Biological Thermodynamics

"Classical thermodynamics… is the only physical theory of universal content concerning which I am convinced that, within the framework of applicability of its basic contents, will never be overthrown"

Albert Einstein

Thermodynamics is fundamental to the development and applications of biophysical methods!

Biological Thermodynamics

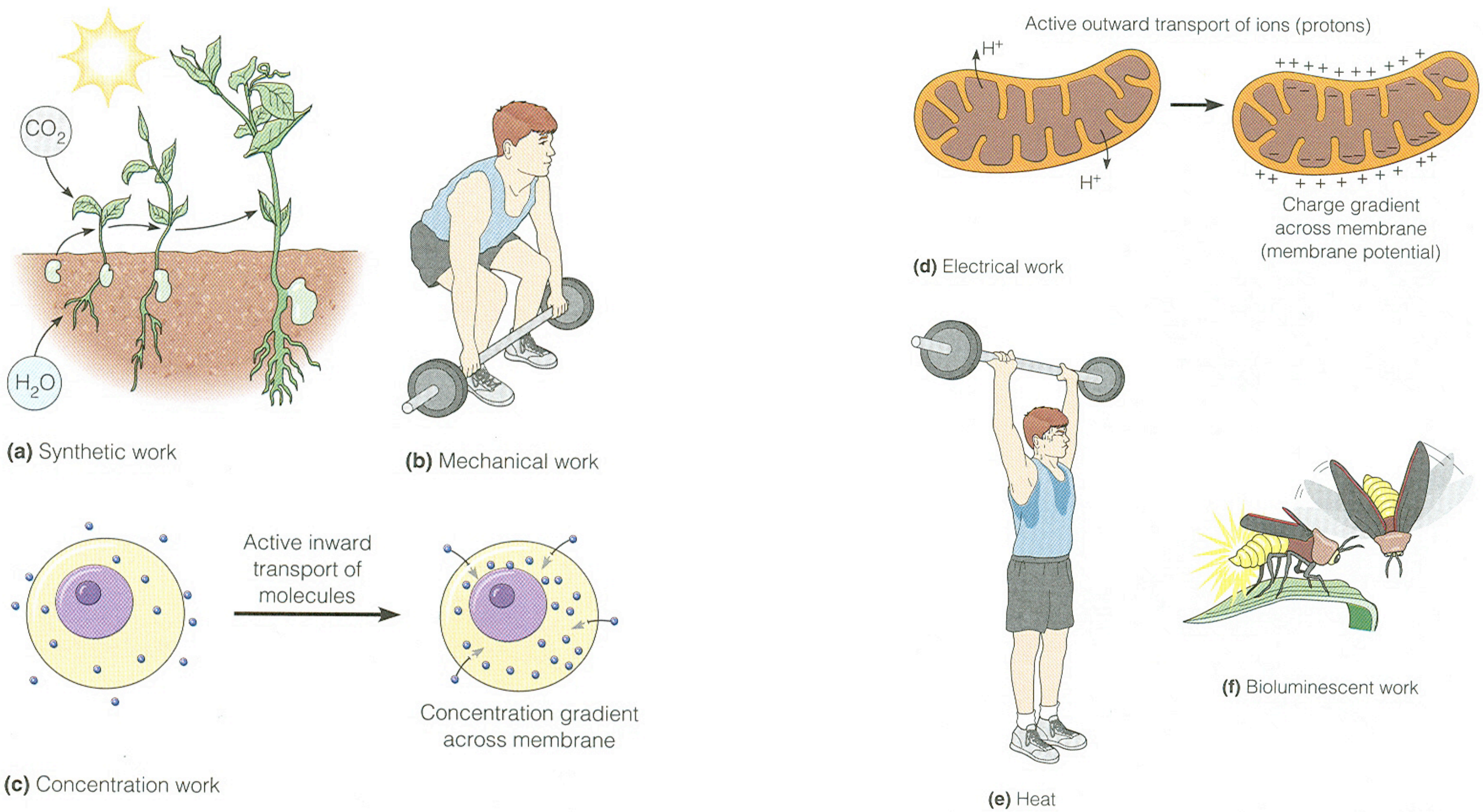
What is energy?

"…the term **energy** is difficult to define precisely, but one possible definition might be the capacity to produce an effect"

Encyclopædia Britannica

Biological Thermodynamics

Biological work



(a) Synthetic work

(b) Mechanical work

(c) Concentration work

(d) Electrical work

(e) Heat

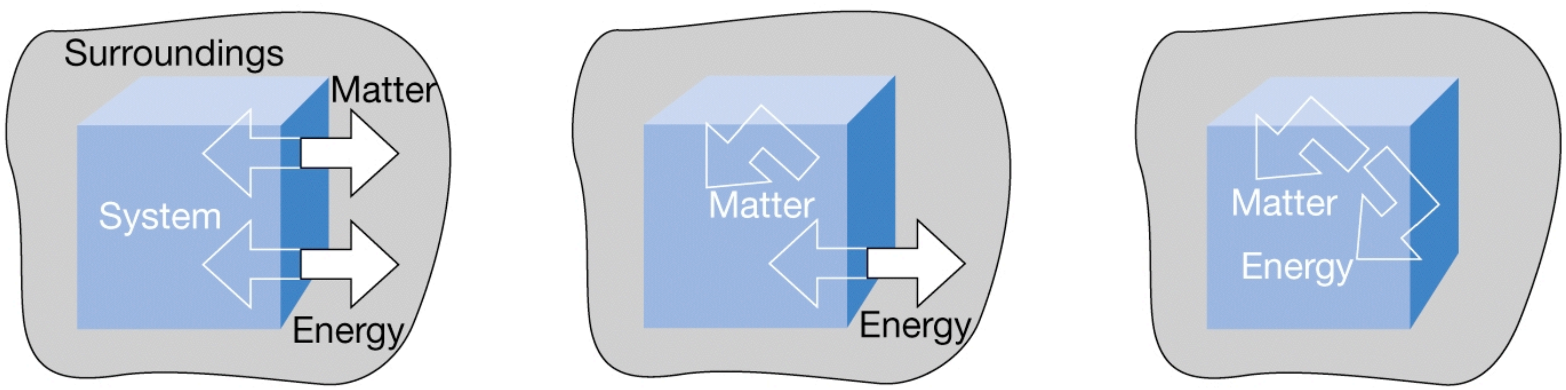
(f) Bioluminescent work

Biological Thermodynamics

System and Surroundings

A **system** is defined as the matter within a defined region of space (i.e., reactants, products, solvent)

The matter in the rest of the universe is called the **surroundings**



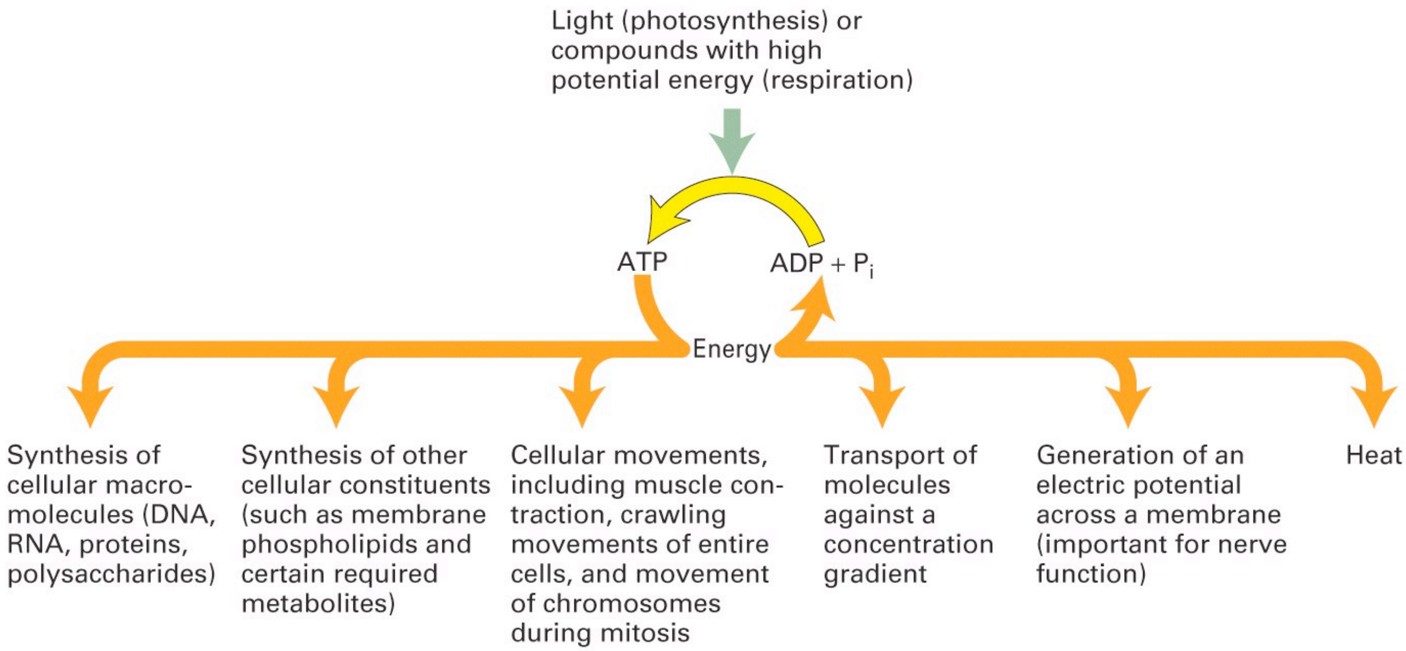
(a) Open (b) Closed (c) Isolated

The **First** Law of thermodynamics

The Energy is conserved

The total energy of a system and its surroundings is constant

In any physical or chemical change, the total amount of energy in the universe remains constant, although the form of the energy may change.



Biological Thermodynamics

Internal Energy (U)

Is the energy within the system

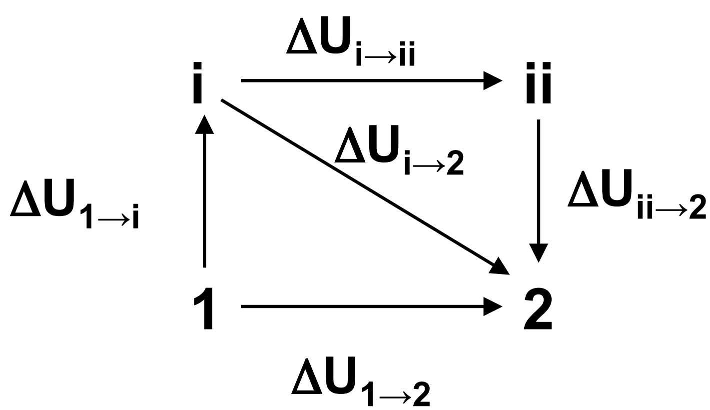
The **internal energy** of a system is the total kinetic energy due to the motion of molecules (translational, rotational, vibrational) and the total potential energy associated with the vibrational and electric energy of atoms within molecules or crystals.

U is a state function, that is, its value depends only on the current **state of the system**

Biological Thermodynamics

Internal Energy (U)

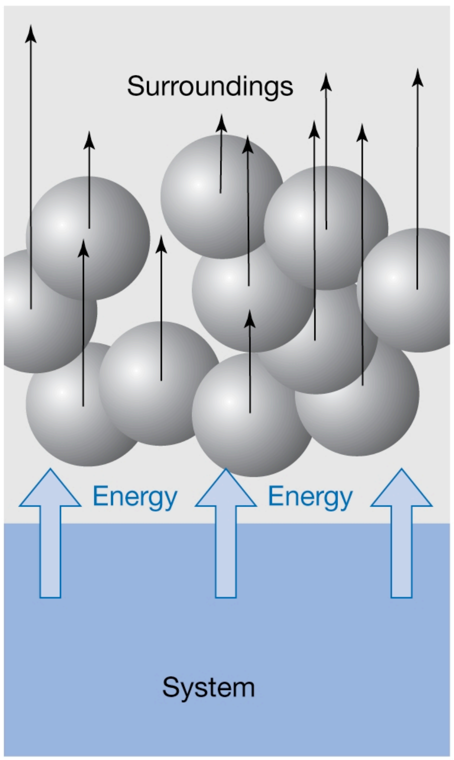
Only **ΔU** *can be measured directly*



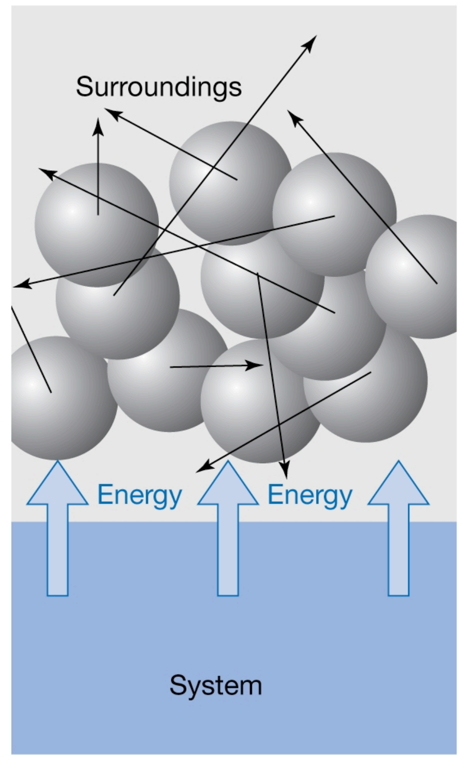
Biological Thermodynamics

Work (W) and Heat (Q)

$$\Delta U = W + Q$$



Work involves the non-random movement of particles

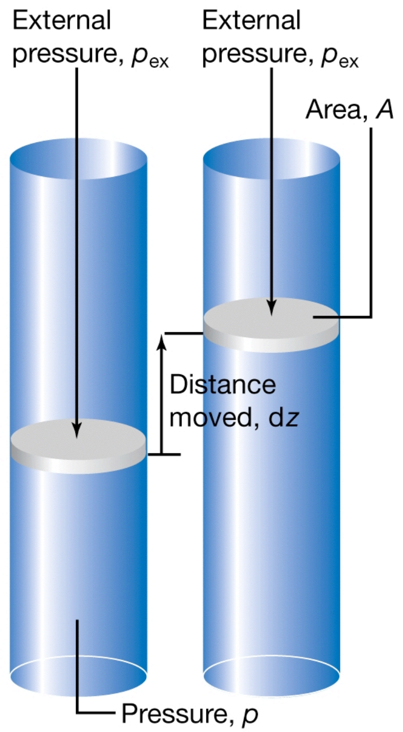


Heat involves the random movement of particles

Biological Thermodynamics

Enthalpy (H)

