

Physical analysis of chromatin and chromosome movements : from classical theories to open challenges.

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The general scope of this workshop mentions the seminal work of Einstein about Brownian motion. One century later, the challenge to physicists is no more to assess the atomic nature of the matter but to extend Einstein theory to *living matter*, with a fundamental questioning about the power and limits of physical modeling for analyzing and understanding intranuclear motions and transport processes, in particular those involving chromatin and chromosome.

Introduction

Part of the discovery of the biologist R. Brown in 1827, while observing the wriggling motion of particles suspended in a fluid, was to preclude an explanation invoking the living nature of the particles : the fact that he used pollen grains intervenes only to fulfill the required (sub)micronic size necessary to observe this purely random thermal motion. Life is today back in the “*investigations about Brownian movement*” [Einstein 1905, 1956], and it is much fruitful to delineate in what respect Einstein theory and its modern developments are relevant inside the cell nucleus. This requires a careful analysis of the *limits of validity and implicit hypotheses* involved in standard diffusion theory, to be compared with the actual context and features of intracellular motions.

Basics on diffusion theory

Several theoretical approaches of diffusion and Brownian motion have been developed since Einstein work, according to the scale of description : they range from deterministic (and reversible) Newtonian equations, at the microscopic level of colliding molecules, to different stochastic modelings, at mesoscopic scales, to the phenomenological (deterministic but irreversible) diffusion equation, at macroscopic scale. They are related bottom-up through well-identified approximations and projection techniques or coarse-grainings, thus forming one consistent diffusion theory, involving a unique parameter D (the diffusion coefficient) [Laguës and Lesne 2003]. We recall the *normal diffusion law* describing the mean-square displacement $R^2(t)$ of a single diffusing object :

$$\begin{aligned} \text{in a membrane (dimension } d = 2) : & \quad R^2(t) \sim 4Dt \quad \text{as } t \rightarrow \infty \\ \text{in a volume (dimension } d = 3) : & \quad R^2(t) \sim 6Dt \quad \text{as } t \rightarrow \infty \end{aligned}$$

(the general formula being $R^2(t) \sim 2dDt$ in dimension d). It is associated with the equation $\partial_t n = D\Delta n$, called the *equation of diffusion* at the level of (noninteracting) particle population, $n(\vec{r}, t)$ being then the concentration at position \vec{r} and time t , and the *Fokker-Planck equation* at the level of a single diffusing particle, $n(\vec{r}, t)$ being then the spatial distribution of probability of this particle at time t (the straightforward identification of these two equations is supported by the law of large numbers provided the particles are uncorrelated). D is thus measured in m^2/s (or related unit $\mu\text{m}^2/\text{s}$, more relevant for chromatin, since D never exceeds $10^{-3} \mu\text{m}^2/\text{s}$ for chromatin regions [Spector 2003], whereas D is around $10^{-10} \text{m}^2/\text{s}$ for proteins, and around $10^{-8} \text{m}^2/\text{s}$ for ions).

Einstein derived a useful relation $D = \mu k_B T$ (now named after him) relating the characteristic thermal energy $k_B T$, the diffusion coefficient D and the mobility μ of the diffusing particle. The inverse μ^{-1} is the friction coefficient of the particle, which might be computed in the hydrodynamic regime, e.g. $\mu^{-1} = 6\pi a \eta$ (*Stokes law*) for a spherical particle of radius a in a fluid of dynamic viscosity η .

