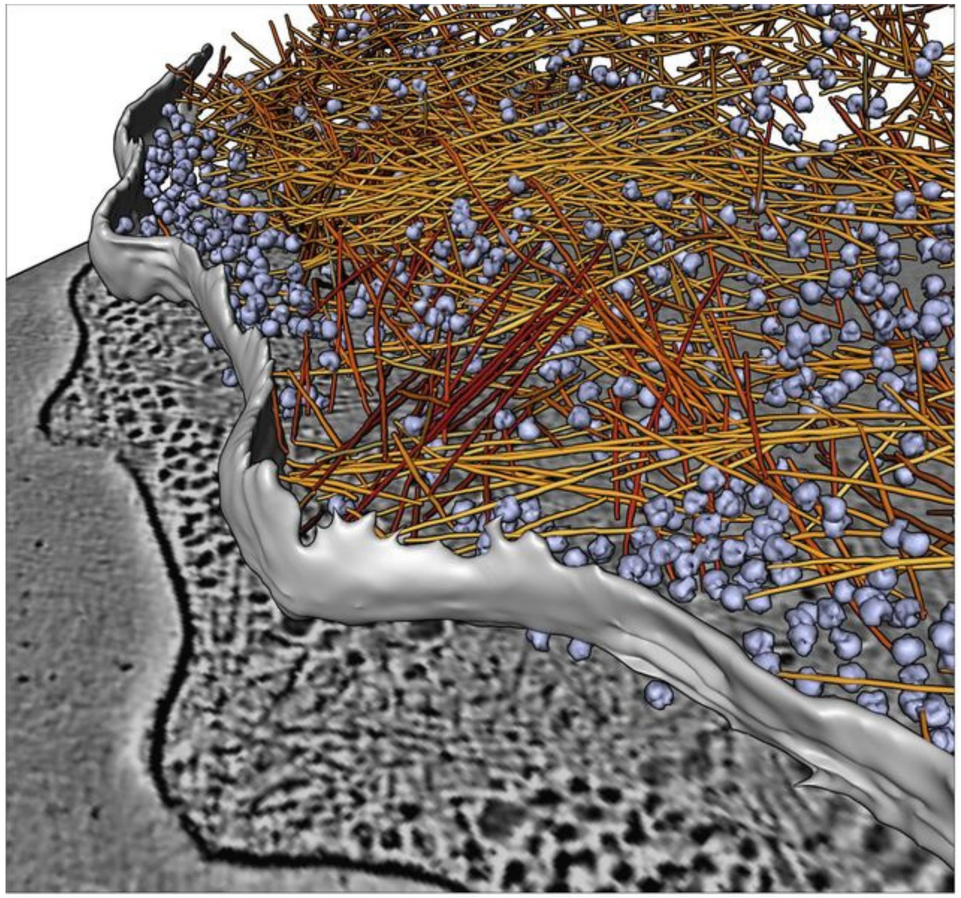
Interactions between proteins, DNA and RNA

