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Supporting Information

Interlocked DNA Nanojoints for Reversible Thermal Sensing

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Supporting Information

1. Experimental

1.1 Materials

All oligodeoxynucleotides (ODNs) were purchased from Microsynth AG and dissolved in ddH_2O. The concentration of each oligo was measured with a Nanodrop 2000 (Thermo Scientific™).

Buffer solutions used in experiments

1x TAE buffer: 40 mM Tris, 20 mM AcOH, 1 mM EDTA.
1x ligase buffer: 40 mM Tris·HCl, 10 mM DTT, 5 mM ATP at pH 7.8
1x DNA Catenane buffer (DC buffer): 10 mM Tris-Cl, 1 mM EDTA, 25 mM $MgCl_2$ (pH 8.0).
1x DNA Catenane buffer with HEPES (HEPES Buffer): 10 mM HEPES, 1 mM EDTA, 25 mM $MgCl_2$ (pH 8.0)
WAX buffer A: 20 mM Tris·HCl at pH 9.0
WAX buffer B: 20 mM Tris·HCl, 1 M NaCl at pH 9.0

1.2 Assembly of the hybridized [2]catenane (Cat^{hyb})

1.2.1 Assembly of the threaded Ring A

The ODNs (0.2 nmol each) used for the assembly of the threaded Ring A (for design and sequences see Figure S1 and Table S2) and NaCl (20 µmol) were mixed in 1x ligase buffer (total volume 200 µL). The solution was annealed in a temperature gradient from 90 °C to 15 °C at a rate of -1 °C/min.

1.2.2 Assembly of the Cat^{hyb}

To complete the catenane assembly, the ODNs (0.2 nmol each) for the assembly of Ring B (see Figure S1 and Table S2) are added into the solution of threaded Ring A and carefully mixed with a pipette. The solution was incubated at 15 °C for at least 4h, then 2 µL T4 DNA ligase (5 U/µl, Thermo Scientific) were added and the solution was incubated at 15 °C for at least 4h.

1.2.3 Conversion of the Cat^{hyb} to the mechanically interlocked DNA [2]catenane (Cat^{mec})

To convert the hybridized catenane to the mechanically interlocked catenane, 0.5 nmol of the corresponding Release ODN (R-ODN, see Table S3) were added into the solution of the Cat^{hyb}; the ratio of R-ODN to the Cat^{hyb} was 5:2. The solution was carefully mixed with a pipette and incubated at 15 °C for at least 4h. After incubation, the Cat^{mec} was purified by HPLC (See 1.3 HPLC Purification).

1.2.4 Conversion of the Cat^{mec} to the Cat^{hyb}

After the purification by HPLC, the concentration of the Cat^{mec} was measured by

Nanodrop 2000 (Thermo Scientific™). To revert the catenane back to its hybridizing state, the corresponding Reverse ODN (RR-ODN, see Table S3) was added into the purified solution of the Cat^{mec}; the ratio of RR-ODN to the Cat^{mec} was 5:2. The solution was carefully mixed with a pipette and incubated at 15 °C for at least 4h.

1.3 Assembly of dsDNA rings

1.3.1 Assembly of the Ring A

The ODNs (0.2 nmol each) used for the assembly of the Ring A (for design and sequences see Figure S1 and Table S4) and NaCl (20 µmol) were mixed in 1x ligase buffer (total volume 200 µL). The solution was annealed in a temperature gradient from 90 °C to 15 °C at a rate of -1 °C/min. Then 2 µL T4 DNA ligase (5 U/µL, Thermo Scientific) were added and the solution was incubated at 15 °C for at least 4h.

1.3.2 Assembly of the Ring B

The ODNs (0.2 nmol each) used for the assembly of the Ring B (for design and sequences see Figure S1 and Table S4) and NaCl (20 µmol) were mixed in 1x ligase buffer (total volume 200 µL). The solution was annealed in a temperature gradient from 90 °C to 15 °C at a rate of -1 °C/min. Then 2 µL T4 DNA ligase (5 U/µL, Thermo Scientific) were added and the solution was incubated at 15 °C for at least 4h.

1.3.3 Purification

The samples were purified by using an Amicon Ultra-0.5 mL centrifugal filter devices (YM-30, Millipore), and washed twice with 1x DC buffer. Concentrations were measured with a Nanodrop 2000 (Thermo Scientific™).

