Dynamics of intracellular processes: physical aspects and modeling

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References in bold ([Name]) refer to lectures that will be given during the workshop.

1 Introduction

1.1 Cell specificity, compared to a physical system

When comparing the cell to a plain physical system, some striking differences appear.

■ **The cell is a Brownian world**

Thermal fluctuations are a key ingredient of all intracellular processes. Their origin is stored kinetic energy (the very meaning of temperature T): at thermal equilibrium, each degree of freedom has a kinetic energy[1] of $kT/2$. The characteristic energy of intracellular processes is thus $k_BT \approx 4.10^{-21}$ J (equal to the work of a 4 pN-force over a distance of 1 nm); it means that any process requiring an energy of a few k_BT can occur spontaneously. At intracellular scales (submicronic), *masses become irrelevant*: inertial terms play no role in the dynamics, i.e. motions can be considered as overdamped[2] and now velocities (instead of accelerations) are proportional to forces. The best visualisation of thermal fluctuations and example of overdamped motion is Brownian motion, i.e. the erratic motion of a particle submitted to innumerable and random collisions with water molecules; we describe in Section 2 the ensuing diffusive motion of the particle [Einstein 1956] [Perrin 1913]. To summarize, *thermal fluctuations give an intrinsic stochasticity to any intracellular process.*

■ **The cell is far from equilibrium**

It means that fluxes of matter, energy and charge continuously run across the cell, coming in and out. In contrast to equilibrium states, non-equilibrium states (even stationary ones) are very sensitive to changes in boundary conditions; in particular, they are controlled by incoming fluxes. This non-equilibrium character is essential for rectification of thermal fluctuations yielding oriented motions (molecular motors [Jülicher], traffic in nuclear pores [Goud]).

■ **The cell is a self-organized dynamic system**

The functional intracellular architectures are not fixed, but result from a dynamical equilibrium between various stochastic influences and processes, that can be summarized in the notion of self-organization[3] [Misteli 2001a]. Intracellular examples are numerous[4]: rafts inside phospholipidic membranes [Bagatolli], nuclear compartments, transmembrane receptors and synapses [Choquet] [Choquet and Triller 2003], mitotic spindle [Karsenti], [Nedelec], [Surrey et al. 2001], cell polarization [Wedlich-Soldner and Li 2003]. At supracellular scales, a remarkable example is pattern formation in bacterial colonies [Budrene and Berg 1991] [Ben-Jacob et al. 2000]. We shall distinguish:
— a restricted meaning of self-organization, corresponding to long-range order (e.g. patterns) arising from short-range (and often very simple) interactions between individuals; it is related to the notion of dissipative structure [Glansdorff and Prigogine 1971];
— an extended meaning, referring to pattern formation without a blueprint or a pointwise program. Patterns (whose first step is a symmetry breaking) emerge from interactions and dynamic collective behavior. Here, self-organization corresponds to cooperative action of different, heterogeneous, yet complex, components. In short, it refers to spontaneously (i.e. without an external monitoring nor

1. In particular, the kinetic energy of a particle of mass m is $\langle mv^2 \rangle = 3kT$ at thermal equilibrium, corresponding to a thermal velocity of about 600 m/s for a water molecule at ambient temperature.

2. Consider for instance an oscillator of displacement x, mass m, friction coefficient γ and elastic constant K. Its evolution is ruled by the equation: $m\ddot{x} + \gamma\dot{x} + Kx = f_{ext}$ when submitted to an external force f_{ext}. This dynamics exhibits two characteristic times $\tau_\pm = [(\gamma \pm \sqrt{\gamma^2 - 4Km})/2m]^{-1}$. If $\gamma^2 \gg Km$, then $\tau_+ \approx m/\gamma \ll \tau_- \approx \gamma/K$: the motion exhibits a fast relaxation mode (characteristic time τ^+) and a slow one (characteristic time τ^-). Assuming that fast relaxation occurs instantaneously at the description scale, the dynamics reduces to the overdamped equation $\gamma\dot{x} + Kx = f_{ext}$, with a single characteristic time $\tau_- \approx \gamma/K$, corresponding to the slow mode.

3. Self-organization requires to define what is an "organized" structure; in biology, the less subjective meaning is perhaps a "functional structure", able to perform a task that the parts cannot achieve in isolation.

4. See also [Eigen 1971], [Schieve and Allen 1982] and [Kauffman 1993] for a general reflection.

an imprinted program) concerted behaviors between several elements or spontaneously concerted sequence of events. It might be extended to spontaneous rhythms [Goldbeter 1996] [Winfree 2000].

■ **The cell is a complex, highly structured medium**

Cell includes different subsystems (nucleus, mitochondria), substructures (compartments, membranes), and molecular tracks (actin filaments, microtubules). Ensuing geometric constraints (confinement, restricted degrees of freedom) and boundary conditions (e.g. across membranes) will evidently strongly influence intracellular processes. Moreover, the cell embeds many network structures at the level of spatial organization (for instance cytoskeleton), of interactions (protein-protein interaction or gene networks), and of dynamics (coupled biochemical reactions). Accordingly, the cell exhibits multiscale organization and dynamics, moreover with feedbacks across the levels preventing any separation of scales.

All these characteristics substantiate the relevance and even the necessity of dynamic, stochastic, and integrated (multiscale) modeling of intracellular processes.

1.2 Importance of single-cell *in vivo* studies

The cell properties briefly described above demonstrate the need of *in vivo* data and experiments. Indeed, spatial organization, localization and actual timing (that cannot be reproduced *in vitro*) will play a key role. Most processes will be either concerted with other cooperative ones or on the contrary faced with competitive ones: only considering the whole set of possible mechanisms might account for the actual behavior, which increases the requirement of *in vivo* studies.

Not only *in vivo* but *single-cell* studies are required, to follow actual microscopic processes. Otherwise only an average record is available, that can be far different from single-cell behavior, if there is a noticeable statistical dispersion, i.e. when there is no typical behavior to be identified with the average one. For instance, symmetries and invariances observed on average measurements (over a cell population) might disappear at the level of single cell (or rather, remain with the status of statistical symmetries) [Wedlich-Soldner and Li 2003]. Investigations at the single-cell level yield quantitative estimate of fluctuations and variability; they evidence robustness of cell function despite individual variability (from cell to cell, place to place, and time to time inside the cell).

