Supplementary Material

Stabilization of Three-Way Junctions of DNA under Molecular Crowding Conditions

Sanjukta Muhuri,[†] Kenta Mimura,[‡] Daisuke Miyoshi,[†,‡] and Naoki Sugimoto[†,‡,*]

FIBER (Frontier Institute for Biomolecular Engineering Research) and FIRST (Faculty of Frontiers of Innovative Research in Science and Technology), Konan University, 7-1-20 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

† FIBER (Frontier Institute for Biomolecular Engineering Research)

‡ FIRST (Faculty of Frontiers of Innovative Research in Science and Technology)

*To whom correspondence should be addressed.

Tel: +81-78-303-1416; Fax: +81-78-303-1495; E-mail: sugimoto@konan-u.ac.jp

Table S1: Thermodynamic parameters and water activity for the formation of TWJ with different concentrations of PEG 200[a]

Ionic condition	DNA	PEG 200 (wt%)	T_m (^oC)	ΔG^o_{37} (kcal/mol)	ΔH^o (kcal/mol)	ΔS^o (cal/mol/K)	ln a_w
	J1	0	60.8±1.3	-3.7±0.2	-52.3±1.8	-156.7±5.6	-0.00319
Na^+		10	57.6±0.7	-3.8±0.2	-60.5±1.2	-183.1±3.3	-0.01756
		20	55.6±0.4	-3.1±0.1	-54.3±2.6	-165.2±7.9	-0.03946
		30	53.2±0.2	-2.7±0.1	-55.2±1.5	-169.1±4.8	-0.06081
		40	49.6±0.5	-1.9±0.1	-48.0±1.8	-148.8±5.6	-0.08936
	J3	0	64.1±0.4	-2.5±0.1	-31.3±0.5	-92.9±1.3	-0.00319
		10	60.5±0.8	-2.3±0.1	-33.1±1.3	-99.3±4.0	-0.01756
		20	55.2±0.8	-1.9±0.1	-33.9±1.2	-102.9±3.8	-0.03946
		30	52.8±0.5	-1.6±0.1	-34.1±1.5	-104.6±4.5	-0.06081
		40	48.4±0.5	-1.2±0.1	-33.8±0.8	-105.0±2.9	-0.08936
	J1	0	69.6±0.8	-5.6±0.3	-58.7±2.6	-171.1±7.4	-0.00321
Na^++Mg^{2+}		10	65.8±0.3	-5.0±0.3	-58.6±2.8	-173.0±8.2	-0.01730
		20	61.7±0.6	-4.1±0.1	-55.1±1.8	-164.6±5.7	-0.03698
		30	58.1±1.5	-3.3±0.2	-51.9 ±2.6	-156.9±8.0	-0.06233
		40	53.4±1.6	-2.3±0.2	-46.5±0.7	-142.3±2.6	-0.08051
	J3	0	67.4±0.4	-4.0±0.2	-45.4±2.4	-133.3±7.1	-0.00321
		10	64.1±0.6	-3.9±0.2	-48.2±1.0	-142.8±2.5	-0.01730
		20	59.8±0.2	-3.1±0.1	-45.6±0.3	-136.9±1.0	-0.03698
		30	56.7±0.8	-2.5±0.1	-42.7±1.3	-129.4±4.3	-0.06233
		40	51.9±1.5	-1.6±0.1	-36.0±2.3	-110.2±8.2	-0.08051

[a] All the experiments were carried out in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing 50 mM NaCl, and 0 or 5 mM $MgCl_2$ with different concentrations of PEG 200. All the parameters listed in the above table are the average values obtained from the curve-fitting analyses of the melting curves with different concentrations of DNA ranging from 1 to 30 μM.

Table S2: Number of water molecules taken up ($-\Delta n_w$) during the formation of TWJs and the each helical duplex arms[a]

Ionic conditions	**J1**[b]	**J1-A**[c]	**J1-B**[c]	**J1-C**[b]	**J1-C'**[c]	**J1-AB**[b]	$\Delta\Delta n_w$[d]	$\Delta\Delta n_w$[e]	$\Delta\Delta n_w$[f]
Na^+	38.3±4.1	28.7±2.1	18.3±2.8	43.7±2.1	36.3±1.6	48.0±3.4	-53.4	-52.4	-45.0
Na^+ and Mg^{2+}	63.2±2.8	38.4±nd	35.5±4.4	52.5±4.3	47.1±2.0	66.9±3.4	-56.2	-63.2	-57.8
	J3[b]	**J3-A**[c]	**J3-B**[b]	**J3-C**[c]	**J3-AC**[b]		$\Delta\Delta n_w$[g]	$\Delta\Delta n_w$[h]	
Na^+	25.0±1.8	26.5±3.2	36.3±3.0	32.1±2.5	38.5±4.1		-49.8	-69.9	
Na^+ and Mg^{2+}	48.5±3.5	31.1±5.9	48.4±1.8	37.6±2.7	78.2±1.8		-78.1	-68.6	

[a] All the experiments were carried out in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing 50 mM NaCl, and 0 or 5 mM $MgCl_2$ with different concentrations of PEG 200 from 0 to 40 wt%. [b] The error values were calculated from the average parameters obtained with three different concentrations of DNA. [C] The error values were obtained from linear fitting errors. [d] $\Delta\Delta n_w$ =[($-\Delta n_w$ for the whole TWJ structure J1) – ($-\Delta n_w$ for all the individual duplex arms J1-C and J1-AB)]. [e] $\Delta\Delta n_w$ =[($-\Delta n_w$ for the whole TWJ structure J1) – ($-\Delta n_w$ for all the individual duplex arms J1-A, J1-B and J1-C)]. [f] $\Delta\Delta n_w$ =[($-\Delta n_w$ for the whole TWJ structure J1) – ($-\Delta n_w$ for all the individual duplex arms J1-A, J1-B and J1-C')]. [g] $\Delta\Delta n_w$ =[($-\Delta n_w$ for the whole TWJ structure J3) – ($-\Delta n_w$ for all the individual duplex arms J3-B and J3-AC)]. [h] $\Delta\Delta n_w$ =[($-\Delta n_w$ for the whole TWJ structure J3) – ($-\Delta n_w$ for all the individual duplex arms J3-A, J3-B and J3-C)].

