Supporting Information

Determining The In-Plane Orientation and Binding Mode of Single Fluorescent Dyes in DNA Origami Structures

Kristina Hübner[1], Himanshu Joshi[2], Aleksei Aksimentiev[2], Fernando D. Stefani[3,4]*

Philip Tinnefeld[1] and Guillermo P. Acuna[5*]*

[1] Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13 Haus E, 81377 München, Germany

[2] Department of Physics, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States

[3] Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2390, C1425FQD Ciudad Autónoma de Buenos Aires, Argentina

[4] Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Güiraldes 2620, C1428EHA Ciudad Autónoma de Buenos Aires, Argentina

[5] Department of Physics, University of Fribourg, Chemin du Musée 3, Fribourg CH-1700, Switzerland

1. Linking chemistry of ATTO 647N to DNA

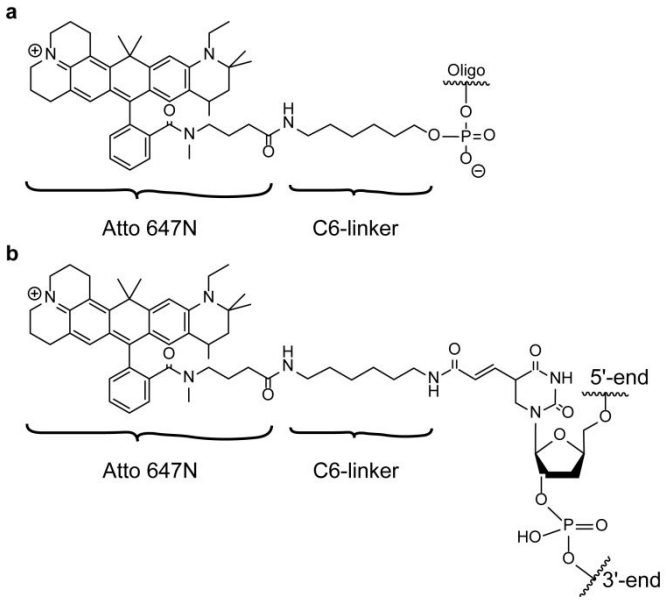

Figure S1: ATTO 647N linked through a C6 linker to DNA, at the 3´-end (a) or internally via a thymine (b).

2. Modulation Data

Table S1: Number of traces that meet the condition of a modulation threshold of M >0.15 for each studied sample with the probed fluorophores.

structure	fluorophore	modulating fraction ($M > 0.15$)
sample 1	ATTO 647N	95 % (out of 301)
	ATTO 643	93 % (out of 72)
	Cy5	95 % (out of 151)
sample 2	ATTO 647N	61 % (out of 303)
	ATTO 643	20 % (out of 319)
	Cy5	88 % (out of 162)
sample 3	ATTO 647N	73 % (out of 224)

