Supporting information

Structural and Functional Characterization of RecG Helicase Enzyme under Dilute and Molecular Crowding Conditions

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Supplementary materials

DNA sequences

A 62-mer DNA oligonucleotide was designed to form a frayed DNA duplex containing non-complementary arms and stem region. This arrangement of bases within the target duplex mimics a replication fork. Truncated sequence constituting only the stem region was also studied separately under similar conditions.

Table S1: Sequences of DNA Used in This Study [a]

abbreviation	Sequences
1. FD-1	5′-TGGGTGAACCTGCAGGTGGGCAAAGATGTCCTAGCAATGTAATCGTCAAGCTTTATGCCGTT-3′
2. FD-2	5′-CAACGGCATAAAGCTTGACGATTACATTGCTAGGACATGCTGTCTAGAGGATCCGACTATCGA−3′
3. Stem-1	5′-ATGTCCTAGCAATGTAATCGTCAAGCTTTATGCCGTT- 3′
4. Stem-2	5′-CAACGGCATAAAGCTTGACGATTACATTGCTAGGACAT-3′

Table S2. Melting temperature of 5 μM RecG evaluated by CD

Ionic conditions	ATP	Melting temperature (°C)	
		0 wt% PEG 200	40 wt% PEG 200
100 mM NaCl	–	45.0	47.0
100 mM NaCl	+	48.0	49.5
100 mM NaCl and 1 mM $MgCl_2$	–	45.5	46.5
100 mM NaCl and 1 mM $MgCl_2$	+	49.0	50.0
5 mM $MgCl_2$	–	47.0	49.5
5 mM $MgCl_2$	+	48.0	50.0

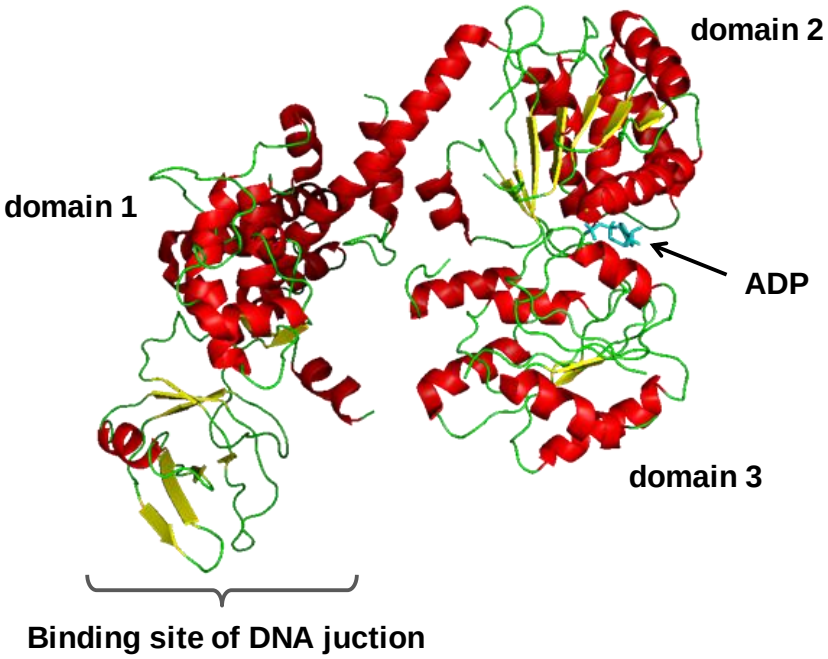

Figure S1. Crystal structure of *Thermotoga maritime* RecG (PDB ID: 1GM5).

