# Supporting Information

# Salt-Induced Conformational Switching of a Flat Rectangular DNA Origami Structure

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## 1. DNA Origami Structure

The DNA origami structure was designed using the CaDNAno software.[1] A 7249-nucleotide long single stranded DNA sequence (scaffold) from the M13mp18 bacteriophage was folded into the designed 71 x 85 nm rectangular shape by the help of short single stranded staple strands. The staple strands were mixed with the scaffold strand in a 10-fold excess. Unmodified staple strands were purchased from IDT, biotinylated strands, nanoparticle binding strands and the ATTO 647N strand were purchased from Eurofins Genomics GmbH. The DNA origami structures were folded using a temperature ramp starting at 70 °C held for 5 min and then the temperature was stepwise decreased with a gradient of 1 °C per minute down to 25 °C. After the folding process, excess staple strands were removed from the sample by gel electrophoresis. Therefore, a 1.5% agarose gel (Biozym LE Agarose) was prepared. A gel electrophoresis chamber was filled with the running buffer (0.5xTAE 11 mM $MgCl_2$) and the pockets of the gel were loaded with the unpurified samples and a 10x BlueJuice™ Gel loading buffer (Thermo Fischer Scientific). The cooled electrophoresis ran at 80 V for 90 min, afterwards the bands containing the purified DNA origami structures were cut out and squeezed with a glass slide to retrieve the purified DNA origami structures. The final concentration of the sample was determined on a Nanodrop 2000 spectralphotometer (Thermo Fischer Scientific).

In order to proof the correct folding and to validate that the rectangular structure of the DNA origami was also stable in the $CaCl_2$ buffer, AFM images were acquired (Figure S1).

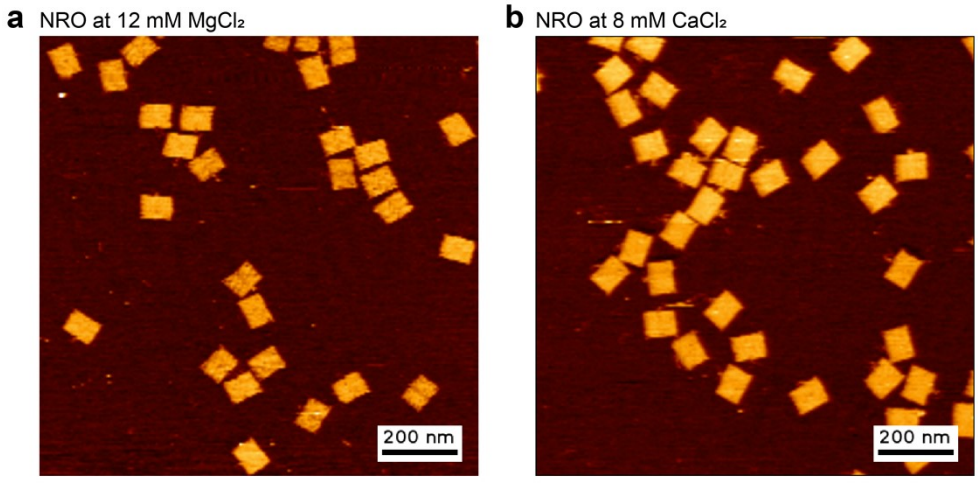


*Figure S1: AFM images of the purified rectangular DNA origami structures (NRO) at 12 mM $MgCl_2$ (a) and at 8 mM $CaCl_2$ (b).*

For the AFM measurements a Nanowizard 3 ultra (JPK, Berlin) equipped with a cantilever (BL-AC-40TS, Olympus, Japan) was used. The mica surface (plano, Berlin) was cleaved with

adhesive tape and incubated for 5 min with 10 µL 0.01 M NiCl2 solution to positively charge the surface. After incubation, the surface was cleaned three times with 300 µL ultrapure water and gently dried under a constant air flow. The DNA origami solution was diluted with AFM-buffer (40 mM Tris, 2 mM EDTA, 12.5 mM $Mg(OAc)_2 \bullet 6\ H_2O$) to a final concentration of 1 nM, added to the mica surface and incubated for 5 min. The mica surface afterwards was washed three times with 300 µL AFM-buffer and filled up to a volume of 1.2 mL AFM-buffer. 3 x 3 µm images were taken with a scanning speed of 1 Hz and a resolution of 512x512 pixels. The images were processed with the JPK data Processing software (JPK, Berlin).

## 2. Gold Nanoparticle Functionalization

Gold nanoparticles (Au NP) were decorated with single stranded DNA sequences for attachment to DNA origami structures. For this purpose, 2 mL of 10 nm Au NPs were mixed with 20 µL 10% Tween20 and 20 µL potassium phosphate buffer (4:5 mixture of 1 M monobasic and dibasic potassium phosphate, Sigma Aldrich). The mixture was heated to 40 °C and 30 µL of 2 nmol thiol-modified single stranded DNA functionalized at the 3'-end ($T_{25}$, Ella Biotech) were added. A salting procedure to reach a final concentration of 750 mM NaCl was carried out by adding a 1xPBS buffer with 3300 mM NaCl every 3 minutes. After salting, unbound DNA strands were removed by centrifugation at 14000 g for 45 min. In this step, the functionalized NPs precipitated and the unbound DNA strands stayed in solution, that was removed after each centrifugation step. The functionalized and concentrated Au NPs were re-diluted after each step with a 1xPBS 10 mM NaCl, 2.11 mM P8709 buffer (Sigma Aldrich). After seven centrifugation steps the particles were kept at high concentration.

## 3. Sample Preparation for Confocal Measurements

For measurements, the DNA origami constructs were immobilized on functionalized coverslips. The coverslips (24 mm x 60 mm, 170 µm thickness; Carl Roth GmbH) were rinsed with Milli-Q water and cleaned in a UV cleaning system (PSD Pro System, Novascan Technologies) for 30 min at 100 °C. After the cleaning, a micro adhesive SecureSeal™ Hybridization Chamber (Grace Bio-Labs) was glued onto the coverslip. The surfaces were incubated first with BSA-biotin (1 mg/mL, Sigma Aldrich) for 10 min and second with neutrAvidin (0.5 mg/mL, Sigma Aldrich). After each incubation step, the surfaces were washed with 1xPBS buffer. The DNA origami structures were immobilized with a concentration of

30 pM in a 1xTAE 12 mM $MgCl_2$ buffer. After incubation of the DNA origami structures the functionalized Au NPs were attached to the DNA origami structures by incubation in a 1xTAE 750 mM NaCl buffer for 30 min. Then unbound NPs were washed out by a 1xTAE 750 mM NaCl buffer. Later the buffer was replaced by the measurement buffer based on a 1xTAE buffer with X mM $MgCl_2$/$CaCl_2$ (X = 12/8, 250, 500, 1000) that also contained trolox/troloxquinone as reducing and oxidizing system (ROXS, 1x TAE, 2 mM trolox/troloxquinone, 1% (w/v) D-(+)-glucose; Sigma Aldrich) as well as glucose/glucose oxidase as oxygen scavenging agent (1 mg/mL glucose oxidase, 0.4% (v/v) catalase (50 µg/mL), 30% glycerol, 12.5 mM KCl in 50 mM TRIS; Sigma Aldrich) to acquire long fluorescence transients.[2,3]

## 4. Sample Preparation for Wide-Field Measurements

For wide-field measurements, LabTek™ chamber slides (Thermo Fisher Scientific Inc.) were incubated with Helmanex overnight and afterwards washed with a 1xPBS buffer. After cleaning, the surface was functionalized using the same protocol for BSA-biotin and neutrAvidin as described above. The DNA origami structures were immobilized by incubating the coverlips with a 30 pM concentration in a 1xTE 12 mM $MgCl_2$ buffer. For measurements, the buffer was exchanged to a 1xTAE buffer containing different concentrations of imager strands (6 bp, ATTO 655 or 7 bp, Cy3B) for the DNA-PAINT experiments (see *Table S1, S2 and S3*).