1.4 HPLC Purification

The HPLC purification was performed by a weak anion exchange, HPLC column (TSKgel DEAE-NPR 4.6 mm x 35 mm, TOSOH). The gradient elution program is listed in Table S1:

Time	WAXA	WAXB	Flow speed
0.0	80 %	20 %	0.4 ml/min
0.1	50 %	50 %	0.4 ml/min
20.1	35 %	65 %	0.4 ml/min
21.0	0 %	100 %	0.4 ml/min
24.0	0 %	100 %	0.4 ml/min
25.0	80 %	20 %	0.4 ml/min

Table S1. The gradient elution program used for HPLC purification

After purification, the different fractions were concentrated by using an Amicon Ultra-0.5 mL centrifugal filter devices (YM-30, Millipore), and washed twice with 1x DC buffer. Concentrations were measured with a Nanodrop 2000 (Thermo Scientific™).

1.5 Fluorescence Detection

All fluorescence detections were performed by an iQ5 Multicolor Real Time PCR Detection System (Bio-Rad).

1.5.1 Thermal conversion of Cat^{hyb} to Cat^{mec}

The PCR was programmed to start with the sample equilibrated at 24 °C and then to run a temperature gradient from 24 ^{o}C to 70 ^{o}C at rate of 0.5 ^{o}C/min. During the temperature increase, the fluorescence intensity was measured every minute.

1.5.2 Conversion of Cat^{mec} to Cat^{hyb}

To test the reversibility of the mechanically interlocked catenane into the hybridized version, the samples were incubated at 65 ^{o}C for 5 min, to allow the Cat^{hyb} to convert into the Cat^{mec}, then the samples were cooled to 24 ^{o}C at rate of -3 ^{o}C/s and incubated at 24 ^{o}C for 1h. After the sample was cooled to 24 °C the fluorescence intensity was measured every 30 seconds in 2 hours.

1.5.3 Thermal reversibility of the Cat^{hyb}

For the temperature cycle experiments the PCR program contains the following steps:

1) incubation of the sample at 42 ^{o}C for 20 mins, allowing the Cat^{mec} to convert into the Cat^{hyb};
2) measurement of fluorescence intensity;
3) heating of the sample from 42 ^{o}C to 60 ^{o}C at rate of 3 ^{o}C/s;
4) incubation of the sample at 60 ^{o}C for 5 mins, allowing the Cat^{hyb} to convert into the Cat^{mec};
5) measurement of the fluorescence intensity;
6) cooling the sample down to 24 ^{o}C at rate of -3 ^{o}C/s.
7) returning to step 1 and repeating for 10 times

Threaded Ring A		
R4B2		5’-phos-CGTTTTTACCGCTTTTTGATACGTGCGGAACCCTGCGGGTGTTCTTTTTTCGCGCTTTTTCCG-3’
RC1-BHQ		5’-ATCAAAAAGCGGTAAAAACGCAAAAACTGGCAAAAAACGTA-BHQ1-3’
RC1-BHQ2 (substitute to RC1-BHQ to label with HEX)		5’-ATCAAAAAGCGGTAAAAACGCAAAAACTGGCAAAAAACGTA-BHQ2-3’
RC2		5’-GCCAAAAAGACGGAAAAAGGAGCCGGAAAAAGCGCGAAAAAAGA-3’
R63-	G7T2	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTTCGCAGAGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G9	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTTCGCCGGGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G5T5	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTAAAAAGCGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G7T3	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTCCTGCTGCTCTTACGTTTTTTGCCAGTTTTTG -3’
R1RM-	G7T2	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCTCTGCGTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G9	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCCCGGCGTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G5T5	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCGCTTTTTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G7T3	5’-phos-TTCTTTCTAGCTGCATTCCACTTGAGCAGCAGGTGACCTTAGAACTGTATGGATATATTCTAC-3’
The remaining ODNs of Ring B		
rm-1		5’- GGTTTGTAAGACGGAATAATGTAGAATATATCCATACAGTTCTAAGGTCA -3’
FAM-rm2		5’-Fam-AGTGGAATGCAGCTAGAAAGAATCAAGATCATAGTCAATCTCTCAGAGACGT-3’
HEX-rm2 (substitute to FAM-rm2 to label with HEX)		5’-HEX-AGTGGAATGCAGCTAGAAAGAATCAAGATCATAGTCAATCTCTCAGAGACGT-3’
RMR2-	G7T2	5’-phos-ATTATTCCGTCTTACAAACCTATGGCGCTTTTTTCGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G9	5’-phos-ATTATTCCGTCTTACAAACCTATGGCGCTTTTTTCGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G5T5	5’-phos-ATTATTCCGTCTTACAAACCTATGGCCCGGCGTACGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G7T3	5’-phos-ATTATTCCGTCTTACAAACCTATGGCCCGGCGTACGTCTCTGAGAGATTGACTATGATCTTGA-3’

