Supplementary Information

DNA origami book biosensor for multiplex detections of cancer-associated nucleic acids

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Figure S1. Blueprint of a DNA origami book biosensor

Depiction of scaffold routing and staple design in a two-dimensional representation. Graphics and sequences were generated using caDNAno software package. The scaffold strand is shown in black. Staple strands labeled with Cy3, Cy5, bh2, or bbq650 are shown in orange. Strands labeled with biotin are shown in purple. Hinge staple strands are shown in red.

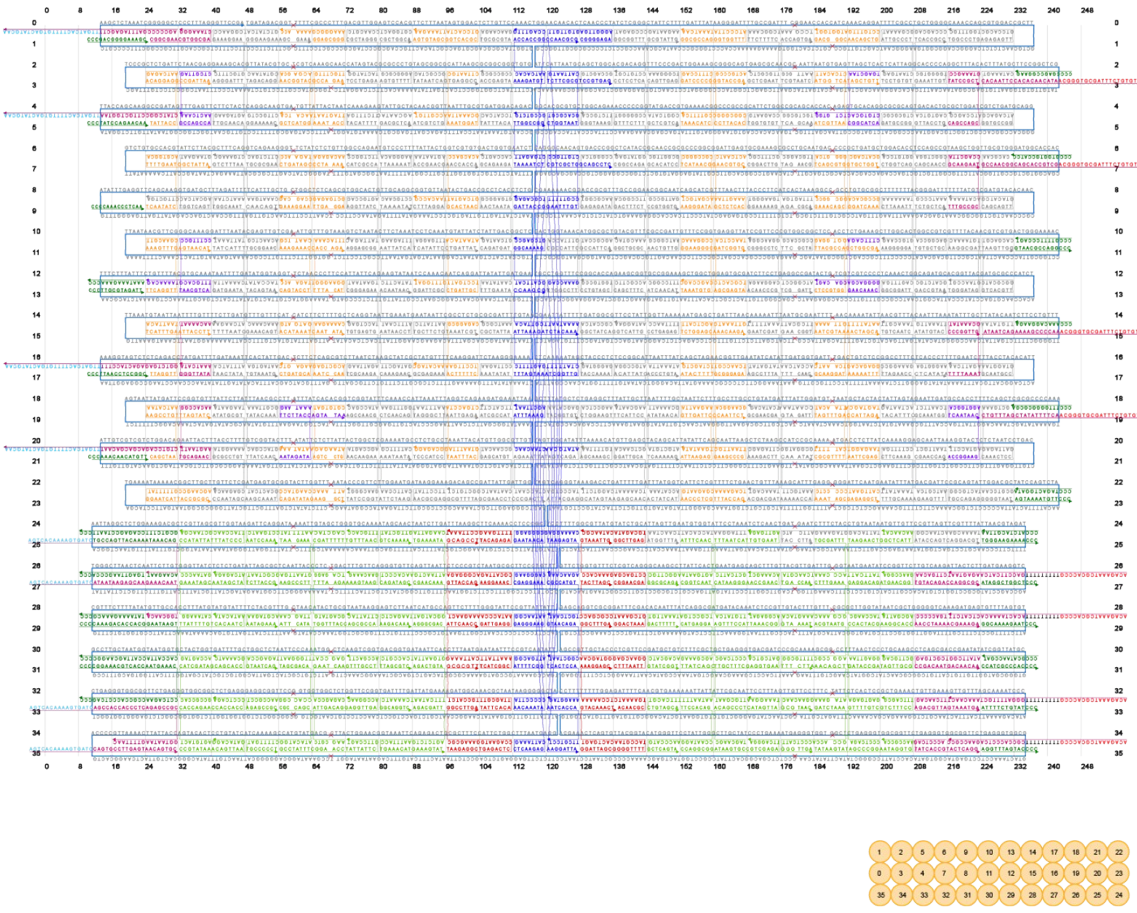


Figure S2. Mapping of a DNA origami book biosensor

The map of a DNA origami book biosensor showing the distance between the positioned fluorophores or quenchers.

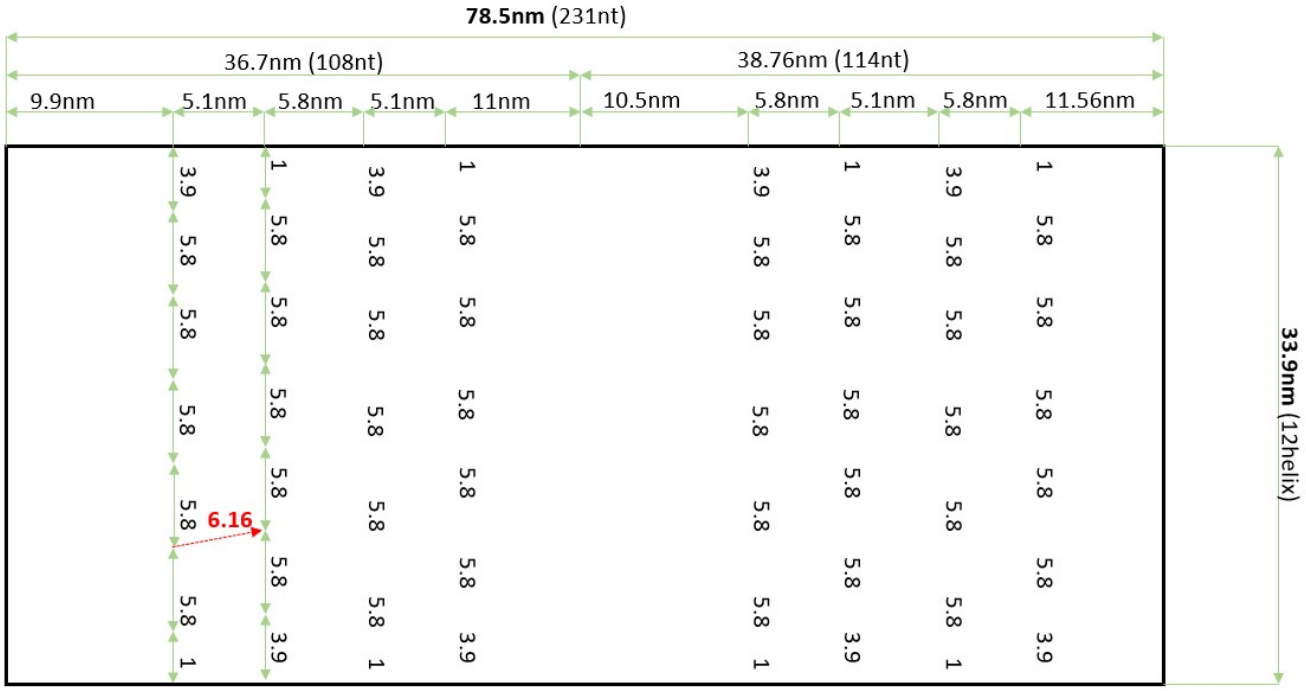


Figure S3. TEM images of DNA origami book biosensors

Structures were self-assembled at 12 mM Mg^{2+} and purified at 8 mM Mg^{2+} and visualized using transmission electron microscopy (TEM) by depositing them on the carbon-coated EM grids (scale bars: 100 nm).

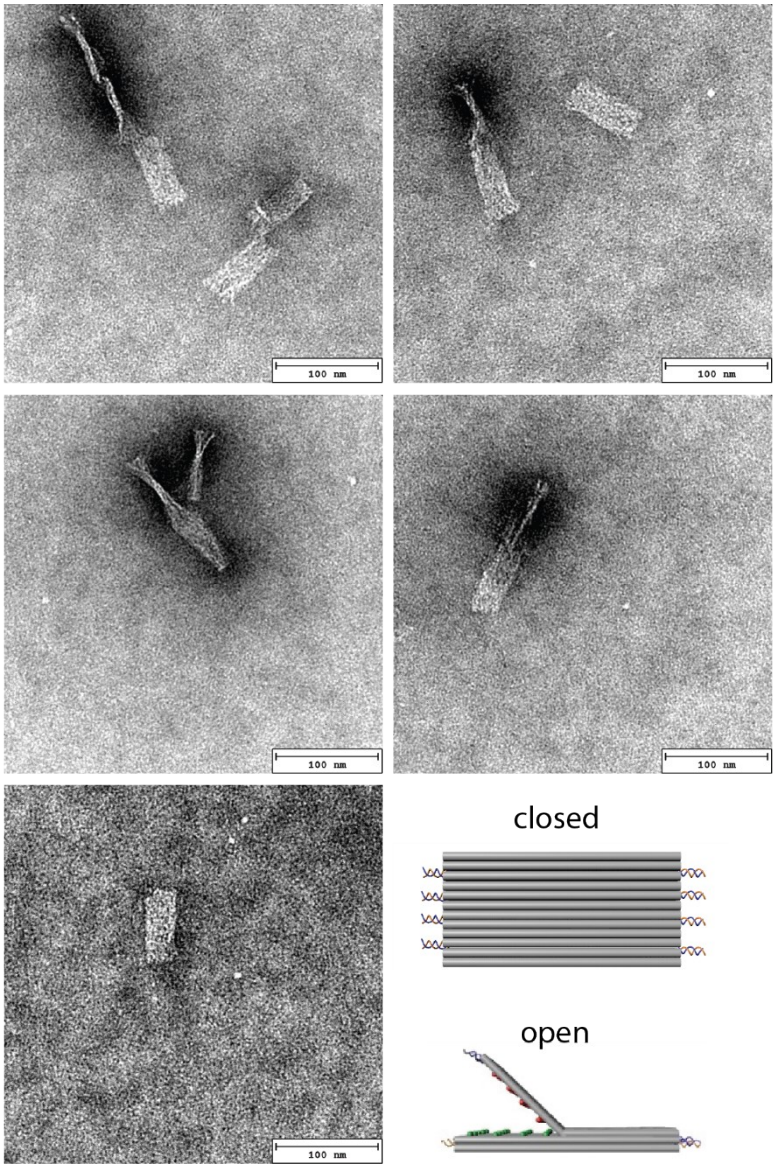


Figure S4. DNA origami book biosensor visualization using atomic force microscopy (AFM)