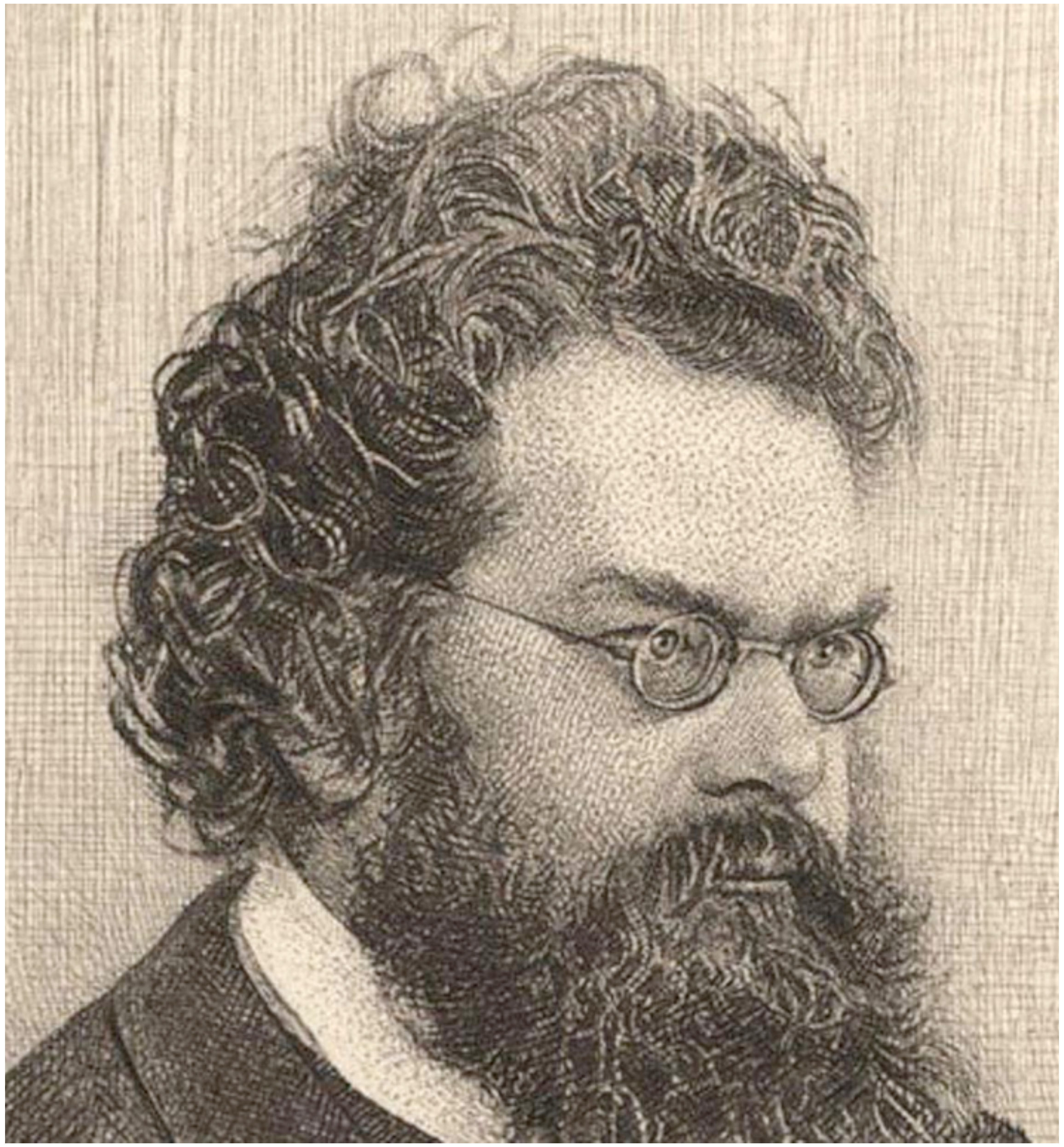
—

The energy coordinate system

Karsten Rippe



The Boltzmann constant



Ludwig Boltzmann

Definition of entropy as a measure of statistical disorder of a system

