PROTEIN FOLDING

Miklós Kellermayer

THE PEPTIDE BOND


amino acid 1   amino acid 2
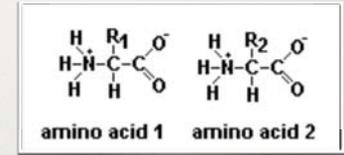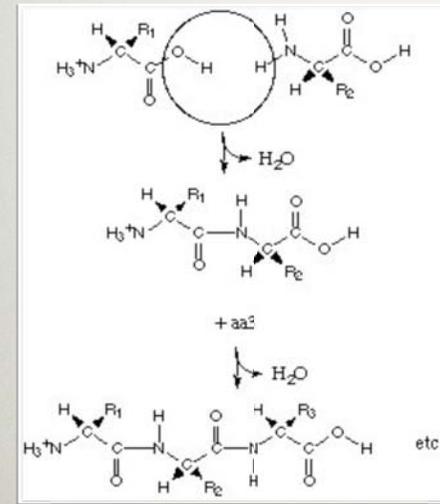
Condensation reaction
followed by the relase of water

PROTEIN STRUCTURE

Primary	Secondary	Tertiary
Amino acid sequence	α-helix β-sheet β-turn	3D structure of single-chain protein
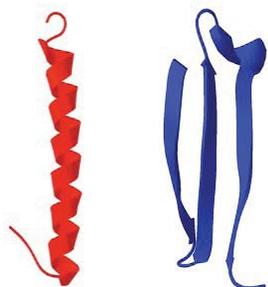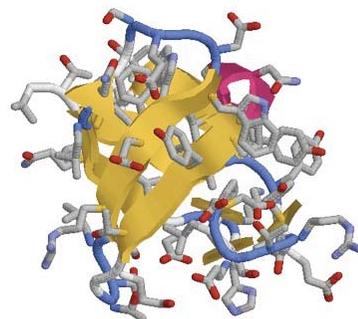
α-helix:
•right handed
•3.4 residue/turn
•H-bridges

β-sheet:
•parallel or
•antiparallel
•H-bridges between distant residues

DISPLAY OF PROTEIN STRUCTURE

wireframe

spacefilling

backbone

ribbon

Myosin S-1
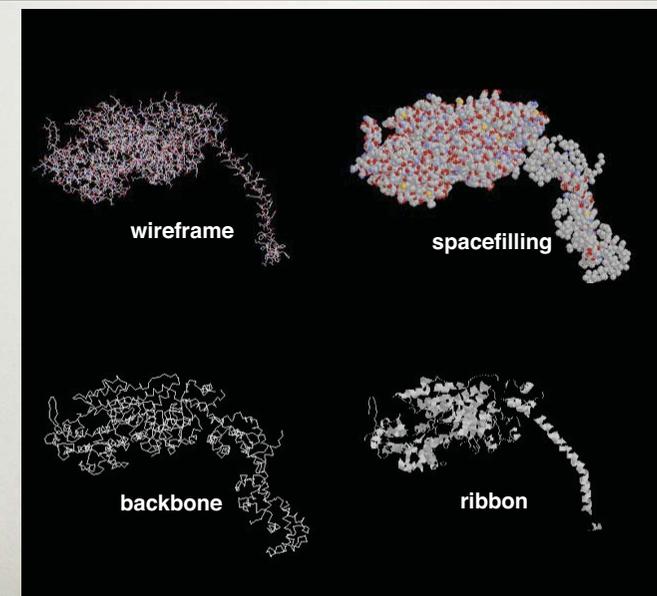
Bonds holding protein structure together

1. Disulfide bridge: between cysteine residues

2. Hydrogen bond: shared proton

3. Salt bridge: between oppositely charged residues

4. Hydrophobic interaction: between hydrophobic residues
   (in the interior of the molecule)

Protein structure classes
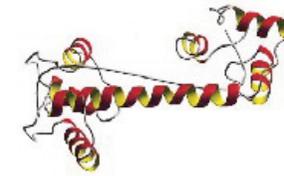
1. All alpha — calmodulin
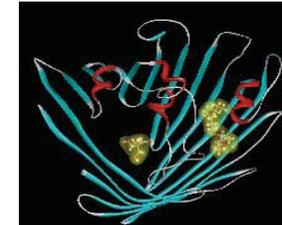
2. All beta — porin

(3. Alfa-beta)
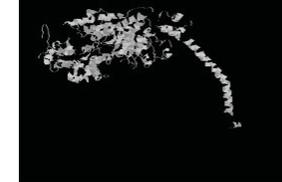
4. Multidomain — myosin

Levinthal's paradox:
Are all conformations explored by the protein molecule?

Planar peptide group
determined by the peptide bond
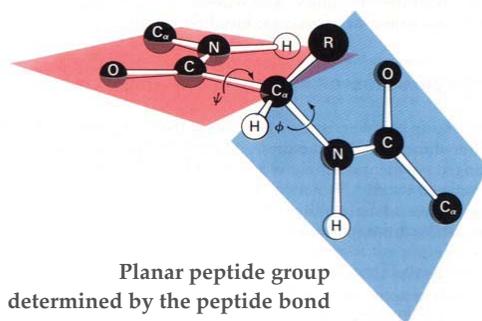
Number of possible protein configurations:

$$i^n$$

i: number of configurations related to the amino acid
n: number of amino acids

Levinthal's paradox:
Are all conformations explored by the protein molecule?

