UNIVERSITÀ DEGLI STUDI DI MILANO

A mechano-statistical perspective of biomolecules in motion: towards building an *in-silico* microscope

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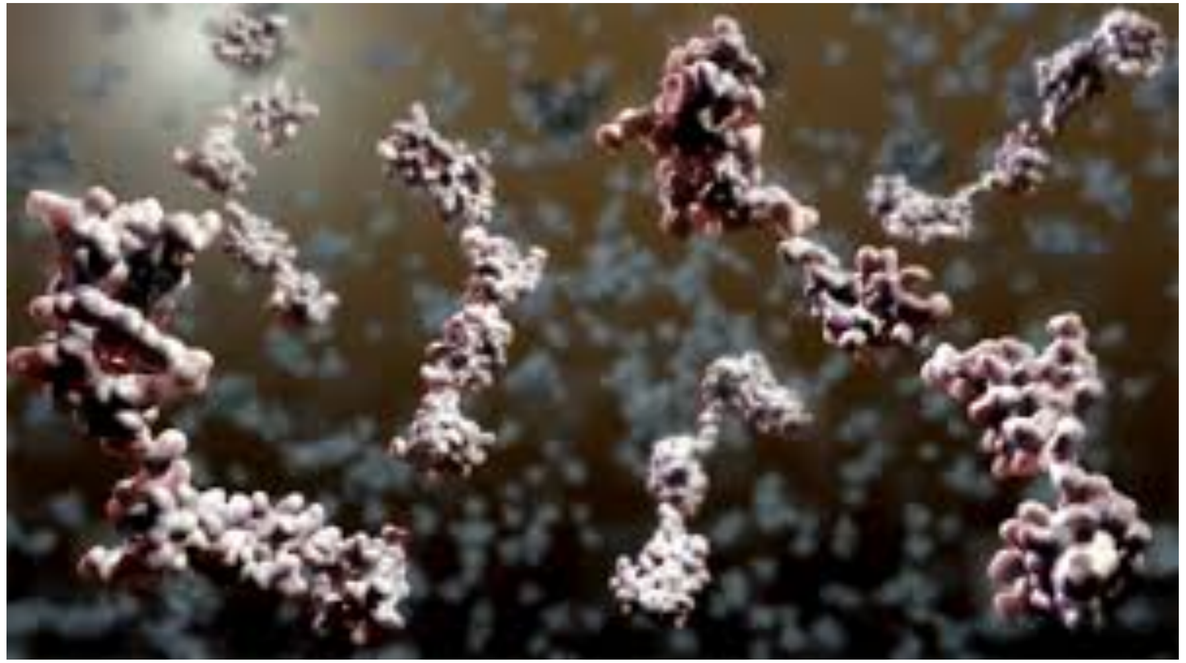
Why a Statical and Mechanical perspective

When we observe/describe biomolecules we generally handle many molecules, made of many atoms, that can:

- change their shape;
- move in 3D space;
- have interactions;



To describe all these we need to know how do they move and interact (mechanics) and how to handle large numbers (statistics)

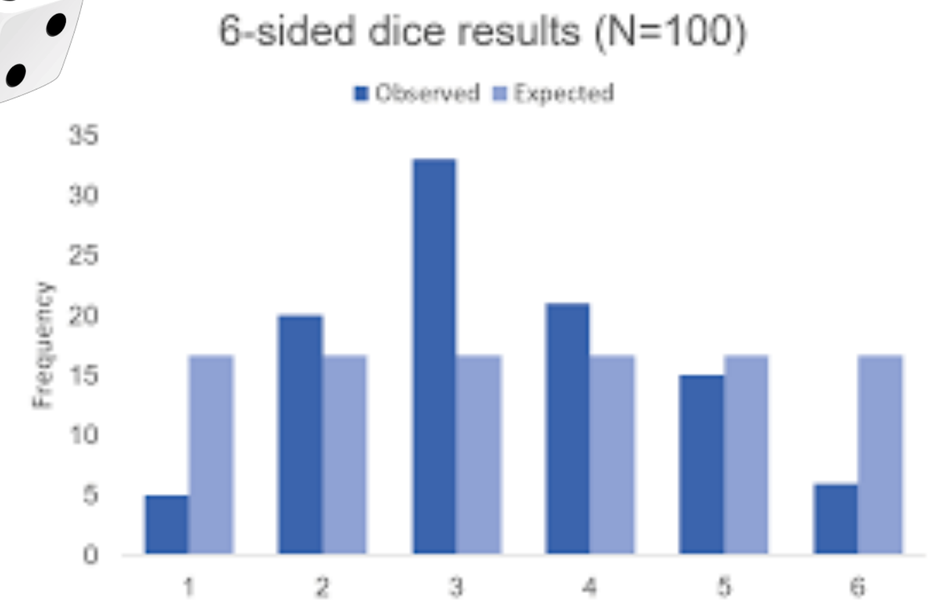
Why a Statical and Mechanical perspective

In the next I want to show you how statistical observations can be interpreted using the language of mechanics: energy.