Table S2. Sequences of ODNs used for Cat^{hyb}. The sequences of ss-region a1 are colored in red, and the sequences of ss-region b1 are colored in blue. The sequences of ss-region b2 are colored in green.

R-ODNs		
For Cat^{hyb}-G7T2	RnG7T2	5’-GGCTCTGCGAAAAAAAGTCCGATATA-3’
For Cat^{hyb}-G9	RnG9	5’-GGCCCGGCGAAAAAAAGTCCGATATA-3’
For Cat^{hyb}-G5T5	RnG5T5	5’-GGCGCTTTTTAAAAAAGTCCGATATA-3’
For Cat^{hyb}-G7T3	RnG7T3	5’-GAGCAGCAGGAAAAAAGTCCGATATA-3’
RR-ODNs		
For Cat^{hyb}-G7T2	RRnG7T2	5’-TATATCGGACTTTTTTTCGCGG-3’
For Cat^{hyb}-G9	RRnG9	5’-TATATCGGACTTTTTTTCGCCG-3’
For Cat^{hyb}-G5T5	RRnG5T5	5’-TATATCGGACTTTTTTAAAAAG-3’
For Cat^{hyb}-G7T3	RRnG7T3	5’-TATATCGGACTTTTTTCCTGC-3’
Negative control		
FAM-control-1	TTGATTCTTTCTAGCTGCATTCCACTGGCTCTGCGTTGACCTTAGAACTG	
Control-BHQ-2	CAGTTCTAAGGTCAACGCAGAGCCTACGTTTTTTGCCAGTTTTTGCGTT	
RC1-BHQ	5’-ATCAAAAAGCGGTAAAAACGCAAAAACTGGCAAAAAAACGTA-BHQ1-3’	
FAM-rm2	5’-Fam-AGTGGAATGCAGCTAGAAAGAATCAAGATCATAGTCAATCTCTCAGAGACGT-3’	

Table S3. Sequences of releasing ODNs, reversing ODNs and the negative control.

Ring A		
R4B2		5’-phos-CGTTTTTACCGCTTTTTGATACGTGCGGAACCCTGCGGGTGTTCTTTTTTCGCGCTTTTTCCG-3’
RC1-BHQ		5’-ATCAAAAAGCGGTAAAAACGCAAAAACTGGCAAAAAACGTA-BHQ1-3’
RC2		5’-GCCAAAAAGACGGAAAAAGGAGCCGGAAAAAGCGCGAAAAAAGA-3’
R63-	G7T2	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTTCGCAGAGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G9	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTTCGCCGGGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G5T5	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTAAAAAGCGCCTTACGTTTTTTGCCAGTTTTTG-3’
	G7T3	5’-phos-GCTCCTTTTTCCGTCTTTTTGGCACTTTTTTCCTGCTGCTCTTACGTTTTTTGCCAGTTTTTG -3’
Ring B		
rm-1		5’- GGTTTGTAAGACGGAATAATGTAGAATATATCCATACAGTTCTAAGGTCA -3’
FAM-rm2		5’-Fam-AGTGGAATGCAGCTAGAAAGAATCAAGATCATAGTCAATCTCTCAGAGACGT-3’
HEX-rm2 (substitute to FAM-rm2 to label with HEX)		5’-HEX-AGTGGAATGCAGCTAGAAAGAATCAAGATCATAGTCAATCTCTCAGAGACGT-3’
R1RM-	G7T2	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCTCTGCGTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G9	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCCCGGCGTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G5T5	5’-phos-TTCTTTCTAGCTGCATTCCACTTGGCGCTTTTTTGACCTTAGAACTGTATGGATATATTCTAC-3’
	G7T3	5’-phos-TTCTTTCTAGCTGCATTCCACTTGAGCAGCAGGTGACCTTAGAACTGTATGGATATATTCTAC-3’
RMR2-	G7T2	5’-phos-ATTATTCCGTCTTACAAACCTATGGCGCTTTTTTCGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G9	5’-phos-ATTATTCCGTCTTACAAACCTATGGCGCTTTTTTCGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G5T5	5’-phos-ATTATTCCGTCTTACAAACCTATGGCCCGGCGTACGTCTCTGAGAGATTGACTATGATCTTGA-3’
	G7T3	5’-phos-ATTATTCCGTCTTACAAACCTATGGCCCGGCGTACGTCTCTGAGAGATTGACTATGATCTTGA-3’

