

Recombination R-triplex: H-bonds contribution to stability as revealed with minor base substitutions for adenine

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Supplementary material:

Figures S1 and S2.

Thermodynamic analysis of the intramolecular triplex formation.

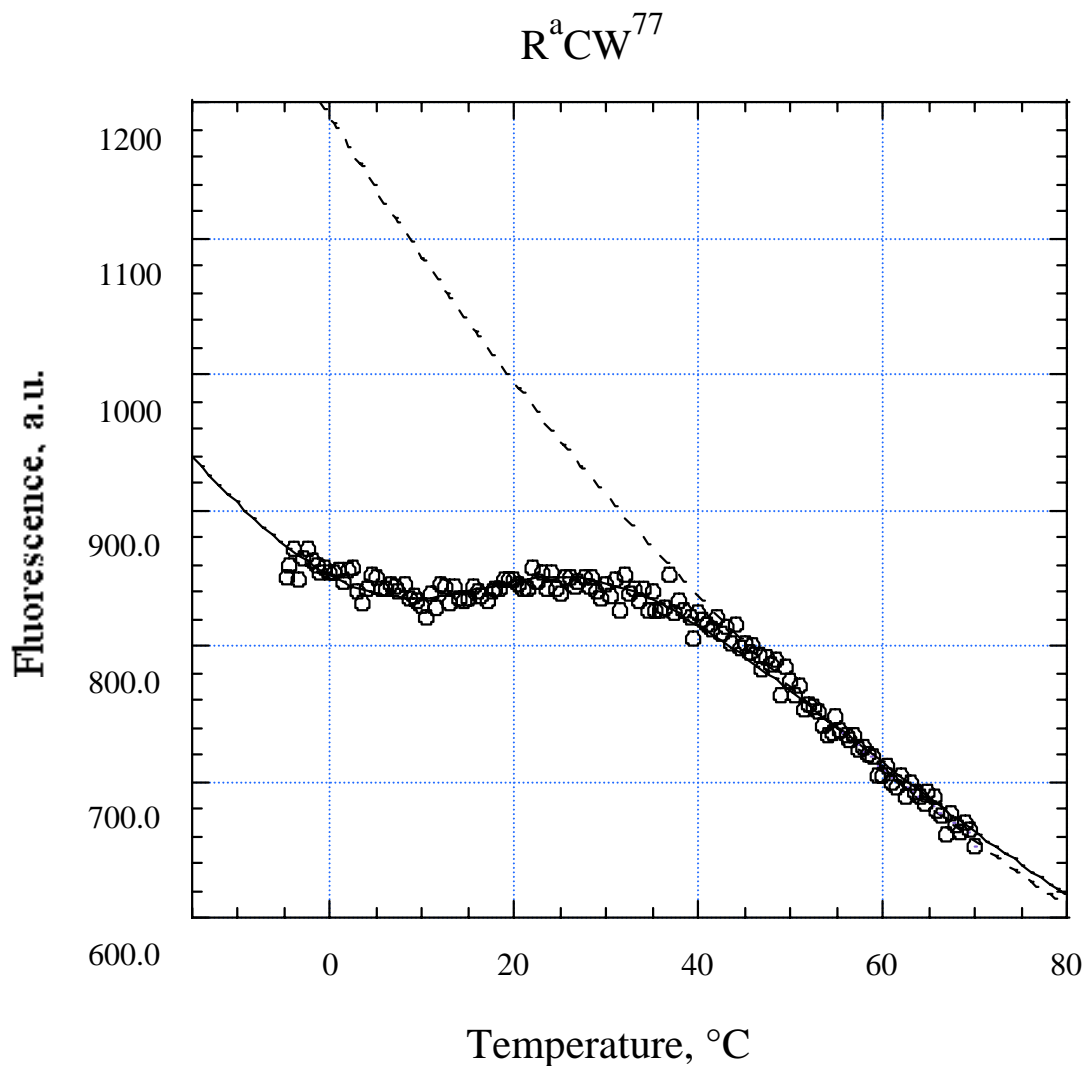


FIGURE S1. Temperature dependence of the fluorescence emission of 2AP incorporated in the oligonucleotide $R^a CW^{77}$ (open circles). Solid curve is the theoretical fit for a two-state model (13). Dashed curve is the theoretical curve describing 2AP fluorescence in the dangling third strand (13). The derived thermodynamic parameters are given in Table 1. The excitation wavelength was 310 nm, emission was measured at 370 nm. Samples contained 1 μ M oligonucleotides, 0.5 M LiCl, 10 mM Tris-HCl buffer, pH 7.6. For details see Materials and Methods.