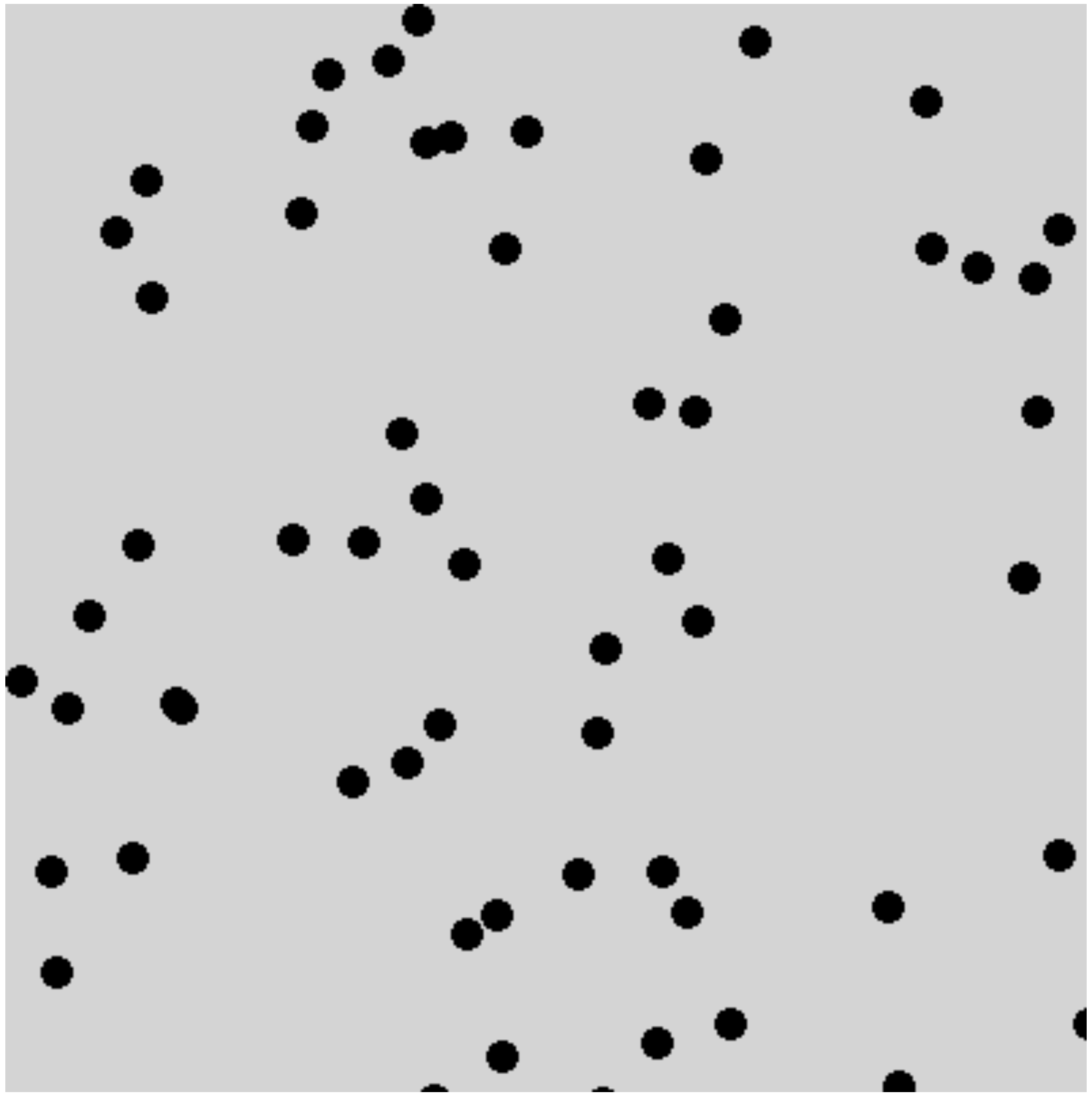
$$S \equiv k_B \ln W$$

Molecular Entropy

Boltzmann Constant

Number of microscopic ways in which a particular outcome (macroscopic state) can be attained