Structures were assembled with 12 mM Mg^{2+} in the closed conformation and absorbed on the mica surface. AFM measurements were performed in tapping mode with AFM NT-MDT (NTEGRA II).

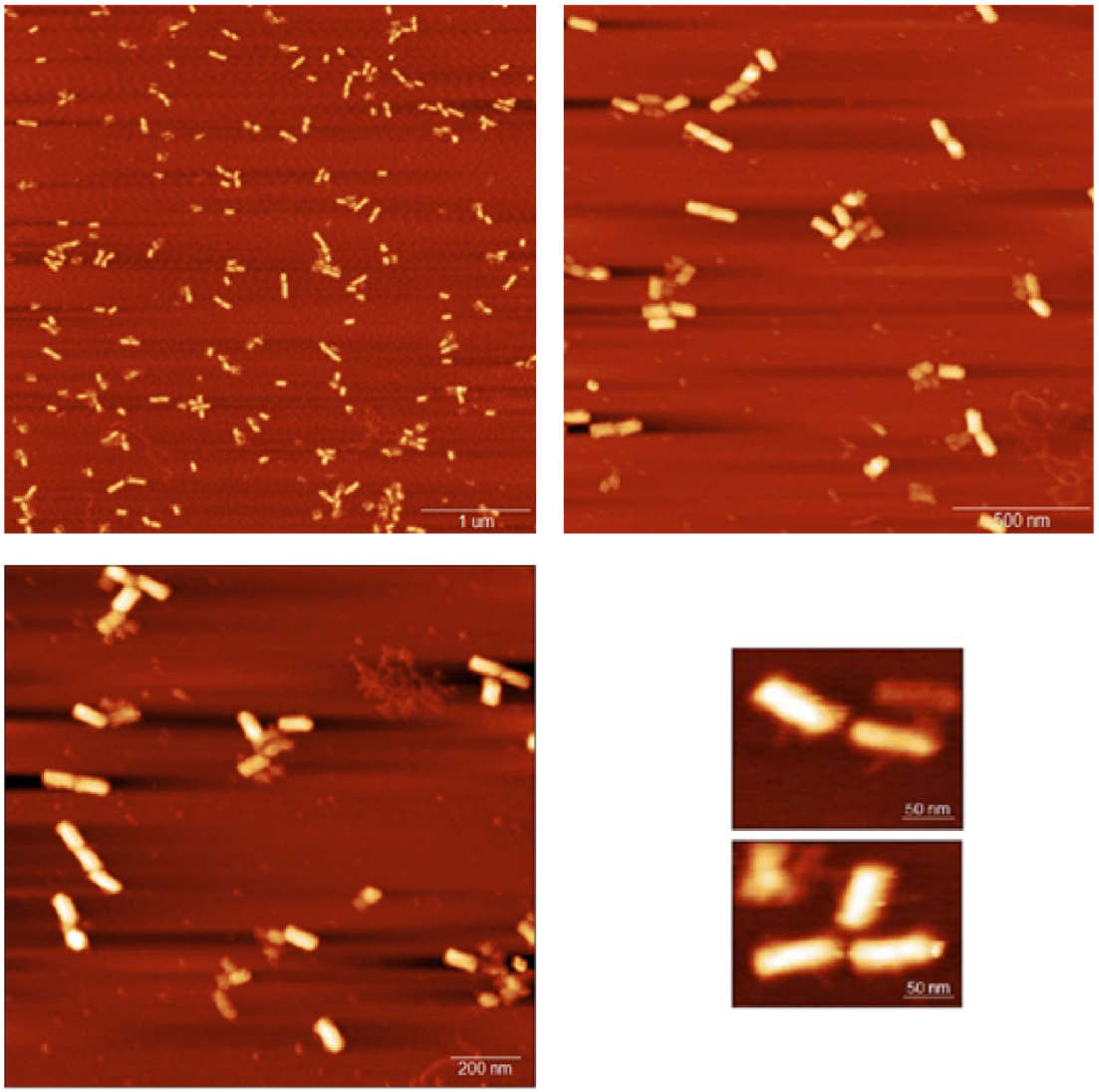


Figure S5. Agarose gel analysis of DNA origami book biosensors

The presence of Mg^{2+} is needed to shield the negatively charged DNA phosphate backbone, for the formation of DNA double helices during the self-assembly process of DNA origami structure.[1, 2] To determine the optimal Mg^{2+} concentration for the self-assembly of the book biosensor, the structures were analyzed by agarose gel electrophoresis. Assembled structures were run on 1.5% agarose gel at 70 V together with a single-stranded M13 scaffold strand and 1 kb ladder. Gel electrophoresis analysis of the self-assembled structures with different Mg^{2+} concentrations. The optimal Mg^{2+} concentration for forming the structure without incorporated fluorophore was greater than 8 mM Mg^{2+} (Figure S5a). Figure S5b demonstrates the gel electrophoresis analysis of the self-assembled structures in the closed state (without fluorophores) at 12 mM Mg^{2+} and purified at 8 mM Mg^{2+} with or without the addition of target oligonucleotides. The gel image shows the differences in the mobility of the structures between open or closed states.

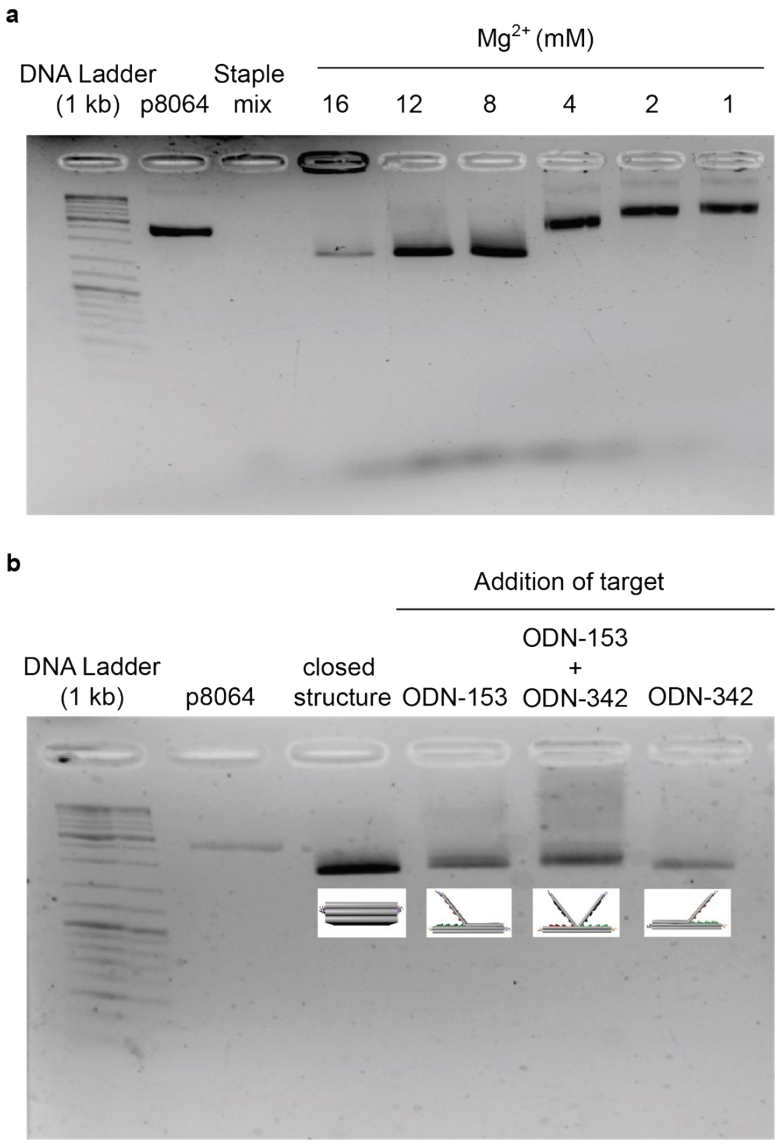


Figure S6. Single-structure study of the incorporation of the fluorophores within DNA origami book biosensor using wide-field fluorescence microscopy

