Very high sensitivity DSC and titration calorimetry applied to the study of proteins

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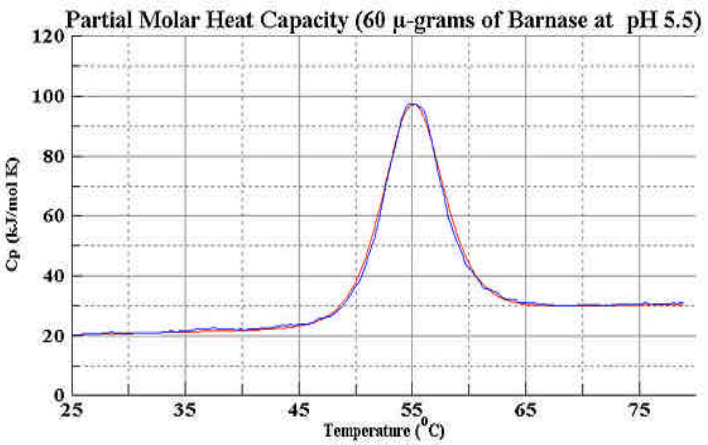
Proteins generally involve small or very small effects when analyzed. This property may have two explanations. First, the heats of reaction, transformation or interaction are weak ; and, above all, the quantities of material involved, may also be reduced to a fraction of milligram which makes the absolute heat to be measured very small.
So, different devices have been developed in the recent years, which explains the increase of very sensitive DSC and titration calorimetry on proteic systems.
More specifically, two types of devices appeared on the market such as:

- Very high sensitivity DSC with power compensation.
- Titration calorimetry.

Very high sensitivity DSC : Nano-DSCII

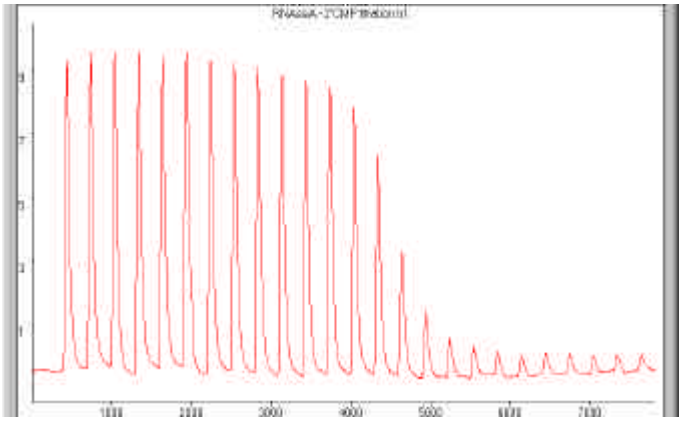
Nano-DSC II is designed specifically for the measurement of absolute heat capacities of biopolymers in dilute solution. It is a power compensation design using a completely solid-state thermostat and is equipped with the user's choice of either capillary or cylindrical cells made from either platinum or 24K gold.
Due to its very low noise (15 nWatts), the Nano-DSC II makes can be used to study the thermal denaturation of most proteins with as little as 50 micrograms or less of sample. The cell having a volume of 300 µL, it corresponds to a concentration of 170 µg / mL.
The thermal denaturation of DNA and RNA may be studied in a similar way.



Denaturation of 60 µg of barnase

Titration calorimetry :

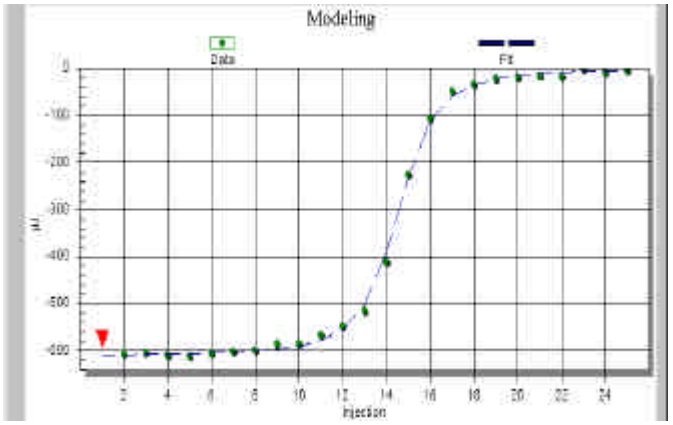
Le Isothermal Titration Calorimeter (ITC) is designed specifically for the study of biopolymer ligand interactions.
The ITC can detect heat effects as small as 0.4 µJ allowing a titration to be done with as little as 1 nanomole of biopolymer.
Equilibrium constants in the range of 10^2 to 10^8 M^{-1} can easily be determined for almost any interaction.
The enclosed example presents the titration of a solution of RNAse by a solution of 2'CMP.
Practically, a volume of 1.3 mL of RNAse is introduced in the cell, then 25 injections of 10 µL each are produced every 300 seconds.
The first 15 injections produce exothermic effects, then these effects become smaller.



Titration of RNAse by 2'CMP

With Bindworks software it is possible to determine :

- Keq : equilibrium constant
- n : stoechiometry
- ΔH : titration enthalpy.



With the same method, it is possible to study :

- enzyme – substrate interactions
- biopolymer – biopolymer interactions
- drug – biopolymer interactions