Molecules in an ideal gas



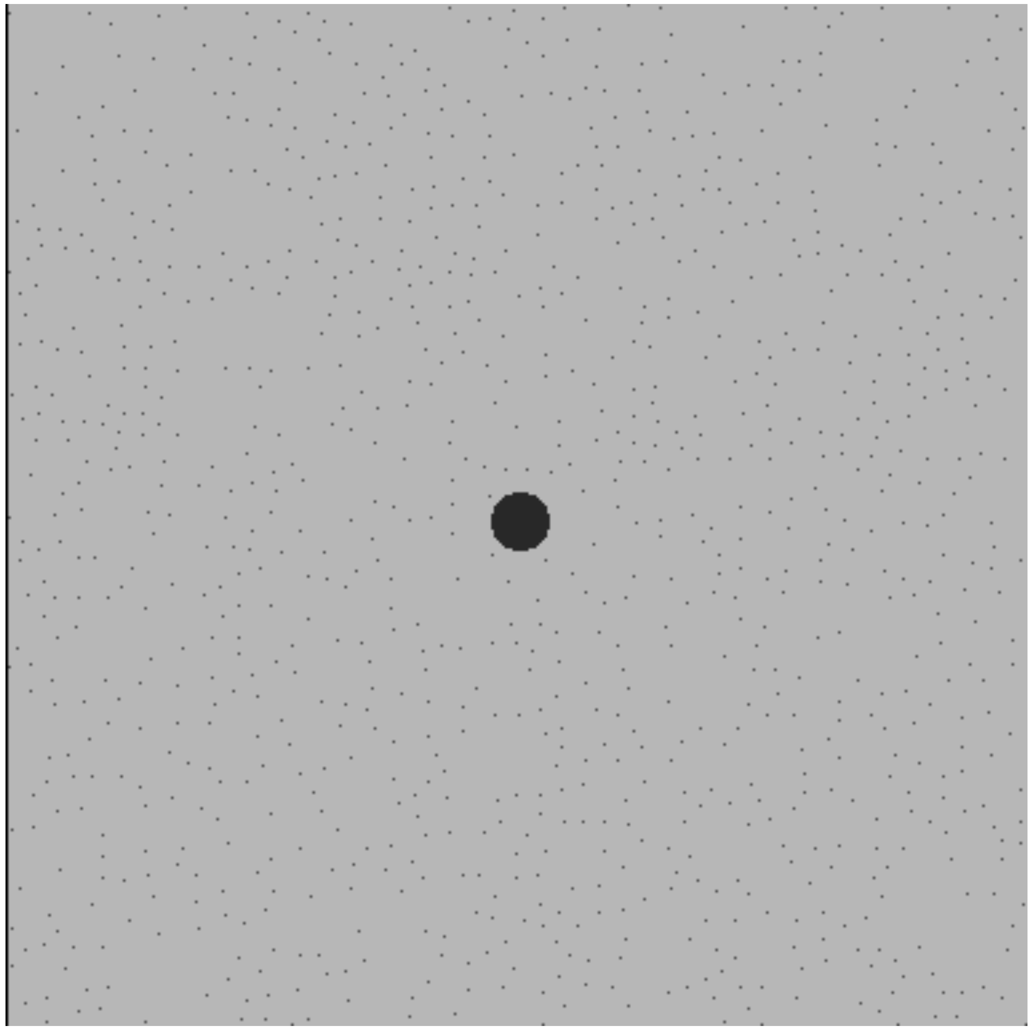
The average kinetic energy of a particle with mass *m* along the x-axis is related to $k_B T/2$

$$\left\langle \frac{m\, v_x^2}{2} \right\rangle = \frac{kT}{2} \qquad \left\langle v_x^2 \right\rangle = \frac{kT}{m}$$

$$\sqrt{\left\langle v_x^2 \right\rangle} = \sqrt{\frac{kT}{m}}$$

A protein like lysozyme (M = 14 kDa) at room temperature would have a speed of $<v_x^2>^{1/2}$ = 13 m/sec **in vacuum**.

Thermal motion defines the energy unit $k_B T$ in the cell
k_B (Boltzmann constant) x T (temperature)



- The macromolecule collides with water molecules and exchange energy $\sim k_B T$
- Macromolecule moves in a “random walk” because collisions are random
- The length of the free path is much smaller than the diameter of the particle.
- The average distance from the starting point is proportional to the square root of time.

$k_B T$ is the energy available for spontaneous reactions

$$P_\mathrm{i} \propto g_i \cdot \exp\left(\frac{-E_\mathrm{i}}{k_\mathrm{B} T}\right)$$

The Boltzmann equation yields the probability P_i to find a molecule with energy E_i

- g_i: number of different states with energy E_i
- k_B: Boltzmann constant
- T: Temperature

probability to find a particle with an energy

- of $k_\mathrm{B}T$ or larger: 0.37 => processes that requires an energy of $k_\mathrm{B}T$ occur spontaneously
- of 10 $k_\mathrm{B}T$ *or larger*: 0.00005 => these processes will not occur spontaneously

at 298 K (25 °C) $k_\mathrm{B}T = 4.1 \cdot 10^{-21}$ J or **$k_\mathrm{B}T$ = 4.1 pN·nm**

$k_\mathrm{B}T$ refers to a single molecule
for 1 mol of particles one has to use $k_\mathrm{B}T \times 6.022 \cdot 10^{23} = RT$

at 25 °C with $R = 8.3\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ => **RT = 2.5 kJ/mol or 0.6 kcal/mol**

Hydrolysis of ATP: 20-30 $k_\mathrm{B}T$/ATP, 12 - 18 kcal/mol or 50 - 70 kJ/mol (physiological conditions)

What is the value of $k_\mathrm{B} \cdot \mathrm{T}$ (or $R \cdot T$)?

