

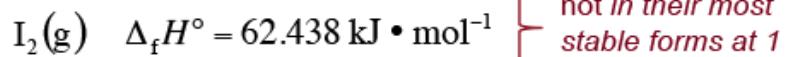
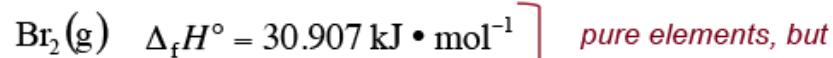
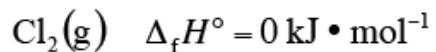
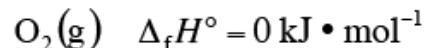
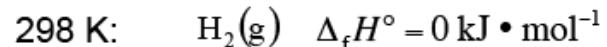
- For a **reversible** process Work on expansion = work of compression (same path)
- For an **irreversible** process Work on expansion \neq work of compression (same path)

Recap

1. $\Delta_r H$ (enthalpy of reaction, *extensive*)
2. Tabulation $\Delta_r H^0$ (Standard reaction enthalpy, *intensive*)
3. $\Delta_f H^0$ (Standard molar enthalpy of formation, *intensive*)

ELEMENTAL HEATS OF FORMATION

To assign specific values for $\Delta_f H^\circ$, the values of $\Delta_f H^\circ$ for *pure elements* in their *most stable forms* at *one bar* and the *temperature of interest* is set to *zero*.



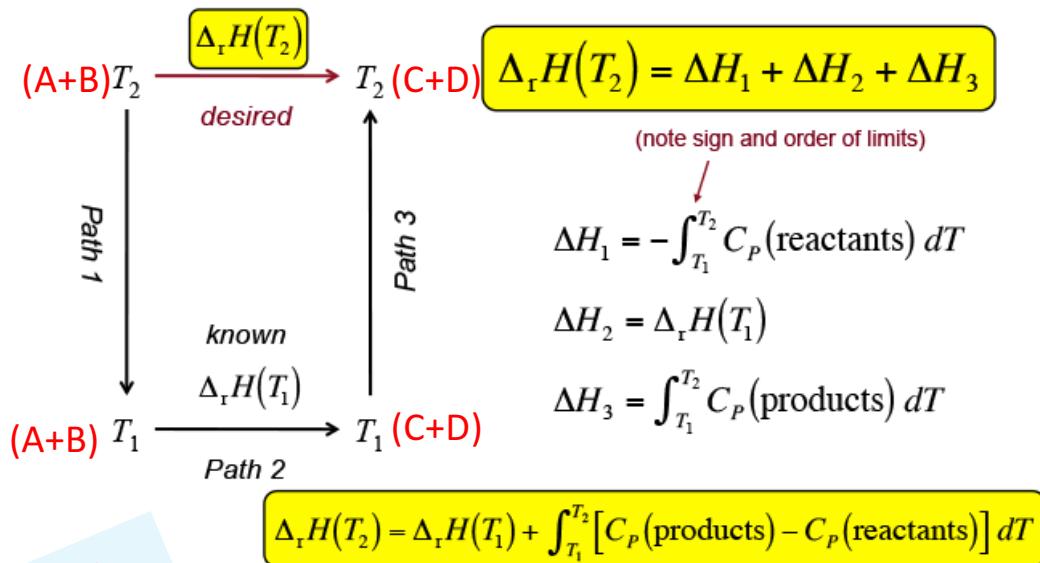
*pure elements, but
not in their most
stable forms at 1
bar and 298 K*