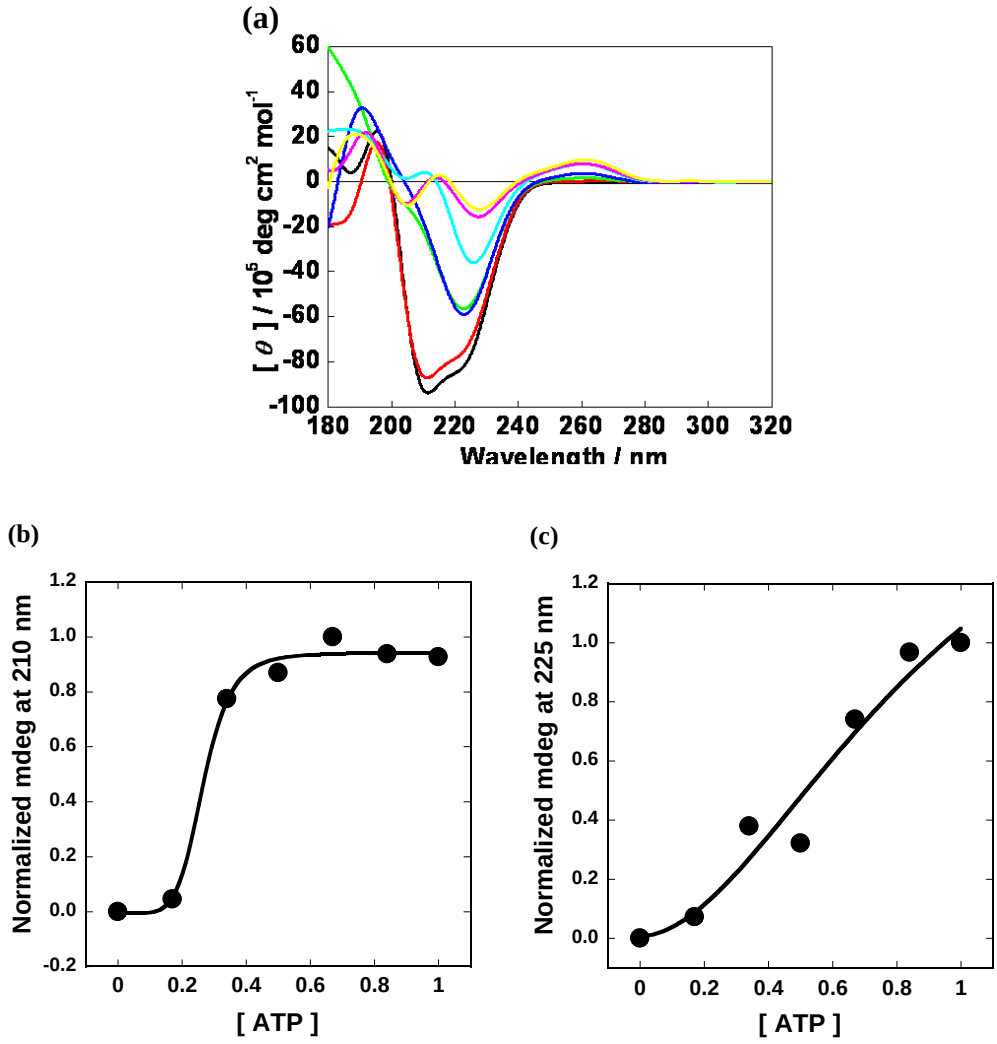

Figure S2. (a) CD spectra of 5 μM RecG. Measurements were carried out at 37 ^{o}C in 30 mM MES buffer (pH 7.0) containing 100 mM NaCl, 1 mM $MgCl_2$, and 0.5 mM Na_2EDTA at 0 wt% PEG 200 without ATP (black), with 0.17 mM ATP (red), 0.34 mM ATP (green), with 0.51 mM ATP (blue), 0.68 mM ATP (cyan), 0.85 mM ATP (pink), and with 1 mM ATP (yellow). (b) and (c) Plots of normalized molar ellipticities at (b) 210 nm and (c) 225 nm versus [ATP] at 0 mM, 0.17 mM, 0.34 mM, 0.51 mM, 0.68 mM, 0.85 mM, and 1 mM. Measurements were carried out at 37 ^{o}C in 30 mM MES buffer (pH 7.0) containing 100 mM NaCl, 1 mM $MgCl_2$, and 0.5 mM Na_2EDTA at 0 wt% PEG 200.

