

Supplementary information

Single Molecule Analysis of Structural Fluctuations in DNA Nanostructures

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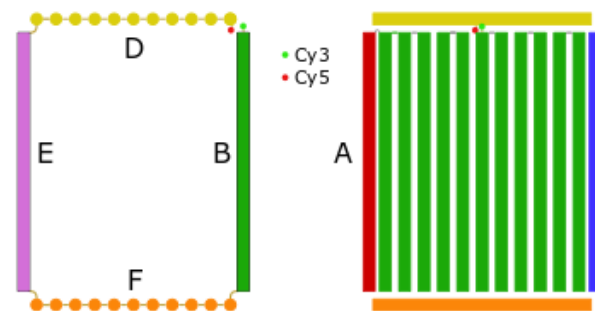


Figure S1: Schematic view of the DNA box showing number of DNA helices and their orientation, including position of the Cy3 and Cy5 fluorophores. Left panel: side view, right panel: front view. Each face is represented with a different color. The letters A-F correspond to how faces were labelled in Ref. [1].

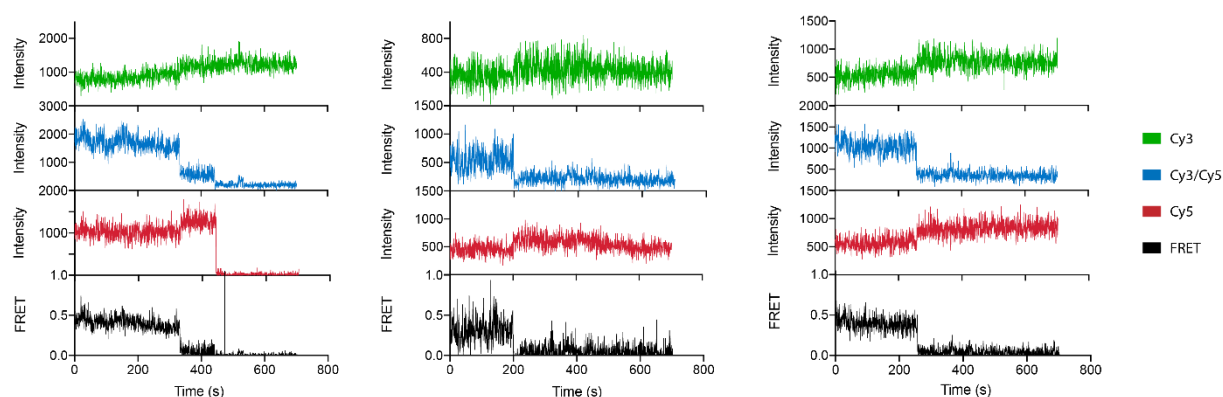


Figure S2: Examples of single molecule time traces of the original DNA box initially in the closed state where DNA keys were added after 100 s. Measurements were performed in the presence of 12.5 mM Mg^{2+} after exposing the sample to 250 mM Mg^{2+} for more than half an hour. The green time trace is Cy3 emission after Cy3 excitation, the blue is Cy5 emission after Cy3 excitation, the red is Cy5 emission after Cy5 excitation and the black is the calculated FRET efficiency. In the Cy5 time trace (red), it can be seen that the intensity increases upon opening of the box.

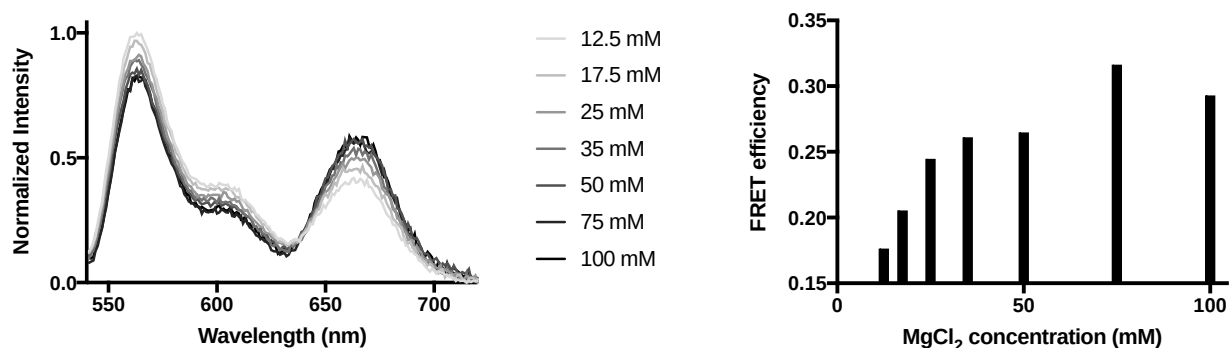


Figure S3: Ensemble FRET data of the original DNA box as a function of Mg^{2+} concentration. Left: Fluorescence spectra. Right: FRET efficiencies.

