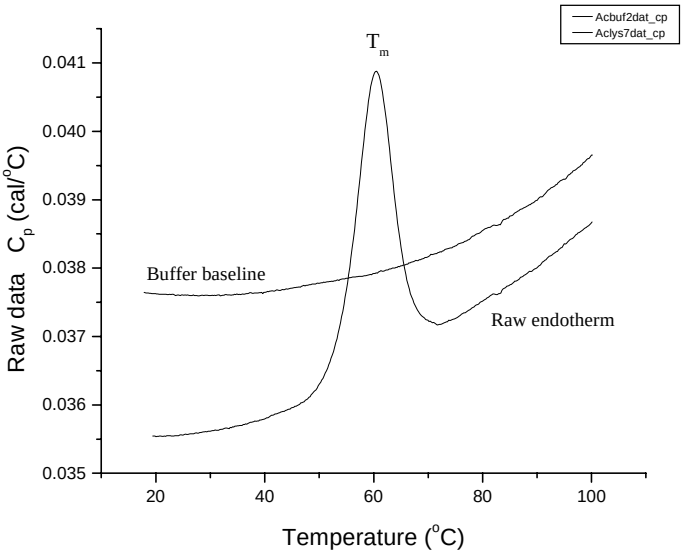
**DSC Interpretation Notes** -- Alan Cooper (Glasgow)

These notes are intended to help in the interpretation, avoiding over-interpretation, of biomolecular DSC data (mainly for proteins). They should be read in conjunction with more comprehensive treatments, some of which are listed at the end.

Solution differential scanning calorimetry (DSC) measures the difference in heat energy uptake between a sample solution and appropriate reference (buffer/solvent) with increase in temperature. A typical experiment comprises one (or more) scans of the sample solution, together with separate control experiments using buffer alone to establish the instrumental baseline. For fully quantitative analysis accurate sample concentrations are required, and parameters such as concentration, scan rate, pH, etc., may be varied.

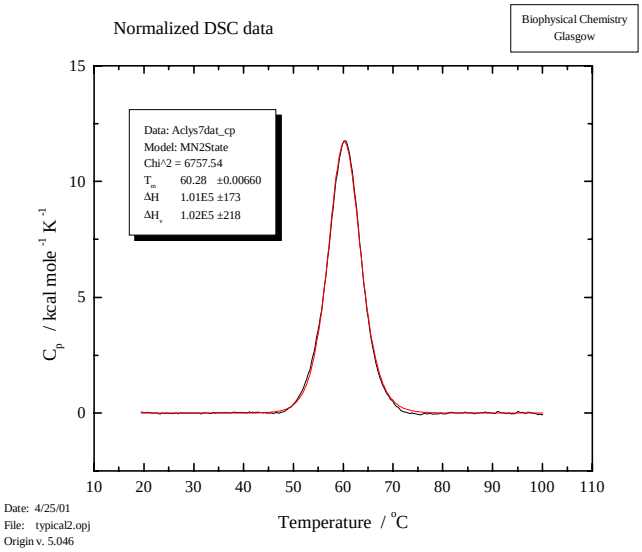
**The ideal world....**

Typical raw DSC data for the thermal unfolding of a simple globular protein in solution. Such scans show the heat energy uptake ($C_p$ endotherm) during the unfolding transition, and are usually (almost) identical upon rescan.




$T_m$ (effectively the transition peak) is defined as the temperature at which 50% of the protein molecules are unfolded or, in an ideal dynamic reversible 2-state equilibrium, the temperature at which any one molecule spends 50% of its time folded and 50% unfolded.

After concentration normalization and baseline correction, such data can usually be fit to a simple 2-state unfolding model (other models available) to give apparent thermodynamic data for the transition. For this kind of model, the enthalpy (heat) of unfolding is estimated in two independent ways...




The <u>calorimetric enthalpy</u> ($\Delta H_{cal}$) is an absolute measurement of the heat energy uptake, given by the <u>area</u> under the transition peak. It depends on the total amount of (active) protein in the calorimeter cell.

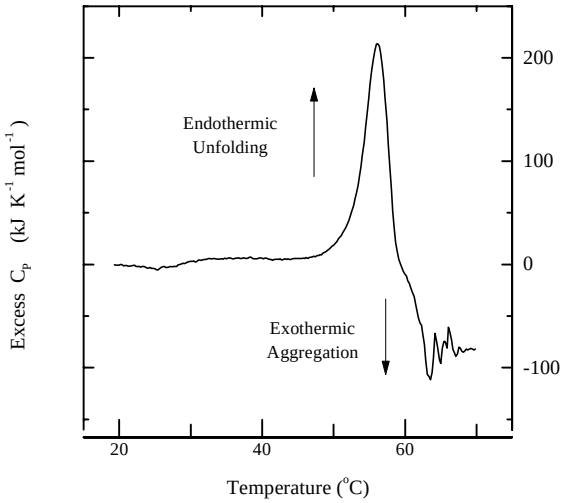
The Van't Hoff enthalpy ($\Delta H_{VH}$) depends on the shape of the endotherm and assumes a true 2-state reversible equilibrium. It is independent of the amount of protein present. It is a measure of the cooperativity of the transition: the greater the cooperativity, the sharper the transition and the greater the $\Delta H_{VH}$ . Broad transitions have low $\Delta H_{VH}$ .