4. Using $\Delta_f H^0 \rightarrow \Delta_r H$

Recap

RELATING $\Delta_r H$ VALUES AT DIFFERENT T

To convert $\Delta_r H$ from T_1 (e.g., 298 K) to T_2 requires C_p



Reactant= A, B
Product = c, D

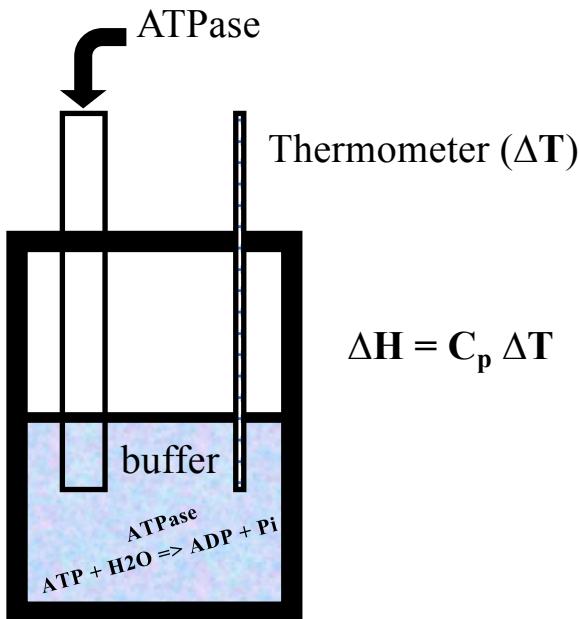
Thermodynamic Cycle

Application of calorimetry (heat measurement) in biology

- Batch Calorimetry
- Scanning calorimetry
- Differential scanning calorimetry
- Isothermal calorimetry

Batch Calorimetry

e.g, Heat change associated with ATP hydrolysis

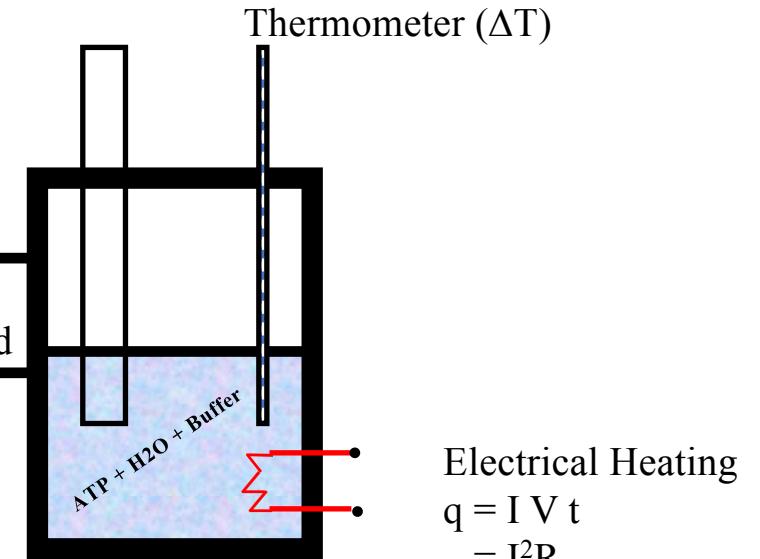


INSULATED VESSEL

- 1. Add ATPase**
- 2. Measure subsequent Temp rise**
- 3. Calculate ΔH**



- Well insulated
- Temp (T) = fixed



C_p is calculated by putting known amount of heat (say electrical heating):

$$C_p = I^2R / \Delta T$$

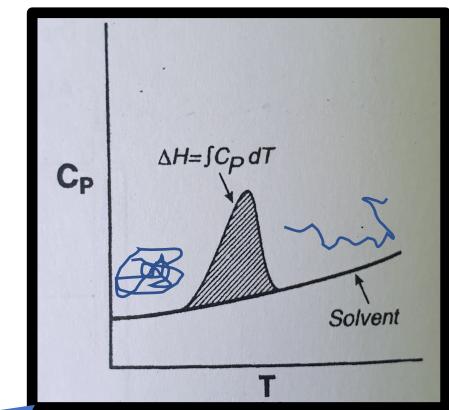
Tabulate,
 $C_p = a + bT + cT^2 + \dots$

Scanning Calorimetry

Measure Heat capacity as a function of temperature
e.g, Study Protein folding/unfolding.

