Supporting Information for

Kinetic and thermodynamic analysis of triplex formation between peptide nucleic acid and double-stranded RNA

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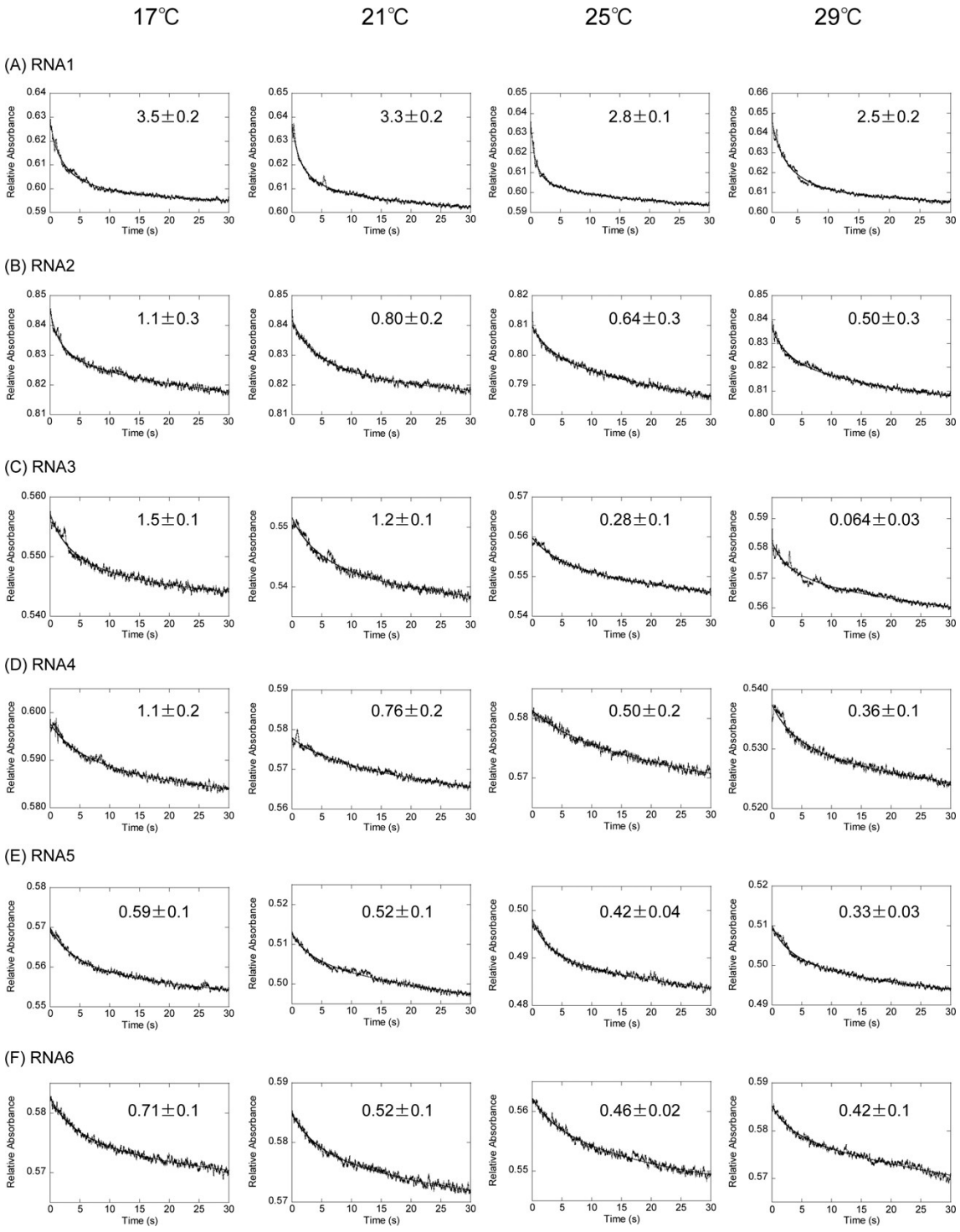

Fig. S1 Temperature dependence of the association kinetics. The absorbance change at 260 nm was monitored from 0−30 s after rapid mixing of 3.0 μM PNA and RNA solutions. The fitting curve for the raw data based on the 1:1 binding model is presented as bold line. The k_{on} values for each target dsRNA are also shown in the figure.

