Core Concepts

- The cellular world is not homogeneous nor isotropic, so simple random walks are not enough

- It’s not only in Quantum Mechanics that the observer changes the system

- The strength of a molecular bond depends on loading rate because thermal fluctuations are a relevant energy scale

- Simulations allow us to solve problems that cannot be done analytically or numerically (unknown potential)

RWs in a potential landscape

Many dynamic problems in cells are more complex than simple Brownian motion:

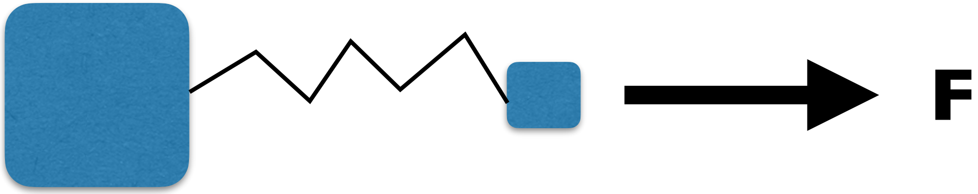
- a bond between two molecules diffusing together in the cytoplasm breaks
- a lipid molecule leaves the membrane and enters bulk solvent
- a chemical reaction converts reactants into products
- a molecular motor pulls a transport vesicle along a filament
- a white blood cells crawls along a vessel wall
- cells adhere by focal adhesion contacts

These problems, and others, can be solved by considering a particle executing a RW in a potential landscape where barriers occur. The interpretation of the potential, the barriers, and the particle (or **reaction coordinate**) varies for each problem but the maths is the same.

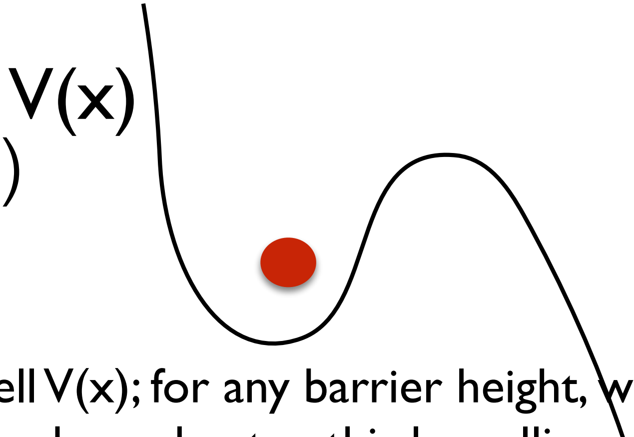
How do we extend our *"RW as a model of Brownian motion"* tool to solve problems like these where there is a combined effect of thermal fluctuations and applied forces?

RWs in a potential landscape

Consider a non-covalent bond binding two molecules



Q. What is the mean bond strength?



$$r \sim \exp(-dV/kT)$$

$$V(f) = V0 - f.x$$

Let the bond be represented as a particle in a potential well V(x); for any barrier height, we can define a mean escape time (~ bond strength) but we can perhaps shorten this by pulling on the particle with a force F(t).

The *overdamped* Langevin equation for this problem is (acceleration term = 0):

$$M.\cancel{d^2x}/dt^2 + \gamma.dx/dt = -dV(x)/dx + F(t) + \sqrt{D}.\Gamma(t)$$

where γ is the friction coefficient, V(x) the potential function, F the applied force, and D the strength of the white noise $\Gamma(t)$ which determines the diffusion rate.

RWs in a potential landscape

We could solve the problem by integrating this stochastic differential equation:

$$x(t + dt) = x(t) + 1/\gamma \, (-dV(x)/dx \; + F(t)).dt + \sqrt{D/\gamma}.\, N(0, 1).\sqrt{dt}$$

with the Recipe:

- Specify the potential landscape V(x)
- Specify the pulling force as a function of time F(t)
- Choose the boundary conditions and initial position x(0)
- Numerically integrate the above Langevin equation until the particle escapes or a maximum number of time steps has passed.

e.g., $V(x) = A.x^2.(1-x)$ and set $F(t) = 0$ and $\gamma = 1$ for simplicity. Start with $x(0) = 0$, and say that the particle has "escaped" when it reaches $x \sim 5$. Repeating the simulation many times with different initial conditions (i.e., random number seed for the generator) allows us to estimate the mean and variance of the escape time. Then we could choose a force F(t) and repeat.