In order to test the incorporation of the dyes and measure the fluorescence signal generated by DNA origami book structures, the structures were functionalized with biotin to allow their immobilization on coverslips covered by biotinylated bovine serum albumin (BSA) and neutravidin. To determine if the incorporation of labelled oligonucleotides within the structure is efficient, we first analyzed the DNA origami book structure with 1 column (5 Cy3 or Cy5), 2 columns (10 Cy3 or Cy5) and 4 columns (20 Cy3 or Cy5). Structures were imaged and the mean intensity of each structure was compared (Figure S6). The increase in the number of fluorophores incorporated within the structure resulted in a linear increase of the mean fluorescence intensity in the absence of self-quenching. This is also reported in earlier studies where it was shown that fluorescence intensity increases linearly with an increasing number of incorporated fluorophores.[1, 3]

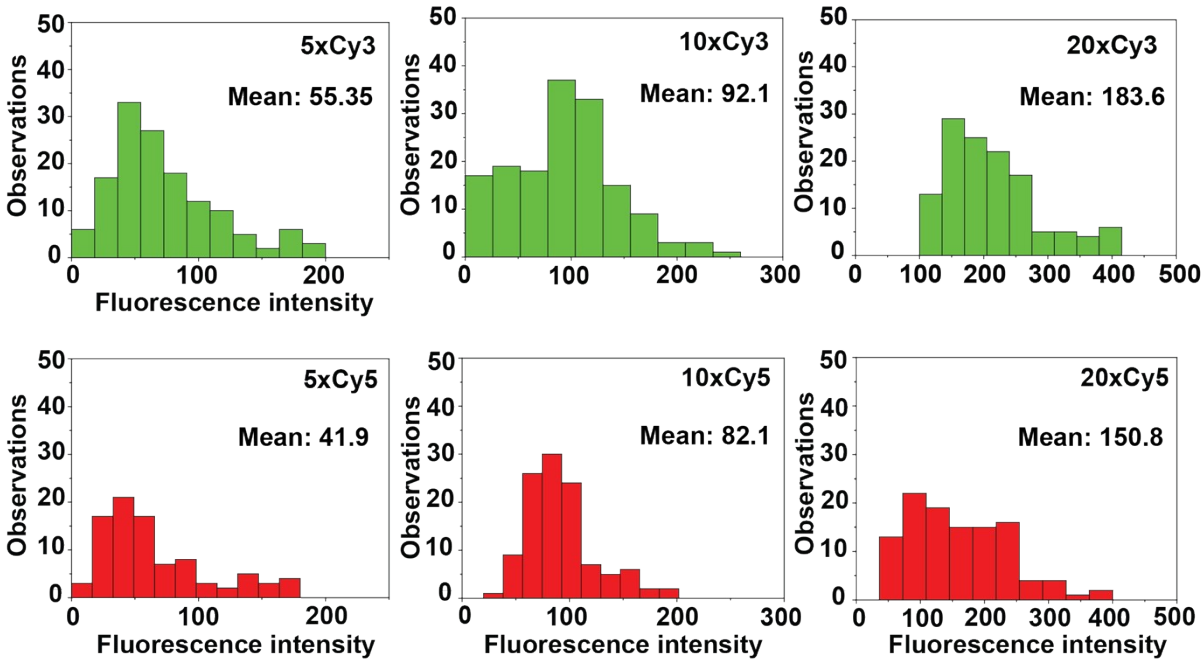


Figure S7. Fluorescence spectroscopy analysis of FRET efficiency

To determine the optimal Mg^{2+} concentration during the self-assembly and purification steps that give the maximum signal difference between open and closed states, we applied the FRET-based detection mechanism by labeling oligonucleotides with Cy3 and Cy5 fluorophores using terminal transferase (TdT) enzyme and incorporated them into DNA origami structure.[4] Ensemble FRET analysis of the DNA origami book biosensors using fluorescence spectroscopy was performed. FRET efficiency of open and closed self-assembled structures with 5 FRET pairs at different Mg^{2+} concentrations (4-16 mM Mg^{2+}) is shown in Figure S7a. Results indicated the optimal signal and the maximum difference between open and closed states (24%) observed when the structure is folded in the presence of 14 mM Mg^{2+}. After defining the optimal salt concentration during self-assembly that gives the maximum signal difference, the optimal Mg^{2+} concentration for purification was determined. All structures were assembled at 14 mM Mg^{2+} in the open and closed states and purified in the presence of Mg^{2+} in the range of 4 mM to 14 mM (Figure S7b). The optimal purification concentration was defined as 10 mM, as it gave the maximum FRET difference between the open and closed states of the device (25%). Higher concentrations of salt are used during self-assembly to decrease repulsion between DNA helices facilitating keeping the DNA origami sensor in the closed state. On the other hand, the lower salt concentration used in the purification step increases the repulsion between DNA helices, which allows the opening of the structure upon ligand binding. We also tested if a higher number of fluorophores incorporated within the structure requires more Mg^{2+}. DNA origami structure with 4 columns (20 fluorophores) was assembled in open and closed states in the range of 12 to 22 mM Mg^{2+} and the optimal signal was found at 16-18 mM Mg^{2+} (Figure S7c), while the purification step was optimal in the range of 10-12 mM Mg^{2+} (Figure S7d).

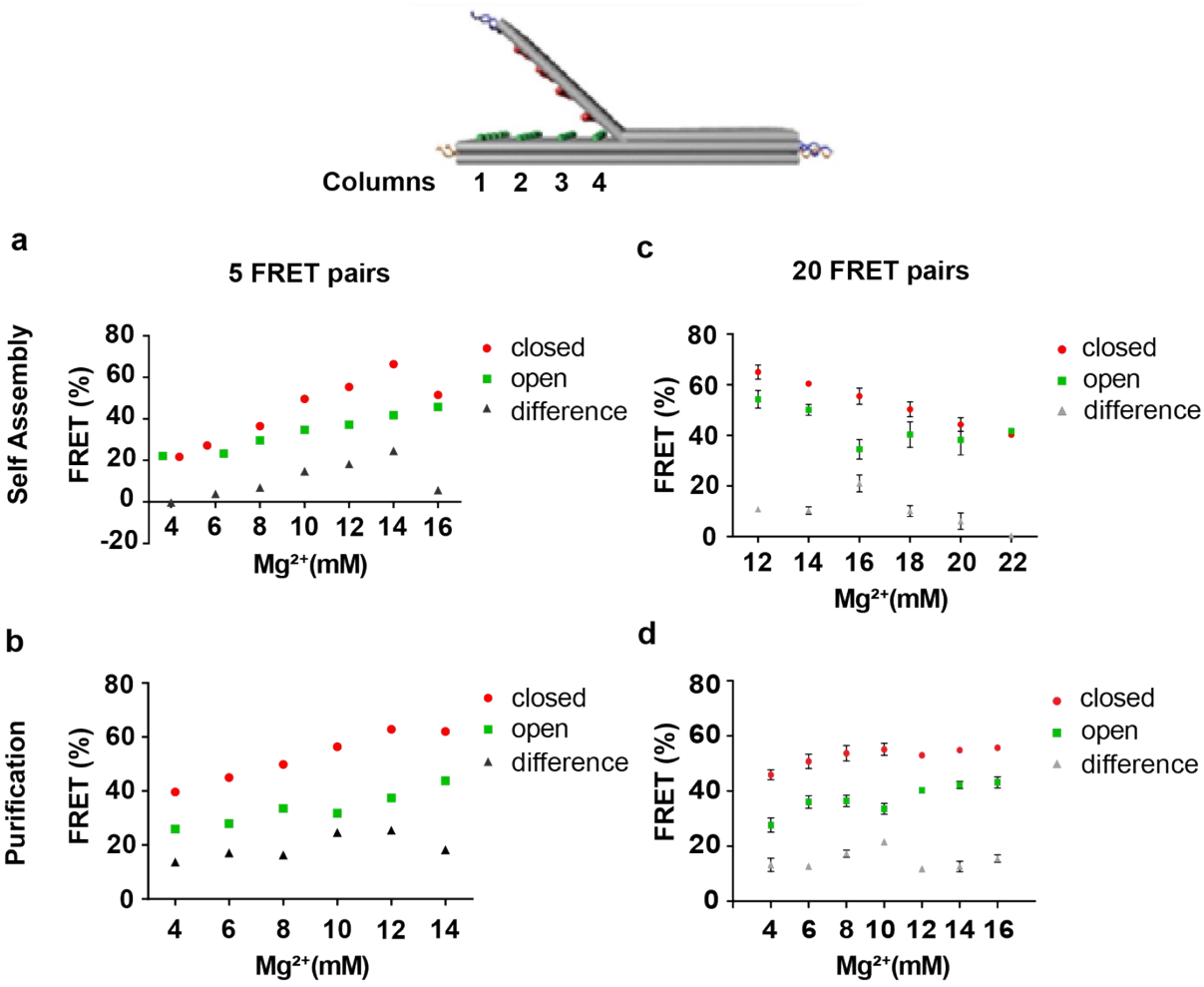

Columns 1 2 3 4
a
5 FRET pairs
Self Assembly
FRET (%)
80
60
40
20
0
-20
4 6 8 10 12 14 16
Mg2+(mM)
closed
open
difference
c
20 FRET pairs
FRET (%)
80
60
40
20
0
12 14 16 18 20 22
Mg2+(mM)
closed
open
difference
b
Purification
FRET (%)
80
60
40
20
0
4 6 8 10 12 14
Mg2+(mM)
closed
open
difference
d
FRET (%)
80
60
40
20
0
4 6 8 10 12 14 16
Mg2+(mM)
closed
open
difference