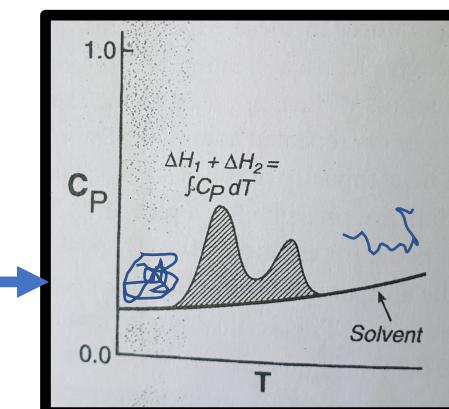
Steps

1. Known amount of heat added (through electrical heating)
2. Measure ΔT
3. **Very small amount of heat (q_p) added so that temp change is small**
4. Process repeated. Heat capacity calculated for each heat increment as,
 $C_p = (q_p / \Delta T)$ and plotted [Cp vs T]



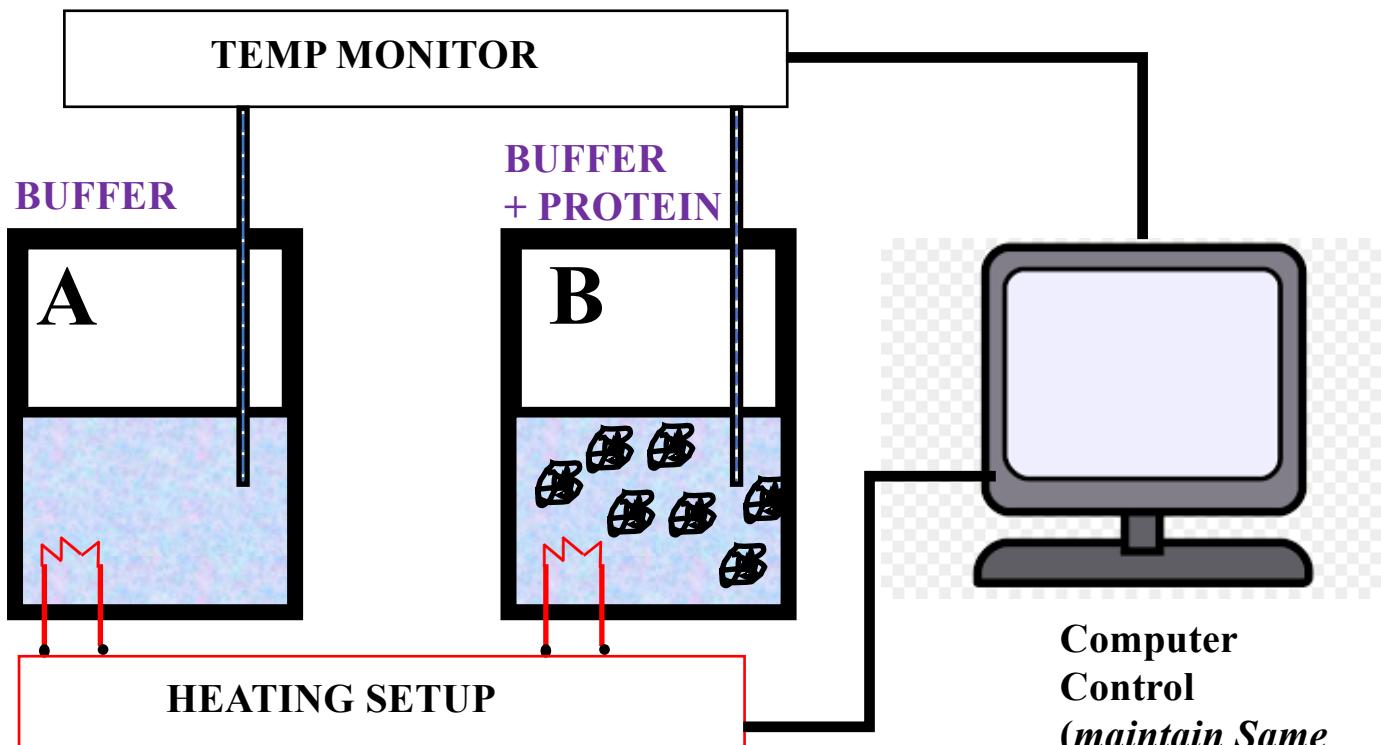
Analysis

- Heat capacity vs Temp smooth curve (slowly rising) for Solvent
- Presence of peak represent denaturation
- The enthalpy change associated with denaturation is $\Delta H = \int C_p dT$
- Protein denaturation may occur in multiple stages ($\Delta H_1, \Delta H_2$)



Differential Scanning Calorimetry

Measure **difference** in Heat capacity as a function of temperature
e.g, Study Protein folding/unfolding.



