Supporting Information for

A DNA walker as fluorescence signal amplifier

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Table of contents

Materials and Methods

Gold nanoparticle functionalization. Gold nanoparticles of 80 nm diameter were purchased from BBI solutions and functionalized with single stranded 25T DNA-oligonucleotides, incorporating a thiol modification on the 5' end (Ella Biotech GmbH). 2 ml of nanoparticle solution was mixed with 20 μl Tween20 (10%, Polysorbate20, Alfa Aesar), 20 μl of potassium phosphate (4:5 mixture of monobasic and dibasic potassium phosphate, Sigma Aldrich) and an excess of the desired oligonucleotide solution (50 nM, 18.4 μl) and stirred overnight. Afterwards, the nanoparticle oligonucleotide mixture was heated to 40 °C and salt was added for an hour in 5 min steps with increasing amounts up to a final concentration of 750 mM using PBS buffer containing 3.3 M NaCl. For purification, the mixture was diluted 1:1 with PBS containing 0.01% Tween20 and 1 mM EDTA and spinned down. The supernatant was pipetted off and the particle pellet was diluted in the PBS buffer mentioned above. This spinning process was repeated 6 times to completely purify nanoparticles from free oligonucleotides.

DNA origami design and folding. The rectangle DNA origami[1,2] (table S2, S3 and S4) consisting of 7249 bp and DNA Origami pillar[3] (table S5) consisting of 8064 bp were designed with the software CaDNAno (http://cadnano.org/)[4]. p7249 and p8064 scaffold was extracted from M13mp18 bacteriophage. All the staple strands were purchased from Eurofins Genomics. For DNA Origami folding, 10 nM scaffold together with a tenfold excess of each staple strand was mixed in 1xTE (10 mM Tris, 1 mM EDTA; pH 8.0) buffer with 14 mM $MgCl_2$. In the annealing process the folding mixture was heated at 65°C and slowly cooled down to 25°C. Afterwards the folded DNA origami was purified from excess staple strands by Amicon filtering (Amicon Ultra—0.5 ml, Ultracel®- 100 K Membrane, Millipore), washed 4 times with 1xTE buffer containing 14 mM $MgCl_2$ and centrifuged each time at 3 krcf speed for 10 min at 20 °C. To recover the DNA origami pillar, the Amicon filter was flipped into a new tube and centrifuged 2 min at 2 krcf speed at 20 °C.

DNA origami preparation on the surface. The DNA origami was immobilized on a glass surface coated with BSA-biotin (Sigma-Aldrich) and Neutravidin (Sigma-Aldrich) by the strong interaction of Neutravidin to the biotins on the base of the DNA origami. For DNA walker in the plasmonic hotspot experiment, the nanoparticle solution was diluted to an absorption of 0.1–0.15 (Nanodrop 2000, Thermo Scientific) with 1xTE containing 660 mM NaCl. Subsequently, the immobilized DNA origami was incubated with the diluted nanoparticle solution for 12 h at 4 °C. Excess nanoparticles were washed by PBS containing 12.5 mM MgCl2 for 3 times.

DNA walker assembly and walking on the DNA origami. 10 nM starting stator of the DNA walker was added to the prepared DNA origami sample for incubation for 1 hour at 20 °C (each volume was 100 μl). Then excess oligos were removed by washing 6 times with PBS containing 12.5 mM $MgCl_2$ and 0.01% Tween 20. Then 10 nM target DNA was added to bind the starting staple for incubation for 1 hour at 20 °C. Excess oligos were removed by washing 6 times with PBS containing 12.5 mM $MgCl_2$ and 0.01% Tween 20. 20 nM (50 nM for 184-step DNA walker) track stator of DNA walker were added for incubation for 1.5 hour at 20 °C. Excess oligos were removed by washing 6 times with PBS containing 12.5 mM $MgCl_2$ and 0.01% Tween 20. 100 μl PBS containing 12.5 mM $MgCl_2$, 10 μl CutSmart® Buffer and 1 μl DNA nicking enzyme Nb.BtsI (both NEW ENGLAND Biolabs, Inc.) was added to the DNA origami sample for incubation for 2 hours at 26-30 °C. PBS containing 330 mM NaCl and 0.02% Paraformaldehyde was added to the sample to inactivate the nicking enzyme for 10 minutes. Excess paraformaldehyde was removed by washing with PBS containing 330 mM NaCl and 0.01% Tween 20 for 6 times. 5 nM imager in PBS containing 330 mM NaCl and 0.01% Tween 20 was added to the sample for incubation for 1 hour at 20 °C. Confocal measurements were performed after washing the sample with PBS containing 330 mM NaCl and 0.01% Tween 20 for 6 times. For confocal measurements with 5-step DNA walker on the rectangle DNA origami, 2 mM Trolox/Trolox quinone was added into the buffer to stabilize the fluorophore[5].

Confocal measurement and analysis. Pulsed Lasers (637 nm, 80 MHz, LDH-D-C-640; 532 80 mHz, LDH-P-FA-530B; both Picoquant) are alternated by an acousto-optical tunable filter (AOTFnc-VIS, AA optoelectronic). Circular polarized light was obtained after a fiber and a linear polarizer (LPVISE100-A, Thorlabs) and a quarter wave plate (AQWP05M- 600, Thorlabs). After passing a dual band dichroic beam splitter (z532/633, AHF), the light is focused by an oil-immersion objective (UPLSAPO 100XO, NA 1.40, Olympus). In the detection path a 50 μm pinhole (Linos) is used. A dichroic beam splitter (640DCXR, AHF) separates between the green (Brightline HC582/75, AHF; RazorEdge LP 532, Semrock) and red (Bandpass ET 700/75m, AHF; RazorEdge LP 647, Semrock) detection channel. Fluorescence was detected by APDs (τ-SPAD 100, Picoquant) and the signals were registered by a TCSPC-card (SPC-830, Becker&Hickl).

Spot finding algorithm. Each scan image has a 10 x 10 μm size with a pixel size of 50 x 50 nm. Each pixel has a total integration time of 2 ms (1 ms per color). We use a custom-made LabView software with a spot finding algorithm to analyze the scans. DNA origamis were marked with three green dyes. Therefore, the spot finding algorithm uses the green excitation green emission channel due to the homogeneous spot size compared to the red excitation red emission channel. To make the analysis as objective as possible we used the same parameters for each scan.

To define a spot, we used three different filters. The first one discriminates the pixels that we take into account. If a pixel has less or equal than 10 photons the algorithm does not take this pixel into account. The second filter discriminates the spot size. If an area of neighboring pixels is between 5 and 70 pixels we will use them for further analysis. This is the expected area size of our PSFs. A bigger area refers to two overlapping (PSF). The third parameter is the Heywood circular factor. Areas with a factor between 1.00 and 1.27 were taken into account. We use the last filter to get rid of PSFs which are cut in half because they are located at the edge of a scan. The remaining spots are analyzed. The program sums up the photons that are in range of a seven-pixel radius from the center of the spot for each channel. Red excitation, red emission was used to determine the intensity per spot.

Monte Carlo simulation. To model the DNA walker, we carried out Monte-Carlo simulations based on a custom written python script. The model is based on the origami sketch in figure 3a. For a given starting position and grid size it simulates the walking steps. For each position the program checks if the walker is in a dead end position. Dead end means that the walker has no neighboring stator where it can migrate because all neighboring stators were exhausted by the restriction enzyme before. For each position the walker has up to six neighbors to which it can walk. The walker cannot walk to positions which are blocked by biotin staples or staples with green dyes. We run 10,000 random walks and calculated the mean step number.

We also used the Monte-Carlo simulations to estimate the rate constant k of the walker. We therefore assumed that the rate constant is identical for a walking step to each of the up to six neighboring strands. The average lifetime of each step thus equals the inverse of the rate constant times the number of intact neighboring stators. The normalized integral of the sum of many simulations vs $1/k$ yields a graph (Figure S4b) of the shape of the kinetic data represented in Figure S5. Adapting k to fit the data yields the rate constants of the walker for the different walker sequences.

Supporting figures

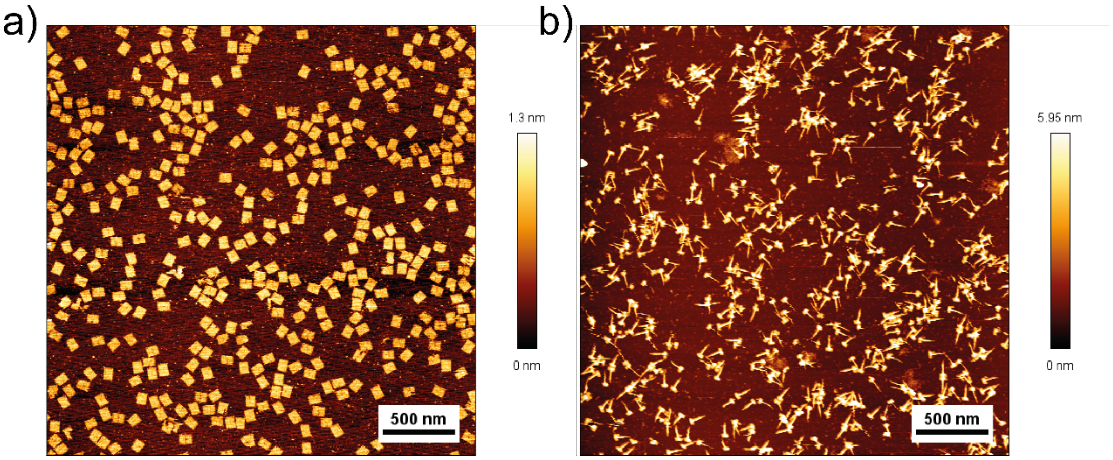

Figure S1. AFM images of rectangle DNA origami (a) and DNA origami pillar (b).