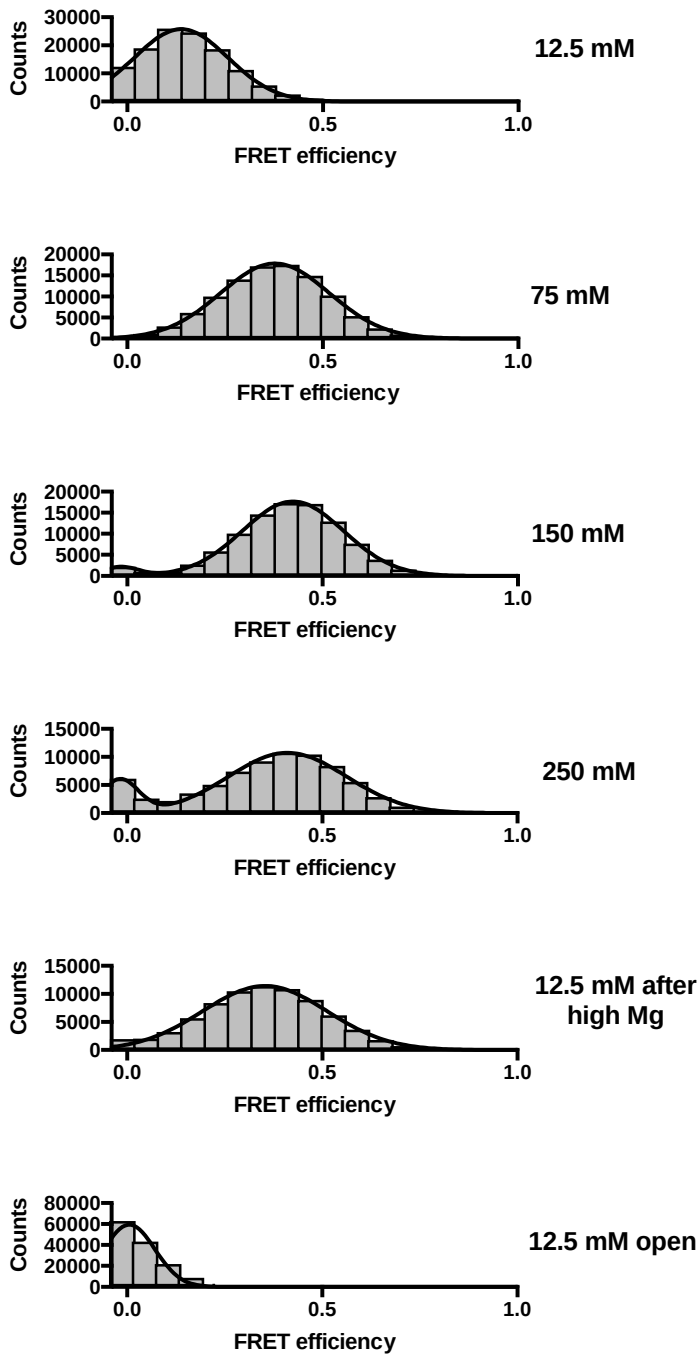


Figure S4: smFRET histograms of the closed original DNA box in the presence of Mg^{2+} at a concentration of 12.5 mM, 75 mM, 150 mM, 250 mM, 12.5 mM after high Mg^{2+} concentration and at 12.5 mM after addition of DNA keys.

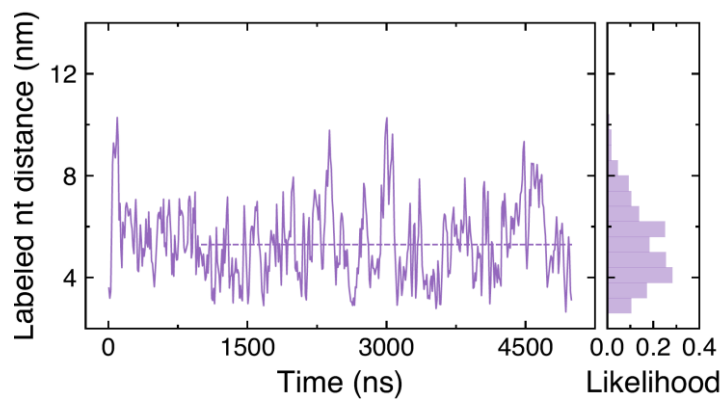


Figure S5: Time series of the distance between fluorescently labeled nucleotides during the BD simulation of the box for the design with fluorophores positioned on neighbouring helices on the front face of the box (Figure 4a). The dashed line shows the average value of the distance for the last 4 μ s of the 5 μ s trajectory. The histogram at the right depicts the distribution of the distance during the same period of time.