Steps

1. Scan Temp UP
2. keep "A" and "B" at same temp
3. Different heat input required (A and B has different heat capacity)

$$\text{Heat flow (rate)} = \frac{dq}{dt} \quad (t = \text{time}, q = \text{heat})$$

$$\text{Rate of Temp (T) change} = \frac{dT}{dt}$$

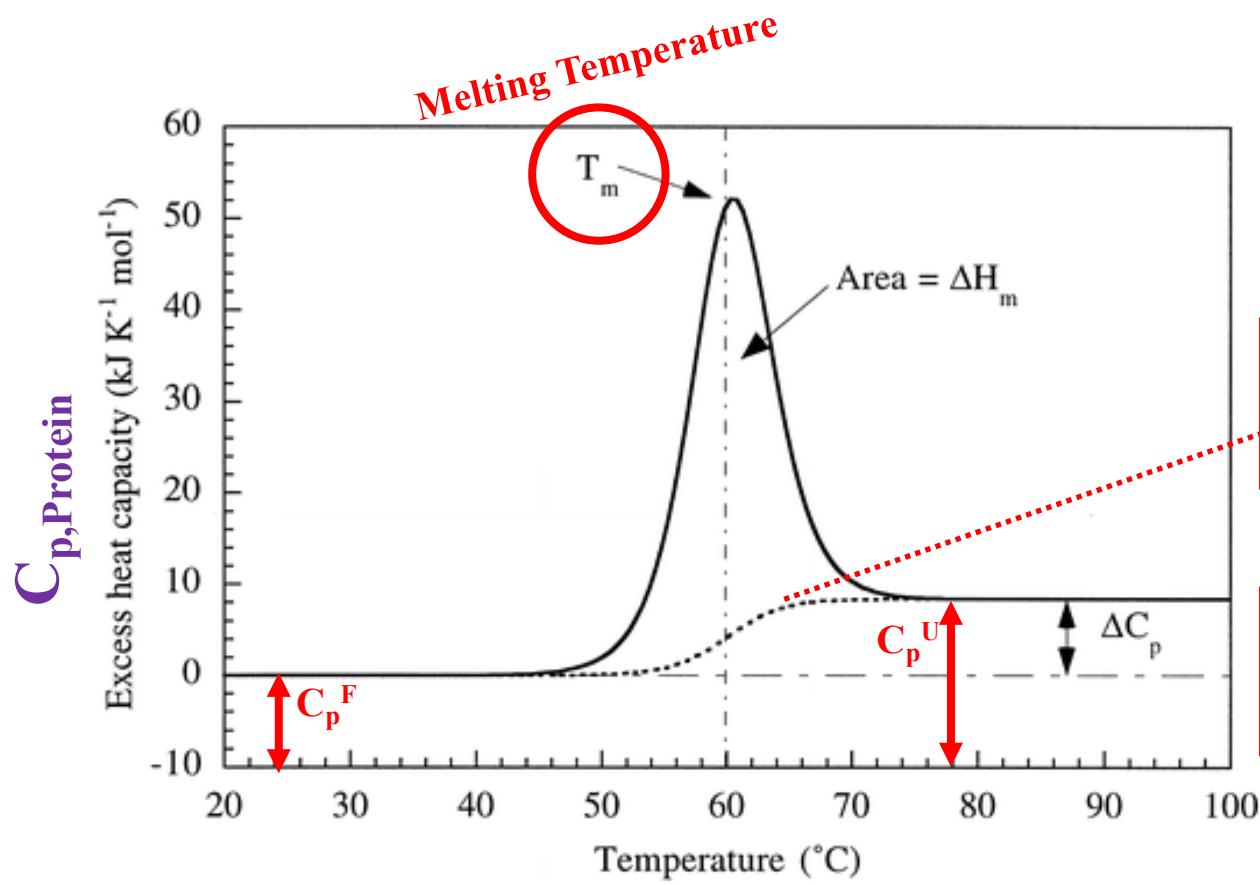
Now,

$$C_{p,A} = \frac{\frac{dq_A}{dt}}{\frac{dT}{dt}} = \frac{dq_A}{dT}$$

$$C_{p,B} = \frac{dq_B}{dT}$$

$$C_{p,B} - C_{p,A} = C_{p, \text{Protein}}$$

Differential (Solvent effect included)



$$\Delta H = \int C_{p,\text{Protein}} dT$$

$$C_p^{F+U} = f^* C_p^F + (1-f)^* C_p^U$$

f = fraction folded

$$\Delta C_p = C_p^U - C_p^F$$

(ALWAYS +Ve)

