

Mono-valent salt corrections for RNA secondary  
structures in the ViennaRNA package

## **Supplementary Data**

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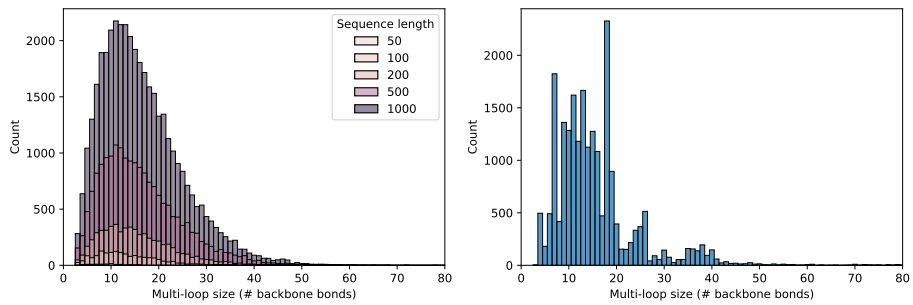


Figure S1: Size distribution of multi-loops. Distribution of multi-loop size  $L$ , number of backbone bonds, among predicted MFE structures (left) and natural RNA structures (right). The MFE structure is computed with `RNAfold` at standard condition on 5 000 uniformly and randomly selected sequences from  $\{A, C, G, U\}^n$  for each length  $n \in \{50, 100, 200, 500, 1\ 000\}$ . In total, 3 582 natural RNA secondary structures featuring multi-loops is downloaded from RNA STRAND database [1]. The distribution is drawn with outlier removal, multi-loop size larger than 80.

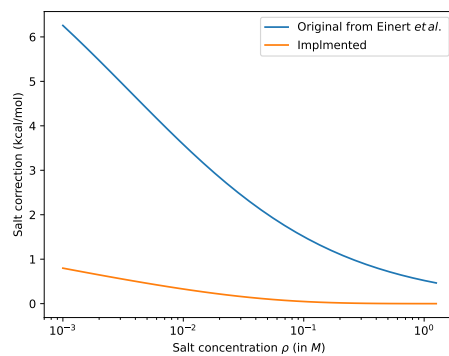


Figure S2: Approximation Error for  $K_0$ . In [2] an approximation for the difference of  $K_0$  at a given concentration and  $1M$  was proposed. However, we noticed that this approximation yields a non-vanishing salt correction at  $1M$ . We therefore used the Cephes library to compute  $K_0$  directly. The panel shows the salt correction of base pair stack at  $37^\circ C$  in the function of salt concentration using the approximation (blue) and the precise computation implemented in ViennaRNA (orange).

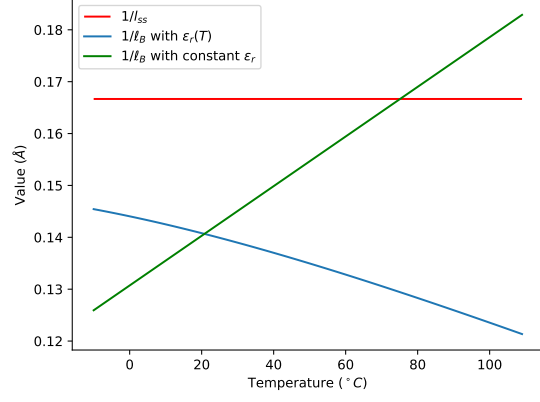


Figure S3: Nonlinear electrostatic effects  $\tau_{ss}$ . In [2], the permittivity (relative dielectric constant)  $\epsilon_r$  of water  $\epsilon_r \approx 80$  is assumed to be temperature independent. This assumption results in a discontinuity of  $\tau_{ss}$  at around  $75^\circ C$ . Incorporating the empirical temperature dependence of  $\epsilon_r$  results in  $1/\ell_B < 1/l_{ss}$ .

