Supplementary Information

Mechanistic model for epigenetic maintenance by methyl-CpG-binding domain proteins

Liuhan Dai[1,2], Alexander Johnson-Buck[1] & Nils G. Walter[1,2*]

[1]Single Molecule Analysis Group, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA

[2]Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI 48109, USA

Corresponding authors

*E-mail: nwalter@umich.edu

The manuscript was written based on contributions from all authors.

All authors have given approval to the final version of the manuscript.

Supplementary Table 1. DNA oligonucleotide names, sequences, and descriptions. All sequences are listed 5′-to-3′.

Name	Sequences	Description
	Cloning	
Halo-hMBD-BF	GGAGGTGGAAGCGGTGAA	Forward primer for linearization of backbone plasmid pBD003_mut_VCP (R155H) for constructing Halo-hMBD, directly purchased from IDT
Halo-hMBD-BR	ATGTATATCTCCTTCTTAAAGTTAA	Reverse primer for linearization of backbone plasmid pBD003_mut_VCP (R155H) for constructing Halo-hMBD, directly purchased from IDT
Halo-hMBD-IF	GAAGGAGATATACATATGGCTGAGGACTGGCTGG	Forward primer for PCR amplification of insert hMBD1 MBD from Addgene plasmid #119966 for constructing 81 Halo-hMBD, directly purchased from IDT
Halo-hMBD-IR	ACCGCTTCCACCTCCATGGGCCTTGGGGGCTGG	Reverse primer for PCR amplification of insert hMBD1 MBD from Addgene plasmid #119966 for constructing Halo-hMBD, directly purchased from IDT
	Target	
M7	GTCTTCCTGCTGATGCAATC/iMe-dC/GCTAGGT/iMe-dC/G/iMe-dC/GAGTCTC/iMe-dC/GC/iMe-dC/G/iMe-dC/GAGAGGGC/iMe-dC/GG	Fully Methylated 55 nt BCAT1 promoter forward strand, with 7 methyl-CpGs, directly purchased from IDT
M4b	GTCTTCCTGCTGATGCAATCCGCTAGGT /iMe-dC/ G /iMe-dC/GAGTCTC/iMe-dC/GC/iMe-dC/GCGAGAGGGCCGG	Methylated 55 nt BCAT1 promoter forward strand, with 4 methyl-CpGs, directly purchased from IDT
M4a	GTCTTCCTGCTGATGCAATCCGCTAGGT /iMe-dC/ G /iMe-dC/ GAGTCTCCGC /iMe-dC/ G /iMe-dC/ GAGAGGGCCGG	Methylated 55 nt BCAT1 promoter forward strand, with 4 methyl-CpGs, directly purchased from IDT
M3b	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTC/iMe-dC/GC/iMe-dC/GCGAGAGGGC/iMe-dC/GG	Methylated 55 nt BCAT1 promoter forward strand, with 3 methyl-CpGs, directly purchased from IDT
M3a	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTC/iMe-dC/GC/iMe-dC/G/iMe-dC/GAGAGGGCCGG	Methylated 55 nt BCAT1 promoter forward strand, with 3 methyl-CpGs, directly purchased from IDT

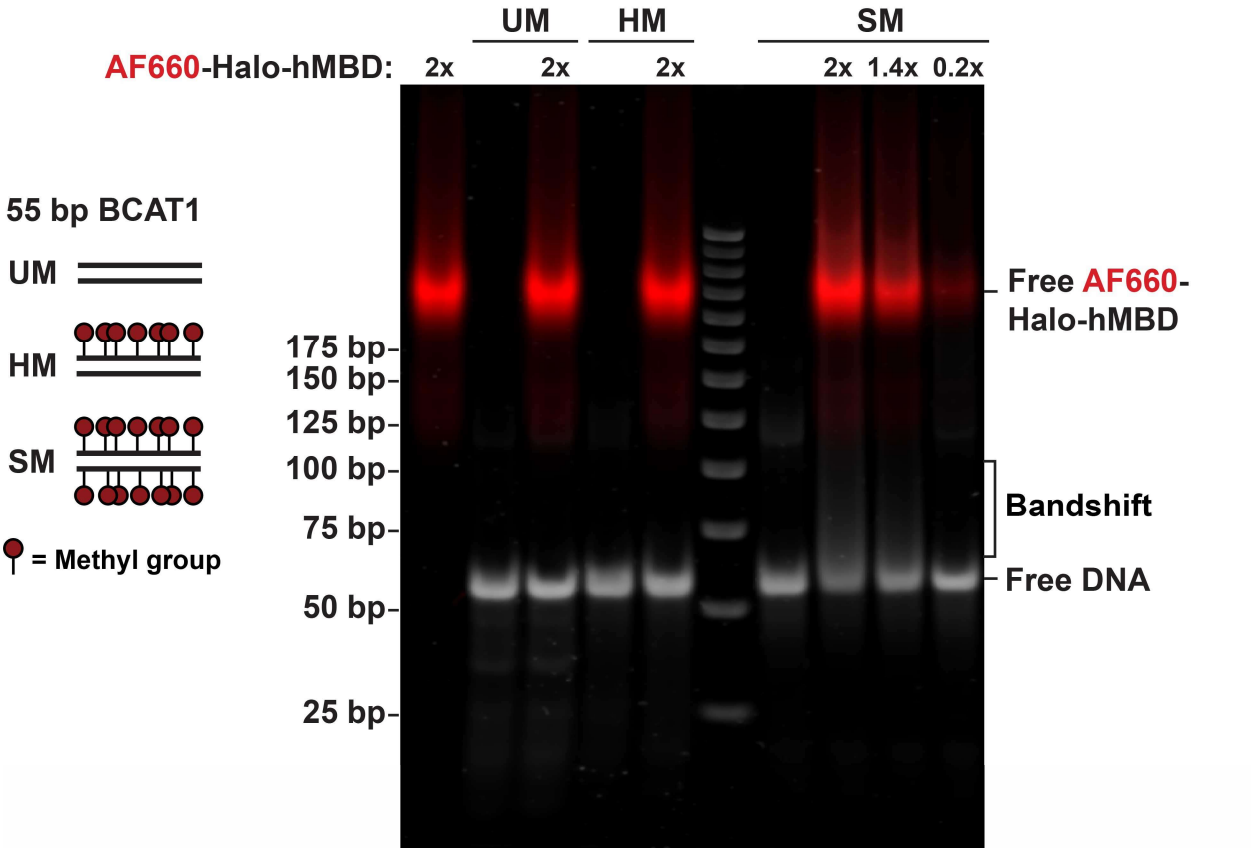
M2c	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTCCGC/iMe-dC/GCGAGAGGGC/iMe-dC/GG	Methylated 55 nt BCAT1 promoter forward strand, with 2 methyl-CpGs, directly purchased from IDT
M2b	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTC/iMe-dC/GCCGCGAGAGGGC/iMe-dC/GG	Methylated 55 nt BCAT1 promoter forward strand, with 2 methyl-CpGs, directly purchased from IDT
M2a	GTCTTCCTGCTGATGCAATCCGCTAGGT/iMe-dC/G/iMe-dC/GAGTCTCCGCCGCGAGAGGGCCGG	Methylated 55 nt BCAT1 promoter forward strand, with 2 methyl-CpGs, directly purchased from IDT
M1b	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTCCGCCGCGAGAGGGC/iMe-dC/GG	Methylated 55 nt BCAT1 promoter forward strand, with single methyl-CpGs, directly purchased from IDT
M1a	GTCTTCCTGCTGATGCAATC/iMe-dC/GCTAGGTCGCGAGTCTCCGCCGCGAGAGGGCCGG	Methylated 55 nt BCAT1 promoter forward strand, with single methyl-CpGs, directly purchased from IDT
M0	GTCTTCCTGCTGATGCAATCCGCTAGGTCGCGAGTCTCCGCCGCGAGAGGGCCGG	Unmethylated 55 nt BCAT1 promoter forward strand, directly purchased from IDT
	Auxiliary probes	
A39m	C/iMe-dC/GGCCCTCT/iMe-dC/G/iMe-dC/GG/iMe-dC/GGAGACT/iMe-dC/G/iMe-dC/GACCTAG/iMe-dC/GGATT	Fully methylated auxiliary probe, 39 nt in total, fully complementary to target sequence, with no “branch” motif, with 7 methyl-CpGs, directly purchased from IDT
A39O	CTTATCTGTTCCGGCCCTCTCGCGGCGGAGACTCGCGACCTAGCGGATT	49 nt auxiliary probe, with 39 nt complementary to target sequence, with a 10 nt overhang sequence “CTTATCTGTT”, directly purchased from IDT
A39	CCGGCCCTCTCGCGGCGGAGACTCGCGACCTAGCGGATT	39 nt auxiliary probe, fully complementary to target sequence, directly purchased from IDT
A30	TCGCGGCGGAGACTCGCGACCTAGCGGAT	30 nt auxiliary probe, fully complementary to target sequence, directly purchased from IDT
A25	GCGGAGACTCGCGACCTAGCGGATT	25 nt auxiliary probe, fully complementary to target sequence, directly purchased from IDT
A17	TCGCGACCTAGCGGATT	17 nt auxiliary probe, fully complementary to target

		sequence, directly purchased from IDT
A17O	CTTATCTGTTTCGCGACCTAGCGGATT	27 nt auxiliary probe, with 17 nt complementary to target sequence, with a 10 nt overhang sequence "CTTATCTGTT", directly purchased from IDT
	Capture probes	
CPO	/5Biosg/ATAATTAATAGCATCAGCAGGAAGAC	26 nt 5' biotinylated capture probe, with 16 nt complementary to target sequence, with a 10 nt overhang sequence "ATAATTAATA", directly purchased from IDT
CP	GCATCAGCAGGAAGAC/3BioTEG/	16 nt 3' biotinylated capture probe, fully complementary to target sequence, directly purchased from IDT
	Others	
M7_rev	C/iMe-dC/GGCCCTCT/iMe-dC/G/iMe-dC/GG/iMe-dC/GGAGACT/iMe-dC/G/iMe-dC/GACCTAG/iMe-dC/GGATTGCATCAGCAGGAAGAC	Fully Methylated 55 nt BCAT1 promoter reverse strand, with 7 methyl-CpGs, fully complementary to M7, directly purchased from IDT
M0_rev	CCGGCCCTCTCGCGGCGGAGACTCGCGACCTAGCGGATTGCATCAGCAGGAAGAC	Unmethylated 55 nt BCAT1 promoter reverse strand, fully complementary to M7, directly purchased from IDT

