Supplementary Material (ESI) for Chemical Communications

Supporting information

Thermodynamic Stability of Hoogsteen and Watson-Crick Base Pairs in the Presence of Histone H3-Mimicking Peptide

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Materials and Methods

Materials. DNA oligonucleotides of high performance liquid chromatography (HPLC) purification grade, Q-strand, T-strand, and W-strand, were purchased from Hokkaido System Science (Sapporo, Japan). Histone H3-mimicking peptide [ARTKQTARKSTGGKAGY] and the control peptide [KTRARATQGTSKGKAGY] were synthesized by standard F-moc chemistry on the solid phase. The peptide was purified by HPLC and the purity of the compound was checked by MALDI-TOF-MS (Figure S9). The concentration of the peptide was determined by measuring the absorbance of Tyr at the C-terminal at 280 nm at 25°C. [http://www.sigmaaldrich.com/life-science/custom-oligos/custom-peptides/learning-center/concentration-calculation.html].

Single-strand concentrations of DNA oligonucleotides were determined by measuring the absorbance at 260 nm at a high temperature using a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) connected to a thermoprogrammer. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data using the nearest-neighbor approximation [#E. G. Richards, Use of tables in calculation of absorption, optical rotator dispersion and circular dichroism of polyribonucleotides. #G. D. Fasman, Ed. Handbook of Biochemistry and Molecular Biology, 3rd ed., CRC Press: Cleveland, OH, 1975; Vol. 1, pp 596-603.]. A.R grade polyethylene glycol 200 (PEG 200), NaCl, and Na_2HPO_4 were obtained from Wako Chemicals, and A.R grade Na_2EDTA was purchased from Alfa Aesar.

Steady-state Fluorescence Measurements. Steady-state fluorescence experiments were performed by utilizing a JASCO FP 6500 spectrofluorometer (JASCO, Hachioji, Japan).

Experiments were carried out at 25°C in a 3-mm path-length quartz cuvette for 5 μM peptide in pH 7.0 buffer [100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4] in the presence or absence of 100 μM H3 peptide at 0 and 20 wt% PEG 200. The temperature of the cell holder was regulated by a JASCO ETC-273T temperature controller. Before measurements, the samples were heated to 90°C, cooled at a rate of 1°C min^{-1}, and incubated at 25°C for 30 min. Excitation and emission slit width were 3 nm each and the samples were excited at 275 nm and the emission was recorded in a range of 280 nm to 400 nm. To study the DNA-peptide binding interaction, DNA concentrations were varied in a range of 0.5 μM to 40 μM for a fixed concentration of peptide (5 μM). The fluorescence intensity change at 303 nm vs. the DNA concentration was fitted to the following equation for one binding site to evaluate the equilibrium parameters [Rippe, K. *B. I. F. Futura* **1997**, *12* 20-26]:

$$F = F_0 + \Delta F(K_a[\mathrm{H3}] + K_a[\mathrm{DNA}] + 1 - (K_a[\mathrm{H3}] + K_a[\mathrm{DNA}] + 1)^2 - 4K_a^2[\mathrm{H3}][\mathrm{DNA}])^{1/2}) / 2K_a[\mathrm{H3}] + (1 - \Delta F)[\mathrm{DNA}] \quad (1)$$

Thermodynamic Analysis. UV absorbances of different samples were recorded with a Shimadzu 1700 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a temperature controller. Melting curves of DNA structures were obtained by measuring the UV absorbance at 260 or 295 nm in buffer [100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4, pH 7.0] in the presence or absence of 100 μM H3 peptide at 0 and 20 wt% PEG 200. The T_m values for 5 μM DNA structures were obtained from the UV melting curves as described previously [Miyoshi, D.; Karimata, H.; Sugimoto, N. *J. Am. Chem. Soc.* **2006**, *128*, 7957–7963]. The heating rates were 0.2°C min^{-1} for the T- and H-strands, and 0.5°C min^{-1} for the W-strand. The thermodynamic parameters (enthalpy

change $\Delta H°$, entropy change $\Delta S°$, and free energy change $\Delta G°$) were evaluated from the fit of the melting curves to a theoretical equation for an intramolecular association as described previously [Miyoshi, D.; Karimata, H.; Sugimoto, N. *J. Am. Chem. Soc.* **2006**, *128*, 7957–7963]. Before measurement, the samples were heated to 90°C, cooled at a rate of 1°C min^{-1}, and incubated at 0°C for 1 h to avoid any non-equilibrium structures.