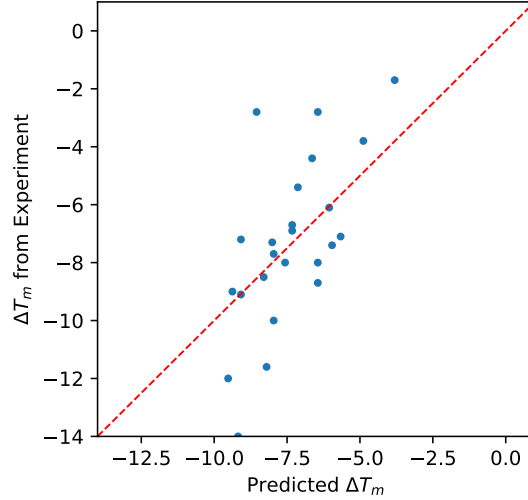


Figure S4: Comparison of experimental and predicted melting temperature corrections  $\Delta T_m$  of DNA duplexes. Experimental DNA duplex melting temperature data is from Table 5 in [3]. Note that only the duplexes with length smaller or equal to 11 are considered.

Due to the lack of DNA energy data at different salt concentrations, the salt correction for DNA duplex initialization is fitted to melting temperature data. The melting temperature corrections from 1.02M for DNA duplexes of length 10 and 11 in Table 2 in [3] are used for fitting. Note that the DNA sequence used is not self-complementary. Let  $A$  and  $B$  be DNA sequences and  $AB$  the corresponding dimer. The dimerization reaction is  $A + B \rightleftharpoons AB$ . The corresponding concentrations are denoted by  $[A]$ ,  $[B]$  and  $[AA]$ , respectively. In equilibrium, we have

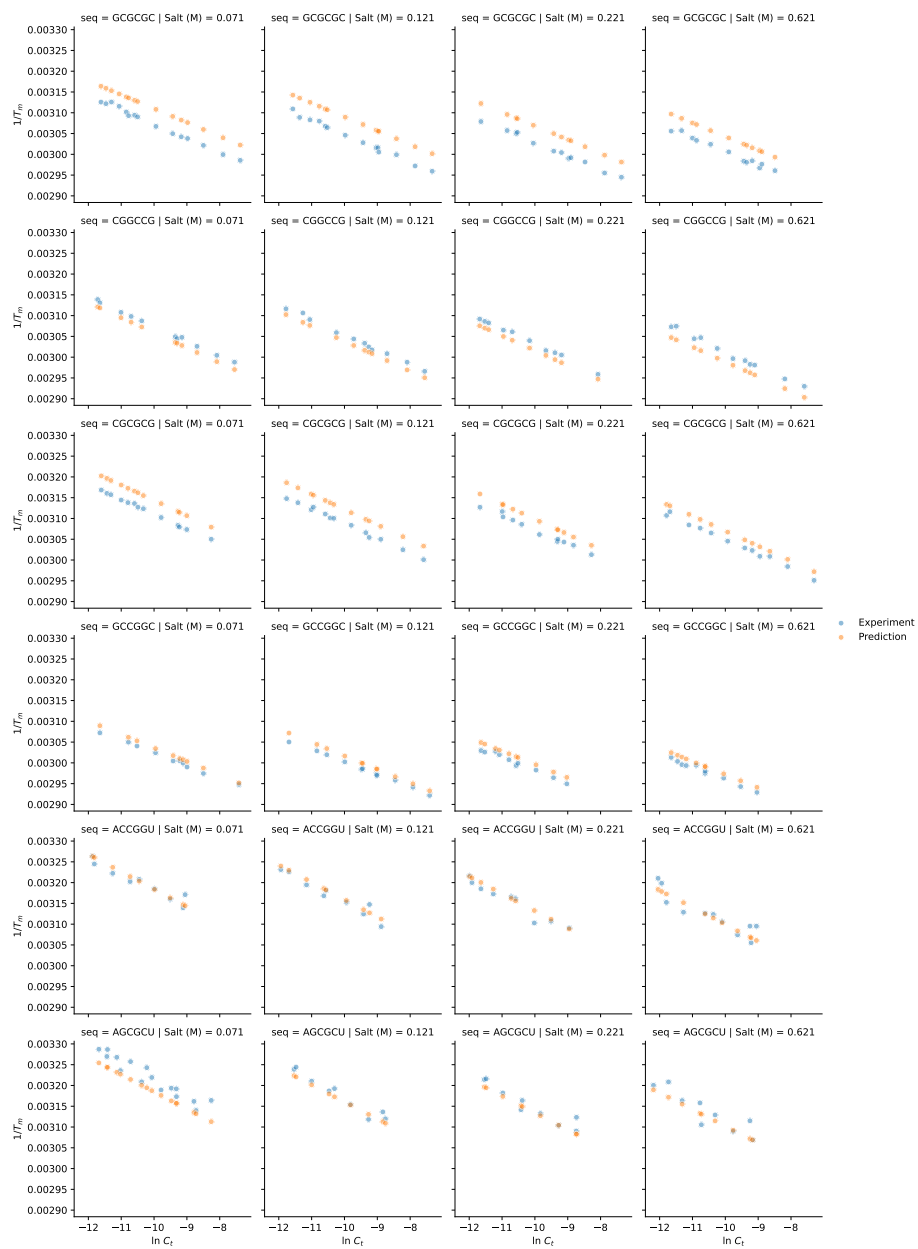
$$\frac{[AB]}{[A][B]} = e^{(G_A + G_B - G_{AB})/RT} \quad (1)$$

