Analysis of Equilibrium Melting Curves and Differential Scanning Calorimetry

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Consider an equilibrium described by the following model:

\[ K_a = \frac{[\text{Products}]}{[\text{Reactants}]} \quad (1) \]

We know that:

\[ \Delta G = -RT \ln K_a \quad (2) \]
\[ \Delta G = \Delta H - T\Delta S \quad (3) \]

What happens to the equilibrium constant as the temperature is varied?

\[ \frac{\partial}{\partial T} \left( \frac{\Delta G}{T} \right)_P = -R \left( \frac{d \ln K_a}{dT} \right)_P \quad (4) \]
\[ \frac{\partial}{\partial T} \left( \frac{\Delta G}{T} \right)_P = -\frac{\Delta H}{T^2} \quad (5) \]
Combining equations (4) and (5) yields the *van’t Hoff equation*.

\[
\Delta H_{vH} = -RT^2 \left( \frac{d \ln K_a}{dT} \right)_p
\]

(6)

\[
\ln K_a = \frac{-(\Delta H - T\Delta S)}{RT}
\]

(7)

\[\Delta H = -10 \text{ kcal/mol}, \quad \Delta S = 0 \text{ cal/mol}\cdot\text{K}\]
\[\Delta H = -5 \text{ kcal/mol}, \quad \Delta S = 16.8 \text{ cal/mol}\cdot\text{K}\]
\[\Delta H = 0 \text{ kcal/mol}, \quad \Delta S = 33.5 \text{ cal/mol}\cdot\text{K}\]
\[\Delta H = 5 \text{ kcal/mol}, \quad \Delta S = 50.3 \text{ cal/mol}\cdot\text{K}\]
\[\Delta H = 10 \text{ kcal/mol}, \quad \Delta S = 67.1 \text{ cal/mol}\cdot\text{K}\]
Analyzing a Thermal Melting Profile: Extraction of a Melting Temperature ($T_m$)

The observable may be optical (e.g., absorbance, fluorescence, circular dichroism, etc.) or another type of spectroscopy (e.g., NMR).
Analyzing the Shape of an Equilibrium Melting Curve to Calculate $\Delta H_{\text{vH}}$

Consider an equilibrium between two distinct species (A and B), which might be two complimentary nucleic acid strands, two protein subunits, or a macromolecule and a small ligand.

$$\text{A} + \text{B} \rightleftharpoons \text{AB}$$

In this case, $C_{\text{tot}} = [\text{A}] + [\text{B}]$ and $[\text{A}] = [\text{B}]$.

One may relate $K_a$ to $\alpha$ and $C_{\text{tot}}$ as follows:

$$K_a = \frac{[\text{AB}]}{[\text{A}][\text{B}]} = \frac{\alpha \left( \frac{C_{\text{tot}}}{2} \right)}{\left( 1 - \alpha \left( \frac{C_{\text{tot}}}{2} \right) \right)^2} = \frac{\alpha}{(1-\alpha)^2 \left( \frac{C_{\text{tot}}}{2} \right)}$$

(8)
Equation (8) implies that both $K_a$ and $\Delta G$ depend on concentration. Note that this dependence vanishes for monomolecular reactions (e.g., the thermal denaturation of a monomeric protein or a nucleic acid hairpin).

At $T_m$, $\alpha = \frac{1}{2}$ by definition.

Thus, $K_a = \left(\frac{C_{\text{tot}}}{4}\right)^{-1}$ at $T_m$

Recall the van’t Hoff equation:

$$\Delta H_{\text{vH}} = -RT^2 \left( \frac{d \ln K_a}{dT} \right)_p$$

Substituting for $K_a$ yields,

$$\Delta H_{\text{vH}} = -RT^2 \frac{d}{dT} \ln \left( \frac{\alpha}{(1-\alpha)\left(\frac{C_{\text{tot}}}{2}\right)} \right)$$
When the smoke clears......

\[ \Delta H_{vH} = -RT^2 \left( \frac{(1+\alpha)}{\alpha(1-\alpha)} \right) \frac{d\alpha}{dT} \]

Evaluating at \( T_m \), where \( \alpha = \frac{1}{2} \) yields:

\[ \Delta H_{vH} = 6RT^2 \left( \frac{d\alpha}{dT} \right) \bigg|_{T=T_m} \]

(9)
Using the integrated form of the van’t Hoff equation,

\[
\ln\left(\frac{K_{T_m}}{K_T}\right) = \frac{\Delta H_{vH}}{R} \left(\frac{1}{T_m} - \frac{1}{T}\right)
\]

and the knowledge that

\[
K_a = \left(\frac{C_{\text{tot}}}{4}\right)^{-1}
\]

at \(T_m\),

one can calculate \(K_a\) at any temperature.

