Probing Multiple Binding Modes of DNA Hybridization: A Comparison between Single-molecule Observations and Ensemble Measurements

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Supporting Information

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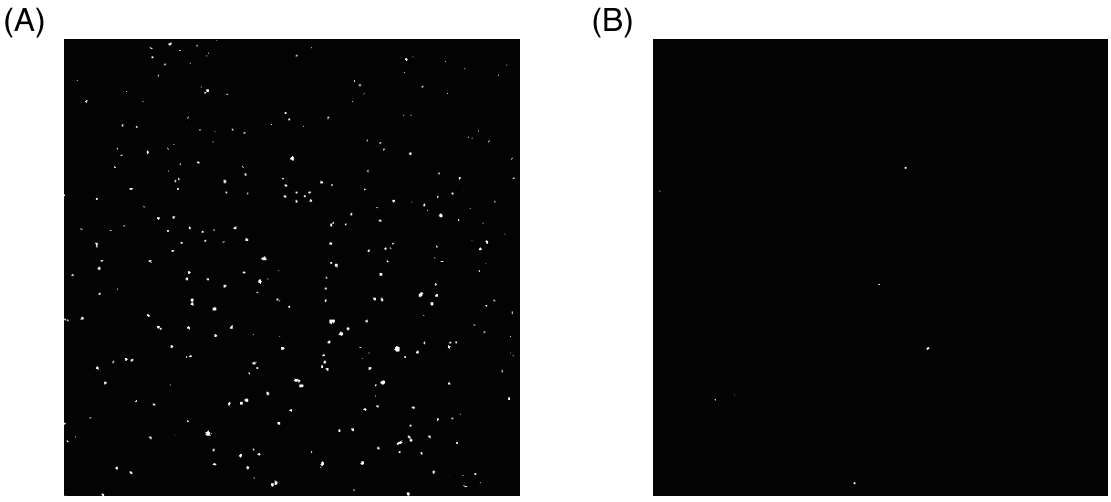


Figure S-1. CCD-camera images for DNA hybridization of Cy3-labeled DNAs (8 mer) with (A) the prove-DNA (8 mer) and (B) no prove-DNA on the glass plate of the reaction cell in single-molecule observation by TIRFM. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 °C.

