Supplementary information:

Multi-Fluorophore DNA Origami Beacon as a Biosensing Platform

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Figure S1. Comparison of the three designs.

(a) Two structures were designed with single-layer sheets and one structure was designed with double layer sheets. Two of the structures have the helices running parallel to their hinge region - a horizontal direction when the structures are positioned on the surface. One structure has the helices running perpendicular to the hinge and are positioned vertically with respect to the surface. The structures were named according to these features: single-layer vertical (SV), single-layer horizontal (SH) and double-layer horizontal (DH). (b, c) SV and SH structures are based on the same scaffold strand pathway resulting in sharing more than half of the staple strands. The difference between the structures is the position of the foot (green) either perpendicularly to the helix direction (SV) or along the helix direction (SH). All the structures have a loop due to the circularity of the scaffold strand. The loop is colored blue. While SV's loop is placed at the foot and does not affect the opening of the device, the SH's loop is placed at the top connecting two sheets near the sensor modules. However, the loop is 96 bps and is long enough allow opening of the device. The loop of the DH structure is also positioned between the two sheets although at the bottom next to the foot. With a length of 37 bps it should be sufficiently long for proper opening of the device. (d) There are 5 fluorophore rows with 12 fluorophores per row in each fluorophore array in the SV structure adding up to a total of 60 FRET pairs. The SH structure has 8 rows with 7 fluorophores per row with 56 FRET pairs in total. The DH structure is more compact due to its double-layered sheets and has only 6 rows with 5 fluorophores per row that adds up to 30 FRET pairs in total.

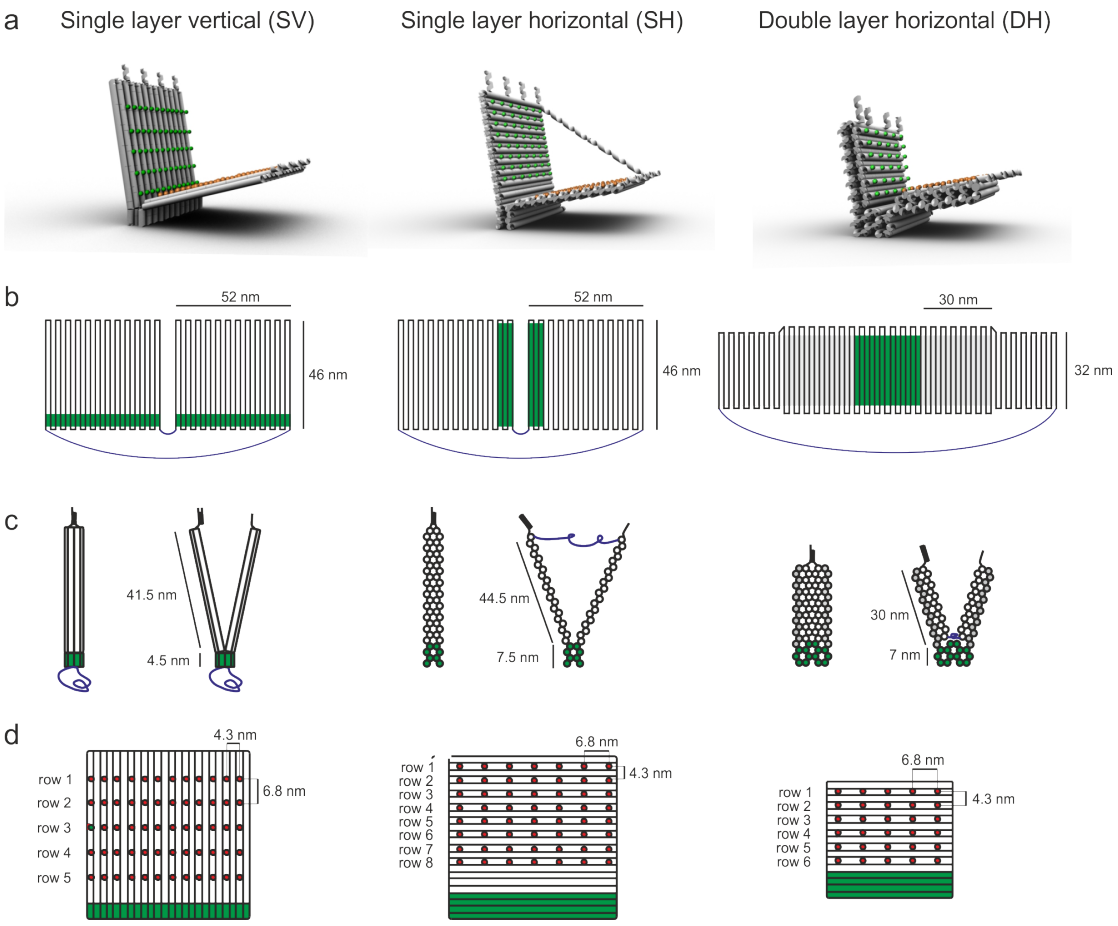

Figure S2. Blueprint of a SH sensor device.

The scaffold strand path is shown in light blue.

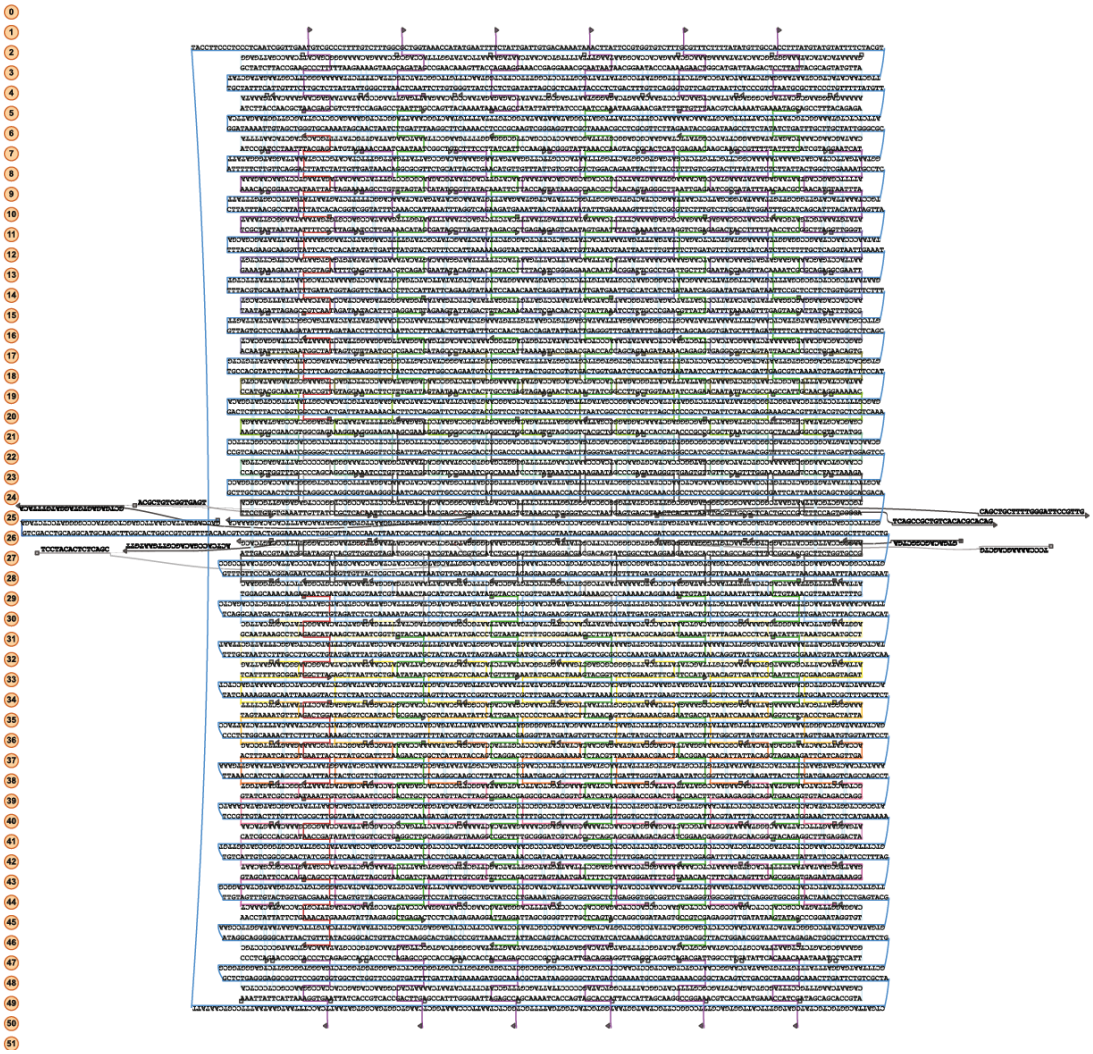

Figure S3. Blueprint of a SV sensor device.

The scaffold strand path is shown in light blue. Strands labelled with biotin shown in purple. Hinge staple strands are shown in red. Sensor module complementary strands are shown in the figure.

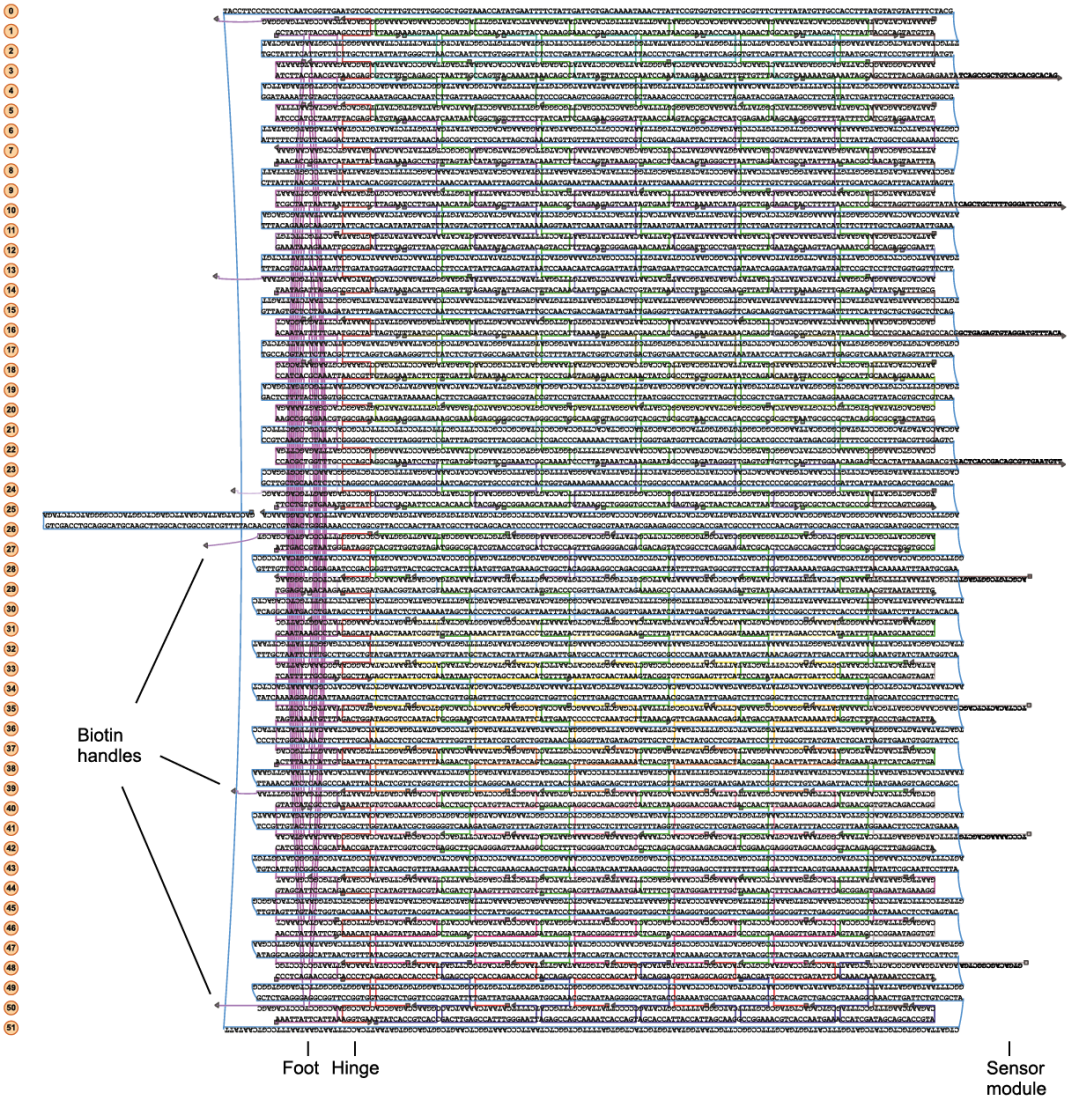

Figure S4. Blueprint of a DH sensor device.

Scaffold strand is shown in light blue.

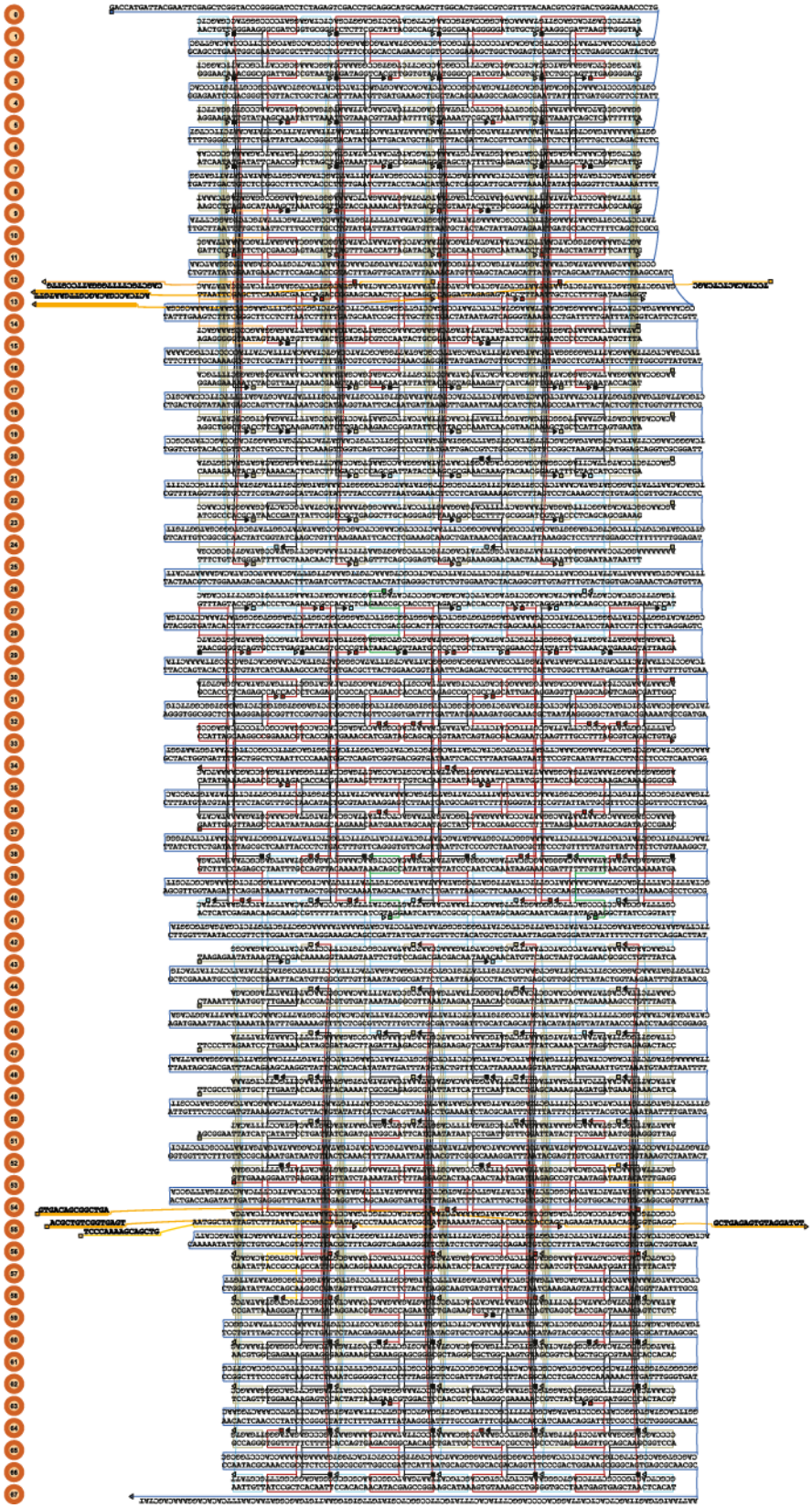

Figure S5. Electrophoretic mobility of self-assembled structures.