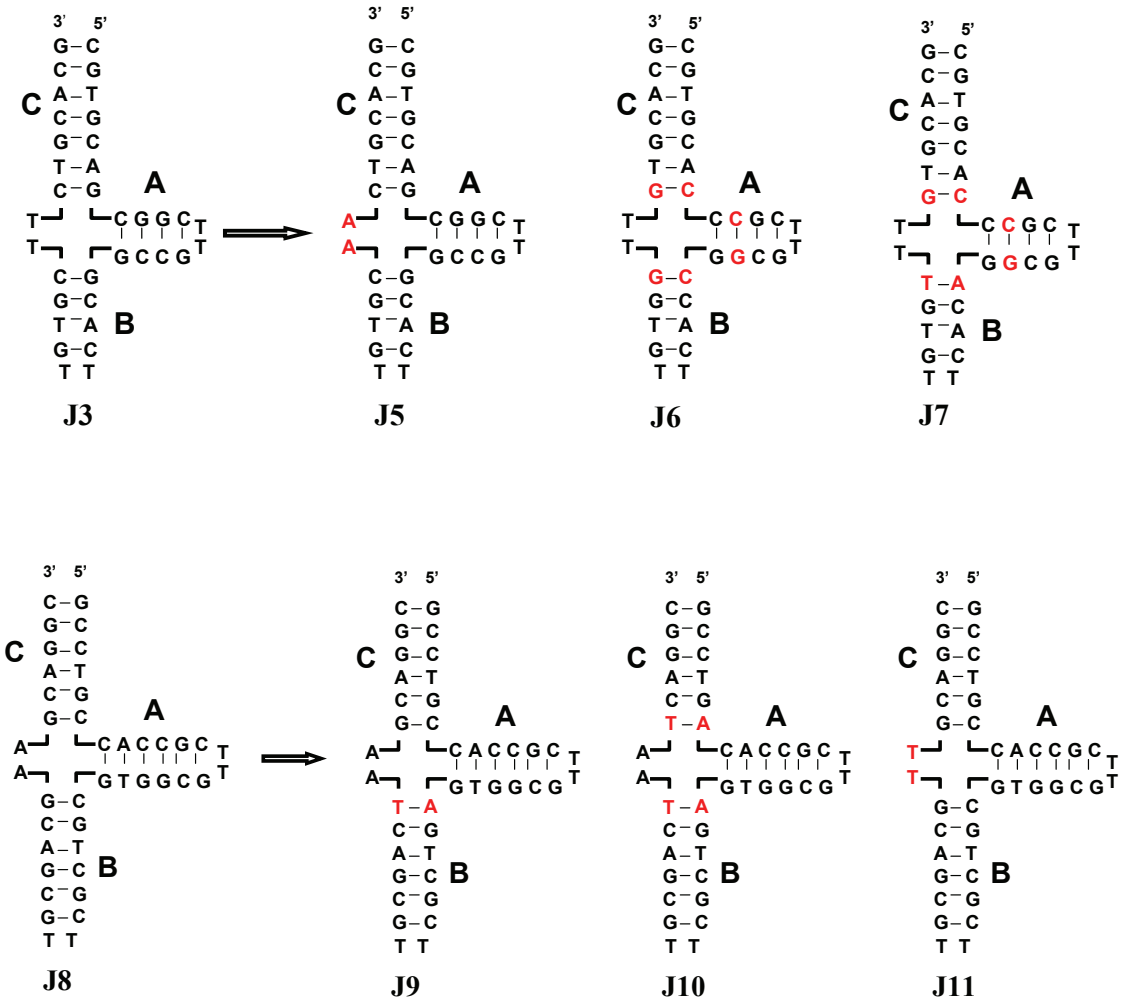


Figure S1: Schematic representation of the sequences of the TWJs designed for this study. Sequences from **J5** to **J7** are designed from the three way junction of **J3** by systematic changes of the base pair near the branch point and also the penultimate position of the helical arm **A**. Sequences from **J9** to **J11** are similarly designed from three way junction of **J8.** All the changed base pairs are marked with red color in each case.

