTTTTC TTATCTACATA

A3	17[32]19[31]P3	TGCATCTTTCCCAGTCACGACGGCCTGCAG TTTCTTCATTA
A4	15[32]17[31]P1	TAATCAGCGGATTGACCGTAATCGTAACCG TTATACATCTA
A5	13[32]15[31]P2	AACGCAAAATCGATGAACGGTACCGGTTGA TTATCTACATA
A6	11[32]13[31]P3	AACAGTTTTGTACCAAAAACATTTTATTTC TTTCTTCATTA
A7	9[32]11[31]P1	TTTACCCCAACATGTTTTAAATTTCCATAT TTATACATCTA
A8	7[32]9[31]P2	TTTAGGACAAATGCTTTAAACAATCAGGTC TTATCTACATA
A9	5[32]7[31]P1	CATCAAGTAAAACGAACTAACGAGTTGAGA TTATACATCTA
A10	3[32]5[31]P3	AATACGTTTGAAAGAGGACAGACTGACCTT TTTCTTCATTA
A11	1[32]3[31]BLK	AGGCTCCAGAGGCTTTGAGGACACGGGTAA
A12	0[47]1[31]BLK	AGAAAGGAACAACTAAAGGAATTCAAAAAAA
B1	23[32]22[48]BLK	CAAATCAAGTTTTTTGGGGTCGAAACGTGGA
B2	22[47]20[48]P3	CTCCAACGCAGTGAGACGGGCAACCAGCTGCA TTTCTTCATTA
B3	20[47]18[48]P1	TTAATGAACTAGAGGATCCCCGGGGGGTAACG TTATACATCTA
B4	18[47]16[48]P2	CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA TTATCTACATA
B5	16[47]14[48]P3	ACAAACGGAAAAGCCCCAAAAACACTGGAGCA TTTCTTCATTA
B6	14[47]12[48]P1	AACAAGAGGGATAAAAATTTTTAGCATAAAGC TTATACATCTA
B7	12[47]10[48]P2	TAAATCGGGATTCCCAATTCTGCGATATAATG TTATCTACATA
B8	10[47]8[48]P3	CTGTAGCTTGACTATTATAGTCAGTTCATTGA TTTCTTCATTA
B9	8[47]6[48]P1	ATCCCCCTATACCACATTCAACTAGAAAAATC TTATACATCTA
B10	6[47]4[48]P2	TACGTTAAAGTAATCTTGACAAGAACCGAACT TTATCTACATA
B11	4[47]2[48]P1	GACCAACTAATGCCACTACGAAGGGGGTAGCA TTATACATCTA
B12	2[47]0[48]BLK	ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT
C1	21[56]23[63]BLK	AGCTGATTGCCCTTCAGAGTCCACTATTAAAGGGTGCCGT
C2		
C3		
C4	15[64]18[64]BLK	GTATAAGCCAACCCGTCGGATTCTGACGACAGTATCGGCCGCAAGGCG
C5	13[64]15[63]P2	TATATTTTGTCATTGCCTGAGAGTGGAAGATT TTATCTACATA
C6	11[64]13[63]P3	GATTTAGTCAATAAAGCCTCAGAGAACCCTCA TTTCTTCATTA
C7	9[64]11[63]P1	CGGATTGCAGAGCTTAATTGCTGAAACGAGTA TTATACATCTA
C8	7[56]9[63]BLK	ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG
C9		
C10		
C11	1[64]4[64]BLK	TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGAGGTCAATC
C12	0[79]1[63]BLK	ACAACTTTCAACAGTTTCAGCGGATGTATCGG
D1	23[64]22[80]BLK	AAAGCACTAAATCGGAACCCTAATCCAGTT
D2	22[79]20[80]P3	TGGAACAACCGCCTGGCCCTGAGGCCCGCT TTTCTTCATTA
D3	20[79]18[80]P1	TTCCAGTCGTAATCATGGTCATAAAAGGGG TTATACATCTA
D4	18[79]16[80]P2	GATGTGCTTCAGGAAGATCGCACAATGTGA TTATCTACATA
D5	16[79]14[80]P3	GCGAGTAAAAATATTTAAATTGTTACAAAG TTTCTTCATTA
D6	14[79]12[80]P1	GCTATCAGAAATGCAATGCCTGAATTAGCA TTATACATCTA
D7	12[79]10[80]P2	AAATTAAGTTGACCATTAGATACTTTTGCG TTATCTACATA

D8	10[79]8[80]P3	GATGGCTTATCAAAAAGATTAAGAGCGTCC TTTCTTCATTA
D9	8[79]6[80]P1	AATACTGCCCAAAAGGAATTACGTGGCTCA TTATACATCTA
D10	6[79]4[80]P2	TTATACCACCAAATCAACGTAACGAACGAG TTATCTACATA
D11	4[79]2[80]P3	GCGCAGACAAGAGGCAAAAGAATCCCTCAG TTTCTTCATTA
D12	2[79]0[80]BLK	CAGCGAAACTTGCTTTCGAGGTGTTGCTAA
E1	21[96]23[95]BLK	AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC
E2	19[96]21[95]P2	CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC TTATCTACATA
E3	17[96]19[95]P3	GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC TTTCTTCATTA
E4	15[96]17[95]P1	ATATTTTGGCTTTCATCAACATTATCCAGCCA TTATACATCTA
E5	13[96]15[95]P2	TAGGTAAACTATTTTTGAGAGATCAAACGTTA TTATCTACATA
E6	11[96]13[95]P3	AATGGTCAACAGGCAAGGCAAAGAGTAATGTG TTTCTTCATTA
E7	9[96]11[95]P1	CGAAAGACTTTGATAAGAGGTCATATTTCGCA TTATACATCTA
E8	7[96]9[95]P2	TAAGAGCAAATGTTTAGACTGGATAGGAAGCC TTATCTACATA
E9	5[96]7[95]P3	TCATTCAGATGCGATTTTAAGAACAGGCATAG TTTCTTCATTA
E10	3[96]5[95]P1	ACACTCATCCATGTTACTTAGCCGAAAGCTGC TTATACATCTA
E11	1[96]3[95]P2	AAACAGCTTTTTGCGGGATCGTCAACACTAAA TTATCTACATA
E12	0[111]1[95]BLK	TAAATGAATTTTCTGTATGGGATTAATTTCTT
F1	23[96]22[112]BLK	CCCGATTTAGAGCTTGACGGGGAAAAAGAATA
F2	22[111]20[112]P3	GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT TTTCTTCATTA
F3	20[111]18[112]P1	CACATTAAAATTGTTATCCGCTCATGCGGGCC TTATACATCTA
F4	18[111]16[112]P2	TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC TTATCTACATA
F5	16[111]14[112]P3	TGTAGCCATTAAAATTCGCATTAAATGCCGGA TTTCTTCATTA
F6	14[111]12[112]P1	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA TTATACATCTA
F7	12[111]10[112]P2	TAAATCATATAACCTGTTTAGCTAACCTTTAA TTATCTACATA
F8	10[111]8[112]P3	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT TTTCTTCATTA
F9	8[111]6[112]P1	AATAGTAAACACTATCATAACCCTCATTGTGA TTATACATCTA
F10	6[111]4[112]P2	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC TTATCTACATA
F11	4[111]2[112]P3	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA TTTCTTCATTA
F12	2[111]0[112]BLK	AAGGCCGCTGATACCGATAGTTGCGACGTTAG
G1	21[120]23[127]BLK	CCCAGCAGGCGAAAAATCCCTTATAAATCAAGCCGGCG
G2		
G3		
G4	15[128]18[128]BLK	TAAATCAAAATAATTCGCGTCTCGGAAACCAGGCAAAGGGAAGG
G5	13[128]15[127]P2	GAGACAGCTAGCTGATAAATTAATTTTTGT TTATCTACATA
G6	11[128]13[127]P3	TTTGGGGATAGTAGTAGCATTAAAAGGCCG TTTCTTCATTA
G7	9[128]11[127]P1	GCTTCAATCAGGATTAGAGAGTTATTTTCA TTATACATCTA
G8	7[120]9[127]BLK	CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA
G9		
G10		
G11	1[128]4[128]BLK	TGACAACTCGCTGAGGCTTGCATTATACCAAGCGCGATGATAAA
G12	0[143]1[127]BLK	TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA

H1	21[160]22[144]P3	TCAATATCGAACCTCAAATATCAATTCCGAAA TTTCTTCATTA
H2	19[160]20[144]P3	GCAATTCACATATTCCTGATTATCAAAGTGTA TTTCTTCATTA
H3	17[160]18[144]P2	AGAAAACAAAGAAGATGATGAAACAGGCTGCG TTATCTACATA
H4	15[160]16[144]P3	ATCGCAAGTATGTAAATGCTGATGATAGGAAC TTTCTTCATTA
H5	13[160]14[144]P3	GTAATAAGTTAGGCAGAGGCATTTATGATATT TTTCTTCATTA
H6	11[160]12[144]P2	CCAATAGCTCATCGTAGGAATCATGGCATCAA TTATCTACATA
H7	9[160]10[144]P3	AGAGAGAAAAAAAATGAAAATAGCAAGCAAACT TTTCTTCATTA
H8	7[160]8[144]P3	TTATTACGAAGAACTGGCATGATTGCGAGAGG TTTCTTCATTA
H9	5[160]6[144]P2	GCAAGGCCTCACCAGTAGCACCATGGGCTTGA TTATCTACATA
H10	3[160]4[144]P3	TTGACAGGCCACCACCAGAGCCGCGATTTGTA TTTCTTCATTA
H11	1[160]2[144]P3	TTAGGATTGGCTGAGACTCCTCAATAACCGAT TTTCTTCATTA
H12	0[175]0[144]BLK	TCCACAGACAGCCCTCATAGTTAGCGTAACGA
A1	23[128]23[159]BLK	AACGTGGCGAGAAAGGAAGGGAAACCAGTAA
A2	22[143]21[159]P1	TCGGCAAATCCTGTTTGATGGTGGACCCTCAA TTATACATCTA
A3	20[143]19[159]P2	AAGCCTGGTACGAGCCGGAAGCATAGATGATG TTATCTACATA
A4	18[143]17[159]P1	CAACTGTTGCGCCATTCGCCATTCAAACATCA TTATACATCTA
A5	16[143]15[159]P1	GCCATCAAGCTCATTTTTTAACCACAAATCCA TTATACATCTA
A6	14[143]13[159]P2	CAACCGTTTCAAATCACCATCAATTCGAGCCA TTATCTACATA
A7	12[143]11[159]P1	TTCTACTACGCGAGCTGAAAAGGTTACCGCGC TTATACATCTA
A8	10[143]9[159]P1	CCAACAGGAGCGAACCAGACCGGAGCCTTTAC TTATACATCTA
A9	8[143]7[159]P2	CTTTTGCAGATAAAAACCAAAATAAAGACTCC TTATCTACATA
A10	6[143]5[159]P1	GATGGTTTGAACGAGTAGTAAATTTACCATTA TTATACATCTA
A11	4[143]3[159]P1	TCATCGCCAACAAAGTACAACGGACGCCAGCA TTATACATCTA
A12	2[143]1[159]P2	ATATTCGGAACCATCGCCCACGCAGAGAAGGA TTATCTACATA
B1	23[160]22[176]BLK	TAAAAGGGACATTCTGGCCAACAAAGCATC
B2	22[175]20[176]P3	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA TTTCTTCATTA
B3	20[175]18[176]P1	ATTATCATTCAATATAATCCTGACAATTAC TTATACATCTA
B4	18[175]16[176]P2	CTGAGCAAAAATTAATTACATTTTGGGTTA TTATCTACATA
B5	16[175]14[176]P3	TATAACTAACAAAGAACGCGAGAACGCCAA TTTCTTCATTA
B6	14[175]12[176]P1	CATGTAATAGAATATAAAGTACCAAGCCGT TTATACATCTA
B7	12[175]10[176]P2	TTTTATTTAAGCAAATCAGATATTTTTTGT TTATCTACATA
B8	10[175]8[176]P3	TTAACGTCTAACATAAAAACAGGTAACGGA TTTCTTCATTA
B9	8[175]6[176]P1	ATACCCAACAGTATGTTAGCAAATTAGAGC TTATACATCTA
B10	6[175]4[176]P2	CAGCAAAAGGAAACGTCACCAATGAGCCGC TTATCTACATA
B11	4[175]2[176]P3	CACCAGAAAGGTTGAGGCAGGTCATGAAAG TTTCTTCATTA
B12	2[175]0[176]BLK	TATTAAGAAGCGGGGTTTTGCTCGTAGCAT
C1	21[184]23[191]BLK	TCAACAGTTGAAAGGAGCAAATGAAAAATCTAGAGATAGA
C2		
C3		
C4	15[192]18[192]BLK	TCAAATATAACCTCCGGCTTAGGTAACAATTTCATTTGAAGGCGAATT
C5	13[192]15[191]P1	GTAAAGTAATCGCCATATTTAACAAAACTTTT TTATACATCTA

C6	11[192]13[191]P2	TATCCGGTCTCATCGAGAACAAGCGACAAAAG TTATCTACATA
C7	9[192]11[191]P1	TTAGACGGCCAAATAAGAAACGATAGAAGGCT TTATACATCTA
C8	7[184]9[191]BLK	CGTAGAAAATACATACCGAGGAAACGCAATAAGAAGCGCA
C9		
C10		
C11	1[192]4[192]BLK	GCGGATAACCTATTATTCTGAAACAGACGATTGGCCTTGAAGAGCCAC
C12	0[207]1[191]BLK	TCACCAGTACAAACTACAACGCCTAGTACCAG
D1	23[192]22[208]BLK	ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG
D2	22[207]20[208]P2	AGCCAGCAATTGAGGAAGGTTATCATCATTTT TTATCTACATA
D3	20[207]18[208]P3	GCGGAACATCTGAATAATGGAAGGTACAAAAT TTTCTTCATTA
D4	18[207]16[208]P1	CGCGCAGATTACCTTTTTTAATGGGAGAGACT TTATACATCTA
D5	16[207]14[208]P2	ACCTTTTTATTTTAGTTAATTTCATAGGGCTT TTATCTACATA
D6	14[207]12[208]P3	AATTGAGAATTCTGTCCAGACGACTAAACCAA TTTCTTCATTA
D7	12[207]10[208]P1	GTACCGCAATTCTAAGAACGCGAGTATTATTT TTATACATCTA
D8	10[207]8[208]P2	ATCCCAATGAGAATTAACTGAACAGTTACCAG TTATCTACATA
D9	8[207]6[208]P3	AAGGAAACATAAAGGTGGCAACATTATCACCG TTTCTTCATTA
D10	6[207]4[208]P1	TCACCGACGCACCGTAATCAGTAGCAGAACCG TTATACATCTA
D11	4[207]2[208]P2	CCACCCTCTATTCACAAACAAATACCTGCCTA TTATCTACATA
D12	2[207]0[208]BLK	TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG
E1	21[224]23[223]BLK	CTTTAGGGCCTGCAACAGTGCCAATACGTG
E2	19[224]21[223]P1	CTACCATAGTTTGAGTAACATTTAAAATAT TTATACATCTA
E3	17[224]19[223]P2	CATAAATCTTTGAATACCAAGTGTTAGAAC TTATCTACATA
E4	15[224]17[223]P3	CCTAAATCAAAATCATAGGTCTAAACAGTA TTTCTTCATTA
E5	13[224]15[223]P1	ACAACATGCCAACGCTCAACAGTCTTCTGA TTATACATCTA
E6	11[224]13[223]P2	GCGAACCTCCAAGAACGGGTATGACAATAA TTATCTACATA
E7	9[224]11[223]P3	AAAGTCACAAAATAAACAGCCAGCGTTTTA TTTCTTCATTA
E8	7[224]9[223]P1	AACGCAAAGATAGCCGAACAAACCCTGAAC TTATACATCTA
E9	5[224]7[223]P2	TCAAGTTTCATTAAAGGTGAATATAAAAGA TTATCTACATA
E10	3[224]5[223]P3	TTAAAGCCAGAGCCGCCACCCTCGACAGAA TTTCTTCATTA
E11	1[224]3[223]P1	GTATAGCAAACAGTTAATGCCCAATCCTCA TTATACATCTA
E12	0[239]1[223]BLK	AGGAACCCATGTACCGTAACACTTGATATAA
F1	23[224]22[240]BLK	GCACAGACAATATTTTTGAATGGGGTCAGTA
F2	22[239]20[240]P2	TTAACACCAGCACTAACAACTAATCGTTATTA TTATCTACATA
F3	20[239]18[240]P3	ATTTTAAAATCAAAATTATTTGCACGGATTCG TTTCTTCATTA
F4	18[239]16[240]P1	CCTGATTGCAATATATGTGAGTGATCAATAGT TTATACATCTA
F5	16[239]14[240]P2	GAATTTATTTAATGGTTTGAAATATTCTTACC TTATCTACATA
F6	14[239]12[240]P3	AGTATAAAGTTCAGCTAATGCAGATGTCTTTC TTTCTTCATTA
F7	12[239]10[240]P1	CTTATCATTCCCGACTTGCGGGAGCCTAATTT TTATACATCTA
F8	10[239]8[240]P2	GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA TTATCTACATA
F9	8[239]6[240]P3	AAGTAAGCAGACACCACGGAATAATATTGACG TTTCTTCATTA
F10	6[239]4[240]P1	GAAATTATTGCCTTTAGCGTCAGACCGGAACC TTATACATCTA