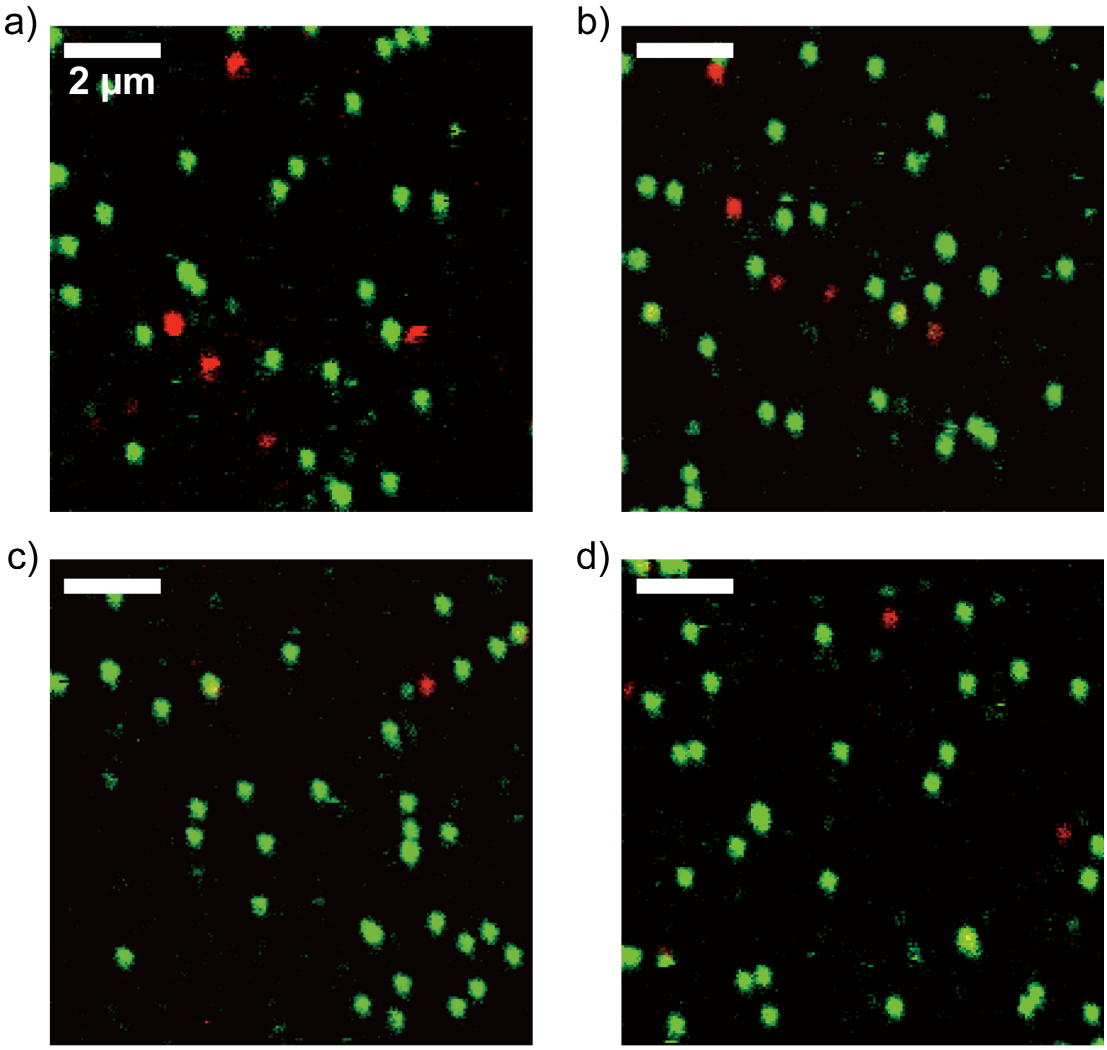

Figure S2. Confocal images from five-step DNA walker. a),b) Stators are separated by 6 nm in DNA walker. a) DNA walker without nicking enzyme, b) DNA walker without target DNA. c),d) DNA walker with 36 nm stator separation. c) DNA walker without nicking enzyme, d) DNA walker without target DNA.

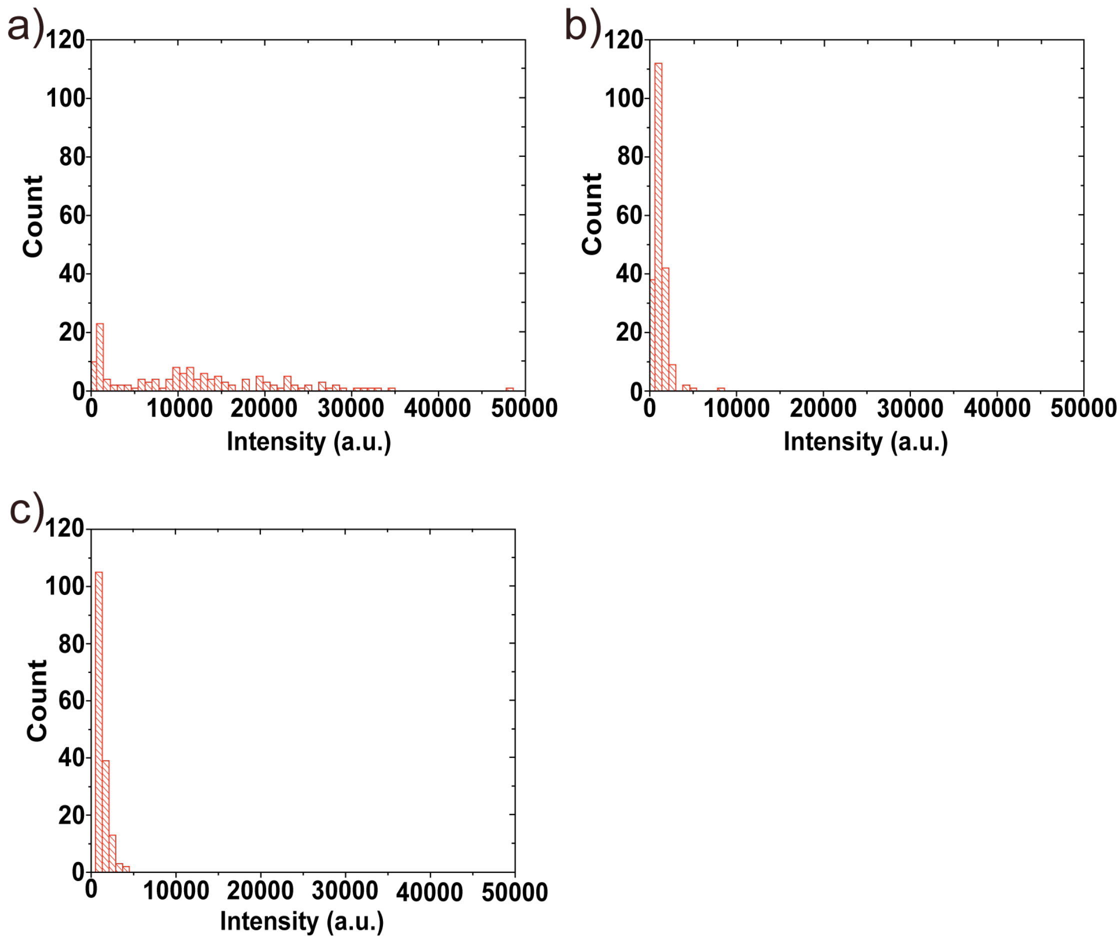

Figure S3. Intensity histograms of ATTO647N from DNA walker with 184 stators. a) DNA walker with target DNA and nicking enzyme. b) DNA walker without target DNA. c) DNA walker without nicking enzyme.

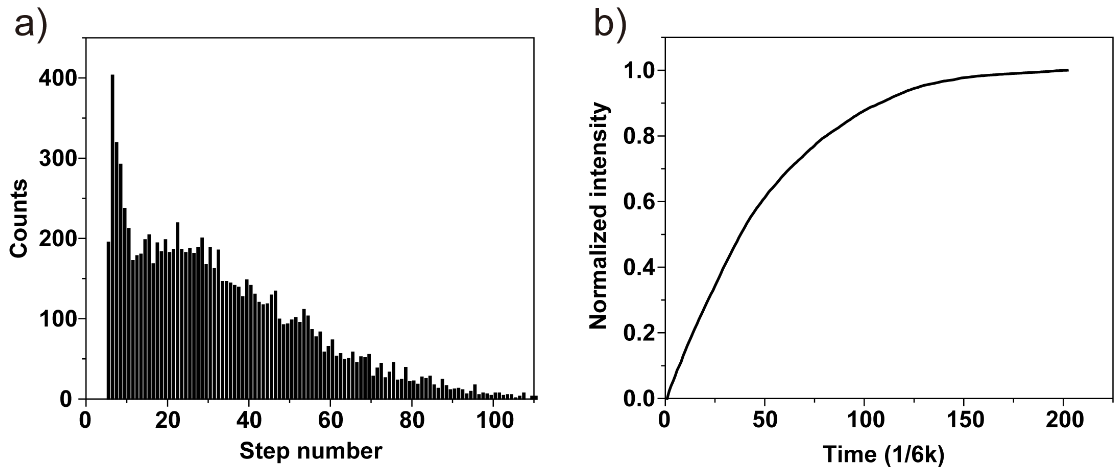

Figure S4: Simulations of DNA walker**.** a) Step number histogram obtained from 10,000 simulations. The peak around 6 steps is related to walkers stuck in the corner close to the position of the starting strand. The fact that this feature is not reproduced in the measurements (compare figure 3f) might indicate that the walker proceeds with some lower probability to strands slightly further away than the nearest neighbors, as discussed in ref.[6]. b) Plot of normalized intensity versus walking time from 10,000 simulations. The rate constant is assumed identical for a walking step to each of the up to six neighboring strands. The average lifetime of each step thus equals the inverse of the rate constant k times the number of intact neighboring stators. The walking time is the sum of lifetime of each step in units of $1/6k$ ($1/6k$ is used because the maximum rate per step is 6k when six intact stators are found around the walker). To determine the rate constant for walking, k is varied so that the graph of Figure S4b fits the experimental data of Figure S5.

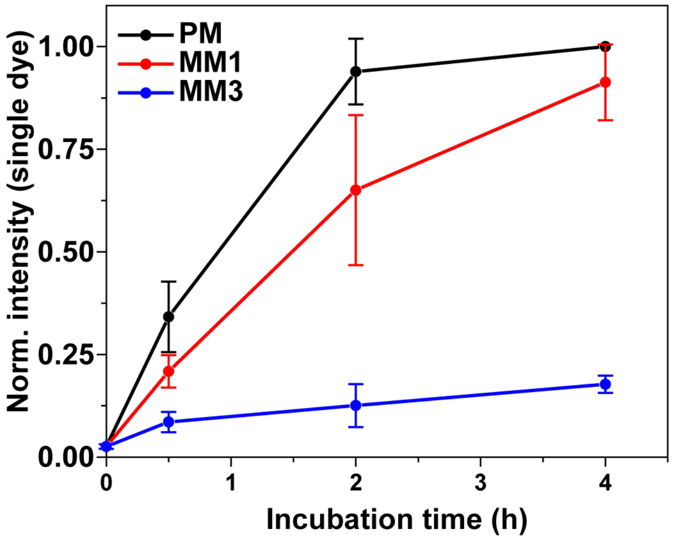

Figure S5. Normalized mean intensities of DNA origamis as a function of the time a DNA walker was exposed to active nicking enzyme (intensities normalized to the fluorescence intensity of the complementary target DNA after 4 hours). DNA walkers with perfect complementary target DNA (PM), one (MM1) and three mismatches (MM3) of nucleotides of target DNA were incubated with nicking enzyme, the reaction was stopped by adding 0.02% PFA after different incubation times. Error bars are standard deviations from three independent measurements. With the aid of simulations, we determined the rate constant for walking to be 0.0024 s^{-1} for PM,0.0013 s^{-1} (MM1) and smaller than 10^{-4} s^{-1} (MM3).

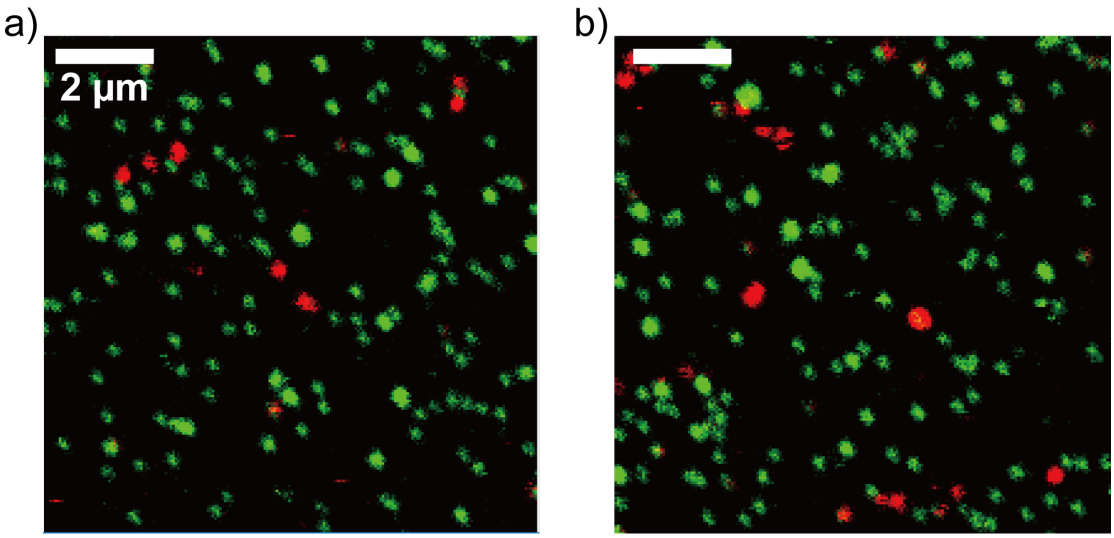

Figure S6. Confocal images of DNA walker without nicking enzyme (a) and without target DNA (b) in the plasmonic hotspot. Image size is 10×10 μm.