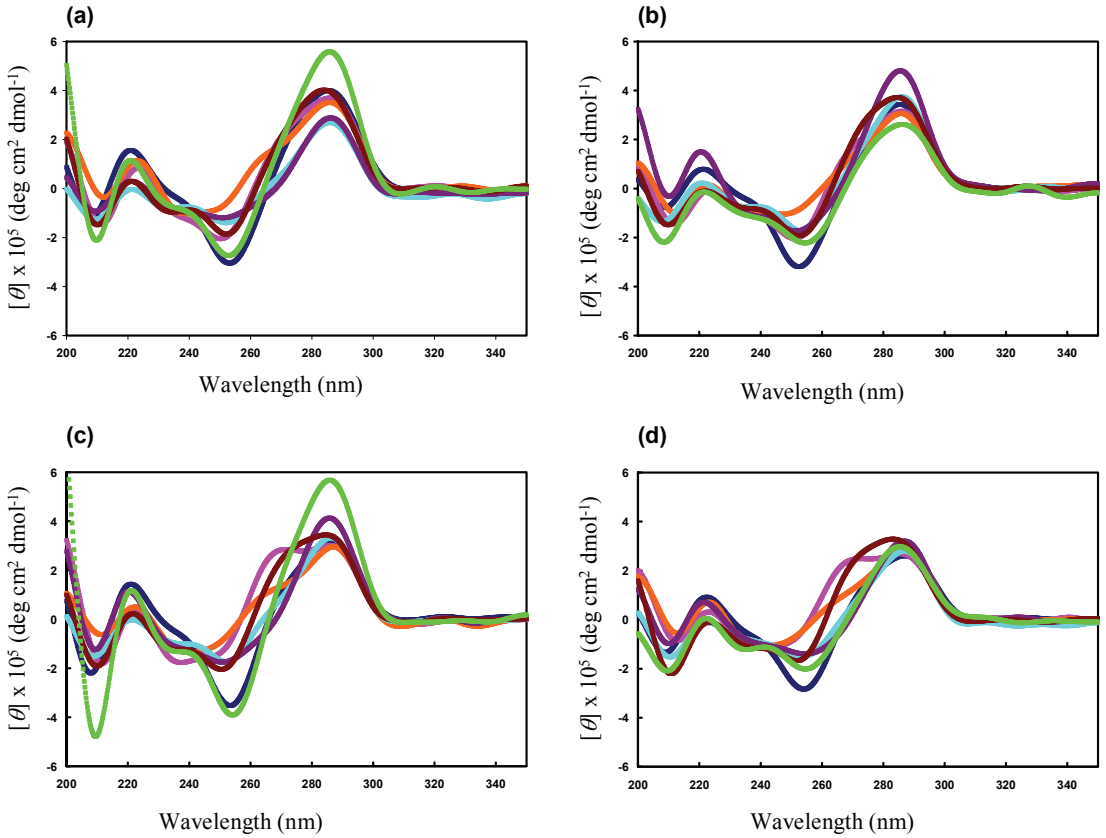


Figure S2: CD spectra of 6 μM J5 (dark blue), J6 (pink), J7 (orange), J8 (green), J9 (light blue), J10 (purple), and J11 (brown) at 4°C in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (a) 50 mM NaCl, (b) 50 mM NaCl and 20 wt% PEG 200, (c) 50 mM NaCl and 5 mM $MgCl_2$, and (d) 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200.

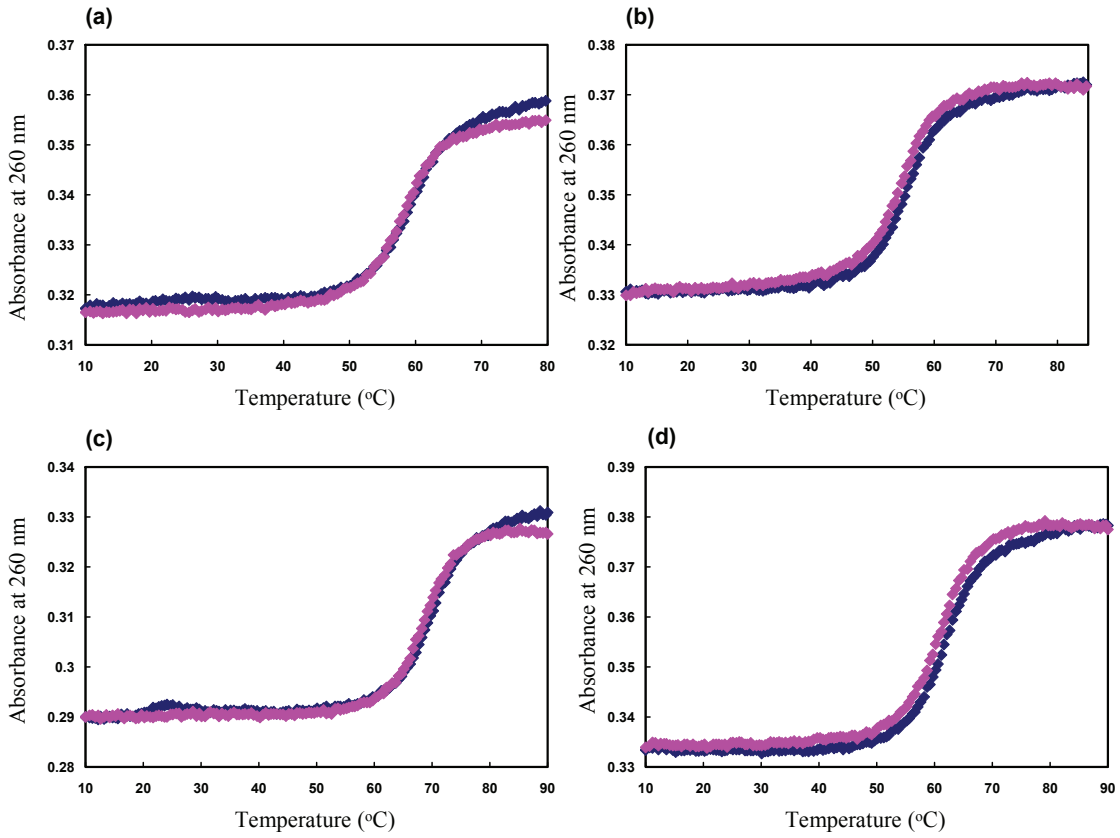


Figure S3: UV melting (blue) and annealing (pink) curves for 1 μM J1 in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (a) 50 mM NaCl, (b) 50 mM NaCl and 20 wt% PEG 200, (c) 50 mM NaCl and 5 mM $MgCl_2$, and (d) 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200. Melting and annealing were assessed by UV absorbance at 260 nm with both melting and annealing rates of 0.5°C/min.