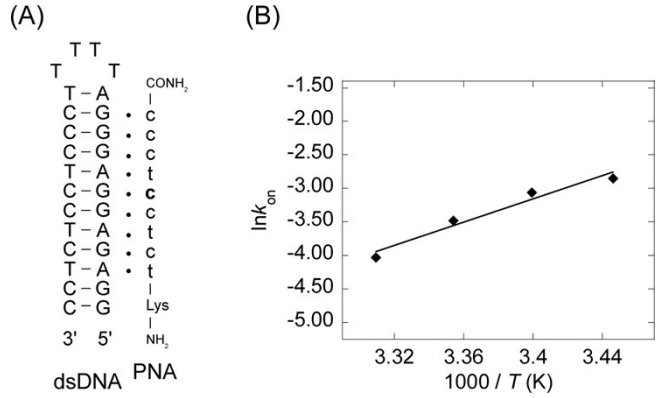

Fig. S2 (A) dsDNA and PNA sequences. (B) Arrhenius plot for the PNA−dsDNA triplex formation. Stopped-flow measurements for 3.0 μM PNA and dsDNA were performed by the same method for the PNA−dsRNA triplex formation.

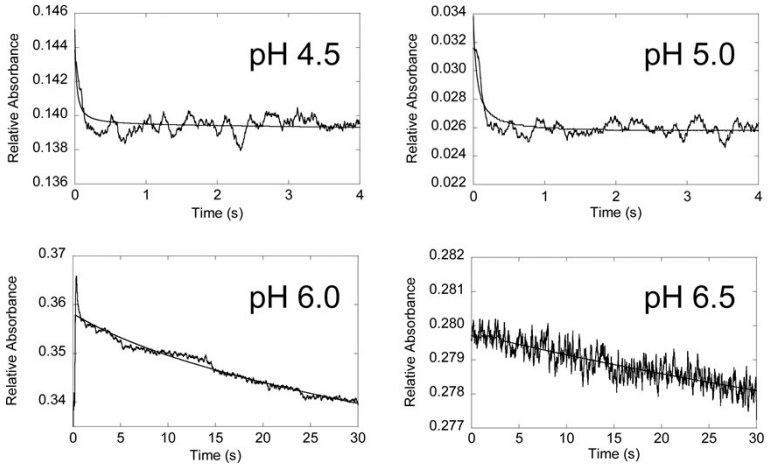

Fig. S3 pH dependence of the association kinetics for 3.0 μM PNA−RNA1 triplex. The absorbance change at 260 nm in 10 mM sodium acetate buffer solutions (pH 4.5, 5.0) or 10 mM sodium phosphate buffer solutions (pH 6.0, 6.5) at 25°C is shown.

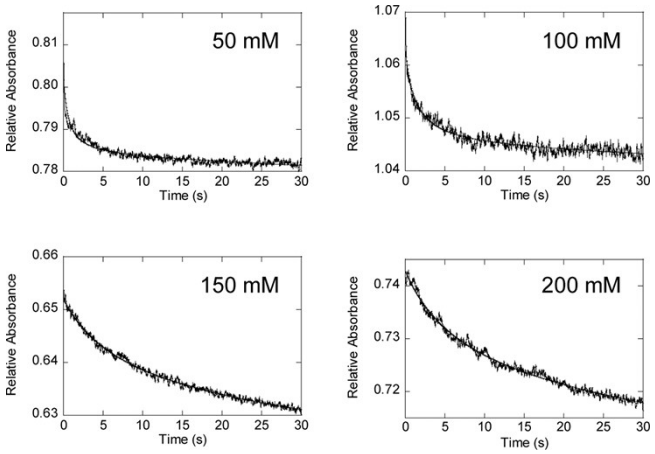

Fig. S4 Salt concentration dependence of the association kinetics for 3.0 μM PNA−RNA1 triplex. The absorbance change at 260 nm in 10 mM sodium acetate buffer solution in the presence of 50, 100, 150, and 200 mM NaCl at 25°C is shown.