The free energy ΔG

- **At constant pressure *P* and constant temperature *T* the system is described by the Gibbs free energy:**

$$G \equiv H - T\,S \qquad\qquad \Delta G = \Delta H - T\,\Delta S$$

- ***H* is the enthalpy or heat content of the system, *S* is the entropy of the system**
- **a reaction occurs spontaneously only if $\Delta G < 0$**
- **at equilibrium $\Delta G = 0$**
- **for $\Delta G > 0$ the input of energy is required to drive the reaction**

ΔG of a reaction in equilibrium

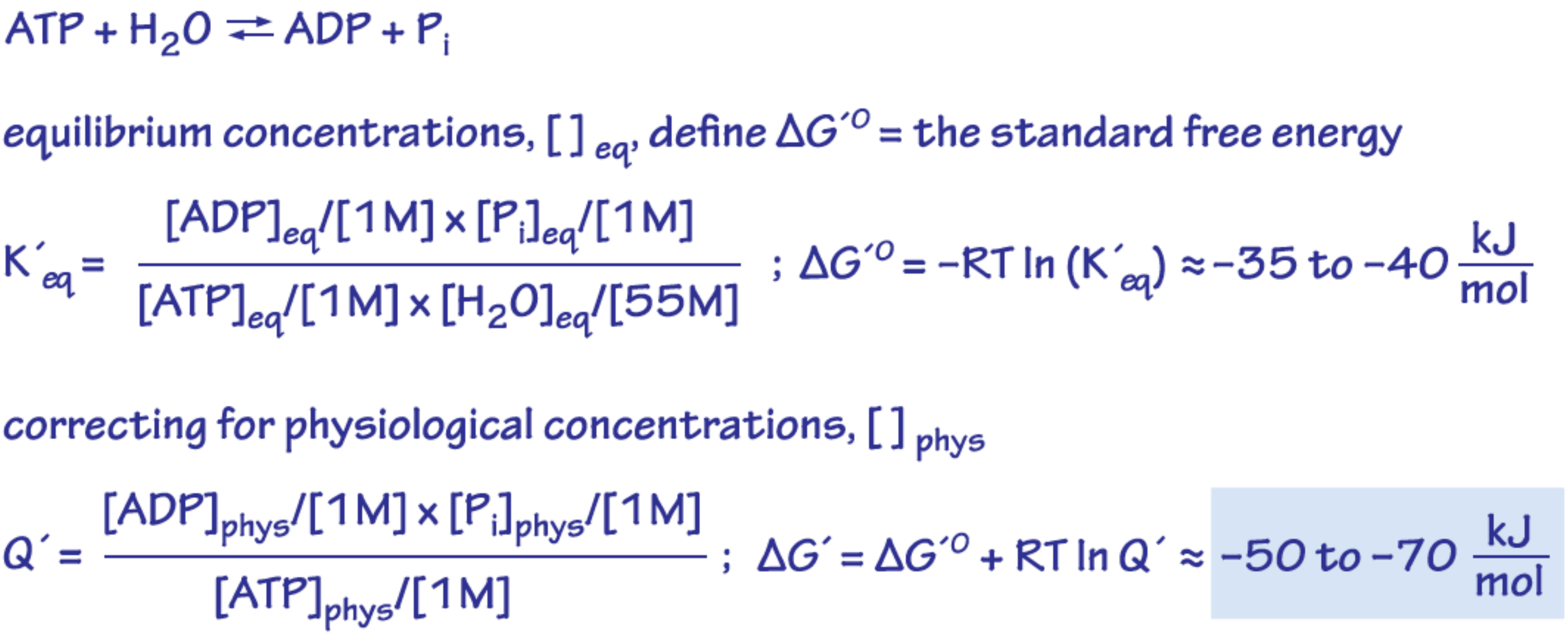
$$aA + bB + \ldots \rightleftarrows gG + hH \ldots$$

$$0 = \Delta G^0 + RT \ln\left(\frac{[G]^g[H]^h \ldots}{[A]^a[B]^b \ldots}\right)_{Eq}$$

$$\boxed{\Delta G^0 = -RT \ln\left(\frac{[G]^g[H]^h \ldots}{[A]^a[B]^b \ldots}\right)_{Eq} = -RT \ln K}$$

$$K = \left(\frac{[G]^g[H]^h \ldots}{[A]^a[B]^b \ldots}\right)_{Eq} = \exp\left(\frac{-\Delta G^0}{RT}\right)$$