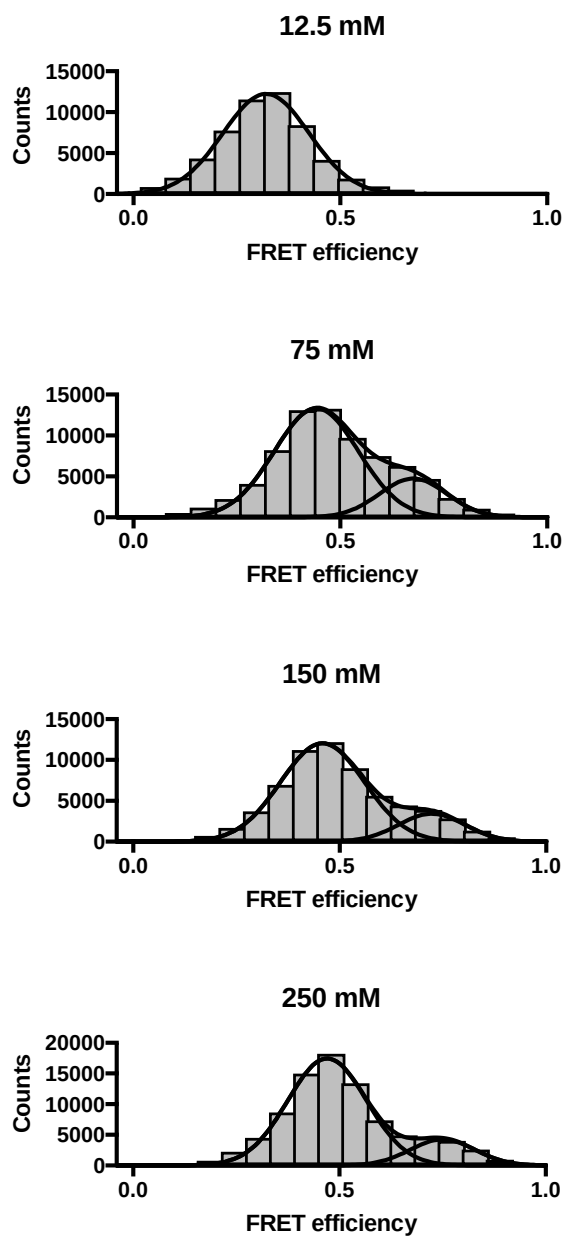


Figure S6: smFRET histograms of the closed DNA box with fluorophores positioned on neighbouring helices on the front face of the box (see Figure 4a). Measurements were performed in the presence of 12.5 mM, 75 mM, 150 mM and 250 mM MgCl_2 .

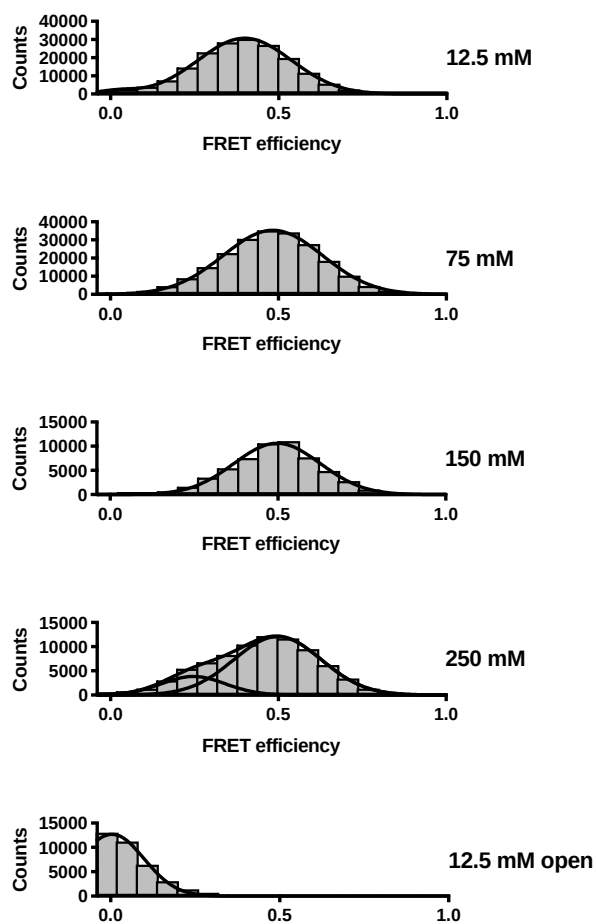


Figure S7: smFRET histograms of the modified closed DNA box (Figure 5a) in the presence of Mg^{2+} at a concentration of 12.5 mM, 75 mM, 150 mM, 250 mM, and at 12.5 mM after the additions of keys. The last histogram is based on 54 molecules