Water Activity Measurements and evaluation of the number of water molecules. The water activities were determined by the osmotic stressing method via vapor phase osmometry using a model 5520XR pressure osmometer (Wescor, Utah, U.S.A). Intramolecular duplex, triplex, or G-quadruplex formation by a single strand (A_{unfold}) in an aqueous solution containing cosolute (CS) and sodium ion (Na^+) can be represented by the following reaction:

$$A_{unfold} \rightleftharpoons A_{fold} + \Delta n_w H_2O + \Delta n_{cs} CS + \Delta n_{Na+} Na^+ \qquad (2)$$

where, Δn_w, Δn_{cs}, and Δn_{Na+} are the numbers of water molecules, osmolyte, and sodium ion released on the structure (A_{fold}) formation, respectively. At a fixed temperature and pressure, the observed equilibrium constant (K_{obs}) for the duplex formation is thus given as:

$$\frac{d\ln K_{obs}}{d\ln a_W} = -\left(\Delta n_w + \Delta n_{cs} \left(\frac{d\ln a_{cs}}{d\ln a_W} \right) + \Delta n_{Na^+} \left(\frac{d\ln a_{na}^+}{d\ln a_W} \right) \right) \qquad (3)$$

where, a_w, a_{cs}, and a_{Na+} are the activities of water, cosolute and sodium ion, respectively. Slope of the plots of ln K_{obs} (an observed equilibrium constant) vs ln a_w (water activity) determined by osmotic pressure measurements is approximately equal to the constant term $-\Delta n_w$, which is the number of water molecules taken up on formation of a structure.

Table S1. Melting temperature evaluated from UV melting curves with 5 μM DNA strand concentration.

Condition	Melting temperature (°C)					
	T-strand (H bp[a])	**T-strand (W bp[a])**	**T2-strand (H bp[b])**	**T2-strand (W bp[b])**	**Q-strand**	**W-strand**
None	33.9	67.8	33.5	70.5	24.6	67.4
+100 μM H3	36.7	67.7	38.0	70.5	30.1	67.3
+100 μM control peptide	35.0	67.0	38.0	70.5	28.0	67.5
+20 wt% PEG200	37.1	61.9	38.0	64.5	33.1	62.2
+ 100 μM H3 + 20 wt% PEG 200	40.2	61.8	44.0	65.0	33.2	62.4
+100 μM control peptide + 20 wt% PEG200	40.0	63.0	42.5	63.5	31.0	62.5

[a] H bp indicates Hoogsteen base pair.

[b] W bp indicates Watson-Crick base pair.

