

Calculation of T_m for Oligonucleotide Duplexes

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The melting temperature of an oligonucleotide duplex (T_m) refers to the temperature at which the oligonucleotide is 50% annealed to its exact complement. Due to the extreme cooperativity seen in DNA hybridization and melting, this means that 50% of the molecules are single-stranded (SS) while 50% of the molecules are in the double-stranded (DS) form. All mathematical modeling of T_m used here assumes this simple two-state model, which seems to experimentally hold true for most short oligo sequences. Accurate estimation of the T_m of an oligonucleotide probe-target duplex is important for a wide variety of applications including PCR, hybridization, sequencing, and antisense/RNAi applications. In the absence of destabilizing agents such as urea or formamide, the T_m of an oligonucleotide will depend upon three major factors:

1. Oligonucleotide concentration (C_i): High DNA concentrations favor duplex formation and increase T_m (See Figure 1).
2. Salt concentration: The T_m increases with higher ionic concentrations of the solvent due to the stabilizing effects that cations have on DNA duplex formation. More cations bind to duplex DNA than to the component single strands. Different cations may have different effects on T_m . Most T_m research is done using Na^+ as the primary cation; from a T_m standpoint, sodium and potassium are functionally interchangeable. Divalent cations (such as Mg^{++}) also stabilize DNA hybrids (increase T_m) but their effects are quantitatively much different from monovalent cations. Magnesium effects are currently under investigation at IDT and we hope to add terms to correct for Mg^{++} ions in buffers to our calculations in the near future.
3. Oligonucleotide sequence: Generally, sequences with higher fraction of G-C base pairs, $f(\text{G}\bullet\text{C})$, have a higher T_m than do AT-rich sequences. However, the T_m of an oligo is not simply the sum of AT and GC base content. Base stacking interactions must also be taken into account such that the actual specific sequence must be known to accurately predict T_m . The effects of neighboring bases as contributed through base stacking are called "nearest neighbor effects" which are mathematically accounted for by calculations made using experimentally determined nearest neighbor (NN) thermodynamic parameters.

For oligos of the length ranges used today, the best method to estimate the T_m of an oligonucleotide probe–target duplex (which is used at IDT in all of our web calculators and on our oligo spec sheets), takes into account all the above factors, including nearest-neighbor interactions (Breslauer et al., 1986; Santa-Lucia et al., 1998; Sugimoto et al., 1995, 1996; Xia et al., 1998), salt concentration, and oligo concentration. Assuming that the concentration of the oligonucleotide probe is much higher than concentration of DNA target, the following thermodynamic relationship can be used to predict T_m ,

$$T_m (\text{Kelvin}) = \frac{\Delta H^\circ}{\Delta S^\circ + R \ln C_t} \quad [1]$$

where the changes in standard enthalpy (ΔH°) and entropy (ΔS°) associated with duplex formation are calculated from nearest-neighbor thermodynamic parameters (1, 4, and 5). “R” is the ideal gas constant ($1.987 \text{ cal.K}^{-1}.\text{mole}^{-1}$), and C_t is the molar concentration of oligonucleotide probe. Historically, the nearest-neighbor parameter set of Breslauer et al. (1986) has been used to estimate the melting temperatures of oligonucleotide duplexes. However, several improved NN parameter sets have been published in recent years which offer better accuracy (Santa-Lucia et al., 1998; Sugimoto et al., 1995, 1996; Xia et al., 1998). In agreement with the detailed analysis of Owczarzy et al. (1997), our research lab at IDT finds that the newest sets of nearest-neighbor thermodynamic parameters for DNA, RNA, and DNA/RNA duplexes provide the most accurate predictions of melting temperatures. Therefore, these parameter sets are used to estimate T_m in all calculations done at IDT.