Probabilities are estimated using histograms: the problem of sampling relative and absolute probabilities

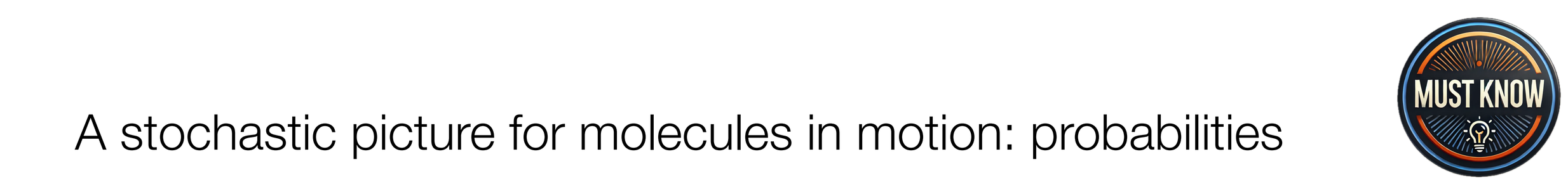
Stochastic events are estimated by SAMPLING. Sampling means exploring a probability distribution looking at many outcomes organised as histograms.

Absolute probability needs to have informations on all outcomes, relative probabilities are just frequency ratios:



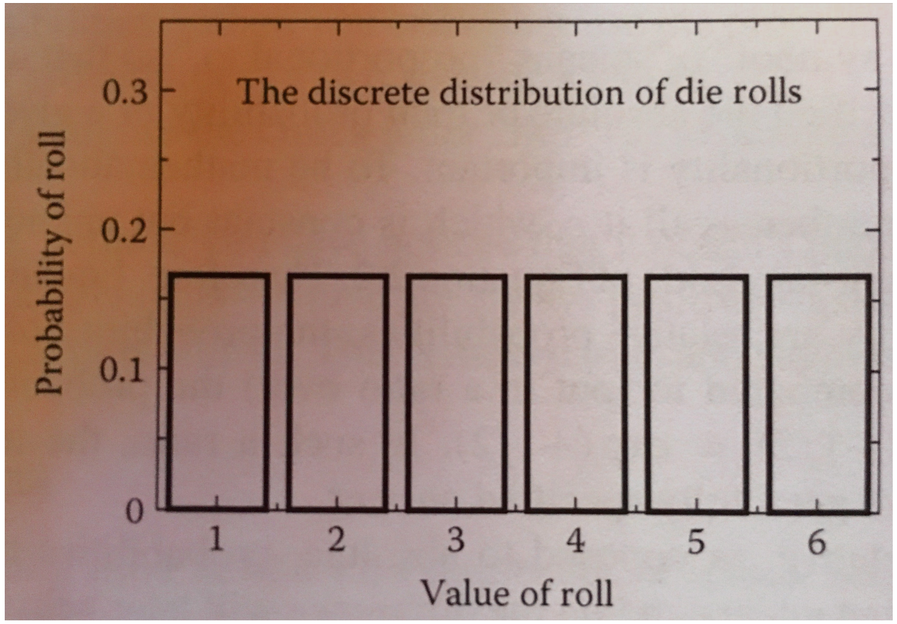
Absolute probability: p(i) = count(i)/(sum_k(count(k)))
Relative probability: p(j)/p(i) = count(j)/count(i)

A stochastic picture for molecules in motion: probabilities



Stochastic means randomly determined, events happen by chance, by observing many events one can learn **what is the probability of the different outcomes** that is one can learn their **probability distribution**.

If some outcome is more likely than another one could think in terms of energy that that outcome is favourable in **energy**.



