Melting Curve Mathematics

Consider the hybridization of the Watson and Crick strands of a duplex. For simplicity, we will consider only non-self-complementary DNA. The equilibrium between ssDNA and dsDNA is:

$$W + C \Leftrightarrow W \bullet C \quad (1.1)$$

We define C_T, the total strand concentration, as follows:

$$C_T = [W] + [C] + 2[W \bullet C] = 2[W] + 2[W \bullet C] \quad (1.2)$$

Then we define α as the fraction of total DNA in double-stranded form:

$$\alpha \equiv \frac{2[W \bullet C]}{C_T} = \frac{C_T - 2[W]}{C_T} = 1 - 2\frac{[W]}{C_T}, \text{ so } [W] = \frac{(1-\alpha)C_T}{2} \quad (1.3)$$

Then we have the following equation for the equilibrium constant Keq:

$$Keq = \frac{[W \bullet C]}{[W][C]} = \frac{\alpha C_T / 2}{[(1-\alpha)C_T / 2]^2} = \frac{2\alpha}{(1-\alpha)^2 C_T} \quad (1.4)$$

Note that when $\alpha = 0.5$, the DNA is half "melted". The temperature at which this occurs is denoted Tm, the melting temperature. At this temperature,

$$Keq = 4/C_T. \quad (1.5)$$

Solving equation (1.4) for α using the quadratic formula and choosing the physically realizable root, we get

$$\alpha = \frac{1 + C_T Keq - \sqrt{1 + 2C_T Keq}}{C_T Keq} \quad (1.6)$$

The van't Hoff relationship is

$$Keq = \exp\left(-\frac{\Delta G^0}{RT}\right) = \exp\left(-\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}\right), \quad (1.7)$$

Substituting (1.7) into (1.6) gives us a very complicated formula for α as a function of C_T, ΔH^0, ΔS^0, and T

$$\alpha(C_T, \Delta H^0, \Delta S^0, T), \text{ not shown} \quad (1.8)$$

The optical absorbance at 260 nm of the sample is given by

$$A_{260} = \sum \varepsilon \text{cl} = (\varepsilon_W [W] + \varepsilon_C [C] + \varepsilon_{WC} [W \bullet C]) \quad (1.9)$$

The physical basis of the melting curve technique is that $\varepsilon_{WC} < \varepsilon_W + \varepsilon_C$, i.e. dsDNA exhibits hypochromism. In practice, we do not need to measure the individual extinction coefficients, although the concentrations of the two strands should be about the same. The extinction coefficients are, however, temperature dependent, and this must be accounted for in fitting/simulating the sloping baselines at low and high temperatures. We absorb the extinction coefficients into the measured absorbances at the beginning and end of the melting curve, where the DNA is exclusively ds and ss respectively. The derivation is simple but tedious, giving

$$A_{260}(T) = \alpha\left[A_{260}(T_{\min}) + \tfrac{1}{2} m_{ds}(T - T_{\min})\right] + (1-\alpha)\left[\left(A_{260}(T_{\max}) - \tfrac{1}{2} m_{ss}(T_{\max} - T)\right)\right] \quad (1.10)$$

where $A_{260}(T_{min})$ and $A_{260}(T_{max})$ are the measured absorbances at temperatures T_{min} and T_{max}, and m_{ss} and m_{ds} are the slopes representing the temperature dependence of the extinction coefficients of the ssDNA and dsDNA respectively. These are typically fit to the observed data.

Substituting (1.8) into (1.10) gives us a huge expression for the observed/predicted A_{260} as a function of thermodynamic variables, experiment-dependent absorbances, and empirically fit slopes. The Tm and the ΔG^0 at 37C, which are experimentally the most reliable, are calculated from ΔH^0 and ΔS^0 and (1.5), and the derivative curve numerically from the $A_{260}(T)$. This is all the math coded in the associated spreadsheet.