Gel electrophoresis analysis of the self-assembled structures showed a slight difference in mobility between closed and open DH and SV structures but no difference of SH structures (Fig. A and B). (A) Structures (120 fmol) were assembled at 8 mM Mg^{2+} and run on 1% agarose gel together with single stranded M13 scaffold strand (418 fmol) and 1 kb ladder. The constructs were run for 4 hours at 70 V in 8°C buffer. SHC – single-layer horizontal closed, SHO – single-layer horizontal open, SVC – single-layer vertical closed and SVO – single-layer vertical open. (B) DHC – double-layer horizontal closed, DHO –double layer horizontal open. The constructs were run for 4 hours at 70 V in 8°C buffer on 1% agarose gel. (C) SV structures were assembled and purified at 12 and 24 mM Mg^{2+}. Constructs were run on 0.8% agarose gel for 3.5 hours at 65V in 8°C buffer.

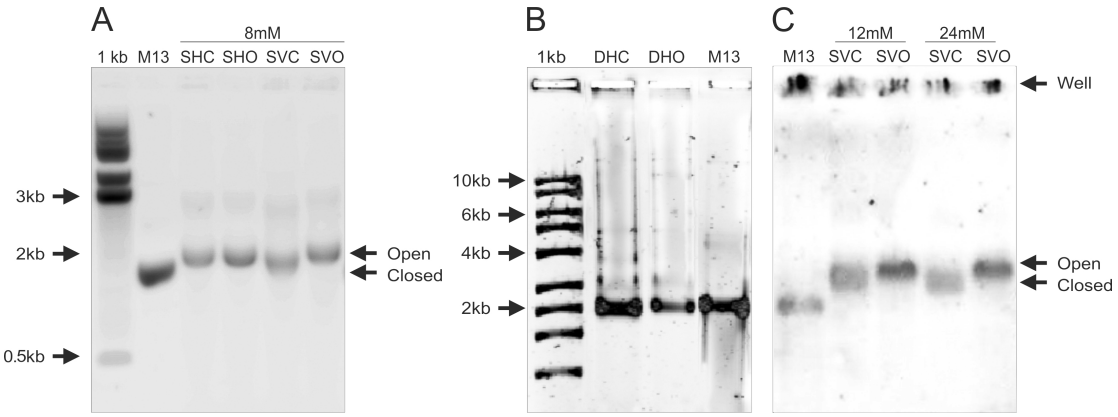

Figure S6. SV device assembled in closed and open state at different Mg^{2+} concentrations.

Higher Mg^{2+} concentration during self-assembly results in more structures adsorbing to the mica in a closed conformation. (A) Closed, 12 mM Mg^{2+}. (B) Closed, 24 mM Mg^{2+}. (C) Open 12 mM Mg^{2+}. (D) Open, 18 mM Mg^{2+}. Scale bars are 200 nm.

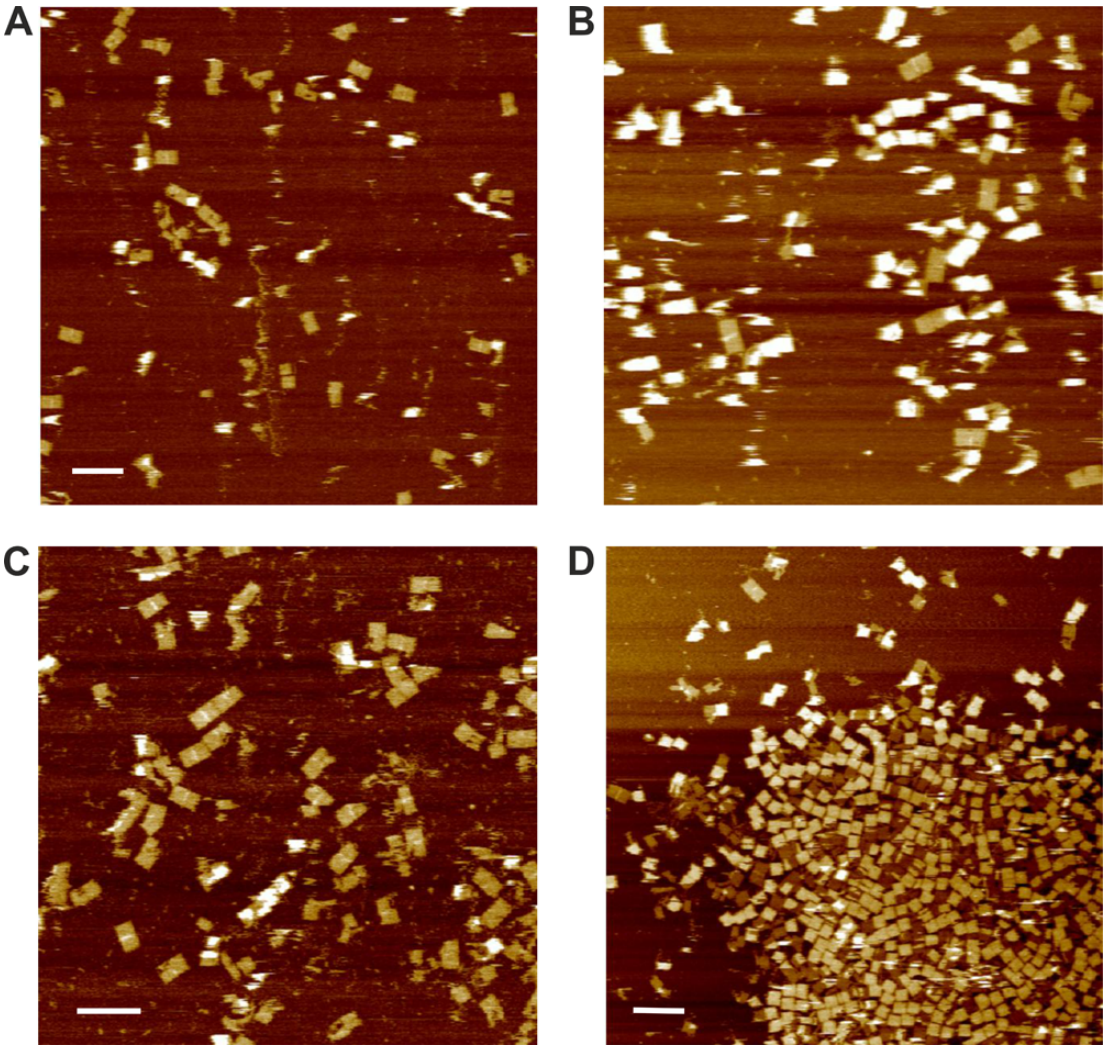

Figure S7. DH structure TEM images.

Double-layer structures were assembled in closed and open conformation and visualized using TEM. Almost all of the structures assembled in closed conformation had a square-like shape corresponding to a device in a closed state. Some of the structures adhered on the foot as shown in the sketch. Scale bars 20 nm. Few structures assembled in open conformation adhered to the surface in open state and were visualized as a rectangle with a darker protruding foot in the middle; the majority of the structures adhered to the surface in a closed conformation. Some of the structures appeared to be lying on one panel while the other panel was pointing up as shown in the sketch. DH device measured 34.3 ± 3.1 nm by 37.3 ± 2.8 nm and 33.7 ± 2.5 nm by 60.0 ± 3.8 nm (20 closed and 3 open) compared with theoretical values of 32 x 37 nm and 32 x 67 nm for closed and open states, respectively. Scale bars 20 nm.

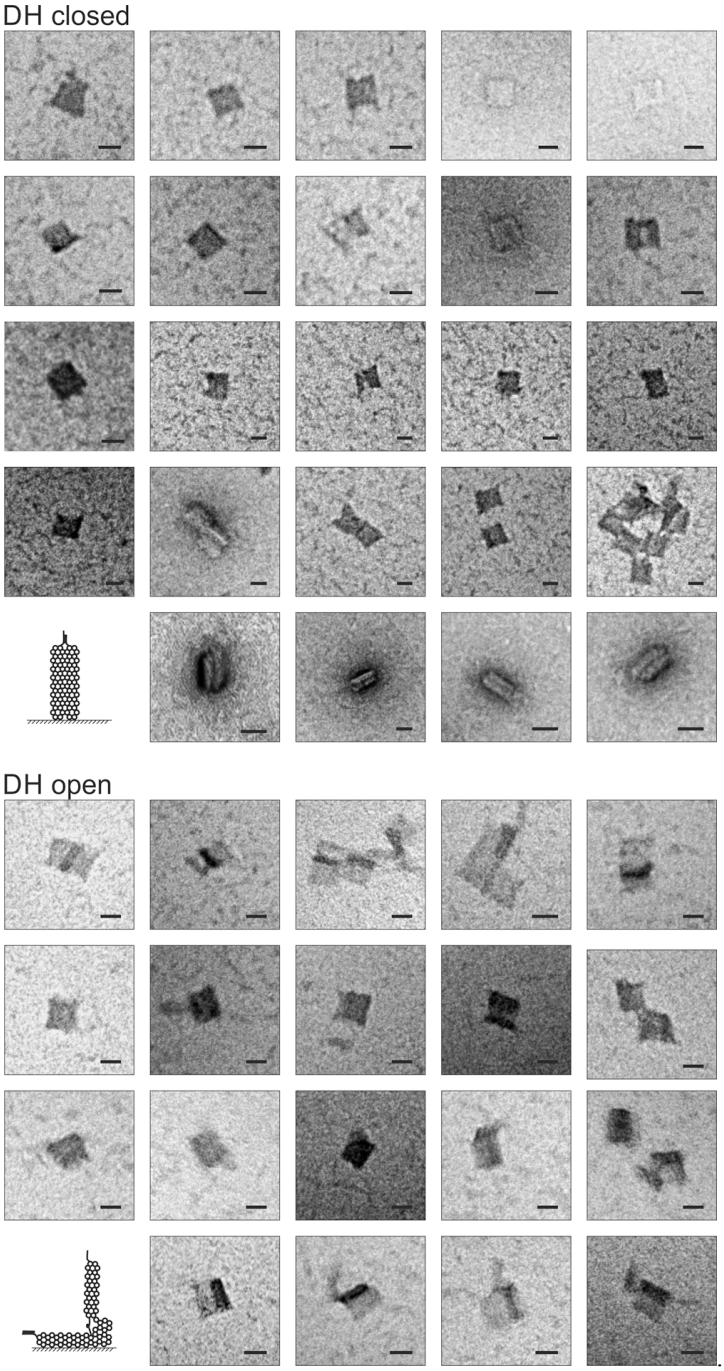

Figure S8. AFM of SH structure.

The structure was assembled in a standard DNA origami buffer with 12 mM Mg^{2+} and visualized using atomic force (AFM) or transmission electron microscopy (TEM) both in closed and open states. The SH device measured 54.2 ± 4.4 nm by 48.4 ± 2.9 nm and 51.0 ± 2.5 nm by 86.4 ± 1.7 nm in the closed and open states (20 closed and 4 open) compared with theoretical values of 52 x 46 nm and 52 x 89 nm. Only few structures were adsorbed to the surface in open conformation even when assembled without locks. Scale bars are 20 nm for the top panel and 200 nm for the bottom panel.

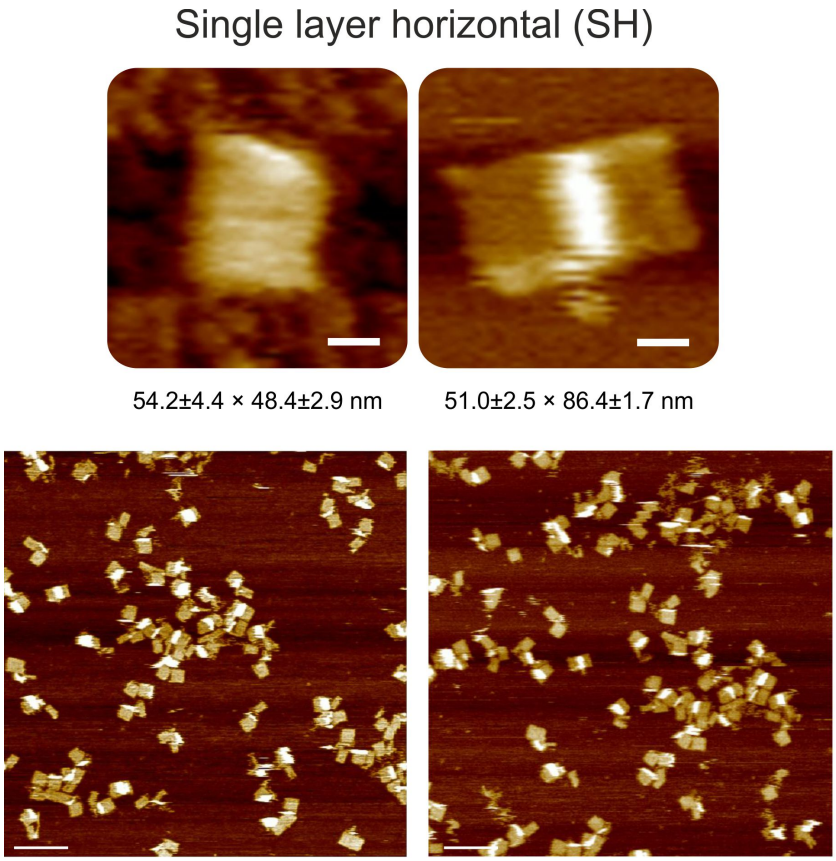

Figure S9. Ensemble FRET.

Dependence on Mg^{2+} concentration during self-assembly and purification was tested for all the structures (panel f and g, numbering continued from Fig. S1). To test the conformation of the structures in solution, devices were assembled at optimal self-assembly concentration and then purified at optimal Mg^{2+}concentration: The SV structure was assembled at 20 mM Mg^{2+} and purified at 12 mM Mg^{2+}. The rows in the device closer to the foot have higher FRET efficiency than the rows closer to the sensor modules both in the closed and open conformations (panel h, SV rows). The FRET difference is decreasing from the row closest to the sensor modules (ΔFRET≈ 0.3) to the row closest to the foot (ΔFRET< 0.1) corresponding to a smaller change in distance closer to the foot. The SH device assembled at 25 mM Mg^{2+} and purified at 18 mM Mg^{2+} has a high FRET efficiency in the closed conformation for the row which is closest to the sensor module and for the row that is closest to the foot (panel h, SH rows). The FRET efficiency was lowest for rows 4 and 5 in the middle of the structure. The row closest to the sensor modules has the largest FRET difference (ΔFRET≈ 0.4). From there FRET difference decreases reaching its minimum at row 4 (ΔFRET< 0.1), and then increases again to ΔFRET≈0.15 for the row closest to the foot. The results suggest that the SH structure is bending to form a "O" shape when seen from the side with the helices in the middle being further apart than the helices in the top and bottom. The DH structure assembled at 20 mM Mg^{2+} and purified at 10 mM Mg^{2+} has ΔFRET≈ 0.1 for top three rows next to the sensor modules, a smaller ΔFRET < 0.1 for the 4th row and no FRET change for the two rows next to the foot (panel h, DH rows). DH structure self-assembly yield in closed state was probably much lower compared with SV and SH devices due to the more flexible hinges connecting foot and sheet structures. This would explain the lower FRET efficiency achieved for the DH structure.

