Supporting Information for:

The role of cytosine methylation in regulating the topology and liquid-liquid phase separation of DNA G-quadruplexes

Mitsuki Tsuruta, Sumit Shil, Shinya Taniguchi, Keiko Kawauchi, Daisuke Miyoshi*

Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan.

* Correspondence and requests for materials should be addressed to Daisuke Miyoshi

(email: miyoshi@konan-u.ac.jp).

This file includes

Supplementary Table S1 – S3

Supplementary Figures S1 – S16

Table S1 Chromosome number, start sites, and gene symbol of $(GGGGCC)_4$

Chromosome number	Start site	Gene symbol*
chr1	**110607470**	**KCNA2**
chr1	**150579772**	**MCL1**
chr2	**168247289**	**STK39**
chr4	**2387058**	
chr4	**82561547**	
chr4	**186144723**	**FAM149A**
chr5	**177371074**	**RGS14**
chr6	**43021678**	**RRP36**
chr6	**109483543**	**ZBTB24**
chr7	**123748477**	**AC006333.2**
chr9	**129569211**	
chr9	**129666157**	
chr9	**131394270**	**PRRC2B**
chr9	**136327775**	**GPSM1**
chr10	**46337044**	**AGAP14P**
chr10	**46816336**	**AGAP13P**
chr11	**910938**	**CHID1**
chr11	**2884860**	**CDKN1C**
chr12	**51217720**	**POU6F1**
chr12	**54082575**	**AC023794.5**
chr13	**106917584**	
chr13	**113585151**	**TFDP1**
chr16	**1153342**	**CACNA1H**
chr16	**1614313**	**CRAMP1**
chr16	**49492476**	**ZNF423**
chr17	**38454151**	**AC244153.1**
chr19	**613554**	**HCN2**
chrX	**53625858**	**HUWE1**
chrX	**154341585**	

*Gene symbols are written when $(GGGGCC)_4$ sites are identified in the promoter region of genes.

Table S2. DNA duplex sequences used in this study

Abbreviation	Sequence (5'-3')*
Duplex-0Me	GGCTGCGGTC / GACCGCAGCC
Duplex-8Me	GG<u>C</u>TG<u>C</u>GGT<u>C</u> / GA<u>CC</u>G<u>C</u>AG<u>CC</u>

*<u>C</u> indicates the position of 5-methylcytosine.

Table S3. DNA sequences used in this study

Abbreviation	Sequence (5'-3')*
P1	TGAGGGTGGGTAGGGTGGGTAA
abasic P1	TGAGGGXGGGXXGGGXGGGTAA
Tetramer G4	TTGGGG

*X indicates the position of abasic site.

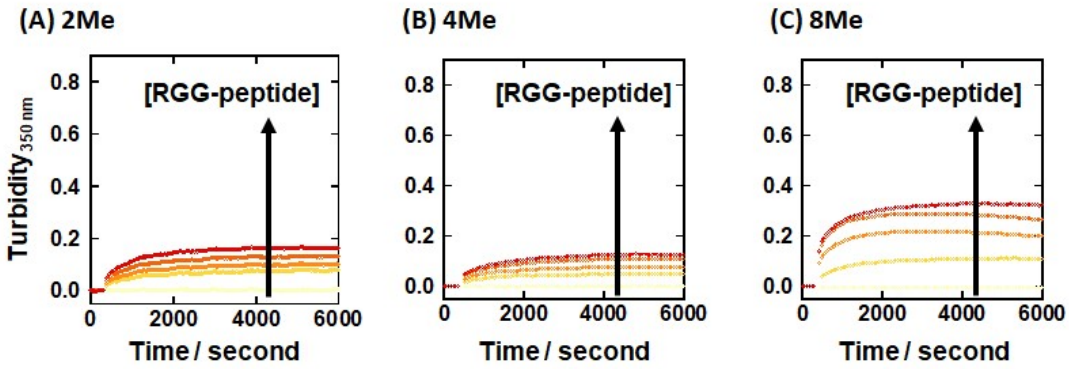


Figure S1. Turbidity changes at 350 nm of 5 μM DNAs [(A) 2Me, (B) 4Me, and (C) 8Me] with various concentrations (0, 1, 3, 5, 10, and 15 μM) of RGG-peptide in the KCl buffer at 25°C.