F11	4[239]2[240]P2	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT TTATCTACATA
F12	2[239]0[240]BLK	GCCCGTATCCGGAATAGGTGTATCAGCCCAAT
G1	21[248]23[255]BLK	AGATTAGAGCCGTCAAAAAACAGAGGTGAGGCCTATTAGT
G2		
G3		
G4	15[256]18[256]BLK	GTGATAAAAAGACGCTGAGAAGAGATAACCTTGCTTCTGTTCGGGAGA
G5	13[256]15[255]P1	GTTTATCAATATGCGTTATACAAACCGACCGT TTATACATCTA
G6	11[256]13[255]P2	GCCTTAAACCAATCAATAATCGGCACGCGCCT TTATCTACATA
G7	9[256]11[255]P3	GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA TTTCTTCATTA
G8	7[248]9[255]BLK	GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA
G9		
G10		
G11	1[256]4[256]BLK	CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGGGAACCAG
G12	0[271]1[255]BLK	CCACCCTCATTTTCAGGGATAGCAACCGTACT
H1	23[256]22[272]BLK	CTTTAATGCGCGAACTGATAGCCCCACCAG
H2	22[271]20[272]BLK	CAGAAGATTAGATAATACATTTGTCGACAA
H3	20[271]18[272]P3	CTCGTATTAGAAATTGCGTAGATACAGTAC TTTCTTCATTA
H4	18[271]16[272]P1	CTTTTACAAAATCGTCGCTATTAGCGATAG TTATACATCTA
H5	16[271]14[272]P2	CTTAGATTTAAGGCGTTAAATAAAGCCTGT TTATCTACATA
H6	14[271]12[272]P3	TTAGTATCACAATAGATAAGTCCACGAGCA TTTCTTCATTA
H7	12[271]10[272]P1	TGTAGAAATCAAGATTAGTTGCTCTTACCA TTATACATCTA
H8	10[271]8[272]P2	ACGCTAACACCCACAAGAATTGAAAATAGC TTATCTACATA
H9	8[271]6[272]P3	AATAGCTATCAATAGAAAATTCAACATTCA TTTCTTCATTA
H10	6[271]4[272]P1	ACCGATTGTCGGCATTTTCGGTCATAATCA TTATACATCTA
H11	4[271]2[272]P2	AAATCACCTTCCAGTAAGCGTCAGTAATAA TTATCTACATA
H12	2[271]0[272]BLK	GTTTTAACTTAGTACCGCCACCCAGAGCCA

Supplementary Table 10 | Barcode IDs and combinations of frequencies to achieve 124 colors

Barcode ID	Red (P1 handle)	Green (P2 handle)	Blue (P3 handle)
0	0	0	0
1	0	0	3
2	0	0	9
3	0	0	22
4	0	0	44
5	0	3	0
6	0	3	3
7	0	3	9
8	0	3	22
9	0	3	44
10	0	9	0
11	0	9	3
12	0	9	9
13	0	9	22
14	0	9	44
15	0	22	0
16	0	22	3
17	0	22	9

18	0	22	22
19	0	22	44
20	0	44	0
21	0	44	3
22	0	44	9
23	0	44	22
24	0	44	44
25	3	0	0
26	3	0	3
27	3	0	9
28	3	0	22
29	3	0	44
30	3	3	0
31	3	3	3
32	3	3	9
33	3	3	22
34	3	3	44
35	3	9	0
36	3	9	3
37	3	9	9
38	3	9	22
39	3	9	44
40	3	22	0
41	3	22	3
42	3	22	9
43	3	22	22
44	3	22	44
45	3	44	0
46	3	44	3
47	3	44	9
48	3	44	22
49	3	44	44
50	9	0	0
51	9	0	3
52	9	0	9
53	9	0	22
54	9	0	44
55	9	3	0
56	9	3	3
57	9	3	9
58	9	3	22
59	9	3	44
60	9	9	0
61	9	9	3
62	9	9	9
63	9	9	22
64	9	9	44
65	9	22	0
66	9	22	3
67	9	22	9
68	9	22	22
69	9	22	44
70	9	44	0
71	9	44	3
72	9	44	9
73	9	44	22
74	9	44	44
75	22	0	0
76	22	0	3
77	22	0	9
78	22	0	22
79	22	0	44
80	22	3	0
81	22	3	3
82	22	3	9
83	22	3	22
84	22	3	44

85	22	9	0
86	22	9	3
87	22	9	9
88	22	9	22
89	22	9	44
90	22	22	0
91	22	22	3
92	22	22	9
93	22	22	22
94	22	22	44
95	22	44	0
96	22	44	3
97	22	44	9
98	22	44	22
99	22	44	44
100	44	0	0
101	44	0	3
102	44	0	9
103	44	0	22
104	44	0	44
105	44	3	0
106	44	3	3
107	44	3	9
108	44	3	22
109	44	3	44
110	44	9	0
111	44	9	3
112	44	9	9
113	44	9	22
114	44	9	44
115	44	22	0
116	44	22	3
117	44	22	9
118	44	22	22
119	44	22	44
120	44	44	0
121	44	44	3
122	44	44	9
123	44	44	22
124	44	44	44

Supplementary Note 1: Design of rectangular DNA origami

The DNA origami we used for the 40 and 120 binding site experiments was based on the original flat, rectangular structure[7]. For both the 40 and 120 binding sites, the same core staples were used, and the same 40 staples were modified in the case of all four species. All DNA-PAINT handle extensions added to the staple strands can be found in **Supplementary Tables 3** and **4**. We used the same basic structure for the 124 barcoded origami species as well.