*Table S1: Concentrations of imager strands used at different $MgCl_2$ concentrations.*

| **c($MgCl_2$) [mM]** | **c(imager strand) [pM]** |
|---|---|
| 12 | 250 |
| 50 | 1000 |
| 100 | 1000 |
| 250 | 250 |
| 500 | 500 |
| 750 | 500 |
| 1000 | 1000 |

*Table S2: Concentrations of imager strands used in the two-color experiments at different salt concentrations.*

| **c($MgCl_2$) [mM]** | **c(red imager strand) [pM]** | **c(green imager strand) [pM]** |
|---|---|---|
| 12 | 1000 | 750 |
| 500 | 3000 | 1500 |

*Table S3: Concentrations of imager strands used at different $MgCl_2$ concentrations.*

| **c($MgCl_2$) [mM]** | **c(imager strand) [pM]** |
|---|---|
| 12 | 250 |
| 500 | 1000 |

## 5. Confocal Setup

Confocal measurements were performed on a home-built confocal setup based on an inverted Olympus IX81 microscope. For excitation, a 78 MHz pulsed white light laser (SuperK Extreme, NKT Photonics) was used. The wavelength was set to 639 nm and 532 nm by an AOTF (201608R, Crystal Technology Inc.). To alternate the red and green excitation a second AOTF (AA.AOTF.nsTN, A-Opto-Electronic) was used. The excitation laser passed through a neutral density filter (ndF, OF 0-2, Thorlabs) and was coupled into a polarization maintaining single-mode fiber (PM-Fiber, P1-488PM-FC-2, Thorlabs). After the fiber, the laser was sent through a linear polarizer (LPVISE100-A, Thorlabs) and a lambda quarter waveplate (AQWP05M-600, Thorlabs) to obtain circularly-polarized light. The laser was focused on the sample by an immersion oil objective (UPlanSApo 100x, NA = 1.4, WD = 0.12 mm, Olympus). Scanning of the sample in x and y direction was performed by a piezo stage (P-517.3CL, E-501.00, Physik Instrumente GmbH & Co. KG). Fluorescence light was collected by the same objective and separated from the excitation light by a dichroic mirror (DS, zt532/640rpc, Chroma). The transmitted light was focused onto a 50 µm pinhole (Linos) to filter out scattered laser light. Green and red fluorescence signals were split into two pathways by a dichroic mirror. Each signal was filtered (RazorEdge 647, Semrock Inc. for the red channel; BrightLine HC 582/75, Semrock Inc. for the green channel) and focused by a lens onto the active area of an APD (Avalanche Photo Diode, SPCM, AQR 14, Perkin Elmer). A TSCPC system (Hydra Harp 400, PicoQuant) was used for time-correlated single-photon counting. Data Processing was performed by a custom-written LabVIEW software (National Instruments).

## 6. Wide-Field Setup

DNA-PAINT measurements were performed on a home-built wide-field microscope based on an inverted Olympus IX71 microscope. As excitation source, a 644 nm diode laser (ibeam smart, Toptica Photonics) was used that was spectrally cleaned-up by a filter (Brightline HC 650/13, Semrock). The laser was focused in the back-focal plane of the objective (UPLXAPO 100X, numerical aperture (NA) = 1.45, working distance (WD) = 0.13, Olympus). The light was directed to the sample situated on top of a nosepiece stage (IX2-NPS, Olympus). The nosepiece stage together with an actively stabilized optical table (TS-300, JRS Scientific Instruments) stabilized the sample for the measurements. Emission light was collected by the same objective as for excitation and directed through a dichroic beamsplitter (Dual Line zt532/ 640 rpc, AHF Analysentechnik) to separate from excitation light. The emission light was filtered (ET 700/75, Chroma) before it was focused onto an EMCCD camera (iXon X3, DU-897, Andor). For data acquisition, the open source imageJ software Micro-Manager was used.[4]

## 7. Reference and Incubation Studies

Reference measurements of the DNA origami structures without any attached NP were carried out to study whether high salt concentrations of magnesium (Figure S2a) or calcium (Figure S3b) influenced the fluorescence lifetime of the fluorescent dye. The fluorescence lifetime distributions measured for the different reference assemblies all showed the same distributions and therefore no influence of the high salt concentration on the dye alone was seen.

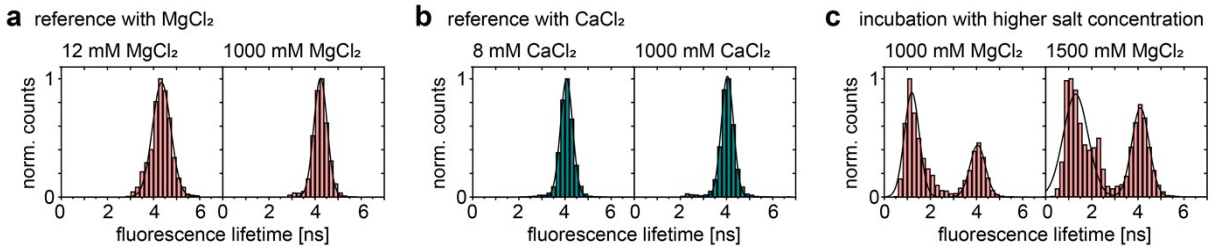


*Figure S2: Reference measurements of the fluorescence lifetime of ATTO 647N in DNA origami structures without Au NP at low (12 mM/ 8 mM) and high salt (1000 mM) for $MgCl_2$ (a) and $CaCl_2$ (b). Fluorescence lifetime histogram for DNA origami structures with an attached 10 nm Au NP incubated at 1500 mM $MgCl_2$ (c).*

To study whether salt concentrations higher than 1000 mM $MgCl_2$ had an influence on the fluorescence lifetime distributions and hence the rolling-up of the DNA origami structures an

experiment with a concentration of 1500 mM $MgCl_2$ was performed. Figure S2c shows no impact of the higher salt concentration.

## 8. Calcium to Magnesium and *vice versa* Experiments

A memory effect in DNA was observed in a salt exchange experiment. We immobilized DNA origami structures on a glass surface and attached a nanoparticle in a 1xTAE buffer containing 12 mM $MgCl_2$. After immobilization, we performed experiments in a 1xTAE buffer containing 8 mM and 1000 mM $CaCl_2$ (Figure S3a i) and ii)). Contrary to our expectations the observed fluorescence lifetime distributions were not reproducing the $MgCl_2$ experiments as only a minor quenched population showed up (Figure S3a ii). To ensure the quality of our samples, we changed the buffer to 1000 mM $MgCl_2$, but a quenched fluorescence lifetime population was not observed (Figure S3a iii)). Even washing with a buffer containing 2 M NaCl (Figure S3a iv)) or overnight incubation in 1 M $MgCl_2$ (Figure S3a v)) could not show the expected fluorescence lifetime quenching in the histograms.

The ion exchange measurement was also performed in the opposite direction to first prove that the DNA origami structures were able to undergo the structural change observed in the fluorescence lifetime quenching. The measurements with 12 mM and 1000 mM $MgCl_2$ (Figure S3b i and ii)) reproduced well the effect shown in Figure 2 in the manuscript. After proving the functionality of the DNA origami structures, a recovery of the flat state by a change to 8 mM $CaCl_2$ was shown (Figure S3b iii)). Now a titration experiment with $CaCl_2$ was performed to reproduce the histograms obtained with $MgCl_2$. Therefore, fluorescence intensity transients were taken at 8 and 1000 mM $CaCl_2$ and converted into fluorescence lifetime transients summed up in fluorescence lifetime histograms in Figure S3b iv) and v). This time a small fraction of DNA origami structures was showing a rolling-up as indicated by the quenched fluorescence lifetime population. Still, the fraction of rolled-up DNA origami structures was lower than in the magnesium experiments.