Energy

Conformation

N
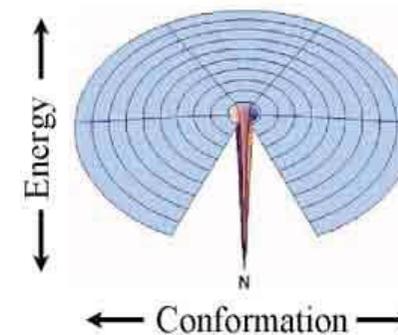
„Folding funnel"
Protein folding diseases

Pathology:
-Alzheimer's disease,
-Familial amyloidotic neuropathy (FAP)
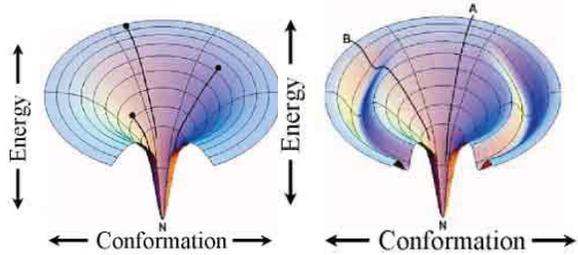
**In the living cell, chaperones assist protein folding**
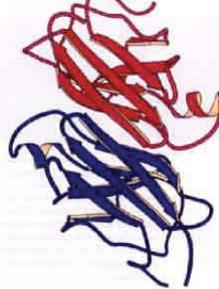
Beta-fibrils: Insoluble aggregates

Methods of protein unfolding (denaturation)

- Heat
- Chemical agent
- Mechanical force

Mechanical unfolding of a single protein with atomic force microscope
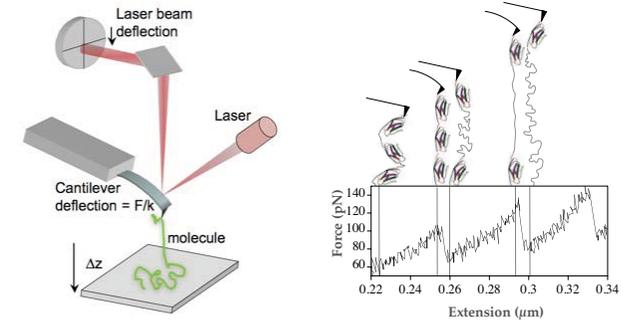
Mechanical unfolding of a beta-sheet protein

Steps of computer-simulated domain unfolding
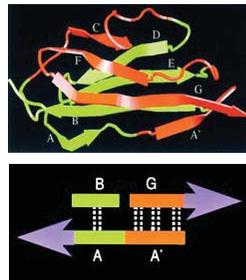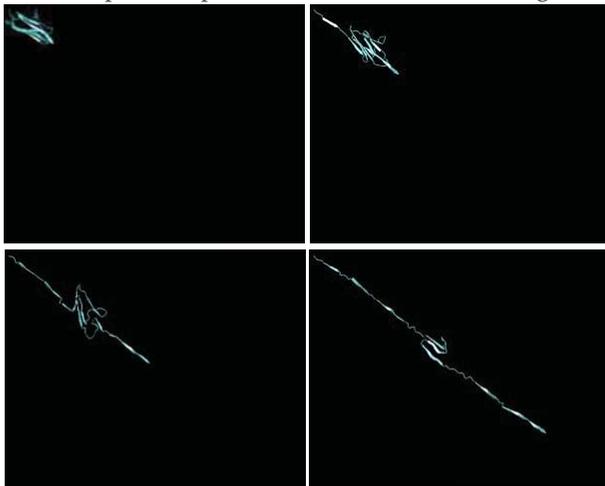
Basis of mechanical stability:

H-bridges between the first and last ß-strands of the domain

Mechanical unfolding of titin I27 domain

Mechanical stability provided by shear pattern of H-bond patch

Force spectrum of $I27_{12}$

200 pN

100 nm
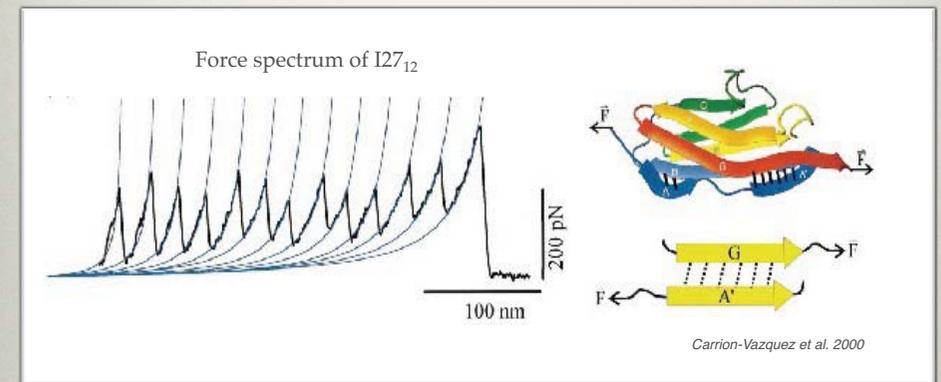
Carrion-Vazquez et al. 2000

Mechanical unfolding of C2A domain

Low mechanical stability due to zipper pattern of H-bond patch

Force spectrum of $C2A_9$

100 pN

100 nm

Carrion-Vazquez et al. 2000
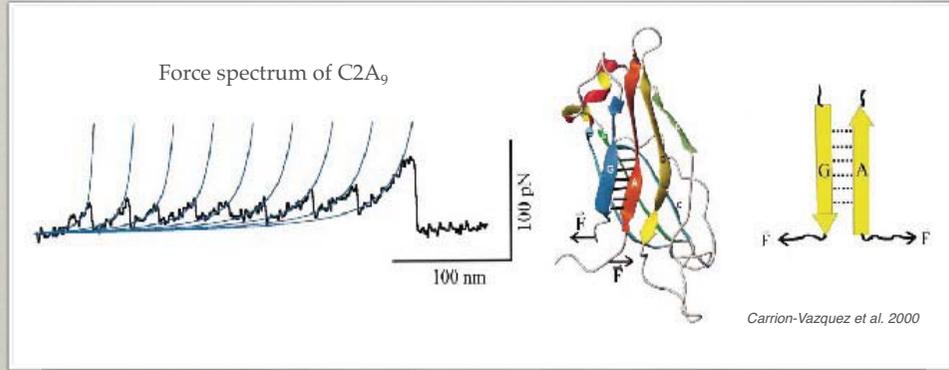
Mechanical stability in proteins

Principle of parallel bond coupling

Gecko foot stickiness: Bristles (setae) coupled in parallel
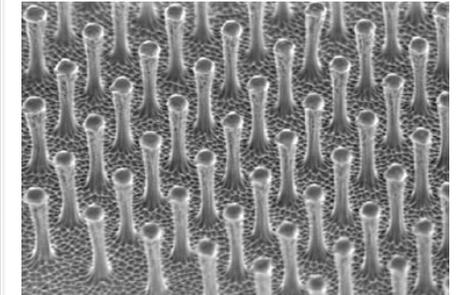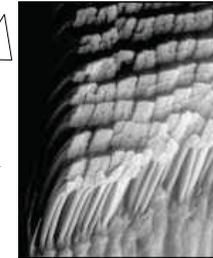
Artificial gecko foot

Thermodynamics

Outline

- Thermodynamic system.
- Characterization of the system. Thermodynamic variables.
- Microscopic structure of the system - internal energy.
- Equilibrium, reversibility.
- Zeroth Law of thermodynamics.
- Change, reversible change, thermodynamic processes.
- First Law of thermodynamics. Change in internal energy. Energy is conserved!
- Enthalpy.
- Second Law of thermodynamics.
- Direction of spontaneous processes. Entropy. Statistical interpretation. Thermodynamic probability. Phenomenological interpretation of entropy.
- Third Law of thermodynamics.
- Helmholtz- and Gibbs-free energy. Significance of free energy and entropy.
- Chemical potential.
- Irreversible thermodynamics, transport phenomena.
- Diffusion, Brown-movement.
- Osmosis.

WHAT IS THERMODYNAMICS?

Definition: branch of physics studying the effects of changes in temperature, pressure and volume (energy changes).

Therme (Gr) heat, *dynamis* (Gr) power.

heat source — heat sink — Work
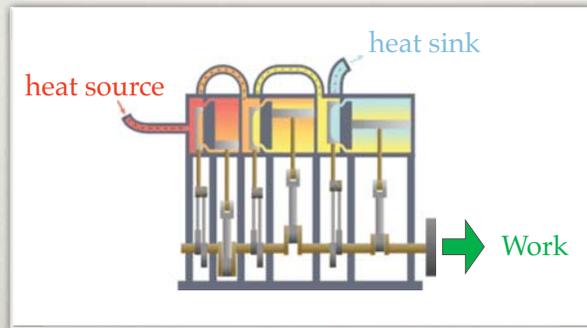
THERMODYNAMICS

"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

- Its importance stems from its generality.
- It reveals the driving forces behind natural phenomena.
- Due to its generalizations, it has abstract, complex and difficult-to-understand concepts.

"Thermodynamics is a funny subject. The first time you go through it, you don't understand it at all. The second time you go through it, you think you understand it, except for one or two small points. The third time you go through it, you know you don't understand it, but by that time your are so used to it, it doesn't bother you any more."

-Arnold Sommerfeld

SHORT HISTORY OF THERMODYNAMICS

Otto von Guericke (1650) first vacuum pump.

Robert Boyle (1660) Boyle's law: $pV=constant$.

Joseph Louis Gay-Lussac (1802) universal gas law: $pV=nRT$.

Sadi Carnot (1824) father of thermodynamics. Concept of "work".

William Thomson (Lord Kelvin) (1849) name of "thermodynamics".

Rudolf Clausius (1850) concepts of enthalpy, entropy.

James Clerk Maxwell (1871) statistical thermodynamics, concept of equilibrium.

Ludwig Boltzmann (1875) statistical interpretation of entropy ($S=k\ln W$).

Willard Gibbs (1876) concept of free energy (useful work).

Lars Onsager (1929) irreversible thermodynamics, Onsager's linear relationship.