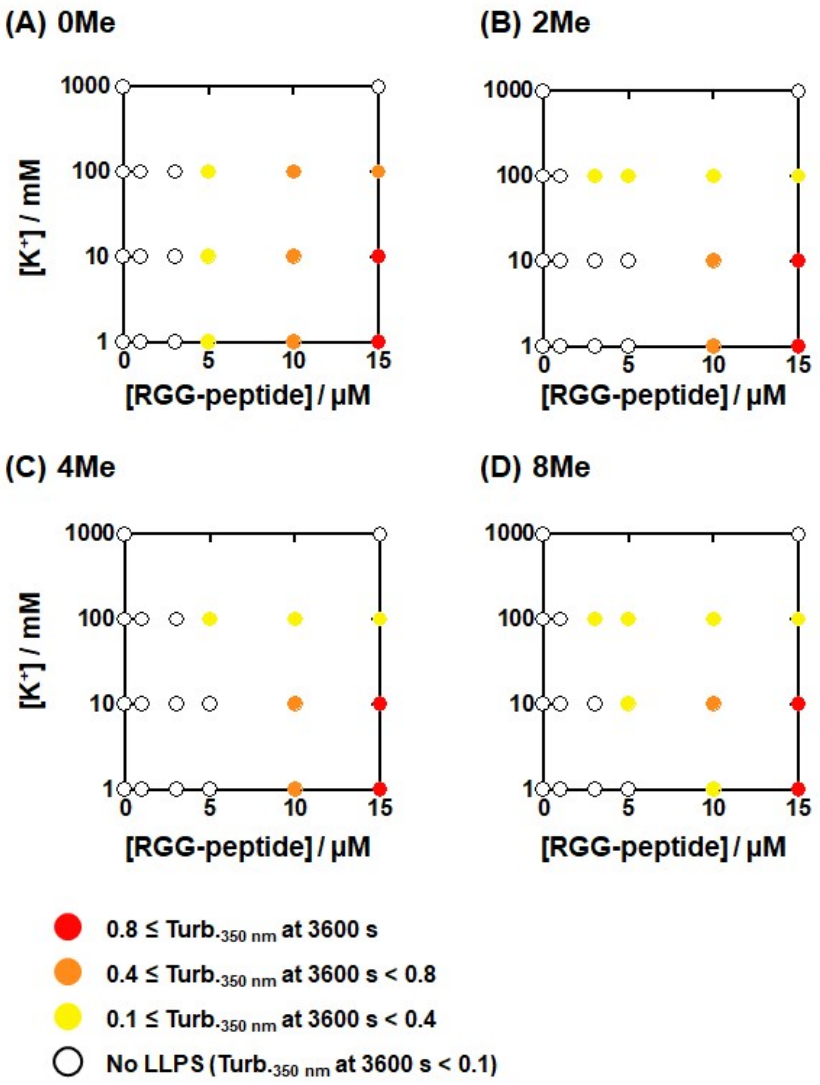


Figure S2. Phase diagram for mixtures of 5 μM DNAs and various RGG-peptide concentrations (0, 1,3, 5, 10, and 15 μM) in 10 mM K_2HPO_4 (pH 7.0) and 1 mM K_2EDTA with various concentrations (1, 10, 100, and 1000 mM) of KCl at 25 ˚C. The circle colors indicate the values of $turbidity_{350\ nm}$ at 3600 s [below 0.1 (white), 0.1 to 0.4 (yellow), 0.4 to 0.8 (orange), and above 0.8 (red)]

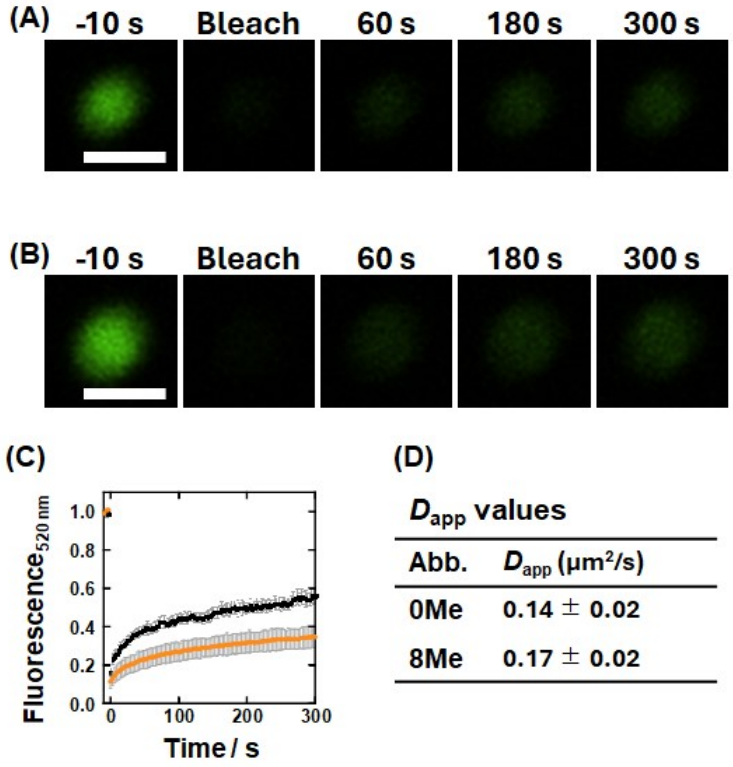


Figure S3. Confocal fluorescence microscopy images for droplets formed by 5 μM FAM-tagged 0Me (A) or 8Me (B) in the presence of 5 μM RGG-peptide at room temperature. Scale bar = 1 μm. Negative time indicates droplets before bleaching. (C) FRAP curves for a mixture of 5 μM DNAs [0Me (black) and 8Me (orange)] with 5 μM RGG-peptide. (D) The apparent diffusion coefficient (D_{app}) of respective DNAs within droplet evaluated by exponential fitting (N = 3).