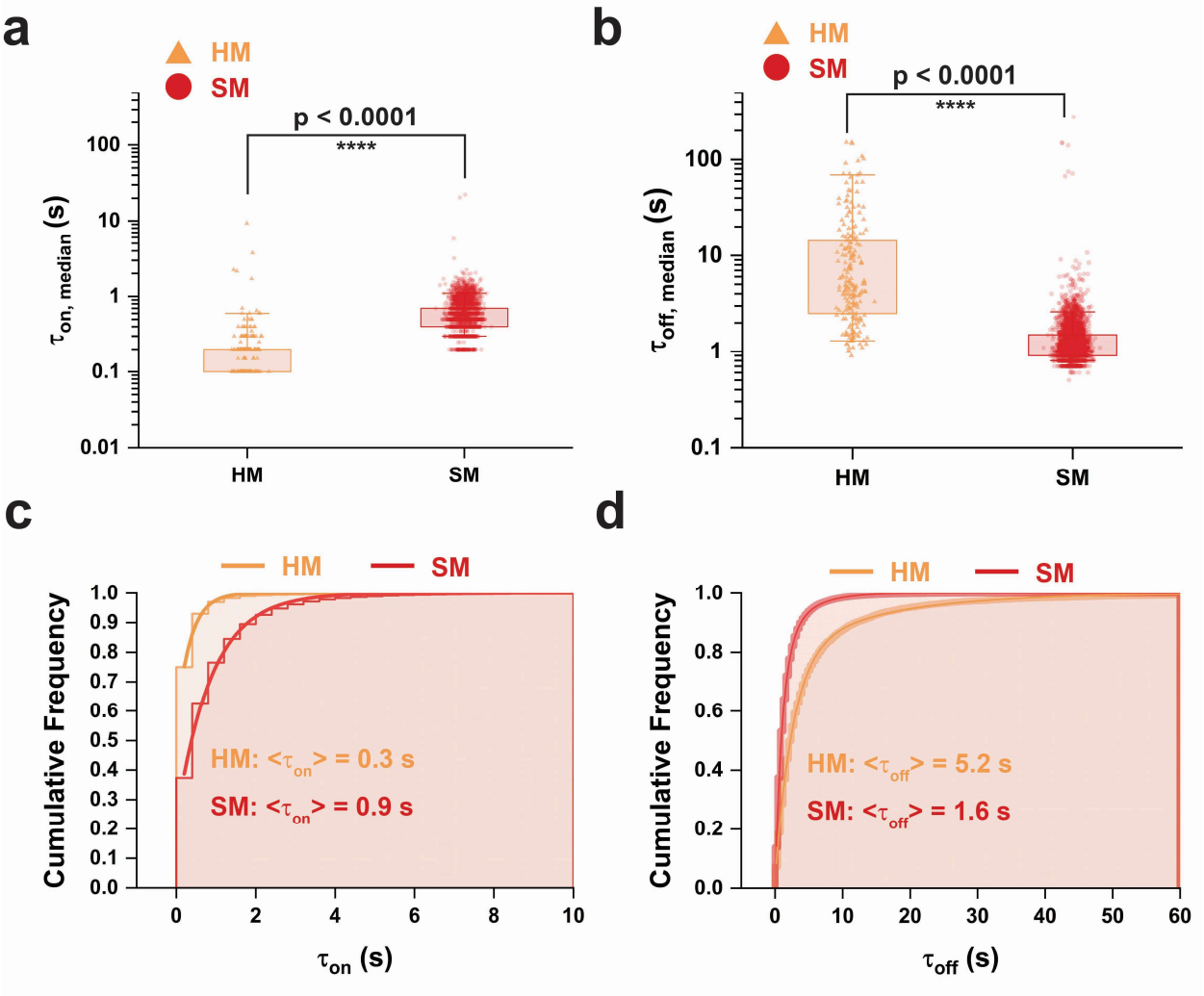
Supplementary Table 2. Parameter sets for trace generation and analysis.

Trace Generation Parameters	
use fluctuation map?	2 ('N_{b+d} map')
Stdfactor	5
start frame	1
end frame	3000
edgePx	20
Percentilecut	0.95
ROI size (pixels)	5

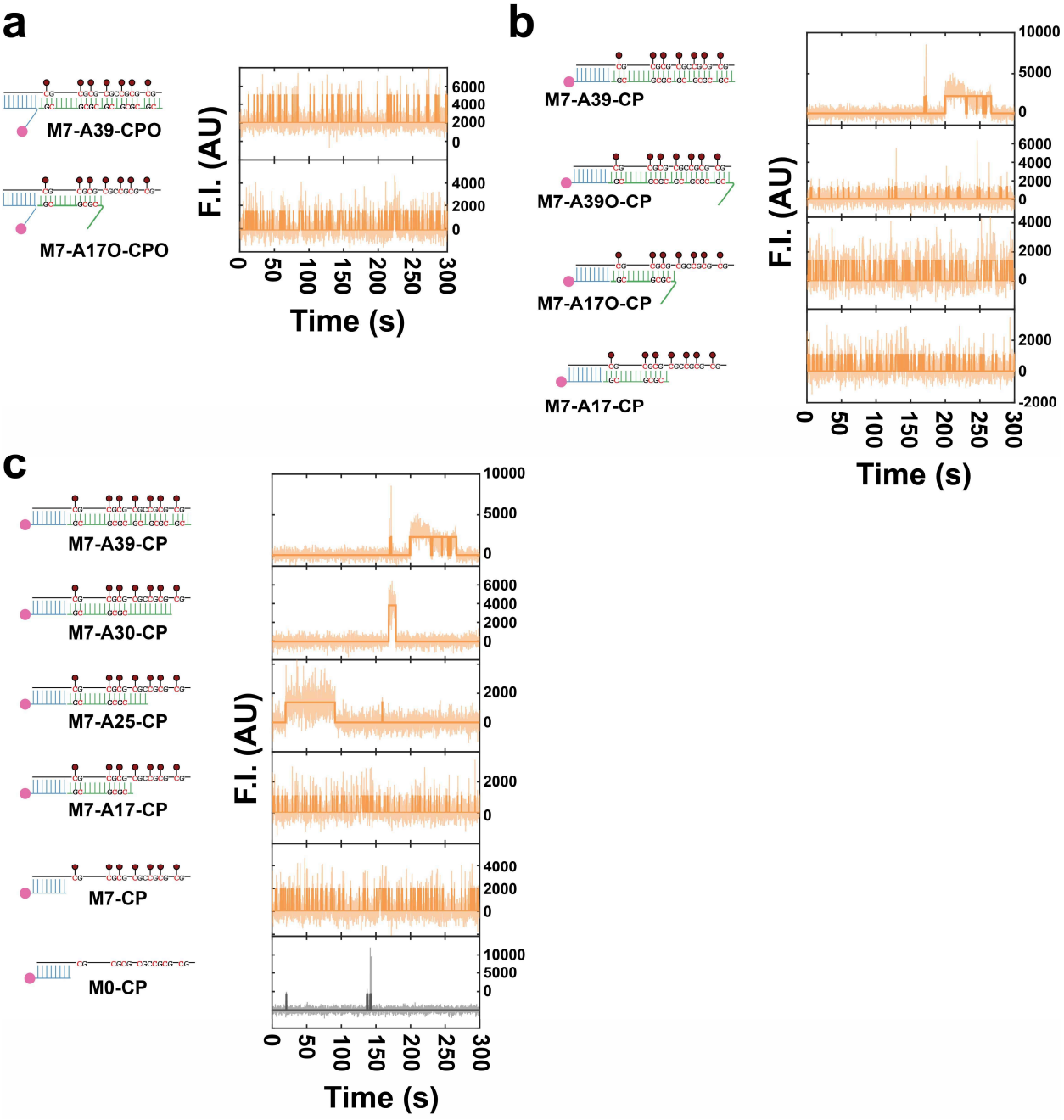
Trace Fitting Parameters	
start frame	1
end frame	3000
exposure time (s)	0.1
Smoothframes	1
remove_single_frame_events	FALSE
Ithresh	1000
SNthresh	2



