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

Supplementary Data

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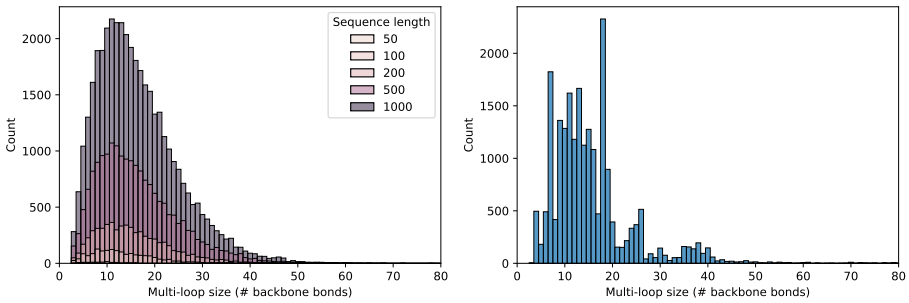


Figure S1: Size distribution of multi-loops. Distribution of multi-loop size L, number of backbone bonds, among predicted MFE structures (left) and natural RNA structures (right). The MFE structure is computed with `RNAfold` at standard condition on 5 000 uniformly and randomly selected sequences from $\{A, C, G, U\}^n$ 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.

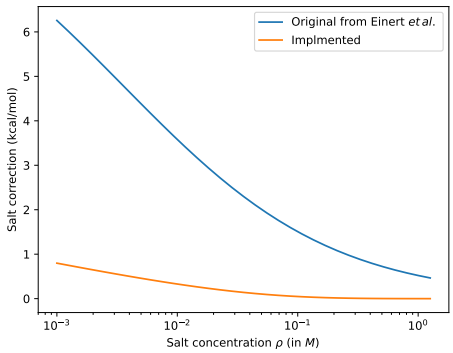


Figure S2: Approximation Error for K_0. In [2] an approximation for the difference of K_0 at a given concentration and $1M$ was proposed. However, we noticed that this approximation yields a non-vanishing salt correction at $1M$. We therefore used the Cephes library to compute K_0 directly. The panel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

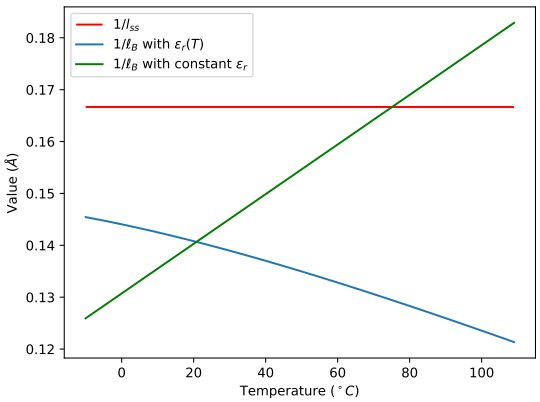


Figure S3: Nonlinear electrostatic effects τ_{ss}. In [2], the permittivity (relative dielectric constant) ϵ_r of water $\epsilon_r \approx 80$ is assumed to be temperature independent. This assumption results in a discontinuity of τ_{ss} at around $75\,^{\circ}C$. Incorporating the empirical temperature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

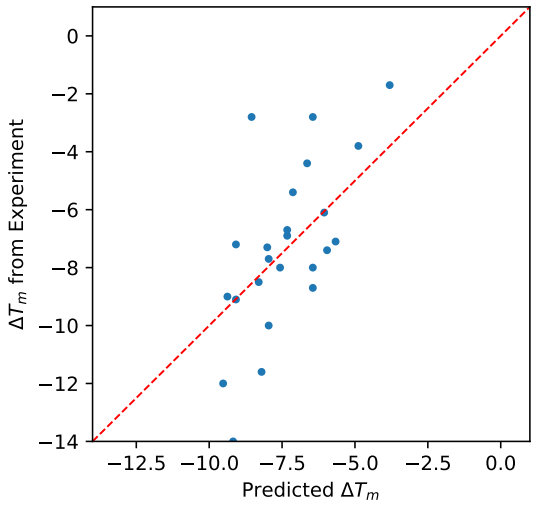


Figure S4: Comparison of experimental and predicted melting temperature corrections ΔT_m of DNA duplexes. Experimental DNA duplex melting temperature data is from Table 5 in [3]. Note that only the duplexes with length smaller or equal to 11 are considered.

