

Multiscale Modeling

Introduction:

To understand the concept of model and the practice of modeling we must consider the process of scientific discovery. Scientific discovery involves:

- Formulation of hypotheses to explain observed phenomena.
- Design and execution of experiments to test hypotheses.
- Feedback from experimental results to derive laws and theories.

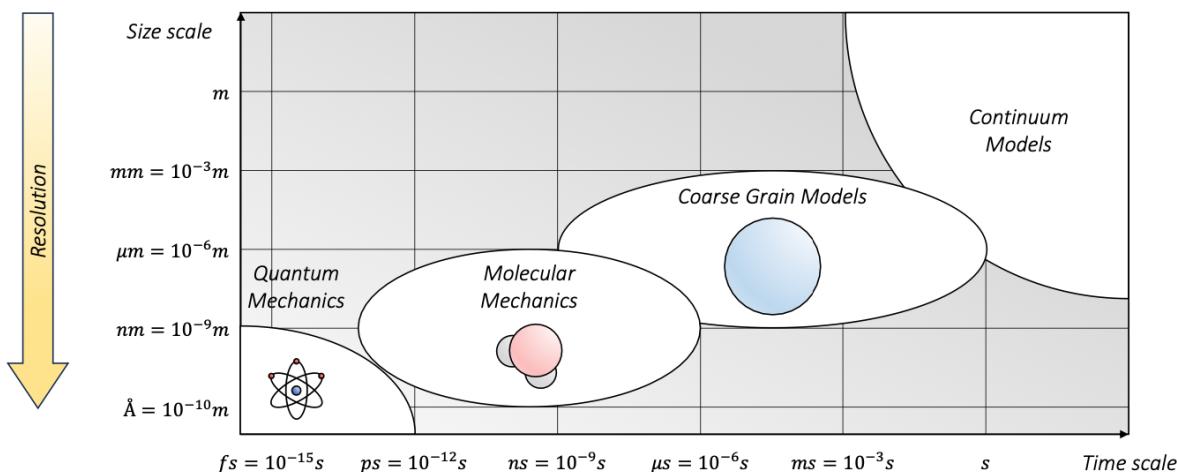
To formulate hypotheses and perform experiments we always make use of models.

- **Models:** abstractions of real-world systems, capturing features of reality deemed to be essential to describe the phenomenon under investigation.
- **Modeling:** scientific method of formulating a simplified imitation of a real situation with the preservation of its essential features.

We also define:

- **System:** specific portion or aspect of reality that is being modeled. It is represented by a collection of interrelated objects.
- **Objects:** elemental unit upon which observation can be made but whose internal structure is either unknown or not relevant to the problem.
- **Model:** description of a system in terms of constitutive objects and the relationships among them.

Depending on the size of the system we are considering we have different fields of modeling:



- **Variables:** independent physical quantities used in the formal description of the state of the system (degrees of freedom)
- **Observables:** any physical quantity that can be experimentally measured and predicted by the model
- **Parameters:** constants figuring in the mathematical equations. These should have a physical meaning and should be amenable to experiment/theory. The more detailed the description of reality, the smaller the need for additional empirical quantities (parameters).

Unlike most artificial systems or physical phenomena, which have been the subject of mathematical modelling for decades, biological systems are unique in several respects and as such they pose a number of specific challenges:

1. **Complexity.** Behaviour of systems composed of different interconnected parts that, as a whole, exhibit properties that do not obviously arise from the properties of the individual parts (emerging properties).
2. **Heterogeneity.** Biological systems are made of different types of components, and they show a large variability in composition between different individuals.
3. **Hierarchy.** Phenomena in biological systems typically take place over a wide range of length scales and time scales.

A problem in science is said to be “**multiscale**” when it involves phenomena taking place at distinct length- and/or time- scales spanning several orders of magnitude, and when these phenomena all play key roles in the problem so that we cannot model using a single scale.

When scales can be safely separated (**separation of timescales**), it is possible to reasonably model a biological phenomenon using single scale models. Otherwise, specific modeling techniques must be devised to integrate the scales (multiscale modeling).

The previous considerations concerning modelling do not imply any technical details regarding the way in which the mathematical equations are solved.

Most of the time, formal models are formulated in terms of **Ordinary Differential Equations** (ODE) or **Partial Differential Equations** (PDE). Depending on the way the mathematical equations are solved, we have:

<i>Analytical models</i>	<i>Numerical models</i>
<ul style="list-style-type: none">• Exact (under the approximations introduced by the abstraction process)• They can be solved only for simple cases (e.g. simple geometries)	<ul style="list-style-type: none">• Approximated• They can be used to solve complicated initial-value and boundary-value problems

Analytical models → **Lattice models**.

All numerical methods have in common the discretization of the independent variables (typically time and space) and the transformation of continuous derivatives into their discontinuous counterpart: the finite difference quotient.

By doing so, the continuous problem, expressed by differential equations with an infinite number of unknowns, i.e. function values, is replaced by discrete algebraic problem with a finite number of unknowns parameters which can be calculated in an approximate fashion.

With these definitions in place, more precisely, we can distinguish:

- **Numerical modelling**: entire procedure of model formulation and code programming
- **Simulation**: numerical experimentation of the model under certain initial-value and boundary-value conditions.

Molecular mechanics:

We start by defining the quantum mechanical model of the atom. Using quantum mechanics means to be able to solve the Schrödinger equation:

$$\hat{H} \Psi(x) = E \Psi(x)$$

This is the common time-independent equation. As output, we will obtain:

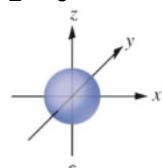
- the wavefunction of electrons.
- the probability of the electrons to be located at a given region in space and energy levels.

It is an eigenvalues equation, meaning that it will give out infinite solutions.

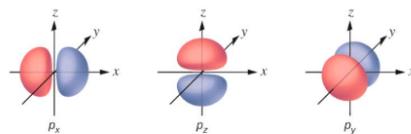
The wavefunction describing the stationary state of an electron in an atom is called an atomic orbital. The atomic orbital is a function that describes the place of a single electron. Each orbital is specified by three quantum numbers which act as the "address" of the electron atom:

1. Primary (or principal) quantum number, n , 1,2,3,...
- (The first quantum number can take integer values and indicate the energy levels of the e- that we are considering, i.e. if an e- has a $n=3$ then it has a higher energy level than an e- with $n=1$)
2. Second (or azimuthal) quantum number, l , 0,1, 2,...,(n-1)
- (The second quantum number describes the density distribution of the e- around the atom (shapes of the orbital), the values that it can assume are bounded to the first quantum number, i.e. if $n=1$ then $l=0$, if $n=2$ then $l=0,1$)
3. Magnetic quantum number, m_l , -l, ..., 0, ..., +1
- (The magnetic quantum number describes the component of the angular momentum along the z-axis, its values are bounded to the secondary quantum number)

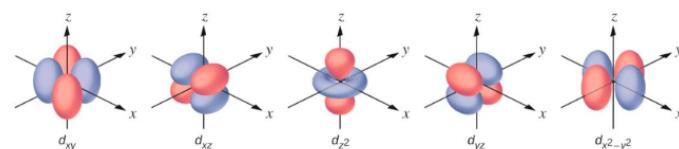
- $L = 0$



- $L = 1$



- $L = 2$



4. Spin quantum number, s , $\frac{1}{2}$

(The fourth quantum number described the intrinsic property of the e- which is the spin)

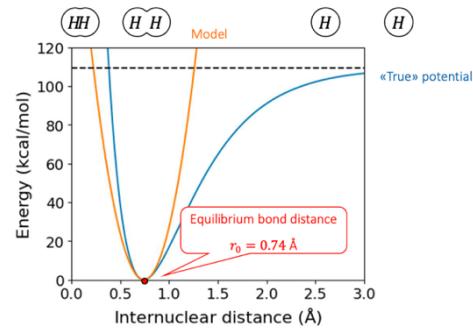
5. Spin magnetic, m_s , -s, +s

(The fifth quantum number describes the component of the spin angular momentum along the z-axis)

We do not deal with isolated atoms, we need to understand how we can model chemical bonds. Most of the time chemical bonds are of a kind called **covalent bonds**. This kind of bond is formed when two atoms share their unpaired e- in the **valence shell**.

How covalent bonds are formed? When two atoms approach one another, the shape of their orbitals changes, in certain conditions we can obtain wavefunctions that are perturbed. The energy levels of these perturbed atomic wavefunctions can drop to energies lower than the sum of the energy of the isolated atoms and for these decreasing, the two atoms are prone to form a stable chemical bond.

In the graph on the right: in blue (true potential) is represented how the energy changes as a function of the distance between the two hydrogen atoms. This plot can be obtained by solving the Schrödinger equation for every possible spatial distance that the two atoms can reach. When we arrive at a distance of 3 Å we reach a plateau, meaning that further than these the two atoms do not interact. But when getting near, the two wavefunctions will perturbate each other, these perturbations will decrease the energy level until a minimum possible level which is called equilibrium bond distance, in this example the **equilibrium bond distance** is 0.74 Å. If we start to push together the two atoms that are now bonded, we observe that the potential energy will rise to infinity, this happens because at a certain point if we try to get the atoms closer the e-clouds of the atoms will try to repulse, until the nuclei of the atoms will arrive to shield themselves.



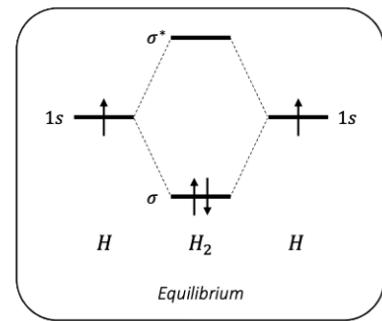
In orange, we can model the wave function as a **harmonic function**, we then are computing an approximation of the true model. In some cases, the approximation can be tolerated. Harmonical models are used to model covalent bonds. The harmonic function to describe a covalent bond is a simple equation which is a parabola:

$$\text{Harmonic potential: } V_{\text{stretch}} = \frac{1}{2} k_{\text{stretch}} (\mathbf{r} - \mathbf{r}_0)^2$$

It is very important which are the variables and parameters of the harmonic model, the variable is \mathbf{r} which is the bond distance, \mathbf{r}_0 is the **equilibrium bond distance**, the third quantity is the k_{stretch} is a farse constant that describes the curvature of the parabola at the minimum, it describes how narrow the parabola is.

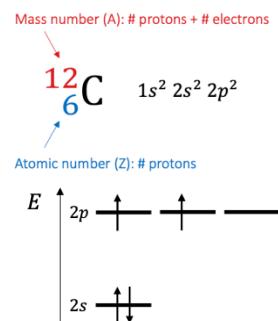
It is also very important which are the variable and parameters of the model, the variable r_0 is the equilibrium bond distance, the second quantity is the k_{stretch} is constant which describes the curvature of the parabola at the minimum it describes how narrow the parabola is.

This is the **electron configuration energy diagram**, in which the e- of the two hydrogen atoms are depicted. We have the e- in the same orbitals, the arrow up indicates the spinning of the e-. When the isolated atoms are approaching, these two orbitals collapse, and they generate two others: one is on a low energy level with respect to the initial orbitals the other is instead on a higher level. The lower level orbital is called **bond orbital** while the higher level orbital is called **anti-bond orbital**. If the antiorbital is being populated, you are not doing the right thing because it has a much higher energy level.

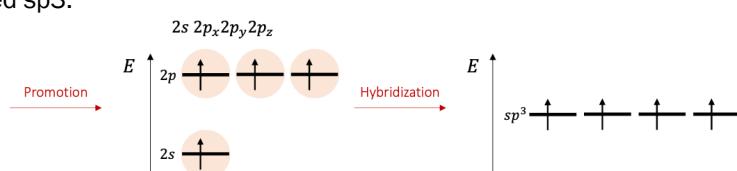


When we are building different atoms, we have also to consider their geometry. The geometry that describes the formation of molecules can be explained in terms of the valence orbitals of the involved atoms.

Ex. Consider a carbon atom (C). There is the symbol, and beside it, there is the electronic configuration, i.e. two e- are in the 1s orbital, the other two in the 2s, etc. Each of them has a higher energy level.



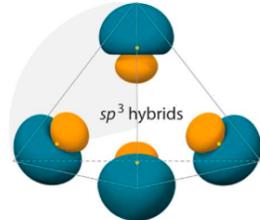
This is the configuration of the valence e- (e- in the highest energy level orbital) which are more prone to change and form bonds. If you look at one of C this is how they are distributed.



These orbitals will have a tetrahedral shape with a new angle bond that in this case will be equal to 109.5° .

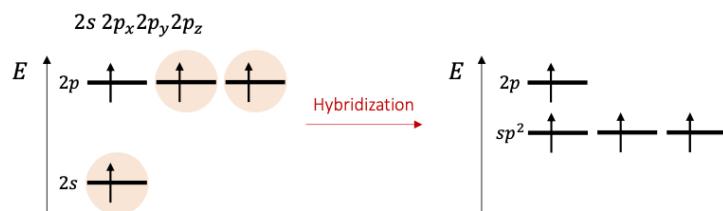
To describe the distance between the orbitals with this new hybridization, we can have another harmonic function which is:

$$V_{\text{bend}} = \frac{1}{2} k_{\text{bend}} (\alpha - \alpha_0)^2$$

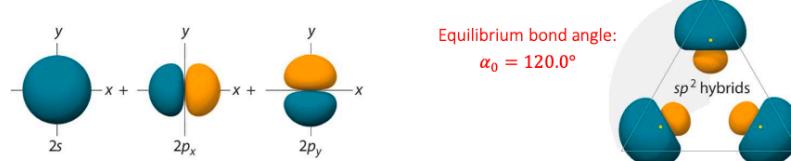


Where, α_0 is the equilibrium bond angle. Whenever we change this angle, we should expect an increase in energy.

Sp3 orbitals are just one of the many possibilities that we can have. Another possible kind of orbital can be to mix just 3 orbitals. If we mix only the yellow orbitals, we obtain sp2 orbitals and one of the kind 2p which has not been touched by the process of hybridization.

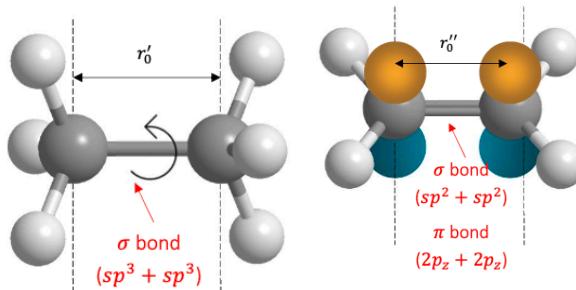


The distance from the central atom here is instead of 120° . The shape resembles more the one of the s orbital. To describe the distance between the orbitals we have the same equation that we saw before for the sp3.



The value of K_{bend} can be obtained by experimental observation or mechanical methods.

The formation of the hybrids is also important from another perspective. We now consider ethane molecules and acetylene molecules.



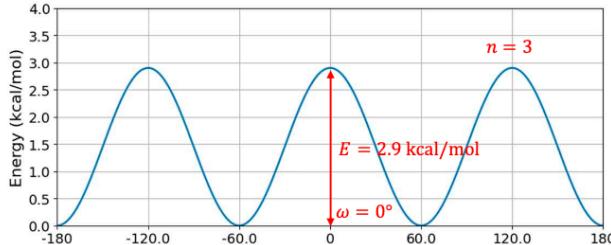
In the case of the first molecule, the orbitals are hybridized as sp3 while for the second they are hybridized as sp3. By comparing the two molecules, we can observe that the acetylene (the second) molecule is shrunk, and the equilibrium distance of the Csp2 and Csp2 chemical bonds is lower than that we find in the methane molecule (the first) Csp3 and Csp3. This is due to the different orbitals' hybridization.

For each C atom, we have a 2p orbital in which there is one e- that is allowed to make an additional bond. This is why acetylene has a double bond.

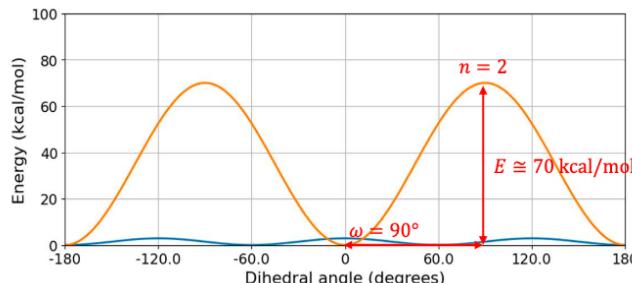
We can now distinguish two kinds of bonds:

- **σ bonds**, formed by two sp^3 orbitals. They are rotationally symmetric meaning that these bonds can be subjected to thermal rotation. The σ bond can rotate around the tetrahedral angle and by that there is a change in the energy.

We can have a function describing the energy levels with respect to the tetrahedral angle of the molecule. We can observe that the highest energy level is found when the atoms are eclipsed and not staggered.



- **π bonds**, formed by two $2p_z$ orbitals. They are not rotationally symmetric, whenever we rotate a tetrahedral angle which has a double bond, the two π bonds are going to break. And because of the breaking of the bonds, the energy required to rotate a portion of the same molecule will be much higher.

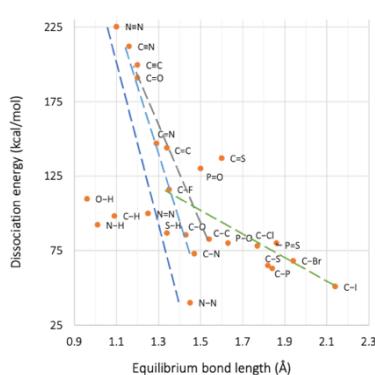


(In blue is plotted the energy of the molecule of ethane)

How can we model the behavior of molecules dependent on the orbitals? In this case, we cannot use a harmonic function, the error of approximating would be too high. We use a **cosine or sine function**.

$$V_{\text{tors}} = \frac{E}{2} [1 + \cos(n\theta - \omega)]$$

Here the variable is θ whose value corresponds to the tetrahedral angle. The first parameter is the **energy barrier** which in the case of ethane is 2.9 kcal/mol. In the case of ethane, its energy barrier can be overcome efficiently by thermal energy. In the case of the acetylene molecule, we have an energy barrier of about 70 kcal/mol, these cannot be overcome by simply thermal energy, if we want to rotate the bond, we have to break the π bonds and insert energy. The n is the **multiplicity** that corresponds to the number of the H atoms. The third is the so-called **phase angle ω** .



In the graph on the right, we see the relationship between bond length and bond strength in common bonds.

As we said if we increase the number of bonds, the **equation bond length decreases**, and the **dissociation energy increases**.

In **heteronuclear diatomic molecules**, the electron distribution in the covalent bond is not symmetrical between the atoms, this results in a **polar bond**.

To describe this typology of chemical bonds we need to describe first the concept of **electron negativity**. The electronegativity, χ , of an element is the ability of its atoms to draw electrons to itself when it is part of a compound. Electronegativity scales for individual elements:

- Based on heteronuclear bond dissociation energies, $E(A-B)$ (Pauling):

$$\Delta E = E(A - B) - \frac{1}{2} [E(A - A) + E(B - B)]$$

$$\text{Relative scale } |\chi_A - \chi_B| = 0.102 \times \Delta E (\text{kJ/mol})$$

C	N	O	F
2.5	3.0	3.5	4.0
P	S	Cl	
2.1	2.5	3.0	
	Br		2.8
	I		2.5

The energy that we must give in order to dissociate two atoms. (A and B are the pure covalent energies of two different species, A and B can be the same). Using the concept of ΔE Pauling derived the **relative scale**.

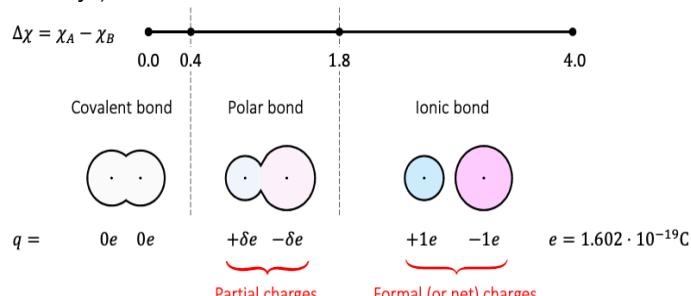
The fluorine is the most electron negativity element, and its electron negativity is equal to 4.

- Based on the **ionization energy**, E_{i1} , and the **electron affinity**, E_{ea} , of an atom (Mulliken): We only have an absolute scale because the values that we consider are specific for the elements in consideration.

$$\chi = \frac{1}{2} (E_{i1} + E_{ea})$$

- High E_{i1} means that the atom will not release electrons readily.
- High E_{ea} means that the atom is energetically favorable to acquire electrons (by convention, for exothermic reactions $E_{ea} > 0$).

It is possible to relate the electronegativity difference $\Delta\chi$ between atoms in a bond and the percent of ionic character of the bond ("ionicity").



The differences $\Delta\chi$ dictate what kind of bond we have. After 1.8 the electron negativity is so high that one atom will directly acquire the electrons of another.

This reflects on another property of the molecules which is related to the **partial charges** of the atoms. In covalent bonds, the e- are evenly shared by the two atoms, there will be no preference for the valence of the electrons. The difference in partial charge is 0. In a polar bond instead, there is a partial density. E is the elementary charge of the e- which is equal to 1.602×10^{-19} C. In the case of the ionic bond, we will have a +1 or -1 charge, we have **formal (net) charges**. In the case of a polar bond, these are **partial charges** of the atom involved.

Ionicity based on Pauling's empirical relationship:

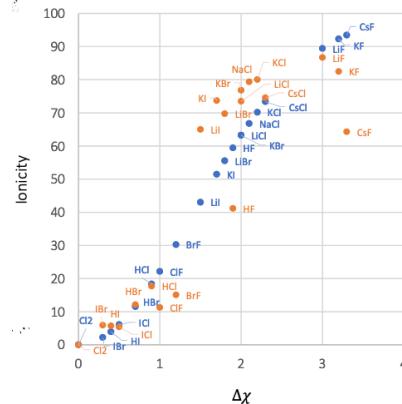
$$\text{I\%} = \left[1 - e^{-\frac{1}{4}\Delta\chi^2} \right] \cdot 100$$

Ionicity based on the knowledge of the experimental bond electric dipole moment:

$$\text{I\%} = \frac{\mu_{\text{obs}}}{\mu_{\text{calc}}} \cdot 100$$

- μ_{obs} : measured dipole moment.
- μ_{obs} : dipole moment calculated assuming a pure ionic character and the actual bond length d.

Which can derive two plots of the kind:



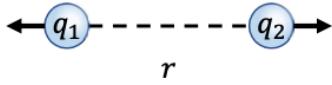
In a polar bond, there is the formation of the so-called **dipole**. The dipole is a vector that has its own direction (from the less negative element to the most negative one) and it has its own magnitude.

$$\text{Bond dipole moment } (\text{C} \cdot \text{m}) \quad \leftarrow \quad \mu = q \cdot d$$

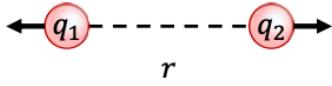
Ionicity can also be expressed on the knowledge of **experimental bond electric dipole moment**.