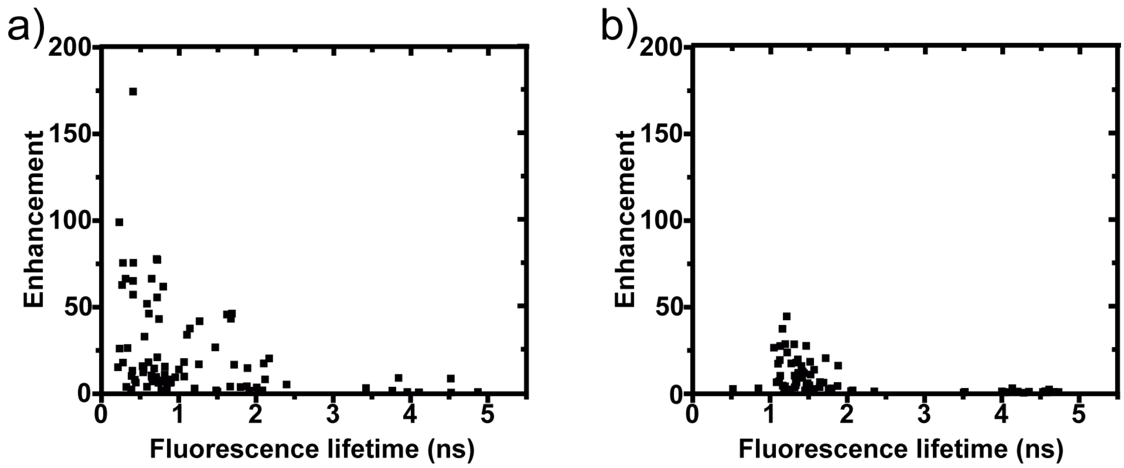

Figure S7. Reference measurements of the DNA walker in the hotspot of the optical antenna. The imager strands were directly hybridized to the 5 stators in the absence of quenchers in the hotspot. This means that up to five dye molecules can be present per antenna in the plasmonic hotspot. The co-localized spots were first selected after imaging and then excited with 640 nm to record fluorescence transients. The fluorescence transients with unquenched fluorescence lifetime and single bleaching step were chosen as reference for the calculation of fluorescence enhancement in a) and b). a) Fluorescence enhancement versus fluorescence lifetime plot for the nanoantenna with two binding sites for gold nanoparticles. Three populations can be assigned to antennas without nanoparticle (fluorescence lifetime $\tau > 3$ ns), antennas with one nanoparticle ($1 < \tau < 3$ ns) and antennas with two nanoparticles ($\tau < 1$ ns). This assignment is based on a reference measurement of an antenna that only offers one binding site for a nanoparticle (b)). For this "monomer" sample, the monomer population is almost exclusively found with fluorescence lifetime values between 1 ns and 2.5 ns.

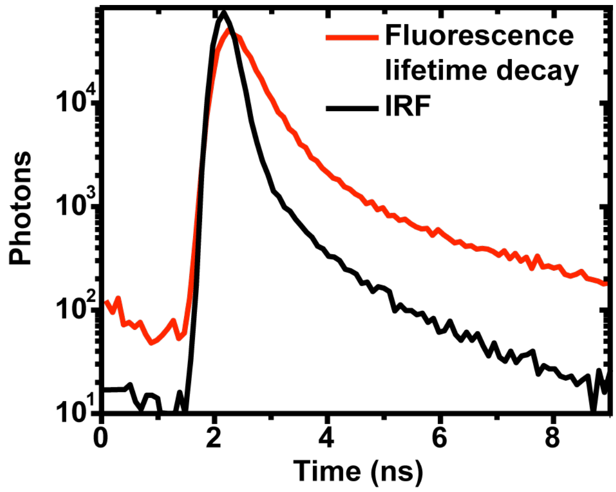

Figure S8. Fluorescence decays from the fluorescence transient in Figure 5d. Fluorescence lifetime is 0.44 ns after deconvolution the instrument response function (IRF) with FluoFit from PicoQuant (www.picoquant.com).

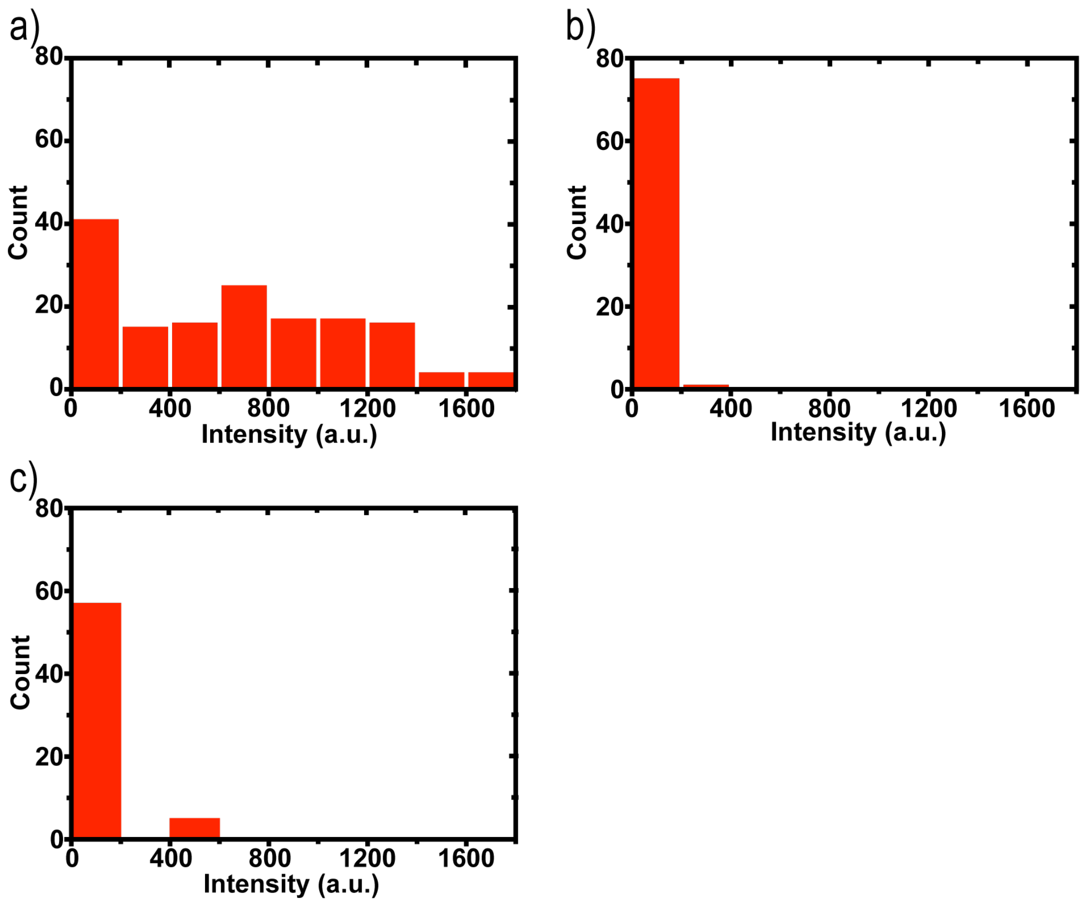

Figure S9. Intensity histograms of ATTO647N from DNA walker on the DNA origami nanopillar without gold nanoparticles. a) DNA walker with nicking enzyme and target DNA, b) DNA walker without nicking enzyme, c) DNA walker without target DNA.

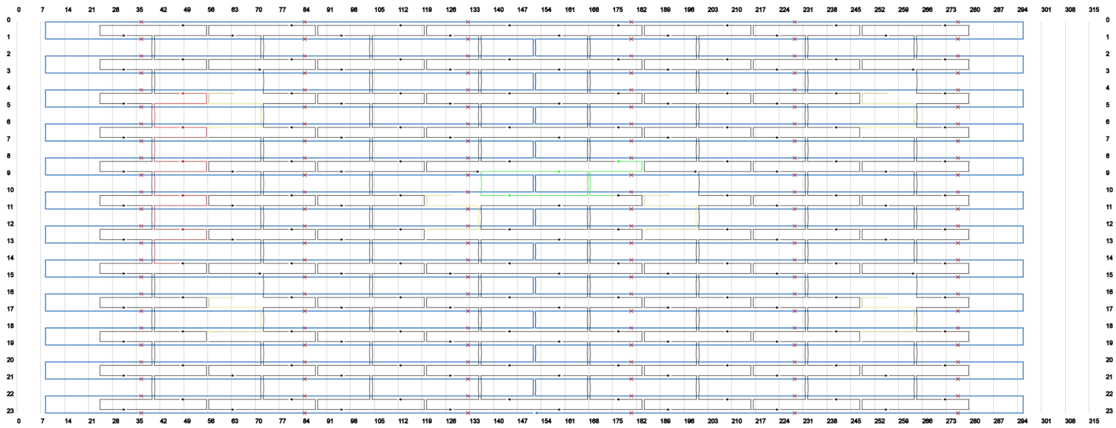

Figure S10. Scaffold/staple layout of rectangle DNA origami for 5-step DNA walker with 6 nm step length. Biotin modified strands were colored in yellow. Capture strands for ATTO532 labelled DNA were green colored. Capture strands for stators were red colored.

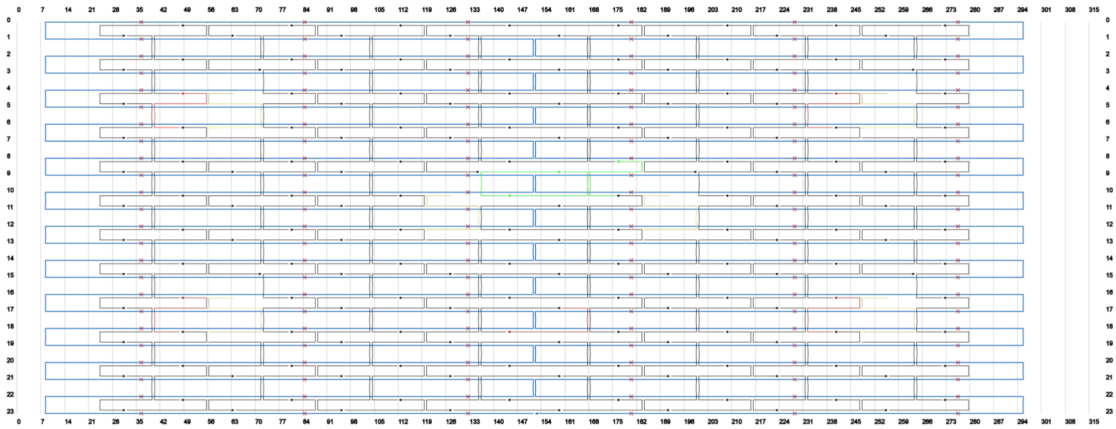

Figure S11. Scaffold/staple layout of rectangle DNA origami for 5-step DNA walker with 36 nm step length. Biotin modified strands were colored in yellow. Capture strands for ATTO532 labelled DNA were green colored. Capture strands for stators were red colored.

