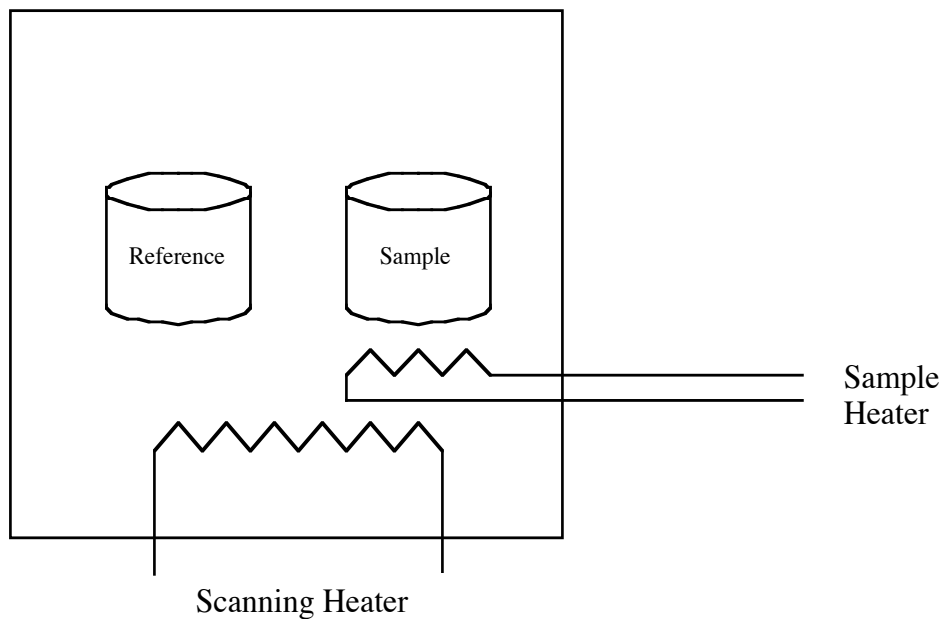


## Modern Biochemical Calorimetry- What are DSC and ITC?

### Differential Scanning Calorimetry (DSC)

DSC can be used to characterize the energetics of a structural transition in a macromolecule, such as the unfolding of a protein or the melting of duplex DNA. It provides the temperature at which this transition occurs, which is known as the melting or midpoint temperature  $T_m$ . It also provides the heat or enthalpy  $\Delta H$  associated with the transition. Careful studies of the dependence of  $\Delta H$  on temperature can be used to also obtain the change in heat capacity that occurs with unfolding or melting, i.e. the  $\Delta C_p$ . Knowledge of these three parameters permits a complete characterization of the stability of the macromolecule using the Gibbs-Helmholtz equation (which will be covered when we talk about free energy).