Theoretical works more recently extended beyond normal diffusion and also describe correlated, trapped or otherwise constrained motions, accounting for *anomalous diffusion laws* $R^2(t) \sim t^\gamma$ with $\gamma \neq 1$. One speaks of *sub-diffusion* if $\gamma < 1$ (trapping with no finite characteristic trapping time, diffusion on a fractal substrate) and of *super-diffusion* if $\gamma > 1$ (arbitrarily long deterministic steps, steric hindrance as in self-avoiding walks) [Bouchaud and Georges 1990] [Havlin and Ben Avraham 2002]. Let us detail more explicitly two instances. Anomalous diffusion might originate in an *anomalous distribution of the size of elementary steps* (i.e. steps occurring in a unit time); in case when steps of all sizes might occur, one obtains a super-diffusive motion. More precisely, if the step-size distribution behaves as $p(a) \sim a^{-(1+\mu)}$ at large sizes a , then $R^2(t) \sim t^{2/\mu}$. One recovers normal diffusion for $\mu > 2$. Another origin for anomalous diffusion is an *anomalous distribution of time steps* (e.g. in case of a transient trapping). In case when steps of all durations might occur, while the step-size is fixed or normally distributed (square average a^2), one obtains a sub-diffusive motion. More precisely, if the step-duration distribution behaves as $p(\tau) \sim \tau_0^\alpha \tau^{-(1+\alpha)}$ at large durations τ then $R^2(t) \sim a^2(t/\tau_0)^\alpha$ for $\alpha < 1$ (where τ_0 is some reference time). One recovers normal diffusion for $\alpha > 1$, and gets $R^2(t) \sim a^2(t/\tau_0)[\log(t/\tau_0)]^{-1}$ for the special case $\alpha = 1$ [Bouchaud and Georges 1990].

At least, a merit of theoretical approaches is to bring out some key features and caveats :

- Diffusion equation and the associated Fick law $\vec{j} = -D\nabla n$ are statistical expressions : there is *no diffusion force* acting on the particles. It is only in a mesoscopic modeling that one can introduce an effective “Langevin force” accounting for the effect of thermal noise.
- There is *no way to define a diffusion velocity*; the naive definition $v(t) \equiv R(t)/t$ yields an apparent velocity $v(t) \sim 1/\sqrt{t}$ that strongly varies with the time-scale of observation (it is physically truncated for $t \rightarrow 0$ at a value equal to the thermal velocity of the particle).
- The trajectory, at mesoscopic scale where one does not distinguish individual water molecules, is *essentially random* : its noisiness is the very core of the phenomenon. Nevertheless, care should be taken to distinguish fully random motion (independent isotropic elementary steps) and more general randomness. *Stochasticity does not preclude some time correlations*, and the whole range of behaviors from purely random to deterministic can be encountered.

Limitations of standard theory

Intracellular motions are most often out of the scope of standard theory, for various reasons :

- relevant times are *finite* (in contrast to the asymptotic regime $t \rightarrow \infty$ of the diffusion laws) ;
- only *small numbers* of particles are involved and their density is too high to ignore their mutual interactions (in contrast to the large population of independent particles underlying the Fick law) ;
- intracellular medium exhibits a complicated geometry and motions are likely to be *strongly confined*; moreover there is a possible alternative between 1-dimensional (on filaments or DNA), 2-dimensional (on membranes) and 3-dimensional (in the bulk) motions [Stanford et al. 2000] ;
- another difference with the plain solvent considered in Brownian motion theory is intracellular (or intranuclear) crowding ; moreover water is presumably *partly structured* (in contrast to the plain solvent considered in Brownian motion theory) ;
- diffusing objects are charged, hydrated and able to undergo conformational transitions ;
- competition with chemical reactions is to be taken into account : it might induce an uptake or a trapping of the diffusing species (“*reaction-limited diffusion*”) ;
- diffusion is also “positively” coupled with equilibrium chemical reactions (*reaction-diffusion*)

processes, inducing pattern formation and wave propagation);

- thermal diffusion is alternatively coupled with *nonequilibrium* chemical reactions (ATP-hydrolysis, translocations), generating what is called *active processes*. It points out the importance that a cell and its nucleus are open systems travelled across by various fluxes (energy, matter, charges).

Experimental data analysis

A main point to be underlined is that analysis of experimental data on chromatin and chromosome movements *requires a prior modeling* of these movements, in order to turn observations into meaningful parameters about the local organization and transport mechanisms. Using fluorescent probes also requires a model for the interaction between the probe and the investigated medium, to extract local informations from the motion of the probe. Let us illustrate this point for typical experimental techniques (not speaking of optical biases in data acquisition, photobleaching, substrate uptake and spurious complexation of the probes, among other difficulties).

Although invaluable to form a proper intuition on the phenomena, direct visual observation (e.g. of “painted chromosomes”) has in other respects a limited value : for instance, it appears to be difficult to distinguish steady states and stable equilibrium states : if fluxes (in and out the observed region) are not visualized, both cases will correspond to a seemingly fixed image with no apparent motion.