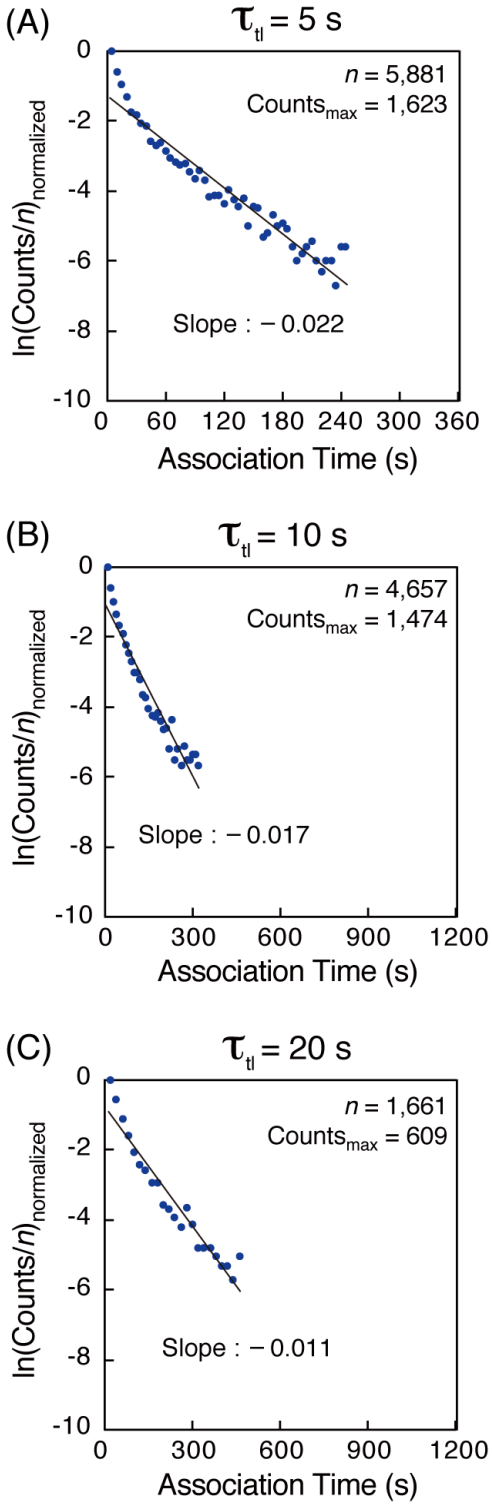


Figure S-2. Histogram analyses of association time for single-molecule hybridization of 8 mer-8 mer DNA based on time-lapse images obtained at an interval of (A) 5 s, (B) 10 s, and (C) 20 s. Each vertical axis was normalized with the maximum value of (Counts/*n*) of 1. Slope values were obtained according to eq. 6. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 ºC.

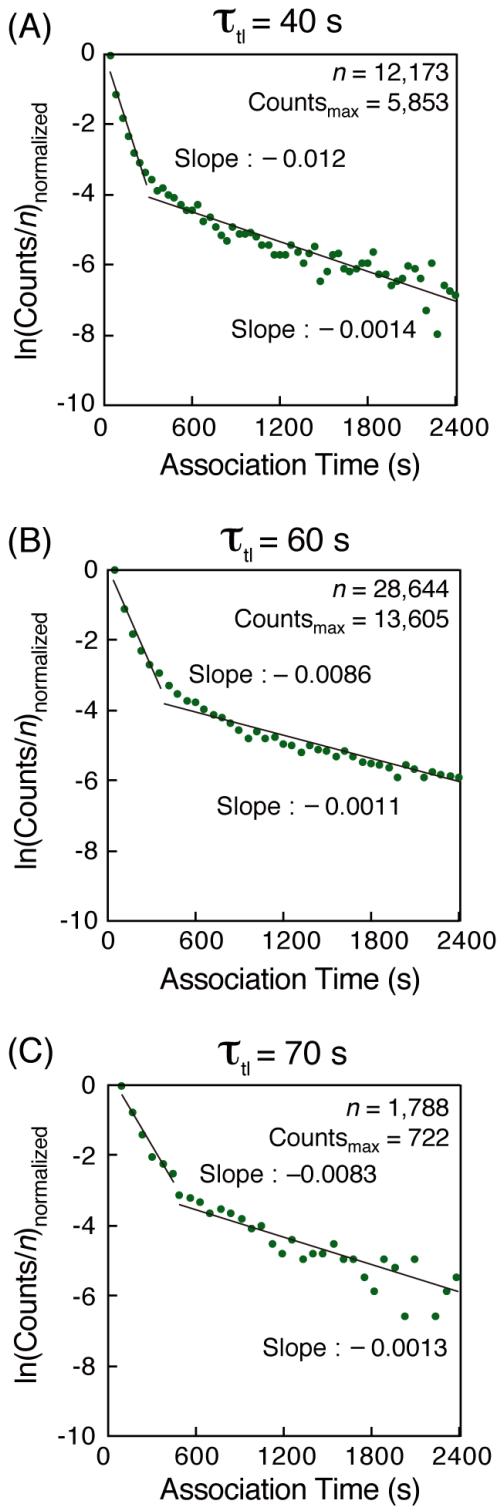


Figure S-3. Histogram analyses of association time for single-molecule hybridization of 12 mer-12 mer DNA based on time-lapse images obtained at an interval of (A) 40 s, (B) 60 s, and (C) 70 s. Each vertical axis was normalized with the maximum value of (Counts/*n*) of 1. Slope values were obtained according to eq. 6. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 ºC.

