

Engineering modular and tunable single-molecule sensors by decoupling sensing from signal output

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Supplementary Text

Detailed DNA origami folding procedure

16 μL of unmodified staples was mixed with 4 μL of modified staples, 5 μL of FoB20 and 25 μL of p8064 scaffold (produced in-house). Modified staples consisted of the desired modified staples and the respective unmodified staples to replace the modified staples that were not desired in the respective experiment. An exemplary recipe for the modified master mix for the 2 $\times$ 13 sample from Fig. 2 is given below:

- Red and green dye strands (2 $\times$ 1 μL)
- Row 4 closing staples (4 $\times$ 1 μL)
- Row 1-3 staples without closing interactions (12 $\times$ 1 μL)
- Biotin 1-4 (4 $\times$ 1 μL)
- Stabilization staples without stabilizing interactions (4 $\times$ 1 μL)

We used two slightly different versions of the DNA origami design. Version 1 was used in the experiments described in Fig. 3, Fig. 4a-c, Fig. 4e, and Fig. 5 in the main text, while Version 2 was used in the experiments described in Fig. 1, Fig. 2, Fig. 4d, and Fig. 4f. Additionally, for the PDGF-BB sensor, the '1 nt spacer green dye' and the '3 nt spacer red dye' (Tables S3 and S4) were used. The staples needed for Version 1 of the sensor are described in Tables S1, S3, S5-S7 and the staples needed for Version 2 of the sensor are described in Tables S2, S4, S5 and S8.

The main differences between the two versions included different strengths of DNA-DNA closing interactions (11 bp for Version 1 and 13 bp for Version 2). Version 1 of the nanosensor required at least four 11 bp DNA-DNA closing interactions to achieve a stable closed state. Therefore, Version 2 of the sensor with more stable 13 bp DNA-DNA closing interactions was designed which required only two closing interactions (on each side of the nanostructure) and provided a better platform to explore the effects of multivalency on the response window of the nanosensor (Figure 2). Additionally, in the Version 2 of the sensor we optimized the staple routing, especially around the positions where the sensing elements and the hybridizing FRET pair were incorporated to ensure the highest incorporation efficiency and most stable placement of these key interactions. Finally, since compared to Version 1, Version 2 of the nanosensor was also used at varying ionic strength conditions we have also optimized the linker length (to 3 nt + 3 nt) that was used for the hybridizing FRET pair to achieve high FRET in the closed of the sensor independently of the imaging buffer (Fig. S8).

Estimation of incorporation efficiency of fluorophore labelled staple strands forming the FRET pair

To determine the incorporation efficiency of fluorophore labelled staple strands, the samples were additionally filtered to ensure that all fluorophore labelled staple strands are removed and nanosensors were immobilized on BSA/biotin surfaces as described in the Methods section. Then, confocal microscopy scans were acquired with alternating laser excitation (ALEX, alternating 532

nm and 640 nm lasers every 100 μ s). We manually counted spots in all channels. The incorporation efficiencies were then determined using the following equations (neglecting double negatives):

$$Incorp. eff._{Green staple} = \frac{N_{Spots (Green exc. Green em.)} + N_{Spots (Green exc. Red em.)}}{N_{Spots (Green exc. Green em.)} + N_{Spots (Green exc. Red em.)} + N_{Spots (Red exc. Red em.)}}$$

$$Incorp. eff._{Red staple} = \frac{N_{Spots (Red exc. Red em.)} + N_{Spots (Green exc. Red em.)}}{N_{Spots (Green exc. Green em.)} + N_{Spots (Green exc. Red em.)} + N_{Spots (Red exc. Red em.)}}$$

By analyzing the closed nanosensor sample (shown in Fig. 1) we estimated incorporation efficiencies of 96% for the staple containing the acceptor fluorophore (ATTO647N) and 84% for the staple containing the donor fluorophore (ATTO542). We would like to note that incomplete incorporation of the staple carrying the donor fluorophore has no influence on the sensor performance because our analysis uses FRET channel data upon 532 nm excitation and nanosensors that do not contain the donor fluorophore would simply not be excited. The incomplete incorporation of the acceptor dye, on the other hand, leads to an additional 4% of “green only” spots in the FRET channel slightly lowering the FRET contrast that can be achieved when only FRET channel data is analyzed.

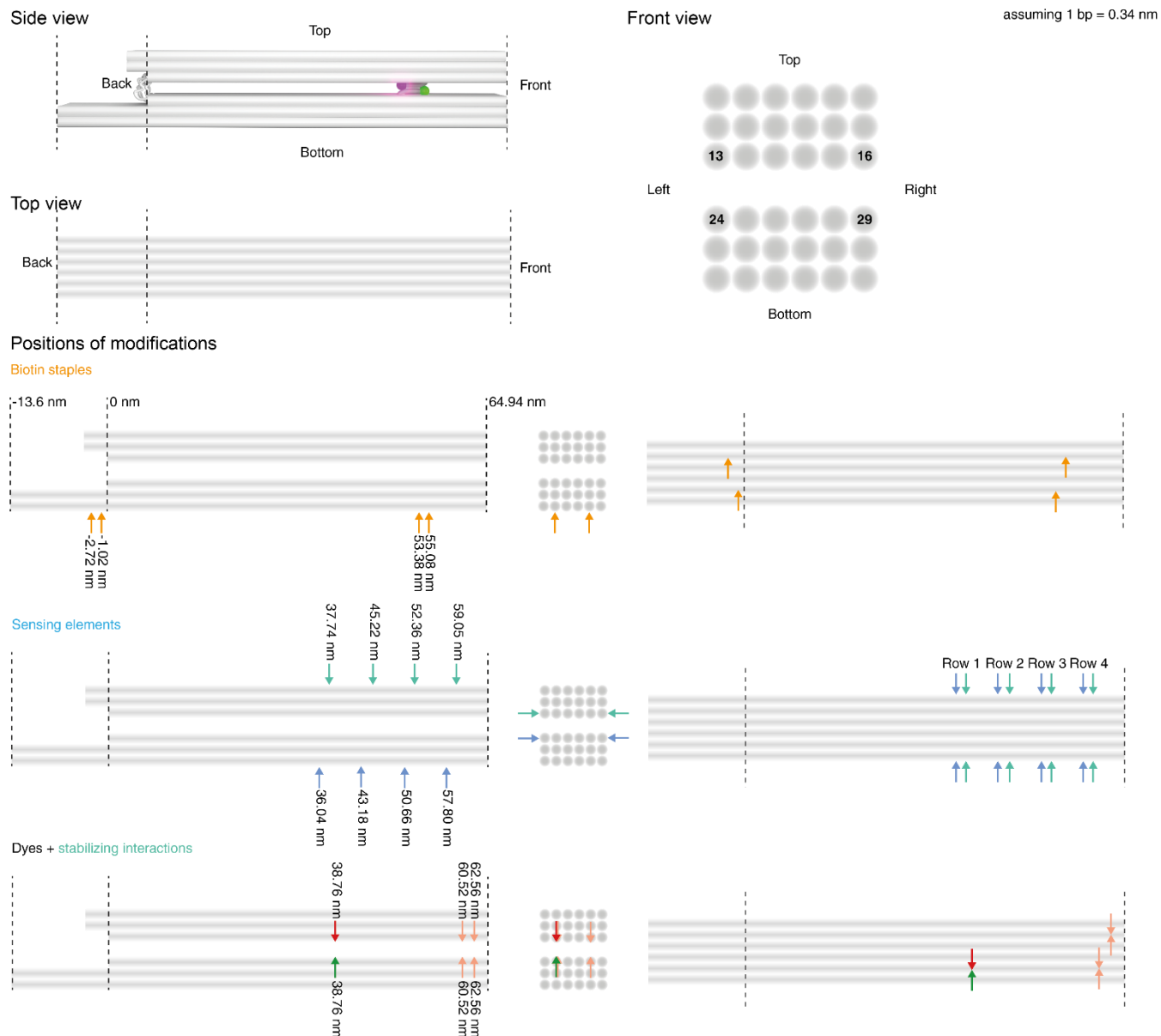


Fig. S1. Depiction of the placement of the modifications (FRET pair, sensing elements, biotin staples used for immobilization as well as stabilizing interactions) in the nanostructure. The numbering in the front view represents the helix numbering in caDNAno and are also used to indicate the positions of the sensing elements in Tables S3, S4, S6, S7 and S8.

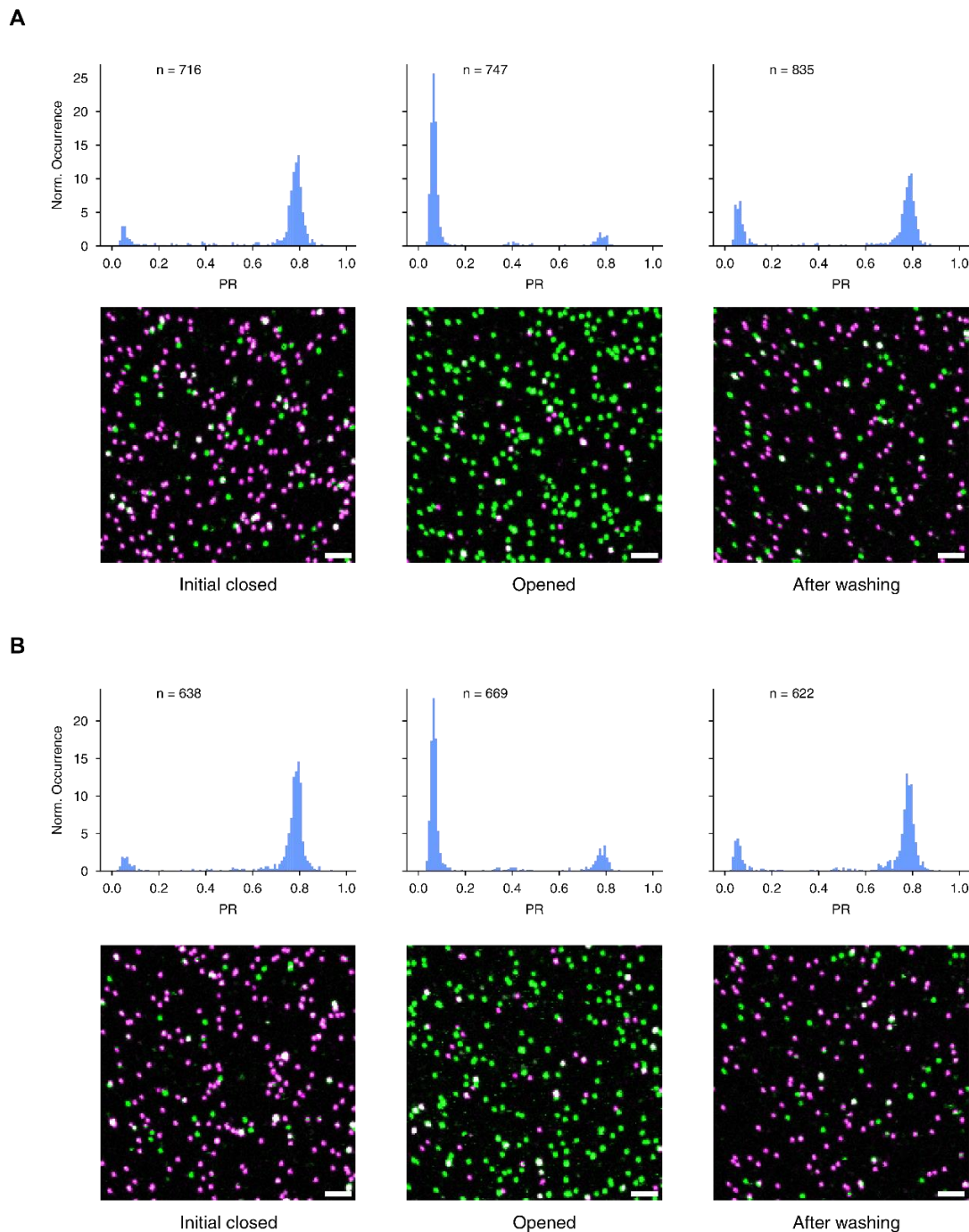


Fig. S2. Reclosing of the hinge nanostructure with two closing interactions measured in the presence of 50 mM (**a**) and 400 mM (**b**) NaCl. Samples were opened by incubating them with 100 μ M opening strands for 1h. Afterwards, they were washed at least 10 times with the respective buffer and scans were taken after approximately 190 hours to allow for complete equilibration. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m).