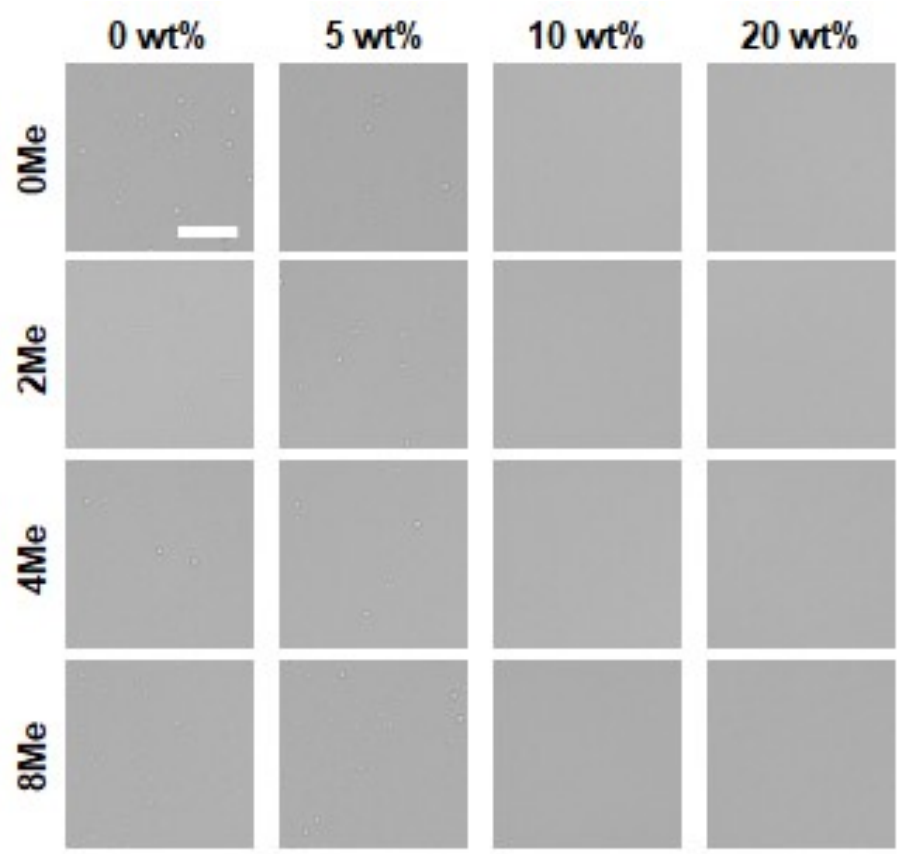


Figure S4. DIC images of droplets formed by 5 μM DNAs (0Me, 2Me, 4Me, and 8Me] with 5 μM RGG-peptide in the presence of various concentrations (0, 5, 10, and 20 wt%) of 1,6-hexanediol in the KCl buffer at room temperature. Scale bar = 10 μm

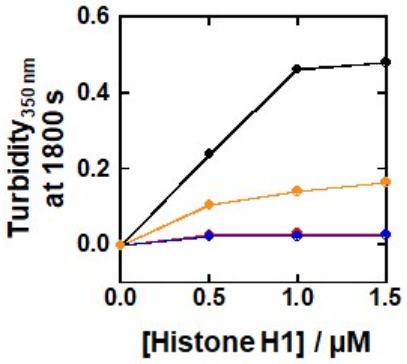


Figure S5. Turbidity$_{350\ nm}$ at 1800 s of 3.00 μM various DNAs (0Me; black, 2Me; red, 4Me; blue, 8Me; orange) with 0.00, 0.25, 0.50, 1.00, and 1.50 μM Histone H1 in the KCl buffer at 25 °C.