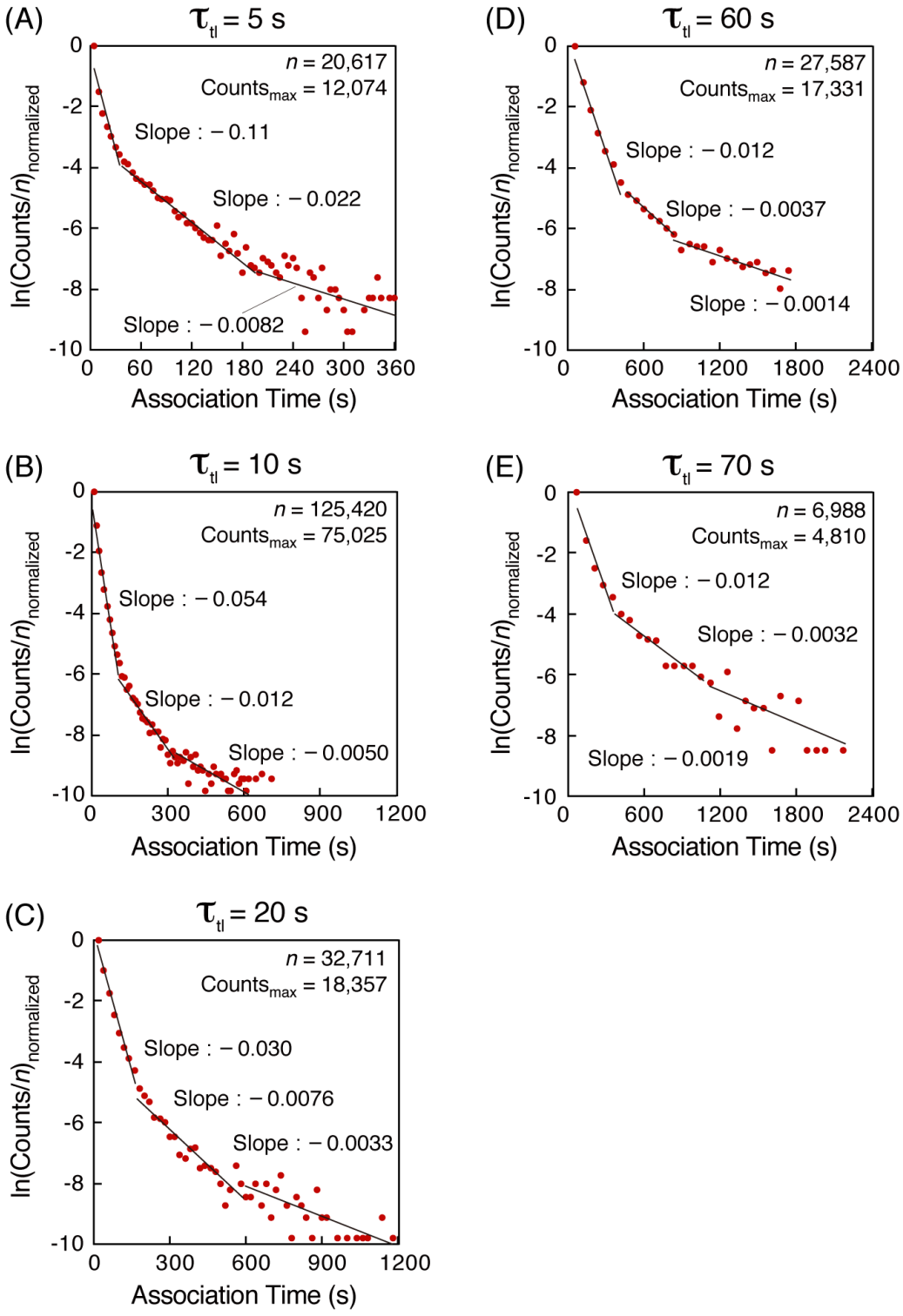
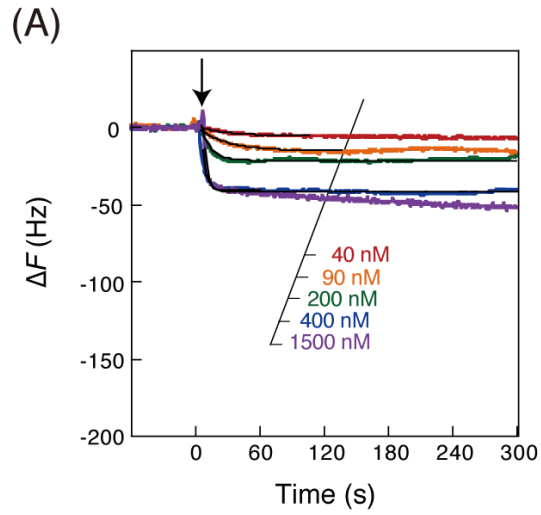
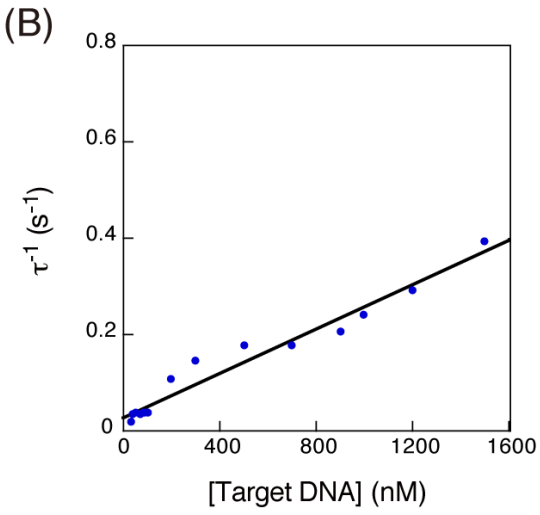
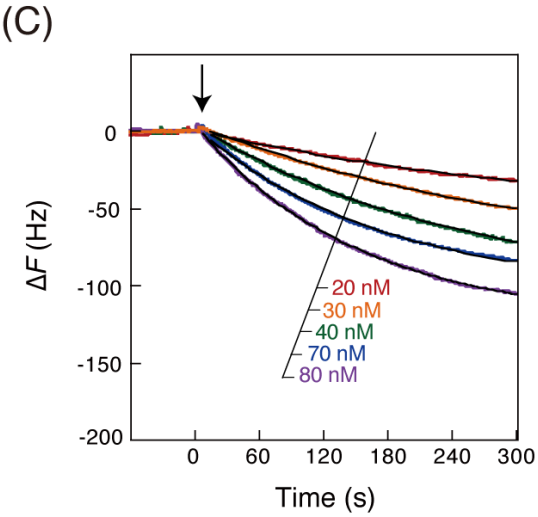
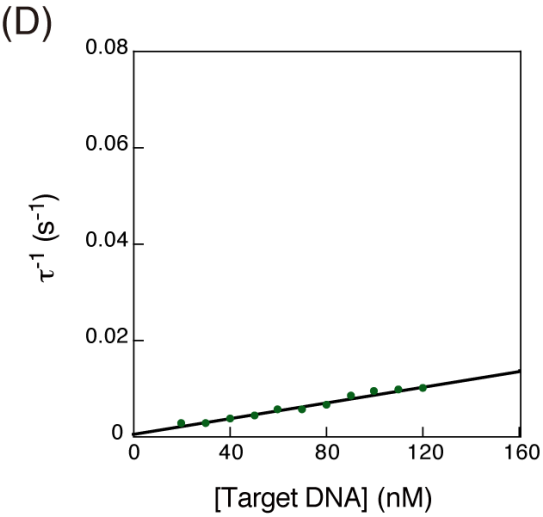