Supplementary Note 2: RNA-FISH probe design

To the 3'-end of the probe's complementary region we added either a P3+ meta-stable handle sequence (in the case of MKI67) or a P3+ and a P1+ meta-stable handle sequence (in the case of TFRC)[8]. By hybridizing meta-stable imager strands to these handles we were able to identify favorable planes in the cell and could also use the acquired diffraction limited signals as an initial control to overlay with the subsequently acquired DNA-PAINT data.

Name	Sequence
MKI67_P3Plus_120_1	probe-TT-TCTTCATTAGCG-TT-TCTTCATTA-TT-TCTTCATTA
TFRC-P3+_P1+_40-1	probe-TT-TCTTCATTAGCG-TT-TT-ATACATCTACGG

In the case of MKI67 probes, the complementary region to the mRNA is in small letters, followed by a P3+ meta-stable handle (12 bp) and two P3 transient DNA-PAINT handle sequences (9bp) at the 3' end. The TFRC probes are elongated on

the 3’ end with a P3+ meta-stable handle (12 bp) and a P1+ meta-stable handle (12bp). The handle sequences are separated from each other by thymine (T) spacers. (See **Supplementary Table 5** and **6** for all probes).

Supplementary Note 3: Sequence of MKI67 mRNA variant 2 used for probe design, 11427 bp

