Physics of Life

PHYS-468

Calorimetry

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Thermodynamics

The first law of thermodynamics:
*"**Energy cannot be destroyed.**"*
The change of the internal energy *U* is
the supplied heat *q*, minus the work done by system.

$$\Delta U = q - p\Delta V$$

The second law of thermodynamics:
"**Entropy is always increasing.**"

$$\Delta S \geq 0$$

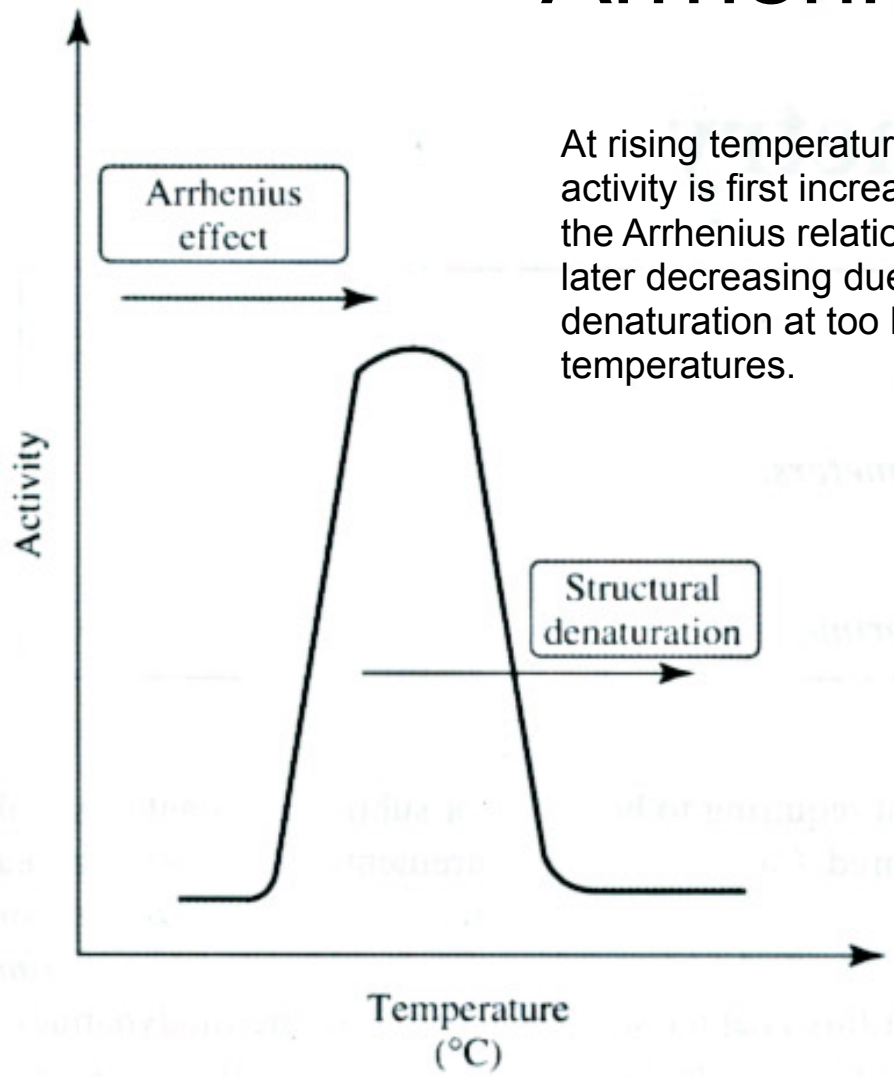
Enthalpy H = Inner energy U of a system, plus energy to make room for system (pV).

$$H = U + pV$$

Gibbs free energy G = thermodynamic potential that measures the work that a system can do
(at constant temperature and pressure).

$$G = H - TS$$

Arrhenius Effect



At rising temperature, a reaction activity is first increasing due to the Arrhenius relationship, but later decreasing due to protein denaturation at too high temperatures.

Arrhenius Effect: Activities are higher at higher temperatures.

$$k = A \cdot e^{-\frac{E_a}{RT}}$$

k = rate constant
A = frequency factor (a constant)
E_a = activation energy
R = universal gas constant
T = temperature [Kelvin]

Fig. 8.1. Effect of temperature on an enzyme-catalyzed reaction. Temperature increases the ratest of chemical reactions, including rates of enzyme-catalyzed reactions, because it has an exponential effect due to the Arrhenius relationship.
However, structural denaturation of proteins also is more severe at higher temperature, which leads to inactivation of enzymes.
These two effects together produce the the temperature profile of enzymes.

Rule of thumb:
Reaction speed doubles if the experiment is repeated 10º Celsius hotter.

Temperature: 0 ºKelvin = -273.15 ºCelsius

k = rate constant
A = frequency factor (a constant)
E_a = activation energy
R = universal gas constant
T = temperature

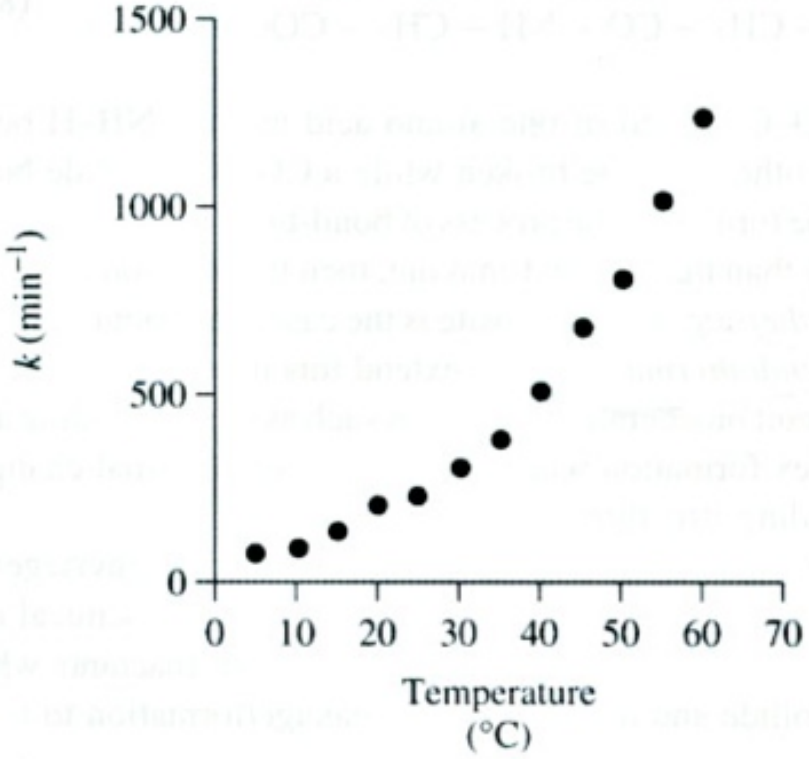
Arrhenius Plot

$$k = A \cdot e^{-\frac{E_a}{RT}}$$

$$E_a = -RT \cdot \ln(k/A)$$
$$= -RT \cdot \ln k + RT \cdot \ln A$$
$$= -RT \cdot 2.303 \cdot \log k + constant$$

$$\log k + constant = -E_a/2.303R \cdot \frac{1}{T}$$

Plot “log k” over 1/Temperature. The slope of the resulting plot gives you the activation energy of the reaction.