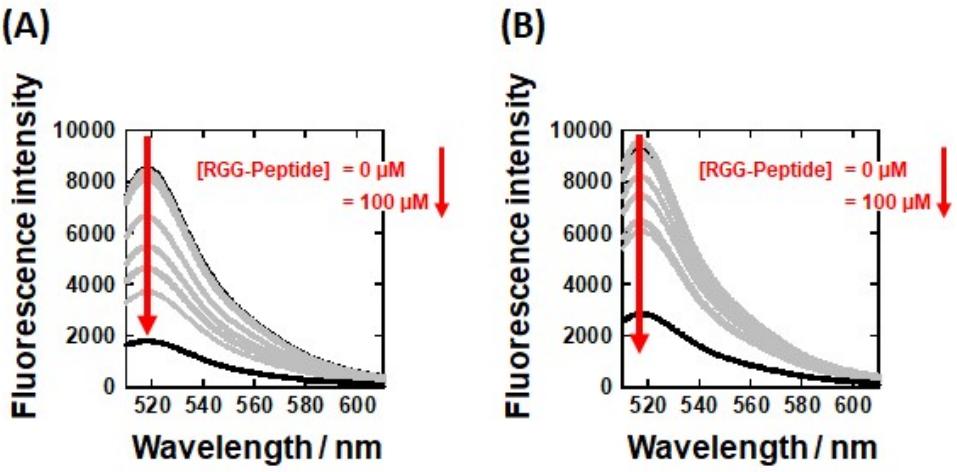


Figure S6. Fluorescence spectra of 50 nM FAM-tagged 0Me (A) and FAM-tagged 8Me (B) with various concentration (0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μM) of RGG-peptide in the KCl buffer at 25 °C.

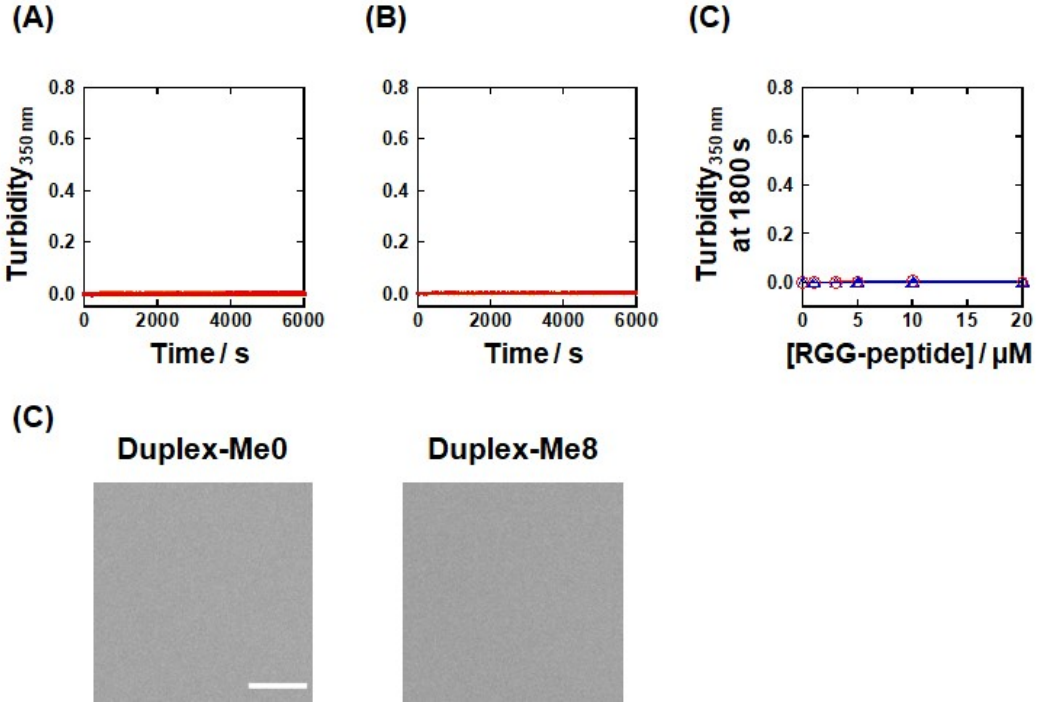


Figure S7. Turbidity changes at 350 nm for a mixture of 5 μM DNAs (Duplex-Me0: A, Duplex-Me8: B) and various concentrations of RGG-peptide (0, 1, 3, 5, 10, and 20 μM) in a buffer containing 100 mM KCl, 1 mM K_2EDTA, and 10 mM K_2HPO_4 (pH 7.0) at 25°C. (C) Turbidity at 1800 s for 5 μM DNAs (Duplex-Me0: red circle, Duplex-Me8: blue triangle) against various RGG-peptide concentrations (0, 1, 3, 5, 10, and 20 μM). (D) DIC images for the mixture of 20 μM Duplex-Me0 and Duplex-Me8 with 20 μM RGG-peptide in a buffer containing 100 mM KCl, 1 mM K_2EDTA, and 10 mM K_2HPO_4 (pH 7.0) at room temperature. Scale bar = 10 μm