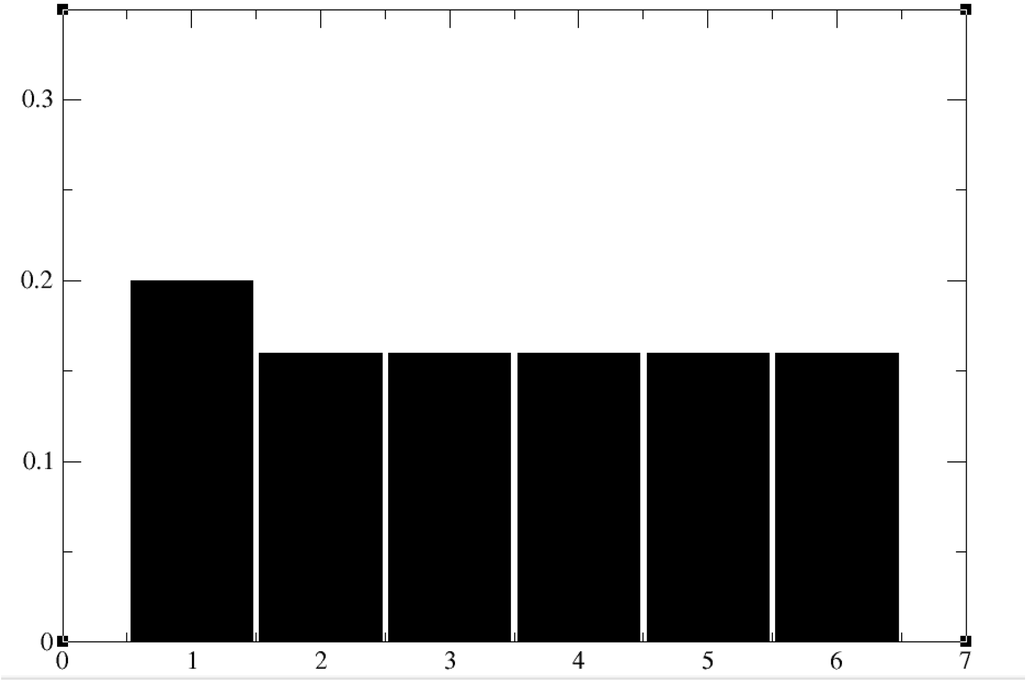
p(j)=1/6 with j=1 to 6 **Discrete**

$$\sum_{j=1}^{6} p(j) = 1$$

Normalised
When all possibilities are accounted for the probability is 100%

A stochastic picture for molecules in motion: biases

If your dice is biased towards a given number, let's say 1, then your histogram will be higher at 1. In terms of energy you can think that the state 1 is energetically more favourable.



$$\sum_{j=1}^{6} p(j) = 1$$

Normalised
When all possibilities are accounted for the probability is 100%

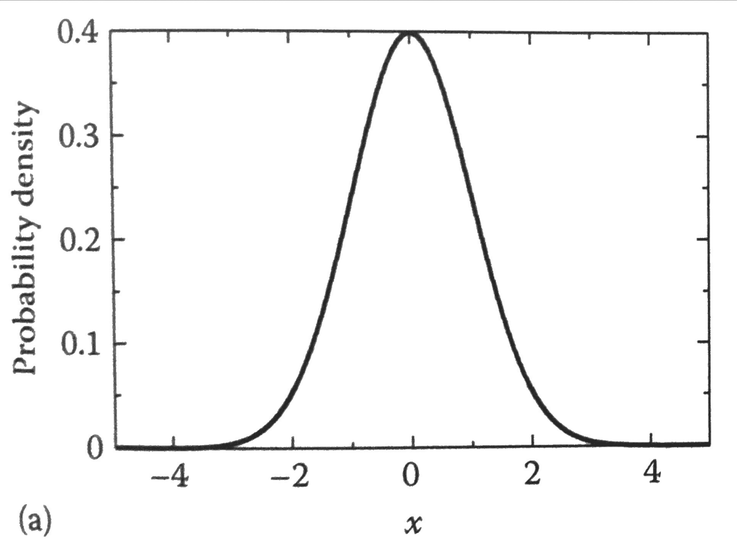
To bias a dice you need to change some of its mechanical properties, that generally speakin will have to do with it being thronw under the influence of gravity

Continuos Probability Distributions are also estimated using histograms

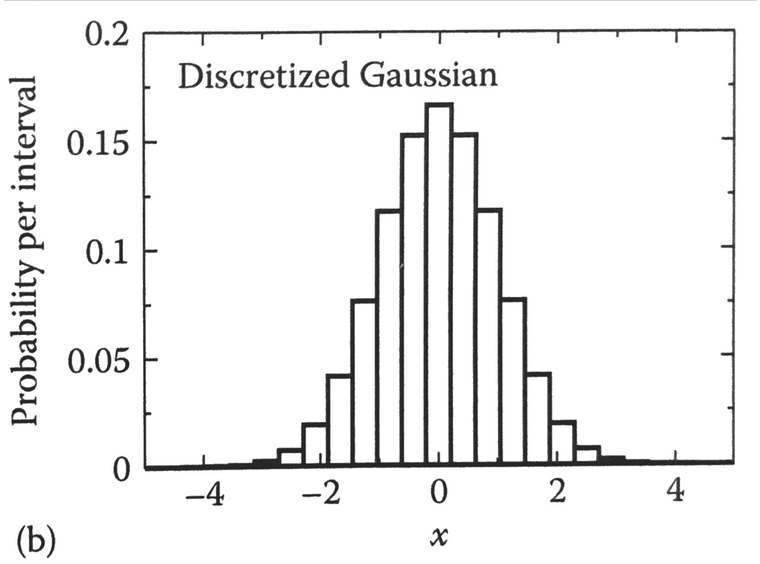
$$\rho_G(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-<x>)^2}{2\sigma^2}}$$

Gaussian

Even if we know only proportionality this can be enough to know make comparisons: the probability of p(0)/p(1). This is clear if you think about histograms.



Density, they are in units^{-1}



To calculate a probability one multiples for a small interval.

Average, Standard Deviation, Standard Error of the Mean

In practice we **estimate** averages using sums:

$$< f > \doteq \frac{1}{N}\sum_{i=1}^{N} f(x_i)$$

Variance and Standard deviation:

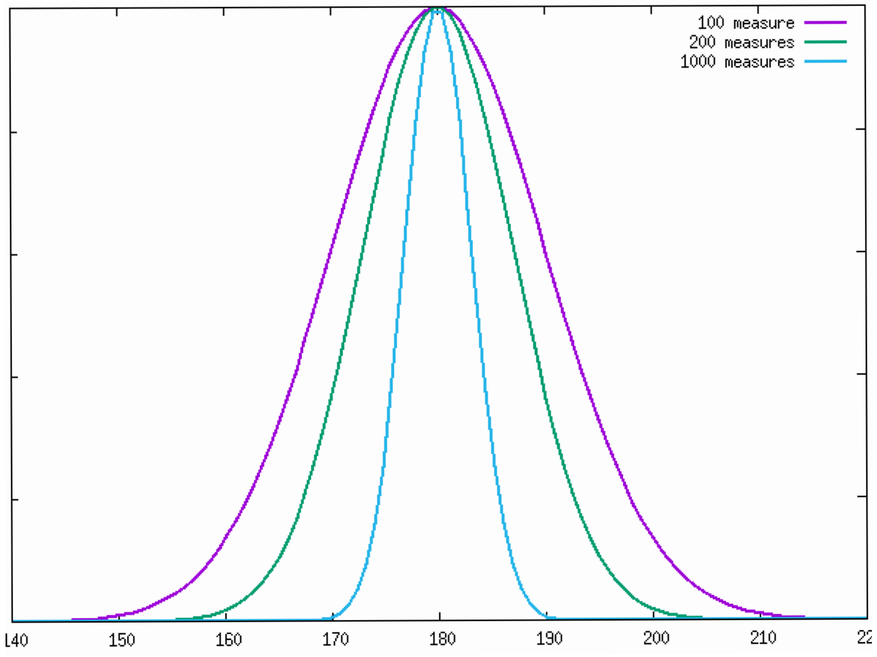
$$\sigma^2 = var(f) = < (f - < f >)^2 > = \int dx (f(x) - < f >)\rho(x) \doteq \frac{1}{N-1}\sum_{i=1}^{N}(f(x_i) - < f >)^2$$

Tell us about the width of the distribution ρ(x).

Standard error:

$$\text{Std-err} = \sqrt{\frac{var(f)}{N}} = \frac{\sigma}{\sqrt{N}} = \sqrt{\frac{1}{N^2 - N}\sum_{i=1}^{N}(f(x_i) - < f >)^2}$$

Tell us about **accuracy of our estimate of the average,** the idea is that our average value that we estimate from multiple observations has a resolution with the shape of a Gaussian with the width of the standard error.

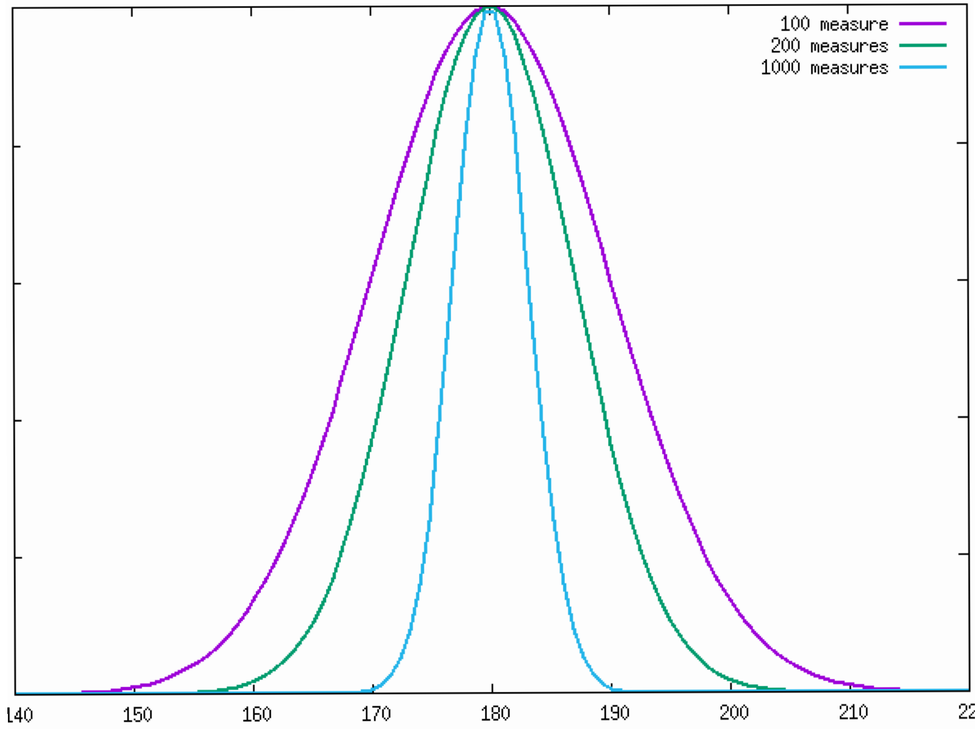


Average, Standard Deviation, Standard Error of the Mean

$$< f > \doteq \frac{1}{N}\sum_{i=1}^{N} f(x_i) \qquad \sigma^2 = var(f) =< (f - < f >)^2 >= \int dx (f(x) - < f >)\rho(x) \doteq \frac{1}{N-1}\sum_{i=1}^{N}(f(x_i) - < f >)^2$$

$$\text{Std-err} = \sqrt{\frac{var(f)}{N}} = \frac{\sigma}{\sqrt{N}} = \sqrt{\frac{1}{N^2 - N}\sum_{i=1}^{N}(f(x_i) - < f >)^2}$$

The average of a random process is always distributed as a gaussian of width std-err and so you can set confidence intervals: (±1std-err is 68%, ±2std-err 95%, ±3std-err 99%, …). This is called the theorem of the central limit.