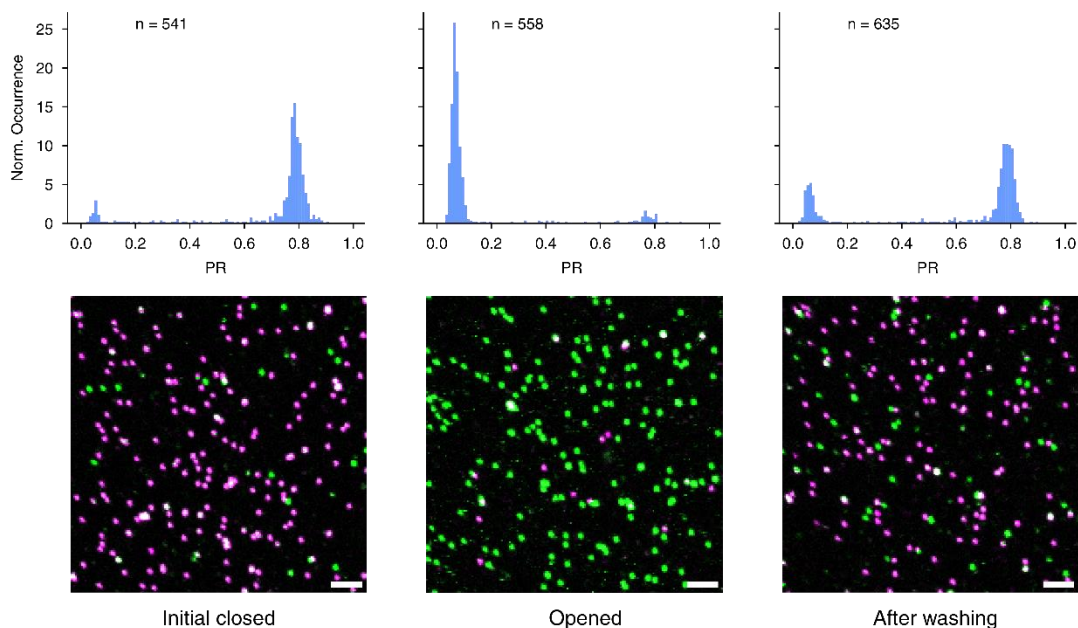
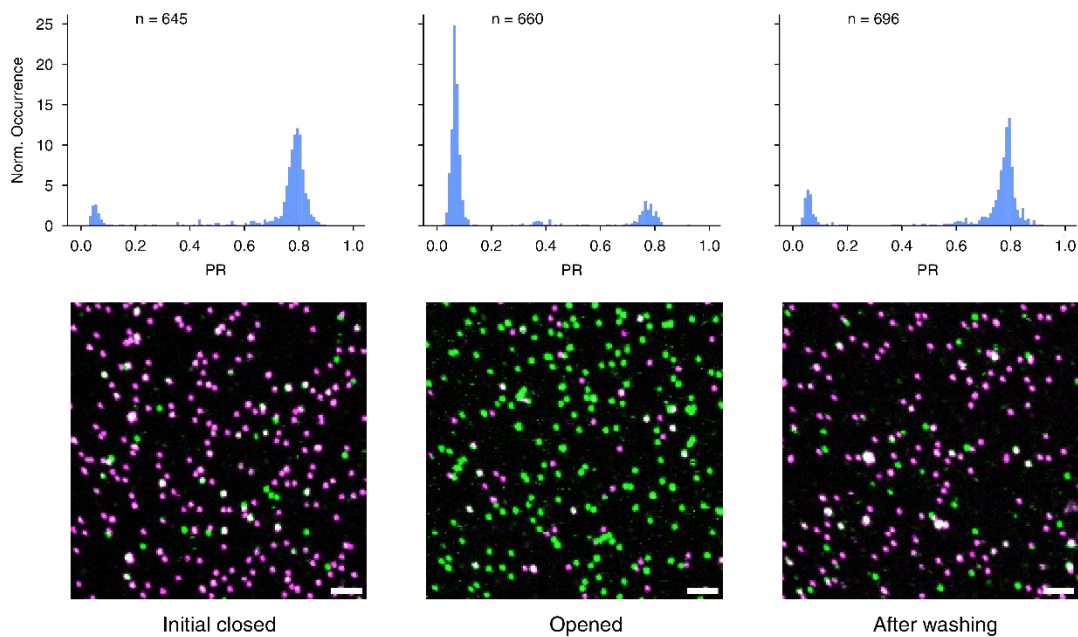
A**B**

Fig. S3. Reclosing of the hinge nanostructure with four closing interactions measured in the presence of 50 mM (a) and 400 mM (b) NaCl. Samples were opened by incubating them with 100 μM opening strands for 1h. Afterwards, they were washed at least 10 times with the respective buffer and scans were taken after approximately 190 hours to allow for complete equilibration. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μm).

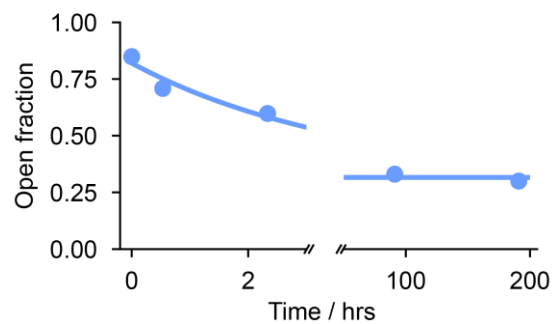
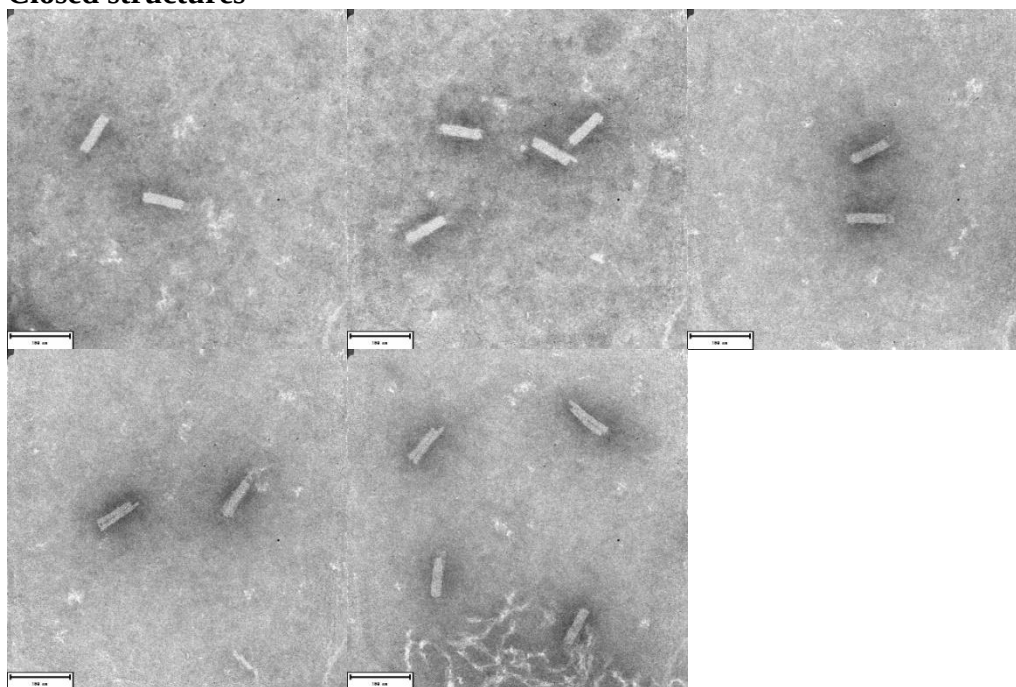


Fig. S4. Reclosing kinetics of the hinge nanostructure with two closing interactions measured in the presence of 50 mM NaCl. Samples were opened by incubating them with 100 μ M opening strands for 1h. Afterwards, they were washed at least 10 times with the respective buffer and scans were taken at different time points (as shown in Fig. S2) after washing and the fraction of open sensors was obtained analysing at least 746 single nanosensors for each time point.

Closed structures



Open structures

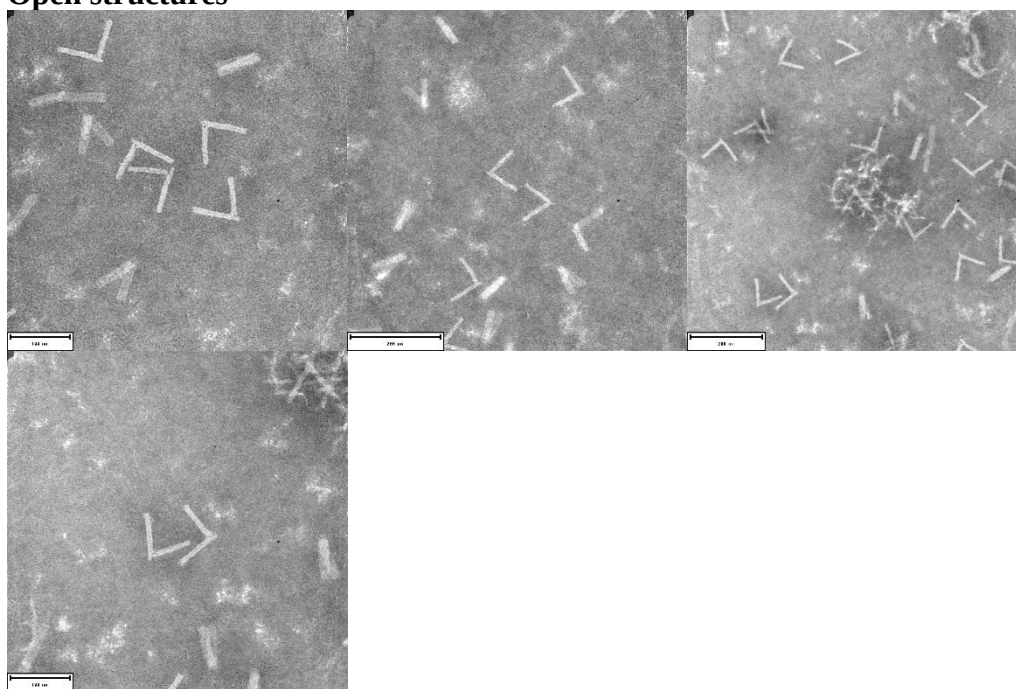


Fig. S5. Full field of view of the TEM micrographs used for the collage shown in Fig. 1b.

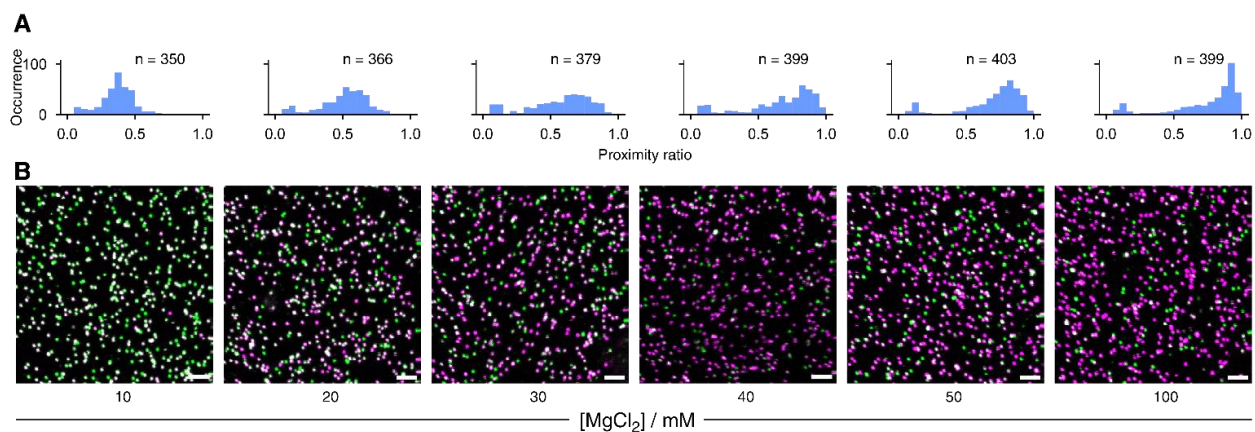


Fig. S6. FRET proximity ratios (**a**) and single molecule confocal scans (**b**) obtained for Version 1 of the nanosensor with a FRET pair consisting of ATTO532 and ATTO647N incorporated directly onto the hinge arms (Table S5) at different ionic strengths (increasing concentrations of MgCl_2). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with the 532 nm laser (scale bar: 2 μm (scale bar: 2 μm)).

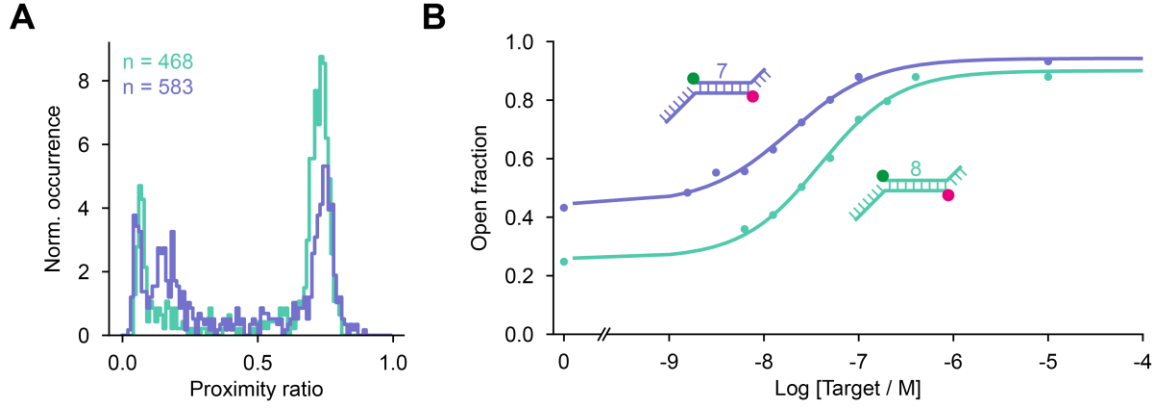


Fig. S7. Effect of the hybridizing FRET pair interaction strength (7 bp vs. 8 bp) on the nanosensor (Version 2, two 13 bp closing interactions) closing (**a**) and response window (**b**). At least 8 bp interaction is required to ensure stable FRET pair hybridization in the closed state. Decreasing the complementary region of the FRET pair to from 8 bp to 7 bp leads to lower stability of nanosensor closed state leading to nanosensor opening at lower concentration ranges (a shift of $K_{1/2}$ and nH from 39 ± 5 nM and 1.05 ± 0.11 to 18 ± 3 nM and 0.96 ± 0.12 , respectively). However, that also comes at the price of a greatly reduced FRET contrast due to lower FRET pair closing in the closed sensor state.