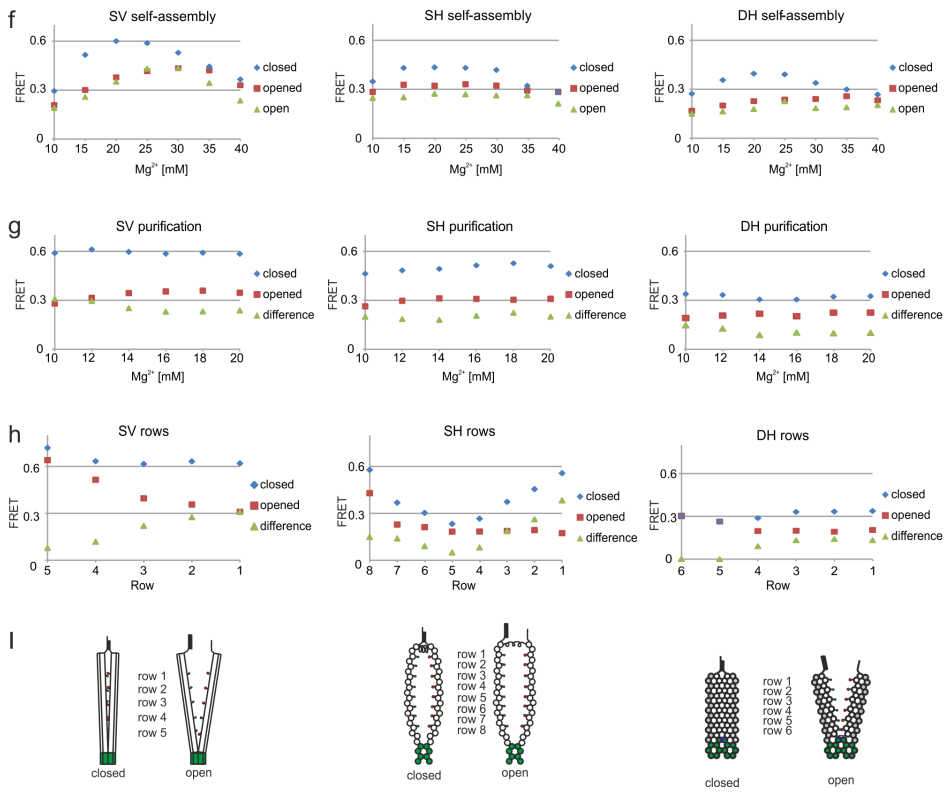

Figure S10. One-pot TdT labelling of oligo row with either Cy3 or Cy5 conjugated ddUTP.

Twelve oligos at the time corresponding to one row of the fluorophore array were labelled using the terminal transferase enzyme with either Cy3 or Cy5 conjugated ddUTP. Labelling efficiency was assessed using 18% denaturing polyacrylamide gel electrophoresis where 5 pmol of unlabelled (U) and labelled product (designated as Cy3 or Cy5 on the image) were run at 20W for 1.5 hour. First imaged for Cy3 and Cy5 labelled products, then stained with Sybr Gold and imaged again for unlabelled oligos. Overlay image: green –unlabelled oligos, yellow –Cy3 labelled oligos and red –Cy5 labelled oligos. Two bands corresponding to oligos of two different length in the mix (see table S2). Labelling with Cy3-ddUTP seemed to be more efficient with a yield of approximately 90-95%. Cy5-ddUTP labelling was observed to be less efficient yielding around 75-80% of conjugated product.

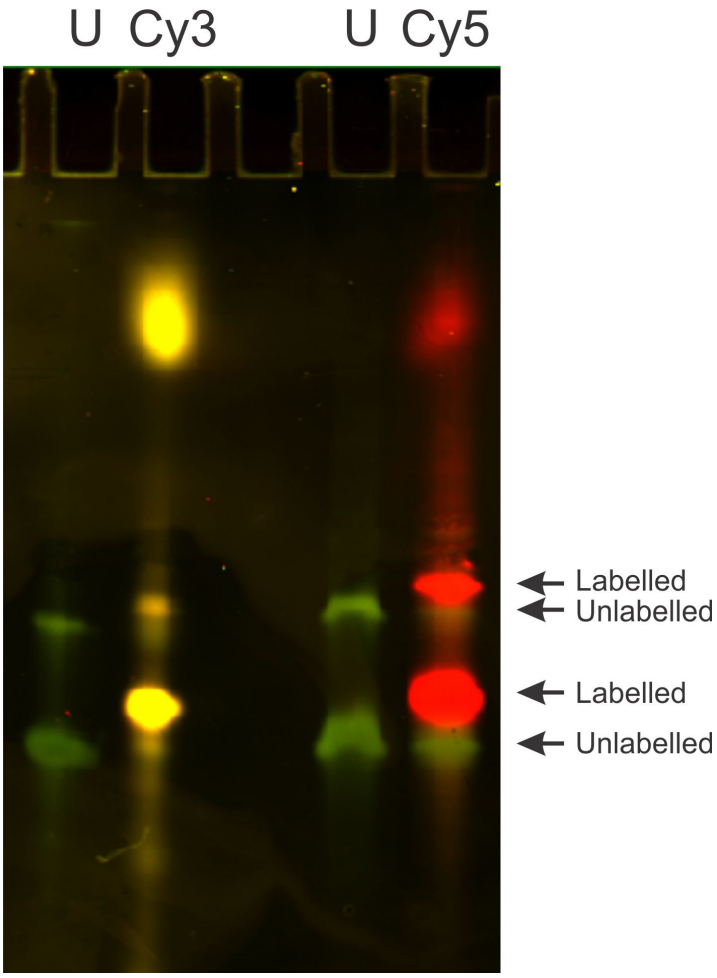

Figure S11. Mg^{2+} concentration during self-assembly and purification of SV device.

SV device with 48 FRET pairs (4 rows closest to the sensor modules were labelled) was assembled at various Mg^{2+} concentrations (left graph). Structure assembled at 30 mM Mg^{2+} was then purified using buffers with different Mg^{2+} concentrations (right graph).

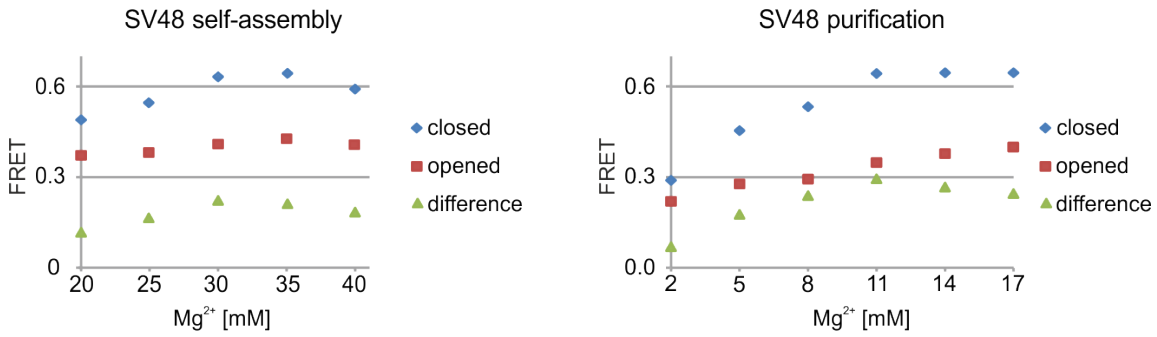

Figure S12. SV device without and with a hinge.

SV structures were assembled without hinge and with a hinge. Structures with a hinge were achieved by removing staple strands ("Removable hinge" module in Table S2) shown in red (Fig. S3) resulting in a 7-nucleotide single stranded hinge consisting of only scaffold strand. Samples were assembled with different rows with one row per device. FRET efficiency was measured for closed and open states. Addition of the 7-nucleotide hinge adds flexibility and better opening but reduces FRET efficiency in a closed conformation resulting in a lower ΔFRET compared to structure without hinge.

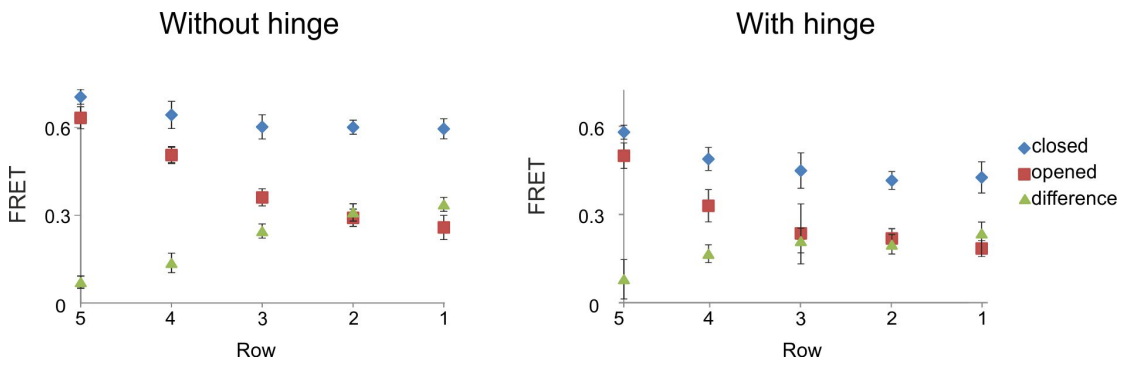

Figure S13. Sequential opening of SH, SV and DH devices.

One FRET pair was positioned at three places between two neighbouring sensor modules in an SH device. All four sensor modules were designed to detect different sequences. Keys were added one at a time in sequence: 1, 2, 3 and 4, where 0 corresponds to no keys added. FRET efficiency was measured before and after addition of the keys. Structures were labelled with 25 (DH) and 24 (SV) FRET pairs. Keys were added one at a time in the sequence shown below each graph, where 0 corresponds to no key added.

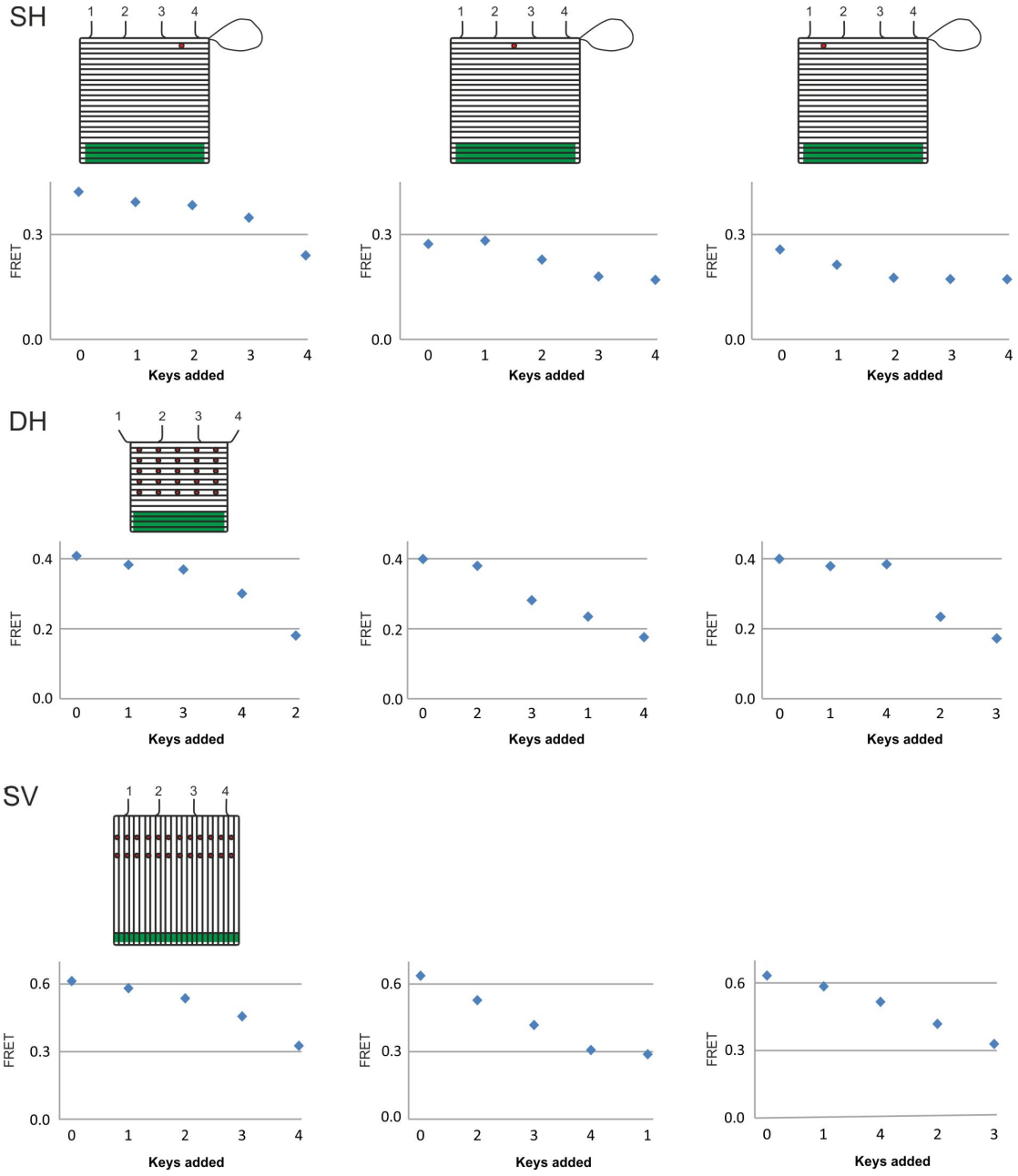

Figure S14. Concentration detection limit.

Various concentrations of oligonucleotides complementary to sensor modules were added to the sample of the sensor device with 48 FRET pairs. FRET change was recorded and the change of the sample with only buffer added was subtracted.

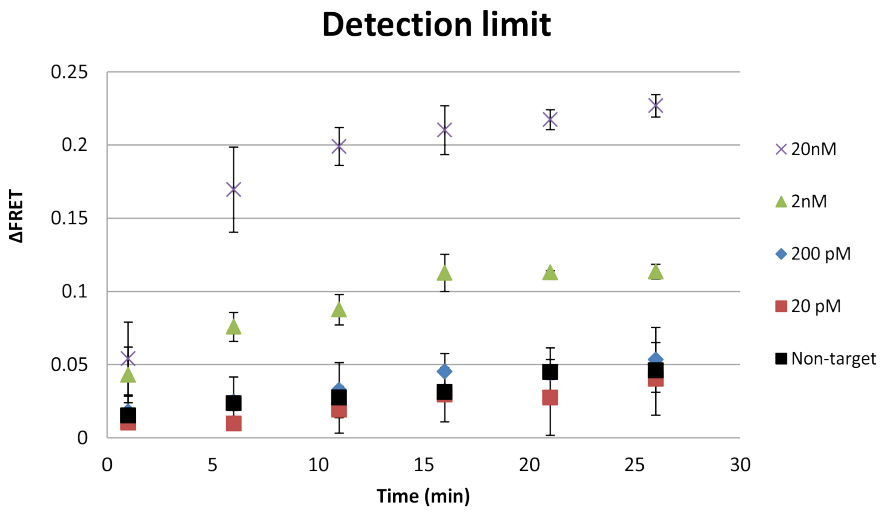

Figure S15. Overview of single particle experiments using Zeiss LSM 700.