Ionic bonds are formed when there is a total transfer of an electron from the valency orbital of one atom to the other. This causes a full separation of charges with the generation of ions (and loss of the molecular identity). In contrast to the covalent bond, the ionic bond can be considered simply from a classical electrostatics point of view. In this case, whenever we are talking about classical electrostatic we should focus on **Coulomb's law** (1785), which defines the force that two points, carrying electric charge q_1 and q_2 repel or attract each other at a distance r :

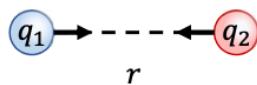
(for convention positive charges are depicted in blue, while negative one are depicted in red)



$$\text{Scalar form} \quad f_{1,2} \propto \frac{q_1 \cdot q_2}{r^2} \quad f_{2,1} \propto \frac{q_1 \cdot q_2}{r^2} \quad f_{1,2} = f_{2,1}$$



The third Newton's law implies that the two forms are true. This is the easiest way to look at the forces, but recall that they are actually vectors, this is why we show them with arrows, so they can be represented also as vector:



$$\text{Vector form} \quad \mathbf{f}_{1,2} \propto \frac{q_1 \cdot q_2}{r^2} \hat{\mathbf{r}}_{1,2} \quad \mathbf{f}_{2,1} \propto \frac{q_1 \cdot q_2}{r^2} \hat{\mathbf{r}}_{2,1} \quad \mathbf{f}_{1,2} = -\mathbf{f}_{2,1}$$

The concept of proportionality is derived by the coulomb's constant.

$$k = \frac{1}{4\pi\epsilon_0\epsilon}$$

When we were talking about the elementary charge of electrons, we used Coulomb to give a dimension to it. ϵ_0 is the permittivity of vacuum:

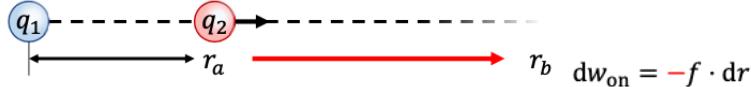
$$\epsilon_0 = 8.854 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$$

Dielectric constant:

- Vacuum, $\epsilon = 1$
- Non polar medium, $\epsilon = 2 - 4$
- Polar medium (water), $\epsilon = 78.54$

When we are considering the electrostatic forces in the vacuum this is the only information that we need, but when the charges are placed in a medium that is different from the vacuum the ϵ changes (polarizability). In a vacuum, we can set the ϵ to 1. In a polar medium, the epsilon is 80 times weaker than the one in the vacuum.

Ionic bonds are quantified by the so-called **bonding energy** which is the work required to move an ion from its equilibrium place to an infinite distance from the counter ion. We will store the energy in the ionic bond. To define the bonding energy we first have to talk about a **work differential** which can be calculated as the product of the force and a corresponding distance differential:



dw_{on} is the differential work made on the system. dr is the positive increasing separation. The presence of the $-$ sign is a convention.

The sign of the work differential depends on the point of view of consideration, and this is a matter of definition. If we consider charges of the opposite sign, $f < 0$ for every distance, and:

- If charges are **moved towards** each other, $dr < 0$, then: $dw_{on} < 0$
- If charges are **moved away** from each other, $dr > 0$, then: $dw_{on} > 0$

The work required to move the charges from r_a to r_b is:

$$w_{on} = \int_{r_a}^{r_b} dw_{on} = \int_{r_a}^{r_b} -\frac{q_1 \cdot q_2}{4\pi\epsilon_0 r^2} \cdot dr = -\frac{q_1 \cdot q_2}{4\pi\epsilon_0} \left[-\frac{1}{r} \right]_{r_a}^{r_b} = \frac{q_1 \cdot q_2}{4\pi\epsilon_0} \left(\frac{1}{r_b} - \frac{1}{r_a} \right) \xrightarrow{r_b = \infty} w_{on} = -\frac{q_1 \cdot q_2}{4\pi\epsilon_0 r_a}$$

We integrate the work differential from the first position to the second position. If we set r_b to infinity, what we obtain is the final definition of the work which is the bonding energy.

Ex. Compute the bonding energy of two atoms, one of Na, and the second of Cl.

- $R_{Na}=0.98 \text{ \AA}$
- $R_{Cl}=1.81 \text{ \AA}$

```
# Calculate the bonding energy between two atoms: the first is Sodium (Na): 0.98\AA, Chlorine (Cl): 1.81\AA
import numpy as np
e=1.602e-19 #C
e0=8.854e-12 #C^2 N^-1 m^-2
# need to change the \AA to m, 1 \AA = 0.1 nm
rNa=0.098e-9 #m
rCl=0.181e-9 #m
NA=6.022e23 #mol^-1

W=-(e**e)/(4*np.pi*e0*(rNa+rCl))
print(W)

print(W*NA/1000) # force in Kjoule/mol
print(W*NA/1000/4.184) # this is the force in Kcal/mol
```

Result: 8.267447363377319e-19 (Nm) → Joule

Let's do the dimensional analysis of this :

$$\frac{[C][N][m]^2}{[C]^2[m]}$$

it becomes Nm

In the case of an ionic bond, we are estimating an energy that is 120 Kcal/mol

8.267447363377319e-19
497.8656802225821
118.9927533992787

The electrostatic energy of two point charges equals the work required to move the charges from an infinite distance to the distance r :

$$w_{\text{on}} = -\Delta V$$

$$V(r) = \frac{q_1 \cdot q_2}{4\pi\epsilon_0 r}$$

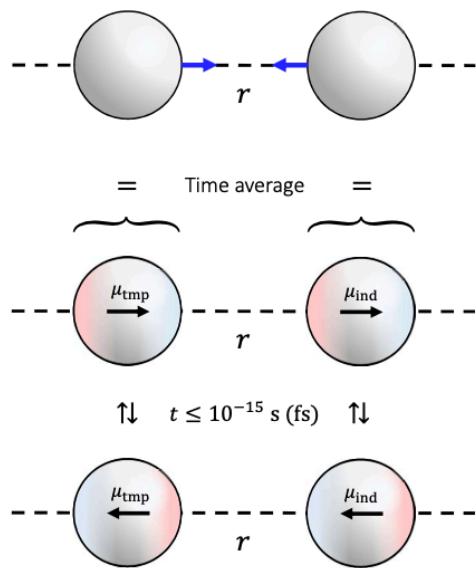
Coulomb potential

The electrostatic nature of ionic bonds indicates that, in contrast to covalent bonds, no bond angle is predicted (radial dependence on r).

- Decreases with the increase of the distance.

Differently from the covalent bond, the electrostatic interaction is not directional.

Van der Waals interactions are universal forces between individual atoms, whole molecules, or supramolecular particles which are not simply based on electrostatic interactions. They are based on electromagnetic interactions, occurring by the fluctuation of charges.



Among this van der Waals interaction, we have London's **forces or dispersion forces**, which are quantum mechanical in nature as they involve interactions between rapidly fluctuating dipoles resulting from the movement of the outer-valence-shell electrons of an atom or molecule.

The energy and attractive force between units become more negative as the separation distance decreases until the electron clouds of the respective units begin to interact.

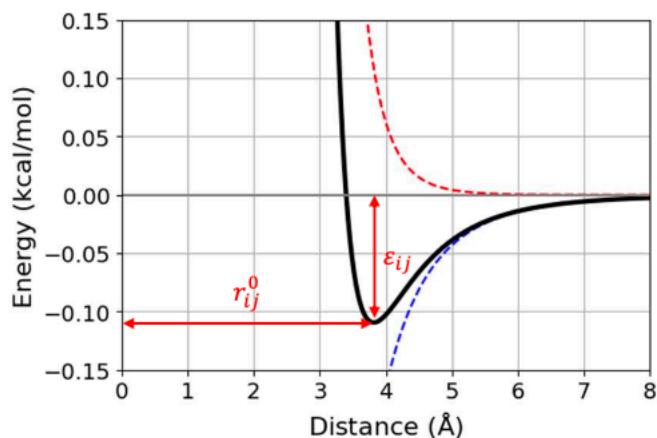
Instantaneously we can have the formation of a temporary dipole due to the fluctuation of charges around the nucleus, this dipole changes its direction. There would be an attractive force on the two atoms. These are the forces beyond the fact that noble gases can be found in a liquid state.

At a certain point, the two molecules will not collapse in a single point. How can we model the interactions, we use the so-called **12-6 Lennard-Jones potential model**: the important thing to remember is that.

$$V_{\text{LJ}}(r_{ij}) = \epsilon_{ij} \left[\left(\frac{r_{ij}^0}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{ij}^0}{r_{ij}} \right)^6 \right]$$

Repulsive term Attractive term

Parameters $\begin{cases} r_{ij}^0: \text{equilibrium distance} \\ \epsilon_{ij}: \text{well depth} \end{cases}$



The fact that the repulsive term increases by 12 times the distance means that it increases much faster than the attractive term. At the same time, the attractive term goes to 0 very much faster than the electrostatic interaction.

The shape of the potential should remind us of a chemical bond but remember: the energy is hundreds of kcal in this case -0.15 kcal, very weak interactions.

We should not underestimate this kind of interaction, they occur among every atom regardless of their charge. Electrostatic interaction instead occurs only when there is a charge.

When looking at the equation we have two parameters to quantify the Van der Waals interactions.

The force field in molecular mechanics:

The most general expression of a conventional force field is the following:

$$V(l, \alpha, \theta, r) = \left[\sum_{\text{bonds}} V_{\text{stretch}}(l) + \sum_{\text{angles}} V_{\text{bend}}(\alpha) + \sum_{\text{dihedrals}} V_{\text{torsion}}(\theta) \right] + \left[\sum_{\text{pair}} (V_{\text{ele}}(r) + V_{\text{vdw}}(r)) \right]$$

Sum over all bonds Sum over all bond angles Sum over all dihedral angles Sum over all pairs
 bonds angles dihedrals pair
 ↓ ↓ ↓ ↓
 Potential energy of the molecule Stretching energy of a chemical bond Bending energy of a bond angle Torsional energy of a dihedral angle Electrostatic energy between atoms Van der Waals energy between atoms
 ↓ ↓ ↓ ↓ ↓
 ● "bonded" terms ● "non-bonded" terms

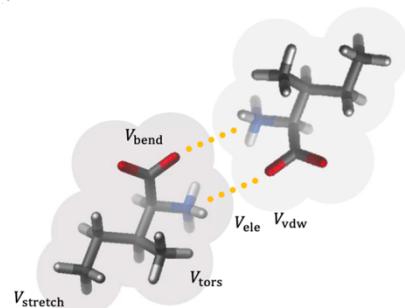
We see that the potential energy is expressed as a sum of simpler terms, each of which is describing a specific contribution with a clear physical interpretation (**principle of additivity**).

Each of these terms involves:

- **independent variables** (l, α, θ, r)
- **parameters**: empirical coefficients that quantify the relationship between the energy and the value assumed by the independent variables.

During force field development, these parameters are derived for reproducing experimental observables of small molecules or properties computed at the QM level of theory.

The parameters are then used for describing more complex systems (**principle of transferability**).



Energy and equilibrium:

There are four main forms of energy relevant to biological systems:

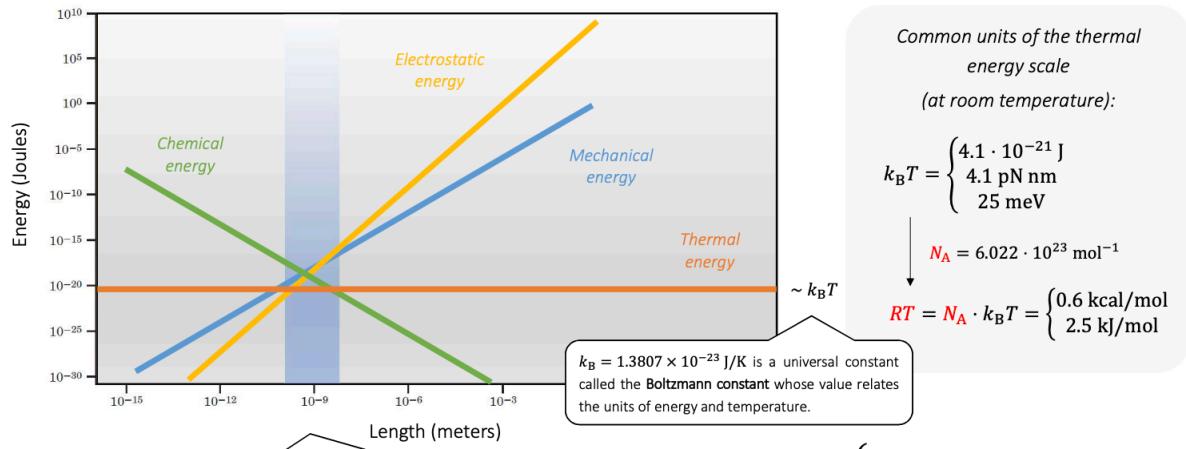
- **Mechanical energy**
- **Chemical energy**
- **Electromagnetic energy**
- **Thermal energy**

Mechanical, chemical, and electromagnetic energy are due to deterministic forces which imply the entity of causality, on the other hand, thermal energy is caused by stochastic forces which are caused by causality or randomness.

Energy can be converted. There are two modalities for transferring energy:

- **Heat** is the spontaneous flow of energy from one object to another caused by a different temperature between them.
- **Work** any other transfer of energy into or out of the system.

These terms work in a macroscopic word.



This plot represents the different kinds of energy scaled as a function of the size of the system that we are considering. This plot allows us to understand what is happening at a microscopic and molecular level.

For example, the higher the size of the system you want to bend, the higher would be the mechanical energy required. The same goes for the electrostatic energy, the larger the size at which electrostatic interaction the higher the length.

The chemical energy instead is important on a very short scale, if you stretch a bond at some point the chemical energy will decrease.

The thermal energy instead, is **scale-invariant**, the entity of thermal energy is proximate to the order of $k_B T$. k_B is a universal constant, the **Boltzmann constant**. This value links the energy and the temperature. Depending on the unit of measure of the Boltzmann constant it can have many different values. Whenever we multiply the k_B for the Avogadro's constant what will be obtain is RT , where R is the universal gas constant and T is the temperature.

What is important to understand from the graph is that there is a specific land scale at which every of the four energy has the same size. The size corresponds to a nm scale. It is very easy for biological machinery to convert one energy into another and to use thermal energy which is useful for the cell.

Several processes taking place in biological systems can be described in the form of a **minimization problem**. In minimization problems, we seek the least value of some energy function with respect to some independent variables. Reaching the minimum of such energy function is equivalent to reaching an **equilibrium condition**. We can have two main types of equilibrium:

- **Mechanical equilibrium** is reached when there are no unbalanced forces. The equilibrium condition is represented by the **Newton's first law of motion**. (useful when we want to model the static property of a molecule.)

$$\sum_i \mathbf{f}_i = 0 \quad \left\{ \begin{array}{l} \mathbf{f} = f_x \hat{\mathbf{i}} + f_y \hat{\mathbf{j}} + f_z \hat{\mathbf{k}} \\ f = \sqrt{f_x^2 + f_y^2 + f_z^2} \end{array} \right.$$

Forces are vectors • Unit vectors

- **Thermodynamical equilibrium** is reached when the average properties of a macroscopic system ($p, V, T, U, H, A, G, S, \dots$) do not change over time. The following conditions must be satisfied:
 1. Mechanical equilibrium.
 2. Thermal equilibrium.
 3. Chemical equilibrium.

Chemical equilibrium is a **dynamic equilibrium** meaning that the chemical individuals (the molecules) might change over time, but their concentrations do not. Thus, the equilibrium is reached when the concentration of reactants and products won't change over time.

The problem of reaching mechanical equilibrium can be formulated in terms of the minimization of a suitable energy function. The energy function is the **potential energy**, and the functional relationship between forces and potential energy is:

$$f(x) = - \frac{dV(x)}{dx}$$

For example, having a spring, the velocity of a spring is determined by an equation:

Hooke's law $V(x) = \frac{1}{2} k(x - x_0)^2$

Not every processes in biology can be modeled using harmonic potential energy functions. Still, locally, the harmonic function can be a reasonable approximation. If we model the system as a harmonic function we can lose something. . We can approximate any function using the **Taylor's series expansion**:

$$V(x) = V(x_0 + \Delta x) \approx V(x_0) + \underbrace{\left(\frac{dV}{dx}\right)_0}_{:= 0} \Delta x + \frac{1}{2} \underbrace{\left(\frac{d^2V}{dx^2}\right)_0}_{= 0} \Delta x^2 + \dots$$

$$V(x) = V(x_0 + \Delta x) \approx \frac{1}{2} \left(\frac{d^2V}{dx^2}\right)_0 \Delta x^2$$

Equilibrium demands that $\left(\frac{dV}{dx}\right)_0 = 0$, since at the equilibrium point there are no unbalanced forces

Force constant or stiffness of the «spring»
 $k = \left(\frac{d^2V}{dx^2}\right)_0$

The Taylor's expansion around the minimum can be approximated to the second equation.

In most general cases we have a multidimensional problem because there are several independent variables.

Consider a function y which depends upon N variables $\{x_i\}$:

$$y(x_1, x_2, \dots, x_N) = y(\{x_i\})$$

The notation $\{\dots\}$ indicates a set of objects

What choice of values $\{x_i\}$ renders the function $y(\{x_i\})$ a minimum or a maximum?

In this case, like the nondimensional one, we have to take a look at the partial derivative of the function with respect to each variable of the function.

$$\frac{\partial y}{\partial x_i} = 0 \quad (i = 1, 2, \dots, N)$$

The partial derivative tells us how a function changes when we make a small deviation in one of the variables in the function while leaving others constant.

$$\frac{\partial y(x_1, x_2)}{\partial x_1} = \lim_{\Delta x_1 \rightarrow 0} \frac{y(x_1 + \Delta x_1, x_2) - y(x_1, x_2)}{\Delta x_1};$$

$$\frac{\partial y(x_1, x_2)}{\partial x_2} = \lim_{\Delta x_2 \rightarrow 0} \frac{y(x_1, x_2 + \Delta x_2) - y(x_1, x_2)}{\Delta x_2}$$

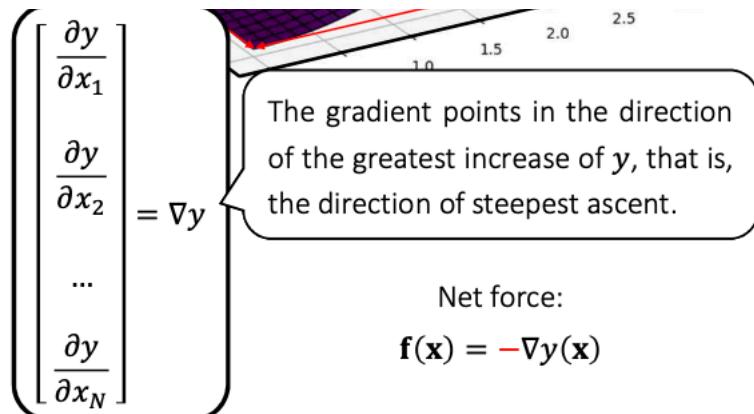
Partial derivatives can be arranged in a vector, called the gradient of a function, ∇ :

Each element of the gradient tells us the partial derivative of the function with respect to the different independent variables that the system might have.

Like in the case of the nondimensional problem, we were looking at the negative of the potential energy to find the restoring force, we now do the same but with every partial derivative. What we obtain is the **net force**.

We know that for a very simple function, we can also use mathematical analysis tools to find out the position of the minimum, but in general it is not possible

to use these because molecules, are highly dimensional so it is very difficult to get the minimum for the system using simple analytical tools.



The complexity of the potential energy is due to the fact the molecules are described by many atoms and each of them has an impact on the total potential energy of the system.

We need to have a numerical tool that is able to get us as close as possible to the minimum with respect to the initial conformation.

The simplest minimization method is the so-called **steepest descent method**. It is a numerical method, which means that the method works iteratively. The method moves on the potential energy surface iteratively, and, for each step, takes the direction parallel to the net force.

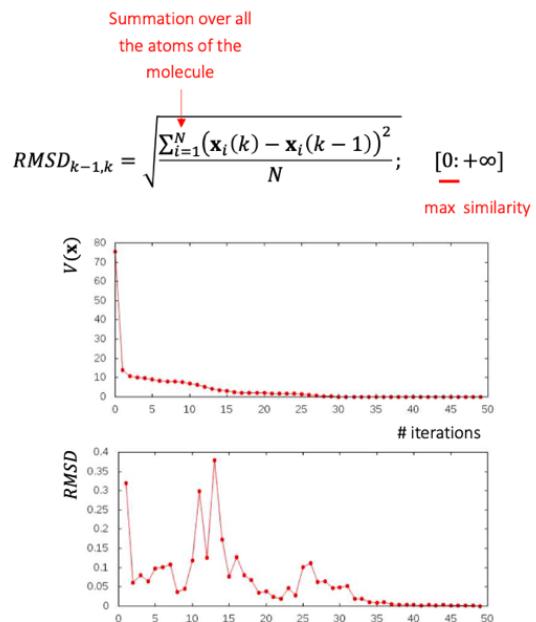
$$\mathbf{x}_{k+1} = \mathbf{x}_k + \lambda_k \frac{\mathbf{f}_k}{\|\mathbf{f}_k\|}$$

● direction
● step size

The energy minimization algorithm may stop when (**convergence criteria**):

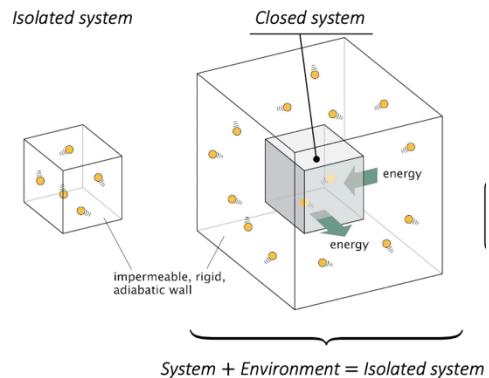
1. The maximum number of iterations defined by the user has been reached
2. The change in energy (or forces) between subsequent iterations is smaller than a user-defined threshold
3. The change in conformation between subsequent iterations is smaller than a user-defined threshold

There is another way to specify that we reached the minimum. Monitoring the change of conformation of the molecule, if we are close to the minimum, means that the conformation should not move much. The change in conformation can be measured with a formula called **RMSD (root mean square dimension)**. The limiting values that RMSD contains are 0, up to infinity.



Thermodynamical potentials:

For our purposes, it is very important the second law of thermodynamics, which states that in **isolated systems** the value of the state variable **entropy** always increases in every spontaneous process (when reached the equilibrium in an isolated system the entropy is at its maximum). However, the entropy of a non-isolated system may decrease at the expense of the environment.



In a closed system, the temperature is constant, while in an isolated system, the energy is constant.

In closed systems and opened systems, we can identify an “energy function”, the **free energy**, that reaches a minimum at equilibrium. Since the equilibrium condition is reached whenever we reach the minimum, the free energy is therefore called a **thermodynamic potential**. The free energy is divided in two components:

$$F = \langle E \rangle - TS$$

- **$\langle E \rangle$** , which is the energy that can be exchanged with the environment and that can be used to perform work.
- **TS** , which is the product of the temperature (T) and the entropy of the system (S)

Entropy:

Entropy is a measure of the microscopic degeneracy of a macroscopic state.

We need to define many different concepts, to do so we use as an example the roll of a pair of dice, we define them:

- **Microstate:** an individual outcome of the roll, e.g.(4,1)
- **Microstate:** the resulting total of the roll, e.g. "5"
- **Multiplicity, Ω :** number of microstates corresponding to a given microstate, e.g. $\Omega(5) = 4$,
 $\Omega_{\text{tot}} = 36$

If we know the microstate, we also know its macrostate, but the reverse is not true, because we can have several ways to obtain the same microstate. The probability of a given microstate is: $p(x) = \frac{\Omega(x)}{\Omega_{\text{tot}}}$.

The assumption of this example is that the dice are fair, so each individual outcome is equally probable.

There can be a relation between the multiplicity and the entropy, this relationship is defined by an equation, and in our case it takes the name of **Boltzmann entropy**:

$$S = k_B \ln \Omega(E)$$

Entropy of a macrostate
(thermodynamic concept)

Multiplicity: total number of
microstates with energy E

(k_B is the Boltzman constant that we already have seen.)

This is the microscopic definition of entropy: if we know the number of microstates, we can calculate the total entropy.

We are assuming that all microstates have the same energy, this is called in mechanics **equal a-priori probability**, and therefore the same probability of being visited. Since we are talking about probabilities, we can introduce a normalization condition:

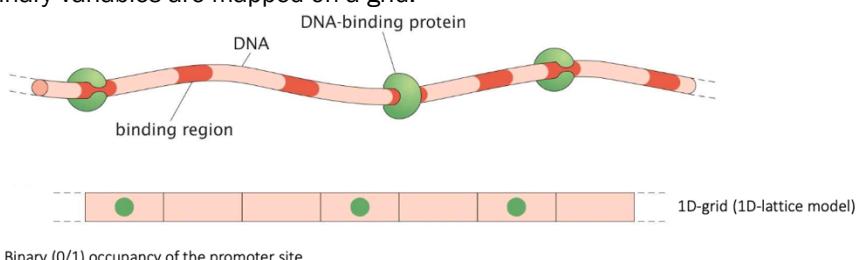
$$\sum_i^{\Omega(E)} p_i = 1 \rightarrow p_i = \frac{1}{\Omega(E)}$$

An isolated system will spontaneously evolve towards the most likely equilibrium state, i.e. the microstate with the highest multiplicity Ω :

$$S_{\text{eq}} = \max_{\Omega}(S)$$

Entropy is the driving force of several cellular processes (molecular recognition, hydrophobic effect, osmosis, etc.)