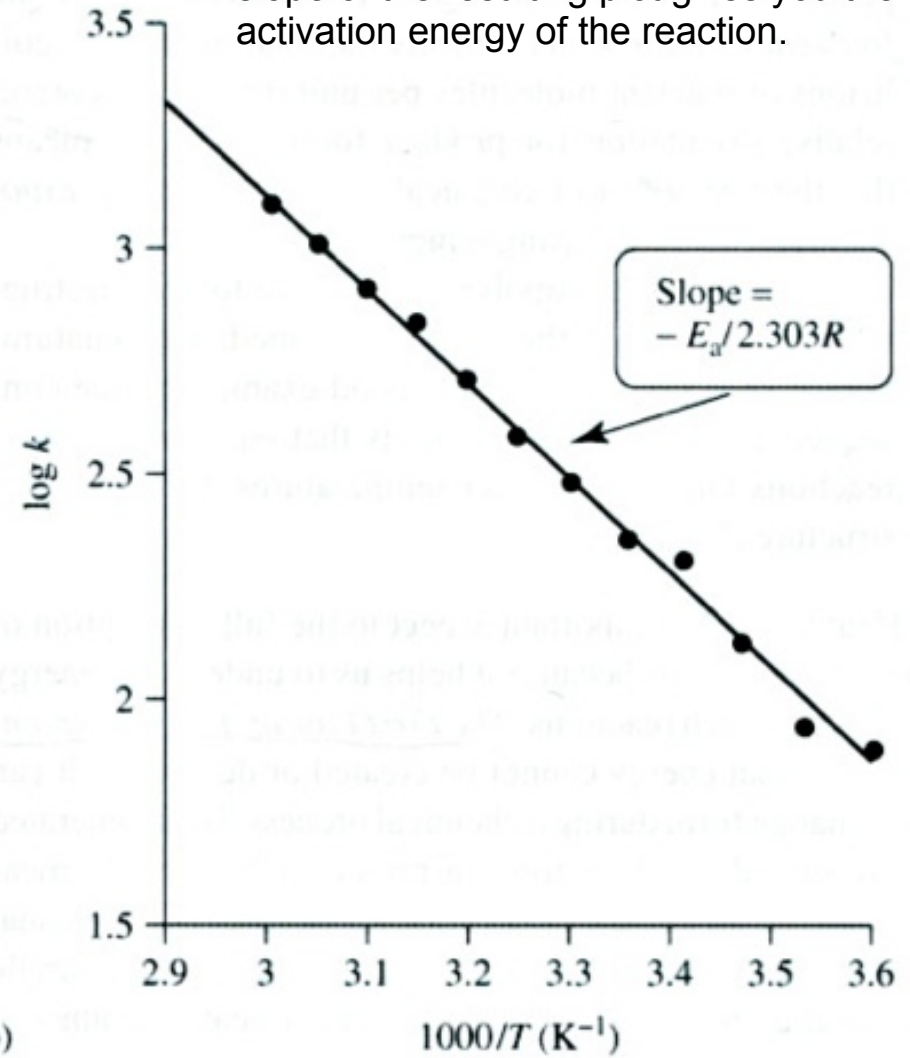


Figure 8.2. Arrhenius plot of a chemical reaction. (a) Chemical reactions depend exponentially on temperature. (b) The activation energy (E_a) can be calculated from the slope of an Arrhenius plot.

Potential Energy

The Potential Energy determines the reaction speed. It first has to be overcome, before the reaction can proceed.
The total reaction can be exothermic (negative change in Enthalpy) or endothermic (positive change in Enthalpy).

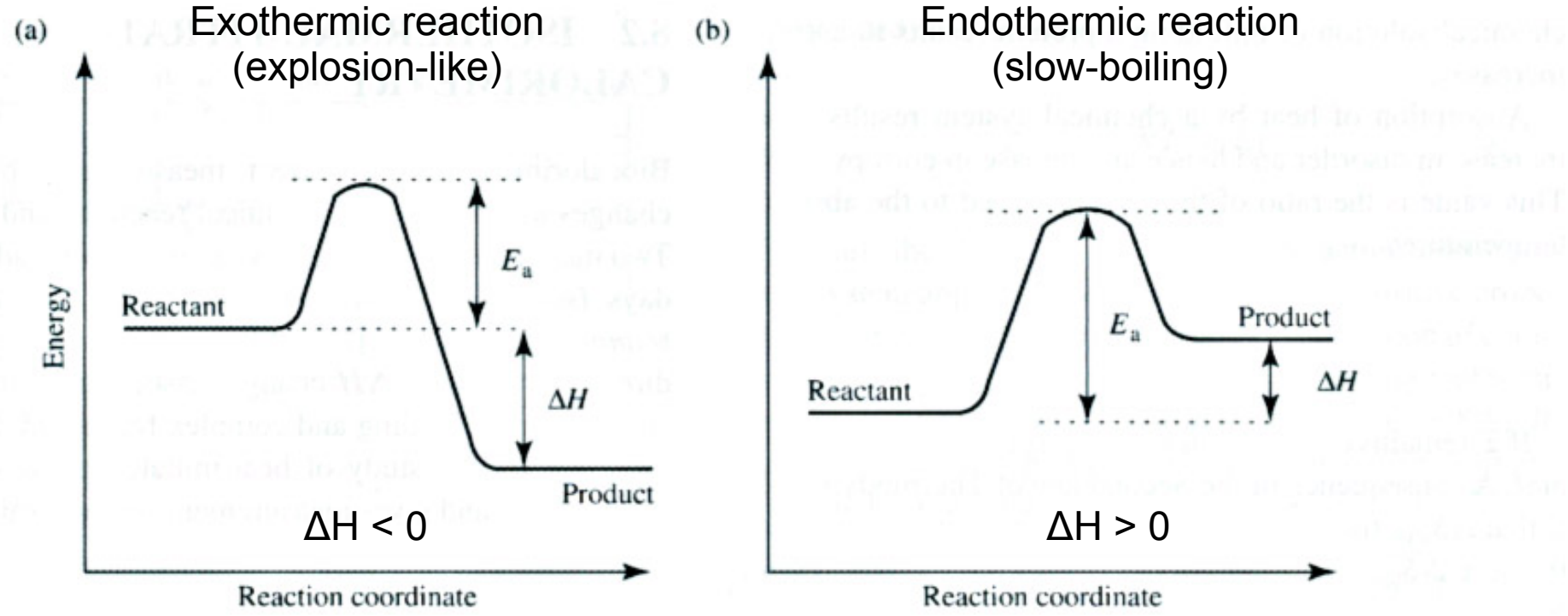


Figure 8.3. Potential energy diagrams of reactions. Potential energy diagrams of reactions are shown. The activation energy barrier is denoted by E_a while the enthalpy of the reaction is shown by ΔH.

ΔH = H(Product) - H(Reactant)

DSC – Differential Scanning Calorimetry

DSC – Differential Scanning Calorimetry

- DSC measures the heat required to raise the temperature of a sample.

- Usage:
  - Raise temperature in Sample and Reference cuvette
  - Measure energy needed to raise temperatures.
  - Difference between energies needed on both sides is the energy absorbed by molecules in the sample.

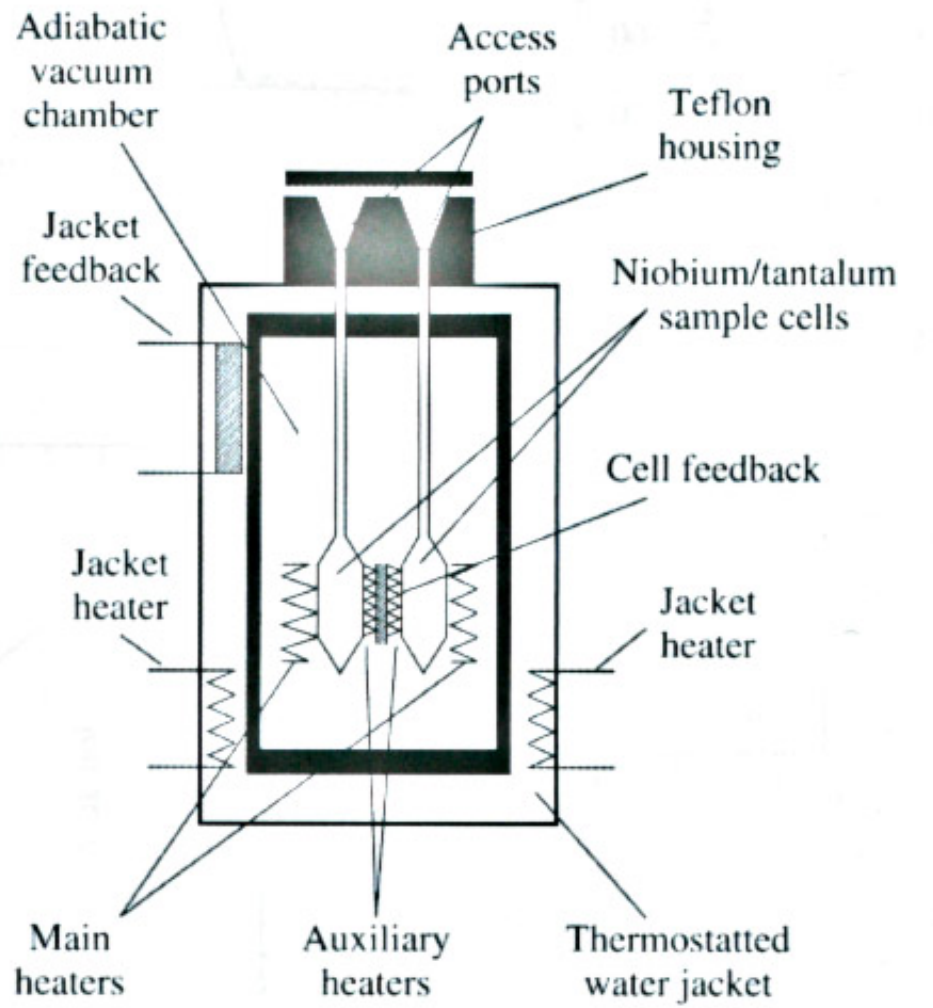


Figure 8.7. Outline design of a differential scanning calorimeter. The outline design of a Microcal MC-2 calorimetric unit is shown. Reprinted from Ladbury and Chowdhry (1998). *Biocalorimetry: Applications of Calorimetry in the Biological Sciences*. Copyright John Wiley & Sons Limited.

