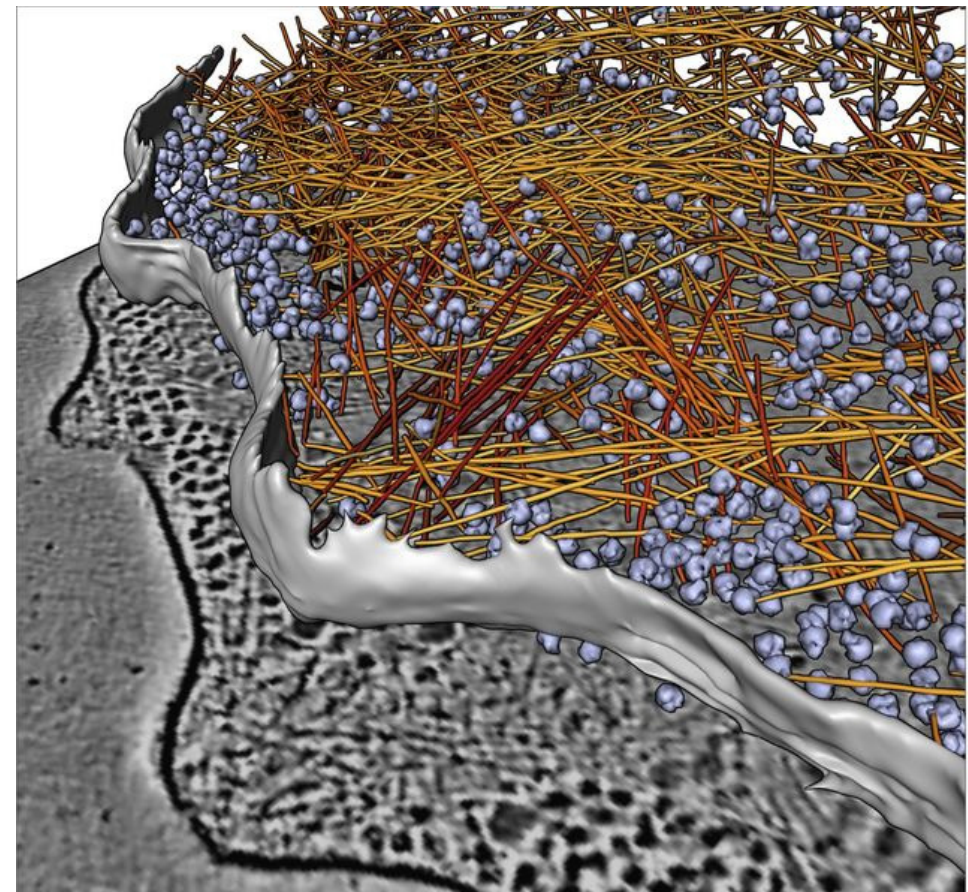


Interactions between proteins, DNA and RNA

—
The energy coordinate system

Karsten Rippe



The Boltzmann constant



Ludwig Boltzmann

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

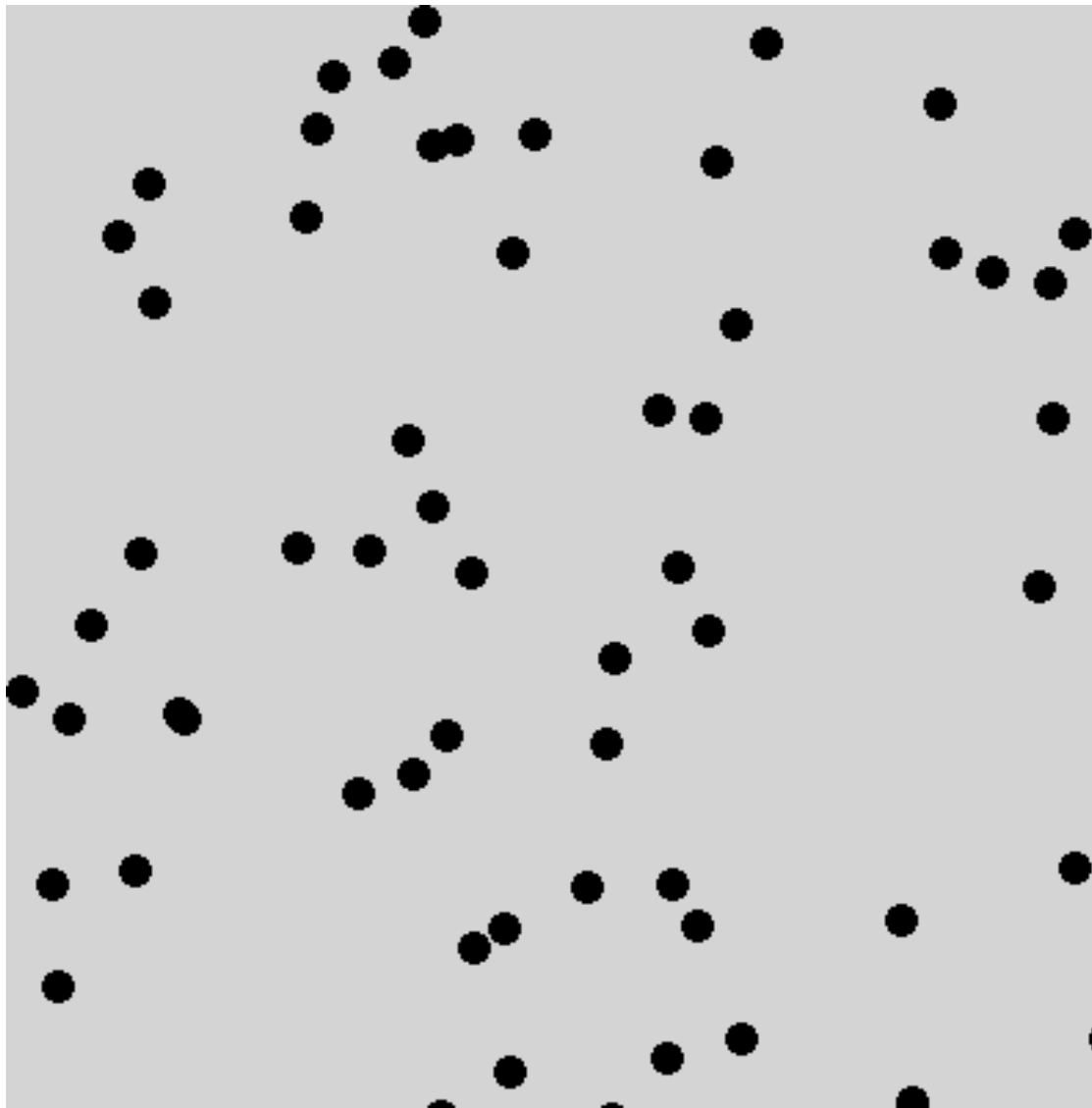