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

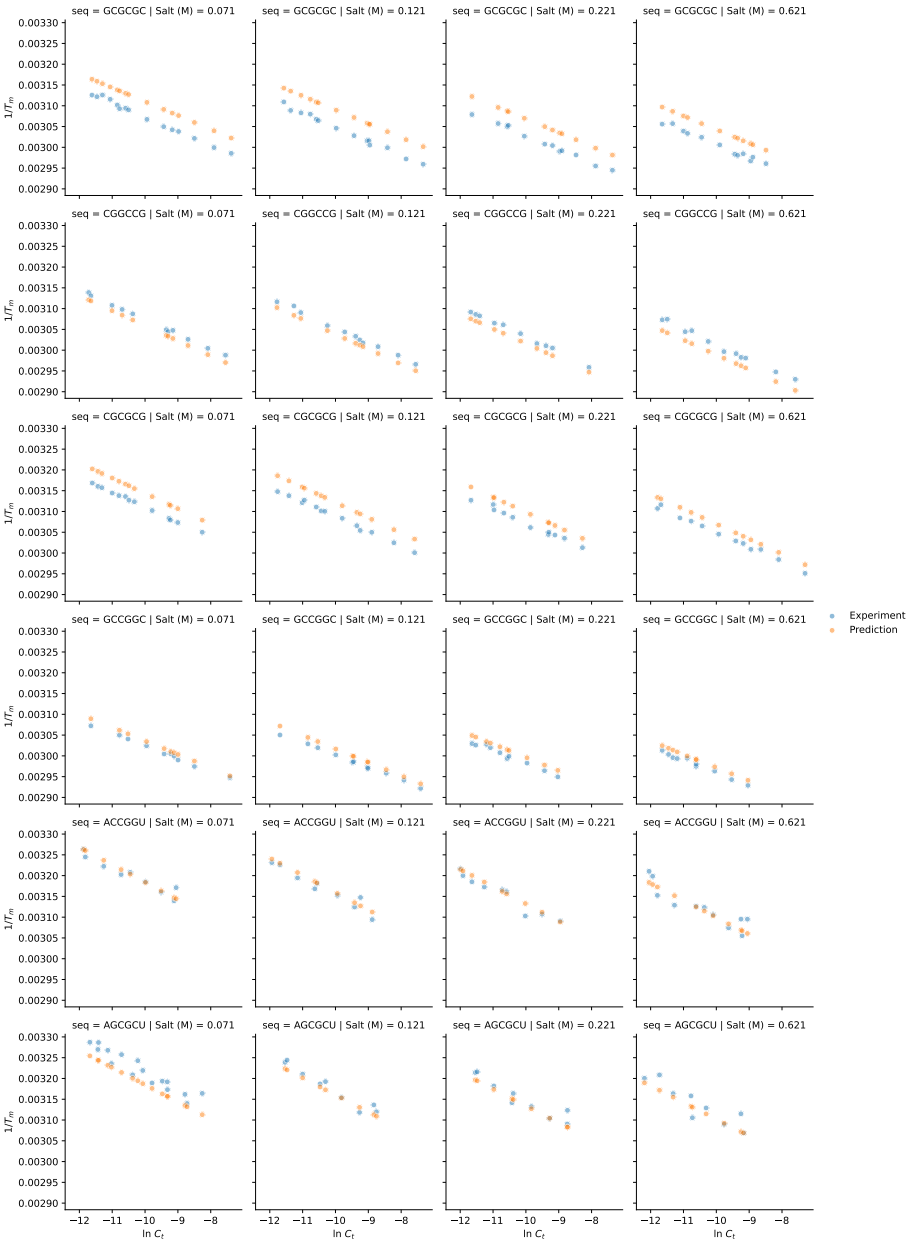
$$\frac{[AB]}{[A][B]} = e^{(G_A+G_B-G_{AB})/RT} \tag{1}$$