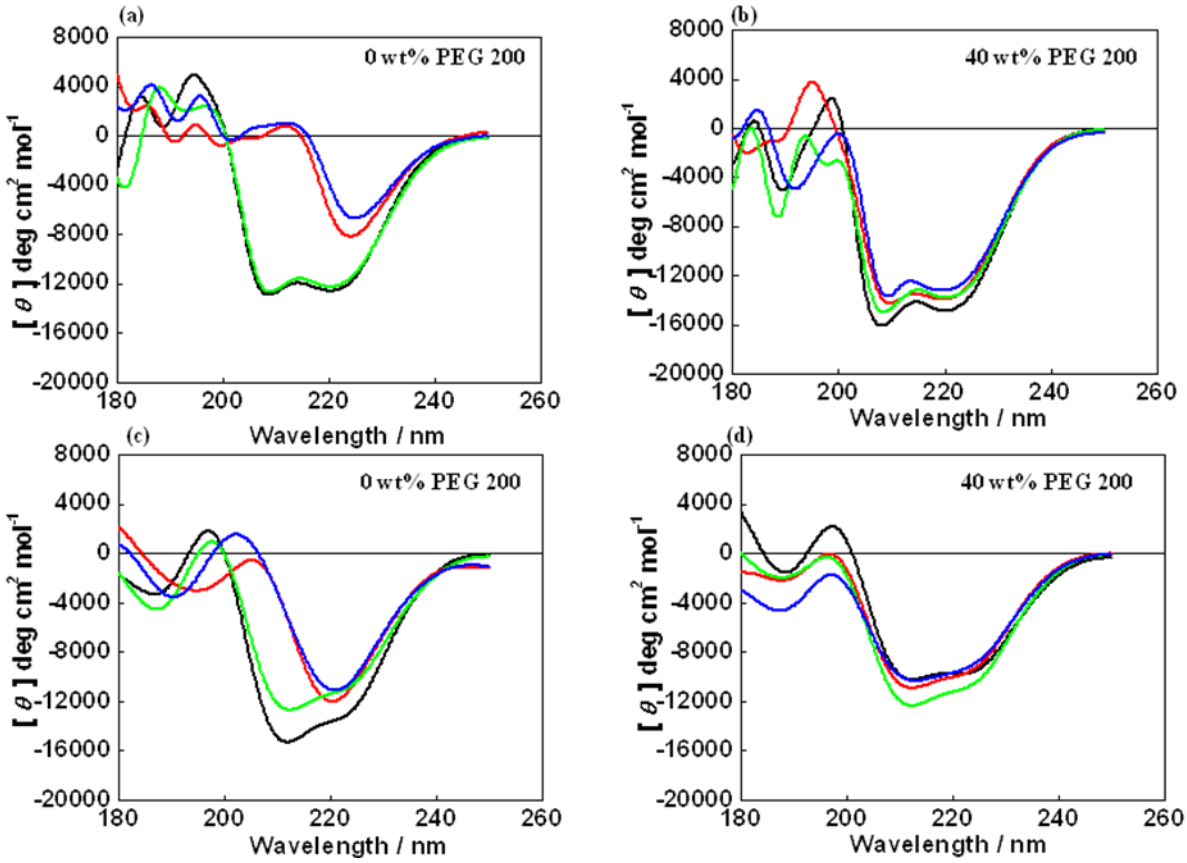

Figure S3. (a) CD spectra of 5 μM RecG. Measurements were carried out in 30 mM MES buffer (pH 7.0) containing (a) 100 mM NaCl, 1 mM $MgCl_2$, and 0.5 mM Na_2EDTA at 0 wt% PEG 200 without ATP at 4 ^oC (black) and at 37 ^oC (green) or with ATP at 4 ^oC (red) and 37 ^oC (blue). (b) Measurements were carried out in 100 mM NaCl, 1 mM $MgCl_2$, and 0.5 mM Na_2EDTA at 40 wt% PEG 200 without ATP at 4 ^oC (black) and 37 ^oC (green) or with ATP at 4 ^oC (red) and 37 ^oC (blue). (c) Measurements were carried out in 5 mM $MgCl_2$ and 0.5 mM Na_2EDTA at 0 wt% PEG 200 without ATP at 4^oC (black) and 37 ^oC (green) or with ATP at 4 ^oC (red) and 37 ^oC (blue). (d) Measurements were carried out in 5 mM $MgCl_2$ and 0.5 mM Na_2EDTA at 40 wt% PEG 200 without ATP at 4 ^oC (black) and 37 ^oC (green) or with ATP at 4 ^oC (red) and 37 ^oC (blue).