In an ideal world (but see below) $\Delta H_{cal} = \Delta H_{VH}$

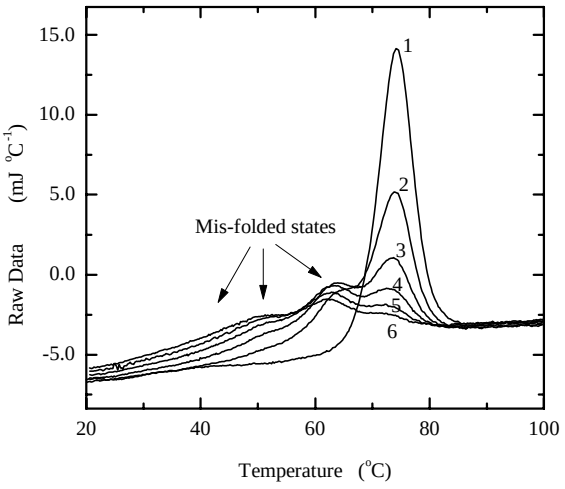
Notes: (a) Units: For experiments done using Microcal instruments with Origin software, enthalpies are usually given in calories per mole (1 cal = 4.184 J). (b) The error estimates for $T_m$ and $\Delta H$ given by the Origin fitting routines are usually highly optimistic, and do not reflect the real experimental uncertainties.

**The real world....**

Unfolded protein is sticky stuff, and many (most?) will aggregate upon thermal denaturation, especially at physiological pH. This aggregation is normally exothermic and irreversible, causing distortion of the DSC endotherm, with noisy traces at higher temperature due to convection of clumpy aggregates in the DSC cell. Such aggregation is difficult to control or predict. It is a kinetic phenomenon, likely to depend on protein concentrations, DSC scan rates, etc. The distortion/sharpening of the DSC peak leads to lower values for apparent $T_m$, lower values for $\Delta H_{cal}$ and higher estimates for $\Delta H_{VH}$ .




Even in the absence of aggregation, thermal unfolding is rarely completely reversible, since exposure of the unfolded polypeptide to higher temperatures can lead to improper refolding, proline isomerization, de-amidation, or other chemical changes that give rise to mis-folded forms. These can be seen in subsequent re-scans:-



**Why does $\Delta H_{cal} \neq \Delta H_{VH}$ ?**

Agreement between $\Delta H_{cal}$ and $\Delta H_{VH}$ depends on a number of factors such as the accuracy of the (active) protein concentration, the validity of the 2-state approximation, reversibility of the transition, etc. Discrepancies betwen the two enthalpy estimates can sometimes be instructive.

$\Delta H_{cal} > \Delta H_{VH}$ may arise if:-

- the protein concentration has been under-estimated
- the unfolding is not 2-state, but involves unfolding intermediates or independent domains
- the protein concentration has been expressed in the wrong molar units (e.g. as moles of dimer, when the cooperative unit for unfolding is the monomer)
- baseline correction is wrong

$\Delta H_{cal} < \Delta H_{VH}$ may arise if:-

- the protein concentration has been over-estimated
- the concentration may be correct, but not all the protein is correctly folded
- the protein concentration has been expressed in the wrong molar units (e.g. as moles of monomer, when the appropriate cooperative unit for unfolding is the dimer)
- the DSC peak is artificially sharpened (distorted) by irreversible/aggregation effects
- baseline correction is wrong

... and so on – see further reading for more on this complex topic.

Further reading:


[1] A. Cooper, Microcalorimetry of protein-protein interactions in J. E. Ladbury and B. Z. Chowdhry (Eds.), Biocalorimetry: the applications of calorimetry in the biological sciences. Wiley, (1998) p 103-111.

[2] A. Cooper, Thermodynamic analysis of biomolecular interactions, Curr. Opin. Chem. Biol. (1999) 3, 557-563.

[3] A. Cooper, Microcalorimetry of protein-DNA interactions in A. Travers and M. Buckle (Eds.), DNA-Protein Interactions. Oxford University Press, Oxford, (2000) p 125-139.

[4] A. Cooper, M. A. Nutley, A. Wadood, Differential scanning microcalorimetry in S. E. Harding and B. Z. Chowdhry (Eds.), Protein-Ligand Interactions: hydrodynamics and calorimetry. Oxford University Press, Oxford New York, (2000) p 287-318.

[5] A. Cooper, C. M. Johnson, Introduction to Microcalorimetry and Biomolecular Energetics in C. Jones, B. Mulloy and A. H. Thomas (Eds.), Microscopy, Optical Spectroscopy, and Macroscopic Techniques. Humana Press, Totowa, NJ, (1994) p 109-124.

[6] A. Cooper, C. M. Johnson, Differential Scanning Calorimetry in C. Jones, B. Mulloy and A. H. Thomas (Eds.), Microscopy, Optical Spectroscopy, and Macroscopic Techniques. Humana Press, Totowa, NJ, (1994) p 125-136.

[7] A. Cooper, C. M. Johnson, Isothermal Titration Microcalorimetry in C. Jones, B. Mulloy and A. H. Thomas (Eds.), Microscopy, Optical Spectroscopy, and Macroscopic Techniques. Humana Press, Totowa, NJ, (1994) p 137-150.

[8] S. C. Gill, P. H. Vonhippel, Calculation of Protein Extinction Coefficients from Amino-Acid Sequence Data, Anal. Biochem. (1989) 182, 319-326.

[9] V. V. Plotnikov, J. M. Brandts, L. N. Lin, J. F. Brandts, A new ultrasensitive scanning calorimeter, Anal. Biochem. (1997) 250, 237-244.

[10] J. M. Sturtevant, Calorimetric studies of biopolymers, Protein Sci. (1996) 5, 391-394.