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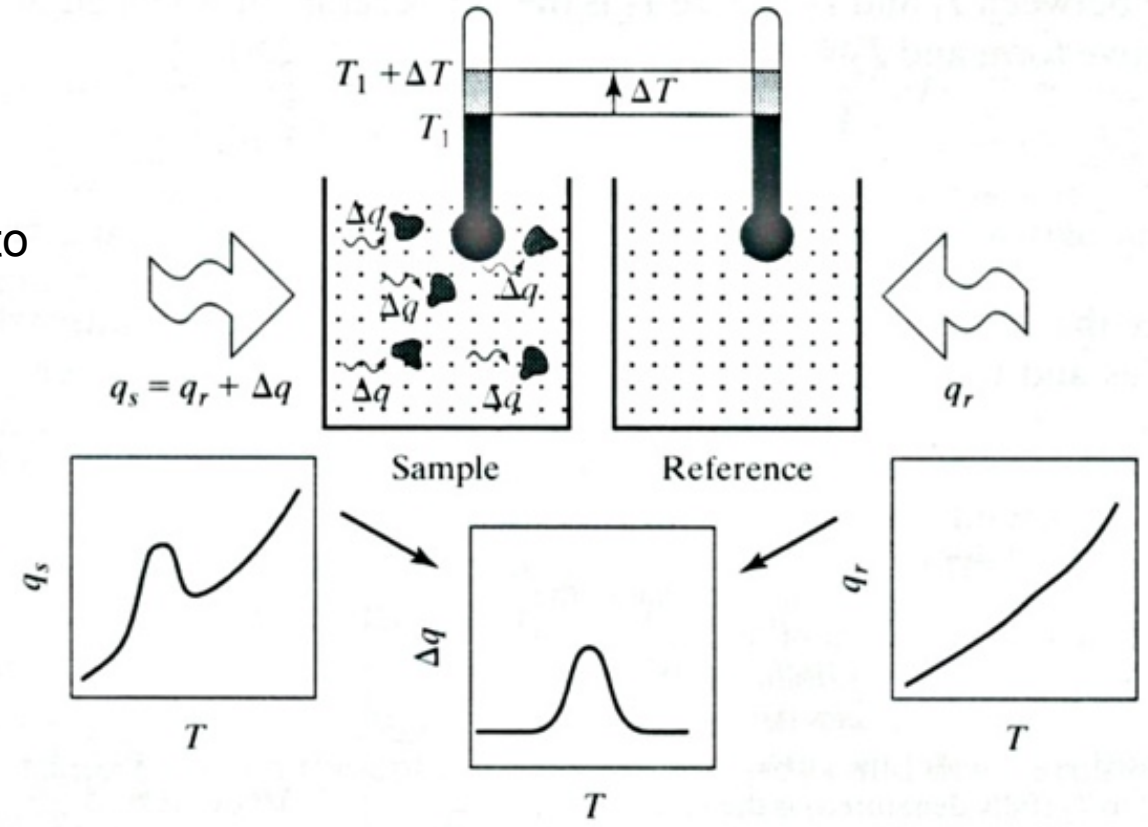
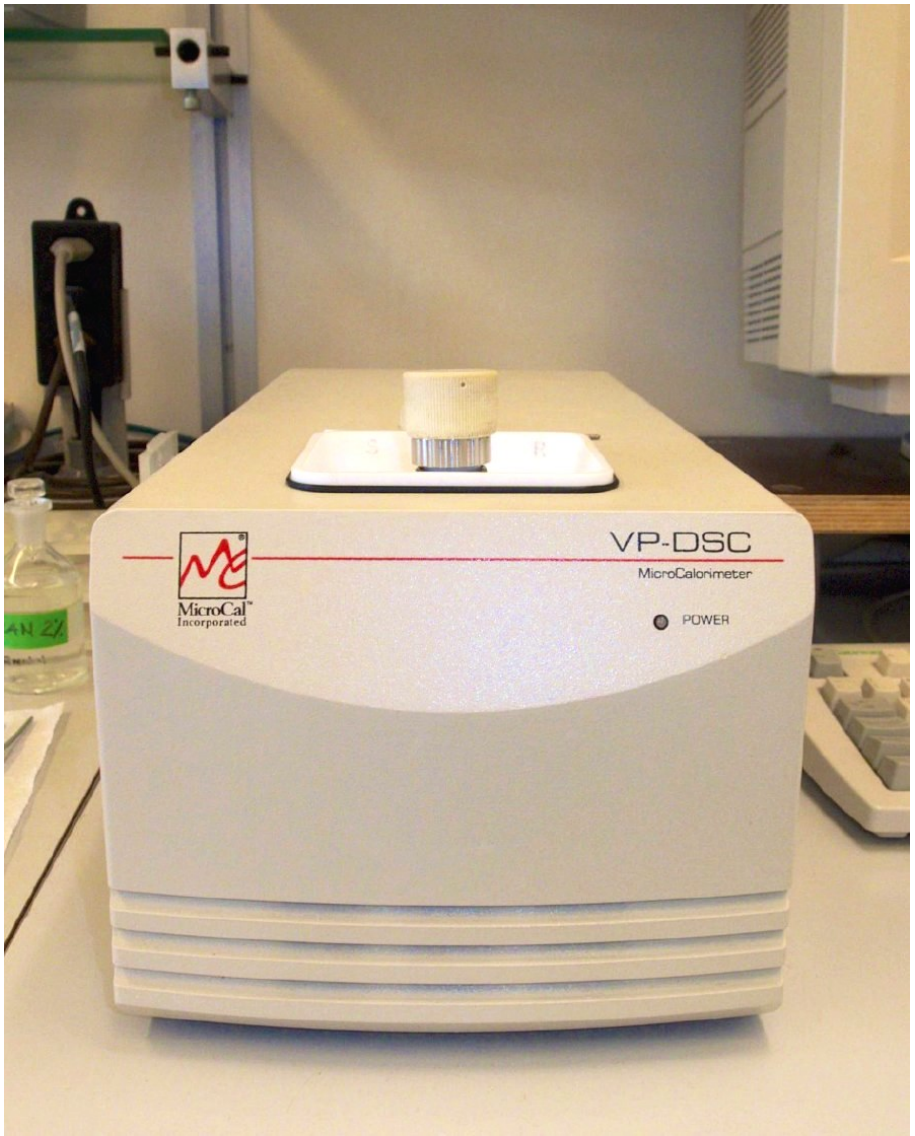


Figure 2.7 Experimental setup for a differential scanning calorimetry experiment. The amount of heat required to increase the temperature by the same increment (ΔT) of a sample cell (q_s) is higher than that required for the reference cell (q_s) by the excess heat absorbed by the molecules in the sample (Δq). The resulting DSC scans with the reference subtracted from the sample shows how this excess heat changes as a function of temperature.