\(\Delta G\) and \(\Delta S\) may then be determined using equations (2) and (3).
Calculating $\Delta H_{vH}$ from the Concentration Dependence of $T_m$

For thermal melting reactions with molecularities $> 1$, $K_a$ depends on concentration.

Recall the following:

$$\Delta G = -RT \ln K_a = \Delta H - T \Delta S \quad \text{and} \quad K_a = \left( \frac{C_{\text{tot}}}{4} \right)^{-1} \quad \text{at } T_m$$

Thus,

$$\Delta H - T_m \Delta S = RT_m \ln \left( \frac{C_{\text{tot}}}{4} \right)^{-1}$$

Dividing by $T_m \Delta H$ and rearranging yields,

$$\frac{1}{T_m} = \frac{R}{\Delta H} \ln C_{\text{tot}} + \frac{\Delta S - R \ln 4}{\Delta H} \quad \text{(10)}$$
\[
\text{y-intercept} = \frac{\Delta S - R \ln 4}{\Delta H}
\]

slope = \frac{R}{\Delta H}
Differential Scanning Calorimetry (DSC)

- Calorimeters are instruments that allow the direct and quantitative measurement of heat.

- A differential scanning calorimeter allows one to measure continuously the heat capacity of a system as a function of temperature.

1. Description of Method
2. Information Content
3. Data Analysis
1. Description of Method

**Problem:**
How does one accurately measure the heat capacity \( (C_p) \) effects associated with thermally-induced transitions of biological macromolecules dissolved in aqueous buffer?
What is the contribution of a protein to the heat capacity of a solution?

Calorimeter Cell Volume, \( V \approx 1 \text{ ml} \)
Protein Concentration, \( C = 0.1 \text{ mM} \)
Protein MW = 50 kD
Specific Volume, \( v = 0.72 \text{ ml/gm} \)
Specific Heat Capacity, \( \text{Cp} = 0.4 \text{ cal/K\cdotgm} \)
\( \rho_{\text{H}_2\text{O}} = 1.0000 \text{ gm/ml} \)
The specific heat capacity of water is 1.0 cal/K\cdotgm

- The contribution of *water* to the solution heat capacity is:
  
  \[ 1.0 \text{ cal/K\cdotgm H}_2\text{O} \times (0.9964 \text{ gm H}_2\text{O}/1.0014 \text{ gm solution}) = 0.9950 \text{ cal/K\cdotgm solution} \]

- The contribution of *protein* to the solution heat capacity is:
  
  \[ 0.4 \text{ cal/K\cdotgm protein} \times (0.0050 \text{ gm protein}/1.0014 \text{ gm solution}) = 0.0020 \text{ cal/K\cdotgm solution} \]

99.8% of the solution heat capacity is due to water and only 0.2% is due to the protein dissolved in the water.
A Typical DSC Used to Study Biological Systems

- Computer system with pre-installed interface card
- Pressure Handle
- Spill plate
- Nano II DSC
- Power switch and indicator LEDs
- Users Manual and software
How Does the DSC Work?

I. The sample and reference cells (6,7) are heated by resistive heaters or peltier devices (4).

II. A thermopile (8) detects differences in temperature between the sample and reference cells. These temperature differences are on the order of $10^{-6}$ °C.

III. The cell feedback heaters (9,10) attempt to null this temperature difference, with the energy employed to equalize the cell temperatures being proportional to the heat capacity difference ($\Delta C_p$) between the cells.

IV. When the macromolecule undergoes its thermally-induced transition, some of the energy from the sample cell's heater drives the transition rather than increasing the cell temperature. Thus, the sample cell temperature lags behind that of the reference cell, a difference that is nulled by the sample cell feedback heater.
2. Information Content

Heat Capacity

When a body absorbs a finite quantity of heat, \( Q \), its temperature rises by a finite amount (\( \Delta T \)). The average heat capacity (\( C_{\text{avg}} \)) over this temperature range is defined as:

\[
C_{\text{avg}} = \frac{Q}{T}
\]

The instantaneous heat capacity at \( T \) is given by:

\[
C = \frac{dQ}{dT}
\]

For \( C \) to be path independent, either pressure (\( P \)) or volume (\( V \)) must be fixed. Recall that

\[
dU = dQ + dW
\]

where \( dU \) and \( dW \) are the instantaneous changes in internal energy and work, respectively.
If only P-V work is occurring, then

\[ C_v = \left( \frac{dQ}{dT} \right)_v = \left( \frac{dU}{dT} \right)_v \]

\[ C_p = \left( \frac{dQ}{dT} \right)_p = \left( \frac{dU - PdV}{dT} \right)_v = \left( \frac{dH}{dT} \right)_p \]  \hspace{1cm} (11)

Note that in aqueous solution, \( C_p \approx C_v \), since volume changes tend to be negligible (i.e., \( PdV \approx 0 \)).