Chemical Reviews 1997 97 (5), 1251-1268

That plot may have multiple hump
: Protein denaturation may occur in multiple stages

CAUTION !

ΔH associated with protein unfolding often interpreted in molecular terms (viz., H-bonds, electrostatics, hydrophobic interactions).

PLEASE NOTE: These interpretations are not inherent in thermodynamic quantities (Thus do not give explicit information at molecular level).

Bottom line: Scrutinize very critically before claiming atomic insights.

Differential Scanning Calorimetry (APPLICABILITY)

- High T_m = High Stability
- Optimize stable construct of protein
- Buffer and storage optimization

<https://www.youtube.com/watch?v=2CU3uvjKXlk>

Isothermal Calorimetry (ITC)

Protein Ligand Binding. $P + L \rightleftharpoons PL$

$$K_a = \frac{[PL]}{[P][L]}$$

Association
constant

Equilibrium concentrations
[PL] = complex
[P] = free protein
[L] = free ligand

Dissociation
constant $K_d = (1/K_a) = \frac{[P][L]}{[PL]}$

Fraction of ligand bound:

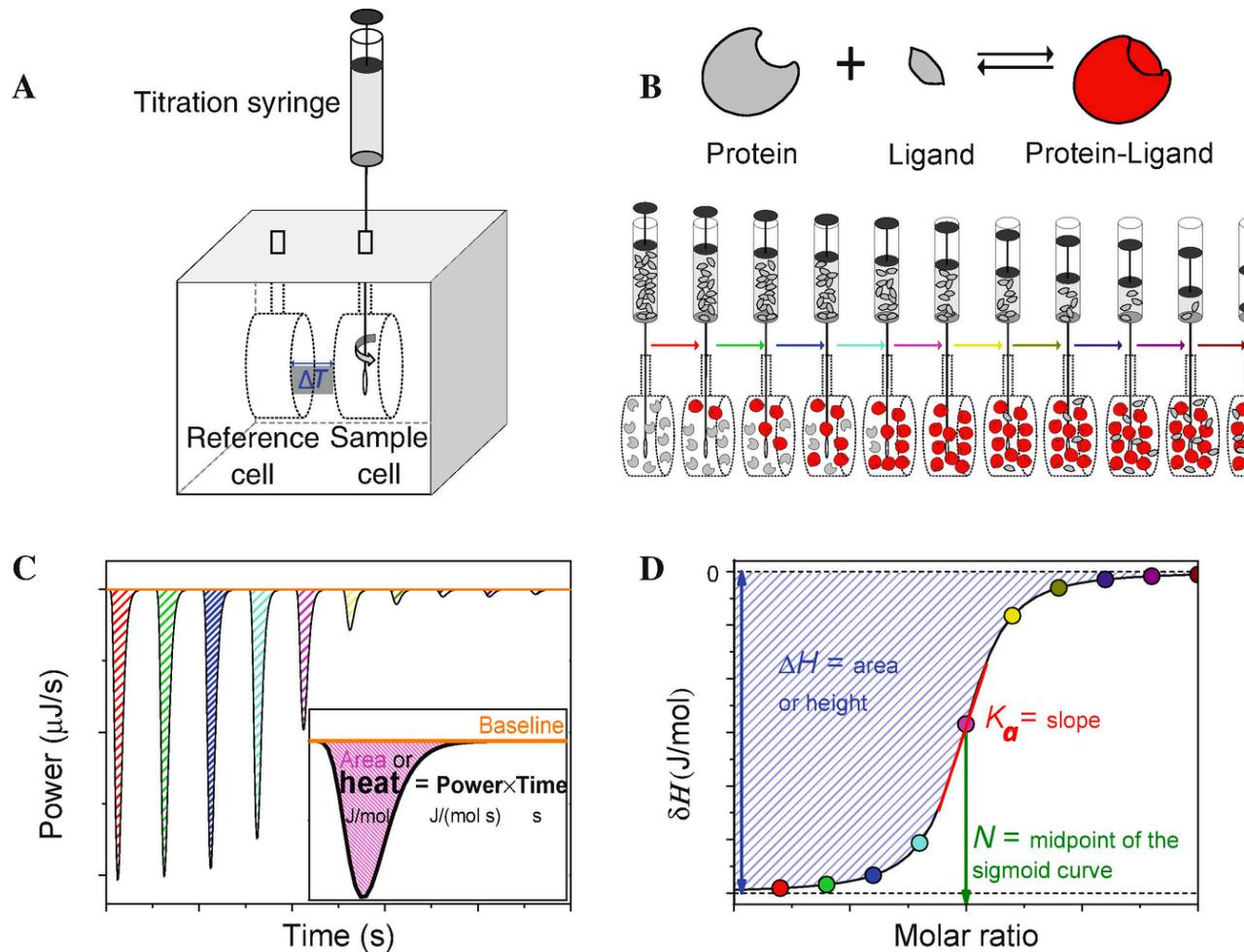
$$f = \frac{[PL]}{[P]_{total}} = \frac{[PL]}{[P]_{total}}$$