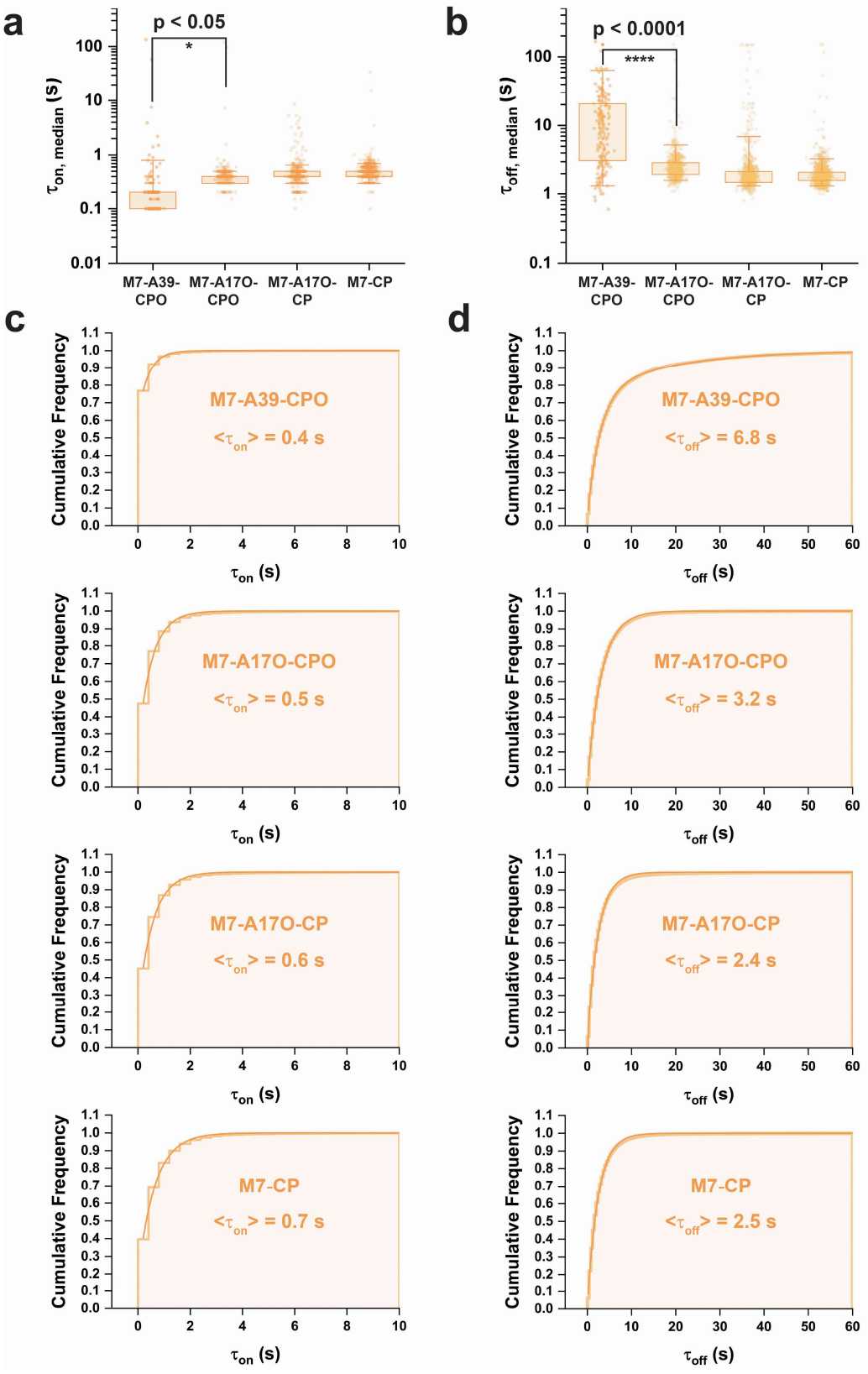
Supplementary Fig. 1. | EMSA of AF660-Halo-hMBD binding to three types of 55 bp BCAT1 promoter substrates. SM (symmetrically methylated DNA), HM (hemimethylated DNA), and UM (unmethylated DNA). AF660-Halo-hMBD was mixed with 100 nM DNAs at different molar ratios in 10% glycerol, 50 mM Tris-HCl pH 8.0 at room temperature in dark for 2 h. 5% PAGE was prepared in 50 mM Tris Acetate pH 7.5. Electrophoresis was running in 50 mM Tris Acetate pH 7.5 at 4°C with approximately 15 V/cm for 3 h. Gel was stained with SYBR Gold and visualized using both Cy5 and Cy2 fluorescence on Typhoon Biomolecular Imager.



Supplementary Fig. 2 | Dwell time comparison between SM and HM in Fig. 1. a,b Comparison of $\tau_{on,median}$ and $\tau_{off,median}$ distributions respectively between SM and HM. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. P-values are assessed using single-tailed unpaired t-test. **c,d** Cumulative dwell time distributions and their exponential fittings of τ_{on} and τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. In panel c, mean bound time, $\langle\tau_{on}\rangle$, is calculated by fitting a single-exponential decay function. In panel d, mean unbound time, $\langle\tau_{off}\rangle$, is calculated by fitting a double-exponential decay function and taking the weighted average of individual components.

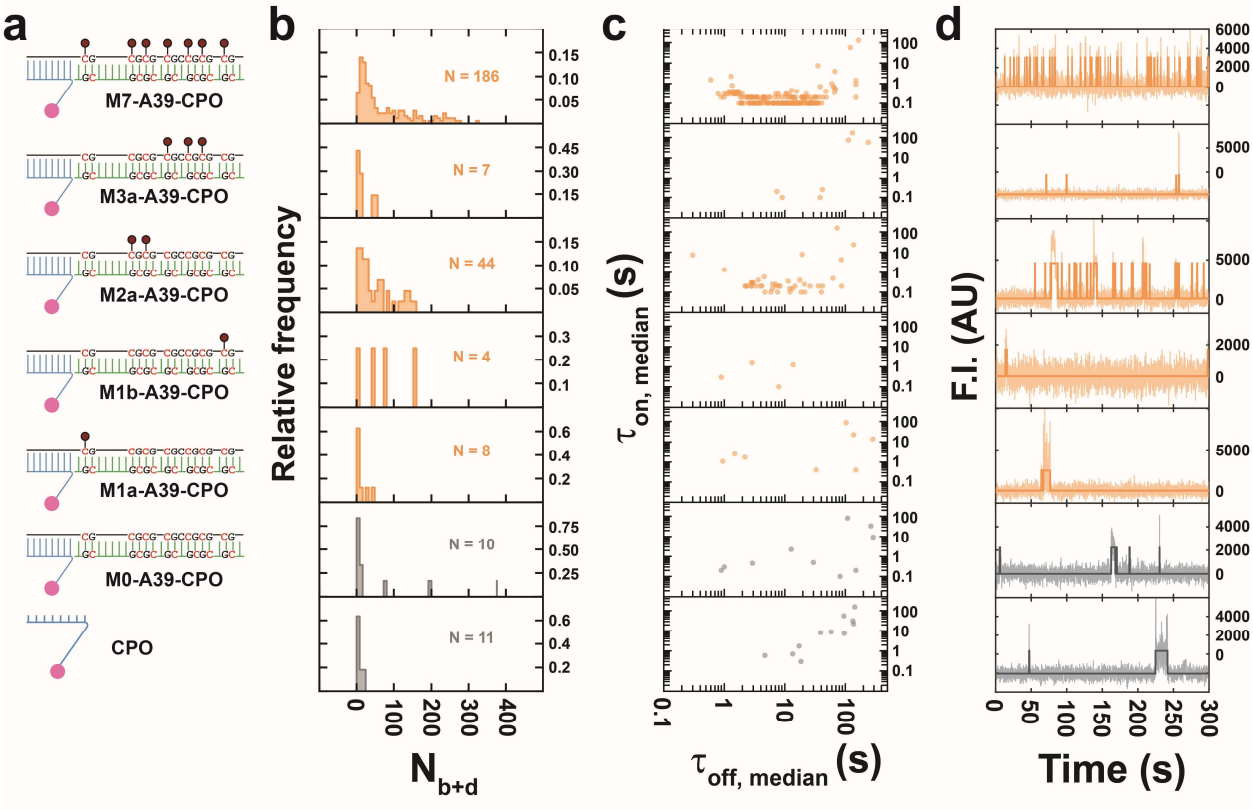


Supplementary Fig. 3 | Representative intensity-time traces of constructs in Fig. 2. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.