In FRAP studies (*Fluorescence Recovery After Photobleaching*, [Misteli 2001]), it is necessary to evaluate the bounded fraction (contributing to fluorescence but not to fluorescence recovery, which occurs exclusively thanks to the diffusion of new particles into the photobleached region). It is also essential to discriminate, in the very procedure of data exploitation, between 1D or 3D diffusion [Stanford et al. 2000], or between plain normal diffusion, possibly with an effective coefficient (homogenized model, [Siggia et al 2000]), biased diffusion and active motions. This is done by indirect inference, by determining which model best fits the data.

In SPT studies (*Single Particle Tracking*), the trajectory $\vec{r}(t)$ of a single particle is observed at $N + 1$ discrete times $0, \Delta t, \dots, N\Delta t$; for the sake of simplicity, Δt will be fixed in what follows and t will denote the integer label of the recording time.

The mean-square displacement $R^2(t)$ is computed as a time average (i.e. by averaging the squares distances $|\vec{r}(t+n) - \vec{r}(n)|^2$ obtained when scanning the trajectory, by varying n [Saxton and Jacobson 1997]. It is to note that this definition makes sense only when there is no average motion, i.e. no drift : $\langle \vec{r}(t) - \vec{r}(0) \rangle = 0$. More generally, the mean-square displacement is defined as a *variance* :

$$R^2(t) \equiv \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle - |\langle \vec{r}(t) - \vec{r}(0) \rangle|^2 = \langle |\vec{r}(t) - \vec{r}(0) - \langle \vec{r}(t) - \vec{r}(0) \rangle|^2 \rangle \quad (1)$$

$$\approx \frac{1}{N-t+1} \sum_{n=0}^{N-t} |\vec{r}(t+n) - \vec{r}(n)|^2 \quad (2)$$

Biased diffusion corresponds to the superimposition of a deterministic drift, with velocity \vec{v} , and a diffusive motion of diffusion coefficient D . At short times, the diffusive component is overwhelming (hence the slope of $\log t \rightarrow \log R^2(t)$ should be equal to 1) whereas the drift is the long-term leading behavior (hence the slope of $\log t \rightarrow \log R^2(t)$ should be equal to 2 at long times); the crossover is observed for $2dDt \sim v^2 t^2$, namely around $t_c \sim 2dD/v^2$. More precisely, the drift is evidenced from the SPT data by computing the average motion $\vec{m}(t) \equiv \langle \vec{r}(t) - \vec{r}(0) \rangle$, and observing a linear region, of slope v_i in the plot $t \rightarrow m_i(t)$ for each component $i = 1, \dots, d$ (the dimension is $d = 3$ in case of intranuclear motions).

Confinement (also known as *constrained diffusion* [Marshall et al. 1997]) reflects in a saturation of the mean-square displacement as a function of t : $R^2(t) \approx R_\infty^2 = cte$ for large t . Typically, one observes a short-time diffusive regime, where the slope of $t \rightarrow R^2(t)$ equals $2dD$ (in spatial dimension d) and a crossover towards a long-term saturation to a constant value. If the motion

is actually a free diffusion in a limited region, the motion at short times corresponds to the same diffusive motion as in an infinite medium (the particle does not yet experience the presence of the boundaries), and the crossover should occur around $t_c = R_\infty^2/2dD$. Moreover, for $t \gg t_c$, the distribution of the diffusing particles is uniform in the bounded region; in case of a spheric region in dimension d (i.e. a disk in $d = 2$ or a sphere in $d = 3$), a simple computation allows to relate its radius r to the saturation value of the mean-square displacement : $r = R_\infty \sqrt{(d+2)/d}$.

Multiscale analysis of the trajectories allows to evidence the superimposition of different diffusion regimes, of possibly different natures, for instance the existence of a confined diffusive motion, of coefficient D_{micro} , in regions that themselves diffuse, with a coefficient D_{macro} ; in other cases, the coefficient D_{macro} might arise as an effective coefficient accounting for characteristic (finite) trapping time when the regions are fixed and the diffusing particles jump randomly across their boundaries [Fujiwara et al. 2002].