Six images of fluorescence emission with two minutes interval were recorded after donor excitation at 555 nm for every series; Cy3 and Cy5 fluorescence emissions are represented by green and red colours. Yellow colour is a result of red and green colours overlay. Random DNA and target DNA were added after second image was recorded. A part of each image shown here is 10.6×10.6 µm. The first series, called "control", is used to control if fluorophores are bleaching and if the signal changes during the measuring time. In the second series "random DNA" that is non-complementary to the sensor modules oligonucleotide is added after the second frame in a final concentration of 2.5 µM. "After DNA control" series was recorded to see if addition of random DNA and new buffer would affect the fluorescent emission of the sample in a longer time perspective and at a different spot on the slide. The fourth series "keys DNA" were used to investigate the addition of target DNA sequences complimentary to the sensor modules after the second frame (final concentration of 2.5 µM). The last series were recorded to demonstrate that the emission profile changed everywhere in the flow cell as a result of opening of the device.

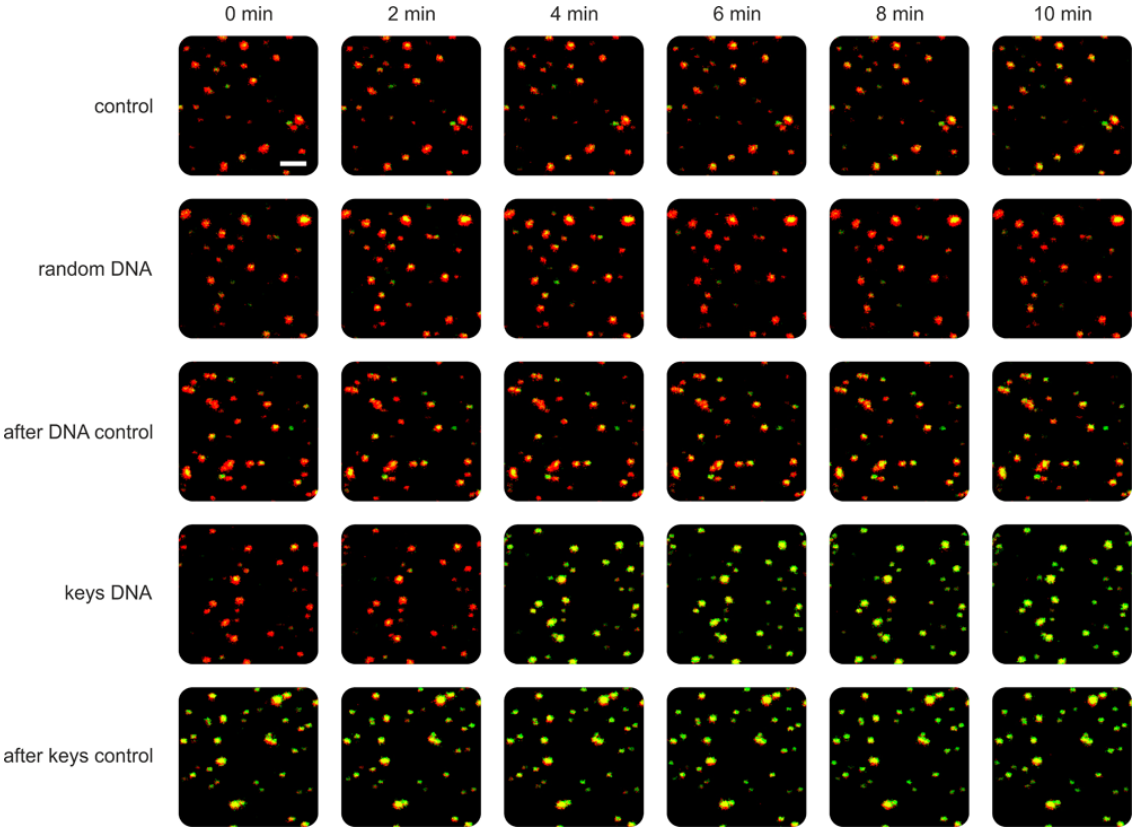

Figure S16. Tall rectangle blueprint.

Staple strands along the edges were omitted from a staple mix to make non-aggregating origami structures. One of the columns consisting of 16 staple strands was labelled with biotinylated nucleotide. Four columns (16 staple each) were labelled with ddUTP-Cy5 conjugates.

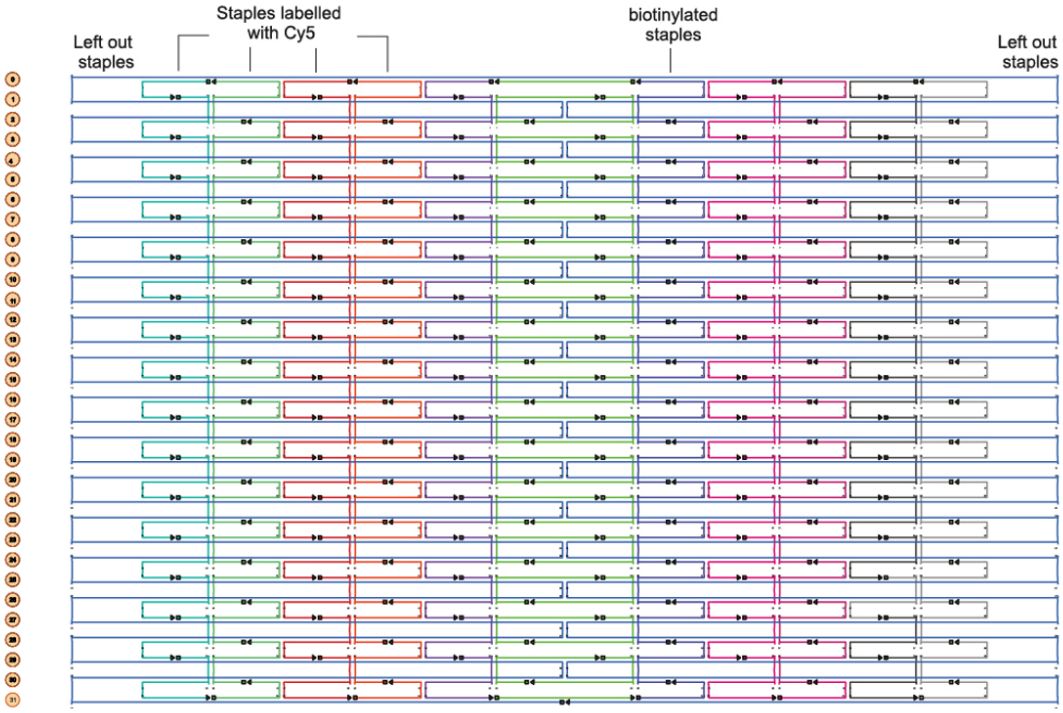

Figure S17. Single particle analysis.

Signals were grouped according to their direct excited Cy5 fluorescence intensity (blue histogram) into several groups: single – consisting of single device particles, double – particles consisting of two devices, multiple – particles consisting of multiple devices and was further grouped into small multiple (approx. 3-5 devices), medium (5-9 devices) and large (10-11 devices). Performance of each group is summarized as average FRET (graph) and FRET distribution after second (red histogram - before addition of target DNA) and third (green histogram -after addition of target DNA). Interestingly, only the most intense signals –multiple large group - showed poor change between closed and open conformations. These spots probably represent large aggregates of poorly assembled structures. Fortunately, this group is very small consisting of 23 particles that represent a very small fraction of total amount of particles - approximately 1.8% by number. Although, the group represents 6.6% of total direct excited Cy5 fluorescence by intensity indicating that the yield of the self-assembly is 93.4%.

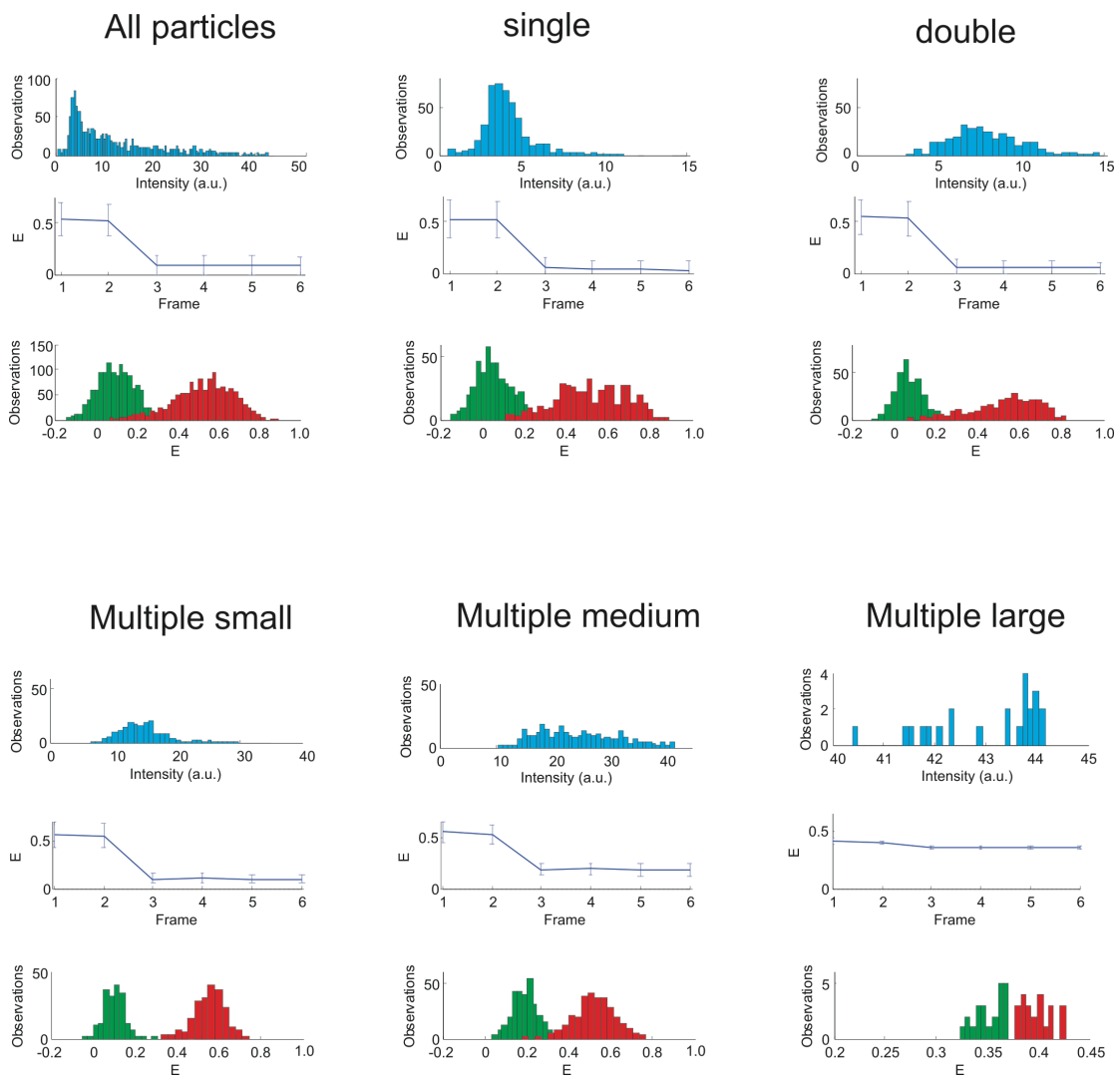

Figure S18. Comparison of ensemble vs single device detection limits.

Comparison of the ensemble experiment data to the single device experiment: various concentrations of oligonucleotides complementary to sensor modules were added to the sample of sensor device with 48 FRET pairs, FRET difference after 6 min is shown in the graphs. Standard deviation for ensemble measurements comes from tree independent measurements, while in the single device experiment deviation represents deviation between hundreds of detected devices in the sample.

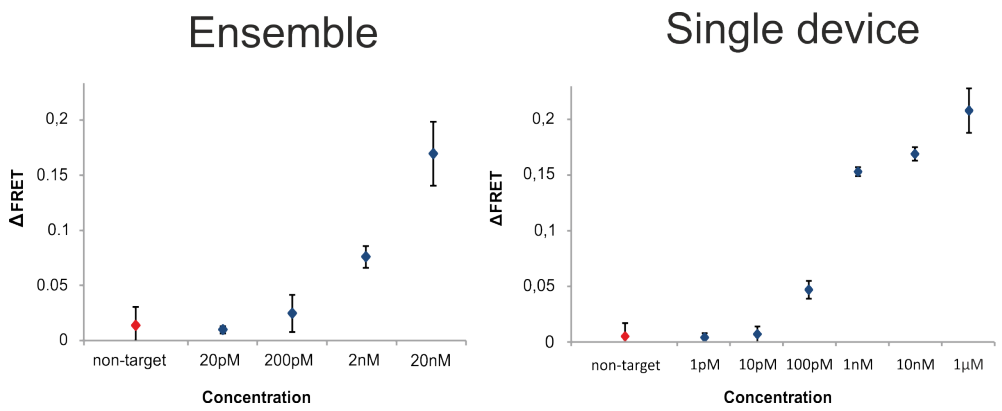

Table S1. SH staple strand sequences.

All SH structures were assembled using the complete set of the listed oligos. Oligos are divided into different modules. Fluorophore rows of 1st sheet are labelled with Cy3 and fluorophore rows on the second sheet are labelled with Cy5. Sensor module contains lock strand with complementary parts coloured in red.