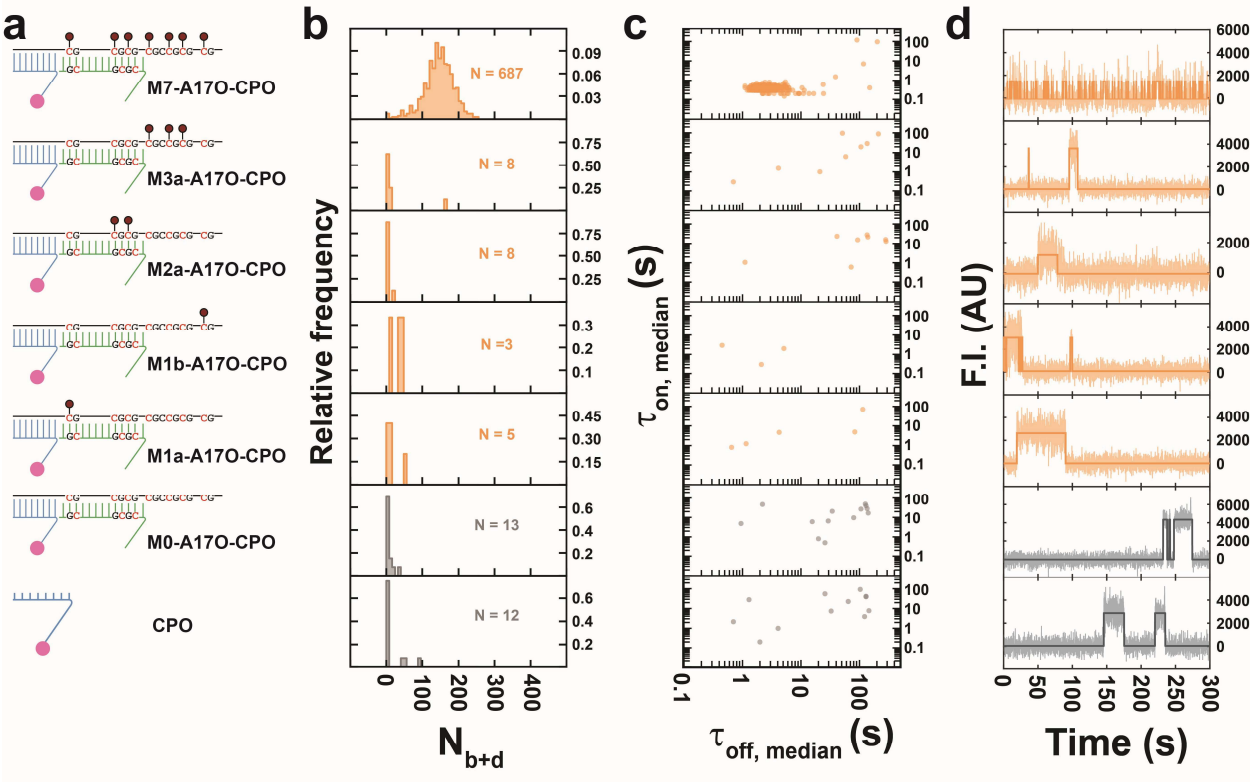


Supplementary Fig. 4 | Dwell time comparison and mean dwell time calculations of M7-A39-CPO, M7-A17O-CPO, M7-A17O-CP and M7-CP. a,b Boxplots of $\tau_{on,median}$ and $\tau_{off,median}$

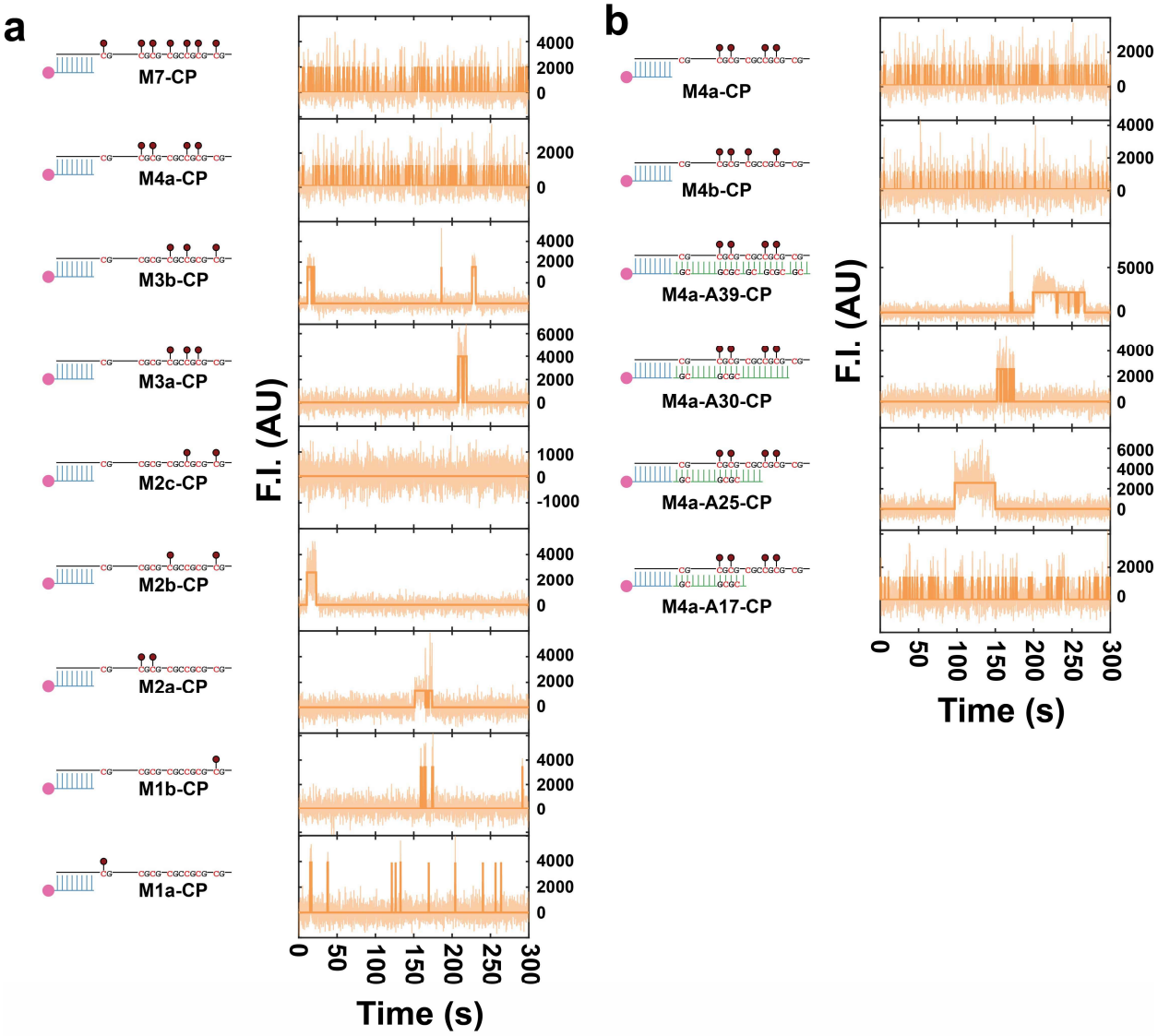
distributions respectively. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. P-values are assessed using single-tailed unpaired t-test. **c,d** Cumulative dwell time distributions and their exponential fittings of τ_{on} and τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Mean dwell times, $\langle\tau_{on}\rangle$ and $\langle\tau_{off}\rangle$, are calculated by fitting a single-exponential decay function except that $\langle\tau_{off}\rangle$ of M7-CP is calculated by fitting a double-exponential decay function and taking the weighted average of individual components.



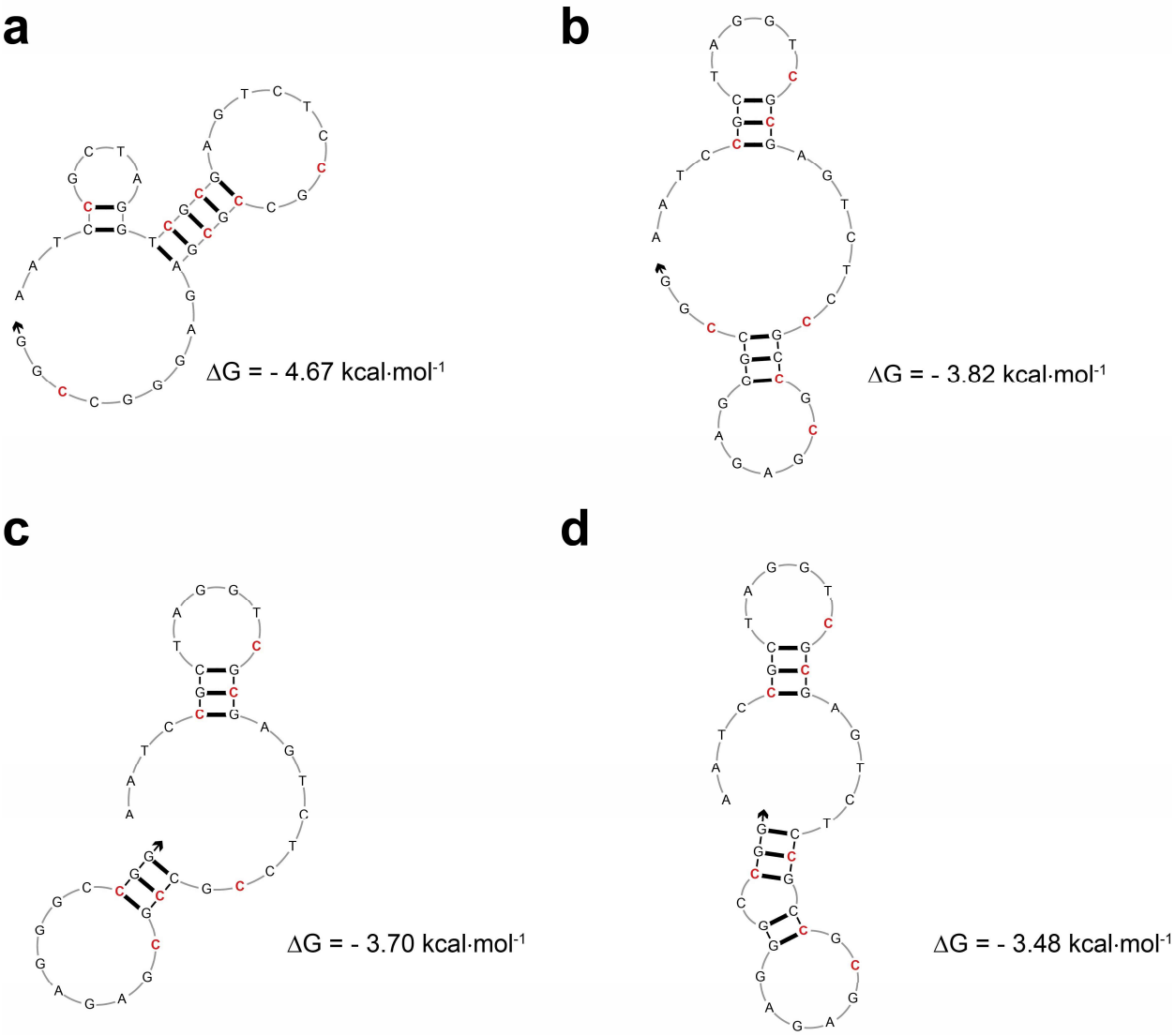
Supplementary Fig. 5 | Binding kinetics of single internal overhang-containing constructs modulated by clustering of methyl-CpG sites. a Constructs of M7-A39-CPO, M3a-A39-CPO, M2a-A39-CPO, M1b-A39-CPO, M1a-A39-CPO, M0-A39-CPO and CPO. **b** N_{b+d} distributions. N, the number of detected molecules of one field of view (FOV). **c** Median dwell time distributions. **d** Representative intensity-time traces. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