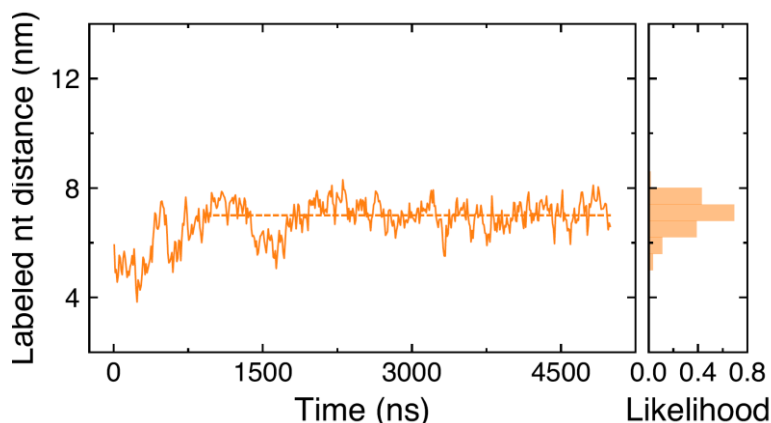


Figure S8: Time series of the distance between fluorescently labeled nucleotides during a BD simulation of the closed linked box design. Donor position here is the same as in the original box design. The dashed line shows the average value of the distance from the last 4 μ s of the 5 μ s trajectory. The histogram at the right depicts the distribution of the distance during the same period of time.

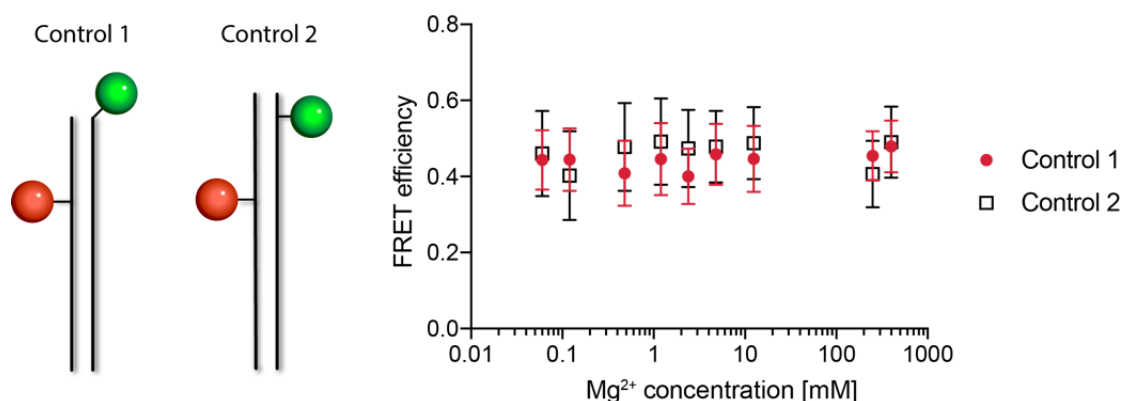


Figure S9: Schematic view of the double stranded DNA controls showing the two different positions of the acceptor, left panel. FRET efficiency as a function of Mg^{2+} concentration extracted from single molecule FRET data, right panel.

References:

[1] E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf and J. Kjems, *Nature*, 2009, **459**, 73-76.

Movie 1: BD simulations of the closed original box design. The Cy3 and Cy5 fluorophores are shown as bright green and red spheres, respectively.

Movie 2: BD simulations of closed original (left; blue) and linked (right; orange) box designs. The top and bottom rows show the systems from above and from the side, respectively. Double-stranded DNA helices are shown using a large-diameter tube representation and single-stranded DNA is shown with a narrow bond representation. The Cy3 and Cy5 fluorophores are shown as bright green and red spheres, respectively.

DNA sequences and DNA origami box designs (see also additional supporting files)

Sequences for control DNA oligonucleotides

The following sequences were used for results presented in Fig. S8.

Sequence:	Sample name:
[Cy3]GTAATCATGGTCATAGTACCGAGCTCGAATTCTT-biotin	Control 1
AAGAATTCGAGCTCGGTAC[Cy5]TATGACCATGATTAC	Control 1
GACT[Cy3]GTAATCATGGTCATAGTACCGAGCTCGAATTCTT-biotin	Control 2
AAGAATTCGAGCTCGGTAC[Cy5]TATGACCATGATTACAGTC	Control 2

DNA origami box

We describe here three different DNA origami designs:

1. A design similar to the original DNA box design.
2. A design where the helices on face B have been linked.
3. A design where the Cy5 dye of the FRET pair has been moved to the neighboring helix on the same face (face B).

All designs and complete oligo sequences are included as Supplementary Files in their proper data formats, i.e. CSV and Excel files for tabulated data (oligo sequences), and Cadnano2 files for the Cadnano2 designs.

Here, in this Supplementary Information document, we only list the full set of oligo sequences for a single, primary design. The other designs are then described by the oligos that have been added or removed compared to this primary design. These differences can be reproduced by running a `diff` comparison between the oligo sequence files listed in Supplementary Files zip archive.

#1: Primary design

The primary design, denoted `invFseam-4bio-pair`, is identical to the original DNA Box design, with the following changes:

- The seam of face F has been inverted so that the nicks between staple strands face outwards.
- The biotin-labelled staples are marked in orange-red (#f74308).