	Start	End	**Fluorophore rows: 1st sheet**
8th	7[55]	9[54]	TCCTAATTTCCTGAACAAGAAAAAACAC
	7[76]	9[75]	AAACCAATTGTTTATCAACAATTAGAAA
	7[97]	9[96]	TCTTTCCTTCAGCTAATGCAGACATATG
	7[118]	9[117]	ACGGGTATCGACGACAATAAACTACCAG
	7[139]	9[138]	GCACTCATGGTAAAGTAATTCTCAACAG
	7[160]	9[159]	GCCGTTTTGAATATAAAGTACCAATCGC
	7[181]	9[180]	GGAATCATATTTTCGAGCCAGTCAACAT
7th	9[55]	11[54]	CGGAATCAATAAGGCGTTAAATTCGCTA
	9[76]	11[75]	AAGCCTGTGAAATACCGACCGTTTAGAA
	9[97]	11[96]	CGTTATACCTGACCTAAATTTACGATAG
	9[118]	11[117]	TATAAAGCTATTTTAGTTAATTTGAGAA
	9[139]	11[138]	TAGGGCTTGCGAGAAAACTTTTTTATCA
	9[160]	11[159]	CATATTTATCCAATCGCAAGACGAGACT
	9[181]	11[180]	GTAATTTAATATGTAAATGCTGGGCTTA
6th	11[55]	13[54]	TTAATTAAAACCTTGCTTCTGTGAAATA
	11[76]	13[75]	TCCTTGAAAATCAATATATGTGTTTTCA
	11[97]	13[96]	CTTAGATTTTTTAATGGAAACAGAATAT
	11[118]	13[117]	GAGTCAATCAATTTCATTTGAATTTACA
	11[139]	13[138]	AAATCATAAACAAAATTAATTAACGGAT
	11[160]	13[159]	ACCTTTTTTGATGAAACAAACAGAATAC
	11[181]	13[180]	GGTTGGGTAATTACCTGAGCAACGCAGA
5th	13[55]	15[54]	AAGAAATTAATTATTTGCACGTTAATAG
	13[76]	15[75]	GGTTTAACGGTTAGAACCTACCAGATAA
	13[97]	15[96]	ACAGTAACTATACTTCTGAATAAGAAGT
	13[118]	15[117]	TCGGGAGATATAATCCTGATTGCAATTC
	13[139]	15[138]	TCGCCTGACAGATGATGGCAATATCCTT
	13[160]	15[159]	CAAGTTACTATCATCATATTCCAATTTT
	13[181]	15[180]	GGCGAATTAACCACCAGAAGGAATTATC
4th	15[55]	17[54]	ATTAGAGCTCTTTAGGAGCACTACAATA
	15[76]	17[75]	TACATTTGTGAGGAAGGTTATCTAGTCT
	15[97]	17[96]	ATTAGACTAAATCAACAGTTGAATAGCC
	15[118]	17[117]	GACAACTCATCAATATCTGGTCTAAAAA
	15[139]	17[138]	TGCCCGAAACCTCAAATATCAACAGCAG
	15[160]	17[159]	AAAAGTTTTCTAAAGCATCACCTGAGGC
	15[181]	17[180]	ATTTTGCGGAGCCAGCAGCAAACGCCTG
3rd	17[55]	19[54]	TTTTTGAACGTAAGAATACGTGCCATCA
	17[76]	19[75]	TTAATGCGAGAACCCTTCTGACGTAGCA
	17[97]	19[96]	CTAAAACAGACATTCTGGCCAAGTAATA
	17[118]	19[117]	TACCGAACCACACGACCAGTAAGTAGAA

	17[139]	19[138]	AAGATAAATACATTGGCAGATTGCCTTG
	17[160]	19[159]	GGTCAGTAAATCGTCTGAAATGACAATA
	17[181]	19[180]	CAACAGTGAATACCTACATTTTGCAACA
2nd	19[55]	21[54]	CGCAAATTCACCGAGTAAAAGAAAGCCG
	19[76]	21[75]	ATACTTCTTGTTTTTATAATCAAAGGAA
	19[97]	21[96]	ACATCACTGTACGCCAGAATCCGGAGCG
	19[118]	21[117]	GAACTCAAAAGGGATTTTAGACGCAAGT
	19[139]	21[138]	CTGGTAATAGCTAAACAGGAGGCGCGTA
	19[160]	21[159]	TTACCGCCCCTCGTTAGAATCAGCGCTT
	19[181]	21[180]	GGAAAAACCGAGCACGTATAACGGCGCG
1st	21[55]	23[54]	GCGAACGTGATTTAGAGCTTGACCACGC
	21[76]	23[75]	GGGAAGAAGGAACCCTAAAGGGGGCGAA
	21[97]	23[96]	GGCGCTAGGGTGCCGTAAAGCATGGTTC
	21[118]	23[117]	GTAGCGGTAATCAAGTTTTTTGCCCTTA
	21[139]	23[138]	ACCACCACCACTACGTGAACCAGCCCGA
	21[160]	23[159]	AATGCGCCCCGTCTATCAGGGCTGTTCC
	21[181]	23[180]	TACTATGGCCAACGTCAAAGGGTCCACT

Fluorophore rows: 2nd sheet

1st	30[56]	28[57]	GTCATTGCTGGAGCAAACAAGATTCTCC
	30[77]	28[78]	GAGATCTATGAACGGTAATCGTAGCGAG
	30[98]	28[99]	GGAGAGGGGCATGTCAATCATACTTTCA
	30[119]	28[120]	TCTAGCTGCGGTTGATAATCAGGTCTGG
	30[140]	28[141]	ATCAATATCCAAAAACAGGAAGACGCCA
	30[161]	28[162]	GCCGGAGAAAGCAAATATTTAACTCATT
	30[182]	28[183]	AGATTCAAACGTTAATATTTTGATTAAA
2nd	32[56]	30[57]	AGAATTAGGCAATAAAGCCTCATATCAG
	32[77]	30[78]	TAAATCATAAGCTAAATCGGTTTTTTGA
	32[98]	30[99]	AGTAGTAGAAACATTATGACCCAATGCC
	32[119]	30[120]	GGTGGCATCTTTTGCGGGAGAAAACCGT
	32[140]	30[141]	ATTTGGGGTTTCAACGCAAGGAATCACC
	32[161]	30[162]	AACCTGTTTTTTAGAACCCTCAAGAAAG
	32[182]	30[183]	TACATTTCTAAATGCAATGCCTAGGTAA
3rd	34[56]	32[57]	CTCCTTTTTCATTTTTGCGGATAGGCAA
	34[77]	32[78]	ATTAGAGAAGCTTAATTGCTGAATCCAA
	34[98]	32[99]	AGCAAACTGCTGTAGCTCAACAACTAAT
	34[119]	32[120]	TTCAAAGCAATATGCAACTAAATGAAAA
	34[140]	32[141]	ATATCGCGGTCTGGAAGTTTCAATTTTC
	34[161]	32[162]	AGGAAGCCTAACAGTTGATTCCGTCAAT
	34[182]	32[183]	GATTGCATGCGAACGAGTAGATATTAGA
4th	36[56]	34[57]	TTTGCCAGTAGTAAAATGTTTATAATTG
	36[77]	34[78]	AGAGGCTTTAGCGTCCAATACTGTCAGG
	36[98]	34[99]	ACGATAAACGTCATAAATATTCACCGGA
	36[119]	34[120]	ATAACCCTCCCCCTCAAATGCTTCGAGC

	36[161]	34[162]	ACGCCAAAATAAATCAAAAATCATTAAG
	36[140]	34[141]	CATAGTAAGTTCAGAAAACGAGCTTCAA
	36[182]	34[183]	TTCAACTATACCCTGACTATTAAAAGCG
5th	38[56]	36[57]	GAGATGGTACTTTAATCATTGTAGAAGT
	38[77]	36[78]	AACGAGTACTTATGCGATTTTAATAGCG
	38[98]	36[99]	TGCCCTGAGCTCATTATACCAGCAGACG
	38[119]	36[120]	GCTCATTCGTTGGGAAGAAAAAACTATC
	38[140]	36[141]	CCAAATCATAATAAAACGAACTACGAGG
	38[161]	36[162]	CAAGAACCCAACATTATTACAGTACATA
	38[182]	36[183]	CTTCATCAGATTCATCAGTTGAACCACA
6th	40[56]	38[57]	AAGTACAAGTATCATCGCCTGAGGGCTT
	40[77]	38[78]	GATTATACTGTCGAAATCCGCGCACCAG
	40[98]	38[99]	CACTCATCCCATGTTACTTAGCAAGGCT
	40[119]	38[120]	AGGCAAAAAGGCGCAGACGGTCAAAGCT
	40[140]	38[141]	GGCACCAAAGGGAACCGAACTGCATTAC
	40[161]	38[162]	AATACGTATTGAAAGAGGACAGTCTTGA
	40[182]	38[183]	AAGTTTCCGTGTACAGACCAGGGCTGAC
7th	42[56]	40[57]	CCGACAATCATCGCCCACGCATGAAACA
	42[77]	40[78]	AGCTTGATATATTCGGTCGCTGCCCAGC
	42[98]	40[99]	TTCGAGGTCAGGGAGTTAAAGGCTAAAA
	42[119]	40[120]	GTATCGGTTGCGGGATCGTCACCGAAAG
	42[140]	40[141]	CTCCAAAAAGCGAAAGACAGCATACGAA
	42[161]	40[162]	TGAAAATCGAGGGTAGCAACGGGGGTAA
	42[182]	40[183]	CGAATAATGGCTTTGAGGACTAATGAGG
8th	44[56]	42[57]	TACAAACTGTAGCATTCCACAGGTTGCG
	44[77]	42[78]	AACACTGATCATAGTTAGCGTATTAAAC
	44[98]	42[99]	CAATAGGAAAAGTTTTGTCGTCCTTGCT
	44[119]	42[120]	CATTTTCAACGTTAGTAAATGATTAATT
	44[140]	42[141]	CACCCTCAGTATGGGATTTTGCAAAAGG
	44[161]	42[162]	AACCGCCACTTTCAACAGTTTCTCACGT
	44[182]	42[183]	GTTTAGTATGAGAATAGAAAGGGAATTG

Structural strands

9[126]	5[132]	CAACGCTGTCCAGATAAACCAGTTTTAGAATCCAA
9[168]	5[174]	ACAACGCAATAAGATATTTTCTCAGATAAAATAGC
9[84]	5[90]	TTAGTATACGCGCCCAATAATAAATCAATAATTTG
10[111]	6[105]	TCATCTTAAATTCTAACATGTTATCATTTGCGGGA
10[153]	6[147]	AAAGAACAATTGAGGACAAAACGAGAACGGTATTC
14[132]	10[126]	TCATCAAAACAATACATTTAAAGTGAATTCAAATA
14[174]	10[168]	GCGGAATAAAATCGAAGAAGAAACCTCCATGCAAA
14[90]	10[84]	ATGGAAGGTCAGATGTACATAAACATAGATGGTTT
15[105]	11[111]	TTACAAATTTGGATAGTACCTTTACCTTAAGACGC
15[147]	11[153]	CGTTATTTGATTATTTGCTTTTCAAGAAGGTCTGA

19[126]	15[132]	ACTATCGCACCAGTGAACCACACCCTCAGTATTAA
19[168]	15[174]	AGCCATTGACGCTCTTAACACTGAAAAAGAGTAAC
19[84]	15[90]	TTGATTACAGAGATCGAACTGAAGGAATAGGATTT
20[111]	16[105]	AGGAACGTGCCTGATAAAAGGTCGCCATAGTTGGC
20[153]	16[147]	GAGCGGGATCCAGAGATTATTACAGAGGTTGCTGA
28[174]	30[168]	AAATCAGATTGTAAAAGGGTG
29[105]	33[111]	TGTACCCATAAATTTGTAATACAATTCTTGTTTTA
29[147]	33[153]	ATTGTATCAGTCAATAAAAATTAGCTATTTCCATA
31[126]	35[132]	GCCTTTACGCGAGCGTACGGTTTTTAATTTAAACA
31[168]	35[174]	TATATTTGCAAATGCAATTCTCAAAAAGAGGTCTT
31[84]	35[90]	GTACCAACATTAACATATAATCCAACAGGCGGAAT
34[111]	38[105]	GAACCAGATTGAATCGTTTACTCAGGACAGTGAAT
34[153]	38[147]	CGAAAGAAATGACCAGGAATTAACGGAAGGATATT
36[132]	40[126]	GAGCAACTCTACGTACGTAACAATCATACCTAAAA
36[174]	40[168]	ATGCAGAGTAGAAAAGAGTAAATGAACGATTAAAC
36[90]	40[84]	AACCAAAAGAACTGCGAGAAAACCTGCTTTTGACC
39[105]	42[105]	CGGAACGGAATACACCGCTTTTTATCAG
39[147]	42[147]	ACCAACTATGCCACTCGGAACTCCAAAA
41[126]	45[132]	CCTCAGCGGAGCCTATTTTCTGAGCCACCTCAGTA
41[168]	45[174]	CTACAGAAATTTTTAGCGGAGCCGCCACAGTATAG
41[84]	45[90]	AGGCTTGGAATTTCACGATCTACCCATGCTGAGAC
43[105]	46[105]	TTTCCAGGGGATAGATTAGGATAAGTTT
43[147]	46[147]	TAAACAACCCTCAGGCCGTCGGCGTCAT
49[49]	4[56]	AAATTATTCATTAAAACCGCCTACCGAAGAAACAA
10[69]	6[63]	GTGATAATAATTACAGATAAGTACGAGCTTGCACC
15[63]	11[69]	CGTCAATATATCAAGCGTAGAAGTGAATTTTTCCC
20[69]	16[63]	GTGAGGCAACCGTTCTGAAAGTGGCTATTAAAATA
29[63]	33[69]	GAATCGACAAAGGCGAGCATAACAGGCAGGCTTAG
34[69]	38[63]	GTACCTTGACTGGATTGCAAAGAATTACGTAAATT
39[63]	42[63]	TAAATTGCAAGCGCAACCGATACCGATA
43[63]	46[63]	ACAGCCCGTTTCGTAAACATGTAAACAG
23[118]	25[120]	TAAATCAAGGTGGTTTTTCTTTGTAAAGCCT
23[160]	25[162]	AGTTTGGAGGCCAACGCGCGGGTTGCGTTGC
23[55]	25[56]	TGGTTTGCAGAGAGTTGCAGCATTCCTGTG
23[76]	25[78]	AATCCTGTGCCCTTCACCGCCTCGCTCACAA
25[100]	21[111]	GAAGCATAAAGTTCACCAGGCAAAATGGGTCGAGGCGCTG
25[121]	20[126]	GGGGTGCCTAATGCGCCAGAAGAATATCACCCACACGCTGCCGATTA
25[142]	21[153]	AACTCACATTAAGAGAGGCTGAGTGTGATGGCCACCCGCC
25[163]	20[168]	GCTCACTGCCCGATGAATCACAAGAGCGAAAAAGCTACAGGTGCTTT
25[79]	20[84]	TTCCACACAACAGCTGATTTTGATGGCTAAATCAGCGAAATGAGAAG
26[120]	28[105]	ACGCCAGCTGGCGAAATCTGCCATAGCCAG
26[162]	28[147]	CAACTGTTGGGAAGGGGATCGCAAATAGGA
26[56]	26[42]	CAGTCACGACGTTGT
26[78]	28[63]	TTGGGTAACGCCAGGGGATAGGTCGTCGGA

28[119]	26[121]	CCTTCCTGGTTTGAGGGGACGACTATT
28[140]	26[142]	TCAAAAATATCGGCCTCAGGAACGATC
28[161]	26[163]	TTTTAACCCTCCAGCCAGCTTTCTGCG
28[182]	26[184]	TTTTTGTTCGCTTCTGGTGCCGAAGCG
28[77]	26[79]	TAACAACCCACGTTGGTGTAGATTAAG
28[98]	26[100]	TCAACATTATCGTAACCGTGCAGGGGG

Sensor modules

23[139]	25[222]	GATAGGGTGGTTTGCGTATTGGGAGTGAGCTcagctgcttttgggattccgttg
23[181]	25[206]	ATTAAAGATGCCAGCTGCATTACTTTCCAGTtcagccgctgtcacacgcacag
23[97]	25[236]	CGAAATCGTGAGACGGGCAACATACGAGCCGgctgagagtgtaggatgtttaca
24[41]	21[69]	acgctgtcggtgagtTGAAATTGTTATCGGCCCTGCCCAGCAAGCCCCCGGCGAGA
26[213]	27[174]	gtgacagcggctgaCCATTCGCCATTCAGGCCGGCAC
26[228]	30[126]	tcccaaaagcagctgGGTGCGGGCCTCTTCGCGACAGTAATTCGCAAAAGCCGATATTC
26[244]	30[84]	tcctacactctcagcATGTGCTGCAAGGCGATGGGCGCAAATGTGAAAACTATAGCTAT
28[56]	27[41]	GTGGGAACATTGACCGTAATGGTTTTCCactcaccgacagcgttgaatgtt