Correlations in the motion can be measured from the SPT recording : at time-scale k (i.e. investigating correlations between steps of duration k), the time-correlation function can be estimated as

$$C_k(t) = \langle [\bar{r}(t+k) - \bar{r}(t)].[\bar{r}(k) - \bar{r}(0)] \rangle - |\langle \bar{r}(k) - \bar{r}(0) \rangle|^2 \quad (3)$$

where as above, $\langle . \rangle$ is computed in practice as a time-average along the trajectory. Finite-range correlations reflect in an exponential behavior $C(t) \sim e^{t/\tau_c}$, defining the correlation time τ_c , roughly estimating the range of the correlations. In this case, correlations can be integrated in an effective diffusion coefficient $D_{eff} = D \sum_t C_1(t)$ (larger than D in case of constructive correlations, smaller than D in case of anti-correlations) [Lesne 1998]. Long-range correlations (with a large or even diverging correlation time) typically reflect in a power-law behavior for $C(t)$, and the diffusion is anomalous [Lesne 1998].

More general instances of *anomalous diffusion* can be evidenced from SPT studies. In this case, prior clue about diffusion law helps, since the same data can possibly be explained, say, with an effective coefficient D_{eff} or an anomalous diffusion law with exponent $\gamma < 1$ (unless a specially long recording and error-less measurements are available, allowing to discriminate with certainty the normal power law $R^2(t) \sim 2dDt$ and an anomalous power law for $R^2(t)$). It is besides to be underlined that *estimating faithfully a power-law behavior is in general a very delicate matter* [Laguës and Lesne 2003].

A prerequisite of all the above statistical estimates is the *stationarity* of the recorded motion : it is often valuable to discard the first steps (as a rule of a thumb, for a normal diffusion motion, an initial segment of the trajectory of duration equal to a few correlation times should be discarded, since the correlation time coincide with the relaxation time, i.e. the duration required to reach a stationary statistics). Moreover, stationarity can be checked by computing the averages of relevant observables in a moving window of duration $n \ll N$, and by testing the time-independence of the local averages thus obtained.

Computing the *error bars* requires a model for the joint statistics (at multiple-time) . For independent steps (purely random walk), the relative error in the estimation of averages decreases as $1/\sqrt{N}$. If the motion is time-correlated, with a characteristic time N_c (in the time-unit of the recording), the error now decreases as $\sqrt{N_c/N}$; the intuitive explanation of this result is that one now observes only N/N_c independent steps [Bouchaud and Georges 1990].

Let us conclude this section with a wide-scope guideline : general method to the prior (or rather simultaneous) validation of the diffusion model used to interpret the data is the *surrogate data method* : the supposed model is simulated, generating a sample of associated motions; then the statistics of various observables describing this motion is compared to the measured data, and a statistical test is performed to check whether the modeling hypothesis can be accepted or has to be rejected in favor of another modeling.

Towards theoretical approaches to intracellular diffusion and transport processes

The above list of intranuclear specificities leading to the failure of *standard* diffusion theory does not necessarily preclude all theoretical attempts to describe and even explain intranuclear motions. On the contrary, it should be taken as a set of specifications, providing guidelines for such an enterprise. Namely, it delineates biological features to be essentially taken into account. Moreover, theoretical approaches have to somehow include the biological functions for the realization of which the involved components have specifically adapted during Evolution.

To cite but one successful example, modeling *active processes* (e.g. motion of motor proteins) illustrates such a theoretical achievement. It requires to couple thermal motion in an asymmetric energy landscape (scanned by the motor as it moves along its substrate, typically actine or microtubules) with a nonequilibrium chemical reaction (currently ATP-hydrolysis). It is now acknowledged that *both an oriented substrate and nonequilibrium fluctuations* are necessary to break detailed balance and get an oriented motion : ATP hydrolysis fuels the entropy cost of an otherwise thermally activated motion [Jülicher et al. 1997]. Theoretical investigations then allow to bring out organization principles, e.g. the collective behavior of an assembly of motor proteins [Jülicher and Prost 1995].

To summarize, diffusive motion reveals the obstacles encountered, thus might provide an indirect (i.e. requiring an underlying modeling to be interpreted quantitatively) access to the organized structure of the cell or cell nucleus. Major challenges, both in theoretical investigations and modeling for data analysis purposes, come from the fact that this organization is *dynamic* (thus requiring a multiscale modeling, accounting from motions and rearrangements at different space and time scales) and *functional* (thus requiring to describe the interplay between diffusive motion, various binding events and active processes).

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