Christopher Jarzynski (1997) Jarzynski's equality. Free-energy change can be calculated from average irreversible work: $e^{-\Delta G/k_B T} = \left\langle e^{-W/k_B T} \right\rangle$

THERMODYNAMIC SYSTEM

Economic system

Social system

Financial system

Chromatin
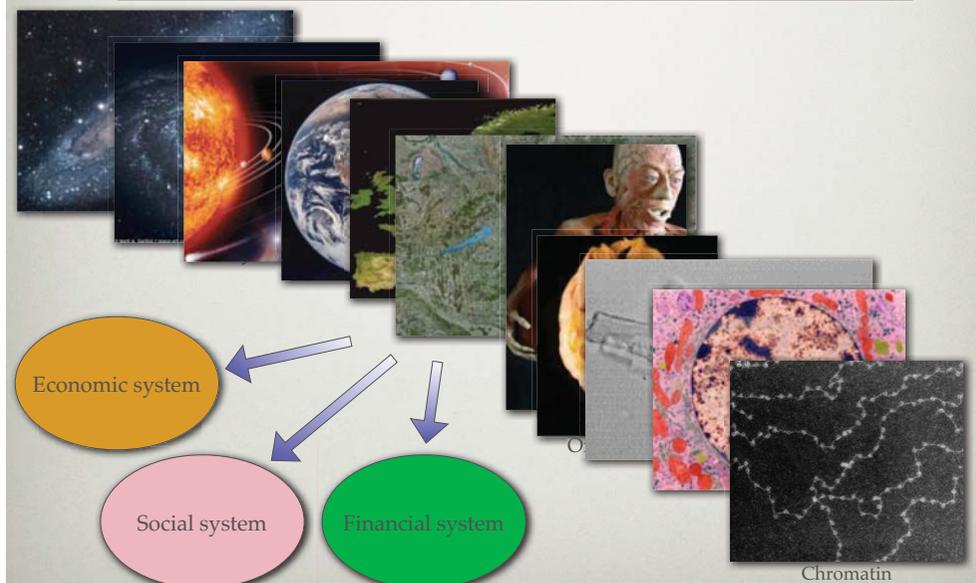
## ABSTRACTION OF THE THERMODYNAMIC SYSTEM

*Definition:* the thermodynamic system is the part of nature under investigation.
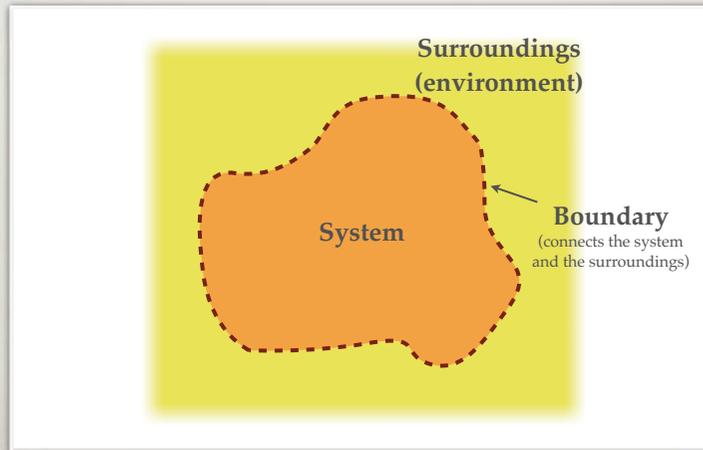
THE THERMODYNAMIC SYSTEM INTERACTS WITH THE SURROUNDINGS

Exchange of matter and energy may occur across the boundary.
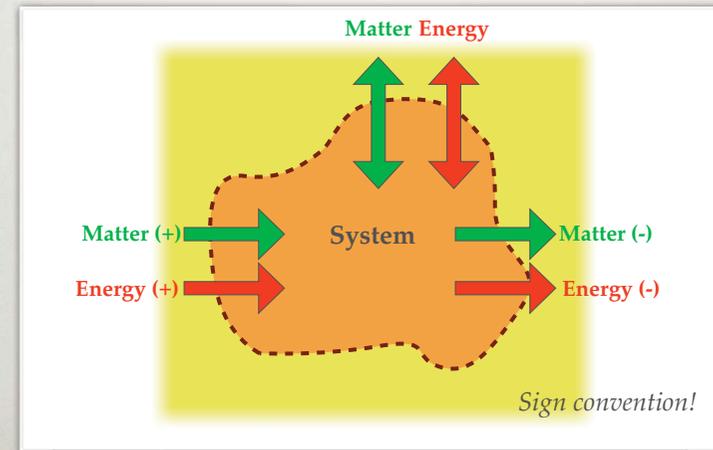
THE THERMODYNAMIC SYSTEM INTERACTS WITH THE SURROUNDINGS