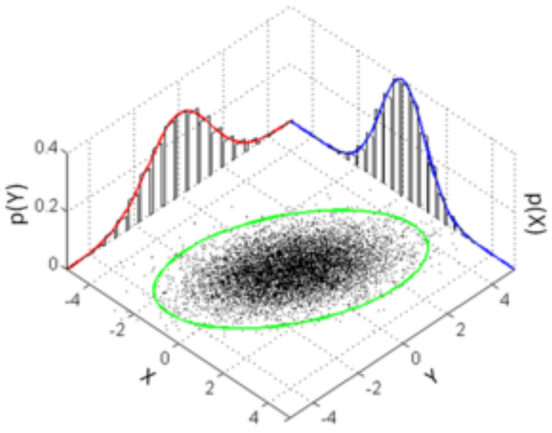
Multidimensional distribution function: projection and correlation

Probability distributions can be more than 1D. For example the probability distribution of a molecule around a receptor may be studied in terms of (x,y,z) coordinates of the center-of-mass, in this case would be 3D.

$\int \rho(x,y)dxdy = 1$ Normalisation

How can we show a multi dimensional distribution in less dimensions? By projecting it:

$\rho(x) = \int \rho(x,y)dy$ Projection/Marginalisation



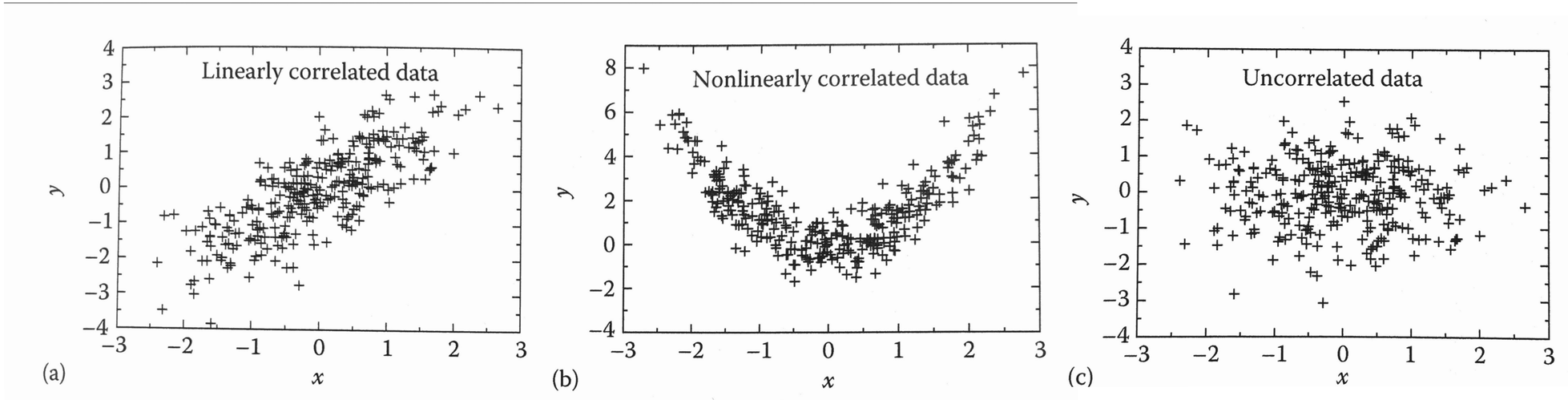
$$< x >= \int dxdyx\rho(x,y)$$

$$< y >= \int dxdyy\rho(x,y)$$

$$< xy > \int dxdyxy\rho(x,y)$$

By looking at the data can you think of a projection that is more important to understand the data?

Multidimensional distribution function: correlation



$$< x >= \int dxdyx\rho(x,y)$$

$$< y >= \int dxdyy\rho(x,y)$$

$$< xy > \int dxdyxy\rho(x,y)$$

If two variables are independent than

$$\rho(x,y) = \rho(x)\rho(y) \quad \textbf{So}$$

$$< xy >=< x >< y >$$

So we have correlation if

$$< xy > - < x >< y > \neq 0$$

Pearson correlation coefficient:

$$r = \frac{< xy > - < x >< y >}{\sigma_x \sigma_y}$$

Statistical Mechanics: the Boltzman distribution gives the probability to observe a molecule in a given conformation

What is the connection between statistics and mechanics?

That is the Boltzmann equation: $$pdf(x,v) \equiv \rho(x,v) \propto \exp\left[\frac{-E(x,v)}{k_B T}\right] = \exp\left[\frac{-U(x)}{k_B T}\right] \exp\left[\frac{-K(v)}{k_B T}\right]$$

Let's look at this pdf in more detail: $$pdf(x) \equiv \rho(x) \propto \exp\left[\frac{-U(x)}{k_B T}\right]$$

$$pdf(v) \equiv \rho(v) \propto \exp\left[\frac{-K(v)}{k_B T}\right] = \exp\left[\frac{-\frac{1}{2}mv^2}{k_B T}\right]$$

Conformations and velocities are independent. This means that we can study them separately. The distribution of the velocities does not affect the distribution of the configurations. Furthermore the distribution of the velocity is Gaussian, so it is easy to integrate it.