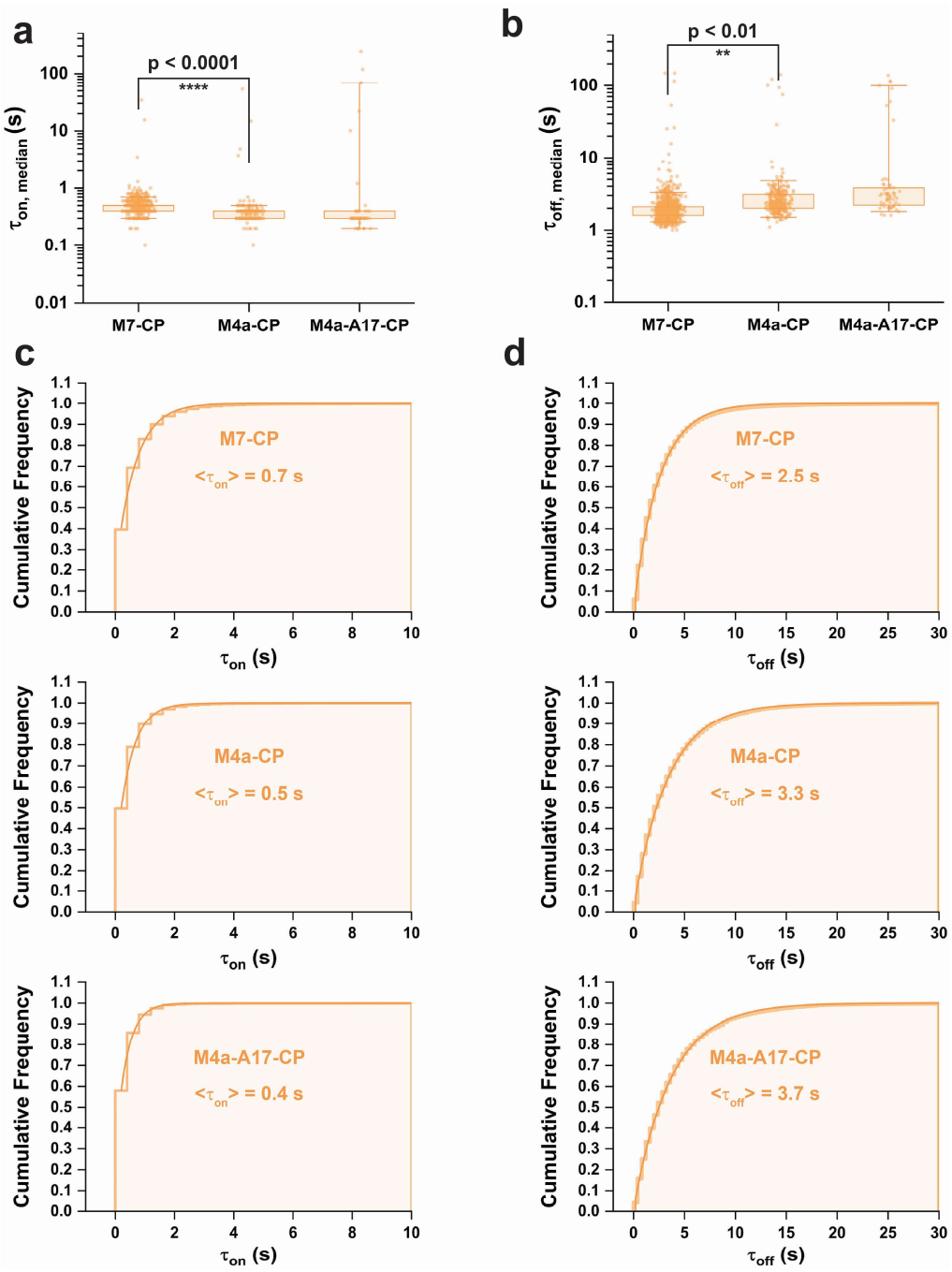
Supplementary Fig. 6 | Binding kinetics of double overhang-containing constructs modulated by clustering of methyl-CpG sites. a Constructs of M7-A17O-CPO, M3a-A17O-CPO, M2a- A17O-CPO, M1b- A17O-CPO, M1a-A17O-CPO, M0-A17O-CPO and CPO. **b** N_{b+d} distributions. N, the number of detected molecules of one field of view (FOV). **c** Median dwell time distributions. **d** Representative intensity-time traces. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



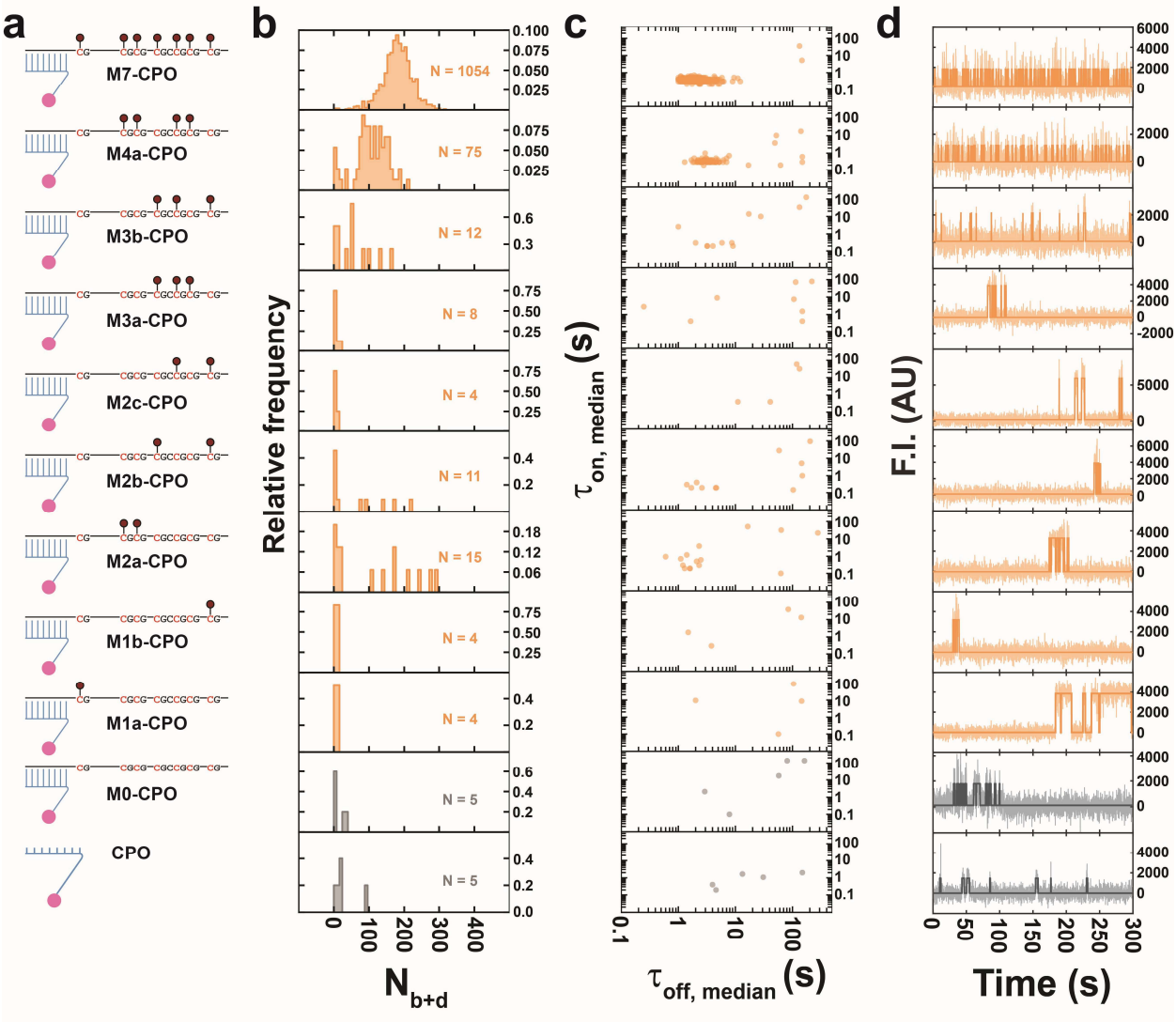
Supplementary Fig. 7 | Representative intensity-time traces of constructs in Fig. 3. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