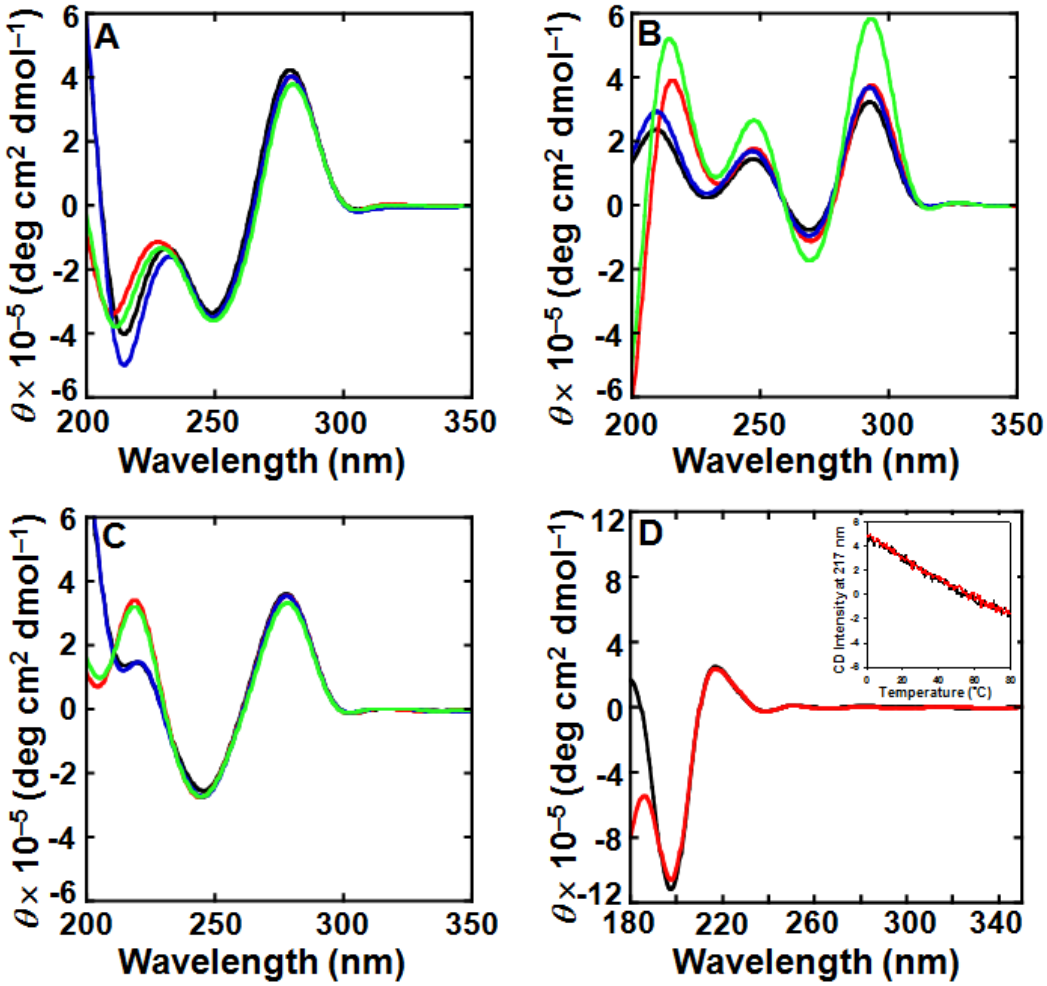

Fig. S1 CD spectra of 20 μM of T-strand (A), Q-strand (B), and W-strand (C) in buffer containing NaCl (100 mM), Na_2EDTA (1 mM), and Na_2HPO4 (10 mM, pH 7.0) without any additives (black lines), 100 μM H3 peptide (red lines), 20 wt% PEG 200 (blue lines), and a combination of 100 μM H3 peptide and 20 wt% PEG 200 (green lines). (D) CD spectra of 100 μM of H3 peptide in the presence of 0 wt% (black line) and 20 wt% (red line) PEG 200 in the same buffer. Inset: CD melting curves of 100 μM of H3 peptide at 217 nm in presence of 0 wt% (black line) and 20 wt% (red line) PEG 200.