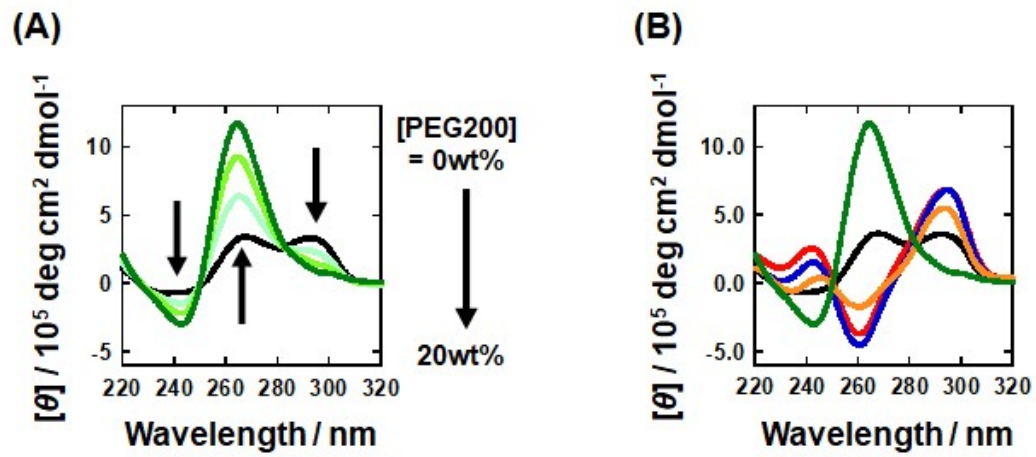


Figure S8. (A) CD spectra of 0Me in a buffer containing 100 mM KCl, 10 mM K_2HPO_4 (pH 7.0), 1 mM K_2EDTA in the presence of 0, 5, 10, and 20 wt% PEG200. (B) . CD spectra of 10 μM DNAs (0Me; black, 0Me in 20 wt% PEG200, green, 2Me; red, 4Me; blue, 8Me; orange) in a buffer containing 100 mM KCl, 1 mM K_2EDTA, and 10 mM K_2HPO_4 (pH 7.0) at 25˚C.

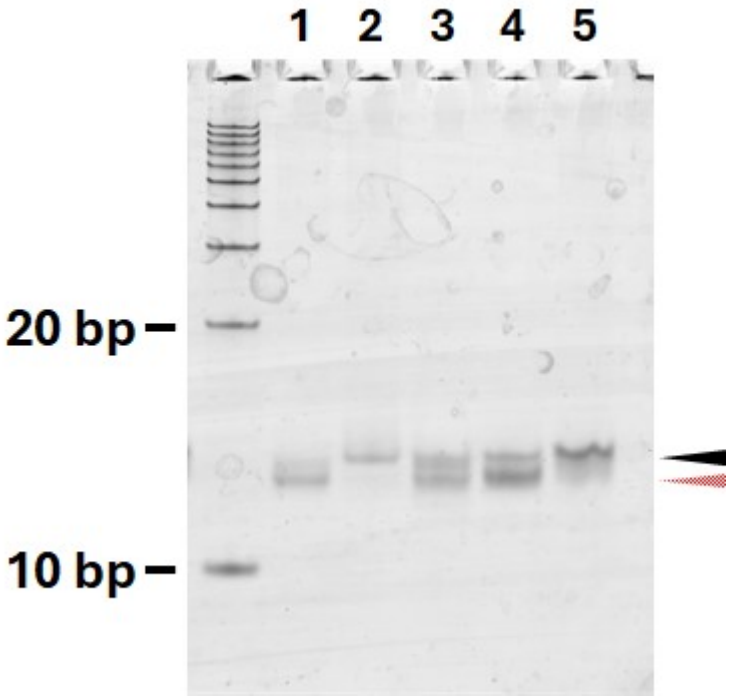


Figure S9. 18% native-PAGE of 0Me, 2Me, 4Me, and 8Me with 0 or 20 wt% PEG200 in a buffer containing 100 mM KCl, 10mM K_2HPO_4 (pH 7.0), and 1 mM K_2EDTA. 10 bp ladder was loaded in the leftmost lane. Black and red allow head indicate parallel and antiparallel topologies, respectively. Lane1: 0Me, Lane 2: 0Me with 20 wt% PEG200, Lane 3: 2Me, Lane 4: 4Me, Lane 5: 8Me