1.3 Bridging biology and physics

■ **What do we mean by "dynamics"?**

The term of "dynamics" covers here a large variety of time-dependent processes:

— a first, plain, meaning refers to *any process varying in time*, either transient or periodic, either thermally activated (i.e. occurring spontaneously at high enough temperature) or active (i.e. fuelled by ATP-hydrolysis or fed by fluxes of matter or charges). Some examples are biochemical rhythms like circadian rhythm, mitotic oscillator, calcium or glycolytic oscillations. The viewpoint of dynamical systems for modeling these processes and analyzing the data is then very fruitful [Jülicher] [Goldbeter 1996] [Keener and Sneyd 1998] [Murray 2002];

— a second meaning refers to *continuing creation/destruction* of intracellular components and *assembling/disassembling* of complexes and organites. Among numerous examples, we may cite dynamic instability of microtubules [Dogterom and Leibler 1993], actine polymerization, cytoskeleton architecture [Amblard];

— dynamics should also include *conformational transitions* of macromolecules (RNA loops, allosteric enzymes, ionic channels or carriers) or complexes (nucleosomes and chromatin fiber, ribosomes). Here, yet the *kinematics* deserves attention;

— dynamics finally encompasses all kinds of *transport phenomena*: in various dimensions, active or passive, biased, mediated, gated, confined, coupled with chemical reactions (see Section 2).

■ **What do we mean by "modeling"?**

As for the term "dynamics", the terms "model" and "modeling" are used with different shades:

— a *close analytical formula to fit data*, classify them, compare them, or summarize them. It might acquire the status of (phenomenological) law if it is sufficiently simple and general. It is all the more valuable that the number of free parameters to be fitted from the data is small;

— a *framework built from hypotheses*, allowing to make predictions whose agreement with experimental data validate these hypotheses;

— a consistent framework *bridging scales and levels of organization*, allowing to interpret (macroscopic, *in vivo*) data in terms of microscopic mechanisms and parameters, for instance those involved in molecular modelization or determined by specific *in vitro* experiments.

■ **What role for physics in intracellular studies**?

— *Imaging techniques*, but that's not our main point (although of invaluable practical interest!).

— Description of *some key ingredients*: electron transfer, proton transfer, bonding (hydrophobic, ionic), electrostatics (interactions, persistence length, screening), molecular structures, membrane properties, macromolecular conformations, normal modes, kinetic constants, energies and forces involved... the list is far from complete;

— Identification of *some generic mechanisms*: for instance, the diffusive instability first introduced by Turing as a basic mechanism of pattern formation, stochastic resonance, or the effect of feedback loops. Here, the aim is to describe the features that physics alone can yet explain, without invoking any specific biological mechanism or component [Turing 1952]. It might serve as a basis to appreciate the influence, either essential or secondary, of additional biological ingredients.

— *Integrated multiscale modeling*, at the same time holist and reductionist: from DNA up to chromosome, actine network, cooperative molecular motors, pattern formation,... again the list is all but closed.

— *Functional effective approach*: we here mark out physical approaches including the biological specificity, namely the use of physical laws and concepts, but involving effective parameters and embedded within functional approaches (i.e. rooted in the biological function) and following the logic of evolutionary optimization. For instance, natural selection allows to *assume adaptation and optimal performances* which is a way to account for Evolution and natural selection in modeling the biological systems of today.

2 Thermal fluctuations and transport phenomena

2.1 Many different modes of transport

■ **Thermal diffusion and Brownian motion**

A typical example of thermal diffusion is the Brownian motion of a particle in a large vessel of water ([Perrin 1913], see also Fig. 4 below). Such normal diffusion is associated with a diffusion law $R^2(t) \sim 2dDt$ where (conventionnally) the dimension d of the space appears in front of the diffusion coefficient[5] D and $R(t)$ is the (root-mean-square) distance travelled by the particle at time t from its starting point [Laguës and Lesne 2003]. In the cell, such diffusion is encountered:

— in dimension 1: proteins sliding along DNA [Shimamoto 1999]. A debated issue is how a factor finds its target, either hopping (3-dimensional) or sliding (1-dimensional) [Stanford et al. 2000].

5. To grasp some figures, $D \approx 10^{-12}$ m^2/s for proteins to 10^{-10} m^2/s for a water molecule.

— in dimension 2, within membranes [Choquet] [Cognet] [Bagatolli];

— in dimension 3; it is then possibly confined (see Fig. 5). Normal diffusion can be generalized to a porous medium, by introducing an effective (smaller) diffusion coefficient accounting for the reduction of accessible space, provided the pores have a finite characteristic size a; such an homogeneization is thus relevant at scales larger than a [Siggia et al. 2000], [Nicholson 2001].

■ **Electrodiffusion**

Electrodiffusion, i.e. the superimposition of random thermal diffusion and deterministic drift induced by an electric field, is mainly encountered in transmembrane transport [6] through pores and gated ionic channels [Hille 1992]. The field is here induced by the ionic concentration gradient across the membrane (ionic concentrations differ on each side of the membrane, see Fig. 1) [Goldman 1943] [Hodgkin and Katz 1949] [Keener and Sneyd 1998]. It is worth noticing the key role of membranes in slowing down thermal motions of ions and yielding processes at almost macroscopic time-scale (microsecond for an action potential, directly induced by ionic currents across the axon membrane, i.e. molecular events) [Eisenberg 2000].

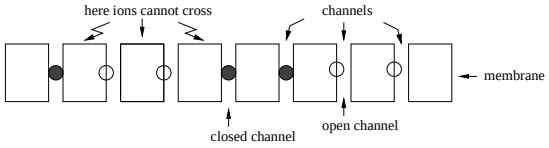

Figure 1: Sketch of a phospholipidic membrane with ionic channels and their gates (voltage-dependent or controlled by binding of a specific ligand) represented as black circles when closed.

■ **Anomalous diffusion**

This term covers all diffusive motions that do not satisfy the normal diffusion law. Typically, $R^2(t) \sim t^\gamma$ with $\gamma \neq 1$. This exponent modification reflects a dramatic change in the motion [Bouchaud and Georges 1990] [Shlesinger et al. 1999] [Laguës and Lesne 2003] that might originates in:

— fractal substrate, for instance diffusion in a porous medium with pores of all sizes (then $\gamma < 1$) or highly disordered medium [Havlin and Ben Avraham 2002];

— trapping and arbitrarily long residence times (then $\gamma < 1$);

— steps of all sizes, what is called a Levy flight (then $\gamma > 1$) [Ott et al. 1990] [Klafter et al. 1996]);

— correlated motion ($\gamma > 1$) or anti-correlated motion ($\gamma < 1$).

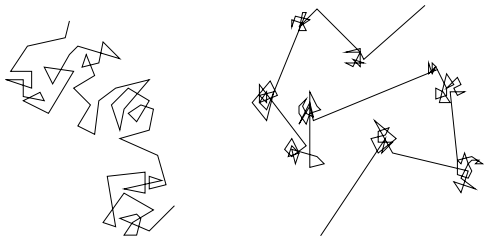

Figure 2: Sketch of a typical individual trajectory for (Left) Brownian motion (normal diffusion, $\gamma = 1$) and (Right) Levy flight (anomalous diffusion, $\gamma > 1$) with arbitrarily long jumps or arbitrarily long periods of deterministic motion.