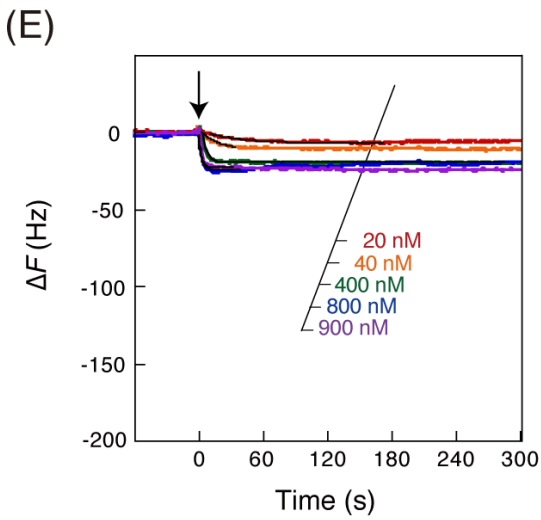
Figure S-4. Histogram analyses of association time for single-molecule hybridization of dA_{12}-dT_{12} DNA based on time-lapse images obtained at an interval of (A) 5 s, (B) 10 s, (C) 20 s, (D) 60 s, and (E) 70 s. Each vertical axis was normalized with the maximum value of (Counts/*n*) of 1. Slope values were obtained according to eq. 6. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 ºC.