DSC – Differential Scanning Calorimetry

DSC Instrument



DSC – Differential Scanning Calorimetry

$$C_p = dq / dT$$

C_p = heat capacity at constant pressure

H = enthalpy

U = internal energy

V = volume

p = pressure

q = heat

$$H = U + pV$$

$$\Delta H = \Delta U + p\Delta V + V\Delta p$$

$$q := \Delta U + p\Delta V$$

The first law of thermodynamics:
"Energy cannot be destroyed."
The change of the internal energy U is
the supplied heat q, minus the work done by system.

$$\Delta U = q - p\Delta V$$

$$\Delta H = q + V\Delta p$$

If: $\Delta p = 0$, then:

$$\Delta H = q$$

ΔH can therefore be measured in an isobaric calorimeter by measuring q.

$$C_p = dH / dT \quad ; \text{if } \Delta p = 0$$

DSC – Differential Scanning Calorimetry

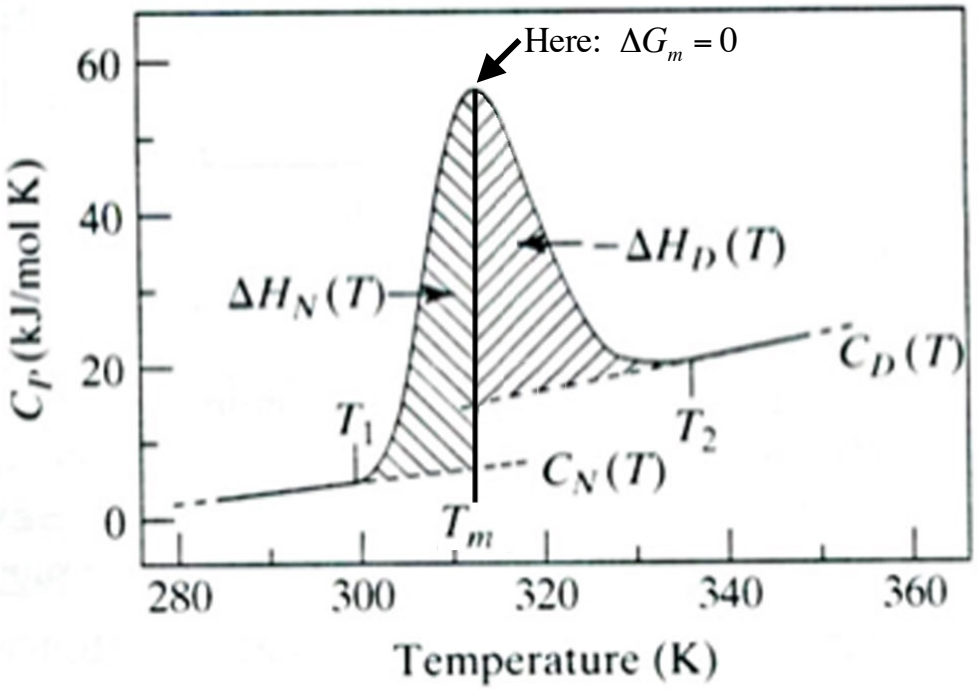
Start with a folded protein of heat capacity C_N.
Heat sample until protein denatures at the melting temperature T_m, where [denatured]=[native].
End with unfolded protein of heat capacity C_D.

[ ] := *concentration of*

The average calorimetric enthalpy associated with protein unfolding is:

$$\langle \Delta H \rangle \propto \int_{T_1}^{T_2} C_P \cdot dT$$

Figure 2.8 DSC for protein denaturation shows the heat capacity (C_P) changing from that of the native form [$C_N(T)$] to that of denatured form [$C_D(T)$]. At the point of transition is the excess heat capacity associated with denaturation. The integrated areas under the curve from T_1 (fully native) to T_2 (fully denatured) is the enthalpy for denaturation. At the midpoint temperature (T_m), the protein is half native and half denatured: thus the associated enthalpies for the folded native [$\Delta H_N(T)$] and denatured [$\Delta H_D(T)$] protein are equal and of opposite signs. [Adapted from S.-I. Kidokoro and A. Wada (1987), *Biopolymers* **26**, 213–229.]



DSC of ubiquitin

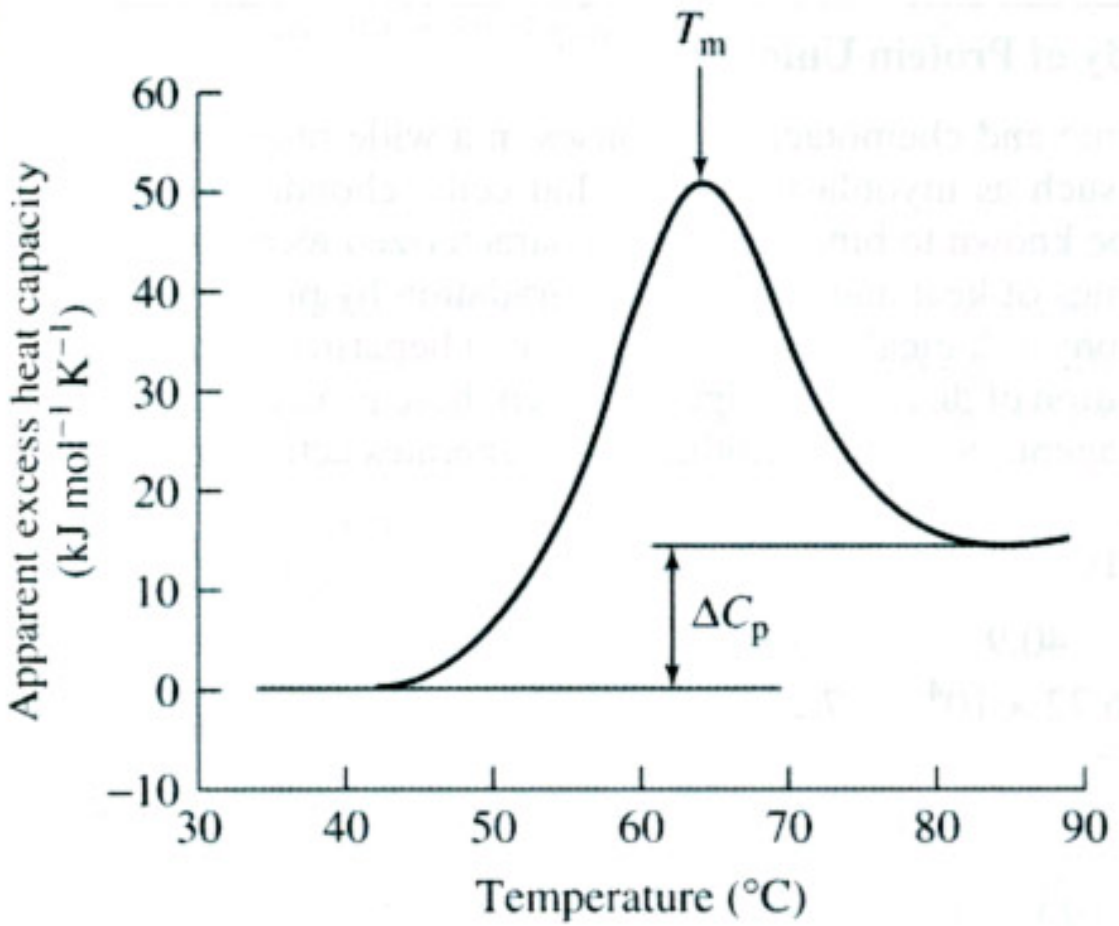
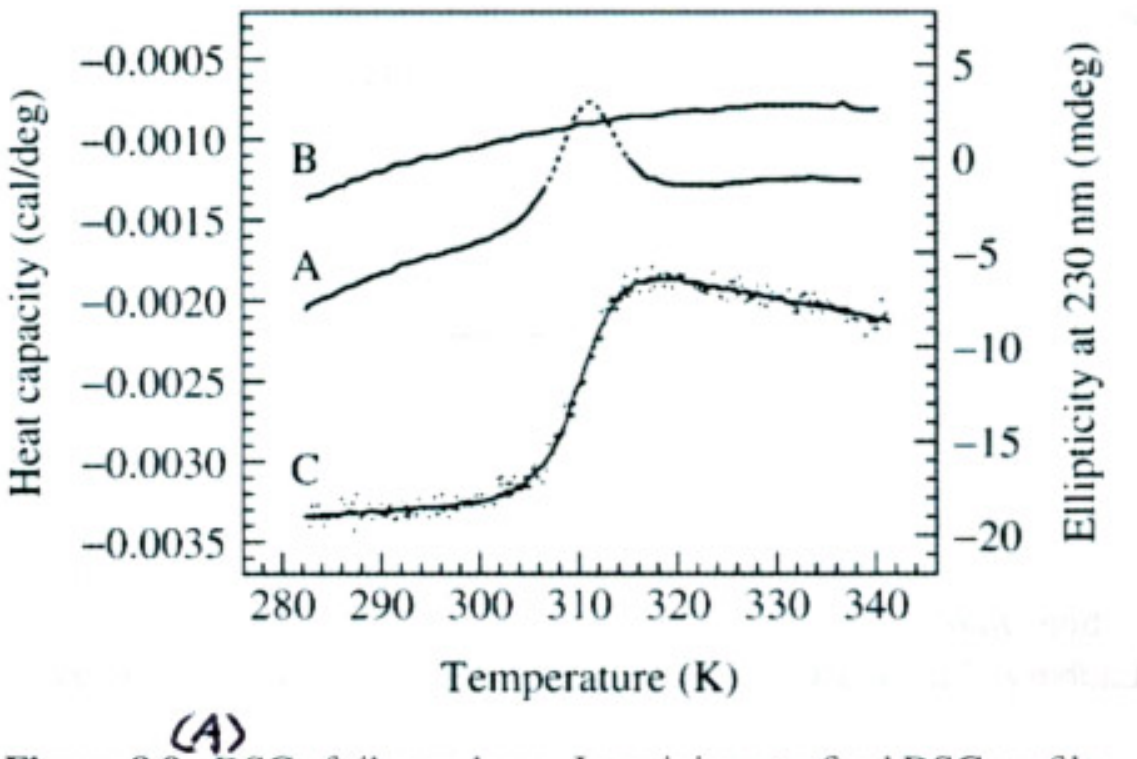


Figure 8.8. DSC of ubiquitin. A typical DSC result for the thermal denaturation of ubiquitin (5 mg/ml) is shown. The melting temperature is denoted by T_m while the change in specific heat (ΔC_p) is calculated as shown Reprinted from Ladbury and Chowdhry (1998). *Biocalorimetry: Applications of Calorimetry in the Biological Sciences*. Copyright John Wiley & Sons Limited.