Or, we could do a simulation …

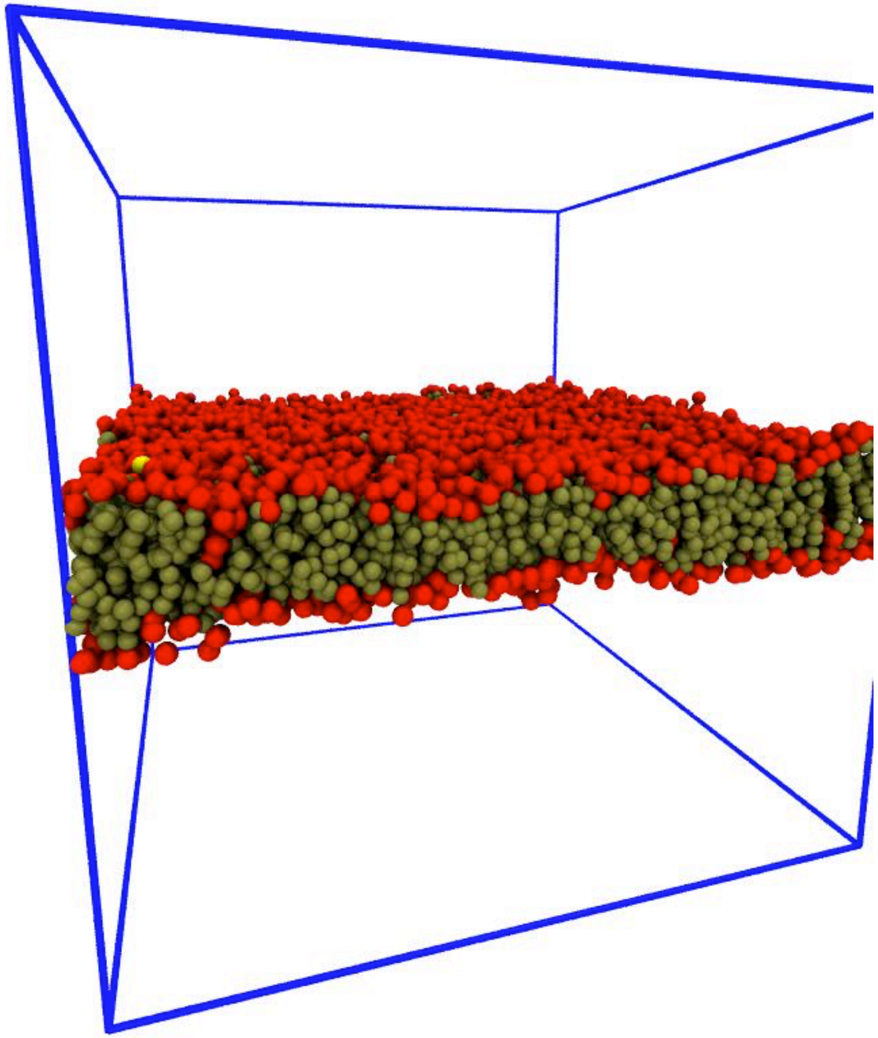
Pulling a lipid out of a membrane

(see dmpci.fs1 on moodle, lecture 3)

Note that F(t) can have different forms:

$F(t)$ = constant
$F(t) = k(t - t0)$

We don't know the form of V(x) for the membrane but the simulation implements it for us



Assisted escape from a potential well

If a particle takes a certain, average time to escape from a potential well in the quasi-equilibrium case, how does this time increase or decrease if we apply a pulling force to help it?

(Q. What does your intuition tell you before looking at next slides!)

Clearly, the mean escape time is the product of two terms:

Prob. escapes in t, t+dt = prob. survives to time t * prob. escapes in t, t+dt

Consider the radioactive decay of particles.