Table S4. Sequences of ODNs used for Ring A and Ring B. The sequences of ss-region a1 are colored in red, and the sequences of ss-region b1 are colored in blue. The sequences of ss-region b2 are colored in green.

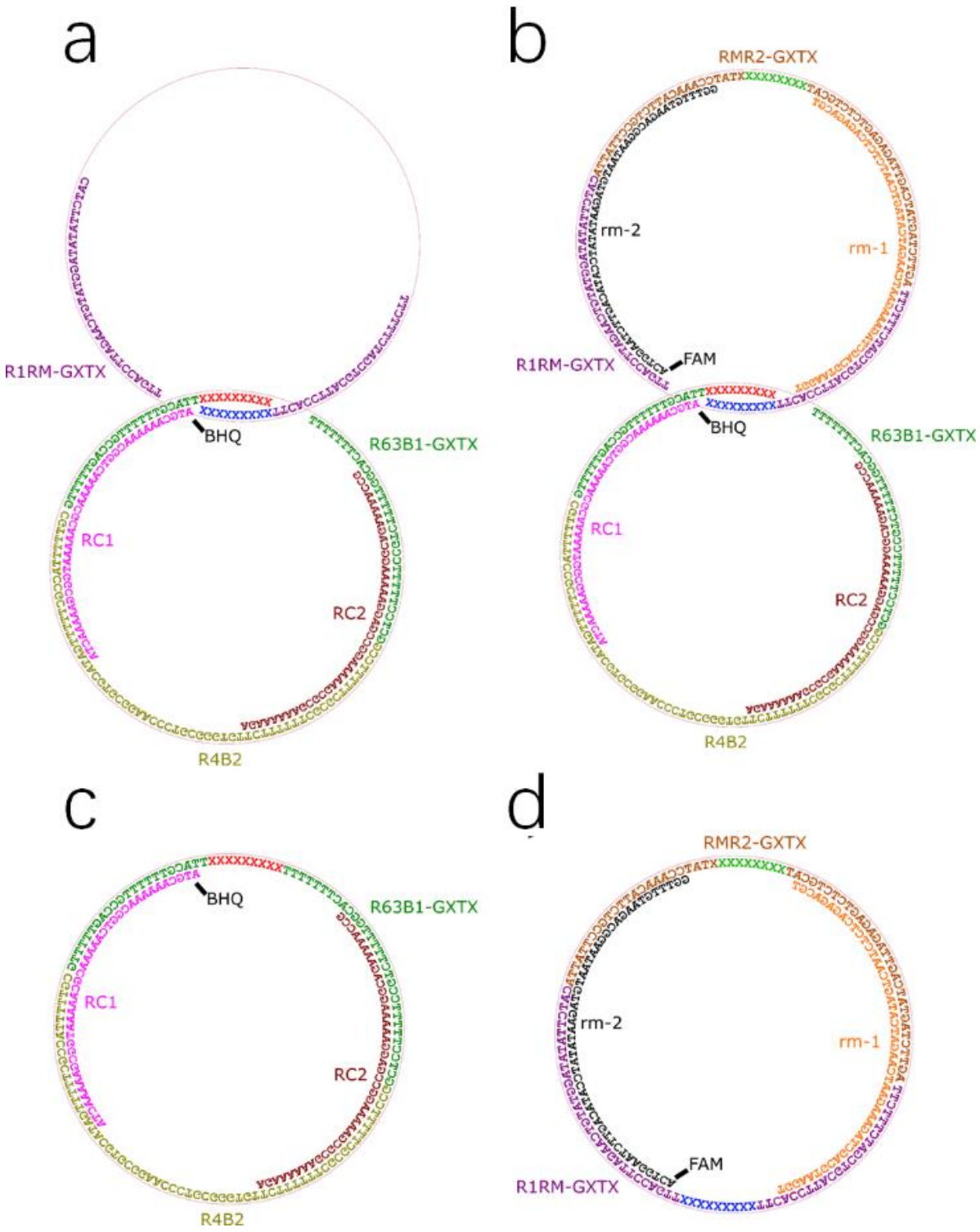


Figure S1. ODNs used for the threaded Ring A, Cat^{hyb}, Ring A and Ring B. (a) Structure of the threaded Ring A; (b) Structure of