$$S \equiv k_B \ln W$$

Molecular Entropy

Boltzmann Constant

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

Molecules in an ideal gas



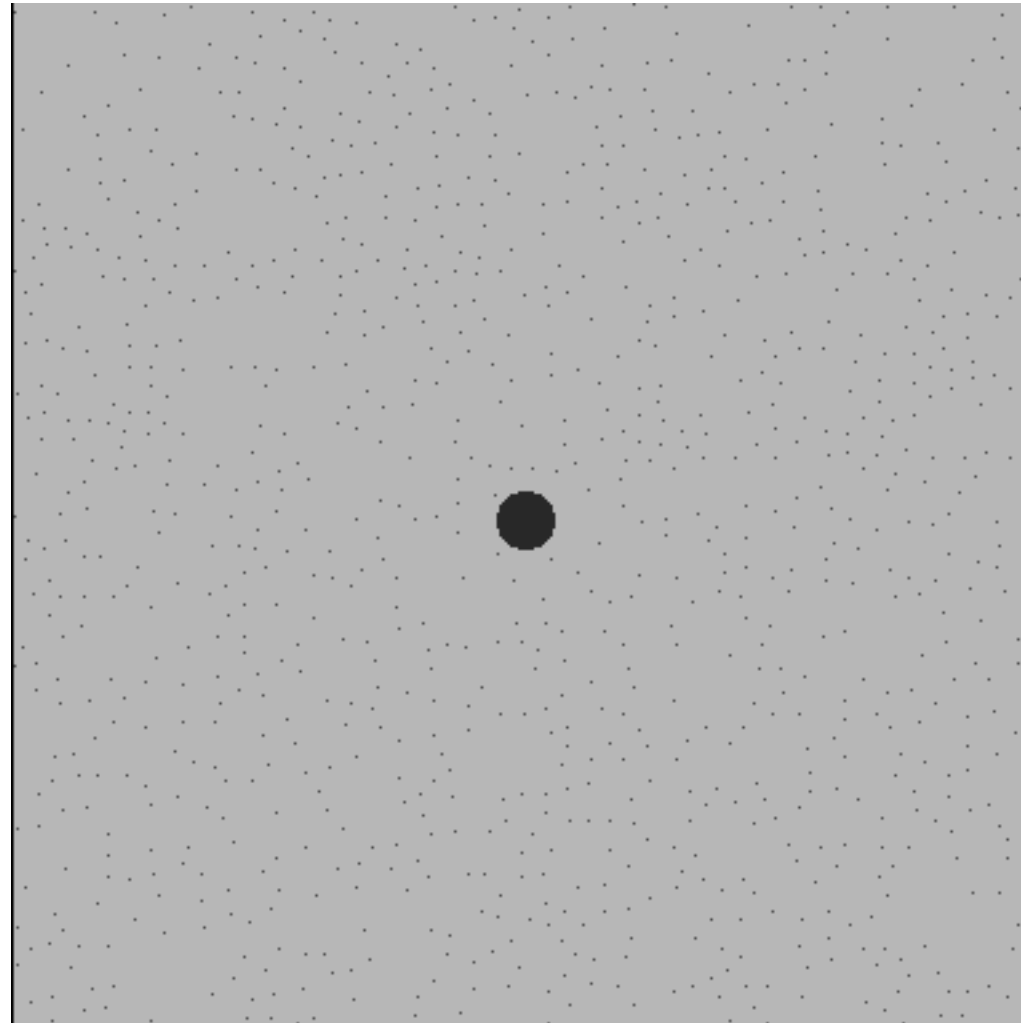
The average kinetic energy of a particle with mass m along the x-axis is related to $k_B T/2$