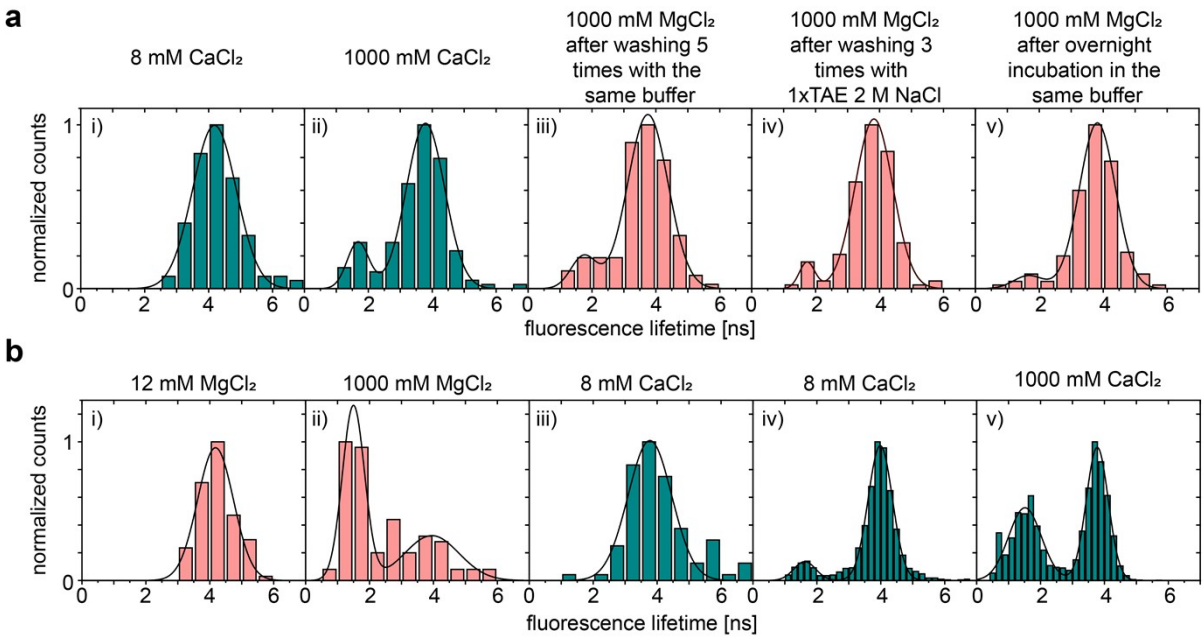


*Figure S3: (a) Fluorescence lifetime distributions of ATTO 647N from scan images for i) 8 mM $CaCl_2$ and ii) 1000 mM $CaCl_2$. After the calcium measurements the buffer was exchanged to magnesium with different incubation methods: iii) washing 5 times with 1000 mM $MgCl_2$, iv) washing 3 times with 2 M NaCl then adding 1000 mM $CaCl_2$, v) overnight incubation with 1000 mM $CaCl_2$. (b) Fluorescence lifetime distributions from scan images for i) 8 mM $MgCl_2$ and ii) 1000 mM $MgCl_2$. The high magnesium concentration measurement was recovered with 8 mM $CaCl_2$ (iii). After switching between the rolled up and open state the sample was treated with calcium ions for two measurements, distributions (iv) an (v) show the histograms from fluorescence lifetime transient for 8 mM $CaCl_2$ and 1000 mM $CaCl_2$.*

These measurements showed that it is crucial to decide which incubation step is done in which buffer and in which order. We assume this effect to be related to ion species binding stably to DNA inducing a kind of memory effect in the DNA. Once an ion is bound tightly, it influences the binding of other ions in the vicinity and can hinder ions of different kind to interact with the DNA the way they would do in the absence of the first bound ion.

Finally, we found that an attachment of the nanoparticle in 1xTAE with 750 mM NaCl and afterwards several washing steps and overnight incubation in 1xTAE with 8 mM $CaCl_2$ for the calcium titration experiments helped to overcome this kinetic blocking of the conformational changes upon ion exchange.

## 9. Distance Calculation

We calculated the distances of the red fluorescent dye to the gold nanoparticle's surface in the flat and rolled-up state of the DNA origami structure. For all calculations we used the theorem of Pythagoras. In a relaxed geometry (Figure S4a) the fluorophore was 33 nm away from the NP which is out of the range for energy transfer between the fluorescent dye and the NP. As the DNA origami structure rolls up the dye approached the NP and the fluorescence lifetime gets quenched. We assumed two possible axes of rolling-up of the DNA origami structure. One axis was parallel to the DNA origami helices (black dashed line in Figure S4a) and the other possible axis was a diagonal axis (green dashed line in Figure S4a) on the DNA origami rectangle. The distance between the ATTO 467N and the NP's surface for parallel rolling-up was calculated with a $D_{NP,\ dye}$ of 12.2 nm. In a diagonal rolling-up geometry the edge with the ATTO 647N bound is not directly approaching the opposing edge, but is shifted. This geometric point we calculated to be 30% of the of the long axis. A diagonal rolling-up leads to a different diameter of the formed DNA origami tube. Based on this assumption, a dye nanoparticle distance of $D_{NP,\ dye}$ of 8.3 nm was calculated for the diagonal rolling-up.

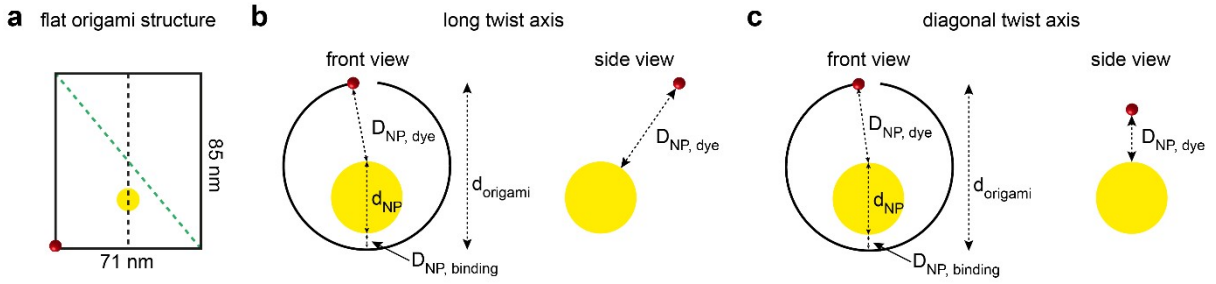


*Figure S4: Schematic of the DNA origami rectangle with dimensions of 71 x 85 nm (a). The two lines are indicating possible directions of bending, which are along the DNA helices (black dashed line) or diagonal (green dashed line). The yellow circle is presenting the gold nanoparticle. (b) Sketches of the front and side view of the rolled-up DNA origami structure along the parallel axis with a DNA tube diameter of $d_{origami}$ of 22.6 nm and a calculated distance between the dye and nanoparticle $D_{NP,\ dye}$ of 12.2 nm. (c) Sketches of the front and side view of the rolling-up of the DNA origami structure along the diagonal axis with $d_{origami}$ 24.3 nm and $D_{NP,\ dye}$ of 8.3 nm. In both geometries the diameter of the nanoparticle $d_{NP}$ is 10 nm and the nanoparticle binding distance between particle and DNA origami structure $D_{NP,\ binding}$ is 4 nm.*

If the measured data is compared to earlier work[5], the fluorescence lifetime at a distance of 8.3 nm is quenched by 62%. For ATTO 647N this quenching would result in a fluorescence lifetime of 1.5 ns. This value is close to the measured fluorescence lifetime of 1.3 ns. The

quenching in rolling-up along the parallel axis of the DNA origami structure in turn would only lead to a quenching of 39% and thus a fluorescence lifetime of 2.9 ns would be expected.