Figure S8. Single-structure analysis of the DNA origami book biosensor

Fluorescence images were recorded 6 min after the addition of the target. a) A representative image of the FRET-based detection method. The image shows the emission profiles of single DNA origami book sensors after donor excitation at 532 nm; the green circle and red circle represent Cy3 and Cy5 emissions for the same DNA origami book biosensor, respectively. b) A representative image of the quenching-based detection method (Cy3+bh2). It shows the emission after excitation at 532 nm; the green circle represents a single DNA origami structure. c) A representative image of the quenching-based detection method (Cy5+bb650q). It shows the emission after excitation at 640 nm; the red circle represents a single DNA origami structure.

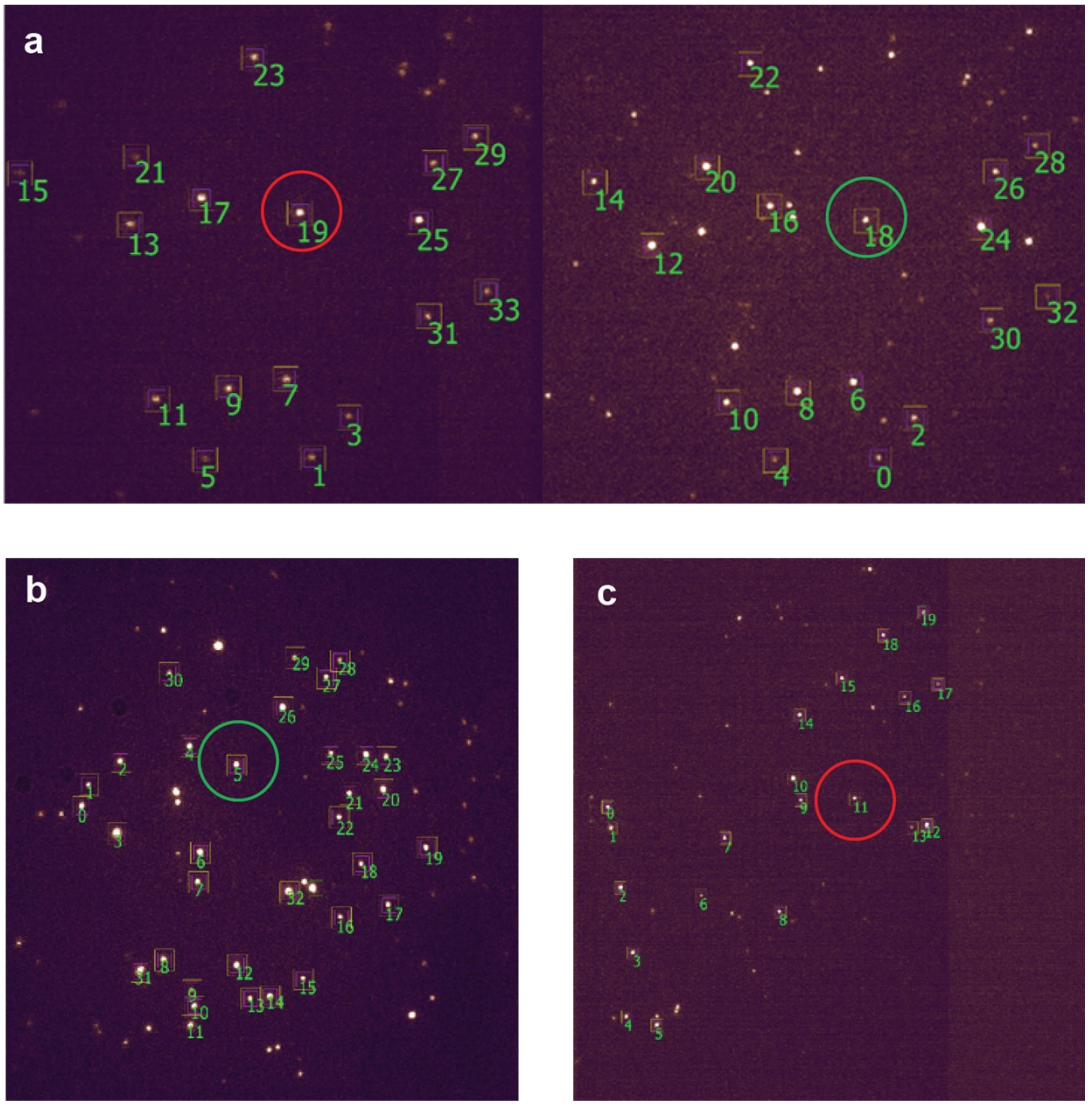


Figure S9. Effect of amount and position of the locks within DNA origami book biosensor on FRET efficiency

Schematic representation of the positioning of locks within DNA origami book biosensor. Four different structures with 15 FRET pairs were assembled with different combinations of locks: a) combination of all four locks, b) with 3 locks (1, 3 and 4), c) 2 locks at the edges (1 and 4) and d) 2 locks in the middle (2 and 3). Detection of 100 pM of target DNA, ODN-153 was performed using these structures. Results were plotted into histograms where red and green represent FRET efficiency distribution before (0 min) and after (6 min) the addition of target DNA.

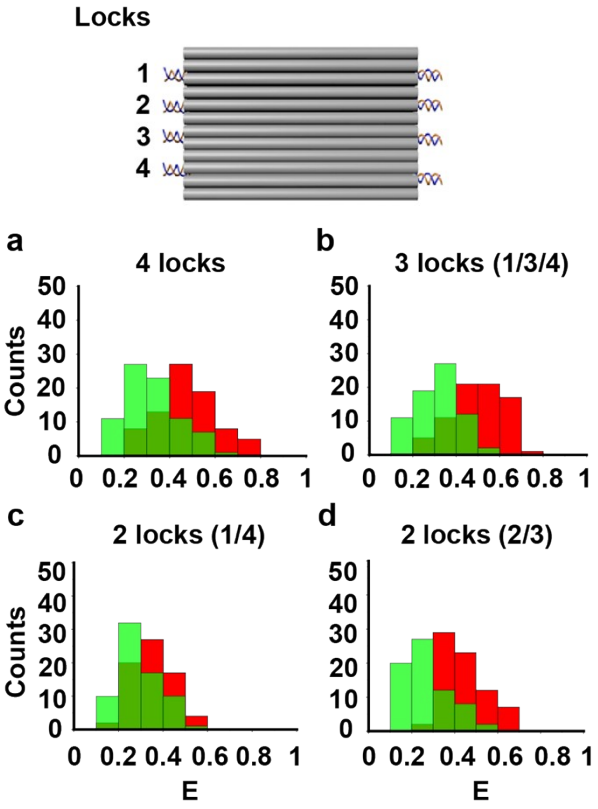


Figure S10. Single-structure study of the limit of detection for multisensing of DNA targets

The detection limit of the book biosensor with 10 quenching pairs on both sides and with the addition of 2 DNA targets at the same time. Left: Opening at the left side of DNA origami book upon addition of ODN-153. Right: Opening at the right side of DNA origami book upon addition of ODN-342. The red and green histograms represent fluorescence intensity increase after the addition of the target of interest due to the opening of the structure. A yellow histogram represents fluorescence intensity in the closed state.

