Differential Scanning Calorimetry

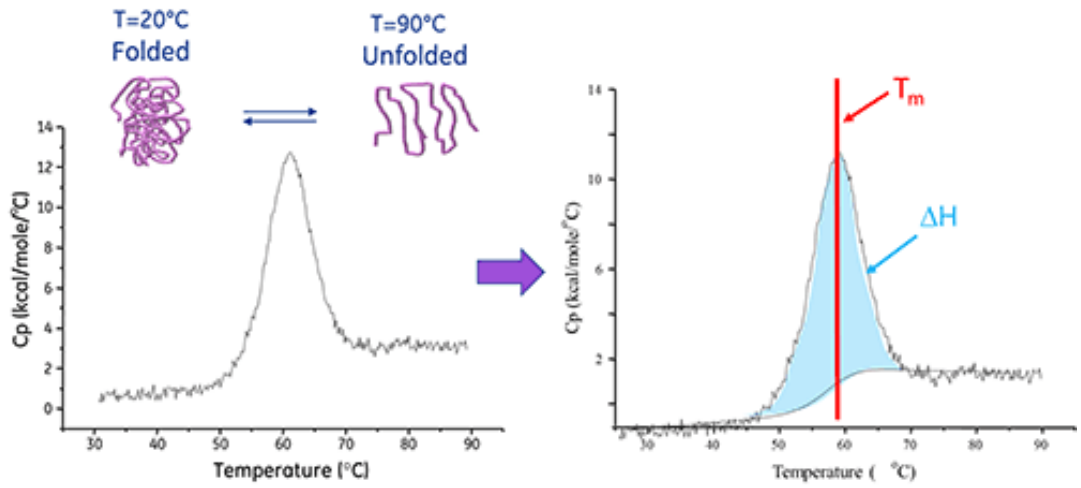
Differential Scanning Calorimetry (DSC) is a technique used to characterize the stability of a protein or other biomolecule directly in its native form. It does this by measuring the heat change associated with the molecule's thermal denaturation when heated at a constant rate.

Heat Capacity & Melting Transitions

First order vs. two-state transitions.

What is Excess Heat Capacity?

Water, DPPC (vesicle), Ribonuclease A (typical globular protein), DNA (RNA).



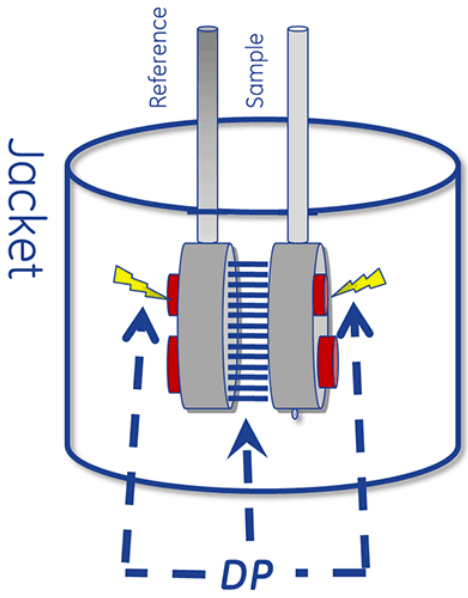
Two Types of DSCs

Standard DSCs

- Used for synthetic polymers and other materials exhibiting thermally induced transitions
- Small sample volume = typically µl (mg)
- Rapid scanning of T = 10 – 50 C/min
- Wide temperature range = -50 to +500 C

Biological Micro-DSCs

- Used for proteins, nucleic acids, and lipid assemblies
- Large sample volume = typically 0.5 to 1.0 ml (g)
- Slow scanning of T = 0.1 – 2 C/min
- Narrow temperature range = 0 to 100 C



Obtaining Thermodynamic Parameters: The Simple Two-State Model

$K = [D]/[N]$

$\Delta G^\circ = - RT \ln K$

$\Delta H^\circ = - R \left[\partial(\ln K)/\partial(1/T)\right]_p$

$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$

$\Delta C_p^\circ = (\partial \Delta H^\circ/\partial T)_p = T(\partial \Delta S^\circ/\partial T)_p$

Independent Transitions

Assuming that a protein (or other macromolecule) is composed of a number of independent structural domains A, B, C, ..., each of which can transition between the folded and unfolded forms (A = A', B = B', ...)

$$H = H_N + f_{A'}\Delta H_A + f_{B'}\Delta H_B + \ldots \quad , C_p = (\delta H/\delta T)$$

$$C_p = C_{pN} + [f_{A'}\Delta C_{pA} + \Delta H_A (\delta f_{A'}/\delta T)] + \ldots$$

$$C_p = C_{pN} + [(K_A/(1 + K_A))\,\Delta C_{pA} + (K_A/(1 + K_A)^2)\,\Delta H_A\,\Delta H_A^*/(RT^2)] + \ldots$$

The above equation is perfectly general and can be applied to either two-state or non-two-state transitions so long as all of the parameters are evaluated at the same temperature T. (For a "two-state" transition, $\Delta H_A = \Delta H_A^*$.)