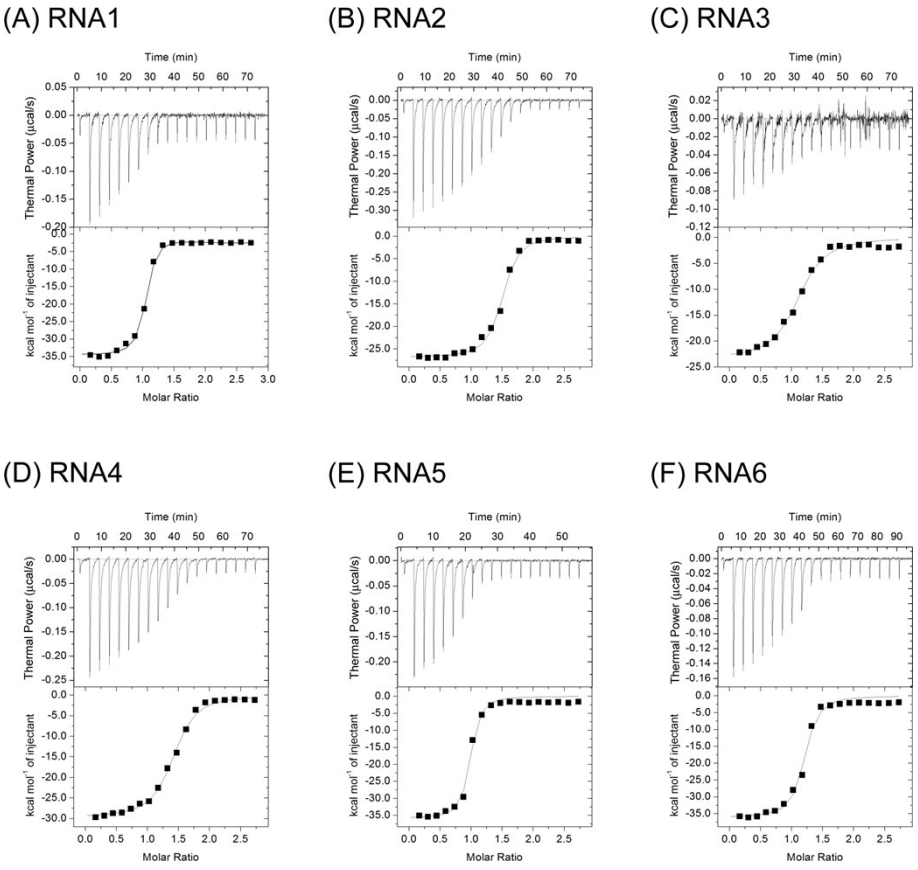

Fig. S5 ITC profiles for the titration of the PNA (70 μM in syringe) with RNA1−RNA6 (5 μM in cell) at 25°C.

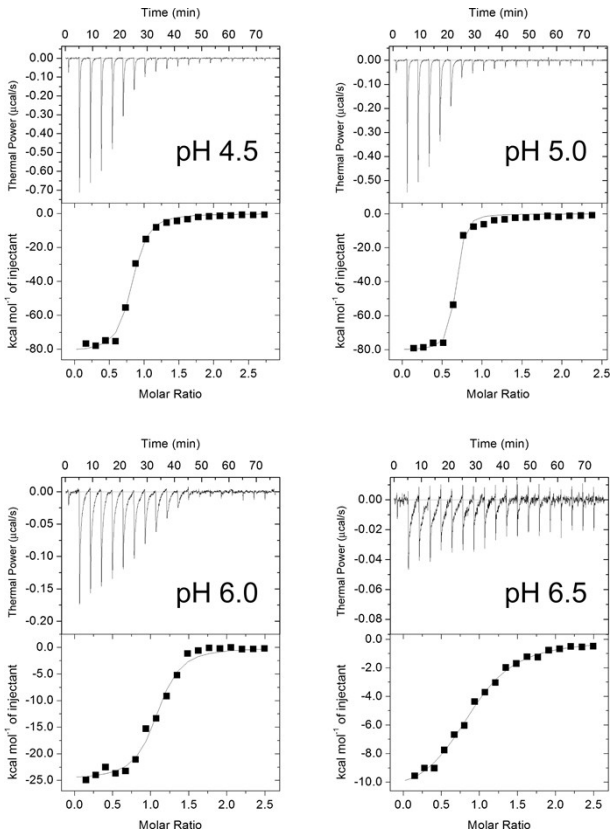

Fig. S6 ITC profiles for the titration of the PNA (70 μM) with RNA1 (5 μM) at pH 4.5 and pH 5.0 or of the PNA (140 μM) with RNA1 (10 μM) at pH 6.0 and pH 6.5 at 25°C.