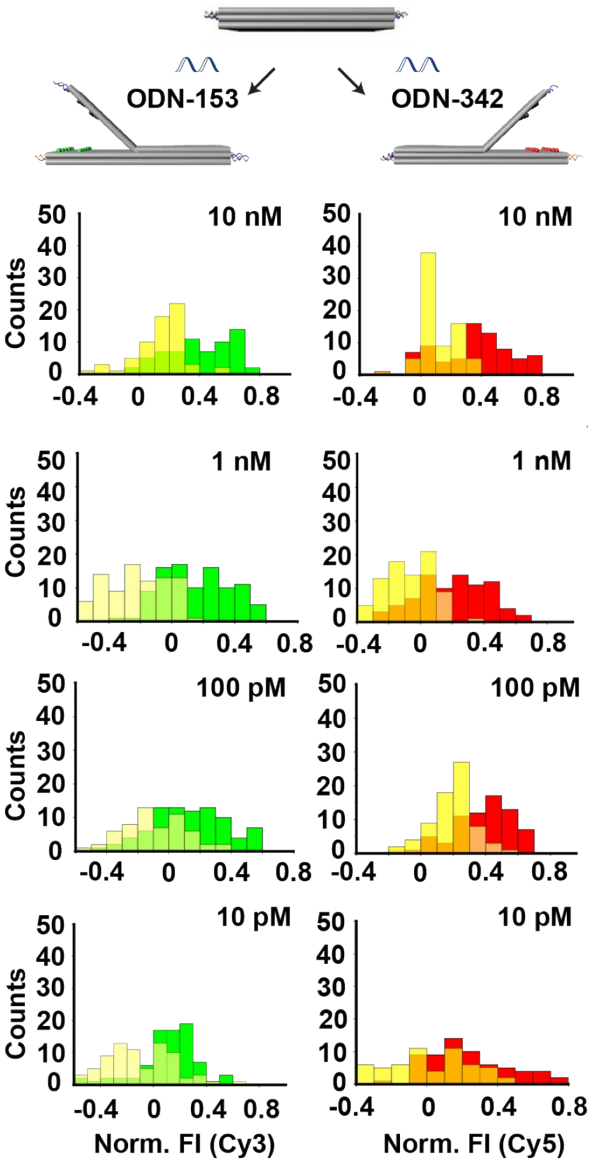
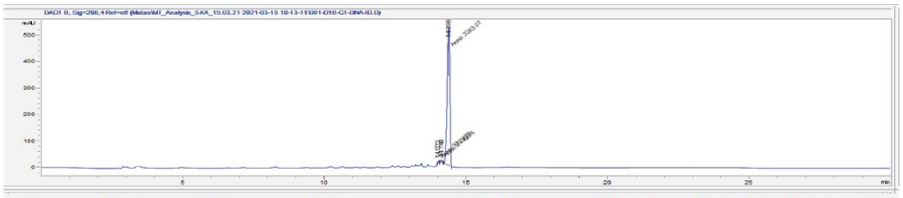


Figure S11. Terminal transferase (TdT) labeling of oligonucleotides with Cy3 and Cy5

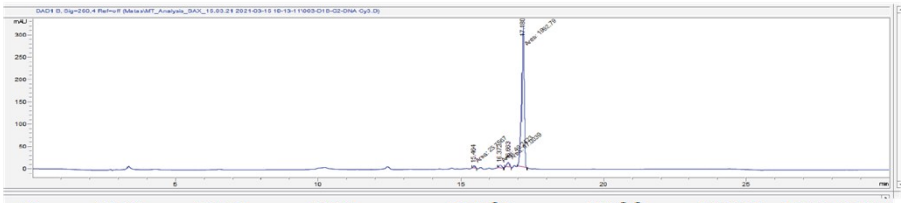
Five oligonucleotides from each column were labeled with either Cy3 or Cy5-conjugated ddUTP using TdT enzyme. The efficiency of labeling was analyzed using HPLC. Results showed that labeling of oligonucleotides with Cy3 or Cy5 was efficient with an approximate yield of 93%.

a DNA_oligo



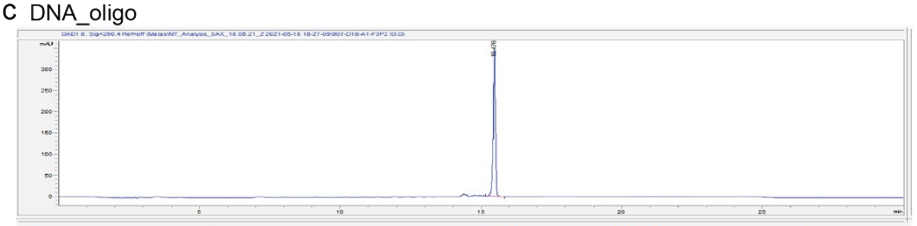
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.023	MM	33.3	10.3	0.0541	0.996	0.756
2	14.14	MM	27.4	10.9	0.042	0.820	0.998
3	14.396	MM	3283.1	521.1	0.105	98.184	1.491

b DNA_Cy3



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.464	MM	25.8	5.5	0.0776	1.207	0.85
2	16.372	MM	49.2	6.6	0.124	2.307	0.686
3	16.663	MM	67	10.7	0.1044	3.139	1.502
4	17.18	MM	1992.8	316.9	0.1048	93.348	1.359

c DNA_oligo



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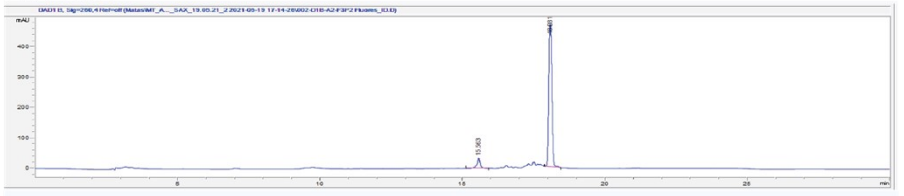
1	15.476	BB	1783.5	349.7	0.0705	100.000	0.971

d DNA_Cy5



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.563	BV R	254.3	33.4	0.1044	6.887	1.155
2	18.081	BB	3438.3	467.9	0.1177	93.113	0.885

Table S1: DNA origami book biosensor staple strand sequences

All structures were assembled using the list of oligonucleotides reported below. They are divided into different sections based on the purpose of usage.

Start	End	Core staples
0[55]	2[40]	GTCTATCATCGGAACCGAAAGGAAGTGCTTTC
0[87]	2[72]	CGTGGACTCCAACGTCCGCTAGGGTACTATGG
0[111]	2[104]	GAACAAGATGCGCGTACCGCCGCG
0[151]	2[136]	AAGAATAGCCCGAGATGGCGGTTTCCTGTCGT
0[183]	2[168]	TCCGAAATCGGCAAATTCTTTTCTTGCGCTC
0[215]	2[200]	CCCAGCAGGCGAAAATATTGCCCTCTAATGAG
1[216]	0[216]	TGGCCCTGAGAGAGTTGTCCACGCTGGTTTGC
2[135]	4[128]	GCCAGCTGGGGTAAAGTTCTGCCA
2[231]	4[216]	CATAAAGTGGTGCCGGATCAGACGATCCAGCG
3[40]	6[40]	AGGGATTTAGTAGAAGTTGCAACACTGAAAGC
3[72]	6[72]	TCCTGAGATTCTTTGATACATTTTTTCTGGCC
3[104]	6[104]	ACCGAGTATCCATCACTATTTACACACCAGTC
3[136]	6[136]	CCTCCTCACCGGGGGTGTTTCTTTGGTATGAG
3[168]	6[168]	GCTCGAATCCAGAATGTGGTGTGTGCTTTCGC
3[200]	6[200]	TCCTGTGTCTGCGCGCGATGCCGGGGTGTCCA
4[55]	1[63]	CTTGCCTGTAGACAGGCGTATAACGGGAAGAAAGCGAAA
4[87]	1[87]	AGCAATACAGTGTTTTAGGGCGCGCGCTGGCA
4[151]	1[151]	CGGTCATACAGTTGAGTCGGGAAAGCGTATTG
4[183]	1[183]	TGCTGCGGTCGTAATCAATTGCGACCAGTGA
4[215]	1[215]	CAGTGTCAGAAATTGTTGGGGTGCTCACCGCC
5[88]	3[103]	ATCGTCTGGCCGCTACTATAATCAGTGAGGCC
6[39]	8[24]	GTAAGAATTGGTCAGTAGCATCACCTTGCTGA
6[135]	8[128]	CCGGGTCAGAGAGATAAACGCGGT
6[230]	8[216]	CACGCAACAGCAGTTGTACATCGACATAAAA
7[40]	10[40]	GTCTTTAAAAATCTAATGGCAAATTATTAAAT
7[72]	10[72]	CATCGCCAAGTGCCACAGGTTATCTATTAGAC
7[104]	10[104]	GCAGAAGAGTGAGGCGAACTAATAGTCAATAG
7[136]	10[136]	CGGCCAGATTCCGGCAGACTTTCTAAACGTAC
7[168]	10[168]	CGGACTTGTGAAGGGTGGAAAAAGGGAACGGA
7[200]	10[200]	CTGGTCAGTAAAAAAACTTAAATTGTGCCAAG
8[55]	5[55]	CAAATGAATGCGCGAACTTCTGACGGAAAAAC
8[87]	5[87]	CCTGCAACTTAAAAATAAGGGACAGACGCTCA
8[151]	5[151]	GATTGCCGGCACATCCGCGGTTGCGCTCGTCA
8[183]	5[183]	TTTAGTGATAGAACGTTGCAGGCTCAGCAA
8[215]	5[215]	AAATCCCGCAGCAACCCGGCTGGAGTTACCTG
9[88]	7[103]	CTTTAGGAAGTAATAAACCGAACGAACCACCA
10[135]	12[128]	AGCGCCATCCTGTAGCTGGTGCCG
10[231]	12[216]	CACGACGTGTCACGTTGGCGCATCGTAACCGT
11[40]	14[40]	TATCATTTCAAAATTAGATGAATAATCAAGAA
11[72]	14[72]	AGGAGCGGTGAATAATCGGGAGAATACCTGAG