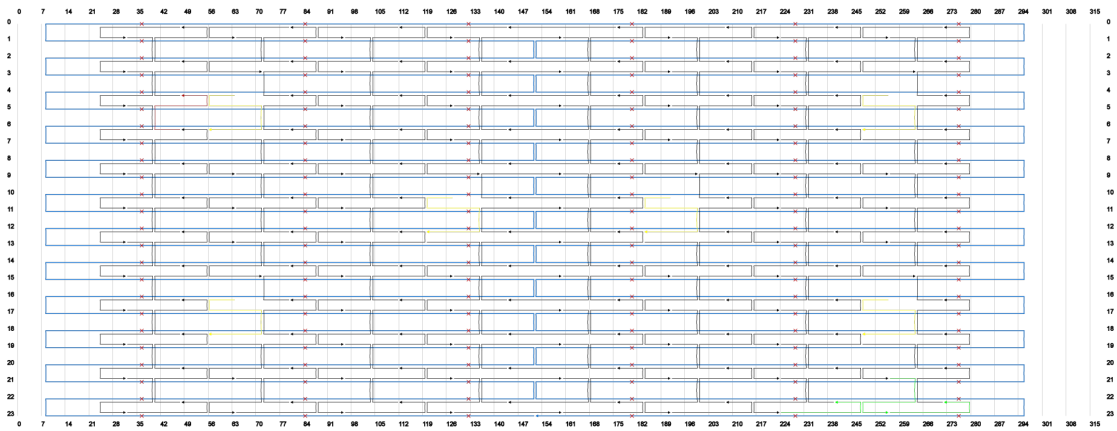

Figure S12. Scaffold/staple layout of rectangle DNA origami for 184-step DNA walker with 6 nm step length. Biotin modified strands were colored in yellow. Capture strands for ATTO550 labelled DNA were green colored. Capture strands for stators were colored in red.

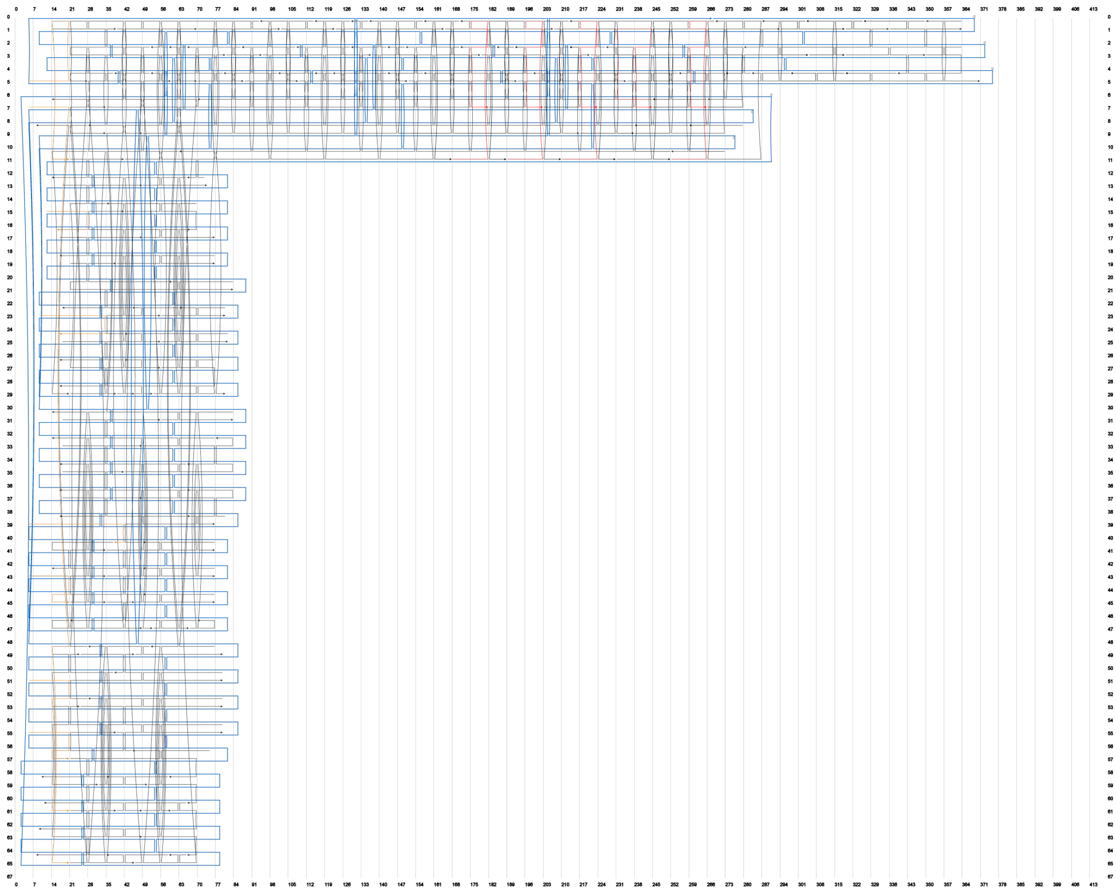

Figure S13. Scaffold/staple layout of nanopillar origami. Biotin modified strands were colored in yellow. Capture strands for stators were colored in red.

Tables for DNA sequences

Table S1. Modified target sequences for mismatch experiments

no mismatch	AGAATATAAAGCAGTGAAAATA
1 mismatch	AGAATATAAAGCAGTGAAACTA
2 mismatches	AGAATATAAAGCAGTGAATCTA
3 mismatches	AGAATATAAAGCAGTGATTCTA

Note: Nucleotides in green color represent recognition sequence of Nb.BtsI. Nucleotides in red color represent mismatches of target sequence.

Table S2. DNA sequence used for the DNA walker

Imager for 5-step DNA walker on rectangle DNA origami and nanopillar
GAGTTA GATGAAG ATAGCAGTGAAAATA-ATTO647N

Target DNA for 5-step DNA walker on rectangle DNA origami and nanopillar
GATGAAG ATAGCAGTGAAAATA
Starting stator for 5-step DNA walker on rectangle DNA origami and nanopillar
BBQ650-TATTTTCACTGCTATCTTCATCTAACTC CTACTACACTCACTT
Stator sequence for 5-step DNA walker on rectangle DNA origami and nanopillar
BBQ650-TATTTTCACTGCTATCTTCATCTAACTCCACAATTCAATACAA
ATTO532 labelled DNA sequence
GTGATGTAGGTGGTAGAGGAA-ATTO532
Starting stator sequence for 184-step DNA walker on rectangle DNA origami
BBQ650-TATTTTCACTGCTTTATATTCTTTCTTTACTTCACTCTCACTTCACTCT
Stator sequence for 184-step DNA walker on rectangle DNA origami
BBQ650-TATTTTCACTGCTTTATATTCTTTCTTTGTGATGTAGGTGGTAGAGGAA
Imager for 184-step DNA walker on rectangle DNA origami
AAAGAAAGAATATAAAGCAGTGAAAATA－ATTO647N
Target DNA for 184-step DNA walker on rectangle DNA origami
AGAATATAAAGCAGTGAAAATA
ATTO550 labelled DNA sequence
AAAAAAAAAAAAAAA-ATTO550

Note: Nucleotides in green color represent recognition sequence of Nb.BtsI.

Table S3. Staples of the rectangle origami for the 5-step DNA walker (5' to 3'end)

Sequence	Note
AGTATAAAGTTCAGCTAATGCAGATGTCTTTC	
CTTTAATGCGCGAACTGATAGCCCCACCAG	
TCCACAGACAGCCCTCATAGTTAGCGTAACGA	
GATGGTTTGAACGAGTAGTAAATTTACCATTA	
TTTACCCCAACATGTTTTAAATTTCCATAT	
ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG	
GTATAGCAAACAGTTAATGCCCAATCCTCA	
TTTTATTTAAGCAAATCAGATATTTTTTGT	
TCACCGACGCACCGTAATCAGTAGCAGAACCG	
AGAAAGGAACAACTAAAGGAATTCAAAAAAA	
GCCCGTATCCGGAATAGGTGTATCAGCCCAAT	
TCACCAGTACAAACTACAACGCCTAGTACCAG	
ATCCCAATGAGAATTAACTGAACAGTTACCAG	
CGATAGCATTGAGCCATTTGGGAACGTAGAAA	
TTCCAGTCGTAATCATGGTCATAAAAGGGG	
GATTTAGTCAATAAAGCCTCAGAGAACCCTCA	
CGCGCAGATTACCTTTTTTAATGGGAGAGACT	
TTAAAGCCAGAGCCGCCACCCTCGACAGAA	
TGTAGAAATCAAGATTAGTTGCTCTTACCA	
AAATTAAGTTGACCATTAGATACTTTTGCG TTTTGTATTGAATTGTG	Capture strand for stator strand
ATATTCGGAACCATCGCCCACGCAGAGAAGGA	

CTCGTATTAGAAATTGCGTAGATACAGTAC	
GCTATCAGAAATGCAATGCCTGAATTAGCA TTTTGTATTGAATTGTG	Capture strand for stator strand
TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG	
GTAATAAGTTAGGCAGAGGCATTTATGATATT	
GCCCTTCAGAGTCCACTATTAAAGGGTGCCGT	
TCATCGCCAACAAAGTACAACGGACGCCAGCA	
ATCCCCCTATACCACATTCAACTAGAAAAATC	
TTAACGTCTAACATAAAAACAGGTAACGGA TTCCTCTACCACCTACATCAC	Capture strand for atto532 labelled DNA
CATCAAGTAAAACGAACTAACGAGTTGAGA	
AGGAACCCATGTACCGTAACACTTGATATAA	
AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC	
TCAAATATAACCTCCGGCTTAGGTAACAATTT	
GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT	
AAAGCACTAAATCGGAACCCTAATCCAGTT	
CTACCATAGTTTGAGTAACATTTAAAATAT	
TGAAAGGAGCAAATGAAAAATCTAGAGATAGA	
GACCAACTAATGCCACTACGAAGGGGGTAGCA	
CGAAAGACTTTGATAAGAGGTCATATTTCGCA	
ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG	
CTTTTGCAGATAAAAACCAAAATAAAGACTCC	
CACCAGAAAGGTTGAGGCAGGTCATGAAAG	
TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA	Biotin modification on 5’
TCAAGTTTCATTAAAGGTGAATATAAAAGA	
CGGATTGCAGAGCTTAATTGCTGAAACGAGTA	
TGACAACTCGCTGAGGCTTGCATTATACCA	
CCTGATTGCAATATATGTGAGTGATCAATAGTTTTTGTATTGAATTGTG	Capture strand for stator strand (control experiment)
AATTGAGAATTCTGTCCAGACGACTAAACCAA	
TATTAAGAAGCGGGGTTTTGCTCGTAGCAT	
GTACCGCAATTCTAAGAACGCGAGTATTATTT	
AGGCTCCAGAGGCTTTGAGGACACGGGTAA	
ATTATCATTCAATATAATCCTGACAATTAC	
GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA	
TTTTCACTCAAAGGGCGAAAAACCATCACC	
AGCCAGCAATTGAGGAAGGTTATCATCATTTT	
TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC	
TAAATCAAAATAATTCGCGTCTCGGAAACC	
CATTTGAAGGCGAATTATTCATTTTTGTTTGG	
TCAATATCGAACCTCAAATATCAATTCCGAAA	
TAAGAGCAAATGTTTAGACTGGATAGGAAGCC	
CAAATCAAGTTTTTTGGGGTCGAAACGTGGA	
ATTACCTTTGAATAAGGCTTGCCCAAATCCGC	

CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGATTTTGTATTGAATTGTG	Capture strand for stator strand (control experiment)
CAGCAAAAGGAAACGTCACCAATGAGCCGC	
AACAAGAGGGATAAAAATTTTTAGCATAAAGC	
CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCG	
AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	Biotin modification on 5’
TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGA	
CTGAGCAAAAATTAATTACATTTTGGGTTA	
GTTTTAACTTAGTACCGCCACCCAGAGCCA	
GAATTTATTTAATGGTTTGAAATATTCTTACC	
TCGGCAAATCCTGTTTGATGGTGGACCCTCAA	
AAATCACCTTCCAGTAAGCGTCAGTAATAA	
ACCTTTTTATTTTAGTTAATTTCATAGGGCTT	
CTGTAGCTTGACTATTATAGTCAGTTCATTGA	
GTTTATCAATATGCGTTATACAAACCGACCGTGTGATAAA	
CAGAAGATTAGATAATACATTTGTCGACAA	
AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGT	
TTATACCACCAAATCAACGTAACGAACGAG TTAAGTGAGTGTAGTAG	Capture strand for stator strand
CCACCCTCATTTTCAGGGATAGCAACCGTACT	
TAAATCATATAACCTGTTTAGCTAACCTTTAA	
CCTAAATCAAAATCATAGGTCTAAACAGTA	
CCAATAGCTCATCGTAGGAATCATGGCATCAA	
CCCGATTTAGAGCTTGACGGGGAAAAAGAATA	
AACGTGGCGAGAAAGGAAGGGAAACCAGTAA	
ACAACATGCCAACGCTCAACAGTCTTCTGA	
AGAGAGAAAAAAATGAAAATAGCAAGCAAACT TTCCTCTACCACCTACATCAC	Capture strand for atto532 labelled DNA
AAGGAAACATAAAGGTGGCAACATTATCACCG	
TTAATGAACTAGAGGATCCCCGGGGGGTAACG	
ATTATACTAAGAAACCACCAGAAGTCAACAGT	
ACGCTAACACCCACAAGAATTGAAAATAGC	
CAACTGTTGCGCCATTCGCCATTCAAACATCA	
AGCGCGATGATAAATTGTGTCGTGACGAGA	
GCGGATAACCTATTATTCTGAAACAGACGATT	
TGGAACAACCGCCTGGCCCTGAGGCCCGCT	
TATAACTAACAAAGAACGCGAGAACGCCAA	
AACACCAAATTTCAACTTTAATCGTTTACC	
TTAGGATTGGCTGAGACTCCTCAATAACCGAT	
TTAGTATCACAATAGATAAGTCCACGAGCA	
ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGG	
ACACTCATCCATGTTACTTAGCCGAAAGCTGC	
CATGTAATAGAATATAAAGTACCAAGCCGT	
CATAAATCTTTGAATACCAAGTGTTAGAAC	

TAAATGAATTTTCTGTATGGGATTAATTTCTT	
AAACAGCTTTTTGCGGGATCGTCAACACTAAA	
AGGCAAAGGGAAGGGCGATCGGCAATTCCA	
GCCTTAAACCAATCAATAATCGGCACGCGCCT	
CACATTAAAATTGTTATCCGCTCATGCGGGCC	
ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	Biotin modification on 5’
GCCATCAAGCTCATTTTTTAACCACAAATCCA	
CAGCGAAACTTGCTTTCGAGGTGTTGCTAA	
GGCCTTGAAGAGCCACCACCCTCAGAAACCAT	
CCAACAGGAGCGAACCAGACCGGAGCCTTTAC TTCCTCTACCACCTACATCAC	Capture strand for atto532 labelled DNA
AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAA	
GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC	
TATATTTTGTCATTGCCTGAGAGTGGAAGATTGTATAAGC	
GAGGGTAGGATTCAAAAGGGTGAGACATCCAA	
GCGAAAAATCCCTTATAAATCAAGCCGGCG	
ATATTTTGGCTTTCATCAACATTATCCAGCCA	
AATGGTCAACAGGCAAGGCAAAGAGTAATGTG	
AACGCAAAGATAGCCGAACAAACCCTGAAC	
CTTATCATTCCCGACTTGCGGGAGCCTAATTT	
GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA	
GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC	Biotin modification on 5’
GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT	
ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA	
CTTTAGGGCCTGCAACAGTGCCAATACGTG	
AGAAAACAAAGAAGATGATGAAACAGGCTGCGTTTTGTATTGAATTGTG	Capture strand for stator strand (control experiment)
GACAAAAGGTAAAGTAATCGCCATATTTAACAAAACTTTT	
TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT	
CACAACAGGTGCCTAATGAGTGCCCAGCAG	
AACAGTTTTGTACCAAAAACATTTTATTTC	
ATACCCAACAGTATGTTAGCAAATTAGAGC	
GCGAGTAAAAATATTTAAATTGTTACAAAG	
TTCTACTACGCGAGCTGAAAAGGTTACCGCGC	
TTGACAGGCCACCACCAGAGCCGCGATTTGTA	
CGGATTCTGACGACAGTATCGGCCGCAAGGCGATTAAGTT	Biotin modification on 5’
ATTTTAAAATCAAAATTATTTGCACGGATTCG	
CTCCAACGCAGTGAGACGGGCAACCAGCTGCA	
TTTAGGACAAATGCTTTAAACAATCAGGTC	
CTTTTACAAAATCGTCGCTATTAGCGATAG	
GCGCAGACAAGAGGCAAAAGAATCCCTCAG	
AATAGTAAACACTATCATAACCCTCATTGTGA	
GAGAAGAGATAACCTTGCTTCTGTTCGGGAGAAACAATAA	Biotin modification on 5’

CAACCGTTTCAAATCACCATCAATTCGAGCCA	
GCAATTCACATATTCCTGATTATCAAAGTGTA	
TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA	
TAAATCGGGATTCCCAATTCTGCGATATAATG	
AAGGCCGCTGATACCGATAGTTGCGACGTTAG	
CGTAAAACAGAAATAAAAATCCTTTGCCCGAAAGATTAGA	
GATGTGCTTCAGGAAGATCGCACAATGTGA TTTTGTATTGAATTGTG	Capture strand for stator strand
AACGCAAAATCGATGAACGGTACCGGTTGA	
GAAATTATTGCCTTTAGCGTCAGACCGGAACCTTTTGTATTGAATTGTG	capture strand for stator strand (control experiment)
GCCGTCAAAAAACAGAGGTGAGGCCTATTAGT	
GATGGCTTATCAAAAAGATTAAGAGCGTCC TTTTGTATTGAATTGTG	Capture strand for stator strand
AATACTGCCCAAAAGGAATTACGTGGCTCA	
ACCGATTGTCGGCATTTTCGGTCATAATCA	
CCACCCTCTATTCACAAACAAATACCTGCCTA	
TACCGAGCTCGAATTCGGGAAACCTGTCGTGCAGCTGATT	
GCAAGGCCTCACCAGTAGCACCATGGGCTTGA	
TAATCAGCGGATTGACCGTAATCGTAACCG	
TTAACACCAGCACTAACAACTAATCGTTATTA	
TCATTCAGATGCGATTTTAAGAACAGGCATAG	
AAGTAAGCAGACACCACGGAATAATATTGACG	
CTTAGATTTAAGGCGTTAAATAAAGCCTGT	
TTATTACGAAGAACTGGCATGATTGCGAGAGG	
TACGTTAAAGTAATCTTGACAAGAACCGAACTTTAAGTGAGTGTAGTAG	Capture strand for stator strand (starting position)
GCGGAACATCTGAATAATGGAAGGTACAAAAT	
GTCGACTTCGGCCAACGCGCGGGGTTTTTC	
ACAACTTTCAACAGTTTCAGCGGATGTATCGG	
GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA	
ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT	
TGCATCTTTCCCAGTCACGACGGCCTGCAG	
ACAAACGGAAAAGCCCCAAAAACACTGGAGCA	
ATCGCAAGTATGTAAATGCTGATGATAGGAAC	
CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC	
AAAGTCACAAAATAAACAGCCAGCGTTTTA	
AAGCCTGGTACGAGCCGGAAGCATAGATGATG	
TGTAGCCATTAAAATTCGCATTAAATGCCGGA	
AATACGTTTGAAAGAGGACAGACTGACCTT	
AATAGCTATCAATAGAAAATTCAACATTCA	
GCACAGACAATATTTTTGAATGGGGTCAGTA	
GCGAACCTCCAAGAACGGGTATGACAATAA	
GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA	
TAGGTAAACTATTTTTGAGAGATCAAACGTTA	

TAAAAGGGACATTCTGGCCAACAAAGCATC	

Table S4. Staples of the rectangle DNA origami for 184-step DNA walker

Sequence	Note
AGTATAAAGTTCAGCTAATGCAGATGTCTTTCTTCCTCTACCACCTACATCAC	
CTTTAATGCGCGAACTGATAGCCCCACCAGTTTTTTTTTTTTTTT	Capture strand for ATTO550 labelled DNA
TCCACAGACAGCCCTCATAGTTAGCGTAACGATTCCTCTACCACCTACATCAC	
GATGGTTTGAACGAGTAGTAAATTTACCATTATTCCTCTACCACCTACATCAC	
TTTACCCCAACATGTTTTAAATTTCCATATTTCCTCTACCACCTACATCAC	
ACCCTTCTGACCTGAAAGCGTAAGACGCTGAGTTCCTCTACCACCTACATCAC	
GTATAGCAAACAGTTAATGCCCAATCCTCATTCCTCTACCACCTACATCAC	
TTTTATTTAAGCAAATCAGATATTTTTTGTTTCCTCTACCACCTACATCAC	
TCACCGACGCACCGTAATCAGTAGCAGAACCGTTCCTCTACCACCTACATCAC	
AGAAAGGAACAACTAAAGGAATTCAAAAAAATTCCTCTACCACCTACATCAC	
GCCCGTATCCGGAATAGGTGTATCAGCCCAATTTCCTCTACCACCTACATCAC	
TCACCAGTACAAACTACAACGCCTAGTACCAGTTCCTCTACCACCTACATCAC	
ATCCCAATGAGAATTAACTGAACAGTTACCAGTTCCTCTACCACCTACATCAC	
CGATAGCATTGAGCCATTTGGGAACGTAGAAATTCCTCTACCACCTACATCAC	
TTCCAGTCGTAATCATGGTCATAAAAGGGGTTCCTCTACCACCTACATCAC	
GATTTAGTCAATAAAGCCTCAGAGAACCCTCATTCCTCTACCACCTACATCAC	
CGCGCAGATTACCTTTTTTAATGGGAGAGACTTTCCTCTACCACCTACATCAC	
TTAAAGCCAGAGCCGCCACCCTCGACAGAATTCCTCTACCACCTACATCAC	
TGTAGAAATCAAGATTAGTTGCTCTTACCATTCCTCTACCACCTACATCAC	
AAATTAAGTTGACCATTAGATACTTTTGCGTTCCTCTACCACCTACATCAC	
ATATTCGGAACCATCGCCCACGCAGAGAAGGATTCCTCTACCACCTACATCAC	
CTCGTATTAGAAATTGCGTAGATACAGTACTTCCTCTACCACCTACATCAC	
GCTATCAGAAATGCAATGCCTGAATTAGCATTCCTCTACCACCTACATCAC	
TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCGTTCCTCTACCACCTACATCAC	
GTAATAAGTTAGGCAGAGGCATTTATGATATTTTCCTCTACCACCTACATCAC	
GCCCTTCAGAGTCCACTATTAAAGGGTGCCGTTTCCTCTACCACCTACATCAC	
TCATCGCCAACAAAGTACAACGGACGCCAGCATTCCTCTACCACCTACATCAC	
ATCCCCCTATACCACATTCAACTAGAAAAATCTTCCTCTACCACCTACATCAC	
TTAACGTCTAACATAAAAACAGGTAACGGATTCCTCTACCACCTACATCAC	
CATCAAGTAAAACGAACTAACGAGTTGAGATTCCTCTACCACCTACATCAC	
AGGAACCCATGTACCGTAACACTTGATATAATTCCTCTACCACCTACATCAC	
AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCCTTCCTCTACCACCTACATCAC	
TCAAATATAACCTCCGGCTTAGGTAACAATTTTTCCTCTACCACCTACATCAC	
GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTCCTCTACCACCTACATCAC	
AAAGCACTAAATCGGAACCCTAATCCAGTTTCCTCTACCACCTACATCAC	
CTACCATAGTTTGAGTAACATTTAAAATATTTCCTCTACCACCTACATCAC	
TGAAAGGAGCAAATGAAAAATCTAGAGATAGATTCCTCTACCACCTACATCAC	
GACCAACTAATGCCACTACGAAGGGGGTAGCATTCCTCTACCACCTACATCAC	