Cat^{hyb}; (c) Structure of Ring A; (d) Structure of Ring B. The sequences of ss-region a1 are shown in red, and the sequences of ss-region b1 are shown in blue. The sequences of ss-region b2 are marked in green.

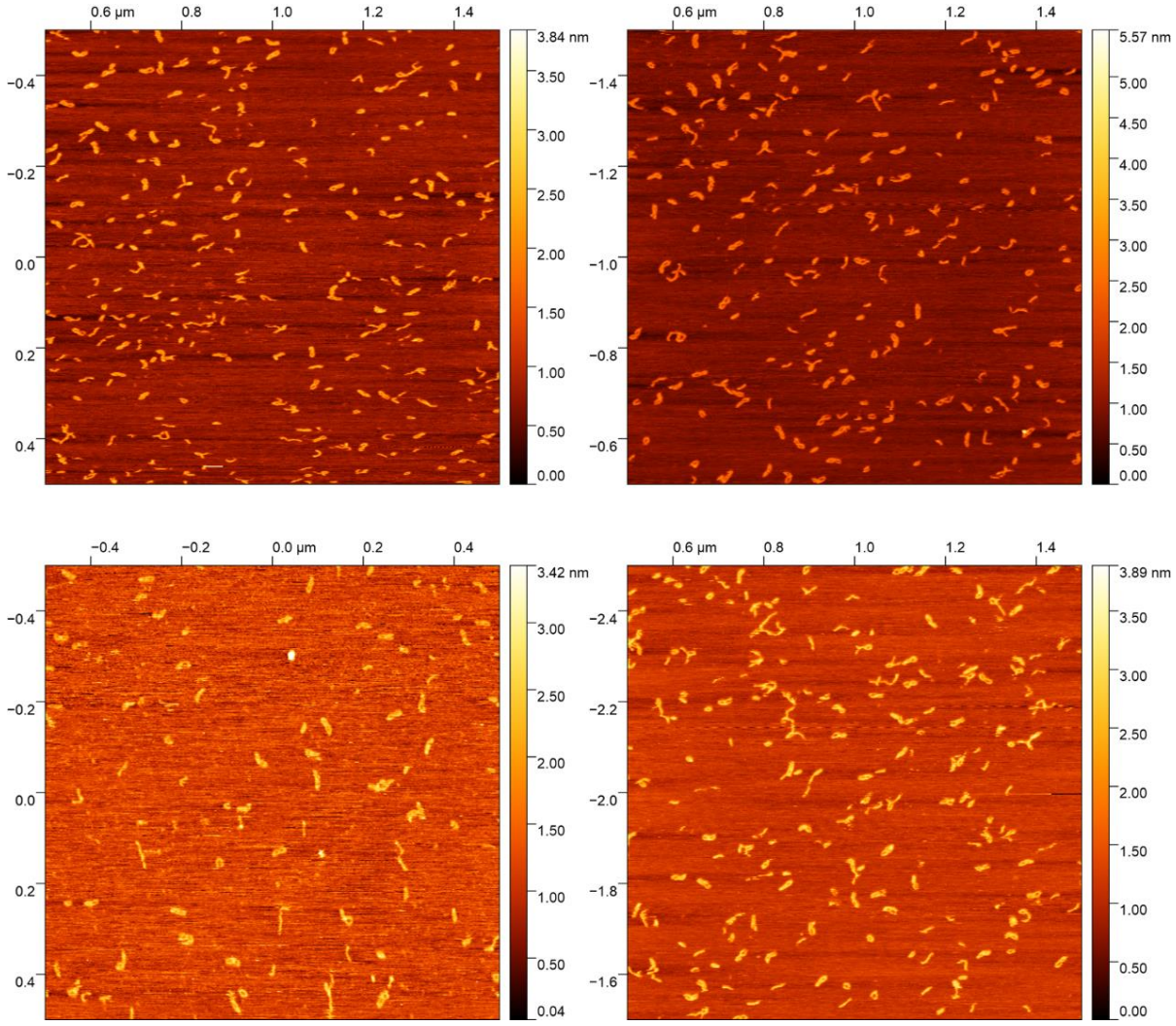


Figure S2. AFM images performed in intermitting contact mode in water of the Cat^{hyb}. Image size: 1 × 1 μm^2 with 512 x 512 pixels.