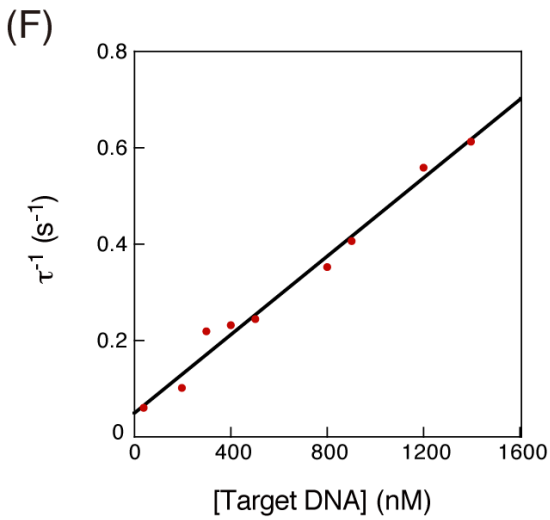

(A)
ΔF (Hz)
0
-50
-100
-150
-200
0
60
120
180
240
300
Time (s)
40 nM
90 nM
200 nM
400 nM
1500 nM


(B)
τ-1 (s-1)
0.8
0.6
0.4
0.2
0
0
400
800
1200
1600
[Target DNA] (nM)


(C)
ΔF (Hz)
0
-50
-100
-150
-200
0
60
120
180
240
300
Time (s)
20 nM
30 nM
40 nM
70 nM
80 nM


(D)
τ-1 (s-1)
0.08
0.06
0.04
0.02
0
0
40
80
120
160
[Target DNA] (nM)


(E)
ΔF (Hz)
0
-50
-100
-150
-200
0
60
120
180
240
300
Time (s)
20 nM
40 nM
400 nM
800 nM
900 nM


(F)
τ-1 (s-1)
0.8
0.6
0.4
0.2
0
0
400
800
1200
1600
[Target DNA] (nM)

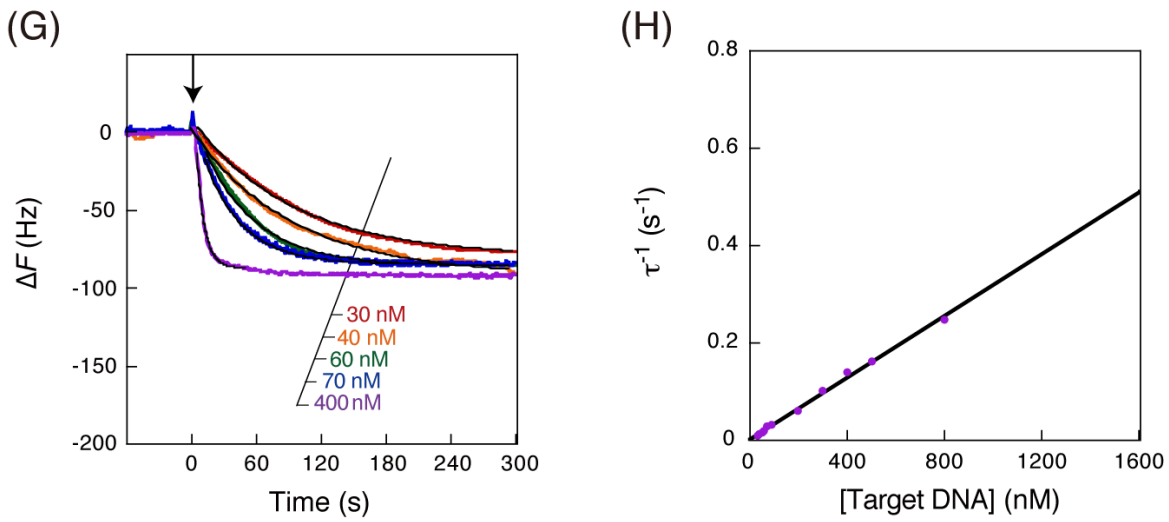


Figure S-5. Typical time courses of frequency of a QCM plate and kinetic analysis according to eq. 11 for observation of DNA hybridizations of (A, B) 8 mer-8 mer, (C, D) 12 mer-12 mer, (E, F) dA_{12}-dT_{12}, and (G, H) $d(ATG)_4$-$d(CAT)_4$. The fitting curves according to eqs. 9 and 10 were overwriting on each plot with a solid black line (A, C, E, and G). Experimental condition: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) at 20 ºC.