## 10. Dynamics of a Holliday Junction

To study the influence of different salt concentrations on dynamic structures and on 3D DNA origami structures a Holliday Junction on the surface ($HJ_{only}$) and in a force clamp[6] DNA origamis structure ($HJ_{FC}$) was analyzed. In recent years, Holliday junctions have become a reference to study DNA dynamics by single-molecule FRET and its sensitivity to environmental conditions.[7,8] The Holliday Junction contains four different oligonucleotides and fluctuates between two stacked conformations. Two out of four oligonucleotides were labeled with dyes (Cy3/Cy5) which can non-radiatively transfer energy via FRET from the donor (Cy3) to the acceptor dye (Cy5). Switching between the two conformations was visualized by FRET changes between a high-FRET (h. FRET) and a low-FRET state (l. FRET; Figure S5a). It is generally known that the kinetics of switching is strongly slowed down with increasing concentration of bivalent ions.[9] We immobilized HJs via neutrAvidin-biotin interactions on BSA-biotin coated coverslips (Figure S54a). Representative FRET transients are shown in Figure S5b for low (12.5 mM, rose) and high (1000 mM, red) magnesium concentration and directly revealed the reduced rate of the transitions.

Next, we incorporated the HJ into a DNA origami force clamp[6,10] in which two arms of the HJ were connected to a rigid DNA origami frame (Figure S5c). Depending on the length of the connecting single-stranded DNA regions, an entropic force is exerted on the HJ yielding a preference for one conformational state (here the high-FRET state) over the other. The applied approximately 4 pN yielded an increased fraction of the high-FRET state directly visible in the representative transients of Figure S5d both at low and high $Mg^{2+}$ concentration.

For statistical analysis, we histogrammed all the dwell times measured and calculated the equilibrium constant *K* (Figure S5e) which is given by:

$$K_{HJ,\ c(MgCl_2)} = \frac{t_{h.\ FRET,\ HJ,\ c(MgCl_2)}}{t_{l.\ FRET,\ HJ,\ c(MgCl_2)}} \qquad (1)$$

*K* illustrates that the higher salt concentration shifted the equilibrium to the low-FRET state which was observed for the $HJ_{only}$ and $HJ_{FC}$ samples. Interestingly, K changed by a factor R of roughly 1.5 for both samples with R calculated as

$$R_{HJ,\ c(MgCl_2)} = \frac{K_{HJ,\ 12.5\ mM\ MgCl2}}{K_{HJ,\ FC,1\ M\ MgCl2}} \qquad (2)$$

(see Figure S5g).

The fact that the kinetics and equilibrium constant changed similarly for the pure HJ and the HJ in the DNA origami force clamp indicated that no significant change in the structure of the force clamp itself occurred which would have an influence on the applied force. Overall, this experiment indicated that $Mg^{2+}$ concentration did not substantially alter the structure and properties of a functional, rigid 3D DNA origami structure and showed that such devices might be functional over a broad range of ion concentration.

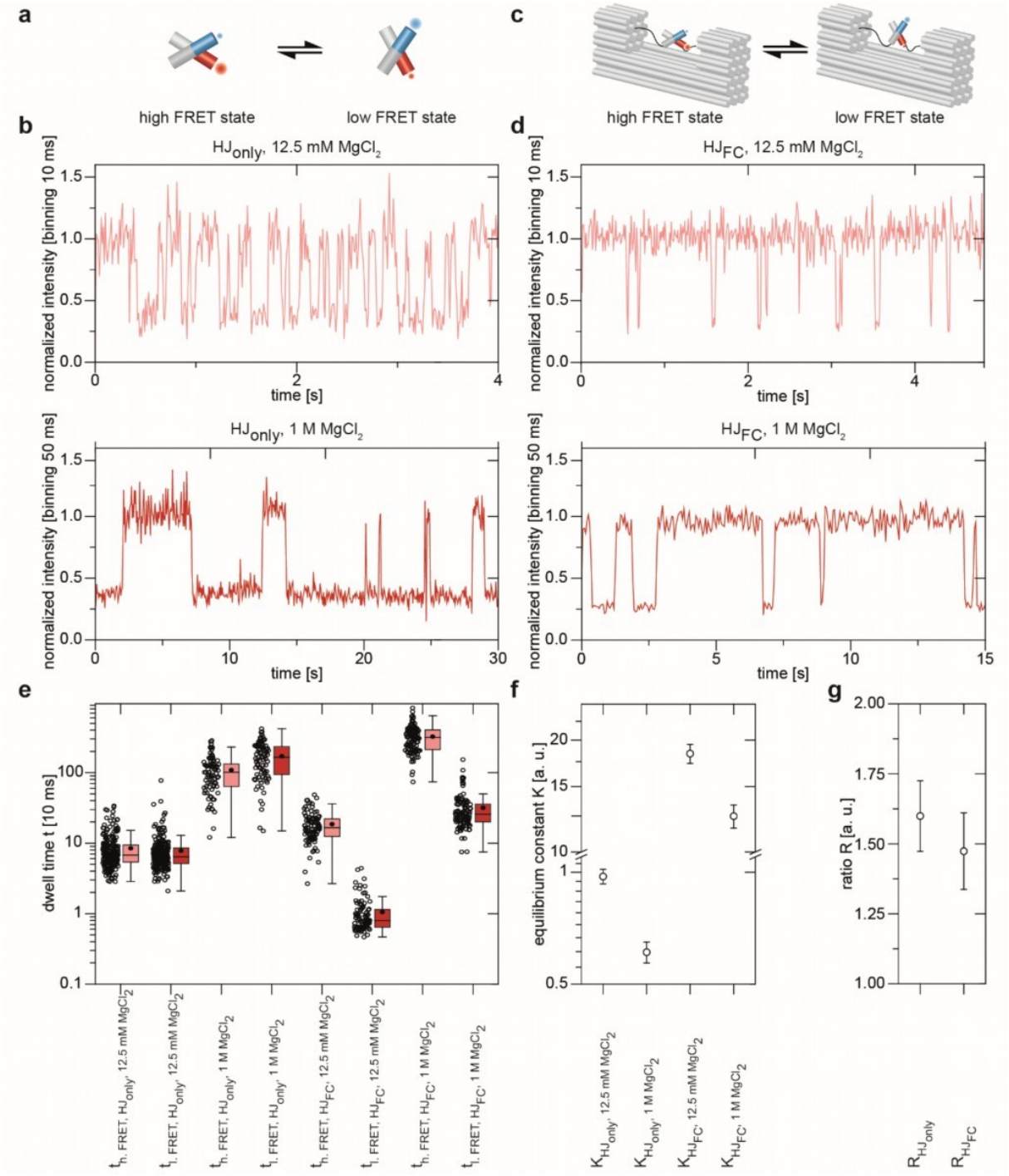

*Figure S5: Illustration of the HJ only ($HJ_{only}$) and HJ in the DNA origami force clamp ($HJ_{FC}$) for observation of the salt influence. HJ only moving between the high- and low-FRET state (a) and in the FC (c). Exemplary transients of (a) are shown in (b) and of (c) in (d) with the salt concentrations of 12.5 mM and 1 M $MgCl_2$. (e) shows the dwell time t for every species obtained from a correlation analysis. The equilibrium constant between high- and low-FRET state shows a shift in the presence of higher salt concentration. By calculating the ratio R between 12.5 mM and 1 M $MgCl_2$ no significant difference is observed which is a clear indicator that high salt concentration does not have an impact on a rigid 3D DNA origami structure and dynamics.*

Holliday Junction Preparation: Sequences for the Holliday Junction are shown in *Table S4*.