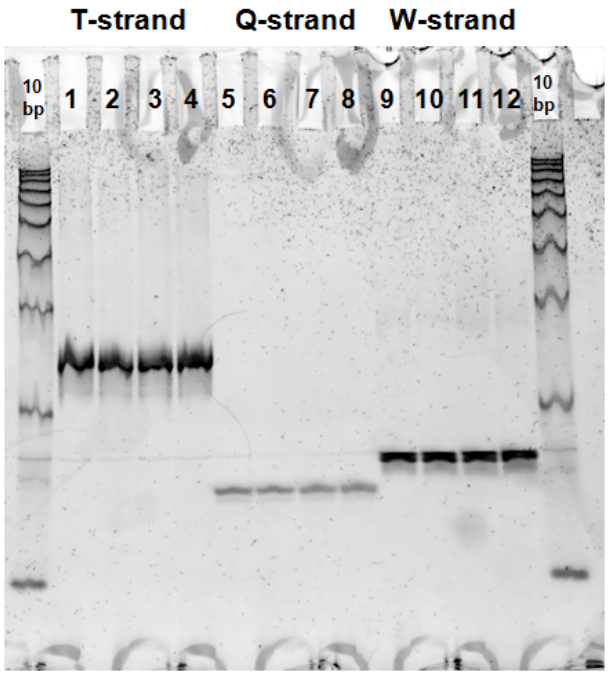

Fig. S2 Non-denaturing polyacrylamide gel electrophoresis of 5 μM T-strand (lanes 1-4), Q-strand (lanes 5-8), and W-strand (lanes 9-12) in buffer containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 (pH 7.0). Lanes 1, 5, 9: without any additives. Lanes 2, 6, 10: 100 μM H3 peptide. Lanes 3, 7, 11: 20 wt % PEG 200. Lanes 4, 8, 12: combination of 100 μM H3 peptide and 20 wt% PEG 200.

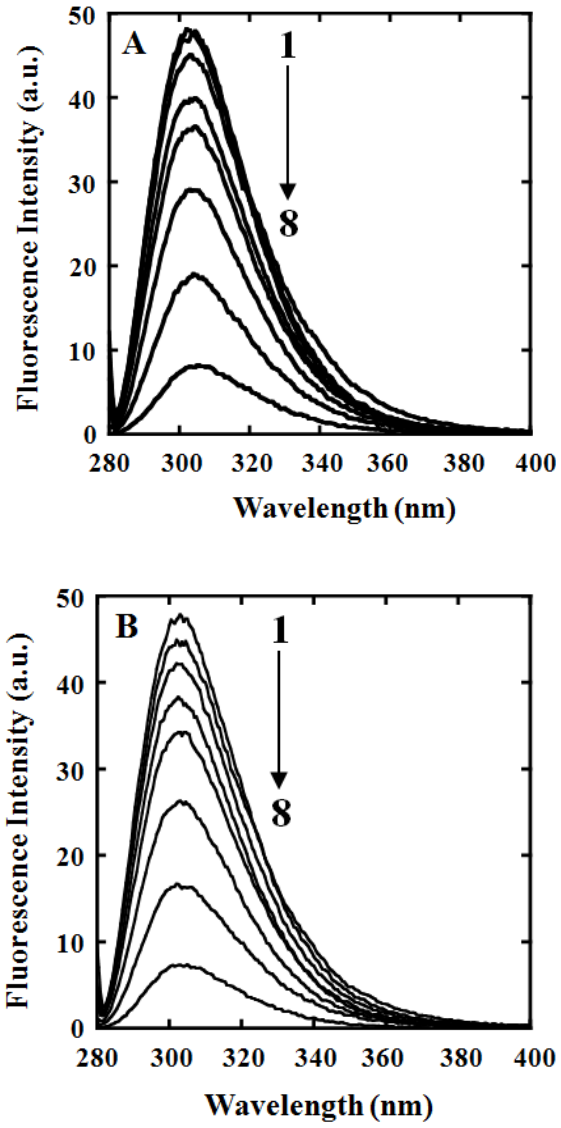

Fig. S3 Emission spectra of H3 with Q-strand (A) and W-strand (B) in buffer containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 (pH 7.0). [H3] = 5 μM and [DNA] = (1) 0 μM, (2) 0.5 μM, (3) 1 μM, (4) 2.5 μM, (5) 5 μM, (6) 10 μM, (7) 20 μM, or (8) 40 μM. λ_{ex} = 275 nm at 25°C.