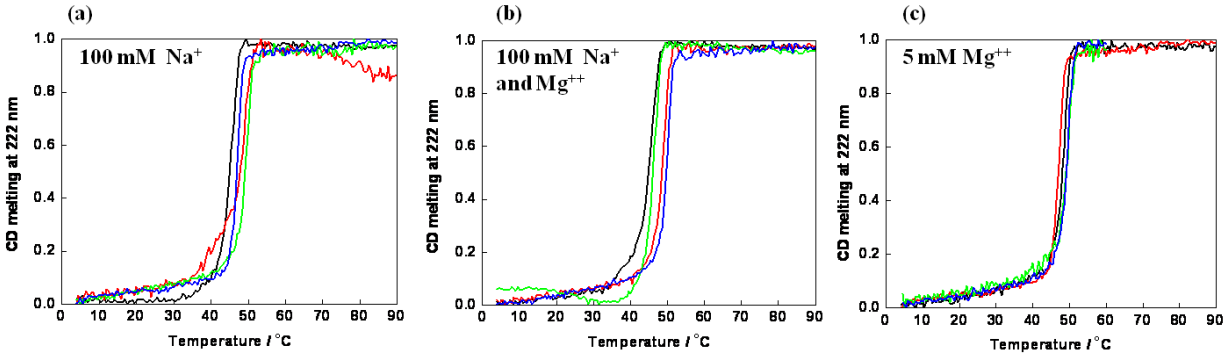

Figure S4. CD melting curves at 222 nm of 5 μM RecG. Measurements were carried out in 30 mM MES buffer (pH 7.0) and 0.5 mM Na_2EDTA at 0 wt% PEG 200 without ATP (black), in 0 wt% PEG 200 with 1 mM ATP (red), in 40 wt% PEG 200 without ATP (green), and in 40 wt% PEG 200 with 1 mM ATP (blue). Buffer contained (a) 100 mM NaCl, (b) 100 mM NaCl and 1 mM $MgCl_2$, and (c) 5 mM $MgCl_2$.

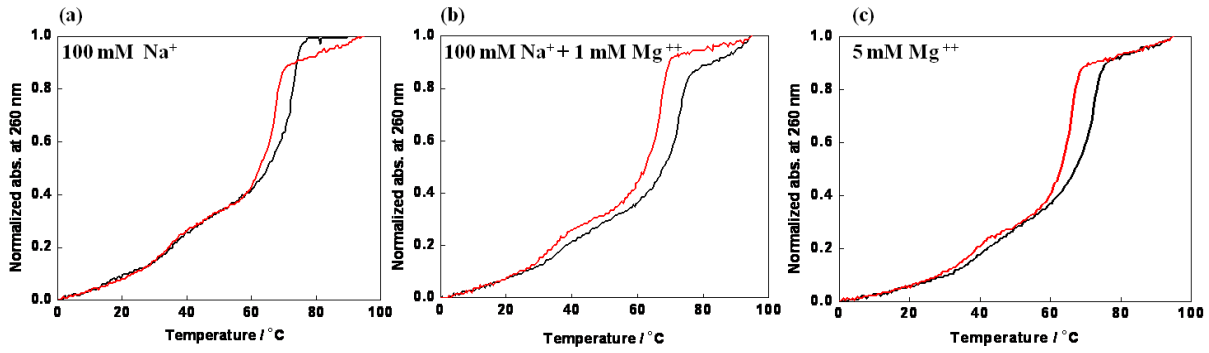

Figure S5. Normalized UV melting curves of 1 μM DNA frayed duplex. Measurements were carried out in 30 mM MES buffer (pH 7.0) at 0 wt% PEG 200 (black) and 40 wt% PEG 200 (red) containing (a) 100 mM NaCl, (b) 100 mM NaCl and 1 mM $MgCl_2$, or (c) 5 mM $MgCl_2$.