Let n(t) = number of particles present at time t, and let $n(0) = n_0$

and let λ be the decay rate, i.e., the number of particles that decay in t, t + dt is dn(t):

$$dn(t) = -\lambda . n(t) . dt$$

This integrates to give the number of particles still undecayed at time t:

$$n(t) = n_0 \exp(-\lambda . t)$$

What if the decay rate depends on time so that $\lambda = \lambda(t)$?

The number of particles that survive up to time t is now (why?):

$$n(t) = n_0 \exp\left(-\int_0^t \lambda(t') \, dt' \right)$$

and we define the probability of surviving to time t as $n(t) / n_0$.

We can use this result to calculate the mean strength of a bond, or rather its lifetime.

Mean bond lifetime under a pulling force

If the instantaneous probability of a particle escaping from a well is $\lambda(t)$, then the probability that it escapes in the interval $t, t + dt$ is:

$$p(t).dt = \lambda(t).\exp(-\int \lambda(t')\, dt') \,.\, dt$$

The mean escape time is defined by:

$$<T> = \int t.\, p(t).dt$$

But $p(t)$ is the derivative of a function so we can integrate by parts to get the mean escape time:

$$<T> = \int_0^\infty dt \,.\, \exp(-\int_0^t \lambda(t')\, dt')$$

where we interpret $\lambda(t)$ as the time-dependent rate of bond breakage. We need a model to relate this to a pulling force $F(t)$, e.g.,

$$\lambda(t) = \exp(-dV/kBT).\, \exp(F(t).\, x_b \,/\, k_B T) = \exp(-(dV - f.x)/kBT)$$

where $F(t)$ is a pulling force and x_b is a length related to the change in the potential (i.e., $\lambda(t)$)

U Seifert, Rupture of multiple parallel molecular bonds under dynamic loading, PRL 84:2750 (2000)

Mean escape time - simple cases

Given the expression for the mean escape time:

$$<T> = \int dt \, . \exp\left(-\int_0^t \lambda(t') \, dt' \right)$$

where $\lambda(t')$ is the time-dependent escape rate (units of 1/time).

What is <T> for the following functional forms of the escape rate (t_0 = constant)

1) $\lambda(t) = \lambda_0$ = constant $<T> = 1/\text{lambda0}$

2) $\lambda(t) = t / t_0^2$. $<T> = \sqrt{(\pi/2)}\, t_0$

3) $\lambda(t) = 1 / (t + t_0)$, infinite

(Classroom derivations: what do you get for <T> in these cases?)

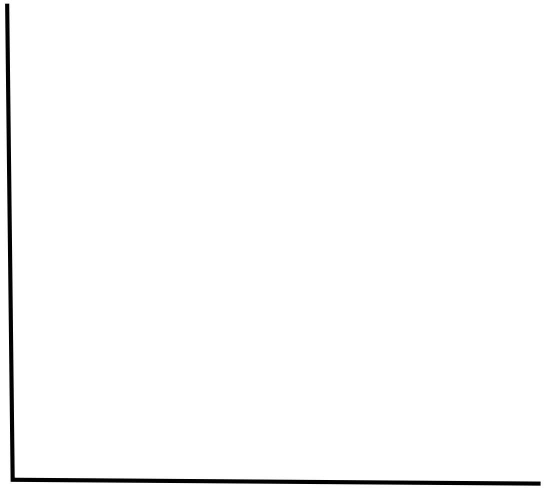
NB It's surprisingly hard to find other "simple" expressions for $\lambda(t)$ that can be integrated analytically, e.g., try $\lambda(t) = t^2 / t_0^3$

Can pulling make the lifetime increase?

$$\langle T \rangle = \int_0^\infty dt \, . \, \exp\left(-\int_0^t \lambda(t')\, dt' \right)$$

It seems counter-intuitive that pulling on a particle in a potential well should increase the time required for it to escape.