So for all practical purposes we can just ignore the velocity: $$pdf(x) \equiv \rho(x) \propto \exp\left[\frac{-U(x)}{k_B T}\right]$$

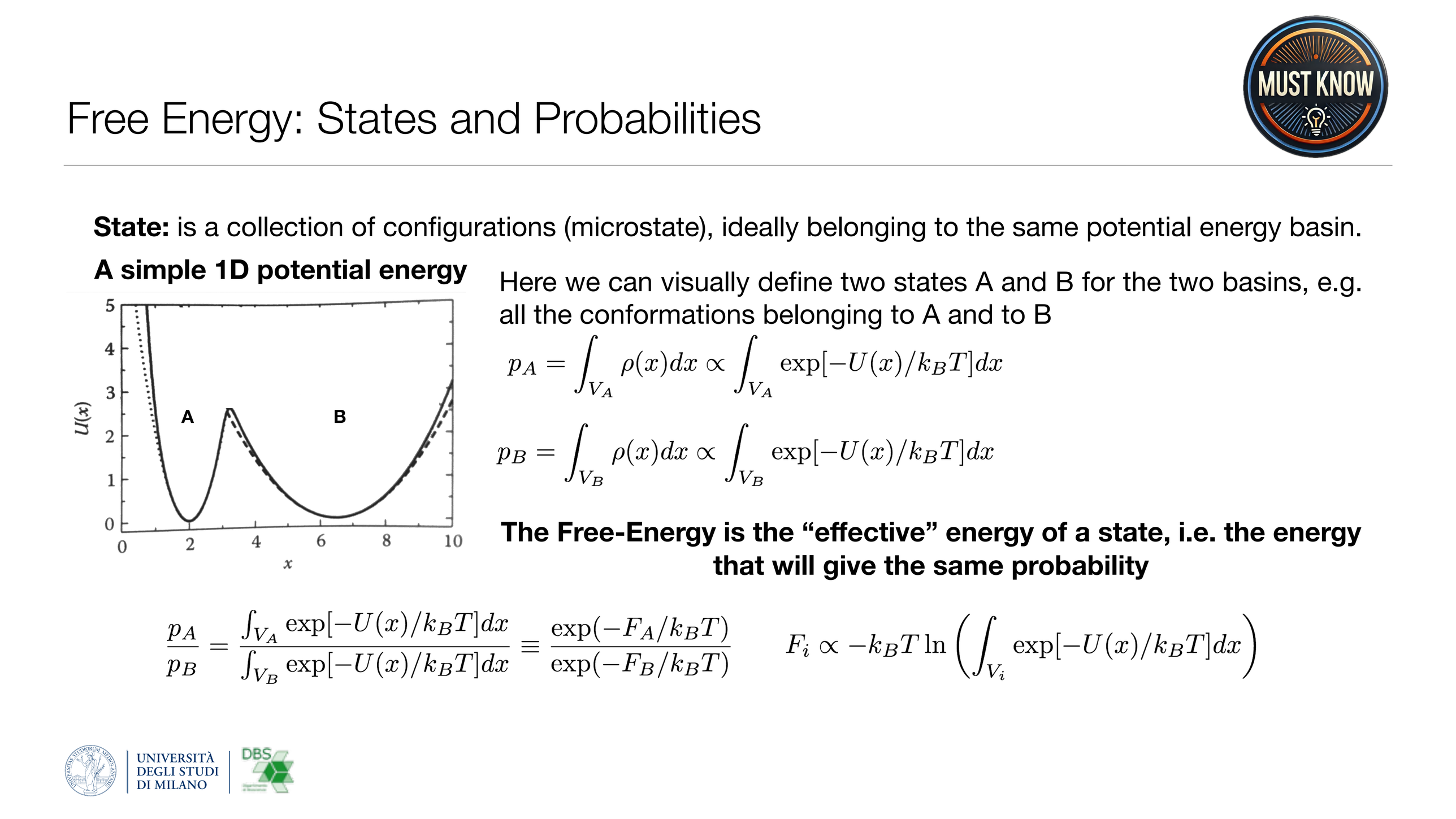
This gives a link between the energy of a conformation and the probability of observing it

Statistical Mechanics: Ergodicity

1. **A single molecule (at constant temperature) will move through all the possible conformations at random due to the collisions with the solvent. Each conformation is gonna be populated accordingly to the energy and temperature (the interaction with environment).**
2. **If at a given time one stops the movie of many many copies of the same system in the same condition the picture taken will display conformations according to the same statistics.**

The collection of conformations in both cases (that is the sampling) is usually called an ensemble of conformations. This ensemble is characterised first of all by the conditions in which it has been acquired (constant temperature, pressure or volume, constant number of particles, ecc)

Free Energy: States and Probabilities



State: is a collection of configurations (microstate), ideally belonging to the same potential energy basin.