*Table S4: Sequences of the $HJ_{only}$.*

| Oligonucleotide | Sequence (5'->3') |
|---|---|
| r | CCCACCGCTCGGCTCAACTGGG |
| x | biotin-TTTCCCAGTTGAGCGCTTGCTAGGG |
| b | Cy5-CCCTAGCAAGCCGCTGCTACGG |
| h | Cy3-CCGTAGCAGCGCGAGCGGTGGG |

For the folding of the HJ an oligonucleotide solution with the ratio of 1:2:2:4 (x:r:b:h) in 1xTAE with 50 mM NaCl is heated to 70 °C and slowly cooled down at a rate of 1 °C/min until room temperature is reached.

DNA origami force clamp preparation: The preparation protocol and the sequences of the 4 pN force clamp were described in ref.[6]. To remove the excess of oligonucleotides gel electrophoreses was used. Therefore, 50 mL of 1.5% agarose gel (Biozym LE Agarose) were prepared in a 1xTAE buffer containing 12.5 mM $MgCl_2$ (Sigma Aldrich). The DNA origami solution was diluted with 10x BlueJuice™ Gel loading buffer (Thermo Fischer Scientific) and filled into the gel. The cooled gel ran for 2 h at 60 V, was cut with a scalpel and squeezed with a glass coverslip. The concentration of the DNA origami structure was determined with a Nanodrop 2000 spectralphotometer (Thermo Fischer Scientific).

Measurement procedure: For the measurements, a flow chamber was first incubated with BSA-biotin (1 mg/mL, Sigma Aldrich) for 10 min. After three times washing with a 1xTAE buffer with 12.5 mM $MgCl_2$ (Sigma Aldrich) the sample was incubated with neutrAvidin (1 mg/mL, Sigma Aldrich) for 10 min and washed again three times with 1xTAE buffer containing 12.5 mM $MgCl_2$ (Sigma Aldrich). Finally, the diluted DNA sample with a concentration of

25 pM was added. After adding the oxygen scavenging (1 mg/mL glucose oxidase, 0.4% (v/v) catalase (50 µg/mL), 30% glycerol, 12.5 mM KCl in 50 mM TRIS; Sigma Aldrich) and ROX system with the respective $MgCl_2$ concentration (1x TAE, 2 mM trolox/troloxquinone, 1% (w/v) D-(+)-glucose; Sigma Aldrich) the sealed sample was measured on a home-built confocal microscope (fluorescence confocal microscope II[11]).[2,3] For the measurements, a laser power between 2 and 3 µW for the green and 1 µW for the red excitation source were used. For PIE[12] measurements the laser repetition rate was set to 80 MHz. The measured data was evaluated with a home-written Labview software and the dwell times were extracted from the correlation of the FRET channel (green excitation – red detection).

## 11. DNA Origami ssDNA Dtrands

*Table S5: Unmodified ssDNA strands.*

| Sequence (5'→3') | Number |
|---|---|
| AAGGCCGCTGATACCGATAGTTGCGACGTTAG | 1 |
| TCGAATTCGGGAAACCTGTCGTGCAGCTGATT | 2 |
| GATGGTTTGAACGAGTAGTAAATTTACCATTA | 3 |
| CGAAAGACTTTGATAAGAGGTCATATTTCGCA | 4 |
| TTAACGTCTAACATAAAAACAGGTAACGGA | 5 |
| TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA | 6 |
| AACAAGAGGGATAAAAATTTTTAGCATAAAGC | 7 |
| AGCCAGCAATTGAGGAAGGTTATCATCATTTT | 8 |
| GATGGCTTATCAAAAAGATTAAGAGCGTCC | 9 |
| GCGGATAACCTATTATTCTGAAACAGACGATT | 10 |
| CTGAGCAAAAATTAATTACATTTTGGGTTA | 11 |
| TGTAGAAATCAAGATTAGTTGCTCTTACCA | 12 |
| CAAATCAAGTTTTTTGGGGTCGAAACGTGGA | 13 |
| TAAGAGCAAATGTTTAGACTGGATAGGAAGCC | 14 |
| TCCACAGACAGCCCTCATAGTTAGCGTAACGA | 15 |
| ACCGATTGTCGGCATTTTCGGTCATAATCA | 16 |
| TTTAGGACAAATGCTTTAAACAATCAGGTC | 17 |
| AGTATAAAGTTCAGCTAATGCAGATGTCTTTC | 18 |
| ACATAACGGGAATCGTCATAAATAAAGCAAAG | 19 |
| CAGAAGATTAGATAATACATTTGTCGACAA | 20 |
| AAGTAAGCAGACACCACGGAATAATATTGACG | 21 |
| GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA | 22 |
| GAATTTATTTAATGGTTTGAAATATTCTTACC | 23 |
| ACAACATGCCAACGCTCAACAGTCTTCTGA | 24 |
| AATACTGCCCAAAAGGAATTACGTGGCTCA | 25 |
| GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC | 26 |
| GCCGTCAAAAAACAGAGGTGAGGCCTATTAGT | 27 |
| ACCTTTTTATTTTAGTTAATTTCATAGGGCTT | 28 |
| TCAATATCGAACCTCAAATATCAATTCCGAAA | 29 |
| GTGATAAAAAGACGCTGAGAAGAGATAACCTT | 30 |
| TTTTCACTCAAAGGGCGAAAAACCATCACC | 31 |
| GAAATAAAAATCCTTTGCCCGAAAGATTAGA | 32 |
| GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | 33 |
| GCCCGTATCCGGAATAGGTGTATCAGCCCAAT | 34 |
| CAACTGTTGCGCCATTCGCCATTCAAACATCA | 35 |
| TTAACACCAGCACTAACAACTAATCGTTATTA | 36 |
| GTATAGCAAACAGTTAATGCCCAATCCTCA | 37 |
| AATACGTTTGAAAGAGGACAGACTGACCTT | 38 |
| TTGACAGGCCACCACCAGAGCCGCGATTTGTA | 39 |
| AATAGTAAACACTATCATAACCCTCATTGTGA | 40 |