Free energy of ATP hydrolysis under physiological conditions



$$ATP + H_2O \rightleftarrows ADP + P_i$$

equilibrium concentrations, $[\,]_{eq}$, define $\Delta G'^{o}$ = the standard free energy

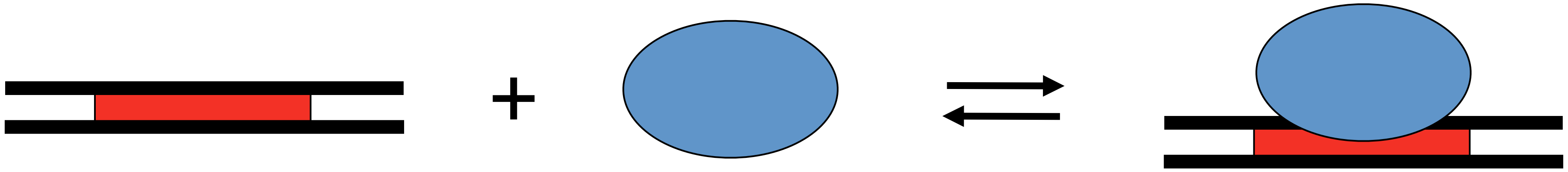
$$K'_{eq} = \frac{[ADP]_{eq}/[1M] \times [P_i]_{eq}/[1M]}{[ATP]_{eq}/[1M] \times [H_2O]_{eq}/[55M]} \; ; \; \Delta G'^{o} = -RT\ln(K'_{eq}) \approx -35 \text{ to } -40 \, \frac{kJ}{mol}$$

correcting for physiological concentrations, $[\,]_{phys}$

$$Q' = \frac{[ADP]_{phys}/[1M] \times [P_i]_{phys}/[1M]}{[ATP]_{phys}/[1M]} \; ; \; \Delta G' = \Delta G'^{o} + RT\ln Q' \approx -50 \text{ to } -70 \, \frac{kJ}{mol}$$

or 20-30 $k_B T$

The mass equation law for binding of a protein P to its DNA D



$$D_{\text{free}} + P_{\text{free}} \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

binding of the first proteins with the dissociation constant K_1

D_{free}, concentration free DNA; P_{free}, concentration free protein

$$\text{binding constant } K_{\text{B}} = \frac{1}{\text{dissociation constant } K_{\text{D}}}$$

How fast is binding or dissociation

$$\mathrm{AB}\ \underset{k_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftarrows}}\ \mathrm{A+B}$$

k_{off} in s^{-1} is the reaction rate constant for dissociation

k_{on} in $M^{-1}\ s^{-1}$ is the reaction rate constant for binding

$$\frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} = K_{\mathrm{d}}$$

relation to the equilibrium dissociation constant

$$\frac{1}{k_{\mathrm{off}}} = \tau$$

life time of the complex

$$\frac{d[\mathrm{AB}]}{dt} = \mathrm{k_{on}} \cdot [\mathrm{A}] \cdot [\mathrm{B}] - \mathrm{k_{off}} \cdot [\mathrm{AB}]$$

rate equation for complex formation,

can be solved but it is already difficult

k_{on} cannot be higher than 10^8 - 10^9 $M^{-1}\ s^{-1}$ for a diffusion controlled reaction