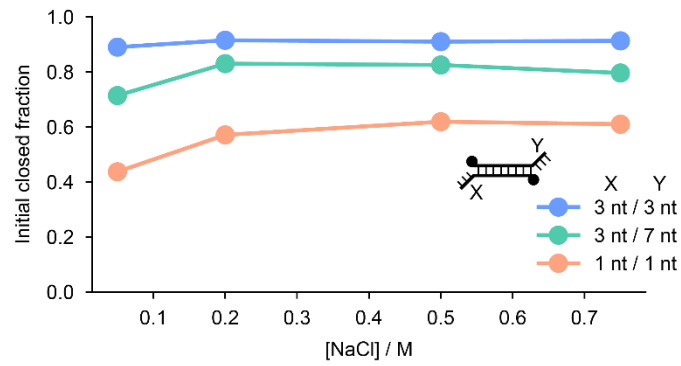


Fig. S8. Effect of the length of the linkers in the hybridizing FRET pair (Version 2 of the nanosensor, two 13 bp closing interactions, 8 bp interaction in the FRET pair) on the fraction of initially closed FRET pairs in the closed sensor state over varying concentrations of NaCl in the imaging buffer. The hybridizing FRET pair containing 3 nt + 3 nt linkers was chosen for further experiments as it led to the highest fraction of closed sensors independently of imaging conditions.

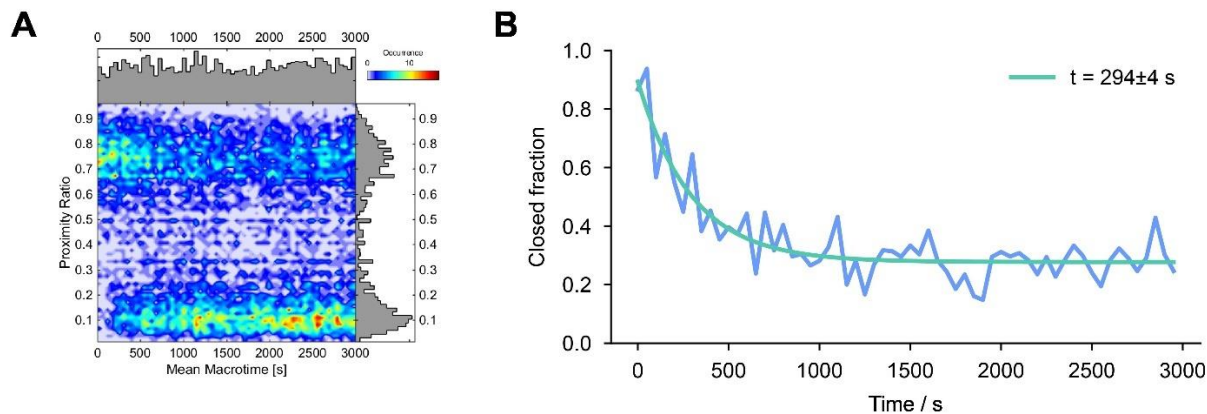


Fig. S9. Nanosensor (as shown in Fig. 1) opening kinetics measured in the presence of 1 μ M 15 bp DNA target strand. **(a)** Proximity ratio of the single nanosensors diffusing through the confocal volume measured at monitored over time after addition of the opening target strand. **(b)** Average fraction of closed sensors over time (averaged over 50 second time bins, blue) extracted from data in (a) and fitted to a single exponential decay function (green).

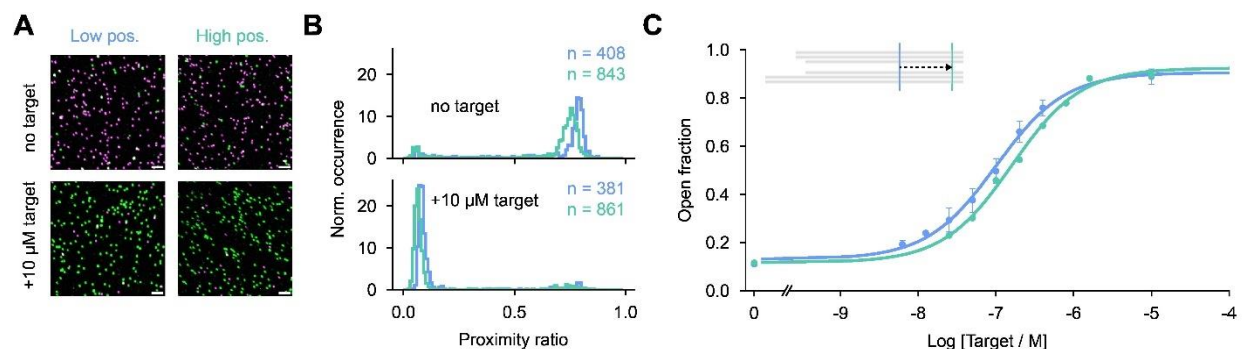


Fig. S10. Effect of the FRET pair position on the nanosensor (Version 2, two 13 bp closing interactions) arms on the FRET contrast that can be achieved as well as the response window of the resulting nanosensors. Two different positions for placing the hybridizing FRET pair (lower positions (closer to the hinge vertex, blue) and higher position (further away from the hinge vertex, green) were compared. **(a)** Single-molecule confocal scans obtained for closed nanosensors (no opening target, top panel) and open nanosensor states (in the presence of 10 μ M 15 bp opening target, lower panel). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m (scale bar: 2 μ m)). **(b)** FRET proximity ratio histograms obtained from analysis of single molecule confocal scans for the two nanosensor designs. **(c)** Response window of the two nanosensor designs obtained by quantifying the number of opened and sensors for different 15 bp DNA target concentrations. We obtain a $K_{1/2}$ of 100 ± 10 nM and a n_H of 0.98 ± 0.08 for the low dye position and a $K_{1/2}$ of 160 ± 19 nM and a n_H of 0.98 ± 0.09 for the high dye position. The titration curve of the low position construct shows mean values of three independent measurements with the error bars corresponding to the standard deviation, while the titration curve of the high position construct shows a single measurement.

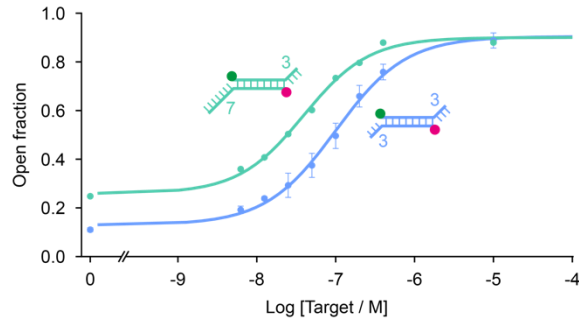


Fig. S11. Effect of the lengths of the linkers (3 nt + 7 nt vs 3 nt + 3 nt) used for hybridizing 8 bp FRET pair (Version 2, two 13 bp closing interactions) response window. Increasing the linker length on one side of the FRET pair from 3 nt to 7 nt results in nanosensor opening at lower concentration (a shift of $K_{1/2}$ and n_H from 100 ± 10 nM and 0.98 ± 0.08 to 39 ± 5 nM and 1.05 ± 0.11 , respectively). However, also at the price of slightly reduced FRET contrast due to lower FRET pair closing in the closed sensor state. The titration curve of the 3 nt + 3 nt construct shows mean values of three independent measurements with the error bars corresponding to the standard deviation, while the titration curve of the 3 nt + 7 nt construct shows a single measurement.

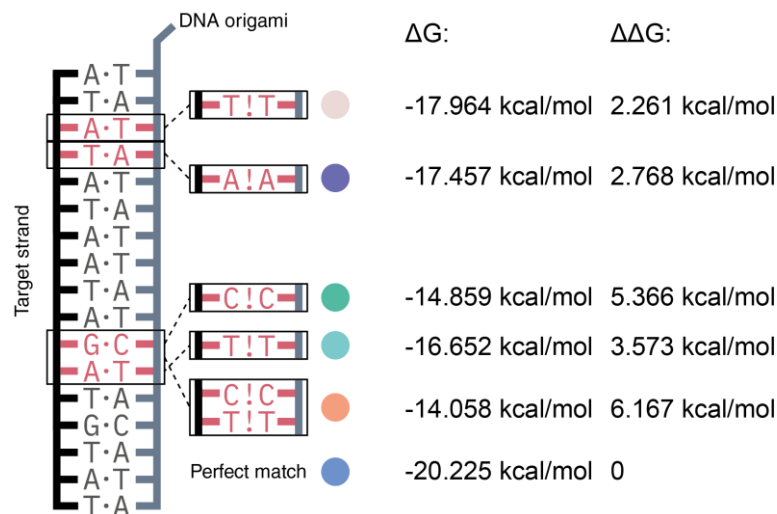


Fig. S12. Binding free energies (ΔG) for the different off-targets as well as for the perfect match as shown in Fig. 3 in the main text, as calculated by NUPACK at 25°C in a buffer containing 750 mM NaCl and 5 mM MgCl₂. $\Delta\Delta G$ is the difference in binding free energy when compared to the perfectly matched target.

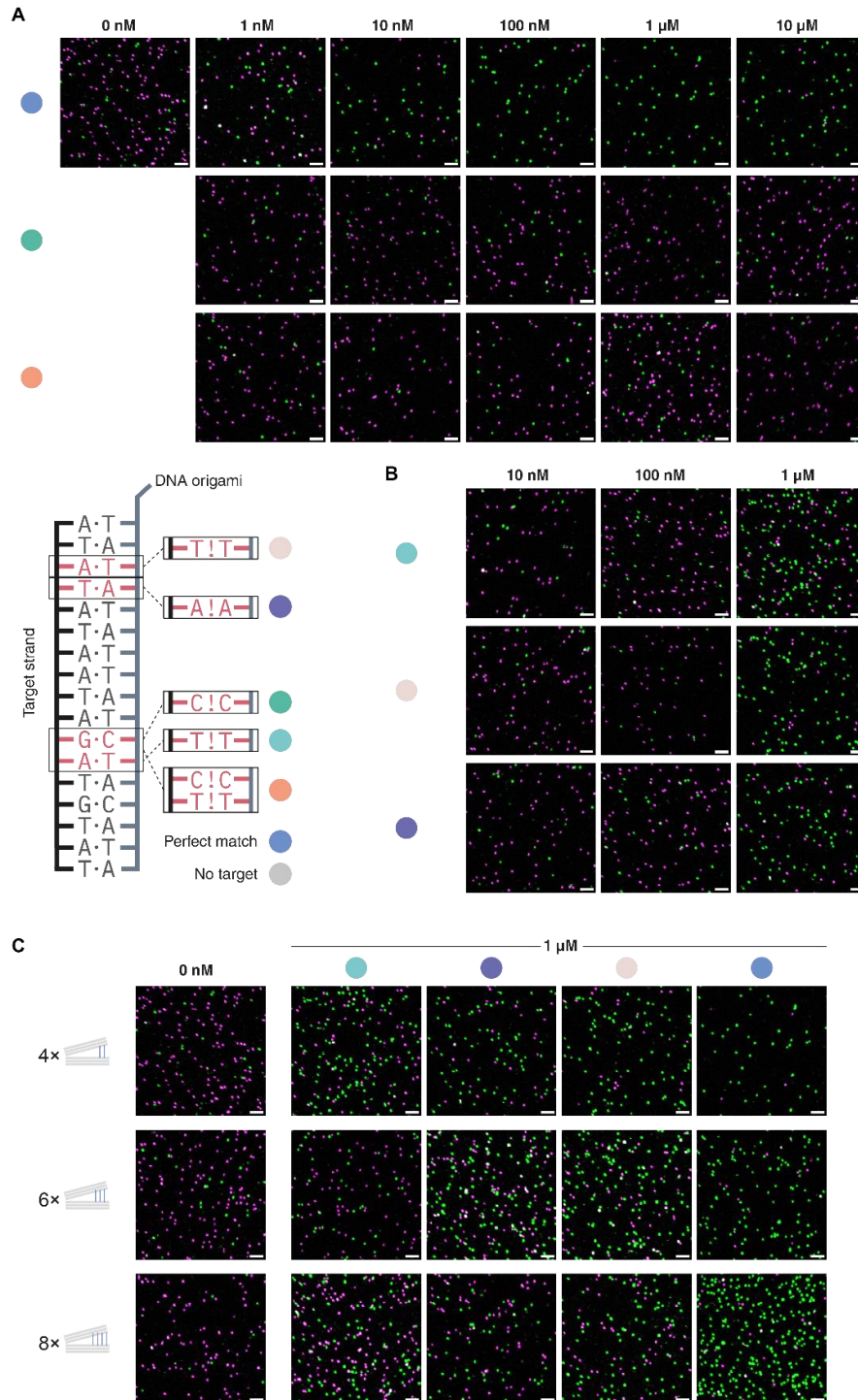


Fig. S13. Exemplary confocal microscopy scan images of the experiments shown in Fig. 3B (a), Fig. 3C (b) and Fig. 3D (c). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m).

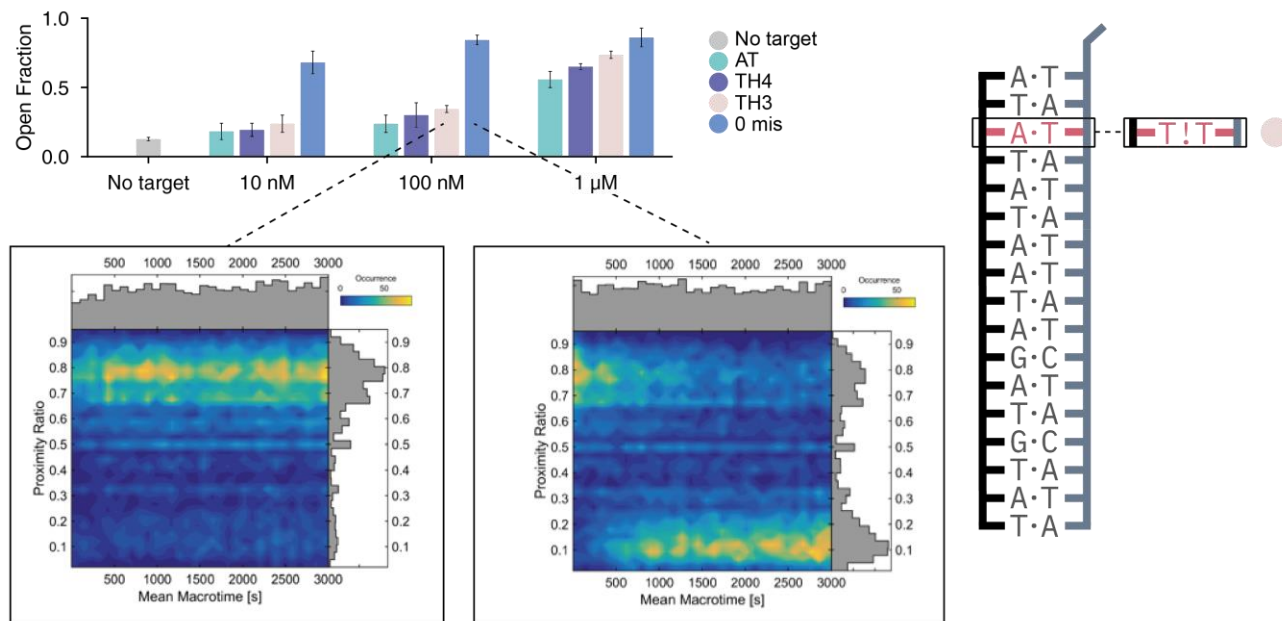


Fig. S14. Nanosensor opening kinetics (proximity ratio vs. time) obtained for 100 nM of the target containing a single nucleotide mismatch (left) and 100 nM of perfectly matched target (right) measured at 1 nM concentration of DNA origami nanosensor containing 4 closing interactions.