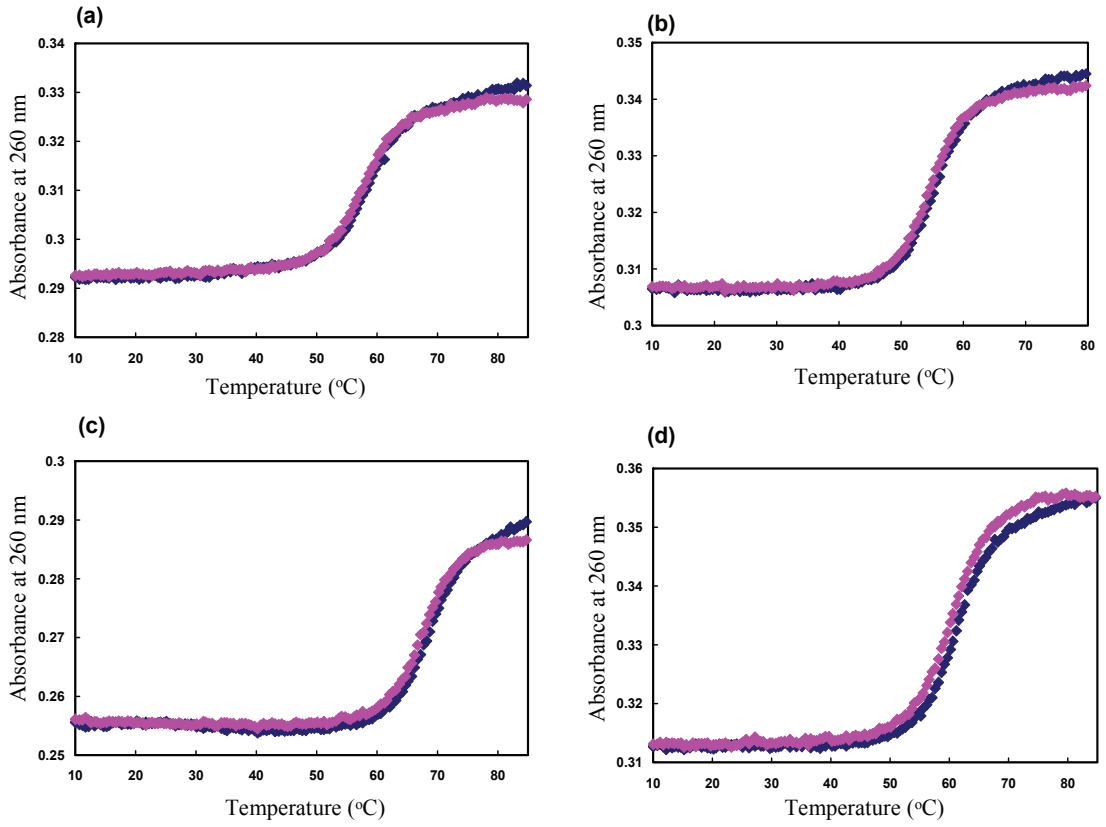


Figure S4: UV melting (blue) and annealing (pink) curves for 1 μM J2 in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (a) 50 mM NaCl, (b) 50 mM NaCl and 20 wt% PEG 200, (c) 50 mM NaCl and 5 mM $MgCl_2$, and (d) 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200. Melting and annealing were assessed by UV absorbance at 260 nm with heating and cooling rates of 0.5°C/min