■ **Active processes**

Typical examples of active (i.e. ATP-consuming) processes are the oriented motion of motor protein along filaments [Jülicher 2003] or the transport of vesicles along axons towards synapses by kinesin. Such oriented motions require the conjunction of spatial asymmetry and non-equilibrium fluctuations. Free-energy balance is ensured by ATP-hydrolysis: energy is mainly supplied by thermal energy, but the Maxwell daemon achieving oriented motion (entropic cost) feeds on ATP.

6. Other modes of transmembrane transport are (passive) carriers, whose main mechanism is joint translocation, or (active) pumps, that are a special instance of motor proteins [Mentré 1995] [Keener and Sneyd 1998].

■ **Propagation phenomena**

Here it is not matter but some kind of excitation that travels, as in macroscopic wave propagation. A typical mechanism is provided by local chemical reactions supplemented with diffusion ensuring the coupling of neighboring points. Main examples are action potential propagation and other reaction-diffusion phenomena [Keener and Sneyd 1998] [Murray 2002]. Another mechanism is translocation, for instance charge translocation in proton transfer [Vuilleumier and Borgis 1998].

2.2 Various models and theoretical frameworks according to the scale

Let us briefly sketch the different theoretical approaches[7] accounting for diffusive motion, mainly in the case of plain 3-dimensional thermal diffusion. Their validity and relevance depend on the space and time scales of the description, and they should be chosen accordingly in practical situations (modeling from experimental data, simulations).

■ Historically the first model is the macroscopic deterministic irreversible **diffusion equation**:

$$\partial_t n = D\Delta n \tag{1}$$

where $n(\vec{r},t)$ is the local instantaneous concentration of diffusing particles. This equation comes from the conservation law[8] $\partial_t n + \vec{\nabla}.\vec{j} = 0$ and the phenomenological Fick law $\vec{j} = -D\vec{\nabla}n$ introducing the diffusion coefficient D (linear response theory and continuous medium approximation). This diffusion equation is to be supplemented with boundary conditions. A source term can be added on the right hand side when there is a supply of matter in the system. It is to note that the Fick law does not involve an actual force: it is a statistical law[9]; there is no "diffusion force" at the microscopic level, and each diffusing particle totally ignores what are doing the other particles of the considered population. Two simple extensions are also of relevance in cellular biology.

Electrodiffusion: the constitutive equation (number density n, individual charge $q = Ze$) now writes:

$$\vec{j} = -D\vec{\nabla}n + q\vec{E}n/\gamma \tag{2}$$

to be plugged into the still valid conservation law. The first term in right hand side is the Fick law (effective "statistical" law); the second one corresponds to the Ohm law (involving an actual electric force $q\vec{E}$ and linear response relating force and velocity through mobility $1/\gamma$).

Chemotaxis: the constitutive equation here includes a chemotactic term:

$$\vec{j}_{chem} = \chi(a)\; n\; \vec{\nabla}n \tag{3}$$

where the additional field $a(\vec{r},t)$ represents the local concentration of the chemo-attractant.

■ At microscopic scales, deterministic reversible **Newton equations** apply, describing particle motion as an alternation of free flights and elastic collisions (modeling short-range pair interactions), Fig. 3.

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7. It is to note, from a theoretical physics viewpoint, that diffusion is an exemplary instance where all approaches available at different scales to describe many-particle systems can be consistently and explicitly related. In particular, the diffusion coefficient D appearing in all the different models is actually *one and the same quantity*. We refer to [Laguës and Lesne 2003] for a detailed exposition of these approaches and their links.

8. The variation of the number of particles contained in a small volume $\mathcal{V}$ is due to currents in and out, i.e. flux across the boundary $\delta\mathcal{V}$ of $\mathcal{V}$ (no creation nor annihilation of matter):
$\partial_t \int_{\mathcal{V}} n(\vec{r},t)d^3\vec{r} = -\oint_{\delta\mathcal{V}} \vec{j}.d\vec{S} = -\int_{\mathcal{V}} \vec{\nabla}.\vec{j}(\vec{r},t)d^3\vec{r}$ where the second equality follows from Stokes formula.

9. Of similar microscopic origin and statistical nature is the osmotic pressure, inducing water motion across semi-permeable membranes [Einstein 1956].

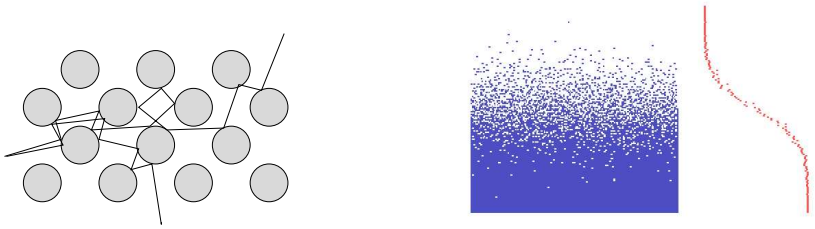

Figure 3: (Left) microscopic deterministic model of diffusive transport (Lorentz gas model [Dorfman 1999]) where a light particle experiences numerous elastic collisions on spherical scatterers. Defocussing character of the collisions induces molecular chaos, in turn generating a diffusive motion and supporting a statistical approach. (Right) observation at time t of the microscopic simulation (implementing random walks) of diffusion in a semi-infinite box, starting from a step in $x = 0$ at time $t = 0$. (Extreme right) average profile $n(x,t)$ (vertical axis represents the spatial coordinate x whereas concentration $n(x,t)$ spans values from 0 to 1 horizontally); $n(x,t)$ tends to the solution of the diffusion equation $\partial_t n = \partial^2_{xx} n$ as the number of particles tends to infinity.

■ In between, various **mesoscopic stochastic descriptions** have been developed, bridging atomic and observation scales:

— *kinetic theory* reducing to Boltzmann equation[10] when correlation between particles can be neglected (what is called "Boltzmann approximation") [Dorfman 1999];

— *Green-Kubo relation* relating D to the velocity autocorrelation of the particle [Kubo et al. 1991]: $D = (1/d) \int_0^\infty \langle \vec{v}(t).\vec{v}(0) \rangle dt$ (in dimension d);

— *master equation* [Schnakenberg 1971], [Van Kampen 1981] and its numerical implementation, cellular automata [Ermentrout and Edelstein-Keshet 1993] [Chopard and Droz 1998];

— *discrete random walks* and their continuous counterpart, Wiener processes (Fig. 4);

— *Langevin equation*, leading to Einstein relation $\gamma D = k_B T$ [Einstein 1956] where γ is friction coefficient of the particle, $1/\gamma$ its mobility and k_B the Boltzmann constant. In particular, for a spherical particle of radius a diffusing in a fluid of dynamic viscosity η, Stokes law: $\gamma = 6\pi a\eta$ leads to $D = k_B T/6\pi a\eta$ (or $D = RT/6\pi a\eta \mathcal{N}_{Av}$, that is the relation which allowed Perrin to measure the Avogadro number $\mathcal{N}_{Av}$, thus providing a strong support to atomic theory of matter).