DSC of ribonuclease



(A)

Figure 8.9. DSC of ribonuclease. In arriving at a final DSC profile, the baseline (B) must be subtracted from the thermal denaturation profile of the protein (in this case RNAase from *Bacillus amyloliquifaciens*). Circular dichroism (C) confirms that the melting temperature (T_m) occurs at the peak of the DSC signal. Fersht, A. (1999). *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*. W.H. Freeman & Co, reproduced with permission.

A: DSC of ribonuclease
B: DCS baseline (buffer)
C: CD(222nm) melting curve, for comparison

ITC – Isothermal Titration Calorimetry

ITC – Isothermal Titration Calorimetry

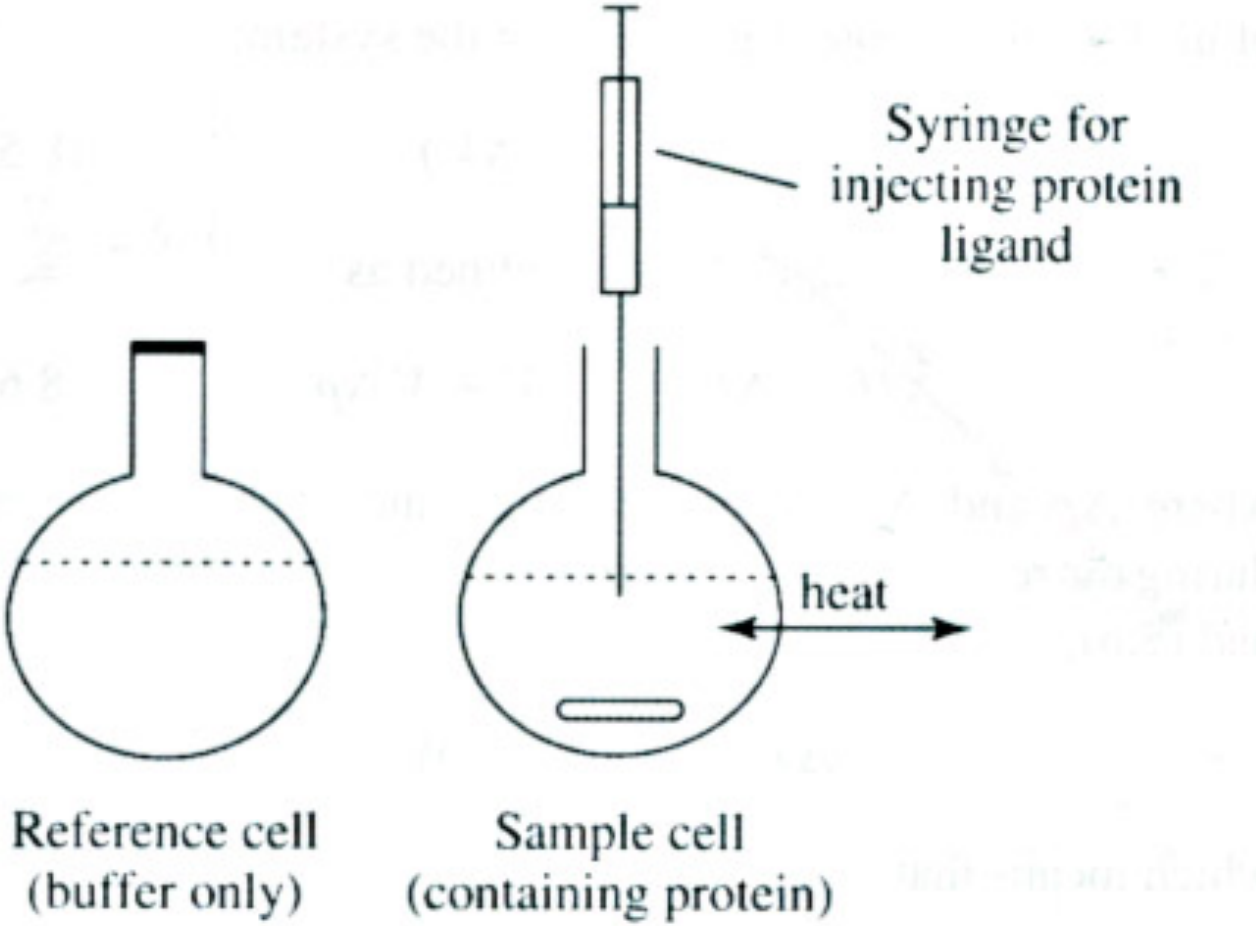
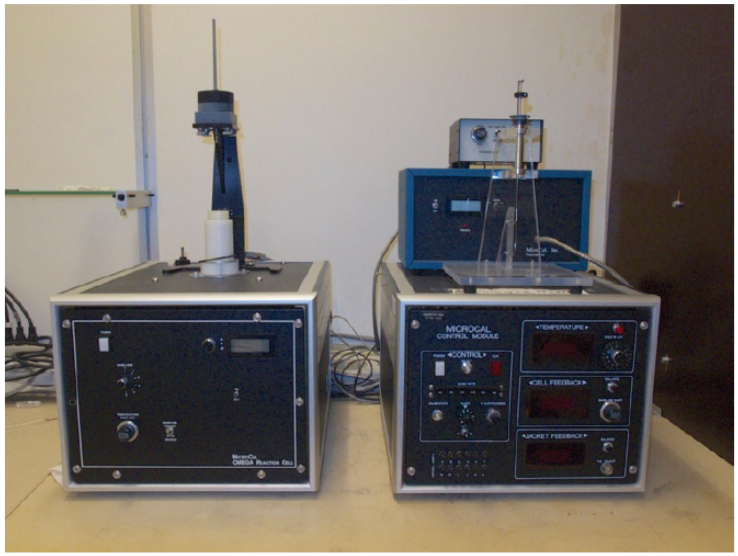
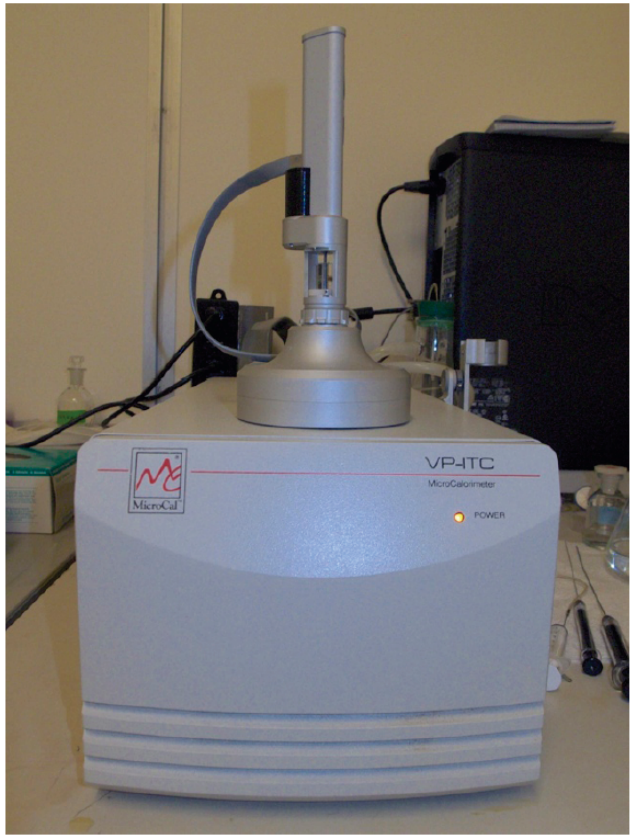