The oligo sequences for the primary design are:

Start	End	Sequence	Len	Color	Pool-name
16[263]	17[225]	[Cy3]GTAATCATGGTCATAGTACCGAGCTCGAATTCTT	34	#ff00ff	FRET-pair
16[280]	22[240]	[Cy5]AGCGAAAGACAGCATCGGAACGAGGGT	27	#ff00ff	FRET-pair
0[191]	2[192]	CAAAATCGACCTACCATATCAAAATCCTGATT	32	#03b6a2	foldA
0[223]	2[224]	GGATTTCGCCAGAAATAAAGAAATTAACCACCA	32	#03b6a2	foldA
2[191]	4[192]	ATCAGATGGATTAGAAAGTATTAGATTGAGGA	32	#03b6a2	foldA
2[223]	4[224]	GAAGGAGCGACAACCTCGTATTAAGTGGTCAG	32	#03b6a2	foldA
3[176]	1[175]	CATTTGAGATGGCAATTCATCAATTAATGGAA	32	#03b6a2	foldA
3[208]	1[207]	AACAATTCGGAATTATCATCATTTATTTGTC	32	#03b6a2	foldA
3[240]	1[239]	CCGAACGTTTTGCGGAACAAAGAGCGTAGAT	32	#03b6a2	foldA
4[191]	6[192]	AGGTTATCGGCGGTCTAGTATTAATAATGCGC	32	#03b6a2	foldA
4[223]	6[224]	TTGGCAAACACGCTGAGAGCCAGACAGACAA	32	#03b6a2	foldA
5[176]	3[175]	AGAGGTGATAAAATATCTTTAGGAAGATAATA	32	#03b6a2	foldA
5[208]	3[207]	CAACAGTGTCAACAGTTGAAAGGAACCTTACA	32	#03b6a2	foldA
5[240]	3[239]	GAAAAATCAACCCTCAATCAATATTCCTTTC	32	#03b6a2	foldA
6[191]	8[192]	GAAGTATAAATGGATTATTTACATCCAGAAC	32	#03b6a2	foldA
6[223]	8[224]	TATTTTGTACACGACCAAGTAATAAGAAGTCT	32	#03b6a2	foldA
7[176]	5[175]	ATCGTCTGAGCCCTAAACATCGCGATAAAAC	32	#03b6a2	foldA
7[208]	5[207]	TTCACGAGAATGGCTATTAGTCTTACCGCTG	32	#03b6a2	foldA
7[240]	5[239]	CATTCTGGCGTAAGAATACGTGGCCAGCAAAAT	32	#03b6a2	foldA
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8[223]	10[224]	AAACTATCTAAAAGAGTCTGTCCAGGTTGCTT	32	#03b6a2	foldA
9[176]	7[175]	AATCCTGACGCCAGCCATTGCAACGACGCTCA	32	#03b6a2	foldA
9[208]	7[207]	CCACCGAGGGCCTTGCTGGTAATATTGGCAGA	32	#03b6a2	foldA
9[240]	7[239]	ATTAACCGTCACTTGCTGAGTAGAAAAAGGGA	32	#03b6a2	foldA
12[151]	14[152]	TCACCAATCGGCAAAATCCCTTAGGCGCTGA	32	#cc0000	foldB
12[183]	14[184]	TCTATCAGCCGAGATAGGGTTGAGACGGGCA	32	#cc0000	foldB
13[136]	11[135]	TTCCGAAATCAAGTTTTTGGGGTAAGGGGA	32	#cc0000	foldB
13[168]	11[167]	AAGAATAGGGCGATGGCCCACTACGAAAGCCG	32	#cc0000	foldB
13[200]	11[199]	CCAGTTTGCCAACGTCAAAGGGCGAAGAAAGC	32	#cc0000	foldB
14[151]	16[152]	GAGAGTTGCGCTTTCCAGTCGGGACGAGCCGG	32	#cc0000	foldB
14[183]	16[184]	ACAGCTGATGCATTAATGAATCGGATTGTTAT	32	#cc0000	foldB
15[136]	13[135]	TCACTGCCAGCAAGCGGTCCACGGATGGTGG	32	#cc0000	foldB
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17[136]	15[135]	GTCACGACAGTGTAAGCCTGGGGCGTTGCGC	32	#cc0000	foldB
17[168]	15[167]	AGCTTGCAAATTCACACAACATAAACCTGTC	32	#cc0000	foldB
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24[111]	26[112]	TCATATATAATCATACAGGCAAGGTTTAGCTA	32	#57bb00	foldC
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25[128]	23[127]	TAGCAAAACAAGGATAAAAAATTTTATGATAT	32	#57bb00	foldC
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28[111]	30[112]	GAGTACCTATCAGGTCTTTACCTCGGAATCG	32	#57bb00	foldC
28[143]	30[144]	CCGGAAGCAGCAAAGCGGATTGCAAAAATGTT	32	#57bb00	foldC
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24[239]	26[240]	CCTAAAACCTCTTGACCCCCAGCGGATTTGTA	32	#b8056c	foldD
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5[80]	4[64]	TAACATAACCTCCGGCTTAGGTTTTTCATCG	32	#7300de	foldF
5[96]	7[95]	CTGATGCACTTTTCAAATATATTGGTTTGA	32	#7300de	foldF
6[47]	4[48]	TTTCCTTAGCACTCATCGAGAACAATTACCGC	32	#7300de	foldF