Biotin anchors

4[132]	2[127]	GAGGGTACAATAATTAAGTT
4[174]	1[192]	GCATTAGTCCTTATAAAGGT
4[90]	2[85]	ATTGAGTCAGATAGACCAGC
5[105]	1[133]	AACAGCCTCAGAGACAGAAGGAATAGA
5[147]	2[148]	TTTGTTTTGAACACAAGAACTAAACGC
5[63]	1[88]	AACGAGCAGAGCAAGCCCTTTGCGACA
46[132]	49[131]	GATACAGGACAGGAGTCATAGAGCACC
46[174]	50[162]	CTGAATTACAAACACCTTTAGACCATC
46[90]	49[89]	CCTTGAGAGAGCCGAAATCACGACTTG
47[105]	50[100]	ACCAGAGTTTGCCAAGAGCC
47[147]	49[152]	AGACGATGCGTTTTGCCGGA
47[63]	50[57]	ACCCTCACCACCGGAGGTGA

Foot

2[105]	47[97]	AAATTCATATGGTTTCCGAACAATAATCACCACCAG
2[126]	47[118]	TATTTTGTCACAATCAAACCGAATTAGCGCCGCCGC
2[147]	47[139]	AAAGACACCACGGAAAACGGAAATTTTCGGGTTGAG
2[168]	47[160]	GGCAACATATAAAAGGGCATGACTGTAGCTGGCCTT
2[188]	47[181]	GAAAATACATACATTACGCAGAAGTTTGAATAAAT
2[63]	47[55]	TTCAACCGATTGAGGGCTATCTTCCCTCACCCTCAG
2[84]	47[76]	GCCAAAGACAAAAGGTTAAGAAAGAGCCAGAGCCAC
4[118]	44[120]	GCTAATAATATTATCTGGTAATTAGCGGGGTTTTGCACCCT
4[139]	44[141]	AAAGTCAATAAGAATTTTGATCCAGGCGGATAAGTAACCGC
4[160]	44[162]	AATTAACAACGTCACCAGTAAAGAGGGTTGATATACCTCAG
4[181]	44[183]	GGGAAGCAGCCTTTGCAGTCTCCCGGAATAGGTGTCAGGAG
4[55]	44[57]	TGAAATAATCTTACCCCCTGCAACCTATTATTCTGCACCAG

4[76]	44[78]	AATAATAGTCTTTCGCCCGTAAAAGTATTAAGAGGTACCGT
4[97]	44[99]	CACAAGACCAGTTAGTCAGTGTCCTCAAGAGAAGGCAAGCC
47[119]	7[117]	CAGCATTGAGTGTATTATCCCCGAACCTCCCGACTCCAAGA
47[140]	7[138]	GCAGGTCACATGGCACGATTTTAAGAACGCGAGGCAGTACC
47[161]	7[159]	GATATTCTACCGTTAAAATGATAGAAGGCTTATCCAAGCAA
47[182]	7[180]	CCTCATTGGAAAGCACAGAGACAATAGCAAGCAAAATCGTA
47[56]	7[54]	AACCGCCTTAATGCCAACGCTCAGCTACAATTTTAATCCCA
47[77]	7[75]	CACCCTCTAACAGTCAGAGCCGATTAGTTGCTATTATGTAG
47[98]	7[96]	AACCACCTAACGGGCAAAATAGGTTTTGAAGCCTTCGGCTG
49[111]	4[119]	AGCAAAATCACCAGTCCCCCTTGGAAACGATTGAGC
49[132]	4[140]	ATTACCATTAGCAAGCATCGGCTACCCAACCTGAAC
49[153]	4[161]	AACGTCACCAATGAACGTCAGATTAAGACACGGGAG
49[174]	4[182]	GATAGCAGCACCGTACAGAATCTATGTTAAAAAACA
49[69]	4[77]	ATTATCACCGTCACCCGGAACCAAGTAAGTAAGCCC
49[90]	4[98]	AGCCATTTGGGAATTTCTTTTCAAGTTACGATAACC

Table S2. SV staple strand sequences.

SV structures were assembled using the complete set of the listed oligos. SV structures with the hinge were assembled without the "Removable hinge" module, resulting in a 7 base single stranded scaffold linker between the foot and the sheet structures that was acting as a flexible hinge. Fluorophore rows of 1st sheet are labelled with Cy3 and fluorophore rows on the second sheet are labelled with Cy5. Sensor module contains lock strand with complementary parts coloured in red. Biotin anchor module lists oligos that were labelled with biotinylated ddUTP at the 5'end.

	Start	End	**Fluorophore rows: 1st sheet**
1st	4[174]	3[159]	GCATTAGTCCTTATAAAGGTGGCAACATATAAAAGGGCATG
	3[160]	5[159]	ATTAAGACACGGGAGAATTAACAACGTC
	5[160]	7[159]	AAAAATGATAGAAGGCTTATCCAAGCAA
	7[160]	9[159]	GCCGTTTTGAATATAAAGTACCAATCGC
	9[160]	11[159]	CATATTTATCCAATCGCAAGACGAGACT
	11[160]	13[159]	ACCTTTTTTGATGAAACAAACAGAATAC
	13[160]	15[159]	CAAGTTACTATCATCATATTCCAATTTT
	15[160]	17[159]	AAAAGTTTTCTAAAGCATCACCTGAGGC
	17[160]	19[159]	GGTCAGTAAATCGTCTGAAATGACAATA
	19[160]	21[159]	TTACCGCCCCTCGTTAGAATCAGCGCTT
	21[160]	23[159]	AATGCGCCCCGTCTATCAGGGCTGTTCC
	23[160]	25[159]	AGTTTGGAGGCCAACGCGCGGGTTGCGT
2nd	5[147]	3[138]	TTTGTTTTGAACACAAGAACTAAACGCAAAGACACCACGGAAAACGGA
	3[139]	5[138]	ATACCCAACCTGAACAAAGTCAATAAGA
	5[139]	7[138]	AACGATTTTAAGAACGCGAGGCAGTACC
	7[139]	9[138]	GCACTCATGGTAAAGTAATTCTCAACAG
	9[139]	11[138]	TAGGGCTTGCGAGAAAACTTTTTTATCA
	11[139]	13[138]	AAATCATAAACAAAATTAATTAACGGAT
	13[139]	15[138]	TCGCCTGACAGATGATGGCAATATCCTT
	15[139]	17[138]	TGCCCGAAACCTCAAATATCAACAGCAG
	17[139]	19[138]	AAGATAAATACATTGGCAGATTGCCTTG
	19[139]	21[138]	CTGGTAATAGCTAAACAGGAGGCGCGTA
	21[139]	23[138]	ACCACCACCACTACGTGAACCAGCCCGA
	23[139]	25[138]	GATAGGGTGGTTTGCGTATTGGGAGTGA
3rd	4[132]	3[117]	GAGGGTACAATAATTAAGTTTATTTTGTCACAATCAAACCG
	3[118]	5[117]	AGGAAACGATTGAGCGCTAATAATATTA
	5[118]	7[117]	TTTATCCCCGAACCTCCCGACTCCAAGA
	7[118]	9[117]	ACGGGTATCGACGACAATAAACTACCAG
	9[118]	11[117]	TATAAAGCTATTTTAGTTAATTTGAGAA
	11[118]	13[117]	GAGTCAATCAATTTCATTTGAATTTACA
	13[118]	15[117]	TCGGGAGATATAATCCTGATTGCAATTC
	15[118]	17[117]	GACAACTCATCAATATCTGGTCTAAAAA
	17[118]	19[117]	TACCGAACCACACGACCAGTAAGTAGAA
	19[118]	21[117]	GAACTCAAAAGGGATTTTAGACGCAAGT
	21[118]	23[117]	GTAGCGGTAATCAAGTTTTTTGCCCTTA
	23[118]	25[117]	TAAATCAAGGTGGTTTTTCTTTGTAAAG
4th	5[105]	3[96]	AACAGCCTCAGAGACAGAAGGAATAGAAAATTCATATGGTTTCCGAAC

	3[97]	5[96]	AAAGTTACGATAACCCACAAGACCAGTT
	5[97]	7[96]	ACAAAATAGGTTTTGAAGCCTTCGGCTG
	7[97]	9[96]	TCTTTCCTTCAGCTAATGCAGACATATG
	9[97]	11[96]	CGTTATACCTGACCTAAATTTACGATAG
	11[97]	13[96]	CTTAGATTTTTTAATGGAAACAGAATAT
	13[97]	15[96]	ACAGTAACTATACTTCTGAATAAGAAGT
	15[97]	17[96]	ATTAGACTAAATCAACAGTTGAATAGCC
	17[97]	19[96]	CTAAAACAGACATTCTGGCCAAGTAATA
	19[97]	21[96]	ACATCACTGTACGCCAGAATCCGGAGCG
	21[97]	23[96]	GGCGCTAGGGTGCCGTAAAGCATGGTTC
	23[97]	25[96]	CGAAATCGTGAGACGGGCAACATACGAG
5th	4[90]	3[75]	ATTGAGTCAGATAGACCAGCGCCAAAGACAAAAGGTTAAGA
	3[76]	5[75]	AAAGTAAGTAAGCCCAATAATAGTCTTT
	5[76]	7[75]	CCAGAGCCGATTAGTTGCTATTATGTAG
	7[76]	9[75]	AAACCAATTGTTTATCAACAATTAGAAA
	9[76]	11[75]	AAGCCTGTGAAATACCGACCGTTTAGAA
	11[76]	13[75]	TCCTTGAAAATCAATATATGTGTTTTCA
	13[76]	15[75]	GGTTTAACGGTTAGAACCTACCAGATAA
	15[76]	17[75]	TACATTTGTGAGGAAGGTTATCTAGTCT
	17[76]	19[75]	TTAATGCGAGAACCCTTCTGACGTAGCA
	19[76]	21[75]	ATACTTCTTGTTTTTATAATCAAAGGAA
	21[76]	23[75]	GGGAAGAAGGAACCCTAAAGGGGGCGAA

Fluorophore rows: 2nd sheet

5th	30[77]	28[78]	GAGATCTATGAACGGTAATCGTAGCGAG
	32[77]	30[78]	TAAATCATAAGCTAAATCGGTTTTTTGA
	34[77]	32[78]	ATTAGAGAAGCTTAATTGCTGAATCCAA
	36[77]	34[78]	AGAGGCTTTAGCGTCCAATACTGTCAGG
	38[77]	36[78]	AACGAGTACTTATGCGATTTTAATAGCG
	40[77]	38[78]	GATTATACTGTCGAAATCCGCGCACCAG
	42[77]	40[78]	AGCTTGATATATTCGGTCGCTGCCCAGC
	44[77]	42[78]	AACACTGATCATAGTTAGCGTATTAAAC
	46[77]	44[78]	TGCCCGTAAAAGTATTAAGAGGTACCGT
	48[77]	46[78]	CAGAGCCAGAGCCACCACCCTCTAACAG
	49[70]	48[78]	TTATCACCGTCACCCGGAAC
4th	28[98]	26[99]	TCAACATTATCGTAACCGTGCAGGGGGA
	30[98]	28[99]	GGAGAGGGGCATGTCAATCATACTTTCA
	32[98]	30[99]	AGTAGTAGAAACATTATGACCCAATGCC
	34[98]	32[99]	AGCAAACTGCTGTAGCTCAACAACTAAT
	36[98]	34[99]	ACGATAAACGTCATAAATATTCACCGGA
	38[98]	36[99]	TGCCCTGAGCTCATTATACCAGCAGACG
	40[98]	38[99]	CACTCATCCCATGTTACTTAGCAAGGCT
	42[98]	40[99]	TTCGAGGTCAGGGAGTTAAAGGCTAAAA
	44[98]	42[99]	CAATAGGAAAAGTTTTGTCGTCCTTGCT
	46[98]	44[99]	GGTCAGTGTCCTCAAGAGAAGGCAAGCC

	48[98]	46[99]	CATAATCACCACCAGAACCACCTAACGG
	46[90]	48[99]	CCTTGAGAGAGCCGAAATCACGACTTGAGCCATTTGGGAATTTCTTTT
3rd	28[119]	26[120]	CCTTCCTGGTTTGAGGGGACGACTATTA
	30[119]	28[120]	TCTAGCTGCGGTTGATAATCAGGTCTGG
	32[119]	30[120]	GGTGGCATCTTTTGCGGGAGAAAACCGT
	34[119]	32[120]	TTCAAAGCAATATGCAACTAAATGAAAA
	36[119]	34[120]	ATAACCCTCCCCCTCAAATGCTTCGAGC
	38[119]	36[120]	GCTCATTCGTTGGGAAGAAAAAACTATC
	40[119]	38[120]	AGGCAAAAAGGCGCAGACGGTCAAAGCT
	42[119]	40[120]	GTATCGGTTGCGGGATCGTCACCGAAAG
	44[119]	42[120]	CATTTTCAACGTTAGTAAATGATTAATT
	46[119]	44[120]	ACTGGTAATTAGCGGGGTTTTGCACCCT
	48[119]	46[120]	TATTAGCGCCGCCGCCAGCATTGAGTGT
	47[105]	48[120]	ACCAGAGTTTGCCAAGAGCCAGCAAAATCACCAGTCCCCCT
2nd	28[140]	26[141]	TCAAAAATATCGGCCTCAGGAACGATCG
	30[140]	28[141]	ATCAATATCCAAAAACAGGAAGACGCCA
	32[140]	30[141]	ATTTGGGGTTTCAACGCAAGGAATCACC
	34[140]	32[141]	ATATCGCGGTCTGGAAGTTTCAATTTTC
	36[161]	34[162]	ACGCCAAAATAAATCAAAAATCATTAAG
	38[140]	36[141]	CCAAATCATAATAAAACGAACTACGAGG
	40[140]	38[141]	GGCACCAAAGGGAACCGAACTGCATTAC
	42[140]	40[141]	CTCCAAAAAGCGAAAGACAGCATACGAA
	44[140]	42[141]	CACCCTCAGTATGGGATTTTGCAAAAGG
	46[140]	44[141]	CTTTTGATCCAGGCGGATAAGTAACCGC
	48[140]	46[141]	CATTTTCGGGTTGAGGCAGGTCACATGG
	46[132]	48[141]	GATACAGGACAGGAGTCATAGAGCACCATTACCATTAGCAAGCATCGG
1st	28[161]	26[162]	TTTTAACCCTCCAGCCAGCTTTCTGCGC
	30[161]	28[162]	GCCGGAGAAAGCAAATATTTAACTCATT
	32[161]	30[162]	AACCTGTTTTTTAGAACCCTCAAGAAAG
	34[161]	32[162]	AGGAAGCCTAACAGTTGATTCCGTCAAT
	36[140]	34[141]	CATAGTAAGTTCAGAAAACGAGCTTCAA
	38[161]	36[162]	CAAGAACCCAACATTATTACAGTACATA
	40[161]	38[162]	AATACGTATTGAAAGAGGACAGTCTTGA
	42[161]	40[162]	TGAAAATCGAGGGTAGCAACGGGGGTAA
	44[161]	42[162]	AACCGCCACTTTCAACAGTTTCTCACGT
	46[161]	44[162]	TCCAGTAAAGAGGGTTGATATACCTCAG
	48[161]	46[162]	ACTGTAGCTGGCCTTGATATTCTACCGT
	47[147]	48[162]	AGACGATGCGTTTTGCCGGAAACGTCACCAATGAACGTCAG
			Structural strands
	9[126]	5[132]	CAACGCTGTCCAGATAAACCAGTTTTAGAATCCAA
	9[168]	5[174]	ACAACGCAATAAGATATTTTCTCAGATAAAATAGC
	9[84]	5[90]	TTAGTATACGCGCCCAATAATAAATCAATAATTTG
	10[111]	6[105]	TCATCTTAAATTCTAACATGTTATCATTTGCGGGA
	10[153]	6[147]	AAAGAACAATTGAGGACAAAACGAGAACGGTATTC