| Sequence (5'→3') | Number |
|---|---|
| GAAATTATTGCCTTTAGCGTCAGACCGGAACC | 41 |
| CTTTTACAAAATCGTCGCTATTAGCGATAG | 42 |
| GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC | 43 |
| AACAGTTTTGTACCAAAAACATTTTATTTC | 44 |
| AAGGAAACATAAAGGTGGCAACATTATCACCG | 45 |
| AAGCCTGGTACGAGCCGGAAGCATAGATGATG | 46 |
| ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG | 47 |
| ACAACTTTCAACAGTTTCAGCGGATGTATCGG | 48 |
| CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA | 49 |
| ATCGCAAGTATGTAAATGCTGATGATAGGAAC | 50 |
| CATCAAGTAAAACGAACTAACGAGTTGAGA | 51 |
| TAAAAGGGACATTCTGGCCAACAAAGCATC | 52 |
| AGAGAGAAAAAAATGAAAATAGCAAGCAAACT | 53 |
| AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC | 54 |
| GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA | 55 |
| TACGTTAAAGTAATCTTGACAAGAACCGAACT | 56 |
| CCCGATTTAGAGCTTGACGGGGAAAAAGAATA | 57 |
| TAAATCAAAATAATTCGCGTCTCGGAAACC | 58 |
| ATCCCCCTATACCACATTCAACTAGAAAAATC | 59 |
| TAAAACGAGGTCAATCATAAGGGAACCGGATA | 60 |
| AGGCTCCAGAGGCTTTGAGGACACGGGTAA | 61 |
| TCACCAGTACAAACTACAACGCCTAGTACCAG | 62 |
| GCGAAAAATCCCTTATAAATCAAGCCGGCG | 63 |
| TTTACCCCAACATGTTTTAAATTTCCATAT | 64 |
| ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGG | 65 |
| GATGTGCTTCAGGAAGATCGCACAATGTGA | 66 |
| CAGCGAAACTTGCTTTCGAGGTGTTGCTAA | 67 |
| GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT | 68 |
| TTCATTACGTCAGGACGTTGGGAAATGCAGAT | 69 |
| CATGTAATAGAATATAAAGTACCAAGCCGT | 70 |
| TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | 71 |
| AAATTAAGTTGACCATTAGATACTTTTGCG | 72 |
| GCACAGACAATATTTTTGAATGGGGTCAGTA | 73 |
| CATTTGAAGGCGAATTATTCATTTTTGTTTGG | 74 |
| CCACCCTCATTTTCAGGGATAGCAACCGTACT | 75 |
| CTTTAATGCGCGAACTGATAGCCCCACCAG | 76 |
| ATTATCATTCAATATAATCCTGACAATTAC | 77 |
| GCGAGTAAAAATATTTAAATTGTTACAAAG | 78 |
| CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC | 79 |
| TTAGGATTGGCTGAGACTCCTCAATAACCGAT | 80 |
| TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC | 81 |
| ATTATACTAAGAAACCACCAGAAGTCAACAGT | 82 |
| ATTTACCGGGAACCAGAGCCACCACTGTAGCGC | 83 |
| TGACAACTCGCTGAGGCTTGCATTATACCA | 84 |

| Sequence (5'→3') | Number |
|---|---|
| CAGCAAAAGGAAACGTCACCAATGAGCCGC | 85 |
| GACCAACTAATGCCACTACGAAGGGGGTAGCA | 86 |
| GCCCTTCAGAGTCCACTATTAAAGGGTGCCGT | 87 |
| GGCCTTGAAGAGCCACCACCCTCAGAAACCAT | 88 |
| GTACCGCAATTCTAAGAACGCGAGTATTATTT | 89 |
| CTTAGATTTAAGGCGTTAAATAAAGCCTGT | 90 |
| TCAAATATAACCTCCGGCTTAGGTAACAATTT | 91 |
| GTCGACTTCGGCCAACGCGCGGGGTTTTTC | 92 |
| GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA | 93 |
| GTTTATCAATATGCGTTATACAAACCGACCGT | 94 |
| TTATTACGAAGAACTGGCATGATTGCGAGAGG | 95 |
| TGAAAGGAGCAAATGAAAAATCTAGAGATAGA | 96 |
| TGTAGCCATTAAAATTCGCATTAAATGCCGGA | 97 |
| CGCGCAGATTACCTTTTTTAATGGGAGAGACT | 98 |
| AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGT | 99 |
| AGGAACCCATGTACCGTAACACTTGATATAA | 100 |
| GTTTTCAAGGGAGGGAAGGTAAAGTTTATTT | 101 |
| AAAGTCACAAAATAAACAGCCAGCGTTTTA | 102 |
| AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAA | 103 |
| AACACCAAATTTCAACTTTAATCGTTTACC | 104 |
| TTAAAGCCAGAGCCGCCACCCTCGACAGAA | 105 |
| GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT | 106 |
| AACGTGGCGAGAAAGGAAGGGAAACCAGTAA | 107 |
| GCAATTCACATATTCCTGATTATCAAAGTGTA | 108 |
| TTAATGAACTAGAGGATCCCCGGGGGGTAACG | 109 |
| ATCCCAATGAGAATTAACTGAACAGTTACCAG | 110 |
| AACGCAAAATCGATGAACGGTACCGGTTGA | 111 |
| GCTATCAGAAATGCAATGCCTGAATTAGCA | 112 |
| CCAATAGCTCATCGTAGGAATCATGGCATCAA | 113 |
| ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA | 114 |
| GTTTTAACTTAGTACCGCCACCCAGAGCCA | 115 |
| GCCTTAAACCAATCAATAATCGGCACGCGCCT | 116 |
| TGCATCTTTCCCAGTCACGACGGCCTGCAG | 117 |
| TATTAAGAAGCGGGGTTTTGCTCGTAGCAT | 118 |
| ACGCTAACACCCACAAGAATTGAAAATAGC | 119 |
| GCGAACCTCCAAGAACGGGTATGACAATAA | 120 |
| AAATCACCTTCCAGTAAGCGTCAGTAATAA | 121 |
| TTTTATTTAAGCAAATCAGATATTTTTTGT | 122 |
| AATGGTCAACAGGCAAGGCAAAGAGTAATGTG | 123 |
| AGAAAGGAACAACTAAAGGAATTCAAAAAAA | 124 |
| TATAAGCCAACCCGTCGGATTCTGACGACAG | 125 |
| GCCATCAAGCTCATTTTTTAACCACAAATCCA | 126 |
| CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGA | 127 |
| ACACTCATCCATGTTACTTAGCCGAAAGCTGC | 128 |

| Sequence (5'→3') | Number |
|---|---|
| CTCGTATTAGAAATTGCGTAGATACAGTAC | 129 |
| TAAATCATATAACCTGTTTAGCTAACCTTTAA | 130 |
| TAATCAGCGGATTGACCGTAATCGTAACCG | 131 |
| ACAAACGGAAAAGCCCCAAAAACACTGGAGCA | 132 |
| TCGGCAAATCCTGTTTGATGGTGGACCCTCAA | 133 |
| CTTTTGCAGATAAAAACCAAAATAAAGACTCC | 134 |
| TCATTCAGATGCGATTTTAAGAACAGGCATAG | 135 |
| GTAATAAGTTAGGCAGAGGCATTTATGATATT | 136 |
| CATAAATCTTTGAATACCAAGTGTTAGAAC | 137 |
| TCATCGCCAACAAAGTACAACGGACGCCAGCA | 138 |
| ATATTTTGGCTTTCATCAACATTATCCAGCCA | 139 |
| CCTAAATCAAAATCATAGGTCTAAACAGTA | 140 |
| TCACCGACGCACCGTAATCAGTAGCAGAACCG | 141 |
| CGATAGCATTGAGCCATTTGGGAACGTAGAAA | 142 |
| ATTTTAAAATCAAAATTATTTGCACGGATTCG | 143 |
| TATATTTTGTCATTGCCTGAGAGTGGAAGATTG | 144 |
| TCAAGTTTCATTAAAGGTGAATATAAAAGA | 145 |
| CTGTAGCTTGACTATTATAGTCAGTTCATTGA | 146 |
| CAACCGTTTCAAATCACCATCAATTCGAGCCA | 147 |
| TTAGTATCACAATAGATAAGTCCACGAGCA | 148 |
| CTACCATAGTTTGAGTAACATTTAAAATAT | 149 |
| CTCCAACGCAGTGAGACGGGCAACCAGCTGCA | 150 |
| CTTATCATTCCCGACTTGCGGGAGCCTAATTT | 151 |
| GCTTCTGTTCGGGAGAAACAATAACGTAAAACA | 152 |
| GCAAGGCCTCACCAGTAGCACCATGGGCTTGA | 153 |
| CGGATTGCAGAGCTTAATTGCTGAAACGAGTA | 154 |
| CACATTAAAATTGTTATCCGCTCATGCGGGCC | 155 |
| ATTACCTTTGAATAAGGCTTGCCCAAATCCGC | 156 |
| GCGGAACATCTGAATAATGGAAGGTACAAAAT | 157 |
| TAAATGAATTTTCTGTATGGGATTAATTTCTT | 158 |
| CACAACAGGTGCCTAATGAGTGCCCAGCAG | 159 |
| AAACAGCTTTTTGCGGGATCGTCAACACTAAA | 160 |
| GACAAAAGGTAAAGTAATCGCCATATTTAACAAAACTTTT | 161 |
| TAGGTAAACTATTTTTGAGAGATCAAACGTTA | 162 |
| GCGCAGACAAGAGGCAAAAGAATCCCTCAG | 163 |
| TTCCAGTCGTAATCATGGTCATAAAAGGGG | 164 |
| TAAATCGGGATTCCCAATTCTGCGATATAATG | 165 |
| CACCAGAAAGGTTGAGGCAGGTCATGAAAG | 166 |
| AAAGCACTAAATCGGAACCCTAATCCAGTT | 167 |
| AATTGAGAATTCTGTCCAGACGACTAAACCAA | 168 |
| ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT | 169 |
| CTTTAGGGCCTGCAACAGTGCCAATACGTG | 170 |
| TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA | 171 |
| TGTCACAATCTTACCGAAGCCCTTTAATATCA | 172 |