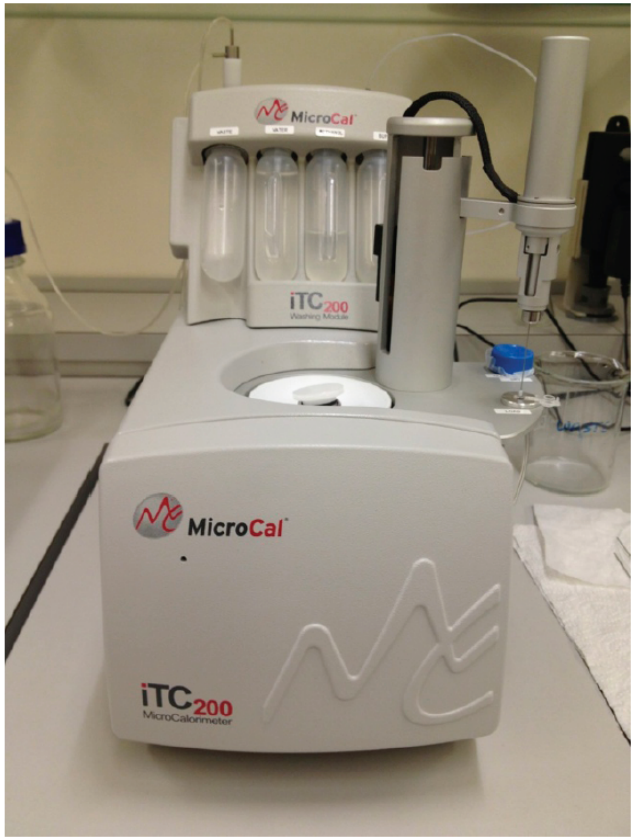
Figure 8.4. Outline design of an isothermal titration calorimeter. Addition of a substance such as a protein ligand into the sample cell causes a release or absorption of a small amount of heat. This is detected and analyzed electronically. Several such injections are made during a typical experiment.

ITC – Isothermal Titration Calorimetry

ITC Instruments







ITC – Isothermal Titration Calorimetry

A simple chemical reaction of a ligand binding to a protein is described as:

$$P + L \underset{}{\overset{K_a}{\rightleftharpoons}} PL$$

P = Protein
L = Ligand
PL = Protein with ligand bound

The heat released from the reaction is described as:

$$q = \Delta H(T) \cdot n_{PL} = \Delta H(T) \cdot V \cdot [PL]$$

Using:

$$[PL] = K_a[P][L]$$

$$[PL] + K_a[PL][L] = K_a[P][L] + K_a[PL][L]$$

$$[PL] + K_a[PL][L] = K_a[P_T][L]$$

$$[PL] \cdot (1 + K_a[L]) = [P_T]K_a[L]$$

$$[PL] = [P_T]\left(\frac{K_a[L]}{1 + K_a[L]}\right)$$

q = released heat from a reaction
$\Delta H(T)$ = enthalpy change at a particular temperature T
n_{PL} = number of moles of reaction product PL
V = volume of reaction
$[P]$ = concentration of protein P
$[L]$ = concentration of ligand L
$[PL]$ = concentration of reaction product PL
$[P_T] = [P] + [PL]$ = total protein concentration

We obtain:

$$q = \Delta H(T) \cdot V \cdot [P_T]\left(\frac{K_a[L]}{1 + K_a[L]}\right)$$

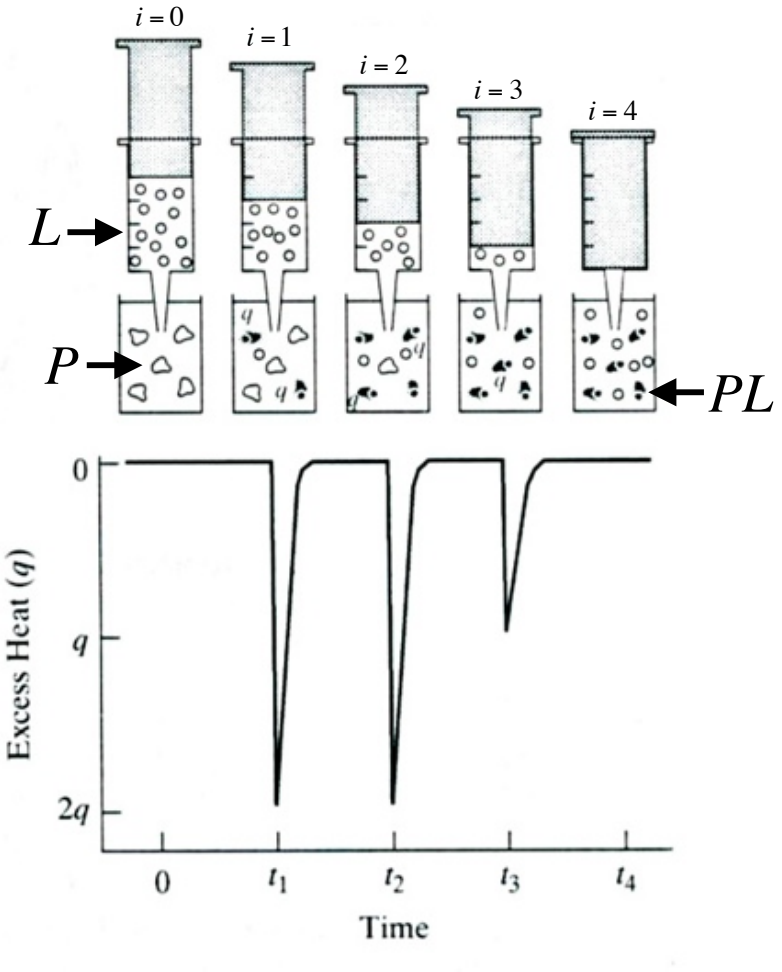
We can measure q.
We know $T, V, [P_T], [L]$.
We want ΔH and also K_a.
One equation for two variables doesn't work.

ITC – Isothermal Titration Calorimetry

Solution: We add the ligand in many small amounts, and measure the heat during each injection.

We then measure the heat in each peak *"i"* in an ITC measurement, and obtain *"i"* equations:

Figure 2.10 Isothermal titration calorimetry (ITC) involves incremental addition over time of a ligand (circles) into a solution of a protein. At each titration point, formation of a complex results in the release or absorption of heat (q), which is the heat of complex formation. At the initial time intervals (t_1 and t_2), the amount of free protein is sufficient to bind ligand according to the concentrations of free ligand and protein in the solution. In later intervals, however, the amount of free protein becomes limiting until, at the end of the titration (t_4), there is no protein available to form a complex and, therefore, no excess heat is absorbed or released, even with the added ligand.



$$q_i = \Delta H(T) \cdot V \cdot \left([PL]_i - [PL]_{i-1} \right)$$

$$[PL] = [P_T]\left(\frac{K_a[L]}{1 + K_a[L]}\right)$$

$$q_i = \Delta H(T) \cdot V \cdot [P_T]\left(\frac{K_a[L]_i}{1 + K_a[L]_i} - \frac{K_a[L]_{i-1}}{1 + K_a[L]_{i-1}}\right)$$

q_i = released heat after injection i

$[PL]_i$ = concentration of reaction product PL after injection i

$[L]_i$ = concentration of injected ligand after injection i

ITC – Isothermal Titration Calorimetry

We obtain a series of ITC peaks, which give a series of heats q_i.
This gives a series of equations:

$$q_i = \Delta H(T) \cdot V \cdot [P_T] \left(\frac{K_a [L]_i}{1 + K_a [L]_i} - \frac{K_a [L]_{i-1}}{1 + K_a [L]_{i-1}} \right)$$

We can measure q_i.

We know $T, V, [P_T], [L]_n$.

We want ΔH and K_a.

We have many values of q_i, which corresponds to many equations, so that we can fit those two variables.

Fitting can be done with a computer program that adapts the values for K_a and ΔH, until the equations correspond to the observed measurements for q_i.