Defining the thermodynamic system has consequences for processes.
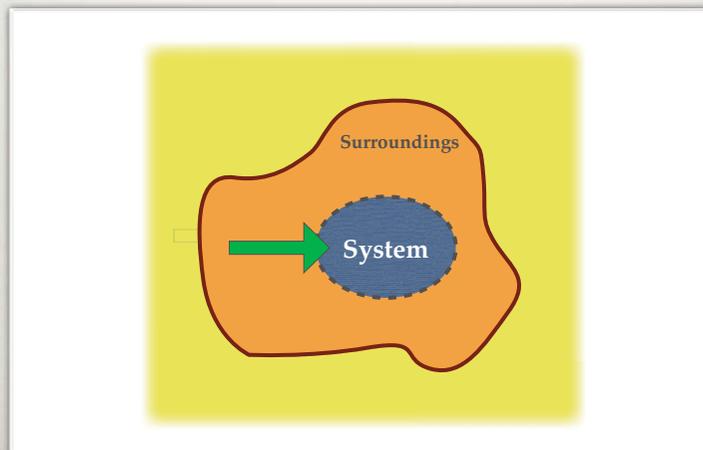

TYPES OF THERMODYNAMIC SYSTEMS
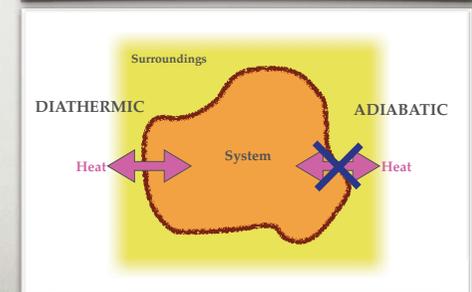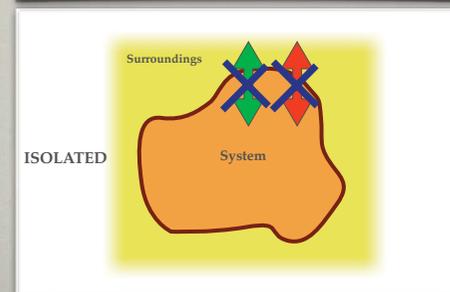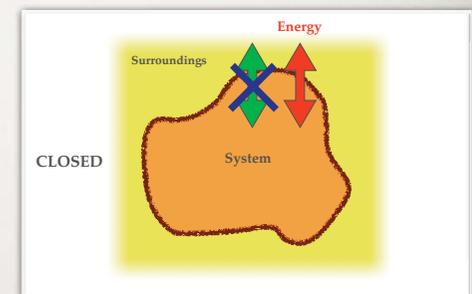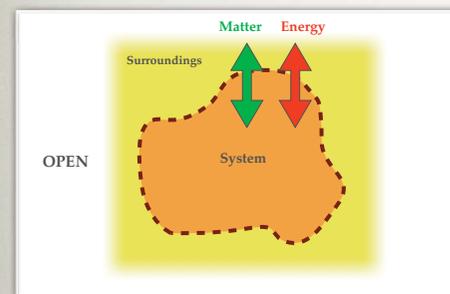
CHARACTERIZATION OF THE THERMODYNAMIC SYSTEM

Macroscopic characterization: state variables - explicitly determine the *state* of the system.

Pressure: p
Volume: V
Temperature: T
Concentration: c
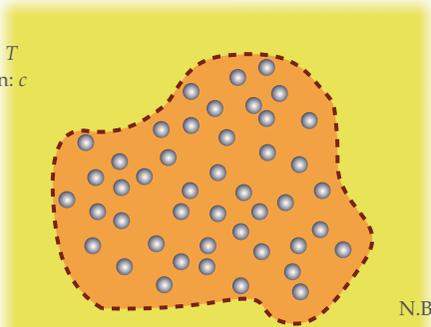
N.B.: universal gas law
$$pV = nRT$$

CHARACTERIZATION OF THE THERMODYNAMIC SYSTEM

Macroscopic characterization: extensive and intensive variables
Extensive variables: their value is proportional to the size of the system
Intensive variables: their value is independent of the size of the system

Extensive variable	Intensive variable	Product: energy change
Volume (V)	Pressure (p)	Volumetric work (pΔV)
Matter (n)	Chemical potential (μ)	Work of material transport ($\mu\Delta n$)
Charge (Q)	Electric potential (ϕ)	Electric work ($\phi\Delta Q$)
Entropy (disorder, S)	Temperature (T)	Heat ($T\Delta S$)

CHARACTERIZATION OF THE THERMODYNAMIC SYSTEM

Microscopic characterization: internal energy (E)

$$E = E_{el} + E_{vib} + E_{rot} + E_{other}$$
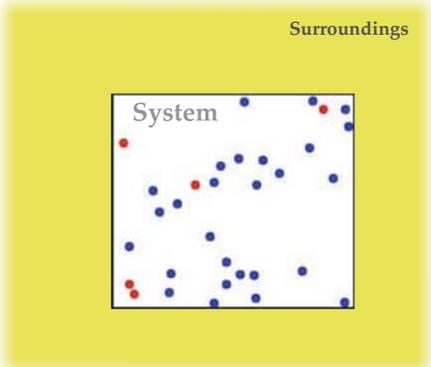
Surroundings

System

Internal energy does not contain the potential and kinetic energy of the macroscopic body.

INTERNAL ENERGY (E) IS "STATE FUNCTION"

State functions: single-value functions of the state variables (independent variables) of the system.
Change in the state functions depend only on the initial and final states of the system; it is independent of the path.