$$\Delta U = W + Q$$



$$Q_P = \Delta U - W$$

$$\Downarrow$$

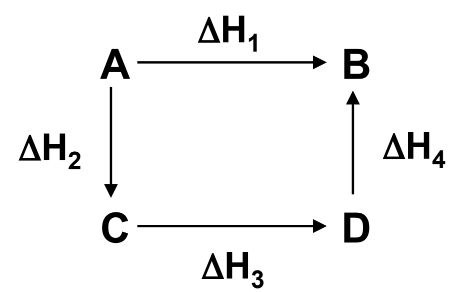
$$Q_P = \Delta U - P(V_2 - V_1)$$

$$\Downarrow$$

$$Q_P = \Delta U - P(\Delta V)$$

$$\Downarrow$$

$$Q_P = \Delta H$$

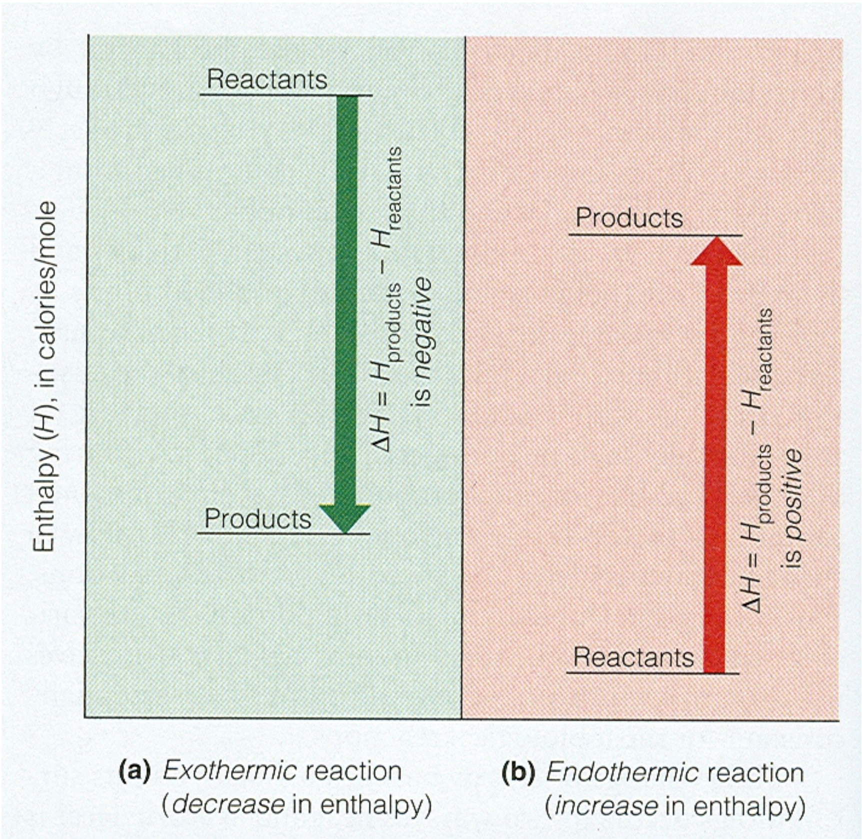


Enthalpy is a state function

The enthalpy is the heat absorbed or emitted by a system at constant pressure.

Biological Thermodynamics

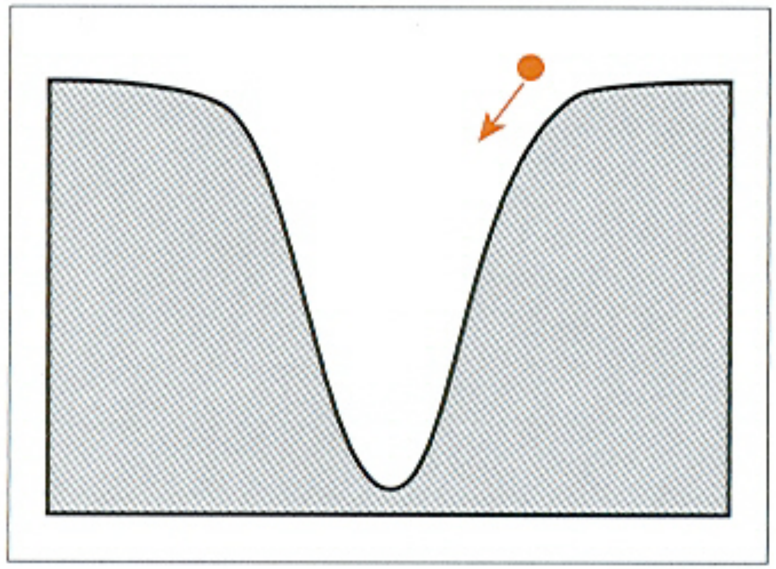
Enthalpy change (ΔH)



ΔH during a chemical reaction is the heat absorbed or released in the breaking and formation of bonds

Biological Thermodynamics

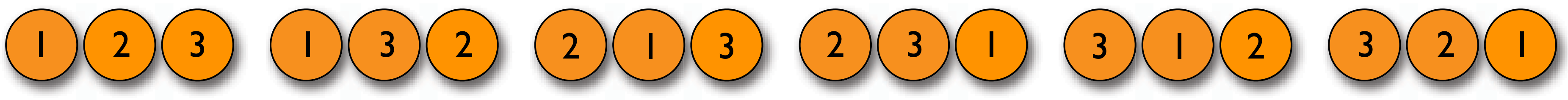
When is a reaction spontaneous?



Things tend to want to roll downhill: ΔH tends to be negative

Biological Thermodynamics

When is a reaction spontaneous?

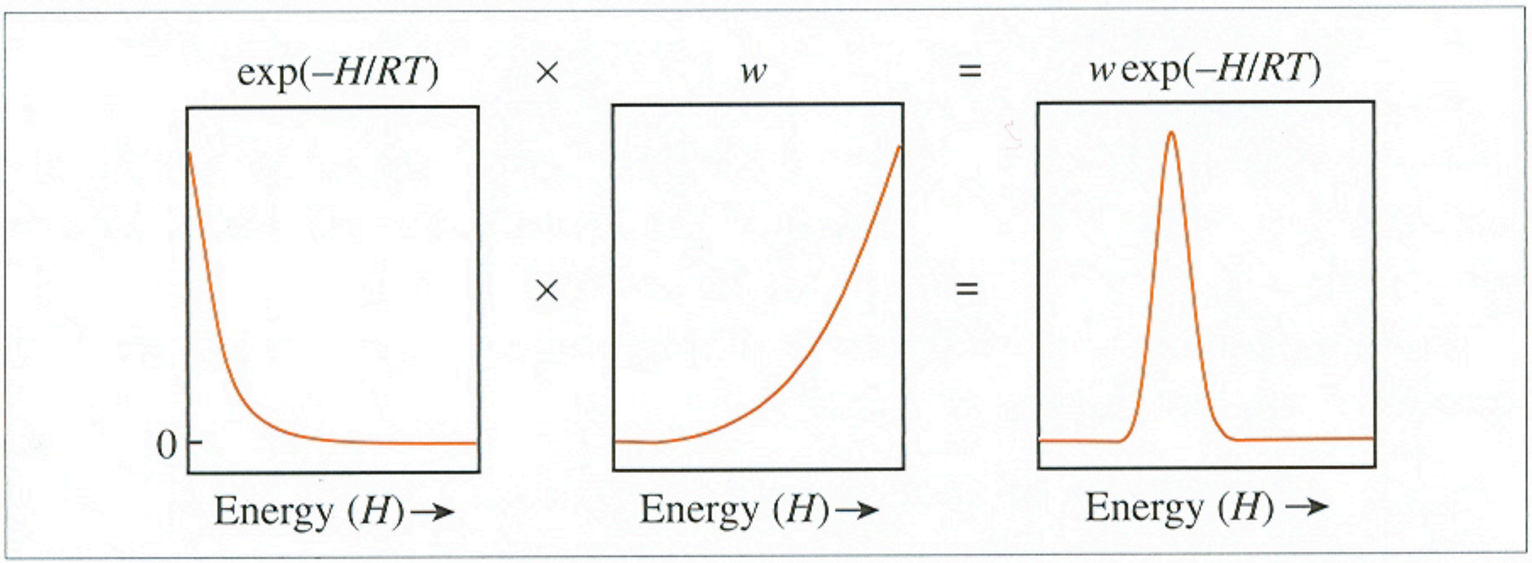


The real situation must involve a balance between energy and probability.

Biological Thermodynamics

Boltzmann probability

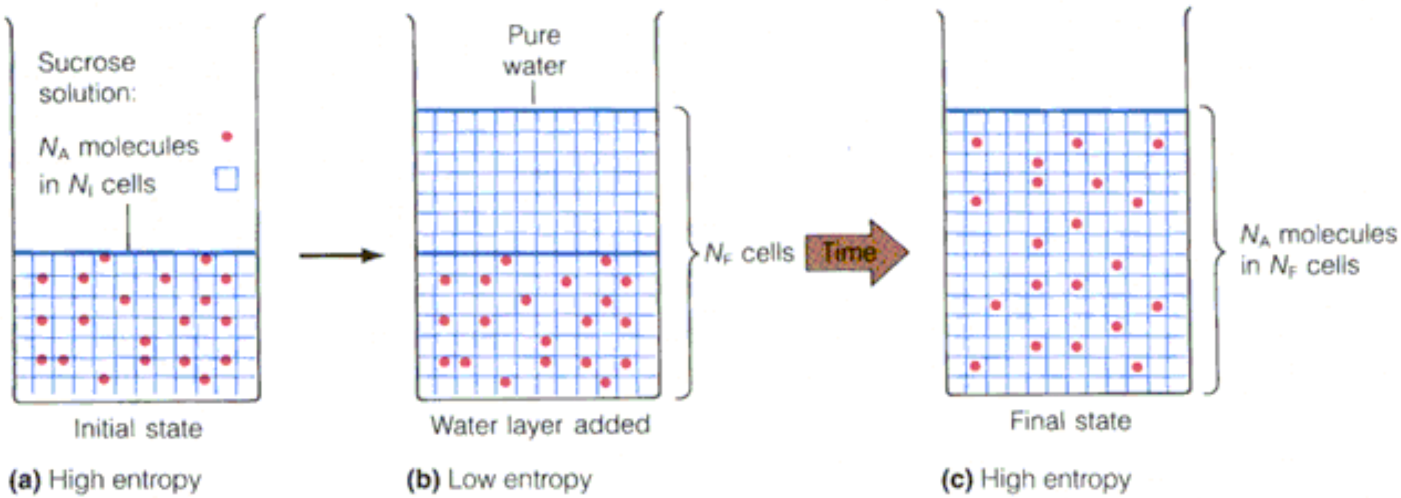
$p(H) = w \exp(-H/RT)$



Biological Thermodynamics

Entropy (S) - a measure of the order of the system

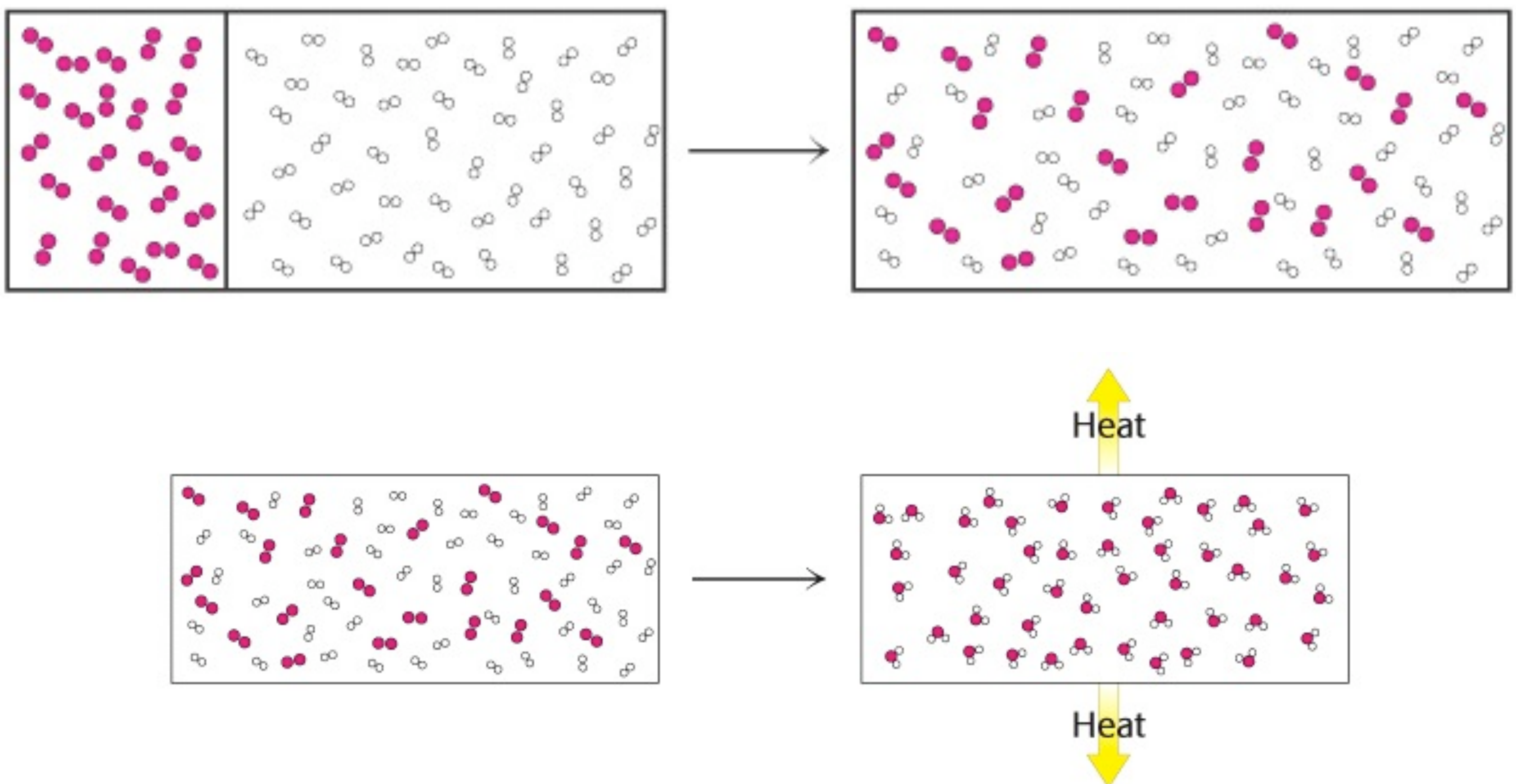
$$S = k \ln N$$



Low Entropy	High Entropy
Ice, at 0°C	Water, at 0°C
A diamond, at 0 K	Carbon vapor, at 1,000,000 K
A protein molecule in its regular, native structure	The same protein molecule in an unfolded, random coil state
A Shakespearean sonnet	A random string of letters
A bank manager's desk	A professor's desk

The **Second** Law of thermodynamics

The total entropy of a system and its surroundings always increases for a spontaneous process