For a reversible process in a closed system, \( dS = dQ/T \). Thus \( C_v \) and \( C_p \) may be written as

\[ C_v = T \left( \frac{dS}{dT} \right)_v \quad \text{and} \quad C_p = T \left( \frac{dS}{dT} \right)_p \]  \hspace{1cm} (12)

Thus, through heat capacity measurements, one may determine the enthalpy, entropy, and free energy of a system.
3. Data Analysis

Typical DSC Profile of a Protein

\[ \Delta C_p \]
From equation (11), it follows that

$$\Delta H_{T_{\text{max}}} = \int C_p \, dT$$

Thus, the area under the baseline-corrected curve yields the enthalpy of the process at $T_{\text{max}}$. 

$$\int_{T_{\text{min}}}^{T_{\text{max}}} C_p \, dT = \Delta H_{T_{\text{max}}}$$
The difference between the pre- and post-transition baselines is the $\Delta C_p$ for the process (typically large for protein denaturation and negligible for nucleic acid denaturation).

Knowledge of $\Delta C_p$ allows one to calculate $\Delta H$ at any temperature.

$$\Delta H_T = \Delta H_{T_{\text{max}}} - \int_{T_{\text{initial}}}^{T_{\text{final}}} C_p \,dT$$
The area under the baseline-corrected curve of $C_p/T$ versus $T$, which can be derived from the experimental DSC curve, yields the entropy of the process at $T_{\text{max}}$.

$$\Delta S_{T_{\text{max}}} = \int \frac{C_p}{T} \, dT$$
Cooperativity or Stateness of a Transition

The model-dependent (usually "two-state") van't Hoff transition enthalpy ($\Delta H_{vH}$) can be extracted from the shape of or directly from DSC curves.

For a bimolecular process,

$$\Delta H_{vH} = 6R(T_{max})^2 \left[ \frac{(C_p)_{T_{max}}}{\Delta H_{cal}} \right]$$

- If $\Delta H_{vH} = \Delta H_{cal}$, the transition proceeds in 2-state manner.
- If $\Delta H_{vH} < \Delta H_{cal}$, the transition involves a population of intermediate states that contribute thermodynamically.
- If $\Delta H_{vH} > \Delta H_{cal}$, intermolecular cooperation (e.g., aggregation) is implicated.

$\Delta H_{vH}/\Delta H_{cal}$ provides a measure of the fraction of the macromolecule that melts as a single thermodynamic entity (i.e., the size of the cooperative unit).
Influence of cisplatin intrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex

(differential scanning calorimetry/thermodynamics/crosslink-induced conformational/structural changes/DNA recognition)

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Cross-link formation enthalpically destabilizes the host duplex.

Cross-link formation does not alter the two-state nature or cooperativity of the melting transition.

Table 1. Thermodynamic parameters for formation of the GG20 and cis-Pt-GG20 duplexes

<table>
<thead>
<tr>
<th>Duplex</th>
<th>$T_m, \degree C$</th>
<th>$\Delta H^\circ, \text{kcal/mol duplex}$</th>
<th>$\Delta S^\circ, \text{cal/K-mol duplex}$</th>
<th>$\Delta G^\circ25,\degree \text{ kcal/mol duplex}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG20</td>
<td>$70.2 \pm 0.2$</td>
<td>$-152 \pm 8$</td>
<td>$-151 \pm 8$</td>
<td>$-20.9 \pm 2.1$</td>
</tr>
<tr>
<td>cis-Pt-GG20</td>
<td>$61.8 \pm 0.2$</td>
<td>$-135 \pm 7$</td>
<td>$-136 \pm 12$</td>
<td>$-14.6 \pm 1.5$</td>
</tr>
</tbody>
</table>
Berenil Binding to Higher Ordered Nucleic Acid Structures: Complexation with a DNA and RNA Triple Helix

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Table 3: Calorimetric and van't Hoff Transition Enthalpies for the Berenil-Free and Berenil-Saturated Poly(dA)•2Poly(dT) and Poly(rA)•2Poly(rU) Triplexes and for the Corresponding Berenil-Free and Berenil-Saturated Poly(dA)•Poly(dT) and Poly(rA)•Poly(rU) Duplexes