The first example that we can exploit in order to better understand the concept of entropy is the **lattice model of DNA-protein binding**. First, what is a lattice model? A **lattice model** is a kind of discrete-states model where binary variables are mapped on a grid.



We want to understand the role of entropy in the context of DNA-binding proteins. Entropy, in this case, is related to the number of distinct ways of arranging the bound proteins (nonspecifically) along the entire DNA molecule.

We imagine our DNA molecule has M binding sites, N of which are occupied by the protein. We assume that the binding energies for the promoter sites are the same.

$$S = k_B \ln \Omega(N; M)$$

Multiplicity: number of ways of rearranging N proteins on the M binding sites.

The entropy is proportional to the log of the number of microstates which itself is a function of the M binding site domain and the N proteins.

How can we arrange N objects into M bins?

- For the first of the N proteins we have M options
 - For the second of the N proteins we have $M - 1$ options
 - ...
 - For the last of the N proteins we have $M - (N - 1)$ options
- The total number of ways is: $\Omega(N; M) = M \cdot (M - 1) \cdot (M - 2) \cdot \dots \cdot (M - N + 1)$

$$\begin{aligned} M \cdot (M - 1) \cdot (M - 2) \cdot \dots \cdot (M - N + 1) \cdot (M - N) \cdot (M - N - 1) \cdot (M - N - 2) \cdot \dots \cdot 1 &= M! \\ \Downarrow & \\ \Omega(N; M) &= \frac{M!}{(M - N)!} \end{aligned}$$

We have ignored the fact that these are not distinct configurations, as the protein binding on a particular site is indistinguishable. To correct for this overcounting we must divide by the number of rearrangements of those N proteins on the occupied sites, which is, following the same argument as above, $N \cdot N-1 \cdot N-2 \cdot \dots \cdot 1 = N!$ The multiplicity corresponds to the binomial coefficient:

$$\Omega(N; M) = \frac{M!}{N! (M - N)!}$$

So we can write the entropy as:

$$S = k_B \ln \Omega(N; M) = k_B \ln \left(\frac{M!}{N! (M - N)!} \right)$$

If $M \gg N$, as it is usually the case, we can invoke the **Stirling's approximation**:

$$\ln N! \approx N \ln N - N$$

This approximation works only on the scale of the large number, which is important because we can get rid of a factorial. We now have a better look at the Stirling's approximation.

- The Stirling's approximation:

$$\begin{aligned} \ln AB &= \ln A + \ln B \\ \ln N! &= \ln[N \cdot (N - 1) \cdot (N - 2) \cdot \dots \cdot 1] \quad \Rightarrow \quad \ln N! = \sum_{n=1}^N \ln n \end{aligned}$$

- Replacing the sum with the appropriate integral:

$$\ln N! = \sum_{n=1}^N \ln n \cong \int_1^N \ln x \, dx = N \ln N - N - (1 \ln 1 - 1) \cong N \ln N - N$$

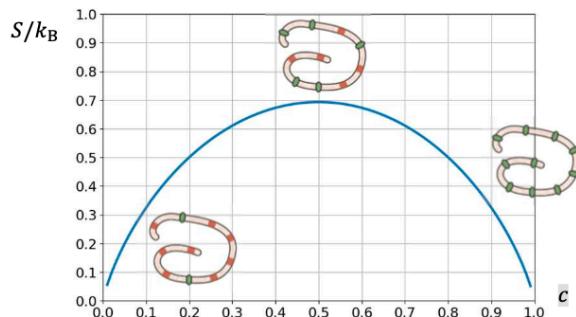
- A more refined derivation of the Stirling's approximation gives:

$$\ln N! \approx N \ln N - N + \ln \sqrt{2\pi N}$$

Invoking the Stirling approximation and by introducing a more convenient concentration variable $C = \frac{N}{M}$ and performing a tedious path of algebraic steps we can obtain a new relationship for entropy:

$$S \approx -M k_B [c \ln c + (1 - c) \ln(1 - c)]$$

The number of different ways of arranging the two species depends upon their relative numbers. The entropy is maximal when half of the sites are occupied. This situation reflects that this occupation permits the largest number of distinct arrangements. These can be plotted:

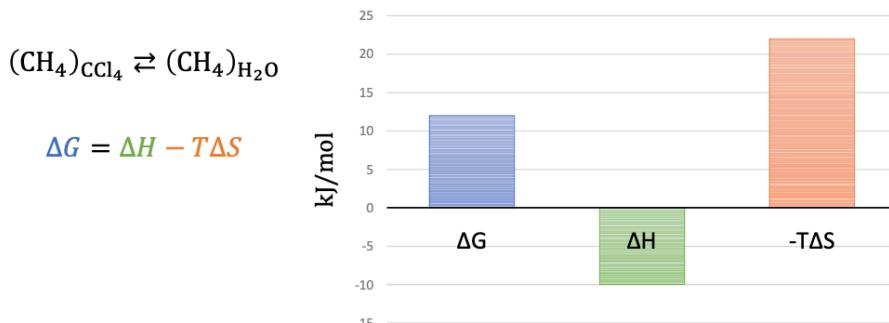


The entropy is plotted related to the concentration. Entropy is very low when we have very few sites that are occupied, and when all the available sites are occupied. The maximum value of the entropy is the situation where half of the sites are occupied, and the others are empty.

To gain a little more practice in the use of the entropy idea, we consider a toy model of one of the most important molecular driving forces in biological systems, namely, the **hydrophobic effect**.

First, we need to understand why hydrocarbon molecules do not dissolve appreciably in water ($\Delta G (\Delta F) > 0$).

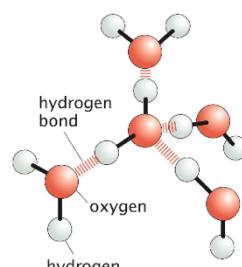
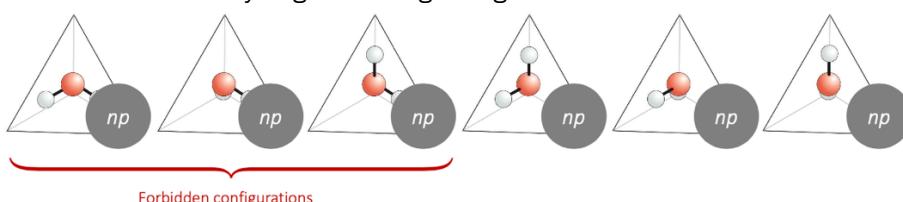
Experiments indicate that the transfer of a hydrocarbon molecule from a non-polar solvent to water is often exothermic ($\Delta H < 0$). Therefore, the fact that dissolving is not a spontaneous process, must mean that the entropy change is negative ($\Delta S < 0$).



The origin of the decrease in entropy that prevents hydrocarbons from dissolving in water is the formation of a polyhedral solvent cage around the molecule called a **clathrate**. The formation of this cage decreases the entropy of the system because the water molecules lose part of their configurational freedom (decrease of multiplicity).

A given water molecule can be idealized as having neighbors arranged in a tetrahedral structure. Water molecules form a dynamic network of hydrogen bonds, where each oxygen, on average, makes hydrogen bonds with 2-3 out of the 4 water molecules surrounding it.

We can discretize the continuum of possible orientations available to a water molecule to 6 distinct hydrogen-bonding configurations.



When we add a non-polar solute to the water solution, the non-polar molecule will replace one of the water molecules that we can find in the vertexes. Whenever we do that, we end up in a situation where we have three of the possible configurations become forbidden.

We end up with just 3 microstates, the change in entropy that we have can be expressed by taking the difference of the entropy of the system when we have a non-polar molecule is added to water minus the entropy in which we only have the water molecules. The change of entropy, in this case, is equal to:
The entropic contribution to the free energy cost to place a non-polar molecule in water is:

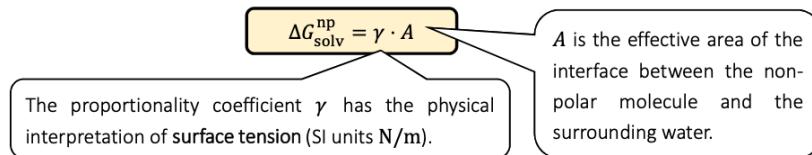
$$\left. \begin{array}{l} \text{The entropy of a single water molecule is: } S_{\text{wat}} = k_B \ln(\Omega_{\text{wat}}) = k_B \ln(6) \\ \text{When a non-polar molecule is added: } S_{\text{wat+np}} = k_B \ln(\Omega_{\text{wat+np}}) = k_B \ln(3) \end{array} \right\} \quad \Delta S_{\text{hydrophobic}} = S_{\text{wat+np}} - S_{\text{wa}} = k_B \ln \left(\frac{\Omega_{\text{wat+}}}{\Omega_{\text{wat}}} \right) = k_B \ln \frac{3}{6} = -k_B \ln 2$$

$$\Delta G_{\text{hydrophobic}}(n) = -T \Delta S_{\text{hydrophobic}}(n) = n \cdot k_B T \ln 2$$

Where n is the number of water molecules adjacent the nonpolar molecule

One particularly useful way of characterizing such result is to say that the presence of nonpolar molecules incurs some **free-energy cost per unit area**, $\gamma_{\text{hydrophobic}}$.

Therefore, the free energy cost to embed a given non-polar molecule in water (non-polar contribution to the solvation-free energy) is:



We can combine the two results that we have obtained:

$$\Delta G_{\text{hydrophobic}}(n) = n \cdot k_B T \ln 2 \Rightarrow n \cdot k_B T \ln 2 = \gamma \cdot A \Rightarrow \gamma = \frac{n \cdot k_B T \ln 2}{A}$$

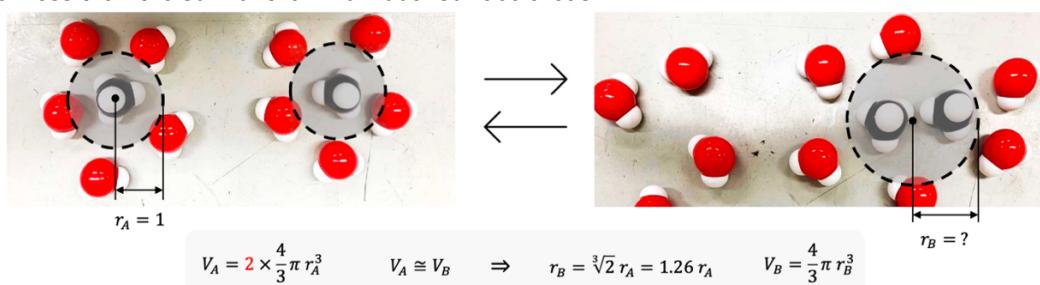
Using the simple estimate that **10 water molecules** cover an area of approximately 1 nm^2 , we can estimate the free energy cost per unit area:

$$\gamma = \frac{n \cdot k_B T \ln 2}{A} \approx \frac{10 \cdot k_B T \cdot 0.7}{1 \text{ nm}^2} \approx 7 k_B T / \text{nm}^2 \quad \left\{ \begin{array}{ll} \bullet \text{ «Small» non-polar molecule like O}_2: & A = 0.1 - 0.2 \text{ nm}^2 \Rightarrow \Delta G_{\text{solv}}^{\text{np}} \approx 1 k_B T & \checkmark \\ \bullet \text{ «Large» non-polar molecule like n-octane:} & A = 2.0 - 2.5 \text{ nm}^2 \Rightarrow \Delta G_{\text{solv}}^{\text{np}} \approx 15 k_B T & \times \end{array} \right.$$

Each addition of a new molecule of octane to water costs the same amount of free energy additively.

Hydrophobic effect:

The free energy penalty of dissolving a non-polar molecule in water is approximately proportional to the non-polar surface area. However, when non-polar molecules cluster together, the total surface area of the cluster is much less than the sum of their individual surface areas.



$$\begin{aligned} \Delta G_{\text{solv},A}^{\text{np}} &= \gamma \cdot A_A = \gamma \cdot 2 \times (4\pi r_A^2) = \\ &\cong \gamma \cdot 25 r_A^2 \quad \xrightarrow[r_A := 1]{} \Delta G_{\text{solv},A}^{\text{np}} \approx \gamma \cdot 25 \end{aligned} \quad \begin{aligned} \Delta G_{\text{solv},B}^{\text{np}} &= \gamma \cdot A_B = \gamma \cdot (4\pi r_B^2) = \\ &\cong \gamma \cdot 12.5 r_B^2 \quad \xrightarrow[r_b := 1.26]{} \Delta G_{\text{solv},B}^{\text{np}} \approx \gamma \cdot 20 \end{aligned}$$

Non-polar molecules stick together in a polar medium to minimize their water-exposed non-polar surface area. The net effect of the formation of large clusters of hydrophobic molecules is then a decrease in the organization of the solvent and therefore a net increase in the entropy of the system (**hydrophobic effect**).

The Boltzmann distribution:

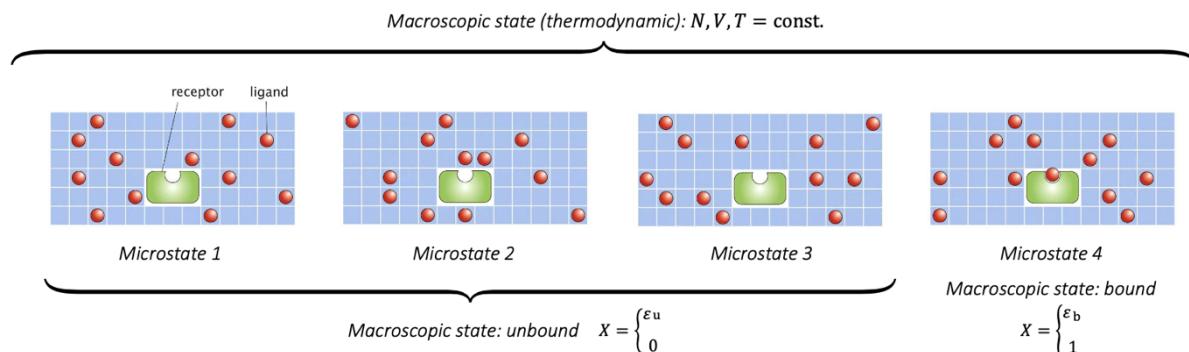
Statistical mechanics is a theoretical framework for a quantitative description of thermodynamic processes at the molecular level (at different scales). Thermodynamic state functions are interpreted through the concept of microstates and macrostates. From a thermodynamic standpoint, a **microstate** or **thermodynamic state**, is specified by a certain number of macroscopic variables (or **state variables**) such as pressure, temperature, volume, etc. more in general, it is a collection of Ω number of microstates. A microstate instead, is described by microscopic variables, such as particle positions, particle velocities, spin orientations, etc. depending on the degrees of freedom of the system.

By definition, **multiple microstates can correspond to the same microstate**.

Microstates compatible with a given microstate are different possible ways that the system can achieve that microstate. Statistical mechanics allows calculating the probability of observing each microstate under a set of conditions representing the definition of the microstate under investigation.

We are often interested in determining the likelihood that our system of interest will adopt a state characterized by some **internal state variable X**. Typically, X is a variable that we can measure or control with experiments. Depending on the values adopted by such an internal state, we can distinguish between relevant macrostates of our systems that are still represented by a collection of microstates, for example:

- Folded state/unfolded state of a protein,
- Closed state/open state of a channel,
- Bound state/unbound state of a protein-ligand complex,



More formally, a microstate is characterized by a **probability distribution** of the system among all the possible microstates. The probability distribution is defined as a list of the probabilities of all the microstates that are compatible with the given definition of macrostate.

$$\{p_i\} = \{p_1, p_2, \dots, p_\Omega\}$$

Where $p_1, p_2, \dots, p_\Omega$ indicates the Ω th microstate's probability.

The probability distribution is considered normalized:

$$\sum_i p_i = 1$$

The **internal energy** of the system in a given microstate is the **weighted average** of the energy over all the microstates:

$$\langle E \rangle = U = \sum_i p_i E_i$$

(internal energy includes contributions from both potential and kinetic energy)

The corresponding entropy is given by the **Gibbs entropy**:

$$S(\{p_i\}) = -k_B \sum_i p_i \ln p_i$$

For the special case of the **isolated system** ($N, V, E = \text{const.}$), $p_i = \frac{1}{\Omega}$:

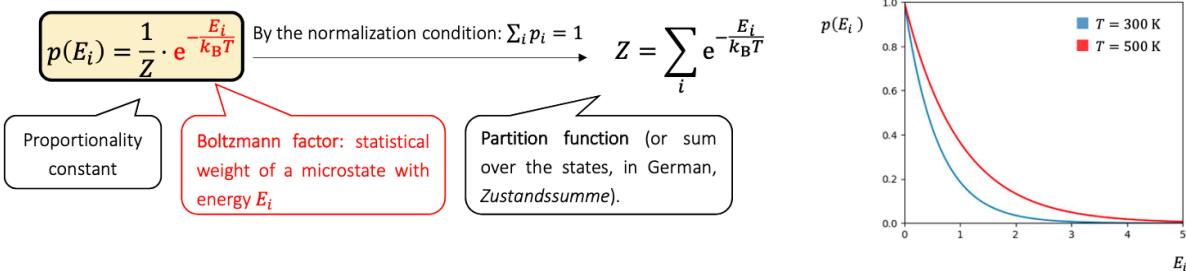
The internal energy: $\langle E \rangle = \sum_i^{\Omega} \frac{1}{\Omega} E = \frac{\Omega}{\Omega} \cdot E = E$

The Gibbs entropy: $S(\{p_i\}) = -k_B \sum_i^{\Omega} \frac{1}{\Omega} \ln \left(\frac{1}{\Omega} \right) = -k_B \frac{\Omega}{\Omega} \cdot \ln \left(\frac{1}{\Omega} \right) = +k_B \ln(\Omega)$

(what is the microstate's probability in a non-isolated systems)

The Boltzmann distribution:

It can be shown that, at fixed number of particles, volume, and temperature (closed system, N, V, T=const.), at the equilibrium, the most probable distribution of microstates with energy levels E_1, E_2, \dots , is the **Boltzmann distribution** (or canonical distribution). According to the Boltzmann distribution, the probability of visiting a microstate with energy E_i is:



This allows us to assess the probability (or statistical weight) of observing different macrostates of the system described by different values of a given microscopic parameter. For example, the statistical weight of a macrostate I with energy E that can be realized in Ω number of ways can be expressed as:

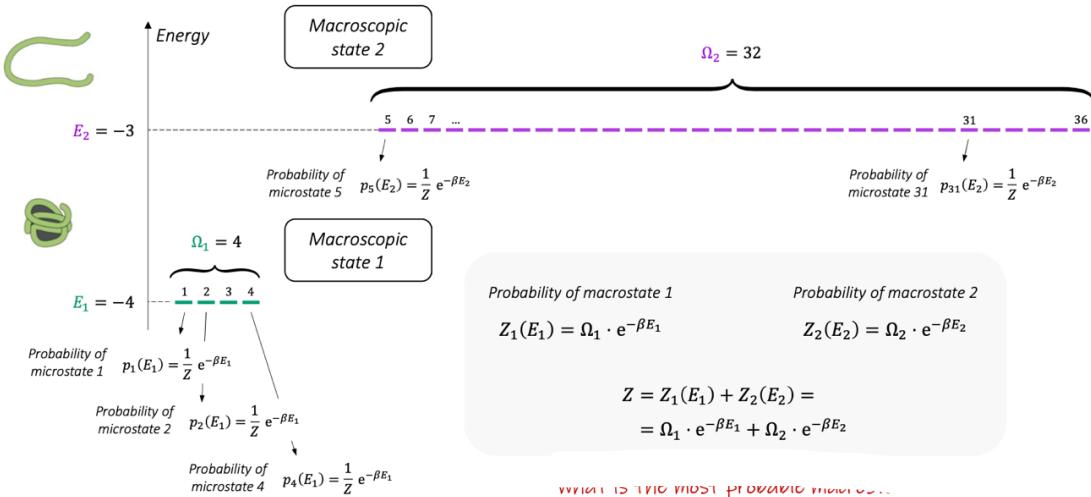
$$\text{Probability of the macrostate (partial partition function)} \quad Z_I(E) = \sum_{\Omega(E)} p(E) = \sum_{\Omega(E)} e^{-\frac{E}{k_B T}} \longrightarrow Z_I(E) = \Omega(E) \cdot e^{-\frac{E}{k_B T}}$$

Probability of microstates with the same energy E

It is convenient to introduce the notation:

$$\beta = \frac{1}{k_B T} \quad p(E_i) = \frac{1}{Z} \cdot e^{-\beta E_i}$$

Consider a system that can be found in two distinct macrostates characterized by a different energy and multiplicity:



(what is the most probable microstate)

The Helmholtz free energy:

The Helmholtz free energy at a given temperature T can be obtained by considering energy and entropy together.

$$F = \langle E \rangle - TS$$

Internal energy (U) of the macrostate given its distribution $\{p_i\}$

Entropy of the macrostate given its distribution $\{p_i\}$

This definition does not require the macrostate to be in thermodynamic equilibrium. In other words, for any distribution $\{p_i\}$, we can still define and compute the corresponding value of $\langle E \rangle, S$, and F . In fact, the **equilibrium free energy** is obtained by minimizing the free energy with respect to all possible distributions $\{p_i\}$:

$$F_{\text{eq}} = \min_{\{p_i\}} (F)$$

The distribution that leads to the minimum free energy is the Boltzmann distribution

In contrast, a **non-equilibrium microstate** has an arbitrary distribution $\{p_i\}$ that leads to a free energy higher than the minimum value F_{eq} .

A special case is a **metastable macrostate**, that has a free energy higher than F_{eq} but lower than that of neighboring macrostates (or, in other words, a local free energy minimum).