Model for Two-State Independent Transitions

Including ΔC_p Effects:

$$C_{p,T} = C_{pN,T} + [(K_{A,T}/(1 + K_{A,T}))\,\Delta C_{pA} + (K_{A,T}/(1 + K_{A,T})^2)\,\Delta H_{A,T}^2/(RT^2)] + \ldots$$

$$C_{pN,T} = B_0 + B_1T + B_2T^2 + \ldots$$

$$\Delta H_{A,T} = \Delta H_{mA} + C(T - T_{mA})$$

$$K_{A,T} = \exp\{(-\Delta H_{mA}/RT)(1-(T/T_{mA})) - (\Delta C_{pA}/RT)(T - T_{mA} - T\ln(T/T_{mA})\}$$

Curve fitting will yield values for: B_0, B_1, T_{mA}, ΔH_{mA}, ΔC_{pA}, T_{mB}, ΔH_{mB}, and ΔC_{pB}.

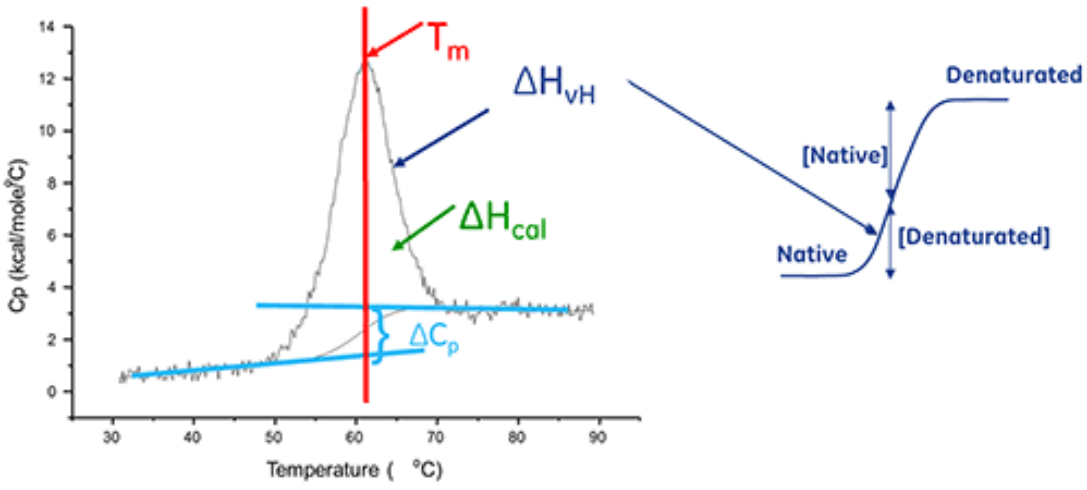
Model for Two-State Independent Transitions

Excluding ΔC_p Effects:

$C_{p,T} = (K_{A,T}/(1 + K_{A,T})^2)\ \Delta H_{A,T}^2/(RT^2)] + \ldots$

$K_{A,T} = exp\ \{(-\Delta H_{mA}/RT)(1-(T/T_{mA}))$

Curve fitting will yield values for: T_{mA}, ΔH_{mA}, T_{mB}, and ΔH_{mB}.



Model for Non-Two-State Independent Transitions

Excluding ΔC_p Effects:

$$C_{p,T} = (K_{A,T}/(1 + K_{A,T})^2)\, \Delta H_{mA}\, \Delta H_{mA}^*/(RT^2)] + \ldots$$

$$K_{A,T} = \exp\{(-\Delta H_{mA}^*/RT)(1-(T/T_{mA}))$$

Curve fitting will yield values for: T_{mA}, ΔH_{mA}, ΔH_{mA}^*, T_{mB}, ΔH_{mB}, and ΔH_{mB}^*.