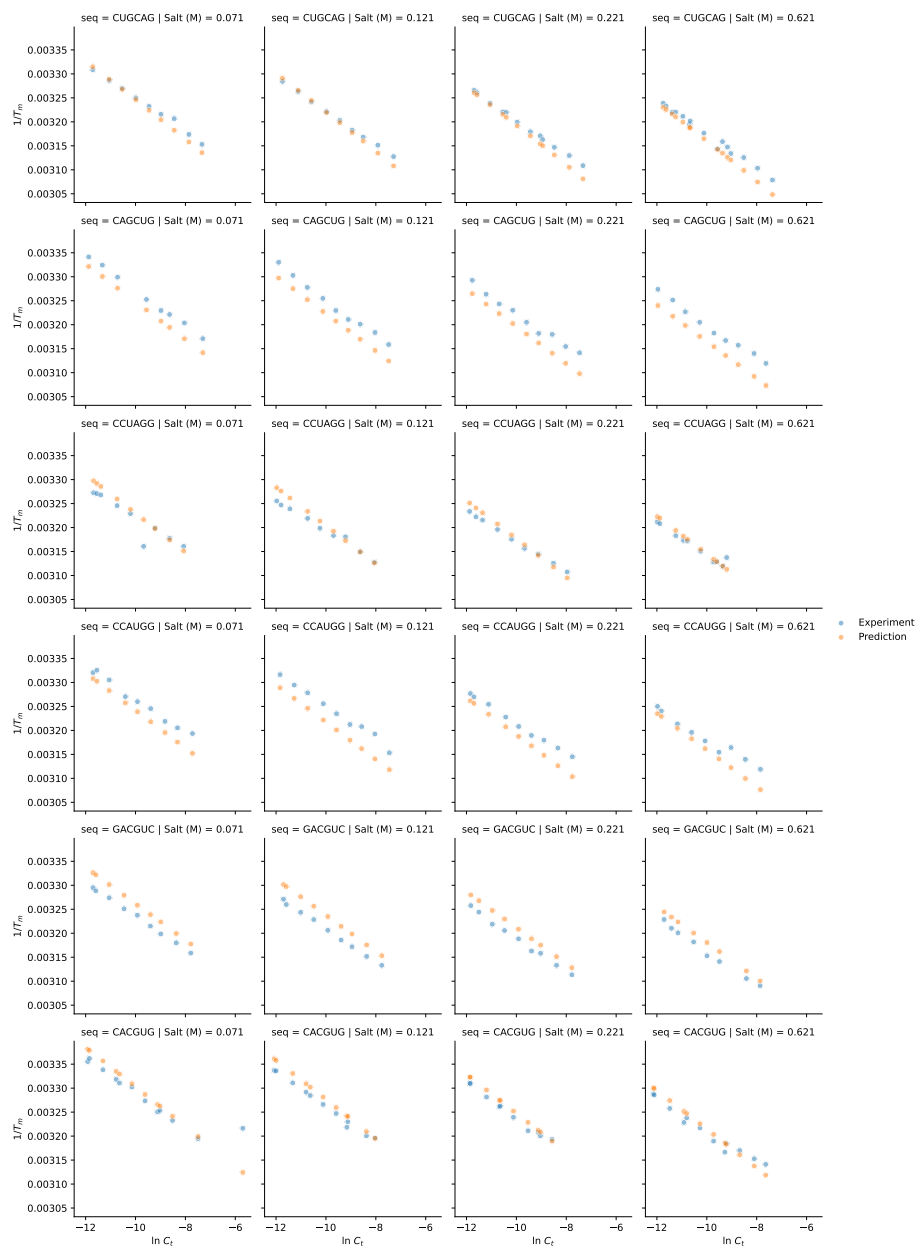
where  $G_A$ ,  $G_B$ , and  $G_{AB}$  are the *ensemble* free energies of  $A$ ,  $B$ , and  $AA$ , respectively. The melting temperature  $T_m$  is the temperature at which half of  $A$  and  $B$  form the dimer  $AB$ , i.e., where  $[A] = [B] = [AA] = c/4$  with  $c$  is the initial concentration of the single strand. Equ.(1) then yields