11[104]	14[104]	CAGATGATCAATATAATTTGAATAAAATCGCG
11[136]	14[136]	GGCTGCGCACCGCTTCCAGCTTTCAATAGGAA
11[168]	14[168]	CGGGCCTCCAGGAAGAACAACCCGAAATTTTT
11[200]	14[200]	AAGGGGGACAGTTTGAGGCGGATTTAAACGTT
12[55]	9[55]	TACCATATTGCGGAACACAACTCGCAACAGTT
12[87]	9[87]	TATACTTCAATTATCATTTAGAAGTAAAATAT
12[151]	9[151]	TTTCCGGCAACTGTTGAATCGGCGCCGTGGTG
12[183]	9[183]	ATCGGCCTTTCGCTACCGCCACGAGACGCA
12[215]	9[215]	GCATCTGCTGTGCTGCGACGGCCATCTGCTCA
13[88]	11[103]	GGATTCGCTTTGAGGATCATATTCCTGATTAT
13[120]	11[135]	CTGGCCTTGTTTACCACGCCATTCGCCATTCA
13[216]	11[231]	TGGGATAGTGTAAAACAAGGCGATTAAGTTGG
14[135]	16[128]	CGCCATCATACCAAAAGAGGGTAG
14[231]	16[216]	TTGTATAAGCAATGCCGTAGGTAAAGATTCAA
15[40]	18[40]	TTTTAATGATTTATCATAACTATATAAGAATA
15[72]	18[72]	TGTGAGTGAGATTAAGTCGCAAGAATACCGAC
15[104]	18[104]	CGCTATTACCTTAGAAAAATATATTTCATCTT
15[128]	18[136]	GGCTATCAGGTCATTGATGCCGGAACATTATGAAGCAATA
15[168]	18[168]	GAATCGATTATGATATAGCCTTTATCATACAG
15[200]	18[200]	TGTCAATCGAAAGGCCTTAGAACCACTAATAG
16[55]	13[55]	AATAGTGAGAAACAGTAAACAAACTACAGTAA
16[87]	13[87]	GATAGCTTAATAACCTATTTCAATACAATAAC
16[151]	13[151]	ATAAATTACCTGAGAGTTTTAACCATCAACAT
16[183]	13[183]	ACCATCAAGAACGGTTTCGCATTTCGGATT
16[215]	13[215]	AAGGGTGAATATGTACTTAAATTGGACCGTAA
17[88]	15[103]	GCGAGAAAAATTATTCTGCTTCTGTAAATCGT
18[135]	20[128]	AAGCCTCAAGCAAAGCTGTTTTAA
18[230]	20[216]	GCTGAAACAAACTCCGATTAGAGAGTACCTT
19[40]	22[40]	ATATGCGTAAAAGGTAGCGCCTGTCGAGAACA
19[72]	22[72]	AGCCAACGAGCCAGTAAACAAGAAGAACGGGT
19[104]	22[96]	ATCGCCATGCCAACATGAGCATGTCAATAATCGGCTGTCT
19[128]	22[128]	CTGGAAGTTTCATTCCGCTCAACAGGATTGCATCAAAAATCAGGTCTT
19[168]	22[168]	GCGAACGACTTAGAGCAAAGACTTTAAACAGT
19[200]	22[200]	TACATTTCCCTTTTGACTTCAAAGTAAATATT
20[55]	17[55]	GTACCGACTATACAAAGCGTTAAATGTAAATG
20[87]	17[87]	CATTTTCGCTCAACAGGGTTTGAACAAAGAAC
20[151]	17[151]	ATGCTGTAATATAACAGCAAAATTACCCTGTA
20[183]	17[183]	GCGGATGGGTAGATTCCAATAAATTTCAAC
20[215]	17[215]	TAATTGCTGCAAATGGTCAATTCTCTCATATA
21[88]	19[103]	TCCCATCCAATTTAATTAGGGCTTAATTGAGA
22[95]	23[119]	TTCCTTATAACGCGAGGCGTTTTAGCGAACCTCCCGACTT
22[127]	19[127]	TACCCTGACTACCAATAGAAATTATAGTCAGAGAGCATAATACGGTGT
22[231]	23[231]	GCGTCCAATACTGCGGTGCCAGAGGGGGTAAT
23[40]	21[55]	CCAATAGCAAGCAAATGCACTCATTTATCAAC
23[72]	21[87]	TATCCGGTATTCTAAGCATTCCAAAAATAATA
23[120]	21[151]	ATTACGAGGCATAGTAAGAGCAACACTATCATACCATAAATCAAAAAG
23[168]	21[183]	ACGACGATAAAAACCAAAATGCTTCAAATAT
23[200]	21[215]	TTTGCAAAAGAAGTTTAATCGTCACGAACCAG

24[167]	25[151]	TTTAGGAATACCACATTCAACTAAATGGTTTA
24[199]	25[183]	TATTACAGGTAGAAAGATTCATCTACCTTA
24[223]	25[223]	TAAAACGAACTAACGGATACCAGTCAGGACGT
2[39]	4[32]	CTCGTTAGGCCAGCCAAACTCAAA
21[216]	19[223]	ACCGGAAGAGGTGGCATCAATAAC
10[39]	12[24]	CCTTTGCCTAACGTCATTTGCACGTAAAACAG
26[39]	24[32]	GATAACCCCCATATTATTTATCCCTTACCAACGCTAACGA
26[71]	24[72]	TCAGAGGCGATTTTTTGTTTAACTTTGCACC
26[167]	24[168]	GCTCATTCTTTAATCATTGTGAATAGTTGAGA
26[199]	24[200]	TATTCATTTAAGAACTGGCTCATTAACAACAT
33[56]	35[55]	CGCCAGCATACATGGCTTTTGATTGCCCCCT
33[88]	35[95]	AGACGATTAGTCTCTGAATTTACCTGAAACATGAAAGTAT
1[64]	0[56]	GGAGCGGGAAAGGGCGAAAAACC
1[88]	0[88]	AGTGTAGCGGTCACGCGTCCACTATTAAAGAA
18[167]	20[152]	GCAAGGCAGAAGCCCGTTAATTGCTGAATATA
18[199]	20[184]	TAGTAGCAAATTCGAGTAAGAGGTCATTTTT
22[39]	23[39]	AGCAAGCCGTTTTTATGGAATCATTACCGCGC
22[71]	23[71]	ATTAAACCAAGTACCCAGATATAGAAGGCT
22[167]	23[167]	TCAGAAAACGAGAATGAACCCTCGTTTACCAG
22[199]	23[199]	CATTGAATCCCCCTCAAATAGCGAGAGGCT
14[39]	16[32]	AACAAAATGGGTTATAAAATCATA
1[24]	0[32]	CCGGCGAACGTGGCGACTAAAGGG
5[216]	3[223]	CAGCCAGCGTAAAGCCTATCCGCT
9[216]	7[223]	TTTGCCGCCCAGCTTAGCAAGAAT
17[216]	15[223]	TTTTAAATGCAAATATCCCGGTTG
18[39]	20[32]	AACACCGGTGCAGAACAAGTAATT
26[215]	27[223]	CTTGACAATGTACAGACCAGGCGC
28[215]	29[223]	TGACCCCCAACCTAAAACGAAAGA
30[223]	31[223]	ATATTCGGTCGCTGAGCCGACAATGACAACAA
32[215]	33[223]	TTCAACAGAGACGTTAGTAAATGA
34[215]	35[223]	ACCCTCAGTATCACCGTACTCAGG

Start	**End**	**foot staples**
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3[112]	1[127]	AAAGATGTAATCACACACCACCGGCCAACGCG
4[127]	34[120]	GCACGCGTAATCACCATTCGTCCT
5[120]	3[135]	CTGGTAATCATTAATGTCTTCGCGTCCGTGAG
7[112]	5[119]	TAAAATCTCTGAGATTTTGGCCGG
8[127]	32[120]	CCGTTTTTTCACTCCAAAAGGTAG
9[112]	7[135]	GATTACCGGAATTTGTCTGTTGCCCGTCGCTGGCAGCCTC
12[127]	30[120]	GAAACCAGGTAACAGACGGCTAAT
16[127]	28[120]	CTATTTTTCGCCATGTTGCTCAAT
17[112]	13[119]	TTTAGTAAATCGGTTGAAAATAATTCGTTACACCAAGCGT
20[127]	26[120]	ATATGCAATAAGAGTAAGAACAAA