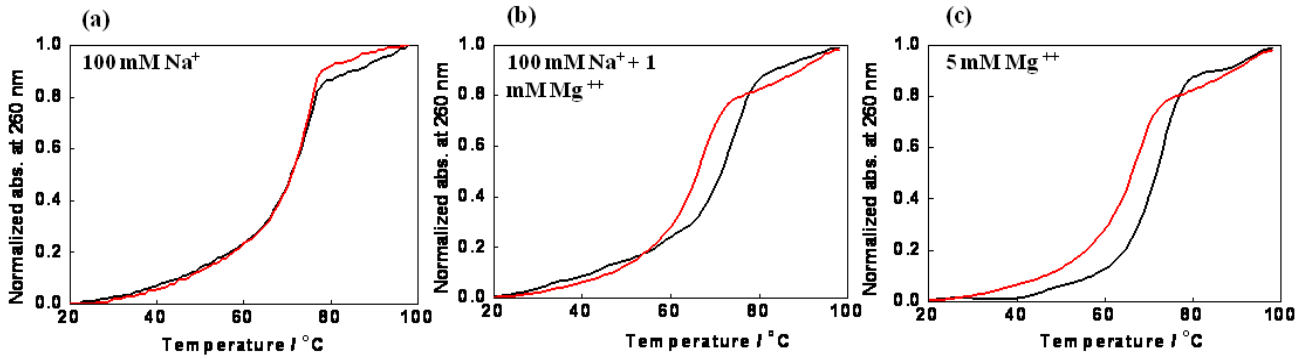

Figure S6. Normalized UV melting curves of 1 μM stem region of frayed duplex. Measurements were carried out in 30 mM MES buffer (pH 7.0) at 0 wt% PEG 200 (black) and 40 wt% PEG 200 (red) containing (a) 100 mM NaCl, (b) 100 mM NaCl and 1 mM $MgCl_2$, or (c) 5 mM $MgCl_2$.