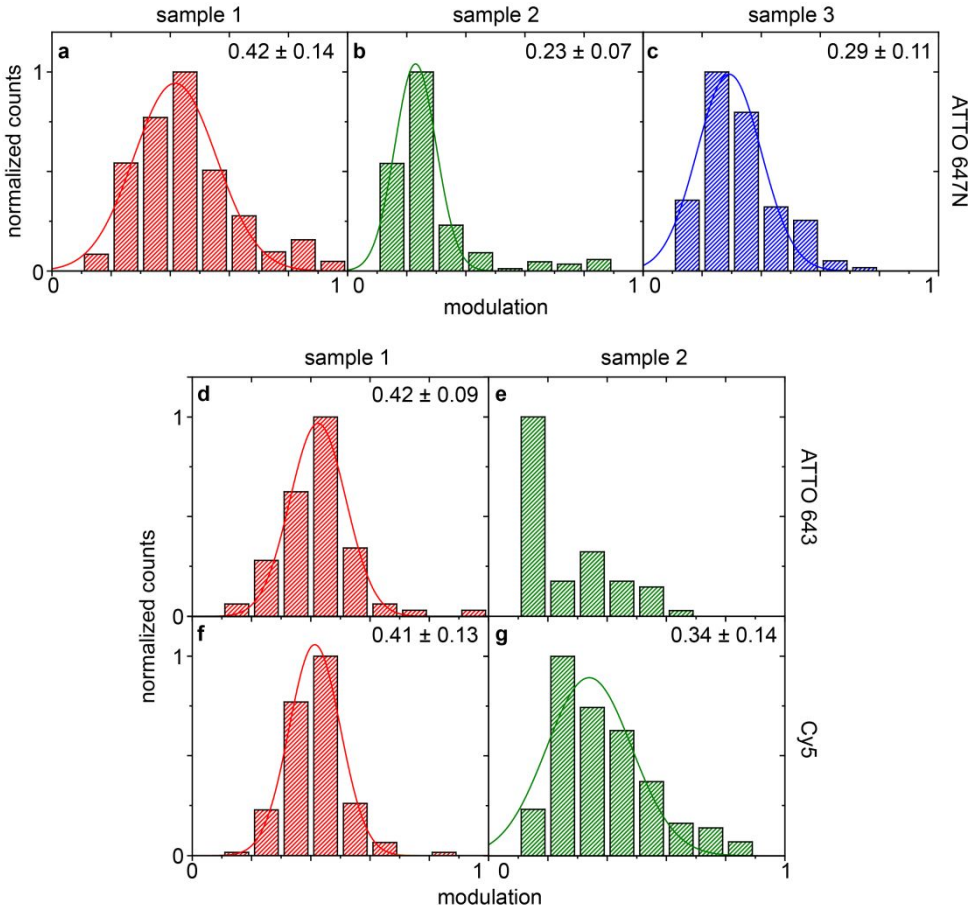

Figure S2: Modulation distributions with standard deviation of the three measured samples with the ATTO 647N dye (a-c). Modulation data for sample 1 and 2 with the dyes ATTO 643 (d-e) and Cy5 (f-g).

3. DNA-PAINT Data

Due to the design of the DNA origami rectangle an upside down binding of the structure to the functionalized surface is possible. The chiral DNA-PAINT pattern on the DNA origami structure enables to distinguish flipped from non-flipped structures. Figure S 3a shows a schematic of the chiral DNA-PAINT pattern and figure S 3b shows an image with the two different binding possibilities, facing with the top up (orange square) or down (green square).

Furthermore, we can make a distance analysis of the measured DNA origami structures showing that the measured distances (figure S 3c) fit well to the designed distances. For this kind of analysis the super resolution data were first processed with the open source software Picasso[1]. The localization files were exported for further analysis with self-written Labview software.

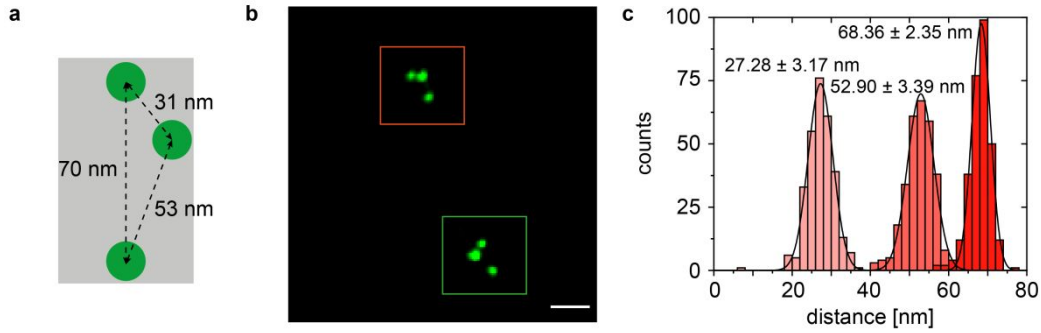

Figure S3: DNA-PAINT super resolution sample design and data. Showing a schematic of the asymmetric DNA-PAINT pattern (a) and super-resolved images of the DNA-PAINT measurements (b, scale bar 100 nm), where structures lying with the top up (orange square) or down (green square) can be distinguished. A histogram (c) is showing the measured distances for the asymmetric pattern for 297 molecules.

4. ATTO 647N dye orientation of flipped and non-flipped DNA origami structures

If both populations, non-flipped and flipped origami structures distinguished by the super resolved images in figure S3, are plotted separately the histograms draw similar distributions. This indicates that the orientation of the DNA origami structure on the surface does not has an influence on the ATTO 647N dye sticking to the DNA. The histograms are plotted in figure S 4 and fitted with Gaussian distribution functions to extract the mean orientation with their standard deviations.