Recalling that:

$$\begin{cases} \langle E \rangle = \sum_i p_i E_i \\ S(\{p_i\}) = -k_B \sum_i p_i \ln p_i \end{cases}$$

We have that:

$$F = \langle E \rangle - TS$$

$$\begin{aligned} F &= \sum_i p_i E_i + k_B T \sum_i p_i \ln p_i = \\ &= \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot E_i + k_B T \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot \ln \left(\frac{e^{-\frac{E_i}{k_B T}}}{Z} \right) = \\ &= \sum_i \frac{E_i \cdot e^{-\frac{E_i}{k_B T}}}{Z} + k_B T \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot \ln \left(e^{-\frac{E_i}{k_B T}} \right) - k_B T \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot \ln(Z) = \\ &= \sum_i \frac{E_i \cdot e^{-\frac{E_i}{k_B T}}}{Z} + k_B T \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot \left(-\frac{E_i}{k_B T} \right) - k_B T \sum_i \frac{e^{-\frac{E_i}{k_B T}}}{Z} \cdot \ln(Z) = \\ &= \sum_i \cancel{\frac{E_i \cdot e^{-\frac{E_i}{k_B T}}}{Z}} - \sum_i \cancel{\frac{E_i \cdot e^{-\frac{E_i}{k_B T}}}{Z}} - k_B T \ln(Z) \end{aligned}$$

Free energy expressed in terms of the partition function:

$$F = -k_B T \ln(Z)$$

To find the most stable microstate at a given temperature T we must compare the free energy of the individual states:

$$E_2 = -3 \quad \Omega_2 = 32 \quad S_2 = +\ln(32) = 3.466 \quad F_2 = E_2 - TS_2 = -3 - T \cdot 3.466$$

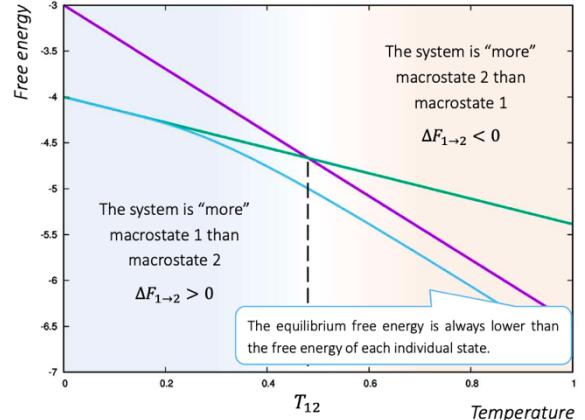
$$E_1 = -4 \quad \Omega_1 = 4 \quad S_1 = +\ln(4) = 1.386 \quad F_1 = E_1 - TS_1 = -4 - T \cdot 1.386$$

The free energy of the whole system composed of macrostate 1 and macrostate 2 at equilibrium and at a given temperature:

$$F_{\text{eq}} = -k_B T \ln(Z) \quad Z = Z_1(E_1) + Z_2(E_2) = \Omega_1 \cdot e^{-\frac{(E_1)}{k_B T}} + \Omega_2 \cdot e^{-\frac{(E_2)}{k_B T}} = 4 \cdot e^{\frac{4}{T}} + 32 \cdot e^{\frac{3}{T}}$$

At any given temperature we can compute the relative free energy $\Delta F_{1 \rightarrow 2}$ as a ratio between partial partition functions:

$$\Delta F_{1 \rightarrow 2} = F_2 - F_1 = -k_B T \ln\left(\frac{Z_2(E_2)}{Z_1(E_1)}\right) + k_B T \ln\left(\frac{\Omega_1}{\Omega_2}\right) = -k_B T \ln\left(\frac{Z_2(E_2)}{Z_1(E_1)}\right) \left\{ \begin{array}{l} \text{Probability of macrostate 2} \\ \text{Probability of macrostate 1} \end{array} \right\} \left\{ \begin{array}{l} \text{Any free energy} \\ \text{difference is a} \\ \text{probability ratio!} \end{array} \right\}$$



Crossover temperature (phase transition):

$$\Omega_1 \cdot p(E_1) = \Omega_2 \cdot p(E_2) = 0.5$$

$$\Delta F_{1 \rightarrow 2} = 0$$

We see now an informal derivation of the Boltzmann distribution:

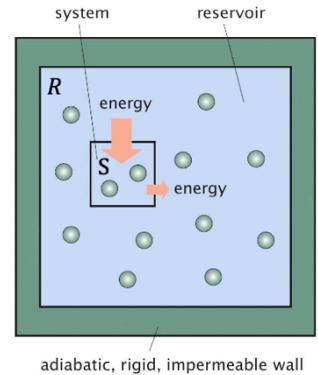
We assume that the system of interest can only exchange heat with its surroundings. System S has energy E_S , system R has an energy E_R , and the total energy is:

$$E_T = E_S + E_R$$

System R is taken to be much larger than system S, so that:

$$\left\{ \begin{array}{l} N_R \gg N_S \\ V_R \gg V_S \\ E_R \gg E_S \end{array} \right.$$

By construction, system R acts as a **thermal reservoir**, a system that is allowed to exchange energy with system S without its energy appreciably.



- The probability of finding the system S in a certain microstate i with energy E_i is proportional to the number of states Ω_S available when the system is in that state.
 $p_i \propto \Omega_S(E_i)$
- Since the total system ($E_S + E_R$) is isolated, this probability is equal to the number of microstates Ω_R of the heat bath for the energy ($E_r - E_i$).
 $p_i \propto \Omega_R(E_r - E_i)$

By the microscopic definition of entropy:

$$S = k_B \ln \Omega(E); \quad \Omega(E) = e^{\frac{S}{k_B}} \Rightarrow p_i \propto e^{\frac{S_R(E_T - E_i)}{k_B}}$$

From thermodynamics:

$$\left(\frac{\partial S}{\partial E} \right)_{N,V} = \frac{1}{T}$$

We now expand the entropy as a Taylor expansion truncated at the second order:

$$S_R(E_T - E_i) \cong S_R(E_T) - \frac{\partial S}{\partial E} E_i \Rightarrow p_i \propto e^{\frac{1}{k_B} [S_R(E_T) - \frac{1}{T} E_i]}$$

$$\cong S_R(E_T) - \frac{1}{T} E_i$$

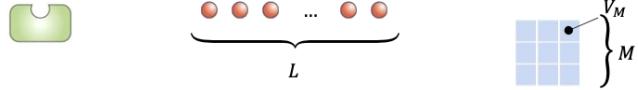
$$p_i \propto \underbrace{e^{\frac{S_R(E_T)}{k_B}}}_{\text{Constant}} \cdot e^{-\frac{E_i}{k_B T}} \Rightarrow p_i \propto e^{-\frac{E_i}{k_B T}}$$

Boltzmann factor

Lattice models of ligand-receptor binding:

We want to compute the probability that a receptor (p_b) by a ligand as a function of the number of ligands (concentration). We consider:

- A single receptor with one binding site
- L ligand molecules in the system
- M lattice sites in solution each with volume V_M
- For simplicity, we ignore any internal degrees of freedom of the ligand and the receptor, i.e. they are modeled as rigid bodies.



We have 2 classes of (macro)states:

1. **Unbound state** in which L ligands have the energy ε_{sol} .
2. **Bound state** in which $(L - 1)$ ligands have the energy ε_{sol} . The bound ligand will have the energy ε_b .

States and weights diagram			
STATE	ENERGY	MULTIPLICITY	TOTAL STATISTICAL WEIGHT
	$L \cdot \varepsilon_{sol}$	$\Omega_u = \frac{M!}{L! (M-L)!}$	$Z_u = \Omega_u \cdot e^{-\beta L \varepsilon_{sol}}$
	$(L-1) \cdot \varepsilon_{sol} + \varepsilon_b$	$\Omega_b = \frac{M!}{(L-1)! [M-(L-1)]!}$	$Z_b = \Omega_b \cdot e^{-\beta [(L-1)\varepsilon_{sol} + \varepsilon_b]}$

The probability of receptor occupancy can be written as a ratio of the weights of the favorable outcomes and the weights of all the outcomes:

$$p_b = \frac{Z_b}{Z_b + Z_u} \longrightarrow Z_b = \frac{M!}{(L-1)! [M-(L-1)]!} \cdot e^{-\beta [(L-1)\varepsilon_{sol} + \varepsilon_b]} = \underbrace{\frac{M!}{(L-1)! [M-(L-1)]!}}_{\text{Multiplicity: number of microstates that share the same Boltzmann Factor}} \cdot \underbrace{e^{-\beta \varepsilon_b} \cdot e^{-\beta (L-1)\varepsilon_{sol}}}_{\text{Statistical weight of a single microstate dictated by the Boltzmann factor}}$$

$$Z_u = \frac{M!}{L! (M-L)!} \cdot e^{-\beta L \varepsilon_{sol}}$$

$$Z = Z_b + Z_u$$

$$p_b = \frac{e^{-\beta \varepsilon_b} \cdot \frac{M!}{(L-1)! [M-(L-1)]!} \cdot e^{-\beta (L-1)\varepsilon_{sol}}}{\frac{M!}{L! (M-L)!} \cdot e^{-\beta L \varepsilon_{sol}} + e^{-\beta \varepsilon_b} \cdot \frac{M!}{(L-1)! [M-(L-1)]!} \cdot e^{-\beta (L-1)\varepsilon_{sol}}}$$

Clearly, by construction, we also have:

$$\left\{ \begin{array}{l} p_u = \frac{Z_u}{Z_b + Z_u} \\ p_b + p_u = 1 \end{array} \right.$$

If $M \gg L$, then:

$$\frac{M!}{(M-L)!} \approx M^L$$

$$p_b = \frac{e^{-\beta \varepsilon_b} \cdot \frac{M!}{(L-1)! [M-(L-1)]!} \cdot e^{-\beta(L-1)\varepsilon_{sol}}}{\frac{M!}{L! (M-L)!} \cdot e^{-\beta L \varepsilon_{sol}} + e^{-\beta \varepsilon_b} \cdot \frac{M!}{(L-1)! [M-(L-1)]!} \cdot e^{-\beta(L-1)\varepsilon_{sol}}}$$

$$p_b \approx \frac{\frac{e^{-\beta \varepsilon_b} \cdot \frac{M^{L-1}}{(L-1)!} \cdot e^{-\beta(L-1)\varepsilon_{sol}}}{\frac{M^L}{L!} \cdot e^{-\beta L \varepsilon_{sol}} + e^{-\beta \varepsilon_b} \cdot \frac{M^{L-1}}{(L-1)!} \cdot e^{-\beta(L-1)\varepsilon_{sol}}}}{\times \frac{L!/M^L \cdot e^{\beta L \varepsilon_{sol}}}{L!/M^L \cdot e^{\beta L \varepsilon_{sol}}}}$$

$$p_b \approx \frac{(L/M) \cdot e^{-\beta[\varepsilon_b - \varepsilon_{sol}]}}{1 + (L/M) \cdot e^{-\beta[\varepsilon_b - \varepsilon_{sol}]}}$$

Introducing: $\Delta\varepsilon = [\varepsilon_b - \varepsilon_{sol}]$

Standard state concentration: $c_0 = \frac{1}{V_M}$

$c = \frac{L}{M \cdot V_M}$

$\left(\frac{L}{M}\right) = \left(\frac{c}{c_0}\right)$

$p_b \approx \frac{(c/c_0) \cdot e^{-\beta\Delta\varepsilon}}{1 + (c/c_0) \cdot e^{-\beta\Delta\varepsilon}}$

Numerator:

$$e^{-\beta \varepsilon_b} \cdot \frac{M^{L-1}}{(L-1)!} \cdot e^{-\beta(L-1)\varepsilon_{sol}} \cdot \frac{L!}{M^L} \cdot e^{\beta L \varepsilon_{sol}} = \frac{L}{M} \cdot e^{-\beta[\varepsilon_b + L\varepsilon_{sol} - L\varepsilon_{sol}]} = \frac{L}{M} \cdot e^{-\beta[\varepsilon_b - \varepsilon_{sol}]}$$

Denominator:

$$\frac{M^L}{L!} \cdot e^{-\beta L \varepsilon_{sol}} \cdot \frac{L!}{M^L} \cdot e^{\beta L \varepsilon_{sol}} + \frac{L}{M} \cdot e^{-\beta[\varepsilon_b - \varepsilon_{sol}]} = 1 + \frac{L}{M} \cdot e^{-\beta[\varepsilon_b - \varepsilon_{sol}]}$$

- In this equation we recognize:
- 1. The independent variable, c
- 2. The parameters of the model:
 - o V_M or the standard state concentration, c_0
 - o The energy difference, $\Delta\varepsilon$, which we expect to be negative
- The parameters must be derived from experiments or previous knowledge

This result goes under different names depending upon the field (such as Langmuir adsorption isotherm or a Hill equation with Hill coefficient $n = 1$).

$$p_b = \frac{Z_b}{Z_b + Z_u} \longrightarrow p_b \approx \frac{(c/c_0) \cdot e^{-\beta\Delta\varepsilon}}{1 + (c/c_0) \cdot e^{-\beta\Delta\varepsilon}}$$

- To make an estimate of the parameters:

$$V_M = ? \quad c_0 = \frac{1 \text{ mol}}{1 \text{ l}} = \frac{1 \text{ mol}}{1 \text{ dm}^3} \quad \begin{cases} 1 \text{ nm} \equiv 10^{-9} \text{ m} \equiv 10^{-8} \text{ dm} \\ 1 \text{ dm}^3 \equiv (10^8 \text{ nm})^3 \equiv 10^{24} \text{ nm}^3 \end{cases}$$

$$c_0 = \frac{1 \text{ mol}}{10^{24} \text{ nm}^3} \equiv \frac{10^{-24} \text{ mol}}{1 \text{ nm}^3} \quad N_A = \frac{6.022 \cdot 10^{23} \text{ molecules}}{\text{mol}}$$

$$c_0 = \frac{10^{-24} \text{ mol}}{1 \text{ nm}^3} \cdot \frac{6.022 \cdot 10^{23} \text{ molecules}}{\text{mol}} = 0.6022 \frac{\text{molecules}}{\text{nm}^3}$$

$$V_M = \frac{1}{c_0} \cong 1.66 \frac{\text{nm}^3}{\text{molecule}} = 1660 \frac{\text{\AA}^3}{\text{molecule}} \quad \approx 12 \text{\AA} \uparrow \boxed{\text{cube}}$$

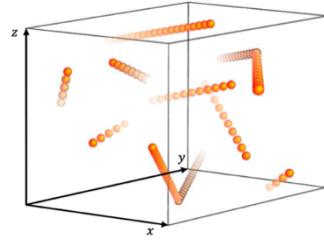
An interesting concentration of ligand is the one for which the two terms in the denominator are approximately equal. Equality of these two terms roughly amounts to the statement that the entropy lost in stealing one of the ligands from solution to bind it to the receptor is just made up for by the energetic gain ($\Delta\varepsilon$) associated with binding the ligand to the receptor. This concentration corresponds to having half occupancy ($p_b = 0.5$). At low concentrations, the entropic contribution is dominant, while at high enough concentrations, the energetic contribution prevails.

Thermally activated process:

What are the mean properties of the molecules at the equilibrium state? Consider a box that contains gas molecules of uniform mass (m) which have at the beginning of the experiment the same velocity (v).

The kinetic energy per molecule is the same and it can be calculated as:

$$\left. \begin{array}{l} K_1 = \frac{1}{2}mv_1^2 \\ K_2 = \frac{1}{2}mv_2^2 \\ \dots \\ K_N = \frac{1}{2}mv_N^2 \end{array} \right\} K_1 = K_2 = \dots = K_N$$



But this state where the particles do not interact with each other is highly improbable. This situation will change instantaneously as the molecules exchange their energy by collision with each other. Very soon, a great number of microstates will be populated.

Even under equilibrium conditions the energy of the individual molecules will constantly change. Still, after a sufficiently long period of time, the statistical distribution of energy will become **stationary** (i.e. time independent), meaning that the kinetic energy will fluctuate but the average value would be constant. The time required to achieve the stationary state is called **relaxation time** (equilibrium time).

The Maxwell-Boltzmann distribution:

The fraction of molecules f with **velocity components** v_x, v_y, v_z is proportional to an exponential function of their kinetic energy:

Fraction of molecules

$$f(\mathbf{v}) = c \cdot e^{-\frac{K}{k_B T}}$$

$$K = \frac{1}{2}mv_x^2 + \frac{1}{2}mv_y^2 + \frac{1}{2}mv_z^2$$

$$\rightarrow f(\mathbf{v}) = c \cdot e^{-\frac{(\frac{1}{2}mv_x^2 + \frac{1}{2}mv_y^2 + \frac{1}{2}mv_z^2)}{k_B T}} = c \cdot e^{-\frac{mv_x^2}{2k_B T}} \cdot e^{-\frac{mv_y^2}{2k_B T}} \cdot e^{-\frac{mv_z^2}{2k_B T}}$$

C is our new **proportionality factor**, and we are interested in the way it can be approximated.

$(f(\mathbf{v})dv_x dv_y dv_z)$ is the fraction of molecules in the velocity range of the kind:

$$(v_x: v_x + dv_x), (v_y: v_y + dv_y), (v_z: v_z + dv_z)$$

This fraction can be factorized into three components, one for each of the arbitrary axis:

$$f(\mathbf{v}) = f(v_x) \cdot f(v_y) \cdot f(v_z) \quad \text{with} \quad f(v_i) = c^{1/3} \cdot e^{-\frac{mv_i^2}{2k_B T}}$$

We can use the index i to show that this factorization applies to every axis.

To determine c , we note that a molecule must have a velocity somewhere in the range $-\infty < v_i < +\infty$, and by integrating in that specific range of space, the product of the fraction of molecules showing a specific velocity with the infinitesimal of the velocity, we obtain:

$$\int_{-\infty}^{+\infty} f(v_i) dv_i = 1 \quad \text{by substitution} \quad c^{1/3} \int_{-\infty}^{+\infty} e^{-\frac{mv_i^2}{2k_B T}} dv_i = 1 \quad \rightarrow c^{1/3} \sqrt{\frac{2\pi k_B T}{m}} = 1$$

\Downarrow

$$c = \left(\frac{m}{2\pi k_B T} \right)^{\frac{3}{2}} \equiv \left(\frac{M}{2\pi R T} \right)^{\frac{3}{2}}$$

$$\int_{-\infty}^{+\infty} e^{-ax^2} dx = \frac{\pi}{a}$$

Molar mass: $M = N_A \cdot m$

We observe then that c is related to the mass of the atoms and to the temperature. But, if we are considering molar units we can represent the same quantity with respect of the molar mass instead of the single atoms mass. When we are considering molar units the $k_B T$ becomes RT .

What we obtained can be represented in this way:

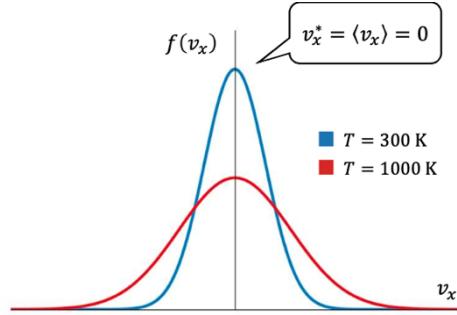
Fraction of molecules

$$f(v_x) = \left(\frac{M}{2\pi RT} \right)^{\frac{1}{2}} \cdot e^{-\frac{Mv_x^2}{2RT}}$$

This can be plotted. In this function we have an exponential term that was inherited by the very first equation, this term is however very different with respect that of the Boltzmann distribution. In fact, by having the square of the velocity at the exponent, we will have the shape of a Gaussian distribution.

The blue plot shows us the plot under equilibrium condition. At this condition very few molecules will have a high velocity, most of the others will have a velocity component that is picked around 0. This result is expected because our initial assumption of the box in which our gas is, is that the box is fixed in the space, it is not moving.

As we said this distribution depend on the mass of the atoms and on the temperature. If we increase the temperature of the system what we will obtain is again a Gaussian distribution but the probability of having molecules with a high velocity will increase, red plot.



Instead, we want to have the probability that a molecule will have a velocity in the range:

$(v_x : v_x + dv_x), (v_y : v_y + dv_y), (v_z : v_z + dv_z)$ is:

$$f(\mathbf{v}) dv = f(v_x)f(v_y)f(v_z) dv_x dv_y dv_z = \left(\frac{M}{2\pi RT} \right)^{\frac{3}{2}} \cdot e^{-\frac{Mv_x^2}{2RT}} \cdot e^{-\frac{Mv_y^2}{2RT}} \cdot e^{-\frac{Mv_z^2}{2RT}} dv_x dv_y dv_z =$$

Probability density of velocities

$$= \left(\frac{M}{2\pi RT} \right)^{\frac{3}{2}} \cdot e^{-\frac{Mv^2}{2RT}} dv_x dv_y dv_z$$

Volume of a spherical shell

$$4\pi v^2 dv$$

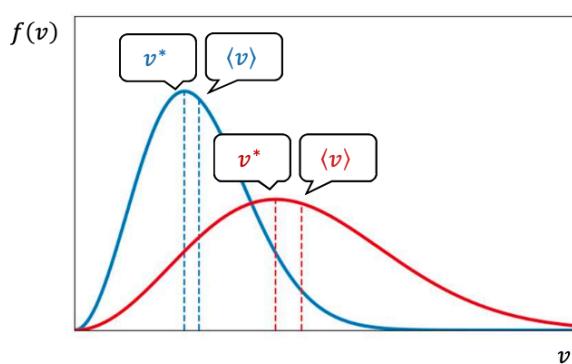
What we obtain is the **probability density of velocities**.

The square of the velocity is equal to the sum of the square of each component of the single axis.

From the probability density of velocities, we can calculate the **probability density of speeds** regardless of the orientation. Remember that the **speed** is a scalar value of the velocity vector.

$$f(\mathbf{v}) = 4\pi v^2 f(\mathbf{v}) = 4\pi \left(\frac{M}{2\pi RT} \right)^{\frac{3}{2}} v^2 \cdot e^{-\frac{Mv^2}{2RT}}$$

At the exponent we still have the square of the velocity so again if we plot this, we should obtain a Gaussian distribution, but in this case it will not be centered in the 0, but it will be skew.



The average molecular kinetic energy:

From the probability density of speed, we can calculate the average kinetic energy of the molecules:

$$\langle K \rangle = \frac{1}{2} m \langle v^2 \rangle = \frac{3}{2} k_B T$$

This microscopic kinetic energy is often called thermal energy. The triangular brackets indicate an average. We can have this transformation:

$$\begin{aligned} \frac{1}{2} m \langle v^2 \rangle &= \int_{v=0}^{v=\infty} \left(\frac{1}{2} m v^2 \right) f(v) dv = \\ &= \int_{v=0}^{v=\infty} \left(\frac{1}{2} m v^2 \right) \cdot 4\pi \left(\frac{m}{2\pi k_B T} \right)^{\frac{3}{2}} v^2 \cdot e^{-\frac{mv^2}{2k_B T}} dv = \\ &= 4\pi \left(\frac{m}{2\pi k_B T} \right)^{\frac{3}{2}} \cdot \left(\frac{1}{2} m \right) \int_{v=0}^{v=\infty} v^2 \cdot v^2 \cdot e^{-\frac{mv^2}{2k_B T}} dv = \frac{3}{2} k_B T \end{aligned}$$

$$\int_0^{+\infty} x^4 e^{-ax^2} dx = \frac{3}{8} \left(\frac{\pi}{a^5} \right)^{1/2}$$

And by recalling that the velocity distribution must be isotropic, meaning that the entire system is not traveling along the space.

$$\langle v^2 \rangle = \langle v_x^2 + v_y^2 + v_z^2 \rangle = \langle v_x^2 \rangle + \langle v_y^2 \rangle + \langle v_z^2 \rangle$$

We should have satisfied this condition:

$$\langle v_x^2 \rangle = \langle v_y^2 \rangle = \langle v_z^2 \rangle$$

And thanks to it we can obtain an **average kinetic energy per degree of freedom**:

$$\frac{1}{2} m \langle v_x^2 \rangle = \frac{1}{2} m \langle v_y^2 \rangle = \frac{1}{2} m \langle v_z^2 \rangle = \frac{1}{2} k_B T$$

We only have three external degrees of freedom around the three arbitrary axis. We do not have an internal degree of freedom, we can think at a molecule as a rigid body.

This result is one example of the principle of **equipartition of energy** that states that, the average energy associated with each degree of freedom that is described by a quadratic term in the energy is the same and equal to $k_B T / 2$ per molecule.

The kinetic energy of the system is the average kinetic energy per molecule multiplied by the number of molecules.

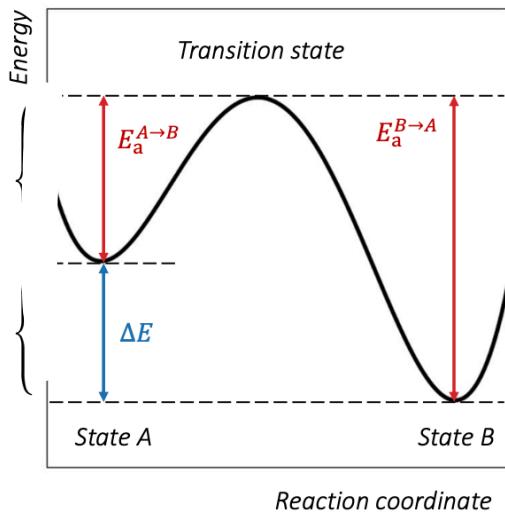
$$K = N_A \cdot \langle K \rangle = N_A \cdot \frac{3}{2} k_B T = \frac{3}{2} RT$$

As always if we multiplied k_B by the Avogadro's constant, we obtain R.

In conclusion we can say that the thermal energy scale is of the order of $k_B T$, and it will be distributed equally to all the degrees of freedom of the system as $\frac{1}{2} k_B T$.

The Activation Energy:

A **thermally activated** process is one in which a system must overcome an energy barrier to go forward. When considering the transformations a biological system can undergo, the range of available possibilities is depicted in terms of an **energy landscape**.



In this plot, on the y axis we have the energy, while on the x axis we have the **reaction coordinates**, which indicate the state of the reaction that we are considering, for example we are in the state in which a bond is forming or breaking.