TACCGGGCGGAGGTGAGCGCGGCGCCGGCTCCTCCTGCGGCGGACTTTGGGTGCGACTTGACGAGCGGTGGTTCGACAAGTGGCCTTGCGGGCCGGATCGTCCCAGTGGAAG
AGTTGTAAATTTGCTTCTGGCCTTCCCCTACGGATTATACCTGGCCTTCCCCTACGGATTATACTCAACTTACTGTTTAGAAAATGTGGCCCACGAGACGCCTGGTTACTAT
CAAAAGGAGCGGGGTCGACGGTCCCCACTTTCCCCTGAGCCTCAGCACCTGCTTGTTTGGAAGGGGTATTGAATGTGACATCCGTATCCAGCTTCCTGTTGTGTCAAAACAA
CATTGCAAAATTGAAATCCATGAGCAGGAGGCAATATTACATAATTTCAGTTCCACAAATCCAACACAAGTAAATGGGTCTGTTATTGATGAGCCTGTACGGCTAAAACATG
GAGATGTAATAACTATTATTGATCGTTCCTTCAGGTATGAAAATGAAAGTCTTCAGAATGGAAGGAAGTCAACTGAATTTCCAAGAAAAATACGTGAACAGGAGCCAGCACG
TCGTGTCTCAAGATCTAGCTTCTCTTCTGACCCTGATGAGAGTGAGGGAATACCTTTGAAAAGAAGGCGTGTGTCCTTTGGTGGGCACCTAAGACCTGAACTATTTGATGAA
AACTTGCCTCCTAATACGCCTCTCAAAAGGGGAGAAGCCCCAACCAAAAGAAAGTCTCTGGTAATGCACACTCCACCTGTCCTGAAGAAAATCATCAAGGAACAGCCTCAAC
CATCAGGAAAACAAGAGTCAGGTTCAGAAATCCATGTGGAAGTGAAGGCACAAAGCTTGGTTATAAGCCCTCCAGCTCCTAGTCCTAGGAAAACTCCAGTTGCCAGTGATCA
ACGCCGTAGGTCCTGCAAAACAGCCCCTGCTTCCAGCAGCAAATCTCAGACAGAGGTTCCTAAGAGAGGAGGGAGAAAGAGTGGCAACCTGCCTTCAAAGAGAGTGTCTATC
AGCCGAAGTCAACATGATATTTTACAGATGATATGTTCCAAAAGAAGAAGTGGTGCTTCGGAAGCAAATCTGATTGTTGCAAAATCATGGGCAGATGTAGTAAAACTTGGTG
CAAAACAAACACAAACTAAAGTCATAAAACATGGTCCTCAAAGGTCAATGAACAAAAGGCAAAGAAGACCTGCTACTCCAAAGAAGCCTGTGGGCGAAGTTCACAGTCAATT
TAGTACAGGCCACGCAAACTCTCCTTGTACCATAATAATAGGGAAAGCTCATACTGAAAAAGTACATGTGCCTGCTCGACCCTACAGAGTGCTCAACAACTTCATTTCCAAC
CAAAAAATGGACTTTAAGGAAGATCTTTCAGGAATAGCTGAAATGTTCAAGACCCCAGTGAAGGAGCAACCGCAGTTGACAAGCACATGTCACATCGCTATTTCAAATTCAG
AGAATTTGCTTGGAAAACAGTTTCAAGGAACTGATTCAGGAGAAGAACCTCTGCTCCCCACCTCAGAGAGTTTTGGAGGAAATGTGTTCTTCAGTGCACAGAATGCAGCAAA
ACAGCCATCTGATAAATGCTCTGCAAGCCCTCCCTTAAGACGGCAGTGTATTAGAGAAAATGGAAACGTAGCAAAAACGCCCAGGAACACCTACAAAATGACTTCTCTGGAG
ACAAAAACTTCAGATACTGAGACAGAGCCTTCAAAAACAGTATCCACTGCAAACAGGTCAGGAAGGTCTACAGAGTTCAGGAATATACAGAAGCTACCTGTGGAAAGTAAGA
GTGAAGAAACAAATACAGAAATTGTTGAGTGCATCCTAAAAAGAGGTCAGAAGGCAACACTACTACAACAAAGGAGAGAAGGAGAGATGAAGGAAATAGAAAGACCTTTTGA
GACATATAAGGAAAATATTGAATTAAAAGAAAACGATGAAAAGATGAAAGCAATGAAGAGATCAAGAACTTGGGGGCAGAAATGTGCACCAATGTCTGACCTGACAGACCTC
AAGAGCTTGCCTGATACAGAACTCATGAAAGACACGGCACGTGGCCAGAATCTCCTCCAAACCCAAGATCATGCCAAGGCACCAAAGAGTGAGAAAGGCAAAATCACTAAAA
TGCCCTGCCAGTCATTACAACCAGAACCAATAAACACCCCAACACACAAAACAACAGTTGAAGGCATCCCTGGGGAAAGTAGGTGTGAAAGAAGAGCTCCTAGCAGTCGG
CAAGTTCACACGGACGTCAGGGGAGACCACGCACACGCACAGAGAGCCAGCAGGAGATGGCAAGAGCATCAGAACGTTTAAGGAGTCTCCAAAGCAGATCCTGGACCCAGCA
GCCCGTGTAACTGGAATGAAGAAGTGGCCAAGAACGCCTAAGGAAGAGGCCCAGTCACTAGAAGACCTGGCTGGCTTCAAAGAGCTCTTCCAGACACCAGGTCCCTCTGAGG
AATCAATGACTGATGAGAAAACTACCAAAATAGCCTGCAAATCTCCACCACCAGAATCAGTGGACACTCCAACAAGCACAAAGCAATGGCCTAAGAGAAGTCTCAGGAAAGC
AGATGTAGAGGAAGAATTCTTAGCACTCAGGAAACTAACACCATCAGCAGGGAAAGCCATGCTTACGCCCAAACCAGCAGGAGGTGATGAGAAAGACATTAAAGCATTTATG
GGAACTCCAGTGCAGAAACTGGACCTGGCAGGAACTTTACCTGGCAGCAATAGACTACAGACTCCTAAGGAAAAGGCCCAGGCTCTAGAAGACCTGGCTGGCTTTAAAG
AGCTCTTCCAGACTCCTGGTCACACCGAGGAATTAGTGGCTGCTGGTAAAACCACTAAAATACCCTGCGACTCTCCACAGTCAGACCCAGTGGACACCCCAACAAGCACAAA
GCAACGACCCAAGAGAAGTATCAGGAAAGCAGATGTAGAGGGAGAACTCTTAGCGTGCAGGAATCTAATGCCATCAGCAGGCAAAGCCATGCACACGCCTAAACCATCAGTA
GGTGAAGAGAAAGACATCATCATATTTGTGGGAACTCCAGTGCAGAAACTGGACCTGACAGAGAACTTAACCGGCAGCAAGAGACGGCCACAAACTCCTAAGGAAGAGGCCC
AGGCTCTGGAAGACCTGACTGGCTTTAAAGAGCTCTTCCAGACCCCTGGTCATACTGAAGAAGCAGTGGCTGCTGGCAAAACTACTAAAATGCCCTGCGAATCTTCTCCACC
AGAATCAGCAGACACCCCAACAAGCACAAGAAGGCAGCCCAAGACACCTTTGGAGAAAAGGGACGTACAGAAGGAGCTCTCAGCCCTGAAGAAGCTCACACAGACATCAGGG
GAAACCACACACACAGATAAAGTACCAGGAGGTGAGGATAAAAGCATCAACGCGTTTAGGGAAACTGCAAAACAGAAACTGGACCCAGCAGCAAGTGTAACTGGTAGCAAGA
GGCACCCAAAAACTAAGGAAAAGGCCCAACCCCTAGAAGACCTGGCTGGCTTGAAAGAGCTCTTCCAGACACCAGTATGCACTGACAAGCCCACGACTCACGAGAAAACTAC
CAAAATAGCCTGCAGATCACAACCAGACCCAGTGGACACACCAACAAGCTCCAAGCCACAGTCCAAGAGAAGTCTCAGGAAAGTGGACGTAGAAGAAGAATTCTTCGCACTC
AGGAAACGAACACCATCAGCAGGCAAAGCCATGCACACACCCAAACCAGCAGTAAGTGGTGAGAAAAACATCTACGCATTTATGGGAACTCCAGTGCAGAAACTGGACCTGA
CAGAGAACTTAACTGGCAGCAAGAGACGGCTACAAACTCCTAAGGAAAAGGCCCAGGCTCTAGAAGACCTGGCTGGCTTTAAAGAGCTCTTCCAGACACGAGGTCACACTGA