CGAAAGACTTTGATAAGAGGTCATATTTCGCATTCCTCTACCACCTACATCAC	
ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAGTTCCTCTACCACCTACATCAC	
CTTTTGCAGATAAAAACCAAAATAAAGACTCCTTCCTCTACCACCTACATCAC	
CACCAGAAAGGTTGAGGCAGGTCATGAAAGTTCCTCTACCACCTACATCAC	
TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA	Biotin modification on 5’
TCAAGTTTCATTAAAGGTGAATATAAAAGATTCCTCTACCACCTACATCAC	
CGGATTGCAGAGCTTAATTGCTGAAACGAGTATTCCTCTACCACCTACATCAC	
TGACAACTCGCTGAGGCTTGCATTATACCATTCCTCTACCACCTACATCAC	
CCTGATTGCAATATATGTGAGTGATCAATAGTTTCCTCTACCACCTACATCAC	
AATTGAGAATTCTGTCCAGACGACTAAACCAATTCCTCTACCACCTACATCAC	
TATTAAGAAGCGGGGTTTTGCTCGTAGCATTTCCTCTACCACCTACATCAC	
GTACCGCAATTCTAAGAACGCGAGTATTATTTTTCCTCTACCACCTACATCAC	
AGGCTCCAGAGGCTTTGAGGACACGGGTAATTCCTCTACCACCTACATCAC	
ATTATCATTCAATATAATCCTGACAATTACTTCCTCTACCACCTACATCAC	
GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAATTCCTCTACCACCTACATCAC	
TTTTCACTCAAAGGGCGAAAAACCATCACCTTCCTCTACCACCTACATCAC	
AGCCAGCAATTGAGGAAGGTTATCATCATTTTTTCCTCTACCACCTACATCAC	
TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCCTTCCTCTACCACCTACATCAC	
TAAATCAAAATAATTCGCGTCTCGGAAACCTTCCTCTACCACCTACATCAC	
CATTTGAAGGCGAATTATTCATTTTTGTTTGGTTCCTCTACCACCTACATCAC	
TCAATATCGAACCTCAAATATCAATTCCGAAATTCCTCTACCACCTACATCAC	
TAAGAGCAAATGTTTAGACTGGATAGGAAGCCTTCCTCTACCACCTACATCAC	
CAAATCAAGTTTTTTGGGGTCGAAACGTGGATTCCTCTACCACCTACATCAC	
ATTACCTTTGAATAAGGCTTGCCCAAATCCGCTTCCTCTACCACCTACATCAC	
CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGATTCCTCTACCACCTACATCAC	
CAGCAAAAGGAAACGTCACCAATGAGCCGCTTCCTCTACCACCTACATCAC	
AACAAGAGGGATAAAAATTTTTAGCATAAAGCTTCCTCTACCACCTACATCAC	
CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGTTCCTCTACCACCTACATCAC	
AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	Biotin modification on 5’
TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGATTCCTCTACCACCTACATCAC	
CTGAGCAAAAATTAATTACATTTTGGGTTATTCCTCTACCACCTACATCAC	
GTTTTAACTTAGTACCGCCACCCAGAGCCATTCCTCTACCACCTACATCAC	
GAATTTATTTAATGGTTTGAAATATTCTTACCTTCCTCTACCACCTACATCAC	
TCGGCAAATCCTGTTTGATGGTGGACCCTCAATTCCTCTACCACCTACATCAC	
AAATCACCTTCCAGTAAGCGTCAGTAATAATTCCTCTACCACCTACATCAC	
ACCTTTTTATTTTAGTTAATTTCATAGGGCTTTTCCTCTACCACCTACATCAC	
CTGTAGCTTGACTATTATAGTCAGTTCATTGATTCCTCTACCACCTACATCAC	
GTTTATCAATATGCGTTATACAAACCGACCGTGTGATAAATTCCTCTACCACCTACATCAC	
CAGAAGATTAGATAATACATTTGTCGACAATTCCTCTACCACCTACATCAC	
AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGTTTCCTCTACCACCTACATCAC	
TTATACCACCAAATCAACGTAACGAACGAGTTCCTCTACCACCTACATCAC	
CCACCCTCATTTTCAGGGATAGCAACCGTACTTTCCTCTACCACCTACATCAC	
TAAATCATATAACCTGTTTAGCTAACCTTTAATTCCTCTACCACCTACATCAC	

CCTAAATCAAAATCATAGGTCTAAACAGTATTCCTCTACCACCTACATCAC	
CCAATAGCTCATCGTAGGAATCATGGCATCAATTCCTCTACCACCTACATCAC	
CCCGATTTAGAGCTTGACGGGGAAAAAGAATATTCCTCTACCACCTACATCAC	
AACGTGGCGAGAAAGGAAGGGAAACCAGTAATTCCTCTACCACCTACATCAC	
ACAACATGCCAACGCTCAACAGTCTTCTGATTCCTCTACCACCTACATCAC	
AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTTCCTCTACCACCTACATCAC	
AAGGAAACATAAAGGTGGCAACATTATCACCGTTCCTCTACCACCTACATCAC	
TTAATGAACTAGAGGATCCCCGGGGGGTAACGTTCCTCTACCACCTACATCAC	
ATTATACTAAGAAACCACCAGAAGTCAACAGTTTCCTCTACCACCTACATCAC	
ACGCTAACACCCACAAGAATTGAAAATAGCTTCCTCTACCACCTACATCAC	
CAACTGTTGCGCCATTCGCCATTCAAACATCATTCCTCTACCACCTACATCAC	
AGCGCGATGATAAATTGTGTCGTGACGAGATTCCTCTACCACCTACATCAC	
GCGGATAACCTATTATTCTGAAACAGACGATTTTCCTCTACCACCTACATCAC	
TGGAACAACCGCCTGGCCCTGAGGCCCGCTTTCCTCTACCACCTACATCAC	
TATAACTAACAAAGAACGCGAGAACGCCAATTCCTCTACCACCTACATCAC	
AACACCAAATTTCAACTTTAATCGTTTACCTTCCTCTACCACCTACATCAC	
TTAGGATTGGCTGAGACTCCTCAATAACCGATTTCCTCTACCACCTACATCAC	
TTAGTATCACAATAGATAAGTCCACGAGCATTCCTCTACCACCTACATCAC	
ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGGTTCCTCTACCACCTACATCAC	
ACACTCATCCATGTTACTTAGCCGAAAGCTGCTTCCTCTACCACCTACATCAC	
CATGTAATAGAATATAAAGTACCAAGCCGTTTCCTCTACCACCTACATCAC	
CATAAATCTTTGAATACCAAGTGTTAGAACTTCCTCTACCACCTACATCAC	
TAAATGAATTTTCTGTATGGGATTAATTTCTTTTCCTCTACCACCTACATCAC	
AAACAGCTTTTTGCGGGATCGTCAACACTAAATTCCTCTACCACCTACATCAC	
AGGCAAAGGGAAGGGCGATCGGCAATTCCATTCCTCTACCACCTACATCAC	
GCCTTAAACCAATCAATAATCGGCACGCGCCTTTCCTCTACCACCTACATCAC	
CACATTAAAATTGTTATCCGCTCATGCGGGCCTTCCTCTACCACCTACATCAC	
ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	Biotin modification on 5’
GCCATCAAGCTCATTTTTTAACCACAAATCCATTCCTCTACCACCTACATCAC	
CAGCGAAACTTGCTTTCGAGGTGTTGCTAATTCCTCTACCACCTACATCAC	
GGCCTTGAAGAGCCACCACCCTCAGAAACCATTTCCTCTACCACCTACATCAC	
CCAACAGGAGCGAACCAGACCGGAGCCTTTACTTCCTCTACCACCTACATCAC	
AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAATTCCTCTACCACCTACATCAC	
GCTTTCCGATTACGCCAGCTGGCGGCTGTTTCTTCCTCTACCACCTACATCAC	
TATATTTTGTCATTGCCTGAGAGTGGAAGATTGTATAAGCTTCCTCTACCACCTACATCAC	
GAGGGTAGGATTCAAAAGGGTGAGACATCCAATTCCTCTACCACCTACATCAC	
GCGAAAAATCCCTTATAAATCAAGCCGGCGTTCCTCTACCACCTACATCAC	
ATATTTTGGCTTTCATCAACATTATCCAGCCATTCCTCTACCACCTACATCAC	
AATGGTCAACAGGCAAGGCAAAGAGTAATGTGTTCCTCTACCACCTACATCAC	
AACGCAAAGATAGCCGAACAAACCCTGAACTTCCTCTACCACCTACATCAC	
CTTATCATTCCCGACTTGCGGGAGCCTAATTTTTCCTCTACCACCTACATCAC	
GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCATTCCTCTACCACCTACATCAC	
GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC	Biotin modification on 5’