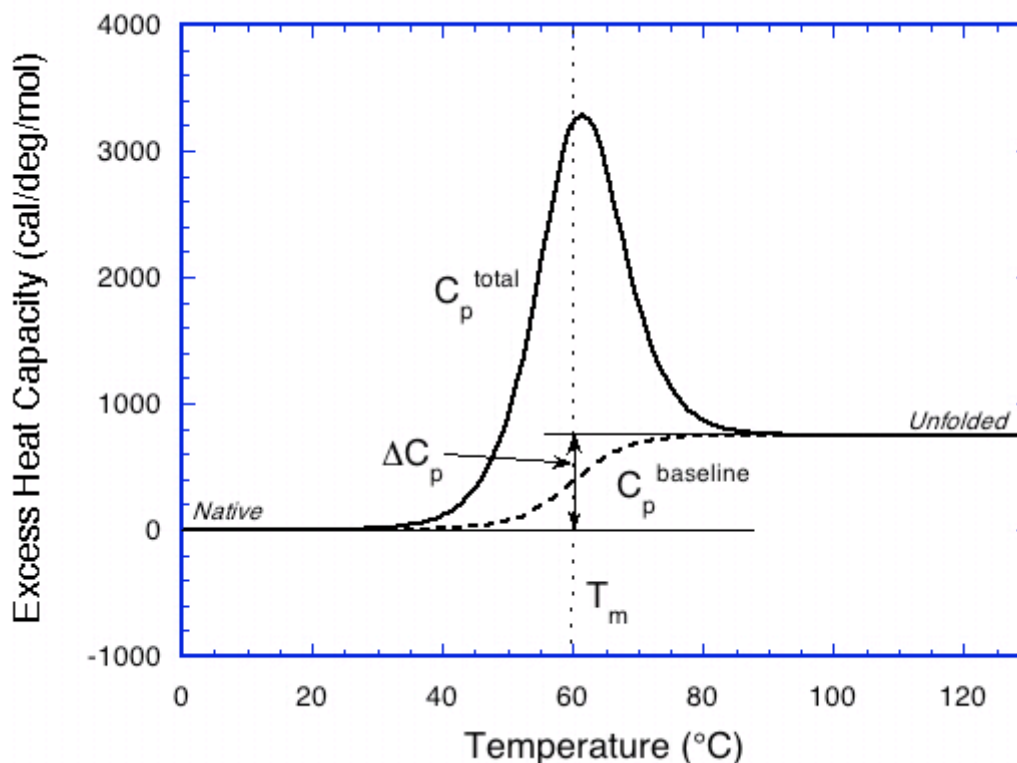
A DSC instrument is composed of two cells of approximately 1 ml volume each (made of a metal such as tantalum or gold) that are thermally isolated from the environment with insulation or a vacuum. One cell contains buffer (reference cell) and the other contains buffer plus the macromolecule (the sample, which we will assume here to be a protein). Typical sample concentrations are about 1 mg/ml.



Initially the reference and sample cells are equilibrated at the desired starting temperature, e.g. 5 °C. The sample heater is computer controlled and when the scan is started, sufficient current is applied to slowly raise the temperature of the cells at a constant rate, typically on the order of 1 deg/min. As the temperature of the sample enters a region where an endothermic transition starts to occur, some of the heat flowing into the sample cell will be used to drive the transition instead of raising the temperature. At this point the sample cell has an anomalous increase in heat capacity. A minute

difference in temperature is sensed between the two cells as the temperature of the sample cell begins to lag behind that of the reference. The computer is programmed to apply a current to the sample heater to maintain the sample cell at the same temperature as the reference. The heat applied to the sample cell represents the excess heat required to drive the endothermic structural transition in the sample. The raw data is the heat flow into the sample cell, in cal/min. If this is divided by the scan rate (deg/min) the result is cal/deg which is the heat capacity. This can be scaled to the molar heat capacity if it is divided by the number of moles of substance in the sample cell. With a continued increase in temperature the structural transition will be driven to completion, and the heat capacity will return to a value similar to that observed at the beginning. The heat capacity will not be identical however, because the structural transition almost always leads to a change in heat capacity,  $\Delta C_p$ , which is the difference between the baselines observed before and after the transition.