We know, $[P]_{total} = [P] + [PL]$

$$\begin{aligned} f &= \frac{[PL]}{[P] + [PL]} = \frac{[PL]}{[P] + [PL]} = \frac{\left(\frac{[P][L]}{K_d}\right)}{[P] + \left(\frac{[P][L]}{K_d}\right)} \\ &= \frac{[L]}{K_d + [L]} \end{aligned}$$

f (fraction of ligand bound) = $\frac{[L]}{K_d + [L]}$

Isothermal Calorimetry (ITC)



Steps

1. FIX the temperature of the Cell (ISOTHERMAL)
2. Add sample and Stirr
3. Measure Power/ Heat rate ($\frac{dq}{dT}$, t = time)
unit ($\mu\text{J}/\text{Sec}$)
4. Estimate area (power vs time plot)

Information

- Binding affinity (slope of the isotherm) $\Rightarrow K_a = (1/K_d)$
- stoichiometry (middle point of the isotherm)

Why inhibitor is strongest binder ?

ITC → K_d

Drug discovery

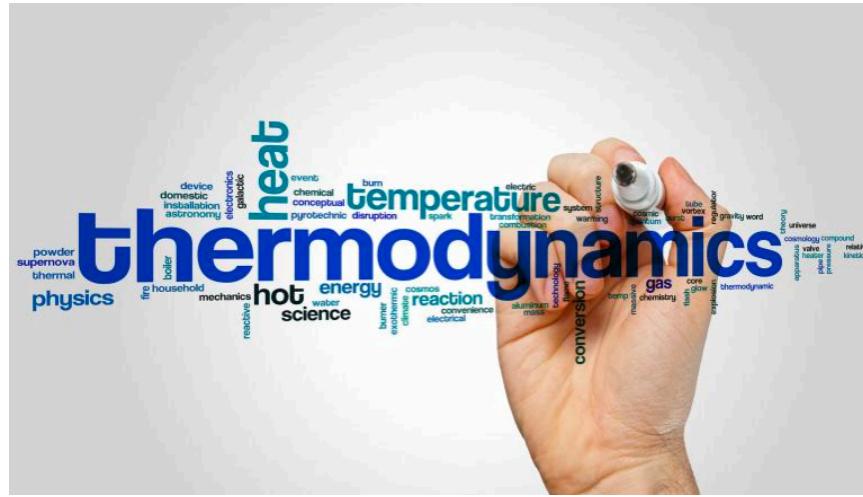
Lecture 0 (Slide 6)

How Biomolecules Recognize Right from Wrong ?

Drug discovery:
Which one is best L1 or L2 ?

6

<https://www.youtube.com/watch?v=oIpWcWKNXI>



Next: 1st law for open system