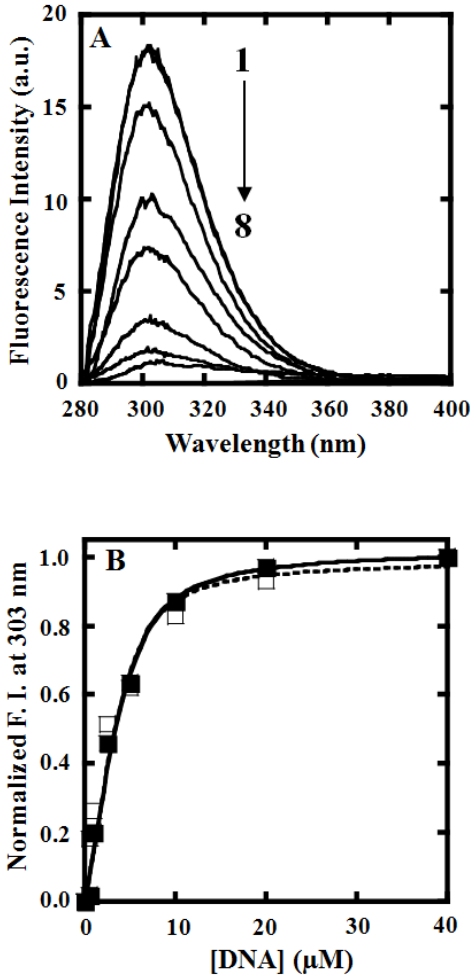

Fig. S4 Emission spectra of control peptide with T-strand in pH 7.0 buffer containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 at 25°C. [control peptide] = 5 μM and [T-strand] = (1) 0 μM, (2) 0.5 μM, (3) 1 μM, (4) 2.5 μM, (5) 5 μM, (6) 10 μM, (7) 20 μM, or (8) 40 μM. λ_{ex} = 275 nm. (**B**) Normalized fluorescence intensity of 5 μM control peptide with different T-strand concentrations in the absence (filled symbols) and presence (open symbols) of PEG 200.

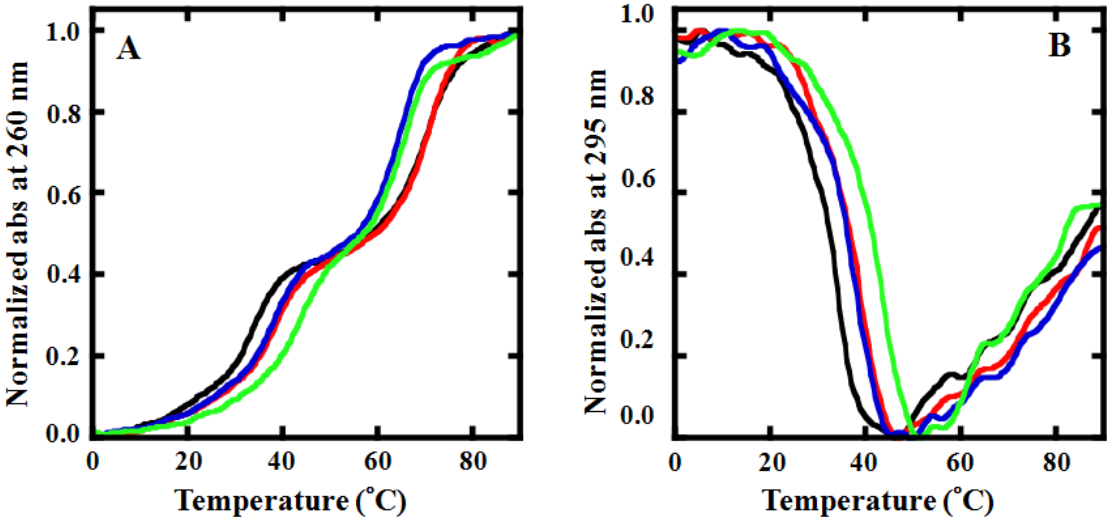

Fig. S5 Normalized UV melting curves of 5 μM DMT at 260 nm (**A**) and 295 nm (**B**) in buffers containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 (pH 7.0), without any additives (black lines), and with 100 μM H3 peptide (red lines), 20 wt% PEG 200 (blue lines), and a combination of 100 μM H3 peptide and 20 wt% PEG 200 (green lines).