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

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

The fitting yields the salt correction for DNA duplex initialization

$$g_{\text{init}}(\rho) = a\ln(\frac{\rho}{\rho_0}) \qquad \text{with} \quad a = -0.58389\text{kcal/mol}. \tag{3}$$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seq = GCGCGC | Salt (M) = 0.621
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seq = CGGCCG | Salt (M) = 0.121
seq = CGGCCG | Salt (M) = 0.221
seq = CGGCCG | Salt (M) = 0.621
seq = CGCGCG | Salt (M) = 0.071
seq = CGCGCG | Salt (M) = 0.121
seq = CGCGCG | Salt (M) = 0.221
seq = CGCGCG | Salt (M) = 0.621
seq = GCCGGC | Salt (M) = 0.071
seq = GCCGGC | Salt (M) = 0.121
seq = GCCGGC | Salt (M) = 0.221
seq = GCCGGC | Salt (M) = 0.621
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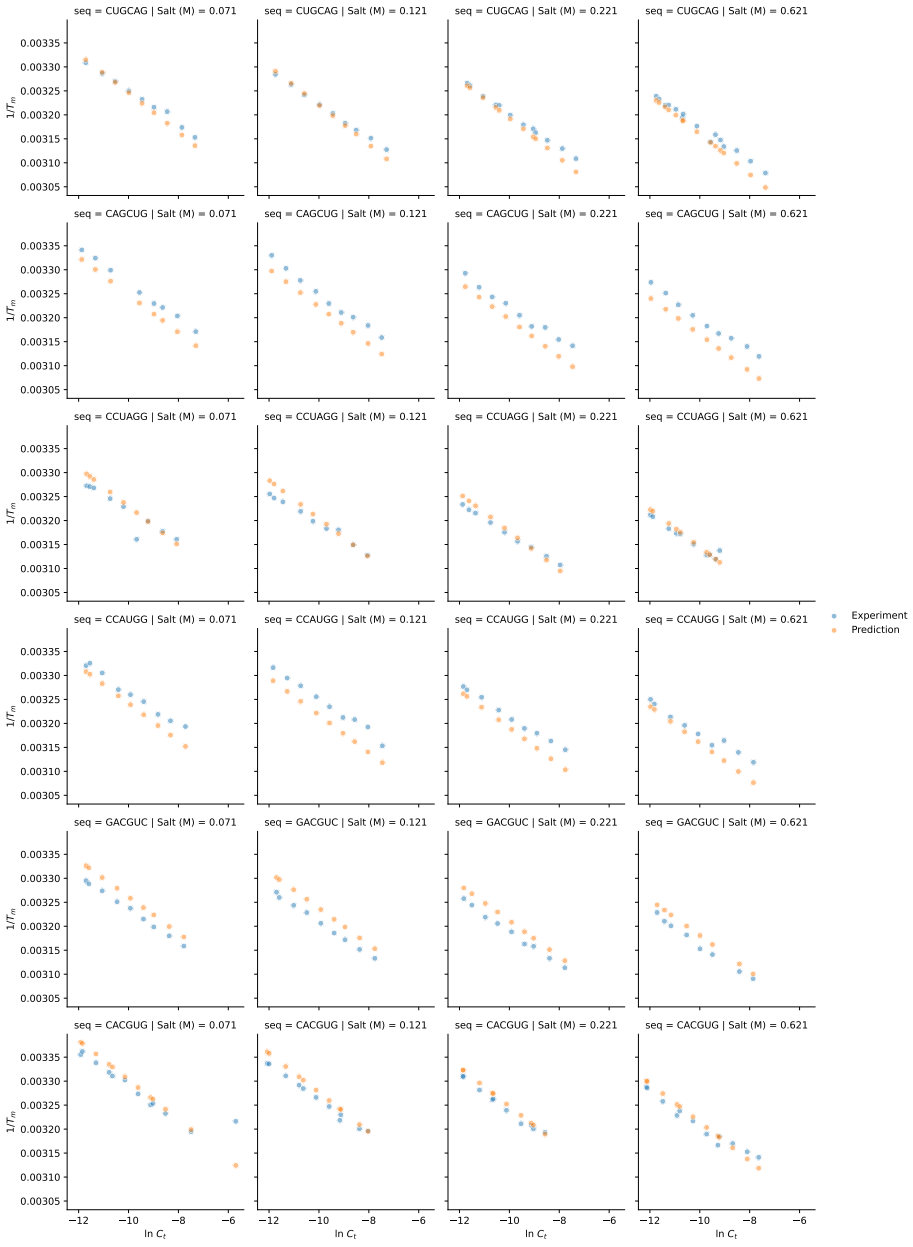
seq = ACCGGU | Salt (M) = 0.121
seq = ACCGGU | Salt (M) = 0.221
seq = ACCGGU | Salt (M) = 0.621
seq = AGCGCU | Salt (M) = 0.071
seq = AGCGCU | Salt (M) = 0.121
seq = AGCGCU | Salt (M) = 0.221
seq = AGCGCU | Salt (M) = 0.621
$1/T_m$
$\ln C_t$
Experiment
Prediction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seq = CAGCUG | Salt (M) = 0.221
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seq = CCUAGG | Salt (M) = 0.071
seq = CCUAGG | Salt (M) = 0.121
seq = CCUAGG | Salt (M) = 0.221
seq = CCUAGG | Salt (M) = 0.621
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seq = CCAUGG | Salt (M) = 0.121
seq = CCAUGG | Salt (M) = 0.221
seq = CCAUGG | Salt (M) = 0.621
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seq = GACGUC | Salt (M) = 0.121
seq = GACGUC | Salt (M) = 0.221
seq = GACGUC | Salt (M) = 0.621
seq = CACGUG | Salt (M) = 0.071
seq = CACGUG | Salt (M) = 0.121
seq = CACGUG | Salt (M) = 0.221
seq = CACGUG | Salt (M) = 0.621
$1/T_m$
0.00335
0.00330
0.00325
0.00320
0.00315
0.00310
0.00305
−12
−10
−8
−6
ln C_t
Experiment
Prediction