— *Fokker-Planck equation* for the probability distribution $P(\vec{r},t)$, recovering diffusion equation by identification (supported by the law of large numbers) of $P(\vec{r},t)$ with the local concentration $n(\vec{r},t)$ [Lemarchand and Vidal 1988].

Summary and discussion in the context of chemical reactions can be found in [Arnold 1980].

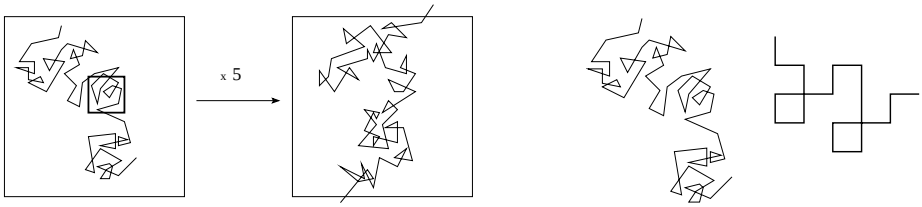

Figure 4: (Left) self-similarity of Brownian motion trajectories, yet observed by Perrin on his experimental recordings (performed with different sampling frequencies, see [Perrin 1913]). (Right) discrete random walk model mimicking Brownian motion on a lattice.

10. It writes $[\partial_t + \vec{v}.\vec{\nabla}_{\vec{r}} + \vec{a}.\vec{\nabla}_{\vec{v}}] f_1(\vec{r},\vec{v},t) =$ collision term where $\vec{a}(\vec{r},t)$ is a field of acceleration (for instance $\vec{a}(\vec{r},t) \equiv \vec{g}$ if gravity is relevant, or $\vec{a}(\vec{r},t) = q\vec{E}/m$ for a particle of mass m and charge q in an electric field $\vec{E}$).

■ More generally [11], **Langevin equations** are stochastic equations: $dz/dt = f(z) + \xi$ where $\xi(t)$ is a white noise, i.e. a Gaussian process defined through its moments $\langle \xi(t) \rangle = 0$ and $\langle \xi(t)\xi(s) \rangle = \delta(t-s)$. These equations are used to account for the influence of thermal fluctuations on systems evolving according to $dz/dt = f(z)$ at zero temperature (i.e. in the absence of fluctuations). In particular, such description of "Brownian motion in a potential $U(z)$" is at the basis of Kramers rate theory [Kramers 1940] [Hänggi et al. 1990], allowing to compute the characteristic time $\tau_K \sim e^{\Delta U/k_B T}$ (or rate $1/\tau_K$) to jump over an energy barrier ΔU. It led to the development of landscape paradigm [Sherrington 1997], specially fruitful to study protein conformations and folding [Frauenfelder 2002] [Frauenfelder et al. 2001].

2.3 Data analysis and modeling from imaging experiments

It is now possible to observe specifically fluorescent tagged proteins or molecules with a high space and time resolution, and to determine their dynamical properties in living cells or in minimally reconstituted systems (FRAP, SPT, FCS) [Engelborghs] [Bagatolli]. It is certainly a vain challenge to discuss generally and theoretically the issue of intracellular data analysis and the fruitfulness, if not the need, of joint physical modeling. I'll then restrict to a few guidelines and caveats.

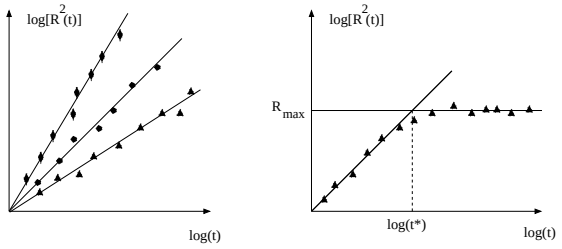

Figure 5: Sketch of the time dependence of the root--mean-square displacement $R(t)$. (Left) in case of diffusion law $R^2(t) \sim t^\gamma$, corresponding to linear slopes in a log-log plot $\log R^2(t) = \gamma \log t + c$; normal diffusion is associated with $\gamma = 1$ (circles) whereas diffusion is said to be anomalous if $\gamma \neq 1$ (subdiffusive if $\gamma < 1$, triangles, or superdiffusive if $\gamma > 1$, diamonds). (Right) in case of confined motion; the crossover time t^* is related to the linear size R_{max} of the domain through $R_{max}^2 \sim Dt^*$ (in case of normal diffusion).

■ **Trajectory analysis**

It is now possible to record single trajectories, either those of proteins of interest properly tagged with a fluorescent tail [12], either those of tracer particles, for instance fluorescent beads used as probes reflecting the fluctuations and motions of their environment [Le Goff et al. 2002a]. >From such recordings, one computes the root-mean-square displacement $R(t)$ with $R^2(t) \equiv \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle$ (or $R^2(t) \equiv \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle - \langle \vec{r}(t) - \vec{r}(0) \rangle^2$ in case of a biased motion); the ensuing analysis is presented on Fig. 5 [Cognet]. The main difficulties lie in finite-time and finite-size effects, that can yield marked discrepancies with respect to pure scaling law $R(t) \sim t^{\gamma/2}$. These discrepancies can be meaningful if a quantitative analysis is available and if their origin is well-identified. Indeed, another difficulty is the superimposition of several specific mechanisms that could account for the observed data. General caveats about empirical determination of scaling behavior (crossover, sub-classes, see [Laguës and Lesne 2003]) are described on Fig. 6.

11. Langevin equations can also be used to account for thermal fluctuations in spatially extended systems; let us cite for instance the equation describing the longitudinal fluctuations $r(s,t)$ of an actin filament (arc-length s):
$\eta \partial r/\partial t = \kappa \partial^4 r/\partial s^4 + f$ with $\langle f_i(s,t) f_j(s',t') \rangle = \delta_{ij}\delta(s-s')\delta(t-t')\eta k_B T_{act}$ where η is the dynamic viscosity of the medium and κ an elastic constant; it is to note that with $T_{act} = T$ at thermal equilibrium whereas $T_{act} \gg T$ if the actin filament is coupled to molecular motors (myosin, here) [Le Goff et al. 2002b].