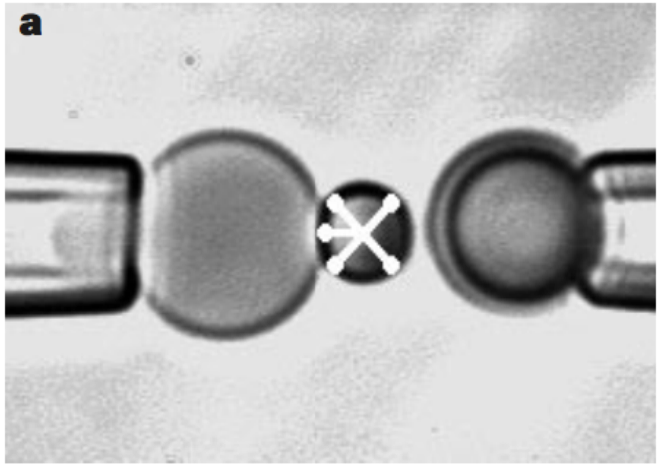
Q. How could it happen?



Dynamic force spectroscopy

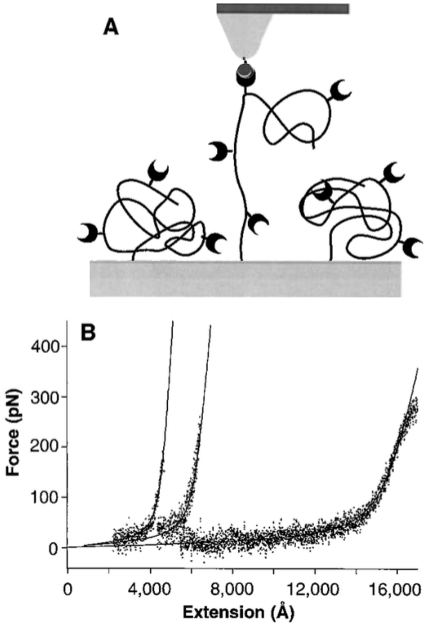
In the 1990s, people started to use the AFM and optical tweezers to explore the strength of molecular bonds. Because these bonds are very weak, there must be a way of applying tiny (pN) forces.

One technique used an inflated vesicle held in a micropipette to apply the force.