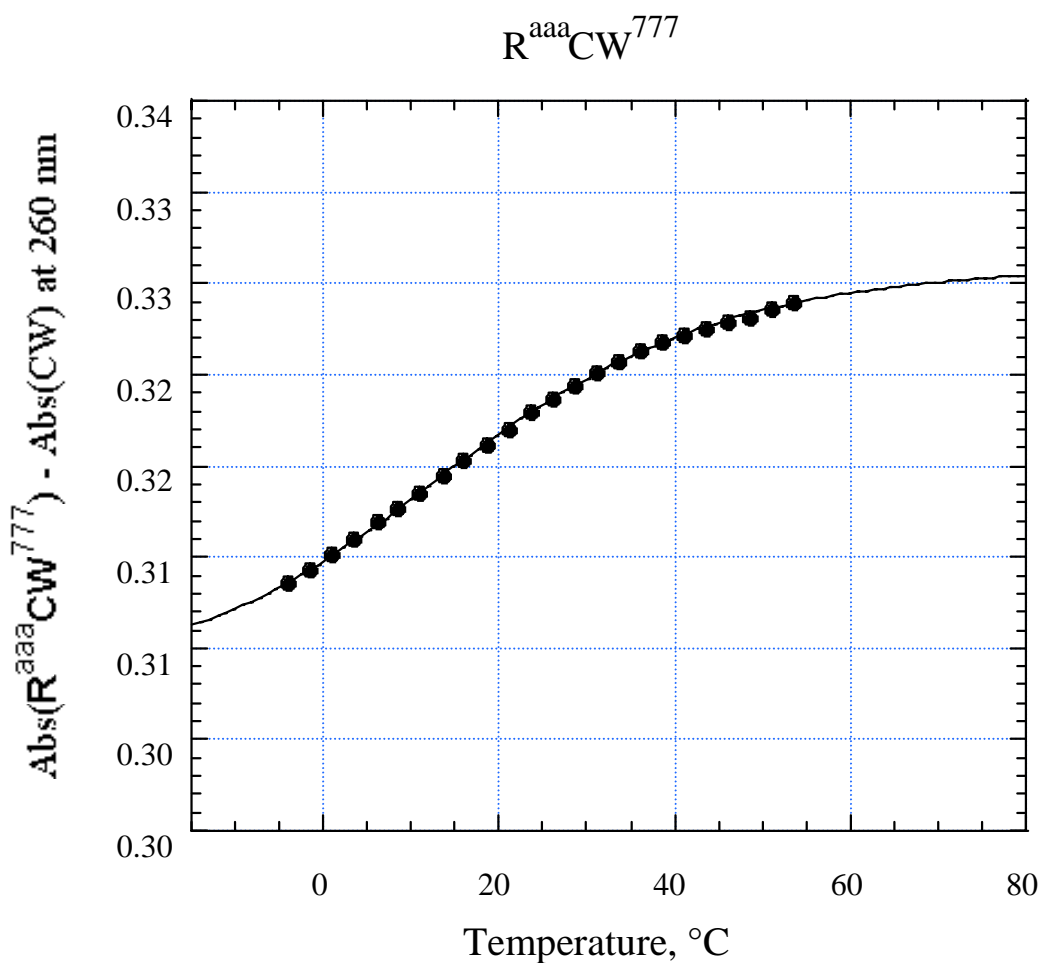


FIGURE S2. Determination of the thermodynamic parameters of the $R^{aaa}CW^{777}$ triplex formation from the UV absorption at 260 nm. The shown data (solid circles) are the result of subtraction of the CW contribution from the melting profile of the $R^{aaa}CW^{777}$ (not shown). The curve represents the best theoretical fit for a two-state model (see Materials and Methods and Ref. 13). The derived thermodynamic parameters are given in Table 1.

Thermodynamic analysis of the intramolecular triplex formation (from Ref. 13).

We performed a thermodynamic analysis for the intramolecular binding of the dangling third strand of the triplex forming oligonucleotide to its double helical CW part. We define such a process as ‘triple helix formation.’ The transition curve for triplex formation was detected by two experimental techniques. One is the conventional melting curve acquired by measuring the hyperchromicity of the UV absorbance at 260 nm. All nucleotide bases of the third strand absorbing light at 260 nm contribute to this curve, whereas the transition curve measured by 2AP fluorescence emission reflects the status of a single triplet 2AP*(T·A).

The transition curve corresponds to the relationship

$$A(t) = B(t) + [C(t) - B(t)] \cdot \sigma(t) \quad (1)$$

where t is the temperature ($^{\circ}\text{C}$), $B(t)$ and $C(t)$ represent the temperature dependencies of the measured optical signal of the third strand in the bound and the dangling (‘single-stranded’) states, respectively, and $\sigma(t)$ is shown in equation 2:

$$\sigma(t) = 1 / \{1 + \exp[\Delta H/R(1/T - 1/T_m)]\} \quad (2)$$

where ΔH is the transition enthalpy, T is the absolute temperature ($t + 273.15$) and T_m is the melting temperature (K).

For UV melting curves, $B(t)$ and $C(t)$ are defined by $a(1 - bt)$, the linear dependencies of extinction coefficients of the bases in the bound third strand and in the dangling third strand, respectively.

In the case of the fluorescence melting curve $B(t) = a \cdot c \cdot (1 - bt)$, where c is a fraction of fluorescing 2AP in the bound third strand at 0°C . $C(t) = d \cdot (e^{-nt} + m) / (1 + m)$ describes an exponential quenching of 2AP in a dangling third ‘single strand’ due to collision events with solvent. Parameters d , n and m were experimentally determined by ‘melting’ the R^{2AP} single strand, and these values were used during fitting the theoretical curve (equation 1) to the experimental fluorescence melting curve.