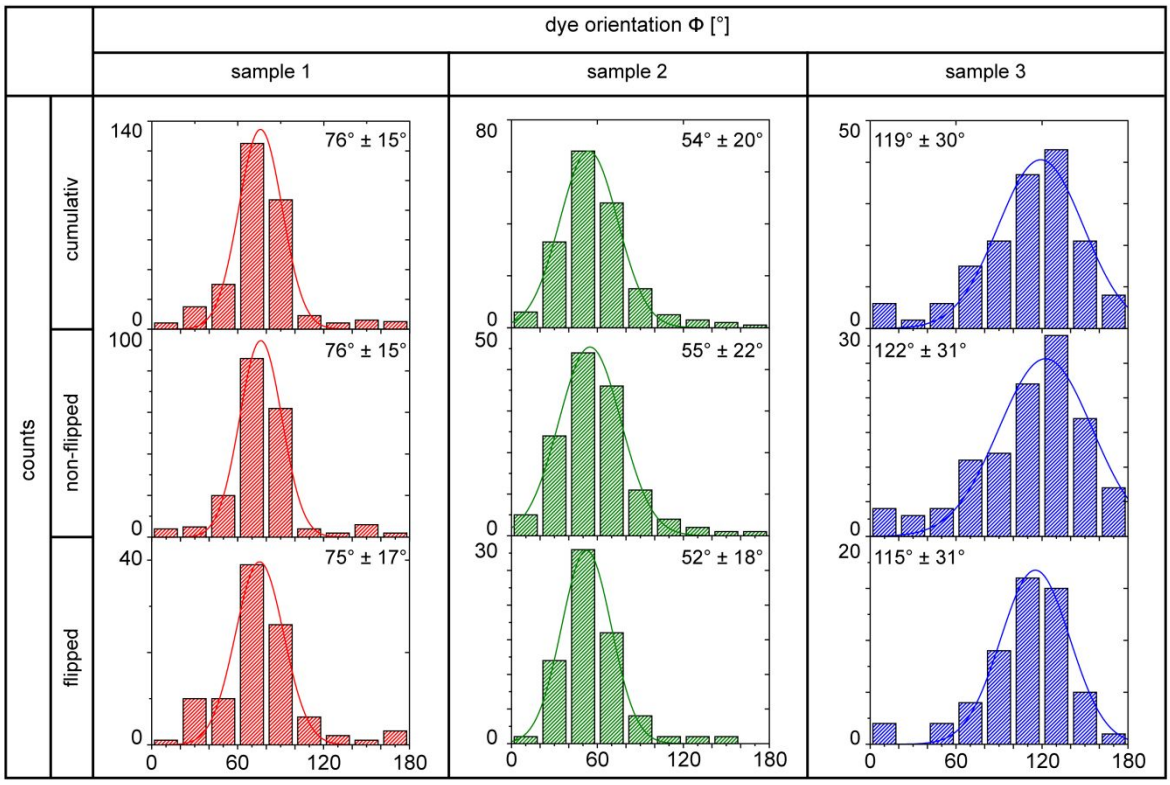

Figure S4: ATTO 647N dye orientation distributions with standard deviations of the three samples. Separated Histograms of non-flipped and flipped samples show the same distributions as the combined histograms in the first row.