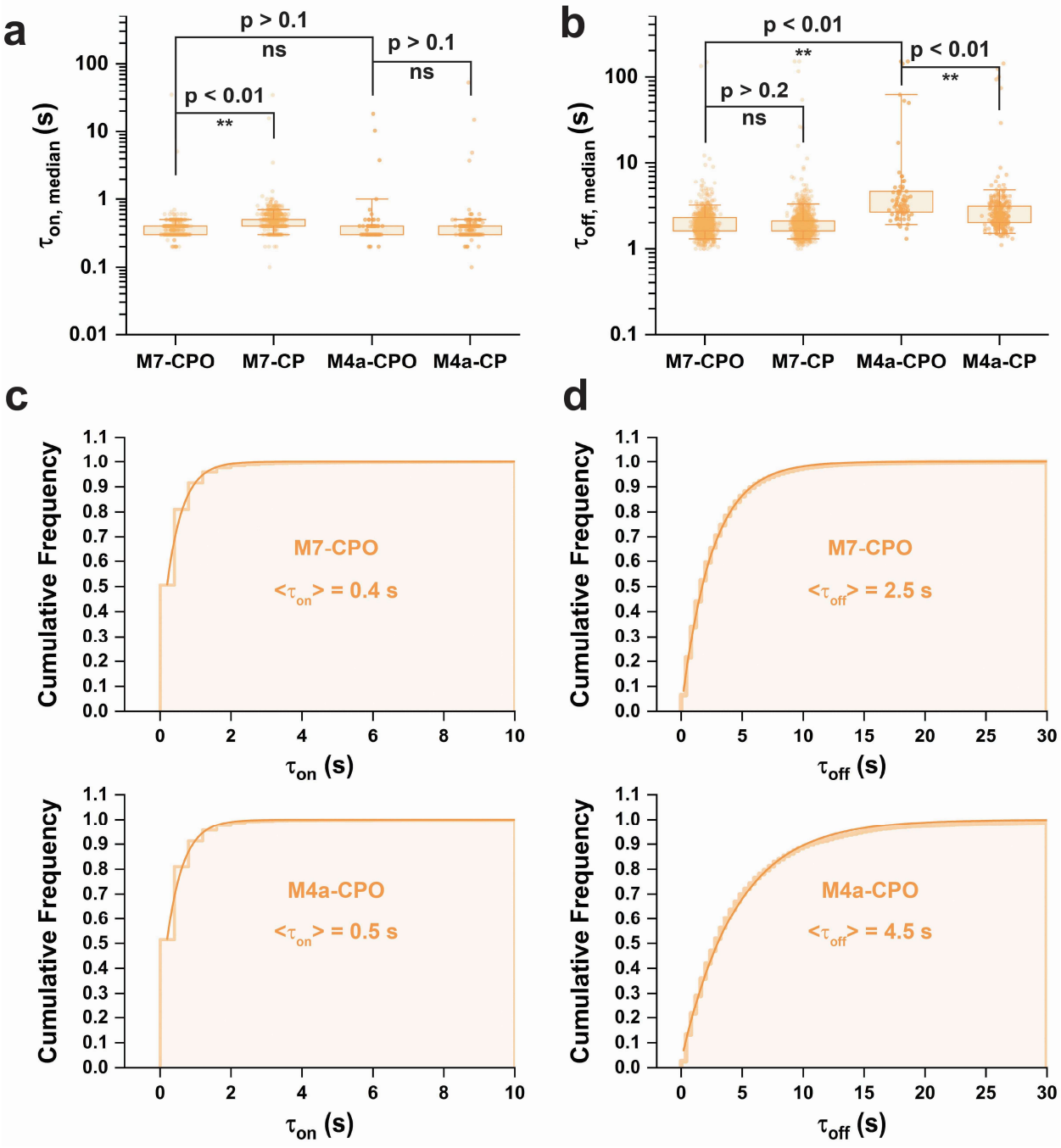
Supplementary Fig. 8 | Predicted secondary structures and calculated free energy by IDT. The top 4 structures are shown here. Input parameters for "HAIRPIN" prediction: Sequence, AATC**CG**CTAGGT**CGCG**AGTCTC**CG**C**CGCG**AGAGGGC**CG**G; Target type, DNA; Oligo Conc, 0.01 μM; Na^+ Conc, 20 mM; Mg^{2+} Conc, 0 mM; dNTPs Conc, 0 mM; Suboptimality, 50%; Sequence type, Linear; Temperature, 22 °C. Max Foldings, 20; Start position, 0; Stop position, 0.



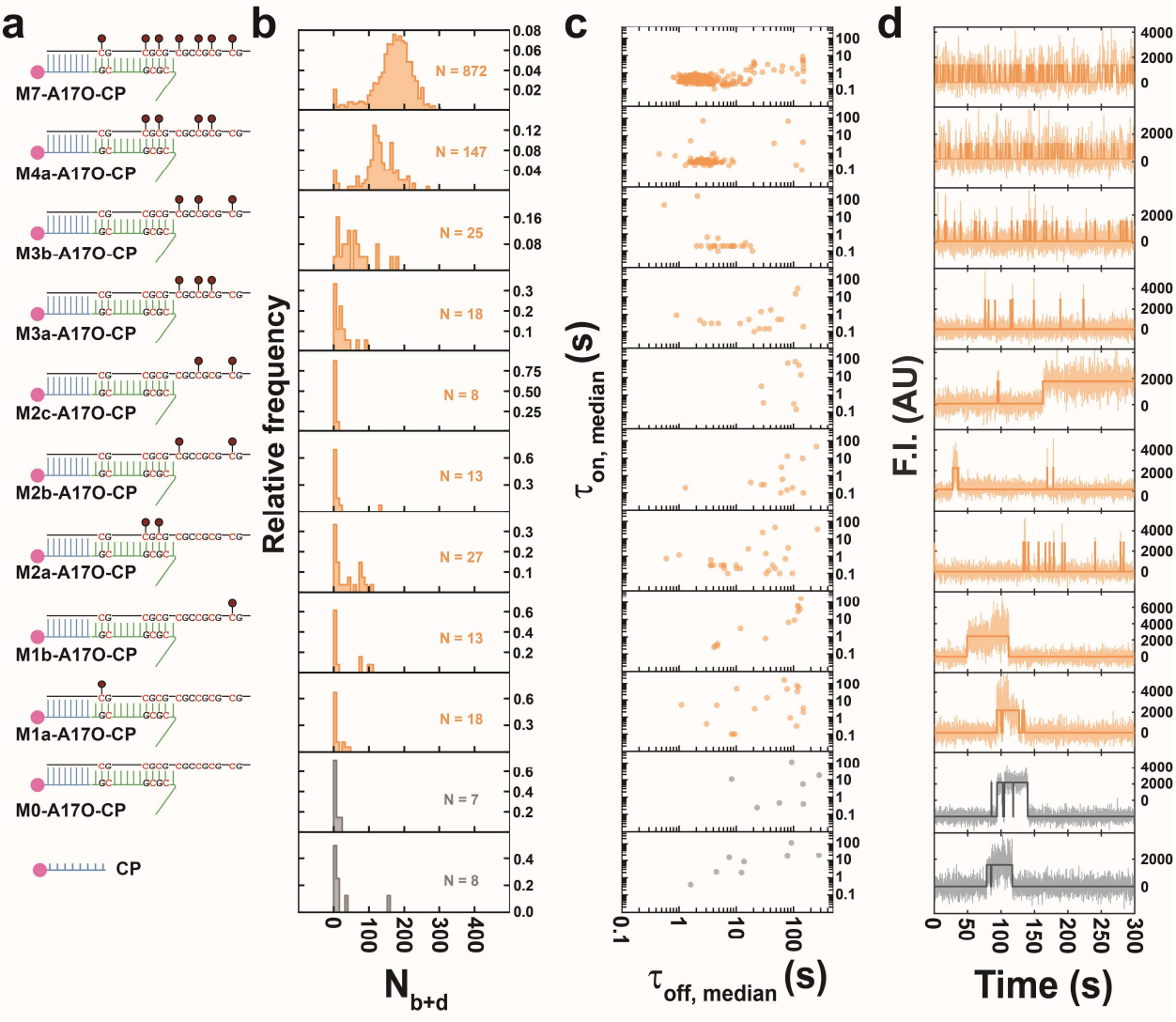
Supplementary Fig. 9 | Dwell time comparison and mean dwell time calculations of M7-CP, M4a-CP and M4a-A17-CP. a,b Boxplots of $\tau_{on,median}$ and $\tau_{off,median}$ distributions respectively. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. For hypothesis testing, the datasets of M4a-CP are 96% winsorized to tolerate interference of outliers. P-values are assessed using single-tailed unpaired t-test. **c,d** Cumulative dwell time distributions and their exponential fittings of τ_{on} and τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Mean dwell times, $\langle\tau_{on}\rangle$ and $\langle\tau_{off}\rangle$, are calculated by fitting a single-exponential decay function.