7[96]	9[95]	AATACCGAAACACCGGAATCATAAGTTATACA	32	#7300de	foldF
8[47]	6[48]	GCGCTGTAATAATATCCCATCTCGGCTGTC	32	#7300de	foldF
8[63]	9[79]	TCAGCTAAAGACGACGACAATAAAGTTTAGTA	32	#7300de	foldF
9[80]	8[64]	TCATATGCTTACTAGAAAAGCCTCAACATGT	32	#7300de	foldF
2[63]	3[79]	TGCGGGAGGGCGTTTTAGCGAACCAGCGATAG	32	#f74308	foldF-biotin
3[80]	2[64]	CTTAGATTGAATCCTTGAAAACATCCCCACT	32	#f74308	foldF-biotin
6[63]	7[79]	TCAATAATAATTTACGAGCATGTATCTGACCT	32	#f74308	foldF-biotin
7[80]	6[64]	AAATTTAATTAGTTAATTTTCATCTGAAACCAA	32	#f74308	foldF-biotin
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42[199]	0[224]	AAATAAGTTAAGTGCCGTTTCGGGAGAAACAATAAC	35	#f7931e	linkABCE
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2[47]	35[119]	GCCTTAAAAATTTTATCCTGAATCTAATTTGCTTTTAGTAAGCA	44	#333333	linkE-DF
30[295]	29[287]	TTTCGTCACCAAGTACACCTCATAG	24	#333333	linkE-DF
32[239]	33[223]	TTAATTTCTTTTATTGAGCGCTAATATCACAAAGTCAGAGGGTA	44	#333333	linkE-DF
32[295]	39[215]	CAGAACCGTTTTACCGCTCCCTCAGAGCAGAGCCA	36	#333333	linkE-DF
35[120]	33[119]	GATAGCCGAGCCCTTTTAAAGAAATTTTGTTAAC	36	#333333	linkE-DF
35[216]	31[223]	TATGTTAGTTTTAACTTTAATCATTGTGTTTTGCTCATT	40	#333333	linkE-DF
37[120]	0[40]	CAAAAGGGCATATGGTTTACCAGCTTTTCAGTTACA	36	#333333	linkE-DF
37[216]	35[215]	TTTGGGAATTTTCAAACGTAGAAAATACTTACGCAG	36	#333333	linkE-DF
39[120]	37[119]	TCAGACTGACAGAATCAAGTTTGCTTTTGCCAAAGA	36	#333333	linkE-DF
39[216]	37[215]	CCACCGGATTTTTAGAGCCAGCAAAATTTGAGCCA	36	#333333	linkE-DF
41[216]	31[295]	CATGGCTTTTTCCACCTCTTTCAGGGATAGCAAG	36	#333333	linkE-DF
42[119]	1[95]	TTATTCTGTTTTTAAACAATTTCAATTTATCAATAT	36	#333333	linkE-DF
43[112]	42[120]	AAACATGAAAGTATTAGGAACCTA	24	#333333	linkE-DF
43[216]	41[215]	AAGTATAGTTTTTGATGATACAGGAGTGCGTCATA	36	#333333	linkE-DF
0[39]	1[31]	AAATAAACAGCCATATTTTTTTTTTGACAC	28	#888888	linkF-BAC
0[159]	2[160]	ATTACCTGTTTTGGATTATACTTCTGAAATAATCCT	36	#888888	linkF-BAC
1[32]	30[80]	CCAGCTACTCAAGATTAGTTGCTATTTTTAGAAGGCTTTTTTTTAACGAGAATCAAATGC	60	#888888	linkF-BAC
1[104]	0[112]	GAATAACCTTGCTTCTTTTAAACAAAAT	28	#888888	linkF-BAC
2[159]	2[104]	GATTGTTTTTTGTAATCGTCGCTATT	27	#888888	linkF-BAC
3[104]	3[121]	TCAATAGTGAATTTATTT	18	#888888	linkF-BAC
3[150]	4[160]	TTGATTAGAGCCGTCAATGCACTAAC	26	#888888	linkF-BAC
4[159]	4[104]	AACTAATATTTCAAATCATAGGTCTG	27	#888888	linkF-BAC
5[32]	26[80]	CAAGTACCTCATTCCAAGAACGGGTTTTAGTCCTGATTTTTTTTAGTAGTAAAAAGGTG	60	#888888	linkF-BAC
5[104]	5[121]	AATCCAATCGCAAGACTT	18	#888888	linkF-BAC
5[150]	6[160]	TTCGAACCACCAGCAGAACATTAAAA	26	#888888	linkF-BAC
6[159]	6[104]	ATACCGAATTTAAAGAACGCGAGAAAA	27	#888888	linkF-BAC