5. All-atom molecular dynamics simulations

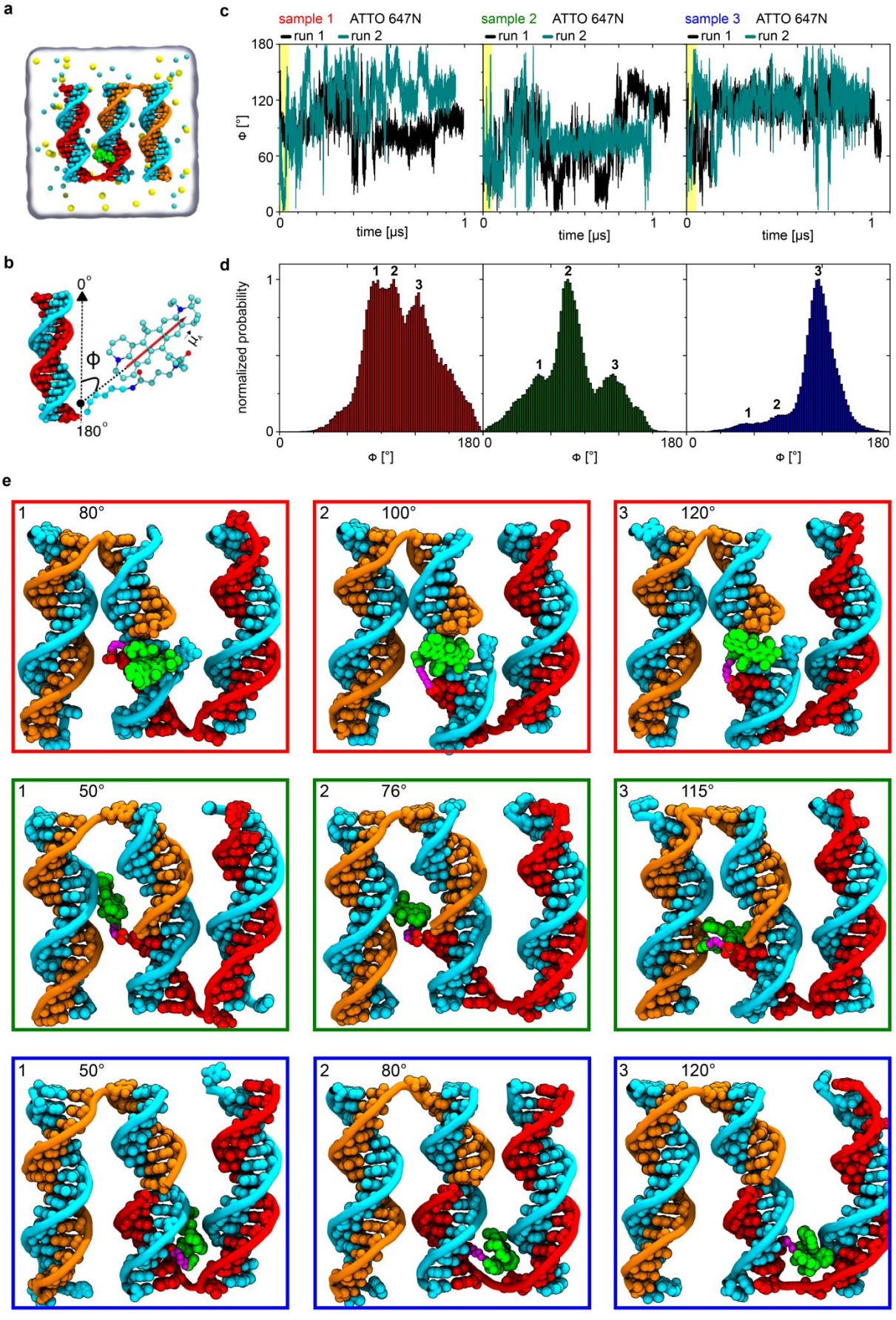
a
b
0°
Φ
180°
c
sample 1 ATTO 647N
sample 2 ATTO 647N
sample 3 ATTO 647N
run 1
run 2
Φ [°]
time [µs]
d
normalized probability
Φ [°]
e
1 80°
2 100°
3 120°
1 50°
2 76°
3 115°
1 50°
2 80°
3 120°

Figure S5: (a) A representative snapshot of the fully assembled all-atom model of the ATTO 647N dye-conjugated DNA system. The template strand of the DNA origami structure is shown in cyan, the staple strand carrying the dye molecule is shown in red and the other staple strand is shown in orange. The atoms of the dye molecule are shown using green spheres whereas the atoms of the C6 anchor between the dye and the DNA are shown using magenta spheres. Sodium, potassium and chloride ions are shown using yellow, tan and light cyan spheres, respectively. The volume occupied by water molecules is represented by a semi-transparent white surface. (b) Schematics illustrating the definition of the angle (ϕ) between the helical axis of the DNA and the dipole moment of the ATTO 647N dye molecule. (c) The angle between the helical axis of the DNA and the dye molecule's dipole as a function of simulation time for sample 1, 2, and 3. The first 50 ns of each trace (yellow rectangle) were excluded from the histogram analysis. (d). Histograms of the ϕ angles observed in the MD simulations of samples 1-3 with the preferred orientations marked (1-3). (e) Microscopic configurations of the simulation systems corresponding to the preferred orientations of the dye labelled 1-3 in panel d. Red, green and blue boundary box indicates microscopic configurations extracted from the MD trajectories of sample 1, 2, and 3, respectively.