12. In this aim, the protein coding sequence is modified in the genome, in order to supplement the actual protein with a GFP (Green Fluorescent Protein) tail, enough small not to modify the properties and behaviour of the wild protein and allowing to visualize it thanks to its intrinsic fluorescence [Li][Misteli 2001b].

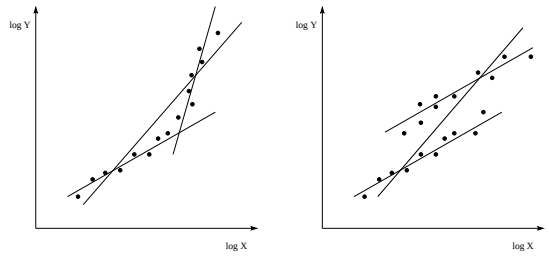

Figure 6: Caveats in experimental determination of a scaling law $Y \sim X^a$. (Left) ignoring a crossover ($Y \sim X^{a_1}$ if $X < X^*$ and $Y \sim X^{a_2}$ if $X > X^*$) or (Right) ignoring the existence of different subclasses ($Y \sim A_1 X^a$ and $Y \sim A_2 X^a$) yields a meaningless effective exponent a_{eff} (slope of the bold line).

■ **Exploitation of FRAP data**

Other observation techniques, for instance FRAP[13], are analyzed through indirect inference, by comparison and fit with the prediction of theoretical models, numerically implemented (random walk modeling, cellular automata, Brownian dynamics). The analysis thus yields at the same time a set of hypotheses about the nature and mechanisms of the observed transport phenomenon and the values of the parameters involved: *there is no model-free way of analyzing FRAP data.*

The requirement of an underlying dynamic modeling of the observed process is not restricted to diffusion and FRAP data. Modeling is essential to bridge experimental data with mechanisms, i.e. to interpret quantitatively the data in terms of biological processes and their parameters (shapes and sizes, concentrations, velocities, rates and affinities, interactions).

■ **Different kinds of modeling:**

Modeling can be roughly classified into:

— *statistical modeling*, allowing to tackle quantitatively with intrinsic variability of the observed processes, mainly by computing moments ($\langle X^n \rangle$ or derived quantities) of observables X, fitting the distribution $P(X)$ and performing statistical tests on X values;

— *dynamic, more often stochastic, modeling*; models of this class aim not only at quantifying the observed variability/robustness of the processes but also at explaining it, starting from underlying mechanisms ruling the system evolution and associated dynamical equations;

— *numerical simulations*, aiming at validating a consistent explanatory framework (biological objects involved, interactions and mechanisms at work) by comparing simulation results and experimental data. It might not only validate a scenario and its hypotheses, but also evidence the characteristic scales and control parameters, and determine the range of scale and parameter values in which the observed behavior is expected to occur. More generally, it allows to perform a sensitivity analysis with respect to any modification/perturbation of the system or scenario (in particular, it allows to vary parameters one by one, independently, with almost no restriction on their range, which is experimentally impossible, or to add a chosen amount of noise). They also give access to processes too complex to be handle analytically or with the help of intuition and basic principles: let us quote for instance simulation of encounters [Gabdoulline and Wade 1998] and target location [Jülicher and Bruinsma 1998] [von Hippel and Berg 1989], or collective behavior of molecular motors and filaments [Surrey et al. 2001].

■ **Relevance of generic models:**

In physics, high value is put on generic models. First, they are simple enough to lend themselves to computation (sometimes analytically) and numerical simulations. More deeply, their very status is to capture the principles and essential mechanisms, notwithstanding details, specific features, and

13. Fluorescence Recovery After Photobleaching. Proteins of interest or tracers particles are tagged with fluorescent probes. Their fluorescence is irreversibly photobleached (by an appropriate laser pulse) over a small region at a given time. Recovery of fluorescence in this region reflects the arrival of unbleached particles from outside the region, giving a direct access (quantitative through the measurement of fluorescence intensity) to particle flux at this point, and an indirect access to particle motion and its parameters [Misteli 2001b].

individual exceptions. Detailed modeling is generally not enough robust to be faithfully explanative, since somehow arbitrarily kept details might unduly play a key role while other let apart are ignored.

The price to pay for robustness and structural stability is that generic models do not yield quantitatively accurate predictions, hence might be hard to compare with and validate from data, except if the ensuing behavior satisfies scaling laws, or exhibits thresholds, bifurcations or other qualitative features that can be clearly identified hence checked.

In the biological context, the relevance of investigating generic mechanisms is to determine what can be yet explained by physical and chemical features, without invoking specific biological ingredients and adapted mechanisms. It is then a fundamental, much debated issue to appreciate the point to which specific details and individual peculiarities of each process, in each context (e.g. in each organism) are only devoted to fine-tuning, or rather required to stabilize and regulate it, or on the contrary essential to achieve the biological function.

3 Integrated modeling: some exemplary achievements

It is not the place to develop all the achievements of integrated modeling of intracellular phenomena involving physical concepts and ingredients; I shall only recall the most acknowledged biological processes, mainly self-organizing structures and transport mechanisms, which have been, or can be, modelled in terms of dynamical equations or reproduced by computational models.

■ **Electrodiffusion and transmembrane transport**

The first step is the phenomenological modeling of electrodiffusion (Fick law supplemented with Ohm law, § 2.2) [Goldman 1943] [Hodgkin and Katz 1949]. It has been followed by a microscopic, stochastic modeling of gating and kinetics of ionic channels [Hille 1992] [Destexhe et al. 1994]. This second step allows to express the parameters involved in electrodiffusion equation as a function (possibly time-dependent or controlled by an auxiliary process) of the microscopic structure and conformation of ionic channels. It thus bridges the level of observation with the underlying molecular level at which excitation and regulation actually occur.

■ **Propagation of action potentials**:

The foundation of the theoretical study of neural activity is the famous space-clamped[14] Hodgkin-Huxley equation describing the time evolution of the membrane potential[15] of an axon. Hodgkin and Huxley obtained it as a four-variable fit of careful and numerous experimental recordings guided with qualitative arguments [Hodgkin and Huxley 1952]. The three auxiliary variables appearing in this equation have been afterwards interpreted (see previous point) at a microscopic level within a probabilistic approach of channel gating. Then, considering a spatial dependence for membrane potential and ionic concentrations, and the presence of currents along the axon, Hodgkin-Huxley equation has been supplemented by a diffusive coupling term between neighboring values of the potential [Keener and Sneyd 1998]. An argument of scale separation led to a simplified dimensionless model, FitzHugh-Nagumo equation [FitzHugh 1961] [Nagumo et al. 1962] for a variable $u(x,t)$ related to the membrane potential and a variable $v(x,t)$ related to a membrane conductance (the slowest varying one, the two other conductances involved in Hodgkin-Huxley equation beeing eliminated thanks to a quasi-stationary approximation):

$$\begin{cases} \partial_t u = 3u - u^3 - v + D\partial^2_{xx} u & (x \text{ along the axon}) \\ \partial_t v = \epsilon(v - \Gamma u) \end{cases} \tag{4}$$

When the parameter Γ is smaller than 1, there is only one stable fixed point, see Fig. 7, right), and this equation is a generic model of excitable medium (as neurons or cardiac cells): the response

14. "Space-clamp" refers to an experimental setup (a shunt) ensuring spatial homogeneity: the membrane potential and ionic concentrations are forced to be spatially uniform all over the time.