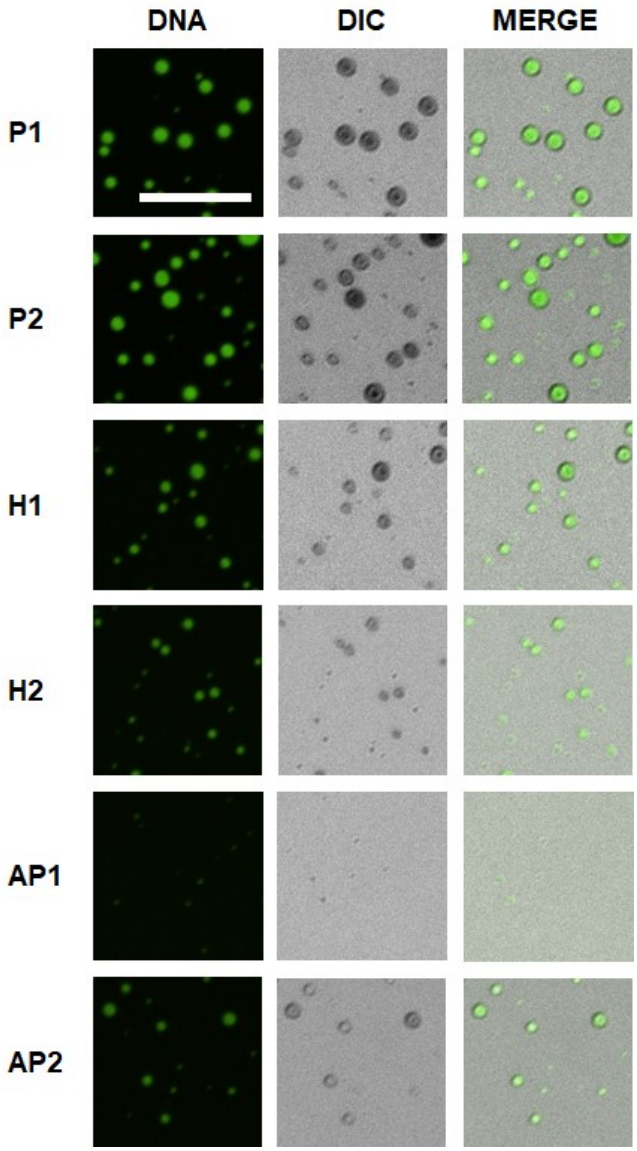


Figure S10. Confocal fluorescence/DIC microscopy images for droplets formed by 5 μM P1, P2, H1, H2, AP1, and AP2 in the presence of 5 μM FAM-tagged RGG-peptide in the KCl buffer at room temperature. Images were captured 30 min after mixing DNA and RGG-peptide. Scale bar = 10 μm.

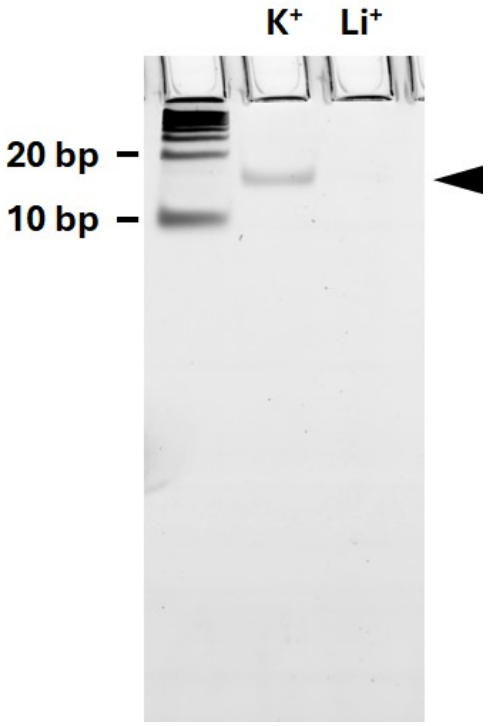


Figure S11. 20% native-PAGE of 40 μM tetramer G4 in the presence of 100 mM K^+ or Li^+. 10 bp ladder was loaded in the leftmost lane.

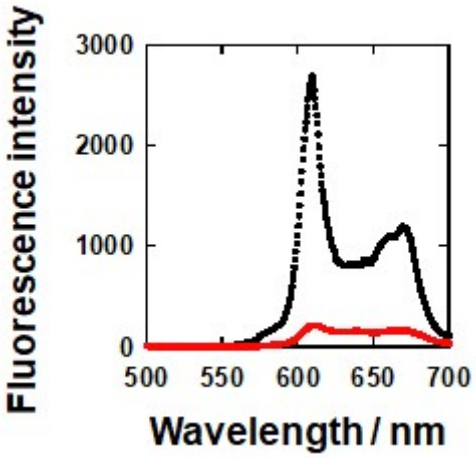


Figure S12. Fluorescence spectra of 40 μM tetramer G4 with 5 μM NMM in the presence of 100 mM KCl (black) or LiCl (red).