Biological Thermodynamics

The Gibbs free energy (ΔG)

$$\Delta S_{total} = \Delta S_{system} + \Delta S_{surroundings}$$

$$\Downarrow \quad \Delta S_{surroundings} = -\Delta H_{system}/T$$

$$\Delta S_{total} = \Delta S_{system} - \Delta H_{system}/T$$

$$\Downarrow$$

$$-T\Delta S_{total} = \Delta H_{system} - T\Delta S_{system}$$

$$\Downarrow$$

$$\boxed{\Delta G = \Delta H_{system} - T\Delta S_{system}}$$

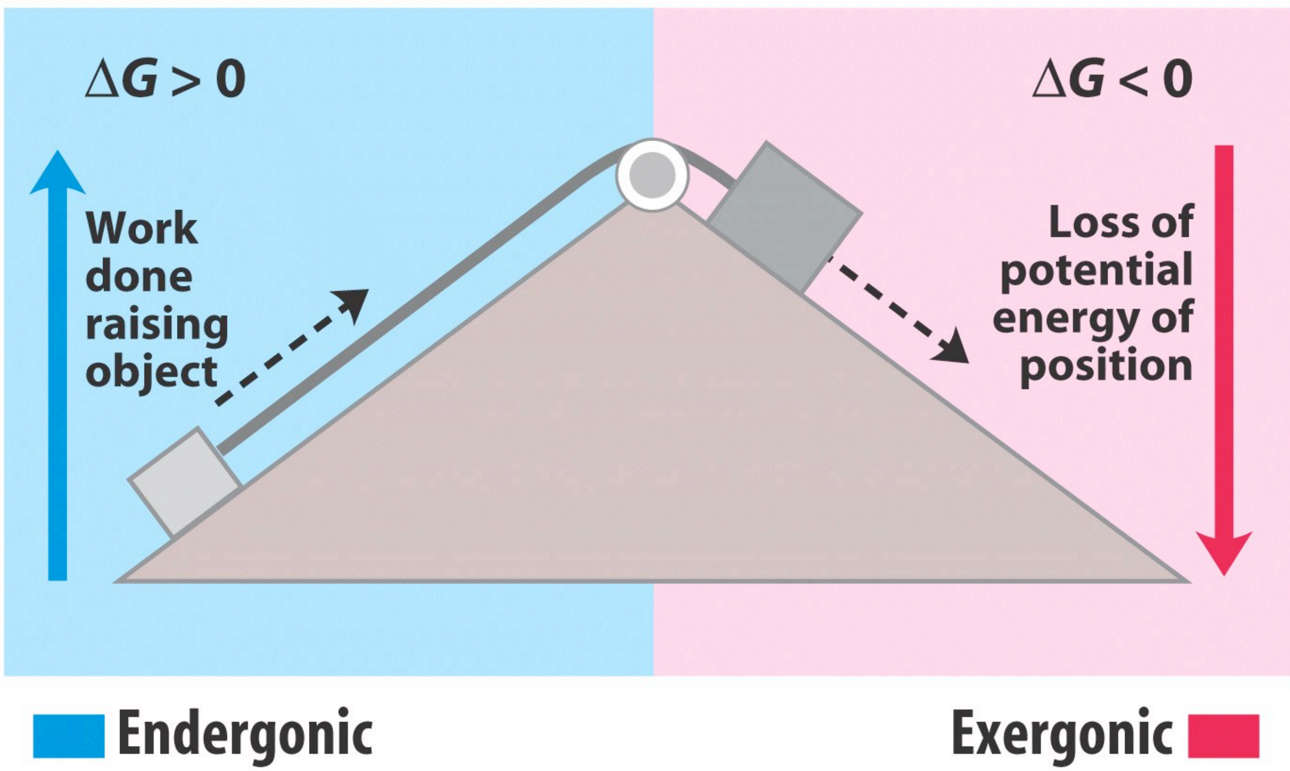
For a reaction to be spontaneous, the entropy of the universe, ΔS_{total}, must increase

$\Delta S_{system} > \Delta H_{system}/T$ or $\Delta G = \Delta H_{system} - T\Delta S_{system} < 0$

The **free energy** must be **negative** for a reaction to be **spontaneous!**

Biological Thermodynamics

The Gibbs free energy (ΔG)

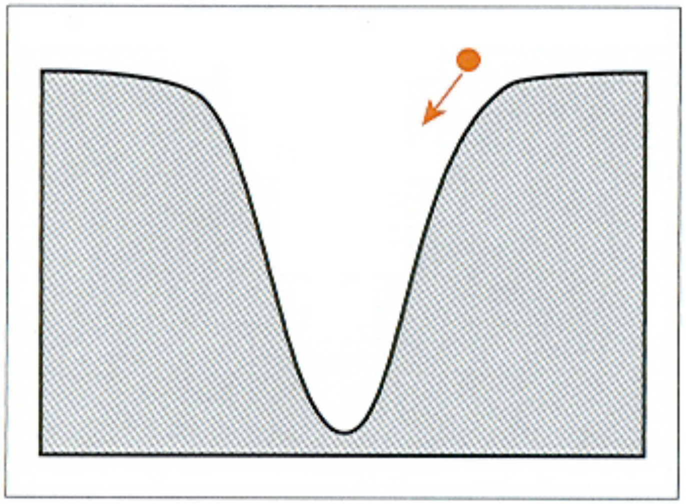


Energy coupling links reactions

Biological Thermodynamics

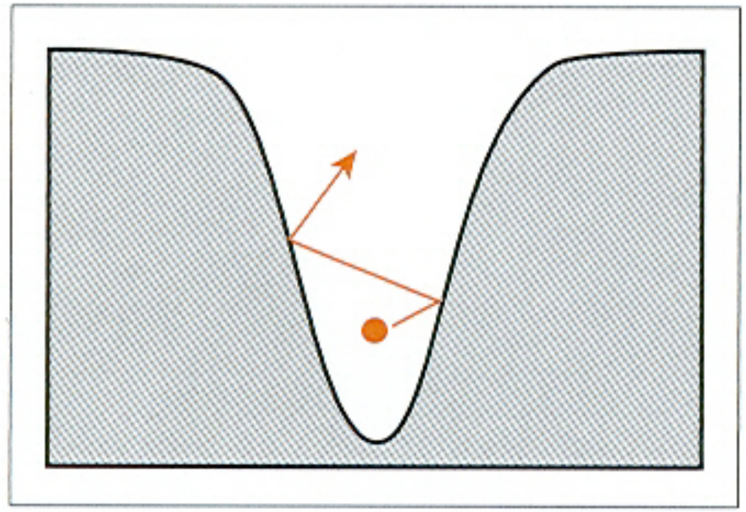
The Gibbs free energy (ΔG)

Enthalpy



Things tend to want to roll downhill: Δ**H tends to be negative**

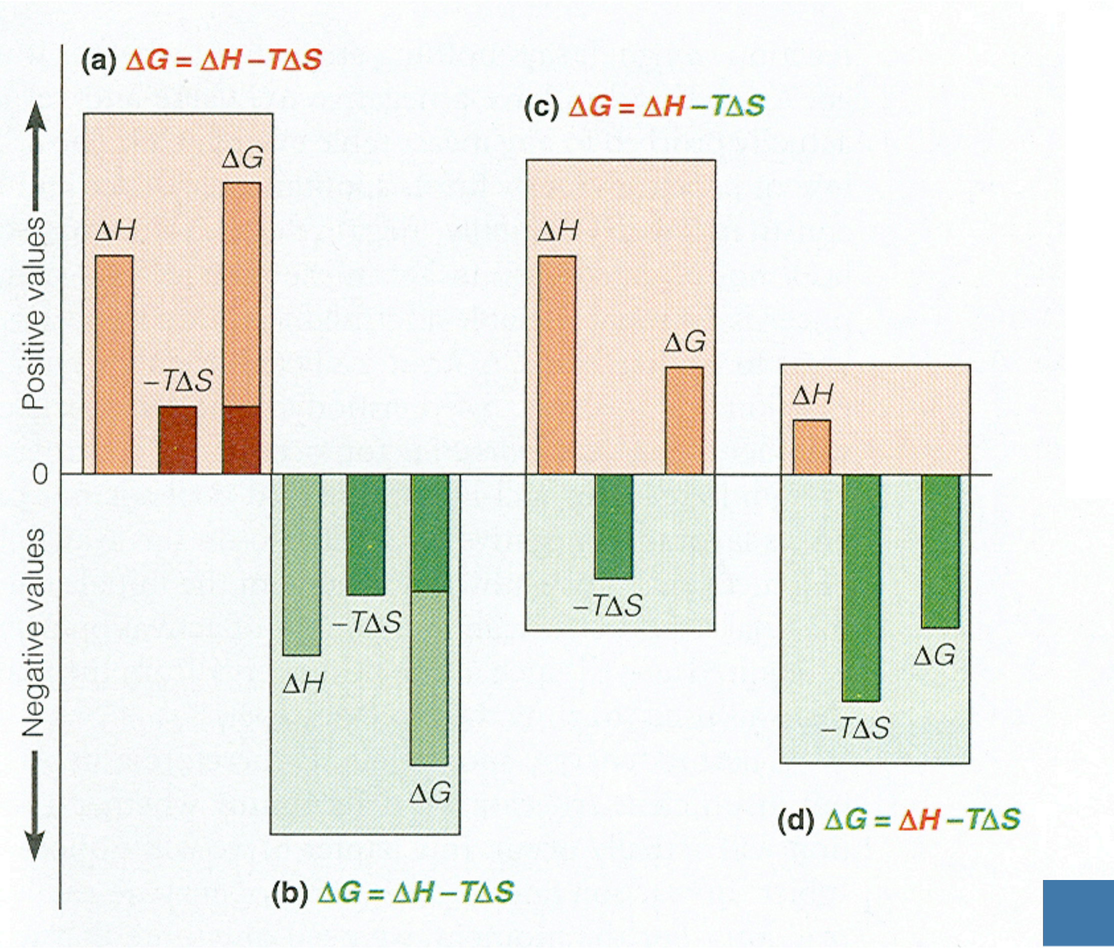
Entropy



Thermal (Brownian) motion tends to kick things uphill: Δ**S tends to be positive**

Biological Thermodynamics

The Gibbs free energy (ΔG)



If ΔG is ...	The process is ...
Negative	Thermodynamically favored
Zero	Reversible; at equilibrium
Positive	Thermodynamically unfavored; reverse process is favored

ΔH	ΔS	Low T	High T
+	+	ΔG positive; not favored	ΔG negative; favored
+	−	ΔG positive; not favored	ΔG positive; not favored
−	+	ΔG negative; favored	ΔG negative; favored
−	−	ΔG negative; favored	ΔG positive; not favored

Biological Thermodynamics

$$\Delta G = \Delta H - T\Delta S$$

The Enthalpic term

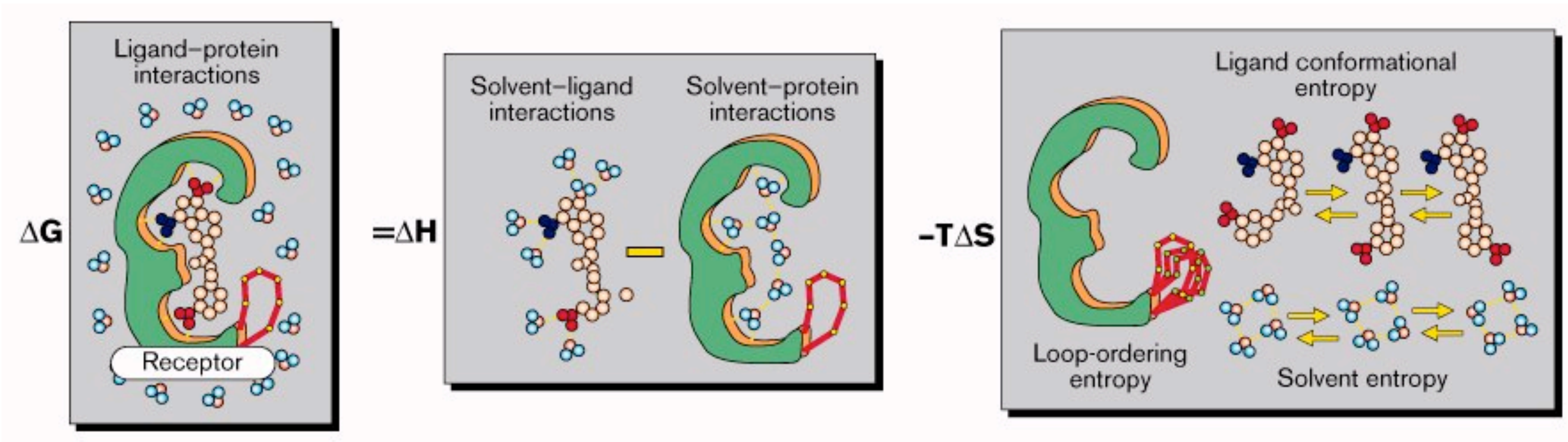
- Changes in bonding
  - van der Waals
  - Hydrogen bonding
  - Charge interactions

The Entropic term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

Biological Thermodynamics

$$\Delta G = \Delta H - T\Delta S$$



Biological Thermodynamics

Quantitative description of protein-ligand interactions

$$P + L \rightleftharpoons PL$$

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

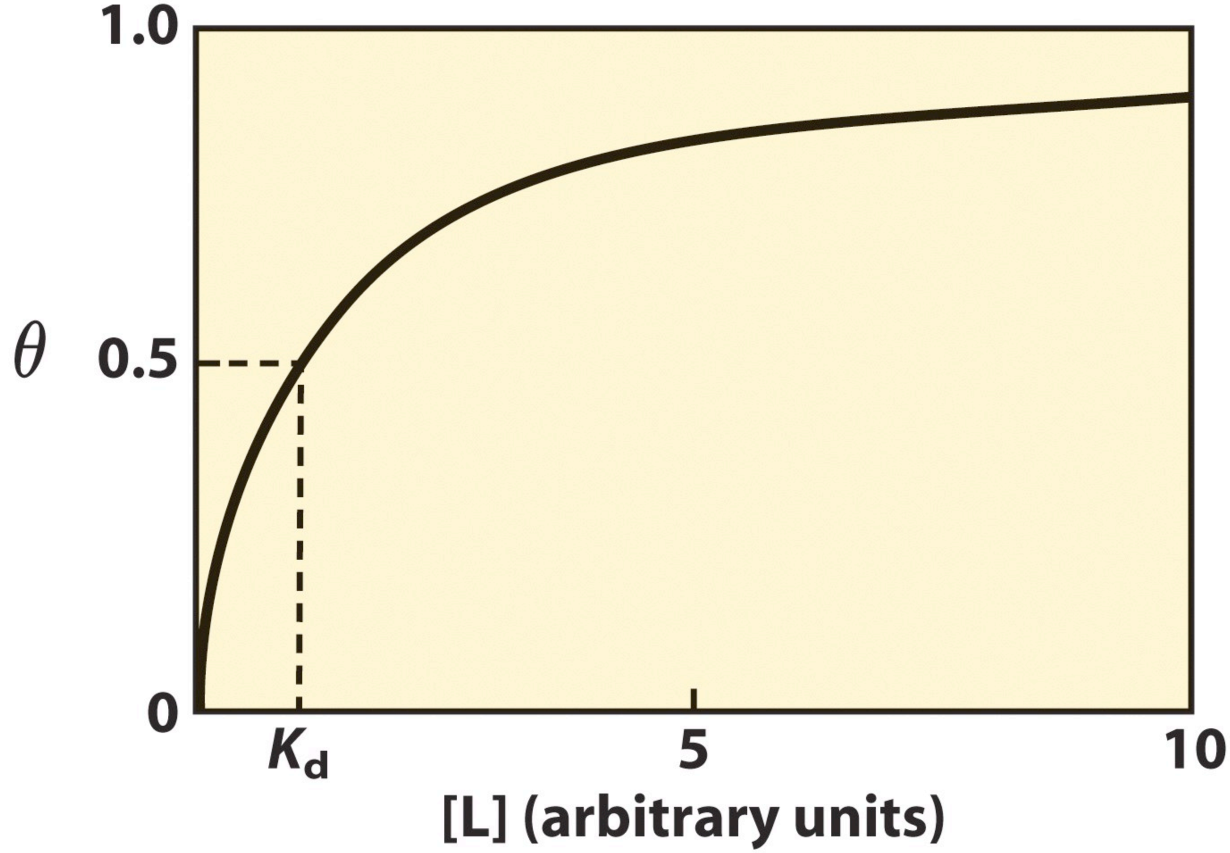
fraction, θ $$\theta = \frac{[PL]}{[PL]+[P]} = \frac{\text{binding sites occupied}}{\text{total binding sites}}$$

$$\theta = \frac{K_a[L][P]}{K_a[L][P]+[P]} = \frac{K_a[L]}{K_a[L]+1} = \frac{[L]}{[L]+\frac{1}{K_a}}$$