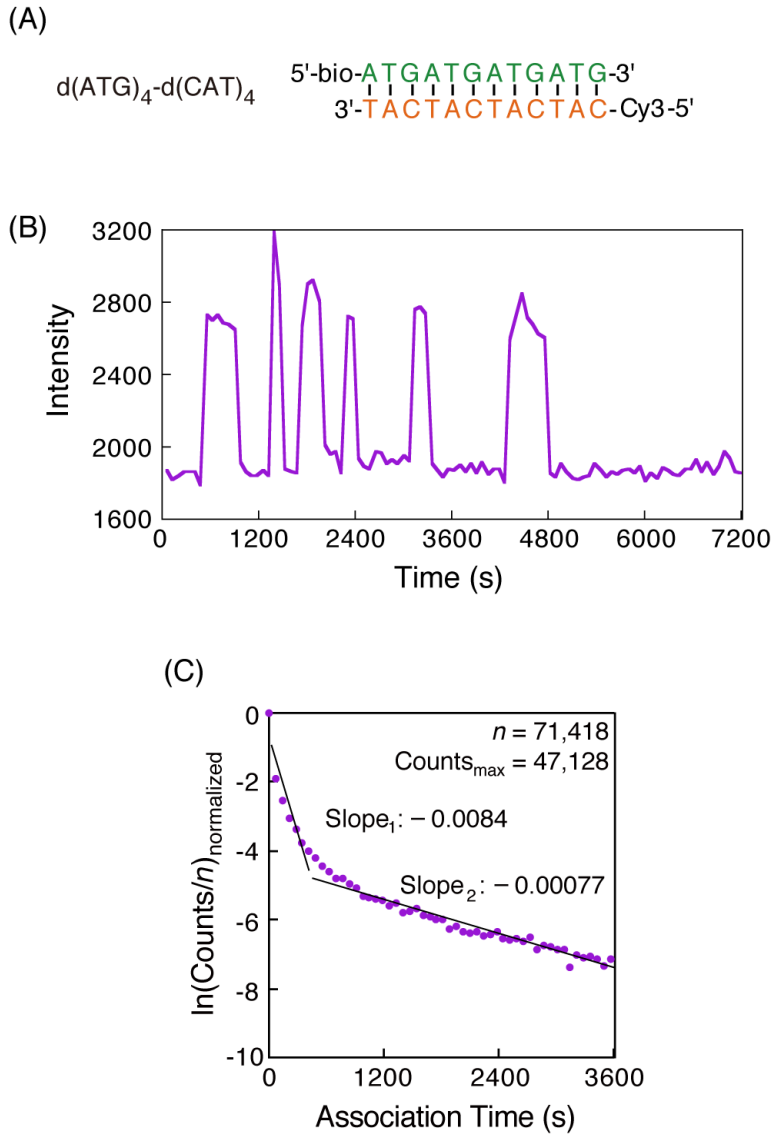


Figure S-6. (A) DNA sequence and modifications (bio-: biotinylated and -Cy3: Cy3-labeled) for block-like DNA hybridization. (B) Time course of fluorescent intensity of bright spots corresponding to the binding and dissociation of single-molecule hybridization in $d(ATG)_4$-$d(CAT)_4$ DNA. (C) Histogram analyses of association time for single-molecule hybridization of $d(ATG)_4$-$d(CAT)_4$ DNA. Time-lapse images were obtained at an interval of 70 s. The vertical axis was normalized with the maximum value of (Counts/*n*) of 1. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 °C.