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

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

A protein like lysozyme ($M = 14$ kDa) at room temperature would have a speed of $\langle v_x^2 \rangle^{1/2} = 13$ 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

$$P_i \propto g_i \cdot \exp\left(\frac{-E_i}{k_B T}\right)$$

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 $k_B T$ or larger: 0.37 $\Rightarrow$ processes that requires an energy of $k_B T$ occur spontaneously
- of 10 $k_B T$ or larger: 0.00005 $\Rightarrow$ these processes will not occur spontaneously

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

$k_B T$ refers to a single molecule

for 1 mol of particles one has to use $k_B T \times 6.022 \cdot 10^{23} = RT$

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

Hydrolysis of ATP: 20-30 $k_B T$ /ATP, 12 - 18 kcal/mol or 50 - 70 kJ/mol (physiological conditions)

What is the value of $k_B \cdot T$ (or $R \cdot T$)?

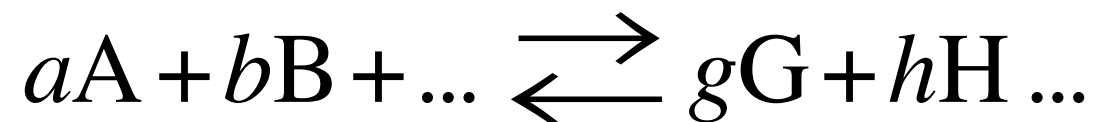
The free energy ΔG

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

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

- 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



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

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

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

Free energy of ATP hydrolysis under physiological conditions



equilibrium concentrations, $[]_{eq}$, define $\Delta G'^0$ = the standard free energy

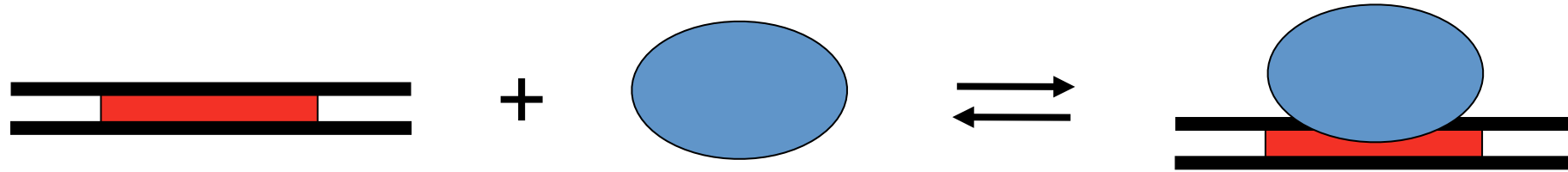
$$K'_{eq} = \frac{[\text{ADP}]_{eq}/[1\text{M}] \times [\text{P}_i]_{eq}/[1\text{M}]}{[\text{ATP}]_{eq}/[1\text{M}] \times [\text{H}_2\text{O}]_{eq}/[55\text{M}]} ; \Delta G'^0 = -RT \ln (K'_{eq}) \approx -35 \text{ to } -40 \frac{\text{kJ}}{\text{mol}}$$