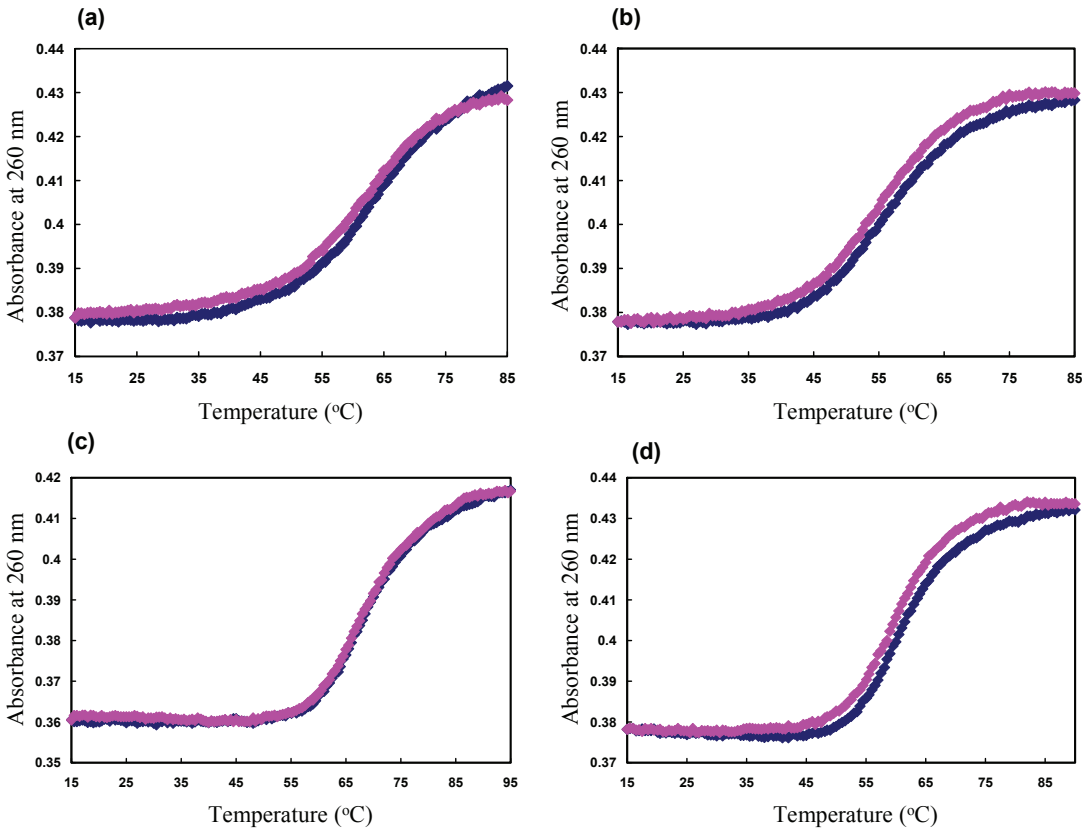


Figure S5: UV melting (blue) and annealing (pink) curves for 1 μM of J3 in buffers 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (a) 50 mM NaCl, (b) 50 mM NaCl and 20 wt% PEG 200, (c) 50 mM NaCl and 5 mM $MgCl_2$, and (d) 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200. Melting and annealing were assessed by UV absorbance at 260 nm with heating and cooling rates of 0.5°C/min

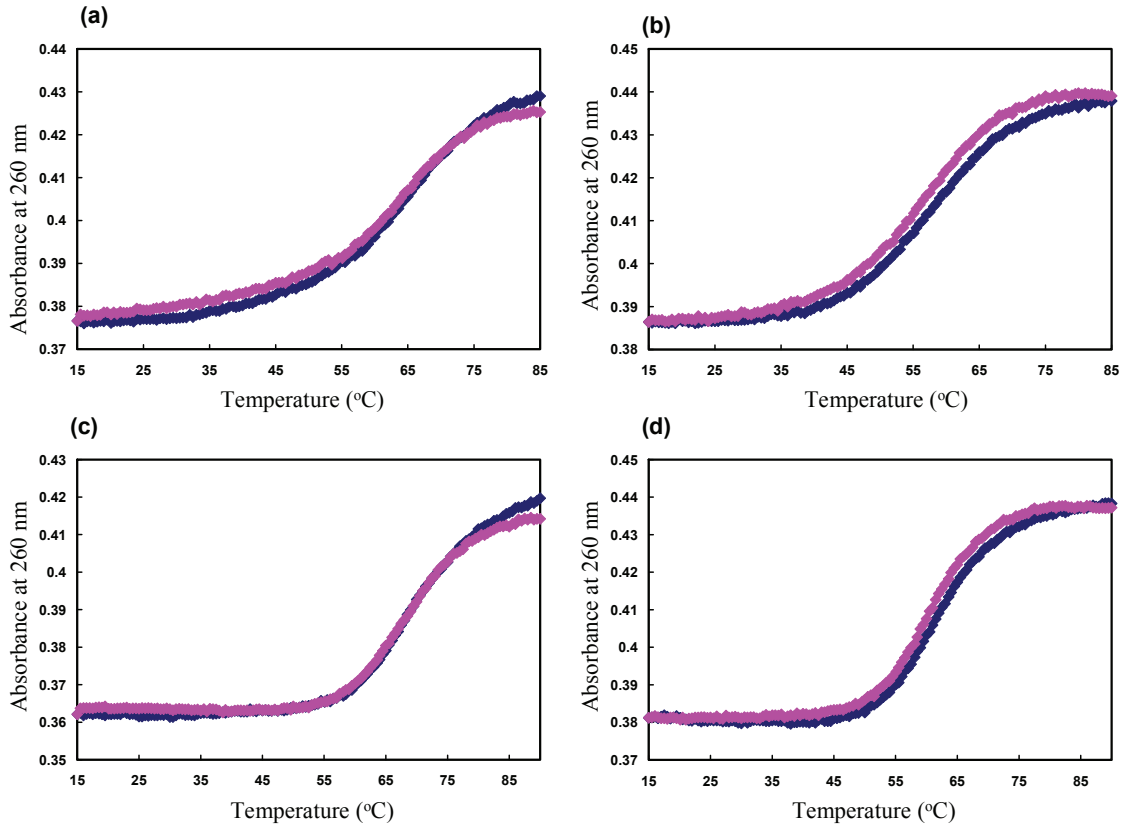
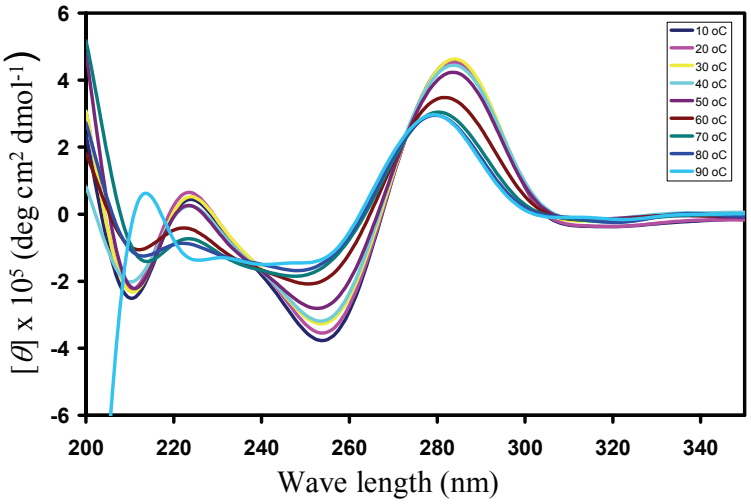