| Sequence (5'→3') | Number |
|---|---|
| CCAACAGGAGCGAACCAGACCGGAGCCTTTAC | 173 |
| AATAGCTATCAATAGAAAATTCAACATTCA | 174 |
| TGGAACAACCGCCTGGCCCTGAGGCCCGCT | 175 |
| TTATACCACCAAATCAACGTAACGAACGAG | 176 |
| ATATTCGGAACCATCGCCCACGCAGAGAAGGA | 177 |
| TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG | 178 |
| TATAACTAACAAAGAACGCGAGAACGCCAA | 179 |
| ATACCCAACAGTATGTTAGCAAATTAGAGC | 180 |
| TATCGGCCGCAAGGCGATTAAGTTTACCGAGC | 181 |
| GATTTAGTCAATAAAGCCTCAGAGAACCCTCA | 182 |
| TTTATCAGGACAGCATCGGAACGACACCAACC | 183 |
| CCACCCTCTATTCACAAACAAATACCTGCCTA | 184 |
| AACGCAAAGATAGCCGAACAAACCCTGAAC | 185 |
| AGCGCGATGATAAATTGTGTCGTGACGAGA | 186 |
| CCTGATTGCAATATATGTGAGTGATCAATAGT | 187 |
| TTCTACTACGCGAGCTGAAAAGGTTACCGCGC | 188 |
| AGGCAAAGGGAAGGGCGATCGGCAATTCCA | 189 |
| AGAAAACAAAGAAGATGATGAAACAGGCTGCG | 190 |

*Table S6: Modified ssDNA strands.*

| Sequence (5'→3') | Number |
|---|---|
| biotin - GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC | 26 |
| biotin - TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA | 171 |

### 11.1 Energy Transfer Assembly

*Table S7: Replaced ssDNA strands for confocal measurements.*

| Sequence (5'→3') | Number |
|---|---|
| ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT - ATTO 647N | 169 |
| GATGGCTTATCAAAAA - ATTO 532 - GATTAAGAGCGTCC | 9 |
| AAATTAAGTTGACCATTAGATACTTTTGCGAAAAAAAAAAAAAAA AAAAA | 72 |
| GCTATCAGAAATGCAATGCCTGAATTAGCAAAAAAAAAAAAAAA AAAAA | 112 |
| AATGGTCAACAGGCAAGGCAAAGAGTAATGTGAAAAAAAAAAAA AAAAAAA | 123 |
| ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGGCCAAATAA | 65 |
| AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAATCAGGAT | 103 |

Oligonucleotide sequence on nanoparticles (5'→3'):

TTTTTTTTTTTTTTTTTTTTTTTT-Thiol

### 11.2 One Color DNA-PAINT Sample

*Table S8: Replaced ssDNA strands for one color DNA-PAINT measurements.*

| **Sequence (5'→3')** | **Number** |
|---|---|
| AAGGCCGCTGATACCGATAGTTGCGACGTTAGTTAAATGCCCG | 1 |
| TCTAAAGTTTTGTCGTCTTTCCAGCCGACAATTAAATGCCCG | 6 |
| AGCCAGCAATTGAGGAAGGTTATCATCATTTTTTAAATGCCCG | 8 |
| GCGGATAACCTATTATTCTGAAACAGACGATTTTAAATGCCCG | 10 |
| CAAATCAAGTTTTTTGGGGTCGAAACGTGGATTAAATGCCCG | 13 |
| TCCACAGACAGCCCTCATAGTTAGCGTAACGATTAAATGCCCG | 15 |
| CAGAAGATTAGATAATACATTTGTCGACAATTAAATGCCCG | 20 |
| GCCGTCAAAAAACAGAGGTGAGGCCTATTAGTTTAAATGCCCG | 27 |
| TCAATATCGAACCTCAAATATCAATTCCGAAATTAAATGCCCG | 29 |
| TTTTCACTCAAAGGGCGAAAAACCATCACCTTAAATGCCCG | 31 |
| GCCCGTATCCGGAATAGGTGTATCAGCCCAATTTAAATGCCCG | 34 |
| TTAACACCAGCACTAACAACTAATCGTTATTATTAAATGCCCG | 36 |
| GTATAGCAAACAGTTAATGCCCAATCCTCATTAAATGCCCG | 37 |
| ACCCTTCTGACCTGAAAGCGTAAGACGCTGAGTTAAATGCCCG | 47 |
| ACAACTTTCAACAGTTTCAGCGGATGTATCGGTTAAATGCCCG | 48 |
| TAAAAGGGACATTCTGGCCAACAAAGCATCTTAAATGCCCG | 52 |
| AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCCTTAAATGCCCG | 54 |
| GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATTAAATGCCCG | 55 |
| CCCGATTTAGAGCTTGACGGGGAAAAAGAATATTAAATGCCCG | 57 |
| AGGCTCCAGAGGCTTTGAGGACACGGGTAATTAAATGCCCG | 61 |
| TCACCAGTACAAACTACAACGCCTAGTACCAGTTAAATGCCCG | 62 |
| GCGAAAAATCCCTTATAAATCAAGCCGGCGTTAAATGCCCG | 63 |
| CAGCGAAACTTGCTTTCGAGGTGTTGCTAATTAAATGCCCG | 67 |
| GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTAAATGCCCG | 68 |
| GCACAGACAATATTTTTGAATGGGGTCAGTATTAAATGCCCG | 73 |
| CCACCCTCATTTTCAGGGATAGCAACCGTACTTTAAATGCCCG | 75 |
| CTTTAATGCGCGAACTGATAGCCCCACCAGTTAAATGCCCG | 76 |
| TTAGGATTGGCTGAGACTCCTCAATAACCGATTTAAATGCCCG | 80 |
| TGACAACTCGCTGAGGCTTGCATTATACCATTAAATGCCCG | 84 |
| GACCAACTAATGCCACTACGAAGGGGGTAGCATTAAATGCCCG | 86 |
| GCCCTTCAGAGTCCACTATTAAAGGGTGCCGTTTAAATGCCCG | 87 |
| TGAAAGGAGCAAATGAAAAATCTAGAGATAGATTAAATGCCCG | 96 |
| AGGAACCCATGTACCGTAACACTTGATATAATTAAATGCCCG | 100 |
| GCCCGAGAGTCCACGCTGGTTTGCAGCTAACTTTAAATGCCCG | 106 |
| AACGTGGCGAGAAAGGAAGGGAAACCAGTAATTAAATGCCCG | 107 |
| ACCTTGCTTGGTCAGTTGGCAAAGAGCGGATTAAATGCCCG | 114 |
| GTTTTAACTTAGTACCGCCACCCAGAGCCATTAAATGCCCG | 115 |
| TATTAAGAAGCGGGGTTTTGCTCGTAGCATTTAAATGCCCG | 118 |
| AAATCACCTTCCAGTAAGCGTCAGTAATAATTAAATGCCCG | 121 |
| AGAAAGGAACAACTAAAGGAATTCAAAAAAATTAAATGCCCG | 124 |