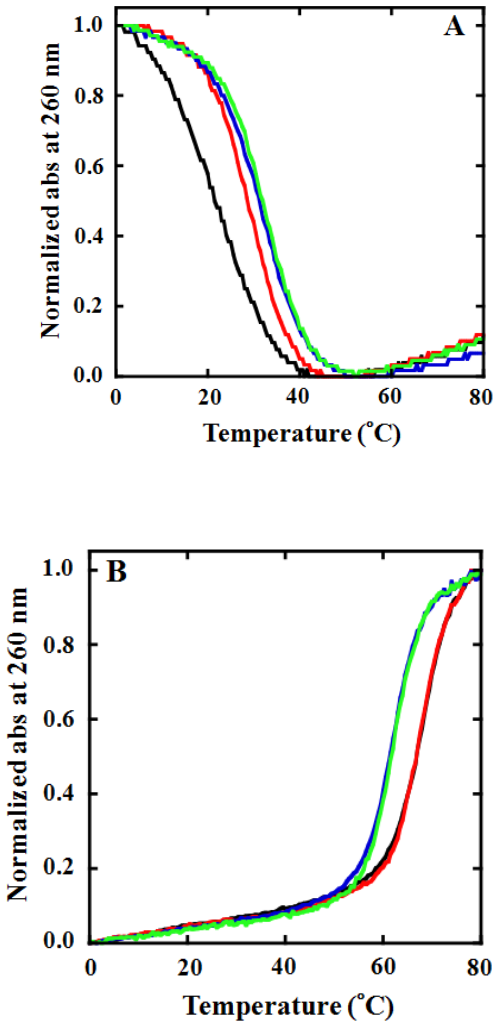

Fig. S6 Normalized UV melting curves of 5 μM Q-strand at 295 nm (A) and W-strand at 260 nm (B) in a buffer containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 (pH 7.0) without any additives (black lines), 100 μM H3 peptide (red lines), 20 wt% PEG 200 (blue lines), and a combination of 100 μM H3 peptide and 20 wt% PEG 200 (green lines).