ITC – Isothermal Titration Calorimetry

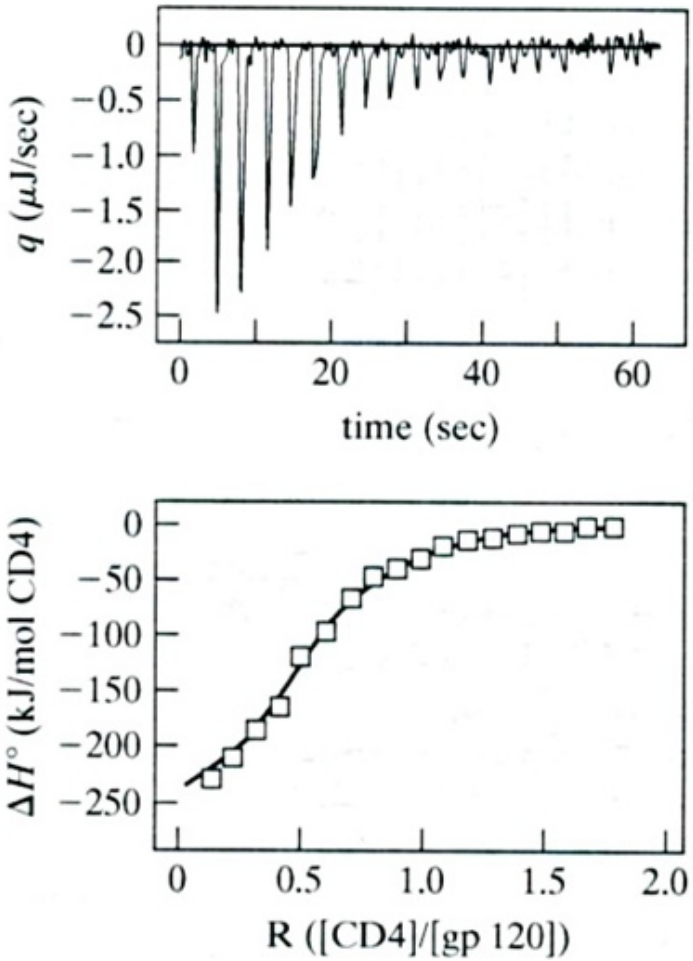
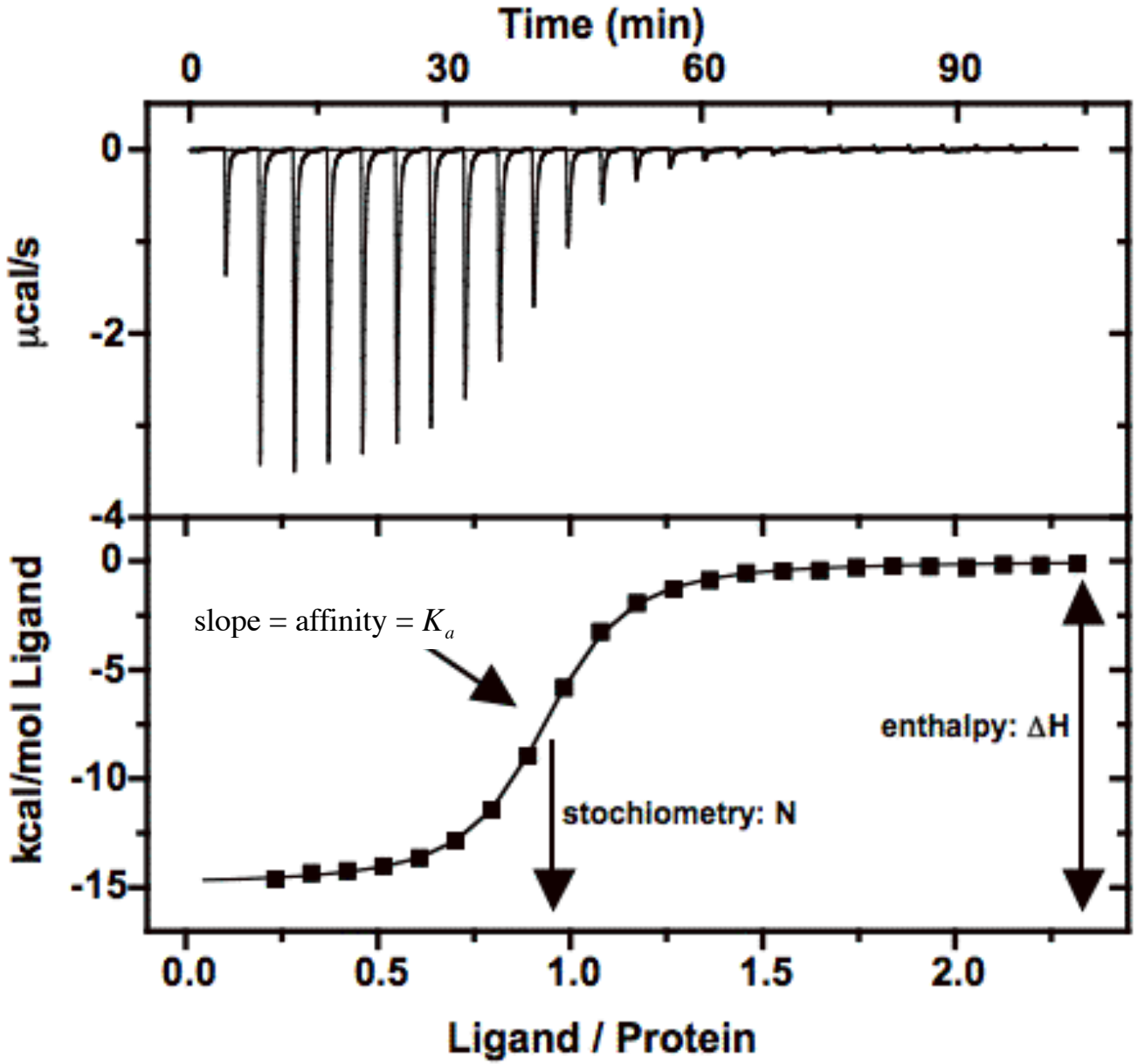


Figure 2.11 The thermochemistry of the binding reaction between core binding domain of the exterior glycoprotein (gp120) from the HIV-1 virus and the CD4 receptor of the targeted host cell. In this ITC study, the heat change over time (top graph) is integrated and normalized to the amount of the CD4 receptor injected. This yields the enthalpy change ($\Delta H^0 = -263$ kJ/mol) and an association constant of $K_a = 5 \times 10^6\ \mathrm{M}^{-1}$ when the resulting titration curve (bottom graph) is fit using Eq. 2.82 and 2.83. [Data from Myszka et al. (2000), *Proc. Natl., Acad. Sci., USA* **97**, 9026–9031.]

A software can fit a graph as in Fig. 2.11 above with the equations for q_i and to obtain ΔH and K_a.

$$R_{L/P} = [L_T]_i / [P_T]$$

Isothermal Titration Calorimetry (ITC)



ITC – Isothermal Titration Calorimetry

Measure q_i, determine $\Delta H(T)$ and K_a.

Use that to calculate $\Delta G(T)$, using: $\Delta G(T) = -RT \ln K_a$

And calculate $\Delta S(T)$, using: $\Delta G(T) = \Delta H(T) - T\Delta S(T)$

or: $$\Delta S(T) = \frac{\Delta G(T) - \Delta H(T)}{T}$$

The heat capacity C_p associated with the binding reaction can be determined from the slope of *H* vs. *T, using:* $$C_P = \frac{dH}{dT}$$

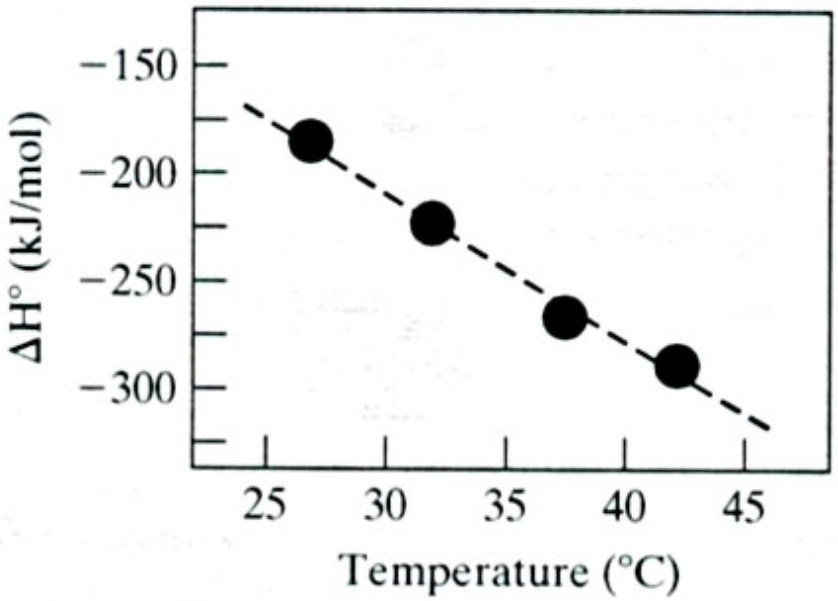


Figure 2.12 The heat capacity of the HIV-1 gp120 and CD4 receptor binding reaction in Figure 2.11 is determined by measuring the change in enthalpy over a range of temperatures. A $C_p = -7.5$ kJ/mol° is calculated from the slope when ΔH^0 is plotted relative to the temperature. [Data from Myszka et al. (2000) *Proc. Natl., Acad. Sci., USA* **97**, 9026–9031.]