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

$$Q' = \frac{[\text{ADP}]_{phys}/[1\text{M}] \times [\text{P}_i]_{phys}/[1\text{M}]}{[\text{ATP}]_{phys}/[1\text{M}]} ; \Delta G' = \Delta G'^0 + RT \ln Q' \approx -50 \text{ to } -70 \frac{\text{kJ}}{\text{mol}}$$

or $20\text{-}30 k_B T$

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



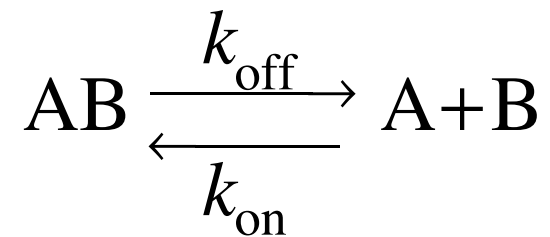
$$D_{\text{free}} + P_{\text{free}} \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} DP \quad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

binding of the first proteins with the dissociation constant K_1

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

$$\text{binding constant } K_B = \frac{1}{\text{dissociation constant } K_D}$$

How fast is binding or dissociation



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

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

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

relation to the equilibrium dissociation constant

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

life time of the complex

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

rate equation for complex formation,
can be solved but it is already difficult

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

Our energy and time coordinate system

K_d (M)	concentration scale	ΔG (kcal/mol)	k_{off} (s⁻¹)	complex life time	Binding interaction
for k _{on} = 10 ⁵ M ⁻¹ s ⁻¹					
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 μM	-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

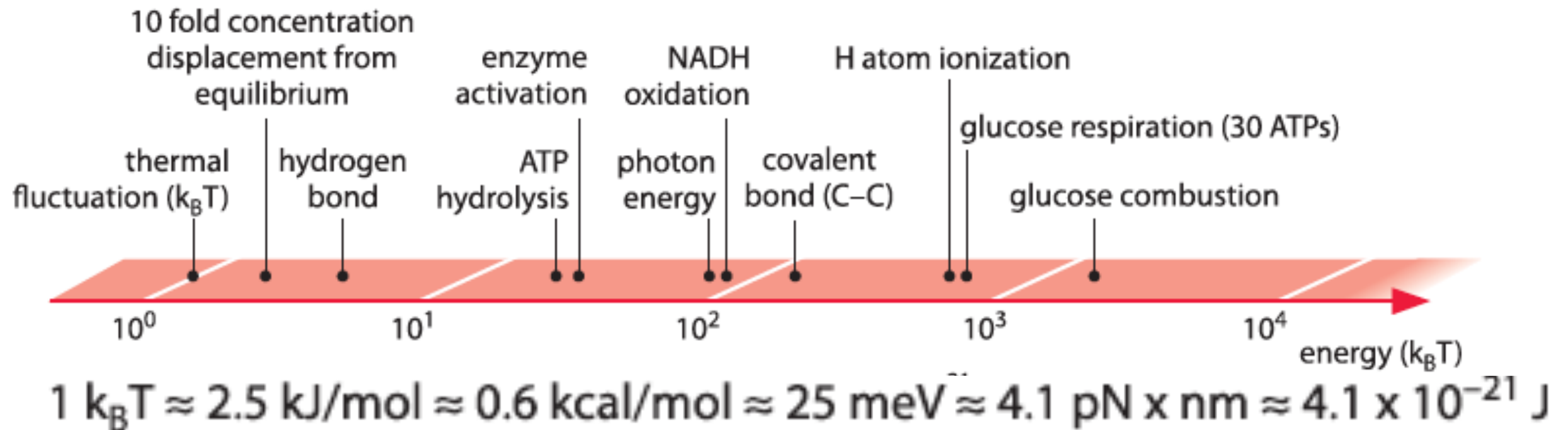
0.6 kcal/mol or 2.5 kJ/mol thermal energy at 25 °C: enough to break non-covalent bonds

=> 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



Energy scales

- $k_B T$ (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 $k_B T$
- 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