Biological Thermodynamics

Quantitative description of protein-ligand interactions

$$\theta = \frac{[L]}{[L] + \frac{1}{K_a}}$$



$$K_d = \frac{1}{K_a}$$

$$\theta = \frac{[L]}{[L] + K_d}$$

Biological Thermodynamics

Quantitative description of protein-ligand interactions

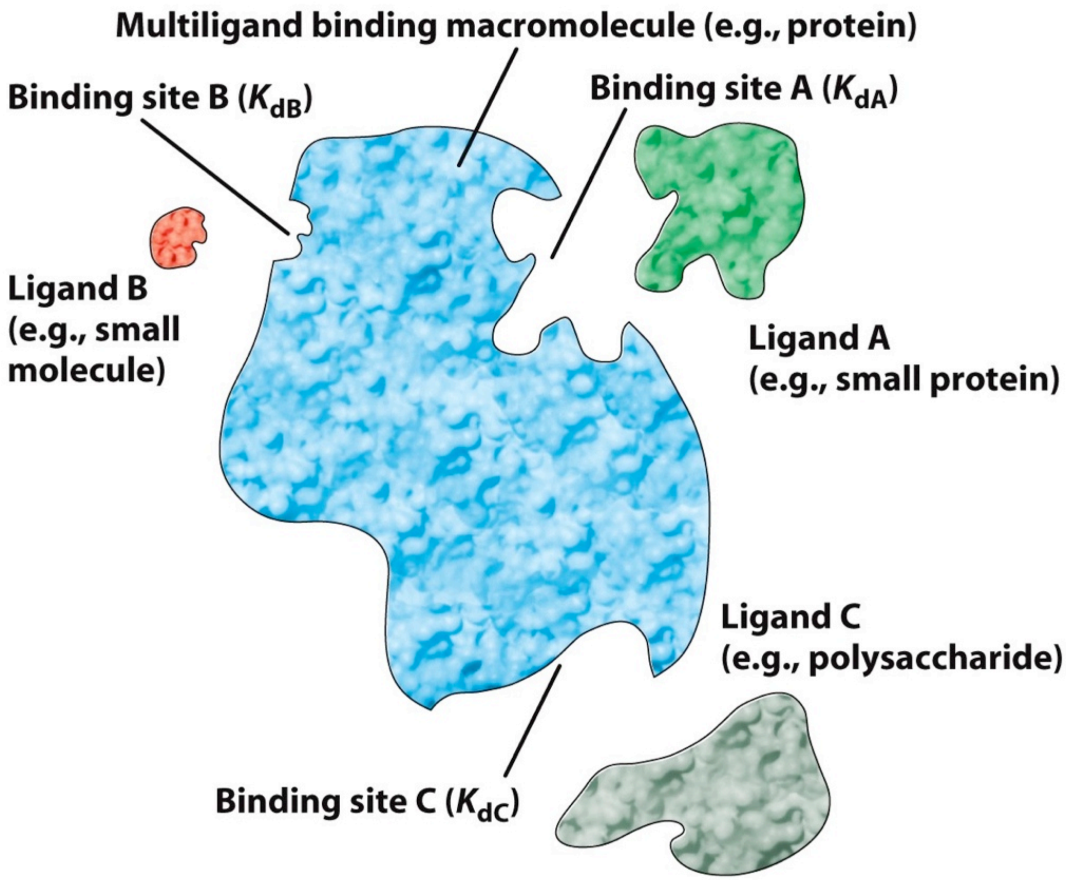
$\Delta G = -RT \ln K_{eq}$ $\quad\quad$ $K_{eq} = 10^{-\Delta G/1.36}$

Relation between ΔG and K_{eq} (at 25°C)

	$\Delta G^{o\prime}$	
K'_{eq}	kcal mol^{-1}	kJ/mol^{-1}
10^{-5}	6.82	28.53
10^{-4}	5.46	22.84
10^{-3}	4.09	17.11
10^{-2}	2.73	11.42
10^{-1}	1.36	5.69
1	0	0
10	−1.36	−5.69
10^{2}	−2.73	−11.42
10^{3}	−4.09	−17.11
10^{4}	−5.46	−22.84
10^{5}	−6.82	−28.53

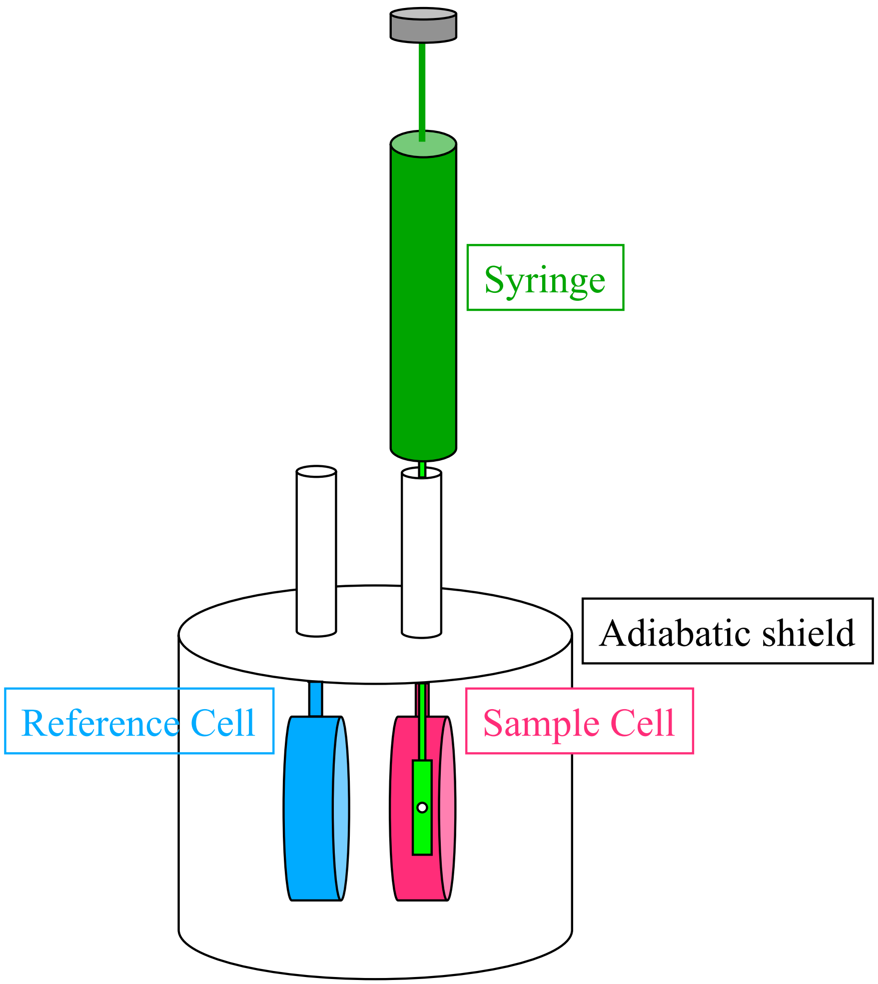
Biological Thermodynamics

Quantitative description of protein-ligand interactions



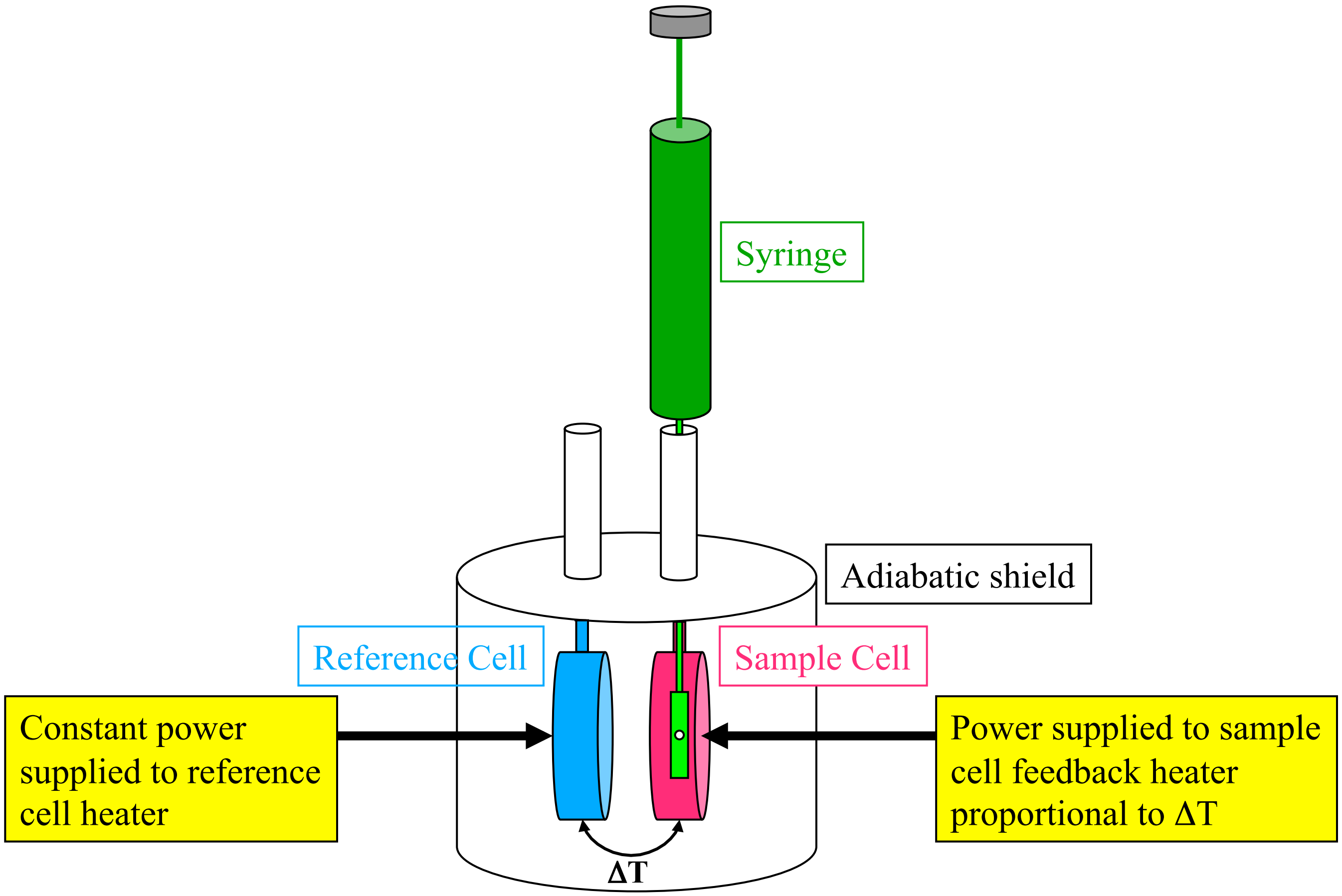
Isothermal Titration Calorimetry (ITC)

A single experiment is sufficient to obtain all of the thermodynamic components



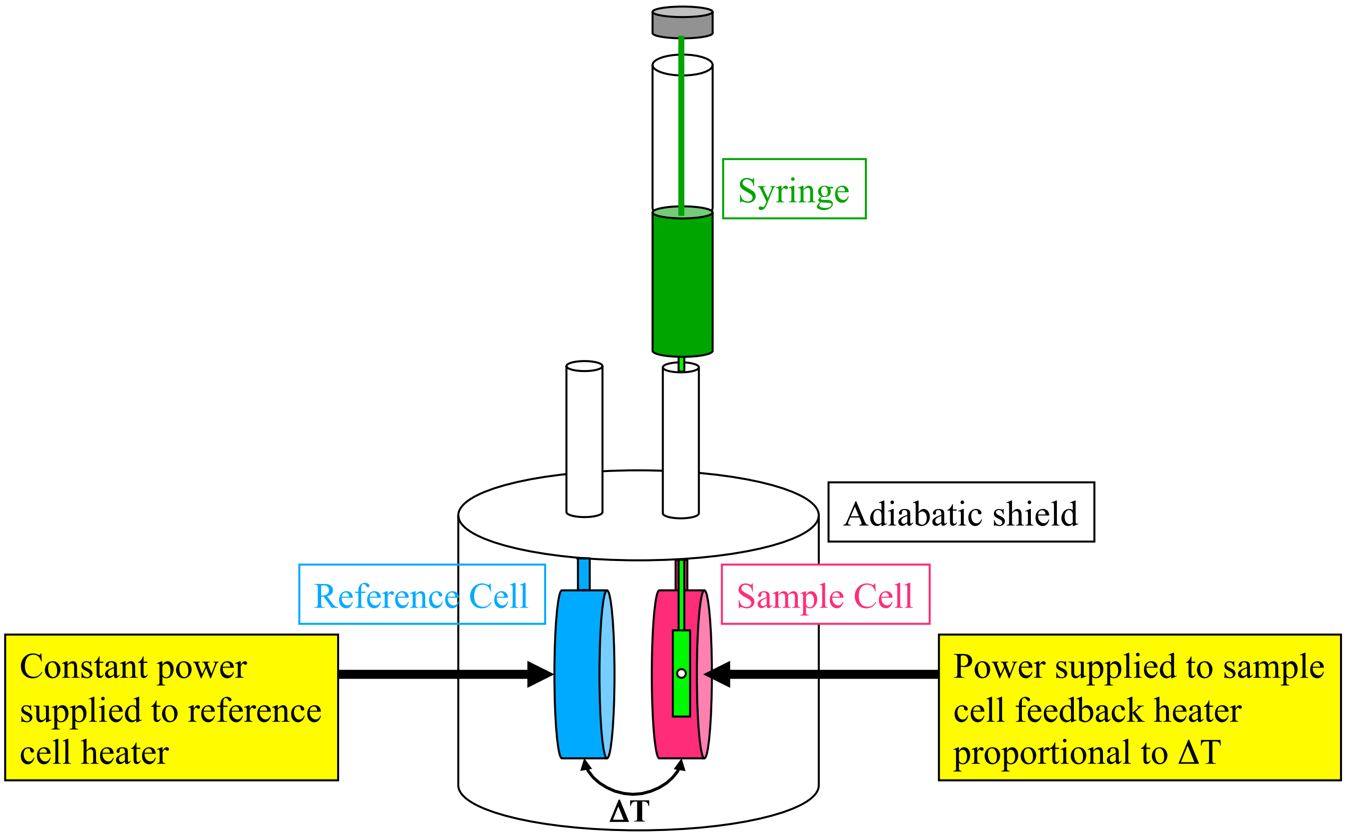
Isothermal Titration Calorimetry (ITC)