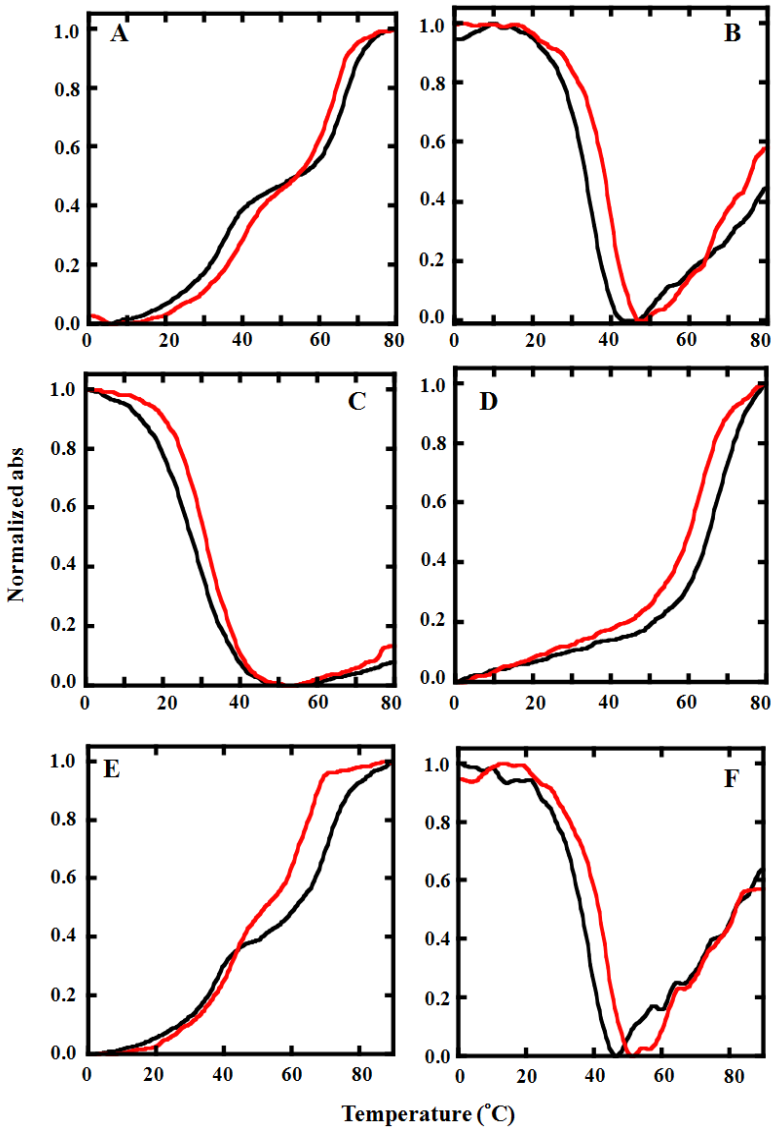

Fig. S7 Normalized UV melting curves of 5 μM T-strand at 260 nm (**A**), T-strand at 295 nm (**B**), Q-strand at 295 nm (C), W-strand at 260 nm (D) DMT at 260 nm (E), and DMT at 295 nm in a buffer containing 100 mM NaCl, 1 mM Na_2EDTA, and 10 mM Na_2HPO_4 (pH 7.0) with 100 μM control peptide (black lines) and a combination of 100 μM control peptide and 20 wt% PEG 200 (red lines).

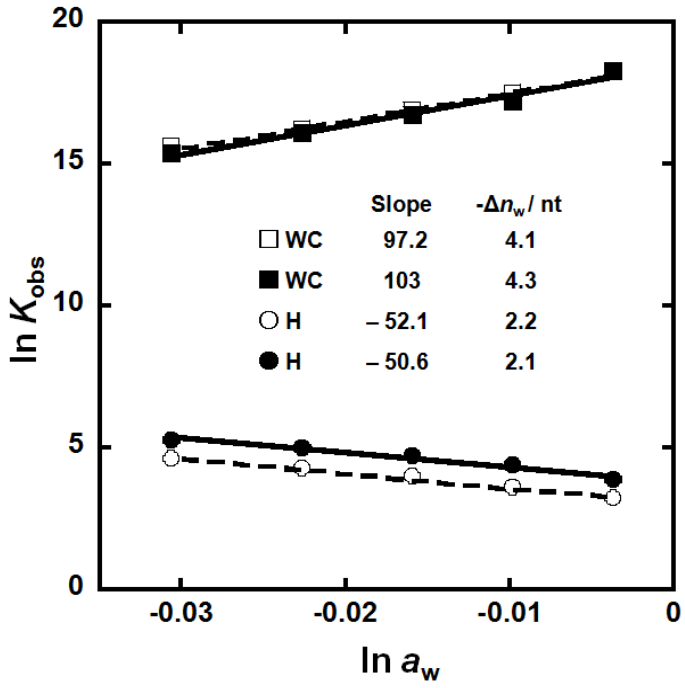

Fig. S8 Plot of ln *K*obs versus ln a_w for Hoogsteen (H) and Watson-Crick (WC) base pair formations in the T-strand, in the absence of H3-peptide (open symbols), and in the presence of H3-peptide (filled symbols). Circles and squares indicate H and WC base pairs, respectively. The slope of these plots is approximately equal to the constant term $-\Delta n_w$, which is the number of water molecules taken up on formation of a structure (S. Nakano, H. Karimata, T. Ohmichi, J. Kawakami and N. Sugimoto, *J. Am. Chem. Soc.*, 2004, **126**, 14330-14331).