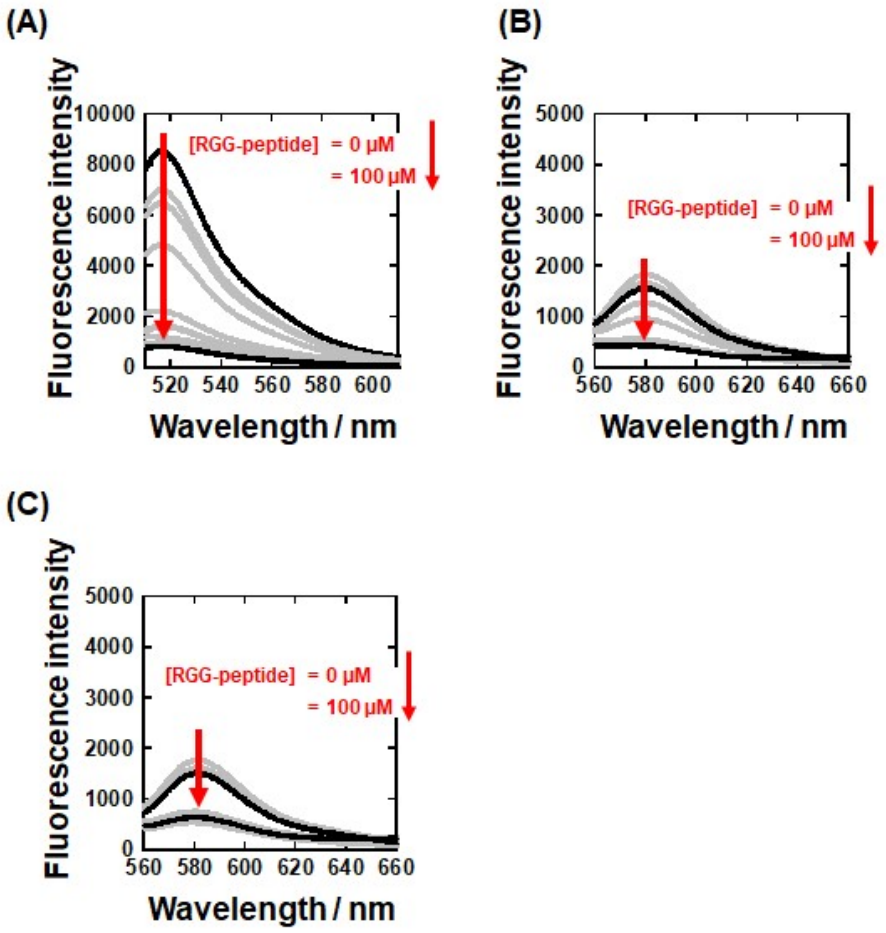
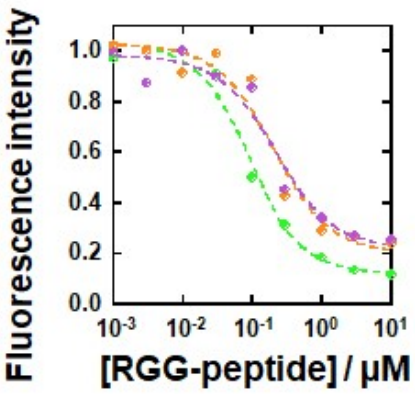


Figure S13. Fluorescence spectra of 50 nM FAM-tagged P1 (A), TAMRA-tagged H1 (B), or TAMRA-tagged AP1 (C) with various concentrations (0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μM) of RGG-peptide in the KCl buffer at 25 °C.

(A)



(B)

K_d values

Abb.	K_d (μM)
P1	0.07 ± 0.01
H1	0.20 ± 0.05
AP1	0.14 ± 0.03

Figure S14. (A) The Plots of relative fluorescence for 50 nM FAM-tagged P1 (green), TAMRA-tagged H1 (orange), or TAMRA-tagged AP1 (purple) as a function of RGG-peptide concentration. All measurements were carried out in KCl buffer at 25 °C. (B) The K_d values of P1, H1, and AP1 with RGG-peptide in the KCl buffer at 25 ˚C.