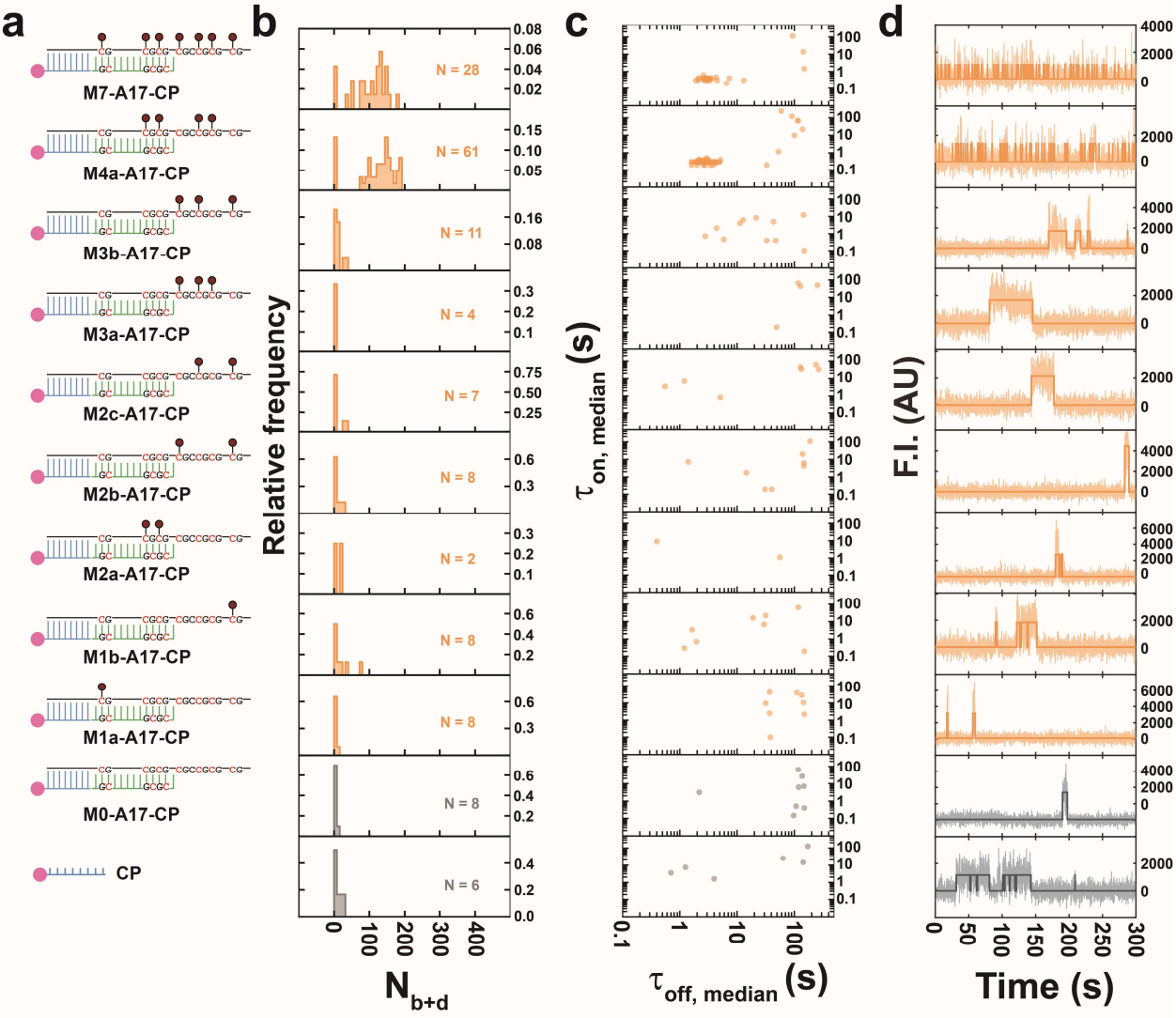
Supplementary Fig. 10 | Interplay between overhangs and secondary structure. a Constructs of M7-CPO, M4a-CPO, M3b-CPO, M3a-CPO, M2c-CPO, M2b-CPO, M2a-CPO, M1b-CPO and M1a-CPO, M0-CPO and CPO. **b** N_{b+d} distributions. N, the number of detected molecules of one field of view (FOV). **c** Median dwell time distributions. **d** Representative intensity-time traces. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



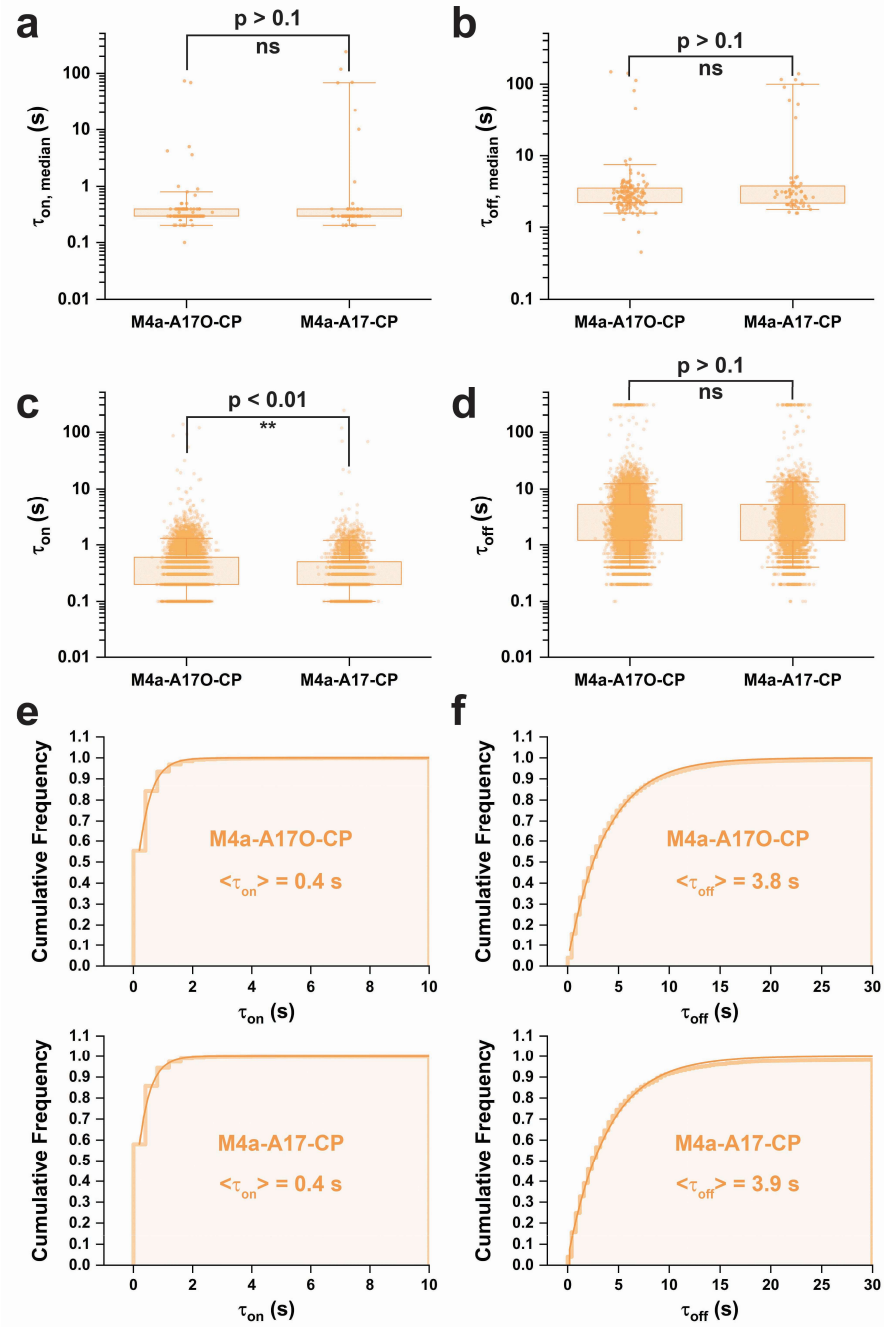
Supplementary Fig. 11 | Dwell time comparison and mean dwell time calculations of M7-CPO, M7-CP, M4a-CPO and M4a-CP. a,b Boxplots of $\tau_{on,median}$ and $\tau_{off,median}$ distributions respectively. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. For hypothesis testing, the datasets of M4a-CPO and M4a-CP are 96% winsorized to tolerate interference of outliers. P-values smaller than 0.05 are assessed using single-tailed unpaired t-test and P-values higher than 0.05 are assessed using two-tailed unpaired t-test. **c,d** Cumulative dwell time distributions and their exponential fittings of τ_{on} and τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Mean dwell times, $\langle\tau_{on}\rangle$ and $\langle\tau_{off}\rangle$, are calculated by fitting a single-exponential decay function.