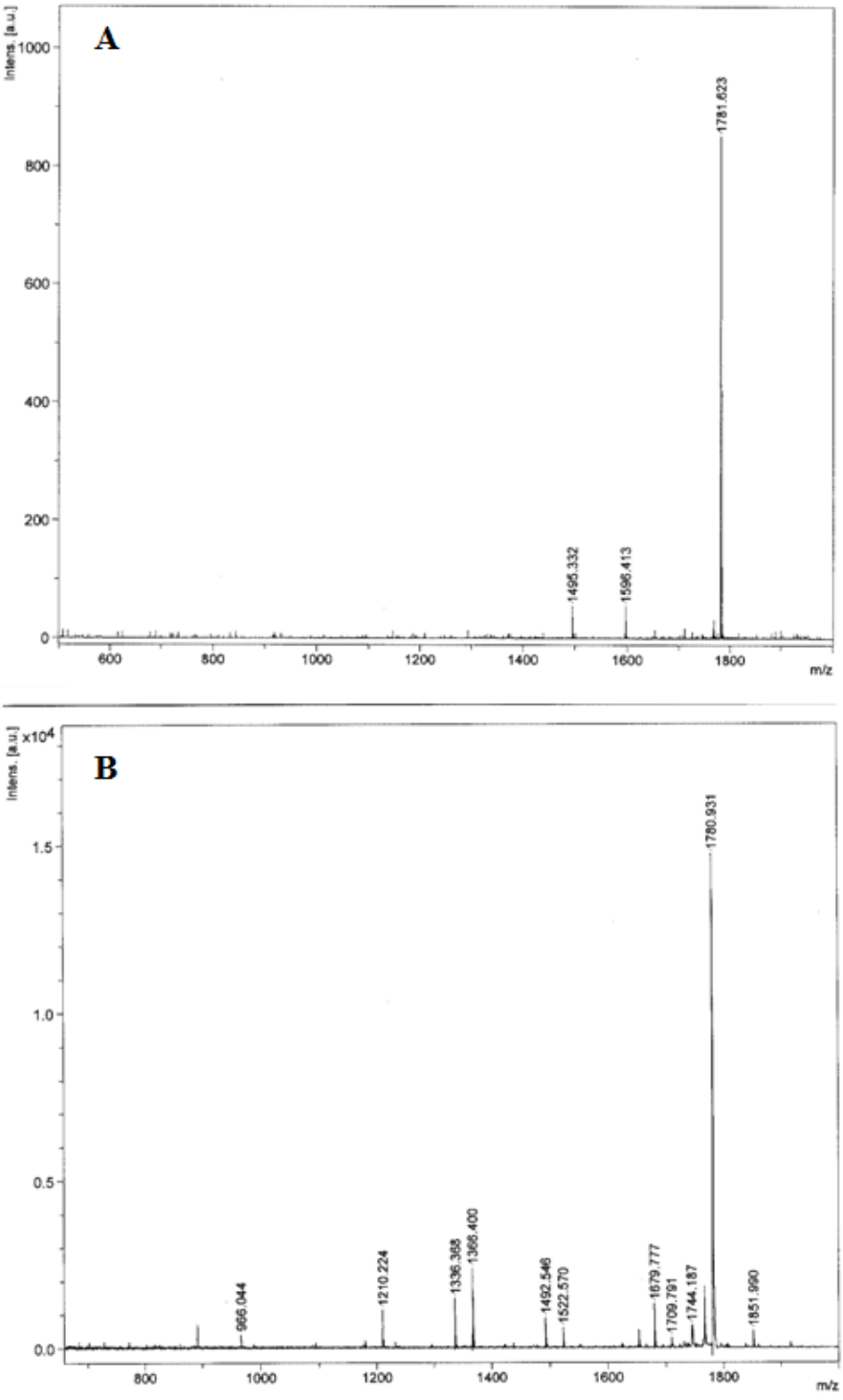

Fig. S9 Mass spectra of H3 peptide (A) and control peptide (B).