24[127]	18[112]	CAAAAGGAGCGGGAGGGAATAACACTAACAACATTTAAAGAGCTTAAT
26[119]	15[127]	CAGGGAAGCGAGGAAAGAGTTTTCATTAAAGATCTACAAA
28[119]	10[112]	AATAACGGGAGGGAAGGCATTCATGGCAAAAGGTCGAGCC
30[119]	8[112]	ATTGACGGATTTTCGGTCGCAGAG
32[119]	4[112]	CCCCCTTAAACAAATAGCCGTCTG
34[119]	0[112]	CATTAAAGCTCAAGAGCCAGTTTG

Start	**End**	**hinge staples**
25[104]	24[96]	TACAGAGATTTTGAAGCCTTAAAT
33[137]	31[135]	ACAACGCTGAAAATCTCCAAAAAAAAGGAGC
35[96]	33[103]	TAAGAGGCTGAGACTCCCAGAATGGAAAGCGCGGCCTTGA
35[128]	33[136]	GGATTAGCGGGGTTTTTACCGTAACACTGAGTGTACAAACT
25[136]	24[128]	GGCTTGAGTGCAGATACATAACGC
27[104]	25[103]	AAGGAAACCGCATTAGACGGGAGAGCAGCCTT
27[136]	25[135]	CGGAACGACCTGACGAGAAACACCGTAAATTG
29[104]	27[103]	GATTGAGGAATACCCAAAAGAACTGTTACCAG
29[136]	27[135]	GGACTAAAGTCGAAATCCGCGACCTACTTAGC
31[104]	29[103]	TCATCGGCAAATTATTCATTAAAGATTCAACC
31[136]	29[135]	CTTTAATTGGAACGAGGGTAGCAAGGCTTTGA
33[104]	31[103]	TATTCACATTAGCGTTTGCCATCTGCGCGTTT

Start	**End**	**staples with biotin**
21[216]	19[223]	ACCGGAAGAGGTGGCATCAATAAC
2[39]	4[32]	CTCGTTAGGCCAGCCAAACTCAAA
10[39]	12[24]	CCTTTGCCTAACGTCATTTGCACGTAAAACAG
21[56]	19[71]	AATAGATAAAATAAGTTCTTACCAGTATAA
2[199]	4[184]	TGAGCTAACGGCATCACTGTGCACTCTGTGG
10[199]	12[184]	CTTTCAGAGAACAAACGGGGACGACGACAGT

Start	**End**	**endcap staples**
1[10]	1[24]	**CCC**GACGGGGAAAGC
2[245]	2[232]	**CCCC**CGAGCCGGAAG
5[10]	5[24]	**CCC**TATCCAGAACAA
6[245]	6[231]	**CCC**GTGGTGCCATCC
9[11]	9[23]	**CCCC**CAAACCCTCAA
10[245]	10[232]	**CCCC**GTTTTCCCAGT
11[232]	11[245]	GTAACGCCAGG**CCCC**
12[23]	12[10]	AAATAAAGAAA**CCCC**
13[11]	13[23]	**CCCC**TTGCGTAGATT
14[245]	14[232]	**CCCC**AAACAGGAAGA

17[10]	17[24]	**CCC**TTAACCTCCGGC
18[245]	18[231]	**CCC**TTTGGGGCGCGA
21[11]	21[24]	**CCC**AAACAACATGTT
22[245]	22[232]	**CCCC**TAGACTGGATA
23[232]	23[245]	AGTAAAATGTT**CCCC**
24[31]	24[8]	GCGTCTTTCCAGAGCCTAATT**CCC**
24[237]	24[224]	**CCCC**ATCTACGTTAA
25[224]	25[237]	TGGGAAGAAAA**CCCC**
26[23]	26[8]	TGAGTTAAGCCCA**CCC**
27[224]	27[237]	ATAGGCTGGCT**CCCC**
28[23]	28[8]	TATAAAAGAAACG**CCC**
29[8]	30[8]	**CCC**CAAAGACACCACGGAATAAGTCACCATTACCATTAGCAAGGC**CCC**
29[224]	29[237]	GGCAAAAGAAT**CCCC**
30[237]	30[224]	**CCCC**GCATAACCGAT
31[8]	32[8]	**CCC**CGGAAACGTCACCAATGAAACCCTCAGAACCGCCACCCTCAG**CCC**
31[224]	31[237]	CCATCGCCCAC**CCCC**
33[224]	33[237]	ATTTTCTGTAT**CCCC**
35[224]	35[237]	AGGTTTAGTAC**CCCC**

Start	**End**	**Cy3_staples column 1**
28[39]	26[40]	CATAAAGGGAAATAGCAATAGCTAATCAGAGA
30[39]	28[40]	ACCAGTAGTTATTTTGTCACAATCAATACATA
32[39]	30[40]	GCCGCCACCATCGATAGCAGCACCGCAAAATC
34[39]	32[40]	AGTGTACTCACCAGAACCACCACCCCCTCAGA
35[32]	34[40]	CCGTATAAACAGTTAAGATACAGG
		Cy3_staples column 2
24[71]	25[55]	CAGCTACAATTTTATCCTGAATCAATCCAAA
25[56]	27[55]	TAAGAAAGTAATTGAGCGCTAATTCTTACCG
27[56]	29[55]	AAGCCCTTTTAGCAAACGTAGAAAATAGAAA
29[56]	31[55]	ATTCATATTGGGAATTAGAGCCAGTAATCAG
31[56]	33[55]	TAGCGACACCACCGGAACCGCCTAGAGCCGC
		Cy3_staples column 3
28[71]	26[72]	GCAGTATGTTTAAGAAAAGTAAGGAACAAAG
30[71]	28[72]	GAGCCATTGGTTTACCAGCGCCACTTATTAC
32[71]	30[72]	CAGAGCCAGAATCAAGTTTGCCTACCGACTT
34[71]	32[72]	AAGCGTCATTGACAGGAGGTTGAACCGGAAC
35[56]	34[72]	GCCTATTTCGGAACCTATTATTCGTTCCAGT
		Cy3_staples column 4
24[95]	25[87]	CAAGATTAGTTGCTATGTCAAAAA
25[88]	27[87]	TGAAAATAATTAACTGAACACCCTCAGATAGC
27[88]	29[87]	CGAACAAAGGCATGATTAAGACTCAAGACAAA
29[88]	31[87]	AGGGCGACGTGAATTATCACCGTCTTAGCGTC
31[88]	33[87]	AGACTGTATTTCATAATCAAAATCGGCAGGTC

		Cy3_staples column 5 (FRET) or Cy5_staples column 4 (quenching)
25[152]	27[151]	ATTTCAACAGTGAATAAGGCTTGCGGCGCAGA
27[152]	29[151]	CGGTCAATTCGCCTGATAAATTGTGACTTTTT
29[152]	31[151]	CATGAGGAAGCGAAAGACAGCATCGTATCGGT
31[152]	33[151]	TTATCAGCAATAATTTTTTCACGTCTGTAGCA
33[152]	35[151]	TTCCACAGCCAATAGGAACCCATGGCTCAGTA
		Cy3_staples column 6 (FRET) or Cy5_staples column 3 (quenching)
28[167]	26[168]	TTGTATCACATAAGGGAACCGAACACAAAGCT
30[167]	28[168]	CCCTCAGCAGTTTCCATTAAACGGACGGAGAT
32[167]	30[168]	TTGCGAATTTGCTTTCGAGGTGAAGATCGTCA
34[167]	32[168]	TAGCAAGCACAGCCCTCATAGTTATAAAGGAA
35[152]	34[168]	CCAGGCGGATAAGTGCCGTCGAGATTCAGGGA
		Cy3_staples column 7 (FRET) or Cy5_staples column 2 (quenching)
25[184]	27[183]	TGCGATTTACCCAAATCAACGTATGACCAA
27[184]	29[183]	CTTTGAAAGCGAAACAAAGTACAGTAAAAT
29[184]	31[183]	ACGTAATGAGGCCGCTTTTGCGGTTTCTTA
31[184]	33[183]	AACAGCTTATAGAAAGGAACAACGCGTAAC
33[184]	35[183]	GATCTAAAGCCACCACCCTCATTGGGTTGA
		Cy3_staples column 8 (FRET) or Cy5_staples column 1 (quenching)
28[199]	26[200]	TACCAAGCGAGGACAGATGAACGGGAACCGGA
30[199]	28[200]	GGAGTTAACCACTACGAAGGCACCAGCGATTA
32[199]	30[200]	GAGTGAGAGATACCGATAGTTGCGGCTTGCAG
34[199]	32[200]	CCCTCAGAGTTTTGTCGTCTTTCCTTTCAGCG
35[184]	34[200]	TATAAGTATAGCCCGGAATAGGTGAACCGCCA

