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 equilibrium, each degree of freedom has a kinetic energy 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 [1] 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" [Misteli 2001a]. Intracellular examples are numerous: rafts inside phospholipidic membranes [Bagatolli], nuclear compartments, synapses [Choquet], mitotic spindle [Karsenti], [Nedelec], [Surrey et al. 2001], cell polarization [Wedlich-Soldner and Li 2003]. 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 emerge from interactions and dynamic collective behaviors. In short, self-organization refers to spontaneously (i.e. without an external monitoring nor an imprinted program) concerted behaviors between several elements.

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

Cell includes different subsystems (nucleus, mitochondria), substructures (compartments, membranes), 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.

1. Consider for instance an oscillator of position 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 evolution of the slow mode.

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 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 or invariances observed on the 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 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*. 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 [Goldbeter 1996] [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] and actine polymerization;
— 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) data in terms of microscopic mechanisms and parameters.

■ **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 [Turing 1952], 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.
— *Integrated multiscale modeling*, at the same time holist and reductionist: from DNA up to chromosome, actine network, cooperative molecular motors, pattern formation...
— *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 in the modeling.

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]. Such normal diffusion is associated with a diffusion law $R^2(t) \sim Dt$ where D is the diffusion coefficient 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].
— in dimension 2, within membranes [Choquet] [Cognet] [Bagatolli];
— in dimension 3; it is then possibly confined (see Fig. 3). It 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 a description is thus relevant at a scale 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 through pores and gated ionic channels. The field is here induced by the ionic concentration gradient across the membrane [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 yield processes at almost macroscopic time-scale (microsecond for an action potential).

■ **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, that might originates in [Bouchaud and Georges 1990] [Shlesinger et al. 1999] [Laguës and Lesne 2003]:
— fractal substrate, for instance diffusion in a porous medium with pores of all sizes (then $\gamma < 1$);
— trapping and arbitrarily long residence times (then $\gamma < 1$);
— steps of all sizes, what is called Levy flight (then $\gamma > 1$) [Ott et al. 1990] [Klafter et al. 1996]);
— correlated motion ($\gamma > 1$) or anti-correlated motion ($\gamma < 1$).

■ **Active processes**

Typical examples of active (i.e. ATP-consuming) processes are the oriented motion of motor protein along filaments [Jülicher 2003] or 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.

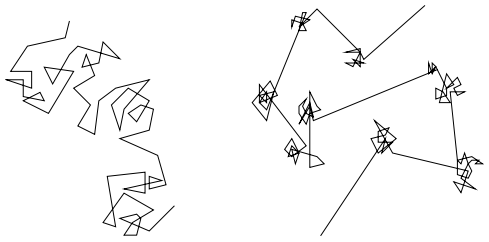

Figure 1: Sketch of a typical individual trajectory for (Left) Brownian motion (normal diffusion, $\gamma = 1$) and (Right) Levy flight (anomalous diffusion, $\gamma > 1$).

■ **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 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 [Laguës and Lesne 2003], and they should be chosen accordingly in practical situations (modeling from experimental data, simulations).

■ Historically the first model is the **diffusion equation**: $\partial_t n = D\Delta n$ where $n(\vec{r},t)$ is the local instantaneous concentration of diffusing particles. This macroscopic deterministic irreversible equation comes from the conservation law $\partial_t n + \vec{\nabla}.\vec{j} = 0$ 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; 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 extensions are of relevance in cellular biology.

Electrodiffusion: the constitutive equation (number density n, individual charge $q = Ze$) now writes $\vec{j} = -D\vec{\nabla}n + q\vec{E}n/\gamma$ to be plugged into the still valid conservation law. The first term in r.h.s. 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 $\vec{j}_{chem} = \chi(a)\, n\, \vec{\nabla}n$ where a is 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. 2.

■ In between, various **mesoscopic stochastic descriptions** have been developed, bridging atomic and observation scales:
— kinetic theory reducing to Boltzmann equation[2] 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;

2. 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 $\vec{a}(\vec{r},t) = q\vec{E}/m$ in an electric field $\vec{E}$ where q is the particle charge).

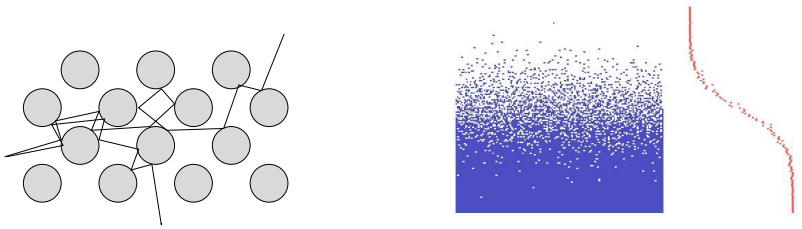

Figure 2: (Left) microscopic deterministic model of diffusive transport (Lorentz gas model) where a light particle experiences numerous elastic collisions on spherical scatterers. Defocussing character of the collisions induces molecular chaos, in turn generating a diffusive motion and supporting a statistical approach. (Right) microscopic simulation of diffusion (random walks) and spatially average profile, tending to the solution of diffusion equation as the number of particles tends to infinity.

— 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 concentration $n(\vec{r}, t)$.

■ More generally, **Langevin equations**: $dz/dt = f(z) + \xi$ where $\xi(t)$ is a white noise (defined through its moments $\langle \xi(t) \rangle = 0$ and $\langle \xi(t)\xi(s) \rangle = \delta(t-s)$) are used to account for the influence of thermal fluctuations on systems evolving according to $dz/dt = f(z)$ at zero temperature. Such description of "Brownian motion in a potential" is at the basis of Kramers rate theory [Kramers 1940] [Hänggi et al. 1990], allowing to compute the characteristic time $\tau_K \sim e^{\Delta U/k_B T}$ 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 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.