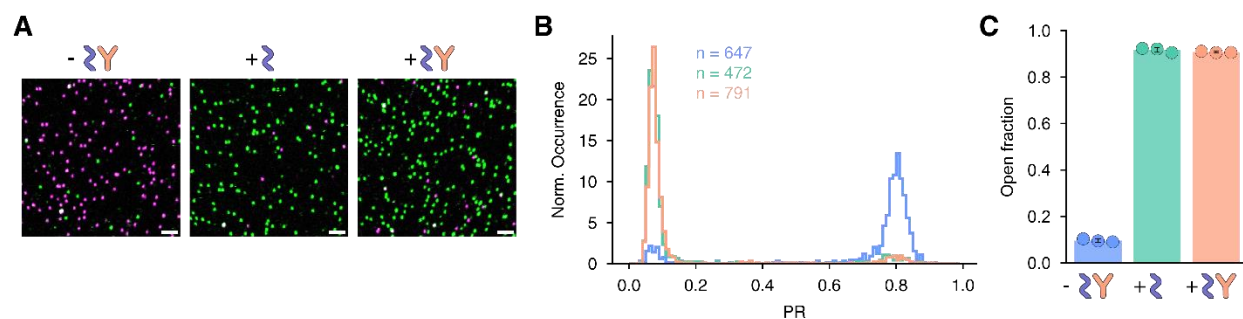


Fig. S15. Control experiment for nanosensor containing no Dig recognition elements. In this case, opening of the sensor is observed even after incubation (100 nM, 30 min) with anti-Dig antibodies. **(a)** Exemplary confocal microscopy scans. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μm). **(b)** Proximity ratio histograms for one experiment. **(c)** Fraction of open sensors. The bar centers show the mean value of three independent measurements. The error bar represents the standard deviation obtained from these independent measurements.

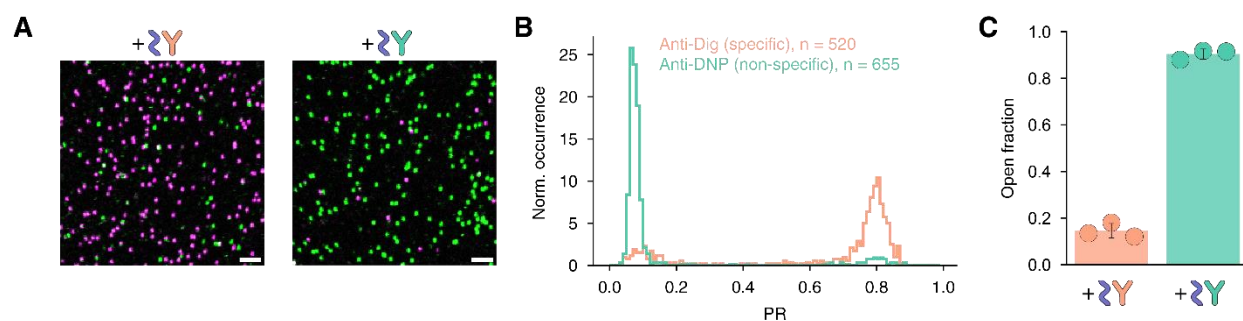


Fig. S16. Comparison of the extent of opening for sensor with Dig antigens when incubated with 100 nM of the corresponding specific antibody (Anti-Dig, orange) to 100 nM of a non-specific antibody (Anti-DNP, green). **(a)** Exemplary confocal microscopy scan images. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m). **(b)** Proximity ratio histograms for one experiment. **(c)** Fraction of open sensors. The bar centers show the mean value of three independent measurements. The error bar represents the standard deviation obtained from these independent measurements.

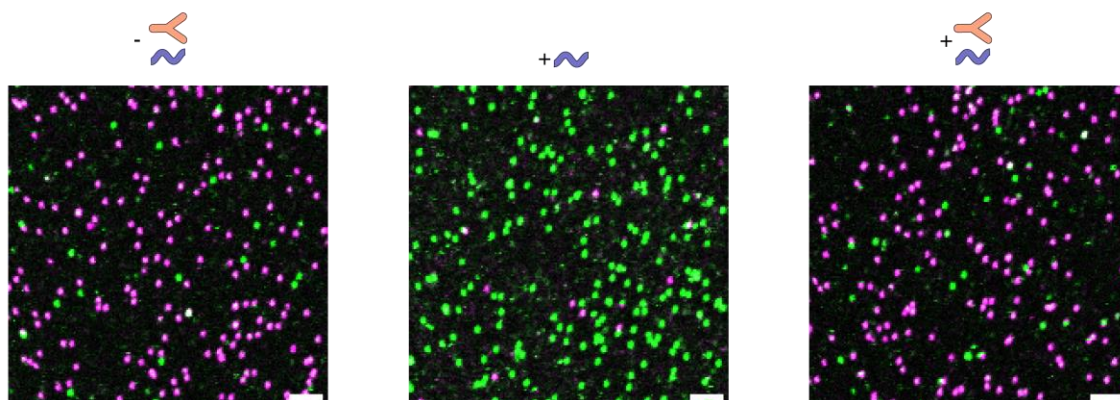


Fig. S17. Exemplary confocal microscopy scans for the data acquired for the antibody sensor (data shown in Fig. 4c before addition of the target (left), upon the addition of DNA opening strand alone (middle) and upon incubation of 100 nM antiDig antibody and addition of DNA opening strands (right) in heparin plasma. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m).

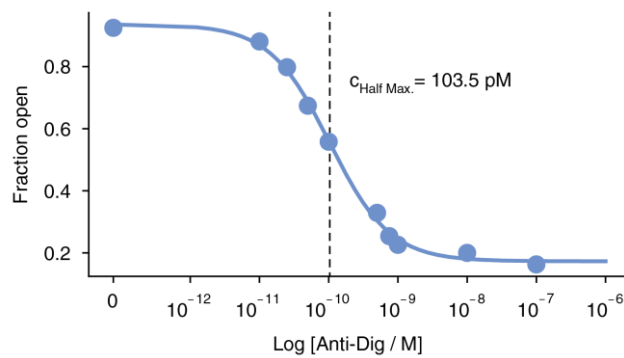


Fig S18. Estimation of the response window of the anti-Dig antibody nanosensor. We obtained a $c_{\text{Half-Max.}}$ value of 103.5 pM.

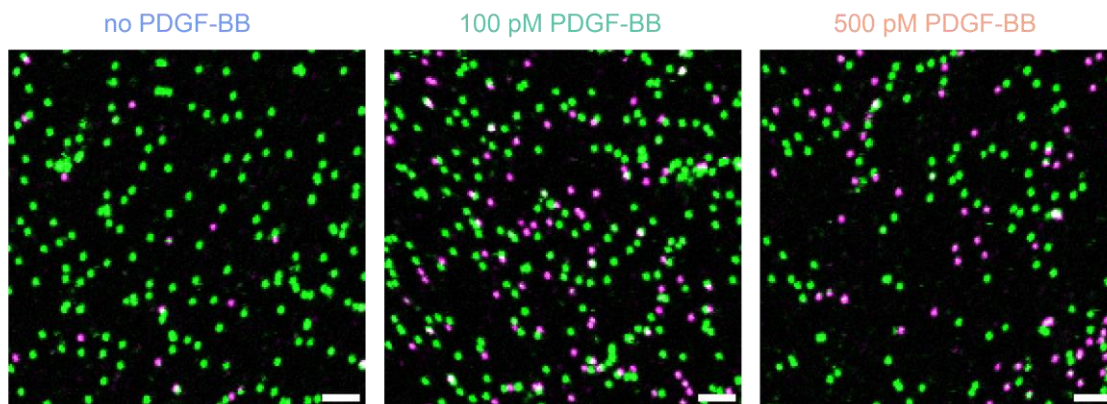


Fig S19. Exemplary confocal microscopy scans of the nanosensor for the detection of different diagnostically relevant concentrations of PDGF-BB (Fig. 4d). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m).

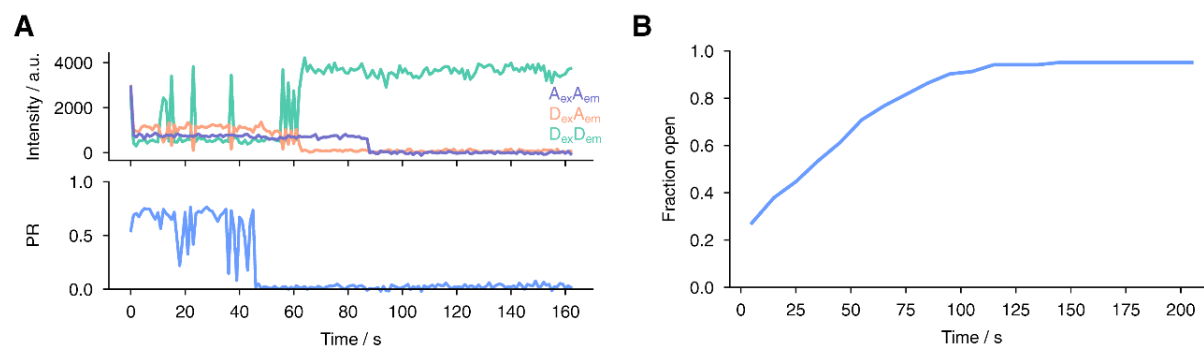


Fig. S20. Kinetics of the XhoI restriction enzyme reaction. **(a)** Exemplary single-molecule FRET trace and **(b)** Average of 103 single-molecule transients of the cleavage reaction of the closing interactions (66 units/mL XhoI, 10 min incubation) monitored by the DNA origami sensor. The FRET transients of the single DNA origami sensors were recorded on a wide-field microscope.

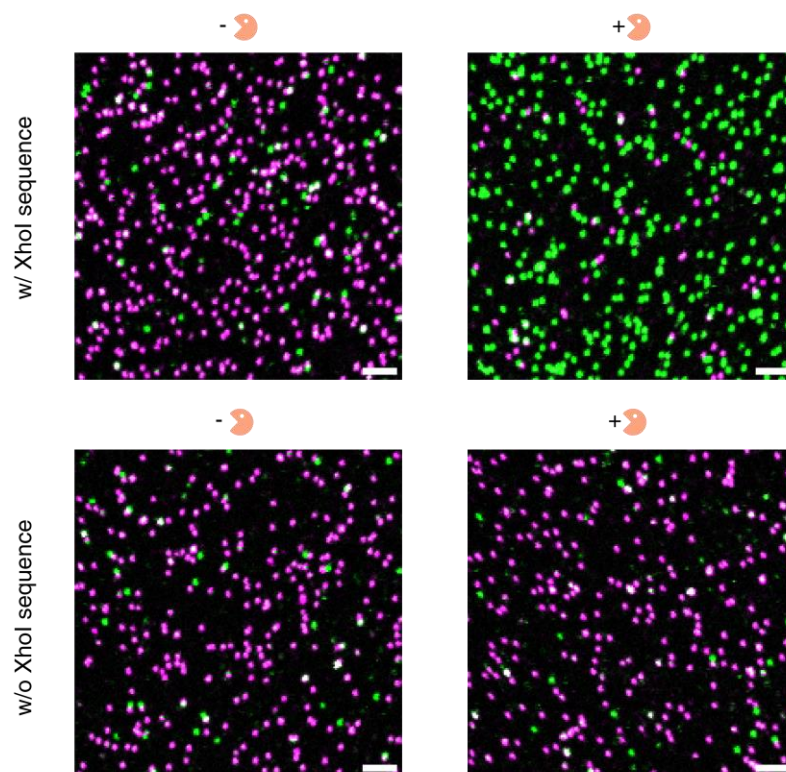


Fig. S21. Exemplary confocal microscopy scan images obtained for the nuclease nanosensor (shown in Fig. 4e). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μm).

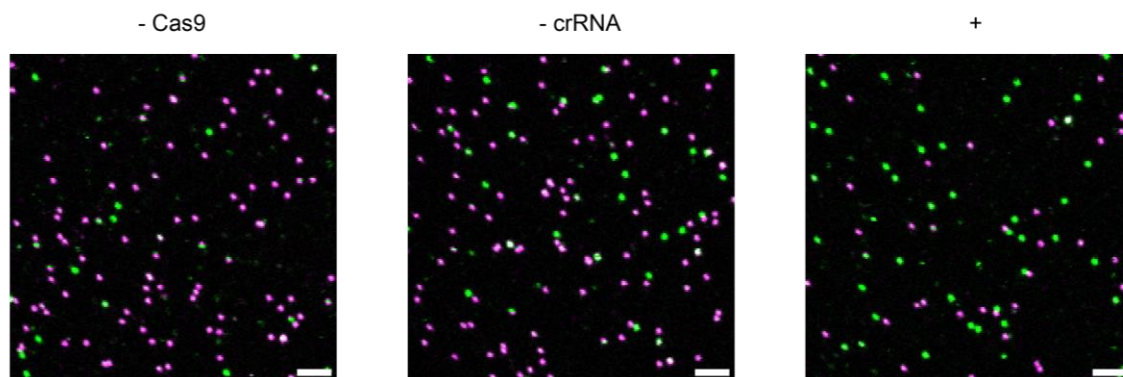


Fig. S22. Exemplary confocal microscopy scan images obtained for the Cas9-based RNA nanosensor as shown in Fig. 4f and 4g in the absence of Cas9 enzyme (left), in the absence of target crRNA (middle) and in the presence of full RNP (right). Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m).