15. i.e. the difference of electric potential between intra- and extra-cellular sides of the axon membrane.

to an enough large perturbation is first an amplification of this perturbation, before relaxation towards the equilibrium state occurs. Another time-scale separation illustrated on Fig. 7 (membrane potential variations are far faster than conductance variations, which reflects in $\epsilon \ll 1$) allows to analyze the behavior of the solutions. and to describe explicitly the propagation of action potentials[16], i.e. localized supra-threshold excitations whose profile is sketched on Fig. 7, left. This analysis, leading to quantitative results about observable neural activity, relies on deep and rigorous mathematical studies of singular perturbations[17] and propagating fronts. It allows to relate features of spike recordings with microscopic properties of the axon membrane and stimuli.

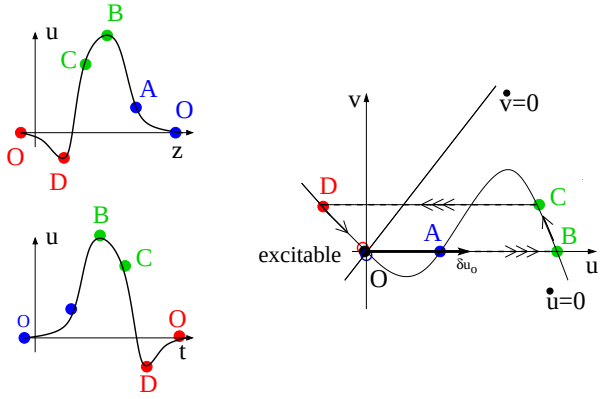

Figure 7: (Right) phase portrait of the space-clamped FitzHugh-Nagumo equation in the case of an excitable regime: O is the only fixed point, it is stable but a perturbation $\delta u > \delta u_0 = OA$ is first amplified before relaxing to O, in four stages: first (OB), a fast amplification of u while v remains constant, then (BC) a slow drift of v along the curve $\dot{u} = 0$ (quasi-stationary approximation for u, slaved to the variation of v), then (CD) a fast relaxation of u at constant v, and a final (DO) slow relaxation of v. (Left) profile of an action potential (in time, at a given point, or in space, at a given time); letters corresponds to those indicated on the phase portrait.

■ Reaction-diffusion and Turing structures

In a now celebrated paper, Turing introduced a reaction-diffusion model[18] accounting for pattern formation under some general hypotheses [Turing 1952]. It involves[19]a self-enhanced activator u diffusing far slowly than an inhibitor v: the control parameter appears to be the ratio $d = D_v/D_u$ of diffusion coefficients. Turing mechanism is also called a "diffusive instability", occuring only if $d > d_c$ where d_c is some critical value of the control parameter. Turing model thus describes a dynamic instability of the homogenenous situation, with spatially uniform concentrations. The range of characteristic lengths of the pattern is prescribed by the dynamics; further selection among this range occurs due to boundary conditions [Cross and Hohenberg 1993]. Turing structures are an exemplary instance of emergent behavior: in isolation, both the diffusion and the set of coupled chemical reactions smooth out any inhomogeneity and restore the homogeneous equilibrium concentrations. This model has remained almost ignored till its first observation in a real system [Boissonade et al. 1995]. Numerous variants have been developed [Koch and Meinhardt 1994] to explain pattern formation inside the cell or at the level of cell population, with internal tuning of the parameters (kinetic rates, cell characteristics) [Eldar] [Felix] [Ben Jacob et al. 2000]. It thus bridges intracellular and multicellular processes. It is to be underlined that Turing structures are

16. One shows that this equation admits a propagating solution $u(x,t) = \tilde{u}(x - ct)$: its profile $\tilde{u}(z)$ is stationary in a frame moving at constant velocity c. This special solution is called an action potential or a spike; it is emitted as soon as the membrane experiences a supra-threshold excitation.

17. This refers to the case when equation exhibits a small parameter ϵ with $\epsilon = 0$ corresponding to a qualitatively different behavior, that cannot be used as a starting point for a perturbation approach with respect to ϵ.

18. i.e. a spatially extended dynamical model in which a set of chemical reaction is supplemented with diffusion of each species.

19. It writes explicitly: $\begin{cases} \partial_t u = f(u,v) + \partial_{xx} u \\ \partial_t v = g(u,v) + d\ \partial_{xx} v \end{cases}$ where $u(x,t)$ and $v(x,t)$ are the local concentrations at time t. Let us give some feeling of the mechanism [Murray 2002]. The self-enhancing local activator u is associated with a positive feedback loop achieving a nonlinear amplification of stochastic variations, and acting as a switch. The fast-diffusing inhibitor induces a negative feedback loop, typically associated with regulatory processes [Thomas and Kaufman 2001], and contributing to the spatial oscillations. Turing structures also occurs with a long-range inhibitor, which can be foreseen, since long-range inhibition and fast diffusion both correspond to a long-range coupling kernel $K(.)$ in the general expression $\int K(x-y)v(y,t)dy$ of the spatial coupling, reducing to $D_v \partial_{xx} v$ in the special instance of coupling through molecular diffusion [Laguës and Lesne 2003].

an instance of dissipative structure, occuring in an open system, continuously fed with reactive species. They belong to the general class of self-organized structures, accounting for spontaneous symmetry breaking, due to a dynamic instability, and global pattern formation from local rules.

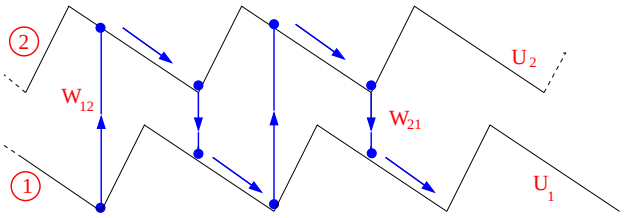

Figure 8: Scheme of the concerted mechanisms (random switch between potential energy landscape U_1 and U_2 with rates W_{12} and W_{21}, superimposed to thermal diffusion in these landscapes) yielding the oriented motion of a molecular motor, see text and equation (5).