The amount of power (in millijoules per sec required to maintain a constant temperature difference between the reaction cell and the reference cell is measured



Isothermal Titration Calorimetry (ITC)

The amount of power (in microjoules per sec required to maintain a constant temperature difference between the reaction cell and the reference cell is measured



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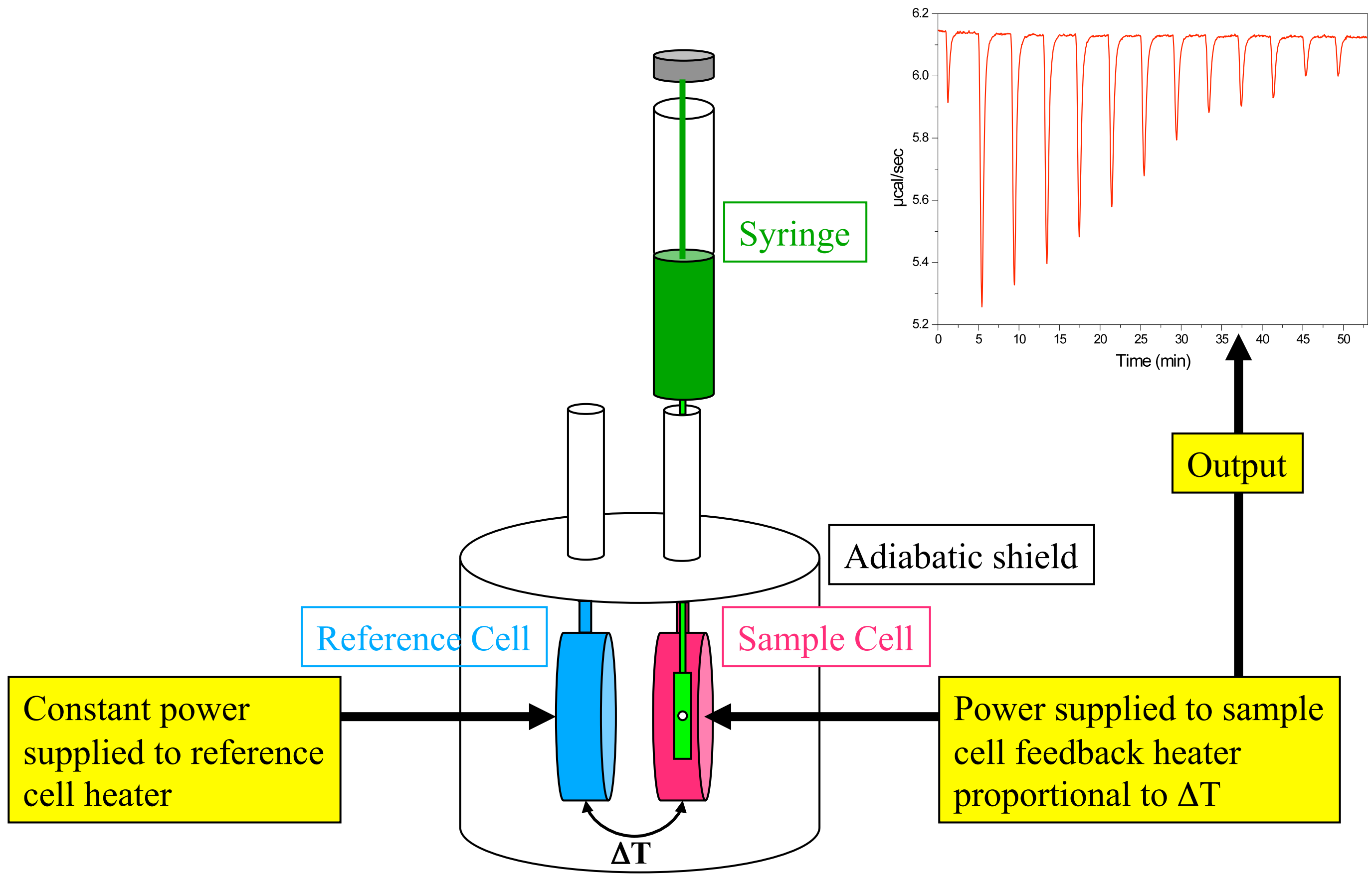

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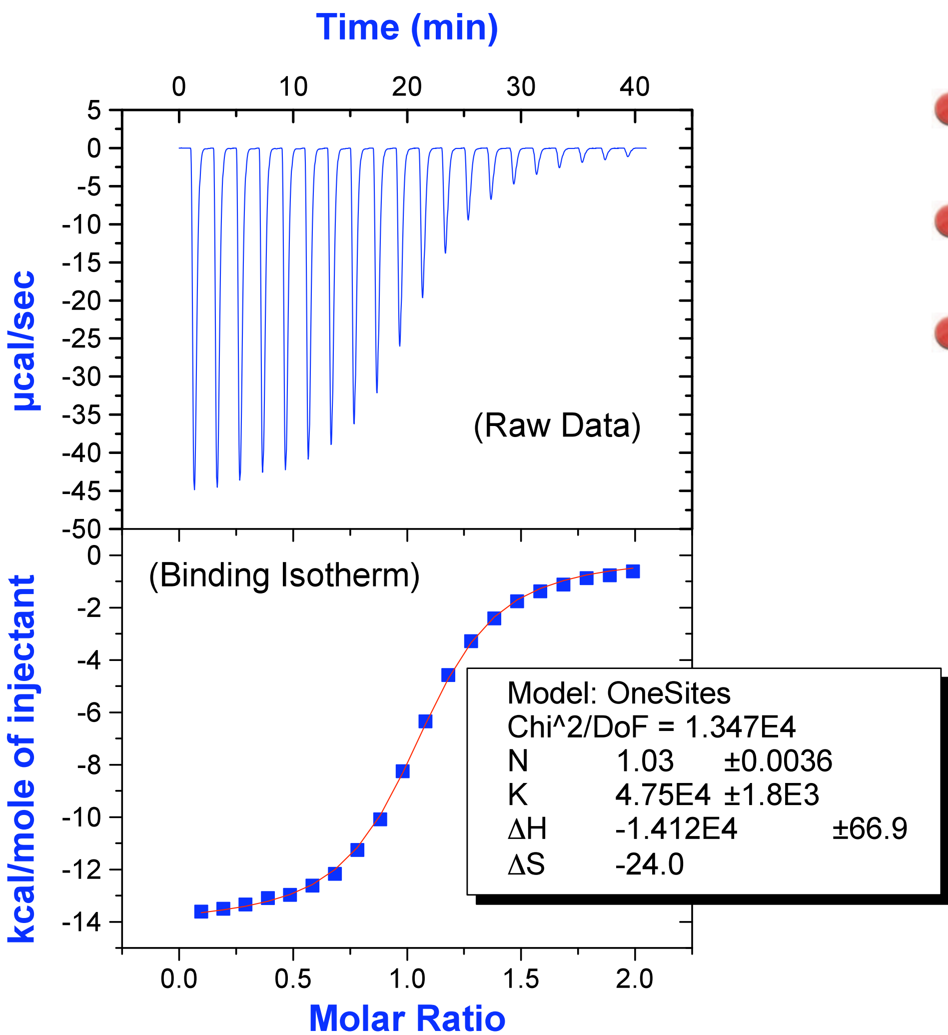
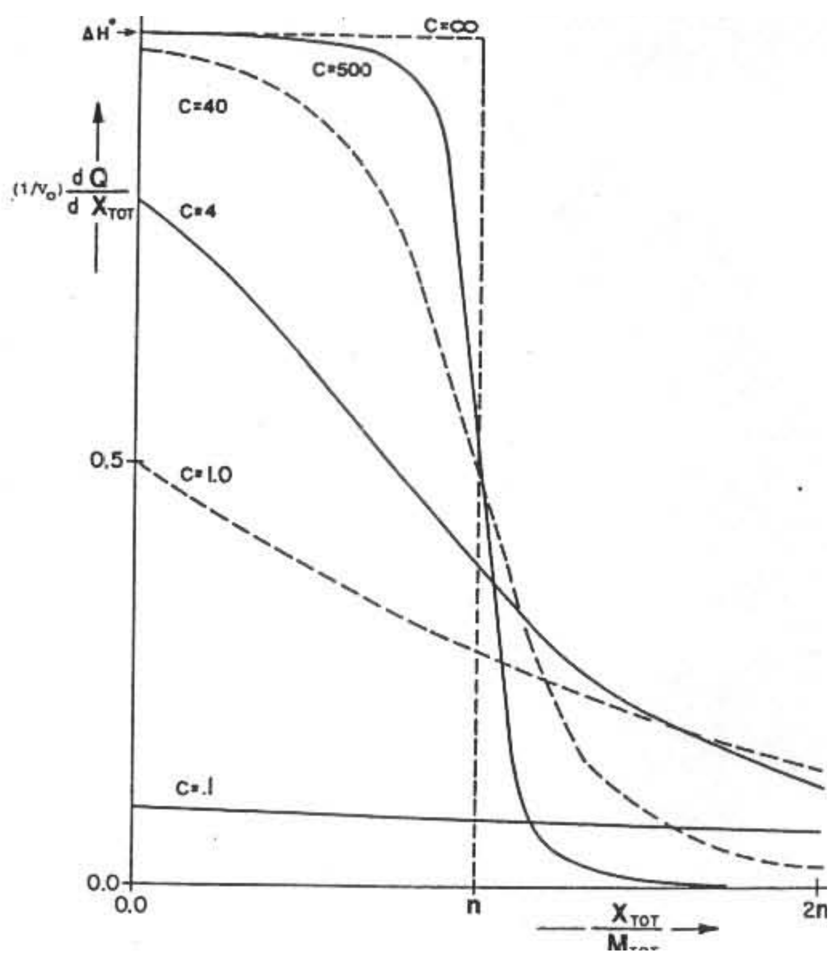


- Exothermic reaction: "negative" peak on ITC
- Endothermic reaction: "positive" peak on ITC
- Heat absorbed or generated during titration directly proportional to amount of bound ligand

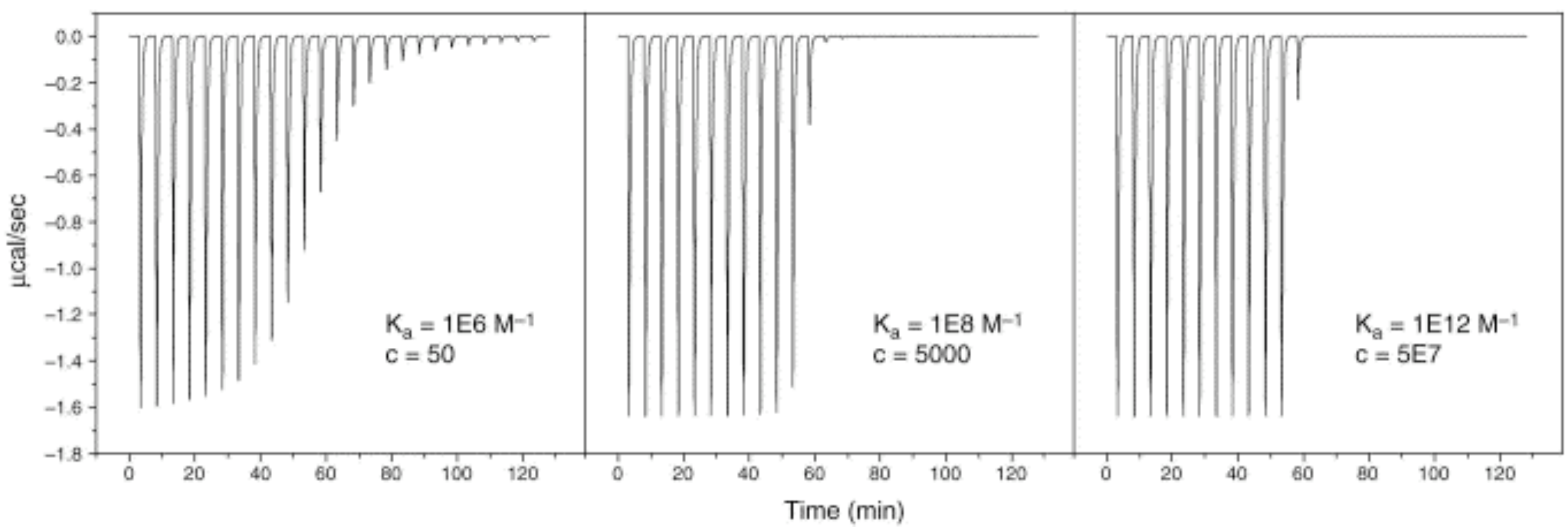
Isothermal Titration Calorimetry (ITC)

Simulated binding isotherms for various c values.



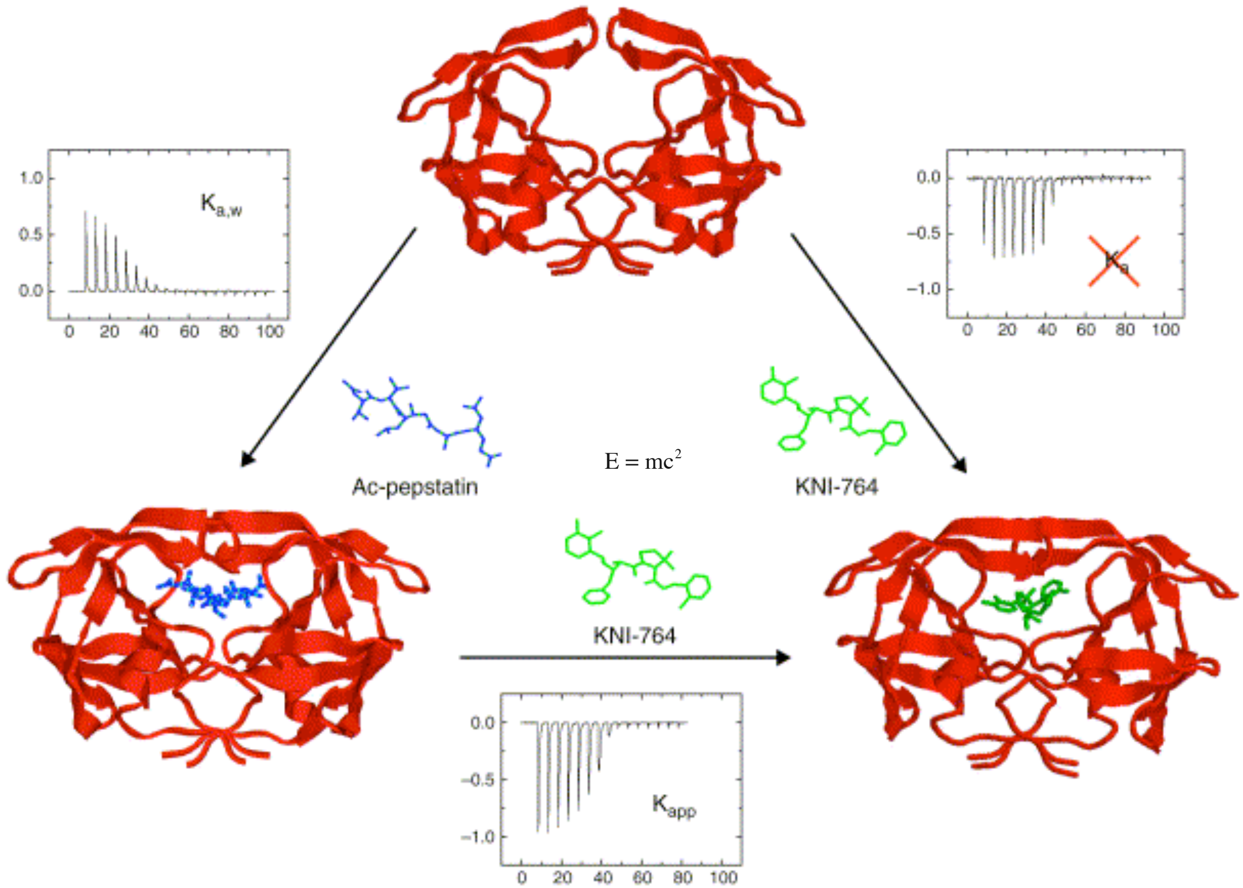
Isothermal Titration Calorimetry (ITC)

Simulated binding isotherms for various c values.