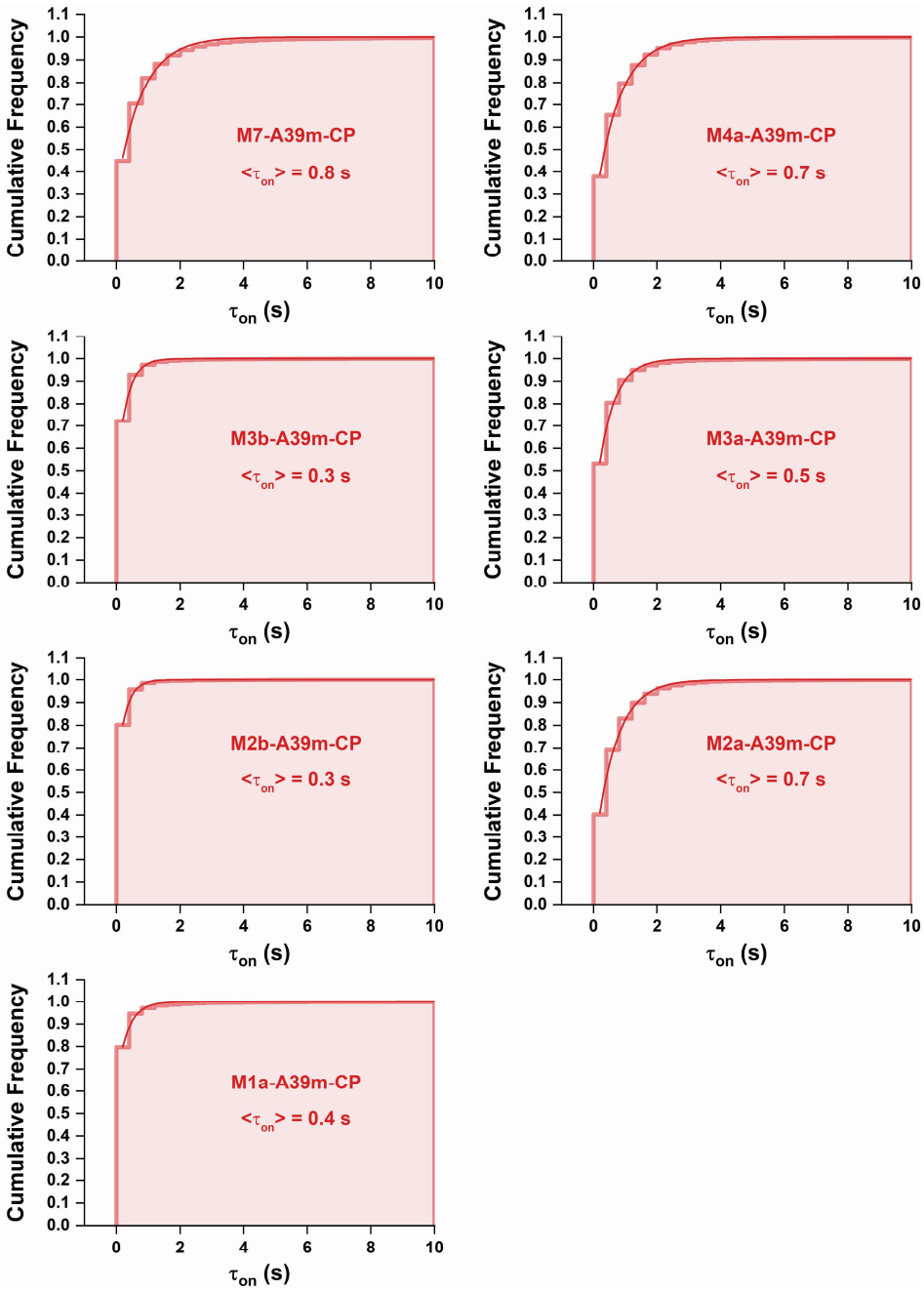
Supplementary Fig. 12 | Binding kinetics of bifurcating hemimethylated DNA modulated by clustering of methyl-CpG sites. a Constructs of M7-A17O-CP, M4a-A17O-CP, M3b--A17O-CP, M3a--A17O-CP, M2c--A17O-CP, M2b--A17O-CP, M2a--A17O-CP, M1b--A17O-CP and M1a-A17O-CP, M0-A17O-CP and CP. **b** N_{b+d} distributions. N, the number of detected molecules of one field of view (FOV). **c** Median dwell time distributions. **d** Representative intensity-time traces. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



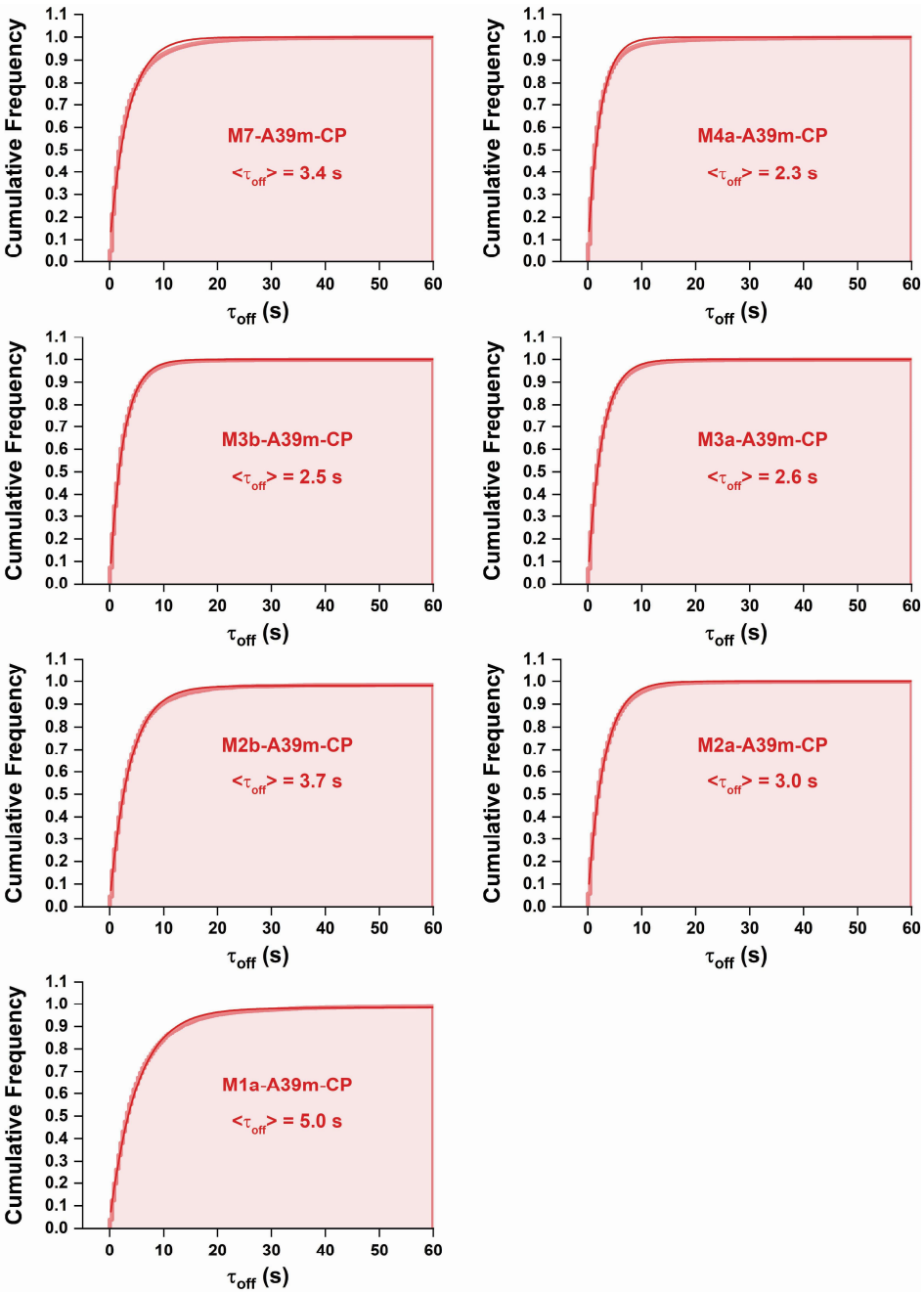
Supplementary Fig. 13 | Binding kinetics of overhang-free partially exposed hemimethylated DNA modulated by clustering of methyl-CpG sites. a Constructs of M7-A17-CP, M4a-A17-CP, M3b--A17-CP, M3a--A17-CP, M2c--A17-CP, M2b--A17-CP, M2a--A17-CP, M1b--A17-CP and M1a-A17-CP, M0-A17-CP and CP. **b** N_{b+d} distributions. N, the number of detected molecules of one field of view (FOV). **c** Median dwell time distributions. **d** Representative intensity-time traces. Semi-transparent lines in the background are raw traces and solid lines are idealized traces by hidden Markov modeling. F.I., fluorescence intensity; AU, arbitrary unit.



Supplementary Fig. 14 | Dwell time comparison and mean dwell time calculations of M4a-A17O-CP and M4a-A17-CP. a,b Boxplots of $\tau_{on,median}$ and $\tau_{off,median}$ distributions respectively. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. For hypothesis testing, all datasets are 96% winsorized to tolerate interference of outliers. P-values are assessed using two-tailed unpaired t-test. **c,d** Boxplots of τ_{on} and τ_{off} distributions respectively. Boxes are drawn from Q1 to Q3 with whiskers from 5% percentile to 95% percentile. P-values are assessed using Mann-Whitney U test. **e,f** Cumulative dwell time distributions and their exponential fittings of τ_{on} and τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Mean dwell times, $\langle\tau_{on}\rangle$ and $\langle\tau_{off}\rangle$, are calculated by fitting a single-exponential decay function.



Supplementary Fig. 15 | Mean bound time calculations of constructs in Fig. 4. Cumulative dwell time distributions and their exponential fittings of τ_{on} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Mean bound time, $\langle\tau_{on}\rangle$, is calculated by fitting a single-exponential decay function.



Supplementary Fig. 16 | Mean unbound time calculations of constructs in Fig. 4. Cumulative dwell time distributions and their exponential fittings of τ_{off} of individual events of detected molecules. Step horizontal lines are cumulative frequency counts and solid curves are fitting curves. Except for M2c-A39m-CP, M2b-A39m-CP and M1a-A39m-CP, mean unbound time, $\langle\tau_{off}\rangle$, is calculated by fitting a single-exponential decay function. For M2c-A39m-CP, M2b-A39m-CP and M1a-A39m-CP, mean unbound time, $\langle\tau_{off}\rangle$, is calculated by fitting a double-exponential decay function and taking only the primary component since the secondary component is marginal (< 5% compared to the primary component) and way too big (> 180 s).