■ Active processes and molecular motors

Oriented motion at molecular scale requires spatial asymmetry and non-equilibrium fluctuations. An example is provided by motor proteins able to move along a filament (actin or microtubules), carry a cargo or exert a force. The interaction potential between the motor protein and the filament is assumed to switch between two landscapes $U_1(x)$ and $U_2(x)$ according to the conformation of the protein (Fig. 8). The protein conformational transition (with stochastic rates W_{12} and W_{21}) and associated change in the potential energy landscape are induced by coupling with a far-from-equilibrium chemical reaction, ATP-hydrolysis. Biased diffusive motion (Brownian motion in the interaction potential) takes place in between transitions [Jülicher et al. 1997] [Vicsek 2001] [Jülicher]. Modeling relies on Fokker-Plack equations coupled with the switching mechanism:

$$\begin{cases} \partial_t P_1(x,t) = \nabla(D\nabla P_1 + \beta P_1 \nabla U_1) + P_2 W_{21} - P_1 W_{12} \\ \\ \partial_t P_2(x,t) = \nabla(D\nabla P_2 + \beta P_2 \nabla U_2) + P_1 W_{12} - P_2 W_{21} \end{cases} \tag{5}$$

The coupling with this far from equilibrium chemical reaction reflects in the fact that detailed balance is not satisfied ($P_2 W_{21} \neq P_1 W_{12}$), otherwise no oriented motion would emerge, despite asymmetry of the filament (actin, microtubule). Another example of oriented motion is provided by ionic pumps, like NaK-ATPases, that maintain a ionic concentration step across cellular membranes and, in the case of neurons, restore it after the emission of an action potential.

Let us also note that molecular motors is one example of the various mechanisms leading to *order from noise* in the cell; let us give a sample of these mechanisms:
— annealing where noise allows to pass barriers, leading to more organized states, as shaking a vessel full of small hard beads allows to pack them in a more ordered and compact fashion (a typical example is provided by protein folding and more generally, conformational transitions of macromolecules);
— stochastic resonance, e.g. in neural activity [Gammaitoni et al. 1998] [Schmid et al. 2001];
— any active process or rectification of thermal fluctuations;
— stochastic coherence (oscillators coupled to each other through coupling to a common noise).

■ Still in progress...

This above list is only a small sample; it should be supplemented with numerous other examples:
— calcium waves and calcium signalling [Goldbeter 1996],
— Mitotic oscillator [Goldbeter 1996]
— Mitotic spindle and other assemblies of motors and filaments [Karsenti] [Nedelec] [Jülicher]
— membrane internal organization (rafts, transmembranes receptors)[Choquet] [Cognet] [Bagatolli];
— nuclear processes: chromatin dynamic organization, nuclear domains, nuclear trafficking [Goud].

4 Limitations, caveats and open issues

Basic physical properties and models of transport and intracellular processes should be adapted to account from biological specificity of the cell and intracellular medium, briefly recalled in § 1.1; a short list follows for illustration. Other difficulties, and ways out, will be presented in [Lavalette].

■ **Crowding**

The interior of the cell is crowded with numerous organites, filaments, macromolecules and other objects, of large size and likely to interact with their neighborhood; intracellular processes are thus spatially localized and highly coupled, each with those occuring nearby [Luby-Phelps 2000]. Actually, *the intracellular medium is all but a dilute solution: usual thermochemical notions and relations fail* [Lavalette]. For instance, cytoplasm has presumably a gel-like structure; spaces remaining for pure water are of so small size (equal to the thickness of only a few hydration shells) that water is almost everywhere structured and should be regarded more as an additional ligand than as a plain solvent [Mentré 1995]. Also of importance is the role of counter-ions in the vicinity of charged domains, leading to complex electrostatics properties [Gelbart et al. 2000] with possible cooperative effects between counter-ions condensation (Manning condensation) and conformational transitions of macromolecules. Crowding moreover induces superimposition and mutual influence of chemical reactions and binding, physical bonding, steric hindrance and competitive modes of transport, preventing from investigating elementary processes in isolation.

■ **Non-equilibrium**

Another difficulty preventing from a straightforward application of thermodynamic relations comes from the *failure of local thermodynamic equilibrium and linear response hypotheses*, often encountered in intracellular processes. There is no longer equipartition of thermal energy and fluctuation-dissipation theorem is no longer valid. Coupling with far from equilibrium chemical reaction (typically the ubiquitous ATP-hydrolysis) might achieve selective excitation of some modes which breaks energy equipartition and gives an effective temperature to this mode far higher to the ambient temperature [Le Goff et al. 2002b]. Actually, in non-equilibrium situations, the very notion of temperature is ill-defined. These features strenghten the importance to work with *in vivo* data: it is difficult, if not impossible, to reproduce *in vitro* a non-equilibrium regime; for instance, far from equilibrium systems are sensitive to boundary processes. As a matter of fact, the situation is not even clear-cut: many processes are partly at equilibrium, partly far from equilibrium, so that usual thermodynamics (at equilibrium) is sometimes valid, sometimes a proper first approximation, and sometimes totally irrelevant. For instance, the native configuration of a protein is at equilibrium whereas motor protein functioning is essentially far from equilibrium.

■ **Compartmentalization and spatial organization**

The cell interior is an highly organized medium, with networks of filaments, complexes and macromolecular assemblies, compartments. This structured character —actually a functional organization yet to be fully understood— strongly affects chemical reaction kinetics: strong discrepancies between enzyme functioning *in vitro* and in organized medium, as the living cell, have been evidenced [Thellier et al. 2003]. Moreover, due to compartmentalization or yet with localization, most intracellular processes involved only a small number of molecules, leading to strong fluctuations and failure of mean field chemical kinetics) [Barkai and Leibler 2000] [Gonze et al. 2001]. For instance, a μM solution in a volume of 1 μ^3 corresponds to about 600 molecules (and less than 1 molecule for a nM solution): *the meaning of concentrations and gradients is thus highly questionable*, and it would undoubtedly be more relevant to reason at the level of single molecules.

■ **Complex kinetics**

Biochemical reactions are yet very rich, exhibiting as a rule nonlinear features like auto-catalysis

or enzymatic catalysis [Goldbeter 1996] [Cornish-Bowden 2004a]. The additional point to be here underlined is the strong modification of chemical kinetics (with respect to usual mass action and mean field kinetics) due to density fluctuations [Ovchinnikov and Zeldovich 1978], fractal substrate [Berry 2002], confinement [Sanfeld and Steinchen 2003], diffusion acting as a limiting step in the intracellular medium [Thellier et al. 2003] and collective effects in large complexes [Van Holde et al. 2000]. We have yet mentioned the relevance of fluctuations, due to the small number of molecules involved. Also of importance are cooperative effects (for instance binding of dimers [Frey et al. 2004], or the now acknowledged cooperative allostery [Monod et al. 1965]), competition between reactions or between reactions and physical processes (adsorption-desorption, diffusion), and influence of mechanical constraints on binding affinities [Victor et al. 2002].