<table>
<thead>
<tr>
<th>transition</th>
<th>$r_{bt}$ or $r_{bp}$</th>
<th>$\Delta H_{cal}^b$ kcal/mol</th>
<th>$\Delta H_{vH}^c$ kcal/mol</th>
<th>$\Delta H_{vH}/\Delta H_{cal}$ (cooperative unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dA•2(dT) → dA•dT + dT</td>
<td>0</td>
<td>4.1</td>
<td>192</td>
<td>47</td>
</tr>
<tr>
<td>dA•2(dT) → dA•dT + dT</td>
<td>0.30</td>
<td>6.0</td>
<td>181</td>
<td>30</td>
</tr>
<tr>
<td>dA•dT → dA + dT</td>
<td>0</td>
<td>10.4</td>
<td>260</td>
<td>25</td>
</tr>
<tr>
<td>dA•dT → dA + dT</td>
<td>0.23</td>
<td>14.8</td>
<td>276</td>
<td>19</td>
</tr>
<tr>
<td>RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rA•2(rU) → rA•rU + rU</td>
<td>0</td>
<td>2.0</td>
<td>173</td>
<td>87</td>
</tr>
<tr>
<td>rA•2(rU) → rA•rU + rU</td>
<td>0.21</td>
<td>2.4</td>
<td>172</td>
<td>72</td>
</tr>
<tr>
<td>rA•rU → rA + rU</td>
<td>0</td>
<td>8.7</td>
<td>269</td>
<td>31</td>
</tr>
<tr>
<td>rA•rU → rA + rU</td>
<td>0.19</td>
<td>8.8</td>
<td>232</td>
<td>26</td>
</tr>
</tbody>
</table>
A spectroscopic and calorimetric study of the melting behaviors of a "bent" and a "normal" DNA duplex: \([d(GA_4T_4C)]_2\) versus \([d(GT_4A_4C)]_2\)

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**Table 1.** Spectroscopically derived thermodynamic data for the melting of \([d(GA_4T_4C)]_2\) and \([d(GT_4A_4C)]_2\) in 1 M NaCl

<table>
<thead>
<tr>
<th>Duplex</th>
<th>(t_m), °C</th>
<th>(\Delta H_{VH}), kcal/mol</th>
<th>(T\Delta S_{VH}), kcal/mol</th>
<th>(\Delta G^\circ (25^\circ C)), kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>([d(GA_4T_4C)]_2)</td>
<td>49.9</td>
<td>72.4</td>
<td>62.6</td>
<td>9.8</td>
</tr>
<tr>
<td>([d(GT_4A_4C)]_2)</td>
<td>45.6</td>
<td>66.5</td>
<td>57.9</td>
<td>8.6</td>
</tr>
</tbody>
</table>
Table 3. Calorimetric transition temperatures ($T_{\text{max}}$) and transition enthalpies ($\Delta H_{\text{cal}}$) obtained by deconvolution for the premelting and global melting of [d(GA$_4$T$_4$C)$_2$]

<table>
<thead>
<tr>
<th>$T_{\text{max}}$, °C</th>
<th>$\Delta H_{\text{cal(p)}}$, kcal/mol</th>
<th>$\Delta H_{\text{cal(g)}}$, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.0</td>
<td>16.0</td>
<td>72.4</td>
</tr>
<tr>
<td>50.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Study of Strong to Ultratight Protein Interactions Using Differential Scanning Calorimetry†

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Interaction Between a Ligand and a Protein that Undergoes a Thermal Transition

\[ C = K_a[P] \]

**FIGURE 4:** Simulations of DSC curves when the total ligand concentration is half of the protein concentration. The protein has assumed transition parameters of 50 ºC for \( T_0 \), 200 kcal for \( \Delta H(T_0) \), and 3000 cal/deg for \( \Delta C_p \). The \( C \) parameter measures the tightness of binding and is equal to the product of the protein concentration times the binding constant at \( T_0 \). The heat of binding was assumed to be -10 kcal and temperature-independent.
Interaction Between Two Molecules that Each Undergo a Thermal Transition

Figure 7: Simulated DSC curves for two molecules, both of which undergo a two-state thermal unfolding transition. Both transitions were assumed to have $\Delta H(T_0)$ of 150 kcal and $\Delta C_p$ of 2000 cal/deg, while the $T_0$ values are 50 °C for the least stable and 65 °C for the most stable of the two. The assumed values of the interaction constant (M⁻¹) at 50 °C are listed for each scan, and the heat of interaction was -20 kcal in all cases.