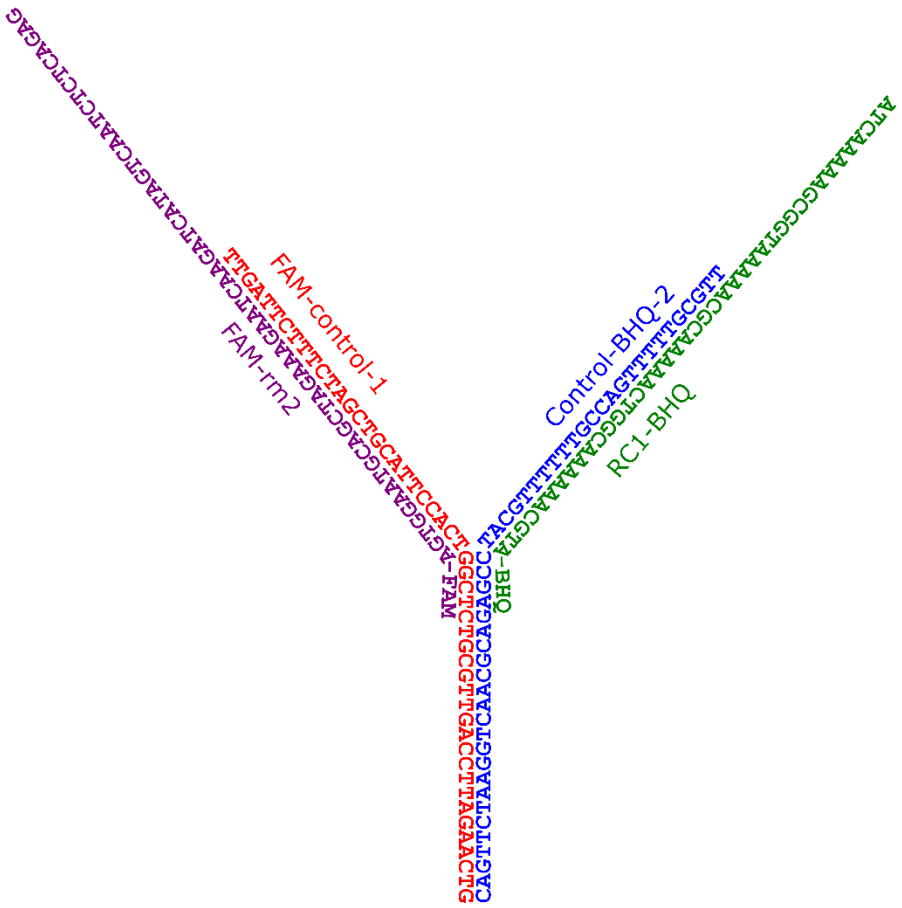


Figure S3. ODNs used for the negative control and its structure.

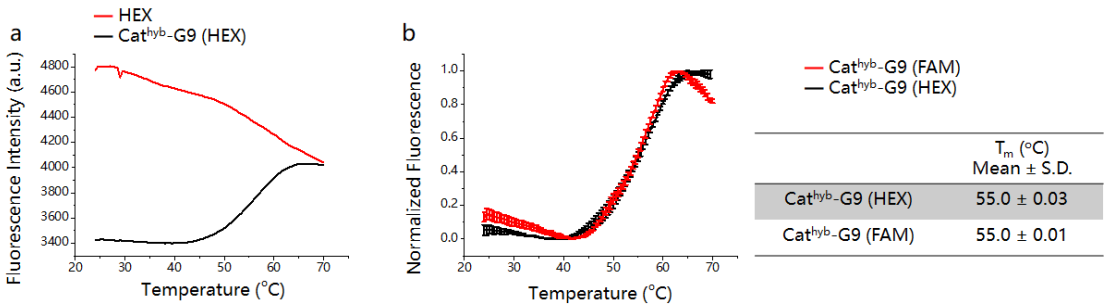
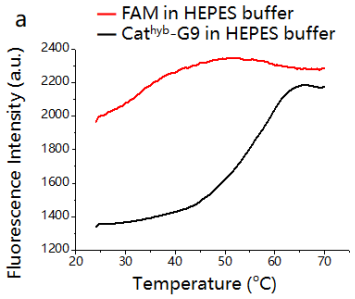
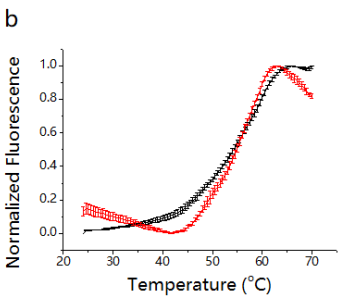
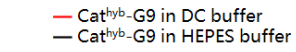