14[132]	10[126]	TCATCAAAACAATACATTTAAAGTGAATTCAAATA
14[174]	10[168]	GCGGAATAAAATCGAAGAAGAAACCTCCATGCAAA
14[90]	10[84]	ATGGAAGGTCAGATGTACATAAACATAGATGGTTT
15[105]	11[111]	TTACAAATTTGGATAGTACCTTTACCTTAAGACGC
15[147]	11[153]	CGTTATTTGATTATTTGCTTTTCAAGAAGGTCTGA
19[126]	15[132]	ACTATCGCACCAGTGAACCACACCCTCAGTATTAA
19[168]	15[174]	AGCCATTGACGCTCTTAACACTGAAAAAGAGTAAC
19[84]	15[90]	TTGATTACAGAGATCGAACTGAAGGAATAGGATTT
20[111]	16[105]	AGGAACGTGCCTGATAAAAGGTCGCCATAGTTGGC
20[153]	16[147]	GAGCGGGATCCAGAGATTATTACAGAGGTTGCTGA
28[174]	30[168]	AAATCAGATTGTAAAAGGGTG
29[105]	33[111]	TGTACCCATAAATTTGTAATACAATTCTTGTTTTA
29[147]	33[153]	ATTGTATCAGTCAATAAAAATTAGCTATTTCCATA
31[126]	35[132]	GCCTTTACGCGAGCGTACGGTTTTTAATTTAAACA
31[168]	35[174]	TATATTTGCAAATGCAATTCTCAAAAAGAGGTCTT
31[84]	35[90]	GTACCAACATTAACATATAATCCAACAGGCGGAAT
34[111]	38[105]	GAACCAGATTGAATCGTTTACTCAGGACAGTGAAT
34[153]	38[147]	CGAAAGAAATGACCAGGAATTAACGGAAGGATATT
36[132]	40[126]	GAGCAACTCTACGTACGTAACAATCATACCTAAAA
36[174]	40[168]	ATGCAGAGTAGAAAAGAGTAAATGAACGATTAAAC
36[90]	40[84]	AACCAAAAGAACTGCGAGAAAACCTGCTTTTGACC
39[105]	42[105]	CGGAACGGAATACACCGCTTTTTATCAG
39[147]	42[147]	ACCAACTATGCCACTCGGAACTCCAAAA
41[126]	45[132]	CCTCAGCGGAGCCTATTTTCTGAGCCACCTCAGTA
41[168]	45[174]	CTACAGAAATTTTTAGCGGAGCCGCCACAGTATAG
41[84]	45[90]	AGGCTTGGAATTTCACGATCTACCCATGCTGAGAC
43[105]	46[105]	TTTCCAGGGGATAGATTAGGATAAGTTT
43[147]	46[147]	TAAACAACCCTCAGGCCGTCGGCGTCAT
49[49]	4[56]	AAATTATTCATTAAAACCGCCTACCGAAGAAACAA
44[182]	42[183]	GTTTAGTATGAGAATAGAAAGGGAATTG
46[174]	48[183]	CTGAATTACAAACACCTTTAGACCATCGATAGCAGCACCGTACAGAAT
23[76]	25[75]	AATCCTGTGCCCTTCACCGCCTCGCTCA
2[188]	3[180]	GAAAATACATACATTACGCA
6[188]	7[180]	CAATAGCAAGCAAAATCGTA
12[188]	13[180]	AATTACCTGAGCAACGCAGA
18[188]	19[180]	AATACCTACATTTTGCAACA
24[188]	25[188]	TGCCAGCTGCATTACTTTCCAGTCGGGA
30[182]	29[188]	AGATTCAAACGTTAATATTTTG
36[182]	35[188]	TTCAACTATACCCTGACTATTA
42[182]	41[188]	CGAATAATGGCTTTGAGGACTA
48[182]	47[188]	CAAGTTTGAATAAATCCTCATT
25[76]	20[84]	CAATTCCACACAACAGCTGATTTTGATGGCTAAATCAGCGAAATGAGAAG
25[97]	21[111]	CCGGAAGCATAAAGTTCACCAGGCAAAATGGGTCGAGGCGCTG
25[118]	20[126]	CCTGGGGTGCCTAATGCGCCAGAAGAATATCACCCACACGCTGCCGATTA
25[139]	21[153]	GCTAACTCACATTAAGAGAGGCTGAGTGTGATGGCCACCCGCC
25[160]	20[168]	TGCGCTCACTGCCCGATGAATCACAAGAGCGAAAAAGCTACAGGTGCTTT
28[77]	26[70]	TAACAACCCACGTTGGTGTAGATTAAGTTGGGTAAC
26[98]	30[84]	TGTGCTGCAAGGCGATGGGCGCAAATGTGAAAACTATAGCTAT

26[119]	28[105]	CGCCAGCTGGCGAAATCTGCCATAGCCAG
26[140]	30[126]	GTGCGGGCCTCTTCGCGACAGTAATTCGCAAAAGCCGATATTC
26[161]	28[147]	AACTGTTGGGAAGGGGATCGCAAATAGGA
27[182]	27[174]	GGTGCCGAAGCGCCATTCGCCATTCAGGCCGGCAC

Sensor modules

3[181]	5[202]	GTATGTTAAAAAACAGGGAAGCAGCCTTTACAGAGAGAATAtcagccgctgtcacacgcacag
9[181]	11[202]	GTAATTTAATATGTAAATGCTGGGCTTAGGTTGGGTTATATcagctgcttttgggattccgttg
15[181]	17[202]	ATTTTGCGGAGCCAGCAGCAAACGCCTGCAACAGTGCCACGgctgagagtgtaggatgtttaca
21[181]	23[202]	TACTATGGCCAACGTCAAAGGGTCCACTATTAAAGAACGTGactcaccgacagcgttgaatgtt
28[209]	27[181]	acgctgtcggtgagtTTCGCATTAAATTTTTGTTCGCTTCT
34[209]	32[183]	tcctacactctcagcGAAGCAAAGCGGATTGCATGCGAACGAGTAGATATTAGA
40[209]	38[183]	tcccaaaagcagctgTTTTCATGAGGAAGTTTCCGTGTACAGACCAGGGCTGAC
46[209]	44[183]	gtgacagcggctgaAGAATGGAAAGCGCAGTCTCCCGGAATAGGTGTCAGGAG

Biotin anchors

2[62]	2[15]	TCAACCGATTGAGGGAG
3[49]	48[17]	GCTATCTTCCCTCAGAGC
12[62]	38[21]	AACCTTGCTTCTGTGAAATAAAGATGGTTTAA
25[49]	27[40]	TTCCTGTAGTCACGACGT
29[56]	24[18]	AACAAGATTCTCCGGGTTTGCAGAGAGTTGCAGCAAGC
39[56]	14[13]	CGCCTGAGGGCTTGAGAAATTAATTATTTGCACGTAAA

Foot

6[55]	42[56]	AATTTTAATCCCATACAAACTGTAGCATTCCACAGGTTGCGC
9[49]	11[62]	AAACACCCGACAATCATCGCCCACGCATGAAACAATAATTAA
9[56]	39[55]	GGAATCAATAAGGCGTTAAATTCGCTATAGTACAAGTATCAT
15[49]	18[56]	TAATAGATTGCCAGTAGTAAAATGTTTATAATTGCTTTTGAACGTAAGA
16[55]	33[62]	GAGCACTACAATATTCCTTTTTCATTTTTGCGGAT
18[55]	30[56]	ATACGTGCCATCACGAATTAGGCAATAAAGCCTCATATCAGG
21[56]	28[49]	CGAACGTGATTTAGAGCTTGACCACGCTTGGGAAC
27[49]	25[62]	ATTGACCGTAATGGTTTTCCCGTGAAAT
32[62]	29[55]	AGGCAAAGCAAATTCACCGAGTAAAAGAAAGCCGGTCATTGCTGGAGCA
37[49]	16[56]	ACTTTAATCATTGTAGAAGTTTTAGAGCTCTTTAG
4[55]	45[62]	TGAAATAATCTTACCCCCTGCAACCTATTATTCTG
44[62]	8[49]	CACCAGTCCTAATTTCCTGAACAAGAAA
47[49]	6[56]	CCCTCAGAACCGCCTTAATGCCAACGCTCAGCTAC

Removable hinge

5[63]	2[63]	AACGAGCAGAGCAAGCCCTTTGCGACAT
10[69]	6[63]	GTGATAATAATTACAGATAAGTACGAGCTTGCACC
15[63]	11[69]	CGTCAATATATCAAGCGTAGAAGTGAATTTTTCCC
20[69]	16[63]	GTGAGGCAACCGTTCTGAAAGTGGCTATTAAAATA
25[63]	21[69]	TGTTATCGGCCCTGCCCAGCAAGCCCCCGGCGAGA
26[69]	28[63]	GCCAGGGGATAGGTCGTCGGA
29[63]	33[69]	GAATCGACAAAGGCGAGCATAACAGGCAGGCTTAG
34[69]	38[63]	GTACCTTGACTGGATTGCAAAGAATTACGTAAATT

39[63]	42[63]	TAAATTGCAAGCGCAACCGATACCGATA
43[63]	46[63]	ACAGCCCGTTTCGTAAACATGTAAACAG
47[63]	49[69]	ACCCTCACCACCGGAGGTGAA

Table S3. DH staple strand sequences.

DH structures were assembled using the complete set of the listed oligos. Fluorophore rows of 1st sheet are labelled with Cy3 and fluorophore row on the second sheet are labelled with Cy5. Sensor module contains lock strand with complementary parts coloured in red. Staples from the 6th fluorophore rows that connect sheets to the foot have unpaired thymidines, marked in green, that should provide extra flexibility for the opening of the structure.

row	Start-end	Fluorophore rows: 1st sheet
1st	17[39]-11[34]	CTAGAGAGGCTTTTGCAAGAGGGGATTAAGCGATTCCC
	17[60]-11[55]	ACGGACGATAAAAACCATTTAGACGCAAGGCGTAGATT
	17[81]-11[76]	TAGCATAACCCTCGTTTTACTGCGAACATCCGATACAT
	17[102]-11[97]	ATTGCATAGTAAGAGCAATTCATTTCTACTAATAACCT
	16[125]-11[118]	AACGCCAAAAGGAATGCTTTAAGCTGAATCATTTG
2nd	19[39]-9[34]	CCTCTGGCTCATTATACGGAAGAAGTCAAATAAGCCTC
	19[60]-9[55]	ACATACCTTATGCGATTAAAACGAGGGTGAGAATCGGT
	19[81]-9[76]	CCCTCAACTTTAATCATATTATTATGTGTAGTATGACC
	19[102]-9[97]	TGCTGGGCTTGAGATGGCATCAGTTTAAATGCGGGAGA
	18[125]-9[118]	ACACCAGAACGAGTACCACATTTTTTAGCGCAAGG
3rd	21[39]-7[34]	ACTAGATGAACGGTGTAAGGCTGGGCCCCAAATCAATA
	21[60]-7[55]	CCCTGACCAACTTTGAAGAGTAATCCCCGGTTCTAGCT
	21[81]-7[76]	CGATCAATCATAAGGGAGATATTCCTAGCATGGAGAGG
	21[102]-7[97]	TTTGCCGGAACGAGGCGCGTAACACGATGAAGAGATCT
	20[125]-7[118]	CGACCTGCTCCATGGTGAATAGTCTGGAGTCATTG
4th	23[39]-5[34]	ATACGAAGGCACCAACCCAAAAGAGGATTCTAGGAAGA
	23[60]-5[55]	TGAGTAAAATACGTAATTCATCTTGTGAGCGATTTAAA
	23[81]-5[76]	GGCGAGGAAGTTTCCATTATACCACAGCTTTATTTTGT
	23[102]-5[97]	ACCGAGGACTAAAGACTTACAACGCGCGTCTAATTTTT
	22[125]-5[118]	AGCAACGGCTACAGCGCCTGAGGAACGCTTTTTTA
5th	25[39]-3[34]	GATGATAGTTGCGCCGAATCGCCCTCAGGCTGGGAACA
	25[60]-3[55]	ACATTTCTTAAACAGCTTATTCGGAGGCAAACGTAATG
	25[81]-3[76]	TAGTCAGCTTGCTTTCGAGGGAGTCCGCTTCGGTGTAG
	25[102]-3[97]	ATTGCCTTTAATTGTATGCGGGATACTCCAGACCGTGC
	24[125]-3[118]	AAAAAAAAGGCTCCGCGAAAGTATCGGCGGGGACG
6th	27[101]-1[97]	GATAACTACAACGCCTGTtCAACTAATAAAACGTGTGCTG
	27[123]-1[118]	CATTGAGTTTCGTCACCtATAATTTGTTTTCCTGGGTAA
	27[39]-1[34]	CCATCGTCTTTCCAGACtTTTCTGTCGAGCTCAACTGTT
	27[59]-1[55]	CTCAGCGTAACGATCTAAtAACAACTCTCTAGAGTGCGGG
	27[81]-1[76]	CCACACAGACAGCCCTCtCGGAGTGGCTTGCACGCCAGC
row		Fluorophore rows: 2nd sheet
1st	51[28]-56[35]	AGCGGAATTATCATTGCGGAACAATATTACAGACA
	50[51]-56[56]	AACCTGATTATCAGATGGTTTGAGCAACAGGGAAAGCG
	50[72]-56[77]	AACTTCATCAATATAATCGAACGTAAATACCGAGATAG
	50[93]-56[98]	ATTGTTTGGATTATACTACTCGTACAATCGTAAAGGGA

