$$T(p) = \frac{dH}{dS - R\ln\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}}$$

We begin with two types of ssDNA, ssDNA1 with initial concentration [ssDNA1] and ssDNA2 with initial concentration [ssDNA2] $\geq$ [ssDNA1]. The when all strands are coiled, we have a final concentration of dsDNA, [dsDNA1] = [ssDNA1] and an excess concentration of ssDNA2 equal to [ssDNA2] $-$ [ssDNA1]. If p represents the proportion of final dsDNA product that is uncoiled so that $(1-p)$[dsDNA] represents the concentration of coiled dsDNA, then p is also the uncoiled proportion of ssDNA1, with p[ssDNA1] and [ssDNA2] $-(1-p)$[ssDNA1] the concentrations of ssDNA1 and ssDNA2 respectively. The expression $\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}$ becomes

$$\frac{1-p}{p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])}$$

.

When $p = 1/2$, at T_M, this is

$$\frac{1}{[\text{ssDNA2}]-1/2[\text{ssDNA1}]).}$$

so since inverting an expression inside a log corresponds to a change of sign outside,

$$T(p) = \frac{dH}{dS - R\ln\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}} = \frac{dH}{dS + R\ln[\text{ssDNA2}]-1/2[\text{ssDNA1}])},$$

the form in TmCalculators and TmPrecalc.

When [ssDNA2] = [ssDNA1], $\frac{1-p}{p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])}$ becomes $\frac{1-p}{p^2[\text{ssDNA1}]}$ so since the log of a quotient is the difference of the logs,

$$T(p) = \frac{dH}{dS - R\ln\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}} = \frac{dH}{dS + R\ln[\text{ssDNA1}] - R\ln\frac{1-p}{p^2}}.$$

In the equal concentrations case, we proceed as follows.

Setting $K = R\ln[\text{ssDNA1}]$ and solving

$$T(p) = \frac{dH}{dS + C(p)} = \frac{dH}{dS + K - R\ln\frac{1-p}{p^2}}$$

for p, we get

$$ap^2 + p - 1 = 0$$

Here p is the proportion uncoiled and $1-p$ is the proportion coiled, and $a = e^{-dG(T)}$, where $G = (dH - TdS - KT)/RT$, where K depends only on probe and target concentrations, and dH and dS depend only upon the nearest neighbor parameters and properties of the oligo under consideration.

Since we know $C(.5)$, we can obtain

$$K = C(.5) + R \ln .5.$$

Dividing by a and setting $v = 1/a$, we solve for the positive root p of the quadratic equation

$$p^2 + vp - v = 0,$$

$$p = \frac{-v + \sqrt{v^2 + 4v}}{2}.$$

Some observations about the dependence of G, a, and v on T.

Since $dH < 0, dS < 0, K < 0$ (depending upon [ssDNA1]), as $T \to 0$, $dG = (dH - TdS - KT)/RT = (\frac{dH}{RT} \frac{-dS-K}{R} =\to -\infty$ so $a \to +\infty$, $v \to 0^+$.

Interestingly, as $T \to +\infty$, $dG \to \frac{-dS-K}{R} > 0$, $a \to a_*$ with $0 < a_* << 1$, $v \to v_*$ with $1 << v_* << +\infty$.

So as $T \to 0$, $p \to 0$ and the fraction coiled $1 - p \to 1$. but as $T \to +\infty$, $p \to p_*$ with $0 < 1 - p_* << 1$. A minimum proportion of DNA remains coiled no matter how great T becomes.

Rewriting

$$p = \frac{-v + \sqrt{v^2 + 4v}}{2}$$

in terms of $w = v + 2$ to complete the square, we obtain

$$p = \frac{-(v+2) + 2 + \sqrt{(v+2)^2 - 4}}{2}$$

or

$$p = 1 - \frac{w - \sqrt{w2 - 4}}{2}$$

from which the fraction coiled, $1 - p$ becomes

$$1 - p = \frac{w}{2} - \sqrt{\frac{w}{2}^2 - 1}$$

where $\frac{w}{2} = \frac{w(T)}{2} = 1 + \frac{1}{2} e^{dG(T)}$. So $w \to 1$ as $T \to 0$. The melting temperature T_M is where this difference is $1/2$. Solving $x - 1/2 = \sqrt{x^2 - 1}$, we get this occurs when $x = w/2 = 5/4$.