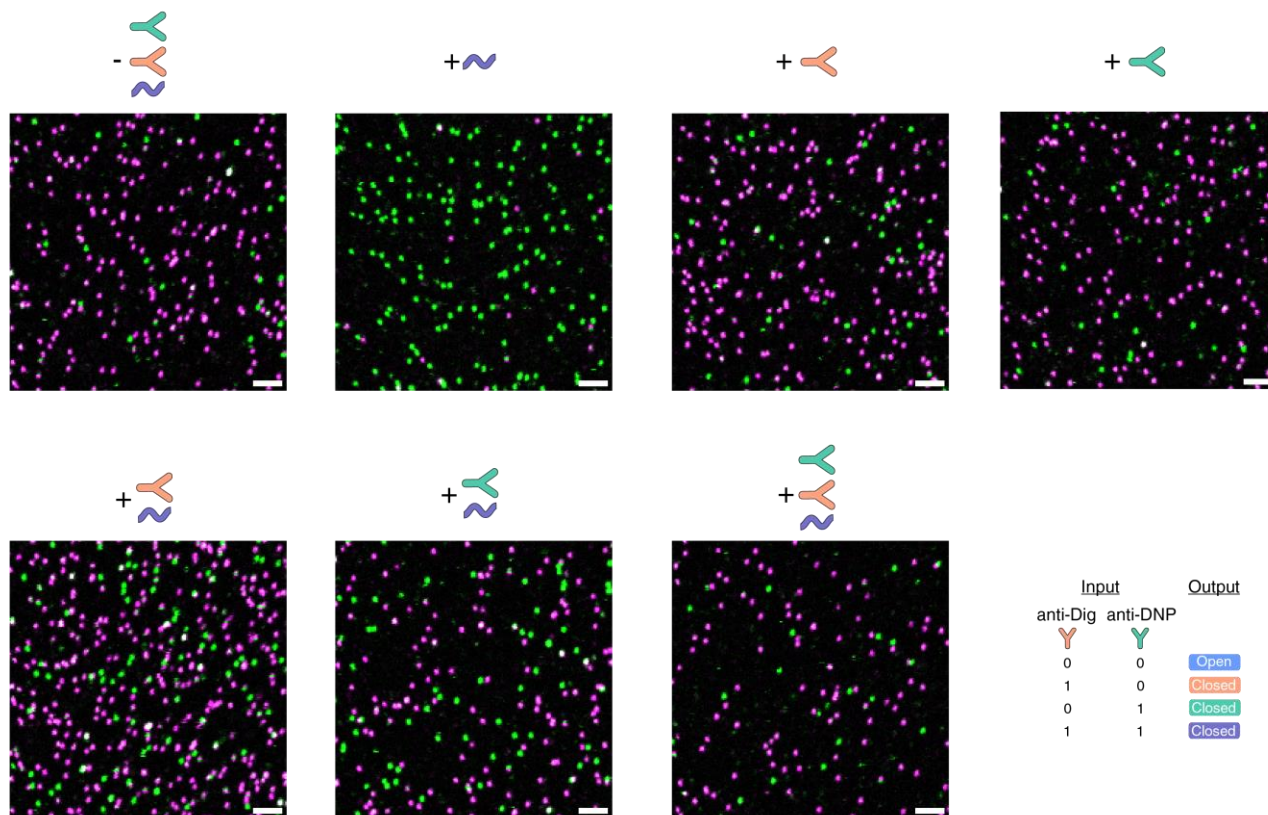


Fig. S23. Exemplary confocal microscopy scan images obtained for the multiplexed sensor for anti-Dig (orange), and anti-DNP (cyan) antibodies (OR gate) as shown in Fig. 5a-c. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m). We note that different fractions of opened nanosensors in the presence of only one antibody could be attributed to a number of reasons, such as different labelling efficiencies of the antigen-labeled DNA strands, different spatial tolerances for bivalent binding distances or different affinities of the two antibodies, ultimately resulting in different degrees of mono- vs. bivalent binding.

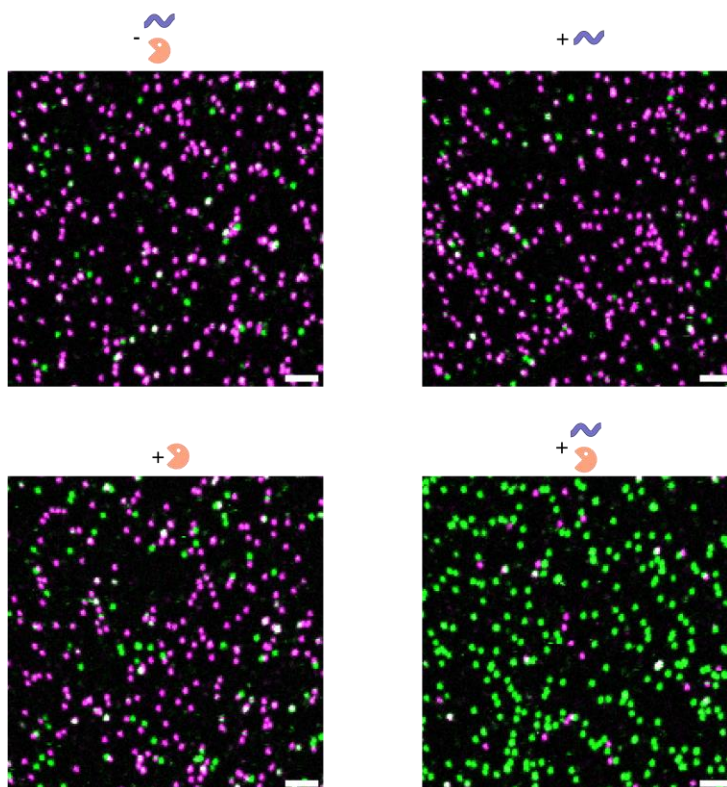


Fig. S24. Exemplary confocal microscopy scan images for the multiplexed nuclease and nucleic acid sensor (AND gate) shown in Fig. 5d-f. Confocal images show merged green (ATTO542 fluorescence) and magenta (ATTO647N fluorescence) channels upon excitation of the sensors with 532 nm laser (scale bar: 2 μ m). We note that the slight increase in the fraction of opened structures in the presence of XhoI is not caused by unspecific activity of the restriction enzyme as confirmed in Fig. S21. We believe it is related to a slightly higher fraction of open nanostructures when the sensors are held together by only two closing interactions upon XhoI cleavage. The contrast of this logic gate could be further optimized using longer/stronger DNA-closing interactions.

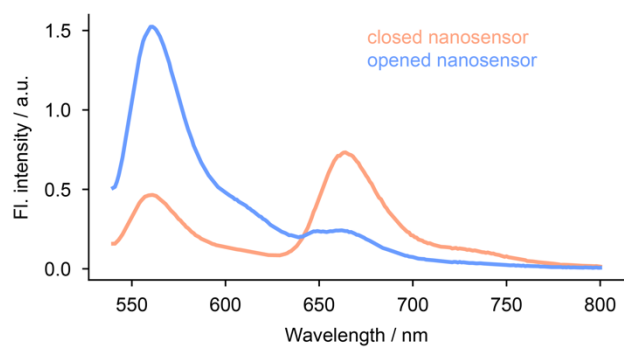


Fig. S25. Ensemble fluorescence spectra of the closed (orange) and open (blue, after addition of 250 μ M 15 bp target strand) nanosensors acquired on the spectrofluorometer at approx. 50 nM DNA nanosensor concentration and excitation at 530 nm.

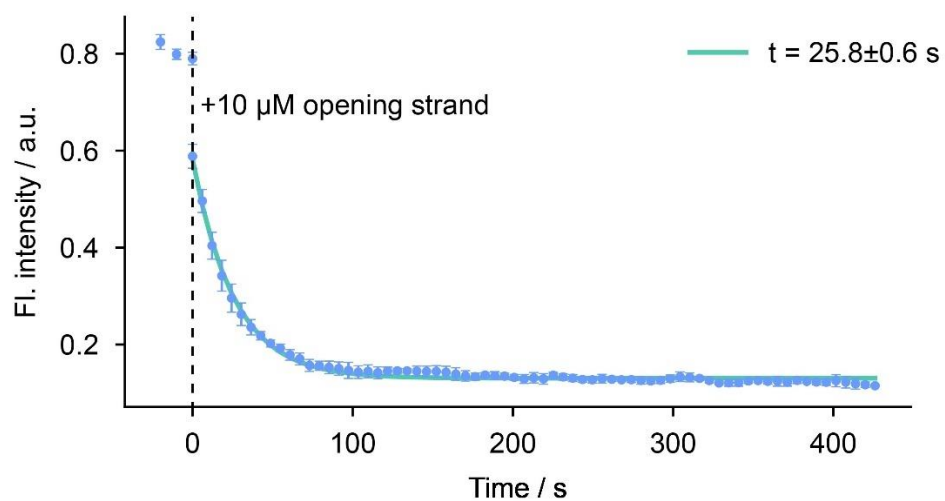


Fig. S26. Nanosensor opening kinetics measured in real-time PCR machine at approx. 50 nM nanosensor concentration upon the addition of 10 μ M 15 bp target opening strand using the FRET emission channel. The reported values are mean values and standard deviation obtained from three independent measurements. The data was fitted to a single exponential decay function to extract sensor opening kinetics.

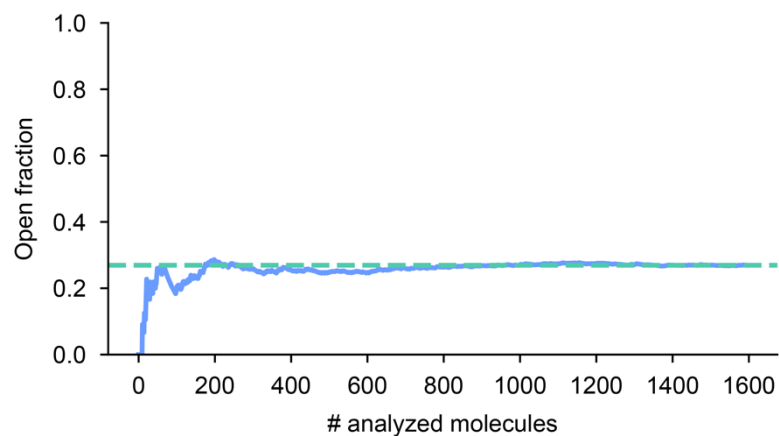


Fig. S28. Effect of the number of single sensors analyzed on the average value of the sensor open fraction that is obtained from confocal single-molecule fluorescence scans indicating that the analysis of several hundred of single molecules leads to a statistically relevant result. The data is measured on the closed nanosensor sample as shown in Fig. S11 (3 nt + 7 nt linkers in the hybridizing FRET pair).

Table S1. Unmodified DNA origami staple sequences used for Version 1 of the nanosensor.

ID	Sequence (5'→3')
1	AAATATCCCATATGTTTCAGGCAAATCCAGTGATGACAGTTGGGCGGTTGTAAATC
2	ATATTACCAAATACCTGTAATTCTAAACTTT
3	GCTGGGAATCAAAGAAACGTAGACGGGAGAATTAAGTGAACACCCA
4	GTCCACTAAATCCCTTTAAACCAAGGCGGGCCTGT
5	ACGTGGACTCCAACGT
6	CAAAGGGCTCAAAAATCAAGCCGTGTATGTTAGCAAAGGTGCTGCGGC
7	GAGCACGTATCCAAATTTACCGCGAACTGGCAAAGAAACGCAAAGACATTAC
8	CGTGGAACCTCAAACCTATCGGAAATGGATAAGAG
9	AAATTAGTAATAACATCATCACAGAATTTAGGCACCG
10	TTTACGAGCGTGGCGTTTTATAGCCGATTTACCAGCGCCAAAGTATC
11	AGGGCAAATTAACCGTTGGCCAACAGAGAATCGCAGAA
12	CAGCCGAAATCGGCAATTAAAGA
13	CACCCAGCAGTGTTTTGAGGCCACCGAGTAAAGCGTAAGAAAGCCAAAAGCCTG
14	TATATAAATCAAAAAGAAT
15	CGCTAAACAACCCTAATTGGAAAAACGCTCATGGGCCAGCCAAGAACAAGGAAAATAG
16	TATAAACAGCCAAAGTCA
17	CTAACTTCTTTGCAGGAGGCCGATTAAAGGGA
18	ACTCCTTAAAGCGCATATTTTTTTGTTTAACGGAAAAACCCAGAACA
19	AAAGCCCAATAGCCAGTAATTATTTACAACAGAGGTGAGGCGGTGCTG
20	GCTTTTGTCAATCAATATTAG
21	CCAGCAGGCGAAAATCCTGTTTGATGGTGGTCAGAATGCGTAC
22	TCCTTTACAGAGAGAATAACATAAAAAACAGGGTTACGCATTTTATTTAGGT
23	AACATATTATTTATCCCAATAA
24	CGCACAGGGTGCACTCTGTCGTAGAAAATACATACATAAAGAAA
25	CCATCGCCGTAAAAAAGC
26	CATTGACCTAAAAATTACCTTCAAATATAATTTTAAAAGTTTGAGTAACA
27	AGCTGTGATAACAAAATTAAGTTGGCACTCGTATT
28	ACCCACCGGAAAAAGAAGGGAAGGTTGAAGTATTAGACTTTACAAACAAAACA
29	AGACGATCCAGCGCAGGTTTTTCACGGTCATAC
30	CACTGCGCGCCTCGGCCTTTAATCGCAGTAAA
31	GCAACCGACAAATCATCGTAGTAATATCGTCTATCACTTTGAC
32	GAATTTTCGAGCAAGCAAAAAGTAGAAGTTTCCTC
33	CATTCCCTTTTTAAGAAAAGTTAAGCCCATCTTACCAACGCTATAGAC
34	CTCTCTGAATAATGGAAGGGAGCGGAGAATTT
35	ATATTCACAAACAAATGTACATCGTTGCTTCTAGACAAAGCATAG
36	GCTCATTTGCCGCCAGAGGGTAAAGTTAAACG
37	CACCAGACGATATCAAAATTATTTGCAATCATTTTCTAAAGCA