Figure S4. Thermal switching from the Cat^{hyb}-G9 to the Cat^{mec}-G9. (a) temperature-dependent fluorescence intensity of 20 µL of 100 nM DNA ring labeled with HEX (red) and 20 µL of 100 nM Cat^{hyb}-G9 labeled with HEX and BHQ2 (black) in DC buffer; (b) temperature-dependent fluorescence intensity of 20 µL of 100 nM Cat^{hyb}-G9 labeled with FAM (red) and 20 µL of 100 nM Cat^{hyb}-G9 labeled with HEX (black) (S.D., n = 3).







	T_m (°C) Mean ± S.D.
In DC buffer	55.0 ± 0.01
In HEPES buffer	54.5 ± 0.02

Figure S5. Thermal switching between the Cat^{hyb}-G9 and the Cat^{mec}-G9 in the HEPES buffer (10 mM, EDTA 1 mM, $MgCl_2$ 25 mM pH=8). (a) temperature-dependent fluorescence intensity of 20 µL of 100 nM DNA ring labeled with 6-FAM (red) and 20 µL of 100 nM Cat^{hyb}-G9 labeled with FAM and BHQ1 (black) in HEPES buffer; (b) temperature-dependent fluorescence intensity of 20 µL of 100 nM Cat^{hyb}-G9 in DC buffer (red) and HEPES buffer (black) (S.D., n = 3).