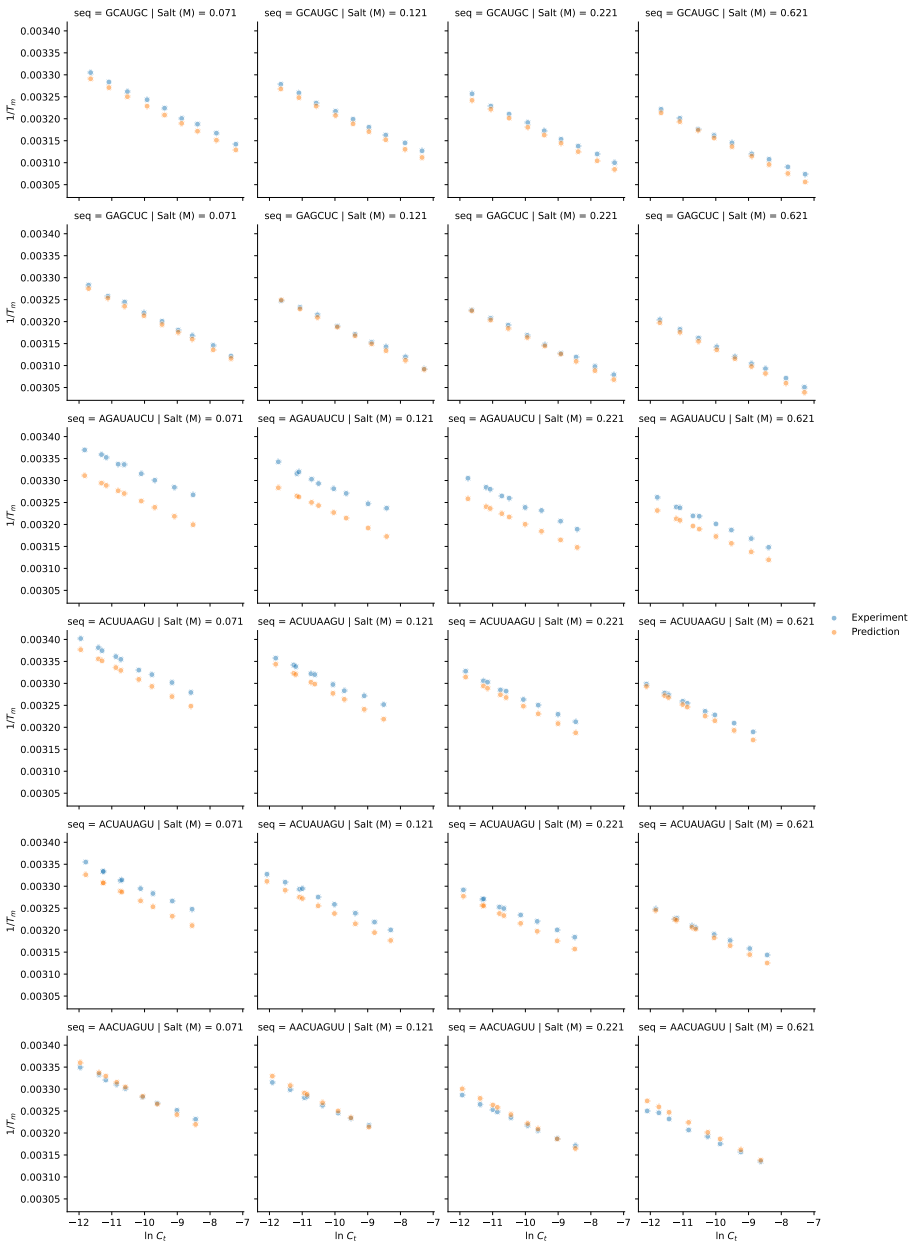

Figure S5: Van t' Hoff plots for 18 duplexes. Plotting $1/T_m$ versus $\ln c$ shows a generally good agreement of between predictions and the experimental data from [4, 5].

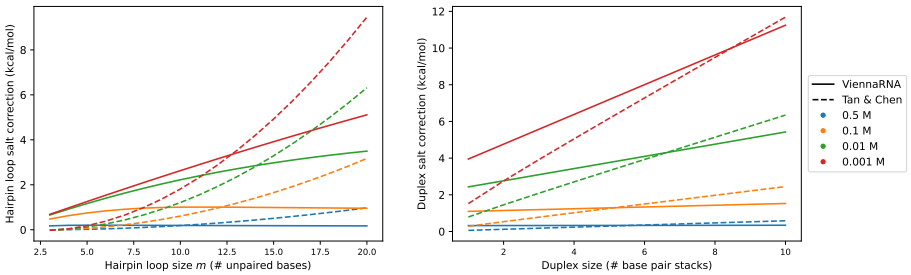

Figure S6: Comparison of salt correction for hairpin loop (left) and duplex (right) with Tan & Chen model (dashed). The salt correction for duplex initialization is included in the one for duplex computed with ViennaRNA. For Tan & Chen model, the correction for hairpin loop is computed using Equ.(9) of [6] with the end-to-end distance $x = 17\,\mathring{A}$, 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	ϵ_0 [F/Å]	$8.854 \cdot 10^{-22}$
Avogadro constant	N_A [mol^{-1}]	$6.022 \cdot 10^{23}$

References

[1] Andronescu, M., Bereg, V., Hoos, H.H., Condon, A.: RNA STRAND: the RNA secondary structure and statistical analysis database. BMC Bioinformatics **9**, 340 (2008). doi:10.1186/1471-2105-9-340