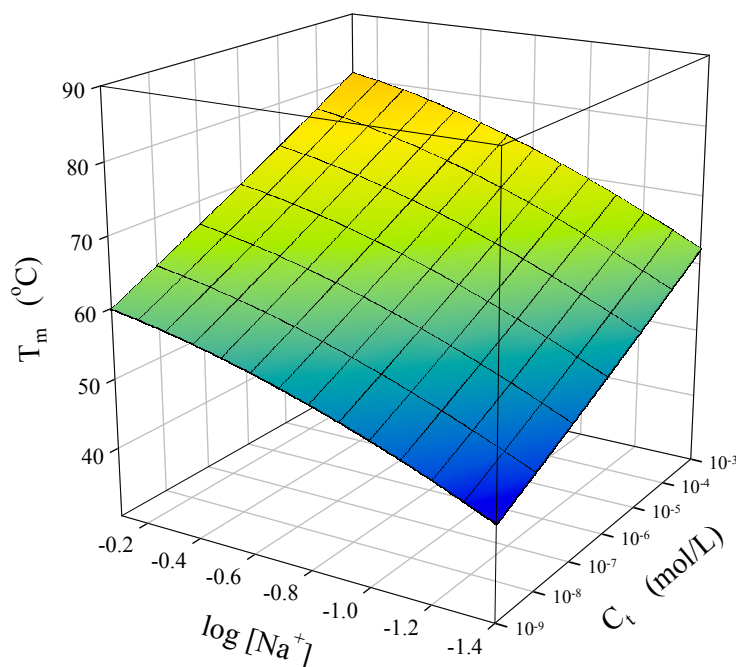


Figure 1. The dependence of T_m on Na^+ ion and DNA concentration for a 20 base pair duplex, 5'-TAGTGGCGATTAGATTCTGC-3'

Use of Equation [1] above results in a calculated T_m for duplexes in 1M Na^+ ion buffers. This estimated T_m must be scaled to a new salt corrected T_m if a use of a different buffer is intended. All of the NN parameters were obtained from DNA melt studies done in 1M Na^+ buffer and this is the default condition used for all calculations. To predict melting temperatures at different ionic concentration, $[\text{Na}^+]$, a variety of T_m salt correction equations have been suggested (Schildkraut and Lifson, 1965; Wetmer, 1991). Our biophysics research group at IDT has been studying the accuracy of T_m prediction algorithms and will be introducing in upcoming years a number of improvements that will increase the accuracy of our ability to predict T_m . In a study that included almost 3000 T_m measurements done on 92 DNA duplexes in a variety of conditions, we recently published a new equation to scale T_m for changes in monovalent cation concentration (Owczarzy et al., 2004):

$$\frac{1}{T_m(\text{Na}^+)} = \frac{1}{T_m(1\text{M})} + (4.29f(\text{G}\bullet\text{C}) - 3.95) \times 10^{-5} \ln[\text{Na}^+] + 9.40 \times 10^{-6} \ln^2[\text{Na}^+] \quad [2]$$

Figure 2 shows that the new salt correction formula Equation [2] is more accurate than any of the previously used T_m salt corrections. Elements that make the new salt correction formula more accurate than previous equations include a quadratic dependence (the salt effect is not linear) and a correction for oligo GC content. For more details, see Owczarzy et al. (2004).

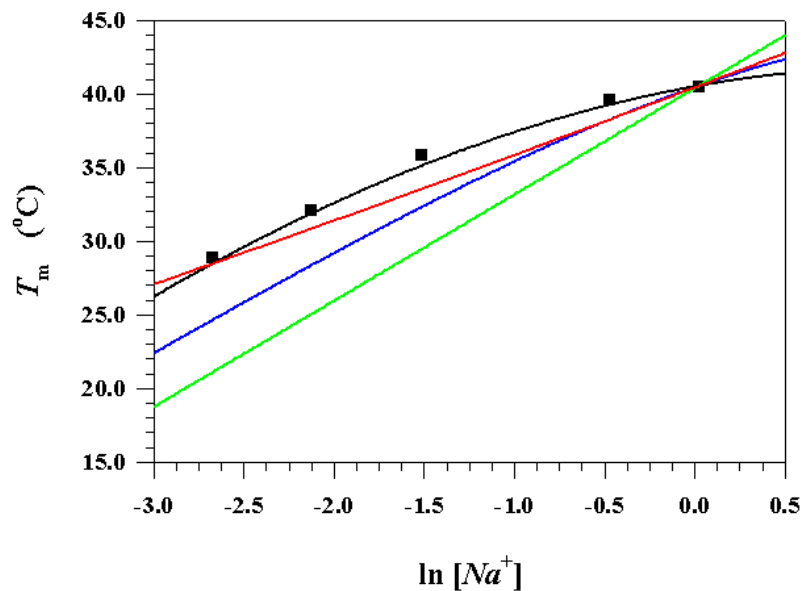


Figure 2. Comparison of some commonly used salt corrections and our new T_m salt correction. Experimentally measured (■) and predicted melting temperatures for DNA duplex oligomer, 5'-CCAACCTTCTT-3' are shown. Salt-corrected melting temperatures from 1 M Na^+ buffer to lower Na^+ concentrations are calculated by new equation [2] (black line), Schildkraut-Lifson salt correction (green line), Wetmur salt correction (blue line) and SantaLucia unified parameters salt correction (red line).

IDT's T_m calculator (OligoAnalyzer 3.0 in SciTools at www.idtdna.com) and the T_m values given on our specification sheets are predicted using the latest nearest-neighbor thermodynamic parameters and our new salt correction. This approach provides oligonucleotide users with the most accurate estimate of T_m currently available with the average error of $\pm 2^\circ\text{C}$.

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