$$T_m = \frac{G_A(T_m) + G_B(T_m) - G_{AA}(T_m)}{R \ln 4 - R \ln c}, \quad (2)$$

The fitting yields the salt correction for DNA duplex initialization

$$g_{\text{init}}(\rho) = a \ln\left(\frac{\rho}{\rho_0}\right) \quad \text{with} \quad a = -0.58389 \text{ kcal/mol}. \quad (3)$$





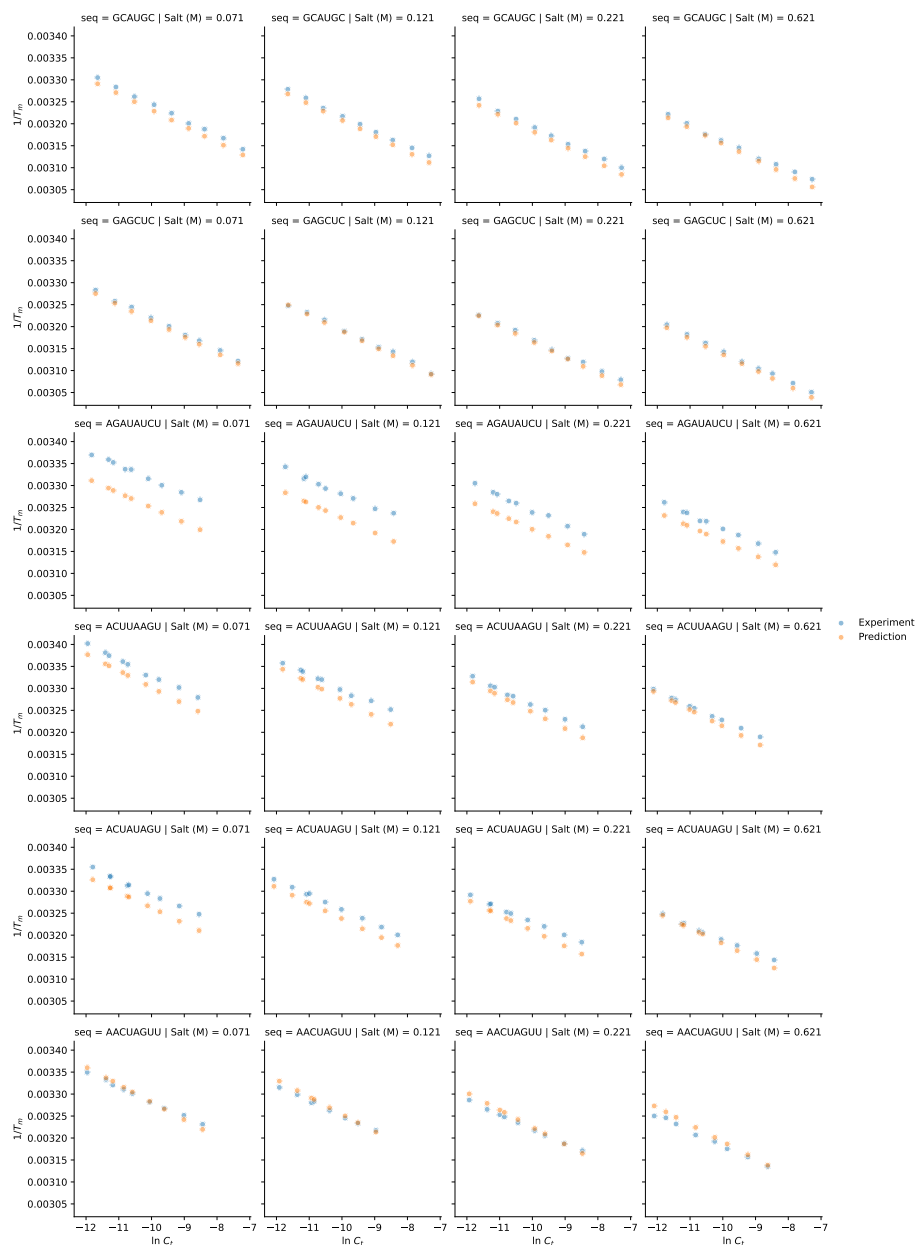


Figure S5: Van t' Hoff plots for 18 duplexes. Plotting  $1/T_m$  versus  $\ln c$  shows a generally good agreement of between predictions and the experimental data from [4, 5].



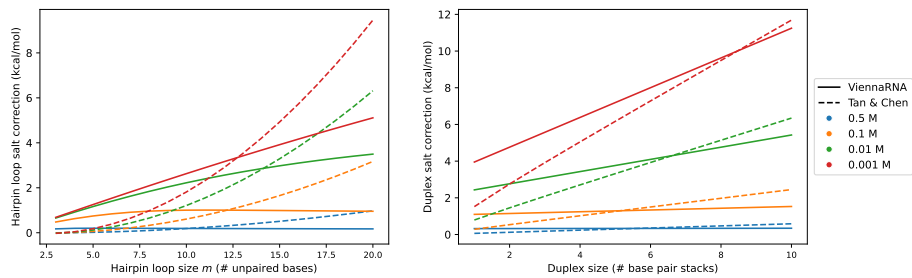


Figure S6: Comparison of salt correction for hairpin loop (left) and duplex (right) with Tan & Chen model (dashed). The salt correction for duplex initialization is included in the one for duplex computed with ViennaRNA. For Tan & Chen model, the correction for hairpin loop is computed using Equ.(9) of [6] with the end-to-end distance  $x = 17 \text{ \AA}$ , while the one for duplex is from Equ.(13) in [7].

Table S1: Numeric values of physical constants.

Constant	Symbol [units]	Value
Unit charge	$e$ [C]	$1.602 \cdot 10^{-19}$
Boltzmann constant	$k_B$ [eV/K]	$8.617 \cdot 10^{-5}$
Vacuum permittivity	$\epsilon_0$ [F/Å]	$8.854 \cdot 10^{-22}$
Avogadro constant	$N_A$ [mol <sup>-1</sup> ]	$6.022 \cdot 10^{23}$

## References

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