[2] Einert, T.R., Netz, R.R.: Theory for RNA folding, stretching, and melting including loops and salt. Biophys. J. **100**, 2745–2753 (2011). doi:10.1016/j.bpj.2011.04.038

[3] Owczarzy, R., You, Y., Moreira, B.G., Manthey, J.A., Huang, L., Behlke, M.A., Walder, J.A.: Effects of sodium ions on DNA duplex oligomers: improved predictions of melting temperatures. Biochemistry **43**(12), 3537–3554 (2004). doi:10.1021/bi034621r

[4] Chen, Z., Znosko, B.M.: Effect of sodium ions on RNA duplex stability. Biochemistry **52**(42), 7477–7485 (2013). doi:10.1021/bi4008275

[5] Ferreira, I., Jolley, E.A., Znosko, B.M., Weber, G.: Replacing salt correction factors with optimized RNA nearest-neighbour enthalpy and entropy parameters. Chem. Phys. **521**, 69–76 (2019). doi:10.1016/j.chemphys.2019.01.016

[6] Tan, Z.-J., Chen, S.-J.: Salt dependence of nucleic acid hairpin stability. Biophys. J. **95**(2), 738–752 (2008). doi:10.1529/biophysj.108.131524

[7] Tan, Z.-J., Chen, S.-J.: RNA helix stability in mixed Na^+/Mg^{2+} solution. Biophys J **92**(10), 3615–3632 (2007). doi:10.1529/biophysj.106.100388