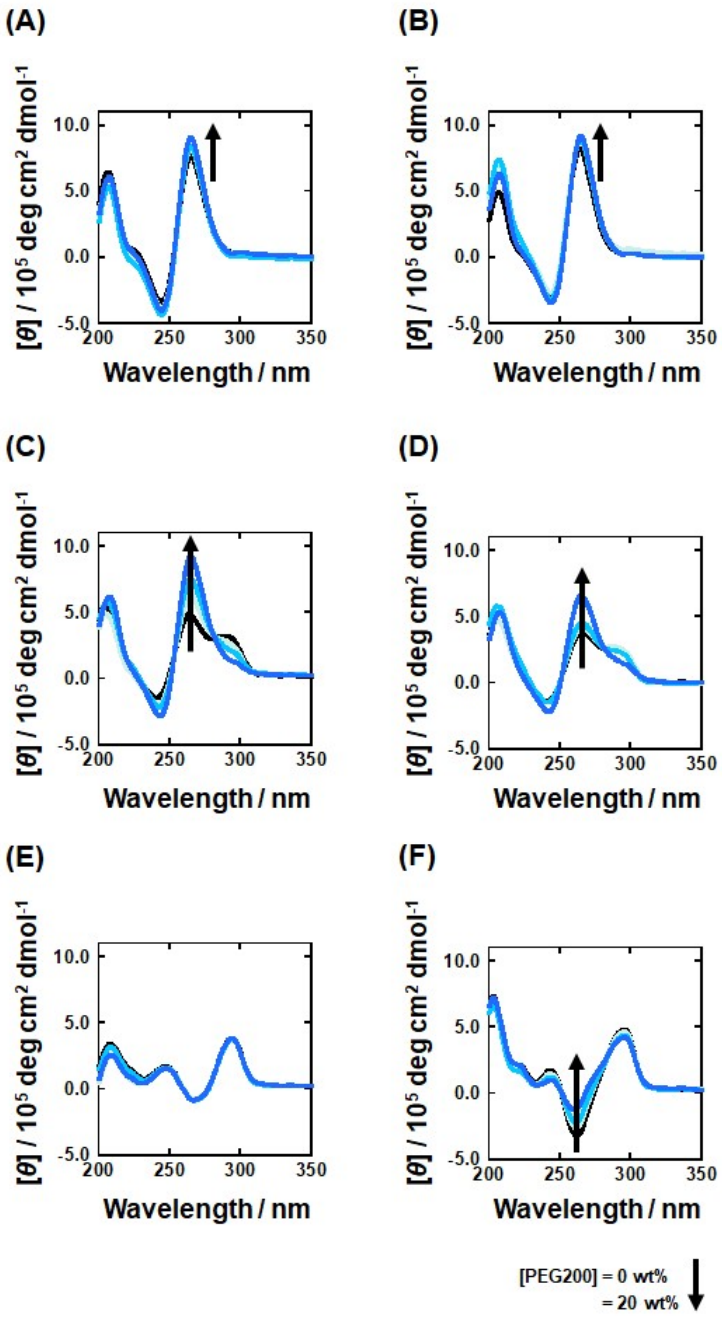


Figure S15. CD spectra for 10 μM various topology forming DNAs [(A) P1, (B) P2, (C) H1, (D) H2, (E) AP1, and (F) AP2] in the presence of various concentrations (0, 5, 10, and 20 wt%) of PEG200 in the KCl buffer at 25 °C.

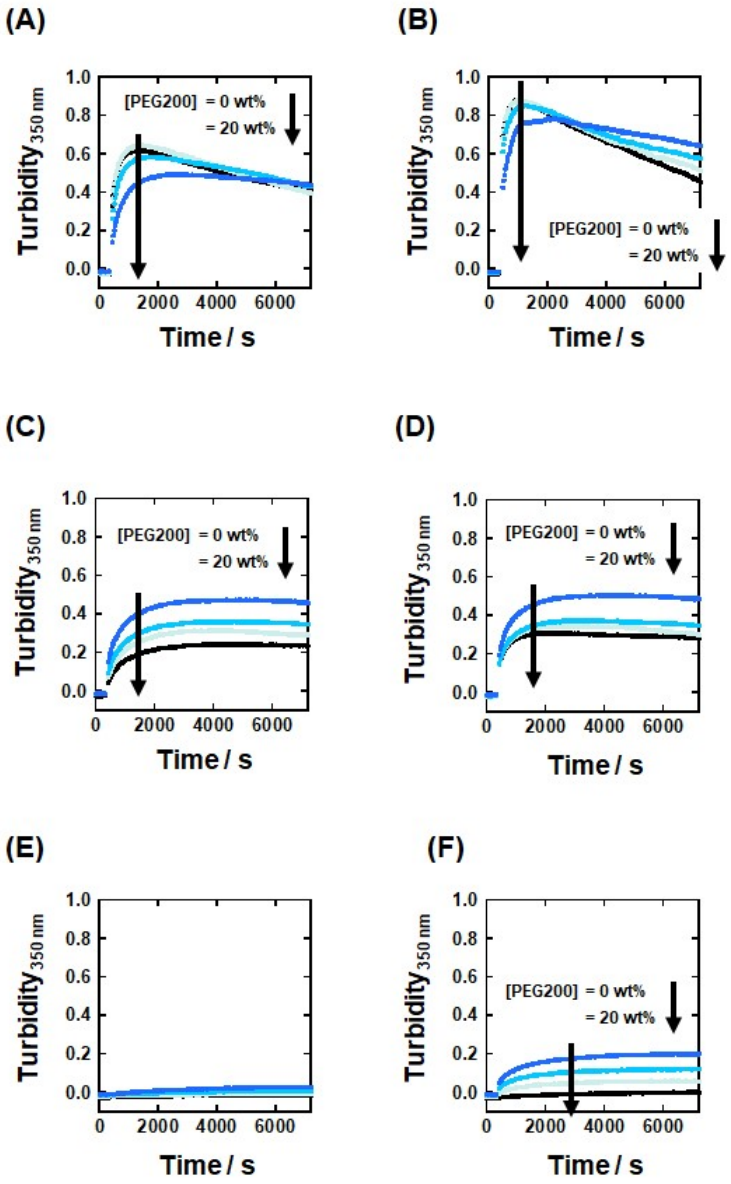


Figure S16. Turbidity for 5 μM various DNAs [(A) P1, (B) P2, (C) H1, (D) H2, (E) AP1, and (F) AP2] with 5 μM RGG-peptide in the presence of various concentrations (0, 5. 10, and 20 wt%) of PEG200 in the KCl buffer at 25 °C.