	50[114]-56[119]	ACGAATGGAAGGGTTAGGACTTTATTACATTCCAGTCA
2nd	49[28]-58[35]	TTCGCCTGATTGCTTTTACATCCGATTAAATATCC
	48[51]-58[56]	AACCCAAGTTACAAAATGAATATAAGGAACGCAAACTA
	48[72]-58[77]	AATAGGCGAATTATTCATTTTCAGTGAGAAGACTTGCC
	48[93]-58[98]	TTTTACCTGAGCAAAAGGAAATAAGTGAGGCTCTTTGA
	48[114]-58[119]	CAATGAAACAAACATCAAAATTATGTCTGTCATTAACC
3rd	47[28]-60[35]	TTCCCTTAGAATCCCTGTAAAAACGTGGTAAACAG
	46[51]-60[56]	TTTCATAGCGATAGCTTTGTGAGTAAGAAAGCGTTAGA
	46[72]-60[77]	GACGACGCTGAGAAGAGAACAGTAGCTAGGGGCACGTA
	46[93]-60[98]	CTGTGAATTTATCAAAATGAATTAGCGGTCAGTACTAT
	46[114]-60[119]	TTATCTGAGAGACTACCATTACATACCACACTAATGCG
4th	45[28]-62[35]	CTAAATTTAATGGTTATTTTATCCAGTTGAAAGCC
	44[51]-62[56]	CATACCGACCGTGTGATGCGAGAAACTATTACCCGATT
	44[72]-62[77]	CGCGCGTTAAATAAGAATCCAATCCAACGTCATCGGAA
	44[93]-62[98]	CAGCGGAATCATAATTAATATGTACGTCTATCGAGGTG
	44[114]-62[119]	CAGAAGCCTGTTTAGTACTTAGGTACTACGTCCAAATC
5th	43[28]-64[35]	TAAGAGAATATAAAAGGCAGAGCCAGGGTTGAGTG
	42[51]-64[56]	TATCAAAAGGTAAAGTAAACAACGACCAGTGAAAGAAT
	42[72]-64[77]	CAACCAGACGACGACAATAATTGATGATTGCGGCAAAA
	42[93]-64[98]	TACCATGTTCAGCTAATCCAACGCCCCTGAGTTTGATG
	42[114]-64[119]	AAAGCGCCTGTTTATCACAAATTCCGGTCCACCCCAGC
6th	40[114]-66[119]	ACCGCTTATCCGGTATTtCTGAACACTCACATGCGCTCA
	40[30]-66[35]	TACACTCATCGAGAACAtAGAACGGAATTGTTGCGTATT
	40[51]-66[56]	CTACGTTTTTATTTTCAtCTGTCTTCCACACAACGCGCG
	40[72]-66[77]	TAGAATCATTACCGCGCtTAGAAACAGCATAAGCTGCAT
	40[93]-66[98]	TTTCAAGCAAATCAGATtCCATCCTGGTGCCTGTCGGGA
		Structural staples
	11[35]-15[45]	AATTCTGTCATTCCGAGCTTCAAAGCGATAAGAGGTAAA
	11[56]-7[66]	TAGTTTGACCATTAAATAAATAAAACATGTAAAGATAAT
	11[77]-15[86]	TTCGCAAACATGTTCAGGATTAGAGAGTGACTATTCAT
	11[98]-7[107]	GTTTAGCTATATTTAAGGTGGATTTCAAAACCCTCCTA
	12[102]-17[108]	AATATATTGCTCCTTTTGACAAAAATCCTCAAATTACGAGTAGG
	12[60]-17[66]	AGTACCGGAAGCAAACTCCAGCGGATCGTCCAAACCAGACGAAC
	13[53]-9[45]	GACGGTGTCTGGAAGTTCGAACGAAAAGAATAAAG
	13[94]-9[86]	TTTAAATGCTGTAGCTCAATGGTCAATAGTAGACT
	27[53]-35[45]	GCCACCACCGTCACCAAATTTGGGAATTAGAGCAA
	27[94]-35[86]	TTTCGTTTGCCACAGAATTTATTCATTAAAGGGAA
	29[101]-27[93]	ATTCGCGGGGTTTTGCTCCAT
	29[38]-34[28]	AGTGGGAATAGGTGTATCGTTTAGTAGAGCCGCCATTAGAAATCAC
	29[60]-27[52]	GTAGGGTTGATATAAGTAACC
	29[80]-32[73]	CTGCGGCGGATAAGTGCCCACCCTCAAAA
	29[91]-40[94]	GGAACCTGGAAAGCATTGACAATACCCACATATGGAGGT

3[108]-25[101]	TTGACTCAGGAATTAAGTCAGTCACGACGTTGAGGA
3[67]-25[59]	GTTTGGTGCCGCTATTATGCCTGCAGGTCGATTCA
30[125]-32[115]	ACAAATAATTAAGATCAAGAGAAGGATTCAATAGGGGTC
31[45]-29[37]	CACCTTTTGATGATACAGGTC
31[88]-29[79]	AGCGCAGTCTCTGAATTCCC
32[108]-29[100]	CCCTGAGGCAGATTAAAGCCAGAATATT
32[114]-36[108]	ATAGCGTCAGGAAGGTAAATATTGGCGCCAAGCAA
32[65]-29[59]	AACCAGAACCCCAGTAAGCGTCATGCCC
32[72]-36[66]	TCAGCAGCACTCACCGTCACCGACTTTATTTCTCC
34[79]-31[87]	TTACGTAATCAGTAGCGATCTTTTCGCC
35[46]-38[52]	AGAAGCAAACCCAATAATAAGAGCCAGA
35[87]-38[95]	AATTAAAGAACTTACCGAAGCCCTTGCG
36[107]-38[115]	TAAAAAGTAAGCAGATAGAAT
36[65]-38[73]	TTAAATGAAATAGCAATACTG
37[28]-40[38]	GAATTGATATCAGAGTCTTTCCAGAGCCATCC
38[114]-33[125]	AACAACGTCAAAAATGACGTTTTAAGGGCGAAGGGAGGACTGTAG
38[51]-40[60]	GGGCCAGTTACAAAATATTT
38[72]-34[80]	AACATATTATTTATCCCTAAATCAATCAATATGAA
38[94]-40[101]	CATTATAAGAAACGATTTTTGC
40[100]-32[109]	GGGTTTACCAACGGAAACAAGTTTGCCTTTAGCC
40[37]-31[44]	TGAAAGAAACGCCAGCACAAGGCCGGAAACGGAACCGCCAC
40[59]-32[66]	GCACGAATAAGTTGAGCCTGAAACCATCGATACCGG
5[46]-25[38]	AATAGTAACAGATTGACGCGCCATGCGATCGGGATCCCCGGGTACATGG
5[88]-25[80]	TTAGGCCTTCCATCGTACCAGCTTAGGGGGAACGGCCAGTGCCAAAGAA
55[35]-58[45]	TTAGTCTTTAATGCACGTGGCACCGCCATGCT
54[118]-50[108]	CAACAGTGCCACGCCAATAGATTCGACATCTGAATTAAA
54[76]-50[67]	AAGCATCACCTTGCAAATATCTTTAAAAATGGCAAGTC
54[79]-59[87]	CTATTAAAAATACCGAAGCCAACATACATTTTAACATCTGTT
56[118]-60[109]	CACGACCAGTAATACTGAAATCAATACTCACCGAGAGG
56[55]-52[46]	TAAGAATGCGAACTCAAATATCAAACCCGGAATTGATC
56[76]-60[66]	AACCCTTCTGACCTAAAAACGAAGAACTGTACGCCCTTT
56[97]-52[87]	CATTCTGCGAACCACAGCAGCAAATGAACAACTAACTTT
58[55]-55[60]	TCGGCCTGCCATTGTAACATTAGGAAGGTTATCTATGAACCTGATAG
58[76]-54[80]	TGAGTAGCTCATGGTATTAATTTTAGGAGCACTAAAAAT
58[97]-55[102]	TTAGTAATGACGCTTTAAATCTAGATTAGAGCCGTTGAGAGCCCAGC
63[108]-42[115]	GGCGAATCCTGAGAGTTGCTTTCCAAATGAGTGAGCTAAAGAA
63[67]-42[73]	ACGTAAATCAAGACGGGTCGGCCAACATACGAGCCGGACAAT
64[45]-42[52]	AGGGTGGTTTTGCGGTTTATCCGCTCACAATTTCCT
64[86]-42[94]	ATCCCTTCACCGTGCCAAGTGTAAAGCCTGGAATT
9[35]-14[28]	AGAGCATTAGCAAAGTAATAGAAGCCCGAAAGACT
9[56]-13[52]	TGTACCACATACAGTGGATAGTGCATCAAAAAGATACCA
9[77]-13[76]	CTGTAATTAGCATTGAATCGTATAGTCAGAAGCAAAACAGGT
9[98]-13[93]	AGCCTTTCATCAATGAATCCCCAGGTCTTTACCCTACC
1[35]-23[38]	GGGAAGGTCGCCATACGC
1[56]-23[59]	CCTCTTCGGAAACCTCGC
1[77]-23[80]	TGGCGAATCCGGCATAAA
1[98]-23[101]	CAAGGCGAGATCGCCGTC
14[118]-13[119]	CATAAATTAAGAGGT

15[46]-19[45]	ATGAAATAGCCGTTAATTTAAGAATCAT
15[87]-20[87]	AAATACACTATAAAGATTTTTAATTAAATCAACAGA
17[109]-21[107]	AATAGTAAATTCATTCATTACTTAGTA
17[67]-21[66]	AACTGTGAATAGAACCGACCGAACCAGC
19[46]-24[45]	CAAAGAGGACAAAACACGCCACTAACCGATATGAT
20[86]-24[88]	CGGAACAAAGTTTTCATCGCTTTTCGG
29[112]-40[115]	TGAAAGTAATCCTCGTCAGACAGGAAACAGACAAAGCGA
29[28]-40[31]	TAACGGGGAGTGTAGCCACCCATACATACATATAAATCT
29[49]-40[52]	TAACAGTACATGGCCTCAGAGGTATGTTCACCACGCCAG
29[70]-40[73]	TTAATGCTACCGTTACCACCAATTAAGATGTCACAAGAT
3[35]-21[38]	AACGGCGACCCGTCATAC
3[56]-21[59]	GGATAGGATTAAATTGAC
3[77]-21[80]	ATGGGCGCTGTAGCAGCG
3[98]-21[101]	ATCTGCCAATAATTGAGA
38[104]-27[100]	CAGGGAATTTAAGATAACGGAGGAGGTTTATTAGCAGG
38[125]-27[122]	ACAGAGAGCCGAACGAAACCGGATTGGCCATTTTCAACC
38[41]-27[38]	GCGCTAAGTTAAGCGTAGAAATCAGAGCCTCCCTCACCG
38[62]-27[58]	ACAAAGTAAGAAACTTACGCACCGCCACCAGAGCCACC
38[83]-27[80]	AGAATTAAGCTATCTGGCATGGAGCCGCCATAATCAGAG
48[44]-43[45]	CTTTTGAAAATCAAATATTGAAATGTAATTTGTAC
48[87]-43[87]	TGGATCAATAGATGCAAATAAACACTAGGGCTTAAA
5[35]-19[38]	TTGTATACAGAAAACTGA
5[56]-19[59]	TTGTAAAATATGTACTTG
5[77]-19[80]	TAAAATTCGTAAAAATTA
5[98]-19[101]	GTTAAATAGAGAATAAGC
50[107]-46[108]	ACAAAGATGATTTCATTTCATAGGTATA
50[66]-46[66]	AGATCGCGCAGCAATATAAGATTAAAAAG
52[45]-48[45]	ATTTCATATTCAGTACCTTTGAATACTTG
52[86]-48[88]	GCCCCTGATTGCGTAGATTTCAATTAA
58[118]-52[115]	GTTGTAGGGATTATCAAA
58[44]-64[46]	GGTAAGGGATCGGGAGCCGAGAAATGACGGGTGGAACAGAT
59[88]-64[87]	TTTTTGACGACGCTGGCGCACTAAAAAGGGCCGAA
60[108]-63[107]	GCGCCGCTGCGTTGGGGTCAG
60[118]-50[115]	CCGCTACTAAAAGATTGC
60[55]-50[52]	ATCAGAGTTTAGACCAGT
60[65]-63[66]	CCTCGAAAGGGGGAGCCAAGA
60[76]-50[73]	TAACGTGAGAATCCGTTT
60[97]-50[94]	GGTTGCTATAATCAAGAA
62[118]-48[115]	AAGTTTTCGTAACCTTAA
62[55]-48[52]	TAGAGCTGGAAGGGGAAT
62[76]-48[73]	CCCTAAAAGCGGGCCATA
62[97]-48[94]	CCGTAAAAAGTGTACCTT
64[118]-46[115]	AGGCGAAATGGCCCTGGG
64[55]-46[52]	AGCCCGAAGAGTCCAACT
64[76]-46[73]	TCCCTTATGGACTCGCAA
64[97]-46[94]	GTGGTTCGAAAAACAATG
66[118]-44[115]	CTGCCCGCAGCAAGTTAC
66[55]-44[52]	GGGAGAGTCTTTTCCCAA

66[76]-44[73]	TAATGAACAACAGCGAAT
66[97]-44[94]	AACCTGTCGCCTGGTCAA
7[108]-3[107]	TCAGGCAAACACAGCTCACATCAAAAGT
7[35]-17[38]	TGATATTGGAGACAAAAT
7[56]-17[59]	GATAAATTTCAAAAACTA
7[67]-3[66]	GCCGTCAATCCGTTAATCATCAACTCAC
7[77]-17[80]	GTAGCTATGAGTAACAGG
7[98]-17[101]	ACAAAGGATATATTTGAG
9[46]-5[45]	CTAAAAGGCCCAACCGTTGATAATAGCA
9[87]-5[87]	TTTGCAATGCCTTTTTGACGGTAATCGCA
21[108]-26[108]	TCATAGGCTTTCTCAGCAAAAAGGAGCGAATAtAGTA
21[67]-26[67]	GATTAAACGGGGCTTGCAGGTGAAGTTTCAGtATA
46[107]-41[108]	ACTCTAGAAATATAAAGGCAGAACTAATATCtATAG
46[65]-41[66]	AACAAATAAGCATATTTATTCTGTTAATCGGtTCGT
	Sensor modules
12[76]-12[12]	TTAAATATGCAACTAAactcaccgacagcgttgaatgtt
55[14]-55[76]	acgctgtcggtgagtCCCTAAAACATCGCCA
55[7]-55[118]	gtgacagcggctgaAGAAGATAAAACAGAG
53[35]-54[10]	GTTGAAATCAATCAATATCTGGTCAGTttttcagctgcttttgggattccgttg
12[144]-12[5]	tcctacactctcagcGATGGCTTAGAGCTTAATTGCTGtcagccgctgtcacacgcac
52[114]-55[143]	CAATAATACATTTGAGGCCGCCTGGTGAGGCgctgagagtgtaggatgtttaca
13[8]-12[28]	tcccaaaagcagctgtttttttCGCGTTTTAATTCATATAAC
	Biotin anchors
26[107]-29[111]	CAAGCAAGCCAGGATTATGAAACA
26[66]-29[69]	GTTAGAACCGCGTCGAGATAAACAG
41[109]-38[105]	AAGTCCCGACTTTGTTTATAAAAA
41[67]-38[63]	AGGTTGCTATAACAGCCACCCTGA
24[44]-29[48]	ACCTTTGCTAtAGTTTTGCCCTCAGATAGCCCCCTTGAG
24[87]-29[90]	TTTAAAAGGAAtAGCATTCCCACCCTAGTACCACTATTTC
43[46]-38[42]	CGACATTCCAtAGCAAGCCAATTTTTAATTTGTAATTGA
43[88]-38[84]	CAAGAGCATGtCCAATAGGAAGCCTAATCCAAAGACGGG

Table S4. Correction factors for single molecule FRET measurements.

Correction factors for Zeiss laser scanning microscope 700 based single molecule measurements.

SV structure labelled with 48 Cy3 molecules

Excitation at	639 nm	555 nm	555 nm	Relative bleed through
Sample nr.	Cy5 em	Cy3 em	Cy5 em	
1	57.5	6487.9	1269.4	0.20
2	57.3	4022.9	768.8	0.19
3	42.6	8120.9	1721.2	0.21
4	31.1	13568.7	3575.4	0.26
5	26.3	11976.9	2710.1	0.23
6	54.3	12252.6	3127.9	0.26
7	30.0	11451.5	2741.6	0.24
D bleed trough (D_{bt}) into Cy5 channel				**0.23**

SV structure labelled with 48 Cy5 molecules

Excitation at	639 nm	555 nm	555 nm	Relative bleed through
Sample nr.	Cy5em	Cy3em	Cy5em	
1	2350.6	20.0	444.7	0.19
2	1815.8	16.4	276.4	0.15
3	1443.2	15.5	249.4	0.17
4	1434.9	14.9	245.6	0.17
5	1766.4	15.9	319.7	0.18
6	1501.1	15.0	249.3	0.17
Direct excitation of A at D wavelength (A_{de})				**0.17**