"A" state

ΔE
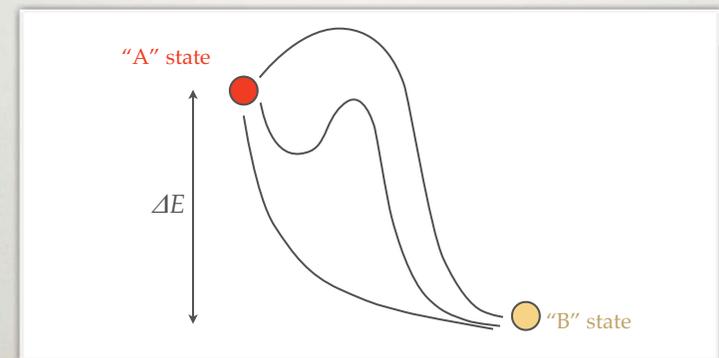
"B" state

Other state functions: enthalpy (H), Helmholtz free energy (F), Gibbs free energy (G), entropy (S)

EQUILIBRIUM

Macroscopic description: intensive variables are identical (between the system and surroundings, or between different parts of the system)

p_k, μ_k, ϕ_k, T_k

p_r, μ_r, ϕ_r, T_r

$p_k, \mu_k, \phi_k, T_k = p_r, \mu_r, \phi_r, T_r$
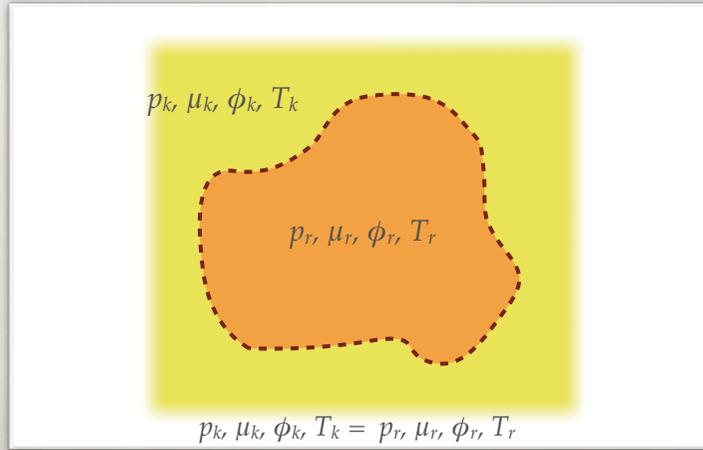
EQUILIBRIUM

Microscopic description: average rates of forward and reverse reactions are equal.

Boundary

Surroundings   System

$k_{forward}$

$k_{backward}$

$$\bar{k}_{forward} = \bar{k}_{backward}$$
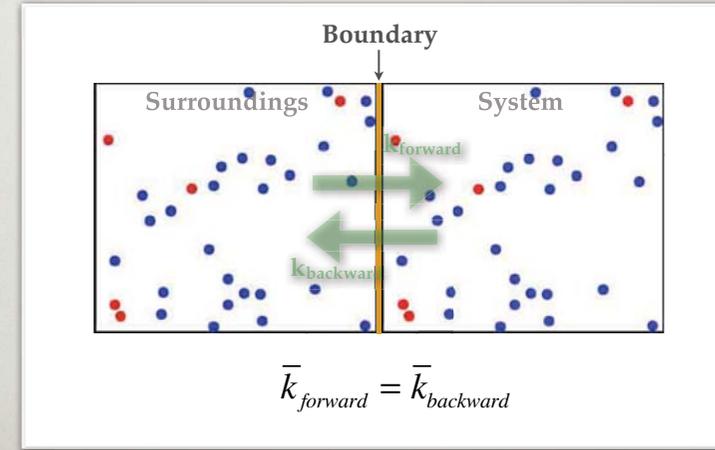
ZEROTH LAW OF THERMODYNAMICS

- If two systems (*A* and *B*) are independently in equilibrium with a third one (*C*), then they are in equilibrium with each other as well.
- Between different points of a system in equilibrium, the intensive variables are equel (there are no thermodynamic currents).

$A = B$

C

p_1, μ_1, ϕ_1, T_1

p_2, μ_2, ϕ_2, T_2

p_3, μ_3, ϕ_3, T_3
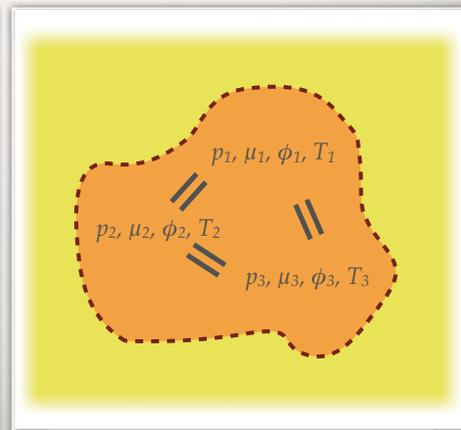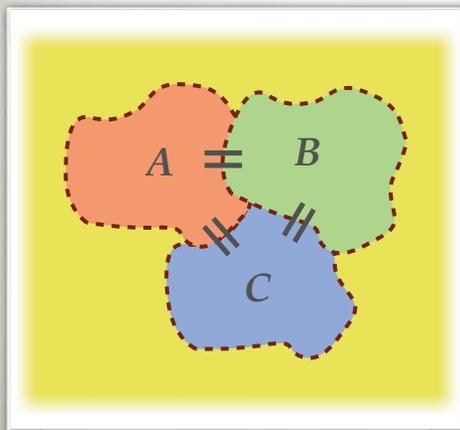
CHANGE

Thermodynamics is interested in the changes within the system.
Changes may be evoked by: heating, work.
Result: internal energy of the system changes.