■ **Collective behavior**

Beyond the case of coupled chemical reactions, collective behaviors are essential in achieving functions and their regulation. Many concerted steps, with many ingredients and competitive or cooperative mechanisms are to be taken into account in their description. The very characteristic feature of collective behaviors is the emergence of novel properties that are not foreseable from the individual properties. Let us cite for instance cooperative molecular motors [Jülicher and Prost 1995] and assembly of molecular motors and filaments [Surrey et al. 2001] [Frey et al. 2004].

■ **Networks**

A special instance of collective behavior is provided by networks of various type [Vandenbunder]; let us cite:
— actual networks of filaments forming the cytoskeleton, coupled with active processes [Amblard];
— protein-protein interaction networks;
— gene networks (regulation of expression of gene A by transcription products of gene B);
— network of coupled chemical reactions [Goldbeter 1996] [Thomas 1998].
Again, such networks (whether structural, functional, or dynamical) are almost impossible to reproduce *in vitro*, further motivating living-cell studies. Networking might achieve amplification of minute stochastic variations, or on the contrary suppress sensitivity to noise. Hence networks play a key role in the variability/robustness and self-organization of intracellular processes.

5 Conclusions and perspectives: a dialogue between biology and physics

The above presentation has put forward the following general points, that might now be used as guidelines in other investigations.

■ **Stochasticity and robustness**

Most intracellular processes involve out-of-equilibrium stochastic interactions. Stochasticity is not inconsistent with reproducibility. On the contrary, stochasticity is essential to get robust behavior. first when considering a large number of individuals and statistical behavior (law of large numbers, for instance) but also, of higher relevance in the cell, thanks to feedbacks, selection or dynamical stabilization (self-organization). A typical example is cellular homeostasis, which is ensured through feedback loops and regulatory networks rather than by deterministic programming, that would be dramatically sensitive to the least perturbation of the program or change in the inputs. More generally, assembly and interactions of imperfect, low-performance, noisy elements lead to a robust, efficient, predictable and reproductible global behavior.

■ **Multiscale organization**

Intracellular processes exhibit a multiscale organization, from atomic scale up to the whole cell scale. Different levels cannot be separated. Upper levels control smaller scales (that is not necessarily preserved nor reproducible *in vitro*). Multiscale organization is currently involved in self-organization. Here, far more, it is involved in the consistency between the different levels with respect to natural selection (optimality at any level). Hence this multiscale organization is a key difference between soft and living matter. This feature again points out the need of living-cells investigations and integrated, multiscale modeling, both to grasp the whole functional processes and, from a practical viewpoint, to bridge different experimental accesses to be unraveled. Actually, modeling should include at the same time a bottom-up way of assembling elementary components and mechanisms, a top-down reasoning by considering the biological function achieved, and up-and-down bridges between components, parts and levels of organization provided by feedbacks loops: this is what we summarize as a "multiscale approach".

■ **Generic modeling and biological details**

Physical modeling more or less relies on the existence of generic principles, mechanisms or architecture whereas biological experiments often put forward the key role of highly specific components. A possible way to reconcile these opposite viewpoints is to consider at the same time the existence of general structural of regulatory (dynamic) principles, as for instance feedbacks loops and multiscale organization mentioned in the previous point, and the highly specific features of their actual implementation (e.g. using different molecules in different contexts).

A major difficulty is that alternative explanations often available, equally plausible, sustaining a necessary interplay between modeling and experiments to discriminate between possible scenarios. Another one is that almost any component involved in a functional process has the ability to suppress or at least modify the function, with no prejudice of its actual basic and causal role in this function (besides, causality itself is quite a meaningless concept in case of a systemic behavior).

■ **Biological specificity**

It reflects at least in the following points:
— involvement of genome;
— imprinting of Evolution, natural selection and ensuing adaptation in present biological systems;
— adapted and sometimes unexpected time scales (for instance, transport might be either far faster than diffusion, thanks to active transport, or far slower than molecular scales, due to membranes)
— joint evolution and adaptation of the different levels of organization typically led to situations where all levels are consistently coupled and cannot be investigated separatedly: upper levels regulate and even shape the lower (smaller) levels [Victor et al. 2002].
In a word, a biological object has *functions rather than properties*, and their proper implementation is ensured by many checkpoints and regulatory loops.

■ **Natural selection**

Natural selection supports to look for optimized/optimal functioning [Kacser 1957] [Kauffman 1993] [Cornish-Bowden 2004b]. In particular, it is interesting to notice the double meaning of adaptation:
— physical adaptation refers to evolution of the state to match evolving external conditions.
— biological adaptation refers to evolution of the matching conditions, of the interactions, to maintain the same internal state despite changing environment.
At a shorter time scale, "natural selection" (jointly with stochasticity) is often at work inside the cell (kinetic race between parallel pathways, competitive inhibition) or between cells (immune systems, and possibly cell differenciation).

■ **A way back from biology towards physics**

In conclusion, while claiming the relevance of physical concepts and modeling tools for understanding intracellular processes, it is also of importance to underline, conversely, that intracellular processes are the most valuable testbed for the development of nonequilibrium statistical mechanics (understanding heat flow and conditions for transport at a microscopic level, conditions for equipartition, extension of fluctuation-dissipation, non-equilibrium temperature ... [Dorfman 1999]) A typical example is the so-called "TASEP" model (totally asymmetric exclusion process, Fig. 9) initially introduced in a biological context [MacDonald et al. 1968] (to account for processing of mRNA templates in the ribosome, and trafficking along bio-filaments), but nowadays providing an analytically soluble toy-model for investigating non-equilibrium statistical mechanics concepts and methods [Derrida 1998] [Frey et al. 2004].

It is actually a dialogue between biology and physics that deserves to be developed.

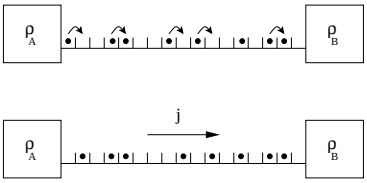

Figure 9: Totally Asymmetric Simple Exclusion Process: the particles jump at random from left to right, with double occupancy forbidden; boundary conditions are prescribed through the densities ρ_A and ρ_B of particle reservoirs, with $\rho_A \neq \rho_B$ enforcing non-equilibrium stationary state.

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