GGAATCAATGACTAACGATAAAACTGCCAAAGTAGCCTGCAAATCTTCACAACCAGACCCAGACAAAAACCCAGCAAGCTCCAAGCGACGGCTCAAGACATCCCTGGGGAAA
GTGGGCGTGAAAGAAGAGCTCCTAGCAGTTGGCAAGCTCACACAGACATCAGGAGAGACTACACACACACACACAGAGCCAACAGGAGATGGTAAGAGCATGAAAGCATTTA
TGGAGTCTCCAAAGCAGATCTTAGACTCAGCAGCAAGTCTAACTGGCAGCAAGAGGCAGCTGAGAACTCCTAAGGGAAAGTCTGAAGTCCCTGAAGACCTGGCCGGCTTCAT
CGAGCTCTTCCAGACACCAAGTCACACTAAGGAATCAATGACTAACGAAAAAACTACCAAAGTATCCTACAGAGCTTCACAGCCAGACCTAGTGGACACCCCAACAAGCTCC
AAGCCACAGCCCAAGAGAAGTCTCAGGAAAGCAGACACTGAAGAAGAATTTTTAGCATTTAGGAAACAAACGCCATCAGCAGGCAAAGCCATGCACACACCCAAACCAGCAG
TAGGTGAAGAGAAAGACATCAACACGTTTTTGGGAACTCCAGTGCAGAAACTGGACCAGCCAGGAAATTTACCTGGCAGCAATAGACGGCTACAAACTCGTAAGGAAAAGGC
CCAGGCTCTAGAAGAACTGACTGGCTTCAGAGAGCTTTTCCAGACACCATGCACTGATAACCCCACGACTGATGAGAAAACTACCAAAAAAATACTCTGCAAATCTCCGCAA
TCAGACCCAGCGGACACCCCAACAAACACAAAGCAACGGCCCAAGAGAAGCCTCAAGAAAGCAGACGTAGAGGAAGAATTTTTAGCATTCAGGAAACTAACACCATCAGCAG
GCAAAGCCATGCACACGCCTAAAGCAGCAGTAGGTGAAGAGAAAGACATCAACACATTTGTGGGGACTCCAGTGGAGAAACTGGACCTGCTAGGAAATTTACCTGGCAGCAA
GAGACGGCCACAAACTCCTAAAGAAAAGGCCAAGGCTCTAGAAGATCTGGCTGGCTTCAAAGAGCTCTTCCAGACACCAGGTCACACTGAGGAATCAATGACCGATGACAAA
ATCACAGAAGTATCCTGCAAATCTCCACAACCAGACCCAGTCAAAACCCCAACAAGCTCCAAGCAACGACTCAAGATATCCTTGGGGAAAGTAGGTGTGAAAGAAGAGGTCC
TACCAGTCGGCAAGCTCACACAGACGTCAGGGAAGACCACACAGACACACAGAGAGACAGCAGGAGATGGAAAGAGCATCAAAGCGTTTAAGGAATCTGCAAAGCAGATGCT
GGACCCAGCAAACTATGGAACTGGGATGGAGAGGTGGCCAAGAACACCTAAGGAAGAGGCCCAATCACTAGAAGACCTGGCCGGCTTCAAAGAGCTCTTCCAGACACCAGAC
CACACTGAGGAATCAACAACTGATGACAAAACTACCAAAATAGCCTGCAAATCTCCACCACCAGAATCAATGGACACTCCAACAAGCACAAGGAGGCGGCCCAAAACACCTT
TGGGGAAAAGGGATATAGTGGAAGAGCTCTCAGCCCTGAAGCAGCTCACACAGACCACACACACAGACAAAGTACCAGGAGATGAGGATAAAGGCATCAACGTGTTCAGGGA
AACTGCAAAACAGAAACTGGACCCAGCAGCAAGTGTAACTGGTAGCAAGAGGCAGCCAAGAACTCCTAAGGGAAAAGCCCAACCCCTAGAAGACTTGGCTGGCTTGAAAGAG
CTCTTCCAGACACCAATATGCACTGACAAGCCCACGACTCATGAGAAAACTACCAAAATAGCCTGCAGATCTCCACAACCAGACCCAGTGGGTACCCCAACAATCTTCAAGC
CACAGTCCAAGAGAAGTCTCAGGAAAGCAGACGTAGAGGAAGAATCCTTAGCACTCAGGAAACGAACACCATCAGTAGGGAAAGCTATGGACACACCCAAACCAGCAGGAGG
TGATGAGAAAGACATGAAAGCATTTATGGGAACTCCAGTGCAGAAATTGGACCTGCCAGGAAATTTACCTGGCAGCAAAAGATGGCCACAAACTCCTAAGGAAAAGGCCCAG
GCTCTAGAAGACCTGGCTGGCTTCAAAGAGCTCTTCCAGACACCAGGCACTGACAAGCCCACGACTGATGAGAAAACTACCAAAATAGCCTGCAAATCTCCACAACCAGACC
CAGTGGACACCCCAGCAAGCACAAAGCAACGGCCCAAGAGAAGCCTCAGGAAAGCAGACGTAGAGGAAGAATTTTTAGCACTCAGGAAACGAACACCATCAGCAGGCAAAGC
CATGGACACACCAAAACCAGCAGTAAGTGATGAGAAAAATATCAACACATTTGTGGAAACTCCAGTGCAGAAACTGGACCTGCTAGGAAATTTACCTGGCAGCAAGAGACAG
CCACAGACTCCTAAGGAAAAGGCTGAGGCTCTAGAGGACCTGGTTGGCTTCAAAGAACTCTTCCAGACACCAGGTCACACTGAGGAATCAATGACTGATGACAAAATCACAG
AAGTATCCTGTAAATCTCCACAGCCAGAGTCATTCAAAACCTCAAGAAGCTCCAAGCAAAGGCTCAAGATACCCCTGGTGAAAGTGGACATGAAAGAAGAGCCCCTAGCAGT
CAGCAAGCTCACACGGACATCAGGGGAGACTACGCAAACACACACAGAGCCAACAGGAGATAGTAAGAGCATCAAAGCGTTTAAGGAGTCTCCAAAGCAGATCCTGGACCCA
GCAGCAAGTGTAACTGGTAGCAGGAGGCAGCTGAGAACTCGTAAGGAAAAGGCCCGTGCTCTAGAAGACCTGGTTGACTTCAAAGAGCTCTTCTCAGCACCAGGTCACACTG
AAGAGTCAATGACTATTGACAAAAACACAAAAATTCCCTGCAAATCTCCCCCACCAGAACTAACAGACACTGCCACGAGCACAAAGAGATGCCCCAAGACACGTCCCAGGAA
AGAAGTAAAAGAGGAGCTCTCAGCAGTTGAGAGGCTCACGCAAACATCAGGGCAAAGCACACACACACACAAAGAACCAGCAAGCGGTGATGAGGGCATCAAAGTATTGAAG
CAACGTGCAAAGAAGAAACCAAACCCAGTAGAAGAGGAACCCAGCAGGAGAAGGCCAAGAGCACCTAAGGAAAAGGCCCAACCCCTGGAAGACCTGGCCGGCTTCACAGAGC
TCTCTGAAACATCAGGTCACACTCAGGAATCACTGACTGCTGGCAAAGCCACTAAAATACCCTGCGAATCTCCCCCACTAGAAGTGGTAGACACCACAGCAAGCACAAAGAG
GCATCTCAGGACACGTGTGCAGAAGGTACAAGTAAAAGAAGAGCCTTCAGCAGTCAAGTTCACACAAACATCAGGGGAAACCACGGATGCAGACAAAGAACCAGCAGGTGAA
GATAAAGGCATCAAAGCATTGAAGGAATCTGCAAAACAGACACCGGCTCCAGCAGCAAGTGTAACTGGCAGCAGGAGACGGCCAAGAGCACCCAGGGAAAGTGCCCAAGCCA
TAGAAGACCTAGCTGGCTTCAAAGACCCAGCAGCAGGTCACACTGAAGAATCAATGACTGATGACAAAACCACTAAAATACCCTGCAAATCATCACCAGAACTAGAAGACAC
CGCAACAAGCTCAAAGAGACGGCCCAGGACACGTGCCCAGAAAGTAGAAGTGAAGGAGGAGCTGTTAGCAGTTGGCAAGCTCACACAAACCTCAGGGGAGACCACGCACACC
GACAAAGAGCCGGTAGGTGAGGGCAAAGGCACGAAAGCATTTAAGCAACCTGCAAAGCGGAAGCTGGACGCAGAAGATGTAATTGGCAGCAGGAGACAGCCAAGAGCACCTA