38	GTCTCATTTGTTTAATGGAGATAAAATTGGCAGATTCACCAGCTTGCCTGTCAGA
39	TATCACCAGTAGCACCACCACG
40	TAATTTTCATTAAAGGTGAATACAAAAGGAGTAGGGCTCCCGACTTCCATCAC
41	CCGGAACACCGGAACGCTTTGAA
42	TAGTGAACAAGAAAAATAACCAATCAAACGGG
43	GTCTGAGAGACTACCTTTTAGA
44	AGATTTGAAATAGAGGCATTAAGTTTAAATAATAACGGAATACCCTG
45	ATCGGTAAATACATATTTATCATATGGACAAAGTTACC
46	CCTAGGAATTGAATGATGAACTTTTACAGCCACCCTCAGAGCCACC
47	CGCCCCCTCTGACCTGAAAAGAGTCTGTGCGGGAGTATCCTGAAATAATA
48	CGCCTGGCCCTGAGACGCGTGCCATAGCGTCTGCTGTAGTCGAGCTTGATAAATT
49	GGGTGGAATTCGTAGCGAGAGATAACAGTCAGCAGCGTAAAGGAATTGCGAATTTTT
50	GGGGTTTCTGCCAGCAGAGTTGCAGAGTAGTA
51	TTCCAGATGCCGGGTACGCAAACGCAACGCAAGAAAACAGGGAGAGATCTTAGGATT
52	CCGAGCTCGTTTTTCGATTTTAAAGAACTGGCAGGGAACCCATGAGGA
53	TGAAACTCGTTTATAGATTTAGTTAA
54	CGGATCCGCCGGGCGCGGTATGCCAACAAATCATACGTC
55	TGGGGAATGCAGCATTTGGGACAA
56	GCATGCGTCCGTGAGCCTCCGCTGATTGTCAACTT
57	AAACCATTCCATGCTTTTGGACTTTTTGAACTGACCAACTTTGAA
58	GGTACGAACGAGCCAGACGAGGTAAATACGAGGCGCAGACGGT
59	GCTGATTGCCGTTCCGCTGCAGCCGAATATAACAATA
60	TCGTGCTAAATCGTTTATGCAACTAATAGTAGCCCGAACTACAGAGGC
61	GCCTGTAATACTATTGTAAATATGGGATCGGATAAGTGCCGTC
62	ACGTAAATCGGTCATTAAATTCTTTCCAGTATAGCCCGGAATAG
63	GGAAAAAATTCGTGTACCAGGAACAACAAAGACAGCATCGGAGGAC
64	TTACCAGTATTCGCCAGACGACGACAGTATCTGATATAAGACGTTA
65	GCGAATCATTTTTAATAAAGCTTTTCACGCCGCTTTT
66	AAAACGAGCGATTAATCTCCGTGGGAACAAAGAGCCACTGA
67	CTCTGAATTTACCGTTACGCAGAAAAGCCCCAGATAAAAATTCTA
68	GTAAGGTACTGGTAATAAGTTGAGAAGGATACAAAGG
69	ATGGCTTTCCGGCACCTGGTGAAGATATTTAATTTGCGGGTTCAA
70	CTGGCACTCCAGCCAGCTTTTGATGATAATAAGCAAGGATAGCTTTTTTCGTC
71	TCTGTAGCAATAGAACGCGACTTTCCAGGAGAGATAACCCACAAGA
72	GTGTATTTTTGAATGGCTAGGAGCACTTATTCATCCTGATTCGCCTCCC
73	CCGTGCAACCGTACTGCGTAACGATCTAAAGAAT
74	CGTCGGATGTTGGGTAACGCCA
75	CCCTGAGAGTATTAAGAG
76	TTAATGAAGATCGTGCCGGAACAGGCAAAGCGCCCCC
77	CAATCATAGTTACAGGAGGTTTAGTACCGCCACCCTTTGGTGTCATC
78	AAAAACGGATAAGCAGCAACCGCAAGATGCGGTATCATTTTCGCGTAA

79	TGGCACAACGCCCCACCCTCAGAACCGCCACCCTCACGGCGGATAGC
80	CAGCGCCAAGCTCCGTCGGTGGTGCCATGGCGCTTTTATATTTTATACATAATAAA
81	TAGCATGTCAATCATAAAATGCAAGTCAAATCCAGGTC
82	AGAATTAGATAGAGCCGGCGCTCACAATTCCACAAGCTGCATACGAACTA
83	TCTACTAACATTAAAGTAACACCACCCTCATTTTCAGGGATAG
84	AACATTTATTTTCGGTCCGTTCTCACGGAAAAAGAGCCA
85	CCCATCCAATGGCAGCATTGAGAGGTGGAGCCGGCCAG
86	AGCCGCACTCAAAGCATAAAGTGTAAGCCCCG
87	AAGAGGTCATTTTTGCGAATCCCCGGTCTTTA
88	CTGAAAGCGGAGACCTTCATCAAGAGTAATCTTGAGAG
89	GGGTAAAGTACAGCAACGGGACTTCAA
90	AACCTTGTTATCGTCACTGTTGCCCTGCTGGTGCTGAATTAAGCTAAC
91	GAGCAACCTAAATCCGCGACCTGCTCCATGTTACTAATCTACGTCAT
92	ATTCAACTTGCCTAATAGCGGGGT
93	AAACATCATAAATATTCATTGGATGGCTACTC
94	AATTGGGCGGCTGGCTTTGCATCAAAAAGATTTTTTAATCTC
95	TAATCATTGACAGATGAACGGTGTA
96	ACGGAACATGTGCGAAAACGAAAGAGGCAAAACGCATAACAATAA
97	AAGGCTTGCCCTGACCAAGAACCCCTGACT
98	CAGACCAGGCGCATATTGAGATGAGACTGGTGTTT
99	AGAGGTGAATTAGTTTTGCCGAGGATCCACTGGTGTTTCAGCGGCA
100	CAATCATATCATTATAACCAAAATAATCATGGAGGTTTCTTTGCTCGTCGGA
101	GAAACAAAGTACAGAGATTTA
102	CGGATATTCATTACCCAAATCAACTGCTCATCTTTAAAC
103	TTTGAACGAGGGTGGTGTCTATGACCCTCCGGCCAAGATAGACTTTTCTCCGGCTT
104	TAAACAAAAGAACCTTATGCTTTTCACCAGTGAGACGGGCAACATCACAGTTAGAGG
105	AGTTTCCAGTCACCCTTGATTCCCATAAAGCTGCCGGACTCGCCATGT
106	CCACTACGTGCAGGGAGTTTGACCATTAGCAAGTCTG
107	ACACTTTGTATCATCGCCTGATAAATTGACATTATGGAATTACGAGC
108	GACCCCCATTGCGCCGGCGCGAGCGCATCAAT
109	ATAAATCAGTACCTTTTTTGATTAATGTGT
110	ATATCGCGAAGAGGAAAAATGTTTGTTTAATTCCCTTCAC
111	GCGGGATCTTAAACGCGATAAAACCAGTCAGTTTGCGTATTGGGCGCCA
112	AGGTTGAAAATCTCCAAAAACAGCCCTAGGAACGCAGATGGGCCGGTGCG
113	TGATTTTCGAGGTGAATTTCCATGTACCTGTGAGCG
114	AGGATTAGAGAAAAATCACTCAAATGTCAGTGAAT
115	CAAACCATCAATATGATATTC
116	GCTCAAAGCGAACCAGACCCAGAAGCCGGAATCGCCAGAACGCAAGCGG
117	AATGCCGGAGAGGACTAGAAGCCTGTTTTAAAAACG
118	CAGTTTCAGCGGAGTGAGATTTTCTGCG
119	AAGGTCGGTTCGCTGAGGCTAAGGCACCAACACTATTAATAAATAATGAA

120	GAAAGGCAATCGATGGTAAAACACAGTTAA
121	TTCGTAAAAGGAAACAGTAGCGACCGGAAACGTCACCAATGGTG
122	TTAATCTAAAATATCTTTATTAGTCTTACTAGAAACGCTCAACGCGA
123	CTATCAGGTCAACCGTTTTTTAGAAATTGCTAGCGGTGC
124	GTAAATGAAATAGAAAAAACATTGGAAGTTTATCCCTTACCCGGGTA
125	GTCGTTTTGTTATTTGAGGGTTCAGGCTGCGCAACTG
126	GCATTCCACAGAAAAGGCTGCAA
127	GTTTCGTCACCAGCGGTTTATAGCATTAATGTTT
128	GCTGAGACTCCTCAATTAACGGGATCAGAAACAGCGGA
129	AGCGGGGTTTTGCTCAGTAC
130	CAGGTTTGCTAAACAGTAGCTATTTTTAAGATTGTCAGGAGTCGTCATAC
131	GAGAGGGTGGCCTCAGATTTTGTGTTTGTGAGGAGCACATCCTCATAACAT
132	GTGTATCTCTGCCAGAATCAGCACGTACAGTGTAGAACGTCAGCGGGCT
133	CTATTATTCTGAAACCCGTATAA
134	AGTCTGGAGCAAACAAGAGCGGAGACATGCCTGAG
135	CGCTGTAACCTCCATGCTGATCTAATGCAGAAC
136	AGAGTTCGCTATTATTATACTATTAAAGCCAGAATGGAAAGCGCA
137	ATCAAAGCCAGCAGCAAAATGTGAGTCCTACCATTTGGCCTTG
138	TCACCTTCAGTATTATTAATTTATAAAGTACATATAATGATTAAG
139	AACCTTTTTAATCAGAAATAATTGACAGGAGTTGAGGCAGGTCGTAATCAGTAC
140	TCATTACATTTTAACGTCCACCAGAA
141	TCGCAATAGTATTATCATCATATTCCTGATTATCTTG
142	ATAAATATTTTAGACACCGCCACGCTCAATCGTCTGCCTT
143	AATCCTGAAGATGATTTAGATTAAGA
144	TTTGGAATTAATTTTCCCTTATATGTAAGGCT
145	AGAAGAATAACCACATAAAAAAATCATAGCAG
146	GCGTAGATCGTTATTCAAACCCCTCAATCAATCCAGC
147	ATACAGTTTCGACAAAATCAACAGTTGAAAAAAC
148	GTACACAAACATGTCATAGCCTTGAGCCATTTGGGAAGAAAATACAACGCCATT
149	CGGATTCGTTCAATTATTCA
150	GCGTTCCAAGATAATCGGCCAGTTTGGAACAAGA
151	GACGACAAACTCATCGTTGCAACATACGAGCATGTAG
152	AAAACATTTTGTGCAACAGTGCCACGCTGAGAATAACGCGAGGTCCAGAC
153	AATCATCTTCTAGCAAGGCAGAATCAAGTTTGCCTCCGCCAGCAAGAAATT
154	TGTACCAGTAATAATACCGAACGAACCAATCTGG
155	TTCAAATATCAATATTGAAAAATGCGGAACAAAGAAACCACCAGAAGGTT
156	ACCGCAGCAAAAAGCGCGTTTTTCATCGGCAGAGCCGCAGATGAAT
157	TAAAGTCACCGACCCCTTATTAGCGTTTCAGA
158	TTTAGTATGAGGCGAATAACAACACTAGCCGTCAATAGATAATACATTTG

Table S2. Unmodified DNA origami staple sequences used for Version 2 of the nanosensor.