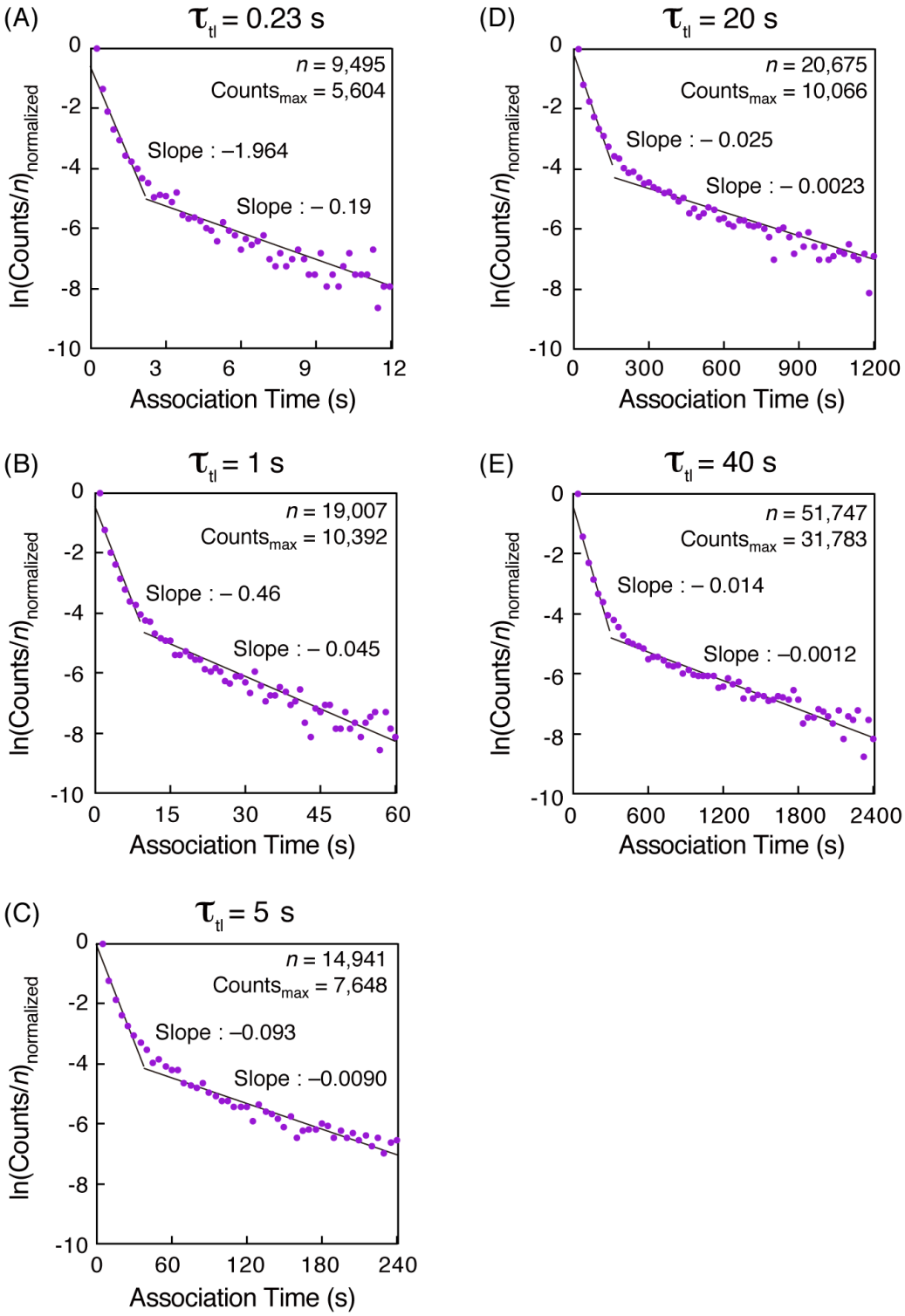


Figure S-7. Histogram analyses of association time for single-molecule hybridization of d(ATG)$_4$-d(CAT)$_4$ DNA based on time-lapse images obtained at an interval of (A) 0.23 s, (B) 1 s, (C) 5 s, (D) 20 s, and (E) 40 s. Each vertical axis was normalized with the maximum value of (Counts/*n*) of 1. Slope values were obtained according to eq. 6. Experimental conditions: 200 mM NaCl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) [Cy3-DNA] = 1 nM at 20 ºC.