So the sharp phase transition is due to the fact that $e^{dG(T)}$ remains very small until the melting temperature then grows quickly to its large positive limit e^{dG_*}. $e^{dG(T)}$ is of the form $e^{a - \frac{b}{T}}$, where $a > 0, b > 0$, so when T is small, the second term is dominant and the result is extremely small, but as T grows, the first term eventually dominates, with

a rapid transition to extremely large values. This function becomes the argument of a function with values near 1 for small values of its argument, and near zero for large values of its argument, hence the form of the melting curves.

In the general case,

$$T(p) = \frac{dH}{dS - R\ln\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}} = \frac{dH}{dS - R\ln\frac{1-p}{p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])}}$$

.

Rearranging again,

$$p([\text{ssDNA2}] - (1-p)[\text{ssDNA1}])e^{-\frac{dH-TdS}{RT}} + p - 1 = 0$$

$$[\text{ssDNA1}])e^{-\frac{dH-TdS}{RT}}p^2 + (1 + ([\text{ssDNA2}] - [\text{ssDNA1}])e^{-\frac{dH-TdS}{RT}}) - 1 = 0$$

$$ap^2 + bp - 1 = 0$$

where $a = [\text{ssDNA1}]e^{-\frac{dH-TdS}{RT}}$ and $b = 1 + ([\text{ssDNA2}] - [\text{ssDNA1}])e^{-\frac{dH-TdS}{RT}})$

We note that when the initial concentrations are equal, the general case reduces to the original equal concentration case, since the expression for b becomes 1, and the expression for a is identical to the expression for a only depends on the smaller initial concentration. Also, since the equal concentration case $K = R\ln[\text{ssDNA1}]$ a may also be written as $e^{-\frac{dH-TdS-KT}{RT}}$, no different than in the equal concentration case, and in that case,

If we let $d = [\text{ssDNA2}] - [\text{ssDNA1}]$ so that $b = 1 + d$, and again dividing through by a, so the quadratic equation becomes monic, $p^2 + \frac{(1+d)}{a} - \frac{1}{a} = 0$, we call $v = \frac{1}{a}$ (which has the same value as before in terms of the initial concentration of ssDNA1 and dH, dS, and R so $p^2 + (1+d)vp - v = 0$ and the melting curve solution is given by

$$p = \frac{-(1+d)v + \sqrt{((1+d)v)^2 + 4v}}{2}.$$

Exercise: Complete the square and try to make analogous transformations to obtain a simpler form for $1 - p$ as in the equal concentration case.

In the equal concentrations case, we are able to compute $T(p)$ for different p by determining $C(p) = K - R\ln\frac{1-p}{p^2}$, and either using the formula $K = R\ln[\text{ssDNA1}]$ or solving for K from the known value of $C(p)$ when $p = .5$, $\frac{C(.5)=R\ln[\text{ssDNA1}]}{2}$ $C(.5) = K - \ln 2$, so $K = C(.5) + \ln 2$. Another form is $C(p) = C(.5) + \ln 2 - R\ln\frac{1-p}{p^2}$ so we only need to know $C(.5)$ the factor used in the melting temperature calculator to compute all other $T(p)$s.

This is not so in the general case. To compute $C(p)$, hence $T(p)$, use

$$T(p) = \frac{dH}{dS - R\ln\frac{[\text{dsDNA}]}{[\text{ssDNA1}][\text{ssDNA2}]}} = \frac{dH}{dS - R\ln\frac{1-p}{p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])}}$$

.

Here, $C(.5) = -R\ln\frac{1-p}{p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])} = R\ln([\text{ssDNA2}] - .5[\text{ssDNA1}])$ is not enough to compute $C(p)$.

Note that $p([\text{ssDNA2}]-(1-p)[\text{ssDNA1}])$ may be rewritten $p^2[\text{ssDNA1}]+p([\text{ssDNA2}]-[\text{ssDNA1}])$

TmCalculators and TmPrecalculators computed $C(.5)$ and for the equal concentration first example, we did use this to compute other T_Ms for parameter inversion. To do this in more generality, "$T(p)$-Precalc" must provide the data necessary to compute the coefficients for melting curve inversion and computing the temperature for an arbitrary fraction uncoiled, p. so TpPrecalc.vi delivers [ssDNA1] and [ssDNA2] − [ssDNA1] along with the salt dependent length factor.

$T(p)$-calculator takes these values and given p, as well as the values of dH and dS and the length of the oligo, computes $T(p)$. The default is $p = .5$, which reduces to a T_M calculator.

Curve.vi takes values of dH, dS and the length of an oligo, combines them with the initial concentrations and salt factor from T(p)-precalc, and for any value of T, computes the coefficients of the quadratic and solves them for $p(T)$.