A simple 1D potential energy

Here we can visually define two states A and B for the two basins, e.g. all the conformations belonging to A and to B

$$p_A = \int_{V_A} \rho(x)dx \propto \int_{V_A} \exp[-U(x)/k_BT]dx$$

$$p_B = \int_{V_B} \rho(x)dx \propto \int_{V_B} \exp[-U(x)/k_BT]dx$$

The Free-Energy is the "effective" energy of a state, i.e. the energy that will give the same probability

$$\frac{p_A}{p_B} = \frac{\int_{V_A} \exp[-U(x)/k_BT]dx}{\int_{V_B} \exp[-U(x)/k_BT]dx} \equiv \frac{\exp(-F_A/k_BT)}{\exp(-F_B/k_BT)}$$

$$F_i \propto -k_BT \ln\left(\int_{V_i} \exp[-U(x)/k_BT]dx\right)$$

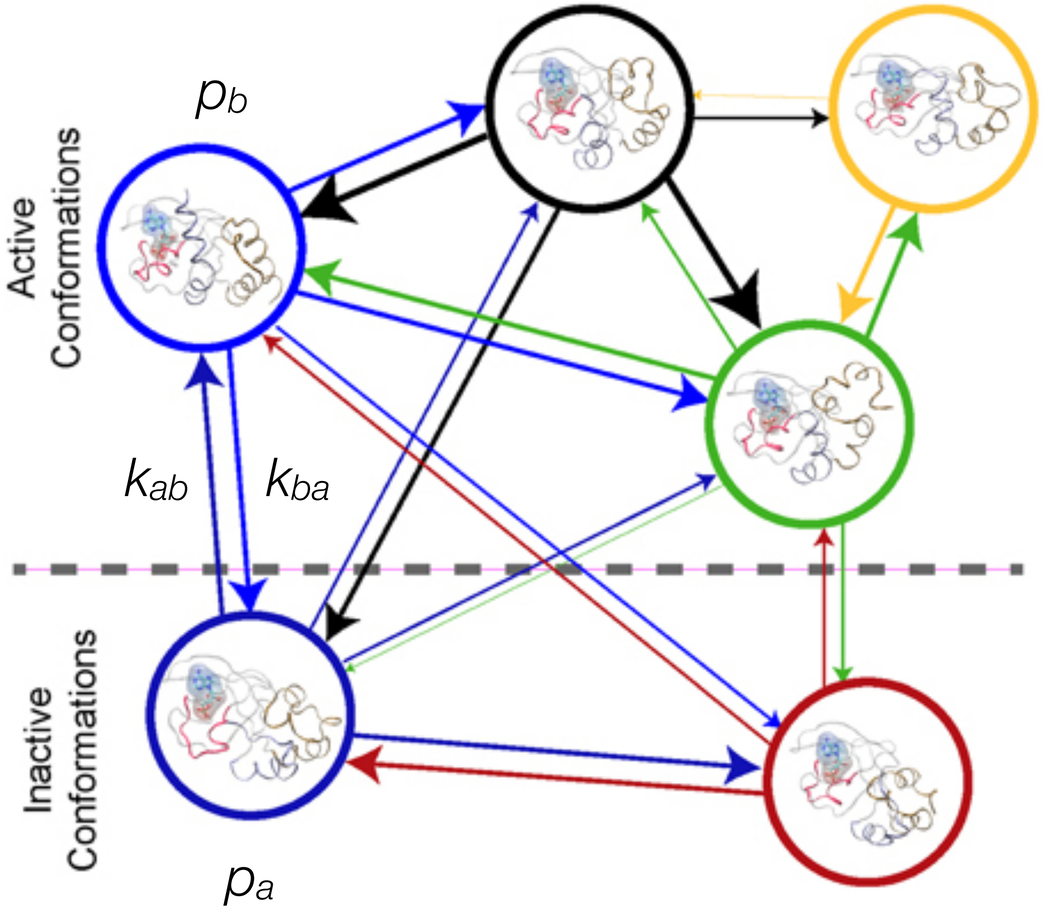
Summary: relative probabilities, energy and time

Microscopic processes are guided by **energy**, between two microscopic state their relative probability is associated exponentially to their energy difference.

Macroscopic processes (made of many microscopic one) are guided by **free-energy**, between two macroscopic state their relative probability is associated exponentially to their free-energy difference, the number of microscopic states making a macroscopic state is the entropy of the macroscopic state.

Macroscopic Processes happen in a given average time that is associated the free-energy barrier separating them

Summary: A stochastic picture of molecules in solution



Macrostate populations are associated with free energy differences:

$$p_i \propto \exp\left(-\frac{G_i}{RT}\right)$$

Exchange rates are associated with free energy barriers:

$$k_{ij} = k_0 \exp\left(-\frac{G_{ij}^{\ddagger}}{RT}\right)$$

Energy for these processes is provided by thermal energy, so essentially by collision with water molecules

An equilibrium bulk experiment ideally capture the contribution from all the states:

$$< O >= \sum_i p_i O_i = \frac{\sum_i O_i \exp\left(\frac{-G_i}{RT}\right)}{\sum_i \exp\left(\frac{-G_i}{RT}\right)}$$

Microscopically:

$$G_i = -RT \ln \int \exp\left(-\frac{U(r)}{RT}\right) \delta(i - i(r)) dr$$

Ligand binding: a molecular example

Here energy and probability are intuitively linked, furthermore, events happen on some **time scale**, so one would like to find a relationship between probability, energy and time scales of events.

$$R + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} RL$$

In a ligand-receptor binding process we can picture the process, at least in some cases, as a probability for R and L to be unbound vs a probability to be bound (RL) and consider the time scale of the process in terms of rates of binding (on) and unbinding (off).

The k_{off} rate is the frequency of unbinding (s^{-1}) so that $k_{off}[RL]$ is the number of unbinding events per second.