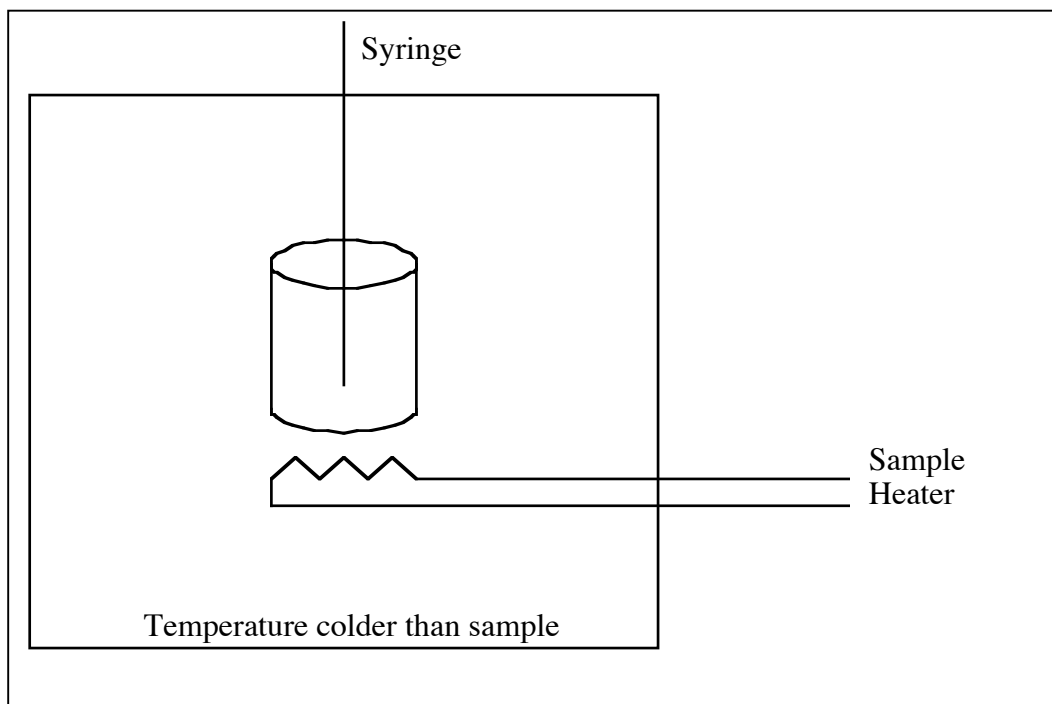
The temperature at the center of the transition (which can be slightly different from the temperature of the maximum) is the midpoint or melting temperature. The area of the peak is the enthalpy of the transition since  $C_p = (\delta H/\delta T)_p$ . The area of the peak is obtained after removal of the contribution below the baselines. A transition from the native baseline to the unfolded baseline is shown below (dashed line) and is defined by a weighted average of the two baselines. The dashed curve (which, of course, cannot be directly observed) is dictated by the temperature dependence of the equilibrium constant (covered later when we talk about free energy, equilibrium, temperature, and the van't Hoff equation).



If the stability of the protein is decreased by lowering the pH, the  $T_m$  will decrease. If the change in heat capacity in going from the folded to unfolded forms is positive, the Kirchhoff equation ( $\Delta C_p = (\delta\Delta H/\delta T)_p$ ) tells us that the change in enthalpy should decrease with decreasing temperature. The dependence of  $\Delta H$  on  $T_m$  provides the  $\Delta C_p$  (see the handout on the Kirchhoff equation).

### **Isothermal Titration Calorimetry (ITC)**

ITC is used to measure the heat (enthalpy) of binding of molecules to biochemical macromolecules, such as the binding of a protein to DNA, or the binding of a drug to a protein receptor. The equipment design is very similar to that of a DSC except that a computer controlled syringe is used to titrate a solution of the binding molecule into the sample cell. The sample cell and reference cells are kept at a constant temperature (thus isothermal) slightly above that of the system. Thus, the surroundings are constantly trying to cool the sample cell but heat is applied to keep it warmer and at a constant temperature (e.g. 37 °C). As a molecule is titrated into the sample cell heat may be absorbed or liberated depending on whether or not binding is endothermic or exothermic. If it is endothermic, more heat will need to be added to the sample cell to keep its temperature constant, while if it is exothermic less heat will need to be added. In both cases the change in heat flow into the sample cell required to maintain a constant temperature represents the heat of binding. As more titrant is added, the binding sites will become filled and once saturation is achieved, no further change will be observed except for a heat associated with dilution. Note that if there is little or no heat change associated with binding, this technique cannot be used to study binding.



Analysis of the ITC provides the number of binding sites (stoichiometry), the heat or enthalpy of binding, as well as the binding constant. We will return to this when we talk in more detail about binding in biochemistry.

The raw data for a typical ITC experiment might look something like that shown below. Each peak represent the heat change associated with an injection of binding molecule (called the ligand) into the macromolecule. The area under each peak is the total heat associated with the binding of all the material in that injection.