ID	Sequence (5'->3')
1	GCTGGAATCAAAGAAACGTAGACGGGAGAATTAACGAACACCCA
2	TCATTACATTTTAACGTCCACCAGAA
3	CAGCCGAAATCGGCAATTAAAGA
4	CAGGTTTGCTAAACAGTAGCTATTTTTAAGATTGTCAGGAGTCGTCATAC
5	CTCTGAATTTACCGTTACGCAGAAAAGCCCCAGATAAAAATTCTA
6	GTACACAAACATGTCATAGCCTTGAGCCATTTGGGAAGAAAATACAACGCCATT
7	CAACATACGAGGCATAAAAATGGTCCGATATATAGCCTTTAATTGTATTA
8	AAGGCTTGCCCTGACCAAGAACCCCTGACT
9	AACATTTATTTTCGGTCCGTTCTCACGGAAAAAGAGCCA
10	TTTAGTATGAGGCGAATAACAACCTAGCCGTCAATAGATAATACATTTG
11	CATTCCCTTTTTAAGAAAAGTAAGCAGAGCGAACCTTAATTGAGATAGAAGAACTGATAGC
12	CGCACAGGGTGCACTCTGTCGTAGAAAATACATACATAAAGAAA
13	AATGGGATAGGTCACGCAGAACCGTGTA
14	GCGTAGATCGTTATTCAAACCCTCAATCAATCCAGC
15	GCGGGATCTTAAACGCGATAAAACCAGTCA
16	TTACCAGTATTCGCCAGACGACGACAGTATCTGATATAAGACGTTA
17	GAATTTGAGCAAGCAAAAAGTAGAACTTTCTCTC
18	GGTACGAACGAGCCAGACGAGGTAAAATACGAGGCGCAGACGGT
19	ATAAATCAGTACCTTTTTTTGATTAATGTGT
20	CACTGCGCGCCTCGGCCTTTAATCGCAGTAAA
21	AGTTTCCAGTCACCCTTGATTCCCATAAAGCTGCCGGACTCGCCATGT
22	GCATGCGTCCGTGAGCCTCCGCTGATTGTCAACTT
23	GCATTCCACAGAAAAGGCTGCAA
24	GAGAGGGTGGCCTCAGATTTTGTTTTGTGAGGAGCACATCCTCATAACAT
25	CGTGGAACCTCAAACCTATCGGAAATGGATAAGAG
26	CCACTACGTGCAGGGAGTTTGACCATTAGCAAGTCTG
27	AAACATCATAAATATTCATTGGATGGCTACTC
28	AACATATTATTTATCCCAATAA
29	ATCGGTAAATACATATTTATCATATGGACAAAGTTACC
30	GTCTGAGAGACTACCTTTTAGA
31	CCGTGCAACCGTACTGCGTAACGATCTAAAGAAT
32	CCCATCCAATGGCAGCATTGAGAGGTGGAGCCGGCCAG
33	TGGCACAACGCCCCACCCTCAGAACC GCCACCCTCACGGCGGATAGC
34	GTCGTTTTGTTATTTGAGGGTTCAGGCTGCGCAACTG
35	GCTTTTGTGACAATCAATATTAG
36	AACCTTGTTATCGTCACTGTTGCCCTGCTGGTGCTGAATTAAGCTAAC
37	AACCTTTTTAATCAGAAATAATTGACAGGAGTTGAGGCAGGTCGTAATCAGTAC
38	AATGCCGGAGAGGACTAGAAGCCTGTTTTAAAAACG

39	AGGGCAAATTAACCGTTGGCCAACAGAGAATCGCAGAA
40	AGCCTAATTTGCCAGTGAGCTA
41	AAACCATTCCATGCTTTTTGGACTTTTTGAACTGACCAACTTTGAA
42	CGGATATTCATTACCCAAATCAACTGCTCATCTTTAAAC
43	ATCAAAGCCAGCAGCAAAATGTGAGTCCTACCATTGGCCTTG
44	TCGTCGCTAAATCGTTTATGCAACTAATAGTAGCCCGAAACTACAGAGGC
45	GAAAGATTCATCAGTTACGGAGATCATCTTT
46	ACACTTTGTATCATCGCCTGATAAATTGACATTATGGAATTACGAGC
47	GCCTGTAATACTATTGTAAATATGGGATCGGATAAGTGCCGTC
48	CAATCATAGTTACAGGAGGTTTAGTACCGCCACCCTTTGGTGTATC
49	CTCTCTGAATAATGGAAGGGAGCGGAGAATTT
50	GGGTAAAGTACAGCAACGGGACTTCAA
51	AAGAGGTCATTTTTGCGAATCCCCGGTCTTTA
52	TAGCATGTCAATCATAAAATGCAAGTCAAATCCAGGTC
53	TTTGAACGAGGGTGGTGTCTATGACCCTCCGGCCAAGATAGACTTTTCTCCGGCTT
54	ACGGAACATGTCGAAAACGAAAGAGGCAAAACGCATAACAATAA
55	AGCCGCACTCAAAGCATAAAGTGTAAGCCCCG
56	CCAGCAGGCGAAAATCCTGTTTGATGGTGGTCAGAATGCGTAC
57	CAATCATATCATTATAACCAAAATAATCATGGAGGTTTCTTTGCTCGTCGGA
58	CTATCAGGTCAACCGTTTTTAGAAATTGCTAGCGGTGC
59	AGTCTGGAGCAAACAAGAGCGGAGACATGCCTGAG
60	ATTTTCGCAAGAAAAATAAGGCCCATTAAAAAAAGGGACAT
61	TGGGGAATGCAGCATTGTTGGGACAA
62	GCTGATTGCCGTTCCGCTGCAGCCGAATATAACAATA
63	ATGGCTTTCCGGCACCTGGTGAAGATATTTAATTTGCGGGTTCAA
64	GTCCACTAAATCCCTTTAAACCAAGGCGGGCCTGT
65	CACCAGACGATATCAAAATTATTTGCAATCATTTTCTAAAGCA
66	TGAAACTCGTTTATAGATTTAGTTAA
67	ATTCAACTTGCCTAATAGCGGGGT
68	AGACGATCCAGCGCAGGTTTTACGGTCATAC
69	TTAATGAAGATCGTGCCGGAACCAGGCAAAGCGCCCCC
70	GGGGTTTTCTGCCAGCAGAGTTGCAGAGTAGTA
71	GTGTATTTTGAATGGCTAGGAGCACTTATTCATCCTGATTCGCCTCCC
72	TCGGGAGAAACAATAAAGGAT
73	AAGGTCGGTCGCTGAGGCTAAGGCACCAACACTATTAATAAATAATGAA
74	ATATTACCAAATACCTGTAATTCTAAACTTT
75	CGCTAAACAACCCTAATTGGA AAAACGCTCATGGGCCAGCCAAGAACAAGGAAAATAG
76	CTAACTTCTTTGCAGGAGGCCGATTAAAGGGA
77	TAAACAAAAGAACCTTATGCTTTTACCAGTGAGACGGGCAACATCACAGTTAGAGG
78	AGAATTAGATAGAGCCGGCGCTCACAATTCCACA
79	TTCAAATATCAATATTGAAAAATGCGGAACAAAGAAACCACCAGAAGGTT

80	CCGGAACACCGGAACGCTTTGAA
81	CGGATTCGTTCAATTATTCA
82	TGTACCAGTAATAATACCGAACGAACCAATCTGG
83	GTCTCATTTGTTTAATGGAGATAAATTGGCAGATTCACCAGCTTGCCTGTCAGA
84	CAGTTTCAGCGGAGTGAGATTTTCTGCG
85	TAGTGAACAAGAAAAATAACCAATCAAACGGG
86	GTTTCGTCACCAGCGGTTTATAGCATTAAATGTTT
87	AGGTTGAAAATCTCCAAAAACAGCCCTAGGAACGCAGATGGGCCGGTGCG
88	CAGACCAGGCGCATATTGAGATGAGACTGGTGTTT
89	AAAGCCCAATAGCCAGTAATTATTTACAACAGAGGTGAGGCGGTGCTG
90	GAGCACGTATCCAAATTTACCGCGAACTGGCAAAGAAACGCAAAGACATTAC
91	CCATCGCCGTAAAAAAGC
92	AAAACATTTTGTGCAACAGTGCCACGCTGAGAATAACGCGAGGTCCAGAC
93	CGGATCCGCCGGGCGCGGTATGCCAACAATCATACGTC
94	GCGAATCATTTTTAATAAAGCTTTTCACGCCGCTTTT
95	AGGATTAGAGAAAAATCACTCAAATGTCAGTGAAT
96	AGCGGGGTTTTGCTCAGTAC
97	AATCATCTTCTAGCAAGGCAGAATCAAGTTTGCCTCCGCCAGCAAGAAATT
98	CAAACCATCAATATGATATTC
99	GCGTTCCAAGATAATCGGCCAGTTTGGAACAAGA
100	GAAACAAAGTACAGAGATTTA
101	CCTAGGAATTGAATGATGAACTTTTACA
102	CATTGACCTAAAAATTACCTTCAAATATAATTTTAAAAGTTTGAGTAACA
103	GCTCAAAGCGAACCAGACCCAGAAGCCGGAATCGCCAGAACGCAAGCGG
104	AAATTAGTAATAACATCATCACACGAATTTAGGCACCG
105	ACCCACCGGAAAAAGAAGGGAAGGTTGAAGTATTAGACTTTACAAACAAAACA
106	TGATTTTCGAGGTGAATTTCCATGTACCTGTGAGCG
107	GTGTATCTCTGCCAGAATCAGCACGTACAGTGTAGAACGTCAGCGGGCT
108	GCTGAGACTCCTCAATTAACGGGATCAGAAACAGCGGA
109	GTAAGGTACTGGTAATAAGTTGAGAAGGATACAAAGG
110	GCTCATTTGCCGCCAGAGGGTAAAGTTAAACG
111	CTGGCACTCCAGCCAGCTTTTGTATGATAATAAGCAAGGATAGCTTTTTTCGTC
112	CAGCGCCAAGCTCCGTCGGTGGTGCCATGGCGCTTTTATATTTTATACATAATAAA
113	GGGTGGAATTCGTAGCGAGAGATAACAGTCAGCAGCGTAAAGGAATTGCGAATTTTT
114	TATAAACAGCCAAAGTCA
115	GGACGTTGGGAAGAAATAGCCGGAACGTAATG
116	AGAGTTCGCTATTATTATACTATTAAAGCCAGAATGGAAAGCGCA
117	TTCGTAAAAGGAAACAGTAGCGACCGGAAACGTCACCAATGGTG
118	AGCTGTGATAACAAAATTAAGTTGGCACTCGTATT
119	CCCTGAGAGTATTAAGAG
120	AAAACGACGGCCAGTTTTTCATCAATAGTAGTCAGCTTGCCAACAACCATCGCCCAGA

121	GGAAAAAATTCGTGTACCAGGAACAACAAAGACAGCATCGGAGGAC
122	TCACCTTCAGTATTATTAATTTATAAAGTACATATAATGATTAAG
123	AGAAGAATAACCACATAAAAAAATCATAGCAG
124	ACCGCAGCAAAAGCGCGTTTTTCATCGGC
125	TCGCAATAGTATTATCATCATATTCCTGATTATCTTG
126	CTGAAAGCGGAGACCTTCATCAAGAGTAATCTTGAGAG
127	ACGTGGACTCCAACGT
128	ATACAGTTTCGACAAAATCAACAGTTGAAAAAC
129	CACCCAGCAGTGTTTTGAGGCCACCGAGTAAAGCGTAAGAAAGCCAAAAGCCTG
130	AGAGGTGAATTAGTTTTGCGGAGGATCCACTGGTGTGTTTCAGCGGCA
131	AATCCTGAAGATGATTTAGATTAAGA
132	TCCTTTACAGAGAGAATAACATAAAAAACAGGGTTACGCATTTTATTTAGGT
133	AGATTTGAAATAGAGGCATTAAGTTTAAATAATAACGGAATACCCTG
134	TTAATCTAAAATATCTTTATTAGTCTTACTAGAAACGCTCAACGCGA
135	GACCCCCATTGCGCCGGCGGAGCGCATCAAT
136	CGCCTGGCCCTGAGACGCGTGCCATAGCGTCTGCTGTAGTCGAGCTTGATAAATT
137	GAGCAACCTAAATCCGCGACCTGCTCCATGTTACTAATCTACGTCAT
138	ATATTCACAAACAAATGTACATCGTTGCTTCTAGACAAAGCATAG
139	ACGTAAATCGGTCATTAAATCCTTCCAGTATAGCCCGGAATAG
140	CAAAGGGCTCAAAAATCAAGCCGTGTATGTTAGCAAAGGTGCTGCGGC
141	CGCCCCCTTCTGACCTGAAAAGAGTCTGTGCGGGAGTATCCTGAAATAATA
142	AAATATCCCATATGTTTCAGGCAAATCCAGTGATGACAGTTGGGCGGTTGTAAATC
143	ATATCGCGAAGAGGAAAAATGTTTGTTTAATTCCCTTCAC
144	CTATTATTCTGAAACCCGTATAA
145	TAAAGTCACCGACCCCTTATTAGCGTTTCAGA
146	TATATAAATCAAAAGAAT
147	TTTACGAGCGTGCGTTTTATAGCCGATTTACCAGCGCCAAAGTATC
148	TTCCAGATGCCGGGTACGCAAACGCAACGCAAGAAAACAGGGAGAGATCTTAGGATT
149	CGCTGTAACCTCCATGCTGATCTAATGCAGAAC
150	AAAAACGGATAAGCAGCAACCGCAAGATGCGGTATCATTTTCGCGTAA
151	GTAAATGAAATAGAAAAAACATTGGAAGTTTATCCCTTACCCGGGTA
152	TCTGTAGCAATAGAACGCGACTTTCCAG
153	AATTGGGCGGCTGGCTTTGCATCAAAAAGATTTTTTAATCTC
154	TAATCATTGACAGATGAACGGTGTA
155	ATAAATATTTTAGACACCGCCACGCTCAATCGTCTGCCTT
156	GCAACCGACAAATCATCGTAGTAATATCGTCTATCACTTTGAC
157	TCTACTAACATTAAAGTAACACCACCCTCATTTTCAGGGATAG
158	ACTCCTTAAAGCGCATATTTTTTGTTTAACGGAAAAACCCAGAACA
159	CCGAGCTCGTTTTTCGATTTTAAAGAACTGGCAGGGAACCCATGAGGA
160	TTTGGAATTAATTTTCCCTTATATGTAAGGCT
161	TAATTTTCATTAAAGGTGAATACAAAAGGAGTAGGGCTCCCGACTTCCATCAC

162	GACGACAACTCATCGTTGCAACATACGAGCATGTAG
163	GAAAGGCAATCGATGGTAAAACACAGTTAA
164	TATCACCAGTAGCACCACCACG
165	CGTCGGATGTTGGGTAACGCCA
166	AAATCCTTTGCCCGAATTCAGGTTAACAATTAGACTG
167	GTCACCTCACCGGAAACAATCG

Table S3. Closing staples and fluorescently labelled staples used in Version 1 of the nanosensor. For the closing interactions, the number of the caDNAno helix is indicated (see Fig. S18). Yellow and Red indicate complementary sequences. Green indicates the toehold used for opening. Blue indicates additional spacer elements.