Our energy and time coordinate system

K_d (M)	**concentration scale**	**ΔG (kcal/mol)**	**k_{off} (s-1)** for $k_{on} = 10^5\ M^{-1}\ s^{-1}$	**complex life time**	**Binding interaction**
10^{-3}	1 mM	-4.1	10^{2}	10 ms	ion-DNA ion-protein
10^{-4}	0.1 mM	-5.5	10^{1}	0.1 sec	
10^{-5}	10 µM	-6.8	1	1 sec	enzyme-ligand (weak)
10^{-6}	1 uM	-8.2	10^{-1}	10 sec	protein-DNA, unspecific
10^{-7}	0.1 µM	-9.5	10^{-2}	100 sec	enzyme-ligand (strong)
10^{-8}	10 nM	-10.9	10^{-3}	16.7 min	
10^{-9}	1 nM	-12.3	10^{-4}	2.8 hours	protein-DNA specific
10^{-10}	0.1 nM	-13.6	10^{-5}	28 hours	
10^{-11}	10 pM	-15	10^{-6}	11.6 days	antibody-antigen
10^{-12}	1 pM	-16.4	10^{-7}	116 days	

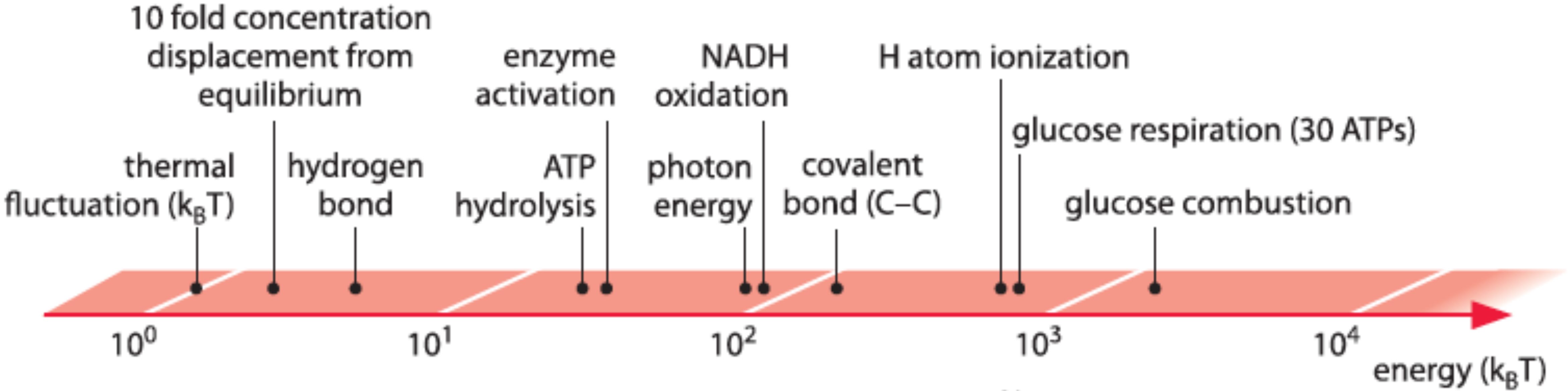
Weak forces maintain macromolecular structure

0.6 kcal/mol or 2.5 kJ/mol thermal energy at 25 °C: enough to break non-covalent bonds
=> Non-covalent interactions constantly forming and breaking

- Van der Waals forces: $k_B T$ (0.4 - 4 kJ/mol), $1/r^6$
- Hydrogen bonds: 2-12 $k_B T$, 6 - 30 kJ/mol, $1/r^3$
- Ionic interactions: 5 kcal/mol physiological, $1/r$
- Covalent bonds: 80-300 $k_B T$, 200-800 kJ/mol, $1/r^{\text{very high}}$
  200 (S-S) 300 (C-C) 740 (C=O) kJ/mol
- Hydrolysis of ATP: 20-30 $k_B T$, 50 - 70 kJ/mol

but noncovalent interactions can occur between macromolecule and water/ions just as well...

Energy scale



$1\ k_BT \approx 2.5$ kJ/mol ≈ 0.6 kcal/mol ≈ 25 meV ≈ 4.1 pN x nm $\approx 4.1 \times 10^{-21}$ J

Energy scales

- k_BT (single molecule) or RT (per mole) reference scale
- Free energy ΔG (equivalent to K_d) for reactions at equilibrium
- Concentration affects binding according to mass equation law
- Entropy from changes of number of states

Macromolecule interactions in water with ions at pH 7

- Non-covalent interactions between macromolecules around k_BT
- H-bonds and ionic interactions compete with water and ions
- Hydrophobic effect can drive interactions
- Entropy changes (e.g., folding of protein) make large contributions