| Sequence (5'→3') | Number |
|---|---|
| CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGTTAAATG CCCG | 127 |
| TCGGCAAATCCTGTTTGATGGTGGACCCTCAATTAAATGCCCG | 133 |
| TCATCGCCAACAAAGTACAACGGACGCCAGCATTAAATGCCCG | 138 |
| CTCCAACGCAGTGAGACGGGCAACCAGCTGCATTAAATGCCCG | 150 |
| TAAATGAATTTTCTGTATGGGATTAATTTCTTTTAAATGCCCG | 158 |
| AAACAGCTTTTTGCGGGATCGTCAACACTAAATTAAATGCCCG | 160 |
| GCGCAGACAAGAGGCAAAAGAATCCCTCAGTTAAATGCCCG | 163 |
| CACCAGAAAGGTTGAGGCAGGTCATGAAAGTTAAATGCCCG | 166 |
| AAAGCACTAAATCGGAACCCTAATCCAGTTTTAAATGCCCG | 167 |
| ACGGCTACAAAAGGAGCCTTTAATGTGAGAATTTAAATGCCCG | 169 |
| CTTTAGGGCCTGCAACAGTGCCAATACGTGTTAAATGCCCG | 170 |
| TGGAACAACCGCCTGGCCCTGAGGCCCGCTTTAAATGCCCG | 175 |
| ATATTCGGAACCATCGCCCACGCAGAGAAGGATTAAATGCCCG | 177 |
| TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCGTTAAATGCCCG | 178 |
| TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGATTAAATG CCCG | 183 |
| CCACCCTCTATTCACAAACAAATACCTGCCTATTAAATGCCCG | 184 |

Imager strand (5'→3'): CGGGCA-ATTO 655

### 11.3 Two Color DNA-PAINT Sample

*Table S9: Replaced ssDNA strands for two color DNA-PAINT measurements.*

| Sequence (5'→3') | Number |
|---|---|
| AAGGCCGCTGATACCGATAGTTGCGACGTTAGTTAAATGCCCG | 1 |
| TCTAAAGTTTTGTCGTCTTTCCAGCCGACAATTAAATGCCCG | 6 |
| TCCACAGACAGCCCTCATAGTTAGCGTAACGATTAAATGCCCG | 15 |
| GCCCGTATCCGGAATAGGTGTATCAGCCCAATTTAAATGCCCG | 34 |
| ACAACTTTCAACAGTTTCAGCGGATGTATCGGTTAAATGCCCG | 48 |
| GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATTAAATGCCCG | 55 |
| TCACCAGTACAAACTACAACGCCTAGTACCAGTTAAATGCCCG | 62 |
| CAGCGAAACTTGCTTTCGAGGTGTTGCTAATTAAATGCCCG | 67 |
| GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTAAATGCCCG | 68 |
| CCACCCTCATTTTCAGGGATAGCAACCGTACTTTAAATGCCCG | 75 |
| TTAGGATTGGCTGAGACTCCTCAATAACCGATTTAAATGCCCG | 80 |
| GACCAACTAATGCCACTACGAAGGGGGTAGCATTAAATGCCCG | 86 |
| AGGAACCCATGTACCGTAACACTTGATATAATTAAATGCCCG | 100 |
| GTTTTAACTTAGTACCGCCACCCAGAGCCATTAAATGCCCG | 115 |
| TATTAAGAAGCGGGGTTTTGCTCGTAGCATTTAAATGCCCG | 118 |
| AAATCACCTTCCAGTAAGCGTCAGTAATAATTAAATGCCCG | 121 |
| AGAAAGGAACAACTAAAGGAATTCAAAAAAATTAAATGCCCG | 124 |

| Sequence (5'→3') | Number |
|---|---|
| TAAATGAATTTTCTGTATGGGATTAATTTCTTTTAAATGCCCG | 158 |
| GCGCAGACAAGAGGCAAAAGAATCCCTCAGTTAAATGCCCG | 163 |
| CACCAGAAAGGTTGAGGCAGGTCATGAAAGTTAAATGCCCG | 166 |
| ACGGCTACAAAAGGAGCCTTTAATGTGAGAATTTAAATGCCCG | 169 |
| ATATTCGGAACCATCGCCCACGCAGAGAAGGATTAAATGCCCG | 177 |
| TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCGTTAAATGCCCG | 178 |
| CCACCCTCTATTCACAAACAAATACCTGCCTATTAAATGCCCG | 184 |
| TCGAATTCGGGAAACCTGTCGTGCAGCTGATTTTTCCTCCTCCT | 2 |
| CAAATCAAGTTTTTTGGGGTCGAAACGTGGATTTCCTCCTCCT | 13 |
| GCCGTCAAAAAACAGAGGTGAGGCCTATTAGTTTTCCTCCTCCT | 27 |
| TCAATATCGAACCTCAAATATCAATTCCGAAATTTCCTCCTCCT | 29 |
| TTTTCACTCAAAGGGCGAAAAACCATCACCTTTCCTCCTCCT | 31 |
| GAAATAAAAATCCTTTGCCCGAAAGATTAGATTTCCTCCTCCT | 32 |
| ACCCTTCTGACCTGAAAGCGTAAGACGCTGAGTTTCCTCCTCCT | 47 |
| TAAAAGGGACATTCTGGCCAACAAAGCATCTTTCCTCCTCCT | 52 |
| AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCCTTTCCTCCTCCT | 54 |
| CCCGATTTAGAGCTTGACGGGGAAAAAGAATATTTCCTCCTCCT | 57 |
| GCGAAAAATCCCTTATAAATCAAGCCGGCGTTTCCTCCTCCT | 63 |
| GCACAGACAATATTTTTGAATGGGGTCAGTATTTCCTCCTCCT | 73 |
| CTTTAATGCGCGAACTGATAGCCCCACCAGTTTCCTCCTCCT | 76 |
| CTGTGTGATTGCGTTGCGCTCACTAGAGTTGCTTTCCTCCTCCT | 79 |
| ATTATACTAAGAAACCACCAGAAGTCAACAGTTTTCCTCCTCCT | 82 |
| GCCCTTCAGAGTCCACTATTAAAGGGTGCCGTTTTCCTCCTCCT | 87 |
| GTCGACTTCGGCCAACGCGCGGGGTTTTTCTTTCCTCCTCCT | 92 |
| TGAAAGGAGCAAATGAAAAATCTAGAGATAGATTTCCTCCTCCT | 96 |
| AACGTGGCGAGAAAGGAAGGGAAACCAGTAATTTCCTCCTCCT | 107 |
| TCGGCAAATCCTGTTTGATGGTGGACCCTCAATTTCCTCCTCCT | 133 |
| CTACCATAGTTTGAGTAACATTTAAAATATTTTCCTCCTCCT | 149 |
| CACAACAGGTGCCTAATGAGTGCCCAGCAGTTTCCTCCTCCT | 159 |
| AAAGCACTAAATCGGAACCCTAATCCAGTTTTTCCTCCTCCT | 167 |
| CTTTAGGGCCTGCAACAGTGCCAATACGTGTTTCCTCCTCCT | 170 |

Imager Strands (5'→3'):

CGGGCA-ATTO 655

AGGAGGA-Cy3B

### 11.4 NRO 6 nm Grid Sample

All strands (except 26 and 171) are extended with the aptamer sequence (TTAAATGCCCG).

Imager Strand (5'→3'): CGGGCA-ATTO 655

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