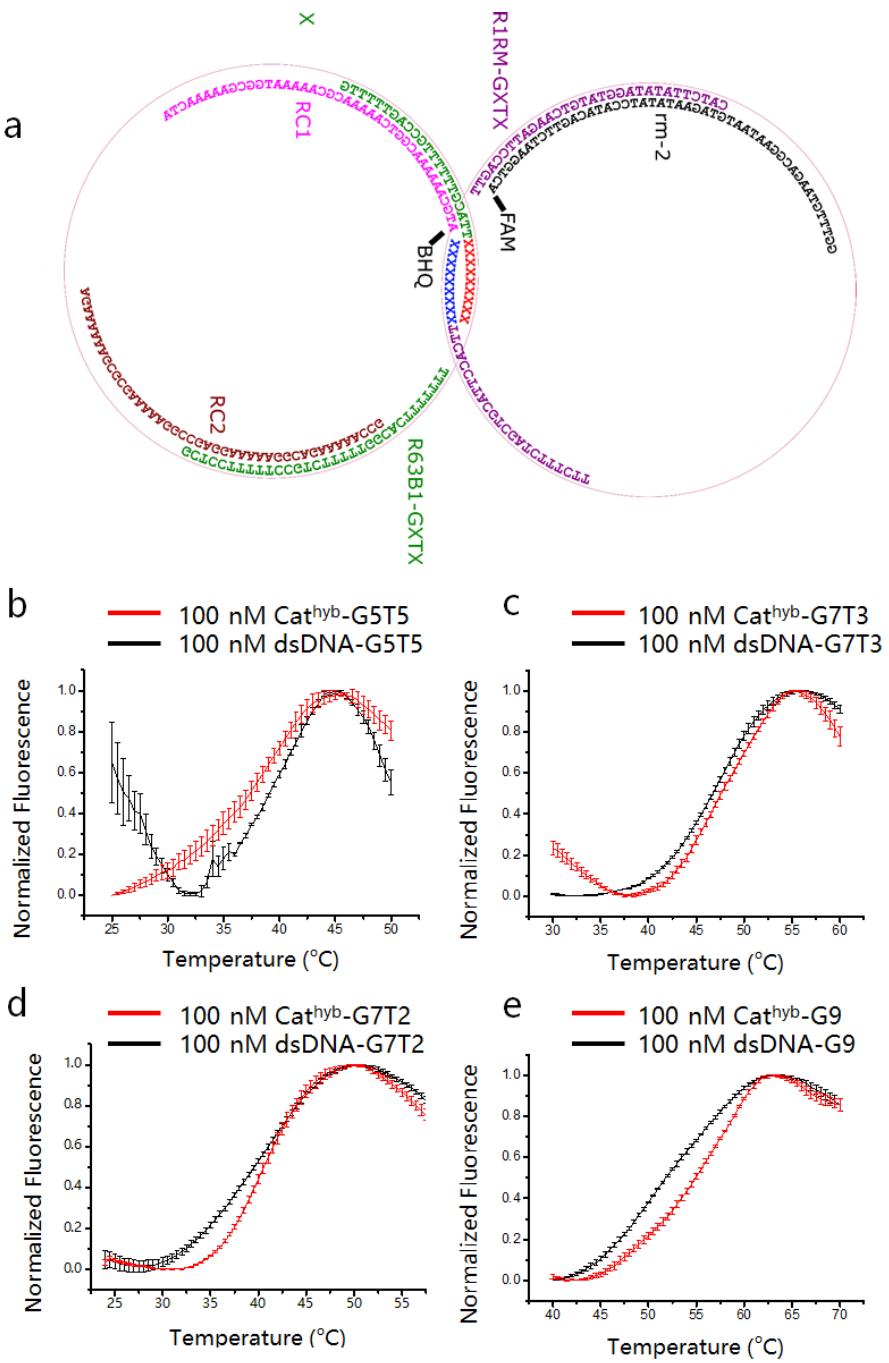


Figure S6. (a) ODNs used for measuring T_m of dsDNA with different HR; (b-e) Melting curves of the Cat^{hyb}s and dsDNA with different HR; (b) 100 nM of the Cat^{hyb}-G5T5 (red) and dsDNA-G5T5 (black) (S.D., n = 3); (c) 100 nM of the Cat^{hyb}-G7T3 (red) and dsDNA-G7T3 (black) (S.D., n = 3); (d) 100 nM of the Cat^{hyb}-G7T2 (red) and dsDNA-G7T2 (black) (S.D., n = 3); (e)100 nM of the Cat^{hyb}-G9 (red) and dsDNA-G9 (black) (S.D., n = 3).

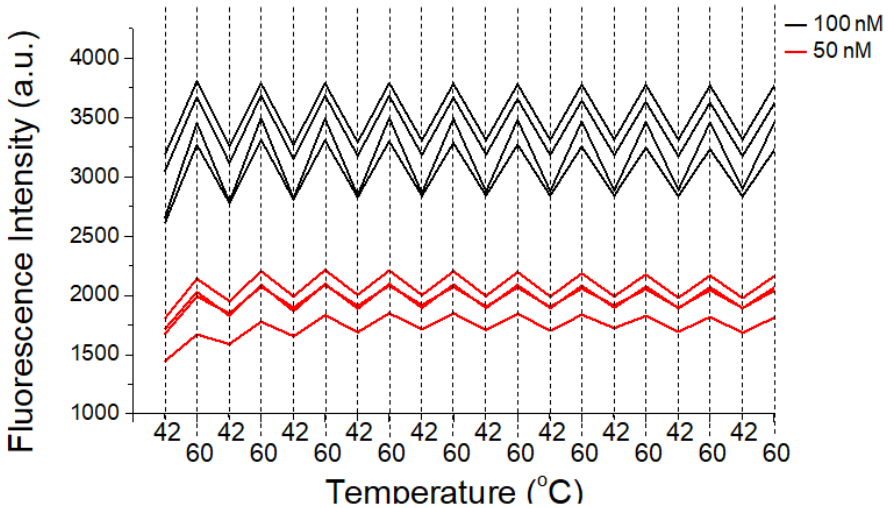


Figure S7. Fluorescence intensity of Cat^{hyb}-G9 with different concentrations at 42 °C and 60 °C. Black: 100 nM, red: 50 nM.