GCCCGAGAGTCCACGCTGGTTTGCAGCTAACTTTCCTCTACCACCTACATCAC	
ACCTTGCTTGGTCAGTTGGCAAAGAGCGGATTCCTCTACCACCTACATCAC	
CTTTAGGGCCTGCAACAGTGCCAATACGTGTTCCTCTACCACCTACATCAC	
AGAAAACAAAGAAGATGATGAAACAGGCTGCGTTCCTCTACCACCTACATCAC	
GACAAAAGGTAAAGTAATCGCCATATTTAACAAAACTTTTTTCCTCTACCACCTACATCAC	
TTGCTCCTTTCAAATATCGCGTTTGAGGGGGTTTCCTCTACCACCTACATCAC	
CACAACAGGTGCCTAATGAGTGCCCAGCAGTTCCTCTACCACCTACATCAC	
AACAGTTTTGTACCAAAAACATTTTATTTCTTCCTCTACCACCTACATCAC	
ATACCCAACAGTATGTTAGCAAATTAGAGCTTCCTCTACCACCTACATCAC	
GCGAGTAAAAATATTTAAATTGTTACAAAGTTCCTCTACCACCTACATCAC	
TTCTACTACGCGAGCTGAAAAGGTTACCGCGCTTCCTCTACCACCTACATCAC	
TTGACAGGCCACCACCAGAGCCGCGATTTGTATTCCTCTACCACCTACATCAC	
CGGATTCTGACGACAGTATCGGCCGCAAGGCGATTAAGTT	Biotin modification on 5’
ATTTTAAAATCAAAATTATTTGCACGGATTCGTTCCTCTACCACCTACATCAC	
CTCCAACGCAGTGAGACGGGCAACCAGCTGCATTCCTCTACCACCTACATCAC	
TTTAGGACAAATGCTTTAAACAATCAGGTCTTCCTCTACCACCTACATCAC	
CTTTTACAAAATCGTCGCTATTAGCGATAGTTCCTCTACCACCTACATCAC	
GCGCAGACAAGAGGCAAAAGAATCCCTCAGTTCCTCTACCACCTACATCAC	
AATAGTAAACACTATCATAACCCTCATTGTGATTCCTCTACCACCTACATCAC	
GAGAAGAGATAACCTTGCTTCTGTTCGGGAGAAACAATAA	Biotin modification on 5’
CAACCGTTTCAAATCACCATCAATTCGAGCCATTCCTCTACCACCTACATCAC	
GCAATTCACATATTCCTGATTATCAAAGTGTATTCCTCTACCACCTACATCAC	
TCTAAAGTTTTGTCGTCTTTCCAGCCGACAATTCCTCTACCACCTACATCAC	
TAAATCGGGATTCCCAATTCTGCGATATAATGTTCCTCTACCACCTACATCAC	
AAGGCCGCTGATACCGATAGTTGCGACGTTAGTTCCTCTACCACCTACATCAC	
CGTAAAACAGAAATAAAAATCCTTTGCCCGAAAGATTAGATTCCTCTACCACCTACATCAC	
GATGTGCTTCAGGAAGATCGCACAATGTGATTCCTCTACCACCTACATCAC	
AACGCAAAATCGATGAACGGTACCGGTTGATTCCTCTACCACCTACATCAC	
GAAATTATTGCCTTTAGCGTCAGACCGGAACCTTCCTCTACCACCTACATCAC	
GCCGTCAAAAAACAGAGGTGAGGCCTATTAGTTTTTTTTTTTTTTTT	Capture strand for ATTO550 labelled DNA
GATGGCTTATCAAAAAGATTAAGAGCGTCCTTCCTCTACCACCTACATCAC	
AATACTGCCCAAAAGGAATTACGTGGCTCATTCCTCTACCACCTACATCAC	
ACCGATTGTCGGCATTTTCGGTCATAATCATTCCTCTACCACCTACATCAC	
CCACCCTCTATTCACAAACAAATACCTGCCTATTCCTCTACCACCTACATCAC	
TACCGAGCTCGAATTCGGGAAACCTGTCGTGCAGCTGATTTTCCTCTACCACCTACATCAC	
GCAAGGCCTCACCAGTAGCACCATGGGCTTGATTCCTCTACCACCTACATCAC	
TAATCAGCGGATTGACCGTAATCGTAACCGTTCCTCTACCACCTACATCAC	
TTAACACCAGCACTAACAACTAATCGTTATTATTCCTCTACCACCTACATCAC	
TCATTCAGATGCGATTTTAAGAACAGGCATAGTTCCTCTACCACCTACATCAC	
AAGTAAGCAGACACCACGGAATAATATTGACGTTCCTCTACCACCTACATCAC	
CTTAGATTTAAGGCGTTAAATAAAGCCTGTTTCCTCTACCACCTACATCAC	
TTATTACGAAGAACTGGCATGATTGCGAGAGGTTCCTCTACCACCTACATCAC	

TACGTTAAAGTAATCTTGACAAGAACCGAACT AGAGTGAAGTGAGAGTGAAGT	Capture strand for stator strand (starting position)
GCGGAACATCTGAATAATGGAAGGTACAAAATTTCCTCTACCACCTACATCAC	
GTCGACTTCGGCCAACGCGCGGGGTTTTTCTTCCTCTACCACCTACATCAC	
ACAACTTTCAACAGTTTCAGCGGATGTATCGGTTCCTCTACCACCTACATCAC	
GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATTCCTCTACCACCTACATCAC	
ACGGCTACAAAAGGAGCCTTTAATGTGAGAATTTCCTCTACCACCTACATCAC	
TGCATCTTTCCCAGTCACGACGGCCTGCAGTTCCTCTACCACCTACATCAC	
ACAAACGGAAAAGCCCCAAAAACACTGGAGCATTCCTCTACCACCTACATCAC	
ATCGCAAGTATGTAAATGCTGATGATAGGAACTTCCTCTACCACCTACATCAC	
CTGTGTGATTGCGTTGCGCTCACTAGAGTTGCTTCCTCTACCACCTACATCAC	
AAAGTCACAAAATAAACAGCCAGCGTTTTATTCCTCTACCACCTACATCAC	
AAGCCTGGTACGAGCCGGAAGCATAGATGATGTTCCTCTACCACCTACATCAC	
TGTAGCCATTAAAATTCGCATTAAATGCCGGATTCCTCTACCACCTACATCAC	
AATACGTTTGAAAGAGGACAGACTGACCTTTTCCTCTACCACCTACATCAC	
AATAGCTATCAATAGAAAATTCAACATTCATTCCTCTACCACCTACATCAC	
GCACAGACAATATTTTTGAATGGGGTCAGTATTTTTTTTTTTTTT	Capture strand for ATTO550 labelled DNA
GCGAACCTCCAAGAACGGGTATGACAATAATTCCTCTACCACCTACATCAC	
GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAATTCCTCTACCACCTACATCAC	
TAGGTAAACTATTTTTGAGAGATCAAACGTTATTCCTCTACCACCTACATCAC	
TAAAAGGGACATTCTGGCCAACAAAGCATCTTCCTCTACCACCTACATCAC	

Table S5. Staples of the DNA origami pillar

Sequence	Note
TGCTAAATCGGGGAGCCCCCGATTTAGAGCTAGCAGAACATT	
TACGGCTGGAGGTGCGCACTCGTCACTGTTTGCTCCCGGCAAAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
CGTACAGGCCCCCTAACCGTCCCCGGGTACCGAGCGTTC	
ATTTGGAAGTTTCATGCCTCAACATGTTTTA	
ATTTCAACCAAAAATTCTACTAATAGTTAGTTTCATTTGGGGCGCGAGC	
CTAAATCGGTCAGAATTAGCAAAATTAAGCAATAAAATAATA	
ACCGCCACCCTCAGAACCCGTACTCTAGGGA	
AGGAATCATTACCGCGTTTTTATAAGTACC	Biotin modification on 5’
TATGACTTTATACATTTTTTTTTAATGGAAACAGTACACCGT	
CGCGCTACAGAGTAATAAAAGGGACATTCTGATAGAACTTAG	
CCTAATTTAACAAACCCTCAATCAATATCTGATTCGCTAATC	
AAATCAGCTCATTTTTTAACCATTTTGTTAAAATTCGCATTA	
TTAGCCCTGACGAGAAACACCAGAAATTGGGGTGAATTATTTTAA	
TGCCCGTATAAACAGTGTGCCTTCTGGTAA	Biotin modification on 5’
GAATTCGTCTCGTCGCTGGGTCTGCAATCCATTGCAACACGG	

GAGAGATAGACTTTACGGCATCAGATGCGTGTTCAGGTTGTG TTGTGGTGTGGTGTGTG	Capture strand for stator strand
TTGGTAGAACATTTAATTAAGCAAC	
TTACCATTAGCAAGGCCTTGAATTAGAGCCAGCCCGACTTGAGC	
TGGCTTTTTACCGTAGAATGGAAAGCG	
ACGCGAGAGAAGGCCATGTAATTTAGGCCAGGCTTAATTGAGAATCGC	
GGCCAACGCGCGGGGAGGGCCCTGTGTTTGA	
AGCAACAAAGTCAGAAATAATATCCAATAATCGGCTCAGGGA	
CCCAGCTACAATGACAGCATTTGAGGCAAGTTGAGAAATGAA	
AATAGAAAAAAATAAACGTCTGAGAGGAATATAAGAGCAACACTATGAT	
CGTAAAAAAAGCCGTGGTGCTCATACCGGCGTCCG	
AGTACCGCATTCCACAACATGTTCAGCCTTAAGGTAAAGTAATTC	
ATTGTTATCTGAGAAGAAACCAGGCAAAGCGCCATTCGTAGA	
CAGCAGCGCCGCTTGTTTATCAGCTTCACGAAAAA	
TATTACGAATAATAAACAAATCAGATATGCGT	
GCTGGTCTGGTCAGGAGCCGGAATCCGCCGTGAACAGTGCCAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
AATACCCCAACATTCATCAAAAATAATTCGCGTCT	
TTGGGCGGCTGATTTCGGCAAAATCCCT	
ATTAGCGGGGTTTTGCTCAGTACCAGGCTGACAACAAGCTG	Biotin modification on 5’
GAGAAGGCATCTGCAATGGGATAGGTCAAAAC	
AAGAAAGCGCTGAACCTCAAATATTCTAAAGGAAAGCGTTCA	
AGACAGCAGAAACGAAAGAGGAAATAAATCGAGGTGACAGTTAAATT	ATTO542 modification on 3’
TTATAAGGGTATGGAATAATTCATCAATATA	
GGAACCATACAGGCAAGGCAAATCAAAAAGACGTAGTAGCAT	
ATTTCCTGATTATCAGATGATGGCTTTAAAAAGACGCTAAAA	
ATTACGAGATAAATGCCAGCTTTGAGGGGACGACGACAG	
GCTGTAGTTAGAGCTTAATTG	
TAAAGCCTCCAGTACCTCATAGTTAGCG	
TAAGTTTACACTGAGTTTCGT	
TGAAAATCCGGTCAATAACCTAAATTTTAGCCTTT	
CAAATTATTCATTTCAATTACCTGAGTA	
GGCGCAGACGGTCAATCATCGAGACCTGCTCCATGTGGT	
TGACCGCGCCTTAATTTACAATATTTTTGAATGGCTATCACACCCCGCTAGGGCAACAGCTGGCGA	
CTGGCATTAGGAGAATAAAATGAAGAAACGATTTTTTGAGTA	
ATCGATGCTGAGAGTCTACAAGGAGAGGGAACGCCAAAAGGA	
TTTAGCGATACCAACGCGTTA	
TGCATTAATGAGCGGTCCACGCTCACTGCGCCACGTGCCAGC	
AGTAGGTATATGCGTTATACA	
TTAACTCGGAATTAGAGTAAATCAATATATGTGAGTGATTCT	
AATTTCTTAAACCCGCTTAATTGTATCGTTGCGGGCGATATA	
GCGAATCAGTGAGGCCACCGAGTAGTAGCAACTGAGAGTTGA	