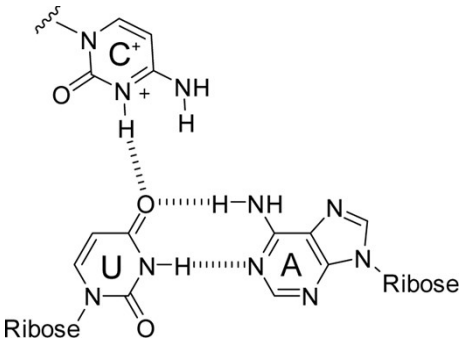

Fig. S7 Possible chemical structure of the partially complementary C^{+}·U−A triplet.

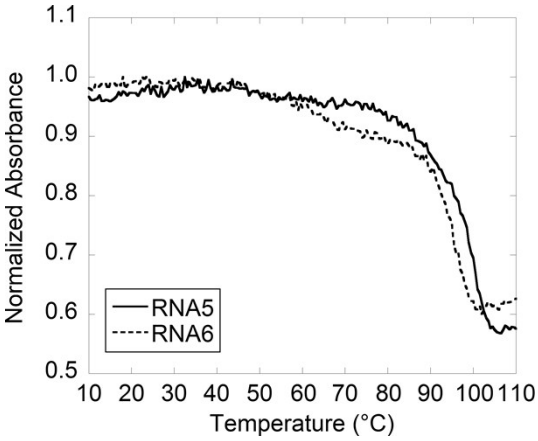

Fig. S8 UV melting curves for 3.0 μM PNA−RNA5 and PNA−RNA6 triplexes.

Table S1 T_m values (±1.5°C) for the triplexes and duplexes[a]

	Triplex	Duplex
RNA1	102[b,c]	97[c]
RNA2	82[c]	97
RNA3	76[c]	92
RNA4	69[c]	92
RNA5	98[b]	91
RNA6	93[b]	89

[a]The T_m values were determined from the melting curves of 3.0 μM PNA−dsRNA or 3.0 μM dsRNA. [b]The merged transition between triplex and duplex. [c]Values from Ref 9a in the main text.

Comments to the T_m values: At pH 5.5, the two melting transitions: triplex ↔ PNA + dsRNA & dsRNA ↔ ssRNA were merged into one transition, and could not be separated by monitoring the absorbance change either at 260 nm or at 300 nm. Furthermore, the T_m values vary among the sequences. These situations made it difficult for us to quantitatively determine the T_m values for the triplex melting (triplex ↔ PNA + dsRNA) and discuss how much the mismatched base pair change the triplex stability in a quantitative manner. However, this can be concluded from Table S1 that the central mismatch (RNA2−RNA4) is more detrimental to the triplex stability than the terminal mismatch (RNA5, RNA6).

UV melting measurements: UV melting experiments were conducted using a UV−Vis spectrophotometer Model UV−2450 (Shimadzu Co. Ltd., Kyoto, Japan) equipped with a thermoelectrically temperature-controlled micro-multicell holder (8 cells). Prior to each melting experiment, the sample solutions containing the annealed RNA and the PNA were put into the cells and covered with paraffin and silicon caps. Absorbance at 260 nm (for duplex) or 300 nm (for triplex) was recorded while increasing temperature from 10 to 110°C with a rate of 0.5°C/min and then corrected by subtracting the absorbance at 320 nm or 400 nm, respectively. Optical path length was 10 mm. The final concentration of each strand was 3.0 μM. Under the condition, we observed a slight hysteresis for the heating and cooling curves. For calculation of the T_m value, the melting (absorbance vs. temperature) curve was baseline-corrected. Then, the first differential processing was executed in the selected region where absorbance sharply changed (TMSPC-8, Shimadzu). The peak point was defined as T_m.