In addition to the simulations of the ATTO 647N dye-conjugated DNA system described in the main text and Figure S5, four 1 μs-long equilibrium all-atom molecular dynamics simulations were performed to study the orientation of a Cy5 dye conjugated to DNA in sample 1 and 2 geometries, two independent simulations for each sample. Supplementary movie S4 and S5 illustrate the simulation trajectories. During the simulations, the Cy5 dyes were observed to sample a wide range of orientations with respect to the DNA axis (Figure S6 a, b). For sample 1, where the next two DNA bases after the attached dye were missing, the Cy5 dye was frequently observed to engage in base stacking interactions with the unpaired nucleotides. For sample 2, where all bases ware paired, the Cy5 dye was observed to transiently bind to minor and major groves of its parent and neighboring DNA helices. The helical structure of the DNA near the dye attachment point was better preserved in sample 2 than in sample 1. Unfortunately, the simulation trajectories were too short to sample Cy5 orientations with enough statistics to make quantitative conclusions about the preferred orientation of the dye.

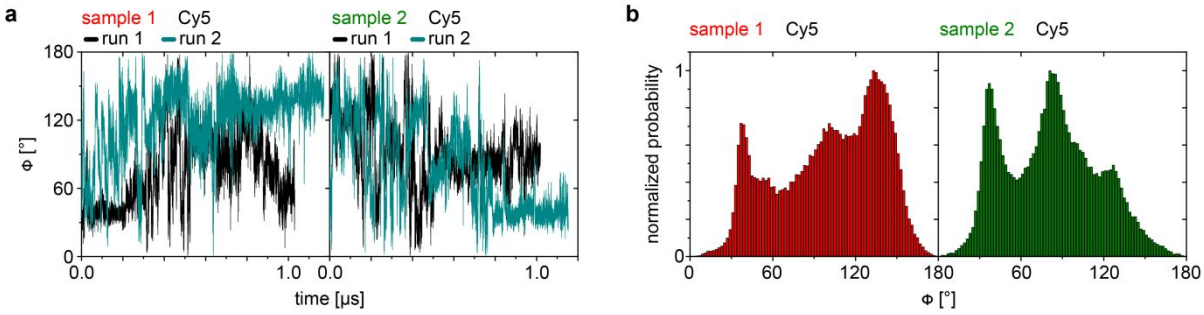

Figure S6: (a) Angle between the helical axis of DNA and the Cy5 molecule's dipole as a function of simulation time for sample 1 and 2. Two independent simulations were performed for each sample. (b) Histograms of the angle distribution for sample 1 and sample 2 simulations. The first 50 ns of each simulation (yellow region in panel a) were excluded from the histogram analysis.

6. Supplementary movies

Supplementary movies S1, S2 and S3 show a 1 μs long MD simulation trajectories of the ATTO 647N dye-conjugated DNA systems corresponding to sample 1, 2 and 3, respectively. Supplementary movies S4 and S5 shows the 1 μs long MD simulation trajectories of the Cy5 dye-conjugated DNA systems corresponding to sample 1 and 2, respectively. The scaffold strand of the DNA origami structure is shown in cyan, the staple strand carrying the dye molecule is shown in red and the other staple strand is shown in orange. The atoms of the dye molecule are shown using green spheres whereas the atoms of C6 molecules (anchor between the dye and DNA) are shown using magenta spheres. Water and counter ions are not shown for clarity.

7. DNA origami ssDNA strands

Table 1: Unmodified ssDNA strands

Sequence (5‘-> 3‘)	**Number**
AGTATAAAGTTCAGCTAATGCAGATGTCTTTC	1
AATACTGCCCAAAAGGAATTACGTGGCTCA	2
ATCCCAATGAGAATTAACTGAACAGTTACCAG	3
TGGAACAACCGCCTGGCCCTGAGGCCCGCT	4
GAGGGTAGGATTCAAAAGGGTGAGACATCCAA	5
TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG	6
CTACCATAGTTTGAGTAACATTTAAAATAT	7
GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT	8
AGAAAACAAAGAAGATGATGAAACAGGCTGCG	9
AAAGCACTAAATCGGAACCCTAATCCAGTT	10
AATTGAGAATTCTGTCCAGACGACTAAACCAA	11
TAGGTAAACTATTTTTGAGAGATCAAACGTTA	12
AGGCAAAGGGAAGGGCGATCGGCAATTCCA	13
CATTTGAAGGCGAATTATTCATTTTTGTTTGG	14
ATACCCAACAGTATGTTAGCAAATTAGAGC	15
CTTTAGGGCCTGCAACAGTGCCAATACGTG	16
TGTAGCCATTAAAATTCGCATTAAATGCCGGA	17
CACCAGAAAGGTTGAGGCAGGTCATGAAAG	18
TTCCAGTCGTAATCATGGTCATAAAAGGGG	19
TCAAGTTTCATTAAAGGTGAATATAAAAGA	20
ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG	21
GCGAAAAATCCCTTATAAATCAAGCCGGCG	22
TTATTACGAAGAACTGGCATGATTGCGAGAGG	23
AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGT	24
AAATCACCTTCCAGTAAGCGTCAGTAATAA	25
CATCAAGTAAAACGAACTAACGAGTTGAGA	26
TTAGGATTGGCTGAGACTCCTCAATAACCGAT	27
AGCGCGATGATAAATTGTGTCGTGACGAGA	28
TGACAACTCGCTGAGGCTTGCATTATACCA	29