TCACAGCGTACTCCGTGGTGAAGGGATAGCTAAGAGACGAGG	
CATTTCGCAAATGTCATCTGCGAACGAGAGATTCACAATGCC	
CATTTGAGATAACCCACGAAACAATG	
CAAAATCACCGGAACCAGAGCCAGATTTTGTCACAATCACAC	
AATATGCAACTACCATCATAGACCGGAACCGC	
CGAGGGTACTTTTTCATGAACGGGGTCATAATGCCGAGCCACCACC	
TTTTTGCGGATGCTCCTAAAATGTTTAGATGAATTTTGCAAAAGAAGTT	
ACGGGCCGATAATCCTGAGAAGTGTTTTTATGGAGCTAACCG	
ACCAGACCGGATTAATTCGAGC	
AGAAAACGAGAATGACCATAAATCTACGCCCCTCAAATGCTTTA	Biotin modification on 5’
GAGCATTTATCCTGAATCAAACGTGACTCCT	
GCATGTAGAAACCAATCCATCCTAGTCCTG	Biotin modification on 5’
AGAAATCGTTAGACTACCTTTTTAAGGCGTTCTGACCTTTTTGCA	
ATTGCGTTGCTGTTATCCGCTCACAATTCCAAACTCACTTGCGTA	
AAGGCTCCAAAAGGAGCCTTTATATTTTTTCACGTGCTACAGTCACCCT	
CCTGCGCTGGGTGGCGAGAAAGGAAGGGAAGGAGCGGGGCCG	
ATAAAGTCTTTCCTTATCACT	
TGAGTAAAGGATAAGTTTAGCTATATCATAGACCATTAGATA	
AAGAAAGCTTGATACCGCCACGCATACAGACCAGGCGCTGAC	
AGTTTATTGTCCATATAACAGTTGATTC	
ATGAAGGGTAAAGTTCACGGTGCGGCCATGCCGGTCGCCATG	
GGCTAAAACTTCAGAAAAGTTTTGCGGGAGATAGAACC	
ACCTGACGGGGAAAGCCGGCGAACCAAGTGTCTGCGCGTTGC	
AGAGAAAATCCAGAGAGTTGCAGCAAATC	
ATCGGTCAGATGATATTCACAAACCAAAAGA	
TAAAACCGTTAAAGAGTCTGTCCATCCAGAAACCACACAATC	
GTCGCGTGCCTTCGAATTGTCAAAG	
ACATAAGTAGAAAAATCAAGAAGCAAAAGAAGATGTCAT	
TTTAGATTCACCAGTCACACGACCGGCGCGTGCTTTCCCAGA	
GGTTTTCCCAGTCATGGGGTCGCAGAAAAACTTAAATTTGCC TTGTGGTGTGGTGTGTG	Capture strand for stator strand
AGCTCTTACCGAAGCCCAATA	
GAACCGCCACCCTCCATATCATACC	
CATCGAGATAACGTCAAACATAAAAGAGCAAAAGAATT	
ACCAACAAACCAAAATTAACAATTTCATTTGAATTACCGAGG	
TTTCCATGGCACCAACCTACGTCATACA	
AGGCTTGCGAGACTCCTCAAGAGAAAAGTATTCGGAAC	
ACTAAAGAGCAACGTGAAAATCTCCACCCACAACTAAAGGAA	
CCGTGTGATAAATAACCTCCGGCTGATG	
ACAACGCCTGTAGCATTTACCGTATAGGAAG	
CTAGTCAGTTGGCAAATCAACAGTCTTTAGGTAGATAACAAA	
ACGTAAGAATTCGTTCTTAGAAGAACTCAAACTATCGGATAA	
GTAAAACGACGGCCCATCACCCAAATCAGCGC	

CTCATCGGGATTGAGTGAGCGAGTAACAACCCGTC	
AATAAAACGAACTATGACCCCACCAAGC	
TCATACATTTAATACCGATAGCCCTAAAACATCGAACGTAACGGCGAAGCACCGTAATAACGCCAG	
AAGGGGGATGTGCTTATTAACAACAGGAAGCACGTCCTTGCT TTGTGGTGTGGTGTGTG	Capture strand for stator strand
TAAGTTGGCATGATTAAAGAA	
CGGAATAGAAAGGAATGCCTTGCTAAACAACTTTCAAC	
CGCGCCGCCACCAGAACAGAGCCATAAAGGTGGAA	
CCAGCCTCCGATCCTCATGCCGGA	
CACGGCAACAATCCTGATATACTT	
GCCCGAGTACGAGCCGGAAGC	
AACAAGAGCCTAATGCAGAACGCGC	
GTTAAAGGAAAGACAGCATCTGCCTATTTAAGAGGCAGGAGGTTTA	
TTCGGTCCCATCGCATAGTTGCGCCGACATGCTTTCGAGGTG	
CCTCGTCTTTCCACCACCGGAACCGCCTCCCTCA	
TGCTGATTGCCGTTGTCATAAACATCGGGCGG	
AAGGCCTGTTTAGTATCATGTTAGCTACCTC	
AGGGAGCCGCCACGGGAACGGATAGGCGAAAGCATCAGCACTCTG	
CTGTATGGGATTACCGTTAGTATCA	
AAATGCGGAAACATCGGTTTTCAGGTTTAACGTCAGATTAAC	
TGAGCAAATTTATACAGGAATAACATCACTTGCCTGAGTCTT	
GAACTGGCTCATTACAACTTTAATCATTCTTGAGATTACTTA	
TGCCATCCCACGCAGGCAGTTCCTCATTGCCGTTTTAAACGAAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
AGTTTCCAACATTATTACATTATAC	
TGAGTGTTCCGAAAGCCCTTCACCGCCTAGGCGGTATTA	
AGTCGCCTGATACTTGCATAACAGAATACGTGGCACAGCTGA	
TTGCGAATAATATTTACAGCGGAGTGAGGTAAAATTTTGAGG	
TTCATCGGCATTTTCGGTCATATCAAAA	
CCTTAAATCAAGATTAGCGGGAGGCTCAAC	Biotin modification on 5’
TAGCCTCAGAGCATACCCTGT	
TTTTCCAGCATCAGCGGGGCTAAAGAACCTCGTAGCACGCCA	
AAGGGATATTCATTACCGTAATCTATAGGCT	
GCGAAACAAAGTGTAAAACACATGGCCTCGATTGAACCA	
CCTCGTTTACCAGAAACCAAA	
ATAACTATATGTAAATGCTTAGGATATAAT	Biotin modification on 5’
GAGAACAATATACAAAATCGCGCAGAGGCGATTCGACAAATCCTTTAAC	
CTTGTAGAACGTCAGCGGCTGATTGCAGAGTTTTTCGACGTTAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
GACAATTACGCAGAGGCATTTTCGAG	
GAGTCTGGATTTGTTATAATTACTACATACACCAC	
TATTGAAAGGAATTGAGGTAG	
GGATGTGGTTTGCCCCAGCAG	

GAGGCCAAGCTTTGAATACCAAGTACGGATTACCTTTTCAAA	
CCCGGTTGATAAAGCATGTCAATC	
GCCAGCAGTTGGGCGCAAATCAGGTTTCTTGCCCTGCGTGGTAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
CCAATGTTTAAGTACGGTGTCCAAC	
TAGCCCGGAATAGGTGTAAGGATAAGTGCCGTCGA	
TTAGTTTGAGTGCCCGAGAAATAAAGAAATTGCGTAGAGATA	
AATATTCATTGAATCCATGCTGGATAGCGTCCAAT	
ACGCGGTCCGTTTTTGGGTAAGTGA	
CTGAATATAGAACCAAATTATTTGCACGTAAAACAACGT	
GCTGGCATAGCCACATTATTC	
CGTACTATGGTAACCACTAGTCTTTAATGCGCGAACTGAATCACGAGCGGCGCGGTCAGGCAAGGC	
ACTAATGCCACTACGAATAAA	
AAACTCACAGGAACGGTACGCCAGTAAAGGGGGTGAGGAACC	
CGTGTCAAATCACCATCTAGGTAATAGATTT	
GGCAACACCAGGGTCTAATGAGTGAGCTCACAACAATAGGGT	
CAAACGGAATAGGAAACCGAGGAATAAGAAATTACAAG	
CCTCATCACCCCAGCAGGCCTCTTCGCTATTACGCCAGTGCC	
TCGTGCCGGAGTCAATAGTGAATTTGCAGAT	
TACATCGACATAAAGCCCTTACACTGGTCGGGTTAAATTTGTAAAAAAAAAAAAAAA	Capture strand for gold nanoparticle
CAAAGCACTAGATAGCTCCATTCAGGCTGCGCAACTGTCTTG	
AAGACAAATCAGCTGCTCATTCAGTCTGACCA	
TGGTGGTTGTTCCAGTTTGGAACA	
CAAGCCGCCCAATAGCAAGTAAACAGCCATATTATTTTGCCATAAC	
TATCAGCAACCGCAAGAATGCCAATGAGCCTGAGGATCTATC	
AGCGCAGCTCCAACCGTAATCATGGTCACGGGAAACCT	
GGGATATTGACGTAGCAATAGCTAAGATAGC	
GATTAAGTTGGGTAAAACAGAATTTTAGAGGAAAACAATATT TTGTGGTGTGGTGTGTG	Capture strand for stator strand
CCATAATGCCAGGCTATCAAGGCCGGAGACATCTA	
AATTGTGTCGAAATCCGCGGCACACAACGGAGATTTGTATCA	
GTAATTAATTTAGAATCTGGGAAGGGCGATCGGTGCGGCAAA	
AATATCGTTAAGAGAGCAAAGCGGATTGTGAAAAATCAGGTCTTT	
AGGACAGATGAACGGTGTAACATAAGGGAACCGAAGAAT	
ATAGCGAGAGGCTATCATAACCAAATCCCAAAGAAAATTTCATCCTCAT	
TATTTAAATTGCAGGAAGATTG	
AAAGATTACAGAACGGGAGAAGGAAACGTCACCAATGAAACCA	
GAAGGAGCGGAATTATCATCATATATCATTTACATAGCACAA	
TTTACCAGTCCCGGCCTGCAGCCCACTACGGGCGCACCAGCT	
CCGACTTGTTGCTAAAATTTATTTAGTTCGCGAGAGTCGTCTTTCCAGA	
CAAGCCCAATAGGAACCACCCTCACCCGGAA	
AGAACTTAGCCTAATTATCCCAAGCCCCCTTATTAGCGTTTGCCA	

TAGCCAGCTTTCATCCAAAAATAAACGT	
AAATGACGCTAAATGGATTATTTACATTGGCGAATACCTGGA	
TAACGACATTTTTACCAGCGCCAAAGAAAGTTACCAGAACCCAAA	
GCGTCCACTATTCCTGTGTGAAATGCTCACTGCC	
ACCGCCAGCTGCTCATTT	
GAGTTAAAAGGGTAATTGAGCGCTAATATCAGAGGAACTGAACACC	
TAACATCCAATAAATGCAAAGGTGGCATCAACATTATGAAAG	
CCGTAATCAGTAGCGACAGAATCTAATTATTCATTAAAAAGG	
GATTAGAGAGTACCTTAACTCCAACAGG	Biotin modification on 5’
CTTACGGAACAGTCAGGACGTTGGGAAGAAA	
CGAACACCAAATAAAATAGCAGCCAAGTTTGCCTTTAGCGTCAGA	
TAATATCAAAGGCACCGCTTCTGGCACT	
AACCGTGTCATTGCAACGGTAATATATTTTAAATGAAAGGGT	
CGCTTTCCAGTTAGCTGTTTAAAGAACGT	
TTCGGGGTTTCTGCCAGGCCTGTGACGATCC	
GGTAATATCCAGAAACGC	
AGCTTTCAGAGGTGGCGATGGCCAGCGGGAATGCGAAAATCC TTAAGTGAGTGTAGTAG	Capture strand for stator DNA (stating position)

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