Isothermal Titration Calorimetry (ITC)

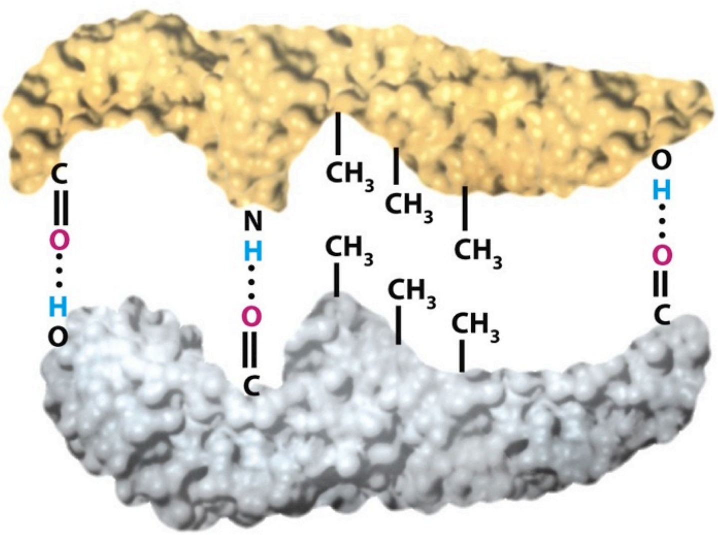
Displacement ITC to measure high affinities

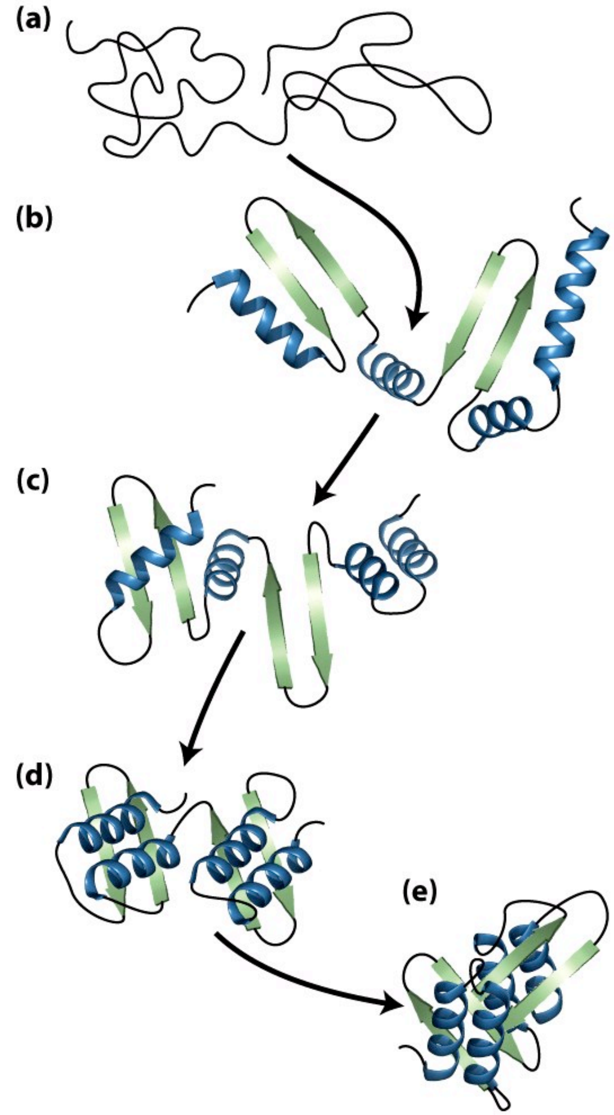


$$K_{app} = \frac{K_a}{1 + K_{a,w}[X]}$$

Protein binding and Protein folding

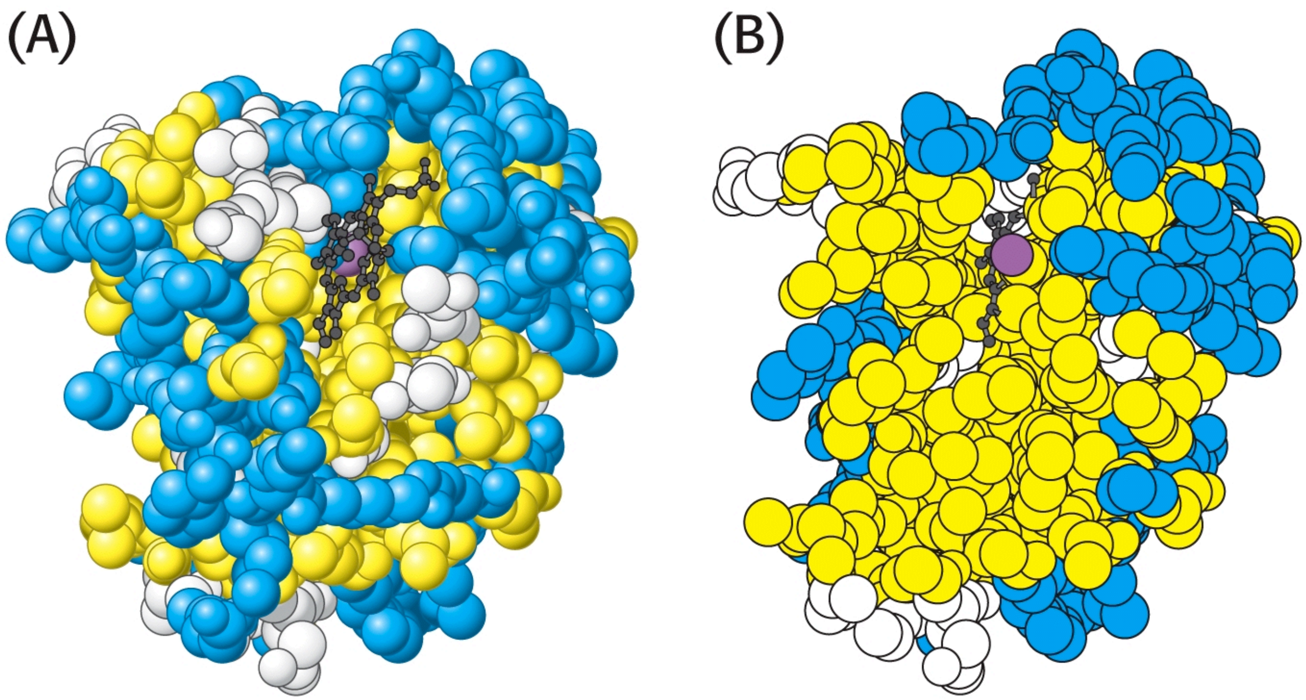
Very similar processes!





Protein folding

Amino acid distribution



space-filling

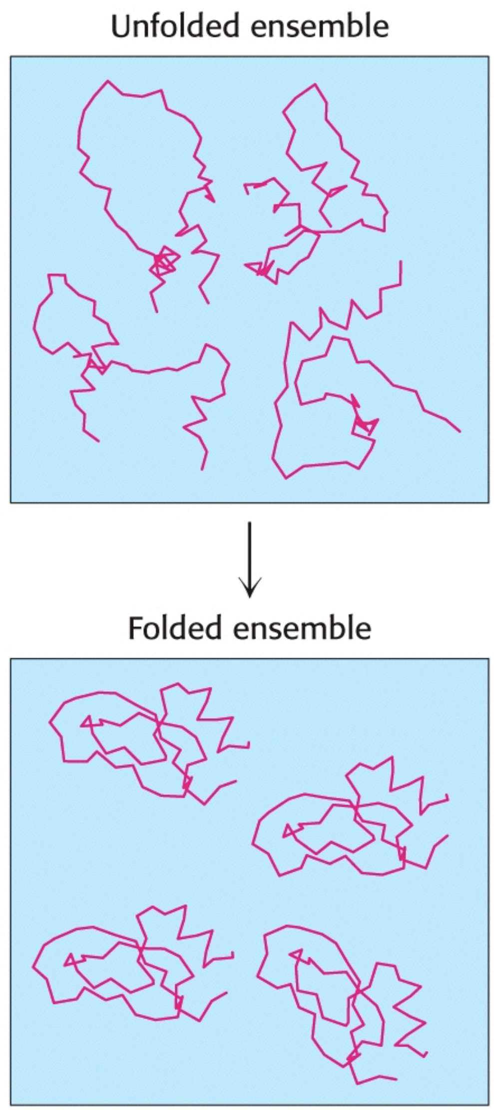
cross-section

nonpolar

polar

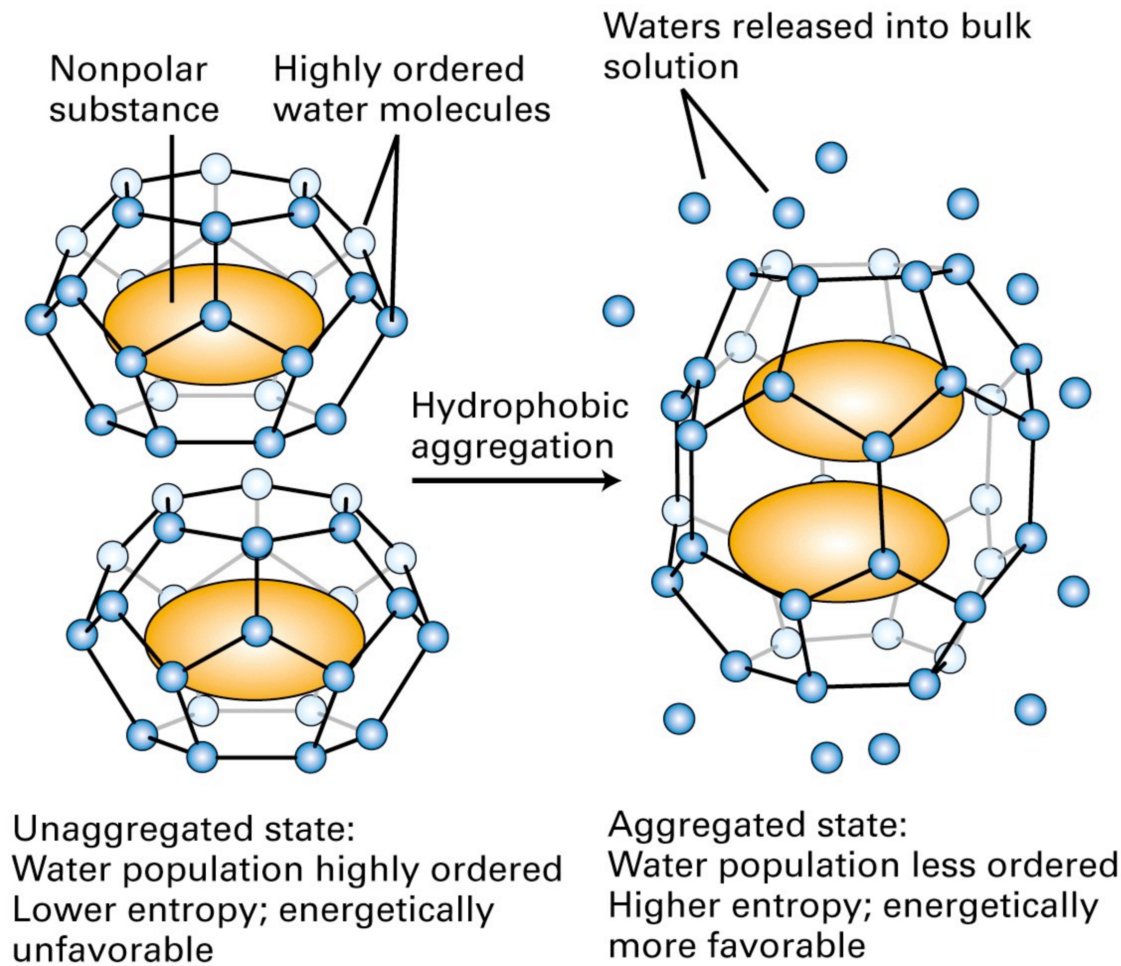
Protein folding

Why do proteins fold?



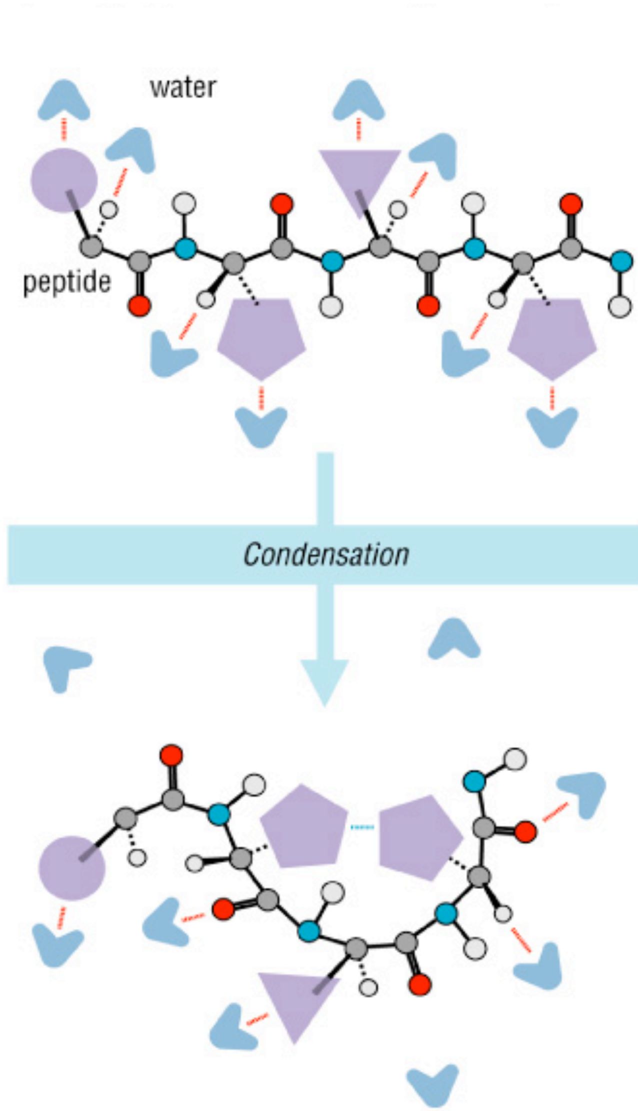
Protein folding

Why do proteins fold?