Surroundings

Heat (*Q*)

System

Work (W):
mechanical
electric
material

Sign of change is +, if heat is given to the system or work is done on the system.
Sign of change is -, if the system gives off heat or does work.
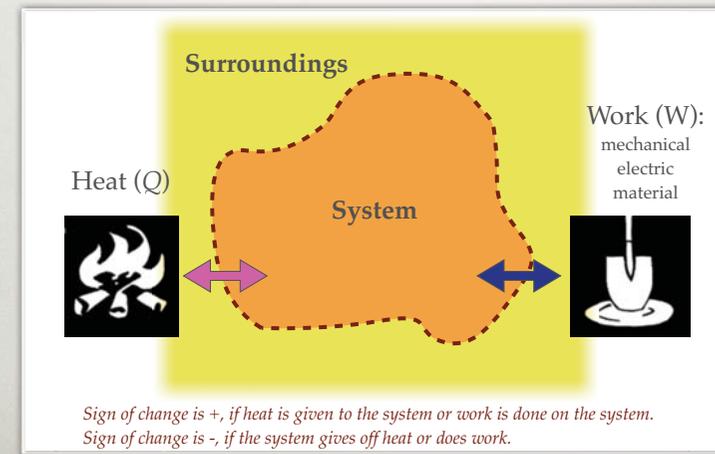
EQUILIBRIUM CHANGE

Definitions:
- Reversible change (reversibly driven proceess).
- Series of reversible (equilibrium, infinitesimal) steps; the sysem is in equilibrium during each step of the process.
- "Infinitely slow", "quasi-static" process.
- There are no permanent (compensating) energy changes in the surroundings (work done by the system and surroundings are equal).

Characteristics: Work done by the system is maximal.

Surroundings

System
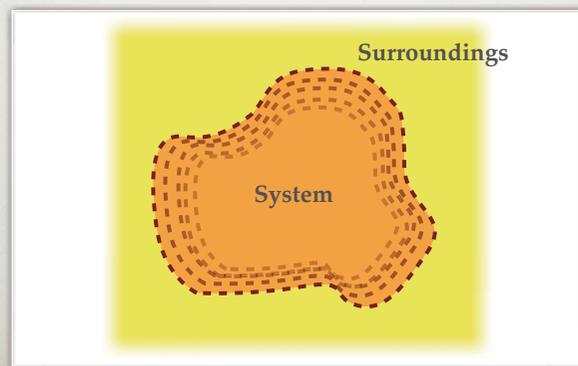
THERMODYNAMIC PROCESSES

- ***Isobaric:*** at constant pressure.
- ***Isochoric (isometric, isovolumetric):*** at constant volume.
- ***Isothermic:*** at constant temperature.
- *Adiabatic:* no heat exchange.
- *Isentropic:* at constant entropy.
- *Isenthalpic:* at constant enthalpy.
- *"Steady state":* at constant internal energy.

FIRST LAW OF THERMODYNAMICS

- Law of conservation of energy.
- Energy may be converted into different forms, but the total energy of the system remains constant.
- The change in the internal energy of the system is the sum of the supplied heat (Q_E) and the work (W) done on the system.

$$E_2 - E_1 = \Delta E$$

Difference between the internal energies of the initial and final stats of the system.

$$\Delta E = Q_E + W$$

Work parts:

Volumetric work
$$W_V = -p\Delta V$$
Negative, because in the case of work done on the system $V_2 - V_1 < 0$.

Electric work:
$$W_Q = \varphi\Delta Q$$

Material transport:
$$W_n = \mu\Delta n$$

Generalized:
$$W^i = y^i_{int}\Delta x^i_{ext}$$

Izochoric process ($\Delta V = 0$):
$$\Delta E = Q_V$$
Change in internal energy is caused only by the heat exchange.

Izobaric process (p=constant):
$$\Delta E = Q_p + W_V = Q_p - p\Delta V$$

ENTHALPY

- "Heat content" (H)
- Total heat absorbed by the system at constant pressure.
- Thermodynamic state function, by the help of which the useful work done at constant pressure may be calculated.
- *enthalpein* (Gr) to heat

$$\Delta E = Q_p + W_V = Q_p - p\Delta V$$
Internal energy change of the system during isobaric process.

Enthalpy:
$$H = E + pV$$
E, p, V: equilibrium internal energy, pressure and volume.

Enthalpy change:
$$\Delta H = \Delta E + p\Delta V$$

Isobaric process:
$$\Delta H = Q_p$$
If there are no other energy changes beside volumetric work.
Q_p = Reaction heat at constant pressure.

Isochoric process (V=constant):
$$\Delta E = Q_V$$
Volumetric work = 0.
Q_V = Reaction heat at onstant volume.

Standard state: 25 °C, 1 atm. Standard enthalpy of compounds ($\Delta H°$): reaction heat of 1 mole of compound measured in standard state, at constant pressure.