In the bottom of the energy wells there are the **stable state**, this are characterized by the so called **depth of minima** which indicate the relative stability of the different states (in this example, state B is much more stable because it is lower than state A).

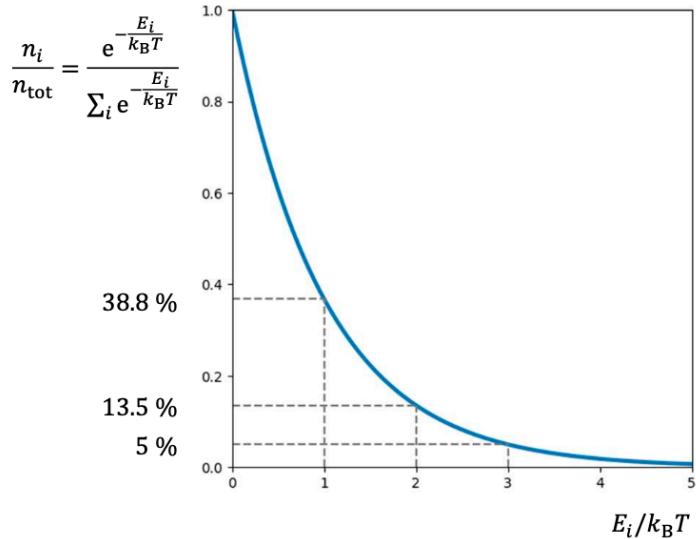
Another value characterizing the states is the **height of barriers**, which indicates the rate of transition between them.

We have two states divided by one barrier. We also have two different kind of **activation energy** E_a because the activation energy required to go from state A \rightarrow state B is different from that of going from state B \rightarrow to state A ($E_a^{A \rightarrow B} \neq E_a^{B \rightarrow A}$).

On the pick of the barrier we have the so called **transition state**, which is a non-stable state.

In the stable state the molecules will be continuously deflected along the reaction coordinate by thermal collisions. The effectiveness (see the passage from one state to another) of these collisions depends on their energy, that is on the ratio $\frac{E_a}{k_B T}$.

We can plot the ratio between the activation energy and the $k_B T$. Obtaining a graph like this:



This plot is an exponential function and it indicates the percentage of molecules that will have a specific energy level. For example, if we will have an activation energy (barrier) twice as the $k_B T$, the number of molecules that will have that value of energy will be only the 13.5%.

The Boltzmann factor, allows the calculation of the relative number of molecules n_i / n_{tot} which are able to overcome the energy barrier.

The rate at which the energy barriers are crossed at a given temperature is described by the empirical model of the **Arrhenius equation**:

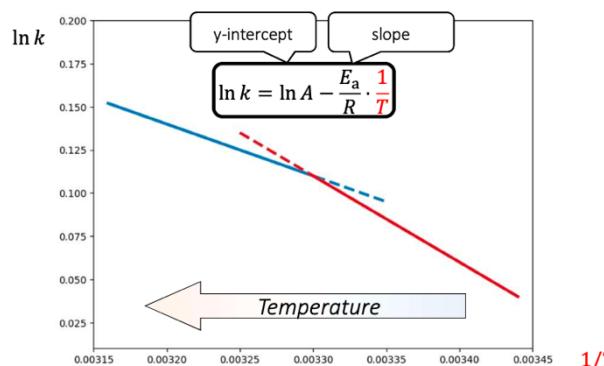
$$k = A \cdot e^{-\frac{E_a}{RT}}$$

Rate constant [s⁻¹]

Pre-exponential factor (empirical parameter with the same units of k)

The rate constant is expressed with the inverse of the second, the pre-exponential factor has the same unit of k. While the exponential factor is dimensionless.

The parameters can be obtained by measuring the changes in the rate as a function of the temperature through the so-called Arrhenius plot:



We can linearize the Arrhenius equation by computing the logarithm at both part of the equations. $\frac{1}{T}$ becomes our independent variable. By this when having the values of the slope and the intercept we can compute the **rate constant** of our process.

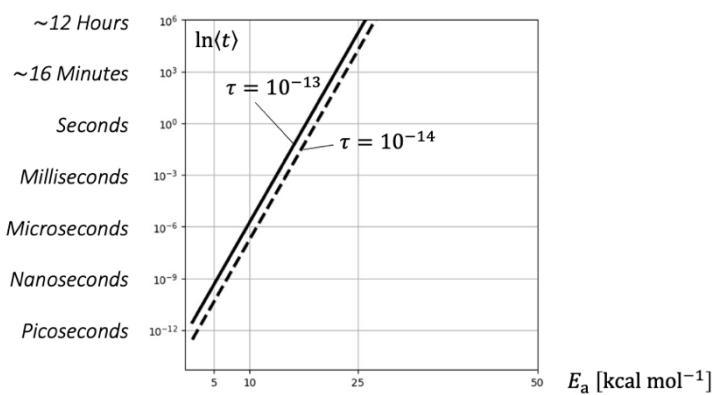
Sometimes if we increase the temperature the slope will change, because at a certain temperature the mechanism change. There is a deviation from linearity.

If we take the reciprocal of the rate constant, $\frac{1}{k}$, it will represent the **average time** (or timescale) required to observe a given process.

$$\frac{1}{k} = \langle t \rangle = \tau \cdot e^{\frac{E_a}{RT}}$$

Time constant [s] ($10^{-14} - 10^{-13}$ s for small molecules)

The τ constant, on the right side of the equation, is very system dependent.



Ex. Breaking of an H bond.

Average energy required to break an H bond is equal to 3 – 5 kcal/mol.

So by using the graph, if we have an energy level of 5 kcal/mol, what we expect to see is the breaking of the H bond to the scale od nanoseconds.

This plot is very important because from the value of the energy we can achieve the value of the time required to observe a process with that specific amount of energy.

Thermal molecular motion:

What are the relevant molecular motions in molecules?

- 1. Vibrations:** oscillations in the bonding distances **between the atoms in a molecule.**

$$v = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} [s^{-1}]$$

Reduced mass:

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

$$v \approx 10^{14} \text{ s}^{-1}$$

$$\langle t \rangle \approx 10^{-1} \text{ s} = 10 \text{ fs}$$

2. **Rotations:** change in the orientation of the entire molecule as a rigid body, or change in the orientation of individual atoms or atomic groups around the axes of their bonds.

Ex. A covalent bond, rotation around its axis, describes a “cone” of rotation. In this way chain molecules can adopt stochastic orientations unless strong attracting or repelling interactions between portions of the molecule are preventing this.

3. Translation: motion of the entire molecule through space.

In the **Freely Joint Chain (FJC) model**, a polymer made of $(n + 1)$ non-interacting monomers A_i ($0 \leq i \leq n$) is described as a chain of n segments:

- With fixed bond length $l = |v_i|$ and varying angles φ and θ .
 - With free orientation ($V(\varphi, \theta) = 0$) and independent of the orientation of the former segment.

With these definitions, the size of the polymer can be described in several ways:

- **Contour length, R_{\max} :** length of the polymer at its maximum extent of elongation.

$$R_{\max} = n \cdot l$$

- **End – to – end distance, R**: distance between the first and the last monomer of the chain.

$$\mathbf{R} = \mathbf{r}_n - \mathbf{r}_0 = \sum_{i=1}^n \mathbf{v}_i \quad R \leq R_{\max} \quad \xrightarrow{\langle \cdot \rangle_{\varphi, \theta}} \quad \langle \mathbf{R} \rangle = \left\langle \sum_{i=1}^n \mathbf{v}_i \right\rangle = \sum_{i=1}^n \langle \mathbf{v}_i \rangle := 0$$

Position vectors that represents the absolute position of the monomers

Bond vector that represents the spatial orientation of the i^{th} segment

Since there is no preferential direction, the average end-to-end distance will be zero

- The simplest non-zero average is represented by the **mean-squared end-to-end distance**:

$$\langle R^2 \rangle = \langle \mathbf{R} \cdot \mathbf{R} \rangle = \left(\sum_{i=1}^n \langle \mathbf{v}_i \rangle \right) \cdot \left(\sum_{j=1}^n \langle \mathbf{v}_j \rangle \right) = \sum_{i=1}^n \sum_{j=1}^n \langle \mathbf{v}_i \cdot \mathbf{v}_j \rangle =$$

Root-mean-squared end-to-end distance

$$= \sum_{i=1}^n \sum_{j=1}^n l^2 \langle \cos \varphi_{ij} \rangle = \sum_{i=1}^n l^2 \underbrace{\langle \cos \varphi_{ii} \rangle}_{=1} + \sum_{j \neq i}^n l^2 \underbrace{\langle \cos \varphi_{ij} \rangle}_{=0} = nl^2$$

$$R_{\text{rms}} = \sqrt{\langle R^2 \rangle} = \sqrt{nl} \quad R_{\text{rms}} = \frac{R_{\max}}{\sqrt{n}}$$

A more precise measure of the size of the polymer is obtained through the radius of gyration:

- **Radius of gyration, R_g** , mean of the square of the distance of all the monomers from the center of mass r_{CM} of the molecule.

$$R_G = \sqrt{\frac{1}{(n+1)} \sum_{i=0}^n (\mathbf{r}_i - \mathbf{r}_{CM})^2}$$

$$\mathbf{r}_{CM} = \frac{1}{(n+1)} \sum_{i=0}^n \mathbf{r}_i$$

For a number of segments $n \rightarrow \infty$, it can be shown that:

$$R_G = \frac{R_{\text{rms}}}{\sqrt{6}} = l \sqrt{\frac{n}{6}}$$

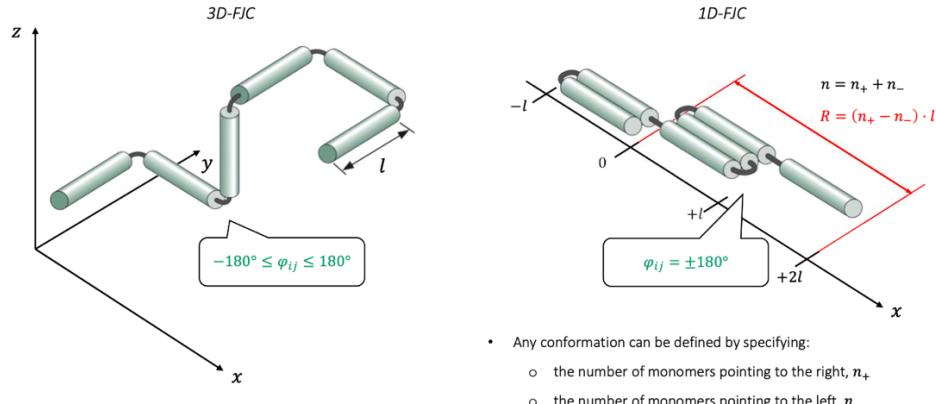
For globular proteins: $R_G \cong 1.51 \cdot R_H$

The radius of gyration of molecules can be measured with experimental techniques like Small-Angle X-Ray Scattering (SAXS). More in general SAXS provides low-resolution information on protein shape, conformation, and the state of its assembly.

Hydrodynamic radius, R_H , radius for which the molecule travels in water like a macroscopic sphere with a hydrophilic surface. In this case the **frictional force** experienced by the sphere of radius R_H moving through a fluid with viscosity η at speed v is:

$$\text{Stokes' law} \quad f = 6\pi \eta R_H v$$

More information regarding the end-to-end distance is carried by its probability distribution. In order to derive the probability distribution of the end-to-end distance, we have to map every possible conformation on a **random walk model**.



A polymer of n monomers can be described by a **random walk model** of n steps. Each step can only be taken to the right or to the left with the same probability:

$$p(n_+) = p(n_-) = 1/2$$

After the walker has made a total of n steps, we ask ourselves what is the probability of having an end-to-end distance corresponding to n_+ steps to the right (and hence $n_- = n - n_+$ steps on the left). Since the probability of each right or left step is $1/2$, the probability of a particular sequence of n left and right steps is:

$$p(n) = (1/2)^n$$

On the other hand, there are many ways of realizing a particular sequence of n_+ steps out of a total of n steps:

$$\text{Multiplicity of a given end-to-end distance:} \quad \Omega(n_+; n) = \frac{n!}{n_+!(n-n_+)!}$$

Therefore, the probability distribution for n_+ is:

$$p(n_+; n) = \frac{n!}{n_+!(n-n_+)!} \cdot \left(\frac{1}{2}\right)^n$$

We convert the probability distribution for n_+ in the probability distribution of the end-to-end distance:

$$p(n_+; n) = \frac{n!}{n_+!(n-n_+)!} \cdot \left(\frac{1}{2}\right)^n$$

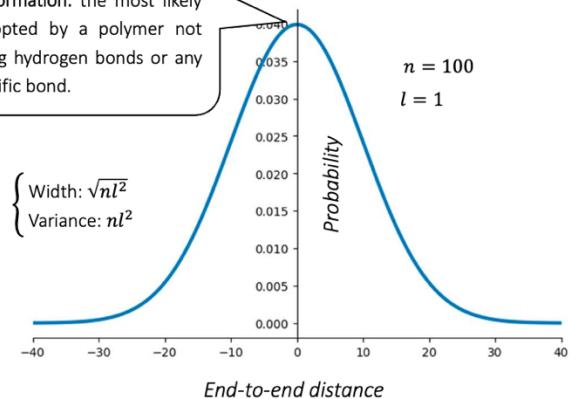
$$(n_+ - n_-) = \frac{R}{l} \quad \downarrow \quad n = n_+ + n_-$$

$$p(R; n) = \frac{n!}{\left(\frac{n}{2} + \frac{R}{2l}\right)! \left(\frac{n}{2} - \frac{R}{2l}\right)!} \cdot \left(\frac{1}{2}\right)^n$$

- Stirling's approximation
- Taylor's expansion
- Several algebraic rearrangements

$$\ln p(R; n) \cong \ln 2 - \frac{1}{2} \ln(2\pi n) - \frac{R}{2nl^2}$$

Random coil conformation: the most likely conformation adopted by a polymer not capable of forming hydrogen bonds or any other type of specific bond.



Discrete probability function

$$p(R; n) = \frac{2}{\sqrt{2\pi n}} \cdot e^{-\frac{R^2}{2n}}$$

↓

Probability density function

$$P_{1D}(R; n) = \frac{1}{\sqrt{2\pi nl^2}} \cdot e^{-\frac{R^2}{2nl^2}}$$

× $\frac{1}{2l}$

$$P_{3D}(\mathbf{R}; n) = \left(\frac{3}{2\pi nl^2}\right)^{3/2} \cdot e^{-\frac{3R^2}{2nl^2}}$$

The random coil is the least structured conformation of a polymer chain and corresponds to the state of greatest entropy:

- Any stretching or compression introduces order and reduces the entropy.
- The formation of a random coil from a more extended conformation is a spontaneous process (provided enthalpy contributions do not interfere).

We can estimate the change in **conformational entropy** when a random coil of a polymer containing n segments of length l is stretched by the probability density function of the end-to-end distance:

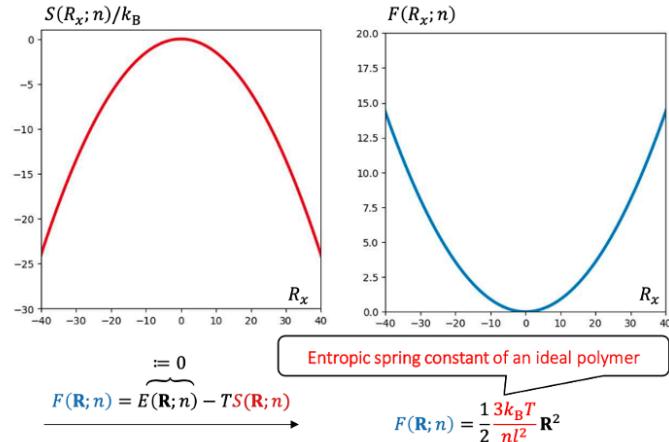
$$S(\mathbf{R}; n) = k_B \ln \Omega(\mathbf{R}; n)$$

$$\downarrow \quad \Omega(\mathbf{R}; n) \propto P_{3D}(\mathbf{R}; n) = \left(\frac{3}{2\pi nl^2} \right)^{3/2} \cdot e^{-\frac{3\mathbf{R}^2}{2nl^2}}$$

$$S(\mathbf{R}; n) = k_B \ln \left[\left(\frac{3}{2\pi nl^2} \right)^{3/2} \cdot e^{-\frac{3\mathbf{R}^2}{2nl^2}} \right] + \text{const.} = \\ = \frac{3}{2} k_B \ln \left(\frac{3}{2\pi nl^2} \right) - \frac{3}{2} k_B \frac{\mathbf{R}^2}{nl^2} + \text{const.}$$

Term that depends on the number of monomers but not on the end-to-end distance

$$S(\mathbf{R}; n) \propto -\frac{3}{2} k_B \frac{\mathbf{R}^2}{nl^2}$$



Brownian motion:

The translational motion due to thermal collision is important not only for single molecules but also for macroscopically visible particles. The fact that macroscopic particles follow chaotic trajectories over time was first observed by the botanist Robert Brown in 1827, and a theoretical description was given by Albert Einstein in 1905.

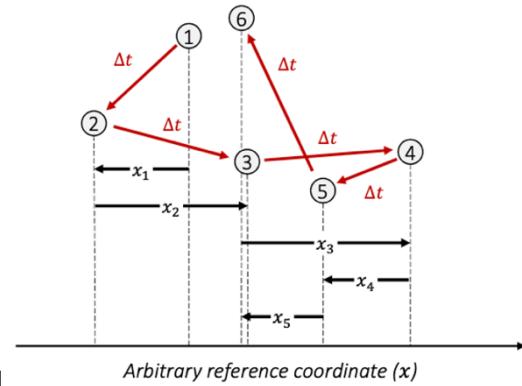
Brownian motion is determined by collisions with the molecules of the surrounding medium resulting from thermal translations, and these collisions are uncorrelated (i.e. e. random) over intervals long enough to be observed. The time interval should not be too small, otherwise we won't be able to observe the motion of the particle.

In particular the motion results from the vector sum of these hits:

The easiest way to track the motion of the particles is always to identify an arbitrary axis and then take notes about the displacement around that axis. For each motion the time interval is the same.

By observing the average position of the particle after a sufficiently long time, there will be no translocation at all. In order to quantify the net motion, the **mean squared displacement** over n time intervals is used (won't be zero):

$$\langle (\Delta x)^2 \rangle = \frac{1}{n} \sum_i x_i^2$$

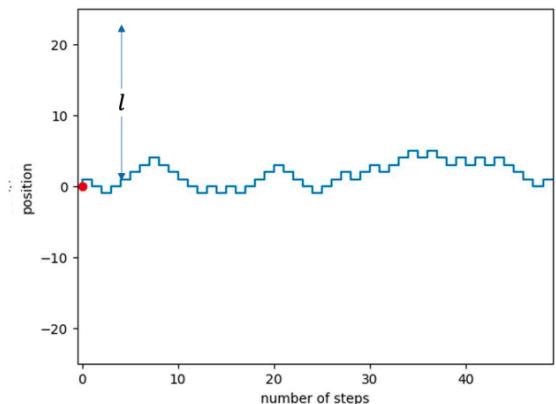


This parameter corresponds to the square of the mean path length:

$$\bar{x} = \sqrt{\langle (\Delta x)^2 \rangle}$$

It is possible to employ a random walk model to describe random motion:

- Consider a particle initially ($t=0$) placed at the position $x=0$. The number of steps can also be considered as time.
- At regular intervals the particle is displaced by one length unit l either in the positive or in the negative direction with the same probability $p=1/2$.
- Construct a sequence of displacement where the probability p at each step is assumed to be independent of the previous displacement (the displacements are statistically independent; it depends on the timescale used for inquiring about the displacement).



The total number of displacements is: $n = n_+ + n_-$

The net displacement is: $(n_+ - n_-)l$

Many sequences of n displacement can lead to the same net displacement. There is a $\Omega(n_+; n)$ number of such ways:

$$\Omega(n_+; n) = \frac{n!}{n_+! n_-!} = \frac{n!}{n_+! (n - n_+)!}$$

Therefore, the probability of a displacement $n_+ - n_-)l$ after n steps independent of the sequence is:

$$p(n_+; n) = \frac{n!}{n_+! (n - n_+)!} \cdot \left(\frac{1}{2}\right)^n$$

It is possible to show that the probability distribution tends to a Gaussian distribution in the limit $n \rightarrow \infty$.

The diffusion coefficient:

The mean-squared displacement increases linearly with the number of steps (or time). The proportionality constant that describes the intensity of the Brownian motion is related to the **diffusion coefficient D** of the considered particle:

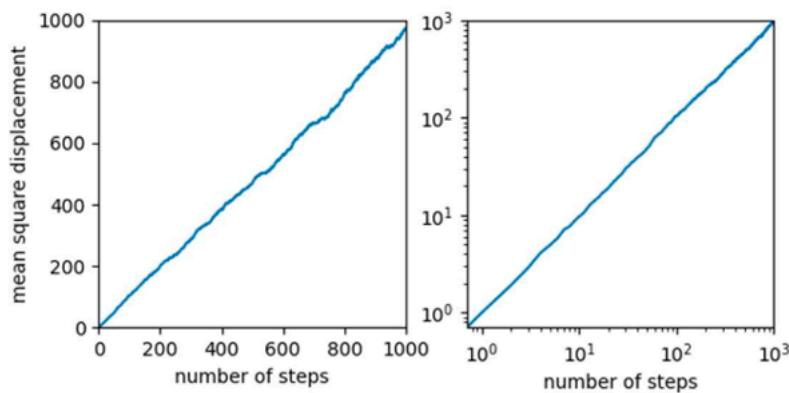
$$\text{Einstein's diffusion law: } \langle (\Delta x)^2 \rangle = 2D \Delta t$$

It can be shown that the diffusion coefficient depends on:

- **Shape** and size of the particle (hydrodynamic radius, R_H).
- **Viscosity** of the medium (η).
- **Temperature**.

$$\text{Stokes-Einstein equation: } D = \frac{k_B T}{6\pi \eta R_H}$$

- For globular protein: $R_G \cong 0.8 \cdot R_H$
- The viscosity of water is: $\eta = 0.00089 \frac{\text{kg}}{\text{s} \cdot \text{m}}$

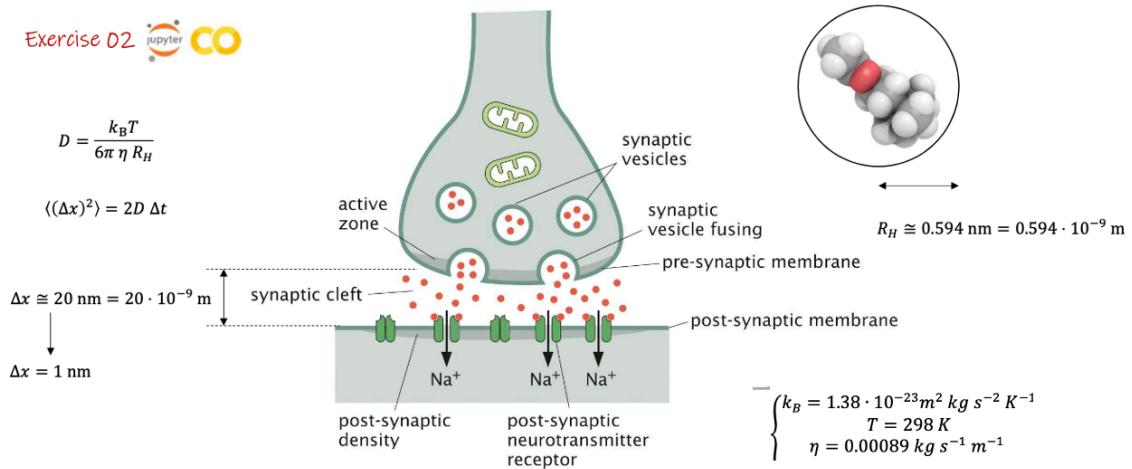


Ex. Diffusion of a neurotransmitter across the synaptic cleft:

Exercise 02  

$$D = \frac{k_B T}{6\pi \eta R_H}$$

$$\langle(\Delta x)^2\rangle = 2D \Delta t$$



Calculate the diffusion coefficient:

$$D = \frac{k_B T}{6\pi \eta R_H} = \frac{1.38 \cdot 10^{-23} \cdot 298}{6\pi \cdot 8.9 \cdot 10^{-4} \cdot 0.594 \cdot 10^{-9}} = 4.13 \cdot 10^{-10}$$

Now:

$$\langle(\Delta x)^2\rangle = 2D \Delta t \rightarrow \Delta t = \frac{\langle(\Delta x)^2\rangle}{2D} = \frac{(20 \cdot 10^{-9})^2}{2 \cdot 4.13 \cdot 10^{-10}} = 4.85 \cdot 10^{-7} \text{ s}$$

And now with $\Delta x = 1 \text{ nm}$

$$\Delta t = \frac{\langle(\Delta x)^2\rangle}{2D} = \frac{(1 \cdot 10^{-3})^2}{2 \cdot 4.13 \cdot 10^{-10}} = 1211 \text{ s} = 20 \text{ min}$$

We can observe that for large cellular distances the diffusion is not so effective.