Figure S6: UV melting (blue) and annealing (pink) curves for 1 μM J4 in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (a) 50 mM NaCl, (b) 50 mM NaCl and 20 wt% PEG 200, (c) 50 mM NaCl and 5 mM $MgCl_2$, and (d) 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200. Melting and annealing were assessed by UV absorbance at 260 nm with heating and cooling rates of 0.5°C/min

(a)



(b)

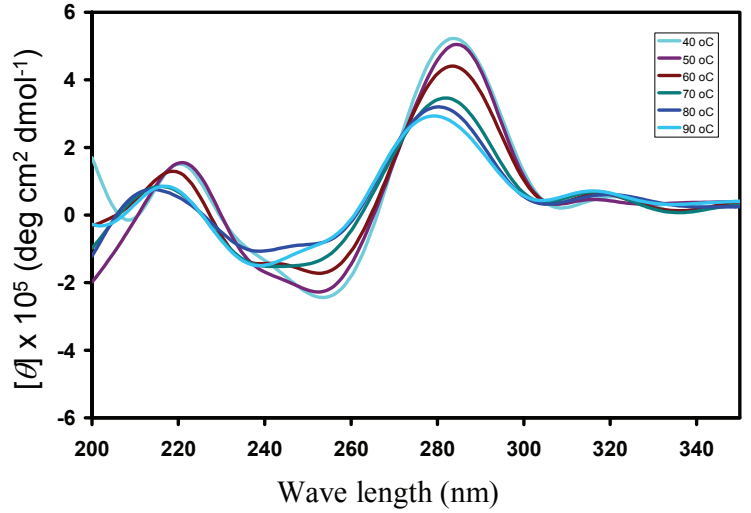


Figure S7: CD melting curves at different temperatures of 6 µM concentration of (a) J1 and (b) J3 in a buffer containing 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0), and 50 mM NaCl.

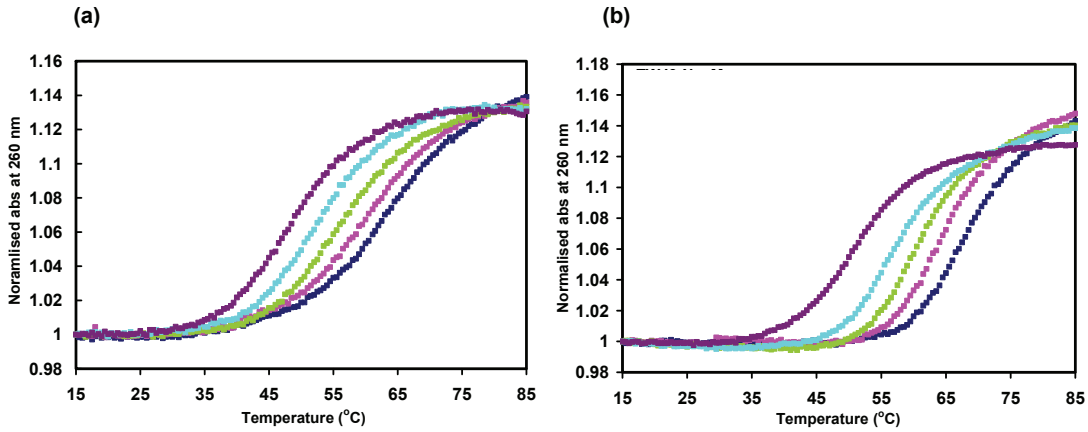


Figure S8: Normalized UV melting curves for 1 μM J3 in the presence of (a) 50 mM NaCl or (b) 50 mM NaCl and 5 mM $MgCl_2$ in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing 0 wt% (dark blue), 10 wt% (pink), 20 wt% (green), 30 wt% (light blue), and 40 wt% (purple) of PEG 200. Melting was assessed by UV absorbance at 260 nm with heating rate of 0.5°C /min.