Sequence (5‘-> 3‘)	Number
TAATCAGCGGATTGACCGTAATCGTAACCG	30
GATGTGCTTCAGGAAGATCGCACAATGTGA	31
ACCGATTGTCGGCATTTTCGGTCATAATCA	32
GCCCTTCAGAGTCCACTATTAAAGGGTGCCGT	33
GCGAACCTCCAAGAACGGGTATGACAATAA	34
CTTTTACAAAATCGTCGCTATTAGCGATAG	35
AAACAGCTTTTTGCGGGATCGTCAACACTAAA	36
AAATTAAGTTGACCATTAGATACTTTTGCG	37
TACCGAGCTCGAATTCGGGAAACCTGTCGTGCAGCTGATT	38
AAGGAAACATAAAGGTGGCAACATTATCACCG	39
CTTAGATTTAAGGCGTTAAATAAAGCCTGT	40
ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA	41
TAAATGAATTTTCTGTATGGGATTAATTTCTT	42
ACAAACGGAAAAGCCCCAAAAACACTGGAGCA	43
ATTATACTAAGAAACCACCAGAAGTCAACAGT	44
CTCGTATTAGAAATTGCGTAGATACAGTAC	45
CAGAAGATTAGATAATACATTTGTCGACAA	46
ATTTTAAAATCAAAATTATTTGCACGGATTCG	47
TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGA	48
TTGACAGGCCACCACCAGAGCCGCGATTTGTA	49
CGTAAAACAGAAATAAAAATCCTTTGCCCGAAAGATTAGA	50
GTTTATCAATATGCGTTATACAAACCGACCGTGTGATAAA	51
CTGAGCAAAAATTAATTACATTTTGGGTTA	52
ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG	53
GTATAGCAAACAGTTAATGCCCAATCCTCA	54
ATATTCGGAACCATCGCCCACGCAGAGAAGGA	55
TTATACCACCAAATCAACGTAACGAACGAG	56
GCTATCAGAAATGCAATGCCTGAATTAGCA	57
TCACCGACGCACCGTAATCAGTAGCAGAACCG	58
ATTATCATTCAATATAATCCTGACAATTAC	59
TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT	60
GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA	61

Sequence (5‘-> 3‘)	Number
CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCG	62
GAAATTATTGCCTTTAGCGTCAGACCGGAACC	63
AGGCTCCAGAGGCTTTGAGGACACGGGTAA	64
ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGG	65
TTAATGAACTAGAGGATCCCCGGGGGGTAACG	66
GCCATCAAGCTCATTTTTTAACCACAAATCCA	67
AAGTAAGCAGACACCACGGAATAATATTGACG	68
AGCCAGCAATTGAGGAAGGTTATCATCATTTT	69
ATTACCTTTGAATAAGGCTTGCCCAAATCCGC	70
CGAAAGACTTTGATAAGAGGTCATATTTCGCA	71
CGATAGCATTGAGCCATTTGGGAACGTAGAAA	72
TCACCAGTACAAACTACAACGCCTAGTACCAG	73
TTAAAGCCAGAGCCGCCACCCTCGACAGAA	74
TCATTCAGATGCGATTTTAAGAACAGGCATAG	75
CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA	76
ACAACATGCCAACGCTCAACAGTCTTCTGA	77
GTAATAAGTTAGGCAGAGGCATTTATGATATT	78
AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAA	79
GATGGCTTATCAAAAAGATTAAGAGCGTCC	80
TAAATCAAAATAATTCGCGTCTCGGAAACC	81
TTAACGTCTAACATAAAAACAGGTAACGGA	82
AACGCAAAGATAGCCGAACAAACCCTGAAC	83
ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT	84
ACACTCATCCATGTTACTTAGCCGAAAGCTGC	85
TTAACACCAGCACTAACAACTAATCGTTATTA	86
GCCGTCAAAAAACAGAGGTGAGGCCTATTAGT	87
ATCGCAAGTATGTAAATGCTGATGATAGGAAC	88
TAAATCATATAACCTGTTTAGCTAACCTTTAA	89
CATGTAATAGAATATAAAGTACCAAGCCGT	90
CCTGATTGCAATATATGTGAGTGATCAATAGT	91
CCTAAATCAAAATCATAGGTCTAAACAGTA	92
TGAAAGGAGCAAATGAAAAATCTAGAGATAGA	93