Start	**End**	**Cy5_staples column 1 (FRET) or bh2_staples column 1 (quenching)**
5[24]	3[39]	ATATTACCAATCAGAGACAGGAGGCCGATTAA
13[24]	11[39]	TTCAGGTTCGAACGTTAAAGTTTGAGTAACAT
17[24]	15[39]	CTTAGGTTTAATTACATCATTTGAATTACCTT
9[24]	7[39]	TCAATATCACGTGGCATTTTGAATGGCTATTA
21[24]	19[39]	TCAGCTAAAATCATAAAAGCCTGTTTAGTATC
		Cy5_staples column 2 (FRET) or bh2_staples column 2 (quenching)
2[71]	4[56]	TTGCTTTGAAATACCTTAGTAATAACATCA
6[71]	8[56]	AACAGAGATTGAGGAGCTGAGAGCCAGCAG
10[71]	12[56]	TTTACAAATTTACATGGAAGGGTTAGAACC
14[71]	16[56]	CAAAAGAAAATCCAAACGCTGAGAAGAGTC
18[71]	20[56]	CGTGTGATAGTCCTGATAAGAGAATATAAA
		Cy5_staples column 3 (FRET) or bh2_staples

		column 3 (quenching)
5[56]	3[71]	GCTCATGGACGAGCAAACGGTACGCCAGAA
9[56]	7[71]	GAAAGGAATAGAACCCTGATAGCCCTAAAA
13[56]	11[71]	CAGTACCTCAATTCGAAAGAAACCACCAGA
17[56]	15[71]	CTGATGCAGATGATGACATAAATCAATATA
21[56]	19[71]	AATAGATAAAATAAGTTCTTACCAGTATAA
		Cy5_staples column 4 (FRET) or bh2_staples column 4 (quenching)
2[103]	4[88]	CTTAATGCAAATGGATGCAAATTAACCGTTGT
6[103]	8[88]	ACACGACCGCACTAACGTCAGTATTAACACCG
10[103]	12[88]	ATAATACACTGATTGCTCCTGATTGTTTGGAT
14[103]	16[88]	CAGAGGCGACTTTTTCTCCTTGAAAACATAGC
18[103]	20[88]	CTGACCTATAATTTACGTAATTTAGGCAGAGG
		Cy5_staples column 5 (FRET) or bb650q_staples column 4 (quenching)
1[152]	0[152]	GGCGCCAGGGTGGTTTATCCCTTATAAATCAA
2[167]	4[152]	ACTGCCCGCCTTACACCGGCGGGCCGTTTTCA
6[167]	8[152]	ACTCAATCGCTCTCACAAAGTTAAACGATGCT
10[167]	12[152]	TAACCTCAAGCGAGTATCGCACTCCAGCCAGC
14[167]	16[152]	GTTAAATCGCGGGAGATCAACCGTTCTAGCTG
		Cy5_staples column 6 (FRET) or bb650q_staples column 3 (quenching)
5[152]	3[167]	TAAACATCCTTTCCAGGATCCCCGGGTACCGA
9[152]	7[167]	AAGGGATACGCCGGGCTCATAACGGAACGTGC
13[152]	11[167]	TAAATGTGCCGGAAACGGAAGGGCGATCGGTG
17[152]	15[167]	ATACTTTTAGCTCATTTCTGGAGCAAACAAGA
21[152]	19[167]	ATTAAGAGAAGAATTAGTTGATTCCCAATTCT
		Cy5_staples column 7 (FRET) or bb650q_staples column 2 (quenching)
1[184]	0[184]	GACGGGCAACAGCTGCCTGTTTGATGGTGGT
2[199]	4[184]	TGAGCTAACGGCATCACTGTGCACTCTGTGG
6[199]	8[184]	GCATCAGCGGATCAAAGCCGCACAGGCGGCC
10[199]	12[184]	CTTTCAGAGAACAAACGGGGACGACGACAGT
14[199]	16[184]	AATATTTTAAAAATTTGGAGACAGTCAAATC
		Cy5_staples column 8 (FRET) or bb650q_staples column 1 (quenching)
5[184]	3[199]	ATCGTTAACTCACATTATGGTCATAGCTGTT
9[184]	7[199]	GAAACAGCGGGGTCATCAGCGTGGTGCTGGT
13[184]	11[199]	CTCCGTGGGGTGGAGTTACGCCAGCTGGCGA
17[184]	15[199]	GCAAGGATGTTAAAAAATCGTAAAACTAGCA
21[184]	19[199]	CGCGTTTTTTAACATTAGTTTGACCATTAGA

Start	End	Locks for ODN-153 (blue) and ODN-342 (orange)

0[31]	0[0]	AGCCCCCGATTTAGAGCTTTTGATCACTTTTGTGACTATGCAA
3[224]	3[255]	CACAATTCCACACAACATAACGGGTGCGATTTCTGTGTGAGA
4[31]	4[0]	CTATCGGCCTTGCTGGTAATTGATCACTTTTGTGACTATGCAA
7[224]	7[255]	GCCAACGGCAGCACCGTCGACGGGTGCGATTTCTGTGTGAGA
15[224]	15[255]	ATAATCAGAAAAGCCCCAAACGGGTGCGATTTCTGTGTGAGA
16[31]	16[0]	GGTCTGAGAGACTACCTTTTTGATCACTTTTGTGACTATGCAA
20[31]	20[0]	CTGTCCAGACGACGACAATTTGATCACTTTTGTGACTATGCAA
19[224]	19[255]	CTGTTTAGCTATATTTTCAACGGGTGCGATTTCTGTGTGAGA
25[0]	26[24]	AGTCACAAAAGTGATCTGCCAGTTACAAAATAAACAGACAAGAAT
26[255]	26[216]	ACAGAAATCGCACCCGTTTTTTTTTGACCTTCATCAAGAGTAAT
27[0]	28[24]	AGTCACAAAAGTGATCATAATAAGAGCAAGAAACAATTGGCAACA
28[255]	28[216]	ACAGAAATCGCACCCGTTTTTTTTACACTAAAACACTCATCTT
33[0]	34[16]	AGTCACAAAAGTGATCAGCCACCACCCTCAGAGCCGCGGTAATAAGTTTTAAC
32[255]	32[216]	ACAGAAATCGCACCCGTTTTTTTTGGGATTTTGCTAAACAACT
35[0]	35[31]	AGTCACAAAAGTGATCCAGTGCCTTGAGTAACAGTGC
34[255]	34[216]	ACAGAAATCGCACCCGTTTTTTTTCGCCACCCTCAGAACCGCC

Locks for miR-21 (left side)

AGCCCCCGATTTAGAGCTTTTTCAACATCAGTCTGATAAGCTA
CTATCGGCCTTGCTGGTAATTTCAACATCAGTCTGATAAGCTA
GGTCTGAGAGACTACCTTTTTTCAACATCAGTCTGATAAGCTA
CTGTCCAGACGACGACAATTTTCAACATCAGTCTGATAAGCTA
ATCAGACTGATGTTGATGCCAGTTACAAAATAAACAGACAAGAATTGAGTTAA
ATCAGACTGATGTTGAATAATAAGAGCAAGAAACAATTGGCAACA
ATCAGACTGATGTTGAAGCCACCACCCTCAGAGCCGCGGTAATAAGTTTTAAC
ATCAGACTGATGTTGACAGTGCCTTGAGTAACAGTGC

Locks for let-7a (right side)

CACAATTCCACACAACATAAACTATACAACCTACTACCTCA
GCCAACGGCAGCACCGTCGAACTATACAACCTACTACCTCA
ATAATCAGAAAAGCCCCAAAACTATACAACCTACTACCTCA
CTGTTTAGCTATATTTTCAAACTATACAACCTACTACCTCA
AGTAGGTTGTATAGTTTTTTTTTGACCTTCATCAAGAGTAAT
AGTAGGTTGTATAGTTTTTTTTACACTAAAACACTCATCTT
AGTAGGTTGTATAGTTTTTTTTGGGATTTTGCTAAACAACT
AGTAGGTTGTATAGTTTTTTTTCGCCACCCTCAGAACCGCC

Table S2: List of the target key oligonucleotide sequences

miRNAs (or DNA analogs)	Sequence
ODN-342	**TCTCACACAGAAATCGCACCCGT (GC%-52.2)**
ODN-153	**TTGCATAGTCACAAAAGTGATC (GC%-40.9)**
let-7a	**UGAGGUAGUAGGUUGUAUAGUU (GC%-36.4)**
miR-21	**UCAACAUCAGUCUGAUAAGCUA (GC%-36.4)**
non-target	**CATCATAATTTTAAAACAATT (GC%-14.2)**

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