7[150]	8[160]	TTGAAATACCTACATTTTAGGAAAAA	26	#888888	linkF-BAC
8[111]	7[121]	TAAGAATACCGTGTGATAAATAAGTT	26	#888888	linkF-BAC
8[159]	10[160]	CGCTCATGTTTTTTAGACAGGAACGGAGGCCGAT	35	#888888	linkF-BAC
9[32]	10[22]	ACAAAAGGGTAATAAGAGAATATATT	26	#888888	linkF-BAC
9[40]	8[48]	TAAAGTAATTCTGTCCTGCAGAAC	24	#888888	linkF-BAC
9[96]	15[119]	AATTCCTATAATTGAGAATCGCCATATTTTTAACTCAC	39	#888888	linkF-BAC
10[61]	20[120]	AGGCAGAGTTTTGGCGCATCGTAACCGTCACGTTGG	36	#888888	linkF-BAC
10[121]	8[112]	TTGCTCAACAGTAGGGCTCCAGTATAAAGCCAACTTTGCGTTAAA	46	#888888	linkF-BAC
12[119]	14[120]	CCGTAAAGTTTTGGCGAAAAATCCTGTTTCTGGTTTG	36	#888888	linkF-BAC
14[119]	10[62]	CCCCAGCATTTTTTAACAACGCCAACATGTAATTT	35	#888888	linkF-BAC
15[120]	16[110]	ATTAATTGTGCCTAATGAGTGAGCTT	26	#888888	linkF-BAC
17[110]	18[120]	TTTTGGGTAACGCCAGGGGCTGCAAGG	26	#888888	linkF-BAC
18[119]	10[40]	CGATTAAGTTTTGCATTTTCGAGCCA	26	#888888	linkF-BAC
22[79]	24[80]	ATCAGAAATTTAAGATTCAAAAGGGTGGAGTAATG	36	#888888	linkF-BAC
24[79]	9[31]	TGTAGGTATTTTCTACTAATTTTTTTACAAGAAATTATCAACAATAGATATTTAAGTACCG	64	#888888	linkF-BAC
26[79]	28[80]	GCATCAATTTTGGCGATGGCTTAGAGCATAAGAGG	36	#888888	linkF-BAC
28[79]	5[31]	TCATTTTTTTTGTTCAGAATTTTTTTTATCCGGGCAAGCAAATCAGATATTTTATTAAC	64	#888888	linkF-BAC
30[79]	32[72]	TTTAAACATTTTAACTAATGCAGATACATAGGAATACCACATTC	44	#888888	linkF-BAC
10[255]	12[216]	GCGCCGCTTTTCGGGCGCTAGGGCGCTTTTTTAAAGAA	39	#aaaa00	lockD-B
12[215]	13[264]	CGTGGACTGAACAAGAGTCCACTATTACAGTGCTTTTGGGATTCCGTTG	49	#aaaa00	lockD-B
14[225]	15[225]	TTGGCGCCAGGGTGGTTTGGCGGTTTGCCTATTGTT	36	#aaaa00	lockD-B
14[258]	22[267]	TCCCAAAAGCAGCTGTTTGGCGGATCGTCACCTCAGC	38	#aaaa00	lockD-B
18[225]	19[263]	TTCAAAGCGCCATTGCGCGGTGCCGAAACCAGGTTTCAACATCAGTCTGATAAGCTA	58	#aaaa00	lockD-B
20[225]	22[176]	TTCTGTAGCCAGCTTTCATTGCGCTCTGGCCTTCTTTACAAAGG	45	#aaaa00	lockD-B
20[257]	24[240]	CAGACTGATGTTGATTAGCAACGGATGAGGAAGTTCCATGGCACCAA	48	#aaaa00	lockD-B
23[280]	22[288]	CACGCATAACCGATATGCCGCTTT	24	#aaaa00	lockD-B
25[288]	24[280]	TGTATCGGAACAGCTTGATACCGA	24	#aaaa00	lockD-B
0[263]	2[256]	ATACAGTAACAGTACCTAACGTCAGATGAATTTTTTTGAGTAA	44	#007200	unlinkD-AC
2[255]	4[256]	CATTATCATATTAATTTTAAAAGTTTTTGAACCTC	36	#007200	unlinkD-AC
4[255]	6[256]	AAATATCATAAAGCATCACCTTGCTTTTCCTCTGA	36	#007200	unlinkD-AC
6[255]	8[256]	CCTGAAAGCCAACAGAGATAGAATTTTTTGATTAG	36	#007200	unlinkD-AC
8[255]	10[256]	TAATAACATTGTAGCAATACTTCTTTTCGCTTAAT	36	#007200	unlinkD-AC
22[175]	24[176]	CTATCAGGCTATTTTGGAGAGATCTTTTACATTATG	36	#007200	unlinkD-AC
22[311]	24[304]	TTGCAGGGAGTTAAAGATTTCGGTCTGAGGCTTTTCGAGGTGA	44	#007200	unlinkD-AC
23[224]	22[216]	ACTTTTTCTACAGAGGCTTTGAG	24	#007200	unlinkD-AC
24[175]	26[176]	ACCCTGTAATCGGTTGTACCAAAATTTAATTCTGC	36	#007200	unlinkD-AC
24[303]	26[304]	ATTTCTTATTTATCAGCTTGCTTTTTTAAAGGAAT	36	#007200	unlinkD-AC
25[224]	23[223]	AACACTCAGAAAGAGGCCAAAAGAAATTTGACTAAAG	36	#007200	unlinkD-AC
26[175]	28[176]	GAACGAGTATAACAGTTGATTCCCTTTTCAAATATC	36	#007200	unlinkD-AC
26[303]	28[304]	TGCGAATAATAGAAAGGAACAACCTTTTTCGTCTTT	36	#007200	unlinkD-AC
27[224]	25[223]	ACCTGCTCTGATAAATTGTGTCGATTTTACACTAA	36	#007200	unlinkD-AC
28[175]	30[176]	GCGTTTTAGAAGCCCGAAAAGACTTTTTTGCAAAAGA	36	#007200	unlinkD-AC
28[303]	30[304]	CCAGACGTACGATCTAAAGTTTTGTTTTGTACCGTA	36	#007200	unlinkD-AC
29[224]	27[223]	CAGACCAGGAAAGAGGACAGATGATTTTAAATCCGCG	36	#007200	unlinkD-AC
30[175]	32[176]	AGTTTTGCATAGCGAGAGGCTTTTTTTTATACCAAG	36	#007200	unlinkD-AC
30[303]	32[296]	AACTGAGCCCAATAGGAACCATTTTTTATGACGCCACCCT	44	#007200	unlinkD-AC
31[224]	29[223]	CAGTGAATATCAACGTAAACAAAGCTTTTACGGTGTA	36	#007200	unlinkD-AC