Sequence (5‘-> 3‘)	Number
GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA	94
CCCGATTTAGAGCTTGACGGGGAAAAAGAATA	95
CATAAATCTTTGAATACCAAGTGTTAGAAC	96
GCGAGTAAAAATATTTAAATTGTTACAAAG	97
AATGGTCAACAGGCAAGGCAAAGAGTAATGTG	98
GACCAACTAATGCCACTACGAAGGGGGTAGCA	99
ACCTTTTTATTTTAGTTAATTTCATAGGGCTT	100
GCAAGGCCTCACCAGTAGCACCATGGGCTTGA	101
CAACTGTTGCGCCATTCGCCATTCAAACATCA	102
GACAAAAGGTAAAGTAATCGCCATATTTAACAAAACTTTT	103
AATACGTTTGAAAGAGGACAGACTGACCTT	104
CAGCGAAACTTGCTTTCGAGGTGTTGCTAA	105
TATAACTAACAAAGAACGCGAGAACGCCAA	106
ATCCCCCTATACCACATTCAACTAGAAAAATC	107
TATTAAGAAGCGGGGTTTTGCTCGTAGCAT	108
CCACCCTCTATTCACAAACAAATACCTGCCTA	109
TCAAATATAACCTCCGGCTTAGGTAACAATTT	110
GATGGTTTGAACGAGTAGTAAATTTACCATTA	111
TATATTTTGTCATTGCCTGAGAGTGGAAGATTGTATAAGC	112
AAAGTCACAAAATAAACAGCCAGCGTTTTA	123
GCGGATAACCTATTATTCTGAAACAGACGATT	124
CAGCAAAAGGAAACGTCACCAATGAGCCGC	125
TCATCGCCAACAAAGTACAACGGACGCCAGCA	126
CTTTTGCAGATAAAAACCAAAATAAAGACTCC	127
CACAACAGGTGCCTAATGAGTGCCCAGCAG	128
TGCATCTTTCCCAGTCACGACGGCCTGCAG	129
CGCGCAGATTACCTTTTTTAATGGGAGAGACT	130
TTTTATTTAAGCAAATCAGATATTTTTTGT	131
GAATTTATTTAATGGTTTGAAATATTCTTACC	132
AACACCAAATTTCAACTTTAATCGTTTACC	133
GCGCAGACAAGAGGCAAAAGAATCCCTCAG	134
GTACCGCAATTCTAAGAACGCGAGTATTATTT	135

Sequence (5‘-> 3‘)	Number
GCGGAACATCTGAATAATGGAAGGTACAAAAT	136
AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC	137
GGCCTTGAAGAGCCACCACCCTCAGAAACCAT	138
TACGTTAAAGTAATCTTGACAAGAACCGAACT	139
AAGGCCGCTGATACCGATAGTTGCGACGTTAG	140
AATAGTAAACACTATCATAACCCTCATTGTGA	141
CGGATTGCAGAGCTTAATTGCTGAAACGAGTA	142
GATTTAGTCAATAAAGCCTCAGAGAACCCTCA	143
CTTATCATTCCCGACTTGCGGGAGCCTAATTT	144
AATAGCTATCAATAGAAAATTCAACATTCA	145
CTTTAATGCGCGAACTGATAGCCCCACCAG	146
AGAAAGGAACAACTAAAGGAATTCAAAAAAA	147
ACAACTTTCAACAGTTTCAGCGGATGTATCGG	148
GCACAGACAATATTTTTGAATGGGGTCAGTA	149
TTCTACTACGCGAGCTGAAAAGGTTACCGCGC	150
CAACCGTTTCAAATCACCATCAATTCGAGCCA	151
TCAATATCGAACCTCAAATATCAATTCCGAAA	152
TAAAAGGGACATTCTGGCCAACAAAGCATC	153
GTCGACTTCGGCCAACGCGCGGGGTTTTTC	154
GCCCGTATCCGGAATAGGTGTATCAGCCCAAT	155
AACGTGGCGAGAAAGGAAGGGAAACCAGTAA	156
GCAATTCACATATTCCTGATTATCAAAGTGTA	157
AAGCCTGGTACGAGCCGGAAGCATAGATGATG	158
CAAATCAAGTTTTTTGGGGTCGAAACGTGGA	159
CTCCAACGCAGTGAGACGGGCAACCAGCTGCA	160
AACGCAAAATCGATGAACGGTACCGGTTGA	161
CCAATAGCTCATCGTAGGAATCATGGCATCAA	162
CCACCCTCATTTTCAGGGATAGCAACCGTACT	163
AGGAACCCATGTACCGTAACACTTGATATAA	164
GTTTTAACTTAGTACCGCCACCCAGAGCCA	165
CCAACAGGAGCGAACCAGACCGGAGCCTTTAC	166
TTTTCACTCAAAGGGCGAAAAACCATCACC	167