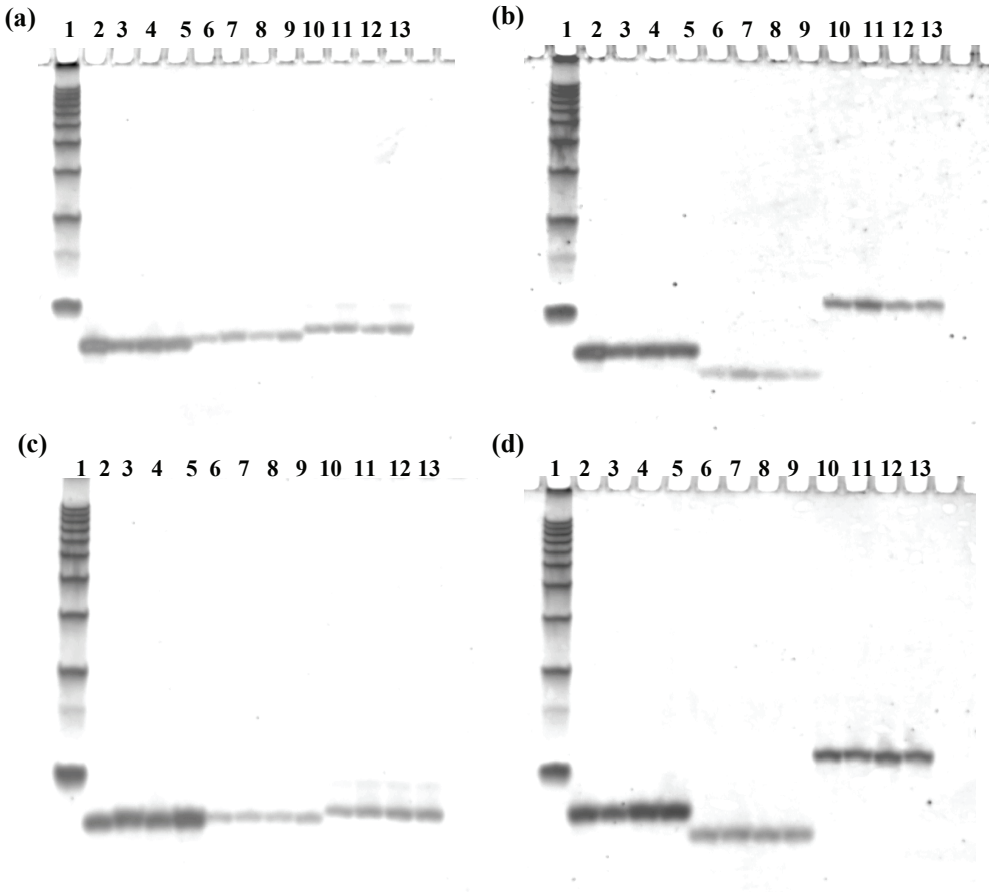


Figure S9: Non-denaturing 15% polyacrylamide gel of (a) 1 μM H (lanes 2-5), 1 μM J1-C (lanes 6-9) and 1 μM J1-AB (lanes 10-13), (b) 1 μM H (lanes 2-5), 1 μM J3-B (lanes 6-9) and 1 μM J3-AC (lanes 10-13), (c) 12 μM H (lanes 2-5), 12 μM J1-C (lanes 6-9) and 12 μM J1-AB (lanes 10-13), and (d) 12 μM H (lanes 2-5), 12 μM J3-B (lanes 6-9) and 12 μM J3-AC (lanes 10-13) in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing 50 mM NaCl (lanes 2, 6 and 10), 50 mM NaCl and 20 wt% PEG 200 (lanes 3, 7 and 11), 50 mM NaCl and 5 mM $MgCl_2$ (lanes 4, 8 and 12), and 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200 (lanes 5, 9 and 13) at 4°C. Lane 1 corresponds to the 10 base pair ladder. H indicates the stable hairpin 5'-GGAC<u>TTCG</u>GTCC-3' with 4 base pair stem as a reference hairpin structure.