ITC – Isothermal Titration Calorimetry

If K_a is very large, i.e., above 10^9 M^{-1}, then $[PL] \approx [L_T]$.

In this case, ITC cannot be applied.

$$P + L \overset{K_a}{\rightleftharpoons} PL$$

Since: $[P] = [P_T] - [P_L]$

when $[L_T] < [P_T]$, then $q_i = const.$

when $[L_T] \geq [P_T]$, then $[P] = 0$

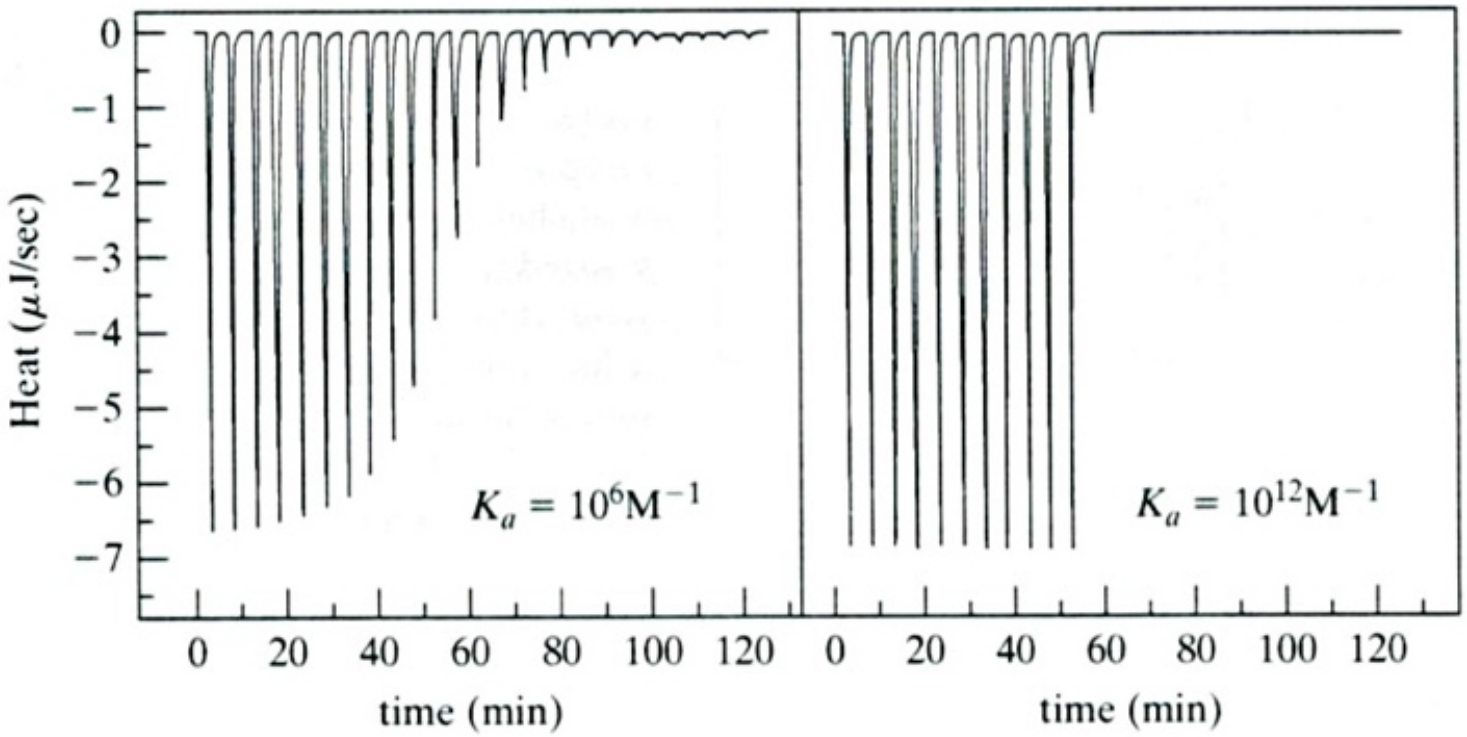


Figure 2.13 The ITC trace of a binding reaction having a measurable association constant ($K_a = 10^6$ M^{-1}) results in a smooth titration curve that can be fit to yield the ΔH^0 and K_a of the reaction. A binding reaction with a very strong association ($K_a = 10^{12}$ M^{-1}), however, shows heats of complex formation that are identical for all intervals where the ligand added is less than the protein in solution, but becomes zero when the concentration of ligand added becomes greater than that of the protein. This results in a very sharp titration curve that cannot be fit by Eqs. 2.82 and 2.83.

ITC – Isothermal Titration Calorimetry

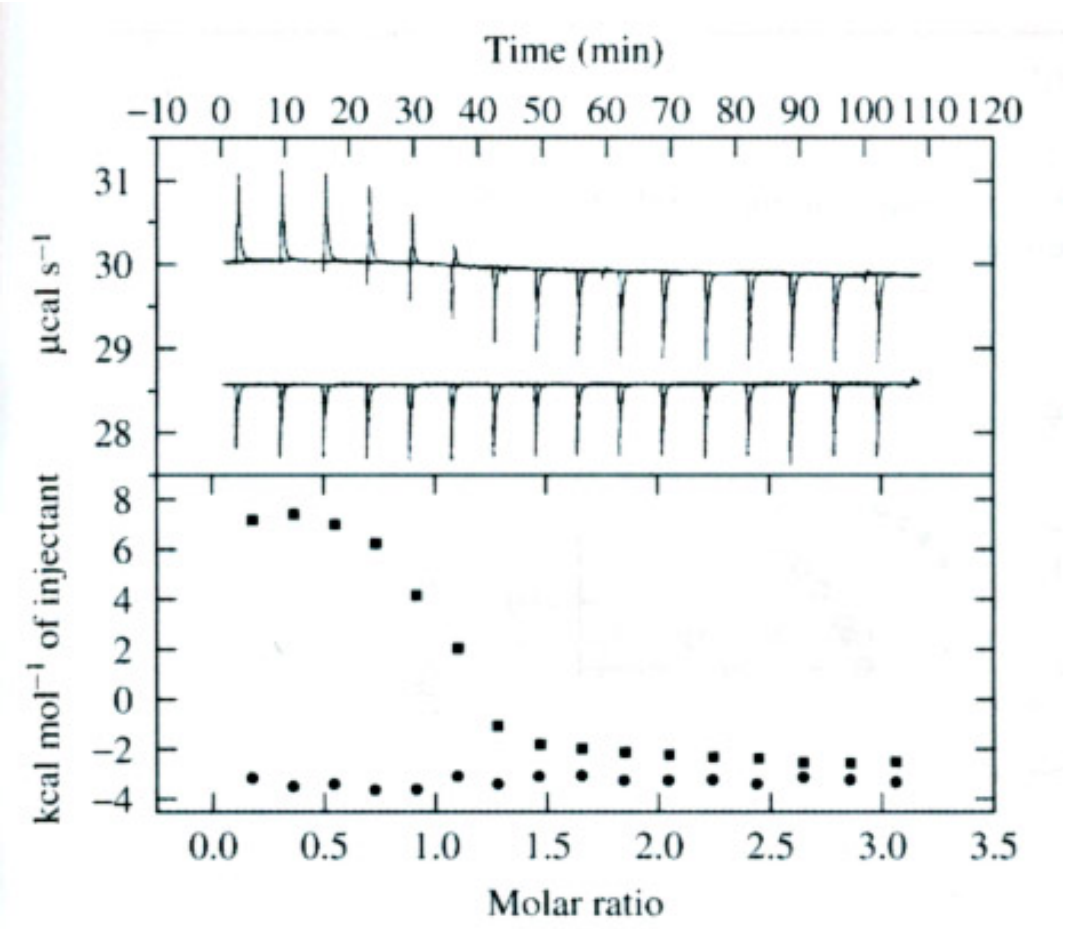


Figure 8.5. A typical ITC experiment. Small aliquots of a drug capable of interacting with DNA are sequentially injected into the calorimeter sample cell which contains DNA. Aliquots of heat resulting from each injection are accurately measured (upper panel). After subtracting the heat of solution for each injection (inverted spikes in upper panel), a binding isotherm may be constructed for this process (square points in lower panel). Reprinted from Ladbury and Chowdhry (1998). Biocalorimetry: Applications of calorimetry in the Biological Sciences. Copyright John Wiley & Sons Limited.

<==== Injection of ligand

<==== Injection of only buffer solution, to obtain baseline.