Lattice models of proteins folding:

First observations about protein were made by Christian Anfinsen, these can be grouped in three main points:

- Proteins can fold reversibly; we can fold and unfold back protein.
- The native structure of globular proteins are thermodynamically stable states.
- Native structures must be global minima of their accessible energy landscape.

Few years later, Cyrus Levinthal, stated that the observations made previously contained a paradox. The Anfinsen hypothesis implied a paradoxical result (**Levinthal's paradox**):

Consider a 100 aa-long chain (99 bonds), and assume that every aa can adopt only 3 conformations. The total number of conformations is: $3^{99} \approx 10^{47}$. Assuming a rate of interconversion between conformations to the order of a picosecond (10^{-12} s), and neglecting any recurrence among already visited conformations, the time required to explore the conformational space would be:

$$10^{47} \times 10^{-12} \text{ s} = 10^{35} \text{ s} \approx 10^{27} \text{ yrs}$$

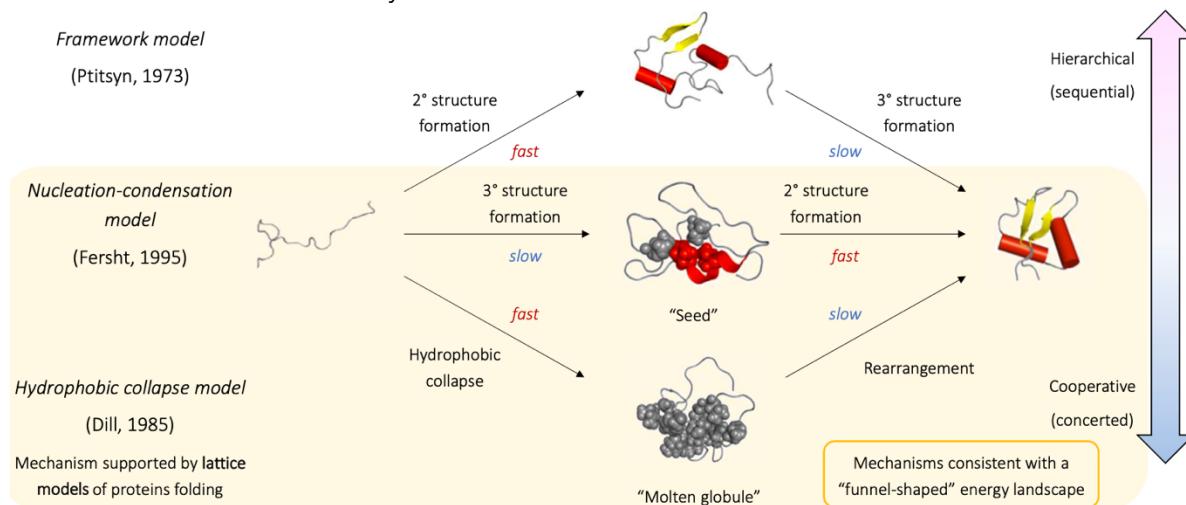
(cfr. Estimated age of the Universe: 14×10^9 yrs)

Experimentally observed folding rates are ranging from microseconds (fast folders) to milliseconds. There is nothing to do to the time required to reach native state if the peptide was moving as a random molecule. Folding cannot be a totally random process. For Levinthal, Proteins must fold through a sequence of stable intermediates, leading to specific “folding pathways” (or mechanisms). Experiments on fast-folding two-state proteins clearly showed that these proteins fold quickly without accumulating into detectable intermediates.

Over the years, several models for protein folding have been devised. They can be summarized into three main models:

1. **Framework model** (Ptitsyn, 1973). We have the formation of a secondary structure; this is a very fast event. Then, a second state in lower energy and here we have the formation of the tertiary structure.
2. **Hydrophobic collapse model** (Dill, 1985). Is opposite to the framework model. We start with the ‘collapse’ of the protein into the **molten globule**, this is a very fast event. Then with a slow process, there is the formation of the tertiary structure. This model’s mechanisms are supported by **lattice models** of proteins folding. Moreover, the mechanisms are consistent with a **funnel shaped** energy landscape.
3. **Nucleation-condensation model** (Fersht, 1995). This last model is a kind of average between the first two. In this model we first have a slow formation of the tertiary structure, then, a second fast formation of the secondary structure.

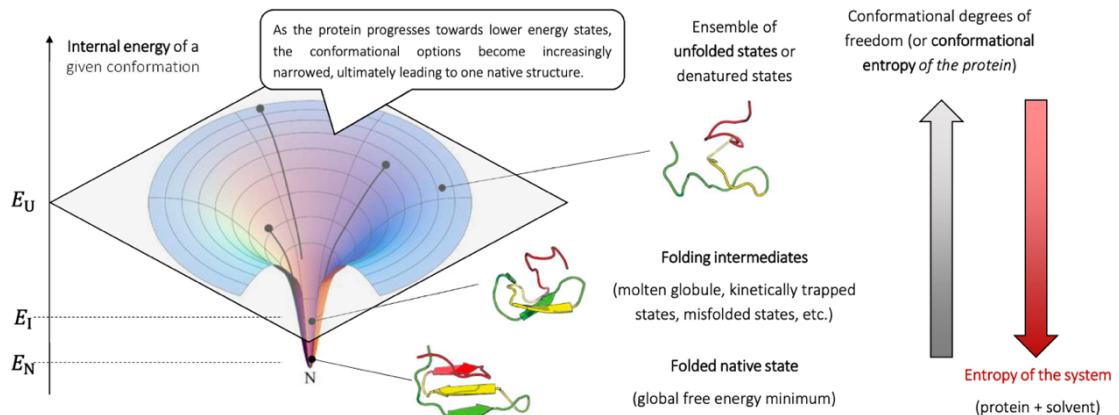
These models expand different levels of **cooperativity** and **hierarchy**. High levels of cooperativity indicates low levels of hierarchy and vice versa.



The hydrophobicity model can be sustained by the lattice models of proteins folding and also its mechanisms are consistent with a **funnel-shape** energy landscape.

Protein folding is a complex problem, it can be understood by resorting to the energetics of protein conformation: the energy landscape. In the case of protein folding, the energy landscape is embodied by the concept of the **folding funnel** (José Nelson Onuchic & coworkers, 1992).

Funnel-shaped energy landscape: folded states must have a strong conformational focus to direct folding to a unique native conformation that must be locally stable and kinetically accessible from not just one path of folding, but from many pathways.



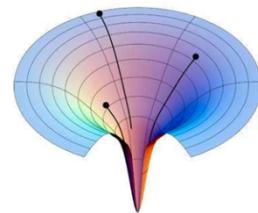
At the bottom of the funnel there is the native conformation of the peptide. Whereas, at the top there is an ensemble of the unfolded states or denatured states.

Another information provided by this kind of landscape is the one given by the radius of the funnel. Given a specific energy level in the funnel, its radius represents a specific **conformational degree of freedom** of the peptide at a that given energy level. By going from the bottom to the top we have that the conformational degrees of freedom increase, and so it does the **conformational entropy** of the protein.

Folding funnel model provides a conceptual framework for understanding the different scenarios of protein folding:

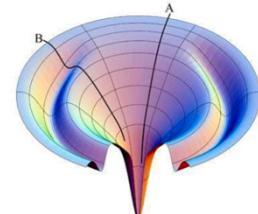
- **Idealized folding funnel:**

An ensemble of conformations can reach the global minimum satisfying Anfinsen's thermodynamic hypothesis. The minimum is reached quickly satisfying Levinthal's concern. Each protein molecule will follow its own route, not a single pathway. The smooth energy surface results in fast folding and two-state (single-exponential) kinetics.



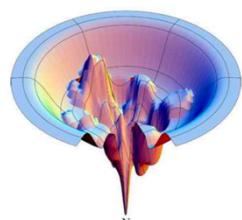
- **Moat folding funnel:**

This kind of funnel represent the situation in which the peptide finds an energy barrier to overcome to proceed along the funnel itself. It represents a **kinetic trap**. In this case "A" route is a funnel-like path, while "B" route the protein must pass through an obligatory folding intermediate.



- **Realistic folding funnel:**

One can distinguish several minima, distinct energy barriers, and multiple kinetic traps. A certain degree of "frustration" (or ruggedness), that is the existence of competing minima separated by large barriers, is expected. The rugged energy surface results in (relatively) slow folding and the folding kinetics is likely multiple-exponential.

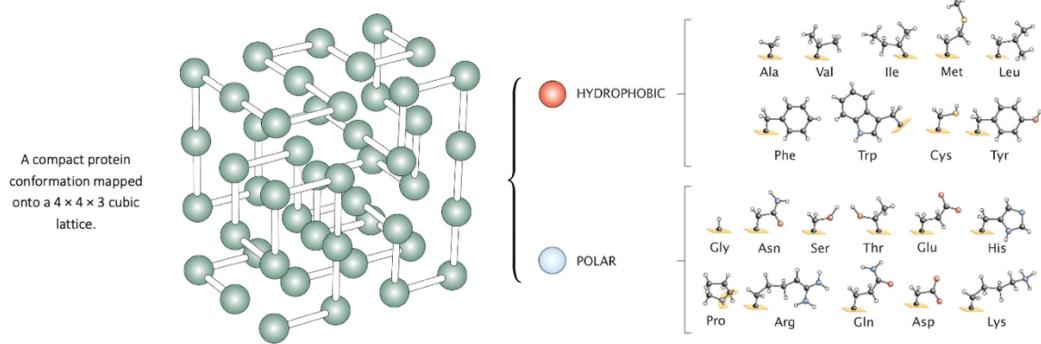


Lattice models of protein folding:

The most basic models of protein folding are defined on a lattice, and they have the following features:

1. Each monomer (amino acid) is represented by a bead located on a node of the lattice.
2. Connectivity between monomers is represented by lines.
3. Sites not occupied by the monomers are considered as occupied by solvent molecules.

HP (hydrophobic polar) models are a sub-set of lattice models which build on the idea that the hydrophobic effect has a dominant role in protein folding. In HP models, the 20 naturally occurring amino acids are replaced with a two-letter alphabet that identifies each amino acid as:

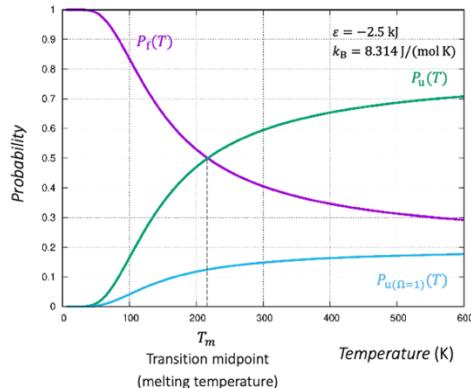


We consider a toy model that consists in 4 monomers mapped on a 2D square lattice. The chain has: 2 **H-monomers** at the ends, 2 **P-monomers** in the middle. This short chain has only 5 possible conformations.

We assign: an **energy gain ϵ** for every contact between H monomers, and a null energy for contacts with either a solvent molecule or a P monomer.

With the partition function we can compute the probabilities for the folded and unfolded states as a function of the temperature.

$$P_f(T) = \frac{e^{-\epsilon/k_B T}}{4 + e^{-\epsilon/k_B T}} \quad P_u(T) = \frac{4}{4 + e^{-\epsilon/k_B T}}$$



The blue line indicate the case in which the unfolded state had only one conformation.

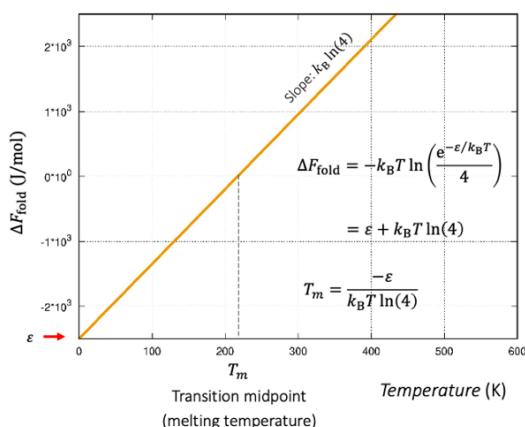
Is it possible to see these cases in another prospective, the one in which the energy difference is given by the single two energies. We can exploit the equation that we obtained and reach a new one where we find the **probability ratio**.

$$\Delta F_{fold} = F_f - F_u = -k_B T \ln(P_f) + k_B T \ln(P_u) = -k_B T \ln\left(\frac{P_f}{P_u}\right)$$

By inserting the values that we have obtained in the first case we find a line equation.

$$\Delta F_{fold} = -k_B T \ln\left(\frac{e^{-\epsilon/k_B T}}{4}\right)$$

And so we can plot this equation and obtain this:

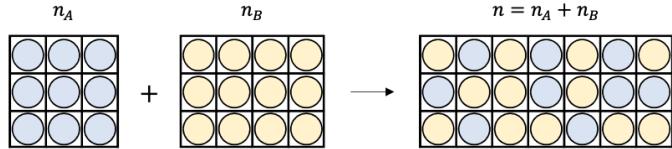


This function is always sensible to the temperature. At T_m we have the **transition midpoint**, before it the folded state is more favorable, after it the most favorable state is the unfolded one.

Chemical equilibrium:

First of proceeding with the chemical equilibrium, is reasonably needed to talk about **entropy of mixing**. Consider a lattice model of a binary mixture, assume that:

- Particles of A and B are of the same size,
- Each occupies one lattice site,
- Together, they completely fill a lattice of n lattice sites.



If we mix the two kind of particles, the multiplicity of states is the number of spatial arrangements of the molecules:

$$\Omega = \frac{n!}{n_A! n_B!}$$

Therefore, the (molar) **entropy of mixing** is defined as:

$$\begin{aligned} S_{\text{mix}} &= R \ln \frac{n!}{n_A! n_B!} \quad \longrightarrow \quad S_{\text{mix}} = R (n \ln n - n_A \ln n_A - n_B \ln n_B) = \\ &\quad \underbrace{\qquad\qquad\qquad}_{\text{Stirling's approximation}} = R (n_A \ln n + n_B \ln n - n_A \ln n_A - n_B \ln n_B) = \\ &\quad = R \left(n_A \ln \frac{n}{n_A} + n_B \ln \frac{n}{n_B} \right) \end{aligned}$$

Once we have this relationship, we can recognize that in order to make progress, is more useful to represent the entropy of mixing in terms of the **mole fraction**, x_i :

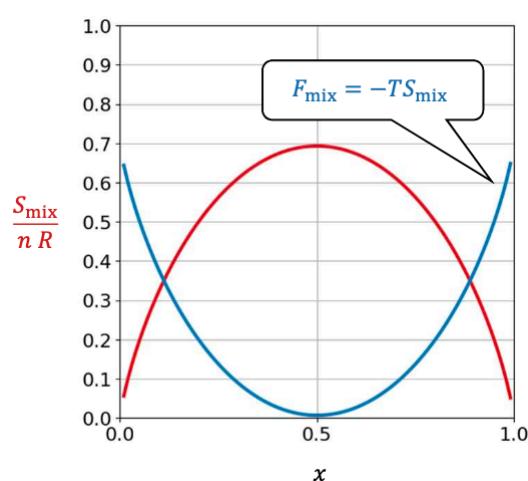
$$S_{\text{mix}} = -R (n_A \ln x_A + n_B \ln x_B)$$

$$x_A = \frac{n_A}{n} \quad x_B = \frac{n_B}{n}$$

If we change the notation by defining a variable x represented by the mole fraction of B , and since the total mole fraction sum of A and B is equal to 1, we can even obtain a more general equation.

$$\frac{S_{\text{mix}}}{n R} = -[(1-x) \ln(1-x) + x \ln x]$$

To be more precise, we are not representing the entropy of mixing. We are representing the ratio between the entropy of mixing and the product of the total number of moles n and R . This equation can be plotted (red plot).



In this plot the x axis indicate the composition of the species B and not the degree of freedom.

We can obtain the **free energy of mixing** F_{mix} by taking the minus of the entropy of mixing multiplied by the temperature. This equation can be plotted as well (blue plot).

Chemical equilibrium:

We start with the simplest chemical equilibrium. We consider a hypothetical reaction where species A, that exists in a specified standard state, is converted to B, also in a standard state. Assume that there is **no mixing** between reactants and products.

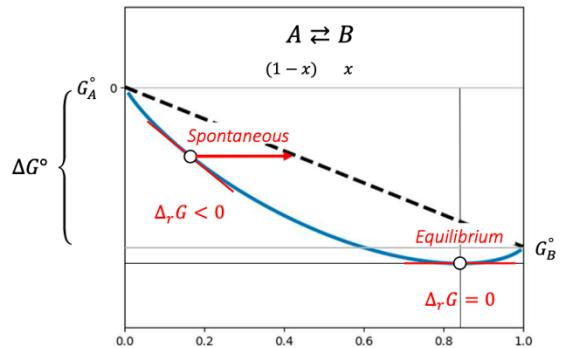


$(1-x) \quad x$

If a substance A is converted to a substance B, whatever it is, the substance B when is pure is associated to a free energy G_B , in this reaction the free energy will change linearly from G_A to G_B . The change in free energy with respect to the mole fraction of B is:

$$\begin{aligned} G_{\text{no-mix}} &= (1-x)G_A + xG_B = G_A + x(G_B - G_A) = \\ &= G_A + x(G_B - G_A) = \\ &\equiv x\Delta G^\circ \quad \left\{ \begin{array}{l} \text{Setting } G_A^\circ = 0 \text{ for convenience} \end{array} \right. \end{aligned}$$

Free energy or *chemical energy* per mole of substance A or B in the standard state



On the x axis we have the composition of the reaction: when the mole fraction of B is equal to 0 we only have the A substance, that for convinience in this case we take as 0. While the reaction progress, A is being converted to B, we will reach a point to which there is only B associated to its free energy value. The free energy of B is on a lower level with respect to that of A, this indicates that the reaction is spontaneous.

Now we complicate the system by letting the two substances to mix.

The free energy now will be the sum of the two single contribution, these contributions are the free energy of the pure substances. The mixing contribution is purely entropic (no energy).

$$\begin{aligned} G &= G_{\text{no-mix}} + G_{\text{mix}} = G_{\text{no-mix}} - TS_{\text{mix}} \\ &= x\Delta G^\circ + RT[(1-x)\ln(1-x) + x\ln x] \end{aligned}$$

From the graph we see that at equilibrium the free energy as a minimum, that is the slope of G with respect to the change in composition is zero:

$$\begin{aligned} \Delta_r G &= \frac{dG}{dx} = \Delta G^\circ + RT[-\ln(1-x) + \ln x] = 0 \\ \text{Reaction free energy} &= \Delta G^\circ + RT \ln \left(\frac{x}{1-x} \right) = 0 \quad \Delta G^\circ = -RT \ln \left(\frac{x_B}{x_A} \right)_{\text{eq}} \end{aligned}$$

When the reaction free energy is < 0 it means that the creation of the products is spontaneous. On the other hand, for values of the reaction free energy > 0 , the reverse reaction is favorable, meaning that from the products there would be the productions of the reactants.

The **reaction free energy** is the slope of the free energy of the process plotted against the extent of the reaction itself.

$$\begin{aligned} \Delta_r G &= \frac{dG}{dx} = \Delta G^\circ + RT[-\ln(1-x) + \ln x] = \\ &= G_B^\circ - G_A^\circ + RT \ln \left(\frac{x_B}{x_A} \right) \end{aligned}$$

Another way to interpret the reaction free energy is as the difference of the **chemical potential** of products and reactants at the composition of the reaction mixture. The equation obtained before can be expanded.

$$= G_B^\circ - G_A^\circ + RT \ln \left(\frac{x_B}{x_A} \right) \equiv \mu_B - \mu_A \quad \begin{cases} \mu_A = \mu_A^\circ + RT \ln x_A \\ \mu_B = \mu_B^\circ + RT \ln x_B \end{cases}$$

\square is the **chemical potential**. It is also called the “escape tendency”, because the higher the value of \square_A , the greater is the tendency of the system to escape from state A and enter to state B.

Generalizing, the chemical potential of a solute at a mole fraction x_i is:

$$\mu_i = \mu_i^\circ + RT \ln x_i$$

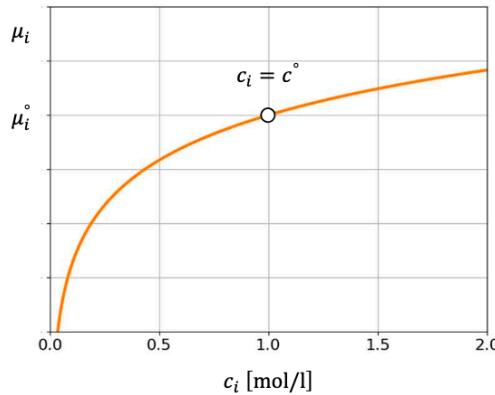
For practical purposes, it is more useful to express the chemical potential in terms of the molar concentration of the solute, c_i :

$$\downarrow \quad x_i \propto \frac{c_i}{c^\circ}$$

$$\mu_i = \mu_i^\circ + RT \ln \left(\frac{c_i}{c^\circ} \right)$$

C° is the **standard molar concentration**, introduced to ensure that the constant of proportionality is dimensionless. Usually, c° is equal to 1 mol/l.

As usual we can see the shape of it. If we increase the concentration of the substances that we have we increase the chemical potentials, there is the tendency of escape state A.



Clearly the chemical potential that we have a 1 mol/l is the c° .

A very important reaction, might find whenever we are dealing with biological reactions is:



The association between two entities A and B, to form a complex (AB). This reaction can represent the binding of two proteins, binding of protein and DNA, etc.

The easiest way to arrive at a operational definition of equilibrium constant for the reaction is to use the concept of chemical potential.

$$\begin{cases} \mu_A = \mu_A^\circ + RT \ln \left(\frac{c_A}{c^\circ} \right) \\ \mu_B = \mu_B^\circ + RT \ln \left(\frac{c_B}{c^\circ} \right) \\ \mu_{AB} = \mu_{AB}^\circ + RT \ln \left(\frac{c_{AB}}{c^\circ} \right) \end{cases}$$

From this the reaction free energy is equal to:

$$\begin{aligned}\Delta_r G &= \mu_{AB} - \mu_A - \mu_B = \\ &= \overset{\circ}{\mu}_{AB} + RT \ln \left(\frac{c_{AB}}{c^\circ} \right) - \overset{\circ}{\mu}_A - RT \ln \left(\frac{c_A}{c^\circ} \right) - \overset{\circ}{\mu}_B - RT \ln \left(\frac{c_B}{c^\circ} \right) = \\ &= \overset{\circ}{\mu}_{AB} - \overset{\circ}{\mu}_A - \overset{\circ}{\mu}_B + RT \ln \left(\frac{c_{AB}}{c^\circ} \right) - RT \ln \left(\frac{c_A}{c^\circ} \right) - RT \ln \left(\frac{c_B}{c^\circ} \right) = \\ &= \Delta G^\circ + RT \ln \left(\frac{c_{AB}}{c_A \cdot c_B} \cdot \frac{c^\circ}{c^\circ} \right)\end{aligned}$$

By replacing in the equation, the chemical potential, we obtain:

$$\Delta_r G = \Delta G^\circ + RT \ln \left(\frac{c_{AB} \cdot c^\circ}{c_A \cdot c_B} \right)$$

At equilibrium $\Delta_r G = 0$:

$$\Delta G^\circ = -RT \ln \underbrace{\left(\frac{c_{AB} \cdot c^\circ}{c_A \cdot c_B} \right)}_{K_{eq}}$$

K_{eq} is the **equilibrium constant**.