AGGAAAAGGCCCAACCCCTGGAAGATCTGGCCAGCTTCCAAGAGCTCTCTCAAACACCAGGCCACACTGAGGAACTGGCAAATGGTGCTGCTGATAGCTTTACAAGCGCTCC
AAAGCAAACACCTGACAGTGGAAAACCTCTAAAAATATCCAGAAGAGTTCTTCGGGCCCCTAAAGTAGAACCCGTGGGAGACGTGGTAAGCACCAGAGACCCTGTAAAATCA
CAAAGCAAAAGCAACACTTCCCTGCCCCCACTGCCCTTCAAGAGGGGAGGTGGCAAAGATGGAAGCGTCACGGGAACCAAGAGGCTGCGCTGCATGCCAGCACCAGAGGAAA
TTGTGGAGGAGCTGCCAGCCAGCAAGAAGCAGAGGGTTGCTCCCAGGGCAAGAGGCAAATCATCCGAACCCGTGGTCATCATGAAGAGAAGTTTGAGGACTTCTGCAAAAAG
AATTGAACCTGCGGAAGAGCTGAACAGCAACGACATGAAAACCAACAAAGAGGAACACAAATTACAAGACTCGGTCCCTGAAAATAAGGGAATATCCCTGCGCTCCAGACGC
CAAAATAAGACTGAGGCAGAACAGCAAATAACTGAGGTCTTTGTATTAGCAGAAAGAATAGAAATAAACAGAAATGAAAAGAAGCCCATGAAGACCTCCCCAGAGATGGACA
TTCAGAATCCAGATGATGGAGCCCGGAAACCCATACCTAGAGACAAAGTCACTGAGAACAAAAGGTGCTTGAGGTCTGCTAGACAGAATGAGAGCTCCCAGCCTAAGGTGGC
AGAGGAGAGCGGAGGGCAGAAGAGTGCGAAGGTTCTCATGCAGAATCAGAAAGGGAAAGGAGAAGCAGGAAATTCAGACTCCATGTGCCTGAGATCAAGAAAGACAAAAAGC
CAGCCTGCAGCAAGCACTTTGGAGAGCAAATCTGTGCAGAGAGTAACGCGGAGTGTCAAGAGGTGTGCAGAAAATCCAAAGAAGGCTGAGGACAATGTGTGTGTCAAGAAAA
TAAGAACCAGAAGTCATAGGGACAGTGAAGATATTTGACAGAAAAATCGAACTGGGAAAAATATAATAAAGTTAGTTTTGTGATAAGTTCTAGTGCAGTTTTTGTCATAAAT
TACAAGTGAATTCTGTAAGTAAGGCTGTCAGTCTGCTTAAGGGAAGAAAACTTTGGATTTGCTGGGTCTGAATCGGCTTCATAAACTCCACTGGGAGCACTGCTGGGCTCCT
GGACTGAGAATAGTTGAACACCGGGGGCTTTGTGAAGGAGTCTGGGCCAAGGTTTGCCCTCAGCTTTGCAGAATGAAGCCTTGAGGTCTGTCACCACCCACAGCCACCCTAC
AGCAGCCTTAACTGTGACACTTGCCACACTGTGTCGTCGTTTGTTTGCCTATGTCCTCCAGGGCACGGTGGCAGGAACAACTATCCTCGTCTGTCCCAACACTGAGCAGGCA
CTCGGTAAACACGAATGAATGGATGAGCGCACGGATGAATGGAGCTTACAAGATCTGTCTTTCCAATGGCCGGGGGCATTTGGTCCCCAAATTAAGGCTATTGGACATCTGC
ACAGGACAGTCCTATTTTTGATGTCCTTTCCTTTCTGAAAATAAAGTTTTGTGCTTTGGAGAATGACTCGTGAGCACATCTTTAGGGACCAAGAGTGACTTTCTGTAAGGAG
TGACTCGTGGCTTGCCTTGGTCTCTTGGGAATACTTTTCTAACTAGGGTTGCTCTCACCTGAGACATTCTCCACCCGCGGAATCTCAGGGTCCCAGGCTGTGGGCCATCACG
ACCTCAAACTGGCTCCTAATCTCCAGCTTTCCTGTCATTGAAAGCTTCGGAAGTTTACTGGCTCTGCTCCCGCCTGTTTTCTTTCTGACTCTATCTGGCAGCCCGATGCCAC
CCAGTACAGGAAGTGACACCAGTACTCTGTAAAGCATCATCATCCTTGGAGAGACTGAGCACTCAGCACCTTCAGCCACGATTTCAGGATCGCTTCCTTGTGAGCCGCTGCC
TCCGAAATCTCCTTTGAAGCCCAGACATCTTTCTCCAGCTTCAGACTTGTAGATATAACTCGTTCATCTTCATTTACTTTCCACTTTGCCCCCTGTCCTCTCTGTGTTCCCC
AAATCAGAGAATAGCCCGCCATCCCCCAGGTCACCTGTCTGGATTCCTCCCCATTCACCCACCTTGCCAGGTGCAGGTGAGGATGGTGCACCAGACAGGGTAGCTGTCCCCC
AAAATGTGCCCTGTGCGGGCAGTGCCCTGTCTCCACGTTTGTTTCCCCAGTGTCTGGCGGGGAGCCAGGTGACATCATAAATACTTGCTGAATGAATGCAGAAATCAGCGGT
ACTGACTTGTACTATATTGGCTGCCATGATAGGGTTCTCACAGCGTCATCCATGATCGTAAGGGAGAATGACATTCTGCTTGAGGGAGGGAATAGAAAGGGGCAGGGAGGGG
ACATCTGAGGGCTTCACAGGGCTGCAAAGGGTACAGGGATTGCACCAGGGCAGAACAGGGGAGGGTGTTCAAGGAAGAGTGGCTCTTAGCAGAGGCACTTTGGAAGGTGTGA
GGCATAAATGCTTCCTTCTACGTAGGCCAACCTCAAAACTTTCAGTAGGAATGTTGCTATGATCAAGTTGTTCTAACACTTTAGACTTAGTAGTAATTATGAACCTCACATA
GAAAAATTTCATCCAGCCATATGCCTGTGGAGTGGAATATTCTGTTTAGTAGAAAAATCCTTTAGAGTTCAGCTCTAACCAGAAATCTTGCTGAAGTATGTCAGCACCTTTT
CTCACCCTGGTAAGTACAGTATTTCAAGAGCACGCTAAGGGTGGTTTTCATTTTACAGGGCTGTTGATGATGGGTTAAAAATGTTCATTTAAGGGCTACCCCCGTGTTTAAT
AGATGAACACCACTTCTACACAACCCTCCTTGGTACTGGGGGAGGGAGAGATCTGACAAATACTGCCCATTCCCCTAGGCTGACTGGATTTGAGAACAAATACCCACCCATT
TCCACCATGGTATGGTAACTTCTCTGAGCTTCAGTTTCCAAGTGAATTTCCATGTAATAGGACATTCCCATTAAATACAAGCTGTTTTTACTTTTTCGCCTCCCAGGGCCTG
TGGGATCTGGTCCCCCAGCCTCTCTTGGGCTTTCTTACACTAACTCTGTACCTACCATCTCCTGCCTCCCTTAGGCAGGCACCTCCAACCACCACACACTCCCTGCTGTTTT
CCCTGCCTGGAACTTTCCCTCCTGCCCCACCAAGATCATTTCATCCAGTCCTGAGCTCAGCTTAAGGGAGGCTTCTTGCCTGTGGGTTCCCTCACCCCCATGCCTGTCCTCC
AGGCTGGGGCAGGTTCTTAGTTTGCCTGGAATTGTTCTGTACCTCTTTGTAGCACGTAGTGTTGTGGAAACTAAGCCACTAATTGAGTTTCTGGCTCCCCTCCTGGGGTTGT
AAGTTTTGTTCATTCATGAGGGCCGACTGCATTTCCTGGTTACTCTATCCCAGTGACCAGCCACAGGAGATGTCCAATAAAGTATGTGATGAAATGGTCTTAAAAAAAAAAA
AAA