Name-Helix ID	Sequence (5'→3')
Row1-top16	ATACATCTATTATATATTGAGGGTAATTGAGCGCTGAAACCGATATC CGGTAACA
Row1-top13	ATACATCTATTATATATTCCACCACCAGAGCCGTTAGC
Row1-bot24	AATAGATGTATTTATATATTGCGGGACGTTGGGAAGAAATAGCCGG AACGTAATG
Row1-bot29	AATAGATGTATTTATATATTTTGGGAAGGGCGATGCATCGTAA
Row2-top13	ATACATCTATTATATATTTTCAAGCCTAATTTGCCAGTGAGCTA
Row2-top16	ATACATCTATTATATATTTTACCCTCATTTTCGCAAGAAAAATAAGGC CCATTAAAAAAGGGACAT
Row2-bot24	AATAGATGTATTTATATATTTTCGGCCAACGCGCGGGGAGAG
Row2-bot29	AATAGATGTATTTATATATTTTGGCCTCTTCGCTATTACCCACGGGAAT AATTCGCAGGCAAGCCAA
Row3-top13	ATACATCTATTATATATTTTATTGAGTAAGCAGAGCGAACCTTAATT GAGATAGAAGAACTGATAGC
Row3-top16	ATACATCTATTATATATTTTACCTCGGGAGAAACAATAAAGGAT
Row3-bot24	AATAGATGTATTTATATATTTTCGTGCCCCAACATACGAGGCATAAAA TGGTCCGATATATAGCCTTTAATTGTATTA
Row3-bot29	AATAGATGTATTTATATATTTTCTGAATGGGATAGGTCACGCAGAAC CGTGTA
Row4-top13	ATACATCTATTATATATTTTATAGAGCAAGAAACAATGAGCTATCTAG CCTTAAACCAGTATAATAC
Row4-top16	ATACATCTATTATATATTTTTCAGAGCCGCCACCCTGCCATCTTCCT GAGCATCATAATTTAATG
Row4-bot24	AATAGATGTATTTATATATTTTCTTGAAAGATTCATCAGTTACGGAG ATCATCTTT
Row4-bot29	AATAGATGTATTTATATATTTTGTGCAAGCGGCCAGTTTTCATCAATA GTAGTCAGCTTGCCAACAACCATCGCCAGA
Green_dye_non_hybridizing	AATTCTCAGAGCAATTCTGATGGGTAATCATAGCTGTTTCCTGTG- [ATTO542]
Red_dye_non_hybridizing	GTTAGAATCAGAGCGGTACAAAATAGAAGGCTGGAAAC- [ATTO647N]
Red_dye_1nt_spacer	GTTAGAATCAGAGCGGTACAAAATAGAAGGCTGGAAACTCTGACGG C-[ATTO647N]
Green_dye_1nt_spacer	AATTCTCAGAGCAATTCTGATGGGTAATCATAGCTGTTTCCTGTGTG CCGTCAG-[ATTO542]

Table S4. Modified (closing, stabilization and fluorescently labelled) staples used in Version 2 of the sensor. For the closing interactions, the number of the caDNA helix is indicated (see Fig. S18). Yellow and Red indicate complementary sequences. Green indicates the toehold used for opening. Blue indicates additional spacer elements.

Name-Helix ID	Sequence (5'→3')
Row1-top13	ATATCACGAGCACATCAGTACGAGGGTAATTGAGCGCTGAAACCGATATCCGGTAACA
Row1-top16	GTGCTCGTGATATTTTTCCACCACCAGAGCCGTTAGC
Row1-bot24	GTGCTCGTGATATTTTGCGGTTTGCGTATTGGGCGCCA
Row1-bot29	ATATCACGAGCACATCAGTACTTGGGAAGGGCGATGCATCGTAA
Row2-top13	ATATCACGAGCACATCAGTACTCAGAGAGATAACCCACAAGA
Row2-top16	GTGCTCGTGATATTTTTTTTACCCTCAGAGCCGCAGATGAAT
Row2-bot24	GTGCTCGTGATATTTTTTTTTCGGCCAACGCGCGGGGAGAG
Row2-bot29	ATATCACGAGCACATCAGTACGGCCTCTTCGCTATTACCCACGGGAATAATTTCGAGGCAAGCCAA
Row3-top13	ATATCACGAGCACATCAGTACTTATTGAGTTAAGCCCATCTTACCAACGCTATAGAC
Row3-top16	GTGCTCGTGATATTTTTTTTTTACCGCCACCCTCAGAGCCACC
Row3-bot24	GTGCTCGTGATATTTTTTTTTTTCGTGCCAGCTGCATACGAACCTA
Row3-bot29	ATATCACGAGCACATCAGTACTTCTGGCGAAAGGGGGATGTGC
Row4-top13	ATATCACGAGCACATCAGTACTTTAGAGCAAGAAACAATGAGCTATCTAGCCTTAAACCAGTATAATAC
Row4-top16	GTGCTCGTGATATTTTTTTTTTTTCAGAGCCGCCACCCTGCCATCTTCTGAGCATCATAATTTAATG
Row4-bot24	GTGCTCGTGATATTTTTTTTTTTTCTTCCAGTCGGGAAACCTGT
Row4-bot29	ATATCACGAGCACATCAGTACTTTTGCAAGGCGATTAATCTCCGTGGGAACAAAGAGCCACTGA
5bp_stabilisation_1	CATTGCACCCACGCAACCAGCTTGACGTTGTTTTTAGACC
5bp_stabilisation_2	TTAATTGCGTTGCGCTCACTGCCTTTTTACAC
5bp_stabilisation_3	AACGGTACGCCAGAATCCTGAGATACAATTTGTTTTGATACCGATTTTTGTGTG
5bp_stabilisation_4	TTGAGGGAATATTGACGGAAATTACAAAATCATTTTTGGTCT
Red_dye_3nt_spacer	GTTAGAATCAGAGCGGTACAAAATAGAAGGCTGGAAACCTTCTGACGGC-[ATTO647N]
Green_dye_3nt_spacer	AATTCTCAGAGCAATTCTGATGGGTAATCATAGCTGTTTCCTGTGTTTGCCTGTCAG-[ATTO542]
Red_dye_7nt_spacer	GTTAGAATCAGAGCGGTACAAAATAGAAGGCTGGAAACTTTTTTTCTGACGGC[ATTO647N]

Red_dye_7nt_spacer_7bp _interaction	GTTAGAATCAGAGCGGTACAAAATAGAAGGCTGGAAAC TTTTTTCTG ACGGT[ATTO647N]
Green_dye_high_position	CATTGCACCCACGCAACCAGCTTGACGTTG TTTTTTTTT GCCGTCAG[AT TO532]
Red_dye_high_position	TTGAGGGAATATTGACGGAAATTACAAAATCA TTTTTTTTCTGACGGC[ATTO647N]

Table S5. Biotinylated staples for immobilization of DNA origami nanostructures used in both versions of the sensor. Blue indicates additional spacer elements.

Name	Sequence (5'->3')
Biotin1	5'Biotin- TTTATTGCCGGTTGATAGTCAGTGCCTTGAGTAACAGTGCATGAA
Biotin2	5'Biotin- TTTATTATAGTGGAAGCAATAGAGCTTACCCTCATATATTTTGTAC
Biotin3	5'Biotin-TTTCAAACTCTTCCTGTTGACCGT
Biotin4	5'Biotin-TTTATACACCGCCAAAATACAGGTA

Table S6. DNA-DNA closing interactions used for XhoI nanosensors. Shown in purple is restriction site of XhoI. The number of the caDNAno helix is indicated (see Fig. S18).

Name-Helix ID	Sequence (5'→3')
Row1-top 13	ATA CTCGAG TTATATATTGAGGGTAATTGAGCGCTGAAACCGATATCCGGTAA CA
Row1-top16	ATA CTCGAG TTATATATT CCACCACCAGAGCCGTTAGC
Row1-bot24	A ACTCGAG TATTTATATATTGCGGGACGTTGGGAAGAAATAGCCGGAACGTAA TG
Row1-bot29	A ACTCGAG TATTTATATATT TTGGGAAGGGCGATGCATCGTAA
Row2-top 13	ATA CTCGAG TTATATATTTTCAAGCCTAATTTGCCAGTGAGCTA
Row2-top16	ATA CTCGAG TTATATATTTTACCCTCATTTTCGCAAGAAAAATAAGGCCCATTA AAAAAGGGACAT
Row2-bot24	A ACTCGAG TATTTATATATTTTCGGCCAACGCGCGGGGAGAG
Row2-bot29	A ACTCGAG TATTTATATATTTGGCCTCTTCGCTATTACCCACGGAATAATT CGCAGGCAAGCCAA
Row3-top 13	ATA CTCGAG TTATATATTTTATTGAGTAAGCAGAGCGAACCTTAATTGAGATA GAAGAACTGATAGC
Row3-top16	ATA CTCGAG TTATATATTTTACCTCGGGAGAAACAATAAAGGAT
Row3-bot24	A ACTCGAG TATTTATATATTTTCGTGCCCAACATACGAGGCATAAAATGGTCC GATATATAGCCTTTAATTGTATTA
Row3-bot29	A ACTCGAG TATTTATATATTTTCTGAATGGGATAGGTCACGCAGAACCGTGTA
Row4-top 13	ATA CTCGAG TTATATATTTTTCAGAGCCGCCACCCTGCCATCTTCCTGAGCATC ATAATTTAATG
Row4-top16	ATA CTCGAG TTATATATTTTTCAGAGCCGCCACCCTGCCATCTTCCTGAGCAT CATAATTTAATG
Row4-bot24	A ACTCGAG TATTTATATATTTTCTTGAAAGATTCATCAGTTACGGAGATCATC TTT
Row4-bot29	A ACTCGAG TATTTATATATTTTTCGCAAGCGGCCAGTTTTCATCAATAGTAGTC AGCTTGCCAACAACCATCGCCCAGA

Table S7. Antigen modified staples that were used in the sensor for anti-Dig and anti-DNP antibodies. The number of the caDNA helix is indicated (see Fig. S18). Blue indicates additional spacer element.

Name-Helix ID	Sequence (5'->3')
Row1-top16	[DNP]TGAGGGTAATTGAGCGCTGAAACCGATATCCGGTAACA
Row1-top13	[DNP]TCCACCACCAGAGCCGTTAGC
Row1-bot24	[DNP]TGCGGGACGTTGGGAAGAAATAGCCGGAACGTAATG
Row1-bot29	[DNP]TTTGGGAAGGGCGATGCATCGTAA
Row4-top13	[DIG]TTTAGAGCAAGAAACAATGAGCTATCTAGCCTTAAACCAGTATAATAC
Row4-top16	[DIG]TTTTCAGAGCCGCCACCCTGCCATCTTCCTGAGCATCATAATTTAATG
Row4-bot24	[DIG]TTTCTTGAAAGATTCATCAGTTACGGAGATCATCTTT
Row4-bot29	[DIG]TTTGTGCAAGCGGCCAGTTTTTCATCAATAGTAGTCAGCTTGCCAACAACCA TCGCCCAGA

Table S8. Staples with PDGF-BB aptamer sequence (green) that were used in the sensor for PDGF-BB. The number of the caDNAno helix is indicated (see Fig. S18). Blue indicates additional spacer element.

Name-Helix ID	Sequence (5'->3')
4_1 bot aptamer 3T	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTCTTCCAGTCGGGAA ACCTGT
4_2 bot aptamer 3T	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTGTCAAGGCGATTAAT CTCCGTGGGAACAAAGAGCCACTGA
4_2 top aptamer 3T	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTTCAGAGCCGCCACCC TGCCATCTTCCTGAGCATCATAATTTAATG
4_1 top aptamer 3T	CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTAGAGCAAGAAACAAT GAGCTATCTAGCCTTAAACCAGTATAATAC

Table S9. DNA and RNA strands used for the RNA detection assay using Cas9.

Name	Sequence (5'->3')
Alt-R™ CRISPR-Cas9 crRNA	UCGCGUAUCCGUCUGAAUAAGUUUUAGAGCUAUGCU
DNA substrate containing Cas9 recognition sequence and key motif	CAGTACCTTTATTCAGACGGATACGCGACGGGAAGGGTTTCCC TTCCCGtcgcgtatccgtctgaataaAGGTACTGATGTGCTCGTGATAT

Table S10. Sequences of opening strands used in different sensor constructs. Red indicates the mismatch.

Name	Sequence (5'->3')
15 bp interaction (Version 2)	ACTGATGTGCTCGTG
17 bp interaction (Version 2)	GTACTGATGTGCTCGTG
17 bp interaction (Version 1)	ATATATAATAGATGTAT
17 bp mismatch GC (Version 1)	ATATATAATA C ATGTAT
17 bp mismatch AT (Version 1)	ATATATAATAG T TGTAT
17 bp mismatch GC+AT (Version 1)	ATATATAATA CT TGTAT
17 bp mismatch Toehold AT (Version 1)	AT TT TATAATAGATGTAT
17 bp mismatch Toehold TA (Version 1)	ATA A ATAATAGATGTAT

Table S11. Fit results as well as buffer compositions for the titration data shown in Fig. 2.

Sample	Buffer	K_{1/2}	pH
2x13	10 mM Tris, 10 mM MgCl ₂ , 50 mM NaCl	100.08 ± 10.43 nM	0.9782 ± 0.084
2x13	10 mM Tris, 10 mM MgCl ₂ , 200 mM NaCl	195.05 ± 12.36 nM	0.8709 ± 0.041
2x13	10 mM Tris, 10 mM MgCl ₂ , 400 mM NaCl	601.19 ± 19.57 nM	0.8075 ± 0.019
2x13 stabilized	10 mM Tris, 10 mM MgCl ₂ , 50 mM NaCl	704.97 ± 64.84 nM	0.7997 ± 0.049
4x13	10 mM Tris, 10 mM MgCl ₂ , 50 mM NaCl	1087.31 ± 85.60 nM	1.5459 ± 0.1524
6x13	10 mM Tris, 10 mM MgCl ₂ , 50 mM NaCl	2025.14 ± 102.35 nM	1.7335 ± 0.1255