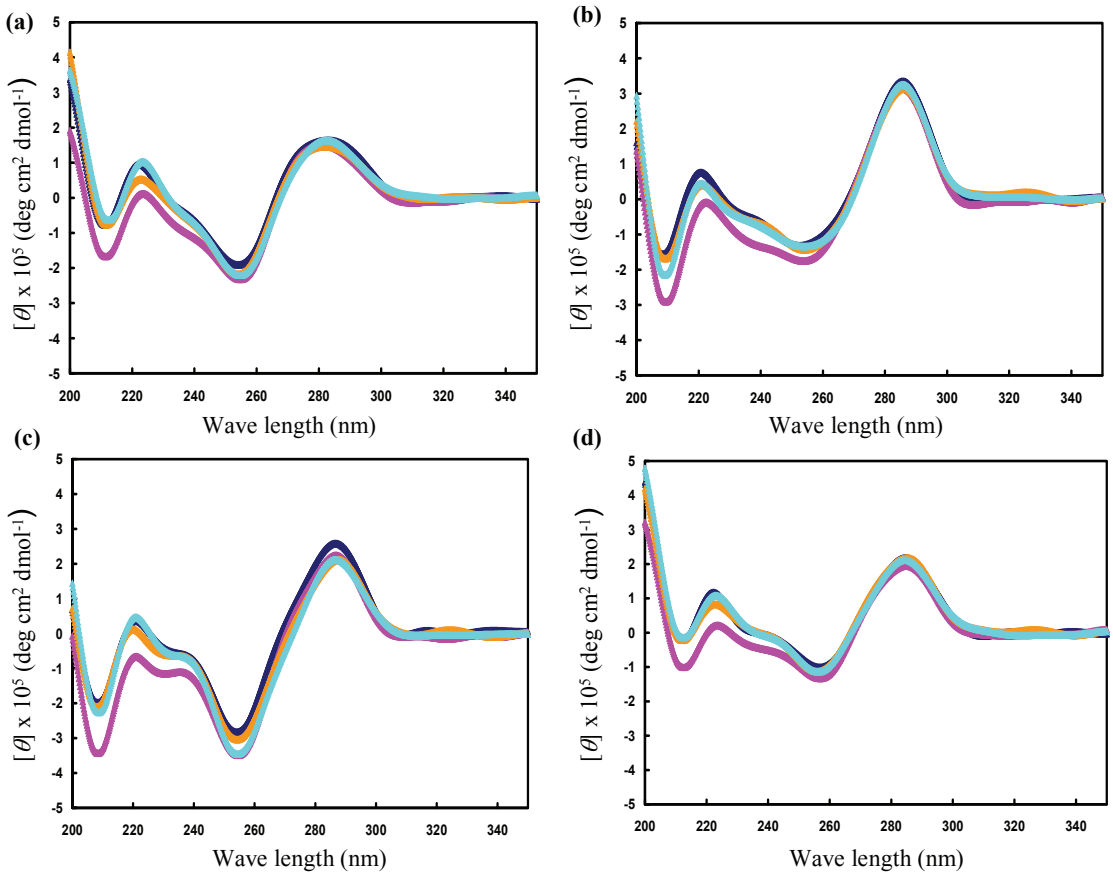


Figure S10: CD spectra of 12 μM (a) J1-C, (b) J1-AB, (c) J3-AC, and (d) J3-B at 4°C in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing 50 mM NaCl (dark blue), 50 mM NaCl and 20 wt% PEG 200 (pink), 50 mM NaCl and 5 mM $MgCl_2$ (orange), and 50 mM NaCl, 5 mM $MgCl_2$, and 20 wt% PEG 200 (light blue).

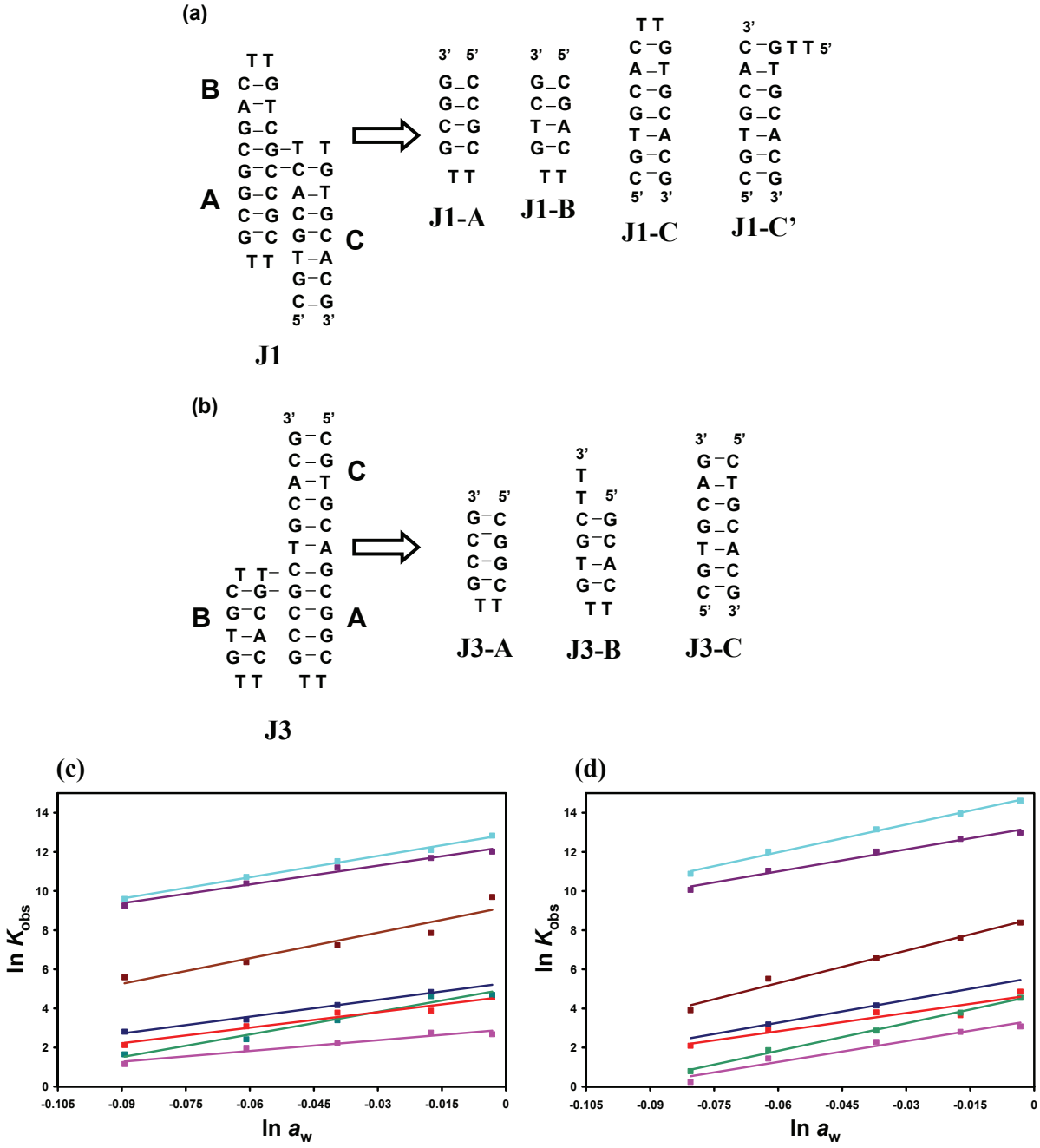


Figure S11: Schematic representation of different arms of (a) J1 and (b) J3. Plots of ln K_{obs} versus ln a_w for the formation of J1-A (blue), J1-B (pink), J1-C (brown), J1-C' (purple), J3-A (red), J3-B (green), and J3-C (light blue) in buffers of 20 mM Na-cacodylate, 0.5 mM Na_2EDTA (pH 7.0) containing (c) 50 mM NaCl, and (d) 50 mM NaCl and 5 mM $MgCl_2$ at 37°C.