Supplementary Note 4: Sequence of TFRC mRNA variant 4 used for probe design, 4695 bp

ACGCACAGCCCCCCTGGGGGCCGGGGGCGGGGCCAGGCTATAAACCGCCGGTTAGGGGCCGCCATCCCCTCAGAGCGTCGGGATATCGGGTGGCGGCTCGGGACGGAGGACG
CGCTAGTGTTCTTCTGTGTGGCAGTTCAGAATGATGGATCAAGCTAGATCAGCATTCTCTAACTTGGCTGCTGAATGAAAATTCATATGTCCCTCGTGAGGCTGGATCTCAA
AAAGATGAAAATCTTGCGTTGTATGTTGAAAATCAATTTCGTGAATTTAAACTCAGCAAAGTCTGGCGTGATCAACATTTTGTTAAGATTCAGGTCAAAGACAGCGCTCAAA
ACTCGGTGATCATAGTTGATAAGAACGGTAGACTTGTTTACCTGGTGGAGAATCCTGGGGGTTATGTGGCGTATAGTAAGGCTGCAACAGTTACTGGTAAACTGGTCCATGC
TAATTTTGGTACTAAAAAAGATTTTGAGGATTTATACACTCCTGTGAATGGATCTATAGTGATTGTCAGAGCAGGGAAAATCACCTTTGCAGAAAAGGTTGCAAATGCTGAA
AGCTTAAATGCAATTGGTGTGTTGATATACATGGACCAGACTAAATTTCCCATTGTTAACGCAGAACTTTCATTCTTTGGACATGCTCATCTGGGGACAGGTGACCCTTACA
CACCTGGATTCCCTTCCTTCAATCACACTCAGTTTCCACCATCTCGGTCATCAGGATTGCCTAATATACCTGTCCAGACAATCTCCAGAGCTGCTGCAGAAAAGCTGTTTGG
GAATATGGAAGGAGACTGTCCCTCTGACTGGAAAACAGACTCTACATGTAGGATGGTAACCTCAGAAAGCAAGAATGTGAAGCTCACTGTGAGCAATGTGCTGAAAGAGATA
AAAATTCTTAACATCTTTGGAGTTATTAAAGGCTTTGTAGAACCAGATCACTATGTTGTAGTTGGGGCCCAGAGAGATGCATGGGGCCCTGGAGCTGCAAAATCCGGTGTAG
GCACAGCTCTCCTATTGAAACTTGCCCAGATGTTCTCAGATATGGTCTTAAAAGATGGGTTTCAGCCCAGCAGAAGCATTATCTTTGCCAGTTGGAGTGCTGGAGACTTTGG
ATCGGTTGGTGCCACTGAATGGCTAGAGGGATACCTTTCGTCCCTGCATTTAAAGGCTTTCACTTATATTAATCTGGATAAAGCGGTTCTTGGTACCAGCAACTTCAAGGTT
TCTGCCAGCCCACTGTTGTATACGCTTATTGAGAAAACAATGCAAAATGTGAAGCATCCGGTTACTGGGCAATTTCTATATCAGGACAGCAACTGGGCCAGCAAAGTTGAGA
AACTCACTTTAGACAATGCTGCTTTCCCTTTCCTTGCATATTCTGGAATCCCAGCAGTTTCTTTCTGTTTTTGCGAGGACACAGATTATCCTTATTTGGGTACCACCATGGA
CACCTATAAGGAACTGATTGAGAGGATTCCTGAGTTGAACAAAGTGGCACGAGCAGCTGCAGAGGTCGCTGGTCAGTTCGTGATTAAACTAACCCATGATGTTGAATTGAAC
CTGGACTATGAGAGGTACAACAGCCAACTGCTTTCATTTGTGAGGGATCTGAACCAATACAGAGCAGACATAAAGGAAATGGGCCTGAGTTTACAGTGGCTGTATTCTGCTC
GTGGAGACTTCTTCCGTGCTACTTCCAGACTAACAACAGATTTCGGGAATGCTGAGAAAACAGACAGATTTGTCATGAAGAAACTCAATGATCGTGTCATGAGAGTGGAGTA
TCACTTCCTCTCTCCCTACGTATCTCCAAAAGAGTCTCCTTTCCGACATGTCTTCTGGGGCTCCGGCTCTCACACGCTGCCAGCTTTACTGGAGAACTTGAAACTGCGTAAA
CAAAATAACGGTGCTTTTAATGAAACGCTGTTCAGAAACCAGTTGGCTCTAGCTACTTGGACTATTCAGGGAGCTGCAAATGCCCTCTCTGGTGACGTTTGGGACATTGACA
ATGAGTTTTAAATGTGATACCCATAGCTTCCATGAGAACAGCAGGGTAGTCTGGTTTCTAGACTTGTGCTGATCGTGCTAAATTTTCAGTAGGGCTACAAAACCTGATGTTA
AAATTCCATCCCATCATCTTGGTACTACTAGATGTCTTTAGGCAGCAGCTTTTAATACAGGGTAGATAACCTGTACTTCAAGTTAAAGTGAATAACCACTTAAAAAATGTCC
ATGATGGAATATTCCCCTATCTCTAGAATTTTAAGTGCTTTGTAATGGGAACTGCCTCTTTCCTGTTGTTGTTAATGAAAATGTCAGAAACCAGTTATGTGAATGATCTCTC
TGAATCCTAAGGGCTGGTCTCTGCTGAAGGTTGTAAGTGGTCGCTTACTTTGAGTGATCCTCCAACTTCATTTGATGCTAAATAGGAGATACCAGGTTGAAAGACCTTCTCC
AAATGAGATCTAAGCCTTTCCATAAGGAATGTAGCTGGTTTCCTCATTCCTGAAAGAAACAGTTAACTTTCAGAAGAGATGGGCTTGTTTTCTTGCCAATGAGGTCTGAAAT
GGAGGTCCTTCTGCTGGATAAAATGAGGTTCAACTGTTGATTGCAGGAATAAGGCCTTAATATGTTAACCTCAGTGTCATTTATGAAAAGAGGGGACCAGAAGCCAAAGACT
TAGTATATTTTCTTTTCCTCTGTCCCTTCCCCCATAAGCCTCCATTTAGTTCTTTGTTATTTTTGTTTCTTCCAAAGCACATTGAAAGAGAACCAGTTTCAGGTGTTTAGTT
GCAGACTCAGTTTGTCAGACTTTAAAGAATAATATGCTGCCAAATTTTGGCCAAAGTGTTAATCTTAGGGGAGAGCTTTCTGTCCTTTTGGCACTGAGATATTTATTGTTTA
TTTATCAGTGACAGAGTTCACTATAAATGGTGTTTTTTTAATAGAATATAATTATCGGAAGCAGTGCCTTCCATAATTATGACAGTTATACTGTCGGTTTTTTTTAAATAAA
AGCAGCATCTGCTAATAAAACCCAACAGATACTGGAAGTTTTGCATTTATGGTCAACACTTAAGGGTTTTAGAAAACAGCCGTCAGCCAAATGTAATTGAATAAAGTTGAAG
CTAAGATTTAGAGATGAATTAAATTTAATTAGGGGTTGCTAAGAAGCGAGCACTGACCAGATAAGAATGCTGGTTTTCCTAAATGCAGTGAATTGTGACCAAGTTATAAATC
AATGTCACTTAAAGGCTGTGGTAGTACTCCTGCAAAATTTTATAGCTCAGTTTATCCAAGGTGTAACTCTAATTCCCATTTTGCAAAATTTCCAGTACCTTTGTCACAATCC
TAACACATTATCGGGAGCAGTGTCTTCCATAATGTATAAAGAACAAGGTAGTTTTTACCTACCACAGTGTCTGTATCGGAGACAGTGATCTCCATATGTTACACTAAGGGTG
TAAGTAATTATCGGGAACAGTGTTTCCCATAATTTTCTTCATGCAATGACATCTTCAAAGCTTGAAGATCGTTAGTATCTAACATGTATCCCAACTCCTATAATTCCCTATC
TTTTAGTTTTAGTTGCAGAAACATTTTGTGGTCATTAAGCATTGGGTGGGTAAATTCAACCACTGTAAAATGAAATTACTACAAAATTTGAAATTTAGCTTGGGTTTTTGTT
ACCTTTATGGTTTCTCCAGGTCCTCTACTTAATGAGATAGTAGCATACATTTATAATGTTTGCTATTGACAAGTCATTTTAACTTTATCACATTATTTGCATGTTACCTCCT
ATAAACTTAGTGCGGACAAGTTTTAATCCAGAATTGACCTTTTGACTTAAAGCAGAGGGACTTTGTATAGAAGGTTTGGGGGCTGTGGGGAAGGAGAGTCCCCTGAAGGTCT
GACACGTCTGCCTACCCATTCGTGGTGATCAATTAAATGTAGGTATGAATAAGTTCGAAGCTCCGTGAGTGAACCATCATTATAAACGTGATGATCAGCTGTTTGTCATAGG
GCAGTTGGAAACGGCCTCCTAGGGAAAAGTTCATAGGGTCTCTTCAGGTTCTTAGTGTCACTTACCTAGATTTACAGCCTCACTTGAATGTGTCACTACTCACAGTCTCTTT
AATCTTCAGTTTTATCTTTAATCTCCTCTTTTATCTTGGACTGACATTTAGCGTAGCTAAGTGAAAAGGTCATAGCTGAGATTCCTGGTTCGGGTGTTACGCACACGTACTT
AAATGAAAGCATGTGGCATGTTCATCGTATAACACAATATGAATACAGGGCATGCATTTTGCAGCAGTGAGTCTCTTCAGAAAACCCTTTTCTACAGTTAGGGTTGAGTTAC
TTCCTATCAAGCCAGTACGTGCTAACAGGCTCAATATTCCTGAATGAAATATCAGACTAGTGACAAGCTCCTGGTCTTGAGATGTCTTCTCGTTAAGGAGATGGGCCTTTTG

GAGGTAAAGGATAAAATGAATGAGTTCTGTCATGATTCACTATTCTAGAACTTGCATGACCTTTACTGTGTTAGCTCTTTGAATGTTCTTGAAATTTTAGACTTTCTTTGTA
AACAAATGATATGTCCTTATCATTGTATAAAAGCTGTTATGTGCAACAGTGTGGAGATTCCTTGTCTGATTTAATAAAATACTTAAACACTGAAAAAAAAAAA

Supplementary References

1. Hoops, S.; Sahle, S.; Gauges, R.; Lee, C.; Pahle, J.; Simus, N.; Singhal, M.; Xu, L.; Mendes, P.; Kummer, U., COPASI--a COmplex PAthway SImulator. *Bioinformatics* **2006,** *22* (24), 3067-74.

2. Jungmann, R.; Avendano, M. S.; Dai, M.; Woehrstein, J. B.; Agasti, S. S.; Feiger, Z.; Rodal, A.; Yin, P., Quantitative super-resolution imaging with qPAINT. *Nat Methods* **2016,** *13* (5), 439-42.

3. Schnitzbauer, J.; Strauss, M. T.; Schlichthaerle, T.; Schueder, F.; Jungmann, R., Super-resolution microscopy with DNA-PAINT. *Nat Protoc* **2017,** *12*, 1198-1228.

4. Stahl, E.; Martin, T. G.; Praetorius, F.; Dietz, H., Facile and scalable preparation of pure and dense DNA origami solutions. *Angew Chem Int Ed Engl* **2014,** *53* (47), 12735-40.

5. Agasti, S. S.; Wang, Y.; Schueder, F.; Sukumar, A.; Jungmann, R.; Yin, P., DNA-barcoded labeling probes for highly multiplexed Exchange-PAINT imaging. *Chem Sci* **2017,** *8* (4), 3080-3091.

6. Campello, R. J. G. B.; Moulavi, D.; Sander, J. In *Density-Based Clustering Based on Hierarchical Density Estimates*, Berlin, Heidelberg, Springer Berlin Heidelberg: Berlin, Heidelberg, 2013; pp 160-172.

7. Rothemund, P. W. K., Folding DNA to create nanoscale shapes and patterns. *Nature* **2006,** *440* (7082), 297-302.

8. Schueder, F.; Strauss, M. T.; Hoerl, D.; Schnitzbauer, J.; Schlichthaerle, T.; Strauss, S.; Yin, P.; Harz, H.; Leonhardt, H.; Jungmann, R., Universal Super-Resolution Multiplexing by DNA Exchange. *Angew Chem Int Ed Engl* **2017,** *56* (14), 4052-4055.