Sequence (5‘-> 3‘)	Number
TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA	168
TCGGCAAATCCTGTTTGATGGTGGACCCTCAA	169
TCCACAGACAGCCCTCATAGTTAGCGTAACGA	170
AGAGAGAAAAAAATGAAAATAGCAAGCAAACT	171
TAAGAGCAAATGTTTAGACTGGATAGGAAGCC	172

Table 2: Modified ssDNA strands.

Sequence (5‘ ->3‘)	Number
Biotin strands	
Biotin-TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA	173
Biotin-GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC	174
Biotin-ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	175
Biotin-AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	176
Biotin-GAGAAGAGATAACCTTGCTTCTGTTCGGGAGAAACAATAA	177
Biotin-CGGATTCTGACGACAGTATCGGCCGCAAGGCGATTAAGTT	178
DNA-PAINT functionalized strands	
ACGCTAACACCCACAAGAATTGAAAATAGCTTAAATGCCCG	179
TTTAGGACAAATGCTTTAAACAATCAGGTCTTAAATGCCCG	180
TGTAGAAATCAAGATTAGTTGCTCTTACCATTAAATGCCCG	181
AACAGTTTTGTACCAAAAACATTTTATTTCTTAAATGCCCG	182
ATATTTTGGCTTTCATCAACATTATCCAGCCATTAAATGCCCG	183
GCCTTAAACCAATCAATAATCGGCACGCGCCTTTAAATGCCCG	184
GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAATTAAATGCCCG	185
GCTTTCCGATTACGCCAGCTGGCGGCTGTTTCTTAAATGCCCG	186

Sequence (5‘ ->3‘)	**Number**
TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCCTTAAATGCCCG	187
GCCCGAGAGTCCACGCTGGTTTGCAGCTAACTTTAAATGCCCG	188
TTTACCCCAACATGTTTTAAATTTCCATATTTAAATGCCCG	189
AACAAGAGGGATAAAAATTTTTAGCATAAAGCTTAAATGCCCG	190
CTGTAGCTTGACTATTATAGTCAGTTCATTGATTAAATGCCCG	191
CTGTGTGATTGCGTTGCGCTCACTAGAGTTGCTTAAATGCCCG	192
CACATTAAAATTGTTATCCGCTCATGCGGGCCTTAAATGCCCG	193
GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCATTAAATGCCCG	194

Table 3: Replaced ssDNA strands.

Sequence (5‘ ->3‘)	**Replaced Staple Number**
Strands for sample 1	
CGAAAGACTTTGATAAGAGGTCATATTTCG-ATTO 647N	71
Strands for sample 2	
CGAAAGACTTTGATAAGAGGTCATATTTCG- ATTO 647N	71
CAAATGGTCAACAGGCAAGGCAAAGAGTAATGTG	89
Strands for sample 3	
TAAGAGCAAATGTTTAGACTGGATAG-dT ATTO 647N-AAGCC	172

Imager strand*:* CGGGCAT-ATTO 542

References

(1) Schnitzbauer, J.; Strauss, M. T.; Schlichthaerle, T.; Schueder, F.; Jungmann, R. Super-Resolution Microscopy with DNA-PAINT. *Nature protocols* **2017**, *12*, 1198–1228.