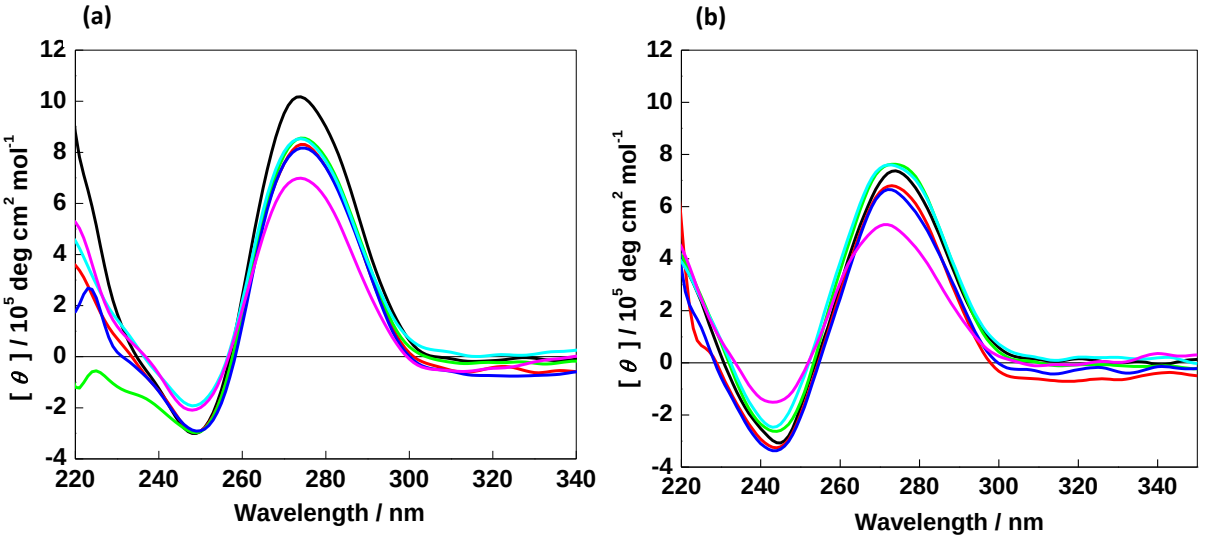

Figure S7. CD spectra of 1 μM FD-1 (a) and FD-2 (b). Measurements were carried out at 37 ^{o}C in 30 mM MES buffer (pH 7.0) containing 100 mM Na^{+}, 0.5 mM Na_2EDTA, at 0 wt% PEG 200 (black) or at 40 wt% PEG 200 (red), 100 mM Na^{+}, 1 mM Mg^{++} at 0 wt% PEG 200 (green), or at 40 wt% PEG 200 (blue), 5 mM Mg^{++} at 0 wt% PEG 200 (cyan), or 5 mM Mg^{++} at 0 wt% PEG 200 (pink).

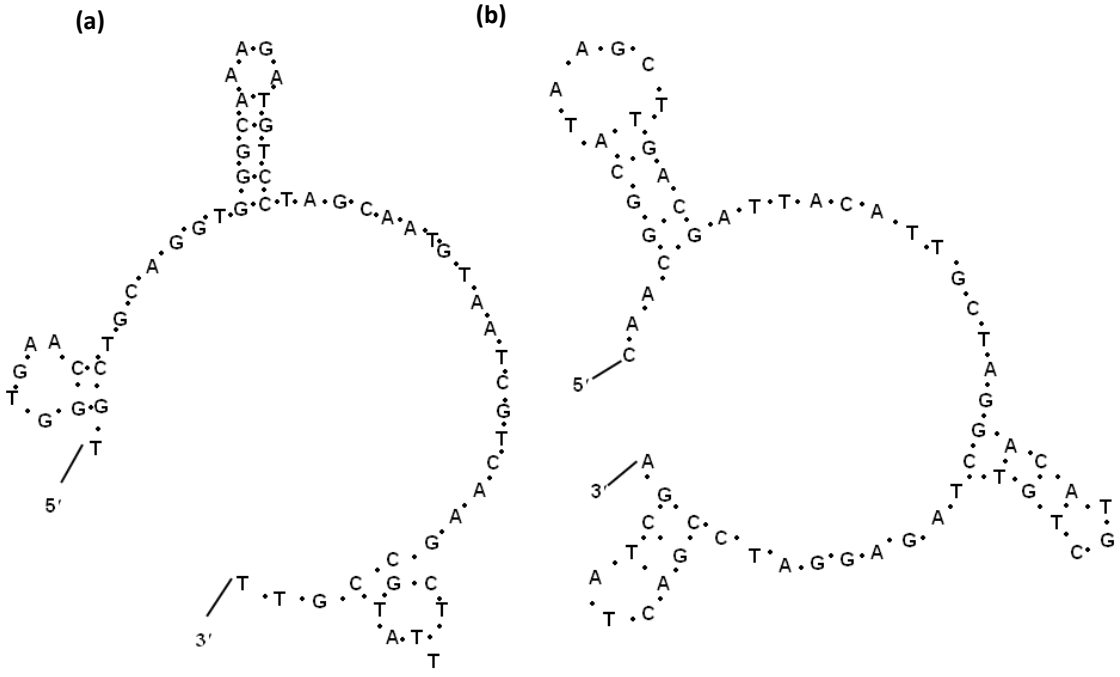

Figure S8. Schematic representation using M fold of predicted monomers of FD-1 (a) and FD-2 (b).