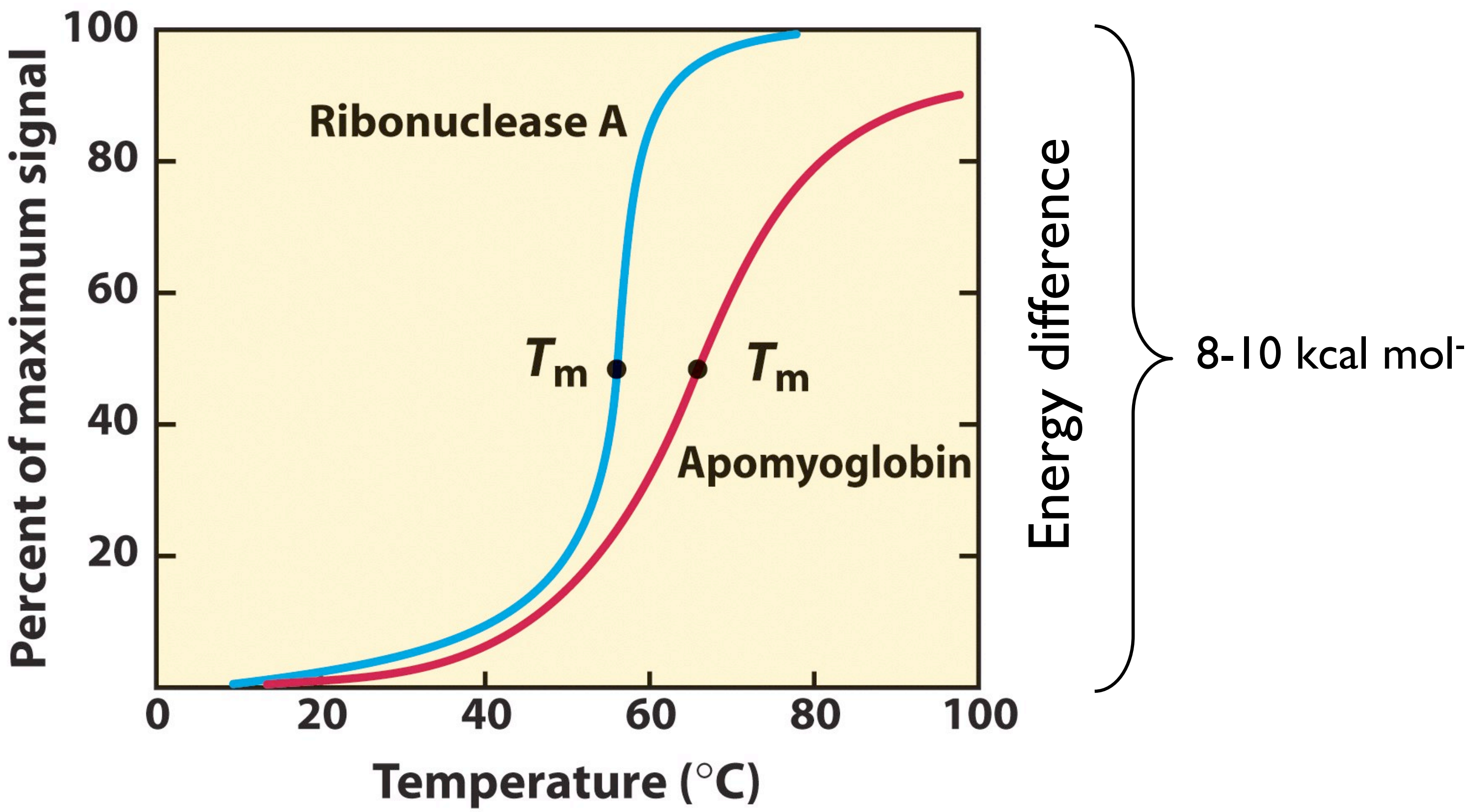
Protein folding

Why do proteins fold?



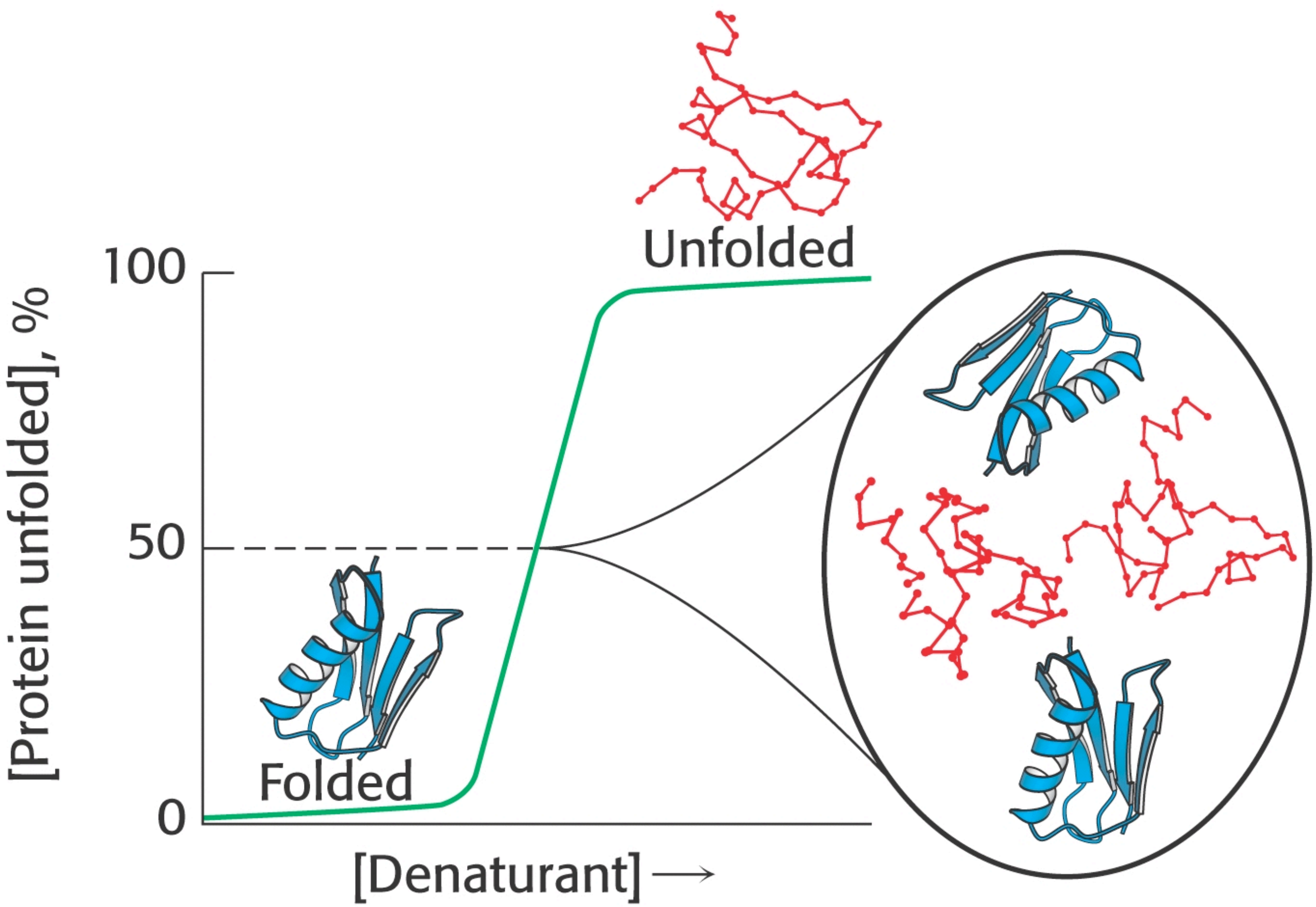
Protein folding

Loss of protein structure results in **loss of function**



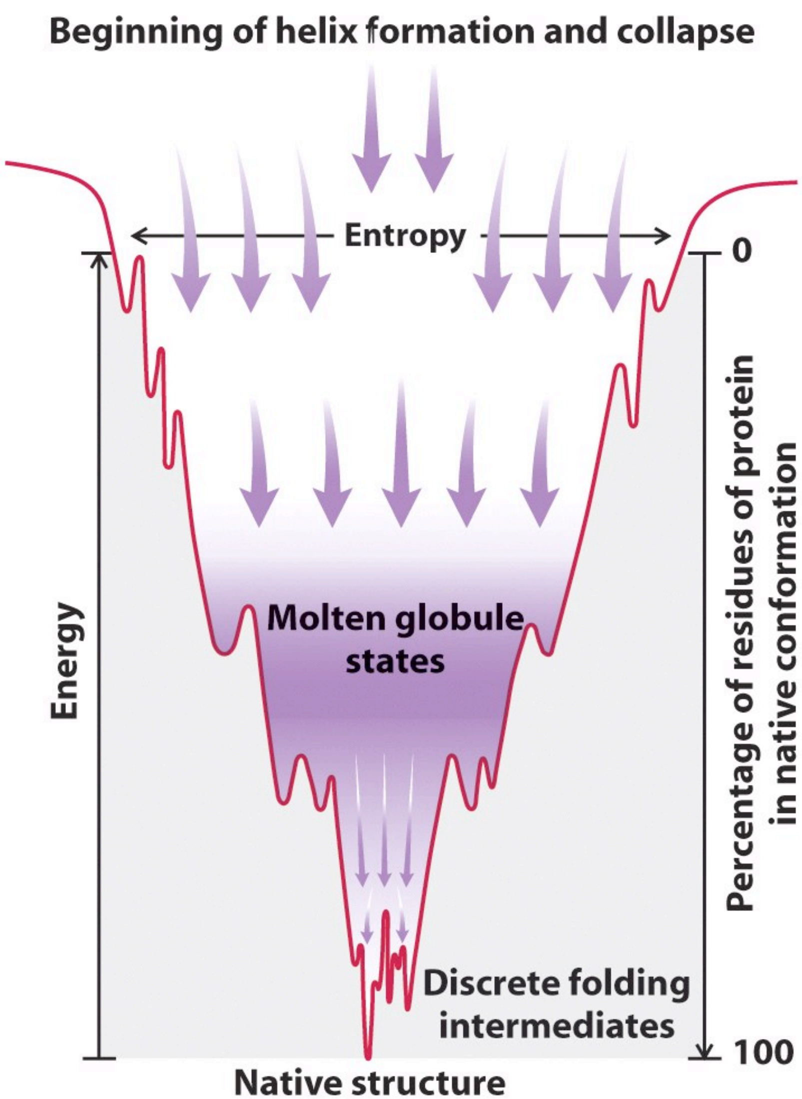
Protein folding

Protein folding is highly **cooperative**



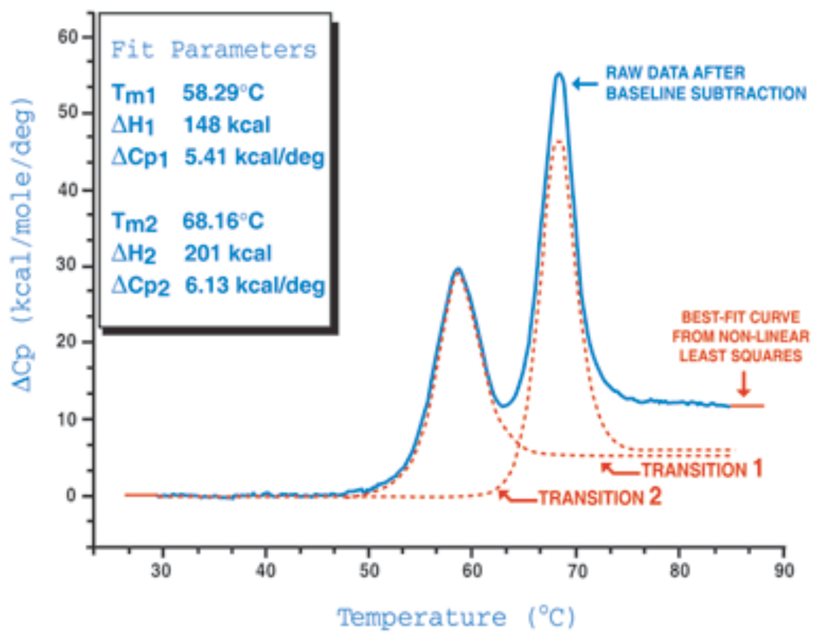
Protein folding

Free energy funnel



Differential Scanning Calorimetry (DSC)

DSC directly measures heat changes that occur in biomolecules during controlled increase or decrease in temperature, making it possible to study materials in their native state

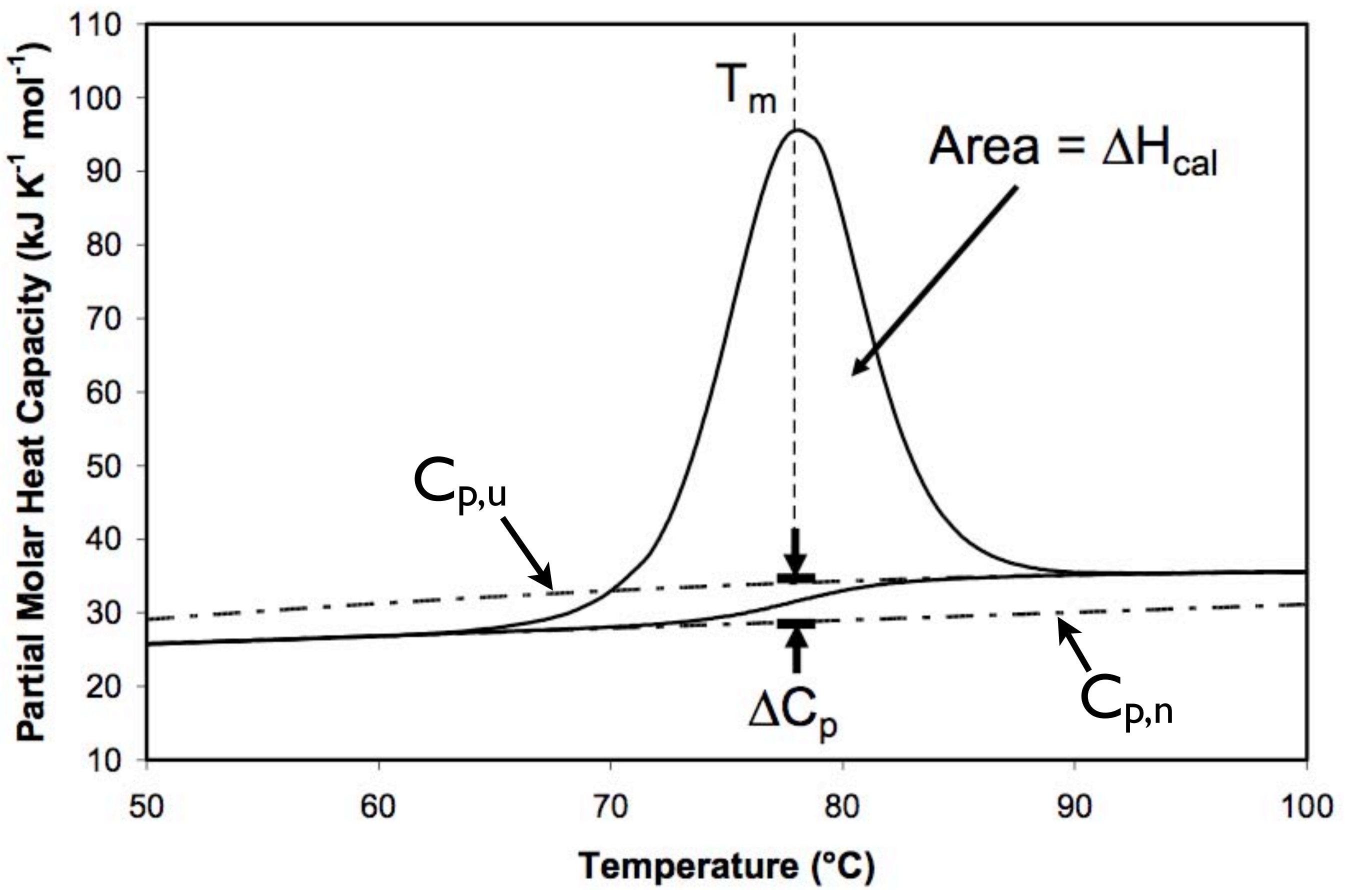


$$\Delta C_p = \frac{\Delta H_2 - \Delta H_1}{T_2 - T_1}$$

DSC measures the enthalpy (ΔH) of unfolding due to heat denaturation.