SECOND LAW OF THERMODYNAMICS

- "Law of entropy."
- Heat flows spotaneously from regions of high to low temperature.
- In an isolated system only such processes occur spontaneously which tend to equilibrate the respective intensive variables.
- During spontaneous processes entropy increases. Thermodynamic equilibrium is characterizd by entropy maximum.
- Spontaneous processes proceed towards the most probably state (irreversible).
- Statistical law.

The fallen and broken flower pot does not spontaneously return to its original place.

The essentially endless heat content of oceans cannot be used to spontaneously produce useful work.

ENTROPY

- *Entropy (S):* unavailability of the system to do useful work. State function (units: *J/K*).
- Measure of "unusable" energy.
- Measure of thermodynamic probability.
- Measure of disorder.
- *entrepein* (Gr) to turn around.

Phenomenologic description:

Energy change during thermodynamic processes (interactions):
$$\Delta E = \sum_i y_{int}^i \Delta x_{ext}^i$$
Product of intensive variables (*y*) and extensive variable-changes (*Δx*).

Energy change during thermal processes (thermal ineractions):
$$Q_E = T\Delta S$$
Intensive variable: temperature (*T*)
Extensive variable: entropy change (*ΔS*)

Entropy change:
$$\Delta S = \frac{\Delta Q}{T}$$
Measure of heat exchanged during reversible (equlibrium) isothermal process.

ENTROPY

- In isolated system, during temperature equilibration entropy always increases.

Isolated system

subsystem 1: T_1, S_1 subsystem 2: T_2, S_2

Heat-conducting wall
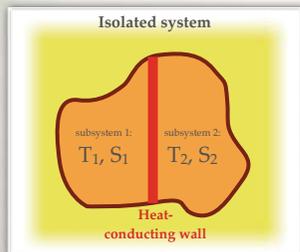
$$\Delta E = \Delta(E_1 + E_2) = \Delta E_1 + \Delta E_2 = 0$$
In an isolated system internal energy is constant ($\Delta E = 0$).

$$\Delta E_1 = T_1 \Delta S_1 \qquad \Delta E_2 = T_2 \Delta S_2$$
Energy changes during thermal interaction.

$$\Delta S = \Delta S_1 + \Delta S_2$$
Entropy is an extensive variable.

$$\Delta S = \frac{\Delta E_1}{T_1} + \frac{\Delta E_2}{T_2} = \Delta E_1 \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

$$T_1 > T_2 \ \text{ or } \ T_1 < T_2 : \Delta S > 0$$
If the initial temperatures are different, entropy increases.

$$T_1 = T_2 \ : \ \Delta S = 0$$
If the initial temperatures are equal, entropy does not change.

S / process / Equilibrium / t
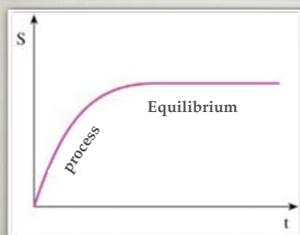
STATISTICAL INTERPRETATION OF ENTROPY

- *Macrostate:* state of the system defined by state variables (e.g., *p, V, T*).
- *Microstate:* internal structre of the system (position, velocity of every particle)
- In the *most probable macrostate* the number of microstates is the greatest.
- Number of microstates describing a given macrostate: *thermodynamic probability (Ω)*.

subsystem 1 / subsystem 2 / **transmissible wall** / a b c d
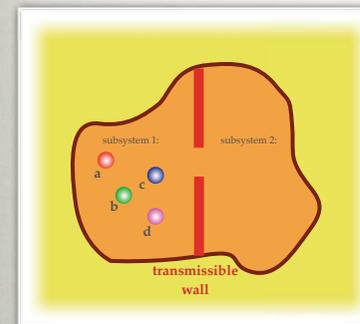
| Macrostate	number of particles in subsystem 1	structure of subsystem 1	Number of microstates (Ω)
A	4	abcd	1
B	3	abc,abd,acd,bcd	4
C	2	ab, ac, ad, bc, bd, cd	6
D	1	a, b, c, d	4
E	0	-	1

Entropy:
$$S = k_B \ln \Omega$$
k_B = Boltzmann's constant (1,38 x 10^{-23} J/K)

ENTROPY IS THE MEASURE OF DISORDER
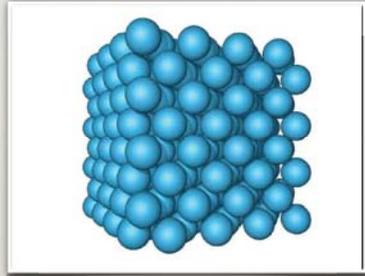
Ordered crystal:
Low entropy
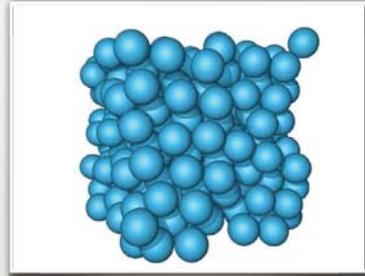
Disordered liquid:
High entropy

THIRD LAW OF THERMODYNAMICS

- Nernst's law.
- The entropy of mon-component, crystallizing material at 0 K temperature is 0.

At 0 K thermal motions halt, there are no thermal crystal faults.

$$\Omega = 1$$

Number of microstates is 1.

Because of mono-component system, only ony type of molecular arrangement is possible.

$$S = k_B \ln 1 = 0$$