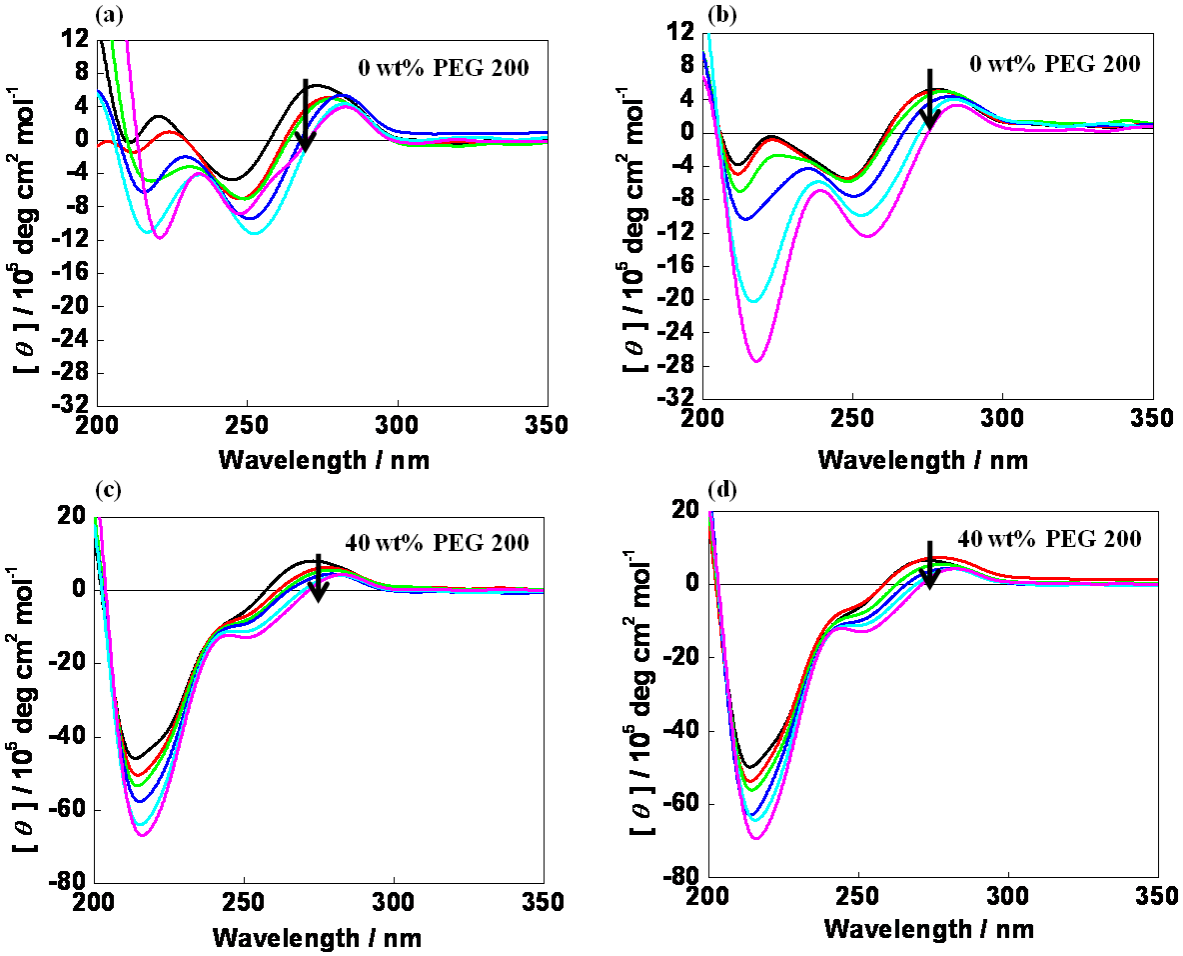

Figure S9. CD spectra of 1 μM frayed duplex. Measurements were carried out at 37 ^{o}C in 30 mM MES buffer (pH 7.0), 0.5 mM Na_2EDTA, titrated with 0 nM RecG and 0 mM ATP (black), 50 nM RecG and 0.1 mM ATP (red), 100 nM RecG and 0.2 mM ATP (green), 150 nM RecG and 0.3 mM ATP (blue), 200 nM RecG and 0.4 mM ATP (cyan), and 250 nM RecG and 0.5 mM ATP (pink), containing (a) 100 mM NaCl and 1 mM $MgCl_2$ at 0 wt% PEG 200, (b) 100 mM NaCl and 1 mM $MgCl_2$ at 40 wt% PEG 200, (c) 5 mM $MgCl_2$ at 0 wt% PEG 200, and (d) 5 mM $MgCl_2$ at 40 wt% PEG 200.