The k_{on} represent instead two processes (1) collision and (2) complexation. So this is the collision frequency times the complexation probability, this means that binding depends on concentration, indeed k_{on} is measured in (s^{-1} M^{-1}). $k_{on}[R][L]$ is the number of binding events per second, while $k_{on}[L]$ is the frequency of binding for a given concentration of ligand (excess of ligand).

A stochastic picture: ligand binding

$$R + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} RL$$

If we start from a solution of free [R] and [L] we start forming RL with rate k_{on}, once we have some [RL] this can also dissociate with rate k_{off}, after some time we will be at equilibrium that is that the concentration of [R], [L] and [RL] will not change anymore.

$$K_d \equiv \frac{k_{off}}{k_{on}} = \frac{[R][L]}{[RL]} = C^0 \frac{p_{unbound}}{p_{bound}}$$

The **dissociation constant** is can then be defined as the ratio between k_{off} and k_{on}, it is measured in (M), where lower values means stronger binding (i.e. lower concentrations of ligand are enough to bind)

$$C^0 = 1\ M$$

$$\Delta G_{\text{binding}} = k_{\text{B}} T \ln\left(\frac{K_{\text{d}}}{C^0}\right) = k_{\text{B}} T \ln\left(\frac{k_{\text{off}}}{k_{\text{on}} C^0}\right)$$

$$= k_B T \ln\left(\frac{p_{unbound}}{p_{bound}}\right)$$

This is the binding free-energy that is the amount of work that can be extracted or that must be provided for the binding reaction to happen, and you see is related to the rates as well as to the probabilities

A stochastic picture: protein folding

$$U \underset{k_u}{\overset{k_f}{\rightleftharpoons}} F$$

The k_u rate is the frequency of unfolding (s^{-1}) so that $k_u[P]$ is the number of unfolding events per second.

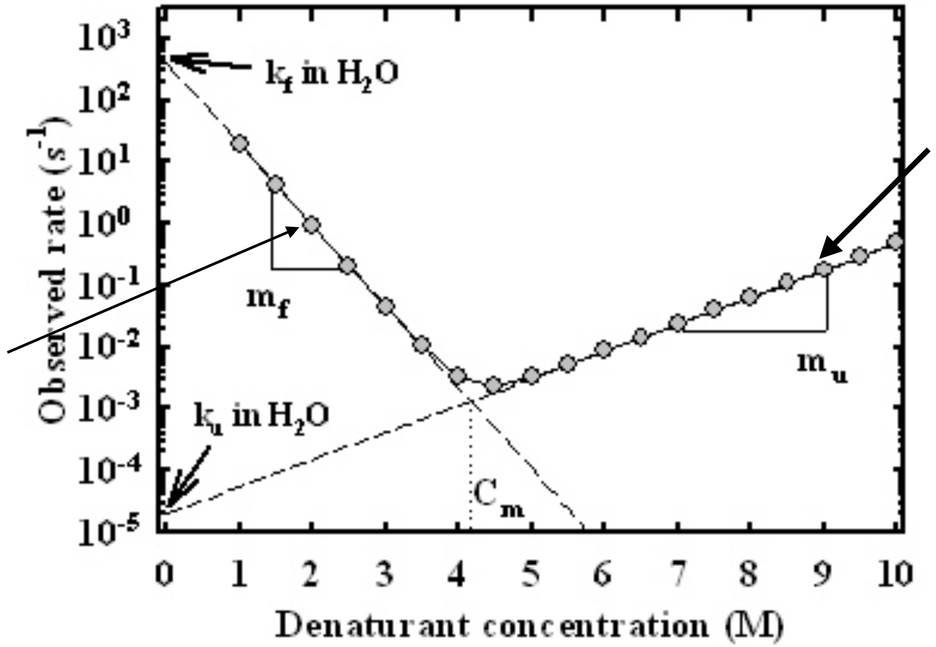
The k_f rate is the frequency of folding (s^{-1}) so that $k_f[P]$ is the number of folding events per second.

Their ratio define the $K_D=k_u/k_f$. This can used to calculate the difference in free energy:

$$\Delta G_{Eq} = RT \ln K_D$$

The two rates can be measured by changing the concentration of denaturants by a stopped-flow and monitoring fluorescence.

Protein in high-conc of denaturant is mixed with buffer to a target final low concentration of denaturant



Protein in buffer is mixed with denaturant to a target final high concentration of denaturant

At high C all the protein unfold $k_{obs}=k_u$ at low C all the protein fold so $k_{obs}=k_f$

The common theme of Structural Bioinformatics: sampling and energy (or score)

In the next lectures we will see how the problem of SAMPLING and the problem of the ENERGY or SCORING FUNCTION, are interconnected and always present. These are the problem to cope with when doing MD simulation, structure prediction, docking, protein design, …

You can think at the next lectures as to specific strategies to cope with these two problems when trying to address specific problems. All the algorithms we will see are related to them (MD, MC, EM, ML/AI, …)

Questions:

- How do you calculate the average outcome of a stocastic process?
- What is the difference between the standard deviation and the standard error of the mean?
- Which is the probability distribution function of the average of a process?
- What is the difference between the absolute probability of an event and the relative probability of that event with respect to another?
- What is the relationship between the probability of a macrostate and its free-energy?
- What is the relantionship between the probability of a configuration and its energy?
- What is the free energy change of a process?
- …