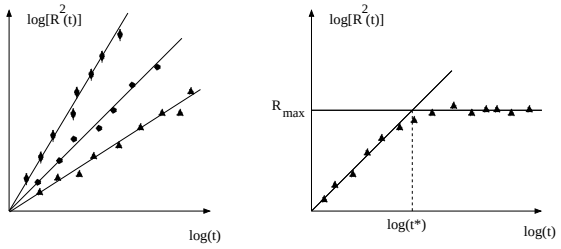

Figure 3: Sketch of the time dependence of the root-mean-square displacement $R(t)$. (Left) in case of diffusion law $R^2(t) \sim t^\gamma$, corresponding to linear slopes in a log-log plot $\log R^2(t) = \gamma \log t + c$; normal diffusion is associated with $\gamma = 1$ (circles) whereas diffusion is said to be anomalous if $\gamma \neq 1$; (Right) in case of confined motion; the crossover time t^* is related to the linear size R_{max} of the domain through $R_{max}^2 \sim Dt^*$ (for normal diffusion).

■ **Trajectory analysis**

It is now possible to record single trajectories, either those of proteins of interest properly tagged with a fluorescent tail [3], 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 records, one computes the root-mean-square displacement $R(t)$ with $R^2(t) \equiv \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle$

3. 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].

(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. 3 [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...) are described on Fig. 4 [Laguës and Lesne 2003].

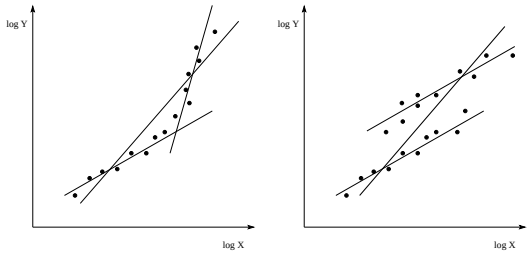

Figure 4: Caveats in experimental determination of a scaling law $Y \sim X^a$. (Left) ignoring a crossover ($Y \sim X^{a_1}$ if $X < X^*$ and $Y \sim X^{a_2}$ if $X > X^*$) or (Right) ignoring the existence of different subclasses ($Y \sim A_1 X^a$ and $Y \sim A_2 X^a$) yields a meaningless effective exponent a_{eff} (slope of the bold line).

■ **Exploitation of FRAP data**

Other observation techniques, for instance FRAP[4], 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 data with mechanisms, i.e. to interpret quantitatively the data in terms of biological processes and their parameters (rates, velocities, concentrations, sizes).

■ **Relevance and limits 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 fundamentally, they capture the principles and essential mechanisms, notwithstanding details, specific features, and individual exceptions. Detailed modeling is generally not enough robust to be really explanative, since somehow arbitrarily kept details might unduly play a key role while other let apart are ignored. The main flaw of generic models is that they 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 faithfully be 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.

Nevertheless, at the stage of integrated modeling (modeling of whole functional processes), biological specificity should be taken into account. It reflects mainly 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);
— multiscale organization: joint evolution and adaptation of the different levels of organization typically led to situations where all levels are consistently coupled and cannot be investigated

4. 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. Recovery of fluorescence in this region reflects the arrival of unbleached particles from outside, 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].

separatedly: upper levels regulate and even shape the lower (smaller) levels [Victor et al. 2002]. 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 unravelled.

A last point deserves some emphasis: the investigations of stochastic models, derived from the basic models presented in § 2.2, demonstrate that 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 this program or change in the inputs.

3 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 then spatially localized and highly coupled, each with these occuring nearby [Luby-Phelps 2000]. Actually, the intracellular medium is all but a dilute solution [Lavalette]. 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 [Gelbart et al. 2000] with possible cooperative effects between counter-ions condensation (Manning condensation) and conformational transitions of macromolecules. In consequence, usual thermochemical notions and relations fail. Crowding moreover induces a superimposition of chemical reactions and binding, physical bonding, steric hindrance and competitive modes of transport.

- **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, compartmentalization is associated with confinement, so that 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. Confinement also affects kinetic rates [Sanfeld and Steinchen 2003].

- **Non-equilibrium**

  The difficulty here 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 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 nonequilibrium situations, the very notion of temperature is ill-defined. This feature strenghtens the importance to work with *in vivo* data: it is difficult, if not impossible, to reproduce *in vitro* a nonequilibrium regime.

■ **Collective behavior**

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. Their most remarkable feature is the emergence of novel properties 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]:
— 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 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 the sensitivity to noise, hence plays a key role in the variability/robustness and self-organization of intracellular processes.

4 Perspectives: a dialogue between biology and physics

While claiming the relevance of physical concepts and modeling tools for understanding intracellular processes, it is also of importance to underline, conversely, intracellular processes are the most valuable testbed for the development of nonequilibrium statistical mechanics. A typical example is the so-called "TASEP" model (totally asymmetric exclusion process, Fig. 7) 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 developped.

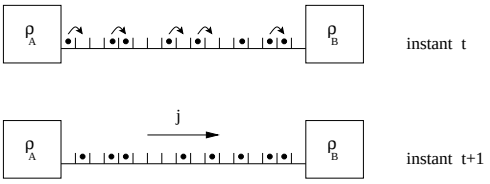

Figure 7: Totally Asymmetric Simple Exclusion Process: the particles jump at random from left to right, with double occupancy forbidden; boundary conditions are prescribed through the densities ρ_A and ρ_B of particle reservoirs, with $\rho_A \neq \rho_B$ enforcing non-equilibrium stationary state.

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