#2: Design with end-linked helices:

Locked box where the helix ends of face B has been linked together to reduce flexibility, with a FRET pair between face B and lid D (but the dye on face B is internal on the staple to keep the ends linked). Created to eliminate the effect of flexible helix ends (casually referred to as helix "fingers").

Remove these staple strands:	
Sequence:	Pool name:
[Cy3]GTAATCATGGTCATAGTACCGAGCTCGAATTCTT	FRET-pair
CGTGGACTGAACAAGAGTCCACTATTTCAGCTGCTTTTGGGATTCCGT	
TG	lockD-B
TCCCAAAAGCAGCTGTTTGCGGGATCGTCACCCTCAGC	lockD-B
TTGGCGCCAGGGTGGTTTGCGGGTTTGCCTATTGTT	lockD-B
TTCAAAGCGCCATTCGCCGGTGCCGAAACCAGGTTTCAACATCAG	
TCTGATAAGCTA	lockD-B
CACGCATAACCGATATGCCGCTTT	lockD-B

Replace with these staple strands:	
Sequence:	Pool name:
GCGTATTGTT[Cy3]TTGTAATCATGGTCATAGTACCGAGCTCGAATTC	FRET-pair
TTTTCAAAGCGC	
CACGCATAACCGATATGCCGCTTTTTCAGCTGCTTTTGGGATTCCGTT	
G	lockD-B
TCCCAAAAGCAGCTGTTGGCGCCAGGGTGGTTTGCGGGTTT	lockD-B
CGTGGACTGAACAAGAGTCCACTATT	lockD-B
CATTCGCCGGTGCCGAAACCAGGTTTCAACATCAGTCTGATAAGCT	
A	lockD-B
TGCGGGATCGTCACCCTCAGC	lockD-B

#3: Design with FRET pair on helix ends on the same face:

Locked box with FRET pair between helix ends of face B. Created to investigate and quantify the magnitude of the flexibility of the helix ends ("fingers").

Remove these staple strands:	
Sequence:	Pool name:
[Cy5]AGCGAAAGACAGCATCGGAACGAGGGT	FRET-pair
TTGGCGCCAGGGTGGTTTGCGGGTTTGCCTATTGTT	lockD-B
Replace with these staple strands:	
Sequence:	Pool name:
AGCGAAAGACAGCATCGGAACGAGGGT	lockD-B
TTGGCGCCAGGGTGGTTTGCGGGTTTGCCTATTGTT[Cy5]	FRET-pair