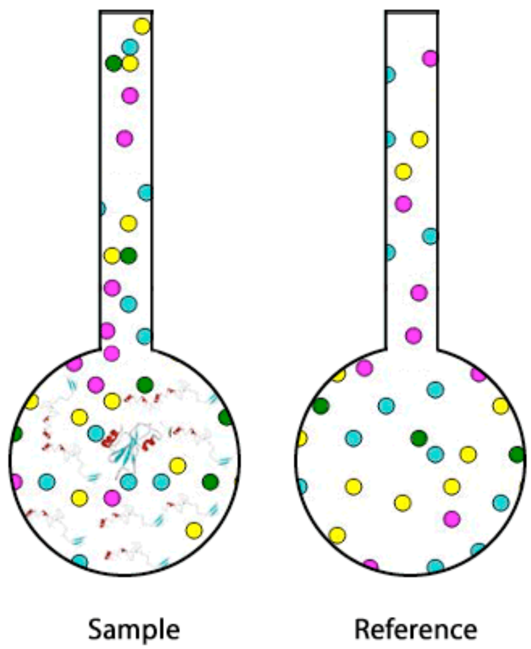
Differential Scanning Calorimetry (DSC)

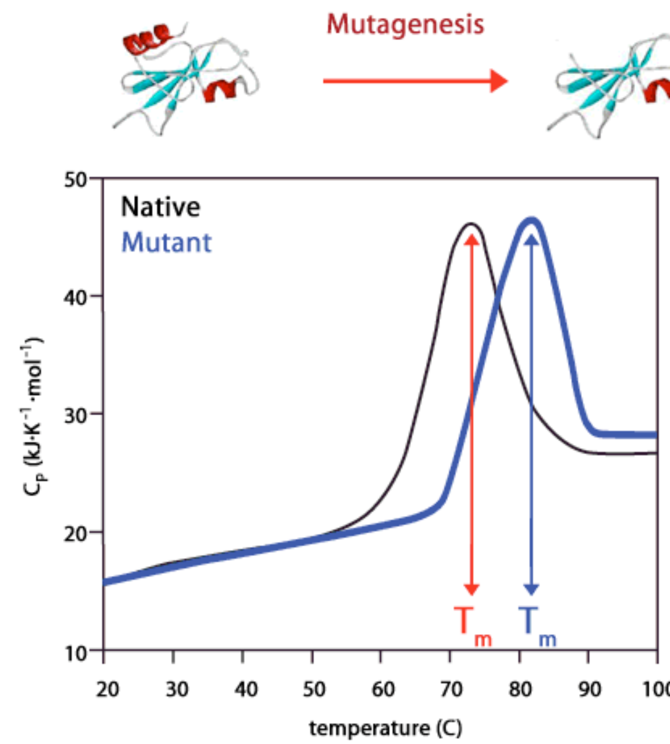
In a single thermal unfolding experiment, DSC can directly measure and allow calculation of all the thermodynamic parameters characterizing a biological molecule



Differential Scanning Calorimetry (DSC)

Increases in thermal stability observed by T_m shifting





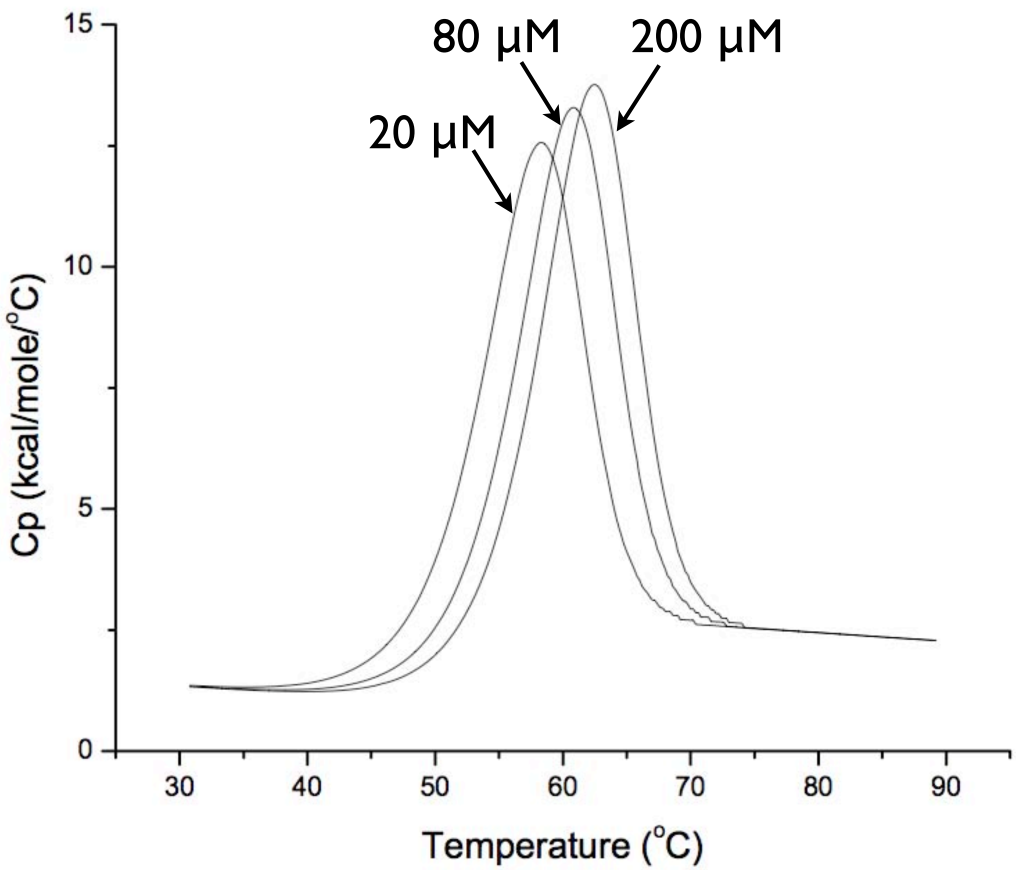
100% Unfolded Protein

T_m shift reflects increased stability

Differential Scanning Calorimetry (DSC)

Concentration dependence

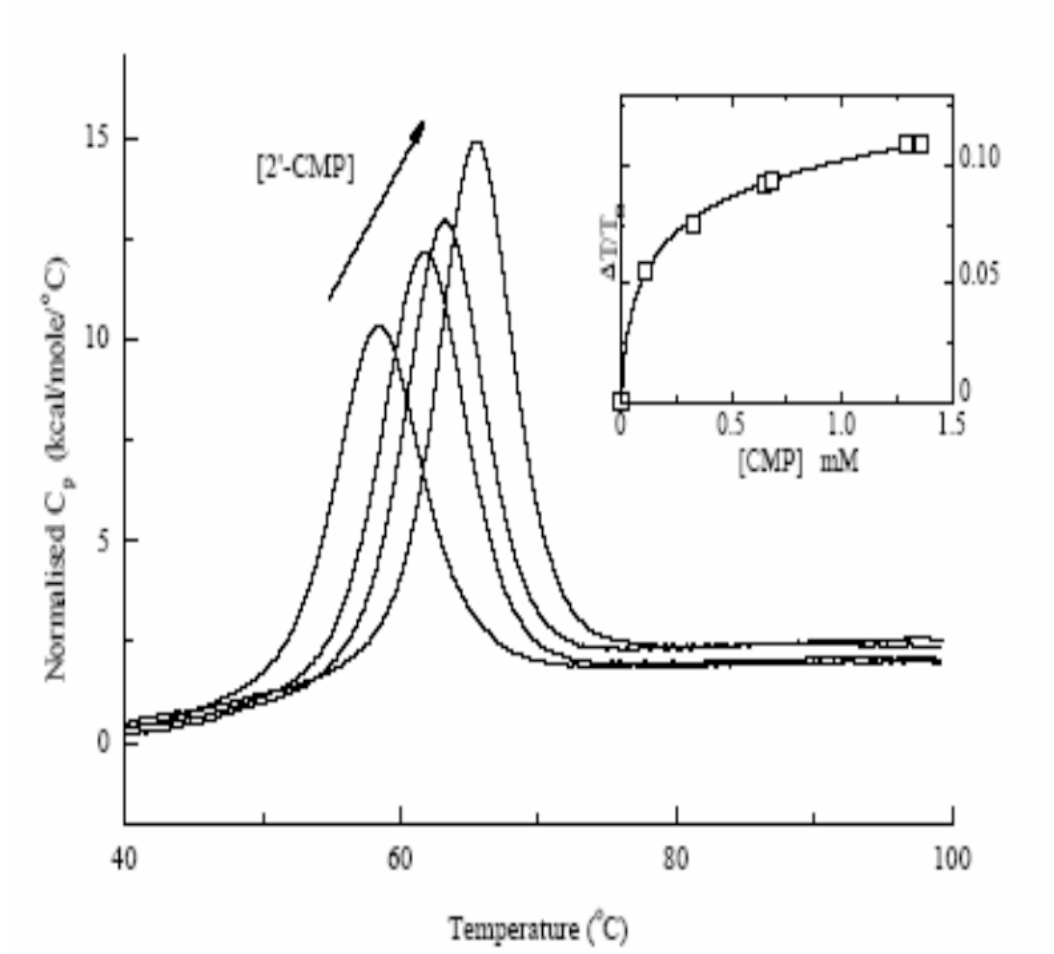
$$N_2 \Leftrightarrow 2U$$



Differential Scanning Calorimetry (DSC)

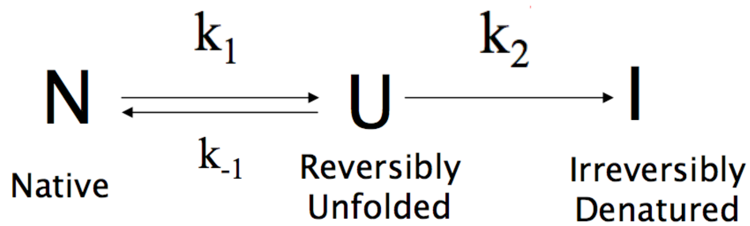
Ligand binding

RNase with increasing [2′-CMP]

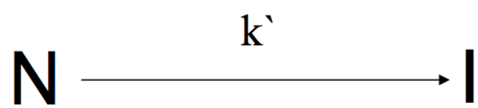


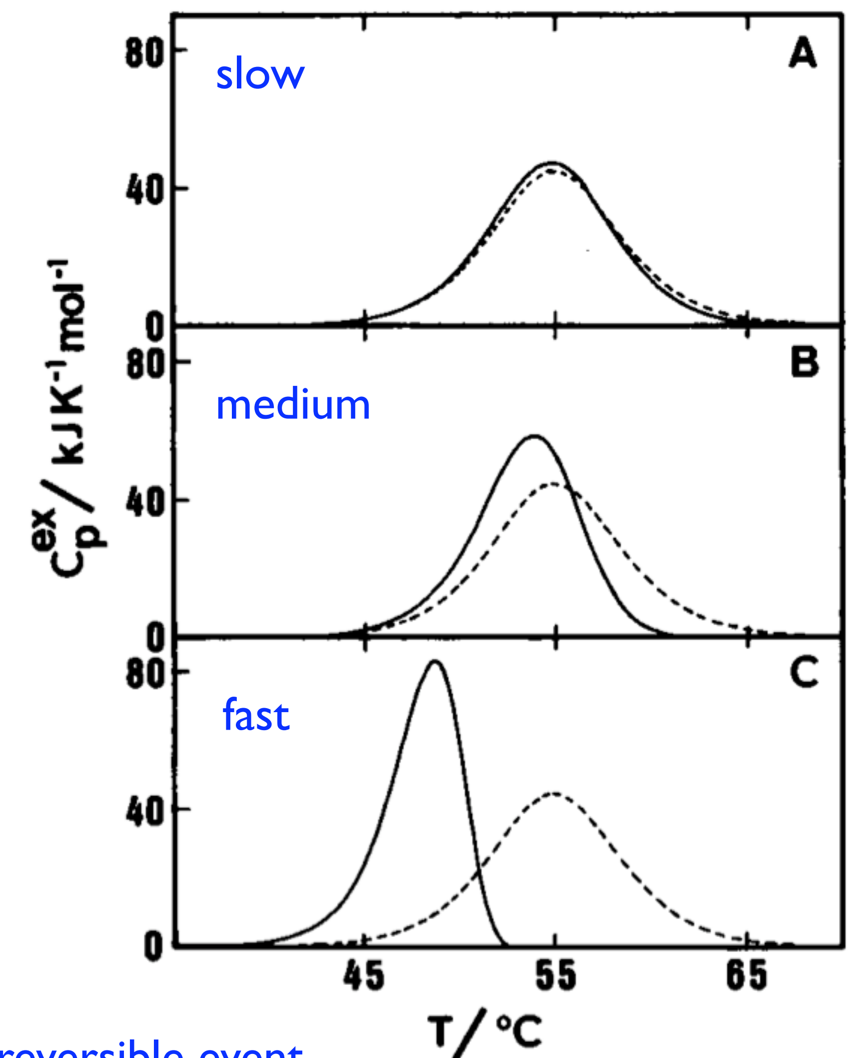
Differential Scanning Calorimetry (DSC)

Protein folding intermediates



If $k_2 >> k_{-1}$ then





kinetics of the irreversible event

Differential Scanning Calorimetry (DSC)

- Ideal for stability and folding studies
- Provides insights into mechanisms of unfolding and refolding
- Monitors reversibility of thermal processes.
- Study molecules in their native state without labeling. Can be use with solutions that interfere with optical methods including turbid or colored solutions or particulate suspensions.
- Monitors conformational energetics of proteins and biopolymers

Surface Plasmon Resonance (SRP)

Measuring binding kinetics

$$K_d = \frac{k_{off}}{k_{on}}$$