Depending on the field we are in, we can find the equilibrium constant expressed in different ways. For example: very often we find the **association or the dissociation constant** instead of the equilibrium one.

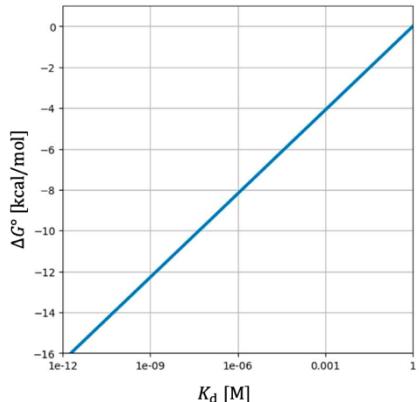
Association constant	Dissociation constant
$K_a = \left(\frac{c_{AB}}{c_A \cdot c_B} \right)_{eq}$	$K_d = \frac{1}{K_a}$
$[M^{-1}]$	$[M]$

By multiplying the association constant for the standard molar concentration we obtain the equilibrium constant.

$$\Delta G^\circ = -RT \ln(K_a \cdot c^\circ) \equiv +RT \ln \left(\frac{K_d}{c^\circ} \right)$$

The greater the association constant, the more spontaneous is the process A + B to obtain AB. The higher is the dissociation constant, the more spontaneous is the dissociation of the species AB to obtain A and B. Higher the association constant the smaller the dissociation constant would be.

The relationship between the free energy at equilibrium and the dissociation constant is not linear, but here it looks since it was plotted in logarithmic units.



Electrostatics:

Amino acids with ionizable side chains impart important properties to proteins:

- Modulation of the charges on these amino acids, e.g. by pH, may result in significant changes in protein conformation ultimately leading to protein denaturation;
- Charge alteration by phosphorylation and dephosphorylation of Ser, Thr, and Tyr is key to inducible protein-protein interactions, which underlie the switch-like response of signal transduction networks;
- Certain kinds of post-translational charge-altering modifications, such as acetylation of Lys, may damp protein-DNA association, most notably in the nucleosome;
- Nucleic acids and cell membranes have surface charges, thus binding of proteins to these targets is expected to be strongly influenced by electrostatic interactions o Net charges have also profound effects on processes such as the conduction of ions through transmembrane channels;
- Charged residues play a role in the binding of metals (e.g., Ca^{2+}) or charged ligands (e.g., ATP) to specific sites of proteins;

The hydrophobic interactions of nonpolar residues are the main driving force for the folding of proteins and several other processes. Hydrophobic interactions are aspecific, while electrostatic interactions are important because they happen between positive and negative charges, so they confer specificity. Charge-charge interactions can be strong even at long-range distance and can be crucial in determining the association rate constants.

Small recap on the Coulomb's law:

It describes the quantitative relationship between the magnitude of two-point charges which are separated by a certain distance r . The force decreases linearly with the distance.

There's a specific relationship between forces and potential energy (coulombic/electrostatic potential energy). Potential energy decreases linearly with the distance (not the square). It is long range for this fact, i.e. it decreases slowly.

Force	Potential energy
$f = \frac{1}{4\pi\epsilon_0\varepsilon} \cdot \frac{q_1 \cdot q_2}{r^2}$	$V(r) = \frac{1}{4\pi\epsilon_0\varepsilon} \cdot \frac{q_1 \cdot q_2}{r}$

If the forces are ions or whatever is charged inside the solution, the orientation of surrounding water molecules will shield the charge of the molecules themselves: this is why the dielectric constant in polar mediums such as water is so high. Considering molecule like water, the overall charge of this is neutral but there are present two local charges, leading to a dipole and the formation of a dipole moment.

Every charge placed in space generates an **electric field \mathbf{E}** which is characterized by the gradient of its electric potential, ϕ .

The **electric field** is defined as the net force in a given point in space due to a fixed distribution of charges per unit charge:

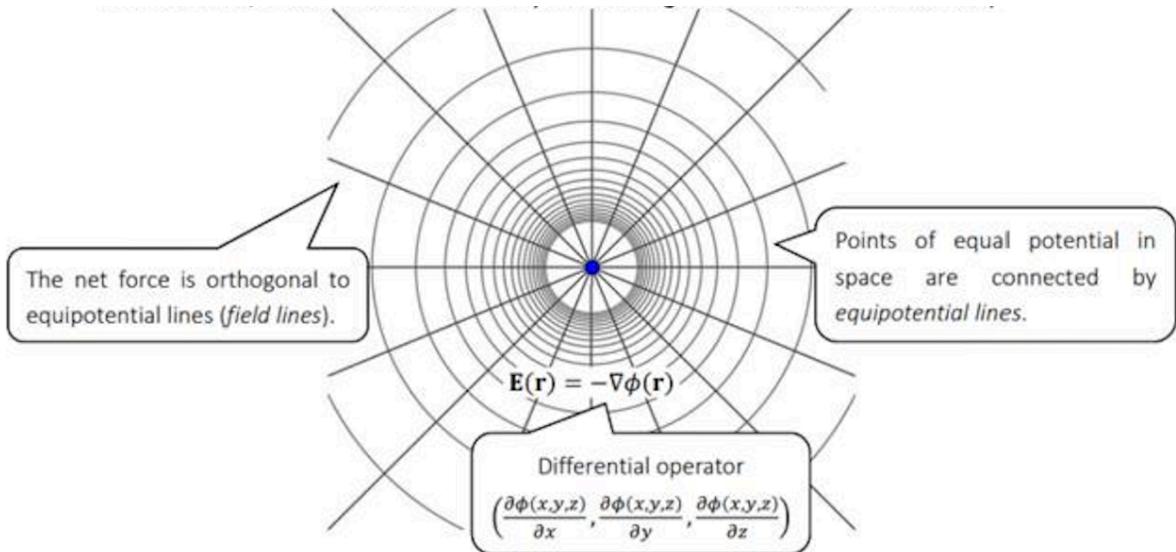
$$\mathbf{E}(\mathbf{r}) = \frac{\mathbf{f}(\mathbf{r})}{q_0}$$

- This is a **vectorial quantity** meaning that for every point we can assign a vector, and it gives an idea of the forces acting on that point (due to this charge distribution).
- q_0 is a positive net charge and is a **unit charge** (an electron charge).

The **electric potential** is the work required to move a unit charge from an infinite distance to a given point in space:

$$\phi(\mathbf{r}) = \frac{V(\mathbf{r})}{q_0} \quad \left[1\text{V} = \frac{1\text{J}}{1\text{C}} \right]$$

- It is not dependent on the magnitude of the charges we are considering.
- The unit of measure of the electric potential is the **Volt**.
- It is a scalar quantity: once we have the distribution of charges, for every point of space we have a certain value of the electric potential.



Circles represent points of equal potential in space (since they are at the same distance from the observed charge): called **equipotential lines**.

Field lines represent the direction of the electric field: the direction of the force that another positive charge would feel. These are directed to the outside, depart from the central charge.

The relationship between the electric field and electric potential can be described by the equation:

$$\mathbf{E}(\mathbf{r}) = -\nabla\Phi(\mathbf{r})$$

If a third point charge is added to the system: compute 3 times the pairwise interaction between all the possible combination of two charges:

$$V_{\text{tot}} = V_{12} + V_{13} + V_{23} = k \frac{q_1 q_2}{r_{12}} + k \frac{q_1 q_3}{r_{13}} + k \frac{q_2 q_3}{r_{23}}$$

So for a set of N point charges, it holds the pairwise additivity:

$$V_{\text{tot}} = k \sum_{i=1}^{N-1} \sum_{j=i+1}^N \frac{q_i q_j}{r_{ij}}$$

We can express the electric potential energy in terms of the potential. The **electric potential energy**: the product of the magnitude of charge 1 and the potential caused by charge 2, equal to product of magnitude of charge 2 and the potential due to charge 1.

$$V_{12} = \underbrace{\frac{1}{4\pi\epsilon_0\epsilon}}_k \cdot \frac{q_1 q_2}{r_{12}}$$

Expressing in terms of the potential:
 $\phi_1 = k \frac{q_2}{r_{12}} \quad \phi_2 = k \frac{q_1}{r_{12}}$

Potential at point 2 due to charge 1
 $V_{12} = q_1 \phi_1 = q_2 \phi_2 = \frac{1}{2} (q_1 \phi_1 + q_2 \phi_2)$

Potential at point 1 due to charge 2

So in the case of the system with N charges:

$$V_{\text{tot}} = \frac{1}{2} \sum_i^N q_i \phi_i$$

Where:

$$\phi_i = \frac{1}{4\pi\epsilon_0\epsilon} \sum_{j \neq i}^N \frac{q_j}{r_{ij}}$$

ϕ is the electric potential felt by charge i due to the presence of all the charges in the system.

For a general continuous charge distribution we replace the summation over the point charges with an integral over the space:

$$V_{\text{tot}} = \frac{1}{2} \int \rho(\mathbf{r}) \phi(\mathbf{r}) d\mathbf{r} \quad \text{Where: } \phi(\mathbf{r}) = \frac{1}{4\pi\epsilon_0\epsilon} \int \frac{\rho(\mathbf{r}')}{r} d\mathbf{r}'$$

With discrete charges the equation is formally correct, while with continuous it is not (we need to take into account a correction factor).

The electrostatic potential energy of continuous charge distributions can be evaluated with the double integral:

$$V(\rho_1(\mathbf{r}), \rho_2(\mathbf{r})) = \frac{1}{2} \iint \frac{\rho_1(\mathbf{r}') \cdot \rho_2(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|} d\mathbf{r}' d\mathbf{r}$$

While the equations for the energy of discrete distributions are exact (sum over all charges except i), the conversion to integrals includes the interaction of charge i with himself (self-energy).

A very simple model of the ion-solvent interactions was proposed by Born:

- The ion is regarded as a charged sphere (not just a point charge, but has a given radius)
- The solvent is considered a dielectric continuum, that is a structureless medium only characterized by its value of **dielectric constant**.

This model provides an approximated representation of the solvent, as the effects of the molecular structure in the solvent are not accounted for. In spite of this, the model is useful in providing a measure of the very strong electrostatic ion-solvent interactions.

The electric potential at a distance \mathbf{R} from the center of the sphere in vacuum is:

$$\phi(R) = \frac{1}{4\pi\epsilon_0} \cdot \frac{q}{R}$$

The elementary work required to bring up an element of charge dq from infinity to a distance \mathbf{R} is:

$$dw(R) = \frac{1}{4\pi\epsilon_0} \cdot \frac{q}{R} dq$$

Therefore, the total work required to charge up a sphere of radius \mathbf{R} from zero to q is:

$$w(R) = \int_0^q \frac{1}{4\pi\epsilon_0} \cdot \frac{q}{R} dq = \frac{1}{2} \cdot \frac{1}{4\pi\epsilon_0} \cdot \frac{q^2}{R}$$

It can be generalized for a dielectric medium:

$$E = \frac{1}{8\pi\epsilon_0\epsilon} \cdot \frac{q^2}{R} \equiv \Delta F$$

Which is the energy stored in the surface of the sphere.

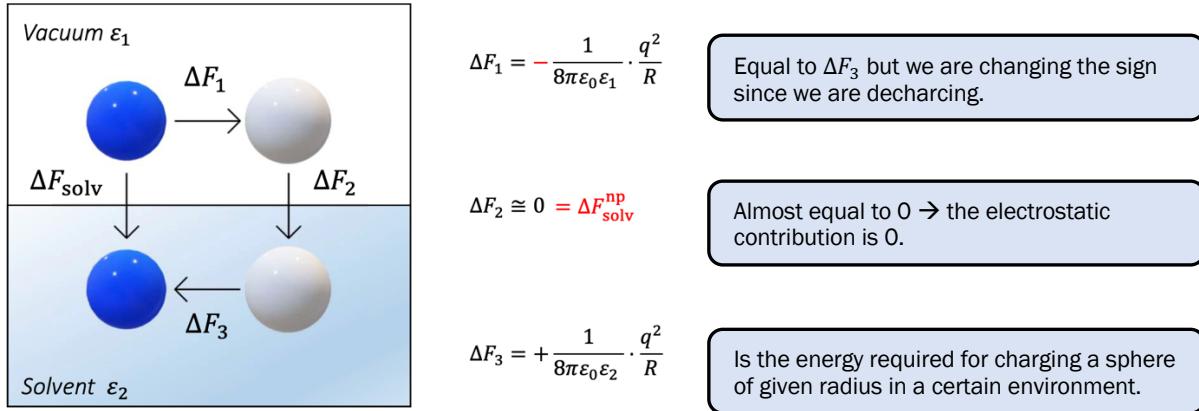
Ion solvation free energy:

The solvation free energy is the free energy required for transferring a solute from vacuum to the solvent and is a general problem that can be addressed through the framework of continuum electrostatics.

We want to estimate the electrostatic contribution to the solvation free energy for an ion having a charge q and radius R .

This kind of problem can be conveniently addressed with a suitable thermodynamic cycle composed by the following steps:

1. “Discharging” the ion in vacuum
2. Transferring the “neutral” ion from vacuum to water
3. “Recharging” the ion in water



If we consider $\Delta F_{\text{solv}}^{\text{el}}$ as the sum of the 3 Δs:

$$\Delta F_{\text{solv}}^{\text{el}} = \Delta F_1 + \Delta F_2 + \Delta F_3 = -\frac{1}{8\pi\epsilon_0\epsilon_1} \cdot \frac{q^2}{R} + 0 + \frac{1}{8P\epsilon_0\epsilon_2} \cdot \frac{q^2}{R} = -\frac{q^2}{8\pi\epsilon_0 R} \left(\frac{1}{\epsilon_1} - \frac{1}{\epsilon_2} \right)$$

Since $\epsilon_1 = 1$, the equation is conveniently written as (**Born model** of solvation, 1920):

$$\Delta F_{\text{solv}}^{\text{el}} = -\frac{q^2}{8\pi\epsilon_0 R} \left(1 - \frac{1}{\epsilon_2} \right)$$

This equation only holds for spherical geometries!

Solvation free energy has both electrostatic and non-electrostatic contribution. For an ion the electrostatic are the most important ones, but ideally we are missing one part: the non-electrostatic contribution factor that is present in the ΔF_2 (but since these are ions the difference is so high that is negligible)

Continuum models of aqueous solutions:

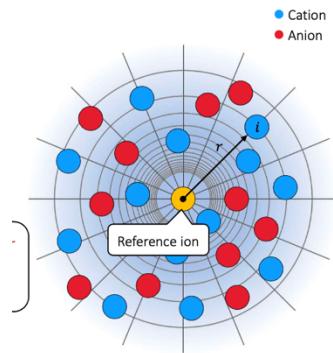
Whenever we talk about charged molecule in solution, we need to consider that in solution there are not just that molecules, but even ions. Is a matter for increasing the realism of the solvent. We need to understand how to treat the electrolyte solutions.

Consider an arbitrarily chosen reference ion taken as the center of polar coordinates:

- It attracts ions with opposite charge (**counter-ions**),
- It repels ions with the same charge (**co-ions**).

After a while a certain **ionic cloud** or **ionic atmosphere** will be generated.

In this way an electric field builds up generated by the electrical potential as a function od the distance $\phi(r)$



The charge carried by the ions is described by the **charge number** (or valence) z_i ($z_{Na^+} = +1$; $z_{Ca^{2+}} = +2$; ...)

The **electrostatic energy** of an ion at a particular point in space with an electric potential $\phi(r)$ is:

$$V(r) = \underbrace{(z_i \cdot e) \cdot \phi(r)}_{\text{Electric charge}} \quad \boxed{\text{Electric potential at distance } r \text{ from the reference ion}} \quad 1e = 1.602 \cdot e^{-19} \text{ C}$$

The **concentration** $c_i(r)$ of an ion i at a point with electrostatic potential $\phi(r)$ can be calculated using the Boltzmann's law of energy distribution:

$$c_i(r) = c_{i,0} \cdot e^{-\frac{V(r)}{k_B T}} = c_{i,0} \cdot e^{-\frac{z_i e \phi(r)}{k_B T}}$$

- $c_{i,0}$ is the concentration of the ion far away from the influence of the reference ion, which means in the bulk solution.
- $\phi(r)$, the electrostatic potential at the point r depends itself on the concentration of the ions in the ionic cloud.

The electric potential determined by a given charge distribution in space can be obtained by solving the **Poisson equation**, a second-order partial differential equation which is a direct consequence of the **Gauss' law**:

<i>Gauss' law</i>		<i>Poisson equation</i>
$\left(\frac{dE_x(x, y, z)}{dx} + \frac{dE_y(x, y, z)}{dy} + \frac{dE_z(x, y, z)}{dz} \right) = \frac{\rho(\mathbf{r})}{\epsilon_0 \epsilon}$		
$\nabla \cdot \mathbf{E}(\mathbf{r}) = \frac{\rho(\mathbf{r})}{\epsilon_0 \epsilon}$	$\xrightarrow{\text{Gradient operator}}$	$\nabla \cdot [\nabla \phi(\mathbf{r})] = -\frac{\rho(\mathbf{r})}{\epsilon_0 \epsilon}$
Divergence operator		Divergence operator

Differential form of the Gauss' law: it provides a local relation between the **electric field** and the **charge density** ($\rho(r)$) enclosed in a differential volume element. It is equivalent to the Coulomb law in that both provide a relationship between a charge distribution and the electric field.

In this equation we have:

- On the right side, as numerator we have the distribution of charges and as denominator the product of permittivity of free space and permittivity of the material in which we are (in the case of vacuum ϵ is negligible)
- On the left, the sum of the component of the electric field along every possible way.

If we combine the equation, we have just described with the gradient operator what we obtain is the **Poisson equation**.

The Poisson equation provides a local relation between the **electrostatic potential** and the **charge density** enclosed a differential volume element.

From the Poisson equation:

$$\text{Laplacian operator} \quad \nabla^2 \phi(\mathbf{r}) = -\frac{\rho(\mathbf{r})}{\epsilon_0 \epsilon}$$

$$\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \right) \phi(\mathbf{r}) = -\frac{\rho(\mathbf{r})}{\epsilon_0 \epsilon}$$

The charge density ρ , measured in $\frac{c}{m^3}$, can be calculated from the ion concentration c_i of the ions in the cloud. The density of ions in the solution can be represented in this way:

The summation runs over the different kinds of ions in the solution. The product of the Avogadro's number (N_A) and the elementary charge (e) is referred as the **Faraday's constant**, F . We can then of course take out the faraday's constant and write the density of the charges in a more compact way.

By combining the definition of charge density of the ions in the solution with the Poisson equation we obtain the so-called **Poisson-Boltzmann equation**:

Poisson-Boltzmann equation

$$\nabla^2 \phi(\mathbf{r}) = -\frac{F}{\epsilon_0 \epsilon} \sum_{i=1}^N c_i(\mathbf{r}) z_i$$

$c_{i,0} \cdot e^{-\frac{z_i e \phi(\mathbf{r})}{k_B T}}$

The $c_i(r)$ is related to the Boltzmann factor so the equation can be converted in this:

$$\nabla^2 \phi(\mathbf{r}) = -\frac{F}{\epsilon_0 \epsilon} \sum_{i=1}^N c_{i,0} z_i \cdot e^{-\frac{z_i e \phi(\mathbf{r})}{k_B T}}$$

From this equation we would like to find the electrical potential $\phi(r)$ but we find it on both side of the equation. In fact the PB is a **non-linear** partial differential equation, which can be solved analytically only under simplified conditions:

1. A solution containing a single kind of ions, $N = 1$;

2. A very low electric potential so that $z_i e \phi(r) \ll k_B T$, and therefore $e^{-\frac{z_i e \phi(r)}{k_B T}} \cong 1 - \frac{z_i e \phi(r)}{k_B T}$

The latter is called the Debye-Hückel approximation, and the resulting equation is the **linearized Poisson-Boltzmann equation**.

In the case of a single kind of ion, the linearized PB equation is:

$$\nabla^2 \phi(\mathbf{r}) = \kappa^2 \phi(\mathbf{r})$$

$\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \right)$

$\kappa = \sqrt{\frac{e^2 N_A}{\epsilon_0 \epsilon k_B T} \sum_{i=1}^N c_{i,0} z_i^2} = \sqrt{\frac{F^2}{\epsilon_0 \epsilon R T} \sum_{i=1}^N c_{i,0} z_i^2} \rightarrow \kappa = \sqrt{\frac{2 F^2 I}{\epsilon_0 \epsilon R T}}$

Debye-Hückel parameter $[m^{-1}]$

Ionic strength of the solution:
 $I = \frac{1}{2} \sum_{i=1}^N c_{i,0} z_i^2$

This equation can be applied in two situations:

1. When there are ions on one side of charged plane,
2. When there is a central ion at the origin of the coordinates and the field has spherical symmetry (Debye-Hückel model).

In the latter case, the Laplacian operator is expressed in spherical coordinates, and the linearized PB equation becomes:

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial \phi(r)}{\partial r} \right) = \kappa^2 \phi(r) \quad \xrightarrow{\text{of the form:}} \quad \phi(r) = A \frac{e^{-\kappa r}}{r} \quad \xrightarrow{\text{Screened Coulomb potential}} \quad \phi(r) \approx \frac{1}{4\pi\epsilon_0 \epsilon} \cdot \frac{z_I e}{r} e^{-\kappa r} = \frac{1}{4\pi\epsilon_0 \epsilon} \cdot \frac{q}{r} e^{-\kappa r}$$

The Debye-Hückel model corresponds to a screened Coulomb potential.

$$\begin{aligned} \phi(r) &= \frac{1}{4\pi\epsilon_0 \epsilon} \cdot \frac{q}{r} e^{-\kappa r} & \phi(r) &= \frac{1}{4\pi\epsilon_0 \epsilon} \cdot \frac{q}{r} \\ \downarrow & & \downarrow & \\ V(r) &= q' \cdot \phi(r) & V(r) &= \frac{1}{4\pi\epsilon_0 \epsilon} \cdot \frac{q \cdot q'}{r} \end{aligned}$$

$$\kappa = \sqrt{\frac{2F^2 I}{\epsilon_0 \epsilon RT}} \quad \xrightarrow{300 \text{ K}} \quad \kappa \cong 3.246 \sqrt{I}$$

[dm^{3/2} mol^{-1/2} nm⁻¹] [mol dm⁻³]

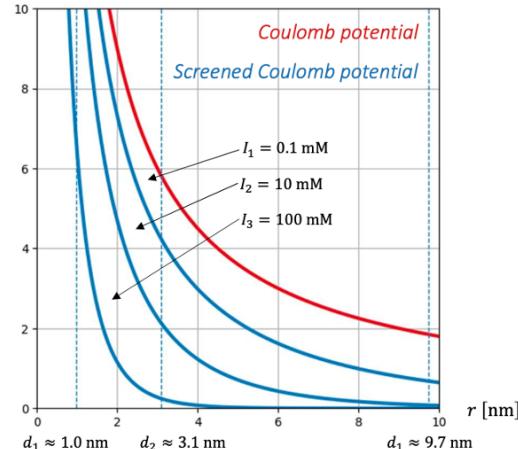
On the right we have the canonical Coulomb potential.

Since has κ the units of [m^{-1}], its reciprocal $1/\kappa$ is therefore a measure of a distance, the so called (**Debye length**).

The **Debye length** emerges as a measure of the thickness of a the ionic cloud that screens the Coulomb potential of the reference ion. For distances greater than the Debye length, the reference ion and its associated screening cloud are effectively neutral, and the charge distribution of the medium can be considered as uniform. **The higher the ionic strength, the smaller the Debye length.** For ionic strength $I = 100 \text{ mM} = 0.1 \text{ M}$, the Debye screening length is $\approx 1 \text{ nm}$.

The model on the right captures that in solution, when there is a charge forming an ionic cloud, this will screen the charge itself. For example, we take a reference charge, if we put a negative ion at 10 nm away the interaction between the two charges will be damped with respect to the simple coulomb potential.

The higher the ionic strength the more efficient will be the screening of the potential.