R. Merkel et al. Nature 397:50 (1999)

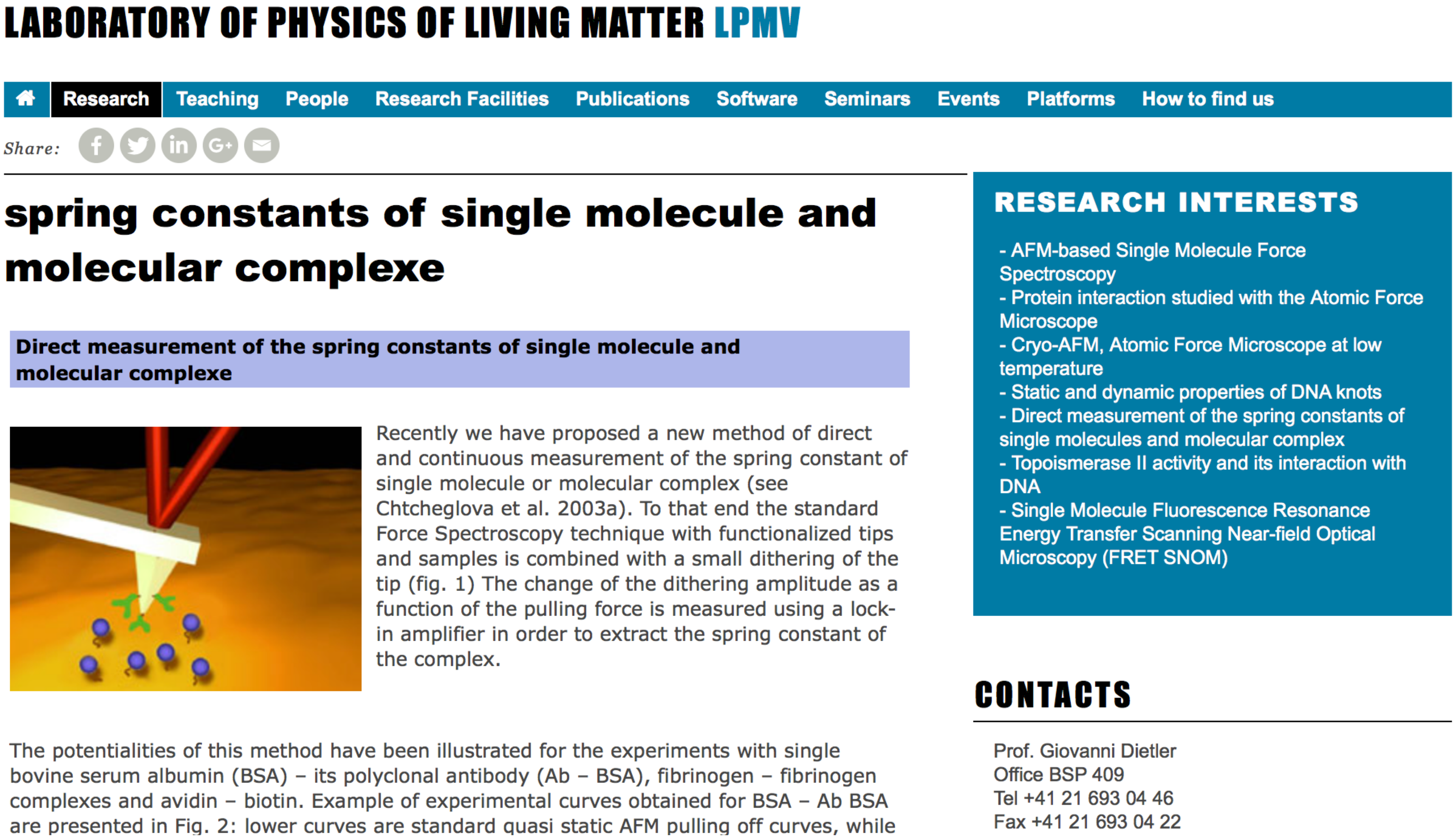
Another, used AFM



M. Rief et al. Science 275:1295 (1997)

They found that the bond strength has a strong entropic component and can exhibit several barriers in the free energy before finally separating. These barriers appear because of the interplay between random thermal motion, the shape of the potential, and the time dependence of the applied force.

Single-molecule spring constant experiments



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spring constants of single molecule and molecular complexe

Direct measurement of the spring constants of single molecule and molecular complexe

Recently we have proposed a new method of direct and continuous measurement of the spring constant of single molecule or molecular complex (see Chtcheglova et al. 2003a). To that end the standard Force Spectroscopy technique with functionalized tips and samples is combined with a small dithering of the tip (fig. 1) The change of the dithering amplitude as a function of the pulling force is measured using a lock-in amplifier in order to extract the spring constant of the complex.

The potentialities of this method have been illustrated for the experiments with single bovine serum albumin (BSA) – its polyclonal antibody (Ab – BSA), fibrinogen – fibrinogen complexes and avidin – biotin. Example of experimental curves obtained for BSA – Ab BSA are presented in Fig. 2: lower curves are standard quasi static AFM pulling off curves, while

RESEARCH INTERESTS

- AFM-based Single Molecule Force Spectroscopy
- Protein interaction studied with the Atomic Force Microscope
- Cryo-AFM, Atomic Force Microscope at low temperature
- Static and dynamic properties of DNA knots
- Direct measurement of the spring constants of single molecules and molecular complex
- Topoismerase II activity and its interaction with DNA
- Single Molecule Fluorescence Resonance Energy Transfer Scanning Near-field Optical Microscopy (FRET SNOM)

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Dynamic force spectroscopy

Typical experimental questions are:

- How does the strength of a molecular bond (or, equivalently, the mean time for rupture) vary under an applied force? What about a time-varying applied force?
- If a force is applied to two vesicles that are linked by many bonds connected in parallel (or series), how does the rupture time vary with numbers of bonds?

To solve these questions, we can write down a Langevin equation and numerically integrate it, essentially doing a set of simulations of individual experiments.

$$\gamma . dx/dt = -dV(x)/dx + F(t) + \sqrt{D} . \Gamma(t)$$