The ringer's solution:

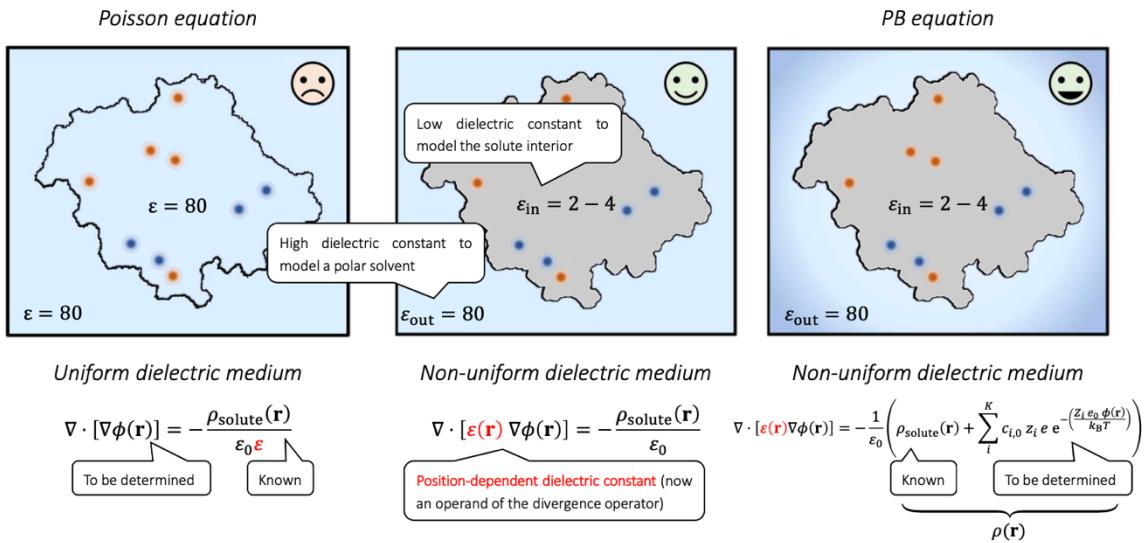
Consider a **physiological Ringer's solution** a solution of several salts dissolved in water with the purpose of creating an isotonic solution to the body fluids:

NaCl	105 mM
KCl	5 mM
Na ₂ HPO ₄	25 mM
CaCl	2 mM

Compute the Debye length at the temperature T=300K:

$$\begin{aligned} I &= \frac{1}{2} [c_{Na^+} \cdot z_{Na^+}^2 + c_{K^+} \cdot z_{K^+}^2 + c_{Ca^{2+}} \cdot z_{Ca^{2+}}^2 + c_{Cl^-} \cdot z_{Cl^-}^2 + c_{HPO_4^{2-}} \cdot z_{HPO_4^{2-}}^2] = \\ &= \frac{1}{2} [0.155 \cdot 1^2 + 0.0005 \cdot 2^2 + 0.002 \cdot 2^2 + 0.114 \cdot (-1^2) + 0.025 \cdot (-2^2)] = \\ &= 0.191 \text{ mol/dm}^3 \\ \kappa &\cong 3.246\sqrt{I} = 1.419 \text{ nm}^{-1}, d \approx 0.705 \text{ nm} \end{aligned}$$

With the Poisson equation we have the theoretical framework for computing the electrostatic potential of biomolecules in a continuum solvent model.



The structure of water and ion hydration:

The term "structure" is used to describe the arrangement of atoms or molecules in space.

In a medium, the number density of atoms is given by: $\rho_0 = N/V$

The number density $\rho(r)$ at a distance r from a reference atom is:

$$\rho(r) = g(r) \rho_0 \quad \Leftrightarrow \quad g(r) = \frac{\rho(r)}{\rho_0}$$

Radial distribution function a form of probability density

"Local" density

"Bulk" density

The radial distribution can be seen also as the ratio between the local density and the bulk density.

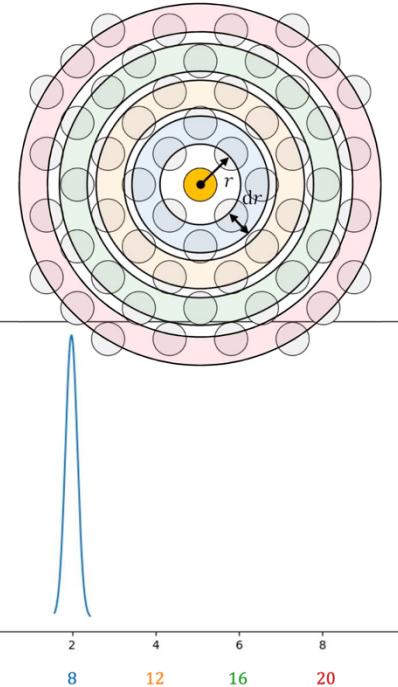
To compute the radial distribution function:

- Pick a reference atom;
- For every value of r , we construct a spherical shell with thickness dr ;
- Count the number of atoms in every shell;

$$g(r) = \frac{1}{\rho_0} \cdot \frac{(N(r + dr))}{V_{\text{shell}}(r + dr)}$$

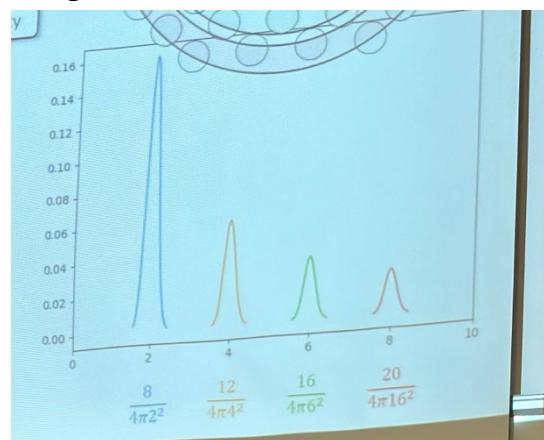
$V_{\text{shell}}(r + dr) \cong 4\pi r^2 dr$

The bracket means that the process is repeated for every atom of the sample (average value)



On the x axis of the graph is the radius, on the y axis there's the number of atoms.

By dividing the number of atoms for the volume of the shell, we obtain a sort of real density and because of this the graph changes.

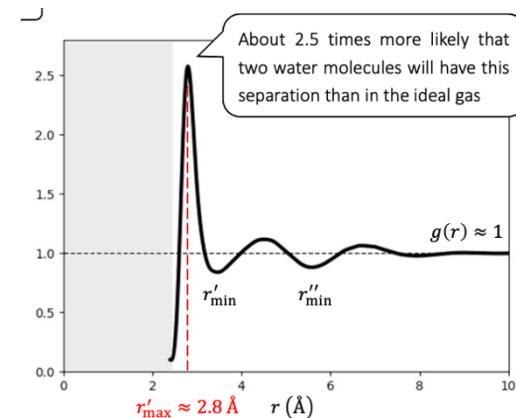


After a certain time the atoms will move away from their original position, that is why there is the average value in the equation.

Ex. Oxygen-oxygen radial distribution function of water measured at room temperature:

- At short distances (less than the atomic diameter) $g(r)$ is zero.
- At large distances $g(r)$ tends to the ideal gas value, indicating that there is no long-range structure.

The reason why at large distances the $g(r)$ tends to 1, is due to the fact that the local density is so far away from the reference atom such that it is impossible to show a visible structure.



The radial distribution function can be understood as a probability density function. 2.8 Å, why? Because the ideal distance of H bonds between hetero atoms is 2.8 Å and 3.2 Å.

the two minima are due to the formation of different shells of water molecules around the reference atom.

The first pick of oxygen atoms in water molecules is due to the presence of the formation of hydrogen bonds between the molecules. At about 2.8 Å we will have the **global maximum**, that represent the higher probability to find another oxygen atom with respect to the reference one.

After the first pick, we can expect a minima. After the minima there would be the formation of another pick of density corresponding to another shell, then another minima. After the last minima there will be another pick, after this however, there won't be a minima because the series is interrupted by the fact that we are so far away from the central atom that the information on the structure are lost.

$$g(r) = \frac{1}{\rho_0} \cdot \frac{\langle N(r + dr) \rangle}{V_{\text{shell}}(r + dr)} \quad \Rightarrow \quad \langle N(r + dr) \rangle = \rho_0 g(r) 4\pi r^2 dr$$

$$\int_0^\infty \rho_0 g(r) 4\pi r^2 dr = (N - 1)$$

So what happens if we integrate until the first minima? What we obtain is the so called **coordination number**:

$$CN = \int_0^{r'_{\min}} \rho_0 g(r) 4\pi r^2 dr$$

When a substance is dissolved in water, the arrangement of molecules near the solute is different from that far away. The alteration of local structure induced by the solute depends on the nature and strength of its interaction with water. In particular, the structure of water is influenced by:

- The size and shape of the solute molecule
- The charge of the solute

Like nonpolar solutes, ions can cause the structuring of water molecules, but the ordering that is induced by ions is different. While the structure around non-polar solutes is driven by the maximization of water-water hydrogen bonds, the structure around ions is driven by the electrostatic interaction of the ion with the nearby water molecules:

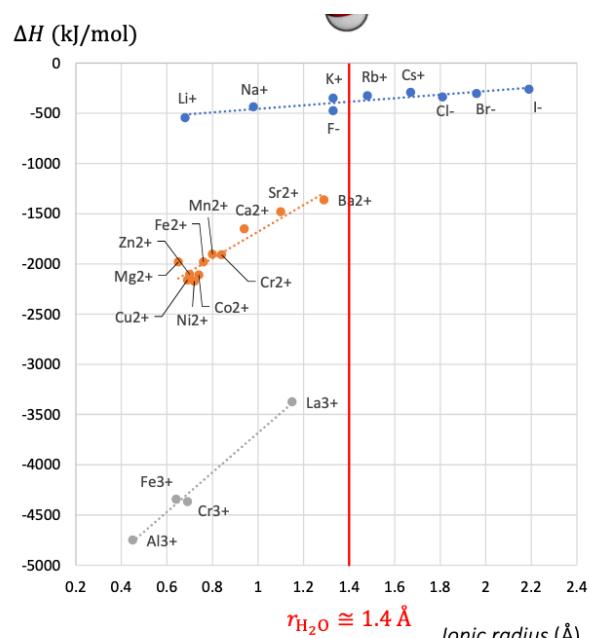
- Oxygen is the negative end of a dipole, so they are attracted to positive ions
- Hydrogens are the positive ends of a dipole, so they are attracted to negative ions.

Inserting non polar solutes strengthens water-water hydrogen bonds while inserting ions can either strengthen or weaken water-water hydrogens bonds (**entropic effects**)

The electrostatic mechanism of water ordering depends not just on the charge of the ion but on its charge density, and a high charge density results either from a high charge on an ion or a small ionic radius. Water molecules bind to small or multivalent ions very tightly (**enthalpic effect**).

Can be seen from a plot, the basically three classes grouped by their ions charged number 1, 2, 3 if we move from 1 to 2 basically we are changing the size enthalpy of the enthalpic effect.

When the radius decreases the enthalpic contribution to the solution energy increases, it is increasing the charge density, meaning that the ions are more favorable to stay in water.



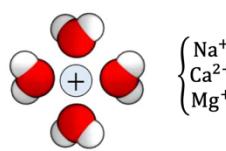
The electric field of ions causes the orientation of water molecules in the proximity of the ion itself, determining a hydration shell.

This orientating force from the electric field of the ion is in competition with the influence of other water molecules and thermal noise. The strength of the electric field decreases with increasing distance from the center of the charge.

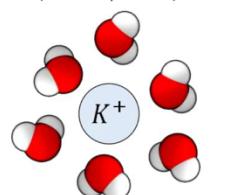
Therefore, at least two regions at different distances from the ion can be considered:

- **Primary hydration shell:** a region with a small number of water molecules that are strongly oriented by the electric field of the ion.
- **Secondary hydration shell:** a region where the electric field is always too weak to fully orient water molecules, but it is strong enough to perturb the normal water structure.

Structure-making ionic hydration
(*kosmotrope* ions) Structure-breaking ionic hydration
(*chaotrope* ions)

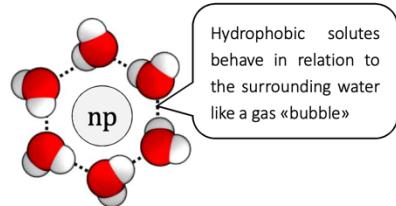


Ions with high charge density strongly orient water's dipoles and cause water molecules to break hydrogen bonds.



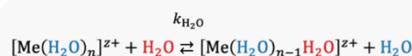
Ions with low charge density cause less electrostatic ordering and allow more hydrogen bonding between water molecules.

Hydrophobic hydration



Neutral solutes cause a high degree of first layer water-water hydrogen bonding.

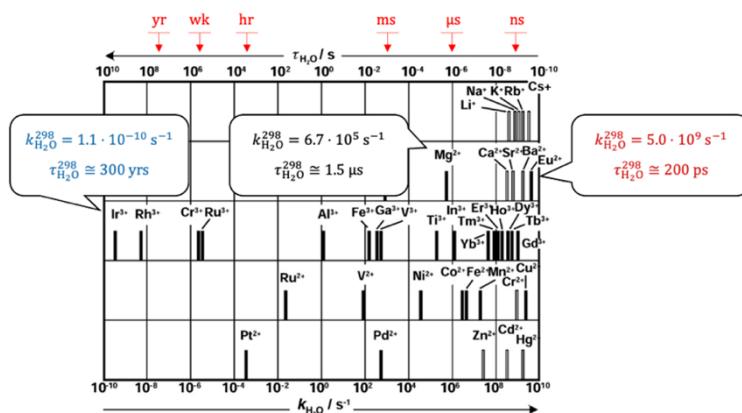
The water exchange between the first and second coordination shell around a metal ion in aqueous solution is the simplest ligand substitution:



Water exchange rate = $n k_{H_2O} [Me(H_2O)_n]^{z+}$

Mean residence time of a particular water molecule in the first coordination shell:

$$\tau_{H_2O} = \frac{1}{k_{H_2O}}$$



The values of $k_{H_2O} [s^{-1}]$ and $\tau_{H_2O} [s]$ vary over almost 20 orders of magnitude! In some cases, the solute ion and its near-neighbor shell of water molecules can be considered as a single chemical species.

Biological membranes:

the cell membrane is a well-organized structure fulfilling a broad spectrum of physiological functions:

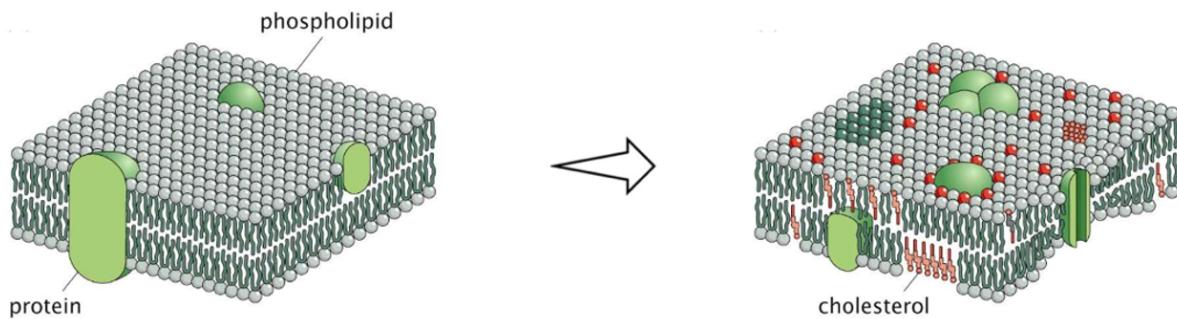
- As a **mechanical structure**, it guarantees the integrity of the cell and influences its shape and movements as well as the displacement of organelles.
- As a **surface**, it forms a dynamic matrix for enzymatic reactions, receptor processes, and immunological recognition.
- As an **electrically isolating leaflet** it contains a plethora of various passive and active devices, controlling membrane potential as well as near-membrane electrodynamic conditions.
- As a **barrier of diffusion**, it controls the ionic composition of the cytoplasm by highly specific channels and transporters.

Considering the spontaneous orientation of molecules in **phase boundaries** is crucial for understanding the mechanism of self-organization and stability of biological membranes.

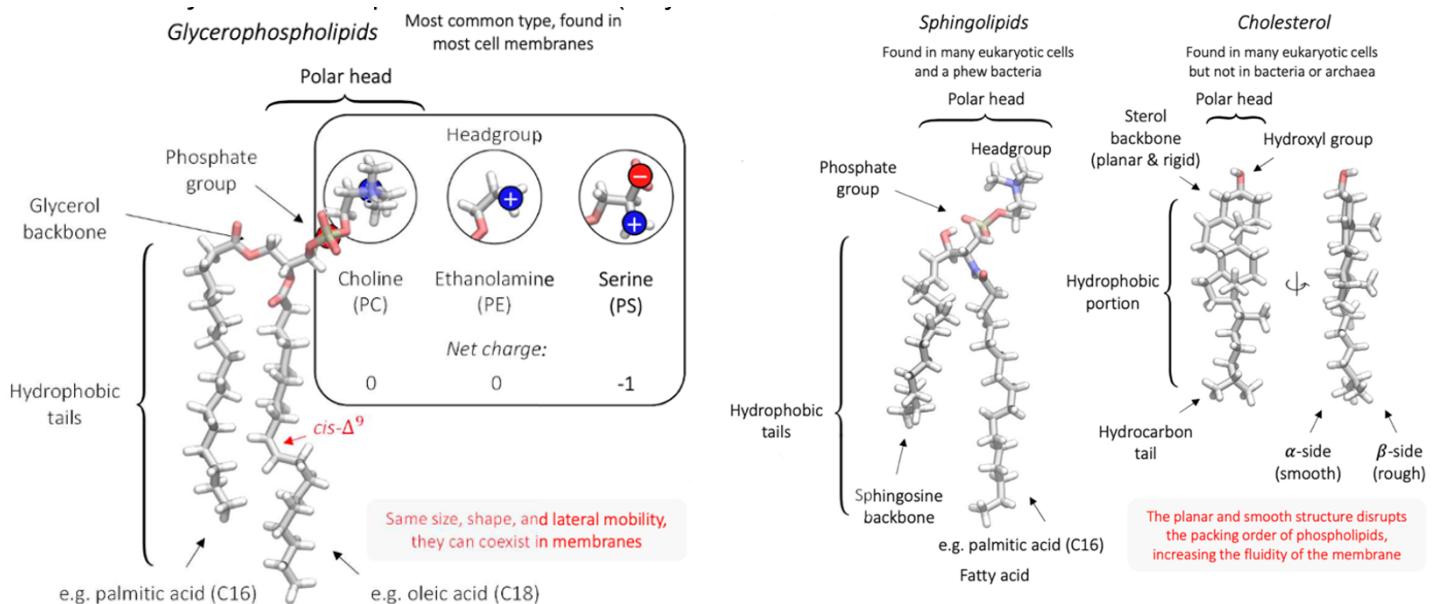
From a mechanical standpoint, membranes are characterized by an extremely tiny aspect ratio, with lateral dimensions typically orders of magnitude larger than their thickness (<5nm). Such shape is determined by the physical properties of the 2 layers of **phospholipids** which are the main constituents of the bilayer.

From the fluid mosaic of Singer and Nicolson (1977) to the latest models, a growing awareness has acknowledged a great deal of structural heterogeneity:

- In the early fluid mosaic hypothesis, the membrane was envisioned as a two-dimensional fluid of phospholipids studded with occasional proteins.
- In the newest hypothesis instead, phospholipids with different chemical characteristics and cholesterol can self-associate to form subdomains (ex. lipids rafts). Proteins also may tend to self-associate within the plane of the membrane and, furthermore, can distort the membrane by locally altering its thickness or composition.

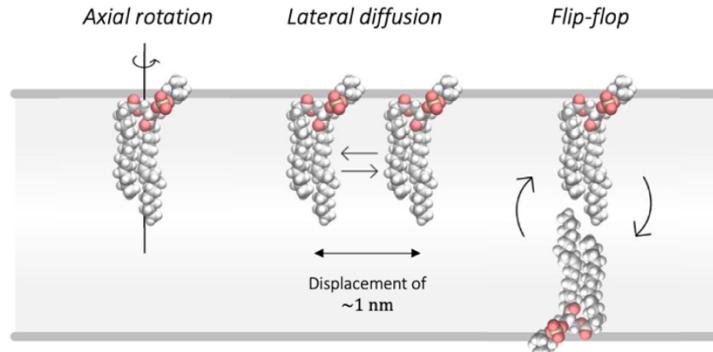


There are few major classes of lipids in membranes (they all are **amphiphilic molecules**):



The mechanical properties of the biological membranes are very important for understanding a number of cell physiological functions, such as cell movement, division, vesiculation, and membrane fusion.

The motion of lipids in biological membranes can be decomposed into:



The mobility of proteins, in contrast, is strongly limited by their fixation on the cytoskeleton.

There are three main classes of **membrane deformations**:

- **In-plane stretching**, change in surface area ΔA .
- **Out-of-plane stretching**, change in thickness, ΔW .
- **Bending**, change in curvature.

In-plane stretching, the energy cost to extend the membrane area A_0 by the amount ΔA :

$$E_{\text{stretch}}^{\parallel} = \frac{K_a}{2} \cdot \frac{\Delta A^2}{A_0}$$

Area-stretch modulus (energy per area):
 $K_a \approx 55 - 70 \text{ } k_B T/\text{nm}^2$
 $230 - 290 \text{ mN/m}$

To find a relationship between the membrane properties of macroscopic materials, K_A (constant of proportionality) must be divided by the thickness of the membrane ($w_0 \approx 4.0 \text{ nm}$):

$$Y = \frac{K_a}{w_0} \approx 62.5 \cdot 10^6 \text{ N/m}^2 \equiv 62.5 \cdot 10^6 \text{ MPa}$$

Young modulus: specific modulus of elasticity

$$\sigma = Y \frac{\Delta A}{A}$$

Stress (force/area)

Strain

$$Y_{\text{steel}} \equiv 2 \cdot 10^5 \text{ MPa}$$

$$Y_{\text{rubber}} \equiv 1 \cdot 10^5 \text{ MPa}$$

Overall the membrane is not particularly stretchable, it undergoes rupture when it is expanded by more than 2-5% of its area.

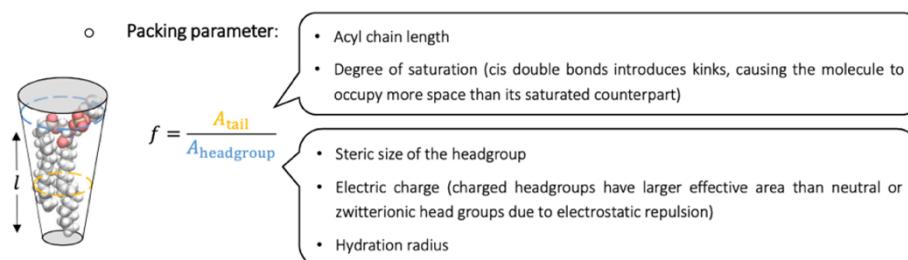
In bending, the energy cost to bend the membrane per unit area (mean curvature, $1/R$):

$$e_{\text{bend}} \approx \frac{K_b}{2} \cdot \frac{1}{R^2}$$

Bending modulus: $K_b \approx 10 - 20 \text{ } k_B T$

A spontaneous curvature of the membrane occur whenever there is a difference in area between the two leaflets:

1. Differential distribution of charged molecules depending on the nature of the ionic group (amino group vs. quaternary ammonium cation).
2. Differential packing due to anisotropic shape of the lipids



We can also have **protein-induced** membrane deformations. We can envision two kind of these deformations. In both cases, the free energy cost of membrane deformation can be calculated in the form of a free energy density (free energy per deformed area of the membrane). In the case of the hydrophobic mismatch, where there is a free energy penalty associated with “gluing” the hydrophobic lipid tails to the hydrophobic region of the membrane protein, the free energy density increases as the square of the hydrophobic mismatch. Similarly, proteins with conical shape will bend the bilayer in their vicinity, introducing a midplane bending which increases quadratically as a function of the bilayer midplane bending angle. In general, the membrane has both a Gaussian and a spontaneous curvature that may or may not stabilize a preferential state of the channel.

